Supplementary Information

Targeting CD40 enhances antibody- and CD8-mediated protection against respiratory syncytial virus infection

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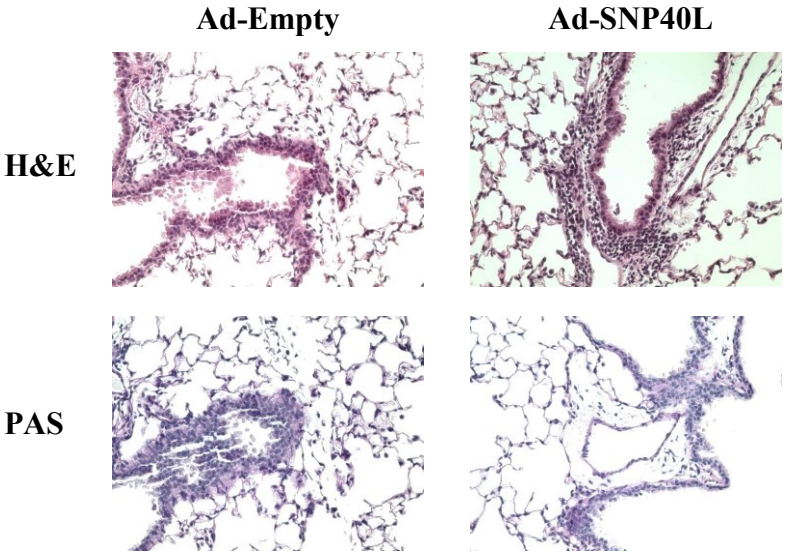
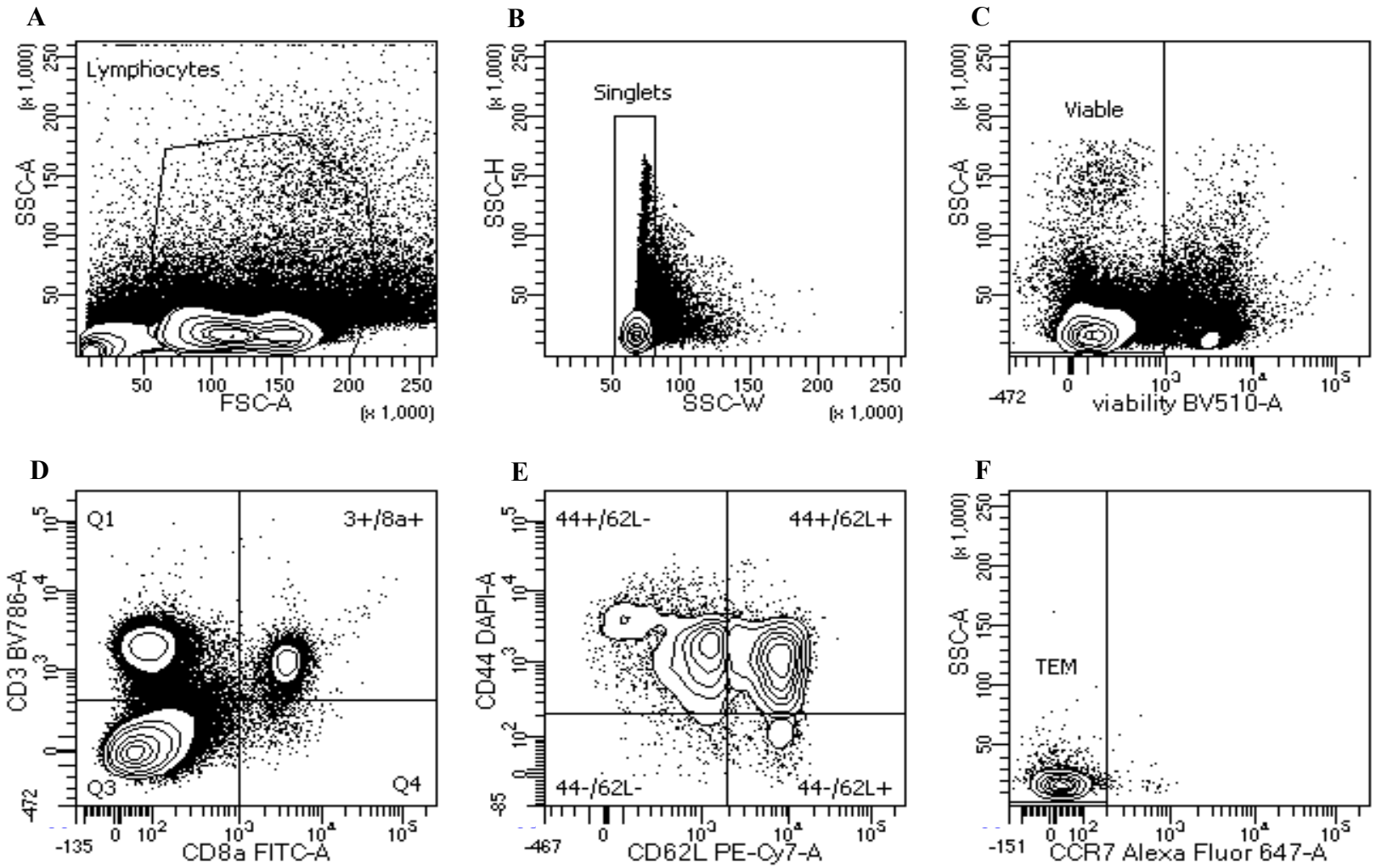
Supplementary Table 1: Primers used for cloning and sequencing of Ad-SF and Ad-SF40L constructs.

