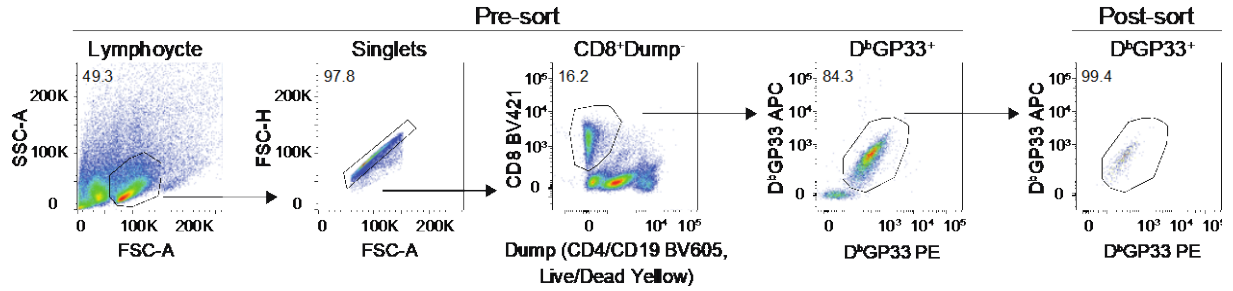**Supplementary information**

PD-1-*cis* IL-2R agonism yields better effectors from stem-like CD8 T cells

In the format provided by the authors and unedited
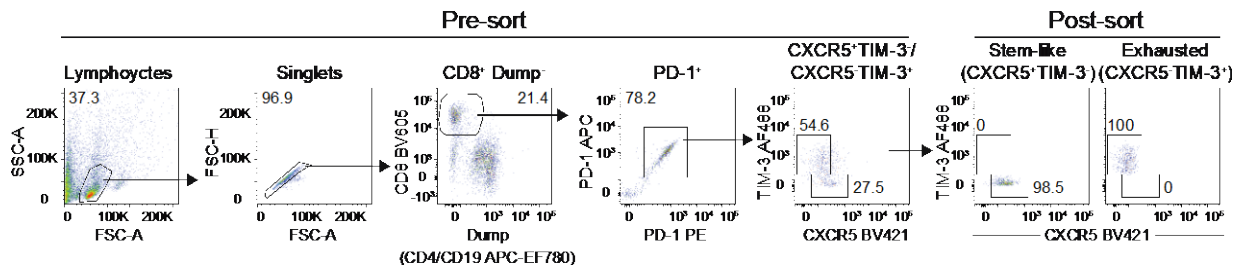
Cell Sorting

Supplementary Figure 1. Gating strategy for sorting D^{b}GP33$^+$ CD8$^+$ T cells in Fig. 2c and Extended Data Fig. 5g, h. Splenocytes isolated from chronically LCMV-infected mice were gated on lymphocytes, singlets, live CD8$^+$ T cells, and D^{b}GP33$^+$ CD8$^+$ T cells were sorted.
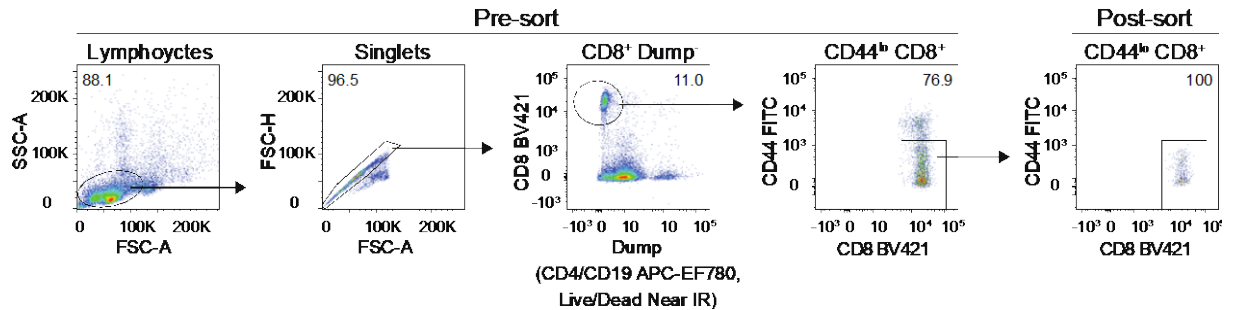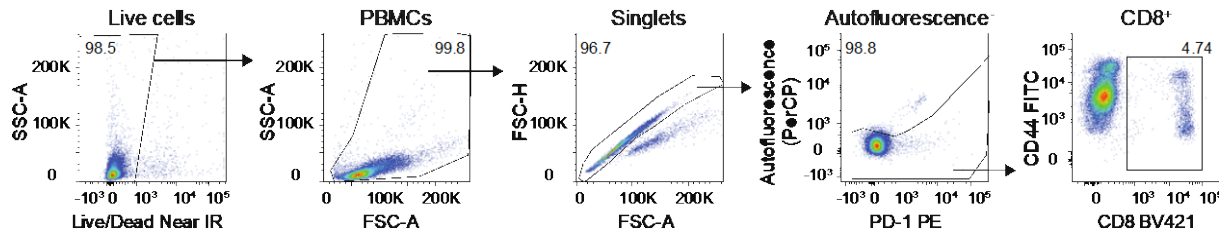

Supplementary Figure 2. Gating strategy for sorting naive CD8$^+$ T cells in Fig. 2c. Splenocytes isolated from uninfected C57BL6/J mice were gated on lymphocytes, singlets, live CD8$^+$ T cells, and CD44^{lo} CD8$^+$ T cells were sorted. EF, eFluor.
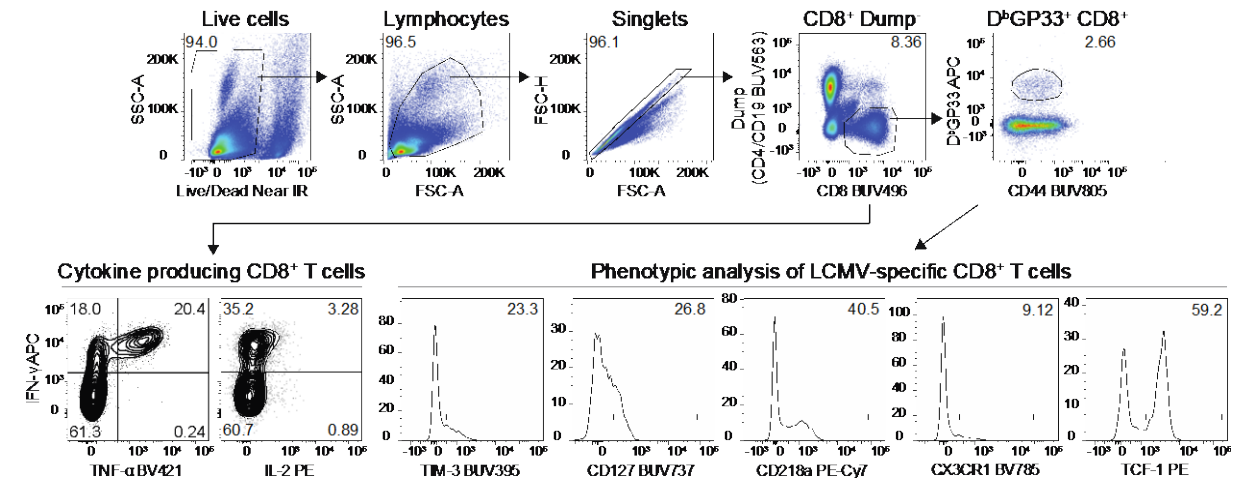

Supplementary Figure 3. Gating strategy for sorting two PD-1$^+$ CD8$^+$ T-cell subsets in Fig. 2f-h and Extended Data Fig. 6f, g. Splenocytes isolated from chronically LCMV-infected mice were gated on lymphocytes, singlets, CD8$^+$ T cells, PD-1$^+$ CD8$^+$ T cells, and PD-1$^+$CXCR5$^+$TIM-3$^-$ and PD-1$^+$CXCR5$^-$TIM-3$^+$ CD8$^+$ T cells were sorted. EF, eFluor; AF, Alexa Fluor.

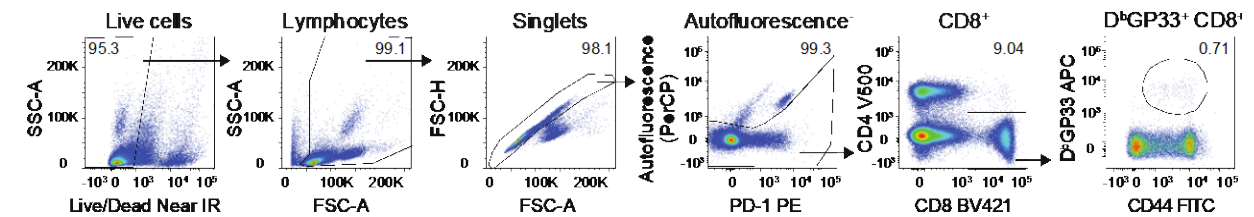**T cell analysis on CD8+ T cells**

Supplementary Figure 4. Gating strategy for CD8+ T cells in Extended Data Fig. 2b-e. PBMCs isolated from chronically LCMV-infected mice were gated on live PBMCs, singlets, autofluorescence- cells, and CD8+ T cells.
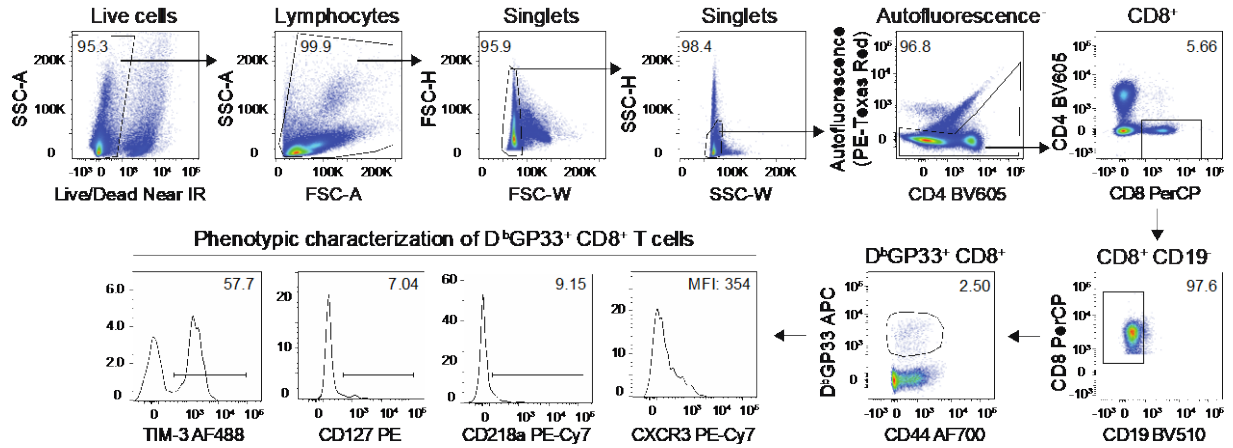
T cell analysis on LCMV-specific CD8+ T cells

Supplementary Figure 5. Gating strategy for LCMV-specific CD8+ T cells in Fig. 2a, b, d, and Extended Data Fig. 6a, b, d, e. Cells isolated from various tissues in chronically LCMV-infected mice were gated on live cells, lymphocytes, singlets, CD8+ T cells, and LCMV-specific D^bGP33+ CD8+ T cells were identified**.** Cytokine producing LCMV-specific CD8+ T cells and LCMV-specific tetramer+ CD8+ T cells were analyzed.

