# Supporting Information

## Stone et al. 10.1073/pnas.1712514114

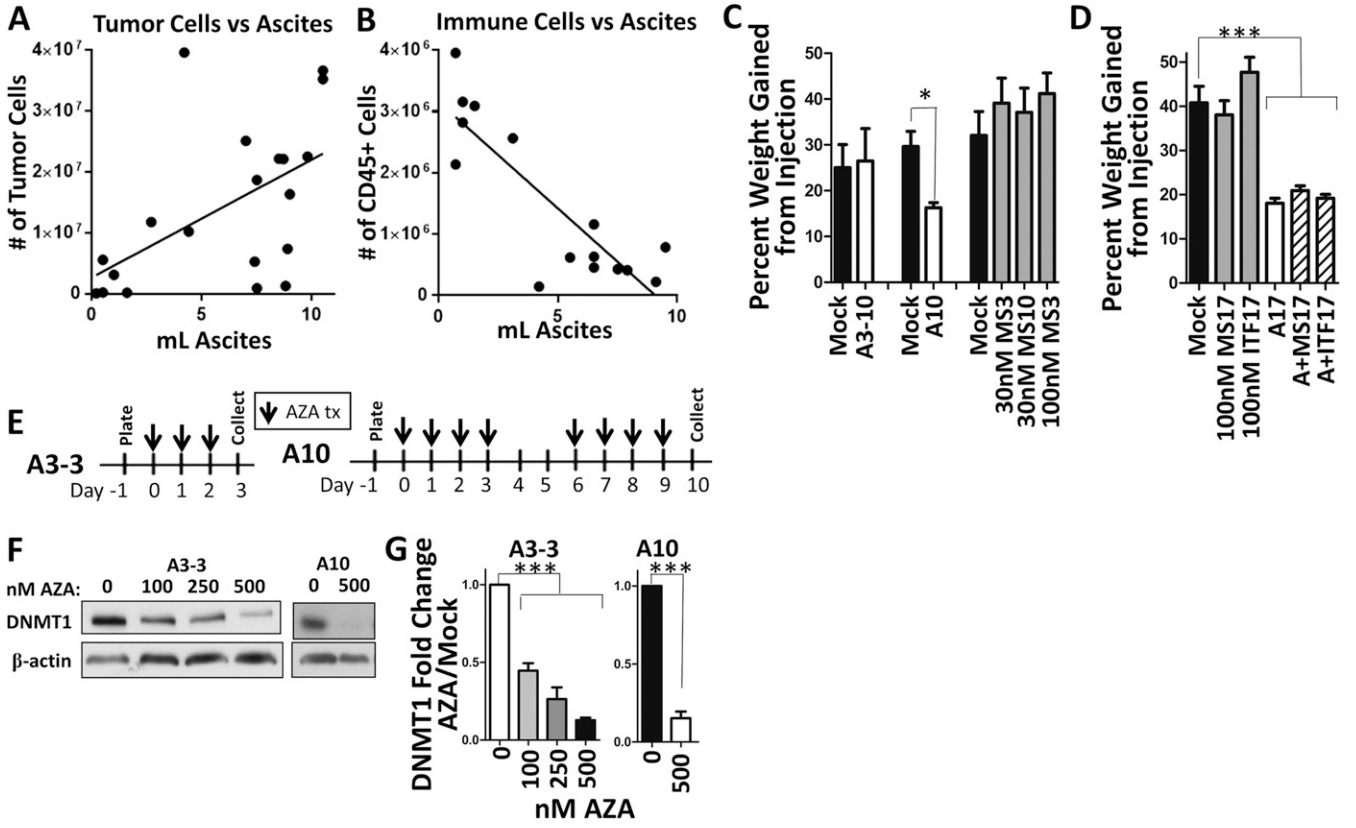



**Fig. S1.** Ascites volume is a reflection of tumor burden, weight gain in mice mirrors ascites development, and increasing doses of AZA lead to degradation of DNMT1. (*A* and *B*) Cells (250,000) were injected into mice, and ascites was drained 5 to 5.5 wk later. (*A*) GFP$^+$ tumor cells were counted by flow cytometry after removing immune cells separated by a Percoll gradient; $n = 20$ mice. The slope is significantly nonzero ($P = 0.0126$). (*B*) Cells from the ascites fluid were stained by flow cytometry to identify live CD45$^+$ cells; $n = 15$ mice. The slope is significantly nonzero ($P < 0.0001$). (*C* and *D*) Percent weight gained by the mice in Fig. 1 *B* and *C*. A10, MS3, MS10: $n = 7$ to 30 mice, three biological replicates; A3-10: $n = 9$ mice, two biological replicates; MS17, ITF17, A17, A+MS17, and A+ITF17: $n =$ 9 or 10 mice, one biological replicate. Statistical outliers were removed using Peirce's criterion, and significance was determined by Mann–Whitney *t* test. (*E*) Treatment schematic for collection of cells treated with 500 nM AZA (A) at day 3 (A3) or day 10 (A10). (*F*) Representative Western blot of DNMT1 levels at day 3 or 10. (*G*) Quantification of DNMT1 Western blots; $n = 3$. $^*P < 0.05$, $^{***}P < 0.001$.

