Supplementary Information
(Supplementary Methods and Results)

Multi-site Assessment of Precision and Reproducibility of Multiple Reaction Monitoring-based Measurements of Proteins in Plasma

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Supplementary Methods: SOP for NCI CPTAC Consortium-Wide Multiple Reaction Monitoring (MRM) Experiment

Experimental Design and Statistics Verification Studies Working Group

Aims: The study described in this SOP is designed to accomplish the following three aims:

1. To assess transferability, reproducibility and analytical performance of MRM-based assays across CPTAC sites for the purpose of quantifying target proteins in human plasma.

2. To generate a data set for the WG statisticians to define and refine metrics of LOD, LOQ, accuracy, and precision for an MRM-based assay designed to quantify target proteins in human plasma.

3. To deliver to the proteomics community a set of reagents and protocols for MRM-based assays for the quantitation of target proteins in plasma.

Background: This study is designed to satisfy the objectives stated above. In order to isolate variability in the quantitative measurements introduced by sample preparation and instrumental analysis, a 3-part study is outlined below. Each part will entail monitoring signature peptides derived from seven target proteins (SOP Table A) that will be quantitatively assayed by LC-MRM/MS against a background of human K_2EDTA plasma. All experiments will consist of a 9-point standard curve ranging in concentration from 500 to 1 fmol/µL. In addition, synthetic signature internal standard (IS) peptides uniformly labeled with a $^{13}C/^{15}N$ amino acid at their C-terminus will be spiked into all plasma samples at a constant concentration of 50 fmol/µL.

Study I: Addition of [$^{12}C/^{14}N$] and [$^{13}C/^{15}N$] synthetic peptides into diluted, digested human plasma.

In Study I, synthetic [$^{12}C/^{14}N$] and [$^{13}C/^{15}N$] signature peptides will be spiked into digested, human K_2EDTA plasma and analyzed by LC-MRM/MS. All sample preparation will be performed at NIST prior to distribution of the sample kits. Results from Study I will yield the optimum LOD/LOQ for each peptide because the design isolates the variability of recovery due to protein digestion and subsequent sample handling.

Study II: Single site digestion of target proteins spiked into diluted, digested plasma

In Study II, human K_2EDTA plasma and an equimolar mixture of the seven target proteins will be digested **separately** with trypsin at a single site (NIST). The target protein digest mixture will be added to diluted, digested plasma at equivalent concentrations used in Study I. Similarly, the same concentration of IS peptides (internal standard) will also be added (50 fmol/µL). Bulk digestion of the target proteins will control for unknown digestion efficiency across decreasing concentration points. Results from Study II will yield LOD/LOQs that take into account variability of digestion efficiency and peptide recovery.

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Study III: Simulation of a verification study across CPTAC sites

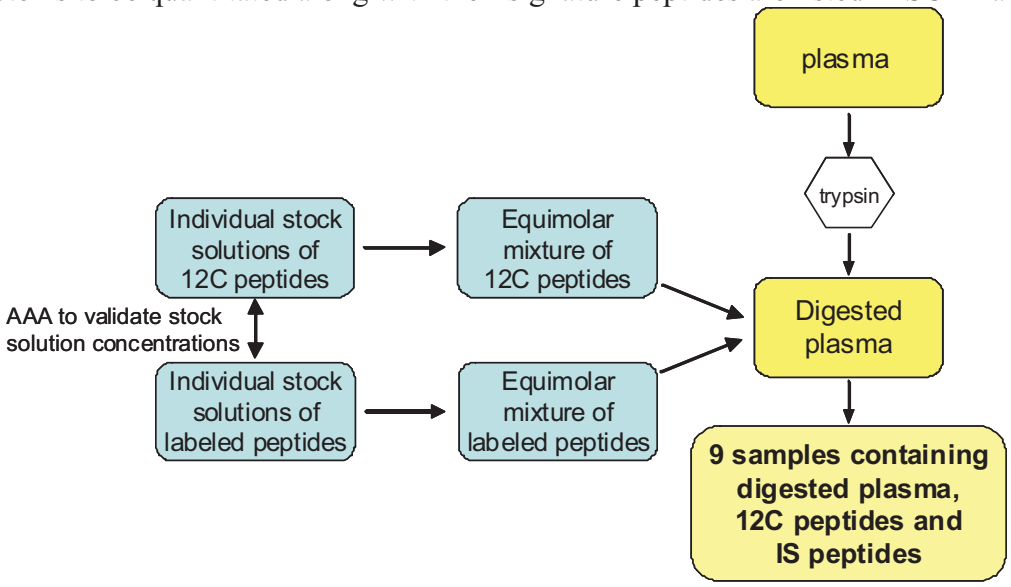
In Study III, the seven target proteins will be spiked into human K_2EDTA plasma prior to digestion. Preparation of the identical standard curve as applied in Studies I and II will be performed at NIST prior to distribution of the sample kits. A detailed SOP for digestion, subsequent sample handling, and instrumental analysis will be included for each lab. **Each participating CPTAC site must strictly follow the specifications of the SOP as outlined.** Results from Study III will mimic a "real world" verification study in which each site is responsible for sample preparation. Study III also rigorously tests the transferability and reproducibility of MRM-based assays for target proteins in plasma across multiple institutions.

Data from each of the studies will be analyzed similarly. The experimentally determined molar concentration of the spiked peptide or protein will be estimated and compared to its theoretical value for accuracy. A logarithmic plot of response versus known concentration from each of the three 9-point standard curves will be used to evaluate the linearity of the MRM measurement across the range of spiked peptide or protein concentrations thus providing evidence of a quantitative measurement process. Replicate analyses of the spiked plasma samples will provide estimates of assay precision (standard deviation and % CV), plus LOQ and LOD will be determined at defined signal-to-noise ratios (S/N). Blank runs of digested plasma with labeled peptides will provide estimates of chemical background levels in the absence of signature peptide peaks. Furthermore, an estimate of carryover will be determined by running a series of gradient HPLC washout runs. Finally, variation across CPTAC sites will be assessed for each of these characteristic analytical metrics.

CPTAC Study I: Addition of [^{12}C/^{14}N] and [^{13}C/^{15}N] synthetic peptides into diluted, digested human plasma.

Methods: Sample Preparation for Study I

All sample preparation and distribution of sample kits to participating CPTAC laboratories will be performed at NIST. SOP Figure A illustrates the sample preparation workflow. The seven target proteins to be quantitated along with their signature peptides are listed in SOP Table A.



SOP Figure A. Sample preparation workflow for Study I. (IS, internal peptide standards)

SOP **Table A.** Target proteins and their [$^{12}C/^{14}N$] signature peptides.

Target Protein	Species	Signature Peptide		
		Identifier	Sequence*	MH+ (mono)
prostate specific antigen (PSA)	human	bi0037	LSEPAELTDAVK	1272.7
		bi0161	IVGGWE<u>C</u>EK	1077.5
horseradish peroxidase (HRP)	horseradish	bi0166	SSDLVALSGGHTFGK	1475.7
leptin (LEP)	murine	bi0167	INDISHTQSVSAK	1399.7
myelin basic protein (MBP)	bovine	bi0169	HGFLPR	725.4
		bi0170	YLASASTMDHAR	1322.6
myoglobin (MYO)	equine	bi0171	LFTGHPETLEK	1271.7
aprotinin (APR)	bovine	bi0173	AGL<u>C</u>QTFVYGG<u>C</u>R	1488.7
C-reactive protein (CRP)	human	bi0231	ESDTSYVSLK	1128.5
		bi0202	GYSIFSYATK	1136.5
		ni0001	YEVQEVFTKPQLWP	1820.9

* cysteines are S-carboxyamidomethylated

Appendix A outlines the digestion procedure that will be employed for the bulk digestion of non-depleted plasma. Urea will be used as denaturant in all digestions and cysteine residues will be alkylated using iodoacetamide. After digestion and off-line desalting, digests will be lyophilized to dryness and resuspended in an aqueous solution containing 3% acetonitrile and 5% formic acid. A dilution of the final reconstituted samples will reduce the acetonitrile and formic acid to <1 % and 0.6 %, respectively No additional sample clean-up will be required.

Two kits containing all the necessary samples required to implement the SOP will be prepared by NIST: a tuning kit and a sample kit.

The tuning kit for Study I will contain:

- an equimolar mixture of the labeled IS peptides and unlabeled signature peptides for chromatographic and MS optimization

The sample kit for Study I will contain:

- a quality control (QC) mixture composed of the unlabeled and labeled synthetic peptides
- unspiked digested plasma
- digested plasma spiked with the isotopically labeled IS peptides
- digested plasma spiked with labeled IS peptides at a constant level and [$^{12}C/^{14}N$] synthetic peptides spanning a concentration range of 2.5 orders of magnitude

Details of each kit's contents according to sample type (tuning, QC, and plasma containing) are as follows:

A. Tuning Samples for Study I

a. 500 fmol/µL mixture of all 11 unlabeled signature peptides and 11 labeled internal standard peptides
   i. one 50 µL aliquot supplied
   ii. supplied in 1 % formic acid in water

B. QC Sample (Sample I-QC)

a. Equimolar mixture of the 11 unlabeled and 11 labeled synthetic peptides at 50 fmol/µL
   i. one 25 µL aliquot supplied
   ii. supplied in 1 % formic acid in water

C. Plasma Samples for Study I

a. Unspiked, digested human plasma (Sample I-Blank)
   i. one 25 µL aliquot supplied
   ii. plasma diluted approximately 60-fold to a total plasma protein concentration (prior to digestion) of approximately 1 µg/µL
   iii. supplied in 1 % formic acid in water (after desalting by SPE)

b. Digested human plasma spiked with labeled IS peptides (Sample I-A)
   i. two 25 µL aliquots supplied
   ii. plasma diluted approximately 60-fold to a total plasma protein concentration (prior to digestion) of approximately 1 µg/µL
   iii. 11 labeled IS peptides spiked at a concentration of 50 fmol/µL
   iv. supplied in 1 % formic acid in water (after desalting by SPE)

c. Digested human plasma spiked with 11 unlabeled synthetic peptides and 11 labeled IS peptides (Samples I-B to I-J)
   i. one 25 µL aliquot of each spike level supplied
   ii. each spiked plasma sample has been diluted to a total plasma protein concentration (prior to digestion) of approximately 1 µg/µL
   iii. 11 labeled IS peptides spiked at a concentration of 50 fmol/µL
   iv. Unlabeled synthetic peptides are spiked in at the following approximate concentrations:

Sample (Study I)	Spiked [$^{12}C/^{14}N$] peptide Concentration (fmol/µL)
I -J	500
I -I	275
I -H	151

I -G	83
I -F	46
I -E	25
I -D	8.55
I -C	2.92
I -B	1.00

v. supplied in 1 % formic acid in water (after desalting by SPE)

MRM Transitions for Studies I, II and III):
Solutions of each synthetic signature peptide were provided previously for optimization of instrument parameters at all CPTAC sites. SOP Table B lists the transitions for all labeled and unlabeled peptides for all 4000 QTRAP users. SOP Table C lists the transitions used for the Quantum mass spectrometer. Additional supplementary tables will report optimized instrument parameters for each individual, participating 4000 QTRAP instrument (*i.e.,* declustering potential [DP], collision energy [CE], and collision cell exit potential [CXP]) and for the participating Quantum instrument (*i.e.,* collision energy [CE]).

SOP Table B. MRM transitions and instrument parameters used on the seven 4000 QTRAP mass spectrometers (Studies I, II, and III). Boldface type indicates labeled peptide internal standard with labeled amino acid in red. Values in red, blue and black indicate 1st, 2nd, and 3rd MRM transitions, respectively, for each peptide pair.

Protein	Signature Peptide*	Group Name	MH+ (mono)	z (Q1)	MRM Transitions		Fragment Ion Type	Identifier
					Q1	Q3		
APR	AGLCQTFVYGGCR	bi0173	1488.7	2	744.8	858.39	y7	173tr1_A
				2	744.8	959.44	y8	173tr2_A
				2	744.8	1087.50	y9	173tr3_A
	AGLCQTFVYGGCR	**bi0081**	**1493.7**	**2**	**747.3**	**863.41**	y7	173tr1_IS
				2	**747.3**	**964.46**	y8	173tr2_IS
				2	**747.3**	**1092.50**	y9	173tr3_IS
LEP	INDISHTQSVSAK	bi0167	1399.7	3	467.2	586.80	$y11^{2+}$	167tr1_A
				3	467.2	643.82	$y12^{2+}$	167tr2_A
				3	467.2	720.39	y7	167tr3_A
	INDISHTQSVSAK	**ni0101**	**1407.3**	**3**	**469.9**	**590.81**	$y11^{2+}$	167tr1_IS
				3	**469.9**	**647.83**	$y12^{2+}$	167tr2_IS
				3	**469.9**	**728.40**	y7	167tr3_IS
MYO	LFTGHPETLEK	bi0171	1271.7	3	424.6	506.26	$y9^{2+}$	171tr1_A
				3	424.6	579.79	$y10^{2+}$	171tr2_A
				3	424.6	716.38	y6	171tr3_A
	LFTGHPETLEK	**ni0102**	**1279.7**	**3**	**427.2**	**510.27**	$y9^{2+}$	171tr1_IS
				3	**427.2**	**583.80**	$y10^{2+}$	171tr2_IS

				3	**427.2**	**724.40**	y6	171tr3_IS
MBP	HGFLPR	bi0169	726.4	2	363.7	385.26	y3	169tr1_A
				2	363.7	532.32	y4	169tr2_A
				2	363.7	589.35	y5	169tr3_A
	HGFLPR	**ni0104**	**732.4**	**2**	**366.7**	**391.28**	y3	169tr1_IS
				2	**366.7**	**538.34**	y4	169tr2_IS
				2	**366.7**	**595.37**	y5	169tr3_IS
	YLASASTMDHAR	bi0170	1322.6	3	441.5	488.22	$y9^{2+}$	170tr1_A
				3	441.5	523.74	$y10^{2+}$	170tr2_A
				3	441.5	817.36	y7	170tr3_A
	YLASASTMDHAR	**ni0105**	**1328.6**	**3**	**443.5**	**491.23**	$y9^{2+}$	170tr1_IS
				3	**443.5**	**526.75**	$y10^{2+}$	170tr2_IS
				3	**443.5**	**823.38**	y7	170tr3_IS
PSA	IVGGWECEK	bi0161	1077.5	2	539.3	808.33	y6	161tr1_A
				2	539.3	865.35	y7	161tr2_A
				2	539.3	964.42	y8	161tr3_A
	IVGGWECEK	**bi0067**	**1082.5**	**2**	**541.7**	**808.33**	y6	161tr1_IS
				2	**541.7**	**865.35**	y7	161tr2_IS
				2	**541.7**	**969.44**	y8	161tr3_IS
	LSEPAELTDAVK	bi0037	1272.7	2	636.8	775.42	y7	37tr1_A
				2	636.8	846.46	y8	37tr2_A
				2	636.8	943.51	y9	37tr3_A
	LSEPAELTDAVK	**ni0107**	**1280.7**	**2**	**640.8**	**783.43**	y7	37tr1_IS
				2	**640.8**	**854.47**	y8	37tr2_IS
				2	**640.8**	**951.52**	y9	37tr3_IS
HRP	SSDLVALSGGHTFGK	bi0166	1475.7	3	492.6	703.35	y7	166tr1_A
				3	492.6	790.38	y8	166tr2_A
				3	492.6	974.51	y10	166tr3_A
	SSDLVALSGGHTFGK	**ni0108**	**1483.8**	**3**	**495.3**	**711.37**	y7	166tr1_IS
				3	**495.3**	**798.40**	y8	166tr2_IS
				3	**495.3**	**982.52**	y10	166tr3_IS
CRP	ESDTSYVSLK	bi0231	1128.5	2	564.8	609.36	y5	231tr1_A
				2	564.8	696.39	y6	231tr2_A
				2	564.8	797.44	y7	231tr3_A
	ESDTSYVSLK	**ni0109**	**1136.6**	**2**	**568.8**	**617.37**	y5	231tr1_IS
				2	**568.8**	**704.41**	y6	231tr2_IS
				2	**568.8**	**805.45**	y7	231tr3_IS
	GYSIFSYATK	bi0202	1136.3	2	568.8	716.36	y6	202tr1_A
				2	568.8	829.45	y7	202tr2_A
				2	568.8	916.48	y8	202tr3_A

	GYSIFSYATK	**ni0110**	**1144.6**	**2**	**572.8**	**724.38**	y6	202tr1_IS
				2	**572.8**	**837.46**	y7	202tr2_IS
				2	**572.8**	**924.49**	y8	202tr3_IS
	YEVQGEVFTKPQLWP	ni0001	1820.9	2	911.0	1053.49	b9	01_tr1_A
				2	911.0	1181.58	b10	01_tr2_A
				2	911.0	1519.78	b13	01_tr3_A
	YEVQGEVFTKPQLWP	**ni0111**	**1826.9**	**2**	**914.0**	**1053.49**	b9	01_tr1_IS
				2	**914.0**	**1181.58**	b10	01_tr2_IS
				2	**914.0**	**1525.80**	b13	01_tr3_IS

- C denotes carboxyamidomethylated cysteine;
- Eight of the 11 IS peptides have been synthesized with a single, uniformly labeled [$^{13}C/^{15}N$] amino acid at the C-terminus, one IS peptide (group name ni0111) has been synthesized with a single uniformly labeled [^{13}C] leucine, two IS peptides have been synthesized with a uniformly labeled [^{13}C] valine (group names bi0081 and bi0067; latter two peptides containing carboxyamidomethylated cysteines).
- The column named "identifier" refers to transition notifications used in a MultiQuant method that was distributed to each participating 4000 QTRAP site.

SOP Table C. MRM transitions used on one TSQ Quantum Ultra mass spectrometer (Studies I, II, and III). Boldface type indicates labeled peptide internal standard with labeled amino acid in red. Values in red, blue and black indicate 1st, 2nd, and 3rd MRM transitions, respectively, for each peptide pair.

Protein	Signature Peptide*	Group Name	MH+ (mono)	z (Q1)	MRM Transitions		Fragment Ion Type	Identifier
					Q1	Q3		
APR	AGLCQTFVYGGCR	bi0173	1488.7	2	744.8	711.23	y6	744_1
				2	744.8	858.39	y7	744_2
				2	744.8	959.44	y8	744_3
	AGLCQTFVYGGCR	**bi0081**	**1493.7**	**2**	**747.3**	**716.53**	y6	747_1
				2	**747.3**	**863.41**	y7	747_2
				2	**747.3**	**946.46**	y8	747_3
LEP	INDISHTQSVSAK	bi0167	1399.7	3	467.2	586.80	$y11^{2+}$	467_1
				3	467.2	643.82	$y12^{2+}$	467_2
				3	467.2	720.39	y7	467_3
	INDISHTQSVSAK	**ni0101**	**1407.3**	**3**	**469.9**	**590.81**	$y11^{2+}$	469_1
				3	**469.9**	**647.83**	$y12^{2+}$	469_1
				3	**469.9**	**728.40**	y7	469_1
MYO	LFTGHPETLEK	bi0171	1271.7	3	424.6	506.26	$y9^{2+}$	424_1
				3	424.6	579.79	$y10^{2+}$	424_2
				3	424.6	716.38	y6	424_3
	LFTGHPETLEK	**ni0102**	**1279.7**	**3**	**427.2**	**510.27**	$y9^{2+}$	427_1
				3	**427.2**	**583.80**	$y10^{2+}$	427_2
				3	**427.2**	**724.40**	y6	427_3
MBP	HGFLPR	bi0169	726.4	2	363.7	455.24	b4	363_1

				2	363.7	532.32	y4	363_2
				2	363.7	589.35	y5	363_3
	HGFLPR	**ni0104**	**732.4**	**2**	**366.7**	**455.24**	b4	366_1
				2	**366.7**	**538.34**	y4	366_2
				2	**366.7**	**595.37**	y5	366_3
	YLASASTMDHAR	bi0170	1322.6	3	441.5	523.74	$y10^{2+}$	441_1
				3	441.5	730.33	y6	441_2
				3	441.5	817.36	y7	441_3
	YLASASTMDHAR	**ni0105**	**1328.6**	**3**	**443.5**	**526.75**	$y10^{2+}$	443_1
				3	**443.5**	736.35	y6	443_2
				3	**443.5**	**823.38**	y7	443_3
PSA	IVGGWECEK	bi0161	1077.5	2	539.3	808.33	y6	539_1
				2	539.3	865.35	y7	539_2
				2	539.3	964.42	y8	539_3
	IVGGWECEK	**bi0067**	**1082.5**	**2**	**541.7**	**808.33**	y6	541_1
				2	**541.7**	**865.35**	y7	541_2
				2	**541.7**	**969.44**	y8	541_3
	LSEPAELTDAVK	bi0037	1272.7	2	636.8	646.38	y6	636_1
				2	636.8	846.46	y8	636_2
				2	636.8	943.51	y9	636_3
	LSEPAELTDAVK	**ni0107**	**1280.7**	**2**	**640.8**	654.39	y6	640_1
				2	**640.8**	**854.47**	y8	640_2
				2	**640.8**	**951.52**	y9	640_3
HRP	SSDLVALSGGHTFGK	bi0166	1475.7	3	492.6	703.35	y7	492_1
				3	492.6	790.38	y8	492_2
				3	492.6	974.51	y10	492_3
	SSDLVALSGGHTFGK	**ni0108**	**1483.8**	**3**	**495.3**	**711.37**	y7	495_1
				3	**495.3**	**798.40**	y8	495_2
				3	**495.3**	**982.52**	y10	495_3
CRP	ESDTSYVSLK	bi0231	1128.5	2	564.8	609.36	y5	564_1
				2	564.8	696.39	y6	564_2
				2	564.8	797.44	y7	564_3
	ESDTSYVSLK	**ni0109**	**1136.6**	**2**	**568.8**	**617.37**	y5	568_1
				2	**568.8**	**704.41**	y6	568_2
				2	**568.8**	**805.45**	y7	568_3
	GYSIFSYATK	bi0202	1136.3	2	568.8	716.36	y6	568_1
				2	568.8	829.45	y7	568_2
				2	568.8	916.48	y8	568_3
	GYSIFSYATK	**ni0110**	**1144.6**	**2**	**572.8**	**724.38**	y6	572_1
				2	**572.8**	**837.46**	y7	572_2

				2	**572.8**	**924.49**	y8	572_3
	YEVQGEVFTKPQLWP	ni0001	1820.9	2	911.0	805.37	b7	911_1
				2	911.0	1016.56	y8	911_2
				2	911.0	1053.49	b9	911_3
	YEVQGEVFTKPQLWP	**ni0111**	**1826.9**	**2**	**914.0**	805.37	b7	914_1
				2	**914.0**	1022.58	y8	914_2
				2	**914.0**	1053.49	b9	914_3

- C denotes carboxyamidomethylated cysteine
- Eight of the 11 IS peptides have been synthesized with a single, uniformly labeled [$^{13}C/^{15}N$] amino acid at the C-terminus, one IS peptide (ni0111) has been synthesized with a single uniformly labeled [^{13}C] leucine, two IS peptides have been synthesized with a uniformly labeled [^{13}C] valine (bi0081 and bi0067; latter two peptides containing carboxyamidomethylated cysteines).

HPLC Chromatography Conditions for Studies I, II and III):
Individual CPTAC sites are expected to implement these HPLC conditions for the duration of the study. Packing material indicated below was provided previously. Please note that customized 1 µL sample loops made out of PEEKsil will be provided to each site for use on an Eksigent LC system. A flow diagram and column packing instructions are provided in Appendix D and Appendix E, respectively.

- Column: PicoFrit 75 µm ID (PF360-75-10-N-5, www.newobjective.com) slurry-packed with ReproSil-Pur C18-AQ, 3µm, 120Å to a length of 12 cm
- Mobile phases: (A) 0.1% Formic acid (v/v); (B) 90% Acetonitrile / 0.1% Formic acid (v/v)
- Flow rate: 200 nL/min on an Agilent or 300 nL/min on an Eksigent NanoLC system
- Injection volume: 1 µL
- Loop for Eksigent LC: 1 µL PEEKsil loop provided in sample kit, 100 µm ID PEEKsil
- Injection Amount: ≤ 1µg total protein on-column
- Gradient[##]: 3 – 20% B in 3 min, 20 – 60% B in 35 min, 60 – 90% B in 2 min, 90% B for 4 min

[##]**Agilent Notes**: 1 µL pickup with vial/bottom well sensing on. Flow rate was increased to 600 nL/min at 3% B for 15 minutes with injector valve in mainpass in order to flow sample through needle/loop onto column prior to start of gradient. Flow rate was reduced to 200 nL/min just prior to start of gradient. Needle was washed in flushport for 15 sec. Cleanup of the needle/loop is essential to decrease carryover. However, injection of solvent B into mainpass can affect equilibration of the system. A 20 minute hold at 3% B with the valve in mainpass (beginning or end of the program is critical for proper re-equilibration. Autosampler settings were as follows:

A 20 min hold in mainpass at initial conditions is **essential** to re-equilibrate mainpass prior to injection of sample.

	#	Command
	1	DRAW def. amount from sample, def. speed, def. offset
	2	WAIT 0.05 min
	3	NEEDLE wash in flush port, 15.0 sec
	4	WAIT 0.05 min
	5	INJECT
	6	REMOTE Startpulse
600nL/min →	7	WAIT 15.00 min
	8	VALVE bypass
	9	VALVE mainpass
	10	VALVE bypass
	11	VALVE mainpass
	12	VALVE bypass
Buffer B	13	REPEAT 3 times*
Buffer B	14	DRAW max. amount from Vial 2, def. speed, def. offset
Buffer B	15	EJECT max. amount into seat, def. speed
Buffer B	16	END REPEAT
Buffer A	17	REPEAT 5 times*
Buffer A	18	DRAW max. amount from Vial 1, def. speed, def. offset
Buffer A	19	EJECT max. amount into seat, def. speed
Buffer A	20	END REPEAT

*Can be increased to 5x injection of solvent B and 8x injection of solvent

Eksigent/Tempo Notes: HPLC conditions for this CPTAC Studies I-III.

Autosampler Program with Standard Injection*

#	Function	Command		
1	Output	1-Off		
2	Output	2-Off		
3	Valve	Injector Load		
4**	Aspirate	10 µL Reagent-1	Speed:1	Height:5
5	Aspirate	2 µL Sample	Speed:1	Height:2
6**	Aspirate	2.3 µL Reagent-1	Speed:1	Height:5
7	Output	2-On		
8	Valve	Injector Inject		
9	Dispense	14.3 µL Waste	Speed:5	Height:0
10 **	Needle Wash	200 µL		
11	End			

Flow Table with Standard Injection*

#	Time (min)	%A	%B
1	0	97	3
2	5	97	3
3	8	80	20
4	43	40	60
5	45	10	90
6	49	10	90
7	50	97	3
8	80	97	3

**Full loop injection with loop in line throughout gradient*
***Reagent-1 = Buffer A; Needle Wash = Buffer B*

NOTE: Total protein greater than 1 µg injected onto nanoLC columns can result in poor chromatographic peak shape and poor reproducibility from run to run. The plasma samples (Samples I-A through I-J, and I-Blank) have been diluted such that a 1 µL injection results in approximately 1.0 µg of total protein on-column. Therefore, they should be analyzed without any additional dilution.

Triple Quadrupole Mass Spectrometer Instrumental Operating Parameters for Study I:
The instrument conditions described below are target values for the 4000 QTRAP mass spectrometers. Participating CPTAC sites that use a mass spectrometer other than a 4000 QTRAP will need to adjust and report the operating parameters unique to their system. Each participating CPTAC site will be required to record all mass spectrometer instrumental operating conditions in the SOP Instrumental Parameters Excel spreadsheet (see additional supplementary tables).

- Ion Source voltage: 2200
- Curtain gas: 20
- Nebulizer gas: 5 psi
- IHT: 150
- DP, CE, CXP: Optimized and recorded for each signature peptide via infusion or flow injection. **NOTE: The same DP, CE and CXP values are to be used for each [^{12}C/^{14}N] / [^{13}C/^{15}N] peptide pair on a 4000 QTRAP.**
- Dwell time: 10 msec for 66 transitions (0.99 sec for entire cycle)
- Q1/Q3 resolution: Unit/Unit

Sample Analysis Run Order for Study I:
Samples will be run in the following sequence:

Run Order	# of Injections	Sample Name	(original name*)
1	1	I -Blank	(7.1-Blank)
2	4	I -A	(7.1-A)
3	4	I -B	(7.1-B)
4	4	I -C	(7.1-C)
5	4	I -D	(7.1-D)
6	4	I -E	(7.1-E)
7	4	I -F	(7.1-F)
8	4	I -QC	(7.1-QC)
9	4	I -G	(7.1-G)
10	4	I -H	(7.1-H)
11	4	I -I	(7.1-I)
12	4	I -J	(7.1-J)
13	4	I -A	(7.1-A)
14	4	gradient wash out runs	
15	4	I -A	(7.1-A)

[*Study 7.1 was the previous/original name of the study that in the manuscript is referred to as Study I, thus all raw data file names/sample names were saved under the name 7.1 at all sites.]

Initially, a single injection of unspiked digested plasma (Sample I-Blank) will be run for column conditioning purposes. Then four replicate injections of digested plasma spiked with the labeled IS peptides (Sample I-A) followed by four replicate injections of each sample containing digested plasma, target [$^{12}C/^{14}N$] peptides and the labeled IS peptides (Samples I-B through I-J) will be run as well as the QC sample (Sample I-QC). Samples I–B through I-J will be analyzed in order of increasing concentration of the target proteins. Next, column carryover effects will be assessed by performing four gradient wash runs bracketed by four replicate injections of Sample I-A. To estimate the carryover effect it is important to record the information in each replicate for #13 and #14 in the order the replicate runs were performed. In addition, between Samples I-F and I-G one replicate injection of Sample I-QC will be analyzed to assess system performance midway through the assay. The estimated turn-around time for each MRM run is 80 minutes. Thus, a total of 54 runs will require a continuous run time of 72 hours or approximately 3 days.

Data Recording and Analysis for Study I:
Participating CPTAC laboratories implementing the SOP with a 4000 QTRAP mass spectrometer will receive a MultiQuant method to be applied to their data files. Sites using the TSQ Quantum Ultra will implement the SOP by using Xcalibur software to create the instrument methods and processing of data files will utilize SRM Workflow (prototype software from ThermoFisher Scientific). Default parameters in MultiQuant are to be used for peak integration. Splitting factor can be adjusted as needed from 2 (default) to 1 or 0 to improve integration when necessary. Retention time should be adjusted for each analyte/IS pair. The relative area ratios of the three transitions per peptide are to be evaluated manually for matrix interferences (*i.e.,* co-eluting peptides with precursor/product transitions that may reside within the mass width of Q1 and Q3).

To facilitate transfer of data between MultiQuant and statistical packages, the column "Sample Names" in MultiQuant and Analyst is standardized across all CPTAC sites. This column is generated through the batch setup and should be cut and pasted into Analyst (4000 QTRAP users) from SOP Table D. The row names are constructed by concatenating "Study Number", "Sample Number", "Injection Number" with "," in between.

SOP Table D: Sample Names for Batch setup in Analyst: Study I.

Sample name for Study I (previous/original Study name was 7.1*)	Copy column starting here and paste into "Sample Name" column in Analyst
7.1, Blank,01	
7.1, A1, 01	
7.1, A1, 02	
7.1, A1, 03	
7.1, A1, 04	
7.1, B, 01	
7.1, B, 02	
7.1, B, 03	
7.1, B, 04	
7.1, C, 01	

7.1, C, 02
7.1, C, 03
7.1, C, 04
7.1, D, 01
7.1, D, 02
7.1, D, 03
7.1, D, 04
7.1, E, 01
7.1, E, 02
7.1, E, 03
7.1, E, 04
7.1, F, 01
7.1, F, 02
7.1, F, 03
7.1, F, 04
7.1, QC, 01
7.1, G, 01
7.1, G, 02
7.1, G, 03
7.1, G, 04
7.1, H, 01
7.1, H, 02
7.1, H, 03
7.1, H, 04
7.1, I, 01
7.1, I, 02
7.1, I, 03
7.1, I, 04
7.1, J, 01
7.1, J, 02
7.1, J, 03
7.1, J, 04
7.1, A2, 01
7.1, A2, 02
7.1, A2, 03
7.1, A2, 04
7.1, gradient wash out, 01
7.1, gradient wash out, 02
7.1, gradient wash out, 03
7.1, gradient wash out, 04
7.1, A3, 01
7.1, A3, 02
7.1, A3, 03
7.1, A3, 04

[*Study 7.1 was the previous/original name of the study that in the manuscript is referred to as Study I, thus all raw data file names/sample names were saved under the name 7.1 at all sites]

The following variables need to be included when exporting the data from MultiQuant:

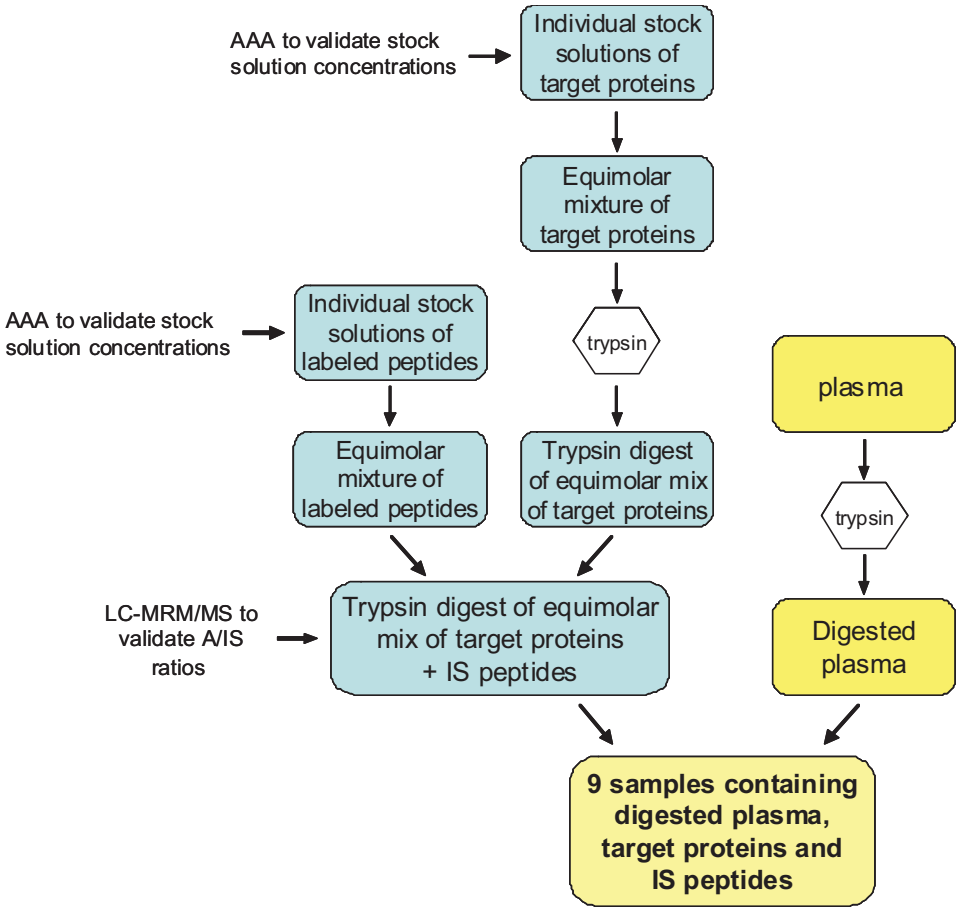
Sample.Name
Component.Name
Component.Group.Name
Area
Height
Retention.Time
Signal / Noise
IS.Name
IS Area
IS Height
IS Retention Time
IS Signal / Noise

In MultiQuant, first select (left click) “All Analytes” in the left pane titled “Components and Groups”. Columns in the “Results Table” can be selected for display by right clicking in the blank line above the row of column names. Right clicking will bring up a list of choices, one of which is "Column settings...". Choose this one and it will bring up a list of all possible MultiQuant columns, with a "Visible" column for selecting the variables. The above column names should be ticked. Additional names can be ticked but the above names are required. Then left click “File”, select “Export” and slide over to first selection “Results Table…”. Export dialog box opens, use the default selections “Export only visible columns” and “Export all rows” and hit OK. In “Save As” dialog box, give name of file as “**CPTAC Study 7.1 siteXX – dateYY**” [Study 7.1 was the original name of the study that in the manuscript is referred to as Study I], where siteXX is name of your site, and dateYY is e.g. “18 Jul 2008”. This creates a tab delimited text file.

Once data analysis has been completed each CPTAC participating laboratory will forward their data in the tab delimited text file to a CPTAC study biostatistician. Statistical analysis of the results will be performed in collaboration with Experimental Design and Statistics Verification Studies Working Group statisticians.

CPTAC Study II: Single site digestion of target proteins spiked into diluted, digested plasma:

Methods: Sample Preparation for Study II
All sample preparation and distribution of sample kits to participating CPTAC laboratories will be performed at NIST. SOP Figure B illustrates the sample preparation workflow. The seven target proteins to be quantitated are listed in SOP Table A along with their signature peptides.



SOP Figure B. Sample preparation workflow for Study II (A, analyte (target) peptides; IS, internal standard peptides).

Appendices A and B outline the digestion procedure that will be employed for the bulk digestion of non-depleted plasma and the digestion of the equimolar mixture of target proteins, respectively. Urea will be used as denaturant in both digestions and cysteine residues will be alkylated using iodoacetamide. After digestion and off-line desalting, digests will be lyophilized to dryness and resuspended in an aqueous solution containing 3% acetonitrile and 5% formic acid. A dilution of the final reconstituted samples will reduce the acetonitrile and formic acid to <1 % and 0.6 %, respectively No additional sample clean-up will be required.

Two kits containing all the necessary samples required to implement the SOP will be prepared by NIST: a tuning kit and a sample kit.

The tuning kit will contain:

- an equimolar mixture of the labeled IS peptides and unlabeled signature peptides for chromatographic and MS optimization

The sample kit will contain:

- a quality control (QC) mixture composed of equimolar amounts of the target [$^{12}C/^{14}N$] and [$^{13}C/^{15}N$] peptides
- unspiked digested plasma
- digested plasma spiked with the isotopically labeled IS peptides
- digested plasma spiked with labeled IS peptides at a constant level and the target protein digest spanning a concentration range of 2.5 orders of magnitude

Details of each kit's contents according to sample type (tuning, QC, and plasma containing) are as follows:

D. Tuning Samples
   a. 500 fmol/µL mixture of all 11 unlabeled signature peptides and 11 labeled internal standard peptides
      i. one 50 µL aliquot supplied
      ii. supplied in 1 % formic acid in water

E. QC Sample (Sample II-QC)
   a. Tryptic digest of the target protein mixture, 50 fmol/µL, spiked with 50 fmol/µL of the 11 labeled IS peptides
      i. one 25 µL aliquot supplied
      ii. supplied in 1 % formic acid in water (after desalting by SPE)

F. Plasma Samples
   a. Unspiked, digested human plasma (**Sample II-Blank**)
      i. one 25 µL aliquot supplied
      ii. plasma diluted approximately 60-fold to a total plasma protein concentration (prior to digestion) of approximately 1 µg/µL
      iii. supplied in 1 % formic acid in water (after desalting by SPE)
   b. Digested human plasma spiked with labeled IS peptides (**Sample II-A**)
      i. two 25 µL aliquots supplied
      ii. plasma diluted approximately 60-fold to a total plasma protein concentration (prior to digestion) of approximately 1 µg/µL
      iii. 11 labeled IS peptides spiked at a concentration of 50 fmol/µL
      iv. supplied in 1 % formic acid in water (after desalting by SPE)

c. Digested human plasma spiked with digested target proteins and labeled IS peptides (**Samples II-B to II-J**)
   i. one 25 µL aliquot of each spike level supplied
   ii. each spiked plasma sample has been diluted to a total plasma protein concentration (prior to digestion) of approximately 1 µg/µL
   iii. 11 labeled IS peptides spiked at a concentration of 50 fmol/µL
   iv. Target proteins are spiked in at the following concentrations:

Sample (Study II)	Spiked Protein Concentration (fmol/µL)
II -J	500
II -I	275
II -H	151
II -G	83
II -F	46
II -E	25
II -D	8.55
II -C	2.92
II -B	1.00

   v. supplied in 1 % formic acid in water (after desalting by SPE)

MRM Transitions for Study II:
SOP Table B lists the MRM transitions for all labeled and unlabeled signature peptides for all 4000 QTRAP users. SOP Table C lists the transitions used for the TSQ Quantum Ultra mass spectrometer. Additional supplementary tables will report optimized instrument parameters for each individual, participating 4000 QTRAP instrument (*i.e.,* declustering potential [DP], collision energy [CE], and collision cell exit potential [CXP]) and for the participating Quantum instrument (*i.e.,* collision energy [CE]).

HPLC Chromatography Conditions for Study II:
Described above and identical to those conditions used for Study I.

NOTE: Total protein greater than 1 µg injected onto nanoLC columns can result in poor chromatographic peak shape and poor reproducibility from run to run. The plasma samples (Samples II-A through II-J, and II-Blank) have been diluted such that a 1 µL injection results in approximately 1.0 µg of total protein on-column. Therefore, they should be analyzed without any additional dilution.

Triple Quadrupole Mass Spectrometer Instrumental Operating Parameters for Study II:
Described above and identical to those conditions used for Study I.

Sample Analysis Run Order for Study II:
Samples will be run in the following sequence:

Run Order	# of Injections	Sample Name	(original name*)
1	1	II -Blank	(7.2-Blank)
2	4	II -A	(7.2-A)
3	4	II -B	(7.2-B)
4	4	II -C	(7.2-C)
5	4	II -D	(7.2-D)
6	4	II -E	(7.2-E)
7	4	II -F	(7.2-F)
8	4	II -QC	(7.2-QC)
9	4	II -G	(7.2-G)
10	4	II -H	(7.2-H)
11	4	II -I	(7.2-I)
12	4	II -J	(7.2-J)
13	4	II -A	(7.2-A)
14	4	gradient wash out runs	
15	4	II -A	(7.2-A)

[*Study 7.2 was the previous/original name of the study that in the manuscript is referred to as Study II, thus all raw data file names/sample names were saved under the name 7.2 at all sites.]

Initially, a single injection of unspiked digested plasma (Sample II-Blank) will be run for column conditioning purposes. Then four replicate injections of digested plasma spiked with the labeled IS peptides (Sample II-A) followed by four replicate injections of each sample containing digested plasma, target digested proteins and the IS labeled peptides (Samples II-B through II-J) will be run as well as the QC sample (Sample II-QC). Samples II-B through II-J will be analyzed in order of increasing concentration of the target proteins. Next, column carryover effects will be assessed by performing four gradient wash runs bracketed by four replicate injections of Sample II-A. To estimate the carryover effect it is important to record the information in each replicate for #13 and #14 in the order the replicate runs were performed. In addition, between Samples II-F and II-G four replicate injections of Sample II-QC will be analyzed to assess system performance midway through the assay. The estimated turn-around time for each MRM run is 80 minutes. Thus, a total of 57 runs will require a continuous run time of 76 hours or approximately 3.2 days.

Data Recording and Analysis for Study II:
Participating CPTAC laboratories implementing the SOP with a 4000 QTRAP mass spectrometer will receive a MultiQuant method to be applied to their data files. Sites using the TSQ Quantum Ultra will implement the SOP by using Xcalibur software to create the instrument methods and processing of data files will utilize SRM Workflow (prototype software from ThermoFisher Scientific). Default parameters in MultiQuant are to be used for peak integration. Splitting factor can be adjusted as needed from 2 (default) to 1 or 0 to improve integration when necessary. Retention time should be adjusted for each analyte/IS pair. The relative area ratios of the three transitions per peptide are to be evaluated manually for matrix interferences (*i.e.,* co-

eluting peptides with precursor/product transitions that may reside within the mass width of Q1 and Q3).

To facilitate transfer of data between MultiQuant and statistical packages, the column "Sample Names" in MultiQuant and Analyst is standardized across all CPTAC sites. This column is generated through the batch setup and should be cut and pasted into Analyst (4000 QTRAP users) from SOP Table E. The row names are constructed by concatenating "Study Number", "Sample Number", "Injection Number" with "," in between.

SOP Table E: Sample Names for Batch setup in Analyst: Study II

Sample name for the Study II (previous/original Study name was 7.2*)	Copy column starting here and paste into "Sample Name" column in Analyst
7.2, Blank,01	
7.2, A1, 01	
7.2, A1, 02	
7.2, A1, 03	
7.2, A1, 04	
7.2, B, 01	
7.2, B, 02	
7.2, B, 03	
7.2, B, 04	
7.2, C, 01	
7.2, C, 02	
7.2, C, 03	
7.2, C, 04	
7.2, D, 01	
7.2, D, 02	
7.2, D, 03	
7.2, D, 04	
7.2, E, 01	
7.2, E, 02	
7.2, E, 03	
7.2, E, 04	
7.2, F, 01	
7.2, F, 02	
7.2, F, 03	
7.2, F, 04	
7.2, QC, 01	
7.2, QC, 02	
7.2, QC, 03	
7.2, QC, 04	
7.2, G, 01	
7.2, G, 02	
7.2, G, 03	
7.2, G, 04	
7.2, H, 01	

7.2, H, 02
7.2, H, 03
7.2, H, 04
7.2, I, 01
7.2, I, 02
7.2, I, 03
7.2, I, 04
7.2, J, 01
7.2, J, 02
7.2, J, 03
7.2, J, 04
7.2, A2, 01
7.2, A2, 02
7.2, A2, 03
7.2, A2, 04
7.2, gradient wash out, 01
7.2, gradient wash out, 02
7.2, gradient wash out, 03
7.2, gradient wash out, 04
7.2, A3, 01
7.2, A3, 02
7.2, A3, 03
7.2, A3, 04

[*Study 7.2 was the previous/original name of the study that in the manuscript is referred to as Study II, thus all raw data file names/sample names were saved under the name 7.2 at all sites.]

The following variables need to be included when exporting the data from MultiQuant:

Sample.Name
Component.Name
Component.Group.Name
Area
Height
Retention.Time
Signal / Noise
IS.Name
IS Area
IS Height
IS Retention Time
IS Signal / Noise

In MultiQuant, first select (left click) “All Analytes” in the left pane titled “Components and Groups”. Columns in the “Results Table” can be selected for display by right clicking in the blank line above the row of column names. Right clicking will bring up a list of choices, one of which is "Column settings...". Choose this one and it will bring up a list of all possible MultiQuant columns, with a "Visible" column for selecting the variables. The above column

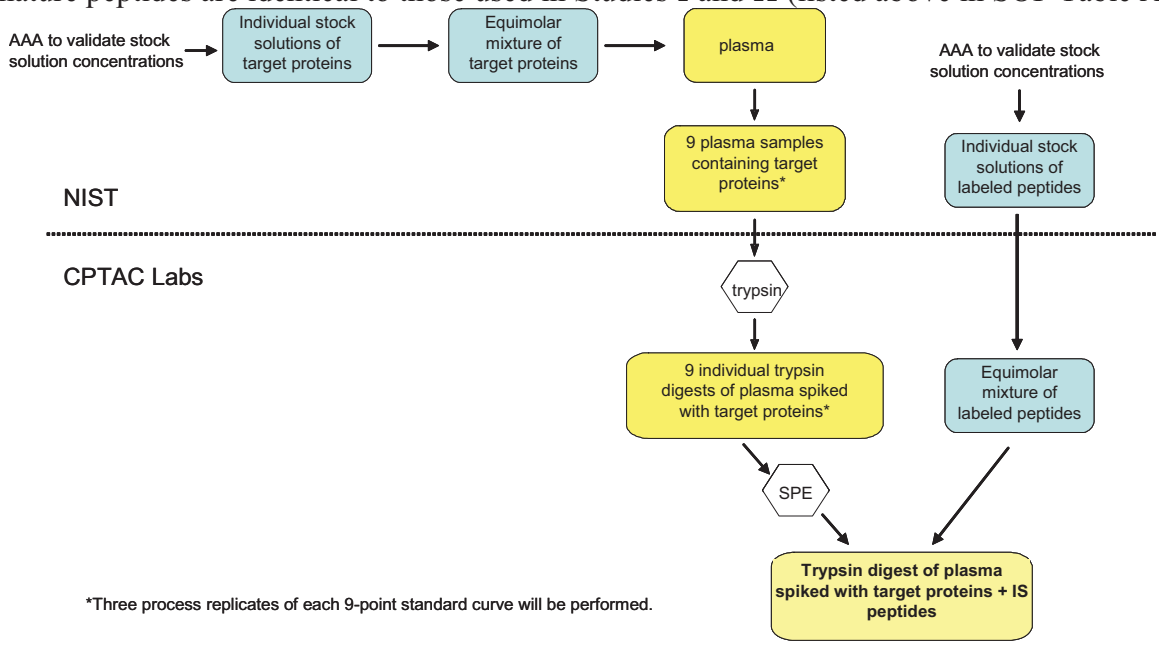
names should be ticked. Additional names can be ticked but the above names are required. Then left click "File", select "Export" and slide over to first selection "Results Table…". Export dialog box opens, use the default selections "Export only visible columns" and "Export all rows" and hit OK. In "Save As" dialog box, give name of file as "**CPTAC Study 7.2 siteXX – dateYY**" [Study 7.2 was the original name of the study that in the manuscript is referred to as Study II], where siteXX is name of your site, and dateYY is e.g. "18 Jul 2008". This creates a tab delimited text file.

Once data analysis has been completed each CPTAC participating laboratory will forward their data in the tab delimited text file to a CPTAC study biostatistician. Statistical analysis of the results will be performed in collaboration with Experimental Design and Statistics Verification Studies Working Group statisticians.

CPTAC Study III: Simulation of a verification study across CPTAC sites.

Methods: Sample Preparation for Study III

SOP Figure C illustrates the sample preparation workflow. A 9-point standard curve (prepared in triplicate) will be generated at NIST by spiking plasma with an equimolar mixture of the target proteins prior to trypsin digestion. The seven target proteins to be quantitated along with their signature peptides are identical to those used in Studies I and II (listed above in SOP Table A).



SOP Figure C. Sample preparation workflow for Study III (IS, internal standard peptides).

Appendix C describes the sample preparation performed at NIST for Study III. The sample kit for Study III will contain:

- an equimolar mixture of the labeled IS peptides and unlabeled signature peptides for chromatographic and MS optimization and validation
- a quality control (QC) mixture composed of an equimolar mixture of the 11 signature and 11 internal standard peptides
- unspiked and undigested plasma
- undigested plasma spiked with the seven target proteins
- an equimolar mixture of the 11 labeled internal standard peptides
- proteomics-grade trypsin

Details of each kit's contents according to sample type (tuning, QC, and plasma containing) are as follows:

G. Tuning Sample for Study III

a. 500 fmol/µL mixture of all 11 unlabeled signature peptides and 11 labeled internal standard peptides
   i. one 50 µL aliquot supplied
   ii. supplied in 1 % formic acid in water

H. QC Sample (Sample III-QC)

a. Equimolar mixture of the 11 unlabeled and 11 labeled synthetic peptides at 50 fmol/µL
   i. three 35 µL aliquots supplied
   ii. supplied in 1 % formic acid in water

I. Plasma Samples for Study III

a. Unspiked, undigested human plasma (**Sample III-Blank**)
   i. three 35 µL aliquots supplied
b. Undigested human plasma (**Sample III-A**)
   i. three 35 µL aliquots supplied
c. Undigested human plasma spiked with the seven target proteins (**Samples III-B to III-J**)
   i. three 35 µL aliquots of each spike level supplied

Sample (Study III)	Spiked Target Protein Concentration (fmol/µL)
III -J	30,000
III -I	16,500
III -H	9,060
III -G	4,980
III -F	2,760
III -E	1,500
III -D	513
III -C	175
III -B	60

J. IS Peptide Mixture

a. 500 fmol/µL equimolar mixture of all 11 labeled internal standard peptides
   i. three 150 µL aliquots supplied
   ii. supplied in 1 % formic acid in water

K. Proteomics Grade Trypsin

a. Twelve vials of Promega Trypsin Gold (Catalog # V5280), each containing 100 µg of lyophilized trypsin

Digestion and Off-line Desalting Procedures for Three Process Replicates of Study III:
The following protocols are to be used for the digestion of Sample III-Blank and Samples III-A through III-J (11 samples per process replicate). Urea will be used as denaturant in all digestions and cysteine residues will be alkylated using iodoacetamide. After digestion, samples will be desalted off-line via Oasis HLB cartridges, lyophilized to dryness and resuspended in an aqueous solution containing 3% acetonitrile and 5% formic acid. A dilution of the final reconstituted samples will reduce the acetonitrile and formic acid to ~1 % and 0.1 %, respectively, prior to LC-MRM/MS.

I. Chemical Reagents and Recommended Sources

The following chemicals are NOT provided in the sample kit from NIST:

1. Urea - Sigma Ultra, product # U0631.
2. DL-1,4-Dithiothreitol [DTT]- Pierce "no-weigh dithiothreitol", product # 20291 (pre-weighed in 7.7 mg aliquots to be used fresh for each experiment).
3. Iodoacetamide - Sigma, product # A3221-10VL (box of 10 pre-weighed ampules of 56 mg iodoacetamide)
4. Tris base -any source; however, needs to have been purchased within 1 year
5. Water, HPLC grade (Fisher, W5-1 or equivalent)
6. Acetonitrile, HPLC grade (Fisher, A998-1 or equivalent)
7. Formic Acid (EMD Suprapur)
8. Hydrochloric acid, certified grade (Fisher, 7647-01-0, or equivalent)

Note that Promega Trypsin Gold mass spectrometry grade (4 x 100 μg per vial, Product # V5280) will be provided in the sample kit from NIST.

II. Reagent Preparation for Plasma and Target Protein Digestions

A. 1 M Tris, pH 8.0 - 250 mL

1. To a 500 mL beaker, add 30.3 g solid Tris-base.
2. Add 150 mL deionized water and stir until dissolved.
3. Adjust pH of solution to 8.0 with concentrated HCl (12 M).
4. Transfer solution to 250 mL or 500 mL graduated cylinder and bring volume to 250 mL with deionized water.

B. 9M urea, 300 mM Tris, pH 8.0 Stock - 50 mL
(This solution must be prepared fresh for each process replicate.)

1. To a 100 mL beaker, add 27 g solid urea
2. Add 15 mL of water and 15 mL of 1 M Tris, pH 8.0 (Reagent A).
3. Add stir bar and place beaker in larger beaker of warm water. Stir until dissolved. **Keep temperature at or below 37 °C. Do not overheat.**
4. Measure pH and adjust to 8.0 if necessary.

5. Transfer to 50 or 100 mL graduated cylinder and bring volume to 50 mL with deionized water.
6. Store this stock for preparation of digestion buffer containing DTT (Reagent C).

C. 9 M Urea, 300 mM Tris, pH 8.0, 20 mM DTT for digestion of plasma - 1 mL
(This solution must be prepared fresh for each process replicate.)

1. Remove 960 µL of the urea/tris solution (Reagent B) and place in a 1.5 mL Eppendorf tube.
2. Withdraw a separate aliquot of 100 µL of the urea/tris solution (Reagent B)) and add to one "no-weigh" tube of DTT (7.7 mg).
3. Solubilize the DTT, which will result in a final concentration of 0.5 M .
4. Add 40 µL of the DTT solution to the 960 µL aliquot of urea/tris solution and vortex.

D. 0.5 M Iodoacetamide (IAM)
(Prepare immediately before use and keep out of light)

1. To one 56 mg vial of iodoacetamide, add 605 µL of water.
2. Mix until dissolved.

E. 100 mM Tris, pH 8.0 – 100 mL

1. Add 10 mL of 1 M Tris, pH 8.0 stock (Reagent A) to a 100 mL graduated cylinder.
2. Add water to a final volume of 100 mL.

F. 5% formic acid (v/v), 3% acetonitrile (v/v) in water - 100 mL

1. In a 100 mL graduated cylinder, add 3 mL acetonitrile. Add water to the 90 mL mark.
2. Add 5 mL neat formic acid.
3. Add water to the 100 mL mark.
4. Mix by transferring into a larger vessel and store in a clean glass container.

III. Digestion Protocol for Plasma Samples

A. Preparation of Samples III-Blank and III-A through III-J (11 samples per process replicate)

1. Combine 25 µL of plasma (35 µL is provided in each vial) with 50 µL of 9M urea, 300 mM Tris, pH 8.0 containing 20 mmol/L DTT (Reagent C) in a 1.5 mL Eppendorf tube. Repeat for all samples (III-Blank and III-A through III-J).
2. Incubate for 30 min at 37 °C.
3. Add 6.5 µL of 0.5 M IAM (Reagent D) to each tube, to yield an IAM concentration of 40 mM.
4. Alkylate at room temperature for 30 min in the dark.
5. Add 736.5 µL of 100 mM Tris, pH 8.0 (Reagent E) to each tube to decrease the urea concentration to 0.55 M.

6. Remove a 50 µL aliquot from each tube as the "before" sample for SDS-PAGE analysis of the digest. Freeze at -80 ° C until needed.
7. Dissolve each of 4 vials (100 µg per vial) of Promega Trypsin Gold in 100 µL of 100 mM Tris, pH 8.0 (Reagent E) and then combine. (Keep trypsin solution on ice and use quickly after preparation to avoid autolysis). Add 28.3 µL to each digest with gentle mixing (do not vortex) to achieve a 1:50 enzyme-to-substrate ratio for ≈ 1413 µg (or 1.4 mg) total protein present.
8. Incubate overnight (18 h) at 37 °C.
9. Remove a 50 µL aliquot from each digest as the "after" sample for SDS-PAGE analysis of the digest and store at -80 °C until needed.
10. Add 7.5 µL of concentrated formic acid to each digest to quench the digestion for a final acid concentration of 1%.

B. Offline Desalting of Digest Solutions via Oasis HLB SPE Cartridges

Each digest requires off-line desalting using Waters Oasis HLB 1 cc, 30 mg cartridges (Product # WAT094225, box of 100). A vacuum manifold (Product # WAT200677) and vacuum source will be required for the cartridges. Each participating lab is responsible for ordering the necessary equipment. Part numbers were provided previously to help each lab procure these items.

Please note that flow rate on the vacuum manifold cannot be tightly controlled if using typical house vacuum, and that multiple cartridges can flow at different rates. For optimum use, it is important that the SPE cartridges not be allowed to dry during the procedure. Therefore, it is strongly recommended that no more than 3 digests be desalted simultaneously. Total turnaround time to desalt 11 samples (2 cartridges in parallel) should take approximately 1.5 - 2 hours (15-20 minutes per processing).

1. Condition cartridge with 3 x 400 µL of 0.1 % formic acid in 80 % ACN.
2. Equilibrate cartridge with 4 x 400 µL of 0.1 % formic acid in 100 % water.
3. Reduce flow rate by lowering vacuum. A slower flow rate during sample loading, washing and eluting will minimize sample loss and maximize salt removal.
4. Add sample to cartridge.
5. Wash cartridge with 4 x 400 µL of 0.1 % formic acid in 100 % water.
6. Elute plasma digest peptides with 3 x 400 µL 0.1 % formic acid in 80 % acetonitrile into 1.7 mL Eppendorf tubes.
7. Freeze eluates on dry ice or at -80 °C for approximately 1 hour.
8. Dry samples to dryness via vacuum centrifugation. Do not dry overnight. Drying time should be approximately 2-4 hr.

Samples can be stored lyophilized at -80 °C until ready for MRM analysis.

C. Sample Reconstitution and $^{13}C/^{15}N$ IS Peptide Spikes
(To be performed just prior to executing LC-MRM/MS.)

1. Reconstitute dried and desalted plasma digests with 25 µL of 5 % formic acid, 3 % acetonitrile, 92% water and vortex.
2. Add 637.2 µL of 3% acetonitrile, 0.1% formic acid to each sample to achieve 2 µg/µL digest solution for the plasma samples.
3. Remove a 50 µL aliquot of the 2 µg/µL stock and place into separate Eppendorf tubes for each sample. Store remaining solution at -80 °C.
4. Thaw the $^{13}C/^{15}N$ IS peptide mixture provided in the sample kit (500 fmol/uL stock concentration; One stock tube of $^{13}C/^{15}N$ IS peptide mixture per process replicate is provided.) and add 600 µL of water to the 150 µL of $^{13}C/^{15}N$ IS peptide mixture in the tube. Add 50 µL of this 100 fmol/µL $^{13}C/^{15}N$ IS peptide mixture to each sample, so that the final $^{13}C/^{15}N$ peptide concentration is 50 fmol/µL and the plasma digest concentration is 1 µg/µL. **It is important to add the 50 µL of $^{13}C/^{15}N$ peptide mixture into the 50 µL of desalted plasma sample digest to minimize adsorptive losses that may occur if the $^{13}C/^{15}N$ peptide mixture were added to the Eppendorf tube first.**

MRM Transitions for Study III:
Solutions of each synthetic signature peptide were provided previously for optimization of instrument parameters at all CPTAC sites. Target peptides are identical to those used in Studies I and II. The same instrument method that was used for these two studies should be used for Study III. **SOP** Table B lists the MRM transitions for all labeled and unlabeled signature peptides for all 4000 QTRAP users. SOP Table C lists the transitions used for the TSQ Quantum Ultra mass spectrometer. Additional supplementary tables will report optimized instrument parameters for each individual, participating 4000 QTRAP instrument (*i.e.,* declustering potential [DP], collision energy [CE], and collision cell exit potential [CXP]) and for the participating Quantum instrument (*i.e.,* collision energy [CE]).

HPLC Chromatography Conditions for Study III:
Described above and identical to those conditions used for Studies I and II.

NOTE: Total protein greater than 1 µg injected onto nanoLC columns can result in poor chromatographic peak shape and poor reproducibility from run to run. The instructions for reconstitution of all plasma samples (Samples III-A through III-J, and III-Blank) yield solutions that are 1 µg/µL such that a 1 µL injection results in approximately 1.0 µg of total protein on-column.

Triple Quadrupole Mass Spectrometer Instrumental Operating Parameters for Study III:
Described above and identical to those conditions used for Studies I and II.

Sample Analysis Run Order for Study III:
Samples for each process replicate will be run in the following sequence:

Run Order	# of Injections	Sample Name	(original name*)
1	1	III -Blank	(7.3-Blank)
2	4	III -A	(7.3-A)
3	4	III -B	(7.3-B)
4	4	III -C	(7.3-C)
5	4	III -D	(7.3-D)
6	4	III -E	(7.3-E)
7	4	III -F	(7.3-F)
8	4	III -QC	(7.3-QC)
9	4	III -G	(7.3-G)
10	4	III -H	(7.3-H)
11	4	III -I	(7.3-I)
12	4	III -J	(7.3-J)
13	4	III -A	(7.3-A)
14	4	gradient wash out runs	
15	4	III -A	(7.3-A)

[*Study 7.3 was the previous/original name of the Study that in the manuscript is referred to as Study III, thus all raw data file names/sample names were saved under the name 7.3 at all sites.]

Initially, a single injection of unspiked digested plasma (Sample III-Blank) will be run for column conditioning purposes. Then four replicate injections of digested plasma spiked with the labeled IS peptides (Sample III-A) followed by four replicate injections of each sample containing digested plasma, target [$^{12}C/^{14}N$] peptides and the IS labeled peptides (Samples III-B through III-J) will be run as well as the QC sample (Sample III-QC). Samples III-B through III-J will be analyzed in order of increasing concentration of the target proteins. Next, column carryover effects will be assessed by performing four gradient wash runs bracketed by four replicate injections of Sample III-A. To estimate the carryover effect it is important to record the information in each replicate for samples #13 and #14 in the order the replicate runs were performed. In addition, between Samples III-F and III-G four replicate injections of Sample III-QC will be analyzed to assess system performance midway through the assay. The estimated turn-around time for each MRM run is 80 minutes. Thus, a total of 57 runs will require a continuous run time of 76 hours or approximately 3.2 days for each process replicate.

Data Recording and Analysis for Study III:
Participating CPTAC laboratories implementing the SOP with a 4000 QTRAP mass spectrometer will receive a MultiQuant method to be applied to their data files. Sites using the TSQ Quantum Ultra will implement the SOP by using Xcalibur software to create the instrument methods and processing of data files will utilize SRM Workflow (prototype software from ThermoFisher Scientific). Default parameters in MultiQuant are to be used for peak integration. Splitting factor can be adjusted as needed from 2 (default) to 1 or 0 to improve integration when necessary. Retention time should be adjusted for each analyte/IS pair. The relative area ratios of the three transitions per peptide are to be evaluated manually for matrix interferences (*i.e.,* co-

eluting peptides with precursor/product transitions that may reside within the mass width of Q1 and Q3).

To facilitate transfer of data between MultiQuant and statistical packages, the column "Sample Names" in MultiQuant and Analyst is standardized across all CPTAC sites. This column is generated through the batch setup and should be cut and pasted into Analyst (4000 QTRAP users) from SOP Table F. The row names are constructed by concatenating "Study Number", "Sample Number", "Injection Number" with "," in between.

SOP Table F: Sample Names for Batch setup in Analyst: Study III.

Sample name for the Study III (previous/original Study name was 7.3*)	Copy column starting here and paste into "Sample Name" column in Analyst
7.3, Blank,01	
7.3, A1, 01	
7.3, A1, 02	
7.3, A1, 03	
7.3, A1, 04	
7.3, B, 01	
7.3, B, 02	
7.3, B, 03	
7.3, B, 04	
7.3, C, 01	
7.3, C, 02	
7.3, C, 03	
7.3, C, 04	
7.3, D, 01	
7.3, D, 02	
7.3, D, 03	
7.3, D, 04	
7.3, E, 01	
7.3, E, 02	
7.3, E, 03	
7.3, E, 04	
7.3, F, 01	
7.3, F, 02	
7.3, F, 03	
7.3, F, 04	
7.3, QC, 01	
7.3, QC, 02	
7.3, QC, 03	
7.3, QC, 04	
7.3, G, 01	
7.3, G, 02	
7.3, G, 03	
7.3, G, 04	

7.3, H, 01
7.3, H, 02
7.3, H, 03
7.3, H, 04
7.3, I, 01
7.3, I, 02
7.3, I, 03
7.3, I, 04
7.3, J, 01
7.3, J, 02
7.3, J, 03
7.3, J, 04
7.3, A2, 01
7.3, A2, 02
7.3, A2, 03
7.3, A2, 04
7.3, gradient wash out, 01
7.3, gradient wash out, 02
7.3, gradient wash out, 03
7.3, gradient wash out, 04
7.3, A3, 01
7.3, A3, 02
7.3, A3, 03
7.3, A3, 04

[*Study 7.3 was the previous/original name of the Study that in the manuscript is referred to as Study III, thus all raw data file names/sample names were saved under the name 7.3 at all sites.]

The following variables need to be included when exporting the data from MultiQuant:

Sample.Name
Component.Name
Component.Group.Name
Area
Height
Retention.Time
Signal / Noise
IS.Name
IS Area
IS Height
IS Retention Time
IS Signal / Noise

In MultiQuant, first select (left click) “All Analytes” in the left pane titled “Components and Groups”. Columns in the “Results Table” can be selected for display by right clicking in the blank line above the row of column names. Right clicking will bring up a list of choices, one of which is "Column settings...". Choose this one and it will bring up a list of all possible

MultiQuant columns, with a "Visible" column for selecting the variables. The above column names should be ticked. Additional names can be ticked but the above names are required. Then left click “File”, select “Export” and slide over to first selection “Results Table…”. Export dialog box opens, use the default selections “Export only visible columns” and “Export all rows” and hit OK. In “Save As” diaglog box, give name of file as “**CPTAC Study 7.3 siteXX – dateYY**” [Study 7.3 was the original name of the study that in the manuscript is referred to as Study III], where siteXX is name of your site, and dateYY is e.g. “18 Jul 2008”. This creates a tab delimited text file.

Once data analysis has been completed each CPTAC participating laboratory will forward their data in the tab delimited text file to a CPTAC study biostatistician. Statistical analysis of the results will be performed in collaboration with Experimental Design and Statistics Verification Studies Working Group statisticians.

HPLC SOP Checklist			
HPLC Parameters and Chromatography			**Notes**
□	Autosampler temperature	4-10 °C	
□	Column dimensions	12cm x 75µm, 10µm tip	
□	Column packing material	Reprosil 3 µm	
□	# of columns to pack	3 (initial column and 2 backup columns)	
□	Column temperature	Room temperature	
□	Mobile phase A	0.1% (v/v) formic acid in water	
□	Mobile phase B	90% (v/v) acetonitrile 0.1% (v/v) formic acid	
□	Injection volume	1.0 µL	
□	Loop (Eksigent only)	100 µm PEEKsil	
□	Flow rate for gradient	300 nL/min (Eksigent) 200 nL/min (Agilent)	
□	Autosampler Method	above	
□	80 min HPLC program	above	

Mass Spectrometer SOP Checklist			
Source/Gas parameters			**Notes**
□	Spray voltage	2200 ± 200 V	
☐	Curtain gas (4000 Q Trap)	20	
☐	Nebulizer gas (GS1) (4000Q)	5 ± 2 V	
☐	IHT or capillary temp	150 °C	
☐	Tube lens		
Compound Parameters			**Notes**
☐	Total # of MRM transitions	66	
☐	# of transitions per peptide	3	
☐	Modification of instrument method to reflect changes in the masses of transitions		
☐	DP, CE, CXP, tube lens	Same for labeled/ unlabeled peptide pairs	
☐	Dwell Time	10 ms	
MS detector			**Notes**
☐	Duration (min)	80	
☐	Cycle time (ms)	0.99 sec	
☐	Q1/Q3 Resolution	unit/unit	
☐	Settling Time	0	
☐	Pause between mass ranges	5 ms	

Appendix A: Digestion protocol performed at NIST for bulk digestion of human plasma to be used in Studies I and II.

a. Combine 1 mL of plasma with 2 mL of 9M urea, 150 mmol/L Tris, pH 8.0 containing 30 mmol/L DTT in a 50 mL tube (final concentrations of 6M urea, 100 mmol/L Tris, pH 8.0, 20 mmol/L DTT).
b. Incubate for 30 min at 37 °C
c. Add 260 µL of 0.5 mol/L iodoacetamide in water to yield an IAM concentration of 40 mmol/L
d. Alkylate at room temperature for 30 min in the dark
e. Add 25.7 mL of 100 mmol/L Tris, pH 8.0
f. Remove a 10 µL aliquot as the "before" sample for the SDS-PAGE analysis of the digest.
g. Dissolve 1 mg of Promega Trypsin Gold in 1 mL 100 mM Tris, pH 8.0 and add to digest with gentle mixing. (to achieve a 1:50 enzyme-to-substrate ratio for the ≈ 60 mg total protein present)
h. Incubate overnight (18 h) at 37 °C
i. Remove one 10 µL aliquot for SDS-PAGE.
j. Add 300 µL of concentrated formic acid to quench the digestion for a final concentration of 1%.
k. Perform SPE desalting using a Waters Oasis HLB vac 6 g (35 cc) extraction cartridge:
    i. Condition cartridge with 3 x 20 mL of 0.1 % formic acid in 80 % ACN
    ii. Equilibrate cartridge with 4 x 20 mL of 0.1 % formic acid in 100 % water
    iii. Add sample to cartridge
    iv. Wash cartridge with 4 x 20 mL of 0.1 % formic acid in 100 % water
    v. Elute plasma digest peptides with 3 x 20 mL 0.1 % formic acid in 80 % ACN
    vi. Lyophilize to dryness
l. Reconstitute dried and desalted plasma digest with 12 mL of 5 % formic acid, 3 % acetonitrile, 92% water
m. Add 48 mL of water for final concentrations of 1 % formic acid, 0.6 % acetonitrile, and 1 µg/µL of total protein.
n. Take one 25 µL aliquot for LC-MS analysis.

Appendix B: Digestion protocol performed at NIST for bulk digestion of target proteins to be used in Study II.

a. Co-lyophilize 150 µL of each 100 pmol/µL protein stock solution into a 2 mL polypropylene centrifuge tube.
b. Reconstitute with 150 µL of 100 mmol/L Tris, pH 8.0, 5 mmol/L DTT, 6 mol/L urea.
c. Heat for 30 min at 37 °C.
d. Add 4.5 µL of 0.5 mol/L IAM in water to obtain 15 mmol/L IAM in the sample.
e. Alkylate for 30 min at room temperature in the dark.
f. Add 745 µL of 100 mmol/L Tris, pH 8.0 to dilute the urea concentration to 1 mol/L.
g. Add 60 µL of 1 µg/µL Promega Gold trypsin in 100 mmol/L Tris (to achieve a 1:50 enzyme-to-substrate ratio for the ≈ 3 mg total protein present).
h. Heat to 37 °C overnight (≈ 18h).
i. Add 10 µL of concentrated formic acid to yield a concentration of 1 % formic acid in the digest
j. Perform SPE desalting using a Waters Oasis HLB Vac 30 mg (1 cc) extraction cartridge:
    i. Condition cartridge with 3 x 0.5 mL of 0.1 % formic acid in 80 % ACN
    ii. Equilibrate cartridge with 4 x 0.5 mL of 0.1 % formic acid in 100 % water
    iii. Add sample to cartridge
    iv. Wash cartridge with 4 x 0.5 mL of 0.1 % formic acid in 100 % water
    v. Elute plasma digest peptides with 3 x 0.5 mL 0.1 % formic acid in 80 % ACN
    vi. Lyophilize to dryness
k. Reconstitute the lyophilized desalted digest with 60 µL of 5% formic acid, 3 % ACN, 92% water
l. Add 240 µL water to yield a digestion mixture with 50 pmol/µL of each of the seven target proteins in 1 % formic acid, 0.6 % acetonitrile

Appendix C. Preparation of the Study III samples

o. To prepare Sample III-J, 3566 µL of an equimolar mixture of the seven target proteins (50 pmol/µL) will be freeze-dried into a 15 mL polypropylene tube and then reconstituted with 5943 µL of human pooled K_2EDTA plasma; 75 x 35 µL aliquots will be dispensed into 500 µL screw-capped polypropylene tubes.

p. To prepare Sample III-G through III-B, the dilution scheme outlined in SOP Table G below will be followed:

SOP Table G. Dilution scheme for the preparation of Sample III-I to III-B

Sample	Volume of Sample Used	Volume Plasma Used
Sample III-I	3223 µL of Sample III-J	2637 µL
Sample III-H	3140 µL of Sample III-I	2579 µL
Sample III-G	2999 µL of Sample III-H	2457 µL
Sample III-F	2735 µL of Sample III-G	2200 µL
Sample III-E	2216 µL of Sample III-F	1861 µL
Sample III-D	1357 µL of Sample III-E	2610 µL
Sample III-C	1247 µL of Sample III-D	2404 µL
Sample III-B	932 µL of Sample III-C	1788 µL

q. For Samples III-I through III-B, 75 x 35 µL aliquots will be dispensed into 500 µL screw-capped polypropylene tubes.

r. For Sample III-A and Sample III-Blank, 75 x 35 µL of the plasma pool will be aliquotted into 500 µL screw-capped polypropylene tubes.

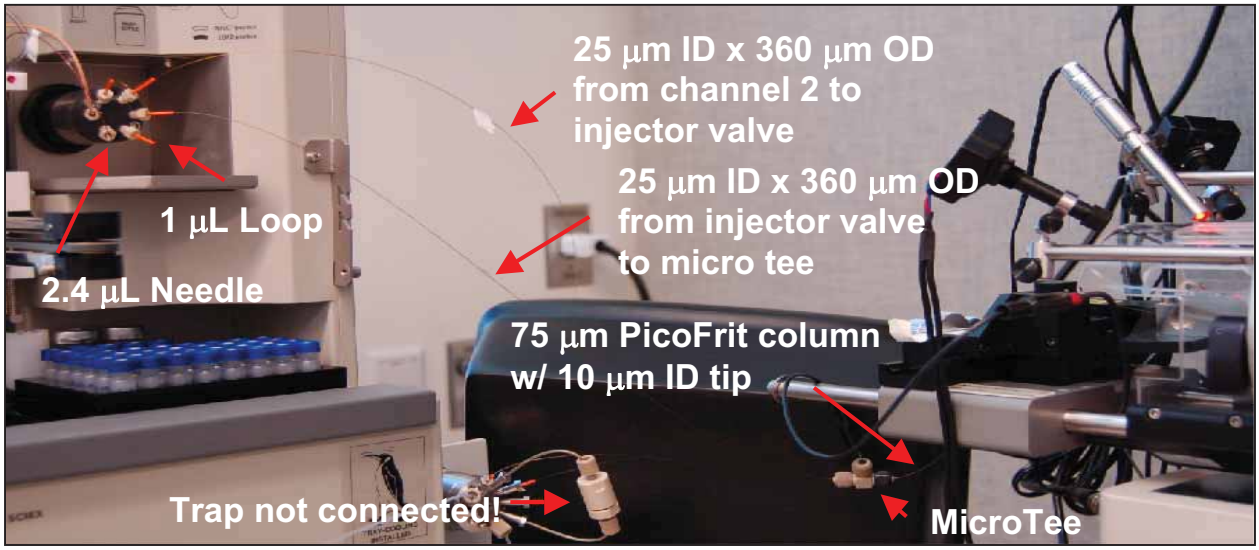
s. To prepare Sample III-QC, approximately 90 µL of the 1 pmol/µL equimolar mixture of the 11 signature peptides (light) will be combined with 90 µL of a 1 pmol/µL equimolar mixture of the 11 IS peptides (heavy) and then diluted with 1620 µL of 1 % formic acid in water to yield a 50 fmol/µL equimolar mixture of the 11 signature and 11 IS peptides; 50 x 35 µL aliquots will be dispensed into 500 µL screw-capped polypropylene tubes.

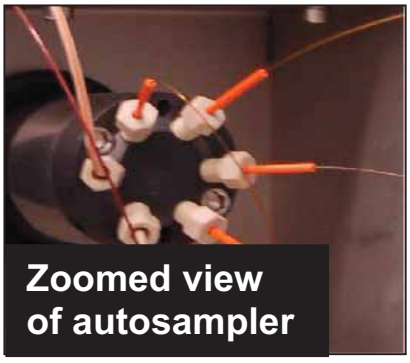
t. All samples will be stored at -80 °C.

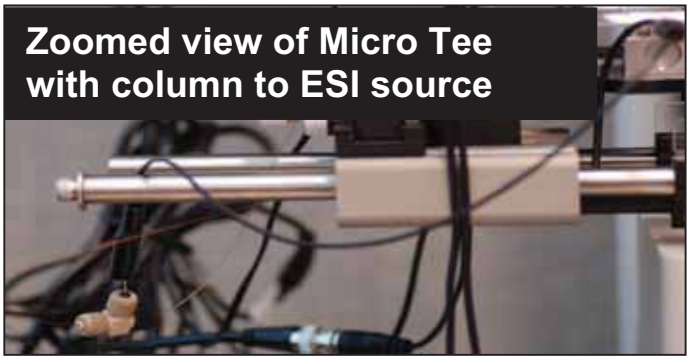
Appendix D: Typical HPLC Setup at one of the CPTAC sites

All fused silica tubing was purchased from Polymicro Technologies. Lengths of tubing are dependent upon the distance between the LC and electrospray source, and are minimized to reduce delay volume.

Note that 1 µL fused silica loops have been replaced with custom 1 µL PEEKsil.







Appendix E: Guidelines for Packing 75 μm ID NanoLC Columns

1. Prepare slurry of 3 μm Reprosil material in 70% acetonitrile/30% isopropanol in borosilicate glass vial with stir bar.
2. Sonicate ~5 minutes
3. Wash Self Pack PicoFrit column with 70% acetonitrile/30% isopropanol for ~1-2 minutes.
4. Place slurry into a stainless steel pressure vessel on top of stir plate and connected to Helium (set to 500 psi).
5. Pack to desired length (12 cm) by pressurizing vessel.
6. After column has reached the appropriate length, turn off He and replace slurry with 0.1% formic acid.
7. Complete packing and wash/equilibrate with 0.1% formic acid for ~15-20 min.
8. Condition column with appropriate QC standard and/or blank gradient.

Supplementary Table 1A: Overview of Experimental Metadata for Studies I, II, and IIIa-c from all eight CPTAC sites including seven 4000 QTRAPs and one TSQ Quantum Ultra. Chromatographic and Mass Spectrometric Details

Mass Spectrometric Parameters:

	CPTAC sites:	site @52	site @56	site @86	site @95	site @73	site @19	site @54	site @65
Mass Spectrometer		4000 QTRAP	4000 QTRAP	4000 QTRAP	4000 QTRAP	4000 QTRAP	4000 QTRAP	4000 QTRAP	TSQ Quantum Ultra
Source/Gas Parameters	**recommended in SOP**								
Spray voltage	2200 ± 200 V								
Study I		2450	2200	2200	3000	2300	2400	2200	1000
Study II		2450	2200	2200	3000	2300	2500	2200	1000
Study IIIa-c		2450	2200	2200 (IIIa) 2300 (IIIb and IIIc)	2200	2300	2500	2200	1200
Curtain gas (4000 QTRAP)	20								
Study I		12	20	20	20	20	20	20	N/A
Study II		12	20	20	20	20	20	20	N/A
Study IIIa-c		12	20	20	20	20	20	20	N/A
Nebulizer gas (GS1) (4000 QTRAP)	5 ± 2								
Study I		14	3	10	35	4	4	12	N/A
Study II		14	3	5	35	4	8	12	N/A
Study IIIa-c		14	3	5 (IIIa) 20 (IIIb and IIIc)	35	4	7	12	N/A
IHT or capillary temperature	150 °C								
Study I		150	150	150	150	150	150	150	capillary temperature of 210 ºC
Study II		150	150	150	150	150	150	150	capillary temperature of 210 ºC
Study IIIa-c		150	150	150	150	150	150	150	capillary temperature of 210 ºC
Compound Parameters									
Total # of MRM transitions	66	66	66	66	66	66	66	66	66
# of transitions per peptide	3	3	3	3	3	3	3	3	3
Modification of instrument method to reflect changes in the masses of transitions		//	//	//	//	//	//	//	//
DP, CE, CXP (tube lens for Quantum)	Same for labeled/ unlabeled peptide pairs	yes (Suppl. Tables)	yes (Suppl. Tables)	yes (Suppl. Tables)	yes (Suppl. Tables)	yes (Suppl. Tables)	yes (Suppl. Tables)	yes (Suppl. Tables)	yes (Suppl. Tables)
Dwell Time	10 ms	10 ms	10 ms	10 ms	10 ms	10 ms	10 ms	10 ms	Scan time = 15 ms (achieving 8-10 scans across peak)

	CPTAC sites:	site @52	site @56	site @86	site @95	site @73	site @19	site @54	site @65
MS detector									
MS Acquisition Duration (min)	80	80	80	80 min	80	80	80	80	85 min
Cycle time (ms)	0.99 sec	0.99 sec	0.99 sec	0.89 sec	0.99 sec	0.99 sec	0.99 sec	0.9903 sec	0.99 sec
Q1/Q3 Resolution	unit/unit	unit/unit	unit/unit	unit/unit	unit/unit	unit/unit	unit/unit	unit/unit	unit/unit (0.7 FWHM)
Settling Time	0	0	0	0	0	0	0	0	N/A
Pause between mass ranges	5 ms	5.007 ms	5 ms	3.5 ms	5 ms	5 ms	5 ms	5.005 ms	N/A
CAD Gas	not defined	high	medium	8	high	high	medium	medium	Argon
Quantum spec. Parameters									
Tube Lens (Quantum)		N/A	N/A	N/A	N/A	N/A	N/A	N/A	Determined during AutoTune
Q2 gas pressure (Quantum)		N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.5 mTorr
capillary offset voltage (Quantum)		N/A	N/A	N/A	N/A	N/A	N/A	N/A	35 V
skimmer offset voltage (Quantum)		N/A	N/A	N/A	N/A	N/A	N/A	N/A	- 5 V

Software

MS software	4000 QTRAP Analyst version	Analyst v1.4.2	Analyst v1.5	Analyst v1.4.2	Analyst v1.4.2	Analyst v1.5	Analyst v1.5	Analyst v1.4.2	//
MS software	Quantum Xcalibur version	//	//	//	//	//	//	//	Quantum v1.4.1/ Xcalibur v2.0.6
Data analysis software	ABI Multiquant version	Multiquant v1.0	Multiquant v1.0.0.1	Multiquant v1.0	Multiquant v1.0.0.1	Multiquant v1.0.0.1	Multiquant v1.0.0.1	Multiquant v1.0	//
Data analysis software	Thermo / others	//	//	//	//	//	//	//	SRM Builder (beta test versions -- Thermo)

Footnote:
Chromatographic and Mass Spectrometric Parameters were recommended in the Standard Operating Procedure (SOP)
Slight Deviations to the SOP were allowed in order for each site to optimize their platforms. All such SOP deviations (if applicable) are documented in this table.

HPLC Parameters and Chromatography:

HPLC gradient described in SOP:

Gradient Step	Solvent % A	Solvent % B	Duration [min]
1	97	3	0
2	97	3	5
3	80	20	8
4	40	60	43
5	10	90	45
6	10	90	49
7	97	3	50
8	97	3	80

	CPTAC sites:	site @52	site @56	site @86	site @95	site @73	site @19	site @54	site @65
HPLC Vendor		Eksigent	Eksigent	Eksigent	Agilent	Eksigent	Eksigent	Eksigent	Eksigent
1D / 2D HPLC system		nano LC-2D	nano LC-2D	nano LC-2D	Agilent 1100 Nanosystem	nano LC-2D	nano LC-2D	nano LC-2D	nano LC 1D Plus
Parameter	**recommended in SOP**								
Autosampler temperature	4-10 °C	10-12 °C	4-10 °C	4-10 °C	5 °C	9 °C	8 °C	15 °C	10 °C
Column dimensions	12cm x 75µm, 10µm tip	12cm x 75µm, 10µm tip	12cm x 75µm, 10µm tip	12cm x 75µm, 10µm tip	12cm x 75µm, 10µm tip	12cm x 75µm, 10µm tip	12cm x 75µm, 10µm tip	12cm x 75µm, 10µm tip	11cm x 75µm, 10µm tip
Column packing material	Reprosil 3 µm	ReproSil-Pur C18-AQ	ReproSil-Pur C18-AQ	ReproSil-Pur C18-AQ	ReproSil-Pur C18-AQ	ReproSil-Pur C18-AQ	ReproSil-Pur C18-AQ	ReproSil-Pur C18-AQ	ReproSil-Pur C18-AQ
# of columns to pack	3 (initial column and 2 backup columns)	yes	yes	yes	yes	yes	yes	yes	yes
Column blank manufacturer (for "handpacking")		PicoFrit 75 µm ID (New Objective), hand packed	PicoFrit 75 µm ID (New Objective), hand packed	PicoFrit 75 µm ID (New Objective), hand packed	PicoFrit 75 µm ID (New Objective), hand packed	IntegraFrit 75 µm ID (New Objective), hand packed, coupled to SilicaTip 10µm emitter (New Objective)	PicoFrit 75 µm ID (New Objective), hand packed	PicoFrit 75 µm ID (New Objective), hand packed	PicoFrit 75 µm ID (New Objective), hand packed
Column temperature	Room temperature	yes	yes	yes	yes	yes	yes	yes	yes
Mobile phase A	0.1% (v/v) formic acid in water	0.1% (v/v) FA	0.1% (v/v) FA	0.1% (v/v) FA	0.1% (v/v) FA	0.1% (v/v) FA	0.1% (v/v) FA	0.1% (v/v) FA	0.1% (v/v) FA
Mobile phase B	90% (v/v) acetonitrile 0.1% (v/v) formic acid	90% (v/v) ACN, 0.1% (v/v) FA	90% (v/v) ACN, 0.1% (v/v) FA	90% (v/v) ACN, 0.1% (v/v) FA	90% (v/v) ACN, 0.1% (v/v) FA	90% (v/v) ACN, 0.1% (v/v) FA	90% (v/v) ACN, 0.1% (v/v) FA	90% (v/v) ACN, 0.1% (v/v) FA	90% (v/v) ACN, 0.1% (v/v) FA
Injection volume	1.0 µL	1.0 µL	1.0 µL	1.0 µL	1.0 µL	1.0 µL	1.0 µL	1.0 µL	1.0 µL
Injection protocol		full loop injection	full loop injection	full loop injection	1µL injected from 8 µL loop	full loop injection	full loop injection	full loop injection	full loop injection
Loop (Eksigent only)	100 µm Peeksil	100 µm Peeksil	100 µm Peeksil	100 µm Peeksil	8 µL capillary loop	100 µm Peeksil	100 µm Peeksil	100 µm Peeksil	100 µm Peeksil
Flow rate for gradient	300 nL/min (Eksigent) 200 nL/min (Agilent)	300 nL/min //	300 nL/min //	300 nL/min //	// 200 nL/min (Agilent)	300 nL/min //	300 nL/min //	300 nL/min //	300 nL/min //
Autosampler Method	as described in SOP	yes	yes	yes	yes	yes	yes	yes	yes
80 min HPLC program	see above (as described in SOP)	yes	yes	5 min extended wash after the gradient (for studies I, II, and IIIa); for study IIIb and IIIc 10 min extended wash after the gradient	at beginning of each run there was a 20 minute equilibration of the column at a flow rate of 600 nL/min	yes	yes	yes	yes
additional comments		//	//	//	//	Study I,II,III included a 1cm packed column (same material) in front of the nanocolumn	//	//	The column length was 11 cm length fitting the specific nanospray set-up at this site without having to reconfigure , i.e., re-configuration of electrodes, etc.)

Footnote:
Chromatographic and Mass Spectrometric Parameters were recommended in the Standard Operating Procedure (SOP)
Slight Deviations to the SOP were allowed in order for each site to optimize their platforms. All such SOP deviations (if applicable) are documented in this table.

Supplementary Table 1B: **Overview of MRM Transitions from all eight CPTAC sites including seven 4000 QTRAPs and one TSQ Quantum Ultra, and Lists of specific Instrument Parameters: Declustering Potential (DP), Collision Energy (CE), and Collision Cell Exit Potential (CXP).**

MRM Transitions and DP/CE/CXP Values

4000 QTRAP at CPTAC site @52

Protein	Signature Peptide	Identifier	MH+ (mono)	z (Q1)	MRM Transitions Q1	MRM Transitions Q3	DP	CE	CXP	Fragment Ion Type
aprotinin (APR)	AGLCamcQTFVYGGCamcR	bi0173	1488.7	2	744.8	858.4	100	40	12	y7
				2	744.8	959.4	100	40	14	y8
				2	744.8	1087.5	100	38	17	y9
	AGLCamcQTFVYGGCamcR	**bi0081**	**1493.7**	**2**	**747.3**	**863.4**	**100**	**40**	**12**	y7
				2	**747.3**	**964.4**	**100**	**40**	**14**	y8
				2	**747.3**	**1092.5**	**100**	**38**	**17**	y9
leptin (LEP)	INDISHTQSVSAK	bi0167	1399.7	3	467.2	586.8	76	21	10	$y11^{2+}$
				3	467.2	643.8	76	23	10	$y12^{2+}$
				3	467.2	720.39	76	29	10	y7
	INDISHTQSVSAK	**ni0101**	**1407.3**	**3**	**469.9**	**590.81**	**76**	**21**	**10**	$y11^{2+}$
				3	**469.9**	**647.83**	**76**	**23**	**10**	$y12^{2+}$
				3	**469.9**	**728.4**	**76**	**29**	**10**	y7
myoglobin (MYO)	LFTGHPETLEK	bi0171	1271.7	3	424.6	506.3	71	19	8	$y9^{2+}$
				3	424.6	579.8	71	20	8	$y10^{2+}$
				3	424.6	716.4	71	25	10	y6
	LFTGHPETLEK	**ni0102**	**1279.7**	**3**	**427.2**	**510.27**	**71**	**19**	**8**	$y9^{2+}$
				3	**427.2**	**583.8**	**71**	**20**	**8**	$y10^{2+}$
				3	**427.2**	**724.4**	**71**	**25**	**10**	y6
myelin basic protein (MBP)	HGFLPR	bi0169	726.4	2	363.7	385.3	70	23	16	y3
				2	363.7	532.3	70	23	12	y4
				2	363.7	589.4	70	23	8	y5
	HGFLPR	**ni0104**	**732.4**	**2**	**366.7**	**391.3**	**70**	**23**	**16**	y3
				2	**366.7**	**538.3**	**70**	**23**	**12**	y4
				2	**366.7**	**595.4**	**70**	**23**	**8**	y5
	YLASASTMDHAR	bi0170	1322.6	3	441.5	488.2	90	21	12	$y9^{2+}$
				3	441.5	523.7	90	19	16	$y10^{2+}$
				3	441.5	817.4	90	24	14	y7
	YLASASTMDHAR	**ni0105**	**1328.6**	**3**	**443.5**	**491.23**	**90**	**21**	**12**	$y9^{2+}$
				3	**443.5**	**526.75**	**90**	**19**	**16**	$y10^{2+}$
				3	**443.5**	**823.38**	**90**	**24**	**14**	y7
prostate specific antigen (PSA)	IVGGWECamcEK	bi0161	1077.5	2	539.3	808.3	81	27	12	y6
				2	539.3	865.4	81	26	14	y7
				2	539.3	964.42	81	27	16	y8
	IVGGWECamcEK	**bi0067**	**1082.5**	**2**	**541.7**	**808.33**	**81**	**27**	**12**	y6
				2	**541.7**	**865.35**	**81**	**26**	**14**	y7
				2	**541.7**	**969.44**	**81**	**27**	**16**	y8
	LSEPAELTDAVK	bi0037	1272.7	2	636.8	775.4	91	39	11	y7
				2	636.8	846.4	91	37	12	y8
				2	636.8	943.4	91	29	15	y9
	LSEPAELTDAVK	**ni0107**	**1280.7**	**2**	**640.8**	**783.43**	**91**	**39**	**11**	y7
				2	**640.8**	**854.47**	**91**	**37**	**12**	y8
				2	**640.8**	**951.52**	**91**	**29**	**15**	y9
horseradish peroxidase (HRP)	SSDLVALSGGHTFGK	bi0166	1475.7	3	492.6	703.35	76	27	10	y7
				3	492.6	790.38	76	27	12	y8
				3	492.6	974.51	76	26	15	y10
	SSDLVALSGGHTFGK	**ni0108**	**1483.8**	**3**	**495.3**	**711.37**	**76**	**27**	**10**	y7
				3	**495.3**	**798.4**	**76**	**27**	**12**	y8
				3	**495.3**	**982.52**	**76**	**26**	**15**	y10
C-reactive protein (CRP)	ESDTSYVSLK	bi0231	1128.5	2	564.8	609.4	81	27	8	y5
				2	564.8	696.4	81	27	10	y6
				2	564.8	797.4	81	29	12	y7
	ESDTSYVSLK	**ni0109**	**1136.6**	**2**	**568.8**	**617.37**	**81**	**27**	**8**	y5
				2	**568.8**	**704.41**	**81**	**27**	**10**	y6
				2	**568.8**	**805.45**	**81**	**29**	**12**	y7
	GYSIFSYATK	bi0202	1136.3	2	568.8	716.4	76	30	10	y6
				2	568.8	829.5	76	27	12	y7
				2	568.8	916.5	76	25	14	y8
	GYSIFSYATK	**ni0110**	**1144.6**	**2**	**572.8**	**724.38**	**76**	**30**	**10**	y6
				2	**572.8**	**837.46**	**76**	**27**	**12**	y7
				2	**572.8**	**924.49**	**76**	**25**	**14**	y8
	YEVQGEVFTKPQLWP	ni0001	1820.9	2	911	1053.5	106	34	16	b9
				2	911	1181.6	106	33	18	b10
				2	911	1519.8	106	37	18	b13
	YEVQGEVFTKPQLWP	**ni0111**	**1826.9**	**2**	**914**	**1053.49**	**106**	**34**	**16**	b9
				2	**914**	**1181.58**	**106**	**33**	**18**	b10
				2	**914**	**1525.8**	**106**	**37**	**18**	b13

4000 QTRAP at CPTAC site @56

MH+ (mono)	z (Q1)	MRM Transitions Q1	MRM Transitions Q3	DP	CE	CXP	Fragment Ion Type
1488.7	2	744.8	858.39	120	35	16	y7
	2	744.8	959.44	120	38	16	y8
	2	744.8	1087.5	120	40	16	y9
1493.7	**2**	**747.3**	**863.41**	**120**	**35**	**16**	y7
	2	**747.3**	**964.46**	**120**	**38**	**16**	y8
	2	**747.3**	**1092.5**	**120**	**40**	**16**	y9
1399.7	3	467.2	586.8	61	20	12	$y11^{2+}$
	3	467.2	643.82	61	20	12	$y12^{2+}$
	3	467.2	720.39	61	20	12	y7
1407.3	**3**	**469.9**	**590.81**	**61**	**20**	**12**	$y11^{2+}$
	3	**469.9**	**647.83**	**61**	**20**	**12**	$y12^{2+}$
	3	**469.9**	**728.4**	**61**	**20**	**12**	y7
1271.7	3	424.6	506.26	66	18	12	$y9^{2+}$
	3	424.6	579.79	66	19	10	$y10^{2+}$
	3	424.6	716.38	66	24	13	y6
1279.7	**3**	**427.2**	**510.27**	**66**	**18**	**12**	$y9^{2+}$
	3	**427.2**	**583.8**	**66**	**19**	**10**	$y10^{2+}$
	3	**427.2**	**724.4**	**66**	**24**	**13**	y6
726.4	2	363.7	385.26	70	23	16	y3
	2	363.7	532.32	70	22	16	y4
	2	363.7	589.35	70	22	14	y5
732.4	**2**	**366.7**	**391.28**	**70**	**23**	**16**	y3
	2	**366.7**	**538.34**	**70**	**22**	**16**	y4
	2	**366.7**	**595.37**	**70**	**22**	**14**	y5
1322.6	3	441.5	488.22	61	20	14	$y9^{2+}$
	3	441.5	523.74	61	17	14	$y10^{2+}$
	3	441.5	817.36	61	23	13	y7
1328.6	**3**	**443.5**	**491.23**	**61**	**20**	**14**	$y9^{2+}$
	3	**443.5**	**526.75**	**61**	**17**	**14**	$y10^{2+}$
	3	**443.5**	**823.38**	**61**	**23**	**13**	y7
1077.5	2	539.3	808.33	75	27	14	y6
	2	539.3	865.35	75	25	14	y7
	2	539.3	964.42	75	27	16	y8
1082.5	**2**	**541.7**	**808.33**	**75**	**27**	**14**	y6
	2	**541.7**	**865.35**	**75**	**25**	**14**	y7
	2	**541.7**	**969.44**	**75**	**27**	**16**	y8
1272.7	2	636.8	775.42	75	39	15	y7
	2	636.8	846.46	75	39	15	y8
	2	636.8	943.51	75	31	15	y9
1280.7	**2**	**640.8**	**783.43**	**75**	**39**	**15**	y7
	2	**640.8**	**854.47**	**75**	**39**	**15**	y8
	2	**640.8**	**951.52**	**75**	**31**	**15**	y9
1475.7	3	492.6	703.35	70	27	14	y7
	3	492.6	790.38	70	27	14	y8
	3	492.6	974.51	70	24	16	y10
1483.8	**3**	**495.3**	**711.37**	**70**	**27**	**14**	y7
	3	**495.3**	**798.4**	**70**	**27**	**14**	y8
	3	**495.3**	**982.52**	**70**	**24**	**16**	y10
1128.5	2	564.8	609.36	86	23	10	y5
	2	564.8	696.39	86	31	12	y6
	2	564.8	797.44	81	31	14	y7
1136.6	**2**	**568.8**	**617.37**	**86**	**23**	**10**	y5
	2	**568.8**	**704.41**	**86**	**31**	**12**	y6
	2	**568.8**	**805.45**	**81**	**31**	**14**	y7
1136.3	2	568.8	716.36	91	31	16	y6
	2	568.8	829.45	51	27	18	y7
	2	568.8	916.48	71	25	16	y8
1144.6	**2**	**572.8**	**724.38**	**91**	**31**	**16**	y6
	2	**572.8**	**837.46**	**51**	**27**	**18**	y7
	2	**572.8**	**924.49**	**71**	**25**	**16**	y8
1820.9	2	911	1053.49	80	35	25	b9
	2	911	1181.58	80	32	25	b10
	2	911	1519.78	80	36	25	b13
1826.9	**2**	**914**	**1053.49**	**80**	**35**	**25**	b9
	2	**914**	**1181.58**	**80**	**32**	**25**	b10
	2	**914**	**1525.8**	**80**	**36**	**25**	b13

Footnote: Instrument Parameters for MRM transitions were optimized for maximum transmission efficiency and sensitivity for individual instruments by infusion of unlabeled signature peptides.
Optimized declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) are reported for 4000 QTRAP instruments for each MRM transition along with the corresponding instrument used at each site.
For the TSQ Quantum Ultra the collision energy (CE) is reported for each MRM transition. Boldface type indicates labeled peptide internal standard with labeled amino acid in red.

