SUPPLEMENTAL MATERIALS

Figure S1. The 1D proton NMR spectra of HDAC6 ZnF-UBP and HDAC6 ZnF-UBP RY mutant.

Figure S2. Gel filtration elution profiles of HDAC6 ZnF-UBP, ubiquitin and ubiquitin mutants.

Figure S3. The mass spectrometry of the commercial ubiquitin-AFC sample.

Figure S4. CFTR-ΔF508 protein aggregates contain unanchored ubiquitin generated by deubiquitinase ataxin-3.

Figure S5. Characterization of siRNA-mediated ataxin-3 knockdown in culture.

Figure S6. Ataxin-3 knockdown did not affect the total levels of CFTR-ΔF508 expression.

Figure S7. Human Ataxin1-82Q-GFP forms aggresomes in the presence of Ataxin-3.

Figure S8. DUB proteins USP5 or USP13 did not localize to the aggresome.

Table S1. Summary of the gel filtration and dynamic light scattering (dls) results.

Supplementary Figure Legends

FIGURE S1. The 1D proton NMR spectra of HDAC6 ZnF-UBP and HDAC6 ZnF-UBP RY mutant. The HDAC6 ZnF-UBP RY mutant proton spectrum (B) showed similar dispersed signals as wild type HDAC6 ZnF-UBP proton spectrum (A), indicating that that RY mutation did not interfere with the proper folding and this mutant is a folded protein.

FIGURE S2. Gel filtration elution profiles of HDAC6 ZnF-UBP, ubiquitin and ubiquitin mutants. (A) Comparison of gel filtration profiles of HDAC6 ZnF-UBP in mixture of wild-type ubiquitin and ubiquitin Ub75 mutant. A 1:1 mixture of HDAC6 ZnF-UBP and wild-type ubiquitin migrated at an apparent mass of 15.8 kDa indicating a complex formation, while the mixture of HDAC6 and Ub75 migrated at the mass as Ub75 or HDAC6 ZnF-UBP alone. (B) Comparison of gel filtration profiles of HDAC6 ZnF-UBP in mixture of wild-type ubiquitin and other ubiquitin C-terminal mutants. Only the

mixture of wild-type ubiquitin and HDAC6 Zn-UBP showed a lower elution volume, indicating interactions between the two proteins. None of the ubiquitin mutants interacted with HDAC6 ZnF-UBP.

FIGURE S3. The mass spectrometry of the commercial ubiquitin-AFC sample. The peak of 8776 Da is ubiquitin-AFC, and the peak of 8565 is unmodified ubiquitin.

FIGURE S4. CFTR-ΔF508 protein aggregates contain unanchored ubiquitin generated by deubiquitinase ataxin-3. Unanchored ubiquitin was co-localized with ataxin-3 in CFTR-ΔF508 aggregates. 293T cells un-transfected or transfected with GFP-CFTR-ΔF508 were treated with either DMSO (A1-5, C1-5) or MG132 (B1-5, D1-5). Cells were labeled for GFP (green), C-terminal ubiquitin (red), and DAPI (blue). In un-transfected cells, unanchored ubiquitin was well dispersed throughout the cells (A2). When GFP- CFTR-ΔF508 was expressed at high levels (arrows in C1-5) or low levels (arrowheads in C1-5), ubiquitin was recruited together with CFTR protein. Upon MG132 treatment (D1-5), CFTR aggregates were observed in perinuclear locations, indicating aggresome formation. Unanchored ubiquitin was co-localized with ataxin-3 in CFTR-ΔF508 aggregates (arrows in D1'-D5') or non-CFTR aggregates (arrowheads in D1'-D5'). When cells are transfected with ataxin-3 siRNA, GFP-CFTR-ΔF508 showed a diffuse signal (arrows in E1 and F1), indicating that aggresome formation was hindered. Scale bars in D1 for A-D, D1' for D', F1 for E-F: 10 μm.

FIGURE S5. Characterization of siRNA-mediated ataxin-3 knockdown in culture. Densitometric quantification of ataxin-3 levels in A549 cells transfected with scramble or ataxin-3 specific siRNA, using Image-J software. Raw intensities of ataxin-3 were averaged for triplicates of each condition and normalized to Actin levels.

FIGURE S6. Ataxin-3 knockdown did not affect the total levels of CFTR-ΔF508 expression. The level of GFP-CFTRΔF508 were measured in cells that transfected with either scrambled or ataxin-3 RNAi and treated with either DMSO or MG132. Actin was used as a loading control. Knockdown of Ataxin 3 did not affect the total level of GFP-CFTRF508 in a cell treated with either DMSO or MG132.

