

Supporting Information

Comparative analysis of cleavage specificities of immobilized porcine pepsin and Nepenthesin II under HDX conditions.

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Supporting Material Figure Legends

Figure S1. Venn diagram analysis of three LC-MSMS replicates of all eleven proteins between NepII and pepsin mediated cleavage events.

Figure S2. Fasta sequence of 11 proteins.

Figure S3 P4-P4' Frequencies. Percentage of amino acid occupation for all positions P4 to P4' with pepsin (top panels) and NepII (bottom panels) for "Unique Protein Sites" (left panels) and "All Peptides Sites" (right panels). Sum of all columns in each of the 4 tables is equal to 100%.

Figure S4. The frequency at which an amino acid was found in the entire peptide was subtracted from the frequency at which it was found in the positions P4 to P4' in order to highlight amino acids that are enriched (positive numbers; red) or depleted (negative numbers; blue) at specific positions. Values near 0 suggest amino acids that appear at a position no more often than are present in the entire peptide. Data presented as in Supplementary Figure 4, pepsin (top panels) and NepII (bottom panels) for "Unique Protein Sites" (left panels) and "All Peptides Sites" (right panels).

Figure S5. Background subtracted profiles (y-axis) of amino acids (top) at different positions (P4 to P4') x-axis were grouped per protein (right) and enzyme (NepII blue, Pepsin red). Kolmogorov Smirnov Asymptotic test followed by FDR (5%) to identify amino acids that were cleaved with different rates across pepsin and NepII at each position. Differentially cleaved amino acid-position combinations are highlighted with yellow graph background.

Figure S6. Sequence coverage maps of proteins tested. The analysis for each protein with both NepII and pepsin was conducted in triplicate and merged. Unique peptides were preserved and rendered using MSTools (1) so as to visualize maximum coverage with minimal convolution. Some of these maps are necessarily large due to protein sequence length and readers are encouraged to zoom in on regions of interest. Pepsin cleaved peptides are blue and NepII cleaved peptides are red.

(1) Kavan, D. and Man, P. "MSTools - Web based application for visualization and presentation of HXMS data" Int. J. Mass Spectrom. 2011, 302: 53-58.
<http://dx.doi.org/10.1016/j.ijms.2010.07.030>.

Figure S7. Background subtracted prevalence of amino acids present at the P1 site depending on charge state. Three different subsets from the same dataset were selected, those peptides with charge state(s) of: +1, +2 through +7, and +1 through +7. The frequency of different amino acids at the P1 site was remarkably similar for unique peptides (A) and all peptides (B) in the three different subsets regardless of digestion enzyme.

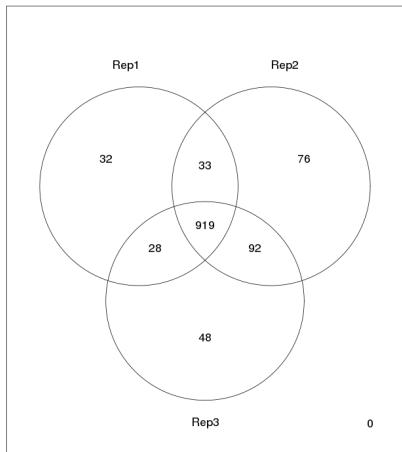
Figure S8. Sequence coverage maps for PPAR γ with +1, +2 through +7, and +1 through +7 charge states. unique peptides. Triplicate datasets were merged and unique peptides were preserved. Though there are many peptides in the +1 map, the visual similarity of the +2 through +7 and +1 through +7 maps highlights how the number of unique peptides is not altered significantly by adding +1 charge peptides.

	+1 to +7	+2 to +7	+1 to +7	+2 to +7
	Pepsin		Nep II	
Total peptides	16,910	15,321	27,124	24,347
Total unique peptides	1,962	1,725	2,993	2,522
Avg. length of unique peptides	16.07	17.28	14.71	16.07

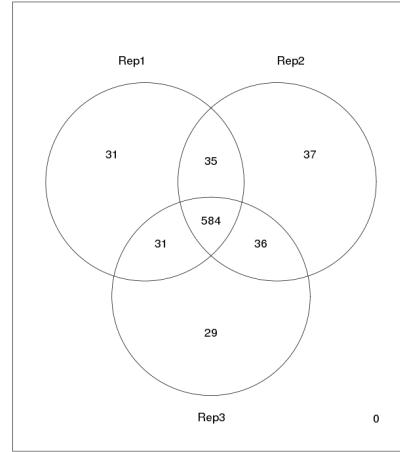
Table S1. Summary statistics of peptides cleaved by pepsin or nep II with respect to charge state.

PPAR γ

NepII

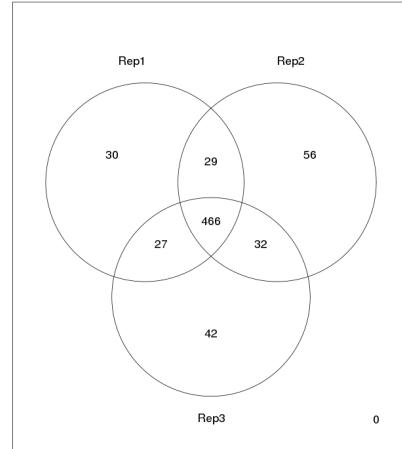


Pepsin

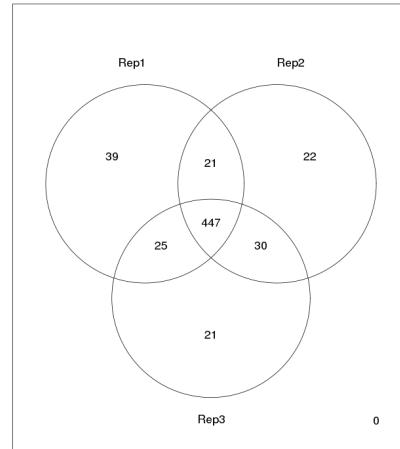


RXR α

NepII

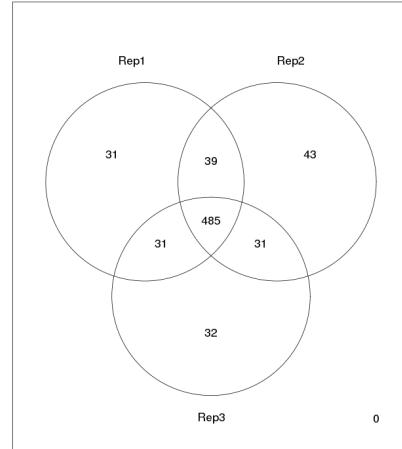


Pepsin

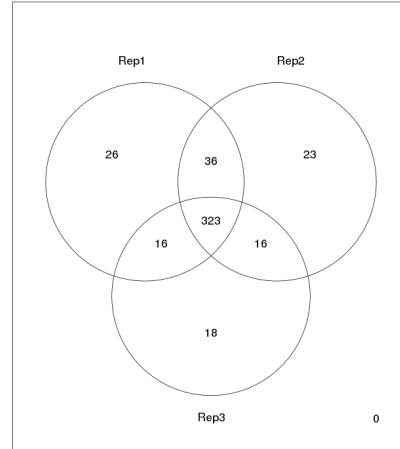


RXR β

NepII

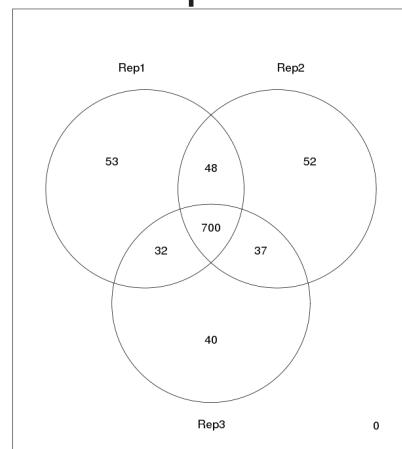


Pepsin



RXR γ

NepII



Pepsin

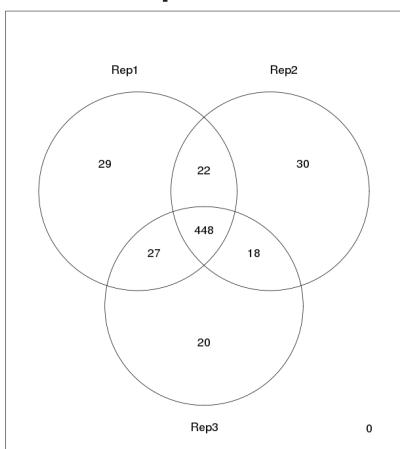
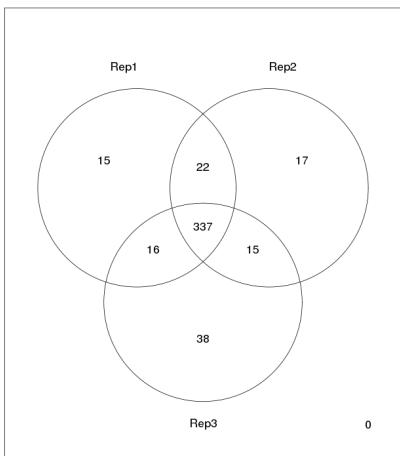


