# HPV E6 oncoprotein targets histone methyltransferases for modulating specific gene transcription

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**Supplemental materials include Supplemental Materials and Methods, Supplemental References, Supplemental Figure Legends and Supplemental Figures.**

## Supplemental Materials and Methods

### *In vitro* chromatin assembly and transcription assays

These procedures were performed as described (Thomas and Chiang 2005). Briefly, HeLa core histones were mixed with the recombinant human histone chaperone NAP-1 on ice for 30 min, followed by the addition of DNA templates, Drosophila nucleosome assembly factor ACF and ATP. The mixtures were then incubated at 27 ℃ for 4 hr. The formed chromatin was further incubated with p53 and SET7, followed by addition of the HeLa nuclear extract, NTPs and the p△MLT, the control plasmid which does not contain any p53 binding site.

### Antibodies

Commercial Abs used for western are against p53 (DO-1) (sc-126, Santa Cruz), CARM1 (A300-421A, Bethyl), PRMT1 (sc-13392, Santa Cruz), SET7 (04-805, Millipore), actin (MAB1501, Millipore), β-tubulin (MAB1637, Millipore), HA

(MMS-101R, Covance), Flag (M2, Sigma), histone H3 (ab1791, Abcam), and p21 (C-19, Santa Cruz). Commercial Abs for IP and ChIP are against Asy-H3R17me2 (07-214, Millipore), Ace-H4 (06-866, Millipore), Asy-H4R3me2 (07-213, Millipore), p53 (FL-393) (sc-6243, Santa Cruz), Flag (M2, Sigma), HA (MMS-101R, Covance), Normal Mouse IgG (12-371B, Millipore), and SET7 (04-805, Millipore).

**Supplemental References**

Thomas MC, Chiang CM (2005). E6 oncoprotein represses p53-dependent gene activation via inhibition of protein acetylation independently of inducing p53 degradation. *Mol Cell* **17:** 251-264.

**Supplemental Figure Legends**

**Figure S1. si-18E6 efficiently knocks down 18E6 expression in HeLa.** Cells were mock-transfected (lane 1), transfected with scramble RNA (lane 2), or two independent si-18E6 (lanes 3 and 4), followed by RT-PCR for measuring 18E6 mRNA level and western using Abs against the indicated proteins.

**Figure S2. E6 represses histone methylation by CARM1 (A), PRMT1 (B), or SET7 (C) *in vitro*.** Indicated proteins (2 μg of each HMTs, 1, 2, or 5 μg of each E6 and 10 μg of core histones) were mixed with [$^3$H]-SAM (S-adenosyl-L-[methyl-$^3$H] methionine) for the *in vitro* methyltransferase assays. The reaction products were separated by 15% SDS–PAGE. The gels were stained with coomassie blue (upper panel) and analyzed by phosphoimaging (lower panel). The molar ratios of histone H3

to CARM1 to E6 are 1: 0.2: 0.37 (lanes 3, 6 and 9), 1: 0.2: 0.72 (lanes 4, 7 and 10), and 1: 0.2: 1.89 (lanes 5, 8 and 11) in (A), of histone H4 to PRMT1 to E6 are 1: 0.21: 0.24 (lanes 3, 6 and 9), 1: 0.21: 0.48 (lanes 4, 7 and 10), and 1: 0.21: 1.2 (lanes 5, 8 and 11) in (B), of histone H3 to SET7 to E6 are 1: 0.28: 0.37 (lanes 3, 6), 1: 0.28: 0.72 (lanes 4, 7), and 1: 0.28: 1.89 (lanes 5, 8) in (C).

**Figure S3. E6 represses CARM1- and PRMT1-stimulated p53 transcriptional activity.** The luciferase assays are identical to those in Figure 3A but the activities are displayed in different order. The western to indicate the protein levels of all factors involved is shown below the bar chart.

**Figure S4. E6 represses CARM1- and PRMT1-mediated induction of p53 transcriptional activity independently of p53 degradation.** H1299 cells were treated with MG132 and transfected with indicated expression plasmids in the presence of the p21 promoter-driven luciferase construct, followed by luciferase assays (upper bar chart) and western (lower panels).

**Figure S5. 11E6, which does not interfere with p53 protein level, inhibits p21 expression.** U2OS cells were transfected with indicated plasmids for 48 hr and then treated with Adr for another 6 hr, followed by RT-PCR and western.

