Expanded View Figures

Figure EV1. USP36 promotes SUMOylation in cells.

A., B USP36 promotes SUMOylation in cells. 293 (A) and T47D (B) cells transfected with control or Flag-USP36 plasmid were assayed by IB for SUMOylated proteins.
C, D USP36 promotion of SUMOylation is specific. H1299 cells transfected with increasing amounts of Flag-USP36, Flag-JOSD3 (C), or Flag-USP7 (D) were assayed by IB.
E, F USP36 promotes SUMOylation by SUMO1. H1299 cells (E) or HeLa and U2OS cells (F) transfected with Flag-USP36 were assayed by IB using anti-SUMO1 antibody.
G USP36 promotes SUMOylation by exogenous SUMO2. H1299 cells transfected with His-SUMO2 and/or Flag-USP36 were subjected to Ni^{2+}-NTA agarose beads pulldown (PD) followed by IB using anti-SUMO2/3 antibody (top panel). WCL were also directly assayed by IB using indicated antibodies (bottom panel).
H Nucleolar localization of USP36. HeLa cells transfected with Flag-USP36 were stained with anti-Flag (red) and anti-B23 (green) antibodies followed by DAPI (blue).
I USP36 promotes nucleolar SUMOylation. HeLa cells transfected with control or V5-USP36 were fractionated to the cytoplasm (Cyto), nucleoplasm (Np), and nucleolus (No) fractions, followed by IB to detect the indicated proteins.

Source data are available online for this figure.

