Article Title: Expression of the SLAM family of receptors adaptor EAT-2 as a novel strategy for enhancing beneficial immune responses to vaccine antigens

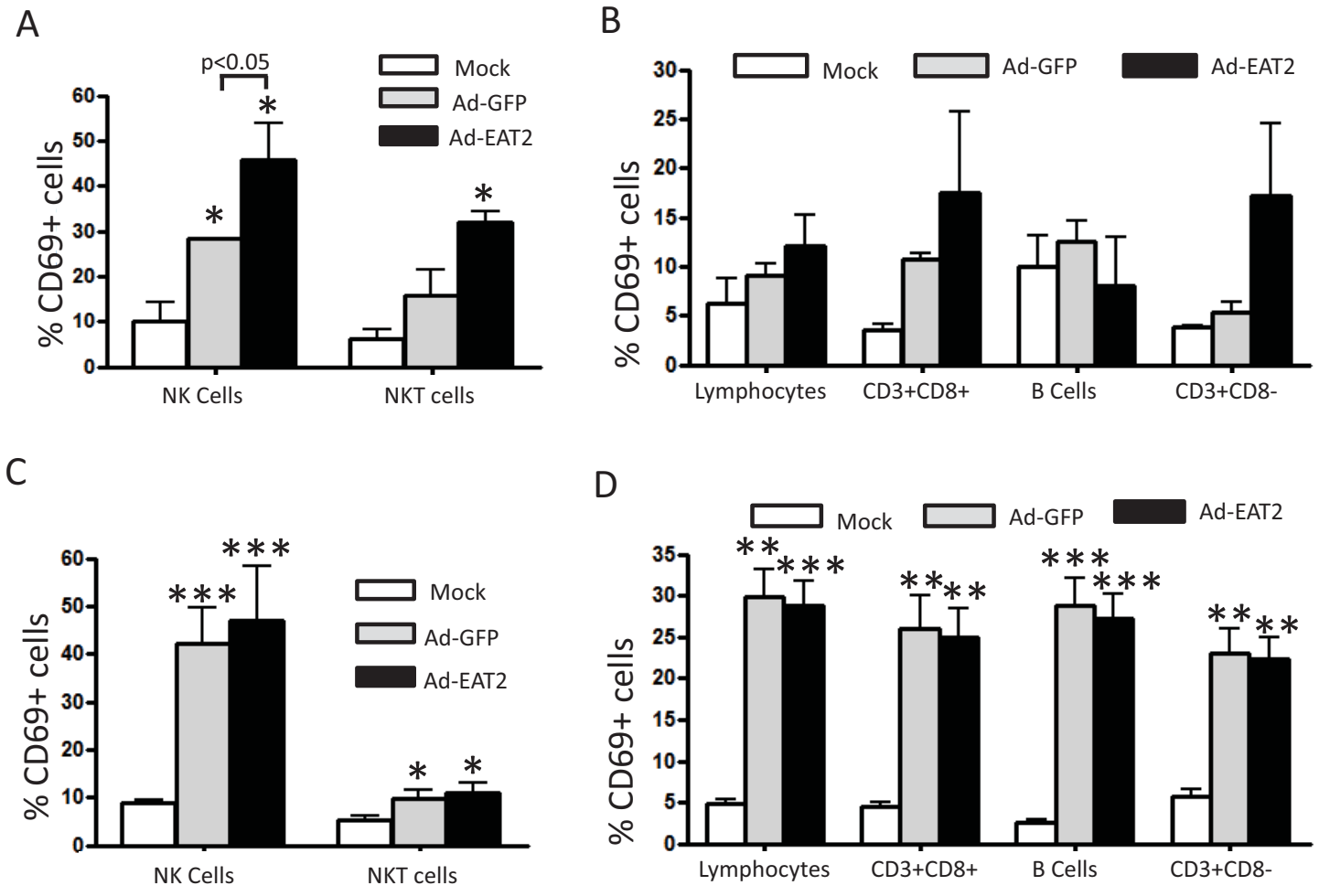
Supplementary Figure 1: Ad-EAT2-mediated activation of innate and adaptive immune cells *in vivo.* C57BL/6 mice (n=4) were either mock injected, or intravenously injected with 7.5 × 10^{10} vps of either Ad-GFP or Ad-EAT2. CD69 expression by PBMCs (a and b) and splenocyte (c and d) derived NK, NKT, $CD3^{+}CD8^{+}$ T cells, $CD3^{+}CD8^{-}$ T cells, and B cells was evaluated 48h after virus injection. PBMCs and Splenocytes were harvested, stained and sorted on a LSRII flow cytometer. The bars represent mean ± SD. Statistical analysis was completed using One Way ANOVA with a student- Newman-Keuls post-hoc test, p<0. 05 was deemed a statistically significant difference. * denotes p<0. 05, ** denotes p<0. 01, *** denotes p<0. 001 statistically different from mock injected animals.

