Supplemental Material Table of Contents

Supplementary Figure 1. Overall study design.

Supplementary Figure 2. Expansion of TEMRA CD8 in KT with biopsy-proven ABMR.

KT were recruited 1 year after their transplantation and with either biopsy-proven ABMR (n=12) or normal histological biopsy (n=52; recipient age and gender matched) (**A**) Absolute number per mL of blood of subsets of CD8 T cells. (**B**) Spearman correlation between the frequency of TEMRA CD8 among CD8 T cells and the percentage of $CD107a^+$ among CD8 T cells. Data represents the median±IQR[25%-75%] and the value (point) of a single patient and one representative patient. The p-values were calculated using the Mann-Whitney test ** $p<0.01$.

Supplementary Figure 3. Migratory properties of memory CD8 T cell (TEMRA and EM): adhesion to activated HDMECs and chemokine screening by transmigration assays. (**A**) Transcriptomic profiling of chemokine receptor expression by EM and TEMRA CD8 T cells according to published RNA-seq data[10]. (**B**) Targeted proteomic analysis of chemokine receptor expression by EM and TEMRA CD8 T cells based on the ImmunoProt database[24]. (**C**) Transmigration of CD8 T cells subsets across TNFα (100 U/ml, O/N)-treated HDMEC monolayers in response to the indicated chemokines and cytokines. (**D**) Impact of CXCL12 (50 ng/ml) on the roundness of CD8 T cell subsets from KT and HVs. The roundness was tracked for all cells during the entire observational period using Fiji- and MATLAB-based scripts. A roundness of 1 is obtained for a perfect circular shape and 0 for a flat shape. (**E**) Migration of Fura-2-labeled CD8 T cell subsets from KT and HV onto TNFα-treated HDMEC monolayers. Average velocity of CD8 T cell subsets is shown. The p-values were calculated using Wilcoxon matched-pairs signed rank test (**A**), nonparametric ANOVA (Kruskal-Wallis) with Dunn's multiple comparisons test (**C**) and the Wilcoxon matched-pairs signed rank test. * $p<0.05$, ** $p<0.01$.

Supplementary Figure 4. Accumulation of TEMRA CD8 T cells in the kidney graft of KT with ABMR. (**A**) GSEA analysis of TEMRA gene signature derived from the literature (**Supplementary Table 3**) in gene expression data of kidney biopsies from kidney transplanted patients with or without ABMR (n= 56 and 168, respectively)[1] (GSE147089). The normalized enrichment score (NES) is the degree to which a gene set is overrepresented, normalized across analyzed gene sets. The nominal P-value (NOM P-val) is the statistical significance of the enrichment score. (**B**) Correlation between expression of *CD8* and TEMRA-associated genes in patients with ABMR (pink) and normal biopsy (shaded gray). Correlation was calculated using nonparametric Spearman correlation.

Supplementary Figure 5. Combined stimulation with CXCL12 and TCR results in the selective activation of memory CD8 T cells (TEMRA and EM). Frequency of $CD69^{+}CD107a^{+}$ among the EM (green dot), TEMRA (red dot) and NAÏVE (black dot) CD8 T cells from KT (**A, B**) or HVs (**B**) after 4 h of stimulation with plate-bound anti-CD3 mAb (2 µg/ml) or aCD3 + CXCL12 (50 ng/ml). Data represent the median (gray histogram) and the value (point) of a single HV. The p-values were calculated using nonparametric ANOVA (Kruskal-Wallis) with Dunn's multiple comparisons test. * $p<0.05$, ** $p<0.01$,**** $p<0.0001$

Supplementary Figure 6. Short-term IL-15 stimulation enhances the selectivity of memory (TEMRA and EM) CD8 T cells to P-selectin. (**A**) CD8 T cell subsets (NAÏVE, black; EM, green; TEMRA, red) were purified from HVs and stimulated, when indicated, O/N with IL-15 (10 ng/ml). Binding of CD8 T cell subsets to P-selectin chimeric proteins was quantified. Data represent the median (gray histogram), the value (point) of a single HV and one representative histogram. The p-values were calculated using nonparametric ANOVA (Kruskal-Wallis) with Dunn's multiple comparisons test. * $p<0.05$, **** $p<0.0001$. (**B**) Gating strategy to analyze the incorporation of ManAc by purified CD8 T cells incubated O/N with Click-IT ManNAz (ManAc) and, when indicated, IL-15 (10 ng/ml). CD8 T cells were then

treated with 2-DG (50 µM, 4 h, 37°C) before the quantification of the incorporation of ManAc after cell permeabilization (intra) or cell fixation (extra). Incorporation of ManAc was quantified after cell permeabilization (Intracellular) or cell fixation (Extracellular).

