

Supplemental Material

Lung endothelial cell antigen cross-presentation to CD8⁺T cells drives malaria-associated lung injury

Claser and Nguee et al.

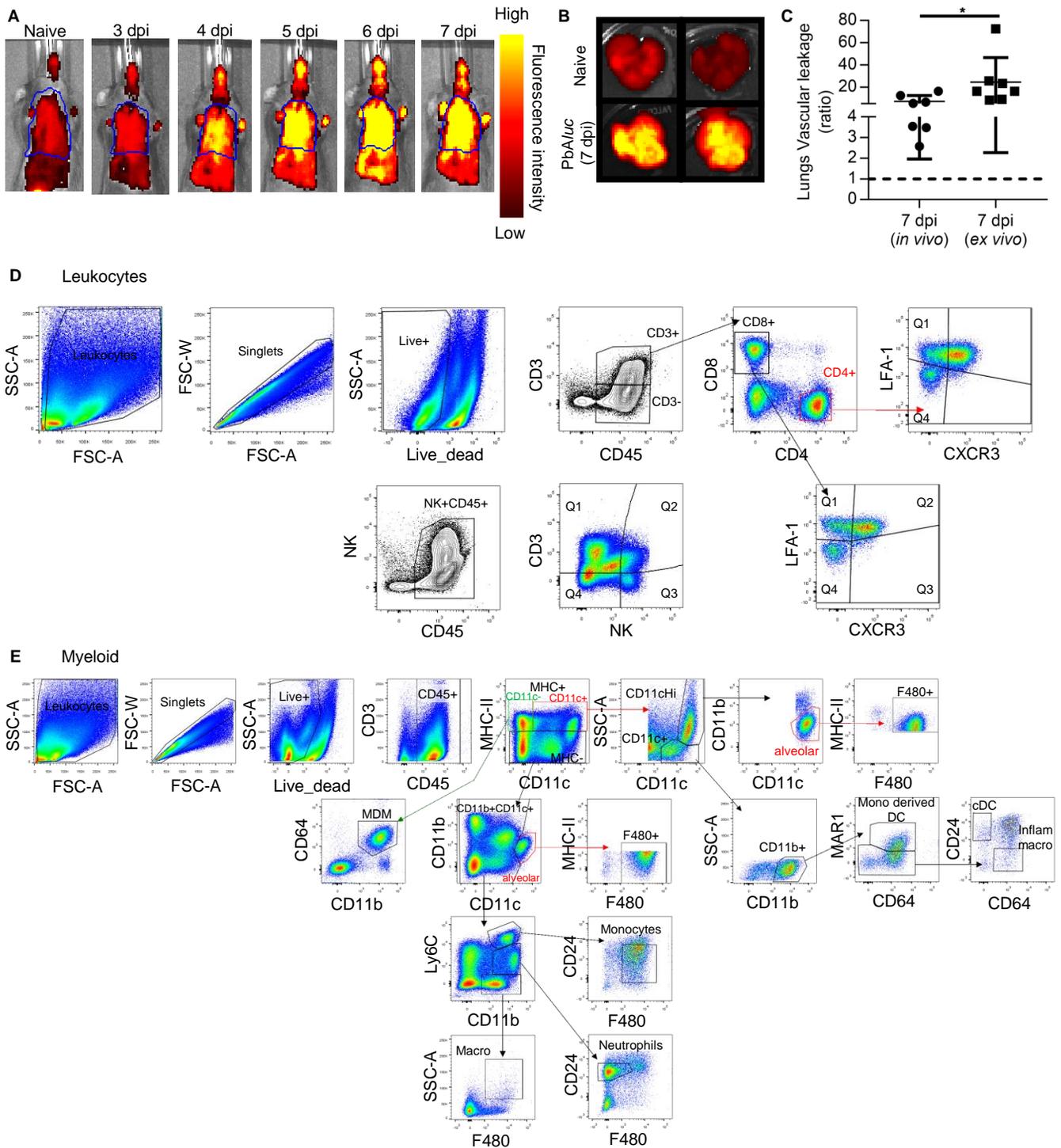
Supplementary Table 1: Cell Number and percentage of immune cells subtypes present in the lungs of PbA/ <i>uc</i> -infected mice on 7 dpi					
	Cell number		Percentage (%)		P Value*
	N (n=9)	PbA (n=11)	N	PbA	
Absolute total cell count	6120000 ± 2369240	5783636 ± 2390512			NS
CD4	127635 ± 72433	175260 ± 78096	16.35	16.42	NS
CD8	83694 ± 47860	184225 ± 107155	10.72	17.26	b
NK	152189 ± 109855	175505 ± 94105	19.49	16.44	NS
Monocytes	60561 ± 30084	131079 ± 111770	7.76	12.28	NS
Neutrophils	290993 ± 249427	140054 ± 128675	37.27	13.12	NS
cDC	5889 ± 4292	1214 ± 1886	0.75	0.11	c
pDC	4202 ± 2339	555.9 ± 549.8	0.54	0.05	d
Mono derived DC	9016 ± 14568	27286 ± 19980	1.15	2.56	b
Macrophages	18150 ± 14590	4891 ± 6493	2.32	0.46	b
Interst Macro	2868 ± 3846	4079 ± 3714	0.37	0.38	NS
MDM	13332 ± 17104	194144 ± 141333	1.71	18.19	d
Alveolar macro	12322 ± 8326	29297 ± 47219	1.58	2.74	NS
<i>Definition of abbreviations:</i> N = naïve; cDC = conventional DC; pDC = plasmacytoid DC; MDM = mono derived macrophages; NS = not significant					
*Significance level of naïve versus PbA/ <i>uc</i> 7 dpi. Cell numbers are indicated in the right-hand end column. Results compiled from 3 independent experiments. Significance levels are indicated as follows: ^a P<0.05; ^b P<0.01; ^c P<0.001; ^d P<0.0001 Unpaired-t test or Mann-Whitney test was used					

