Subtle Dynamic Changes Accompany Hck Activation by HIV-1 Nef and are Reversed by an Antiretroviral Kinase Inhibitor

Thomas E. Wales[1], James M. Hochrein[1], Christopher R. Morgan[1], Lori A. Emert-Sedlak[2],
Thomas E. Smithgall[2], and John R. Engen[1*]

[1]Department of Chemistry & Chemical Biology, Northeastern University, Boston, MA 02115
[2]Department of Microbiology and Molecular Genetics, University of Pittsburgh School of
Medicine, Pittsburgh, PA 15219

Supplementary Information

Table S1

Supplemental Figure Legends

Figures S1-S7

Table S1

Construct Numbering	Hck Numbering	Sequence	Observed m/z	z	Retention time (min)
1-10	84-87	GHHHHHHIIV	633.3234	3	3.25
14-24	90-100	YDYEAIHHEDL	468.872	3	5.55
18-26	94-102	AIHHEDLSF	534.756	2	5.66
25-32	101-108	SFQKGDQM	470.708	2	4.59
36-41	112-118	EESGEW	736.274	1	6.12
42-58	119-135	WKARSLATRKEGYIPSN	495.024	4	4.59
42-59	119-136	WKARSLATRKEGYIPSNY	535.788	4	5.02
59-66	136-143	YVARVDSL	461.749	2	5.5
60-66	137-143	VARVDSL	380.217	2	5.04
60-67	137-144	VARVDSLE	444.742	2	4.8
72-82	149-159	FFKGISRKDAE	433.234	3	4.63
72-91	149-168	FFKGISRKDAERQLLAPGNM	570.313	4	5.77
72-92	149-169	FFKGISRKDAERQLLAPGNML	598.582	4	6.1
73-91	150-168	FKGISRKDAERQLLAPGNM	533.543	4	5.42
73-92	150-169	FKGISRKDAERQLLAPGNML	561.816	4	5.9
76-85	153-162	ISRKDAERQL	608.336	2	6.42
83-91	160-168	RQLLAPGNM	500.273	2	5.71
83-92	160-169	RQLLAPGNML	556.812	2	6.46
96-109	173-186	MIRDSETTKGSYSL	529.928	3	5.2
97-109	174-186	IRDSETTKGSYSL	728.869	2	5.02
110-135	187-212	SVRDYDPRQGDTVKHYKIRTLDNGGF	608.317	5	5.17
116-135	193-212	PRQGDTVKHYKIRTLDNGGF	576.311	4	5.17
136-143	213-220	YISPRSTF	485.751	2	5.63
138-143	215-220	SPRSTF	694.346	1	5.63
147-160	224-237	QELVDHYKKGNDGL	539.273	3	4.75
149-160	226-237	LVDHYKKGNDGL	453.568	3	4.33
150-160	227-237	VDHYKKGNDGL	415.876	3	3.92
161-166	238-243	CQKLSV	677.356	1	4.87
161-169	238-246	CQKLSVPCM	504.735	2	6.07
170-187	247-265	SSKPQKPWEKDAWEIPRE	553.537	4	5.59
170-189	247-267	SSKPQKPWEKDAWEIPRESL	603.57	4	5.94
170-191	247-269	SSKPQKPWEKDAWEIPRESLKL	531.292	5	6.04
190-200	268-278	KLEKKLGAGQF	406.91	3	4.65
190-204	268-282	KLEKKLGAGQFGEVW	563.983	3	6.05
192-204	270-282	EKKLGAGQFGEVW	724.875	2	6.4
205-224	283-302	MATYNKHTKVAVKTMKPGSM	556.542	4	4.25
208-224	286-302	YNKHTKVAVKTMKPGSM	480.759	4	3.91
225-229	303-307	SVEAF	552.26	1	6.01
230-235	308-313	LAEANV	616.324	1	4.77
236-244	314-322	MKTLQHDKL	557.303	2	4.6
245-257	323-335	VKLHAVVTKEPIY	499.629	3	4.98
248-257	326-335	HAVVTKEPIY	578.818	2	4.76
258-262	336-340	IITEF	622.339	1	6.73
262-268	340-346	FMAKGSL	377.197	2	5.3
262-269	340-347	FMAKGSLL	433.74	2	6.24
263-270	341-348	MAKGSLLD	417.722	2	5.3
271-285	349-363	FLKSDEGSKQPLPKL	562.985	3	5.24
272-285	350-363	LKSDEGSKQPLPKL	513.962	3	4.93
273-285	351-363	KSDEGSKQPLPKL	476.265	3	4.85
275-292	353-370	DEGSKQPLPKLIDFSAQI	662.693	3	6.34
292-298	370-376	IAEGMAF	738.344	1	6.78
299-311	377-389	IEQRNYIHRDLRA	561.969	3	4.35
304-313	382-391	YIHRDLRAAN	410.221	3	3.89
314-320	392-398	ILVSASL	702.437	1	6.38
321-326	399-404	VCKIAD	648.333	1	4.22
321-329	399-407	VCKIADFGL	483.257	2	6.93

Table S1 (continued)

Construct Numbering	Hck Numbering	Sequence	Observed m/z	z	Retention time (min)
330-346	408-424	ARVIEDNEYTAREGAKF	683.6565	3	5.01
330-337	408-415	ARVIEDNE	473.232	2	4.06
339-36	417-424	TAREGAKF	440.233	2	4.52
347-358	425-436	PIKWTAPEAINF	693.868	2	6.99
362-367	440-445	TIKSDV	331.687	2	4.16
362-370	440-448	TIKSDVWSF	541.776	2	7.13
377-393	455-471	IVTYGRIPYPGMSNPEV	631.657	3	6.47
377-396	455-474	IVTYGRIPYPGMSNPEVIRA	745.068	3	6.13
380-393	458-471	YGRIPYPGMSNPEV	790.379	2	6.06
380-396	458-474	YGRIPYPGMSNPEVIRA	640.663	3	5.86
397-413	475-491	LERGYRMPRPENCPEEL	697.005	3	5.51
401-409	479-487	YRMPRPENC	583.266	2	4.89
401-413	479-491	YRMPRPENCPEEL	545.257	3	5.69
421-432	499-510	WKNRPEERPTFE	530.267	3	4.75
432-438	510-516	EYIQSVL	851.45	1	6.73
433-438	511-516	YIQSVL	722.4	1	6.5

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Intact mass analysis of Hck-YEEI following incubation with ATP. (**A**) Electrospray mass spectra of recombinant Hck-YEEI before (- ATP) and after (+ ATP) incubation with ATP/Mg^{++}. Lines are shown above the spectrum to indicate the masses of the "base" protein peak as well as the phosphorylated forms (+80 Da per phosphate). The peak indicated with "base" is acetylated (+42 Da); dotted lines show the positions of the three deacetylated species. (B). Tryptic phosphopeptide mapping of Hck-YEEI. The mass spectrum of the tryptic peptide at the C-terminal tail bearing Tyr527 is shown at the top (+3 charge state). The spectrum remains the same in the presence or absence of ATP. Mass spectra of the tryptic peptides containing activation loop Tyr416 are shown in the bottom panels (+2 charge state). The unphosphorylated and phosphorylated peptides elute at different times chromatographically.

