cancers

Supplementary Materials: TGFβ Imprinting During Activation Promotes Natural Killer Cell Cytokine Hypersecretion

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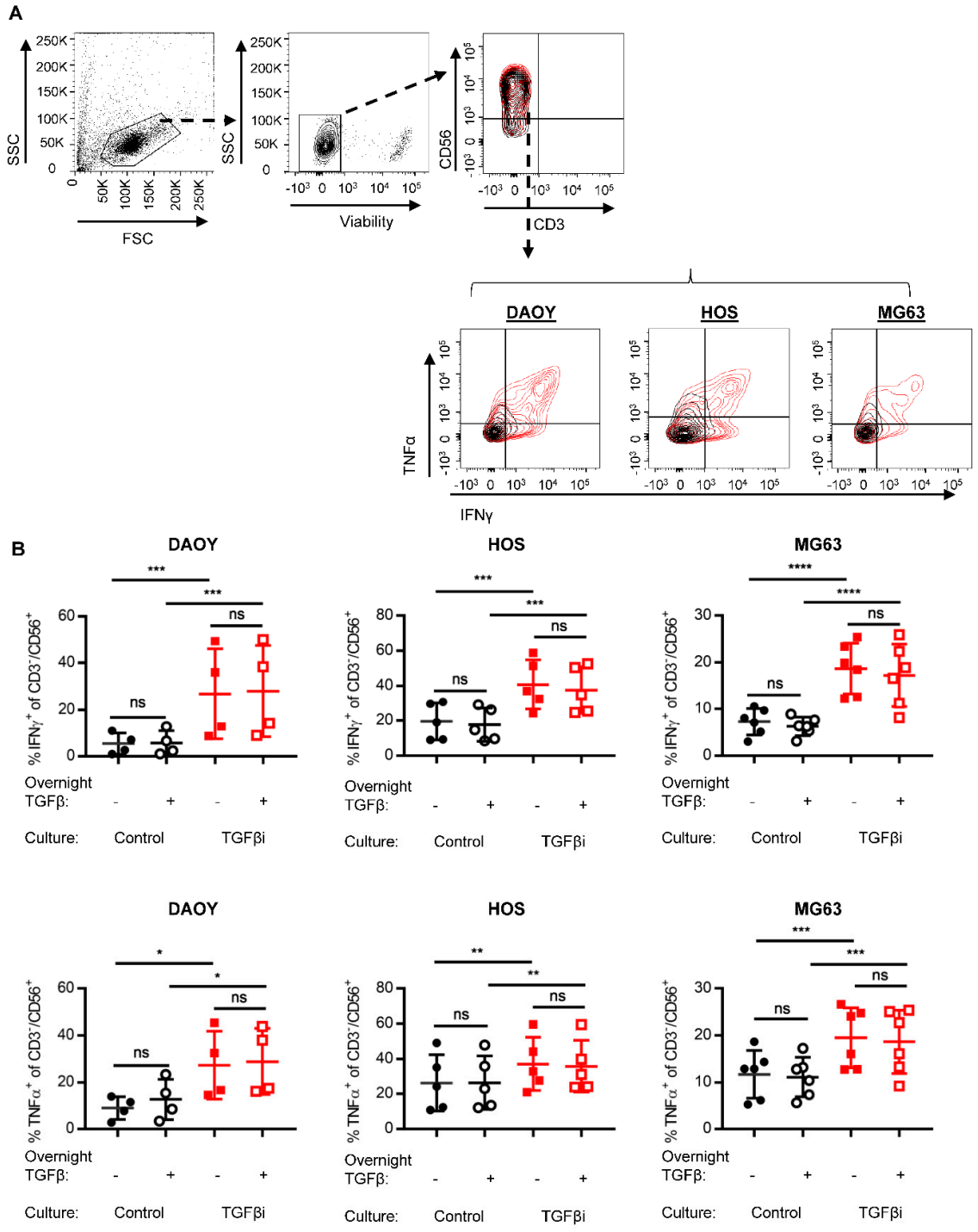

