Supplemental information_Lan et al.

PolyQ expansions in ATNX7 affect solubility but not activity of the SAGA deubiquitinating module

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Supplemental Figure 1. (A-D) Silver stainings of recombinant USP22 and different subcomplexes of DUBm eluted from HA agarose beads after USP22 affinity purifications to verify the expression of DUBm subunits and the purity of these recombinant proteins for control DUB assays. '‡' denotes degradation products of ATXN7L3 or USP22. '#' on left of bands in (D) indicates the DUBm subunits purified in different combinations. USP22 WT or catalytic mutant was tagged with HA; ATXN7-24Q or 92Q NT, ATXN7L3 and ENY2 were tagged with FLAG.

Supplemental Figure 2. Both ATXN7L3 and ENY2 are required for the activation of DUB activity of USP22. ATXN7-24Q NT further stimulates the DUB activity of DUBm. (A) *In vitro* DUB assay with total mononucleosomes as substrate, shows that ATXN7-24Q NT promotes the enzymatic activity of DUBm on histone H2A. (B) Ub-AMC hydrolysis assay using the indicated complexes. As a negative control, Ub-AMC was incubated with DUB buffer alone. (C-F) Equal amounts of purified complexes described in Supplemental Fig. 2 were incubated with 0, 1uM or 5 uM ubiquitin vinyl sulfone (Ub-VS), a suicide substrate of deubiquitinases at 37^{o}C for 2 hrs. This compound can form a covalent bond only with active form of USP22, which is characterized by a ~7 kDa shift of the enzyme using immunoblotting with anti-HA antibody. The stronger activity the deubiquitinase possesses, the more robust the shift is. USP22 alone shows only poor activity. Quantification of USP22-Ub-VS was normalized to HA-USP22

signal by using ImageJ program (D, F). USP22 WT or catalytic mutant was tagged with HA; ATXN7-24Q NT, ATXN7L3 and ENY2 were tagged with FLAG.

Supplemental Figure 3. Formation of nuclear inclusions in astrocytes expressing mutant ATXN7-92Q was observed by immunofluorescence staining of infected astrocytes with anti-FLAG antibody. Upon co-overexpression of ATXN7L3 and ENY2, the number of nuclear inclusions caused by ATXN7-92Q in human astrocytes was clearly decreased. Nuclei were stained with DAPI (blue). Samples were imaged using a laser spectral confocal microscope (Leica STP6000). 10x and 40x objectives were used. Scale bars: 10 μm.

