Supplementary figure legends

S1.

(A) Microscopic pictographs of HeLa cells stably expressing Htt-GFP with NES (upper columns) or NLS (lower columns) containing various Q stretches (left Q = 25, middle Q = 46, and right Q = 72) by GFP direct fluorescence. Scale bar = 20 ⌠m. Representative IBs are shown by arrows.

(B) Immunoblot of stable NES and NLS HeLa cell lines. Htt-GFP expression was detected by anti-GFP antibody.

(C) Filter retardation assay of NES and NLS HeLa stable cell lines. Htt-GFP aggregates retained on cellulose acetate membrane were detected by anti-GFP antibody.

(D) San1p ubiquitylates Htt. FLAG-tagged San1p was cotransfected with HA-tagged ubiquitin to NLSQ25, NLSQ46, and NLSQ72 cells for 48 h. After an additional 4 h of 5-⌠M MG-132 treatment, cell lysates were immunoprecipitated by anti-GFP antibody and subjected to immunoblots by anti-HA antibody.

(E) Expression of various UHRF-2s in HeLa cells was detected with indirect immunofluorescence by anti-FLAG antibody and Alexa 488 secondary antibody (upper panels). Wt (left panels) and RING mutant (middle panels) showed nuclear localization. _NLS mutant was expressed at the cytoplasm (right panels). Lower panels show nuclear staining by SYTOX orange. Scale bar = 20 ⌠m.

S2.

(A) Recombinant RING domain mutant lacks *in vitro* ligase activity. Anti-FLAG immunoblot of the postreaction mixture is shown to detect ubiquitylation.

(B) UHRF-2 _NLS retains *in vitro* ligase activity. Anti-FLAG immunoblot of the postreaction mixture is shown to detect ubiquitylation.

(C) Soluble amount of Htt-GFP was measured upon UHRF-2 expression. Immunoblot analysis (upper bands) and its band intensity measurement revealed little effect of UHRF-2 on soluble amount of Htt-GFP. Bars indicate SE.

