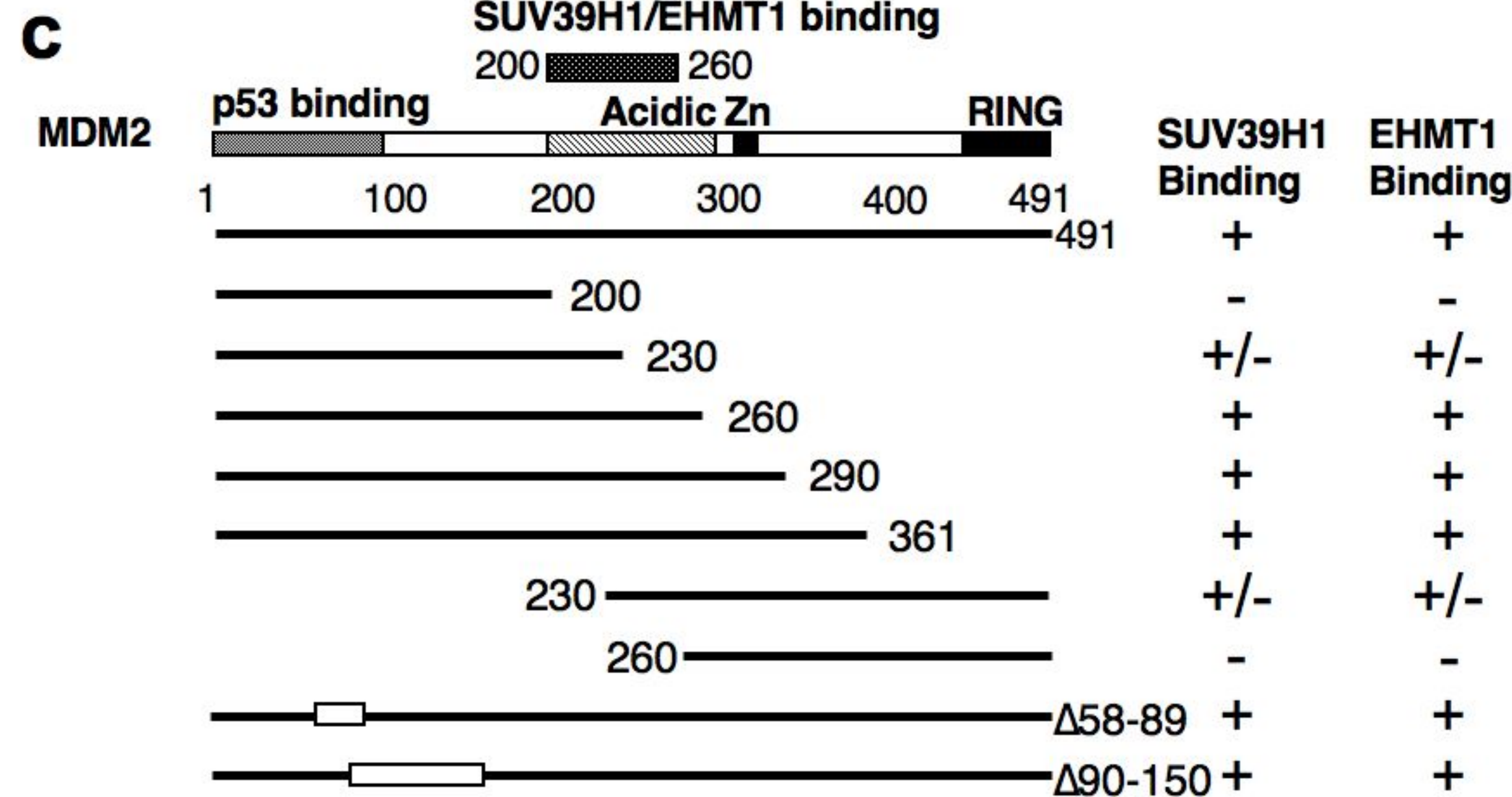
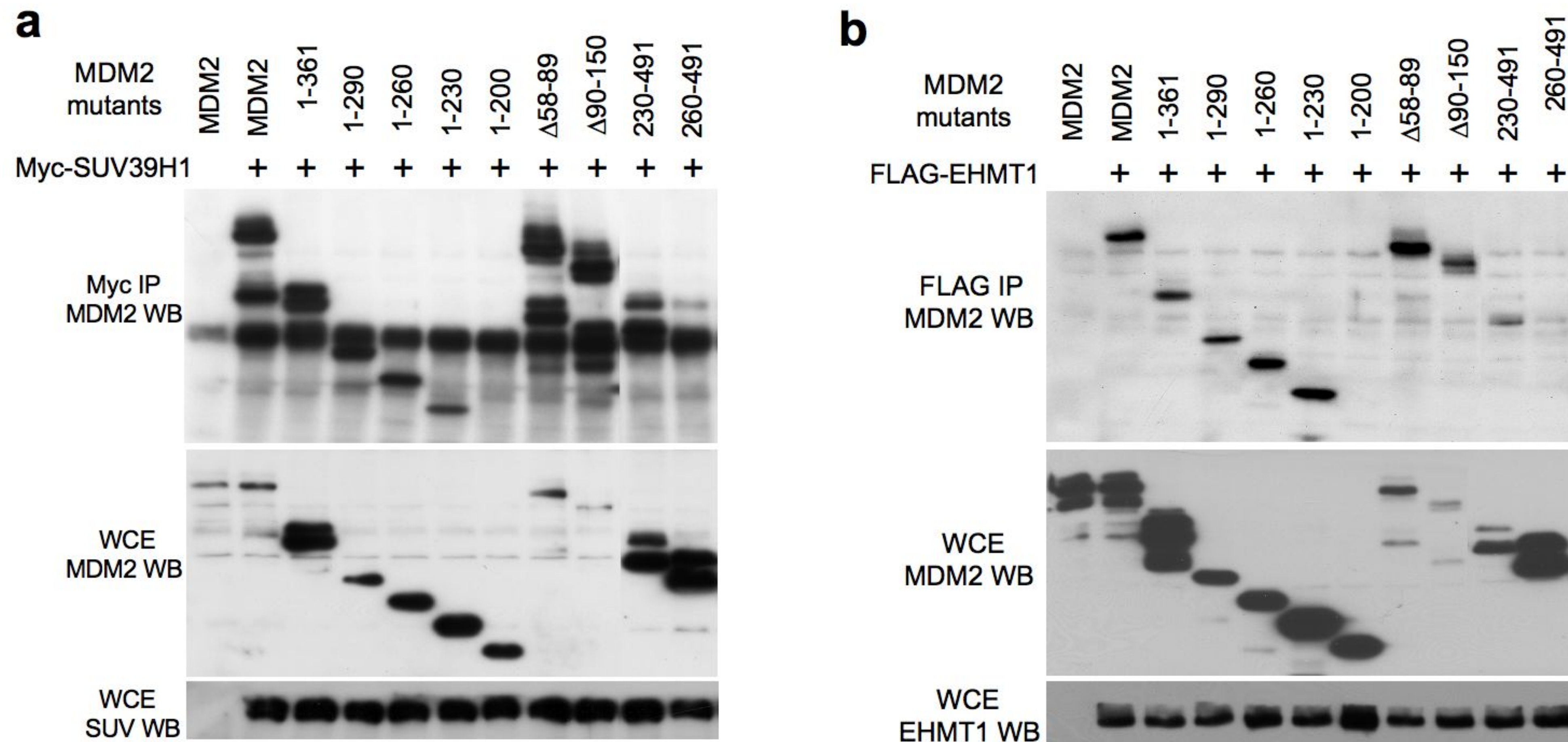
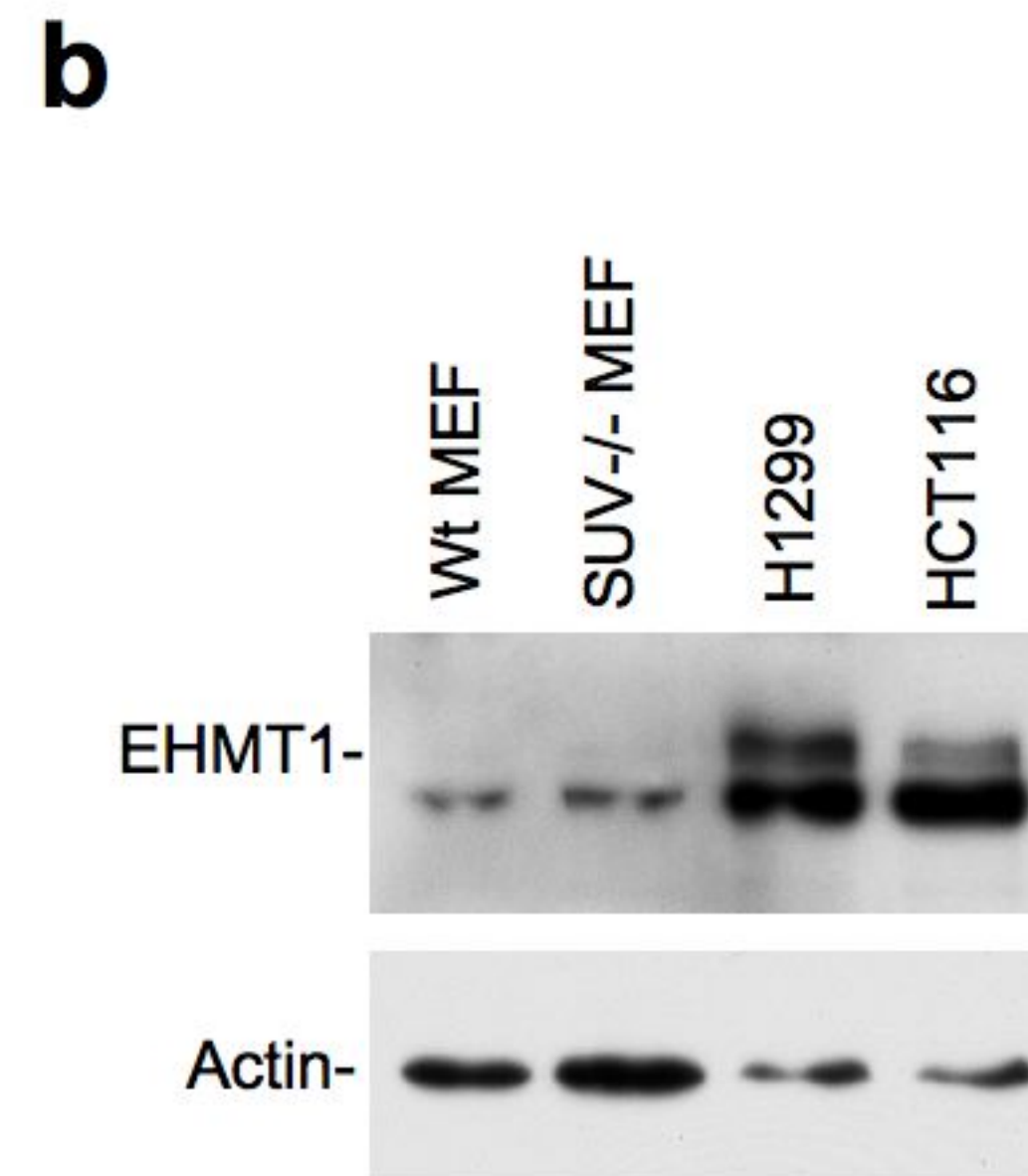
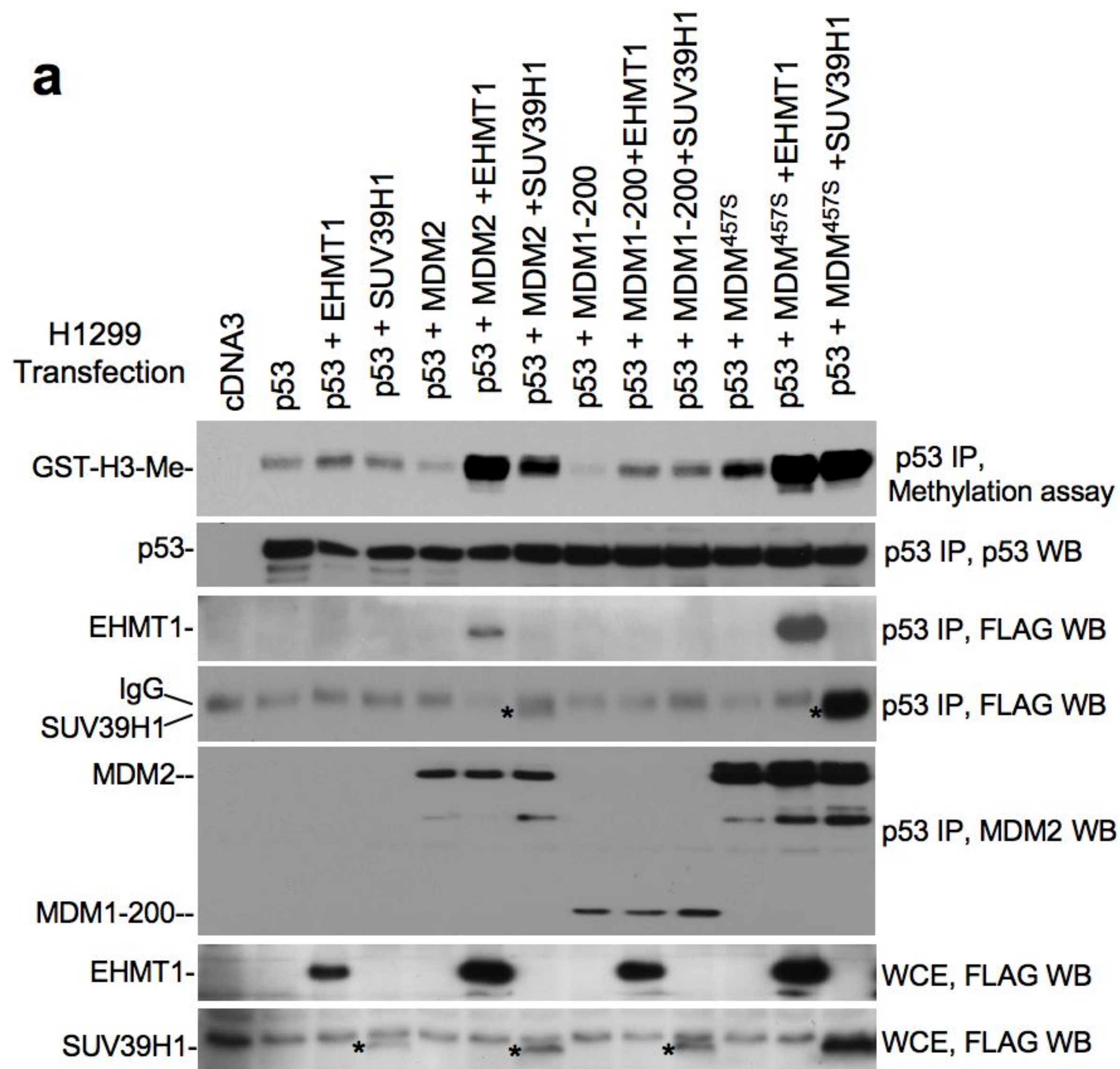


Supplemental Figure 1. MDM2 binds to the pre-SET domain of SUV39H1. (a) H1299 cells were transfected with MDM2 and FLAG-SUV39H1 deletion mutants. SUV39H1 mutants were immunoprecipitated using anti-FLAG antibody, and probed for coprecipitation of MDM2 using a polyclonal MDM2 