Figure S2. Map of the peptic peptides identified for Hck-YEEI and monitored during HX experiments. The peptides followed in the HX experiments cover 86% of the linear sequence. The phosphorylated peptide found only in the Hck-YEEI + ATP and + ATP/Nef samples is indicated by the red bar. Numbering in based on the position of each amino acid in the recombinant Hck-YEEI construct used for HX MS analysis (see also Table S1).

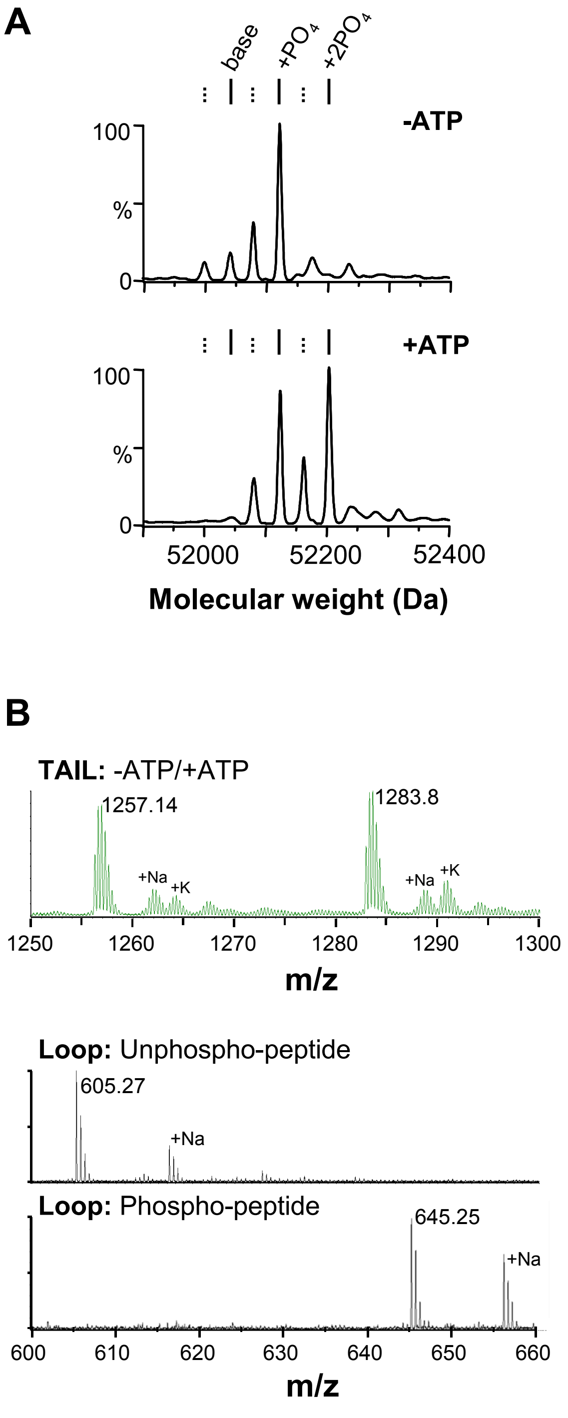
Figure S3. Example mass spectra for deuterium exchange into the peptides shown in Figure 5. In each panel, exchange into Hck-YEEI is shown at the left, into Hck-YEEI + Nef + ATP in the middle and Hck-YEEI + Nef + DFP-4AB at the right.

Figure S4. Deuterium incorporation plots comparing Hck-YEEI with both Hck-YEEI + ATP and Hck-YEEI + DFP-4AB. Amino acids included in each peptide are shown at the top and correspond to the sequence of Hck-YEEI numbered as per the X-ray crystal structure (PDB: 1QCF). Numbers in parenthesis correspond to the amino acid location in the recombinant Hck-YEEI construct used for HX MS analysis. For example, the first peptide contains the N-terminal His-tag followed by three Hck-derived amino acids. Table S1 provides the amino acid sequences of each peptide. Data are the average of two separate HX MS experiments.

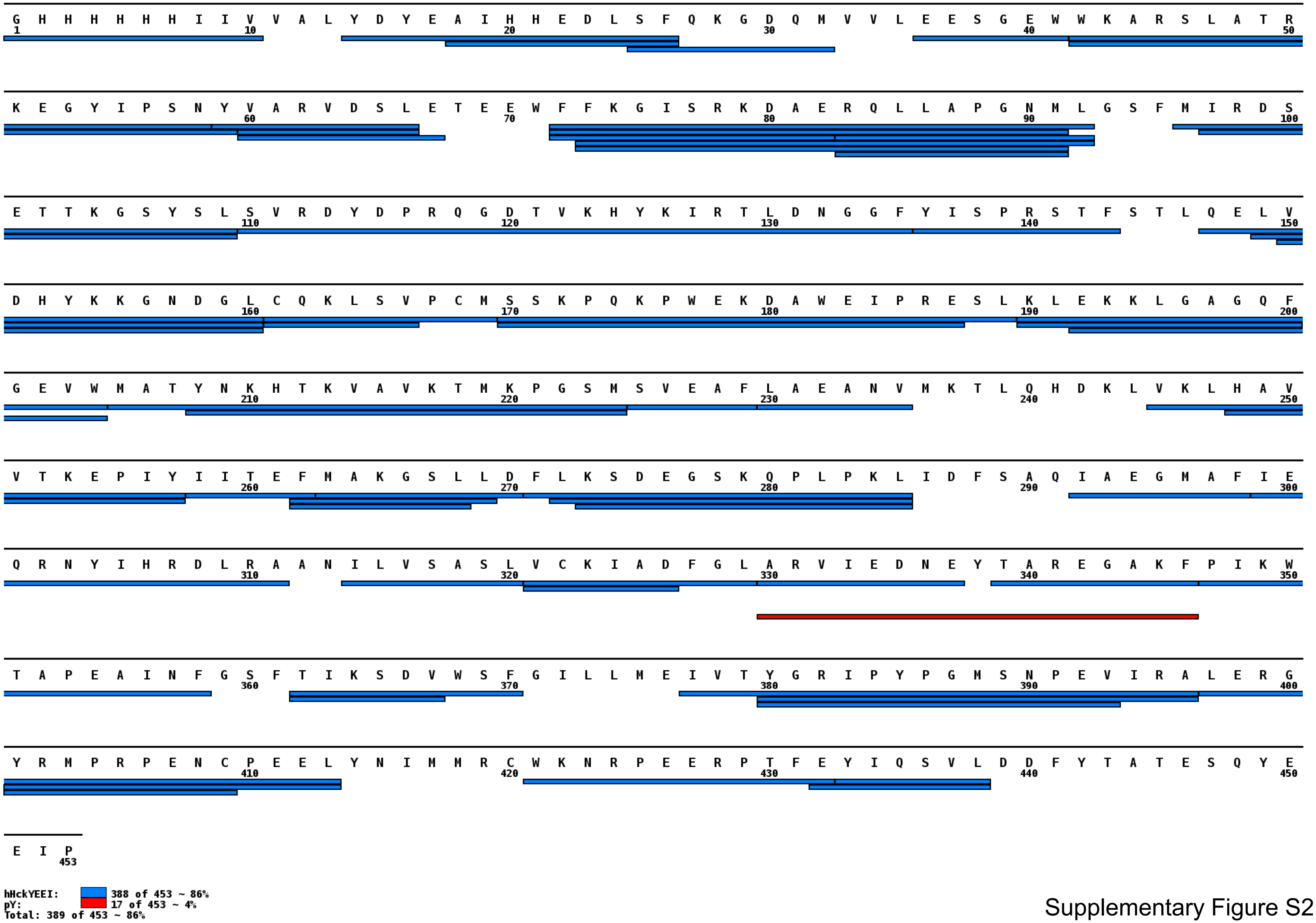
Figure S5. Changes to deuterium incorporation in Hck-YEEI in the presence of small molecule ligands. Deuterium incorporation plots for Hck-YEEI alone, in the presence of ATP/Mg^{++}, or DFP-4AB. Each graph indicates relative deuterium uptake with time (0.1-1000 min) and all axes labels are the same as those presented for peptide 286-302. Data are shown for the SH3 and SH2 reporter peptides, two peptides near the ATP binding pocket, and three peptides presented in main Figure 5. The sequence numbers that are included on the plots are color matched with their presentation on the cartoon model structure of Hck-YEEI to the right of the plots. The error of measuring the deuterium incorporation for each peptide was ± 0.2 Da and is indicated using error bars for each time point.