**Figure S6. E6 represses adriamycin-induced p21 mRNA level depending on CARM1, PRMT1 and SET7.**

The experiments are identical to those in Figures 3D and 7A but the results are displayed differently.

**Figure S7. Quantification of ChIP results of Figure 4A by real-time PCR using Roche Light Cycler 480 system with 384-well plates.**

**Figure S8. E6 inhibits Asy-H4R3me2 and Asy-H3R17me2 around p53-responsive region of p21 and GADD45 promoters in the presence of Adr and MG132.** U2OS cells with or without 18E6 expression in the presence of MG132 and Adr were subjected to ChIP assays and western analysis.

**Figure S9. Quantification of ChIP results of Figure 6A by real-time PCR using Roche Light Cycler 480 system with 384-well plates.**

**Figure S10. 18E6 does not affect p53 binding to histone-free DNA.** (A) E6 binds to p21 promoter depending on p53 and E6 binding does not reduce p53 association with histone-free DNA. The biotin-labeled DNA containing a 214 bp fragment of p21 promoter from -2389 to -2176 bp with a single p53-binding site (illustrated on top) was linked to strepavidin beads and incubated with cell lysates of H1299 with or without expressing Flag-18E6 or p53. As shown here, p53 is pulled down by beads containing DNA, but not by beads alone, indicating that p53 binds to the DNA fragment (lane 3). While E6 alone did not associate with the DNA (lane 2), it appears in the pulled-down fraction in the presence of p53 (lane 4). β-tubulin is not precipitated by this DNA fragment, suggesting that E6 recruitment is specific. Importantly, E6 binding does not interfere with the DNA binding ability of p53 (lane 4). (B) Cellular histone H3 does not associate with the DNA probe used in (A). p53, but not histone H3, is pulled down by DNA-conjugated beads (lane 3), indicating that

the DNA probe, which can be recognized and bound by p53, maintains a nucleosome-free structure in DAPA reaction.

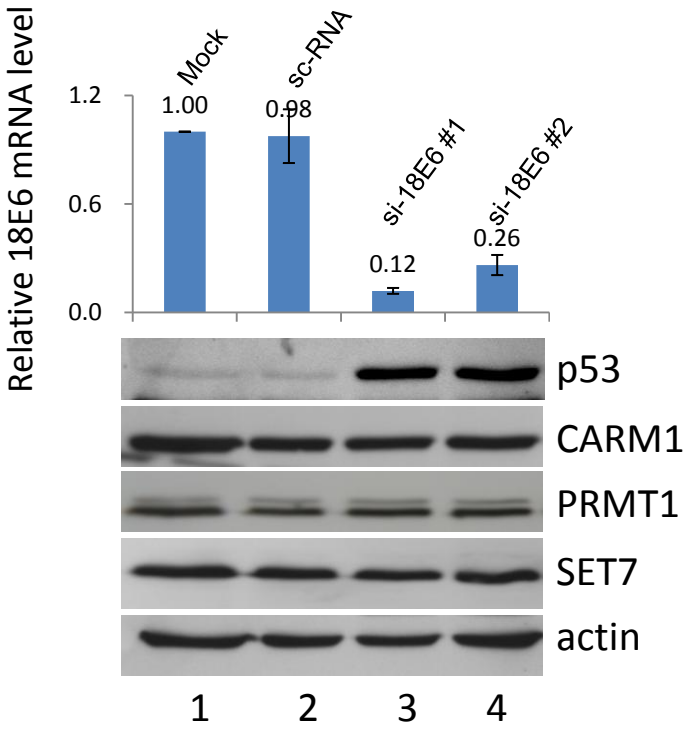
**Figure S11. The enzymatic activity of SET7 is critical for stress-induced p53 stability and its downstream gene expression.** U2OS cells were transfected with control scramble RNA **(sc-RNA)**, siRNA against SET7 (si-SET7), or plasmid encoding methylase-dead SET7 (mt SET7), in the absence (lanes 1 to 4) or presence (lanes 5 to 8) of Adr for 3 hr, followed by western. The protein levels of p53 and p21 were quantified and shown in the bar chart below the western blots.