		4000 QTRAP at CPTAC site @86							4000 QTRAP at CPTAC site @95							4000 QTRAP at CPTAC site @73						
Identifier	MH+ (mono)	z (Q1)	MRM Transitions Q1	Q3	DP	CE	CXP	Fragment Ion Type	MH+ (mono)	z (Q1)	MRM Transitions Q1	Q3	DP	CE	CXP	Fragment Ion Type	MH+ (mono)	z (Q1)	MRM Transitions Q1	Q3	DP	CE	CXP	Fragment Ion Type
		2	744.8	858.39	120	38	20	y7		2	744.8	858.39	76	41	29	y7		2	744.8	858.4	105	40	13	y7
		2	744.8	959.44	120	38	22	y8		2	744.8	959.44	84	45	17	y8		2	744.8	959.4	105	39	13	y8
bi0173	1488.7	2	744.8	1087.5	120	40	24	y9	1488.7	2	744.8	1087.5	76	42	20	y9	1488.7	2	744.8	1087.5	105	39	13	y9
		2	**747.3**	**863.41**	**120**	**38**	**20**	y7		**2**	**747.3**	**863.41**	**76**	**41**	**29**	y7		**2**	**747.3**	**863.4**	**105**	**40**	**13**	y7
		2	**747.3**	**964.46**	**120**	**38**	**22**	y8		**2**	**747.3**	**964.46**	**84**	**45**	**17**	y8		**2**	**747.3**	**964.4**	**105**	**39**	**13**	y8
bi0081	**1493.7**	**2**	**747.3**	**1092.5**	**120**	**40**	**24**	y9	**1493.7**	**2**	**747.3**	**1092.5**	**76**	**42**	**20**	y9	**1493.7**	**2**	**747.3**	**1092.5**	**105**	**39**	**13**	y9
		3	467.2	586.8	61	22	12	$y11^{2+}$		3	467.2	586.8	59	26	19	$y11^{2+}$		3	467.2	586.8	65	22	21	$y11^{2+}$
		3	467.2	643.82	61	22	14	$y12^{2+}$		3	467.2	643.82	56	29	21	$y12^{2+}$		3	467.2	643.82	65	22	11	$y12^{2+}$
bi0167	1399.7	3	467.2	720.39	61	30	16	y7	1399.7	3	467.2	720.39	58	31	20	y7	1399.7	3	467.2	720.39	65	28	18	y7
		3	**469.9**	**590.81**	**61**	**22**	**12**	$y11^{2+}$		**3**	**469.9**	**590.81**	**59**	**26**	**19**	$y11^{2+}$		**3**	**469.9**	**590.81**	**65**	**22**	**21**	$y11^{2+}$
		3	**469.9**	**647.83**	**61**	**22**	**14**	$y12^{2+}$		**3**	**469.9**	**647.83**	**56**	**29**	**21**	$y12^{2+}$		**3**	**469.9**	**647.83**	**65**	**22**	**11**	$y12^{2+}$
ni0101	**1407.3**	**3**	**469.9**	**728.4**	**61**	**30**	**16**	y7	**1407.3**	**3**	**469.9**	**728.4**	**58**	**31**	**20**	y7	**1407.3**	**3**	**469.9**	**728.4**	**65**	**28**	**18**	y7
		3	424.6	506.26	66	18	10	y9 2+		3	424.6	506.26	64	23	16	$y9^{2+}$		3	424.6	506.3	65	19	23	$y9^{2+}$
		3	424.6	579.79	66	20	12	y10 2+		3	424.6	579.79	54	26	10	$y10^{2+}$		3	424.6	579.8	65	19	14	$y10^{2+}$
bi0171	1271.7	3	424.6	716.38	66	24	16	y6	1271.7	3	424.6	716.38	56	25	19	y6	1271.7	3	424.6	716.4	65	23	14	y6
		3	**427.2**	**510.27**	**66**	**18**	**10**	y9 2+		**3**	**427.2**	**510.27**	**64**	**23**	**16**	$y9^{2+}$		**3**	**427.2**	**510.27**	**65**	**19**	**23**	$y9^{2+}$
		3	**427.2**	**583.8**	**66**	**20**	**12**	y10 2+		**3**	**427.2**	**583.8**	**54**	**26**	**10**	$y10^{2+}$		**3**	**427.2**	**583.8**	**65**	**19**	**14**	$y10^{2+}$
ni0102	**1279.7**	**3**	**427.2**	**724.4**	**66**	**24**	**16**	y6	**1279.7**	**3**	**427.2**	**724.4**	**56**	**25**	**19**	y6	**1279.7**	**3**	**427.2**	**724.4**	**65**	**23**	**14**	y6
		2	363.7	385.26	70	22	16	y3		2	363.7	385.26	61	27	12	y3		2	363.7	385.3	70	23	19	y3
		2	363.7	532.32	70	22	16	y4		2	363.7	532.32	64	27	17	y4		2	363.7	532.3	70	22	19	y4
bi0169	726.4	2	363.7	589.35	70	23	11	y5	726.4	2	363.7	589.35	69	26	19	y5	726.4	2	363.7	589.4	70	23	12	y5
		2	**366.7**	**391.28**	**70**	**22**	**16**	y3		**2**	**366.7**	**391.28**	**61**	**27**	**12**	y3		**2**	**366.7**	**391.3**	**70**	**23**	**19**	y3
		2	**366.7**	**538.34**	**70**	**22**	**16**	y4		**2**	**366.7**	**538.34**	**64**	**27**	**17**	y4		**2**	**366.7**	**538.3**	**70**	**22**	**19**	y4
ni0104	**732.4**	**2**	**366.7**	**595.37**	**70**	**23**	**11**	y5	**732.4**	**2**	**366.7**	**595.37**	**69**	**26**	**19**	y5	**732.4**	**2**	**366.7**	**595.4**	**70**	**23**	**12**	y5
		3	441.5	488.22	65	21	9	y9 2+		3	441.5	488.22	51	23	15	$y9^{2+}$		3	441.5	488.2	60	21	13	$y9^{2+}$
		3	441.5	523.74	55	19	10	y10 2+		3	441.5	523.74	54	19	17	$y10^{2+}$		3	441.5	523.7	60	19	14	$y10^{2+}$
bi0170	1322.6	3	441.5	817.36	61	26	18	y7	1322.6	3	441.5	817.36	51	28	15	y7	1322.6	3	441.5	817.4	60	24	14	y7
		3	**443.5**	**491.23**	**65**	**21**	**9**	y9 2+		**3**	**443.5**	**491.23**	**51**	**23**	**15**	$y9^{2+}$		**3**	**443.5**	**491.23**	**60**	**21**	**13**	$y9^{2+}$
		3	**443.5**	**526.75**	**55**	**19**	**10**	y10 2+		**3**	**443.5**	**526.75**	**54**	**19**	**17**	$y10^{2+}$		**3**	**443.5**	**526.75**	**60**	**19**	**14**	$y10^{2+}$
ni0105	**1328.6**	**3**	**443.5**	**823.38**	**61**	**26**	**18**	y7	**1328.6**	**3**	**443.5**	**823.38**	**51**	**28**	**15**	y7	**1328.6**	**3**	**443.5**	**823.38**	**60**	**24**	**14**	y7
		2	539.3	808.33	75	27	20	y6		2	539.3	808.33	64	29	21	y6		2	539.3	808.3	70	27	13	y6
		2	539.3	865.35	75	25	20	y7		2	539.3	865.35	66	29	16	y7		2	539.3	865.4	70	26	13	y7
bi0161	1077.5	2	539.3	964.42	75	28	22	y8	1077.5	2	539.3	964.42	61	29	18	y8	1077.5	2	539.3	964.42	70	27	14	y8
		2	**541.7**	**808.33**	**75**	**27**	**20**	y6		**2**	**541.7**	**808.33**	**64**	**29**	**21**	y6		**2**	**541.7**	**808.33**	**70**	**27**	**13**	y6
		2	**541.7**	**865.35**	**75**	**25**	**20**	y7		**2**	**541.7**	**865.35**	**66**	**29**	**16**	y7		**2**	**541.7**	**865.35**	**70**	**26**	**13**	y7
bi0067	**1082.5**	**2**	**541.7**	**969.44**	**75**	**28**	**22**	y8	**1082.5**	**2**	**541.7**	**969.44**	**61**	**29**	**18**	y8	**1082.5**	**2**	**541.7**	**969.44**	**70**	**27**	**14**	y8
		2	636.8	775.42	75	37	18	y7		2	636.8	775.42	74	40	19	y7		2	636.8	775.4	75	38	11	y7
		2	636.8	846.46	75	38	20	y8		2	636.8	846.46	71	48	14	y8		2	636.8	846.46	75	38	11	y8
bi0037	1272.7	2	636.8	943.51	75	30	22	y9	1272.7	2	636.8	943.51	79	33	17	y9	1272.7	2	636.8	943.51	75	30	14	y9
		2	**640.8**	**783.43**	**75**	**37**	**18**	y7		**2**	**640.8**	**783.43**	**74**	**40**	**19**	y7		**2**	**640.8**	**783.43**	**75**	**38**	**11**	y7
		2	**640.8**	**854.47**	**75**	**38**	**20**	y8		**2**	**640.8**	**854.47**	**71**	**48**	**14**	y8		**2**	**640.8**	**854.47**	**75**	**38**	**11**	y8
ni0107	**1280.7**	**2**	**640.8**	**951.52**	**75**	**30**	**22**	y9	**1280.7**	**2**	**640.8**	**951.52**	**79**	**33**	**17**	y9	**1280.7**	**2**	**640.8**	**951.52**	**75**	**30**	**14**	y9
		3	492.6	703.35	70	26	16	y7		3	492.6	703.35	64	29	18	y7		3	492.6	703.35	69	26	11	y7
		3	492.6	790.38	70	26	17	y8		3	492.6	790.38	59	32	20	y8		3	492.6	790.38	69	27	11	y8
bi0166	1475.7	3	492.6	974.51	70	26	24	y10	1475.7	3	492.6	974.51	54	28	17	y10	1475.7	3	492.6	974.51	69	25	11	y10
		3	**495.3**	**711.37**	**70**	**26**	**16**	y7		**3**	**495.3**	**711.37**	**64**	**29**	**18**	y7		**3**	**495.3**	**711.37**	**69**	**26**	**11**	y7
		3	**495.3**	**798.4**	**70**	**26**	**17**	y8		**3**	**495.3**	**798.4**	**59**	**32**	**20**	y8		**3**	**495.3**	**798.4**	**69**	**27**	**11**	y8
ni0108	**1483.8**	**3**	**495.3**	**982.52**	**70**	**26**	**24**	y10	**1483.8**	**3**	**495.3**	**982.52**	**54**	**28**	**17**	y10	**1483.8**	**3**	**495.3**	**982.52**	**69**	**25**	**11**	y10
		2	564.8	609.36	70	26	12	y5		2	564.8	609.36	64	27	19	y5		2	564.8	609.4	79	26	10	y5
		2	564.8	696.39	70	29	16	y6		2	564.8	696.39	61	29	23	y6		2	564.8	696.4	79	27	11	y6
bi0231	1128.5	2	564.8	797.44	70	28	20	y7	1128.5	2	564.8	797.44	56	30	19	y7	1128.5	2	564.8	797.4	80	28	12	y7
		2	**568.8**	**617.37**	**70**	**26**	**12**	y5		**2**	**568.8**	**617.37**	**64**	**27**	**19**	y5		**2**	**568.8**	**617.37**	**79**	**26**	**10**	y5
		2	**568.8**	**704.41**	**70**	**29**	**16**	y6		**2**	**568.8**	**704.41**	**61**	**29**	**23**	y6		**2**	**568.8**	**704.41**	**79**	**27**	**11**	y6
ni0109	**1136.6**	**2**	**568.8**	**805.45**	**70**	**28**	**20**	y7	**1136.6**	**2**	**568.8**	**805.45**	**56**	**30**	**19**	y7	**1136.6**	**2**	**568.8**	**805.45**	**80**	**28**	**12**	y7
		2	568.8	716.36	70	26	20	y6		2	568.8	716.36	61	27	24	y6		2	568.8	716.4	67	27	11	y6
		2	568.8	829.45	70	26	20	y7		2	568.8	829.45	59	29	22	y7		2	568.8	829.5	67	25	13	y7
bi0202	1136.3	2	568.8	916.48	70	25	22	y8	1136.3	2	568.8	916.48	59	28	16	y8	1136.3	2	568.8	916.5	67	24	14	y8
		2	**572.8**	**724.38**	**70**	**26**	**20**	y6		**2**	**572.8**	**724.38**	**61**	**27**	**24**	y6		**2**	**572.8**	**724.38**	**67**	**27**	**11**	y6
		2	**572.8**	**837.46**	**70**	**26**	**20**	y7		**2**	**572.8**	**837.46**	**59**	**29**	**22**	y7		**2**	**572.8**	**837.46**	**67**	**25**	**13**	y7
ni0110	**1144.6**	**2**	**572.8**	**924.49**	**70**	**25**	**22**	y8	**1144.6**	**2**	**572.8**	**924.49**	**59**	**28**	**16**	y8	**1144.6**	**2**	**572.8**	**924.49**	**67**	**24**	**14**	y8
		2	911	1053.49	85	39	24	b9		2	911	1053.49	96	39	19	b9		2	911	1053.49	95	33	25	b9
		2	911	1181.58	85	40	32	b10		2	911	1181.58	81	36	22	b10		2	911	1181.58	95	34	25	b10
ni0001	1820.9	2	911	1519.78	85	38	38	b13	1820.9	2	911	1519.78	76	37	29	b13	1820.9	2	911	1519.78	95	37	25	b13
		2	**914**	**1053.49**	**85**	**39**	**24**	b9		**2**	**914**	**1053.49**	**96**	**39**	**19**	b9		**2**	**914**	**1053.49**	**95**	**33**	**25**	b9
		2	**914**	**1181.58**	**85**	**40**	**32**	b10		**2**	**914**	**1181.58**	**81**	**36**	**22**	b10		**2**	**914**	**1181.58**	**95**	**34**	**25**	b10
ni0111	**1826.9**	**2**	**914**	**1525.8**	**85**	**38**	**38**	b13	**1826.9**	**2**	**914**	**1525.8**	**76**	**37**	**29**	b13	**1826.9**	**2**	**914**	**1525.8**	**95**	**37**	**25**	b13

Footnote: Instrument Parameters for MRM transitions were optimized for max. transmission efficiency and sensitivity for individual instruments by infusion of unlabeled signature peptides. Optimized declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) are reported for 4000 QTRAP instruments for each MRM transition along with the corresponding instrument used at each site. For the TSQ Quantum Ultra the collision energy (CE) is reported for each MRM transition.

		4000 QTRAP at CPTAC site @19							4000 QTRAP at CPTAC site @54								TSQ Quantum Ultra at CPTAC site @65					
Identifier	MH+ (mono)	z (Q1)	MRM Transitions Q1	MRM Transitions Q3	DP	CE	CXP	Fragment Ion Type	MH+ (mono)	z (Q1)	MRM Transitions Q1	MRM Transitions Q3	DP	CE	CXP	Fragment Ion Type	MH+ (mono)	z (Q1)	MRM Transitions Q1	MRM Transitions Q3	CE	Fragment IonType
		2	744.8	858.40	120	38	23	y7		2	744.8	858.4	111	43	14	y7		2	744.8	711.23	30	y6
		2	744.8	959.4	120	37	23	y8		2	744.8	959.4	111	41	6	y8		2	744.8	858.39	30	y7
bi0173	1488.7	2	744.8	1087.5	120	44	23	y9	1488.7	2	744.8	1087.5	111	39	10	y9	1488.7	2	744.8	959.44	30	y8
		2	**747.3**	**863.4**	**120**	**38**	**23**	y7		**2**	**747.3**	**863.4**	**111**	**43**	**14**	y7		**2**	**747.3**	**716.53**	**30**	y6
		2	**747.3**	**964.4**	**120**	**37**	**23**	y8		**2**	**747.3**	**964.4**	**111**	**41**	**6**	y8		**2**	**747.3**	**863.41**	**30**	y7
bi0081	**1493.7**	**2**	**747.3**	**1092.5**	**120**	**44**	**23**	y9	**1493.7**	**2**	**747.3**	**1092.5**	**111**	**39**	**10**	y9	**1493.7**	**2**	747.3	946.46	30	y8
		3	467.2	586.8	65	20	23	$y11^{2+}$		3	467.2	586.8	81	23	10	$y11^{2+}$		3	467.2	586.8	**20**	$y11^{2+}$
		3	467.2	643.8	65	21	23	$y12^{2+}$		3	467.2	643.8	81	29	12	$y12^{2+}$		3	467.2	643.82	**20**	$y12^{2+}$
bi0167	1399.7	3	467.2	720.4	65	27	23	y7	1399.7	3	467.2	720.39	81	27	8	y7	1399.7	3	467.2	720.39	**20**	y7
		3	**469.9**	**590.8**	**65**	**20**	**23**	$y11^{2+}$		**3**	**469.9**	**590.81**	**81**	**23**	**10**	$y11^{2+}$		**3**	**469.9**	**590.81**	**20**	$y11^{2+}$
		3	**469.9**	**647.8**	**65**	**21**	**23**	$y12^{2+}$		**3**	**469.9**	**647.83**	**81**	**29**	**12**	$y12^{2+}$		**3**	**469.9**	**647.83**	**20**	$y12^{2+}$
ni0101	**1407.3**	**3**	**469.9**	**728.4**	**65**	**27**	**23**	y7	**1407.3**	**3**	**469.9**	**728.4**	**81**	**27**	**8**	y7	**1407.3**	**3**	**469.9**	**728.4**	**20**	y7
		3	424.6	506.3	65	18	23	$y9^{2+}$		3	424.6	506.3	80	19	8	$y9^{2+}$		3	424.6	506.26	**18**	$y9^{2+}$
		3	424.6	579.8	65	19	23	$y10^{2+}$		3	424.6	579.8	80	21	8	$y10^{2+}$		3	424.6	579.79	**18**	$y10^{2+}$
bi0171	1271.7	3	424.6	716.4	65	24	23	y6	1271.7	3	424.6	716.4	80	25	12	y6	1271.7	3	424.6	716.38	**18**	y6
		3	**427.2**	**510.3**	**65**	**18**	**23**	$y9^{2+}$		**3**	**427.2**	**510.27**	**80**	**19**	**8**	$y9^{2+}$		**3**	**427.2**	**510.27**	**18**	$y9^{2+}$
		3	**427.2**	**583.8**	**65**	**19**	**23**	$y10^{2+}$		**3**	**427.2**	**583.8**	**80**	**21**	**8**	$y10^{2+}$		**3**	**427.2**	**583.8**	**18**	$y10^{2+}$
ni0102	**1279.7**	**3**	**427.2**	**724.4**	**65**	**24**	**23**	y6	**1279.7**	**3**	**427.2**	**724.4**	**80**	**25**	**12**	y6	**1279.7**	**3**	**427.2**	**724.4**	**18**	y6
		2	363.7	385.3	70	23	23	y3		2	363.7	385.3	82	29	12	y3		2	363.7	455.24	**20**	b4
		2	363.7	532.3	70	22	23	y4		2	363.7	532.3	82	23	8	y4		2	363.7	532.32	**20**	y4
bi0169	726.4	2	363.7	589.4	70	22	23	y5	726.4	2	363.7	589.4	82	23	10	y5	726.4	2	363.7	589.35	**20**	y5
		2	**366.7**	**391.3**	**70**	**23**	**23**	y3		**2**	**366.7**	**391.3**	**82**	**29**	**12**	y3		**2**	**366.7**	**455.24**	**20**	b4
		2	**366.7**	**538.3**	**70**	**22**	**23**	y4		**2**	**366.7**	**538.3**	**82**	**23**	**8**	y4		**2**	**366.7**	**538.34**	**20**	y4
ni0104	**732.4**	**2**	**366.7**	**595.4**	**70**	**22**	**23**	y5	**732.4**	**2**	**366.7**	**595.4**	**82**	**23**	**10**	y5	**732.4**	**2**	**366.7**	**595.37**	**20**	y5
		3	441.5	488.2	48	20	23	$y9^{2+}$		3	441.5	488.2	76	25	6	$y9^{2+}$		3	441.5	523.74	**18**	$y10^{2+}$
		3	441.5	523.7	48	19	23	$y10^{2+}$		3	441.5	523.7	76	19	8	$y10^{2+}$		3	441.5	730.33	18	y6
bi0170	1322.6	3	441.5	817.4	48	25	23	y7	1322.6	3	441.5	817.4	76	27	14	y7	1322.6	3	441.5	817.36	**18**	y7
		3	**443.5**	**491.2**	**48**	**20**	**23**	$y9^{2+}$		**3**	**443.5**	**491.23**	**76**	**25**	**6**	$y9^{2+}$		**3**	**443.5**	**526.75**	**18**	$y10^{2+}$
		3	**443.5**	**526.7**	**48**	**19**	**23**	$y10^{2+}$		**3**	**443.5**	**526.75**	**76**	**19**	**8**	$y10^{2+}$		**3**	**443.5**	736.35	18	y6
ni0105	**1328.6**	**3**	**443.5**	**823.4**	**48**	**25**	**23**	y7	**1328.6**	**3**	**443.5**	**823.38**	**76**	**27**	**14**	y7	**1328.6**	**3**	**443.5**	**823.38**	**18**	y7
		2	539.3	808.3	65	25	23	y6		2	539.3	808.3	81	37	12	y6		2	539.3	808.33	**26**	y6
		2	539.3	865.4	65	25	23	y7		2	539.3	865.4	81	25	14	y7		2	539.3	865.35	**26**	y7
bi0161	1077.5	2	539.3	964.4	65	27	23	y8	1077.5	2	539.3	964.42	81	27	16	y8	1077.5	2	539.3	964.42	**26**	y8
		2	**541.7**	**808.3**	**65**	**25**	**23**	y6		**2**	**541.7**	**808.33**	**81**	**37**	**12**	y6		**2**	**541.7**	**808.33**	**26**	y6
		2	**541.7**	**865.4**	**65**	**25**	**23**	y7		**2**	**541.7**	**865.35**	**81**	**25**	**14**	y7		**2**	**541.7**	**865.35**	**26**	y7
bi0067	**1082.5**	**2**	**541.7**	**969.4**	**65**	**27**	**23**	y8	**1082.5**	**2**	**541.7**	**969.44**	**81**	**27**	**16**	y8	**1082.5**	**2**	**541.7**	**969.44**	**26**	y8
		2	636.8	775.4	80	35	23	y7		2	636.8	775.4	111	41	12	y7		2	636.8	646.38	29	y6
		2	636.8	846.4	80	35	23	y8		2	636.8	846.4	111	39	14	y8		2	636.8	846.46	**29**	y8
bi0037	1272.7	2	636.8	943.4	80	27	23	y9	1272.7	2	636.8	943.4	111	31	6	y9	1272.7	2	636.8	943.51	**29**	y9
		2	**640.8**	**783.4**	**80**	**35**	**23**	y7		**2**	**640.8**	**783.43**	**111**	**41**	**12**	y7		**2**	**640.8**	654.39	29	y6
		2	**640.8**	**854.5**	**80**	**35**	**23**	y8		**2**	**640.8**	**854.47**	**111**	**39**	**14**	y8		**2**	**640.8**	**854.47**	**29**	y8
ni0107	**1280.7**	**2**	**640.8**	**951.5**	**80**	**27**	**23**	y9	**1280.7**	**2**	**640.8**	**951.52**	**111**	**31**	**6**	y9	**1280.7**	**2**	**640.8**	**951.52**	**29**	y9
		3	492.6	703.35	60	27	23	y7		3	492.6	703.35	81	27	12	y7		3	492.6	703.35	**18**	y7
		3	492.6	790.38	60	27	23	y8		3	492.6	790.38	81	27	14	y8		3	492.6	790.38	**18**	y8
bi0166	1475.7	3	492.6	974.51	60	26	23	y10	1475.7	3	492.6	974.51	81	25	10	y10	1475.7	3	492.6	974.51	**18**	y10
		3	**495.3**	**711.4**	**60**	**27**	**23**	y7		**3**	**495.3**	**711.37**	**81**	**27**	**12**	y7		**3**	**495.3**	**711.37**	**18**	y7
		3	**495.3**	**798.4**	**60**	**27**	**23**	y8		**3**	**495.3**	**798.4**	**81**	**27**	**14**	y8		**3**	**495.3**	**798.4**	**18**	y8
ni0108	**1483.8**	**3**	**495.3**	**982.5**	**60**	**26**	**23**	y10	**1483.8**	**3**	**495.3**	**982.52**	**81**	**25**	**10**	y10	**1483.8**	**3**	**495.3**	**982.52**	**18**	y10
		2	564.8	609.4	70	27	23	y5		2	564.8	609.4	86	27	8	y5		2	564.8	609.36	**22**	y5
		2	564.8	696.4	70	27	23	y6		2	564.8	696.4	86	27	12	y6		2	564.8	696.39	**22**	y6
bi0231	1128.5	2	564.8	797.4	70	28	23	y7	1128.5	2	564.8	797.4	86	31	12	y7	1128.5	2	564.8	797.44	**22**	y7
		2	**568.8**	**617.4**	**70**	**27**	**23**	y5		**2**	**568.8**	**617.37**	**86**	**27**	**8**	y5		**2**	**568.8**	**617.37**	**22**	y5
		2	**568.8**	**704.4**	**70**	**27**	**23**	y6		**2**	**568.8**	**704.41**	**86**	**27**	**12**	y6		**2**	**568.8**	**704.41**	**22**	y6
ni0109	**1136.6**	**2**	**568.8**	**805.5**	**70**	**28**	**23**	y7	**1136.6**	**2**	**568.8**	**805.45**	**86**	**31**	**12**	y7	**1136.6**	**2**	**568.8**	**805.45**	**22**	y7
		2	568.8	716.4	62	26	23	y6		2	568.8	716.4	81	37	12	y6		2	568.8	716.36	**23**	y6
		2	568.8	829.5	62	26	23	y7		2	568.8	829.5	81	27	12	y7		2	568.8	829.45	**23**	y7
bi0202	1136.3	2	568.8	916.5	62	25	23	y8	1136.3	2	568.8	916.5	81	25	8	y8	1136.3	2	568.8	916.48	**23**	y8
		2	**572.8**	**724.4**	**62**	**26**	**23**	y6		**2**	**572.8**	**724.38**	**81**	**37**	**12**	y6		**2**	**572.8**	**724,38**	**23**	y6
		2	**572.8**	**837.5**	**62**	**26**	**23**	y7		**2**	**572.8**	**837.46**	**81**	**27**	**12**	y7		**2**	**572.8**	**837.46**	**23**	y7
ni0110	**1144.6**	**2**	**572.8**	**924.5**	**62**	**25**	**23**	y8	**1144.6**	**2**	**572.8**	**924.49**	**81**	**25**	**8**	y8	**1144.6**	**2**	**572.8**	**924.49**	**23**	y8
		2	911	1053.5	80	35	23	b9		2	911	1053.5	106	33	16	b9		2	911	805.37	28	b7
		2	911	1181.6	80	33	23	b10		2	911	1181.6	106	31	16	b10		2	911	1016.56	28	y8
ni0001	1820.9	2	911	1519.8	80	35	23	b13	1820.9	2	911	1519.8	106	35	16	b13	1820.9	2	911	1053.49	30	b9
		2	**914**	**1053.5**	**80**	**35**	**23**	b9		**2**	**914**	**1053.49**	**106**	**33**	**16**	b9		**2**	**914**	**805.37**	**28**	b7
		2	**914**	**1181.6**	**80**	**33**	**23**	b10		**2**	**914**	**1181.58**	**106**	**31**	**16**	b10		**2**	**914**	**1022.58**	**28**	y8
ni0111	**1826.9**	**2**	**914**	**1525.8**	**80**	**35**	**23**	b13	**1826.9**	**2**	**914**	**1525.8**	**106**	**35**	**16**	b13	**1826.9**	**2**	**914**	**1053.49**	**30**	b9

Footnote: Instrument Parameters for MRM transitions were optimized for max. transmission efficiency and sensitivity for individual instruments by infusion of unlabeled signature peptides. Optimized declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) are reported for 4000 QTRAP instruments for each MRM transition along with the corresponding instrument used at each site. For the TSQ Quantum Ultra the collision energy (CE) is reported for each MRM transition.

Supplementary Table 1C: Comparisons of specific Instrument Parameter Declustering Potential (DP) for all MRM Transitions from seven CPTAC sites with 4000 QTRAP instruments.

MRM Transition DP Value comparison

CPTAC sites: site @52, site @56, site @86, site @95, site @73, site @19, site @54

Protein	Signature Peptide	Identifier	MH+ (mono)	z (Q1)	MRM Transitions Q1	MRM Transitions Q3	Fragment Ion Type	DP	DP	DP	DP	DP	DP	DP
aprotinin (APR)	AGLCamcQTFVYGGCamcR	bi0173	1488.7	2	744.8	858.4	y7	100	120	120	76	105	120	111
				2	744.8	959.4	y8	100	120	120	84	105	120	111
				2	744.8	1087.5	y9	100	120	120	76	105	120	111
				2	**747.3**	**863.4**	y7	**100**	**120**	**120**	**76**	**105**	**120**	**111**
				2	**747.3**	**964.4**	y8	**100**	**120**	**120**	**84**	**105**	**120**	**111**
	AGLCamcQTFVYGGCamcR	**bi0081**	**1493.7**	**2**	**747.3**	**1092.5**	y9	**100**	**120**	**120**	**76**	**105**	**120**	**111**
leptin (LEP)	INDISHTQSVSAK	bi0167	1399.7	3	467.2	586.8	y11 2+	76	61	61	59	65	65	81
				3	467.2	643.8	y12 2+	76	61	61	56	65	65	81
				3	467.2	720.39	y7	76	61	61	58	65	65	81
				3	**469.9**	**590.81**	y11 2+	**76**	**61**	**61**	**59**	**65**	**65**	**81**
				3	**469.9**	**647.83**	y12 2+	**76**	**61**	**61**	**56**	**65**	**65**	**81**
	INDISHTQSVSAK	**ni0101**	**1407.3**	**3**	**469.9**	**728.4**	y7	**76**	**61**	**61**	**58**	**65**	**65**	**81**
myoglobin (MYO)	LFTGHPETLEK	bi0171	1271.7	3	424.6	506.3	y9 2+	71	66	66	64	65	65	80
				3	424.6	579.8	y10 2+	71	66	66	54	65	65	80
				3	424.6	716.4	y6	71	66	66	56	65	65	80
				3	**427.2**	**510.27**	y9 2+	**71**	**66**	**66**	**64**	**65**	**65**	**80**
				3	**427.2**	**583.8**	y10 2+	**71**	**66**	**66**	**54**	**65**	**65**	**80**
	LFTGHPETLEK	**ni0102**	**1279.7**	**3**	**427.2**	**724.4**	y6	**71**	**66**	**66**	**56**	**65**	**65**	**80**
myelin basic protein (MBP)	HGFLPR	bi0169	726.4	2	363.7	385.3	y3	70	70	70	61	70	70	82
				2	363.7	532.3	y4	70	70	70	64	70	70	82
				2	363.7	589.4	y5	70	70	70	69	70	70	82
				2	**366.7**	**391.3**	y3	**70**	**70**	**70**	**61**	**70**	**70**	**82**
				2	**366.7**	**538.3**	y4	**70**	**70**	**70**	**64**	**70**	**70**	**82**
	HGFLPR	**ni0104**	**732.4**	**2**	**366.7**	**595.4**	y5	**70**	**70**	**70**	**69**	**70**	**70**	**82**
	YLASASTMDHAR	bi0170	1322.6	3	441.5	488.2	y9 2+	90	61	65	51	60	48	76
				3	441.5	523.7	y10 2+	90	61	55	54	60	48	76
				3	441.5	817.4	y7	90	61	61	51	60	48	76
				3	**443.5**	**491.23**	y9 2+	**90**	**61**	**65**	**51**	**60**	**48**	**76**
				3	**443.5**	**526.75**	y10 2+	**90**	**61**	**55**	**54**	**60**	**48**	**76**
	YLASASTMDHAR	**ni0105**	**1328.6**	**3**	**443.5**	**823.38**	y7	**90**	**61**	**61**	**51**	**60**	**48**	**76**
prostate specific antigen (PSA)	IVGGWECamcEK	bi0161	1077.5	2	539.3	808.3	y6	81	75	75	64	70	65	81
				2	539.3	865.4	y7	81	75	75	66	70	65	81
				2	539.3	964.42	y8	81	75	75	61	70	65	81
				2	**541.7**	**808.33**	y6	**81**	**75**	**75**	**64**	**70**	**65**	**81**
				2	**541.7**	**865.35**	y7	**81**	**75**	**75**	**66**	**70**	**65**	**81**
	IVGGWECamcEK	**bi0067**	**1082.5**	**2**	**541.7**	**969.44**	y8	**81**	**75**	**75**	**61**	**70**	**65**	**81**
	LSEPAELTDAVK	bi0037	1272.7	2	636.8	775.4	y7	91	75	75	74	75	80	111
				2	636.8	846.4	y8	91	75	75	71	75	80	111
				2	636.8	943.4	y9	91	75	75	79	75	80	111
				2	**640.8**	**783.43**	y7	**91**	**75**	**75**	**74**	**75**	**80**	**111**
				2	**640.8**	**854.47**	y8	**91**	**75**	**75**	**71**	**75**	**80**	**111**
	LSEPAELTDAVK	**ni0107**	**1280.7**	**2**	**640.8**	**951.52**	y9	**91**	**75**	**75**	**79**	**75**	**80**	**111**
horseradish peroxidase (HRP)	SSDLVALSGGHTFGK	bi0166	1475.7	3	492.6	703.35	y7	76	70	70	64	69	60	81
				3	492.6	790.38	y8	76	70	70	59	69	60	81
				3	492.6	974.51	y10	76	70	70	54	69	60	81
				3	**495.3**	**711.37**	y7	**76**	**70**	**70**	**64**	**69**	**60**	**81**
				3	**495.3**	**798.4**	y8	**76**	**70**	**70**	**59**	**69**	**60**	**81**
	SSDLVALSGGHTFGK	**ni0108**	**1483.8**	**3**	**495.3**	**982.52**	y10	**76**	**70**	**70**	**54**	**69**	**60**	**81**
C-reactive Protein (CRP)	ESDTSYVSLK	bi0231	1128.5	2	564.8	609.4	y5	81	86	70	64	79	70	86
				2	564.8	696.4	y6	81	86	70	61	79	70	86
				2	564.8	797.4	y7	81	81	70	56	80	70	86
				2	**568.8**	**617.37**	y5	**81**	**86**	**70**	**64**	**79**	**70**	**86**
				2	**568.8**	**704.41**	y6	**81**	**86**	**70**	**61**	**79**	**70**	**86**
	ESDTSYVSLK	**ni0109**	**1136.6**	**2**	**568.8**	**805.45**	y7	**81**	**81**	**70**	**56**	**80**	**70**	**86**
	GYSIFSYATK	bi0202	1136.3	2	568.8	716.4	y6	76	91	70	61	67	62	81
				2	568.8	829.5	y7	76	51	70	59	67	62	81
				2	568.8	916.5	y8	76	71	70	59	67	62	81
				2	**572.8**	**724.38**	y6	**76**	**91**	**70**	**61**	**67**	**62**	**81**
				2	**572.8**	**837.46**	y7	**76**	**51**	**70**	**59**	**67**	**62**	**81**
	GYSIFSYATK	**ni0110**	**1144.6**	**2**	**572.8**	**924.49**	y8	**76**	**71**	**70**	**59**	**67**	**62**	**81**
	YEVQGEVFTKPQLWP	ni0001	1820.9	2	911	1053.5	b9	106	80	85	96	95	80	106
				2	911	1181.6	b10	106	80	85	81	95	80	106
				2	911	1519.8	b13	106	80	85	76	95	80	106
				2	**914**	**1053.49**	b9	**106**	**80**	**85**	**96**	**95**	**80**	**106**
				2	**914**	**1181.58**	b10	**106**	**80**	**85**	**81**	**95**	**80**	**106**
	YEVQGEVFTKPQLWP	**ni0111**	**1826.9**	**2**	**914**	**1525.8**	b13	**106**	**80**	**85**	**76**	**95**	**80**	**106**

Q1/Q3 transition	across 7 sites range of DP	across 7 sites mean of DP	across 7 sites std. dev. of DP	across 7 sites std. dev. of DP (in %)
744.8 / 858.4	Range: 76.0 - 120.0	Mean: 107.43	StdDev: 16.0	14.9%
744.8 / 959.4	Range: 84.0 - 120.0	Mean: 108.57	StdDev: 13.46	12.4%
744.8 / 1087.5	Range: 76.0 - 120.0	Mean: 107.43	StdDev: 16.0	14.9%
747.3 / 863.4	Range: 76.0 - 120.0	Mean: 107.43	StdDev: 16.0	14.9%
747.3 / 964.4	Range: 84.0 - 120.0	Mean: 108.57	StdDev: 13.46	12.4%
747.3 / 1092.5	Range: 76.0 - 120.0	Mean: 107.43	StdDev: 16.0	14.9%
467.2 / 586.8	Range: 59.0 - 81.0	Mean: 66.86	StdDev: 8.38	12.5%
467.2 / 643.8	Range: 56.0 - 81.0	Mean: 66.43	StdDev: 8.9	13.4%
467.2 / 720.39	Range: 58.0 - 81.0	Mean: 66.71	StdDev: 8.54	12.8%
469.9 / 590.81	Range: 59.0 - 81.0	Mean: 66.86	StdDev: 8.38	12.5%
469.9 / 647.83	Range: 56.0 - 81.0	Mean: 66.43	StdDev: 8.9	13.4%
469.9 / 728.4	Range: 58.0 - 81.0	Mean: 66.71	StdDev: 8.54	12.8%
424.6 / 506.3	Range: 64.0 - 80.0	Mean: 68.14	StdDev: 5.7	8.4%
424.6 / 579.8	Range: 54.0 - 80.0	Mean: 66.71	StdDev: 7.78	11.7%
424.6 / 716.4	Range: 56.0 - 80.0	Mean: 67.0	StdDev: 7.26	10.8%
427.2 / 510.27	Range: 64.0 - 80.0	Mean: 68.14	StdDev: 5.7	8.4%
427.2 / 583.8	Range: 54.0 - 80.0	Mean: 66.71	StdDev: 7.78	11.7%
427.2 / 724.4	Range: 56.0 - 80.0	Mean: 67.0	StdDev: 7.26	10.8%
363.7 / 385.3	Range: 61.0 - 82.0	Mean: 70.43	StdDev: 6.11	8.7%
363.7 / 532.3	Range: 64.0 - 82.0	Mean: 70.86	StdDev: 5.4	7.6%
363.7 / 589.4	Range: 69.0 - 82.0	Mean: 71.57	StdDev: 4.61	6.4%
366.7 / 391.3	Range: 61.0 - 82.0	Mean: 70.43	StdDev: 6.11	8.7%
366.7 / 538.3	Range: 64.0 - 82.0	Mean: 70.86	StdDev: 5.4	7.6%
366.7 / 595.4	Range: 69.0 - 82.0	Mean: 71.57	StdDev: 4.61	6.4%
441.5 / 488.2	Range: 48.0 - 90.0	Mean: 64.43	StdDev: 14.55	22.6%
441.5 / 523.7	Range: 48.0 - 90.0	Mean: 63.43	StdDev: 14.6	23.0%
441.5 / 817.4	Range: 48.0 - 90.0	Mean: 63.86	StdDev: 14.6	22.9%
443.5 / 491.23	Range: 48.0 - 90.0	Mean: 64.43	StdDev: 14.55	22.6%
443.5 / 526.75	Range: 48.0 - 90.0	Mean: 63.43	StdDev: 14.6	23.0%
443.5 / 823.38	Range: 48.0 - 90.0	Mean: 63.86	StdDev: 14.6	22.9%
539.3 / 808.3	Range: 64.0 - 81.0	Mean: 73.0	StdDev: 6.95	9.5%
539.3 / 865.4	Range: 65.0 - 81.0	Mean: 73.29	StdDev: 6.55	8.9%
539.3 / 964.42	Range: 61.0 - 81.0	Mean: 72.57	StdDev: 7.66	10.6%
541.7 / 808.33	Range: 64.0 - 81.0	Mean: 73.0	StdDev: 6.95	9.5%
541.7 / 865.35	Range: 65.0 - 81.0	Mean: 73.29	StdDev: 6.55	8.9%
541.7 / 969.44	Range: 61.0 - 81.0	Mean: 72.57	StdDev: 7.66	10.6%
636.8 / 775.4	Range: 74.0 - 111.0	Mean: 83.0	StdDev: 13.72	16.5%
636.8 / 846.4	Range: 71.0 - 111.0	Mean: 82.57	StdDev: 14.09	17.1%
636.8 / 943.4	Range: 75.0 - 111.0	Mean: 83.71	StdDev: 13.3	15.9%
640.8 / 783.43	Range: 74.0 - 111.0	Mean: 83.0	StdDev: 13.72	16.5%
640.8 / 854.47	Range: 71.0 - 111.0	Mean: 82.57	StdDev: 14.09	17.1%
640.8 / 951.52	Range: 75.0 - 111.0	Mean: 83.71	StdDev: 13.3	15.9%
492.6 / 703.35	Range: 60.0 - 81.0	Mean: 70.0	StdDev: 7.0	10.0%
492.6 / 790.38	Range: 59.0 - 81.0	Mean: 69.29	StdDev: 7.91	11.4%
492.6 / 974.51	Range: 54.0 - 81.0	Mean: 68.57	StdDev: 9.13	13.3%
495.3 / 711.37	Range: 60.0 - 81.0	Mean: 70.0	StdDev: 7.0	10.0%
495.3 / 798.4	Range: 59.0 - 81.0	Mean: 69.29	StdDev: 7.91	11.4%
495.3 / 982.52	Range: 54.0 - 81.0	Mean: 68.57	StdDev: 9.13	13.3%
564.8 / 609.4	Range: 64.0 - 86.0	Mean: 76.57	StdDev: 8.64	11.3%
564.8 / 696.4	Range: 61.0 - 86.0	Mean: 76.14	StdDev: 9.41	12.4%
564.8 / 797.4	Range: 56.0 - 86.0	Mean: 74.86	StdDev: 10.24	13.7%
568.8 / 617.37	Range: 64.0 - 86.0	Mean: 76.57	StdDev: 8.64	11.3%
568.8 / 704.41	Range: 61.0 - 86.0	Mean: 76.14	StdDev: 9.41	12.4%
568.8 / 805.45	Range: 56.0 - 86.0	Mean: 74.86	StdDev: 10.24	13.7%
568.8 / 716.4	Range: 61.0 - 91.0	Mean: 72.57	StdDev: 10.85	14.9%
568.8 / 829.5	Range: 51.0 - 81.0	Mean: 66.57	StdDev: 10.24	15.4%
568.8 / 916.5	Range: 59.0 - 81.0	Mean: 69.43	StdDev: 7.63	11.0%
572.8 / 724.38	Range: 61.0 - 91.0	Mean: 72.57	StdDev: 10.85	14.9%
572.8 / 837.46	Range: 51.0 - 81.0	Mean: 66.57	StdDev: 10.24	15.4%
572.8 / 924.49	Range: 59.0 - 81.0	Mean: 69.43	StdDev: 7.63	11.0%
911 / 1053.5	Range: 80.0 - 106.0	Mean: 92.57	StdDev: 11.19	12.1%
911 / 1181.6	Range: 80.0 - 106.0	Mean: 90.43	StdDev: 11.84	13.1%
911 / 1519.8	Range: 76.0 - 106.0	Mean: 89.71	StdDev: 12.63	14.1%
914 / 1053.49	Range: 80.0 - 106.0	Mean: 92.57	StdDev: 11.19	12.1%
914 / 1181.58	Range: 80.0 - 106.0	Mean: 90.43	StdDev: 11.84	13.1%
914 / 1525.8	Range: 76.0 - 106.0	Mean: 89.71	StdDev: 12.63	14.1%

Footnote: Instrument Parameters for MRM transitions were optimized for maximum transmission efficiency and sensitivity for individual instruments by infusion of unlabeled signature peptides.
Optimized declustering potential (DP) are reported for 4000 QTRAP instruments for each MRM transition along with the corresponding instrument used at each site.
The TSQ Quantum Ultra instrument at CPTAC site @65 is not included in this specific comparison, for TSQ Quantum Ultra instrument parameters see Supplementary Table 1B.
Boldface type indicates labeled peptide internal standard with labeled amino acid in red.

Supplementary Table 1D: Comparisons of specific Instrument Collision Energy (CE) for all MRM Transitions from seven CPTAC sites with 4000 QTRAP instruments.

MRM Transition CE Value comparison

CPTAC sites: site @52, site @56, site @86, site @95, site @73, site @19, site @54

Protein	Signature Peptide	Identifier	MH+ (mono)	z (Q1)	MRM Transitions Q1	MRM Transitions Q3	Fragment Ion Type	CE	CE	CE	CE	CE	CE	CE	Q1/Q3 transition	across 7 sites range of CE	across 7 sites mean of CE	across 7 sites std. dev. of CE	across 7 sites std. dev. of CE (in %)
aprotinin (APR)	AGLCamcQTFVYGGCamcR	bi0173	1488.7	2	744.8	858.4	y7	40	35	38	41	40	38	43	744.8 / 858.4	Range: 35.0 - 43.0	Mean: 39.29	StdDev: 2.56	6.5%
				2	744.8	959.4	y8	40	38	38	45	39	37	41	744.8 / 959.4	Range: 37.0 - 45.0	Mean: 39.71	StdDev: 2.69	6.8%
				2	744.8	1087.5	y9	38	40	40	42	39	44	39	744.8 / 1087.5	Range: 38.0 - 44.0	Mean: 40.29	StdDev: 2.06	5.1%
				2	**747.3**	**863.4**	y7	**40**	**35**	**38**	**41**	**40**	**38**	**43**	747.3 / 863.4	Range: 35.0 - 43.0	Mean: 39.29	StdDev: 2.56	6.5%
				2	**747.3**	**964.4**	y8	**40**	**38**	**38**	**45**	**39**	**37**	**41**	747.3 / 964.4	Range: 37.0 - 45.0	Mean: 39.71	StdDev: 2.69	6.8%
	AGLCamcQTFVYGGCamcR	**bi0081**	**1493.7**	**2**	**747.3**	**1092.5**	y9	38	40	40	42	39	44	39	747.3 / 1092.5	Range: 38.0 - 44.0	Mean: 40.29	StdDev: 2.06	5.1%
leptin (LEP)	INDISHTQSVSAK	bi0167	1399.7	3	467.2	586.8	y11 $^{2+}$	21	20	22	26	22	20	23	467.2 / 586.8	Range: 20.0 - 26.0	Mean: 22.0	StdDev: 2.08	9.5%
				3	467.2	643.8	y12 $^{2+}$	23	20	22	29	22	21	29	467.2 / 643.8	Range: 20.0 - 29.0	Mean: 23.71	StdDev: 3.73	15.7%
				3	467.2	720.39	y7	29	20	30	31	28	27	27	467.2 / 720.39	Range: 20.0 - 31.0	Mean: 27.43	StdDev: 3.6	13.1%
				3	**469.9**	**590.81**	y11 $^{2+}$	**21**	**20**	**22**	**26**	**22**	**20**	**23**	469.9 / 590.81	Range: 20.0 - 26.0	Mean: 22.0	StdDev: 2.08	9.5%
				3	**469.9**	**647.83**	y12 $^{2+}$	**23**	**20**	**22**	**29**	**22**	**21**	**29**	469.9 / 647.83	Range: 20.0 - 29.0	Mean: 23.71	StdDev: 3.73	15.7%
	INDISHTQSVSAK	**ni0101**	**1407.3**	**3**	**469.9**	**728.4**	y7	**29**	**20**	**30**	**31**	**28**	**27**	**27**	469.9 / 728.4	Range: 20.0 - 31.0	Mean: 27.43	StdDev: 3.6	13.1%
myoglobin (MYO)	LFTGHPETLEK	bi0171	1271.7	3	424.6	506.3	y9 $^{2+}$	19	18	18	23	19	18	19	424.6 / 506.3	Range: 18.0 - 23.0	Mean: 19.14	StdDev: 1.77	9.3%
				3	424.6	579.8	y10 $^{2+}$	20	19	20	26	19	19	21	424.6 / 579.8	Range: 19.0 - 26.0	Mean: 20.57	StdDev: 2.51	12.2%
				3	424.6	716.4	y6	25	24	24	25	23	24	25	424.6 / 716.4	Range: 23.0 - 25.0	Mean: 24.29	StdDev: 0.76	3.1%
				3	**427.2**	**510.27**	y9 $^{2+}$	**19**	**18**	**18**	**23**	**19**	**18**	**19**	427.2 / 510.27	Range: 18.0 - 23.0	Mean: 19.14	StdDev: 1.77	9.3%
				3	**427.2**	**583.8**	y10 $^{2+}$	**20**	**19**	**20**	**26**	**19**	**19**	**21**	427.2 / 583.8	Range: 19.0 - 26.0	Mean: 20.57	StdDev: 2.51	12.2%
	LFTGHPETLEK	**ni0102**	**1279.7**	**3**	**427.2**	**724.4**	y6	**25**	**24**	**24**	**25**	**23**	**24**	**25**	427.2 / 724.4	Range: 23.0 - 25.0	Mean: 24.29	StdDev: 0.76	3.1%
myelin basic protein (MBP)	HGFLPR	bi0169	726.4	2	363.7	385.3	y3	23	23	22	27	23	23	29	363.7 / 385.3	Range: 22.0 - 29.0	Mean: 24.29	StdDev: 2.63	10.8%
				2	363.7	532.3	y4	23	22	22	27	22	22	23	363.7 / 532.3	Range: 22.0 - 27.0	Mean: 23.0	StdDev: 1.83	7.9%
				2	363.7	589.4	y5	23	22	23	26	23	22	23	363.7 / 589.4	Range: 22.0 - 26.0	Mean: 23.14	StdDev: 1.35	5.8%
				2	**366.7**	**391.3**	y3	**23**	**23**	**22**	**27**	**23**	**23**	**29**	366.7 / 391.3	Range: 22.0 - 29.0	Mean: 24.29	StdDev: 2.63	10.8%
				2	**366.7**	**538.3**	y4	**23**	**22**	**22**	**27**	**22**	**22**	**23**	366.7 / 538.3	Range: 22.0 - 27.0	Mean: 23.0	StdDev: 1.83	7.9%
	HGFLPR	**ni0104**	**732.4**	**2**	**366.7**	**595.4**	y5	**23**	**22**	**23**	**26**	**23**	**22**	**23**	366.7 / 595.4	Range: 22.0 - 26.0	Mean: 23.14	StdDev: 1.35	5.8%
	YLASASTMDHAR	bi0170	1322.6	3	441.5	488.2	y9 $^{2+}$	21	20	21	23	21	20	25	441.5 / 488.2	Range: 20.0 - 25.0	Mean: 21.57	StdDev: 1.81	8.4%
				3	441.5	523.7	y10 $^{2+}$	19	17	19	19	19	19	19	441.5 / 523.7	Range: 17.0 - 19.0	Mean: 18.71	StdDev: 0.76	4.0%
				3	441.5	817.4	y7	24	23	26	28	24	25	27	441.5 / 817.4	Range: 23.0 - 28.0	Mean: 25.29	StdDev: 1.8	7.1%
				3	**443.5**	**491.23**	y9 $^{2+}$	**21**	**20**	**21**	**23**	**21**	**20**	**25**	443.5 / 491.23	Range: 20.0 - 25.0	Mean: 21.57	StdDev: 1.81	8.4%
				3	**443.5**	**526.75**	y10 $^{2+}$	**19**	**17**	**19**	**19**	**19**	**19**	**19**	443.5 / 526.75	Range: 17.0 - 19.0	Mean: 18.71	StdDev: 0.76	4.0%
	YLASASTMDHAR	**ni0105**	**1328.6**	**3**	**443.5**	**823.38**	y7	**24**	**23**	**26**	**28**	**24**	**25**	**27**	443.5 / 823.38	Range: 23.0 - 28.0	Mean: 25.29	StdDev: 1.8	7.1%
prostate specific antigen (PSA)	IVGGWECamcEK	bi0161	1077.5	2	539.3	808.3	y6	27	27	27	29	27	25	37	539.3 / 808.3	Range: 25.0 - 37.0	Mean: 28.43	StdDev: 3.95	13.9%
				2	539.3	865.4	y7	26	25	25	29	26	25	25	539.3 / 865.4	Range: 25.0 - 29.0	Mean: 25.86	StdDev: 1.46	5.7%
				2	539.3	964.42	y8	27	27	28	29	27	27	27	539.3 / 964.42	Range: 27.0 - 29.0	Mean: 27.43	StdDev: 0.79	2.9%
				2	**541.7**	**808.33**	y6	**27**	**27**	**27**	**29**	**27**	**25**	**37**	541.7 / 808.33	Range: 25.0 - 37.0	Mean: 28.43	StdDev: 3.95	13.9%
				2	**541.7**	**865.35**	y7	**26**	**25**	**25**	**29**	**26**	**25**	**25**	541.7 / 865.35	Range: 25.0 - 29.0	Mean: 25.86	StdDev: 1.46	5.7%
	IVGGWECamcEK	**bi0067**	**1082.5**	**2**	**541.7**	**969.44**	y8	**27**	**27**	**28**	**29**	**27**	**27**	**27**	541.7 / 969.44	Range: 27.0 - 29.0	Mean: 27.43	StdDev: 0.79	2.9%
	LSEPAELTDAVK	bi0037	1272.7	2	636.8	775.4	y7	39	39	37	40	38	35	41	636.8 / 775.4	Range: 35.0 - 41.0	Mean: 38.43	StdDev: 1.99	5.2%
				2	636.8	846.4	y8	37	39	38	48	38	35	39	636.8 / 846.4	Range: 35.0 - 48.0	Mean: 39.14	StdDev: 4.14	10.6%
				2	636.8	943.4	y9	29	31	30	33	30	27	31	636.8 / 943.4	Range: 27.0 - 33.0	Mean: 30.14	StdDev: 1.86	6.2%
				2	**640.8**	**783.43**	y7	**39**	**39**	**37**	**40**	**38**	**35**	**41**	640.8 / 783.43	Range: 35.0 - 41.0	Mean: 38.43	StdDev: 1.99	5.2%
				2	**640.8**	**854.47**	y8	**37**	**39**	**38**	**48**	**38**	**35**	**39**	640.8 / 854.47	Range: 35.0 - 48.0	Mean: 39.14	StdDev: 4.14	10.6%
	LSEPAELTDAVK	**ni0107**	**1280.7**	**2**	**640.8**	**951.52**	y9	**29**	**31**	**30**	**33**	**30**	**27**	**31**	640.8 / 951.52	Range: 27.0 - 33.0	Mean: 30.14	StdDev: 1.86	6.2%
horseradish peroxidase (HRP)	SSDLVALSGGHTFGK	bi0166	1475.7	3	492.6	703.35	y7	27	27	26	29	26	27	27	492.6 / 703.35	Range: 26.0 - 29.0	Mean: 27.0	StdDev: 1.0	3.7%
				3	492.6	790.38	y8	27	27	26	32	27	27	27	492.6 / 790.38	Range: 26.0 - 32.0	Mean: 27.57	StdDev: 1.99	7.2%
				3	492.6	974.51	y10	26	24	26	28	25	26	25	492.6 / 974.51	Range: 24.0 - 28.0	Mean: 25.71	StdDev: 1.25	4.9%
				3	**495.3**	**711.37**	y7	**27**	**27**	**26**	**29**	**26**	**27**	**27**	495.3 / 711.37	Range: 26.0 - 29.0	Mean: 27.0	StdDev: 1.0	3.7%
				3	**495.3**	**798.4**	y8	**27**	**27**	**26**	**32**	**27**	**27**	**27**	495.3 / 798.4	Range: 26.0 - 32.0	Mean: 27.57	StdDev: 1.99	7.2%
	SSDLVALSGGHTFGK	**ni0108**	**1483.8**	**3**	**495.3**	**982.52**	y10	**26**	**24**	**26**	**28**	**25**	**26**	**25**	495.3 / 982.52	Range: 24.0 - 28.0	Mean: 25.71	StdDev: 1.25	4.9%
C-reactive Protein (CRP)	ESDTSYVSLK	bi0231	1128.5	2	564.8	609.4	y5	27	23	26	27	26	27	27	564.8 / 609.4	Range: 23.0 - 27.0	Mean: 26.14	StdDev: 1.46	5.6%
				2	564.8	696.4	y6	27	31	29	29	27	27	27	564.8 / 696.4	Range: 27.0 - 31.0	Mean: 28.14	StdDev: 1.57	5.6%
				2	564.8	797.4	y7	29	31	28	30	28	28	31	564.8 / 797.4	Range: 28.0 - 31.0	Mean: 29.29	StdDev: 1.38	4.7%
				2	**568.8**	**617.37**	y5	**27**	**23**	**26**	**27**	**26**	**27**	**27**	568.8 / 617.37	Range: 23.0 - 27.0	Mean: 26.14	StdDev: 1.46	5.6%
				2	**568.8**	**704.41**	y6	**27**	**31**	**29**	**29**	**27**	**27**	**27**	568.8 / 704.41	Range: 27.0 - 31.0	Mean: 28.14	StdDev: 1.57	5.6%
	ESDTSYVSLK	**ni0109**	**1136.6**	**2**	**568.8**	**805.45**	y7	**29**	**31**	**28**	**30**	**28**	**28**	**31**	568.8 / 805.45	Range: 28.0 - 31.0	Mean: 29.29	StdDev: 1.38	4.7%
	GYSIFSYATK	bi0202	1136.3	2	568.8	716.4	y6	30	31	26	27	27	26	37	568.8 / 716.4	Range: 26.0 - 37.0	Mean: 29.14	StdDev: 3.98	13.6%
				2	568.8	829.5	y7	27	27	26	29	25	26	27	568.8 / 829.5	Range: 25.0 - 29.0	Mean: 26.71	StdDev: 1.25	4.7%
				2	568.8	916.5	y8	25	25	25	28	24	25	25	568.8 / 916.5	Range: 24.0 - 28.0	Mean: 25.29	StdDev: 1.25	5.0%
				2	**572.8**	**724.38**	y6	**30**	**31**	**26**	**27**	**27**	**26**	**37**	572.8 / 724.38	Range: 26.0 - 37.0	Mean: 29.14	StdDev: 3.98	13.6%
				2	**572.8**	**837.46**	y7	**27**	**27**	**26**	**29**	**25**	**26**	**27**	572.8 / 837.46	Range: 25.0 - 29.0	Mean: 26.71	StdDev: 1.25	4.7%
	GYSIFSYATK	**ni0110**	**1144.6**	**2**	**572.8**	**924.49**	y8	**25**	**25**	**25**	**28**	**24**	**25**	**25**	572.8 / 924.49	Range: 24.0 - 28.0	Mean: 25.29	StdDev: 1.25	5.0%
	YEVQGEVFTKPQLWP	ni0001	1820.9	2	911	1053.5	b9	34	35	39	39	33	35	33	911 / 1053.5	Range: 33.0 - 39.0	Mean: 35.43	StdDev: 2.57	7.3%
				2	911	1181.6	b10	33	32	40	36	34	33	31	911 / 1181.6	Range: 31.0 - 40.0	Mean: 34.14	StdDev: 3.02	8.9%
				2	911	1519.8	b13	37	36	38	37	37	35	35	911 / 1519.8	Range: 35.0 - 38.0	Mean: 36.43	StdDev: 1.13	3.1%
				2	**914**	**1053.49**	b9	**34**	**35**	**39**	**39**	**33**	**35**	**33**	914 / 1053.49	Range: 33.0 - 39.0	Mean: 35.43	StdDev: 2.57	7.3%
				2	**914**	**1181.58**	b10	**33**	**32**	**40**	**36**	**34**	**33**	**31**	914 / 1181.58	Range: 31.0 - 40.0	Mean: 34.14	StdDev: 3.02	8.9%
	YEVQGEVFTKPQLWP	**ni0111**	**1826.9**	**2**	**914**	**1525.8**	b13	**37**	**36**	**38**	**37**	**37**	**35**	**35**	914 / 1525.8	Range: 35.0 - 38.0	Mean: 36.43	StdDev: 1.13	3.1%

Footnote: Instrument Parameters for MRM transitions were optimized for maximum transmission efficiency and sensitivity for individual instruments by infusion of unlabeled signature peptides.
Optimized collision energy (CE) are reported for 4000 QTRAP instruments for each MRM transition along with the corresponding instrument used at each site.
The TSQ Quantum Ultra instrument at CPTAC site @65 is not included in this specific comparison, for TSQ Quantum Ultra instrument parameters see Supplementary Table 1B.
Boldface type indicates labeled peptide internal standard with labeled amino acid in red.

Supplementary Table 1E: **Comparisons of specific Instrument Parameter Collision Cell Exit Potential (CXP) for all MRM Transitions from seven CPTAC sites with 4000 QTRAP instruments.**

MRM Transition CXP Value comparison

Protein	Signature Peptide	Identifier	MH+ (mono)	z	MRM Transitions Q1	MRM Transitions Q3	Fragment Ion Type	CPTAC sites: site @52 CXP	site @56 CXP	site @86 CXP	site @95 CXP	site @73 CXP	site @19 CXP	site @54 CXP
aprotinin (APR)	AGLCamcQTFVYGGCamcR	bi0173	1488.7	2	744.8	858.4	y7	12	16	20	29	13	23	14
				2	744.8	959.4	y8	14	16	22	17	13	23	6
				2	744.8	1087.5	y9	17	16	24	20	13	23	10
	AGLCamcQTFVYGGCamcR	**bi0081**	**1493.7**	**2**	**747.3**	**863.4**	y7	**12**	**16**	**20**	**29**	**13**	**23**	**14**
				2	**747.3**	**964.4**	y8	**14**	**16**	**22**	**17**	**13**	**23**	**6**
				2	747.3	1092.5	y9	**17**	**16**	**24**	**20**	**13**	**23**	**10**
leptin (LEP)	INDISHTQSVSAK	bi0167	1399.7	3	467.2	586.8	y11 2+	10	12	12	19	21	23	10
				3	467.2	643.8	y12 2+	10	12	14	21	11	23	12
				3	467.2	720.39	y7	10	12	16	20	18	23	8
	INDISHTQSVSAK	**ni0101**	**1407.3**	**3**	**469.9**	**590.81**	y11 2+	**10**	**12**	**12**	**19**	**21**	**23**	**10**
				3	**469.9**	**647.83**	y12 2+	**10**	**12**	**14**	**21**	**11**	**23**	**12**
				3	**469.9**	**728.4**	y7	**10**	**12**	**16**	**20**	**18**	**23**	**8**
myoglobin (MYO)	LFTGHPETLEK	bi0171	1271.7	3	424.6	506.3	y9 2+	8	12	10	16	23	23	8
				3	424.6	579.8	y10 2+	8	10	12	10	14	23	8
				3	424.6	716.4	y6	10	13	16	19	14	23	12
	LFTGHPETLEK	**ni0102**	**1279.7**	**3**	**427.2**	**510.27**	y9 2+	**8**	**12**	**10**	**16**	**23**	**23**	**8**
				3	**427.2**	**583.8**	y10 2+	**8**	**10**	**12**	**10**	**14**	**23**	**8**
				3	**427.2**	**724.4**	y6	**10**	**13**	**16**	**19**	**14**	**23**	**12**
myelin basic protein (MBP)	HGFLPR	bi0169	726.4	2	363.7	385.3	y3	16	16	16	12	19	23	12
				2	363.7	532.3	y4	12	16	16	17	19	23	8
				2	363.7	589.4	y5	8	14	11	19	12	23	10
	HGFLPR	**ni0104**	**732.4**	**2**	**366.7**	**391.3**	y3	**16**	**16**	**16**	**12**	**19**	**23**	**12**
				2	**366.7**	**538.3**	y4	**12**	**16**	**16**	**17**	**19**	**23**	**8**
				2	**366.7**	**595.4**	y5	**8**	**14**	**11**	**19**	**12**	**23**	**10**
	YLASASTMDHAR	bi0170	1322.6	3	441.5	488.2	y9 2+	12	14	9	15	13	23	6
				3	441.5	523.7	y10 2+	16	14	10	17	14	23	8
				3	441.5	817.4	y7	14	13	18	15	14	23	14
	YLASASTMDHAR	**ni0105**	**1328.6**	**3**	**443.5**	**491.23**	y9 2+	**12**	**14**	**9**	**15**	**13**	**23**	**6**
				3	**443.5**	**526.75**	y10 2+	**16**	**14**	**10**	**17**	**14**	**23**	**8**
				3	**443.5**	**823.38**	y7	**14**	**13**	**18**	**15**	**14**	**23**	**14**
prostate specific antigen (PSA)	IVGGWECamcEK	bi0161	1077.5	2	539.3	808.3	y6	12	14	20	21	13	23	12
				2	539.3	865.4	y7	14	14	20	16	13	23	14
				2	539.3	964.42	y8	16	16	22	18	14	23	16
	IVGGWECamcEK	**bi0067**	**1082.5**	**2**	**541.7**	**808.33**	y6	**12**	**14**	**20**	**21**	**13**	**23**	**12**
				2	**541.7**	**865.35**	y7	**14**	**14**	**20**	**16**	**13**	**23**	**14**
				2	**541.7**	**969.44**	y8	**16**	**16**	**22**	**18**	**14**	**23**	**16**
	LSEPAELTDAVK	bi0037	1272.7	2	636.8	775.4	y7	11	15	18	19	11	23	12
				2	636.8	846.4	y8	12	15	20	14	11	23	14
				2	636.8	943.4	y9	15	15	22	17	14	23	6
	LSEPAELTDAVK	**ni0107**	**1280.7**	**2**	**640.8**	**783.43**	y7	**11**	**15**	**18**	**19**	**11**	**23**	**12**
				2	**640.8**	**854.47**	y8	**12**	**15**	**20**	**14**	**11**	**23**	**14**
				2	**640.8**	**951.52**	y9	**15**	**15**	**22**	**17**	**14**	**23**	**6**
horseradish peroxidase (HRP)	SSDLVALSGGHTFGK	bi0166	1475.7	3	492.6	703.35	y7	10	14	16	18	11	23	12
				3	492.6	790.38	y8	12	14	17	20	11	23	14
				3	492.6	974.51	y10	15	16	24	17	11	23	10
	SSDLVALSGGHTFGK	**ni0108**	**1483.8**	**3**	**495.3**	**711.37**	y7	**10**	**14**	**16**	**18**	**11**	**23**	**12**
				3	**495.3**	**798.4**	y8	**12**	**14**	**17**	**20**	**11**	**23**	**14**
				3	**495.3**	**982.52**	y10	**15**	**16**	**24**	**17**	**11**	**23**	**10**
C-reactive Protein (CRP)	ESDTSYVSLK	bi0231	1128.5	2	564.8	609.4	y5	8	10	12	19	10	23	8
				2	564.8	696.4	y6	10	12	16	23	11	23	12
				2	564.8	797.4	y7	12	14	20	19	12	23	12
	ESDTSYVSLK	**ni0109**	**1136.6**	**2**	**568.8**	**617.37**	y5	**8**	**10**	**12**	**19**	**10**	**23**	**8**
				2	**568.8**	**704.41**	y6	**10**	**12**	**16**	**23**	**11**	**23**	**12**
				2	**568.8**	**805.45**	y7	**12**	**14**	**20**	**19**	**12**	**23**	**12**
	GYSIFSYATK	bi0202	1136.3	2	568.8	716.4	y6	10	16	20	24	11	23	12
				2	568.8	829.5	y7	12	18	20	22	13	23	12
				2	568.8	916.5	y8	14	16	22	16	14	23	8
	GYSIFSYATK	**ni0110**	**1144.6**	**2**	**572.8**	**724.38**	y6	**10**	**16**	**20**	**24**	**11**	**23**	**12**
				2	**572.8**	**837.46**	y7	**12**	**18**	**20**	**22**	**13**	**23**	**12**
				2	**572.8**	**924.49**	y8	**14**	**16**	**22**	**16**	**14**	**23**	**8**
	YEVQGEVFTKPQLWP	ni0001	1820.9	2	911	1053.5	b9	16	25	24	19	25	23	16
				2	911	1181.6	b10	18	25	32	22	25	23	16
				2	911	1519.8	b13	18	25	38	29	25	23	16
	YEVQGEVFTKPQLWP	**ni0111**	**1826.9**	**2**	**914**	**1053.49**	b9	**16**	**25**	**24**	**19**	**25**	**23**	**16**
				2	**914**	**1181.58**	b10	**18**	**25**	**32**	**22**	**25**	**23**	**16**
				2	**914**	**1525.8**	b13	**18**	**25**	**38**	**29**	**25**	**23**	**16**

Q1/Q3 transition	across 7 sites range of CXP	across 7 sites mean of CXP	across 7 sites std. dev. of CXP	across 7 sites std. dev. of CXP (in %)
744.8 / 858.4	Range: 12.0 - 29.0	Mean: 18.14	StdDev: 6.2	34.2%
744.8 / 959.4	Range: 6.0 - 23.0	Mean: 15.86	StdDev: 5.76	36.3%
744.8 / 1087.5	Range: 10.0 - 24.0	Mean: 17.57	StdDev: 5.13	29.2%
747.3 / 863.4	Range: 12.0 - 29.0	Mean: 18.14	StdDev: 6.2	34.2%
747.3 / 964.4	Range: 6.0 - 23.0	Mean: 15.86	StdDev: 5.76	36.3%
747.3 / 1092.5	Range: 10.0 - 24.0	Mean: 17.57	StdDev: 5.13	29.2%
467.2 / 586.8	Range: 10.0 - 23.0	Mean: 15.29	StdDev: 5.53	36.2%
467.2 / 643.8	Range: 10.0 - 23.0	Mean: 14.71	StdDev: 5.15	35.0%
467.2 / 720.39	Range: 8.0 - 23.0	Mean: 15.29	StdDev: 5.5	36.0%
469.9 / 590.81	Range: 10.0 - 23.0	Mean: 15.29	StdDev: 5.53	36.2%
469.9 / 647.83	Range: 10.0 - 23.0	Mean: 14.71	StdDev: 5.15	35.0%
469.9 / 728.4	Range: 8.0 - 23.0	Mean: 15.29	StdDev: 5.5	36.0%
424.6 / 506.3	Range: 8.0 - 23.0	Mean: 14.29	StdDev: 6.55	45.9%
424.6 / 579.8	Range: 8.0 - 23.0	Mean: 12.14	StdDev: 5.24	43.2%
424.6 / 716.4	Range: 10.0 - 23.0	Mean: 15.29	StdDev: 4.46	29.2%
427.2 / 510.27	Range: 8.0 - 23.0	Mean: 14.29	StdDev: 6.55	45.9%
427.2 / 583.8	Range: 8.0 - 23.0	Mean: 12.14	StdDev: 5.24	43.2%
427.2 / 724.4	Range: 10.0 - 23.0	Mean: 15.29	StdDev: 4.46	29.2%
363.7 / 385.3	Range: 12.0 - 23.0	Mean: 16.29	StdDev: 3.86	23.7%
363.7 / 532.3	Range: 8.0 - 23.0	Mean: 15.86	StdDev: 4.81	30.3%
363.7 / 589.4	Range: 8.0 - 23.0	Mean: 13.86	StdDev: 5.34	38.5%
366.7 / 391.3	Range: 12.0 - 23.0	Mean: 16.29	StdDev: 3.86	23.7%
366.7 / 538.3	Range: 8.0 - 23.0	Mean: 15.86	StdDev: 4.81	30.3%
366.7 / 595.4	Range: 8.0 - 23.0	Mean: 13.86	StdDev: 5.34	38.5%
441.5 / 488.2	Range: 6.0 - 23.0	Mean: 13.14	StdDev: 5.34	40.6%
441.5 / 523.7	Range: 8.0 - 23.0	Mean: 14.57	StdDev: 4.89	33.6%
441.5 / 817.4	Range: 13.0 - 23.0	Mean: 15.86	StdDev: 3.53	22.3%
443.5 / 491.23	Range: 6.0 - 23.0	Mean: 13.14	StdDev: 5.34	40.6%
443.5 / 526.75	Range: 8.0 - 23.0	Mean: 14.57	StdDev: 4.89	33.6%
443.5 / 823.38	Range: 13.0 - 23.0	Mean: 15.86	StdDev: 3.53	22.3%
539.3 / 808.3	Range: 12.0 - 23.0	Mean: 16.43	StdDev: 4.72	28.7%
539.3 / 865.4	Range: 13.0 - 23.0	Mean: 16.29	StdDev: 3.77	23.2%
539.3 / 964.42	Range: 14.0 - 23.0	Mean: 17.86	StdDev: 3.39	19.0%
541.7 / 808.33	Range: 12.0 - 23.0	Mean: 16.43	StdDev: 4.72	28.7%
541.7 / 865.35	Range: 13.0 - 23.0	Mean: 16.29	StdDev: 3.77	23.2%
541.7 / 969.44	Range: 14.0 - 23.0	Mean: 17.86	StdDev: 3.39	19.0%
636.8 / 775.4	Range: 11.0 - 23.0	Mean: 15.57	StdDev: 4.61	29.6%
636.8 / 846.4	Range: 11.0 - 23.0	Mean: 15.57	StdDev: 4.35	28.0%
636.8 / 943.4	Range: 6.0 - 23.0	Mean: 16.0	StdDev: 5.66	35.4%
640.8 / 783.43	Range: 11.0 - 23.0	Mean: 15.57	StdDev: 4.61	29.6%
640.8 / 854.47	Range: 11.0 - 23.0	Mean: 15.57	StdDev: 4.35	28.0%
640.8 / 951.52	Range: 6.0 - 23.0	Mean: 16.0	StdDev: 5.66	35.4%
492.6 / 703.35	Range: 10.0 - 23.0	Mean: 14.86	StdDev: 4.56	30.7%
492.6 / 790.38	Range: 11.0 - 23.0	Mean: 15.86	StdDev: 4.38	27.6%
492.6 / 974.51	Range: 10.0 - 24.0	Mean: 16.57	StdDev: 5.38	32.5%
495.3 / 711.37	Range: 10.0 - 23.0	Mean: 14.86	StdDev: 4.56	30.7%
495.3 / 798.4	Range: 11.0 - 23.0	Mean: 15.86	StdDev: 4.38	27.6%
495.3 / 982.52	Range: 10.0 - 24.0	Mean: 16.57	StdDev: 5.38	32.5%
564.8 / 609.4	Range: 8.0 - 23.0	Mean: 12.86	StdDev: 5.84	45.4%
564.8 / 696.4	Range: 10.0 - 23.0	Mean: 15.29	StdDev: 5.59	36.6%
564.8 / 797.4	Range: 12.0 - 23.0	Mean: 16.0	StdDev: 4.58	28.6%
568.8 / 617.37	Range: 8.0 - 23.0	Mean: 12.86	StdDev: 5.84	45.4%
568.8 / 704.41	Range: 10.0 - 23.0	Mean: 15.29	StdDev: 5.59	36.6%
568.8 / 805.45	Range: 12.0 - 23.0	Mean: 16.0	StdDev: 4.58	28.6%
568.8 / 716.4	Range: 10.0 - 24.0	Mean: 16.57	StdDev: 5.83	35.2%
568.8 / 829.5	Range: 12.0 - 23.0	Mean: 17.14	StdDev: 4.78	27.9%
568.8 / 916.5	Range: 8.0 - 23.0	Mean: 16.14	StdDev: 5.11	31.7%
572.8 / 724.38	Range: 10.0 - 24.0	Mean: 16.57	StdDev: 5.83	35.2%
572.8 / 837.46	Range: 12.0 - 23.0	Mean: 17.14	StdDev: 4.78	27.9%
572.8 / 924.49	Range: 8.0 - 23.0	Mean: 16.14	StdDev: 5.11	31.7%
911 / 1053.5	Range: 16.0 - 25.0	Mean: 21.14	StdDev: 4.06	19.2%
911 / 1181.6	Range: 16.0 - 32.0	Mean: 23.0	StdDev: 5.23	22.7%
911 / 1519.8	Range: 16.0 - 38.0	Mean: 24.86	StdDev: 7.29	29.3%
914 / 1053.49	Range: 16.0 - 25.0	Mean: 21.14	StdDev: 4.06	19.2%
914 / 1181.58	Range: 16.0 - 32.0	Mean: 23.0	StdDev: 5.23	22.7%
914 / 1525.8	Range: 16.0 - 38.0	Mean: 24.86	StdDev: 7.29	29.3%

Footnote: Instrument Parameters for MRM transitions were optimized for maximum transmission efficiency and sensitivity for individual instruments by infusion of unlabeled signature peptides.
Optimized collision cell exit potential (CXP) are reported for 4000 QTRAP instruments for each MRM transition along with the corresponding instrument used at each site.
The TSQ Quantum Ultra instrument at CPTAC site @65 is not included in this specific comparison, for TSQ Quantum Ultra instrument parameters see Supplementary Table 1B.
Boldface type indicates labeled peptide internal standard with labeled amino acid in red.

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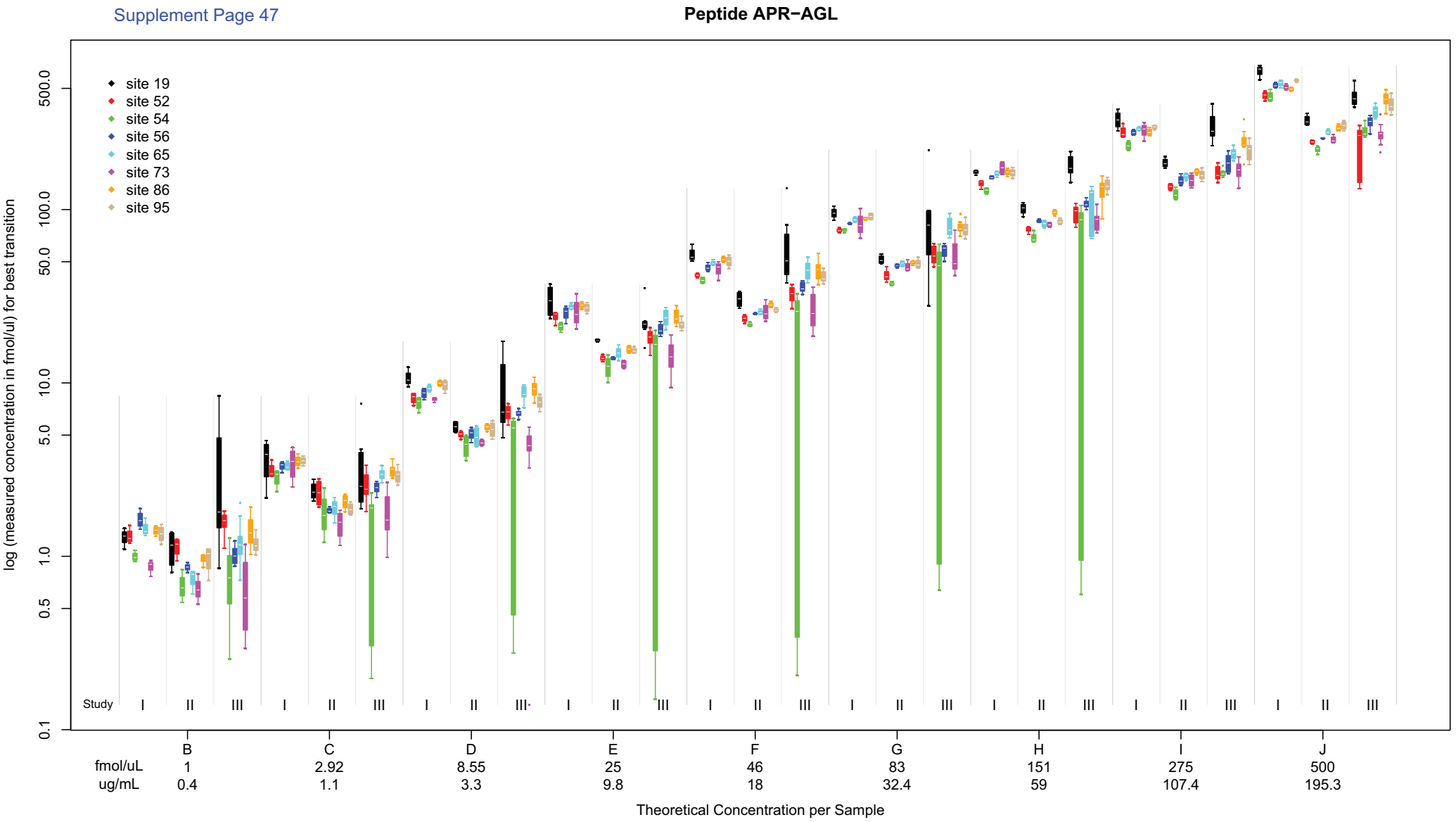
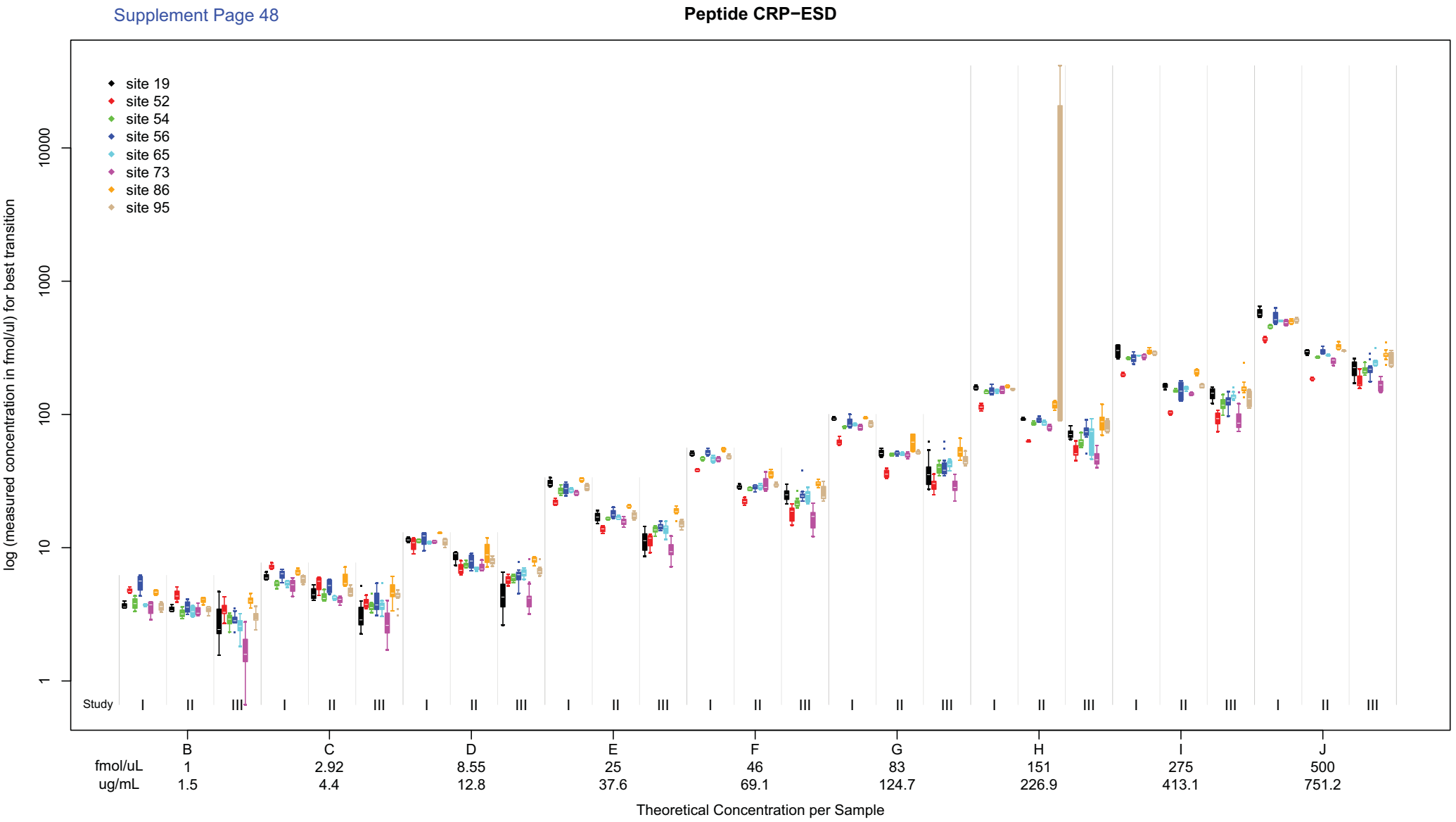
Supplementary Figure 1: Measured concentration in fmol/μL in diluted plasma on log scale across sites and studies I, II, and III for the entire range (1-500 fmol/μL) of spiked in analytes. Protein concentration in μg/mL on x-axis is μg protein equivalent in 1 mL of neat plasma.

Box plots showing theoretical (spike in) concentration (x-axis) vs. measured log concentration (y-axis) for peptides. The plots enable evaluation and comparison of assay reproducibility across different sites and studies. The box plots for studies I and II are based on 4 replicate measurements, while those for study III summarize 12 measurements (4 each from III a, b and c). Data for each site is color coded, and organized by study and concentration. In addition to other observations, the plots clearly show progressively increasing variability, and decreasing recovery (resulting in lower measured concentrations) from study I - III. The box plots show the median as a white horizontal line. The box spans the interquartile range (IQR), with the whiskers extending to 1.5 * IQR. Values beyond 1.5 * IQR are deemed outliers, and shown as separate points.



Nature Biotechnology: doi:10.1038/nbt.1546



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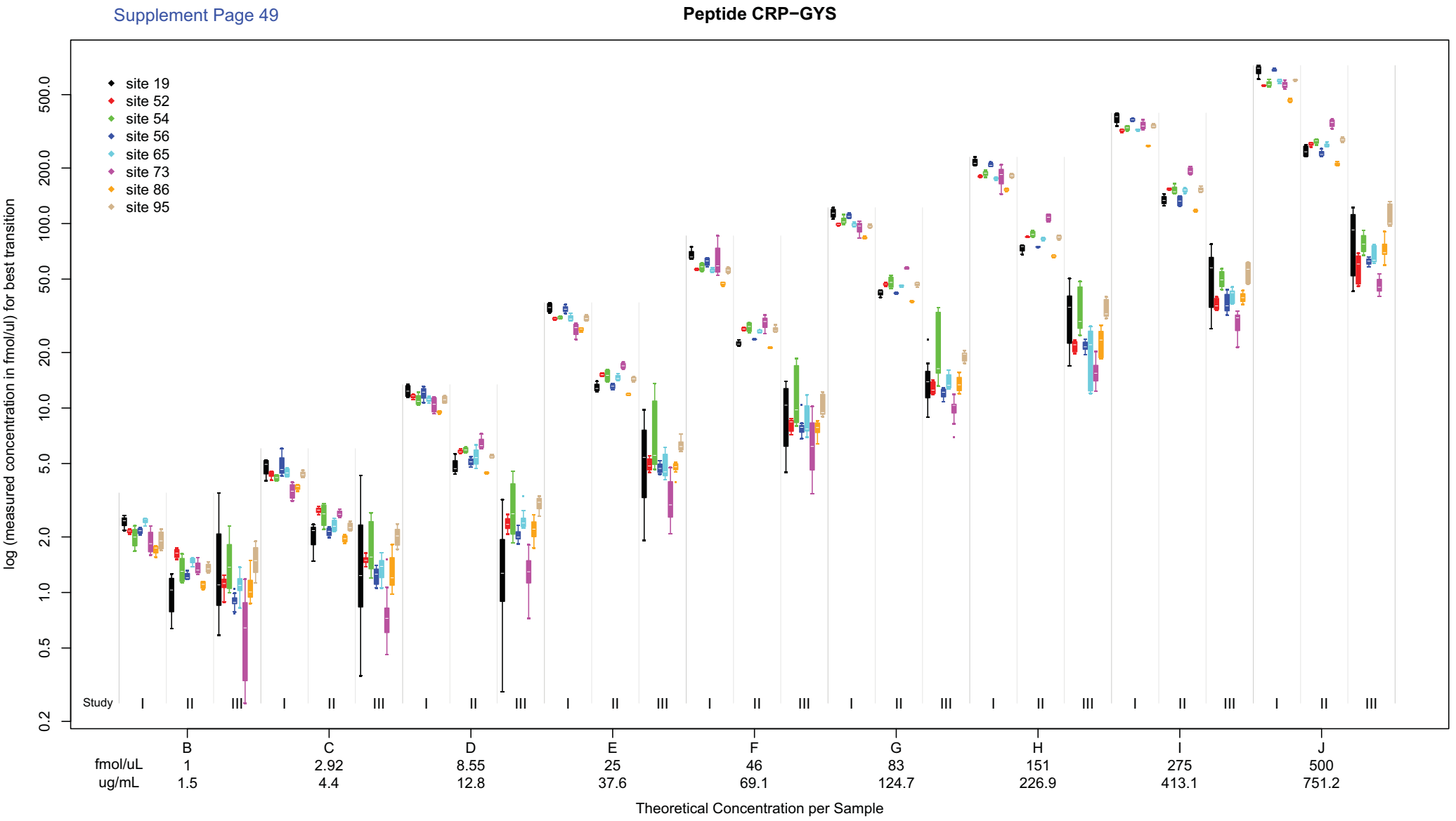
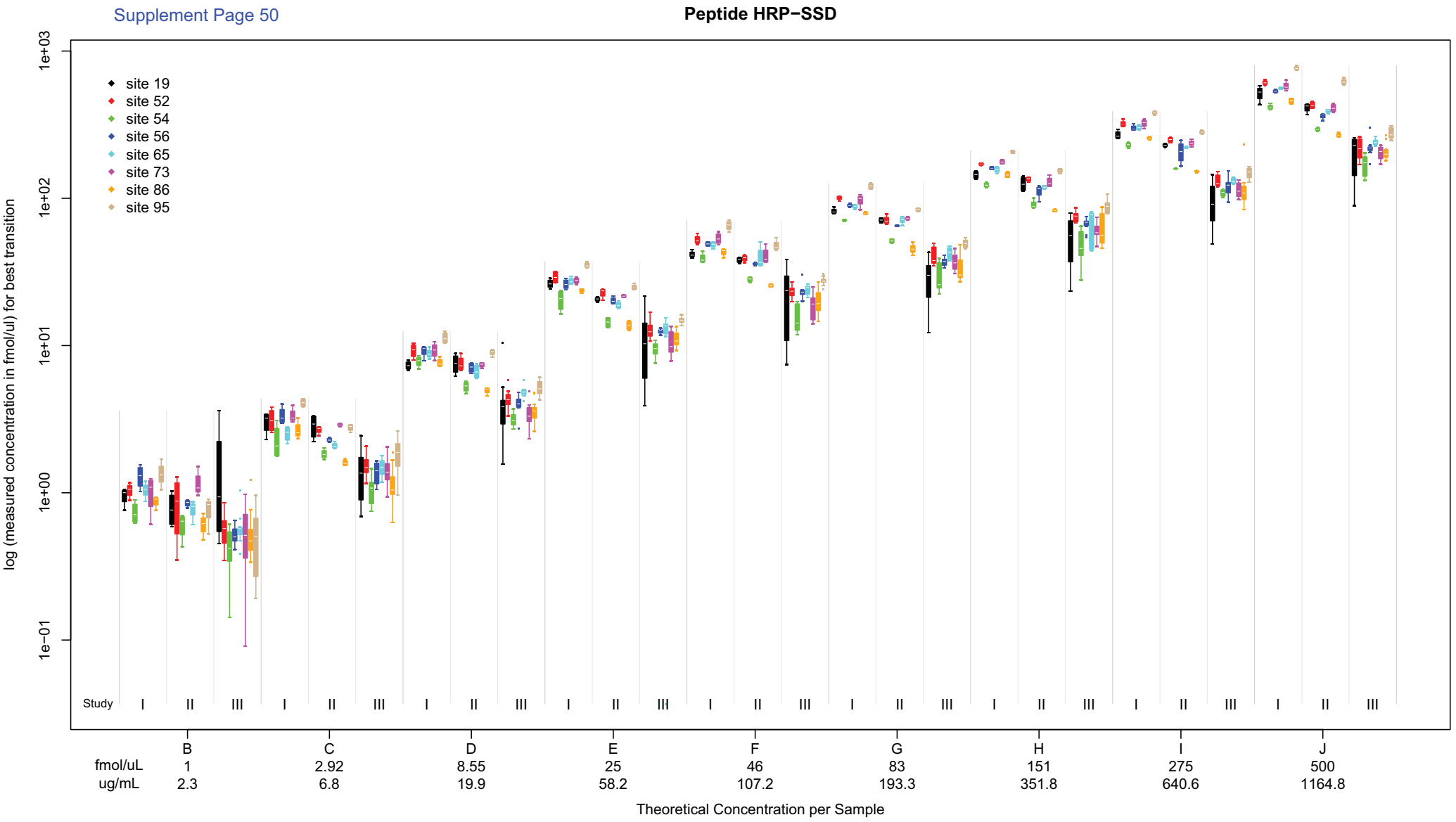


Nature Biotechnology: doi:10.1038/nbt.1546



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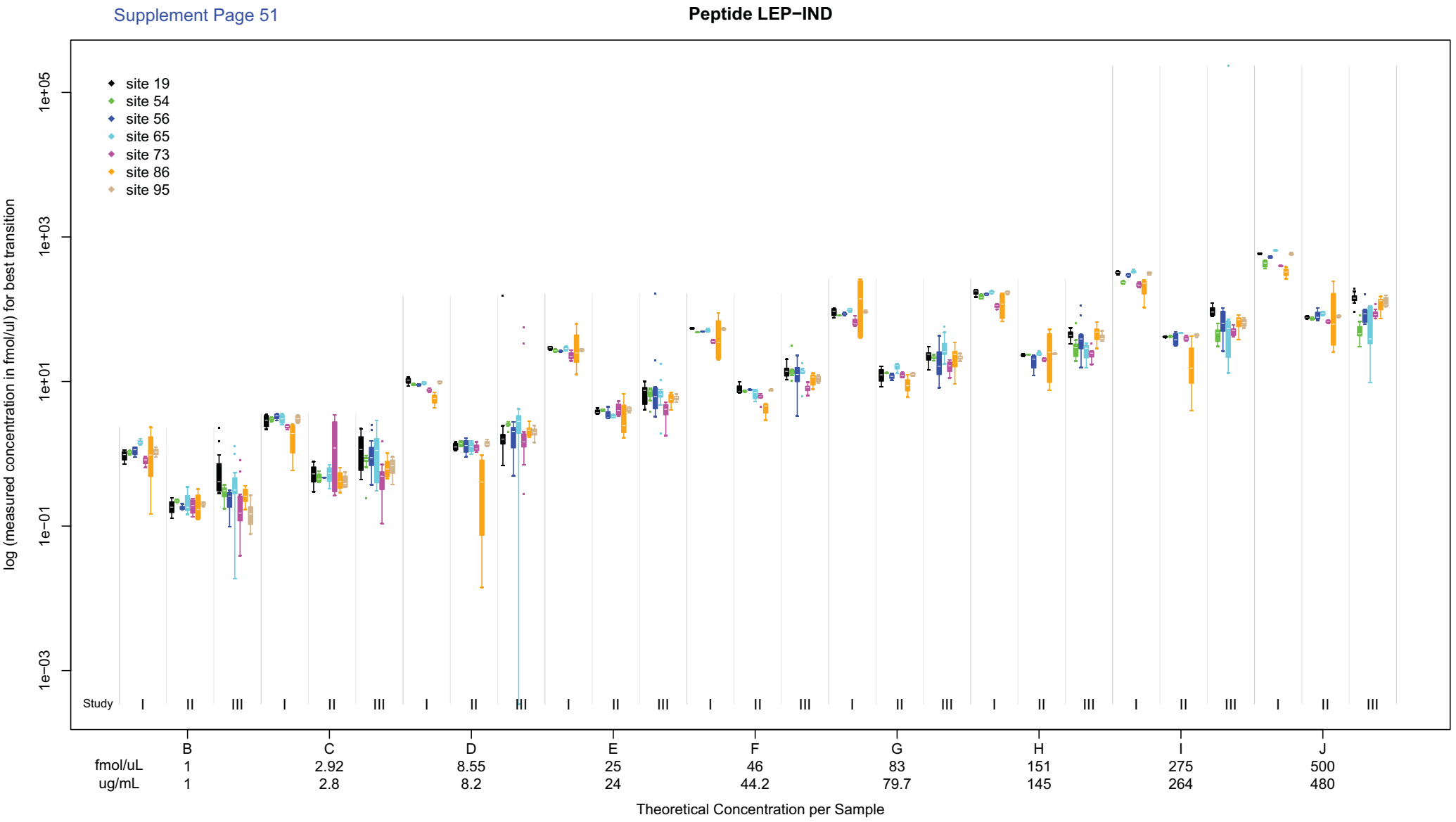

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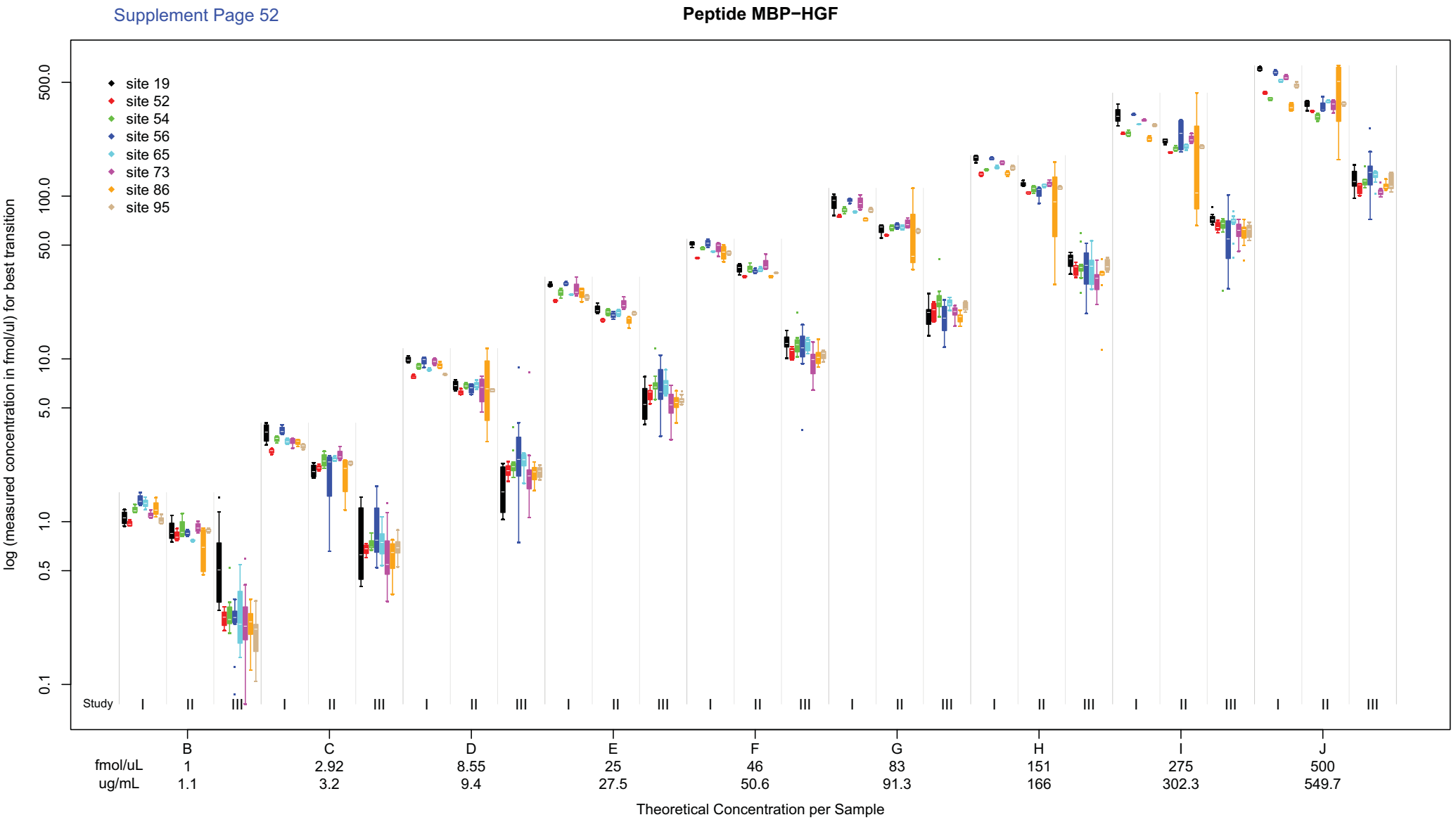
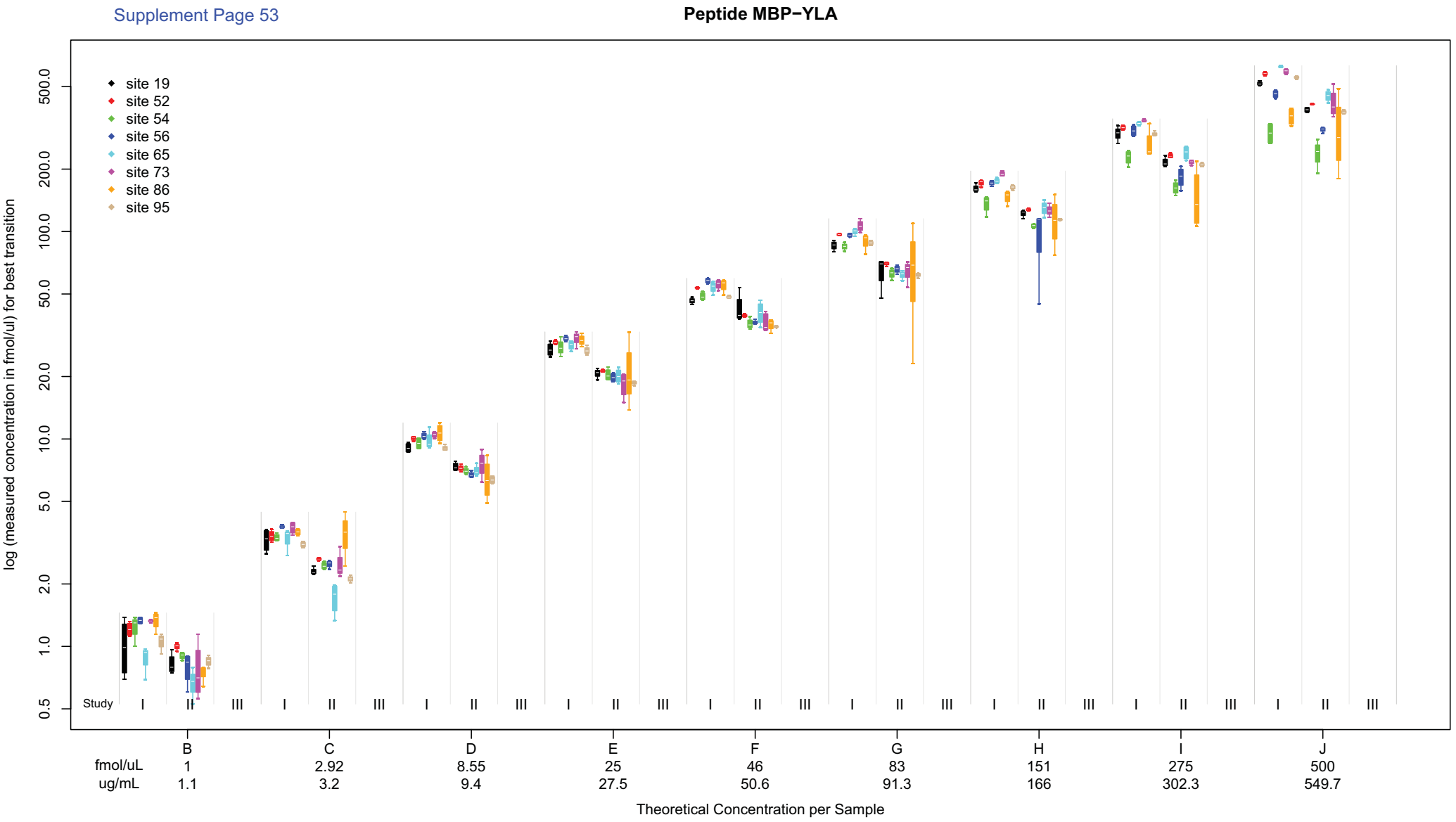


