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Supplemental Information

Dual Role of Ribosome-Binding Domain of NAC as a Potent Suppressor of Protein Aggregation and Aging-Related Proteinopathies

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Figure S1

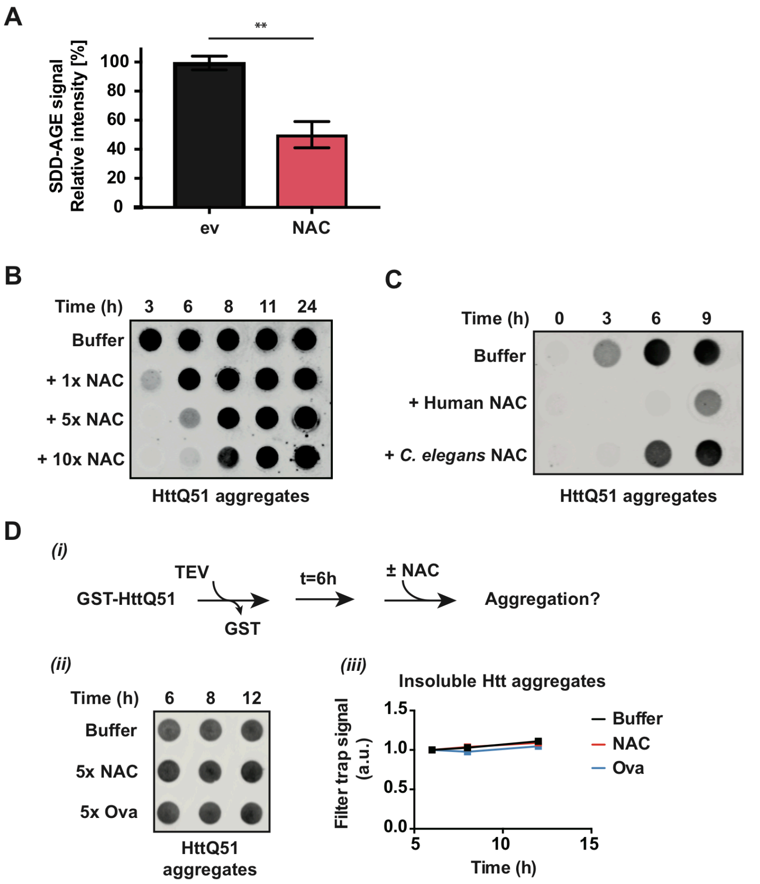

Figure S1. Aggregation suppression of mutant Huntingtin by NAC. Related to Figure 1.

(A) Quantification of PolyQ35::YFP signal on SDD-AGE blots as shown in Figure 1B. Data are represented as mean ± SEM (n = 3). Statistical significance was calculated by Student's t test. ev = empty vector.

(B) *In vitro* filter trap aggregation assay of mutant Huntingtin (mHttQ51) incubated with 1x, 5x or 10x molar excess of human NAC. Aggregation of GST-mHttQ51 was initiated by cleavage of the GST tag using the TEV protease. SDS-insoluble aggregates were detected with an S-tag antibody.

(C) Same assay as in (A) but with either human or *C. elegans* NAC added.

(D) (*i*) Schematic of *in vitro* filter trap aggregation assay of mHttQ51. Experiment was conducted similarly to (A), with the exception of a 6-hour time delay between initiation of aggregation and addition of NAC or Ovalbumin control. (*ii*) SDS-insoluble aggregates of mHttQ51 after addition of 5x molar excess of NAC or ovalbumin (Ova) control. (*iii*) Quantification of SDS-insoluble aggregates shown in (*ii*).

Figure S2

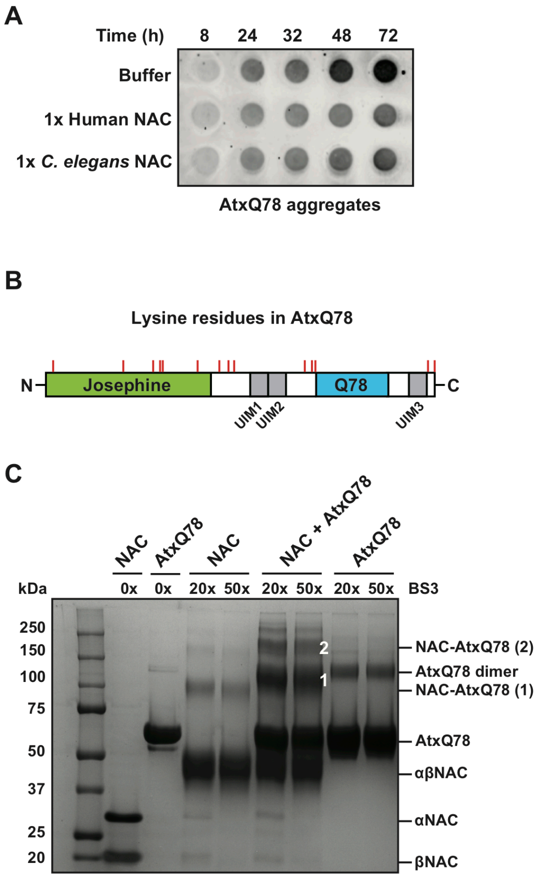

Figure S2. Interaction of NAC with mutant Ataxin-3. Related to Figure 2.
(A) Filter trap aggregation assay of AtxQ78 incubated with an equimolar concentration of either human or *C. elegans* NAC at 37°C for the indicated time. SDS-insoluble aggregates were detected with an anti-His antibody.
(B) Schematic showing the domain architecture of Ataxin-3. All lysine residues carrying optimal functional groups for chemical crosslinking with amine-reactive crosslinkers are highlighted in red. Josephine domain, green. PolyQ78 stretch, blue. UIM = ubiquitin interacting motif.
(C) Coomassie-stained Tris-Tricine gel showing crosslinked protein complexes. NAC and AtxQ78 were crosslinked either with 20x or 50x molar excess of BS3 for 1 hour. NAC and AtxQ78 alone were used as controls. New bands labeled with 1 (NAC-AtxQ78 monomer) and 2 (NAC-AtxQ78 dimer) were excised from the gel and subjected to an in-gel digest for the LC-MS analysis shown in Figure 2C.

