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Supplemental Information

KLRG1^{+} Effector CD8^{+} T Cells Lose KLRG1, Differentiate into All Memory T Cell Lineages, and Convey Enhanced Protective Immunity

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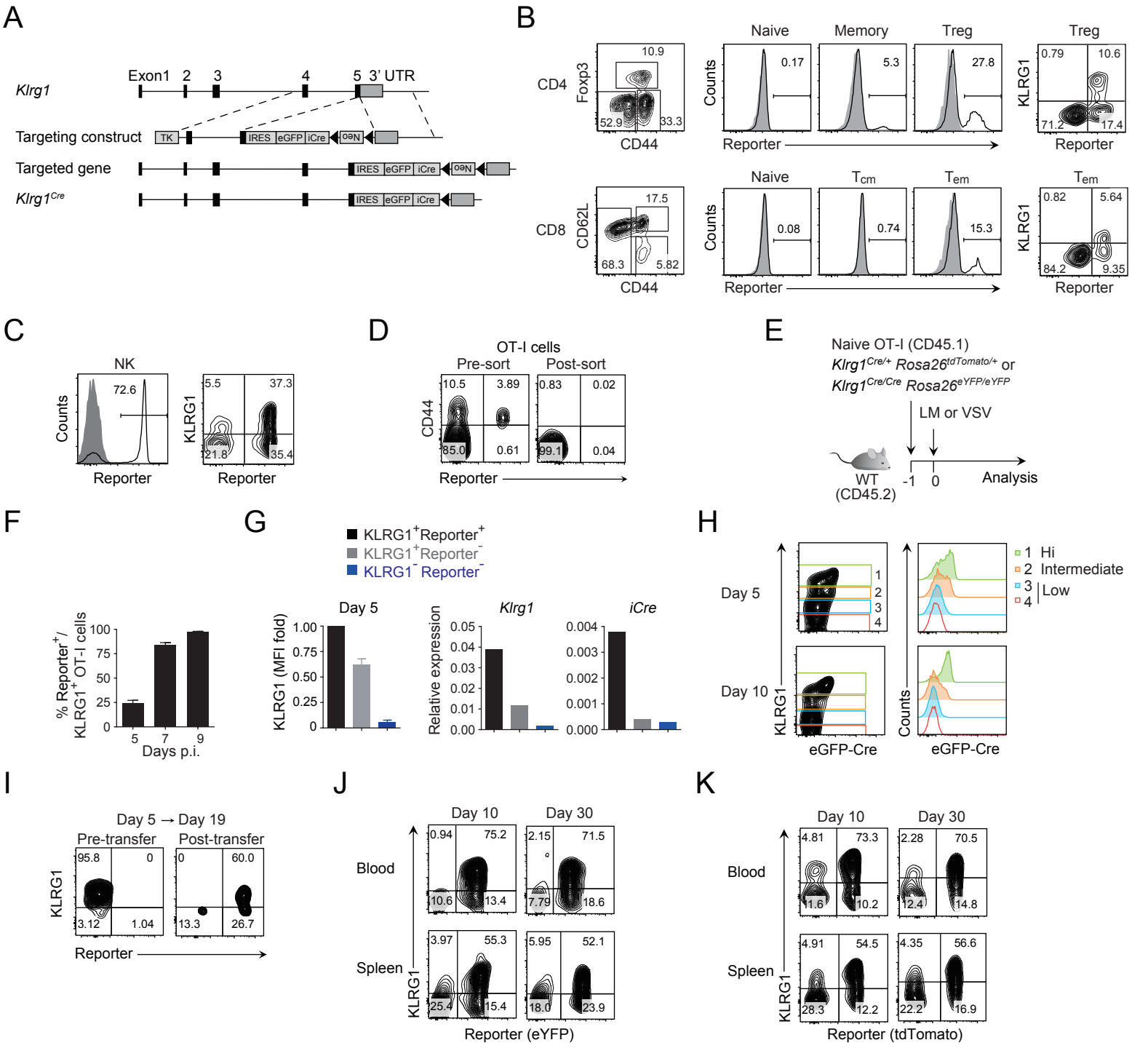
A
Klrg1
Exon1 2 3 4 5 3' UTR
Targeting construct
TK
IRES eGFP iCre
Neo
Targeted gene
Klrg1Cre
B
CD4
Foxp3
CD44
Naive
Memory
Treg
Counts
Reporter
KLRG1
CD8
CD62L
Naive
Tcm
Tem
C
NK
Counts
Reporter
KLRG1
D
OT-I cells
Pre-sort
Post-sort
CD44
Reporter
E
Naive OT-I (CD45.1)
Klrg1Cre/+ Rosa26tdTomato/+ or
Klrg1Cre/Cre Rosa26eYFP/eYFP
LM or VSV
WT
(CD45.2)
-1
0
Analysis
F
% Reporter+/
KLRG1+ OT-I cells
Days p.i.
G
KLRG1+Reporter+
KLRG1+Reporter-
KLRG1- Reporter-
Day 5
KLRG1 (MFI fold)
Klrg1
iCre
Relative expression
H
Day 5
Day 10
KLRG1
Counts
eGFP-Cre
1 Hi
2 Intermediate
3
4 Low
I
Day 5 → Day 19
Pre-transfer
Post-transfer
KLRG1
Reporter
J
Day 10
Day 30
Blood
Spleen
KLRG1
Reporter (eYFP)
K
Day 10
Day 30
Blood
Spleen
KLRG1
Reporter (tdTomato)

