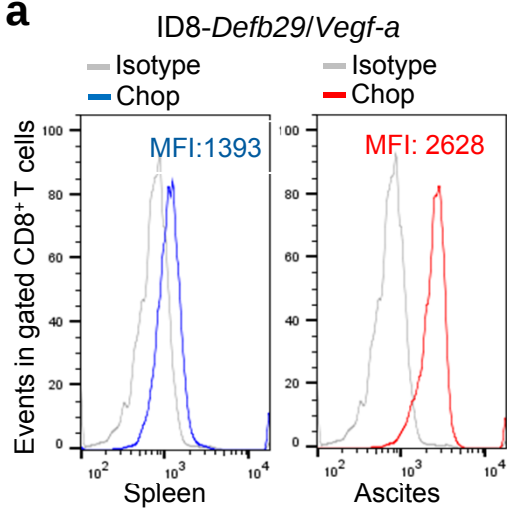
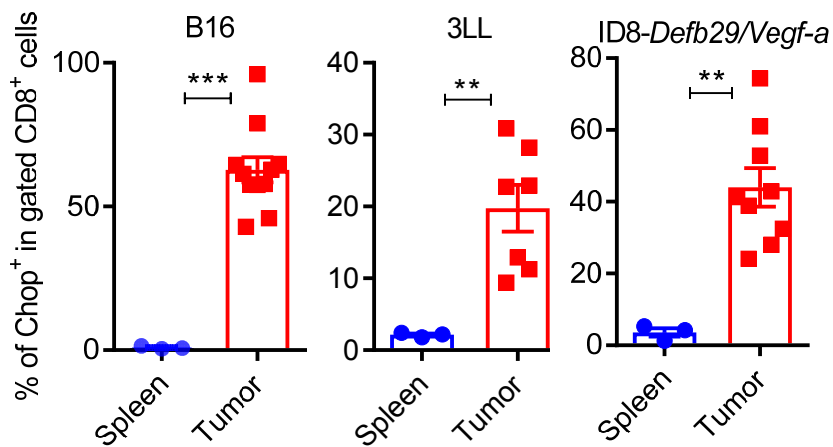
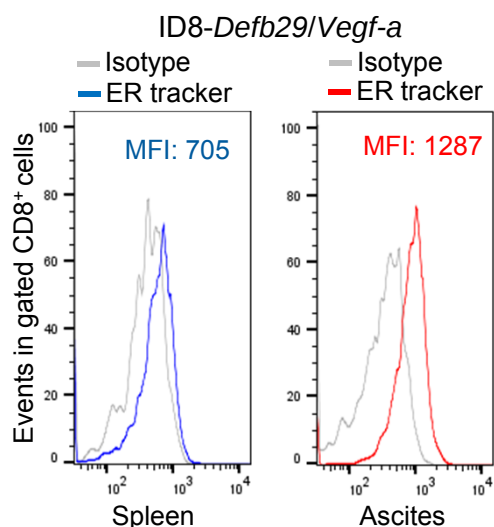
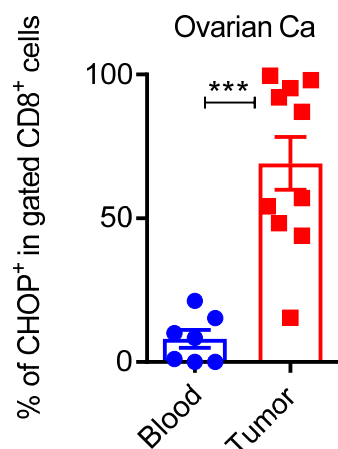
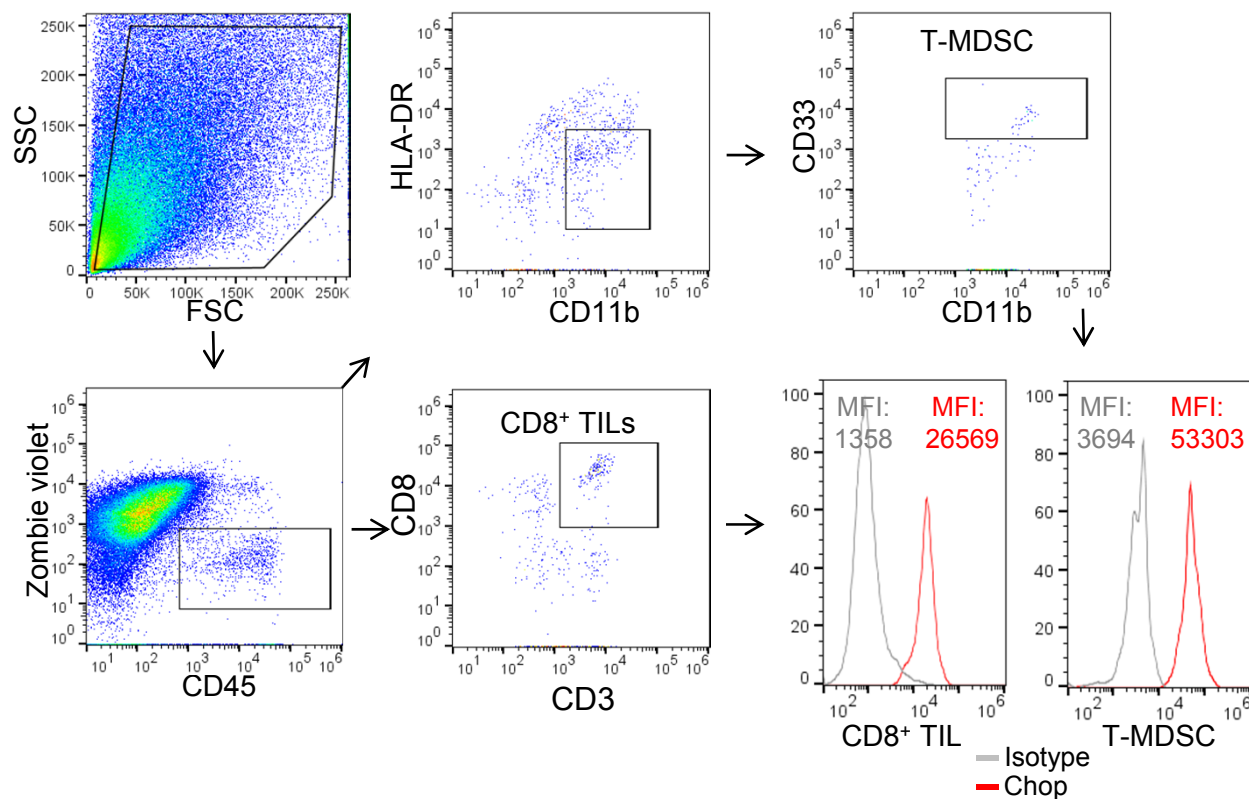


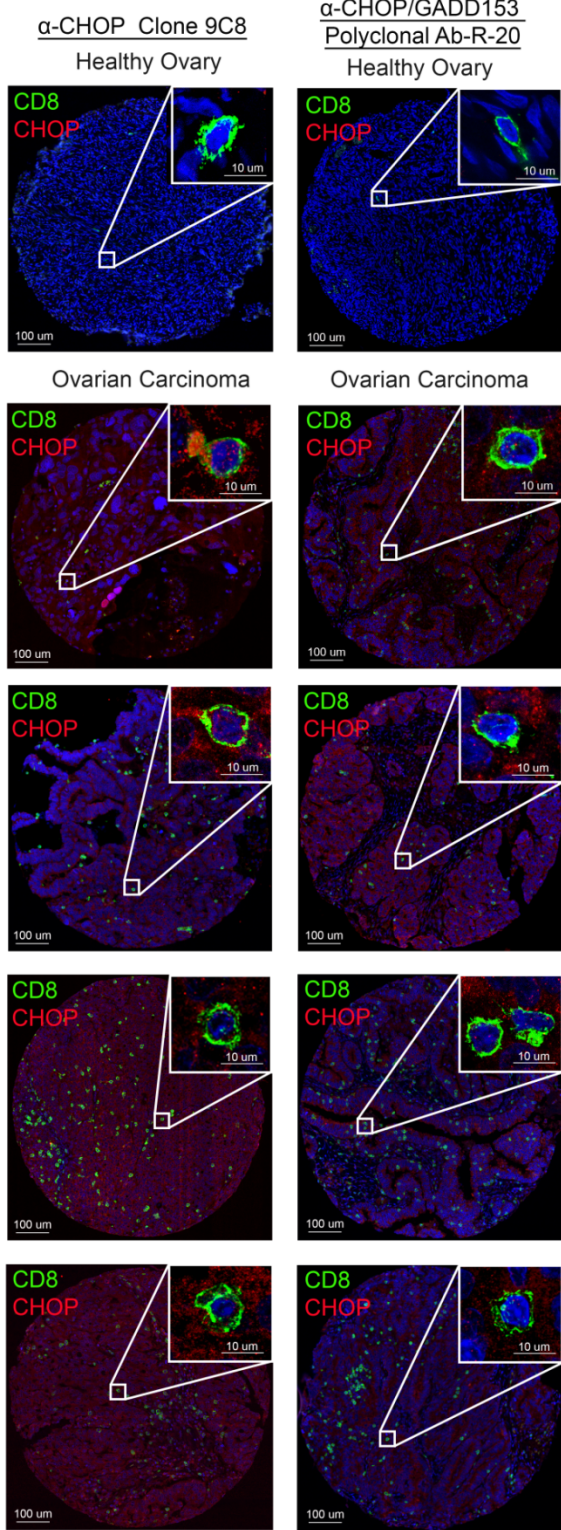
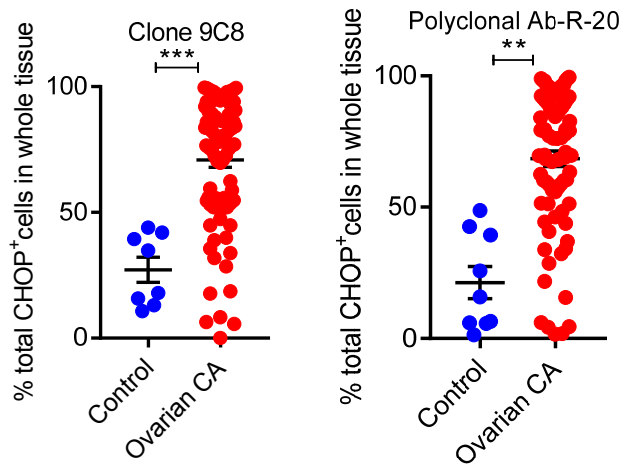
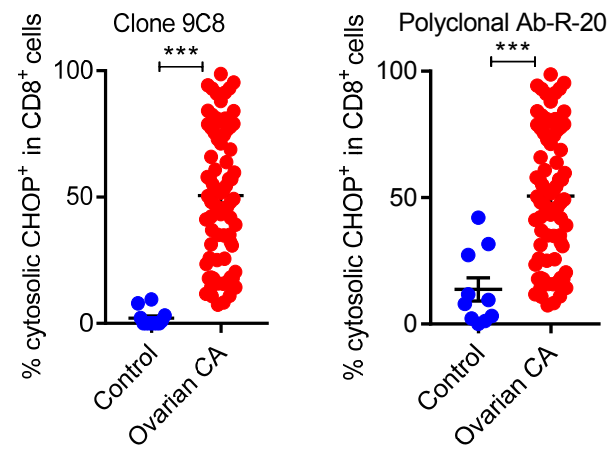
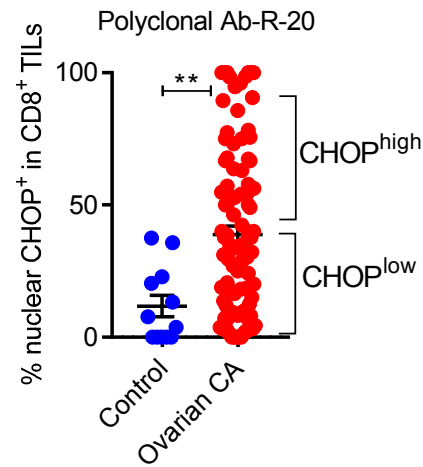
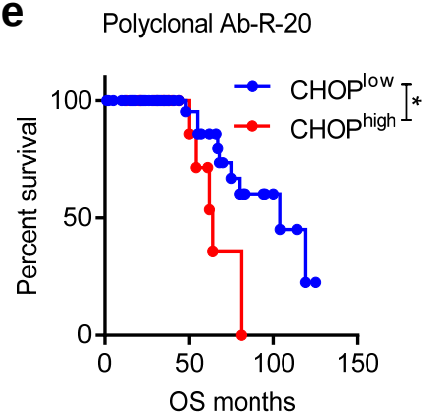
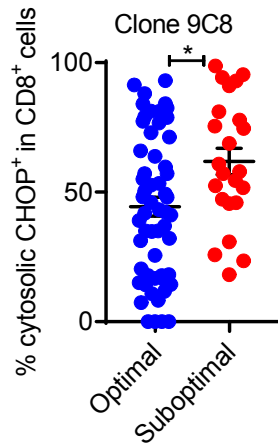
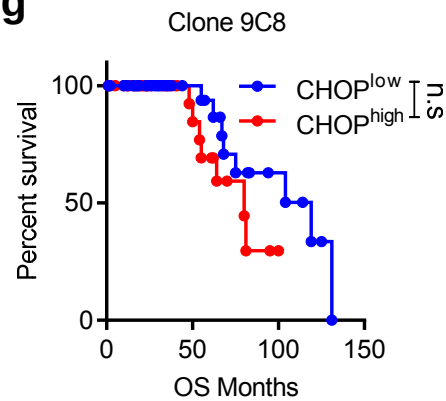
SUPPLEMENTARY INFORMATION

ER stress-induced mediator C/EBP homologous protein thwarts effector T-cell activity in tumors through T-bet repression

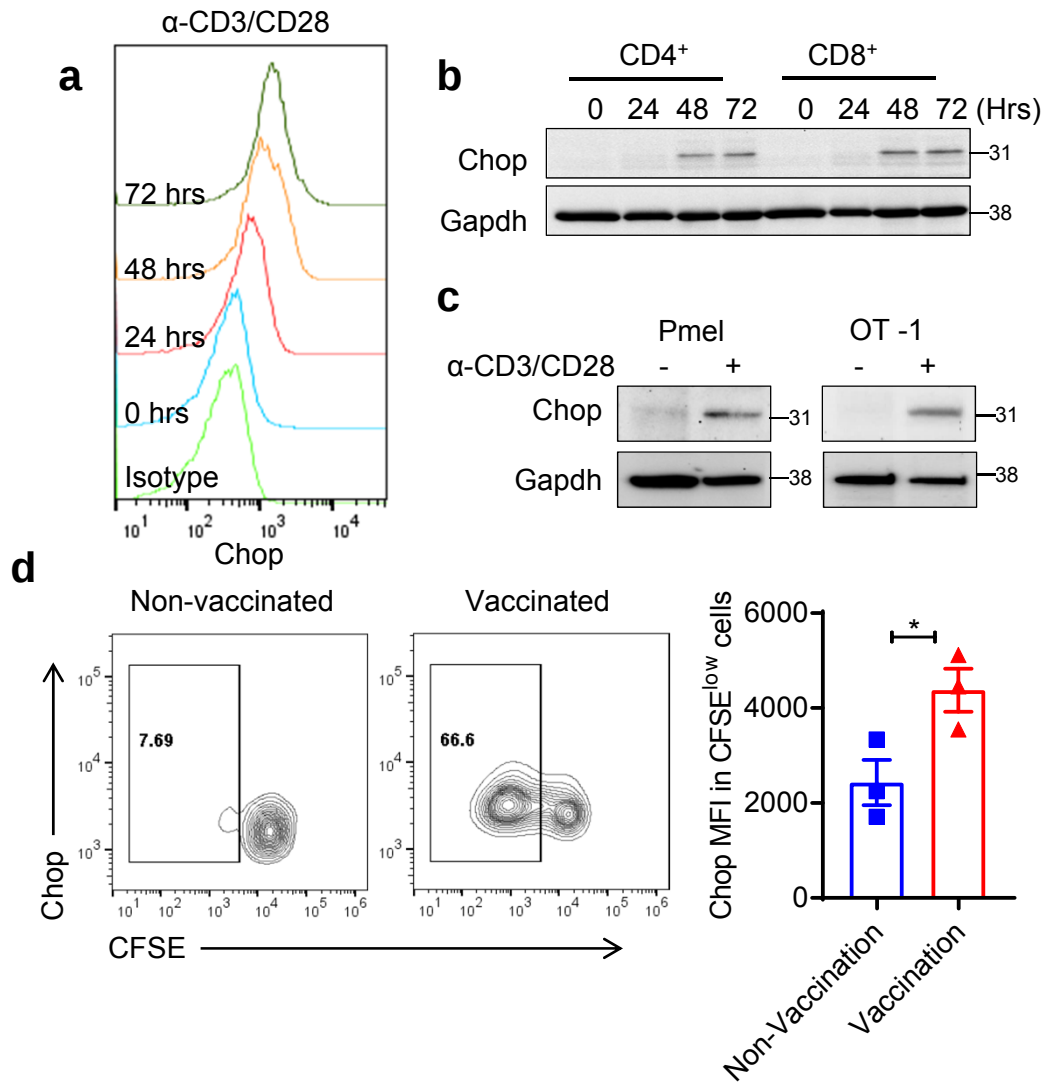
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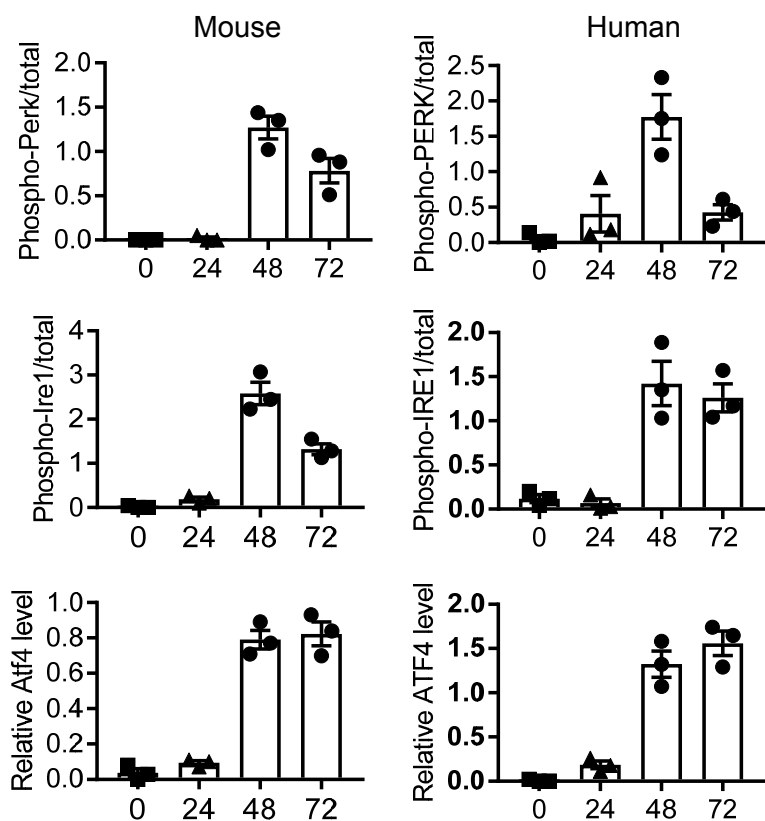
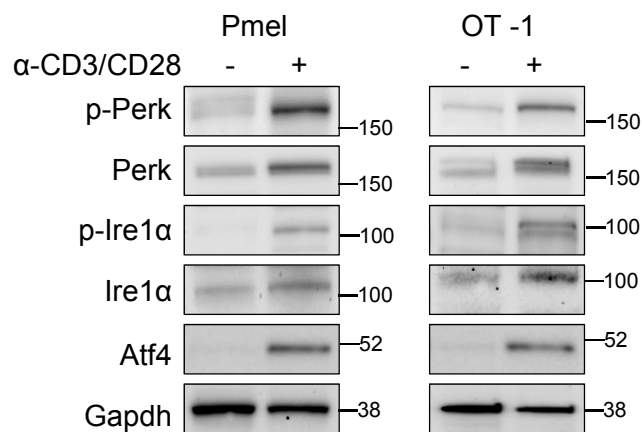
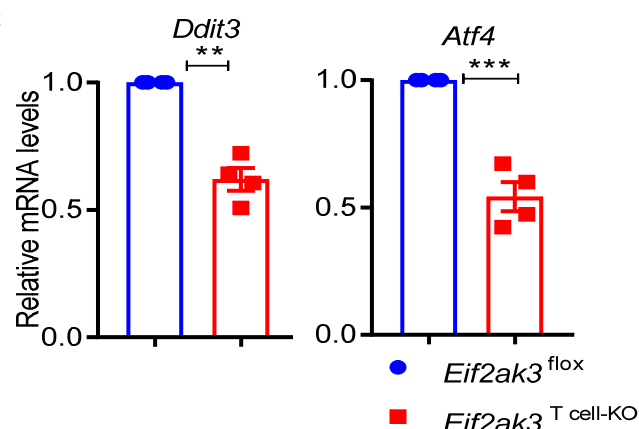
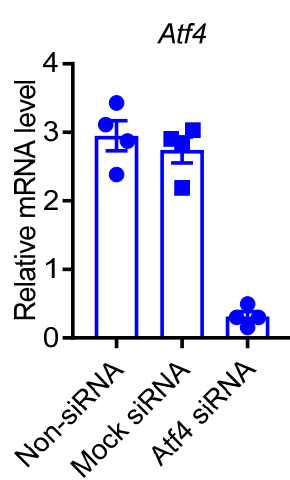
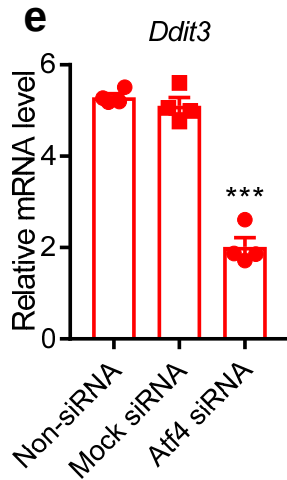
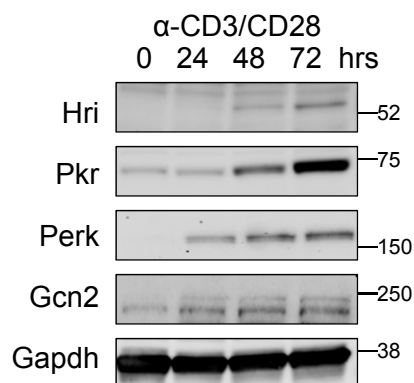
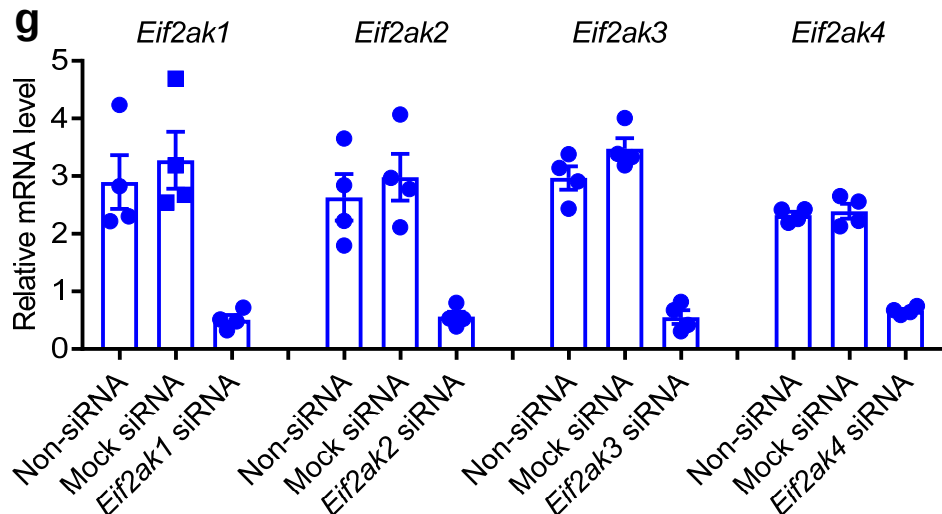
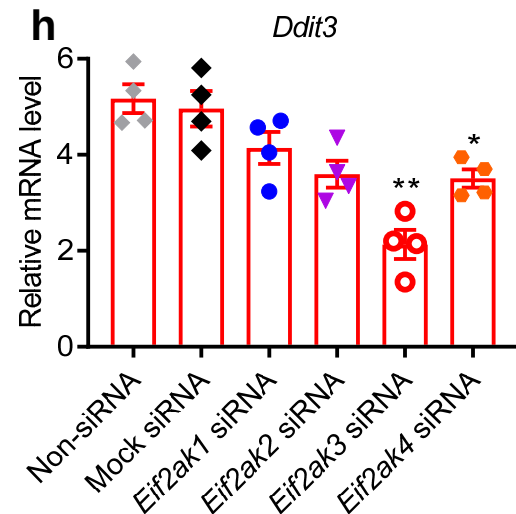