Supplementary Figure 6. Gating strategy for LCMV-specific CD8+ T cells in Extended Data Extended Data Fig. 5c, d. Cells isolated from various tissues in chronically LCMV-infected mice were gated on live cells, lymphocytes, singlets, autofluorescence^- cells, CD8+ T cells, and LCMV-specific D^bGP33+ CD8+ T cells were analyzed**.**
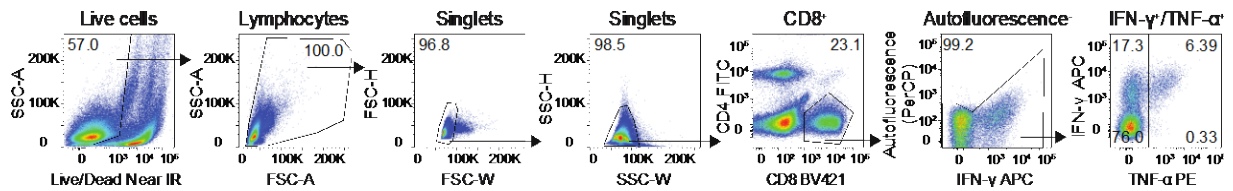
Supplementary Figure 7. Gating strategy for LCMV-specific CD8+ T cells in Extended Data Fig. 1b-d and Extended Data Fig. 5f. Cells isolated from various tissues in chronically LCMV-infected mice were gated on live cells, lymphocytes, singlets, autofluorescence- cells, CD8+ T cells, CD19- CD8+ T cells, and LCMV-specific D^{b}GP33+ CD8+ T cells were analyzed. AF, Alexa Fluor.

Supplementary Figure 8. Gating strategy for cytokine producing CD8+ T cells in Extended Data Fig. 1e, f and Extended Data Fig. 5e. Splenocytes isolated from chronically LCMV-infected mice were gated on live cells, lymphocytes, singlets, CD8+ T cells, autofluorescence- cells, and cytokine producing LCMV-specific CD8+ T cells were analyzed.

T cell analysis on D^bGP33^+ CD8$^+$ T cells, Foxp3$^+$ CD4$^+$ T cells, Foxp3$^-$ CD4$^+$ T cells, and naive CD8$^+$ T cells
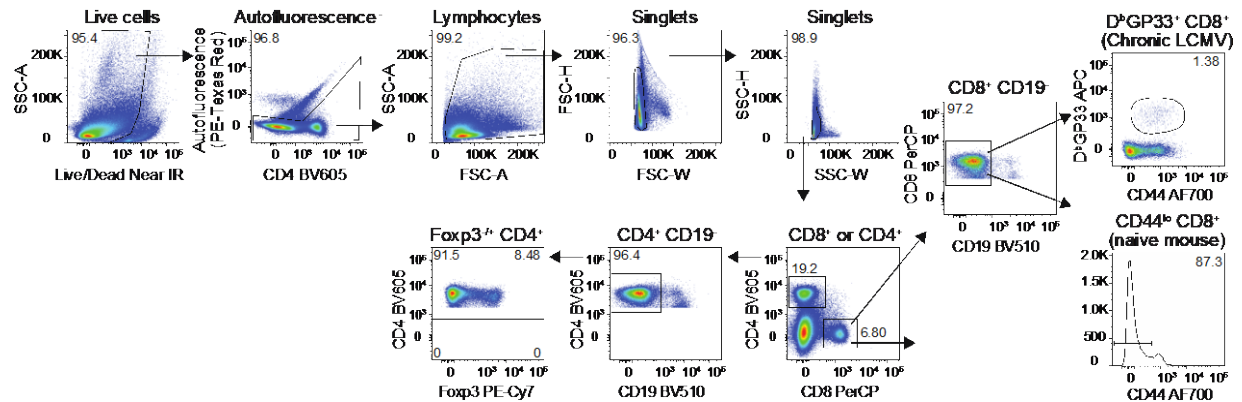
Supplementary Figure 9. Gating strategy for comparing PD-1 expression on D^bGP33^+ CD8$^+$ T cells, Foxp3$^+$ CD4$^+$ T cells, Foxp3$^-$ CD4$^+$ T cells, and naive CD8$^+$ T cells in Extended Data Fig. 5a. Splenocytes isolated from chronically LCMV-infected mice or uninfected C57BL/6J mice were gated on live cells, autofluorescence$^-$ cells, lymphocytes, and CD8$^+$ and CD4$^+$ T cells were identified. For CD8$^+$ T cells, D^bGP33^+ CD8$^+$ T cells in chronically LCMV-infected mice and CD44^{lo} CD8$^+$ T cells in uninfected C57BL/6J mice were included in the analysis. For CD4$^+$ T cells, Foxp3$^+$ CD4$^+$ and Foxp3$^-$ CD4$^+$ T cells were analyzed. AF, Alexa Fluor.

T cell analysis on donor CD45.2⁺ CD8⁺ T cells in adoptive transfer experiments

Supplementary Figure 10. Gating strategy for donor CD45.2⁺ CD8⁺ T cells in Fig. 2g, h and Extended Data Fig. 6f, g. Cells isolated from various tissues in chronically LCMV-infected recipient mice (CD45.1⁺) were gated on live cells, lymphocytes, singlets, autofluorescence⁻ cells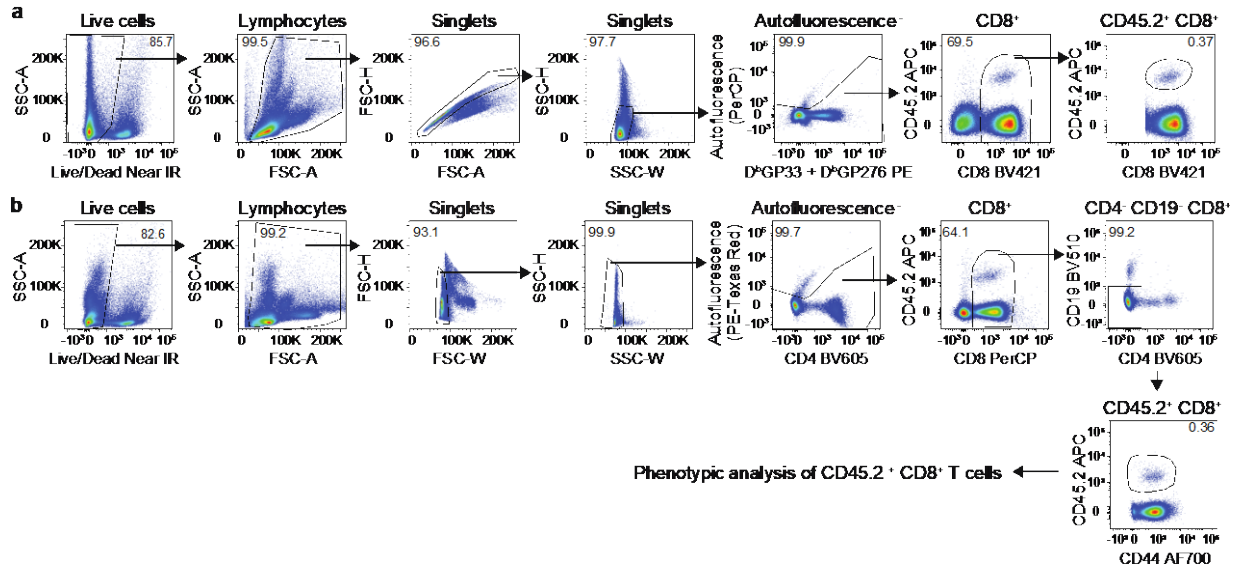, CD8⁺ T cells, and donor CD45.2⁺ CD8⁺ T cells were analyzed for their frequency (**a**) and phenotypic marker expression (**b**). AF, Alexa Fluor.