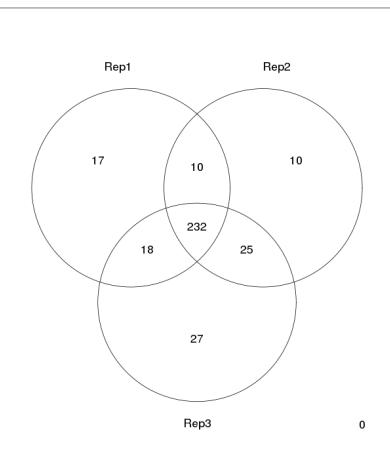
Figure S1

VDR

NepII

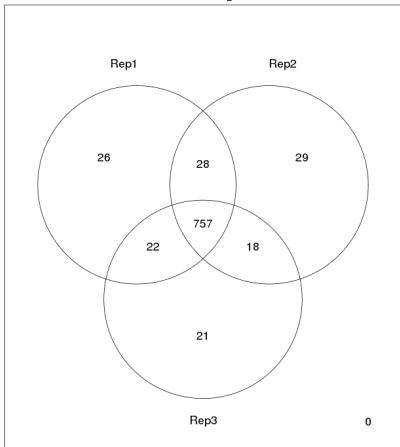


Pepsin

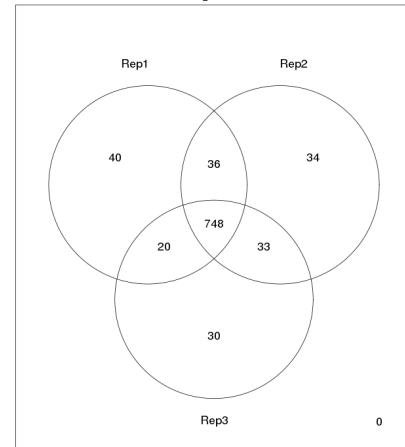


RIG-I
HEL-CTD

NepII

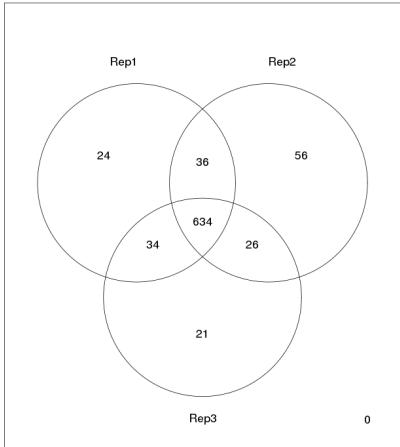


Pepsin

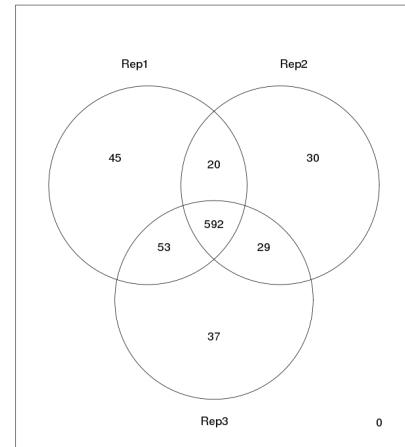


NSP2

NepII

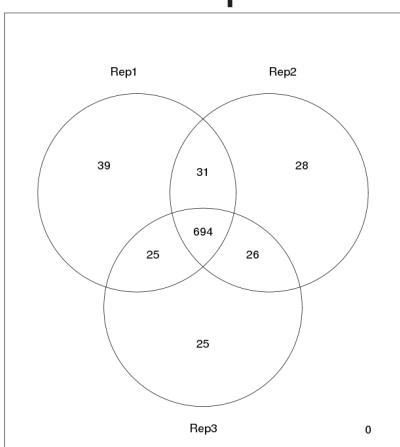


Pepsin



ABHD5

NepII



Pepsin

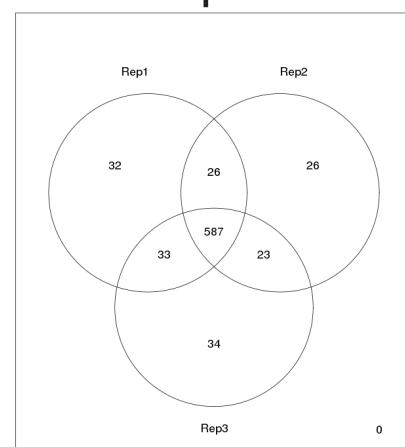
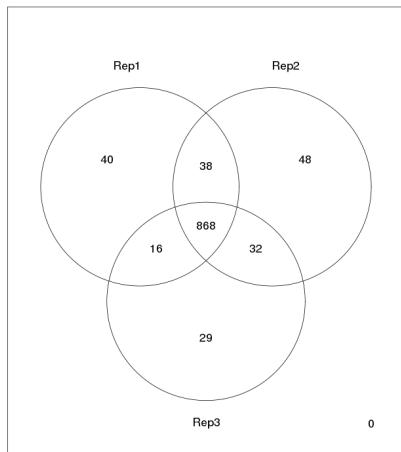


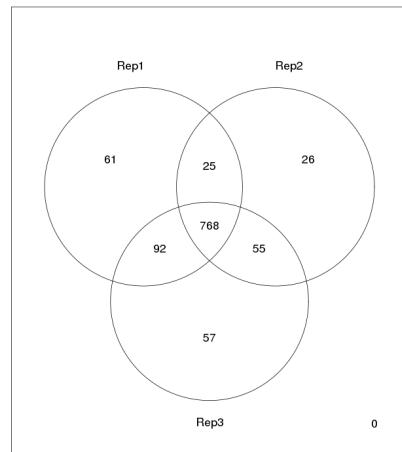
Figure S1

DRH3

NepII

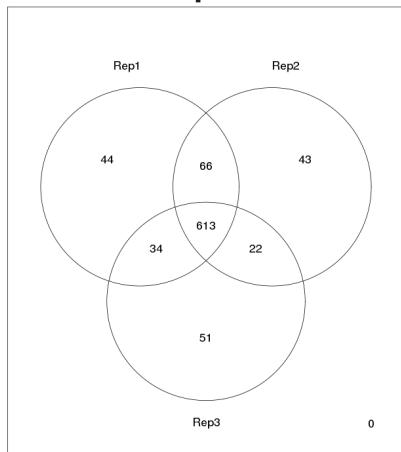


Pepsin



Talin

NepII



Pepsin

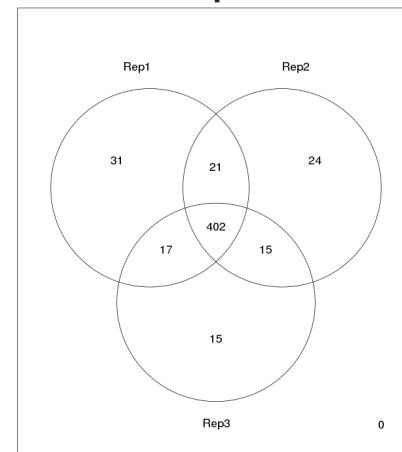


Figure S1

>VDR

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TPLVLEVFGNEIS

>RC2

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>PPAR_g

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PLLQEIYKDLY

>RXRa

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PIDTFLMEMLEAPHQMT

>RXR_g

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>RXRb

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>RORg

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>ABHD5

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>NSP2FL

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>Talin

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>DRH3

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RQHNKQLEMKRFHIQQEPPTKGVAPEAQ

