# Supplemental information

A recombinant bovine adenoviral mucosal vaccine

expressing mycobacterial antigen-85B generates

robust protection against tuberculosis in mice

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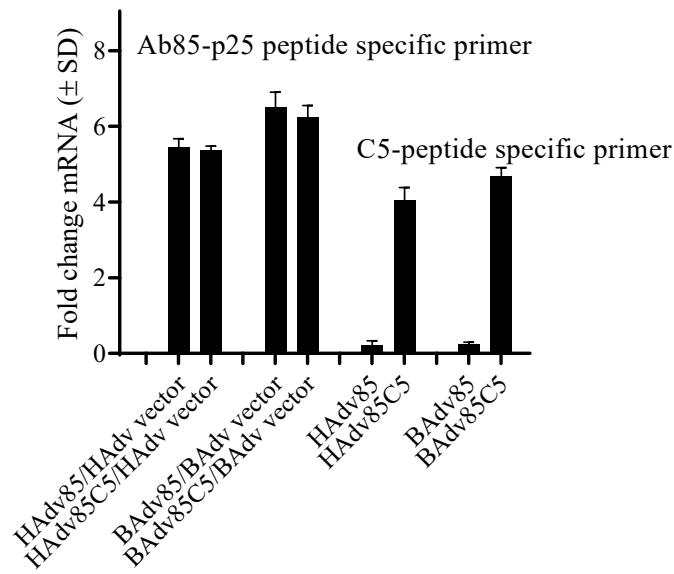
Supplemental Figure- 1a: qPCR validation of Antigen 85B derived p25 and CFP-10 derived C5 related to Fig.1. Primers shown in *Supplemental Fig.8.*
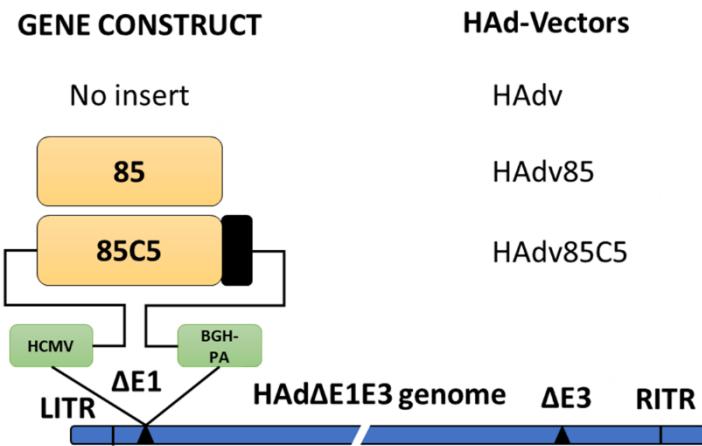
**Supplemental Figure- 1b:** Gene cassette of HAdv constructs related to Fig.1.