antibody. (b) Diagram of SUV39H1 mutants and summary of results in (a).

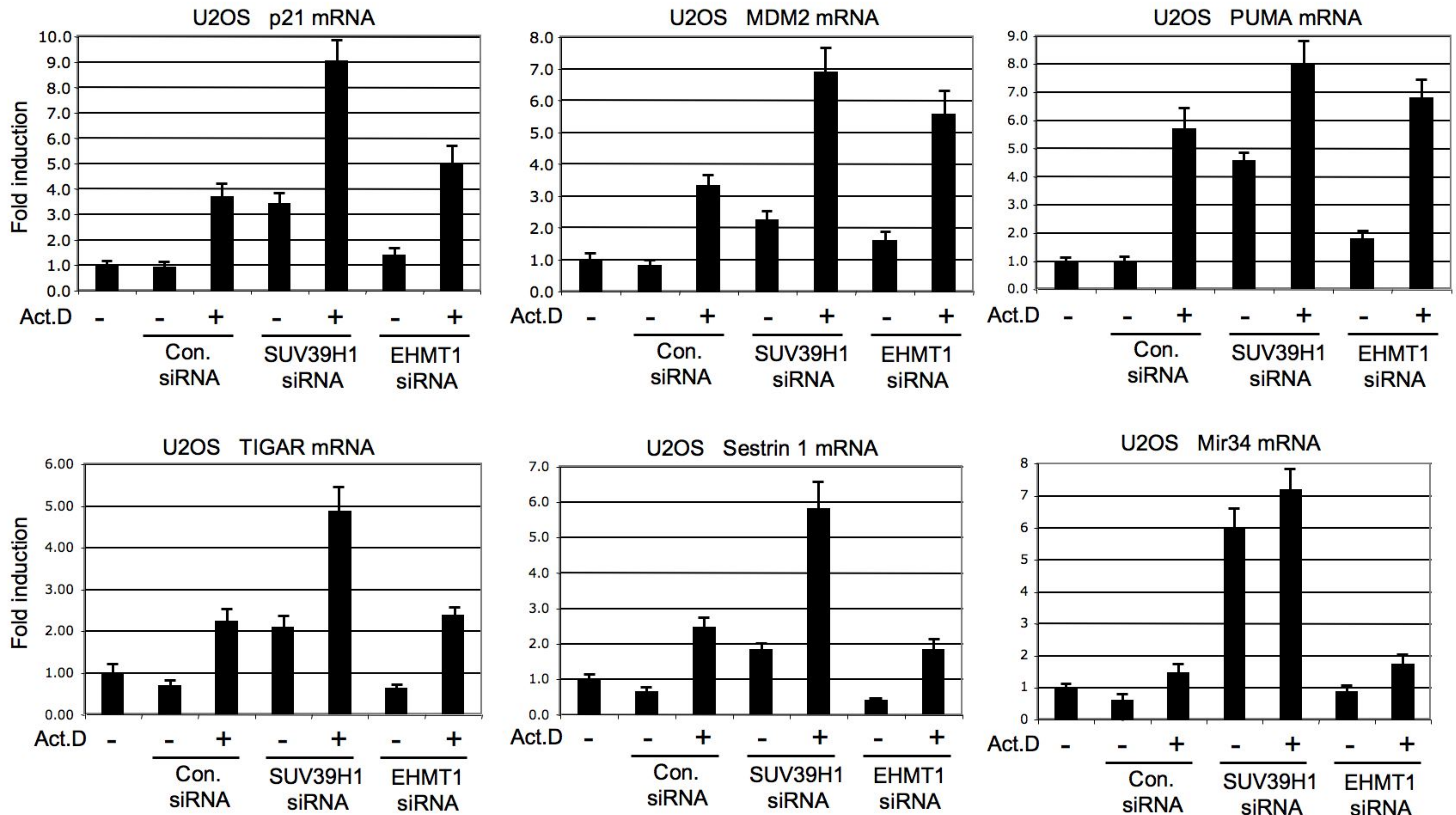


Supplemental Figure 2. MDM2 acidic region is required for SUV39H1 and EHMT1 binding. (a) H1299 cells were transiently transfected with MDM2 deletion mutants and Myc-SUV39H1, immunoprecipitated using anti-Myc antibody, and probed for coprecipitation of MDM2 using a polyclonal MDM2 antibody. (b) H1299 cells were transiently transfected