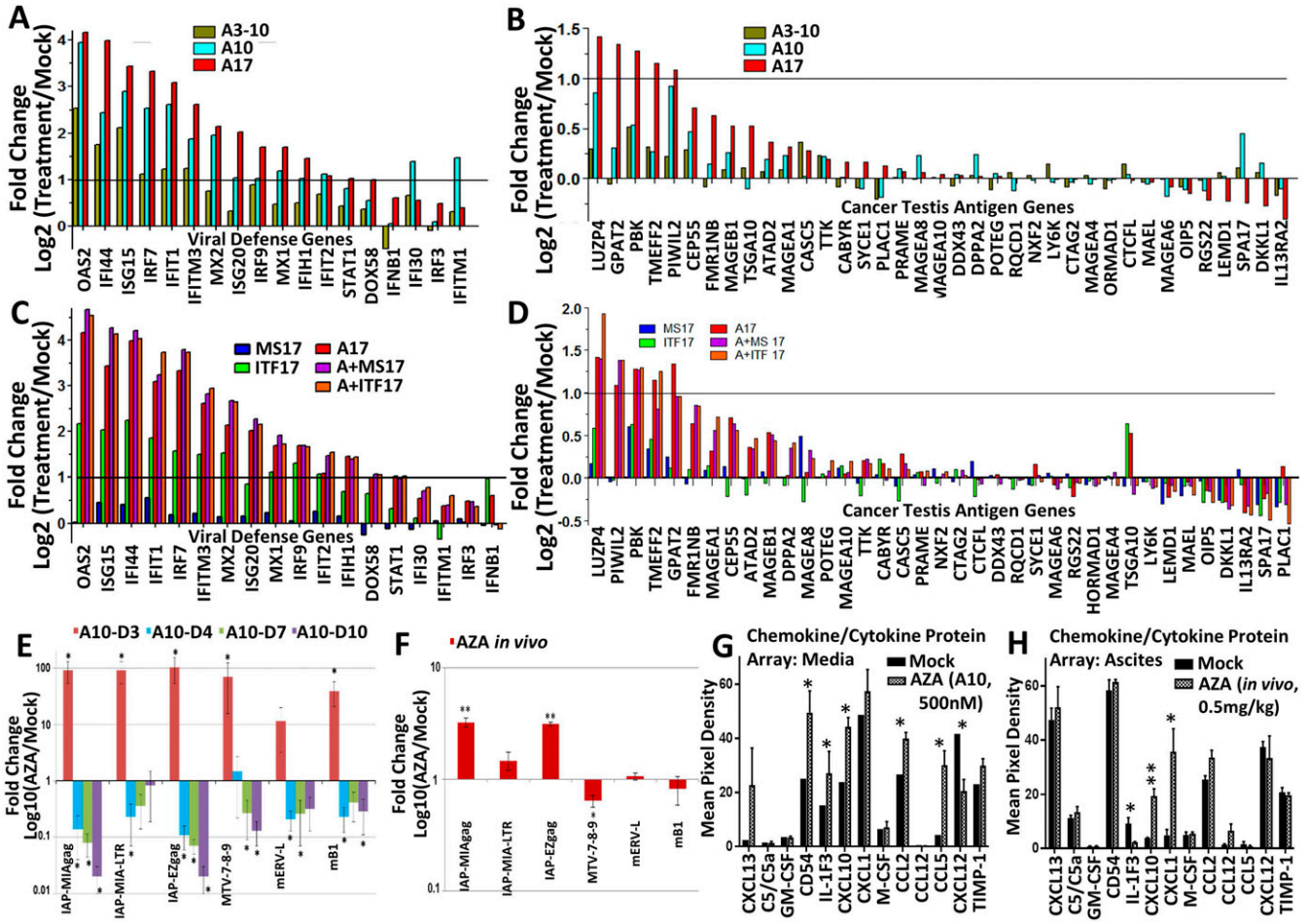



**Fig. S2.** Treatment of tumor epithelial cells with AZA leads to increased expression of viral defense genes, endogenous retroviral transcripts, and chemokine/cytokine proteins. (*A–D*) ID8-VEGF-Defensin cells were treated with A3-10, A10, A17, HDACi17, and A+HDACi17 as shown in Fig. 1*A*. (*A–D*) Expression of viral defense genes (*A* and *C*) and cancer testis antigen genes (*B* and *D*) is shown. The horizontal line at log-twofold change =1 indicates a twofold increase in expression. (*E* and *F*) Mean fold increase of mERV and B1 gene expression levels compared with mock-treated; q-PCR at days 3, 4, 7, and 10 of an A10 treatment schedule; $n = 3$. Mean ± SEM. (*G* and *H*) Protein levels of chemokines and cytokines assessed using the Proteome Profiles Mouse Cytokine Array Kit from R&D Systems; $n = 3$. (*G*) Cells were treated with schedule A10, and media were collected. (*H*) Ascites from mock- or AZA-treated mice was collected at week 4.5 after injection of tumor cells (Fig. S6). $^*P < 0.05$, $^{**}P < 0.01$.

