nature biotechnology

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41587-019-0225-9

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Precision mouse models with expanded tropism for human pathogens

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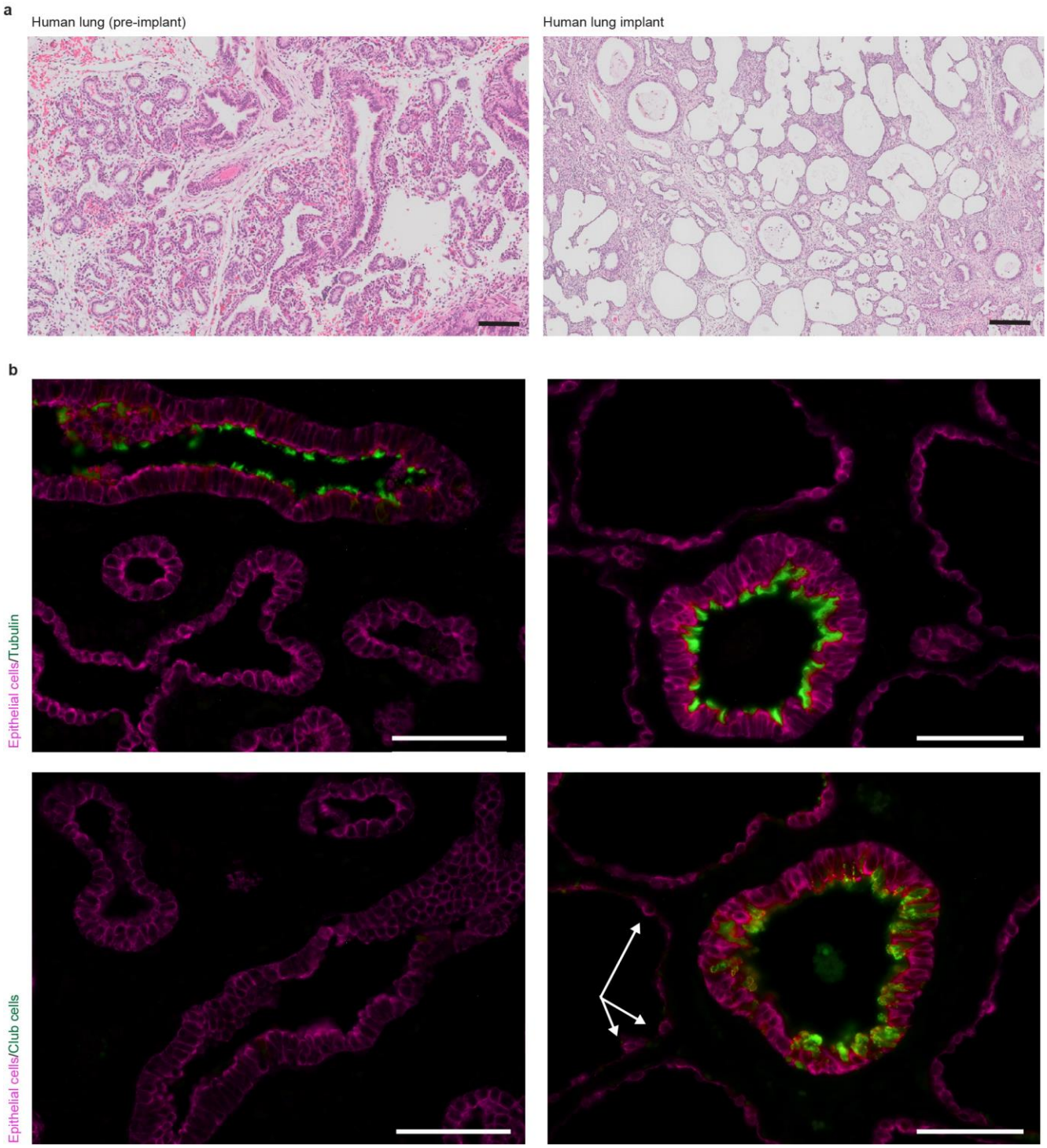
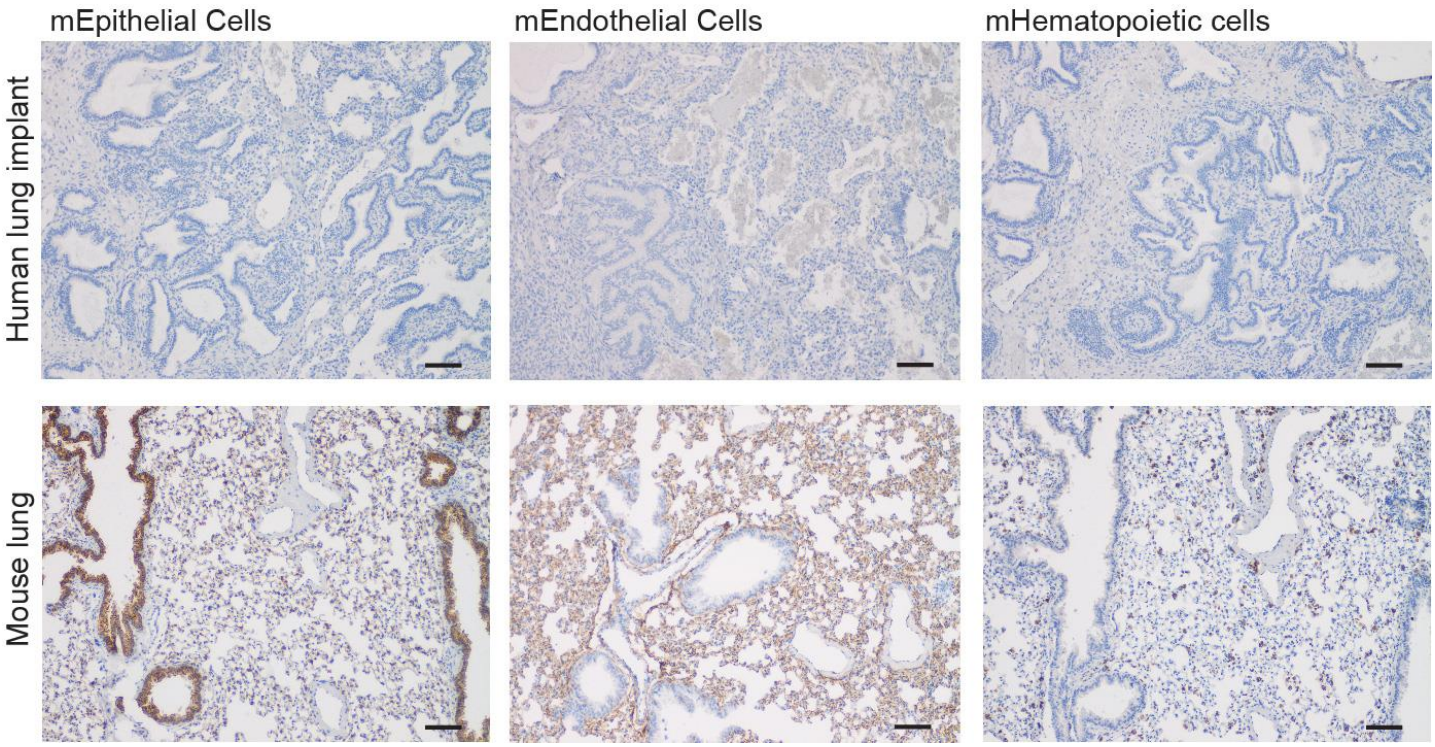


Supplementary Figure 1

Histological analysis of the structure and cellular composition of donor matched human lung tissue pre- and post-implantation.

