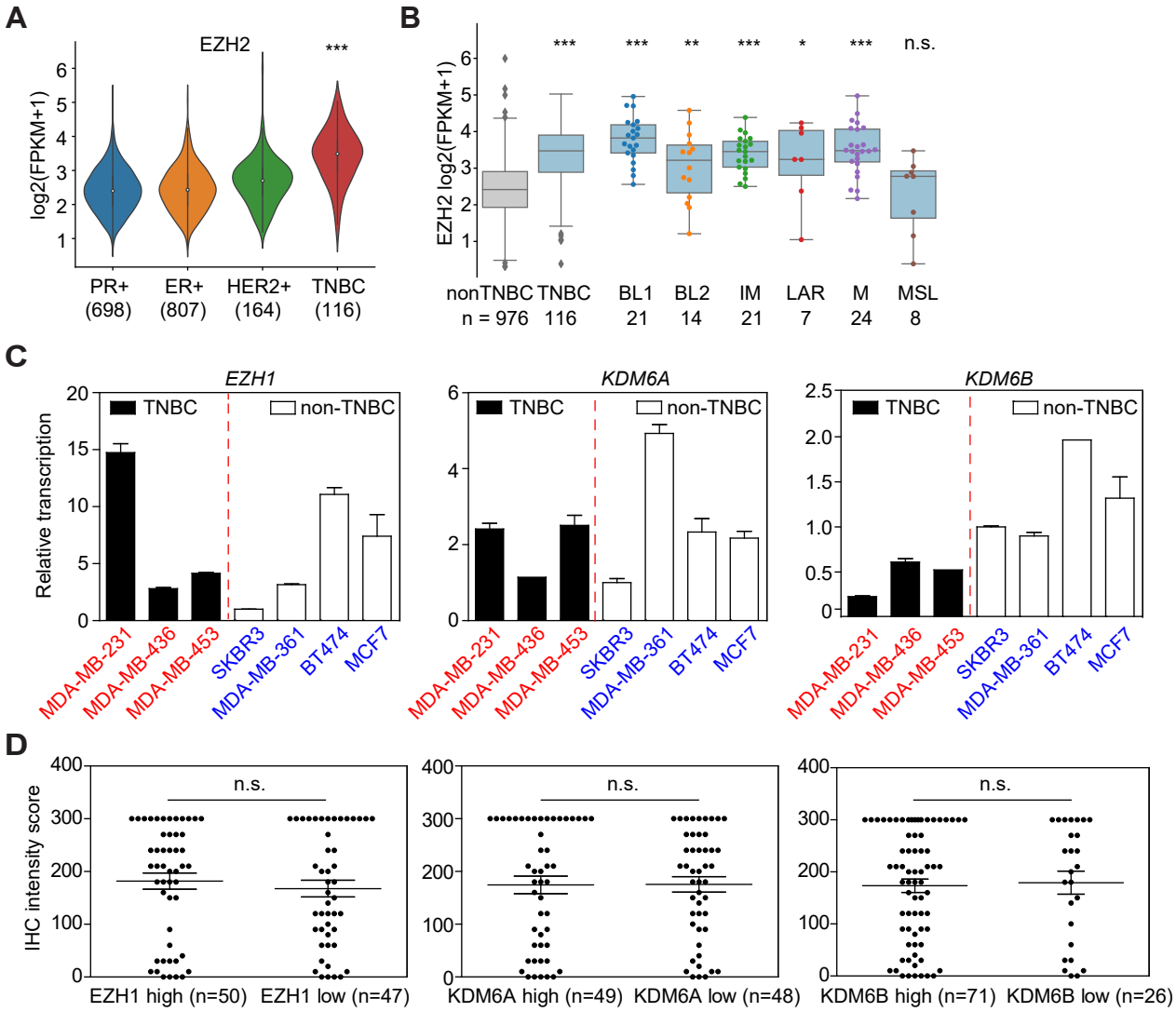

Figure S1. High EZH2 and its target H3K27me3 expression in TNBC predicts poor overall survival of patients

(A) Violin plot showing EZH2 expression in TNBC vs. PR^+, ER^+ and $HER2^+$ breast cancer patients of TCGA-BRCA dataset. (B) Box plot showing EZH2 expression in TNBC and its six subtypes (Lehmann et al., JCI, 2011) vs. non-TNBC. Scale bars indicate mean ± SEM. (C) qRT-PCR analysis showing relative *EZH1*, *KDM6A* and *KDM6B* transcription in indicated cell lines. Scale bars indicate mean ± SD. (D) IHC staining intensity of H3K27me3 in patient samples of EZH1, KDM6A or KDM6B high *vs*. low groups. Scale bars indicate mean ± SEM.