T cell analysis on STAT5 phosphorylation and cis-trans assay
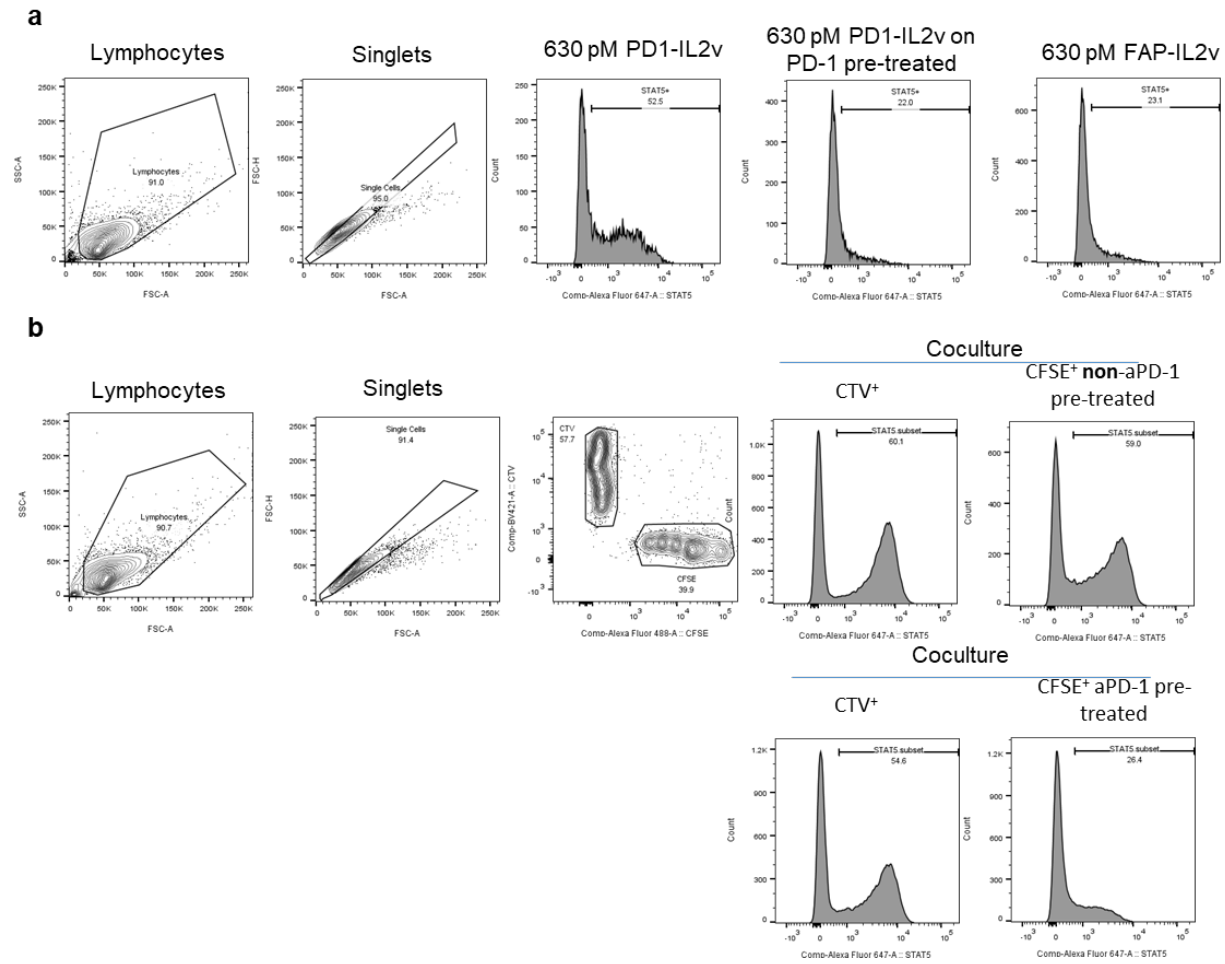
Supplementary Figure 11. Gating strategy for Fig. 1a and b. Sorted 3 days activated PD-1^{+} CD4 T cells from healthy donors were gated on lymphocytes, singlets, positivity for CFSE or CTV and measured for the frequency of STAT-5P^{+} (**a**) and (**b**).

T cell analysis on binding competition on Tregs and Tconv
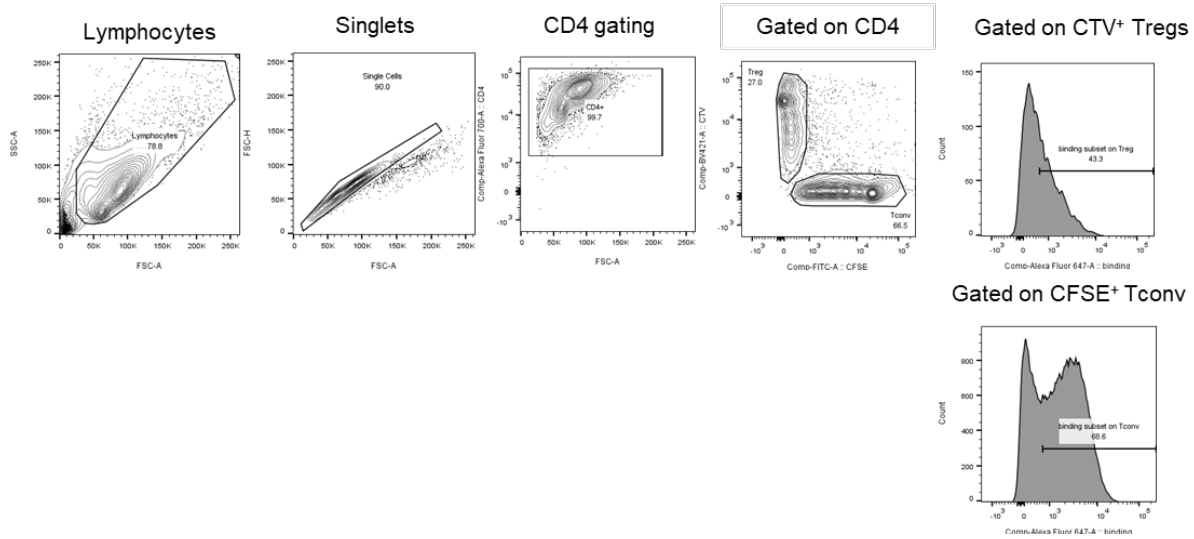
Supplementary Figure 12. Gating strategy for Fig. 1c. Sorted 3 days activated and cocultured Tregs and Tconv from healthy donors were gated on lymphocytes, singlets, CD4$^+$, CFSE$^+$ or CTV$^+$ and measured for the frequency of PGLALA$^+$.

T cell analysis on PD-1 and IL-2Rβ frequencies and quantification
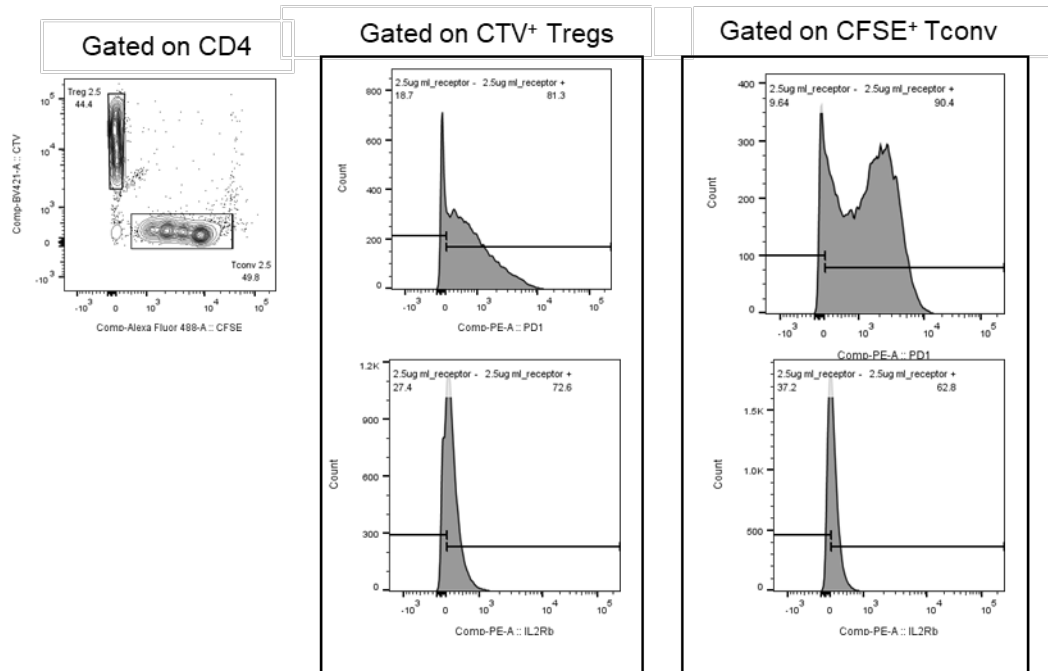
Supplementary Figure 13. Gating strategy for Fig. 1d. Sorted 3 days activated and cocultured Tregs and Tconv from healthy donors were gated on lymphocytes, singlets, CD4$^+$, CFSE$^+$ or CTV$^+$ and measured for the frequency and density of PD-1$^+$ and IL-2Rβ$^+$.

T cell analysis on Treg suppression
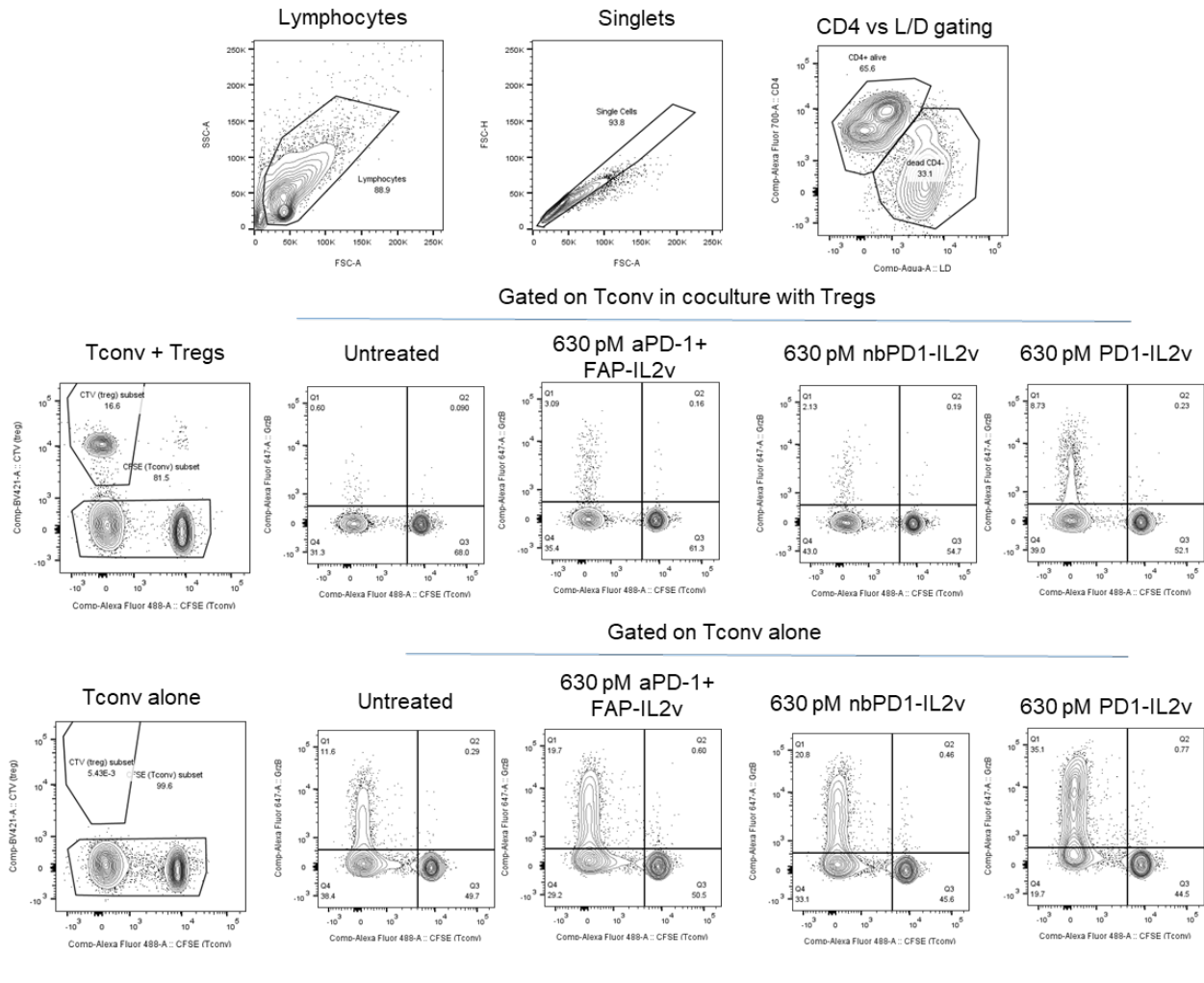
Supplementary Figure 14. Gating strategy for Fig. 1e. Sorted Tregs and Tconv from healthy donors after 5 days coculture were gated on lymphocytes, singlets, live, CD4$^+$, CFSE$^+$ and measured for the frequency of granzyme B$^+$.

