Supplemental Materials

Molecular Biology of the Cell

Hazan *et al*.

Supplemental Figure Legends

Figure S1. Sequence alignment of Deup1 (KC211185) and Cep63 (KC211186). Sequence alignment shows the conserved amino acids between murine Deup1 and murine Cep63. Deup1 and Cep63 share a high level of conserved amino acids in the region necessary for E2F4 binding to Deup1 (residues 1-129), despite the absence of shared coiled-coil domains. Dark shading represents amino acid identity, lighter shading a conservative substitution.

Figure S2. E2F4 interacts with Deup1 but not Cep63. (A) Schematic representation of Deup1 and its paralog, Cep63, with their coiled-coil domains shaded. (B) Cell lysates containing E2F4 and either Flag-Deup1 or Flag-Cep63 were subject to western blotting with α-Flag (upper panel) or α-E2F4 (lower panel) antibodies before (Input) or after immunoprecipitation (IP) with α-Flag antibodies. This shows that E2F4 associates specifically with Deup1, and not Cep63.

Figure S3. Deup1 and SAS6 interact with DP1. (A) Western blots with α-Flag, α-E2F4 and α-HA antibodies from input lysates and α-Flag immunoprecipitates from 293FT cells overexpressing Flag-Deup1, E2F4 and HA-DP1 as designated. (B-C) Western blots with designated antibodies from input lysates and α-HA immunoprecipitates from cells overexpressing HA-DP1 with Flag-Deup1 (B) or SAS6 (C).

Figure S4. E2F4 interacts with the amino-terminal region of Deup1 but not that of Cep63. (A) Schematic representation of full-length and truncated versions of Deup1 and its paralog, Cep63. (B) Cell lysates containing E2F4 and truncated versions of Flag-Deup1 or Flag-Cep63 were subjected to western blotting with α-Flag (upper panel) or α-E2F4 (lower panel) antibodies before (Input) or after immunoprecipitation (IP) with α-Flag antibodies. Western blotting with α-E2F4 shows that the N-terminal region of Cep63 (residues 67-192) does not co-immunoprecipitate E2F4, in contrast to the N-terminal region of Deup1 (residues 1-129). * denotes the heavy chain of IgG.

Figure S5. E2F4 truncation mutants are detected in the cytoplasm. U2OS cells expressing HA-tagged E2F4 wildtype (WT) and the indicated mutants were subjected to immunofluorescence

using an α-HA antibody. Representative fields of cells are shown with nuclear DAPI staining (blue signal) and HA-tagged E2F4 proteins (red signal). Numbers indicate amino acid positions and Δ denotes the residues deleted from full-length protein.

Figure S6. E2F4 truncation mutants colocalize with Deup1 in the cytoplasm. U2OS cells co-expressing Flag-Deup1 and either HA-tagged E2F4 wildtype (WT) or the indicated mutants were subjected to immunofluorescence using antibodies against the HA tag of the E2F4 proteins (red signal) and the Flag tag of Deup1 (green signal), along with nuclear DAPI staining (blue signal), with representative fields shown. These data show overlapping expression of the two proteins indicating that a lack of interaction is not due to nuclear localization of the E2F4 mutants.

Figure S7. E2F4 truncation mutants colocalize with SAS6 in the cytoplasm. U2OS cells co-expressing Flag-SAS6 and either HA-tagged E2F4 wildtype (WT) or the indicated mutants were subjected to immunofluorescence using antibodies against the HA tag of the E2F4 proteins (red signal) and the Flag tag of SAS6 (green signal), along with nuclear DAPI staining (blue signal), with representative fields shown. These data show overlapping expression of the two proteins indicating that a lack of interaction is not due to nuclear localization of the E2F4 mutants.

Figure S8. Phosphorylation of E2F4 contributes to the gel mobility shift detected upon E2F4-Deup1 association. (A) Cell lysates from cells transfected with E2F4 alone or together with Flag-Deup1 were subjected to western blotting with α-E2F4 antibodies after immunoprecipitation (IP) with α-Flag antibodies and treatment with lambda protein phosphatase, as indicated. (B-C) Cell lysates expressing the indicated (B) E2F4 and Flag-Deup1$^{\mathbf{1\text{-}129}}$ or (C) HA-E2F4$^{\mathbf{1\text{-}197}}$ and Flag-Deup1 were subjected to western blotting with the indicated (B) α-Flag or α-E2F4 or (C) α-Deup1 or α-HA antibodies before (Input) or after immunoprecipitation (IP) with α-Flag antibodies and treatment with lambda protein phosphatase, as indicated. These data show that the mobility shift in E2F4 following interaction with full-length or N-terminal (residues 1-129) Deup1 is reduced by phosphatase treatment and that this shift is dependent upon the C-terminus of E2F4, as it is not observed for E2F4$^{\mathbf{1\text{-}197}}$.

Figure S9. E2F4 co-immunoprecipitated with Flag-tagged Deup1 was analyzed by mass spectrometry and one phosphorylated amino acid at Ser-274 was identified. The peptides identified by mass spectrometry following digestion by either trypsin, chymotrypsin or trypsin plus GluC covered 81.2% of E2F4 and are shaded in gray.

Figure S10. Co-expression of Deup1 and E2F4 leads to the C-terminal cleavage of Deup1. (A) Western blotting with the indicated α-E2F4, α-Flag or α-HA antibodies was performed using cell lysates expressing E2F4 and/or double-tagged Deup1 (N-terminal Flag tag and C-terminal HA tag) before (input) or after immunoprecipitation (IP) with α-E2F4 antibodies. The C-terminal tag is lost following co-expression of E2F4, strongly suggesting that the mobility change in Deup1 after co-expression with E2F4 involves removal of the C-terminal domain. (B) Western blotting with the indicated α-Flag or α-HA antibodies was performed using cell lysates expressing E2F4 and/or the indicated C-terminal deletion mutants of double tagged Deup1 before (input) or after immunoprecipitation (IP) with α-E2F4 antibodies. All truncation constructs retained the HA-tag and no downward mobility shift was detected, indicating the cleavage requires sequences within the last 45 amino acids of Deup1, full length Deup1 being 535 amino acids.

Figure S11. Sequence alignment of the N-terminal regions of E2F4, E2F5 and E2F1. Sequence alignment shows the conserved amino acids between murine E2F4$^{\mathbf{1\text{-}197}}$, human E2F5$^{\mathbf{1\text{-}228}}$ and human E2F1$^{\mathbf{105\text{-}305}}$. E2F4 and E2F5 share a higher level of conserved amino acids, in comparison with E2F1. The blue lines denote the boundaries where domains between E2F4 and E2F1 were exchanged to generate the chimeras.

Figure S12. Sequence alignment of E2F family members across species showing the M1 and M2 motifs. Sequence alignments of E2F4, E2F5 and E2F1 in *Homo sapiens* (human), *Mus musculus* (mouse), *Gallus gallus* (chicken), *Xenopus laevis* (frog) and *Danio rerio* (zebrafish), as well as human E2F2, E2F3 and E2F6, with the M1 (red) and M2 (blue) motifs indicated. * denotes the boundary between the DNA binding and dimerization domains.

