Supplemental Data

Modulation of p53 function by SET8-mediated methylation at lysine 382

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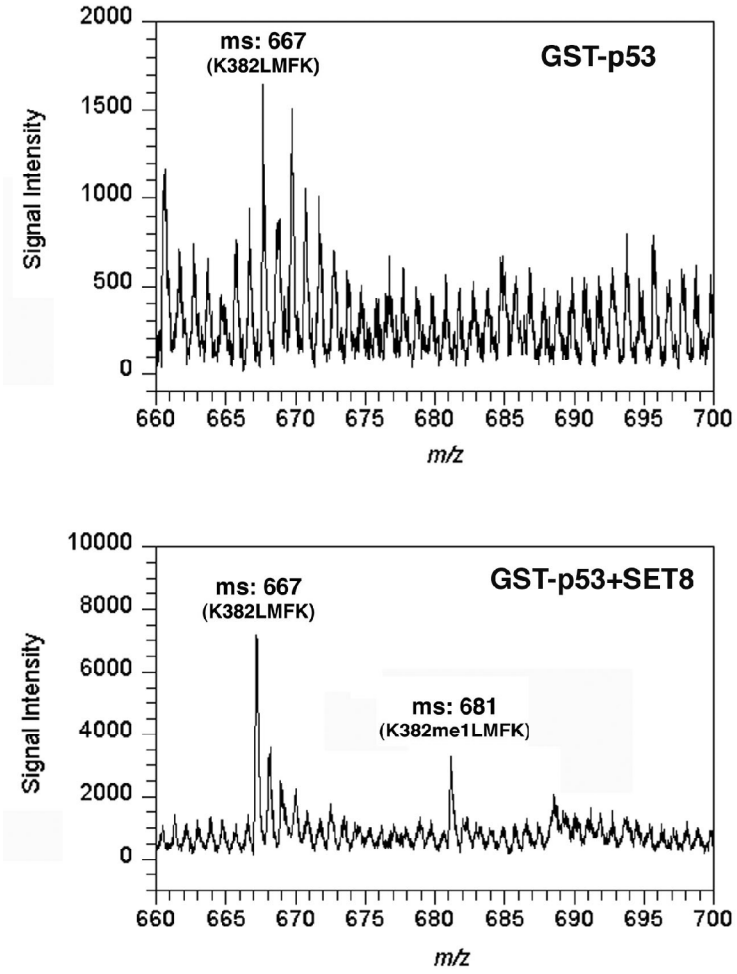

Figure S1. Mass spectrometry analysis of GST-p53 methylated by SET8 in *in vitro* methyltransferase assays
GST-p53 was incubated with SET8 in *in vitro* methyltransferase assays and subject to mass spectrometry analysis. Trypsin-digested peptides containing lysine382 (382-KLMFK-386) are present in peaks containing either unmethylated or monomethylated K382.