Supplementary Figure 1. Chop is upregulated in MDSCs and CD8⁺ TILs from murine and human ovarian tumors. **a.** Chop in splenic CD8⁺ T cells (*left*, n=3) and ascites CD8⁺ TILs (*right*, n=9) from ID8-*Defb29/Vegf-a* ovarian tumor-bearing mice. Chop was detected by FACS and levels indicated by mean fluorescent intensity (MFI). **b.** Percentage of Chop⁺ cells was established by FACS in gated splenic and tumor-associated CD8⁺ T cells from mice bearing: *left*: B16 tumors (n=11); *middle*: 3LL tumors (n=7); *right*: ID8-*Defb29/Vegf-a* ovarian tumors (n=9). **c.** ER tracker in CD8⁺ TILs from ID8-*Defb29/Vegf-a* ovarian tumor ascites, compared with splenic CD8⁺ T cells from the corresponding tumor-bearing mice (n=3). ER tracker was detected by FACS and levels indicated by MFI. **d.** Percentage of Chop⁺ cells by FACS in tumor-associated CD8⁺ T cells from human ovarian carcinoma tissues, compared with matching peripheral blood CD8⁺ T cells from ovarian cancer patients. Bar graphs show mean $\pm$ s.e.m. **e.** Gating strategy for tumor-infiltrating MDSC (T-MDSC, CD45⁺ CD11b⁺ MHC-DR⁻ CD33⁺) and CD8⁺ TILs (CD45⁺ CD3⁺ CD8⁺) from human ovarian carcinoma samples. CHOP levels in T-MDSC vs. CD8⁺ TILs (*lower right*). CHOP expression was detected by FACS