* indicates $p < 0.05$. ** indicates $p < 0.01$. *** indicates $p < 0.001$. n.s. indicates not significant.

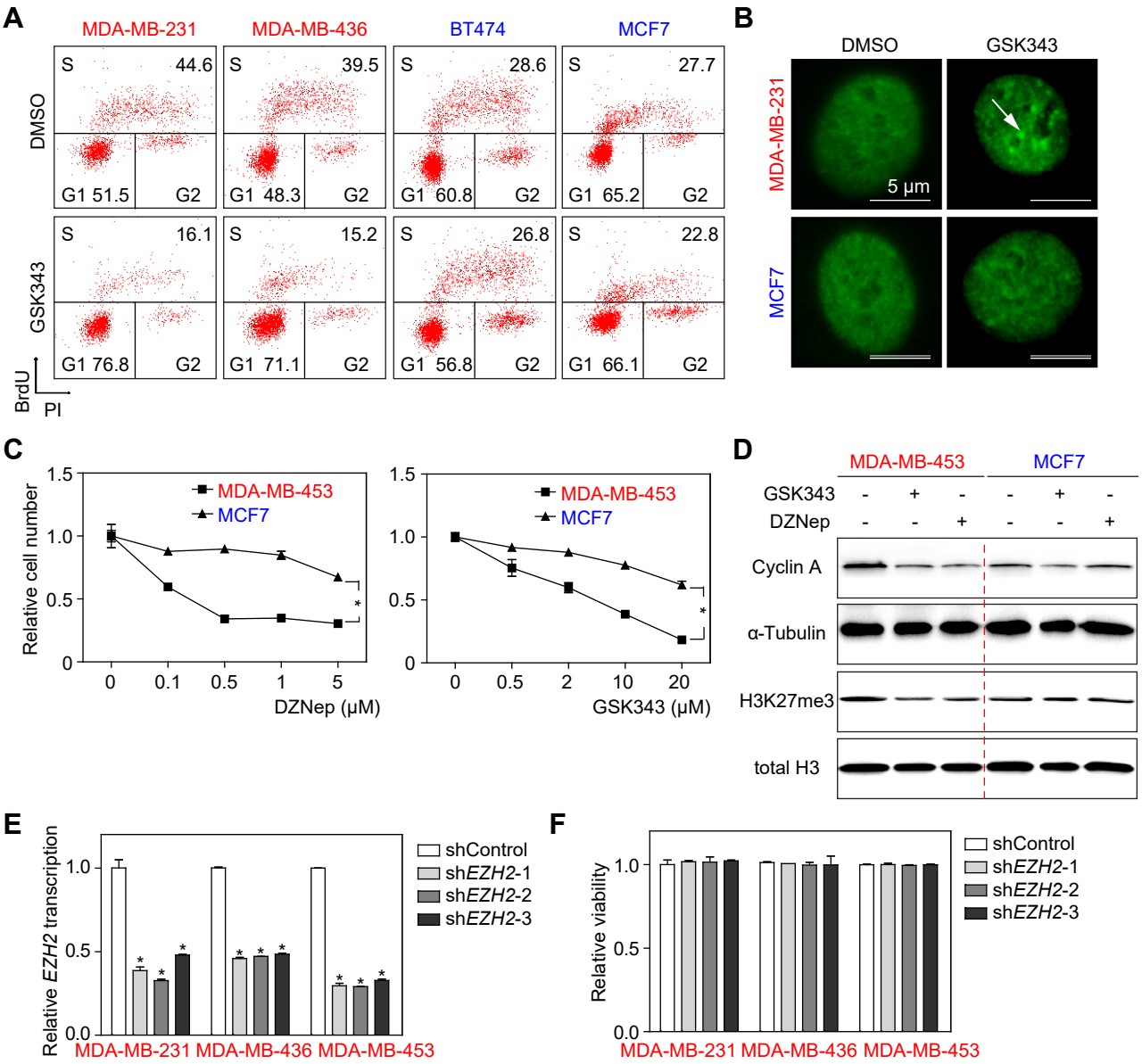

Figure S2. Inhibition of EZH2 selectively suppresses proliferation of TNBC cell lines

