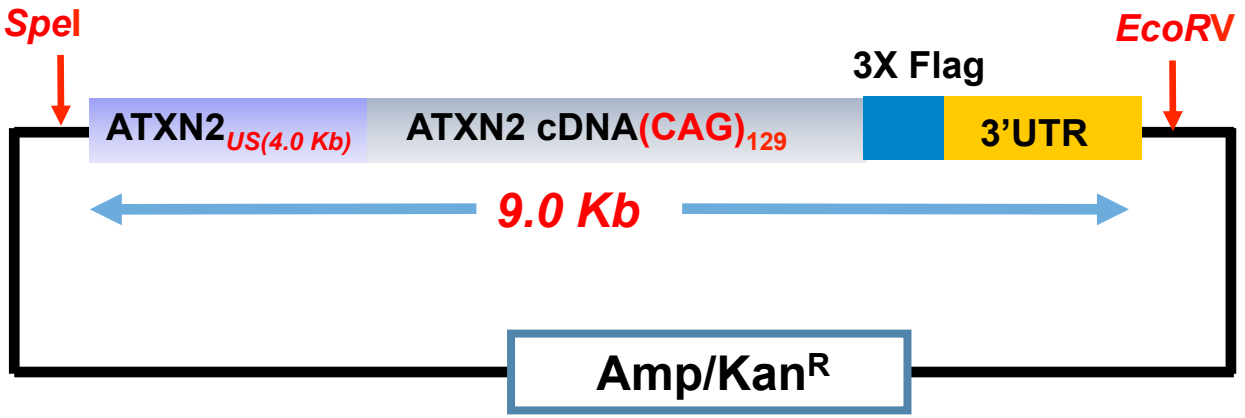
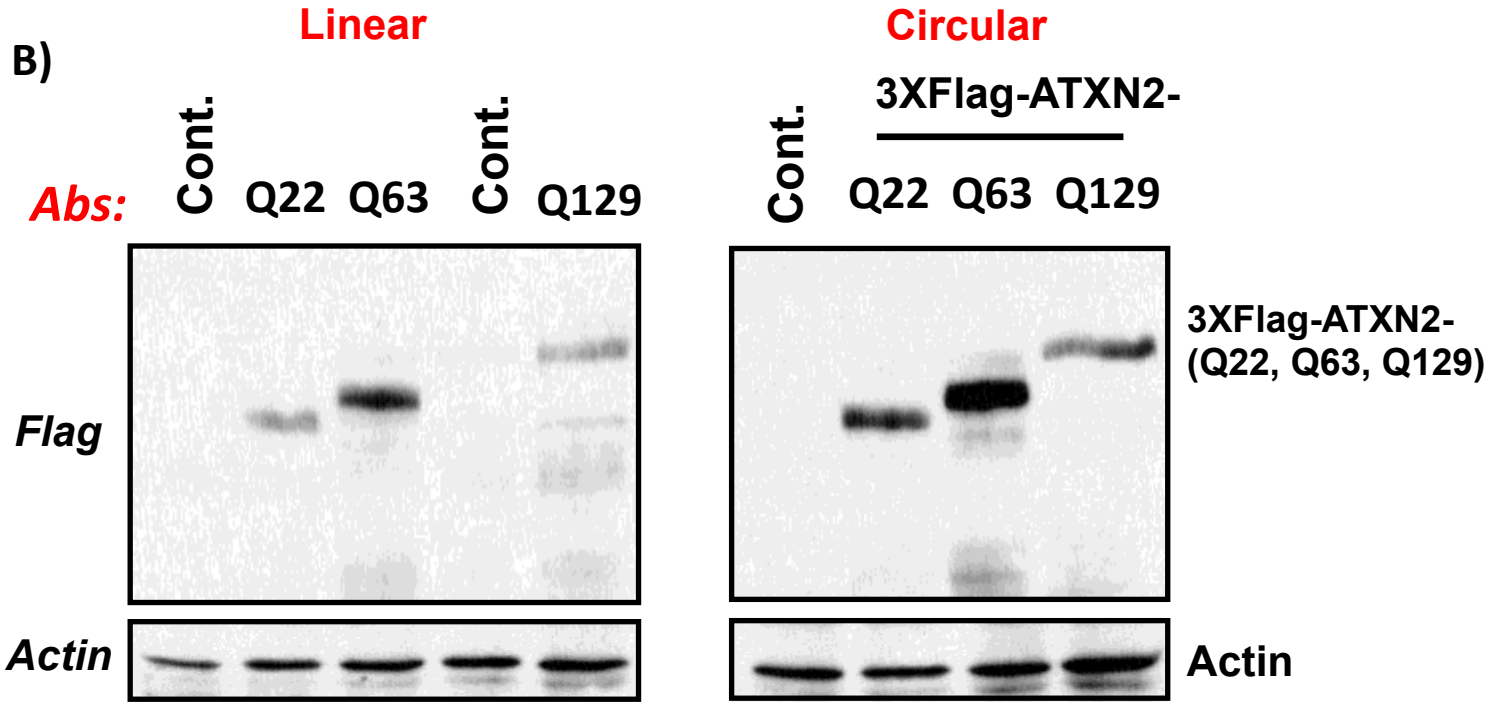
A)

