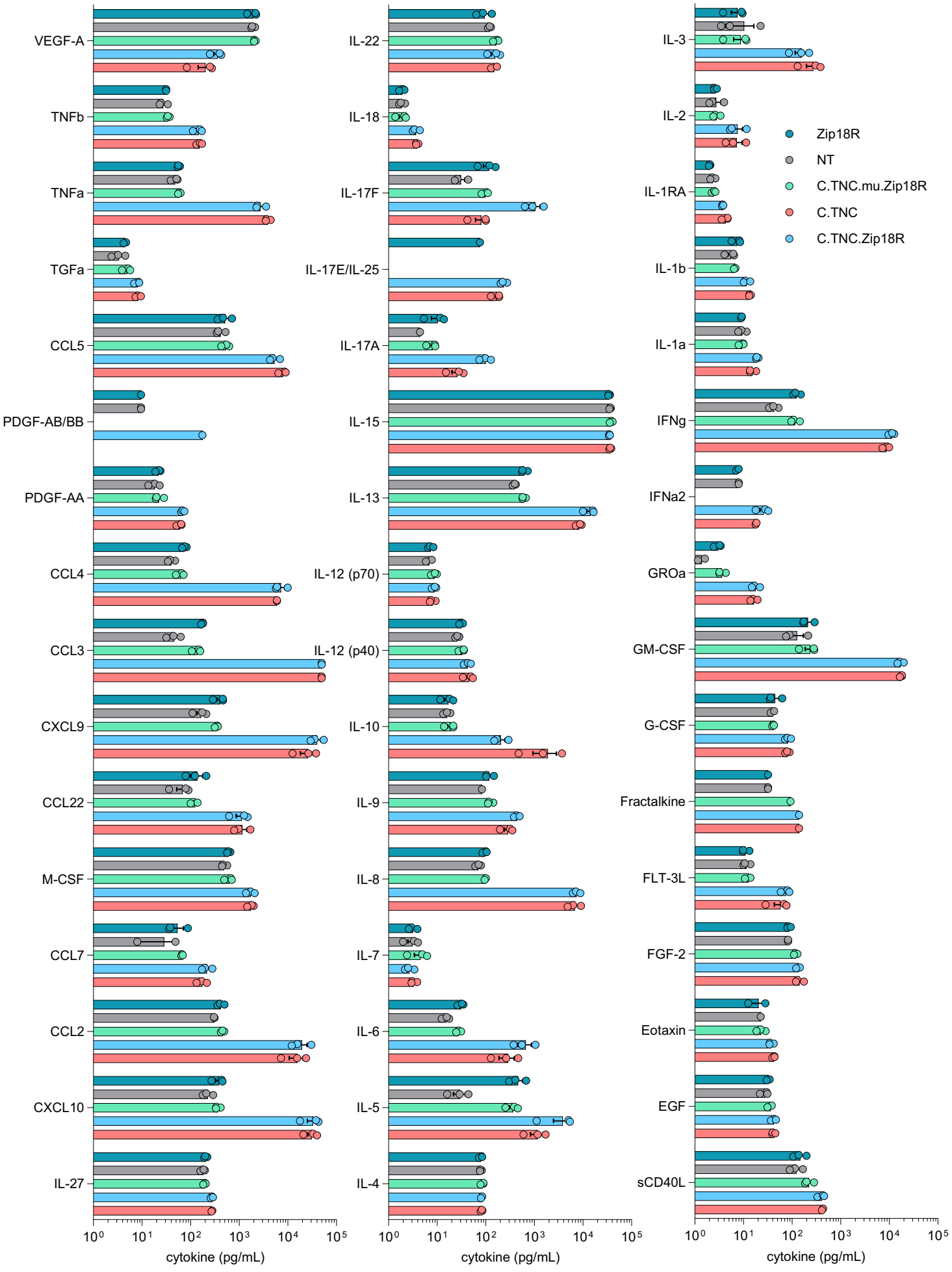
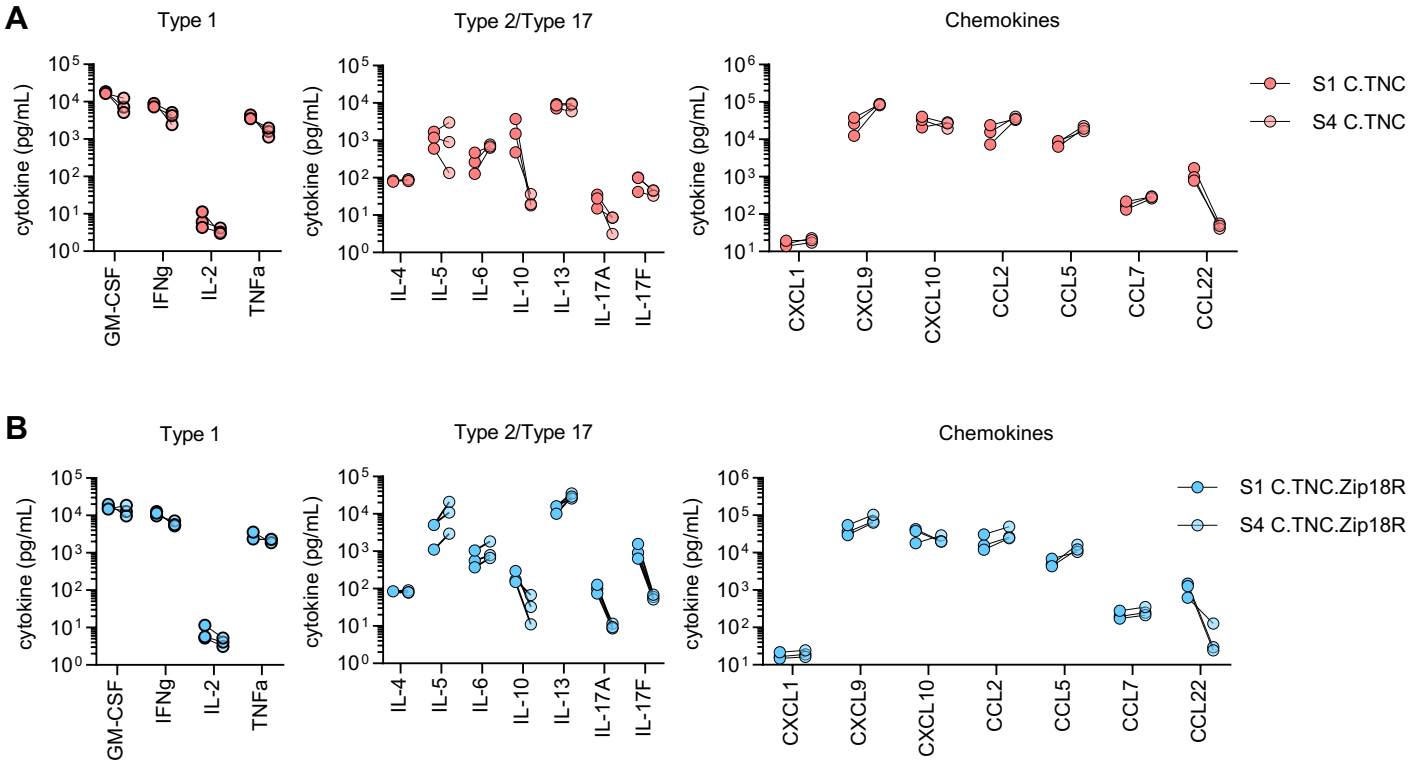
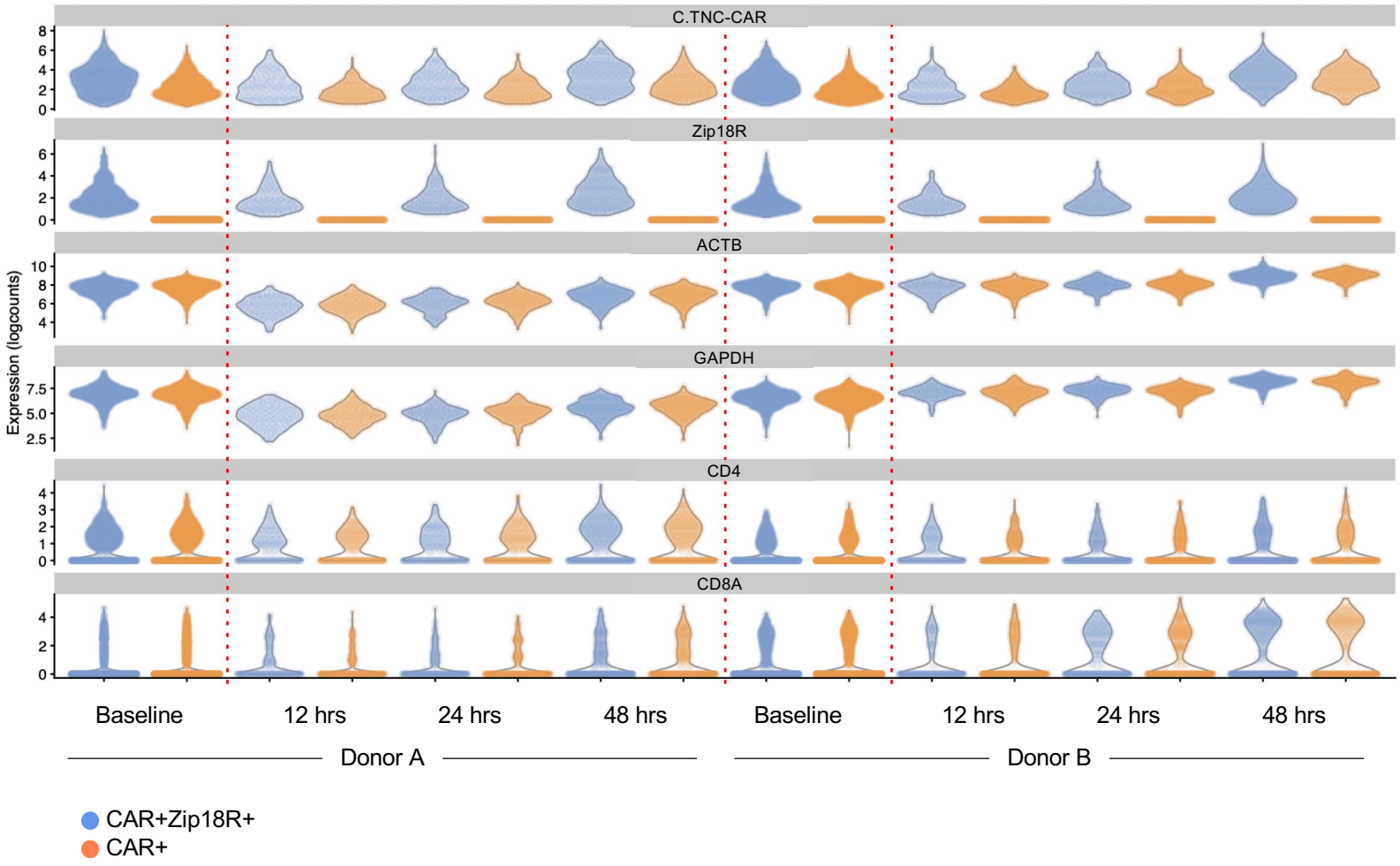
Supplemental Figure 11



