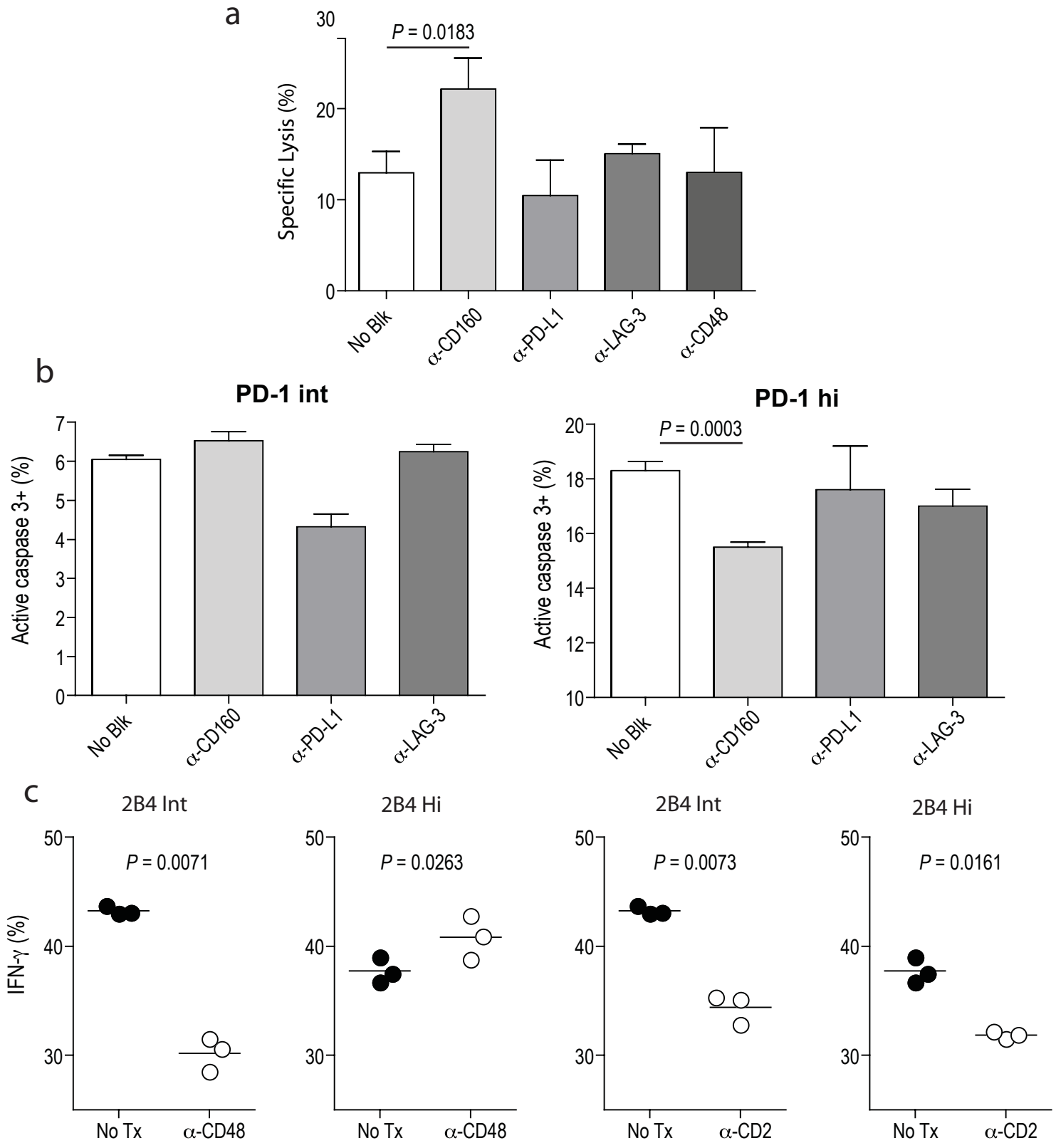
Supplemental Figure 4 Blackburn et al



Supplemental Figure 4. In vitro blockade of inhibitory receptors. (a) A VITAL killing assay was performed using gp33-41 pulsed targets and magnetic bead purified CD8$^+$ splenocytes from LCMV clone 13 infected mice d30 p.i. The assay was performed in the presence or absence of anti-PD-L1 (clone 10F.9G2), anti-CD160 (clone CNX46-3), anti-LAG-3 (clone C9B7W), or anti-CD48 (clone BCM1) with an effector to target ratio of 4:1 for 18h. Representative of four independent experiments with n=3 mice per experiment (b) Splenocytes from clone 13 infected mice were incubated at 37 C for 5 h with or without anti-PD-L1, anti-CD160, or anti-LAG-3 (10ug/ml) and active caspase 3 was detected in the live cell subset by flow cytometry. Representative of two independent experiments with n=3-4 mice per experiment. (c) Splenocytes from clone 13 infected mice were stimulated with LCMV peptides (pooled) and incubated at 37 C for 5 h with or with out anti-CD48 or anti-CD2. Cytokine production was measured by ICS. Representative of two independent experiments with n=3 mice per experiment. Blockade of CD48 on functional LCMV-specific memory CD8$^+$ T cells that are uniformly 2B4^{lo} or 2B4^{int} reduced cytokine production in this assay (data not shown).