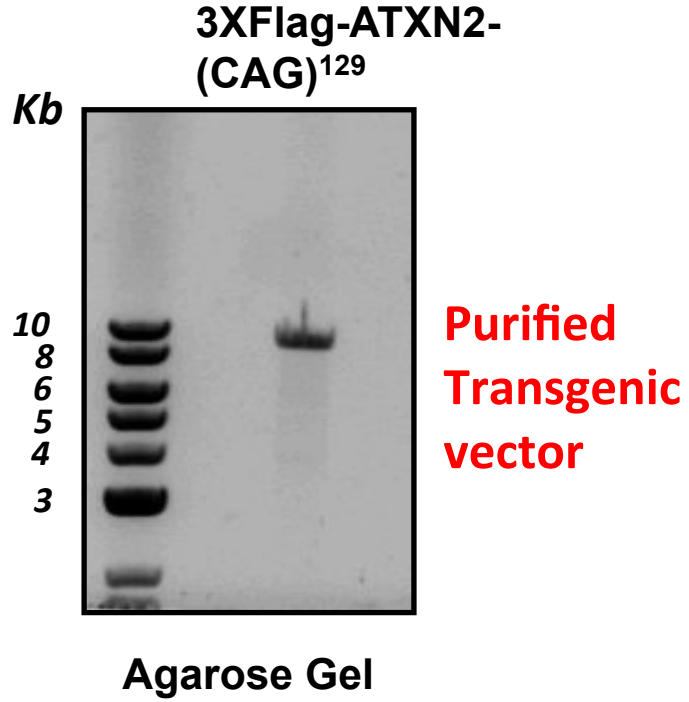



B)




Western blot

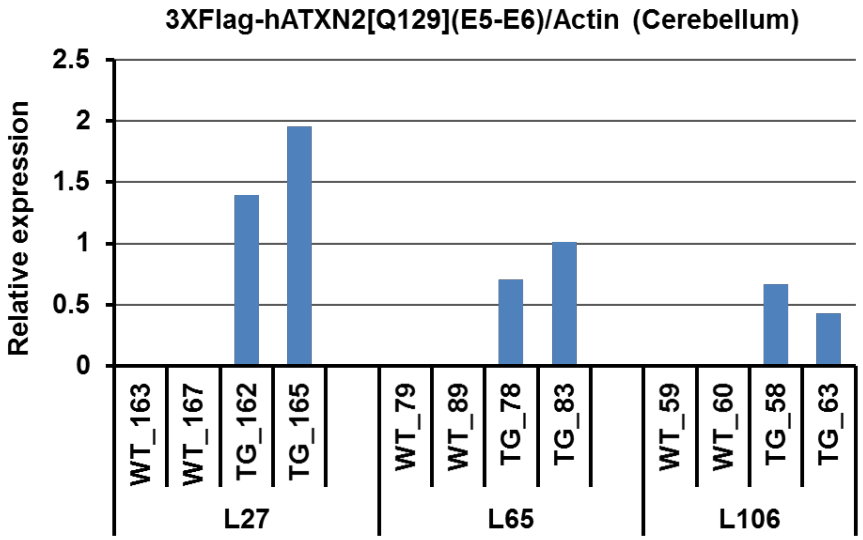
C)