Figure S3

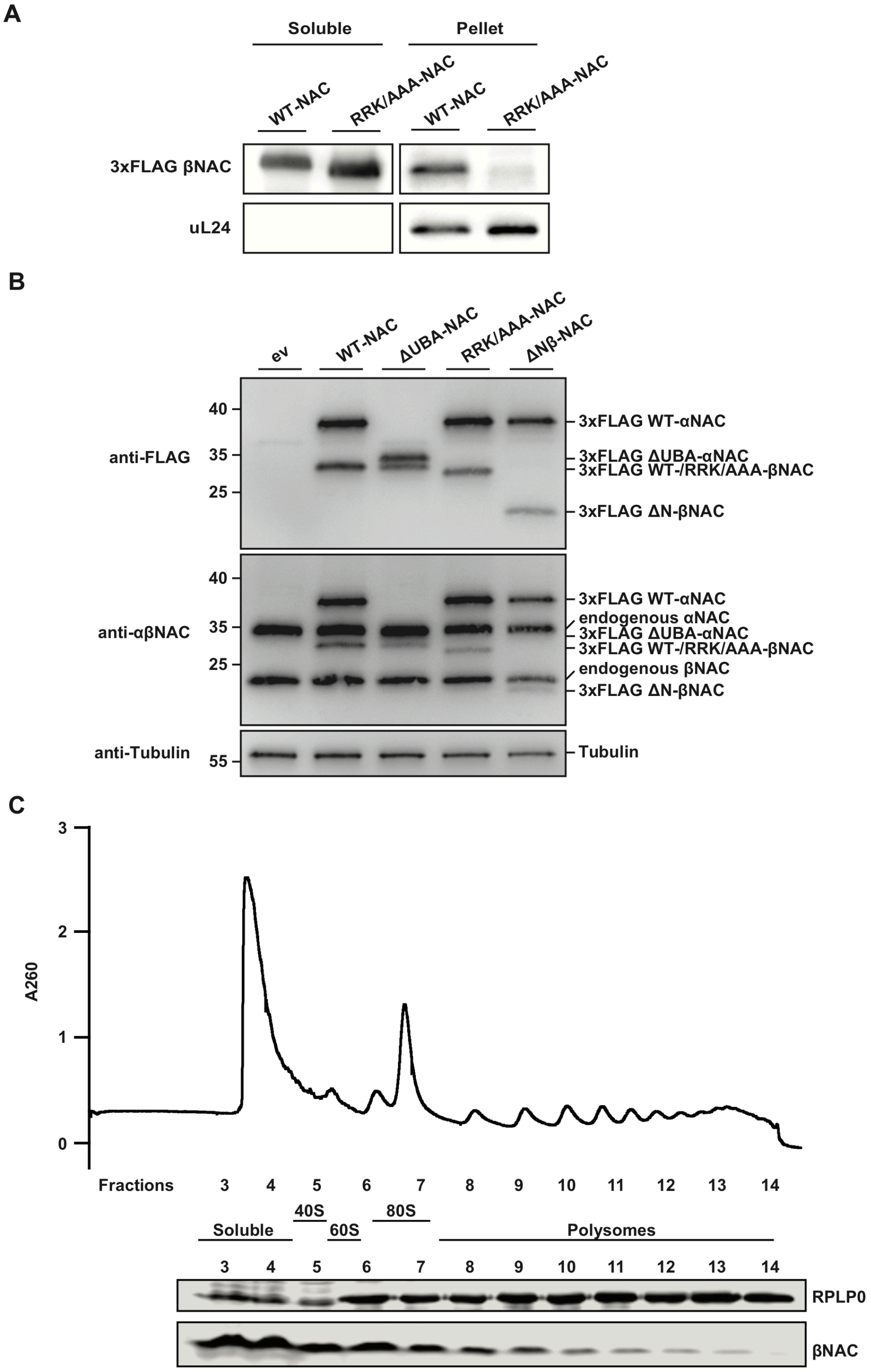

Figure S3. Association of NAC with ribosomes. Related to Figure 3.
(A) Sucrose cushion centrifugation of ribosomes in PolyQ35::YFP *C. elegans* worms overexpressing either 3xFLAG-tagged WT- or RRK/AAA-NAC in muscle cells (*myo-3* promoter). Analysis was performed on day 2 of adulthood. Indicated proteins in the supernatant (Soluble) and ribosomal pellet (Pellet) fractions were analyzed by immunoblotting. uL24 served as a ribosomal marker.
(B) Immunoblot analysis of total levels of endogenous NAC (ubiquitous expression) and overexpressed 3xFLAG-tagged NAC variants in body wall muscles in animals investigated in Figure 3C, D. FLAG immunoblot shows only exogenously overexpressed NAC in muscle cells, while the αβNAC immunoblot shows endogenous and exogenous NAC variants, as indicated. Tubulin served as a loading control.
(C) Polysome profile of HEK293T cells. βNAC levels were assessed by immunoblotting. RPLP0 served as a ribosomal marker.

Figure S4

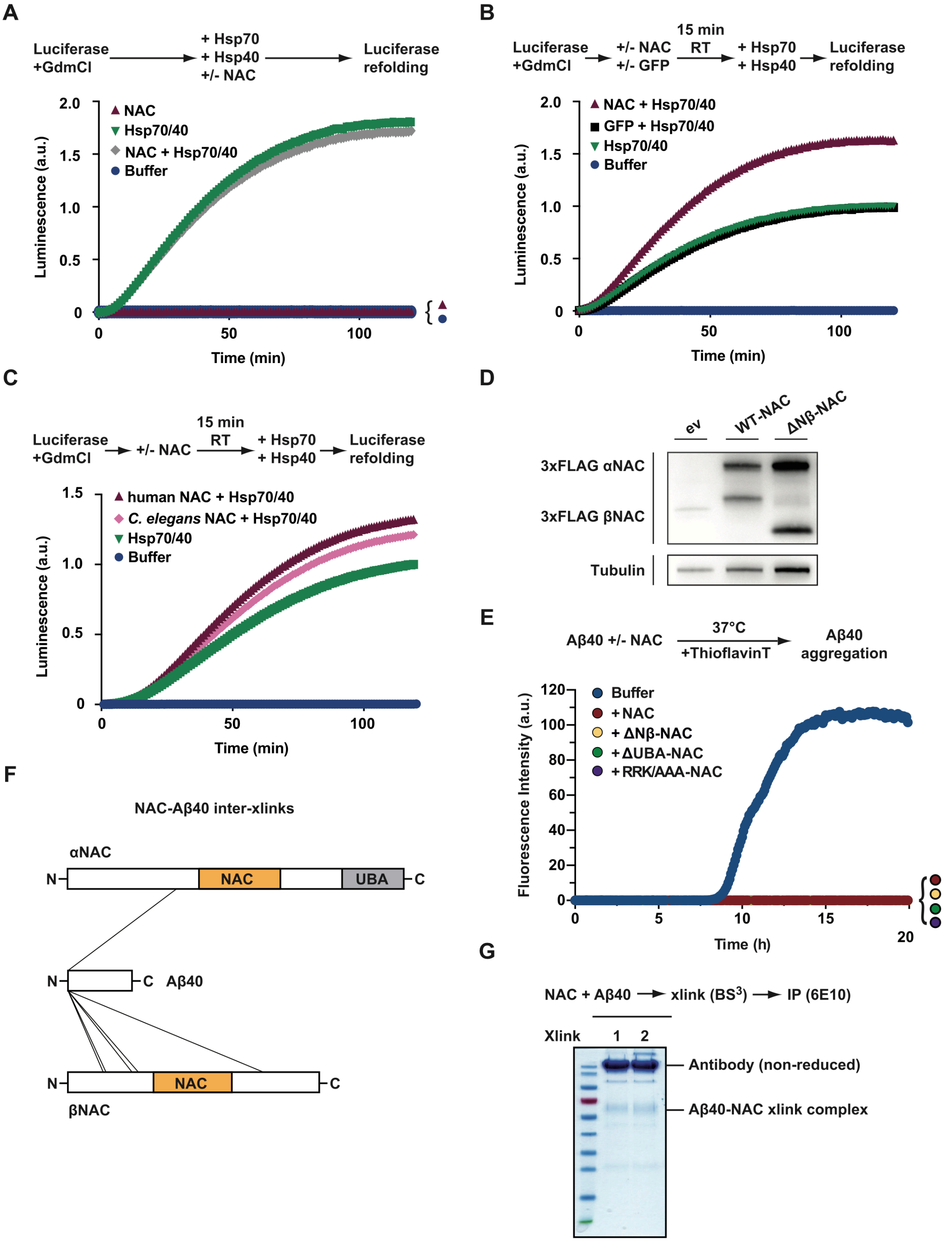

Figure S4. Chaperone activity of NAC on luciferase and Aβ40. Related to Figure 4.