Supplementary Table 2: Antibodies used for Flow Cytometry					
Antibody	Clone	Fluorochrome	Dilution	Catalog number	Supplier
<i>Leukocytes</i>					
CD45	30-F11	APC-Cy7	1:250	557659	BD Pharmingen
CD3e	145-2c11	BV421	1:100	100336	Biolegend
CD8	53-6.7	BV605	1:200	100744	Biolegend
CD4	RM4-5	PE-Cy7	1:200	25-0042-82	eBioscience
NK1.1	Pk136	APC	1:100	17-5941-82	eBioscience
CXCR3	CXCR3-173	PerCP-Cy5.5	1:100	45-1831-82	eBioscience
CD11a (LFA-1)	M17/4	FITC	1:250	11-0111-82	eBioscience
IA^b/IE^b (MHC II)	M5/114.15.2	Alexa700	1:200	56-5321-82	eBioscience
CD11b	M1/70	BV650	1:200	101239	Biolegend
CD11c	N418	PE-Cy7	1:200	25-0114-82	eBioscience
CD24	M1/69	Pacific blue	1:200	48-0242-82	eBioscience
CD64	X54-5/7.1.1	PE	1:100	558455	BD Pharmingen
Ly6C	HK1.4	PerCP-Cy5.5	1:200	45-5932-82	eBioscience
F4/80	BM8	Biotin	1:50	123106	Biolegend
FcεR1 alpha	MAR1	APC	1:50	17-5898-82	eBioscience
BST2	ebio927	FITC	1:200	11-3172-82	eBioscience
<i>Intracellular staining</i>					
IFN-γ	XMG1.2	PerCP-Cy5.5	1:50	45-7311-82	eBioscience
Granzyme B	NGZB	PE-Cy7	1:100	25-8898-82	eBioscience
<i>Endothelial cells</i>					
CD31	390	PE-Cy7	1:50	102418	Biolegend
CD144 (VE-Cad)	BV13	APC	1:100	17-1441-82	eBioscience
CD34	RAM34	Alexa700	1:100	56-0341-82	eBioscience
L-selectin (CD62L)	MEL-14	efluor450	1:100	48-0621-82	eBioscience
H-2D^b (MHC I)	KH95	FITC	1:200	111505	Biolegend
IA^b/IE^b (MHC II)	M5/114.15.2	efluor450	1:200	48-5321-80	eBioscience