Nature Biotechnology: doi:10.1038/nbt.1546



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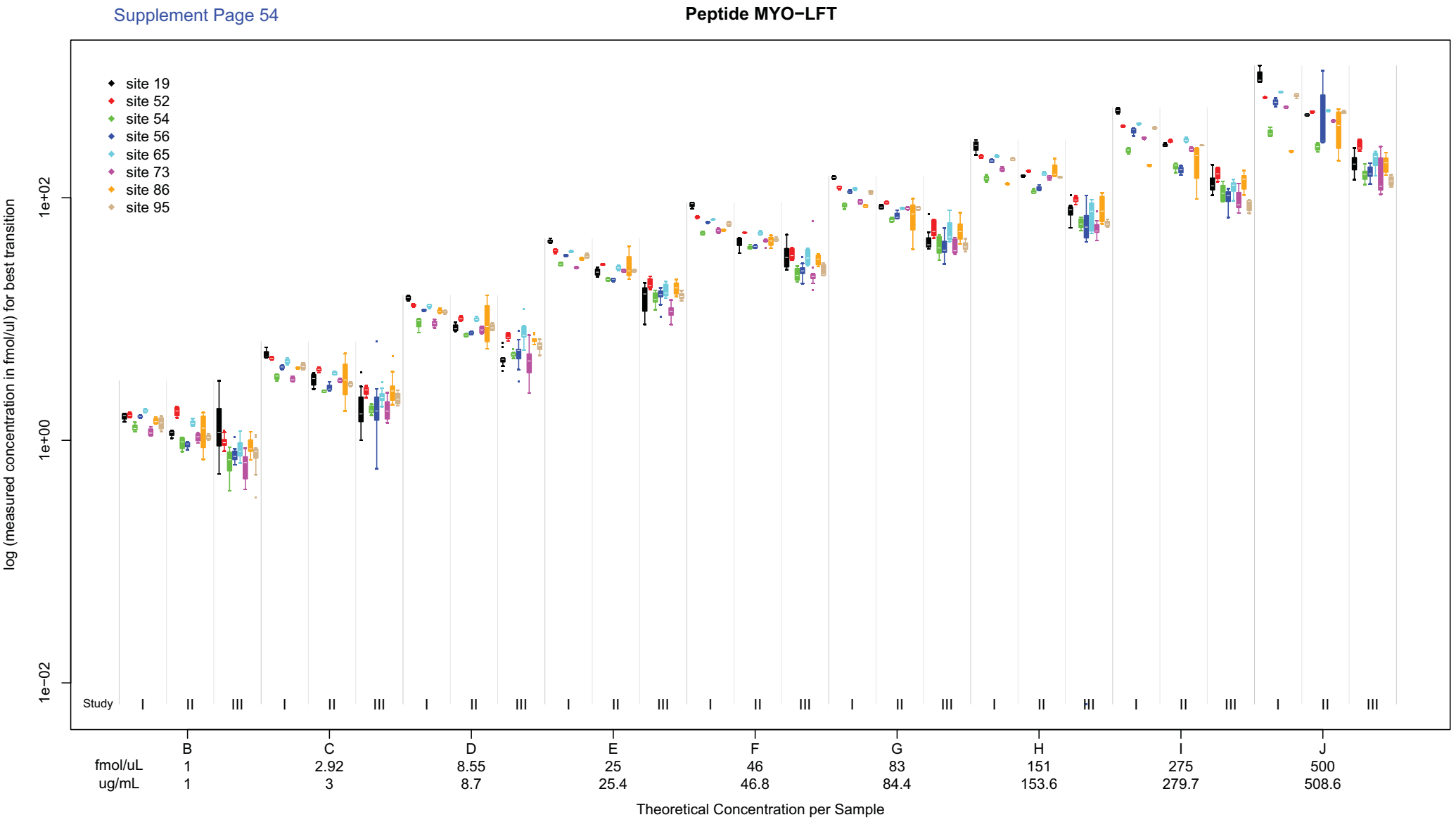

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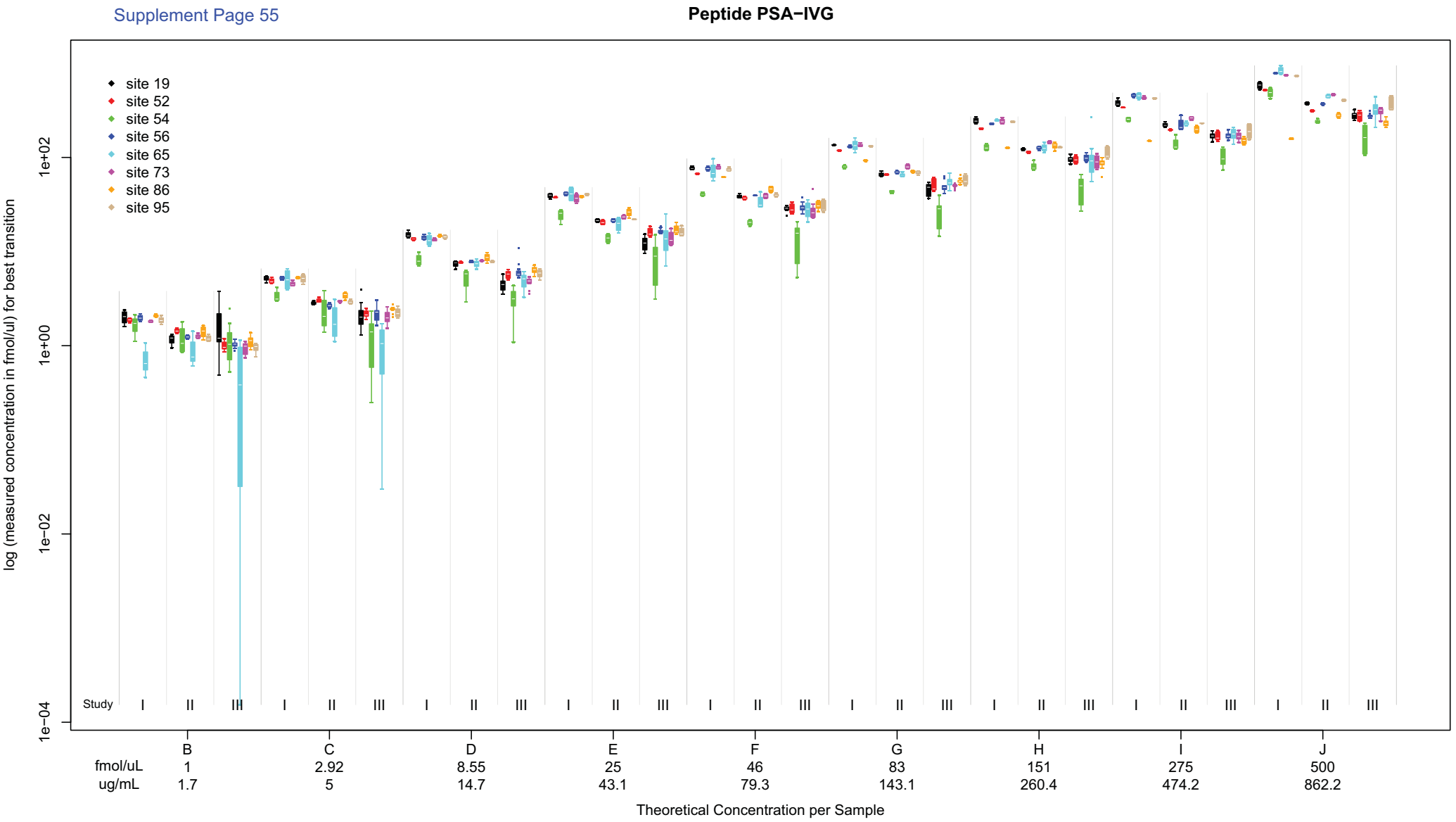

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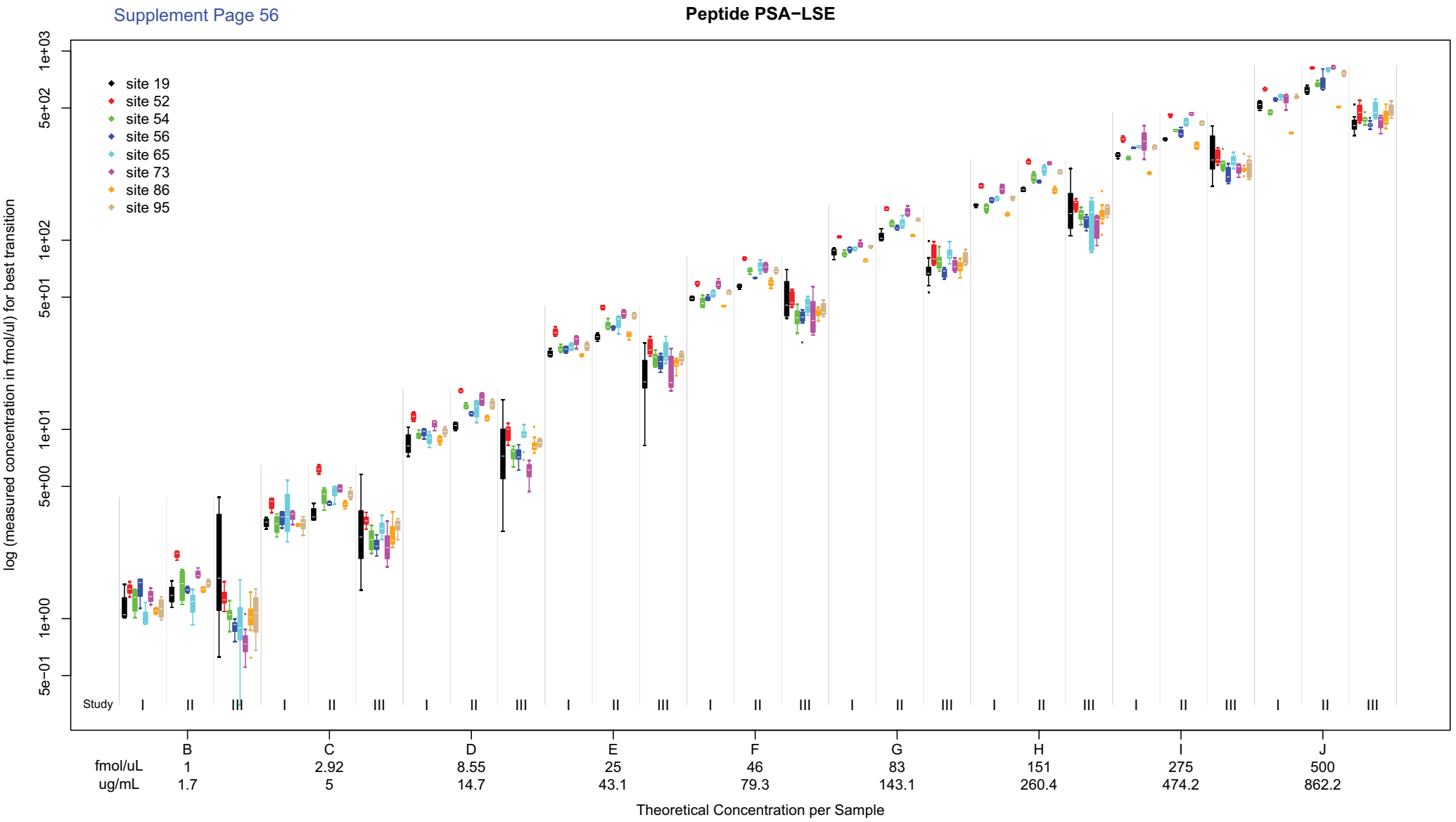


Nature Biotechnology: doi:10.1038/nbt.1546

Supplementary Table 2A: Summary of inter-lab and intra-lab CVs for all peptides calculated across the concentration range of spiked analyte - Study I.
(color code: CV's : 0-10%, CV's : 10-20%, CV's : 20-30%, and CV's ≥ 30%)

Coefficient of variation, Study I															
site	peptide	pro-pept	transition	B (1.0)	C (2.92)	D (8.55)	E (25)	F (46)	G (83)	H (151)	I (275)	J (500)	Min Intra-lab CV	Max Intra-lab CV	Median Intra-lab CV
19	bi0037	PSA-LSE	37tr3_A	20.94%	6.05%	15.56%	4.37%	2.20%	5.99%	1.87%	2.94%	5.17%	1.87%	20.94%	5.17%
52	bi0037	PSA-LSE	37tr3_A	7.53%	8.07%	5.05%	4.98%	2.28%	0.95%	2.35%	3.11%	1.40%	0.95%	8.07%	3.11%
54	bi0037	PSA-LSE	37tr3_A	15.91%	12.11%	4.88%	4.22%	6.76%	4.11%	4.87%	1.74%	2.28%	1.74%	15.91%	4.87%
56	bi0037	PSA-LSE	37tr3_A	15.55%	9.30%	5.35%	4.29%	3.15%	2.62%	2.28%	0.92%	1.72%	0.92%	15.55%	3.15%
65	bi0037	PSA-LSE	37tr2_A	15.41%	32.73%	7.89%	4.23%	3.51%	1.63%	1.79%	1.23%	3.10%	1.23%	32.73%	3.51%
73	bi0037	PSA-LSE	37tr3_A	8.39%	6.98%	5.47%	6.88%	5.00%	3.70%	5.39%	16.48%	8.56%	3.70%	16.48%	6.88%
86	bi0037	PSA-LSE	37tr3_A	3.87%	1.16%	4.32%	1.14%	0.81%	1.28%	1.59%	1.11%	0.68%	0.68%	4.32%	1.16%
95	bi0037	PSA-LSE	37tr3_A	12.86%	9.33%	4.65%	4.33%	2.40%	1.34%	2.12%	2.24%	2.64%	1.34%	12.86%	2.64%
			Min Inter-lab CV	3.87%	1.16%	4.32%	1.14%	0.81%	0.95%	1.59%	0.92%	0.68%			
			Max Inter-lab CV	20.94%	32.73%	15.56%	6.88%	6.76%	5.99%	5.39%	16.48%	8.56%			
			Median Inter-lab CV	14.14%	8.68%	5.20%	4.31%	2.78%	2.12%	2.20%	1.99%	2.46%			
19	bi0161	PSA-IVG	161tr2_A	17.03%	7.10%	8.54%	5.65%	3.52%	0.74%	7.50%	8.78%	8.32%	0.74%	17.03%	7.50%
52	bi0161	PSA-IVG	161tr2_A	4.74%	6.69%	2.72%	1.03%	1.25%	1.51%	1.21%	0.65%	1.20%	0.65%	6.69%	1.25%
54	bi0161	PSA-IVG	161tr1_A	30.73%	16.37%	14.91%	15.02%	4.18%	4.72%	7.94%	5.10%	11.46%	4.18%	30.73%	11.46%
56	bi0161	PSA-IVG	161tr2_A	7.69%	3.53%	5.98%	3.72%	4.48%	2.70%	1.38%	3.64%	1.42%	1.38%	7.69%	3.64%
65	bi0161	PSA-IVG	161tr1_A	43.11%	25.51%	13.94%	17.52%	24.15%	14.66%	1.87%	7.78%	9.70%	1.87%	43.11%	14.66%
73	bi0161	PSA-IVG	161tr2_A	2.34%	6.20%	3.33%	10.80%	4.48%	4.17%	6.51%	3.43%	1.35%	1.35%	10.80%	4.17%
86	bi0161	PSA-IVG	161tr2_A	3.24%	1.97%	2.27%	1.59%	0.77%	1.35%	1.09%	0.65%	1.59%	0.65%	3.24%	1.59%
95	bi0161	PSA-IVG	161tr2_A	8.97%	10.33%	3.73%	1.97%	4.29%	1.12%	1.60%	1.16%	1.67%	1.12%	10.33%	1.97%
			Min Inter-lab CV	2.34%	1.97%	2.27%	1.03%	0.77%	0.74%	1.09%	0.65%	1.20%			
			Max Inter-lab CV	43.11%	25.51%	14.91%	17.52%	24.15%	14.66%	7.94%	8.78%	11.46%			
			Median Inter-lab CV	8.33%	6.89%	4.86%	4.68%	4.24%	2.10%	1.73%	3.53%	1.63%			
19	bi0166	HRP-SSD	166tr2_A	13.64%	17.20%	6.60%	7.14%	5.52%	4.74%	6.10%	6.43%	11.88%	4.74%	17.20%	6.60%
52	bi0166	HRP-SSD	166tr3_A	11.24%	18.53%	11.27%	9.77%	7.49%	3.35%	1.68%	5.38%	3.46%	1.68%	18.53%	7.49%
54	bi0166	HRP-SSD	166tr3_A	17.16%	27.59%	8.51%	16.81%	7.96%	1.61%	3.60%	3.67%	4.15%	1.61%	27.59%	7.96%
56	bi0166	HRP-SSD	166tr2_A	18.03%	13.95%	9.66%	7.93%	3.49%	2.60%	1.99%	4.74%	2.42%	1.99%	18.03%	4.74%
65	bi0166	HRP-SSD	166tr3_A	15.46%	11.98%	7.73%	5.22%	4.72%	3.16%	4.92%	3.48%	1.40%	1.40%	15.46%	4.92%
73	bi0166	HRP-SSD	166tr2_A	28.25%	11.88%	12.06%	4.87%	8.92%	9.69%	3.14%	6.18%	7.15%	3.14%	28.25%	8.92%
86	bi0166	HRP-SSD	166tr3_A	8.89%	14.30%	6.91%	2.68%	6.06%	1.69%	4.30%	1.89%	4.32%	1.69%	14.30%	4.32%
95	bi0166	HRP-SSD	166tr3_A	19.51%	6.42%	8.92%	3.98%	7.80%	3.90%	1.75%	2.84%	3.67%	1.75%	19.51%	3.98%
			Min Inter-lab CV	8.89%	6.42%	6.60%	2.68%	3.49%	1.61%	1.68%	1.89%	1.40%			
			Max Inter-lab CV	28.25%	27.59%	12.06%	16.81%	8.92%	9.69%	6.10%	6.43%	11.88%			
			Median Inter-lab CV	16.31%	14.12%	8.72%	6.18%	6.77%	3.25%	3.37%	4.20%	3.91%			
19	bi0167	LEP-IND	167tr2_A	18.24%	20.85%	11.40%	5.25%	1.83%	13.53%	10.27%	4.69%	1.74%	1.74%	20.85%	10.27%
52	bi0167	LEP-IND													
54	bi0167	LEP-IND	167tr2_A	5.16%	5.53%	2.41%	4.34%	0.94%	1.26%	6.64%	3.84%	11.75%	0.94%	11.75%	4.34%
56	bi0167	LEP-IND	167tr1_A	14.49%	9.29%	2.72%	1.55%	0.83%	3.69%	2.89%	4.57%	2.45%	0.83%	14.49%	2.89%
65	bi0167	LEP-IND	167tr1_A	9.97%	15.62%	3.71%	6.08%	4.68%	4.89%	3.72%	4.95%	1.22%	1.22%	15.62%	4.89%
73	bi0167	LEP-IND	167tr1_A	13.90%	6.42%	4.94%	14.46%	4.26%	15.38%	7.64%	7.23%	1.70%	1.70%	15.38%	7.23%
86	bi0167	LEP-IND	167tr1_A	82.72%	51.78%	19.51%	69.34%	72.37%	82.83%	42.68%	32.95%	15.21%	15.21%	82.83%	51.78%
95	bi0167	LEP-IND	167tr2_A	12.80%	12.52%	3.49%	3.69%	2.62%	3.21%	4.60%	3.47%	3.93%	2.62%	12.80%	3.69%
			Min Inter-lab CV	5.16%	5.53%	2.41%	1.55%	0.83%	1.26%	2.89%	3.47%	1.22%			
			Max Inter-lab CV	82.72%	51.78%	19.51%	69.34%	72.37%	82.83%	42.68%	32.95%	15.21%			
			Median Inter-lab CV	13.90%	12.52%	3.71%	5.25%	2.62%	4.89%	6.64%	4.69%	2.45%			

Coefficient of variation, Study I															
site	peptide	pro-pept	transition	B (1.0)	C (2.92)	D (8.55)	E (25)	F (46)	G (83)	H (151)	I (275)	J (500)	Min Intra-lab CV	Max Intra-lab CV	Median Intra-lab CV
19	bi0169	MBP-HGF	169tr3_A	10.23%	13.89%	4.40%	2.80%	3.57%	12.34%	4.44%	12.79%	2.64%	2.64%	13.89%	4.44%
52	bi0169	MBP-HGF	169tr3_A	4.00%	3.54%	2.61%	1.65%	0.78%	2.07%	2.32%	1.16%	1.65%	0.78%	4.00%	2.07%
54	bi0169	MBP-HGF	169tr3_A	5.15%	4.17%	3.76%	5.67%	1.38%	4.58%	1.07%	3.96%	1.80%	1.07%	5.67%	3.96%
56	bi0169	MBP-HGF	169tr3_A	8.18%	6.03%	6.19%	2.08%	5.12%	3.15%	1.53%	0.82%	2.89%	0.82%	8.18%	3.15%
65	bi0169	MBP-HGF	169tr3_A	8.75%	4.08%	1.69%	0.51%	0.59%	0.94%	1.82%	0.18%	1.73%	0.18%	8.75%	1.69%
73	bi0169	MBP-HGF	169tr3_A	6.32%	6.50%	4.34%	12.48%	8.46%	9.12%	2.07%	1.15%	3.24%	1.15%	12.48%	6.32%
86	bi0169	MBP-HGF	169tr3_A	11.86%	4.51%	4.25%	8.70%	10.78%	2.06%	3.15%	3.43%	5.73%	2.06%	11.86%	4.51%
95	bi0169	MBP-HGF	169tr3_A	6.19%	3.32%	0.85%	3.14%	2.42%	2.55%	2.85%	1.56%	4.27%	0.85%	6.19%	2.85%
			Min Inter-lab CV	4.00%	3.32%	0.85%	0.51%	0.59%	0.94%	1.07%	0.18%	1.65%			
			Max Inter-lab CV	11.86%	13.89%	6.19%	12.48%	10.78%	12.34%	4.44%	12.79%	5.73%			
			Median Inter-lab CV	7.25%	4.34%	4.00%	2.97%	2.99%	2.85%	2.20%	1.36%	2.77%			
19	bi0170	MBP-YLA	170tr2_A	31.76%	12.75%	5.30%	7.69%	3.39%	5.21%	4.41%	8.10%	2.08%	2.08%	31.76%	5.30%
52	bi0170	MBP-YLA	170tr2_A	7.63%	6.24%	2.11%	2.04%	0.70%	1.10%	3.39%	2.38%	1.97%	0.70%	7.63%	2.11%
54	bi0170	MBP-YLA	170tr2_A	13.24%	3.99%	6.42%	9.02%	4.43%	4.95%	9.71%	8.06%	11.80%	3.99%	13.24%	8.06%
56	bi0170	MBP-YLA	170tr2_A	3.85%	1.77%	3.50%	2.77%	2.76%	1.86%	2.61%	6.06%	4.27%	1.77%	6.06%	2.77%
65	bi0170	MBP-YLA	170tr3_A	17.61%	11.97%	10.88%	5.07%	6.24%	3.61%	2.82%	1.95%	0.91%	0.91%	17.61%	5.07%
73	bi0170	MBP-YLA	170tr2_A	1.82%	6.72%	3.39%	7.76%	5.28%	6.54%	2.60%	1.43%	2.74%	1.43%	7.76%	3.39%
86	bi0170	MBP-YLA	170tr2_A	10.13%	3.45%	10.17%	6.27%	7.25%	9.28%	7.21%	17.38%	9.65%	3.45%	17.38%	9.28%
95	bi0170	MBP-YLA	170tr2_A	9.15%	2.87%	3.00%	4.46%	1.25%	2.13%	2.27%	2.51%	1.90%	1.25%	9.15%	2.51%
			Min Inter-lab CV	1.82%	1.77%	2.11%	2.04%	0.70%	1.10%	2.27%	1.43%	0.91%			
			Max Inter-lab CV	31.76%	12.75%	10.88%	9.02%	7.25%	9.28%	9.71%	17.38%	11.80%			
			Median Inter-lab CV	9.64%	5.12%	4.40%	5.67%	3.91%	4.28%	3.10%	4.29%	2.41%			
19	bi0171	MYO-LFT	171tr1_A	6.72%	9.58%	4.35%	3.81%	5.08%	2.53%	11.70%	5.05%	15.73%	2.53%	15.73%	5.08%
52	bi0171	MYO-LFT	171tr1_A	4.85%	2.59%	2.39%	3.71%	1.57%	2.74%	2.56%	1.56%	0.91%	0.91%	4.85%	2.56%
54	bi0171	MYO-LFT	171tr2_A	7.69%	5.68%	11.61%	3.30%	2.65%	5.82%	5.35%	5.06%	7.66%	2.65%	11.61%	5.68%
56	bi0171	MYO-LFT	171tr1_A	2.42%	3.28%	1.50%	1.52%	1.11%	2.64%	3.18%	6.43%	6.57%	1.11%	6.57%	2.64%
65	bi0171	MYO-LFT	171tr3_A	4.01%	5.25%	3.25%	1.36%	0.59%	1.71%	1.63%	1.47%	1.21%	0.59%	5.25%	1.63%
73	bi0171	MYO-LFT	171tr1_A	8.01%	4.49%	6.51%	1.49%	4.10%	3.17%	3.77%	1.86%	1.75%	1.49%	8.01%	3.77%
86	bi0171	MYO-LFT	171tr1_A	6.08%	1.44%	3.90%	2.25%	1.57%	2.53%	1.09%	1.49%	1.25%	1.09%	6.08%	1.57%
95	bi0171	MYO-LFT	171tr1_A	12.90%	5.69%	2.85%	4.17%	3.73%	2.68%	2.35%	2.10%	4.92%	2.10%	12.90%	3.73%
			Min Inter-lab CV	2.42%	1.44%	1.50%	1.36%	0.59%	1.71%	1.09%	1.47%	0.91%			
			Max Inter-lab CV	12.90%	9.58%	11.61%	4.17%	5.08%	5.82%	11.70%	6.43%	15.73%			
			Median Inter-lab CV	6.40%	4.87%	3.58%	2.77%	2.11%	2.66%	2.87%	1.98%	3.33%			
19	bi0173	APR-AGL	173tr2_A	11.24%	29.51%	11.18%	21.77%	10.22%	7.65%	2.76%	12.09%	7.89%	2.76%	29.51%	11.18%
52	bi0173	APR-AGL	173tr3_A	10.53%	10.49%	7.41%	7.88%	2.68%	2.75%	5.17%	8.46%	5.50%	2.68%	10.53%	7.41%
54	bi0173	APR-AGL	173tr2_A	6.46%	12.20%	8.84%	5.60%	4.22%	2.81%	4.01%	6.04%	7.47%	2.81%	12.20%	6.04%
56	bi0173	APR-AGL	173tr2_A	11.74%	6.07%	6.72%	9.70%	5.03%	0.77%	1.65%	3.44%	3.17%	0.77%	11.74%	5.03%
65	bi0173	APR-AGL	173tr3_A	12.72%	5.36%	4.20%	3.95%	3.14%	3.01%	3.51%	2.19%	4.20%	2.19%	12.72%	3.95%
73	bi0173	APR-AGL	173tr3_A	10.75%	21.96%	2.91%	19.92%	10.24%	16.58%	8.29%	9.99%	3.61%	2.91%	21.96%	10.24%
86	bi0173	APR-AGL	173tr2_A	5.98%	7.82%	3.79%	3.90%	3.68%	1.70%	4.44%	4.97%	1.93%	1.70%	7.82%	3.90%
95	bi0173	APR-AGL	173tr2_A	11.24%	5.35%	7.55%	6.18%	7.40%	4.37%	5.81%	2.62%	1.91%	1.91%	11.24%	5.81%
			Min Inter-lab CV	5.98%	5.35%	2.91%	3.90%	2.68%	0.77%	1.65%	2.19%	1.91%			
			Max Inter-lab CV	12.72%	29.51%	11.18%	21.77%	10.24%	16.58%	8.29%	12.09%	7.89%			
			Median Inter-lab CV	10.99%	9.16%	7.07%	7.03%	4.62%	2.91%	4.23%	5.51%	3.91%			

Coefficient of variation, Study I															
site	peptide	pro-pept	transition	B (1.0)	C (2.92)	D (8.55)	E (25)	F (46)	G (83)	H (151)	I (275)	J (500)	Min Intra-lab CV	Max Intra-lab CV	Median Intra-lab CV
19	bi0202	CRP-GYS	202tr3_A	7.69%	11.40%	7.82%	6.11%	7.11%	6.08%	4.94%	7.24%	7.26%	4.94%	11.40%	7.24%
52	bi0202	CRP-GYS	202tr3_A	2.54%	5.05%	2.70%	1.44%	0.96%	1.43%	1.06%	1.66%	0.70%	0.70%	5.05%	1.44%
54	bi0202	CRP-GYS	202tr3_A	13.28%	4.17%	7.41%	1.58%	5.29%	6.42%	3.65%	3.17%	4.03%	1.58%	13.28%	4.17%
56	bi0202	CRP-GYS	202tr3_A	3.89%	15.81%	8.38%	4.51%	5.41%	3.24%	2.59%	1.92%	1.56%	1.56%	15.81%	3.89%
65	bi0202	CRP-GYS	202tr2_A	5.29%	5.77%	3.34%	4.40%	2.47%	2.45%	1.50%	1.05%	2.18%	1.05%	5.77%	2.47%
73	bi0202	CRP-GYS	202tr3_A	16.06%	10.35%	10.23%	8.78%	23.34%	8.65%	14.73%	5.49%	4.57%	4.57%	23.34%	10.23%
86	bi0202	CRP-GYS	202tr3_A	6.24%	3.68%	1.58%	2.34%	2.22%	1.52%	1.57%	0.61%	1.83%	0.61%	6.24%	1.83%
95	bi0202	CRP-GYS	202tr3_A	12.53%	3.81%	4.91%	3.51%	3.46%	1.90%	2.30%	2.29%	1.07%	1.07%	12.53%	3.46%
			Min Inter-lab CV	2.54%	3.68%	1.58%	1.44%	0.96%	1.43%	1.06%	0.61%	0.70%			
			Max Inter-lab CV	16.06%	15.81%	10.23%	8.78%	23.34%	8.65%	14.73%	7.24%	7.26%			
			Median Inter-lab CV	6.97%	5.41%	6.16%	3.96%	4.37%	2.84%	2.45%	2.10%	2.00%			
19	bi0231	CRP-ESD	231tr2_A	5.85%	6.22%	3.82%	7.10%	3.42%	2.47%	3.51%	12.52%	8.43%	2.47%	12.52%	5.85%
52	bi0231	CRP-ESD	231tr3_A	4.60%	5.00%	11.76%	5.45%	2.21%	7.14%	5.27%	2.79%	4.05%	2.21%	11.76%	5.00%
54	bi0231	CRP-ESD	231tr1_A	11.12%	5.66%	2.79%	7.84%	2.49%	1.87%	2.56%	1.78%	2.97%	1.78%	11.12%	2.79%
56	bi0231	CRP-ESD	231tr3_A	15.34%	9.39%	13.67%	10.42%	5.56%	10.82%	8.33%	8.63%	12.92%	5.56%	15.34%	10.42%
65	bi0231	CRP-ESD	231tr2_A	2.24%	5.07%	1.74%	3.33%	5.56%	1.92%	2.72%	0.29%	1.01%	0.29%	5.56%	2.24%
73	bi0231	CRP-ESD	231tr2_A	13.66%	13.07%	2.23%	3.63%	3.47%	4.43%	6.01%	3.73%	5.21%	2.23%	13.66%	4.43%
86	bi0231	CRP-ESD	231tr1_A	4.66%	5.14%	0.76%	2.75%	2.61%	1.22%	1.85%	5.06%	4.12%	0.76%	5.14%	2.75%
95	bi0231	CRP-ESD	231tr1_A	7.35%	7.50%	6.76%	5.60%	3.55%	4.79%	1.42%	3.06%	4.67%	1.42%	7.50%	4.79%
			Min Inter-lab CV	2.24%	5.00%	0.76%	2.75%	2.21%	1.22%	1.42%	0.29%	1.01%			
			Max Inter-lab CV	15.34%	13.07%	13.67%	10.42%	5.56%	10.82%	8.33%	12.52%	12.92%			
			Median Inter-lab CV	6.60%	5.94%	3.30%	5.52%	3.45%	3.45%	3.12%	3.40%	4.39%			

Supplementary Table 2B: Summary of inter-lab and intra-lab CVs for all peptides calculated across the concentration range of spiked analyte - Study II.
(color code: CV's : 0-10%, CV's : 10-20%, CV's : 20-30%, and CV's ≥ 30%)

				Coefficient of variation, Study II											
site	peptide	pro-pept	transition	B (1.0)	C (2.92)	D (8.55)	E (25)	F (46)	G (83)	H (151)	I (275)	J (500)	Min Intra-lab CV	Max Intra-lab CV	Median Intra-lab CV
19	bi0037	PSA-LSE	37tr3_A	13.38%	9.72%	4.81%	3.99%	2.34%	6.60%	2.45%	1.17%	4.58%	1.17%	13.38%	4.58%
52	bi0037	PSA-LSE	37tr3_A	4.83%	4.61%	1.85%	2.07%	1.44%	2.01%	2.30%	1.82%	1.00%	1.00%	4.83%	2.01%
54	bi0037	PSA-LSE	37tr3_A	20.66%	11.49%	3.16%	5.85%	3.14%	2.98%	5.70%	1.28%	3.48%	1.28%	20.66%	3.48%
56	bi0037	PSA-LSE	37tr3_A	3.49%	2.14%	1.99%	2.04%	0.67%	2.17%	1.29%	5.18%	14.52%	0.67%	14.52%	2.14%
65	bi0037	PSA-LSE	37tr2_A	20.98%	9.90%	12.21%	9.58%	6.96%	7.81%	5.12%	4.44%	1.89%	1.89%	20.98%	7.81%
73	bi0037	PSA-LSE	37tr3_A	5.29%	4.62%	7.80%	4.48%	5.35%	5.38%	1.44%	1.37%	1.59%	1.37%	7.80%	4.62%
86	bi0037	PSA-LSE	37tr3_A	3.44%	4.00%	2.76%	4.37%	4.96%	1.12%	3.74%	4.30%	0.59%	0.59%	4.96%	3.74%
95	bi0037	PSA-LSE	37tr3_A	3.65%	6.05%	5.21%	3.16%	3.79%	1.17%	2.25%	2.42%	2.96%	1.17%	6.05%	3.16%
			Min Inter-lab CV	3.44%	2.14%	1.85%	2.04%	0.67%	1.12%	1.29%	1.17%	0.59%			
			Max Inter-lab CV	20.66%	11.49%	12.21%	9.58%	6.96%	7.81%	5.70%	5.18%	14.52%			
			Median Inter-lab CV	5.06%	5.33%	3.98%	4.18%	3.46%	2.57%	2.38%	2.12%	2.42%			
19	bi0161	PSA-IVG	161tr2_A	13.34%	3.94%	8.53%	3.91%	5.18%	5.91%	2.29%	5.56%	2.64%	2.29%	13.34%	5.18%
52	bi0161	PSA-IVG	161tr2_A	5.60%	5.52%	1.73%	5.30%	3.13%	1.52%	2.08%	1.38%	1.87%	1.38%	5.60%	2.08%
54	bi0161	PSA-IVG	161tr1_A	36.67%	45.48%	29.69%	11.18%	6.81%	3.54%	11.15%	17.33%	4.92%	3.54%	45.48%	11.18%
56	bi0161	PSA-IVG	161tr2_A	3.78%	5.52%	2.44%	3.01%	0.70%	2.87%	4.12%	17.24%	2.52%	0.70%	17.24%	3.01%
65	bi0161	PSA-IVG	161tr1_A	46.83%	46.44%	10.04%	17.55%	18.46%	6.31%	10.39%	4.73%	3.94%	3.94%	46.83%	10.39%
73	bi0161	PSA-IVG	161tr2_A	5.68%	2.49%	1.22%	4.11%	5.87%	5.46%	2.60%	3.71%	1.80%	1.22%	5.87%	3.71%
86	bi0161	PSA-IVG	161tr2_A	14.93%	8.24%	10.80%	10.35%	6.97%	2.78%	8.84%	8.32%	4.99%	2.78%	14.93%	8.32%
95	bi0161	PSA-IVG	161tr2_A	7.97%	4.95%	1.45%	0.88%	4.23%	4.39%	1.08%	0.39%	1.98%	0.39%	7.97%	1.98%
			Min Inter-lab CV	3.78%	2.49%	1.22%	0.88%	0.70%	1.52%	1.08%	0.39%	1.80%			
			Max Inter-lab CV	46.83%	46.44%	29.69%	17.55%	18.46%	6.31%	11.15%	17.33%	4.99%			
			Median Inter-lab CV	10.65%	5.52%	5.48%	4.70%	5.53%	3.96%	3.36%	5.15%	2.58%			
19	bi0166	HRP-SSD	166tr2_A	26.72%	19.19%	15.56%	3.28%	4.30%	3.24%	11.51%	2.30%	7.25%	2.30%	26.72%	7.25%
52	bi0166	HRP-SSD	166tr3_A	48.17%	6.05%	12.21%	7.66%	5.03%	6.91%	3.43%	3.70%	5.61%	3.43%	48.17%	6.05%
54	bi0166	HRP-SSD	166tr3_A	20.05%	7.53%	8.33%	8.25%	3.73%	3.41%	7.30%	0.41%	2.58%	0.41%	20.05%	7.30%
56	bi0166	HRP-SSD	166tr2_A	5.37%	3.10%	7.79%	5.48%	2.20%	1.02%	10.65%	17.02%	5.52%	1.02%	17.02%	5.48%
65	bi0166	HRP-SSD	166tr3_A	17.98%	4.57%	11.10%	5.21%	18.48%	7.01%	2.87%	1.21%	2.90%	1.21%	18.48%	5.21%
73	bi0166	HRP-SSD	166tr2_A	20.75%	2.25%	3.81%	2.10%	13.98%	1.81%	8.27%	4.92%	6.29%	1.81%	20.75%	4.92%
86	bi0166	HRP-SSD	166tr3_A	16.36%	4.68%	5.53%	7.01%	2.10%	10.20%	1.65%	1.19%	2.85%	1.19%	16.36%	4.68%
95	bi0166	HRP-SSD	166tr3_A	21.78%	5.54%	4.96%	4.39%	10.51%	2.60%	3.45%	2.21%	4.65%	2.21%	21.78%	4.65%
			Min Inter-lab CV	5.37%	2.25%	3.81%	2.10%	2.10%	1.02%	1.65%	0.41%	2.58%			
			Max Inter-lab CV	48.17%	19.19%	15.56%	8.25%	18.48%	10.20%	11.51%	17.02%	7.25%			
			Median Inter-lab CV	13.36%	5.52%	5.35%	4.43%	4.63%	3.33%	3.39%	3.06%	2.88%			
19	bi0167	LEP-IND	167tr2_A	26.16%	36.87%	12.58%	8.17%	17.89%	25.64%	2.85%	1.96%	4.08%	1.96%	36.87%	12.58%
52	bi0167	LEP-IND													
54	bi0167	LEP-IND	167tr2_A	4.23%	16.28%	8.44%	2.58%	2.07%	1.22%	0.67%	1.51%	3.60%	0.67%	16.28%	2.58%
56	bi0167	LEP-IND	167tr1_A	10.14%		24.26%	21.28%	1.36%	8.65%	26.19%	22.00%	21.14%	1.36%	26.19%	21.21%
65	bi0167	LEP-IND	167tr1_A	47.78%	29.54%	18.52%	4.60%	17.77%	15.26%	5.66%	0.91%	6.13%	0.91%	47.78%	15.26%
73	bi0167	LEP-IND	167tr1_A	25.33%	99.94%	14.48%	21.96%	15.16%	7.37%	4.58%	7.38%	4.41%	4.41%	99.94%	14.48%
86	bi0167	LEP-IND	167tr1_A	47.93%	34.37%	100.09%	69.80%	21.44%	34.71%	77.94%	84.70%	102.31%	21.44%	102.31%	69.80%
95	bi0167	LEP-IND	167tr2_A	6.07%	23.53%	9.70%	7.64%	2.62%	4.74%	1.86%	4.24%	2.95%	1.86%	23.53%	4.74%
			Min Inter-lab CV	4.23%	16.28%	8.44%	2.58%	1.36%	1.22%	0.67%	0.91%	2.95%			
			Max Inter-lab CV	47.93%	99.94%	100.09%	69.80%	21.44%	34.71%	77.94%	84.70%	102.31%			
			Median Inter-lab CV	25.33%	31.96%	14.48%	8.17%	15.16%	8.65%	4.58%	4.24%	4.41%			

				Coefficient of variation, Study II											
site	peptide	pro-pept	transition	B (1.0)	C (2.92)	D (8.55)	E (25)	F (46)	G (83)	H (151)	I (275)	J (500)	Min Intra-lab CV	Max Intra-lab CV	Median Intra-lab CV
19	bi0169	MBP-HGF	169tr3_A	16.60%	9.96%	7.14%	6.61%	6.91%	8.08%	3.73%	3.47%	6.43%	3.47%	16.60%	6.91%
52	bi0169	MBP-HGF	169tr3_A	7.76%	4.00%	4.14%	1.60%	1.22%	0.82%	1.49%	0.79%	0.82%	0.79%	7.76%	1.49%
54	bi0169	MBP-HGF	169tr3_A	15.54%	10.49%	3.83%	4.64%	5.70%	3.31%	4.86%	3.28%	4.82%	3.28%	15.54%	4.82%
56	bi0169	MBP-HGF	169tr3_A	4.12%	44.93%	7.20%	4.53%	3.59%	3.77%	9.94%	23.48%	11.65%	3.59%	44.93%	7.20%
65	bi0169	MBP-HGF	169tr3_A	1.38%	3.13%	5.45%	4.47%	2.76%	3.60%	2.27%	3.57%	1.54%	1.38%	5.45%	3.13%
73	bi0169	MBP-HGF	169tr3_A	7.03%	8.63%	20.87%	7.78%	9.50%	6.63%	3.72%	5.94%	8.12%	3.72%	20.87%	7.78%
86	bi0169	MBP-HGF	169tr3_A	33.51%	29.00%	52.51%	7.09%	1.11%	66.73%	58.41%	96.53%	47.45%	1.11%	96.53%	47.45%
95	bi0169	MBP-HGF	169tr3_A	2.92%	2.45%	2.11%	2.00%	0.77%	2.44%	1.90%	2.12%	2.15%	0.77%	2.92%	2.12%
			Min Inter-lab CV	1.38%	2.45%	2.11%	1.60%	0.77%	0.82%	1.49%	0.79%	0.82%			
			Max Inter-lab CV	33.51%	44.93%	52.51%	7.78%	9.50%	66.73%	58.41%	96.53%	47.45%			
			Median Inter-lab CV	7.39%	9.30%	6.29%	4.59%	3.17%	3.68%	3.72%	3.52%	5.62%			
19	bi0170	MBP-YLA	170tr2_A	11.96%	4.09%	4.80%	5.21%	17.44%	17.73%	3.83%	5.43%	2.78%	2.78%	17.73%	5.21%
52	bi0170	MBP-YLA	170tr2_A	3.82%	1.41%	3.56%	1.06%	1.51%	2.14%	1.22%	2.43%	0.69%	0.69%	3.82%	1.51%
54	bi0170	MBP-YLA	170tr2_A	3.94%	3.92%	3.48%	6.76%	5.95%	5.52%	2.28%	7.24%	15.08%	2.28%	15.08%	5.52%
56	bi0170	MBP-YLA	170tr2_A	17.32%	4.06%	3.45%	4.61%	2.88%	4.11%	35.99%	11.50%	3.58%	2.88%	35.99%	4.11%
65	bi0170	MBP-YLA	170tr3_A	19.83%	17.27%	6.17%	8.23%	12.98%	5.67%	8.03%	7.57%	6.21%	5.67%	19.83%	8.03%
73	bi0170	MBP-YLA	170tr2_A	33.13%	15.46%	14.63%	14.11%	10.28%	11.74%	6.50%	2.49%	16.42%	2.49%	33.13%	14.11%
86	bi0170	MBP-YLA	170tr2_A	9.85%	23.53%	22.66%	37.88%	6.49%	64.39%	26.85%	34.55%	42.19%	6.49%	64.39%	26.85%
95	bi0170	MBP-YLA	170tr2_A	5.95%	3.56%	4.11%	2.15%	1.26%	2.47%	0.73%	1.65%	1.69%	0.73%	5.95%	2.15%
			Min Inter-lab CV	3.82%	1.41%	3.45%	1.06%	1.26%	2.14%	0.73%	1.65%	0.69%			
			Max Inter-lab CV	33.13%	23.53%	22.66%	37.88%	17.44%	64.39%	35.99%	34.55%	42.19%			
			Median Inter-lab CV	10.90%	4.07%	4.45%	5.98%	6.22%	5.60%	5.16%	6.33%	4.89%			
19	bi0171	MYO-LFT	171tr1_A	6.34%	12.83%	7.67%	7.42%	13.05%	3.65%	1.50%	2.72%	1.79%	1.50%	13.05%	6.34%
52	bi0171	MYO-LFT	171tr1_A	9.42%	4.13%	4.09%	1.18%	0.49%	2.19%	1.34%	2.00%	1.86%	0.49%	9.42%	2.00%
54	bi0171	MYO-LFT	171tr2_A	12.32%	1.88%	2.98%	2.73%	3.85%	4.24%	3.89%	7.61%	7.53%	1.88%	12.32%	3.89%
56	bi0171	MYO-LFT	171tr1_A	6.26%	7.84%	2.57%	2.47%	3.81%	7.65%	4.83%	7.15%	84.80%	2.47%	84.80%	6.26%
65	bi0171	MYO-LFT	171tr3_A	7.31%	3.09%	3.91%	4.19%	2.85%	2.09%	2.58%	3.85%	1.69%	1.69%	7.31%	3.09%
73	bi0171	MYO-LFT	171tr1_A	8.13%	3.11%	6.66%	2.71%	6.04%	2.34%	3.51%	2.90%	1.66%	1.66%	8.13%	3.11%
86	bi0171	MYO-LFT	171tr1_A	36.20%	43.14%	45.40%	29.52%	10.16%	35.25%	16.99%	37.20%	40.40%	10.16%	45.40%	36.20%
95	bi0171	MYO-LFT	171tr1_A	4.86%	3.51%	6.16%	2.08%	4.21%	3.29%	1.17%	0.22%	2.51%	0.22%	6.16%	3.29%
			Min Inter-lab CV	4.86%	1.88%	2.57%	1.18%	0.49%	2.09%	1.17%	0.22%	1.66%			
			Max Inter-lab CV	36.20%	43.14%	45.40%	29.52%	13.05%	35.25%	16.99%	37.20%	84.80%			
			Median Inter-lab CV	7.72%	3.82%	5.13%	2.72%	4.03%	3.47%	3.05%	3.37%	2.19%			
19	bi0173	APR-AGL	173tr2_A	24.98%	12.57%	7.76%	1.57%	10.81%	5.99%	7.86%	6.60%	7.36%	1.57%	24.98%	7.76%
52	bi0173	APR-AGL	173tr3_A	12.60%	18.18%	4.50%	3.95%	4.86%	8.66%	4.47%	3.72%	2.42%	2.42%	18.18%	4.50%
54	bi0173	APR-AGL	173tr2_A	18.18%	29.65%	15.82%	15.44%	2.84%	2.13%	7.12%	7.59%	4.59%	2.13%	29.65%	7.59%
56	bi0173	APR-AGL	173tr2_A	5.50%	3.80%	8.27%	1.51%	1.14%	2.00%	1.93%	6.46%	0.69%	0.69%	8.27%	2.00%
65	bi0173	APR-AGL	173tr3_A	13.15%	13.58%	13.98%	8.75%	3.22%	3.33%	4.60%	4.05%	3.12%	3.12%	13.98%	4.60%
73	bi0173	APR-AGL	173tr3_A	16.51%	19.49%	3.72%	5.27%	12.58%	6.84%	2.73%	8.95%	5.10%	2.73%	19.49%	6.84%
86	bi0173	APR-AGL	173tr2_A	7.76%	10.11%	4.38%	4.84%	3.34%	2.67%	3.43%	3.11%	4.07%	2.67%	10.11%	4.07%
95	bi0173	APR-AGL	173tr2_A	18.27%	8.15%	10.84%	3.83%	3.28%	6.36%	3.42%	7.55%	5.88%	3.28%	18.27%	6.36%
			Min Inter-lab CV	5.50%	3.80%	3.72%	1.51%	1.14%	2.00%	1.93%	3.11%	0.69%			
			Max Inter-lab CV	24.98%	29.65%	15.82%	15.44%	12.58%	8.66%	7.86%	8.95%	7.36%			
			Median Inter-lab CV	14.83%	13.08%	8.01%	4.39%	3.31%	4.66%	3.95%	6.53%	4.33%			

				Coefficient of variation, Study II											
site	peptide	pro-pept	transition	B (1.0)	C (2.92)	D (8.55)	E (25)	F (46)	G (83)	H (151)	I (275)	J (500)	Min Intra-lab CV	Max Intra-lab CV	Median Intra-lab CV
19	bi0202	CRP-GYS	202tr3_A	27.25%	18.65%	11.26%	5.84%	3.16%	3.96%	5.22%	6.08%	7.46%	**3.16%**	**27.25%**	6.08%
52	bi0202	CRP-GYS	202tr3_A	6.12%	4.26%	2.31%	2.06%	1.94%	1.81%	0.54%	1.16%	2.56%	**0.54%**	**6.12%**	2.06%
54	bi0202	CRP-GYS	202tr3_A	17.40%	15.08%	3.45%	8.13%	6.38%	7.52%	3.95%	5.79%	3.25%	**3.25%**	**17.40%**	6.38%
56	bi0202	CRP-GYS	202tr3_A	5.18%	5.83%	5.31%	3.31%	0.70%	0.89%	0.51%	6.66%	5.21%	**0.51%**	**6.66%**	5.18%
65	bi0202	CRP-GYS	202tr2_A	13.99%	5.58%	7.72%	2.45%	4.69%	3.20%	1.41%	3.53%	2.47%	**1.41%**	**13.99%**	3.53%
73	bi0202	CRP-GYS	202tr3_A	9.44%	4.44%	8.57%	3.69%	9.37%	1.18%	4.86%	4.72%	5.22%	**1.18%**	**9.44%**	4.86%
86	bi0202	CRP-GYS	202tr3_A	4.92%	4.76%	0.97%	1.36%	0.78%	0.89%	1.23%	1.10%	2.43%	**0.78%**	**4.92%**	1.23%
95	bi0202	CRP-GYS	202tr3_A	6.15%	5.53%	1.89%	2.77%	5.05%	2.73%	2.45%	3.43%	2.80%	**1.89%**	**6.15%**	2.80%
			Min Inter-lab CV	**4.92%**	**4.26%**	**0.97%**	**1.36%**	**0.70%**	**0.89%**	**0.51%**	**1.10%**	**2.43%**			
			Max Inter-lab CV	**27.25%**	**18.65%**	**11.26%**	**8.13%**	**9.37%**	**7.52%**	**5.22%**	**6.66%**	**7.46%**			
			Median Inter-lab CV	7.79%	5.55%	4.38%	3.04%	3.93%	2.27%	1.93%	4.13%	3.03%			
19	bi0231	CRP-ESD	231tr2_A	5.88%	11.81%	10.06%	9.38%	3.67%	6.37%	2.40%	4.43%	4.02%	**2.40%**	**11.81%**	5.88%
52	bi0231	CRP-ESD	231tr3_A	10.94%	13.60%	10.82%	5.43%	5.77%	7.68%	1.88%	2.71%	2.07%	**1.88%**	**13.60%**	5.77%
54	bi0231	CRP-ESD	231tr1_A	8.42%	9.12%	5.70%	2.13%	2.92%	2.06%	3.24%	3.06%	1.70%	**1.70%**	**9.12%**	3.06%
56	bi0231	CRP-ESD	231tr3_A	11.37%	12.81%	13.50%	8.43%	6.46%	3.46%	4.77%	17.99%	7.69%	**3.46%**	**17.99%**	8.43%
65	bi0231	CRP-ESD	231tr2_A	9.86%	3.23%	3.19%	3.28%	4.18%	2.68%	3.96%	3.68%	1.31%	**1.31%**	**9.86%**	3.28%
73	bi0231	CRP-ESD	231tr2_A	10.01%	6.58%	8.75%	7.37%	15.72%	4.76%	4.21%	2.30%	5.53%	**2.30%**	**15.72%**	6.58%
86	bi0231	CRP-ESD	231tr1_A	5.67%	16.15%	21.70%	2.09%	6.40%	21.04%	7.16%	4.95%	6.07%	**2.09%**	**21.70%**	6.40%
95	bi0231	CRP-ESD	231tr1_A	7.13%	9.01%	7.07%	6.80%	3.74%	2.76%	198.26%	3.45%	1.00%	**1.00%**	**198.26%**	6.80%
			Min Inter-lab CV	**5.67%**	**3.23%**	**3.19%**	**2.09%**	**2.92%**	**2.06%**	**1.88%**	**2.30%**	**1.00%**			
			Max Inter-lab CV	**11.37%**	**16.15%**	**21.70%**	**9.38%**	**15.72%**	**21.04%**	**198.26%**	**17.99%**	**7.69%**			
			Median Inter-lab CV	9.14%	10.46%	9.40%	6.12%	4.98%	4.11%	4.09%	3.57%	3.04%			

Supplementary Table 2C: Summary of inter-lab and intra-lab CVs for all peptides calculated across the concentration range of spiked analyte - Study III.
(color code: CV's : 0-10%, CV's : 10-20%, CV's : 20-30%, and CV's ≥ 30%)

				Coefficient of variation, Study III											
site	peptide	pro-pept	transition	B (1.0)	C (2.92)	D (8.55)	E (25)	F (46)	G (83)	H (151)	I (275)	J (500)	Min Intra-lab CV	Max Intra-lab CV	Median Intra-lab CV
19	bi0037	PSA-LSE	37tr3_A	64.98%	42.78%	43.82%	32.58%	23.15%	16.82%	27.49%	23.14%	10.34%	10.34%	64.98%	27.49%
52	bi0037	PSA-LSE	37tr3_A	10.27%	6.75%	9.04%	9.03%	8.66%	11.89%	5.54%	8.03%	9.60%	5.54%	11.89%	9.03%
54	bi0037	PSA-LSE	37tr3_A	10.55%	11.87%	7.48%	7.63%	11.44%	9.96%	6.67%	8.80%	4.45%	4.45%	11.87%	8.80%
56	bi0037	PSA-LSE	37tr3_A	8.37%	7.74%	8.16%	8.24%	9.89%	5.22%	7.00%	9.23%	3.87%	3.87%	9.89%	8.16%
65	bi0037	PSA-LSE	37tr2_A	35.98%	8.73%	11.85%	12.34%	7.95%	8.80%	25.47%	6.23%	9.62%	6.23%	35.98%	9.62%
73	bi0037	PSA-LSE	37tr3_A	17.66%	17.23%	9.85%	20.93%	21.54%	6.48%	13.74%	5.32%	7.94%	5.32%	21.54%	13.74%
86	bi0037	PSA-LSE	37tr3_A	19.37%	14.97%	9.91%	6.55%	5.00%	7.41%	13.98%	7.64%	11.19%	5.00%	19.37%	9.91%
95	bi0037	PSA-LSE	37tr3_A	22.84%	7.61%	3.62%	5.37%	7.29%	7.97%	5.92%	10.36%	7.61%	3.62%	22.84%	7.61%
			Min Inter-lab CV	8.37%	6.75%	3.62%	5.37%	5.00%	5.22%	5.54%	5.32%	3.87%			
			Max Inter-lab CV	64.98%	42.78%	43.82%	32.58%	23.15%	16.82%	27.49%	23.14%	11.19%			
			Median Inter-lab CV	18.52%	10.30%	9.44%	8.64%	9.27%	8.38%	10.37%	8.41%	8.77%			
19	bi0161	PSA-IVG	161tr2_A	58.54%	32.88%	15.19%	16.22%	6.42%	14.45%	7.11%	7.65%	8.63%	6.42%	58.54%	14.45%
52	bi0161	PSA-IVG	161tr2_A	10.53%	8.70%	8.62%	9.51%	10.55%	14.15%	7.63%	9.88%	9.99%	7.63%	14.15%	9.88%
54	bi0161	PSA-IVG	161tr1_A	52.46%	57.30%	31.50%	46.65%	40.31%	31.23%	30.67%	21.34%	35.75%	21.34%	57.30%	35.75%
56	bi0161	PSA-IVG	161tr2_A	7.67%	18.12%	24.95%	5.91%	10.67%	12.69%	7.78%	7.92%	4.85%	4.85%	24.95%	7.92%
65	bi0161	PSA-IVG	161tr1_A	98.60%	59.35%	19.24%	35.89%	17.95%	12.12%	54.23%	13.22%	20.27%	12.12%	98.60%	20.27%
73	bi0161	PSA-IVG	161tr2_A	14.06%	16.23%	10.65%	15.64%	23.54%	4.73%	14.40%	9.53%	9.23%	4.73%	23.54%	14.06%
86	bi0161	PSA-IVG	161tr2_A	12.48%	8.34%	9.38%	9.85%	8.98%	6.03%	10.90%	7.17%	8.06%	6.03%	12.48%	8.98%
95	bi0161	PSA-IVG	161tr2_A	9.86%	10.62%	9.27%	9.10%	13.27%	10.74%	13.29%	14.92%	16.11%	9.10%	16.11%	10.74%
			Min Inter-lab CV	7.67%	8.34%	8.62%	5.91%	6.42%	4.73%	7.11%	7.17%	4.85%			
			Max Inter-lab CV	98.60%	59.35%	31.50%	46.65%	40.31%	31.23%	54.23%	21.34%	35.75%			
			Median Inter-lab CV	13.27%	17.18%	12.92%	12.74%	11.97%	12.41%	12.09%	9.70%	9.61%			
19	bi0166	HRP-SSD	166tr2_A	100.72%	41.92%	80.90%	53.90%	53.51%	33.58%	34.32%	34.96%	30.83%	30.83%	100.72%	41.92%
52	bi0166	HRP-SSD	166tr3_A	25.06%	18.27%	15.01%	13.22%	9.39%	14.05%	7.41%	8.91%	14.64%	7.41%	25.06%	14.05%
54	bi0166	HRP-SSD	166tr3_A	36.66%	21.34%	10.65%	10.35%	20.09%	21.90%	23.75%	5.04%	16.65%	5.04%	36.66%	20.09%
56	bi0166	HRP-SSD	166tr2_A	14.12%	16.16%	13.08%	3.17%	10.68%	5.81%	8.98%	13.37%	14.05%	3.17%	16.16%	13.08%
65	bi0166	HRP-SSD	166tr3_A	28.33%	13.61%	8.64%	32.83%	7.01%	8.89%	24.81%	4.11%	4.64%	4.11%	32.83%	8.89%
73	bi0166	HRP-SSD	166tr2_A	50.79%	22.45%	18.37%	19.40%	18.91%	13.13%	12.13%	11.08%	10.24%	10.24%	50.79%	18.37%
86	bi0166	HRP-SSD	166tr3_A	45.83%	31.88%	18.47%	11.58%	18.72%	21.36%	24.36%	32.69%	13.75%	11.58%	45.83%	21.36%
95	bi0166	HRP-SSD	166tr3_A	51.25%	24.91%	11.56%	4.85%	6.20%	6.24%	10.07%	8.39%	8.40%	4.85%	51.25%	8.40%
			Min Inter-lab CV	14.12%	13.61%	8.64%	3.17%	6.20%	5.81%	7.41%	4.11%	4.64%			
			Max Inter-lab CV	100.72%	41.92%	80.90%	53.90%	53.51%	33.58%	34.32%	34.96%	30.83%			
			Median Inter-lab CV	41.24%	21.89%	14.04%	12.40%	14.70%	13.59%	17.94%	10.00%	13.90%			
19	bi0167	LEP-IND	167tr2_A	92.93%	50.41%	308.42%	28.85%	21.96%	19.32%	15.20%	13.76%	17.64%	13.76%	308.42%	21.96%
52	bi0167	LEP-IND													
54	bi0167	LEP-IND	167tr2_A	25.40%	26.27%	8.33%	18.43%	37.72%	4.60%	38.59%	23.85%	32.54%	4.60%	38.59%	25.40%
56	bi0167	LEP-IND	167tr1_A	30.01%	61.88%	41.15%	223.21%	44.08%	54.39%	60.50%	37.40%	35.82%	30.01%	223.21%	44.08%
65	bi0167	LEP-IND	167tr1_A	81.38%	68.79%	54.89%	51.52%	19.37%	38.54%	23.93%	345.62%	64.68%	19.37%	345.62%	54.89%
73	bi0167	LEP-IND	167tr1_A	94.30%	67.51%	217.10%	25.93%	12.33%	18.23%	16.68%	12.14%	14.13%	12.14%	217.10%	18.23%
86	bi0167	LEP-IND	167tr1_A	24.25%	32.21%	16.98%	17.83%	17.83%	35.37%	27.14%	24.34%	21.92%	16.98%	35.37%	24.25%
95	bi0167	LEP-IND	167tr2_A	38.83%	27.10%	15.46%	7.27%	9.26%	7.47%	10.04%	11.70%	14.81%	7.27%	38.83%	11.70%
			Min Inter-lab CV	24.25%	26.27%	8.33%	7.27%	9.26%	4.60%	10.04%	11.70%	14.13%			
			Max Inter-lab CV	94.30%	68.79%	308.42%	223.21%	44.08%	54.39%	60.50%	345.62%	64.68%			
			Median Inter-lab CV	38.83%	50.41%	41.15%	25.93%	19.37%	19.32%	23.93%	23.85%	21.92%			

				Coefficient of variation, Study III											
site	peptide	pro-pept	transition	B (1.0)	C (2.92)	D (8.55)	E (25)	F (46)	G (83)	H (151)	I (275)	J (500)	Min Intra-lab CV	Max Intra-lab CV	Median Intra-lab CV
19	bi0169	MBP-HGF	169tr3_A	72.06%	49.88%	30.55%	23.30%	10.72%	16.94%	9.94%	6.93%	13.69%	6.93%	72.06%	16.94%
52	bi0169	MBP-HGF	169tr3_A	11.45%	6.70%	8.72%	8.17%	7.42%	11.44%	7.34%	6.06%	7.11%	6.06%	11.45%	7.42%
54	bi0169	MBP-HGF	169tr3_A	32.59%	8.46%	23.03%	21.95%	19.03%	24.52%	23.67%	24.26%	9.79%	8.46%	32.59%	23.03%
56	bi0169	MBP-HGF	169tr3_A	28.53%	38.03%	70.21%	32.76%	28.81%	21.52%	30.05%	36.77%	36.76%	21.52%	70.21%	32.76%
65	bi0169	MBP-HGF	169tr3_A	46.31%	21.37%	11.75%	11.67%	8.48%	5.69%	20.98%	15.76%	8.99%	5.69%	46.31%	11.75%
73	bi0169	MBP-HGF	169tr3_A	54.63%	46.76%	79.96%	20.58%	18.99%	8.83%	17.47%	12.09%	5.50%	5.50%	79.96%	18.99%
86	bi0169	MBP-HGF	169tr3_A	24.47%	22.27%	13.09%	12.72%	11.60%	6.77%	26.01%	15.02%	5.01%	5.01%	26.01%	13.09%
95	bi0169	MBP-HGF	169tr3_A	30.52%	13.62%	7.35%	5.26%	5.74%	6.07%	7.39%	8.83%	11.15%	5.26%	30.52%	7.39%
			Min Inter-lab CV	11.45%	6.70%	7.35%	5.26%	5.74%	5.69%	7.34%	6.06%	5.01%			
			Max Inter-lab CV	72.06%	49.88%	79.96%	32.76%	28.81%	24.52%	30.05%	36.77%	36.76%			
			Median Inter-lab CV	31.55%	21.82%	18.06%	16.65%	11.16%	10.13%	19.22%	13.55%	9.39%			
19	bi0170	MBP-YLA	170tr2_A												
52	bi0170	MBP-YLA	170tr2_A												
54	bi0170	MBP-YLA	170tr2_A												
56	bi0170	MBP-YLA	170tr2_A												
65	bi0170	MBP-YLA	170tr3_A												
73	bi0170	MBP-YLA	170tr2_A												
86	bi0170	MBP-YLA	170tr2_A												
95	bi0170	MBP-YLA	170tr2_A												
			Min Inter-lab CV												
			Max Inter-lab CV												
			Median Inter-lab CV												
19	bi0171	MYO-LFT	171tr1_A	55.68%	39.21%	15.00%	24.76%	21.64%	22.15%	15.14%	20.28%	17.59%	15.00%	55.68%	21.64%
52	bi0171	MYO-LFT	171tr1_A	11.83%	7.76%	5.66%	9.33%	9.18%	14.66%	5.53%	9.89%	8.78%	5.53%	14.66%	9.18%
54	bi0171	MYO-LFT	171tr2_A	24.24%	12.80%	6.63%	14.15%	12.23%	15.73%	7.71%	16.80%	10.34%	6.63%	24.24%	12.80%
56	bi0171	MYO-LFT	171tr1_A	15.13%	73.33%	24.23%	13.57%	12.91%	19.08%	46.40%	14.56%	13.44%	12.91%	73.33%	15.13%
65	bi0171	MYO-LFT	171tr3_A	23.77%	26.40%	30.55%	16.20%	11.94%	28.12%	22.90%	21.69%	23.13%	11.94%	30.55%	23.13%
73	bi0171	MYO-LFT	171tr1_A	23.50%	19.73%	28.28%	11.92%	47.23%	13.41%	15.91%	18.40%	36.46%	11.92%	47.23%	19.73%
86	bi0171	MYO-LFT	171tr1_A	16.12%	31.71%	6.78%	11.47%	8.92%	18.84%	23.81%	15.74%	14.75%	6.78%	31.71%	15.74%
95	bi0171	MYO-LFT	171tr1_A	26.90%	10.97%	8.88%	5.82%	10.73%	7.67%	4.89%	9.44%	8.02%	4.89%	26.90%	8.88%
			Min Inter-lab CV	11.83%	7.76%	5.66%	5.82%	8.92%	7.67%	4.89%	9.44%	8.02%			
			Max Inter-lab CV	55.68%	73.33%	30.55%	24.76%	47.23%	28.12%	46.40%	21.69%	36.46%			
			Median Inter-lab CV	23.64%	23.07%	11.94%	12.74%	12.09%	17.28%	15.52%	16.27%	14.10%			
19	bi0173	APR-AGL	173tr2_A	82.36%	57.91%	55.52%	24.31%	45.18%	59.77%	13.92%	19.31%	12.18%	12.18%	82.36%	45.18%
52	bi0173	APR-AGL	173tr3_A	13.53%	18.30%	8.94%	10.41%	10.05%	11.43%	10.91%	9.09%	30.72%	8.94%	30.72%	10.91%
54	bi0173	APR-AGL	173tr2_A	41.26%	61.22%	68.36%	73.13%	73.66%	73.78%	73.77%	5.00%	7.78%	5.00%	73.78%	68.36%
56	bi0173	APR-AGL	173tr2_A	11.93%	7.29%	4.25%	6.95%	6.72%	7.83%	5.18%	11.21%	7.46%	4.25%	11.93%	7.29%
65	bi0173	APR-AGL	173tr3_A	29.64%	6.61%	9.32%	10.60%	11.41%	12.64%	27.01%	6.87%	7.30%	6.61%	29.64%	10.60%
73	bi0173	APR-AGL	173tr3_A	48.31%	30.48%	31.39%	21.07%	22.51%	20.18%	12.04%	12.12%	12.90%	12.04%	48.31%	21.07%
86	bi0173	APR-AGL	173tr2_A	20.02%	8.42%	10.99%	10.09%	13.62%	9.96%	14.92%	15.40%	9.25%	8.42%	20.02%	10.99%
95	bi0173	APR-AGL	173tr2_A	11.03%	9.00%	7.87%	5.64%	7.51%	8.86%	7.17%	11.47%	9.68%	5.64%	11.47%	8.86%
			Min Inter-lab CV	11.03%	6.61%	4.25%	5.64%	6.72%	7.83%	5.18%	5.00%	7.30%			
			Max Inter-lab CV	82.36%	61.22%	68.36%	73.13%	73.66%	73.78%	73.77%	19.31%	30.72%			
			Median Inter-lab CV	24.83%	13.65%	10.15%	10.51%	12.51%	12.04%	12.98%	11.34%	9.46%			

				Coefficient of variation, Study III											
site	peptide	pro-pept	transition	B (1.0)	C (2.92)	D (8.55)	E (25)	F (46)	G (83)	H (151)	I (275)	J (500)	Min Intra-lab CV	Max Intra-lab CV	Median Intra-lab CV
19	bi0202	CRP-GYS	202tr3_A	61.48%	76.15%	61.95%	49.06%	34.61%	28.11%	34.09%	34.98%	33.45%	28.11%	76.15%	34.98%
52	bi0202	CRP-GYS	202tr3_A	9.36%	4.83%	7.48%	7.12%	7.27%	7.29%	6.25%	6.66%	15.98%	4.83%	15.98%	7.27%
54	bi0202	CRP-GYS	202tr3_A	32.06%	32.38%	33.37%	45.15%	36.85%	41.96%	28.04%	10.50%	11.84%	10.50%	45.15%	32.38%
56	bi0202	CRP-GYS	202tr3_A	9.22%	10.49%	6.60%	5.66%	11.18%	5.80%	5.86%	10.71%	4.24%	4.24%	11.18%	6.60%
65	bi0202	CRP-GYS	202tr2_A	23.31%	16.38%	16.91%	19.11%	18.37%	7.67%	31.63%	8.59%	9.46%	7.67%	31.63%	16.91%
73	bi0202	CRP-GYS	202tr3_A	47.63%	36.11%	24.30%	26.94%	35.30%	13.14%	15.59%	12.68%	9.44%	9.44%	47.63%	24.30%
86	bi0202	CRP-GYS	202tr3_A	16.71%	20.61%	13.01%	6.30%	8.89%	9.49%	16.06%	5.88%	14.40%	5.88%	20.61%	13.01%
95	bi0202	CRP-GYS	202tr3_A	17.21%	11.07%	8.38%	7.62%	13.02%	5.35%	11.12%	11.29%	13.65%	5.35%	17.21%	11.12%
			Min Inter-lab CV	9.22%	4.83%	6.60%	5.66%	7.27%	5.35%	5.86%	5.88%	4.24%			
			Max Inter-lab CV	61.48%	76.15%	61.95%	49.06%	36.85%	41.96%	34.09%	34.98%	33.45%			
			Median Inter-lab CV	20.26%	18.49%	14.96%	13.37%	15.69%	8.58%	15.82%	10.60%	12.75%			
19	bi0231	CRP-ESD	231tr2_A	33.51%	25.98%	28.54%	17.13%	10.29%	28.41%	7.18%	9.32%	13.11%	7.18%	33.51%	17.13%
52	bi0231	CRP-ESD	231tr3_A	12.21%	7.70%	7.15%	11.03%	12.17%	10.30%	11.04%	10.98%	11.59%	7.15%	12.21%	11.03%
54	bi0231	CRP-ESD	231tr1_A	9.59%	9.28%	4.86%	5.63%	8.48%	8.71%	8.54%	11.99%	7.43%	4.86%	11.99%	8.54%
56	bi0231	CRP-ESD	231tr3_A	10.70%	19.02%	12.69%	5.36%	15.88%	20.98%	13.28%	11.06%	13.54%	5.36%	20.98%	13.28%
65	bi0231	CRP-ESD	231tr2_A	14.99%	17.06%	6.64%	9.35%	9.46%	6.22%	23.62%	6.30%	10.14%	6.22%	23.62%	9.46%
73	bi0231	CRP-ESD	231tr2_A	31.91%	24.70%	29.78%	14.43%	18.07%	12.08%	13.03%	22.53%	9.81%	9.81%	31.91%	18.07%
86	bi0231	CRP-ESD	231tr1_A	6.99%	16.32%	5.41%	6.79%	4.64%	11.42%	16.53%	17.79%	9.29%	4.64%	17.79%	9.29%
95	bi0231	CRP-ESD	231tr1_A	10.89%	11.39%	8.35%	5.83%	12.51%	8.59%	9.66%	12.65%	12.30%	5.83%	12.65%	10.89%
			Min Inter-lab CV	6.99%	7.70%	4.86%	5.36%	4.64%	6.22%	7.18%	6.30%	7.43%			
			Max Inter-lab CV	33.51%	25.98%	29.78%	17.13%	18.07%	28.41%	23.62%	22.53%	13.54%			
			Median Inter-lab CV	11.55%	16.69%	7.75%	8.07%	11.23%	10.86%	12.04%	11.53%	10.86%			

Supplementary Figure 2: Intra- and inter-lab variation of assay CV (coefficient of variation) across studies I, II and III, for the entire range (1-500 fmol/µl) of spike in concentrations of the (light) analyte in diluted plasma. Protein concentration in µg/mL on x-axis is µg protein equivalent in 1 mL of neat plasma.

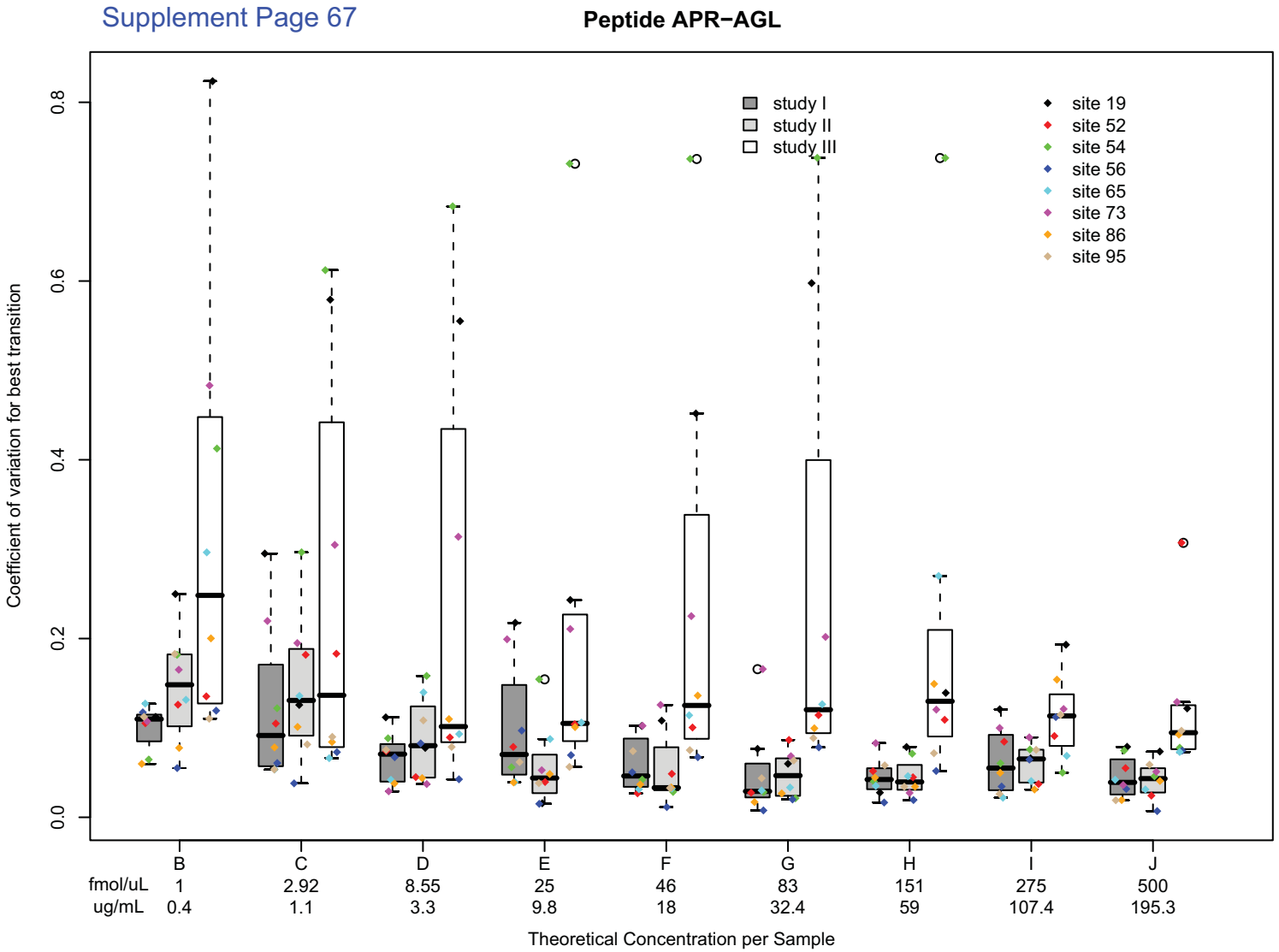
Analyte concentrations are arranged along the x-axis in sample order. At each concentration, three box and whisker plots summarize CV variation for studies I, II and IIIa-c, respectively.

The box plots show inter-lab CV as the median of intra-lab CVs, with the box spanning the interquartile range (IQR), with the whiskers extending to 1.5 * IQR. Values beyond 1.5 * IQR are deemed outliers, and marked by o. Within each box plot, the actual intra-lab CV values for the individual sites are shown with color coded markers. The CV values are calculated based on the single best performing transition (lowest combined CV) across studies I and II. This same transition is also used for study III, in addition to determining LOD and LOQ.

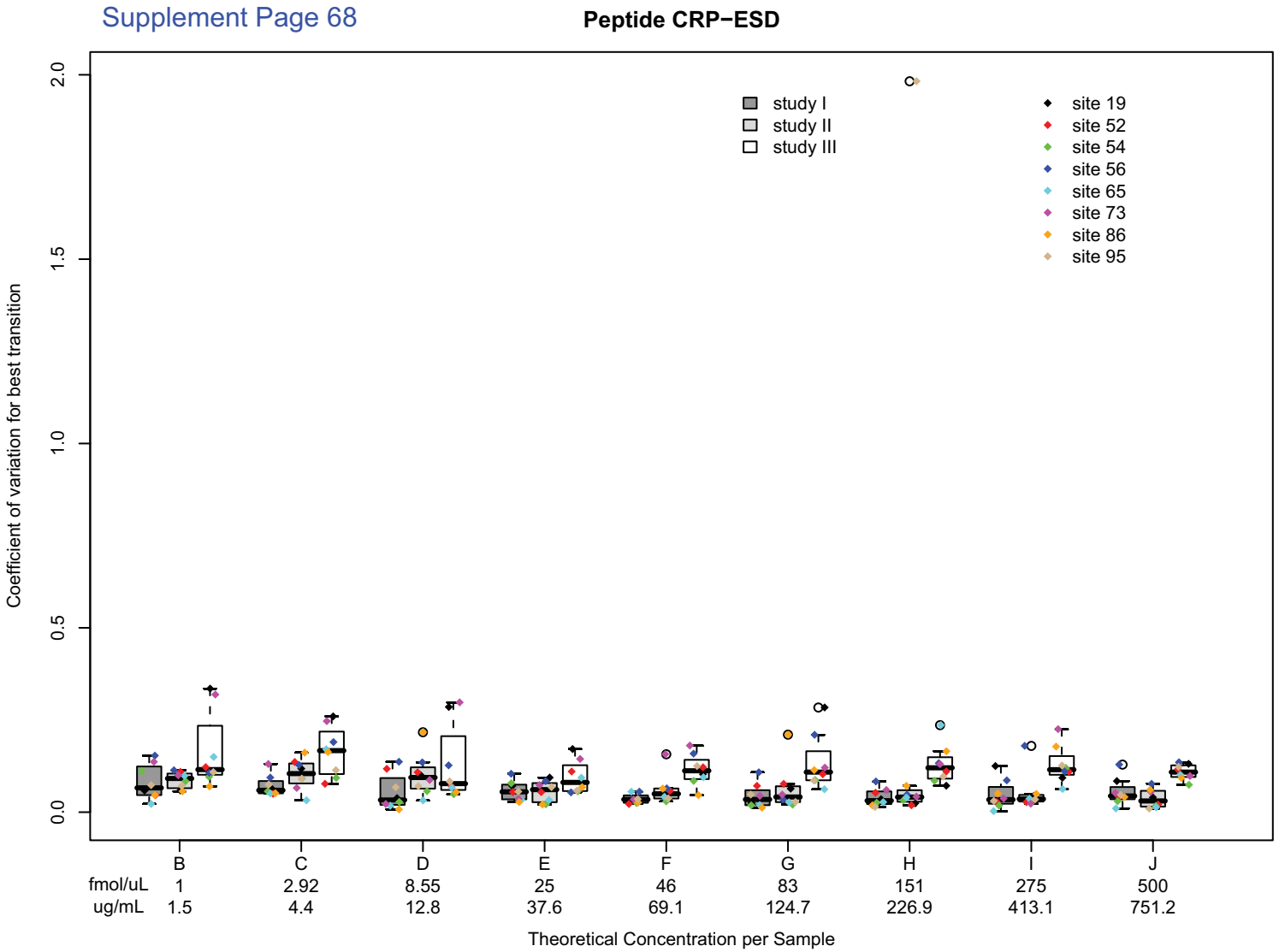
In the plots, the y-axes ranges are different for different peptides to effectively visualize the CV variation for that particular peptide, with some peptides having relatively small inter- and intra-lab variations in comparison.

The placement of color coded site markers (representing the intra-lab variation) is randomly jittered around the vertical axis of the box plot to make it easier to visualize all the sites, even when the CV values are relatively close to each other.

Peptide LEP-IND for site 52 had missing data for the blank runs, and hence the LOD/LOQ could not be calculated, and the best transition is indeterminate, as such, site 52 is missing for LEP-IND. Peptide MBP-YLA was not reliably detected in Study III, and is therefore not shown in Supplementary Figure 2 [in Supplementary Figure 3, the poor quantitation of this peptide is clearly brought out in the MBP-YLA plot].



Nature Biotechnology: doi:10.1038/nbt.1546



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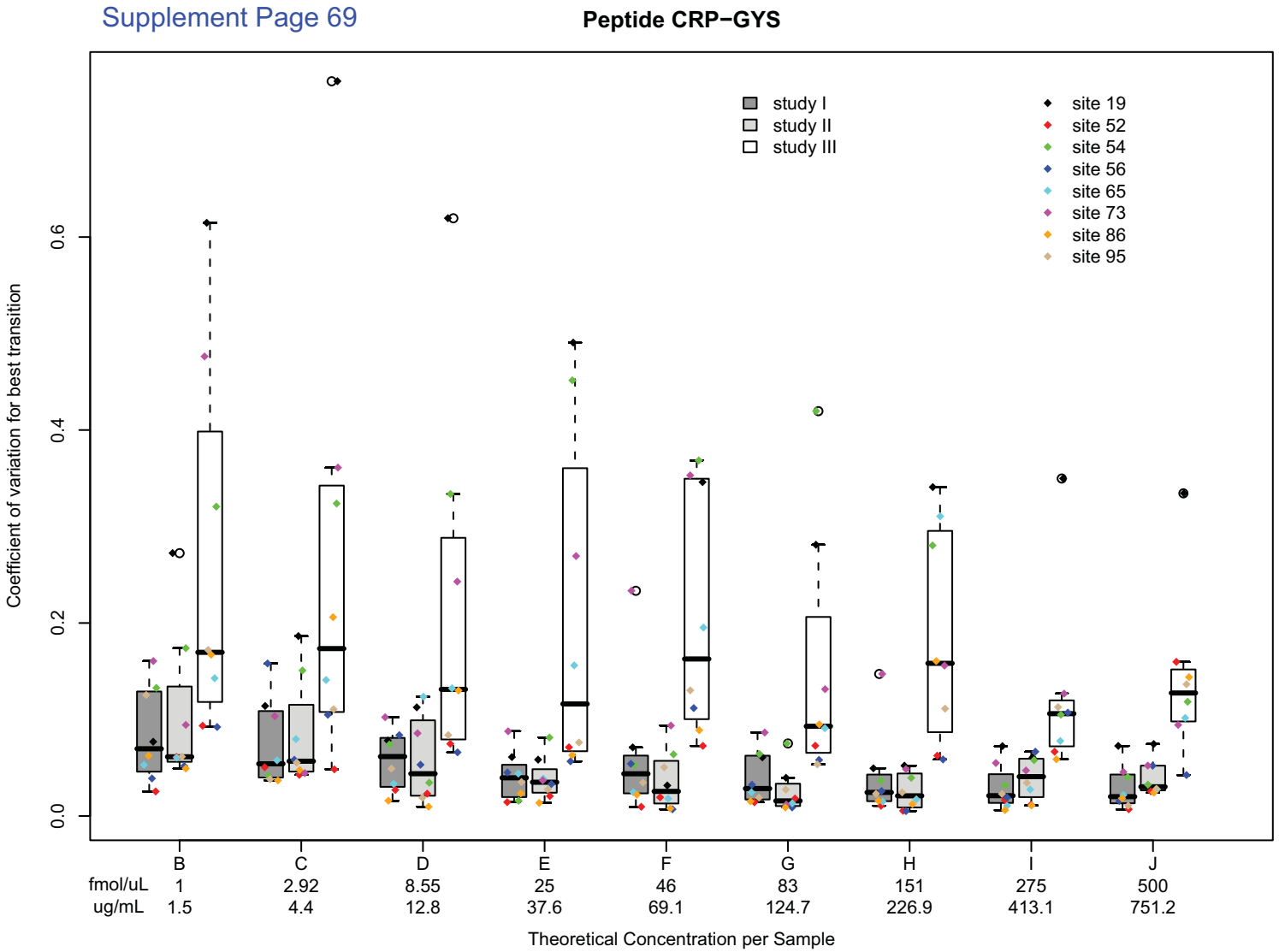

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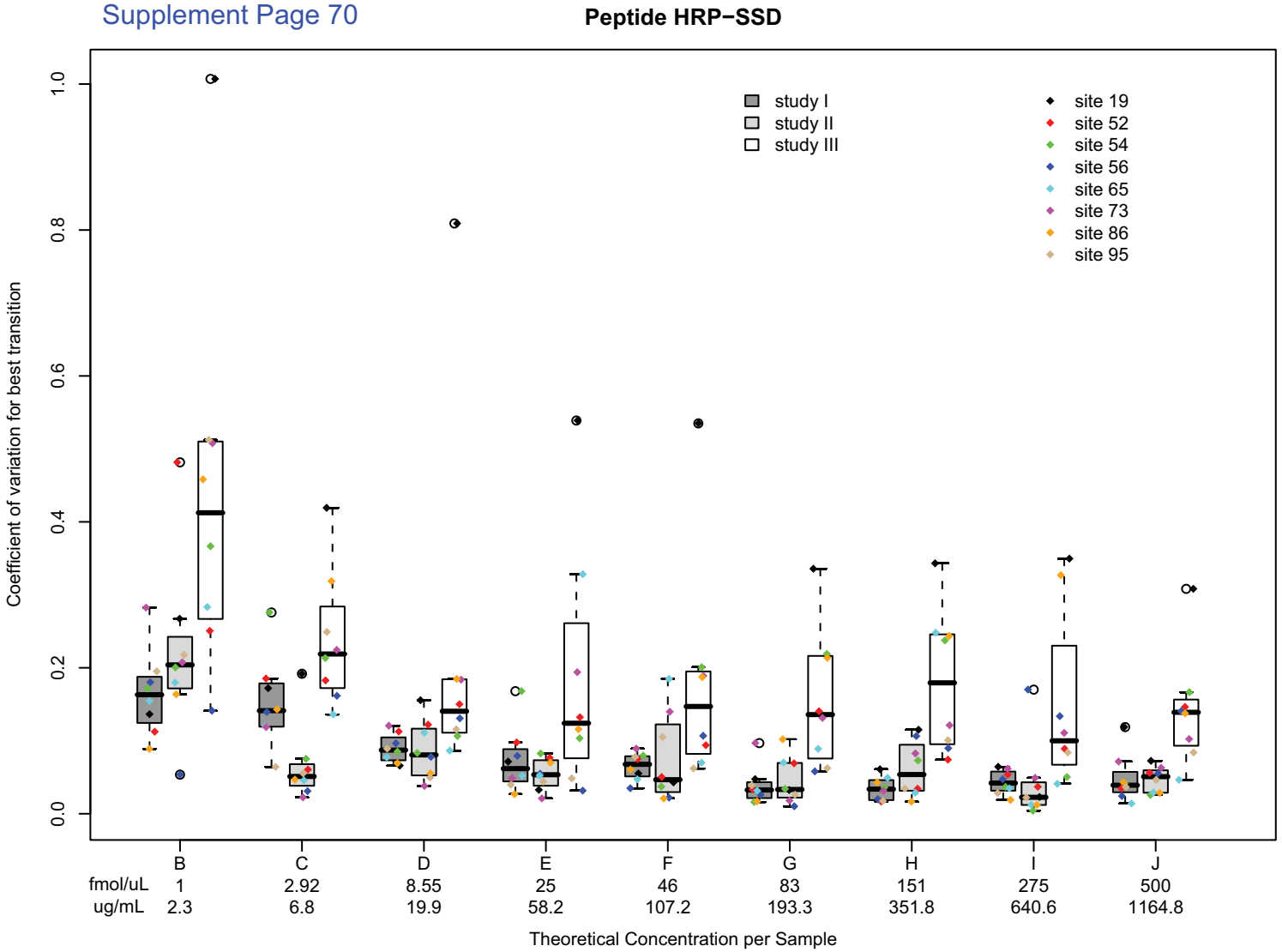

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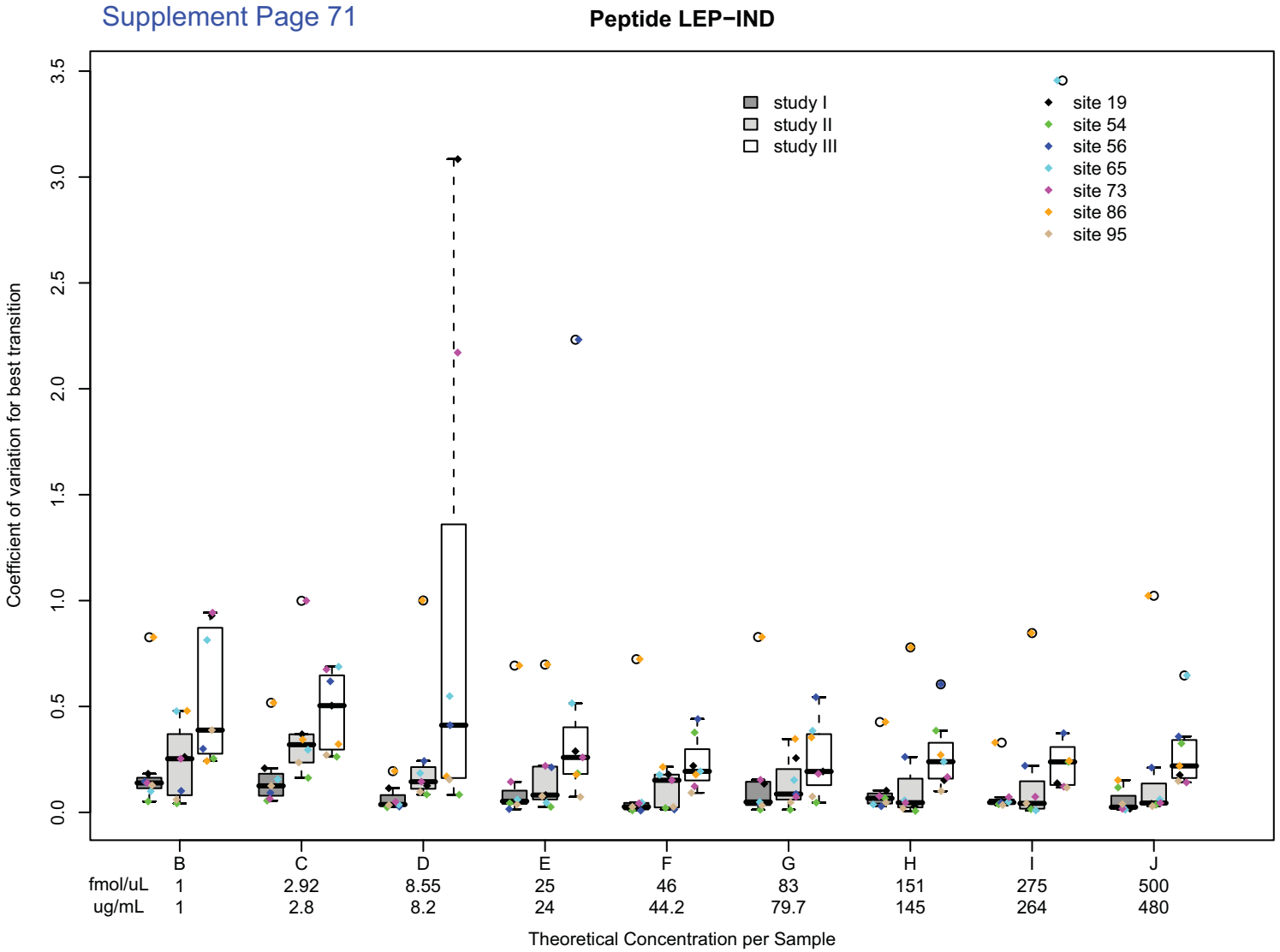

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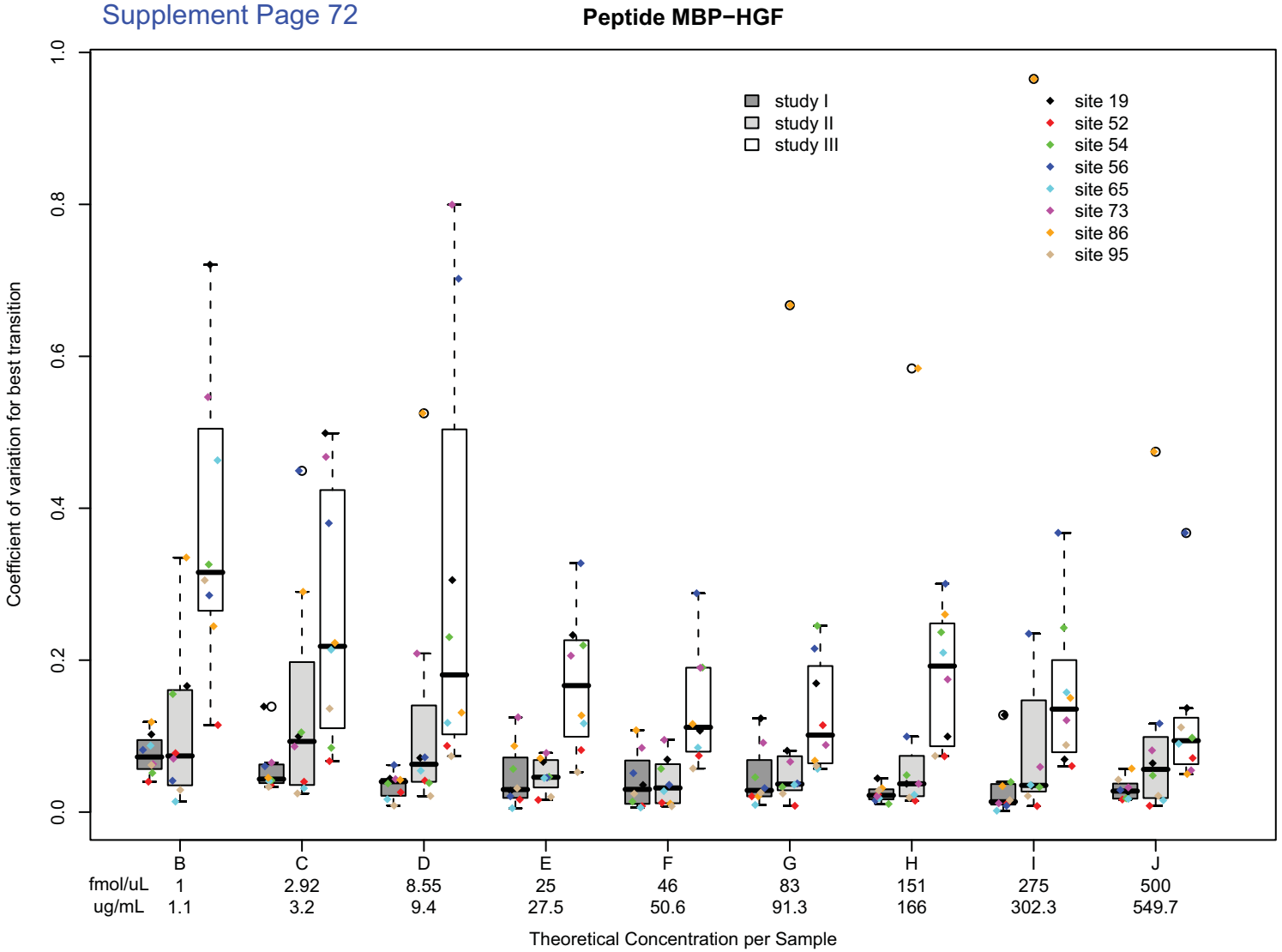

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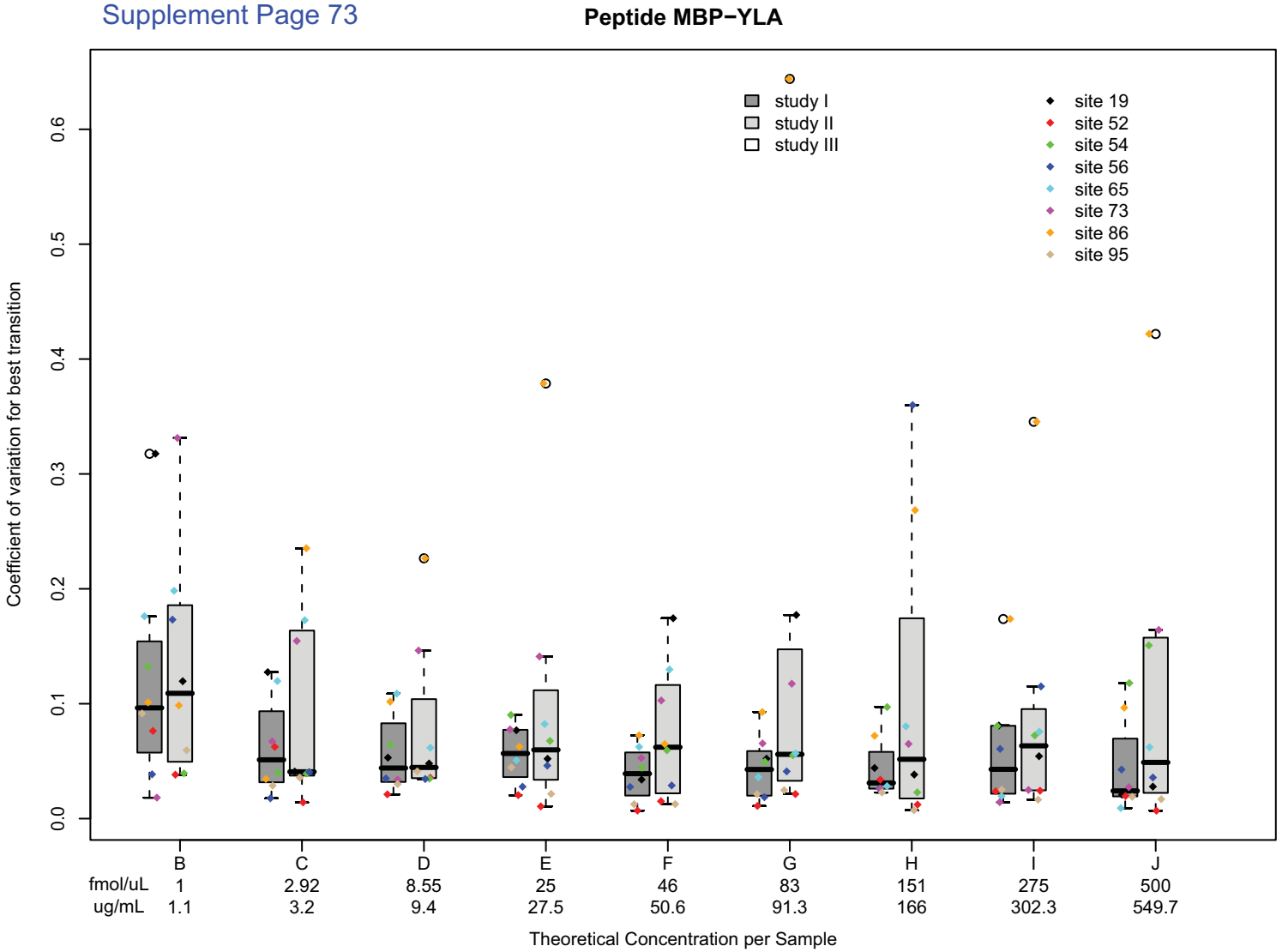