(A) *In vitro* luciferase refolding assays using guanidine-HCl (GdmCl)-denatured substrate. Luciferase (0.02 μM) was incubated with an equimolar concentration of human NAC in the presence and absence of an Hsp70/Hsp40 system (3.2 μM/0.8 μM). Luciferase reactivation was analyzed by luminescence recording over two hours at RT using luciferin as a substrate. a.u. = arbitrary units.

(B) Similar assay as in (A) but luciferase was first preincubated with NAC or GFP control in a 1:1 molar ratio for 15 min at room temperature before adding the Hsp70/Hsp40 chaperone system.

(C) Experiment was conducted similarly to (B) but with NAC from both human and *C. elegans*.

(D) Immunoblot analysis of wild-type NAC and ΔNβ-NAC (FLAG-tagged α- and βNAC) overexpressed in FlucDM-EGFP strain. Tubulin served as loading control.

(E) Aβ40 aggregation (18 μM) was examined in the presence of molar equivalent *C. elegans* NAC constructs (18 μM) with 10 μM thioflavin T (ThT). Samples were incubated quiescently at 37°C for 20 h. Each color indicates replicate assays prepared in the same plate. Aggregation results were baseline subtracted from NAC constructs alone (without Aβ40) or buffer, which did not show aggregation.
(F) Crosslinked NAC-Aβ40 complexes from gel shown in (D) were excised and subjected to an in-gel digest and LC-MS analysis. Schematic shows identified intermolecular crosslinks. See also Table S2.
(G) Coomassie-stained gel showing isolated NAC-Aβ40 complexes crosslinked with BS^3. NAC and Aβ40 (both 18 μM) were incubated at 37°C for two hours and crosslinked using 20x molar excess of BS3 for 30 min. Crosslinked samples were gel-filtrated and NAC-Aβ40 complexes immunoprecipitated using Aβ40 antibody (6E10). Proteins were eluted under non-reducing conditions to avoid antibody splitting into light and heavy chains.

Figure S5

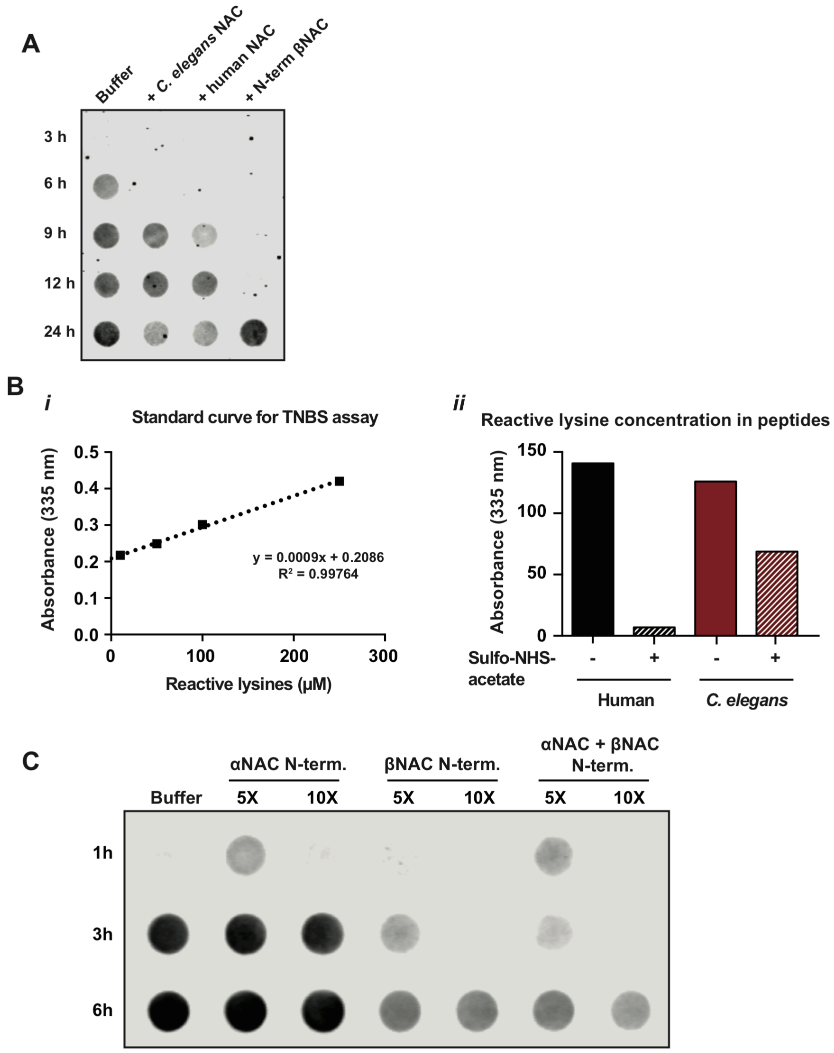

Figure S5. Suppression of PolyQ aggregation by βNAC N-terminus. Related to Figure 5.
(A) In vitro filter trap aggregation assay of PolyQ51 incubated with 5x molar excess of either *C. elegans* NAC, human NAC or the human N-terminal βNAC peptide. Aggregation of GST-PolyQ51 was initiated by cleavage of the GST tag using the TEV protease. SDS-insoluble aggregates were detected with an S-tag antibody.
(B) Quantification of sulfo-NHS-acetate labeling of lysine residues in βNAC N-terminal peptides using the TNBS assay to assess amount of primary amines. (*i*) Standard curve of lysine amino acid with 335 nm absorbance levels. (*ii*) Quantification of primary amine availability with differently treated peptides.
(C) *In vitro* filter trap aggregation assay of mutant Huntingtin (mHttQ51) incubated with 5x or 10x molar excess of human α- or βNAC N-terminal peptides as indicated. SDS-insoluble aggregates were detected with an S-tag antibody.

Figure S6

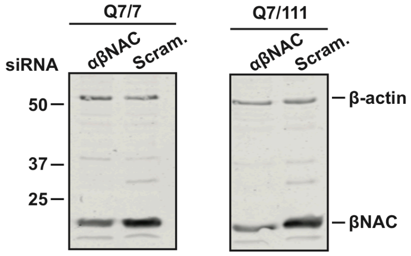

Figure S6. Knockdown efficiency of NAC in Q7/7 and Q7/111 cell lines. Related to Figure 6. Western blot showing βNAC knockdown in siRNA-treated cell lines used in viability assay shown in Figure 6A. β-actin was probed simultaneously as a loading control.

