Supplementary Figure legends

Supplementary Figure 1. *Contraction and differentiation state of ESAT-6$_{4-17}$ specific lung CD4 T cells prior to, during and long-term after 12 weeks treatment with Isoniacid/Rifabutin (INH/RIF) of C57BL/6 mice infected with M. tuberculosis (Mtb) for 6 weeks.*

A) CFU levels in Mtb infected (~100 CFU/lung) C57BL/6 mice prior to, under and after a 12-week INH/RIF treatment period. Total lung CFU was determined by serial plating. Data show mean ± s.d. of 4-16 mice/time-point

B) The number of ESAT-64-17-specific CD4 T cells in perfused lungs was determined by Tetramer pulldown in Mtb infected and treated mice at week 0, 6, 12 and 40 after start of treatment. Symbols, mean ± s.d. of 3-4 mice per time point. The experiment was repeated twice at week 0 & 6 and once at week 12 & 40.

C) Representative flow cytometry plots depicting KLRG1, PD-1 and ICOS expression by ESAT-6 tetramer-binding CD4 T cells after magnetic enrichment in perfused lungs of Mtb infected and treated mice at week 0 to 40 after start of INH/RIF treatment.

Supplementary Figure 2. *Memory cells shortly after a cleared Mycobacterium tuberculosis infection show a higher degree of differentiation than memory cells 3-6 weeks post H56/CAF01 immunization.*

A) Quantitative data showing the expression of KLRG1 vs PD-1, KLRG1 vs ICOS and CXCR3 vs CX3CR1 on ESAT-6 tetramer-specific CD4 T cells primed by a cleared Mtb infection and after three bi-weekly H56/CAF01 vaccinations. Mtb memory show expression on ESAT-6$_{4-17}$-specific cells after tetramer pulldown on perfused lungs following clearance of an Mtb infection by a 12 weeks Isoniacid/Rifabutin treatment schedule. Tetramer pulldown was carried out 0-3 weeks post end of treatment (EoT). H56 memory show the expression on ESAT-6 tetramer binding spleen

cells 3-6 weeks after last (3rd) immunization. Bars show mean percentages of ESAT-6 tetramer +ve CD4 T cells ± s.d. expressing each combination of markers. Values from individual mice are shown by black circles.

Two-way ANOVA with Sidak's multiple comparison test. ** P < 0.01, *** P < 0.001, **** P < 0.0001.

Supplementary Figure 3. *Mycobacterium bovis Bacillus Calmette-Guérin (BCG) drives CD4 memory responses of high differentiation reminiscent of the memory responses after a cleared Mycobacterium tuberculosis infection.*

A), C) & E). CB6F1 (C57BL/6 x Balb/C) mice were infected with *M. tuberculosis* by the aerosol route for 6 weeks and then subjected to 6 weeks treatment with Isoniacid/Rifabutin (INH/RIF) to clear the infection. Ag-specific responses were analyzed after the 6 week treatment period. B), D) & F) CB6F1 (C57BL/6 x Balb/C) mice were immunized once with BCG by s.c. route and Ag-specific responses analyzed 8 weeks post immunization..

TB10.4-specific CD4 T cell responses were analyzed by MHC-II tetramer-based magnetic enrichment using PE-conjugated I-A^d:TB10.4_{70-84} tetramer.

A) Representative flow plots showing tetramer pull-down in spleen (left) and perfused lung (right) in Mtb infected and cleared (6 week chemo) CB6F1 mice using MHC-II I-A^d:TB10.4_{70-84} or irrelevant control tetramer I-A^d:hCLIP.

B) Representative flow plots showing tetramer pull-down in spleen of BCG immunized CB6F1 mice using MHC-II I-A^d:TB10.4_{70-84} or irrelevant control tetramer I-A^d:hCLIP..