(A) BrdU/PI cell-cycle analysis by flow cytometry of indicated cells treated with 2 μM GSK343 and quantification of G1, S, and G2 subpopulations. (B) Immunofluorescent staining with anti-HP1γ antibody of cells as in *D*. Arrow indicates condensed heterochromatin. (C) Relative cell number of MDA-MB-453 and MCF7 cells treated with indicated concentrations of DZNep (*left*) or GSK343 (*right*) for 2 days and compared with cells treated with DMSO. (D) Immunoblot analysis of EZH2 and H3K27me3 of MDA-MB-453 and MCF7 cells treated with 2 μM GSK343, 1 μM DZNep or DMSO for 2 days. α-Tubulin and total H3 were used as loading controls. (E) qRT-PCR analysis of the *EZH2* transcript in cells transduced with sh*EZH2*. (F) Relative viability of cells transduced with sh*EZH2* or shControl at indicated time-points. Viability at day 0 was defined as 100% arbitrarily. Data are shown as mean ± SD. * indicates $p < 0.05$.

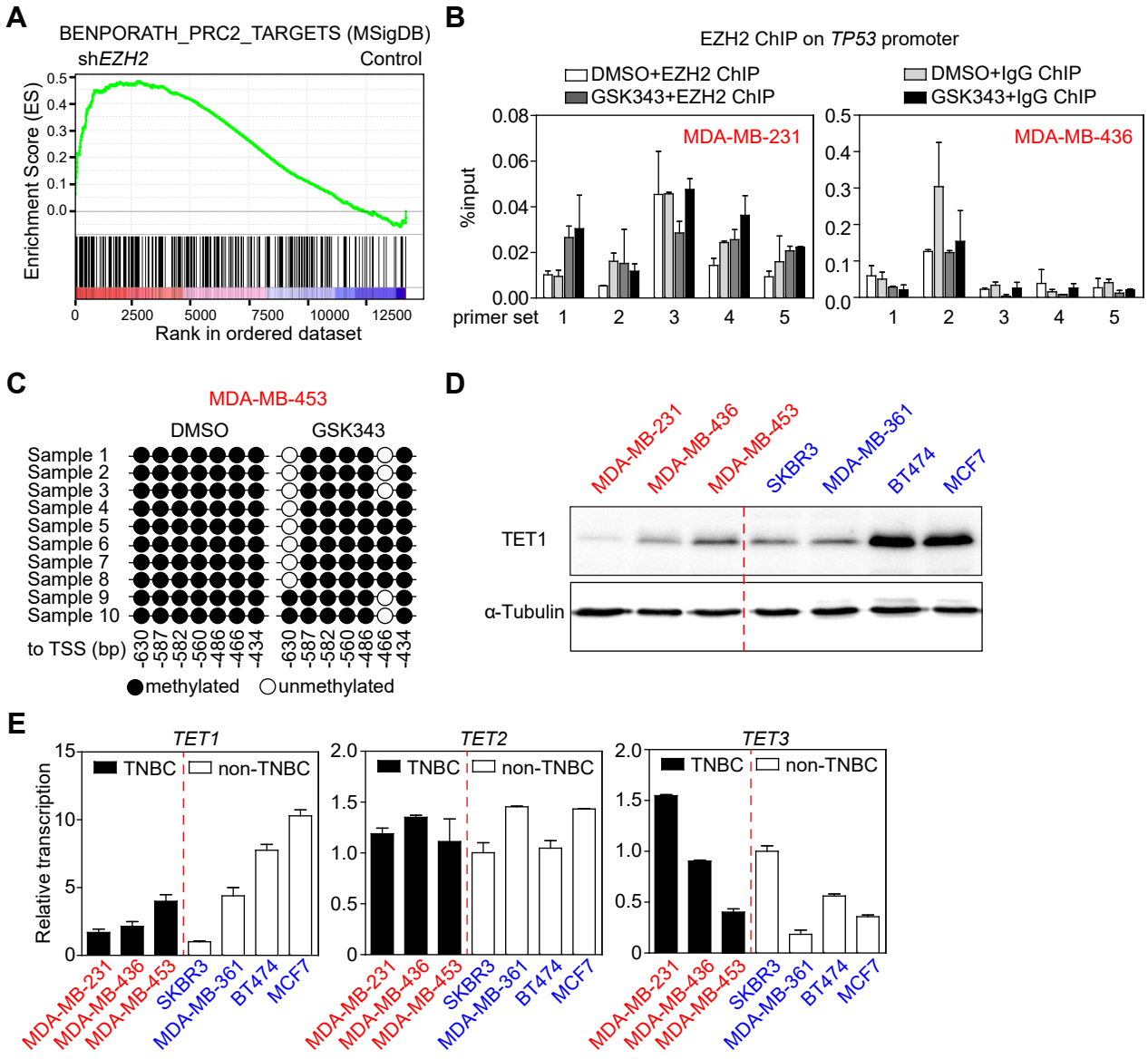

Figure S3. Inhibition of EZH2 elevates p53 and TET1 expression in TNBC

(A) GSEA analysis of PRC2 targets comparing cells with and without EZH2 knock-down. Gene expression profiles are combination of MDA-MB-231, MDA-MB-436 and MDA-MB-453 cells. (B) ChIP-qPCR analysis of MDA-MB-231 and MDA-MB-436 cells treated with GSK343 or DMSO, using an anti-EZH2 antibody and PCR primers specific for *TP53* promoter. Input as internal control and anti-IgG as negative control. (C) Bisulfite sequencing of indicated CpGs at the *TP53* promoter proximal to the transcription start site (TSS) showing methylation status in MDA-MB-453 cells treated with GSK343 or DMSO as control. 10 independent clones were sequenced for each CpG region. (D) Immunoblot showing TET1 expression in indicated cell lines with α-Tubulin as a loading control. (E) qRT-PCR analysis showing relative *TET1*, *TET2* and *TET3* transcription in indicated cell lines. Data are shown as mean ± SD.