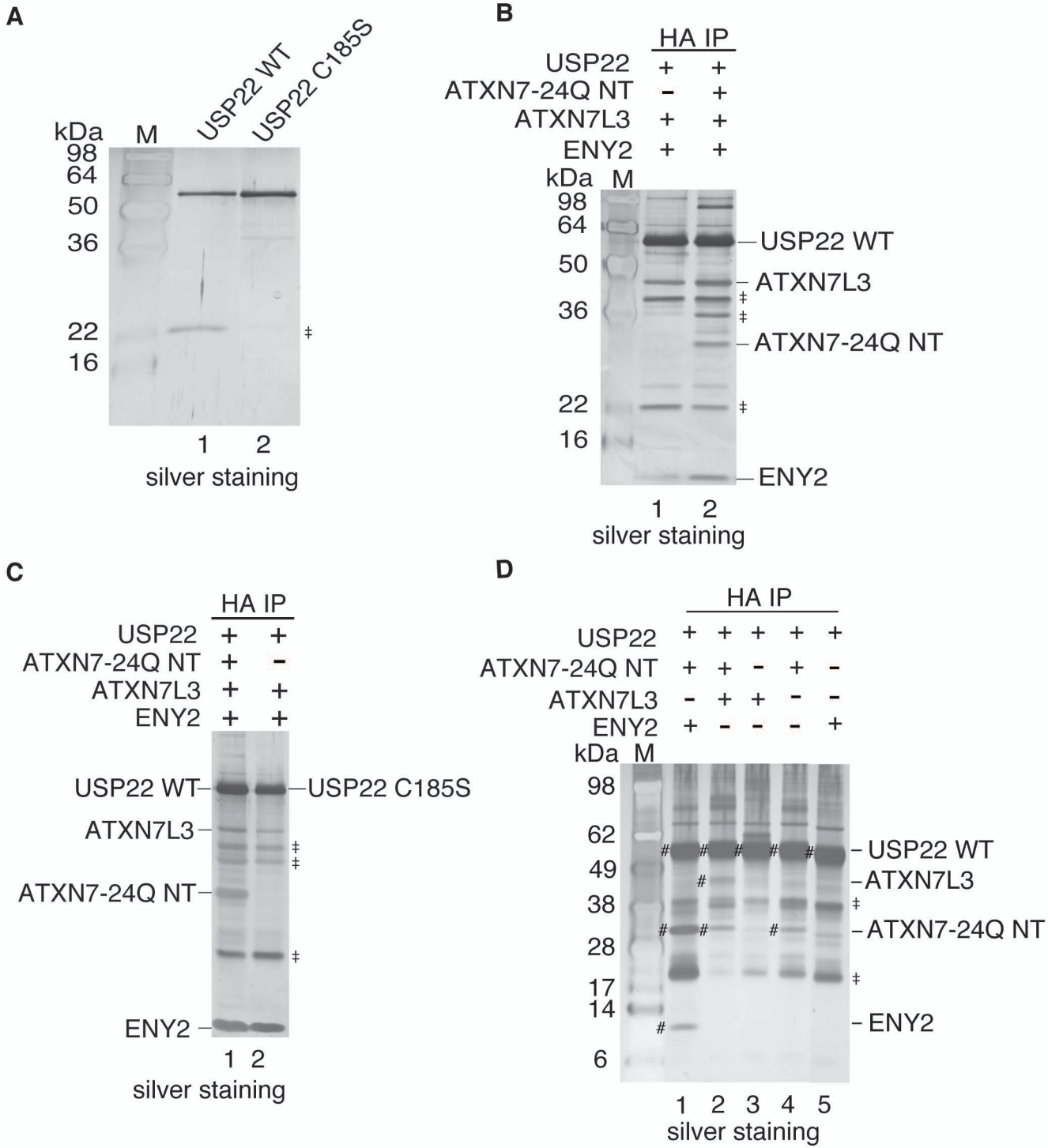
Supplemental Figure 4. (A) Formation of nuclear inclusions in astrocytes expressing mutant ATXN7-92Q was observed by immunofluorescence staining of infected astrocytes with anti-FLAG antibody. Upon co-overexpression of ATXN7L3, the number of nuclear inclusions caused by ATXN7-92Q in human astrocytes was only partially reduced, compared to the overexpression of both ATXN7L3 and ENY2. Anti-V5 staining was performed to show the co-expression of all the proteins analyzed in the same cell. Nuclei were stained with DAPI (blue). Samples were imaged using a laser spectral confocal microscope (Leica STP6000). 10x and 40x objectives were used. Scale bars: 10 μm.

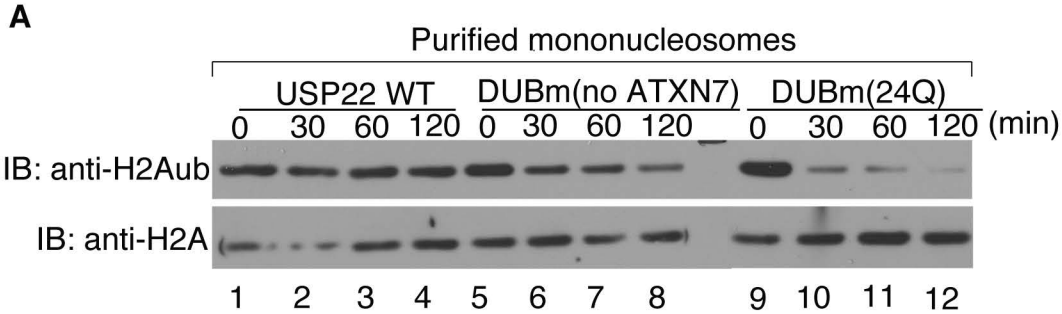
(B) Overexpression of individual subunits of the DUB module, like USP22 and ENY2 does not enhances global H2Bub deubiquitination in astrocytes expressing