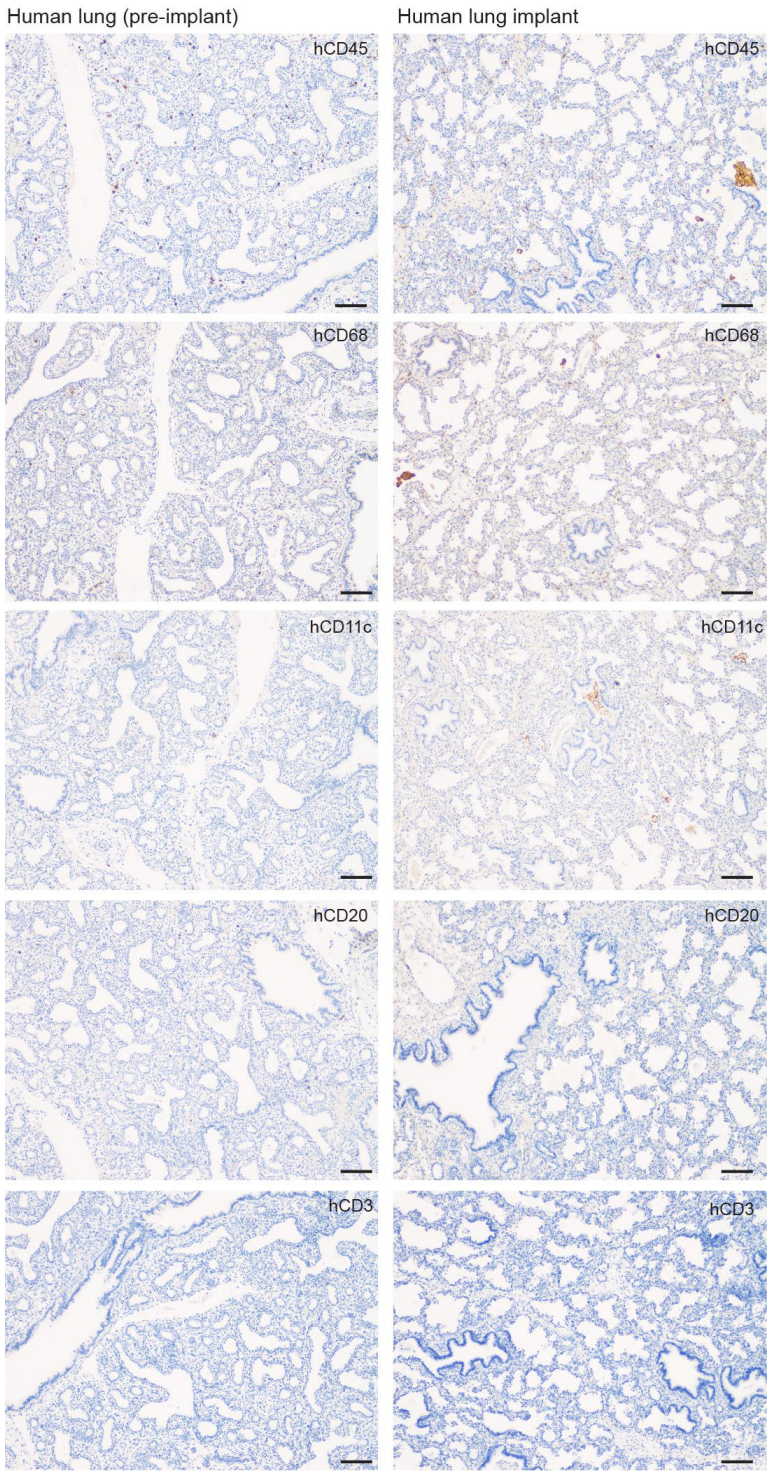
The structure and cellular composition of human lung tissue pre-implantation (n=2 analyzed) and donor matched LoM lung implant harvested 2 months post-implantation (n=4 analyzed) were analyzed by (a) H&E and (b) immunofluorescent staining. In b, co-staining was performed for epithelial cells (cytokeratin 19, magenta) and cilia (alpha-acetylated tubulin, green) or club cells (CC10, green). Arrows show cuboidal cells lining alveoli. In a, scale bars are 100 µm (left panel) and 200 µm (right panel). In b, scale bars are 200 µm (left panels) and 100 µm (right panels).



Supplementary Figure 2

Presence of mouse cells in the human lung implants of LoM and NSG mouse lung.

Immunohistochemical staining for murine epithelial cells, endothelial cells, hematopoietic cells in LoM human lung implants (n=3 analyzed, top panels) and the mouse lung (n=1 analyzed, bottom panels). Images: 10X, scale bars: 100 µm, positive cells: brown.



Supplementary Figure 3

Presence of human immune cells in human lung tissue pre- and post-implantation.

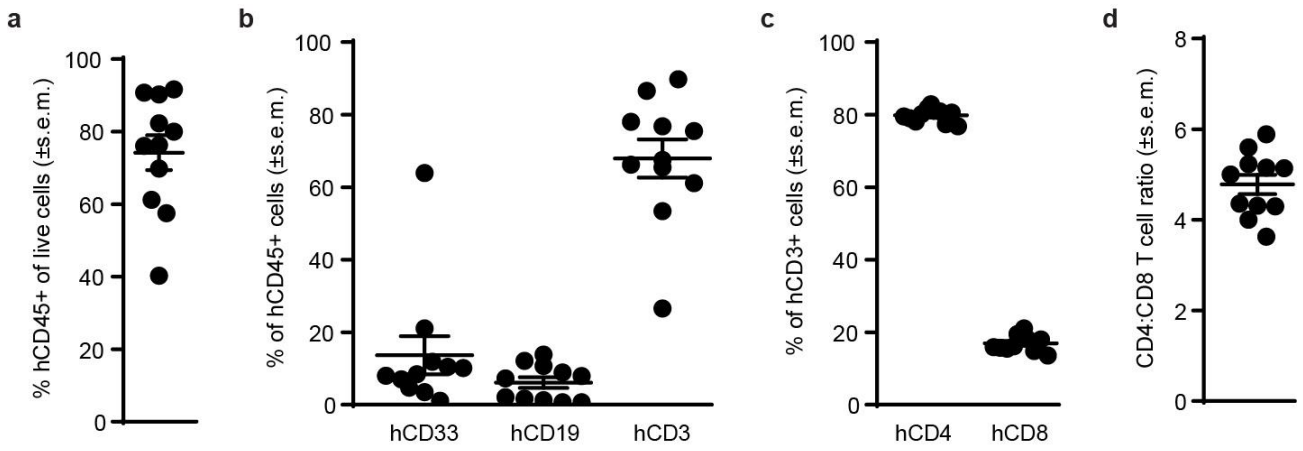
Immunohistochemical staining for human hematopoietic (hCD45) cells including macrophages (hCD68), dendritic cells (hCD11c), B cells (hCD20) and T cells (hCD3) in human donor matched lung tissue pre-implantation (n=1 analyzed, left panels) and two months post-implantation (n=1 analyzed, right panels). Images: 10X, scale bars: 100 µm, positive cells: brown.

Gene	Expression	Gene2	Expression2	Gene3	Expression3	Gene4	Expression4
RL1		UL38	2.38	UL92	2.56	UL148A	
RL8A		UL40	18.23	UL93	13.97	UL148B	
RL9A	11.98	UL41A		UL94	2	UL148C	
RL10		UL42	9.96	UL95		UL148D	
RL11		UL43	9.23	UL96	6.08	UL150	10.78
RL12		UL44	10.88	UL97	3.9	UL150A	8.88
RL13		UL45	2.65	UL98	11.52	TRS1	
UL1		UL46	2.08	UL99		US3	7.55
UL2		UL47	0.96	UL100		US6	
UL4		UL48	14.89	UL102	9.26	US7	
UL5	6.74	UL48A		UL103	3.2	US8	8.7
UL6	9.5	UL49	8.1	UL104	2.73	US9	2.8
UL7		UL50		UL105	9.12	US10	11.25
UL8		UL51		UL111A		US11	1.31
UL9		UL52	7.98	UL112	8	US12	2.24
UL10		UL53	4.43	UL114	11.44	US13	
UL11		UL54	8.93	UL115	2.61	US14	3.85
UL13	1.27	UL55	13.07	UL116	9.49	US15	
UL14	9.87	UL56	9.61	UL117	1.7	US16	
UL15A	5.35	UL57	8.83	UL119		US17	8.03
UL16	11.14	UL69	11.33	UL120		US18	3.01
UL17		UL70	10.86	UL121	4.8	US19	8.43
UL18	1.88	UL71	2.57	UL122	3.15	US20	
UL19		UL72	3.55	UL123	0.93	US21	3.14
UL20		UL73		UL124		US22	3.04
UL21A	4.2	UL74	2.29	UL130		US23	3.62
UL21.5	18.31	UL74A	11.92	UL131A		US24	1.18
UL23	11.91	UL75	7.29	UL132	15.58	US26	11.45
UL24	3.77	UL76	1.77	UL133		US27	2.53
UL25	11.13	UL77		UL135	12.23	US28	7.15
UL26		UL78	7.27	UL136		US29	1.96
UL27	9.92	UL79	4.31	UL138		US30	
UL29	8.53	UL80	11.2	UL139	17.54	US31	3.89
UL30	9.06	UL82	12.17	UL140		US32	
UL30A	6.19	UL83	10.75	UL141		US33A	
UL31	4.24	UL84	12.72	UL142		US33A	
UL32	1.75	UL85	4.23	UL144		US34	
UL33	6.72	UL86	10.94	UL145	4.71	US34A	
UL34	1.87	UL87	8.4	UL146			
UL35	3.22	UL88	7.03	UL147			
UL36	8.81	UL89	10.31	UL147A			
UL37		UL91	10.65	UL148	2.05		

Supplementary Figure 4

In vivo gene expression profile of HCMV-infected LoM is consistent with lytic replication.