C) Quantitative data showing the expression of KLRG1 and PD-1 among TB10.4_{70-84}-specific CD4 T cells in the spleen (left) and perfused lungs (right) of Mtb infected and treated CB6F1 mice. Mean ± s.d. with individual data shown in black circles. TB10.4-specific responses in both spleen and lung (~55%) are dominated by the KLRG1+PD-1- subset.(Spleen ~40%; Lung ~55%).

D) Quantitative data showing the expression of KLRG1 and PD-1 among TB10.4$_{70-84}$-specific CD4 T cells in the spleen of BCG-immunized CB6F1 mice. Mean ± s.d. with individual data shown in black circles. Similar to Mtb infected and treated mice, TB10.4-specific responses in BCG-immunized mice are dominated by the KLRG1+PD-1- subset.

E) Representative flow plots showing KLRG1 vs PD-1 expression among TB10.4$_{70-84}$-tetramer binding CD4 T cells in the spleen (left) and perfused lung (right) of Mtb infected and cleared CB6F1 mice.

F) Representative flow plots showing KLRG1 vs PD-1 expression among TB10.4$_{70-84}$-tetramer binding CD4 T cells in the spleen of BCG-immunized CB6F1 mice.

Supplementary Figure 4. Figure showing gating strategy used in Figures 4 & 5.

Supplementary Figure 5. *Gating strategy in adoptive transfer studies.* **A)** Plot showing composition of ESAT-6 Tet+ CD4 T cells after mixing donor cells from H56 and Mtb memory mice prior to adoptive transfer into Mtb infected recipients. ESAT-6 specific donor cells were transferred in a 1.7:1 ratio of H56 relative to Mtb memory cells. **B)** Gating strategy used for analysis of lung parenchymal homing of co-adoptively transferred donor cells into infected recipients.

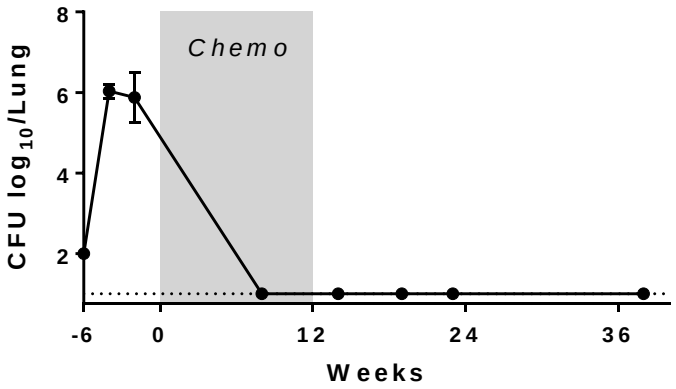
Supplementary Figure 6. *Repeat experiment – Mtb memory provides early, but transient, protection to Mycobacterium tuberculosis infection in contrast to H56 memory.*

C57BL/6 mice were rested for ~half a year prior to aerosol Mtb challenge (~100 CFU/lung). Total lung CFU was determined by serial plating at week 4, 9 and 16 in saline controls (black square), H56 memory (white circle) and Mtb memory (black circle). Data show mean ± s.d. of 8 mice/group at week 4 & 9 and 8-12 mice/group at week 16. Two way ANOVA with Dunnett's multiple comparison test against Saline group. * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$. ns non-significant.

