Supplementary Materials for

An orally bioavailable allele-selective mutant ATXN1 degrader ameliorates motor and cognitive deficits in a humanized SCA1 knock-in model

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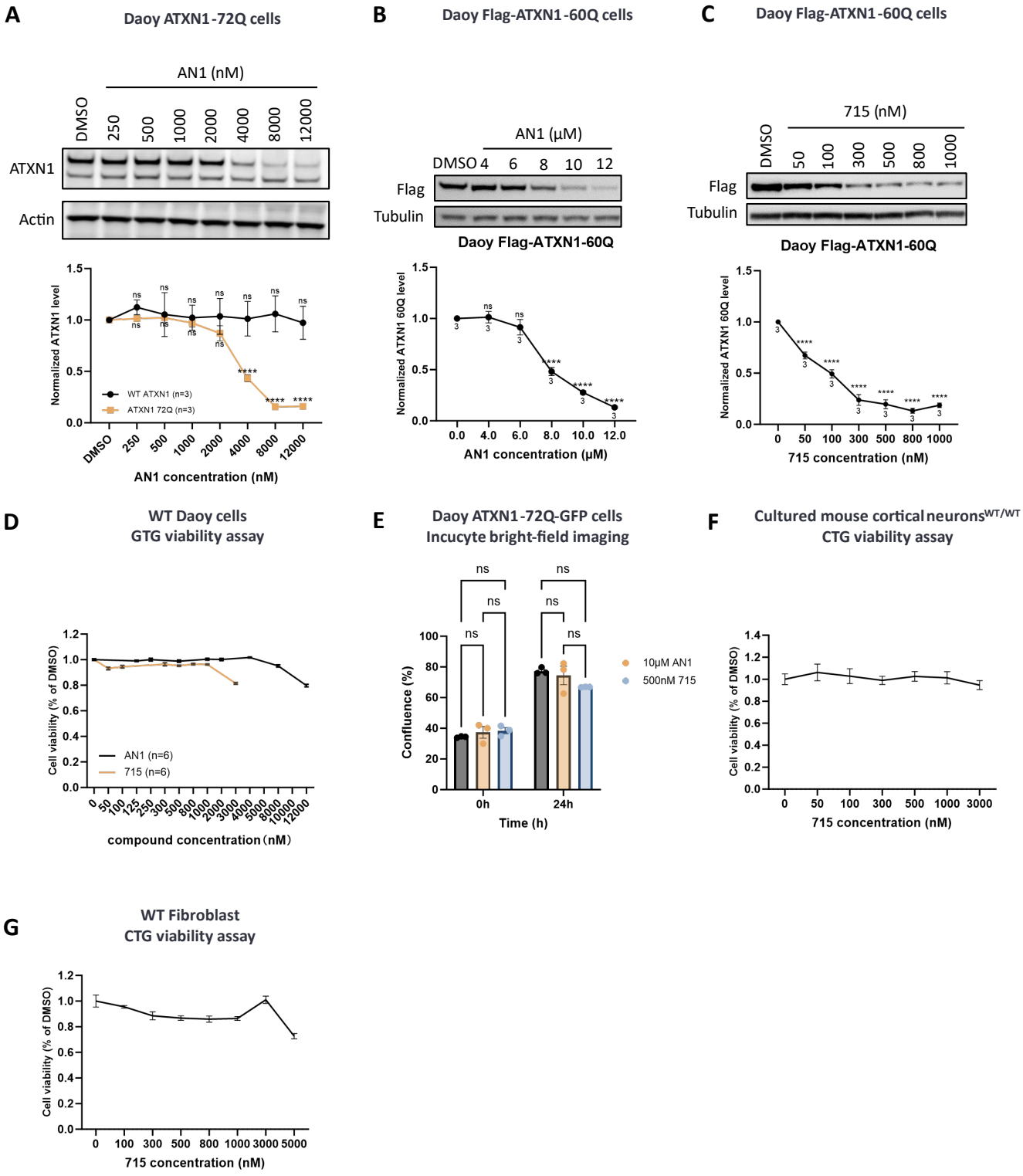


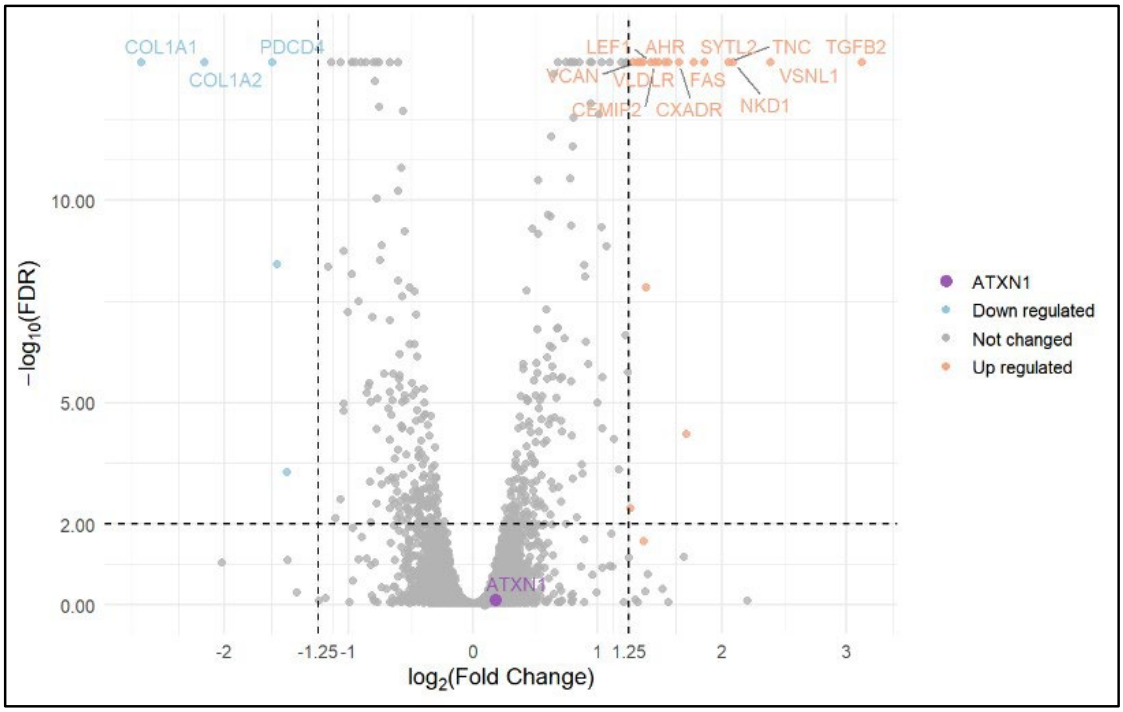
Fig. S1. relevant to Fig. 1. Supplementary characterization of AN1 and 715 activity, cytotoxicity, and proteome-wide selectivity.

(A) Low-dose AN1 response in Daoy ATXN1-72Q cells. **(B-C)** Dose-dependent reduction of ATXN1-60Q by AN1 (B) or 715 (C) in Daoy ATXN1-60Q cells. **(D)** CTG cell viability assay in Daoy cells treated with AN1 or 715. **(E)** Quantification of cell confluence in Daoy ATXN1-72Q-GFP cells treated with DMSO, 10 μM AN1, or 500 nM 715 for 24 h (n = 3 independent biological replicates). **(F)** CTG cell viability assay in WT primary cortical neurons treated with 715 (n = 5

independent biological replicates). **(G)** CTG cell viability assay in WT primary dermal fibroblasts treated with 715 ($n = 5$ independent biological replicates). For all panels, n represents the number of independent biological replicates. Data are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Data were normalized to the corresponding control group. Statistical analyses were performed using one-way ANOVA followed by Dunnett's multiple comparisons test for (A-C), and two-way ANOVA followed by Šídák's multiple comparisons test for (E).

