SUPPLEMENTARY MATERIAL

Supplemental Figure S1. Induction of epitope-specific T cells by sorted adult CD103+ DCs. A) Gating of CD103+ DCs and CD11b+ DCs in the MLN of adult and neonatal mice. Live singlets were gated prior to gating on the CD11c+, Class II^{hi} DC population. This population was further broken down into CD103+ DCs and CD11b+ DCs. B) An example of raw data obtained from an *in vitro* coculture experiment showing the induction of proliferation of $K^dM2_{82\text{-}90}$ and $D^bM_{187\text{-}195}$-specific cells by FACS-sorted CD103+ DCs sorted from the MLN of adult mice on the indicated days post-infection. Mice were infected on different days in order to harvest MLN from all time points post-infection and perform the coculture on one day with the same reporter T cell populations.

Supplemental Figure S2. Frequency and number of CD8+ T cells in RSV-infected adult and neonatal wild-type and Batf3-/- mice. CD8+ T cell frequency and numbers in the lung and MLN 7 days post-infection (A-D) and the frequency and number of CD8+ T cells specific for $D^bM_{187\text{-}195}$ and $K^dM2_{82\text{-}90}$ specific cells in the MLN of wild-type and Batf3-deficient adults and neonates 7 days post-RSV infection (E-H). Data are representative of 3 independent experiments with 5-8 mice/group. P values indicated are from a t-test between wild-type and Batf3-/- mice of the same age.