Figure S1

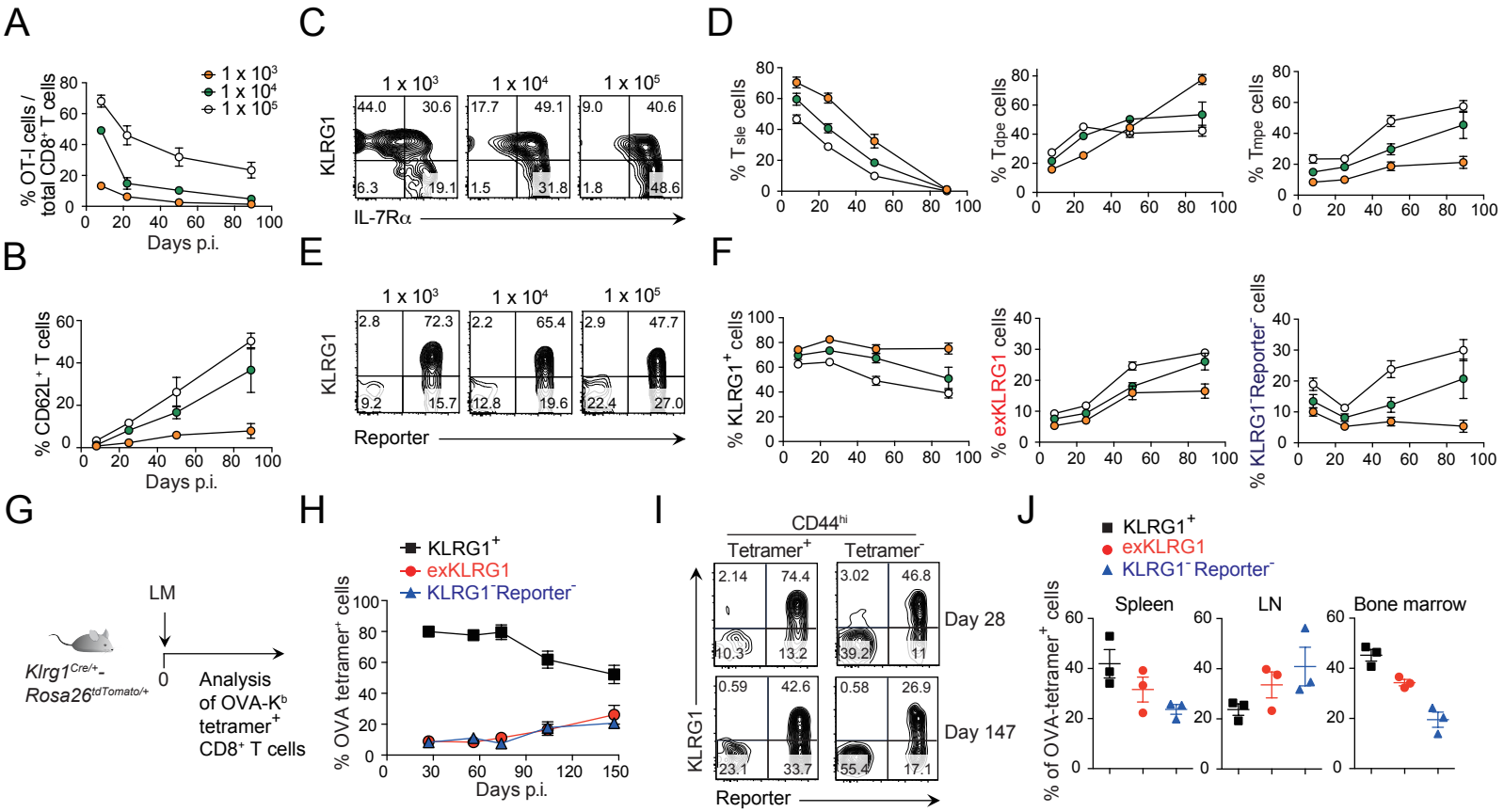
A
% OT-I cells / total CD8+ T cells
1 x 10³
1 x 10⁴
1 x 10⁵
Days p.i.
B
% CD62L+ T cells
Days p.i.
C
1 x 10³
1 x 10⁴
1 x 10⁵
KLRG1
IL-7Rα
44.0
30.6
6.3
19.1
17.7
49.1
1.5
31.8
9.0
40.6
1.8
48.6
D
% Tsle cells
% Tdpe cells
% Tmpe cells
E
KLRG1
Reporter
2.8
72.3
9.2
15.7
2.2
65.4
12.8
19.6
2.9
47.7
22.4
27.0
F
% KLRG1+ cells
% exKLRG1 cells
% KLRG1-Reporter- cells
G
LM
0
Analysis of OVA-Kb tetramer+ CD8+ T cells
Klrg1Cre/+-Rosa26tdTomato/+
H
KLRG1+
exKLRG1
KLRG1-Reporter-
% OVA tetramer+ cells
Days p.i.
I
CD44hi
Tetramer+
Tetramer-
Day 28
Day 147
KLRG1
Reporter
2.14
74.4
10.3
13.2
3.02
46.8
39.2
11
0.59
42.6
23.1
33.7
0.58
26.9
55.4
17.1
J
KLRG1+
exKLRG1
KLRG1- Reporter-
% of OVA-tetramer+ cells
Spleen
LN
Bone marrow

Figure S2

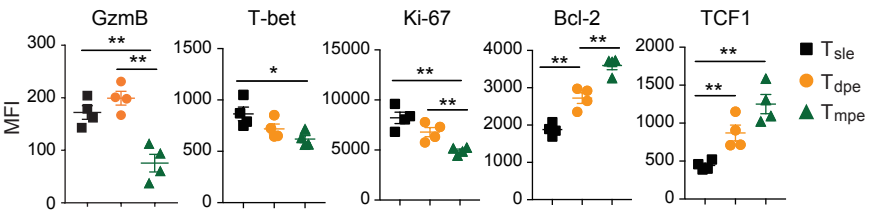
GzmB
T-bet
Ki-67
Bcl-2
TCF1
MFI
300
200
100
0
1500
1000
500
0
15000
10000
5000
0
4000
3000
2000
1000
0
2000
1500
1000
500
0
**
**
*
**
**
**
**
**
**
T sle
T dpe
T mpe

