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Supplemental information

Translation of GGC repeat expansions into a toxic polyglycine protein in NIID defines a novel class of human genetic disorders: The polyG diseases

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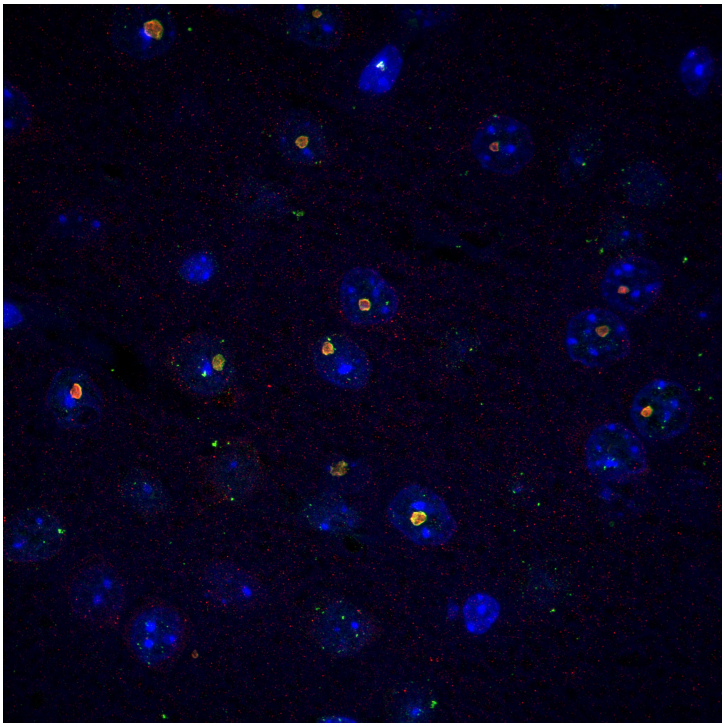
Supplemental Information

Translation of GGC repeat expansions into a toxic polyglycine protein in NIID defines a novel class of human genetic disorders: the polyG diseases.



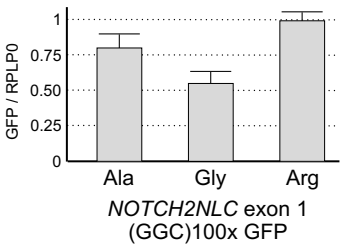
P62 (green) and uN2CpolyG (red)
staining in cortical area of NIID mouse model

A

NOTCH2NLC (N2C) exon1 (GGC)100x *BamHI* GFP (+1 frame, glycine frame):

GCTGAGGCGGCGGCCGAGGAGCGGCGGACTCGGGGCGCGGGGAGTCGAGGCATTTGCGCCTGTGCTTCGGACCGTAGCGCCAGGGCCTGAGCCTTTGAAGCAGGAGGAG
GGGAGGAGAGAGTGGGGCTCCTCTATCGGGACCCCCTCCCC**ATG**TGGATCTGCCCA**GGCGGCGGC…GGCGGCGGC**GACCGAGAAGATGCCCGCCCTGCGCCGCTCTGCT
GTGGGCGCTGCTGGCGCTCTGGCTGTGCTGCGCGACCCCCGCGCATGggatccGTCAGCAAAGGCGAGGAGCTTTTCACCGGGGTTGTTCCCATCCTCGTCGAGCTCGACGGC
GACGTAAACGGCCACAAATTCAGCGTTTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTCACCCTCAAGTTCATCTGCACCACCGGCAAGCTCCCCGTCCCCTGGC
CCACCCTCGTCACCACCCTCACCTACGGCGTCCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGA
GCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG
GACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAA
CATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT
GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAG

B



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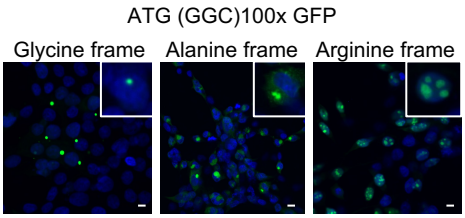

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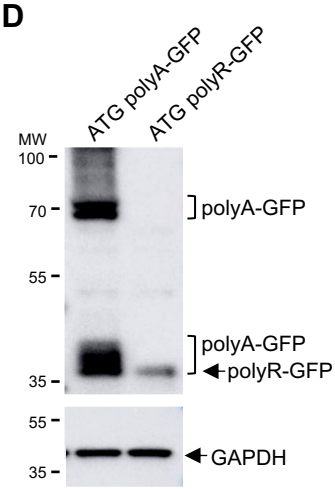


E

Ion Series | Neutral Losses | Precursor Ions

#1	a+	a2+	b+	b2+	Seq.	y+	y2+	#2
1	146.06341	73.53534	174.05833	87.53280	M-Acetyl			20
2	332.14272	166.57500	360.13764	180.57246	W	1587.67171	794.33949	19
3	445.22679	223.11703	473.22170	237.11449	I	1401.59240	701.29984	18
4	605.25744	303.13236	633.25235	317.12981	C-Carbami...	1288.50834	644.75781	17
5	702.31020	351.65874	730.30512	365.65620	P	1128.47769	564.74248	16
6	759.33166	380.16947	787.32658	394.16693	G	1031.42492	516.21610	15
7	816.35313	408.68020	844.34804	422.67766	G	974.40346	487.70537	14
8	873.37459	437.19093	901.36951	451.18839	G	917.38200	459.19464	13
9	930.39606	465.70167	958.39097	479.69912	G	860.36053	430.68390	12
10	987.41752	494.21240	1015.41243	508.20986	G	803.33907	402.17317	11
11	1044.43898	522.72313	1072.43390	536.72059	G	746.31760	373.66244	10
12	1101.46045	551.23386	1129.45536	565.23132	G	689.29614	345.15171	9
13	1158.48191	579.74459	1186.47682	593.74205	G	632.27468	316.64098	8
14	1215.50337	608.25533	1243.49829	622.25278	G	575.25321	288.13025	7
15	1272.52484	636.76606	1300.51975	650.76351	G	518.23175	259.61951	6
16	1329.54630	665.27679	1357.54122	679.27425	G	461.21029	231.10878	5
17	1386.56776	693.78752	1414.56268	707.78498	G	404.18882	202.59805	4
18	1443.58923	722.29825	1471.58414	736.29571	G	347.16736	174.08732	3
19	1558.61617	779.81172	1586.61109	793.80918	D	290.14590	145.57659	2
20					R	175.11895	88.06311	1

M^{Ac}WICPGGGGGGGGGGGGGDR

F

NOTCH2NLC (N2C) exons 1 to 2:

CCAAACTTCGGGCGGCGGCTGAGGCGGCGGCCGAGGAGCGGCGGACTCGGGGCGCGGGGAGTCGAGGCATTTGCGCCTGT
GCTTCGGACCGTAGCGCCAGGGCCTGAGCCTTTGAAGCAGGAGGAGGGGAGGAGAGAGTGGGGCTCCTCTATCGGGACCCC
CTCCCC**ATG**TGGATCTGCCCA**GGCGGCGGC…GGCGGCGGC**GGAGGAGGCGGCGACCGAGAAGATGCCCGCCCTGCGCCG
CTCTGCTGTGGGCGCTGCTGGCGCTCTGGCTGTGCTGCGCGACCCCCGCGCATGgtgagta…intron1…atttttagCATTGCAGTGTC
GAGATGGCTATGAACCCTGTG**TAA**ATGAAGGA**ATG**TGTGTTACCTACCACAATGGCACAGGATACTGCAAgtaagttt…intron2

ATG N2C

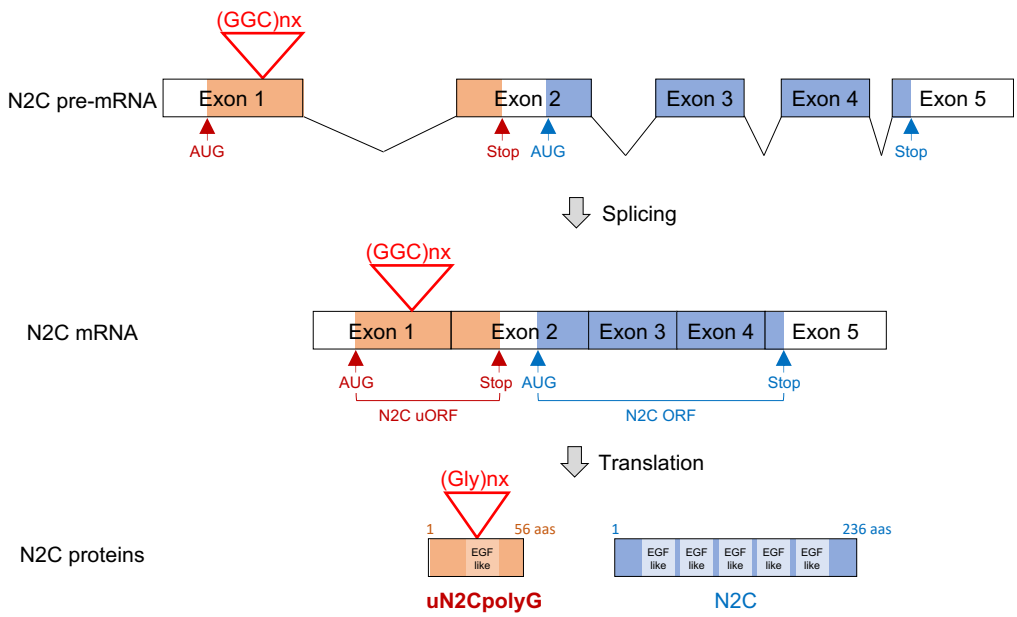


Figure S1. Related to Figure 1.

Figure S1. Related to Figure 1.
***NOTCH2NLC* GGC repeats are translated into polyglycine.**

(**A**) Sequence of the human *NOTCH2NLC* exon 1 with GGC repeats fused to the GFP in the glycine frame and cloned into pcDNA3.

(**B**) RT-qPCR of the GFP and *RPLP0* mRNA expression of HEK293 cells transfected for 24 hours with GGC repeats embedded in the *NOTCH2NLC* exon 1 and fused to the GFP in either the glycine, alanine or arginine frame.

(**C**) GFP fluorescence of HEK293 cells transfected for 24 hours with 100 GGC repeats cloned downstream of an artificial ATG start codon and fused to the GFP in the three possible frames.

(**D**) Immunoblot against the GFP or the GAPDH of HEK293 cells transfected for 24 hours with 100 GGC repeats cloned downstream of an artificial ATG start codon and fused to the GFP in either the alanine or the arginine frame, encoding either a polyalanine or a polyarginine-containing protein, respectively.

(**E**) LC-MS/MS table analysis of the N-terminal peptide from GFP-immunoprecipitated and trypsin digested protein expressed from HEK293 cells transfected with *NOTCH2NLC* exon 1 with GGC repeats and fused to the GFP in the glycine frame.

(**F**) Upper panel, sequence of the human *NOTCH2NLC* exons 1 to 2. Exon sequences are in upper cases, intronic sequences are in grey lower cases. Lower panel, scheme of the *NOTCH2NLC* transcript encoding the upstream (uN2C) and main (N2C) ORFs, which contain 1 and 5 potential EGF-like domains, respectively. Upstream (uN2C) and main (N2C) NOTCH2NLC ORFs are indicated in orange and blue, respectively.