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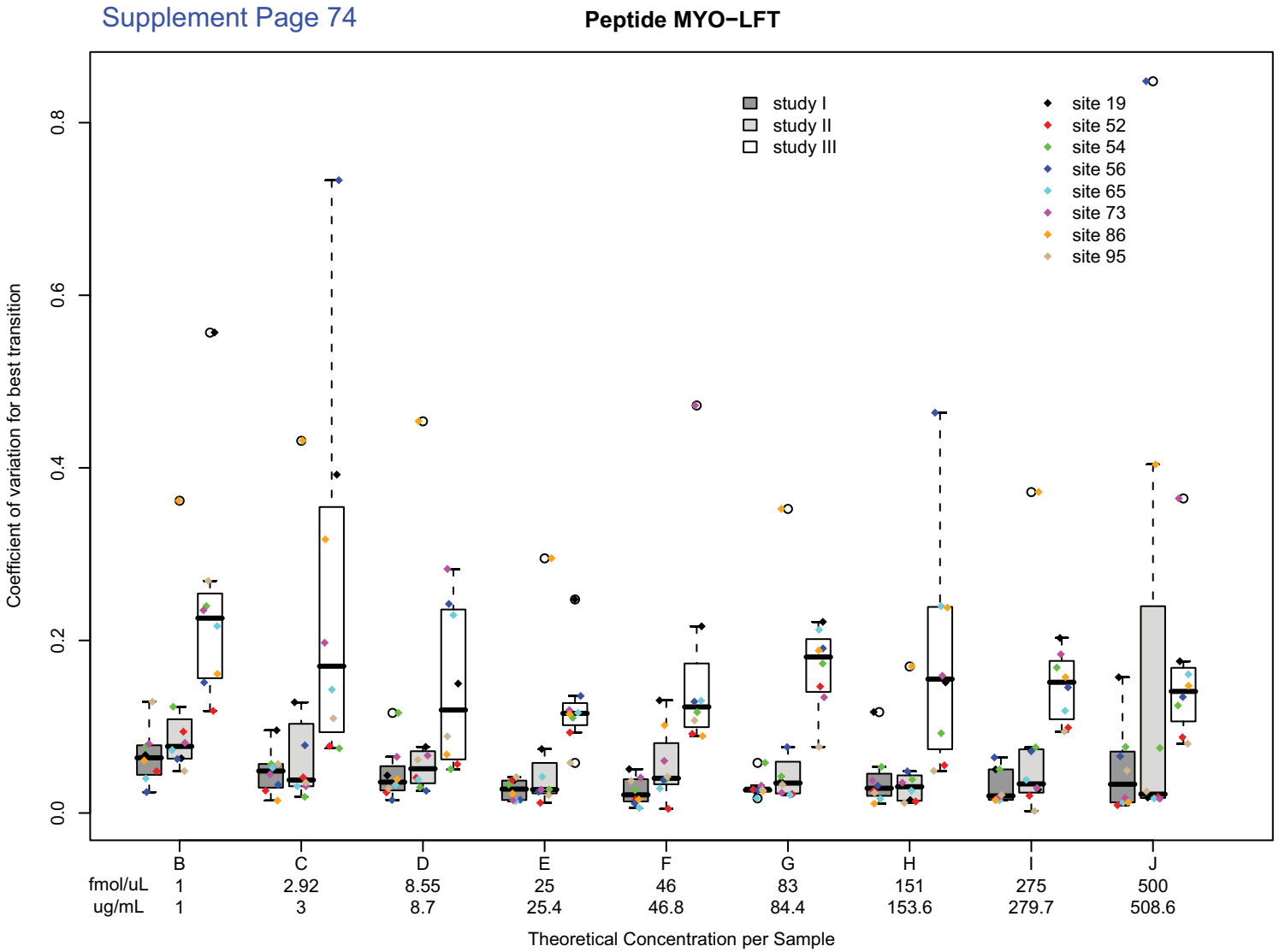

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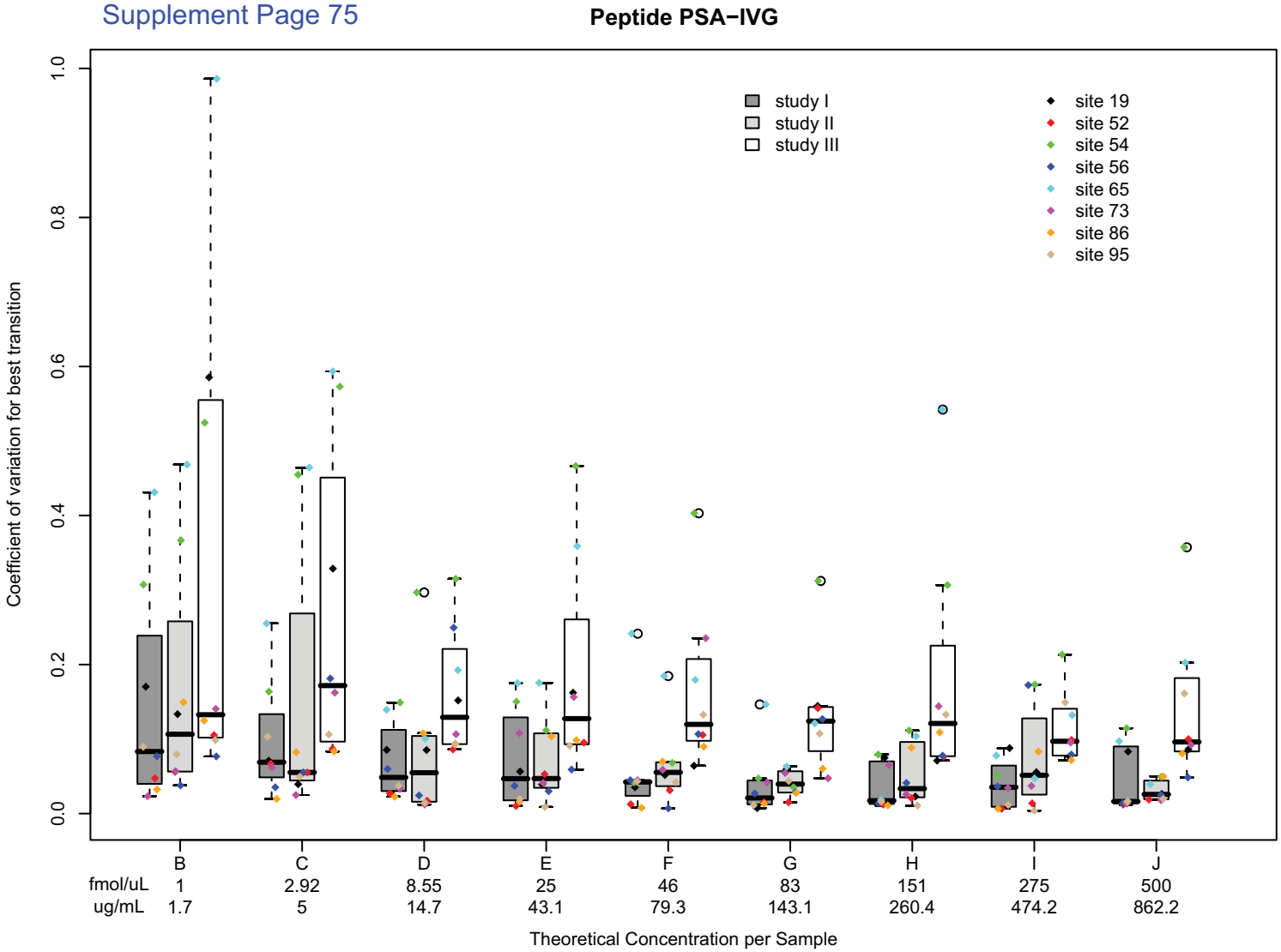

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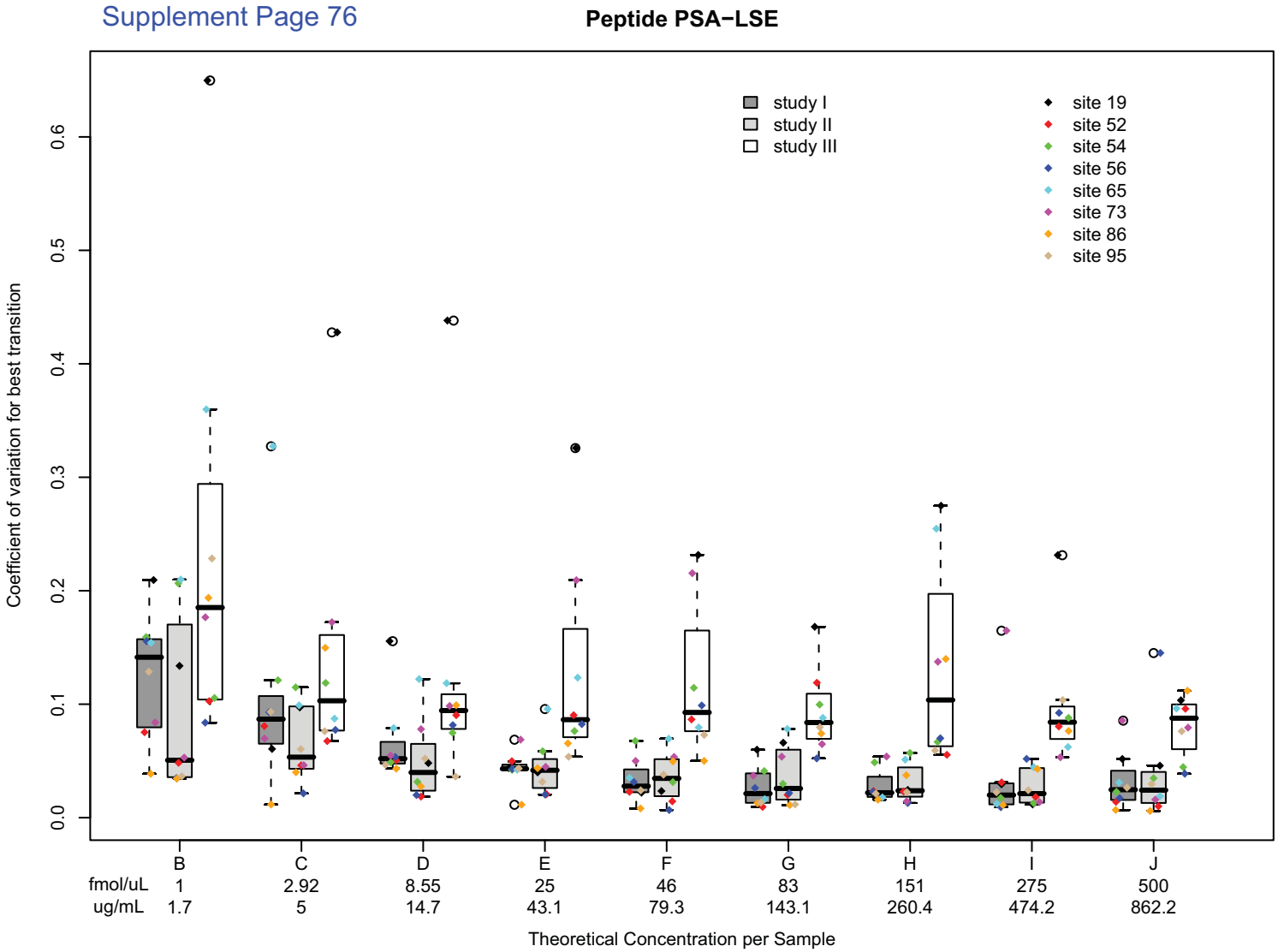


Nature Biotechnology: doi:10.1038/nbt.1546

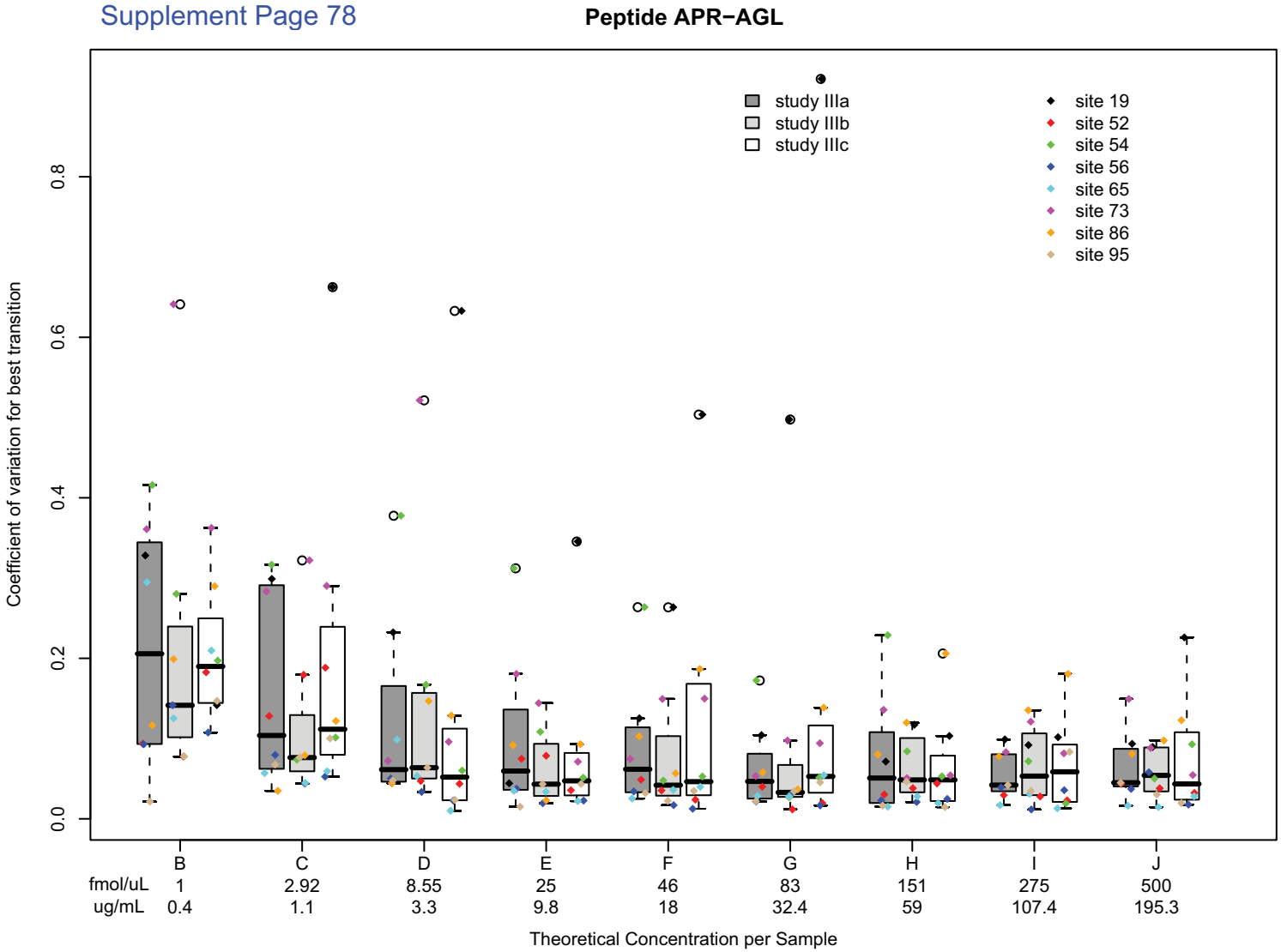
Supplementary Figure 3: Intra- and inter-lab variation of assay CV (coefficient of variation) across studies IIIa, IIIb and IIIc, for the entire range (1-500 fmol/µl) of spike in concentrations of the (light) analyte in diluted plasma. Protein concentration in µg/mL on x-axis is µg protein equivalent in 1 mL of neat plasma.
Analyte concentrations are arranged along the x-axis in sample order. Plots show the study III variation decomposed into the individual process replicates IIIa, IIIb, and IIIc. At each concentration, three box and whisker plots summarize CV variation for studies IIIa-c, respectively.

The box plots show inter-lab CV as the median of intra-lab CVs, with the box spanning the interquartile range (IQR), with the whiskers extending to 1.5 * IQR. Values beyond 1.5 * IQR are deemed outliers, and marked by o. Within each box plot, the actual intra-lab CV values for the individual sites are shown with color coded markers. The CV values are calculated based on the single best performing transition (lowest combined CV) across studies I and II. This same transition is also used for study III, in addition to determining LOD and LOQ.

In the plots, the y-axes ranges are different for different peptides to effectively visualize the CV variation for that particular peptide, with some peptides having relatively small inter- and intra-lab variations in comparison.

The placement of color coded site markers (representing the intra-lab variation) is randomly jittered around the vertical axis of the box plot to make it easier to visualize all the sites, even when the CV values are relatively close to each other.

Peptide LEP-IND for site 52 had missing data for the blank runs, and hence the LOD/LOQ could not be calculated, and the best transition is indeterminate, as such, site 52 is missing for LEP-IND. Peptide MBP-YLA was not reliably detected in Study III, and therefore was not shown in Supplementary Figure 2, however in the following Supplementary Figure 3, the poor quantitation of this peptide is clearly brought out in the MBP-YLA plot.



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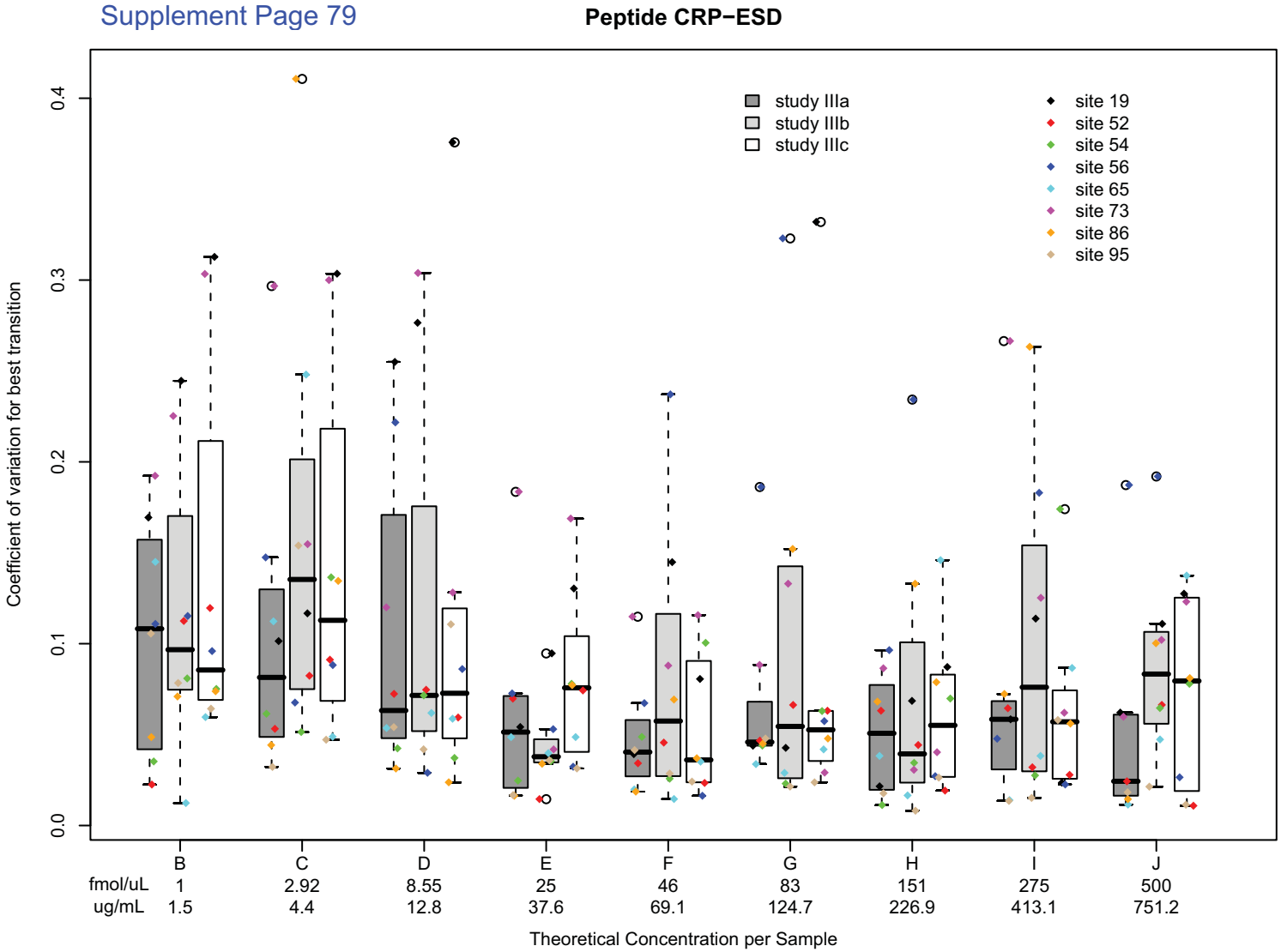

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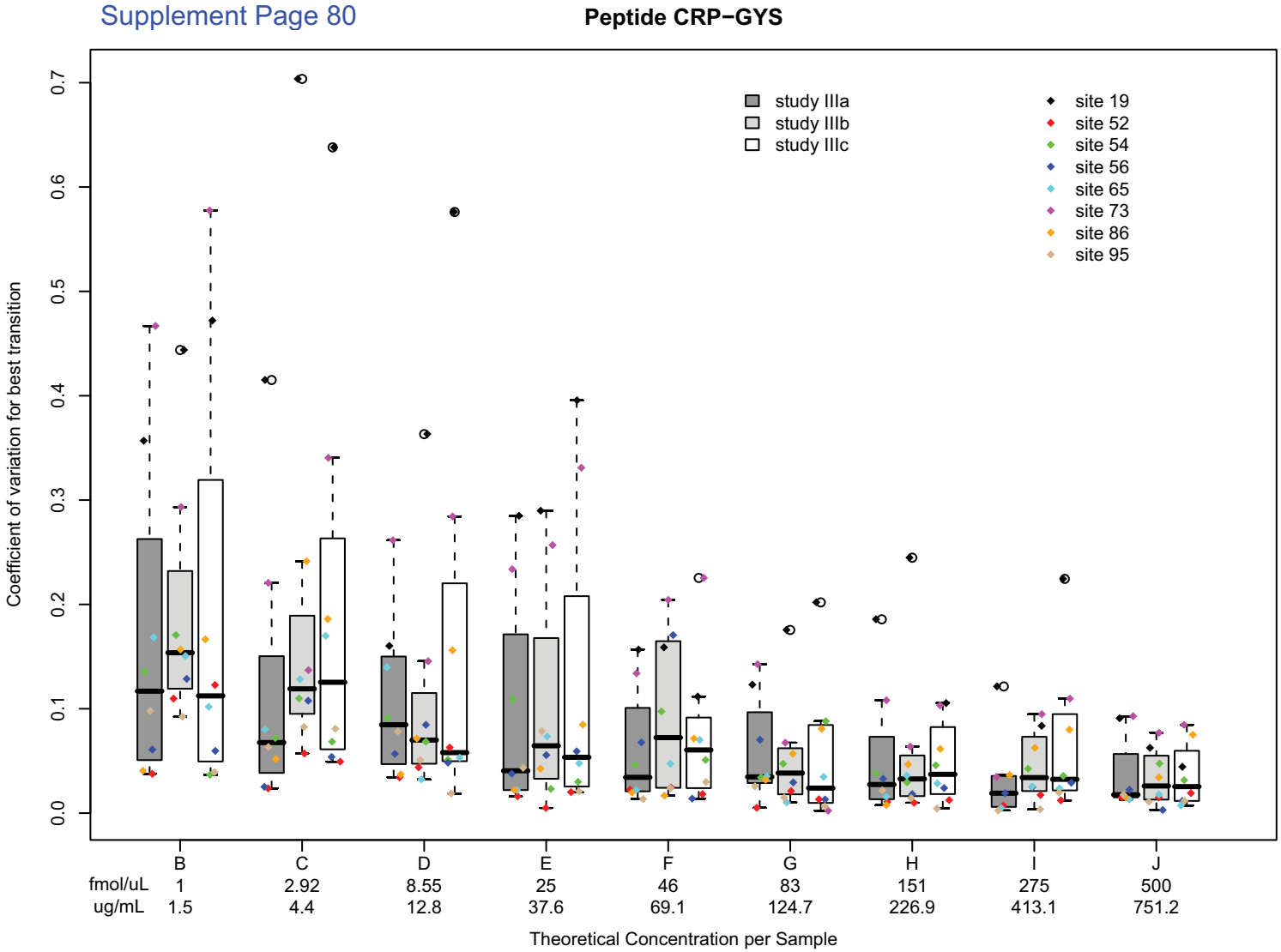

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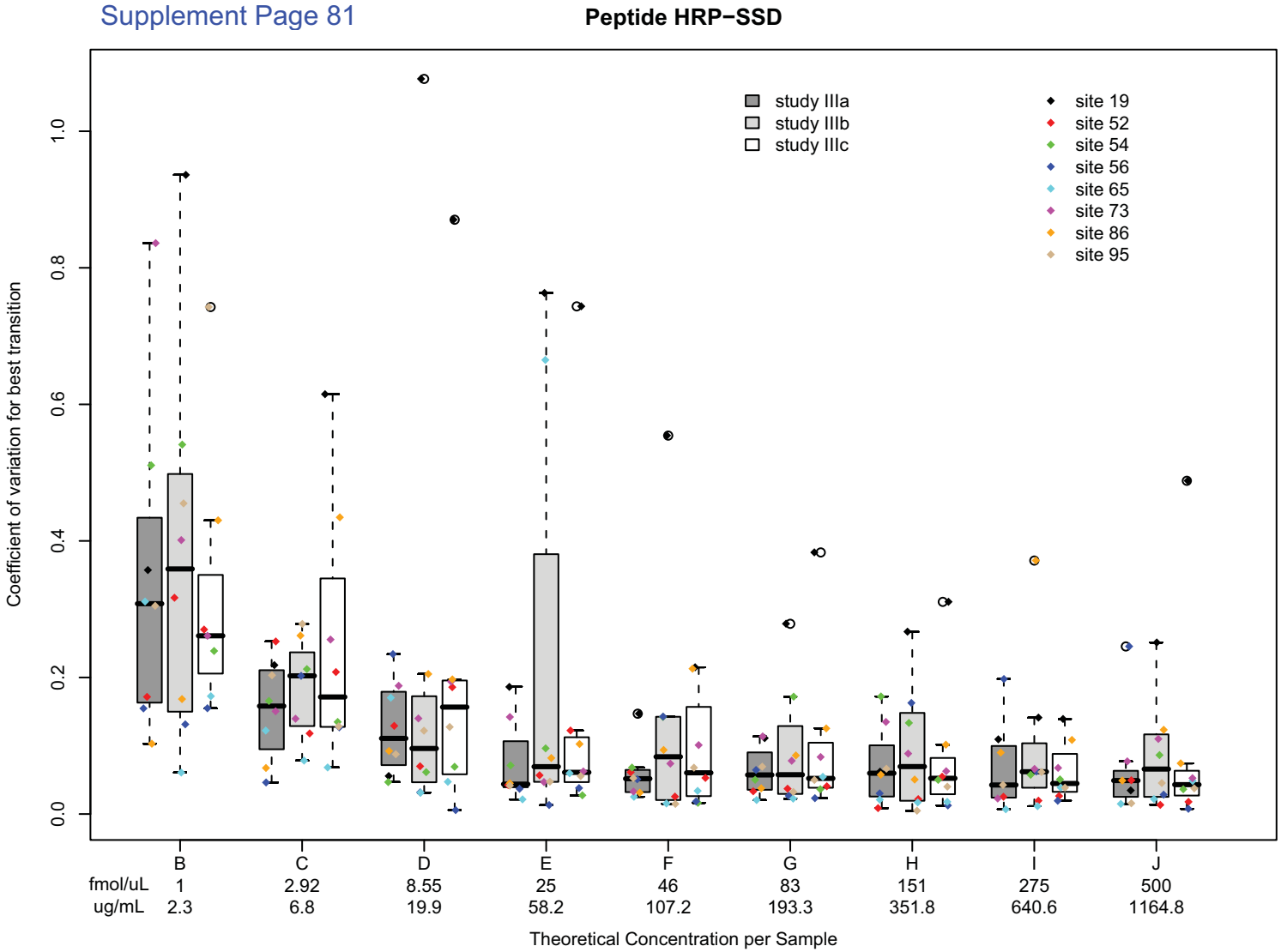

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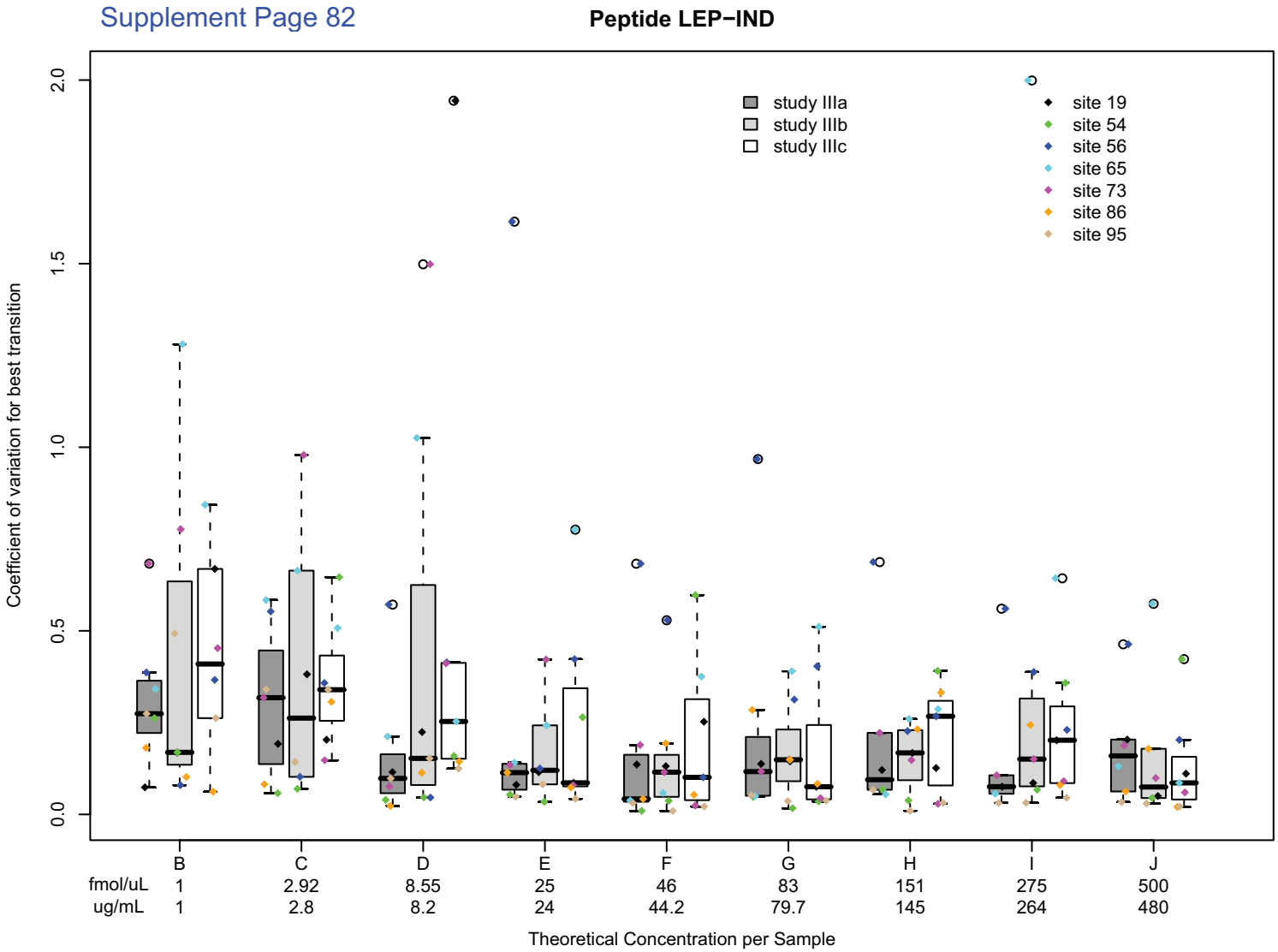

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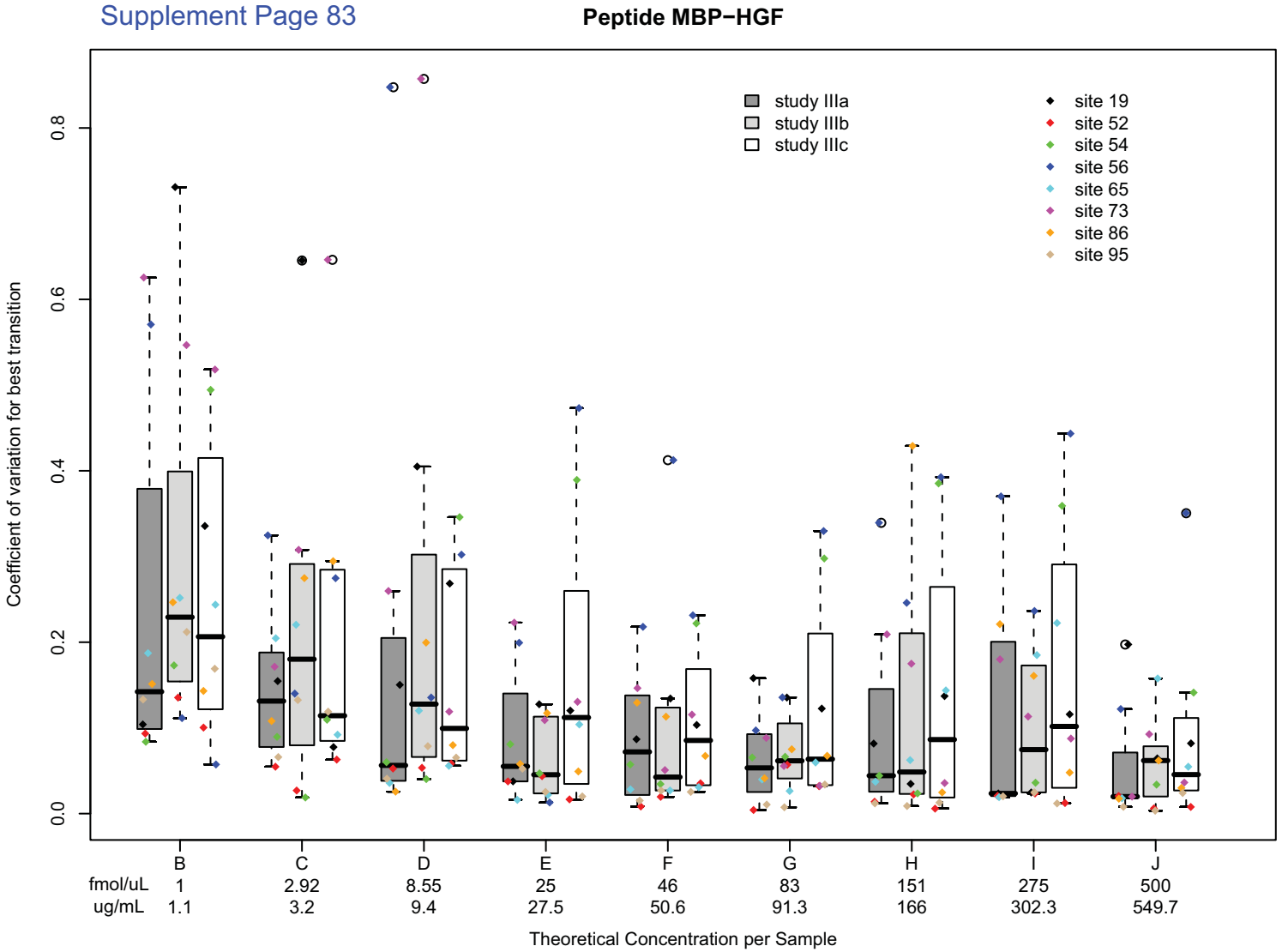
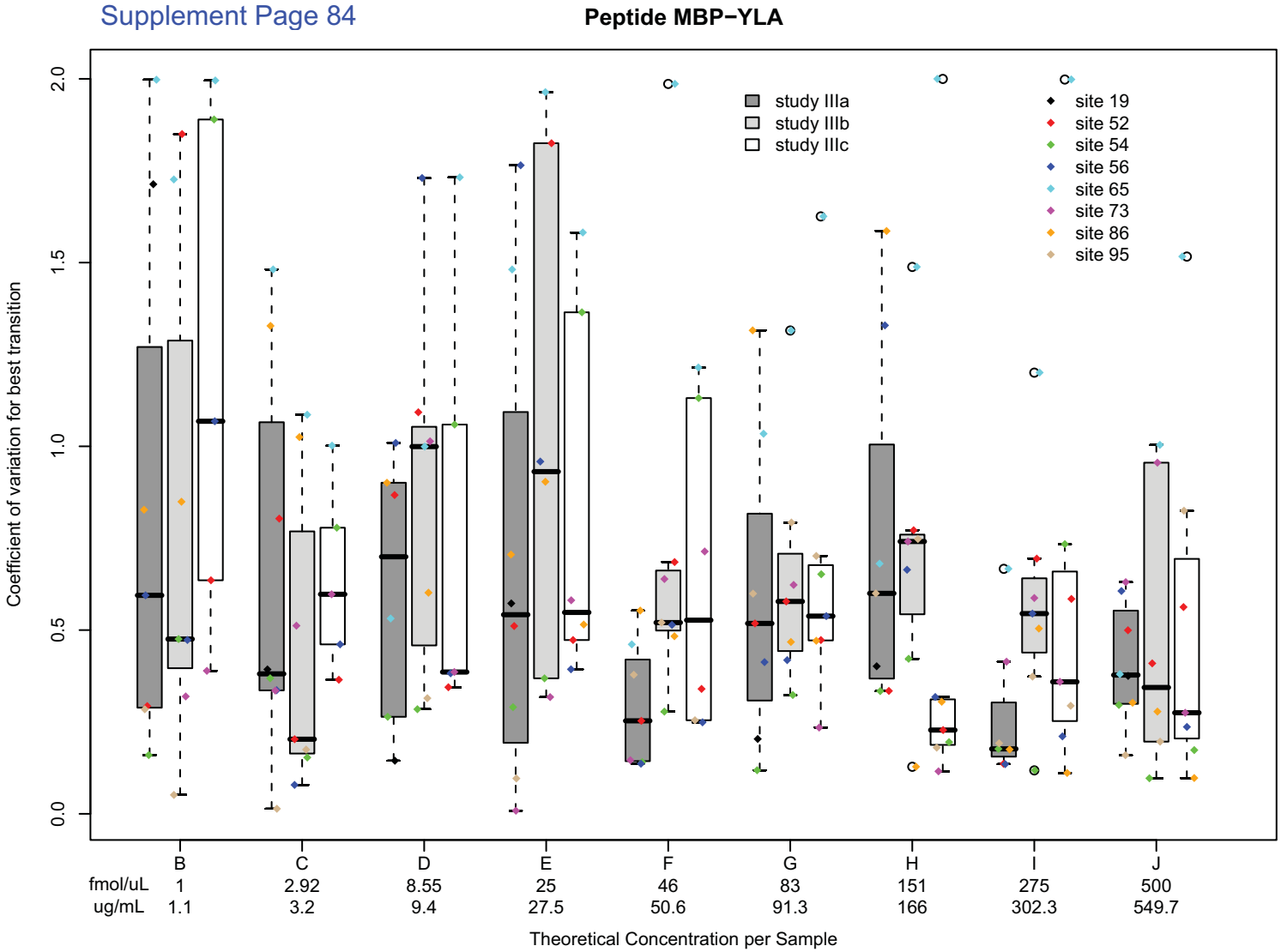


Nature Biotechnology: doi:10.1038/nbt.1546



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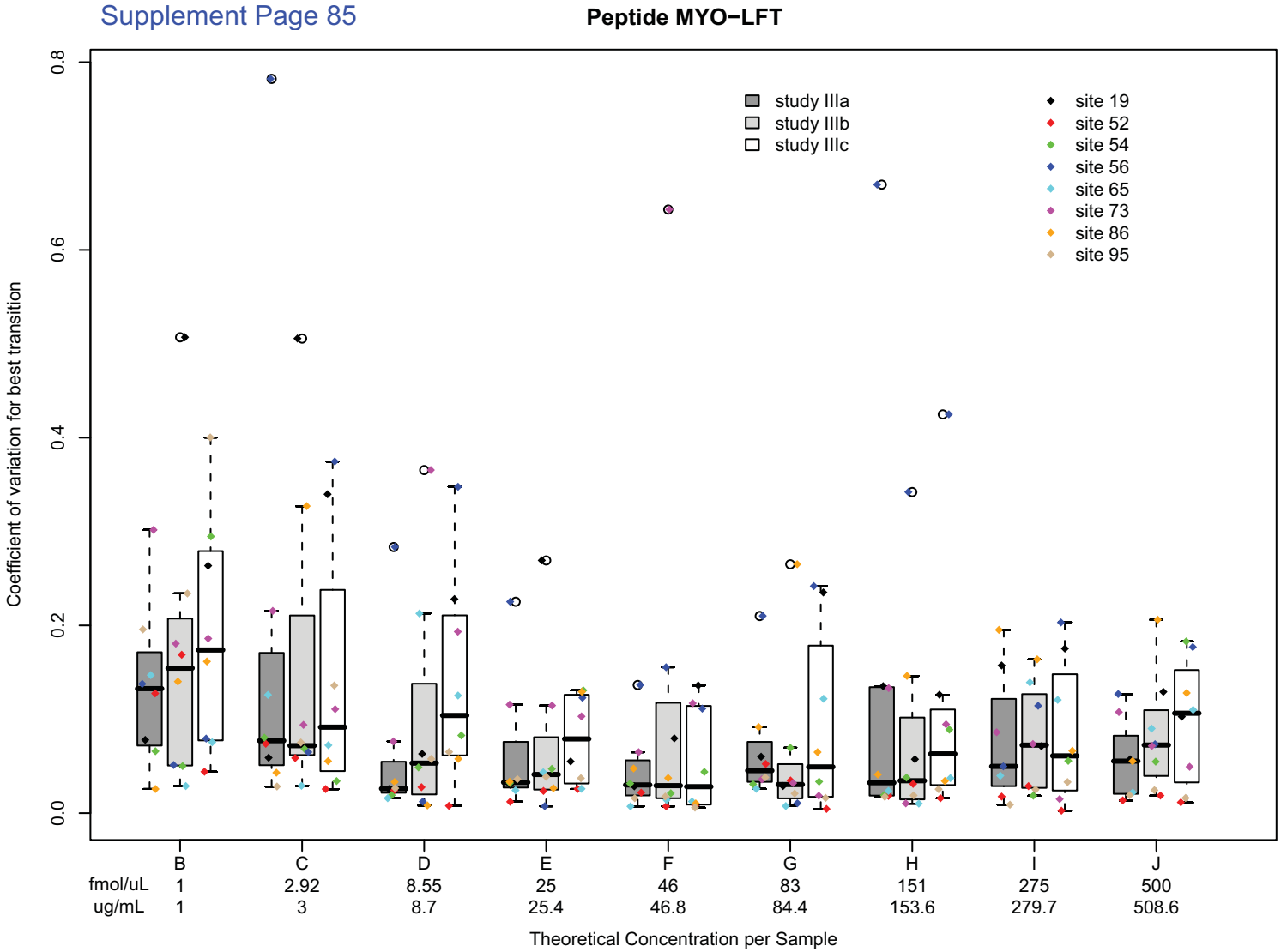

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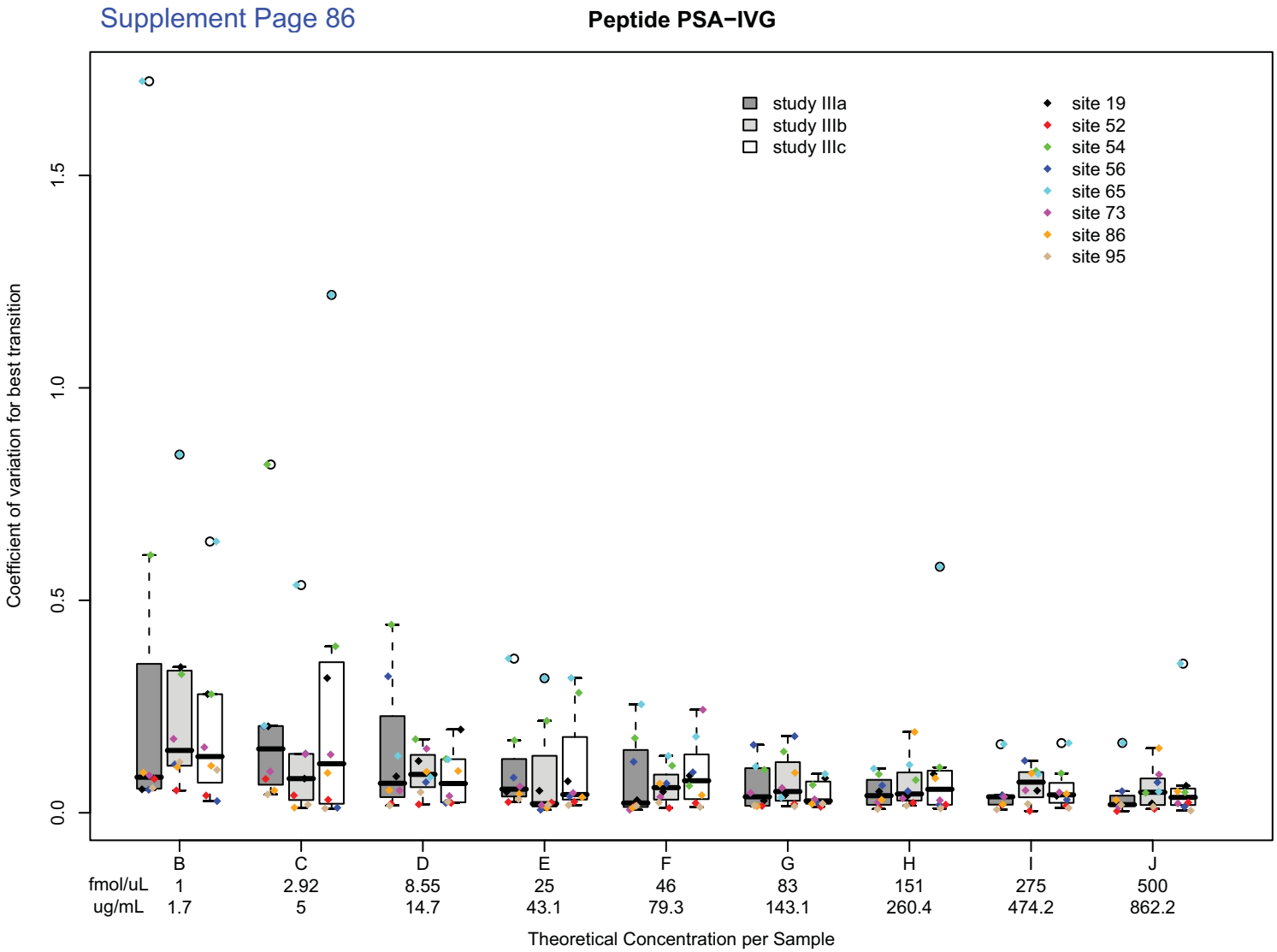

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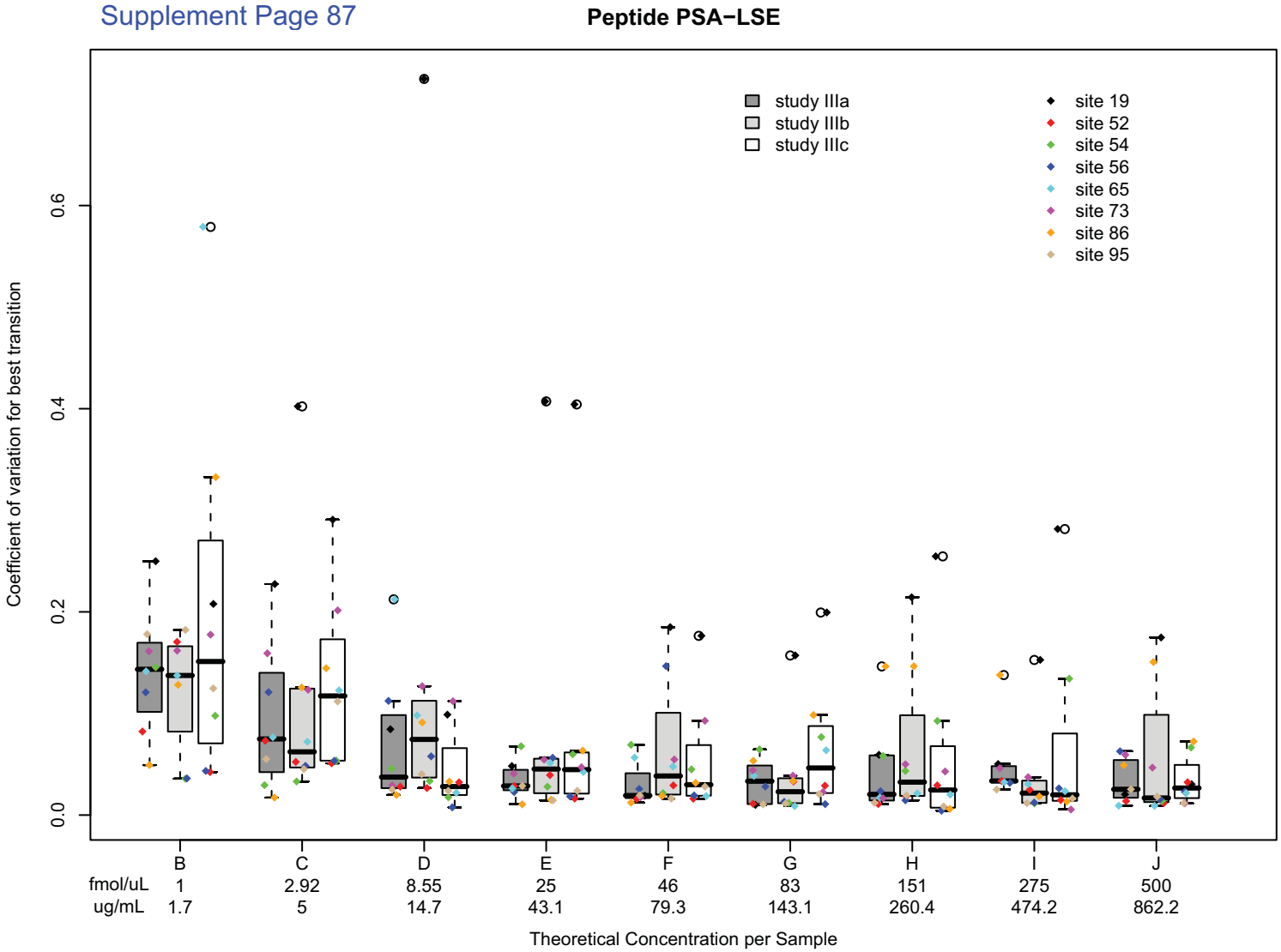

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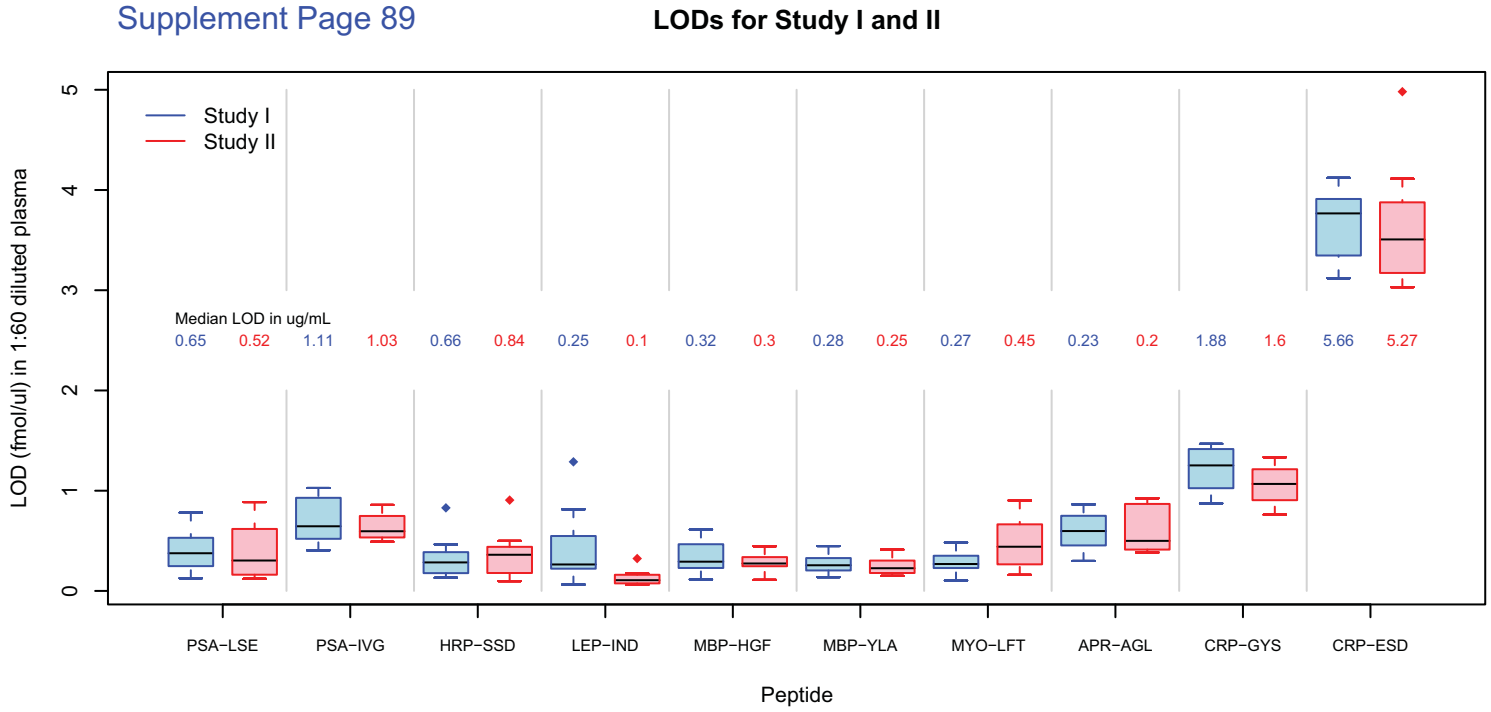
Supplementary Figure 4: Box plot of LOD values for each peptide for Studies I and II. The median LOD values calculated from Studies I and II in 60-fold diluted plasma are shown. The inset values display the conversion of median LOD to µg/mL (µg protein equivalent per 1 mL neat plasma) for each peptide.



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Supplementary Table 3: LOD and LOQ values calculated for each peptide at each site. The MRM transition used for each calculation is shown, along with its fragment ion type.

					Study I		Study II	
Site Code	Peptide Sequence	Peptide Code	Transition * (1, 2, 3)	Fragment Ion Type	LOD (fmol/uL)	LOQ (fmol/uL)	LOD (fmol/uL)	LOQ (fmol/uL)
19	PSA-LSE	bi0037	37tr3_A	y9	0.404	1.21	0.351	1.05
19	PSA-IVG	bi0161	161tr2_A	y7	0.931	2.79	0.622	1.86
19	HRP-SSD	bi0166	166tr2_A	y8	0.280	0.84	0.381	1.14
19	LEP-IND	bi0167	167tr2_A	$y12^{2+}$	0.263	0.79	0.107	0.32
19	MBP-HGL	bi0169	169tr3_A	y5	0.281	0.84	0.289	0.87
19	MBP-YLA	bi0170	170tr2_A	$y10^{2+}$	0.448	1.34	0.221	0.66
19	MYO-LFT	bi0171	171tr1_A	$y9^{2+}$	0.230	0.69	0.324	0.97
19	APR-AGL	bi0173	173tr2_A	y8	0.503	1.51	0.924	2.77
19	CRP-GYS	bi0202	202tr3_A	y8	1.375	4.12	1.041	3.12
19	CRP-ESD	bi0231	231tr2_A	y6	3.739	11.22	3.140	9.42
52	PSA-LSE	bi0037	37tr3_A	y9	0.235	0.70	0.887	2.66
52	PSA-IVG	bi0161	161tr2_A	y7	0.405	1.22	0.764	2.29
52	HRP-SSD	bi0166	166tr3_A	y10	0.207	0.62	0.906	2.72
52	MBP-HGL	bi0169	169tr3_A	y5	0.612	1.84	0.259	0.78
52	MBP-YLA	bi0170	170tr2_A	$y10^{2+}$	0.247	0.74	0.275	0.83
52	MYO-LFT	bi0171	171tr1_A	$y9^{2+}$	0.482	1.45	0.902	2.70
52	APR-AGL	bi0173	173tr3_A	y9	0.694	2.08	0.835	2.50
52	CRP-GYS	bi0202	202tr3_A	y8	0.942	2.83	1.334	4.00
52	CRP-ESD	bi0231	231tr3_A	y7	4.024	12.07	4.981	14.94
54	PSA-LSE	bi0037	37tr3_A	y9	0.594	1.78	0.699	2.10
54	PSA-IVG	bi0161	161tr1_A	y6	0.598	1.79	0.514	1.54
54	HRP-SSD	bi0166	166tr3_A	y10	0.148	0.44	0.226	0.68
54	LEP-IND	bi0167	167tr2_A	$y12^{2+}$	0.064	0.19	0.063	0.19
54	MBP-HGL	bi0169	169tr3_A	y5	0.177	0.53	0.386	1.16
54	MBP-YLA	bi0170	170tr2_A	$y10^{2+}$	0.265	0.80	0.201	0.60
54	MYO-LFT	bi0171	171tr2_A	$y10^{2+}$	0.263	0.79	0.401	1.20
54	APR-AGL	bi0173	173tr2_A	y8	0.423	1.27	0.901	2.70
54	CRP-GYS	bi0202	202tr3_A	y8	1.108	3.32	1.327	3.98
54	CRP-ESD	bi0231	231tr1_A	y5	3.380	10.14	3.373	10.12
56	PSA-LSE	bi0037	37tr3_A	y9	0.781	2.34	0.121	0.36
56	PSA-IVG	bi0161	161tr2_A	y7	0.927	2.78	0.490	1.47
56	HRP-SSD	bi0166	166tr2_A	y8	0.464	1.39	0.098	0.29
56	LEP-IND	bi0167	167tr1_A	$y11^{2+}$	0.282	0.85	0.062	0.19
56	MBP-HGL	bi0169	169tr3_A	y5	0.343	1.03	0.110	0.33
56	MBP-YLA	bi0170	170tr2_A	$y10^{2+}$	0.247	0.74	0.232	0.69
56	MYO-LFT	bi0171	171tr1_A	$y9^{2+}$	0.394	1.18	0.160	0.48
56	APR-AGL	bi0173	173tr2_A	y8	0.805	2.42	0.444	1.33
56	CRP-GYS	bi0202	202tr3_A	y8	1.390	4.17	0.761	2.28
56	CRP-ESD	bi0231	231tr3_A	y7	4.121	12.36	3.641	10.92

Site Code	Peptide Sequence	Peptide Code	Transition * (1, 2, 3)	Fragment Ion Type	Study I		Study II	
					LOD (fmol/uL)	LOQ (fmol/uL)	LOD (fmol/uL)	LOQ (fmol/uL)
65	PSA-LSE	bi0037	37tr2_A	y8	0.348	1.05	0.538	1.61
65	PSA-IVG	bi0161	161tr1_A	y6	1.027	3.08	0.858	2.57
65	HRP-SSD	bi0166	166tr3_A	y10	0.289	0.87	0.374	1.12
65	LEP-IND	bi0167	167tr1_A	$y11^{2+}$	0.814	2.44	0.323	0.97
65	MBP-HGL	bi0169	169tr3_A	y5	0.589	1.77	0.243	0.73
65	MBP-YLA	bi0170	170tr3_A	y7	0.285	0.86	0.331	0.99
65	MYO-LFT	bi0171	171tr3_A	y6	0.105	0.31	0.695	2.08
65	APR-AGL	bi0173	173tr3_A	y8	0.692	2.08	0.414	1.24
65	CRP-GYS	bi0202	202tr2_A	y7	1.467	4.40	1.094	3.28
65	CRP-ESD	bi0231	231tr2_A	y6	3.313	9.94	3.031	9.09
73	PSA-LSE	bi0037	37tr3_A	y9	0.466	1.40	0.256	0.77
73	PSA-IVG	bi0161	161tr2_A	y7	0.692	2.08	0.569	1.71
73	HRP-SSD	bi0166	166tr2_A	y8	0.829	2.49	0.499	1.50
73	LEP-IND	bi0167	167tr1_A	$y11^{2+}$	0.264	0.79	0.174	0.52
73	MBP-HGL	bi0169	169tr3_A	y5	0.291	0.87	0.250	0.75
73	MBP-YLA	bi0170	170tr2_A	$y10^{2+}$	0.162	0.49	0.413	1.24
73	MYO-LFT	bi0171	171tr1_A	$y9^{2+}$	0.274	0.82	0.481	1.44
73	APR-AGL	bi0173	173tr3_A	y9	0.863	2.59	0.411	1.23
73	CRP-GYS	bi0202	202tr3_A	y8	1.441	4.32	1.101	3.30
73	CRP-ESD	bi0231	231tr2_A	y6	3.797	11.39	3.641	10.92
86	PSA-LSE	bi0037	37tr3_A	y9	0.127	0.38	0.177	0.53
86	PSA-IVG	bi0161	161tr2_A	y7	0.484	1.45	0.732	2.20
86	HRP-SSD	bi0166	166tr3_A	y10	0.131	0.39	0.132	0.39
86	LEP-IND	bi0167	167tr1_A	$y11^{2+}$	1.288	3.86	0.148	0.44
86	MBP-HGL	bi0169	169tr3_A	y5	0.293	0.88	0.446	1.34
86	MBP-YLA	bi0170	170tr2_A	$y10^{2+}$	0.371	1.11	0.157	0.47
86	MYO-LFT	bi0171	171tr1_A	$y9^{2+}$	0.227	0.68	0.634	1.90
86	APR-AGL	bi0173	173tr2_A	y8	0.485	1.46	0.384	1.15
86	CRP-GYS	bi0202	202tr3_A	y8	0.874	2.62	0.810	2.43
86	CRP-ESD	bi0231	231tr1_A	y5	3.794	11.38	4.113	12.34
95	PSA-LSE	bi0037	37tr3_A	y9	0.260	0.78	0.147	0.44
95	PSA-IVG	bi0161	161tr2_A	y7	0.556	1.67	0.553	1.66
95	HRP-SSD	bi0166	166tr3_A	y10	0.310	0.93	0.348	1.04
95	LEP-IND	bi0167	167tr2_A	$y12^{2+}$	0.181	0.54	0.088	0.26
95	MBP-HGL	bi0169	169tr3_A	y5	0.115	0.35	0.289	0.87
95	MBP-YLA	bi0170	170tr2_A	$y10^{2+}$	0.138	0.41	0.150	0.45
95	MYO-LFT	bi0171	171tr1_A	$y9^{2+}$	0.308	0.92	0.207	0.62
95	APR-AGL	bi0173	173tr2_A	y8	0.299	0.90	0.555	1.66
95	CRP-GYS	bi0202	202tr3_A	y8	1.130	3.39	1.001	3.00
95	CRP-ESD	bi0231	231tr1_A	y5	3.120	9.36	3.205	9.61

* These transitions are used for calculating LOD and LOQ values for both Study I and II. LOD and LOQ values are first calculated for all transitions for each peptide at each site. The chosen best transition has an LOD with the minimum root mean square deviation from the lowest LODs for both Study I and II.

Supplementary Table 4A: Linear regression-derived parameters: slope, CV(slope), and mean(slope) for Study I.
For Calibration Curve Plots (experimentally determined concentrations and theoretical/spiked-in concentration of signature peptides) see **Supplementary Figure 5**

Study I Parameters

Site	Peptide	Intercept	SE(intercept)	Slope	SE(Slope)	Sigma
site @19	APR-AGL	0.059	0.054	1.309	0.021	0.181
site @52	APR-AGL	0.388	0.027	1.035	0.011	0.083
site @54	APR-AGL	0.221	0.027	0.948	0.011	0.092
site @56	APR-AGL	0.644	0.022	1.130	0.009	0.069
site @65	APR-AGL	0.285	0.046	1.257	0.018	0.113
site @73	APR-AGL	-0.052	0.060	1.208	0.021	0.169
site @86	APR-AGL	0.351	0.030	1.182	0.012	0.109
site @95	APR-AGL	0.354	0.021	1.187	0.008	0.071
site @19	CRP-ESD	2.496	0.038	1.233	0.015	0.146
site @52	CRP-ESD	5.494	0.062	0.920	0.024	0.218
site @54	CRP-ESD	2.853	0.031	1.068	0.012	0.103
site @56	CRP-ESD	2.643	0.033	1.196	0.013	0.120
site @65	CRP-ESD	2.733	0.014	1.118	0.006	0.050
site @73	CRP-ESD	2.690	0.046	1.038	0.018	0.125
site @86	CRP-ESD	5.040	0.028	1.202	0.011	0.098
site @95	CRP-ESD	2.558	0.061	1.218	0.025	0.149
site @19	CRP-GYS	1.139	0.039	1.516	0.015	0.113
site @52	CRP-GYS	1.933	0.030	1.321	0.012	0.103
site @54	CRP-GYS	0.646	0.043	1.283	0.017	0.135
site @56	CRP-GYS	1.027	0.036	1.437	0.014	0.121
site @65	CRP-GYS	1.049	0.020	1.291	0.008	0.067
site @73	CRP-GYS	0.645	0.048	1.297	0.019	0.159
site @86	CRP-GYS	1.116	0.029	1.142	0.011	0.090
site @95	CRP-GYS	0.935	0.018	1.301	0.007	0.064
site @19	HRP-SSD	-0.049	0.051	1.082	0.020	0.163
site @52	HRP-SSD	-0.060	0.029	1.294	0.011	0.101
site @54	HRP-SSD	0.301	0.034	1.026	0.014	0.117
site @56	HRP-SSD	-0.067	0.024	1.194	0.009	0.085
site @65	HRP-SSD	-0.260	0.043	1.097	0.017	0.151
site @73	HRP-SSD	-0.027	0.054	1.248	0.021	0.171
site @86	HRP-SSD	0.017	0.018	1.048	0.007	0.061
site @95	HRP-SSD	-0.163	0.039	1.593	0.016	0.131
site @19	LEP-IND	-0.073	0.072	1.211	0.029	0.261
site @52	LEP-IND	0.041	0.079	1.136	0.032	0.180
site @54	LEP-IND	0.052	0.037	1.110	0.015	0.125
site @56	LEP-IND	0.038	0.024	1.199	0.009	0.070
site @65	LEP-IND	0.222	0.027	1.287	0.011	0.093
site @73	LEP-IND	-0.119	0.048	1.089	0.019	0.162
site @86	LEP-IND	-0.036	0.111	0.968	0.044	0.389
site @95	LEP-IND	-0.156	0.026	1.304	0.010	0.079

Study I Parameters

peptide	inter-lab CV(slope)	inter-lab Mean(slope)
APR-AGL	10.2%	1.157
CRP-ESD	9.9%	1.124
CRP-GYS	8.5%	1.324
HRP-SSD	15.6%	1.198
LEP-IND	9.7%	1.163
MBP-HGF	8.6%	1.161
MBP-YLA	6.5%	1.275
MYO-LFT	16.5%	1.518
PSA-IVG	10.3%	1.658
PSA-LSE	8.6%	1.098

Intercept = y-intercept of linear regression
SE(intercept) = standard error of y-intercept
Slope = slope of linear regression
Sigma = standard deviation about regression line

for details see Methods Section

site @19	MBP-HGF	0.016	0.059	1.287	0.026	0.265
site @52	MBP-HGF	0.146	0.014	1.024	0.006	0.070
site @54	MBP-HGF	0.311	0.025	1.146	0.011	0.086
site @56	MBP-HGF	0.253	0.025	1.255	0.011	0.094
site @65	MBP-HGF	0.270	0.019	1.161	0.008	0.061
site @73	MBP-HGF	0.054	0.034	1.257	0.014	0.140
site @86	MBP-HGF	0.302	0.055	1.054	0.024	0.272
site @95	MBP-HGF	0.071	0.019	1.100	0.009	0.074
site @19	MBP-YLA	0.313	0.031	1.183	0.012	0.104
site @52	MBP-YLA	0.156	0.033	1.252	0.013	0.094
site @54	MBP-YLA	0.179	0.043	1.232	0.017	0.140
site @56	MBP-YLA	0.145	0.040	1.418	0.016	0.136
site @65	MBP-YLA	-0.209	0.027	1.378	0.011	0.084
site @73	MBP-YLA	-0.173	0.064	1.267	0.023	0.202
site @86	MBP-YLA	0.415	0.044	1.221	0.017	0.147
site @95	MBP-YLA	-0.101	0.032	1.248	0.013	0.118
site @19	MYO-LFT	-0.171	0.057	1.985	0.022	0.178
site @52	MYO-LFT	0.203	0.026	1.671	0.010	0.091
site @54	MYO-LFT	0.410	0.053	1.170	0.021	0.149
site @56	MYO-LFT	0.273	0.020	1.445	0.008	0.068
site @65	MYO-LFT	0.254	0.018	1.626	0.007	0.057
site @73	MYO-LFT	0.072	0.057	1.438	0.023	0.209
site @86	MYO-LFT	0.335	0.067	1.293	0.027	0.175
site @95	MYO-LFT	0.107	0.024	1.513	0.010	0.090
site @19	PSA-IVG	0.537	0.129	1.730	0.051	0.411
site @52	PSA-IVG	0.554	0.087	1.537	0.034	0.254
site @54	PSA-IVG	0.803	0.103	1.374	0.040	0.282
site @56	PSA-IVG	0.344	0.053	1.816	0.021	0.182
site @65	PSA-IVG	0.458	0.060	1.800	0.024	0.188
site @73	PSA-IVG	0.311	0.045	1.721	0.018	0.153
site @86	PSA-IVG	0.918	0.112	1.495	0.044	0.284
site @95	PSA-IVG	0.443	0.051	1.795	0.020	0.180
site @19	PSA-LSE	0.009	0.045	1.102	0.016	0.124
site @52	PSA-LSE	0.312	0.033	1.219	0.012	0.100
site @54	PSA-LSE	0.359	0.042	1.031	0.015	0.117
site @56	PSA-LSE	0.514	0.027	1.053	0.010	0.082
site @65	PSA-LSE	-0.121	0.015	1.077	0.005	0.051
site @73	PSA-LSE	0.137	0.050	1.182	0.018	0.144
site @86	PSA-LSE	0.180	0.019	0.939	0.007	0.056
site @95	PSA-LSE	-0.117	0.045	1.178	0.016	0.128

Supplementary Table 4B: Linear regression-derived parameters: slope, CV(slope), and mean(slope) for Study II.
For Calibration Curve Plots (experimentally determined concentrations and theoretical/spiked-in concentration of signature peptides) see **Supplementary Figure 5**

Study II Parameters

Site	Peptide	Intercept	SE(intercept)	Slope	SE(Slope)	Sigma
site @19	APR-AGL	0.261	0.027	0.673	0.010	0.083
site @52	APR-AGL	0.634	0.013	0.519	0.005	0.042
site @54	APR-AGL	0.675	0.018	0.453	0.006	0.051
site @56	APR-AGL	0.364	0.018	0.542	0.007	0.054
site @65	APR-AGL	0.186	0.034	0.637	0.012	0.088
site @73	APR-AGL	0.098	0.011	0.549	0.004	0.032
site @86	APR-AGL	0.317	0.017	0.621	0.006	0.055
site @95	APR-AGL	0.211	0.016	0.603	0.006	0.051
site @19	CRP-ESD	1.849	0.018	0.583	0.006	0.062
site @52	CRP-ESD	5.669	0.058	0.449	0.020	0.181
site @54	CRP-ESD	2.659	0.021	0.557	0.007	0.066
site @56	CRP-ESD	3.395	0.018	0.573	0.006	0.047
site @65	CRP-ESD	2.471	0.012	0.569	0.004	0.037
site @73	CRP-ESD	2.680	0.019	0.516	0.007	0.060
site @86	CRP-ESD	3.528	0.027	0.701	0.010	0.083
site @95	CRP-ESD	2.551	0.035	0.633	0.013	0.087
site @19	CRP-GYS	1.280	0.021	0.498	0.007	0.059
site @52	CRP-GYS	1.451	0.015	0.580	0.005	0.049
site @54	CRP-GYS	0.655	0.019	0.551	0.007	0.059
site @56	CRP-GYS	0.747	0.011	0.511	0.004	0.031
site @65	CRP-GYS	0.695	0.012	0.559	0.004	0.037
site @73	CRP-GYS	0.799	0.024	0.657	0.008	0.074
site @86	CRP-GYS	1.190	0.016	0.466	0.006	0.053
site @95	CRP-GYS	1.125	0.011	0.545	0.004	0.033
site @19	HRP-SSD	-0.079	0.035	0.851	0.012	0.098
site @52	HRP-SSD	0.181	0.023	0.873	0.008	0.072
site @54	HRP-SSD	0.416	0.029	0.676	0.010	0.085
site @56	HRP-SSD	0.061	0.021	0.785	0.008	0.057
site @65	HRP-SSD	0.048	0.029	0.723	0.010	0.095
site @73	HRP-SSD	0.100	0.036	0.828	0.013	0.113
site @86	HRP-SSD	0.070	0.010	0.559	0.004	0.029
site @95	HRP-SSD	-0.159	0.031	1.058	0.011	0.084
site @19	LEP-IND	0.025	0.009	0.146	0.003	0.025
site @52	LEP-IND	0.036	0.014	0.152	0.005	0.033
site @54	LEP-IND	0.060	0.005	0.150	0.002	0.015
site @56	LEP-IND	0.035	0.011	0.156	0.004	0.035
site @65	LEP-IND	0.002	0.008	0.166	0.003	0.023
site @73	LEP-IND	0.047	0.012	0.166	0.004	0.038
site @86	LEP-IND	0.009	0.023	0.113	0.008	0.065
site @95	LEP-IND	0.032	0.005	0.167	0.002	0.015

Study II Parameters

peptide	inter-lab CV(slope)	inter-lab Mean(slope)
APR-AGL	12.6%	0.575
CRP-ESD	13.2%	0.573
CRP-GYS	10.7%	0.546
HRP-SSD	18.7%	0.794
LEP-IND	12.0%	0.152
MBP-HGF	7.4%	0.758
MBP-YLA	6.2%	0.806
MYO-LFT	11.5%	1.012
PSA-IVG	9.9%	0.848
PSA-LSE	9.2%	1.524

Intercept = y-intercept of linear regression
SE(intercept) = standard error of y-intercept
Slope = slope of linear regression
Sigma = standard deviation about regression line

for details see Methods Section

site @19	MBP-HGF	0.122	0.045	0.764	0.016	0.172
site @52	MBP-HGF	0.219	0.012	0.689	0.004	0.054
site @54	MBP-HGF	0.047	0.019	0.781	0.007	0.056
site @56	MBP-HGF	0.155	0.040	0.767	0.015	0.127
site @65	MBP-HGF	0.192	0.015	0.786	0.005	0.041
site @73	MBP-HGF	0.075	0.038	0.852	0.013	0.132
site @86	MBP-HGF	0.047	0.093	0.687	0.034	0.278
site @95	MBP-HGF	0.169	0.011	0.739	0.004	0.033
site @19	MBP-YLA	0.033	0.029	0.860	0.010	0.087
site @52	MBP-YLA	0.189	0.023	0.811	0.008	0.067
site @54	MBP-YLA	0.122	0.030	0.791	0.011	0.083
site @56	MBP-YLA	0.005	0.035	0.797	0.013	0.100
site @65	MBP-YLA	-0.314	0.032	0.875	0.011	0.093
site @73	MBP-YLA	0.403	0.048	0.812	0.017	0.134
site @86	MBP-YLA	0.063	0.086	0.722	0.031	0.247
site @95	MBP-YLA	-0.029	0.023	0.781	0.008	0.065
site @19	MYO-LFT	0.140	0.029	0.995	0.010	0.087
site @52	MYO-LFT	0.484	0.020	1.167	0.007	0.064
site @54	MYO-LFT	0.353	0.043	0.824	0.015	0.117
site @56	MYO-LFT	0.056	0.026	0.888	0.010	0.078
site @65	MYO-LFT	0.123	0.027	1.141	0.010	0.083
site @73	MYO-LFT	0.210	0.030	1.012	0.011	0.088
site @86	MYO-LFT	0.099	0.102	1.012	0.036	0.304
site @95	MYO-LFT	0.040	0.018	1.059	0.007	0.064
site @19	PSA-IVG	0.437	0.050	0.820	0.018	0.142
site @52	PSA-IVG	0.700	0.050	0.773	0.018	0.149
site @54	PSA-IVG	0.803	0.058	0.718	0.021	0.130
site @56	PSA-IVG	0.468	0.029	0.803	0.011	0.095
site @65	PSA-IVG	0.290	0.035	0.888	0.012	0.103
site @73	PSA-IVG	0.325	0.032	0.927	0.011	0.090
site @86	PSA-IVG	0.684	0.068	0.956	0.025	0.194
site @95	PSA-IVG	0.349	0.028	0.897	0.010	0.089
site @19	PSA-LSE	0.017	0.066	1.423	0.024	0.195
site @52	PSA-LSE	1.489	0.036	1.676	0.013	0.120
site @54	PSA-LSE	0.156	0.056	1.559	0.020	0.166
site @56	PSA-LSE	0.062	0.026	1.403	0.010	0.077
site @65	PSA-LSE	-0.253	0.033	1.566	0.012	0.094
site @73	PSA-LSE	0.061	0.035	1.632	0.012	0.109
site @86	PSA-LSE	0.174	0.023	1.286	0.008	0.068
site @95	PSA-LSE	-0.220	0.058	1.648	0.021	0.151

Supplementary Table 4C: Linear regression-derived parameters: slope, CV(slope), and mean(slope) for Study IIIa.
For Calibration Curve Plots (experimentally determined concentrations and theoretical/spiked-in concentration of signature peptides) see **Supplementary Figure 5**

Study IIIa Parameters

Site	Peptide	Intercept	SE(intercept)	Slope	SE(Slope)	Sigma
site @19	APR-AGL	0.179	0.058	0.907	0.020	0.152
site @52	APR-AGL	0.394	0.021	0.583	0.007	0.066
site @54	APR-AGL	0.239	0.003	0.005	0.001	0.009
site @56	APR-AGL	0.302	0.025	0.668	0.008	0.060
site @65	APR-AGL	0.774	0.057	0.786	0.019	0.149
site @73	APR-AGL	0.129	0.034	0.522	0.011	0.094
site @86	APR-AGL	0.412	0.034	0.896	0.012	0.096
site @95	APR-AGL	0.171	0.020	0.859	0.007	0.061
site @19	CRP-ESD	1.015	0.031	0.416	0.011	0.090
site @52	CRP-ESD	2.812	0.029	0.350	0.010	0.101
site @54	CRP-ESD	2.691	0.034	0.408	0.013	0.087
site @56	CRP-ESD	3.663	0.028	0.445	0.010	0.091
site @65	CRP-ESD	1.847	0.023	0.412	0.008	0.059
site @73	CRP-ESD	1.169	0.026	0.321	0.009	0.078
site @86	CRP-ESD	2.888	0.022	0.539	0.007	0.061
site @95	CRP-ESD	2.368	0.026	0.565	0.009	0.076
site @19	CRP-GYS	0.865	0.011	0.105	0.004	0.031
site @52	CRP-GYS	3.177	0.008	0.107	0.003	0.030
site @54	CRP-GYS	1.433	0.027	0.317	0.010	0.079
site @56	CRP-GYS	1.132	0.006	0.127	0.002	0.016
site @65	CRP-GYS	1.063	0.012	0.130	0.004	0.033
site @73	CRP-GYS	0.335	0.008	0.097	0.003	0.023
site @86	CRP-GYS	0.987	0.009	0.142	0.003	0.026
site @95	CRP-GYS	1.047	0.009	0.226	0.003	0.027
site @19	HRP-SSD	0.188	0.035	0.444	0.012	0.096
site @52	HRP-SSD	0.109	0.018	0.437	0.006	0.049
site @54	HRP-SSD	0.220	0.021	0.335	0.008	0.059
site @56	HRP-SSD	0.094	0.018	0.446	0.006	0.049
site @65	HRP-SSD	0.203	0.024	0.437	0.008	0.063
site @73	HRP-SSD	-0.112	0.029	0.374	0.009	0.086
site @86	HRP-SSD	0.047	0.012	0.368	0.004	0.038
site @95	HRP-SSD	-0.193	0.019	0.582	0.006	0.056
site @19	LEP-IND	0.033	0.030	0.223	0.009	0.084
site @52	LEP-IND	0.012	0.011	0.252	0.004	0.037
site @54	LEP-IND	0.067	0.022	0.239	0.008	0.051
site @56	LEP-IND	0.014	0.077	0.264	0.027	0.186
site @65	LEP-IND	0.137	0.024	0.253	0.008	0.065
site @73	LEP-IND	-0.060	0.019	0.213	0.007	0.065
site @86	LEP-IND	-0.002	0.016	0.200	0.005	0.030
site @95	LEP-IND	-0.187	0.013	0.286	0.004	0.035

Study IIIa Parameters

peptide	inter-lab CV(slope)	inter-lab Mean(slope)
APR-AGL	45.8%	0.653
CRP-ESD	19.6%	0.432
CRP-GYS	49.1%	0.156
HRP-SSD	17.6%	0.428
LEP-IND	12.7%	0.241
MBP-HGF	8.5%	0.231
MBP-YLA	154.5%	0.005
MYO-LFT	14.7%	0.517
PSA-IVG	28.6%	0.579
PSA-LSE	8.0%	0.879

Intercept = y-intercept of linear regression
SE(intercept) = standard error of y-intercept
Slope = slope of linear regression
Sigma = standard deviation about regression line

for details see Methods Section

site @19	MBP-HGF	0.032	0.038	0.230	0.011	0.109
site @52	MBP-HGF	0.028	0.007	0.207	0.002	0.026
site @54	MBP-HGF	-0.027	0.011	0.259	0.004	0.031
site @56	MBP-HGF	0.035	0.029	0.234	0.010	0.079
site @65	MBP-HGF	0.220	0.016	0.240	0.006	0.045
site @73	MBP-HGF	0.014	0.031	0.211	0.010	0.083
site @86	MBP-HGF	0.004	0.011	0.216	0.004	0.028
site @95	MBP-HGF	-0.074	0.009	0.248	0.003	0.028
site @19	MBP-YLA	0.171	0.005	0.002	0.002	0.010
site @52	MBP-YLA	0.024	0.001	0.001	0.000	0.003
site @54	MBP-YLA	0.628	0.004	0.000	0.002	0.018
site @56	MBP-YLA	0.025	0.000	0.001	0.000	0.001
site @65	MBP-YLA	-0.001	0.001	0.001	0.000	0.002
site @73	MBP-YLA	0.043	0.007	0.011	0.002	0.018
site @86	MBP-YLA	0.016	0.001	0.001	0.000	0.003
site @95	MBP-YLA	-0.017	0.017	0.018	0.005	0.046
site @19	MYO-LFT	0.361	0.027	0.444	0.009	0.084
site @52	MYO-LFT	0.655	0.029	0.648	0.010	0.079
site @54	MYO-LFT	0.167	0.030	0.596	0.012	0.080
site @56	MYO-LFT	0.339	0.048	0.474	0.017	0.142
site @65	MYO-LFT	0.598	0.044	0.528	0.015	0.094
site @73	MYO-LFT	0.088	0.031	0.474	0.010	0.084
site @86	MYO-LFT	8.402	0.023	0.439	0.008	0.110
site @95	MYO-LFT	0.279	0.064	0.536	0.022	0.224
site @19	PSA-IVG	0.482	0.057	0.551	0.020	0.170
site @52	PSA-IVG	0.400	0.030	0.556	0.010	0.098
site @54	PSA-IVG	0.420	0.025	0.233	0.010	0.077
site @56	PSA-IVG	0.489	0.037	0.609	0.013	0.107
site @65	PSA-IVG	0.131	0.049	0.579	0.017	0.153
site @73	PSA-IVG	0.201	0.043	0.611	0.014	0.125
site @86	PSA-IVG	0.665	0.044	0.668	0.015	0.129
site @95	PSA-IVG	0.133	0.037	0.825	0.012	0.106
site @19	PSA-LSE	0.052	0.050	0.843	0.017	0.143
site @52	PSA-LSE	0.932	0.024	0.904	0.008	0.082
site @54	PSA-LSE	0.033	0.058	0.879	0.023	0.146
site @56	PSA-LSE	0.105	0.019	0.804	0.007	0.054
site @65	PSA-LSE	0.274	0.021	0.898	0.007	0.065
site @73	PSA-LSE	-0.374	0.054	0.807	0.019	0.164
site @86	PSA-LSE	0.124	0.023	0.878	0.008	0.067
site @95	PSA-LSE	-0.270	0.043	1.020	0.015	0.122

Supplementary Table 4D: Linear regression-derived parameters: slope, CV(slope), and mean(slope) for Study IIIb.
For Calibration Curve Plots (experimentally determined concentrations and theoretical/spiked-in concentration of signature peptides) see **Supplementary Figure 5**

Study IIIb Parameters

Site	Peptide	Intercept	SE(intercept)	Slope	SE(Slope)	Sigma
site @19	APR-AGL	0.354	0.233	1.144	0.054	0.406
site @52	APR-AGL	0.967	0.025	0.608	0.008	0.068
site @54	APR-AGL	0.226	0.025	0.558	0.008	0.074
site @56	APR-AGL	0.417	0.031	0.635	0.011	0.094
site @65	APR-AGL	0.187	0.043	0.928	0.015	0.114
site @73	APR-AGL	0.251	0.030	0.517	0.010	0.084
site @86	APR-AGL	0.684	0.037	0.904	0.013	0.102
site @95	APR-AGL	0.340	0.030	0.787	0.010	0.091
site @19	CRP-ESD	1.939	0.032	0.395	0.011	0.096
site @52	CRP-ESD	2.804	0.030	0.422	0.010	0.090
site @54	CRP-ESD	2.457	0.013	0.405	0.004	0.041
site @56	CRP-ESD	2.341	0.028	0.425	0.010	0.080
site @65	CRP-ESD	2.038	0.012	0.476	0.004	0.033
site @73	CRP-ESD	1.362	0.020	0.317	0.007	0.059
site @86	CRP-ESD	3.893	0.035	0.607	0.011	0.089
site @95	CRP-ESD	2.435	0.026	0.461	0.009	0.072
site @19	CRP-GYS	0.864	0.026	0.217	0.009	0.074
site @52	CRP-GYS	2.381	0.009	0.131	0.003	0.027
site @54	CRP-GYS	1.199	0.009	0.176	0.003	0.026
site @56	CRP-GYS	0.891	0.007	0.137	0.002	0.022
site @65	CRP-GYS	0.736	0.010	0.145	0.003	0.027
site @73	CRP-GYS	0.787	0.010	0.098	0.003	0.029
site @86	CRP-GYS	1.183	0.015	0.186	0.005	0.049
site @95	CRP-GYS	1.591	0.009	0.185	0.003	0.029
site @19	HRP-SSD	0.884	0.126	0.275	0.022	0.167
site @52	HRP-SSD	0.455	0.020	0.465	0.007	0.055
site @54	HRP-SSD	0.100	0.024	0.371	0.008	0.070
site @56	HRP-SSD	0.080	0.020	0.444	0.007	0.058
site @65	HRP-SSD	-0.050	0.024	0.497	0.008	0.072
site @73	HRP-SSD	0.075	0.025	0.350	0.007	0.063
site @86	HRP-SSD	0.027	0.027	0.487	0.010	0.080
site @95	HRP-SSD	0.092	0.021	0.534	0.007	0.064
site @19	LEP-IND	0.201	0.041	0.243	0.011	0.100
site @52	LEP-IND	0.054	0.011	0.282	0.004	0.034
site @54	LEP-IND	0.119	0.026	0.222	0.009	0.077
site @56	LEP-IND	0.009	0.024	0.249	0.009	0.068
site @65	LEP-IND	0.664	0.052	0.209	0.018	0.156
site @73	LEP-IND	0.044	0.025	0.200	0.008	0.078
site @86	LEP-IND	-0.036	0.026	0.286	0.009	0.055
site @95	LEP-IND	-0.128	0.011	0.238	0.004	0.032

Study IIIb Parameters

peptide	inter-lab CV(slope)	inter-lab Mean(slope)
APR-AGL	29.0%	0.760
CRP-ESD	19.1%	0.439
CRP-GYS	24.2%	0.159
HRP-SSD	20.7%	0.428
LEP-IND	13.7%	0.241
MBP-HGF	9.3%	0.233
MBP-YLA	154.7%	0.004
MYO-LFT	16.2%	0.458
PSA-IVG	9.9%	0.581
PSA-LSE	11.3%	0.938

Intercept = y-intercept of linear regression
SE(intercept) = standard error of y-intercept
Slope = slope of linear regression
Sigma = standard deviation about regression line

for details see Methods Section

site @19	MBP-HGF	0.208	0.027	0.213	0.008	0.066
site @52	MBP-HGF	0.035	0.007	0.238	0.002	0.030
site @54	MBP-HGF	0.014	0.007	0.245	0.002	0.021
site @56	MBP-HGF	0.062	0.015	0.205	0.005	0.043
site @65	MBP-HGF	-0.107	0.014	0.261	0.005	0.036
site @73	MBP-HGF	0.041	0.028	0.223	0.008	0.079
site @86	MBP-HGF	-0.110	0.038	0.258	0.012	0.124
site @95	MBP-HGF	-0.111	0.008	0.223	0.003	0.022
site @19	MBP-YLA	0.000	0.000	0.000	0.000	0.000
site @52	MBP-YLA	0.017	0.011	0.019	0.004	0.032
site @54	MBP-YLA	0.005	0.001	0.001	0.000	0.002
site @56	MBP-YLA	0.009	0.001	0.001	0.000	0.001
site @65	MBP-YLA	-0.006	0.005	0.008	0.002	0.024
site @73	MBP-YLA	0.809	0.010	0.005	0.003	0.032
site @86	MBP-YLA	0.032	0.001	0.001	0.000	0.001
site @95	MBP-YLA	0.007	0.000	0.000	0.000	0.000
site @19	MYO-LFT	0.557	0.069	0.487	0.021	0.167
site @52	MYO-LFT	2.758	0.025	0.563	0.009	0.078
site @54	MYO-LFT	6.080	0.011	0.332	0.004	0.054
site @56	MYO-LFT	0.209	0.035	0.501	0.012	0.107
site @65	MYO-LFT	5.884	0.041	0.422	0.014	0.136
site @73	MYO-LFT	0.348	0.041	0.397	0.014	0.125
site @86	MYO-LFT	1.335	0.042	0.507	0.015	0.125
site @95	MYO-LFT	0.458	0.056	0.458	0.019	0.179
site @19	PSA-IVG	0.568	0.041	0.504	0.013	0.118
site @52	PSA-IVG	0.348	0.028	0.583	0.009	0.081
site @54	PSA-IVG	0.542	0.057	0.595	0.018	0.119
site @56	PSA-IVG	0.485	0.042	0.565	0.015	0.112
site @65	PSA-IVG	-0.052	0.027	0.669	0.009	0.084
site @73	PSA-IVG	0.500	0.037	0.505	0.012	0.107
site @86	PSA-IVG	0.364	0.047	0.613	0.017	0.144
site @95	PSA-IVG	0.276	0.022	0.615	0.008	0.070
site @19	PSA-LSE	0.433	0.389	1.059	0.053	0.411
site @52	PSA-LSE	0.143	0.032	1.000	0.011	0.100
site @54	PSA-LSE	0.240	0.042	0.955	0.014	0.113
site @56	PSA-LSE	0.175	0.030	0.818	0.010	0.095
site @65	PSA-LSE	-0.217	0.016	0.965	0.006	0.044
site @73	PSA-LSE	-0.102	0.046	0.746	0.015	0.123
site @86	PSA-LSE	-0.090	0.042	1.007	0.016	0.122
site @95	PSA-LSE	-0.452	0.050	0.954	0.017	0.138

Supplementary Table 4E: Linear regression-derived parameters: slope, CV(slope), and mean(slope) for Study IIIc.
For Calibration Curve Plots (experimentally determined concentrations and theoretical/spiked-in concentration of signature peptides) see **Supplementary Figure 5**

Study IIIc Parameters

Site	Peptide	Intercept	SE(intercept)	Slope	SE(Slope)	Sigma
site @19	APR-AGL	1.979	0.334	0.980	0.087	0.490
site @52	APR-AGL	0.588	0.029	0.697	0.010	0.088
site @54	APR-AGL	0.196	0.023	0.634	0.008	0.066
site @56	APR-AGL	0.317	0.029	0.725	0.010	0.080
site @65	APR-AGL	-0.024	0.056	0.978	0.019	0.189
site @73	APR-AGL	-0.012	0.045	0.610	0.014	0.117
site @86	APR-AGL	0.399	0.049	0.936	0.017	0.137
site @95	APR-AGL	0.367	0.030	0.835	0.010	0.086
site @19	CRP-ESD	-0.268	0.028	0.470	0.010	0.100
site @52	CRP-ESD	3.744	0.049	0.406	0.017	0.148
site @54	CRP-ESD	2.188	0.021	0.423	0.007	0.063
site @56	CRP-ESD	2.373	0.014	0.439	0.005	0.035
site @65	CRP-ESD	1.746	0.024	0.493	0.008	0.063
site @73	CRP-ESD	1.032	0.038	0.289	0.012	0.110
site @86	CRP-ESD	3.394	0.026	0.569	0.009	0.074
site @95	CRP-ESD	2.800	0.025	0.480	0.008	0.066
site @19	CRP-GYS	0.623	0.028	0.228	0.010	0.079
site @52	CRP-GYS	3.839	0.010	0.131	0.003	0.033
site @54	CRP-GYS	1.548	0.013	0.167	0.004	0.038
site @56	CRP-GYS	0.747	0.005	0.144	0.002	0.015
site @65	CRP-GYS	1.038	0.013	0.172	0.004	0.036
site @73	CRP-GYS	0.461	0.017	0.099	0.005	0.047
site @86	CRP-GYS	0.996	0.012	0.155	0.004	0.038
site @95	CRP-GYS	1.586	0.009	0.200	0.003	0.028
site @19	HRP-SSD	1.325	0.138	0.238	0.020	0.105
site @52	HRP-SSD	0.150	0.018	0.535	0.006	0.058
site @54	HRP-SSD	-0.033	0.013	0.435	0.005	0.043
site @56	HRP-SSD	-0.014	0.012	0.456	0.004	0.033
site @65	HRP-SSD	-0.081	0.022	0.485	0.007	0.067
site @73	HRP-SSD	0.131	0.048	0.382	0.014	0.114
site @86	HRP-SSD	0.143	0.027	0.399	0.009	0.072
site @95	HRP-SSD	0.372	0.020	0.543	0.007	0.067
site @19	LEP-IND	0.111	0.041	0.288	0.013	0.103
site @52	LEP-IND	0.010	0.010	0.286	0.003	0.030
site @54	LEP-IND	-0.053	0.077	0.215	0.010	0.086
site @56	LEP-IND	-0.049	0.029	0.265	0.010	0.092
site @65	LEP-IND	0.150	0.048	0.204	0.016	0.146
site @73	LEP-IND	0.334	0.024	0.192	0.008	0.061
site @86	LEP-IND	0.079	0.009	0.259	0.003	0.027
site @95	LEP-IND	-0.138	0.009	0.238	0.003	0.026

Study IIIc Parameters

peptide	inter-lab CV(slope)	inter-lab Mean(slope)
APR-AGL	19.6%	0.799
CRP-ESD	18.3%	0.446
CRP-GYS	24.9%	0.162
HRP-SSD	22.8%	0.434
LEP-IND	15.7%	0.243
MBP-HGF	8.5%	0.249
MBP-YLA	230.7%	0.030
MYO-LFT	27.0%	0.536
PSA-IVG	9.4%	0.600
PSA-LSE	12.1%	0.938

Intercept = y-intercept of linear regression
SE(intercept) = standard error of y-intercept
Slope = slope of linear regression
Sigma = standard deviation about regression line

for details see Methods Section

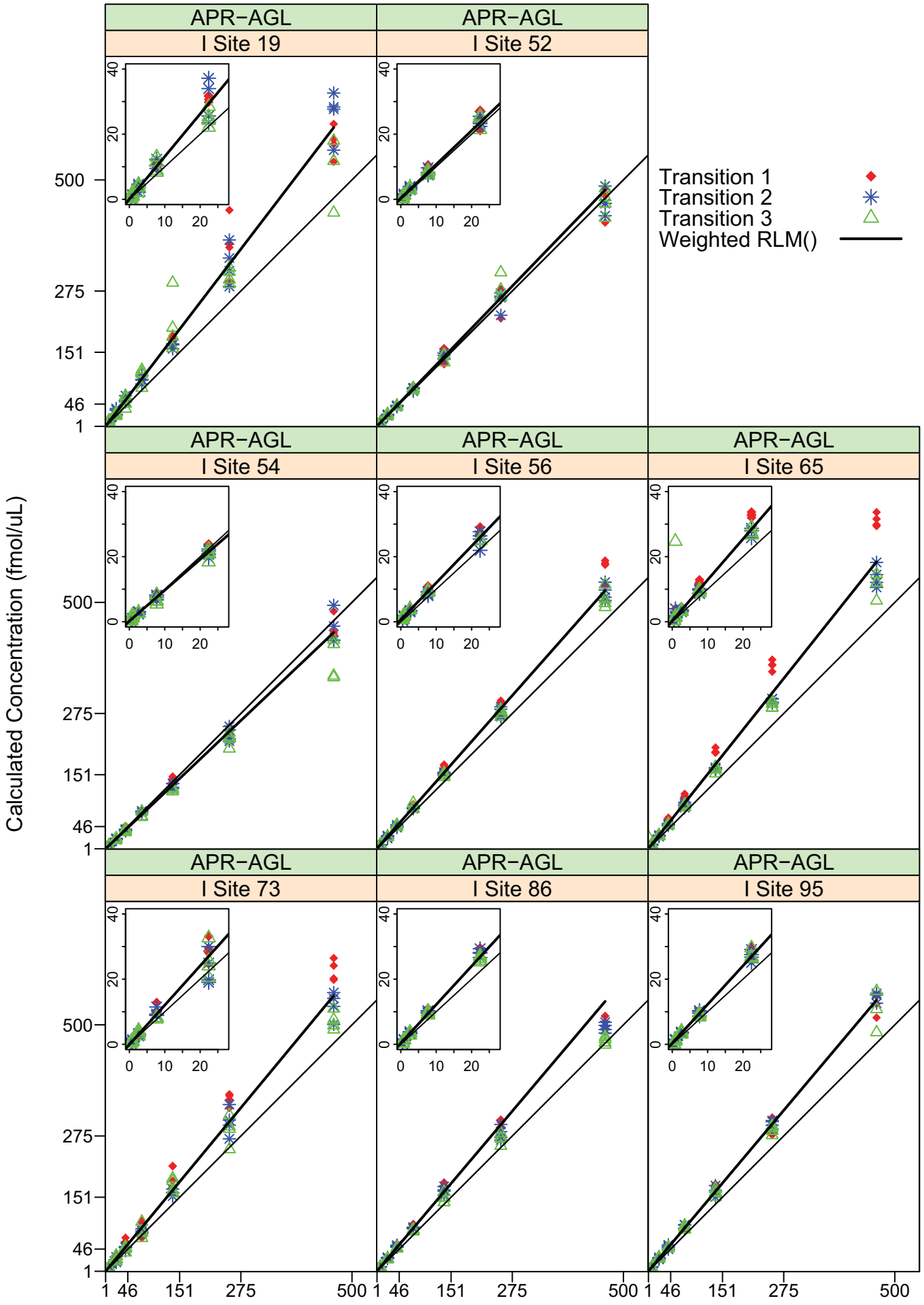
site @19	MBP-HGF	0.471	0.035	0.249	0.012	0.125
site @52	MBP-HGF	0.007	0.006	0.241	0.002	0.025
site @54	MBP-HGF	0.114	0.050	0.266	0.012	0.083
site @56	MBP-HGF	0.007	0.031	0.238	0.010	0.090
site @65	MBP-HGF	-0.107	0.012	0.279	0.004	0.033
site @73	MBP-HGF	0.002	0.041	0.257	0.012	0.108
site @86	MBP-HGF	0.042	0.014	0.242	0.005	0.040
site @95	MBP-HGF	0.008	0.007	0.216	0.002	0.020
site @19	MBP-YLA	8.381	0.352	0.119	0.128	0.694
site @52	MBP-YLA	0.045	0.002	0.003	0.001	0.008
site @54	MBP-YLA	7.486	0.106	0.098	0.036	0.412
site @56	MBP-YLA	0.006	0.000	0.001	0.000	0.001
site @65	MBP-YLA	0.000	0.000	0.000	0.000	0.000
site @73	MBP-YLA	0.085	0.010	0.015	0.003	0.027
site @86	MBP-YLA	0.009	0.000	0.000	0.000	0.000
site @95	MBP-YLA	0.010	0.000	0.000	0.000	0.000
site @19	MYO-LFT	0.615	0.081	0.571	0.027	0.217
site @52	MYO-LFT	0.283	0.033	0.757	0.011	0.099
site @54	MYO-LFT	0.185	0.034	0.484	0.012	0.094
site @56	MYO-LFT	0.213	0.046	0.430	0.016	0.130
site @65	MYO-LFT	0.187	0.068	0.595	0.023	0.195
site @73	MYO-LFT	0.191	0.045	0.391	0.015	0.147
site @86	MYO-LFT	0.308	0.045	0.696	0.015	0.107
site @95	MYO-LFT	3.415	0.028	0.363	0.010	0.098
site @19	PSA-IVG	0.545	0.054	0.538	0.019	0.143
site @52	PSA-IVG	0.506	0.031	0.677	0.011	0.091
site @54	PSA-IVG	0.517	0.067	0.538	0.022	0.143
site @56	PSA-IVG	0.475	0.018	0.569	0.006	0.052
site @65	PSA-IVG	-0.149	0.060	0.646	0.021	0.162
site @73	PSA-IVG	0.192	0.052	0.574	0.016	0.122
site @86	PSA-IVG	0.508	0.044	0.602	0.015	0.131
site @95	PSA-IVG	0.286	0.022	0.658	0.008	0.066
site @19	PSA-LSE	2.146	0.214	0.802	0.065	0.384
site @52	PSA-LSE	0.741	0.032	1.089	0.011	0.097
site @54	PSA-LSE	0.014	0.029	0.894	0.010	0.087
site @56	PSA-LSE	-0.022	0.021	0.884	0.007	0.065
site @65	PSA-LSE	-0.276	0.023	1.096	0.008	0.066
site @73	PSA-LSE	-0.349	0.083	0.863	0.027	0.186
site @86	PSA-LSE	0.043	0.028	0.872	0.009	0.072
site @95	PSA-LSE	-0.037	0.044	1.002	0.015	0.123

Supplementary Figure 5: Linear calibration curves of observed concentrations (y-axis) of the ten signature peptides and the theoretical/spiked-in concentration (x-axis) into solution (buffer or plasma matrix). These regression plots are on a linear / linear scale for all five Studies I, II, III a-c, and all ten peptides with three transitions each from all eight participating sites.