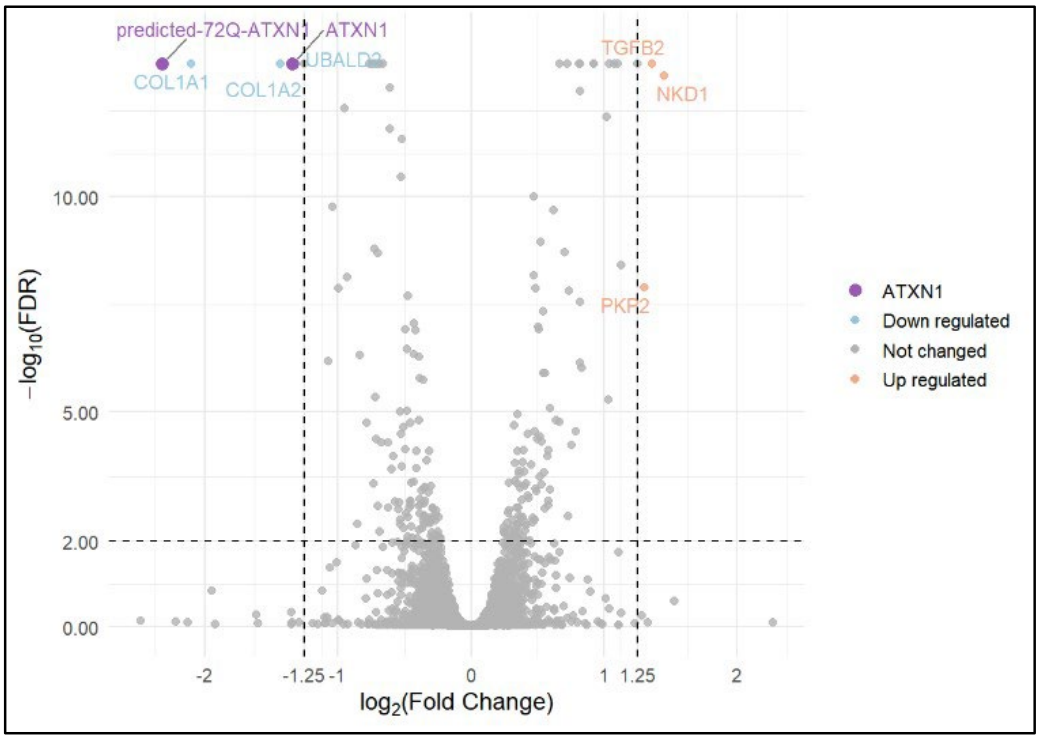
A

WT Daoy cells: 715 vs DMSO proteomics, 24 h



B

Daoy ATXN1-72Q cells: 715 vs DMSO proteomics, 24 h



C

WB-based ATXN1-72Q projection for proteomics

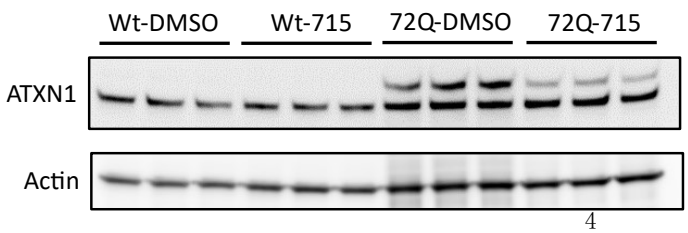


Fig. S2. relevant to Fig. 1. Proteomic profiling of WT and ATXN1-72Q Daoy cells following 715 treatment.

(A, B) Volcano plots showing proteome-wide changes in WT Daoy cells (A) and Daoy ATXN1-72Q cells (B) after treatment with 300 nM 715 for 24 h compared with DMSO controls (n = 3 biological replicates per condition). Differentially abundant proteins were identified using a DEP/limma-based workflow with Benjamini–Hochberg false discovery rate (FDR) correction. Proteins with FDR < 0.05 and $|\log_2(\text{fold change})| > 1.25$ were classified as significantly altered. Selected differentially abundant proteins are labeled, and ATXN1-related signals are highlighted in purple. **(C)** Representative Western blot of the samples analyzed in (B), used to calculate the mutant-to-WT ATXN1 ratio for ATXN1-72Q proteomic abundance projection.

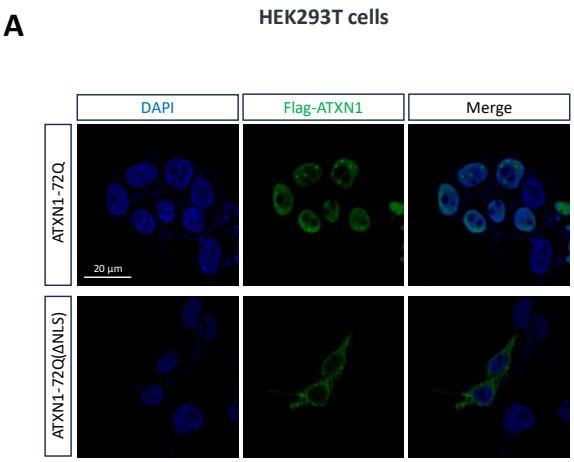


Fig. S3. relevant to Fig. 2. Validation of ATXN1-72Q ΔNLS cytoplasmic redistribution.

(A) Immunofluorescence (IF) staining of overexpressed Flag-ATXN1 72Q and Flag-ATXN1 72Q (ΔNLS) in HEK293T cells. DAPI marks nuclei and Flag staining marks ATXN1 localization.

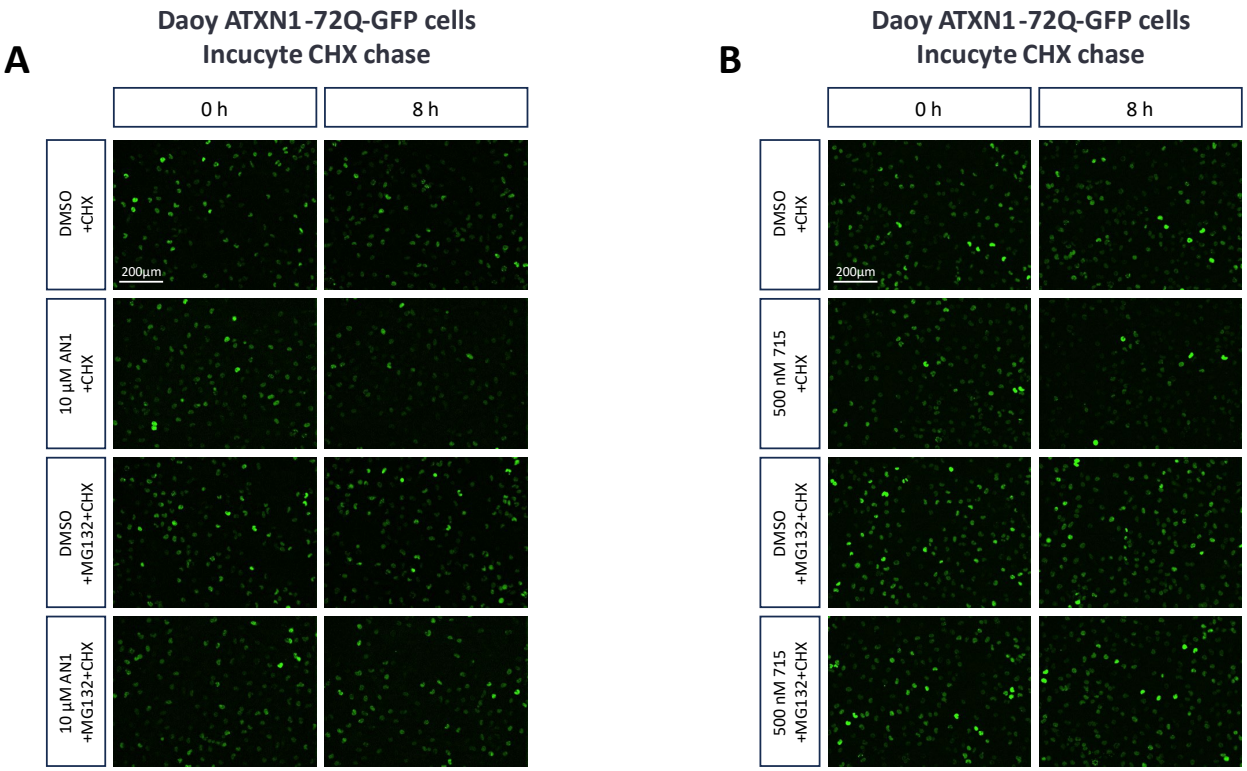


Fig. S4. relevant to Fig. 3. Representative live-cell imaging of ATXN1-72Q-GFP turnover following AN1 or 715 treatment.

(A) Representative Incucyte images of Daoy ATXN1-72Q-GFP cells at the beginning and end of CHX chase following 10 µM AN1 treatment, with or without MG132. **(B)** Representative Incucyte images of Daoy ATXN1-72Q-GFP cells at the beginning and end of CHX chase following 500 nM 715 treatment, with or without MG132.

