Supplementary information

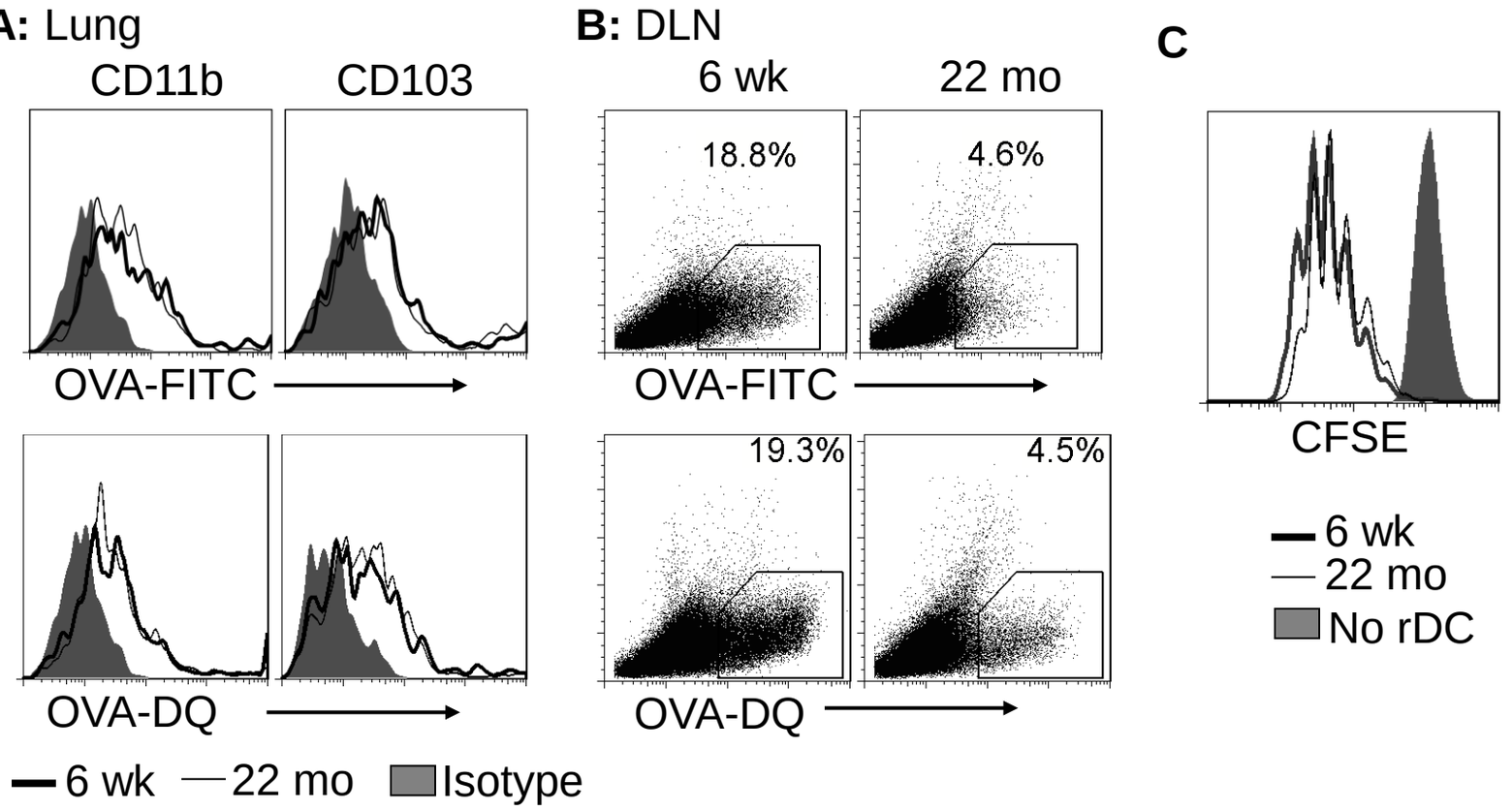
Supplemental Methods

Measurement of anti-SARS-CoV antibody ELISA titers. Whole blood and BALF were collected from SAR-CoV-infected mice at 6 days p.i. and sera were prepared. ELISA titers were measured as follows. 96-well Maxisorp Immuno Plates (Nunc) were coated with 2×10^5 PFU of formaldehyde and UV-inactivated SARS-CoV (BEI Resources). After washing, wells were exposed for 1.5 hours to serially diluted sera or BALF from naïve or infected mice. Wells were washed and then treated sequentially with 1:2000 dilution of HRP-conjugated goat anti-mouse IgG/IgM/IgA secondary antibody (Pierce Biotechnology) for 1 hour at 37 °C and tetramethylbenzidine (K-Blue MAX TMB substrate, Neogen Corporation) for 10 minutes at room temperature. Reactions were stopped with 1.5 M H_2SO_4, and absorbance was read at 450 nm. The total anti-SARS-CoV IgG/IgM/IgA ELISA titer was defined as the highest dilution of the test sample giving a twofold increase over the naïve mice samples.

Measurement of hemagglutination inhibition (HAI) titers in sera and BALF. Whole blood and BALF were collected from IAV-infected mice at 21 days p.i. and sera were prepared. To measure HAI titers, serum and BALF samples were serially diluted two-fold from 1:5 to 1:320, mixed with 4 HA units of IAV for 30 minutes at room temperature, and then incubated with a 1% suspension of chicken red blood cells for 45 minutes at room temperature to visualize the reactions.