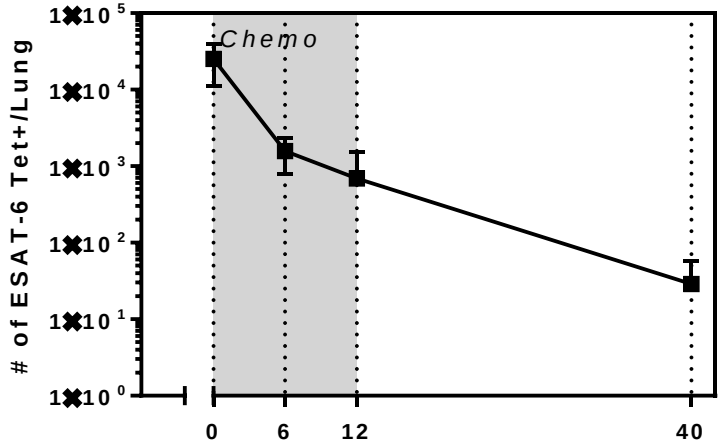
Supplementary Table 1: Table showing the relapse rate after a 12 week Isoniacid/Rifabutin (INH/RIF) treatment started 6 weeks into an Mtb infection. Last column show the number of culture positive lungs (detection limit <10 CFU/lungs) out of lungs examined during treatment and after end of treatment (EoT) – including an almost 1½ year follow up rest period after EoT. Culture positive mice started emerging 11 weeks after end of treatment, with a relapse rate of 12.5%. NB – none of the mice included in the phenotypic characterization (Figure 1 & Supplementary figure 1) were found culture positive as assured by control plating lung homogenates of left lung lobes from all mice included.

Suppl Figure 1

a)



b)



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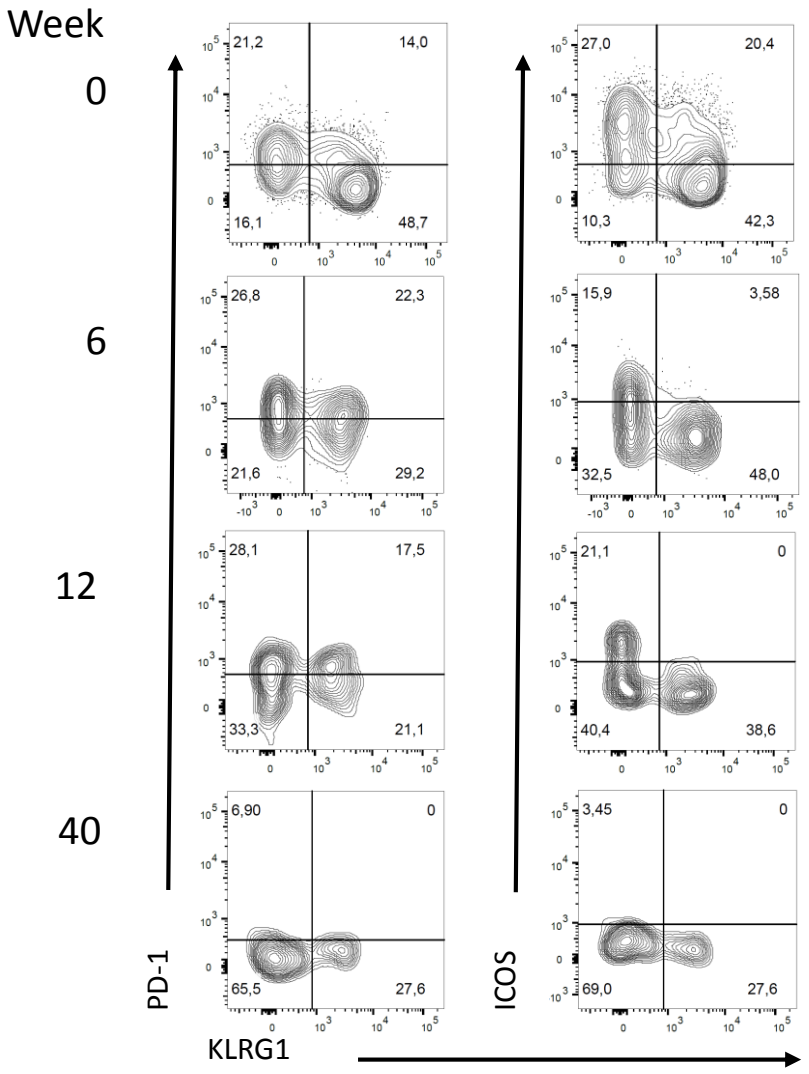
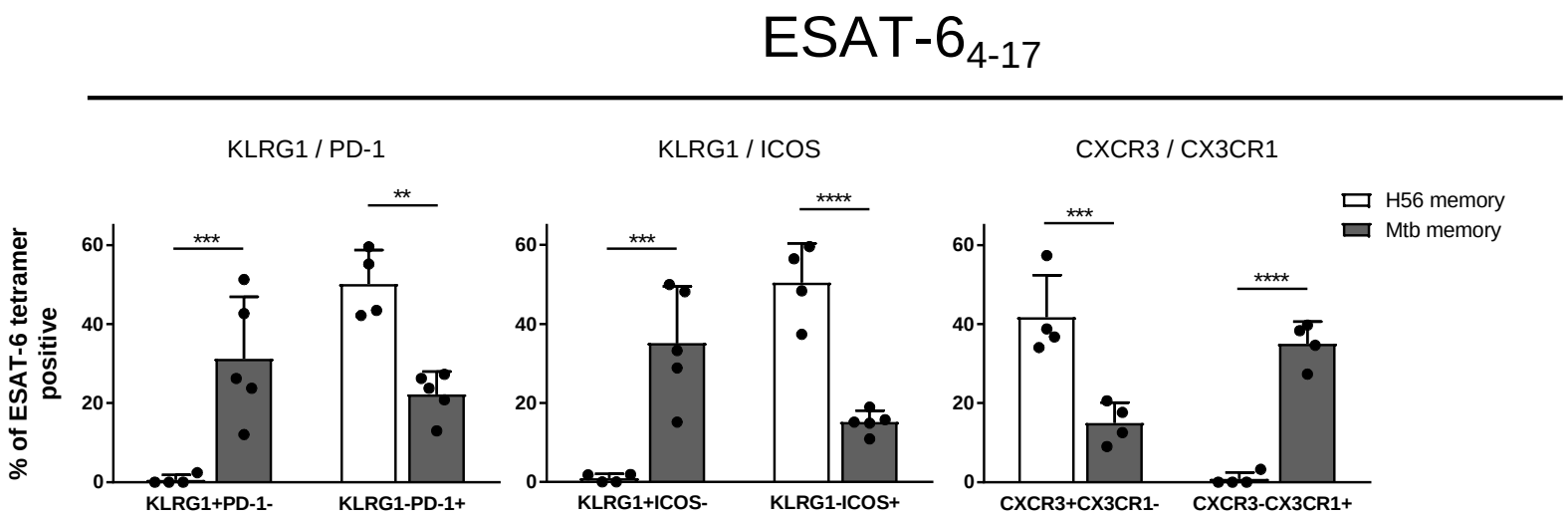