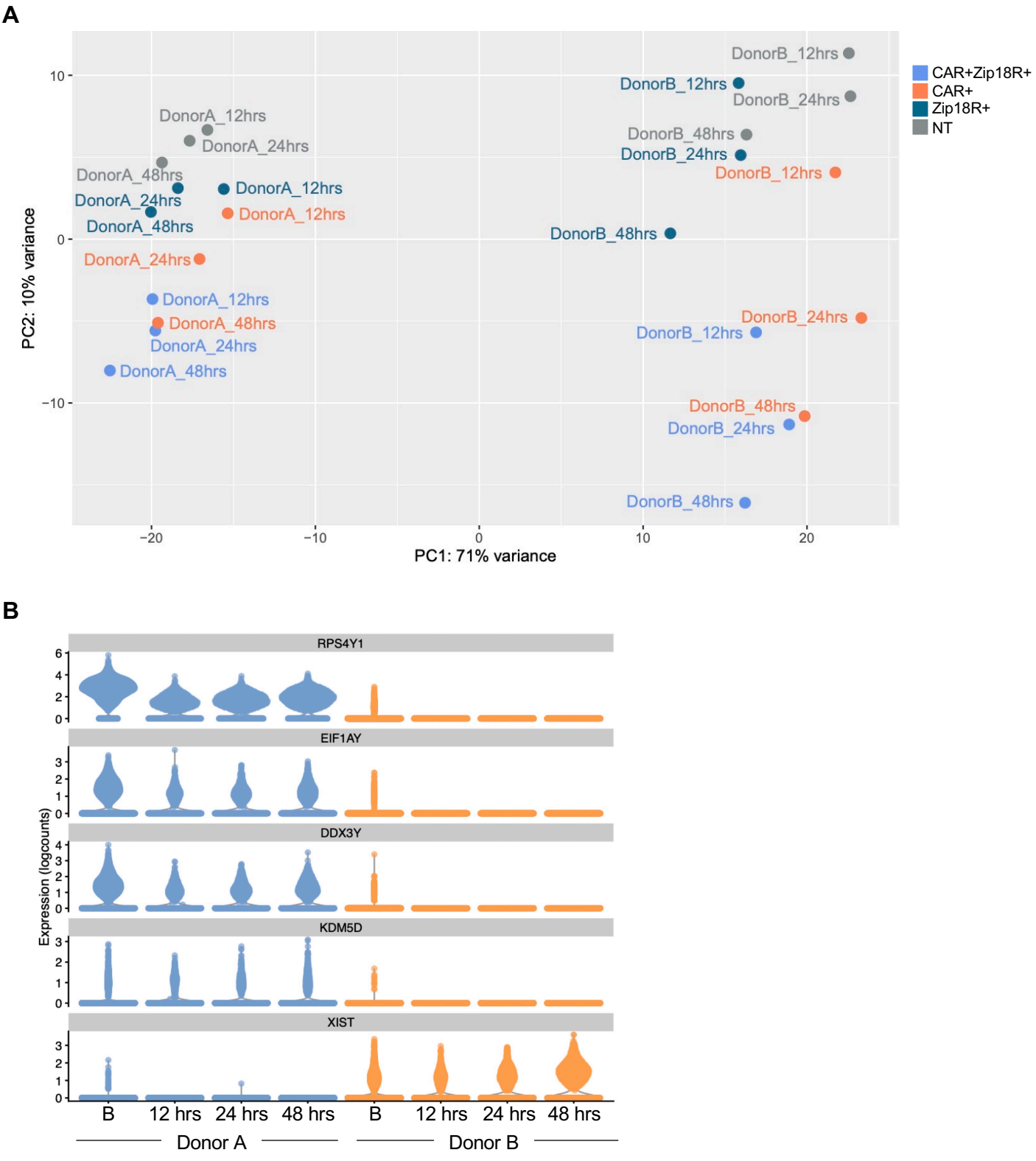
SFig 11. Influence of Zip18R expression in CAR T cells on cytokines, chemokines, and growth factor production post tumor cell stimulation. Extended data set for experiment shown in Figure 4E-G. Cytokines, chemokines, and growth factors determined by 48-plex MILLIPLEX analysis 48 hours post stimulation. CCL-3 and CCL-4 values are capped at maximum assessable value for C.TNC and C.TNC.Zip18R (n=3, undetectable values not reported).



