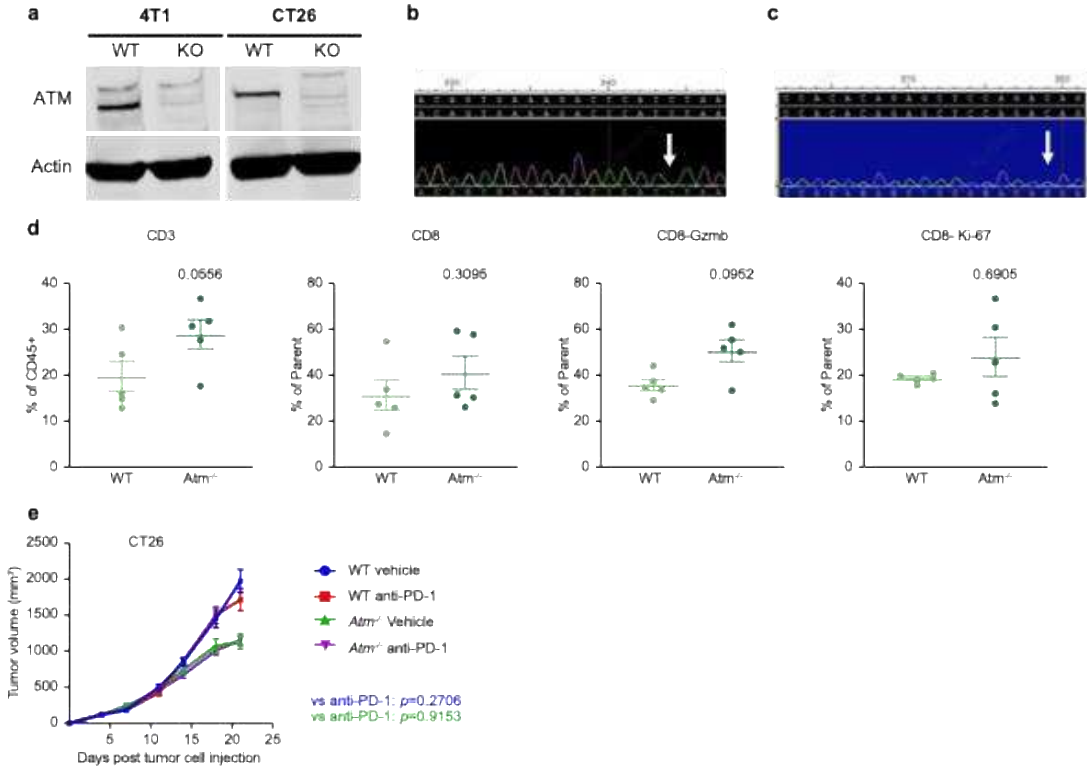
Supplementary Figure 1



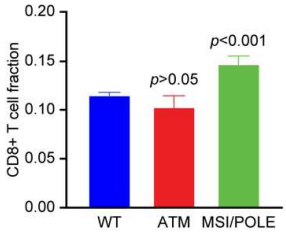
Supplementary Figure 1: ***Atm*** **model validation**. **A**) Western blot showing loss of ATM protein in the *Atm*$^{-/-}$ models from 4T1 and CT26. **B**) PCR Trace for *Atm*$^{-/-}$ 4T1 Clone T3-23. **C**) PCR Trace for *Atm*$^{-/-}$ CT26 Clone5. **D**) Flow cytometry analysis showing no difference in T-cell infiltrate in *Atm*$^{-/-}$ CT26 tumors sacrificed at 16 days (n=5 mice per group, Data represented as Mean ± SEM, two-tailed unpaired Student's t-test). **E**) Tumor growth curve in CT26 model showing no response to anti-PD-1 (n=8 mice per group, Data represented as Mean ± SEM, 2way ANOVA, Sidak's multiple comparisons test).