Table S1

Intra- and intermolecular NAC crosslinks. Related to Figure 2.

Score	m/z	z	M+H+	Calculated	Deviation	Peptide 1	Protein 1	From	To	Peptide 2	Protein 2	From	To	Site 1	Site 2
231	594.99	3	1782.956	1782.953	1.31	[QAK]	alpha-NAC	52	54	[VAEAAGLGDHIDK]	alpha-NAC	39	51	K3	K13
124	593.648	3	1778.929	1778.928	0.47	[VAEAAGLGDHIDKQAK]	alpha-NAC	39	54	0	dead-end	0	0	K13	x0
111	1140.91	3	3420.702	3420.686	4.87	[DDGTVIHFNNPK]	beta-NAC	70	81	[VQTSVPANTFSVTGSADNK]	beta-NAC	82	100	K12	T3
106	464.925	3	1392.76	1392.759	0.33	[KLQAQQEHVR]	beta-NAC	13	22	0	dead-end	0	0	K1	x0
95	550.95	3	1650.834	1650.837	-1.49	[QKEVK]	alpha-NAC	9	13	{MTGSTETR]	alpha-NAC	0	8	K2	{0
83	817.758	3	2451.258	2451.255	1.41	[IEDLTQHAQMSAIENLKPTR]	alpha-NAC	114	133	0	dead-end	0	0	K17	x0
75	711.375	3	2132.111	2132.109	0.61	[VAEAAGLGDHIDKQAKQSR]	alpha-NAC	39	57	1	intrapeptidal	0	0	K13	K16
73	539.313	3	1615.926	1615.928	-1.36	[IKKLQAQQEHVR]	beta-NAC	11	22	1	intrapeptidal	0	0	K2	K3
66	823.088	3	2467.251	2467.25	0.43	[IEDLTQHAQmSAIENLKPTR]	alpha-NAC	114	133	0	dead-end	0	0	K17	x0
66	640.038	3	1918.099	1918.105	-2.95	[SKNILFVINKPDVFK]	alpha-NAC	85	99	0	dead-end	0	0	K2	x0
61	593.647	3	1778.928	1778.928	-0.37	[VAEAAGLGDHIDKQAK]	alpha-NAC	39	54	0	dead-end	0	0	K16	x0
57	632.037	3	1894.097	1894.101	-1.82	[LGLK]	alpha-NAC	69	72	[LANNVTKLGPDGK]	beta-NAC	124	136	K4	K13
52	551.948	3	1653.829	1653.83	-0.35	[VCIRK]	alpha-NAC	80	84	{mTGSTETR]	alpha-NAC	0	8	K5	{0
52	1139.56	3	3416.655	3416.66	-1.62	[DDGTVIHFNNPK]	beta-NAC	70	81	[VQTSVPANTFSVTGSADNK]	beta-NAC	82	100	K12	T3
52	1139.56	3	3416.655	3416.66	-1.62	[DDGTVIHFNNPKVQTSVPANTFSVTGSADNK]	beta-NAC	70	100	0	dead-end	0	0	K12	x0
48	676.735	3	2028.19	2028.189	0.23	[KSKNILFVINKPDVFK]	alpha-NAC	84	99	1	intrapeptidal	0	0	K1	K3
45	602.312	3	1804.92	1804.922	-0.98	[IGGKGTPR]	beta-NAC	23	30	{MTGSTETR]	alpha-NAC	0	8	K4	{0
44	823.085	3	2467.241	2467.25	-3.39	[IEDLTQHAQmSAIENLKPTR]	alpha-NAC	114	133	0	dead-end	0	0	K17	x0
44	817.756	3	2451.254	2451.255	-0.36	[IEDLTQHAQMSAIENLKPTR]	alpha-NAC	114	133	0	dead-end	0	0	K17	x0
43	712.717	3	2136.136	2136.134	0.82	[LGPDGK]	beta-NAC	131	136	{mMDSKAIAERIK]	beta-NAC	0	12	K6	K12
42	817.758	3	2451.259	2451.255	1.59	[IEDLTQHAQMSAIENLKPTR]	alpha-NAC	114	133	0	dead-end	0	0	K17	x0
37	906.168	3	2716.488	2716.495	-2.73	[QITEMLPGILNQLGPESLTHLKK]	beta-NAC	101	123	0	dead-end	0	0	K23	x0
36	911.505	3	2732.502	2732.49	4.16	[QITEmLPGILNQLGPESLTHLKK]	beta-NAC	101	123	0	dead-end	0	0	K22	x0
35	433.451	5	2163.225	2163.229	-2.11	[IGGKGTPR]	beta-NAC	23	30	[KLQAQQEHVR]	beta-NAC	13	22	K4	K1
35	747.379	3	2240.121	2240.131	-4.09	[QVTGVSR]	alpha-NAC	73	79	[DDGTVIHFNNPK]	beta-NAC	70	81	S6	K12
34	607.644	3	1820.917	1820.917	-0.13	[IGGKGTPR]	beta-NAC	23	30	{mTGSTETR]	alpha-NAC	0	8	K4	T2
31	751.723	3	2253.153	2253.162	-3.61	{mTGSTETR]	alpha-NAC	0	8	[QSRSEKKARK]	alpha-NAC	55	64	{0	K6
29	816.777	3	2448.317	2448.313	1.8	[AIAERIK]	beta-NAC	6	12	{mTGSTETRQKEVK]	alpha-NAC	0	13	K7	T2
28	711.375	3	2132.111	2132.109	0.83	[LGPDGK]	beta-NAC	131	136	{mMDSKAIAERIK]	beta-NAC	0	12	K6	K12
26	556.282	3	1666.831	1666.832	-0.36	{mTGSTETRQKEVK]	alpha-NAC	0	13	0	dead-end	0	0	T5	x0
26	772.085	3	2314.24	2314.236	1.82	[QAKQSR]	alpha-NAC	52	57	[DIELVISQANTTR]	alpha-NAC	160	172	K3	T12
25	772.085	3	2314.241	2314.236	2.25	[QAKQSR]	alpha-NAC	52	57	[DIELVISQANTTR]	alpha-NAC	160	172	K3	T12
24	978.107	3	2932.308	2932.319	-3.91	{mTGSTETR]	alpha-NAC	0	8	[GEDEDVPELVGDFDAASK]	beta-NAC	137	154	S4	K18
24	691.408	3	2072.21	2072.201	4.58	[IGGK]	beta-NAC	23	26	[QVTGVSRVCIRKSK]	alpha-NAC	73	86	K4	S6
23	691.408	3	2072.21	2072.212	-0.84	[KAR]	alpha-NAC	61	63	[QVTGVSRVCIRKSK]	alpha-NAC	73	86	K1	S6
22	556.282	3	1666.831	1666.832	-0.36	[EVK]	alpha-NAC	11	13	{mTGSTETRQK]	alpha-NAC	0	10	K3	T5
21	1032.54	3	3095.601	3095.612	-3.42	[SEKKARKLFSK]	alpha-NAC	58	68	[EADNDIVNAIMSLTM}	alpha-NAC	181	196	K11	T14
20	478.29	3	1432.854	1432.852	1.2	[QSR]	alpha-NAC	55	57	[LFSKLGLK]	alpha-NAC	65	72	S2	K8
18	550.277	3	1648.815	1648.821	-3.71	{mTGSTETRQKEVK]	alpha-NAC	0	13	1	intrapeptidal	0	0	{0	K10
18	736.346	3	2207.025	2207.029	-1.93	{MTGSTETR]	alpha-NAC	0	8	{mmDSKAIAER]	beta-NAC	0	10	S4	K5
160	519.74	2	1038.473	1038.477	-3.73	{MTGSTETR]	alpha-NAC	0	8	0	dead-end	0	0	{0	x0
151	379.233	2	757.459	757.457	2.64	[NKAIR]	alpha-NAC	173	177	0	dead-end	0	0	K2	x0
147	527.739	2	1054.472	1054.472	-0.49	{mTGSTETR]	alpha-NAC	0	8	0	dead-end	0	0	{0	x0
134	471.275	2	941.543	941.541	1.56	[IGGKGTPR]	beta-NAC	23	30	0	dead-end	0	0	K4	x0
103	473.289	2	945.57	945.567	3.43	[IGGK]	beta-NAC	23	26	[GTPR]	beta-NAC	27	30	K4	T2
97	471.275	2	941.543	941.541	1.56	[IGGK]	beta-NAC	23	26	[GTPR]	beta-NAC	27	30	K4	T2
96	522.308	2	1043.609	1043.609	0.18	[KLANNVTK]	beta-NAC	123	130	0	dead-end	0	0	K1	x0
91	851.991	2	1702.974	1702.978	-2.29	[NILFVINKPDVFK]	alpha-NAC	87	99	0	dead-end	0	0	K8	x0
90	657.393	2	1313.778	1313.779	-0.76	[LGLKQVTGVSR]	alpha-NAC	69	79	0	dead-end	0	0	K4	x0

