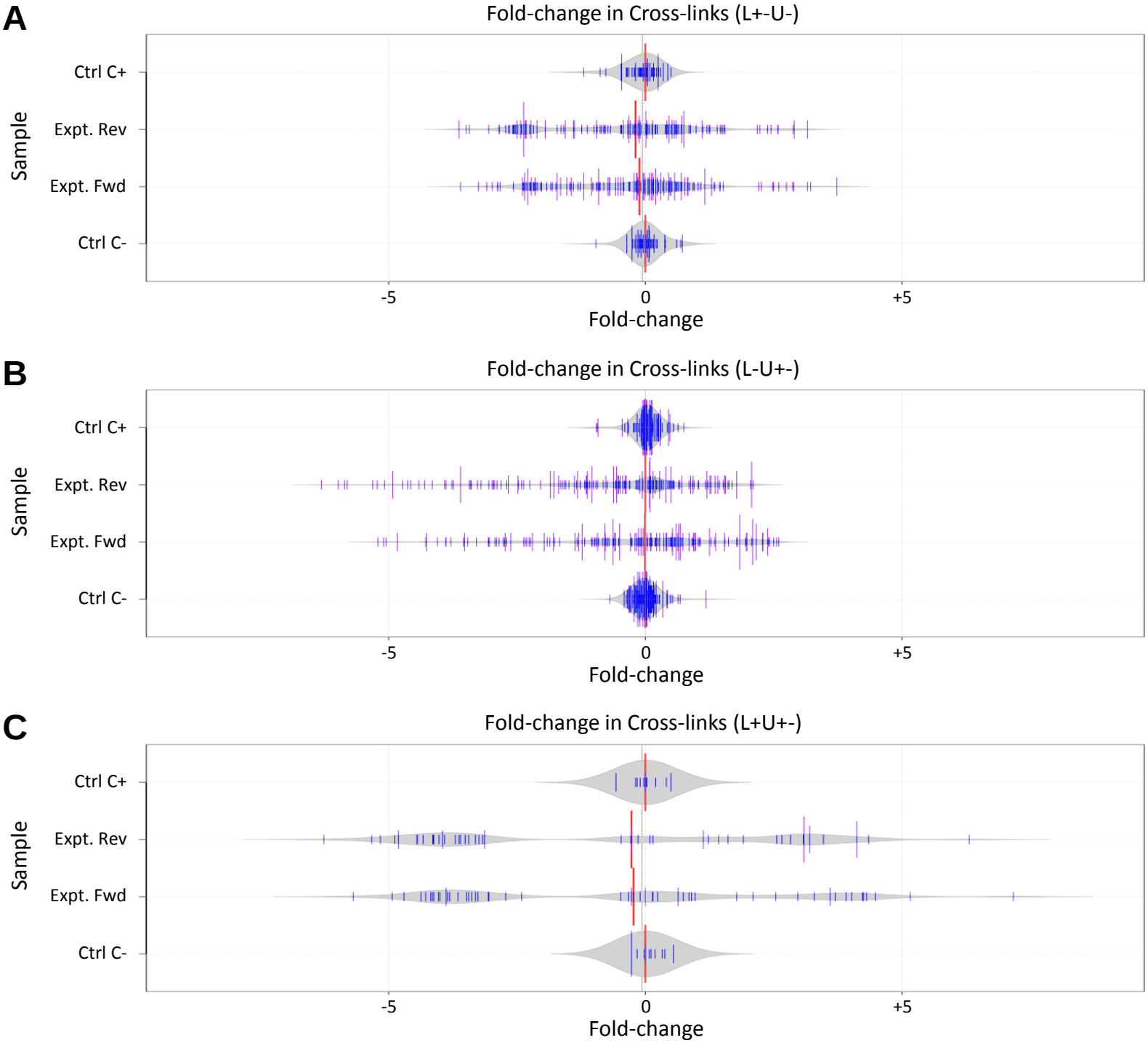


Figure S1a. Bean plots (generated with BoxPlotR) showing the distribution of XiQ quantified fold-change (log_2(H/L ratio)) for cross-links in control and experimental samples for (A) SpyL32P +/- Im7 datasets, (B) SpyL32P +/- 8M Urea datasets, and (C) SpyL32P+Im7 +/- 8M Urea datasets. The fold-change of the reverse-labeled experimental sample is shown as log_2(H/L ratio)*(-1).

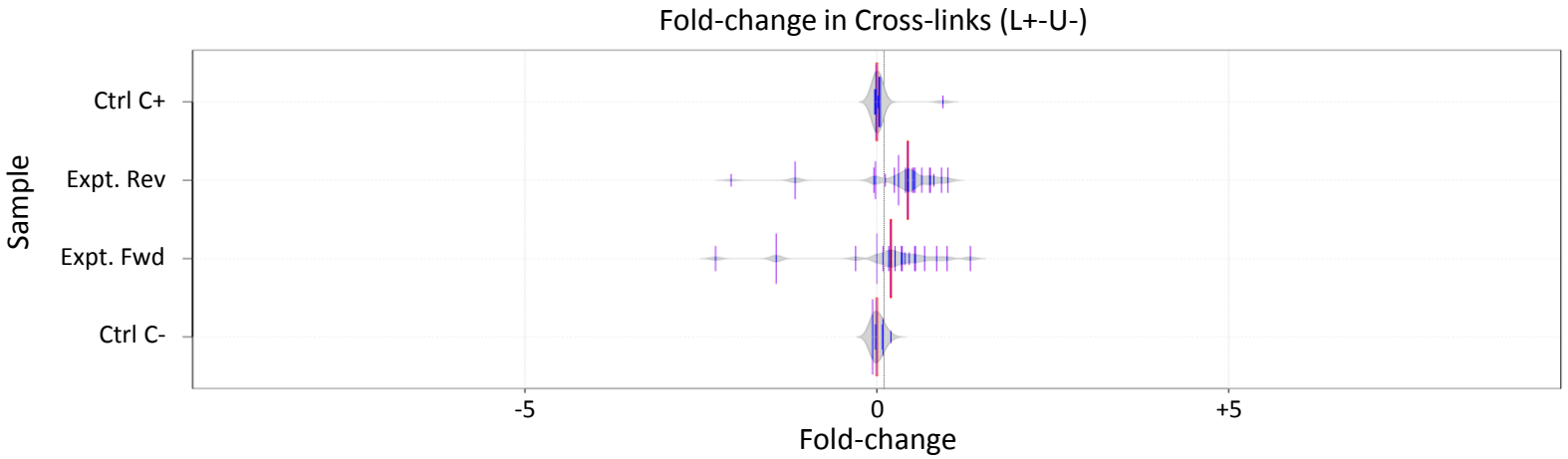


Figure S1b. Bean plots (generated with BoxPlotR) showing the distribution of XiQ quantified fold-change (log_2(H/L ratio)) for cross-links in control and experimental samples for FKBP25 F145A I223P +/- rapamycin datasets. The fold-change of the reverse-labeled experimental sample is shown as log_2(H/L ratio)*(-1).