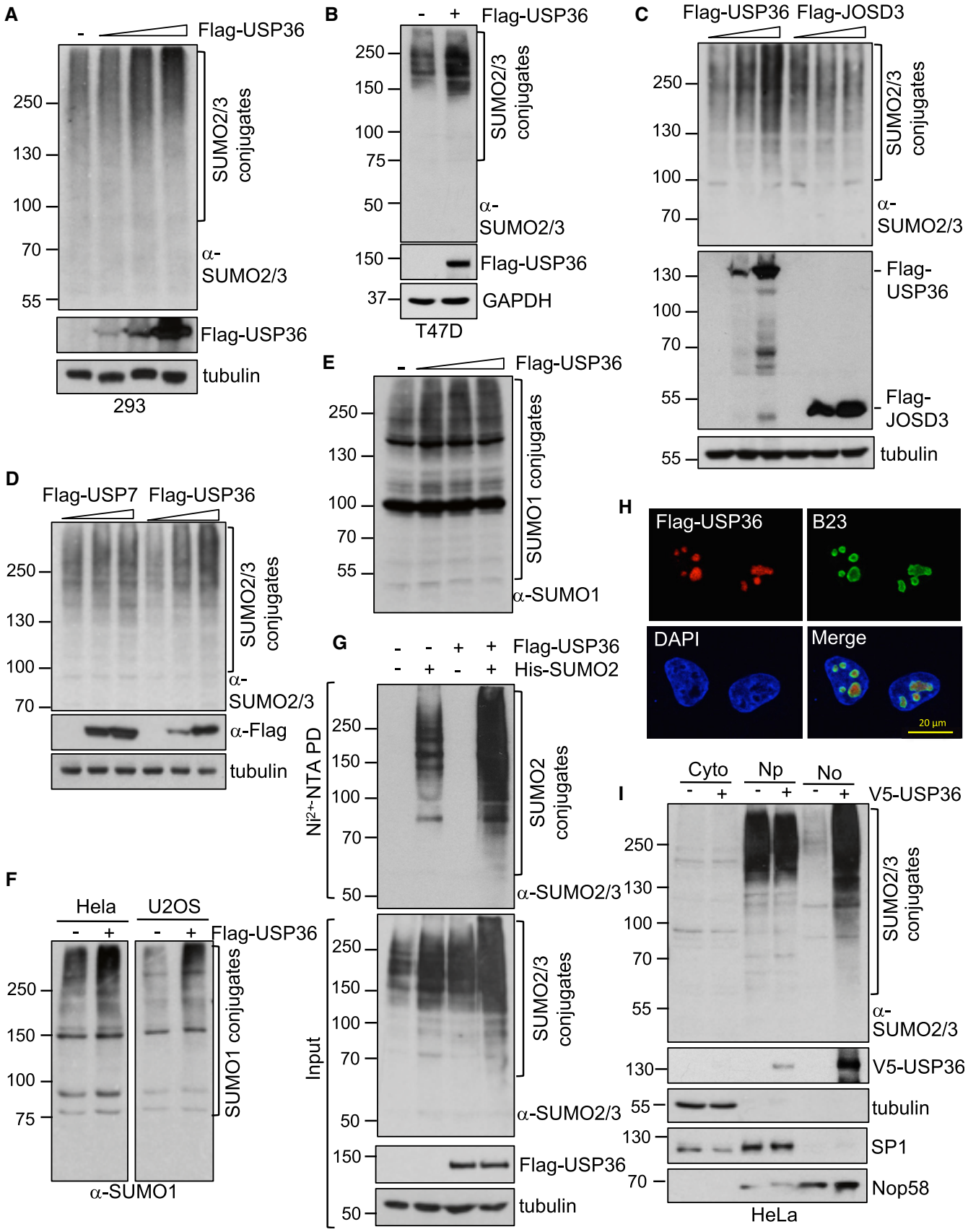

Figure EV1.

Figure EV2. USP36 neither induces the levels of SUMO E1 or E2 nor increases the levels of SUMO proteases.

A USP36 does not increase the levels of SUMO E1 and E2. H1299 cells transfected with control or increased amounts of Flag-USP36 were assayed by IB using antibodies against indicated proteins.

B, C USP36 does not reduce the levels of SUMO proteases. H1299 cells transfected with Flag-USP36 were assayed by IB using antibodies against indicated SENP proteins (B) or with Flag-USPL1 (C) in the presence or absence of V5-USP36 were assayed by IB.

D H1299 cells transfected with V5-Ub together with Flag-USP36 or the indicated mutants were assayed by IB to detect total ubiquitination.

E WT USP36 and the H382A mutant, but not the C131A mutant, promote SUMOylation in the nucleolus. H1299 cells transfected with control or the indicated Flag-USP36 plasmids were subjected to nucleolar isolation, followed by IB. SUMO2/3 conjugates are indicated in the top panel.

F The N-terminus of USP36 interacts with Ubc9 *in vitro*. Purified His-Ubc9 was incubated with GST, GST-USP36$^{1–420}$, GST-USP36$^{421–800}$, or GST-USP36$^{801–1121}$. Bound protein was detected by IB. GST and GST-fusion proteins were shown in the bottom panel by coomassie staining.

G The N-terminal USP36 binds to SUMO in cells. H1299 cells transfected with either control or Flag-USP36$^{1–420}$ plasmid were assayed by IP using anti-Flag, followed by IB with anti-SUMO2/3 antibodies.

H Requirement of the SUMO–Ubc9 backside interaction for USP36's SUMO E3 activity. Recombinant T7-PARP1 protein (0.1 μM) was incubated with SUMO E1 (50 nM, Boston Biochem), Ubc9 (50 nM, WT or the F22A mutant), SUMO2 (4 μM, WT or the D63R mutant) in the presence of USP36$^{1–800}$ (50 nM) and/or ATP (2.5 mM) at 30°C for 5 h and then assayed by IB.

I USP36 interacts with Ubc9 *in vitro*. Purified His-Ubc9 (Wt or the F22A mutant) was incubated with GST or GST-USP36$^{1–800}$. Bound Ubc9 was detected by IB using anti-Ubc9 antibody. GST and GST-fusion proteins were detected by IB with anti-GST.

Source data are available online for this figure.