Figure S13. E2F4$^{\mathbf{1\text{-}197}}$ M1, M2 and M1+2 mutants preserve the ability to bind DP1. Western blots with the indicated α-HA and α-DP1 antibodies were conducted using lysates from cells

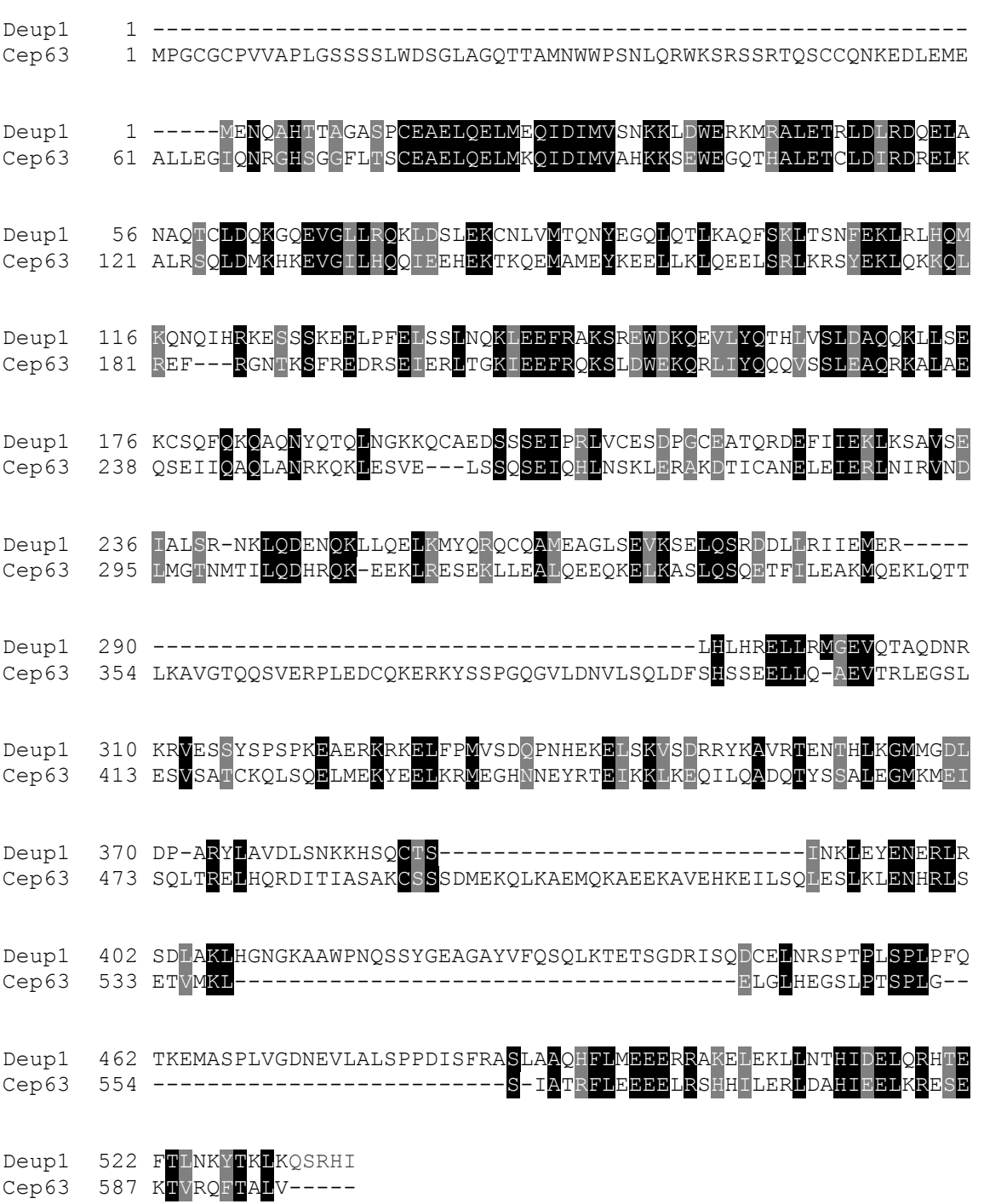
overexpressing DP1 alone or with the indicated wildtype (WT), M1, M2 or M1+2 mutants of HA-E2F4$^{1-197}$ before (input) or after immunoprecipitation (IP) with α-HA antibodies. These data show that tested E2F4 mutants efficiently dimerize with DP1.

Figure S14. E2F4 M1, M2 and M1+2 mutants are detected in the cytoplasm. U2OS cells expressing HA-tagged E2F4 wildtype (WT) and the indicated M1, M2 and M1+2 mutants were subjected to immunofluorescence using an α-HA antibody. Representative fields of cells are shown with nuclear DAPI staining (blue signal) and HA-tagged E2F4 proteins (red signal).

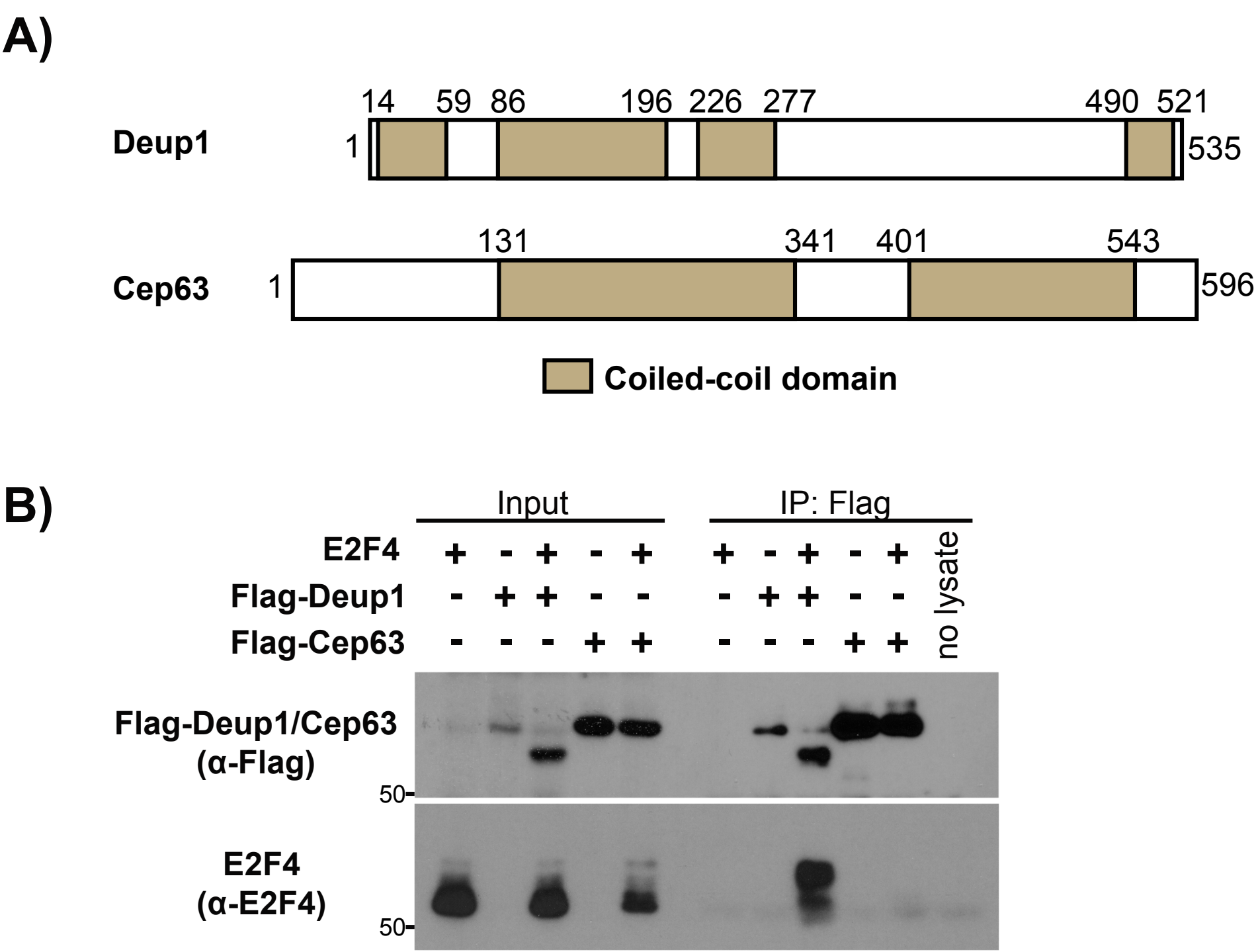
Figure S15. E2F4 M1, M2 and M1+2 mutants co-localize with Deup1 in the cytoplasm. U2OS cells co-expressing Flag-Deup1 and either HA-tagged E2F4 wildtype (WT) or the indicated M1, M2 and M1+2 mutants were subjected to immunofluorescence using antibodies against the HA tag of the E2F4 proteins (red signal) and the Flag tag of Deup1 (green signal), along with nuclear DAPI staining (blue signal), with representative fields shown. These data show overlapping expression of the two proteins indicating that a lack of interaction is not due to nuclear localization of the E2F4 mutants.