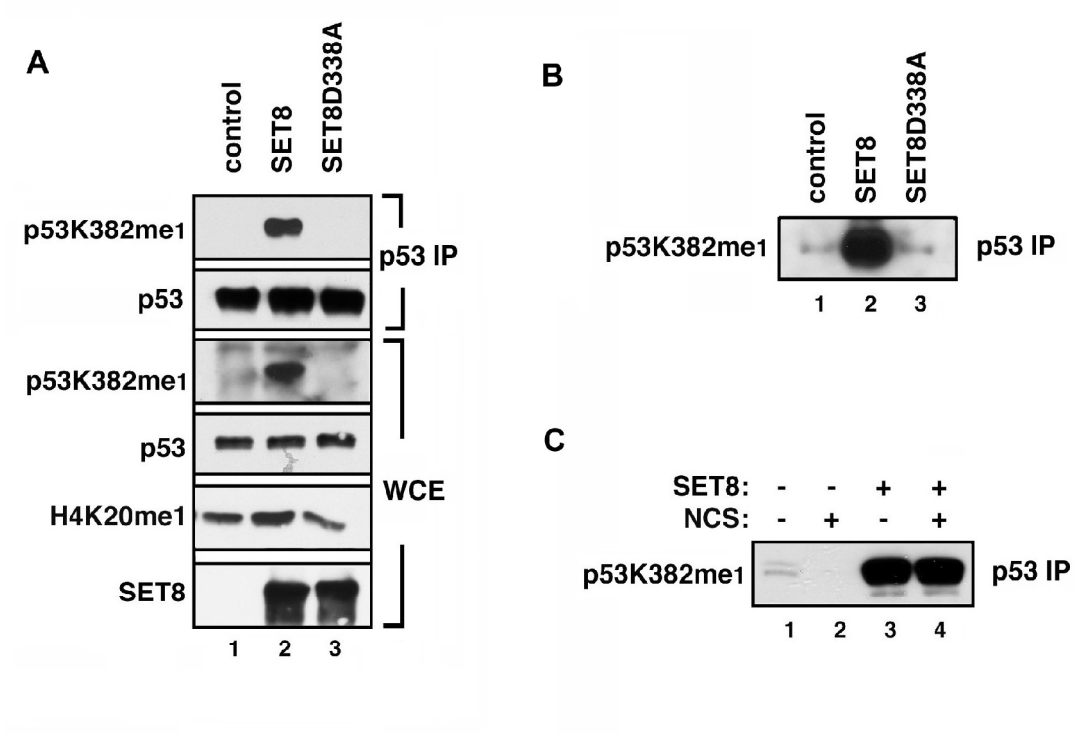

Figure S2. Endogenous p53 is methylated by SET8
(A) Western blot analysis with αp53K382me1 antibody of WCE and p53 IPs from 293T cells expressing SET8, the catalytically inactive SET8D338A mutant, or control vector alone. SET8 and H4K20me1 levels in the WCE are shown. Total p53 in the WCE are shown for equal loading.
(B) Longer exposure of top panel in (A) to show endogenous p53K382me1 levels (Lane 1 and 3).
(C) Longer exposure of top panel of Figure 3C to show endogenous p53K382me1 levels (Lane 1 and 2).