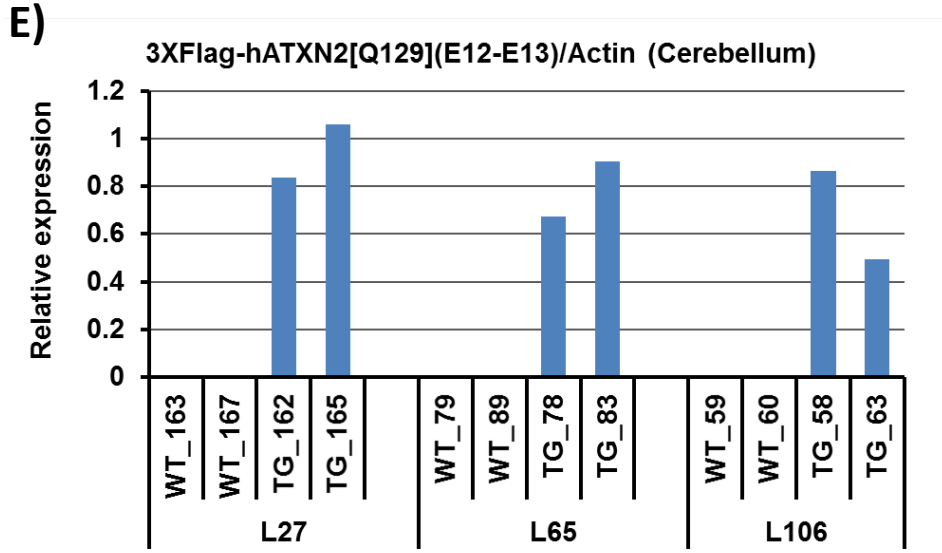

Agarose Gel

# qRT-PCR

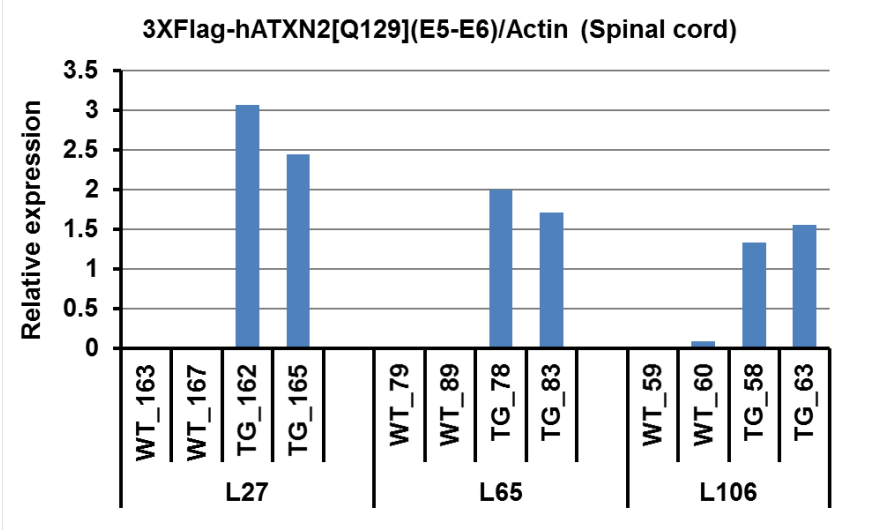
D)


3XFlag-hATXN2[Q129](E5-E6)/Actin (Cerebellum)
Relative expression
2.5
2
1.5
1
0.5
0
WT_163
WT_167
TG_162
TG_165
WT_79
WT_89
TG_78
TG_83
WT_59
WT_60
TG_58
TG_63
L27
L65
L106