Figure S16. E2F4 M1, M2 and M1+2 mutants colocalize with SAS6 in the cytoplasm. U2OS cells co-expressing Flag-SAS6 and either HA-tagged E2F4 wildtype (WT) or the indicated M1, M2 and M1+2 mutants were subjected to immunofluorescence using antibodies against the HA tag of the E2F4 proteins (red signal) and the Flag tag of SAS6 (green signal), along with nuclear DAPI staining (blue signal), with representative fields shown. These data show overlapping expression of the two proteins indicating that a lack of interaction is not due to nuclear localization of the E2F4 mutants.

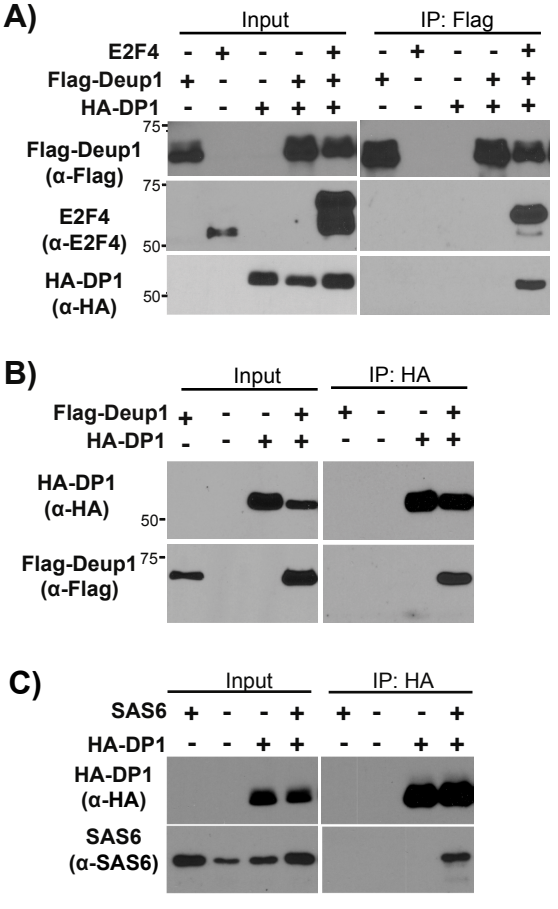
Supplemental Figure 1



Supplemental Figure 2

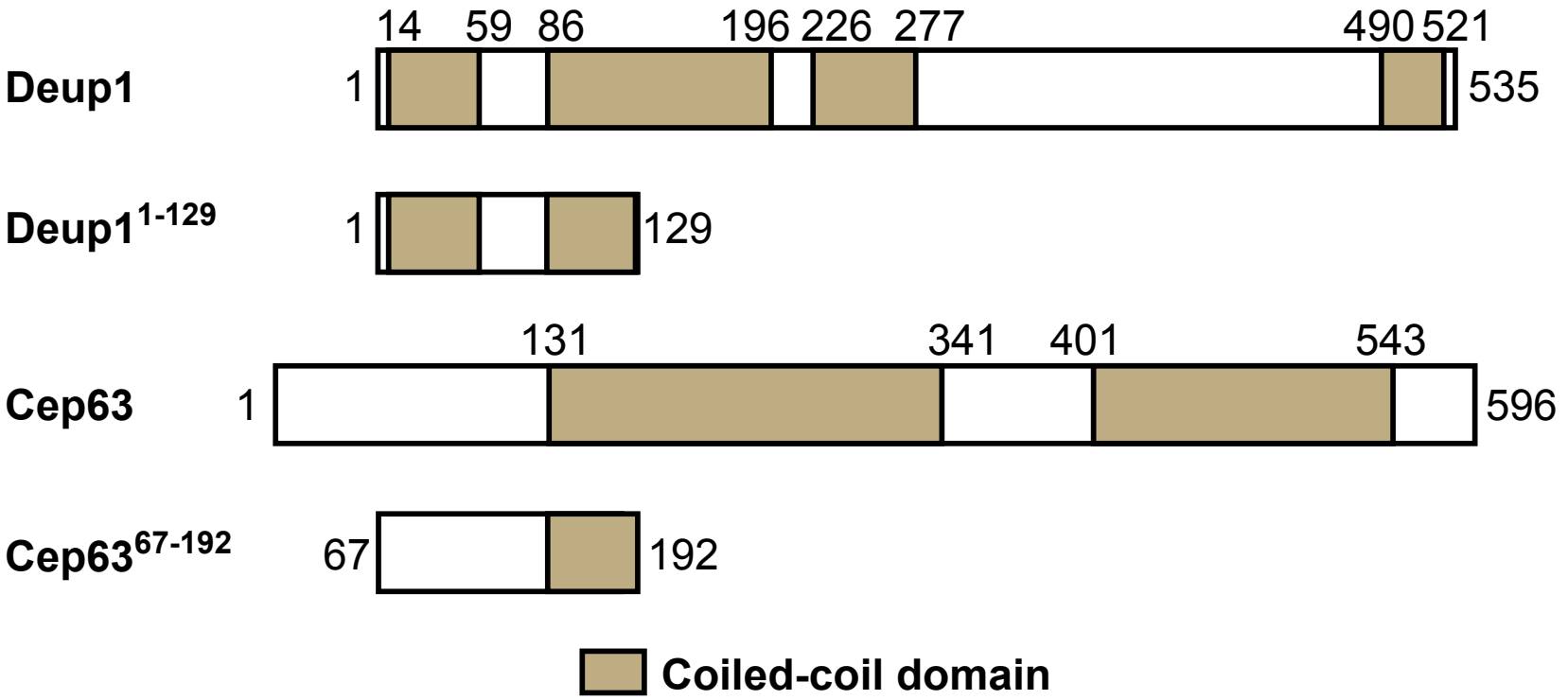


Supplemental Figure 3

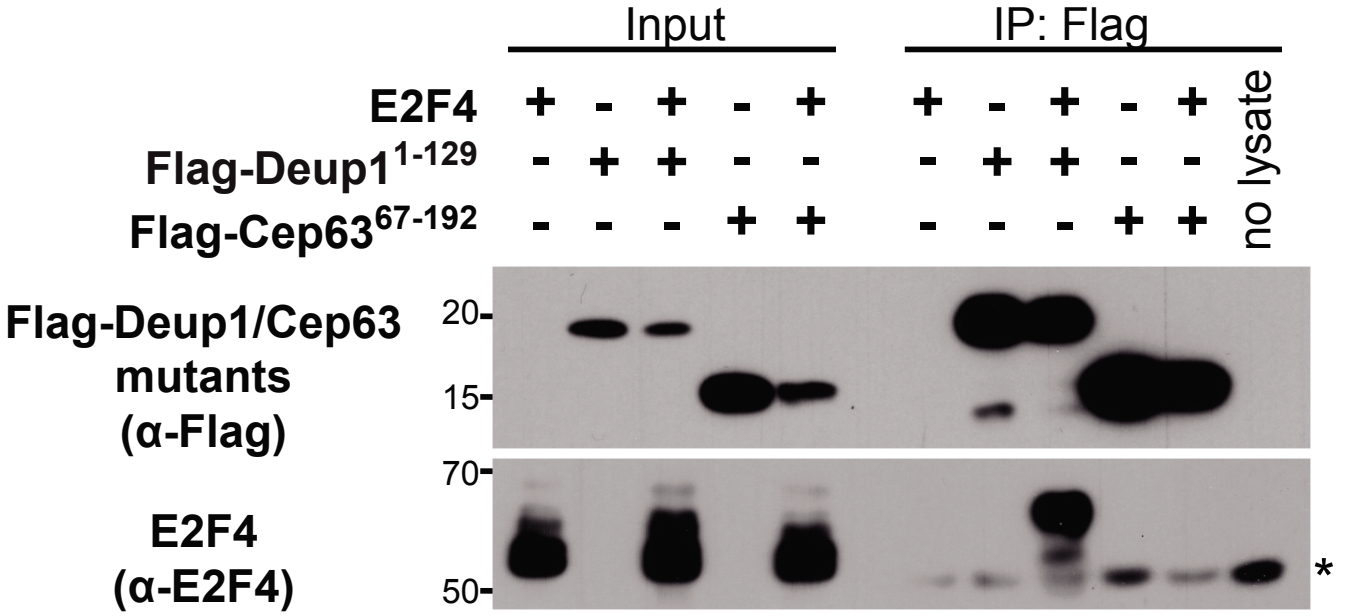


Supplemental Figure 4

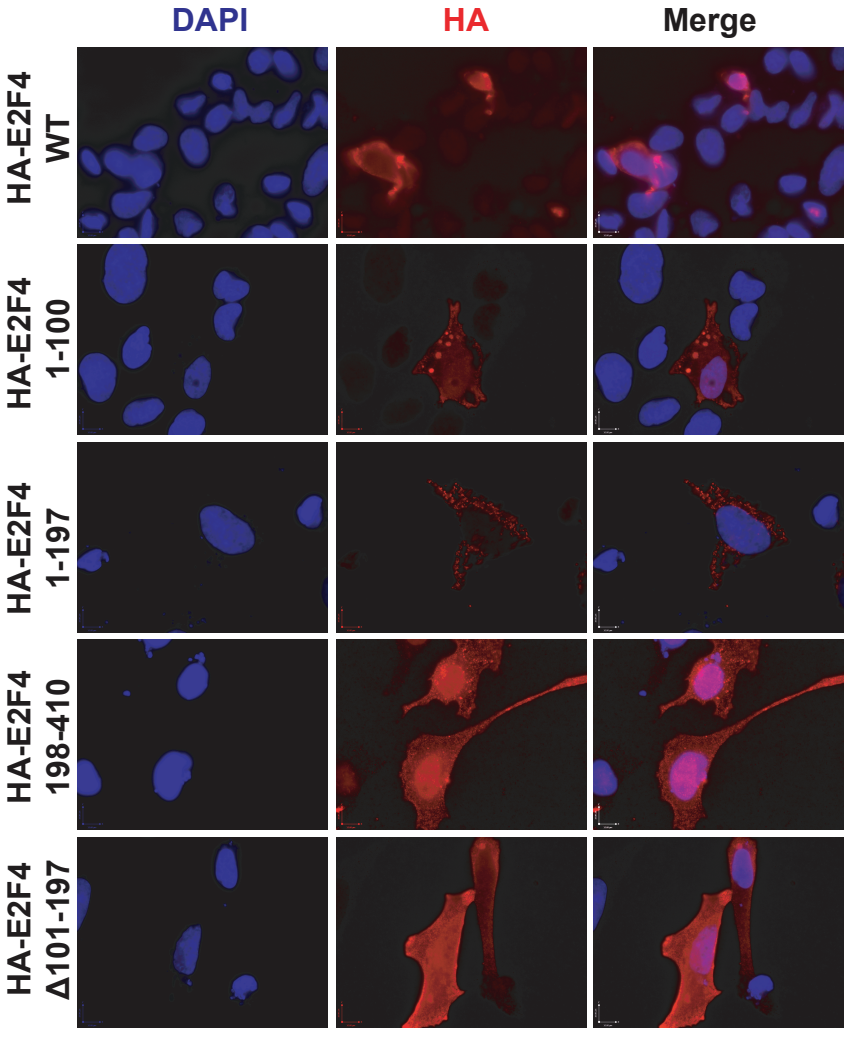
A)



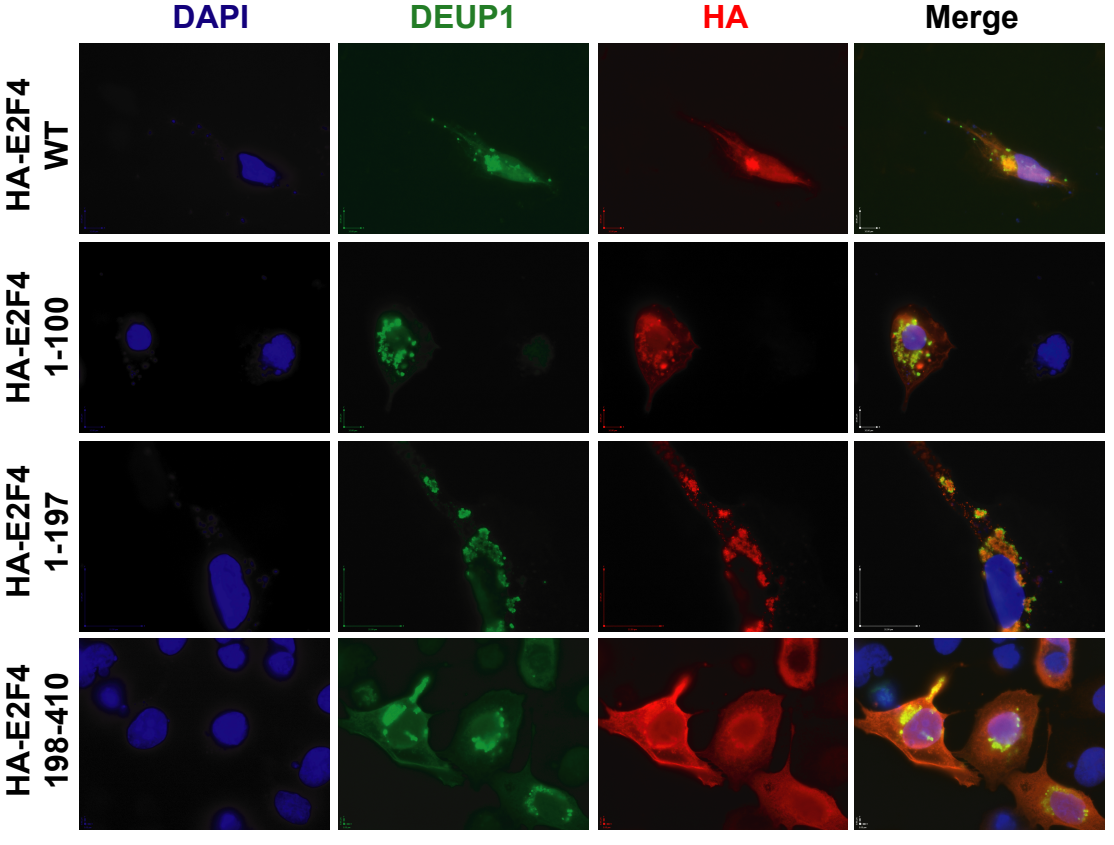
B)



