Cell Reports, Volume *42*

Supplemental information

Lung cDC1 and cDC2 dendritic cells priming naive CD8$^+$ T cells *in situ* prior to migration to draining lymph nodes

Youhui Si, Yihan Wang, Qiaomu Tian, Qiang Wang, Jared M. Pollard, Pramod K. Srivastava, Aaron P. Esser-Kahn, Joel H. Collier, Anne I. Sperling, and Anita S. Chong

Supplementary Materials

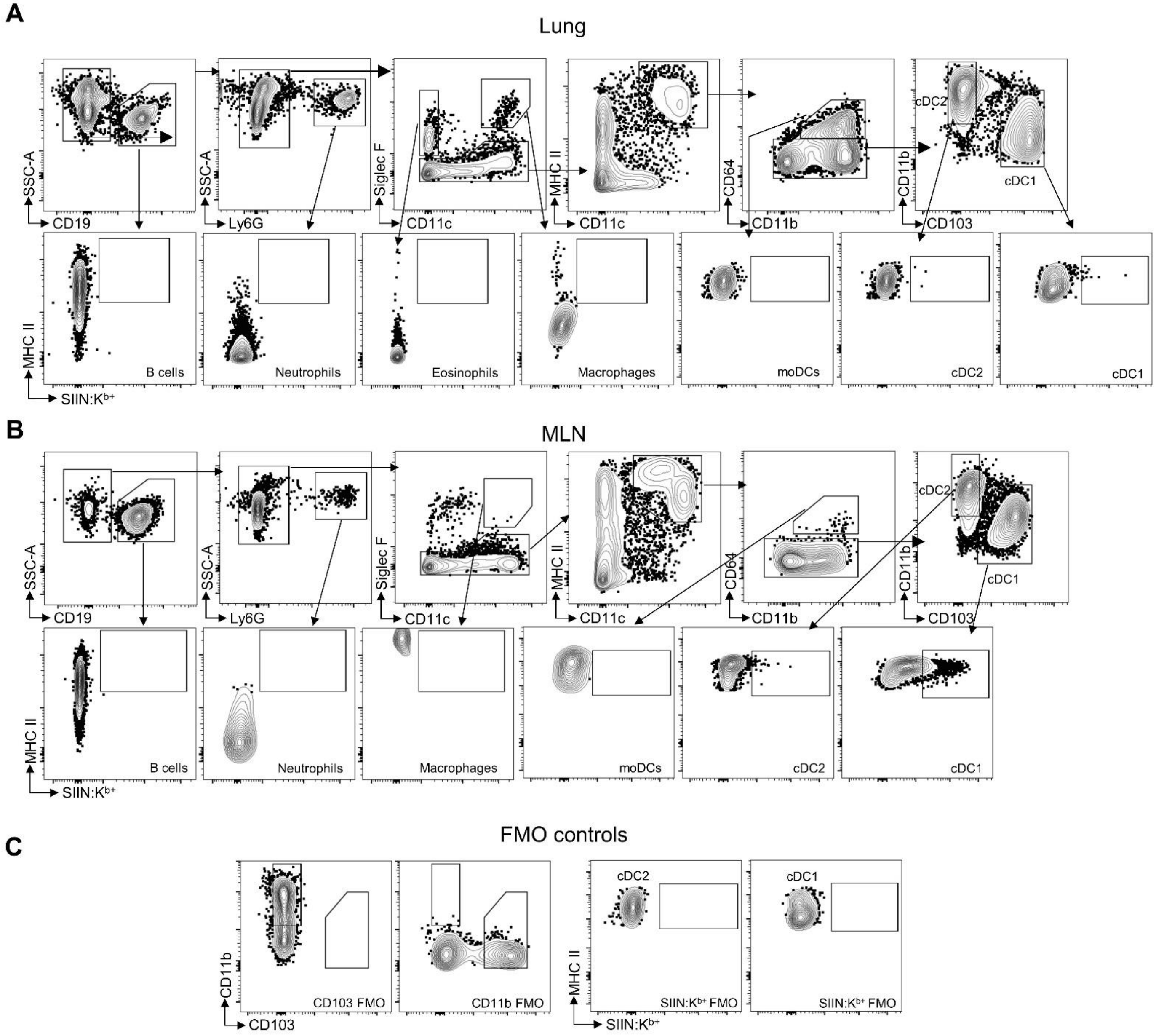

Fig. S1. Gating strategy for SIINFEKL:Kb-presenting cells in lung and mediastinal draining lymph nodes (MLN) after intranasal SIIN-Q11. Related to Figure 3 and 4. Gating strategy to identify SIINFEKL:Kb-presenting cells in lung **(A)** and MLN **(B),** FMO controls **(C).**