and levels indicated by MFI. Representative results from 7 independent patients. In all figures, the bar graphs show the mean $\pm$ s.e.m. *p < 0.05, **p < 0.01, ***p < 0.001 calculated using two-tailed unpaired Student's *t*-test.

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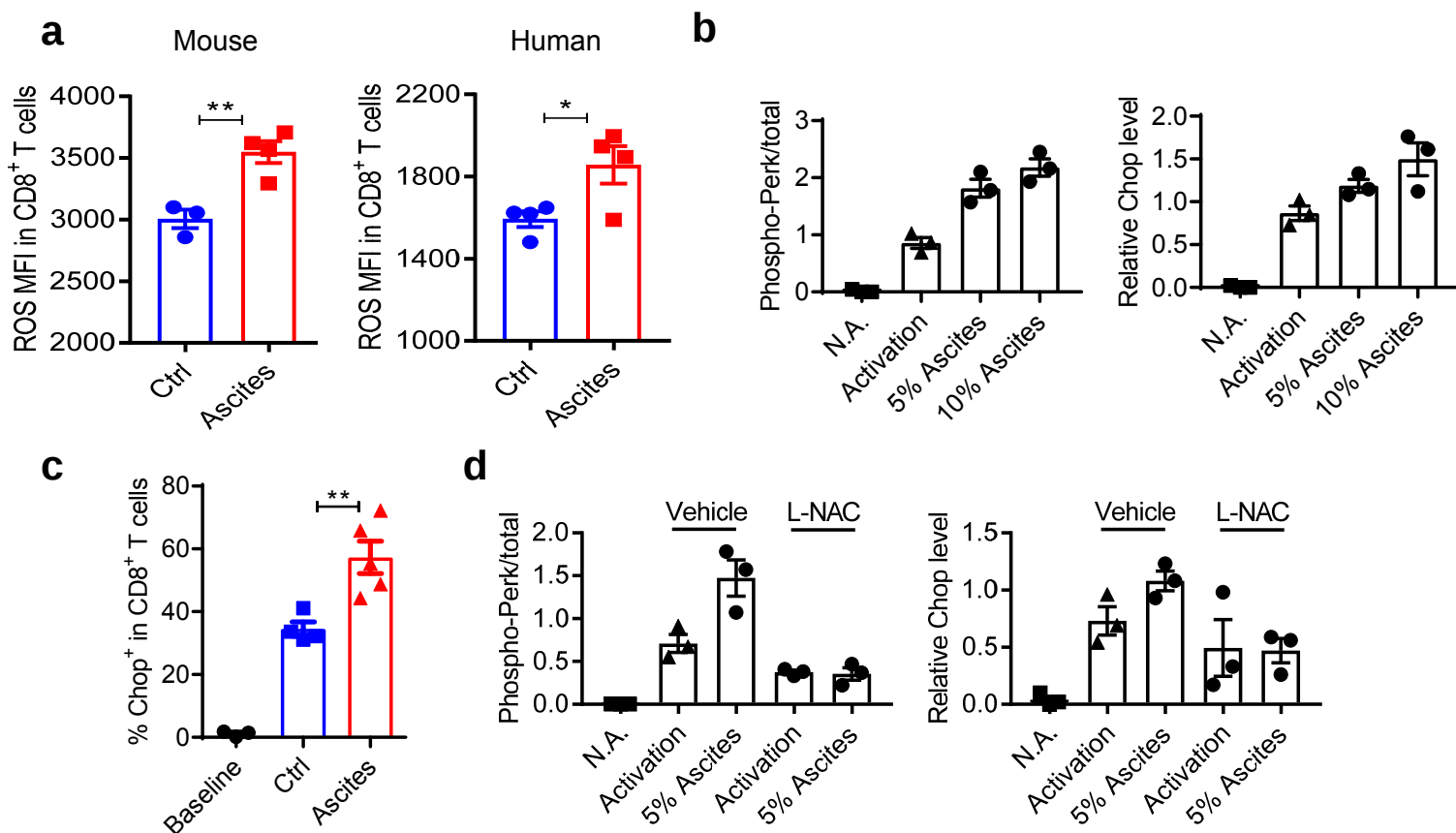
Supplementary Figure 2. CHOP is upregulated in CD8⁺ TILs from ovarian carcinoma patients. **a.** Representative panel illustrating a spectrum of CHOP expression patterns in CD8⁺ TILs from ovarian carcinoma tissues compared to those from healthy ovary tissues. CHOP (red, clone 9C8 (*left*) or polyclonal R-20 (*right*)), CD8 (green) and DAPI (blue) were detected by confocal microscopy. **b.** Percentage of total CHOP⁺ cells (*left*: clone 9C8; *right*: polyclonal Ab-R-20) by non-subjective imaging analysis in a tissue microarray containing ovarian carcinoma tumors and healthy ovary tissues. The graphs show the mean $\pm$ s.e.m.. **c.** Non-subjective results showing a higher frequency of CD8⁺ T cells having cytosolic CHOP in the ovarian carcinoma tumors, compared to those from normal ovarian tissues (*left*: clone 9C8; *right*: polyclonal Ab-R-20). **d.