Figure S3

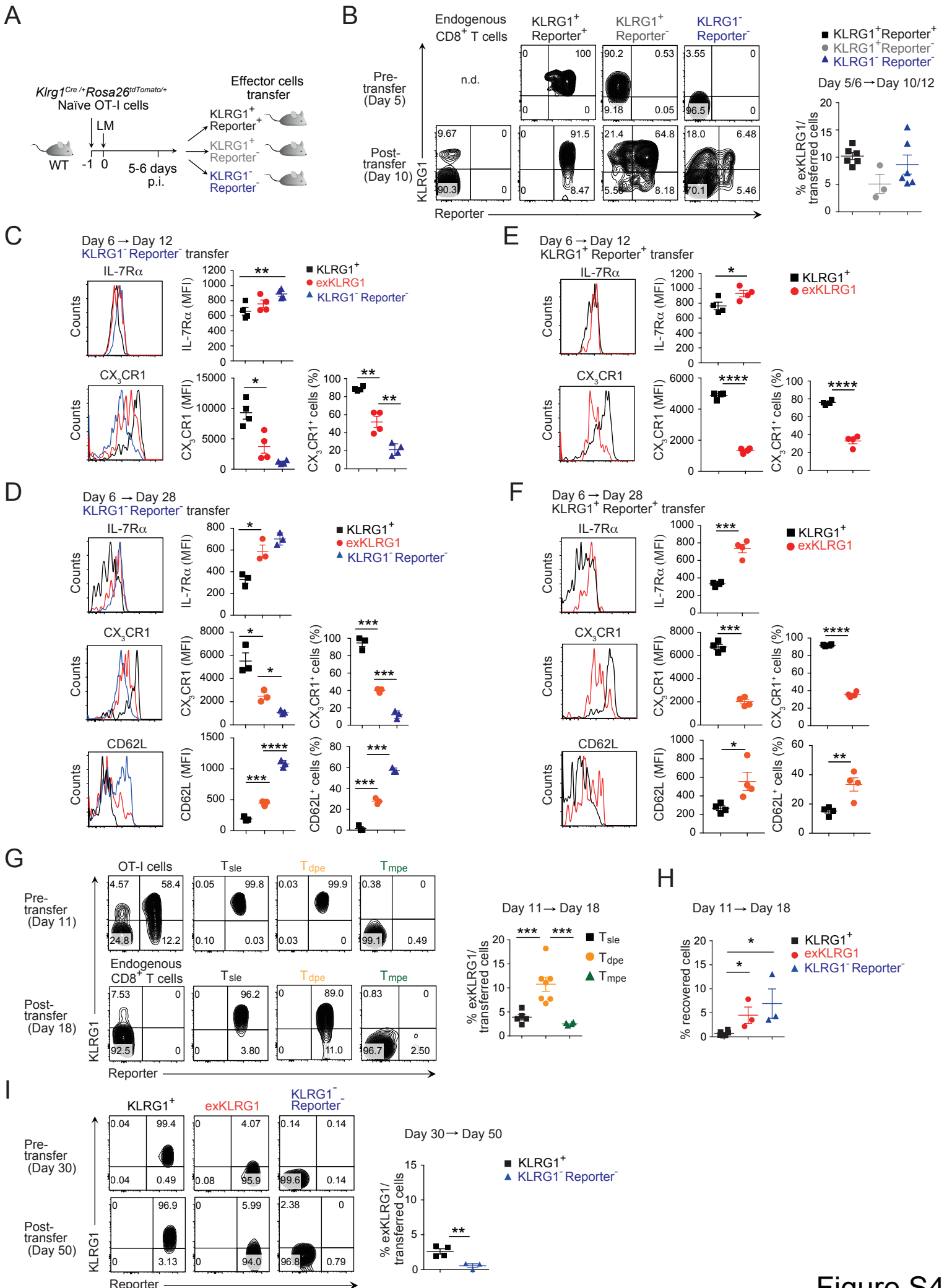
A
Klrg1Cre/+Rosa26tdTomato/+
Naïve OT-I cells
LM
WT
-1 0
5-6 days p.i.
Effector cells transfer
KLRG1+ Reporter+
KLRG1+ Reporter-
KLRG1- Reporter-
B
Endogenous CD8+ T cells
KLRG1+ Reporter+
KLRG1+ Reporter-
KLRG1- Reporter-
Pre-transfer (Day 5)
Post-transfer (Day 10)
n.d.
KLRG1
Reporter
KLRG1+Reporter+
KLRG1+Reporter-
KLRG1- Reporter-
Day 5/6→Day 10/12
% exKLRG1/ transferred cells
C
Day 6 → Day 12
KLRG1- Reporter- transfer
IL-7Rα
Counts
IL-7Rα (MFI)
CX3CR1
CX3CR1 (MFI)
CX3CR1+ cells (%)
KLRG1+
exKLRG1
KLRG1- Reporter-
E
Day 6 → Day 12
KLRG1+ Reporter+ transfer
D
Day 6 → Day 28
KLRG1- Reporter- transfer
CD62L
CD62L (MFI)
CD62L+ cells (%)
F
Day 6 → Day 28
KLRG1+ Reporter+ transfer
G
OT-I cells
Tsle
Tdpe
Tmpe
Pre-transfer (Day 11)
Endogenous CD8+ T cells
Post-transfer (Day 18)
Day 11→ Day 18
% exKLRG1/ transferred cells
H
Day 11→ Day 18
% recovered cells
I
KLRG1+
exKLRG1
KLRG1- Reporter-
Pre-transfer (Day 30)
Post-transfer (Day 50)
Day 30→ Day 50
% exKLRG1/ transferred cells