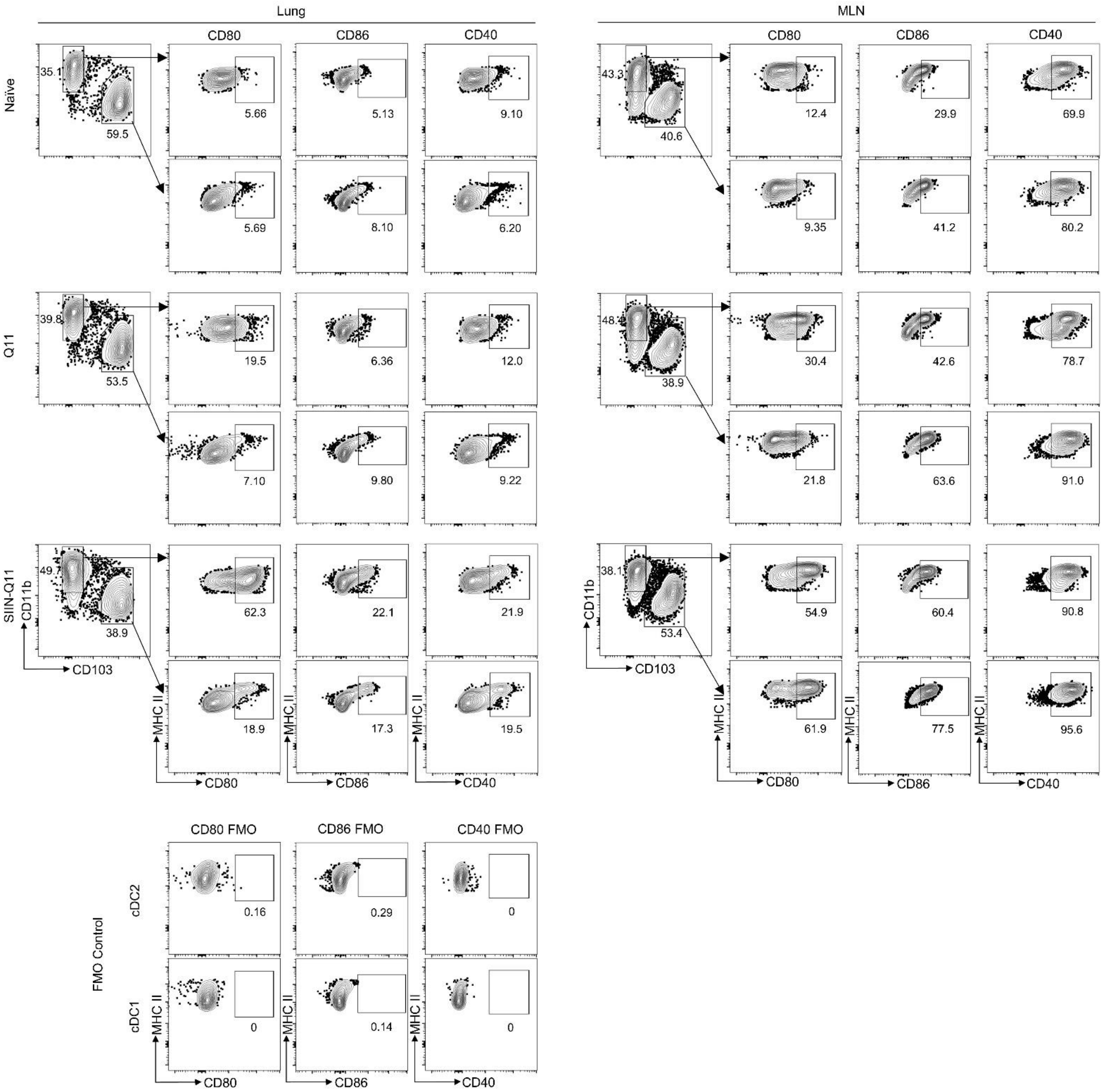

Fig. S2. Representative flow plots of CD80, CD86 and CD40 in lung and MLN after intranasal SIIN-Q11. Related to Figure 3 and 4.