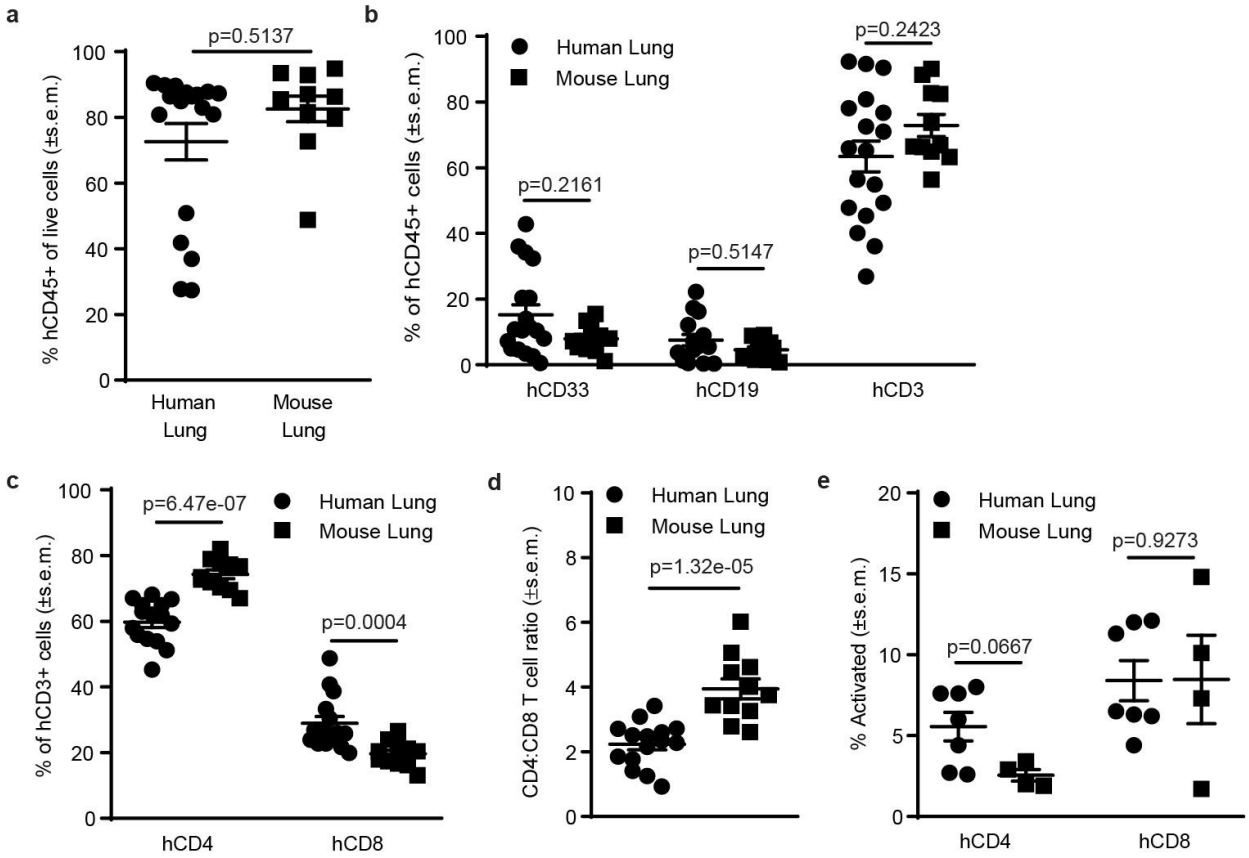
Total RNA was extracted from human lung implants harvested from HCMV TB40/E infected LoM 14 days post exposure (n=2 TB40/E infected implants). Double stranded cDNA ((ds)cDNA) was generated from ribosomal RNA (rRNA) depleted total RNA. HCMV (ds)cDNA was enriched with custom designed biotinylated probes spanning both strands of the entire HCMV genome and sequenced using next generation sequencing. High quality reads were aligned to the HCMV genome, and viral expression was quantified in read per kilobase per million (rpkm). Values show read counts per gene normalized to gene length read (rpkm).



Supplementary Figure 5

Reconstitution of the peripheral blood of BLT-L mice with human innate and adaptive immune cells.

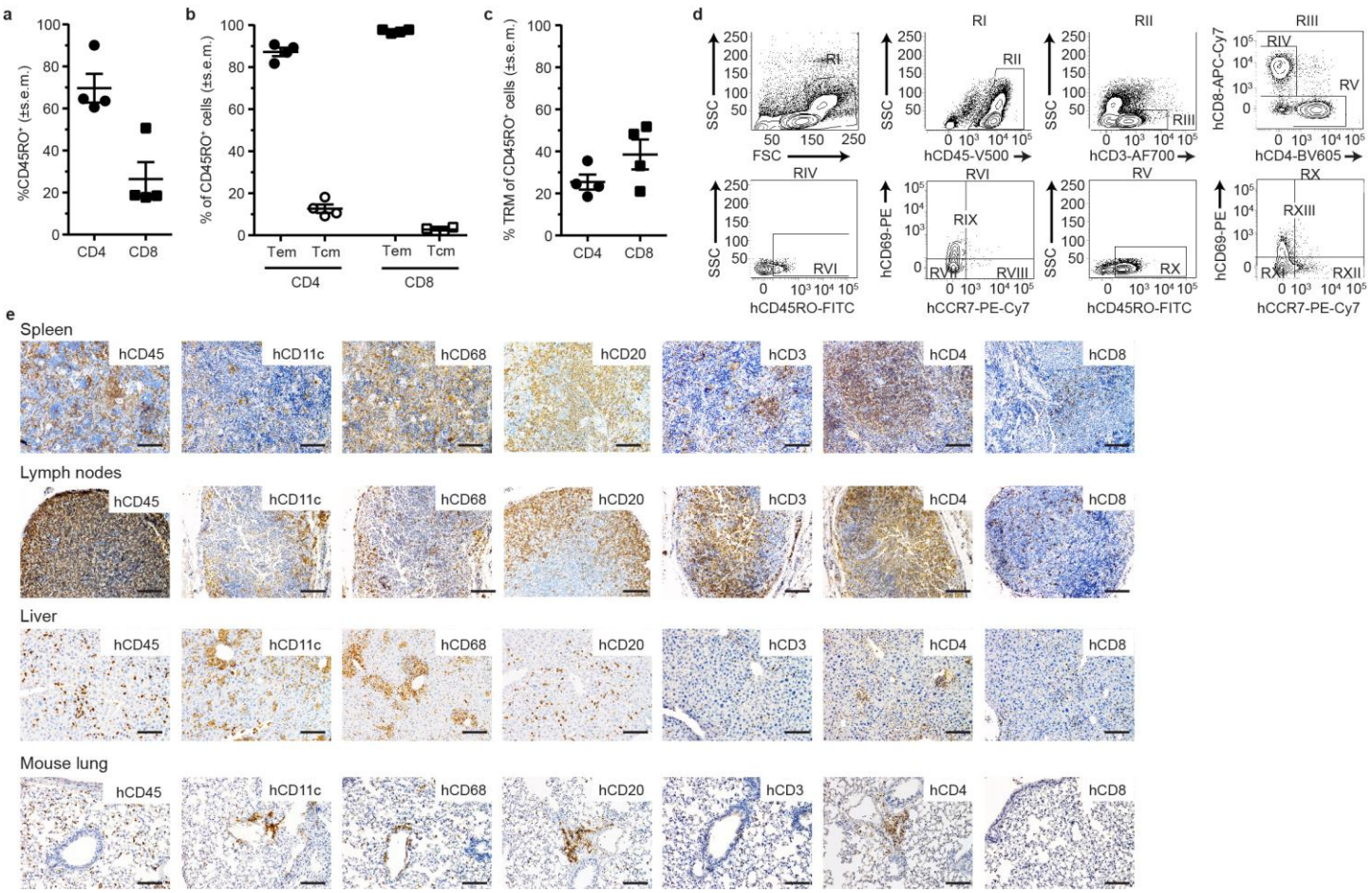
Levels of (a) human hematopoietic cells (hCD45) including (b) human myeloid cells (hCD33), B cells (hCD19) and T cells (hCD3) as well as the (c) levels of $CD4^+$ (hCD4) and $CD8^+$ (hCD8) T cells and (d) ratio of human CD4:CD8 T cells in the peripheral blood of BLT-L mice (n=11, filled circles). Horizontal lines represent mean ± s.e.m.



Supplementary Figure 6

Levels of human immune cells in the human lung implants and mouse lung of BLT-L mice.