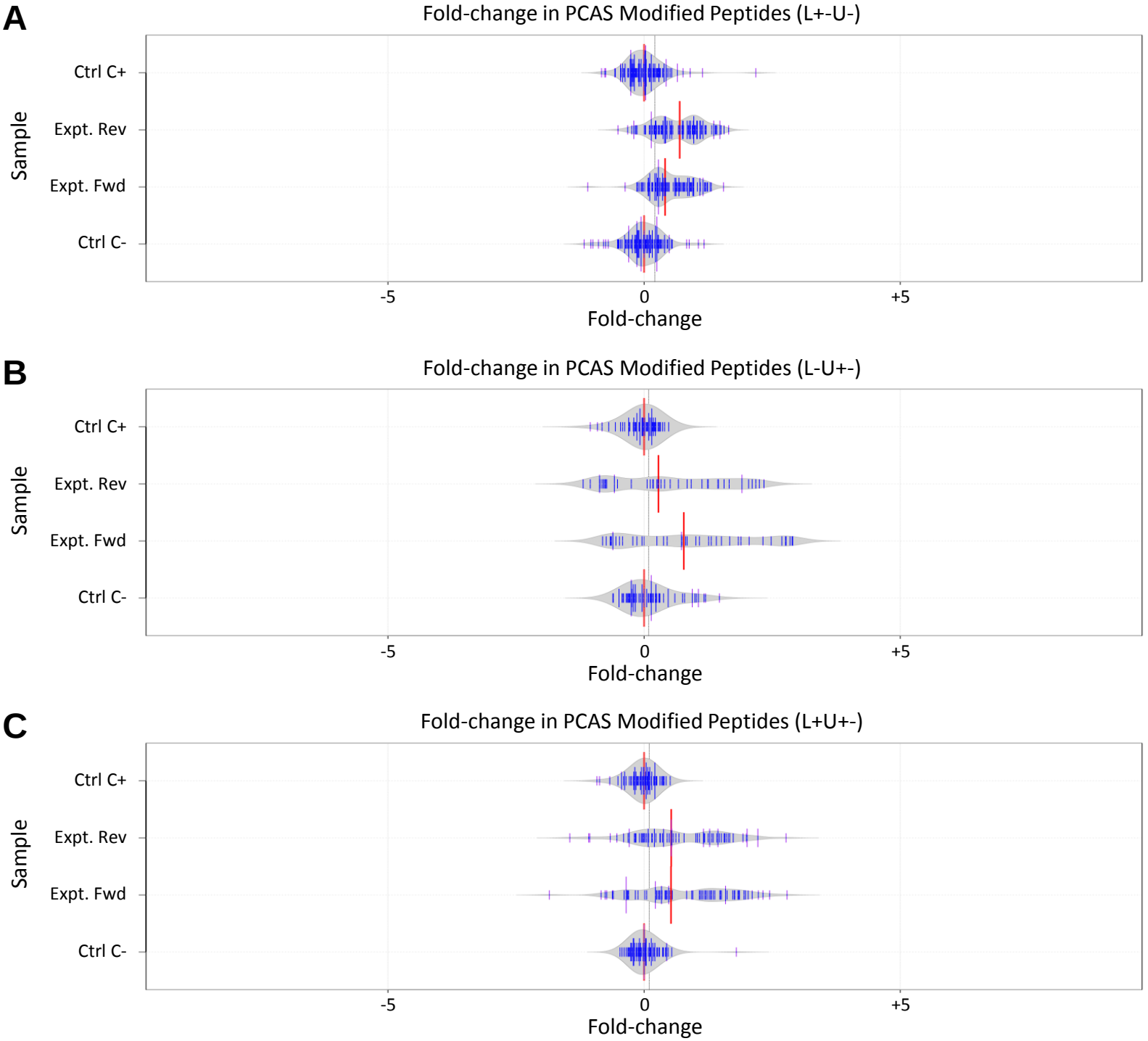


Figure S2a. Bean plots (generated with BoxPlotR) showing the distribution of XiQ quantified fold-change (log_2(H/L ratio)) for PCAS-modified peptides in control and experimental samples for (**A**) SpyL32P +/- Im7 datasets, (**B**) SpyL32P +/- 8M Urea datasets, and (**C**) SpyL32P+Im7 +/- 8M Urea datasets. The fold-change of the reverse-labeled experimental sample is shown as log_2(H/L ratio)*(-1).

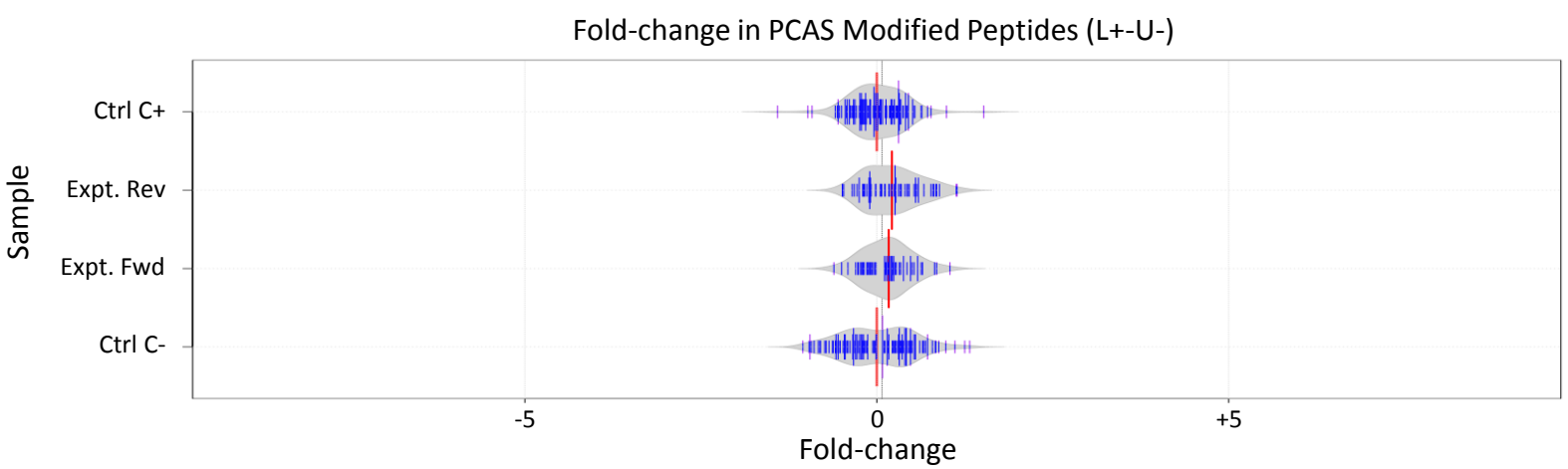


Figure S2b. Bean plots (generated with BoxPlotR) showing the distribution of XiQ quantified fold-change (log_2(H/L ratio)) for PCAS-modified peptides in control and experimental samples for FKBP25 F145A I223P +/- rapamycin datasets. The fold-change of the reverse-labeled experimental sample is shown as log_2(H/L ratio)*(-1).

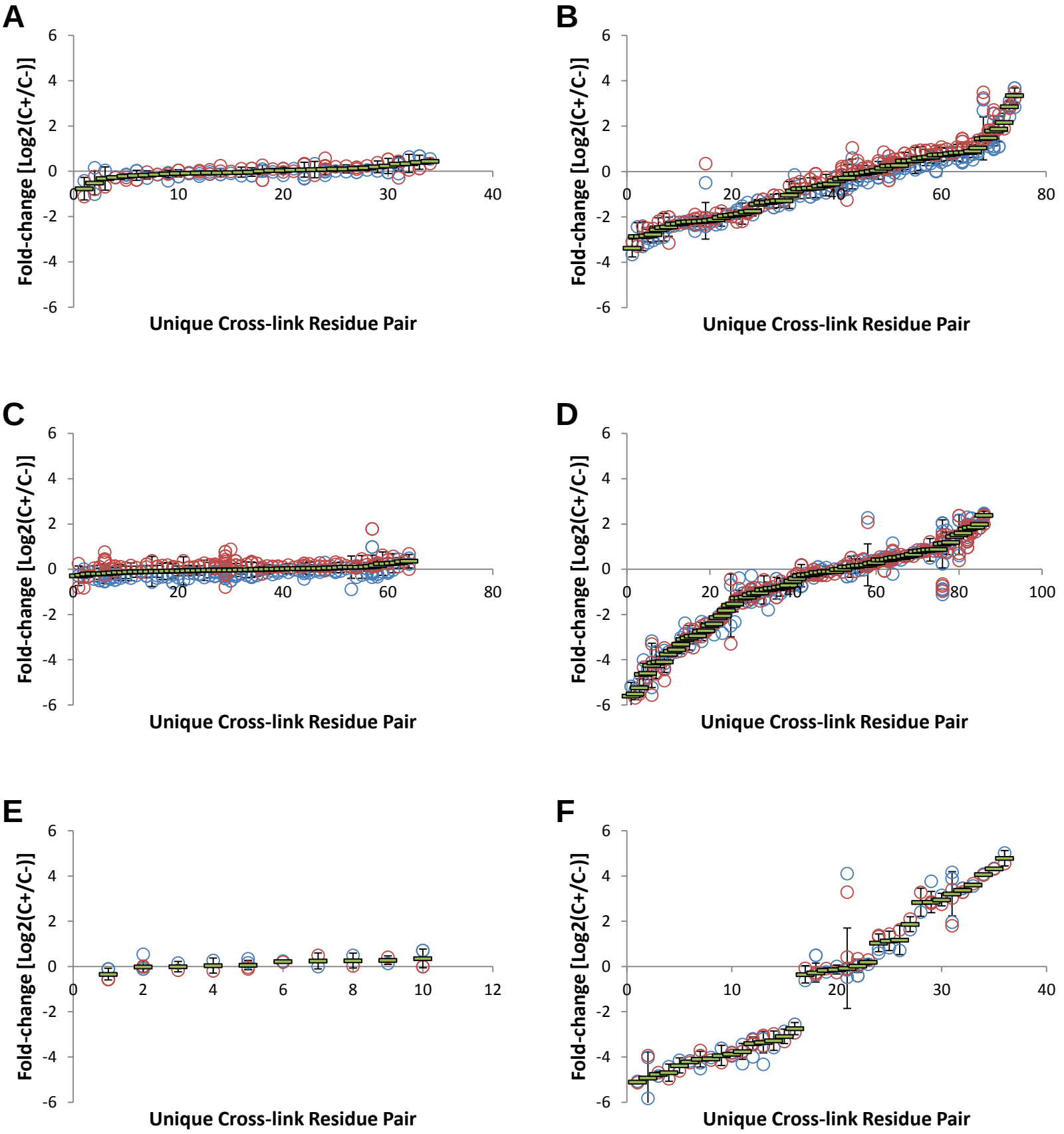


Figure S3a. Range of observed $\log_2$(H/L ratio) for all quantified cross-links. Blue circles represent data for control condition(-) or experimental forward-labeled cross-links and red circles represent data for control condition(+) or experimental reverse-labeled cross-links. Green bars indicate median fold-change. The fold-change of the reverse-labeled experimental sample is shown as $\log_2$(H/L ratio)*(-1). (**A**) SpyL32P condition(+/- Im7) control samples. (**B**) SpyL32P condition(+/- Im7) experimental samples. (**C**) SpyL32P condition(+/- 8M Urea) control samples. (**D**) SpyL32P condition(+/- 8M Urea) experimental samples. (**E**) SpyL32P+Im7 condition(+/- 8M Urea) control samples. (**F**) SpyL32P+Im7 condition(+/- 8M Urea) experimental samples.

