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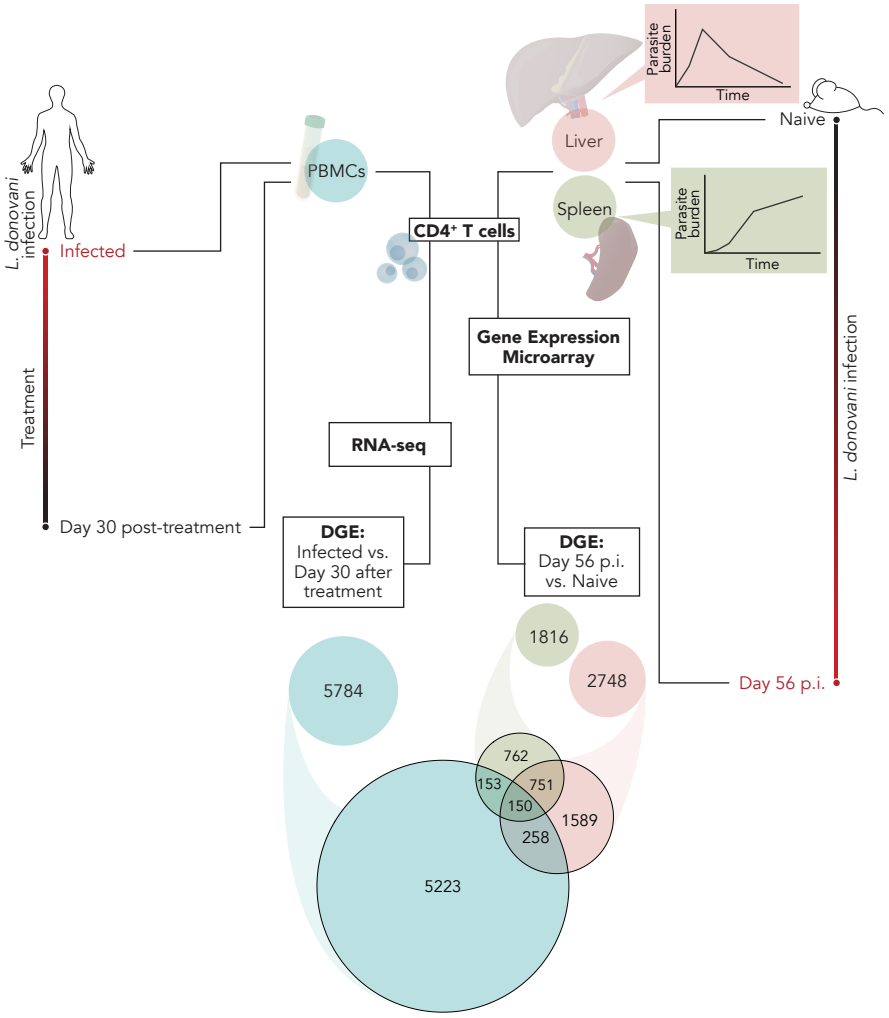
Supplementary information

The NK cell granule protein NKG7 regulates cytotoxic granule exocytosis and inflammation

In the format provided by the authors and unedited

Supplementary Figure 1



Supplementary Figure 1 Defining transcriptomic signature of CD4⁺ T cells during *Leishmania donovani* infection. Schematic showing the approach taken to identify transcriptomic signatures of CD4⁺ T cells in the spleen (*n* = 5 biological replicates) and liver (*n* = 4 naive replicates, each pooled from 4 mice, and 5 infected biological replicates) of mice at day 56 post-infection (p.i.) that are relevant in *L. donovani*-infected patients before and 30 days post-drug treatment (*n* = 9).

Supplementary Table 1. Clinical data from visceral leishmaniasis patients

Variables	Endemic Controls	Visceral Leishmaniasis
n	14	23
Age (years)	31.14±7.94 (29)	26.17±17.79 (22)[a]
Sex (M/F)	08/06	13/10
Duration of illness (days)	N/A	26.82±17.47 (21)
WBC (x 10^3/mm^3, day 0)	N/D	3926.08±1884.43 (3400)
WBC (x 10^3/mm^3, day 30)	N/D	7795.65±2618.72 (7800)
Platelets (x 10^3/mm^3, day 0)	N/D	110260.87±58182.49(98000)
Platelets (x 10^3/mm^3, day 30)	N/D	212608.70±79014.81 (240000)
Splenic enlargement (cm, day 0)	N/A	5.04±3.52 (4)
Splenic enlargement (cm, day 30)	N/A	1.17±1.4 (0)

Abbreviations: N/A, not applicable; N/D, not done.