Suppl Figure 2

a)

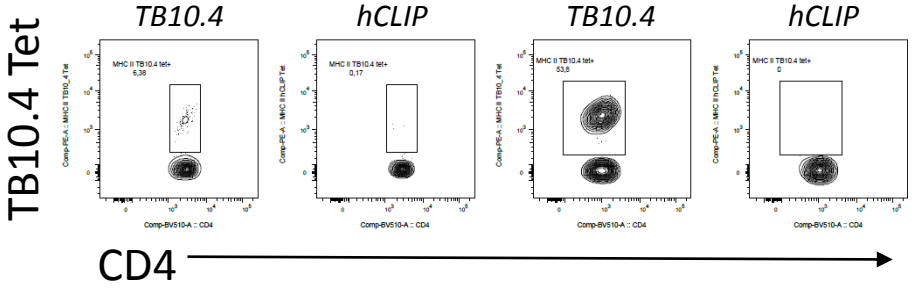


Suppl Figure 3

Mtb (6 wks inf > 6 wks chemo)

BCG (8 wks post-imm)

a)



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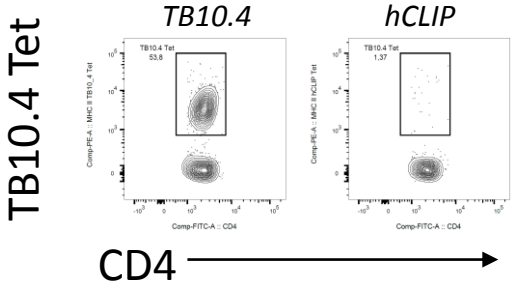