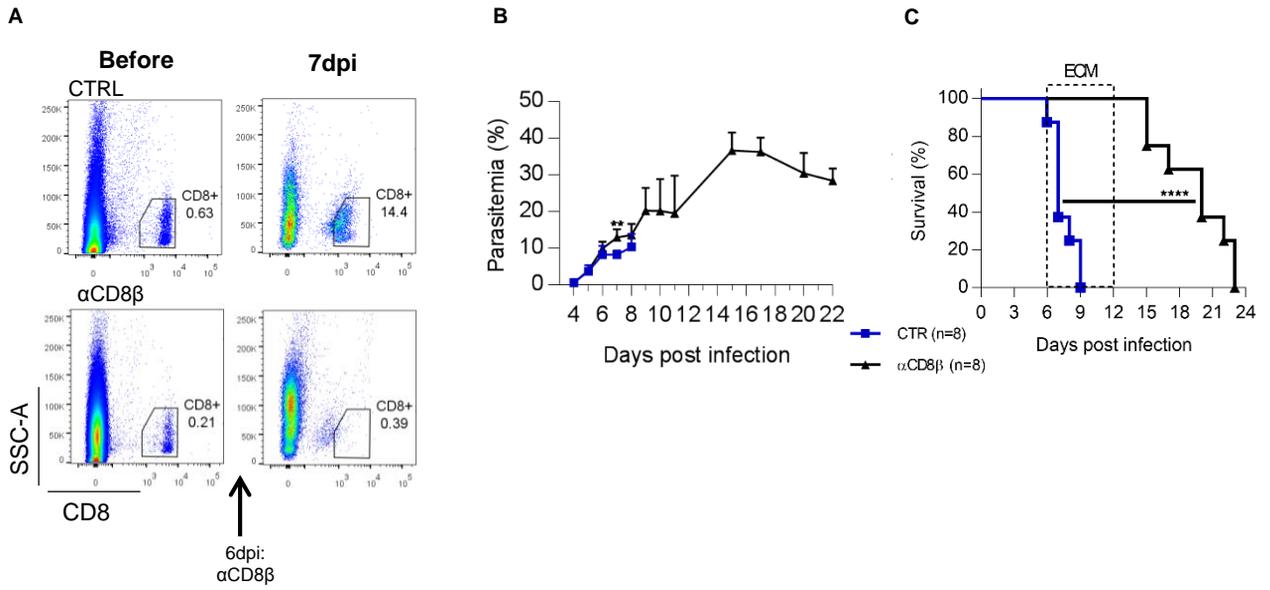
Supplementary Table 3: Antibodies used for CyTOF		
Metal	Antibodies	Clone
Y-89	CD45	Fluidigm
Qdot 655 112/114	CD19	Fluidigm
In-115	CD90	T24B1
Ce-140	Ly6C	HK1.4
Pr-141	CD43	S11
Nd-142	TCR-beta	H57-597
Nd-143	CD49d	PS/2
Nd-144	CD38	90
Nd-145	GITR	DTA-1
Nd-146	CD8a	53-6.7
Sm-147	OX40	OX-86
Nd-148	CD69	H1.2F3
Sm-149	CD4	H129.19
Nd-150	ICOS	C398.4A
Eu-151	CD62L	MEL14
Sm-152	CD127	A7R34
Eu-153	CD49b	DX5
Sm-154	CXCR3	CXCR3-173
Gd-156	CD27	LG.7F9
Gd-157	Ror-gamma T	4G419
Gd-158	KLRG-1	2F1
Tb-159	2B4	m2B4 (B6)458.1
Gd-160	Ki67	11F6
Dy-161	CD25	PC61
Dy-162	PD-1	29F11.1.112
Dy-63	CD122 APC	TM-β1
Dy-164	CD39	5F2
Ho-165	Eomes	7C9B03
Er-166	CD160	7H1
Er-167	CD150 PE	TC15-12F12.2
Er-168	CD272	6A6
Tm-169	CD 103	2 E7
Er-170	TIM-3	B8.2C12
Yb-171	CD73	Ty/23
Yb-172	Sca-1	D7
Yb-173	FOXP3	MF-14
Yb-174	CD24	M1/69
Lu-175	CD44	IM7
Yb-176	Lag-3	C9B7W
Ir-191/193	DNA	
Pt-195	CisPlatin live/dead	