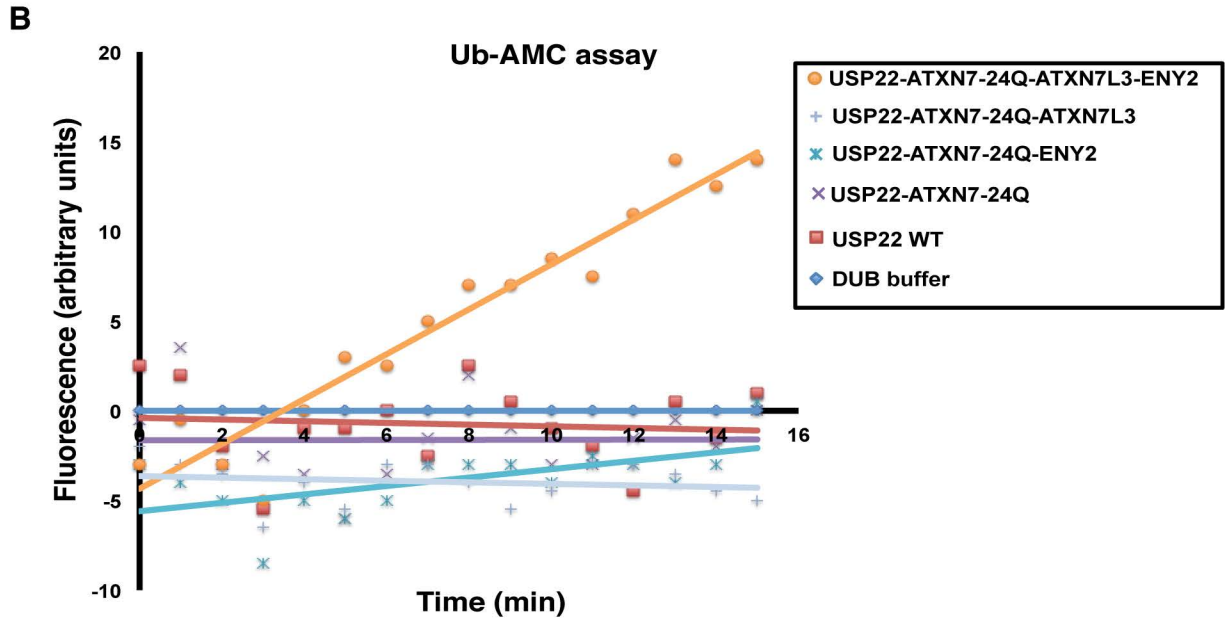
ATXN7-92Q, whereas overexpression of ATXN7L3 does have a dramatic impact. Different sample amounts are analyzed (1x and 2x). H2B immunostaining serves as loading control.

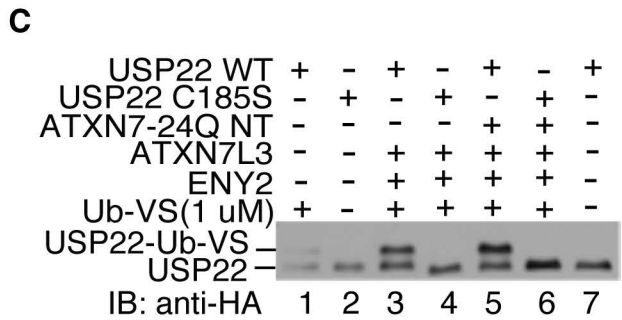
Supplemental Table 1. Primer information