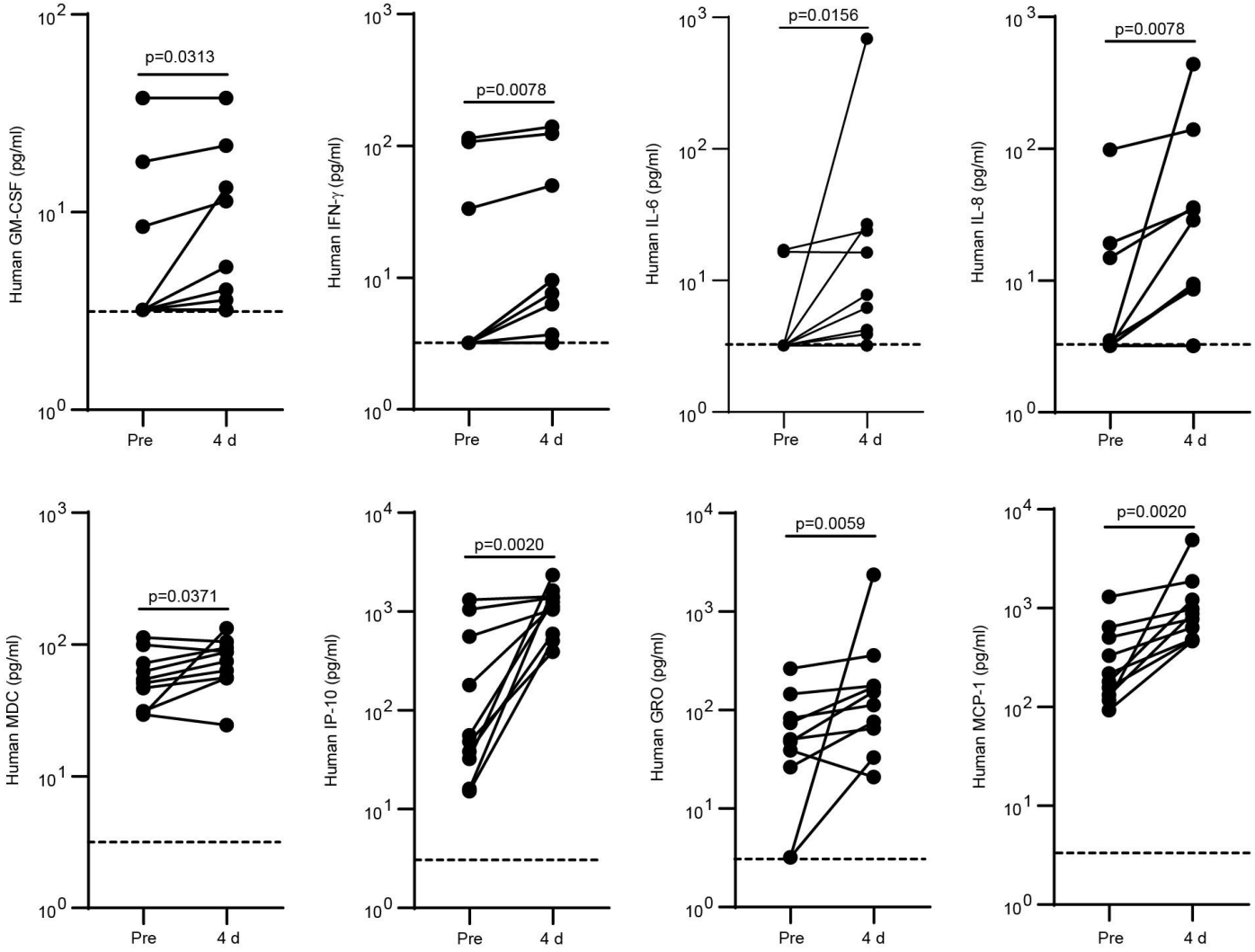
Levels of (a) human hematopoietic cells (hCD45) including (b) human myeloid cells (hCD33), B cells (hCD19) and T cells (hCD3) in the human lung implants (circles; hCD45, hCD33, and hCD3 n=18, hCD19 n=15) and mouse lung (squares, n=11) of BLT-L mice. (c) Levels of $CD4^+$ (hCD4) and $CD8^+$ (hCD8) T cells and (d) ratio of human CD4:CD8 T cells in the human lung implants (circles, n=15) and mouse lungs (squares, n=11) of BLT-L mice. (e) Human $CD4^+$ and $CD8^+$ T cell activation ($CD38^+HLA\text{-}DR^+$) levels in the human lung implant (circles, n=7) and mouse lung (squares, n=4) of BLT-L mice. Horizontal lines represent mean ± s.e.m. Human immune cell levels in the human lung implants and mouse lung were compared with a two-tailed Mann-Whitney test.



Supplementary Figure 7

Systemic presence of human immune cells in BLT-L mice.

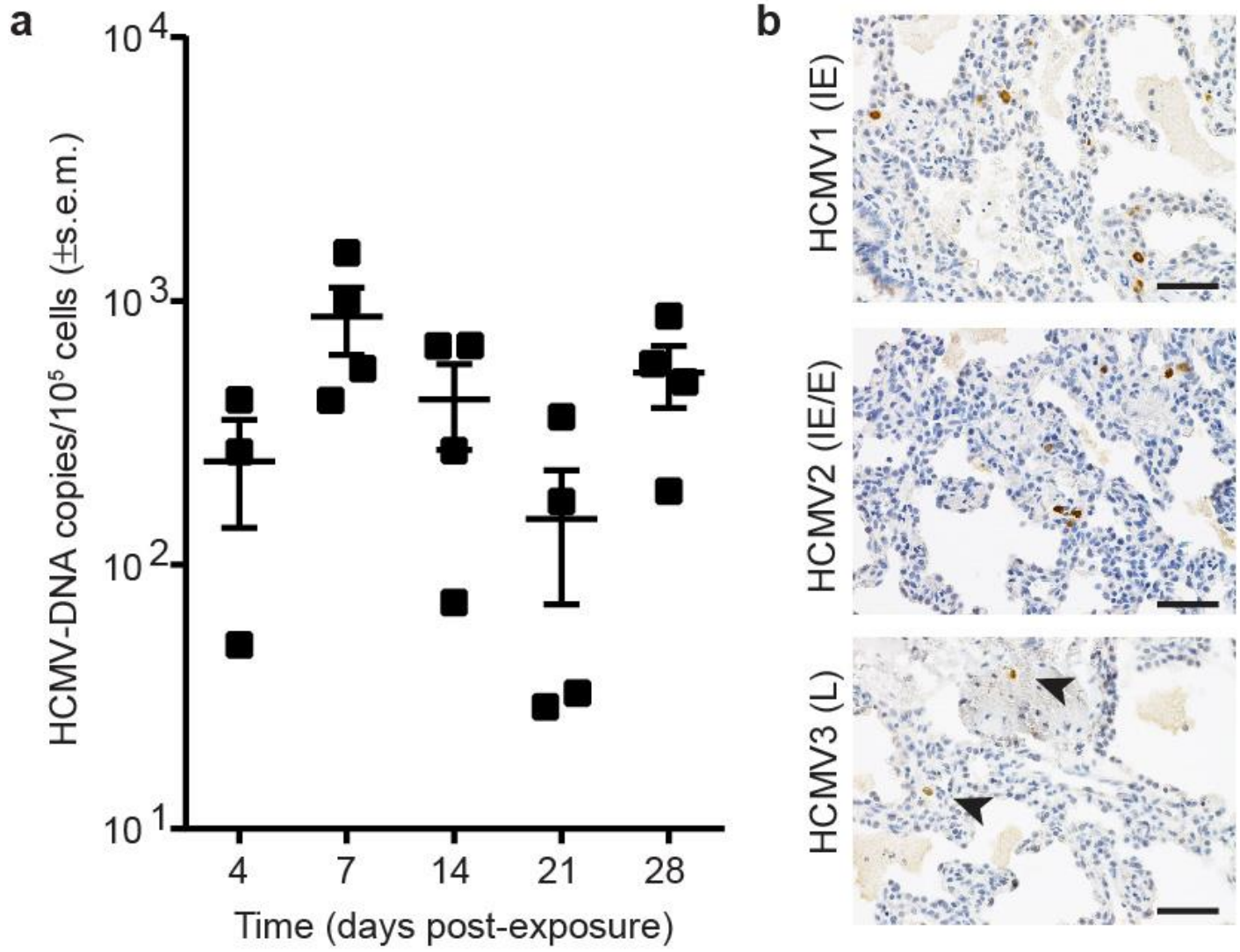
(a-c) The memory phenotype of human T cells in the human lung implants of BLT-L mice (n=4 BLT-L mice, one lung implant per animal). (a) Percent of $CD4^+$ (filled circles) and $CD8^+$ (filled squares) human T cells expressing a memory phenotype ($CD45RO^+$). (b) Percent of memory ($CD45RO^+$) $CD4^+$ (circles) and $CD8^+$ (squares) human T cells expressing an effector memory (Tem, $CCR7^{neg}$, closed symbols) or central memory (Tcm, $CCR7^+$, open symbols) phenotype. (c) Percent of memory ($CD45RO^+$) $CD4^+$ (filled circles) and $CD8^+$ (filled squares) T cells that are tissue-resident (TRM, $CD69^+$). (d) Flow cytometry gating scheme. Regions identify the following human cell populations: RI (live cells), RII (human hematopoietic cells), RIII (T cells), RIV ($CD8^+$ T cells), RV ($CD4^+$ T cells), RVI (memory $CD8^+$ T cells), RVII ($CD8^+$ Tem), RVIII ($CD8^+$ Tcm), RIX ($CD8^+$ TRM), RX (memory $CD4^+$ T cells), RXI ($CD4^+$ Tem), RXII ($CD4^+$ Tcm) and RXIII ($CD4^+$ TRM). In a-c, horizontal lines represent mean ± s.e.m. (e) Human hematopoietic (hCD45) cells including dendritic cells (hCD11c), macrophages (hCD68), B cells (hCD20) and T cells (hCD3, hCD4 and hCD8) in lymphoid (spleen and lymph nodes) and non-lymphoid (liver and mouse lung) of BLT-L mice by immunohistochemical staining (positive cells: brown). Images shown are at 20X magnification and represent three BLT-L mice (scale bars: 100 µm).