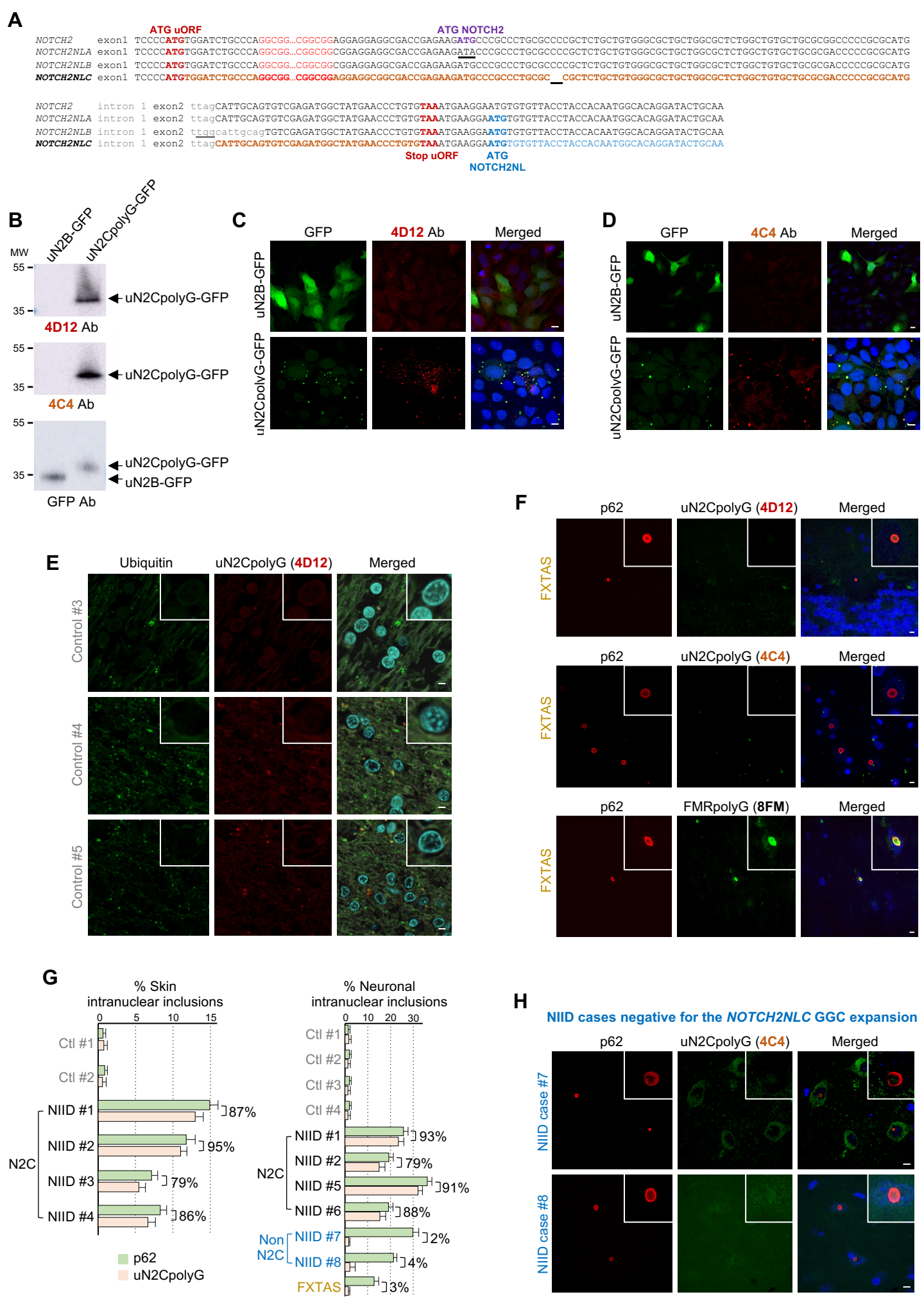


Figure S2. Related to Figure 2.

Figure S2. Related to Figure 2.
uN2polyG is present in NIID nuclear inclusions.

(**A**) Alignment of *NOTCH2* and *NOTCH2NLA*, B and C exons 1 and 2 sequences. Exon sequences are in upper cases, intronic sequences are in grey lower cases. Sequence encoding the *NOTCH2NLC* upstream (uN2C) and main (N2C) ORFs are indicated in orange and blue, respectively. ATG start and stop codons of the potential upstream ORFS are indicated in bold red, while ATG start codons of the NOTCH2NL main ORFs are indicated in bold blue. Of interest, NOTCH2NL proteins are likely cytosolic, as they start in exon 2 and thus lack NOTCH2 peptide signal sequence encoded by the exon 1. Absence of peptide signal in NOTCH2NL proteins is caused by different mechanisms, as the corresponding *NOTCH2* ATG start codon in exon 1 is absent in *NOTCH2NLA* and out of frame in *NOTCH2NLB* and *NOTCH2NLC* due to either mutation of the 3' splice site of *NOTCH2NLB* intron 1 or deletion of 2 nucleotides in *NOTCH2NLC* exon 1. Mutations of *NOTCH2NLA* ATG and *NOTCH2NLB* 3' splice site as well as the 2-nts deletion within *NOTCH2NLC* exon 1 are underlined.

(**B**) Immunoblot of uN2B-GFP or uN2CpolyG-GFP transfected HEK293 cells with 4C4, 4D12 and anti-GFP antibodies.

(**C**, **D**) GFP fluorescence and immunofluorescence of uN2B-GFP and uN2CpolyG-GFP transfected HEK293 cells using either 4D12 (**C**) or 4C4 (**D**) antibody.

(**E**) Control immunofluorescence against uN2CpolyG (4D12 antibody) and ubiquitin on brain sections of control individuals.

(**F**) Immunofluorescence against p62 and either uN2CpolyG (4D12 or 4C4 antibody) or FMRpolyG (8FM antibody) on brain sections of a FXTAS case, carrier of an expansion of CGG repeats within the 5'UTR of the *FMR1* gene.

(**G**) Quantification of p62 or uN2CpolyG-positive intranuclear inclusions per 100 nuclei in skin (left panel) or brain (right panel) sections of control, FXTAS or NIID (carrier or not of an expansion of GGC repeats within *NOTCH2NLC*) individuals. Brackets indicate the percentage of co-localization between p62- and uN2polyG-positive intranuclear inclusions. At least 100 nuclei were counted per individual.

(**H**) Immunofluorescence against p62 and uN2CpolyG (4C4 antibody) on brain sections of two European NIID cases negative for the *NOTCH2NLC* GGC mutation.

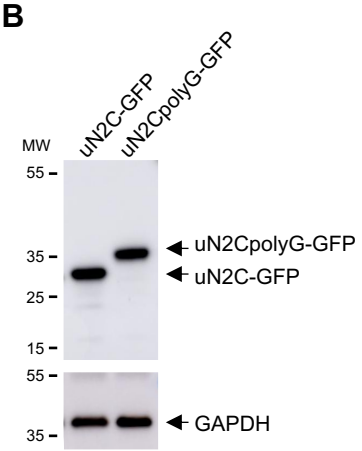
Scale bars, 10 µm. Nuclei were counterstained with DAPI.

A

uN2C HA :
MWICP **(G)12x or 100x** DREDARPAPLCCGRCWRSGCAARPPRMHCSVEMAMNPV GSLYPYDVPDYAA.

uN2C GFP :
MWICP **(G)12x or 100x** DREDARPAPLCCGRCWRSGCAARPPRMHCSVEMAMNPV GSVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPW
PTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGS
VQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK.

B



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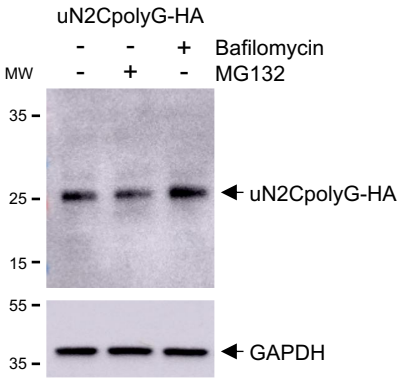

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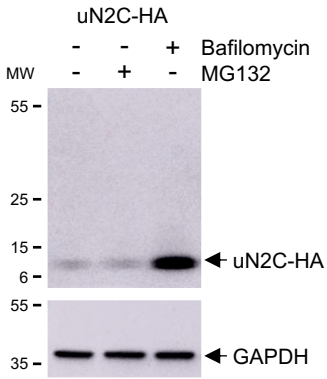