Supplementary Figure 7. Inhibition of glycolysis impairs the generation of functional PSGL-1. CD8 T cell were purified from HVs and stimulated O/N with IL-15 (10 ng/ml) and , when indicated, in the presence of 2-DG (50 µM). Expression by EM and TEMRA of total and functional PSGL-1 was quantified by flow cytometry using KPL-1 and CHO131 anti-PSGL-1 mAb respectively. Data represent the value (point) of a single HV and one representative histogram. The p-values were calculated using nonparametric paired Wilcoxon test. * $p<0.05$.

Target	Fluorochrome	Clone Name	Provider
CD3	BV421	UCHT1	BD Biosciences
CD3	VioBlue	HIT3a	BD Biosciences
CD3		OKT3	Invitrogen
CD3	FITC	UCHT1	BD Biosciences
CD8	VioGreen	BW135/80	Miltenyi
CD8	PE-Cy7	RPA-T8	BD Biosciences
CD8	Purified	C8/144B	Dako
CD28	PE-CF594	CD28.2	BD Biosciences
CD28	Purified	CD28.2	Invitrogen
CD45RA	APC-Vio770	TD611	Miltenyi
CD162	PE	KPL1	BD Biosciences
CD11a	APC	HI111	BD Biosciences
CD49d	FITC	9F10	BD Biosciences
CD162	Purified	CHO131	R&D Systems
PERF1	PE	B-D48	Diaclone
GZMB	PE-C7	QA18A28	Biolegend

Caspase-3	PE	C92 605	BD Biosciences
IgM	PE	C48-6	BD Biosciences
VLA-4	PE-Cy7	9F10	Biolegend
LFA-1	PE	G4325B	Biolegend

Supplementary Table 1. Listing of monoclonal antibodies

Reagent or Resource	Provider
Oligomycin	Sigma
6-Diazo-5-oxo-L-norleucine (DON)	Sigma
2-Deoxy-D-glucose (2-DG)	Sigma
Neuraminidase	Sigma
CXCL10	PeproTech
CXCL9	PeproTech
IL-8	PeproTech
CXCL12 (SDF1α)	R&D Systems
^{10}Panx	R&D Systems
Recombinant human P-selectin	R&D Systems
P2X1 (NF023)	R&D Systems
P2X4 (5-BDBD)	R&D Systems
P2X7 (A438079)	R&D Systems
TNFα	R&D Systems
IL-15	Miltenyi
CD8 T cell Isolation Kit human	Miltenyi
Human REAlease CD8 MicroBead Kit	Miltenyi
TexMacs medium	Miltenyi
Endothelial Cell Growth Medium	Lonza
CCCP	Sigma
123count eBeads™ Counting Beads	Thermo Fisher
Fura2	Thermo Fisher
ApoSENSOR™ ATP Cell Viability Bioluminescence Assay Kit	BioVision
PE-anti-human IgG Fc	Thermo Fisher
Click-iT® Cell Reaction Buffer Kit and alkyne Alexa Fluor® 488	Invitrogen
Click-IT™ ManNAz Metabolic Glycoprotein Labeling Reagent (tetraacetylated N-Azidoacetyl-D-Mannosamine)	Invitrogen

BD Cytofix/Cytoperm™ kit	BD Bioscience
μ-Slide 8 well	ibidi
Poly-L-Lysine precoated μ-Slide 8 well	ibidi
RPMI 1640 medium	Thermo Fisher
FBS	Sigma
Glutamax	Thermo Fisher
Penicillin-Streptomycin-Neomycin Solution	Sigma
IL-2	Biolegend
Trypan blue	Gibco
Ficoll-Paque Plus	GE Healthcare
Cell Proliferation Dye eFluor V450	Thermo Fisher
Fixable Viability Dye 440UV	BD Bioscience
Fc Block	BD Bioscience
OPAL 520	Akoya
DAPI	Invitrogen
TR1, TR2	Impath
Poly-E and HRP-2-Step	Impath
Vectashield® Vibrance™ Antifade Mounting Medium	Vector

Supplementary Table 2. List of reagents

Gene Name	References
CD3D	[2]
CD3E	[2]
CD3G	[2]
CD8A	[2]
CD8B	[2]
TBX21	[2–4]
IFNG	[2]
CX3CR1	[2–6]
GZMB	[2]
GZMH	[2]
GNLY	[2]
PRF1	[2]
FCGR3A	[2–7]
PRDM1	[2]
HK2	[8]
Zeb2	[9]
Ezh2	[8]
Bcl6	[8]
EOMES	[8]
ID3	[8]
TNFa	[2–6]
KLRD1	[10]
IL7R	[10–12]
CD28	[10–12]

Supplementary Table 3. Gene signature of TEMRA CD8 based on a review of literature.