Supplementary Table 4: Cell Number and percentage of immune cells subtypes present in the lungs of PbA/luc-infected mice (CTR) and PbA/luc-infected mice depleted of CD8⁺ T cells (αCD8β) at 6dpi

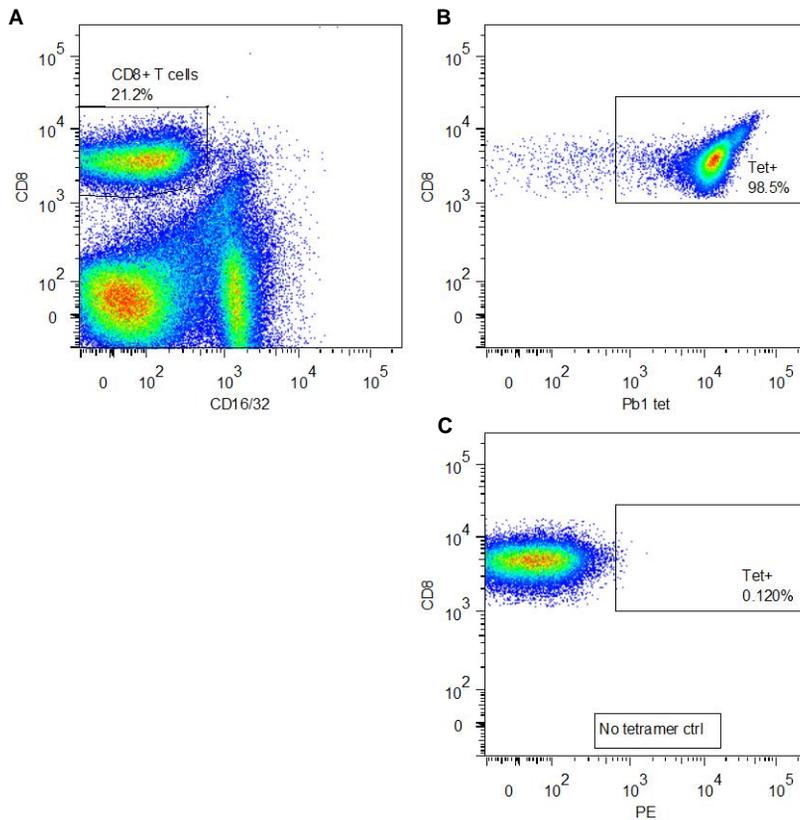
	Cell number		P Value*
	CTR (n=9)	αCD8β (n=12)	
Absolute total cell count	6450000 ± 2314768	6153750 ± 1540447	NS
CD4	165865 ± 71234	185526 ± 63522	NS
CD8	274301 ± 78473	43211 ± 25551	d
NK	177186 ± 80728	244917 ± 72843	NS
Monocytes	72326 ± 62744	180454 ± 93985	b
Neutrophils	180216 ± 228737	170227 ± 133824	NS
cDC	1889 ± 924.6	945.1 ± 409.6	b
Mono derived DC	13074 ± 9304	18953 ± 15126	NS
Macrophages	522.3 ± 416.3	1596 ± 635.3	c
Interst Macro	2968 ± 2226	3971 ± 2841	NS
MDM	112941 ± 89194	232297 ± 80193	b
Alveolar macro	2060 ± 1877	2448 ± 1450	NS
<i>Definition of abbreviations: N = naïve; cDC = conventional DC; MDM = mono derived macrophages; NS = not significant</i>			
*Significance level of CTR versus αCD8β on 7 dpi. Cell numbers are indicated in the right-hand end column. Significance levels are indicated as follows: ^b P<0.01; ^c P<0.001; ^d P<0.0001. Mann-Whitney test was used.			



