

1223 **SUPPLEMENTAL FIGURE LEGENDS**

1224 **Figure S1. CAR Tregs are not cytotoxic towards epithelial cells.** (A) CD80 surface  
1225 expression on monocyte-derived dendritic cells (moDCs) 4 days after co-incubation with  
1226 No Act Tregs, TCR Tregs or CAR Tregs. Representative histogram on the left and  
1227 summary data on the right. (B) CD86 surface expression on moDCs 4 days after co-  
1228 incubation with No Act Tregs, TCR Tregs or CAR Tregs. Representative histogram on the  
1229 left and summary data on the right. (C) Teff cytotoxicity towards target NALM6 cells at  
1230 different effector to target (E:T) ratios. (D) Treg and Teff cytotoxicity towards target CD19-  
1231 A549 cells at different E:T ratios.

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1233 **Figure S2. Inflammatory gene and gene pathways upregulated by CAR activation**  
1234 **in Tregs.** (A) WikiPathways gene set enrichment analysis (GSEA) of CAR Tregs vs. TCR  
1235 Tregs. FDR, false discovery rate. (B) Heatmap of CAR Treg and TCR Treg  
1236 proinflammatory and profibrotic mediator gene expression, as determined by RNA-seq.

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1238 **Figure S3. Chemokine receptor gene expression levels in No Act Tregs, TCR Tregs,**  
1239 **CAR Tregs, No Act Teff, TCR Teff, and CAR Teff.** Violins represent mean  $\pm$  SD of RNA-  
1240 seq values from different blood donors.

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1242 **Figure S4. Cytokine secretion levels by No Act Tregs, TCR Tregs, CAR Tregs, No**  
1243 **Act Teff, TCR Teff, and CAR Teff.** Values represent technical replicates of  
1244 representative experiments. Bars represent mean  $\pm$  SD. One-way ANOVA test with

1245 Tukey's multiple comparison correction. \*\*\*\*,  $p < 0.0001$ ; \*\*\*,  $p < 0.001$ ; \*\*,  $p < 0.01$ ; \*,  $p$   
1246  $< 0.05$ ; ns, not significant.

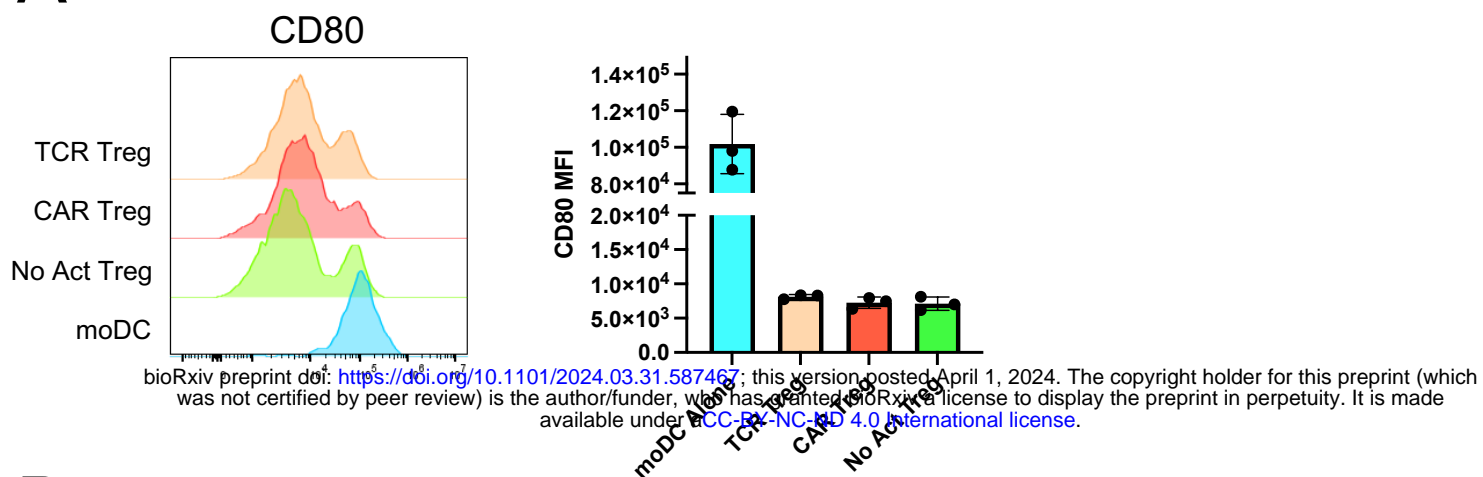
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1248 **Figure S5. CAT Tregs have lower inflammatory gene expression levels than CAR**  
1249 **Tregs.** (A) Heatmap clustered by column (sample) and by row (gene) with top 100 most  
1250 differentially expressed genes between No Act Tregs, TCR Tregs, CAR Tregs, CAT  
1251 Tregs, and PY3 Tregs. (B) Venn diagram with genes upregulated in TCR Tregs, CAR  
1252 Tregs, CAT Tregs, and PY3 Tregs in relation to their respective No Act cell types. Number  
1253 of genes and respective percentage of the total number of genes are indicated in each  
1254 intersection. (C) Venn diagram with genes upregulated in CAT Tregs and in PY3 Tregs.  
1255 (D) Inflammatory, cytotoxic, and chemokine receptor gene expression levels in No Act  
1256 Tregs, TCR Tregs, CAR Tregs, CAT Tregs, and PY3 Tregs. Violins represent mean  $\pm$  SD  
1257 of RNA-seq values from different blood donors.