[a]The data is shown as mean ± SD of aggregated data, and median values are in included in parentheses.

Supplementary Table 5. Composition of in vitro polarisation cocktails

Condition	Composition	Company	Clone	Concentration (1x)
Th0	α-CD3ε	BioLegend*	145-2C11	4 µg/ml
	α-CD28	BioLegend*	37.51	2 µg/ml
	rmIL-2	BioLegend*	-	20 ng/ml
T_H1	α-CD3ε	BioLegend*	145-2C11	4 µg/ml
	α-CD28	BioLegend*	37.51	2 µg/ml
	rmIL-2	BioLegend*	-	20 ng/ml
	rmIL-12 (p70)	BioLegend*	-	10 ng/ml
	α-IL-4	BioLegend*	11B11	10 µg/ml
T_H1 + rIL-27	α-CD3ε	BioLegend*	145-2C11	4 µg/ml
	α-CD28	BioLegend*	37.51	2 µg/ml
	rmIL-2	BioLegend*	-	20 ng/ml
	rmIL-12 (p70)	BioLegend*	-	10 ng/ml
	α-IL-4	BioLegend*	11B11	10 µg/ml
	rmIL-27	BioLegend*	-	100 ng/ml
T_r1	α-CD3ε	BioLegend*	145-2C11	4 µg/ml
	α-CD28	BioLegend*	37.51	2 µg/ml
	rmIL-2	BioLegend*	-	20 ng/ml
	rmIL-27	BioLegend*	-	100ng/ml
T_H2	α-CD3ε	BioLegend*	145-2C11	4 µg/ml
	α-CD28	BioLegend*	37.51	2 µg/ml
	rmIL-2	BioLegend*	-	20 ng/ml
	rmIL-4	BioLegend*	-	40 ng/ml
	α-IFN-γ	BioLegend*	XMG1.2	10 µg/ml
T_H17	α-CD3ε	BioLegend*	145-2C11	4 µg/ml
	α-CD28	BioLegend*	37.51	2 µg/ml
	rmIL-2	BioLegend*	-	20 ng/ml
	rmIL-6	BioLegend*	-	20 ng/ml
	rmIL-1β	eBioscience#	-	10 ng/ml

	rmIL-23	eBioscience[#]	-	10 ng/ml
	rm-TNF	eBioscience[#]	-	10 ng/ml
	rm-TGFβ	BioLegend*	-	1 ng/ml
	α-IFN-γ	BioLegend*	XMG1.2	10 µg/ml
	α-IL-4	BioLegend*	11B11	10 µg/ml
T_r1 + TGFβ	α-CD3ε	BioLegend*	145-2C11	4 µg/ml
	α-CD28	BioLegend*	37.51	2 µg/ml
	rmIL-2	BioLegend*	-	20 ng/ml
	rmIL-27	BioLegend*	-	100 ng/ml
	rm-TGFβ	BioLegend*	-	0.5 µg/ml

*Based on methods previously described in [22]. T_H, T helper; T_{reg}, regulatory T cell; α, purified monoclonal anti-mouse; rmIL, recombinant mouse interleukin; * BioLegend, San Diego CA, USA; [#] eBioscience, Thermo Fisher Scientific, Waltham MA, USA*

Supplementary Table 6. Frequencies of immune cell subsets in WT versus Nkg7$^{-/-}$ mice