Upregulated genes in TEMRA CD8 vs EM CD8

Gene Symbol	log_2FC	AveExpr	P.Value	adj.P.Val
SH2D1B	5,67	1,553	4,44E-07	0,0022
RAMP1	5,17	1,702	9,54E-06	0,0072
LAT2	4,18	2,250	1,05E-05	0,0072
KLRF1	4,01	3,263	3,83E-06	0,0057
FCGR3A	4,01	2,942	1,44E-06	0,0034
FGFBP2	3,87	5,475	4,95E-06	0,0059
NCR1	3,83	3,236	1,43E-06	0,0034
ZEB2-AS1	3,75	1,650	3,01E-04	0,0440
GNLY	3,61	10,829	1,35E-04	0,0282
SPON2	3,45	2,101	3,56E-04	0,0451
KIR3DL2	3,40	1,305	1,49E-04	0,0300
LAIR2	3,40	0,589	3,46E-04	0,0451
KIR3DL1	3,36	0,614	1,65E-04	0,0313
SYNGR1	3,33	1,641	1,57E-05	0,0082
TYROBP	3,17	6,038	2,13E-04	0,0361
CX3CR1	3,09	1,235	4,57E-07	0,0022
PRSS23	3,06	1,597	9,19E-06	0,0072
HAVCR2	3,00	2,797	5,03E-05	0,0154
CD38	2,93	1,507	2,61E-04	0,0409
GSN	2,89	1,716	3,38E-04	0,0451
GPR56	2,78	2,958	2,00E-05	0,0087
AKR1C3	2,54	0,962	3,83E-05	0,0139
FHL3	2,34	1,366	4,67E-05	0,0153
CXXC5	2,29	1,691	2,02E-04	0,0352
TTC38	2,09	3,052	2,04E-04	0,0352
SPINK2	2,09	0,224	1,06E-04	0,0245
CCL4	2,01	5,263	4,57E-05	0,0153
FGR	1,99	3,102	3,49E-04	0,0451
CD160	1,97	1,944	1,79E-04	0,0327
MTSS1	1,97	2,094	8,67E-05	0,0227
LAYN	1,77	0,492	6,97E-05	0,0200
PION	1,76	1,625	3,63E-04	0,0452
UBE2F	1,72	4,388	1,25E-05	0,0074
PTPN12	1,71	1,708	3,08E-04	0,0443
NMUR1	1,54	0,554	9,36E-05	0,0228
IFIT2	1,52	2,793	2,14E-05	0,0087
CERS4	1,51	1,909	9,94E-05	0,0236
PDLIM1	1,50	2,536	4,10E-04	0,0498
IFIT3	1,45	3,120	8,50E-05	0,0227
GNPTAB	1,41	2,380	8,85E-05	0,0227
LIMK1	1,40	1,365	2,68E-04	0,0409
FAM46A	1,39	1,317	8,23E-05	0,0227
TTC28-AS1	1,32	1,955	4,86E-05	0,0154
GEMIN7	1,26	2,982	1,37E-04	0,0282
C5orf56	1,05	5,291	2,67E-04	0,0409

Downregulated genes in TEMRA CD8 vs EM CD8	DYNLT3	-1,00	4,237	3,39E-04	0,0451
	S100A11	-1,00	6,683	3,57E-04	0,0451
	KDSR	-1,14	2,795	3,14E-04	0,0444
	CDC14A	-1,16	3,307	3,43E-04	0,0451
	PLK3	-1,27	2,493	1,33E-04	0,0282
	TMEM154	-1,33	2,298	4,28E-05	0,0150
	INPP4B	-1,35	2,633	2,95E-04	0,0437
	ANO9	-1,38	1,580	2,38E-04	0,0389
	TNIP3	-1,48	1,893	2,50E-04	0,0402
	TC2N	-1,54	4,425	2,81E-04	0,0422
	CENPM	-1,73	2,021	1,11E-04	0,0245
	ANTXR2	-1,91	1,828	2,53E-05	0,0096
	SPON1	-1,93	0,678	3,87E-04	0,0477
	HNRPLL	-1,98	1,937	5,88E-06	0,0062
	FLT4	-2,06	0,072	1,88E-04	0,0336
	NCF4	-2,16	1,192	1,61E-04	0,0310
	AQP3	-2,16	3,847	1,09E-04	0,0245
	CCR5	-2,22	1,929	1,54E-04	0,0304
	HNMT	-2,23	-0,178	1,73E-04	0,0322
	ELOVL4	-2,24	0,219	2,10E-05	0,0087
	AMICA1	-2,25	4,414	9,40E-06	0,0072
	CCR2	-2,35	-0,443	2,31E-04	0,0385
	WDR86-AS1	-2,39	0,991	6,66E-05	0,0197
	GPR183	-2,40	5,542	1,51E-05	0,0082
	IL7R	-2,51	7,638	1,90E-05	0,0087
	FAAH2	-2,52	1,387	3,38E-04	0,0451
	CD28	-2,55	2,409	1,66E-05	0,0083
	GPR15	-2,79	0,750	2,21E-05	0,0087
	SLAMF1	-2,80	1,995	4,19E-06	0,0057
	GZMK	-2,84	7,141	1,14E-05	0,0072
	DPP4	-3,75	2,265	1,13E-05	0,0072
	CCR4	-3,78	0,115	9,12E-05	0,0228
	CCR6	-3,89	1,028	2,87E-06	0,0054