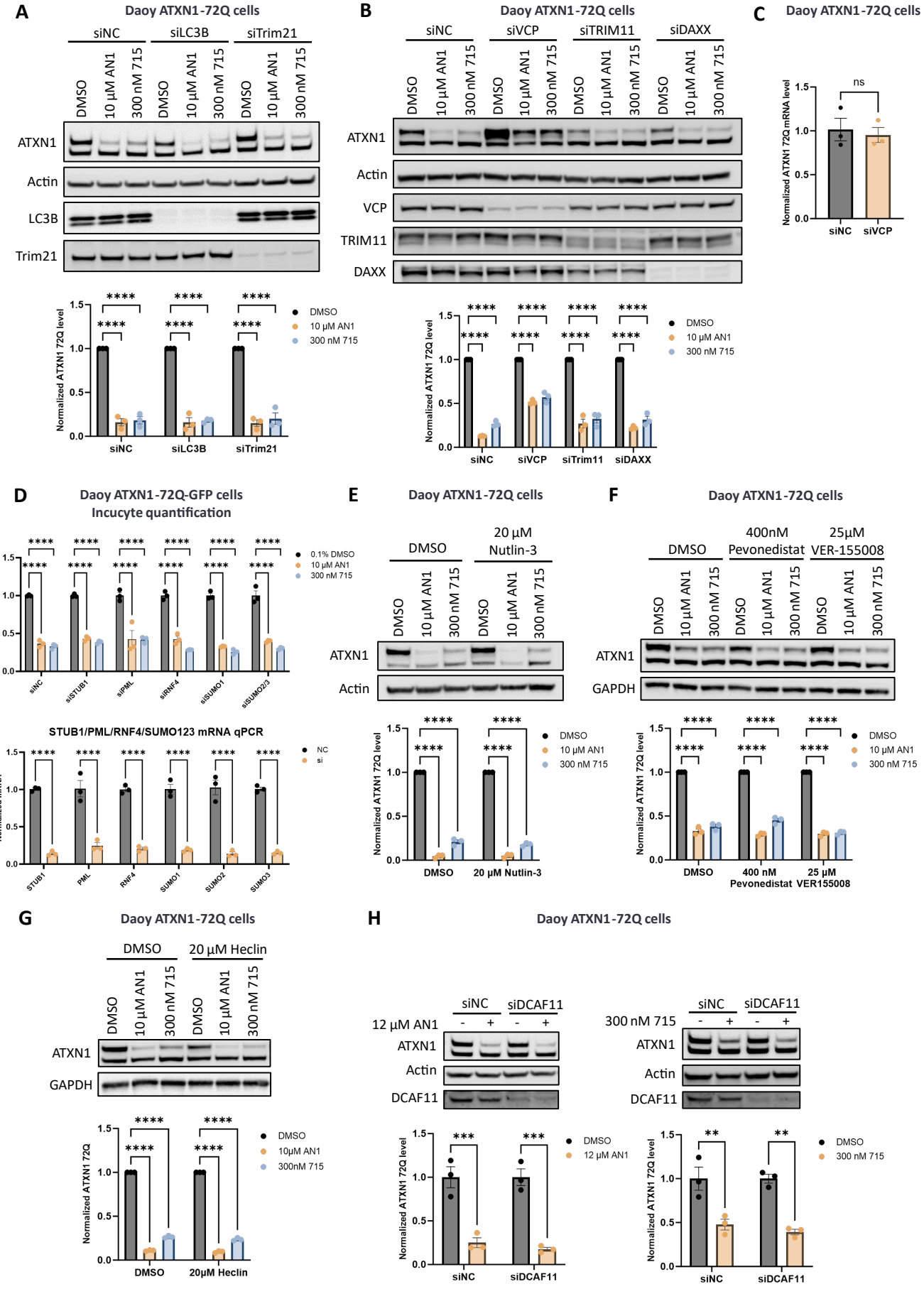


Fig. S5. relevant to Fig. 3. AN1- and 715-mediated mutant ATXN1 degradation was independent of multiple candidate clearance pathways, E3 ligases, chaperones, and unfoldases.

(A) Western blot analysis of 10 μM AN1- and 300 nM 715-mediated ATXN1 reduction following siRNA-mediated knockdown of LC3B or TRIM21 in Daoy ATXN1-72Q cells. **(B)** Western blot analysis of 10 μM AN1- and 300 nM 715-mediated ATXN1 reduction following knockdown of VCP, TRIM11, or DAXX in Daoy ATXN1-72Q cells. **(C)** qPCR analysis of ATXN1-72Q mRNA levels following VCP knockdown. **(D)** Incucyte GFP analysis of 10 μM AN1- and 300 nM 715-mediated ATXN1-72Q-GFP reduction (Top) and qPCR validation (Bottom) following knockdown of STUB1, PML, RNF4, or SUMO1/2/3. **(E)** Western blot analysis of 10 μM AN1- and 300 nM 715-mediated ATXN1 reduction in Daoy ATXN1-72Q cells co-treated with the MDM2 inhibitor Nutlin-3. **(F)** Western blot analysis of 10 μM AN1- and 300 nM 715-mediated ATXN1 reduction in Daoy ATXN1-72Q cells co-treated with the CRL E3 blocker pevonedistat or Hsp70 inhibitor VER-155008. **(G)** Western blot analysis of 10 μM AN1- and 300 nM 715-mediated ATXN1 reduction in Daoy ATXN1-72Q cells co-treated with the HECT E3 inhibitor Heclin. **(H)** Western blot analysis of 12 μM AN1- and 300nM 715-mediated ATXN1 reduction following DCAF11 knockdown in Daoy ATXN1-72Q cells. For all panels, n=3 independent biological replicates. Data are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Data were normalized to the corresponding control group. Statistical analyses were performed using two-tailed unpaired t-test for (C), and two-way ANOVA followed by Šídák's multiple comparisons test for others.

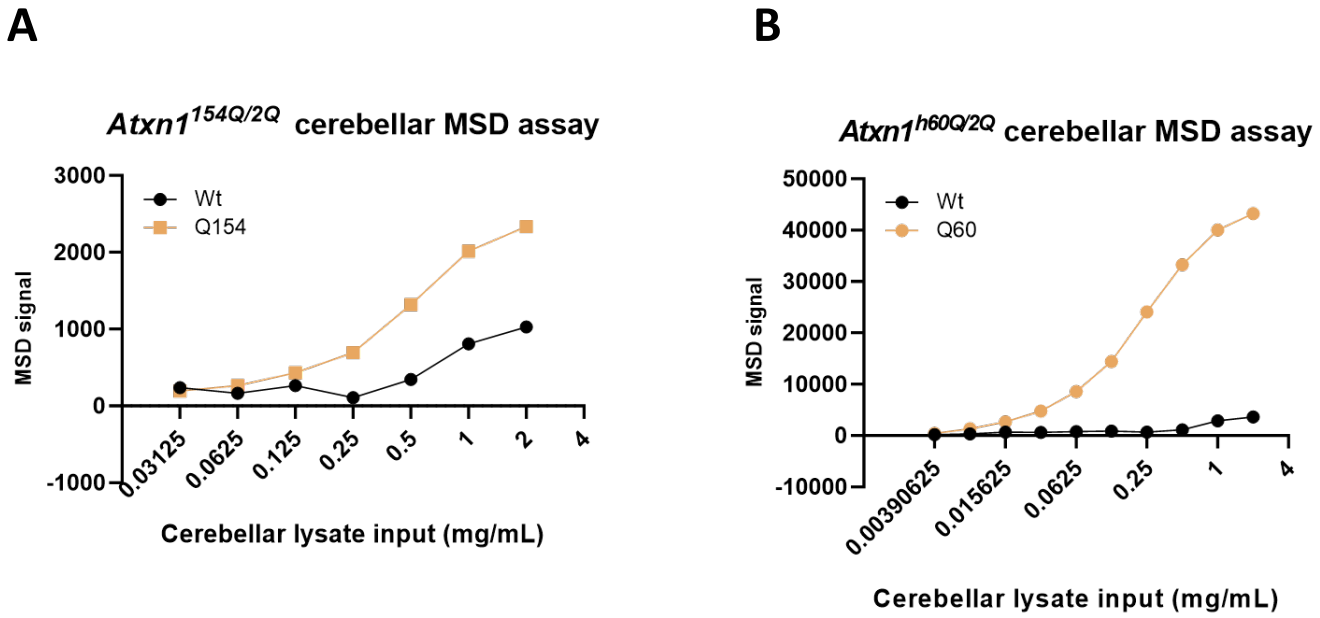
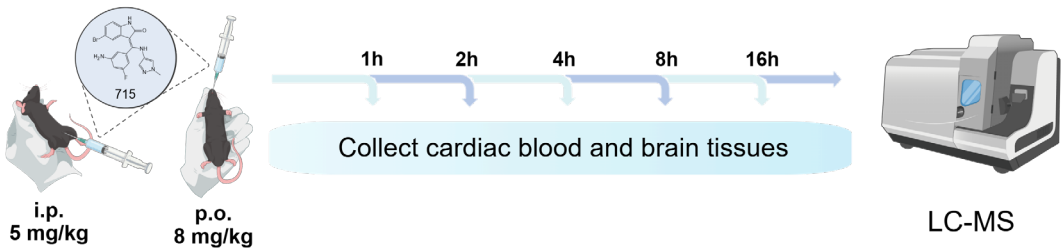


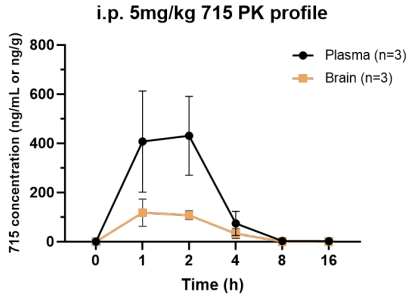
Fig. S6. relevant to Fig. 5. Validation of the linear dynamic range of the mutant ATXN1 MSD assay in SCA1 mouse cerebellar samples.