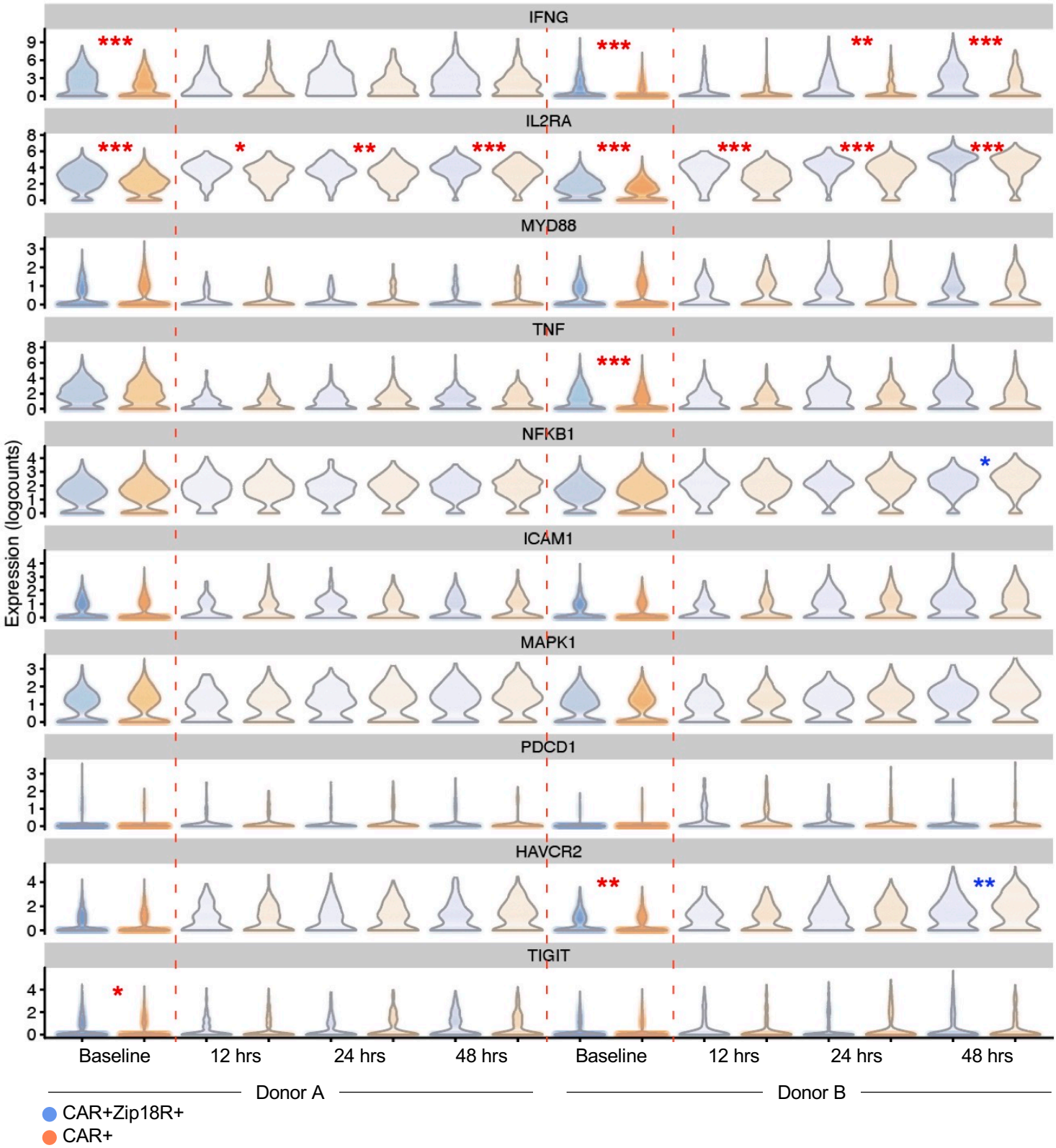
SFig 12. Zip18R.C.TNC-CAR T cells maintain Type 1 effector cytokine production after 4th stimulation *in vitro*. The summary data post 1st and 4th stimulation is shown in Figure 4H-J. Individual Type 1 cytokines, Type 2/Type 17 cytokines, and chemokines for (**A**) C.TNC-CAR and (**B**) C.TNC-CAR.Zip18R T cells. S1: 1st stimulation, S4: 4th stimulation.