Supplemental Figure- 2: DEGs of HAdv85C5 in wt-DCs vs. BAdv85C5 related to Fig.2.
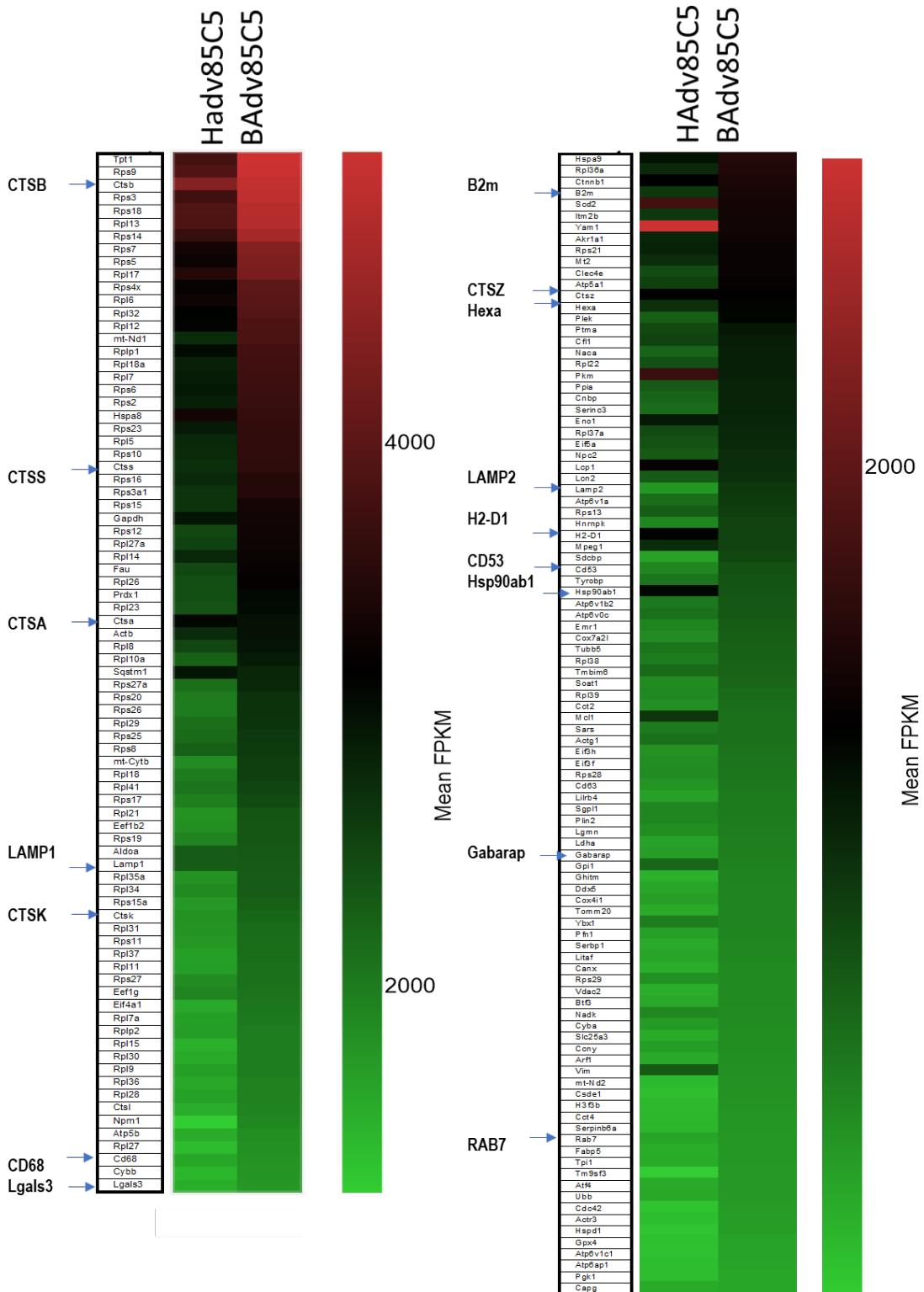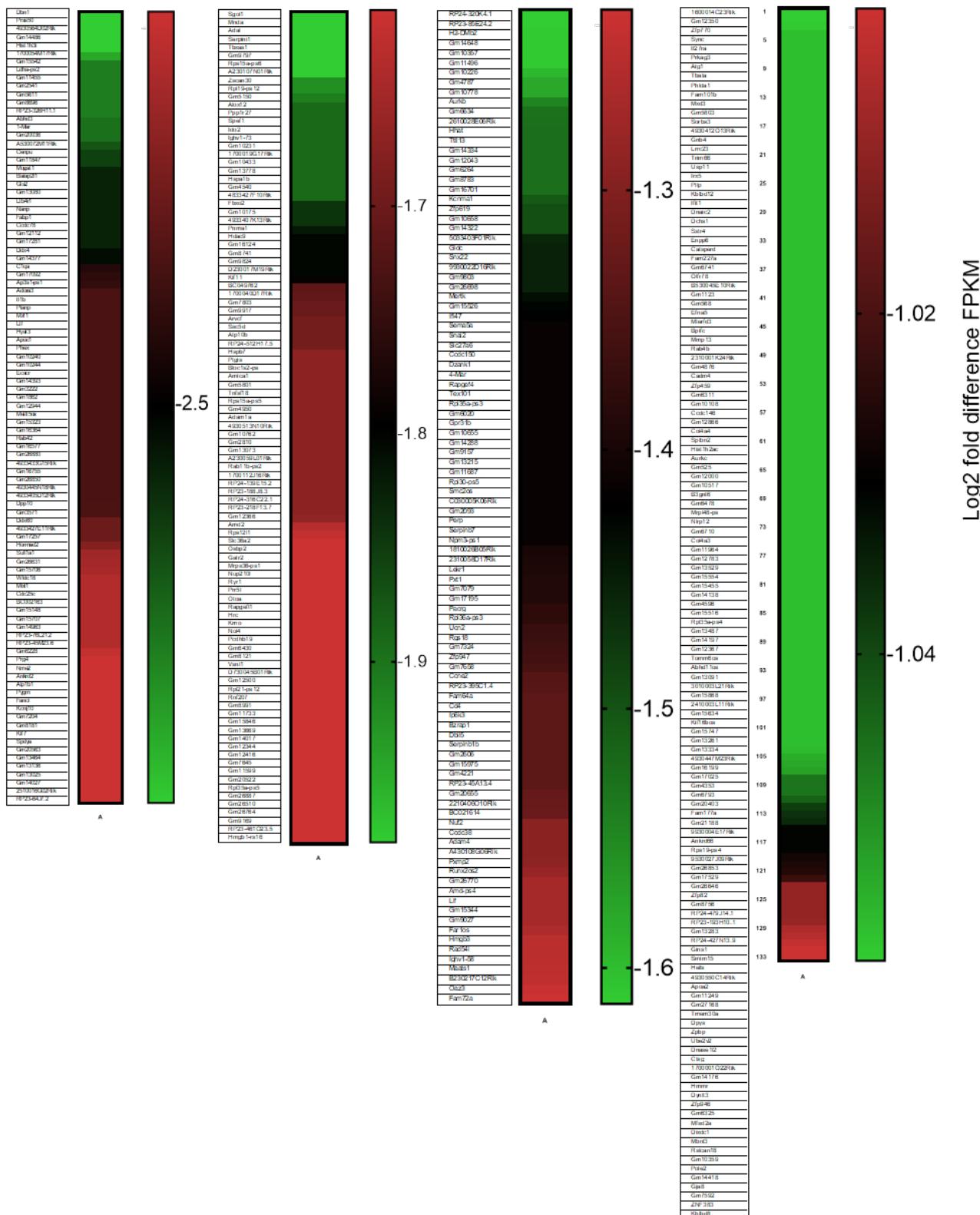
Supplemental Figure- 3: DEGs of BAdv[85C5] in wt-DCs vs. BAdv[85C5] in *ATG7*KODCs, related to Fig.2.