FIGURE S7. Human Ataxin1-82Q-GFP forms aggresomes in the presence of Ataxin-3. Cultured 293T cells were transfected with human Ataxin1-82Q-GFP and either scramble (Lanes 1-6) or Ataxin3 siRNA (Lanes 7-12), and treated with either DMSO (Lanes 1-3, 7-9) or MG132 (lanes 4-6, 10-12). Cell lysates were collected 2 days after transfection. Ataxin1-82Q-GFP aggregates were immunoprecipitated with GFP antibody and resolved by SDS-PAGE and probed for N-terminal Ubiquitin (*A*), C-terminal Ubiquitin (*B*), GFP (C), HDAC6 (*D*) and ataxin-3 (E) and antibody. As shown in *E* (lanes 7-12), ataxin-3 is not completely knocked-down with siRNA, but its reduced levels are enough to significantly reduce the recruitment of HDAC6 *(D*; lanes 7-12) and mono-ubiquitin with exposed C-terminal (*B* ; lanes 7-12) into the aggresome.

FIGURE S8. Other DUB proteins USP5 or USP13 did not localize to the aggresome. A549 cells were transfected with GFP-CFTRΔF508 and treated with MG132 to enhance aggresome formation. Cells were labeled for GFP (Green), USP5 (Red, A2), USP13 (Red, B2) and C-terminal Ubiquitin (Magenta). Neither USP5 nor USP13 colocalized with the aggresome.

Supplementary Table

Table S1. Summary of the Gel Filtration and Dynamic Light Scattering (DLS) Results

	Gel Filtration		Dynamic Light Scattering		Interaction
	Elution Volume (ml)	SEC MW (kDa)	Stokes Radius (nm)	DLS MW (kDa)	with HDAC6 ZnF-UBP
HDAC6 ZnF-UBP	14.48	6.2	1.4	8	n.a.
Ubiquitin and Mutants	14.30	6.4	1.3	6	n.a.
wt Ub + ZnF-UBP	12.73	15.8	2.1	19	Yes
Ub75 + ZnF-UBP	14.48	6.2	1.5	9	No
Ub74 + ZnF-UBP	14.48	6.2	1.4	8	No
Ub G76A + ZnF-UBP	14.27	6.9	1.6	11	No
Ub G75A,G76A + ZnF-UBP	14.48	6.2	1.5	9	No