Supplementary Table 4. Differential expressed genes in TEMRA CD8 vs EM CD8 in KT patients.

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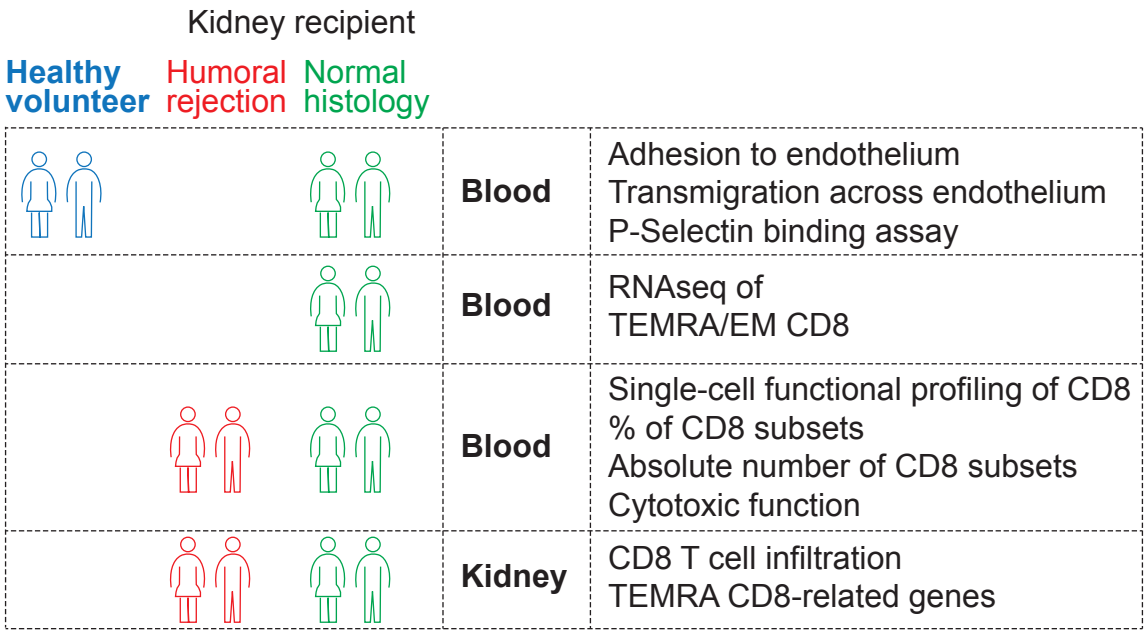
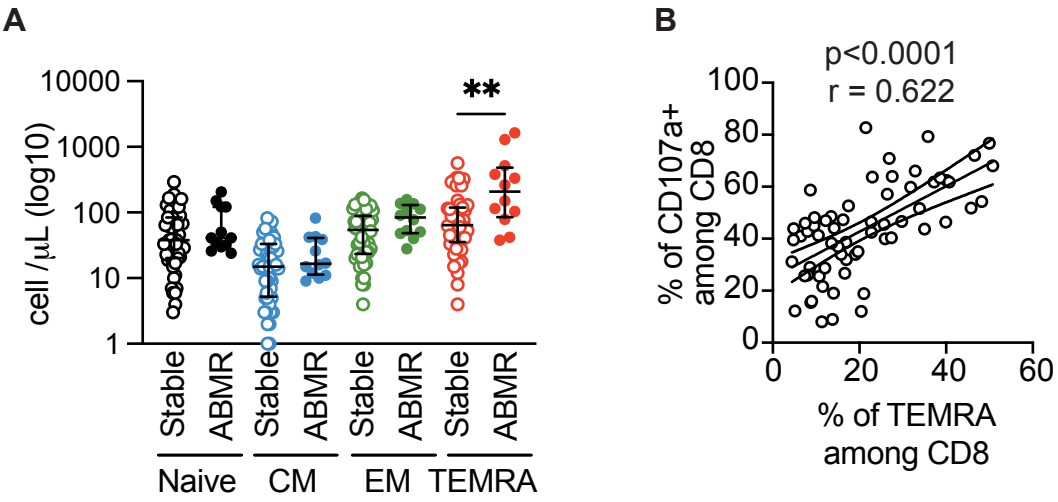