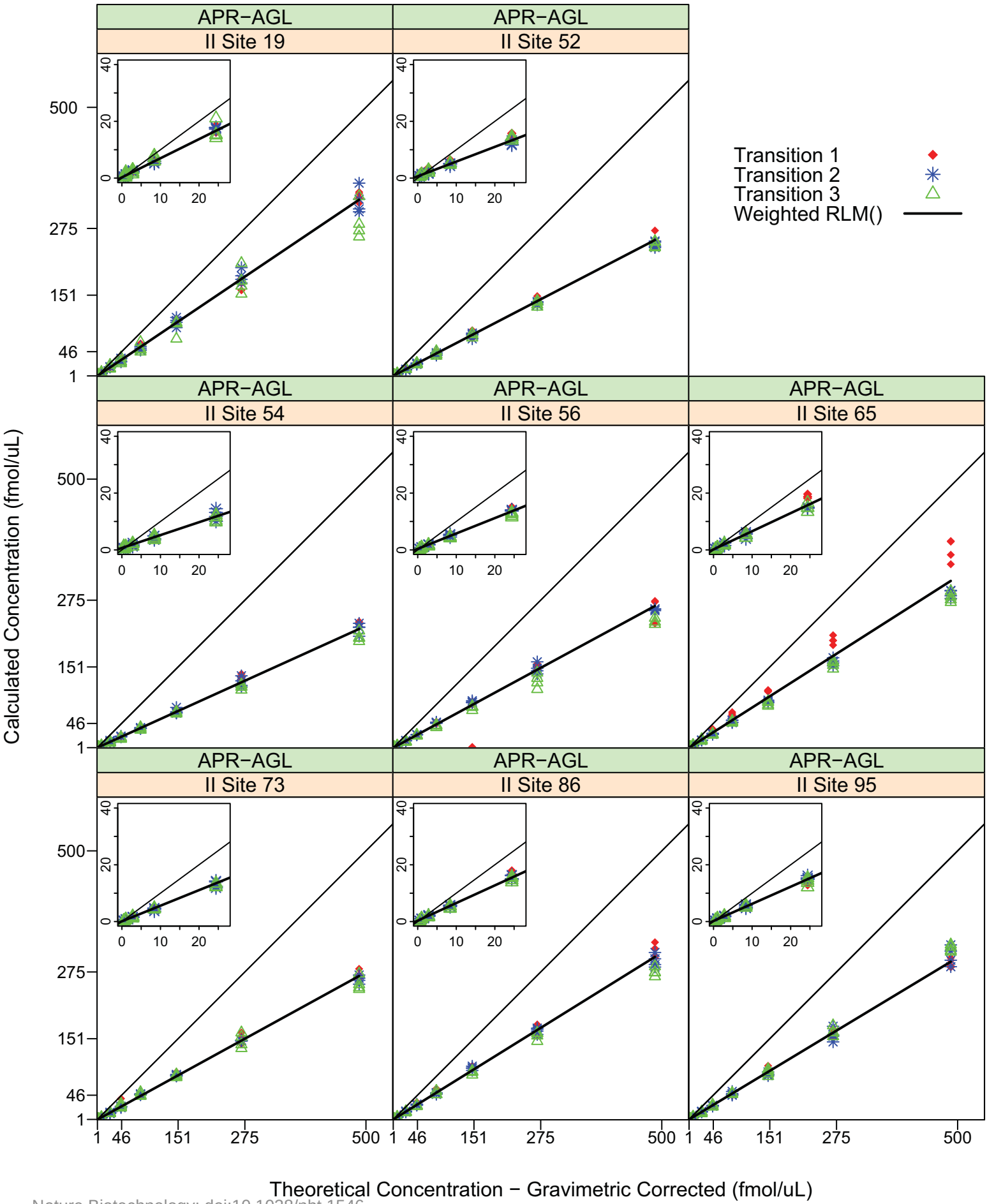
Each of the eight sites were assigned random numerical codes (19, 52, 54, 56, 65, 73, 86, 95) for anonymization purposes. Linear calibration curves are organized by signature peptide, then by experiment (Studies I, II, III a,b,c) and all eight sites are displayed on each page. Study number and anonymization code for each site are displayed right above the individual regression plot (i.e., "I site 19" etc.), as well as the derived protein 3-letter code, the first three amino acids of the peptide sequence (i.e., APR-AGL; for further details see SOP – Supplementary Methods online). Statistical Models for Prediction Curves are described in detail in the Methods section. The linear calibration curves show all three MRM transitions per individual peptide recorded in these studies in different colors. The three letter protein codes stand for APR (aprotinin), CRP (C-reactive protein), HRP (horseradish peroxidase), LEP (leptin), MBP (myelin basic protein), MYO (myoglobin), and PSA (prostate specific antigen). All theoretical/spiked-in concentrations are gravimetrically corrected (see Supplementary Tables 6 A-F).

For better visualization, study I plots are displayed with upper limits for x-axes of 550 fmol/µl and y-axes of 750 fmol/µl, while Studies II and III are displayed with upper limits for x-axes of 550 fmol/µl and y-axes of 600 fmol/µl. For these two plotting options x and y axes length are displayed on isometric scales, where the relation between physical distance on the device and distance in the data scale are forced to be the same for both axes. This ensures that for all main plots for Study I (550 x and 750 y) as well as for Studies II and III (550 x and 600 y), the diagonal is always displayed at 45 degrees, and slopes are directly comparable between all three studies.

Inset plots with an x-axis of 27 fmol/µl and a y-axis of 40 fmol/µl have been added into each plot for each study to better visualize the low end concentration points.



Nature Biotechnology: doi:10.1038/nbt.1546



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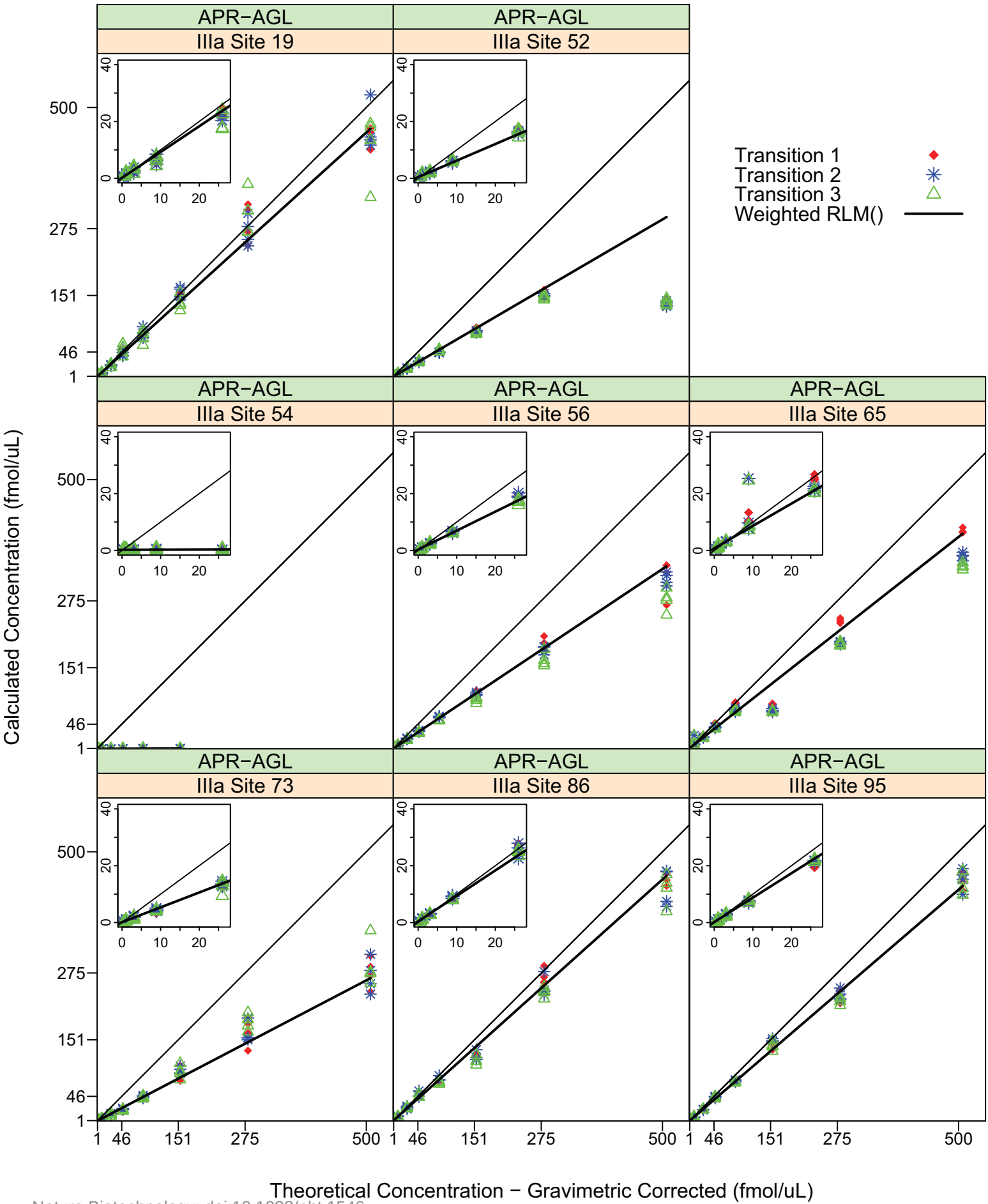

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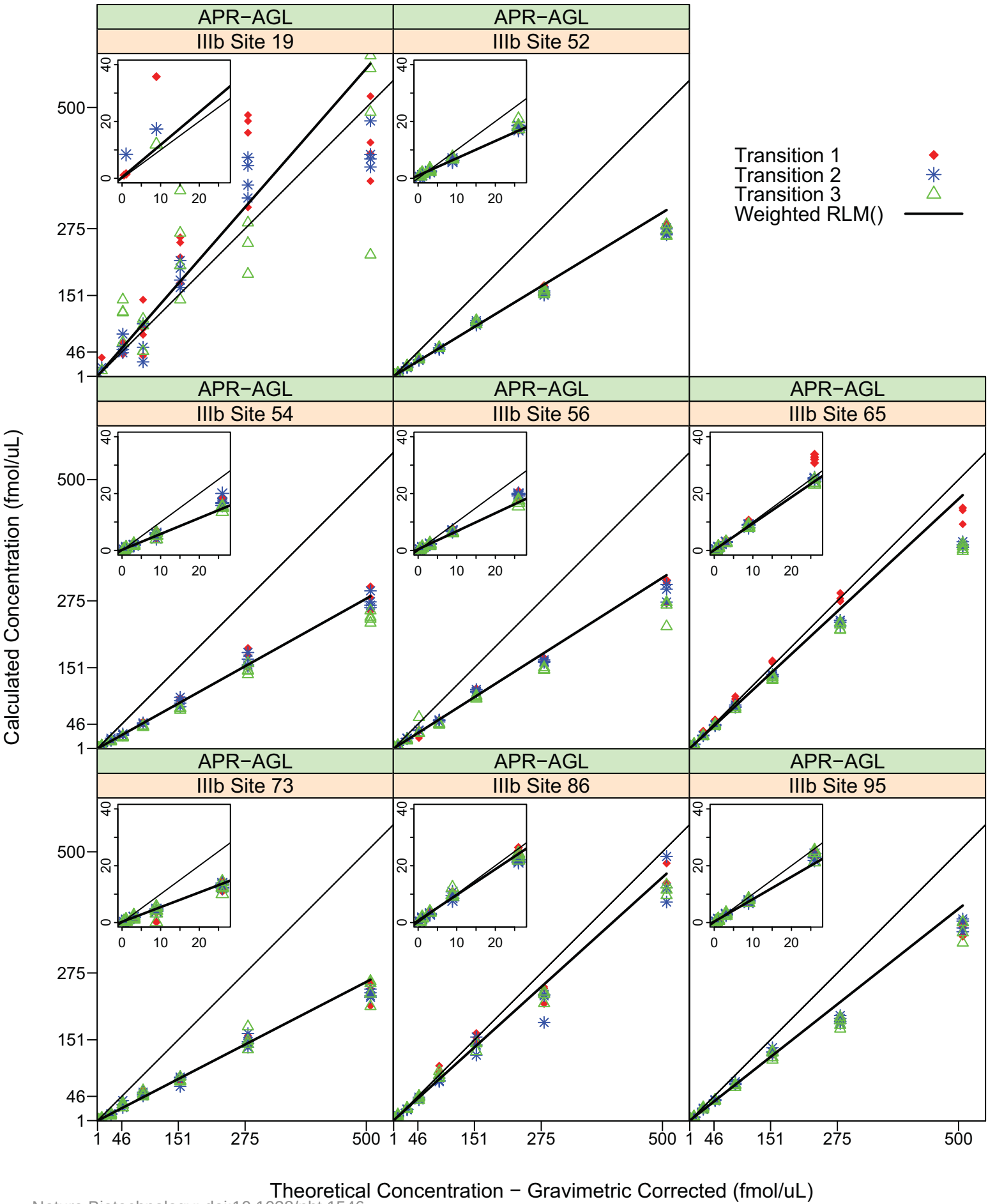

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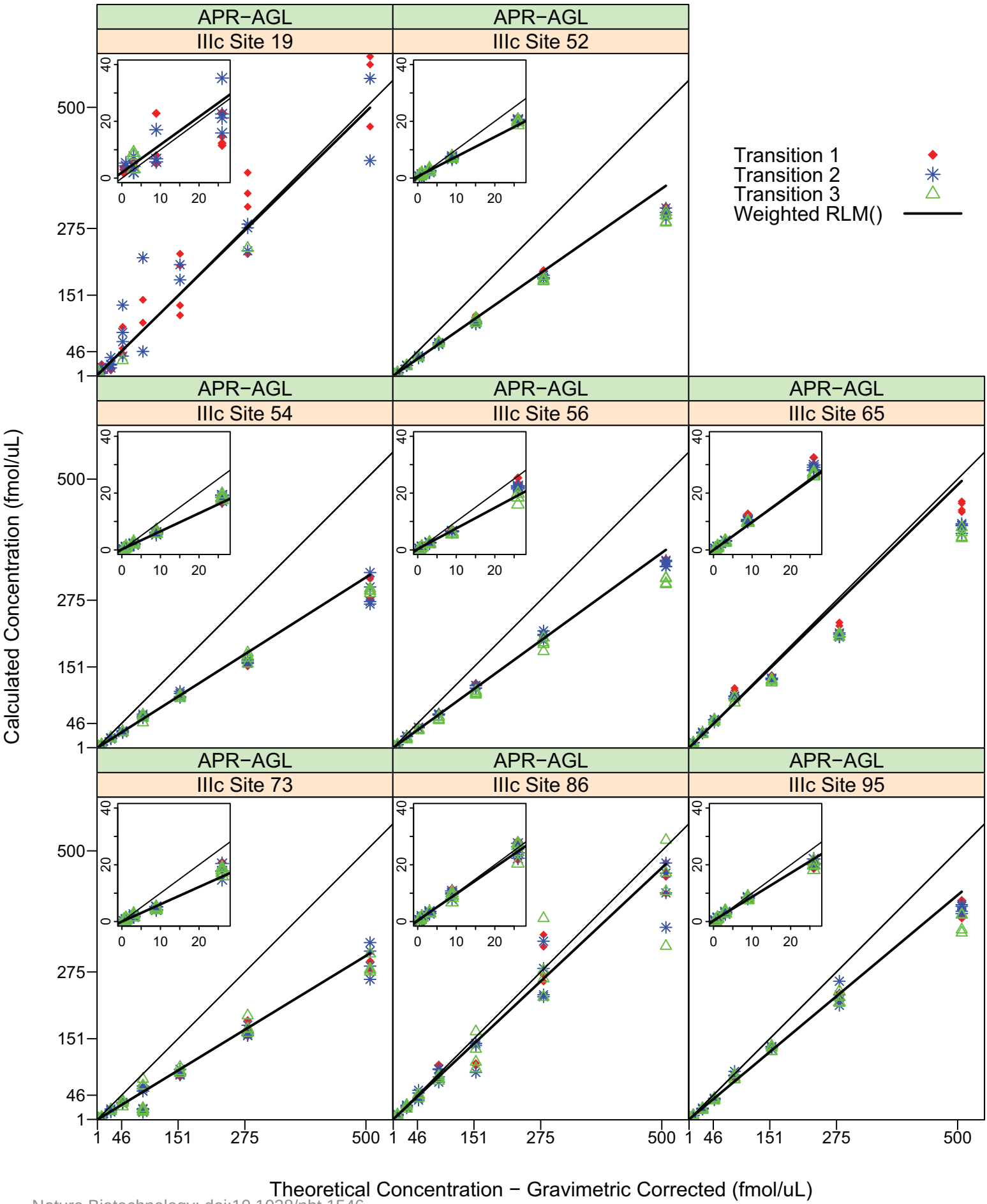

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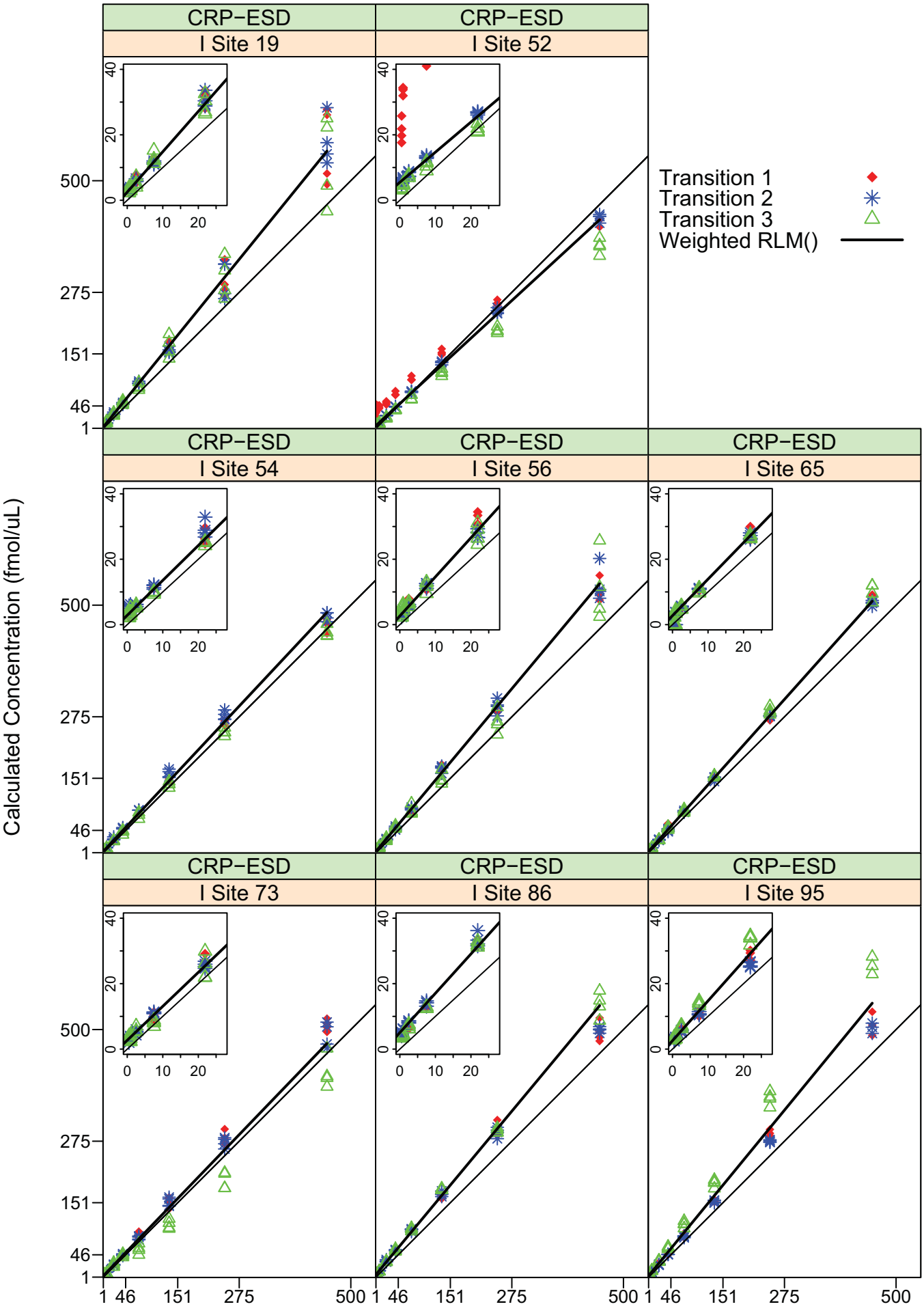

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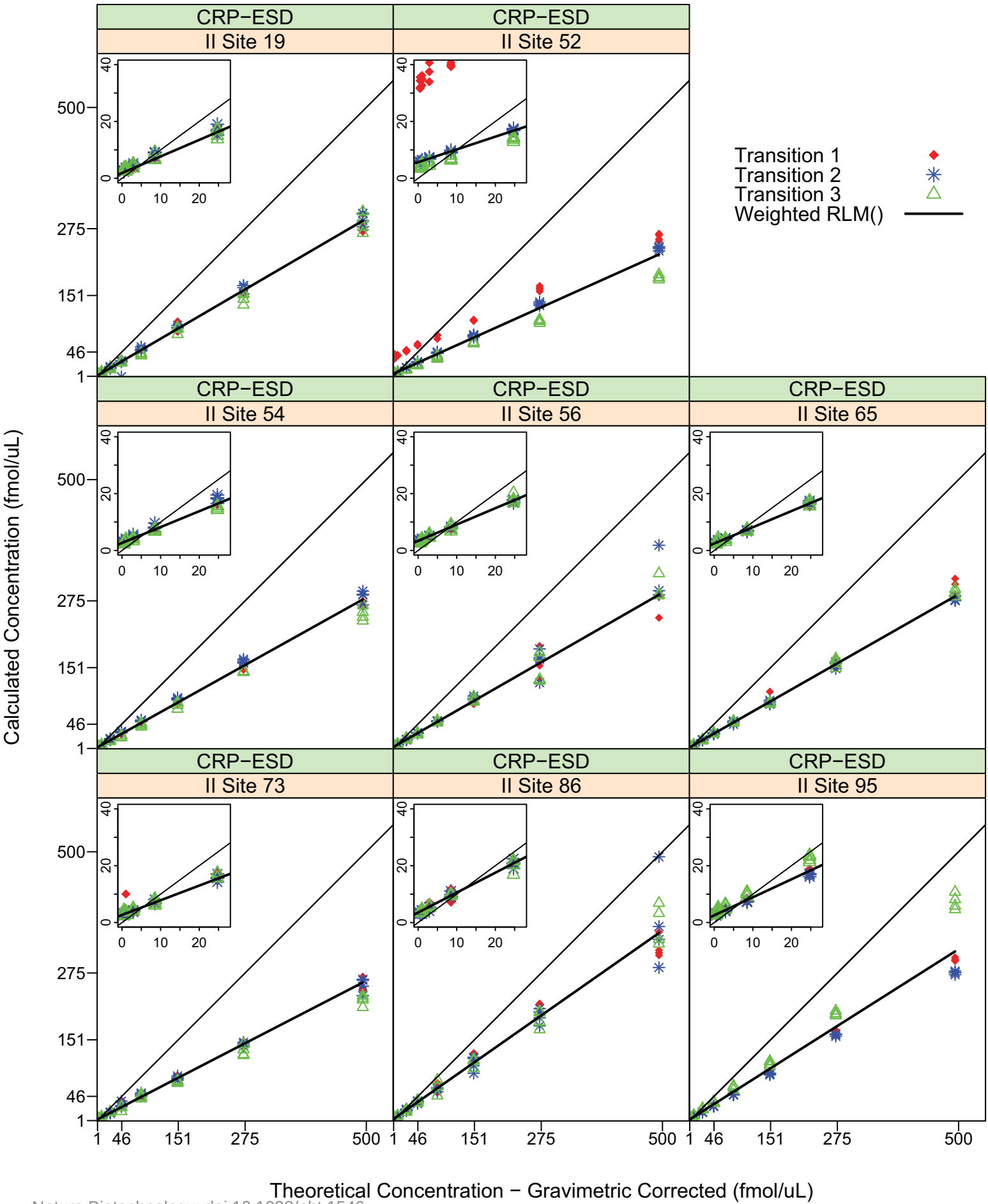

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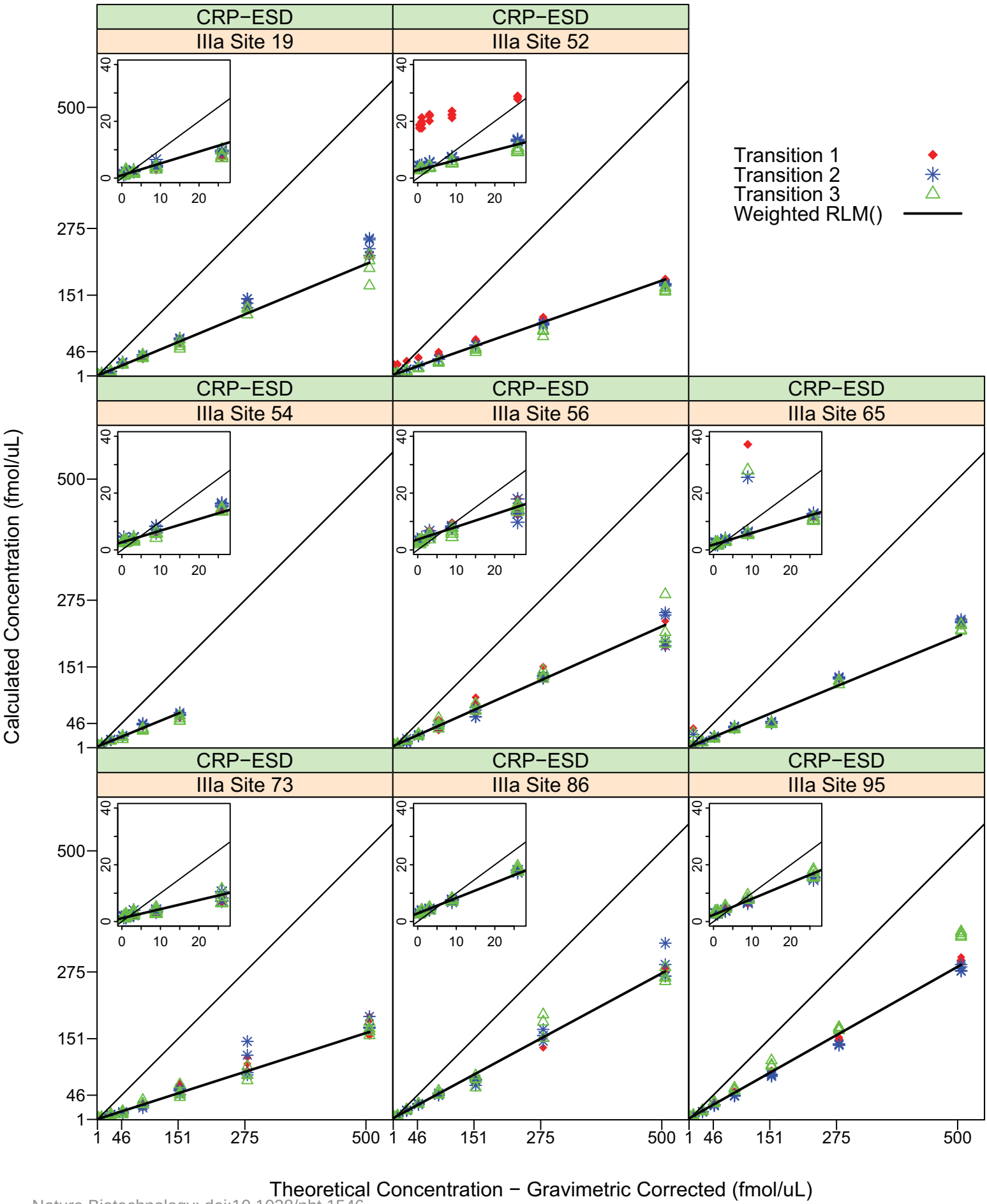

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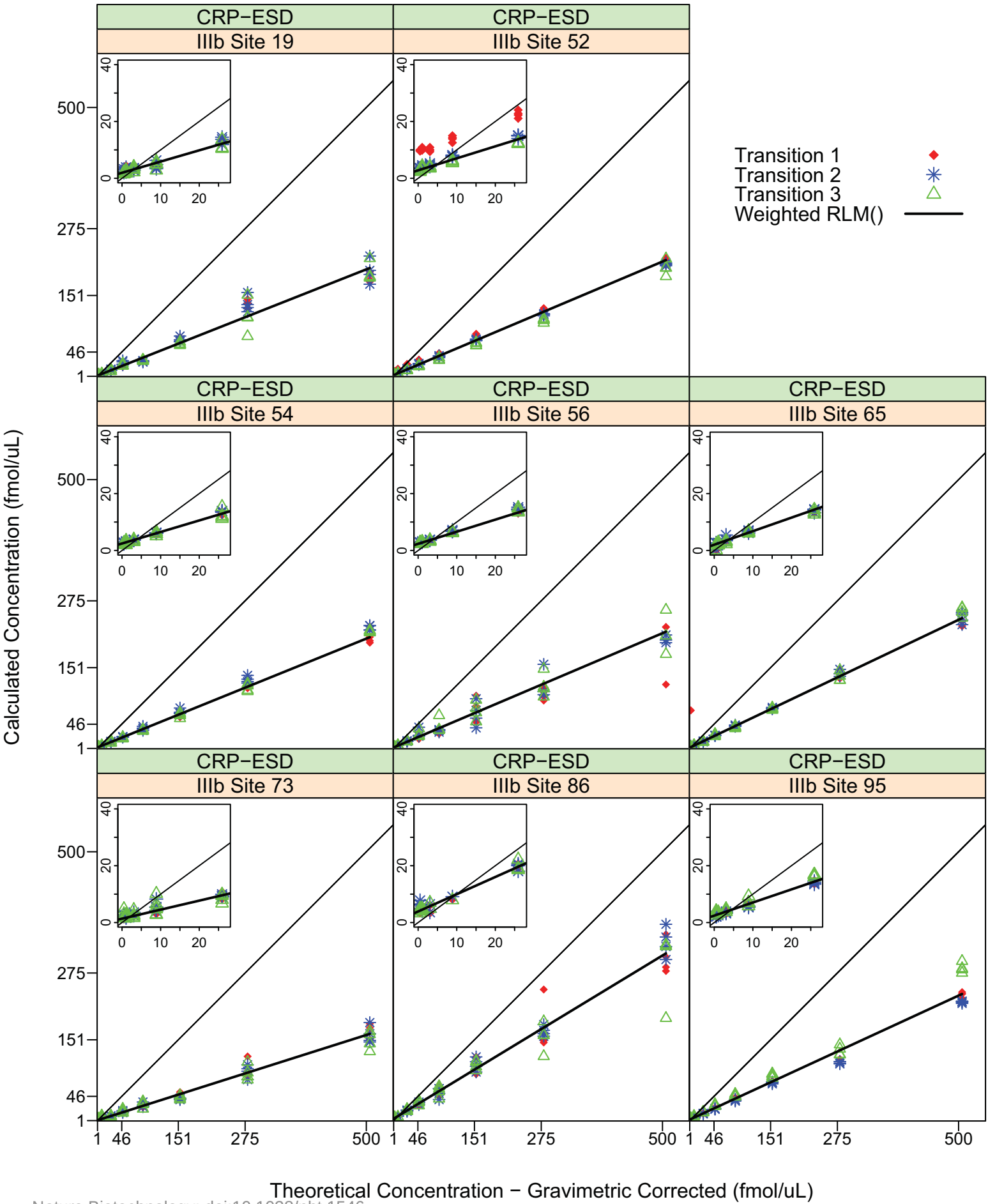

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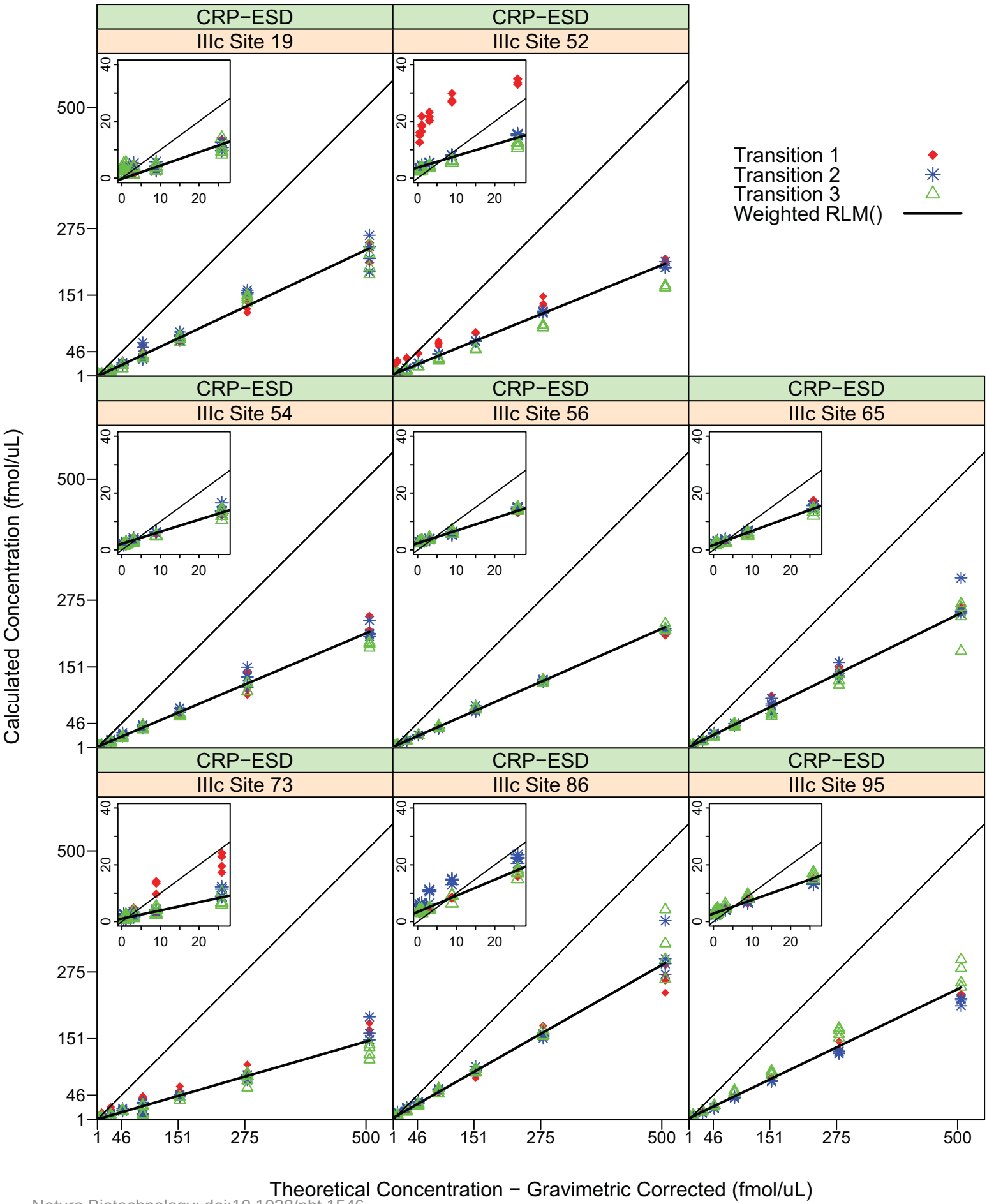

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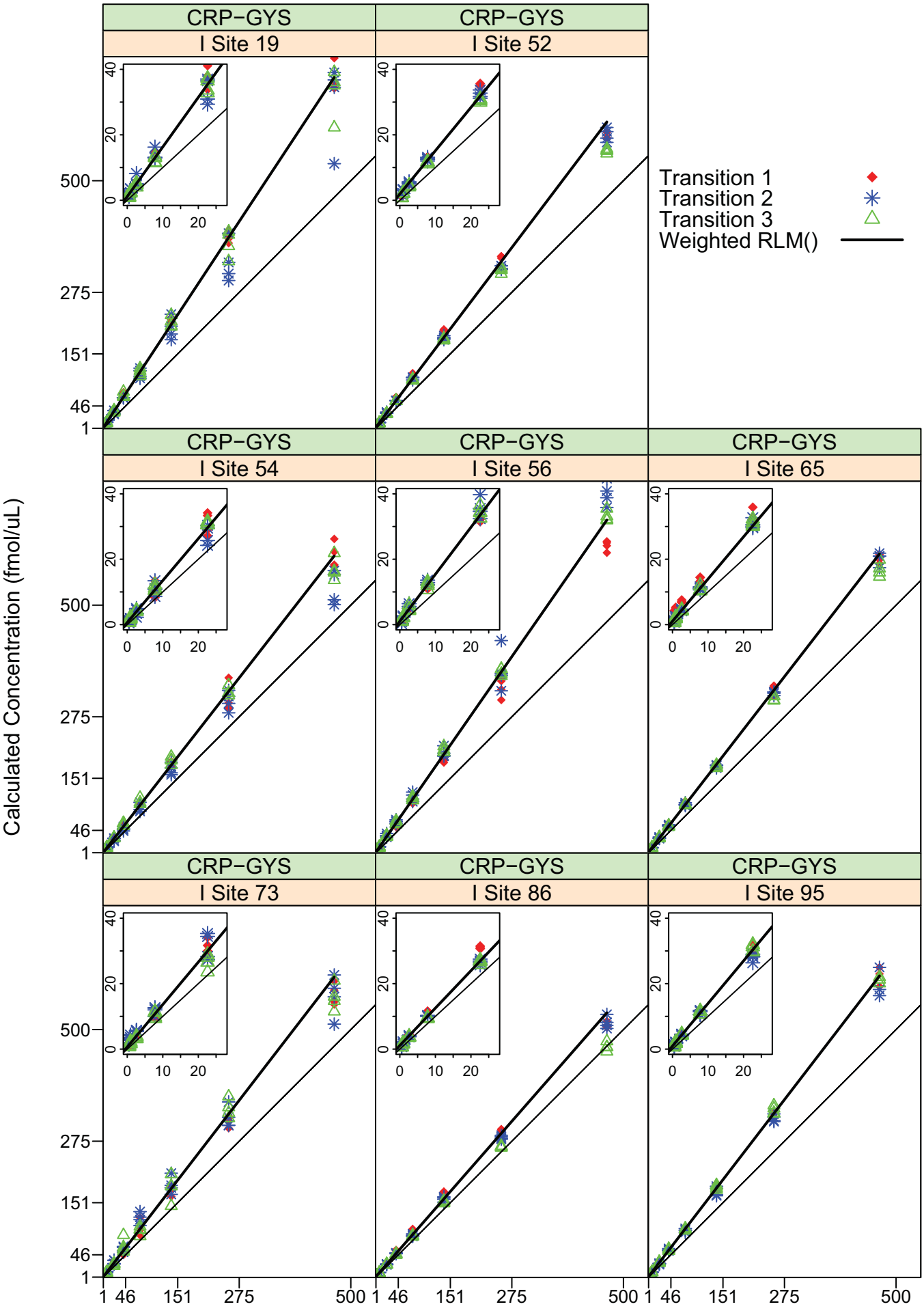

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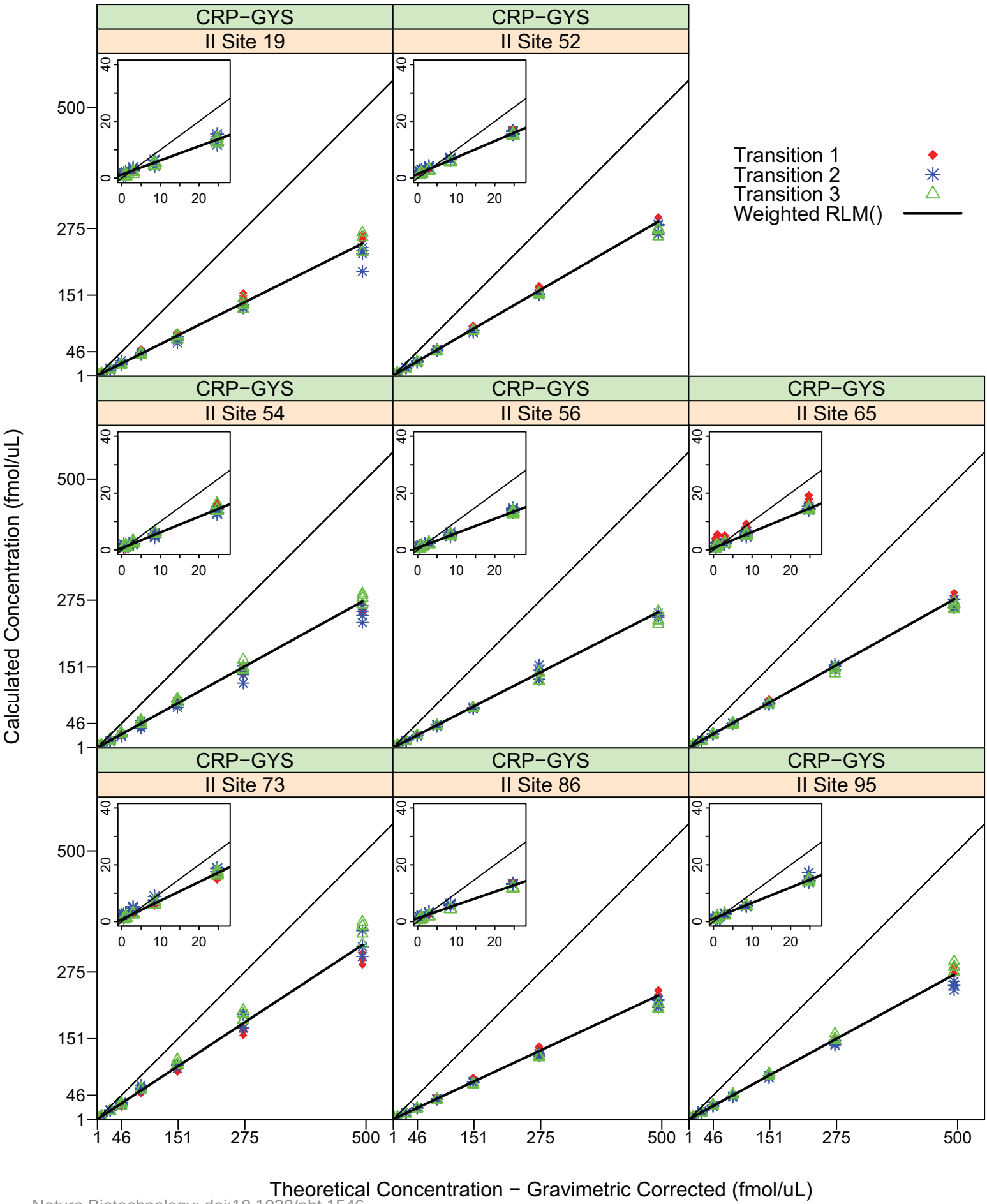

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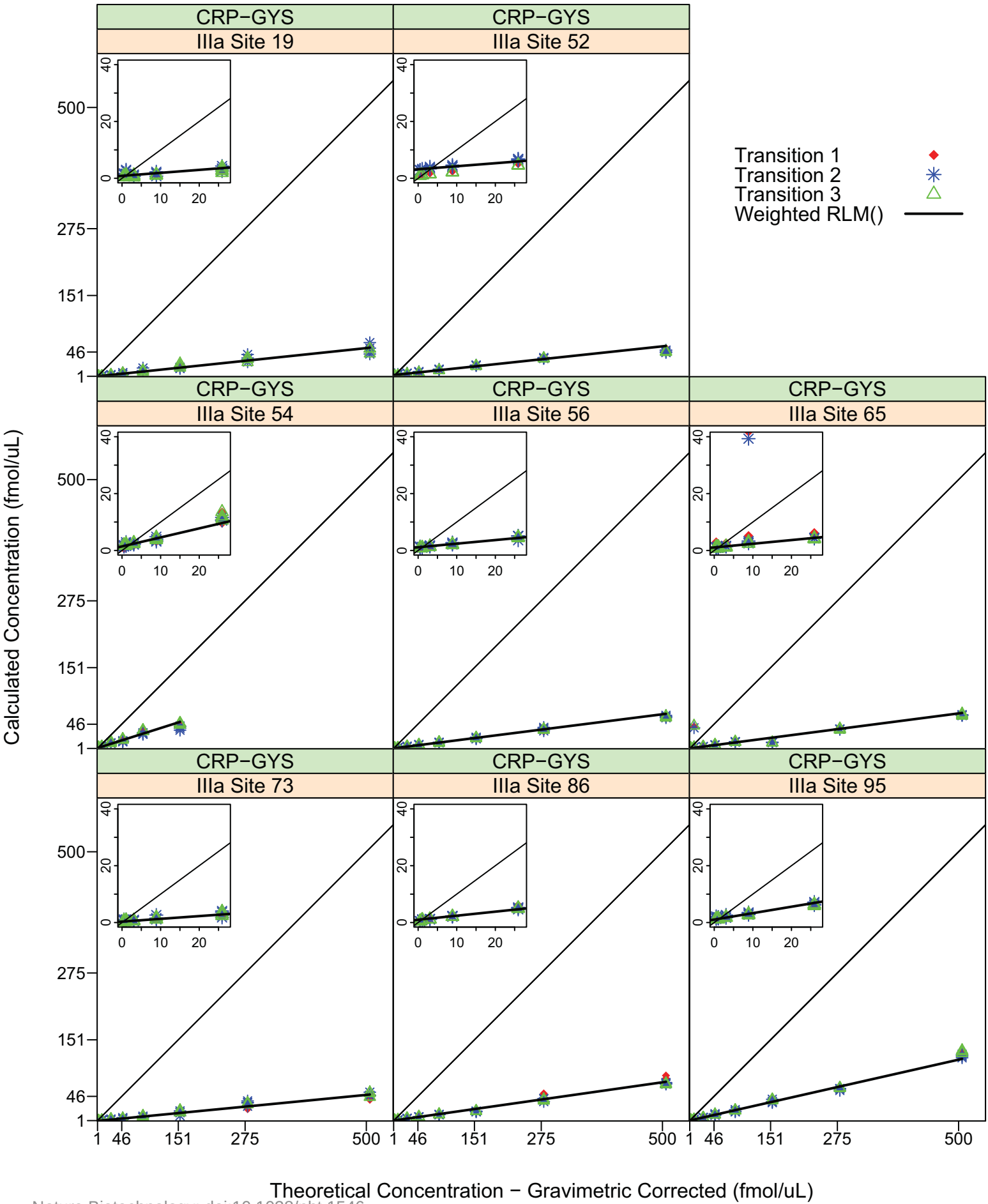

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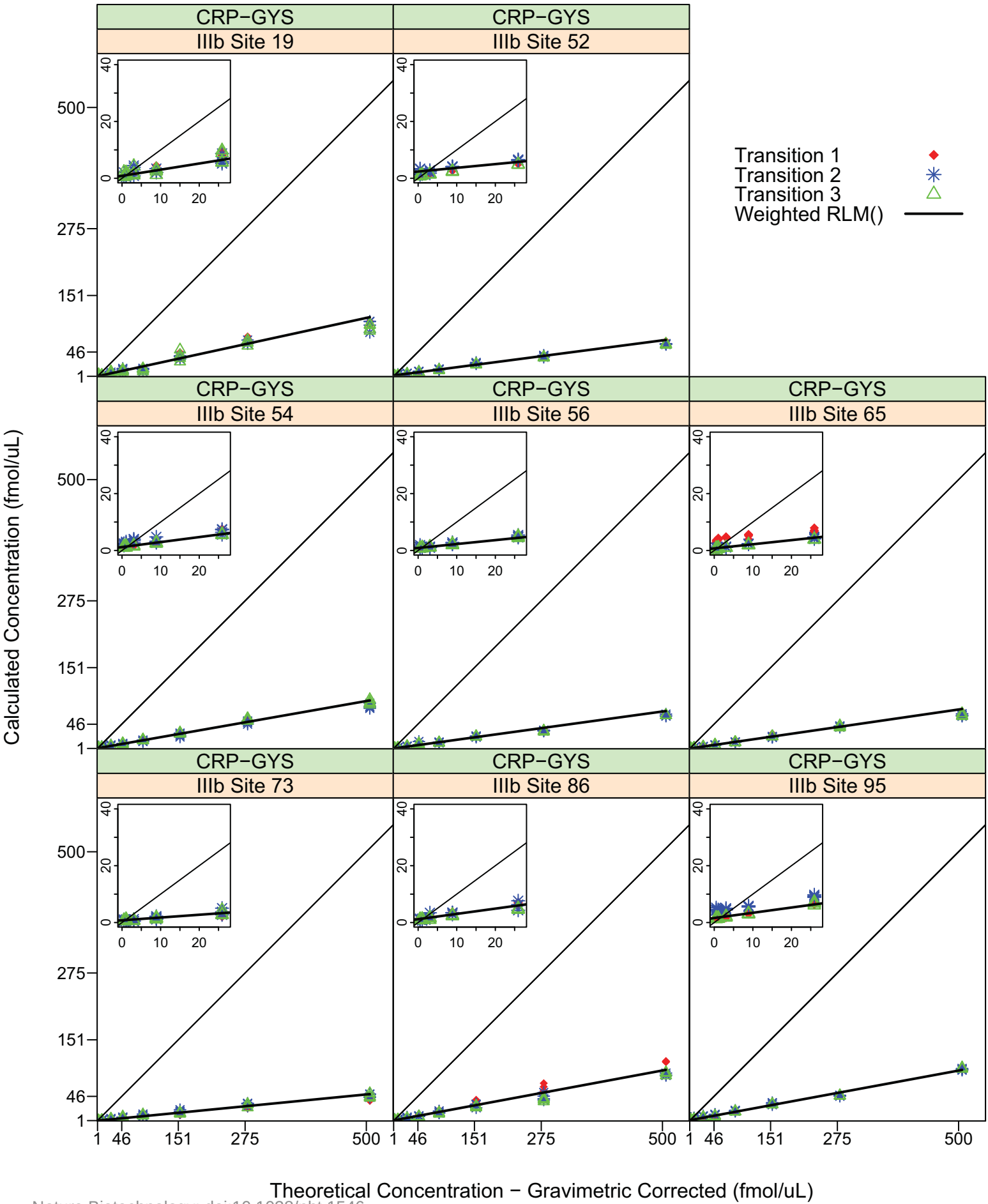

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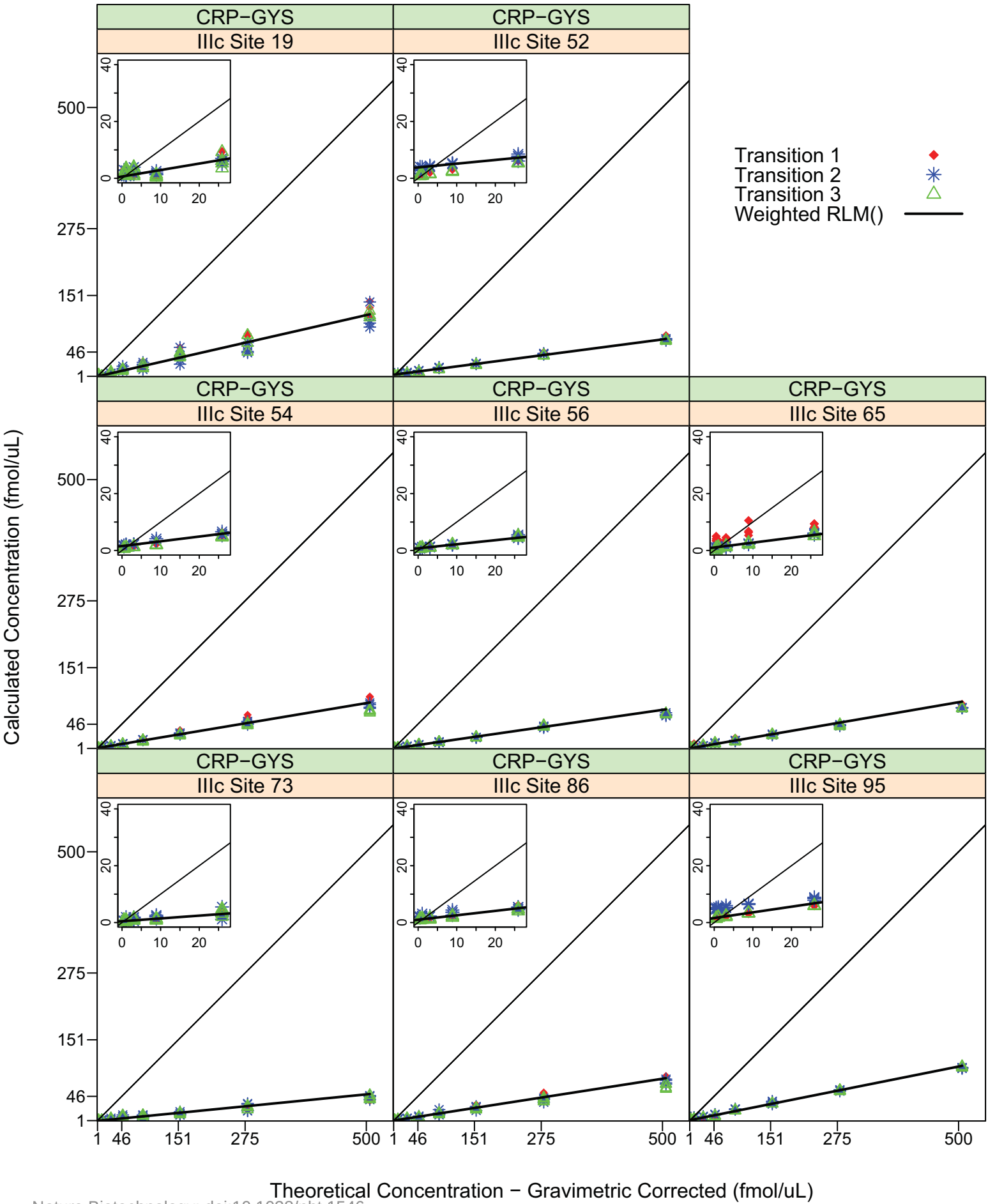

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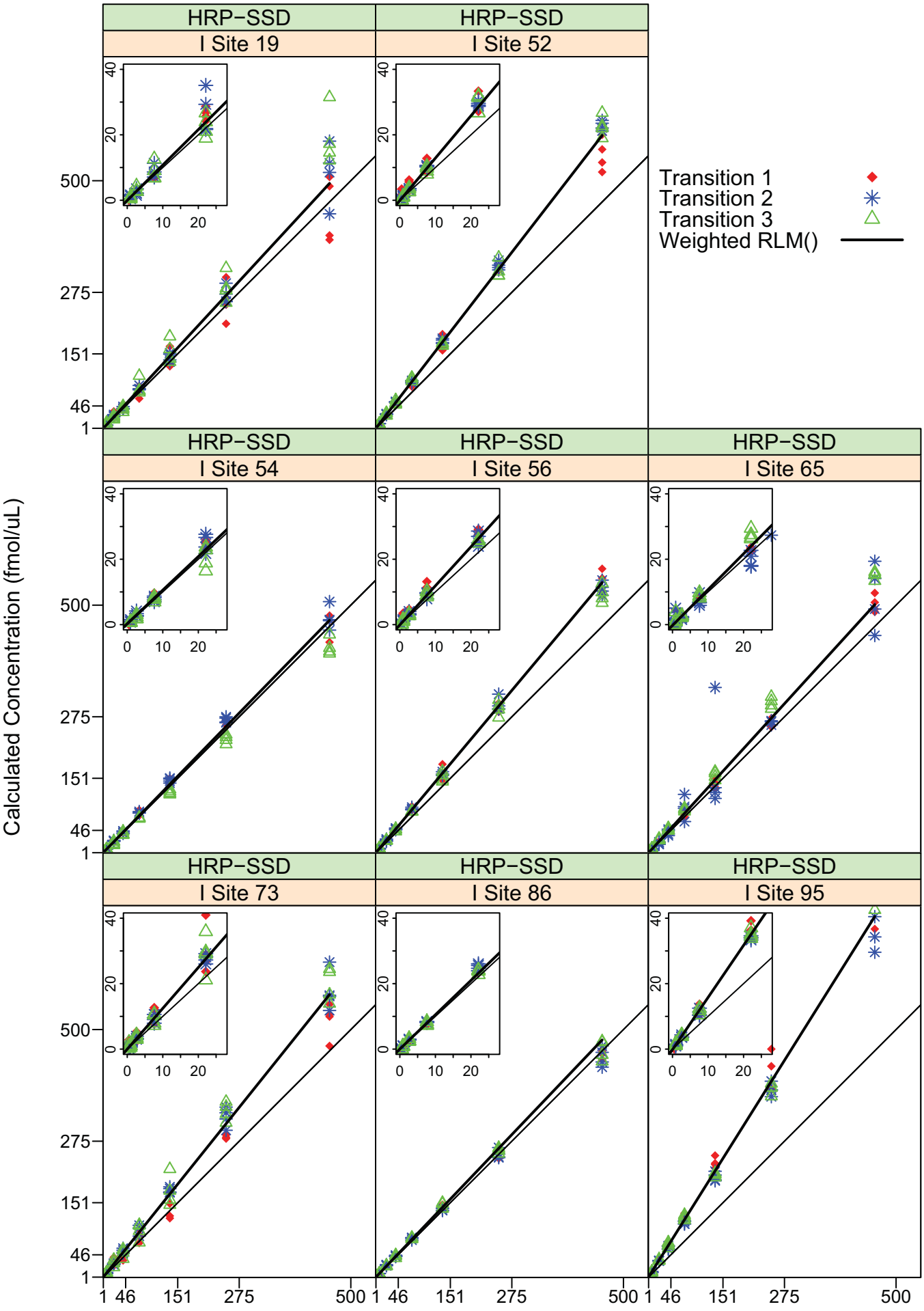

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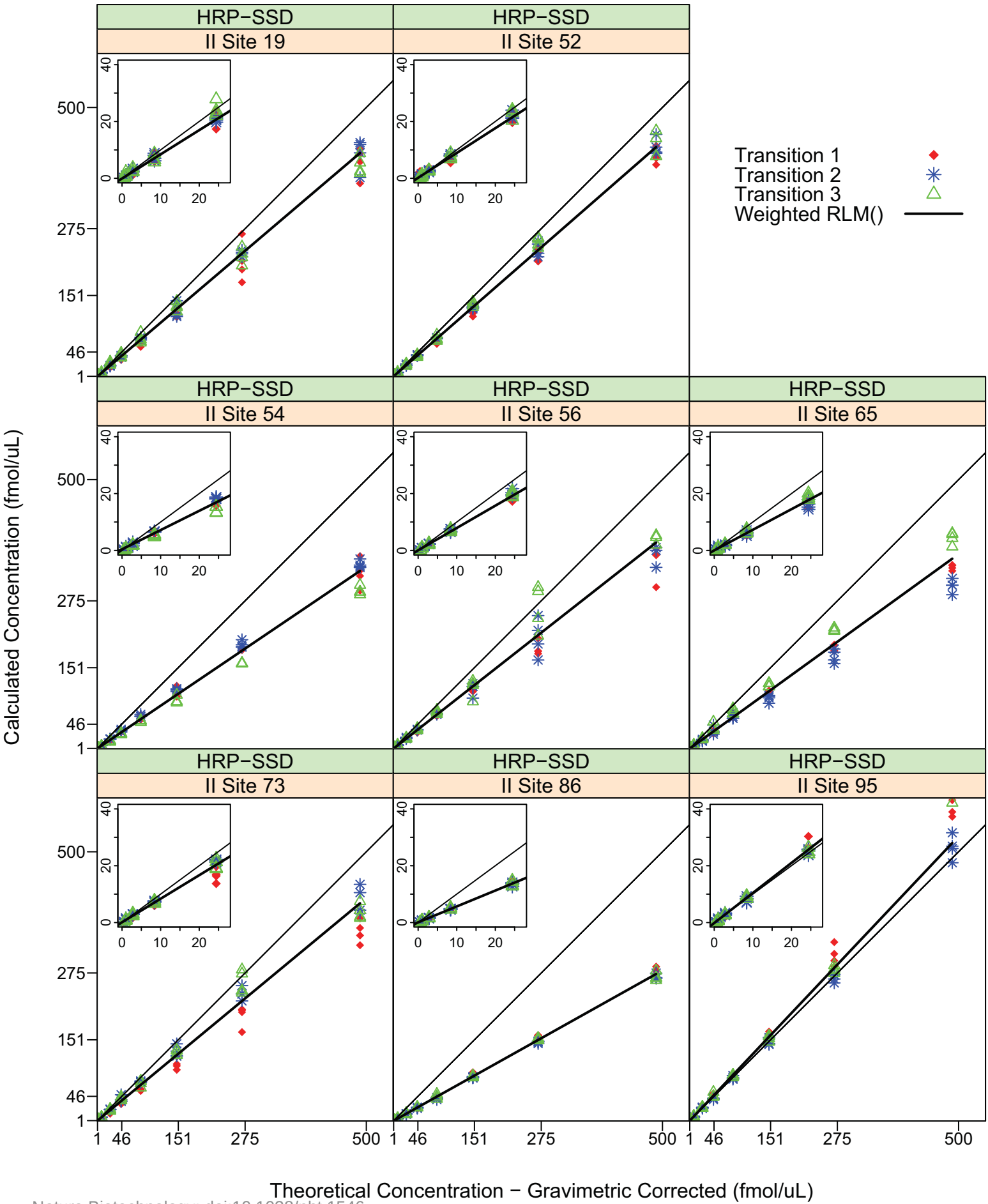

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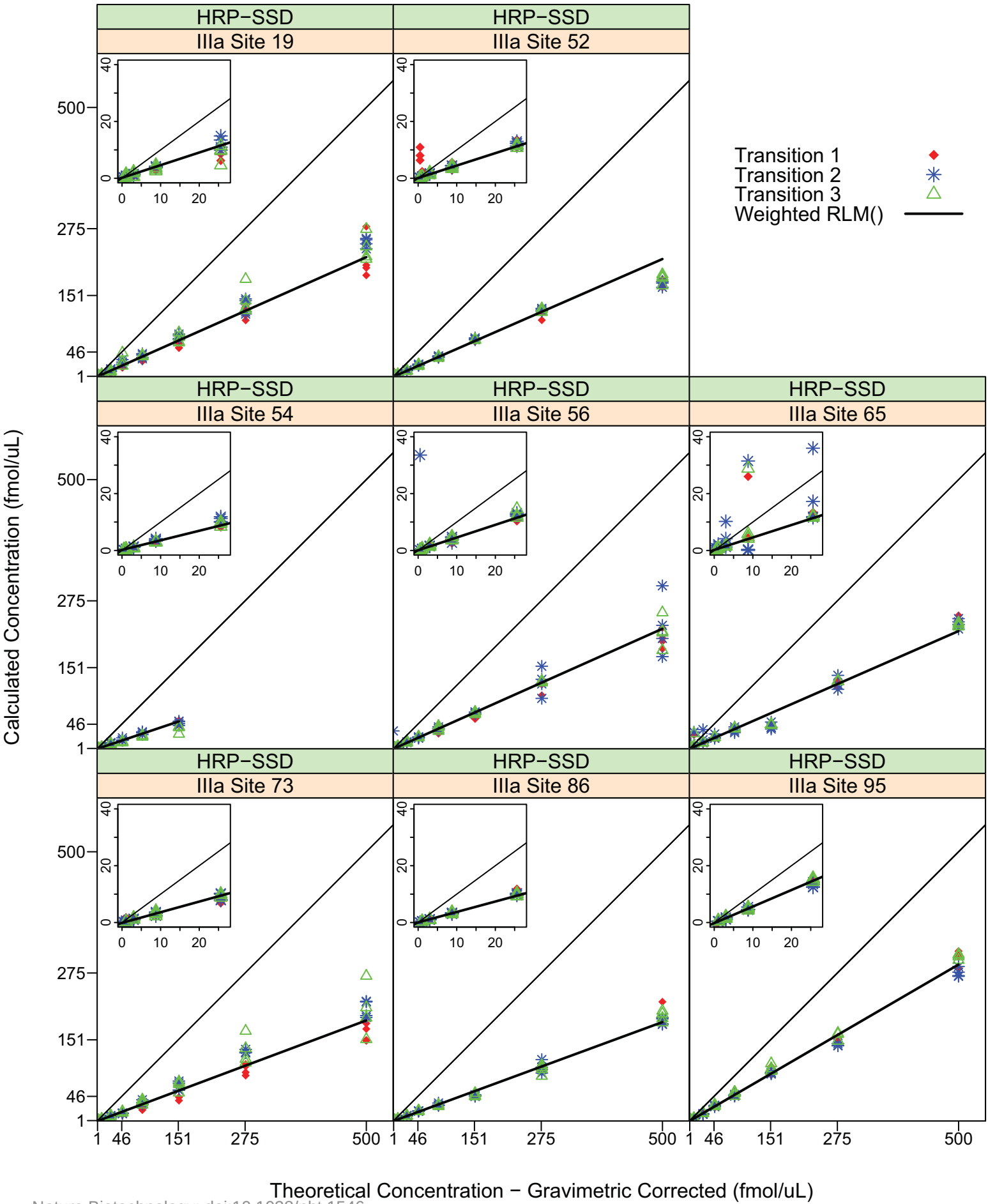

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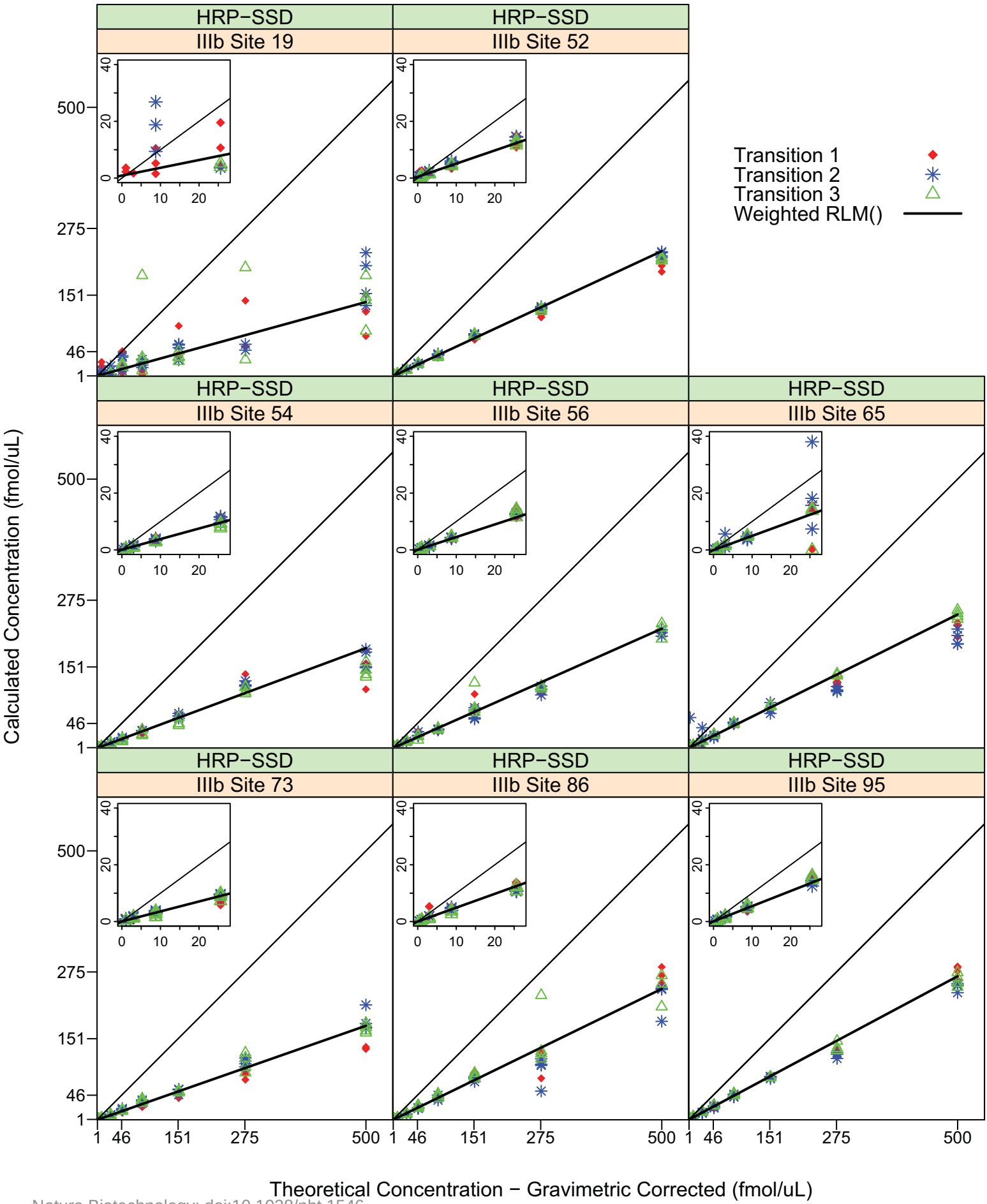

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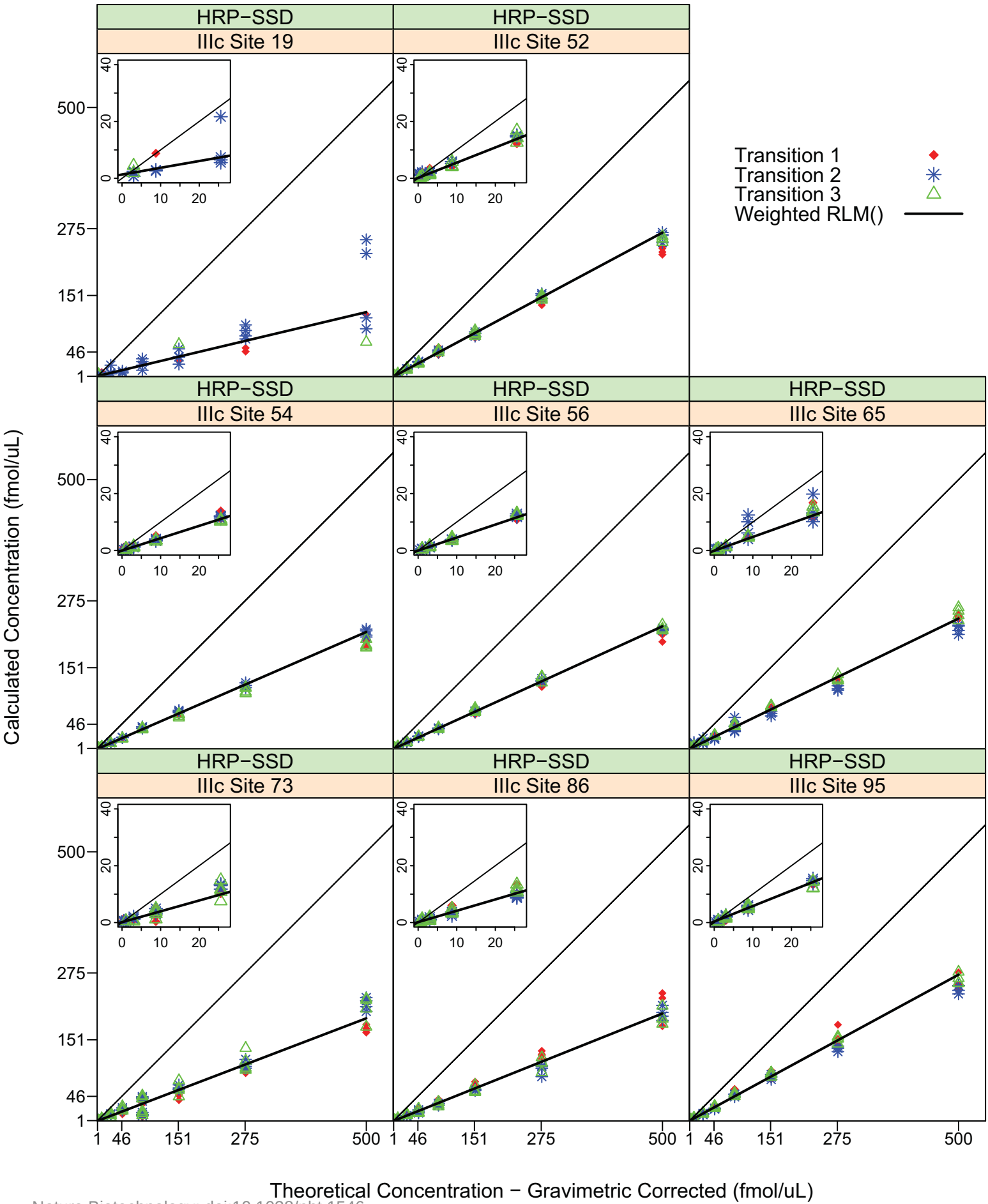

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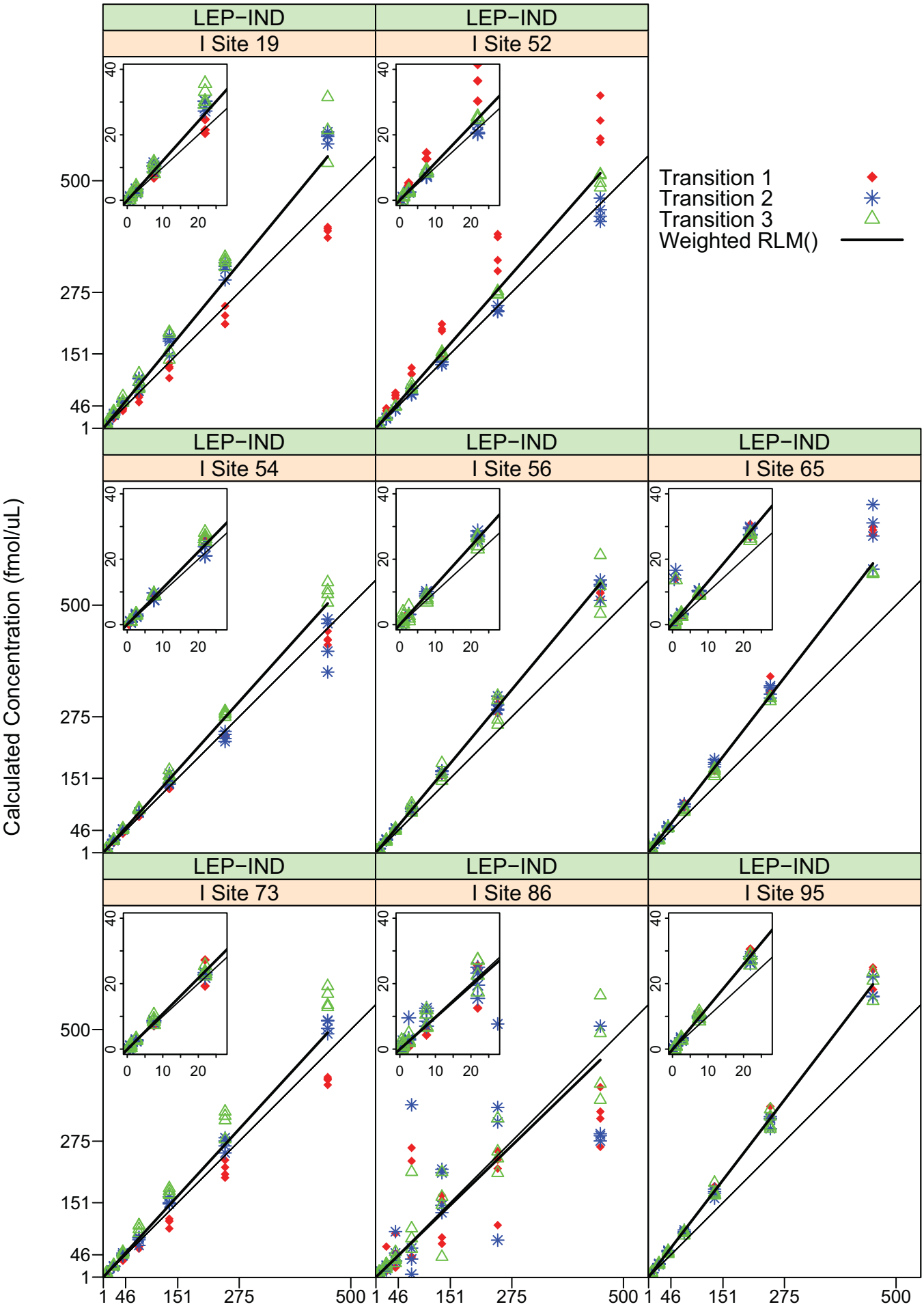

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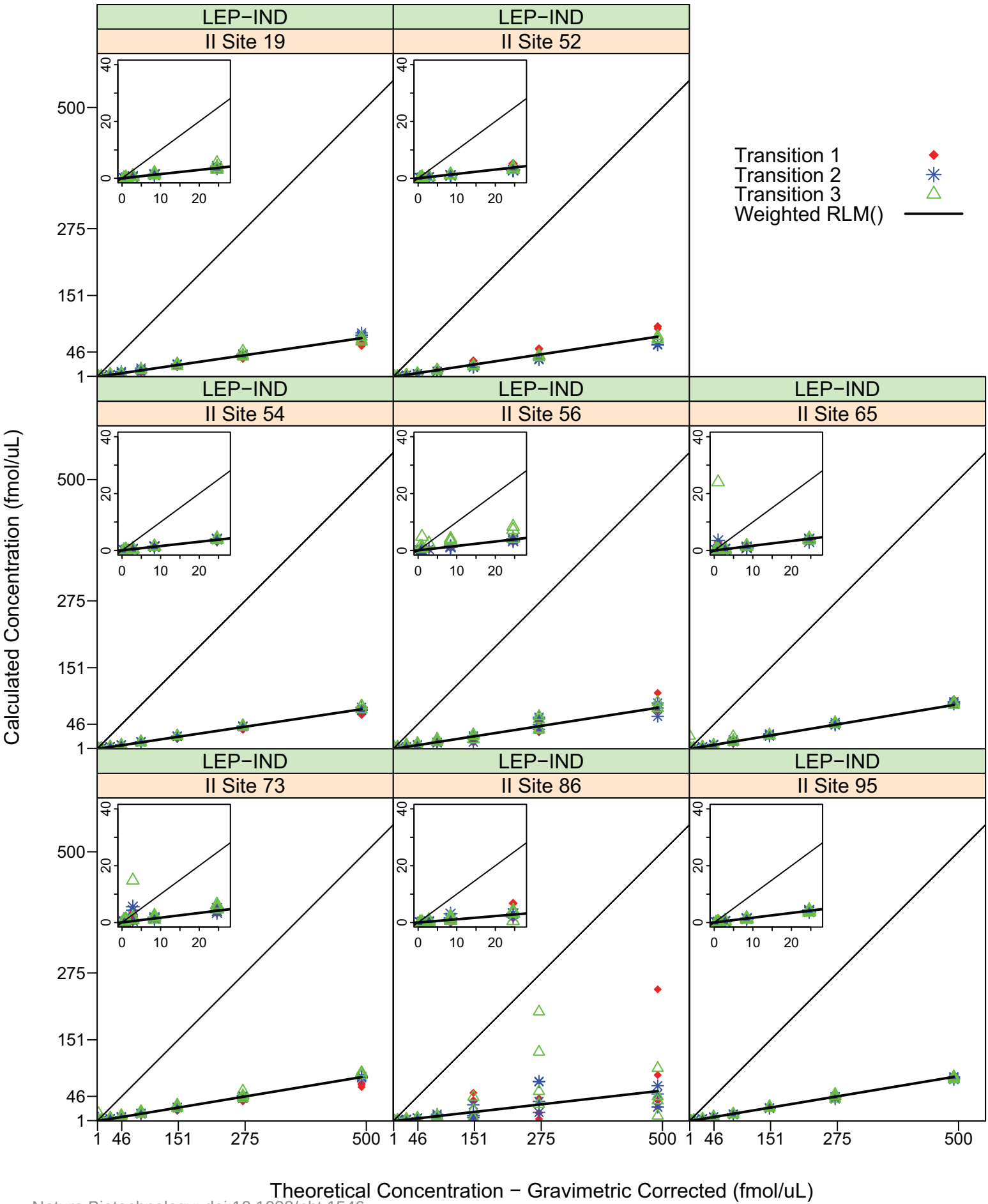

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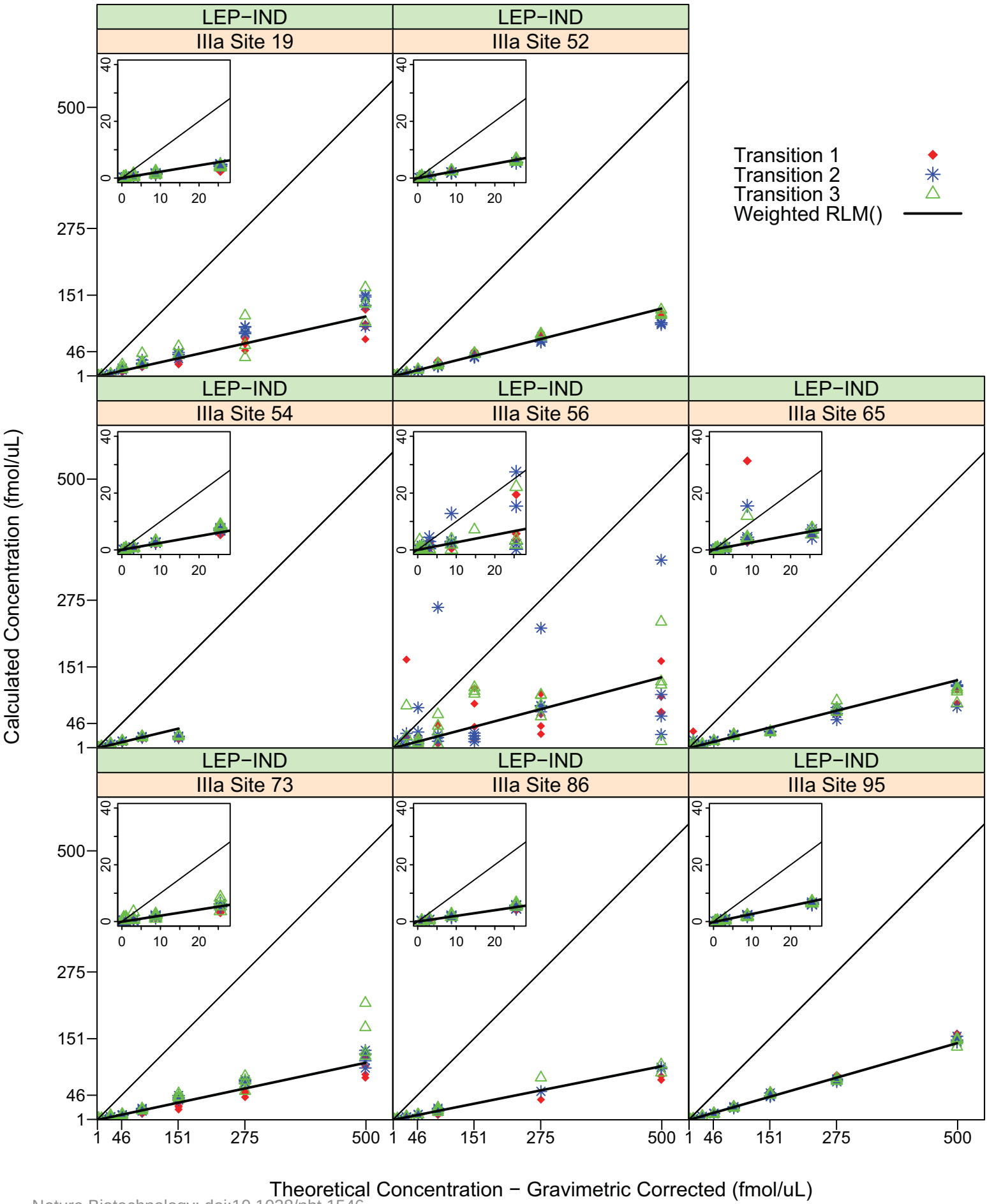

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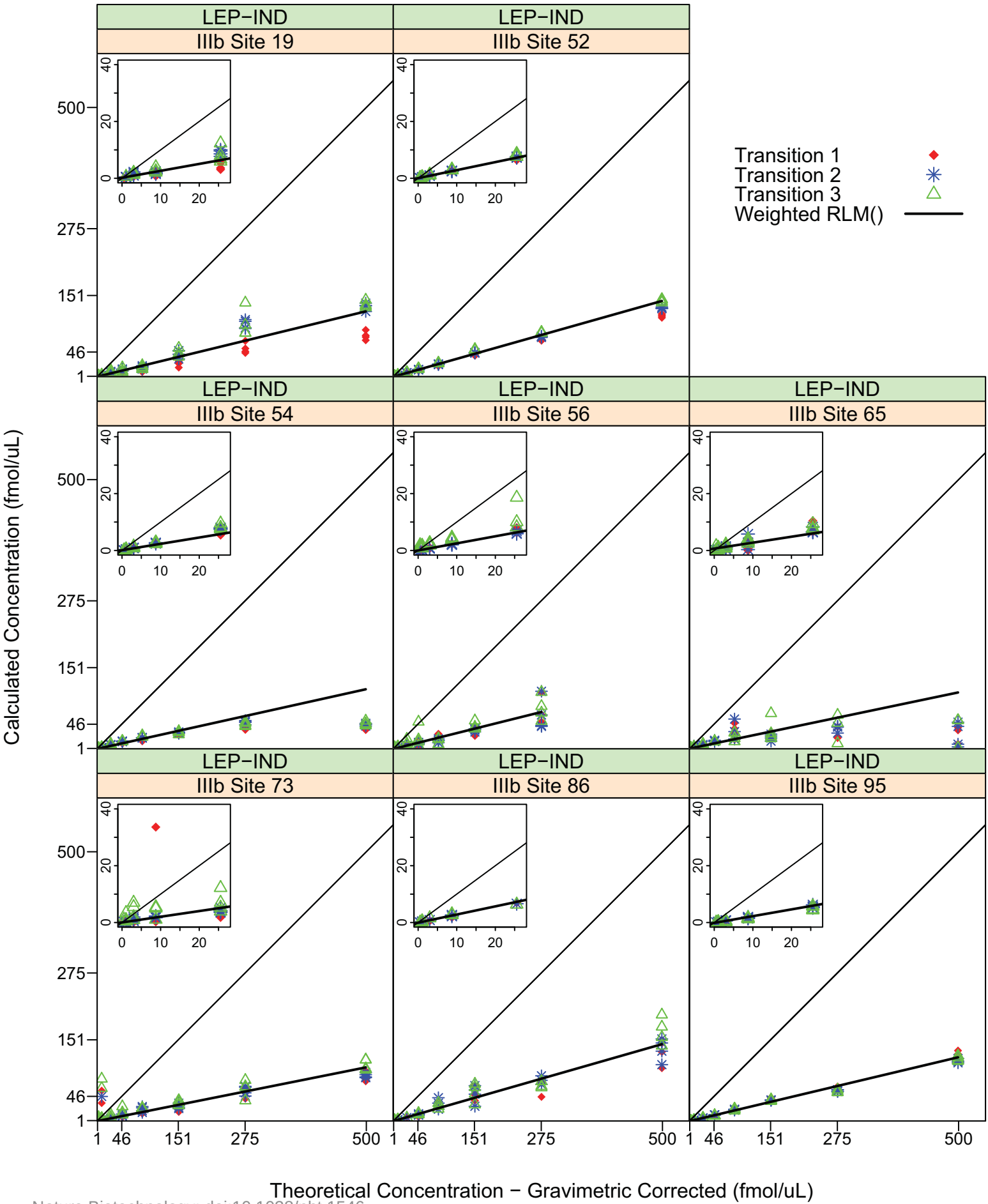

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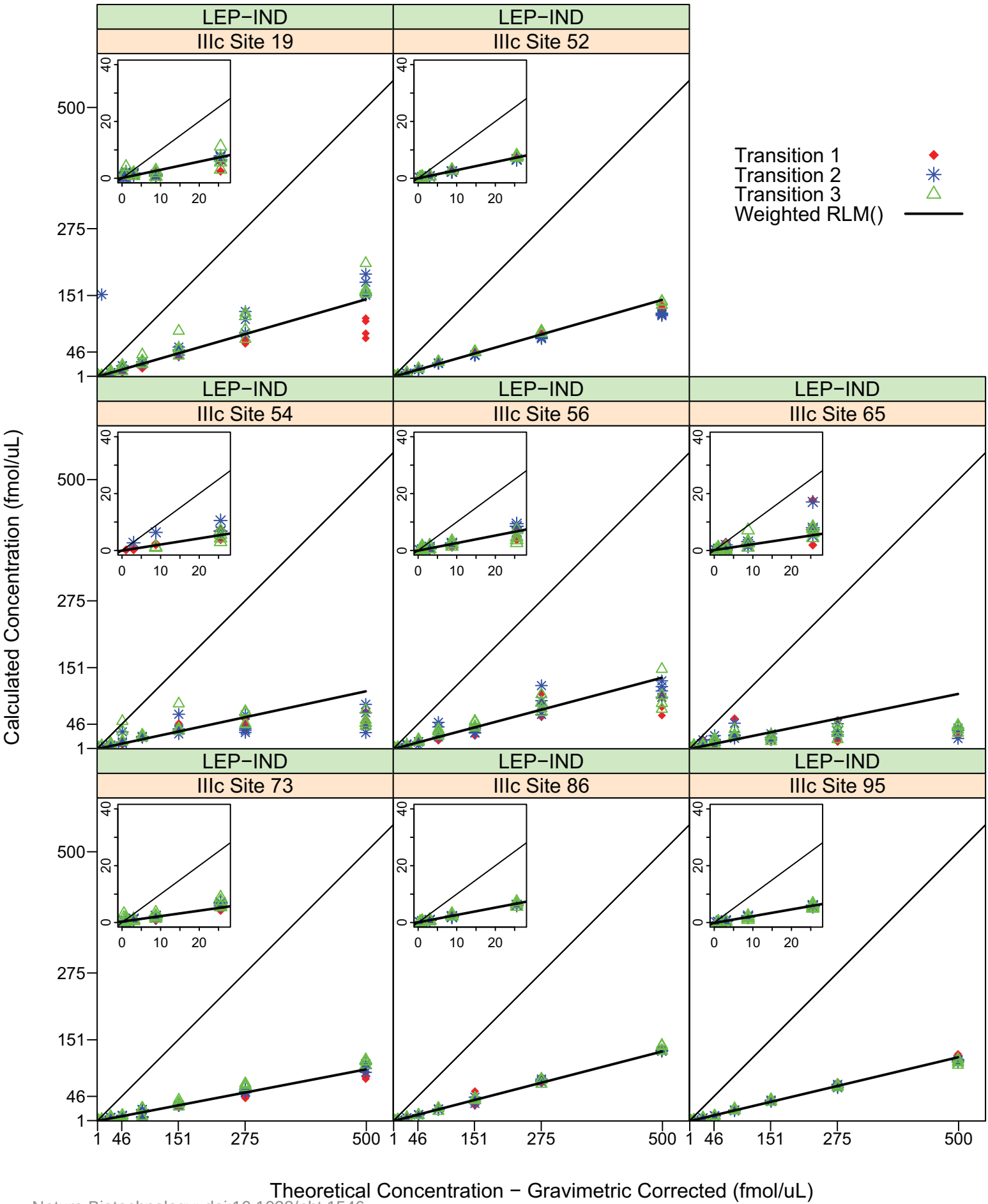

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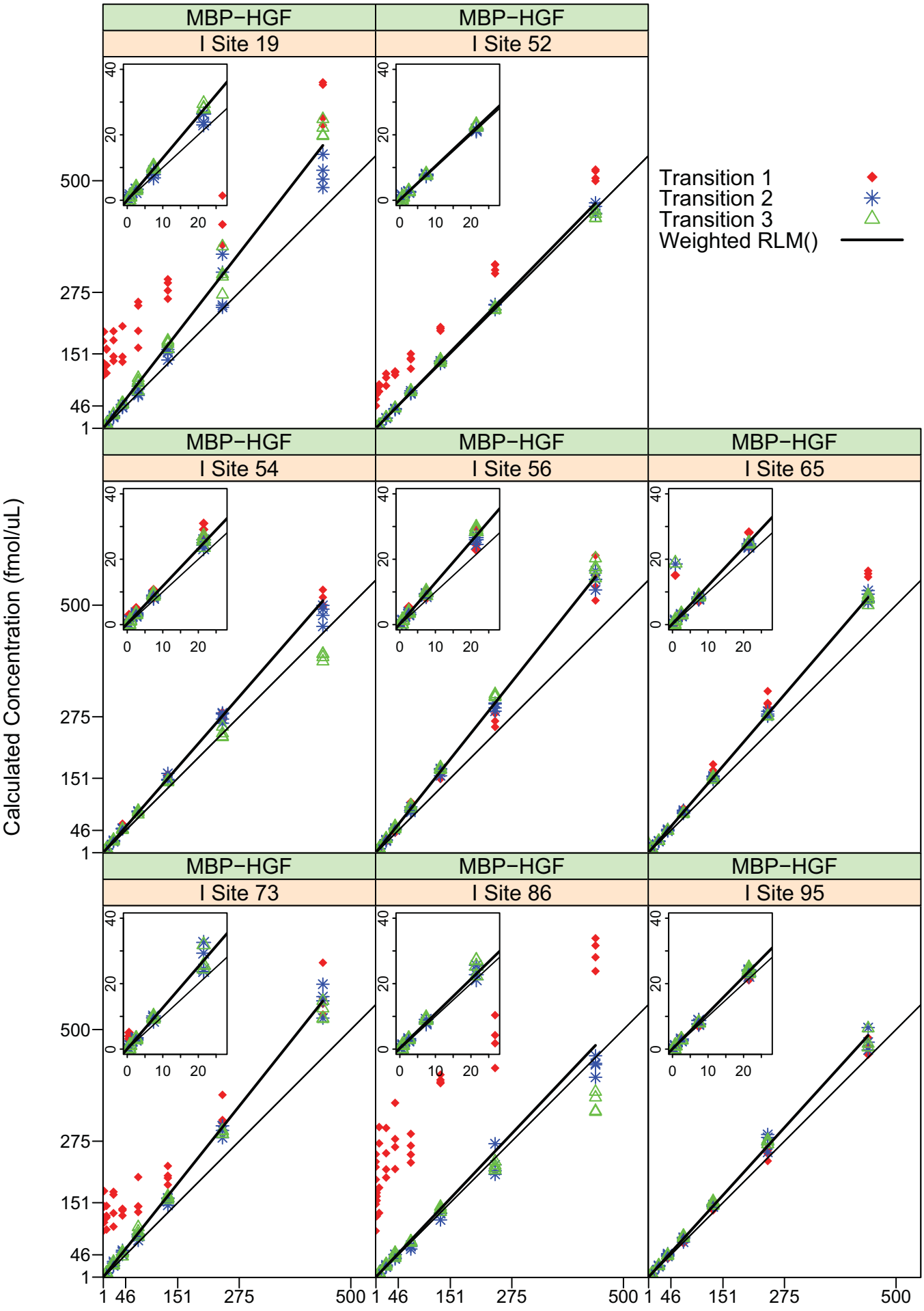

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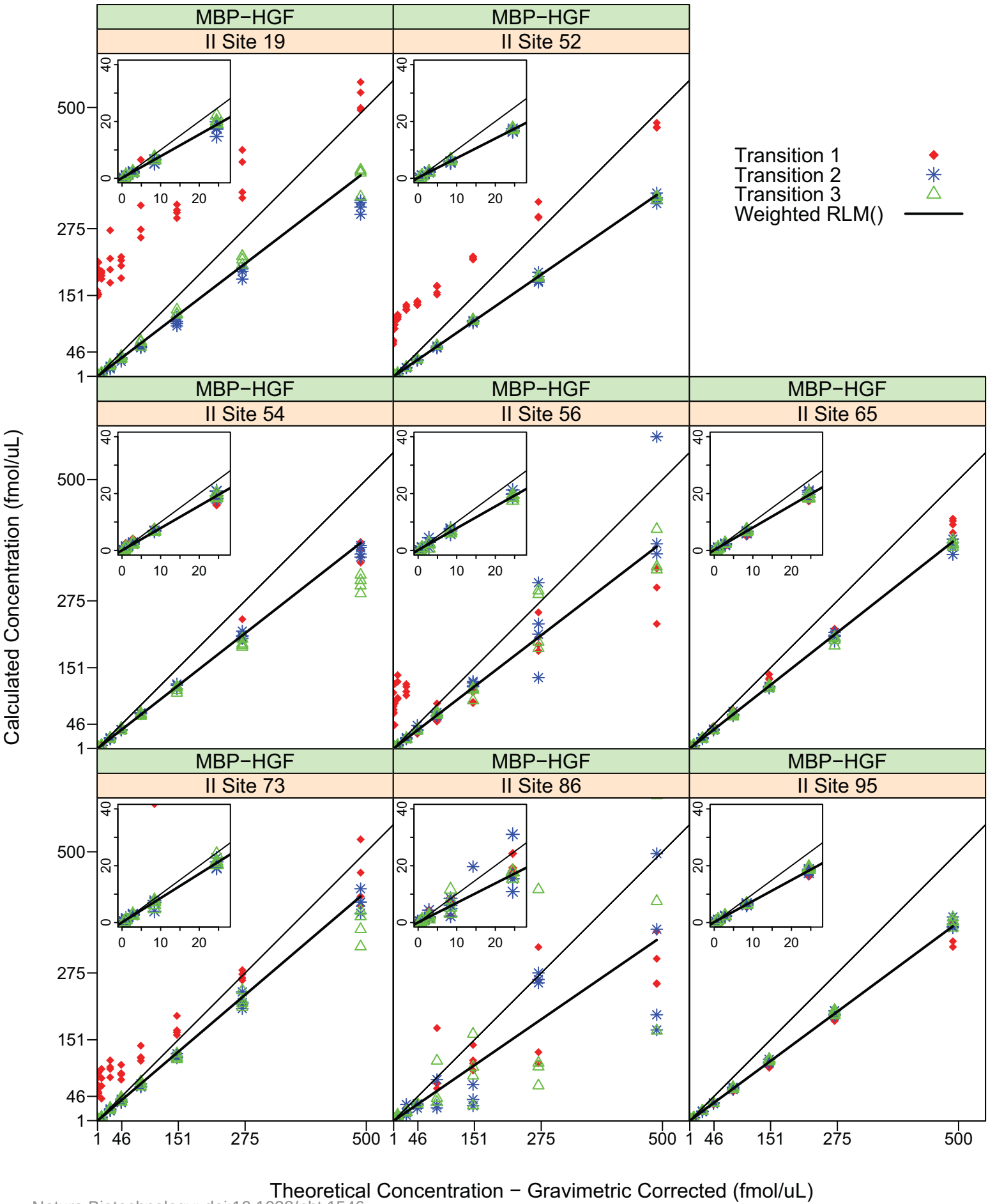