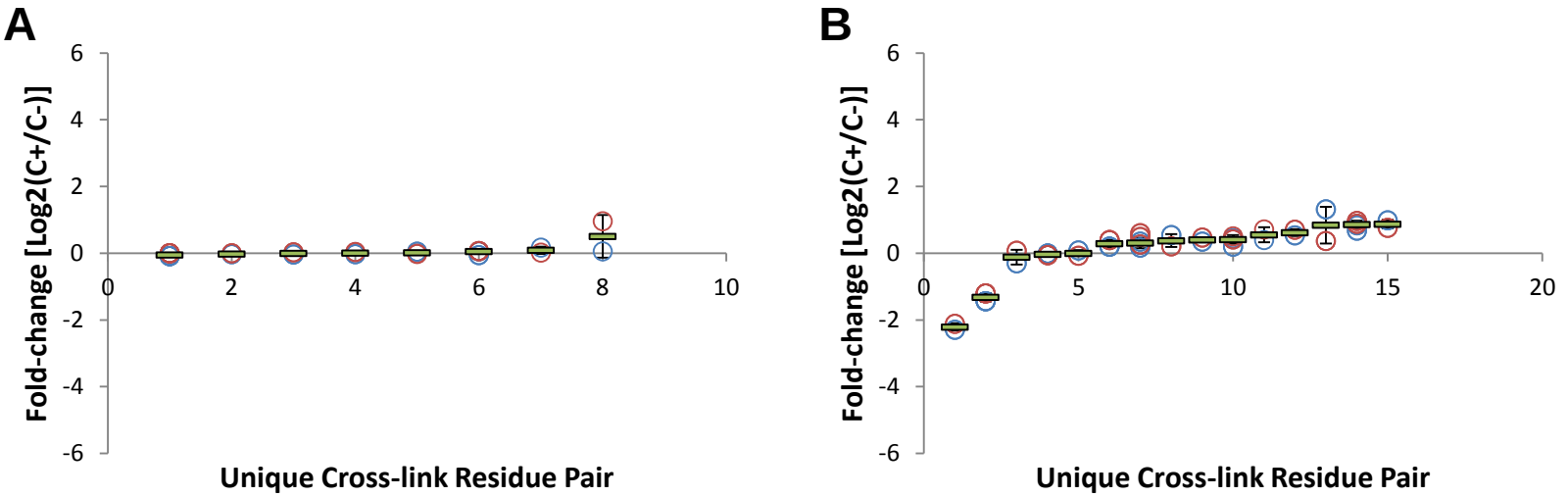


Figure S3b. Range of observed $\log_2$(H/L ratio) for all quantified cross-links. Blue circles represent data for control condition(-) or experimental forward-labeled cross-links and red circles represent data for control condition(+) or experimental reverse-labeled cross-links. Green bars indicate median fold-change. The fold-change of the reverse-labeled experimental sample is shown as $\log_2$(H/L ratio)*(-1). (**A**) FKBP25 F145A I223P condition(+/- rapamycin) control samples. (**B**) FKBP25 F145A I223P condition(+/- rapamycin) experimental samples.

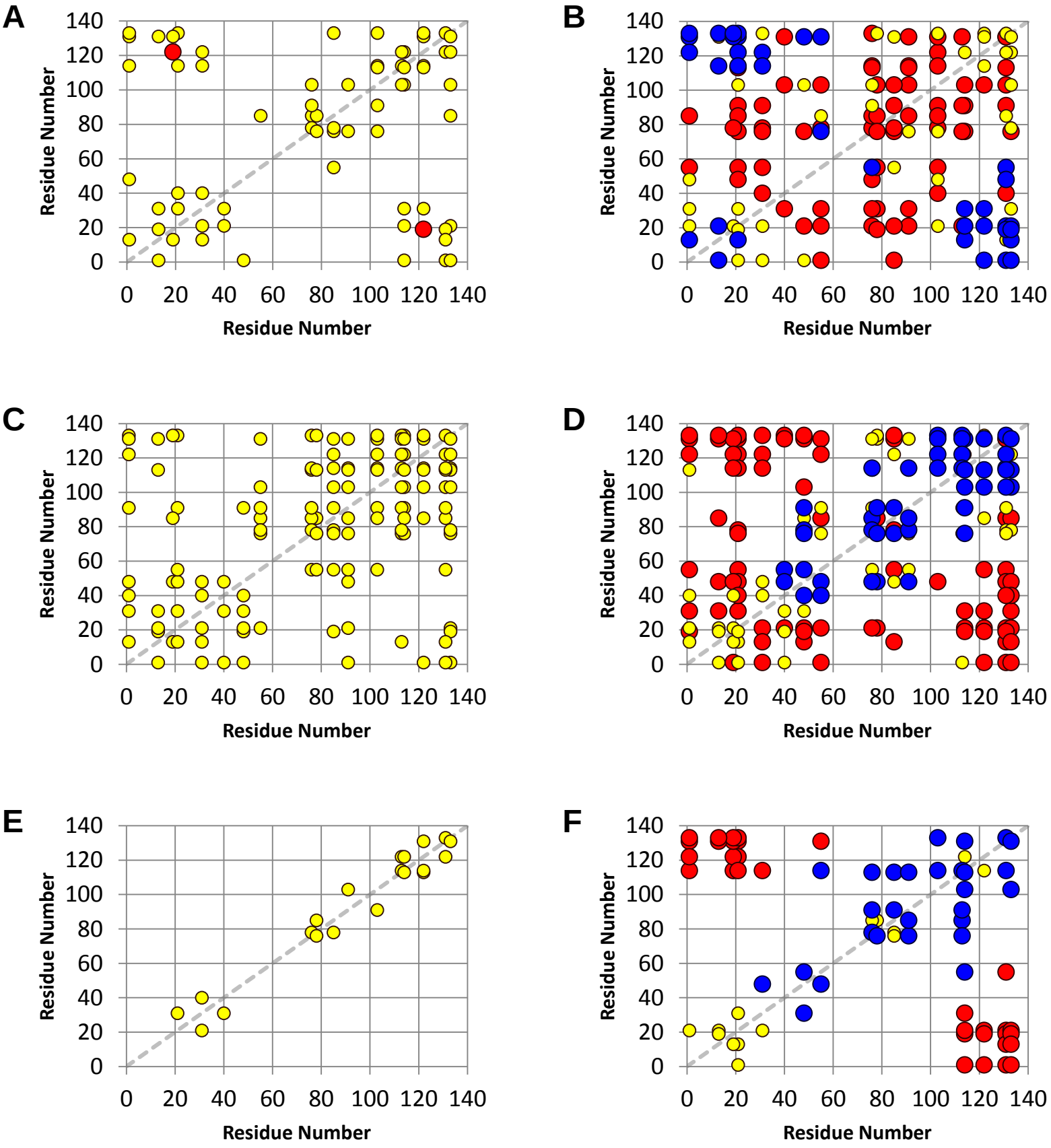


Figure S4a. Cross-link contact maps. (**A**) SpyL32P condition(+/- Im7) control samples. (**B**) SpyL32P condition(+/- Im7) experimental samples. (**C**) SpyL32P condition(+/- 8M Urea) control samples. (**D**) SpyL32P condition(+/- 8M Urea) experimental samples. (**E**) SpyL32P+Im7 condition(+/- 8M Urea) control samples. (**F**) SpyL32P+Im7 condition(+/- 8M Urea) experimental samples. Red circles indicate cross-links are enriched in condition(-). Blue circles indicate cross-links are enriched in condition(+). Yellow circles indicate no significant change between in cross-link abundance between condition(-) and condition(+). Thresholds for significance are reported in supplementary table 1.