**Figure S12. 18E6 associates with CARM1, PRMT1 and SET7 independently of p53.** p53-null H1299 cells transiently expressing HA-tagged HMT, Flag-tagged E6 of 18E6 or both were subjected to IP and western. As shown here, HA Ab, but not IgG, pulls down HA-CARM1 and Flag-18E6 (A), as well as HA-PRMT1 and Flag-18E6 (B). The interaction of Flag-18E6 and the endogenous SET7 is also demonstrated using Flag Ab (C, lanes 1 to 4) or SET7 Ab (lanes 5 to 8) for IP and Abs against indicated proteins for western. These results demonstrate that in the absence of p53, HPV E6 still associates with CARM1, PRMT1, and SET7 in cells.

**Figure S13. SET7 does not stimulate p53 activity in the absence of stress.** (A) The p53-responsive transcription can not be induced by SET7 in *in vitro* transcription experiment (compare lane 3 to 2). (B) The luciferase reporter assays indicate that the ectopically expressed SET7 does not enhance p53-dependent p21 promoter activity in H1299 cells. (C) Over-expressed SET7 does not increase the level of p53-induced p21 mRNA in H1299 (compare lanes 4 and 5 to lanes 2 and 3). (D) ChIP assays show that

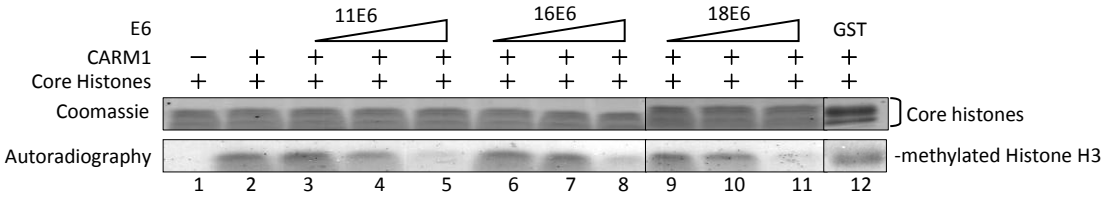
exogenous SET7 does not increase the level of chromatin-bound p53 nor local H3 acetylation (compare lane 2 to 1) in U2OS cells.

**Hsu et al. _Supplementary Figure S1**



# Hsu et al. _Supplementary Figure S2

(A)

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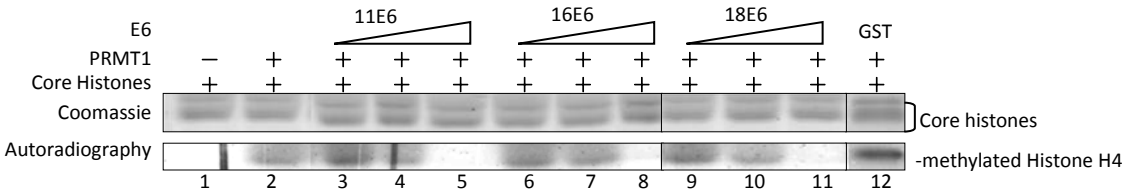


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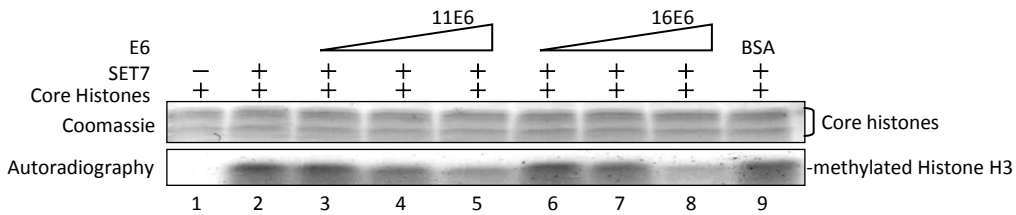