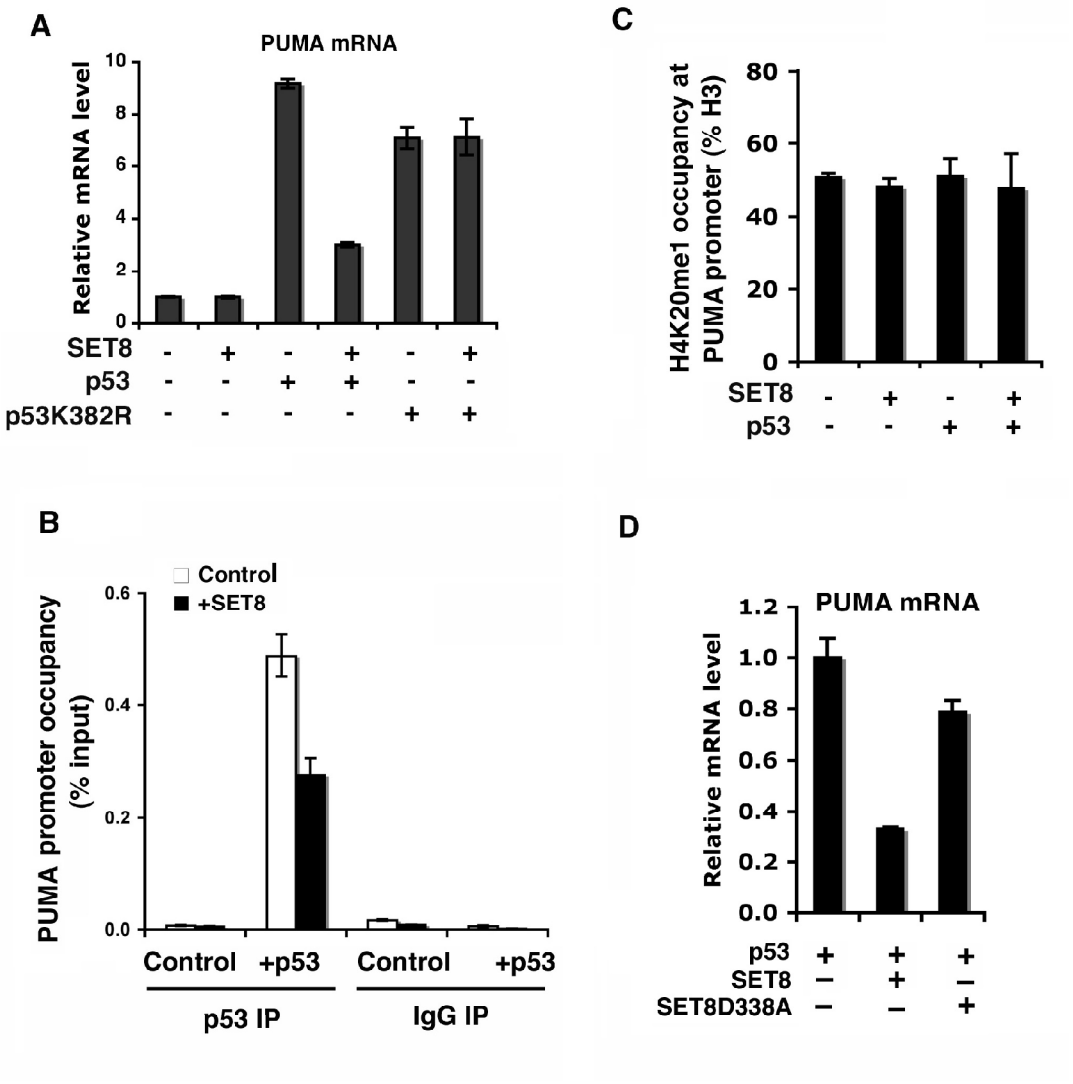

Figure S3. Methylation of p53 at K382 by SET8 suppresses p53 transactivation activity on PUMA

(A) SET8 inhibits induction of *PUMA* transcription by wild-type p53, but does not affect the activity of the p53K382R mutant. Real-time PCR analyses of *PUMA* mRNA levels in H1299 cells transfected with control vector, p53 or p53K382R mutant, ± SET8.

(B) SET8 expression attenuates occupancy of p53 at the *PUMA* promoter. p53 occupancy at the *PUMA* promoter in H1299 cells transfected with control vector or p53, ± SET8 was determined by ChIP analyses. DO-1 antibody was used for p53 ChIP and IgG was used as control. Occupancy values (ChIP/inputX100) were determined by real-time PCR.

(C) SET8 expression does not alter H4K20me1 levels at the *PUMA* promoter. Occupancy of H4K20me1 (H4K20me1 ChIP/H3 ChIPX100) at the *PUMA* promoter was determined as in (B).

(D) SET8 catalytic mutant SETD338A fails to suppress p53 transactivation activity on *PUMA*. Real-time PCR analyses of relative *PUMA* mRNA levels in H1299 cells co-transfected with p53 and SET8, SET8D338A or control vector as indicated. Error bars indicate ± s.e.m. from three experiments.