Figure S2

	Unique pep des es in triple overlap - pepsin									All pep des es in triple overlap - pepsin							
	P4	P3	P2	P1	P1'	P2'	P3'	P4'		P4	P3	P2	P1	P1'	P2'	P3'	P4'
A	6.48	7.32	6.58	8.7	7.72	9.1	5.69	5.79	A	5.97	9.31	6.32	7.58	7.77	11.26	5.77	4.83
R	4.75	0.49	5	0.49	4.75	6.33	5.34	5.74	R	5.08	0.09	4.72	0.09	4.51	7.66	7.07	4.95
N	4.2	3.07	4.45	2.42	3.41	2.67	3.26	4.1	N	3.99	3.29	5.2	1.26	2.54	2.29	1.89	3.62
D	6.33	4.85	5.69	6.08	4.4	4.6	5.79	5.14	D	6.91	3.36	6.44	4.49	4.22	3.45	5.66	5.59
C	1.88	2.92	2.87	3.07	2.03	2.77	2.37	2.52	C	1.15	2.79	2.93	2.65	1.5	2.4	1.38	3.21
Q	4.3	3.61	5.24	4.45	2.82	4.01	4.25	3.91	Q	4.43	2.49	6.15	3.1	2.57	3.03	3.51	3.76
E	7.27	7.37	8.95	11.03	5.98	7.96	7.17	7.17	E	6	5.77	8.74	10.48	4.26	8.67	8.57	6.37
G	4.95	6.63	4.06	4.15	2.42	4.65	4.5	6.78	G	4.82	7.61	4.38	2.18	1.72	4.87	3.11	9.56
H	3.07	0.89	2.13	0.54	2.52	1.29	2.32	3.02	H	3.33	0.54	1.94	0.18	1.99	0.49	2.13	2.69
I	6.33	7.96	6.78	2.42	8.16	6.68	7.32	4.95	I	5.36	10.22	9.52	0.57	10.19	7.77	8.17	5.21
L	8.7	14.14	9.64	21.61	12.46	8.26	12.22	10.14	L	7.89	14.98	7.46	31.51	15.06	6.24	13.41	9.79
K	7.12	2.57	6.23	0.3	5.39	7.91	8.9	7.32	K	8.22	1.62	6.98	0.07	3.92	8.18	9.36	6.91
M	2.27	3.07	2.57	5.14	3.76	2.52	3.56	2.77	M	2.2	2.92	1.99	6.29	2.73	1.73	2.89	2.03
F	4.2	2.77	3.66	8.85	6.97	2.92	3.26	3.36	F	4.09	2.08	3.07	13.02	8.88	1.85	3.18	3.2
P	5.89	6.38	0.59	0.45	3.46	2.32	2.67	6.58	P	7.92	6.74	0.12	0.06	1.94	0.69	1.97	8.36
S	5.39	9.1	8.36	4.75	6.38	7.37	6.82	6.48	S	4.83	9.11	9.72	2.88	4.43	9.08	6.16	6.06
T	5.04	5.89	5.64	5.24	4.15	5.54	5.29	5	T	5.38	6.74	4.65	5.84	3.74	5.48	6.26	5.78
W	0.94	0.64	0.64	1.24	1.19	0.35	0.69	1.04	W	1.3	0.4	0.34	1.04	1.47	0.16	1.08	1.43
Y	4.8	2.67	2.47	5	4.95	3.17	3.31	2.77	Y	5.27	2.5	1.25	4.1	7.27	2.73	2.72	2.02
V	6.08	7.67	8.46	4.06	7.07	9.59	5.24	5.44	V	5.86	7.44	8.09	2.6	9.28	11.98	5.71	4.65
	Unique pep des es in triple overlap - nepII									All pep des es in triple overlap - nepII							
	P4	P3	P2	P1	P1'	P2'	P3'	P4'		P4	P3	P2	P1	P1'	P2'	P3'	P4'
A	4.74	5.97	6.53	7.57	7.93	9.52	6.81	5.06	A	3.88	7.22	5.24	5.74	7.63	13.95	7.25	4.68
R	5.38	5.66	5.66	5.66	4.1	4.5	6.13	5.62	R	4.05	6.04	4.96	6.92	4.74	4.74	7.21	4.25
N	3.9	3.82	3.31	3.7	4.82	3.11	3.58	4.54	N	3.53	4.26	2.09	2.76	4.43	2.76	2.79	3.69
D	5.3	4.34	5.58	7.25	5.89	3.31	6.37	5.62	D	7.35	4.48	4.52	6.2	6.31	1.78	6.12	5.73
C	2.23	2.99	2.95	3.07	2.79	2.99	2.59	2.31	C	1.89	1.82	3.7	2.79	3.08	2.41	1.92	1.94
Q	4.18	4.42	5.26	3.03	3.07	4.46	4.78	4.18	Q	3.19	4.3	7.09	2.61	1.87	4.58	4.91	3.79
E	6.49	7.37	9.2	6.97	5.93	8.2	7.61	7.37	E	5.55	6.99	9.91	5.45	5.08	8.01	6.95	6.67
G	5.69	5.42	3.98	2.55	2.99	5.93	4.9	6.25	G	5.1	7.04	3.52	1.34	1.45	9.03	5.63	7.63
H	2.87	3.19	1.31	1.83	1.55	2.59	3.11	3.35	H	3.35	4.09	0.52	0.79	0.8	1.94	3.09	3.91
I	7.09	5.26	9.32	1.43	7.73	4.06	5.89	5.5	I	6.96	3.65	15.82	0.4	10.04	3.33	6.55	5.15
L	11.11	11.63	10.27	19.28	11.07	8.28	9.28	10.55	L	12.8	12.57	9.09	30.92	12.65	6.91	10.12	11.19
K	5.46	7.73	8.24	12.11	3.82	7.49	7.93	7.01	K	4.82	8.61	6.41	14.81	1.91	6.4	8.52	5.58
M	3.19	3.07	3.27	4.5	3.5	2.51	2.67	3.03	M	3.1	2.23	2.69	3.85	3.47	1.88	1.73	3.96
F	5.06	4.46	1.99	5.81	6.57	4.66	3.35	3.54	F	5.77	4.4	1.18	5.07	8.93	4.19	2.41	2.9
P	4.58	3.98	0.52	0.2	3.58	2.71	3.27	6.09	P	5.82	3.23	0.19	0.02	2.16	1.46	2.61	8.32
S	5.69	5.77	7.33	2.51	6.53	8.44	7.01	6.01	S	5.3	6.87	6.48	1.03	6.23	9.74	7.53	5.62
T	5.66	3.58	5.1	3.27	5.22	5.18	5.18	4.58	T	5.99	2.89	5.31	1.83	5.75	4.84	5.23	4.96
W	1.12	1.08	0.44	0.56	1	1.15	1	0.92	W	1.07	1.15	0.37	0.3	0.6	1.02	0.93	0.69
Y	4.34	4.3	1.23	5.58	4.9	3.5	2.83	2.87	Y	4.72	3.88	0.57	5.65	4.8	3.31	2.13	2.62
V	5.93	5.97	8.52	3.15	7.01	7.41	5.73	5.62	V	5.74	4.27	10.35	1.52	8.08	7.73	6.38	6.71