Supplementary Figure 2: IFNγ production from NK cells 6 and 48 hours after Ads injection. C57BL/6 mice (n=4) were either mock injected, or intravenously injected with 7. 5 × 10^{10} vps of either Ad-GFP or Ad-EAT2 for 6 hpi (a) or 48 hpi (b). Splenocytes were harvested and incubated at 37°C for 5 hours in the presence of Golgi plug. IFNγ intracellular staining was performed and cells were sorted on a LSRII flow cytometer. The bars represent mean ± SD. Statistical analysis was completed using One Way ANOVA with a student- Newman-Keuls post-hoc test, p<0. 05 was deemed a statistically significant difference. * denotes p<0. 05, *** denotes p<0. 001 statistically different from mock injected animals.

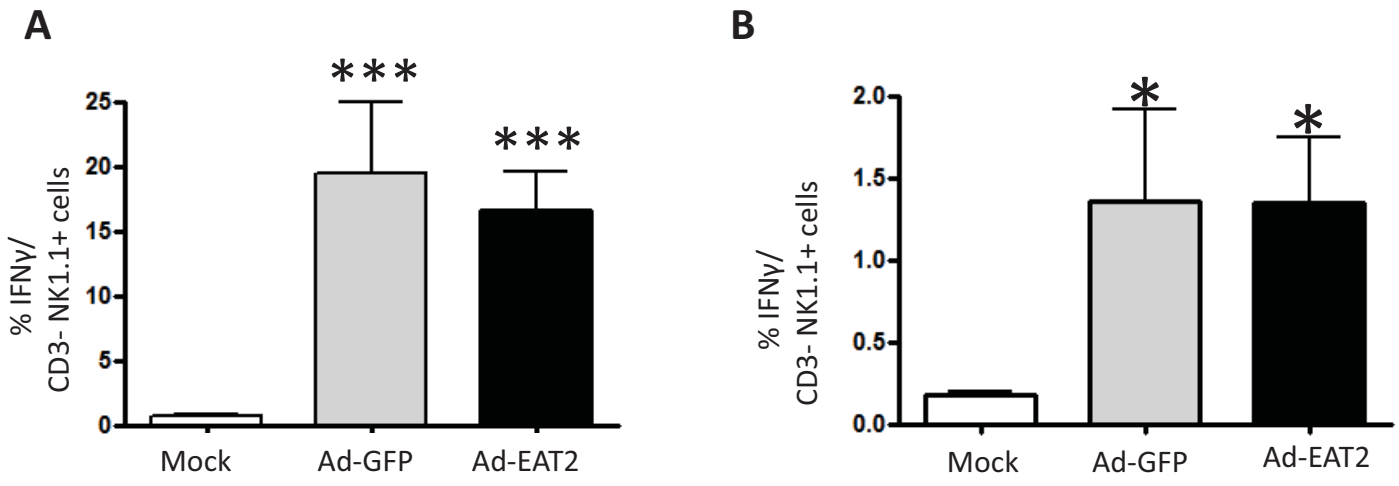
Supplementary Figure 3: Analysis of T cell epitope responses of Balb/c and C57Bl/6 mice to HIV-Gag in Ad-HIV/Gag and Ad-EAT2 co-injected mice. Balb/c (n=6) (a) or C57BL/6 (n=4) (b) mice were co-immunized with equivalent viral particles of Ad-HIV/Gag mixed with either Ad-GFP or Ad-EAT2 (1×10^7 total vps for Balb/c and 1×10^9 total vps for C57Bl/6 mice). At 14 dpi splenocytes were equivalently pooled and IL-2 ELISPOT analysis was carried out by stimulating individual wells *ex vivo* with a pool of 2-4 15mer peptides overlapped by 11, not including peptides included in Figure 4 and 5. SFCs per million splenocytes are shown. The minimal threshold response is indicated by the line above 10.