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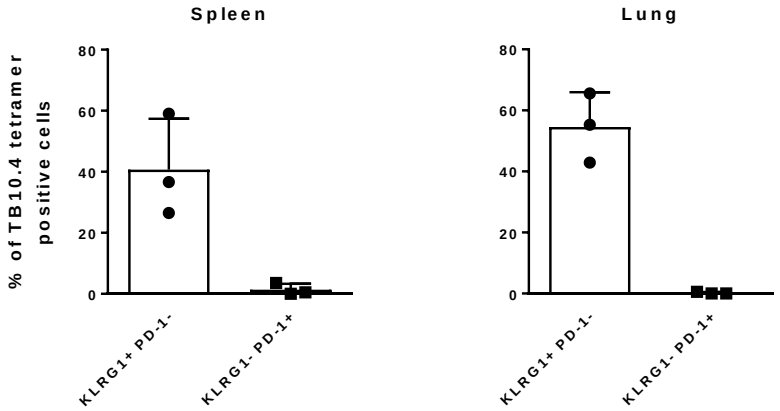

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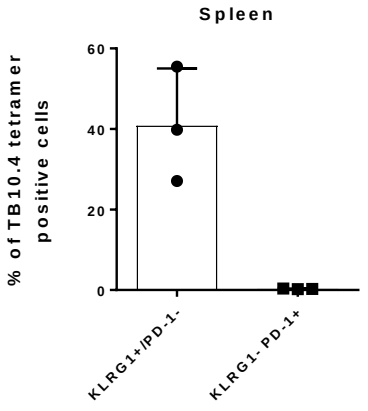

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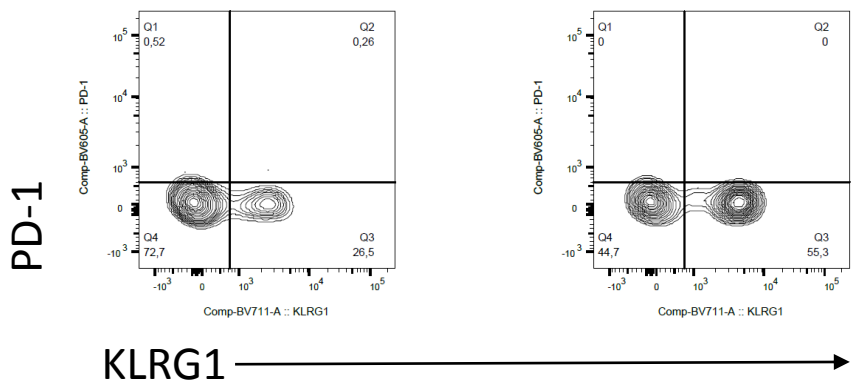

