Zheng et al
Supplemental Figures

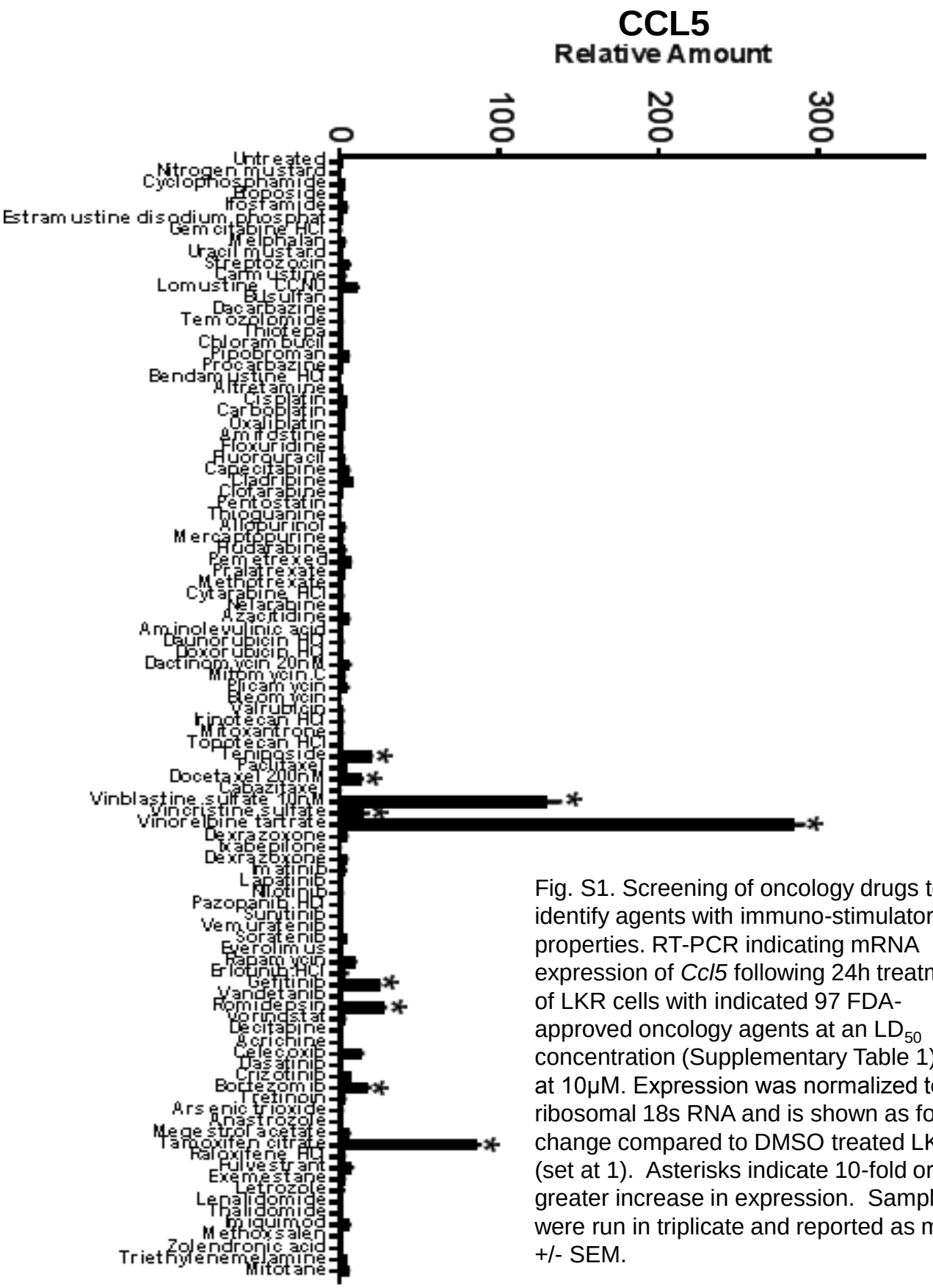


Fig. S1. Screening of oncology drugs to identify agents with immuno-stimulatory properties. RT-PCR indicating mRNA expression of *Ccl5* following 24h treatment of LKR cells with indicated 97 FDA-approved oncology agents at an LD_{50} concentration (Supplementary Table 1) or at 10μM. Expression was normalized to ribosomal 18s RNA and is shown as fold change compared to DMSO treated LKR (set at 1). Asterisks indicate 10-fold or greater increase in expression. Samples were run in triplicate and reported as mean +/- SEM.