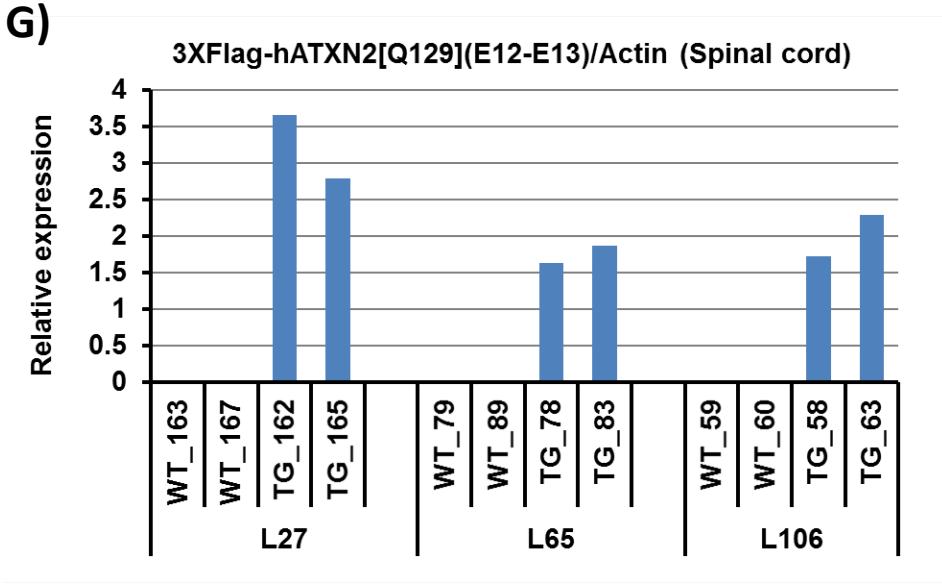
E)


3XFlag-hATXN2[Q129](E12-E13)/Actin (Cerebellum)
Relative expression
1.2
1
0.8
0.6
0.4
0.2
0
WT_163
WT_167
TG_162
TG_165
WT_79
WT_89
TG_78
TG_83
WT_59
WT_60
TG_58
TG_63
L27
L65
L106

F)

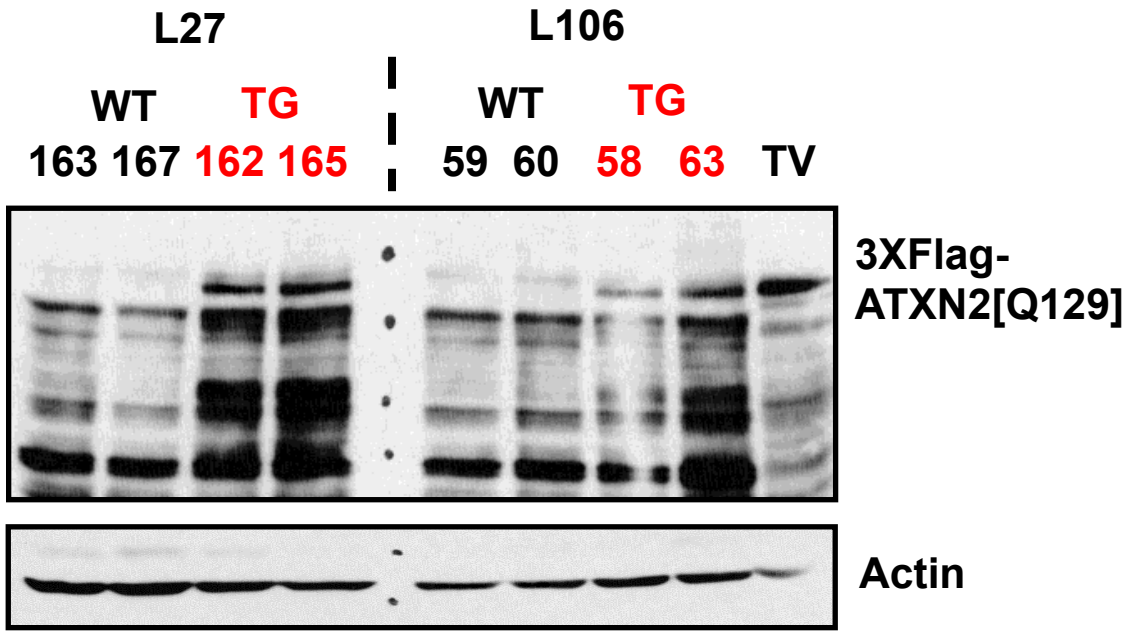

3XFlag-hATXN2[Q129](E5-E6)/Actin (Spinal cord)
Relative expression
3.5
3
2.5
2
1.5
1
0.5
0
WT_163
WT_167
TG_162
TG_165
WT_79
WT_89
TG_78
TG_83
WT_59
WT_60
TG_58
TG_63
L27
L65
L106