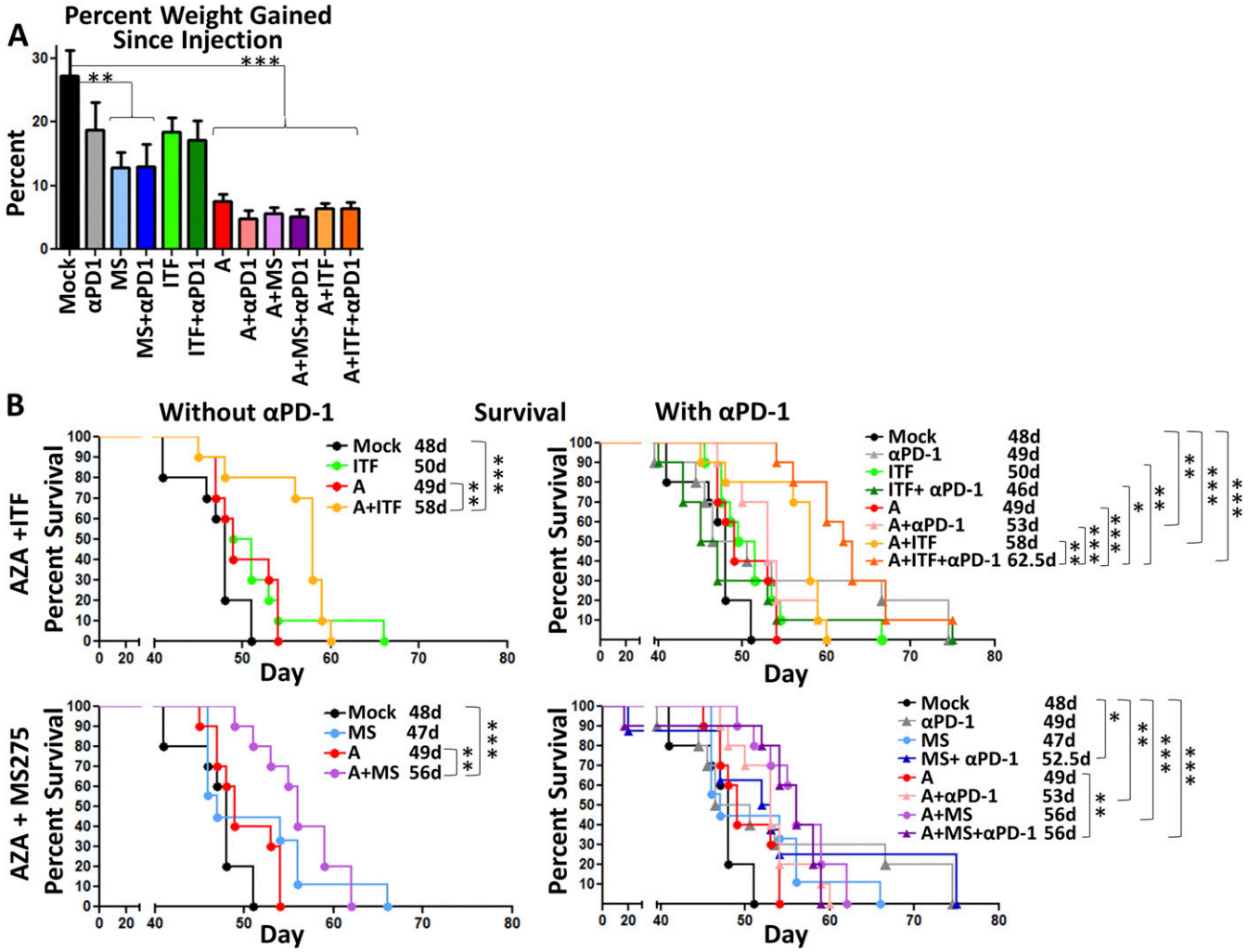



**Fig. S3.** Addition of immune checkpoint inhibition to epigenetic therapy in an intact mouse model increases survival. Mice were treated as described in Fig. 3. (*A*) Percentage of weight gained by the mice in Fig. 3 at week 5 mimics ascites volume; $n = 8$ to 10 mice per group. (*B*) Survival data for all 12 arms of the experiment; $n = 10$ mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