Figure S6. Deuterium incorporation plots comparing Hck-YEEI with both Hck-YEEI + Nef + ATP and Hck-YEEI + Nef + DFP-4AB. Amino acids included in each peptide are shown at the top and correspond to the sequence of Hck-YEEI numbered as per the X-ray crystal structure (PDB: 1QCF). Numbers in parenthesis correspond to the amino acid location in the recombinant Hck-YEEI construct used for HX MS analysis. Peptide sequences are presented in Table S1. Data are the average of two separate HX MS experiments.

Figure S7. Differences in deuteration for the peptides shown in Figure S6. The deuterium levels (D) in Figure S6 were subtracted as shown at the bottom and colored according to the legend shown. Differences of less than 0.5 Da were not considered meaningful and are shown as gray.

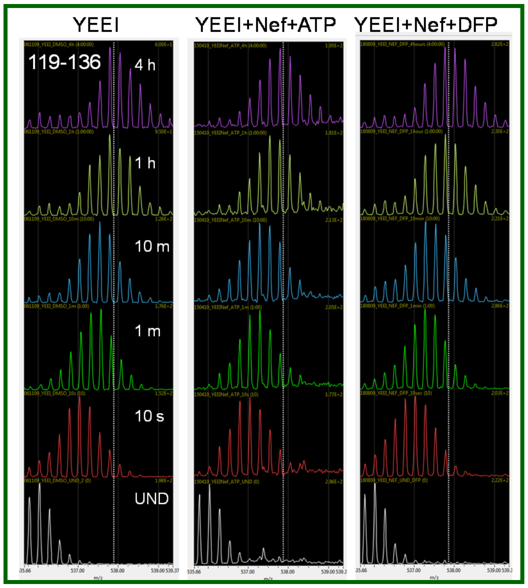


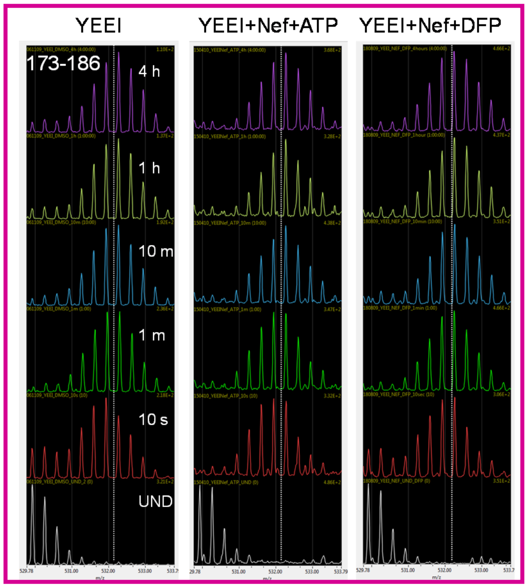
Supplementary Figure S1

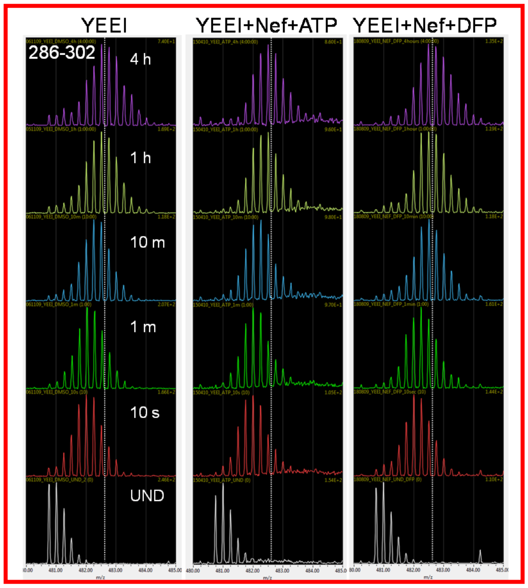