87	540.556	4	2159.201	2159.204	-1.38	[IGGKGTPR]	beta-NAC	23	30	[KLQAQQEHVR]	beta-NAC	13	22	K4	K1
87	851.993	2	1702.98	1702.978	1	[NILFVINKPDVFK]	alpha-NAC	87	99	0	dead-end	0	0	K8	x0
55	657.393	2	1313.778	1313.779	-0.76	[LGLK]	>sp|alpha	69	72	[QVTGVSR]	alpha-NAC	73	79	K4	T3
53	389.739	2	778.47	778.471	-0.92	[KLFSK]	alpha-NAC	64	68	0	dead-end	0	0	K1	x0
52	659.405	2	1317.803	1317.804	-0.69	[LGLK]	alpha-NAC	69	72	[QVTGVSR]	alpha-NAC	73	79	K4	T3
48	891.98	2	1782.952	1782.953	-0.89	[QAK]	alpha-NAC	52	54	[VAEAAGLGDHIDK]	alpha-NAC	39	51	K3	K13
47	541.561	4	2163.222	2163.229	-3.56	[IGGKGTPR]	beta-NAC	23	30	[KLQAQQEHVR]	beta-NAC	13	22	K4	K1
45	855.928	4	3420.689	3420.686	1.03	[DDGTVIHFNNPK]	beta-NAC	70	81	[VQTSVPANTFSVTGSADNK]	beta-NAC	82	100	K12	T3
42	586.376	2	1171.745	1171.745	-0.33	[KLFSKLGLK]	alpha-NAC	64	72	1	intrapeptidal	0	0	K1	K5
41	473.287	2	945.567	945.567	0.66	[IGGK]	beta-NAC	23	26	[GTPR]	beta-NAC	27	30	K4	T2
40	783.872	2	1566.736	1566.743	-4.55	[NETK]	beta-NAC	155	158	[NETKADEQ}	beta-NAC	155	163	K4	T3
36	433.452	5	2163.233	2163.229	1.42	[IGGKGTPR]	beta-NAC	23	30	[KLQAQQEHVR]	beta-NAC	13	22	K4	K1
29	480.293	2	959.579	959.577	2.02	[ALK]	alpha-NAC	178	180	[SEKK]	alpha-NAC	58	61	K3	K3
29	983.996	4	3932.961	3932.959	0.6	[SPGSDTYIIFGEAKIEDLTQHAQmSAIENLKPTR]	alpha-NAC	100	133	0	dead-end	0	0	T6	x0
28	796.984	2	1592.96	1592.964	-2.72	[VCIRK]	alpha-NAC	80	84	[VCIRKSK]	alpha-NAC	80	86	K5	K7
24	562.291	5	2807.424	2807.428	-1.29	{MmDSKAIAER]	beta-NAC	0	10	[TAAADDKKLQSNLK]	beta-NAC	39	52	{0	K8
24	839.19	4	3353.737	3353.741	-1.17	[KKVIHK]	beta-NAC	33	38	[LGPDGKGEDEDVPELVGDFDAASK]	beta-NAC	131	154	K2	K6
23	562.291	5	2807.424	2807.428	-1.29	{mMDSKAIAER]	beta-NAC	0	10	[TAAADDKKLQSNLK]	beta-NAC	39	52	{0	K8
22	983.994	4	3932.954	3932.959	-1.23	[SPGSDTYIIFGEAKIEDLTQHAQmSAIENLKPTR]	alpha-NAC	100	133	0	dead-end	0	0	Y7	x0

Intermolecular AtxQ78-NAC crosslinks.