Supplemental Figure S3. Higher $K^dM2_{82\text{-}90}$-specific responses in the lungs of Batf3-/- deficient neonates are due to the lack of competition from the $D^bM_{187\text{-}195}$-specific response. Wild-type and Batf3-deficient neonatal mice were infected with RSV-N191S, an RSV virus that does not

stimulate a response to the $D^bM_{187-195}$ epitope due to a mutation in the P5 anchor residue. The frequency and number of K^dM2_{82-90}-specific cells were measured by tetramer staining in the lung and MLN 7 days post-infection. Results shown are combined data from two litters of wild-type and two litters of Batf3-/- deficient neonates.

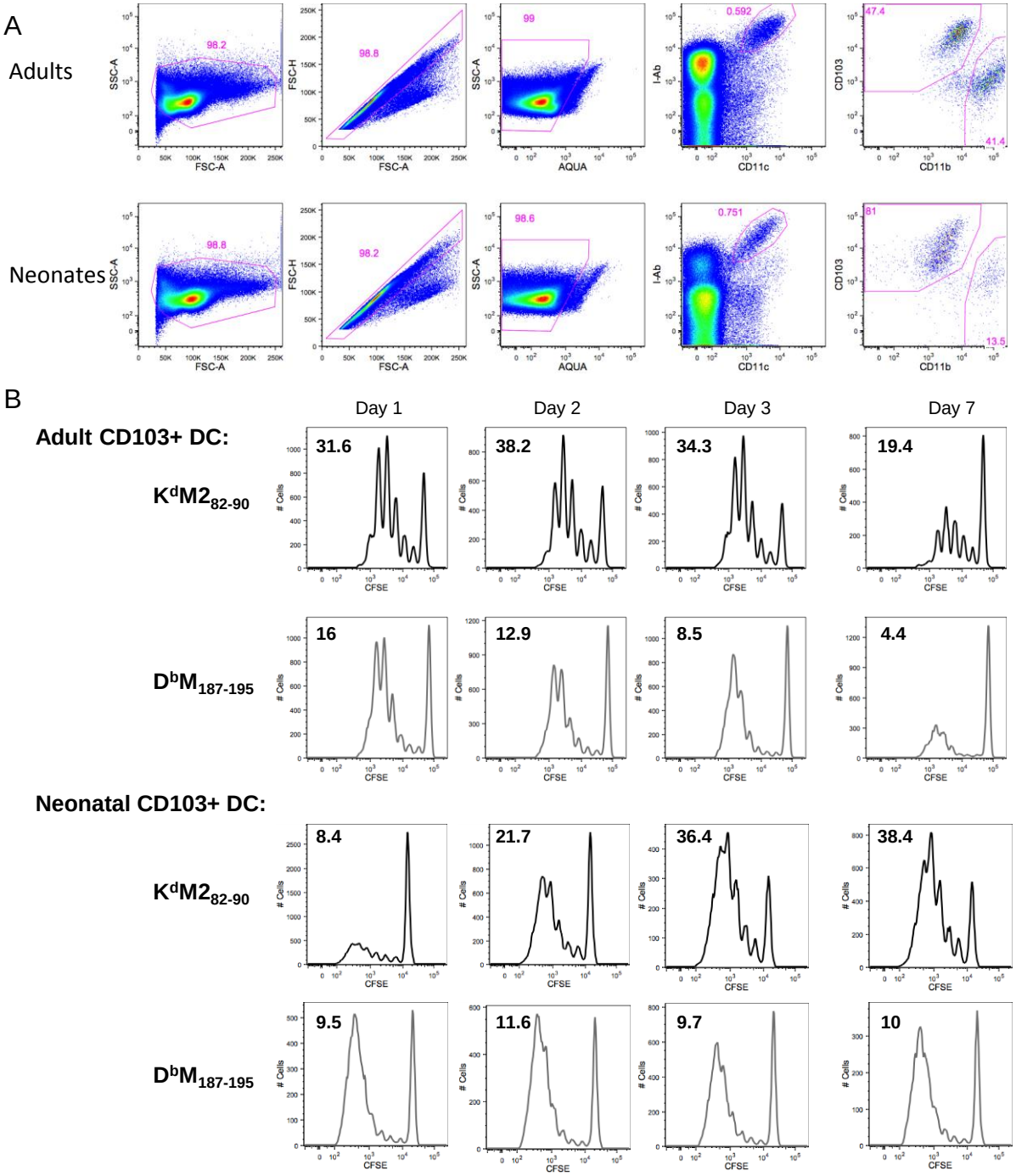
Supplemental Figure S4. Influenza/PR8-infected neonatal mice possess two populations within the CD103+ DC subset. Seven-day-old mice were infected intranasally with 600 $TCID_{50}$ of influenza/PR8. MLN were harvested from naïve mice, and mice at days 1-3 post-infection for surface staining of lung-migratory dendritic cell populations. The sample shown is representative of several pools of MLN from neonatal mice infected with influenza/PR8.