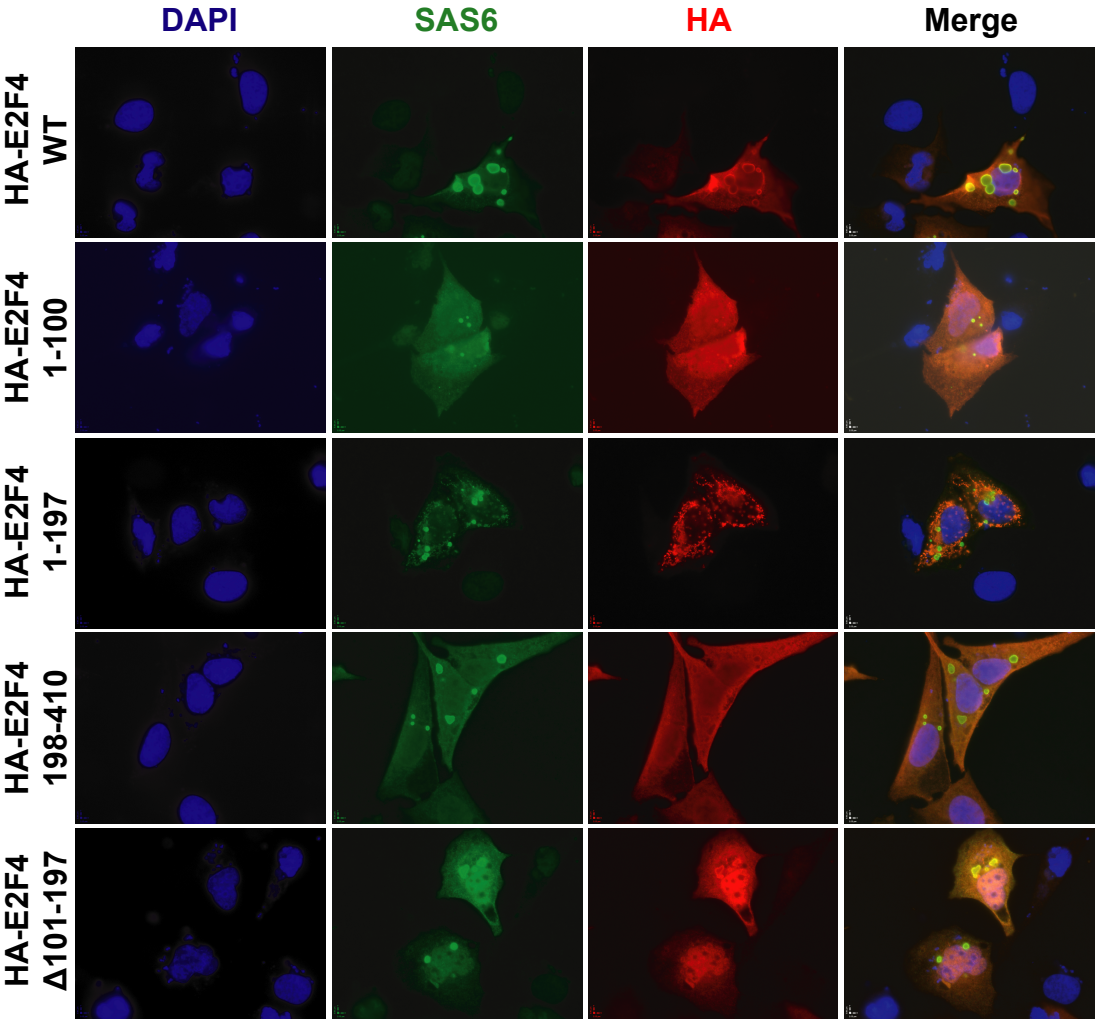
Supplemental Figure 5



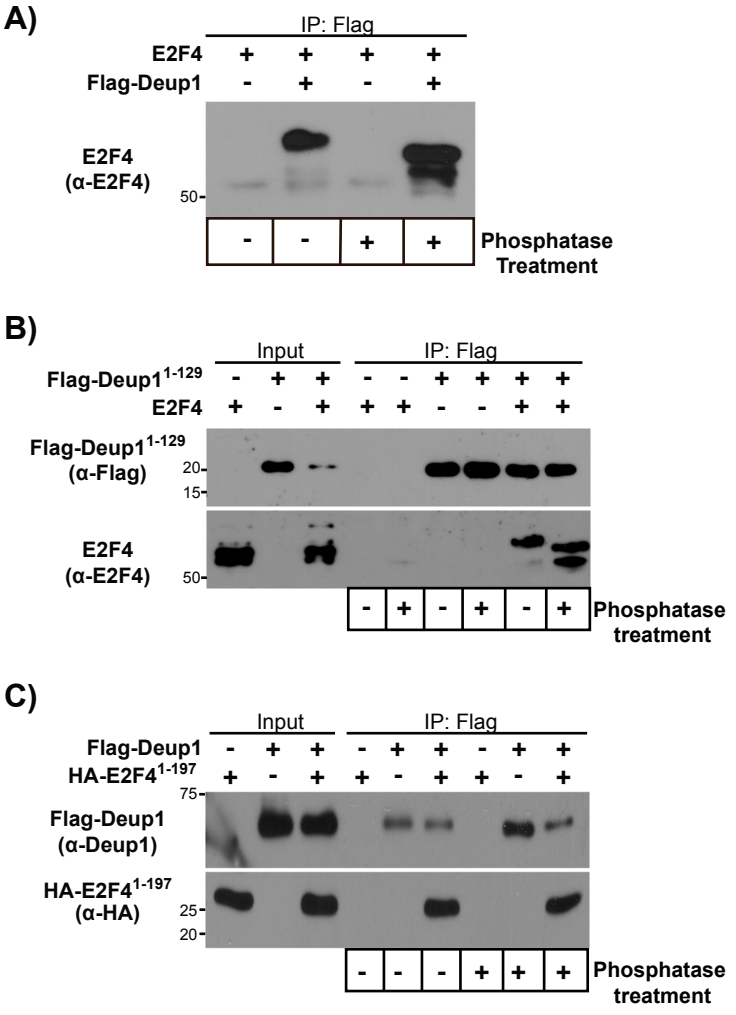
Supplemental Figure 6



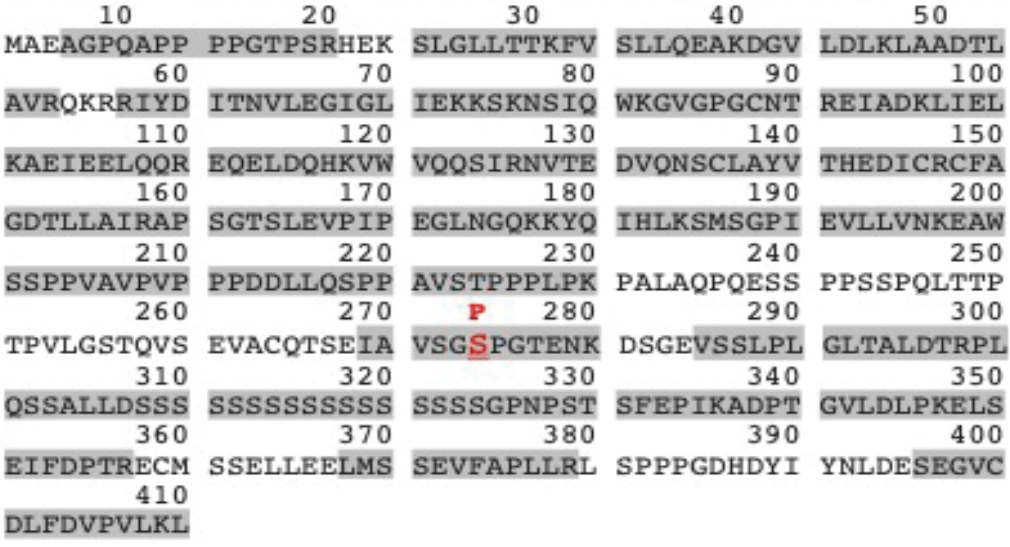
Supplemental Figure 7



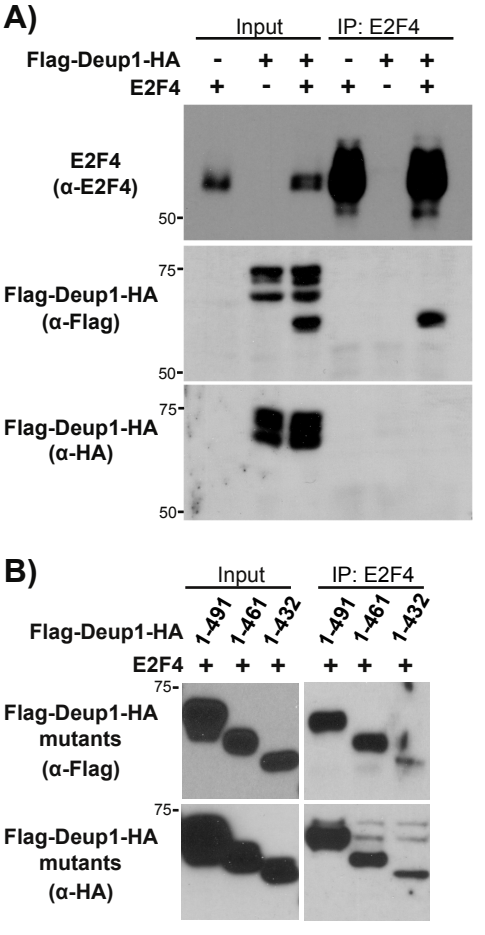
Supplemental Figure 8



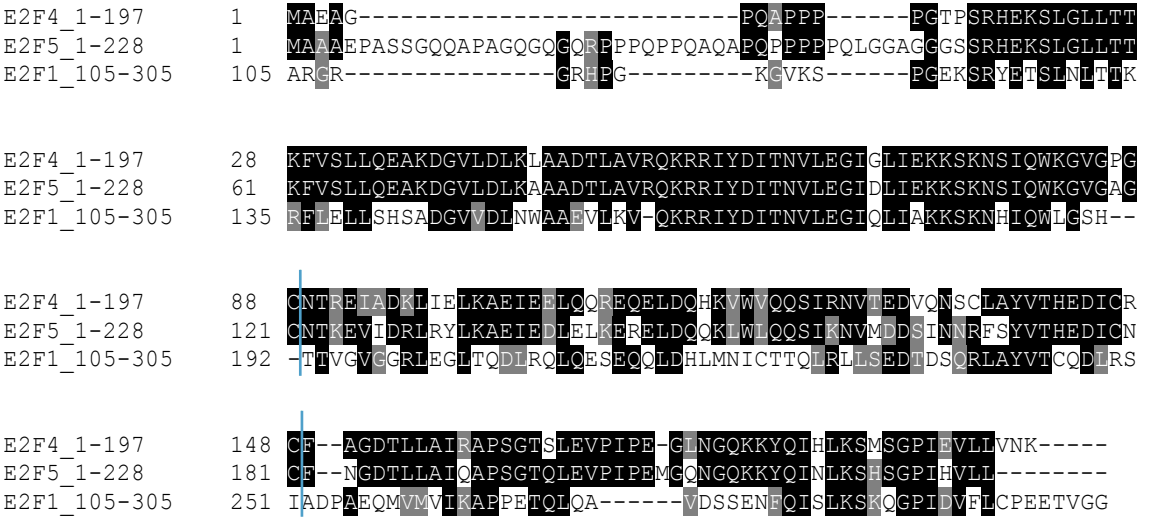
Supplemental Figure 9



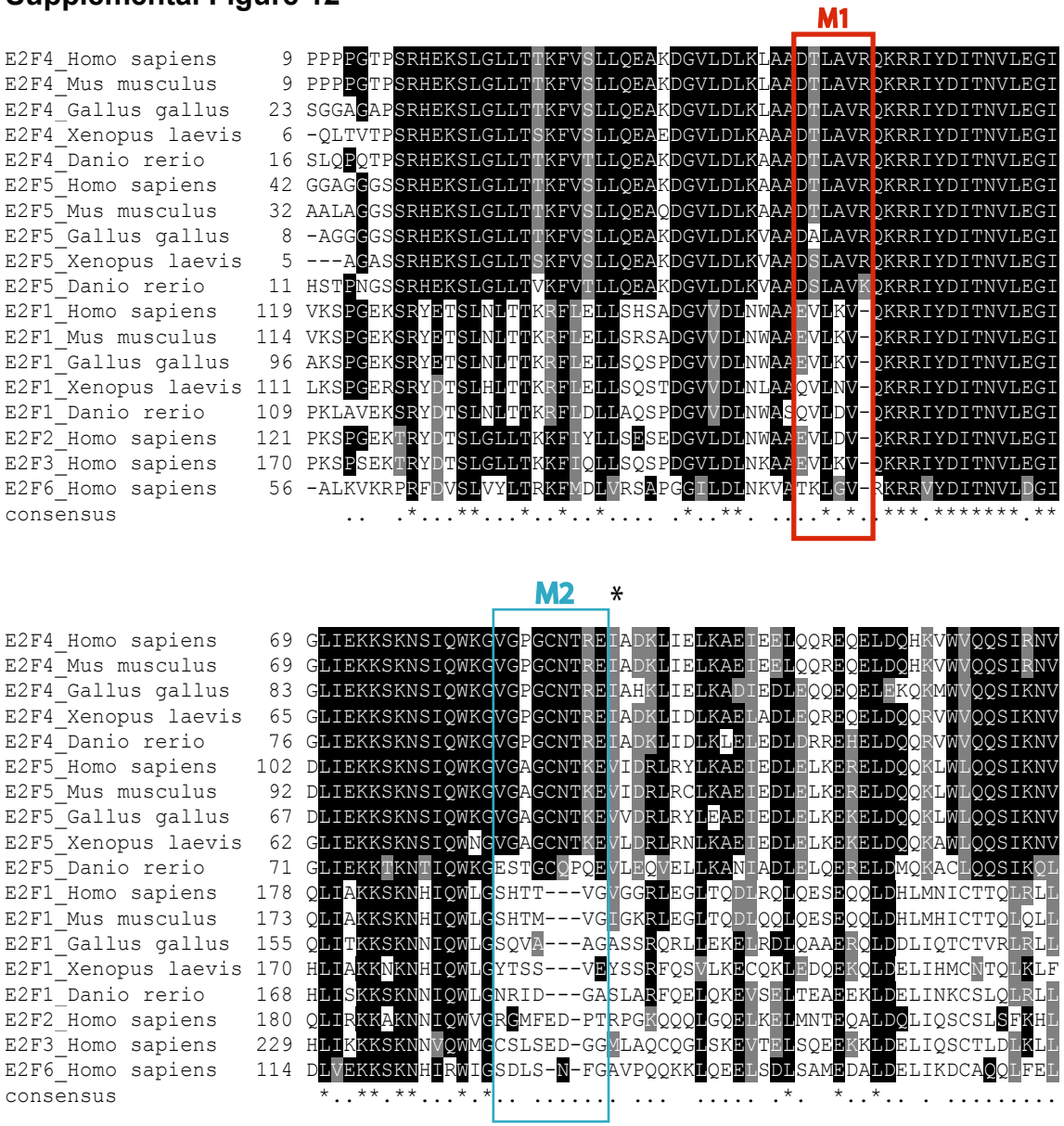
Supplemental Figure 10