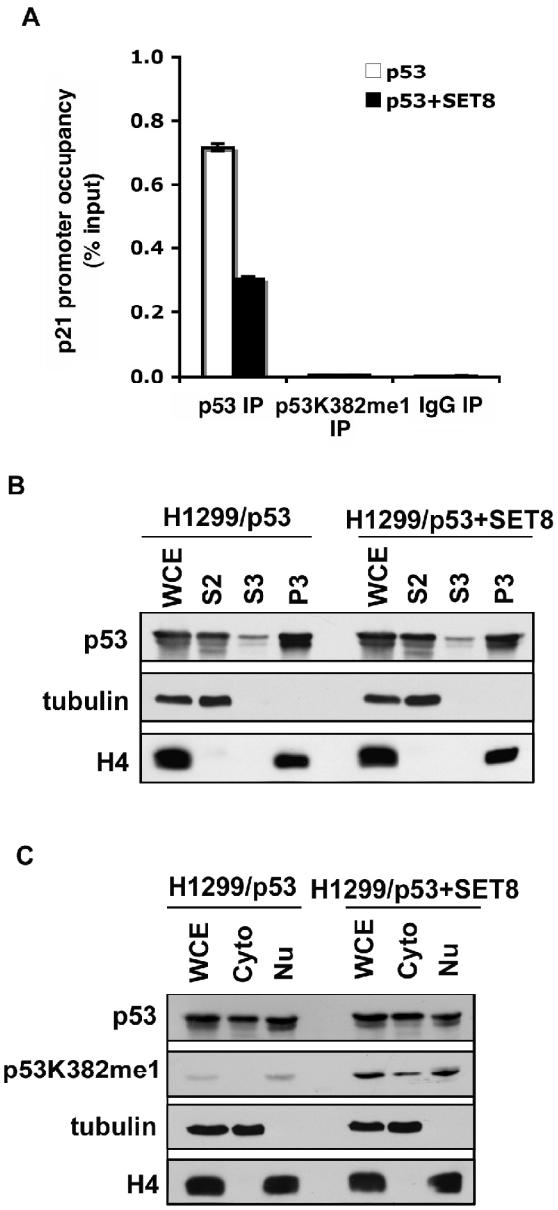

Figure S4. Methylation of p53 at K382 by SET8 does not affect p53 global distribution

(A) Monomethylated p53 at K382 does not occupy *p21* promoter. Total p53 and p53K382me1 occupancy at the p21 promoter in H1299 cells transfected with p53 ± SET8 was determined by ChIP analyses. DO-1 antibody was used for total p53 ChIP, αp53K382me1 was used for p53K382me1 and IgG was used as control. Occupancy values (ChIP/inputX100) were determined by real-time PCR.

(B) p53 distribution is not affected by SET8. H1299 cells as in (A) were fractionated into cytoplasmic extract (S2), nuclear extract (S3) and chromatin (P3) fractions as previously described (Mendez and Stillman, 2000), and were analyzed for the presence of p53. Tubulin served as a marker for the cytoplasmic fraction and histone H4 for chromatin. WCE: whole cell extracts.

(C) SET8 methylates p53 in the nuclei. H1299 cells as in (A) were fractionated into cytoplasmic (Cyto) and nuclear fractions (Nu) as in (B), and were analyzed for the presence of p53 and p53K382me1. Tubulin served as a marker for the cytoplasmic fraction and histone.