Supplementary Figure 8

Increased plasma human cytokine and chemokine levels in BLT-L mice following HCMV exposure.

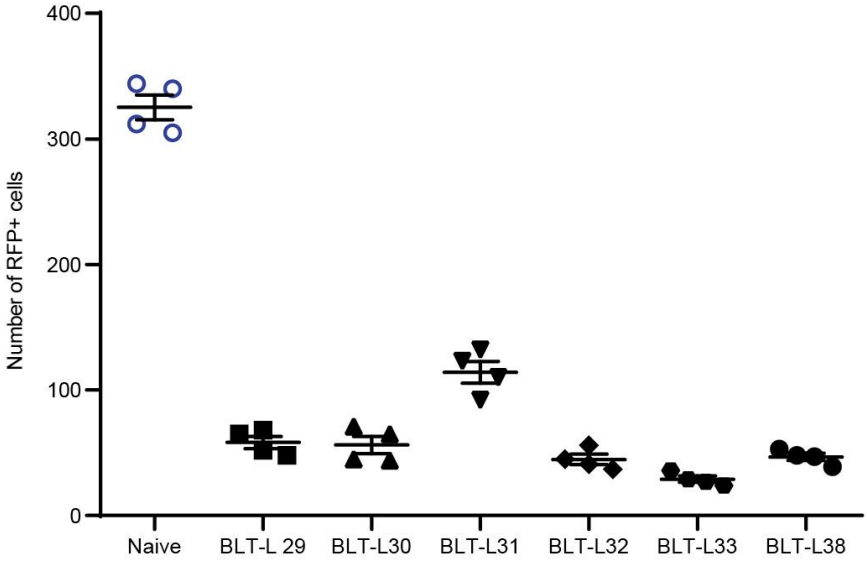
Levels of human GM-CSF IFN-γ, IL-6, IL-8, MDC, IP-10, GRO and MCP-1 in the PB plasma of BLT-L mice (n=10 mice, filled circles) pre and 4 days after HCMV TB40/E inoculation. A value of 3.2 pg/ml was graphed for measurements below the limit of detection of the assay (3.2 pg/ml, shown with a dashed line). Human cytokine and chemokine levels pre and post HCMV inoculation were compared with a two-tailed Wilcoxon matched-pairs signed rank test.



Supplementary Figure 9

In vivo replication of HCMV AD169 in LoM human lung implants.

(a) HCMV-DNA levels in LoM human lung implants at 4, 7, 14, 21 and 28 days post AD169 exposure (day 4: n=3 implants, days 7, 14, 21 and 28: n=4 implants, filled squares). Horizontal lines represent mean ± s.e.m. (b) HCMV immediate early (IE), early (E) and late (L) proteins in the human lung implant of an AD169-infected LoM 21 days post-exposure (n=1 lung implant analyzed, positive cells: brown). Images shown are at 40X magnification (scale bars: 50 µm). Positive cells in the bottom panel are indicated with black arrows.

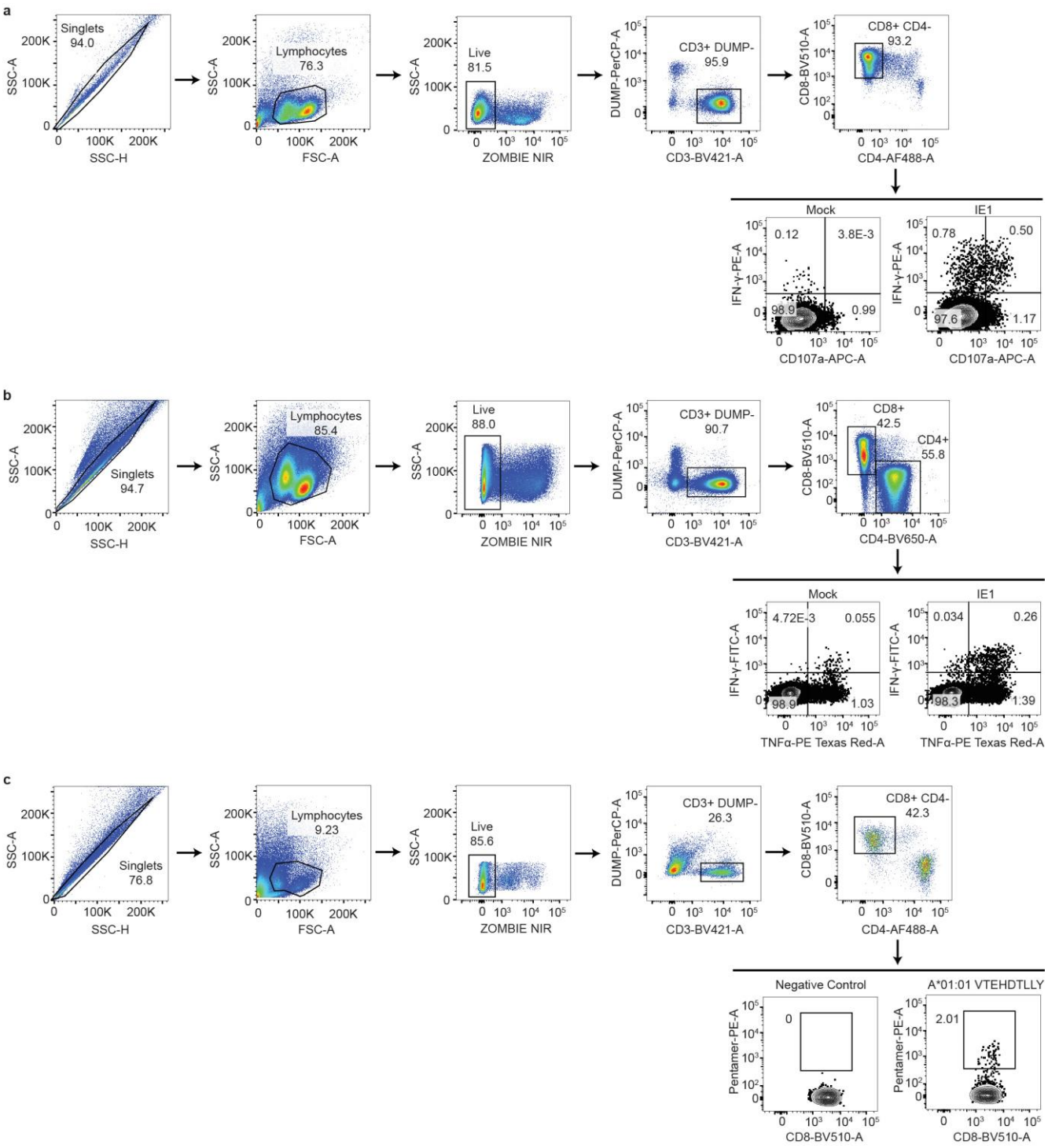


Plasma sample	% Reduction in RFP+ cells
BLT-L29	82%
BLT-L30	83%
BLT-L31	65%
BLT-L32	86%
BLT-L33	91%
BLT-L38	86%

Supplementary Figure 10

Plasma of HCMV-exposed BLT-L mice contains HCMV neutralizing activity.