Figure S4

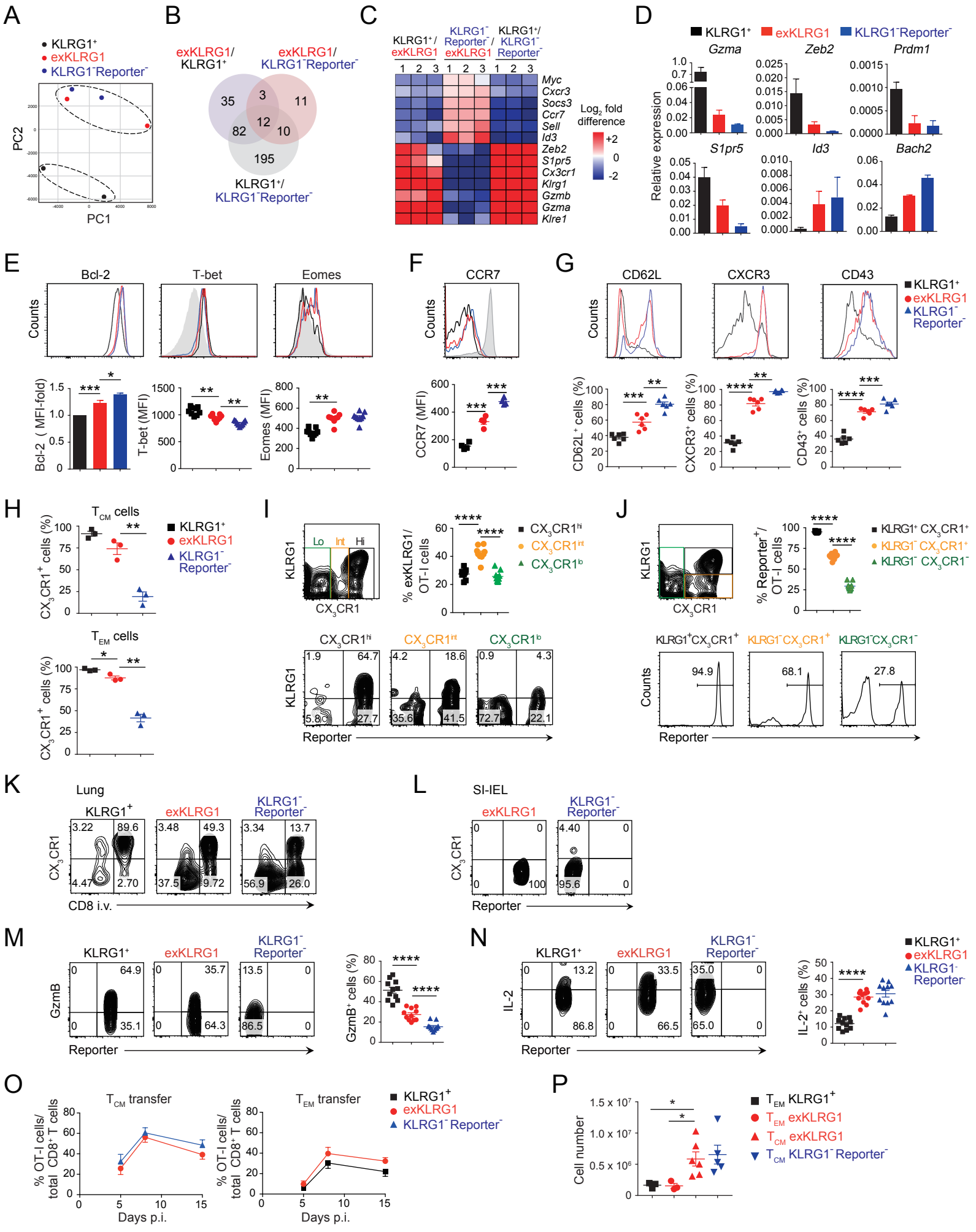
A
KLRG1+
exKLRG1
KLRG1-Reporter-
PC2
PC1
B
exKLRG1/ KLRG1+
exKLRG1/ KLRG1-Reporter-
35
3
11
12
82
10
195
KLRG1+/ KLRG1-Reporter-
C
KLRG1+/ exKLRG1
KLRG1- Reporter-/ exKLRG1
KLRG1+/ KLRG1- Reporter-
1 2 3 1 2 3 1 2 3
Myc
Cxcr3
Socs3
Ccr7
Sell
Id3
Zeb2
S1pr5
Cx3cr1
Klrg1
Gzmb
Gzma
Klre1
Log2 fold difference
+2
0
-2
D
KLRG1+
exKLRG1
KLRG1-Reporter-
Gzma
Zeb2
Prdm1
S1pr5
Id3
Bach2
Relative expression
E
Bcl-2
T-bet
Eomes
Counts
Bcl-2 (MFI-fold)
T-bet (MFI)
Eomes (MFI)
F
CCR7
Counts
CCR7 (MFI)
G
CD62L
CXCR3
CD43
Counts
CD62L+ cells (%)
CXCR3+ cells (%)
CD43+ cells (%)
KLRG1+
exKLRG1
KLRG1- Reporter-
H
TCM cells
TEM cells
CX3CR1+ cells (%)
KLRG1+
exKLRG1
KLRG1- Reporter-
I
KLRG1
CX3CR1
Lo
Int
Hi
% exKLRG1/ OT-I cells
CX3CR1hi
CX3CR1int
CX3CR1lo
Reporter
J
KLRG1
CX3CR1
% Reporter+/ OT-I cells
KLRG1+ CX3CR1+
KLRG1- CX3CR1+
KLRG1- CX3CR1-
Counts
94.9
68.1
27.8
Reporter
K
Lung
KLRG1+
exKLRG1
KLRG1- Reporter-
CX3CR1
CD8 i.v.
L
SI-IEL
exKLRG1
KLRG1- Reporter-
CX3CR1
Reporter
M
KLRG1+
exKLRG1
KLRG1- Reporter-
GzmB
Reporter
GzmB+ cells (%)
N
KLRG1+
exKLRG1
KLRG1- Reporter-
IL-2
Reporter
IL-2+ cells (%)
O
TCM transfer
TEM transfer
% OT-I cells/ total CD8+ T cells
Days p.i.
KLRG1+
exKLRG1
KLRG1- Reporter-
P
Cell number
TEM KLRG1+
TEM exKLRG1
TCM exKLRG1
TCM KLRG1- Reporter-