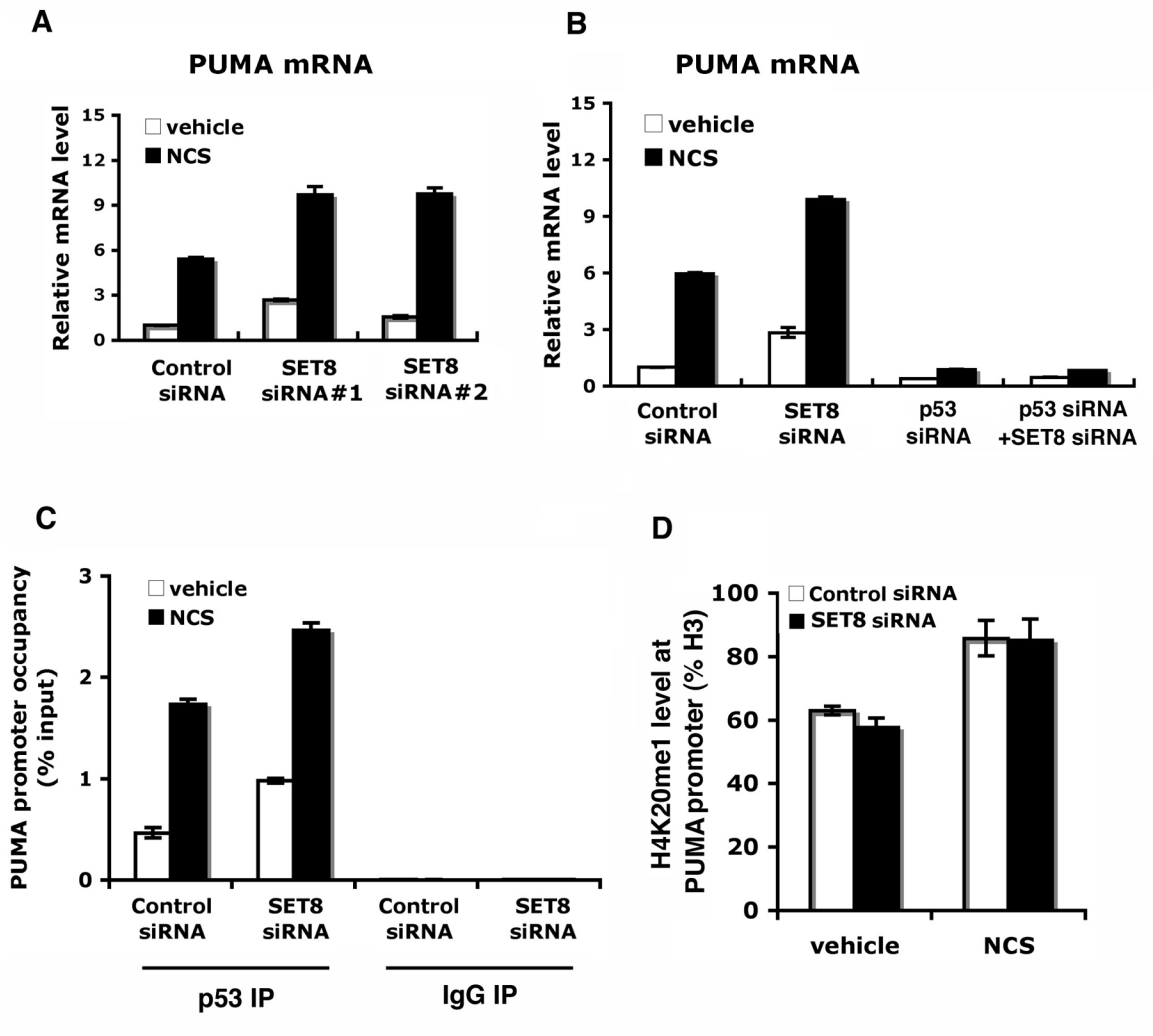

Figure S5. SET8 RNAi augments p53 activity on PUMA gene in response to DNA damage.

(A) Knock-down of SET8 augments expression of *PUMA* mRNA in response to DNA damage. Real-time PCR analyses of *PUMA* mRNA in U2OS cells treated with 0.5 μg/ml NCS (4 hrs) and transfected with control or two different sets of SET8 siRNA as in Figure 5. (B) SET8 regulation of p21 expression is p53-dependent. Real-time PCR analysis of *PUMA* mRNA in U2OS cells as in (Figure 5E). (C) Knock-down of endogenous SET8 augments p53 occupancy at the *PUMA* promoter. ChIP assays as in (Figure 5F) in U2OS cells transfected with control or SET8 siRNA ± NCS treatment. (D) SET8 RNAi does not alter H4K20me1 levels at the *PUMA* promoter. ChIP assays to determine H4K20me1 occupancy at the *PUMA* promoter as in (Figure 5G) in U2OS cells as in (C). Error bars indicate ± s.e.m. from at least three experiments.