G)


3XFlag-hATXN2[Q129](E12-E13)/Actin (Spinal cord)
Relative expression
4
3.5
3
2.5
2
1.5
1
0.5
0
WT_163
WT_167
TG_162
TG_165
WT_79
WT_89
TG_78
TG_83
WT_59
WT_60
TG_58
TG_63
L27
L65
L106

# 3XFlag-ATXN2[Q129] Mice (Cerebellar extract)

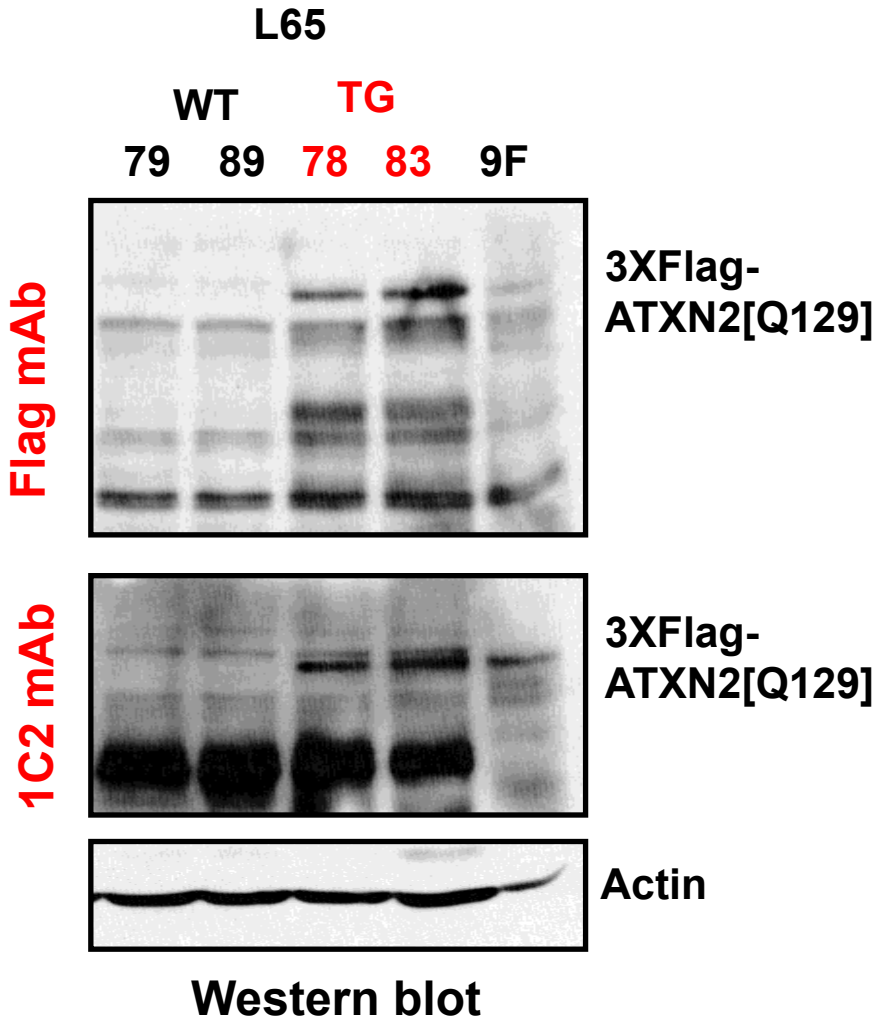
H)




Western blot

Flag and Actin abs

I)