Supplemental Figure S5. Phenotypic comparison of neonatal CD11b+ DCs and adult CD11b+ DCs in the MLN of mice two days post-infection. A) Scatter characteristics and comparison of expression of lineage-defining markers between neonatal and adult CD11b+ DCs. B) Background (FMO)-subtracted median fluorescence intensity (MFI) is presented for CD80, CD86, CD24, CD205, and the MHC Class I molecules K^d and D^b on neonatal and adult CD11b+ DCs. Data are representative of two independent experiments with 3-4 mice/group. * indicates $p<0.05$, *** indicates $p<0.001$.

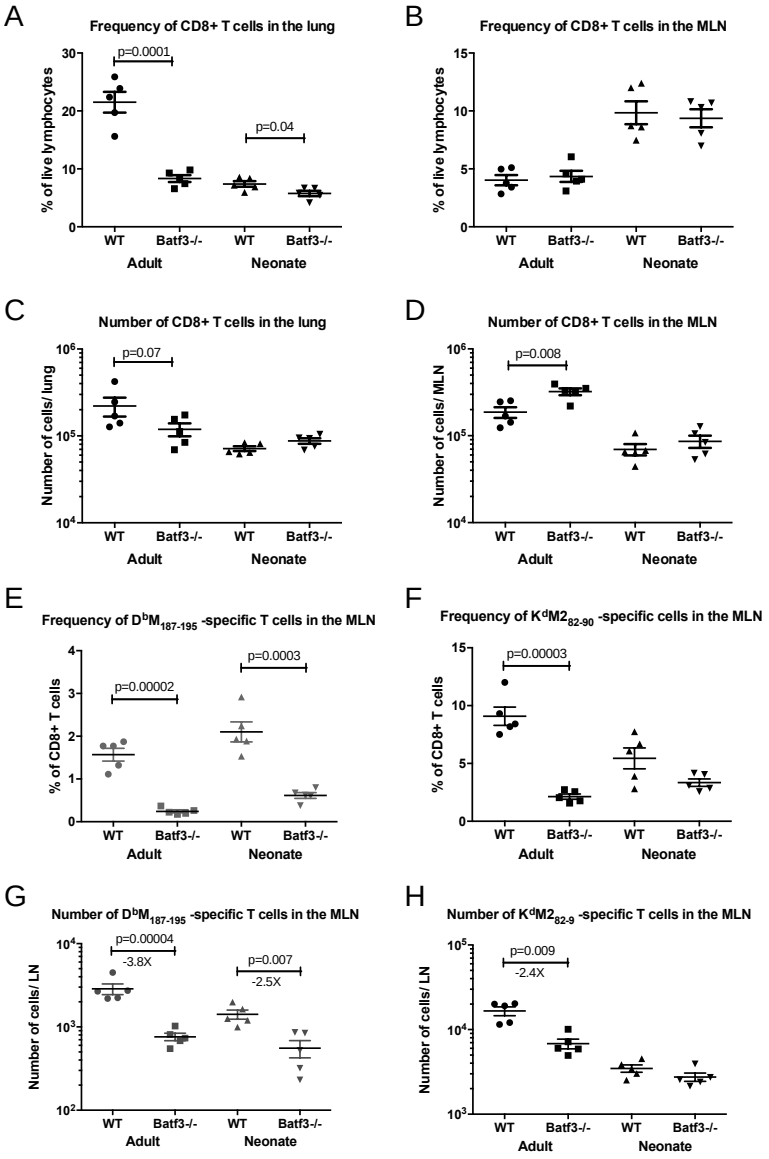
Supplemental Figure S6. Neonatal CD103lo DCs are capable of fully presenting exogenously delivered $M2_{82-90}$ peptide. CD103lo and CD103hi dendritic cells sorted from neonates 2 days post-infection were pulsed with 10^{-6}M or 10^{-8}M of $M2_{82-90}$ (SYIGSINNI) peptide for one hour

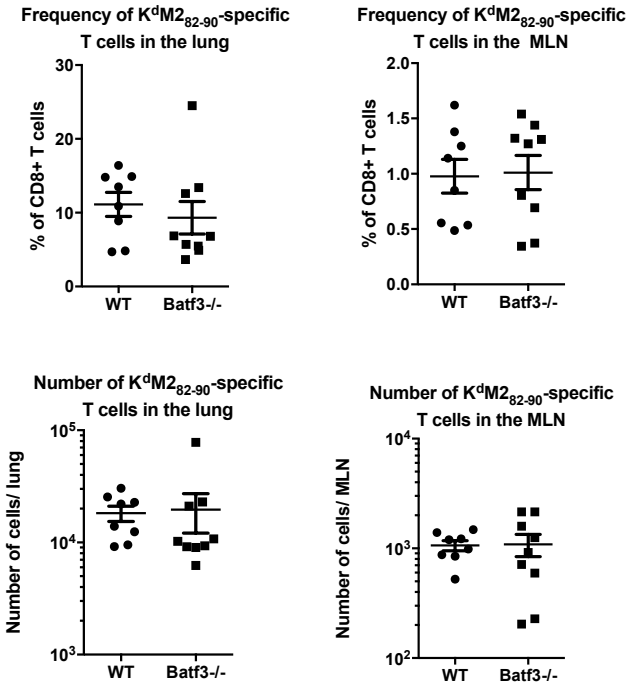
prior to washing and co-culturing with CFSE-labeled K^dM2_{82-90}-specific CD8+ T cells. The percent of transgenic cells induced to proliferate after three days in culture was calculated using Flowjo software.