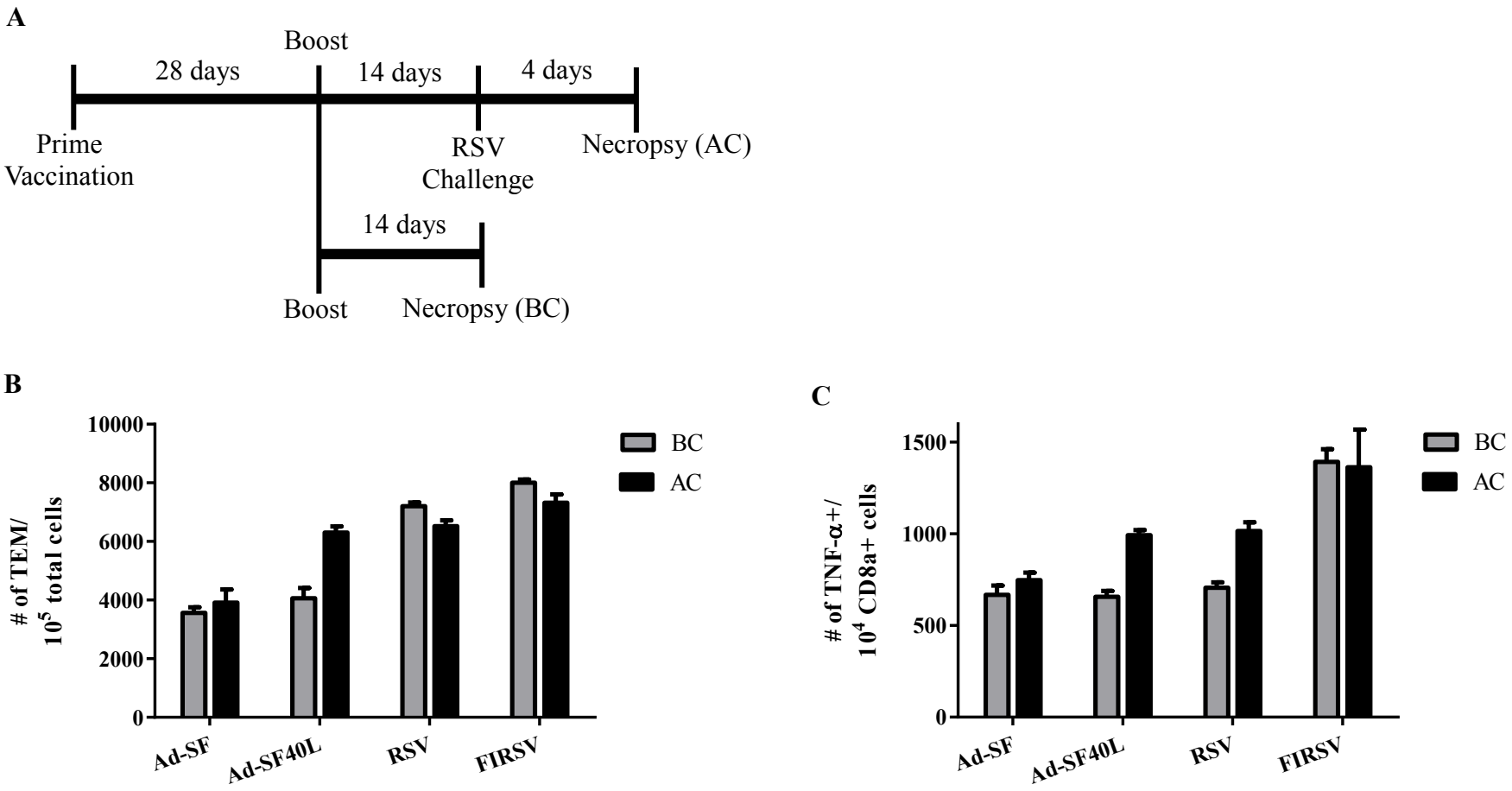
Name	Forward Primer (5' – 3')	Reverse Primer (5' – 3')
Primers for Cloning:		
Ad-SF40L	CACCGCCGCCACCATGCT	TCACAATTTCAACAAGCCAAA
Ad-SF	CACCGCCGCCACCATGCT	TCAATTGCTAAAGGCAATATTATTAATGCCGCTC
Primers for sequencing:		
SF40L-1F	CGCCGCCACCATGCTGCT	
SF40L-2F	AAAGGTAAAGCTCATCAAGCAGGAGC	
SF40L-3F	CAACAAGGCAGTAGTTTCCC	
SF40L-4F	GAGCAACAACGTCCAGATCGTGA	
SF40L-5F	TCTGCCTTCTGAAGTCAACTTGTGCA	
SF40L-6F	TATTAATTTCTATGACCCCCTGG	
SF40L-7F	TGGCTATATACCTGAGGCCCCCA	
SF40L-8F	CAAAGGCGAGGGGTCTCTGT	
SF40L-9F	GGTGACATTTTGCTCAAATCGCG	