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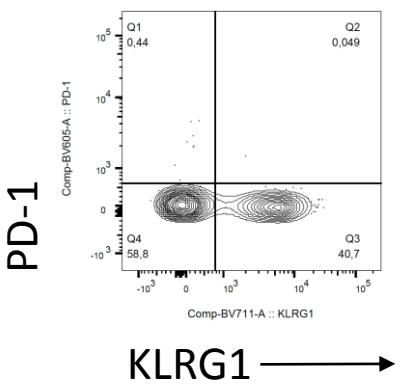
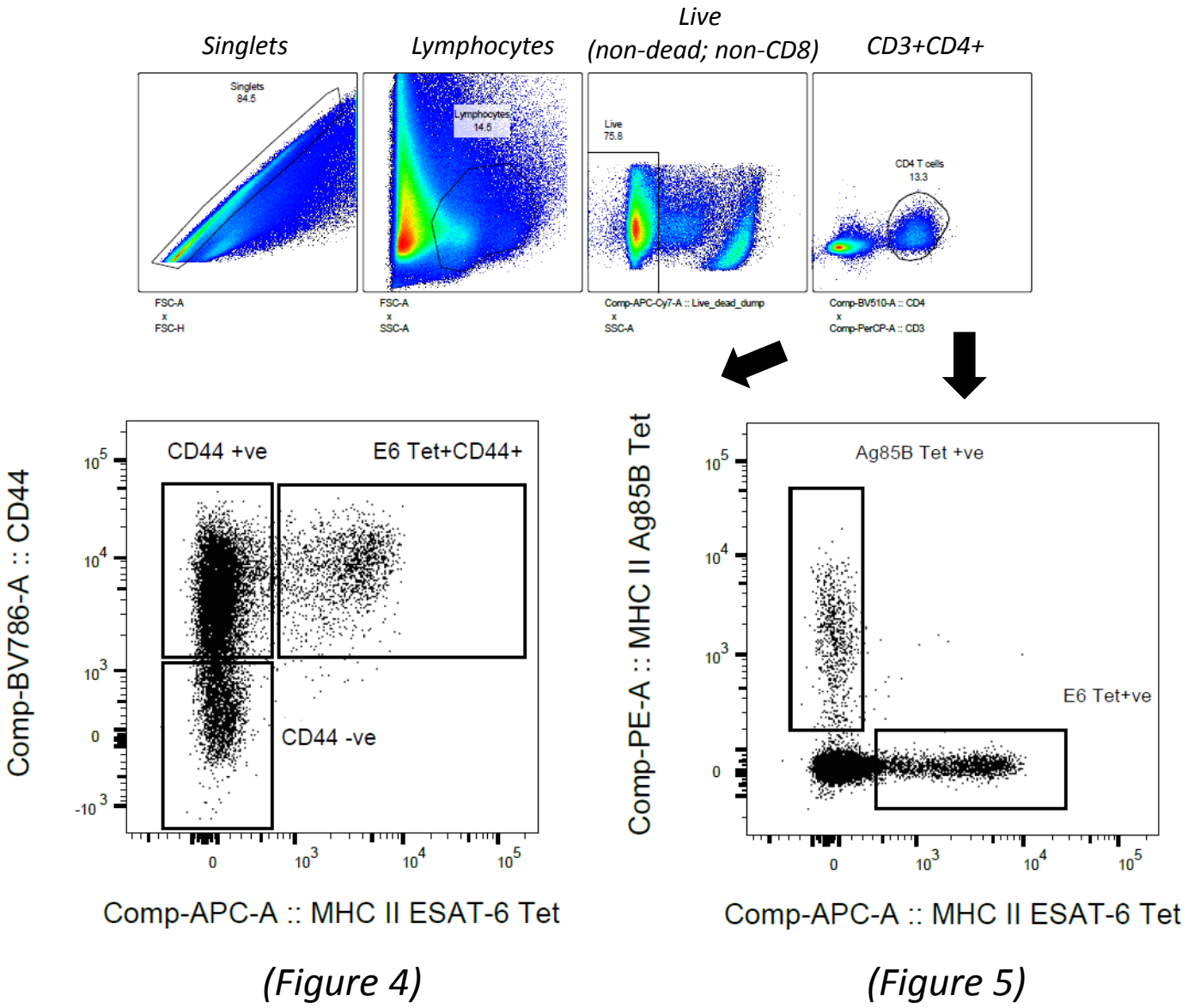


Suppl Figure 4



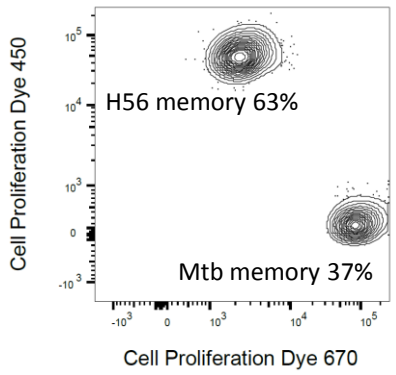
(Figure 4)

(Figure 5)