Supplementary Figure 4: Cellular immune responses after $CD8^+$ T cells depletion in Ad-HIV/Gag and Ad-EAT2 co-immunized mice. At 14 dpi, splenocytes from vaccinated Balb/c mice (1×10^7 total vps) were equivalently pooled (N=6 mice per treatment) and CD8+ cells were depleted using magnetic beads. 5×10^5 splenocytes were added to each well and stimulated with the immunodominant peptide AMQMLKETI. (a) A representative flow cytometric analysis before and after CD8 T cell depletion is shown. Spots from $CD8^+$ un-depleted cells and $CD8^+$ depleted cells were quantified using an automated ELISPOT reader (b and c). %SFC that are CD8- = (#SFCs CD8 dep / #SFCs CD8+)*100 (d). The bars represent mean ± SD. Statistical analysis was completed using student's t-test.

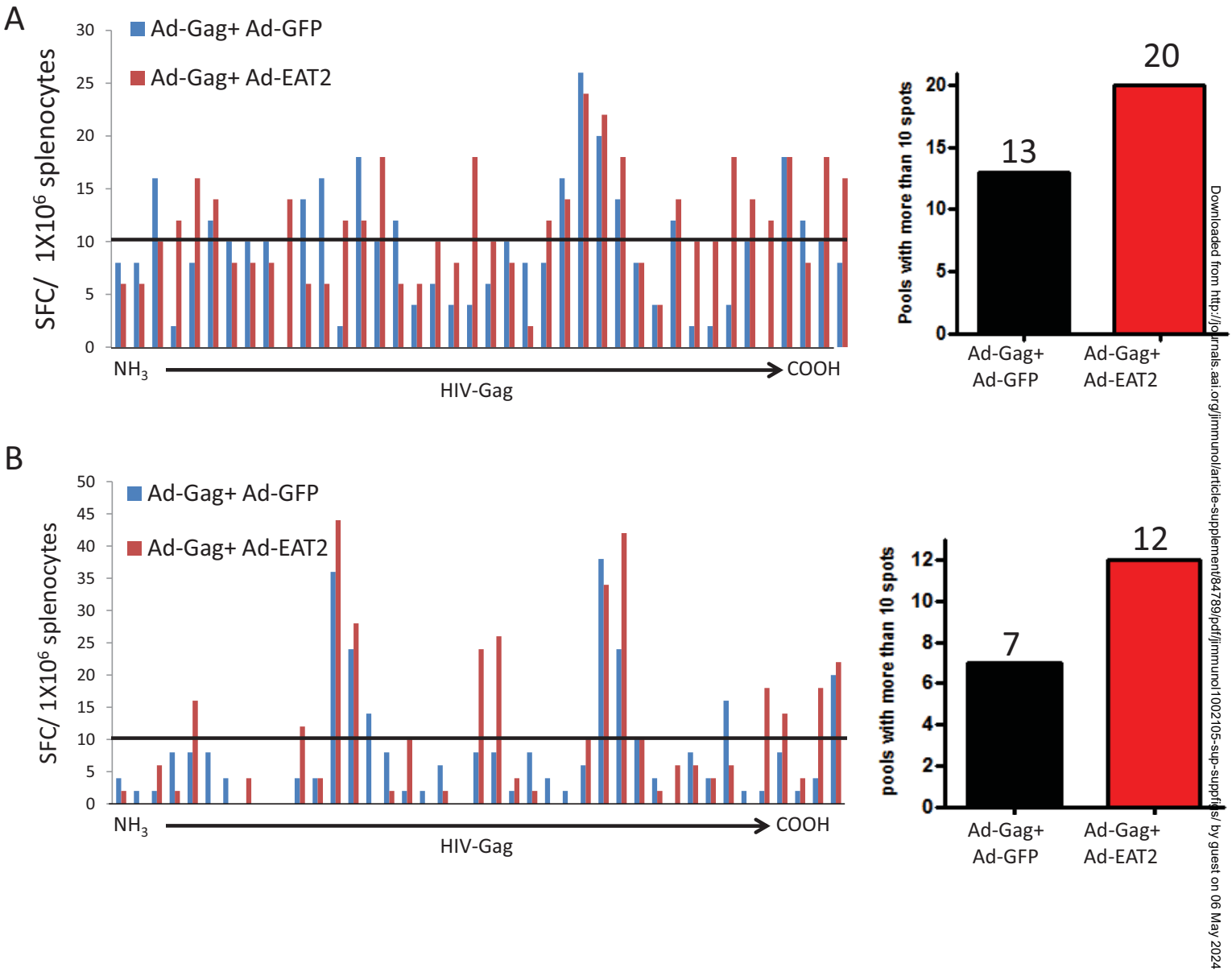
Supplementary Figure: 1



Supplementary Figure: 2



Supplementary Figure: 3



Supplementary Figure: 4