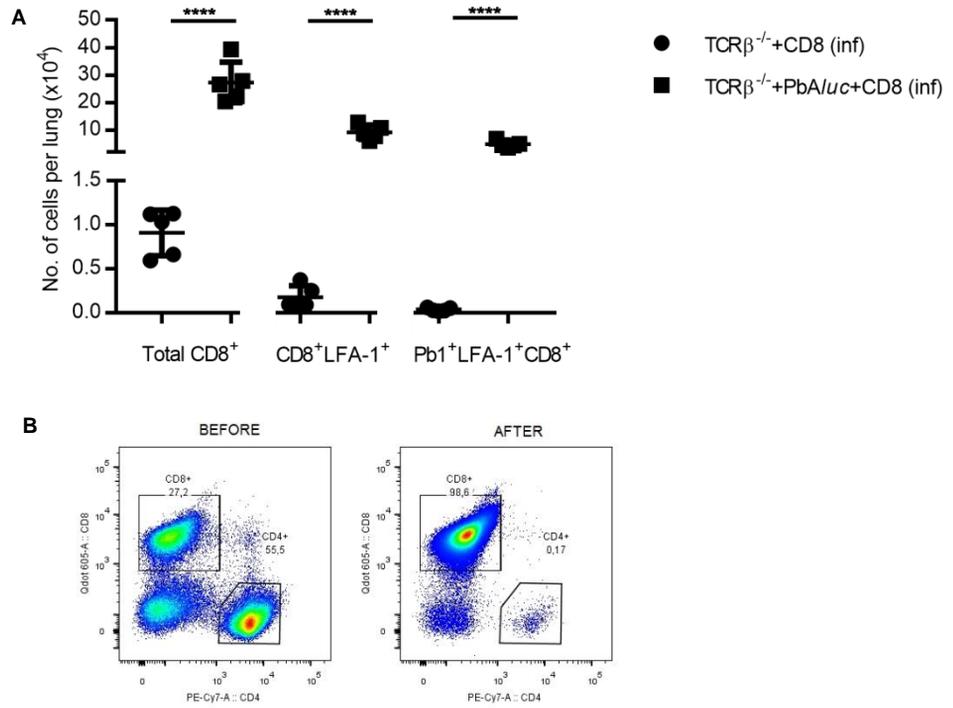
Supplementary Figure 1. Representative images of (A) *in vivo* and (B) *ex vivo* vascular leakiness in the lungs of PbA/luc-infected mice. The blue line outlines the lungs region of interest (ROI) used to quantify the Tracer-653 dye signal intensity. (C) Comparison between *in vivo* and *ex vivo* vascular leakiness in the lungs of PbA/luc-infected mice at 7 dpi. The black dashed line at $y=1$ in (C) represents the ratio of the tracer reading from naive C57BL/6 mice ($n=3$). The data represent the mean \pm SD; * $p<0.05$ by Mann-Whitney test. Gating strategy used to analyze flow cytometry data of (D) leukocytes and (E) myeloids sequestered in the lung tissue presented on Fig 2, Fig 7D-F, Fig 9D-F, Supplementary Fig 6A-C, Supplementary Fig 7A-D.