Heat-inactivated plasma from naïve (n=1, open blue circles) and repeatedly HCMV TB40/E exposed BLT-L mice (n=6, filled symbols) was incubated with HCMV TB40/E expressing RFP for 1 h prior to the addition of virus to epithelial cells (ARPE-19). Epithelial cells were incubated at 37°C with the virus/plasma mixture for 2 h at which time the virus/plasma mixture was removed and fresh media added. Shown is the number of HCMV TB40/E-RFP+ epithelial cells 72 h post-infection in quadruplicate wells. Horizontal lines represent mean ± s.e.m. The percent reduction in TB40/E RFP+ cells compared to wells infected with HCMV pre-treated with naïve control plasma is shown in the table.



Supplementary Figure 11

Gating strategies for the identification of HCMV-specific human T cell responses in BLT-L mice by intracellular cytokine staining (ICS) and pentamer staining.

Representative flow cytometry plots indicating the gating used to detect (a) human $CD8^+$ T cells expressing IFN-γ and CD107a and (b) human $CD4^+$ T cells expressing IFN-γ and TNFα by ICS. (c) Representative flow cytometry plots indicating the gating strategy used to detect HLA class1a-restricted HCMV-specific human $CD8^+$ T cells by pentamer staining.

Supplementary Table 1: LoM human lung implant cell production of human cytokines and chemokines following *ex vivo* LPS stimulation

	Human cytokine/chemokine levels (pg/ml)[a]	
Cytokine/chemokine	Control	LPS[b]
sCD40L	3.3 (±0.1)	3.4 (±0.2)
Eotaxin	11 (±0.1)	11.7 (±0.4)
FGF-2	11.6 (±1.5)	9.4 (±1.8)
Flt-3L	6.5 (±0.7)	6.7 (±0.8)
Fractalkine	38.9 (±3.1)	40.4 (±3.8)
G-CSF	99.4 (±6.8)	1,436 (±588.5)
GM-CSF	21.3 (±5.6)	47.3 (±5.9)
GRO	8,610 (±1,523)	11,479 (±1,577)
IFN-alpha2	8.7 (±1.6)	8.1 (±1.2)
IFN-gamma	<LOD	3.3 (±0.06)
IL-1alpha	<LOD	<LOD
IL-1beta	<LOD	<LOD
IL-1RA	4.6 (±0.6)	4.7 (±0.7)
IL-2	<LOD	<LOD
IL-3	<LOD	<LOD
IL-4	56.9 (±2.7)	71.3 (±2.5)
IL-5	<LOD	<LOD
IL-6	248.9 (±1.5)	538.9 (±101.3)
IL-7	11.9 (±0.9)	16.2 (±1.2)
IL-8	25,466 (±2,919)	23,919 (±3,150)
IL-9	<LOD	<LOD
IL-10	<LOD	3.8 (±0.2)
IL-12p40	4.8 (±0.5)	6.9 (±0.9)
IL-12p70	<LOD	<LOD
IL-13	3.4 (±0.2)	4.8 (±0.7)
IL-15	<LOD	<LOD
IL-17A	<LOD	<LOD
IP-10	29.9 (±1.1)	29.6 (±1.0)
MCP-1	507.9 (±159.1)	1,277 (±481.2)
MCP-3	6.7 (±0.7)	7.8 (±0.7)
MDC	13.6 (±1.3)	21.1 (±0.8)
MIP-1alpha	4.1 (±0.1)	8 (±1.7)
MIP-1beta	5 (±0.1)	7 (±1.2)
PDGF-AA	71.4 (±1.9)	73.8 (±4.4)
PDGF-AB/BB	34.7 (±4.5)	39.2 (±4.65)
RANTES	7.7 (±0.2)	10.2 (±0.8)
TGF-alpha	142.2 (±13.4)	153.5 (±17.9)
TNFalpha	5.2 (±0.8)	9.5 (±1.6)
TNFbeta	<LOD	<LOD
VEGF	169 (±55.4)	195.1 (±64.3)

[a]Mean human cytokine/chemokine levels (pg/ml ± standard error mean). [b]Human cytokine/chemokine levels >10 pg/ml and >1.5 fold control levels are highlighted in blue. LOD: limit of detection (3.2 pg/ml). Cells were isolated from two human lung implants (3 wells per condition/lung implant, represents one experiment).

Supplementary Table 2: BLT-L human lung implant immune cell production of human cytokines and chemokines following *ex vivo* LPS and PMA/ionomycin stimulation

	Human cytokine/chemokine levels (pg/ml)[a]		
Cytokine/chemokine	Control	LPS	PMA/ionomycin
sCD40L	6.4 (±3.2)	6.4 (±3.2)	41 (±7.5)
Eotaxin	8.8 (±1.7)	10 (±1.5)	8.6 (±0.7)
FGF-2	24.7 (±0.8)	21.8 (±0.3)	25.8 (±3.8)
Flt-3L	12.7 (±0.1)	13.4 (±2.2)	13.1 (±1.6)
Fractalkine	56.8 (±5.0)	54.6 (±15.8)	61.3 (±4.8)
G-CSF	45.2 (±12.0)	58 (±10.4)	33.1 (±5.1)
GM-CSF	9.1 (±5.1)	13.8 (±6.2)	175.2 (±36.6)
GRO	889 (±213)	1,150 (±166.3)	961.3 (±215.5)
IFN-alpha2	12 (±0.3)	11.7 (±1.3)	11.6 (±1.3)
IFN-gamma	4.6 (±1.4)	5.7 (±2.1)	1,579 (±262.6)
IL-1alpha	3.8 (±0.6)	5.3 (±2.1)	3.9 (±0.4)
IL-1beta	59.6 (±43.8)	161.9 (±116.2)	190.6 (±52.2)
IL-1RA	17.3 (±1.2)	20.4 (±8.2)	48.3 (±9.9)
IL-2	<LOD	<LOD	892.3 (±185.5)
IL-3	<LOD	<LOD	<LOD
IL-4	17 (±10.6)	15.4 (±12.2)	21.8 (±9.7)
IL-5	<LOD	<LOD	<LOD
IL-6	964.7 (±322.5)	1,552 (±490.2)	236.3 (±55.0)
IL-7	18.6 (±6.8)	19.9 (±7.6)	14.5 (±2.2)
IL-8	5,895 (±2,866)	6,285 (±1,441)	3,876 (±1,205)
IL-9	<LOD	<LOD	4.6 (±0.8)
IL-10	53.1 (±23.4)	87.6 (±28.3)	139.6 (±26.2)
IL-12p40	5.4 (±2.2)	7.4 (±2.0)	4.8 (±0.1)
IL-12p70	<LOD	<LOD	<LOD
IL-13	4.9 (±1.7)	4.2 (±1.0)	75.6 (±9.2)
IL-15	<LOD	3.4 (±0.2)	<LOD
IL-17A	<LOD	<LOD	374 (±33.4)
IP-10	259.1 (±115.6)	327.2 (±106.1)	74.3 (±13.3)
MCP-1	288.8 (±85.1)	398.7 (±70.7)	108.4 (±10.7)
MCP-3	8.4 (±0.3)	10.7 (±0.2)	8 (±1.5)
MDC	10.7 (±2.2)	12.3 (±0.6)	10.5 (±1.3)
MIP-1alpha	1,979 (±841.8)	3,471 (±1,649)	2,039 (±597.9)
MIP-1beta	1,250 (±447.8)	1,547 (±520.2)	1,526 (±163.1)
PDGF-AA	<LOD	<LOD	<LOD
PDGF-AB/BB	6.9 (±2.8)	5.9 (±0.9)	5.3 (±1.2)
RANTES	124.9 (±54.1)	132.7 (±49.4)	551.1 (±114.3)
TGF-alpha	<LOD	<LOD	<LOD
TNFalpha	1,260 (±386.2)	1,915 (±450.6)	2,643 (±396.0)
TNFbeta	<LOD	<LOD	<LOD
VEGF	18.4 (±4.2)	15.4 (±6.0)	23.6 (±5.0)

[a]Mean human cytokine/chemokine levels in control, LPS, and PMA/ionomycin wells (pg/ml ± standard error mean). Human cytokine/chemokine levels >10 pg/ml and >1.5 fold control levels are highlighted in blue or yellow for cells stimulated with LPS or PMA/ionomycin respectively. LOD: limit of detection (3.2 pg/ml). MNC were isolated from BLT-L mouse human lung implants (n=6 mice and 12 lung implants, represents two experiments).