Figure S3

	Unique pep oxes in triple overlap - pepsin										All pep oxes in triple overlap - pepsin								
	P4	P3	P2	P1	P1'	P2'	P3'	P4'		P4	P3	P2	P1	P1'	P2'	P3'	P4'		
A	0.38	1.22	0.48	2.61	1.62	3	-0.41	-0.31		A	-0.03	3.3	0.31	1.57	1.76	5.25	-0.23	-1.18	
R	0.74	-3.51	0.99	-3.51	0.74	2.33	1.34	1.73		R	1.27	-3.72	0.92	-3.71	0.71	3.85	3.26	1.14	
N	-0.46	-1.59	-0.21	-2.24	-1.25	-1.99	-1.4	-0.56		N	-0.65	-1.34	0.56	-3.37	-2.1	-2.35	-2.74	-1.02	
D	0.96	-0.52	0.32	0.71	-0.97	-0.77	0.42	-0.23		D	1.82	-1.73	1.35	-0.6	-0.87	-1.64	0.57	0.5	
C	0.22	1.26	1.21	1.4	0.37	1.11	0.71	0.86		C	-0.27	1.37	1.52	1.24	0.09	0.99	-0.03	1.8	
Q	0.37	-0.32	1.31	0.52	-1.11	0.07	0.32	-0.02		Q	0.58	-1.36	2.3	-0.75	-1.28	-0.82	-0.34	-0.09	
E	1.08	1.18	2.76	4.84	-0.21	1.77	0.98	0.98		E	0.15	-0.07	2.9	4.64	-1.59	2.83	2.72	0.52	
G	-2.06	-0.38	-2.95	-2.85	-4.58	-2.35	-2.5	-0.23		G	-2.23	0.56	-2.68	-4.88	-5.34	-2.19	-3.94	2.5	
H	-0.12	-2.29	-1.06	-2.64	-0.66	-1.9	-0.86	-0.17		H	0.25	-2.54	-1.14	-2.91	-1.09	-2.6	-0.96	-0.39	
I	0.59	2.22	1.04	-3.32	2.42	0.94	1.58	-0.79		I	-0.86	4	3.3	-5.65	3.97	1.55	1.95	-1.01	
L	-0.85	4.59	0.09	12.06	2.91	-1.3	2.66	0.58		L	-1.92	5.17	-2.36	21.7	5.25	-3.58	3.6	-0.02	
K	-0.17	-4.72	-1.07	-7	-1.91	0.62	1.61	0.02		K	1.28	-5.31	0.05	-6.86	-3.01	1.25	2.43	-0.02	
M	0.04	0.83	0.33	2.91	1.52	0.28	1.32	0.53		M	-0.07	0.65	-0.28	4.02	0.46	-0.54	0.62	-0.24	
F	0.58	-0.86	0.03	5.22	3.35	-0.71	-0.36	-0.27		F	0.32	-1.69	-0.69	9.25	5.11	-1.91	-0.59	-0.56	
P	-0.75	-0.25	-6.04	-6.19	-3.17	-4.31	-3.96	-0.05		P	0.68	-0.49	-7.12	-7.17	-5.29	-6.54	-5.26	1.12	
S	-2.44	1.26	0.52	-3.09	-1.46	-0.47	-1.01	-1.36		S	-3.38	0.89	1.51	-5.34	-3.78	0.87	-2.05	-2.16	
T	-0.19	0.65	0.4	0	-1.08	0.3	0.05	-0.24		T	0.05	1.4	-0.69	0.51	-1.6	0.14	0.92	0.44	
W	-0.12	-0.42	-0.42	0.17	0.12	-0.72	-0.37	-0.02		W	0.24	-0.67	-0.73	-0.02	0.41	-0.9	0.02	0.37	
Y	1.92	-0.21	-0.41	2.11	2.06	0.28	0.43	-0.11		Y	2.62	-0.15	-1.4	1.45	4.63	0.08	0.07	-0.63	
V	0.3	1.88	2.67	-1.73	1.28	3.81	-0.55	-0.35		V	0.13	1.72	2.36	-3.12	3.55	6.25	-0.02	-1.08	
	Unique pep oxes in triple overlap - nepII										All pep oxes in triple overlap - nepII								
	P4	P3	P2	P1	P1'	P2'	P3'	P4'		P4	P3	P2	P1	P1'	P2'	P3'	P4'		
A	-1.58	-0.34	0.22	1.25	1.61	3.2	0.49	-1.26		A	-2.65	0.68	-1.3	-0.8	1.09	7.41	0.72	-1.86	
R	0.94	1.22	1.22	1.22	-0.34	0.06	1.69	1.18		R	-0.19	1.8	0.72	2.68	0.5	0.5	2.96	0.01	
N	-0.31	-0.39	-0.91	-0.51	0.61	-1.11	-0.63	0.33		N	-0.57	0.16	-2.02	-1.35	0.32	-1.35	-1.32	-0.41	
D	-0.01	-0.97	0.27	1.94	0.59	-2	1.06	0.31		D	2.22	-0.65	-0.62	1.07	1.17	-3.35	0.99	0.6	
C	0.64	1.39	1.35	1.47	1.2	1.39	1	0.72		C	0.49	0.42	2.3	1.39	1.68	1.01	0.51	0.54	
Q	-0.01	0.23	1.06	-1.17	-1.13	0.27	0.58	-0.01		Q	-0.89	0.22	3.01	-1.47	-2.21	0.5	0.83	-0.29	
E	0.16	1.04	2.87	0.64	-0.39	1.88	1.28	1.04		E	-0.37	1.07	3.99	-0.47	-0.84	2.09	1.03	0.75	
G	-1.21	-1.48	-2.92	-4.35	-3.91	-0.97	-2	-0.65		G	-2.19	-0.25	-3.78	-5.96	-5.84	1.73	-1.66	0.33	
H	-0.23	0.08	-1.79	-1.27	-1.55	-0.51	0.01	0.24		H	0.28	1.02	-2.55	-2.28	-2.28	-1.13	0.01	0.83	
I	1.18	-0.65	3.41	-4.47	1.82	-1.84	-0.01	-0.41		I	0.71	-2.6	9.57	-5.85	3.79	-2.92	0.31	-1.1	
L	1.39	1.91	0.56	9.56	1.35	-1.44	-0.44	0.83		L	2.85	2.63	-0.85	20.98	2.7	-3.03	0.17	1.24	
K	-2	0.27	0.79	4.65	-3.63	0.03	0.47	-0.44		K	-2.09	1.7	-0.51	7.9	-5	-0.51	1.61	-1.33	
M	0.82	0.7	0.9	2.14	1.14	0.15	0.31	0.66		M	0.91	0.03	0.5	1.65	1.28	-0.32	-0.46	1.77	
F	1.85	1.26	-1.21	2.61	3.37	1.45	0.14	0.34		F	2.58	1.21	-2.01	1.87	5.74	0.99	-0.78	-0.29	
P	-2.05	-2.64	-6.11	-6.43	-3.04	-3.92	-3.36	-0.53		P	-1.51	-4.1	-7.14	-7.3	-5.17	-5.87	-4.72	0.99	
S	-2.06	-1.98	-0.43	-5.25	-1.23	0.69	-0.75	-1.74		S	-2.87	-1.3	-1.68	-7.14	-1.94	1.57	-0.64	-2.54	
T	0.59	-1.48	0.03	-1.8	0.15	0.11	0.11	-0.49		T	1.08	-2.02	0.4	-3.07	0.85	-0.06	0.32	0.05	
W	0.4	0.36	-0.28	-0.16	0.28	0.44	0.28	0.2		W	0.38	0.46	-0.32	-0.39	-0.09	0.34	0.24	0	
Y	1.64	1.6	-1.46	2.88	2.2	0.81	0.13	0.17		Y	2.2	1.36	-1.95	3.13	2.27	0.79	-0.39	0.1	
V	-0.17	-0.13	2.42	-2.95	0.91	1.31	-0.37	-0.48		V	-0.38	-1.85	4.23	-4.6	1.96	1.6	0.26	0.59	

