

SUPPLEMENTAL MATERIAL

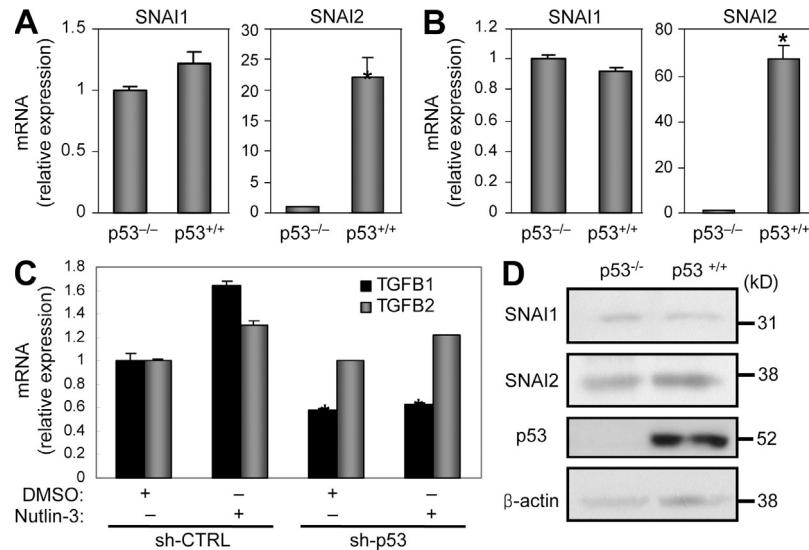
Kim et al., <http://www.jem.org/cgi/content/full/jem.20110235/DC1>

Figure S1. p53-mediated EMT is independent of known EMT activators, such as SNAI1, SNAI2, TWIST (not detected), TGFB1, and TGFB2, except for ZEB1 and ZEB2. (A) Relative mRNA levels of SNAI1 and SNAI2 in RKO (p53^{+/+} and p53^{-/-}) cells. (B) Relative mRNA levels of TGFB1 and TGFB2 in RKO (p53^{+/+} and p53^{-/-}) cells. (C) Relative mRNA levels of TGFB1 and TGFB2 in C3A-sh-CTRL and C3A-sh-p53 cells treated with DMSO or 10μM nutlin-3a for 24 h. (D) Protein levels of SNAI1, SNAI2 and p53 in RKO (p53^{+/+} and p53^{-/-}) cells. A representative experiment out of two independent experiments. (A-C) Data are mean ± SEM of three independent experiments and each is measured in triplicate (**, P < 0.05; *, P ≤ 0.01).