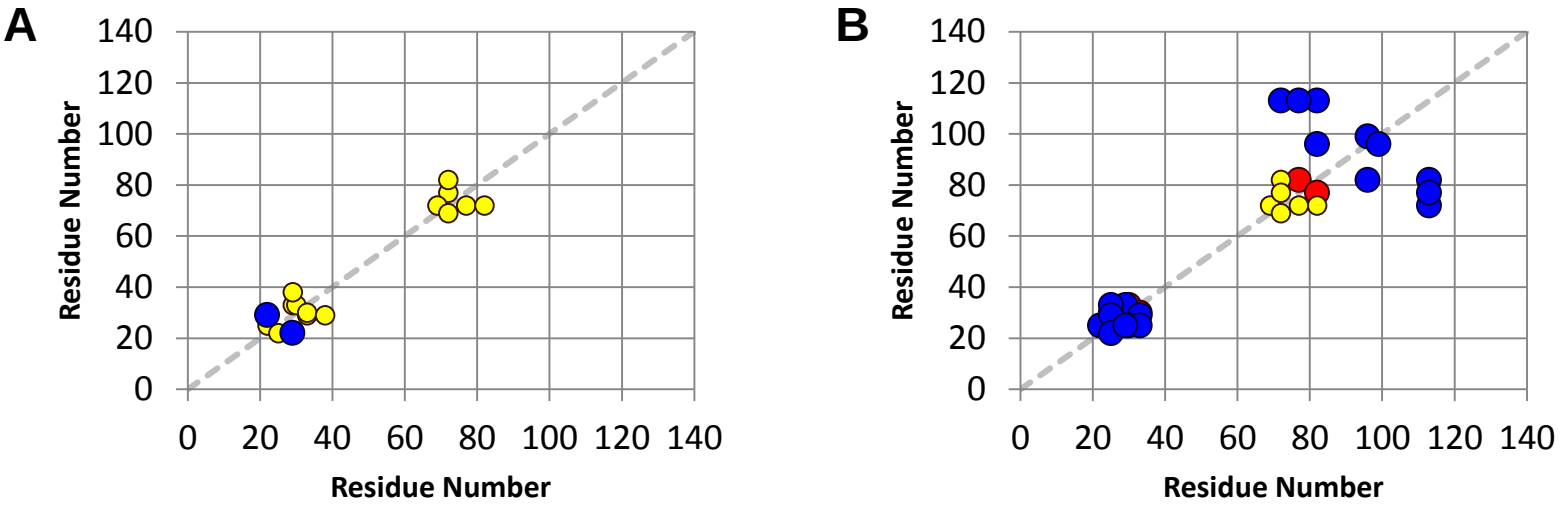


Figure S4b. Cross-link contact maps. (**A**) FKBP25 F145A I223P condition(+/- rapamycin) control samples. (**B**) FKBP25 F145A I223P condition(+/- rapamycin) experimental samples. Thresholds for significance are reported in supplementary table 1.

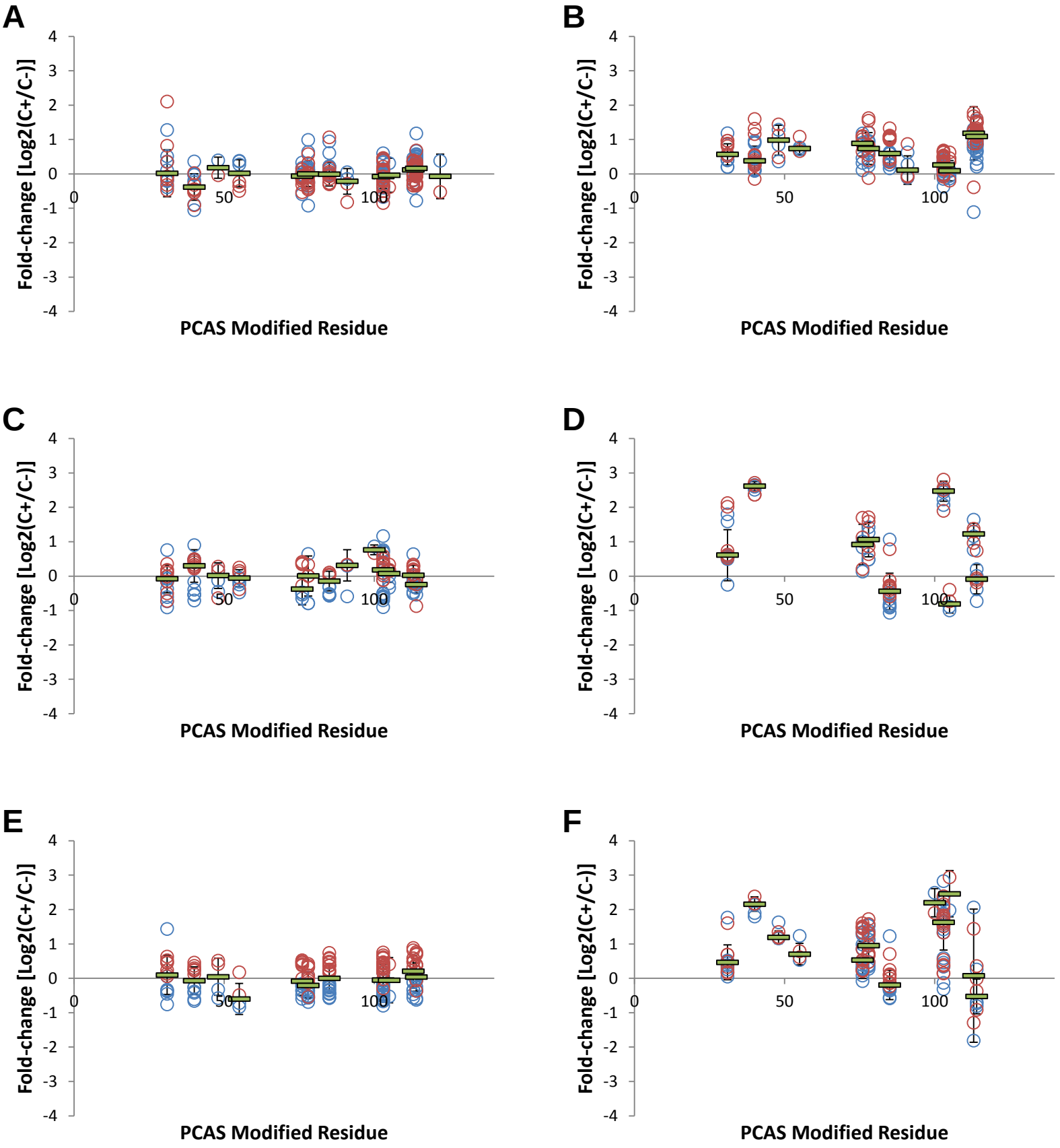


Figure S5a. Range of observed $\log_2$(H/L ratio) for all quantified PCAS-modified peptides. Blue circles represent data for control condition(-) or experimental forward-labeled cross-links and red circles represent data for control condition(+) or experimental reverse-labeled cross-links. Green bars indicate median fold-change. The fold-change of the reverse-labeled experimental sample is shown as $\log_2$(H/L ratio)*(-1). (**A**) SpyL32P condition(+/- Im7) control samples. (**B**) SpyL32P condition(+/- Im7) experimental samples. (**C**) SpyL32P condition(+/- 8M Urea) control samples. (**D**) SpyL32P condition(+/- 8M Urea) experimental samples. (**E**) SpyL32P+Im7 condition(+/- 8M Urea) control samples. (**F**) SpyL32P+Im7 condition(+/- 8M Urea) experimental samples.

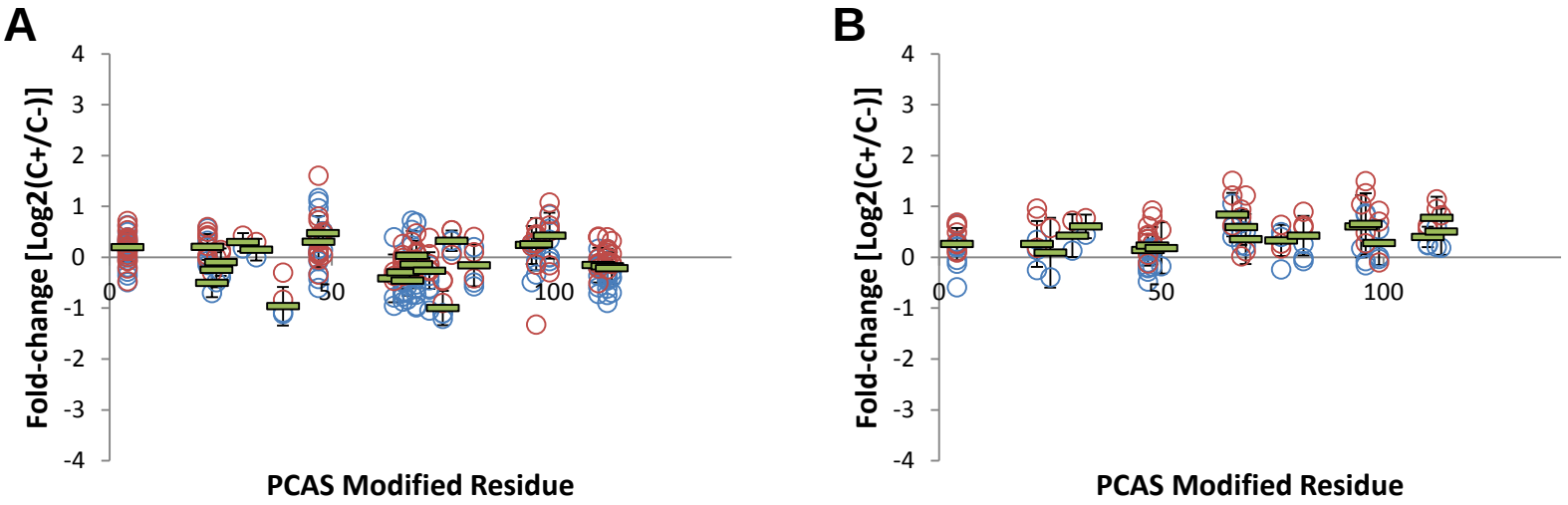


Figure S5b. Range of observed $\log_2$(H/L ratio) for all quantified PCAS-modified peptides. Blue circles represent data for control condition(-) or experimental forward-labeled cross-links and red circles represent data for control condition(+) or experimental reverse-labeled cross-links. Green bars indicate median fold-change. The fold-change of the reverse-labeled experimental sample is shown as $\log_2$(H/L ratio)*(-1). (**A**) FKBP25 F145A I223P condition(+/- rapamycin) control samples. (**B**) FKBP25 F145A I223P condition(+/- rapamycin) experimental samples.