Figure S4

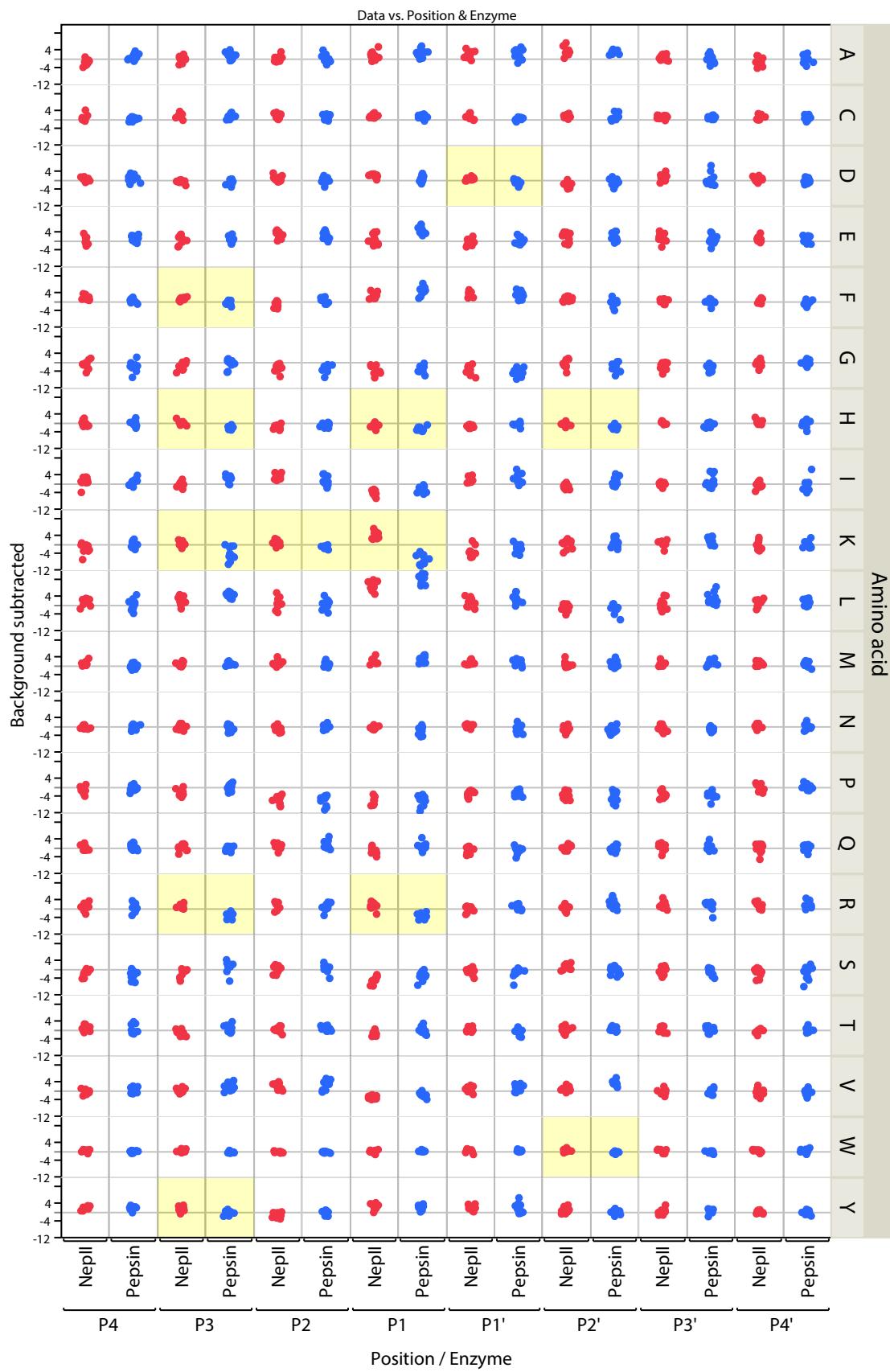
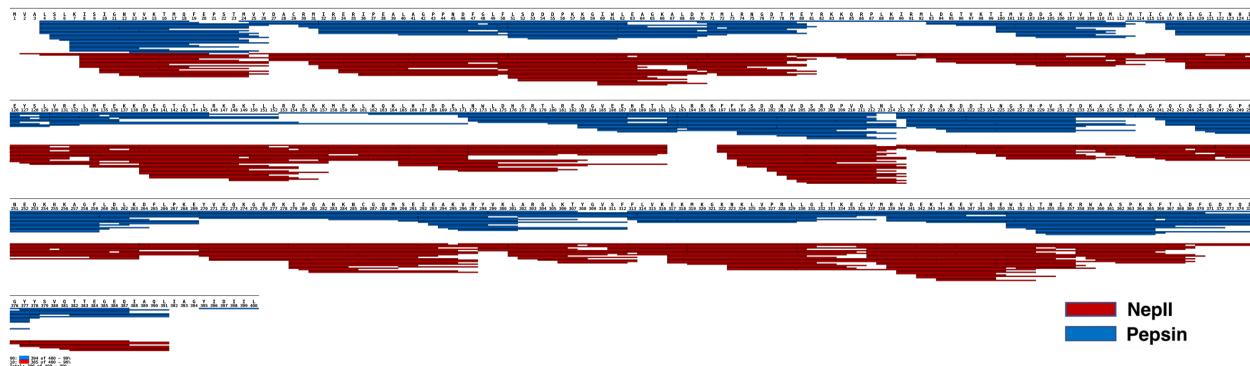
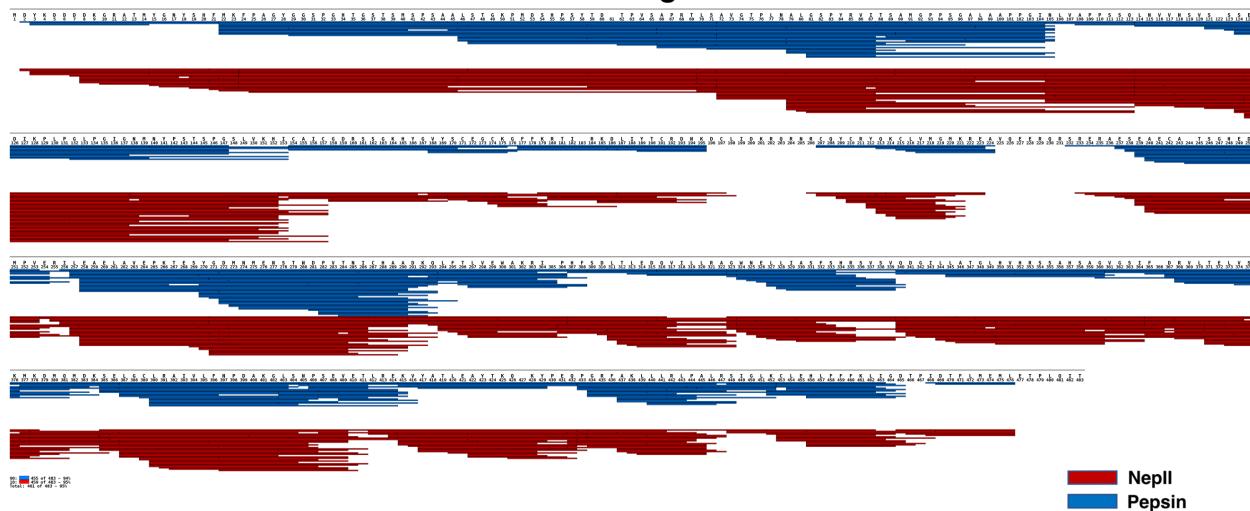


Figure S5

Talin



RXRg



RXRb

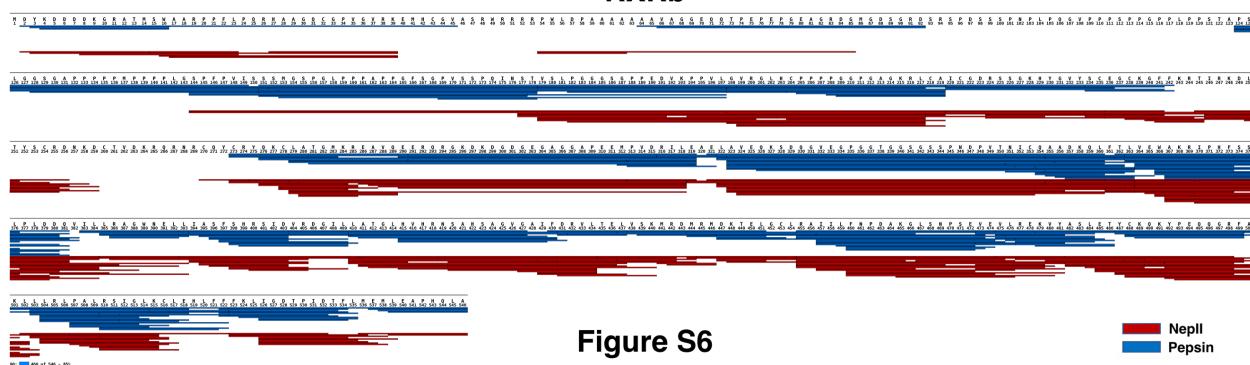
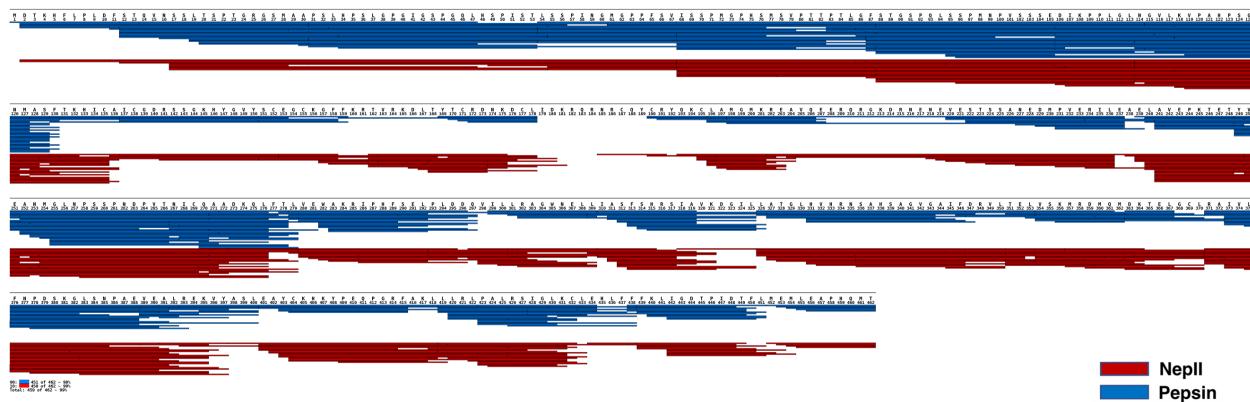
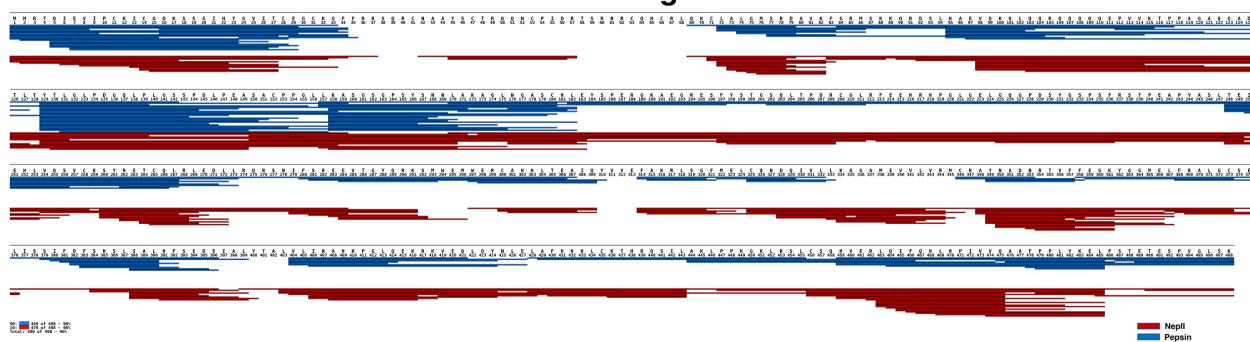