Organ	Cell type	WT	*Nkg7*$^{-/-}$	Mann–Whitney test, *p* value
Lung	**γδ T cells** CD45^{+} TCRβ^{-} TCRγ/δ^{+}	1.155 ± 0.293	1.450 ± 0.272	0.4127
	NK cells CD45^{+} TCRγ/δ^{-} TCRβ^{-} NK1.1^{+}	7.423 ± 1.510	8.334 ± 2.280	0.4127
	CD4^{+} T cells CD45^{+} TCRγ/δ^{-} NK1.1^{-} TCRβ^{+} CD8^{-} CD4^{+}	14.130 ± 2.364	12.960 ± 2.300	0.4127
	CD8^{+} T cells CD45^{+} TCRγ/δ^{-} NK1.1^{-} TCRβ^{+} CD4^{-} CD8^{+}	8.293 ± 1.625	8.886 ± 2.009	0.9048
	B cells CD45^{+} TCRγ/δ^{-} NK1.1^{-} TCRβ^{-} B220^{+}	28.580 ± 2.363	25.660 ± 4.281	0.3095
	Macrophages CD45^{+} TCRγ/δ^{-} NK1.1^{-} TCRβ^{-} B220^{-} MHCII^{+} CD64^{+}	6.788 ± 1.438	6.298 ± 1.756	>0.9999
	cDCs CD45^{+} TCRγ/δ^{-} NK1.1^{-} TCRβ^{-} B220^{-} CD64^{-} MHCII^{hi} CD11c^{+}	1.383 ± 0.217	1.718 ± 0.478	0.4127
	Neutrophils CD45^{+} TCRγ/δ^{-} NK1.1^{-} TCRβ^{-} B220^{-} CD64^{-} CD11c^{-} Ly-6G^{+} Ly-6C^{+}	0.923 ± 0.175	1.692 ± 0.495	0.0635
	Monocytes CD45^{+} TCRγ/δ^{-} NK1.1^{-} TCRβ^{-} B220^{-} CD64^{-} CD11c^{-} Ly-6G^{-} CD11b^{+} Ly-6C^{+}	5.090 ± 2.385	3.098 ± 0.976	0.2857

Spleen	**γδ T cells** $CD45^+$ $TCR\beta^-$ $TCR\gamma/\delta^+$	0.892 ± 0.100	1.060 ± 0.110	0.0635
	NK cells $CD45^+$ $TCR\gamma/\delta^-$ $TCR\beta^-$ $NK1.1^+$	2.790 ± 0.506	2.844 ± 0.688	0.6905
	$CD4^+$ T cells $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^+$ $CD8^-$ $CD4^+$	17.780 ± 2.109	19.480 ± 0.850	0.2143
	$CD8^+$ T cells $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^+$ $CD4^-$ $CD8^+$	9.754 ± 1.760	12.340 ± 1.276	* 0.0476
	B cells $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^-$ $B220^+$	56.900 ± 3.152	52.560 ± 1.276	* 0.0159
	Macrophages $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^-$ $B220^-$ $F4/80^+$ $CD64^+$	1.248 ± 0.357	1.634 ± 0.178	0.2222
	cDCs $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^-$ $B220^-$ $CD64^-$ $MHCII^{hi}$ $CD11c^+$	0.544 ± 0.058	0.580 ± 0.085	0.4603
	Neutrophils $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^-$ $B220^-$ $CD64^-$ $CD11c^-$ $Ly\text{-}6G^+$ $Ly\text{-}6C^+$	0.748 ± 0.344	0.546 ± 0.144	0.3175
	Monocytes $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^-$ $B220^-$ $CD64^-$ $CD11c^-$ $Ly\text{-}6G^-$ $CD11b^+$ $Ly\text{-}6C^+$	0.076 ± 0.008	0.104 ± 0.017	* 0.0159
Thymus	**$CD4^+$** $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $CD8^-$ $CD4^+$	8.584 ± 0.763	8.774 ± 1.278	>0.9999

	CD8$^+$ CD45$^+$ TCRγ/δ$^-$ NK1.1$^-$ CD4$^-$ CD8$^+$	5.198 ± 1.420	3.940 ± 1.288	0.0952
	CD4$^+$ CD8$^+$ CD45$^+$ TCRγ/δ$^-$ NK1.1$^-$ CD4$^+$ CD8$^+$	78.480 ± 1.806	80.680 ± 2.475	0.2222
Bone marrow	**γδ T cells** CD45$^+$ TCRβ$^-$ TCRγ/δ$^+$	0.306 ± 0.042	0.486 ± 0.065	** 0.0079
	NK cells CD45$^+$ TCRγ/δ$^-$ TCRβ$^-$ NK1.1$^+$	1.090 ± 0.171	1.116 ± 0.066	>0.9999
	CD4$^+$ T cells CD45$^+$ TCRγ/δ$^-$ NK1.1$^-$ TCRβ$^+$ CD8$^-$ CD4$^+$	0.500 ± 0.091	0.676 ± 0.105	* 0.0317
	CD8$^+$ T cells CD45$^+$ TCRγ/δ$^-$ NK1.1$^-$ TCRβ$^+$ CD4$^-$ CD8$^+$	0.950 ± 0.219	1.448 ± 0.118	** 0.0079
	B cells CD45$^+$ TCRγ/δ$^-$ NK1.1$^-$ TCRβ$^-$ B220$^+$	34.120 ± 5.591	25.960 ± 2.399	* 0.0159
	Macrophages CD45$^+$ TCRγ/δ$^-$ NK1.1$^-$ TCRβ$^-$ B220$^-$ Ly-6G$^-$ CD64$^+$ F4/80$^+$	8.412 ± 1.608	8.236 ± 1.378	>0.9999
	Ly6C^{hi} monocytes CD45$^+$ TCRγ/δ$^-$ NK1.1$^-$ TCRβ$^-$ B220$^-$ Ly-6G$^-$ CD64$^-$ CD11b$^+$ Ly6C^{hi}	2.686 ± 0.411	3.064 ± 0.571	0.5476
	Ly6C^{lo} monocytes CD45$^+$ TCRγ/δ$^-$ NK1.1$^-$ TCRβ$^-$ B220$^-$ Ly-6G$^-$ CD64$^-$ CD11b$^+$ Ly6C^{lo}	1.600 ± 0.169	1.826 ± 0.231	0.2063