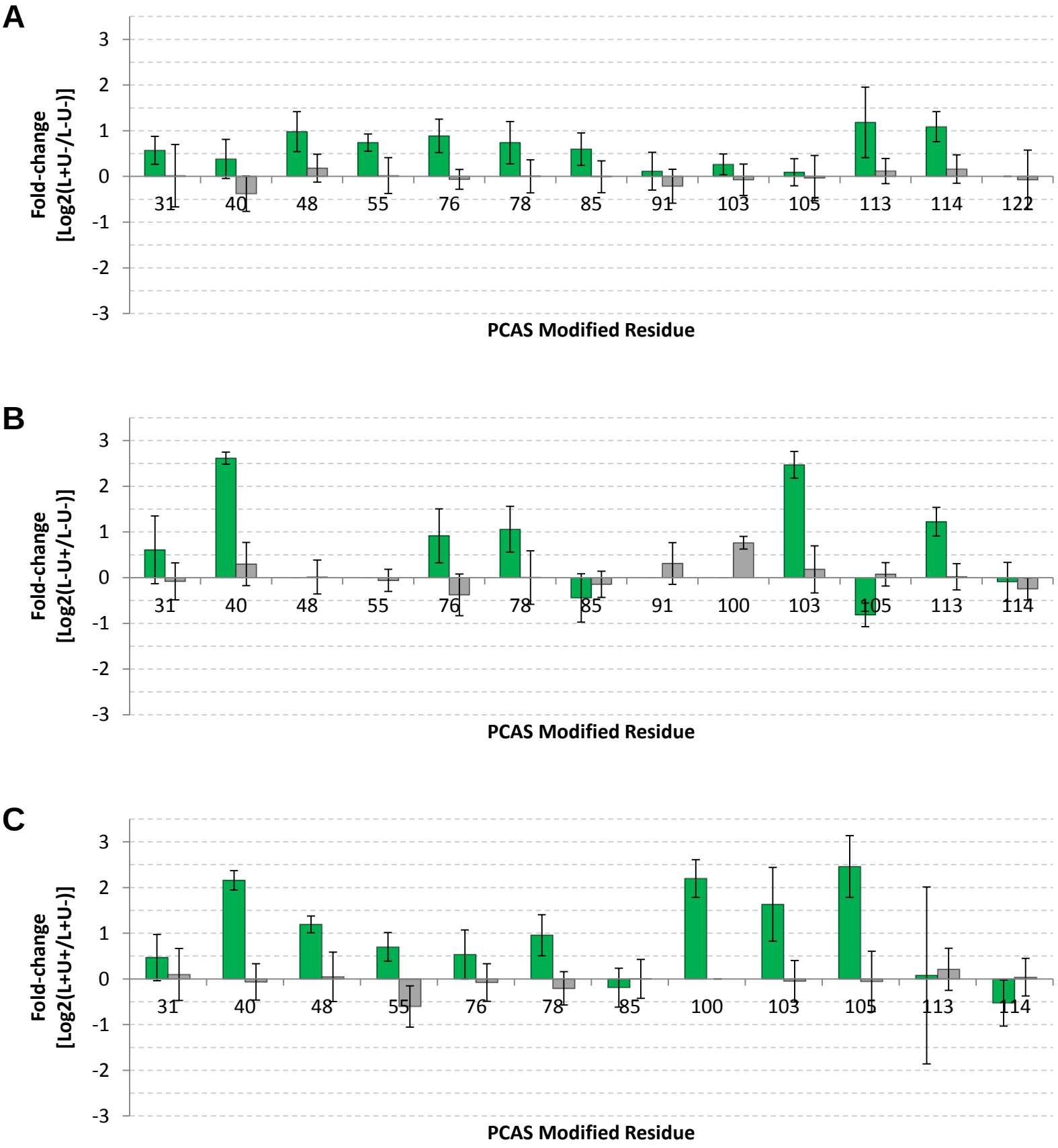


Figure S6a. Unique PCAS-modified residue fold-change column charts. (**A**) SpyL32P condition(+/- Im7) samples. (**B**) SpyL32P condition(+/- 8M Urea) samples. (**C**) SpyL32P+Im7 condition(+/- 8M Urea) samples. Experimental and control sample data are represented by green and grey bars, respectively. Error bars indicate standard error for all identified PCAS-modified peptides with modification on respective residue.

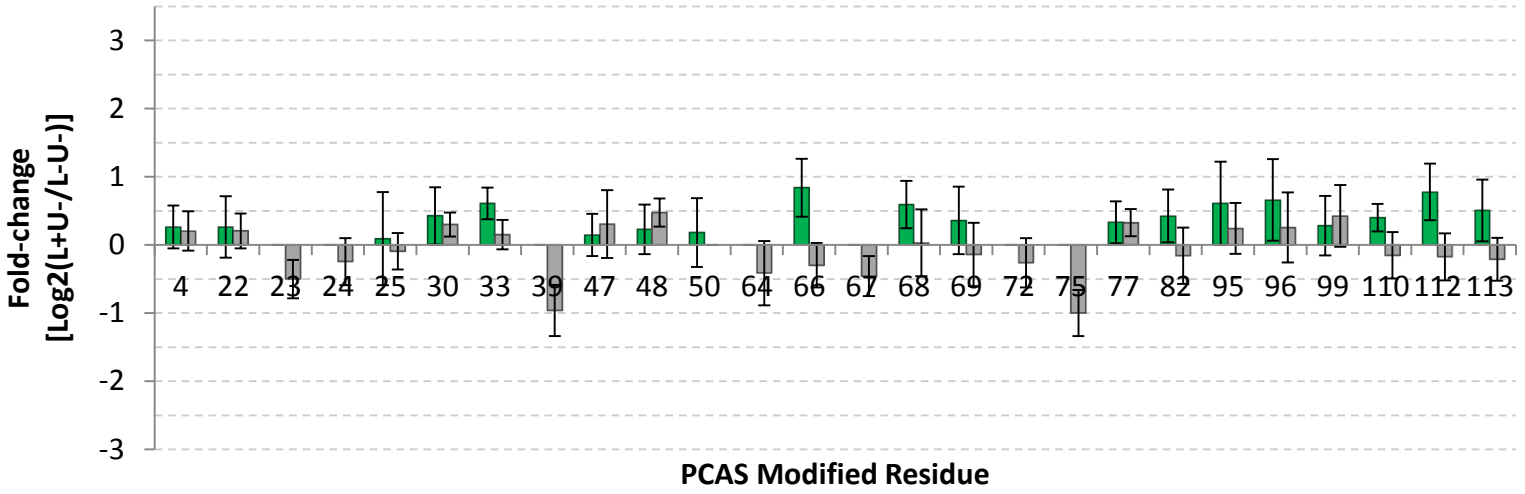


Figure S6b. Unique PCAS-modified residue fold-change column charts for FKBP25 F145A I223P condition(+/- rapamycin) control samples. Experimental and control sample data are represented by green and grey bars, respectively. Error bars indicate standard error for all identified PCAS-modified peptides with modification on respective residue.

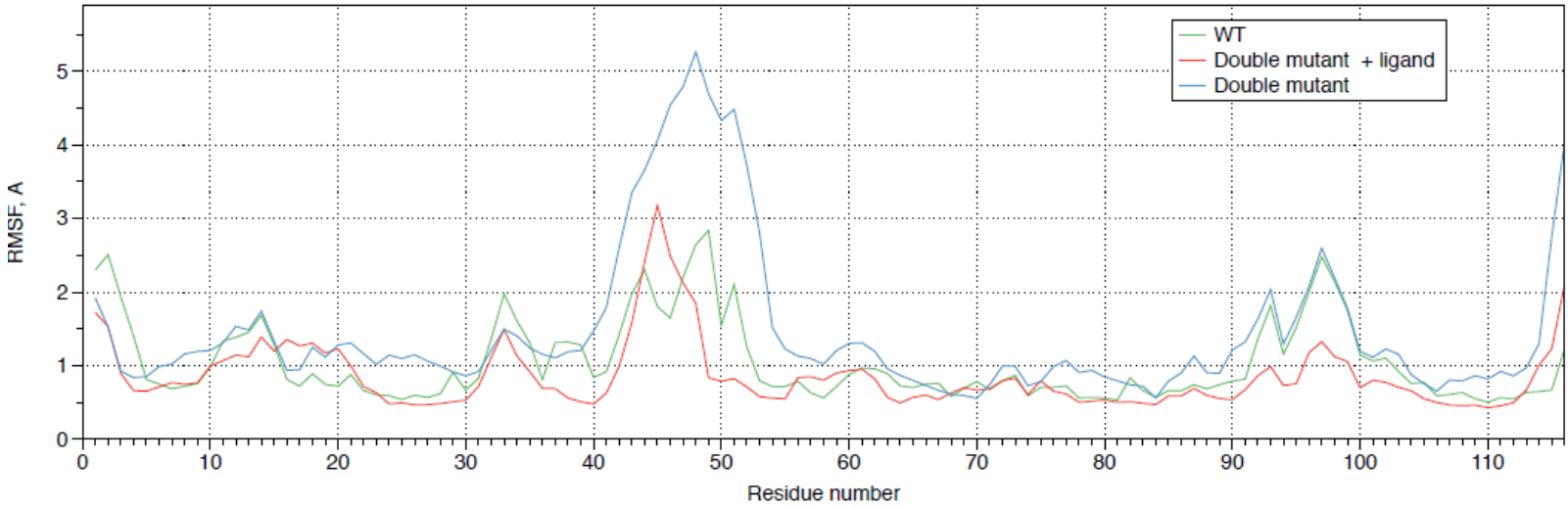


Figure S7. Main chain fluctuations along MD trajectory.