Supplementary Figure 1



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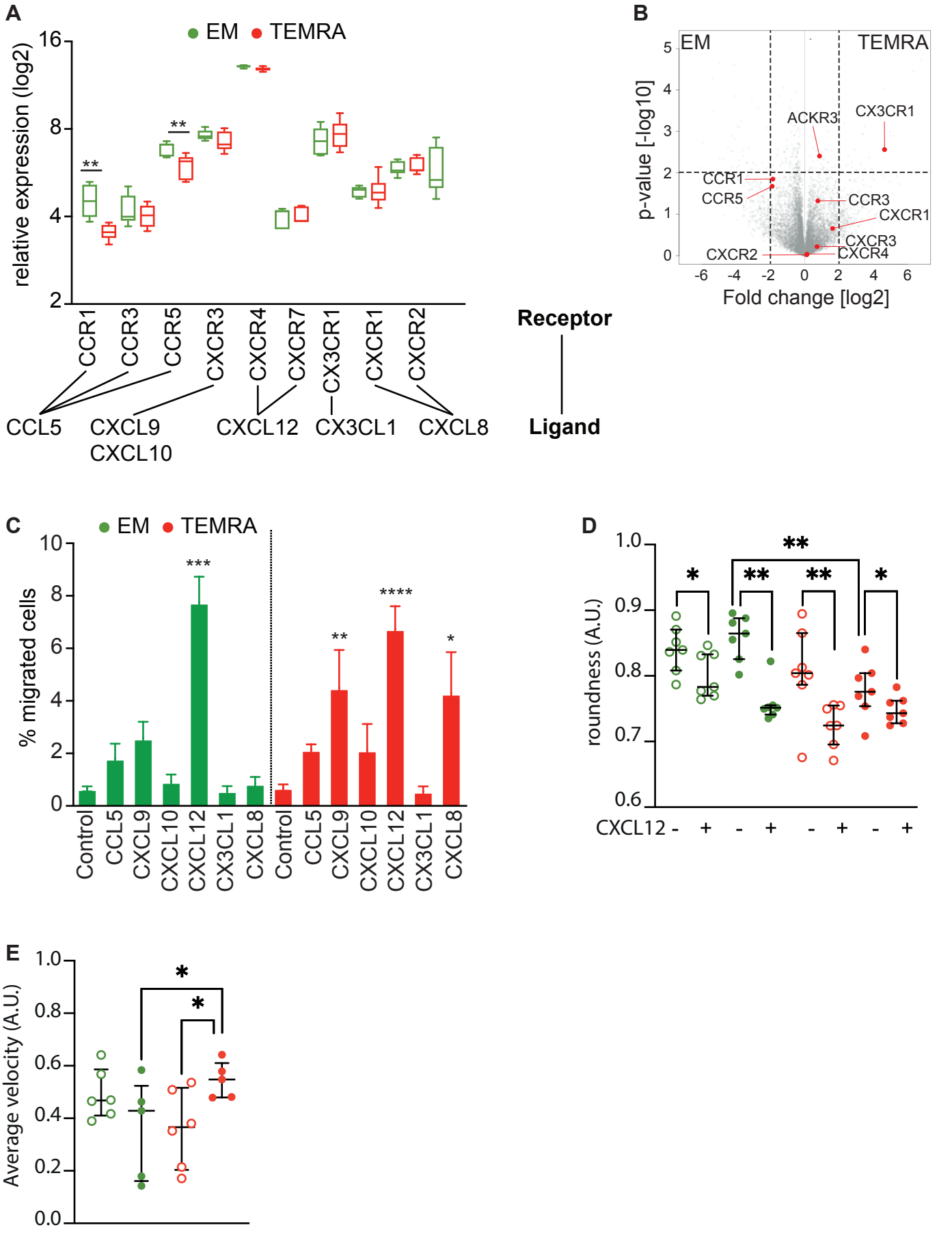