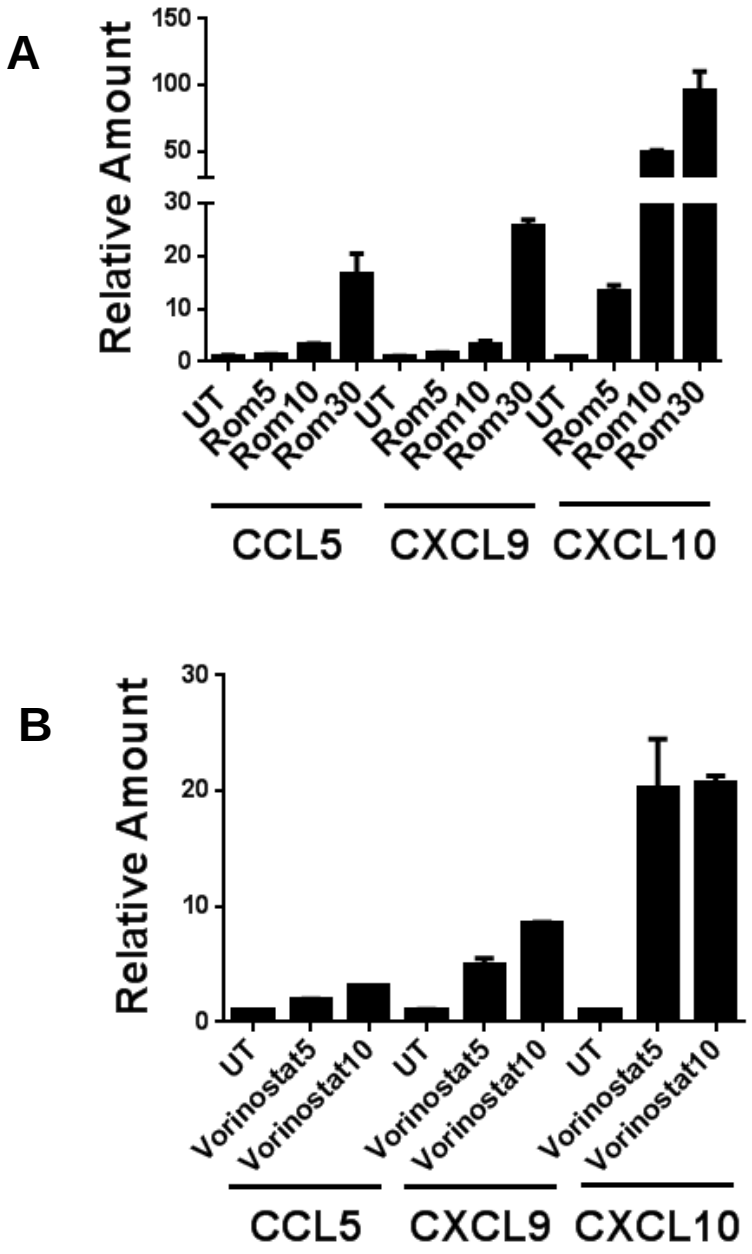


Fig. S2. Expression of *Ccl5*, *Cxcl9* and *Cxcl10* was normalized to ribosomal 18s RNA and is shown as fold change compared to DMSO treated LKR (set at 1). (A) Cells were treated with indicated romidepsin concentrations (nM) for 24 h. (B) Cells were treated with indicated vorinostat concentrations (μM) for 24 h. Samples were run in triplicate and reported as mean +/- SEM.