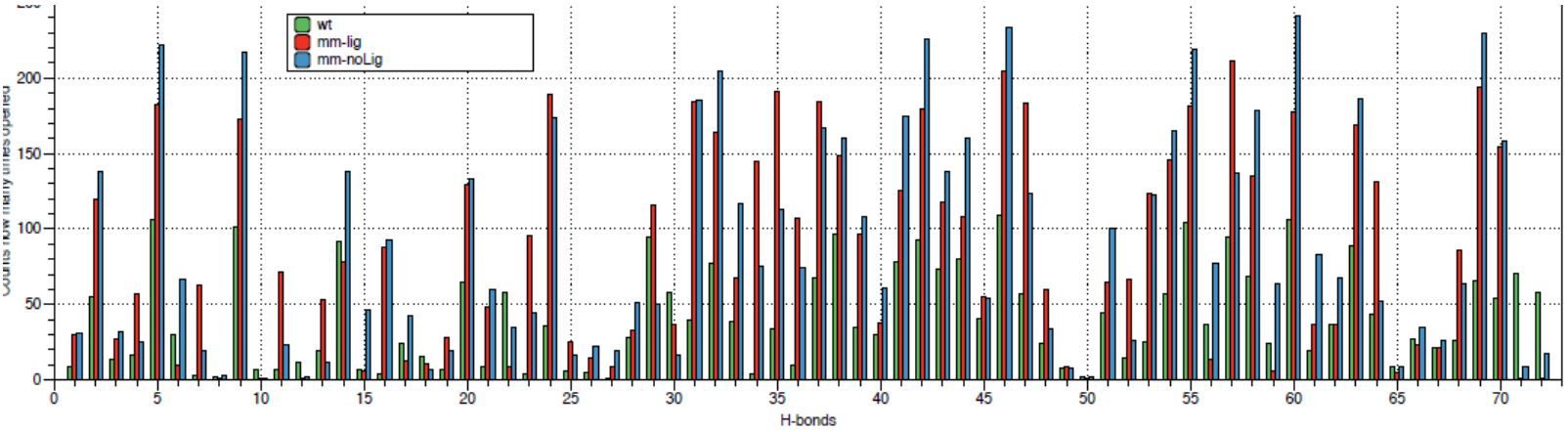


Figure S8. Frequencies of opening along MD trajectory of the backbone amide donor-acceptor pairs.

Table S1. Standard deviation of fold-change [Log2(C+/C-)] Significance thresholds used classification of corresponding log2(H/L ratio) medians as enriched in condition(-), condition(+), or unchanged are calculated as 2x the sample standard deviation.

Experiment	Control Condition(-) and Condition(+) Fold-change [Log_2(C+/C-)] Sample Standard Deviation (s)	Experiment Forward and Reverse Significance Fold-change [Log2(C+/C-)] Threshold (+/-2s)
Spy qCL L+-U-	0.298	0.597
Spy qCL L-U+-	0.234	0.469
Spy qCL L+U+-	0.311	0.622
Spy SM L+-U-	0.373	0.746
Spy SM L-U+-	0.382	0.764
Spy SM L+U+-	0.280	0.561
FKBP qCL L+-U-	0.134	0.268
FKBP SM L+-U-	0.446	0.893
FKBP SM L-U+-	0.721	1.442
FKBP SM L+U+-	0.687	1.374

Table S2. Sample naming convention and conditions.

Sample #	Ligand	Urea	Isotopic Label
01	-	-	Light
02	-	-	Heavy
04	-	+	Light
05	-	+	Heavy
07	+	-	Light
08	+	-	Heavy
10	+	+	Light
11	+	+	Heavy

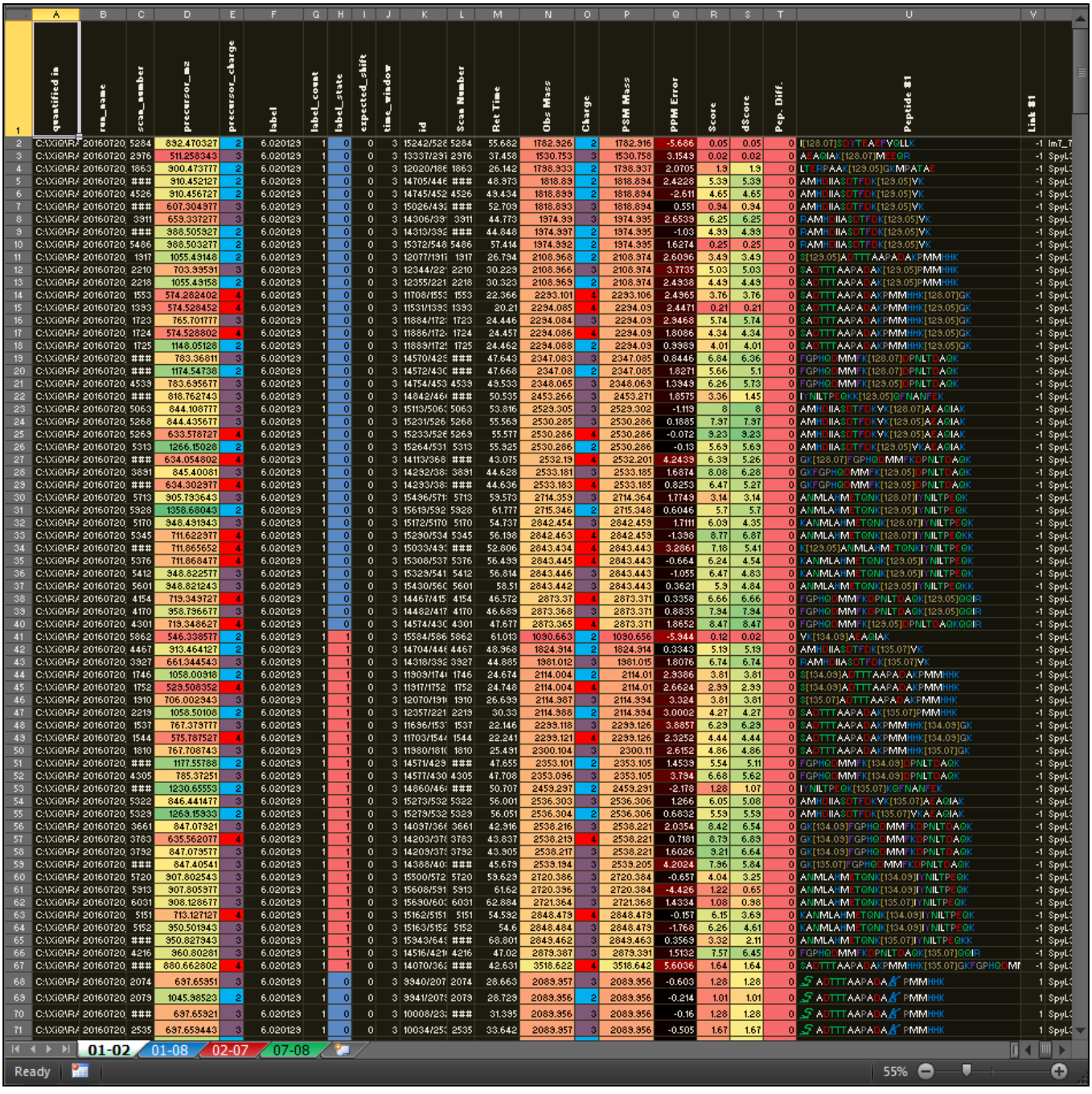
Table S3. Sample combination reference table.

Sample	L+-U-	L-U+-	L+U+-
Ctrl Condition (-)	01-02	01-02	07-08
Experimental Forward Label	01-08	01-05	07-11
Experimental Reverse Label	02-07	02-04	08-10
Ctrl Condition (+)	07-08	04-05	10-11

S. Material. Summary Microsoft Excel workbooks of all quantitative cross-linking and surface modification results to be uploaded.