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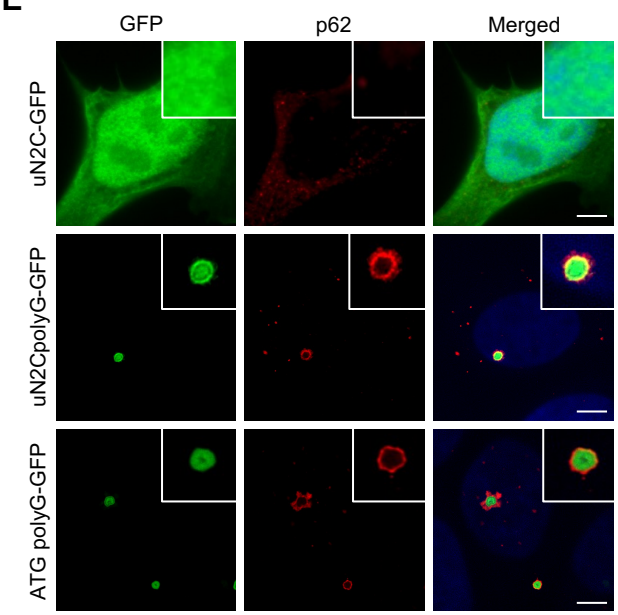
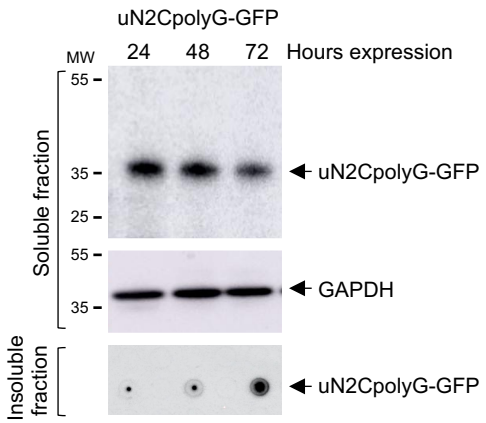
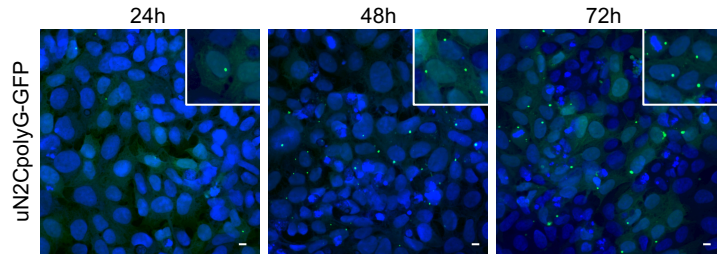


F



G



H

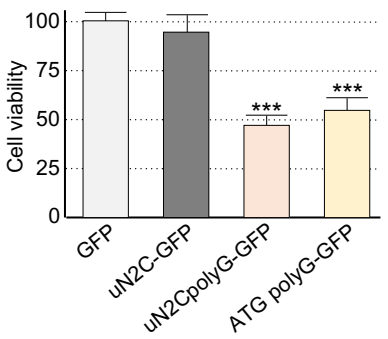


Figure S3. Related to Figure 3.

Figure S3. Related to Figure 3.
Expression of uN2CpolyG is toxic in cell culture.

(**A**) Sequences of the uN2C and uN2CpolyG-HA or -GFP tagged proteins.

(**B**) Immunoblot against the GFP or the GAPDH of proteins extracted from HEK293 cells transfected for 24 hours with uN2C-GFP or uN2CpolyG-GFP.

(**C, D**) Immunoblot against the HA tag or the GAPDH of proteins extracted from HEK293 cells transfected for 24 hours with either uN2CpolyG-HA (C) or uN2C-HA (D) and treated with or without MG132 and/or Bafilomycin A1 for 15 hours.

(**E**) GFP fluorescence and immunofluorescence against p62 of U2OS cells transfected with constructs expressing either the GFP, uN2C-GFP, uN2CpolyG-GFP or ATG polyG-GFP. Scale bars, 5 μm. Nuclei were counterstained with DAPI.

(**F**) Immunoblot on whole cell extract (soluble fraction) or dot blot on urea-treated pellet (insoluble fraction) against the GFP of HEK293 cells transfected with uN2CpolyG-GFP for 24, 48 and 72 hours.

(**G**) GFP fluorescence of U2OS cells expressing uN2CpolyG-GFP for 24, 48 and 72 hours. Scale bars, 10 μm. Nuclei were counterstained with DAPI.

(**H**) Cell viability of GT1-7 neuronal cells transfected for 24 hours with either the GFP, uN2C-GFP, uN2CpolyG-GFP or ATG polyG-GFP. Error bars indicate SEM. Student t-test, *** indicates $p<0.001$.