(A) Linear dynamic range of MSD assay for *Atxn1*$^{154Q/2Q}$ knock-in mice cerebellar samples (background-subtracted). **(B)** Linear dynamic range of the MSD assay for *Atxn1*$^{h60Q/2Q}$ knock-in mice cerebellar samples (background-subtracted).

A



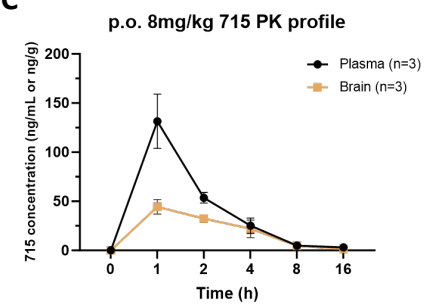
B



Pharmacokinetic parameters of 715 after i.P. administration in mouse

	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{last} (h*ng/mL(g))	AUC_{INF_obs} (h*ng/mL(g))	MRT_{INF_obs} (h)
Plasma	2.77	2.00	431	1136	1145	2.46
Brain	1.40	1.00	118	331	332	2.29

C



Pharmacokinetic parameters of 715 after p.o administration in mouse

	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{last} (h*ng/mL(g))	AUC_{INF_obs} (h*ng/mL(g))	MRT_{INF_obs} (h)
Plasma	1.57	1.00	132	274	282	2.63
Brain	2.12	1.00	44	159	174	3.72

Fig. S7. relevant to Fig. 5-7. Plasma and brain pharmacokinetic profiles of 715 following intraperitoneal versus oral administration.

(A) Experimental design for single-dose pharmacokinetic analysis of 715 in WT mice following i.p. or oral administration. Plasma and brain samples were collected at the indicated time points. Schematic created with BioGDP.com[1]. **(B)** Plasma and brain concentration–time profiles and corresponding pharmacokinetic parameters of 715 following a single i.p. dose of 5 mg/kg. **(C)** Plasma and brain concentration-time profiles and corresponding pharmacokinetic parameters of 715 following a single oral dose of 8 mg/kg. Data are presented as mean ± SEM, and n represents the number of animals analyzed per group.

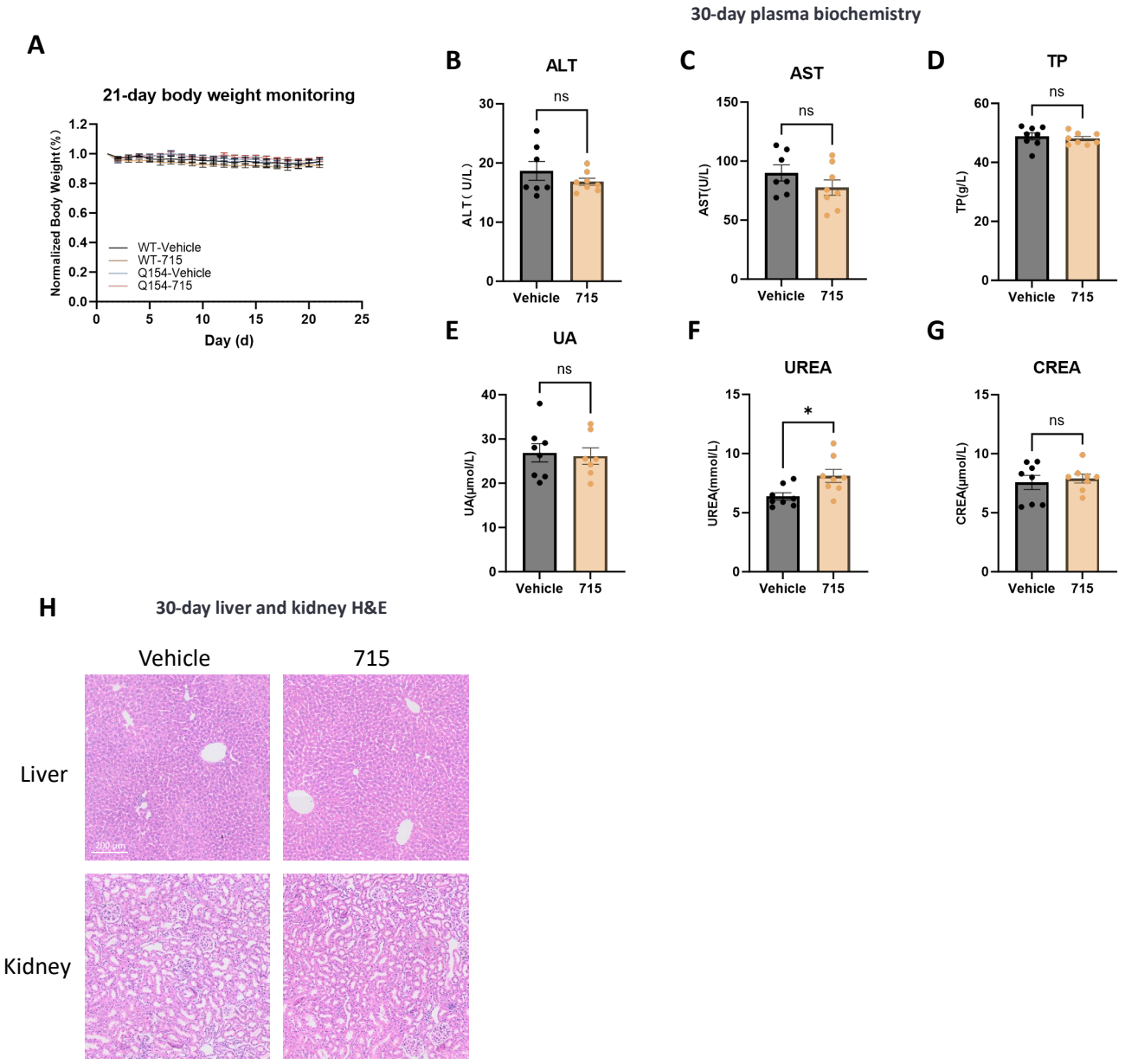
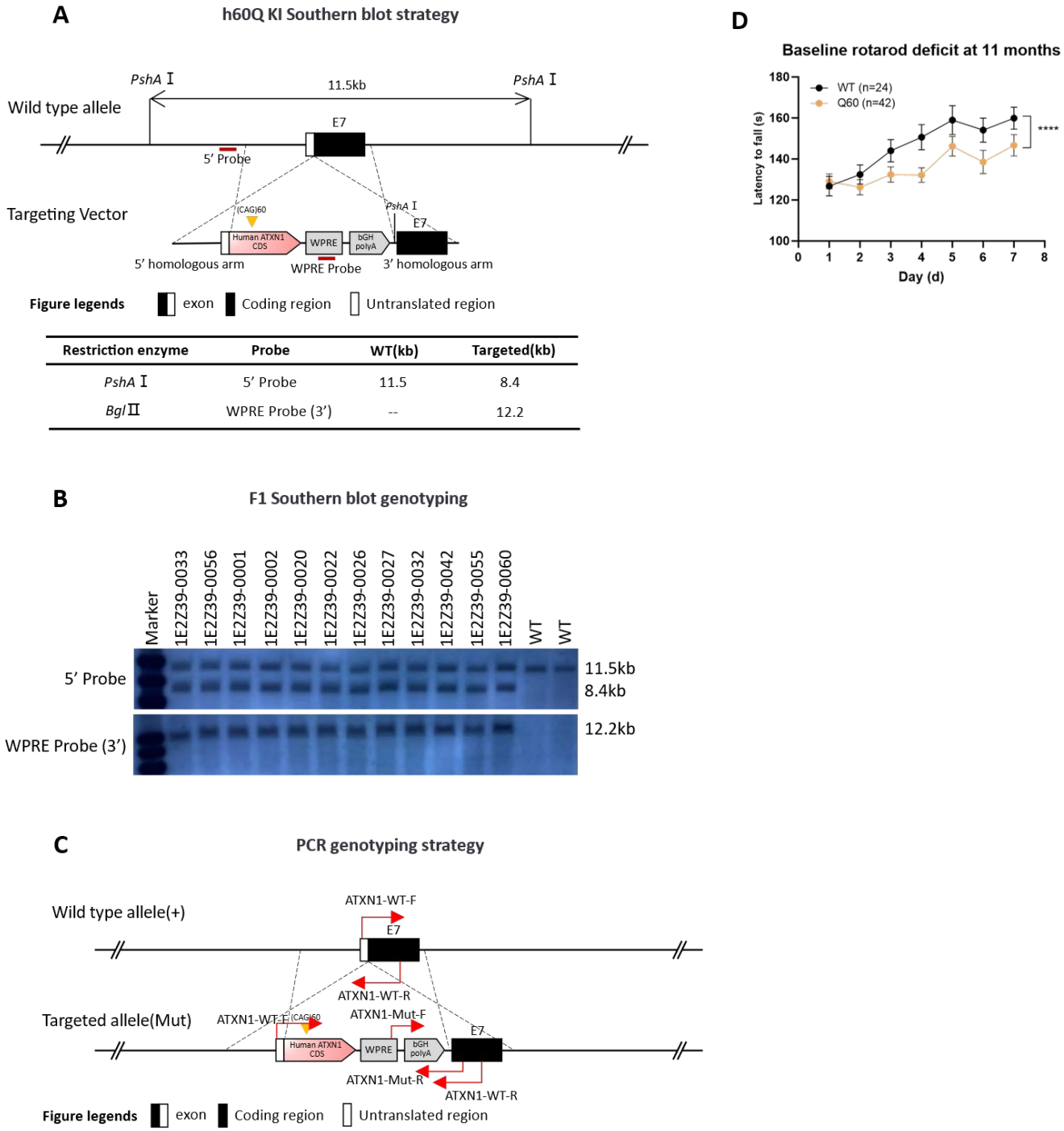