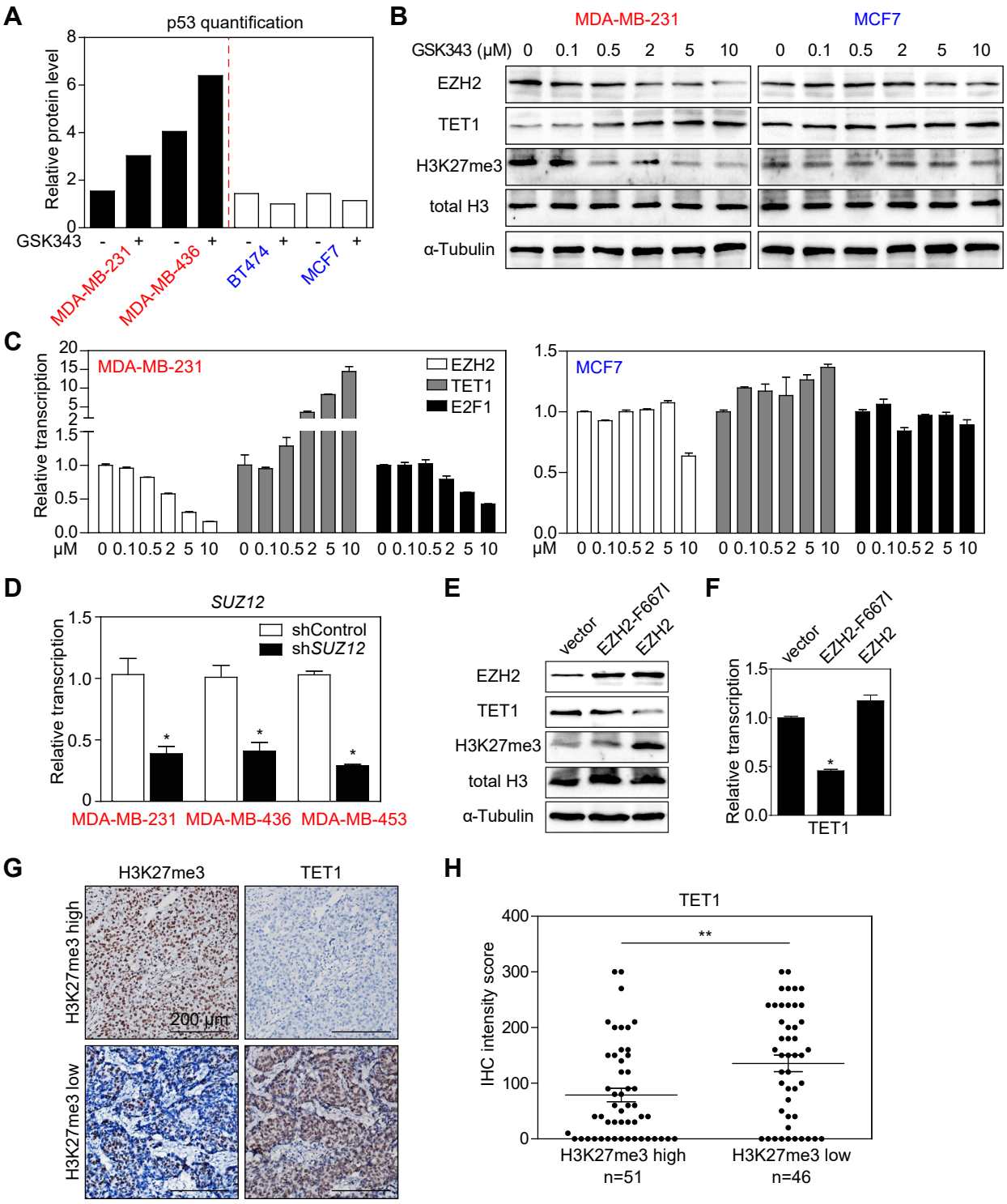

Figure S4. TET1 expression is regulated by H3K27me3

(A) Quantification of p53 immunoblot lanes in Figure 4A. (B) Immunoblot analysis of the indicated proteins in lysates from cells treated with a serial dilution of GSK343 for 2 days and compared with cells treated with DMSO. (C) qRT-PCR analysis of EZH2, TET1 and E2F1 expression in cells as in *B*. (D) qRT-PCR analysis of SUZ12 expression in cells expressing sh*SUZ12*. (E) Immunoblot analysis of the indicated proteins in lysates from MCF7 cells expressing EZH2 or catalytic inactive mutant EZH2-F667I. (F) qRT-PCR analysis of TET1 expression in cells as in *E*. (G) Representative pictures of IHC staining of patient tumor samples showing H3K27me3 and TET1 expression. (H) TET1 IHC staining intensity of patient tumor samples in H3K27me3 high *vs*. low groups. Scale bars indicate mean ± SEM. Data are shown as mean ± SD, unless otherwise indicated. * indicates $p < 0.05$. ** indicates $p < 0.01$.