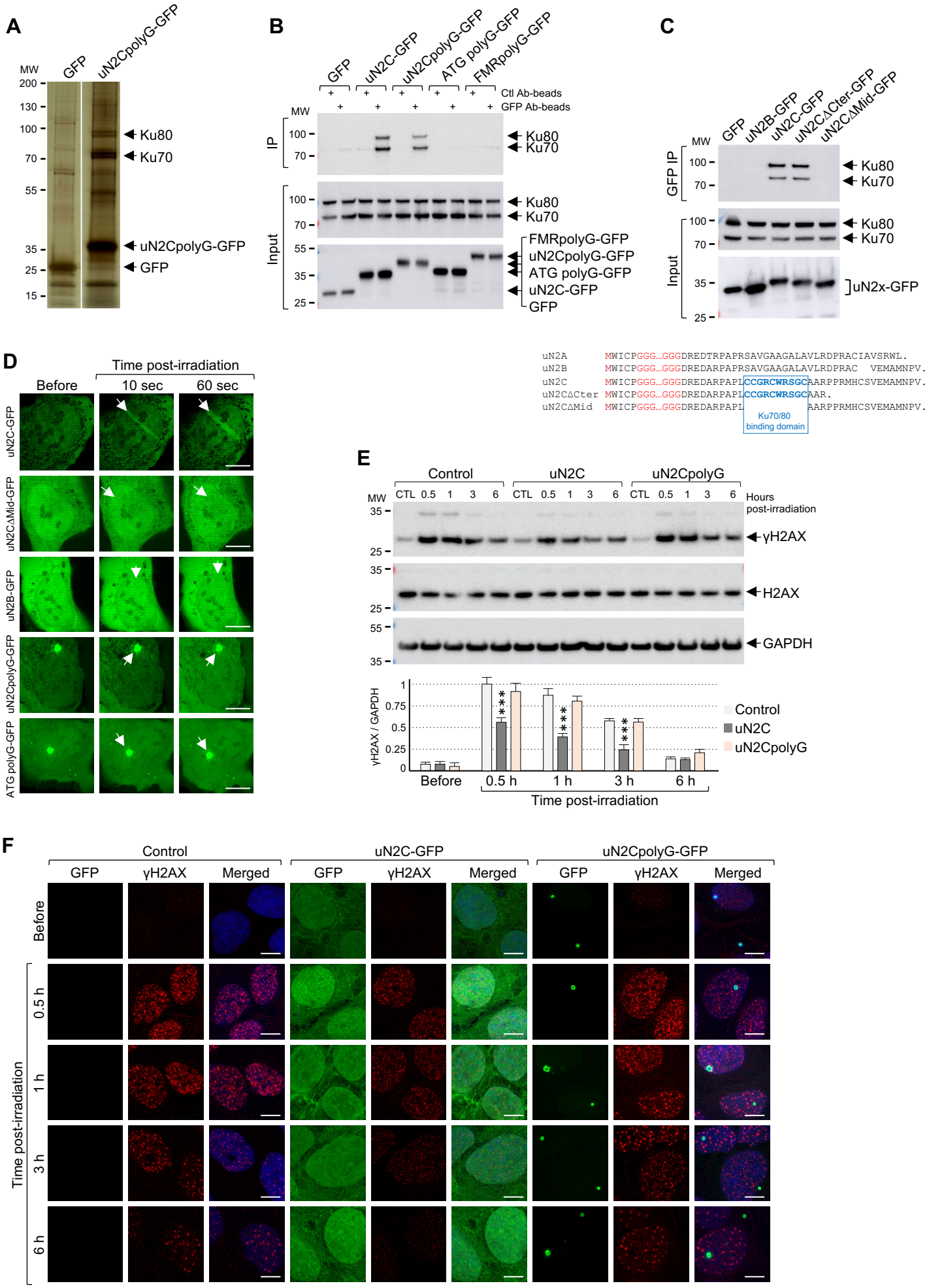


Figure S4. Related to Figure 3.

Figure S4. Related to Figure 3.
The uN2C control protein promotes DNA repair

(**A**) Silver staining of the proteins captured by GFP immunoprecipitation from HEK293 cells transfected for 24 hours with either GFP or uN2CpolyG-GFP.

(**B**) Immunoblot against endogenous Ku70 and Ku80 of either control antibody (anti-HA) or GFP immunoprecipitated proteins (IP) or whole cell lysate (Input) from HEK293 cells transfected for 24 hours with either GFP, uN2C-GFP, uN2CpolyG-GFP, ATG polyG-GFP or FMRpolyG-GFP.

(**C**) Upper panel, immunoblot against endogenous Ku70 and Ku80 of GFP immunoprecipitated proteins or whole cell lysate from HEK293 cells transfected for 24 hours with either GFP, uN2B-GFP or mutants of uN2C-GFP. Lower panel, sequence alignment of the upstream ORFs of *NOTCH2NLA*, *B* and C wild type or mutant constructs. The uN2C amino acid sequence important for binding to Ku proteins is indicated in bold blue.

(**D**) U2OS cells transfected 24 hours with either uN2C-GFP, mutant uN2CΔMid-GFP, uN2B-GFP, uN2CpolyG-GFP or ATG polyG-GFP were subjected to laser micro-irradiation. White arrows indicate laser stripes. Scale bars, 10 μm. Nuclei were counterstained with DAPI.

(**E, F**) Immunoblot against H2AX, γH2AX or the GAPDH (E) or GFP fluorescence and immunofluorescence against γH2AX (F) of control cells or U2OS stably expressing either uN2C-GFP or uN2CpolyG-GFP, X-ray irradiated for 10 minutes, and let to recover for 30 minutes, 1, 3 or 6 hours. Lower panel, quantification of γH2AX / GAPDH ratio before and after X-ray irradiation. Error bars indicate SEM. Student t-test, *** indicates $p<0.001$. Scale bars, 10 μm. Nuclei were counterstained with DAPI.

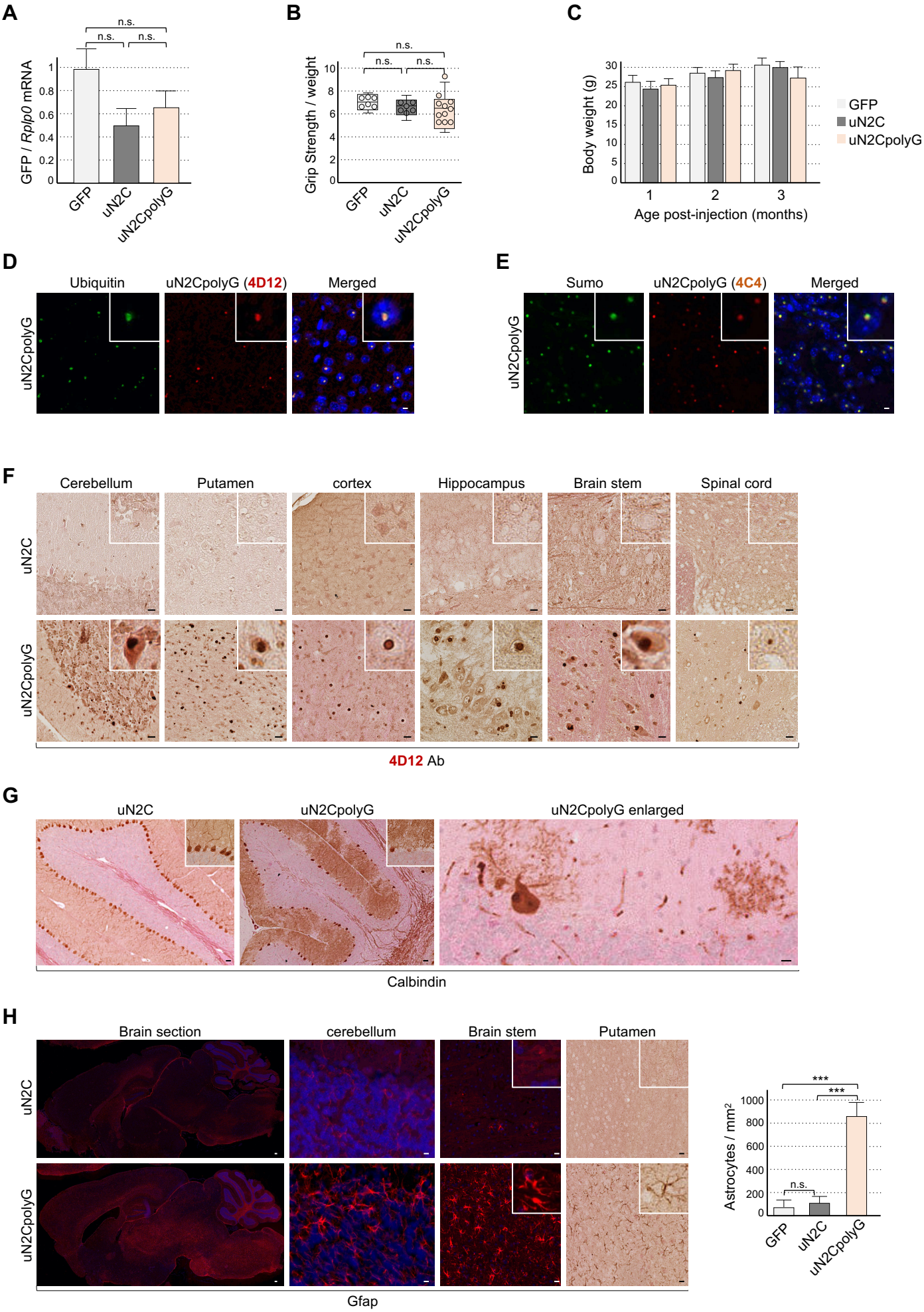


Figure S5. Related to Figure 4.