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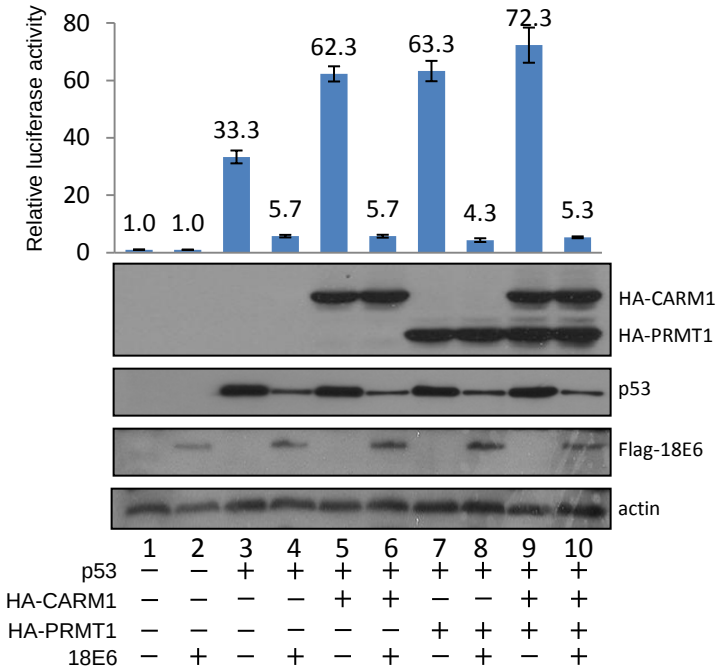

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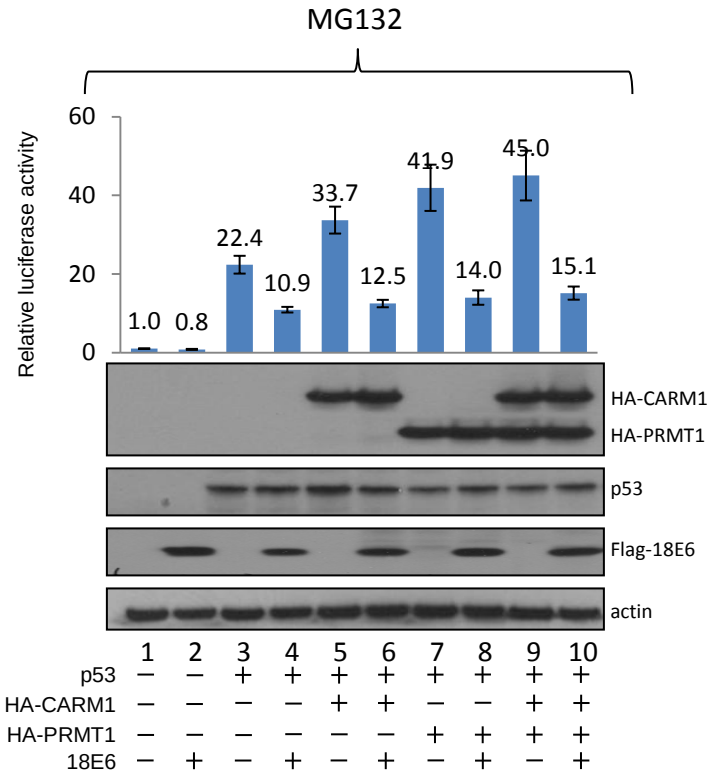

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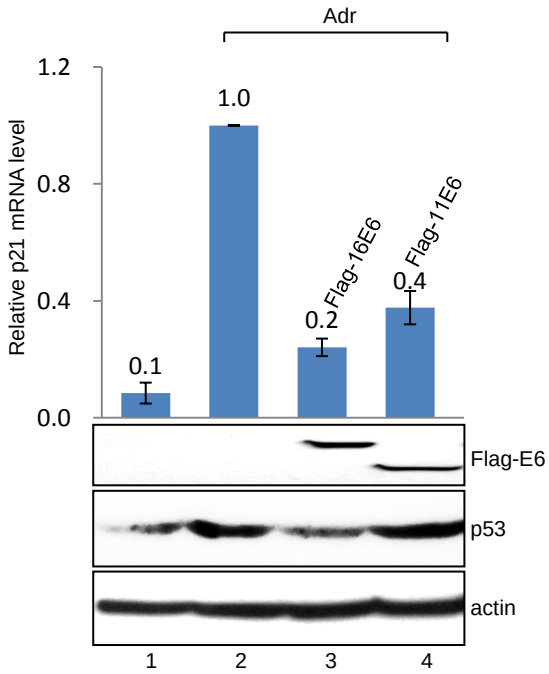