Score	m/z	z	M+H+	Calculated	Deviation	Peptide 1	Protein 1	From	To	Peptide 2	Protein 2	From	To	Site 1	Site 2
45	632.035	3	1894.091	1894.084	3.54	[RKKK]	beta-NAC	31	34	[AIQLSmQGSSR]	Atx3 78Q	269	279	K3	S5
38	1026.208	3	3076.609	3076.594	4.83	[LGLK]	alpha-NAC	69	72	[NISQDMTQTSGTNLTSEELRKR]	Atx3 78Q	280	301	K4	S3
31	1158.192	3	3472.563	3472.554	2.37	[VQQmHRPK]	Atx3 78Q	200	207	[TVEEDENEDVEEDSTGIEEK]	alpha-NAC	140	159	K8	K20
25	772.085	3	2314.24	2314.245	-2.09	[SEKKAR]	alpha-NAC	58	63	[REAYFEKQQQK]	Atx3 78Q	302	312	K3	K7
25	767.06	3	2299.167	2299.165	0.86	[VQQMHRPK]	Atx3 78Q	200	207	{MTGSTETRQK]	alpha-NAC	0	10	K8	T7
21	790.709	3	2370.111	2370.11	0.57	[NETKADEQ}	beta-NAC	155	163	[EAYFEKQQQK]	Atx3 78Q	303	312	K4	K6
15	770.747	3	2310.227	2310.22	3.02	[SEKKAR]	alpha-NAC	58	63	[REAYFEKQQQK]	Atx3 78Q	302	312	K4	Y4
13	772.085	3	2314.241	2314.245	-1.65	[SEKKAR]	alpha-NAC	58	63	[REAYFEKQQQK]	Atx3 78Q	302	312	K4	K11
47	1066.949	5	5330.716	5330.718	-0.39	[LSVTNIPGIEEVNMIK]	beta-NAC	54	69	[NISQDMTQTSGTNLTSEELRKRREAYFEK]	Atx3 78Q	280	308	K16	K29
36	777.121	4	3105.46	3105.469	-2.73	{MMDSK]	beta-NAC	0	5	[NISQDMTQTSGTNLTSEELRK]	Atx3 78Q	280	300	{0	T7
35	777.121	4	3105.463	3105.469	-1.7	{MMDSK]	beta-NAC	0	5	[NISQDMTQTSGTNLTSEELRK]	Atx3 78Q	280	300	{0	S3
32	539.817	2	1078.628	1078.629	-1.58	[EVK]	alpha-NAC	11	13	[TEGKK}	Atx3 78Q	438	443	K3	K5
27	667.123	4	2665.471	2665.483	-4.83	[IGGKGTPR]	beta-NAC	23	30	[KRREAYFEKQQQK]	Atx3 78Q	300	312	T6	K1
24	1020.487	4	4078.928	4078.946	-4.37	[QAKQSR]	alpha-NAC	52	57	{SYYHHHHHHLENLYFQGMESIFHEK]	Atx3 78Q	0	25	K3	K25
20	667.123	4	2665.471	2665.483	-4.83	[IGGKGTPRRK]	beta-NAC	23	32	[REAYFEKQQQK]	Atx3 78Q	302	312	T6	K11
19	791.134	4	3161.513	3161.52	-2.25	[IGGKGTPR]	beta-NAC	23	30	[VLEANDGSGMLDEDEEDLQR]	Atx3 78Q	229	248	K4	S8
19	837.673	4	3347.669	3347.677	-2.16	[QAK]	alpha-NAC	52	54	[TFLQQPSGNmDDSGFFSIQVISNALK]	Atx3 78Q	77	102	K3	S17
18	837.675	4	3347.679	3347.677	0.75	[QAK]	alpha-NAC	52	54	[TFLQQPSGNmDDSGFFSIQVISNALK]	Atx3 78Q	77	102	K3	S22

Intramolecular AtxQ78 crosslinks.

Score	m/z	z	M+H+	Calculated	Deviation	Peptide 1	Protein 1	From	To	Peptide 2	Protein 2	From	To	Site 1	Site 2
106	561.65	3	1682.9	1682.932	-2.25	[LIGEELAQLKEQR]	Atx3 78Q	208	220	0	dead-end	0	0	K10	x0
61	385.21	3	1153.6	1153.621	2.17	[VHKTDLER]	Atx3 78Q	221	228	0	dead-end	0	0	K3	x0
54	799.71	3	2397.1	2397.109	0.61	[NISQDmTQTSGTNLTSEELR]	Atx3 78Q	280	299	0	dead-end	0	0	S10	x0
42	398.23	3	1192.7	1192.672	-1.01	[NDLK]	Atx3 78Q	434	437	[TEGKK}	Atx3 78Q	438	443	K4	T1
38	385.21	3	1153.6	1153.621	2.17	[VHK]	Atx3 78Q	221	223	[TDLER]	Atx3 78Q	224	228	K3	T1
37	396.89	3	1188.6	1188.647	-0.25	[NDLKTEGKK}	Atx3 78Q	434	443	0	dead-end	0	0	K4	x0
30	396.89	3	1188.6	1188.647	-0.25	[NDLK]	Atx3 78Q	434	437	[TEGKK}	Atx3 78Q	438	443	K4	T1
30	794.38	3	2381.1	2381.114	3.94	[NISQDMTQTSGTNLTSEELR]	Atx3 78Q	280	299	0	dead-end	0	0	T12	x0
27	500.62	3	1499.8	1499.848	-0.92	[TEGKK}	Atx3 78Q	438	443	[EQRVHK]	Atx3 78Q	218	223	K5	K6
25	767.06	3	2299.2	2299.165	0.86	[VQQMHRPK]	Atx3 78Q	200	207	{MTGSTETRQK]	alpha-NAC	0	10	K8	T7
22	398.23	3	1192.7	1192.672	0.1	[NDLK]	Atx3 78Q	434	437	[TEGKK}	Atx3 78Q	438	443	K4	K5
18	379.21	3	1135.6	1135.611	2.42	[VHKTDLER]	Atx3 78Q	221	228	1	intrapeptidal	0	0	K3	T4
17	386.55	3	1157.6	1157.646	-2.97	[VHK]	Atx3 78Q	221	223	[TDLER]	Atx3 78Q	224	228	K3	T1
115	577.31	2	1153.6	1153.621	-0.66	[VHKTDLER]	Atx3 78Q	221	228	0	dead-end	0	0	K3	x0
91	577.31	2	1153.6	1153.621	-0.66	[VHK]	Atx3 78Q	221	223	[TDLER]	Atx3 78Q	224	228	K3	T1
83	735.83	2	1470.6	1470.642	0.38	[MAEGGVTSEDYR]	Atx3 78Q	65	76	0	dead-end	0	0	S8	x0
77	735.83	2	1470.6	1470.642	1.24	[MAEGGVTSEDYR]	Atx3 78Q	65	76	0	dead-end	0	0	Y11	x0
75	841.97	2	1682.9	1682.932	0	[LIGEELAQLKEQR]	Atx3 78Q	208	220	0	dead-end	0	0	K10	x0
74	585.82	2	1170.6	1170.636	0.98	[NDLKTEGKK}	Atx3 78Q	434	443	1	intrapeptidal	0	0	K4	K8
73	667.12	4	2665.5	2665.482	-4.33	[VHKTDLER]	Atx3 78Q	221	228	[LIGEELAQLKEQR]	Atx3 78Q	208	220	T4	K10
71	743.82	2	1486.6	1486.637	-0.46	[mAEGGVTSEDYR]	Atx3 78Q	65	76	0	dead-end	0	0	S8	x0
70	573.32	4	2290.3	2290.259	-0.26	[VQQmHRPKLIGEELAQLK]	Atx3 78Q	200	217	0	dead-end	0	0	K8	x0
64	585.82	2	1170.6	1170.636	-1.5	[NDLKTEGKK}	Atx3 78Q	434	443	1	intrapeptidal	0	0	K8	T5
62	569.32	4	2274.3	2274.264	1.02	[VQQMHRPKLIGEELAQLK]	Atx3 78Q	200	217	0	dead-end	0	0	K8	x0
50	596.84	2	1192.7	1192.672	-2.71	[NDLK]	Atx3 78Q	434	437	[TEGKK}	Atx3 78Q	438	443	K4	T1
50	667.12	4	2665.5	2665.482	-4.33	[TDLER]	Atx3 78Q	224	228	LIGEELAQLKEQRVHK	Atx3 78Q	208	223	T1	K16
49	579.33	2	1157.6	1157.646	-0.73	[VHK]	Atx3 78Q	221	223	[TDLER]	Atx3 78Q	224	228	K3	T1
49	667.12	4	2665.5	2665.482	-4.33	[LIGEELAQLK]	Atx3 78Q	208	217	[EQRVHKTDLER]	Atx3 78Q	218	228	K10	T7
44	666.12	4	2661.4	2661.457	-3.24	[LIGEELAQLKEQRVHKTDLER]	Atx3 78Q	208	228	0	dead-end	0	0	T17	x0
43	666.12	4	2661.4	2661.457	-3.24	[TDLER]	Atx3 78Q	224	228	LIGEELAQLKEQRVHK	Atx3 78Q	208	223	T1	K16
43	666.12	4	2661.4	2661.457	-3.24	[VHKTDLER]	Atx3 78Q	221	228	[LIGEELAQLKEQR]	Atx3 78Q	208	220	T4	K10
42	666.12	4	2661.4	2661.457	-3.24	[LIGEELAQLK]	Atx3 78Q	208	217	[EQRVHKTDLER]	Atx3 78Q	218	228	K10	T7
40	727.86	2	1454.7	1454.716	-0.8	[EAYFEKQQQK]	Atx3 78Q	303	312	0	dead-end	0	0	K6	x0
39	729.87	2	1458.7	1458.741	-3.95	[QQQK]	Atx3 78Q	309	312	[EAYFEK]	Atx3 78Q	303	308	K4	K6
36	743.82	2	1486.6	1486.637	1.5	[mAEGGVTSEDYR]	Atx3 78Q	65	76	0	dead-end	0	0	S8	x0
32	727.86	2	1454.7	1454.716	-0.8	[QQQK]	Atx3 78Q	309	312	[EAYFEK]	Atx3 78Q	303	308	K4	K6
27	675.34	2	1349.7	1349.673	0.79	[AIQLSmQGSSR]	Atx3 78Q	269	279	0	dead-end	0	0	S10	x0
27	667.34	2	1333.7	1333.678	-1.88	[AIQLSMQGSSR]	Atx3 78Q	269	279	0	dead-end	0	0	S10	x0
22	475.51	4	1899	1899.038	-1.93	[KRR]	Atx3 78Q	300	302	[EAYFEKQQQK]	Atx3 78Q	303	312	K1	K6