Fig. S8. relevant to Fig. 5. Prolonged intraperitoneal administration of 715 exhibited a favorable in vivo safety and systemic toxicity profile.

(A) Relative body weight changes of WT and *Atxn1*$^{154Q/2Q}$ mice during 21-day i.p. administration of vehicle or 5 mg/kg 715 (n = 8 mice per group). **(B-G)** Plasma biochemical analysis of WT mice following 30-day i.p. administration of vehicle or 715 (n = 8 mice per group). **(H)** Representative hematoxylin and eosin (H&E) staining of liver and kidney sections from WT mice following 30-day i.p. administration of vehicle or 715. For quantitative analyses, n represents the number of animals analyzed per group. Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Statistical analyses were performed using two-tailed unpaired t-test for (B-G).



Restriction enzyme	Probe	WT(kb)	Targeted(kb)
PshA I	5' Probe	11.5	8.4
*Bgl*II	WPRE Probe (3')	--	12.2

Fig. S9. relevant to Fig. 6. Generation, molecular validation, and phenotypic characterization of a novel *Atxn1*$^{h60Q/2Q}$ knock-in mouse model.

(A) Schematic design of the Southern blot probe used for screening and identifying the *Atxn1*$^{h60Q/2Q}$ knock-in line. **(B)** Representative Southern blot analysis for genotype identification of F1 generation *Atxn1*$^{h60Q/2Q}$ mice. **(C)** Genotyping primer design for identification of homozygous and heterozygous *Atxn1*$^{h60Q/2Q}$ mice. **(D)** Baseline accelerating rotarod performance of 11-month-old *Atxn1*$^{h60Q/2Q}$ mice. For quantitative analyses, n represents the number of animals analyzed per group. Data are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Statistical analyses were performed using two-way ANOVA followed by Šídák's multiple comparisons test for (D).

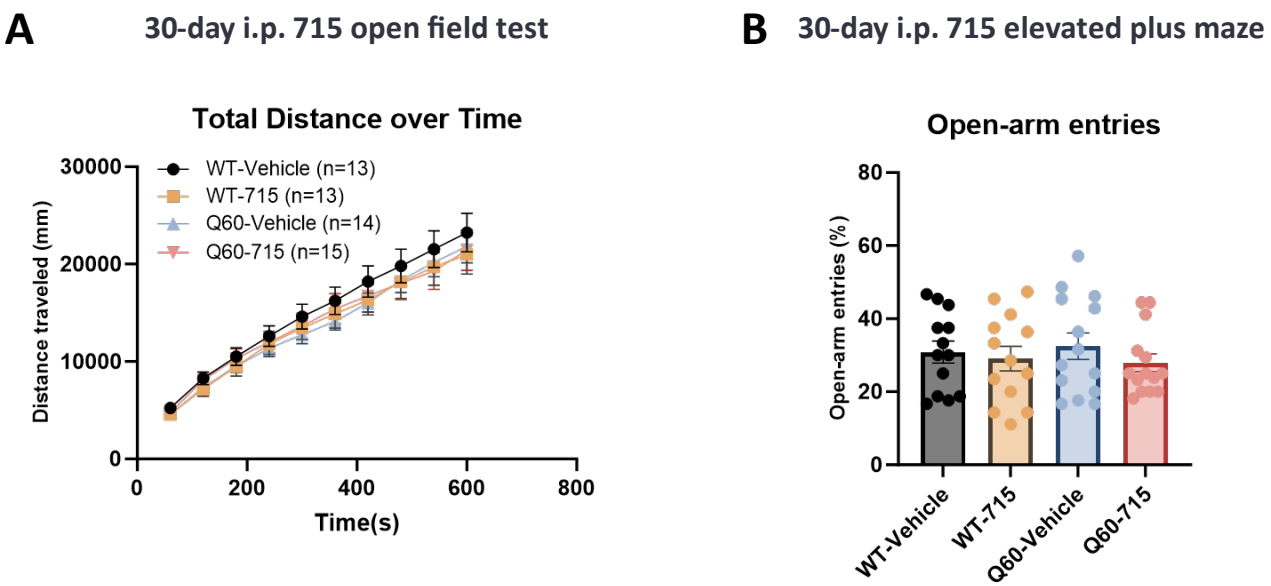


Fig. S10. relevant to Fig. 6. Chronic 715 treatment did not induce hyperactivity or anxiety-like behaviors in the *Atxn1*$^{h60Q/2Q}$ mice.

(A) Open field test analysis of 13-month-old WT and *Atxn1*$^{h60Q/2Q}$ mice following daily i.p. administration of vehicle or 5 mg/kg 715 for 30 days, assessing general locomotor activity and hyperactivity-related behavioral changes. **(B)** Elevated plus maze analysis of 13-month-old WT and *Atxn1*$^{h60Q/2Q}$ mice following daily i.p. administration of vehicle or 5 mg/kg 715 for 30 days, with anxiety-like behavior assessed by the percentage of open-arm entries (WT, n = 13 mice per group; *Atxn1*$^{h60Q/2Q}$, n = 14 mice per group). Data are presented as mean ± SEM, and n represents the number of animals analyzed per group.