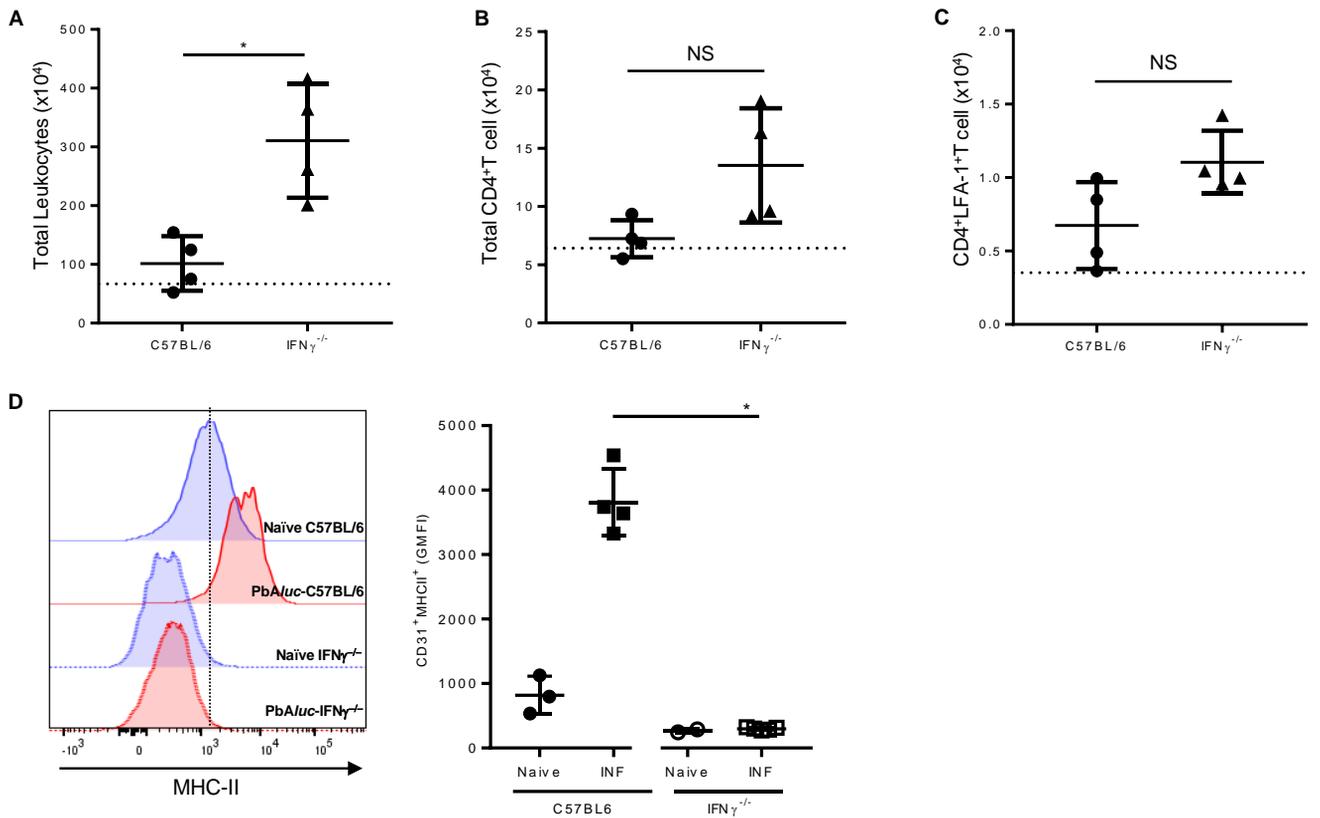
Supplementary Figure 3. Anti-CD8 β depletion efficiency. (A) Flow cytometry dot plots depicting the CD8⁺T cell depletion efficiency in whole blood at 7 dpi in PbA*luc*-infected C57BL/6 mice before and after treatment with a single dose of 0.75mg of anti-mouse CD8 β (α CD8 β) administered on 6dpi. CTR represents PbA*luc*-infected C57BL/6 mice without treatment. (B) Peripheral parasitemia level and (C) survival curve of CTR (n=8) and α CD8 β -treated (n=8) mice. The data represent the mean \pm SD; **p<0.01 by Mann-Whitney test (B) and ****p<0.0001 by log-rank (Mantel-Cox) test (C).