Suppl Figure 5

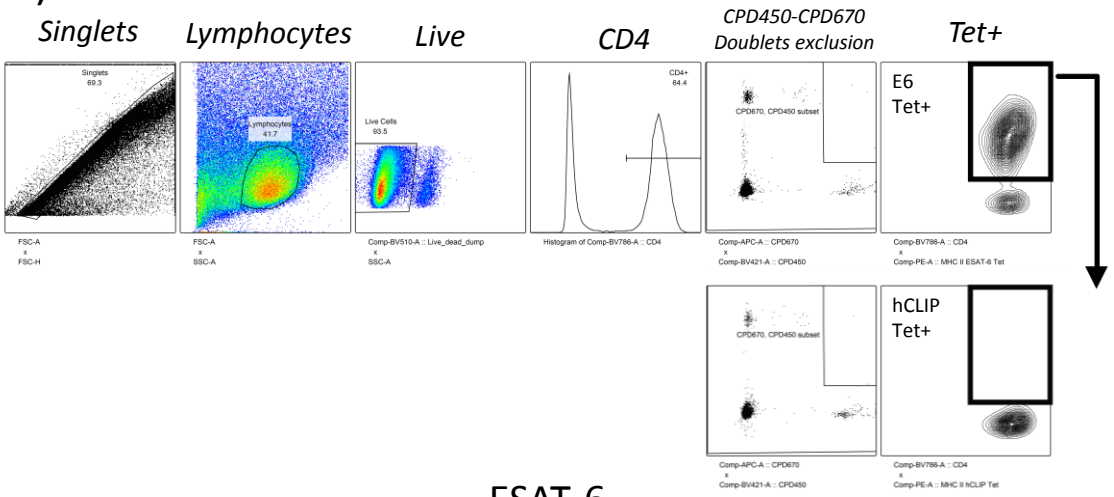
a)

Donor cells
Live E6 Tet+ (Pulldown)
(Singlets > Lymphocytes > Live CD4s > E6 Tet +)

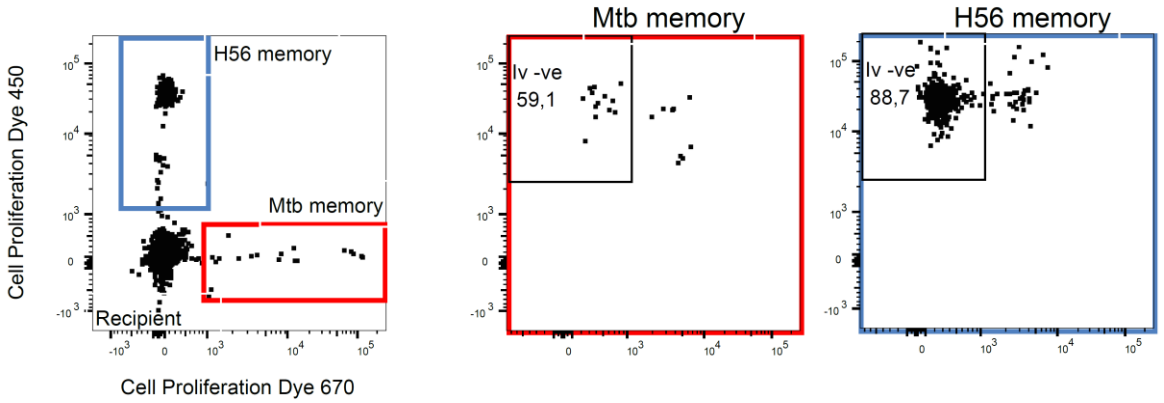


b)

