

Supplementary Information

DNA damage, demethylation and anticancer activity of DNA methyltransferase (DNMT) inhibitors

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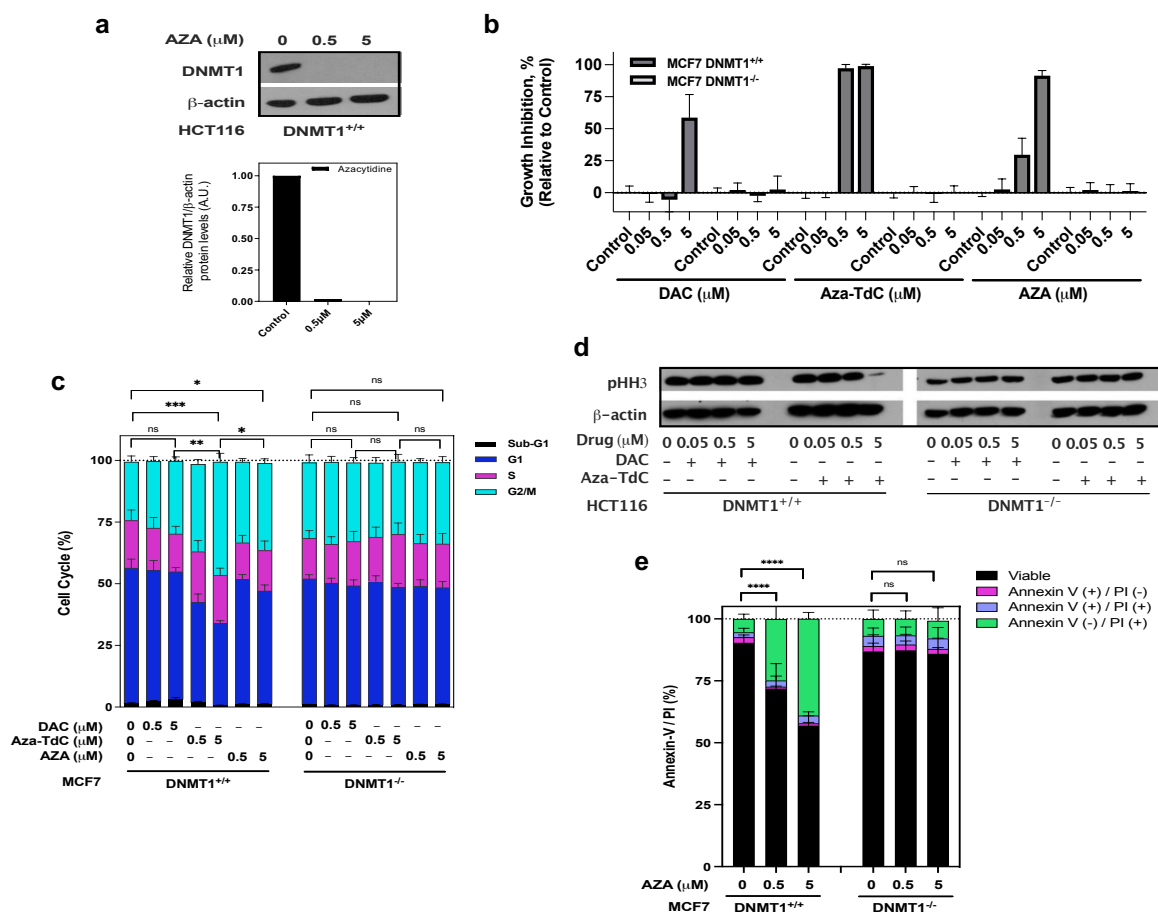
Supplementary Figure 7: Full length Western blot and agarose gel images in Figure 3.

Supplementary Figure 8: Full length Western blot images in Supplementary Figure 1.

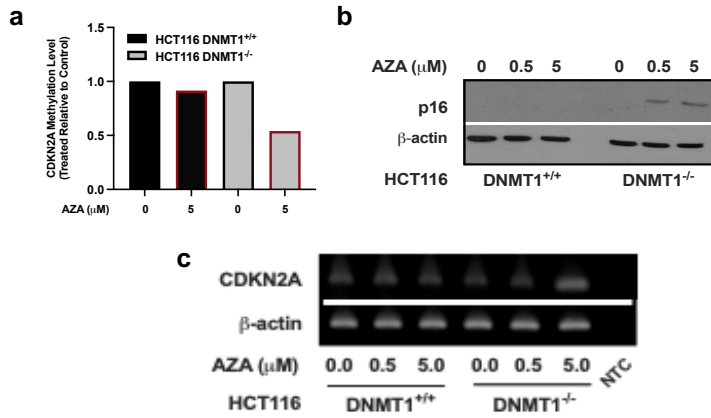
Supplementary Figure 9: Full length Western blot and agarose gel images in Supplementary Figure 2.