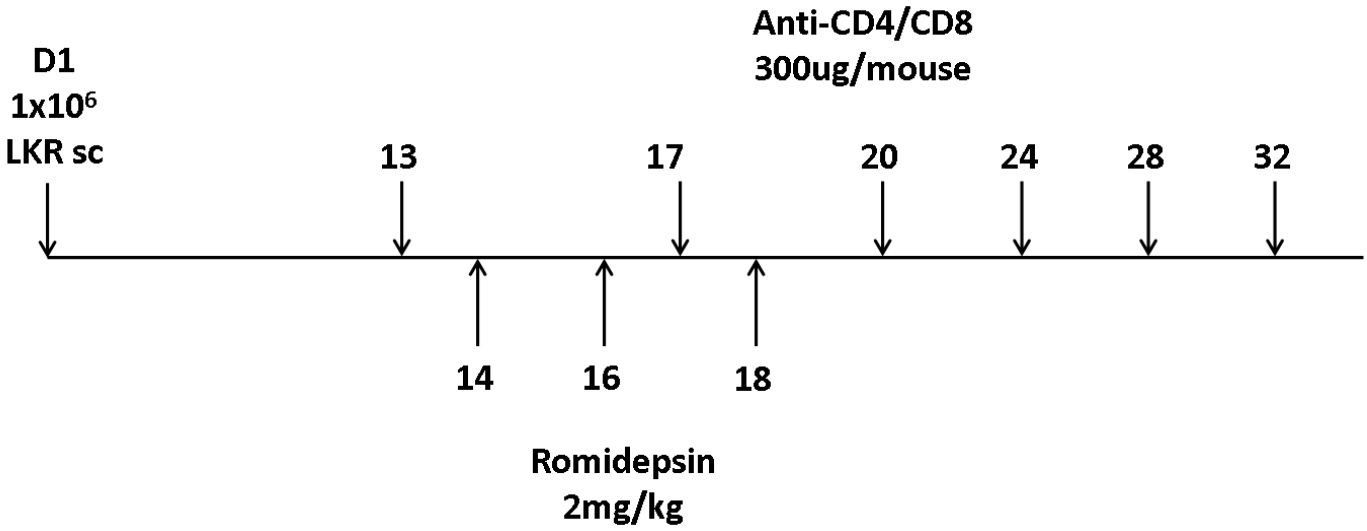


Fig. S3. Treatment regimen of mice receiving i.p. anti-CD4/CD8 and romidepsin.

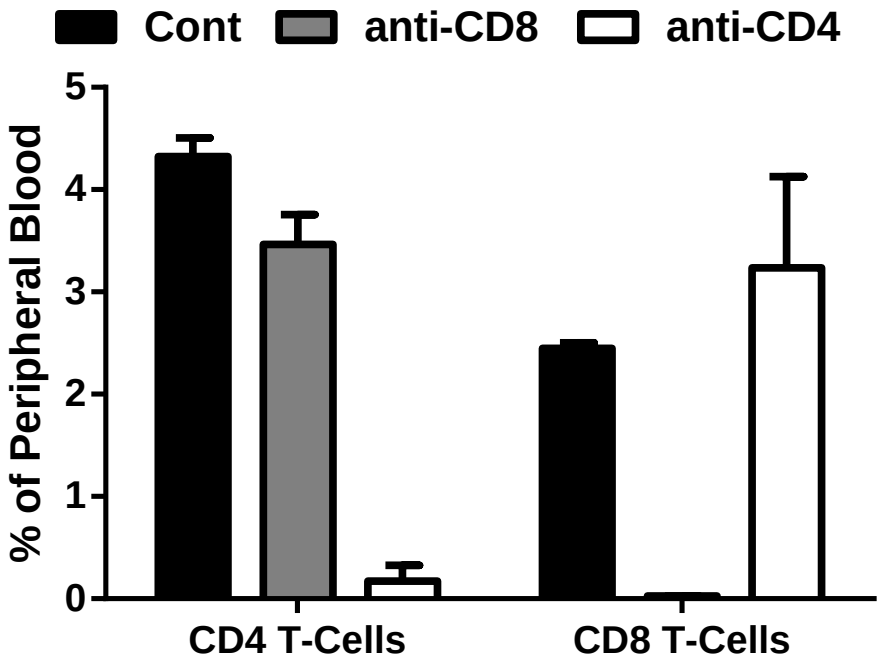


Fig. S4. Percentage of CD4 or CD8 in DAPI- (viable) peripheral blood of mice receiving anti-CD8 or anti-CD4 at 300μg, as indicated.