FIGURE S1

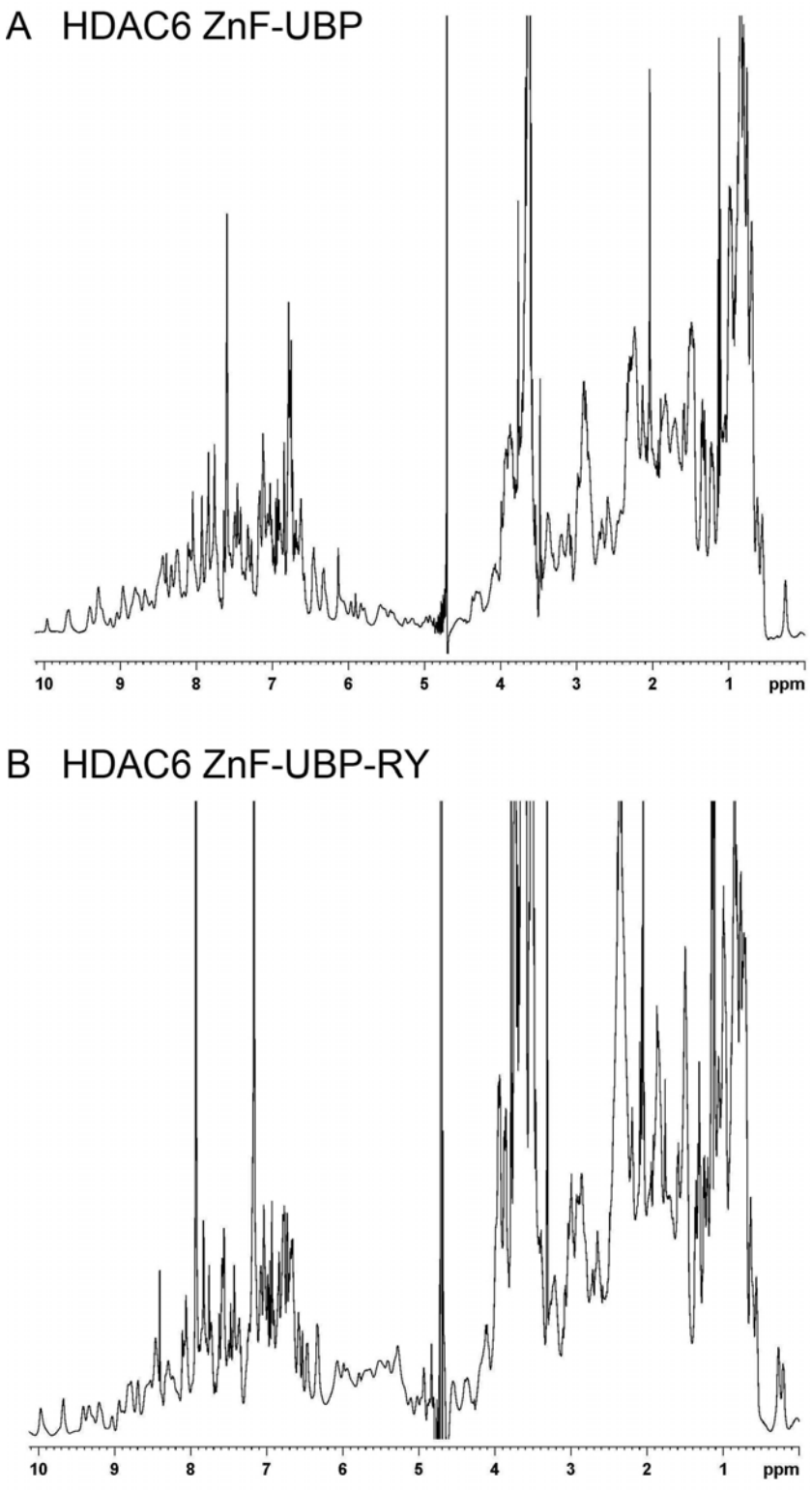

A HDAC6 ZnF-UBP
10 9 8 7 6 5 4 3 2 1 ppm
B HDAC6 ZnF-UBP-RY
10 9 8 7 6 5 4 3 2 1 ppm