Table. Quantitative results of Western blots in Fig. 2f.

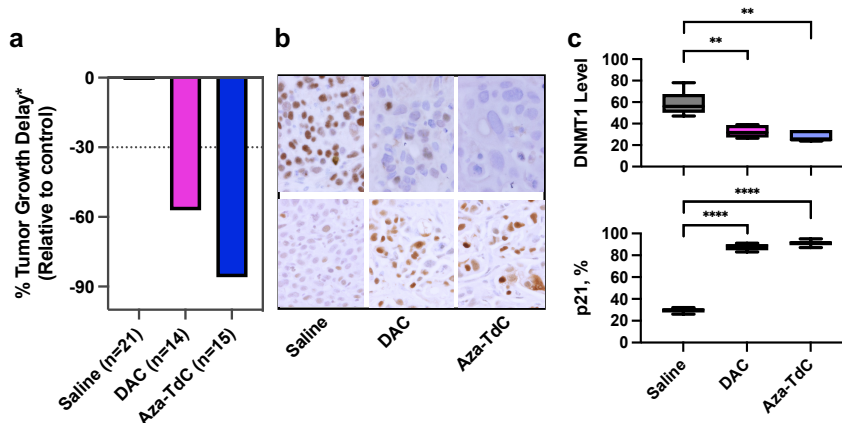
	HCT116 DNMT1 ^{+/+}						HCT116 DNMT1 ^{-/-}					
Drug concentration (μM)	0.00	0.50	5.00	0.00	0.50	5.00	0.00	0.50	5.00	0.00	0.50	5.00
DAC	-	+		-	-	-	-	+	+	-	-	-
Aza-TdC	-	-	-	-	+	+	-	-	-	-	+	+
Ratio of protein level to β-actin												
γH2AX	0.21	0.24	0.23	0.35	0.45	0.41	0.79	0.86	0.85	0.91	0.83	0.63
pCHK1-S345	0.20	0.29	0.30	0.09	0.33	0.31	0.58	0.68	0.73	0.40	0.42	0.42
p21	0.12	0.16	0.13	0.03	0.41	0.42	0.40	0.60	0.56	0.30	0.63	0.49
pCDC2-Y15	0.07	0.16	0.13	0.11	0.23	0.29	0.30	0.32	0.33	0.33	0.38	0.33