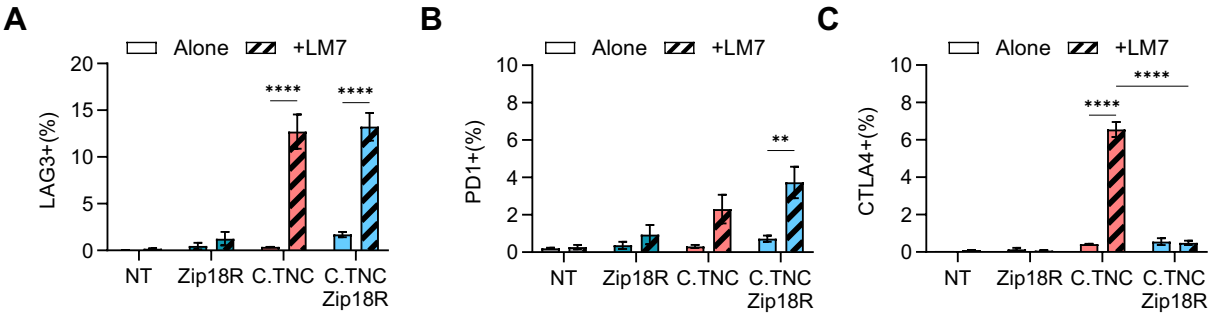
SFig 13. Breakdown of transgene expression. Gene expression in C.TNC-CAR.Zip18R and C.TNC-CAR populations for selected genes: C.TNC-CAR, Zip18R, housekeeping genes (ACTB, GAPDH), CD4, and CD8.



SFig 14. Gender differences in donor selection. (**A**) Principal component analysis of pseudo-bulk data of cell populations from scRNAseq data. (**B**) Gene expression in cell populations for gender-specific genes. B: baseline.