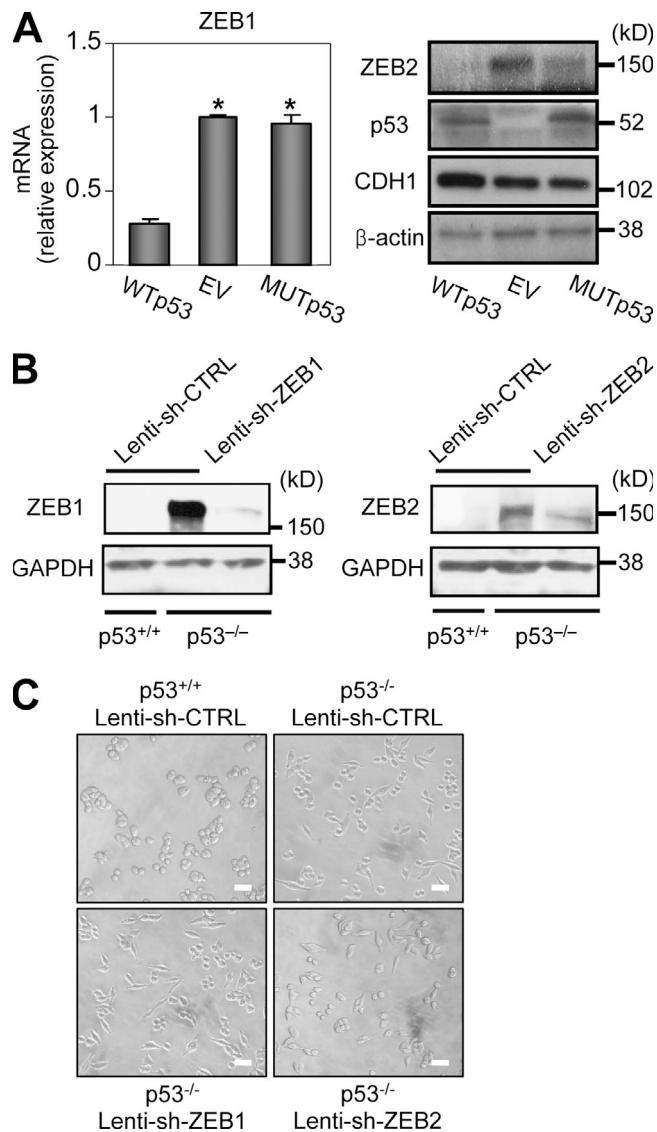


Figure S2. p53-repressed ZEB1 and ZEB2 expression, and effect of single knock-down of ZEB1 or ZEB2 in p53-repressed EMT. (A) Relative mRNA levels of ZEB1 (left), and protein levels of ZEB2, p53, and CDH1 (right) in Hep3B cells stably expressing WT p53 expression vector (WT $p53$), empty vector (EV), or mutant (A161T) p53 expression vector (MUT $p53$). (left) Data are mean \pm SEM of three independent experiments and each measured in triplicate (**, P < 0.05; *, P \leq 0.01). (right) A representative experiment out of two independent experiments. (B) Relative protein levels of ZEB1 or ZEB2 in RKO (p53 $^{+/+}$ and p53 $^{-/-}$) cells stably expressing lentiviral sh-CTRL, sh-ZEB1, or sh-ZEB2. (C) Phase contrast view of RKO (p53 $^{+/+}$ and p53 $^{-/-}$) cells stably expressing lentiviral sh-CTRL, sh-ZEB1 or sh-ZEB2. (B and C) A representative experiment out of two independent experiments. Bars, 50 μ m.