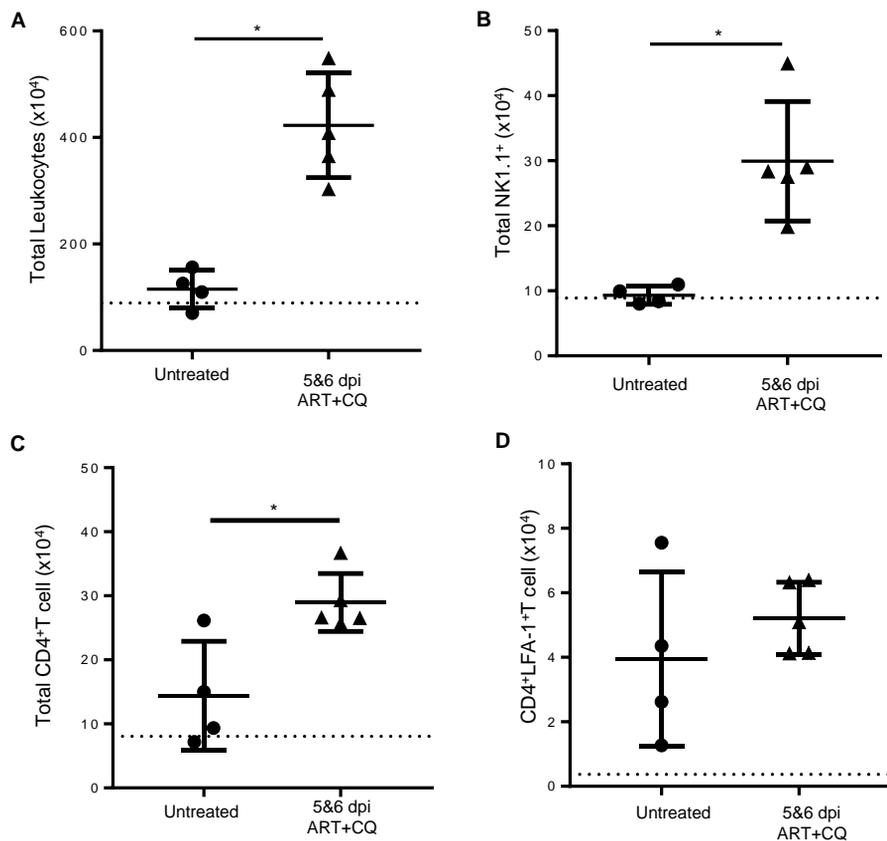
Supplementary Figure 4. Pb1-specific CD8⁺ T cells in transgenic BSL8.4 TCR mice. CD8⁺ T cells of BSL8.4 mice are Pb1-specific. Splenocytes were isolated from an 8.4a^{+/+}8.4b^{+/+}RAG1^{-/-} mice and stained with αCD8, αCD16/32 and Pb1-tetramer (SLLLNAYL-H-2D^b) and analysed by flow cytometry. (A) Gating of CD8⁺ T cells. (B) Tetramer (Tet⁺) staining on gated CD8⁺ T cells. (C) Background signal when Pb1-tetramer was omitted.



Supplementary Figure 5. Donor CD8 $^+$ T cells from infected-C57BL/6 mice migrate into the lungs of TCR $\beta^{-/-}$ only in the presence of PbAluc parasite. (A) The number of total CD8 $^+$, CD8 $^+$ LFA-1 $^+$ and Pb1-specific CD8 $^+$ LFA-1 $^+$ T cells in the lungs of recipient PbAluc-infected (or not) TCR $\beta^{-/-}$ mice that were adoptively transferred with CD8 $^+$ T cells from 7 dpi PbAluc-infected C57BL/6 donor (CD8 (inf)) mice. The data represent the mean \pm SD; **** p <0.0001 by ANOVA with Bonferroni's post-test. (B) Flow cytometry dot plot showing the number of CD8 $^+$ T cells isolated from splenocytes of donor C57BL/6 mice before and after enrichment using a CD8 α^+ T cell isolation kit.