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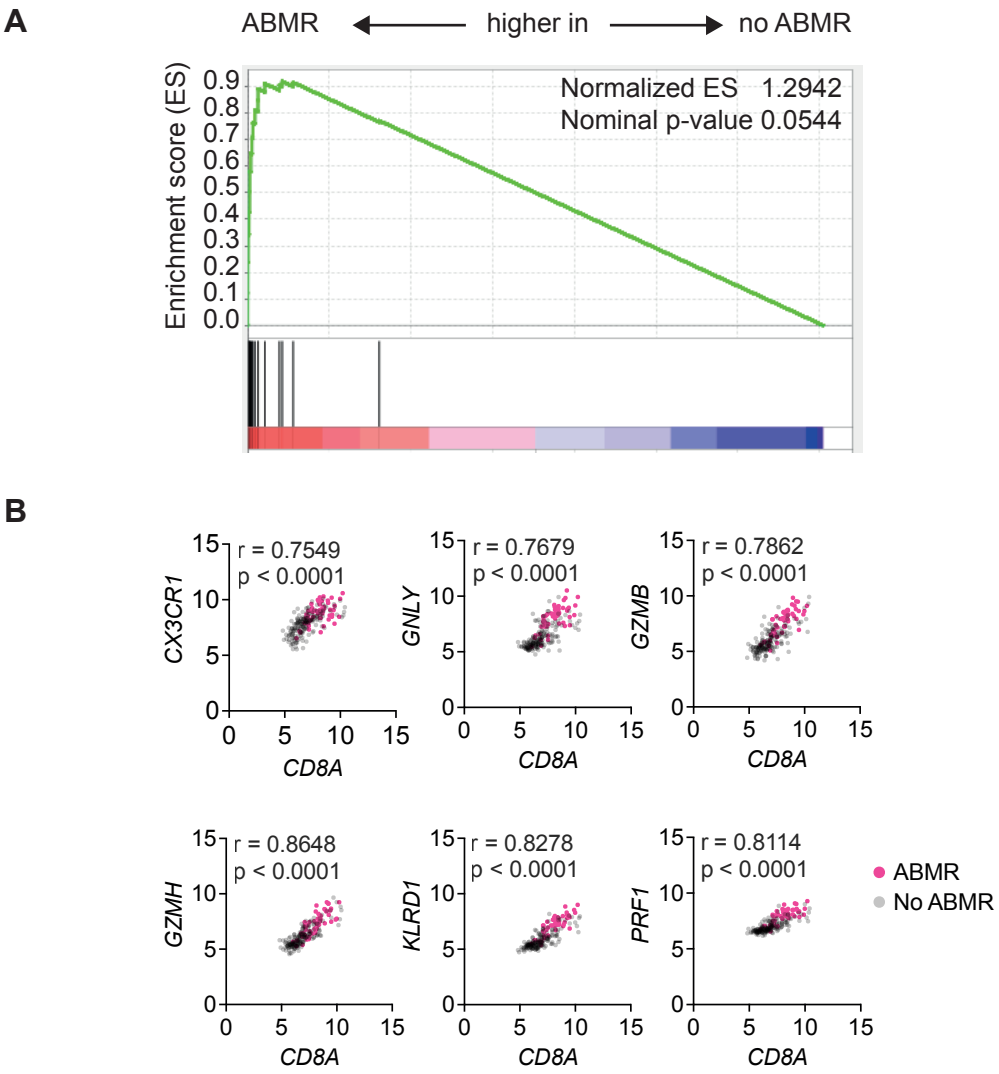