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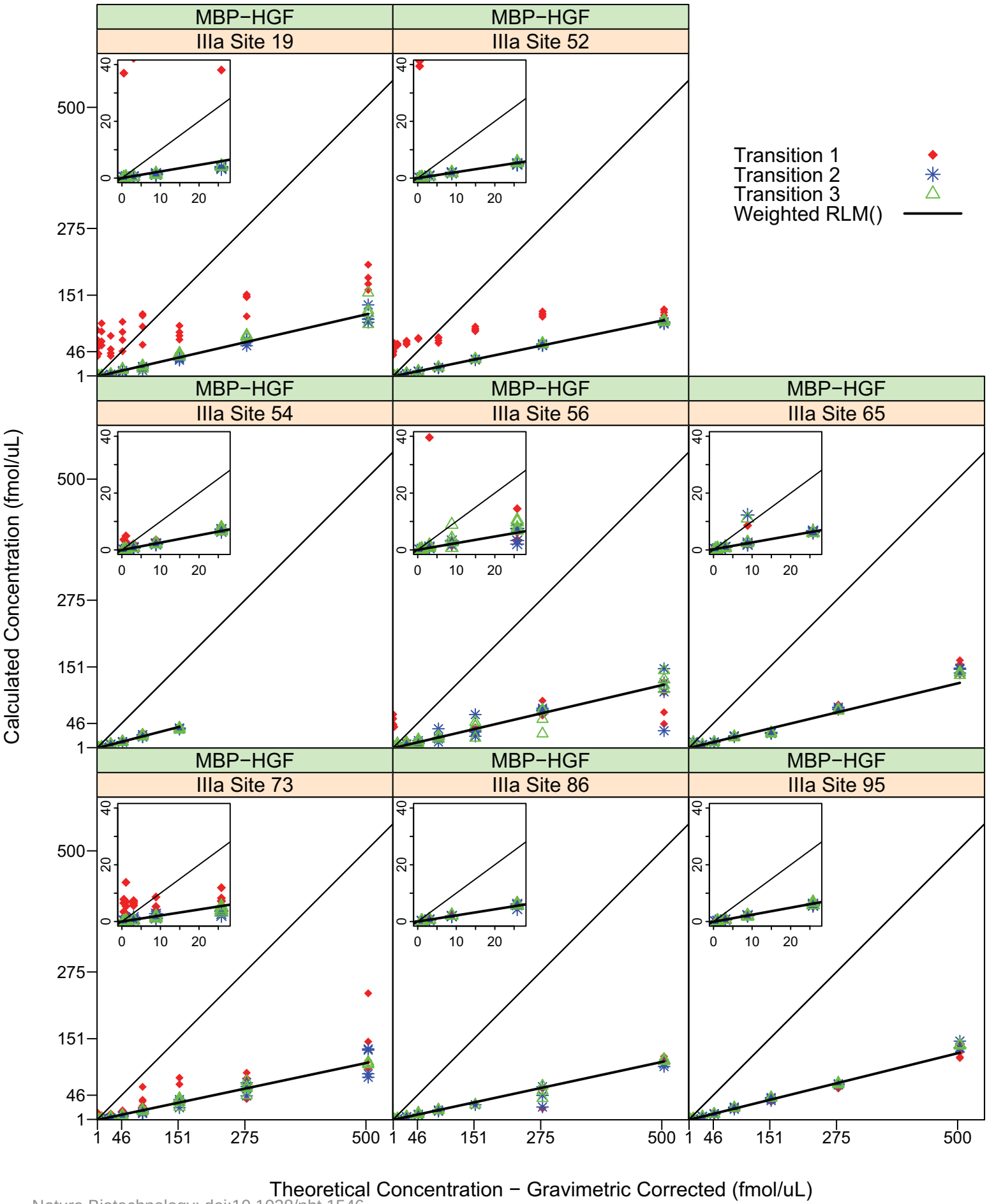

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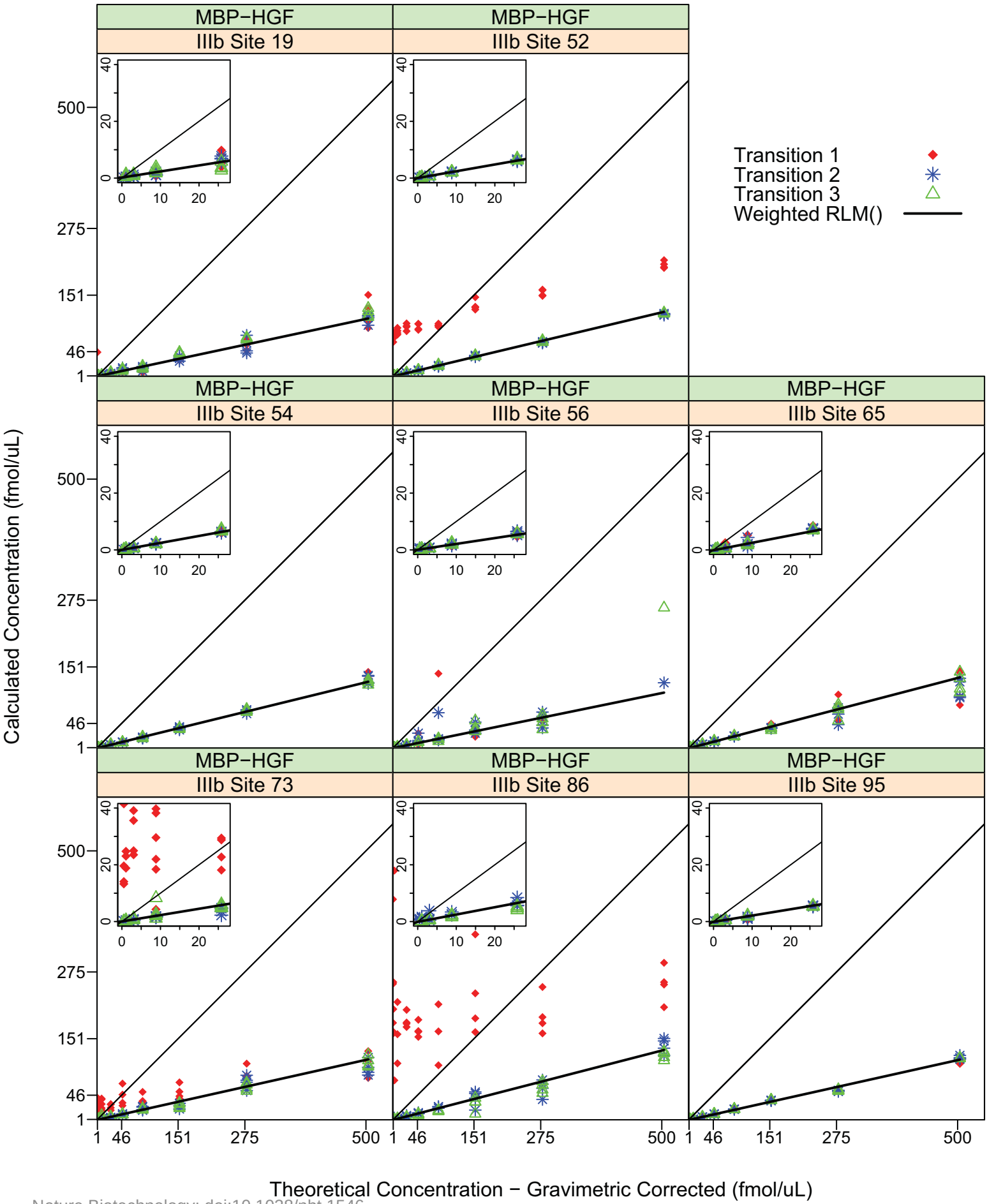

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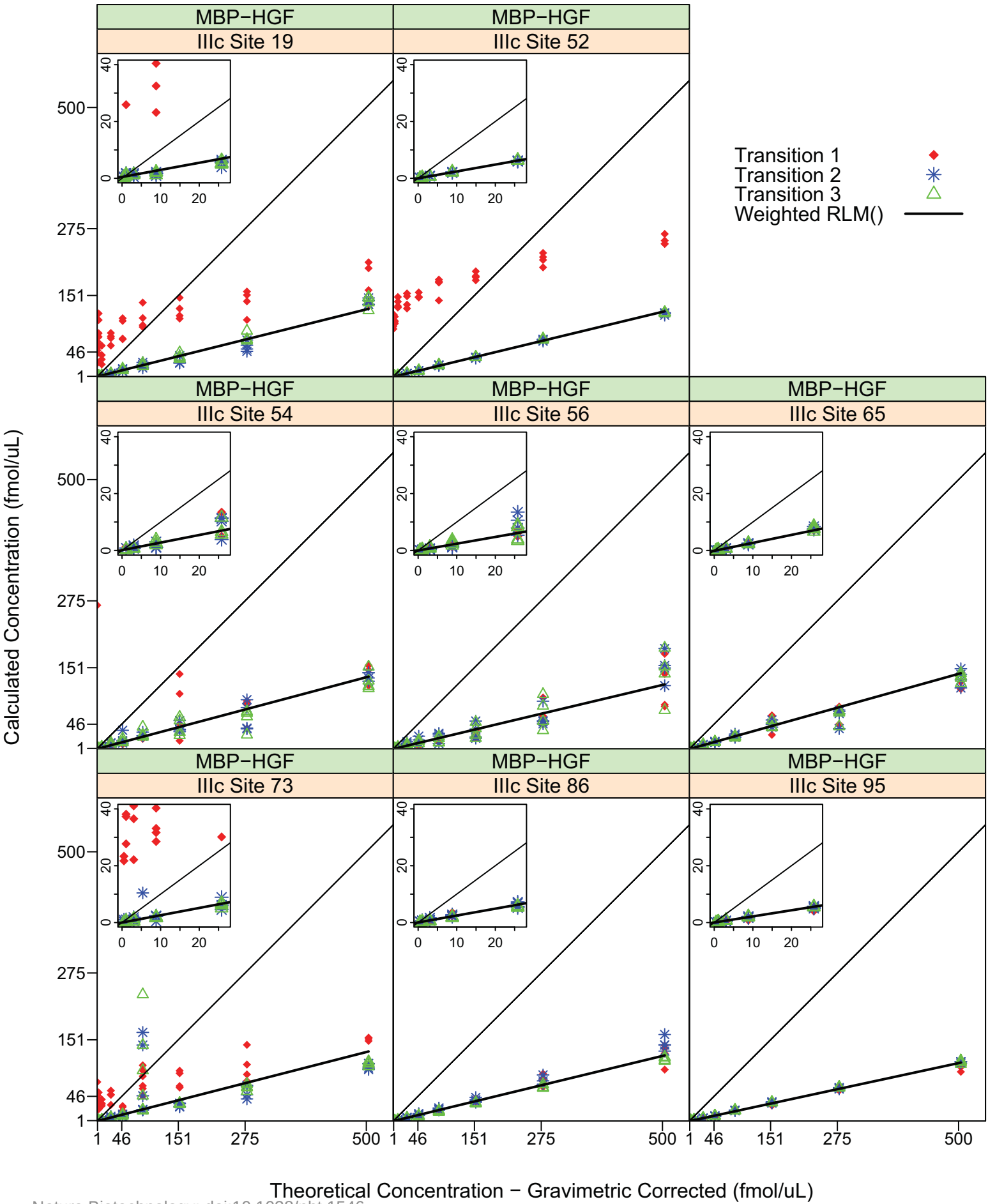

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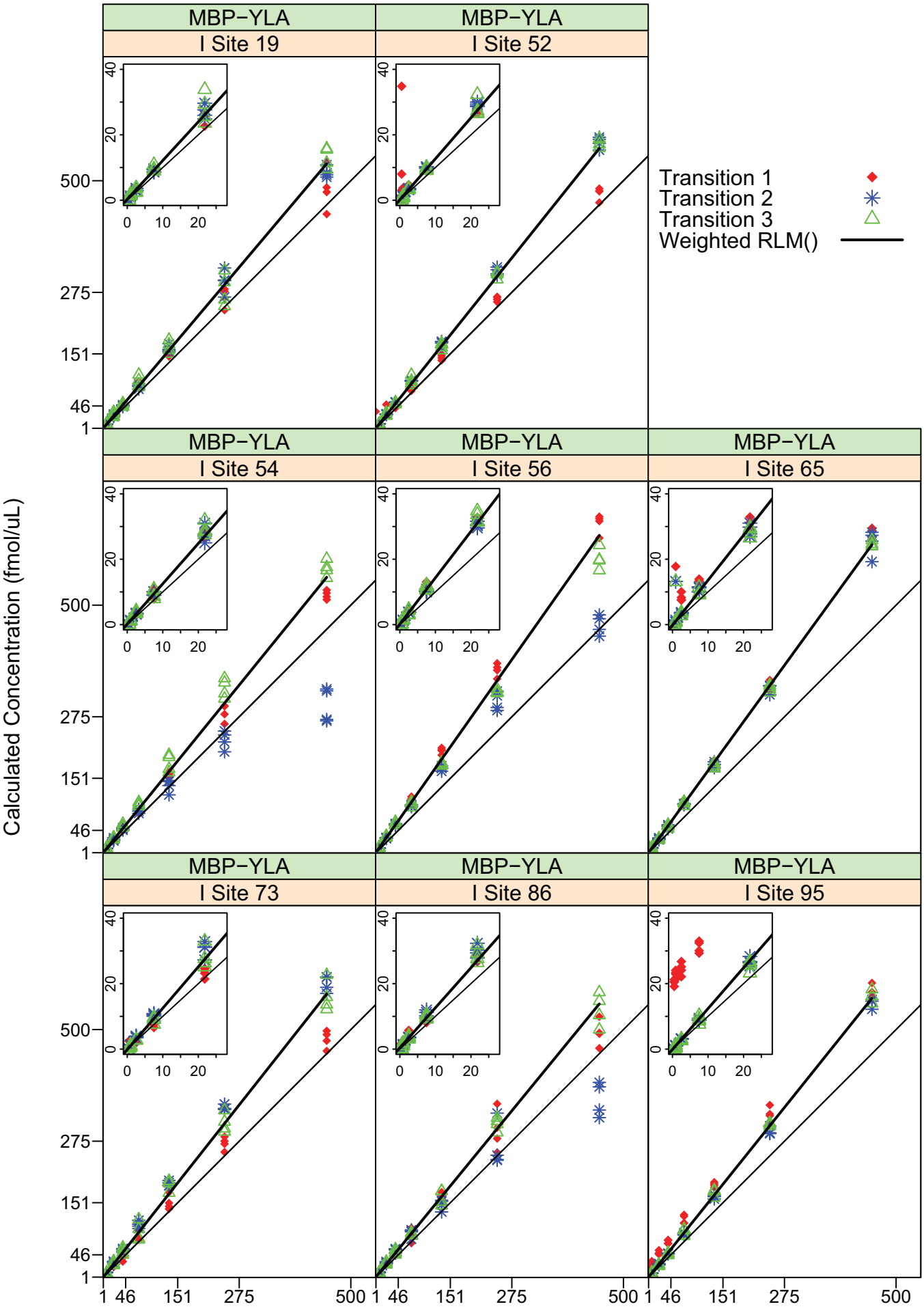

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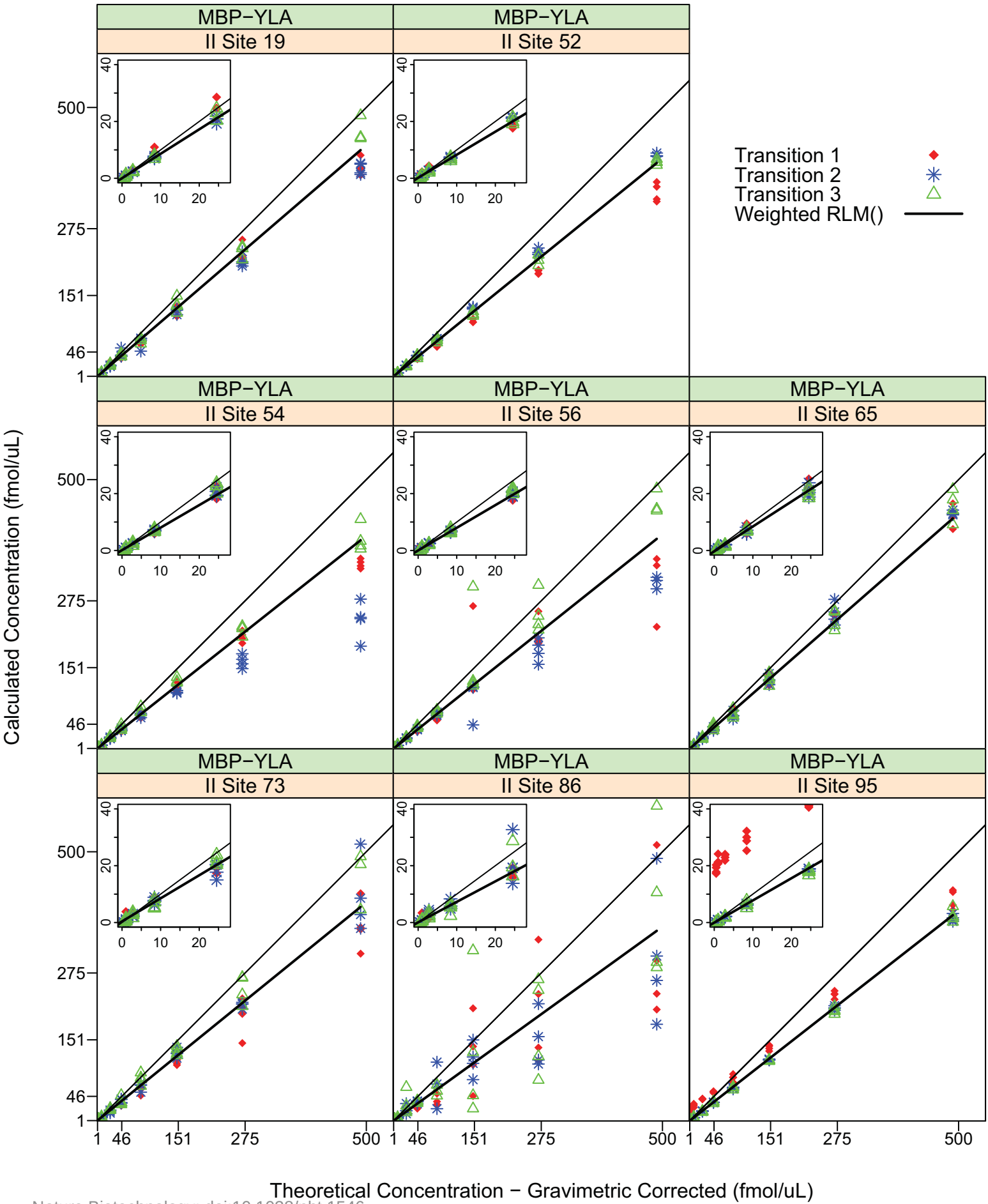

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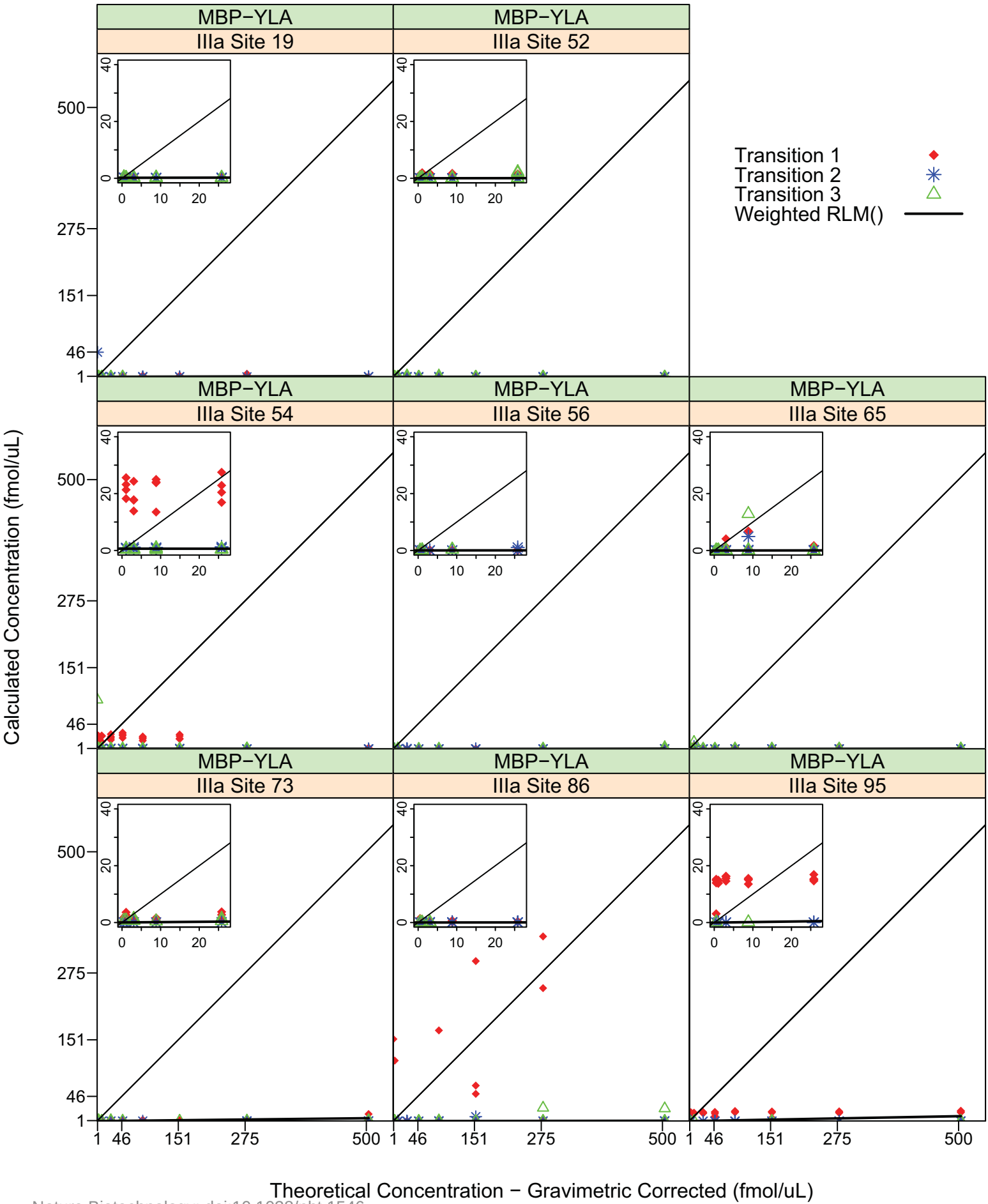

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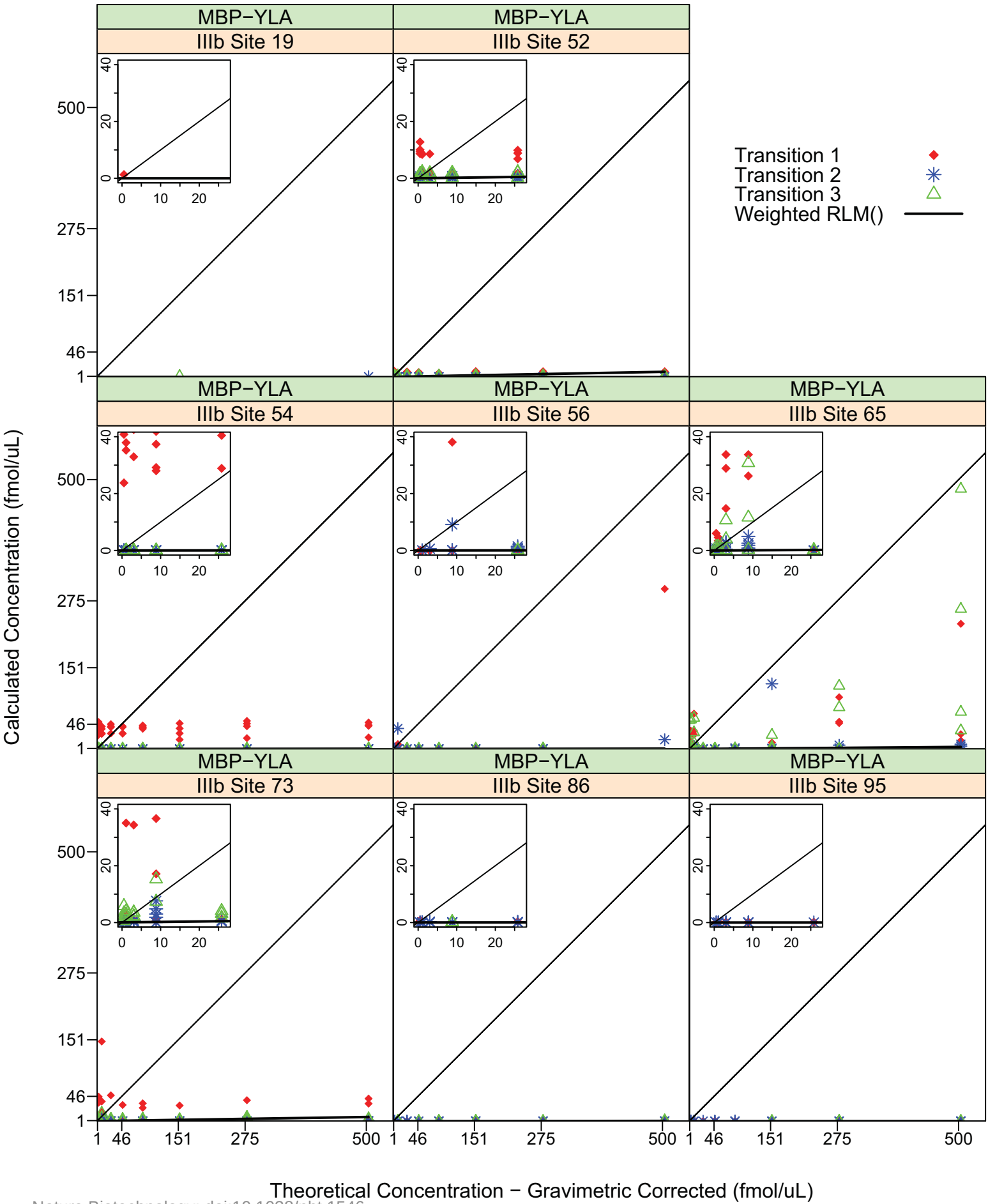

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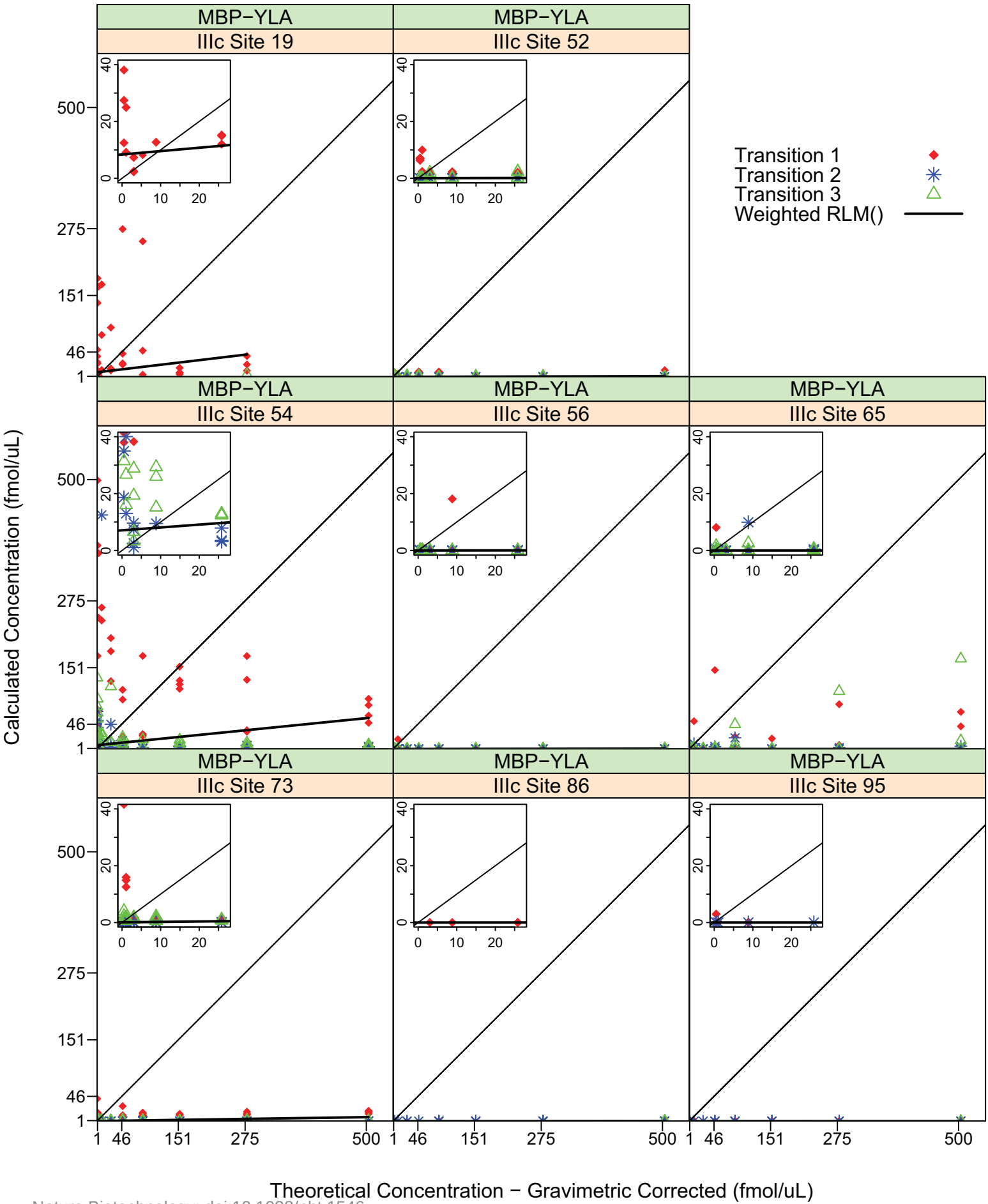

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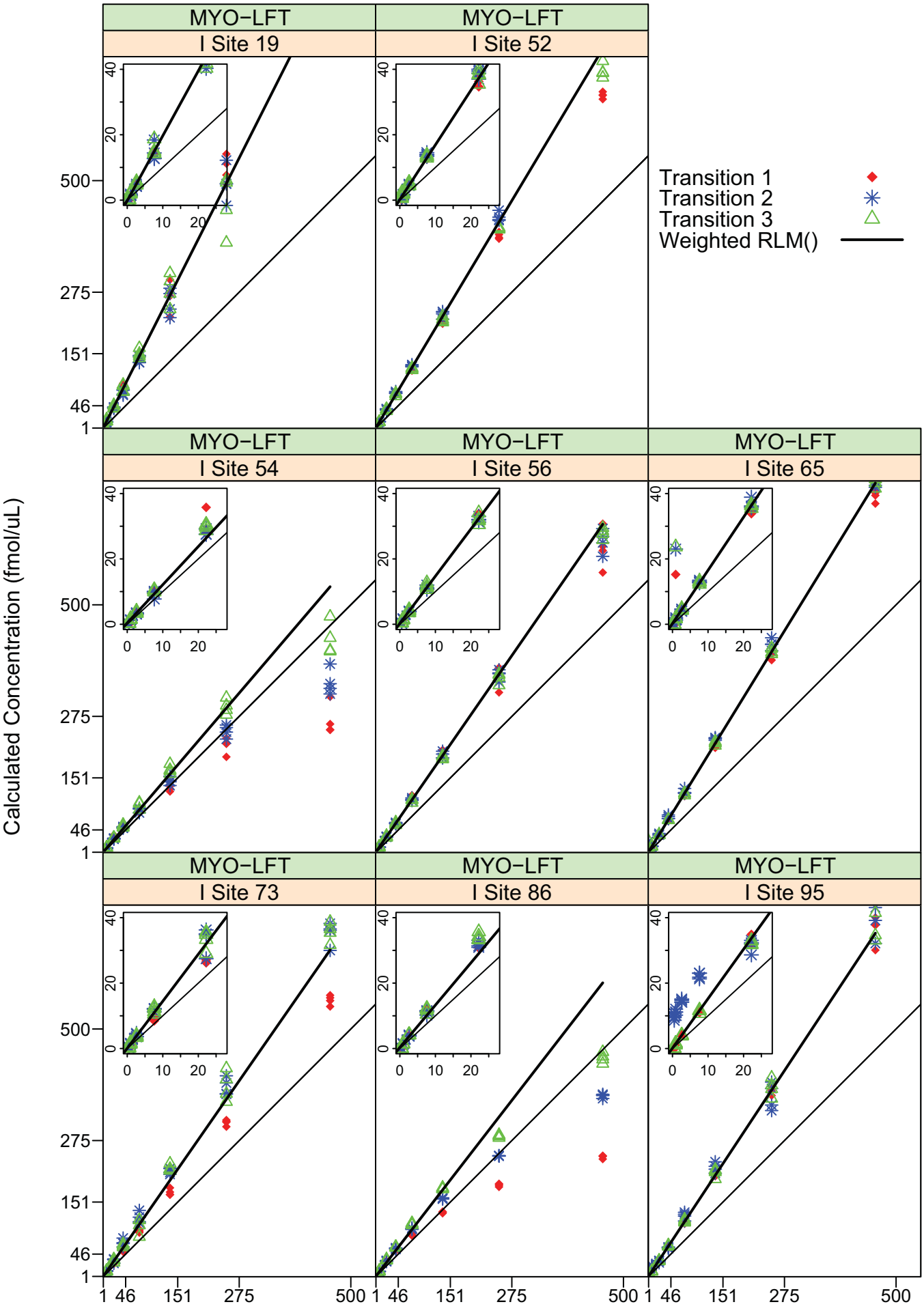

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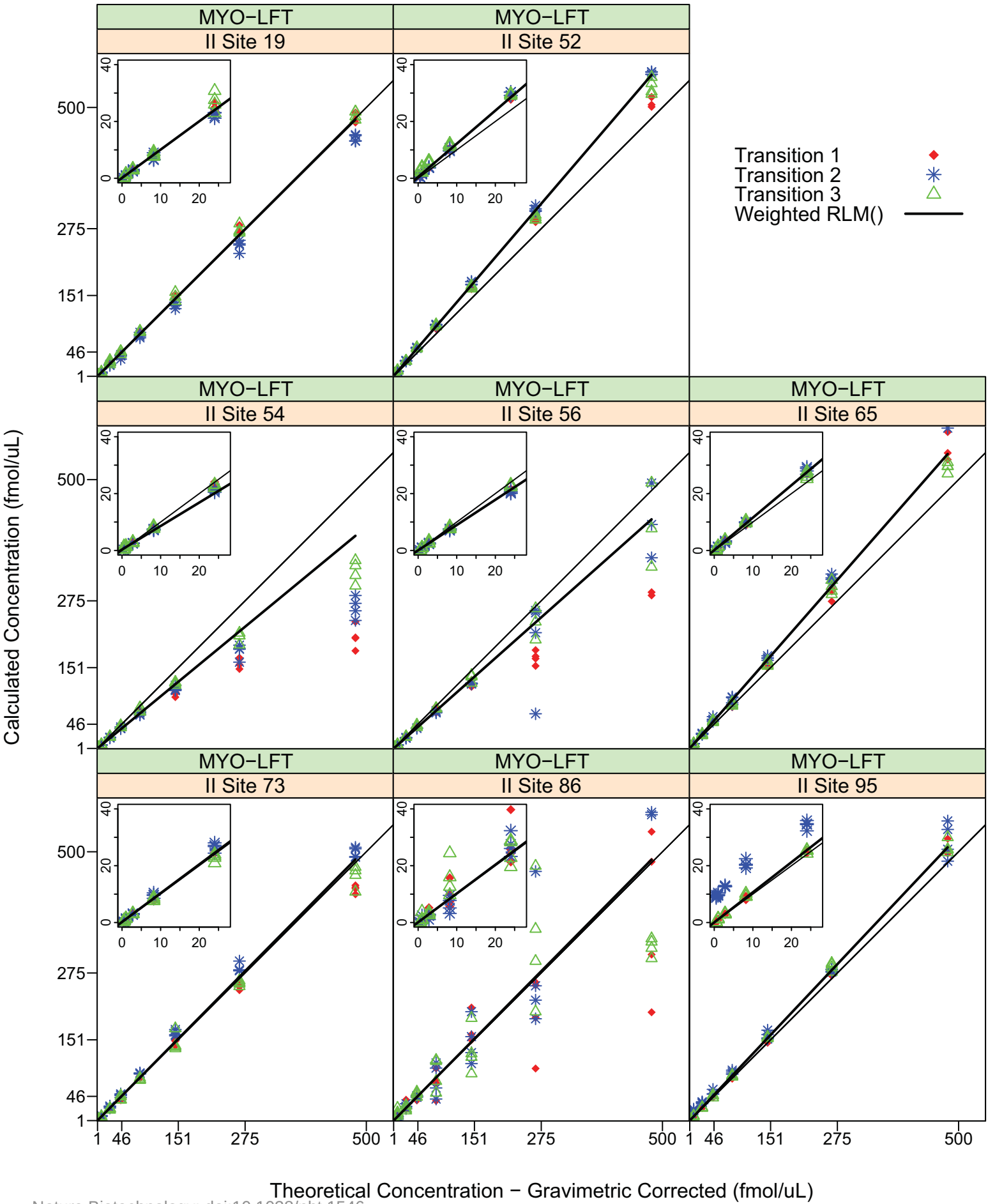

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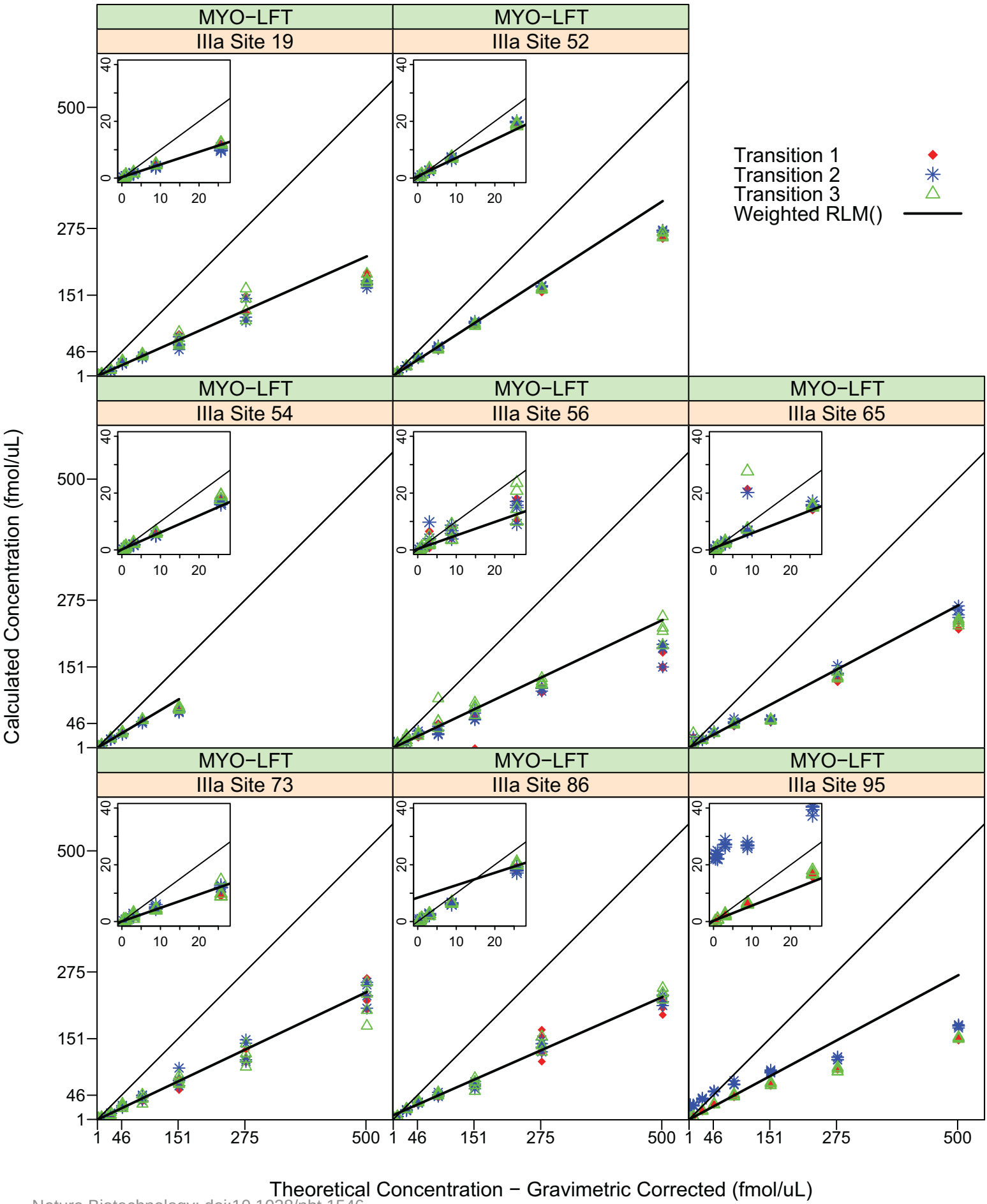

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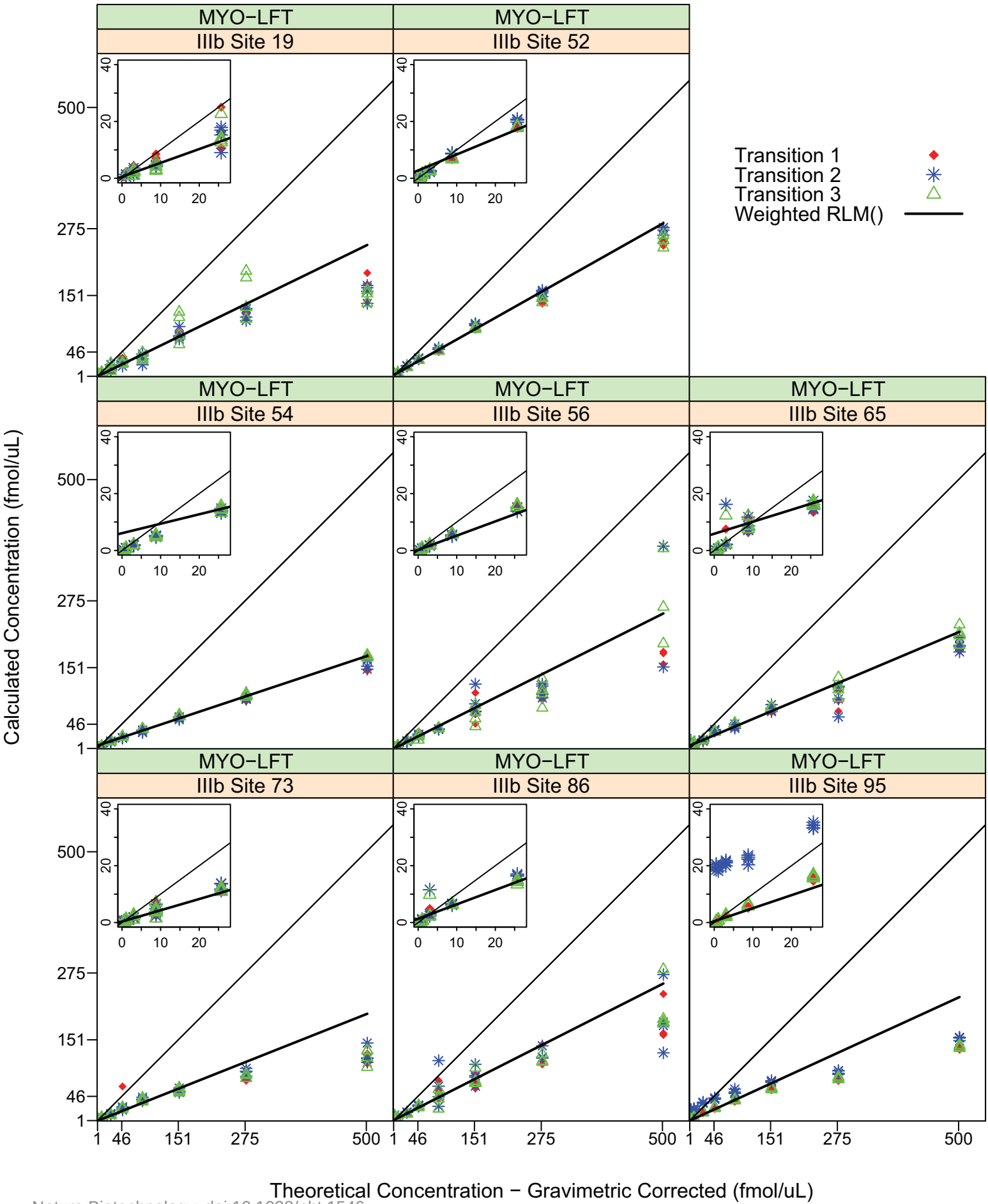

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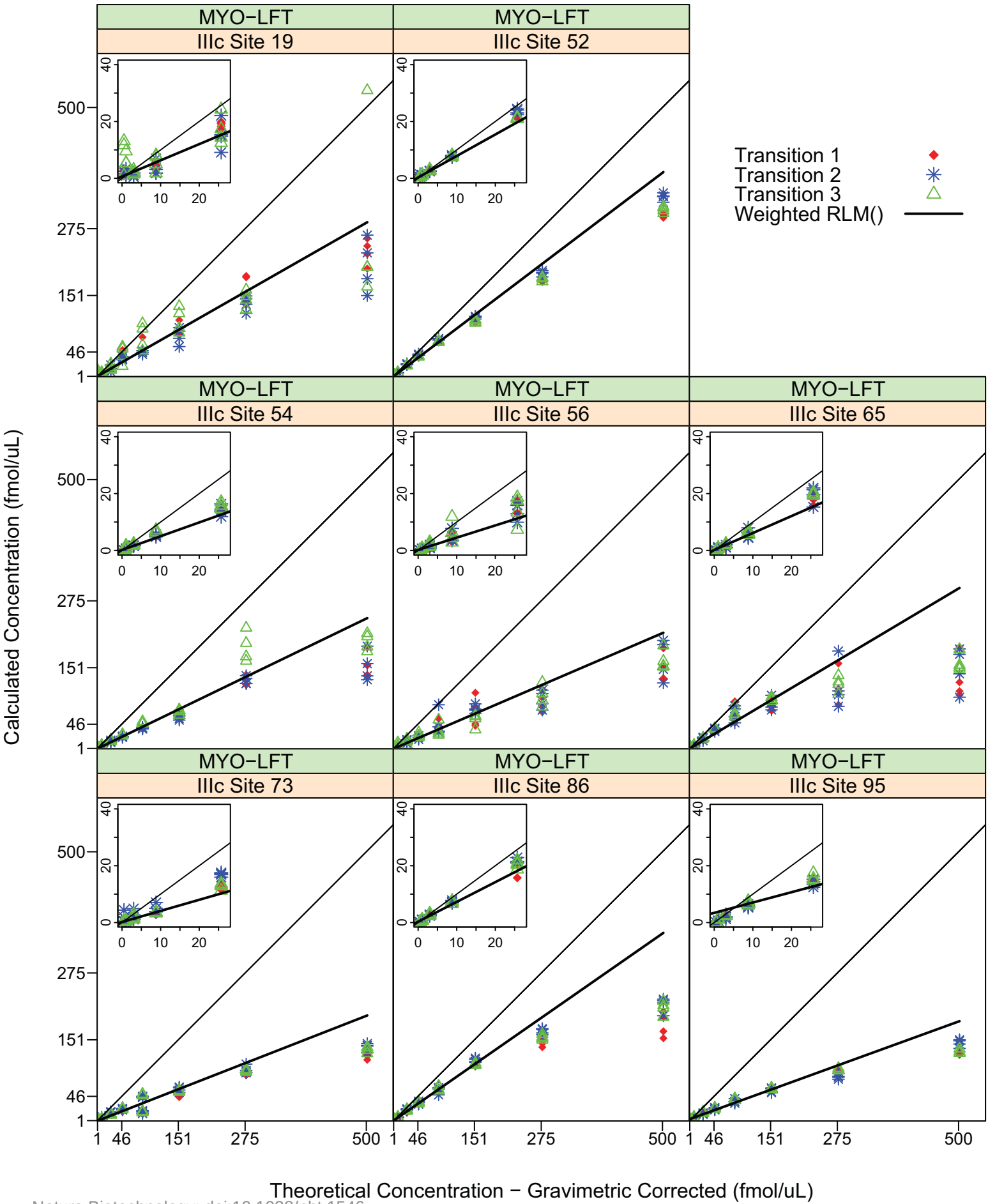

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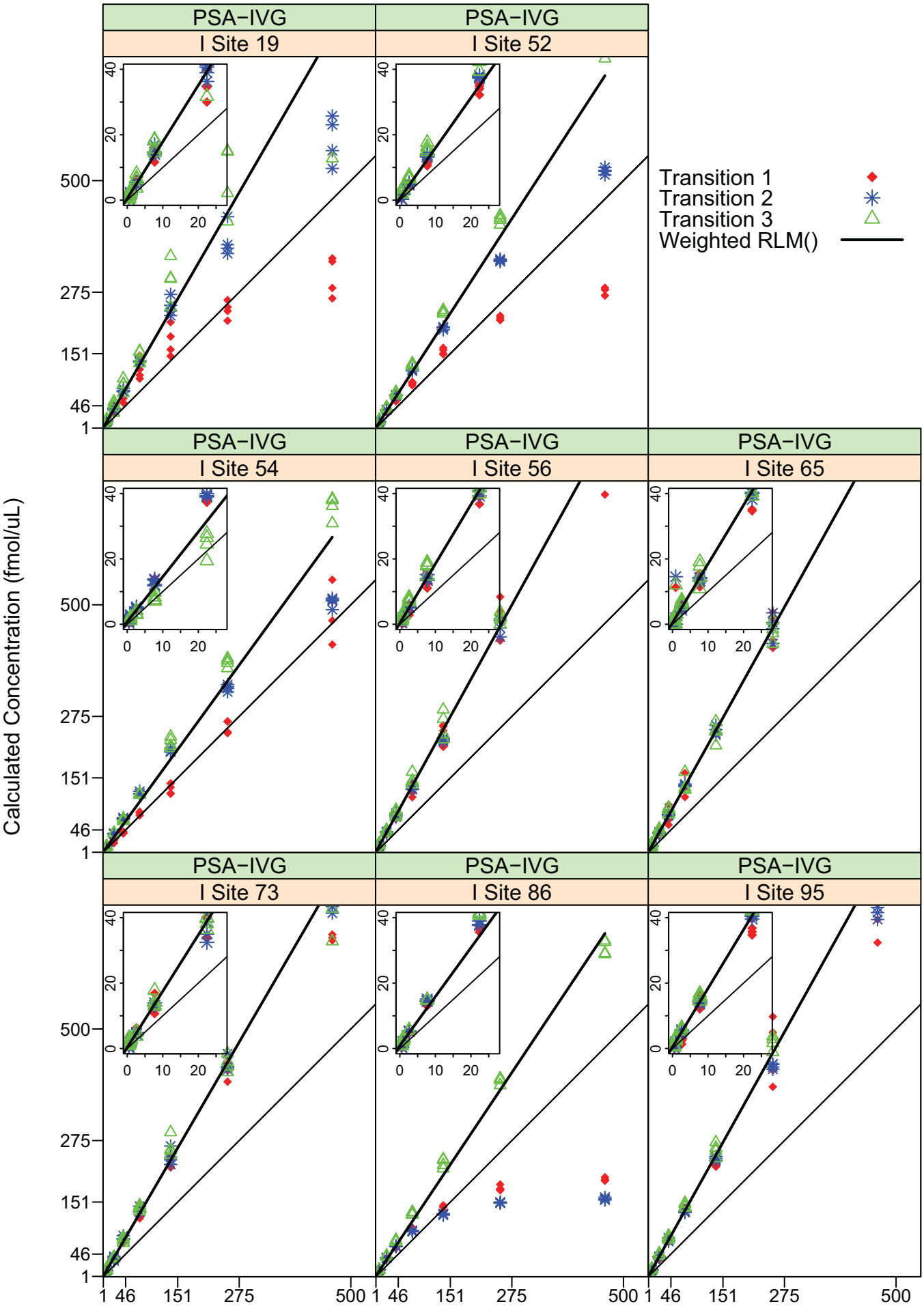

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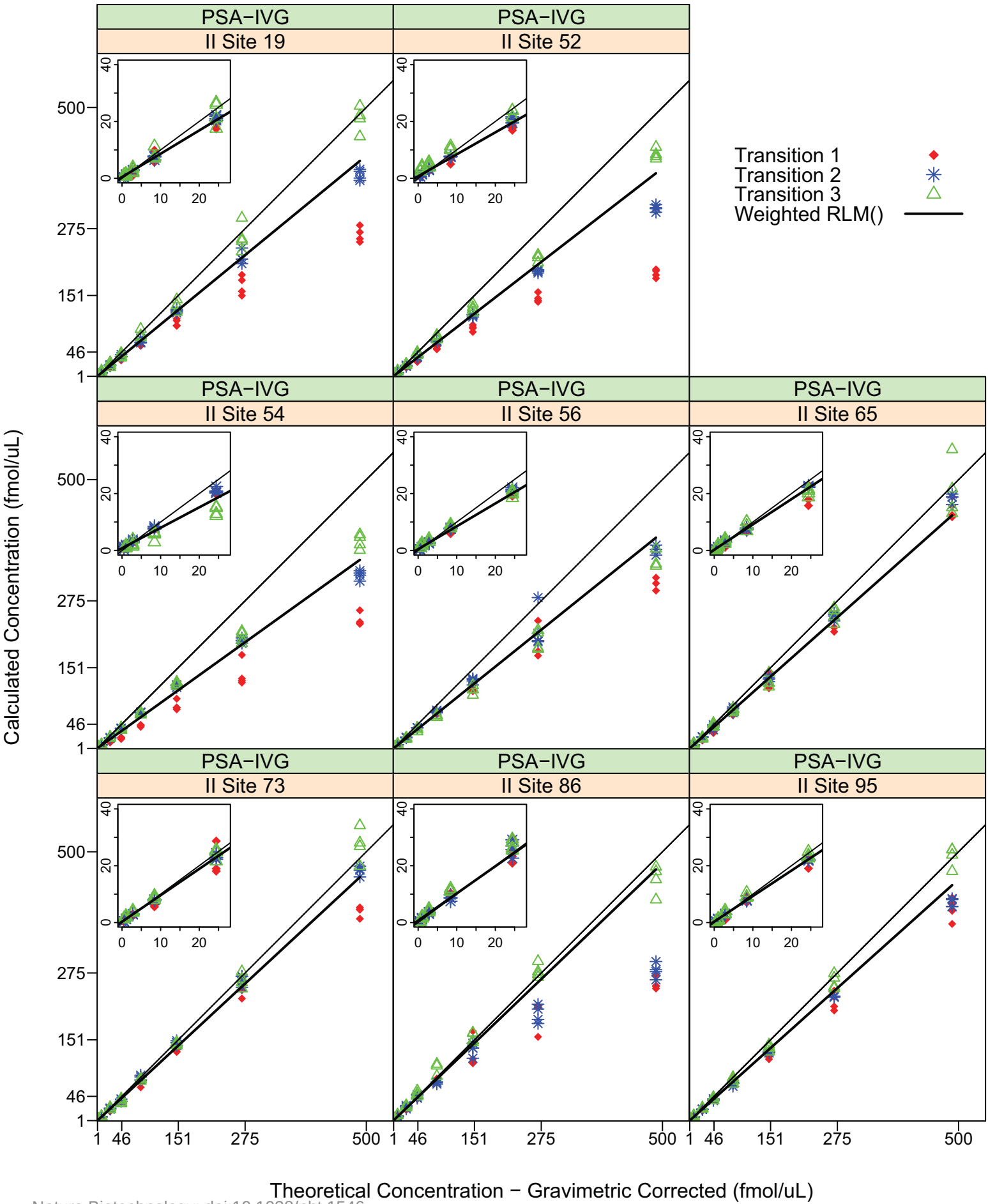

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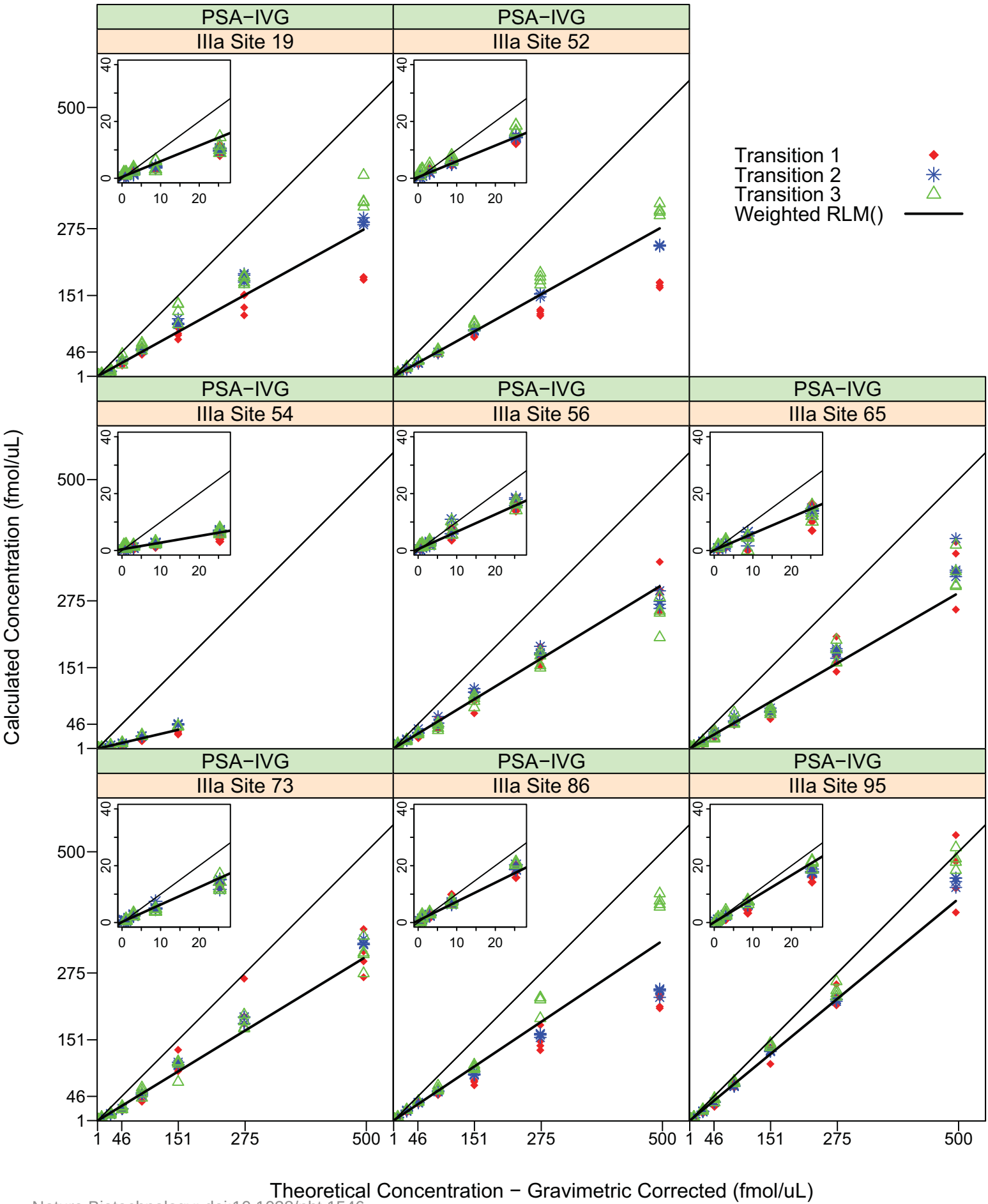

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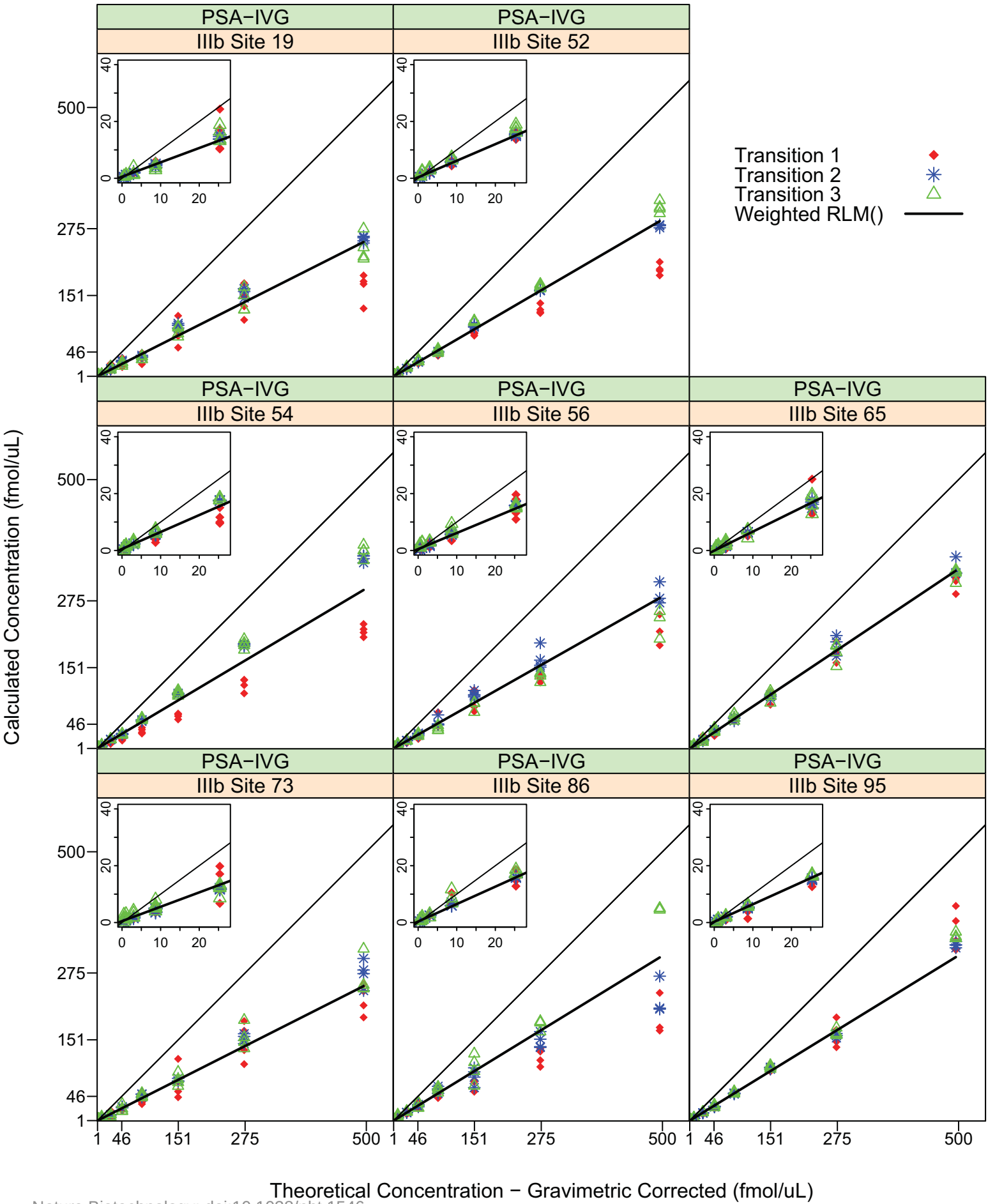

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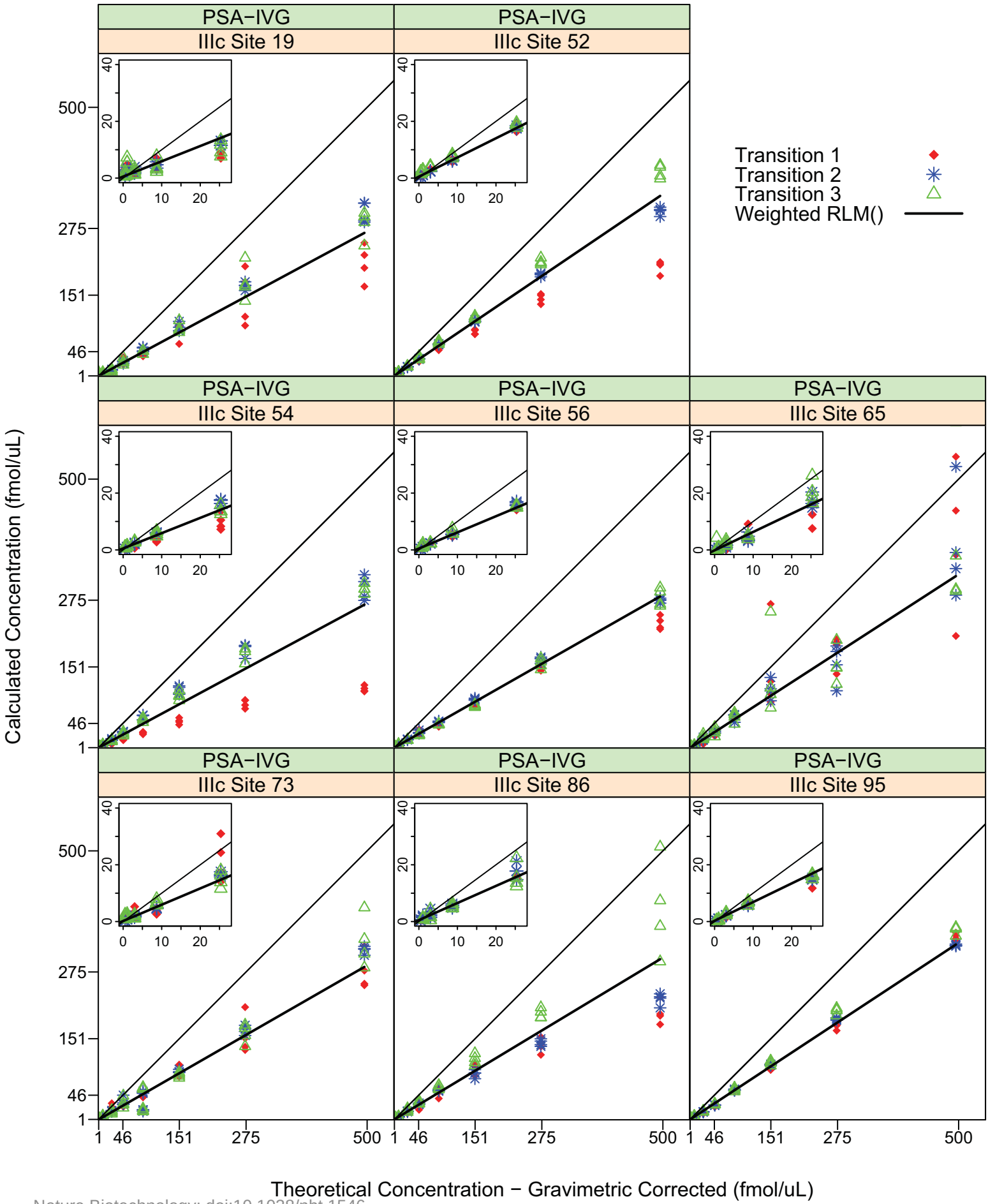

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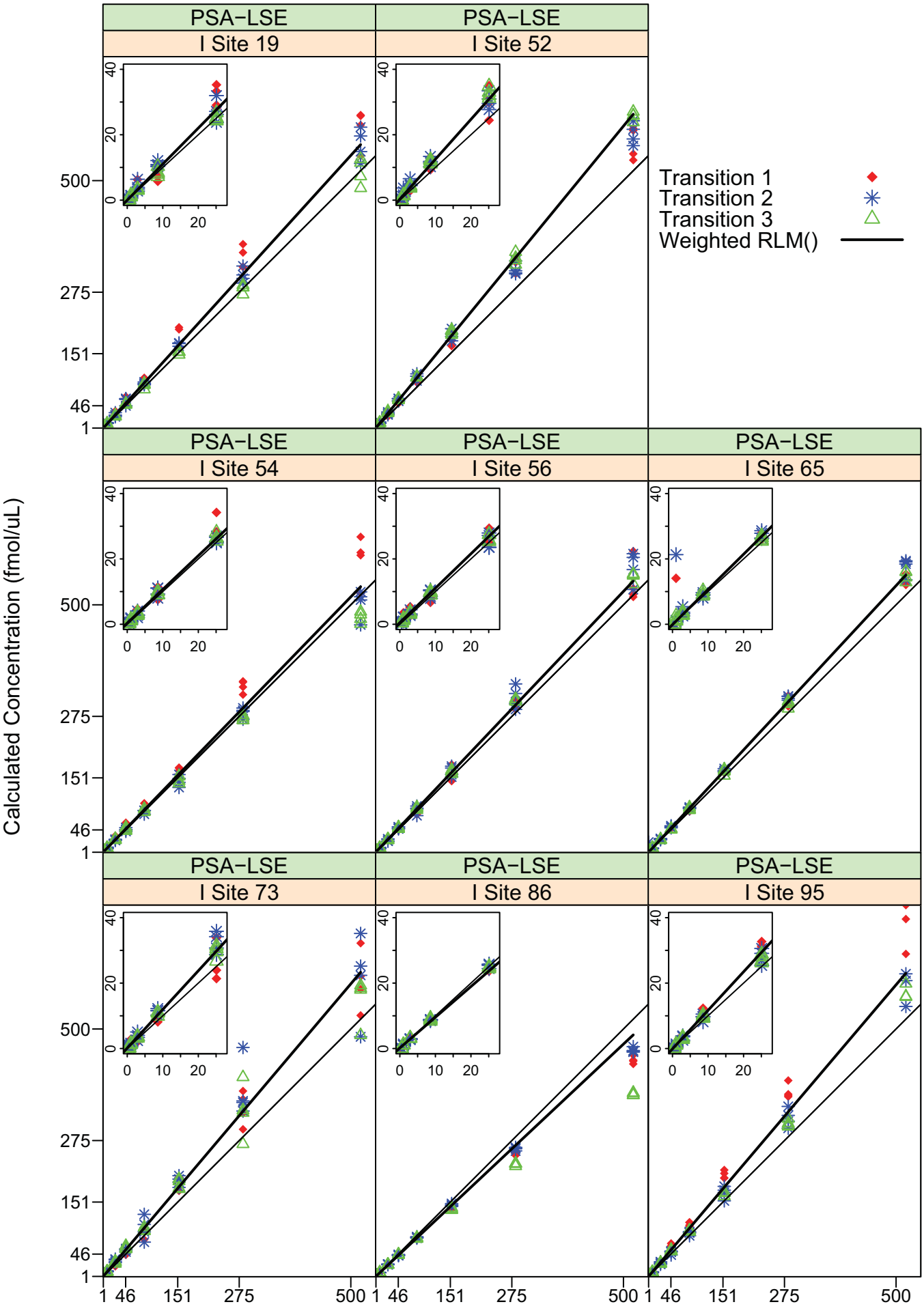

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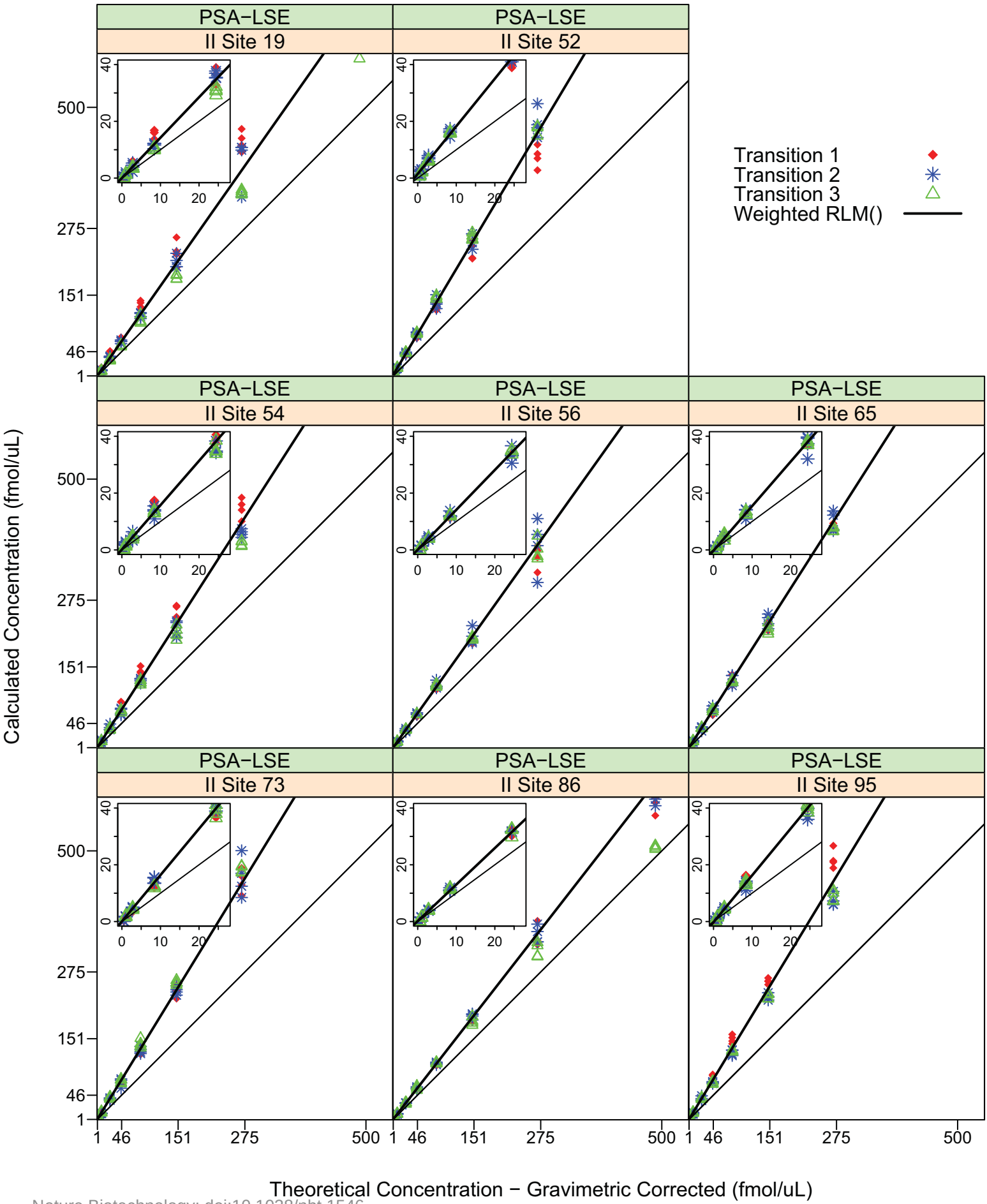

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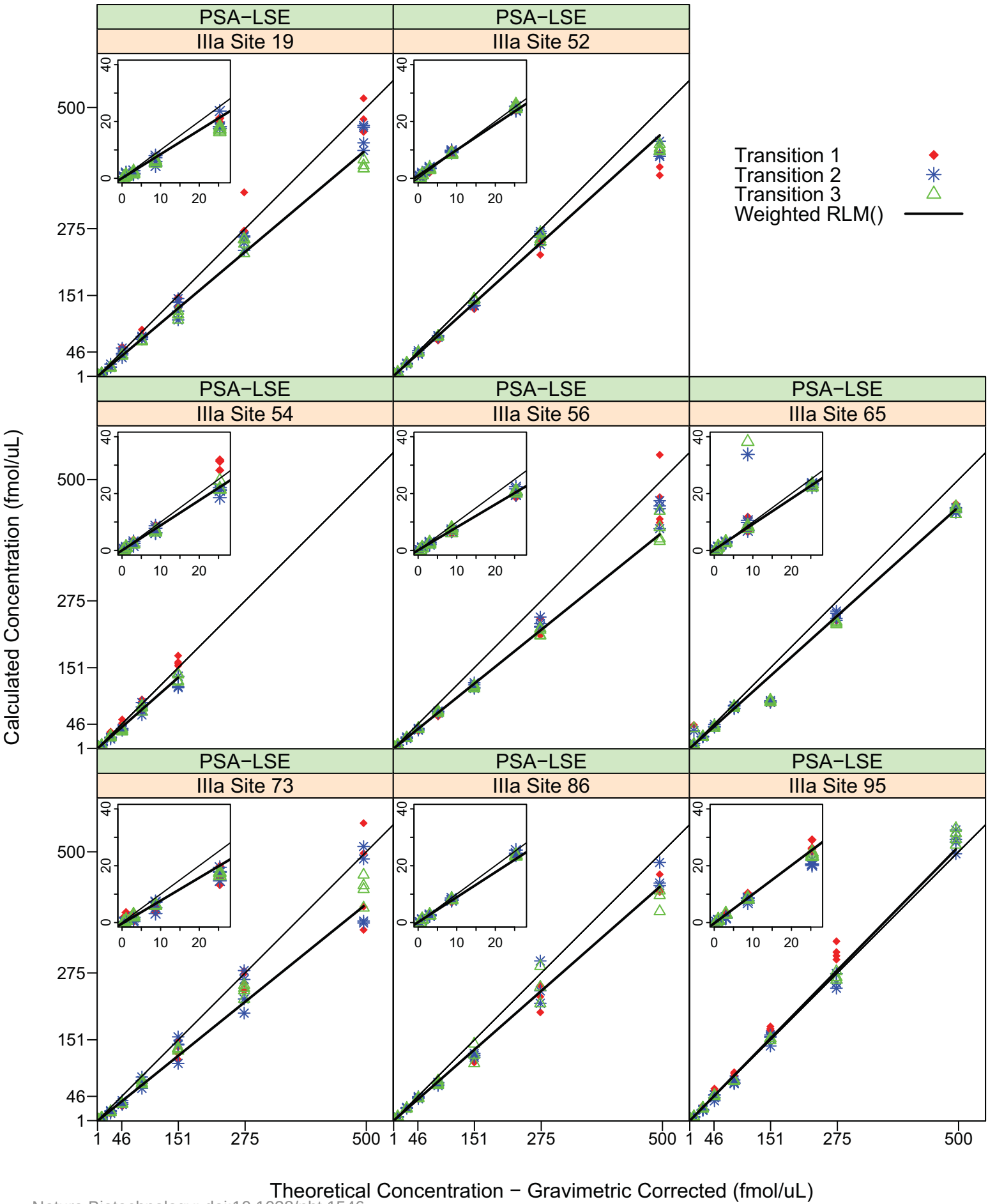

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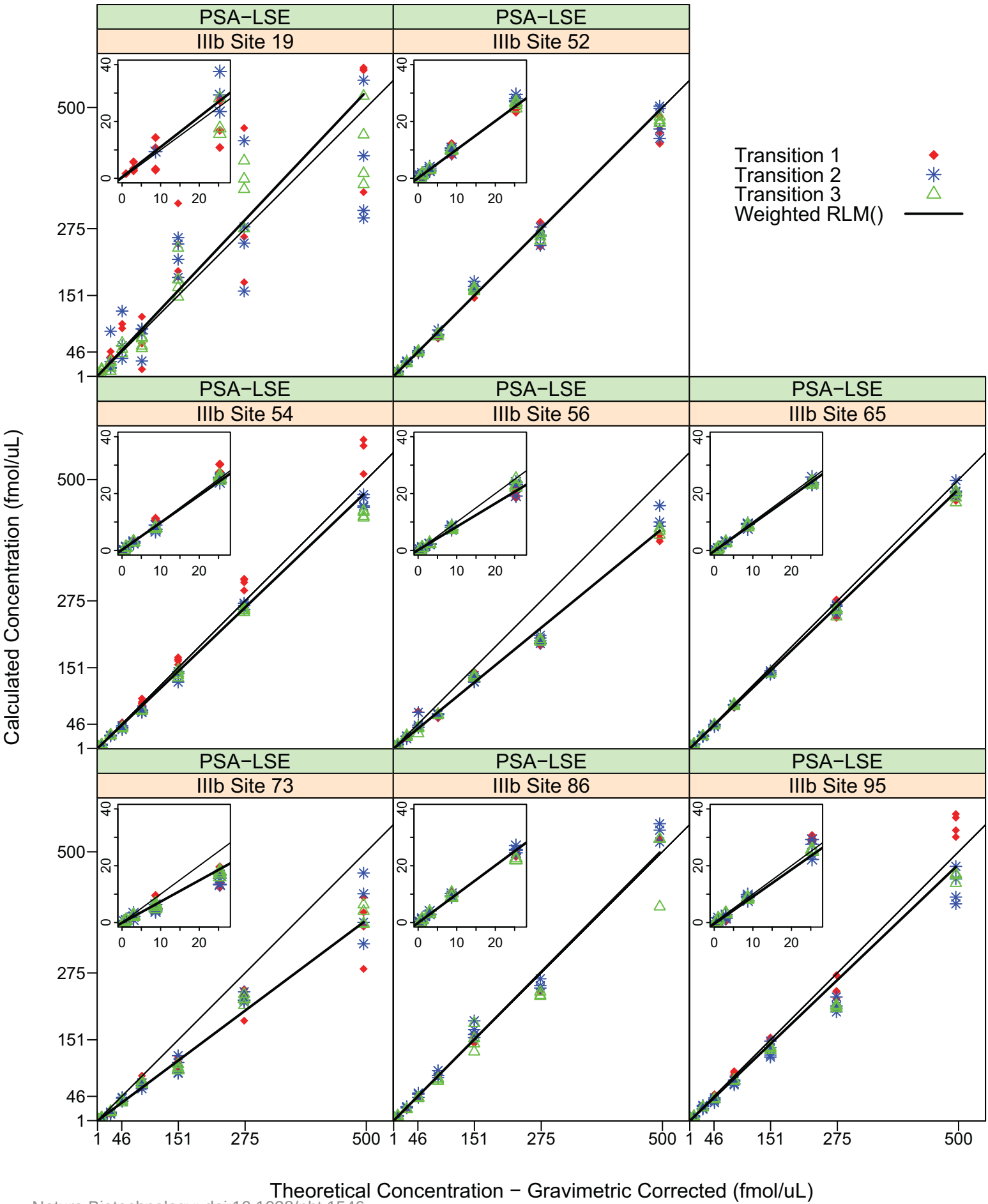

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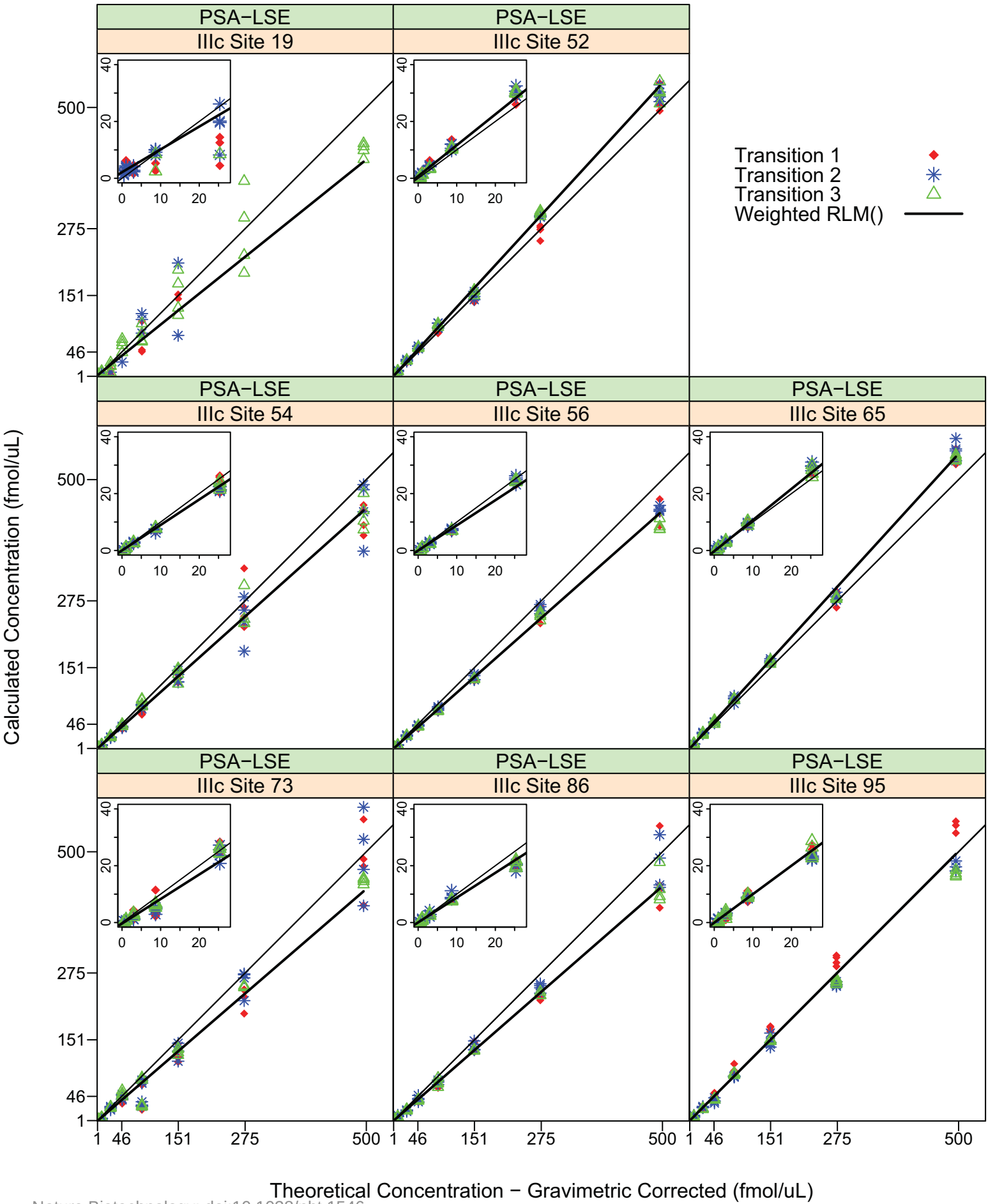
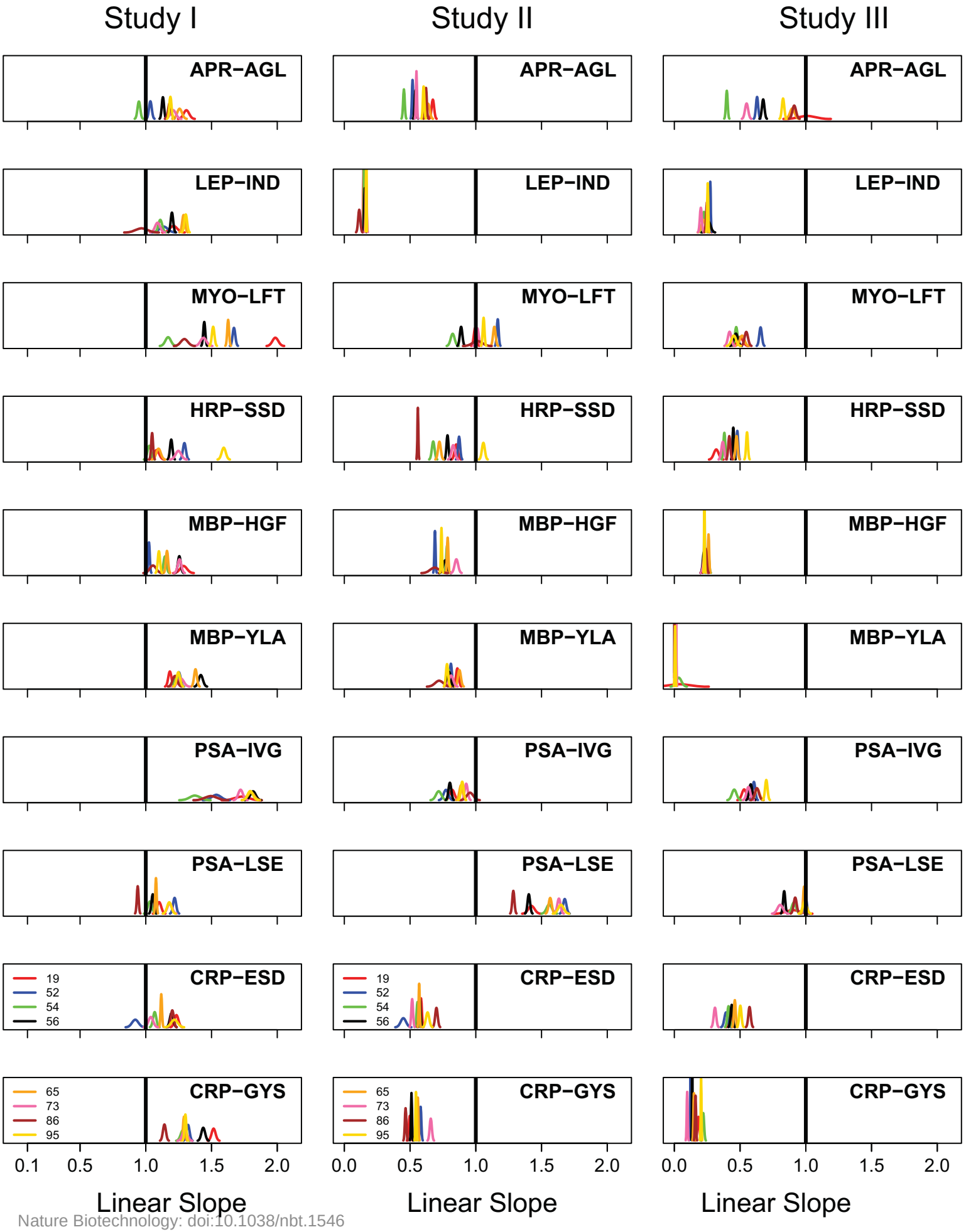


Nature Biotechnology: doi:10.1038/nbt.1546

Supplementary Figure 6: Inter-lab reproducibility of linear calibration curve slopes and percent recovery representation for Study I, II, and IIIa-c. The graphs display the linear slope values for each peptide and are color coded to indicate each participating site. The apex of each Gaussian peak is the linear calibration curve slope point estimate, and the width of each peak represents the standard error of that value. Study I and II data represent single process replicates acquired using quadruplicate injections, while Study III represents the average of three process replicates, also using quadruplicate injections. Note that in most cases, the Gaussian curves overlay well, but the width of their distribution increases from Study I to II to III. In addition, the value of the linear slope decreases with each subsequent study. These data indicate good reproducibility both intra- and inter-laboratory, and visualize the incidence of both sample loss and increased variance with increased sample handling. Peptide MBP-YLA was not observed by any site in Study III. The broad distribution of the APR-AGL and PSA-IVG peptides in Study III for site 54 was due to one process replicate in which the peptide was not detected.



Nature Biotechnology: doi:10.1038/nbt.1546

Supplementary Table 5: % Recovery at individual concentration points (representative examples)
(examples for peptide APR-AGL at sites 52 and 56 for Studies I, II, and IIIa-c)

Study I

spike (fmol/uL)	Peptide	Sample	Transition	Percent Recovery site 52	Percent Recovery site 56
1	APR-AGL	B	1	213.0%	205.0%
2.92	APR-AGL	C	1	138.0%	128.0%
8.55	APR-AGL	D	1	118.0%	130.0%
25	APR-AGL	E	1	110.0%	123.0%
46	APR-AGL	F	1	101.0%	118.0%
95	APR-AGL	G	1	109.0%	121.0%
125	APR-AGL	H	1	107.0%	121.0%
250	APR-AGL	I	1	102.0%	114.0%
500	APR-AGL	J	1	99.0%	123.0%
1	APR-AGL	B	2	149.0%	181.0%
2.92	APR-AGL	C	2	133.0%	128.0%
8.55	APR-AGL	D	2	108.0%	113.0%
25	APR-AGL	E	2	106.0%	113.0%
46	APR-AGL	F	2	102.0%	112.0%
95	APR-AGL	G	2	103.0%	112.0%
125	APR-AGL	H	2	104.0%	112.0%
250	APR-AGL	I	2	102.0%	110.0%
500	APR-AGL	J	2	98.0%	112.0%
1	APR-AGL	B	3	145.0%	213.0%
2.92	APR-AGL	C	3	120.0%	145.0%
8.55	APR-AGL	D	3	106.0%	121.0%
25	APR-AGL	E	3	108.0%	112.0%
46	APR-AGL	F	3	101.0%	115.0%
95	APR-AGL	G	3	102.0%	115.0%
125	APR-AGL	H	3	103.0%	110.0%
250	APR-AGL	I	3	111.0%	110.0%
500	APR-AGL	J	3	98.0%	110.0%

Study II

spike (fmol/uL)	Peptide	Sample	Transition	Percent Recovery site 52	Percent Recovery site 56
1	APR-AGL	B	1	194.0%	102.0%
2.92	APR-AGL	C	1	106.0%	68.0%
8.55	APR-AGL	D	1	67.0%	67.0%
25	APR-AGL	E	1	59.0%	61.0%
46	APR-AGL	F	1	57.0%	58.0%
95	APR-AGL	G	1	55.0%	58.0%
125	APR-AGL	H	1	54.0%	43.0%
250	APR-AGL	I	1	53.0%	57.0%
500	APR-AGL	J	1	52.0%	53.0%
1	APR-AGL	B	2	124.0%	86.0%
2.92	APR-AGL	C	2	62.0%	66.0%
8.55	APR-AGL	D	2	58.0%	61.0%
25	APR-AGL	E	2	51.0%	57.0%
46	APR-AGL	F	2	53.0%	56.0%
95	APR-AGL	G	2	51.0%	59.0%
125	APR-AGL	H	2	52.0%	58.0%
250	APR-AGL	I	2	51.0%	55.0%
500	APR-AGL	J	2	50.0%	53.0%
1	APR-AGL	B	3	114.0%	70.0%
2.92	APR-AGL	C	3	84.0%	56.0%
8.55	APR-AGL	D	3	60.0%	51.0%
25	APR-AGL	E	3	57.0%	51.0%
46	APR-AGL	F	3	52.0%	53.0%
95	APR-AGL	G	3	51.0%	50.0%
125	APR-AGL	H	3	52.0%	51.0%
250	APR-AGL	I	3	50.0%	47.0%
500	APR-AGL	J	3	50.0%	48.0%

Study IIIa

spike (fmol/uL)	Peptide	Sample	Transition	Percent Recovery site 52	Percent Recovery site 56
1	APR-AGL	B	1	98.0%	103.0%
2.92	APR-AGL	C	1	76.0%	89.0%
8.55	APR-AGL	D	1	70.0%	77.0%
25	APR-AGL	E	1	67.0%	73.0%
46	APR-AGL	F	1	63.0%	71.0%
95	APR-AGL	G	1	58.0%	70.0%
125	APR-AGL	H	1	58.0%	67.0%
250	APR-AGL	I	1	55.0%	69.0%
500	APR-AGL	J	1	28.0%	63.0%
1	APR-AGL	B	2	89.0%	90.0%
2.92	APR-AGL	C	2	73.0%	84.0%
8.55	APR-AGL	D	2	67.0%	74.0%
25	APR-AGL	E	2	62.0%	74.0%
46	APR-AGL	F	2	59.0%	70.0%
95	APR-AGL	G	2	53.0%	70.0%
125	APR-AGL	H	2	55.0%	66.0%
250	APR-AGL	I	2	54.0%	66.0%
500	APR-AGL	J	2	27.0%	62.0%
1	APR-AGL	B	3	152.0%	80.0%
2.92	APR-AGL	C	3	73.0%	80.0%
8.55	APR-AGL	D	3	68.0%	72.0%
25	APR-AGL	E	3	62.0%	66.0%
46	APR-AGL	F	3	60.0%	63.0%
95	APR-AGL	G	3	56.0%	62.0%
125	APR-AGL	H	3	53.0%	59.0%
250	APR-AGL	I	3	53.0%	59.0%
500	APR-AGL	J	3	27.0%	54.0%

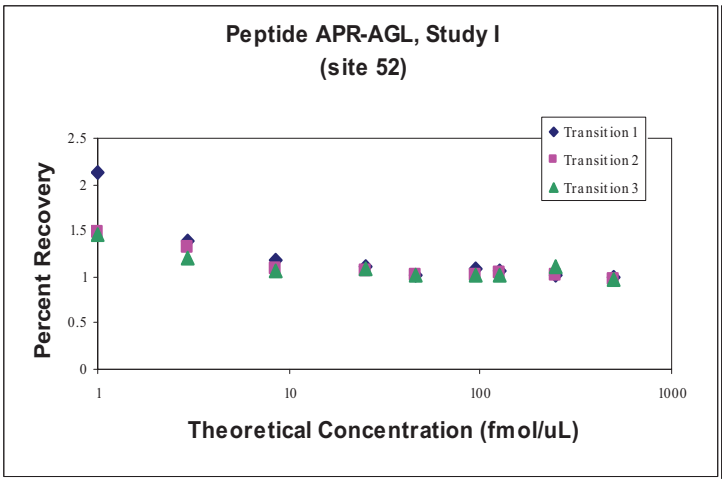
Study IIIb

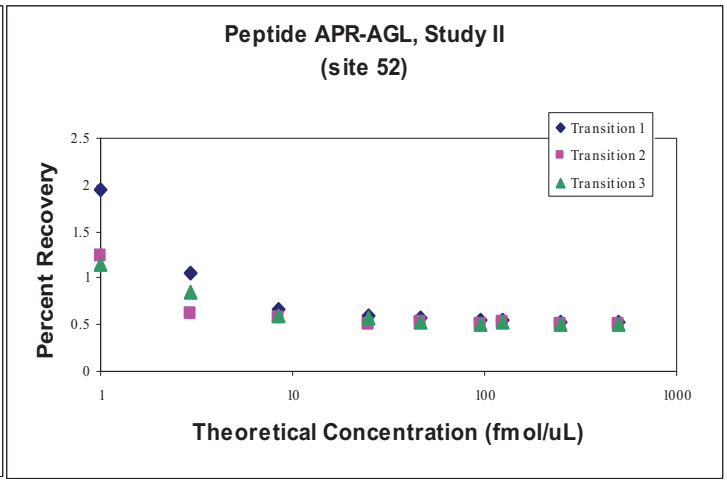
spike (fmol/uL)	Peptide	Sample	Transition	Percent Recovery site 52	Percent Recovery site 56
1	APR-AGL	B	1	96.0%	113.0%
2.92	APR-AGL	C	1	79.0%	78.0%
8.55	APR-AGL	D	1	76.0%	80.0%
25	APR-AGL	E	1	69.0%	73.0%
46	APR-AGL	F	1	67.0%	64.0%
95	APR-AGL	G	1	62.0%	63.0%
125	APR-AGL	H	1	64.0%	72.0%
250	APR-AGL	I	1	59.0%	60.0%
500	APR-AGL	J	1	55.0%	59.0%
1	APR-AGL	B	2	104.0%	99.0%
2.92	APR-AGL	C	2	79.0%	76.0%
8.55	APR-AGL	D	2	71.0%	78.0%
25	APR-AGL	E	2	69.0%	76.0%
46	APR-AGL	F	2	65.0%	72.0%
95	APR-AGL	G	2	61.0%	62.0%
125	APR-AGL	H	2	65.0%	69.0%
250	APR-AGL	I	2	55.0%	58.0%
500	APR-AGL	J	2	53.0%	57.0%
1	APR-AGL	B	3	161.0%	94.0%
2.92	APR-AGL	C	3	90.0%	68.0%
8.55	APR-AGL	D	3	78.0%	70.0%
25	APR-AGL	E	3	72.0%	65.0%
46	APR-AGL	F	3	69.0%	77.0%
95	APR-AGL	G	3	63.0%	54.0%
125	APR-AGL	H	3	65.0%	62.0%
250	APR-AGL	I	3	56.0%	53.0%
500	APR-AGL	J	3	53.0%	50.0%

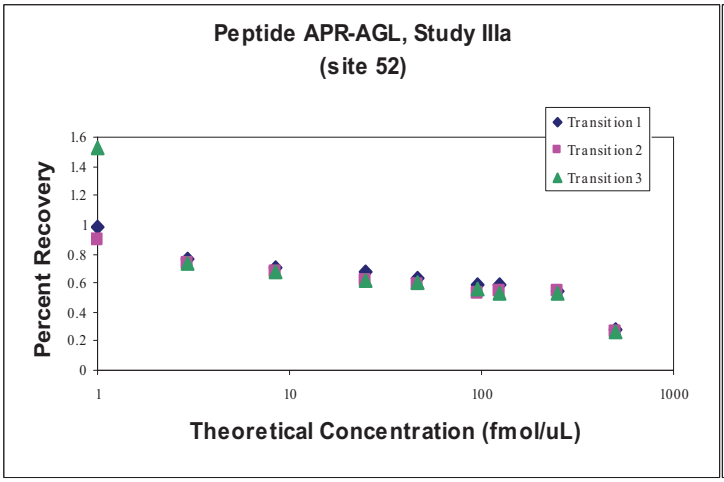
Study IIIc

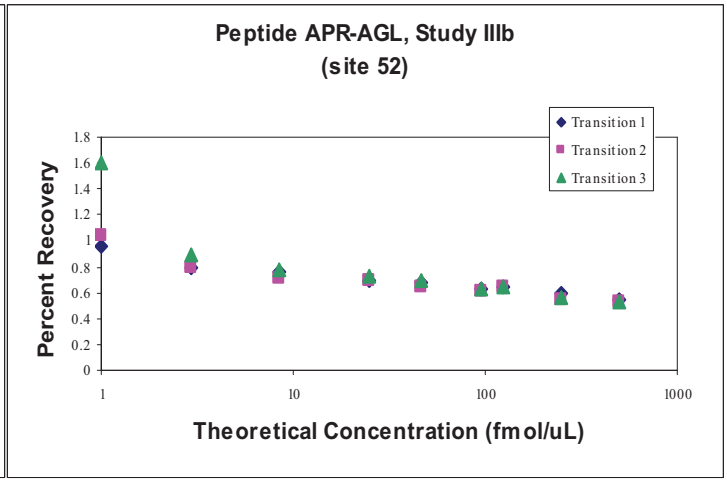
spike (fmol/uL)	Peptide	Sample	Transition	Percent Recovery site 52	Percent Recovery site 56
1	APR-AGL	B	1	138.0%	117.0%
2.92	APR-AGL	C	1	87.0%	91.0%
8.55	APR-AGL	D	1	80.0%	79.0%
25	APR-AGL	E	1	80.0%	90.0%
46	APR-AGL	F	1	80.0%	83.0%
95	APR-AGL	G	1	77.0%	76.0%
125	APR-AGL	H	1	71.0%	77.0%
250	APR-AGL	I	1	68.0%	76.0%
500	APR-AGL	J	1	61.0%	68.0%
1	APR-AGL	B	2	115.0%	102.0%
2.92	APR-AGL	C	2	80.0%	84.0%
8.55	APR-AGL	D	2	81.0%	73.0%
25	APR-AGL	E	2	79.0%	85.0%
46	APR-AGL	F	2	76.0%	80.0%
95	APR-AGL	G	2	71.0%	73.0%
125	APR-AGL	H	2	64.0%	74.0%
250	APR-AGL	I	2	66.0%	75.0%
500	APR-AGL	J	2	60.0%	68.0%
1	APR-AGL	B	3	134.0%	91.0%
2.92	APR-AGL	C	3	89.0%	74.0%
8.55	APR-AGL	D	3	82.0%	64.0%
25	APR-AGL	E	3	76.0%	70.0%
46	APR-AGL	F	3	75.0%	71.0%
95	APR-AGL	G	3	73.0%	65.0%
125	APR-AGL	H	3	66.0%	66.0%
250	APR-AGL	I	3	64.0%	69.0%
500	APR-AGL	J	3	58.0%	61.0%

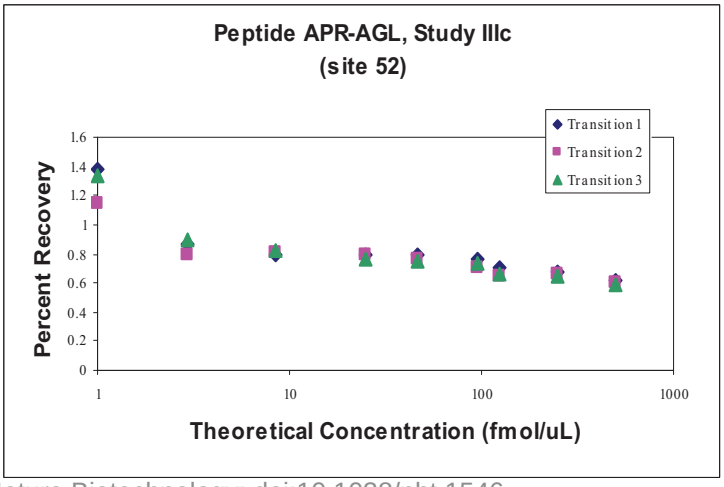
Supplementary Table 5 (graphics site 52): % Recovery at individual concentration points (representative example) (example for peptide APR-AGL at **site 52** for Studies I, II, and IIIa-c)



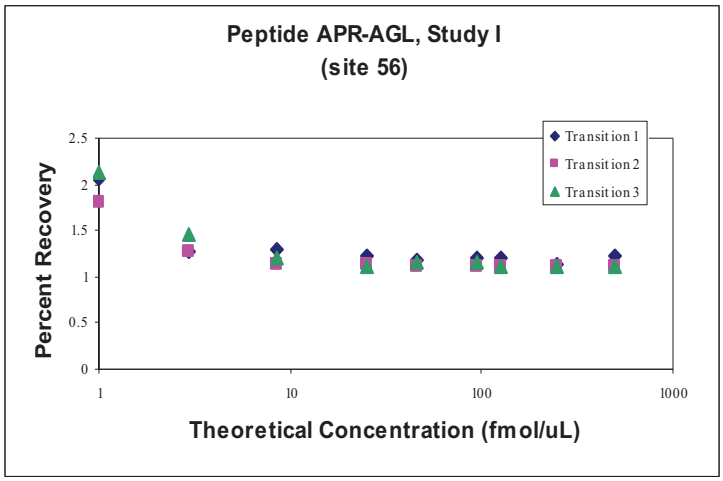


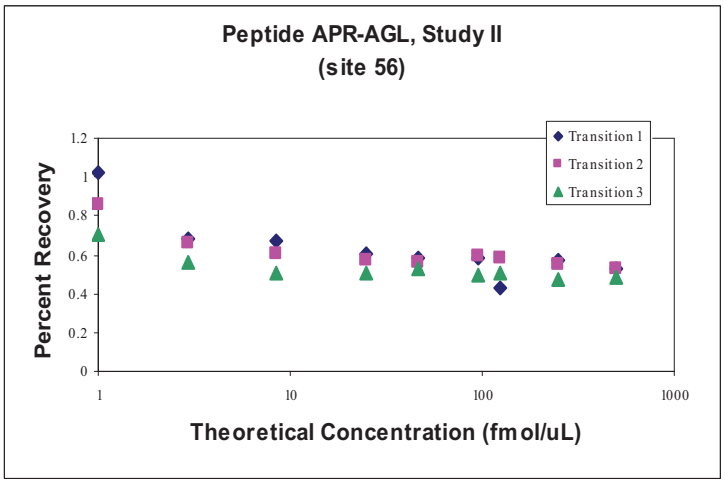


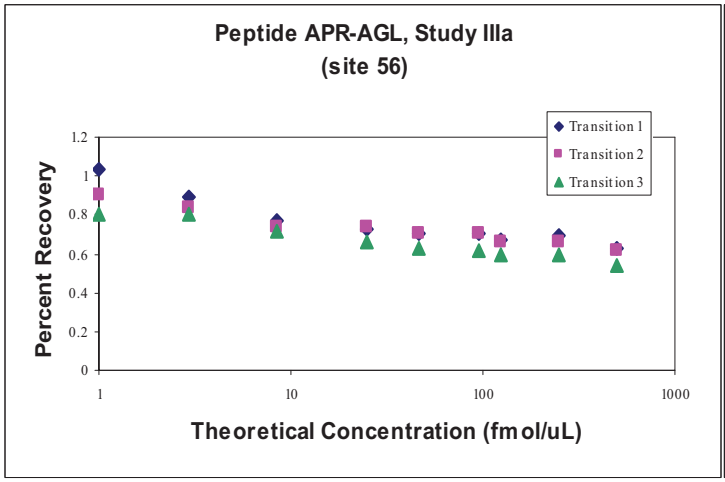


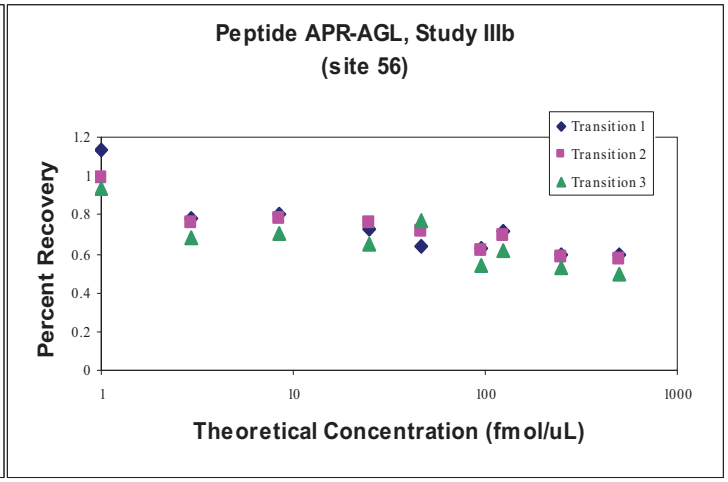


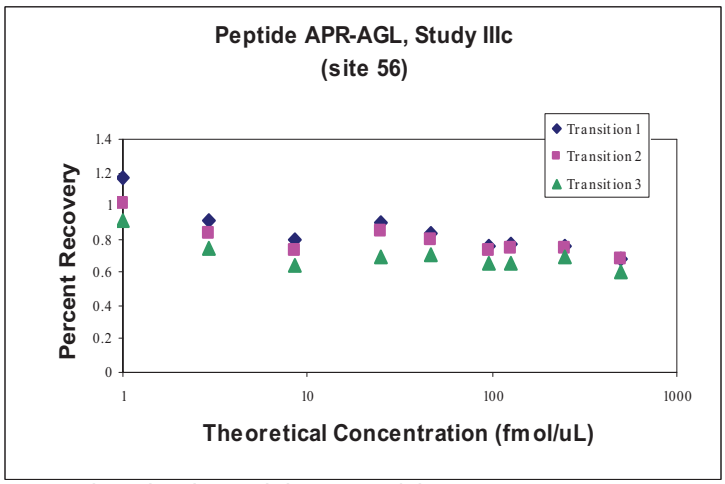
Supplementary Table 5 (graphics site 56): % Recovery at individual concentration points (representative example) (example for peptide APR-AGL at **site 56** for Studies I, II, and IIIa-c)











Supplementary Table 6.A: Concentrations of the isotopically-labeled ($^{13}C/^{15}N$) internal standard peptides spiked into Study I samples

	Concentration, fmol/µL										
Sample	**bi0081**	**ni0101**	**ni0102**	**ni0104**	**ni0105**	**bi0067**	**ni0107**	**ni0108**	**ni0109**	**ni0110**	**ni0111**
I-A	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
I-B	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
I-C	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
I-D	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
I-E	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
I-F	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
I-G	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
I-H	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
I-I	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
I-J	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2

* A legend for the isotopically-labeled ($^{13}C/^{15}N$) internal standard peptide name identifier can be found in the Supplementary Methods - SOP (therein embedded SOP Table A). Target concentrations for spike-in levels were 50 fmol/µL.