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Expression of uN2CpolyG is pathogenic in animal.

(**A**) RT-qPCR analysis of GFP expression relative to *Rplp0* mRNA in brain of AAV2/PHP.eB GFP (n=3), uN2C-GFP (n=3) or uN2CpolyG-GFP (n=3) injected mice.

(**B**) Grip test. Maximal force relative to mouse body weight exerted to releases AAV2/PHP.eB GFP (n=6), uN2C-GFP (n=6) and uN2CpolyG-GFP (n=11) injected male mice holding a grid with their forepaws and tested 3 months post-injection. Box-and-whisker plot, box upper and lower limits represent 25th and 75th percentiles, whiskers represent minimum and maximum values and the horizontal line across the box represents the median.

(**C**) Body weight at 1, 2 and 3 months post-injection of AAV2/PHP.eB GFP (n=6), uN2C-GFP (n=6) and uN2CpolyG-GFP (n=11) injected male mice.

(**D**, **E**) Immunofluorescence against uN2CpolyG using either the 4D12 (D) or 4C4 (E) antibody and ubiquitin (D) or sumo (E) on putamen areas of uN2CpolyG-GFP expressing mice scarified 2 months after AAV injection. Scale bars, 10 µm. Nuclei were counterstained with DAPI.

(**F**) Immunohistochemistry against uN2CpolyG of cerebellum, putamen, cortical, hippocampal, brain stem and spinal cord areas of uN2C-GFP and uN2CpolyG-GFP expressing mice scarified 3 months post AAV injection. Sections were counterstained with hematoxylin and eosin. Scale bars, 20 µm.

(**G**) Calbindin immunohistochemistry of cerebellum areas of uN2C-GFP and uN2CpolyG-GFP expressing mice scarified 4 months post AAV injection. Scale bars, 10 µm.

(**H**) Left panel, immunofluorescence or immunohistochemistry against GFAP of brain sections of uN2C-GFP and uN2CpolyG-GFP expressing mice scarified 3.5 months post AAV injection. IF, nuclei were counterstained with DAPI. IHC, sections were counterstained with hematoxylin and eosin. Scale bars, 10 µm. Right panel, quantification of Gfap-positive cells in the putamen of GFP (n=4), uN2C-GFP (n=4) or uN2CpolyG-GFP (n=4) expressing mice. Bar graphs indicate standard error of the mean (SEM). Student t-test, *** indicates $p<0.001$.

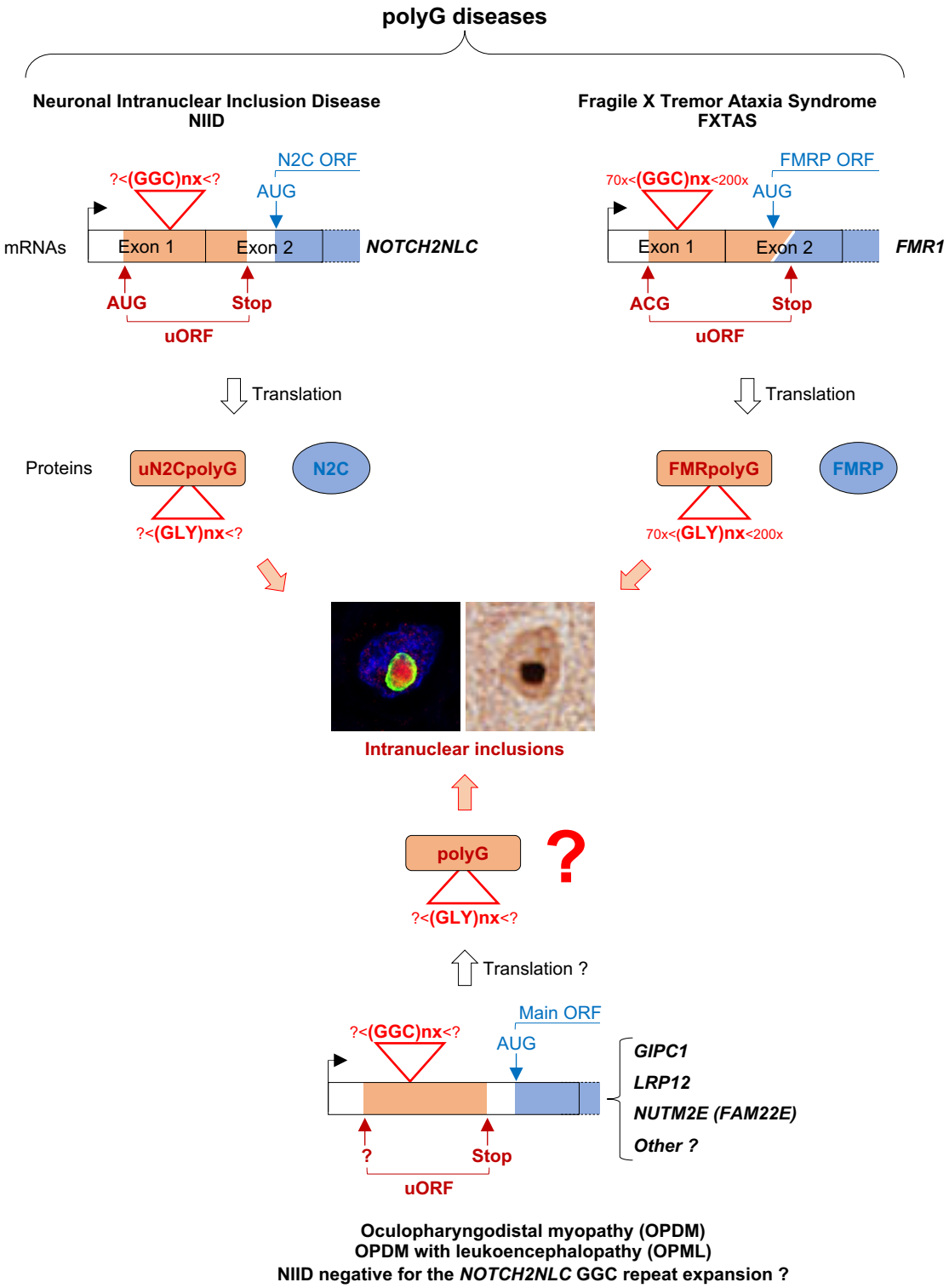

Figure S6. Related to Figure 4.
Model of polyG diseases.