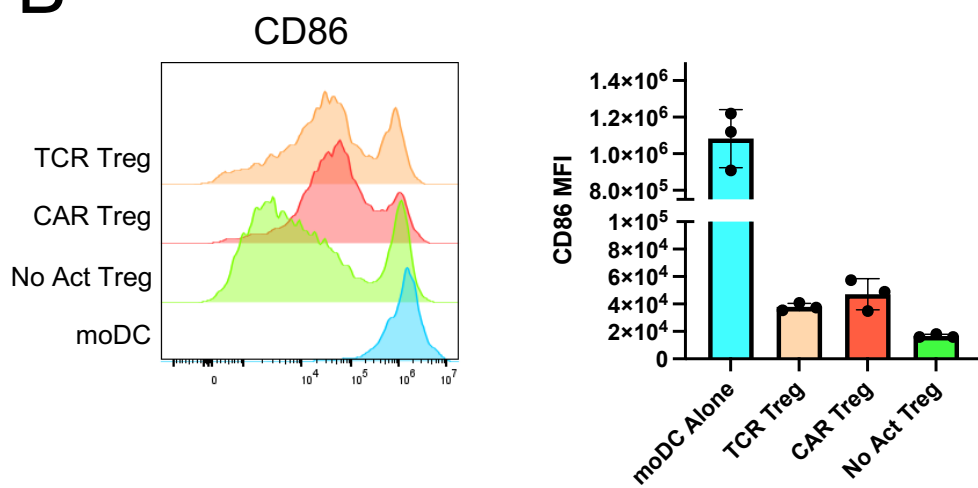
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1259 **Figure S6. Cytokine secretion levels by No Act Tregs, TCR Tregs, CAR Tregs, and**  
1260 **CAT Tregs.** Values are the mean  $\pm$  SD of technical triplicates per blood donor. One-way  
1261 ANOVA test with Tukey's multiple comparison correction. \*\*\*\*,  $p < 0.0001$ ; \*\*\*,  $p < 0.001$ ;  
1262 \*\*,  $p < 0.01$ ; \*,  $p < 0.05$ ; ns, not significant.