T cell analysis on GM-CSF, Granzyme B and IFN-γ measurement
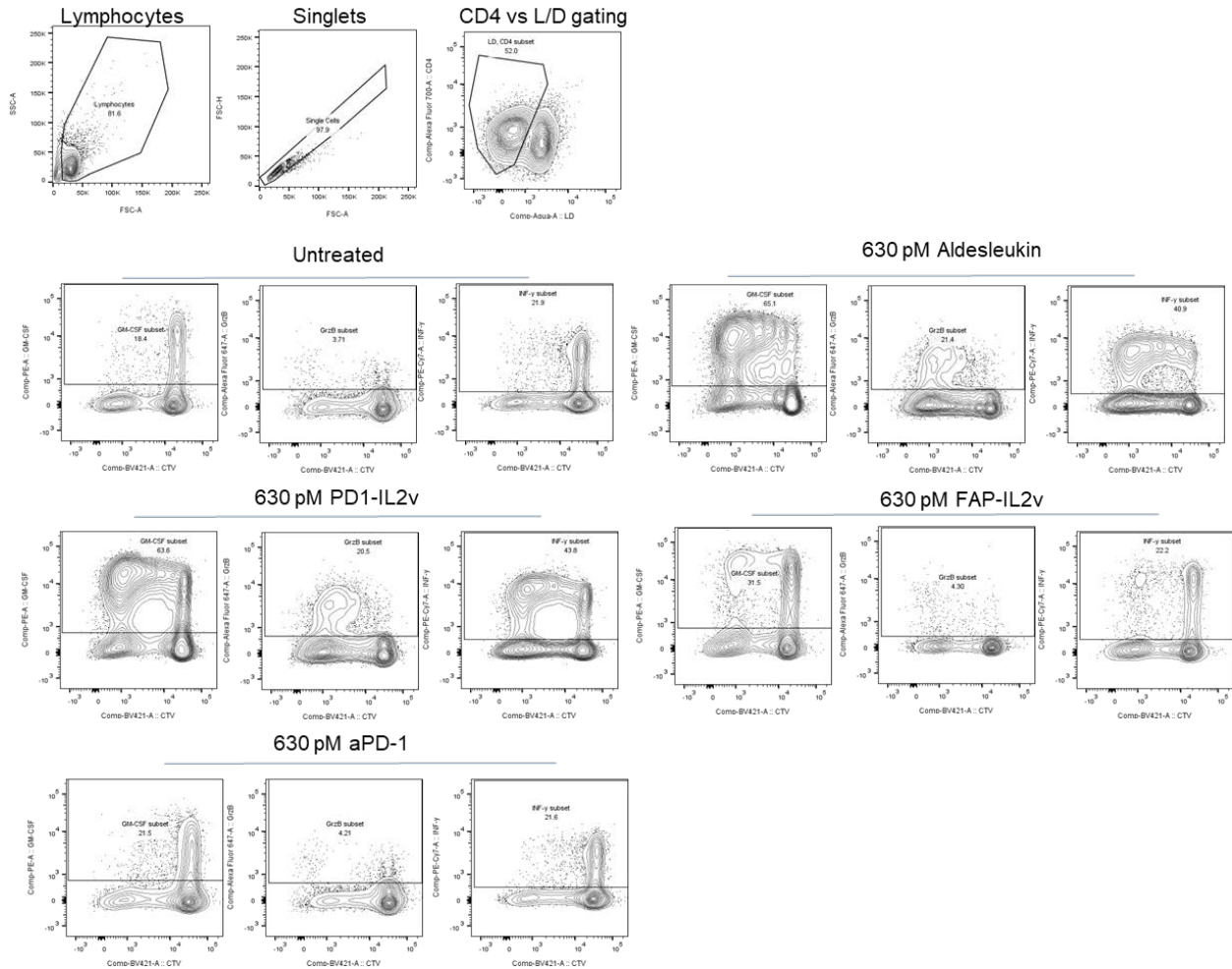
Supplementary Figure 15. Gating strategy for Fig. 1f and EDF4a. Sorted CD4 T cells from healthy donors after 5 days culture were gated on lymphocytes, singlets, live, CD4$^+$, CFSE$^+$ and measured for the frequency of GM-CSF$^+$, granzyme B$^+$ and IFN-γ$^+$.

T cell analysis on CD8:CD4 T cell ratio and differentiation state in tumor and blood
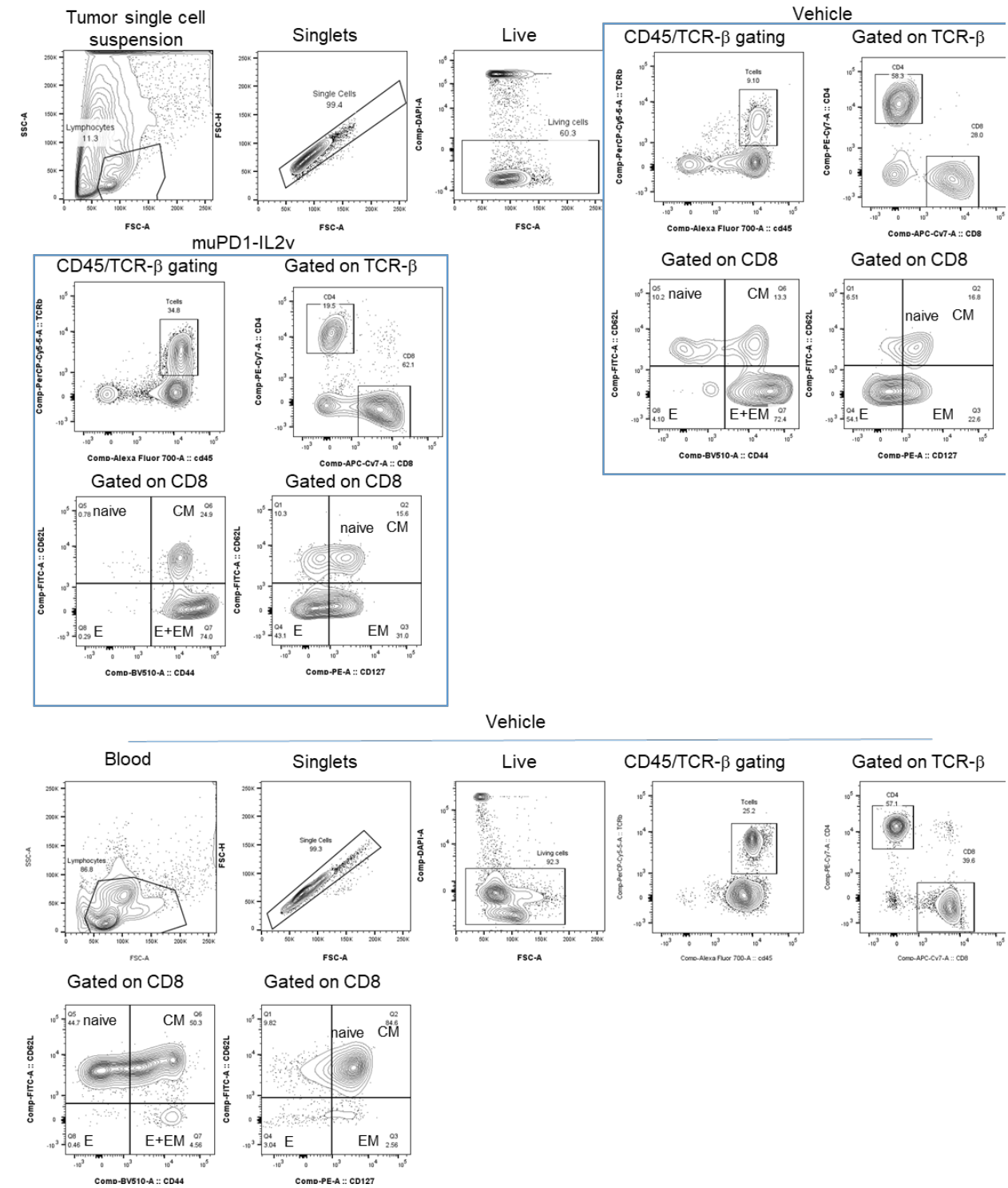
Supplementary Figure 16. Gating strategy for Fig. 3e and f. Single cell suspension of tumor and blood from syngeneic C57BL/6J mice with Panc02 tumors were gated on lymphocytes, singlets, live$^+$, CD45$^+$, TCR-β$^+$, CD8$^+$ and CD4$^+$, and measured for the frequency of naïve, central memory, effector memory and effector.