Figure S1. Related to Figure 1. TGFβi NK cells have increased anti-tumor IFNγ+ and TNFα+ NK cells. (**A**) Representative flow gating depicting IFNγ+ and TNFα+ against DAOY, HOS, and MG63. Control in black, TGFβi in red. (**A**) TGFβi increases anti-tumor IFNγ+ and (**B**) TNFα+ at baseline and in the

presence of additional TGFβ. Individual data points depicted. Lines and Bars are Mean ± SD. Statistical differences were determined by two-way repeated measures ANOVA with Holm-Sidak's multiple comparisons test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

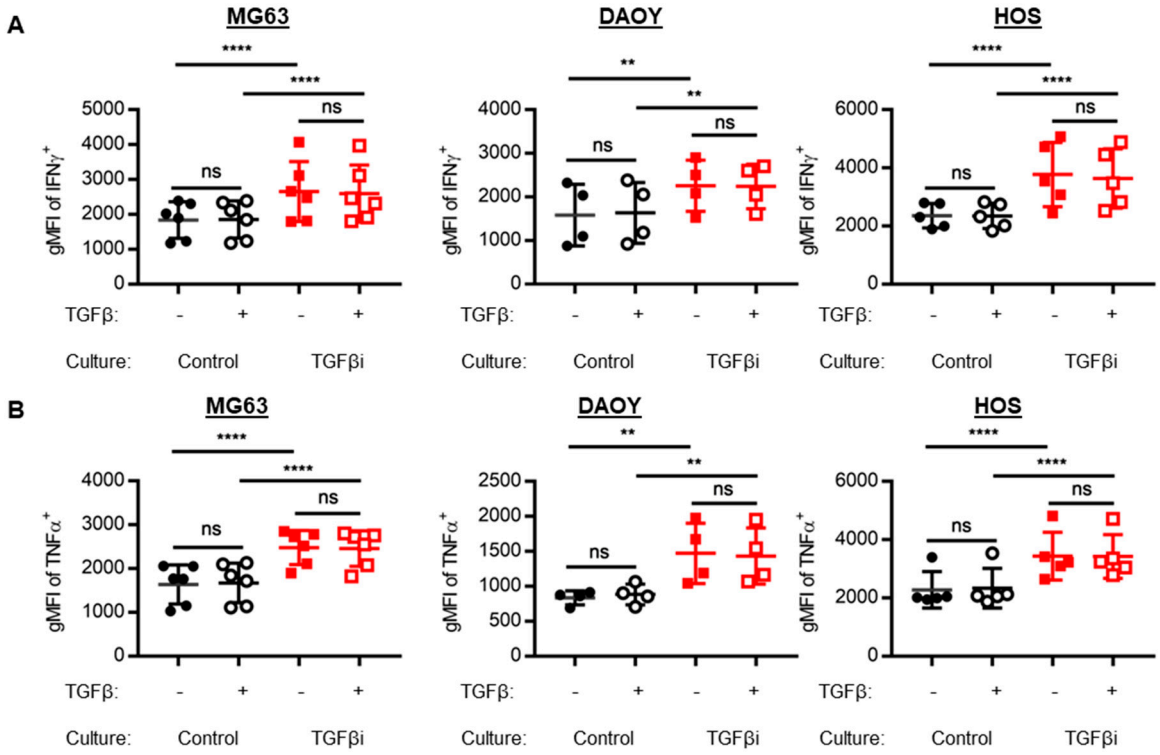

Figure S2. Related to Figure 1. TGFβ imprinting increases intensity of IFNγ and TNFα production. (**A**) TGFβi NK cells have increased geometric mean (gMFI) of IFNγ and (**B**) TNFα. Individual data points depicted. Lines and bars represent Mean ± SD. Statistical differences were determined by two-way repeated measures ANOVA with Holm-Sidak's multiple comparisons test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