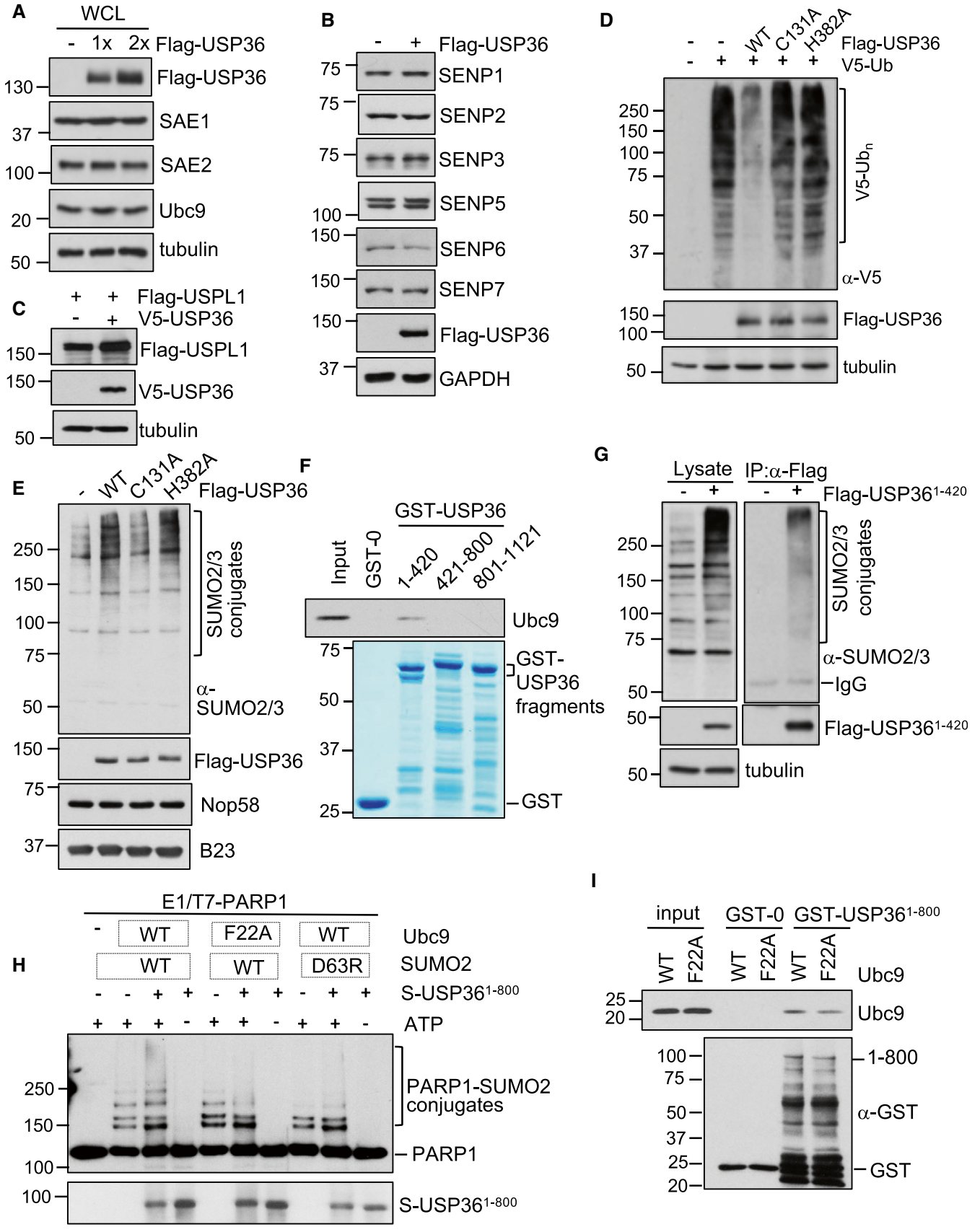

Figure EV2.

Figure EV3. USP36 promotes SUMOylation of Nop58 and Nhp2.

A Candidate proteins whose SUMOylation is increased by USP36. H1299 cells transfected His-SUMO2 together with control or Flag-USP36 plasmids were subjected to Ni^{2+}-NTA purification followed by LC-MS-MS analysis.

B, C USP36 SUMOylates exogenous Nop58. H1299 cells transfected with His-SUMO2 and/or USP36 with WT Nop58 (B) or SUMOylation-defective mutant Nop58 (K457R/K497R, $Nop58^{2KR}$) (C) were subjected to Ni^{2+}-NTA PD followed by IB to detect SUMOylation of Nop58.