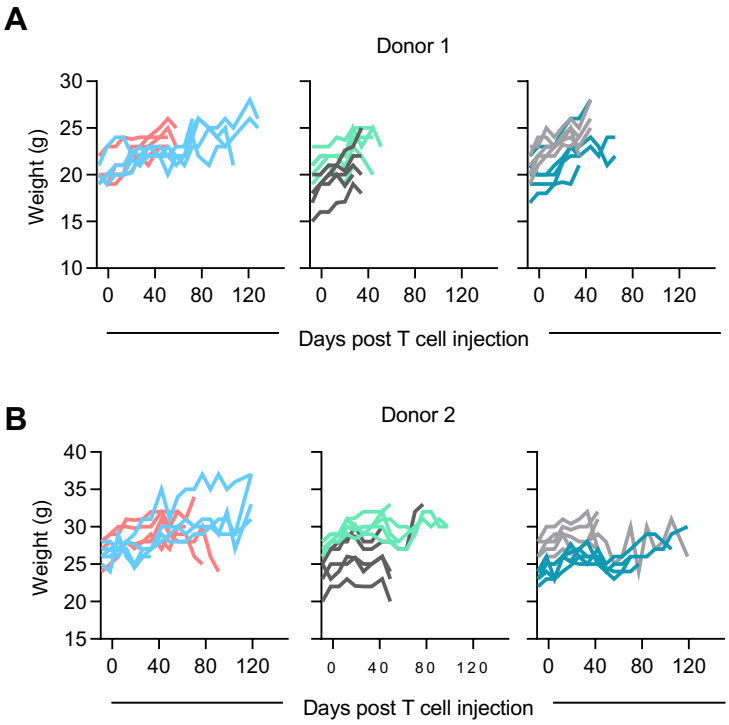
SFig 15. Pathway specific gene expression analysis. Gene expression in cell populations for selected genes involved in T cell activation, T cell exhaustion, and MyD88 signaling. Red asterisks are upregulated in CAR+Zip18R+ compared to CAR+Zip18R-. Blue asterisks are downregulated in CAR+Zip18R+ compared to CAR+Zip18R-. *: adjusted p<0.1; **:adjusted p<0.01; ***:adjusted p<0.001.