A

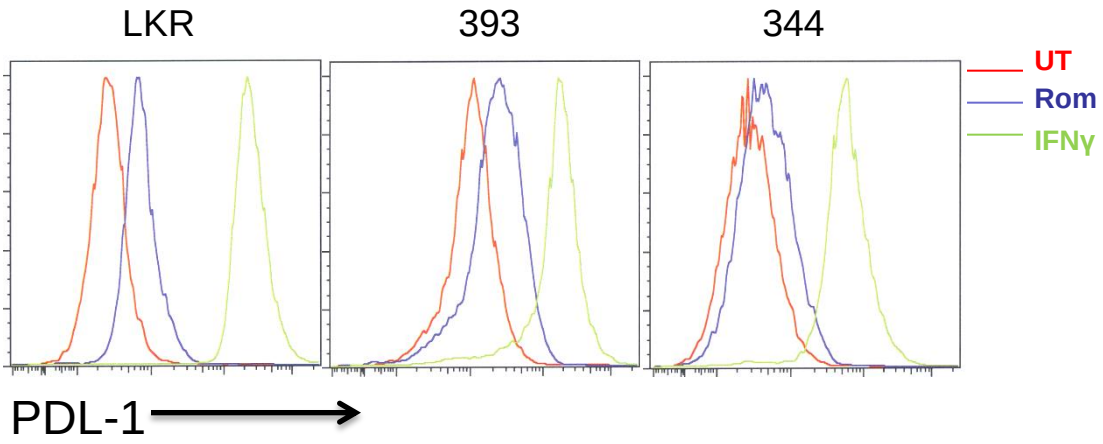


Fig. S5. PD-L1 expression. Cell surface expression of PD-L1 determined by FACS in viable LKR, 393P (393) and 344SQ (344) cells after 24h treatment with 30nM romidepsin or IFNγ as indicated. UT: untreated cells.

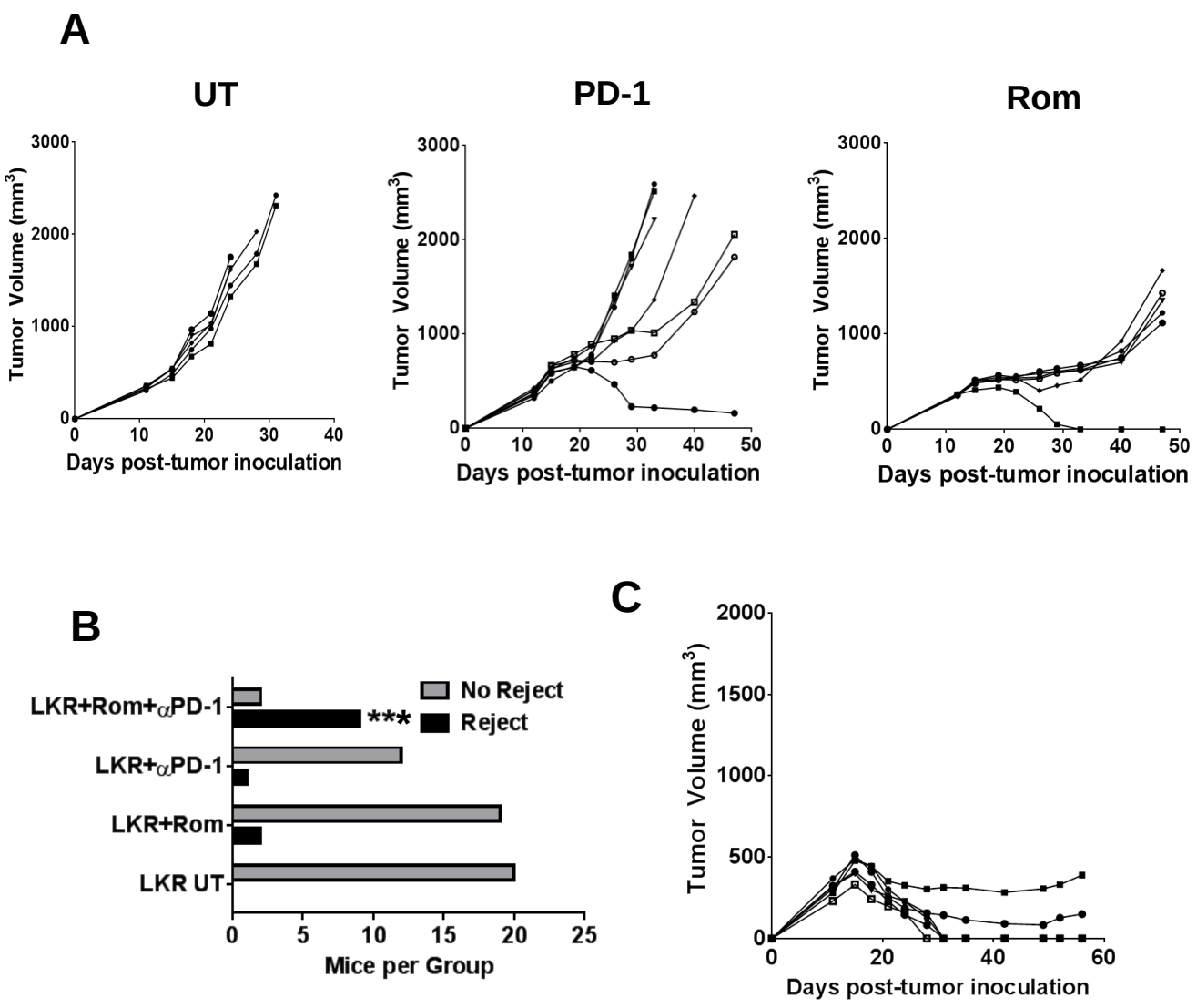
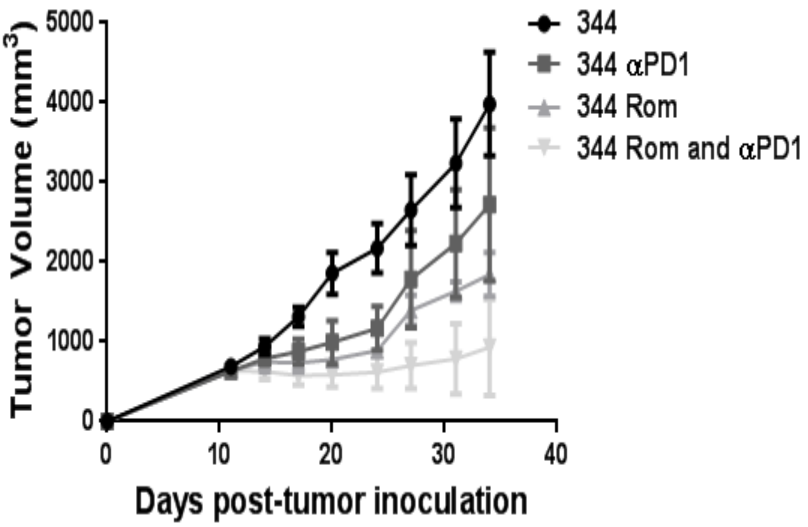