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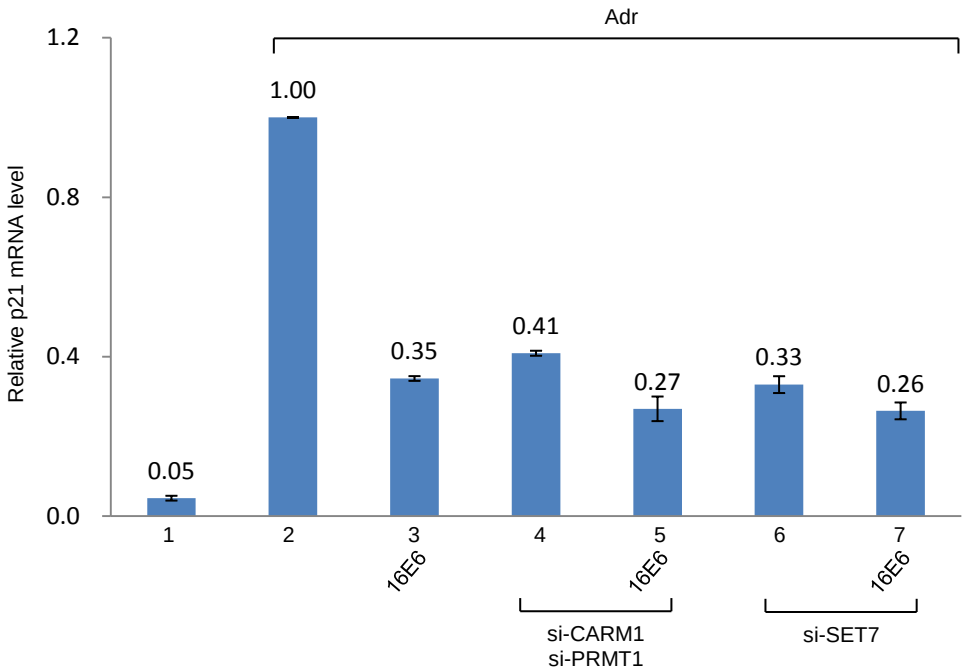

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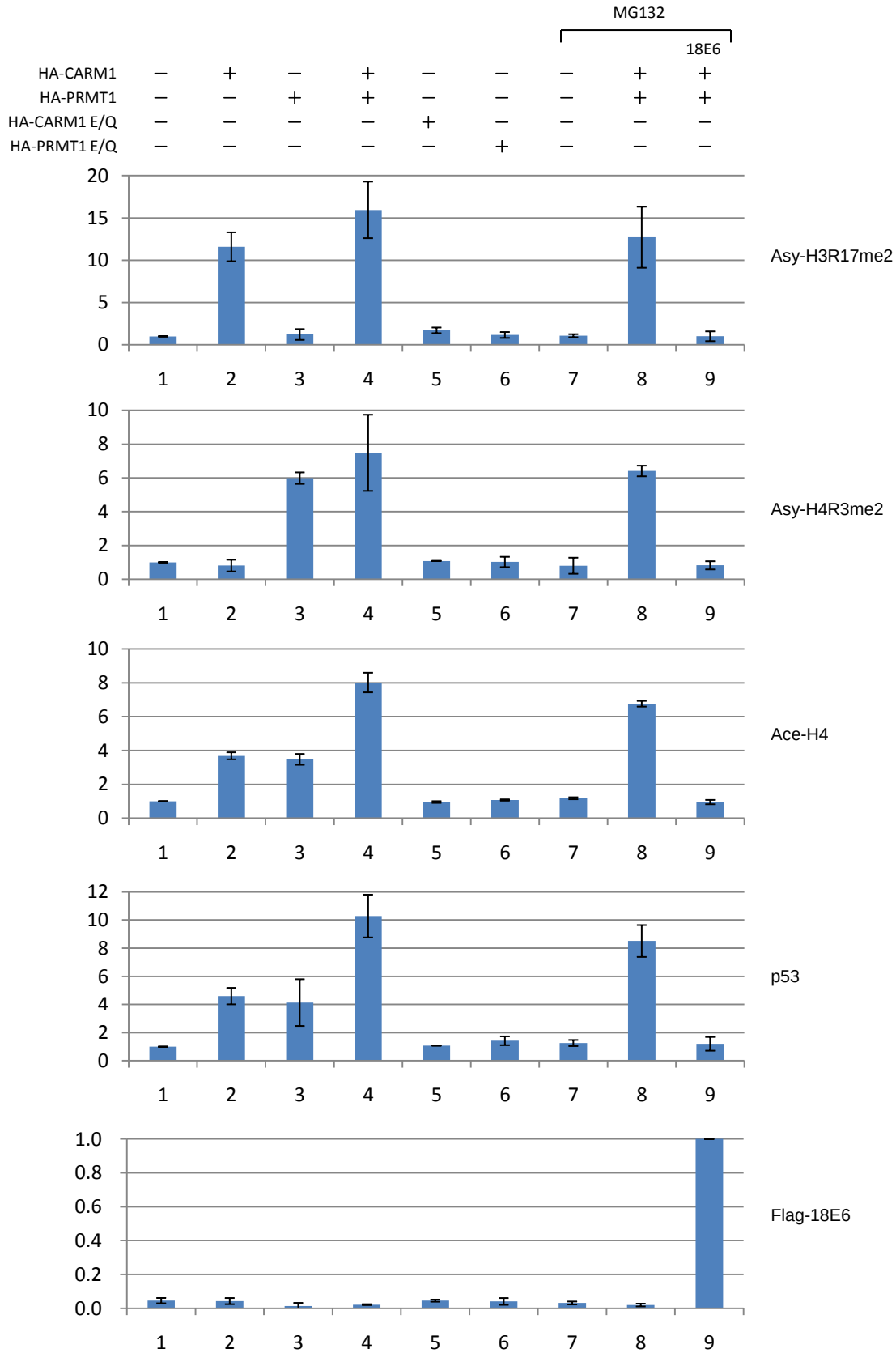