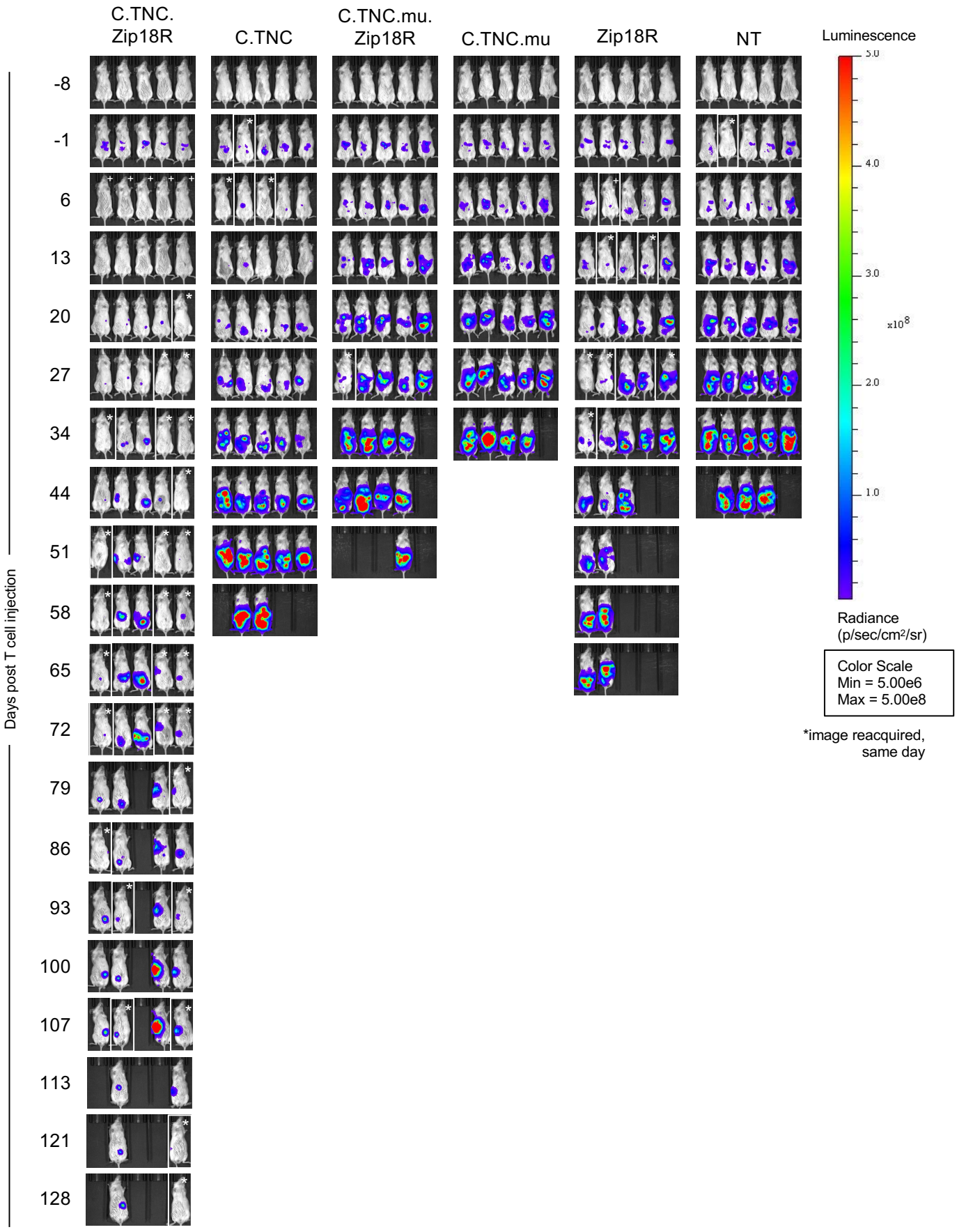
D

	CD28		CD39		CD69		CTLA4		LAG3		PD1		TIM3	
NT:Alone vs. NT:+LM7	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	0.6854
NT:Alone vs. Zip18R:Alone	ns	>0.9999	*	0.0343	ns	0.5708	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	0.6676
NT:Alone vs. Zip18R:+LM7	ns	>0.9999	*	0.0343	ns	0.1516	ns	>0.9999	ns	0.9702	ns	0.9389	***	0.0003
NT:Alone vs. C.TNC:Alone	ns	0.9963	ns	0.1758	ns	0.0898	ns	0.7699	ns	>0.9999	ns	>0.9999	**	0.0059
NT:Alone vs. C.TNC:+LM7	**	0.0024	****	<0.0001	****	<0.0001	****	<0.0001	****	<0.0001	ns	0.0719	****	<0.0001
NT:Alone vs. C.TNC. Zip18R:Alone	ns	>0.9999	****	<0.0001	ns	0.8955	ns	0.4223	ns	0.871	ns	0.9908	****	<0.0001
NT:Alone vs. C.TNC. Zip18R:+LM7	ns	0.5314	****	<0.0001	****	<0.0001	ns	0.5702	****	<0.0001	**	0.001	****	<0.0001
NT:+LM7 vs. Zip18R:Alone	ns	>0.9999	*	0.0123	ns	0.6491	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	>0.9999
NT:+LM7 vs. Zip18R:+LM7	ns	>0.9999	*	0.0123	ns	0.1885	ns	>0.9999	ns	0.984	ns	0.9604	**	0.007
NT:+LM7 vs. C.TNC:Alone	ns	0.9864	ns	0.0657	ns	0.0707	ns	0.8594	ns	>0.9999	ns	>0.9999	ns	0.1451
NT:+LM7 vs. C.TNC:+LM7	**	0.0018	****	<0.0001	****	<0.0001	****	<0.0001	****	<0.0001	ns	0.0854	****	<0.0001
NT:+LM7 vs. C.TNC. Zip18R:Alone	ns	>0.9999	****	<0.0001	ns	0.8417	ns	0.5269	ns	0.9102	ns	0.9956	****	<0.0001
NT:+LM7 vs. C.TNC. Zip18R:+LM7	ns	0.4317	****	<0.0001	****	<0.0001	ns	0.6803	****	<0.0001	**	0.0012	****	<0.0001
Zip18R:Alone vs. Zip18R:+LM7	ns	>0.9999	ns	>0.9999	ns	0.9762	ns	>0.9999	ns	0.9977	ns	0.9827	**	0.0074
Zip18R:Alone vs. C.TNC:Alone	ns	0.582	ns	>0.9999	**	0.0024	ns	0.9306	ns	>0.9999	ns	>0.9999	ns	0.1528
Zip18R:Alone vs. C.TNC:+LM7	***	0.0004	****	<0.0001	****	<0.0001	****	<0.0001	****	<0.0001	ns	0.1112	****	<0.0001
Zip18R:Alone vs. C.TNC. Zip18R:Alone	ns	0.8915	*	0.0485	ns	0.0852	ns	0.6456	ns	0.9698	ns	0.999	****	<0.0001
Zip18R:Alone vs. C.TNC. Zip18R:+LM7	ns	0.0954	****	<0.0001	****	<0.0001	ns	0.7909	****	<0.0001	**	0.0016	****	<0.0001
Zip18R:+LM7 vs. C.TNC:Alone	ns	0.9797	ns	>0.9999	***	0.0004	ns	0.8209	ns	0.9953	ns	0.9694	ns	0.7355
Zip18R:+LM7 vs. C.TNC:+LM7	**	0.0016	****	<0.0001	****	<0.0001	****	<0.0001	****	<0.0001	ns	0.4371	****	<0.0001
Zip18R:+LM7 vs. C.TNC. Zip18R:Alone	ns	0.9999	*	0.0485	*	0.0142	ns	0.4779	ns	>0.9999	ns	>0.9999	ns	0.2063
Zip18R:+LM7 vs. C.TNC. Zip18R:+LM7	ns	0.3974	****	<0.0001	****	<0.0001	ns	0.6302	****	<0.0001	**	0.0091	****	<0.0001
C.TNC:Alone vs. C.TNC:+LM7	*	0.0425	****	<0.0001	****	<0.0001	****	<0.0001	****	<0.0001	ns	0.0934	****	<0.0001
C.TNC:Alone vs. C.TNC. Zip18R:Alone	ns	>0.9999	**	0.0091	ns	0.5882	ns	0.9983	ns	0.9554	ns	0.9972	*	0.0106
C.TNC:Alone vs. C.TNC. Zip18R:+LM7	ns	>0.9999	****	<0.0001	****	<0.0001	ns	>0.9999	****	<0.0001	**	0.0013	****	<0.0001
C.TNC:+LM7 vs. C.TNC. Zip18R:Alone	*	0.0159	ns	0.1256	****	<0.0001	****	<0.0001	****	<0.0001	ns	0.2753	****	<0.0001
C.TNC:+LM7 vs. C.TNC. Zip18R:+LM7	ns	0.3221	ns	>0.9999	ns	0.9998	****	<0.0001	ns	0.9999	ns	0.3904	ns	0.9993
C.TNC. Zip18R:Alone vs. C.TNC. Zip18R:+LM7	ns	0.9891	*	0.0357	****	<0.0001	ns	>0.9999	****	<0.0001	**	0.0047	****	<0.0001