Table S2

Intra- and intermolecular NAC/Aβ40 crosslinks. Related to Figure 4 and Figure S4.

Score	m/z	z	M+H+	Calculated	Deviation	Peptide 1	Protein 1	From	To	Peptide2	Protein 2	From	To	Site 1	Site 2
231	756.419	2	1511.831	1511.832	-0.68	[LAEALPKQSVDGK]	beta-NAC	121	133	0	dead-end	0	0	K7	x0
175	730.889	2	1460.770	1460.767	2.48	[ETIMNQEKLAK]	beta-NAC	3	13	0	dead-end	0	0	K8	x0
169	512.315	3	1534.931	1534.918	8.75	[KVVHR]	beta-NAC	31	35	[IGGKGTAR]	beta-NAC	20	27	K1	K4
169	1149.282	4	4594.107	4594.148	-8.90	[LAEALPKQSVDGKAPLATGEDDDDEVPDLVENFDEASKNEAN}	beta-NAC	121	163	1	intrapeptidal	0	0	K7	K13
164	1156.810	4	4624.217	4624.234	-3.72	[LAEALPKQSVDGK]	beta-NAC	121	133	[APLATGEDDDDEVPDLVENFDEASKNEAN}	beta-NAC	134	163	K7	T5
161	495.954	3	1485.848	1485.850	-0.84	[AKQSR]	alpha-NAC	67	71	[IGGKGTAR]	beta-NAC	20	27	K2	K4
159	571.341	2	1141.675	1141.676	-0.75	[KAMSKLGLR]	alpha-NAC	78	86	1	intrapeptidal	0	0	K5	S4
156	539.954	3	1617.848	1617.844	2.63	[TATADDK]	beta-NAC	36	42	[IGGKGTAR]	beta-NAC	20	27	T1	K4
151	452.790	2	904.572	904.573	-0.50	[KKVVHR]	beta-NAC	30	35	1	intrapeptidal	0	0	K1	K2
151	1153.795	4	4612.157	4612.158	-0.32	[LAEALPKQSVDGKAPLATGEDDDDEVPDLVENFDEASKNEAN}	beta-NAC	121	163	0	dead-end	0	0	K13	x0
150	1156.808	4	4624.211	4624.234	-4.84	[LAEALPKQSVDGK]	beta-NAC	121	133	[APLATGEDDDDEVPDLVENFDEASKNEAN}	beta-NAC	134	163	K7	T5
148	458.268	2	915.528	915.526	2.40	[IGGKGTAR]	beta-NAC	20	27	0	dead-end	0	0	K4	x0
147	852.463	2	1703.919	1703.921	-1.23	[TATADDKKLQFSLK]	beta-NAC	36	49	1	intrapeptidal	0	0	K7	K8
145	1092.481	3	3275.428	3275.423	1.27	[APLATGEDDDDEVPDLVENFDEASKNEAN}	beta-NAC	134	163	0	dead-end	0	0	S24	x0
140	503.259	2	1005.511	1005.510	1.43	[TATADDKK]	beta-NAC	36	43	0	dead-end	0	0	K7	x0
136	559.643	3	1676.914	1676.918	-2.10	[IGGKGTAR]	beta-NAC	20	27	{MDAEFR]	Abeta40	0	6	K4	{0

135	677.733	3	2031.186	2031.189	-1.59	[KSKNILFVITKPDVYK]	alpha-NAC	98	113	1	intrapeptidal	0	0	K1	K3
134	499.607	3	1496.807	1496.807	-0.07	[KVVHR]	beta-NAC	31	35	[TATADDK]	beta-NAC	36	42	K1	T1
134	397.748	2	794.489	794.488	0.59	[KVVHR]	beta-NAC	31	35	0	dead-end	0	0	K1	x0
133	462.710	2	924.413	924.413	-0.35	{MDAEFR]	Abeta40	0	6	0	dead-end	0	0	{0	x0
132	502.942	3	1506.812	1506.812	-0.26	[AKQSR]	alpha-NAC	67	71	{MDAEFR]	Abeta40	0	6	K2	{0
131	512.311	3	1534.919	1534.918	0.62	[KVVHR]	beta-NAC	31	35	[IGGKGTAR]	beta-NAC	20	27	K1	K4
124	566.839	4	2264.333	2264.336	-1.42	[IGGKGTAR]	beta-NAC	20	27	[LAEALPKQSVDGK]	beta-NAC	121	133	K4	K7
123	510.309	2	1019.611	1019.614	-2.62	[KLQFSLK]	beta-NAC	43	49	0	dead-end	0	0	K1	x0
117	543.977	3	1629.917	1629.920	-1.54	[TATADDK]	beta-NAC	36	42	[IGGKGTAR]	beta-NAC	20	27	T1	K4
113	555.619	3	1664.842	1664.842	-0.08	[IGGKGTAR]	beta-NAC	20	27	{MDAEFR]	Abeta40	0	6	K4	{0
112	563.821	4	2252.263	2252.261	0.93	[IGGKGTAR]	beta-NAC	20	27	[LAEALPKQSVDGK]	beta-NAC	121	133	K4	K7
111	503.630	3	1508.877	1508.882	-3.58	[KVVHR]	beta-NAC	31	35	[TATADDK]	beta-NAC	36	42	K1	T1
111	470.707	2	940.406	940.408	-1.74	{mDAEFR]	Abeta40	0	6	0	dead-end	0	0	{0	x0
108	579.340	2	1157.672	1157.671	0.55	[KAmSKLGLR]	alpha-NAC	78	86	1	intrapeptidal	0	0	K5	S4
100	582.651	3	1745.937	1745.939	-1.29	[IGGKGTAR]	beta-NAC	20	27	[TATADDKK]	beta-NAC	36	43	K4	K7
98	384.484	4	1534.913	1534.918	-3.19	[KVVHR]	beta-NAC	31	35	[IGGKGTAR]	beta-NAC	20	27	K1	K4
96	406.979	4	1624.893	1624.902	-5.12	[KVVHR]	beta-NAC	31	35	[TATADDKK]	beta-NAC	36	43	K1	T1
96	499.980	3	1497.926	1497.925	0.87	[AKQSR]	alpha-NAC	67	71	[IGGKGTAR]	beta-NAC	20	27	K2	K4
93	586.676	3	1758.014	1758.015	-0.10	[IGGKGTAR]	beta-NAC	20	27	[TATADDKK]	beta-NAC	36	43	K4	K7
93	350.721	2	700.434	700.435	-1.97	[AKAVR]	alpha-NAC	193	197	0	dead-end	0	0	K2	x0