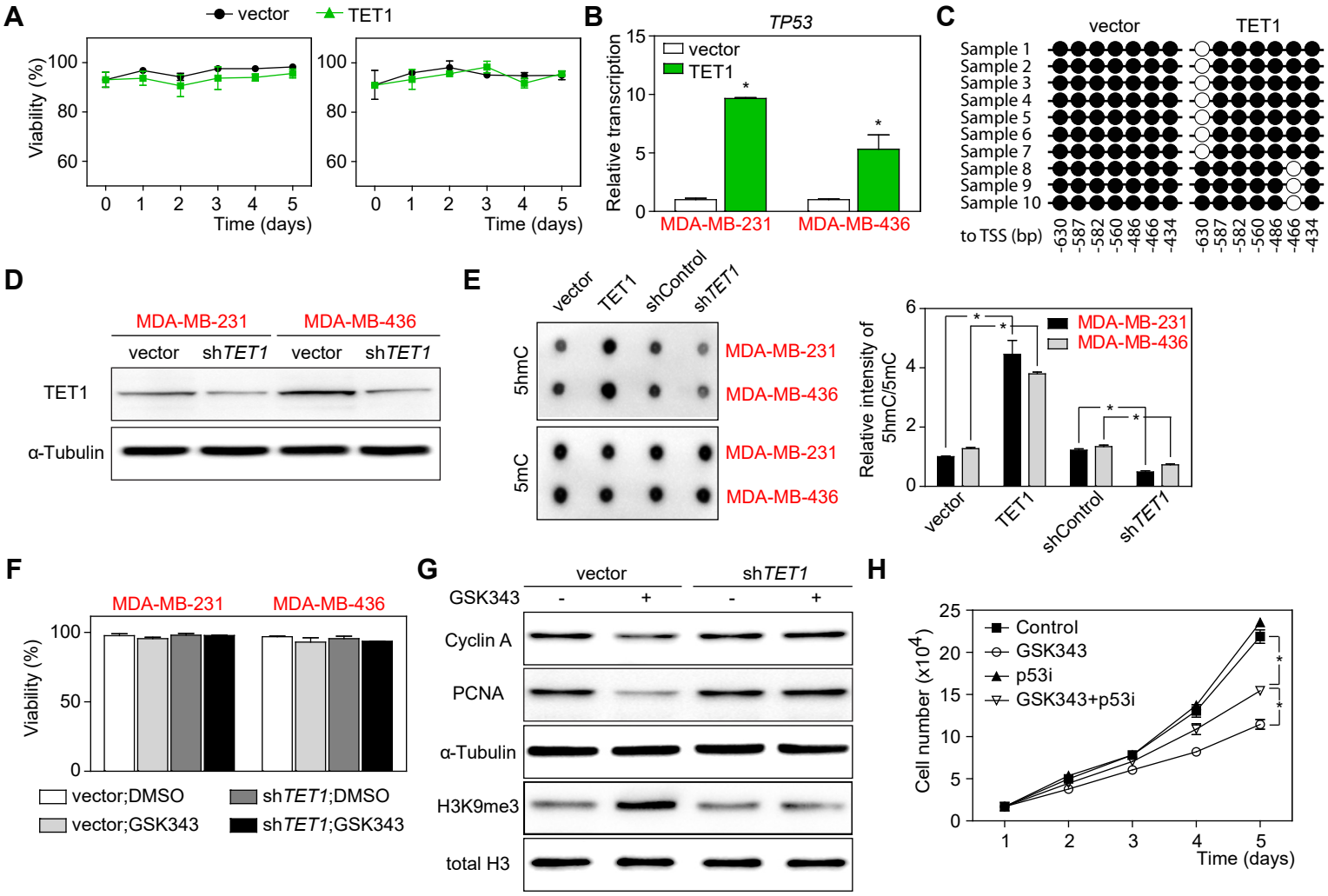

Figure S5. TET1 elevates p53 expression and represses TNBC cell proliferation

(A) Viability of cells expressing TET1 or vector at indicated time points. (B) qRT-PCR analysis showing relative transcription of *TP53* of cells expressing TET1, vector as control. (C) Bisulfite sequencing of indicated CpGs at the *TP53* promoter proximal to the transcription start site (TSS) showing methylation status in MDA-MB-453 cells expressing TET1 or vector as control. 10 independent clones were sequenced for each CpG region. (D) Immunoblot analysis of TET1 expression of MDA-MB-231 and MDA-MB-436 cells expressing sh*TET1* or vector. (E) Dot blot analysis (left) and quantification (right) of global 5hmC and 5mC levels in MDA-MB-231 and MDA-MB-436 cells expressing TET1, sh*TET1* or respective vectors as controls. 5 μg genomic DNA was loaded for detection. (F) Viability of cells expressing sh*TET1* or vector, 2 days after GSK343 or DMSO treatment. (G) Immunoblot analysis of the indicated proteins in cell lysates from cell lines as in *F*. α-Tubulin and total H3 were used as loading controls. (H) Grow curve showing proliferation rate of MDA-MB-231 cells treated with DMSO, 2 μm GSK343, p53 siRNA or 2 μm GSK343+p53 siRNA. Data are shown as mean ± SD. * indicates $p < 0.05$.