FIGURE S2

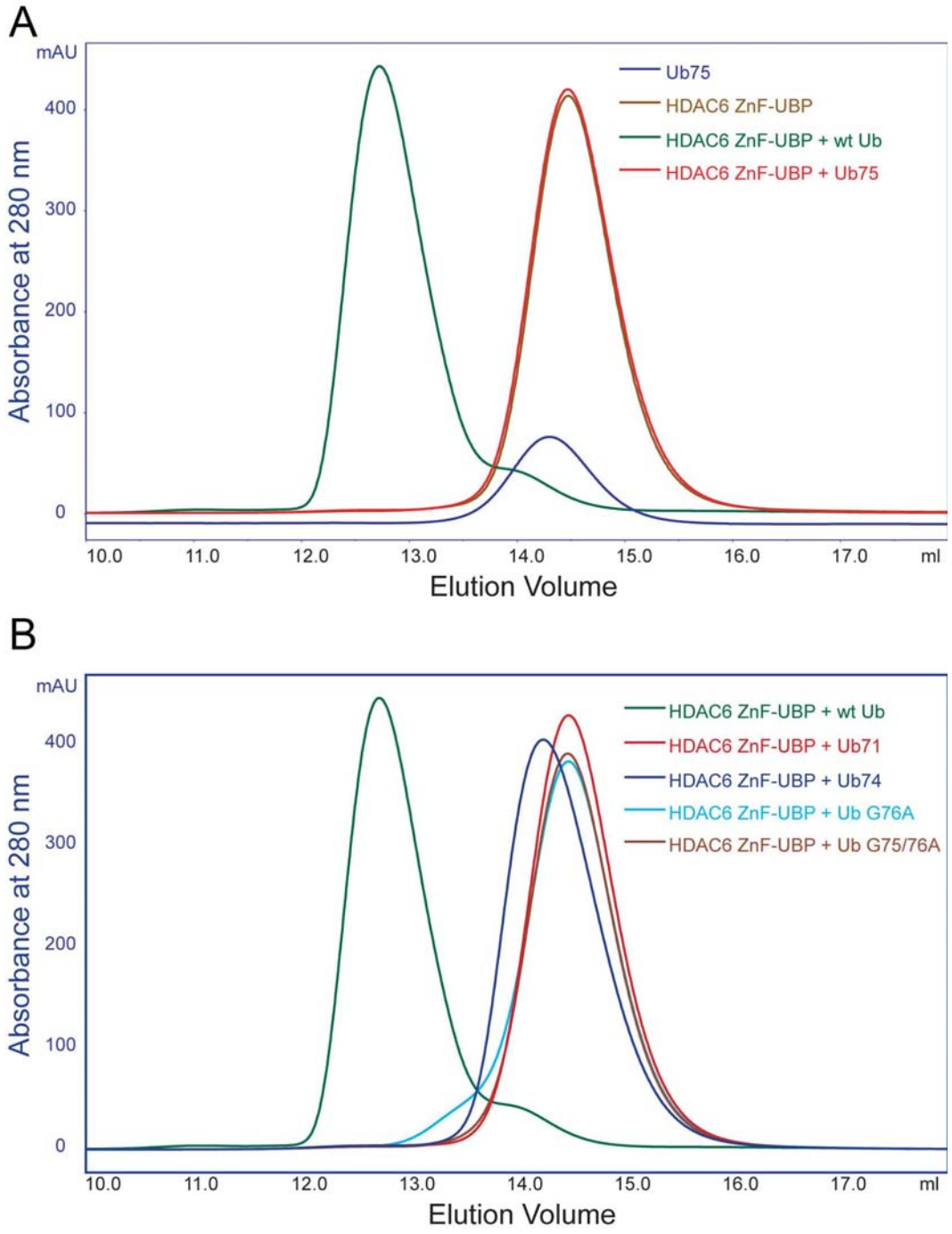

A
mAU
400
300
200
100
0
Absorbance at 280 nm
Ub75
HDAC6 ZnF-UBP
HDAC6 ZnF-UBP + wt Ub
HDAC6 ZnF-UBP + Ub75
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
ml
Elution Volume
B
mAU
400
300
200
100
0
Absorbance at 280 nm
HDAC6 ZnF-UBP + wt Ub
HDAC6 ZnF-UBP + Ub71
HDAC6 ZnF-UBP + Ub74
HDAC6 ZnF-UBP + Ub G76A
HDAC6 ZnF-UBP + Ub G75/76A
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
ml
Elution Volume