D, E USP36 SUMOylates exogenous Nhp2. H1299 cells transfected with His-SUMO2 and/or USP36 with WT Nhp2 (D) or with SUMOylation-defective mutant Nhp2 ($Nop58^{K5R}$) (E) were subjected to Ni^{2+}-NTA PD followed by IB to detect SUMOylation of Nph2.

F USP36 does not SUMOylate nucleolar protein NOLC1. H1299 cells transfected with His-SUMO2, Flag-NOLC1 with or without V5-USP36 were subjected to Ni^{2+}-NTA PD followed by IB to detect SUMOylation of NOLC1.

G, H Co-localization of USP36 with Nop58 and Nhp2 in the nucleolus. HeLa cells were transfected with Flag-USP36 and stained with anti-Flag (green) and anti-Nop58 (red) (G) or transfected with GFP-USP36 (green) and Flag-Nhp2 and then stained with anti-Flag (red) (H).

Source data are available online for this figure.

A

Protein Identified	Numbers of Peptides control	USP36
USP36	0	19
Nop58	3	6
TYY1	3	9
SIN3A	4	11
PRP8	4	9
MEPEC	4	9
XRCC5	4	7
FBLL1	0	3
RRP5	0	3

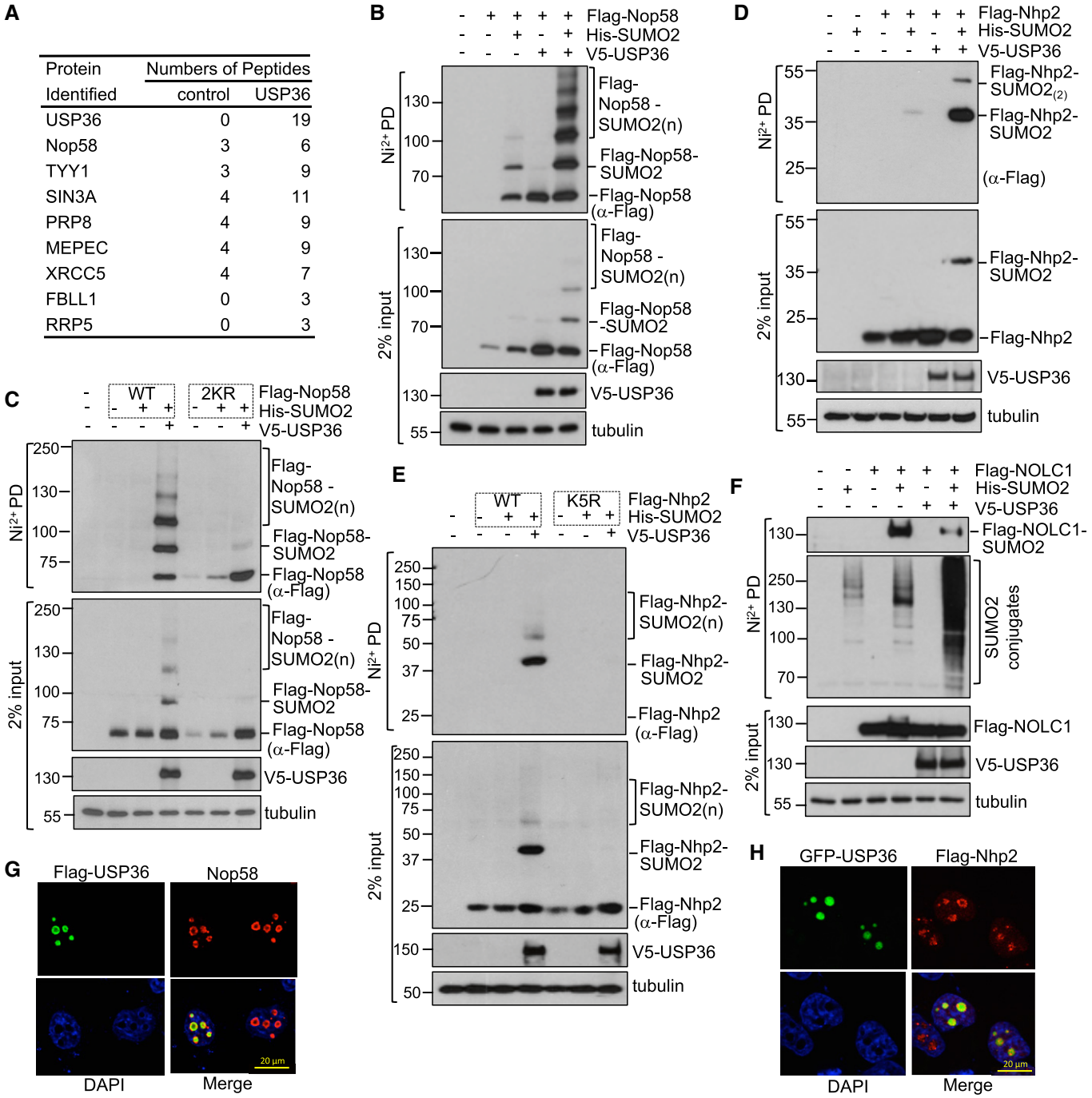

Figure EV3.