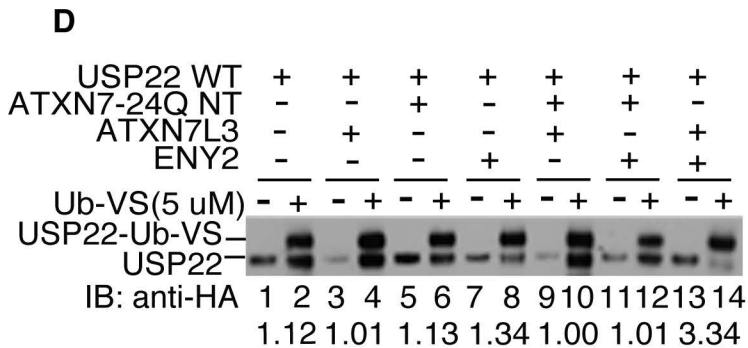
Primer name	Nucleotide sequence
HA-USP22-Mlu I-F	5’ CGACGCGTCGGTAGCTTGGGGCCACC ATG TAC CCA TAC GAT GTT CCA GAT TAC GCT GTG TCC CGG CCA GAG CC 3’
V5-USP22-Mlu I-F	5’ CGACGCGT CGGTAGCTTGGGGCCACC ATGGGTAAGCCTATCCCTAAC CCTCTCCTCGGTCTCGATTCTACG GTG TCC CGG CCA GAG CC 3’
USP22-Nde I-R	5' GGAATTCCATATGGAATTCCCTACTCGTATTCCAGGAACTGTTTG 3'
Flag-ATXN7L3-Mlu I-F	5’ CGAACGCGTCGGTAGCTTGGGGCCACCATG GAT TAC AAG GAT GAC GAC GAT AAG TCG CGAATG AAAATGGAGGAAATGTCTTTGTCTG 3’
V5-ATXN7L3-Mlu I-F	5’ CGACGCGTCGGTAGCTTGGGGCCACCATGGGTAAGCCTATCCCTAACC CTCTCCTCGGTCTCGATTCTACG AAAATGGAGGAAATGTCTTTGTCTG 3’
ATXN7L3-Nde I-R	5' GGAATTC CATATG GAATTCC TCAGTTGATGTCATCATAGATGCTGGG 3'
Flag-ENY2-Mlu I-F	5’ CGAACGCGTCGGTAGCTTGGGGCCACCATG GAT TAC AAG GAT GAC GAC GAT AAG TCG CGAATGGTGGTTAGCAAGATGAACAAAGATG 3’
V5-ENY2-Mlu I-F	5’ CGACGCGTCGGTAGCTTGGGGCCACCATGGGTAAGCCTATCCCTAACC CTCTCCTCGGTCTCGATTCTACG GTGGTTAGCAAGATGAACAAAGATG 3’
ENY2-Nde I-R	5' GGAATTC CATATG GAATTCC TTAAAGGCTGGCATGCTGAGCAAG 3'

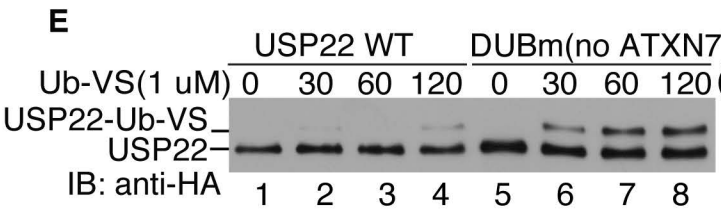

A
kDa
M
USP22 WT
USP22 C185S
98
64
50
36
22
16
‡
1
2
silver staining
B
HA IP
USP22 + +
ATXN7-24Q NT – +
ATXN7L3 + +
ENY2 + +
kDa M
98
64
50
36
22
16
USP22 WT
ATXN7L3
‡
‡
ATXN7-24Q NT
‡
ENY2
1
2
silver staining
C
HA IP
USP22 + +
ATXN7-24Q NT + –
ATXN7L3 + +
ENY2 + +
USP22 WT
USP22 C185S
ATXN7L3
‡
‡
ATXN7-24Q NT
‡
ENY2
1 2
silver staining
D
HA IP
USP22 + + + + +
ATXN7-24Q NT + + – + –
ATXN7L3 – + + – –
ENY2 + – – – +
kDa M
98
62
49
38
28
17
14
6
#
USP22 WT
ATXN7L3
‡
ATXN7-24Q NT
‡
ENY2
1 2 3 4 5
silver staining

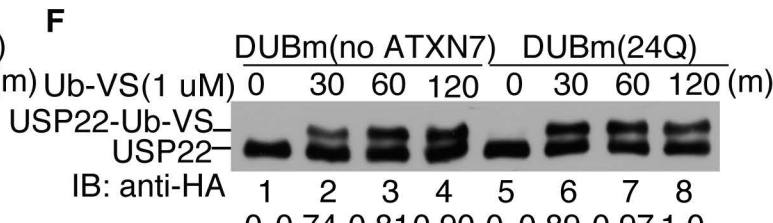

A
Purified mononucleosomes
USP22 WT
DUBm(no ATXN7)
DUBm(24Q)
0 30 60 120 0 30 60 120 0 30 60 120 (min)
IB: anti-H2Aub
IB: anti-H2A
1 2 3 4 5 6 7 8 9 10 11 12