FIGURE S3

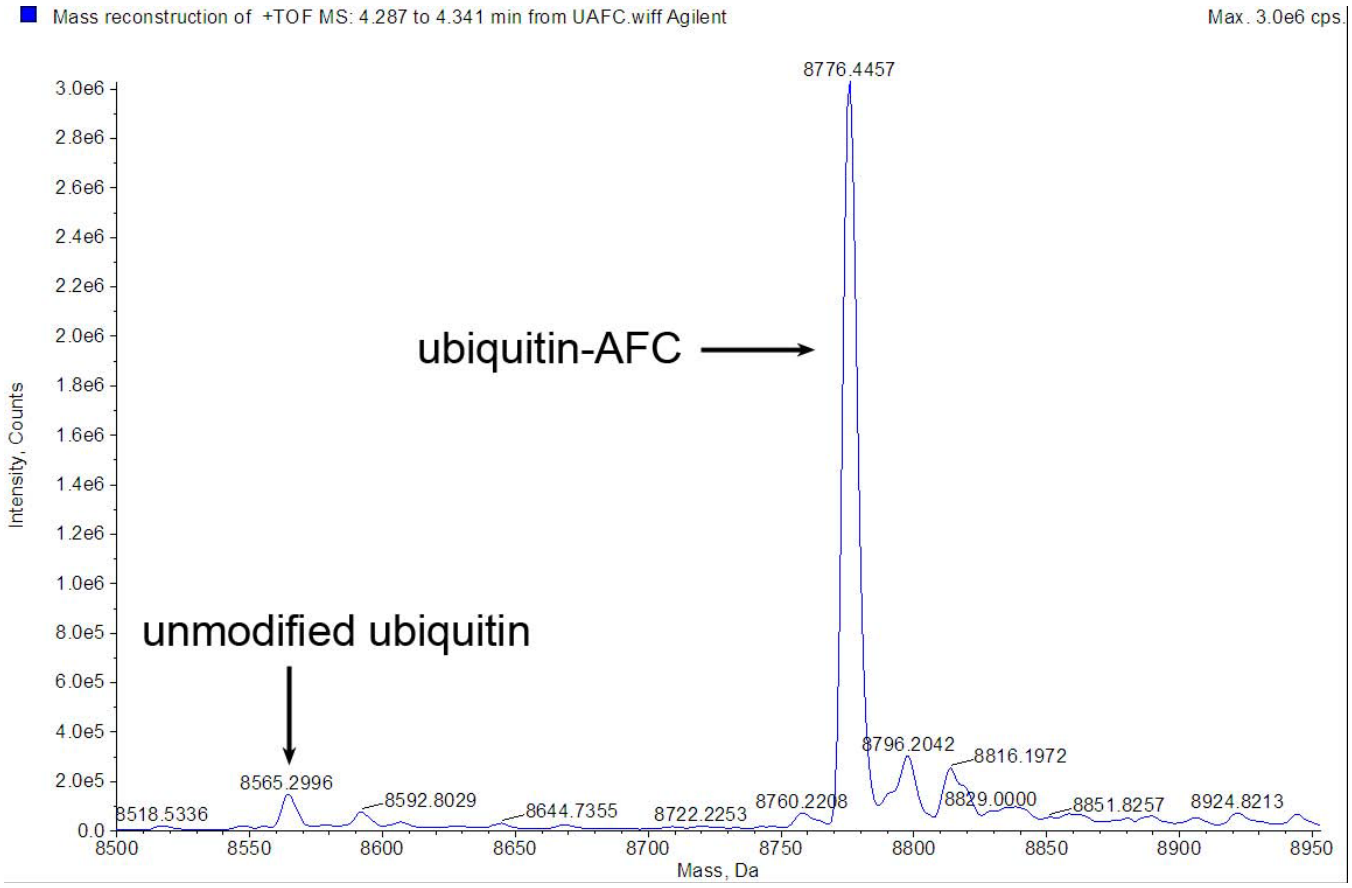

Mass reconstruction of +TOF MS: 4.287 to 4.341 min from UAFC.wiff Agilent
Max. 3.0e6 cps.
Intensity, Counts
Mass, Da
ubiquitin-AFC
unmodified ubiquitin
8776.4457
8796.2042
8816.1972
8565.2996
8592.8029
8644.7355
8722.2253
8760.2208
8829.0000
8851.8257
8924.8213
8518.5336