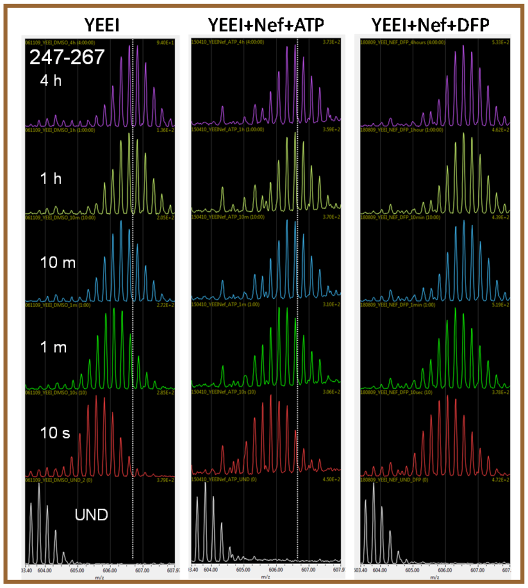
Supplementary Figure S2

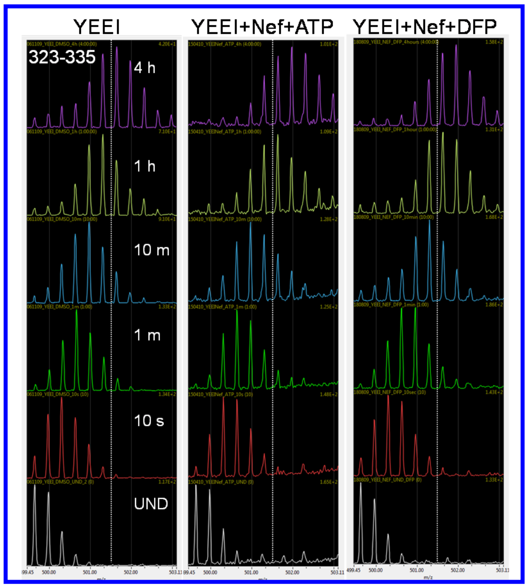
Supplementary Figure S3

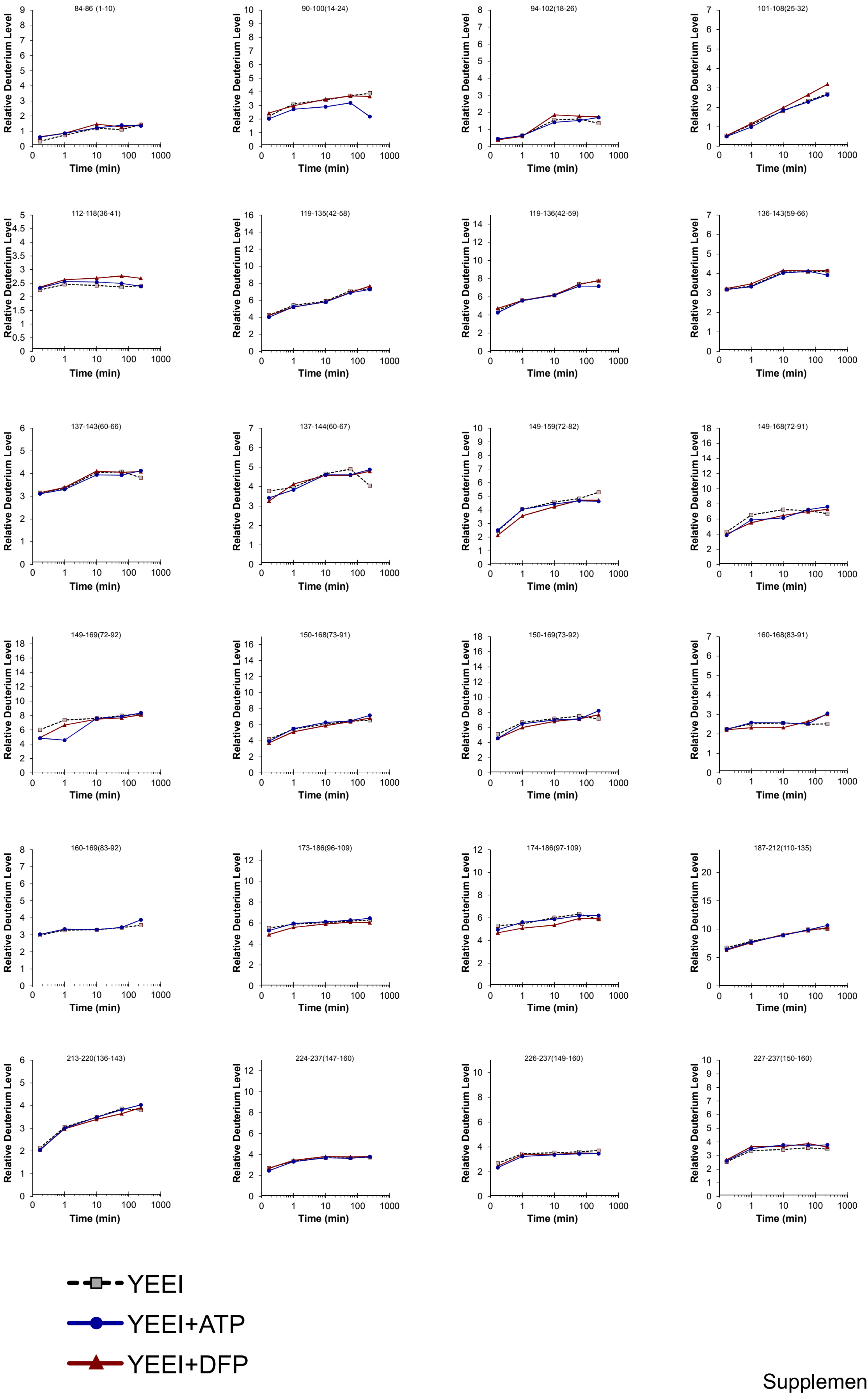




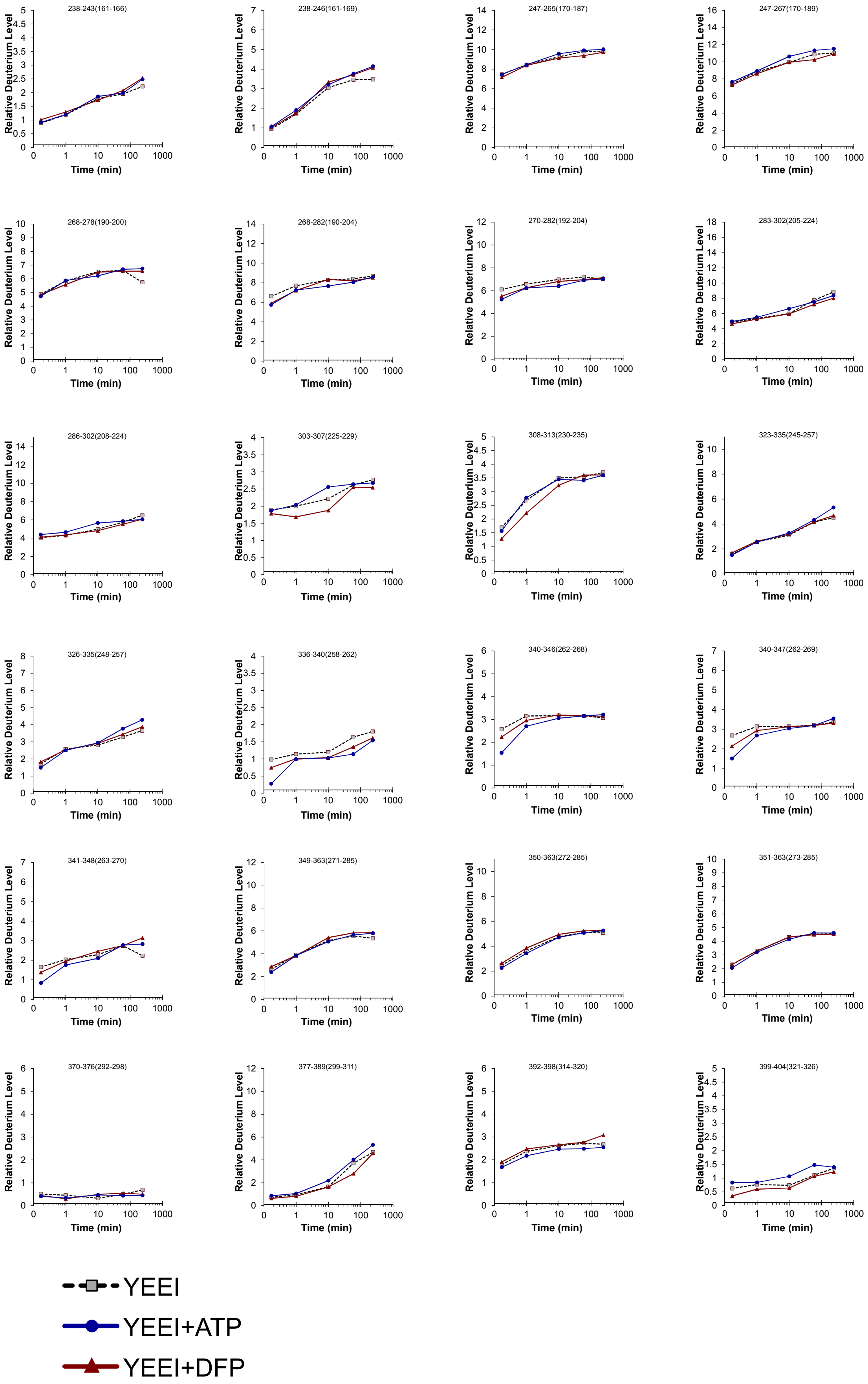




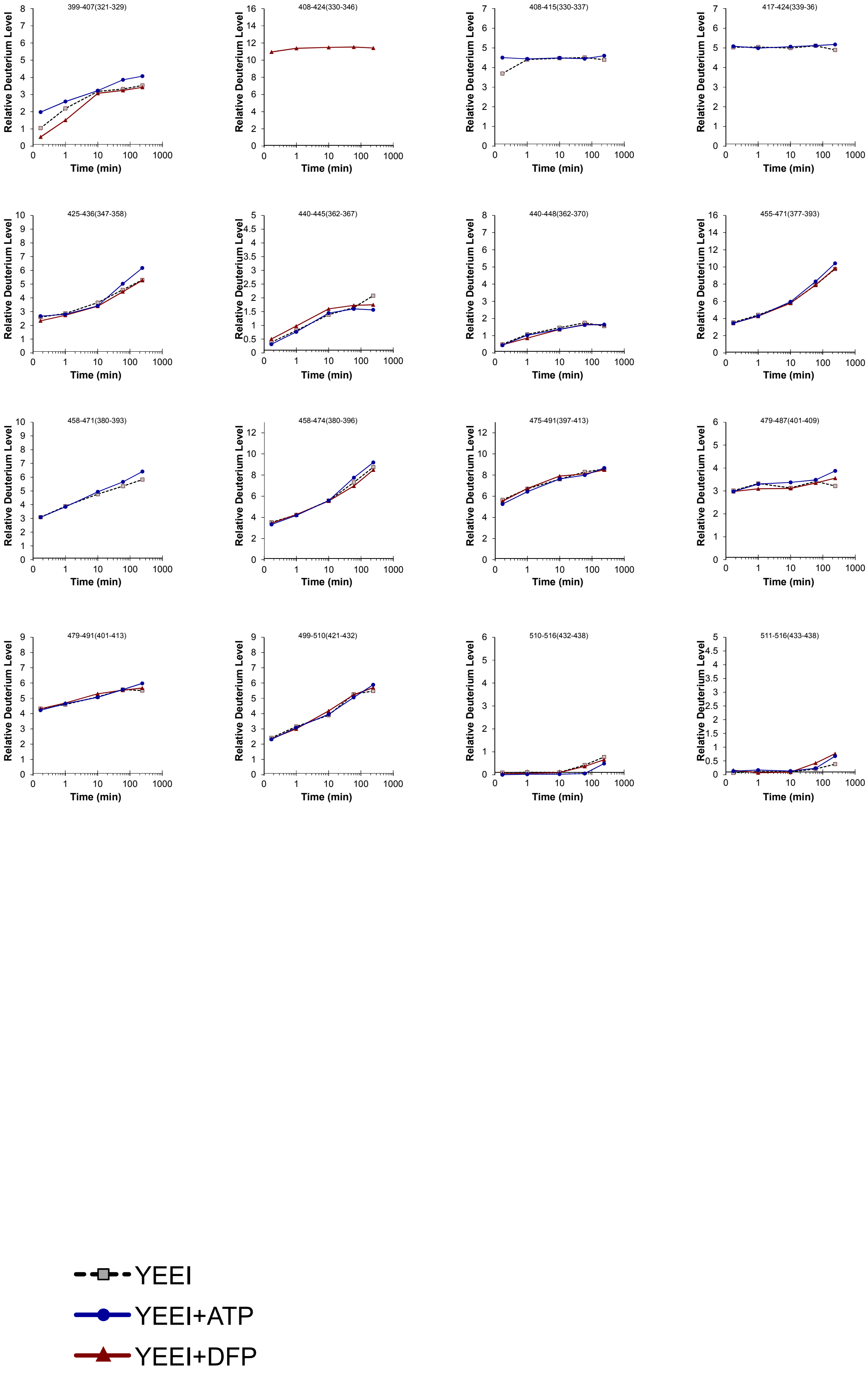




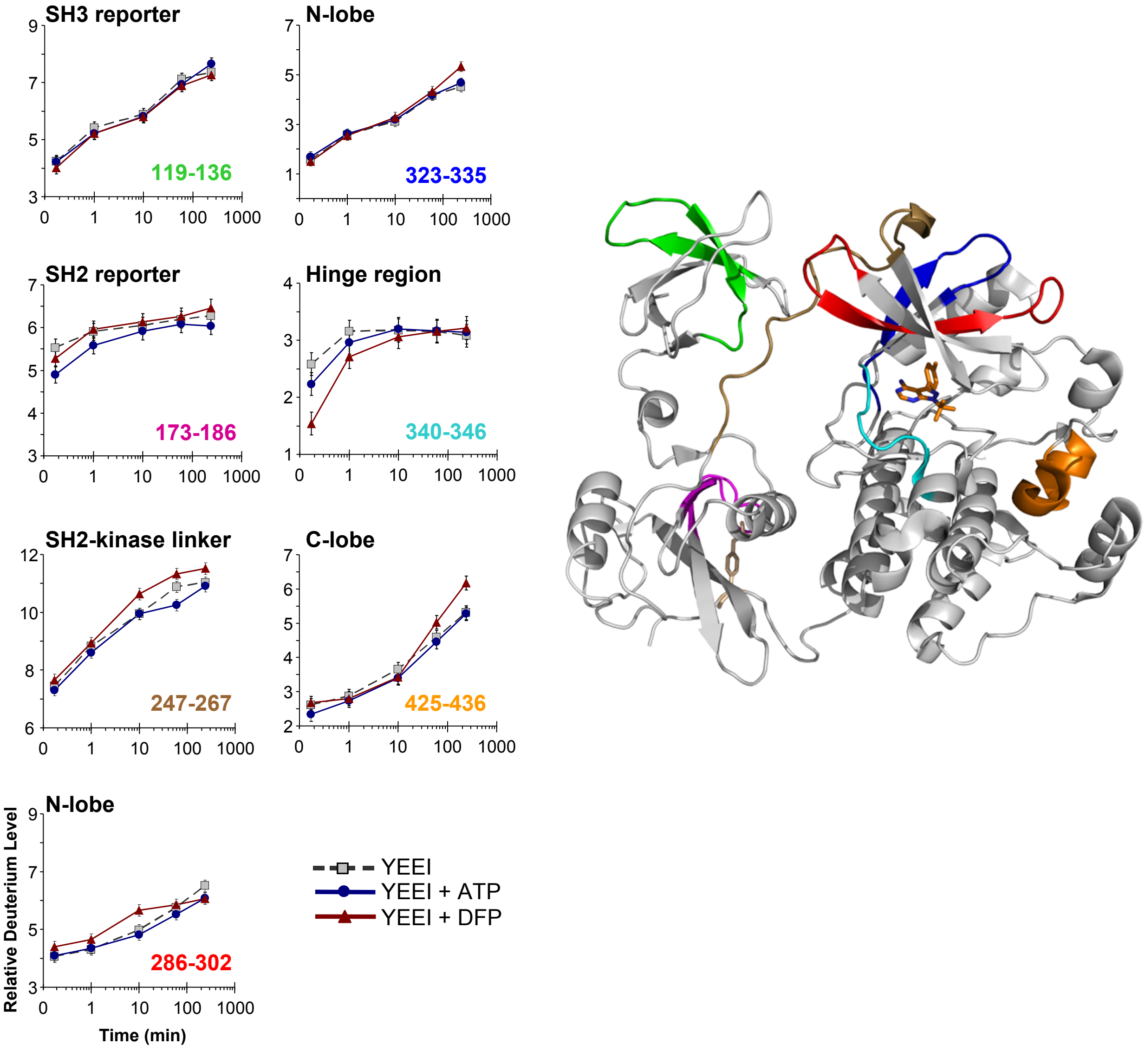
Supplementary Figure S4



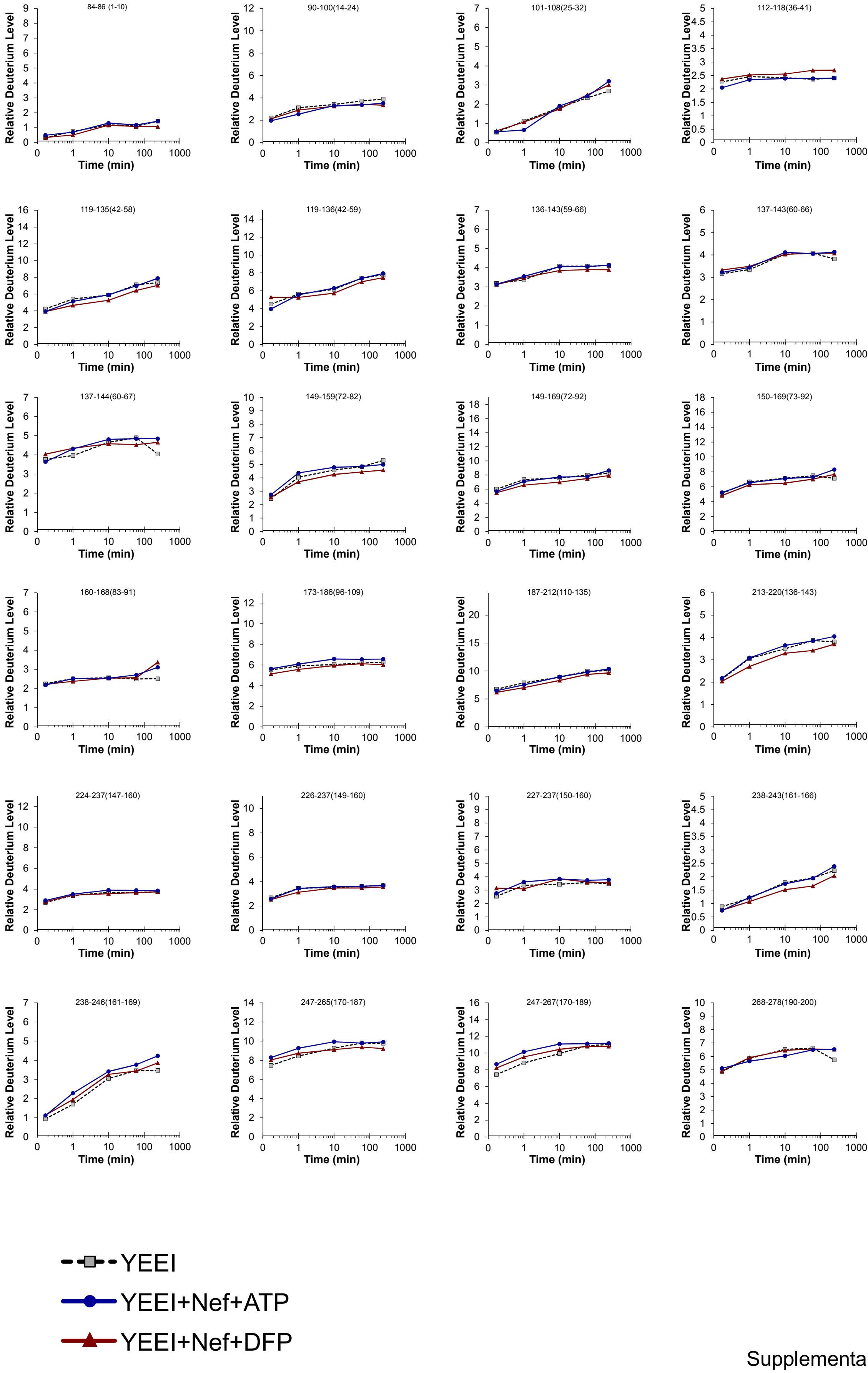
Supplementary Figure S4



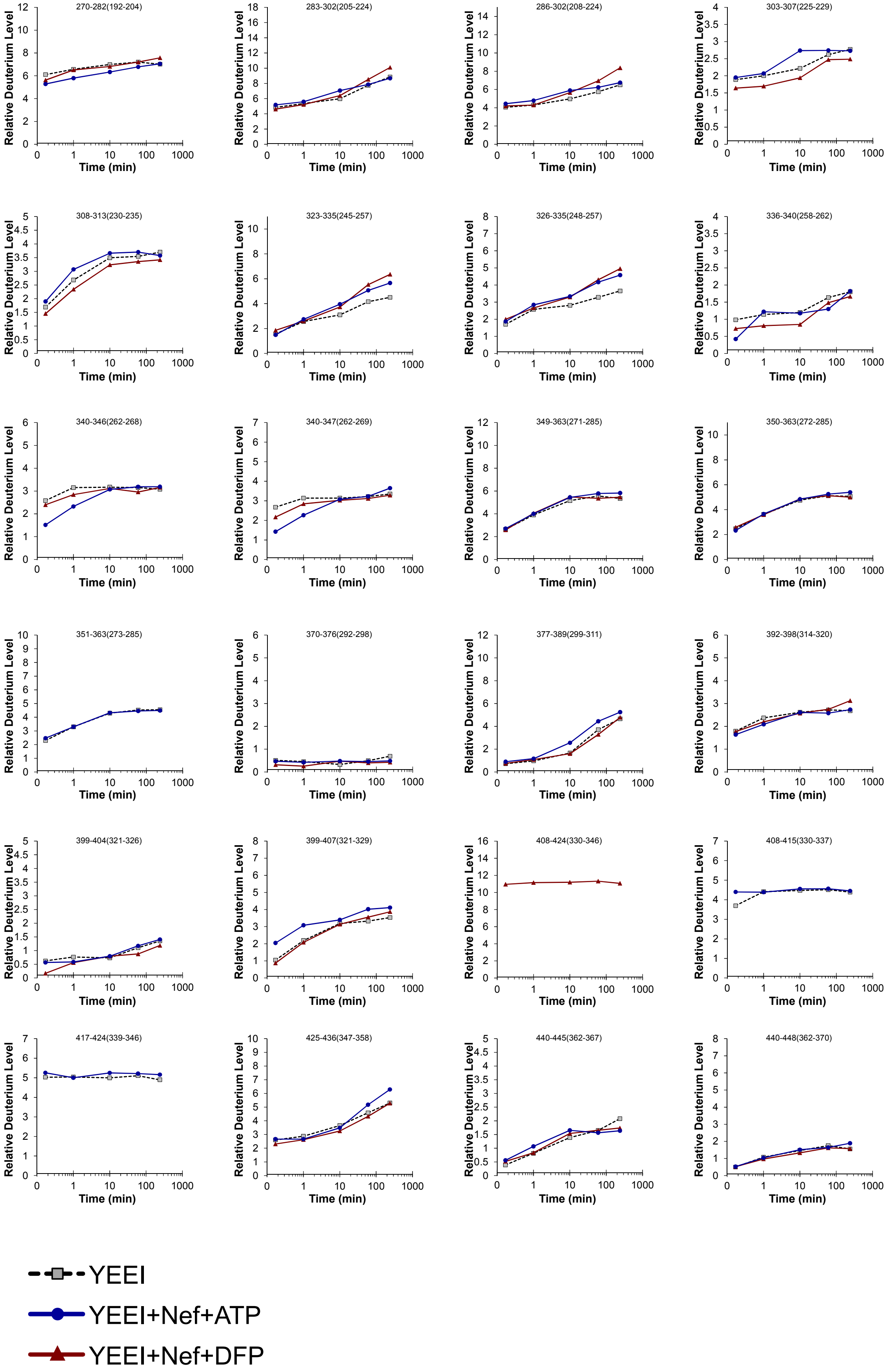
Supplementary Figure S4