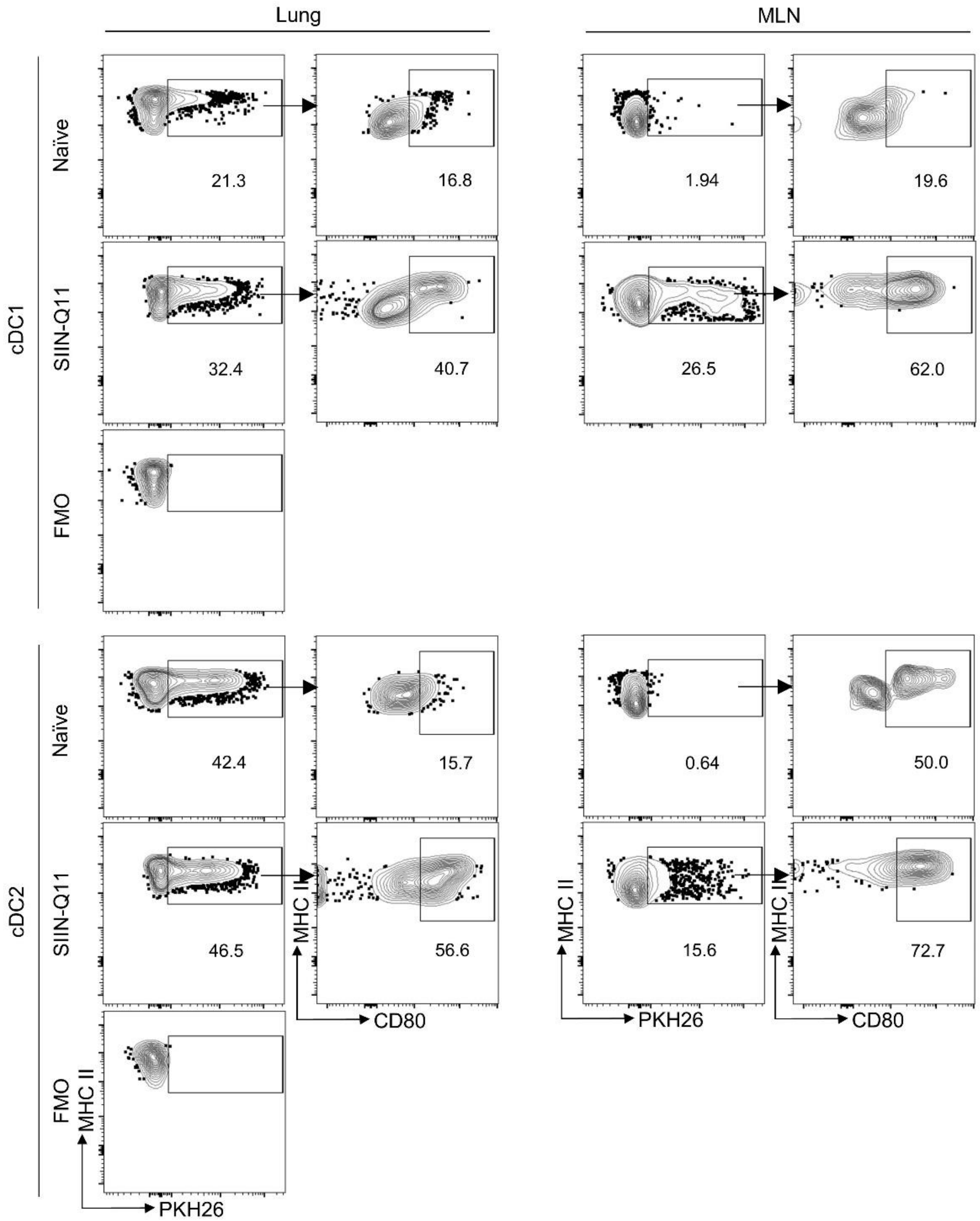

Fig. S3. Representative flow plots of PKH+ DCs and CD80 expression in lung and MLN after intranasal SIIN-Q11. Related to Figure 4.