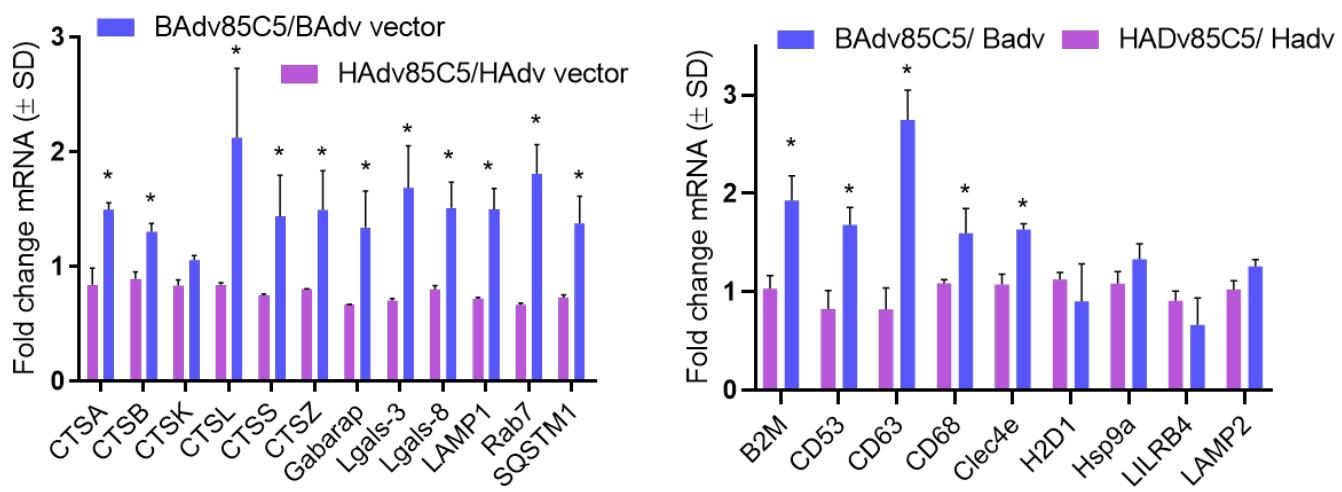
Supplemental Figure- 4: qPCR validation of genes in BAdv[85C5] infected DCs vs. HAdv[85C5] infected DCs, related to Figs. 2.
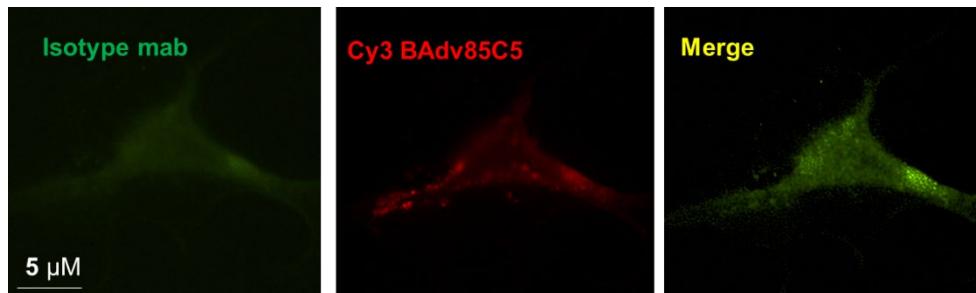