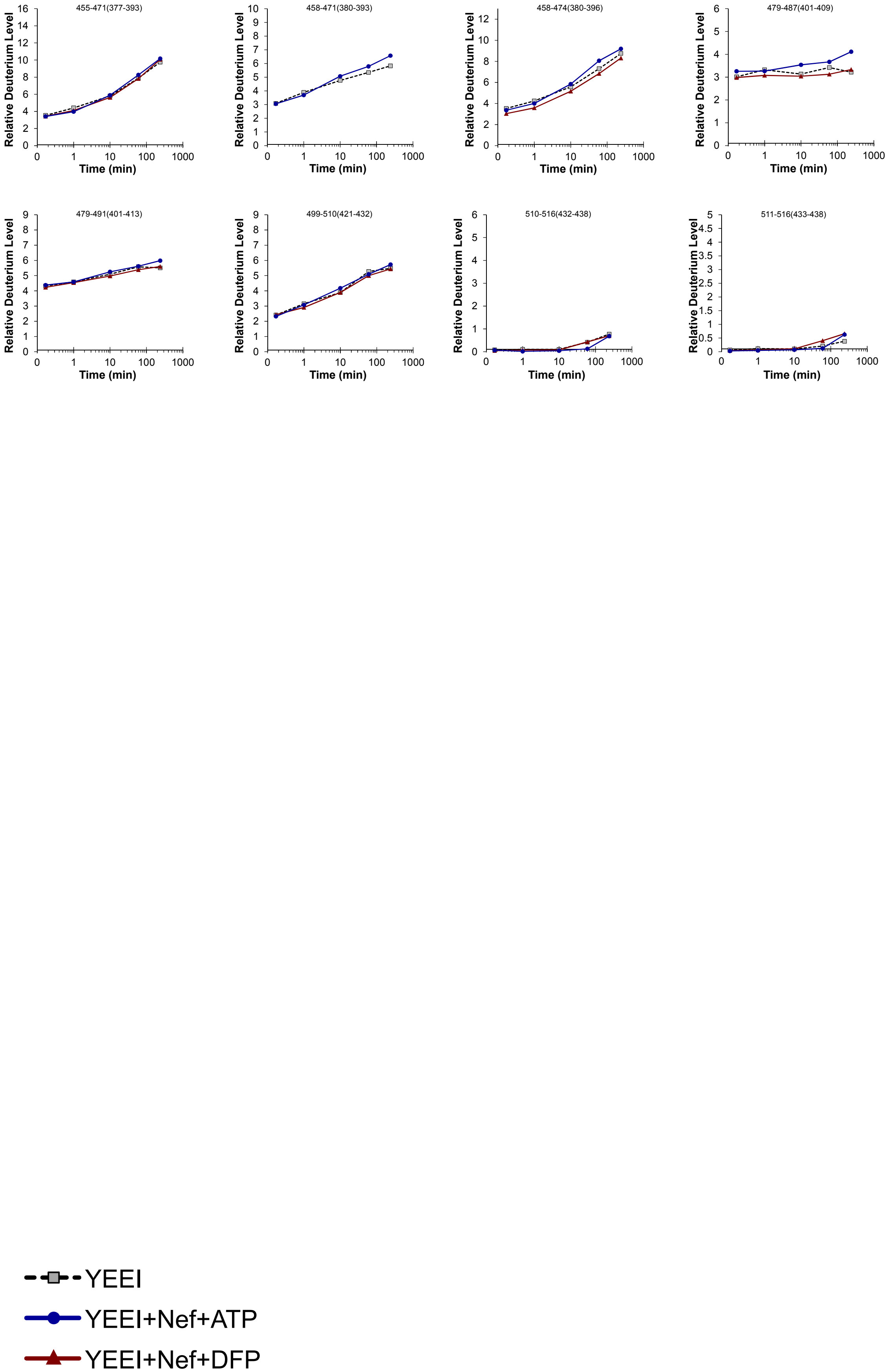
Supplementary Figure S5



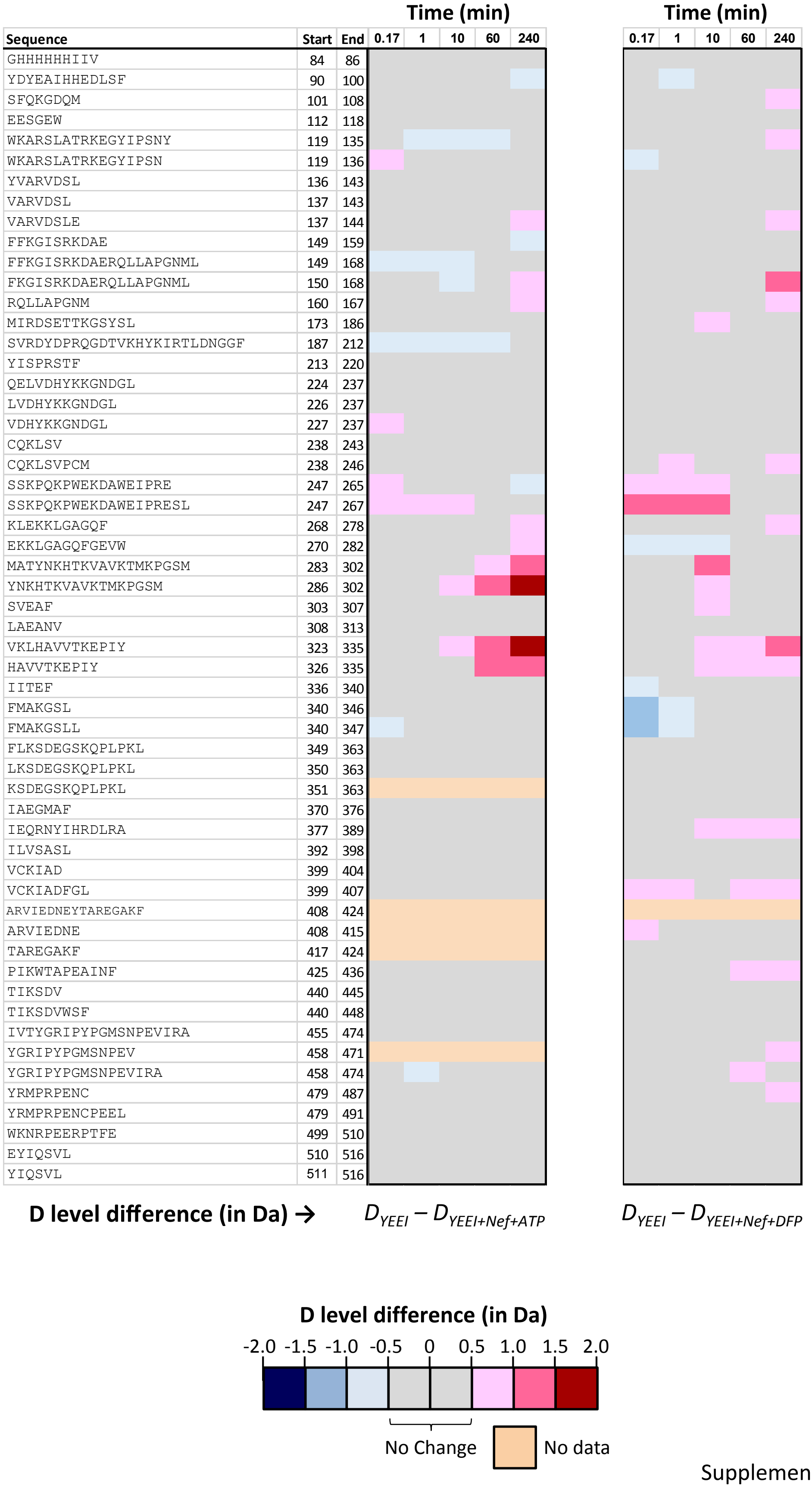
Supplementary Figure S6



Supplementary Figure S6



Supplementary Figure S6



Sequence	Start	End
GHHHHHHIIV	84	86
YDYEAIHHEDLSF	90	100
SFQKGDQM	101	108
EESGEW	112	118
WKARSLATRKEGYIPSNY	119	135
WKARSLATRKEGYIPSN	119	136
YVARVDSL	136	143
VARVDSL	137	143
VARVDSLE	137	144
FFKGISRKDAE	149	159
FFKGISRKDAERQLLAPGNML	149	168
FKGISRKDAERQLLAPGNML	150	168