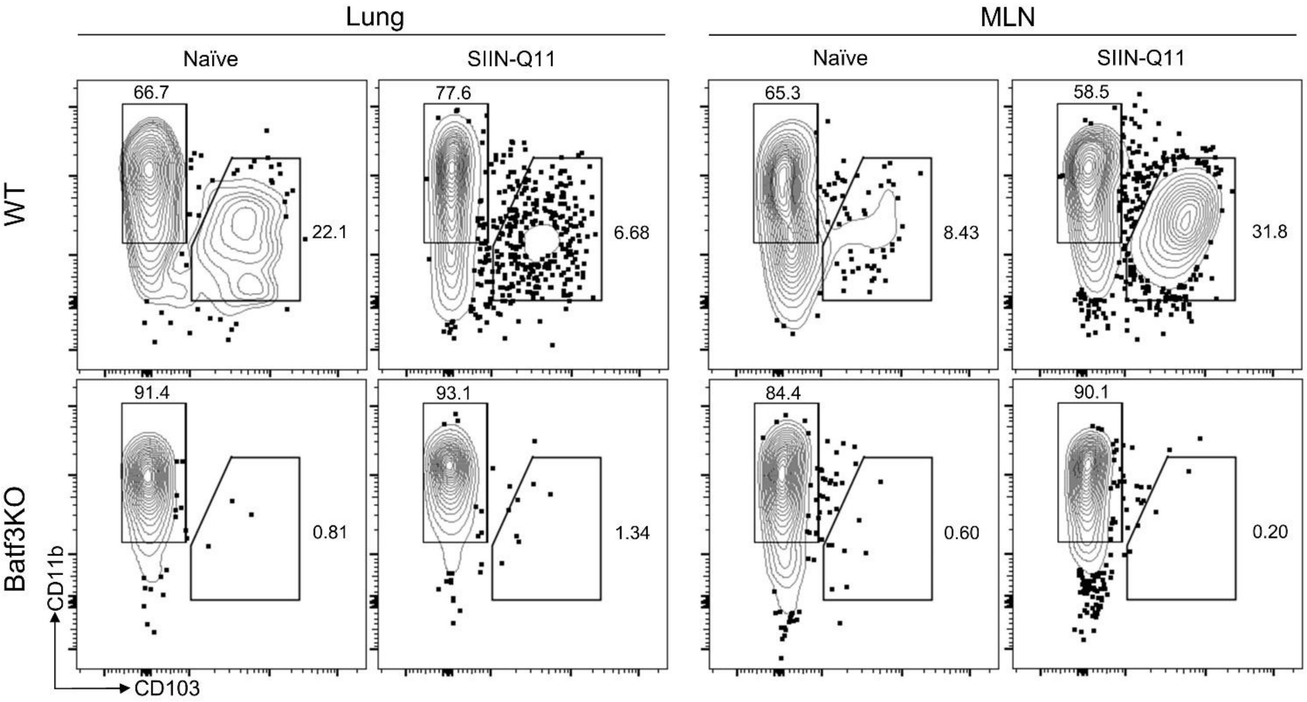

Fig. S4. Verification the absence of cDC1 in Batf3KO mice at baseline and following SIIN-Q11. Related to Figure 6.