** Percentage of nuclear-CHOP⁺ cells (polyclonal Ab-R-20) in tumor-associated CD8⁺ T cells from ovarian carcinoma tissues vs. healthy tissues as in **Fig. 1f**. Graph shows mean $\pm$ s.e.m.. **e.** Overall survival of ovarian tumor patients having high frequency of nuclear-CHOP in CD8⁺ TILs (CHOP^{high}) (n=18) vs. low frequency of nuclear-CHOP in CD8⁺ TILs (nuclear-CHOP^{low}) (n=57) (logrank 4.55, p=0.0329 using the Gehan-Breslow-Wilcoxon test; cutoff was established as in the Methods). Studies were developed using the anti-Chop polyclonal Ab-R-20. **f.** Percentage of CD8⁺ TILs having cytosolic-CHOP (clone 9C8) in ovarian cancer patients that had optimal (n=58) vs. suboptimal (n=23) cytoreductive debulking surgery. **g.** Overall survival in ovarian tumor patients showing high frequency of cytosolic-CHOP in CD8⁺ TILs (CHOP^{high}) (n=37) vs. those having low frequency of cytosolic-CHOP in CD8⁺ TILs (CHOP^{low}) (n=44). CHOP was detected using the anti-Chop 9C8 clone antibody. In the bar graphs showing mean $\pm$ s.e.m., *p < 0.05, **p < 0.01, ***p < 0.001 were calculated using two-tailed unpaired Student's t-test.