	Neutrophils $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^-$ $B220^-$ $Ly\text{-}6G^+$ $Ly\text{-}6C^+$	30.140 ± 2.855	34.960 ± 3.418	0.0952
Blood	**γδ T cells** $CD45^+$ $TCR\beta^-$ $TCR\gamma/\delta^+$	0.808 ± 0.213	1.386 ± 0.232	** 0.0079
	NK cells $CD45^+$ $TCR\gamma/\delta^-$ $TCR\beta^-$ $NK1.1^+$	11.960 ± 4.377	11.580 ± 2.536	0.8968
	$CD4^+$ T cells $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^+$ $CD8^-$ $CD4^+$	16.330 ± 4.123	17.480 ± 1.219	0.9683
	$CD8^+$ T cells $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^+$ $CD4^-$ $CD8^+$	13.220 ± 2.435	15.640 ± 1.682	0.1349
	B cells $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^-$ $B220^+$	36.200 ± 4.047	34.260 ± 2.882	0.4206
	Macrophages $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^-$ $B220^-$ $CD64^+$	2.068 ± 0.262	3.876 ± 0.358	** 0.0079
	Neutrophils $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^-$ $B220^-$ $CD64^-$ $CD11c^-$ $Ly\text{-}6G^+$ $Ly\text{-}6C^+$	3.740 ± 2.980	2.492 ± 0.854	0.8413
	Monocytes $CD45^+$ $TCR\gamma/\delta^-$ $NK1.1^-$ $TCR\beta^-$ $B220^-$ $CD64^-$ $CD11c^-$ $Ly\text{-}6G^-$ $CD11b^+$ $Ly\text{-}6C^+$	0.095 ± 0.028	0.184 ± 0.031	** 0.0079

*n = 4 or 5 mice per group. Data obtained from one experiment. Values represent the mean frequency of each cell subset as a % of $CD45^+$ cells ± standard deviation (SD). Statistical significance was determined using a Mann–Whitney test. * $p < 0.05$, ** $p < 0.01$. cDCs, conventional dendritic cells; NK, natural killer; WT, wild-type.*

Supplementary Table 7. Antibodies used for flow cytometry, polarization cocktails, and immunofluorescence microscopy