Supplementary Table 6.B: Concentrations of the unlabeled signature peptides spiked into Study I samples (NA = none added)

	Concentration, fmol/µL										
Sample	**bi0173**	**bi0167**	**bi0171**	**bi0169**	**bi0170**	**bi0161**	**bi0037**	**bi0166**	**bi0231**	**bi0202**	**ni0001**
I-A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
I-B	0.9	0.9	0.9	0.8	0.9	0.9	1.0	0.9	0.9	0.9	0.9
I-C	2.6	2.5	2.6	2.5	2.5	2.6	2.9	2.6	2.5	2.6	2.7
I-D	7.7	7.5	7.6	7.4	7.5	7.7	8.6	7.6	7.5	7.8	7.9
I-E	22.4	21.9	22.2	21.5	21.8	22.4	25.1	22.1	21.9	22.6	22.9
I-F	41.3	40.4	40.9	39.6	40.3	41.3	46.4	40.8	40.3	41.6	42.3
I-G	74.4	72.9	73.6	71.3	72.5	74.3	83.5	73.4	72.6	75.0	76.1
I-H	136.8	134.0	135.4	131.1	133.4	136.7	153.6	135.1	133.6	137.9	140.0
I-I	251.7	246.5	249.1	241.2	245.4	251.5	282.6	248.5	245.7	253.7	257.6
I-J	463.0	453.4	458.3	443.7	451.4	462.7	519.8	457.1	452.0	466.6	473.8

* A legend for the unlabeled signature peptide name identifier can be found in the Supplementary Methods - SOP (therein embedded SOP Table A). Target concentrations for spike-in levels were the following: I -B (1.00 fmol/µL), I -C (2.92 fmol/µL), I -D (8.55 fmol/µL), I -E (25 fmol/µL), I -F (46 fmol/µL), I -G (83 fmol/µL), I -H (151 fmol/µL), I -I (275 fmol/µL), and I -J (500 fmol/µL).

Supplementary Table 6.C: Concentrations of the isotopically-labeled ($^{13}C/^{15}N$) internal standard peptides spiked into Study II samples

	Concentration, fmol/µL										
Sample	**bi0081**	**ni0101**	**ni0102**	**ni0104**	**ni0105**	**bi0067**	**ni0107**	**ni0108**	**ni0109**	**ni0110**	**ni0111**
II-A	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
II-B	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
II-C	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
II-D	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
II-E	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
II-F	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
II-G	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
II-H	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
II-I	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2
II-J	46.3	44.5	43.9	45.2	45.7	46.0	45.5	45.7	46.8	44.7	45.2

* A legend for the isotopically-labeled ($^{13}C/^{15}N$) internal standard peptide name identifier can be found in the Supplementary Methods - SOP (therein embedded SOP Table A). Target concentrations for spike-in levels were 50 fmol/µL.

Supplementary Table 6.D: Concentrations of the digested proteins spiked into Study II samples (NA = none added)

	Concentration, fmol/µL						
Sample	**APR**	**LEP**	**MYO**	**MBP**	**PSA**	**HRP**	**CRP**
II-A	NA	NA	NA	NA	NA	NA	NA
II-B	1.0	1.0	1.0	1.0	1.0	1.0	1.0
II-C	2.8	2.8	2.8	2.8	2.8	2.8	2.9
II-D	8.4	8.4	8.2	8.4	8.4	8.4	8.5
II-E	24.4	24.6	24.0	24.5	24.4	24.4	24.7
II-F	44.8	45.2	44.1	45.0	44.9	44.9	45.4
II-G	81.1	81.7	79.8	81.4	81.2	81.2	82.1
II-H	147.8	149.0	145.5	148.4	148.0	148.1	149.6
II-I	268.6	270.8	264.5	269.8	269.0	269.2	272.0
II-J	487.3	491.2	479.8	489.4	488.0	488.3	493.5

* Protein name abbreviations: APR (aprotinin), LEP (leptin), MYO (myoglobin), MBP (myelin basic protein), PSA (prostate specific antigen), HRP (horseradish peroxidase), and CRP (C-reactive protein).
Target concentrations for spike-in levels were the following: II -B (1.00 fmol/µL), II -C (2.92 fmol/µL), II -D (8.55 fmol/µL), II -E (25 fmol/µL), II -F (46 fmol/µL), II -G (83 fmol/µL), II -H (151 fmol/µL), II -I (275 fmol/µL), and II -J (500 fmol/µL).

Supplementary Table 6.E: Concentrations of the isotopically-labeled ($^{13}C/^{15}N$) internal standard peptides in the IS peptide mixture distributed with Study III samples

	bi0081	ni0101	ni0102	ni0104	ni0105	bi0067	ni0107	ni0108	ni0109	ni0110	ni0111
IS Peptide Mix, fmol/µL	476.9	458.1	460.2	470.7	457.2	464.2	469.6	469.5	506.8	477.8	447.3

* A legend for the isotopically-labeled ($^{13}C/^{15}N$) internal standard peptide name identifier can be found in the Supplementary Methods - SOP (therein embedded SOP Table A). Target concentrations for spike-in levels were 500 fmol/µL.

Supplementary Table 6.F: Concentrations of the proteins spiked into Study III samples (NA = none added)

	Concentration, fmol/µL						
Sample	**APR**	**LEP**	**MYO**	**MBP**	**PSA**	**HRP**	**CRP**
III-A	NA	NA	NA	NA	NA	NA	NA
III-B	63	62	62	63	62	62	63
III-C	183	180	181	182	179	180	183
III-D	534	525	528	530	521	526	533
III-E	1559	1532	1541	1548	1520	1535	1555
III-F	2879	2829	2845	2859	2807	2835	2872
III-G	5135	5046	5074	5099	5006	5056	5122
III-H	9271	9111	9163	9208	9039	9129	9249
III-I	16817	16525	16619	16701	16394	16559	16777
III-J	30461	29934	30104	30251	29696	29994	30389

* Protein name abbreviations: APR (aprotinin), LEP (leptin), MYO (myoglobin), MBP (myelin basic protein), PSA (prostate specific antigen), HRP (horseradish peroxidase), and CRP (C-reactive protein).
Target concentrations for spike-in levels were the following: II -B (60 fmol/µL), II -C (175 fmol/µL), II -D (513 fmol/µL), II -E (1,500 fmol/µL), II -F (2,760 fmol/µL), II -G (4,980 fmol/µL), II -H (9,060 fmol/µL), II -I (16,500 fmol/µL), and II -J (30,000 fmol/µL).

Footnote for all Supplementary Tables 6 A-F:

For spike in experiments in Studies I-III, gravimetric determination of solution amounts were performed as gravimetric determinations have proven to be much more accurate and precise than volumetric determinations, particularly when total volumes are low. Gravimetric sample preparations were performed using one of two balances (a Mettler Toledo model XP205 and a Mettler Toledo model AX26 balance; 6-place balances).

For instance, for the preparation of the Study I samples, the volume of the heavy peptide mix weighed out was about 1 mL while the volume of the digested, diluted plasma was about 20 mL. In determining both volumes by gravimetry, the solutions were equilibrated to the balance room temperature for a few hours; the weights of aliquots are then converted to volumes using the measured temperature and assumed density. The density of the above solutions was assumed to be the same as that of water (as these solutions were not very concentrated). For Study III sample preparation, we determined the density of the undigested, concentrated plasma, which differs from that of water.

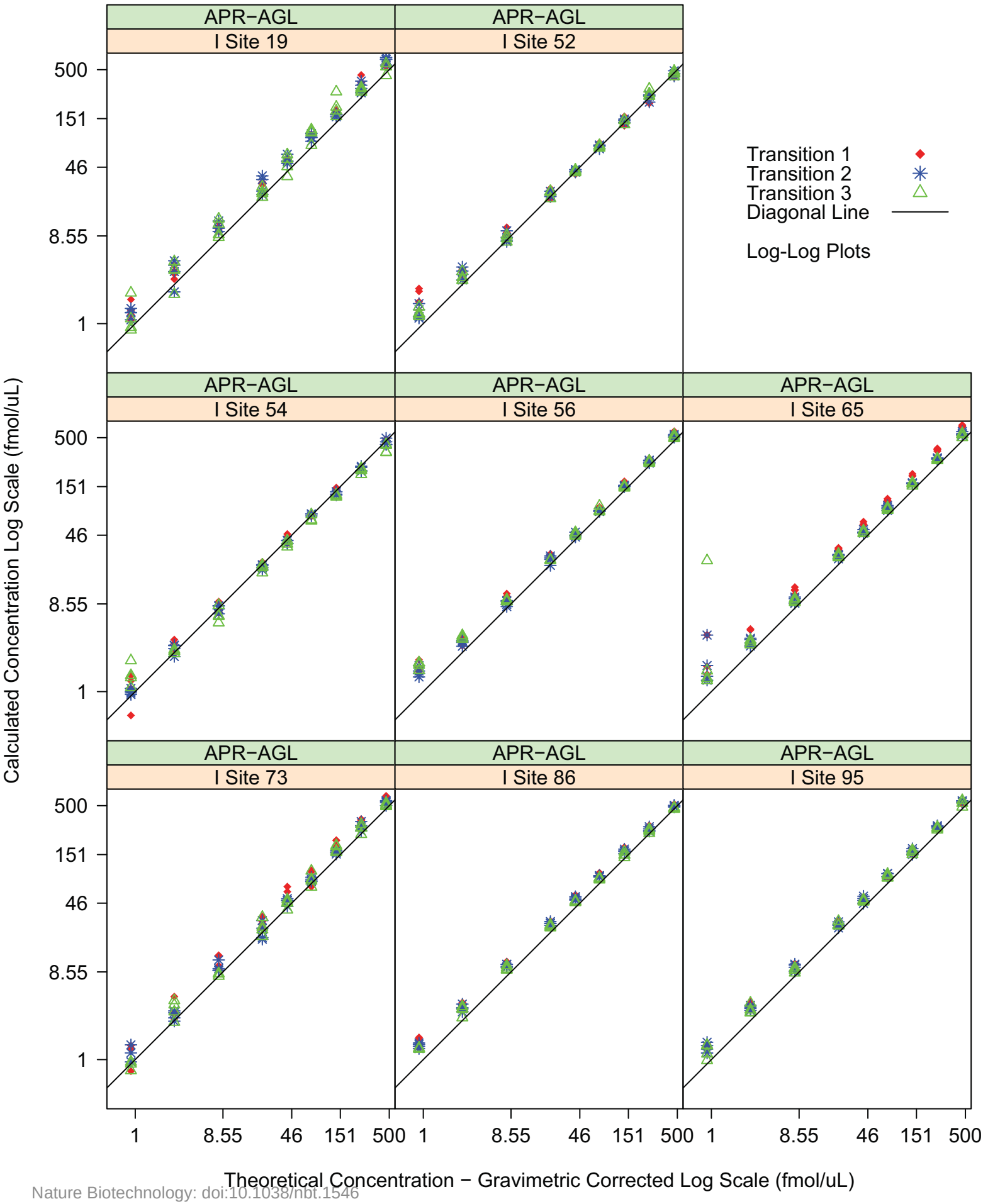
Supplementary Appendix:

Dilution curves represented as **Log-Log Plots**

Dilution curves of calculated concentrations (y-axis, log scale) of the ten signature peptides and the theoretical/spiked-in concentration (x-axis, log scale) into solution (buffer or plasma matrix). These dilution plots are represented on a log / log scale for all five Studies I, II, III a-c, and all ten peptides with three transitions each from all eight participating sites.

Each of the eight sites were assigned random numerical codes (19, 52, 54, 56, 65, 73, 86, 95) for anonymization purposes. The dilution curves are organized by signature peptide, then by experiment (Studies I, II, III a,b,c) and all eight sites are displayed on each page. Study number and anonymization code for each site are displayed right above the individual regression plot (i.e., "I site 19" etc.), as well as the derived protein 3-letter code, the first three amino acids of the peptide sequence (i.e., APR-AGL; for further details see SOP – Supplementary Methods online). The three letter protein codes stand for APR (aprotinin), CRP (C-reactive protein), HRP (horseradish peroxidase), LEP (leptin), MBP (myelin basic protein), MYO (myoglobin), and PSA (prostate specific antigen). All theoretical/spiked-in concentrations are gravimetrically corrected (see Supplementary Tables 6 A-F).

A diagonal is displayed at y=x for all plots, no regression lines are fitted. All data are plotted on a logarithmic scale for the x and y axes.



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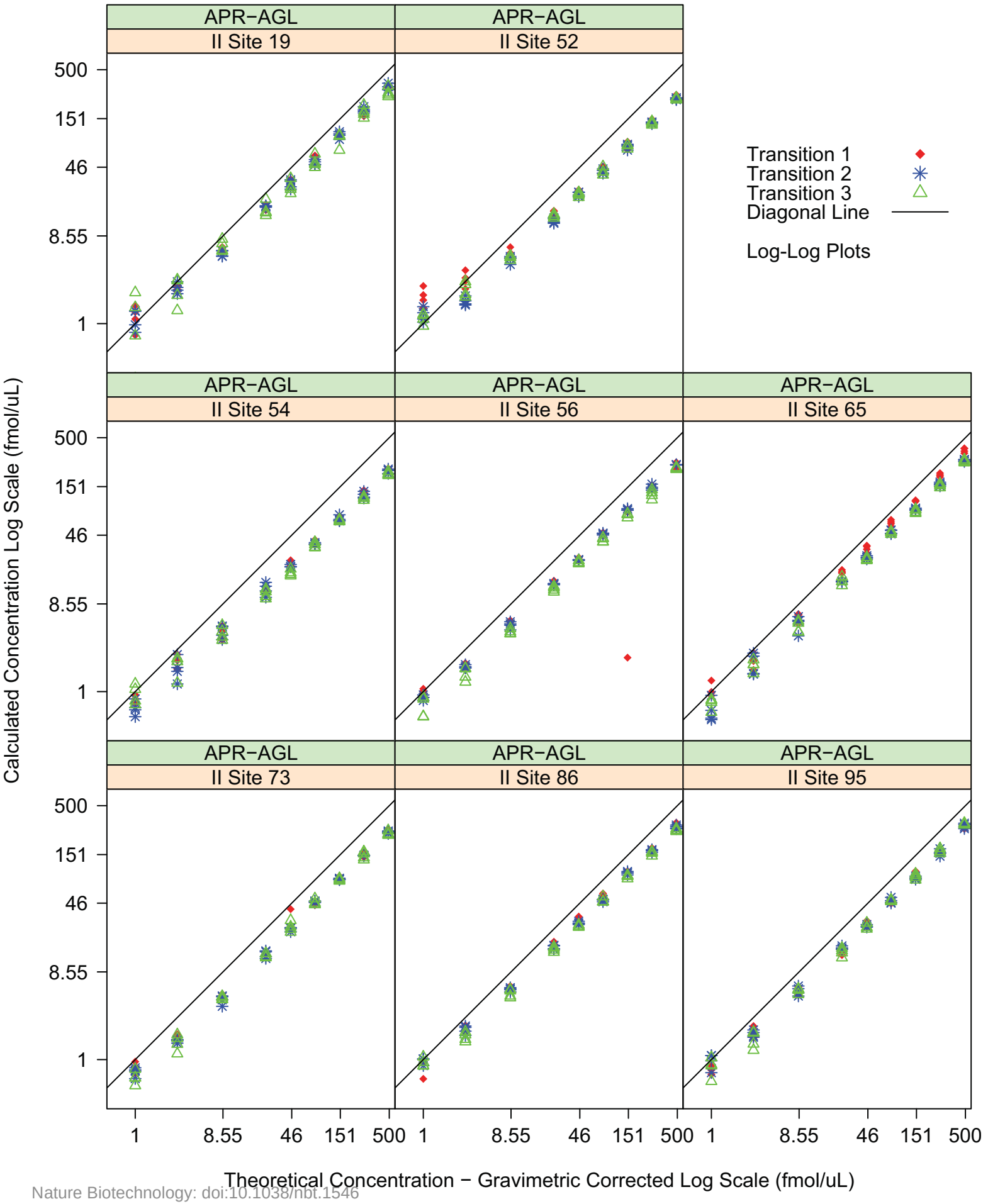

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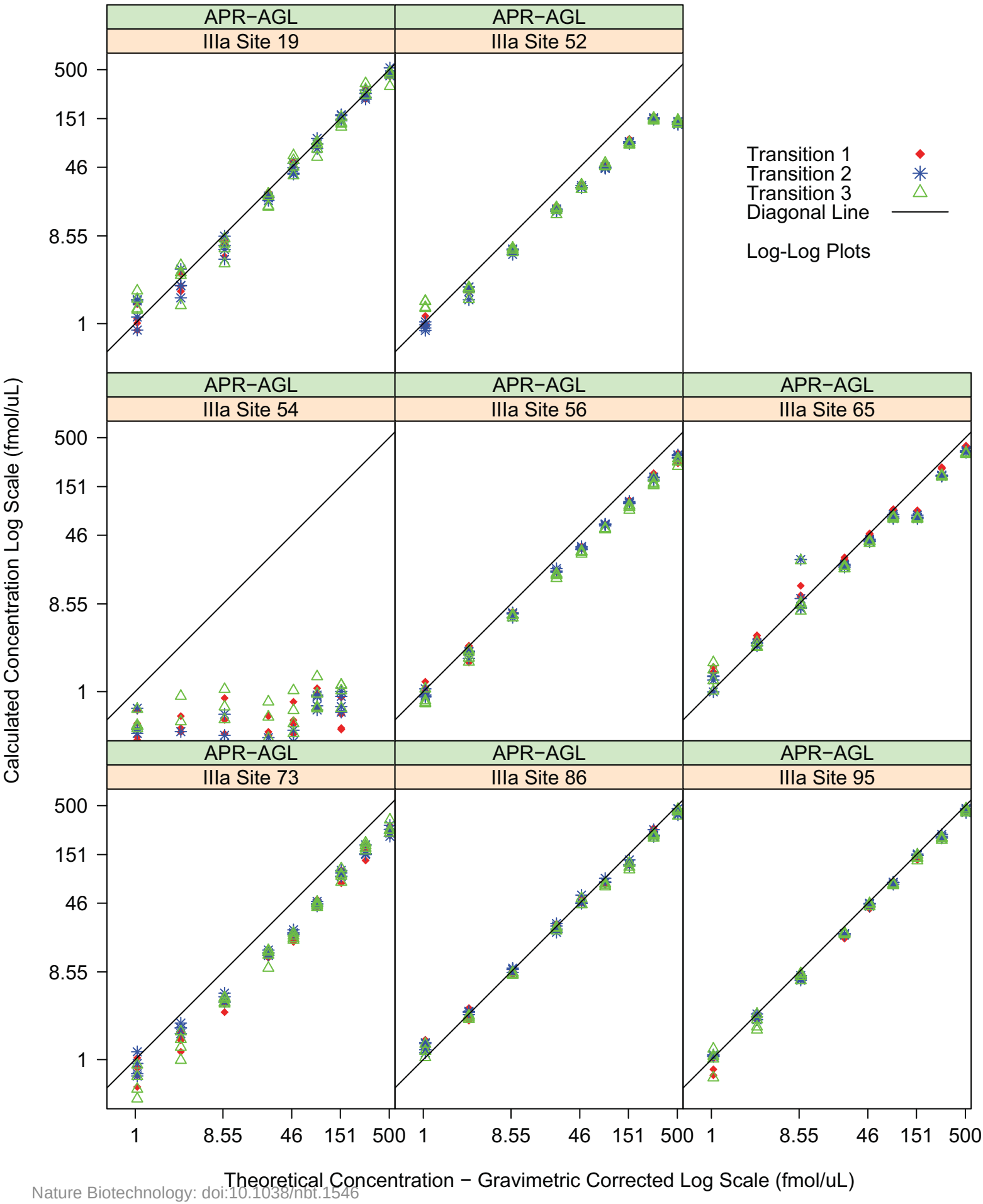

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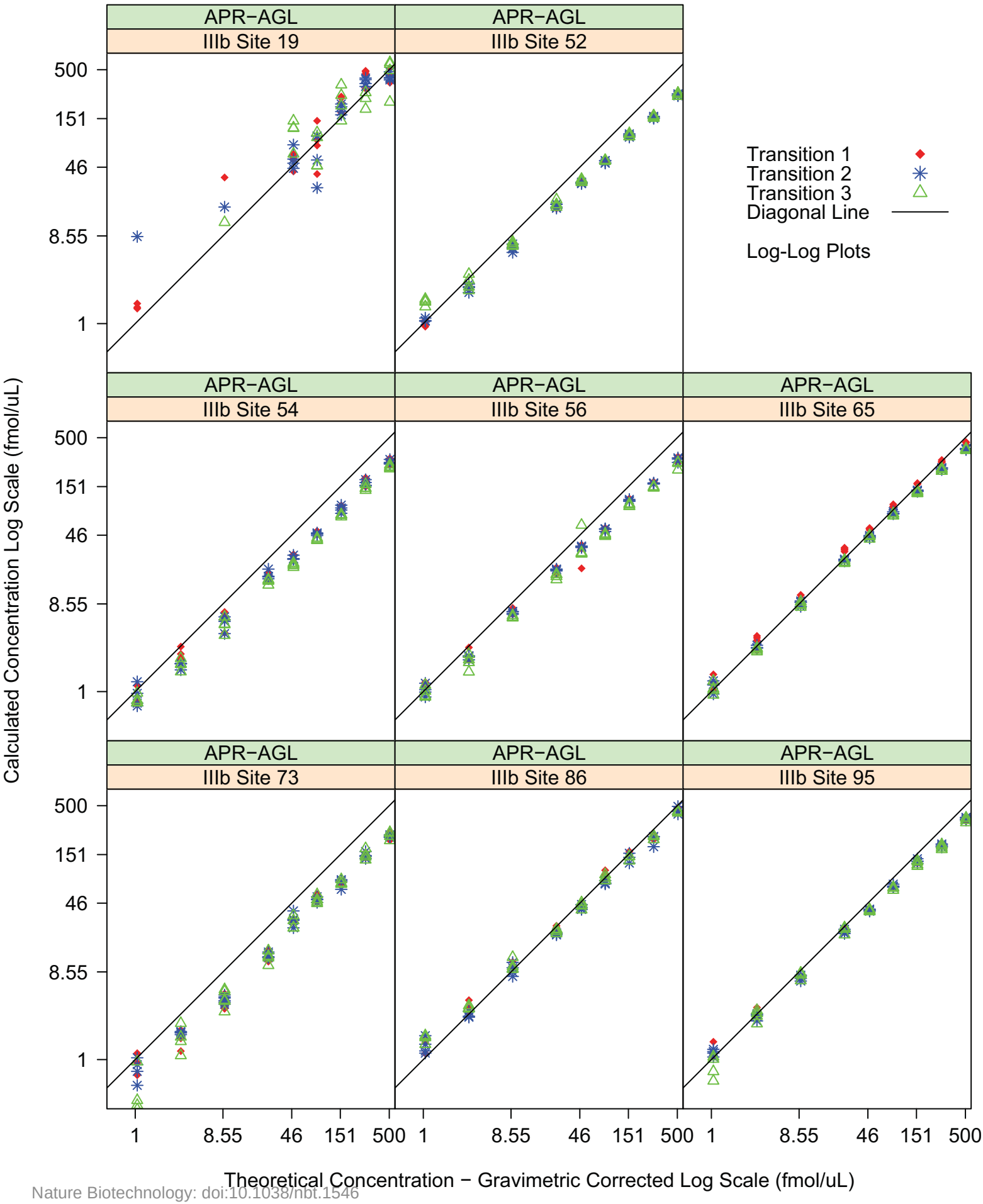

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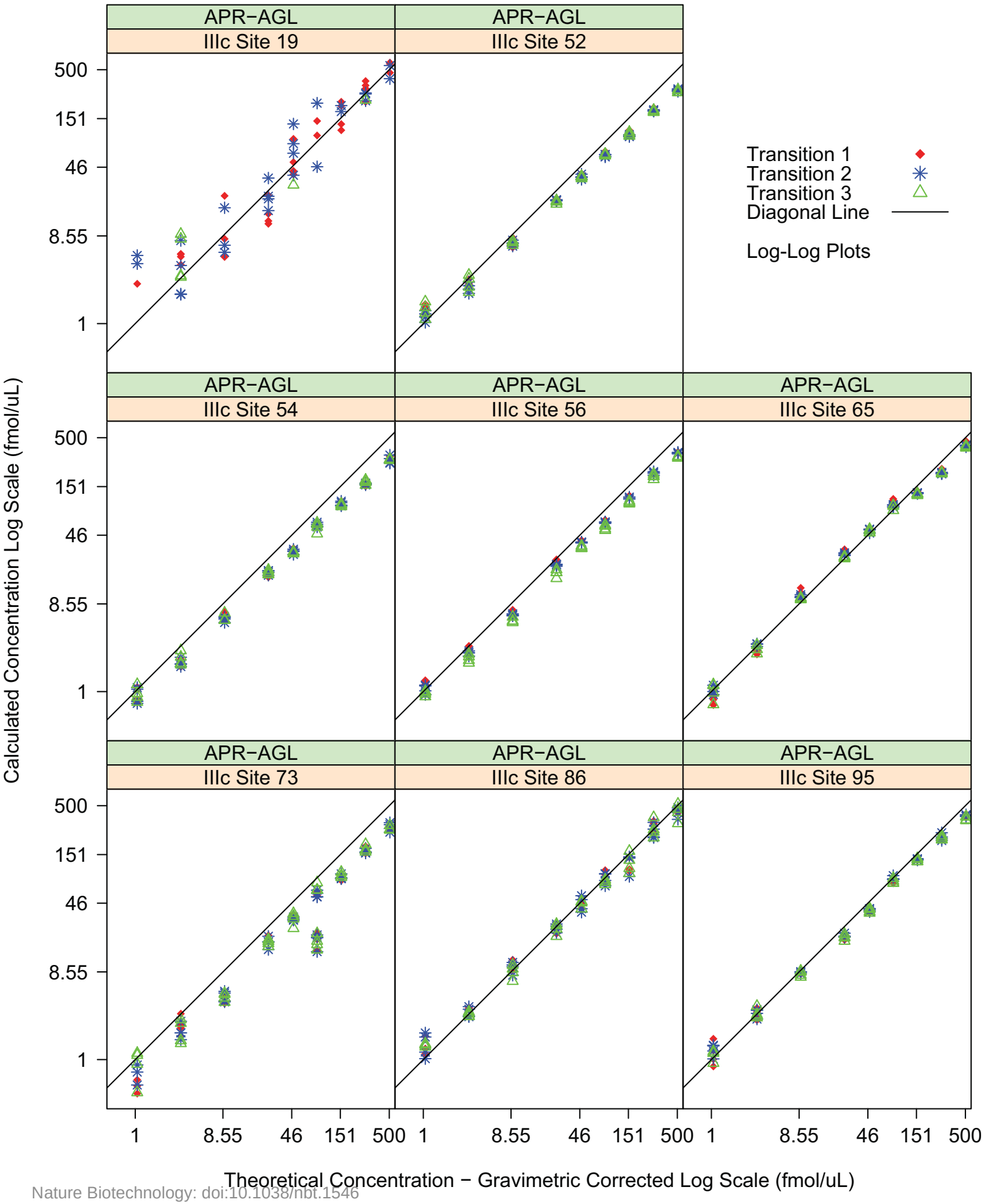

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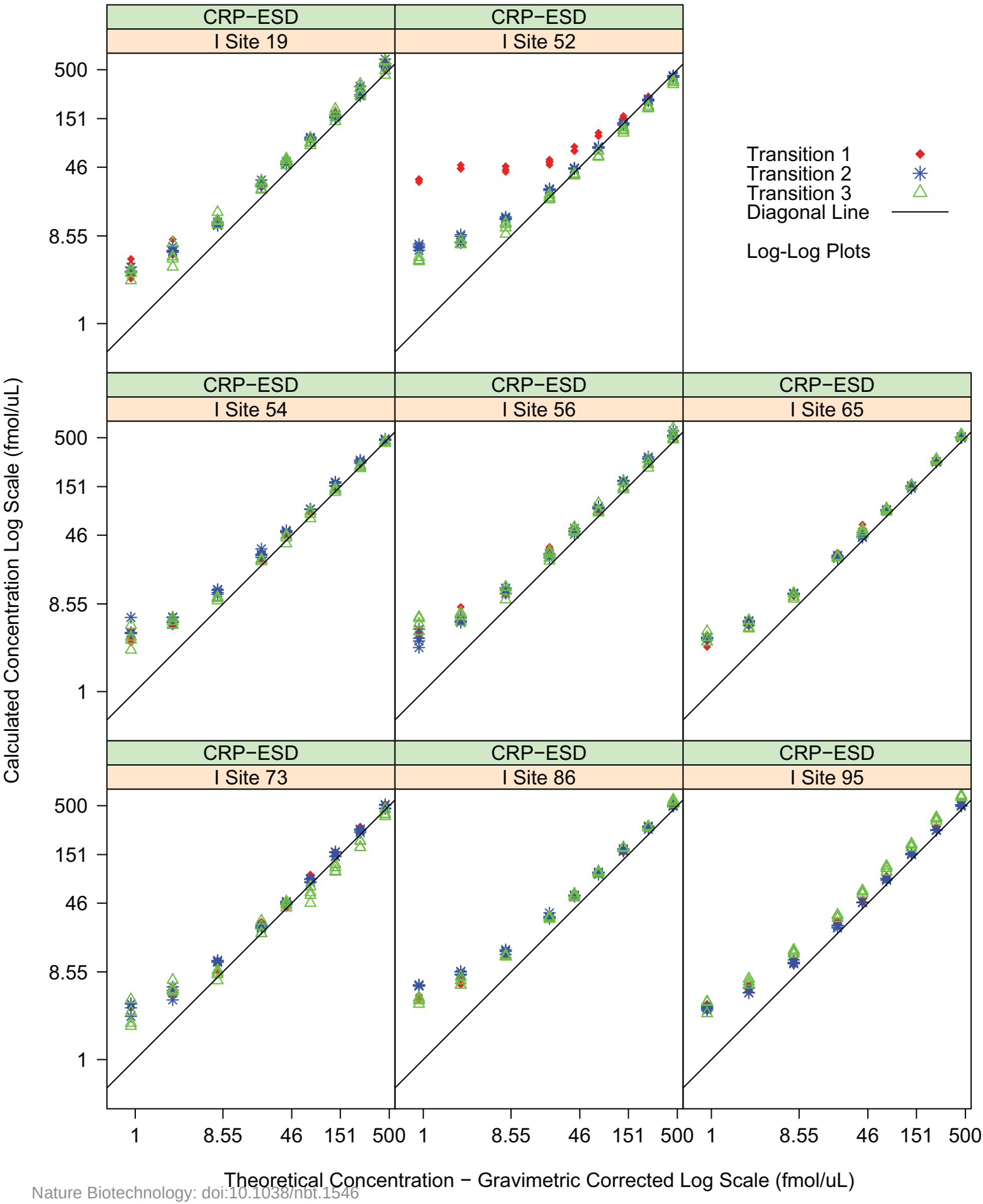

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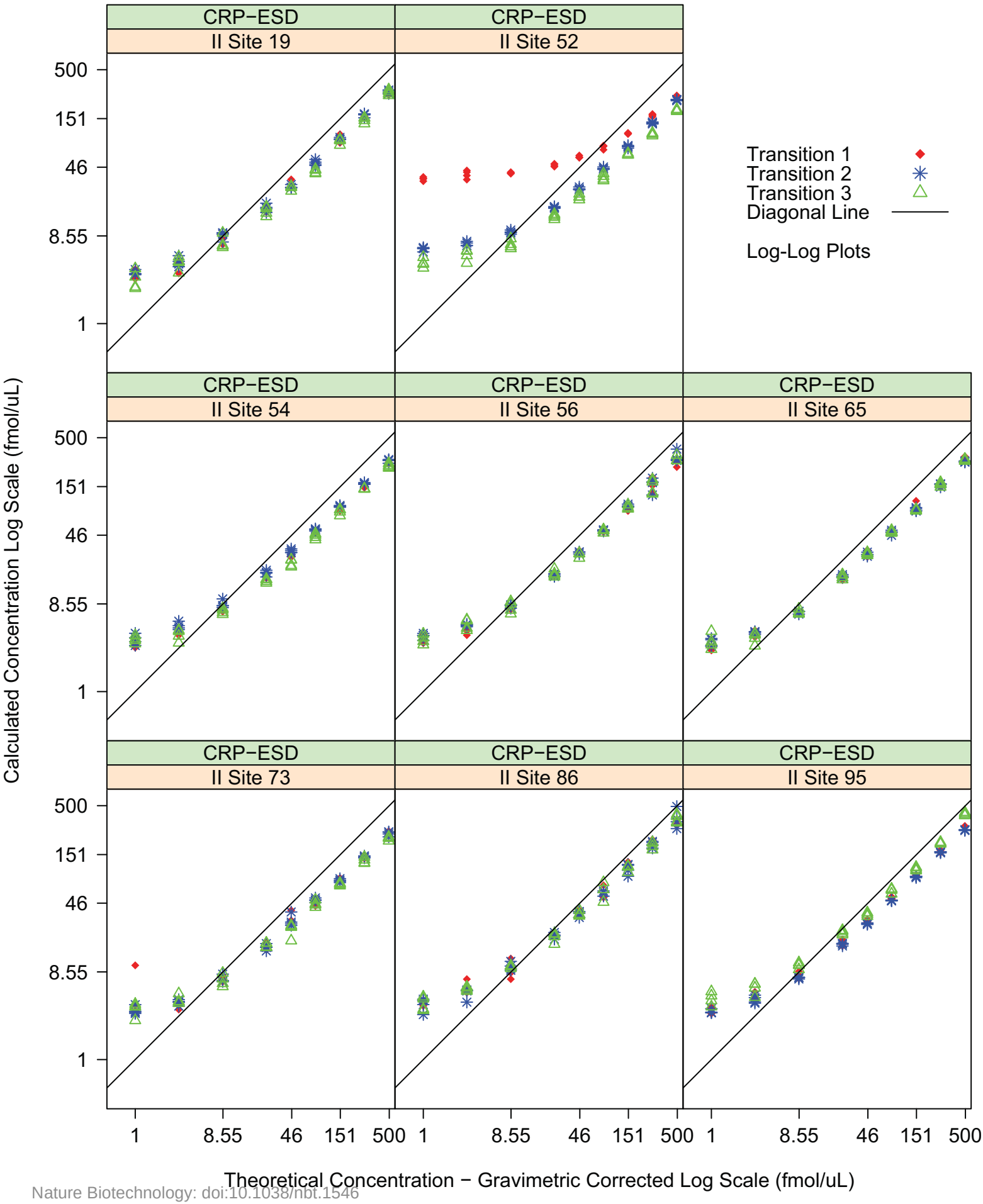

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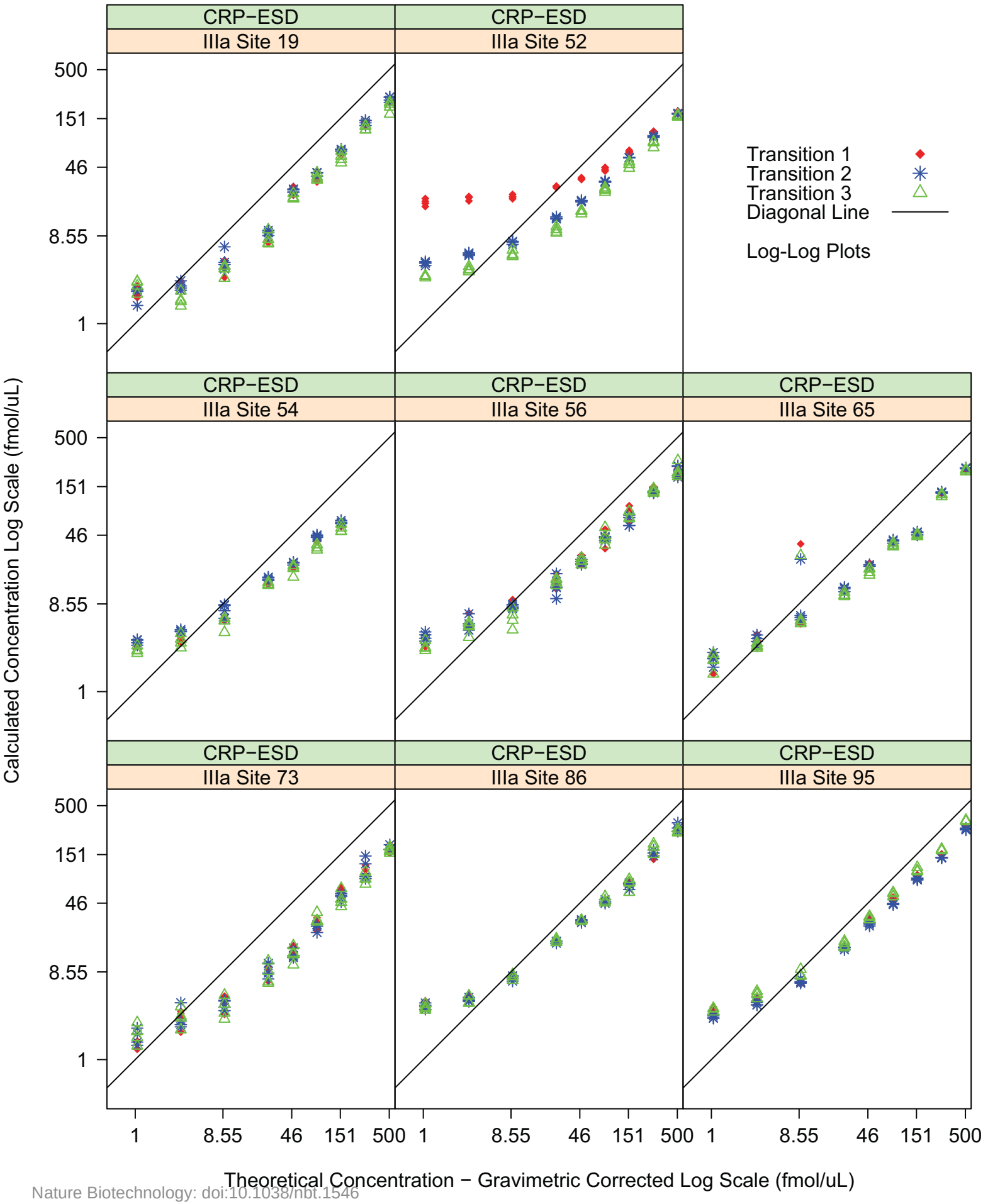

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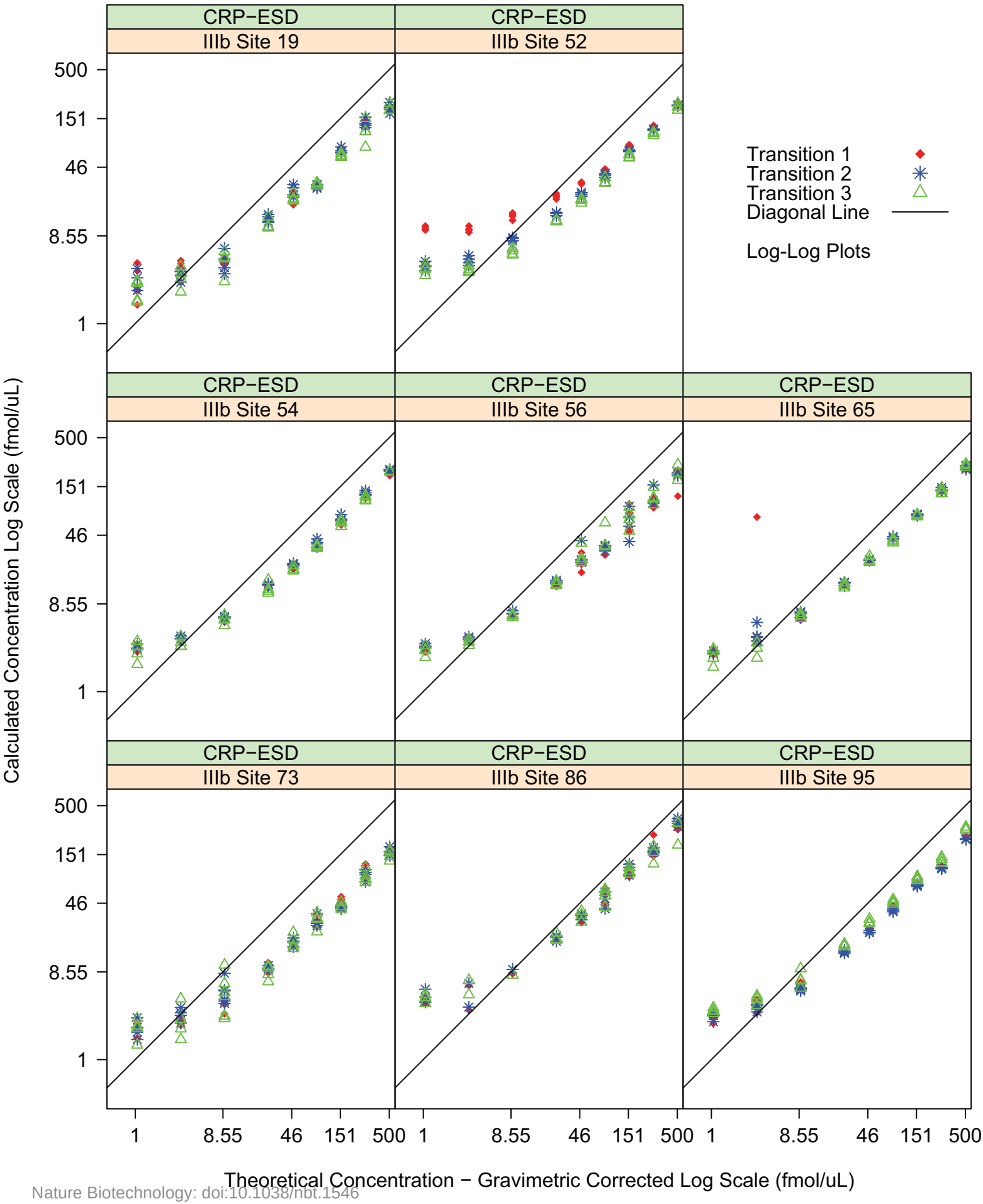

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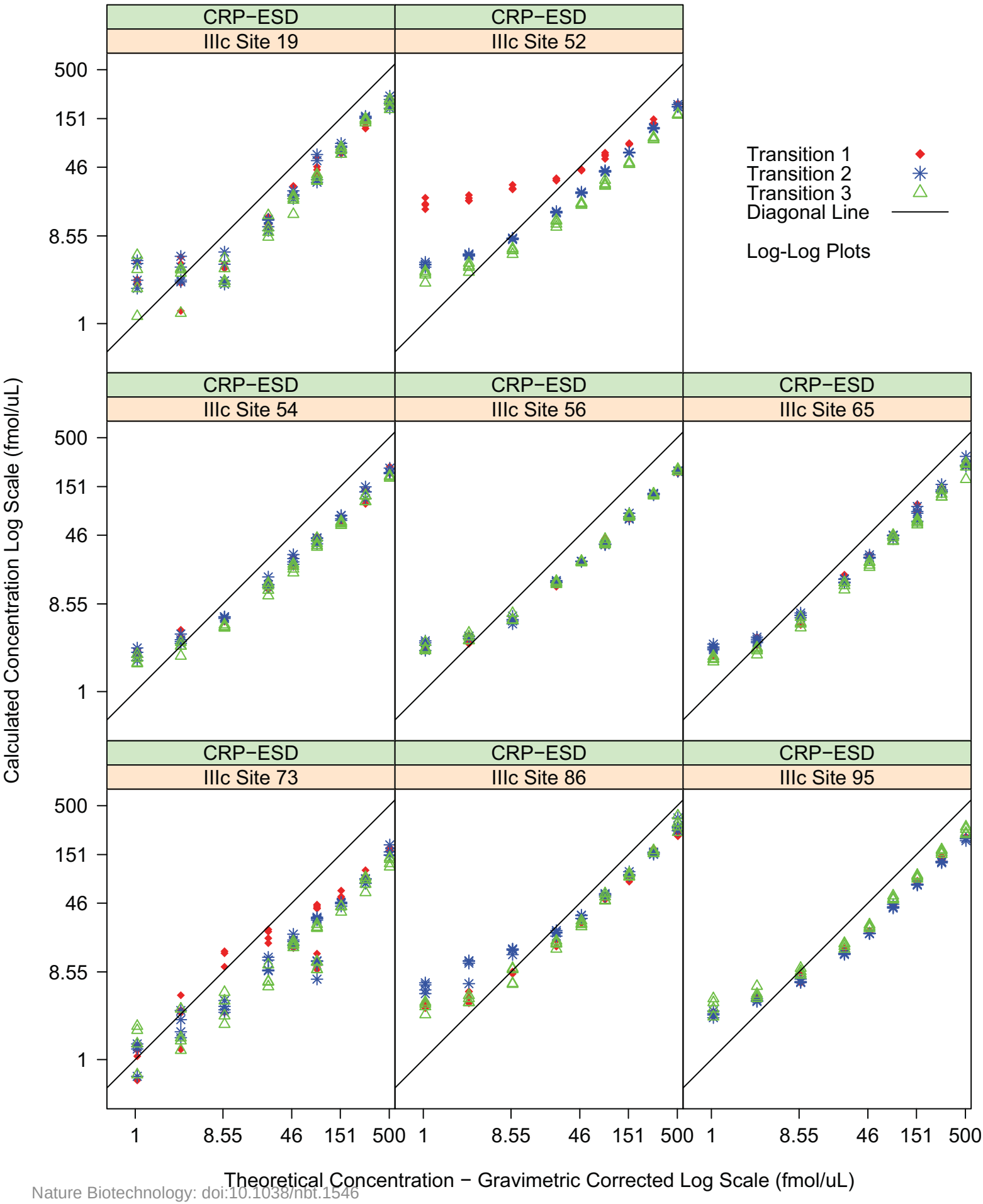

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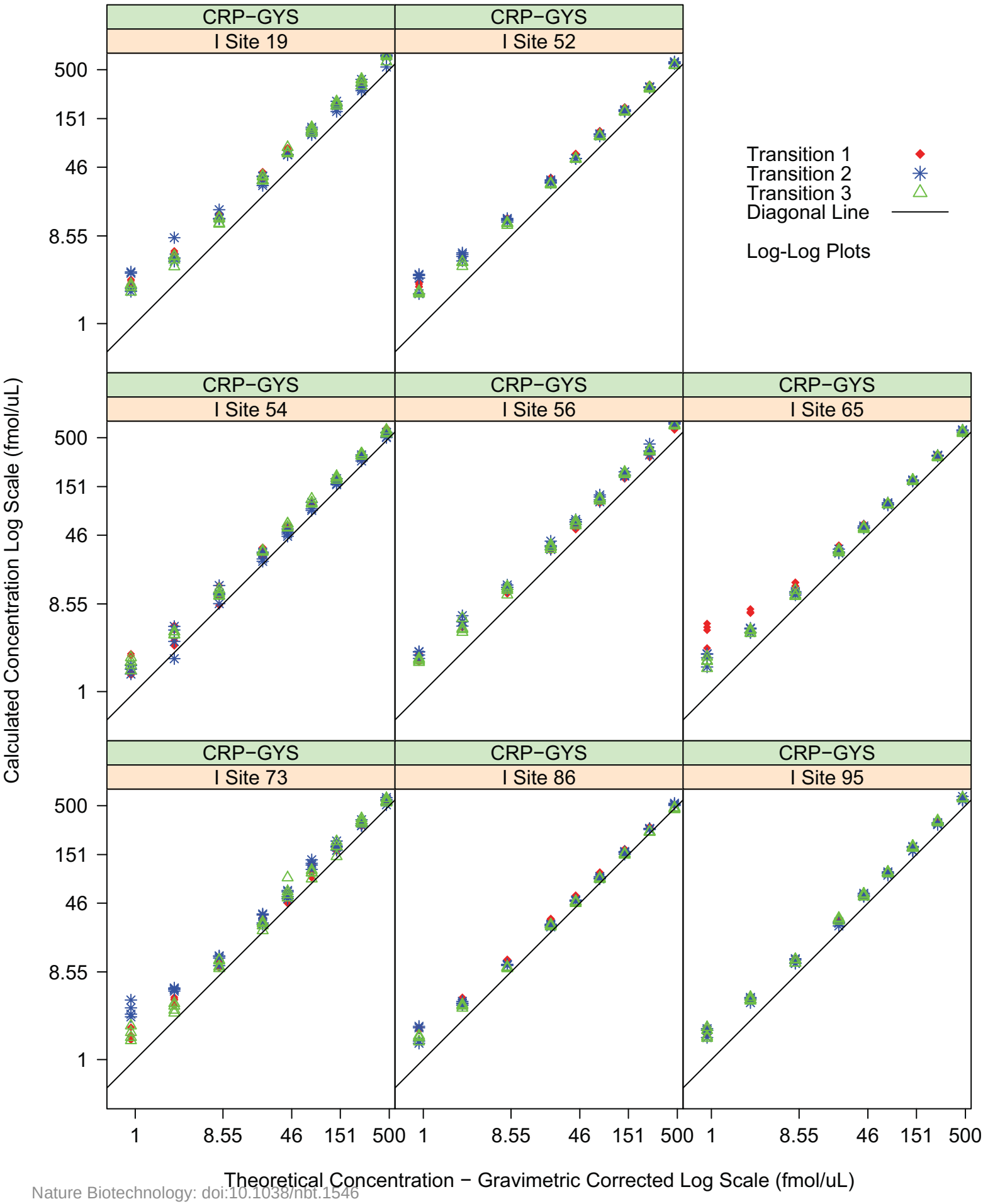

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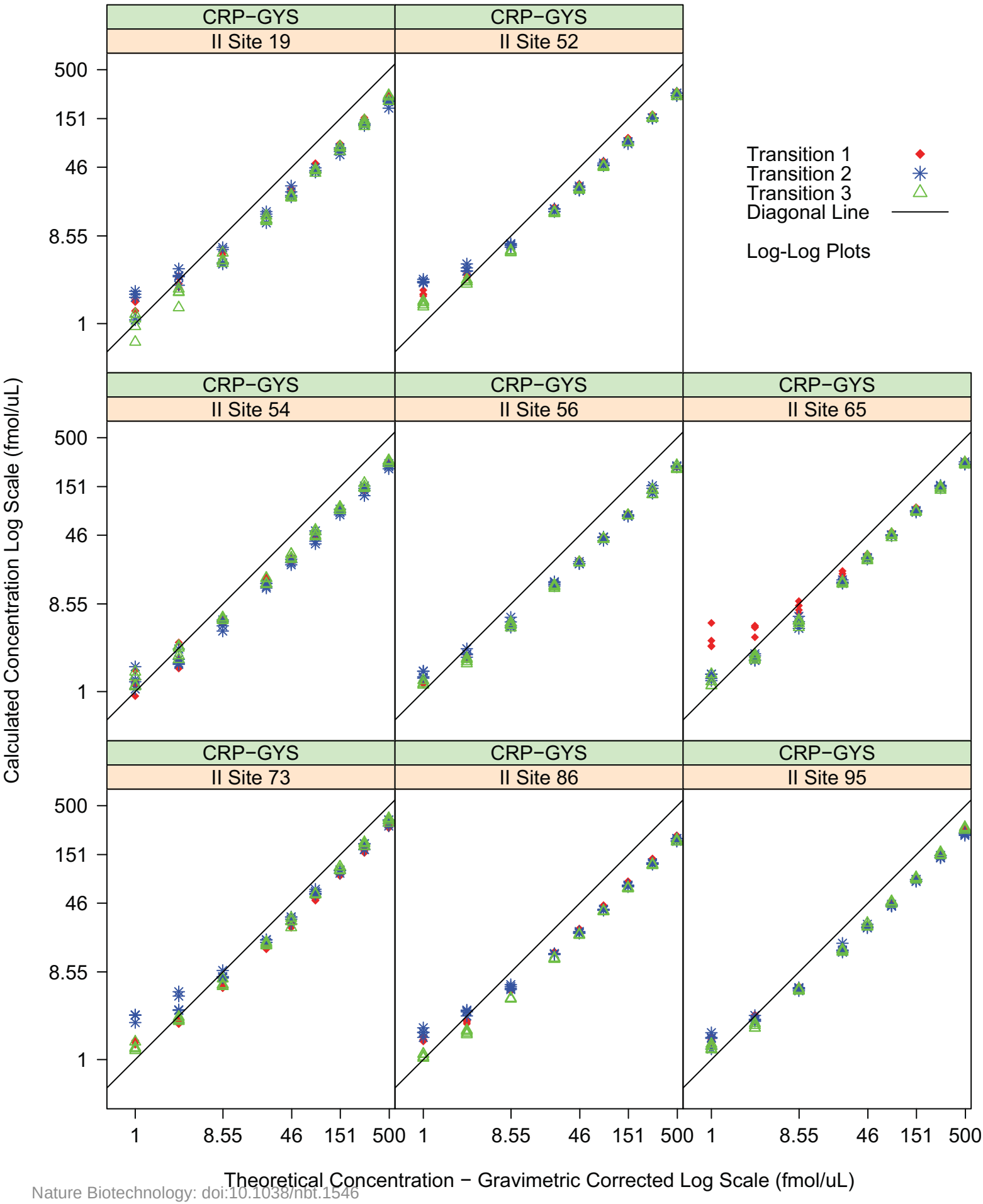

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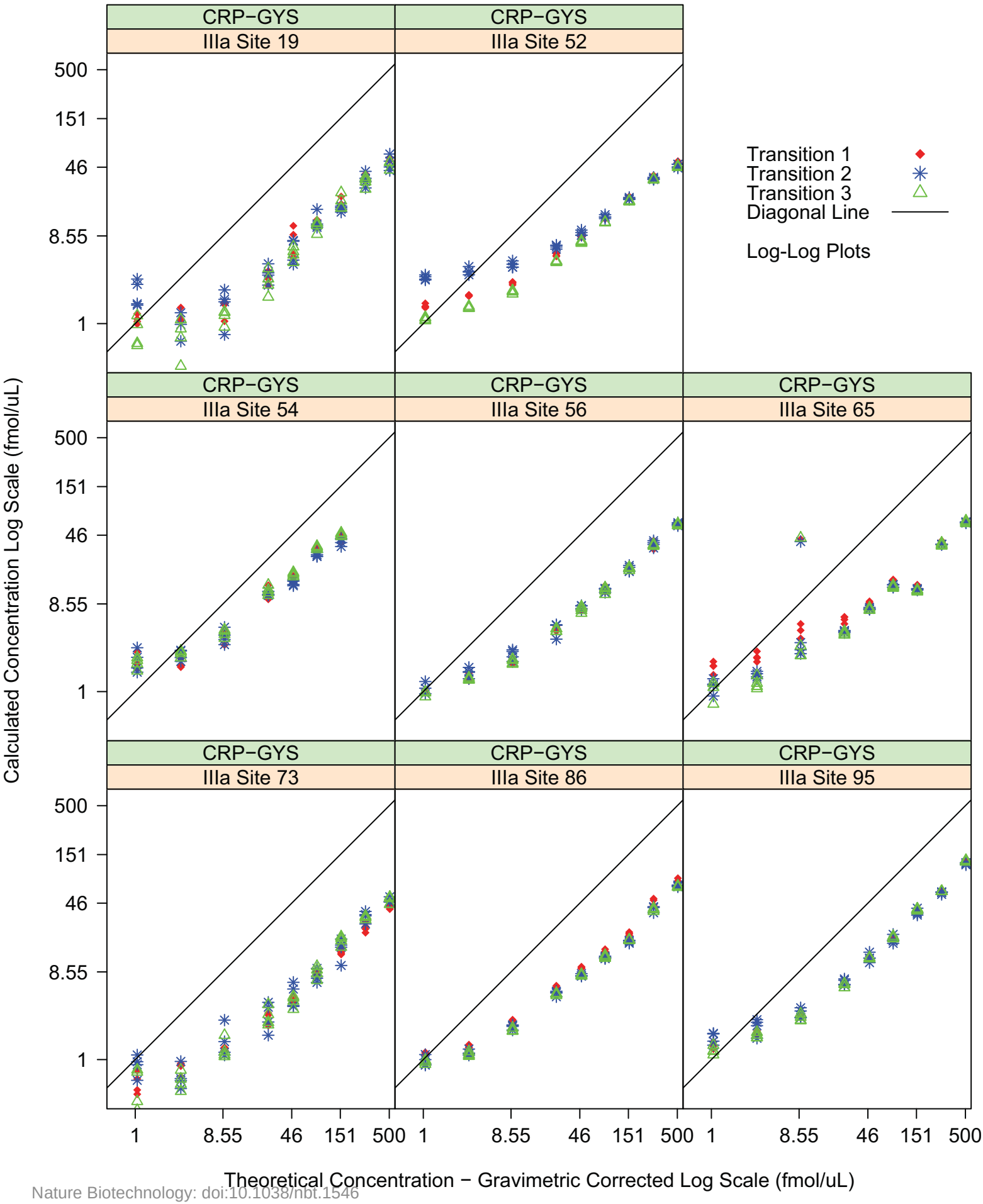

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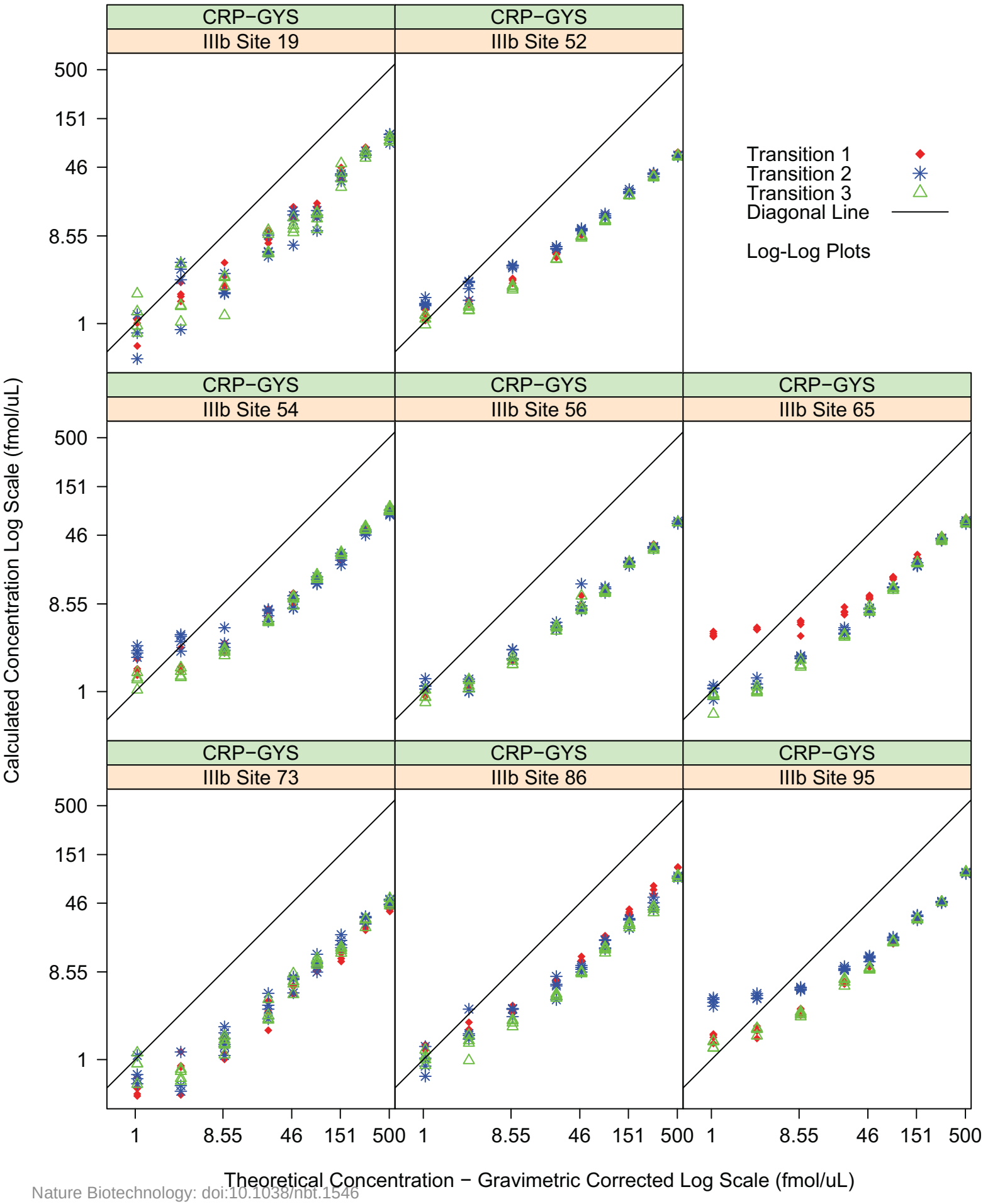

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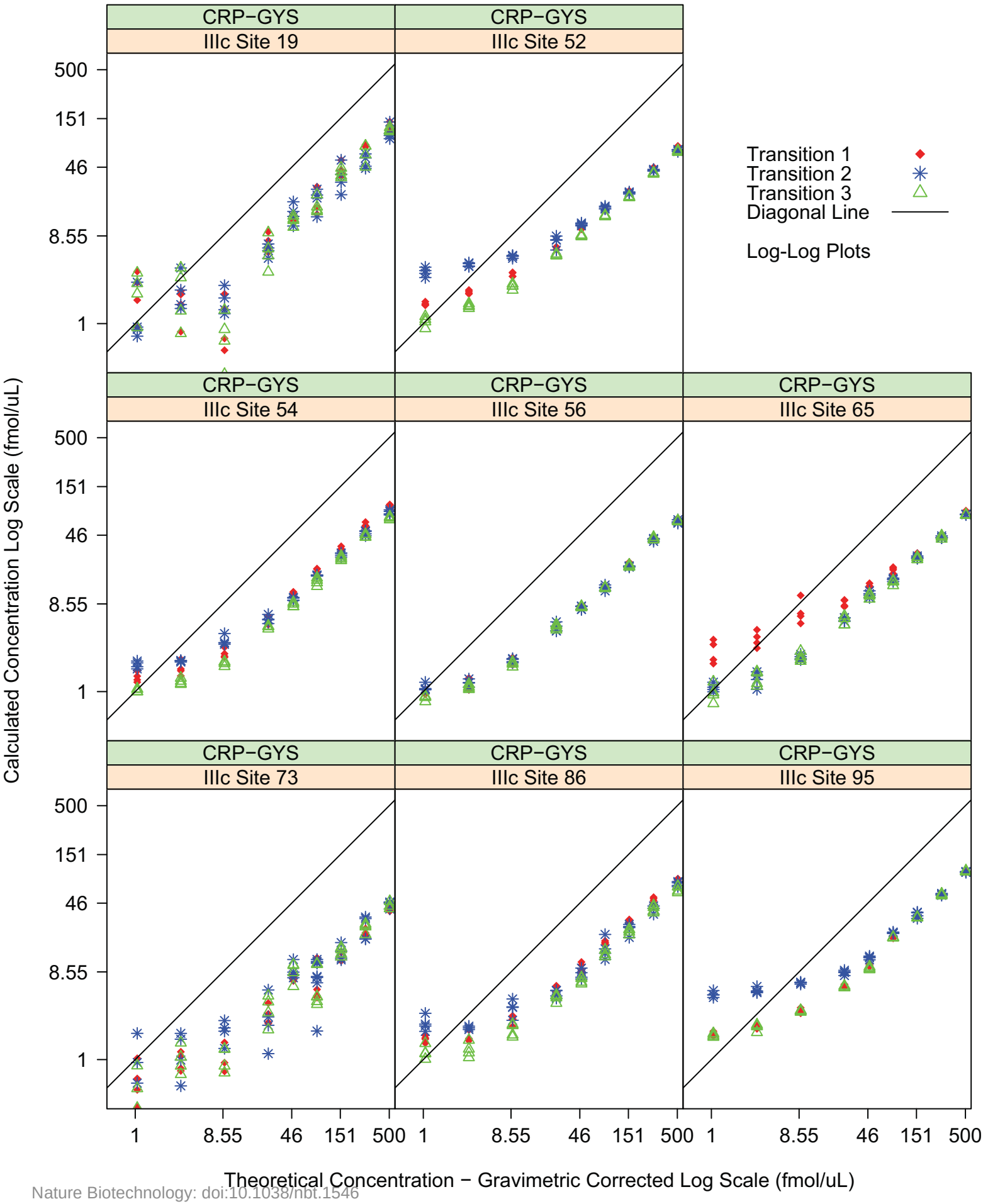

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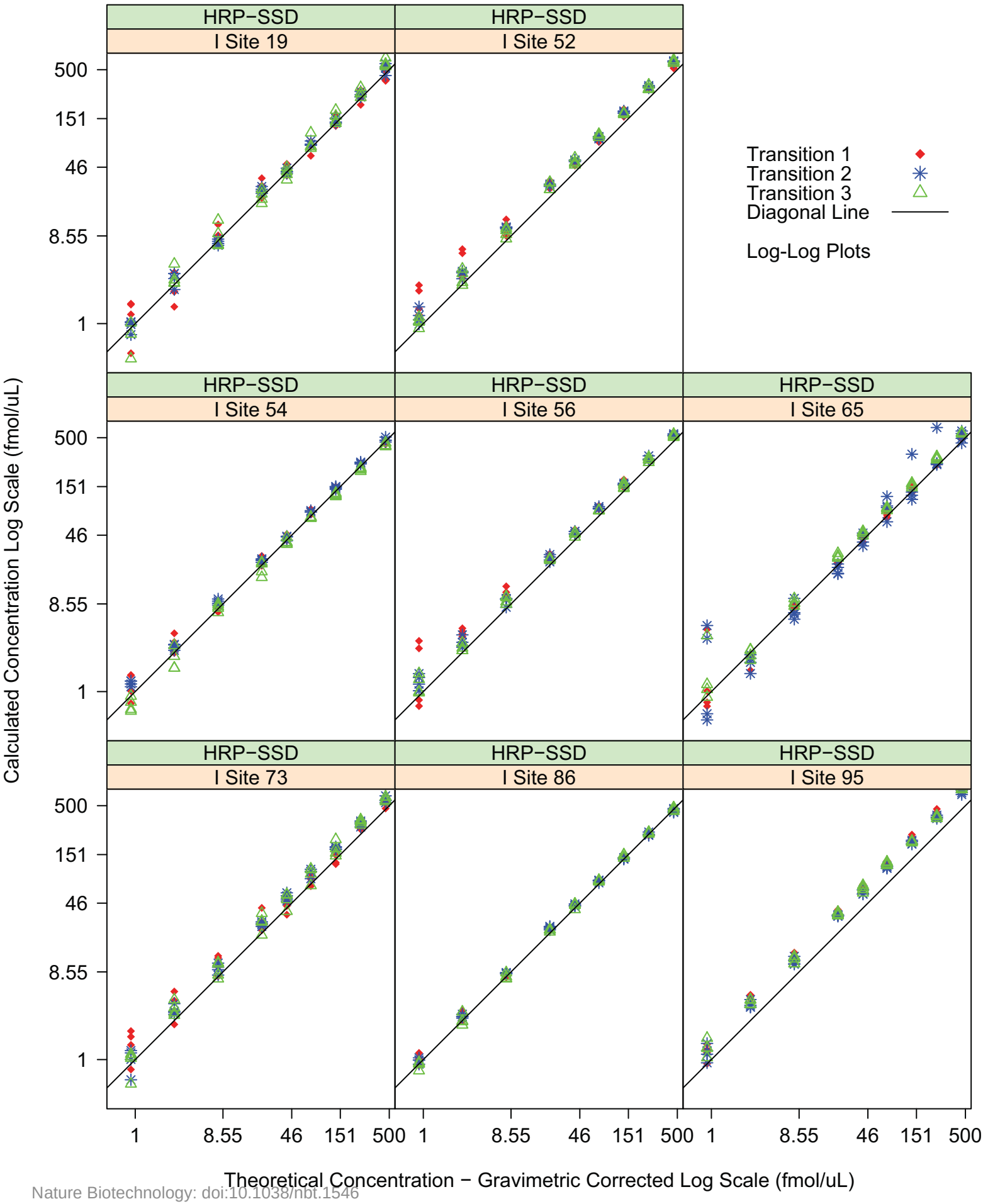

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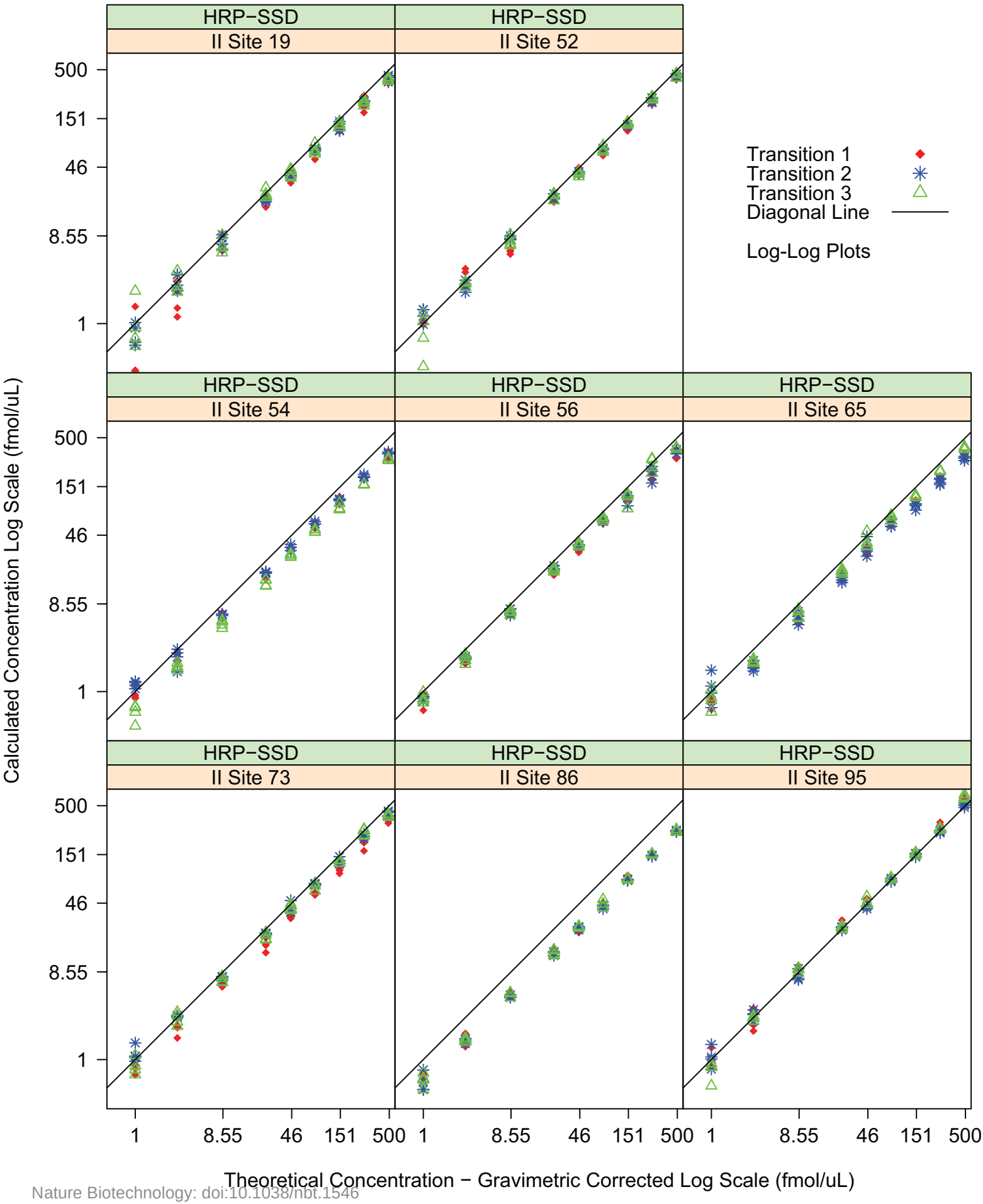

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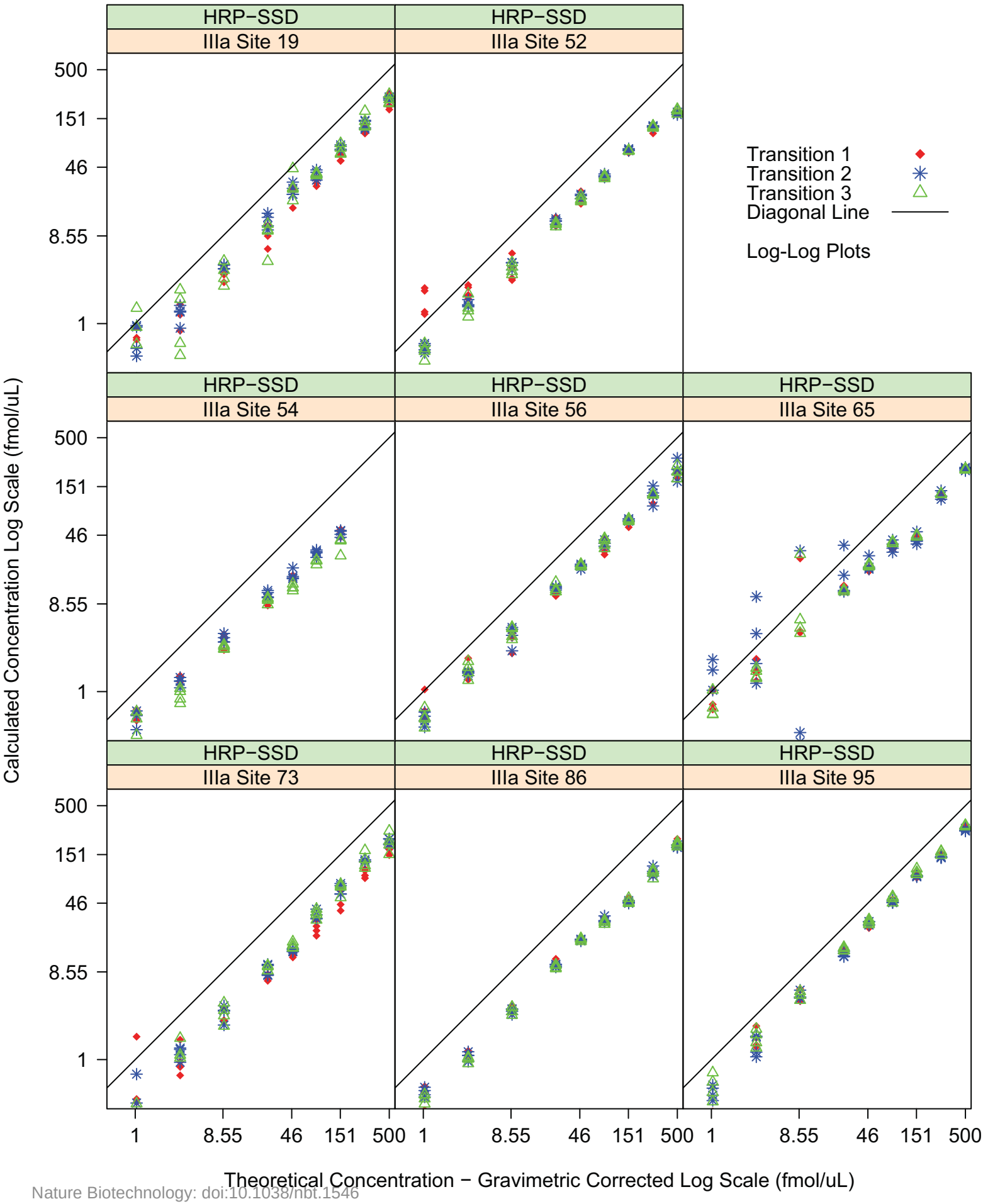

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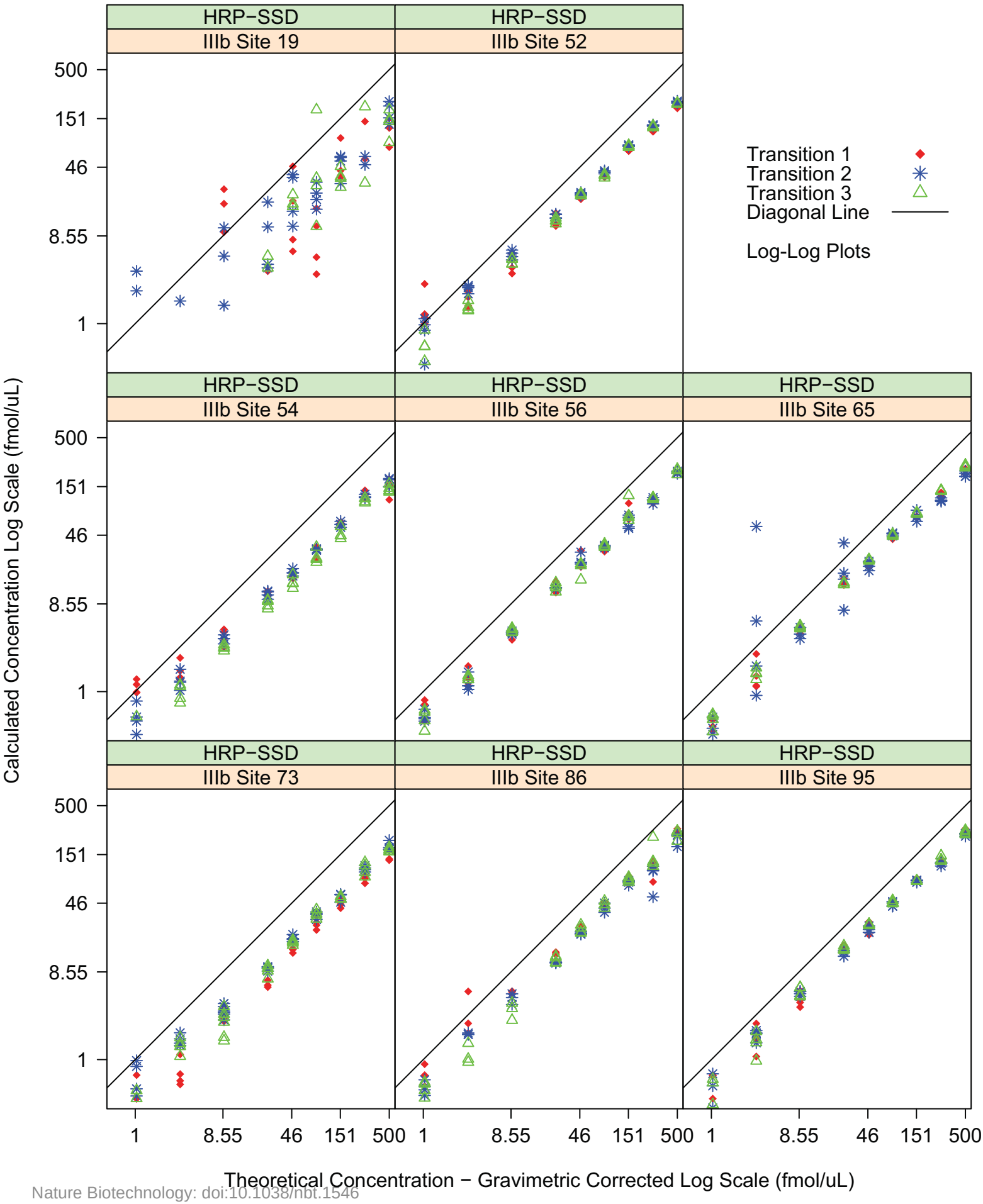

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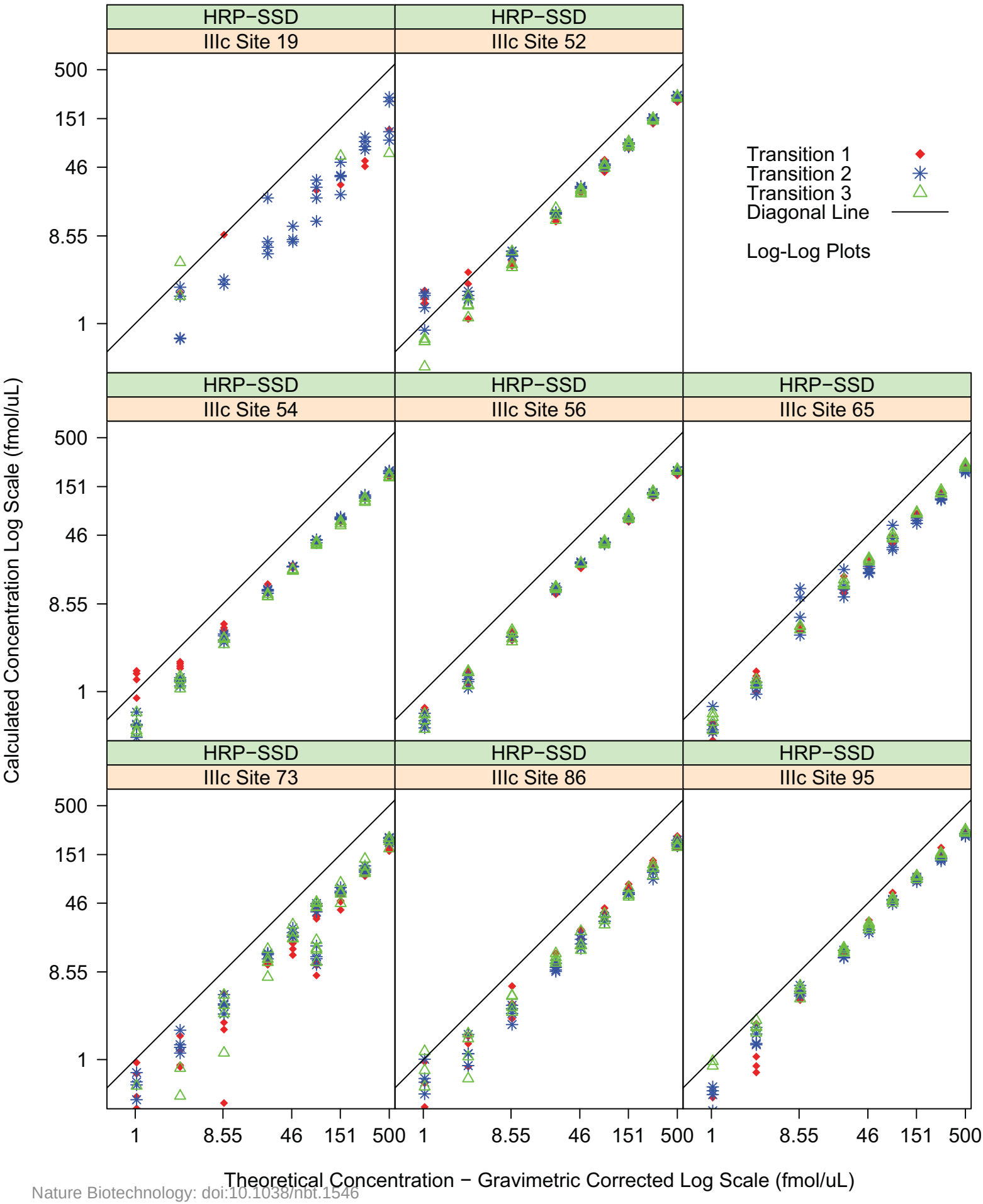

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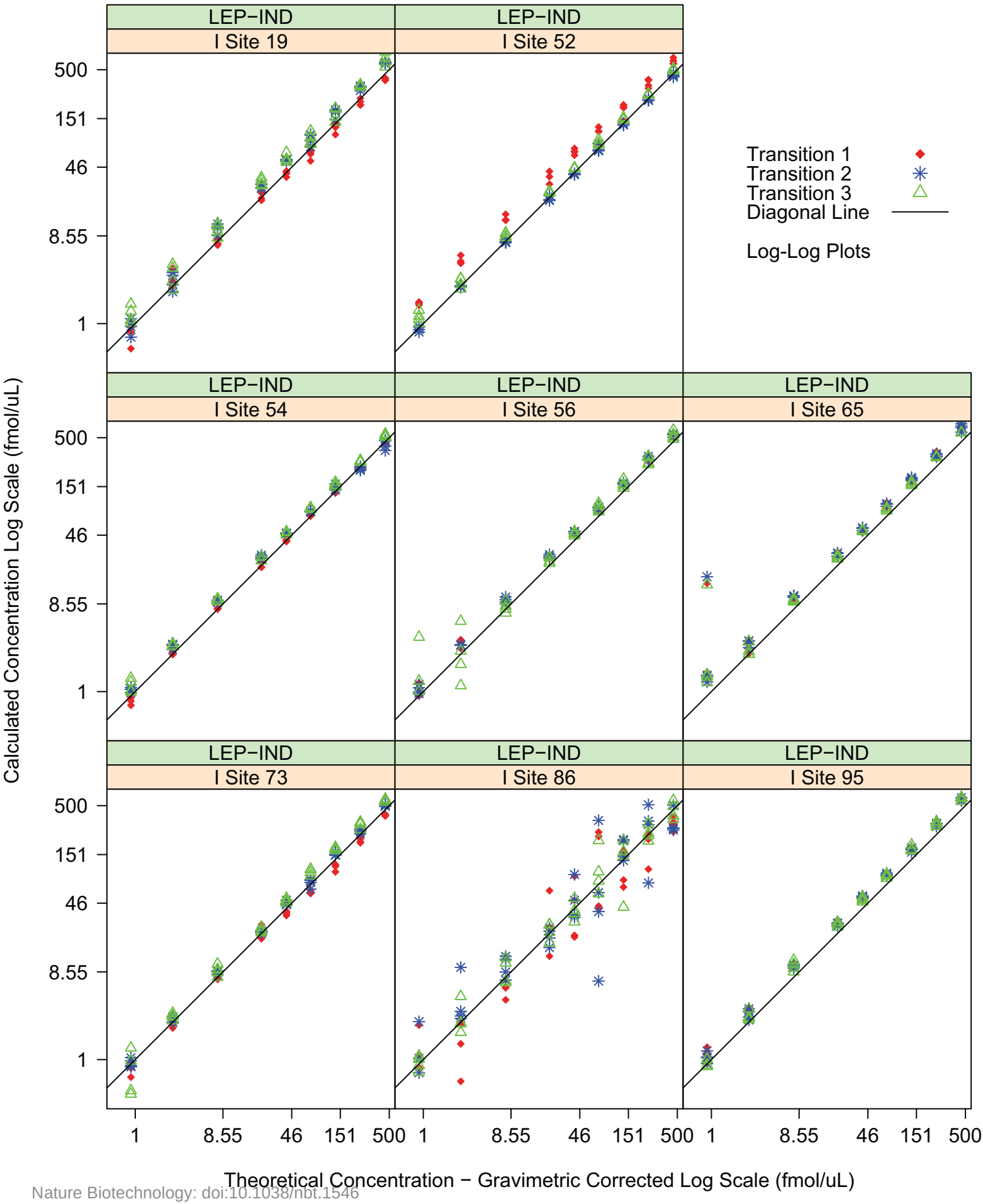

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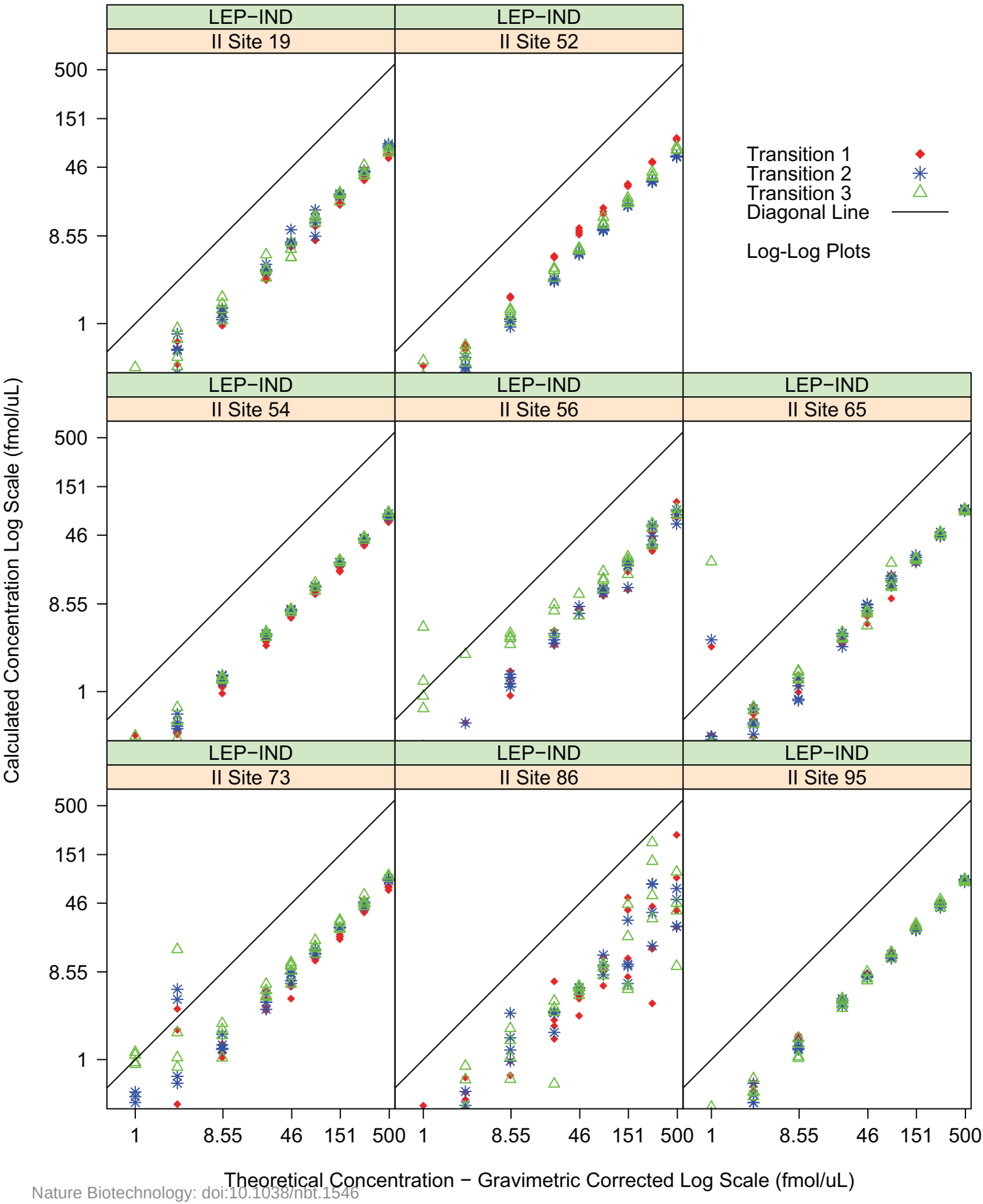

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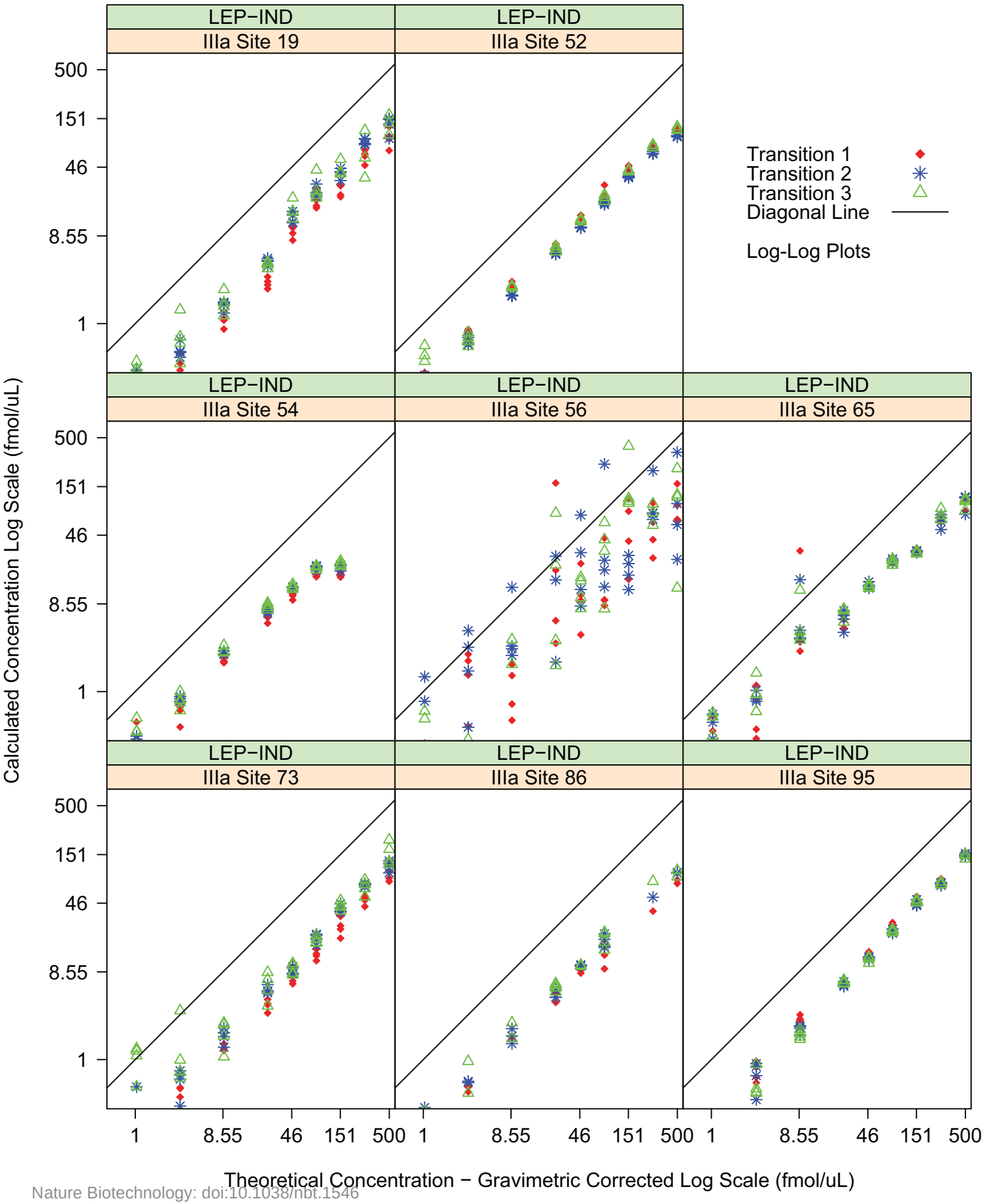