FIGURE S4

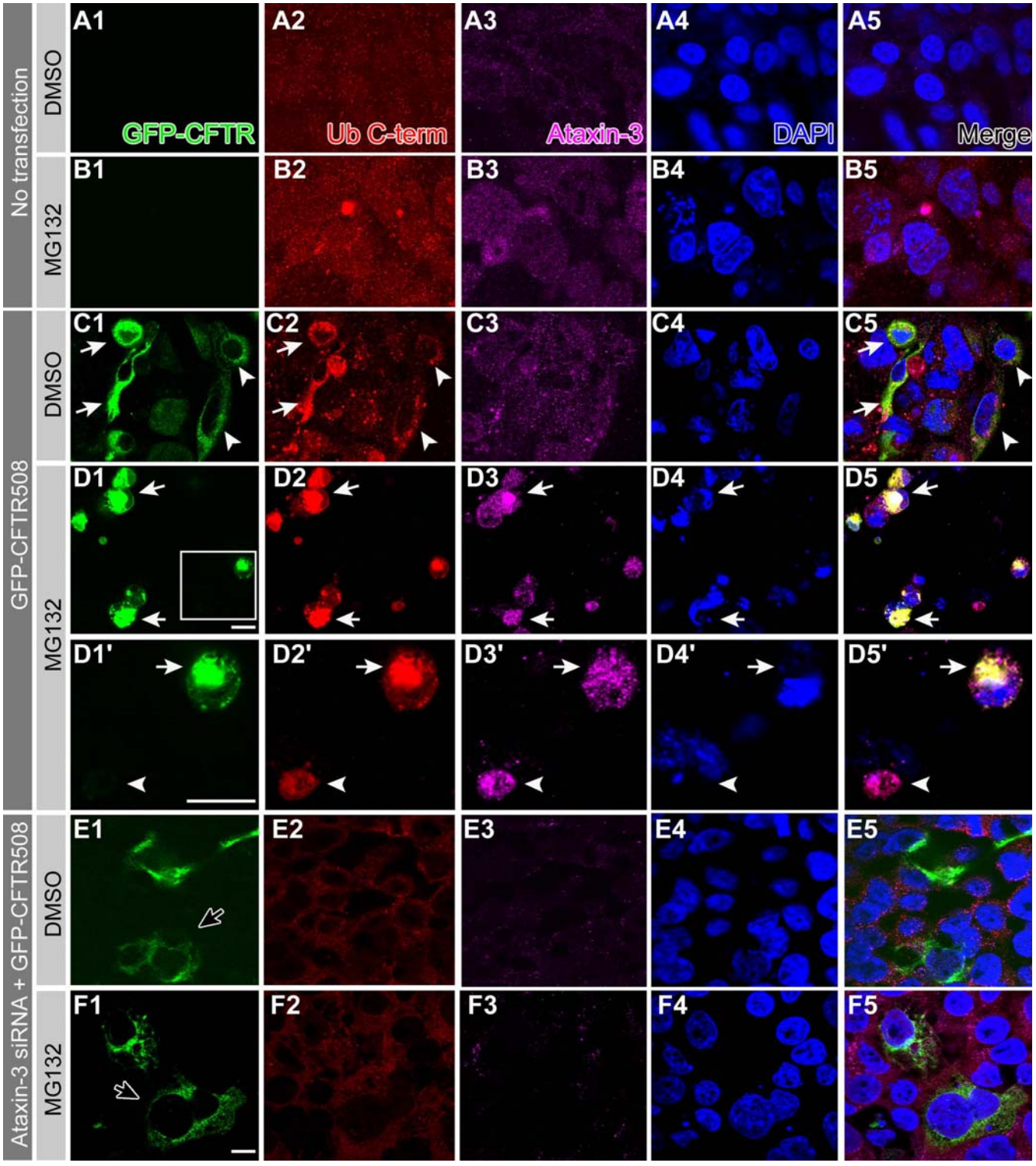

No transfection
GFP-CFTR
Ataxin-3 siRNA + GFP-CFTR508
DMSO
MG132
A1
GFP-CFTR
A2
Ub C-term
A3
Ataxin-3
A4
DAPI
A5
Merge
B1
B2
B3
B4
B5
C1
C2
C3
C4
C5
D1
D2
D3
D4
D5
D1'
D2'
D3'
D4'
D5'
E1
E2
E3
E4
E5
F1
F2
F3
F4
F5