Western blot

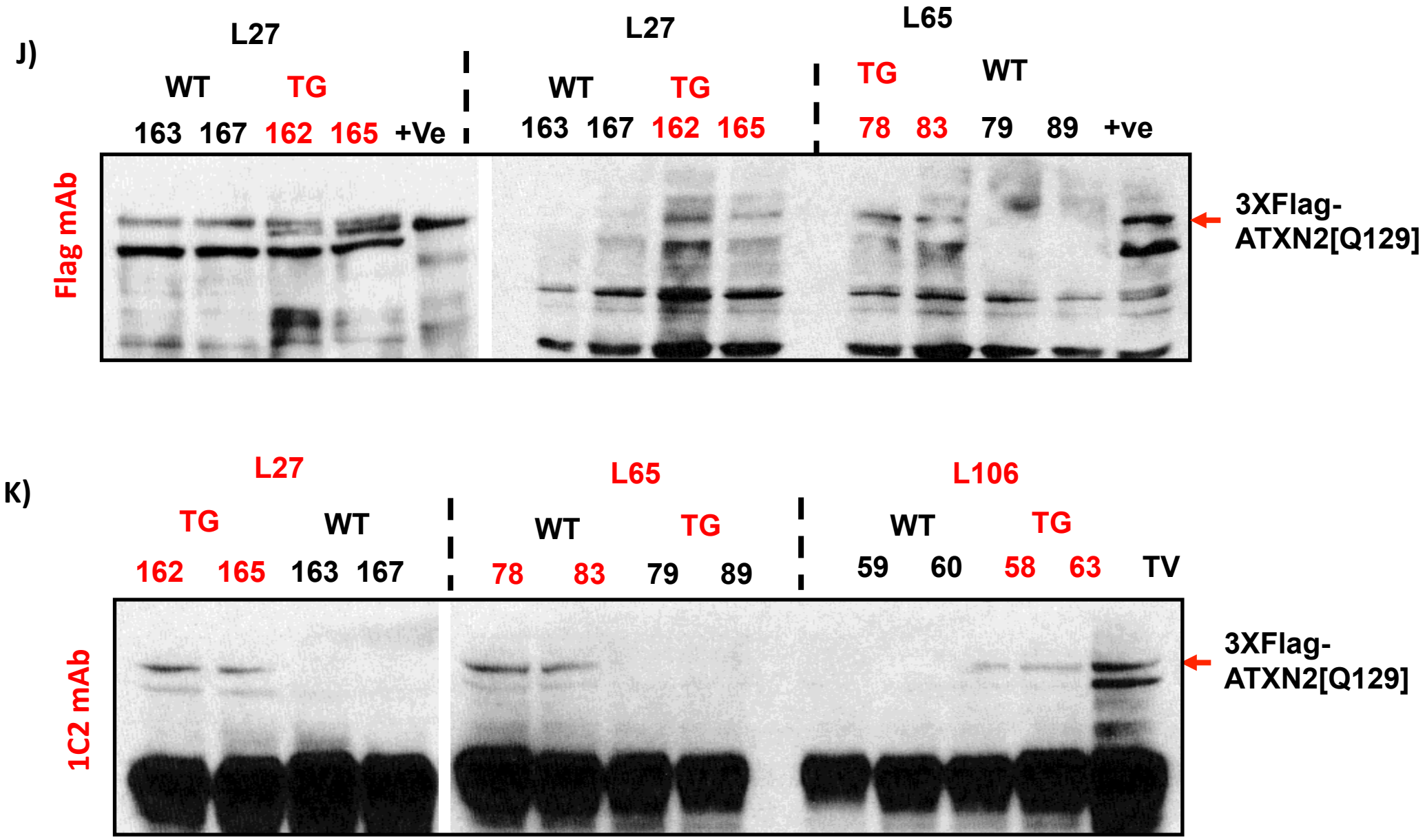

3XFlag-ATXN2[Q129] Mice (Spinal cord extract)
J)
L27
L27
L65
WT
TG
WT
TG
TG
WT
163 167 162 165 +Ve
163 167 162 165
78 83 79 89 +ve
Flag mAb
3XFlag-
ATXN2[Q129]
K)
L27
L65
L106
TG
WT
WT
TG
WT
TG
162 165 163 167
78 83 79 89
59 60 58 63 TV
1C2 mAb
3XFlag-
ATXN2[Q129]
Western blot

**Figure 1. Generation of a 3XFlag-ATXN2[Q129] SCA2 transgenic mouse model.** (A) Schematic representation of 3XFlag-ATXN2[Q129] construct containing ATXN2 cDNA with CAG129 repeats, plus 4 kb 5'-flanking and 3' UTR with 3XFlag sequences.