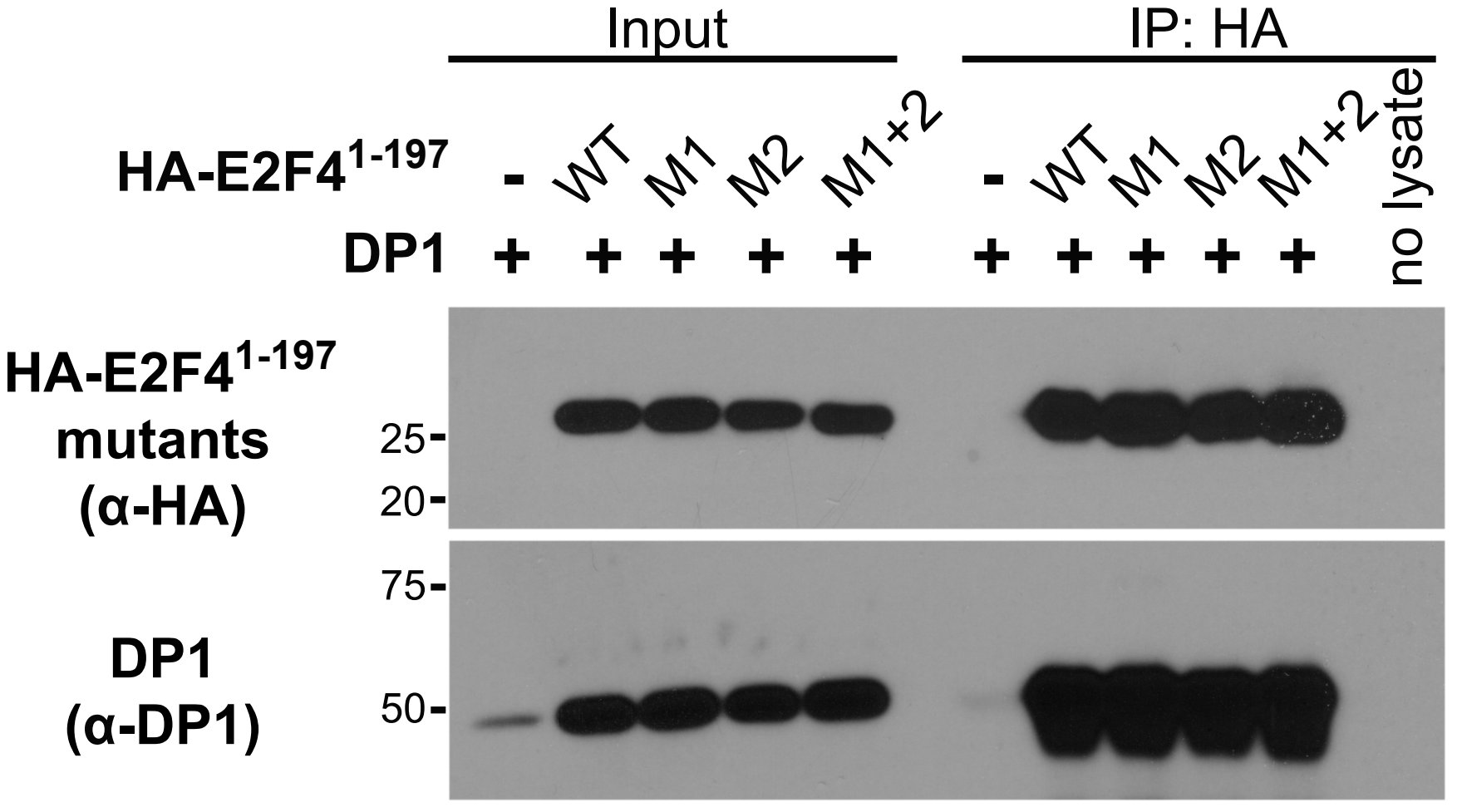
Supplemental Figure 11



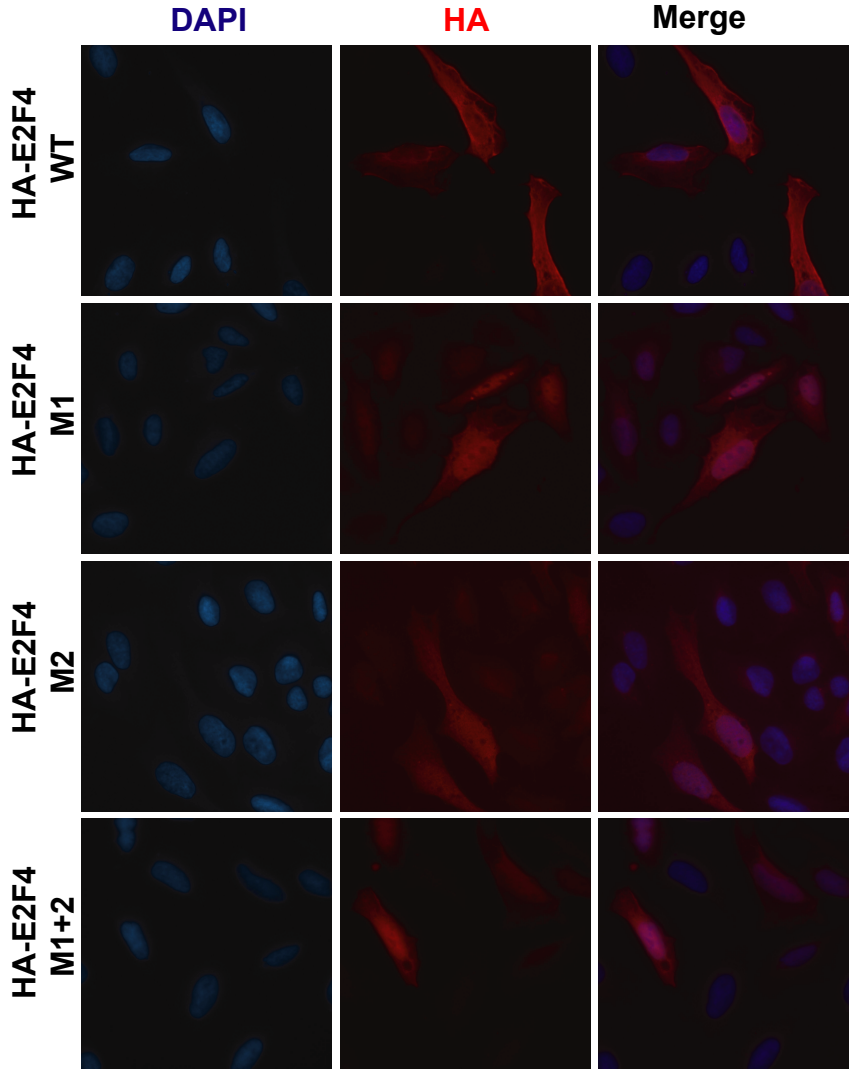
Supplemental Figure 12



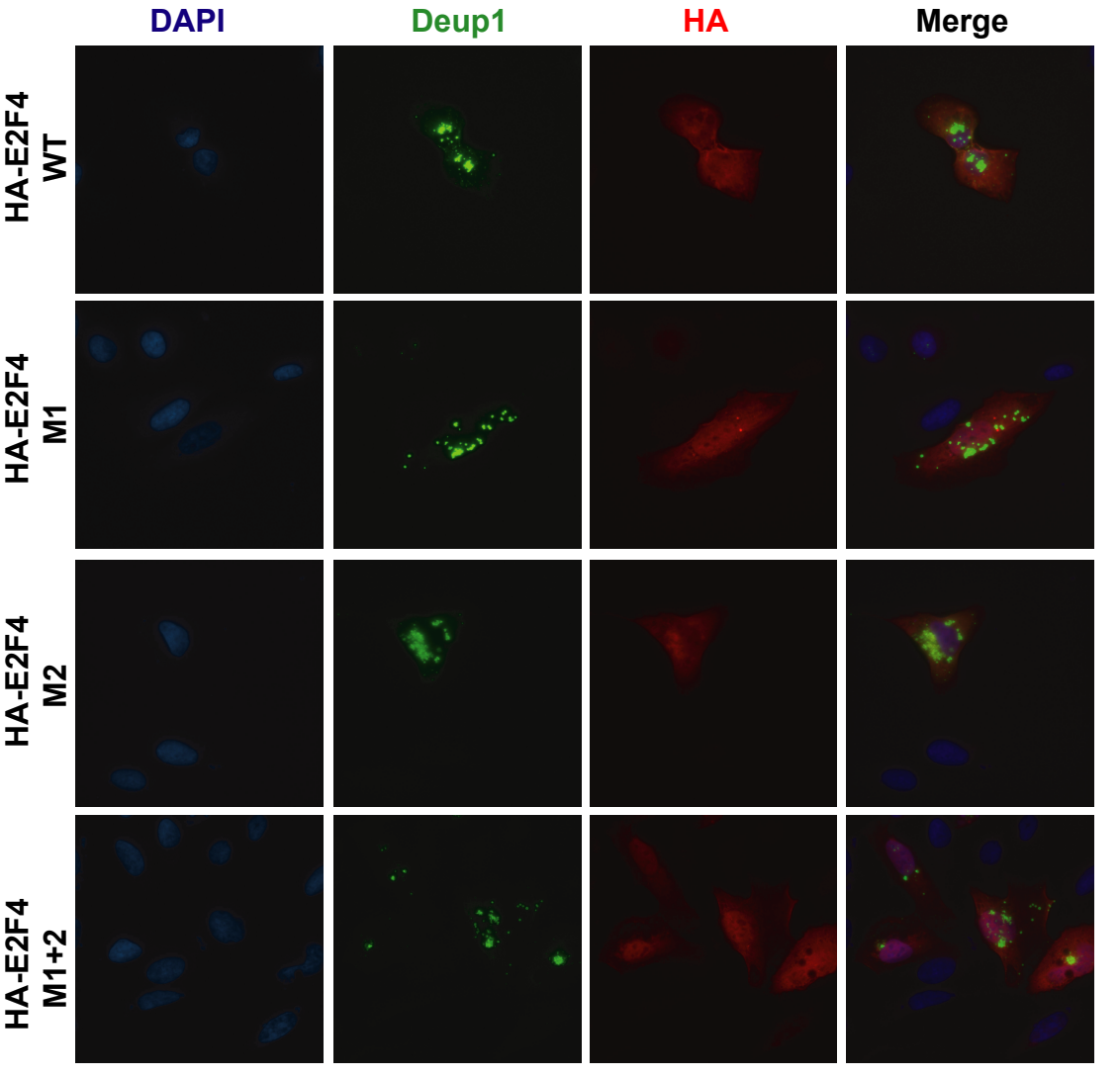
Supplemental Figure 13



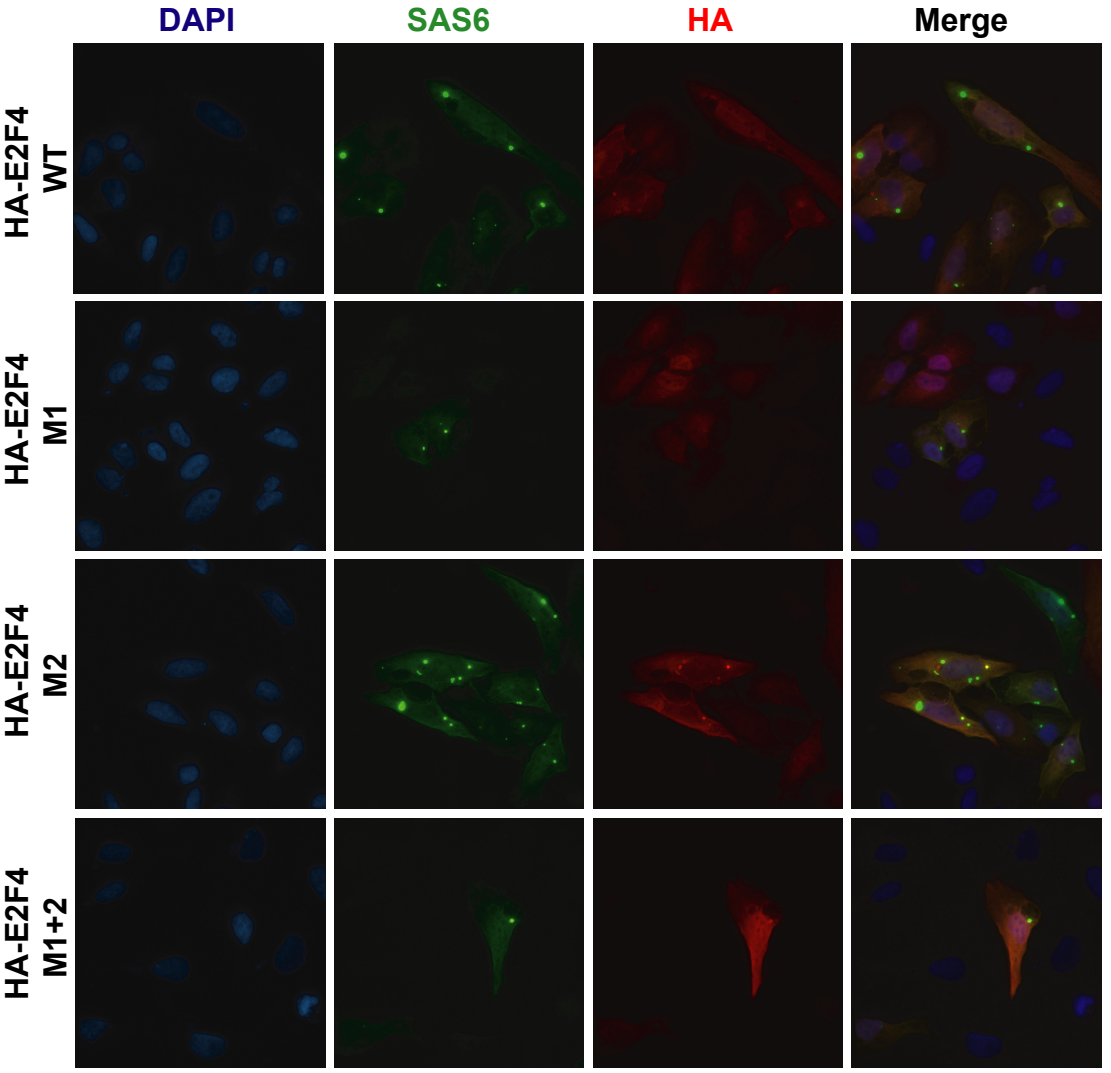
Supplemental Figure 14



Supplemental Figure 15



Supplemental Figure 16



Supplemental Table S1: Sequences of oligonucleotides used for cloning