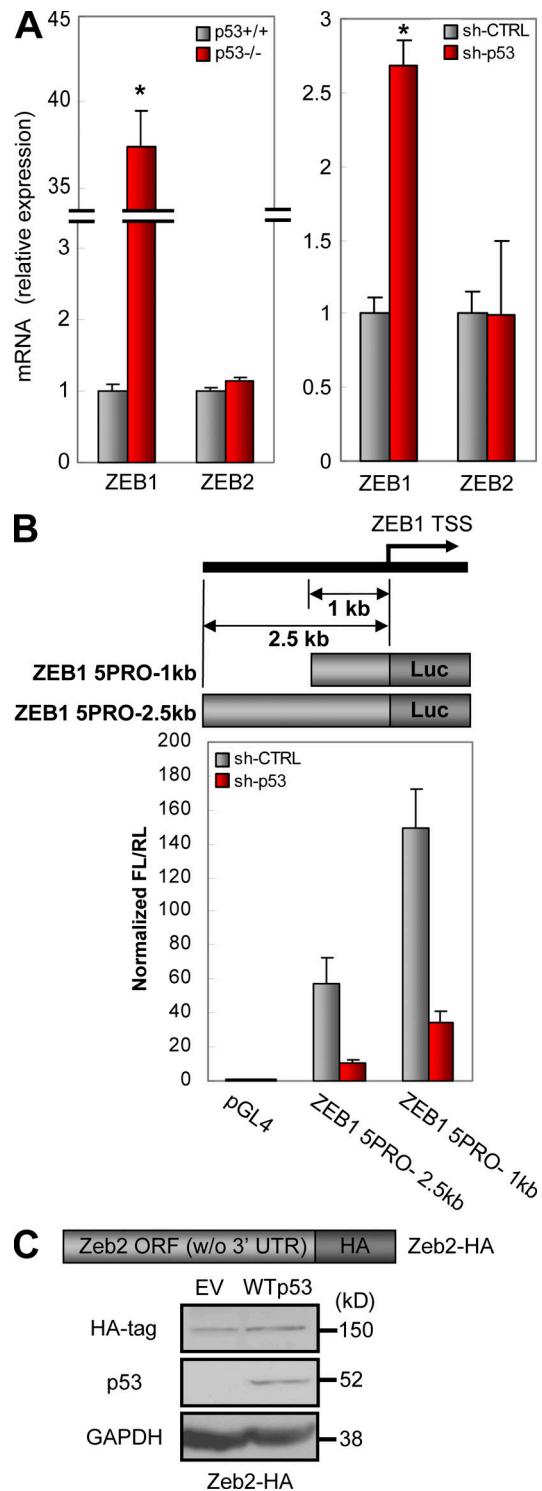


Figure S3. p53 represses ZEB1 and ZEB2 expression in the posttranscriptional level such as mRNA or translational level, but not in transcription or protein level. (A) Relative mRNA levels of ZEB1 and ZEB2 in RKO (p53^{+/+} and p53^{-/-}) cells (left) and C3A (C3A-sh-CTRL and C3A-sh-p53) cells (right). (B) Luciferase assay. ZEB1 5' promoter region (1kb or 2.5kb) was inserted into luciferase vector (pGL4) as shown in the top panel, and luciferase activities were measured in C3A-sh-CTRL and C3A-sh-p53 cells. RL, Renilla luciferase and FL, Firefly luciferase. (C) Western blot analysis. p53^{-/-} RKO cells stably expressing Zeb2-HA (Zeb2 ORF without Zeb2 3'UTR) were transfected with p53 expression vectors (WT p53) or empty vectors (EV). The expression of Zeb2-HA was analyzed using HA antibody. A representative experiment out of two independent experiments. (A, B) Data are mean \pm SEM of three independent experiments and each is measured in triplicate (**, P<0.05; *, P ≤ 0.01).

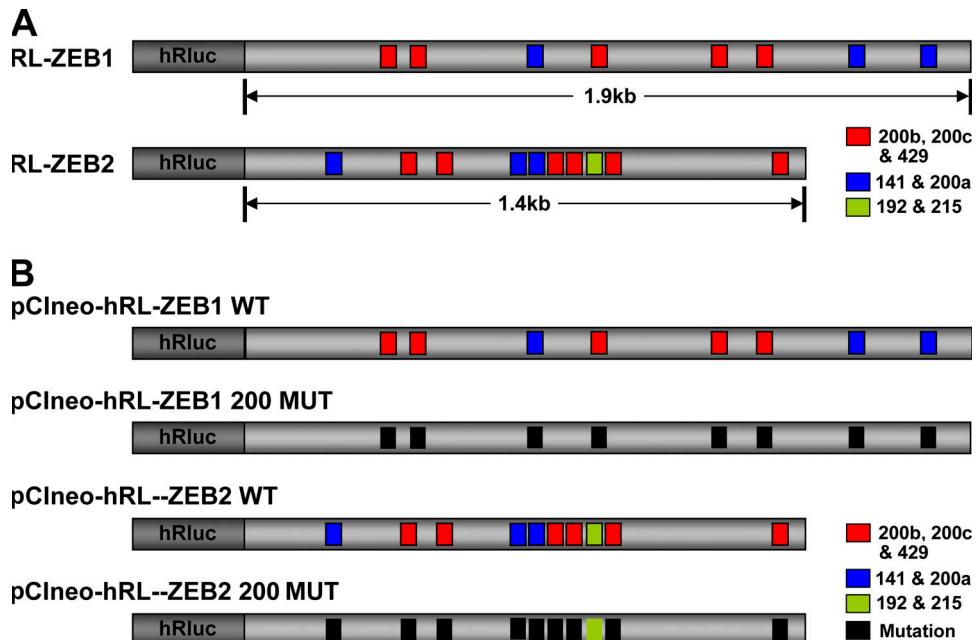


Figure S4. Schematic view of luciferase constructs inserted with the 3' UTR of ZEB1 or ZEB2. (A) Luciferase vectors (psi-CHECK2) were inserted with full-length of ZEB1 or ZEB2 3'UTR (RL-ZEB1 or RL-ZEB2). (B) The constructs, pCneo-hRL-ZEB1 WT, or pCneo-hRL-ZEB2 WT were generated by insertion of full-length ZEB1 or ZEB2 3'UTR into pCneo-hRL vectors. pCneo-hRL-ZEB1 200 MUT and pCneo-hRL-ZEB2 200 MUT were generated by mutations of predicted recognition sites of miR-200 family members.