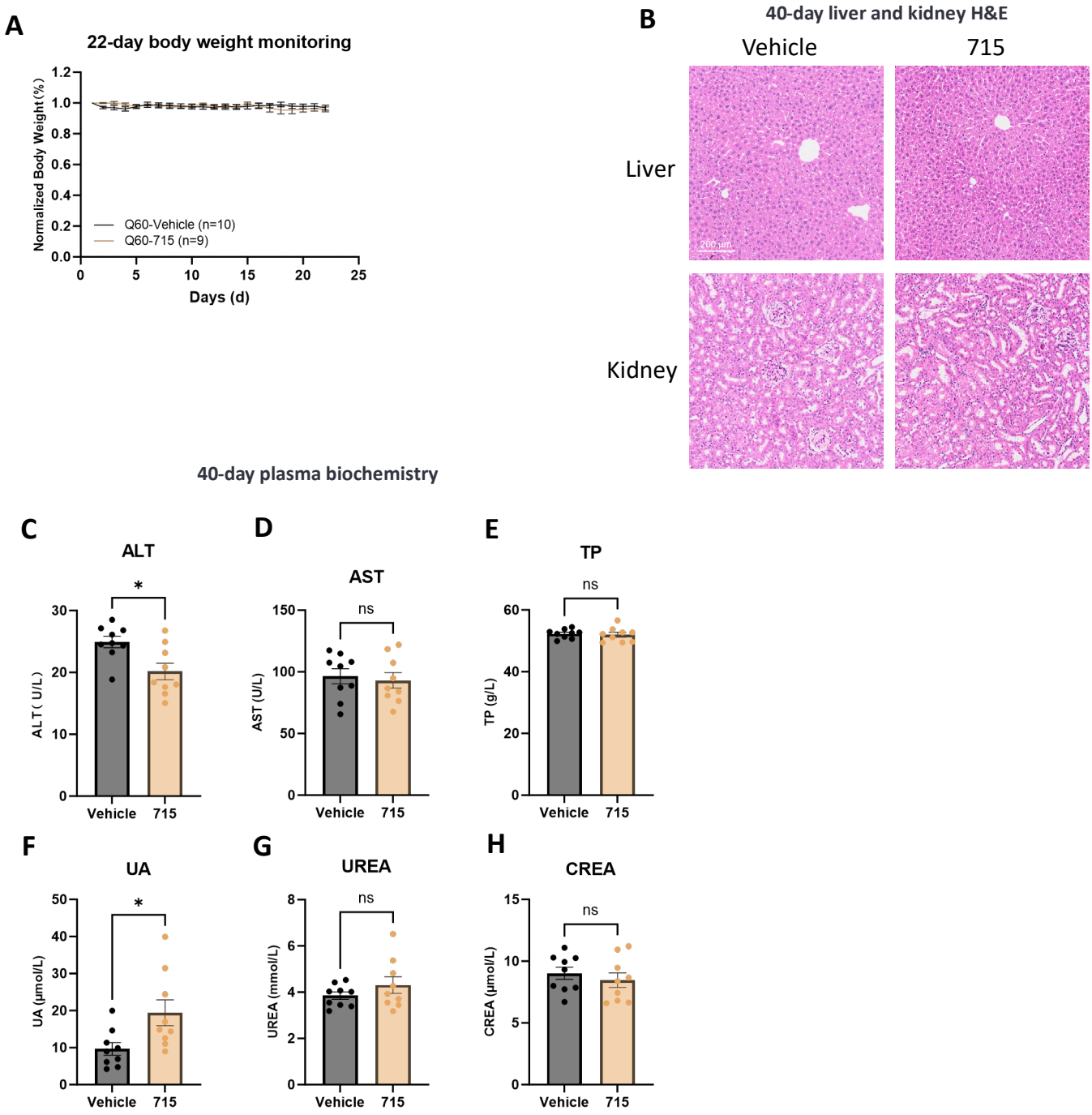


Fig. S11. relevant to Fig. 7. Systemic safety evaluation after prolonged oral 715 administration.

(A) Relative body weight changes of *Atxn1*$^{h60Q/2Q}$ mice during 22-day oral administration of vehicle or 17 mg/kg 715. **(B)** Representative hematoxylin and eosin (H&E) staining of liver and kidney sections from *Atxn1*$^{h60Q/2Q}$ mice following 40-day oral administration of vehicle or 17 mg/kg 715. **(C-H)** Plasma biochemical analysis of *Atxn1*$^{h60Q/2Q}$ mice following 40-day oral administration of vehicle or 17 mg/kg 715 ($n = 9$ mice per group). For quantitative analyses, n represents the number of animals analyzed per group. Data are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Statistical analyses were performed using two-tailed unpaired t-test for (C-H).

Supplementary Tables

Table S1 Genotyping Primer Sequences

Name	Forward (5'-3')	Reverse (5'-3')
Atxn1$^{154Q/2Q}$	GTGAGTTTGGGTCTGGCATC	CCAAAAGTTAGGATCACAGCCC
Atxn1$^{h60Q/2Q}$-WT	CCAATGTGACTCTCTGTTTATCAGAC	GACCCCGGGTTGACTAATCCTGC
Atxn1$^{h60Q/2Q}$-Mut	GCCCTCAGACGAGTCGGATCTC	CACACCCTCGACACAGTGGTTGTC

Table S2 siRNA sequences

Name	Sense (5'-3')	Antisense (5'-3')
siLC3B-1	GUAUGAGAGUGAGAAAGAUTT	AUCUUUCUCACUCUCAUACTT
siLC3B-2	GUUCGGGAUGAAAUUGUCATT	UGACAAUUUCAUCCCGAACTT
siLC3B-3	GCCGUCGGAGAAGACCUUCTT	GAAGGUCUUCUCCGACGGCTT
siTRIM11-1	CUGAUCCUGUCUGAAGACATT	UGUCUUCAGACAGGAUCAGTT
siTRIM11-2	CAGAAGUUGUGCCUAUGGATT	UCCAUAGGCACAACUUCUGTT
siTRIM11-3	CUAUUCAUCUUUCCCGAGATT	UCUCGGGAAAGAUGAAUAGTT
siTRIM11-4	CUAUUACAAUUCCUCGGAATT	UUCCGAGGAAUUGUAAUAGTT
siTRIM21-1	GCAGCACGCUUGACAAUGATT	UCAUUGUCAAGCGUGCUGCTT
siTRIM21-2	AAUAUUGGAUCACAAGGAUTT	AUCCUUGUGAUCCAAUAUUTT
siTRIM21-3	UCUCAGAGCUAGAUCGAAGTT	CUUCGAUCUAGCUCUGAGATT
siTRIM21-4	GAAUGUGCCUUUACAGGACTT	GUCCUGUAAAGGCACAUUCTT
siVCP-1	GAAUAGAGUUGUUCGGAAUTT	AUUCCGAACAACUCUAUUCTT
siVCP-2	GUAGGGUAUGAUGACAUUGTT	CAAUGUCAUCAUACCCUACUU
siVCP-3	GGGCACAUGUGAUUGUUAUTT	AUAACAAUCACAUGUGCCCTT
siDAXX	GGAGUUGGAUCUCUCAGAATT	UUCUGAGAGAUCCAACUCCTT
siBIRC7-1	UGAGGUGCUUCUUCUGCUAUGTT	CAUAGCAGAAGAAGCACCUCATT
siBIRC7-2	UCAGUUCCUGCUCCGGUCAAATT	UUUGACCGGAGCAGGAACUGATT
siBIRC7-3	GAGAGAGGUCCAGUCUGAAAGTT	CUUUCAGACUGGACCUCUCUCTT
siPSMA7-1	GAGGAAGAGACAUUGUUGUTT	ACAACAAUGUCUCUUCCUCTT
siPSMA7-2	GUGCUUUGGAUGACAACGUTT	ACGUUGUCAUCCAAAGCACTT
siPSMA7-3	CCAUUAAGCUGGUGAUCAATT	UUGAUCACCAGCUUAAUGGTT
siSTUB1-1	CCAAGCACGACAAGUACAUTT	AUGUACUUGUCGUGCUUGGTT
siSTUB1-2	CUCUUCGAAUCGCGAAGAATT	UUCUUCGCGAUUCGAAGAGTT
siSTUB1-3	CCUUGUGCUACCUGAAGAUTT	AUCUUCAGGUAGCACAAGGTT