Supplementary Table 3: HLA class I haplotypes of BLT-L mice utilized for T cell immune analysis

	HLA Class I Alleles					
Donor	A	A	B	B	C	C
A	23:01	31:01	35:03	38:01	04:01	12:03
B	03:01	11:01	07:02	35:01	04:01	07:02
C	02:06	11:01	35:01	39:05	04:01	07:02
D	03:01	24:02	07:02	44:02	07:02	16:04
E	02:05	29:02	44:03	58:01	07:18	16:01
F	01:01	01:01	08:01	08:01	07:01	07:01
G	01:01	32:01	18:01	35:08	04:01	07:02
H	01:01	02:01	41:02	57:01	06:02	07:03
I	02:02	33:03	15:10	15:16	03:04	14:12
J	02:01	36:01	08:01	15:03	02:10	07:01

Supplementary Table 4: Detection of HCMV-specific immunity in BLT-L mice exposed to a single inoculation of HCMV.

			At Time of Necropsy		
				HCMV-Specific Immune Response	
Mouse	Donor	Virus	Days Post-Exposure	T Cell Reactivity[a]	Antibody IgM
BLT-L11	B	TB40/E	12	Not detected (P)	Not detected
BLT-L12	B	TB40/E	12	Positive (P) 0.13-0.44%	Not detected
BLT-L13	B	TB40/E	12	Not detected (P)	Not detected
BLT-L14	B	TB40/E	12	Not detected (P)	Not detected
BLT-L15	I	TB40/E	12	Not detected (I)	Not detected
BLT-L16	I	TB40/E	12	Not detected (I)	Not detected
BLT-L17	I	TB40/E	12	Positive (I) 0.07%	Not detected
BLT-L 1	A	TB40/E	14	Positive (E) 191 SFU	Not detected
BLT-L 2	B	TB40/E	21	Not detected (P)	Not detected
BLT-L 3	B	TB40/E	21	Positive (P) 0.10%	Not detected
BLT-L 4	B	TB40/E	21	Positive (P) 0.17%	Positive
BLT-L 5	C	TB40/E	34	Positive (E) 73-160 SFU	Positive
BLT-L 6	A	TB40/E	34	Positive (E) 395 SFU	Positive
BLT-L 7	C	TB40/E	39	Not detected (E)	Not detected
BLT-L 8	A	TB40/E	125	Positive (I) 0.04%	Not detected
BLT-L 9	C	ADrUL131	34	Positive (E) 293-459 SFU	Positive
BLT-L 10	C	ADrUL131	48	Not detected (E)	Not detected
BLT-L55	A	AD169	14	Not detected (E)	Not detected
BLT-L56	J	AD169	15	Positive (E) 254-296 SFU	Not detected
BLT-L57	J	AD169	28	Not detected (E, P)	Positive
BLT-L58	A	AD169	34	Positive (E) 575-789 SFU	Not detected
BLT-L59	A	AD169	47	Not detected (E, I)	Positive
BLT-L60	J	AD169	56	Not detected (E, P)	Not detected
BLT-L61	J	AD169	75	Not detected (I)	Positive
BLT-L62	A	AD169	110	Not detected (I)	Positive

[a]Assay detecting HCMV-specific T cell reactivity: ELISpot (E), intracellular cytokine staining (I), Pentamer staining (P). The magnitude of T cell reactivity is provided as the number of IFN-γ spot forming units (SFU), % IFN-γ and CD107a double positive, or % pentamer positive (or ranges, in case > 1 positive) for ELISpot, intracellular cytokine staining, and pentamer staining respectively. Criteria for T cell assay positivity is detailed in Methods. Positivity threshold for Antibody IgM Index is ≥ 0.5.

Supplementary Table 5: Detection of *ex vivo* HCMV-specific T cells by IFN-γ ELISpot in BLT-L mice exposed to a single inoculation of HCMV.

Fig.	Mouse	Donor	Organ	CMV Antigen	Virus	Cell #	Mock[1]	Antigen[1]	CD45(%)[2]	CD3(%)[2]	SFU/10^6 CD3 T cells[3]
4e	BLT-L5	C	Liver	$pp65_{495-504}$	TB40\E	3×10^5	4.3	20.0	75.1	94.8	73.0
4e	BLT-L9	C	Liver	$pp65_{495-504}$	TB40\E	3×10^5	4.5	96.8	78.9	97.0	402.1
4e	BLT-L5	C	Liver	$pp65_{123-131}$	TB40\E	3×10^5	4.3	22.5	75.1	94.8	84.6
4e	BLT-L9	C	Liver	$pp65_{123-131}$	TB40\E	3×10^5	4.5	71.8	78.9	97.0	293.2
4f	BLT-L1	A	Liver	IE1[4]	TB40\E	3×10^5	32.5	78.8	91.7	88.0	191.6
4f	BLT-L5	C	Liver	IE1[4]	TB40\E	3×10^5	4.3	38.5	75.1	94.8	159.4
4f	BLT-L6	A	Liver	IE1[4]	TB40\E	3×10^5	69.2	139.0	92.1	84.8	296.2
4f	BLT-L9	C	Liver	IE1[4]	TB40\E	3×10^5	4.5	109.8	78.9	97.0	458.6
4f	BLT-L58	A	Liver	IE1[4]	AD169	2×10^5	70.0	180.4	90.3	77.5	783.3
4g	BLT-L6	A	M Lung	IE1[4]	TB40\E	3×10^5	42.0	85.0	80.9	69.6	255.7
4g	BLT-L9	C	M Lung	IE1[4]	TB40\E	3×10^5	5.0	38.2	98.7	96.0	116.1
4g	BLT-L56	J	M Lung	IE1[4]	AD169	3×10^5	44.0	109.0	84	65.3	394.1
4g	BLT-L58	A	M Lung	IE1[4]	AD169	2×10^5	54.8	120.0	79.1	71.7	571.0

[1]Average IFN-γ spot forming units (SFU) of 4 replicate wells. [2]Cell subset frequencies determined by flow cytometric analysis. CD45: $\%CD45^+$ of live cells. CD3: $\%CD3^+$ of live $CD45^+$ cells. [3]$SFU/10^6$ CD3 T cell = ((average of peptide-pulsed wells – average of mock-wells) × (100/%CD45) × (100/%CD3)) × (10^6/input cells). [4]IE1 peptide pool. Positive T cell responses were defined as those > 50 SFU/million CD3 T cells and ≥ 2× mean of negative control wells.

Supplementary Table 6: Detection of HCMV-DNA in in BLT-L mice exposed to a single inoculation of HCMV.

			At Time of Necropsy								
				PCR Detection of HCMV-DNA (+/-)[a]							
Mouse	Donor	Virus	Time (days)	H LNG 1	H LNG 2	BM	SPL	LN	TO	LIV	M LNG	PB
BLT-L11	B	TB40/E	12	+	+	-	-	-	+	-	-	-
BLT-L12	B	TB40/E	12	+	+	-	-	-	-	-	-	-
BLT-L13	B	TB40/E	12	+	+	-	-	-	-	-	-	-
BLT-L14	B	TB40/E	12	+	+	-	-	-	-	-	-	-
BLT-L15	I	TB40/E	12	+	+	-	-	-	-	-	-	ND
BLT-L16	I	TB40/E	12	+	+	-	-	-	-	-	-	ND
BLT-L17	I	TB40/E	12	+	+	-	-	-	-	-	-	ND
BLT-L 1	A	TB40/E	14	+	+	-	+	+	-	ND	ND	-
BLT-L 2	B	TB40/E	21	-	+	-	-	-	-	-	-	-
BLT-L 3	B	TB40/E	21	-	+	-	-	-	-	-	-	-
BLT-L 4	B	TB40/E	21	+	+	-	-	-	-	-	-	-
BLT-L 5	C	TB40/E	34	-	ND	-	-	-	-	-	-	ND
BLT-L 6	A	TB40/E	34	+	-	+	-	+	-	-	+	-
BLT-L 7	C	TB40/E	39	-	-	-	-	-	-	-	-	ND
BLT-L 8	A	TB40/E	125	-	-	-	-	-	-	-	-	-
BLT-L 9	C	ADrUL131	34	+	ND	-	-	-	-	-	-	ND
BLT-L 10	C	ADrUL131	48	-	-	-	-	-	-	-	-	ND
BLT-L55	A	AD169	14	+	-	-	+	ND	-	+	+	ND
BLT-L56	J	AD169	15	-	-	-	-	-	-	-	-	ND
BLT-L57	J	AD169	28	-	-	-	-	-	-	-	+	ND
BLT-L58	A	AD169	34	+	+	-	-	-	-	-	-	ND
BLT-L59	A	AD169	47	-	-	-	-	-	-	-	-	ND
BLT-L60	J	AD169	56	ND	-	-	-	+	-	-	+	ND
BLT-L61	J	AD169	75	ND	ND	ND	ND	ND	ND	ND	ND	ND
BLT-L62	A	AD169	110	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a]Real-time PCR analysis of tissue cells and peripheral blood (PB) for HCMV-DNA: human lung (H LNG; 6×10^4-5×10^6 cells), bone marrow (BM; 1-4×10^6 cells), spleen, (SPL; 1×10^5-4×10^6 cells), lymph node (LN; 5×10^4-1.3×10^6 cells), thymic organoid (TO; 1.7×10^5-4×10^6 cells), liver (LIV; 5×10^4-4×10^6 cells), mouse lung (M LNG; 5×10^4-2.3×10^6 cells) and PB (50-100 µl). ND: not determined.