Fig. S6. (A) Impact of romidepsin (n=6) and anti-PD-1 (n=7) treatment on LKR tumor growth for 47 days. UT: untreated mice (n=5). Mice in which tumor growth is not shown up to 47 days died, were sacrificed for tumor size exceeding IACUC approved levels or moribund status. (B) Combined results from 3 independent experiments were used to determine significance of reject or no reject outcomes by Fisher Exact Test. The different treatment groups are indicated (LKR UT, n=20; LKR Romidepsin, n=21; LKR anti-PD1, n=13; LKR Romidepsin and anti-PD-1, n=11) and p values were determined compared to the untreated group; LKR Romidepsin: p=0.4878; LKR anti-PD-1: p=0.3939; LKR Romidepsin and anti-PD-1 (as indicated) p<0.0001. (C) Impact of romidepsin + anti-PD-1 treatment on LKR tumor growth for indicated days (n=6).



1) 344 (untreated) vs. PD1: p=0.0638

2) 344 (untreated) vs. Rom: p=0.0088

3) 344 (untreated) vs. PD1 and Rom: p=0.0005

4) PD1 vs. PD1 and Rom: p=0.0143

5) Rom vs. PD1 and Rom: p=0.0043

Fig. S7. 129 mice were inoculated s.c. with 10^6 344SQ (344) following which they were treated with 2mg/kg romidepsin on days 14,16,18, with or without 300μg/mouse anti-PD-1 antibody on days 15,17,19 as indicated. Tumor growth over indicated time periods is shown. Measurement of 5 tumors/group are indicated as mean +/- SEM. T-test was used to determine significance of differences in groups, as indicated by p-values above.