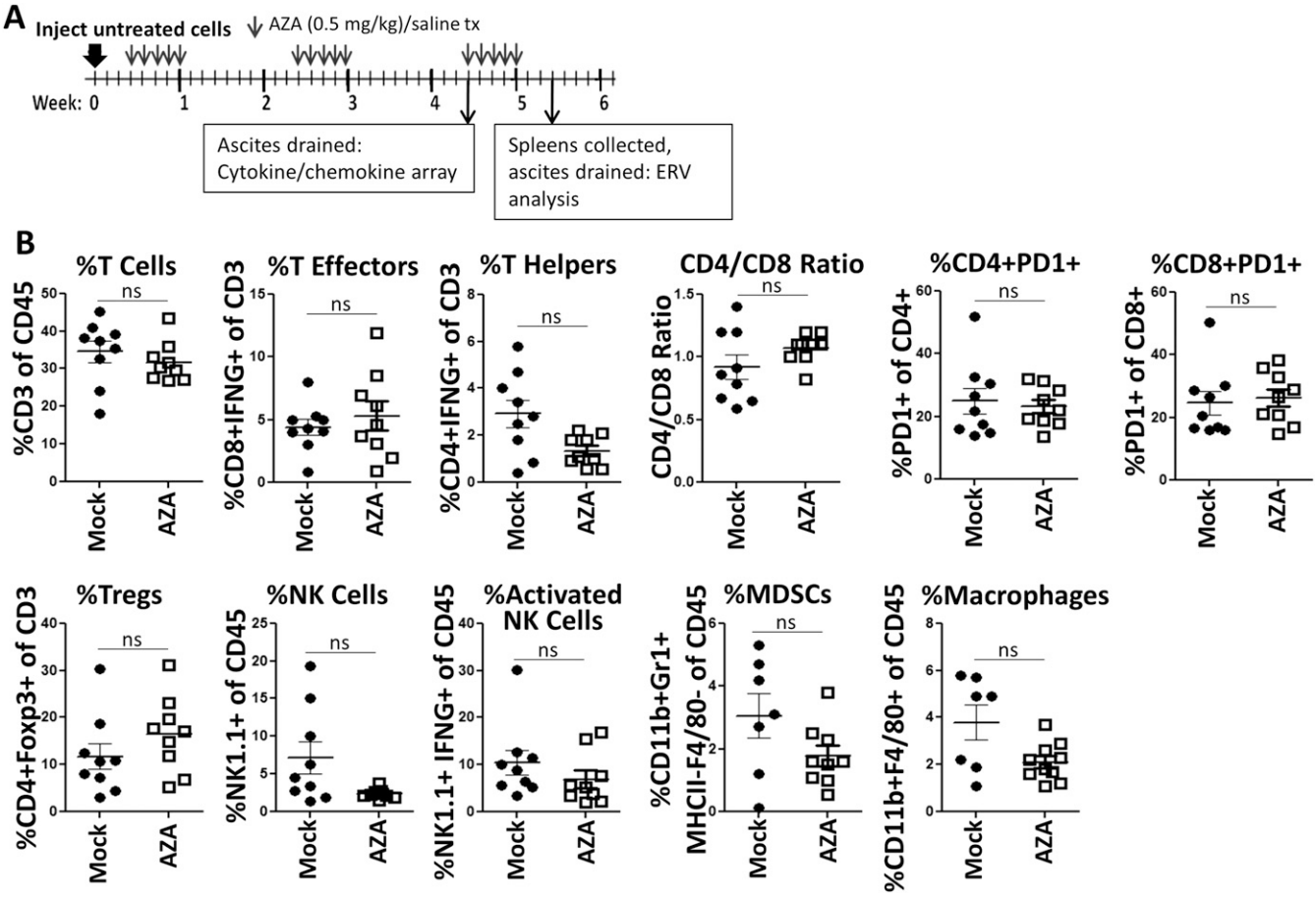



**Fig. S4.** In vivo treatment of mice with AZA does not significantly alter the immune cell populations in the spleen. (*A*) Treatment schematic for mice treated only with mock or AZA. Cells ($2.5 \times 10^6$) were injected i.p. into 8- to 10-wk-old female C57BL/6 mice and treated on the days indicated with an arrow in the schematic (same schedule as Fig. 3). (*B*) Spleens were collected from mock- or AZA-treated tumor-bearing mice at week 5.5. Spleens were filtered and washed to a single-cell suspension, and the cells were analyzed via FACS; $n = 7$ to 9 mice per group.