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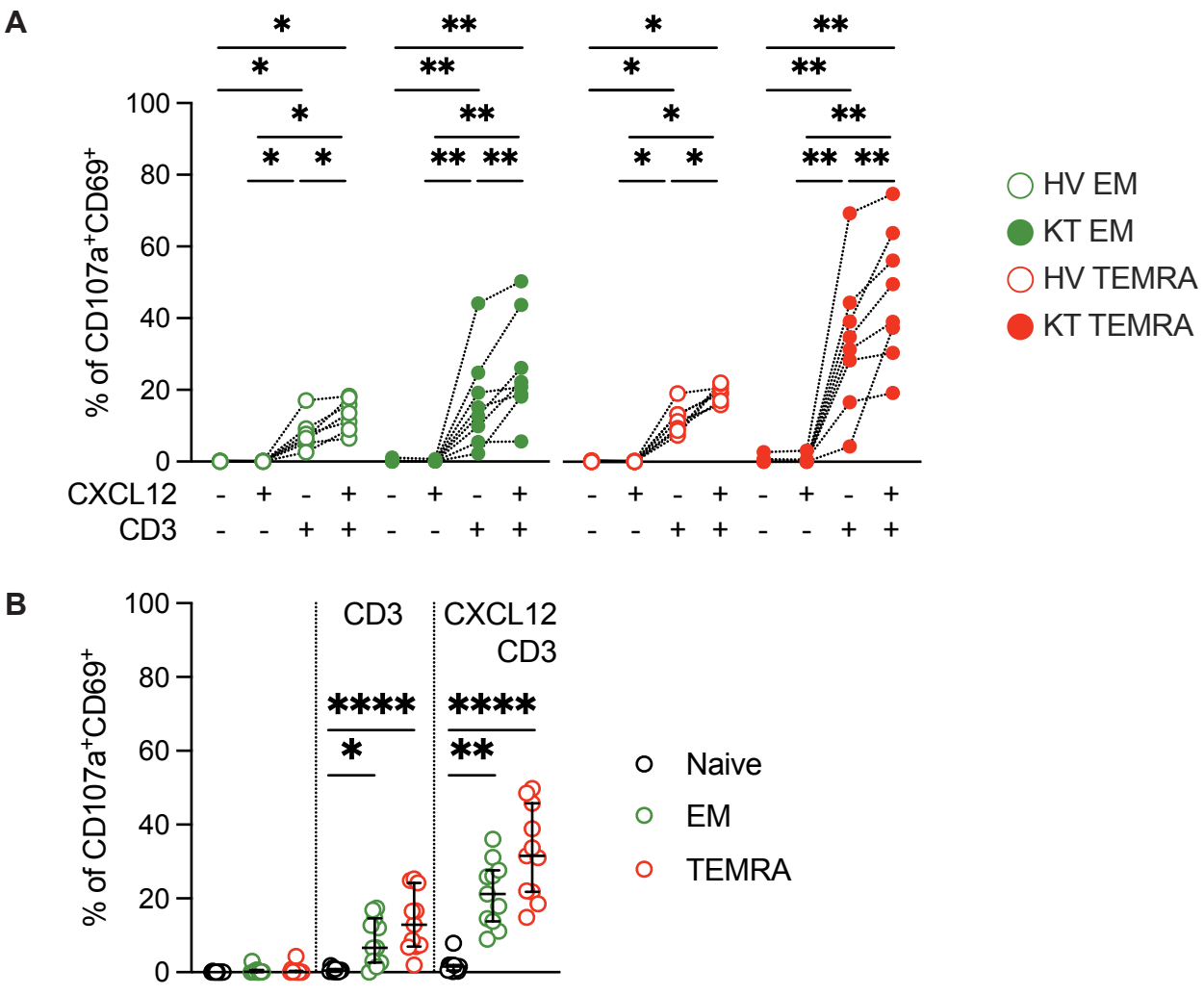
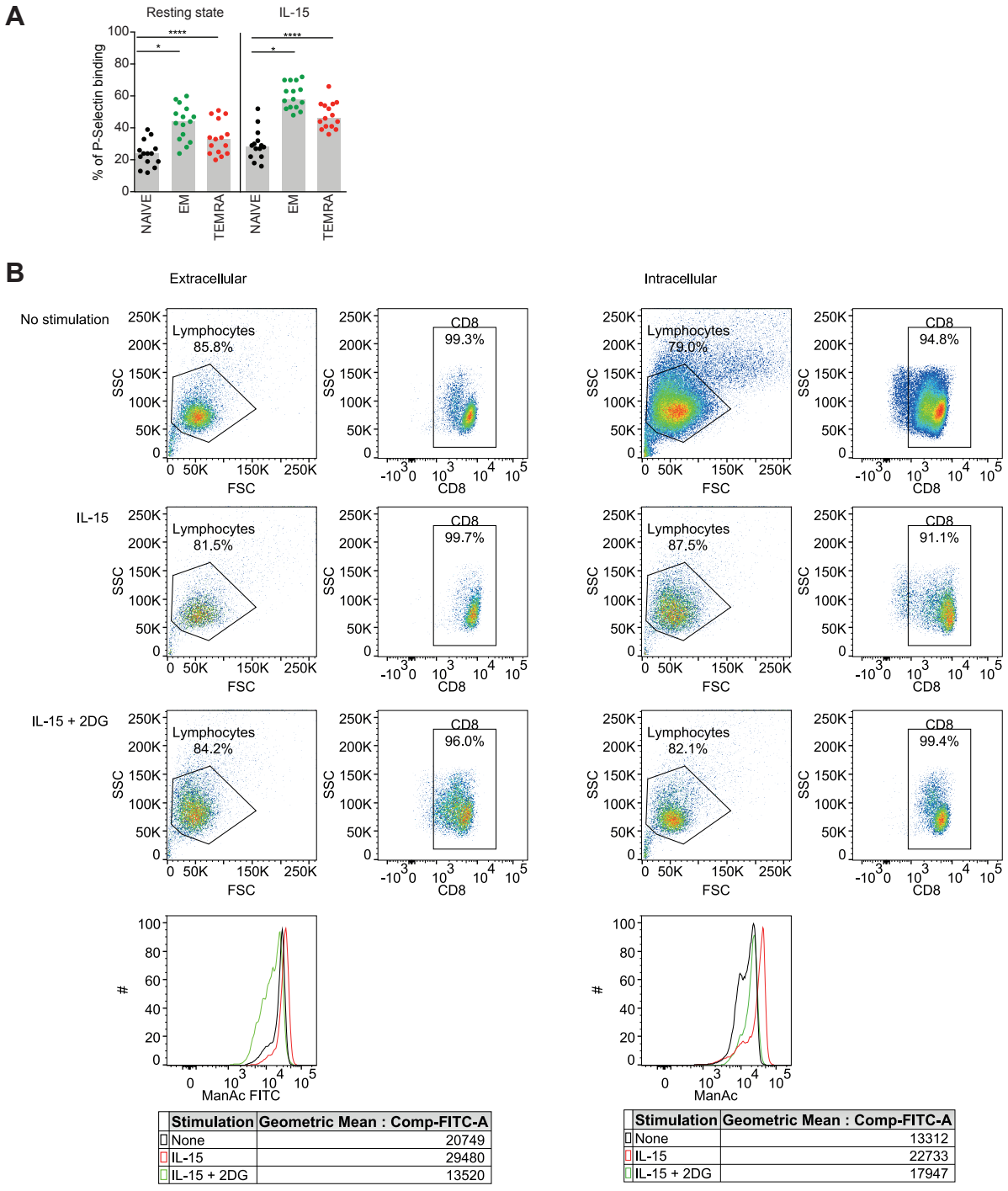