91	578.672	3	1734.001	1734.007	-3.49	[LQFSLK]	beta-NAC	44	49	[TATADDKK]	beta-NAC	36	43	S4	K7
91	574.651	3	1721.938	1721.932	3.64	[TATADDK]	beta-NAC	36	42	[LQFSLKK]	beta-NAC	44	50	T3	K6
88	449.262	2	897.517	897.515	2.32	[IGGKGTAR]	beta-NAC	20	27	1	intrapeptidal	0	0	K4	T6
73	360.702	2	720.397	720.396	0.95	[KAMSK]	alpha-NAC	78	82	0	dead-end	0	0	K1	x0
71	542.304	3	1624.898	1624.902	-2.13	[KVVHR]	beta-NAC	31	35	[TATADDKK]	beta-NAC	36	43	K1	K7
70	544.656	3	1631.954	1631.948	3.52	[LQFSLK]	beta-NAC	44	49	[IGGKGTAR]	beta-NAC	20	27	S4	K4
66	861.468	2	1721.929	1721.932	-1.67	[LQFSLK]	beta-NAC	44	49	[TATADDKK]	beta-NAC	36	43	S4	K7
57	554.072	4	2213.267	2213.271	-1.76	[IGGKGTAR]	beta-NAC	20	27	[ETIMNQEKLAK]	beta-NAC	3	13	K4	K8
51	820.919	2	1640.830	1640.835	-3.01	[LQFSLK]	beta-NAC	44	49	{MDAEFR]	Abeta40	0	6	S4	{0
42	446.264	2	891.521	891.519	3.15	[LQFSLK]	beta-NAC	44	49	0	dead-end	0	0	S4	x0
69	384.485	4	1534.917	1534.918	-0.32	[KVVHR]	beta-NAC	31	35	[IGGKGTAR]	beta-NAC	20	27	K1	K4
117	543.979	3	1629.923	1629.920	2.23	[TATADDK]	beta-NAC	36	42	[IGGKGTAR]	beta-NAC	20	27	T1	K4
68	563.821	4	2252.261	2252.261	-0.13	[IGGKGTAR]	beta-NAC	20	27	[LAEALPKQSVDGK]	beta-NAC	121	133	K4	K7
82	559.643	3	1676.913	1676.918	-2.64	[IGGKGTAR]	beta-NAC	20	27	{MDAEFR]	Abeta40	0	6	K4	{0
112	555.622	3	1664.851	1664.842	4.98	[IGGKGTAR]	beta-NAC	20	27	{MDAEFR]	Abeta40	0	6	K4	{0
80	1049.776	4	4196.082	4196.068	3.33	[LAEALPKQSVDGK]	beta-NAC	121	133	[APLATGEDDDDEVPDLVENFDEASK]	beta-NAC	134	158	K7	T5

Table S3

C. elegans strains. Related to STAR Methods.

DEU117	rmIs132 [*unc-54p*::Q35::YFP]; gamEx17 [*myo-3p*::empty::*unc-54* 3'UTR (50 ng/µl), *myo-2p*::mCherry::*unc-54* 3'UTR (2.5 ng/µl), DNA ladder (100 ng/µl)]
DEU118	rmIs132 [*unc-54p*::Q35::YFP]; gamEx18 [*myo-3p*::3xFLAG::αNAC::*unc-54* 3'UTR (25 ng/µl), *myo-3p*::3xFLAG::βNAC::*unc-54* 3'UTR (25 ng/µl), *myo-2p*::mCherry::*unc-54* 3'UTR (2.5 ng/µl), DNA ladder (100 ng/µl)]
DEU119	rmIs132 [*unc-54p*::Q35::YFP]; gamEx19 [*myo-3p*::3xFLAG::ΔUBA-αNAC::*unc-54* 3'UTR (25 ng/µl), *myo-3p*::3xFLAG::βNAC::*unc-54* 3'UTR (25 ng/µl), *myo-2p*::mCherry::*unc-54* 3'UTR (2.5 ng/µl), DNA ladder (100 ng/µl)]
DEU120	rmIs132 [*unc-54p*::Q35::YFP]; gamEx20 [*myo-3p*::3xFLAG::αNAC::*unc-54* 3'UTR (25 ng/µl), *myo-3p::*3xFLAG::RRK/AAA-βNAC::*unc-54* 3'UTR (25 ng/µl), *myo-2p*::mCherry::*unc-54* 3'UTR (2.5 ng/µl), DNA ladder (100 ng/µl)]
DEU121	rmIs132 [*unc-54p*::Q35::YFP]; gamEx21 [*myo-3p*::3xFLAG::αNAC::*unc-54* 3'UTR (25 ng/µl), *myo-3p*::3xFLAG::ΔNβ-NAC::*unc-54* 3'UTR (25 ng/µl), *myo-2p*::mCherry::*unc-54* 3'UTR (2.5 ng/µl), DNA ladder (100 ng/µl)]
DEU122	*marIs135 [unc-54p::*FlucDM::EGFP]; gamEx22 [*myo-3p*::empty::*unc-54* 3'UTR (50 ng/µl), *myo-2p*::mCherry::*unc-54* 3-UTR (2.5 ng/µl), DNA ladder (50 ng/µl)]
DEU123	*marIs135 [unc-54p::*FlucDM::EGFP]; gamEx23[*myo-3p*::3xFLAG::αNAC::*unc-43* 3'UTR (25 ng/µl), *myo-3p*::3xFLAG::βNAC::*unc-54* 3'UTR (25 ng/µl), *myo-2p*::mCherry::*unc-54* 3'UTR (2.5 ng/µl), DNA ladder (50 ng/µl)]
DEU124	*marIs135 [unc-54p::*FlucDM::EGFP]; gamEx24 [*myo-3p*::3xFLAG::αNAC::*unc-54* 3'UTR (25 ng/µl), *myo-3p*::3xFLAG::ΔNβ-NAC::*unc-54* 3'UTR (25 ng/µl), *myo-2p*::mCherry::*unc-54* 3'UTR (2.5 ng/µl), DNA ladder (50 ng/µl)]