Expanded GGC repeats embedded within upstream ORFs located into *FMR1* and *NOTCH2NLC* 5'UTR are translated into polyglycine containing proteins, FMRpolyG and uN2CpolyG, which are toxic and form p62-positive intranuclear inclusions in FXTAS and NIID, respectively. A similar GGC repeat translation mechanism into yet to discover polyglycine proteins may occur in OPDM and OPML.

Patient	Gender	Age at onset	Duration (months)	Family history	Initial symptom	GGC repeats,	Reference
NIID #1	Female	68	<1	Sporadic	Dizziness & vomiting	102 *NOTCH2NLC*	-
NIID #2	Male	60	12	Sporadic	Paroxysmal encephalopathy	142 *NOTCH2NLC*	Deng et al., 2019
NIID #3	Female	62	60	Familial	Tremor	n.a.	-
NIID #4	Male	63	24	Sporadic	Urinary incontinence	115 *NOTCH2NLC*	-
NIID #5	Male	7	72	Sporadic	Tremor, ataxia,	n.a.	McFadden et al., 2005
NIID #6	Male	30	37	Familial	Muscle weakness	162 *NOTCH2NLC*	Sone et al., 2019
NIID #7	Female	65	3	Sporadic	Memory problems	No expansions *FMR1, NOTCH2NLC*	Cupidi et al., 2019
NIID #8	Male	50	16	Sporadic	Behavioral changes	No expansions *FMR1, NOTCH2NLC*	-
FXTAS	Male	43	22	Familial	Memory problems, aphasia	77 *FMR1*	-
CTL #1	Female	53	-	-	Memory decline	-	-
CTL #2	Female	51	-	-	Urinary difficulties	-	-
CTL #3	Male	66	-	-	Sudden death	-	-
CTL #4	Male	70	-	-	Interstitial pneumonia	-	-
CTL #5	Female	67	-	-	polymyositis	-	-

n.a. Not available

Table S1. Related to Figure 2. Patient information.