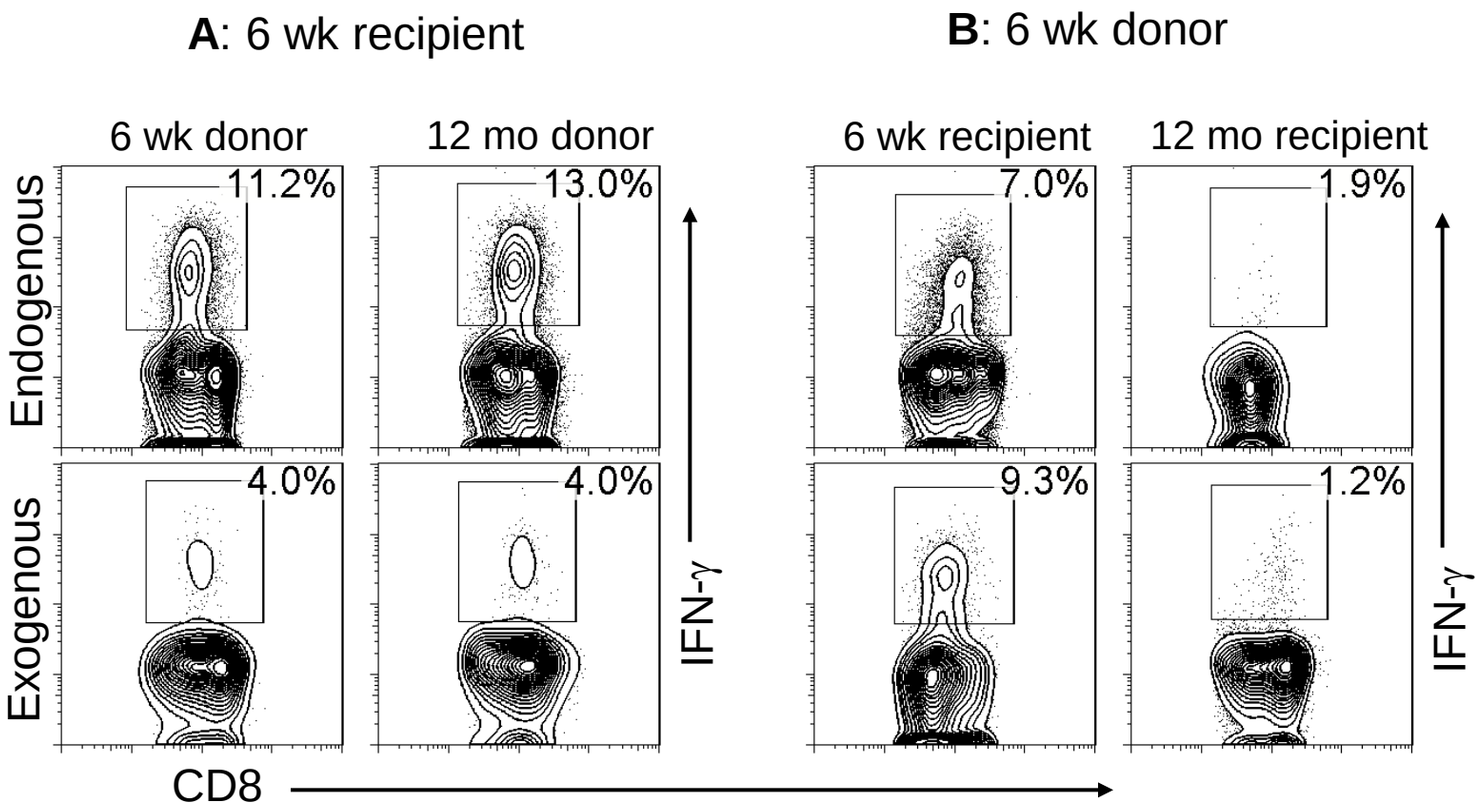
Supplemental Figures

Supplemental Figure 1



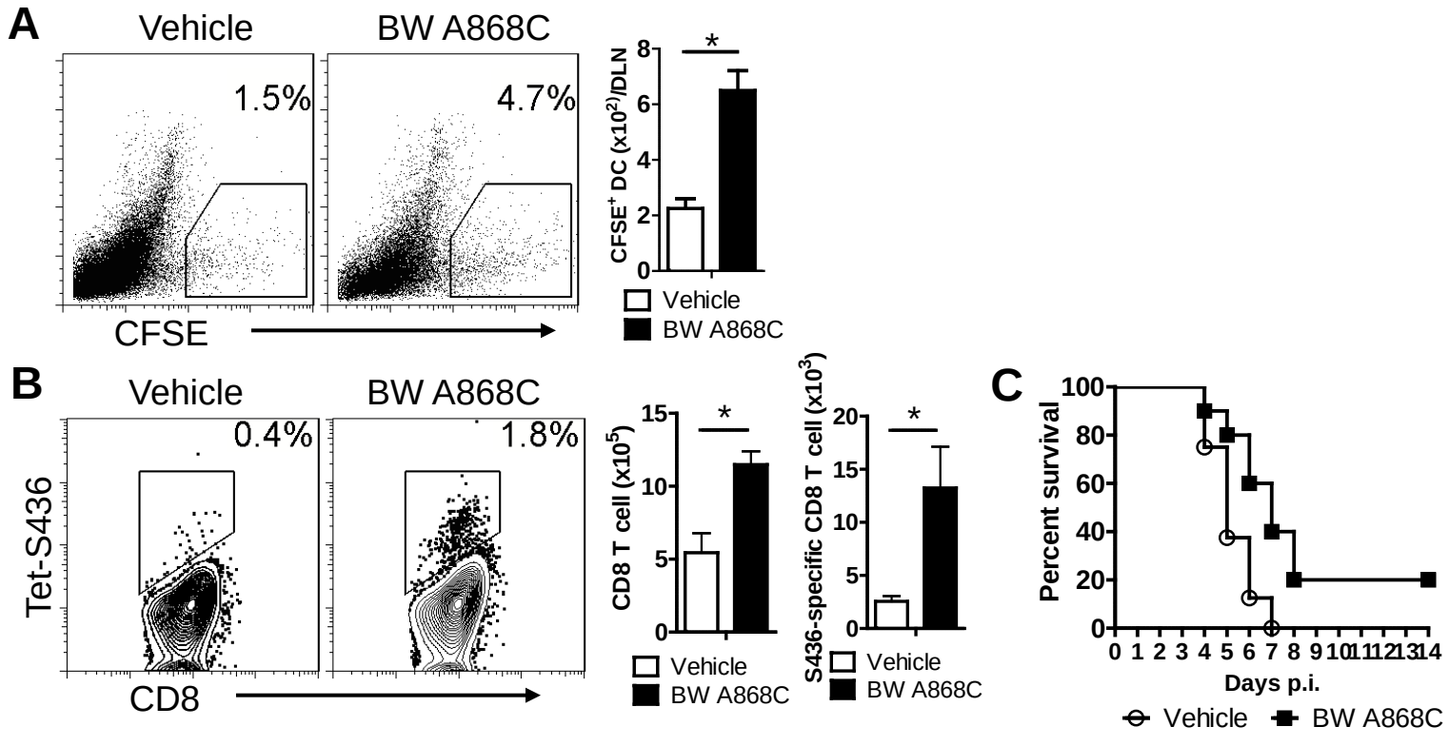
Supplemental Figure 1. Similar antigen uptake, processing and presentation by rDCs from young and aged mice. (**A**) 6-week-old and 22-month-old mice were treated with 10 μg/75 μl OVA-FITC or OVA-DQ i.n. After 1h, lungs were harvested and single cell suspensions prepared. The fluorescence intensities of OVA-FITC or OVA-DQ associated with $CD11c^+MHCII^+CD11b^+$ and $CD11c^+MHCII^+CD103^+$ rDCs are shown. (**B**) 6-week-old and 22-month-old mice were inoculated with 200 μg/75 μl OVA-FITC or OVA-DQ i.n.. After 18 hours, single cell suspensions were prepared from lung DLNs. The percentage of $OVA\text{-}FITC^+$ or $OVA\text{-}DQ^+$ cells within the $CD11c^+MHCII^+$ DC population is shown. 5-6 mice/group/experiment; data are representative of 3 independent experiments. (**C**) $OVA\text{-}FITC^+$ migratory rDCs were sorted from lung DLNs of 6-week-old and 22-month-old mice using a FACSDiva 18 hours after i.n. OVA-FITC treatment. OT-I CD8 T cells were negatively enriched from the spleens of naïve OT-I Tg mice and stained with 2.5 μM CFSE. 1×10^4 $OVA\text{-}FITC^+$ rDCs and 1×10^5 OT-I CD8 T cells were cultured in a volume of 200 μl in round-bottomed 96-well plates (Corning) at 37 ^oC for 4 days in complete RPMI 1640 media. CD8 T cells were analyzed for CFSE dilution by flow cytometry. Data are representative of 3 independent experiments.