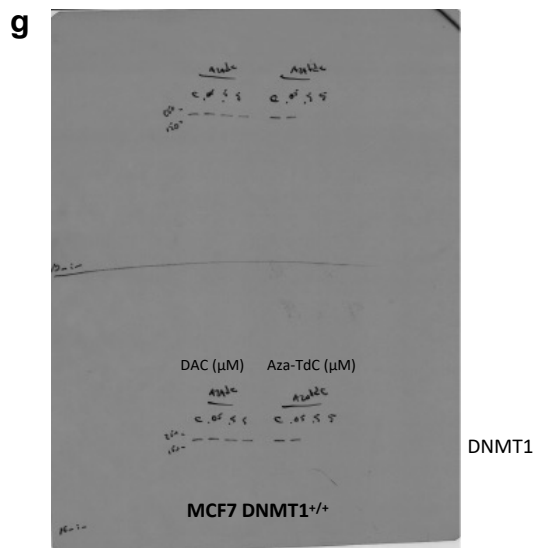
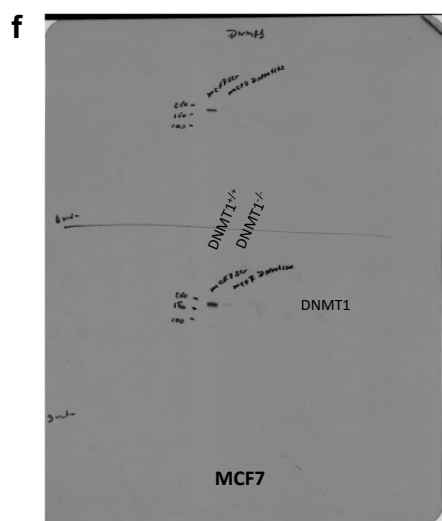
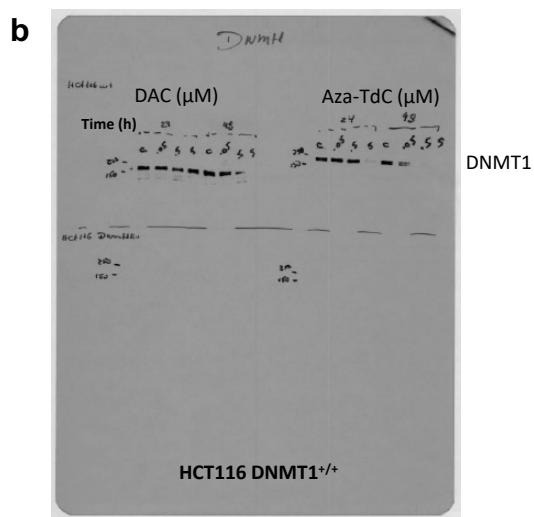
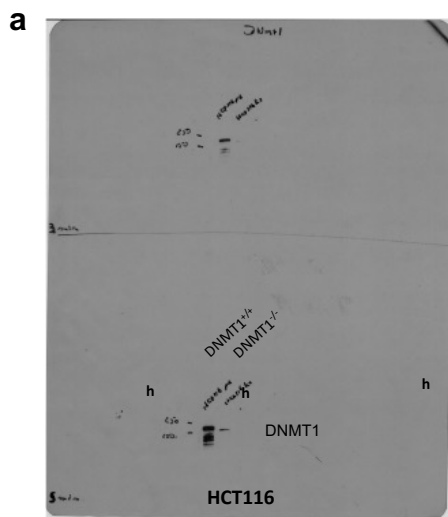
Supplementary Figure 1: Effects of DNMTi on DNMT1, growth, cell-cycle and apoptosis. **a** Inhibition of DNMT1 protein by AZA in HCT116 DNMT1^{+/+} cells. **b** Colony formation inhibition of the MCF7 pair by DAC, aza-TdC and AZA. **c** G₂/M cell-cycle arrest induced by DNMTi in MCF7 cells. **d** Histone H3 phosphorylation (pHH3-S10) after DAC and aza-TdC treatment for 24h in HCT116 pair. **e** Induction of apoptosis and cell death upon exposure to AZA for 96h via Annexin-V/PI apoptosis assay in the MCF7 pair. All samples in the clonogenic, cell-cycle and annexin-V/PI apoptosis tests were assayed in triplicate, with at least three independent experiments performed, and data were shown as mean \pm SD. * P <0.05; ** P <0.01; *** P <0.001; **** P <0.0001; and ns, not significant. PI, propidium iodide. One representative experiment is shown from at least three independent experiments performed for other assays.