Figure S6

RXRa



RORg



RC2

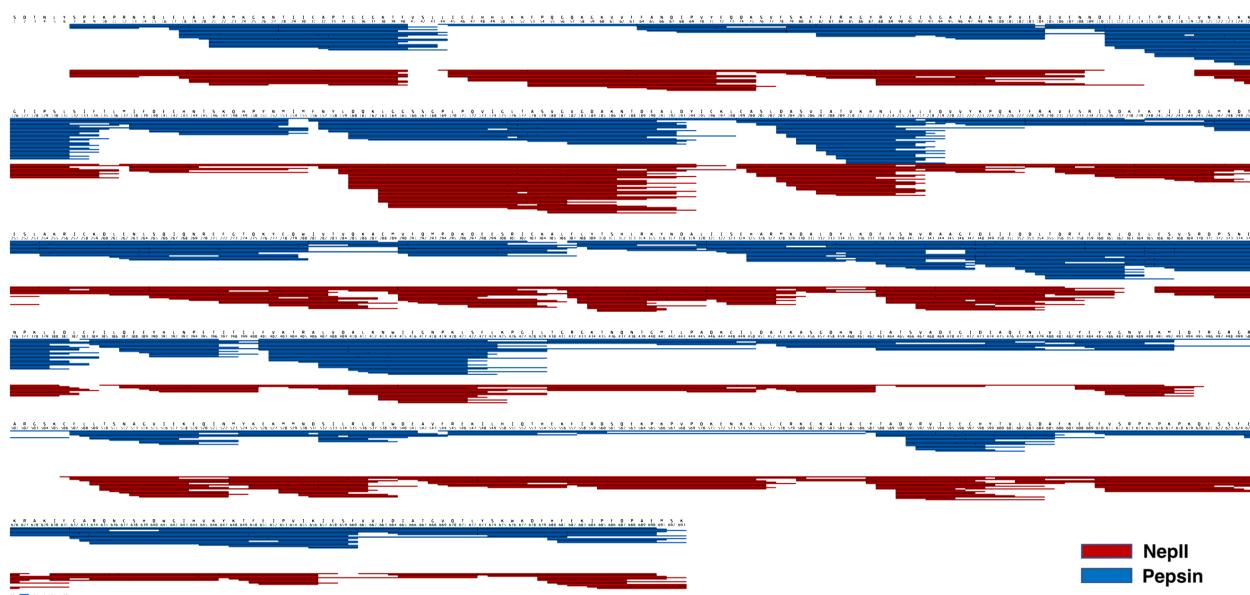
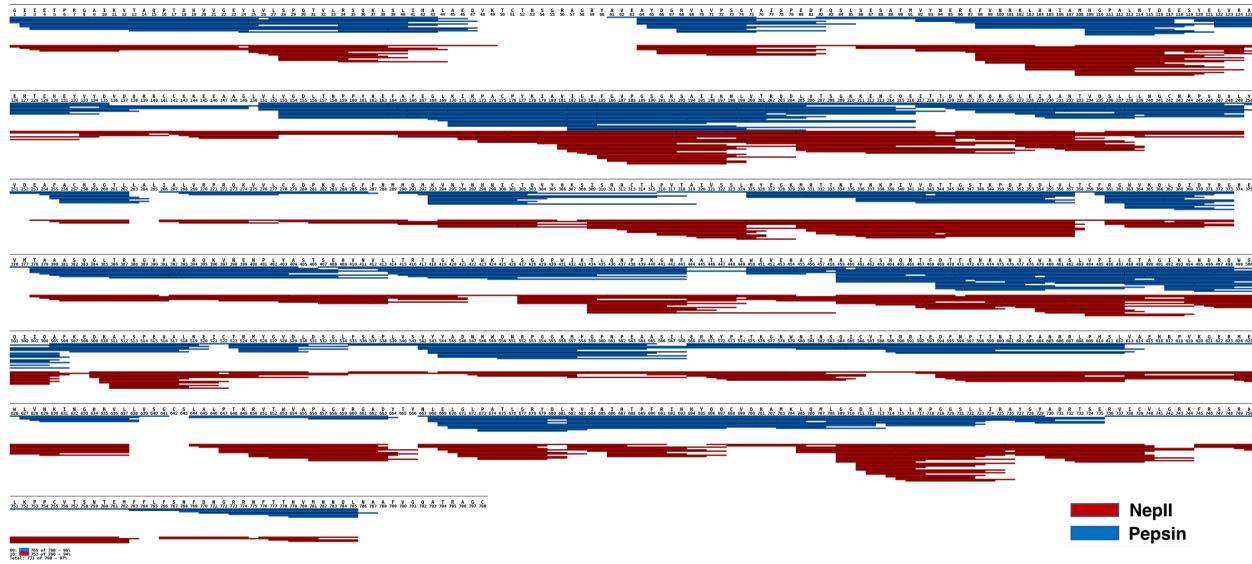


Figure S6

NSP2



DRH3

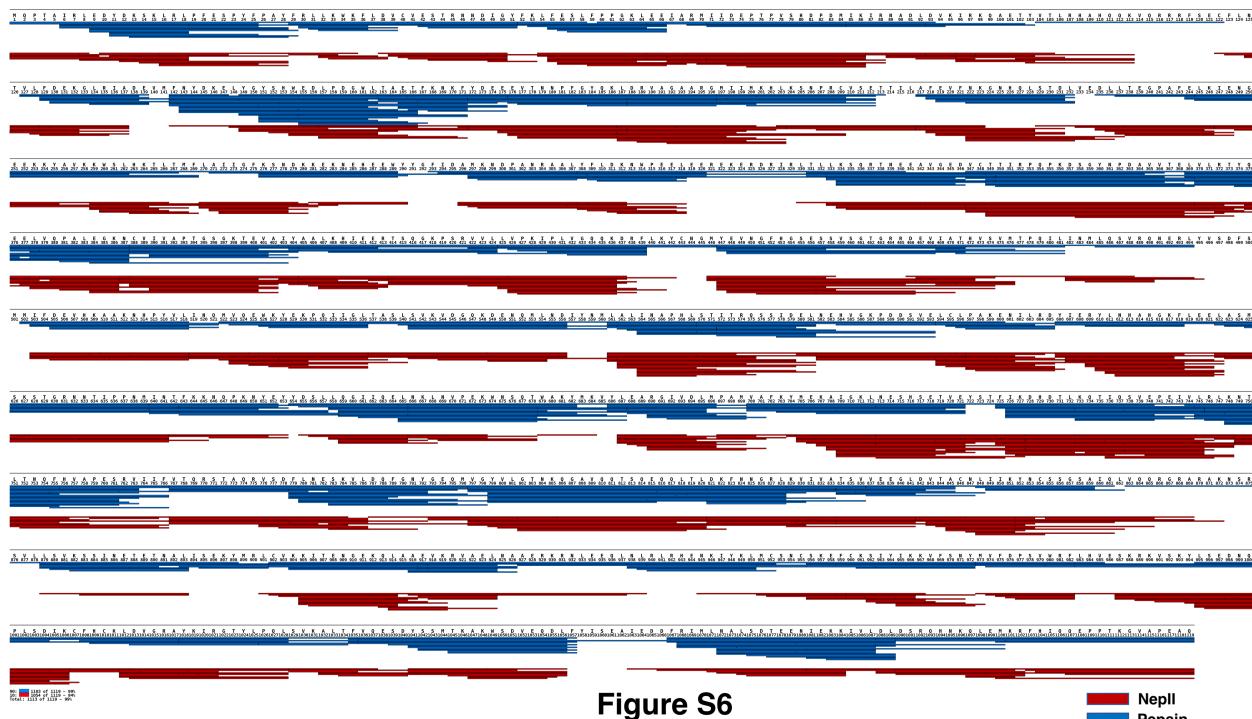
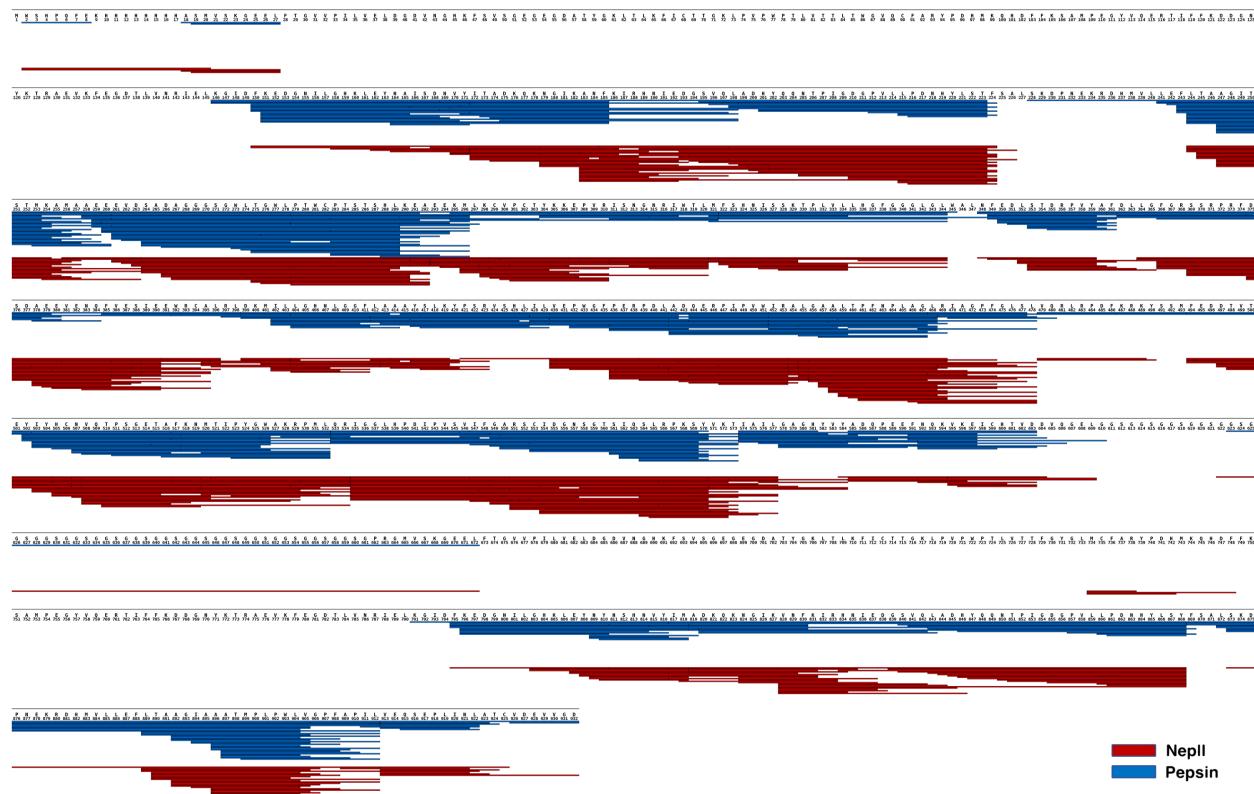