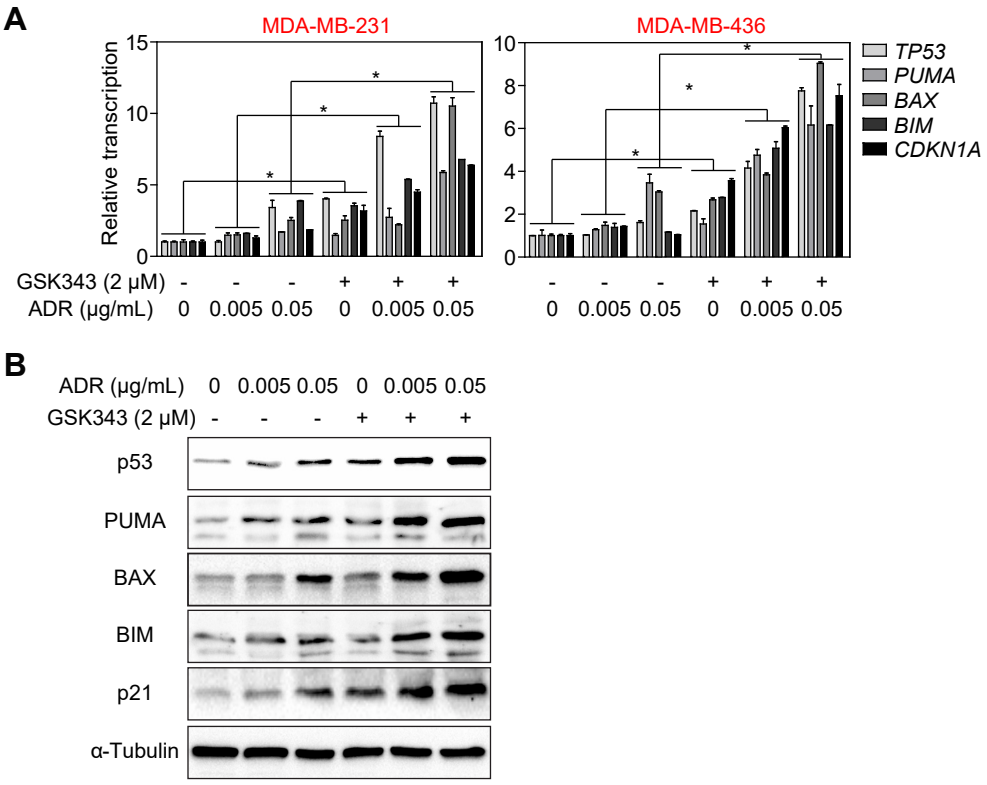

Figure S6. GSK343-treated TNBC cells are sensitive to adriamycin treatment

(A) qRT-PCR analysis of p53, PUMA, BAX, BIM and p21 expression in cells with indicated adriamycin and GSK343 combination treatment. Data are shown as mean ± SD. * indicates $p < 0.05$. (B) Immunoblot analysis of the indicated proteins in lysates from MDA-MB-231 cells treated with indicated adriamycin and GSK343 combination.

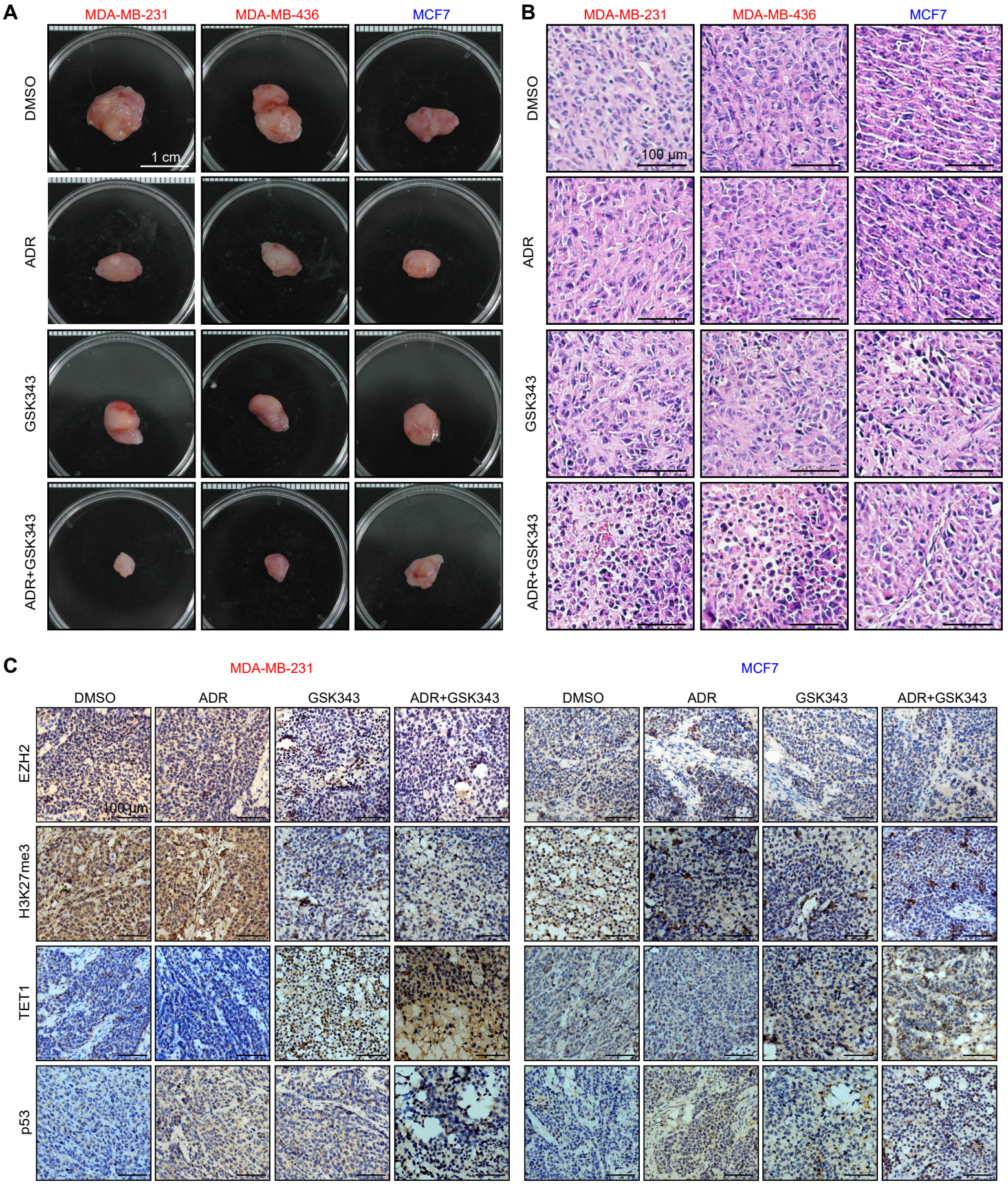

Figure S7. Adriamycin and GSK343 combination treatment represses TNBC xenograft tumor progression