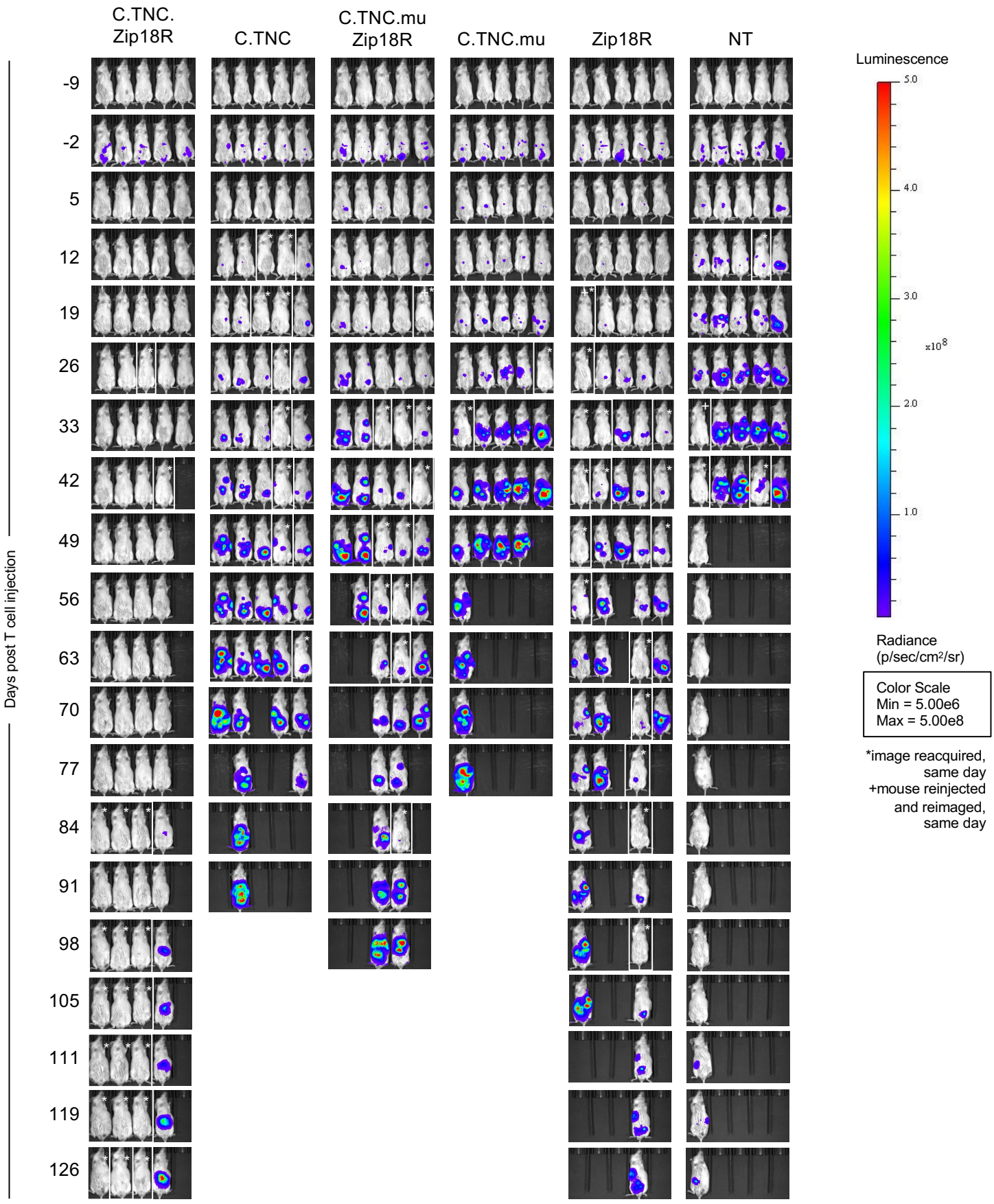
SFig 16. Exhaustion marker expression via flow cytometry. 1x10^6 NT, Zip18R, C.TNC-CAR, and C.TNC-CAR.Zip18R T cells were cultured in media or against LM7 cells at a 2:1 E:T ratio for 48 hours and then collected for flow cytometric analysis. (**A-C**) Expression of (**A**) LAG3, (**B**) PD1, and (**C**) CTLA4 on T cells gated on CD3 (NT), Zip18R+, C.TNC-CAR+, and C.TNC-CAR.Zip18R+ (n=3, mean + SEM), 2-way ANOVA, *:p<0.05, **:p<0.01, ***:p<0.001, ****:p<0.0001. (**D**) P-values from 2-way ANOVA analysis of Fig 5D-H and SFig 16A-C.