Supplemental Figure- 5: Isotype controls related to Fig.3.
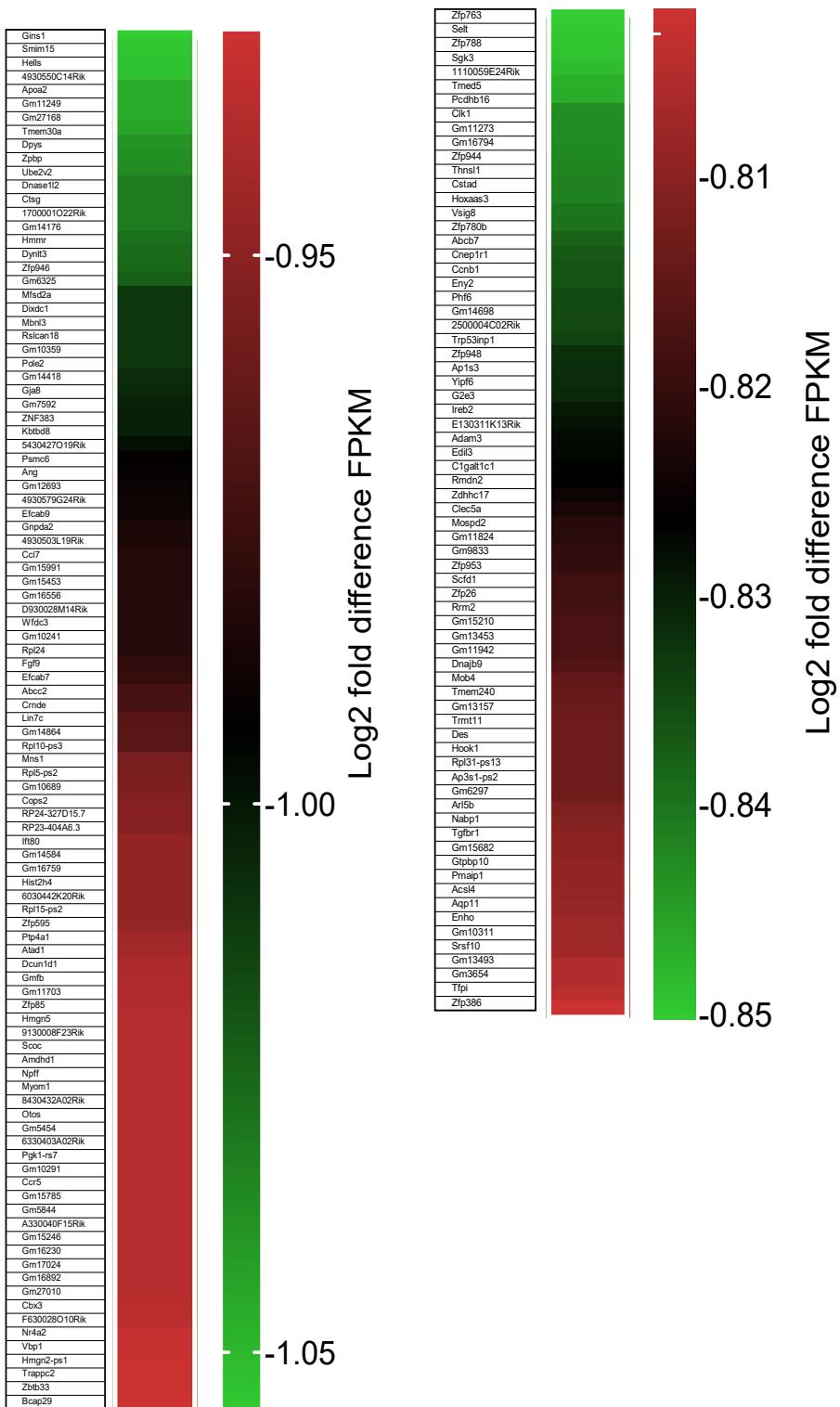
**Supplemental Figure- 6:** DEGs of BAdv[85C5] in C57Bl/6 derived wt-macrophages compared to HAdv[85C5], related to Fig.2.
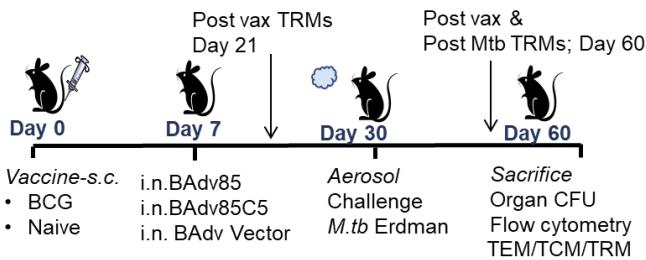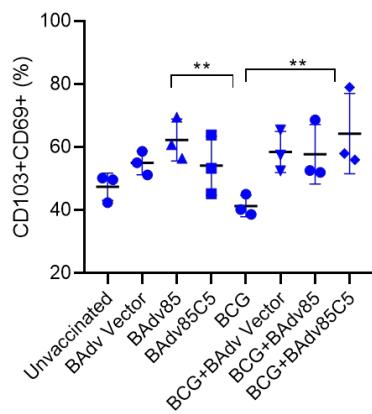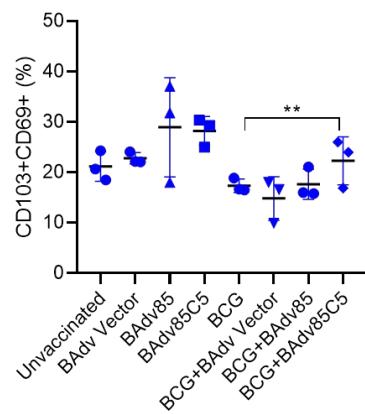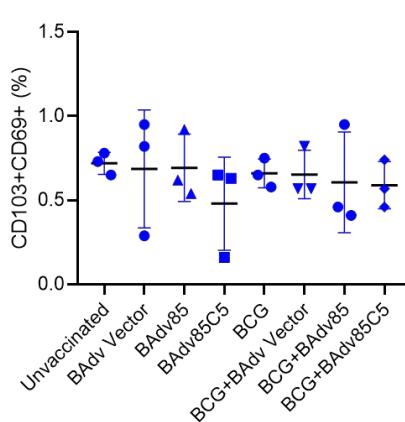
Supplemental Figure- 7: T$_{RMs}$ (CD4$^+$CD103$^+$CD69$^+$) on day 21 for post vaccination and day 60 post Mtb challenge, related to *Fig.7.*

