Supplementary information

Supplementary method. **RT-PCR analysis**

The total RNA was extracted using TRIzol reagent and RT-PCR was carried out with a RT-PCR kit (Invitrogen). The primer sequences for E6-AP and β-actin were as follows: E6-APF, 5'-AACTGAGGGCTGTGGAAATG-3'; E6-APR, 5'-TCCGAAAGCTCAGAACCAGT-3'; ActinF, 5'-TACAGCTTCACCACC-3'; ActinR, 5'- ATGCCACAGGATTTC-3'. PCR conditions for E6-AP and β-actin were same: an initial denaturation step at 94°C for 4 mins and then cycling through 94°C for 30 sec denaturation, 60°C for 30 sec annealing, 72°C for 45 sec extension and a final extension step at 72°C for 5 min. The cycle number for E6-AP was 30 and for β-actin was 23.

Supplementary Fig.S1. **The mRNA level of E6-AP is increased under various stress conditions.**

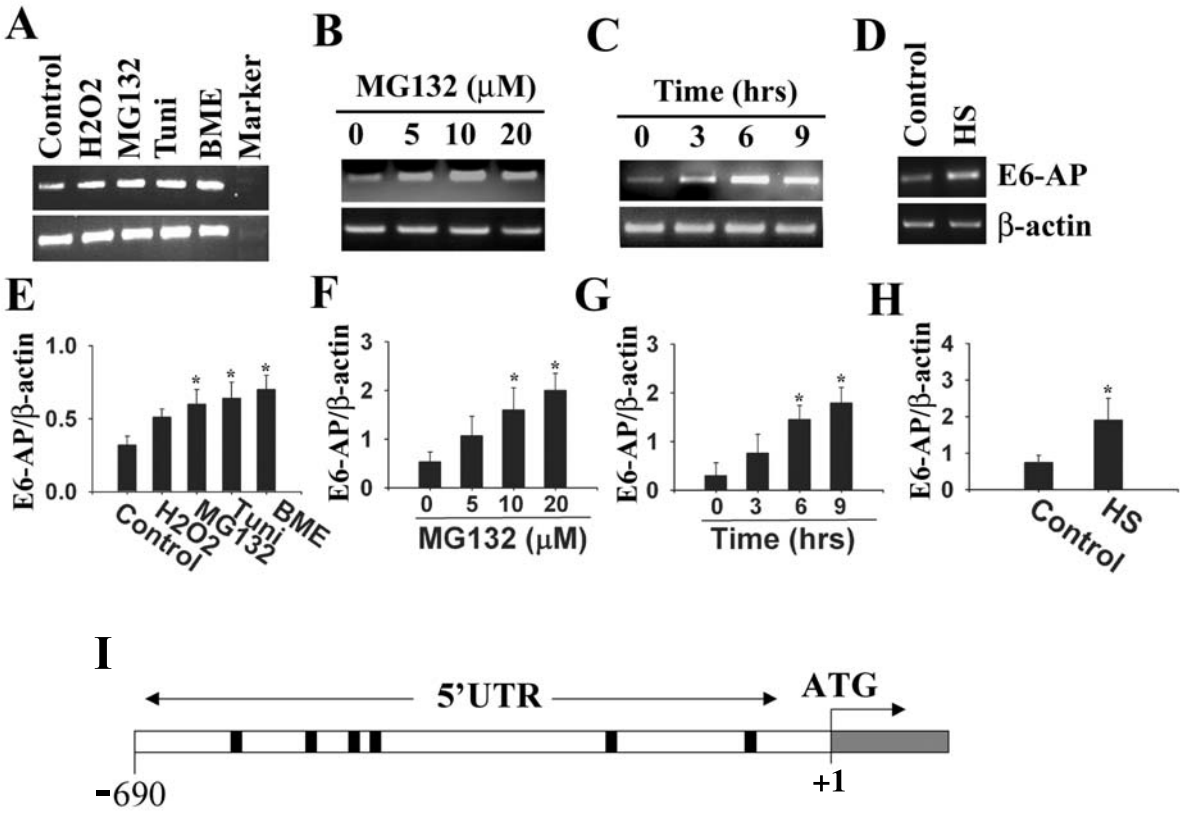
(A) Neuro 2a cells were treated with H_2O_2 (0.25 mM for 2 h), β-mercaptoethanol (5 mM for 2 h), tunicamycin (5 μg/ml for 4 h) and MG132 (5 μM for 4 h). Cells were collected and subjected for RNA extraction followed by RT-PCR analysis of E6-AP and β-actin. (B) Dose-dependent effect of proteasome inhibitor, MG132 (exposed for 4 h) on E6-AP mRNA levels. (C) Time-course studies of the effect of MG132 (5 μM) on E6-AP mRNA levels. (D) Effect of heat stress on E6-AP mRNA levels. Cells were exposed to 45°C for 30 mins and then returned back to the normal incubator for 1 hrs. (E, F, G and H)

Quantitation of the E6-AP band intensities as shown in A, B, C and D respectively collected from three independent experiments using NIH image analysis software. Data were expressed as ratio of E6-AP to β-actin. Values are means ± SD of three independent experiments. *$p<0.05$ as compared to respective control. Tuni, Tunicamycin; BME, β-mercaptoethanol. (I) Schematic diagram of the 5'UTR sequences of *UBE3A* gene. Dark area represents the consensus HSF1 binding sequences (AGAAN).