Figure S6

ABHD5



PPAR γ

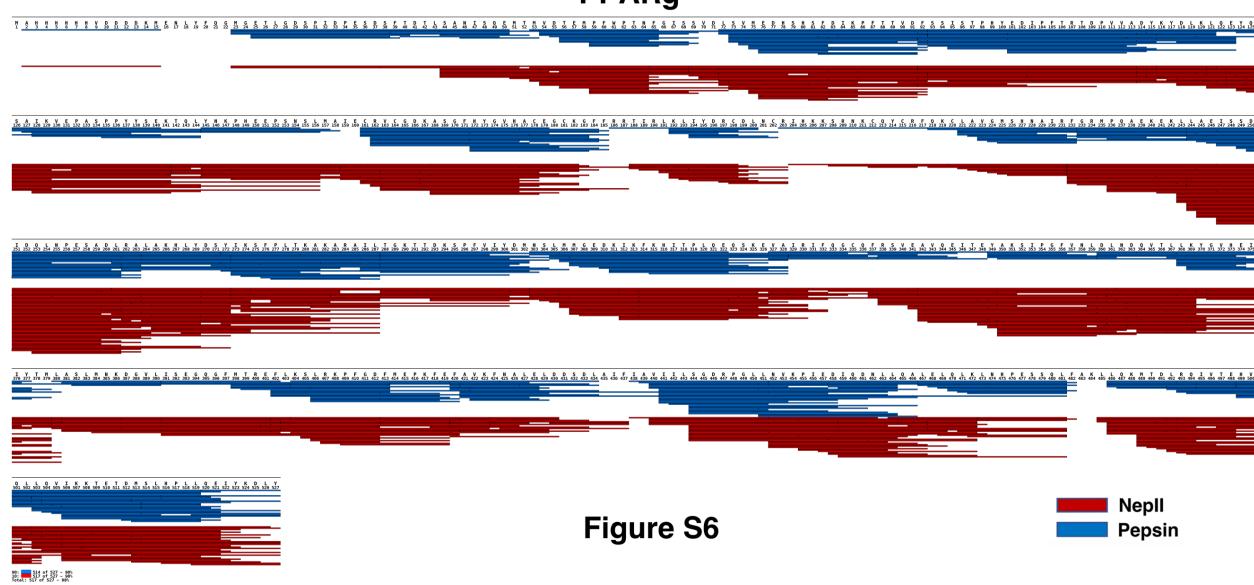


Figure S6

VDR

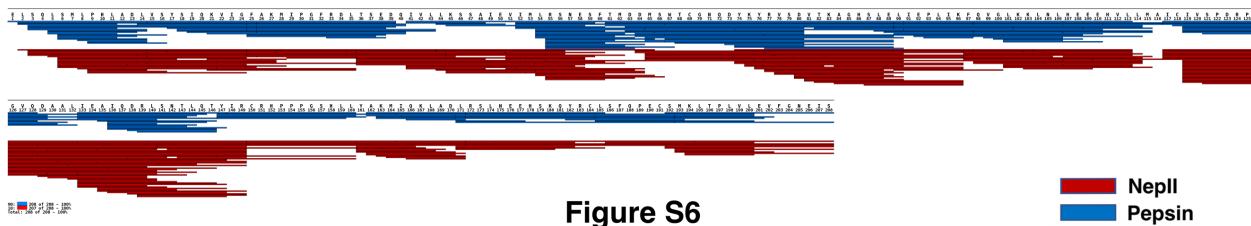
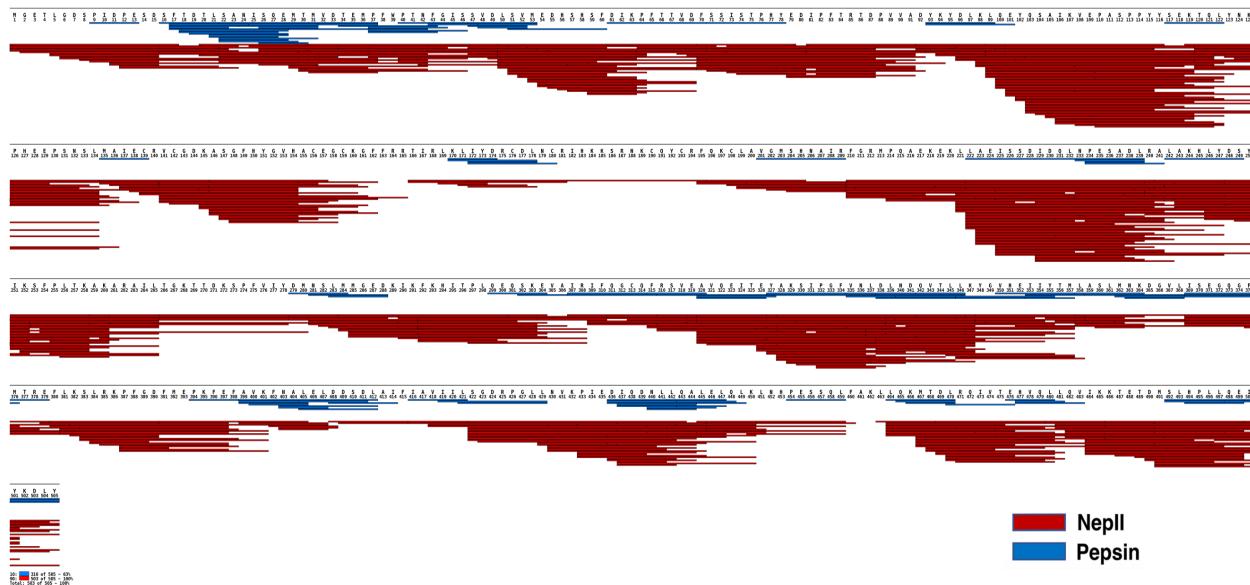