Supplemental Figure 2



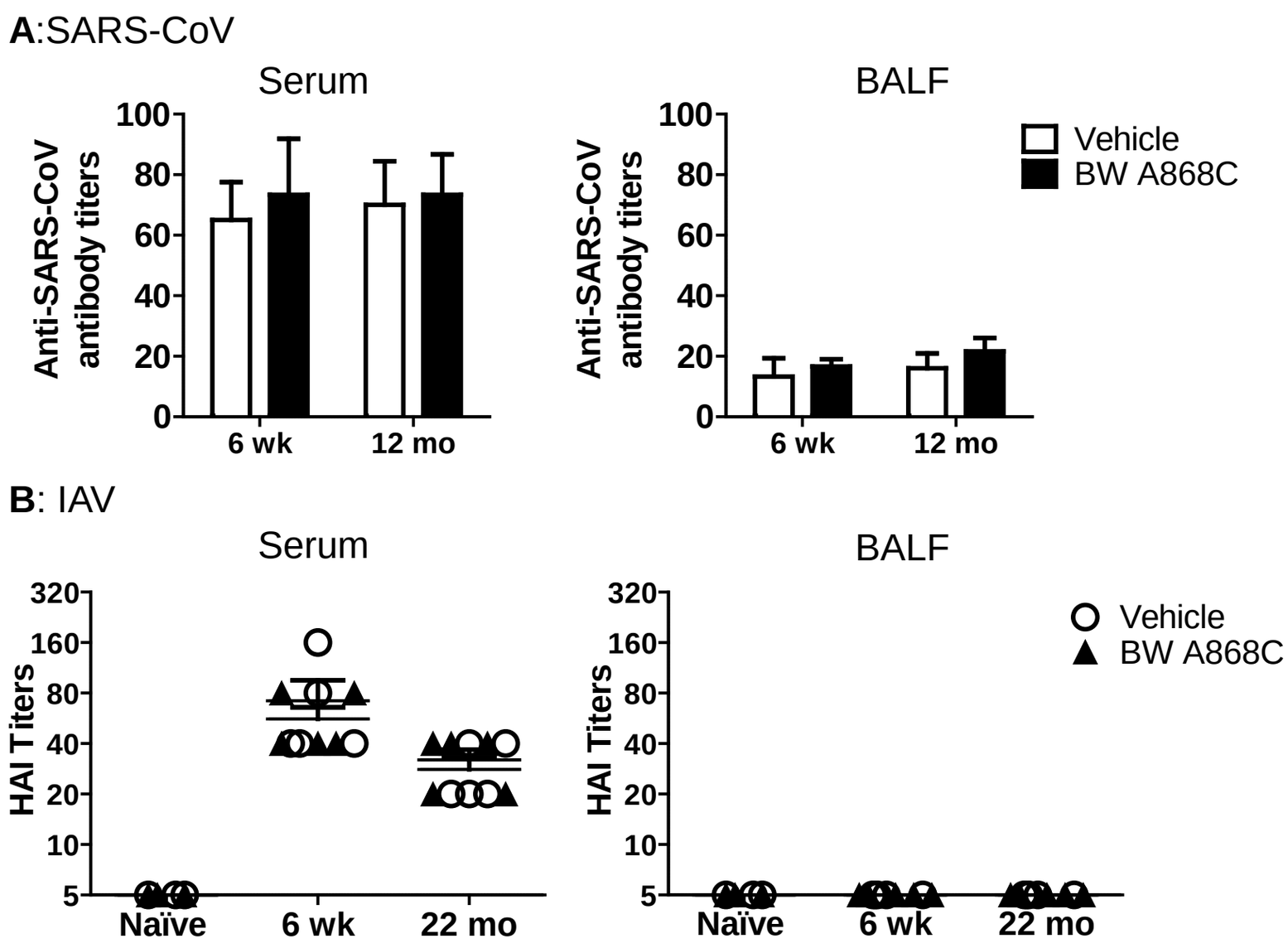
Supplemental Figure 2. Defective SARS-CoV-specific CD8 T cell response in aged mice is not T cell-intrinsic. (**A**) 5×10^7 splenocytes from 6-week-old or 12-month-old (B6-Thy1.2) donor mice were adoptively transferred into 6-week-old B6 (B6-Thy1.1) recipients prior to SARS-CoV infection. (**B**) 5×10^7 splenocytes from 6-week-old (B6-Thy1.1) mice were adoptively transferred into 6-week-old or 12-month-old (B6-Thy1.2) recipient mice prior to SARS-CoV infection. Flow cytometric analysis of endogenous and exogenous epitope S436-specific $CD8^+$ T cell responses assessed by intracellular IFN-γ staining at day 6 p.i. is shown. Numbers represent the percentage of IFN-γ^+ CD8 T cells. 4 mice/group/experiment. Data are representative of 2 independent experiments.