Supplemental Figure S1

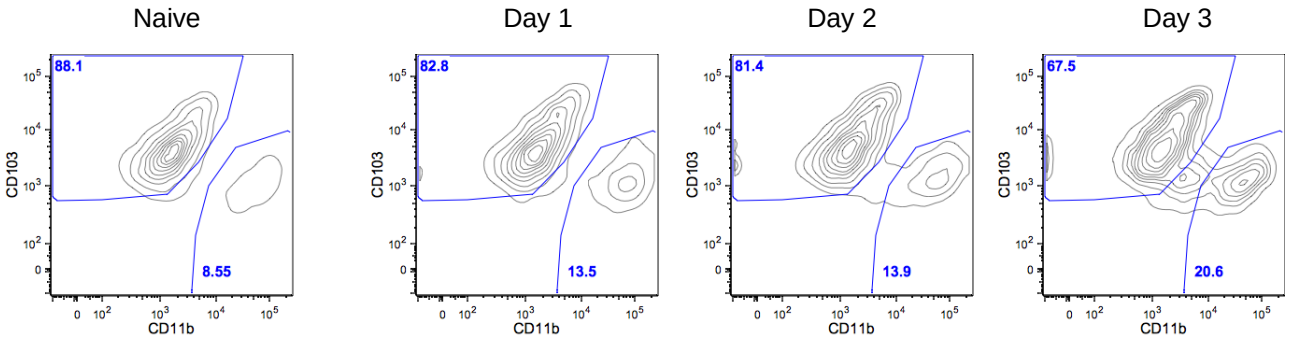


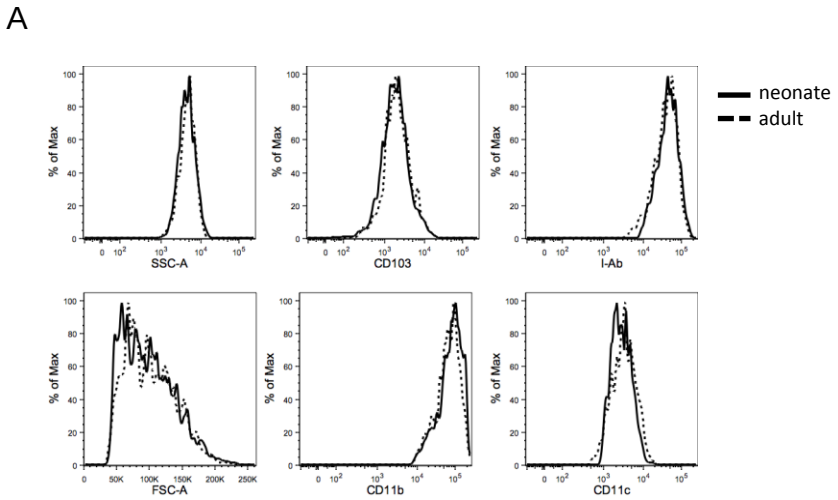
Supplemental Figure S2

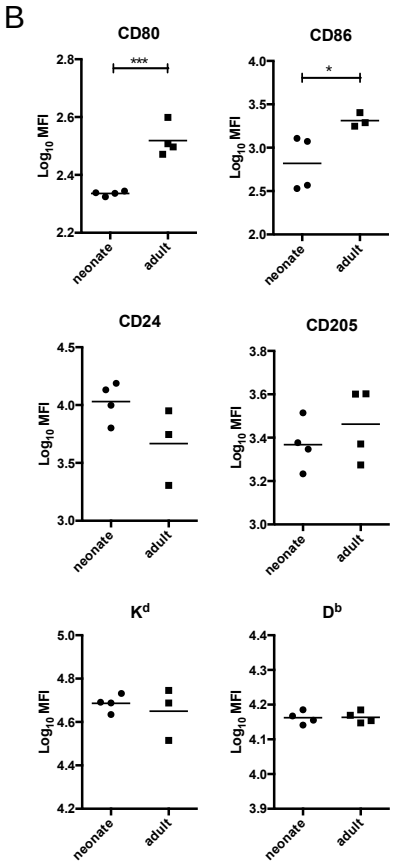



Frequency of KdM2 82-90 -specific T cells in the lung
% of CD8+ T cells
30
20
10
0
WT
Batf3-/-
Frequency of KdM2 82-90 -specific T cells in the MLN
% of CD8+ T cells
2.0
1.5
1.0
0.5
0.0
WT
Batf3-/-
Number of KdM2 82-90 -specific T cells in the lung
Number of cells/ lung
10^5
10^4
10^3
WT
Batf3-/-
Number of KdM2 82-90 -specific T cells in the MLN
Number of cells/ MLN
10^4
10^3
10^2
WT
Batf3-/-

Supplemental Figure S4




A
neonate
adult
% of Max
SSC-A
CD103
I-Ab
FSC-A
CD11b
CD11c


B
CD80
***
CD86
*
CD24
CD205
Kd
Db
Log10 MFI
neonate
adult

Supplemental Figure S6