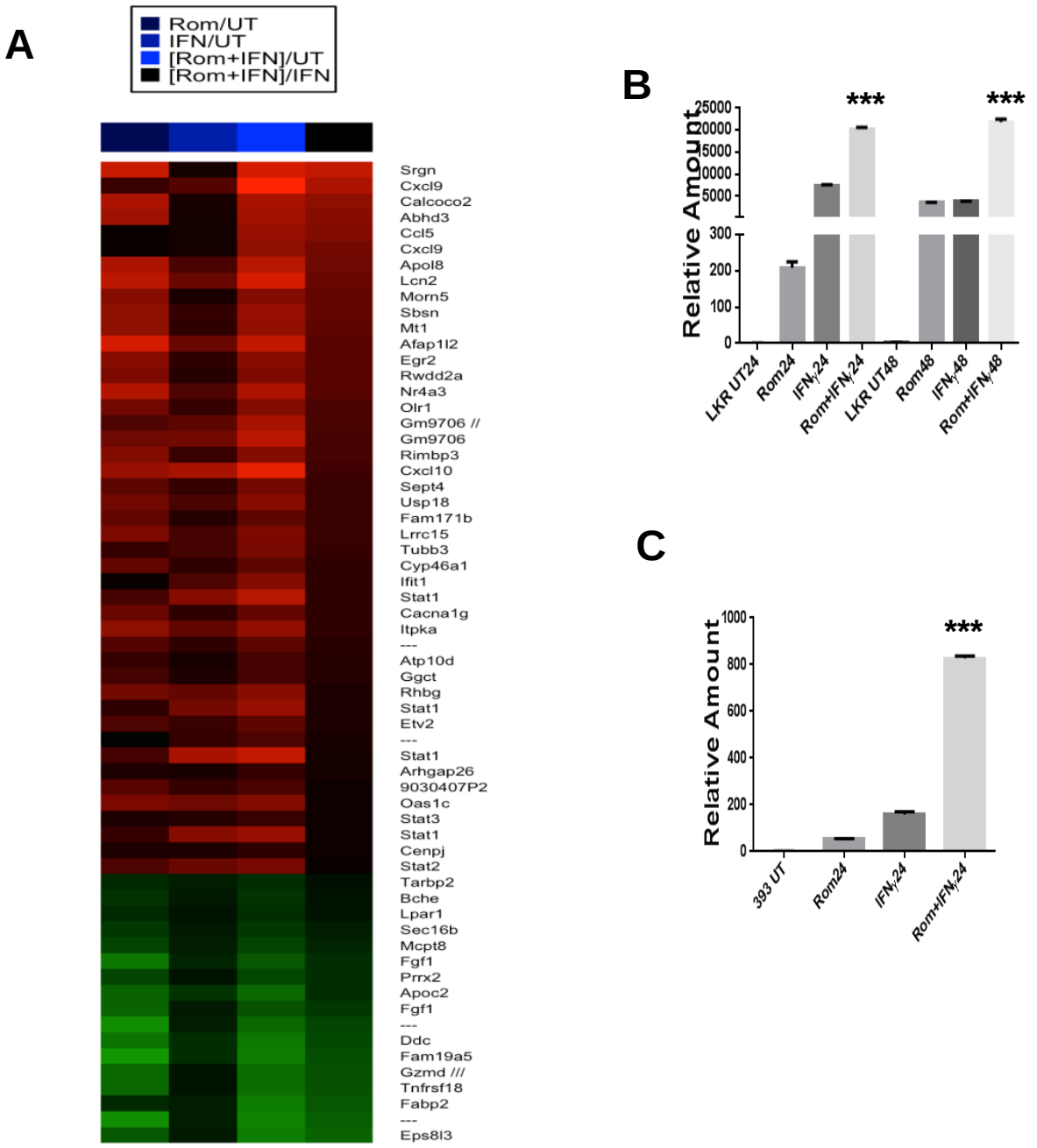


Fig. S8. **(A)** Heatmap analysis using conventional red-green scale of microarray results of LKR cells after IFNγ and/or romidepsin treatment for genes increased after combined vs. single treatments. The fold changes plotted are: treated with Rom over untreated, IFNγ over untreated, both Rom and IFNγ over untreated, and a synergy measure: both Rom and IFNγ over treated with IFNγ (right column). The heatmap rows are sorted in descending order of the synergy measure of Rom and IFNγ over treated with IFNγ (right column). **(B)** CXCL10 mRNA expression was determined in vitro in LKR and **(C)** 393P (393) by RT-PCR after romidepsin (Rom) and/or IFNγ treatment for 24h.