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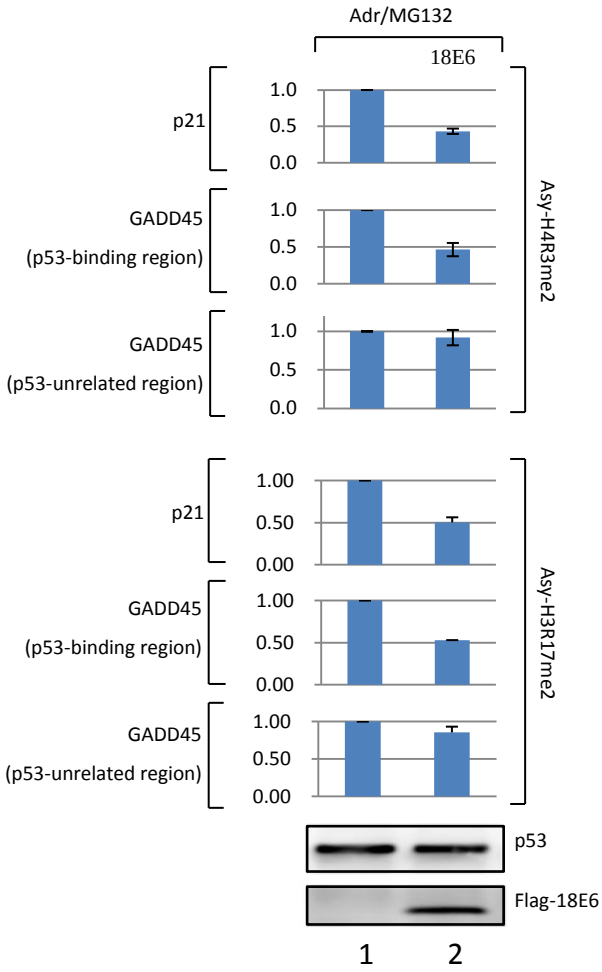