Supplementary Table 7: Detection of HCMV-specific immunity in BLT-L mice exposed to multiple doses of HCMV-TB40/E.

			At Time of Necropsy			
			HCMV-specific Immune Response			
Mouse	Donor	Virus inoculations	T cell reactivity[a]		Antibody IgM	Antibody IgG
BLT-L27	D	3	Positive (I)	0.40%	Positive	Positive
BLT-L28	D	3	Not detected (I)		ND	ND
BLT-L29	D	3	Positive (P)	1.75%	Positive	Positive
BLT-L30	D	3	Not detected (I,P)		Positive	Positive
BLT-L31	E	3	Positive (I)	0.14%	Positive	Positive
BLT-L32	E	3	Positive (I)	0.57%	Positive	Positive
BLT-L33	E	3	Not detected (I)		Positive	Positive
BLT-L34	F	3	Not detected (I)		Positive	Not detected
BLT-L35	F	3	Not detected (I)		Positive	Not detected
BLT-L36	F	3	Not detected (I)		Positive	Not detected
BLT-L37	F	3	Not detected (I)		Positive	Not detected
BLT-L38	G	4	Positive (I,P)	0.13%, 0.13%	Positive	Positive
BLT-L39	H	4	Positive (P)	0.17%	ND	ND
BLT-L40	H	4	Positive (P)	0.07%	ND	ND

[a]Assay detecting HCMV-specific T cell reactivity: intracellular cytokine staining (I), Pentamer staining (P). The magnitude of T cell reactivity is provided as the % IFN-γ and CD107a double positive or % pentamer positive (or ranges, in case > 1 positive) for intracellular cytokine and pentamer staining respectively. ND: not determined. Criteria for T cell assay positivity is detailed in Methods. Positivity threshold for Antibody IgM Index ≥ 0.5 and IgG Index ≥ 0.02.

Supplementary Table 8: Detection of HCMV-specific immunity in BLT-L mice exposed to multiple doses of HCMV-AD169.

			At Time of Necropsy			
			HCMV-specific Immune Response			
Mouse	Donor	Virus inoculations	T cell reactivity[a]		Antibody IgM	Antibody IgG
BLT-L41	E	3	Not detected (I)		Positive	Positive
BLT-L42	E	3	Positive (I)	0.35%	Positive	Positive
BLT-L43	E	3	Positive (I)	0.10%	Positive	Positive
BLT-L44	G	4	Positive (I)	0.26%	Positive	Positive
BLT-L45	G	4	Positive (I)	0.16%	Not detected	ND
BLT-L46	G	4	Positive (I,P)	0.49, 0.02%	Positive	Not detected
BLT-L47	G	4	Positive (I)	0.32%	Positive	Positive
BLT-L48	G	4	Positive (I)	0.16%	Positive	Positive
BLT-L49	G	4	Positive (I)	0.62%	Positive	Positive
BLT-L50	G	4	Positive (I)	0.06%	Positive	Positive
BLT-L51	H	4	Positive (P)	1.91%	ND	ND
BLT-L52	H	4	Positive (I[b],P)	0.05%, 0.08%	ND	ND
BLT-L53	H	4	Positive (I)[b]	0.44%	ND	ND
BLT-L54	H	4	Positive (I,P)	0.20%	ND	ND
BLT-L63	D	3	Not detected (I)		Positive	Positive
BLT-L64	D	3	Positive (I)	0.26%	Not detected	ND
BLT-L65	D	3	Not detected (I,P)		Positive	Not detected

[a]Assay detecting HCMV-specific T cell reactivity: intracellular cytokine staining (I), Pentamer staining (P) with magnitude (or ranges, in case > 1 positive) provided. The magnitude of T cell reactivity is provided as the % IFN-γ and CD107a double positive or % pentamer positive (or ranges, in case > 1 positive) for intracellular cytokine and pentamer staining respectively. ND: Not determined. [b]ICS HCMV-specific $CD4^+$ T cell response. The magnitude of T cell reactivity is provided as the % IFN-γ and TNF-α double positive by intracellular cytokine staining. Criteria for T cell assay positivity is detailed in Methods. Positivity threshold for Antibody IgM Index is ≥ 0.5.

Supplementary Table 9: Detection of HCMV-DNA in BLT-L mice exposed to multiple doses of HCMV-TB40/E.

			At Time of Necropsy								
			PCR Detection of HCMV-DNA (+/-)[a]								
Mouse	Donor	Virus inoculations	H LNG 1	H LNG 2	BM	SPL	LN	TO	LIV	M LNG	PB
BLT-L27	D	3	-	-	-	-	-	-	-	-	ND
BLT-L28	D	3	+	+	-	+	-	-	-	-	ND
BLT-L29	D	3	-	-	-	-	-	-	-	-	ND
BLT-L30	D	3	-	-	-	-	-	-	+	-	ND
BLT-L31	E	3	+	-	-	-	-	-	-	-	-
BLT-L32	E	3	-	-	-	-	-	-	-	-	-
BLT-L33	E	3	-	-	-	-	-	-	-	-	-
BLT-L34	F	3	ND	ND	ND	ND	ND	ND	ND	ND	ND
BLT-L35	F	3	ND	ND	ND	ND	ND	ND	ND	ND	ND
BLT-L36	F	3	ND	ND	ND	ND	ND	ND	ND	ND	ND
BLT-L37	F	3	ND	ND	ND	ND	ND	ND	ND	ND	ND
BLT-L38	G	4	-	-	-	-	-	-	-	-	-
BLT-L39	H	4	ND	ND	ND	ND	ND	ND	ND	ND	ND
BLT-L40	H	4	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a]Real-time PCR analysis of tissue cells and peripheral blood (PB) or HCMV-DNA: human lung (H LNG; 1×10^5-1×10^6 cells), bone marrow (BM; 1-4×10^6 cells), spleen, (SPL; 1-4×10^6 cells), lymph node (LN; 1×10^5-1×10^6 cells), thymic organoid (TO; 4×10^6 cells), liver (LIV; 1×10^5-$1x10^6$ cells), mouse lung (M LNG; 1×10^5-1×10^6 cells) and PB (100 µl). ND: not determined.

Supplementary Table 10: Detection of HCMV-DNA in BLT-L mice exposed to multiple doses of HCMV-AD169.

			At Time of Necropsy								
			PCR Detection of HCMV-DNA (+/-)[a]								
Mouse	Donor	Virus inoculations	H LNG 1	H LNG 2	BM	SPL	LN	TO	LIV	M LNG	PB
BLT-L41	E	3	+	-	-	-	-	-	-	-	-
BLT-L42	E	3	+	+	-	-	-	-	-	-	-
BLT-L43	E	3	-	-	-	-	+	-	-	-	ND
BLT-L44	G	4	-	+	-	-	-	-	-	-	-
BLT-L45	G	4	-	-	-	-	-	-	-	-	-
BLT-L46	G	4	-	+	-	-	-	-	-	-	-
BLT-L47	G	4	-	-	-	-	-	-	-	-	-
BLT-L48	G	4	-	+	-	-	-	-	-	-	-
BLT-L49	G	4	+	-	-	-	-	-	-	-	-
BLT-L50	G	4	+	-	-	-	-	-	-	-	-
BLT-L51	H	4	ND	ND	ND	ND	ND	ND	ND	ND	ND
BLT-L52	H	4	ND	ND	ND	ND	ND	ND	ND	ND	ND
BLT-L53	H	4	ND	ND	ND	ND	ND	ND	ND	ND	ND
BLT-L54	H	4	ND	ND	ND	ND	ND	ND	ND	ND	ND
BLT-L63	D	3	-	-	-	-	-	-	-	-	ND
BLT-L64	D	3	-	-	-	-	-	-	-	-	ND
BLT-L65	D	3	+	-	-	-	-	-	-	-	ND

[a]Real-time PCR analysis of tissue cells and peripheral blood (PB) for HCMV-DNA: human lung (H LNG; 1×10^5-1×10^6 cells), bone marrow (BM; 1-4×10^6 cells), spleen, (SPL; 1-4×10^6 cells), lymph node (LN; 3.1×10^4-1.74×10^6 cells), thymic organoid (TO; 1-4×10^6 cells), liver (LIV; 1×10^5-1×10^6 cells), mouse lung (M LNG; 1×10^5-1×10^6 cells) and PB (50-100 µl). ND: not determined.