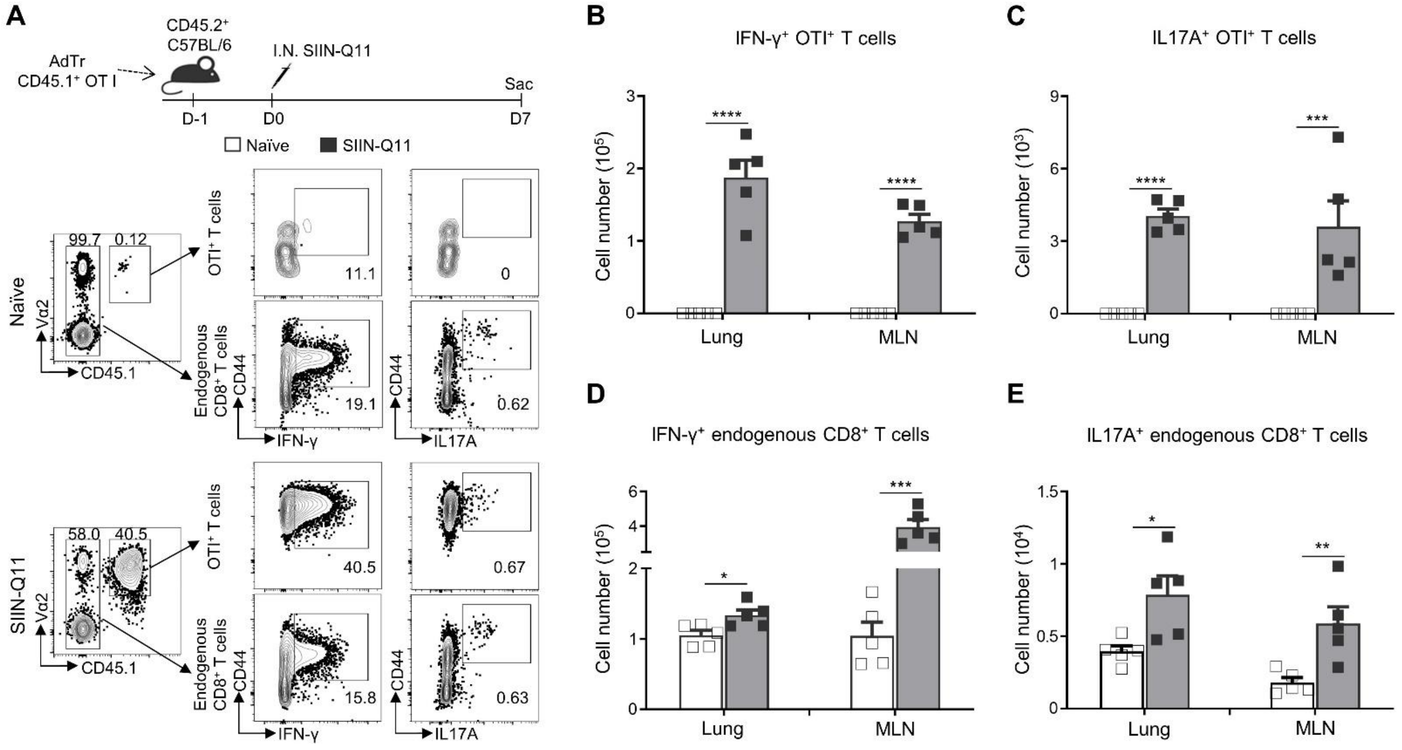

Fig. S5. Intranasal SIIN-Q11 primes IFNγ and IL-17 producing OT-1 and endogenous CD8$^+$ T cells. Related to Figure 2. (A) Representative flow cytometry plots displaying IFNγ- and IL-17-producing OT-1 and endogenous CD8$^+$ T cells in lung. Total numbers of IFNγ producing OT-1 **(B)** and endogenous CD8$^+$ T cells **(D)** in lung and draining mediastinal lymph nodes (MLN). Total numbers of IL-17-producing OT-1 **(C)** and endogenous CD8$^+$ T cells **(E)** from the lung and MLN. Data shown are means ± SEM from two independent experiments. ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$ by two-way ANOVA (B-E).