FIGURE S5

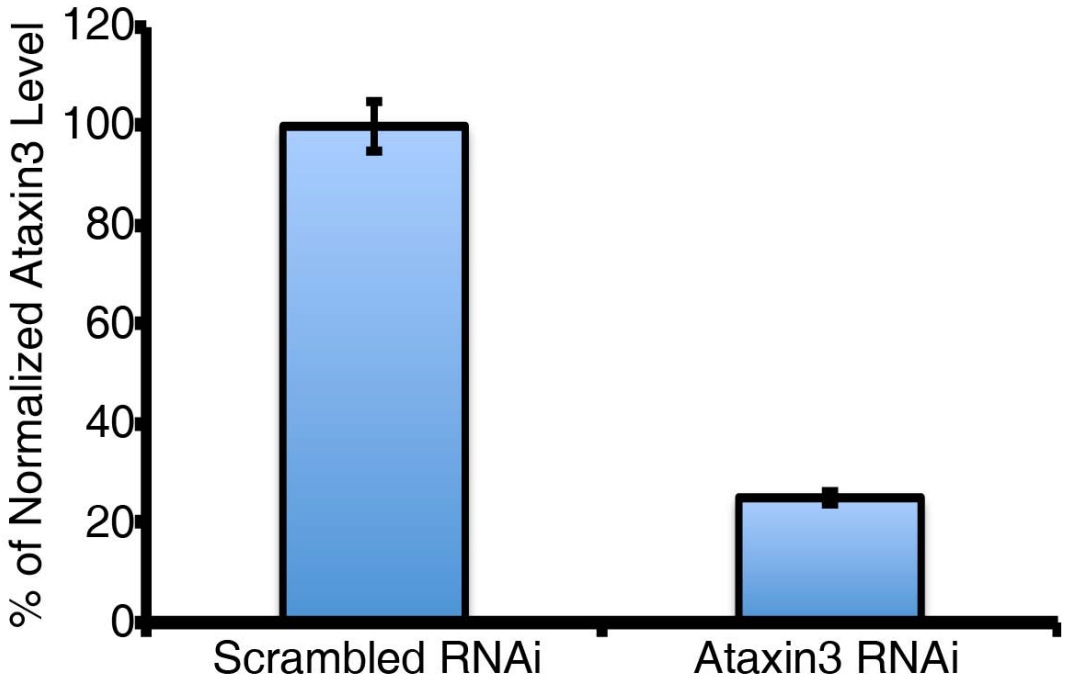

% of Normalized Ataxin3 Level
120
100
80
60
40
20
0
Scrambled RNAi
Ataxin3 RNAi

FIGURE S6

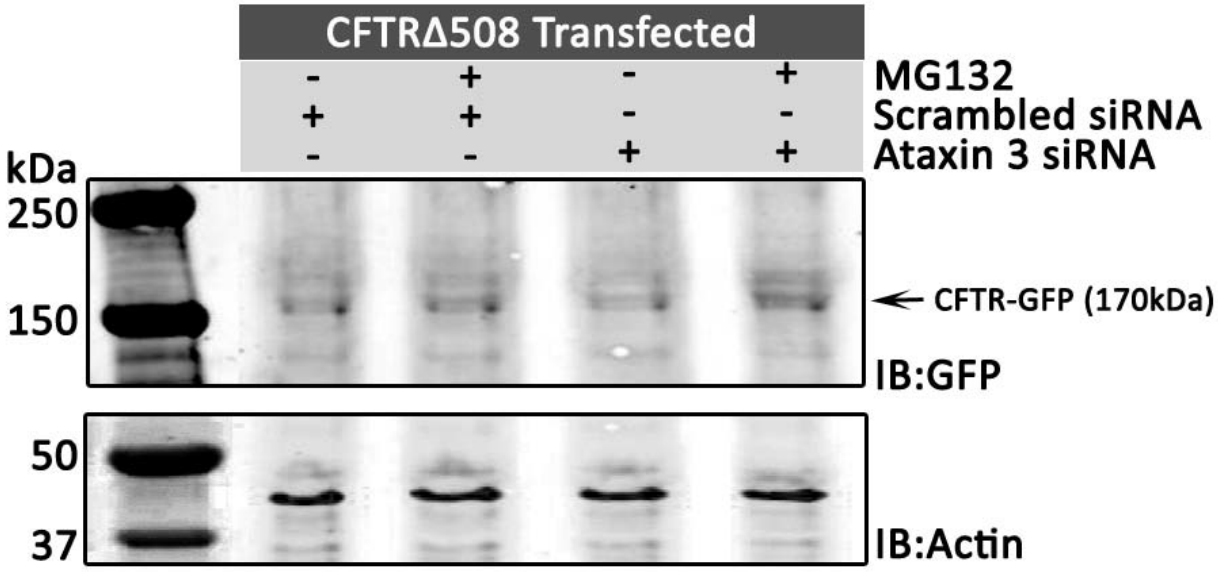

CFTRΔ508 Transfected
- + - +
+ + - -
- - + +
MG132
Scrambled siRNA
Ataxin 3 siRNA
kDa
250
150
CFTR-GFP (170kDa)
IB:GFP
50
37
IB:Actin