Figure S5

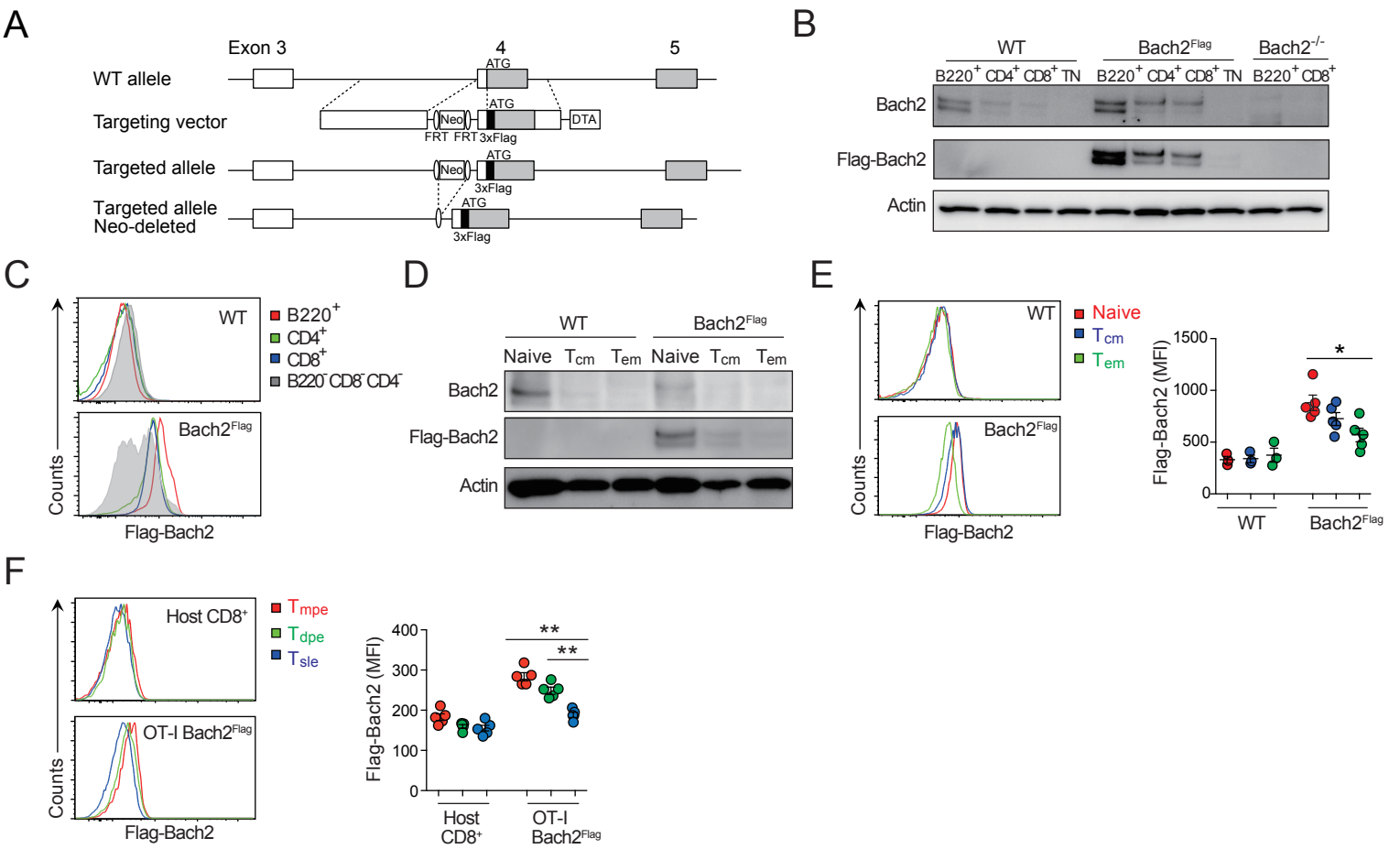
A
Exon 3
4
5
WT allele
ATG
Targeting vector
Neo
DTA
FRT FRT 3xFlag
Targeted allele
3xFlag
Targeted allele
Neo-deleted
B
WT
Bach2Flag
Bach2-/-
B220+ CD4+ CD8+ TN
B220+ CD4+ CD8+ TN
B220+ CD8+
Bach2
Flag-Bach2
Actin
C
WT
Bach2Flag
B220+
CD4+
CD8+
B220-CD8-CD4-
Counts
Flag-Bach2
D
WT
Bach2Flag
Naive Tcm Tem Naive Tcm Tem
Bach2
Flag-Bach2
Actin
E
WT
Bach2Flag
Naive
Tcm
Tem
Counts
Flag-Bach2
Flag-Bach2 (MFI)
1500
1000
500
0
*
WT
Bach2Flag
F
Host CD8+
OT-I Bach2Flag
Tmpe
Tdpe
Tsle
Counts
Flag-Bach2
Flag-Bach2 (MFI)
400
300
200
100
0
**
**
Host CD8+
OT-I Bach2Flag