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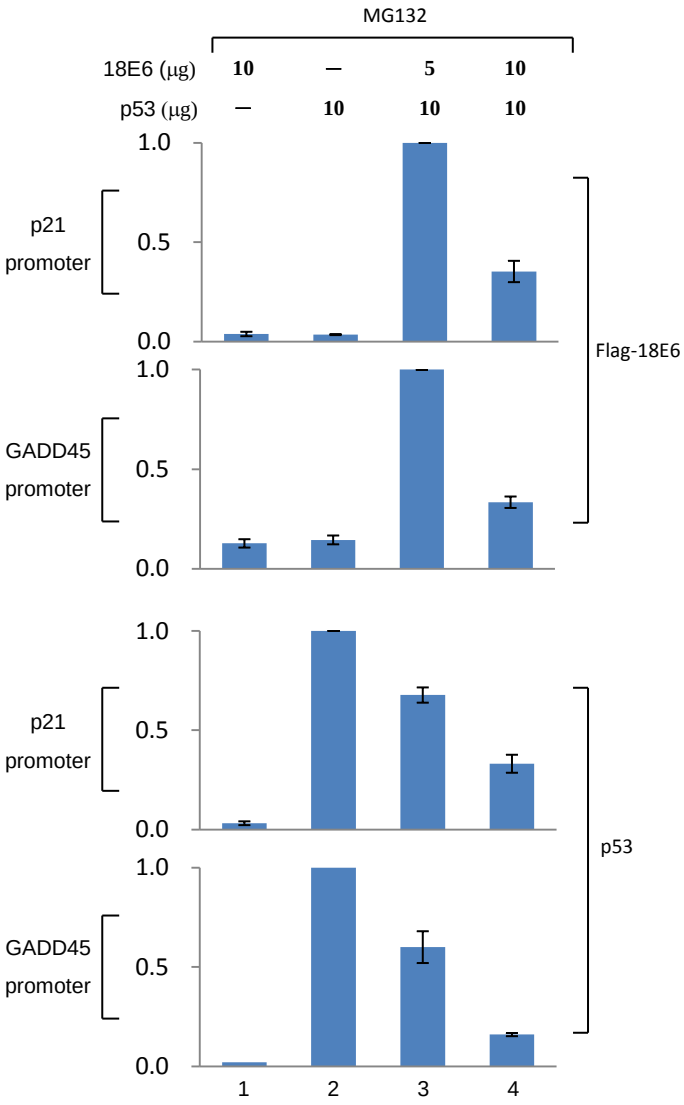
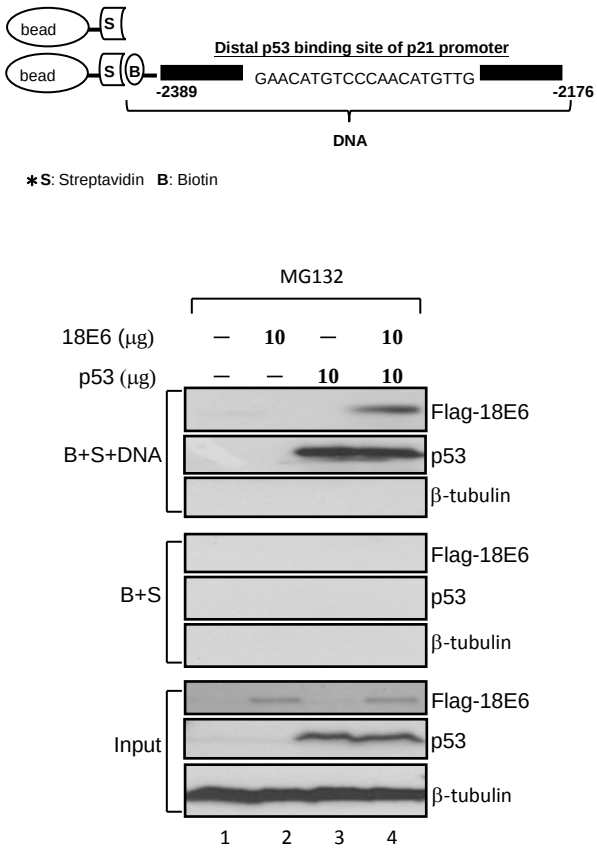


# Hsu et al. _Supplementary Figure S10

(A)

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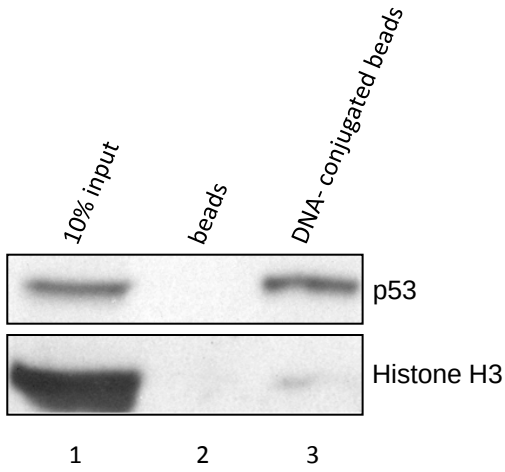