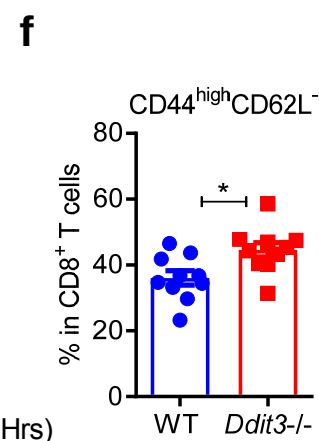
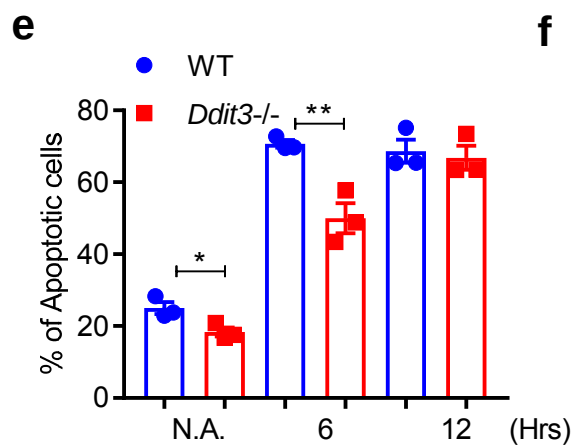
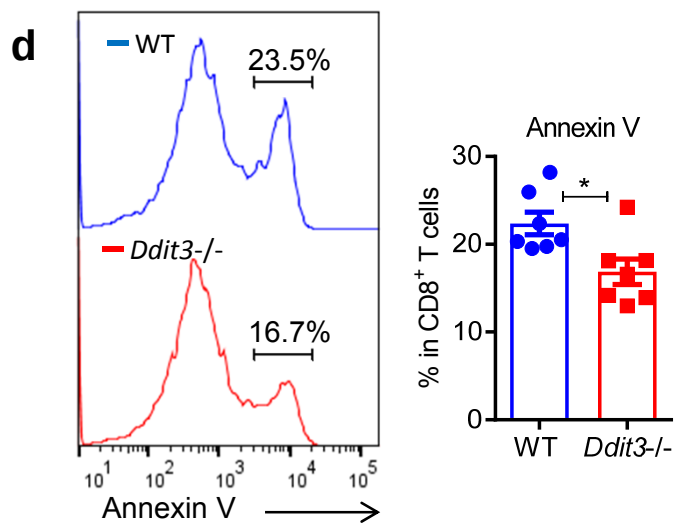
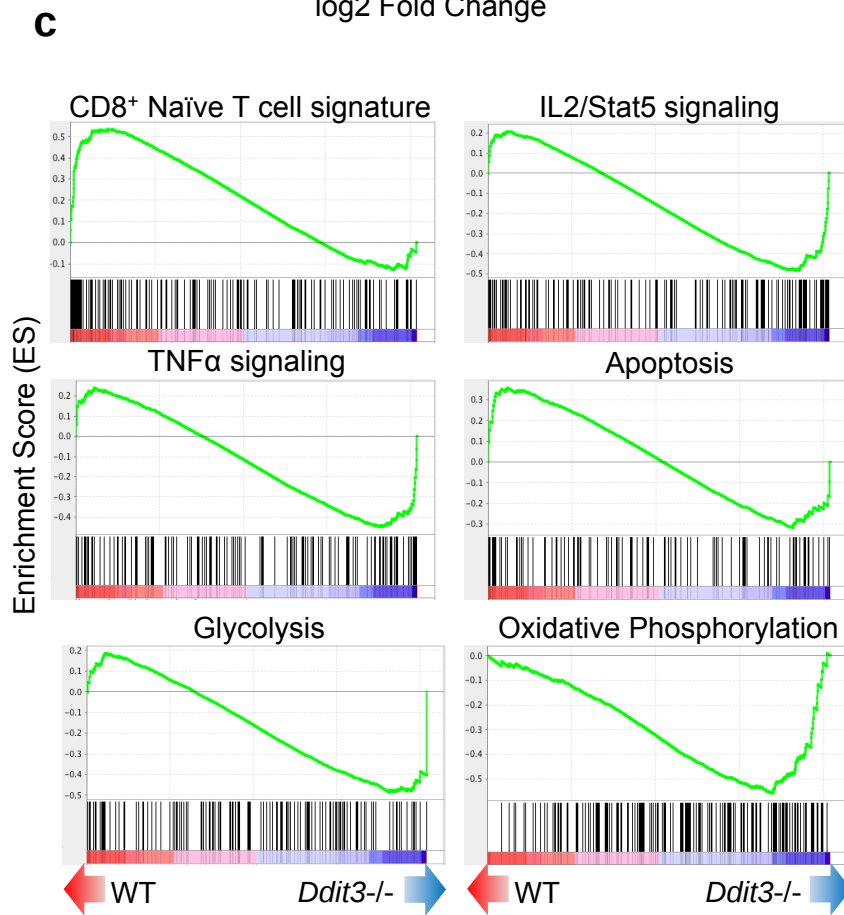
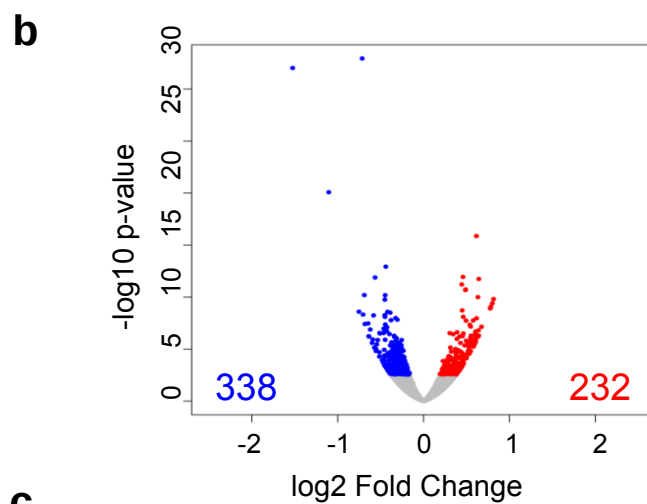
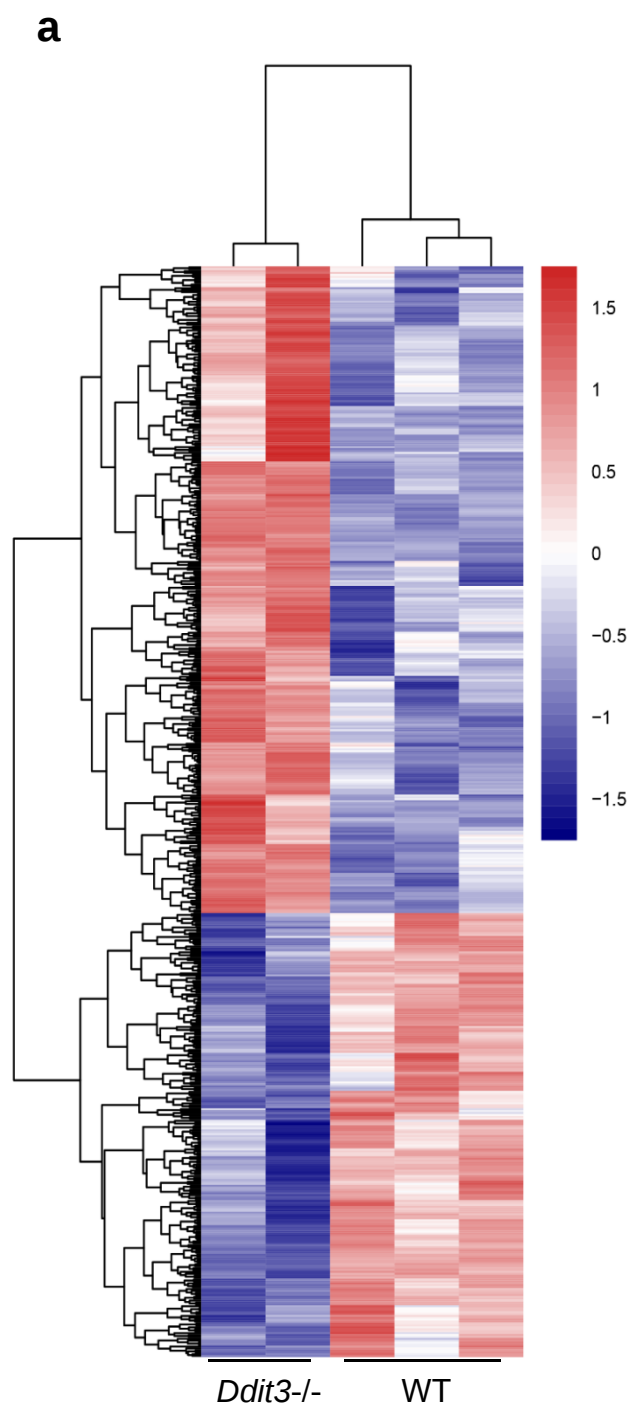
Supplementary Figure 3. Chop is upregulated in stimulated CD8⁺ T cells. **a.** Time-dependent induction of Chop by FACS after CD8⁺ T cell priming with anti-CD3/CD28 (0-72 hours; n=4). **b.** Upregulation of Chop in anti-CD3/CD28-primed CD4⁺ or CD8⁺ T cells (0-72 hours; n=3). **c.** Chop levels in Pmel and OT-1 CD8⁺ T cells stimulated for 48 hours with gp100₂₅₋₃₃ or OVA₂₅₇₋₂₆₄, respectively. (n=3). **d.** Chop expression was assessed by FACS in proliferating transferred Pmel T-cells from gp100₂₅₋₃₃-vaccinated mice (activation driven T cell proliferation) compared to non-vaccinated cohorts (homeostatic T cell division). *Left:* representative gating of CFSE^{diluted} proliferating cells; *right:* Bar graph showing mean $\pm$ s.e.m. of Chop levels (n=3) in gated CD8⁺ CD90.1⁺ CFSE^{diluted} proliferating T cells. *p < 0.05 by two-tailed unpaired Student's *t*-test.

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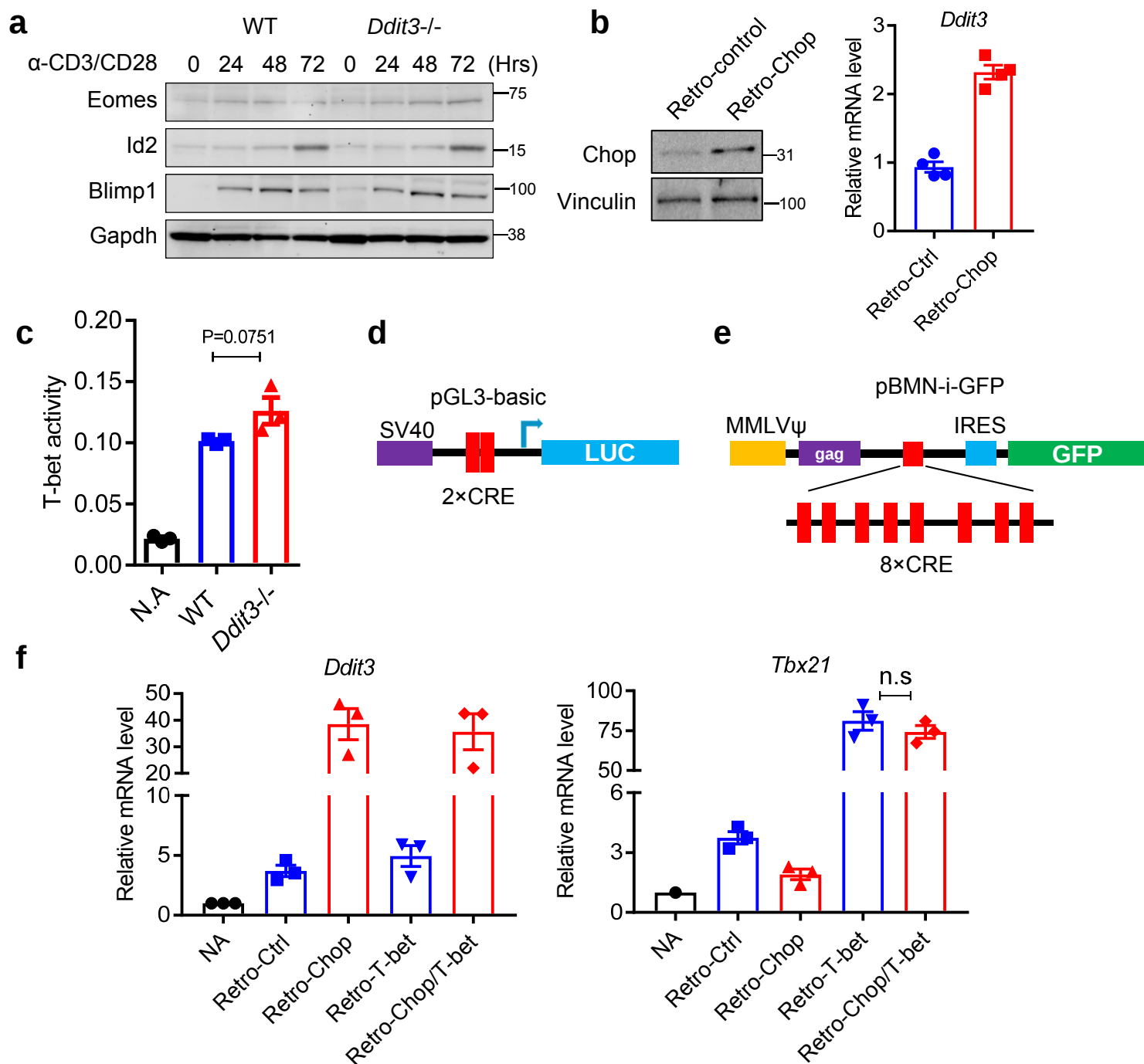