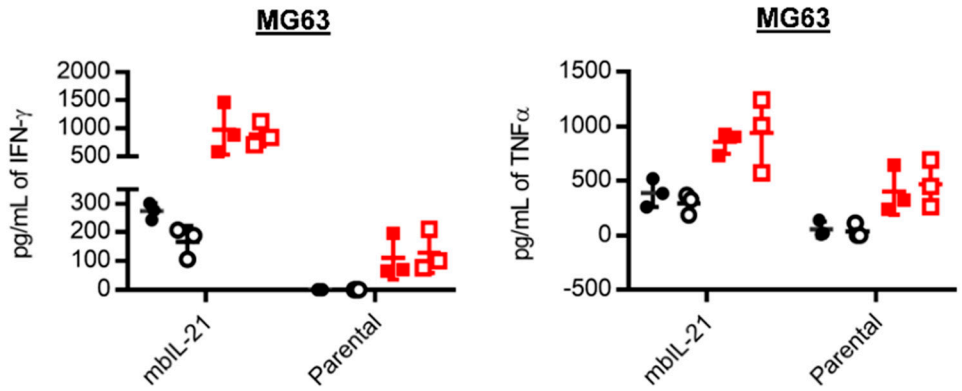

Figure S3. Differential IFNγ and TNFα secretion induced by parental K562 versus K562mbIL-21 feeder cell expansion. NK cells were expanded from identical donors for 2 weeks on either parental K562 or K562mbIL-21 plus IL-2 (Control, black) or IL-2 plus TGFβ (TGFβi, red). At the end of 2 weeks, NK cells were rested overnight in IL-2 only (solid symbols) or IL-2 plus TGFβ (open symbols) and cultured with MG63 for 3 h. Cytokine secretion was measured in the supernatants using CBA analysis ($n = 3$). Individual data points depicted. Lines and bars represent Mean ± SD.