Chimera 1-4-4**:** PCR reaction 1 Forward	AGGCCGGGCCACAGGCGCCGCCGTCCCCGGGGGAGAAGTCACG
Chimera 1-4-4: PCR reaction 1 Reverse	AGCTTGTCAGCGATCTCCCGGGTGCCCACTGTGGTGTGGCTGC
Chimera 1-4-4: PCR reaction 2 Forward	ACCCGGGAGATCGCTGACAAGC
Chimera 1-4-4: PCR reaction 2 Reverse	CGGCGGCGCCTGTGGC
Chimera 4-1-1: PCR reaction 1 Forward	AGGCCGGGCCACAGGCGCCGCCGTCCCCGGGGGAGAAGTCACGC
Chimera 4-1-1: PCR reaction 1 Reverse	ATACTCTAGAGCGGCCGTCACTCAGGGCACAGGAAAACATCGATCG
Chimera 4-1-1: PCR reaction 2 Forward	TGACGGCCGCTCTAGAGTATCCC
Chimera 4-1-1: PCR reaction 2 Reverse	ATTGCAACCTGGCCCGACGC
Chimera 4-4-1: PCR reaction 1 Forward	GAAGACATCTGCAGATGCGCAGACCCTGCAGAGCAGATGG
Chimera 4-4-1: PCR reaction 1 Reverse	ATACTCTAGAGCGGCCGTCACTCAGGGCACAGGAAAACATCGATCG
Chimera 4-4-1: PCR reaction 2 Forward	TGACGGCCGCTCTAGAGTATCCC
Chimera 4-4-1: PCR reaction 2 Reverse	GCATCTGCAGATGTCTTCATGAGTCACG