Supplementary Figure 4. Chop induction in activated CD8⁺ T cells depends on the ER stress-associated kinase Perk. **a.** Densitometry quantitation of immunoblots from **Fig. 2d** and that merges the results from 3 repeats. **b.** UPR mediators in Pmel and OT-1 CD8⁺ T cells stimulated for 48 hours with gp100₂₅₋₃₃ or OVA₂₅₇₋₂₆₄ respectively. (n=3). **c.** *Ddit3* and *Atf4* mRNA levels in stimulated *Eif2ak3*-null and *Eif2ak3*-flox CD8⁺ T cells. (n=4). **d.** *Atf4* mRNA levels in stimulated CD8⁺ T cells transfected with non-targeting siRNA or a pool of specific *Atf4* siRNA. Results are from 4 independent repeats. **e.** *Ddit3* mRNA levels in stimulated CD8⁺ T cells transfected as in (**d**). **f.** Hri, Pkr, Perk, and Gcn2 levels in anti-CD3/CD28-stimulated murine CD8⁺ T cells (0-72 hours). Results are a representative experiment from 3 repeats. **g.** siRNA-based silencing of *Eif2ak1*, *Eif2ak2*, *Eif2ak3*, and *Eif2ak4* upon CD8⁺ T cell priming. Results are from 4 repeats. **h.** *Ddit3* mRNA levels in stimulated CD8⁺ T cells from (**g**). All the bar graphs show mean $\pm$ s.e.m.. *p < 0.05, **p < 0.01, ***p < 0.001 were calculated using two-tailed unpaired Student's *t*-test.