Figure S6

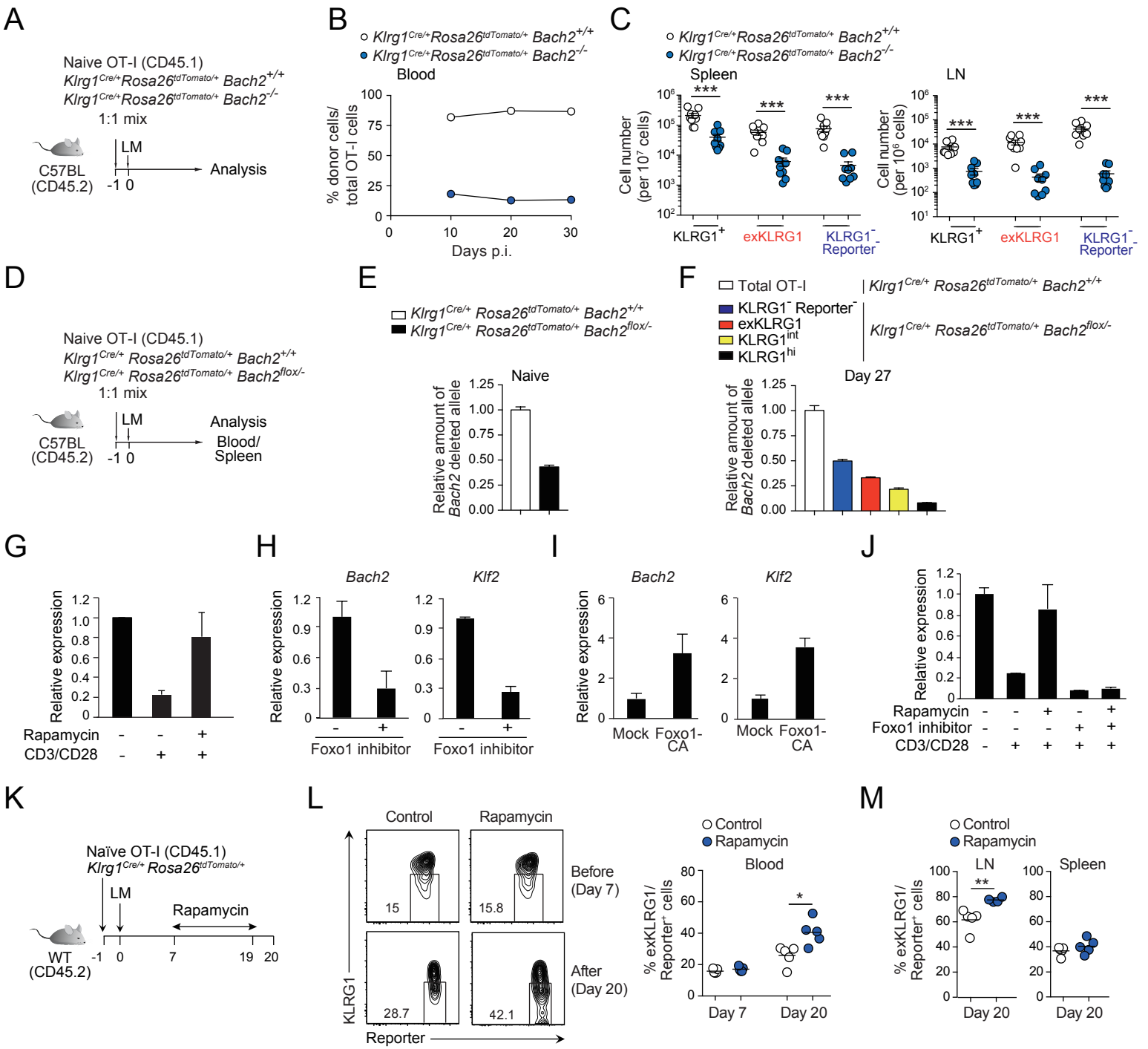
A
Naive OT-I (CD45.1)
Klrg1Cre/+Rosa26tdTomato/+ Bach2+/+
Klrg1Cre/+Rosa26tdTomato/+ Bach2-/-
1:1 mix
C57BL (CD45.2)
LM
-1 0
Analysis
B
Klrg1Cre/+Rosa26tdTomato/+ Bach2+/+
Klrg1Cre/+Rosa26tdTomato/+ Bach2-/-
Blood
% donor cells/ total OT-I cells
Days p.i.
C
Spleen
LN
Cell number (per 10^7 cells)
Cell number (per 10^6 cells)
KLRG1+
exKLRG1
KLRG1- Reporter
D
Naive OT-I (CD45.1)
Klrg1Cre/+ Rosa26tdTomato/+ Bach2+/+
Klrg1Cre/+ Rosa26tdTomato/+ Bach2flox/-
1:1 mix
C57BL (CD45.2)
LM
-1 0
Analysis
Blood/ Spleen
E
Klrg1Cre/+ Rosa26tdTomato/+ Bach2+/+
Klrg1Cre/+ Rosa26tdTomato/+ Bach2flox/-
Naive
Relative amount of Bach2 deleted allele
F
Total OT-I
KLRG1- Reporter-
exKLRG1
KLRG1int
KLRG1hi
Klrg1Cre/+ Rosa26tdTomato/+ Bach2+/+
Klrg1Cre/+ Rosa26tdTomato/+ Bach2flox/-
Day 27
Relative amount of Bach2 deleted allele
G
Relative expression
Rapamycin
CD3/CD28
H
Bach2
Klf2
Relative expression
Foxo1 inhibitor
I
Bach2
Klf2
Relative expression
Mock
Foxo1-CA
J
Relative expression
Rapamycin
Foxo1 inhibitor
CD3/CD28
K
Naïve OT-I (CD45.1)
Klrg1Cre/+ Rosa26tdTomato/+
LM
Rapamycin
WT (CD45.2)
-1 0 7 19 20
L
Control
Rapamycin
KLRG1
Reporter
15
15.8
28.7
42.1
Before (Day 7)
After (Day 20)
Blood
% exKLRG1/ Reporter+ cells
Day 7
Day 20
M
Control
Rapamycin
LN
Spleen
% exKLRG1/ Reporter+ cells
Day 20

Figure S7

Figure S1. Generation and Validation of *Klrg1*^{Cre} Reporter Mice. Related to Figure 1.

(A) Schematic of the generation of *Klrg1*^{Cre} knock-in mice.

(B) Expression of tdTomato in splenic CD44^{hi}Foxp3^{-} (naive), CD44^{hi}Foxp3^{-} (memory) and CD44^{hi}Foxp3^{+} CD4^{+} T_{reg} cells (upper panel). Expression of tdTomato in splenic CD44^{lo}CD62L^{hi} (naïve), CD44^{lo}CD62L^{hi} (Tcm), and CD44^{lo}CD62L^{hi} (Tem) CD8^{+} T cells (lower panel).

(C) Expression of KLRG1 and tdTomato in splenic NK1.1^{+} cells.

(D) Expression of CD44 and tdTomato in OT-I cells before and in naïve CD44^{lo}CD62L^{hi} OT-I cells after fluorescence-activated cell sorting.

(E) Schematic of the OT-I cell transfer and infection experiments. Naïve CD44^{lo}CD62L^{hi} Reporter^{-} OT-I cells from *Klrg1*$^{Cre/+}$*Rosa26*$^{tdTomato/+}$ or *Klrg1*$^{Cre/Cre}$*Rosa26*$^{eYFP/eYFP}$ mice were injected into WT mice and challenged with ovalbumin (OVA)-expressing *Listeria monocytogenes* (LM) or OVA-expressing *Vesicular stomatitis virus* (VSV) one day later.

(F) Expression of the Reporter in KLRG1^{+} OT-I cells following infection with LM.

(G) Mean fluorescence intensity (MFI) of KLRG1 (left graph) and relative expression of *Klrg1* and *iCre* mRNA (normalized to *Actb*) in splenic effector OT-I cell subsets 5 days post infection (p.i.) with LM.

(H) Expression of KLRG1-eGFP-Cre in OT-I cell subsets 5 and 10 days p.i. with LM.

(I) KLRG1^{+} tdTomato^{-} effector OT-I cells were sorted from the spleen on day 5 p.i. with LM and transferred into WT mice. Expression of KLRG1 and tdTomato were analyzed 14 days post transfer.

(J and K) Development of exKLRG1 cells in the blood and spleen of mice transferred with naïve OT-I cells from *Klrg1*$^{Cre/+}$*Rosa26*$^{tdTomato/+}$ (J) or *Klrg1*$^{Cre/Cre}$*Rosa26*$^{eYFP/eYFP}$ mice (K) followed by infection with LM.