Antibody	Company	Clone	Use	Concentration
Alexa Fluor 488 anti-mouse F4/80	BioLegend*	BM8	FC	10 µg/ml
Alexa Fluor 488 anti-mouse FOXP3	BioLegend*	MF-14	FC	10 µg/ml
Alexa Fluor 647 anti-mouse CD4	BioLegend*	RM4-5	IF	5 µg/ml
Alexa Fluor 647 anti-mouse CD107a (LAMP1)	BioLegend*	1D4B	FC	2.5 µg/ml (lung) or 5 µg/ml
			IF	5 µg/ml
Alexa Fluor 647 anti-mouse FOXP3	BioLegend*	MF-14	FC	10 µg/ml
Alexa Fluor 647 anti-mouse IFNγ	BioLegend*	XMG1.2	FC	10 µg/ml
Alexa Fluor 700 anti-mouse CD8α	BioLegend*	53-6.7	FC	2.5 µg/ml
Alexa Fluor 700 anti-mouse/human CD44	BioLegend*	IM7	CS	2.5 µg/ml
Alexa Fluor 700 anti-mouse CD45.1	BioLegend*	A20	FC	5 µg/ml
APC anti-mouse CD4	BioLegend*	GK1.5	FC	1 µg/ml
			CS	2 µg/ml
APC anti-mouse CD11a	BD Biosciences%	2D7	FC	1 µg/ml
CD27 monoclonal antibody, APC	eBioscience#	LG.7F9	FC	1 µg/ml
APC anti-mouse IFN-γ	BioLegend*	XMG1.2	FC	4 µg/ml
APC anti-mouse IL-10	BioLegend*	JES5-16E3	FC	1 µg/ml
APC anti-mouse TCR β chain	BioLegend*	H57-597	FC	1 µg/ml
APC anti-mouse TCR γ/δ	BioLegend*	GL3	FC	2 µg/ml
CD45.2 monoclonal antibody, APC-eFluor 780	eBioscience#	104	FC	0.5 µg/ml
APC/Cy7 anti-mouse NK1.1	BioLegend*	PK136	FC	1 µg/ml
Biotin anti-mouse CD314 (NKG2D)	Miltenyi Biotec^	CX5	FC	0.15 µg/ml
Biotin mouse anti-mouse H-2Kb	BD Biosciences%	AF6-88.5	FC	2.5 µg/ml
BUV395 rat anti-mouse CD4	BD Biosciences%	GK1.5	FC	1 µg/ml
BUV395 mouse anti-mouse CD45.2	BD Biosciences%	104	FC	1 µg/ml
BUV737 hamster anti-mouse TCRβ chain	BD Biosciences%	H57-597	FC	1 µg/ml

Brilliant Violet 421 anti-mouse CD279 (PD-1)	BioLegend*	29F.1A12	FC	5 µg/ml
Brilliant Violet 421 anti-mouse/human CD11b	BioLegend*	M1/70	FC	10 µg/ml
CD335 (NKp46) monoclonal antibody, eFluor 450	eBioscience#	29A1.4	FC	2 µg/ml
Brilliant Violet 421 anti-mouse F4/80	BioLegend*	BM8	FC	2 µg/ml
Brilliant Violet 421 anti-mouse FOXP3	BioLegend*	MF-14	FC	4 µg/ml
Brilliant Violet 605 anti-mouse CD4	BioLegend*	GK1.5	FC CS	1 µg/ml 1 µg/ml
Brilliant Violet 605 anti-mouse CD4	BioLegend*	RM4-5	FC CS	1 µg/ml 1 µg/ml
Brilliant Violet 605 anti-mouse NK1.1	BD Biosciences%	PK136	FC	1 µg/ml
Brilliant Violet 605 anti-mouse Ly-6C	BioLegend*	HK1.4	FC	10 µg/ml
Brilliant Violet 650 anti-mouse/human CD11b	BioLegend*	M1/70	FC	0.25 µg/ml
Brilliant Violet 650 anti-mouse/human CD45R/B220	BioLegend*	RA3-6B2	FC	10 µg/ml
Brilliant Violet 650 rat anti-mouse IFNγ	BD Biosciences%	XMG1.2	FC	2 µg/ml
Brilliant Violet 650 anti-mouse TNFα	BioLegend*	MP6-XT22	FC	2 µg/ml
Brilliant Violet 711 anti-mouse CD45.2	BioLegend*	104	FC	2 µg/ml
Brilliant Violet 785 anti-mouse CD11c	BioLegend*	N418	FC	2.9 µg/ml
Brilliant Violet 785 anti-mouse CD223 (LAG3)	BioLegend*	C9B7W	FC	4 µg/ml
T-bet monoclonal antibody, eFluor 660	eBioscience#	eBio4B10 (4B10)	FC	4 µg/ml
FITC anti-mouse CD4	BioLegend*	GK1.5	FC	2.5 µg/ml
FITC anti-mouse CD8a	BioLegend*	53-6.7	FC	2.5 µg/ml
FITC anti-mouse CD11a	BioLegend*	M17/4	FC	10 µg/ml
FITC anti-mouse CD45.1	BioLegend*	A20	FC	5 µg/ml