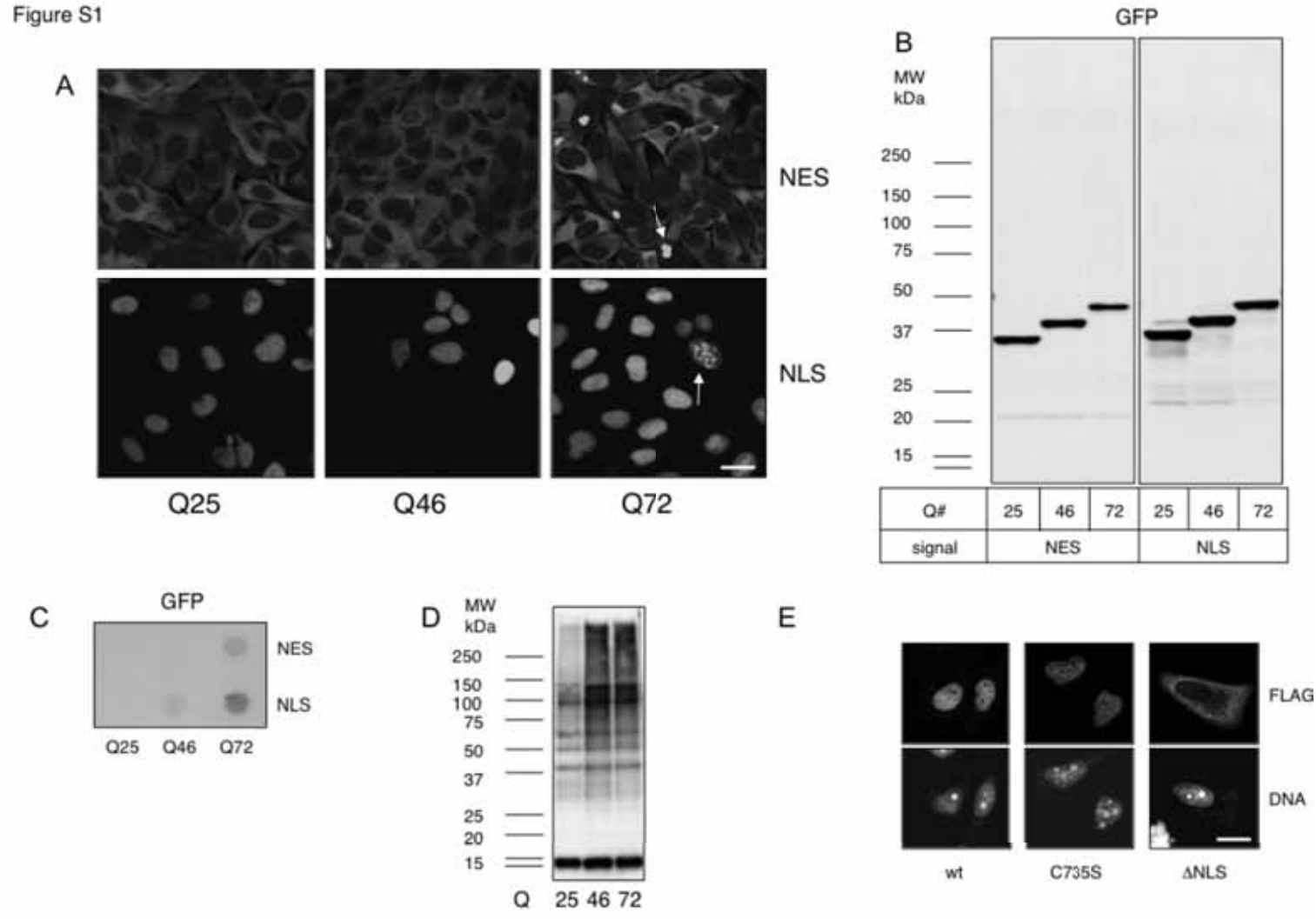
(D) Anti-UHRF-2 antibody specifically recognizes UHRF-2. Specificity of anti-UHRF-2 antibody raised in rabbits was evaluated by immunoblot of recombinant UHRF-1 and UHRF-2. The right panel shows immunoblot by anti-His antibody; the left panel shows immunoblot by anti-UHRF-2 antibody.

(E) Anti-UHRF-2 antibody recognizes endogenous UHRF-2. HeLa cell lysate was subjected to immunoblot by anti-UHRF-2 antibody. Arrow indicates UHRF-2.

(F) UHRF-2 accelerates DRPLA degradation. Wt or RING domain mutant (C735S) UHRF-2 was overexpressed with DRPLAQ19 or DRPLAQ80 in 293T cells and a pulse chase experiment was performed after 48 h. Relative band intensities from SDS-PAGE were normalized to 100% at time = 0 and plotted onto semi-log charts with fitted lines (wt: straight line; C735S: broken line) to calculate the half-life of the proteins. Bars indicate SE.

(G) UHRF-2 antibody recognizes mouse endogenous UHRF-2. Cell lysates from mouse neuro2a cells or human HeLa cells were subjected to immunoblot. The left panel shows immunoblot with peptide-absorbed antibody (peptide +). The right panel shows immunoblot with the antibody (peptide _). Arrow indicates endogenous UHRF-2.