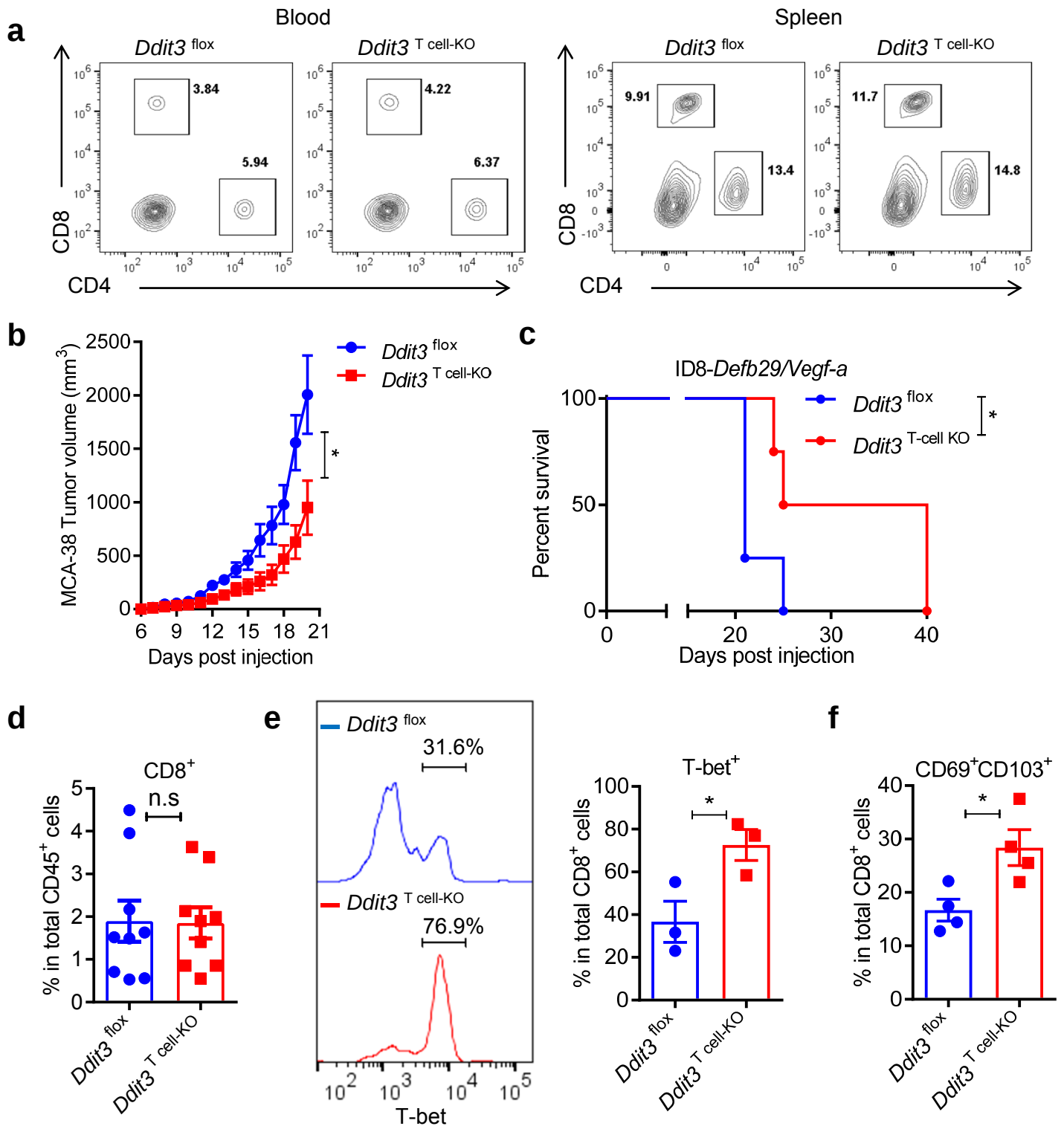
Supplementary Figure 5. ROS-induced Perk activation regulates Chop induction in tumor-exposed CD8⁺ T-cells. **a.** ROS levels were monitored by FACS in mouse and human DHE-labeled CD8⁺ T-cells activated with anti-CD3/CD28 and treated for 24 hours with 5% cell-free ovarian ascites from mice bearing ID8-*Defb29/Vegf-a* ovarian tumors, or 5% primary ascites from patients with ovarian cancer, respectively. (n=4). **b.** Merged densitometry quantitation of immunoblots from **Fig. 2h**. Results are from 3 independent repeats. **c.** Percentage of Chop⁺ cells by FACS in primed CD8⁺ T cells treated with 5% cell-free ID8-*Defb29/Vegf-a* ovarian tumor ascites for 24 hours, compared with vehicle treated CD8⁺ T cells. (n=5). **d.** Densitometry quantitation of immunoblot intensities from **Fig. 2i**. Results are from 3 independent repeats. All bar graphs show the mean $\pm$ s.e.m. *p < 0.05, **p < 0.01 were calculated using two-tailed unpaired Student's *t*-test.