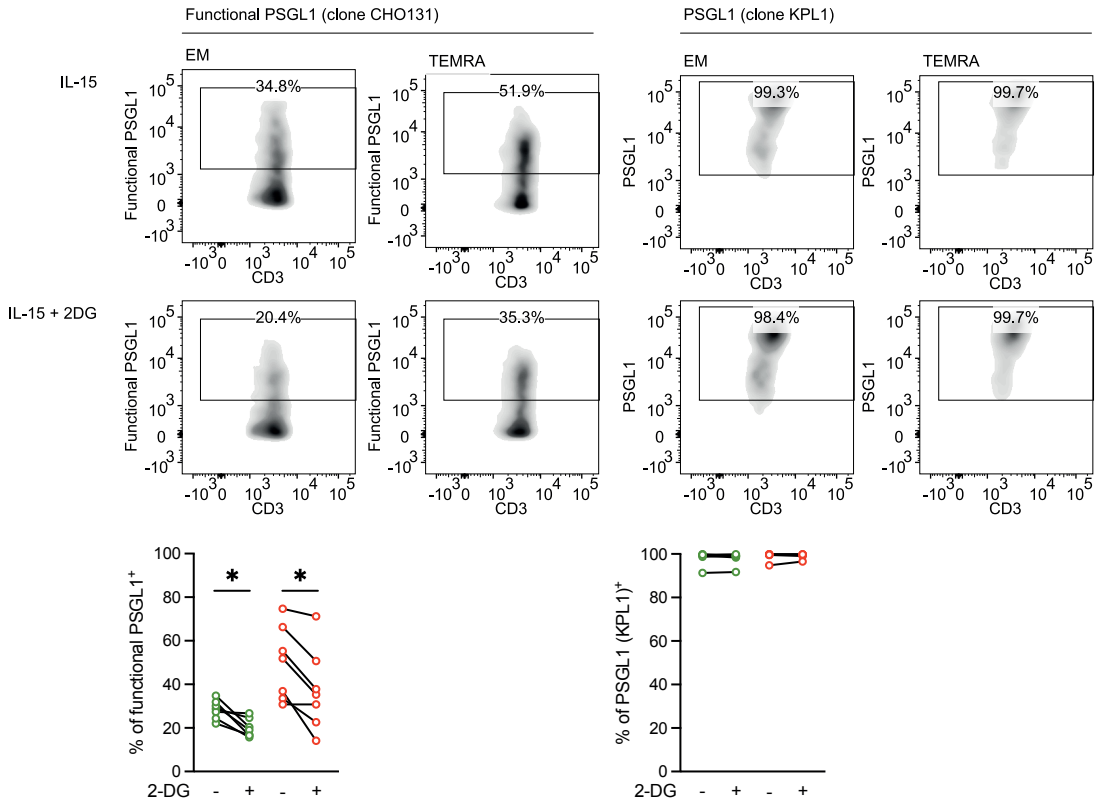
Supplementary Figure 5



	Stimulation	Geometric Mean : Comp-FITC-A
	None	20749
	IL-15	29480
	IL-15 + 2DG	13520

	Stimulation	Geometric Mean : Comp-FITC-A
	None	13312
	IL-15	22733
	IL-15 + 2DG	17947

Supplementary Figure 6



Supplementary Figure 7