GENE	Forward primer 5'->3'	Reverse primer 5'->3'
Ag85b	GATATCGGATCCGCCACC	GCGGCCGCGATATC
Ag85bC5	GATATCGGATCCGCCACC	GCGGCCGCGATATC
β-Actin	CATTGCTGACAGGATGCAGAAGG	TGCTGGAAGGTGGACAGTGAGG
CTSA	TGTTCGGAAGGCTCTCCACATC	CACATCTCCGTTGTAGAGCAGG
CTSB	AGTCAACGTGGAGGTGTCTGCT	GTAGACTCCACCTGAAACCAGG
CTSK	AGCAGAACGGAGGCATTGACTC	CCCTCTGCATTTAGCTGCCTTTG
CTSL	GGAAAATGGAGGTCTGGACTCG	GTGTCATTAGCCACAGCGAACTC
CTSS	GCATAGAGGCAGACGCTTCCTA	CCACTGCTTCTTTCAGGGCATC
CTSZ	GTGTCAGCAACGATGGCATCGA	CCTTGTAGGTGCTGGTCACGAT
GABARAP	CAAAGAGGAGCATCCGTTCGAG	TTGTCCAGGTCTCCTATCCGAG
GAPDH	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG
LGALS3	AACACGAAGCAGGACAATAACTGG	GCAGTAGGTGAGCATCGTTGAC
LGALS8	GAGGAGATCACCTACGACATGC	CGTACAGCAGAACATGCCTTCC
RAB7	GAGCGGACTTTCTGACCAAGGA	CAATCTGCACCTCTGTAGAAGGC
LAMP1	CCAGGCTTTCAAGGTGGACAGT	GGTAGGCAATGAGGACGATGAG
Sqstm1	GCTCTTCGGAAGTCAGCAAACC	GCAGTTTCCCGACTCCATCTGT

Fold change is calculated by using $2^{-\Delta\Delta Ct}$ method

Briefly,

ΔCt = Ct (gene of interest) – Ct (housekeeping gene)

$\Delta\Delta Ct$ = ΔCt (treated sample) – ΔCt (untreated sample)

Fold change = $2^{-\Delta\Delta Ct}$

Supplemental Figure- 8: qPCR primers used in this study; related to *Figs.5 and 6.*