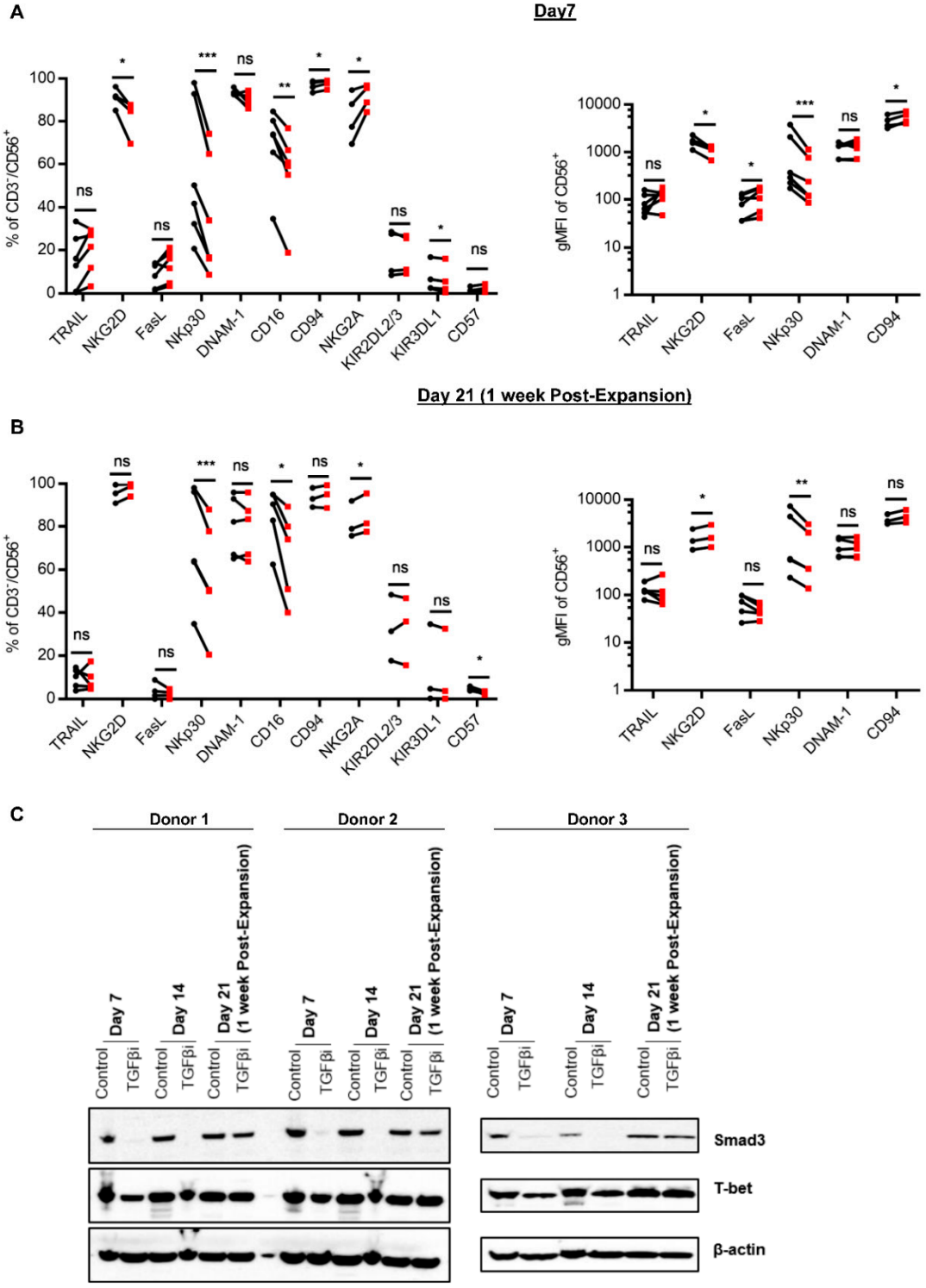

Figure S4. Phenotypic analysis of TGFβi NK cells at Day 7, 14 and 21. Control and TGFβi NK cell receptor repertoire was assessed using flow cytometry at (**A**) Day 7 and (**B**) one week following removal from TGFβ at Day 21 (n = 3–6). (**C**) SMAD3 and T-bet expression was similarly measured by western blot at Day 7, Day 14 and Day 21. Individual data points depicted. Lines connect donors. Statistical differences were determined by paired t-test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$. Related to Figures 4&6.