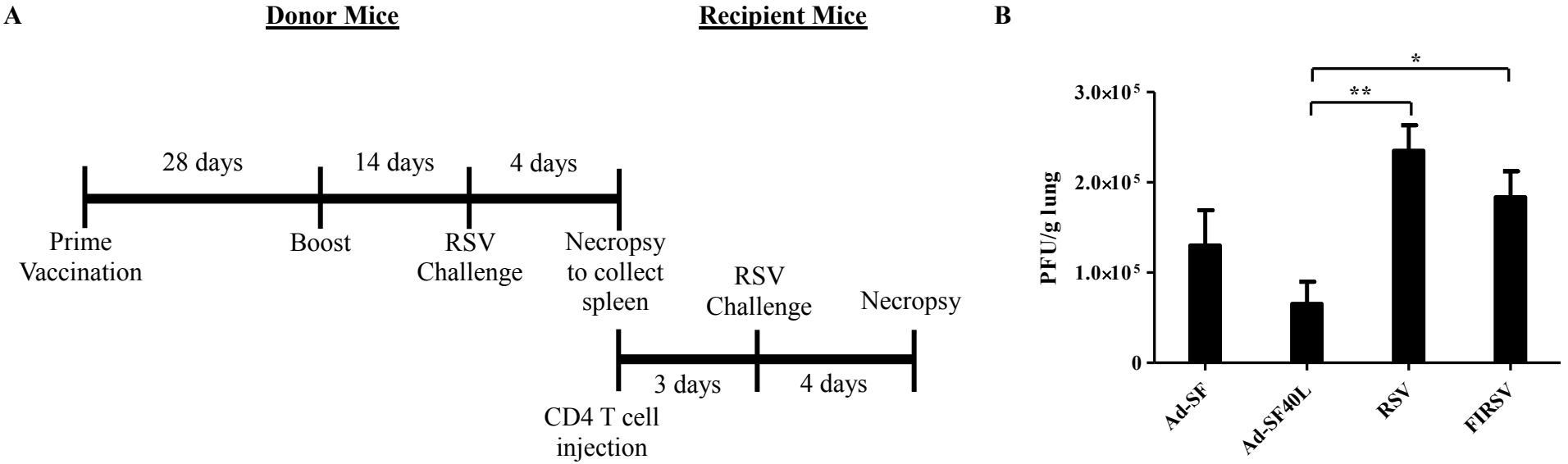
Supplementary Fig. 1: Representative image of H&E and PAS stained lungs of mice post-challenge. BALB/c mice were intranasally immunized twice with control vaccines, Ad-Empty or Ad-SNP40L, to determine the antigen specificity of the observed protection. Lungs were H&E and PAS stained 4 days post challenge for perivascular leukocyte infiltrate and mucus scoring, respectively. Representative images at 40x magnification are shown.