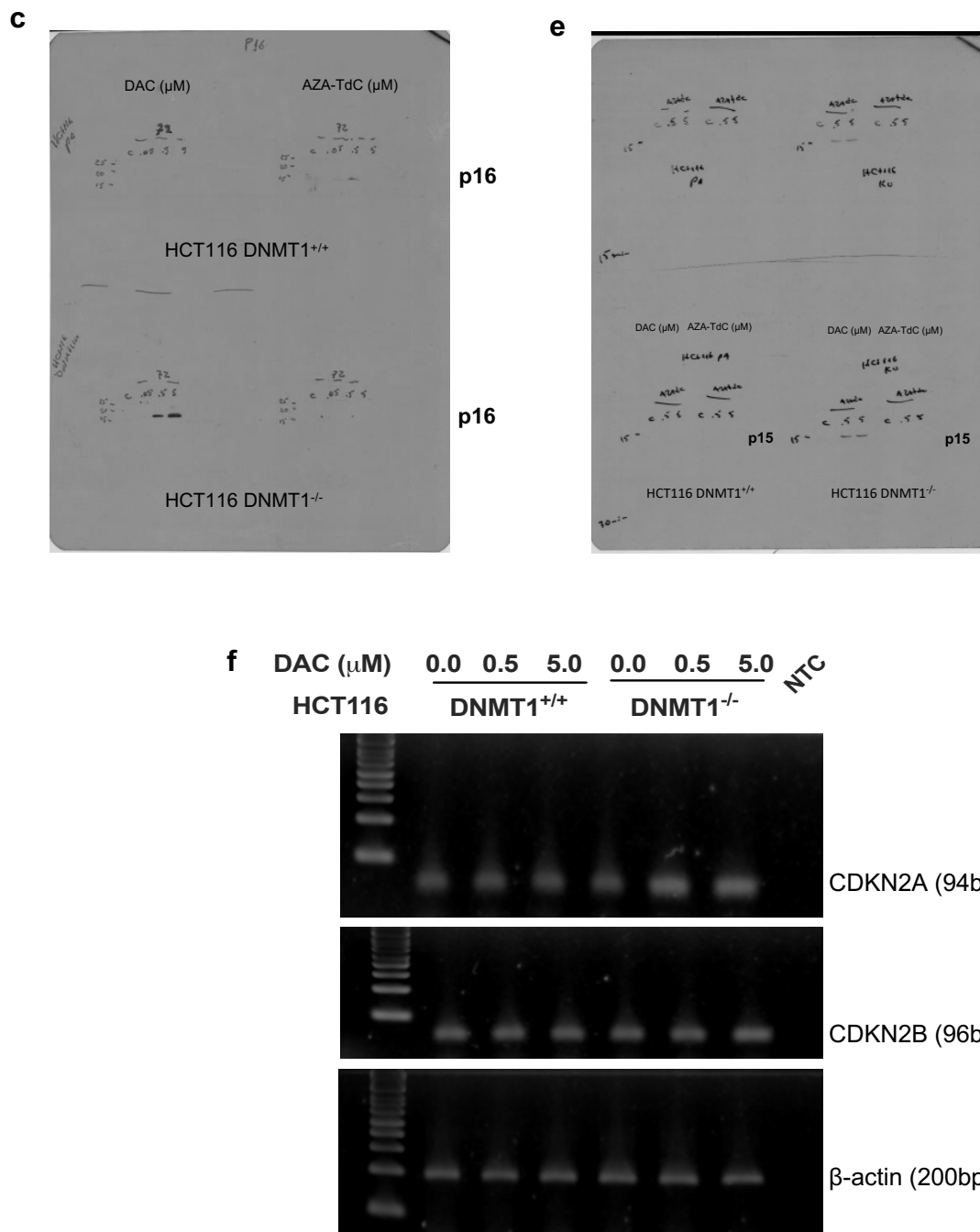
Supplementary Figure 2: Demethylation of *CDKN2A* gene and acetylation of histone H3, and re-expression of p16^{INK4A} by AZA in HCT116 cells. **a** Methylation levels of *CDKN2A* gene promoter after exposure to AZA. **b** p16^{INK4A} protein expression in DNMT1^{-/-} versus DNMT1^{+/+} cells by AZA. **c** Chromatin immunoprecipitation showing modulation of acetylation levels of histone H3 at *CDKN2A* gene locus by AZA in DNMT1^{-/-} versus DNMT1^{+/+} models. NTC, no template control.



Supplementary Figure 3: Tumor growth delay and modulation of DNMT1 and p21 by treatment in bladder PDX tumors. **a** Tumor growth delay (see Methods) by DAC and aza-TdC treatment. Dotted line indicated 30% of tumor growth delay. *The maximal tumor growth delays in DNMT1^{+/+} PDX tumors were observed at day 25 and day 56 of decitabine and aza-TdC treatment, respectively. **b** Representative expression of DNMT1 (upper panel) and p21 (lower panel) from the tumor samples treated with DAC and aza-TdC. Magnification, X200. **c** Modulation of DNMT1 and p21 by treatment (n=4 per group). ** $P < 0.01$, **** $P < 0.0001$. Note: the solid band in the Box-Whisker plot is mean, top and bottom borders of the plot represent 25% of data greater or less than that value, and the whiskers are maximal and minimal values.

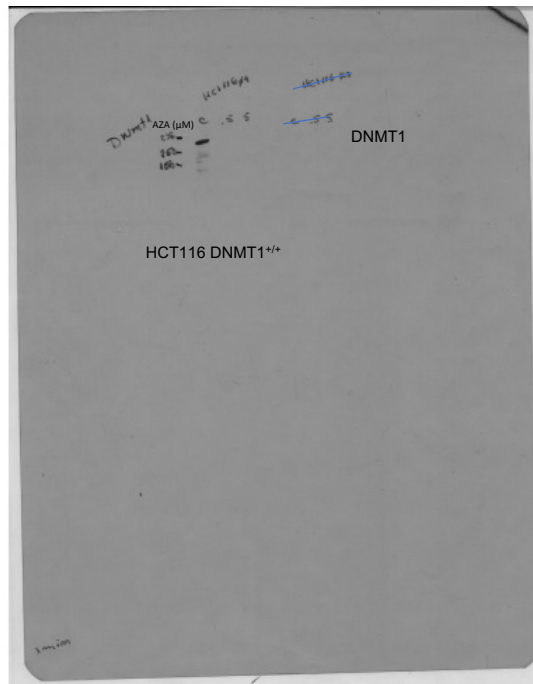


Supplementary Figure 5: Full length Western blot images in Figure 1. **a** Full length blot images in Figure 1a. **b** Full length blot images in Figure 1b. **f** Full length blot images in Figure 1f. **g** Full length blot images in Figure 1g.