Figure S1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25
46
72
25
46
72
signal
NES
NLS
C
GFP
NES
NLS
Q25
Q46
Q72
D
MW
kDa
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20
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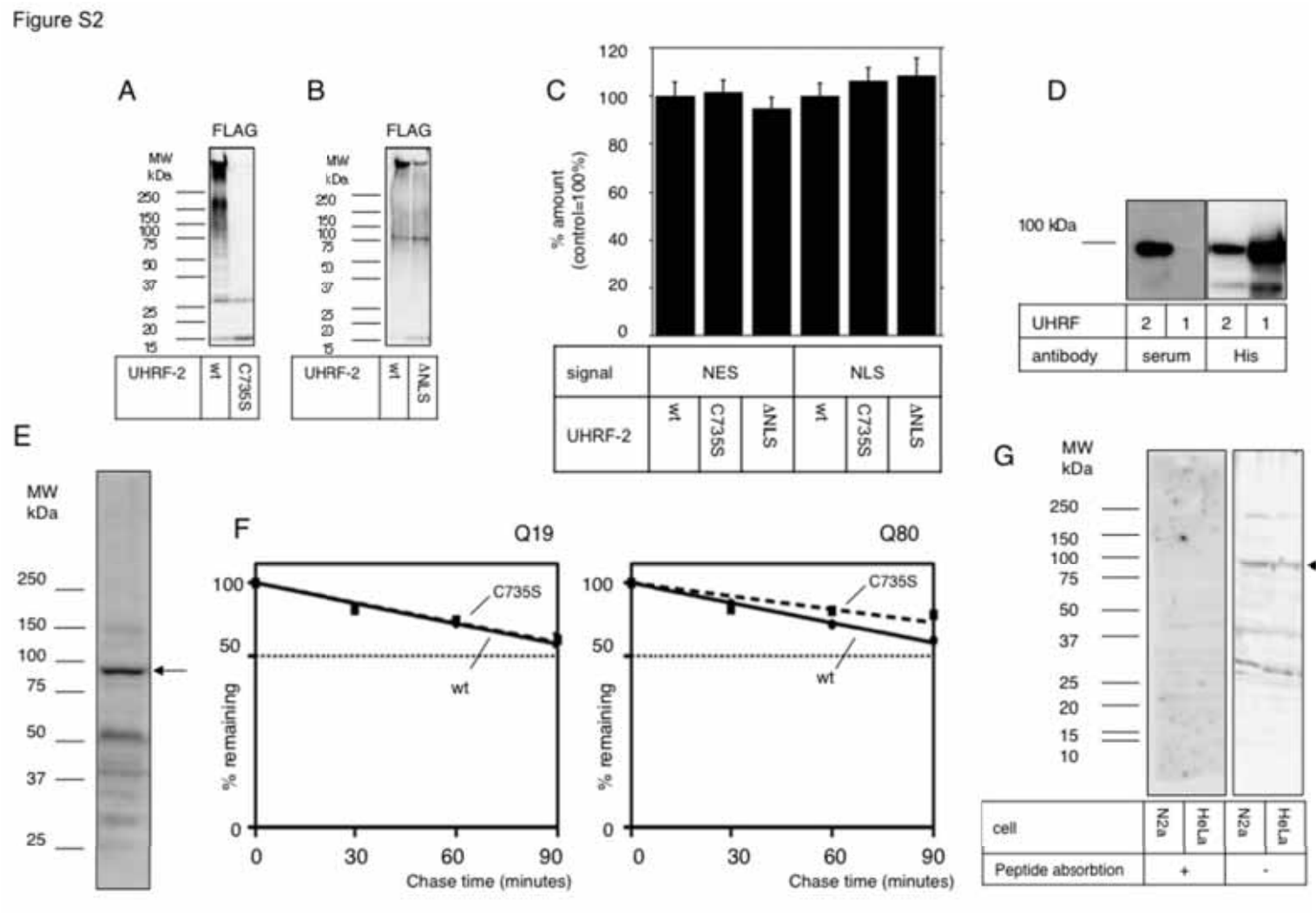
Q
25
46
72
E
FLAG
DNA
wt
C735S
ΔNLS

Figure S2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20
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UHRF-2
wt
C735S
B
FLAG
MW kDa
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150
100
75
50
37
25
20
15
UHRF-2
wt
ΔNLS
C
% amount (control=100%)
120
100
80
60
40
20
0
signal
NES
NLS
UHRF-2
wt
C735S
ΔNLS
wt
C735S
ΔNLS
D
100 kDa
UHRF
2
1
2
1
antibody
serum
His
E
MW kDa
250
150
100
75
50
37
25
F
Q19
Q80
100
50
0
% remaining
C735S
wt
0
30
60
90
Chase time (minutes)
G
MW kDa
250
150
100
75
50
37
25
20
15
10
cell
N2a
HeLa
N2a
HeLa
Peptide absorbtion
+
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