Supplementary Figure 2

a

ATM case	Sample type	Gene	Bi-allelic	cDNA (AA) change	HR/HER2 status
ATM1	Primary	ATM(exon35)	Yes (LOH)	c.5290delC(p.Leu1764Tyrfs*12)	HR+/HER2-
ATM2	Primary	ATM(exon53)	Yes (LOH)	c.7913G>A(p.Trp2638*)	HR+/HER2-
ATM3	Primary	ATM(exon8)	Yes (LOH)	c.1027_1030delGAAA(p.Glu343Ilefs*2)	HR+/HER2+
ATM4	Primary	ATM(exon18)	Yes (LOH)	c.2780T>A(p.Leu927*)	HR+/HER2-
ATM5	Primary	ATM(exon48)	Yes (LOH)	c.7010_7011delGT(p.Cys2337Serfs*35)	HR+/HER2-
ATM6	Primary	ATM(exon49)	Yes (LOH)	c.7271T>G(p.Val2424Gly)	HR+/HER2-
ATM7	Primary	ATM(exon5)	Yes (LOH)	c.381delA(p.Val128*)	HR+/HER2-
ATM8	Primary	ATM(exon8)	Yes (LOH)	c.1027_1030delGAAA(p.Glu343Ilefs*2)	HR+/HER2+
ATM9	Metastasis (Bone)	ATM(exon26)	Yes (LOH)	c.3802delG(p.Val1268*)	HR+/HER2+
ATM10	Metastasis (Liver)	ATM(26, 11q22.3)	Yes (LOH)	-	HR+/HER2-
ATM11	Metastasis (Bone)	ATM(exon53)	Yes (LOH)	c.7913G>A(p.Trp2638*)	HR+/HER2+

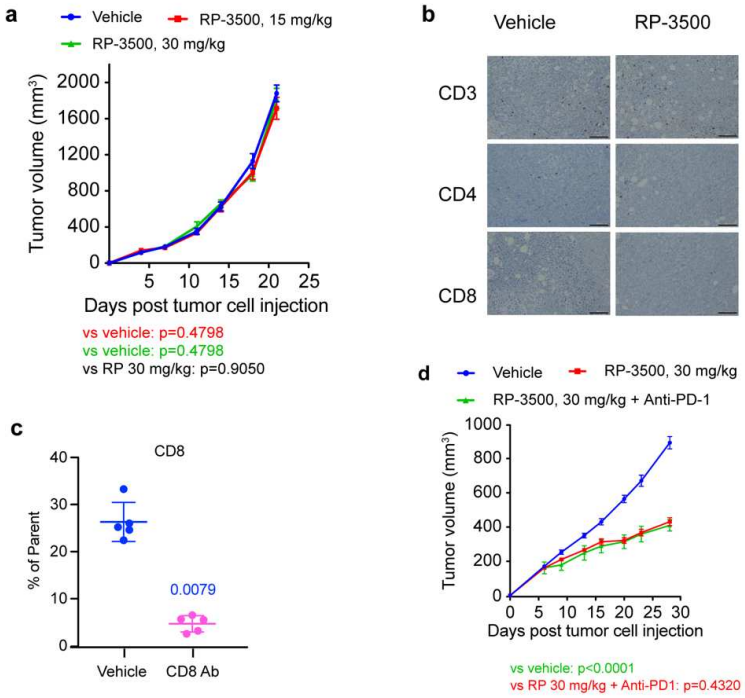
b



Supplementary Figure 2

Supplementary Figure 2: **A)** Details on breast cancer patient cases selected for IHC of T cell markers in the tumor microenvironment. **B**) Evaluation CD8 T cell infiltration by genotype in colorectal cancers reveals no increase in ATM mutated tumors, but higher levels in MSI/POLE altered tumors ($p < 0.001$; Wilcoxon-test).