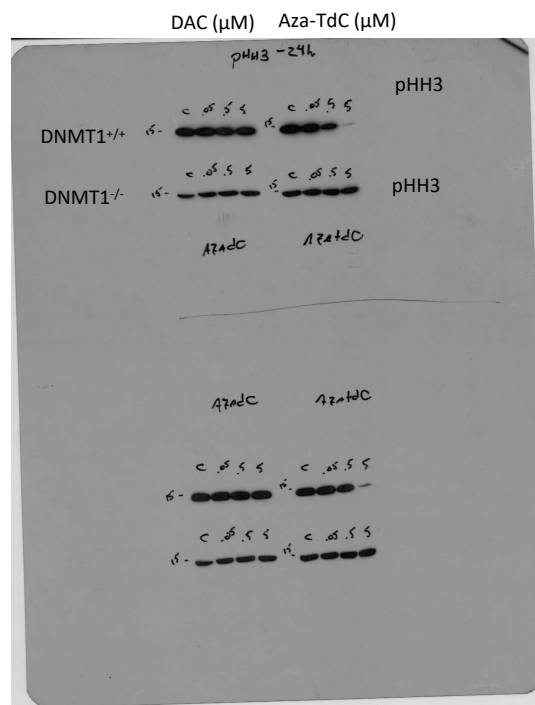


Supplementary Figure 7: Full length Western blot and agarose gel images in Figure 3. **c** Full length Western blot images in Figure 3c. **e** Full length Western blot images in Figure 3e. **f** Unprocessed agarose gel images in Figure 3f.

a

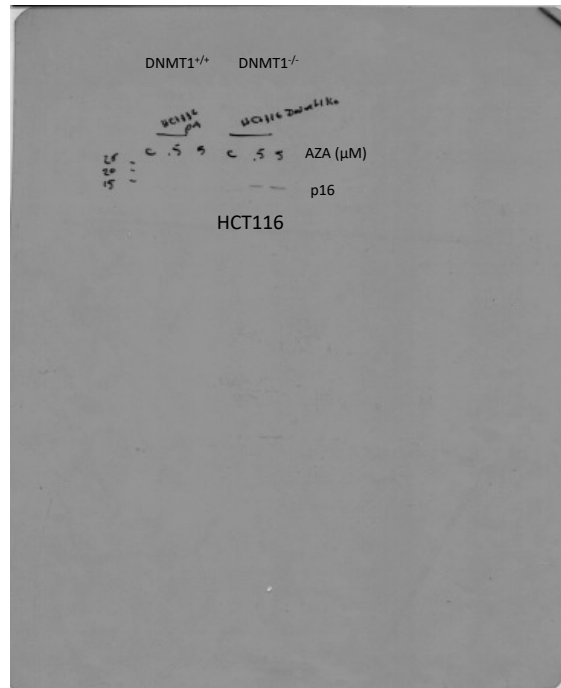


d

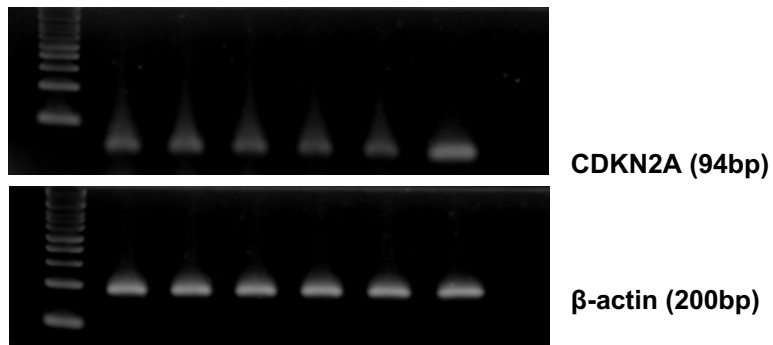


Supplementary Figure 8: Full length Western blot images in Supplementary Figure 1. **a** Full length Western blot images in Supplementary Figure 1a. **d** Full length Western blot images in Supplementary Figure 1d.

b



c DAC (μM) 0.0 0.5 5.0 0.0 0.5 5.0 NTC
HCT116 DNMT1^{+/+} DNMT1^{-/-}



Supplementary Figure 9: Full length Western blot and agarose gel images in Supplementary Figure 2. **b** Full length Western blot images in Supplementary Figure 2b. **c** Original agarose gel images in Supplementary Figure 2c.