Accession	Description	MW [kDa]	Coverage [%]	# Peptides	# PSMs	PSM GFP	PSM uN2CpolyG	uN2cpolyG / GFP
P12956	X-ray repair cross-complementing protein 6 OS=Homo sapiens OX=9606 GN=XRCC6 PE=1 S	70	54	34	460	1	94	94
P13010	X-ray repair cross-complementing protein 5 OS=Homo sapiens OX=9606 GN=XRCC5 PE=1 S	83	76	43	522	1	123	92
P78527	DNA-dependent protein kinase catalytic subunit OS=Homo sapiens OX=9606 GN=PRKDC PE	469	9	24	48	1	36	36
Q16531	DNA damage-binding protein 1 OS=Homo sapiens OX=9606 GN=DDB1 PE=1 SV=1	127	28	23	72	1	41	31
O14744	Protein arginine N-methyltransferase 5 OS=Homo sapiens OX=9606 GN=PRMT5 PE=1 SV=	73	54	26	148	3	32	12
Q9BS26	Endoplasmic reticulum resident protein 44 OS=Homo sapiens OX=9606 GN=ERP44 PE=1 SV	47	48	17	115	3	27	10
P27708	CAD protein OS=Homo sapiens OX=9606 GN=CAD PE=1 SV=3	243	28	42	131	3	26	10
P13639	Elongation factor 2 OS=Homo sapiens OX=9606 GN=EEF2 PE=1 SV=4	95	57	41	211	6	53	9
Q99714	3-hydroxyacyl-CoA dehydrogenase type-2 OS=Homo sapiens OX=9606 GN=HSD17B10 PE=1	27	50	8	39	1	9	9
P61962	DDB1- and CUL4-associated factor 7 OS=Homo sapiens OX=9606 GN=DCAF7 PE=1 SV=1	39	29	8	46	1	12	9
P11586	C-1-tetrahydrofolate synthase, cytoplasmic OS=Homo sapiens OX=9606 GN=MTHFD1 PE=1	102	12	10	34	1	9	9
P30153	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform OS=H	65	42	20	77	2	20	9
P49411	Elongation factor Tu, mitochondrial OS=Homo sapiens OX=9606 GN=TUFM PE=1 SV=2	50	37	13	53	2	14	8
P35579	Myosin-9 OS=Homo sapiens OX=9606 GN=MYH9 PE=1 SV=4	226	12	17	54	1	11	8
Q92945	Far upstream element-binding protein 2 OS=Homo sapiens OX=9606 GN=KHSRP PE=1 SV=	73	37	20	88	2	19	8
Q9H845	Acyl-CoA dehydrogenase family member 9, mitochondrial OS=Homo sapiens OX=9606 GN=	69	16	8	24	1	8	8
Q9BQA1	Methylosome protein 50 OS=Homo sapiens OX=9606 GN=WDR77 PE=1 SV=1	37	33	10	61	2	13	8
P07237	Protein disulfide-isomerase OS=Homo sapiens OX=9606 GN=P4HB PE=1 SV=3	57	23	11	44	1	11	8
P22102	Trifunctional purine biosynthetic protein adenosine-3 OS=Homo sapiens OX=9606 GN=GAR	108	14	9	29	1	8	8
P30048	Thioredoxin-dependent peroxide reductase, mitochondrial OS=Homo sapiens OX=9606 GN	28	51	8	59	2	16	8
O75534	Cold shock domain-containing protein E1 OS=Homo sapiens OX=9606 GN=CSDE1 PE=1 SV=	89	25	15	50	2	13	8
Q9H857	5'-nucleotidase domain-containing protein 2 OS=Homo sapiens OX=9606 GN=NT5DC2 PE=	61	21	10	31	1	10	8
P63151	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform OS=H	52	53	19	74	2	15	7
Q92973	Transportin-1 OS=Homo sapiens OX=9606 GN=TNPO1 PE=1 SV=2	102	13	8	22	1	7	7
P54136	Arginine--tRNA ligase, cytoplasmic OS=Homo sapiens OX=9606 GN=RARS PE=1 SV=2	75	24	11	46	1	10	7
P04843	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 OS=Homo sapien	69	37	15	43	2	14	7
Q13162	Peroxiredoxin-4 OS=Homo sapiens OX=9606 GN=PRDX4 PE=1 SV=1	31	58	13	82	3	19	7
P42771	Cyclin-dependent kinase inhibitor 2A OS=Homo sapiens OX=9606 GN=CDKN2A PE=1 SV=2	17	70	7	33	1	9	7
P31689	DnaJ homolog subfamily A member 1 OS=Homo sapiens OX=9606 GN=DNAJA1 PE=1 SV=2	45	36	9	47	2	12	7
Q5H9R7	Serine/threonine-protein phosphatase 6 regulatory subunit 3 OS=Homo sapiens OX=9606 (	98	28	19	54	2	16	7
P49915	GMP synthase [glutamine-hydrolyzing] OS=Homo sapiens OX=9606 GN=GMPS PE=1 SV=1	77	19	12	29	1	9	7
P63244	Receptor of activated protein C kinase 1 OS=Homo sapiens OX=9606 GN=RACK1 PE=1 SV=	35	50	9	33	1	9	7
Q9Y265	RuvB-like 1 OS=Homo sapiens OX=9606 GN=RUVBL1 PE=1 SV=1	50	28	9	44	1	9	7
P53396	ATP-citrate synthase OS=Homo sapiens OX=9606 GN=ACLY PE=1 SV=3	121	10	8	24	1	7	7
P21333	Filamin-A OS=Homo sapiens OX=9606 GN=FLNA PE=1 SV=4	281	10	17	46	1	9	7
P28482	Mitogen-activated protein kinase 1 OS=Homo sapiens OX=9606 GN=MAPK1 PE=1 SV=3	41	53	15	61	2	13	6
Q93009	Ubiquitin carboxyl-terminal hydrolase 7 OS=Homo sapiens OX=9606 GN=USP7 PE=1 SV=2	128	12	10	25	1	6	6
Q9Y3F4	Serine-threonine kinase receptor-associated protein OS=Homo sapiens OX=9606 GN=STRA	38	46	12	58	2	13	6
O60884	DnaJ homolog subfamily A member 2 OS=Homo sapiens OX=9606 GN=DNAJA2 PE=1 SV=1	46	23	7	23	1	6	6
P67775	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform OS=Homo sapien	36	56	12	61	2	15	6
P27361	Mitogen-activated protein kinase 3 OS=Homo sapiens OX=9606 GN=MAPK3 PE=1 SV=4	43	28	8	30	1	8	6
P45880	Voltage-dependent anion-selective channel protein 2 OS=Homo sapiens OX=9606 GN=VDA	32	43	8	31	1	8	6
P22234	Multifunctional protein ADE2 OS=Homo sapiens OX=9606 GN=PAICS PE=1 SV=3	47	40	14	76	3	16	6
P17812	CTP synthase 1 OS=Homo sapiens OX=9606 GN=CTPS1 PE=1 SV=2	67	26	12	48	2	12	6
Q9UK99	F-box only protein 3 OS=Homo sapiens OX=9606 GN=FBXO3 PE=1 SV=3	55	26	10	33	2	10	6
P13489	Ribonuclease inhibitor OS=Homo sapiens OX=9606 GN=RNH1 PE=1 SV=2	50	36	12	43	2	10	6
O00429	Dynamin-1-like protein OS=Homo sapiens OX=9606 GN=DNM1L PE=1 SV=2	82	19	10	24	1	8	6
P29966	Myristoylated alanine-rich C-kinase substrate OS=Homo sapiens OX=9606 GN=MARCKS PE	32	45	9	54	2	10	6
P49327	Fatty acid synthase OS=Homo sapiens OX=9606 GN=FASN PE=1 SV=3	273	32	50	206	8	46	6
P49366	Deoxyhypusine synthase OS=Homo sapiens OX=9606 GN=DHPS PE=1 SV=1	41	47	11	47	3	16	6
O95831	Apoptosis-inducing factor 1, mitochondrial OS=Homo sapiens OX=9606 GN=AIFM1 PE=1 S	67	20	9	27	1	8	6
Q9UQ80	Proliferation-associated protein 2G4 OS=Homo sapiens OX=9606 GN=PA2G4 PE=1 SV=3	44	28	8	35	1	7	6
P42704	Leucine-rich PPR motif-containing protein, mitochondrial OS=Homo sapiens OX=9606 GN=	158	12	14	29	2	9	5
P31939	Bifunctional purine biosynthesis protein PURH OS=Homo sapiens OX=9606 GN=ATIC PE=1	65	19	7	16	1	5	5
Q15654	Thyroid receptor-interacting protein 6 OS=Homo sapiens OX=9606 GN=TRIP6 PE=1 SV=3	50	26	8	16	1	5	5
P22314	Ubiquitin-like modifier-activating enzyme 1 OS=Homo sapiens OX=9606 GN=UBA1 PE=1 S	118	34	25	110	5	24	5
Q13263	Transcription intermediary factor 1-beta OS=Homo sapiens OX=9606 GN=TRIM28 PE=1 SV	89	37	16	86	3	17	5
Q8IXB1	DnaJ homolog subfamily C member 10 OS=Homo sapiens OX=9606 GN=DNAJC10 PE=1 SV=	91	17	11	31	2	10	5
P25205	DNA replication licensing factor MCM3 OS=Homo sapiens OX=9606 GN=MCM3 PE=1 SV=3	91	22	15	57	2	10	5
Q96QK1	Vacuolar protein sorting-associated protein 35 OS=Homo sapiens OX=9606 GN=VPS35 PE=	92	28	18	58	3	13	5
P29590	Protein PML OS=Homo sapiens OX=9606 GN=PML PE=1 SV=3	98	9	7	16	1	5	5
P10599	Thioredoxin OS=Homo sapiens OX=9606 GN=TXN PE=1 SV=3	12	54	6	67	3	14	5
P55060	Exportin-2 OS=Homo sapiens OX=9606 GN=CSE1L PE=1 SV=3	110	8	7	20	1	6	5
Q96J01	THO complex subunit 3 OS=Homo sapiens OX=9606 GN=THOC3 PE=1 SV=1	39	33	8	22	1	6	5
P26639	Threonine--tRNA ligase, cytoplasmic OS=Homo sapiens OX=9606 GN=TARS PE=1 SV=3	83	19	10	30	2	9	5
P05141	ADP/ATP translocase 2 OS=Homo sapiens OX=9606 GN=SLC25A5 PE=1 SV=7	33	29	8	38	2	9	5
P05023	Sodium/potassium-transporting ATPase subunit alpha-1 OS=Homo sapiens OX=9606 GN=	113	16	12	39	2	11	5
Q13185	Chromobox protein homolog 3 OS=Homo sapiens OX=9606 GN=CBX3 PE=1 SV=4	21	29	5	24	1	6	5
P31040	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial OS=Homo sapie	73	31	12	42	3	12	5

Table S2. Related to Figure 3. Proteins interacting with uN2CpolyG.
Mass spectrometry analysis of GFP-trap immunoprecipitated proteins from HEK293 cells expressing either the GFP or uN2CpolyG-GFP.