T cell analysis on PD-1 and IL-2Rβ frequencies and quantification in tumor and blood
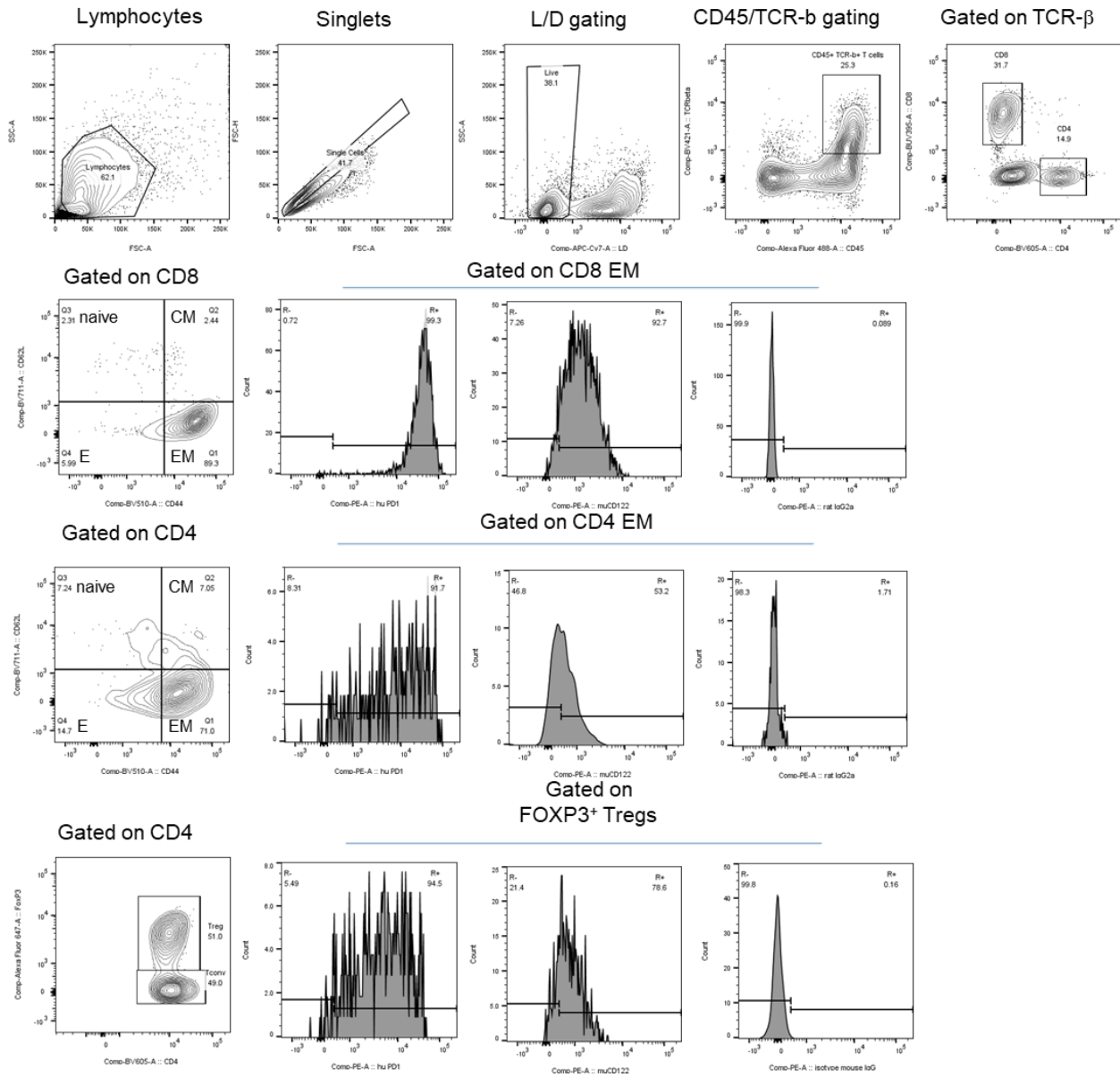
Supplementary Figure 17. Gating strategy for Fig. 3g and EDF7e and g. Single cell suspension of tumor and blood from huPD-1 transgenic mice with Panc02 tumors were gated on lymphocytes, singlets, live$^+$, CD45$^+$, TCR-β$^+$, either CD8$^+$ or CD4$^+$, and measured for the frequency and density of PD-1$^+$ and IL-2Rβ$^+$ on naïve, central memory, effector memory and effector.

T cell analysis on frequency of PD-1$^+$TCF-1$^+$ stem-like and PD-1$^+$TCF-1$^-$ CD8 TILs expressing Granzyme B and/or TIM-3

Supplementary Figure 18. Gating strategy for Fig. 4a-f. Single cell suspension of tumor from syngeneic C57BL/6J mice with Panc02 tumors were gated on lymphocytes, singlets, live$^+$, CD45$^+$, TCR-β$^+$, CD8$^+$ and measured for the frequency of PD-1 and TCF-1 expression and of Granzyme B and TIM-3

Sorting of CD8 TILs
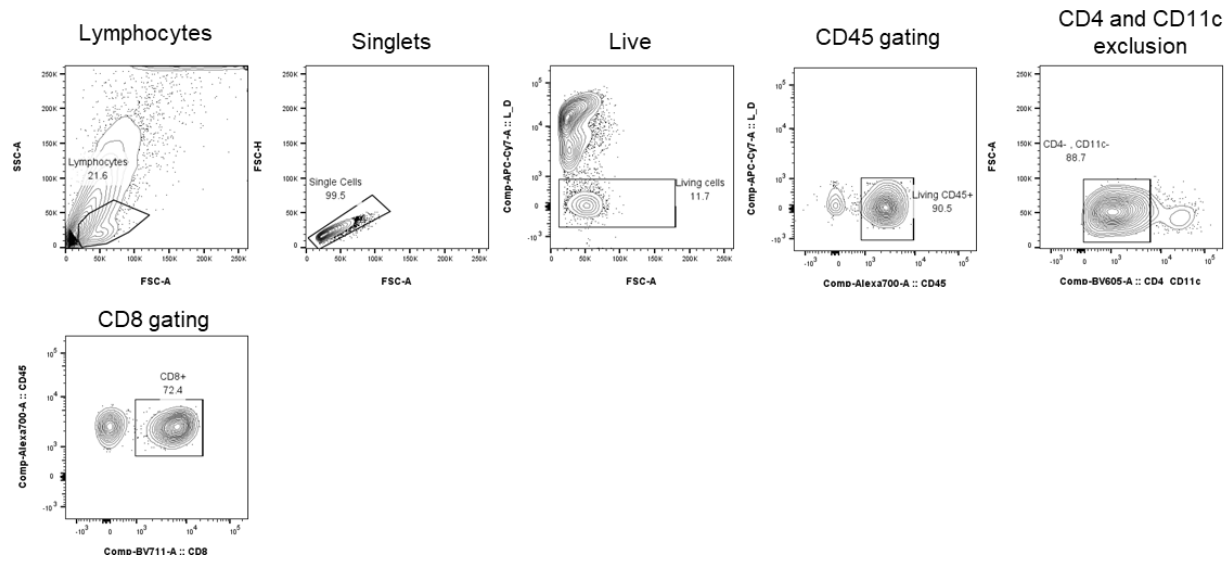
Supplementary Figure 19. Gating strategy for Fig. 4g-k. Single cell suspension of tumor from syngeneic C57BL/6J mice with Panc02 tumors were gated on lymphocytes, singlets, live$^+$, CD45$^+$, TCR-β$^+$, CD8$^+$, CD4$^-$ and CD11c$^-$.

T cell analysis on frequency of OVA-specific PD-1$^+$TCF-1$^+$ stem-like and PD-1$^+$TCF-1$^-$ CD8 TILs expressing Granzyme B and/or TIM-3

Supplementary Figure 20. Gating strategy for Fig. 5b-g. Single cell suspension of tumor from syngeneic C57BL/6J mice with Panc02 tumors were gated on lymphocytes, singlets, live$^+$, CD45$^+$, TCR-β^+, CD8$^+$, OVA$^+$ and OVA$^-$, and measured for the frequency of PD-1 and TCF-1 expression and of Granzyme B and TIM-3.

T cell analysis on restimulated CD8 TILs with OVA peptide (SIINFEKL)

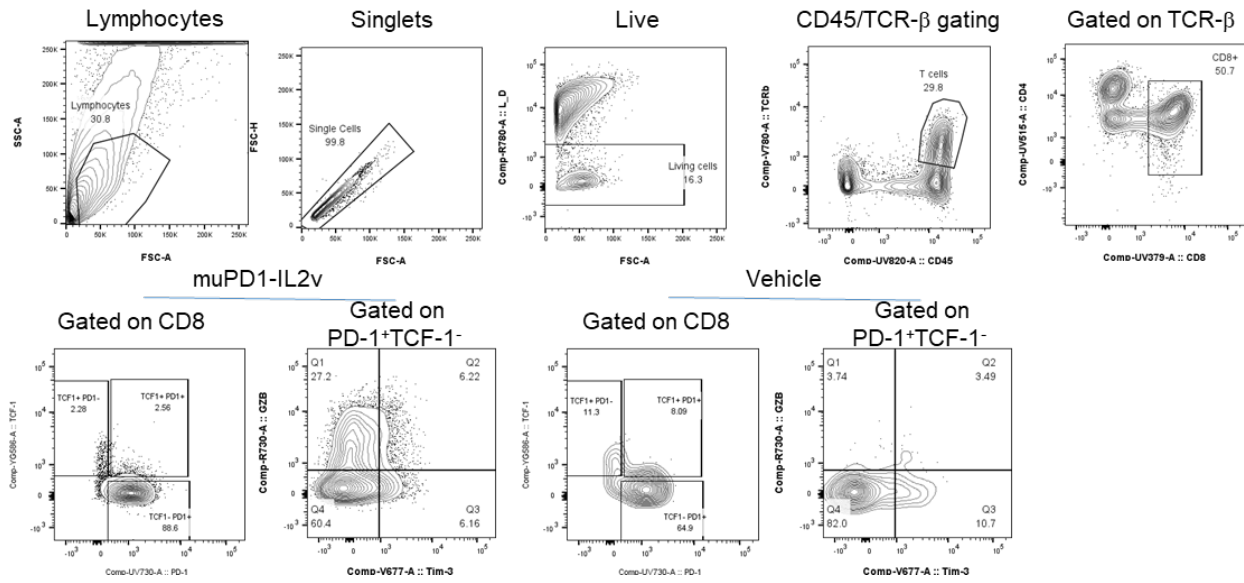**Supplementary Figure 21. Gating strategy for Fig. 5h.** Single cell suspension of tumor from syngeneic C57BL/6J mice with Panc02 tumors were gated on lymphocytes, singlets, CD45$^+$, CD8$^+$, and measured for the frequency of CD107a$^+$ IFN-γ^+.