(B) Western blot analyses revealed the expression of both linear or circular version of 3XFlag-ATXN2[Q129] constructs in SH-SY5Y or HEK293 cells are shown. Cells were transfected with plasmids encoding 3XFlag-tagged cDNAs of human ATXN2 containing Q22 or Q63 or Q129 repeats. Forty-eight hrs post-transfection, cells were harvested and protein extracts were subjected to Western blot analyses using Flag monoclonal antibody. The results demonstrated both circular or linear version of 3XFlag-tagged ATXN2 constructs were expressed as ATXN2 protein. The blots were re-probed for β-Actin as an internal loading control.

(C) Purified linear 3XFlag-ATXN2[Q129] DNA on agarose gel is shown.

(D-G) qRT-PCR analyses revealed expression of 3XFlag-ATXN2[Q129] transcripts in mouse CNS including cerebellum and spinal cord tissues. Synthesized cDNAs from wild-type and mutant mouse tissues of 24 weeks old animals were subjected to qRT-PCR analysis using human ATXN2 specific primers as indicated. The transcript expressions were normalized with mouse Actin. Two animals per group were used for these analyses.

(H-K) Western blot analyses revealed expression of mutant ATXN2 protein (3XFlag-ATXN2[Q129]) in mouse CNS including cerebellum and spinal cord tissues. Protein extracts from wild-type and transgenic mouse cerebella and spinal cord were subjected to Western blot analyses using Flag or 1C2 mAbs. The results demonstrated 3XFlag-ATXN2[Q129] mice expressed human mutant ATXN2 protein in cerebellum and spinal cord. Two animals per group were used for Western blot analyses.

***Generation and characterization of 3XFlag-ATXN2[Q129] mice***

We engineered 3XFlag-tagged human ATXN2[Q129] construct (3XFlag-ATXN2[Q129]) that contained ATXN2 cDNA harboring CAG129 repeats with 4 kb of the 5' flanking genomic sequence and 3' UTR with 3XFlag sequences. The authenticity of this construct was verified by DNA sequence analyses and Western blot analyses by transfecting to SH-SY5Y or HEK293 cells. The CAG tract was mutation-free when sequenced from both strands. In Western blot analyses, Flag monoclonal antibody staining results demonstrated both circular and linear version of 3XFlag-ATXN2[Q129] constructs expressed full-length human mutant ATXN2 protein. The purified linear intact 3XFlag-ATXN2[Q129] DNA was microinjected into FVB fertilized eggs to produce transgenic mice at the University of Utah Mouse Core Facility. Three lines for mutant mice (3XFlag-ATXN2[Q129]) were established and further analyzed. 3XFlag-ATXN2[Q129] were maintained in the FVB background (not sure!!!) and bred and maintained under standard conditions consistent with National Institutes of Health guidelines and approved by the University of Utah, IACUC protocol. In qRT-PCR analyses, 3XFlag-ATXN2[Q129] mice demonstrated the expression of human ATXN2 transcripts throughout the central nervous system (CNS), including cerebellum and spinal cord. To assess protein expression, we performed Western blot analysis using cerebellar and spinal cord extracts of 24 week-old animals with Flag monoclonal antibody. The results showed that 3XFlag-ATXN2[Q129] mice expressed full-length human mutant ATXN2 protein. Furthermore, we confirmed the 3XFlag-ATXN2[Q129] protein expression using 1C2 mAb, an antibody against an expanded polyQ epitope in Western blot analyses. These results demonstrate that human ATXN2 transgene (3XFlag-ATXN2[Q129]) was properly expressed in mutant mice.