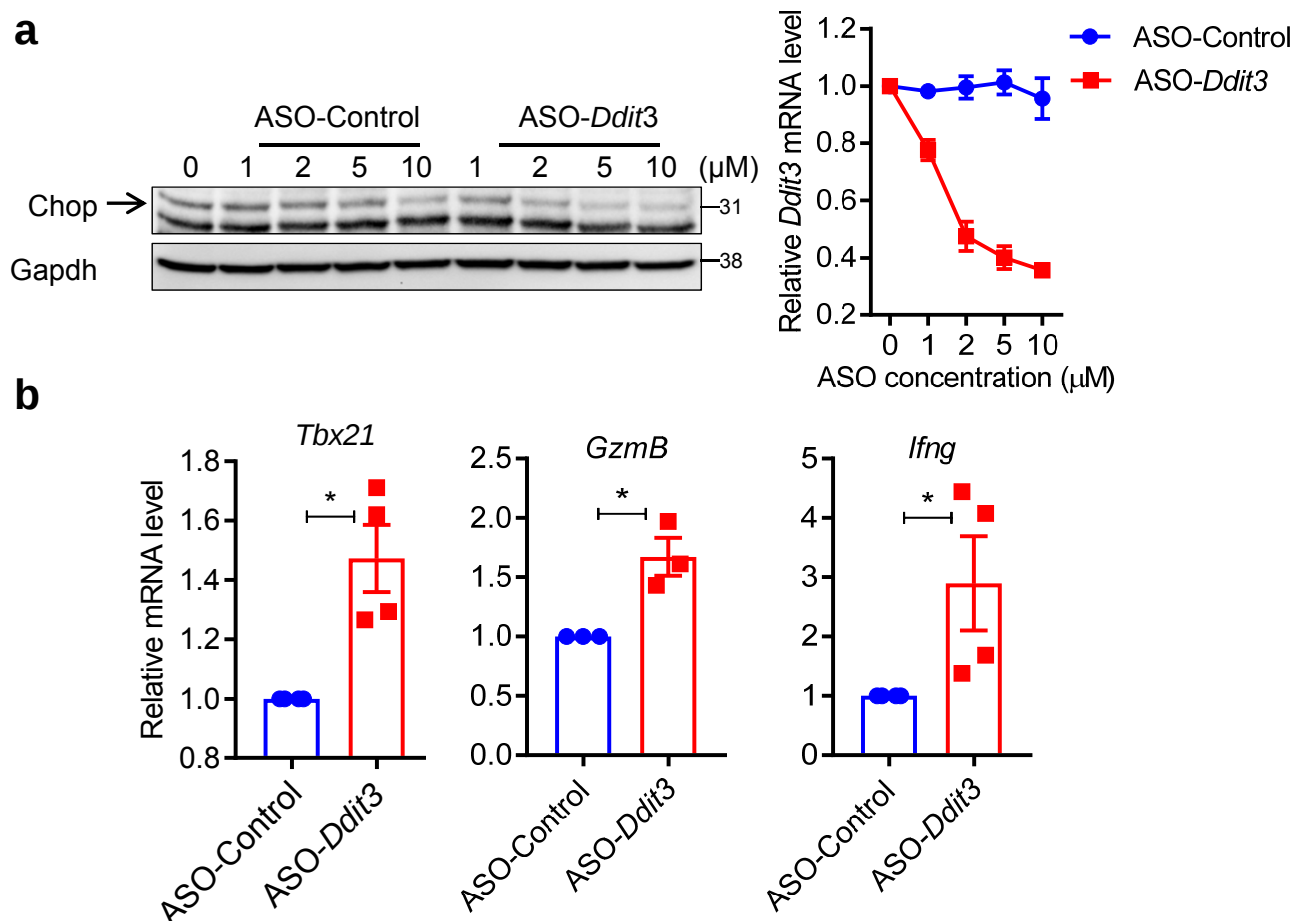
Supplementary Figure 6. Chop plays a primary role in CD8⁺ T cell function. **a-b.** Heatmap (**a**) and plot distribution (**b**) from RNA-Seq detection of activated wild-type (n=3) vs. *Ddit3*^{-/-} (n=2) Pmel CD8⁺ T cells. **c.** GSEA of specific gene signatures in primed wild-type vs. *Ddit3*^{-/-} Pmel CD8⁺ T cells. **d.** Spontaneous frequency of Annexin V⁺ cells in wild-type and *Ddit3*^{-/-} CD8⁺ T cells activated in the presence of anti-CD3/CD28 for 72 hours. *Left*: representative result; *right*: mean $\pm$ s.e.m. from 7 repeats. **e.** Annexin V⁺ cells in wild-type or *Ddit3*^{-/-} CD8⁺ T cells primed *in vitro* for 48 hours and then treated with 1 μ M staurosporine for additional 6 or 12 hours. (n=3). **f.** Merged data from **Fig. 3k** showing the frequency of CD44^{high} CD62L⁻ cells within wild-type or *Ddit3*-null CD8⁺ T-cells. (n=10). *p < 0.05, **p < 0.01 were calculated using two-tailed unpaired Student's *t*-test.



Supplementary Figure 7. Mechanistic and functional interaction between Chop and T-bet in activated CD8⁺ T cells. **a.** Time-course expression of Eomes, Id2, and Blimp1 in wild-type and *Ddit3*^{-/-} CD8⁺ T-cells stimulated for 72 hours with plate bound anti-CD3/CD28. (n=3). **b.** Expression of Chop protein (left, short-time exposure) and *Ddit3* mRNA (right) in primed CD8⁺ T cells infected with control (Retro-ctrl) or *Ddit3*-coding retrovirus (Retro-Chop). (n=3). **c.** DNA-binding activity (TransAM) of T-bet in primed wild-type or *Ddit3*^{-/-} CD8⁺ T cells. **d.** Model of SV40-2CRE-Luc system. **e.** Model of MSCV-8CRE-GFP system. **f.** *Ddit3* and *Tbx21* mRNA in primed CD8⁺ T cells transduced with: 1) CD90.1 and GFP expressing control virus (Retro-Ctrl); 2) *Ddit3*/CD90.1 expressing virus and GFP expressing control virus (Retro-Chop); 3) CD90.1 expressing control virus and *Tbx21*/GFP expressing virus (Retro-T-bet); or 4) Chop/CD90.1 expressing virus and T-bet/GFP expressing virus (Retro-Chop/T-bet). Bar graphs show the mean ± s.e.m. (n=3). **p* < 0.05, ***p* < 0.01, ****p* < 0.001 calculated using two-tailed unpaired Student's *t*-test.



Supplementary Figure 8. Chop intrinsically regulates anti-tumor T cell immunity. **a.** Distribution of CD4⁺ and CD8⁺ T cells in spleen and peripheral blood from *Ddit3*^{flox} and *Ddit3*^{T cell-KO} mice. (n=5). **b.** Tumor growth in *Ddit3*^{flox} (blue) or *Ddit3*^{T cell-KO} (red) mice bearing MCA-38 tumors. Average kinetics $\pm$ s.e.m. of 6 mice/group. **c.** Survival of *Ddit3*^{flox} or *Ddit3*^{T cell-KO} mice bearing ID8-Defb29/Vegf-a ovarian tumors. (n=4; logrank 4.14, p=0.046 using the Gehan-Breslow-Wilcoxon test). **d.** Frequency of CD8⁺ TILs from B16 tumors collected from *Ddit3*^{flox} or *Ddit3*^{T cell-KO} mice. (n= 9 mice/group). **e.** T-bet levels in CD8⁺ TILs from *Ddit3*^{flox} or *Ddit3*^{T cell-KO} mice bearing s.c. B16 tumors. *Left*: representative result; *right*: mean $\pm$ s.e.m. from 3 mice. **f.** Percentage of CD69⁺CD103⁺ resident memory CD8⁺ TILs in B16 tumors isolated from *Ddit3*^{flox} or *Ddit3*^{T cell-KO} mice. (n=4). In the bar graphs, *p < 0.05, **p < 0.01, ***p < 0.001 were calculated using two-tailed unpaired Student's *t*-test.



Supplementary Figure 9. Targeted silencing of Chop in CD8⁺ T cells modulates expression of effector molecules. **a.** Inhibited Chop expression in primed T cells treated with ASO-Control or ASO-Ddit3. *Left:* Representative experiment showing Chop protein levels (n=3); *right:* *Ddit3* mRNA from 3 independent studies. **b.** *Tbx21*, *Gzmb* and *Ifng* mRNA levels (mean $\pm$ s.e.m.) in primed Pmel T cells treated with ASO-Control or ASO-Ddit3. (n=4 for *Tbx21* and *Ifng*, n=3 for *Gzmb*, *p < 0.05 calculated using two-tailed unpaired Student's *t*-test).