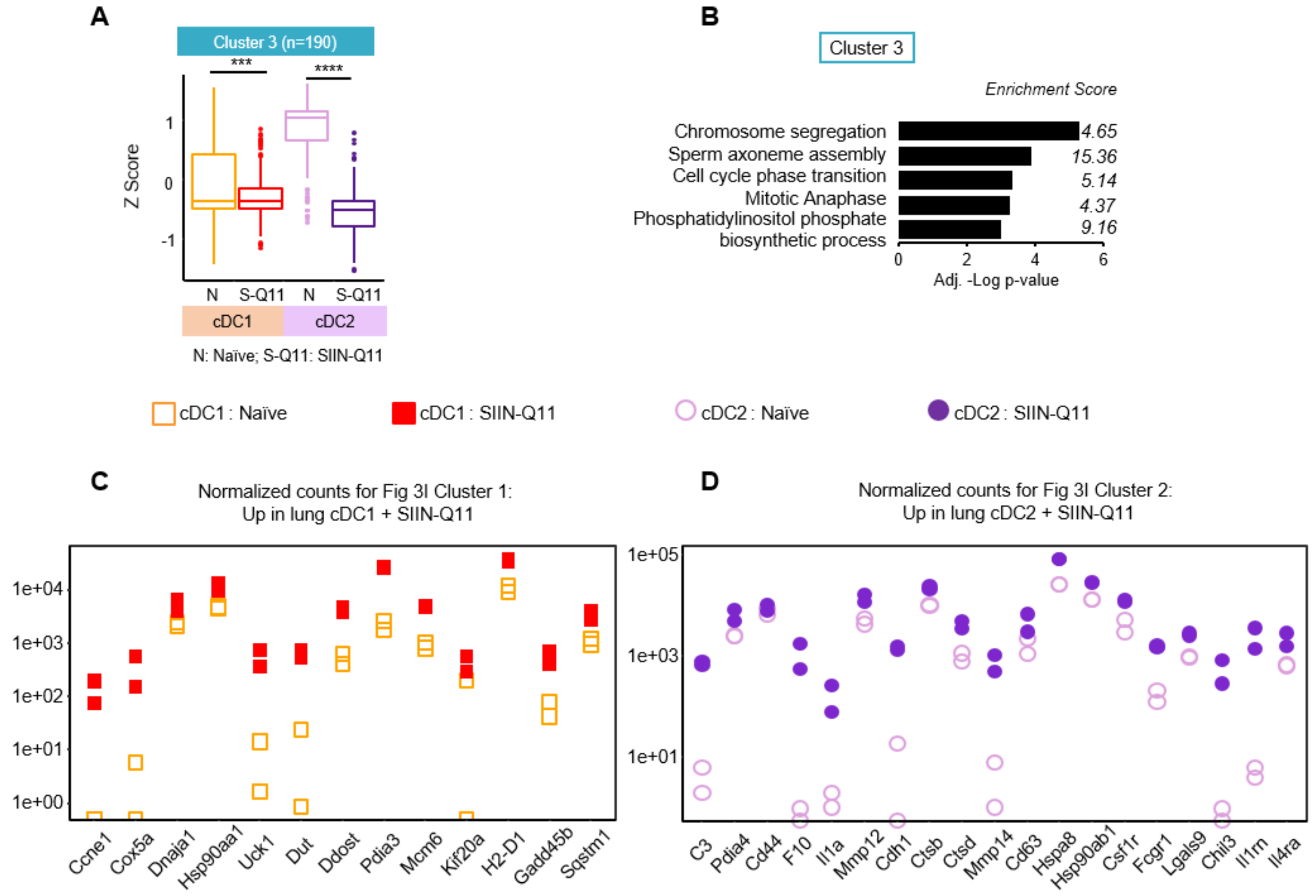

Fig. S6. Transcriptional analysis of cDC1 and cDC2 lung DCs following intranasal SIIN-Q11 immunization. Related to Figure 3. (A) Box plots of the Z scores from Cluster 3 DEGs uniquely upregulated at baseline in cDC2 lung DCs, respectively (From Fig 3I). Significant differences in Z scores between the indicated DC groups were assessed by one-way ANOVA. *P < 0.05, ****P < 0.0001 and ns, not significant. **(B)** Bar charts of Metascape enrichment analysis of DEGs from Clusters 3. **C-D,** Representative differentially expressed genes (DEGs) in lung cDC1 and cDC2 DCs from naïve or SIIN-Q11 immunized mice. Data are presented as normalized counts from Cluster 1 **(C)** and Cluster 2 **(D)** identified in Fig 3I.

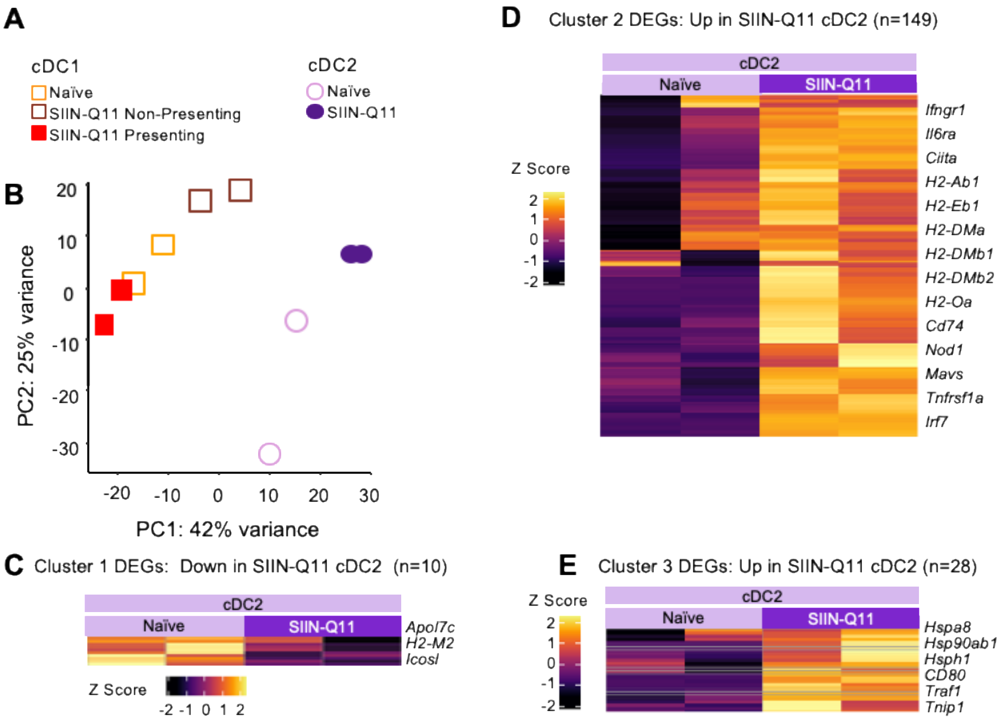

Fig. S7. Transcriptional analysis of cDC2 from MLN following intranasal SIIN-Q11 immunization. Related to Figure 5. Flow-sorted cDC2$^+$, and SIINFEKL-presenting (S-Q11-P) and non-presenting (S-Q11-N) cDC1$^+$ DCs were isolated from the MLN of SIIN-Q11 immunized (S-Q11; 48 h post-immunization) or control naïve mice. **(A)** Groups for **B-E**. **(B)** Principal component analysis (PCA) of RNA-seq data from the 5 DC groups. **(C)** Heat map of Cluster 1 DEGs from Fig 5 that were downregulated in both cDC1 and cDC2 by SIIN-Q11. Genes were filtered based on z-scores >0 for naïve and z-scores <0 for the SIIN-Q11 group. **(D)** Heatmap of Cluster 2 DEGs that were upregulated in non-presenting cDC1 and cDC2 by SIIN-Q11. **(E)** Heatmap of Cluster 3 DEGs that were upregulated in antigen-presenting cDC1 and cDC2 by SIIN-Q11. For **(D)** and **(E)**, genes were filtered based on z-scores z >0 for the SIIN-Q11 group.