RQLLAPGNM	160	167
MIRDSETTKGSYSL	173	186
SVRDYDPRQGDTVKHYKIRTLDNGGF	187	212
YISPRSTF	213	220
QELVDHYKKGNDGL	224	237
LVDHYKKGNDGL	226	237
VDHYKKGNDGL	227	237
CQKLSV	238	243
CQKLSVPCM	238	246
SSKPQKPWEKDAWEIPRE	247	265
SSKPQKPWEKDAWEIPRESL	247	267
KLEKKLGAGQF	268	278
EKKLGAGQFGEVW	270	282
MATYNKHTKVAVKTMKPGSM	283	302
YNKHTKVAVKTMKPGSM	286	302
SVEAF	303	307
LAEANV	308	313
VKLHAVVTKEPIY	323	335
HAVVTKEPIY	326	335
IITEF	336	340
FMAKGSL	340	346
FMAKGSLL	340	347
FLKSDEGSKQPLPKL	349	363
LKSDEGSKQPLPKL	350	363
KSDEGSKQPLPKL	351	363
IAEGMAF	370	376
IEQRNYIHRDLRA	377	389
ILVSASL	392	398
VCKIAD	399	404
VCKIADFGL	399	407
ARVIEDNEYTAREGAKF	408	424
ARVIEDNE	408	415
TAREGAKF	417	424
PIKWTAPEAINF	425	436
TIKSDV	440	445
TIKSDVWSF	440	448
IVTYGRIPYPGMSNPEVIRA	455	474
YGRIPYPGMSNPEV	458	471
YGRIPYPGMSNPEVIRA	458	474
YRMPRPENC	479	487
YRMPRPENCPEEL	479	491
WKNRPEERPTFE	499	510
EYIQSVL	510	516
YIQSVL	511	516

Supplementary Figure S7