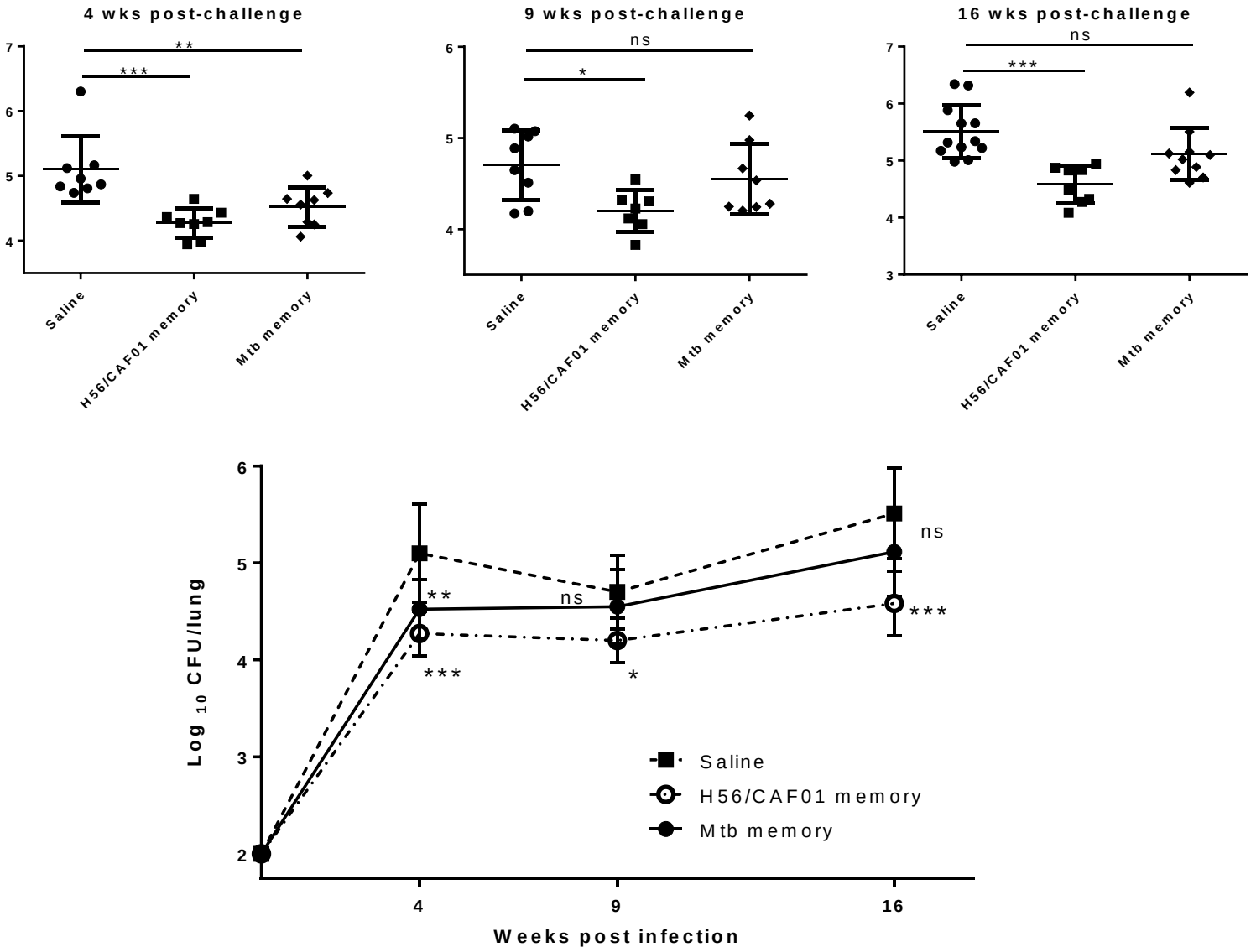
Recipient mice – Gating startegy
E6 vs hCLIP Tet+ (Pulldown)



ESAT-6_{4-17}



Suppl Figure 6



Table

Relapse rate after EoT

(12 weeks INH/RIF treatment started 6 weeks into Mtb infection)

Weeks after start of 12 weeks INH/RIF treatment	Weeks after EoT (End of Treatment – 12 weeks)	# of Culture positive
6		0/8
8		0/14
12	0	0/4
14	2	0/4
19	7	0/16
23	11	2/16
41	29	2/8
84	72	3/18