Name	Sense (5’-3’)	Antisense (5’-3’)
siPML-1	CUCCAAGAUCUAAACCGAGAATT	UUCUCGGUUUAGAUCUUGGAGTT
siPML-2	CACCCGCAAGACCAACAACAUTT	AUGUUGUUGGUCUUGCGGGUGTT
siRNF4-1	CCCUGUUUCCUAAGAACGAAATT	UUUCGUUCUUAGGAAACAGGGTT
siRNF4-2	UAGGCCGAGCUUUGCGGGAAATT	UUUCCCGCAAAGCUCGGCCUATT
siRNF4-3	AAGACUGUUUCGAAACCAACATT	UGUUGGUUUCGAAACAGUCUUTT
siSUMO1-1	UCAAGAAACUCAAAGAAUCTT	GAUUCUUUGAGUUUCUUGATT
siSUMO1-2	GACAGGGUGUUCCAAUGAATT	UUCAUUGGAACACCCUGUCTT
siSUMO1-3	GGUUUCUCUUUGAGGGUCATT	UGACCCUCAAAGAGAAACCTT
siSUMO1-4	GAAUAAAUGGGCAUGCCAATT	UUGGCAUGCCCAUUUAUUCTT
siSUMO2/3-1	CCCAUUCCUUUAUUGUACATT	UGUACAAUAAAGGAAUGGGTT
siSUMO2/3-2	CAGAGAAUGACCACAUCAATT	UUGAUGUGGUCAUUCUCUGTT
siSUMO2/3-3	CAGUUAUGUUGUCGUGUAUTT	AUACACGACAACAUAACUGTT
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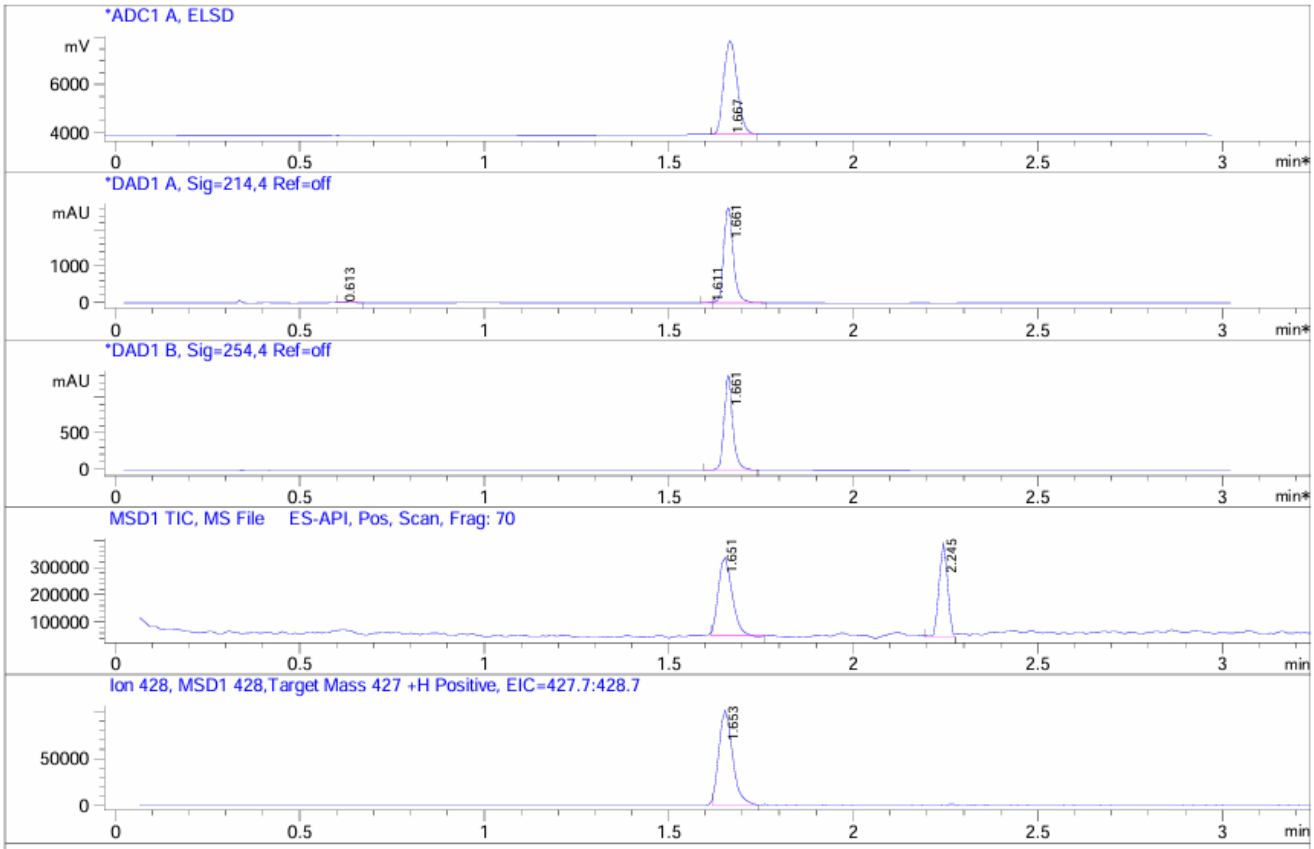
Table S3 qPCR Primer Sequences

Name	Forward (5’-3’)	Reverse (5’-3’)
human ATXN1	CAGAACCAGTACGTCCACATTT	GGCGTATTGCATGACGACCT
Exp. ATXN1 72Q	GGAGGTCTATATAAGCAGAGCTCGTT	CTCGATCTTCTGGGCCTCAAA
human β-ACTIN	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
human BIRC7	TGTCCACAGTGTGCAGGAGACT	GGCACTTTCAGACTGGACCTCT
human PSMA7	CAATGCCATAGGTCGGGGTG	TTTGCCACCTGACTGAACCA
human STUB1	GCCAATCTGCAGCGAGCTTA	CAATGCTGTTCCAGCGCTTC
human PML	ACCCGCAAGACCAACAACAT	CATCCTCGGCAGTAGATGCT
human RNF4	TCAGAAGCGAACTCGGGAAG	GATCAACCACCACAGGCTCT
human SUMO1	ATGTCTGACCAGGAGGCAAA	TCTCACTGCTATCCTGTCCA
human SUMO2	AGGATGGTTCTGTGGTGCAG	TCCTCCATTTCCAACTGTCGT
human SUMO3	AGGAGGGTGTGAAGACAGAGA	ATCTTGAACTGCACCACGGA

715 Compound information and validation

LC/MS Report

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Acq. Operator     : C60-MONITOR                             Inj  : 1
Spec. Reported    : MS Integration                    Inj Volume : 3 ul
Acq. Method       : D:\METHODS\1-POS-3MIN.M
Analysis Method   : D:\METHODS\1-POS-3MIN.M
Sample Info : Easy-Access Method: '1-POS-3MIN'  427.00
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              Flow Rate:2.0ml/min
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              Oven Temperature :45C
              Detection:UV(214,4nm) and MS(ESI,Pos mode,132 to 1500 amu)
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Integration Results for ADC1 A, ELSD

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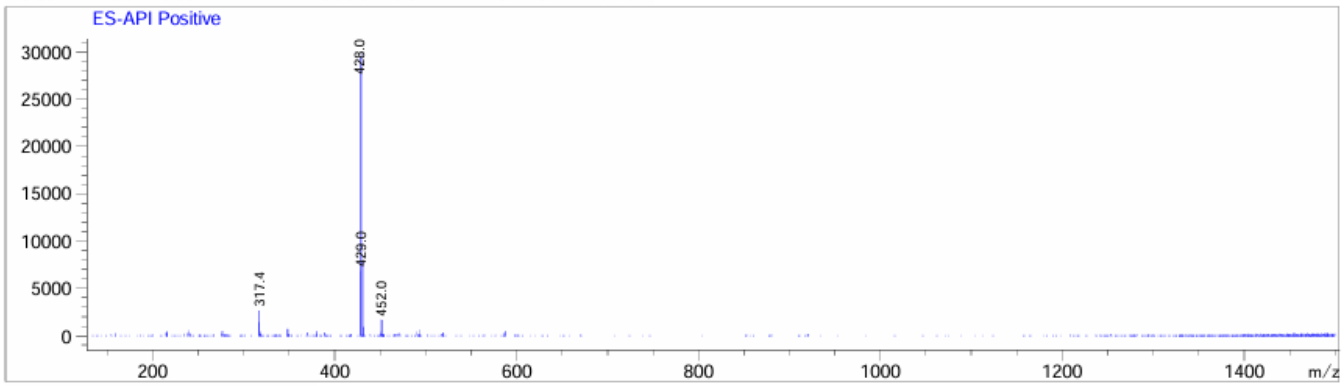
Integration Results for DAD1 A, Sig=214,4 Ref=off

RetTim	Width	Area	Height	Area%
0.61	0.03	14.21	7.29	0.29
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1.66	0.03	4829.71	2646.83	99.20

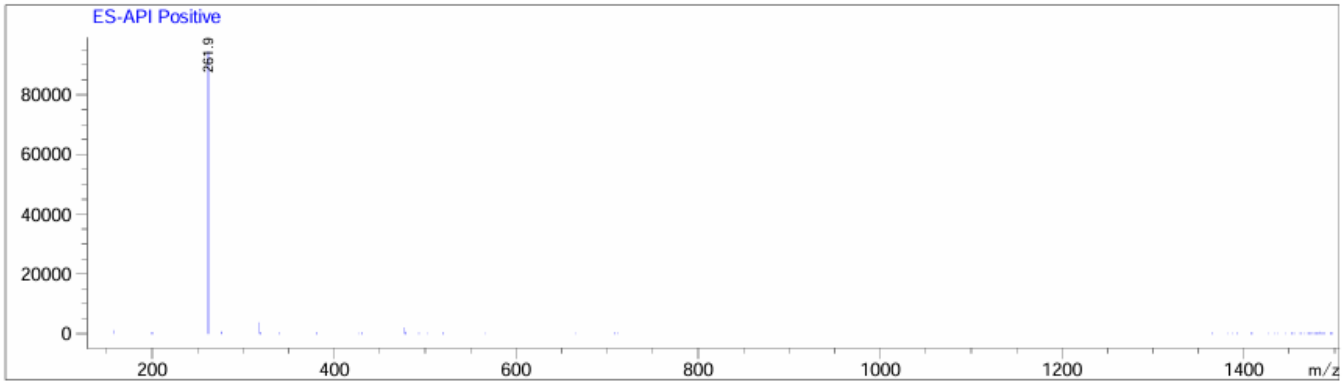
Integration Results for DAD1 B, Sig=254,4 Ref=off