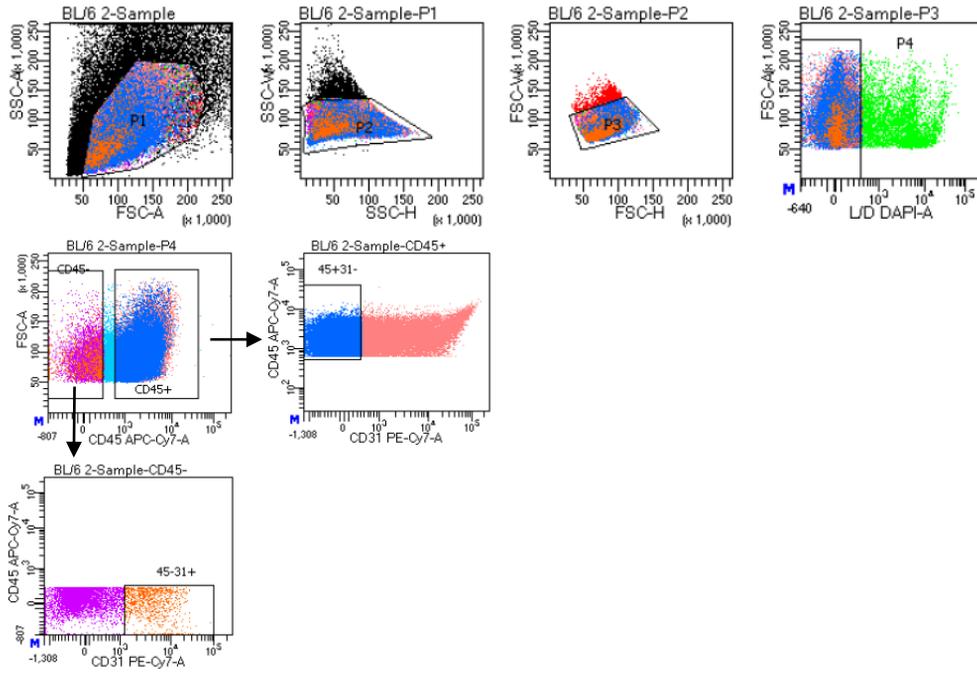


Supplementary Figure 6. Absence of IFN- γ does not affect the migration of CD8⁺ T cells into infected mice. (A) Leukocytes, (B) CD4⁺ T and (C) CD4⁺ LFA-1⁺ T cells in *PbA/luc*-infected C57BL/6 (n=4) and IFN- γ ^{-/-} mice (n=4) at 7dpi. (D) Representative histograms of sorted lung endothelial cells (CD45⁻CD31⁺) from C57BL/6 naïve (n=3) and IFN- γ ^{-/-} naïve (n=3) mice (blue line), and *PbA/luc*-infected C57BL/6 (n=4) and IFN- γ ^{-/-} (n=5) mice (red line) at 7 dpi that were stained for MHC-class II antigen presenting molecules (left). The graph represents the geometric mean fluorescence intensities (GMFI) of MHC-class II on CD31⁺ lung endothelial cells (right) from naïve and infected (INF) mice. The black dotted line in (A-C) represents the value of each respective cell population from naïve mice for quantification of immune-cell populations in the lungs. The data represent the mean \pm SD; *p<0.05, Mann-Whitney test.

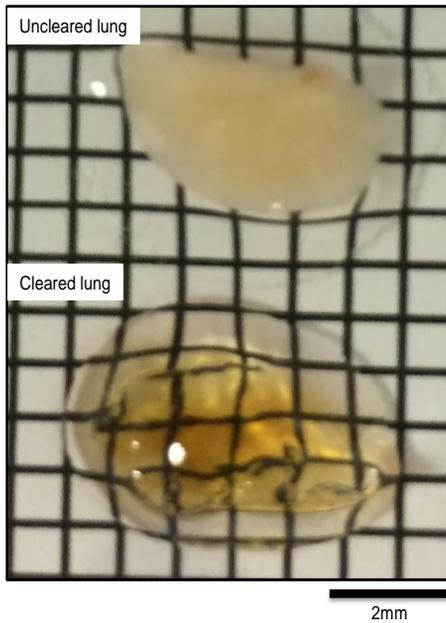


Supplementary Figure 7. Antimalarial treatment enhances leukocytes migration into the lungs of *PbAluc*-infected mice. Comparison between *PbAluc*-infected C57BL/6 mice (untreated) (n=4) and *PbAluc*-infected C57BL/6 mice that were treated with artesunate and chloroquine (ART+CQ) at 5 and 6 dpi (n=5) mice. Analyses were performed at 7 dpi. Total number of (A) leukocytes, (B) NK1.1⁺ cells, (C) CD4⁺T and (D) CD4⁺LFA-1⁺T cells. The black dotted line in (A-D) represents the value of each respective cell population from naïve C57BL/6 mice for quantification of immune-cell populations in the lungs. The data represent the mean \pm SD; *p<0.05, Mann-Whitney test.

A



B



Supplementary Figure 8. (A) Sorting gating strategy of CD45⁺CD31⁻ leukocytes and CD45⁻CD31⁺ endothelial cells. The sorting was done on singlets, DAPI⁺CD45⁺ and CD45⁻, followed by CD45⁺CD31⁻ and CD45⁻CD31⁺. Each sorted population was tested for Pb1 cross-presentation presented on Fig 6, Fig 7G and Fig 9G. L/D = live/dead. (B) Representative image of lung tissue before and after clearance with Benzyl Alcohol Benzyl Benzoate (BABB).