T cell analysis on STAT-5P cis-trans assay with FAP-Superkine-like
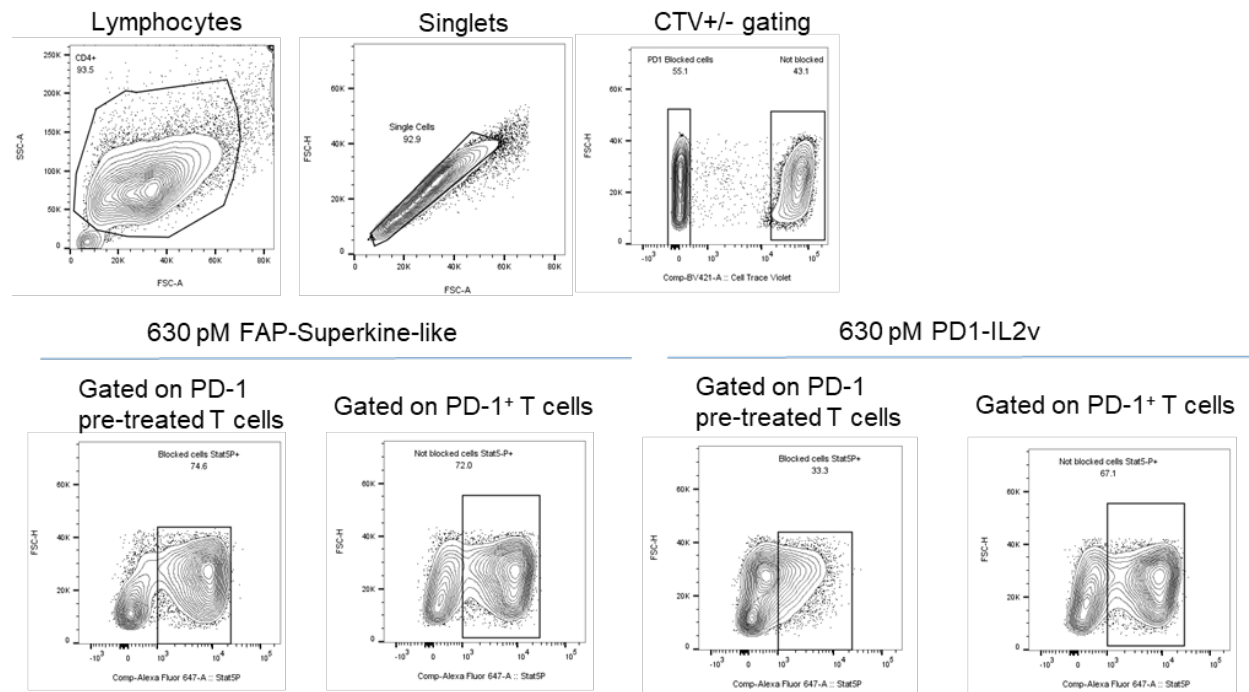
Supplementary Figure 22. Gating strategy for EDF4b. Sorted 3 days activated PD-1⁺ CD4 T cells from healthy donors were gated on lymphocytes, singlets, positivity for CTV and measured for the frequency of STAT-5P⁺ on either PD-1 pre-treated T cells (PD-1 deficient) or PD-1⁺ T cells.

Lymphocyte analysis on preferential binding
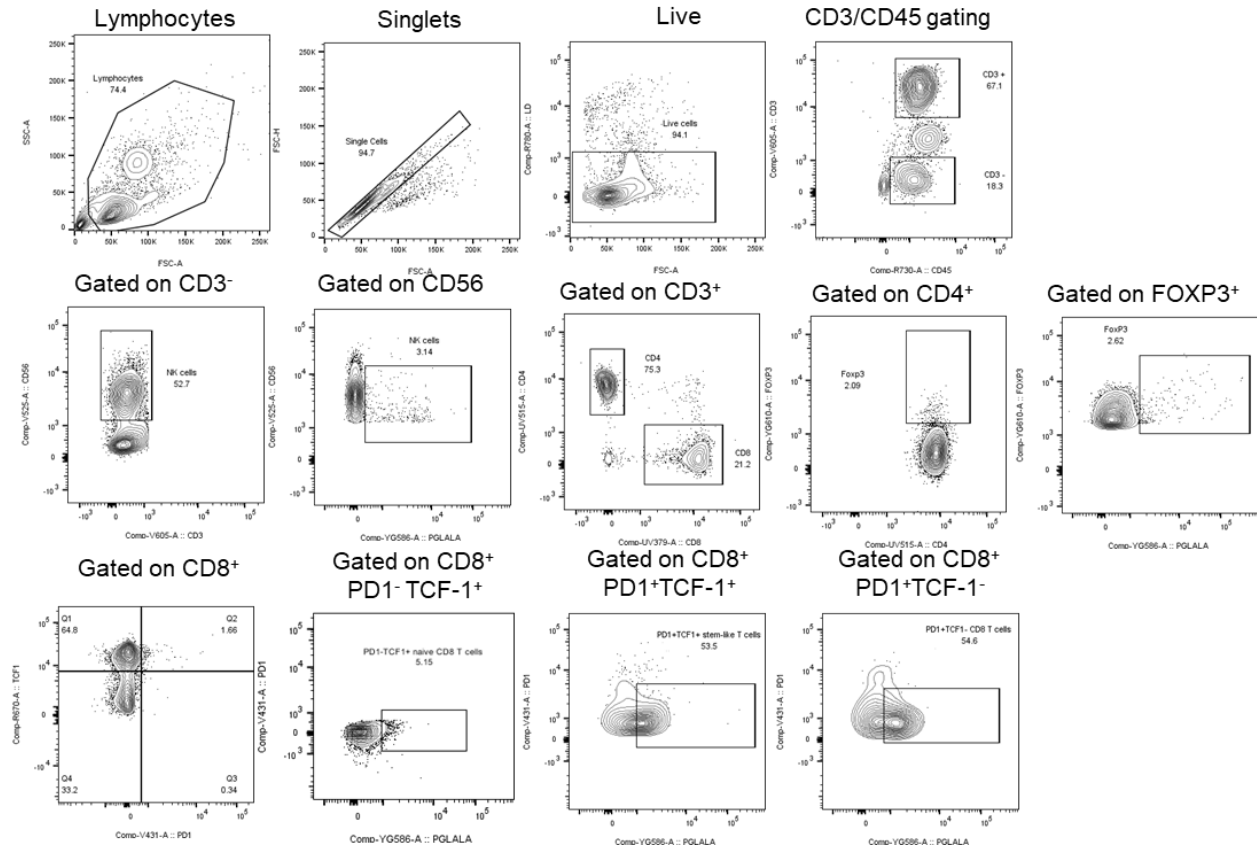
Supplementary Figure 23. Gating strategy for EDF4c. Freshly isolated PBMCs from healthy donors were gated on lymphocytes, singlets, live and either CD3$^-$ CD56$^+$**,** CD4$^+$ FOXP3$^+$, or CD8$^+$ and measured for the co-expression of PD-1 and TCF-1.