FITC anti-mouse CD45.2	BioLegend*	104	FC	2.5 µg/ml
FITC anti-mouse TCR β chain	BioLegend*	H57-597	FC	2.5 µg/ml
LEAF™ Purified anti-mouse CD28	BioLegend*	37.51	IVP	2 µg/ml
LEAF™ Purified anti-mouse IFN-gamma	BioLegend*	XMG1.2	IVP	10 µg/ml
LEAF™ Purified anti-mouse IL-4	BioLegend*	11B11	IVP	10 µg/ml
Pacific Blue anti-human/mouse Granzyme B	BioLegend*	GB11	FC	1 µl/sample
Pacific Blue anti-mouse I-A/I-E	BioLegend*	M5/114. 15.2	FC	1.25 µg/ml
PE anti-mouse CD8a	BioLegend*	53-6.7	FC	1 µg/ml
PE anti-mouse CD62L	BioLegend*	MEL-14	CS	1 µg/ml
PE anti-mouse CD226 (DNAM-1)	BioLegend*	480.1	FC	1 µg/ml
PE anti-mouse Granzyme B	eBioscience#	GB11	FC	0.06 µg/ml
Granzyme B Monoclonal Antibody, PE	Invitrogen&	GB11	FC	1 µl/sample
PE anti-mouse I-A/I-E	BioLegend*	M5/114. 15.2	FC	0.4 µg/ml
PE anti-mouse IL-10	BioLegend*	JES5-16E3	FC	2 µg/ml
PE anti-mouse Perforin	BioLegend*	S16009B	FC	2 µg/ml
Mouse Rae-1 Pan Specific PE-conjugated antibody	R&D Systems@	186107	FC	5 µl/sample
PE Mouse Anti-Stat3 (pY705)	BD Biosciences%	4/P-STAT3	FC	1 µl/sample
PE Mouse Anti-Stat4 (pY693)	BD Biosciences%	38/p-Stat4	FC	1 µl/sample
PE/Cy7 anti-human/mouse/rat CD278 (ICOS)	BioLegend*	C398.4A	FC	4 µg/ml
PE/Cy7 anti-mouse CD8α	BioLegend*	53-6.7	FC	1 µg/ml
PE/Cy7 anti-mouse CD25	BioLegend*	PC61	CS	1 µg/ml
PE/Cy7 anti-mouse CD49d	BioLegend*	R1-2	FC	4 µg/ml
PE/Cy7 anti-mouse F4/80	BioLegend*	BM8	FC	4 µg/ml
PE/Cy7 anti-mouse IFNγ	BioLegend*	XMG1.2	FC	1 µg/ml
PE/Cy7 anti-mouse Ly-6G	BioLegend*	1A8	FC	1 µg/ml

NK1.1 monoclonal antibody, PE-Cyanine7	eBioscience[#]	PK136	FC	1 µg/ml
PE/Cy7 anti-T-bet antibody	BioLegend*	4B10	FC	4 µg/ml
PE/Dazzle 594 anti-mouse CD64 (FcγRI)	BioLegend*	X54-5.71	FC	1 µg/ml
PE/Dazzle 594 anti-mouse CD152	BioLegend*	UC10-4B9	FC	1 µg/ml
PE/Dazzle 594 IFNγ	Biolegend*	XMG1.2	FC	1 µg/ml
PE/Dazzle 594 anti-mouse IL-10	BioLegend*	JES5-16E3	FC	4 µg/ml
PerCP/Cy5.5 anti-mouse CD8α	BioLegend*	53-6.7	FC	1 µg/ml
PerCP/Cy5.5 anti-mouse CD11a/CD18	BioLegend*	LFA-1	FC	1 µg/ml
PerCP/Cy5.5 anti-mouse/human CD11b	BioLegend*	M1/70	FC	1 µg/ml
PerCP/Cy5.5 rat anti-mouse CD38	BD Biosciences[%]	90/CD38	FC	4 µg/ml
PerCP/Cy5.5 anti-mouse CD90.2	BioLegend*	30-H12	CS	2 µg/ml
PerCP/Cy5.5 anti-mouse TCRβ	BioLegend*	H57-597	FC	0.5 µg/ml
TruStain fcX™ (anti-mouse CD16/32)	BioLegend*	93	FC	1.25 µg/ml
Ultra-LEAF™ Purified anti-mouse CD3ε	BioLegend*	145-2C11	IVP	4 µg/ml

**BioLegend, San Diego CA, USA; [#]eBioscience, Thermo Fisher Scientific, Waltham MA, USA; [%]BD Biosciences, San Jose CA, USA; ^Miltenyi Biotec, Bergisch Gladbach, Germany; [&]Invitrogen, Carlsbad CA, USA; [@]R&D Systems, Minneapolis MN, USA; FC, flow cytometry; IF, immunofluorescence; CS, fluorescence-activated cell sorting; IVP, in vitro polarization*