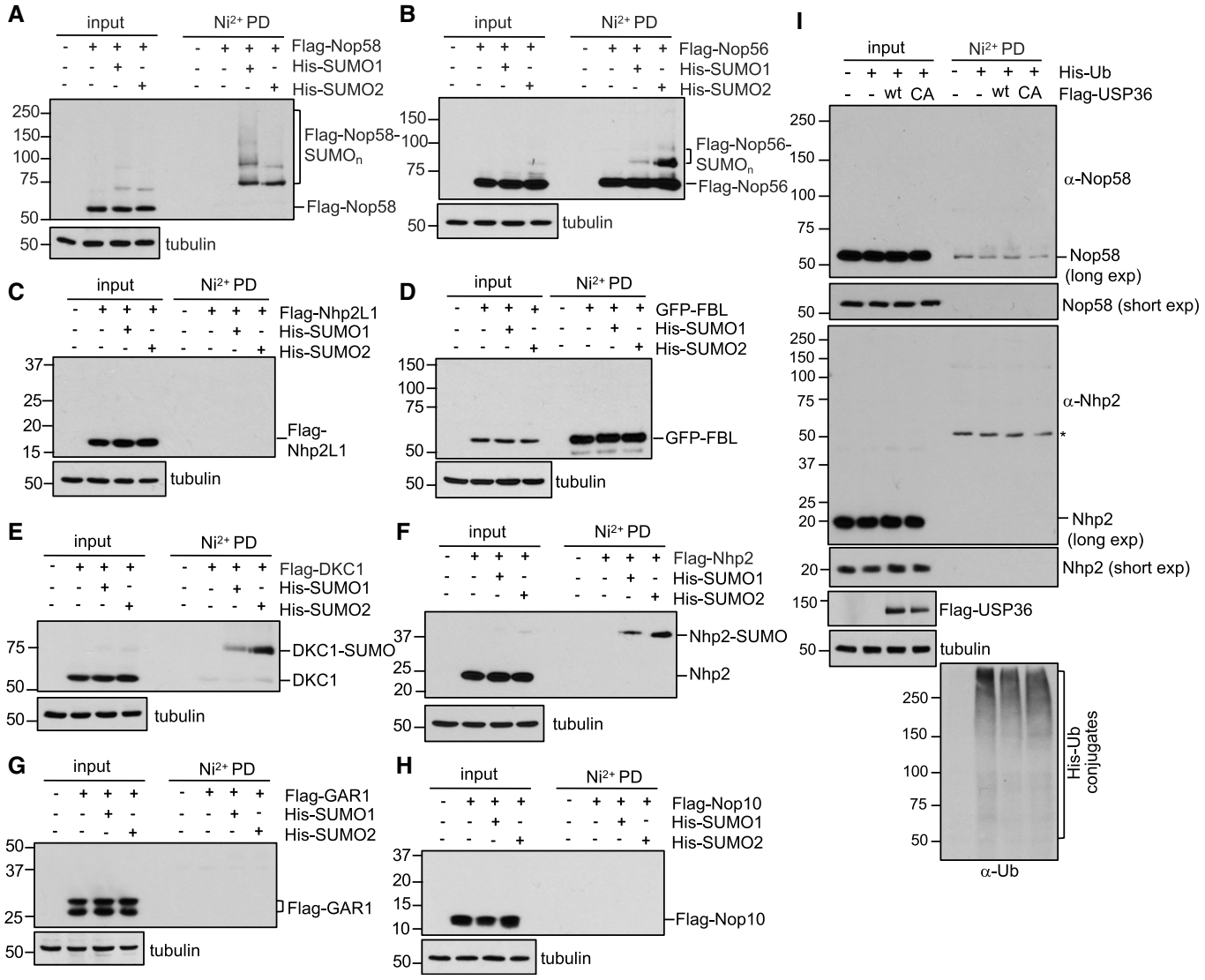

Figure EV4. SUMOylation of snoRNP proteins.

A–H SUMOylation of snoRNP proteins. H1299 cells transfected with the indicated snoRNP proteins together with His-SUMO1 or His-SUMO2 were assayed by Ni^{2+}-NTA PD and IB with anti-Flag (A–C, E–H) or anti-GFP (D) antibody to detect SUMOylated snoRNP proteins.

I Detection of endogenous Nop58 and Nhp2 ubiquitination. H1299 cells were transfected with His-Ub with or without Flag-USP36 (wt or the C131A mutant) and treated with MG132 for 6 h before harvesting. The cells were then subjected to Ni^{2+}-NTA beads pulldown, followed by IB with anti-Nop58 and anti-Nhp2. The total His-Ub conjugates are shown in the bottom. * indicates a non-specific anti-Nhp2 antibody-reacting band.

Source data are available online for this figure.