with MDM2 deletion mutants and FLAG-EHMT1, immunoprecipitated using anti-FLAG antibody, and probed for coprecipitation of MDM2 using a polyclonal MDM2 antibody. (c) Diagram of MDM2 mutants and summary of results in (a) and (b).



Supplemental Figure 3. MDM2 acidic domain is critical for mediating formation of active p53-SUV39H1/EHMT1 complex. H1299 cells were transfected with p53, MDM2, FLAG-SUV39H1, and FLAG-EHMT1. P53 IPs were analyzed for methylase activity. Coprecipitation of SUV39H1 and EHMT1 was confirmed by probing the p53 and MDM2 IP using FLAG antibody. P53 alone did not coprecipitate SUV39H1 or EHMT1. Coexpression of MDM2 but not MDM2-1-200 stimulated the binding and the p53-associated methyltransferase activity. (b) MEFs express low level EHMT1. Identical amounts of whole cell lysate from MEFs and tumor cell lines were analyzed for endogenous EHMT1 by western blot. The cell lines expressed different levels of EHMT1 and actin despite identical total protein loading.

Supplemental Figure 4



Supplemental Figure 4. Knockdown of SUV39H1 and EHMT1 increase p53-mediated transcription. U2OS cells were treated with a second set of siRNA against SUV39H1 and EHMT1 for 48 hours. RNA were purified and analyzed by RT-PCR using primers for indicated p53 target genes. The readout was normalized with GAPDH mRNA level.