Supplementary Fig.S2. **Formation of GFP-CFTR aggresomes upon proteasome inhibition.**

Cos-7 cells were platted onto 2-well chamber slides and transfected with GFP-CFTR plasmid. Thirty-six hours of post transfection, cells were treated with MG132 for 8 h. The cells were then subjected to immunofluorescence staining using γ-tubulin and Hsp70 antibodies. Rhodamine-conjugated secondary antibody was used to stain γ-tubulin and Hsp70. Arrow indicates the GFP-CFTR aggresome and localization of Hsp70 to GFP-CFTR aggresome. Scale bar, 20 μm.

Fig.S1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MG132
Tuni
BME
Marker
B
MG132 (μM)
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C
Time (hrs)
0 3 6 9
D
Control
HS
E6-AP
β-actin
E
E6-AP/β-actin
1.0
0.5
0.0
Control
H2O2
MG132
Tuni
BME
F
E6-AP/β-actin
0 5 10 20
MG132 (μM)
G
E6-AP/β-actin
0 3 6 9
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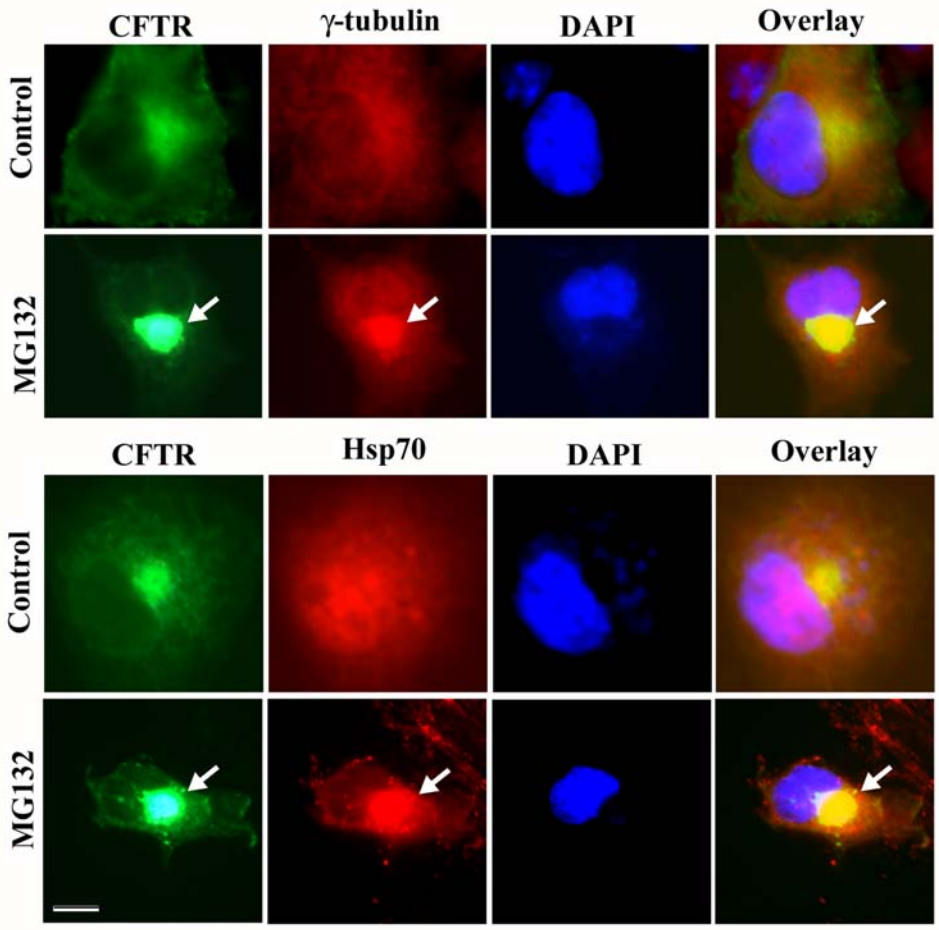
H
E6-AP/β-actin
Control
HS
I
5'UTR
ATG
-690
+1

Fig.S2


CFTR
γ-tubulin
DAPI
Overlay
Control
MG132
CFTR
Hsp70
DAPI
Overlay
Control
MG132