Supplemental Figure 3



Supplemental Figure 3. Treatment with PGD_2 antagonist BW A868C enhances rDC migration and T cell responses in SARS-CoV-infected 22 month old mice. (**A**) 22-month-old mice were i.n. inoculated with 50 μl 8 mM CFSE. Six hours after instillation, mice were infected with SARS-CoV together with BW A868C or vehicle. After 18 hours, single cell suspensions were prepared from lung DLNs. The numbers represent the percentage of $CFSE^+$ cells within the $CD11c^+MHCII^+$ DC population per LN. Total $CFSE^+$ DC numbers per LN are also shown. (**B**) Lung cells were harvested from and 22-month-old B6 mice 6 days after SARS-CoV infection. Tetramer staining for epitope S436, and total numbers of CD8 T cells and tetramer $S436^+$ CD8 T cells are shown. Numbers represent the percentage of $tetramer^+$ CD8 T cells. n=3-4 mice/group/experiment. Data are representative of 3 independent experiments. (**C**) 22-month-old mice were i.n. infected with 1×10^4 PFU MA15 virus. Mortality was monitored daily. n= 8 mice in vehicle group; 10 mice in BW A868C group. *P* value, determined by a Kaplan-Meier survival test, is: $P = 0.017$.

Supplemental Figure 4



Supplemental Figure 4. Treatment with PGD_2 antagonist BW A868C does not enhance antibody responses in SARS-CoV and IAV-infected old mice. (**A**) Sera and BALF from SARS-CoV-infected mice at 6 days p.i. were analyzed for total IgG/IgM/IgA by ELISA as described in Supplemental Materials and Methods. The ELISA titer was defined as the highest dilution of the test sample giving a twofold increase over the naïve mice samples. n=4 mice/group. (**B**) Sera and BALF from IAV-infected mice at 21 days p.i. were serially diluted two-fold from 1:5 to 1:320, mixed with 4 HA units of IAV, and then incubated with a 1% suspension of chicken red blood cells for 45 minutes at room temperature to visualize HAI titers. n=3-5 mice/group.