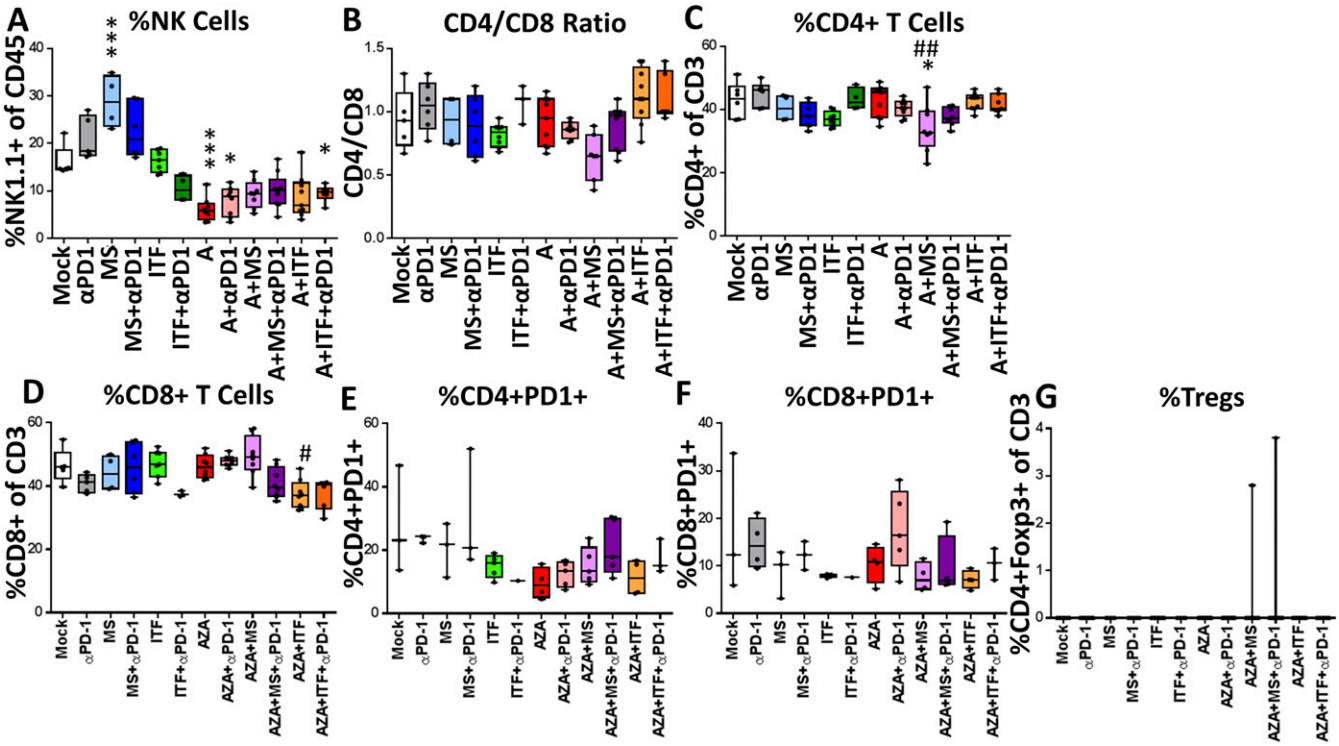
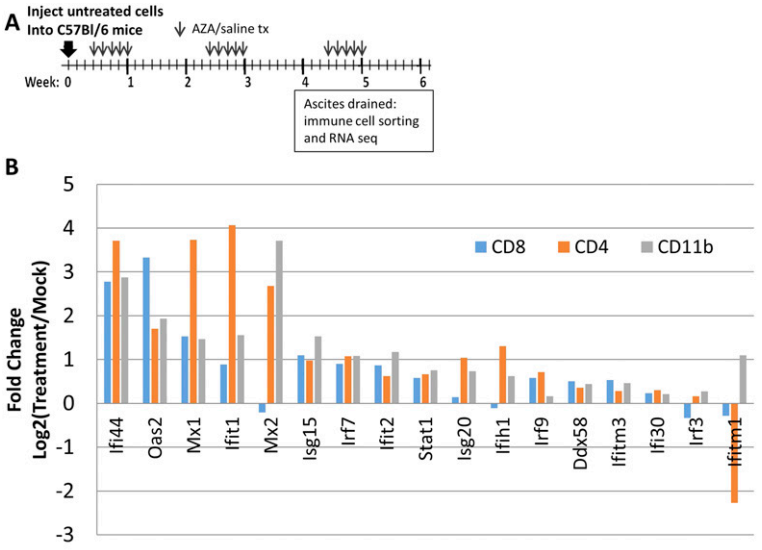



**Fig. S5.** Epigenetic therapy and immune checkpoint inhibition do not have significant effects on some subsets of immune cells. Ascites fluid was drained from the mice in Fig. 3 at week 6.5, and cells were analyzed via FACS. (*A*) % NK cells (NK1.1$^+$) of CD45$^+$ cells. (*B*) CD4$^+$/CD8$^+$ cell ratio. (*C*) % CD4$^+$ of all T cells. (*D*) % CD8$^+$ of all T cells. (*E*) % CD4$^+$PD1$^+$ T cells of CD4$^+$ T cells. (*F*) % CD8$^+$PD1$^+$ T cells of CD8$^+$ T cells. (*G*) % T regulatory cells (CD4$^+$, FoxP3$^+$) of all CD3$^+$ T cells. $n = 4$ to 9 mice per group. $^*P < 0.05$, $^{***}P < 0.001$, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.