(A) Representative photos of xenograft tumor of indicated cells subjected to GSK343, Adriamycin, GSK343+Adriamycin combination or DMSO. (B) H&E staining of tumor sections as in *A*. (C) IHC staining showing EZH2, H3K27me3, TET1 and p53 expression level in MDA-MB-231 and MCF7 cells as in *A*.

Table S1. Antibody list

Antibody	Application	Company	Catalog
EZH2	WB 1:1000; ChIP 1:100; IHC 1:200	Cell signaling technology	5246S
KDM6B	IHC 1:200	Abcam	ab38113
TET1	WB 1:1000; ChIP 1:50; IHC 1:200	Novusbiologicals	NBP2-15135
α-Tubulin	WB 1:2000	Sigma	T5168
total H3	WB 1:2000	Cell signaling technology	4499S
H3K27me3	WB 1:1000; ChIP 1:100; IHC 1:200	Cell signaling technology	9733S
H3K9me3	WB 1:1000	Abcam	ab8898
PCNA	WB 1:1000	Cell signaling technology	2586S
Cyclin A	WB 1:1000	Sigma	C4710
HP1γ	IF 1:500	Cell signaling technology	2619
BrdU-FITC	Flow 1:20	Thermo Fisher Scientific	11-5071-42
total p53	WB 1:500; IHC 1:50	Santa Cruz Biotechnology	sc-126
p53 phospho-S15	WB 1:1000	Cell signaling technology	9284S
$p21^{CIP1}$	WB 1:200	Santa Cruz Biotechnology	sc-397
$p16^{INK4A}$	IHC 1:200	Proteintech	10883-1-AP
$p14^{ARF}$	IHC 1:100	Cell signaling technology	2407S
IgG	ChIP 1:100	Cell signaling technology	2729S
5hmC	Dot blot 1:2000	Genetex	GTX128454
5mC	Dot blot 1:2000	Genetex	GTX128455

Cleaved PARP	WB 1:1000	Cell signaling technology	5625S
Ki67	IHC 1:200	Thermo Fisher Scientific	MA5-14520
HRP-goat-anti-rabbit	WB 1:1000	GE Healthcare	RPN4301
HRP-sheep-anti-mouse	WB 1:2000	GE Healthcare	NA931V

Table S2. ChIP-qPCR primer list

	Forward/Reverse	Sequence
***TP53*-1**	Forward	5’-CGCATCCCGGATCAGATTTC-3’
	Reverse	5’-GCTTGATGGGAACGGGAAAC-3’
***TP53*-2**	Forward	5’-CGAGTCCCGCGGTAATTCTT-3’
	Reverse	5’-TGCAGAAGAGGTGCAAGACC-3’
***TP53*-3**	Forward	5’-TCCACCAATTCTGCCCTCAC-3’
	Reverse	5’-GCCGTAGATACTAACATTTTGGGG-3’
***TP53*-4**	Forward	5’-CAGGCGGATTACTTGCCCTT-3’
	Reverse	5’-AACCCCAATCCCATCAACCC-3’
***TP53*-5**	Forward	5’-GGGTAAGCTCCTGACTGAACT-3’
	Reverse	5’-GCGATTTTCCCGAGCTGAAA-3’
***TET1*-1**	Forward	5’-ATCTTTCCCAGAACAGCCCG-3’
	Reverse	5’-ACCATTCTACCCCCATTCTGC-3’
***TET1*-2**	Forward	5’- AAACTTAGCTCTTCCTGCCCT-3’
	Reverse	5’-TATTTGGCCCTGGACCTCAGT-3’
***TET1*-3**	Forward	5’-TGGTGTTGAGGGCGGTTTTC-3’
	Reverse	5’-AAGAAGGTGCCAGGTCAGAGA-3’
***TET1*-4**	Forward	5’-GCGTTTTGTCTCTCGCTCAAC-3’
	Reverse	5’-ACAGACCTCAGGGAGTGAAG-3’