Name	Date modified	Type	Size
FKBP25_F145A_I223P_qCL_L+-U-.xlsb	11/7/2016 11:12 AM	Microsoft Excel Binary Worksheet	493 KB
FKBP25_F145A_I223P_SM_L+-U-.xlsb	11/6/2016 12:22 PM	Microsoft Excel Binary Worksheet	666 KB
FKBP25_F145A_I223P_SM_L+U+-.xlsb	11/7/2016 11:13 AM	Microsoft Excel Binary Worksheet	378 KB
FKBP25_F145A_I223P_SM_L-U+-.xlsb	11/7/2016 11:13 AM	Microsoft Excel Binary Worksheet	321 KB
SpyL32P_qCL_L+-U-.xlsb	11/7/2016 11:00 AM	Microsoft Excel Binary Worksheet	3,042 KB
SpyL32P_qCL_L+U+-.xlsb	11/7/2016 11:02 AM	Microsoft Excel Binary Worksheet	1,731 KB
SpyL32P_qCL_L-U+-.xlsb	11/7/2016 11:03 AM	Microsoft Excel Binary Worksheet	3,619 KB
SpyL32P_SM_L+-U-.xlsb	11/7/2016 11:08 AM	Microsoft Excel Binary Worksheet	773 KB
SpyL32P_SM_L+U+-.xlsb	11/7/2016 11:08 AM	Microsoft Excel Binary Worksheet	645 KB
SpyL32P_SM_L-U+-.xlsb	11/7/2016 11:09 AM	Microsoft Excel Binary Worksheet	624 KB

e.g.



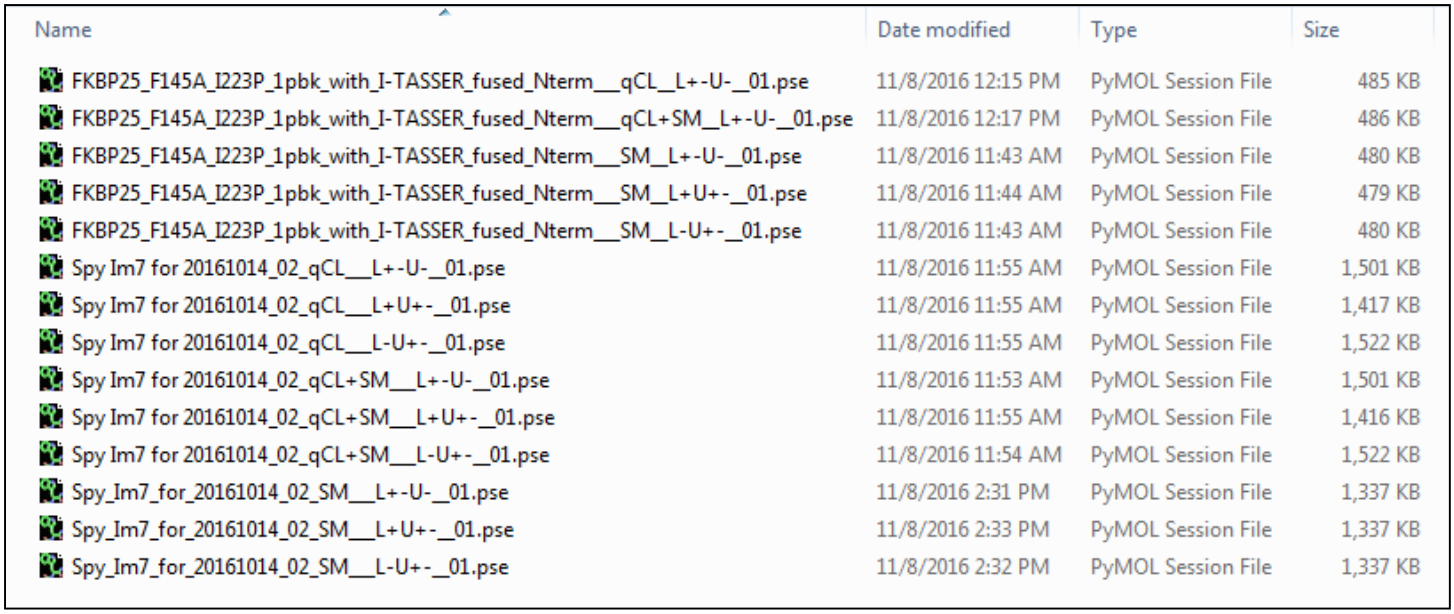
S. Material. Raw data to be uploaded.

e.g.

Name	Date modified	Type	Size
20160426_FKBP25_F145A_I223P_1213.raw	4/28/2016 8:01 PM	Xcalibur Raw File	433,157 KB
20160426_FKBP25_F145A_I223P_1312.raw	4/28/2016 10:09 PM	Xcalibur Raw File	365,506 KB
20160426_FKBP25_F145A_I223P_ctrl.raw	4/28/2016 4:29 PM	Xcalibur Raw File	556,540 KB
20160426_FKBP25_F145A_I223P_rap_ctrl.raw	4/28/2016 5:53 PM	Xcalibur Raw File	342,673 KB
20160620_FKBP25_F145A_I223P_PCASurea_ctrl.raw	6/30/2016 2:10 PM	Xcalibur Raw File	442,843 KB
20160620_FKBP25_F145A_I223P_PCASurea_DH.raw	6/30/2016 12:17 PM	Xcalibur Raw File	439,103 KB
20160620_FKBP25_F145A_I223P_PCASurea_HD.raw	6/30/2016 10:24 AM	Xcalibur Raw File	422,603 KB
20160620_FKBP25_F145A_I223P_PCASurea_urea_ctrl.raw	6/30/2016 3:34 PM	Xcalibur Raw File	438,311 KB
20160620_FKBP25_F145A_I223P_rap_PCASurea_ctrl.raw	6/30/2016 9:12 PM	Xcalibur Raw File	429,375 KB
20160620_FKBP25_F145A_I223P_rap_PCASurea_DH.raw	6/30/2016 7:19 PM	Xcalibur Raw File	408,323 KB
20160620_FKBP25_F145A_I223P_rap_PCASurea_HD.raw	6/30/2016 5:26 PM	Xcalibur Raw File	388,318 KB
20160620_FKBP25_F145A_I223P_rap_PCASurea_urea_ctrl.raw	6/30/2016 10:36 PM	Xcalibur Raw File	412,905 KB
20160720_SpyL32P_qCL_DSA-12C6-13C6_100uM_01-02_TDSol_OT_OT_TN.raw	7/21/2016 1:17 AM	Xcalibur Raw File	577,115 KB
20160720_SpyL32P_qCL_DSA-12C6-13C6_100uM_01-05_TDSol_OT_OT_TN.raw	7/21/2016 4:01 AM	Xcalibur Raw File	530,167 KB
20160720_SpyL32P_qCL_DSA-12C6-13C6_100uM_01-08_TDSol_OT_OT_TN.raw	7/21/2016 6:45 AM	Xcalibur Raw File	448,081 KB
20160720_SpyL32P_qCL_DSA-12C6-13C6_100uM_02-04_TDSol_OT_OT_TN.raw	7/21/2016 9:28 AM	Xcalibur Raw File	625,172 KB
20160720_SpyL32P_qCL_DSA-12C6-13C6_100uM_02-07_TDSol_OT_OT_TN.raw	7/21/2016 12:13 PM	Xcalibur Raw File	519,773 KB
20160720_SpyL32P_qCL_DSA-12C6-13C6_100uM_04-05_TDSol_OT_OT_TN.raw	7/21/2016 2:57 PM	Xcalibur Raw File	750,112 KB
20160720_SpyL32P_qCL_DSA-12C6-13C6_100uM_07-08_TDSol_OT_OT_TN.raw	7/21/2016 5:41 PM	Xcalibur Raw File	414,269 KB
20160720_SpyL32P_qCL_DSA-12C6-13C6_100uM_07-11_TDSol_OT_OT_TN.raw	7/21/2016 8:25 PM	Xcalibur Raw File	380,938 KB
20160720_SpyL32P_qCL_DSA-12C6-13C6_100uM_08-10_TDSol_OT_OT_TN.raw	7/21/2016 11:09 PM	Xcalibur Raw File	435,076 KB
20160720_SpyL32P_qCL_DSA-12C6-13C6_100uM_10-11_TDSol_OT_OT_TN.raw	7/22/2016 1:53 AM	Xcalibur Raw File	832,665 KB
20160720_SpyL32P_SM_PCAS-H4-D4_10mM_01-02_PepSol_OT_OT_TN.raw	7/22/2016 5:04 PM	Xcalibur Raw File	513,535 KB
20160720_SpyL32P_SM_PCAS-H4-D4_10mM_01-05_PepSol_OT_OT_TN.raw	7/22/2016 7:48 PM	Xcalibur Raw File	721,328 KB
20160720_SpyL32P_SM_PCAS-H4-D4_10mM_01-08_PepSol_OT_OT_TN.raw	7/22/2016 10:32 PM	Xcalibur Raw File	697,070 KB
20160720_SpyL32P_SM_PCAS-H4-D4_10mM_02-04_PepSol_OT_OT_TN.raw	7/23/2016 1:16 AM	Xcalibur Raw File	706,956 KB
20160720_SpyL32P_SM_PCAS-H4-D4_10mM_02-07_PepSol_OT_OT_TN.raw	7/23/2016 3:59 AM	Xcalibur Raw File	649,897 KB
20160720_SpyL32P_SM_PCAS-H4-D4_10mM_04-05_PepSol_OT_OT_TN.raw	7/23/2016 6:43 AM	Xcalibur Raw File	684,078 KB
20160720_SpyL32P_SM_PCAS-H4-D4_10mM_07-08_PepSol_OT_OT_TN.raw	7/23/2016 9:27 AM	Xcalibur Raw File	673,614 KB
20160720_SpyL32P_SM_PCAS-H4-D4_10mM_07-11_PepSol_OT_OT_TN.raw	7/23/2016 12:11 PM	Xcalibur Raw File	688,429 KB
20160720_SpyL32P_SM_PCAS-H4-D4_10mM_08-10_PepSol_OT_OT_TN.raw	7/23/2016 2:55 PM	Xcalibur Raw File	632,741 KB
20160720_SpyL32P_SM_PCAS-H4-D4_10mM_10-11_PepSol_OT_OT_TN.raw	7/23/2016 5:40 PM	Xcalibur Raw File	622,478 KB
20160911_FKBP25_F145A_I223P_PCAS_sm_1213.raw	9/12/2016 12:18 AM	Xcalibur Raw File	737,683 KB
20160911_FKBP25_F145A_I223P_PCAS_sm_1312.raw	9/12/2016 3:01 AM	Xcalibur Raw File	749,172 KB
20160911_FKBP25_F145A_I223P_PCAS_sm_ctrl.raw	9/12/2016 5:44 AM	Xcalibur Raw File	696,162 KB
20160911_FKBP25_F145A_I223P_PCAS_sm_rapctrl.raw	9/12/2016 8:27 AM	Xcalibur Raw File	734,700 KB