Mean ± SEM are shown. Data are representative of 2-3 independent experiments with 2-6 mice (B-D, H-K) or are pooled from 2-3 independent experiments with 2-10 mice per time point (F, G).

Figure S2. Development of ExKLRG1 Memory Cells from Different Naïve OT-I Precursor Numbers and from Endogenous OVA-Specific Naïve CD8$^+$ T Cells. Related to Figure 1.

(A and B) 1x10^3, 1x10^4 and 1x10^5 naïve *Klrg1*$^{Cre/+}$*Rosa26*$^{tdTomato/+}$ OT-I cells were injected into WT mice followed by infection with LM the following day. The graph shows the percentage of OT-I cells among total CD8$^+$ T cells (A) and the percentage of CD62L$^+$ cells among transferred OT-I cells (B).

(C and D) Development of Tsle, Tdpe and Tmpe cells after transfer of different naïve OT-I cell numbers followed by infection with LM. The density plots show OT-I cells at day 50 p.i. with LM (C).

(E and F) Development of KLRG1$^+$, exKLRG1 and KLRG1$^-$ Reporter$^-$ cells after transfer of different naïve OT-I cell numbers followed by infection with LM. The density plots show OT-I cells at day 50 p.i. with LM (E).

(G) Schematic of the experimental protocol. *Klrg1*$^{Cre/+}$*Rosa26*$^{tdTomato/+}$ mice were challenged with LM and the development of OVA-K^b tetramer$^+$ exKLRG1 memory CD8$^+$ T cells in the blood was analyzed.

(H and I) Frequency of KLRG1$^+$, exKLRG1 and KLRG1$^-$ Reporter$^-$ cells within OVA-K^b tetramer$^+$ CD44^{hi} CD8$^+$ T cells in the blood. Representative flow cytometry density plots are shown in (I).

(J) ExKLRG1 cells represent a substantial proportion of memory OT-I cells in the spleen, LN and bone marrow 147 days p.i. with LM.

Mean ± SEM are shown. Data are representative of two (A-F) or three (H-J) independent experiments with 3-5 mice.

Figure S3. Tdpe Cells Express Effector- and Memory-Associated Molecules at an Intermediate Level. Related to Figure 3.

Expression of GzmB, T-bet, Ki-67, Bcl-2 and TCF-1 in splenic Tsle, Tdpe, and Tmpe OT-I cell subsets 9-10 days p.i. with LM.

Mean ± SEM are shown. * $P < 0.05$ and ** $P < 0.01$ (unpaired two-tailed Student's *t*-test). Data are representative of two independent experiments with 4 mice.

Figure S4. Developmental Plasticity of CD8$^+$ T cell Subsets Following Bacterial Infection. Related to Figure 4.

(A) Schematic of the infection and effector cell transfer experiments for (A-G).

(B) Expression of KLRG1 and tdTomato in splenic effector OT-I cell subsets 5 days p.i. with LM and 5 days post transfer into WT mice. Development of exKLRG1 cells from three different effector OT-I cell subsets transferred 5 or 6 days p.i. with LM is shown on the right.

(C and D) Expression of CX_3CR1, IL-7Rα and CD62L in effector cell subsets 6 (C) or 22 (D) days post transfer of KLRG1$^-$Reporter$^-$ cells.

(E and F) Expression of CX_3CR1, IL-7Rα and CD62L in effector cell subsets 6 (E) or 22 (F) days post transfer of KLRG1$^+$Reporter$^+$ cells.

(G) Expression of KLRG1 and tdTomato in OT-I cell subsets 7 days post transfer of day 11 effector OT-I cell subsets. The development of exKLRG1 cells from the three different effector OT-I cell subsets transferred 11 days p.i. with LM is shown on the right.

(H) Percentage of recovered cells 7 days post transfer of effector OT-I cell subsets isolated 11 days p.i. with LM.

(I) Expression of KLRG1 and Reporter in OT-I memory cell subsets before (30 days p.i. with LM) and 20 days post transfer.

Mean ± SEM are shown. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ (unpaired two-tailed Student's *t*-test). Data are representative of two independent experiments with 3-4 mice (C-F) or pooled from 2-3 independent experiments (B, G-I).

Figure S5. Molecular Profiling of ExKLRG1 Memory CD8$^+$ T Cells. **Related to Figure 5.**

(A) Principal component analysis of genome-wide expression data in splenic memory OT-I cell subsets 104-110 days p.i. with LM.

(B) Cell-specific and common regulation of genes (> 1.5-fold) in any two subsets of splenic memory OT-I cells, assessed by RNA-seq and presented as a Venn diagram.

(C) Heat map of genes with a difference in gene expression of over 1.5-fold in any two subsets of splenic memory OT-I cells 104-110 days p.i. with LM (samples 1 and 2) and 70 days p.i. with VSV (sample 3).

(D) Analysis of 6 effector or memory signature genes in splenic memory OT-I cell subsets (30+ days p.i. with LM) by quantitative RT-PCR (n=3 biological replicates with 3-4 mice each).

(E) Mean fluorescence intensity (MFI) of Bcl-2, T-bet and Eomes in splenic memory OT-I cell subsets 60 days p.i. with LM.

(F and G) Expression of CCR7, CD62L, CD43 and CXCR3 in splenic memory OT-I cell subsets 27 days (F) or 60-120 days (G) p.i. with LM.

(H) Expression of CX_3CR1 in $KLRG1^+$, exKLRG1 and $KLRG1^-$ $Reporter^-$ Tcm and Tem cell subsets in the blood 30 days p.i. with LM.

(I) Frequency of exKLRG1 cells in effector OT-I cell subsets, defined by CX_3CR1 expression, in the spleen 28 days p.i. with LM.

(J) Flow cytometric analysis of tdTomato expression in effector OT-I cell subsets, defined by KLRG1 and CX_3CR1 expression, in the spleen 28 days p.i. with LM.

(K) Expression of CX_3CR1 in circulating and resident OT-I cell subsets in the lung 60 days p.i. with LM. The circulating cells were identified by i.v. injection of anti-CD8α antibody.

(L) Expression of CX_3CR1 in the SI-IEL 60 days p.i. with LM.

(M and N) Expression of granzyme B (GzmB) (M) and IL-2 (N) in splenic memory OT-I cell subsets 120 days p.i. with LM (n=11 mice).