**RNA sequencing of sorted immune cell populations from ascites fluid showed up-regulation of viral defense genes upon AZA treatment in tumor bearing mice.**

**Fig. S6.** RNA sequencing of murine immune cells sorted from ascites of mice treated with AZA showed up-regulation of viral defense genes. (*A*) Treatment schematic. (*B*) Viral defense gene expression.

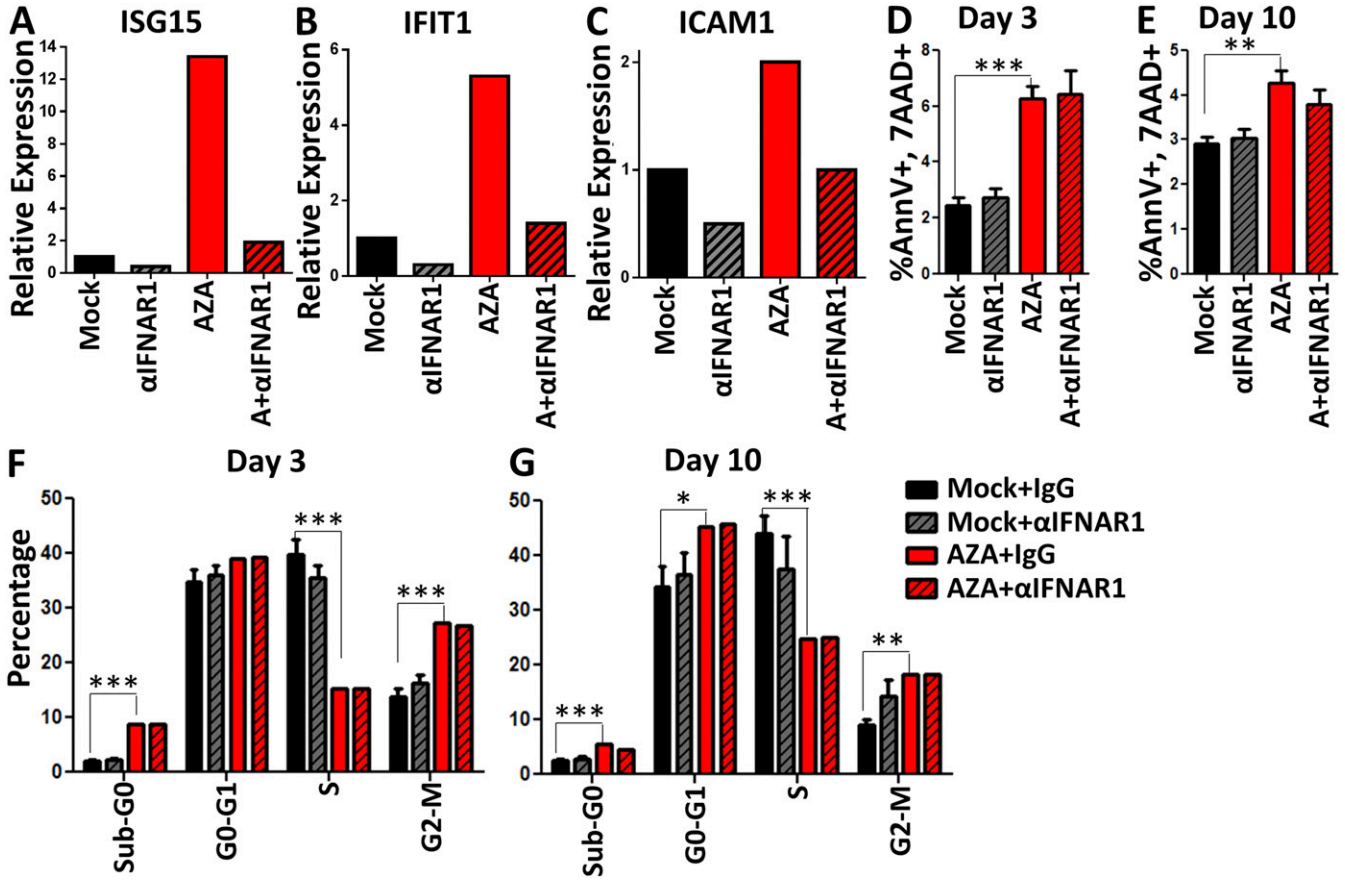



**Fig. S7.** Relative expression of IFN-stimulated genes in cells treated with AZA and anti-IFNAR1 in vitro. (*A–C*) AZA was given at an A3 treatment schedule, and one dose of anti-IFNAR1 (10 μg/mL) was given at day 0. Cells were collected at day 3 for expression analysis via q-RT-PCR. (*D* and *E*) Percentage of annexin $V^+$ and 7-$AAD^+$ apoptotic cells, after an A3 or A10 schedule of 500 nM AZA; $n = 3$. (*F* and *G*) Cell-cycle analysis, determined by BrdU incorporation and 7-AAD staining of DNA content; $n = 3$. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

**Table S1. Mouse embryonic day 16.5 placenta was used as a positive internal control**

| Gene | Primer sequence, 5′-3′ | Accession/ref. no. |
|---|---|---|
| mERVL gag-pol | ACATACCCAGTAATGGTCAGCAC<br>ATTGGTTAGCCAGTACCAAAGGT | 2065208 |
| mErv3* | CATAGCCTCTACCTTCTGTCTGGT<br>AGAGGTCATAGCATTGTAGGGTTC | 261245003 |
| syncytin-A | GATGACATCCACTGCCACAC<br>ATTGTCCGGCTCGAATAGG | AY849973.1 |
| Peg11 (Mart1) (Rtl1) | GAAACAATCAACTCATCCGAGAC<br>AGAGTTCTTGGGCTGACCTTC | NM_184109.1 |