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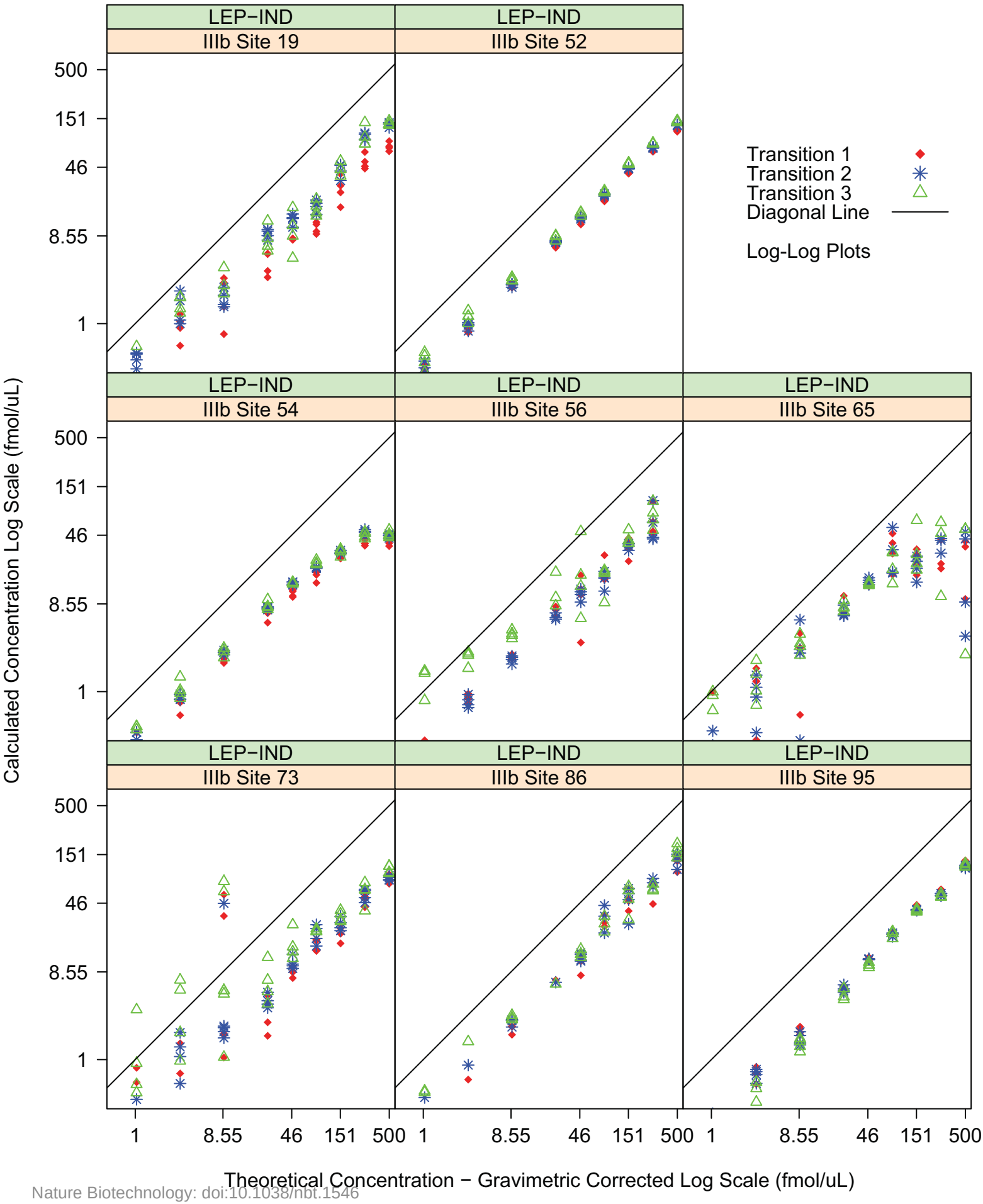

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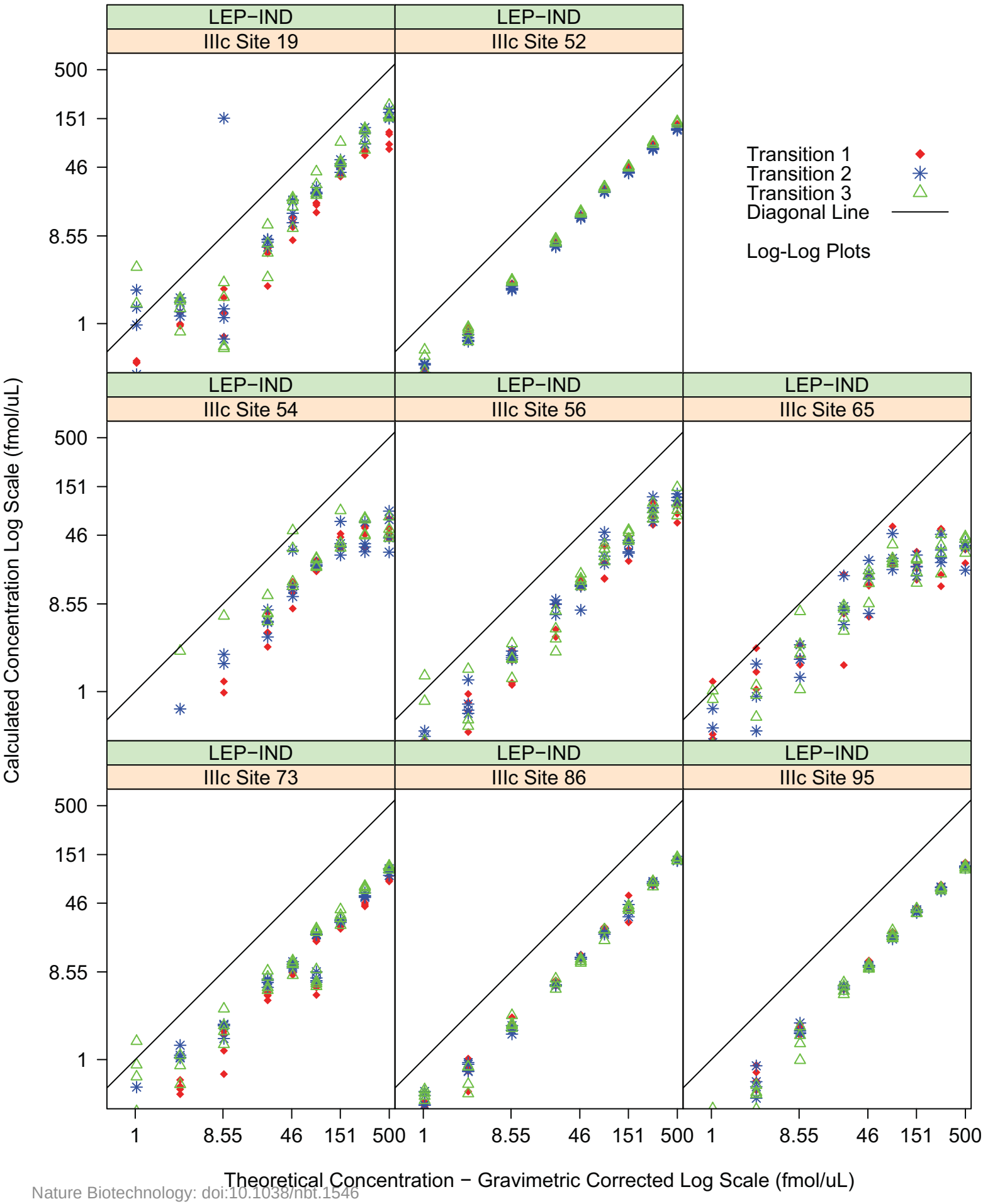

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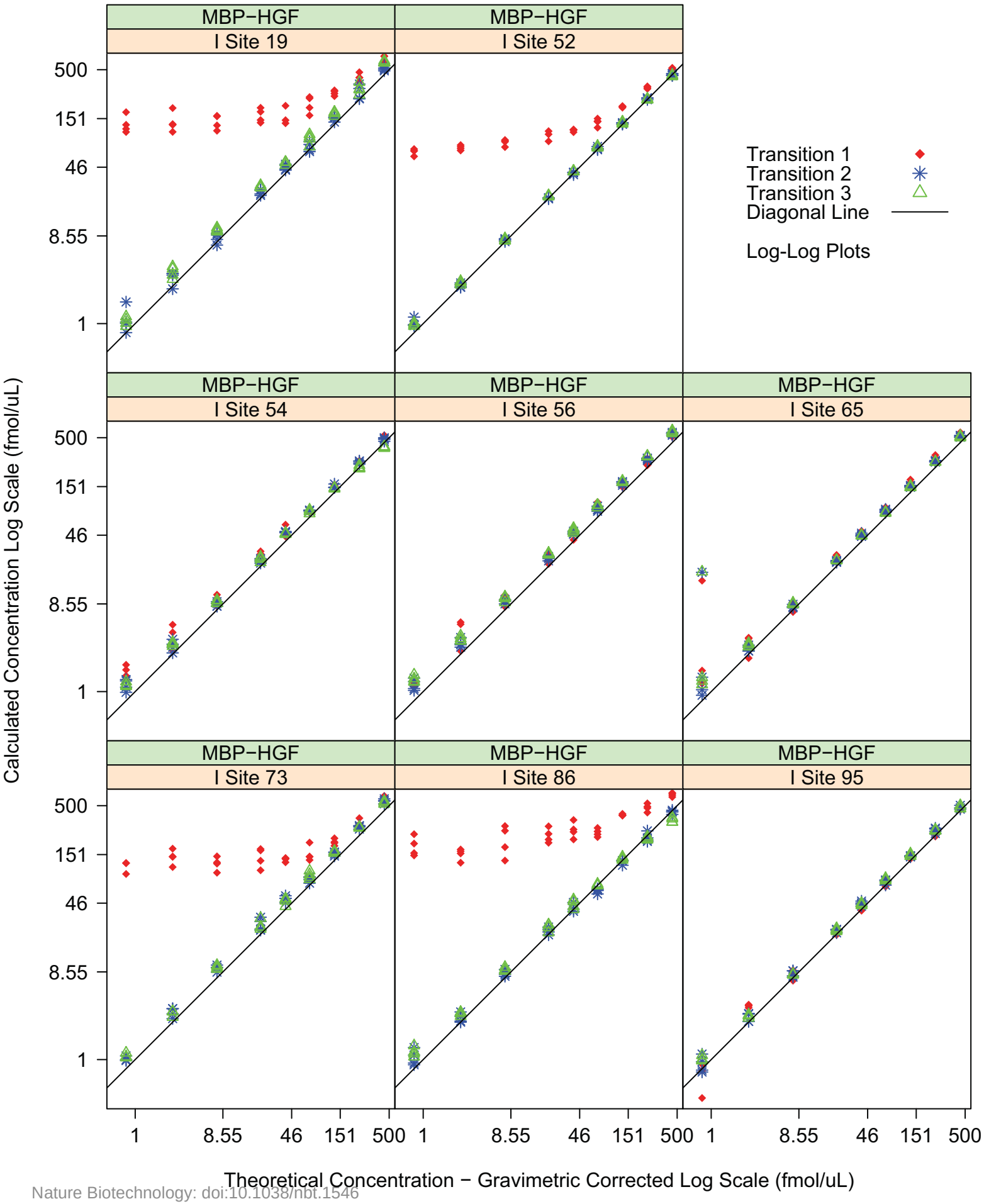

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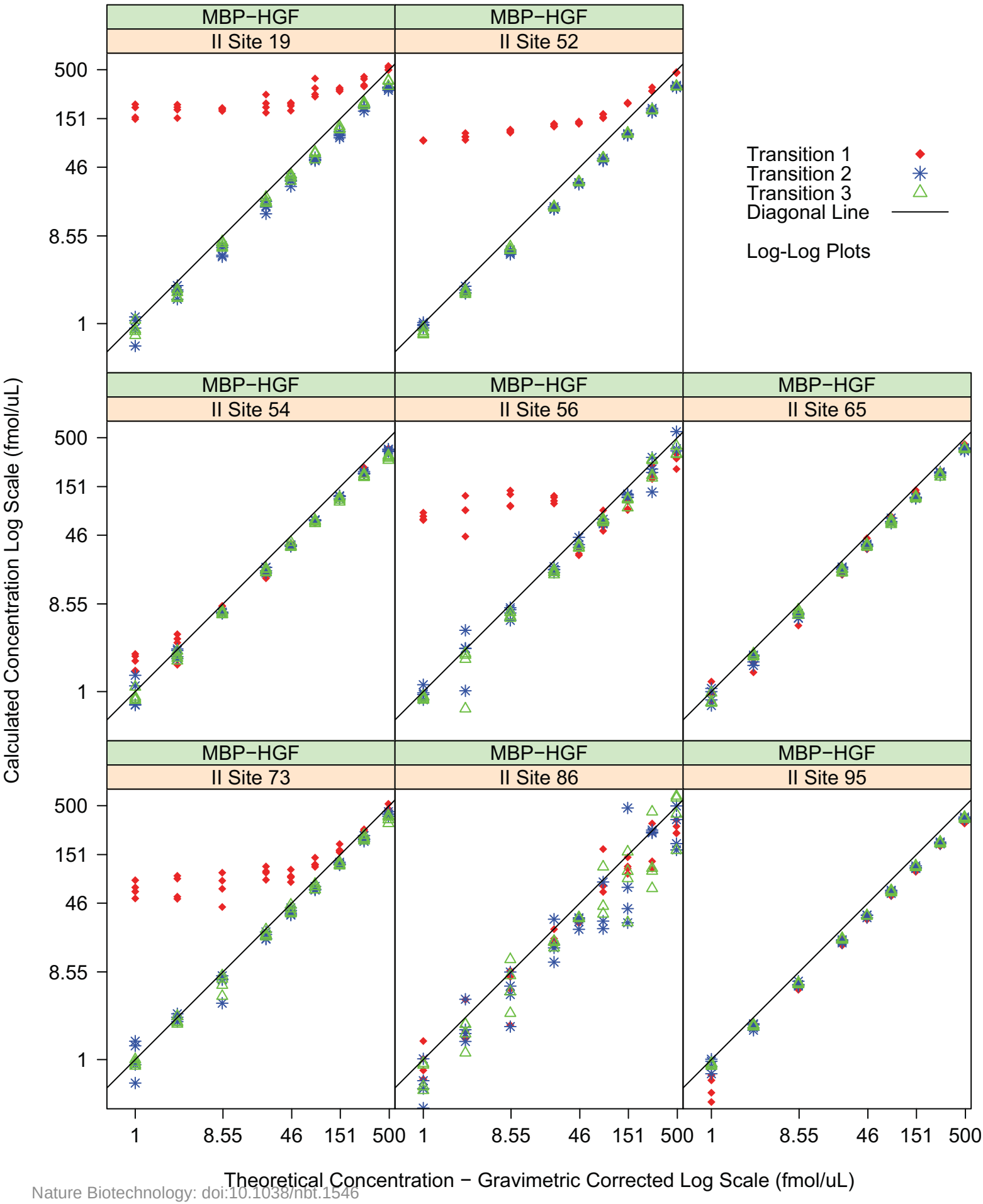

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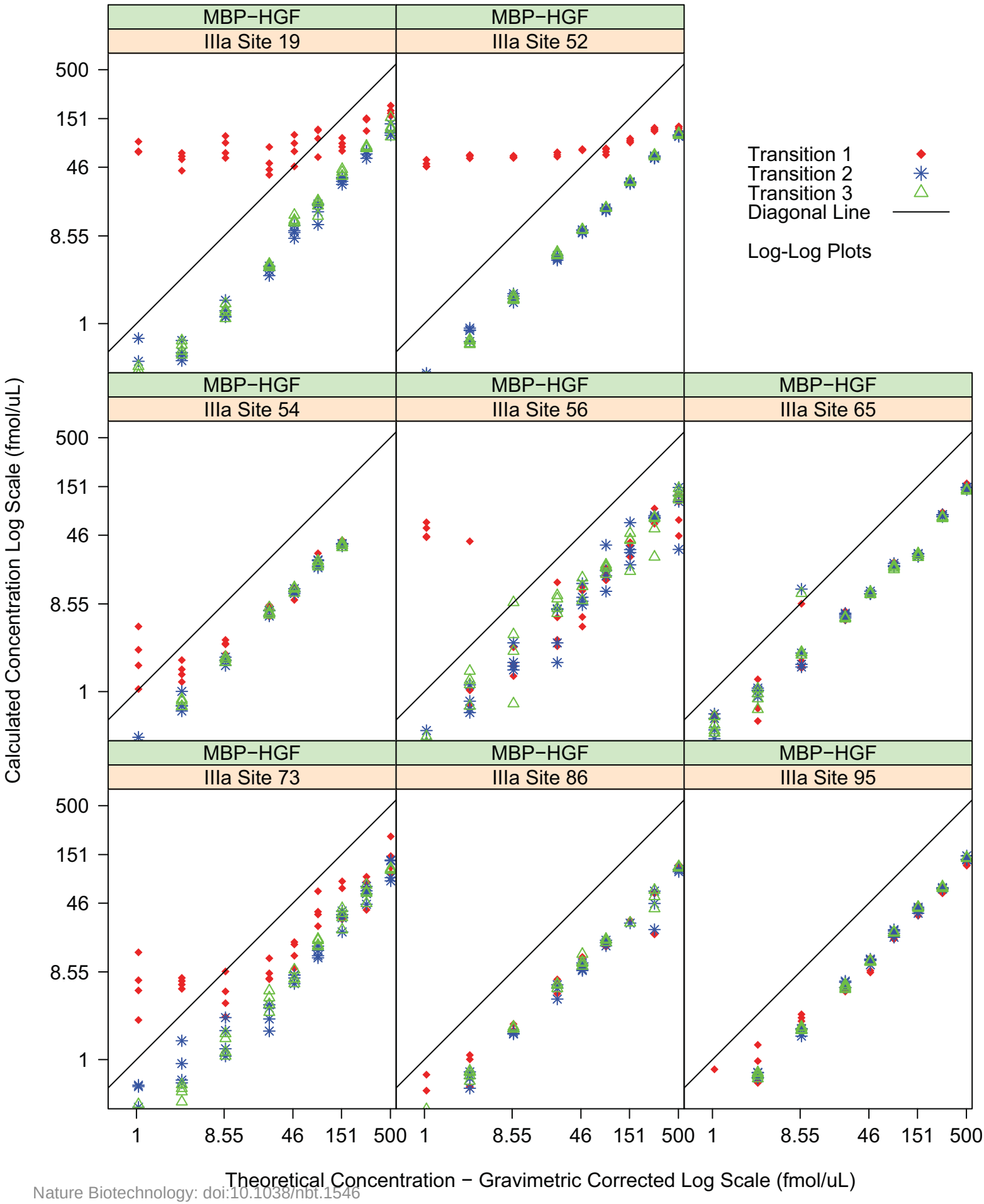

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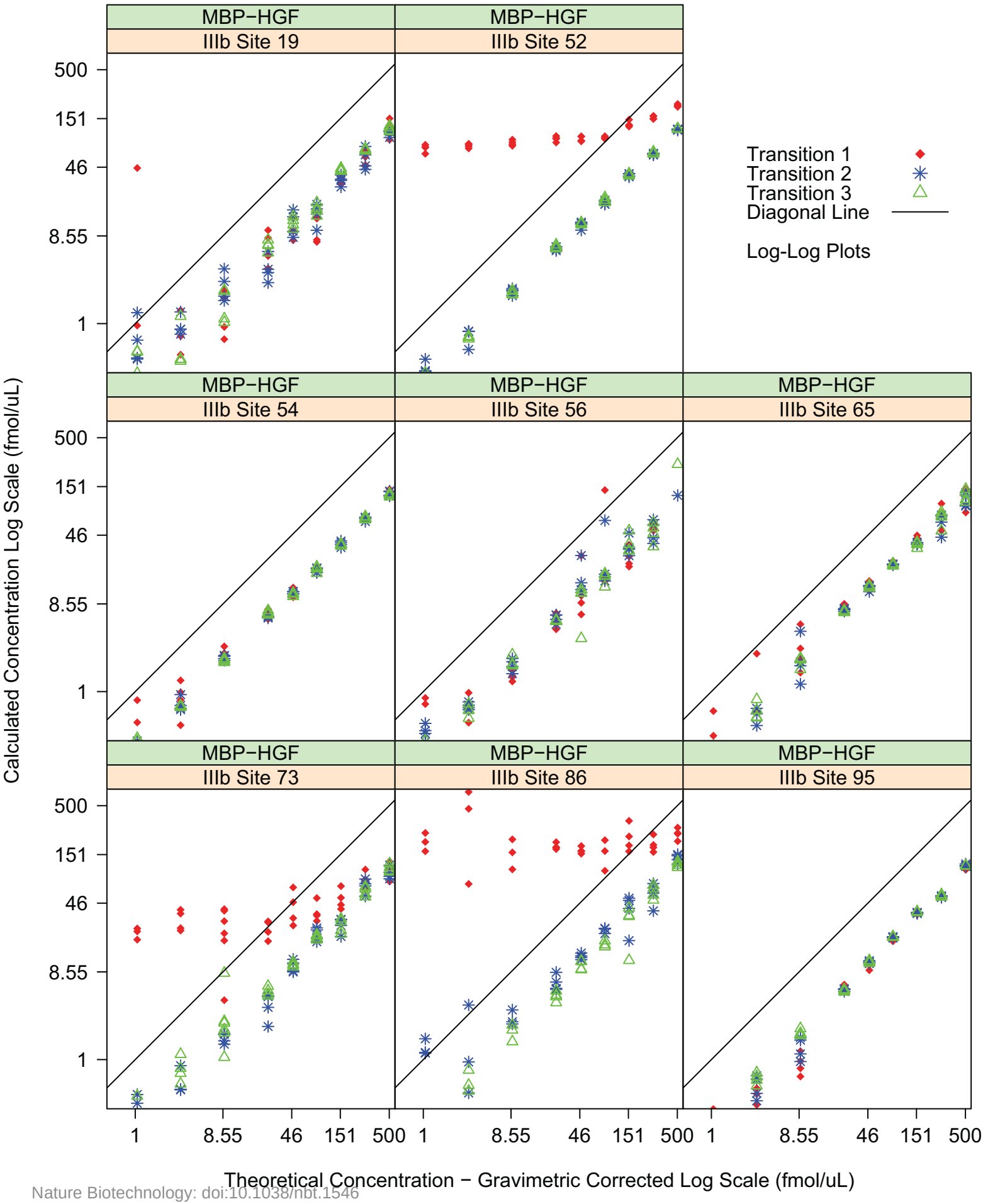

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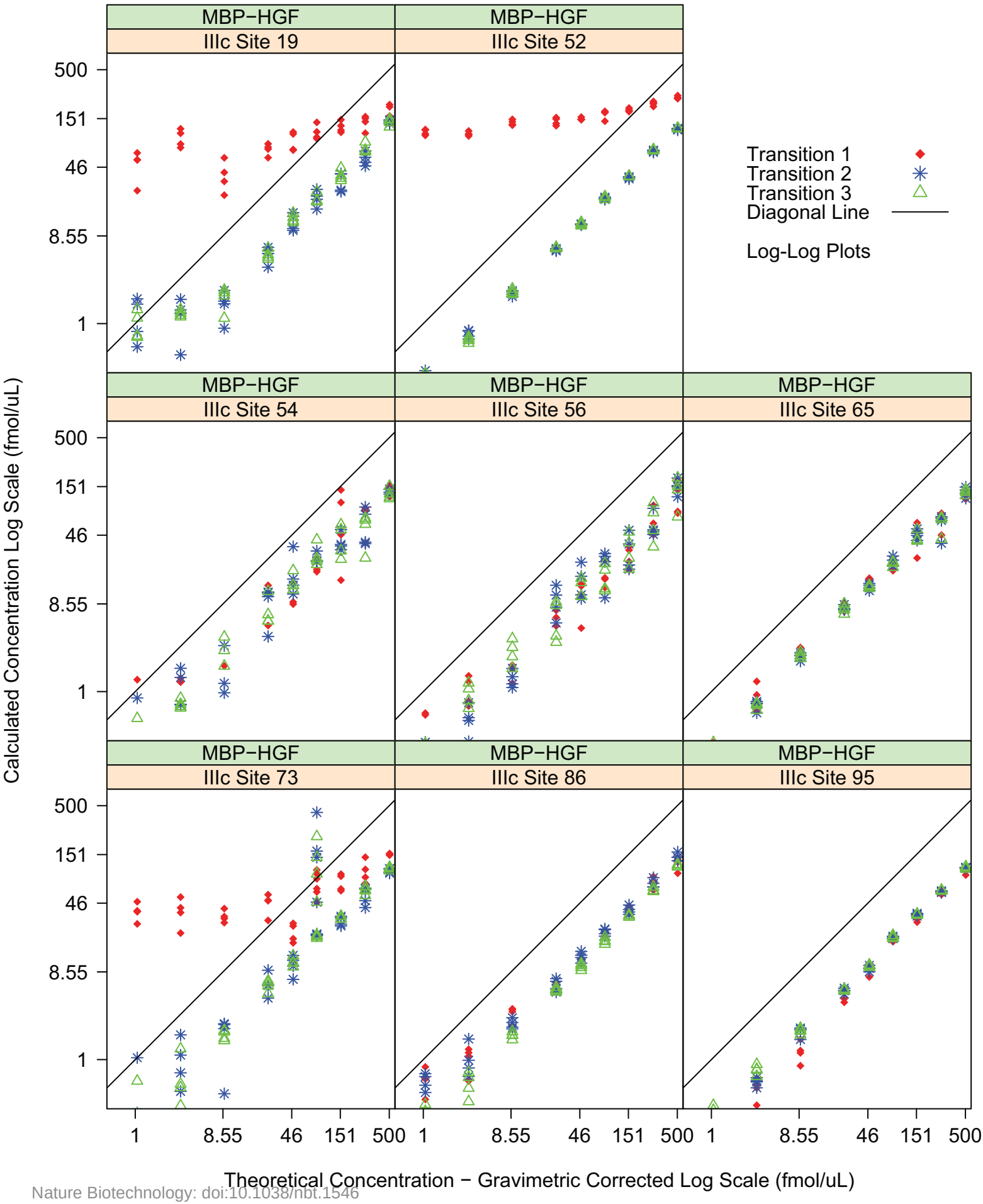

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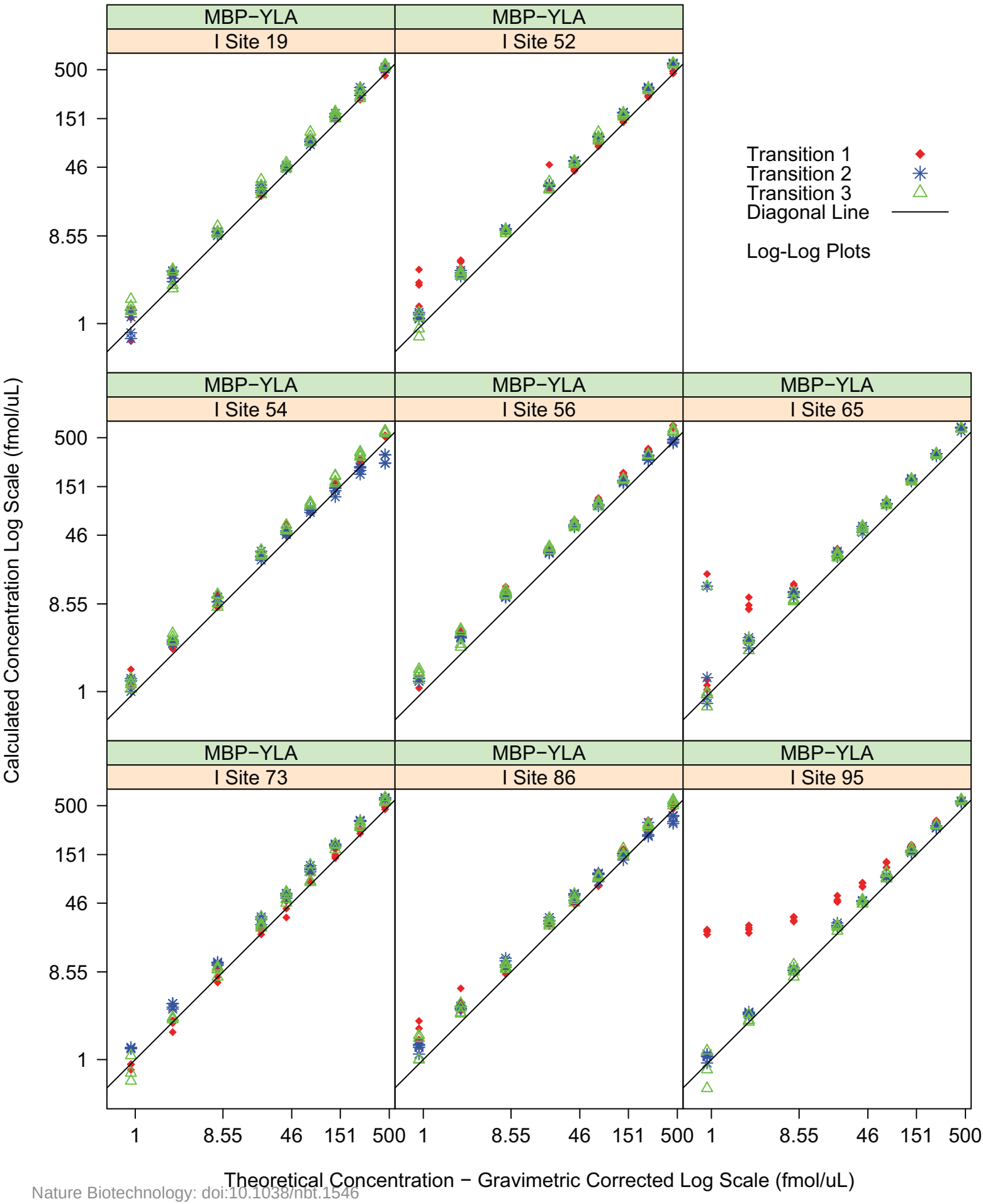

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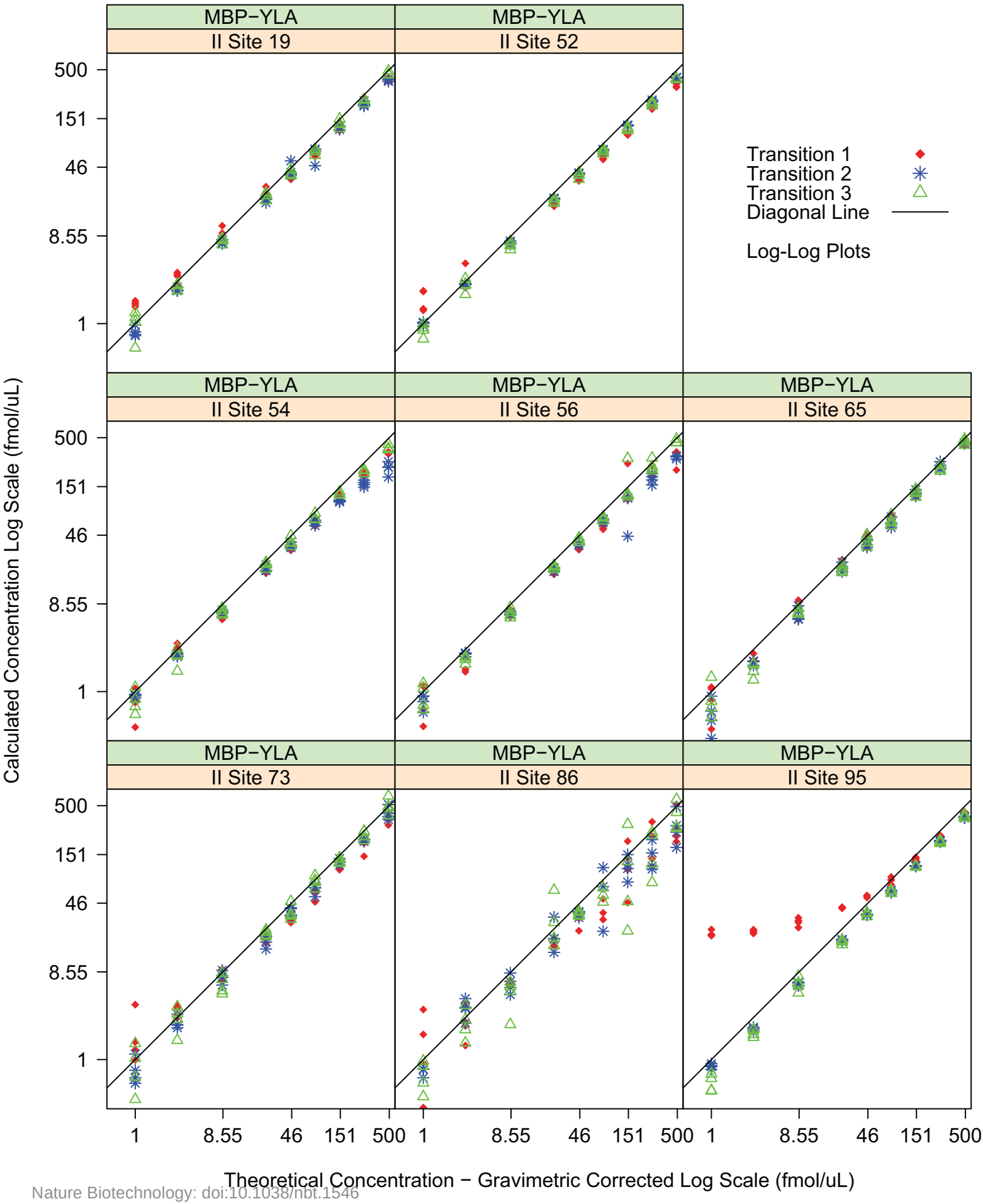

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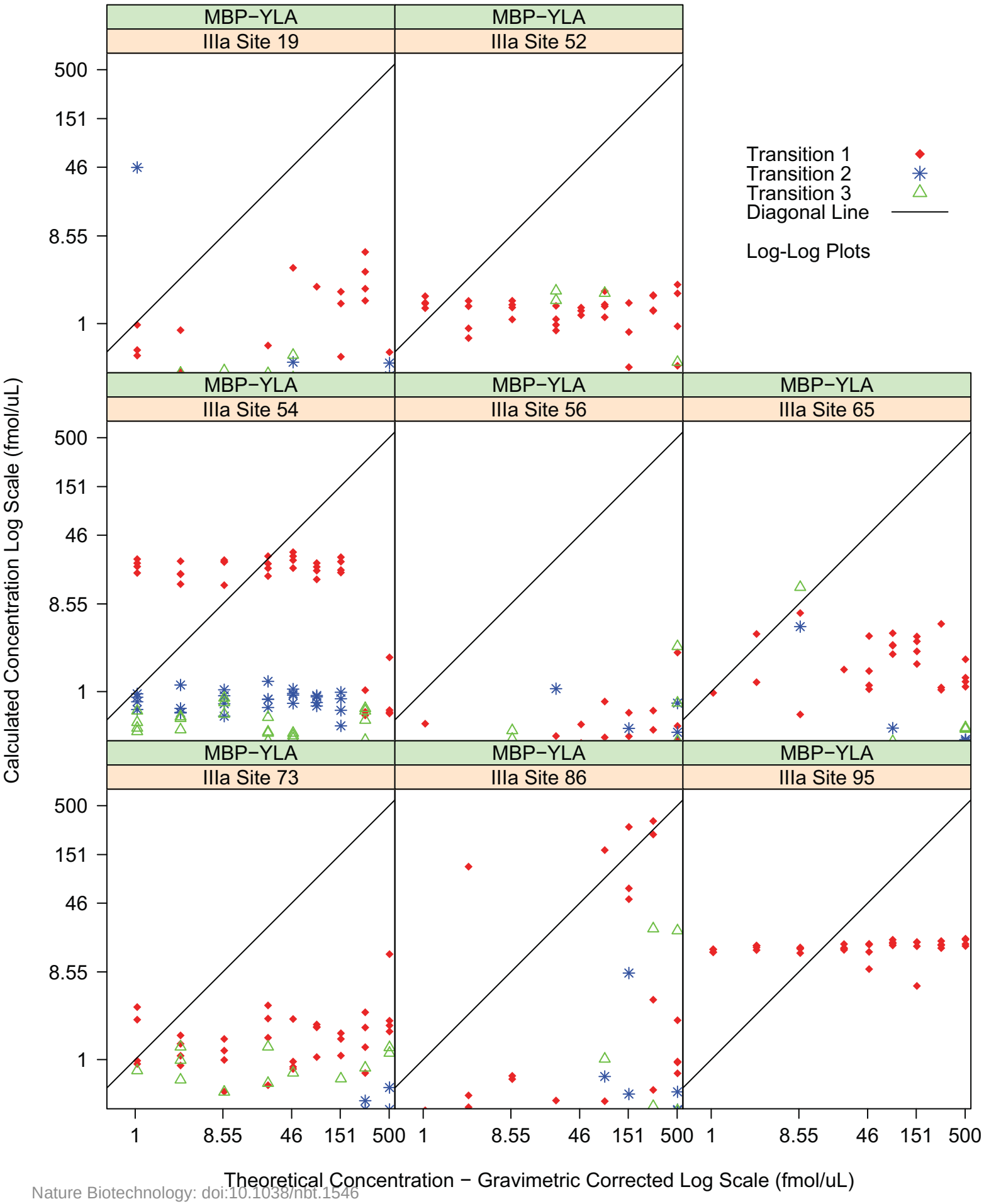

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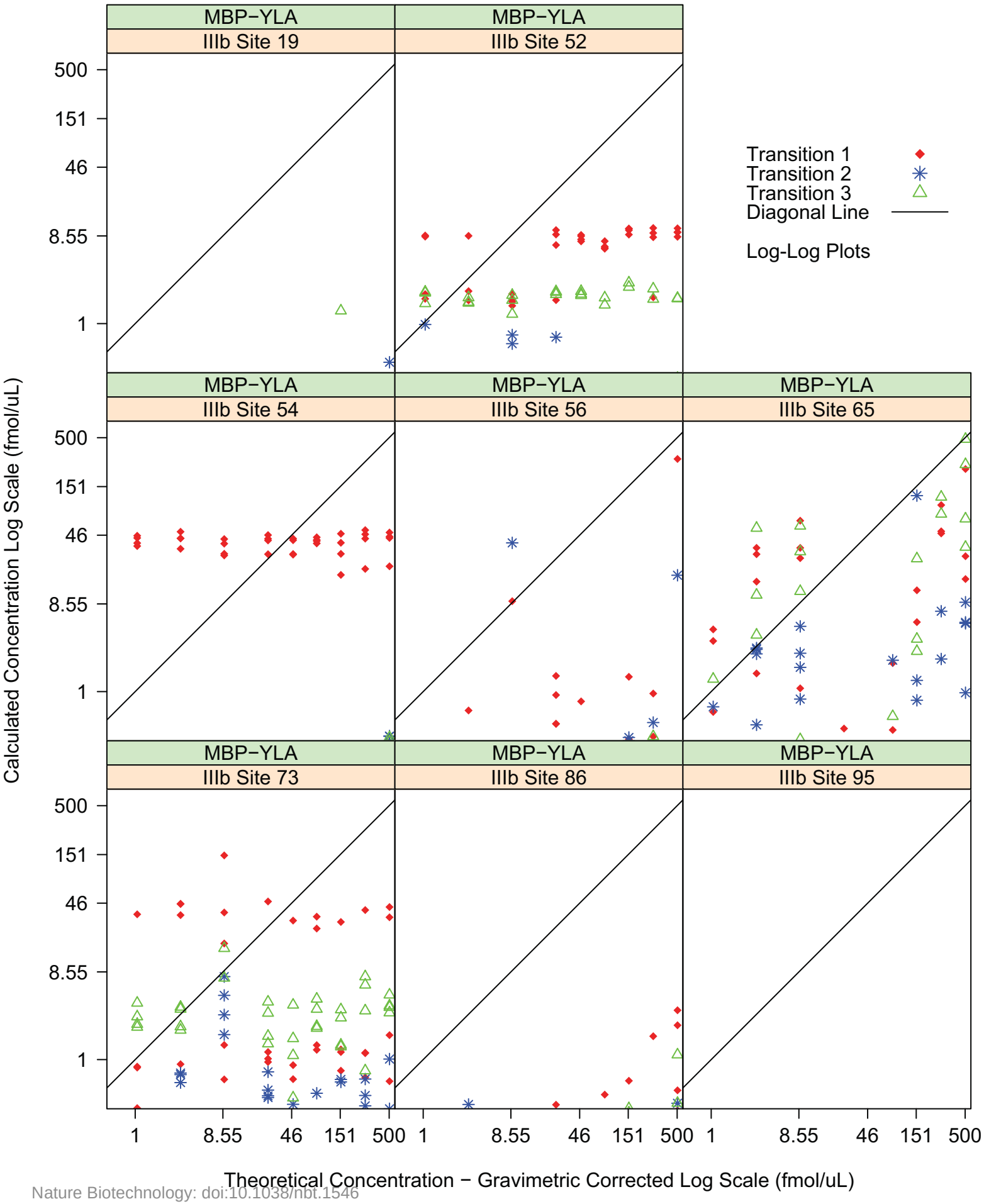

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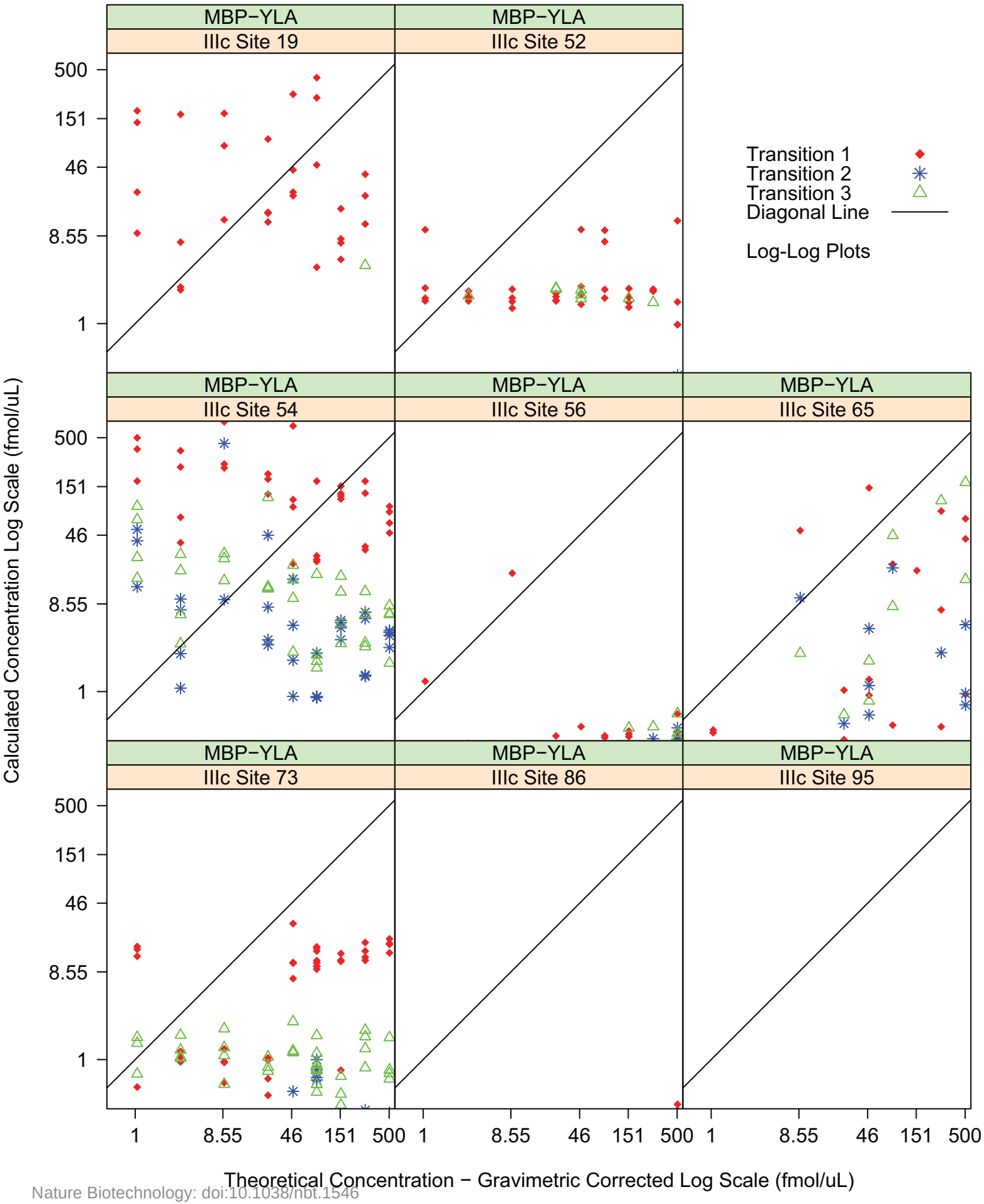

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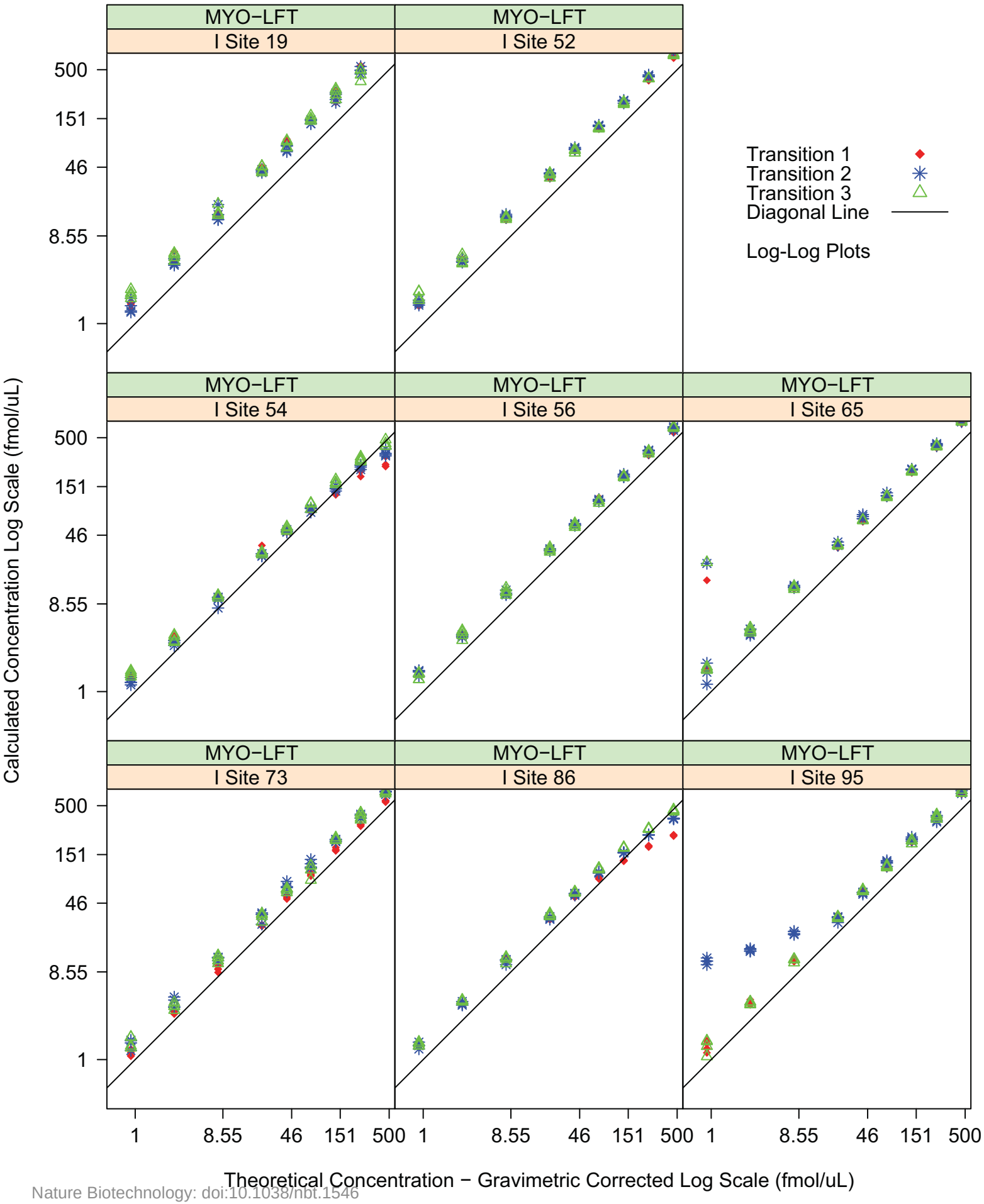

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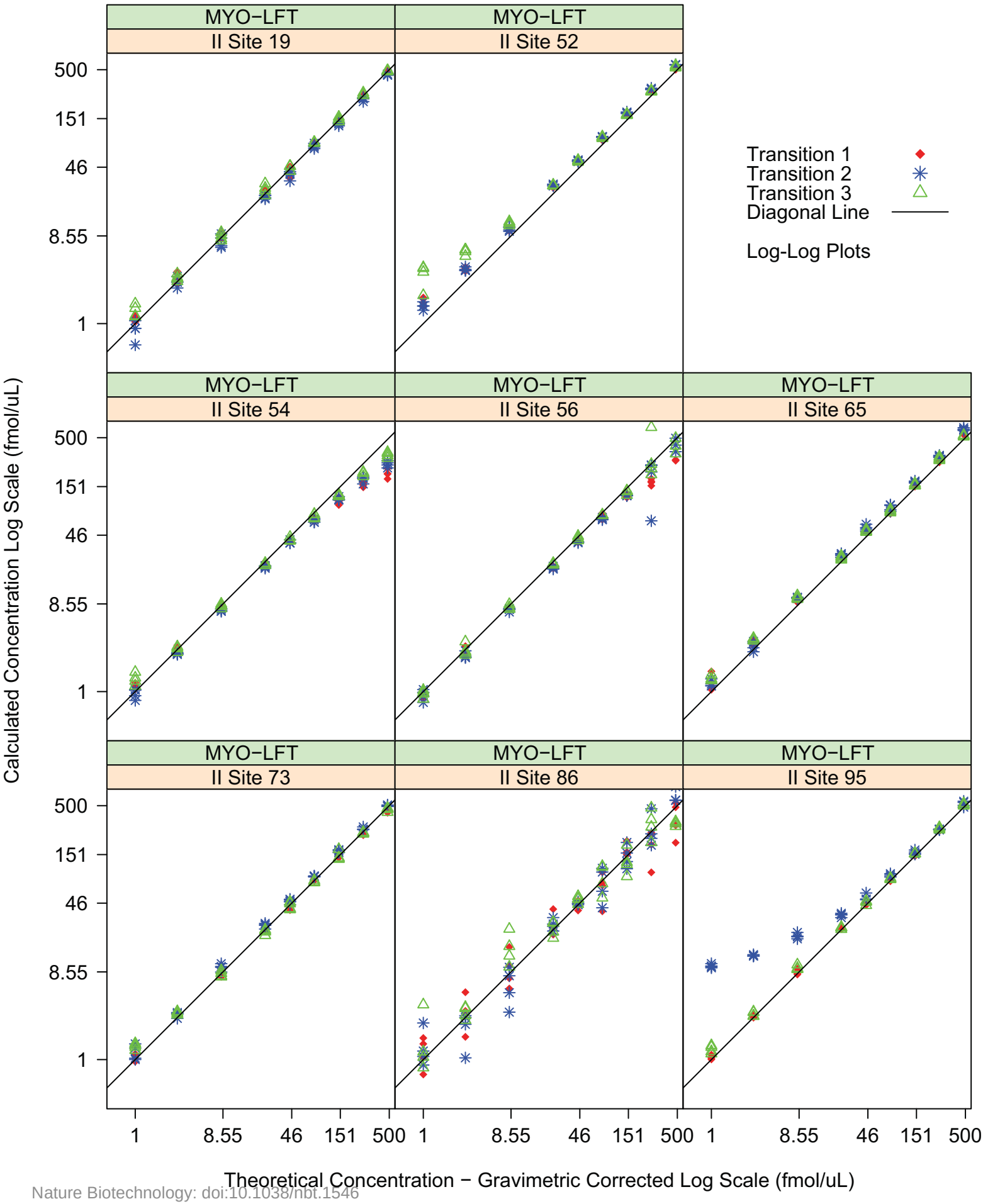

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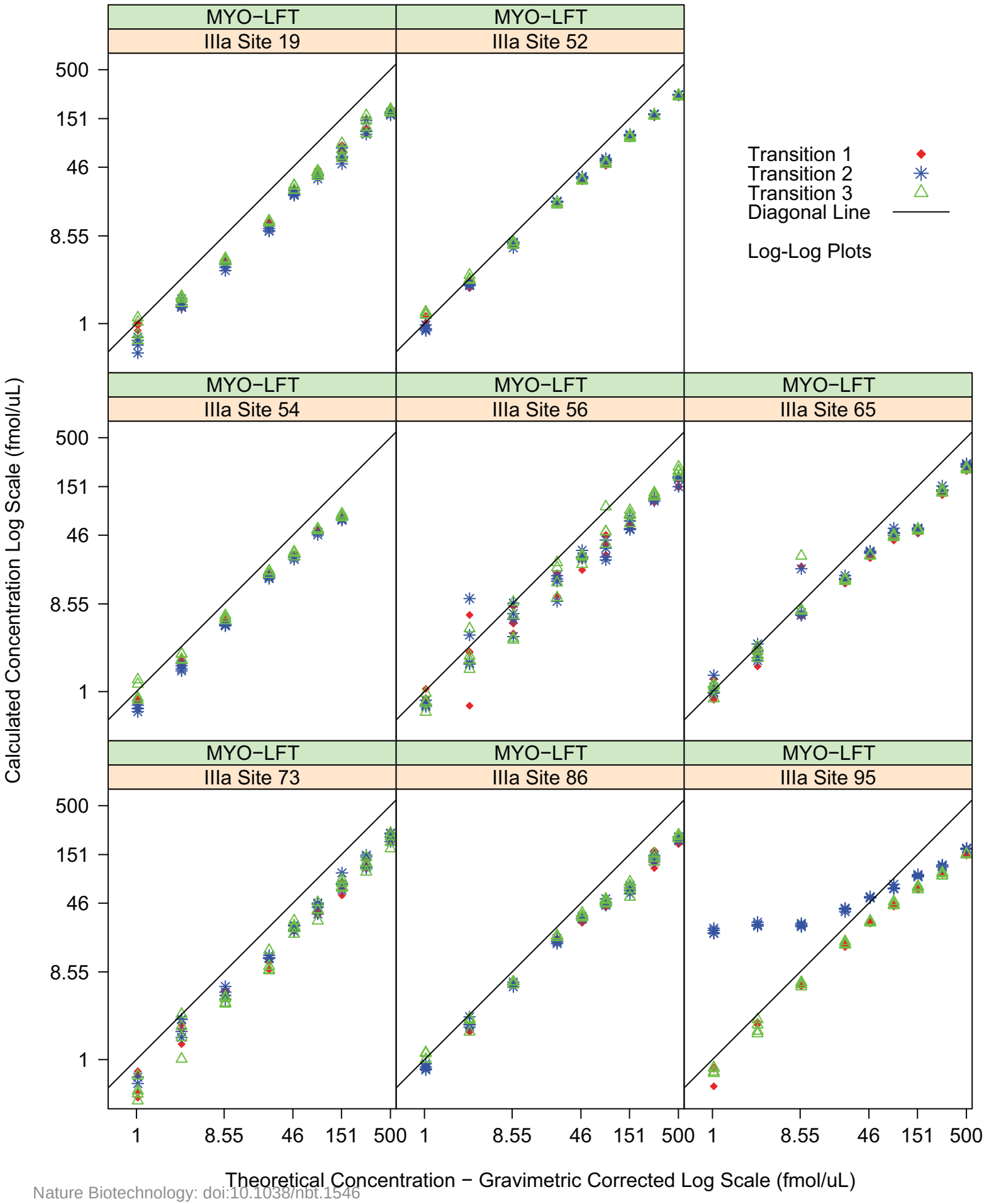

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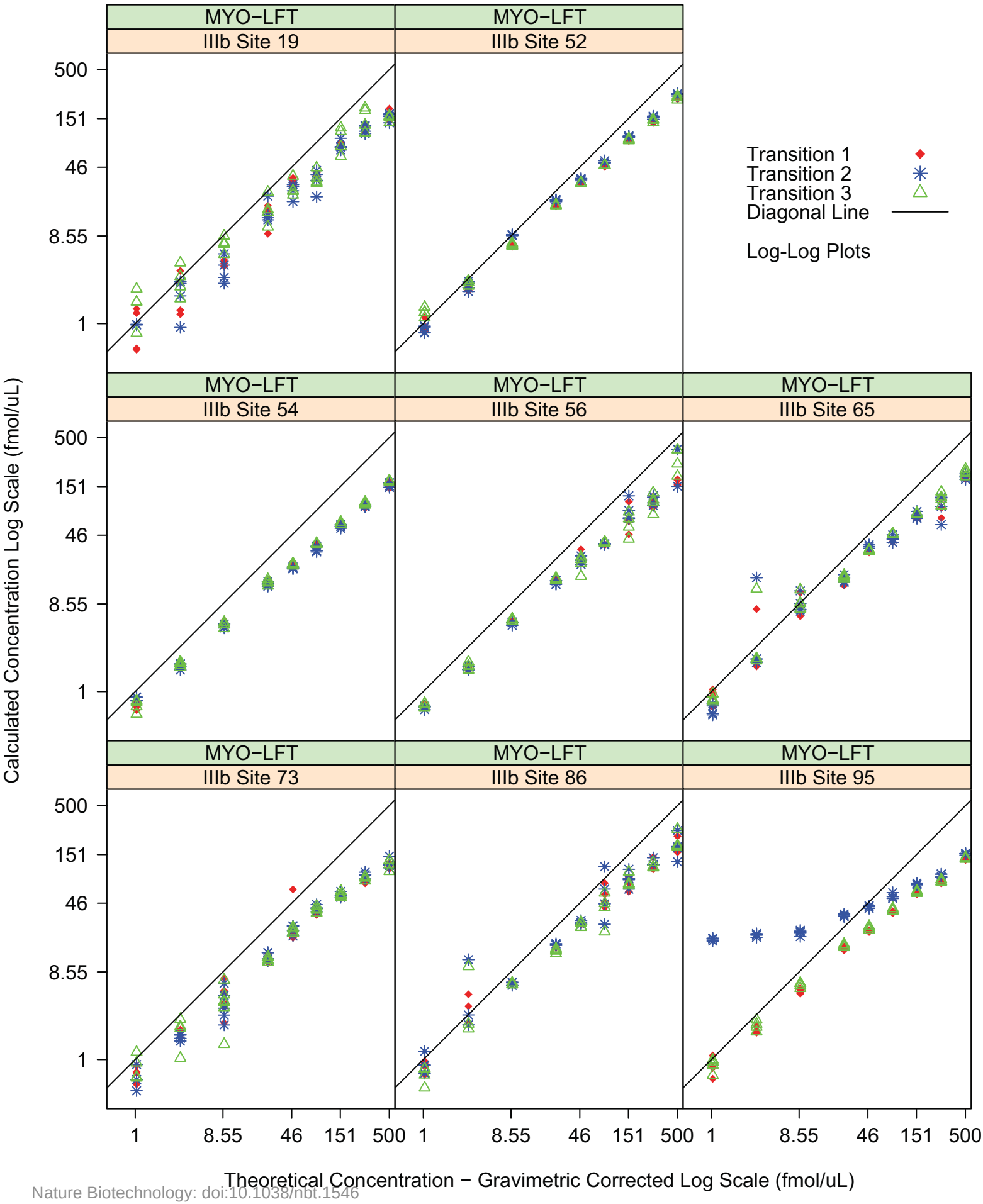

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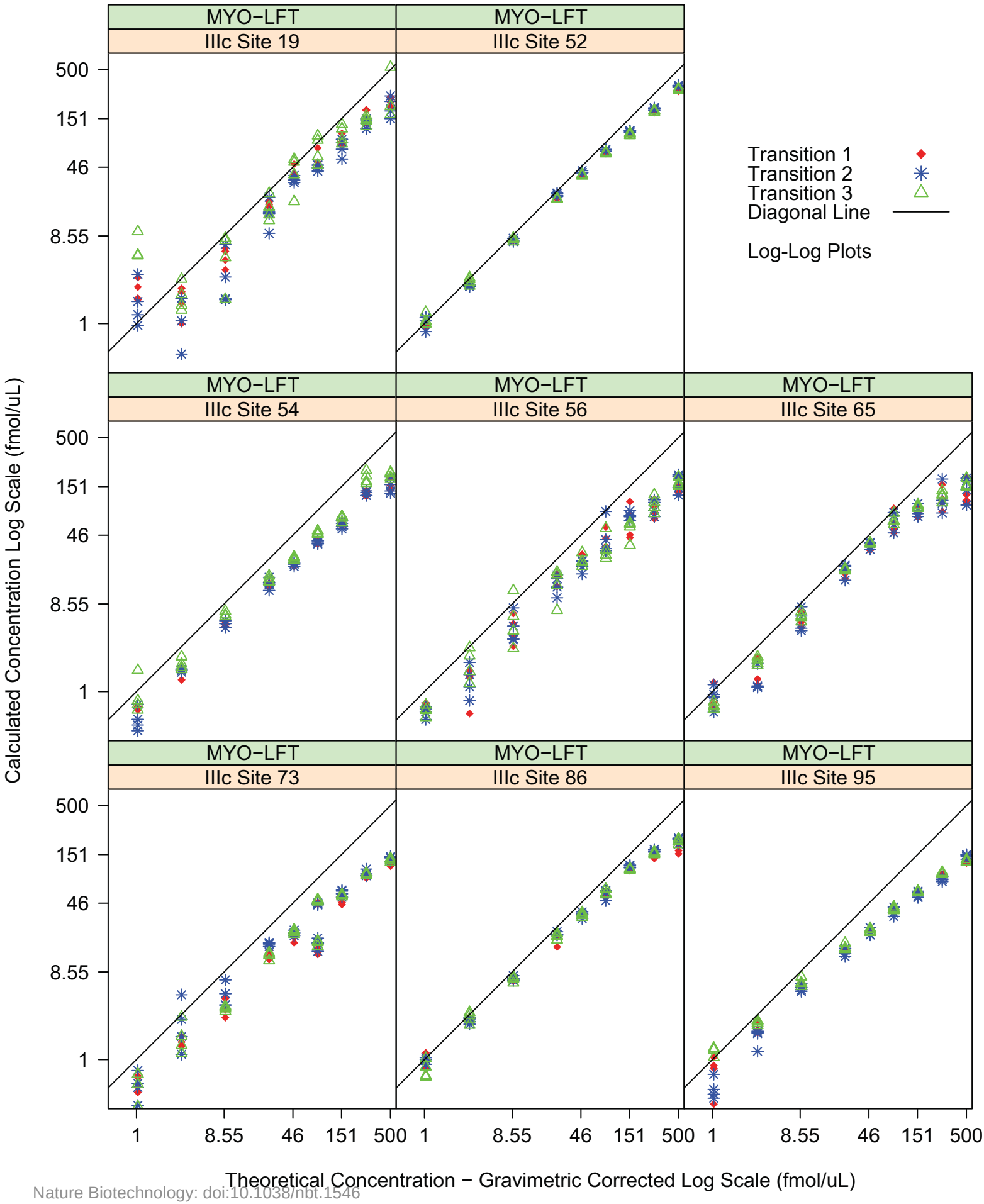

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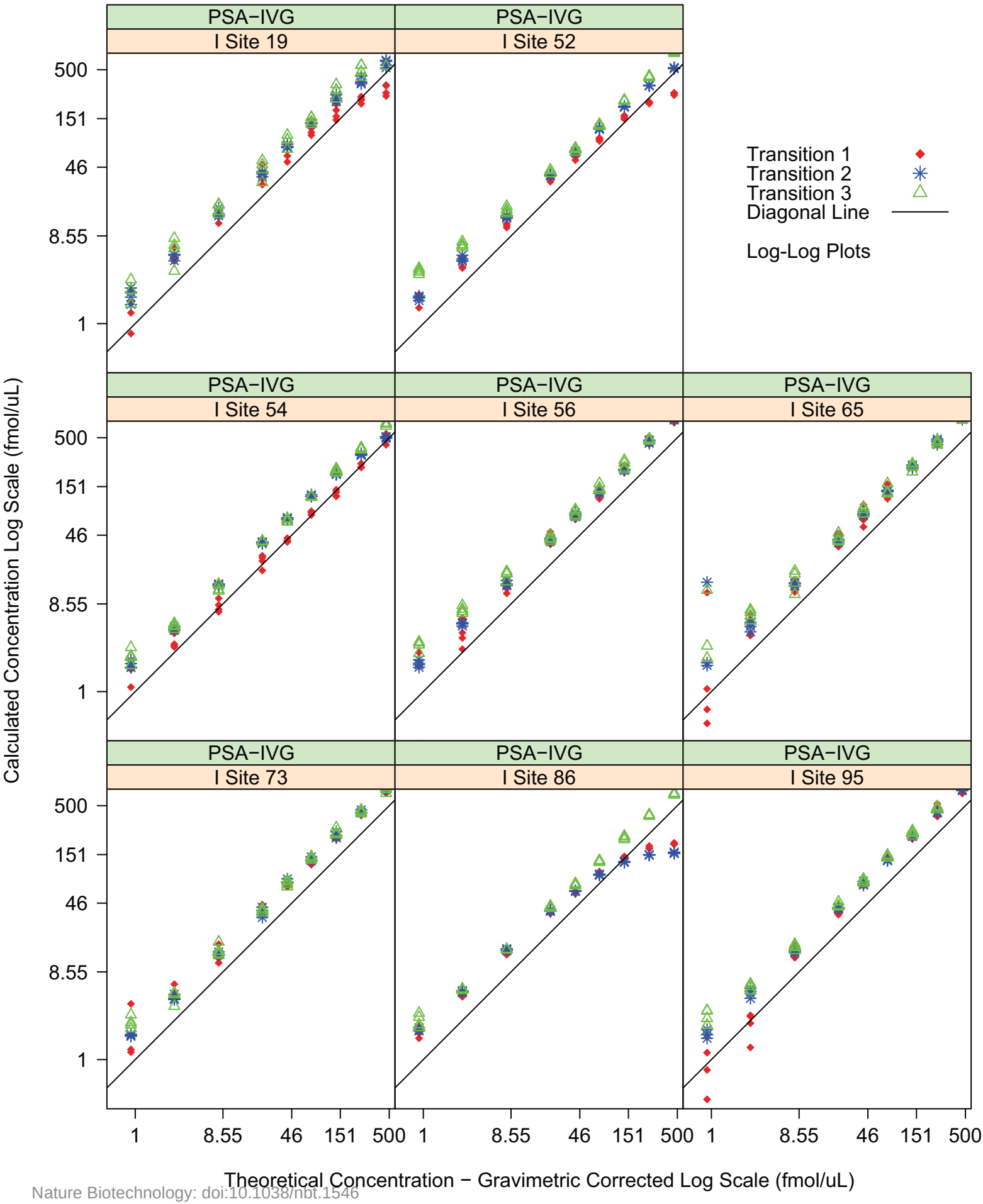

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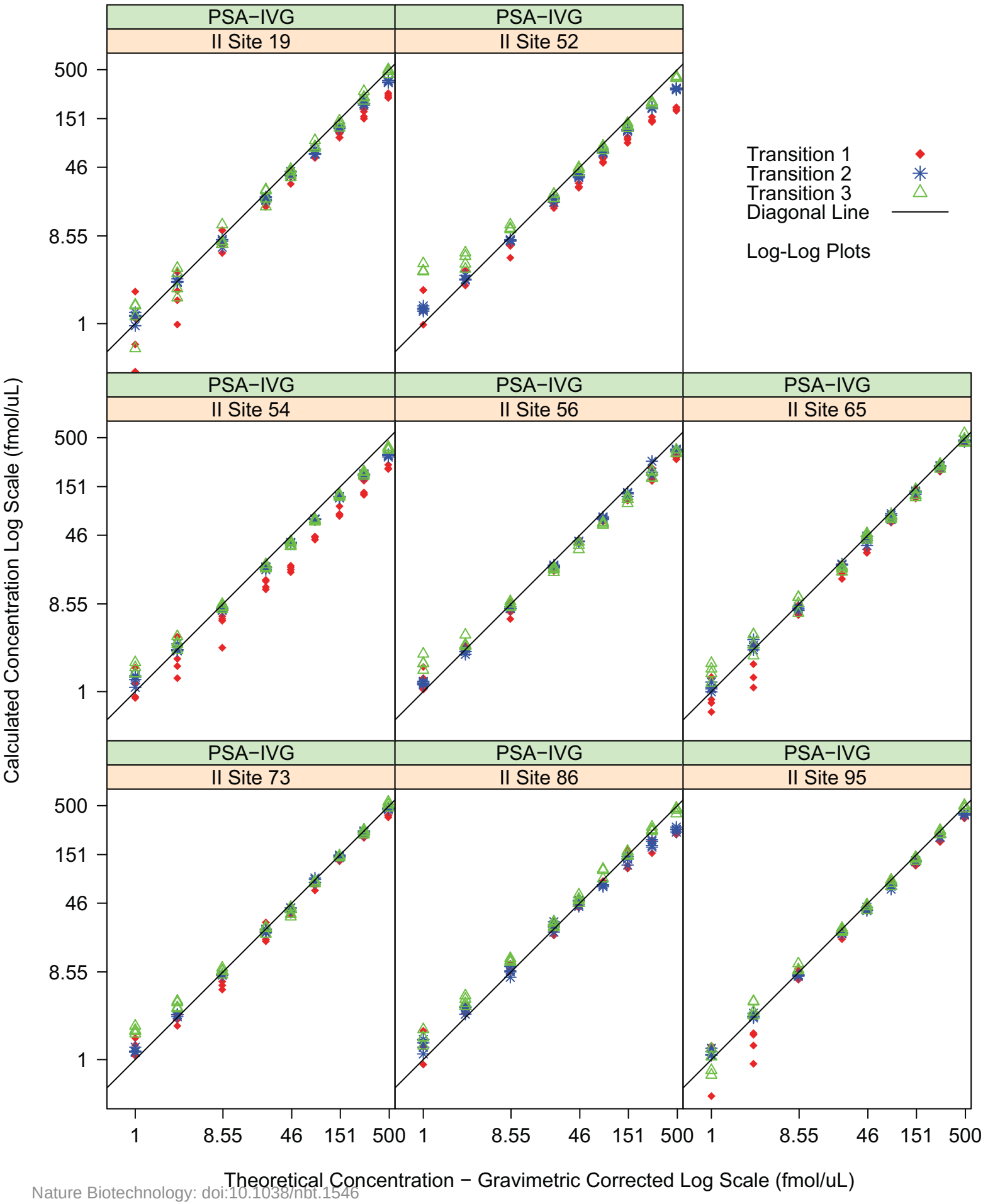

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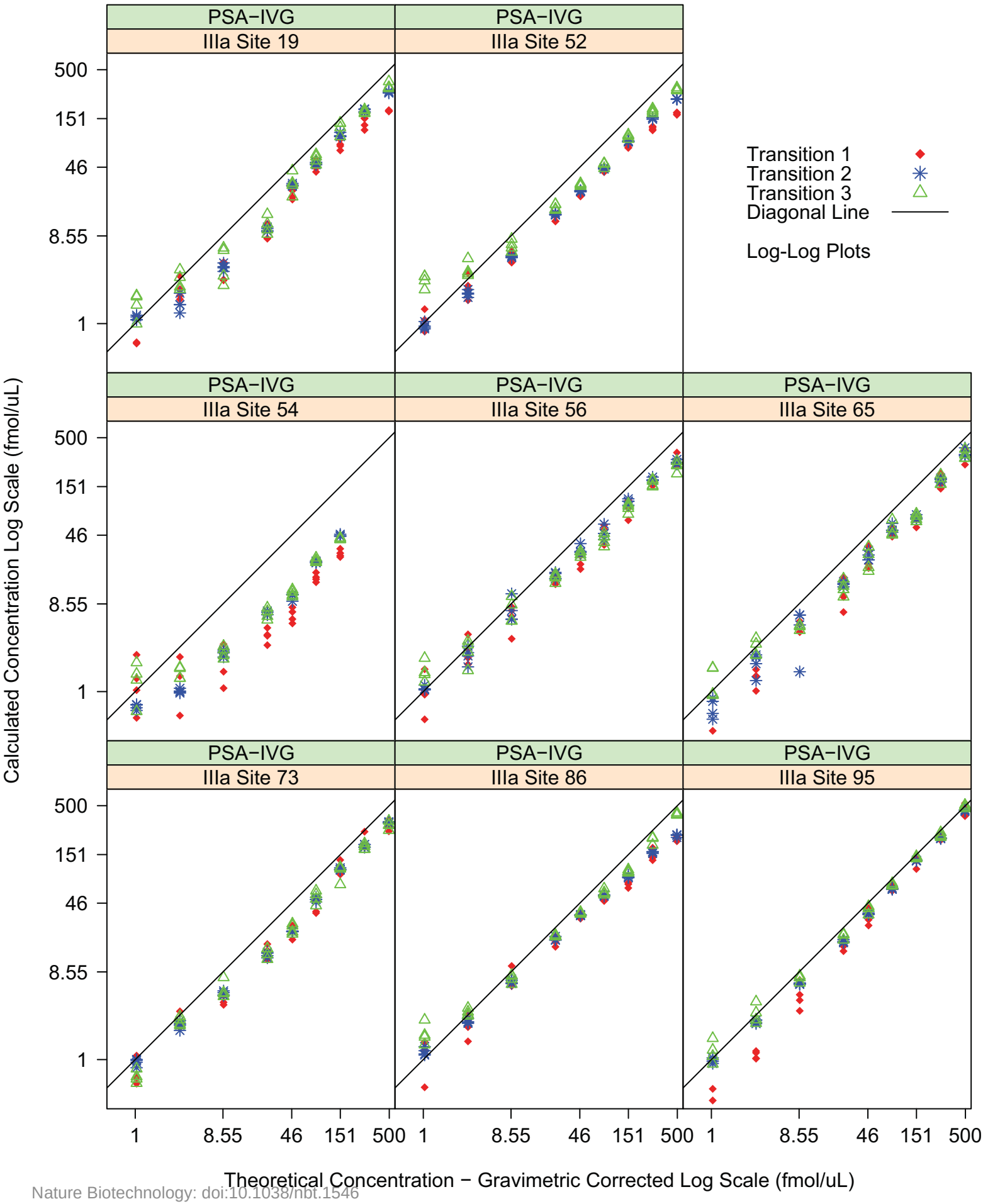

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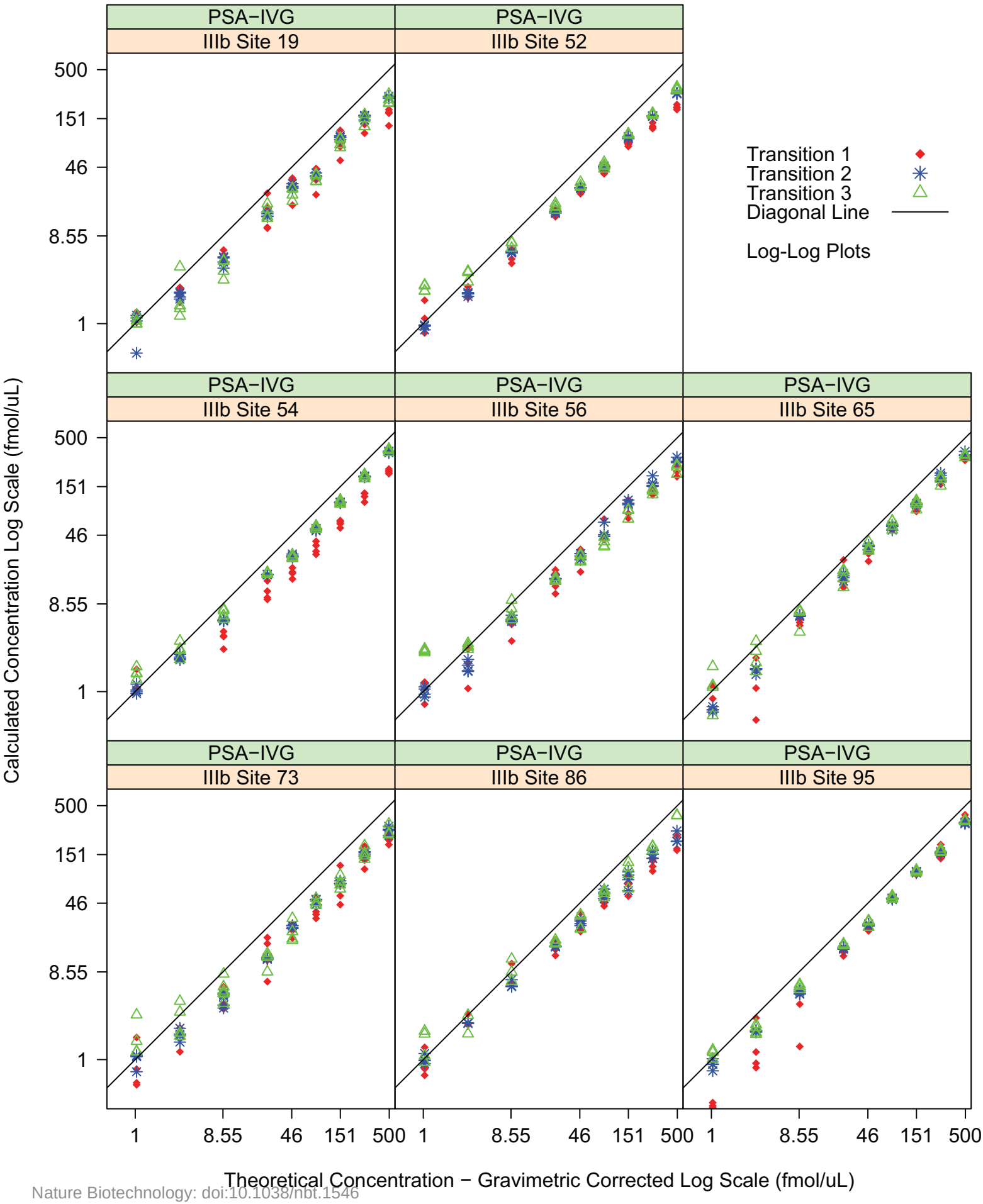

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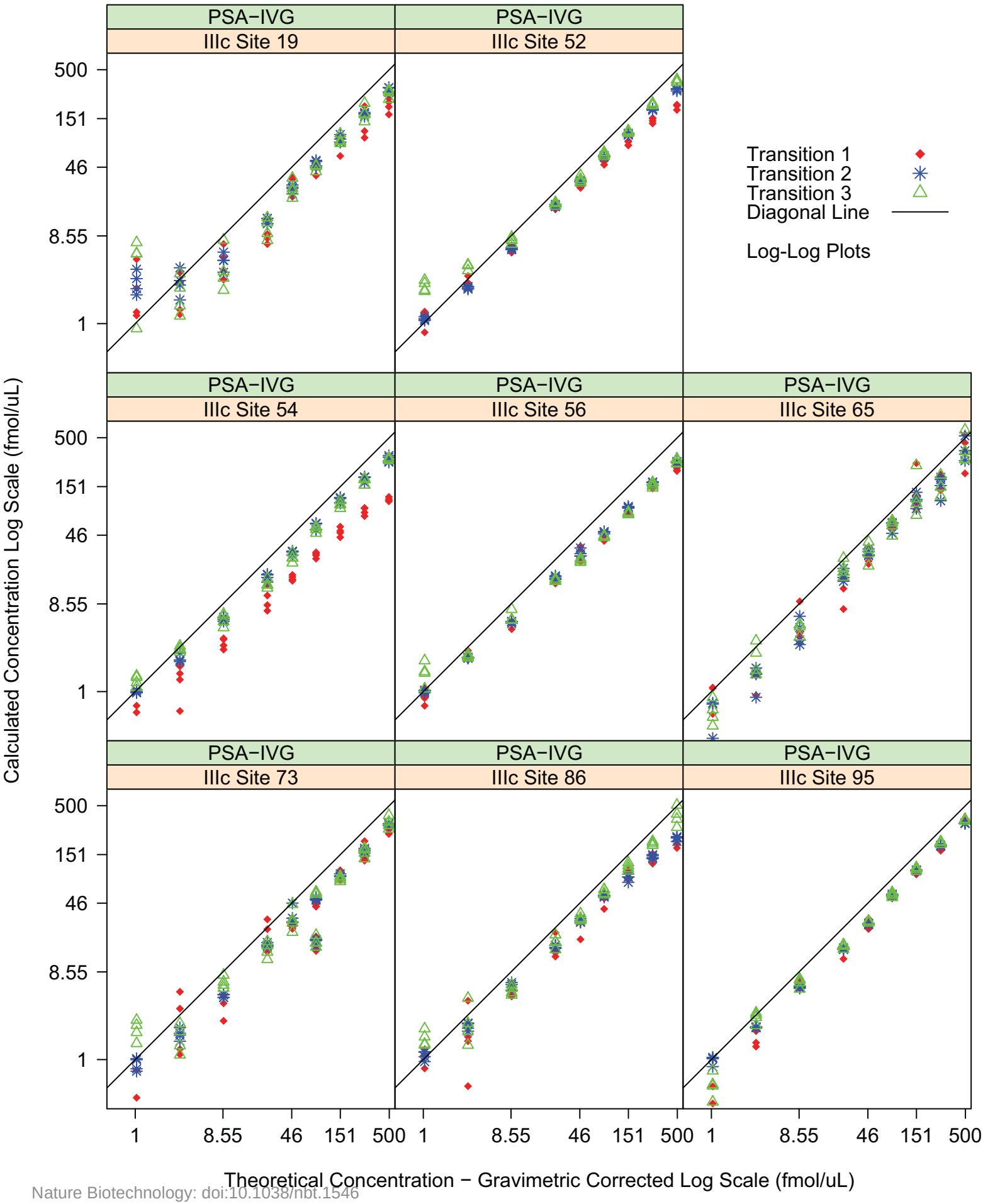

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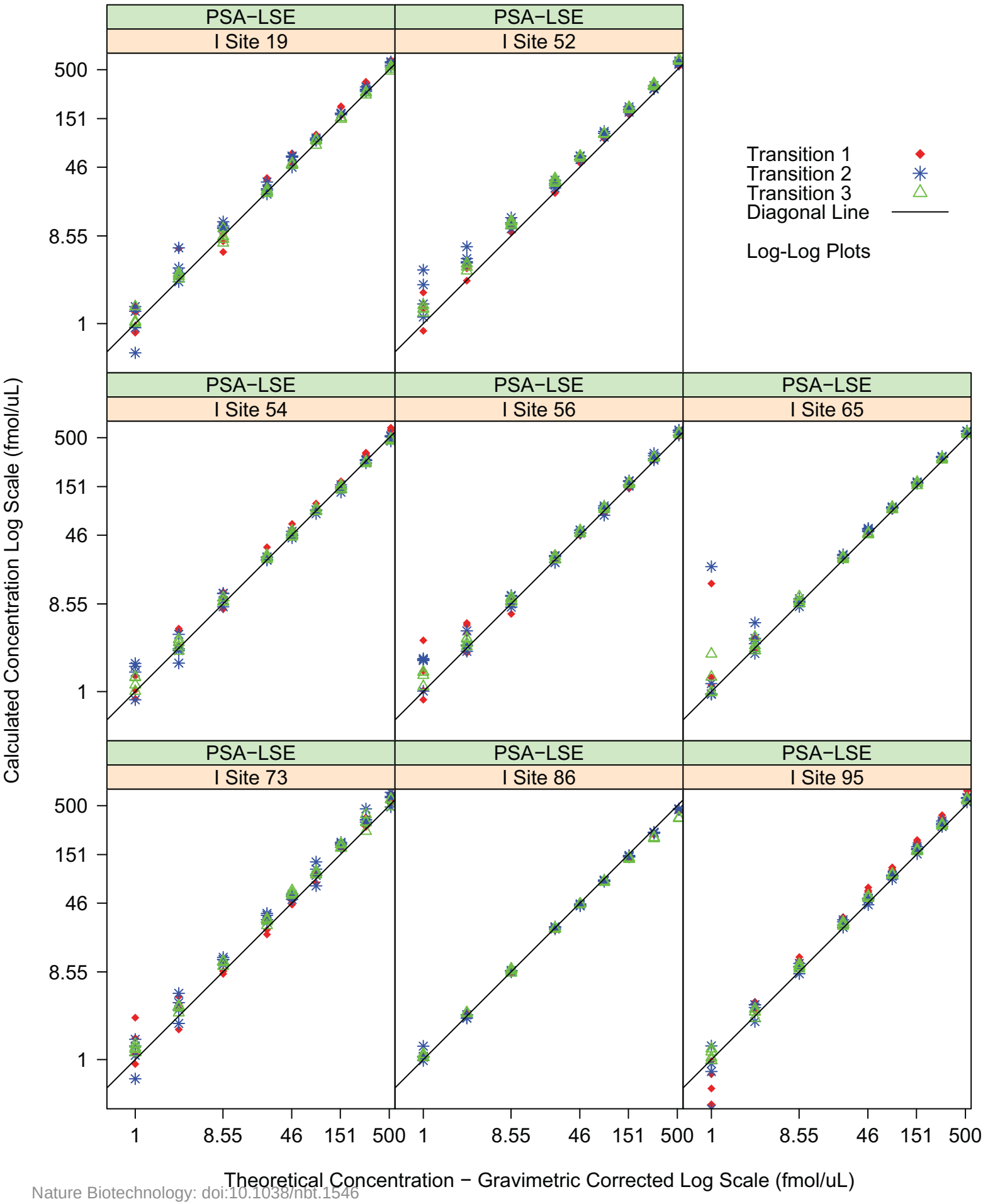

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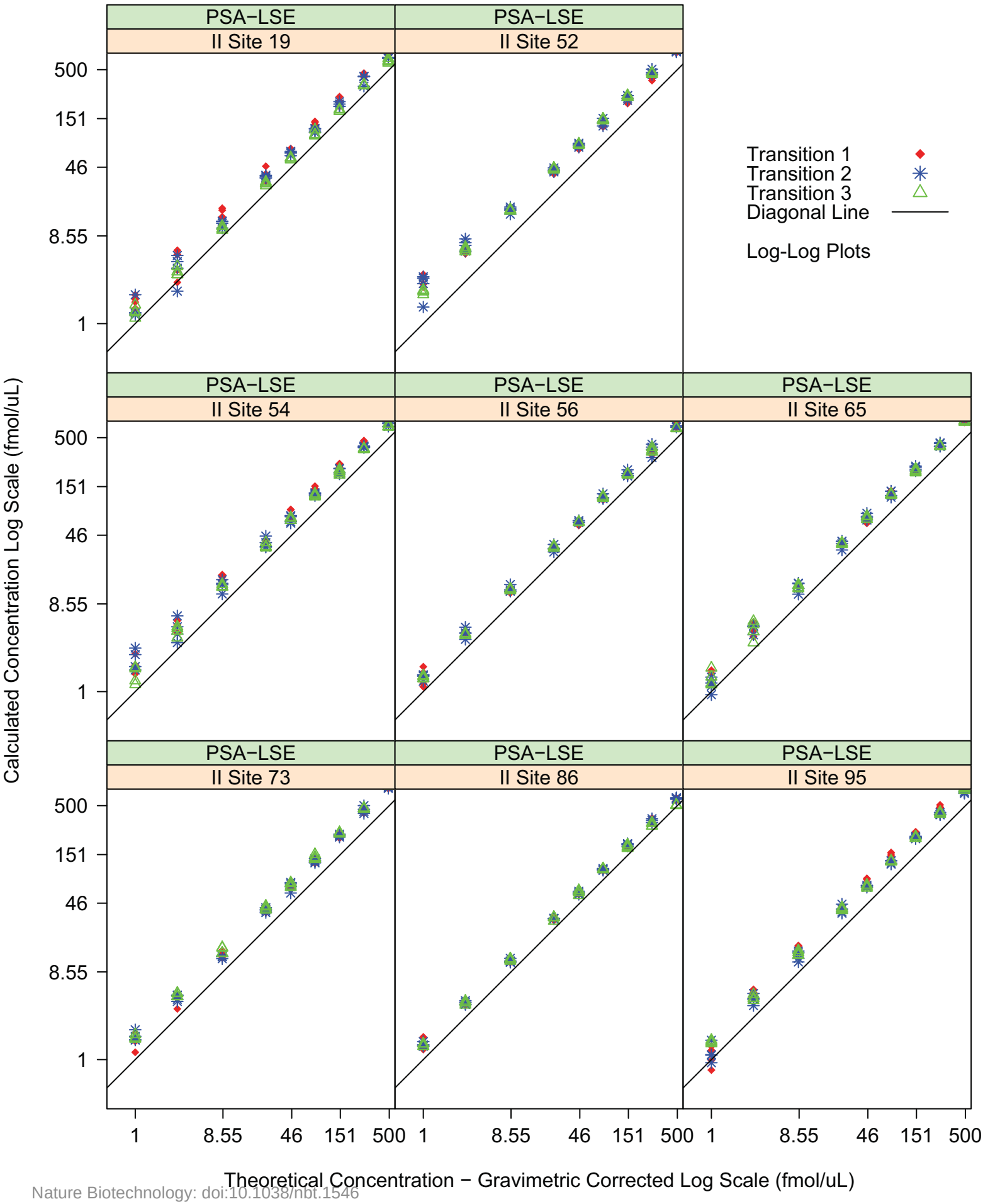

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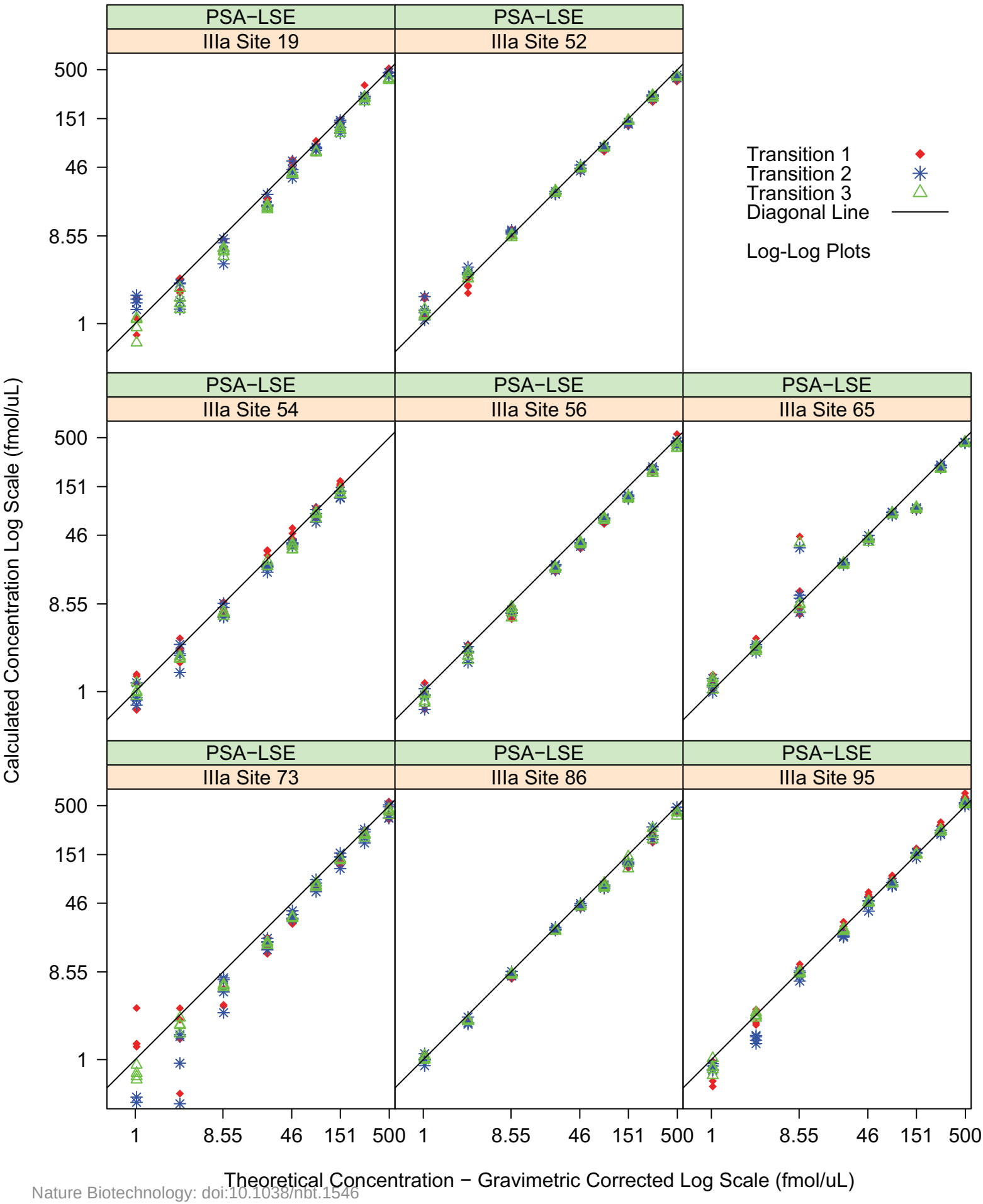

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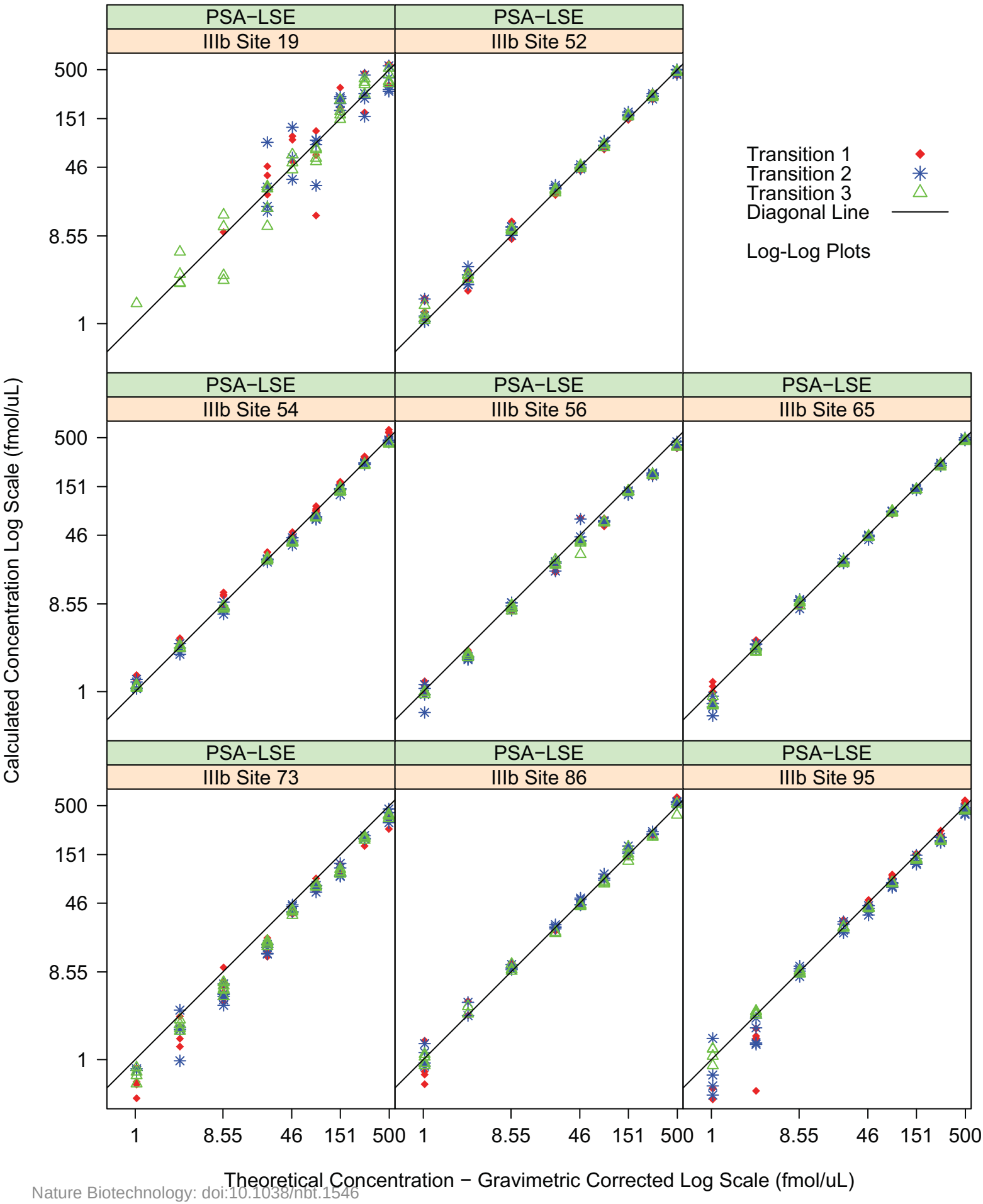

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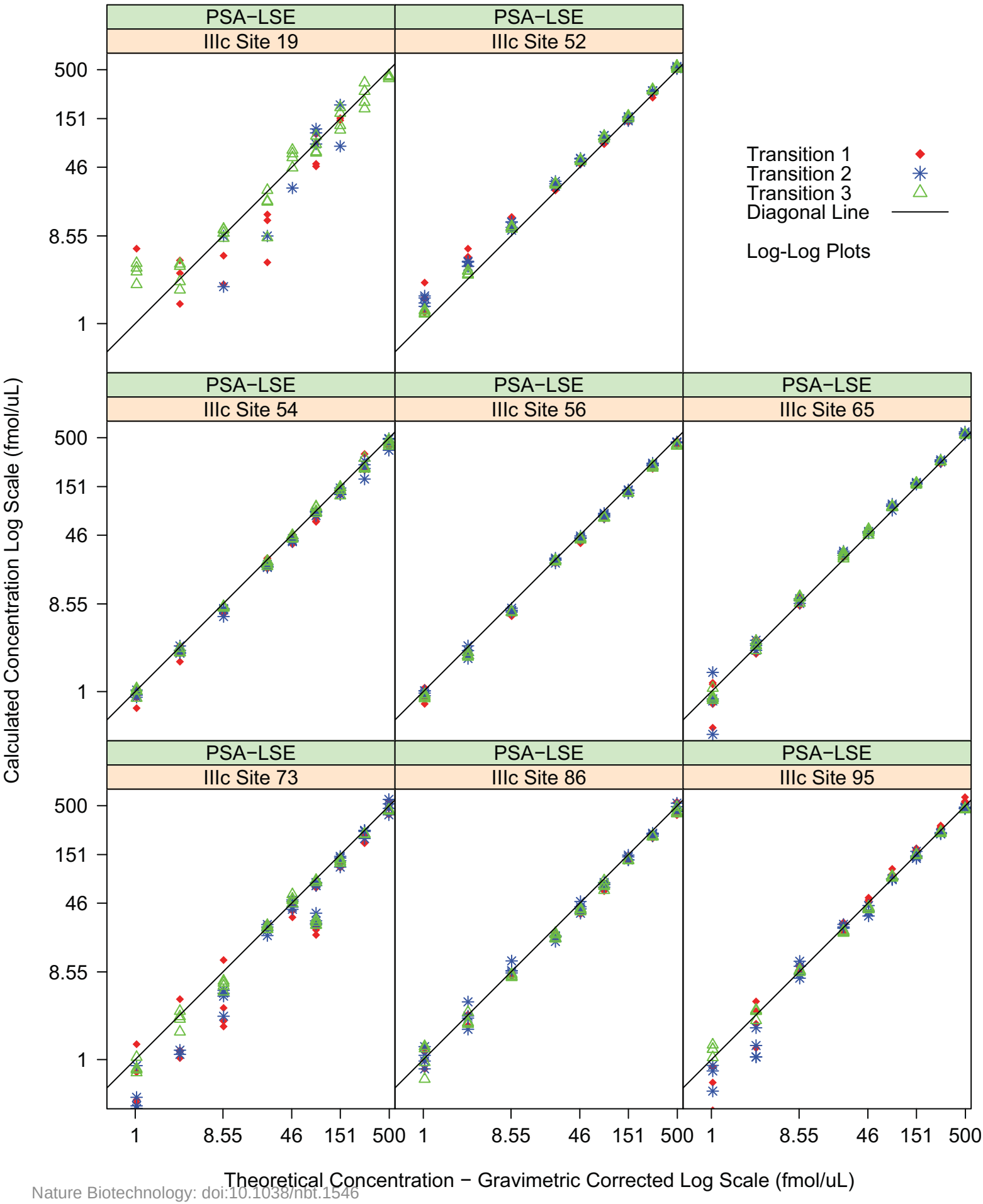

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