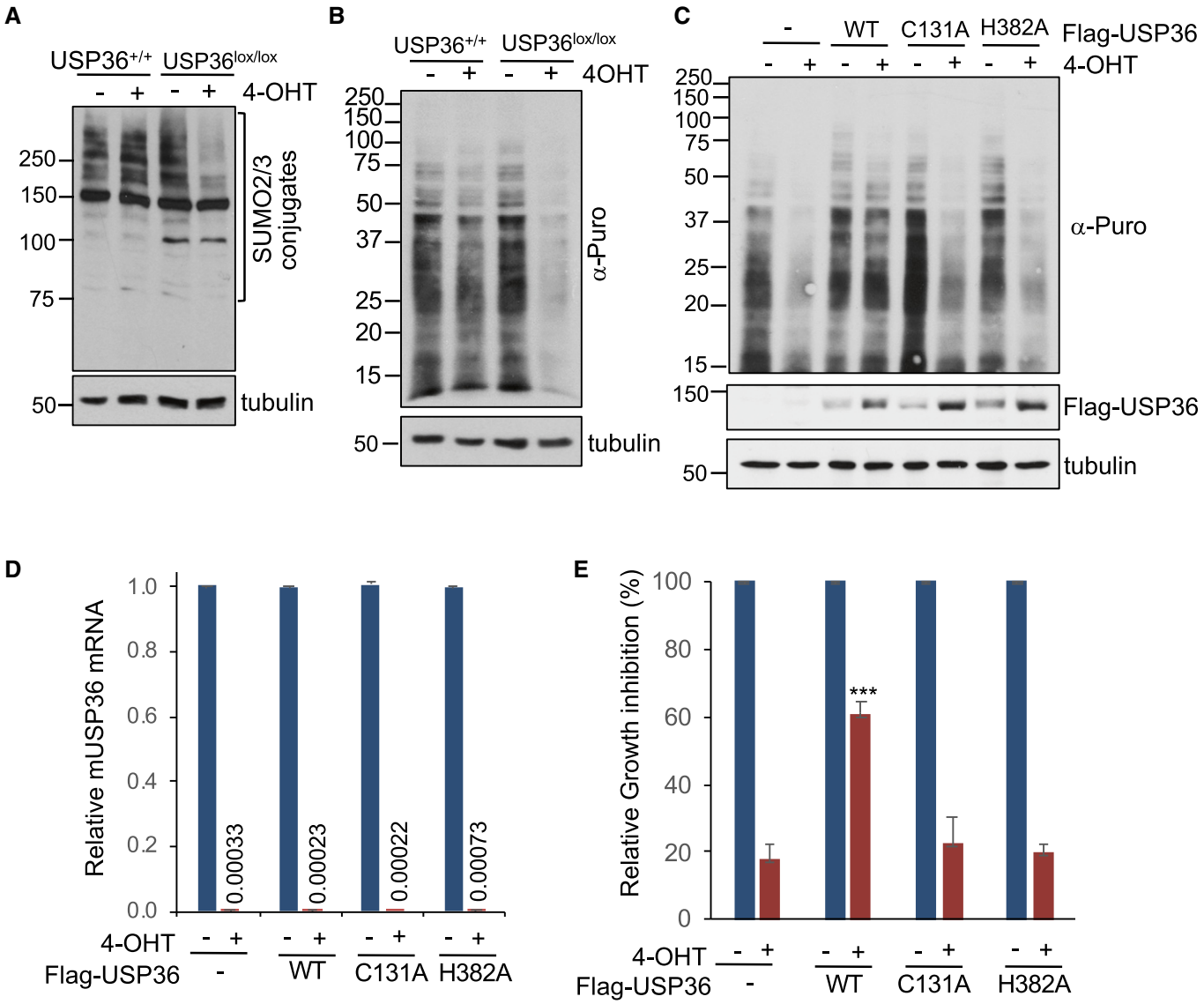

Figure EV5. Deletion of the *USP36* gene inhibits translation and cell growth in MEF cells.

A Deletion of USP36 inhibits SUMOylation in MEF cells. *USP36*$^{+/+}$;*Cre-ER and USP36*$^{lox/lox}$;*Cre-ER* MEF cells were treated with 1 mM 4-OHT or vehicle control for 72 h and assayed for total SUMOylation by IB using anti-SUMO2/3 antibodies.

B Deletion of USP36 inhibits translation in MEF cells. *USP36*$^{+/+}$;*Cre-ER and USP36*$^{lox/lox}$;*Cre-ER* MEF cells were treated with 1 mM 4-OHT or vehicle control for 72 h. The cells were then labeled with 10 μg/ml puromycin for 10 min followed by detection of total protein translation by IB using anti-puromycin antibody.

C, D WT human UPS36 (hUSP36), but not its C131A and H382A mutants, rescues the inhibition of translation by the deletion of endogenous mouse USP36 (mUSP36). *USP36*$^{lox/lox}$;*Cre-ER* MEF cells stably expressing control, WT hUSP36, C131A, or the H382A mutant were treated with 1 mM 4-OHT or vehicle control for 72 h. The cells were then labeled with 10 μg/ml puromycin for 10 min followed by detection of total protein translation by IB using anti-puromycin antibody. The expression of exogenous Flag-hUSP36 was detected by IB with anti-Flag antibody (C). The depletion of endogenous mUSP36 mRNA was detected by RT–qPCR (D). The numbers are average ratios of mUSP36 mRNA expression in cells treated with 4-OHT/ that in cells treated with vehicle control. Data were presented as mean ± SD, $n = 3$ biological replicates.

E WT human UPS36, but not its C131A and H382A mutants, rescues the cell growth inhibition by the deletion of endogenous mUSP36. *USP36*$^{lox/lox}$;*Cre-ER* MEF cells stably expressing control, Flag-hUSP36 (WT, C131A, or the H382A mutant) using lentiviral expression were treated with 1 mM 4-OHT or vehicle control and cultured for 96 h. The percentile inhibition of cell proliferation was determined by cell counting using Countess II (Life Technologies) and compared cells treated with 4-OHT to cells with vehicle control. Data were presented as mean ± SD, $n = 4$ biological replicates. ***$P < 0.001$; compared with empty vector control infected cells treated with 4-OHT (Student's *t*-test).

Source data are available online for this figure.