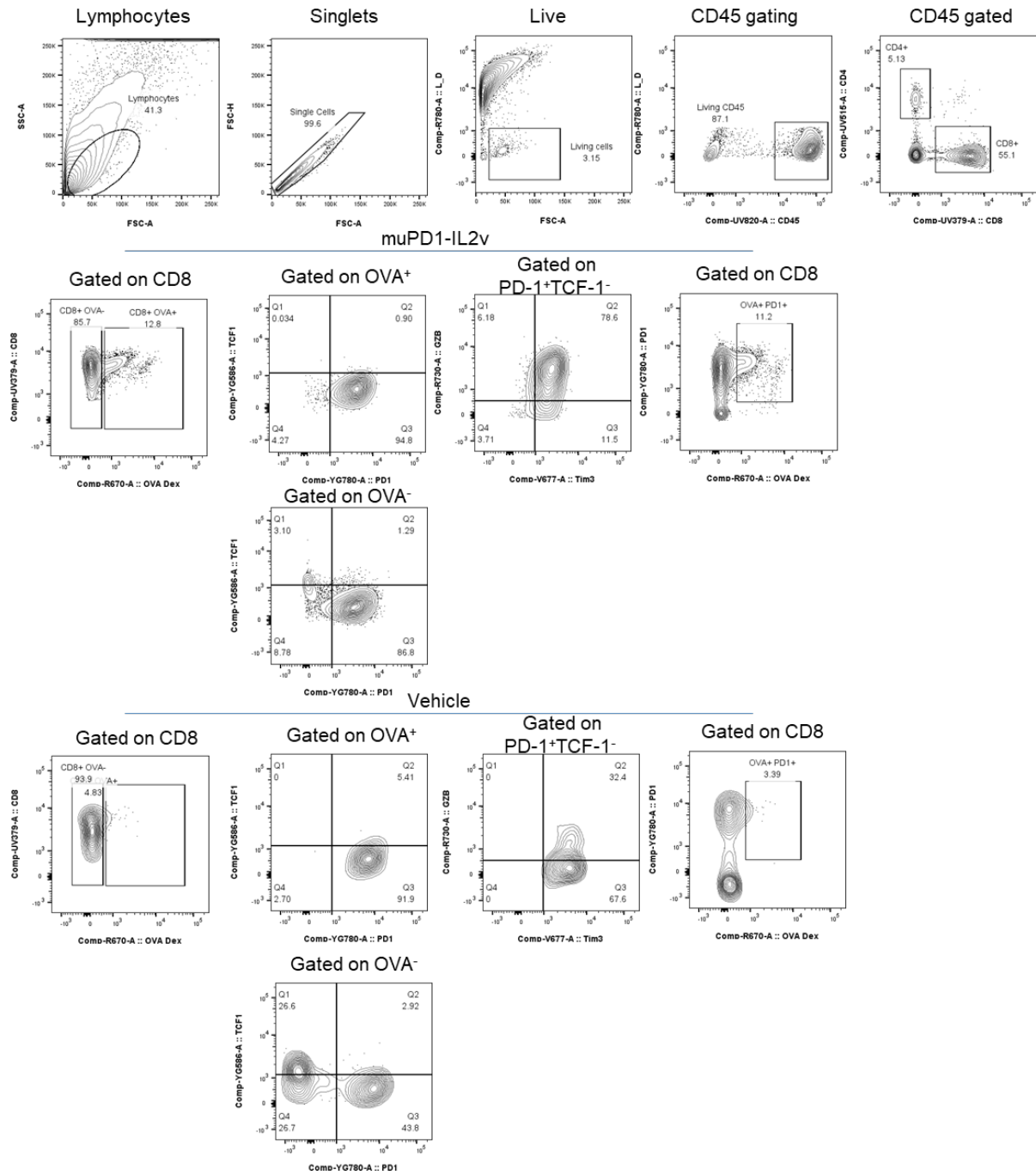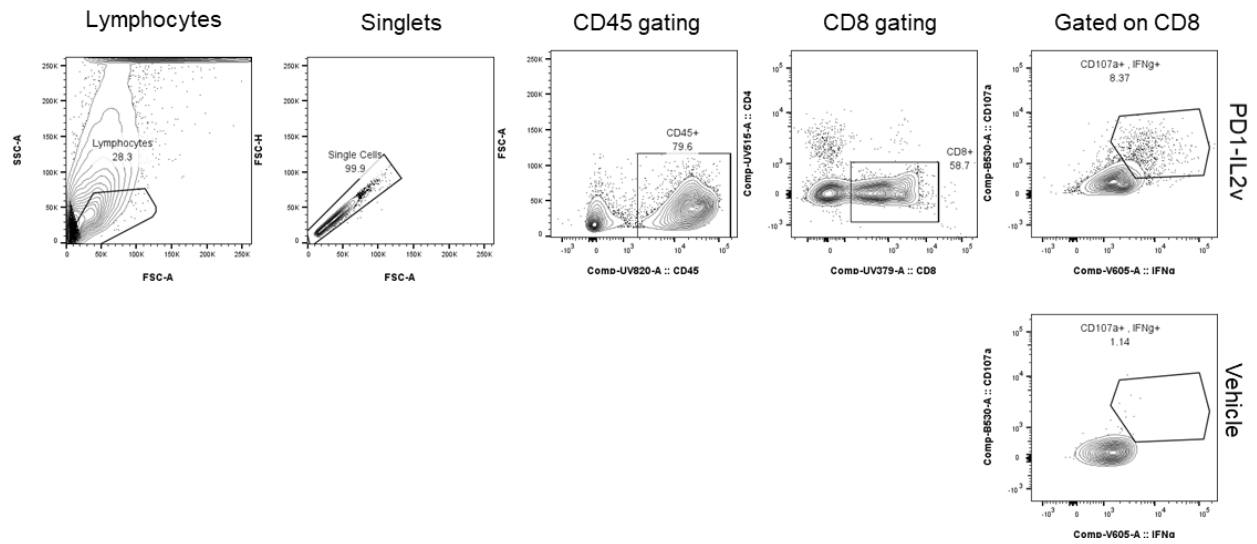**T cell analysis on cytokine secretion by CD8 TILs**
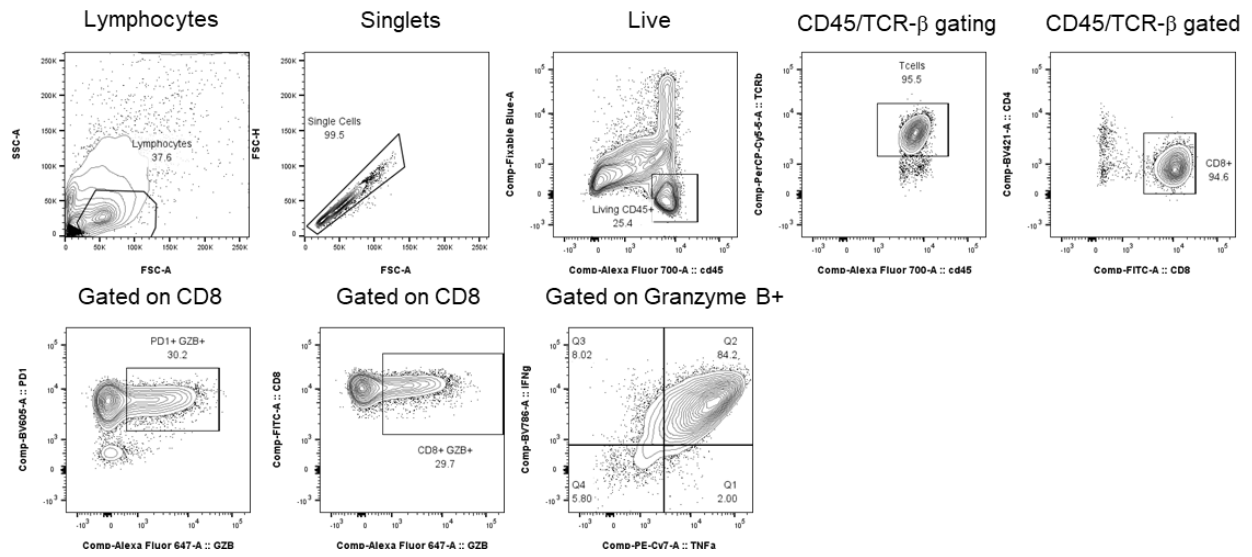
Supplementary Figure 24. Gating strategy for EDF7a-b. Single cell suspension of tumor from syngeneic C57BL/6J mice with Panc02 tumors were gated on lymphocytes, singlets, live, CD45$^+$, TCR-β^+, CD8$^+$ and measured for the frequency of PD-1$^+$ Granzyme B$^+$ and IFN-γ^+ TNF-α^+.

T cell analysis on binding competition with parental anti-PD-1 Alexa Fluor-647
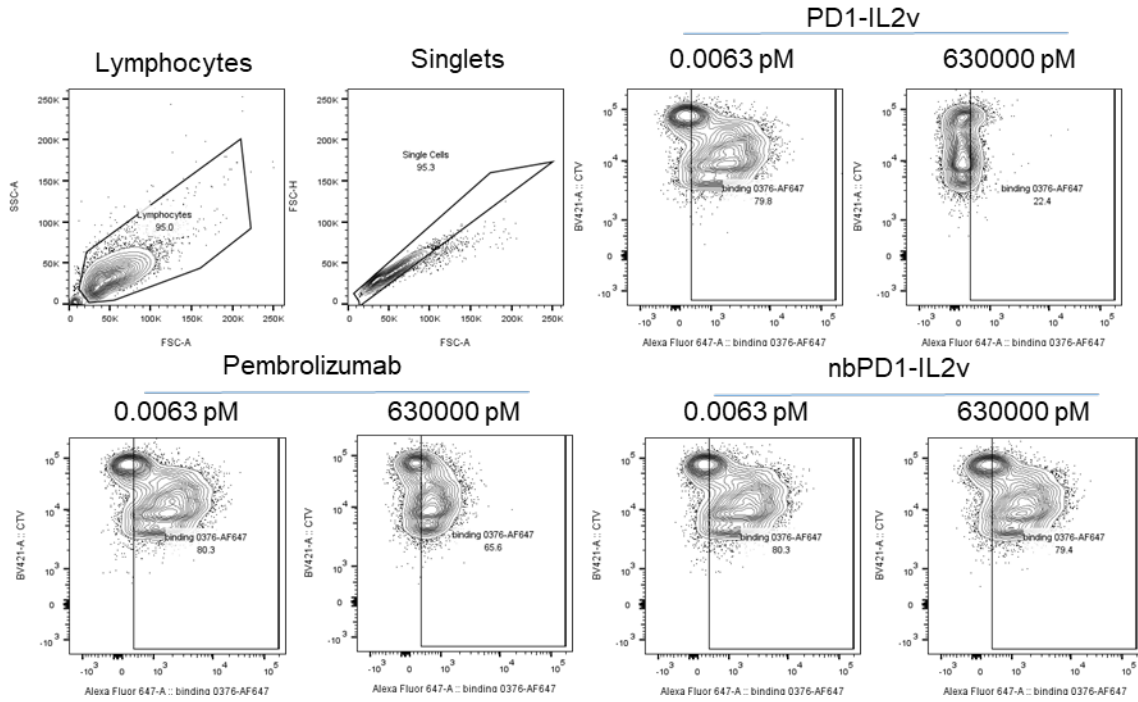
Supplementary Figure 25. Gating strategy for EDF7i. Sorted 3 days activated PD-1^{+} CD4 T cells from healthy donors were gated on lymphocytes, singlets, CTV[+] and measured binding of parental anti-PD-1 Alexa Fluor-647.

Supplementary Table 1
List of antibodies used in the presented study

| Ex-vivo binding on human PBMCs					
Marker	Fluorophore	Clone	#CAT	Company	Dilution/Final Concentration
Live/Dead	APC-Cy7		65-0865-14	eBioscience	1:500
CD45	AF700	HI30	56-9459-42	eBioscience	1:100
CD3	BV605	OKT3	317322	Biolegend	1:100
CD4	BUV496	OKT4	612936	BD Biosciences	1:100
CD8	BUV395	RPA-T8	563795	BD Biosciences	1:100
CD366 (TIM-3)	BV711	F38-2E2	345024	Biolegend	1:20
LAG3 (CD223)	PerCP-Cy5.5	3DS223H	46-2239-42	eBioscience	1:20
TIGIT	BV786	741182	747838	BD Biosciences	1:20
CD218a (IL-18Ra)	FITC	H44	313810	Biolegend	1:100
CD56	BV5480	NCAM16.2	566124	BD Biosciences	1:20
TCR g/d	PE-Cy7	B1	331222	Biolegend	1:50
PG-LALA	PE	NA		Roche	1:650 (2.5ug/ml)
TCF-1 /TCF-7	AF647	C63D9	6709S	Cell Signaling Technologie	1:100
PD-1 IC		D4W2J	86163S	Cell Signaling Technologie	1:100
Goat anti-Rabbit secondary ab	BV421	Polyclonal	565014	BD	1:100
FOXP3	PE-CF594	206D	320126	Biolegend	1:50
In-vitro experiment on human CD4 T cells (incl. T con and Tregs)					
Marker	Fluorophore	Clone	#CAT	Company	Dilution/Final Concentration
Stat5 (pY694)	AF647	47/Stat5 (pY694)	562076	BD	1:20
PD-1	PE	EH12.2H7	329906	Biolegend	2.5ug/ml
IL2Rb	PE	TU27	339006	Biolegend	2.5ug/ml
isotype	PE	MOPC-21	400112	Biolegend	2.5ug/ml
Parental PD-1	AF647	0376	NA	Roche	1ug/ml
PD1-IL2v	AF647	0376-IL2v fused	NA	Roche	1ug/ml
CD4	AF700	RPA-T4	56-0049-42	eBioscience	1:50
live/dead	Aqua Dead Cell Stain	-	L34966	Invitrogen	1:1000
Granzyme B	AF647	GB11	561999	BD Biosciences	1:100
GM-CSF	PE	BVD2-21C11	502306	Biolegend	1:100
IFNγ	PE-Cy7	4S.B3	25-7319-82	eBioscience	1:200
Ex-vivo receptor quantification on mouse TILs and T cells from blood					