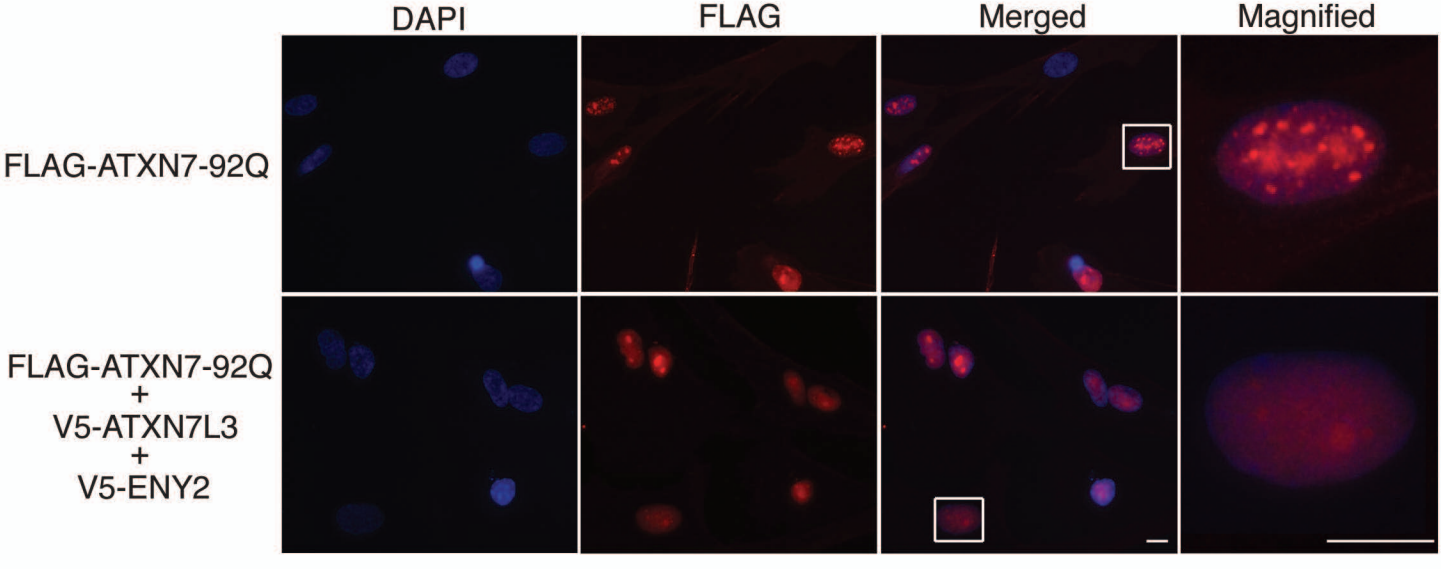

B
Ub-AMC assay
Fluorescence (arbitrary units)
Time (min)
USP22-ATXN7-24Q-ATXN7L3-ENY2
USP22-ATXN7-24Q-ATXN7L3
USP22-ATXN7-24Q-ENY2
USP22-ATXN7-24Q
USP22 WT
DUB buffer