Supplementary Fig. 2: TEM gating strategy. Splenocytes were isolated from immunized BALB/c mice before or after challenge and stained for flow cytometry analysis. A FSC/SSC plot was done to gate for lymphocytes (**A**). Singlets were selected from the lymphocytes (**B**) and further gated for viable cells (**C**). The viable cells were then gated for CD3+CD8α+ (**D**). Next, the CD3+CD8α+ cells were analyzed for CD44+CD62L- (**E**), which were finally gated for CCR7- population (**F**). This CD3+ CD8a+ CD44+ CD62L- CCR7- is denoted as effector/effector memory CD8 T cells or TEM.



Supplementary Fig. 3: Increase in CD8 T cell effector phenotype and function following challenge is unique to Ad-SF40L immunization. (**A**) Schematic diagram of the animal study with BALB/c mice. Mice were necropsied for tissue collection before or after challenge. (**B**) Flow cytometry was used to determine the TEM population (CD3+ CD8a+ CD44+ CD62L- CCR7-) in the spleen. (**C**) Intracellular cytokine staining was done following 4-hour ex-vivo stimulation with F85-93 peptide to analyze the number of CD8α+ TNF-α+ cells in the spleen using flow cytometry. Data shown is mean ± SEM; n = 4 per group representative of 2 separate experiments; *p < 0.05, **p<0.01 (one-way ANOVA with Bonferroni posttest).



Supplementary Fig. 4: CD40L does not enhance CD4 T cell-induced protection. (**A**) Schematic diagram of the animal study timeline. Adoptive CD4 T cell transfer was done from immunized and challenged donor mice into naïve recipient mice. CD4 T cells were isolated from spleens of donor mice using a magnetic bead kit and checked for purity prior to transfer. (**B**) Four days post-challenge, lungs from the recipient mice were collected for viral titer determination using plaque assay. There were no significant differences between Ad-SF and Ad-SF40L group. Data shown is mean ± SEM; n = 4 per group; *p < 0.05, **p<0.01 (one-way ANOVA with Bonferroni posttest).