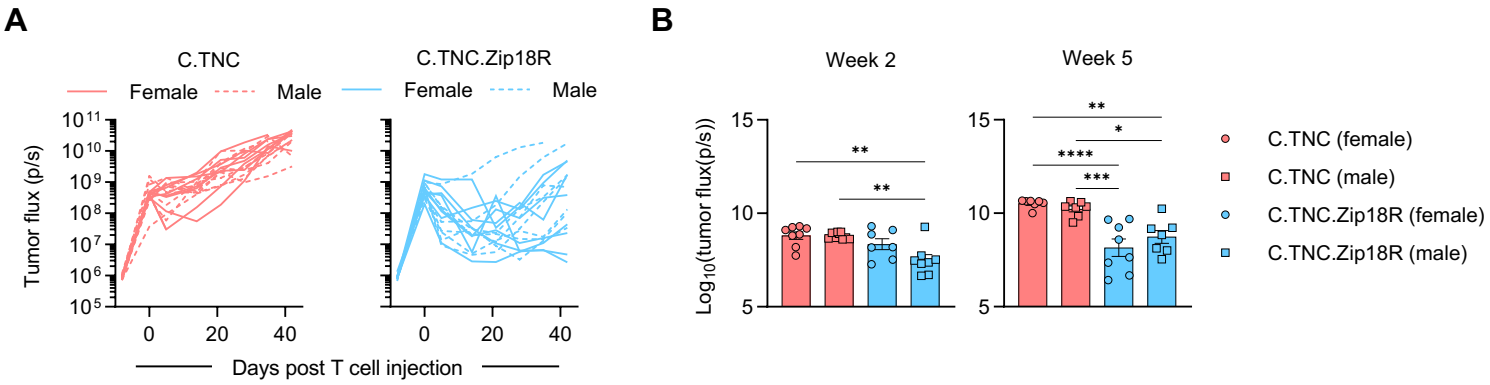
SFig 17. Weight of mice for animal experiment shown Figure 5. Body weights for each cohort measured weekly for (**A**) Donor 1 and (**B**) Donor 2 (n=5 per cohort per donor).



SFig 18. Bioluminescence images for animal experiment shown in Figure 5 – Donor 1. Bioluminescence images of mice.

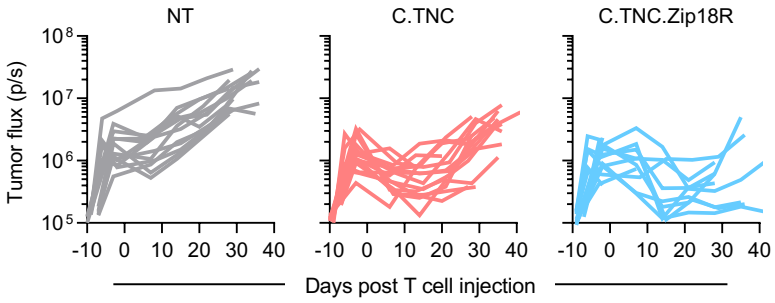


SFig 19. Bioluminescence images for animal experiment shown in Figure 5 – Donor 2. Bioluminescence images of mice.

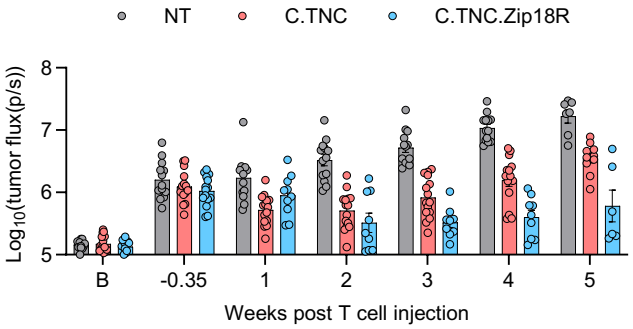


SFig 20. Gender does not impact the antitumor activity of C.TNC-CAR.Zip18R T cells *in vivo*. 1x10^6 LM7.GFP.ffLuc cells were injected intraperitoneally (i.p.), followed by 1x10^6 sorted T cells injected i.p. 7 days later in 5–6-week-old female and male NSG mice. Plots contain data from two biological donors. (**A**) Tumor flux values (n=8-10 per cohort). (**B**) Log-transformed tumor flux values summarized from A at weeks 2 and 5 (n=7-10, mean + SEM). One-way ANOVA, *:p<0.05, **:p<0.01, ***:p<0.001, ****:p<0.0001.

A



B



C

Post T cell injection (weeks)	Comparison	p value
B	NT vs C.TNC	0.9941
	NT vs C.TNC.Zip18R	0.9492
	C.TNC vs C.TNC18R	0.9045
-0.35	NT vs C.TNC	0.5853
	NT vs C.TNC.Zip18R	0.2295
	C.TNC vs C.TNC18R	0.7751
1	NT vs C.TNC	<0.0001
	NT vs C.TNC.Zip18R	0.0639
	C.TNC vs C.TNC18R	0.1151
2	NT vs C.TNC	<0.0001
	NT vs C.TNC.Zip18R	<0.0001
	C.TNC vs C.TNC18R	0.2518
3	NT vs C.TNC	<0.0001
	NT vs C.TNC.Zip18R	<0.0001
	C.TNC vs C.TNC18R	0.0039
4	NT vs C.TNC	<0.0001
	NT vs C.TNC.Zip18R	<0.0001
	C.TNC vs C.TNC18R	<0.0001
5	NT vs C.TNC	<0.0001
	NT vs C.TNC.Zip18R	<0.0001
	C.TNC vs C.TNC18R	<0.0001

SFig 21. C.TNC-CAR.Zip18R T cells show anti-DIPG activity. $1x10^6$ DIPG.YFP.ffLuc cells were injected intracranially (i.c.), followed by $1x10^6$ sorted T cells injected i.c. 7 days later in 10–12-week-old male NSG mice. Plots contain data from two biological donors from 3 experiments. (**A**) Tumor flux values (n=13-15 per cohort). (**B**) Log-transformed tumor flux values summarized from A. (n=6-15, mean + SEM). B: baseline, pre-tumor image; Week -1: Days -2, -3; Week 1: Days 6-8; Week 2: Days 13-15; Week 3: Days 20-22; Week 4: Days 27-29; Week 5: Days 34-36. (**C**) Results from 2-way ANOVA analysis of B.