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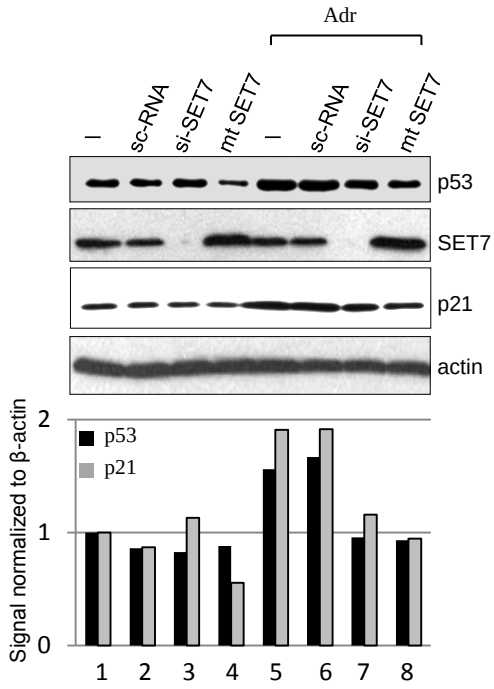

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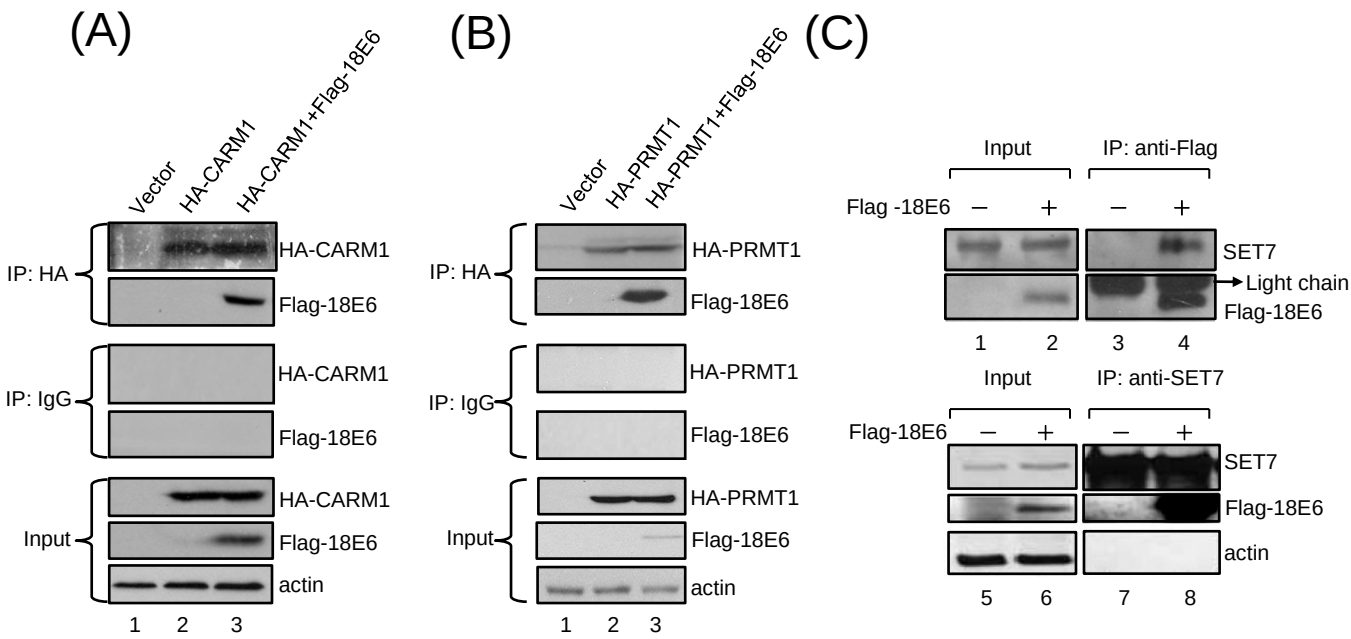

# Hsu et al. _Supplementary Figure S13