FIGURE S7

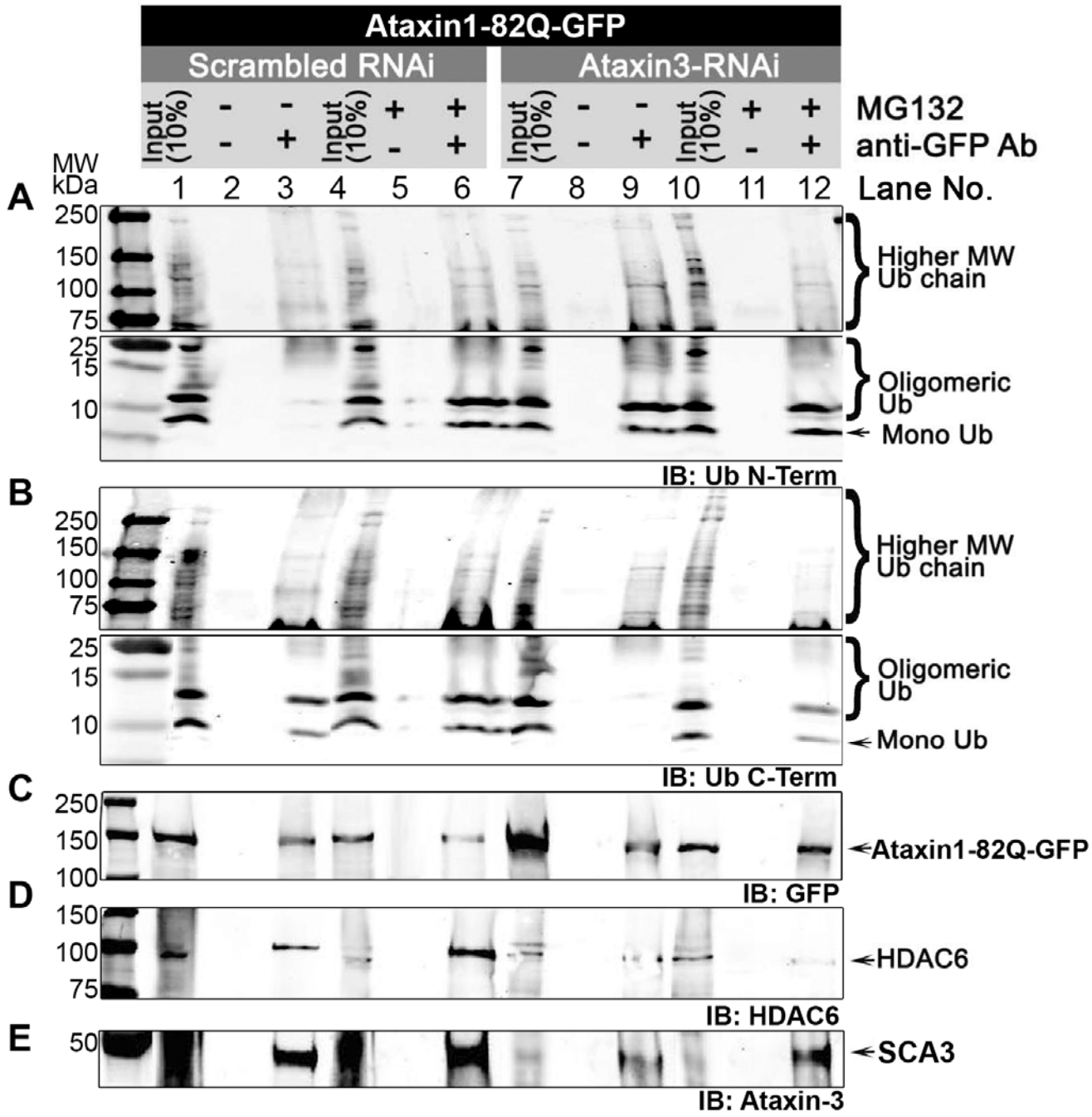
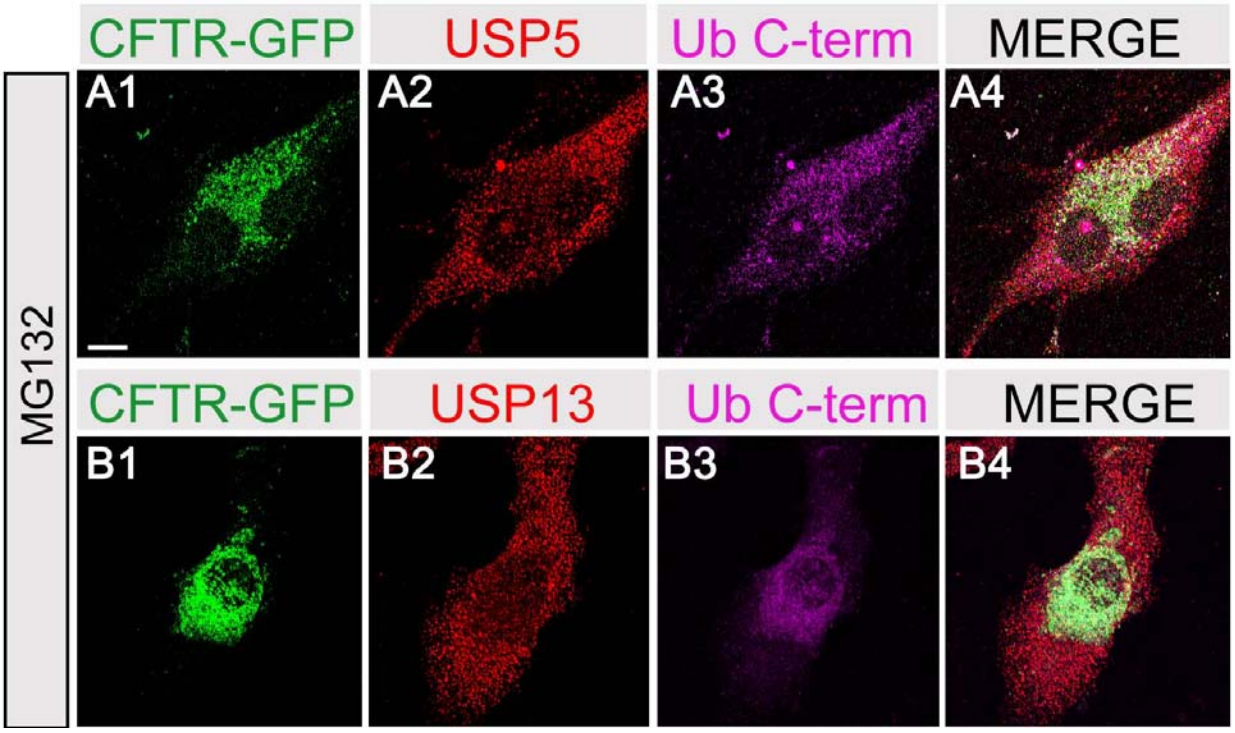

Ataxin1-82Q-GFP
Scrambled RNAi
Ataxin3-RNAi
Input (10%)
MG132
anti-GFP Ab
MW kDa
Lane No.
A
Higher MW Ub chain
Oligomeric Ub
Mono Ub
IB: Ub N-Term
B
Higher MW Ub chain
Oligomeric Ub
Mono Ub
IB: Ub C-Term
C
Ataxin1-82Q-GFP
IB: GFP
D
HDAC6
IB: HDAC6
E
SCA3
IB: Ataxin-3

FIGURE S8


CFTR-GFP
USP5
Ub C-term
MERGE
A1
A2
A3
A4
MG132
CFTR-GFP
USP13
Ub C-term
MERGE
B1
B2
B3
B4