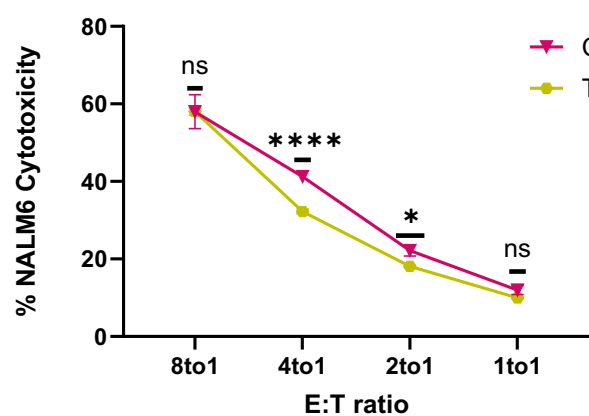
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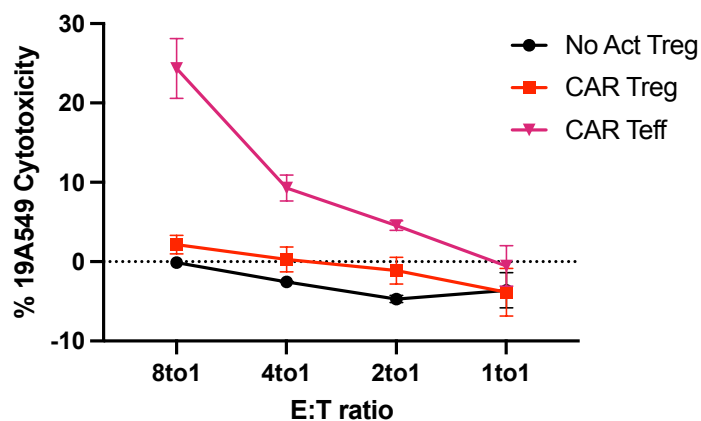
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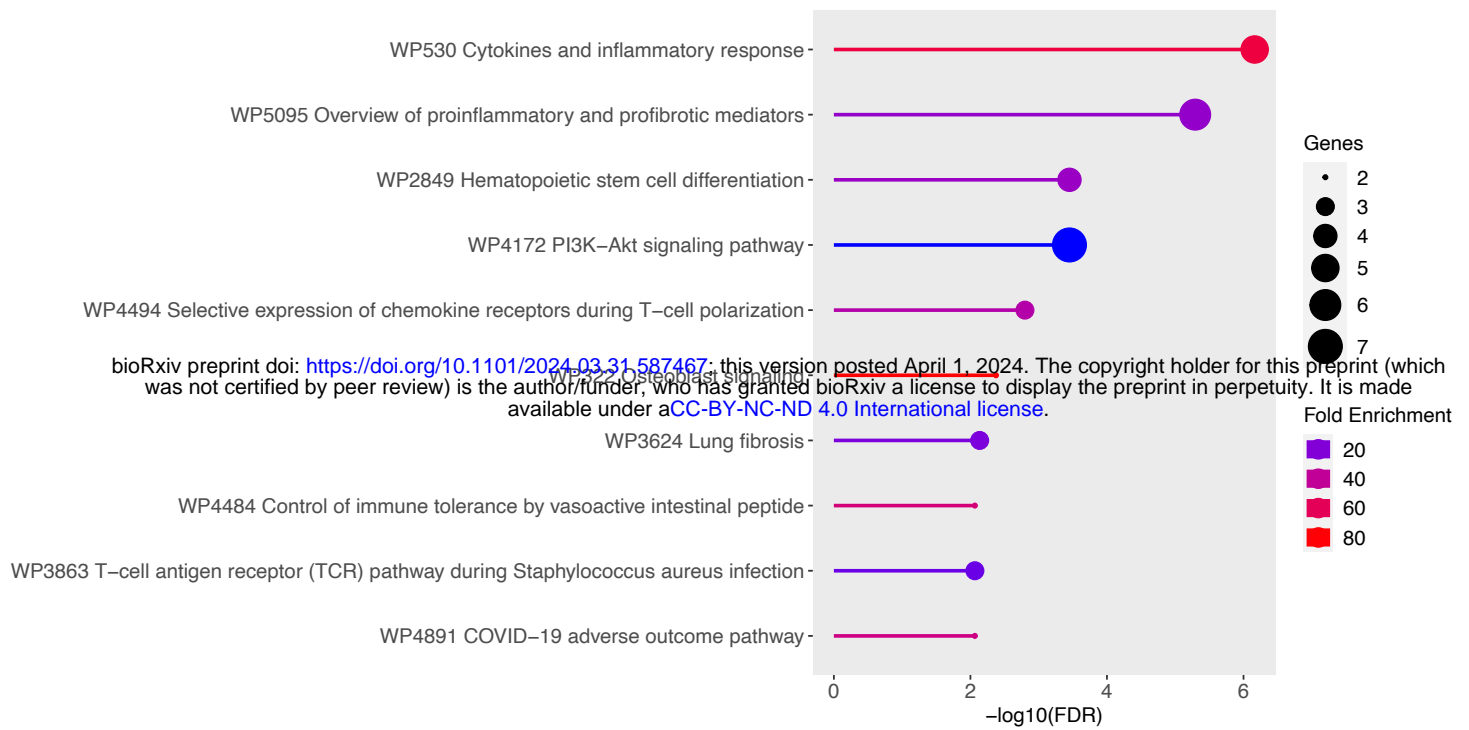
C



D



A



B