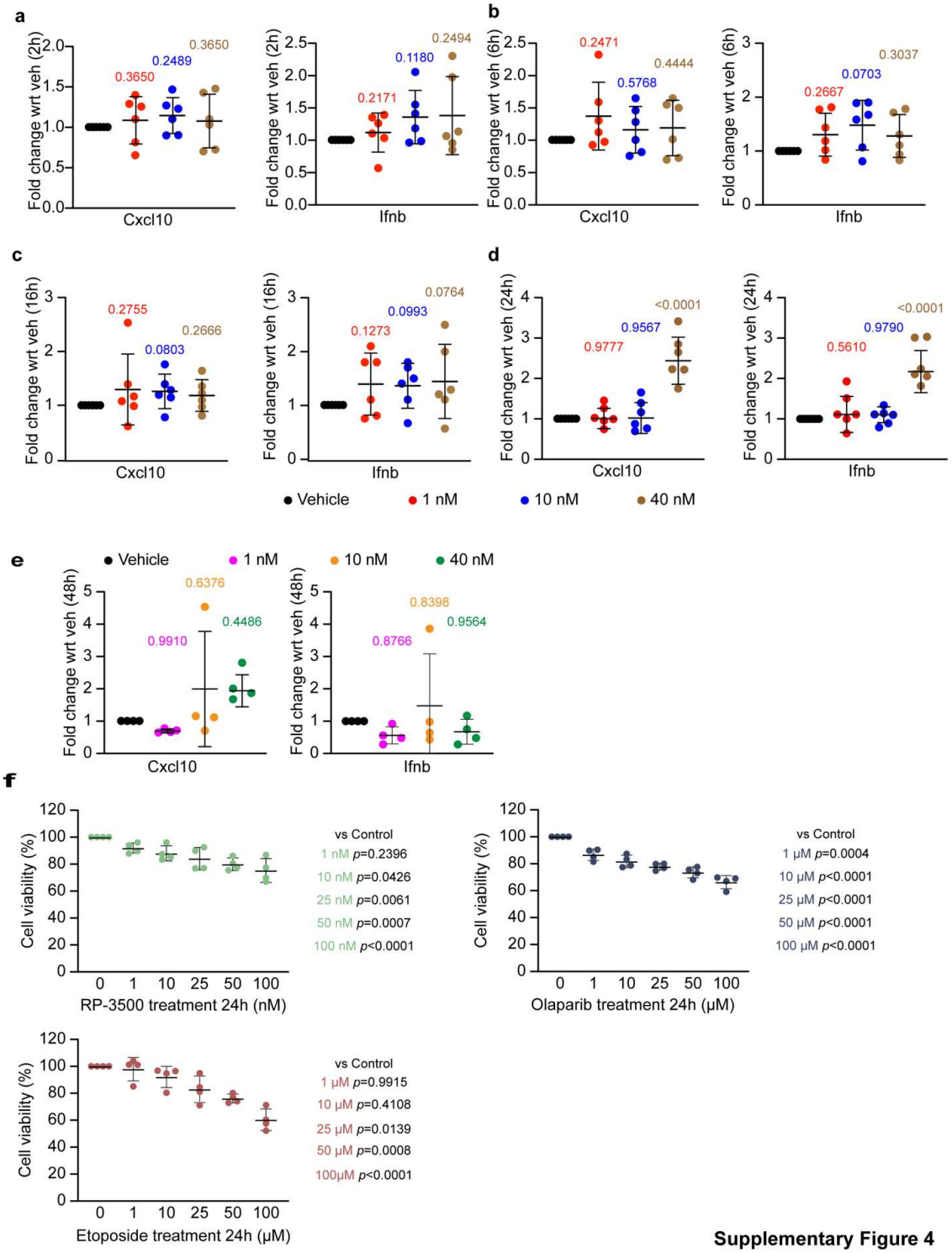
Supplementary Figure 3



Supplementary figure 3

Supplementary Figure 3: **A**) WT 4T1 tumors are not sensitive RP-3500 *in vivo*. **B**) Representative micrographs of T-cell infiltrate corresponding to Figure 3. **C)** Flow cytometry analysis showing significant depletion of CD8+ T-cells in splenic lymphocytes of CD8 Ab treated mice sacrificed at 21 days (n=5 mice per group, Data represented as Mean ± SEM, two-tailed unpaired Student's t-test). **D**) Response to RP-3500 monotherapy or anti-PD-1 combination in another *Atm*$^{-/-}$ 4T1 cell line Clone T3-29 (n=10 mice per group, Data represented as Mean ± SEM, 2way ANOVA).

Supplementary Figure 4



Supplementary Figure 4

Supplementary Figure 4: A) Induction of interferon response in RP-3500 treatment in *Atm*$^{-/-}$ cells. (Data are represented as Mean ± SD, 2way ANOVA, Dunnett's multiple comparisons test). **B-E**). Extended time point for RP-3500 treatment in *Atm*$^{-/-}$ cells. (Data are represented as Mean ± SD, 2way ANOVA, Dunnett's multiple comparisons test). **F**) Cell viability of *Atm*$^{-/-}$ 4T1 treated cells with RP-3500 or Olaparib or Etoposide by CellTiterGlo assay (Data are represented as Mean ± SD, 2way ANOVA, Dunnett's multiple comparisons test).

Supplementary Table 1- Primer sequences for qPCR primers

	Forward	Reverse
Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Ccl5	GCTGCTTTGCCTACCTCTCC	TCGAGTGACAAACACGACTGC
Cxcl10	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTCAA
Ifnb	AGCTCCAAGAAAGGACGAACA	GCCCTGTAGTGTAGGTTGAT
Isg15	GGTGTCCGTGACTAACTCCAT	TGGAAAGGGTAAGACCGTCCT