Marker	Fluorophore	Clone	#CAT	Company	Dilution/Final Concentration
PD-1	PE	29F.1A12	135206	Biolegend	2.5ug/ml
PD-1	PE	EH12.2H7	329906	Biolegend	2.5ug/ml
IL2Rb	PE	5H4	105906	Biolegend	2.5ug/ml
TCRb	BV421	H57-597	109230	Biolegend	1:200
CD8	BV395	53-6.7	565968	BD Biosciences	1:200
CD4	AF488	GK1.5	100406	Biolegend	1:100
CD62L	BV711	MEL-14	104445	Biolegend	1:200
CD44	BV480	IM7	566116	BD Biosciences	1:200
FOXP3	AF647	150D	320014	Biolegend	1:100
Antibodies used for the LCMV-chronic infection					
Marker	Fluorophore	Clone	#CAT	Company	Dilution
CD4	BUV563	RM4-5	741217	BD Biosciences	1:500
CD4	FITC	RM4-5	553046	BD Biosciences	1:500
CD4	V500	RM4-5	560782	Biolegend	1:500
CD4	BV605	RM4-5	100548	Biolegend	1:500
CD4	BV711	RM4-5	100557	Biolegend	1:500
CD4	PE-Cy7	RM4-5	25-0042-82	Thermo Fischer Scientific	1:500
CD4	APC-eFluor 780	RM4-5	47-0042-82	Thermo Fischer Scientific	1:500
CD8a	BUV496	53-6.7	563786	BD Biosciences	1:100
CD8a	BV421	53-6.7	100753	Biolegend	1:150
CD8a	BV605	53-6.7	100744	Biolegend	1:100
CD8a	PerCP	53-6.7	553036	BD Biosciences	1:100
CD19	BUV563	1D3	749028	BD Biosciences	1:150
CD19	BV510	1D3	115546	Biolegend	1:150
CD19	BV605	1D3	115540	Biolegend	1:150
CD19	PE-Cy7	1D3	25-0193-82	Thermo Fischer Scientific	1:150
CD19	APC-eFluor 780	1D3	47-0193-82	Thermo Fischer Scientific	1:150
CD44	BUV805	IM7	741921	BD Biosciences	1:500
CD44	FITC	IM7	561859	BD Biosciences	1:500
CD44	AF700	IM7	56-0441-82	Thermo Fischer Scientific	1:100
CD45.2	APC	104	109814	Biolegend	1:100
CD127	BUV737	SB/199	612841	BD Biosciences	1:100
CD127	PE	A7R34	12-1271-83	Thermo Fischer Scientific	1:100
CD218a	PE	P3TUNYA	12-5183-82	Thermo Fischer Scientific	1:100
CD218a	PE-Cy7	P3TUNYA	25-5183-82	Thermo Fischer Scientific	1:100

CXCR3	PE-Cy7	CXCR3-173	25-1831-82	Thermo Fischer Scientific	1:100
CXCR5	BV421	L138D7	145512	Biolegend	1:50
CX3CR1	BV785	SA011F11	149031	Biolegend	1:500
Foxp3	PE-Cy7	FJK-16s	25-5773-82	Thermo Fischer Scientific	1:250
IL-2	PE	JES6-5H4	554428	BD Biosciences	1:100
IFN-γ	BV421	XMG1.2	505830	Biolegend	1:100
IFN-γ	APC	XMG1.2	554413	BD Biosciences	1:100
PD-1	PE	RMP1-30	109104	Biolegend	1:100
PD-1	APC	RMP1-30	109112	Biolegend	1:100
TCF-1	PE	S33-966	564217	BD Biosciences	1:100
Tim-3	BUV395	5D12	747620	BD Biosciences	1:100
Tim-3	AF488	215008	FAB1529G	R&D systems	1:20
TNF-a	BV421	MP6-XT22	506328	Biolegend	1:100
TNF-a	PE	MP6-XT22	554419	BD Biosciences	1:100
Name		Clone	#Catalogue		
anti-mouse PD-L1 with DAPG mutation		NA	NA	Roche	200 μg/mouse/injection
mouse IgG1 isotype control		MOPC-21	BE0083	BioXcell	200 μg/mouse/injection
anti-mouse CD4		GK1.5	BE0003-1	BioXcell	300 μg/mouse/injection
Antibodies used for TILs characterization in mouse tumor-model					
Marker	Fluorochrome	clone	Cat number	Provider	Dilution
Fixable Viability Dye	eFluor™ 455UV		65-0868-14		1:500
CD45	AF700	30-F11	103128	Biolegend	1:300
TCRb	PercP-Cy5.5	H57-597	109228	Biolegend	1:200
CD8	APC-Cy7	53-6.7	100714	Biolegend	1:200
CD4	PE-Cy7	GK1.5	100422	Biolegend	1:200
CD62L	FITC	MEL-14	104406	Biolegend	1:200
CD127	PE	A7R34	135010	Biolegend	1:100
TCRb	PerCP-Cy5.5	H57-597	109228	Biolegend	1:200
CD4	BV421	GK1.5	100438	Biolegend	1:200
Granzyme B	AF647	GB11	515406	Biolegend	1:100
IFN-g	BV786	XMG1.2	505838	Biolegend	1:100
TNFa	PE-Cy7	MP6-XT22	506306	Biolegend	1:100
FoxP3	BV421	MF-14	126419	Biolegend	1:100
CD39	AF647	Duha59	143808	Biolegend	1:200

Granzyme B	AF700	QA16A02	372222	Biolegend	1:100
ki67	PE-Cy7	16A8	652426	Biolegend	1:300
PD1	PE-Cy7	RMP1-30	109110	Biolegend	1:200
CD25	BV711	RMT3-23	102049	Biolegend	1:200
TIGIT	PE-Dazzle594	1G9	142110	Biolegend	1:100
IFN-g	BV605	XMG1.2	505840	Biolegend	1:100
TNFa	BV421	MP6-XT22	506328	Biolegend	1:100
CD107a	AF488	1D4B	121608	Biolegend	1:100
CD44	BV510	IM7	563114	BD Biosciences	1:200
CD45	BUV805	30-F11	BDB748370	BD Biosciences	1:100
TCRb	BV786	H57-597	742484	BD Biosciences	1:100
CD4	BUV496	RM4-5	612952	BD Biosciences	1:100
CD8	BUV395	53-6.7	563786	BD Biosciences	1:100
PD-1	BUV737	RMP1-30	749306	BD Biosciences	1:100
CD25	PE-CF594	PC61	562694	BD Biosciences	1:100
Tim3	BV650	5D12	747623	BD Biosciences	1:100
TCF1	PE	S33-966	564217	BD Biosciences	1:100
LAG3	BV650	C9B7W	740560	BD Biosciences	1:100
SLAMF6	BV510	13G3	745073	BD Biosciences	1:50
CD218a	FITC	REA947	130-115-703	Miltenyi	1:50
OVA-dextramer H-2 Kb ((SIINFEKL)	APC		JD2163	Immudex	1:100
Rat anti-mouse CD8α InVivoPlus		2.43	BP0061	BioXcell	100 µg/mouse/injection
Feature barcoding					
Marker	Oligo Tag	Clone	Source	Concentration (1 µg/ml)	
CD28	ATTAAGAGCGTGTTG	37.51	TotalSeq-C, BioLegend	1	
CD44	TGGCTTCAGGTCCTA	IM7	TotalSeq-C, BioLegend	1	
CD62L (L-selectin)	TGGGCCTAAGTCATC	MEL-14	TotalSeq-C, BioLegend	1	
CD39	GCGTATTTAACCCGT	Duha59	TotalSeq-C, BioLegend	1	
CD279 (PD-1)	GAAAGTCAAAGCACT	RMP1-30	TotalSeq-C, BioLegend	1	
CD366 (Tim-3)	ATTGGCACTCAGATG	RMT3-23	TotalSeq-C, BioLegend	1	

CD223 (LAG-3)	ATTCCGTCCCTAAGG	C9B7W	TotalSeq-C, BioLegend	1	
CD183 (CXCR3)	GTTCACGCCGTGTTA	CXCR3-173	TotalSeq-C, BioLegend	1	
CD185 (CXCR5)	ACGTAGTCACCTAGT	L138D7	TotalSeq-C, BioLegend	1	
CD127 (IL-7Rα)	GTGTGAGGCACTCTT	A7R34	TotalSeq-C, BioLegend	1	
TIGIT (Vstm3)	GAAAGTCGCCAACAG	1G9	TotalSeq-C, BioLegend	1	
CD25	ACCATGAGACACAGT	PC61	TotalSeq-C, BioLegend	1	
Ly108 (SLAM-F6)	CGATTCTTTGCGAGT	330-AJ	TotalSeq-C, BioLegend	1	
CD137 (4-1BB)	TCCCTGTATAGATGA	17B5	TotalSeq-C, BioLegend	1	
IL-21R	GATTCCGACAGTAGA	4A9	TotalSeq-C, BioLegend	1	
Histology					
Marker	Fluorochrome	clone	Cat number	Provider	Dilution
CD3		SP7	RMAB005	Diagnostic Biosystems	1:100
CD8		4SM15	14-0808-82	eBioscience	1:300
PD-1		Polyclonal	AF1021	R&D Systems	1:250
Granzyme B		Polyclonal	Ab4059	Abcam	1:250
Confocal Imaging					
Marker	Fluorochrome	clone	Cat number	Provider	Dilution
PD-1 IC		D4W2J	86163S	Cell Signaling Technologie	1:100
Goat anti-rabbit IgG (H+L), F(ab')2 Fragment	AF488	Polyclonal	4412S	Cell Signaling Technologie	1:1000
Parental PD-1	Pure	0376	NA	Roche	10ug/ml
PD1-IL2v	AF647	0376-IL2v fused	NA	Roche	1ug/ml
FAP-IL2v	AF647	4B9-IL2v fused	NA	Roche	1ug/ml
Cell sorting					
Marker	Fluorochrome	clone	Cat number	Provider	Dilution
CD45	AF700	30-F11	103128	Biolegend	1:100

CD8	BV711	53-6.7	100748	Biolegend	1:100
CD4	BV605 (Bin channel)	GK1.5	100451	Biolegend	1:100
CD11c	BV605 (Bin channel)	N418	117334	Biolegend	1:100
Live/Dead	APC-Cy7		65-0865-14	eBiosciences	1:500

Supplementary Notes

Acknowledgements