| mMart8 (Cxx1c) | AAGGGCCGGGCCCTGCAGTG<br>CTAGAAGTCCTCATCCTCCTCCCACCCG | 115270961 |
| IAP-MIA14 LTR* | Gacacgtcctaggcgaaatataac<br>Tattgcttacatcttcaggagcaag | M17551.1 |
| IAP-MIA14 gag* | GATCAATTAGCGGAGGTCTCTAG<br>CCAGTCTGTTTCTTCAGAGGAGAA | M17551.1 |
| IAPEZ gag* | Gctctccctagtatgggcaaatat<br>Aatctctctgctctggagtcaaag | AC003993 |
| mMTV 7/8/9* | CTACACTTAGGAGAGAAGCAGC<br>ATGTCTTTGTCTGATGGGCTCAT | M90535.1, X05400.1, M29600.1 |
| B1* (consensus) | GAGGCAGAGGCAGGCGGATT<br>GTTTCTCTGTGTAGCCCTGGC | (65) |
| mGapdh | AGAAACCTGCCAAGTATGATGAC<br>AGACAACCTGGTCCTCAGTGT | 126012538 |
| Mouse β-actin | TTCTTGGGTATGGAATCCTGTGG<br>TGGCATAGAGGTCTTTACGGATG | 145966868 |
| Mouse 18S rRNA | ATGGCCGTTCTTAGTTGGTG<br>GAACGCCACTTGTCCCTCTA | 120444899 |

*Semi-q-PCR; all other genes were done with full q-PCR according to refs. 55 and 56.

**Table S2. Top upstream transcriptional regulators in murine immune cells sorted from ascites of mice treated with AZA**

| $CD8^+$ | $CD4^+$ | $CD11b^+$ |
|---|---|---|
| Ifnb1 | Trim24 | Trim24 |
| Trp53 | Trp53 | Irf7 |
| Alkbh5 | Alkbh5 | Ifn alpha/beta |
| Dysf | Fzd9 | Irf3 |
| Ptger2 | Irf7 | Ptger4 |
| Ptger4 | Ifnar | Ifnar |
| Irgm1 | Irf3 | IL12 |
| Ifng | Dnase2a | Stat1 |
| Irf3 | Stat2 | Nr3c1 |
| Irf7 | Tcf7l2 | Ddx58 |

Ingenuity pathway analysis identified type I IFN pathway-associated genes as top upstream regulators of the transcriptional program in AZA-treated $CD4^+$, $CD8^+$, and $CD11b^+$ cells (Fig. S6*A*).