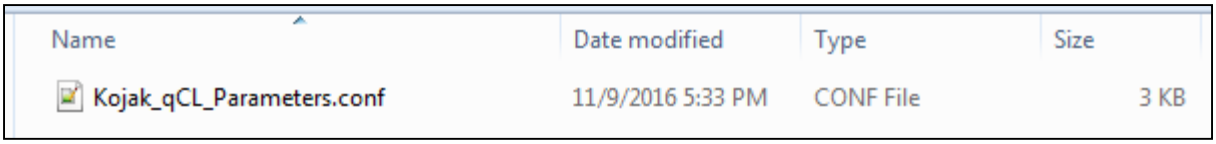
S. Material. PyMOL session files to be uploaded.

e.g.



Name	Date modified	Type	Size
FKBP25_F145A_I223P_1pbk_with_I-TASSER_fused_Nterm__qCL__L+-U-__01.pse	11/8/2016 12:15 PM	PyMOL Session File	485 KB
FKBP25_F145A_I223P_1pbk_with_I-TASSER_fused_Nterm__qCL+SM__L+-U-__01.pse	11/8/2016 12:17 PM	PyMOL Session File	486 KB
FKBP25_F145A_I223P_1pbk_with_I-TASSER_fused_Nterm__SM__L+-U-__01.pse	11/8/2016 11:43 AM	PyMOL Session File	480 KB
FKBP25_F145A_I223P_1pbk_with_I-TASSER_fused_Nterm__SM__L+U+-__01.pse	11/8/2016 11:44 AM	PyMOL Session File	479 KB
FKBP25_F145A_I223P_1pbk_with_I-TASSER_fused_Nterm__SM__L-U+-__01.pse	11/8/2016 11:43 AM	PyMOL Session File	480 KB
Spy Im7 for 20161014_02_qCL__L+-U-__01.pse	11/8/2016 11:55 AM	PyMOL Session File	1,501 KB
Spy Im7 for 20161014_02_qCL__L+U+-__01.pse	11/8/2016 11:55 AM	PyMOL Session File	1,417 KB
Spy Im7 for 20161014_02_qCL__L-U+-__01.pse	11/8/2016 11:55 AM	PyMOL Session File	1,522 KB
Spy Im7 for 20161014_02_qCL+SM__L+-U-__01.pse	11/8/2016 11:53 AM	PyMOL Session File	1,501 KB
Spy Im7 for 20161014_02_qCL+SM__L+U+-__01.pse	11/8/2016 11:55 AM	PyMOL Session File	1,416 KB
Spy Im7 for 20161014_02_qCL+SM__L-U+-__01.pse	11/8/2016 11:54 AM	PyMOL Session File	1,522 KB
Spy_Im7_for_20161014_02_SM__L+-U-__01.pse	11/8/2016 2:31 PM	PyMOL Session File	1,337 KB
Spy_Im7_for_20161014_02_SM__L+U+-__01.pse	11/8/2016 2:33 PM	PyMOL Session File	1,337 KB
Spy_Im7_for_20161014_02_SM__L-U+-__01.pse	11/8/2016 2:32 PM	PyMOL Session File	1,337 KB

S. Material. Kojak configuration.



Name	Date modified	Type	Size
Kojak_qCL_Parameters.conf	11/9/2016 5:33 PM	CONF File	3 KB

e.g.

Kojak_qCL_Parameters.conf

```
# Kojak version 1.5.1 parameter file

threads     =   8

database            =   #Concatenated TARGET-DECOY(seq. reversal) of FKBP25_F145A_I223P or SpyL32P and Im7(7-45)

#   FKBP25_F145A_I223P searches:
#   >FKBP25_F145A_I223P
#   MRGSHHHHHHGLVPRGSMGPPKYTKSVLKKGDKTNFPKKGDVVHCWYTGTLQDGTVADTNIQTSAKKKKNAKPLSFKVGVGKVIRGWDEALLTMSKGEKARLEIEPEWAYGKKGQPDAKIPPNAKLTFEVELVDPD
#   >decoy_FKBP25_F145A_I223P
#   DPDVLEVEFTLKANPPIKADPQGKKGYAWEPEIELRAKEGKSMTLLAEDWGRIVKGVGVKFSLPKANKKKKASTQINTDAVTGDQLTGTYWCHVVDGKKPFNTKDGKKLVSKTYKPPGMSGRPVLGHHHHHHSGRM
#   >SpyL32P
#   SADTTTAAPADAKPMMHHKGKFGPHQDMMFKDPNLTDAQKQQIREIMKGQRDQMKRPPLEERRAMHDIIASDTFDKVKAEAQIAKMEEQRKANMLAHMETQNKIYNILTPEQKKQFNANFEKRLTERPAAKGKMPATAE
#   >Im7_7-45
#   SISDYTEAEFVQLLKEIEKENVAATDDVLDVLLEHFVKIT
#   >decoy_SpyL32P
#   EATAPMKGKAAPRETLRKEFNANFQKKQEPTLINYIKNQTEMHALMNAKRQEEMKAIQAEAKVKDFTDSAIIDHMARREELPPRKMQDRQGKMIERIQQKQADTLNPDKFMMDQHPGFKGKHHMMPKADAPAATTTDAS
#   >decoy_Im7_7-45
#   TIKVFHELLVDLVDDTAAVNEKEIEKLLQVFEAETYDSIS

export_percolator   =   1
export_pepXML       =   1
percolator_version  =   3.0

enrichment      =   0
instrument      =   0
MS1_centroid    =   0
MS2_centroid    =   0
MS1_resolution  =   60000
MS2_resolution  =   30000

cross_link  =   nK  nK  110.0362342 DSA_Light
mono_link   =   nK  128.0706042
mono_link   =   nK  129.0546242
cross_link  =   nK  nK  116.0563632 DSA_Heavy
mono_link   =   nK  134.0907332
mono_link   =   nK  135.0747532

diff_mods_on_xl         =       1
max_mods_per_peptide    =       #1 for FKBP25_F145A_I223P   3 for SpyL32P
mono_links_on_xl        =       #0 for FKBP25_F145A_I223P   1 for SpyL32P
modification            =       M       15.9949

enzyme  =       [KR]|{P}

fragment_bin_offset     =   0.0
fragment_bin_size       =   0.03
ion_series_A            =   0
ion_series_B            =   1
ion_series_C            =   0
ion_series_X            =   0
ion_series_Y            =   1
ion_series_Z            =   0

decoy_filter            =   decoy
isotope_error           =   1
max_miscleavages        =   #5 for FKBP25_F145A_I223P   2 for SpyL32P
max_peptide_mass        =   #6000.0 for FKBP25_F145A_I223P  8000.0 for SpyL32P
min_peptide_mass        =   400.0
max_spectrum_peaks      =   0
ppm_tolerance_pre       =   #6.0 for FKBP25_F145A_I223P 10.0 for SpyL32P
prefer_precursor_pred   =   2
spectrum_processing     =   0
top_count               =   300

truncate_prot_names     =   0
turbo_button            =   1
```