(O and P) Memory Tcm and Tem OT-I cell subsets were transferred into naïve WT mice, followed by infection with LM one day later. Frequency of transferred OT-I cells among total $CD8^+$ T cells in the blood after secondary infection with LM (O). Absolute number of transferred OT-I cells in the spleen 4 days after secondary infection with LM (P).

Mean ± SEM are shown. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ (unpaired two-tailed Student's *t*-test). Data are from two (A, B) or three (C, D) biologically independent samples, each pooled from 3-5 mice, or pooled from 2-3 independent experiments with 7-8 (E), 6 (G) or 11 mice (M, N), or are representative of two independent experiments with 3-8 mice (F, H-L), 7-8 (O) or 3-6 (P) mice.

Figure S6. Generation and Validation of *Bach2*^{flag} Knock-in Mice. Related to Figure 7.

(A) Schematic of the generation of *Bach2*^{flag} knock-in mice.

(B and C) Expression of Flag-Bach2 was determined by western blotting (B) and flow cytometry (C) in purified B220^{+} B cells, CD4^{+} T cells, CD8^{+} T cells and B220^{-}CD4^{-}CD8^{-} triple negative (TN) cells using WT, *Bach2*^{flag} and Bach2$^{-/-}$ mice.

(D and E) *Bach2*^{flag} mice were infected with LM and Flag-Bach2 expression in naïve (CD62L^{hi} CD44^{lo}), Tcm (CD62L^{hi} CD44^{hi}) and Tem (CD62L^{lo} CD44^{hi}) CD8^{+} T cell subsets was analyzed by western blotting (D) and flow cytometry at day 10 p.i. with LM (E). Representative histograms depict the expression of Flag-Bach2 among different CD8^{+} T cell populations in the spleen. WT mice were used as a staining control.

(F) Representative histograms (left) and dot plots (right) of Flag-Bach2 expression in splenic Tsle, Tdpe and Tmpe OT-I cells from *Bach2*^{Flag} mice 10 days p.i. with LM. Host CD8^{+} T cells were used as a control.

Mean ± SEM are shown. * $P < 0.05$, ** $P < 0.01$ (unpaired two-tailed Student's *t*-test). Data are representative of two independent experiments with 3-5 mice (C, E, F), or with pooled samples from 2-4 mice (B, D).

Figure S7. TCR-Induced Downregulation of Bach2 is Regulated by an AKT-mTOR-Foxo1 Pathway. Related to Figure 7.

(A) Schematic of the experimental protocol for (B and C). Naïve *Klrg1*$^{Cre/+}$*Rosa26*$^{tdTomato/+}$*Bach2*$^{+/+}$ and *Klrg1*$^{Cre/+}$*Rosa26*$^{tdTomato/+}$*Bach2*$^{-/-}$ OT-I cells were mixed 1:1, transferred into C57BL/6 mice and infected with LM one day later.

(B) Relative frequency of *Klrg1*$^{Cre/+}$*Rosa26*$^{tdTomato/+}$*Bach2*$^{+/+}$ and *Klrg1*$^{Cre/+}$*Rosa26*$^{tdTomato/+}$*Bach2*$^{-/-}$ OT-I cells in the blood.

(C) Number of KLRG1^{+}, exKLRG1 and KLRG1^{-}Reporter^{-} OT-I cells in the spleen and LN 30 days p.i. with LM.

(D) Schematic of the experimental protocol for (E and F).

(E and F) Quantitative PCR analysis of the floxed *Bach2* allele in naive (E) or memory (F) OT-I cells.

(G) Relative expression of *Bach2* mRNA in $CD8^+$ T cells activated *in vitro* for 24 hours with or without rapamycin.

(H) Relative expression of *Bach2* and *Klf2* mRNA in naïve $CD8^+$ T cells treated *in vitro* for 48 hours with or without Foxo1 inhibitor AS1842856.

(I) *Bach2* and *Klf2* mRNA expression in $CD8^+$ T cells transduced with a retrovirus expressing GFP alone (Mock) or a retrovirus expressing GFP and a constitutively active form of Foxo1 (Foxo1-CA). The *Klf2*, a well characterized target gene of Foxo1, was used as a positive control.

(J) *Bach2* mRNA expression in $CD8^+$ T cells activated *in vitro* for 24 hours with or without rapamycin/Foxo1 inhibitor. Results are normalized to *Gapdh* (control).

(K) Schematic of the experimental protocol for (L and M).

(L) Percentage of exKLRG1 cells within $Reporter^+$ OT-I cells in the blood of LM-infected mice before and after treatment with rapamycin.

(M) Percentage of exKLRG1 cells among $Reporter^+$ donor OT-I cells in the LN and spleen after rapamycin treatment.

Mean ± SEM are shown. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (unpaired two-tailed Student's *t*-test). Data are representative of 2-3 independent experiments (E, F, G-J) or with 9 (B, C) or 5 (L, M) mice.

Movie S1. $KLRG1^+$ Effector $CD8^+$ T Cells Differentiate into Trm cells in the Skin. Related to Figure 2.

Mice were injected with naïve $Klrg1^{Cre/+}Rosa26^{tdTomato/+}$ UBC-GFP^+ OT-I cells followed by subcutaneous injection of soluble OVA into the foot skin 7 days p.i. with LM-OVA. $Reporter^-$ (green) and $Reporter^+$ (yellow = green + red) effector OT-I cells that were recruited to the epidermis and dermis of the foot skin, were analyzed by two-photon microscopy 20 days after the first immunization. The image starts at the epidermis and moves towards the dermis. Nuclei were stained with DAPI (blue). Data are representative of 2 independent experiments with 3-5 mice.

Movie S2. KLRG1^{+} Effector CD8^{+} T Cells Differentiate into Trm Cells Following Immunization with Soluble Antigen in the Skin. Related to Figure 2.

Mice injected with naïve *Klrg1*$^{Cre/+}$*Rosa26*$^{tdTomato/+}$ UBC-GFP^{+} OT-I cells, were injected subcutaneously with soluble OVA into the foot skin 5 days post immunization. Reporter^{-} (green) and Reporter^{+} (yellow = green + red) effector OT-I cells recruited to the epidermis of the foot skin were analyzed by two-photon microscopy 41 days after the first immunization. Data are representative of 3 independent experiments with 3-5 mice.