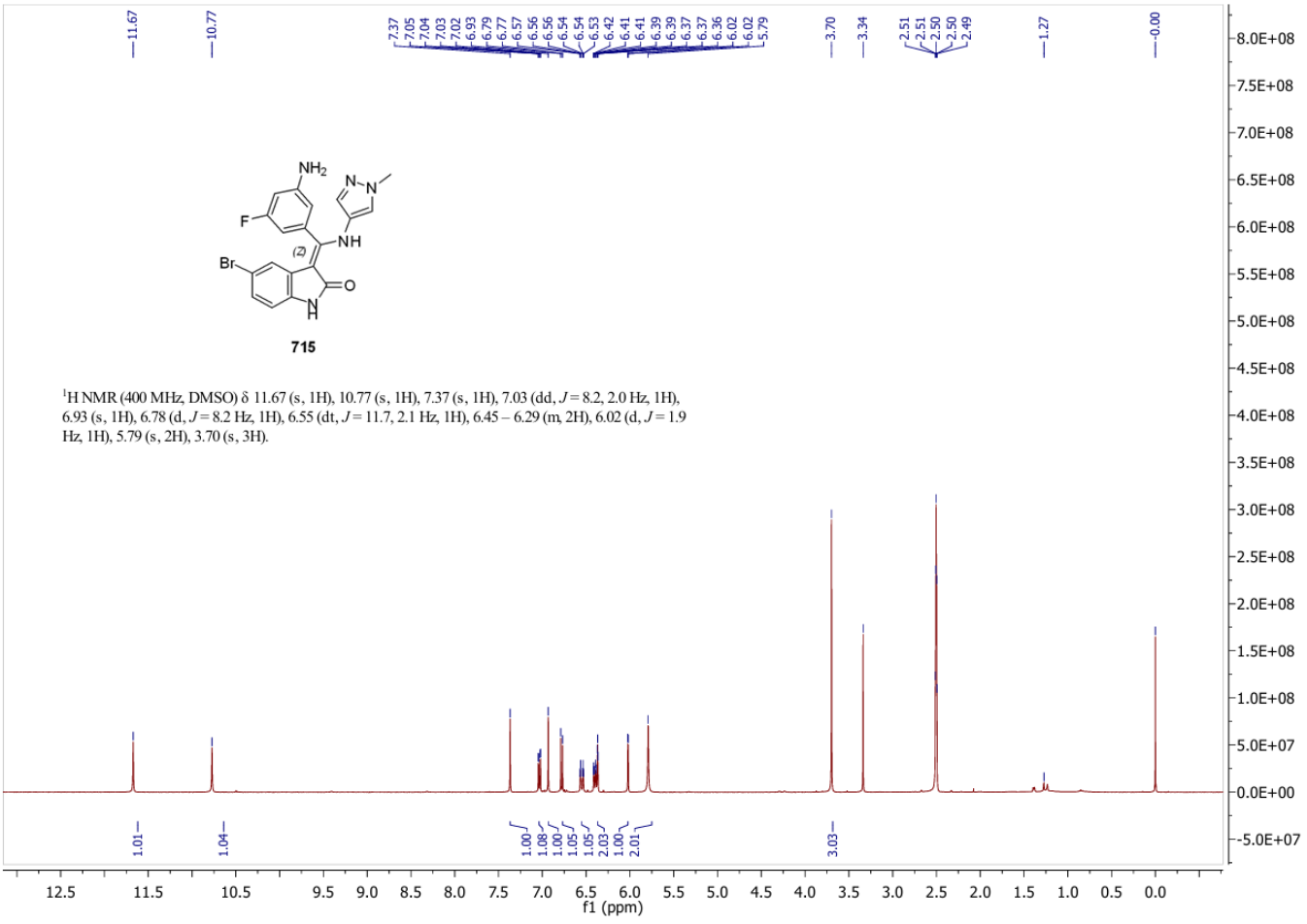
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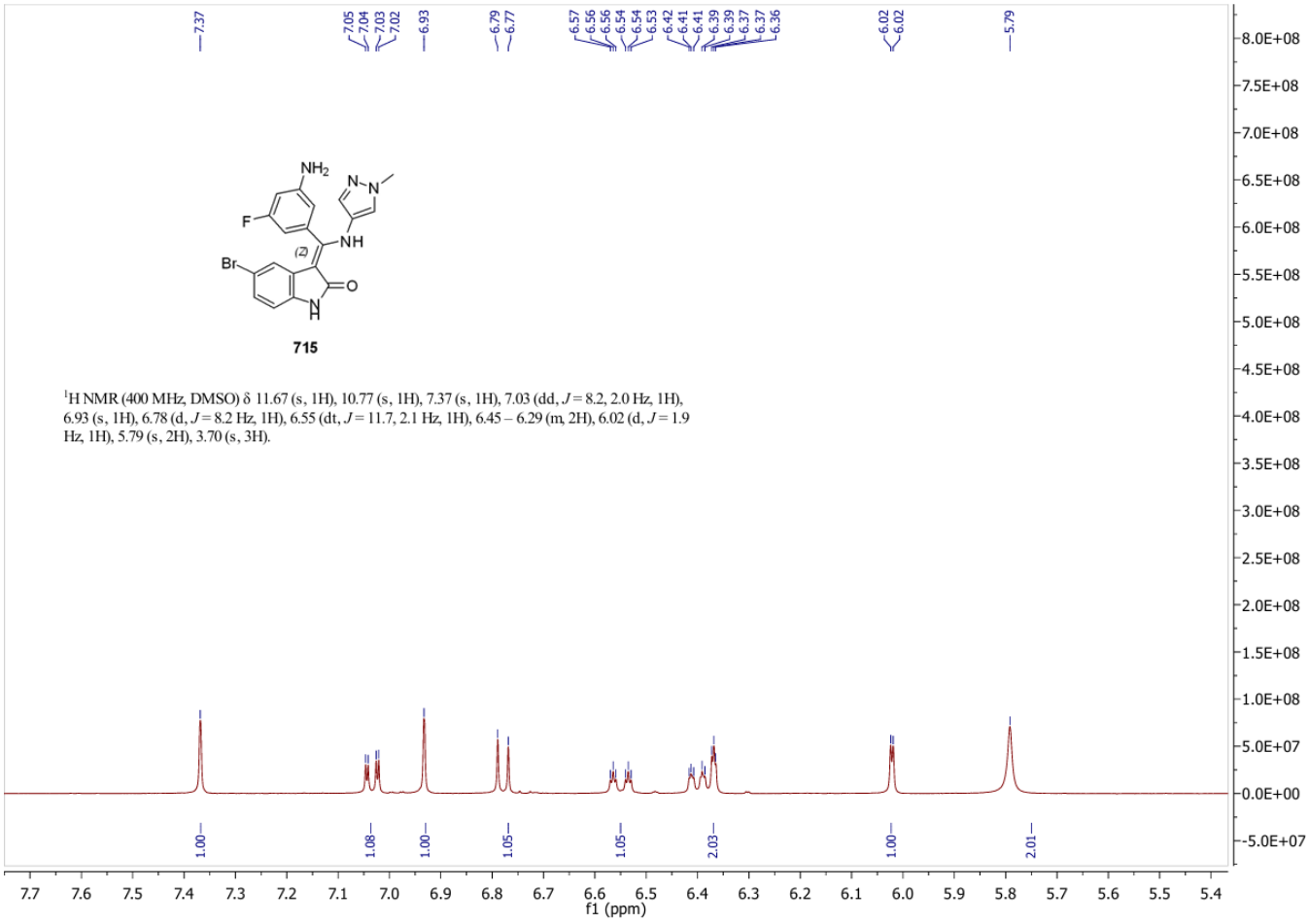


Ret. Time: 2.24




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H
715
1H NMR (400 MHz, DMSO) δ 11.67 (s, 1H), 10.77 (s, 1H), 7.37 (s, 1H), 7.03 (dd, J = 8.2, 2.0 Hz, 1H), 6.93 (s, 1H), 6.78 (d, J = 8.2 Hz, 1H), 6.55 (dt, J = 11.7, 2.1 Hz, 1H), 6.45 – 6.29 (m, 2H), 6.02 (d, J = 1.9 Hz, 1H), 5.79 (s, 2H), 3.70 (s, 3H).
1.01
1.04
1.00
1.08
1.00
1.05
2.03
1.00
2.01
3.03
f1 (ppm)

152


7.37
7.05
7.04
7.03
7.02
6.93
6.79
6.77
6.57
6.56
6.56
6.54
6.54
6.53
6.42
6.41
6.41
6.39
6.39
6.37
6.37
6.36
6.02
6.02
5.79
NH2
F
N
N
NH
(Z)
Br
O
N
H
715
1H NMR (400 MHz, DMSO) δ 11.67 (s, 1H), 10.77 (s, 1H), 7.37 (s, 1H), 7.03 (dd, J = 8.2, 2.0 Hz, 1H), 6.93 (s, 1H), 6.78 (d, J = 8.2 Hz, 1H), 6.55 (dt, J = 11.7, 2.1 Hz, 1H), 6.45 – 6.29 (m, 2H), 6.02 (d, J = 1.9 Hz, 1H), 5.79 (s, 2H), 3.70 (s, 3H).
1.00
1.08
1.00
1.05
1.05
2.03
1.00
2.01
f1 (ppm)

153

715 Compound synthetic route