Fig. S8. Representative DEGs of OT-1 cells harvested from the lungs of naïve or SIIN-Q11 group between WT and Batf3KO mice. Related to Figure 7. Representative DEGs from Cluster 1 up in SIIN-Q11 **(A)**, Cluster 2 down in SIIN-Q11 **(B)**, and Cluster 3 **(C)** in Fig 7D.

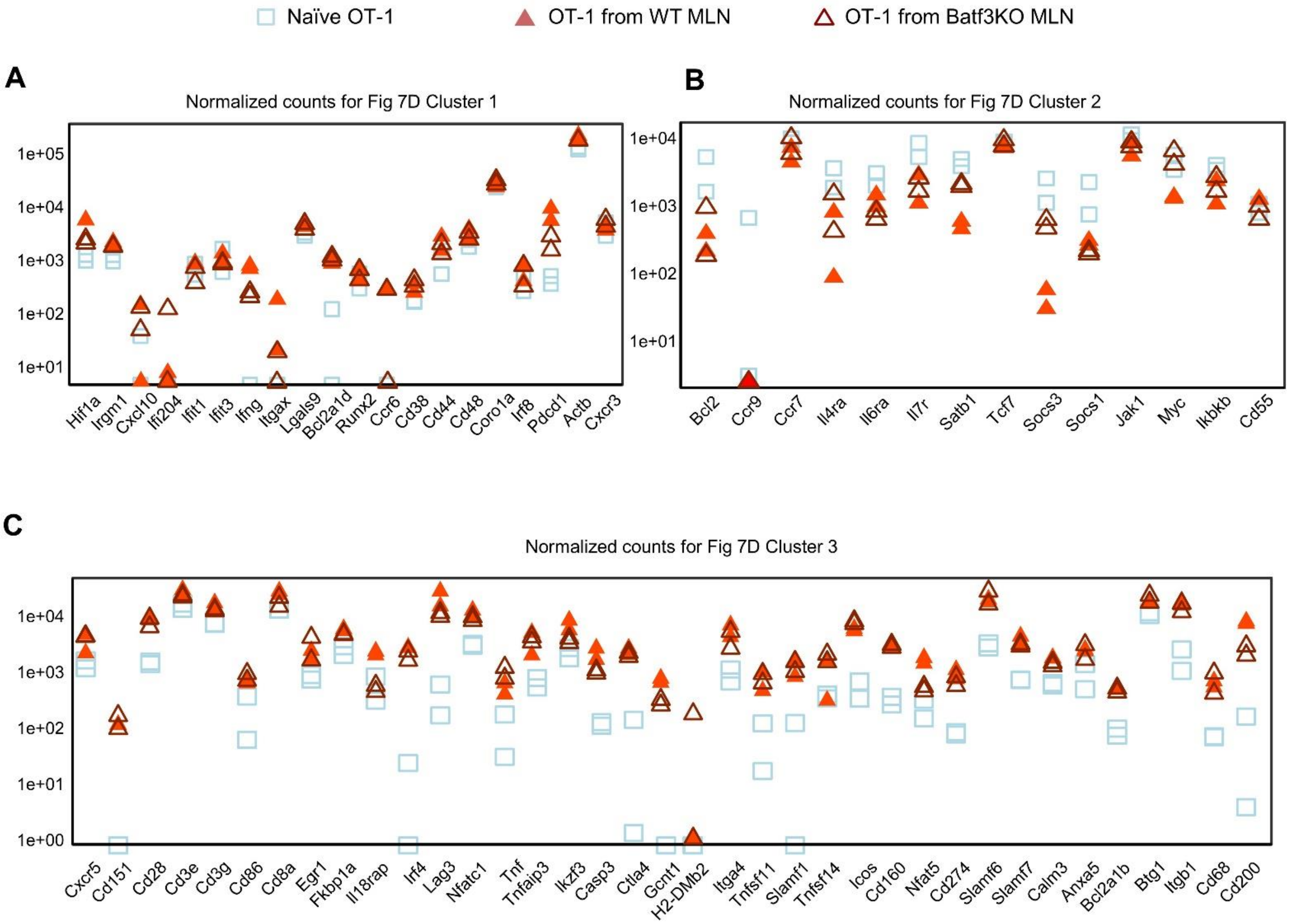

Fig. S9. Representative DEGs of OT-1 cells harvested from the mediastinal MLNs of naïve or SIIN-Q11 immunized WT and Batf3KO mice. Related to Figure 7. Representative genes from Cluster 1 **(A)**, Cluster 2 **(B)**, and Cluster 3 **(C)** in Fig 7D.