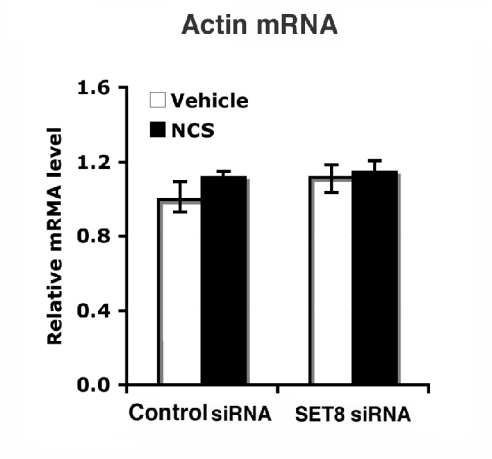

Figure S6. SET8 RNAi does not affect expression of the non-DNA damage-dependent p53 target gene *actin* in response to DNA damage.
Real-time PCR analysis of *Actin* mRNA in U2OS cells transfected with control or SET8 siRNA ± NCS treatments.

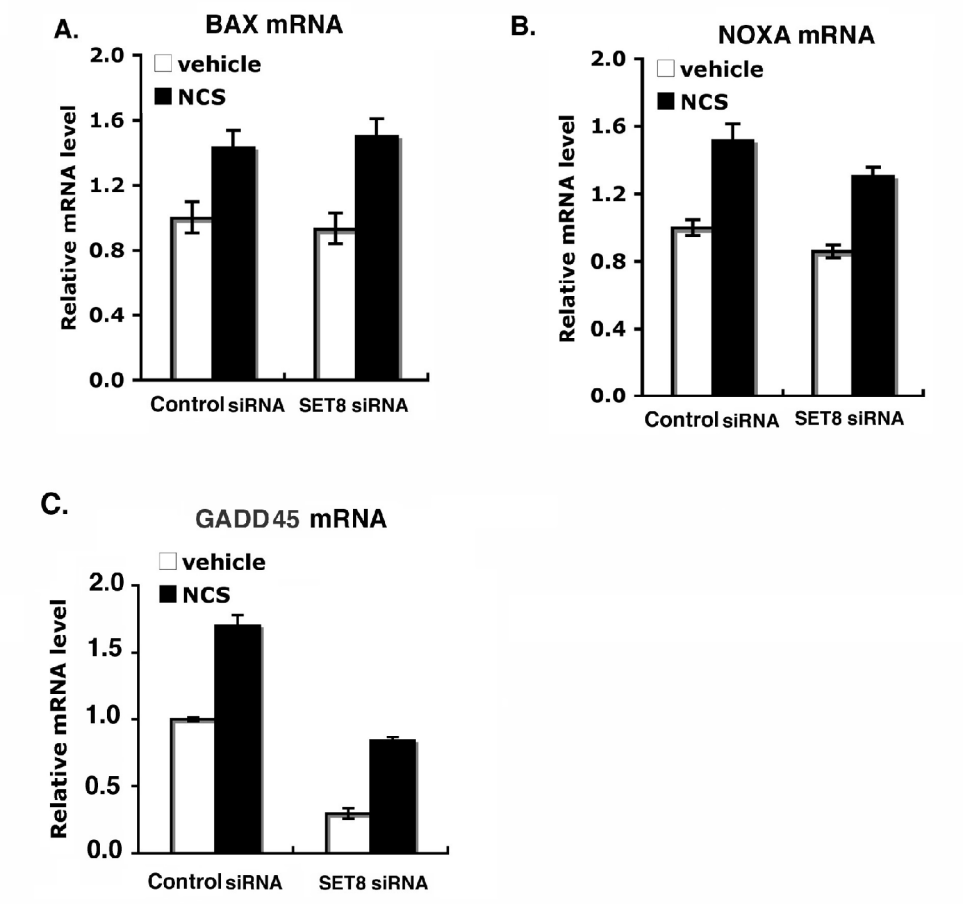

Figure S7. SET8 RNAi decreases *GADD45* expression in response to DNA damage. Real-time PCR analyses of *BAX* (A), *NOXA* (B) and *GADD45*(C) mRNA in U2OS cells transfected with control or SET8 siRNA ± NCS treatments.

References:

Mendez, J., and Stillman, B. (2000). Chromatin association of human origin recognition complex, cdc6, and minichromosome maintenance proteins during the cell cycle: assembly of prereplication complexes in late mitosis. Mol Cell Biol *20*, 8602-8612.