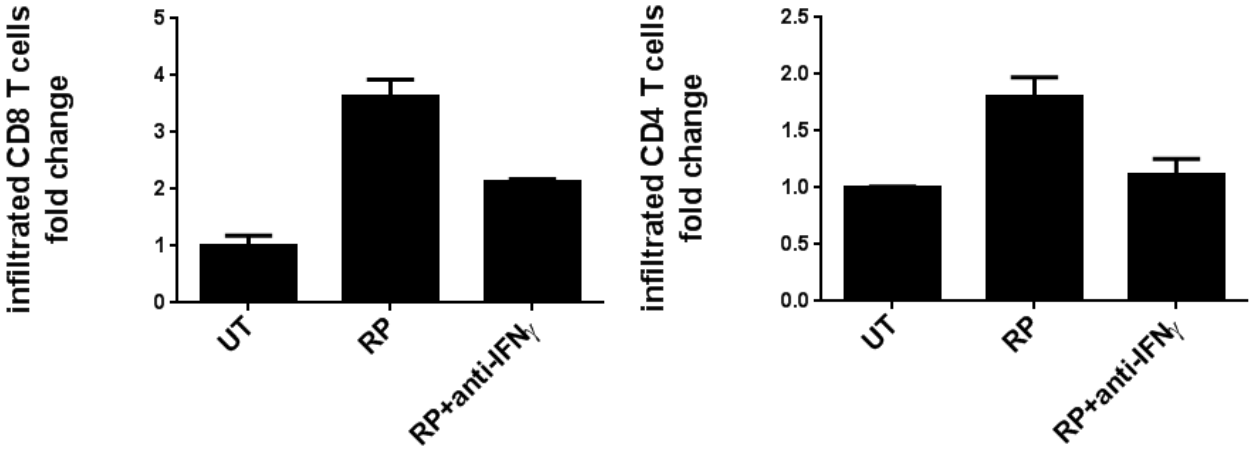


Fig. S9. (A) 129 mice with day 14 tumors were untreated (UT) or treated with romidepsin on days 14 and 16 and anti-PD-1 antibody on days 15 and 17 (RP) after which FACS was used to determine CD4 ($CD3^+CD4^+$) and CD8 ($CD3^+CD8^+$) percentages in total viable cells (day 19). Where indicated, anti-IFNγ antibody (200ug/mouse) was injected 2 days prior to the first treatment. Combined results of 2 independent experiments showing fold increase in presence of CD4 and CD8 T cells in tumors compared to untreated tumors (set at 1) after indicated treatments.