Figure S6

	A			B									
	1 2-7 1-7 pepsin			1 2-7 1-7 nep II									
	P1	P1	P1	P1	P1	P1							
A	1.57	2.4	2.25	0.37	0.74	0.94	A	0.97	1.23	1.29	-0.88	-2.39	-2.2
R	-1.35	-3.99	-3.88	3.16	1.22	1.44	R	-1.29	-4.44	-4.33	2.69	4.4	4.07
N	-2.27	-2.06	-1.66	-0.97	-0.2	-0.29	N	-2.98	-3.03	-3.01	-1.19	-2.1	-2
D	2.3	2.86	3.09	1.79	2.72	2.4	D	0.21	-0.09	0.09	2.94	2.29	2.43
C	-0.29	0.36	0.57	-0.2	1.42	1.12	C	-0.65	0.21	0.11	-0.38	0.58	0.47
Q	-0.35	1.06	1	-1.17	-1.56	-1.34	Q	-2.07	-0.84	-0.95	-1.61	-1.88	-1.89
E	5.77	3.58	3.45	0.46	1.54	1.23	E	5.62	4.41	4.5	-1.7	-0.17	-0.29
G	-1.96	-1.96	-1.81	-2.43	-3.51	-3.07	G	-2.32	-4.36	-4.31	-3.04	-5.07	-4.96
H	-2.56	-3.14	-3.12	-0.69	-1.14	-1.09	H	-2.33	-4.55	-4.47	-1.11	-3.3	-3.16
I	-4.84	-4.33	-3.84	-5.48	-4.33	-3.82	I	-6.38	-5.06	-5.05	-6.14	-5.87	-5.8
L	13.17	12.29	11.4	9.89	7.91	6.73	L	20.65	20.9	21.24	17.07	17.13	17.36
K	-3.04	-6.25	-5.97	7.49	4.48	3.96	K	-2.85	-5.91	-5.91	11.17	13.09	12.79
M	2.86	3.31	3.23	1.24	1.69	1.69	M	2.52	6.34	6.05	1.1	2.3	2.21
F	5.67	5.83	5.05	1.14	2.36	2.45	F	7.89	9.71	9.66	0.81	1.52	1.53
P	-4.49	-5.22	-5.07	-4.82	-6.15	-5.8	P	-4.67	-5.87	-5.83	-5.78	-6.59	-6.54
S	-4.79	-2.55	-2.6	-4.89	-3.89	-3.74	S	-4.96	-4.31	-4.28	-6.01	-6.73	-6.66
T	-1.33	-0.85	-0.88	-2.04	-1.84	-1.78	T	-1.62	-1.49	-1.6	-2.83	-4.24	-4.16
W	0.05	0.11	0.21	-0.1	-0.06	0	W	-0.06	-0.83	-0.79	-0.42	-0.24	-0.24
Y	0.06	1.01	0.95	1.41	1.69	1.63	Y	-0.56	1.95	1.68	-0.1	2.45	2.16
V	-4.16	-2.46	-2.37	-4.15	-3.1	-2.66	V	-5.1	-4	-4.08	-4.58	-5.17	-5.12

Figure S7

PPARy +1



PPARy +2 through +7

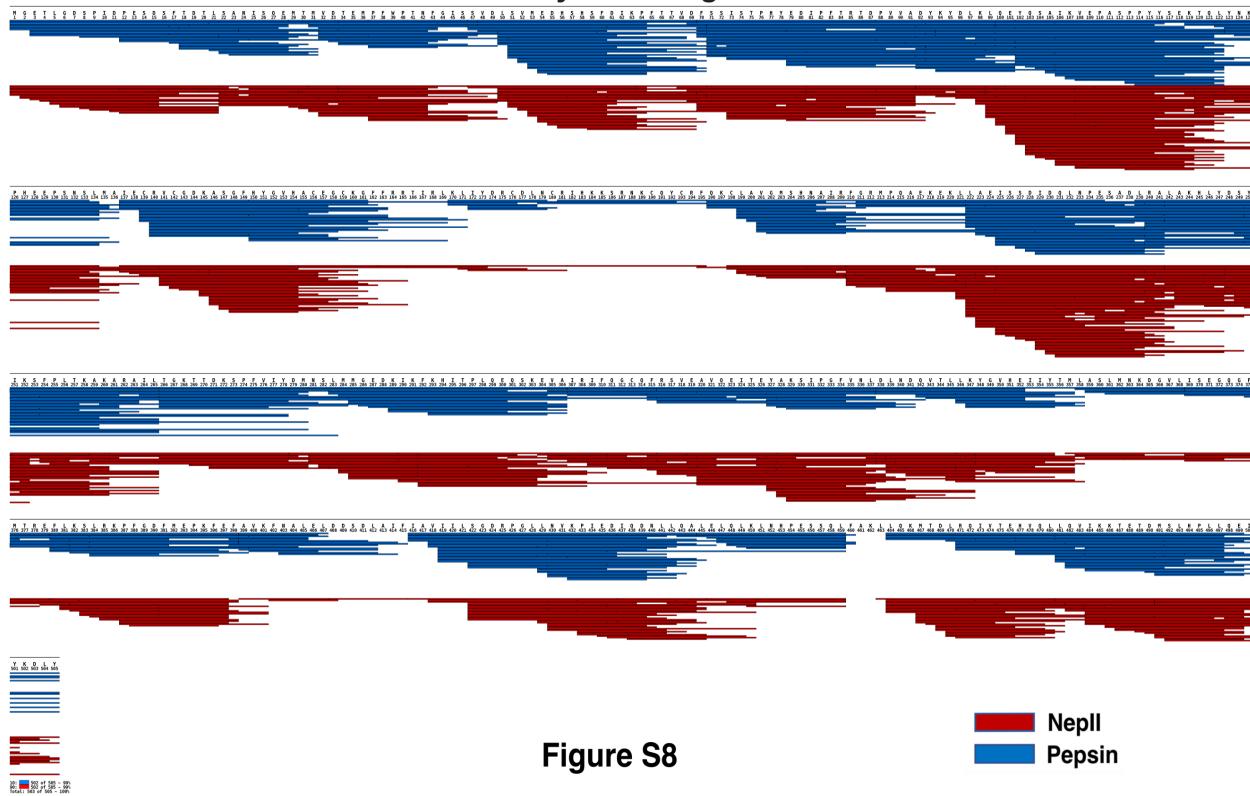


Figure S8

PPAR γ +1 through +7

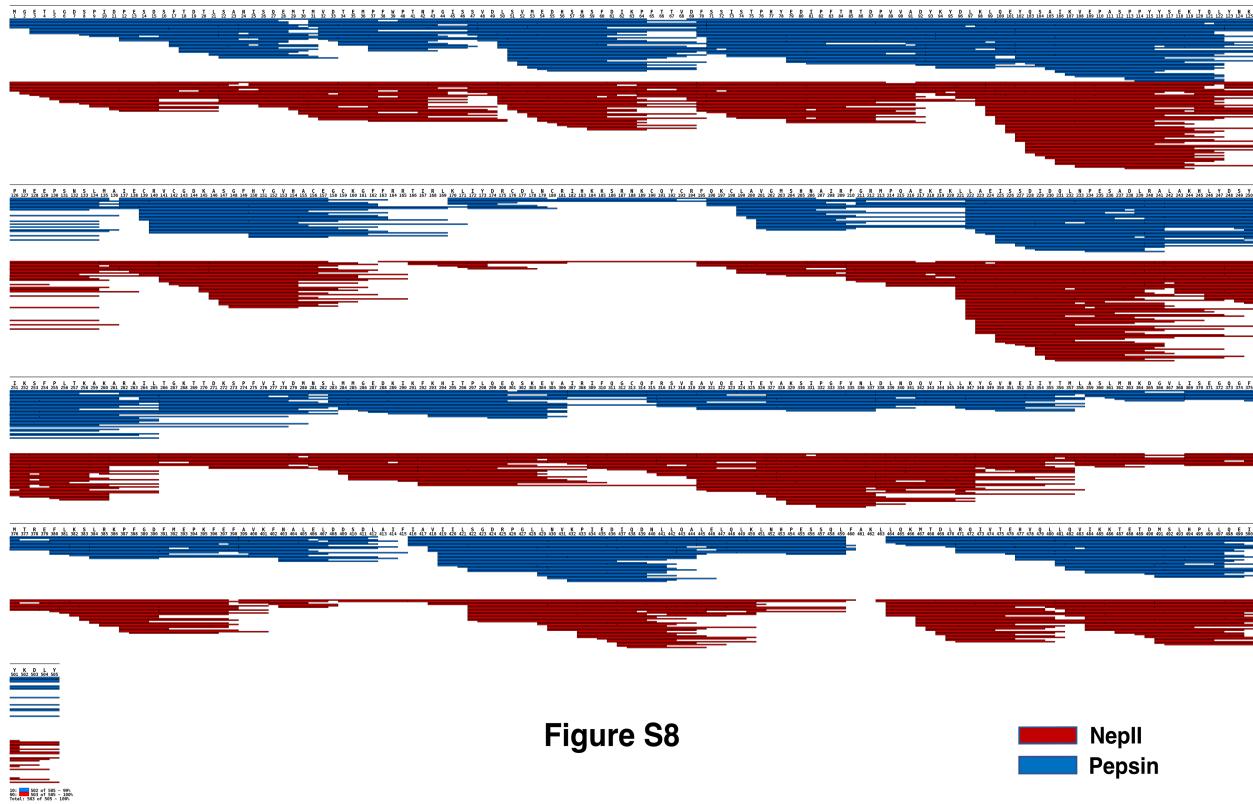


Figure S8