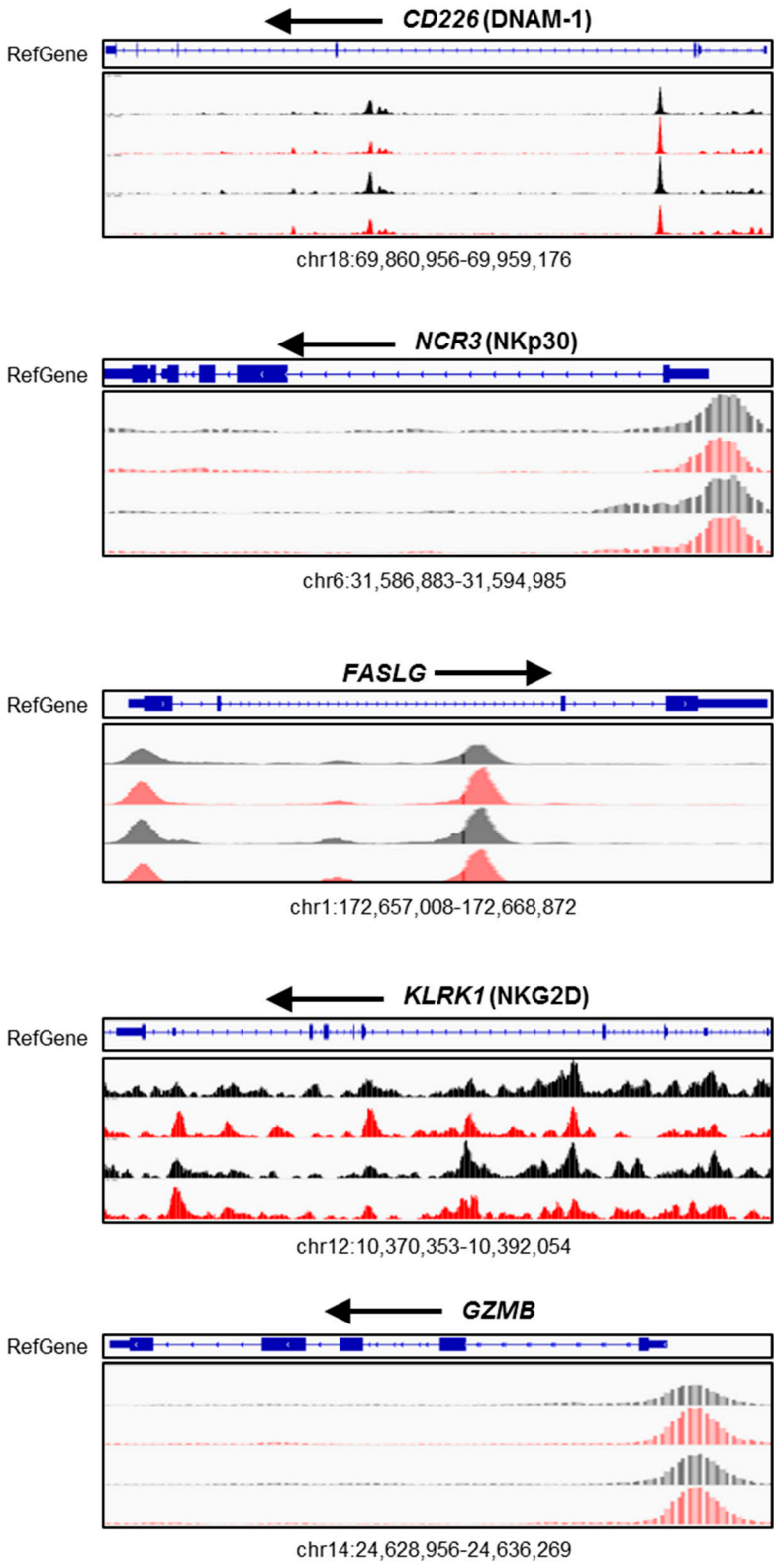

Figure S5. ATAC-seq results for TGFβ imprinted NK cell phenotype. Control in black. TGFβi in red. $n = 2$. Related to Figures 4 & 5.

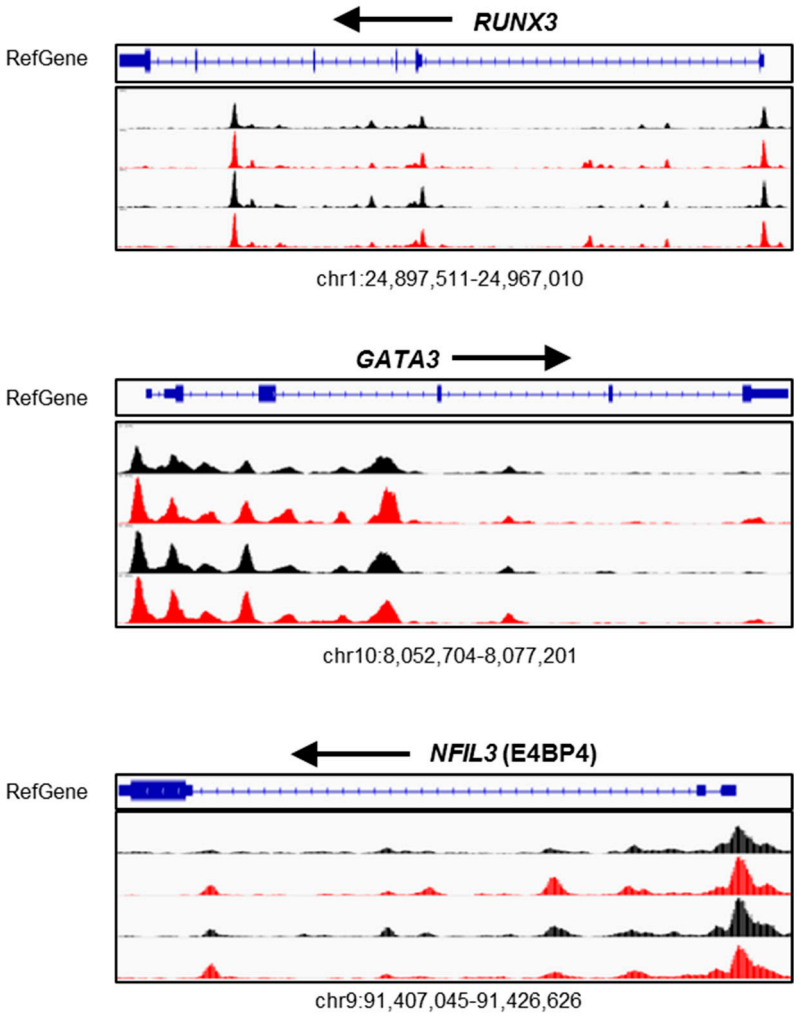

Figure S6. ATAC-seq results for TGFβ imprinted NK cell transcription Factors. Control in black. TGFβi in red. $n = 2$. Related to Figures 6.