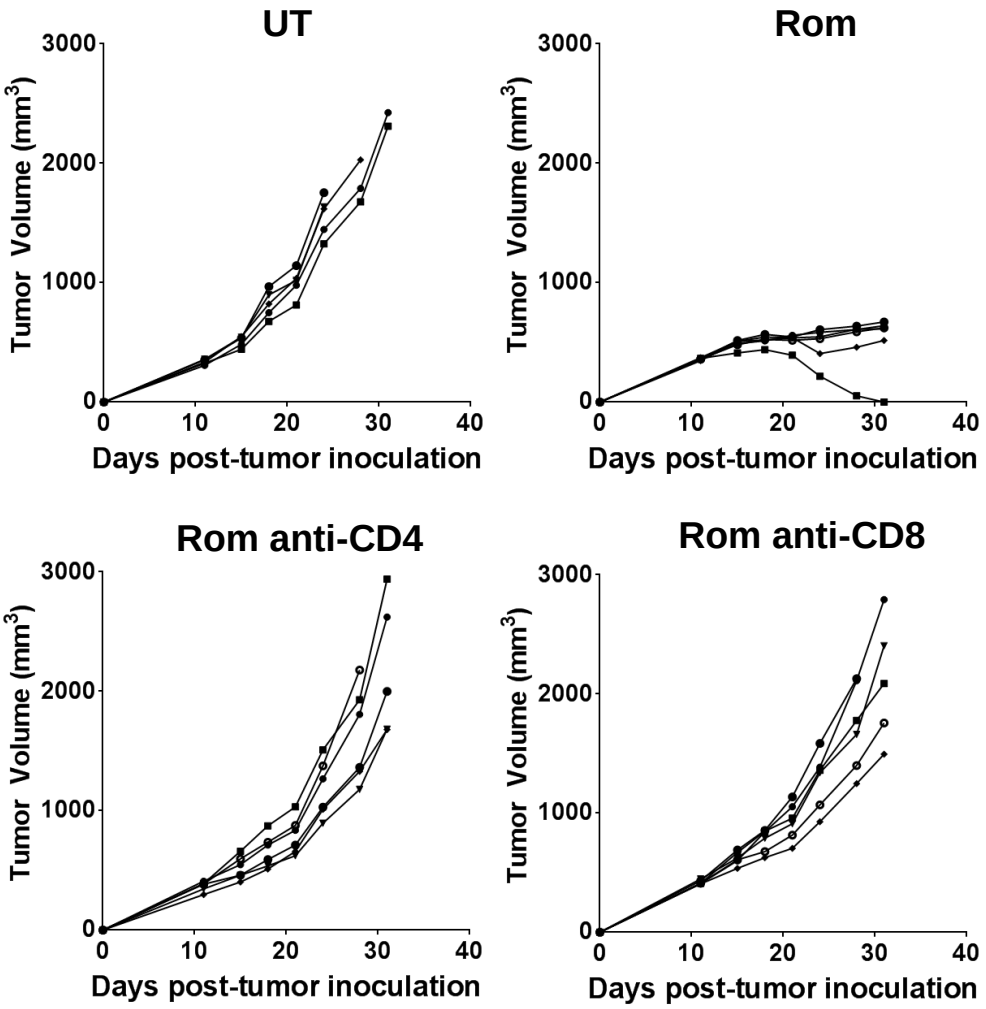


Fig. S10. 129 mice were inoculated s.c. with 10^6 LKR cells. Effect of romidepsin (Rom) treatment (2mg/kg on days 14,16,18) on tumor growth over indicated time periods is shown. Where indicated, depleting antibodies to CD4 or CD8 T cells were injected. UT: untreated mice.

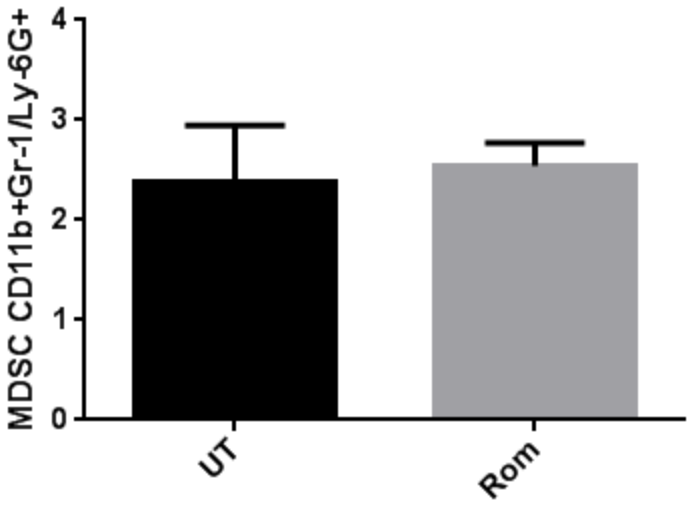


Fig. S11. 129 mice were i.p. injected with 2mg/kg romidepsin twice. 48h after the last injection, MDSC cells ($CD11b^+$ $Gr1^+$ $Ly\text{-}6G^+$) from blood of mice (n=3) were analyzed by FACS.

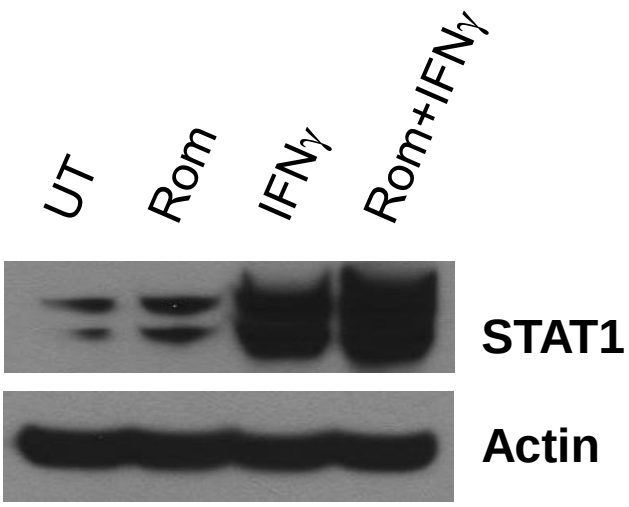


Fig. S12. Western blot analysis of LKR cells treated with Romidepsin (Rom) and/ or IFNγ as indicated. STAT1 levels are shown in whole cell lysates and β-actin levels are indicated as controls