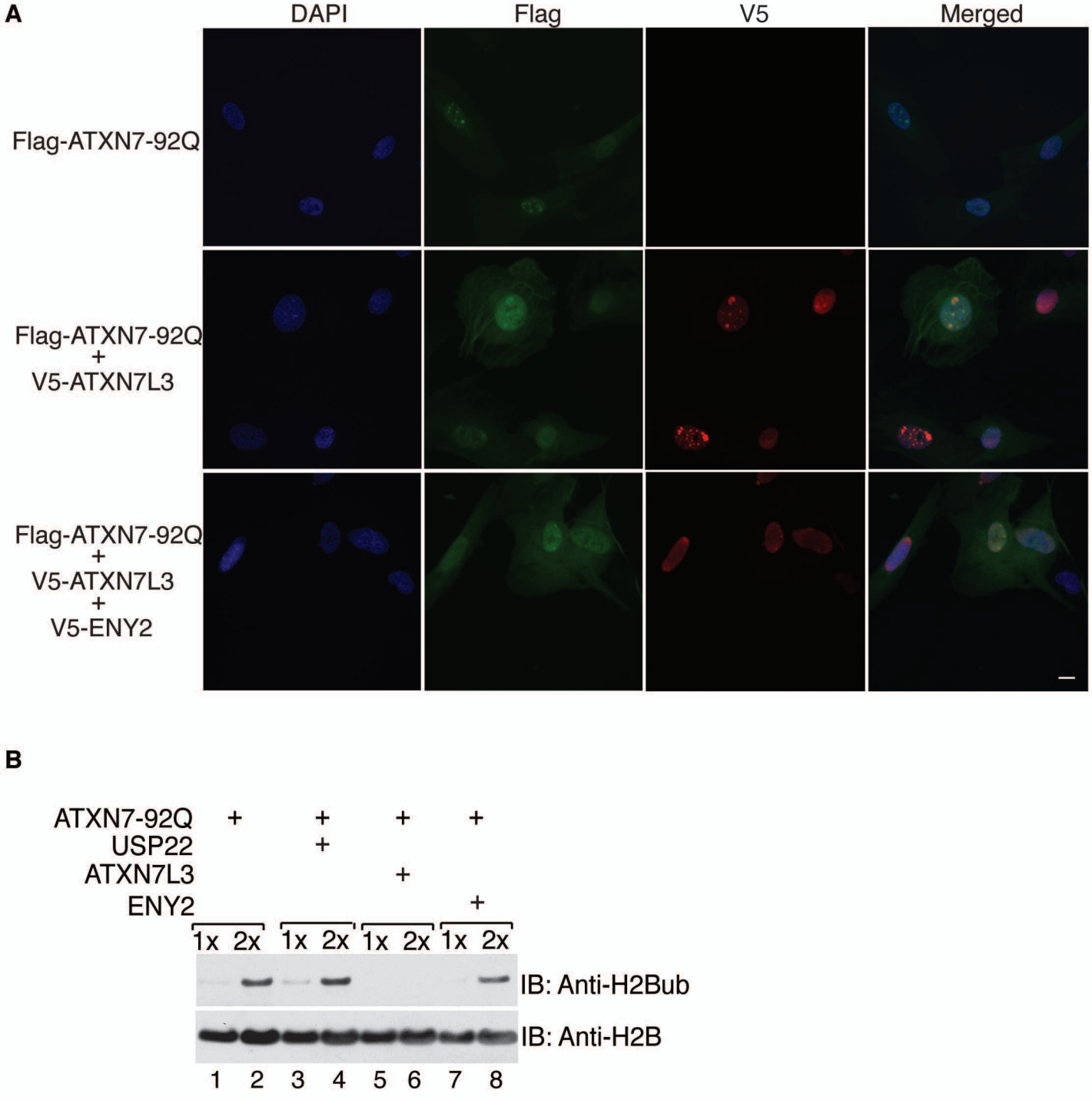

C
USP22 WT
USP22 C185S
ATXN7-24Q NT
ATXN7L3
ENY2
Ub-VS(1 uM)
USP22-Ub-VS
USP22
IB: anti-HA 1 2 3 4 5 6 7


D
USP22 WT
ATXN7-24Q NT
ATXN7L3
ENY2
Ub-VS(5 uM)
USP22-Ub-VS
USP22
IB: anti-HA 1 2 3 4 5 6 7 8 9 10 11 12 13 14
1.12 1.01 1.13 1.34 1.00 1.01 3.34


E
USP22 WT
DUBm(no ATXN7)
Ub-VS(1 uM) 0 30 60 120 0 30 60 120 (m)
USP22-Ub-VS
USP22
IB: anti-HA 1 2 3 4 5 6 7 8


F
DUBm(no ATXN7)
DUBm(24Q)
Ub-VS(1 uM) 0 30 60 120 0 30 60 120 (m)
USP22-Ub-VS
USP22
IB: anti-HA 1 2 3 4 5 6 7 8
0 0.74 0.81 0.90 0 0.89 0.97 1.0


DAPI
FLAG
Merged
Magnified
FLAG-ATXN7-92Q
FLAG-ATXN7-92Q
+
V5-ATXN7L3
+
V5-ENY2


A
DAPI
Flag
V5
Merged
Flag-ATXN7-92Q
Flag-ATXN7-92Q
+
V5-ATXN7L3
Flag-ATXN7-92Q
+
V5-ATXN7L3
+
V5-ENY2
B
ATXN7-92Q + + + +
USP22 +
ATXN7L3 +
ENY2 +
1x 2x 1x 2x 1x 2x 1x 2x
IB: Anti-H2Bub
IB: Anti-H2B
1 2 3 4 5 6 7 8