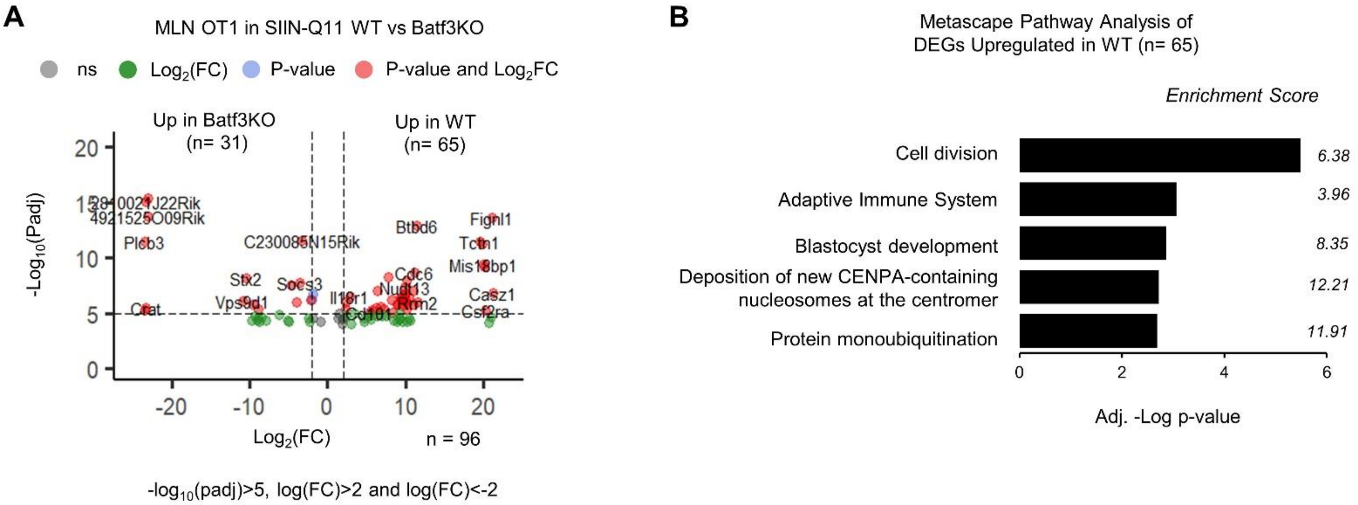

Fig. S10. DEGs induced in OT-1 cells isolated from mediastinal MLNs of WT or Batf3KO mice. Related to Figure 7. (A) Volcano plot of DEGs induced in OT-1 cells harvested from draining MLNs of WT and Batf3KO mice. Genes with log fold greater than or less than 2, and with -log10 (Padj) > 1 are indicated as red. Some of the most significant genes are labeled. **(B)** Pathway enrichment analysis by Metascape of upregulated genes in WT.

Table S2: list of pathways identified by Metascape analysis

Figure #	Pathway Term	Description
Fig. S6B		
Cluster 3	GO:0007059	Chromosome segregation
	GO:0007288	Sperm axoneme assembly
	GO:0044770	Cell cycle phase transition
	Reactome:R-MMU-68882	Mitotic Anaphase
	GO:0046854	Phosphatidylinositol phosphate biosynthetic process
Fig. S10B		
Up in WT	GO:0051301	Cell division
	Reactome:R-MMU-1280218	Adaptive Immune System
	GO:0001824	Blastocyst development
	Reactome:R-MMU-606279	Deposition of new CENPA-containing nucleosomes at the centromere
	GO:0006513	Protein monoubiquitination

Table S3: list of DEGs in MLN cDC2

Figure #	**Gene**
Fig. S7C, D	
Cluster 1	Apol7c,H2-M2,Ets1,Icosl,Tmem176a,Tti2,Tent5c,Zfp398,2700062C07Rik,Pot1b
Cluster 2	Slc2a4rg-ps,Surf2,Pdp2,Rin2,Coq8b,Dhx16,Chmp1b2,Hspa8,Hsp90ab1,H1f4,Gstcd,Hsph1,Ipo4,Bcs1l,Vwa5a,P2rx4,Ddit4,Chst14,Ahsa1,Traf1,Scin,Tnip1,Dnajc12,Rab39b,Nuak2,G2e3,Cd80,Plekha2