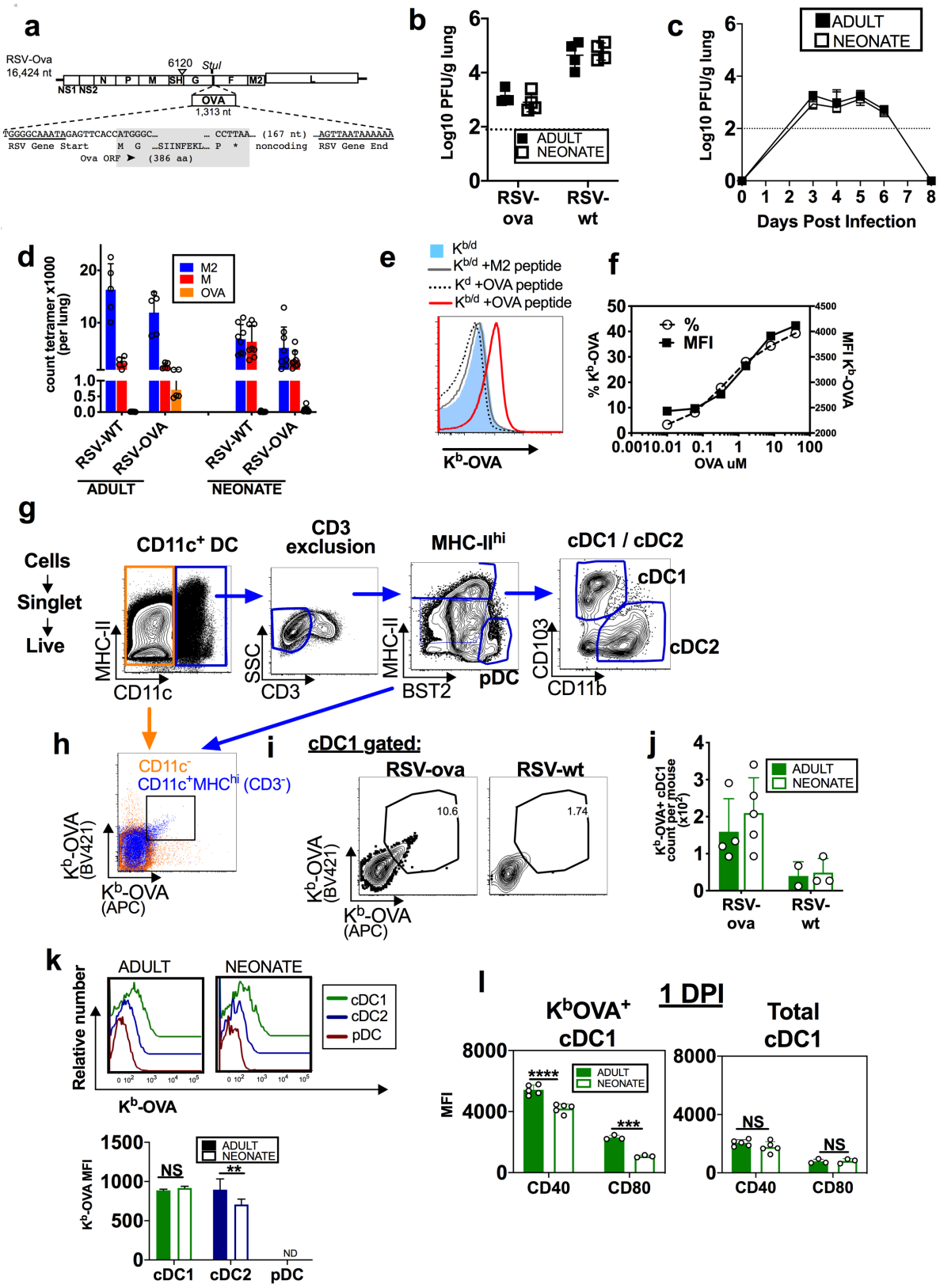
SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURES

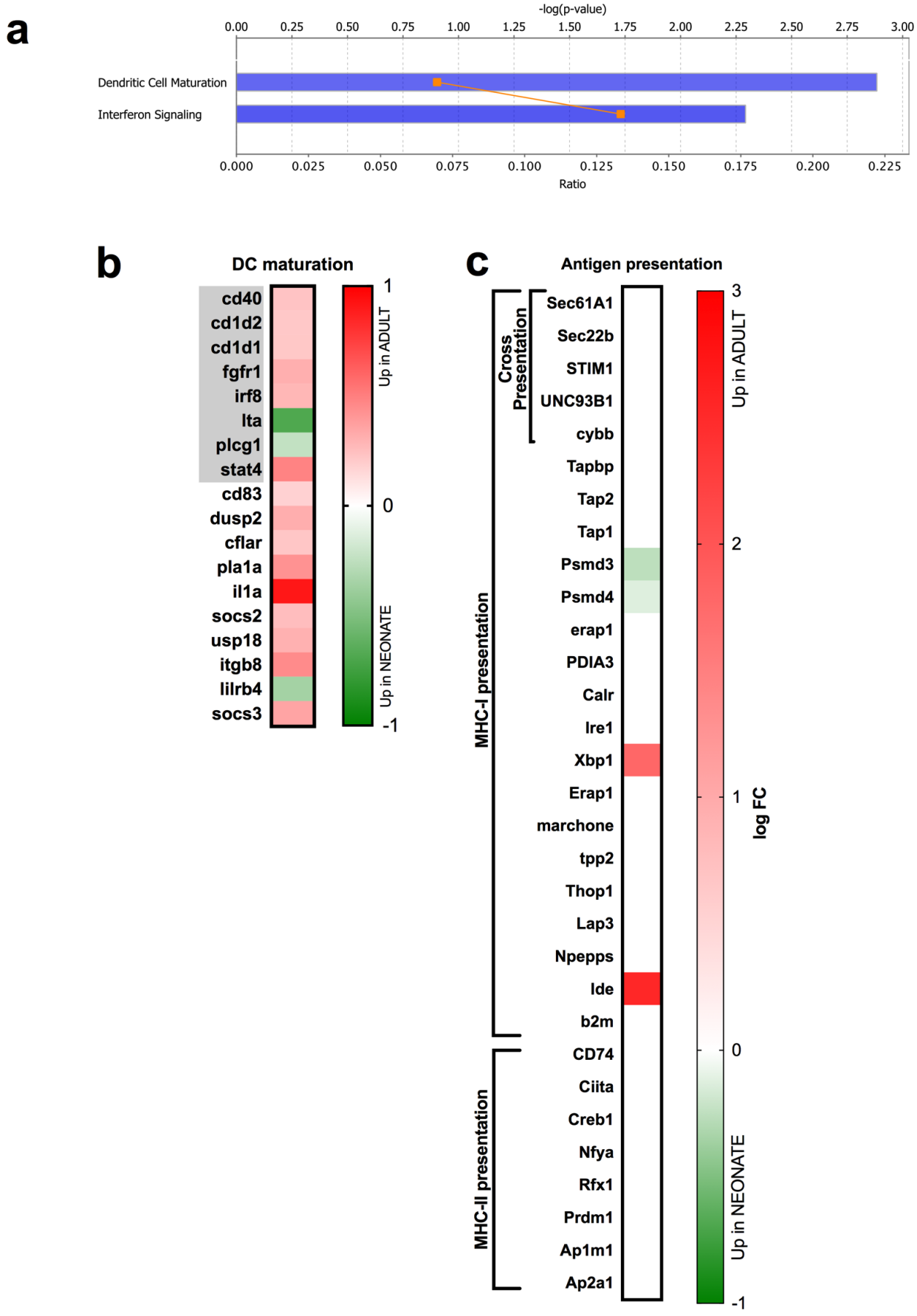
Supplemental Figure 1



Supplemental Fig. 1 Presentation of pathogen-specific MHC-I restricted antigen can be tracked *in vivo* with RSV-ova model a. Schematic overview of the genome organization of RSV-ova: a recombinant version of RSV A2 expressing chicken ovalbumin from an additional gene, inserted between the RSV G and F genes. A 1,313 nucleotide (nt) insert containing a 1,289 nt fragment of the chicken ovalbumin mRNA (Ova, ORF shaded in gray; Genbank Accession number V00383.1), framed by RSV gene start and gene end signals (*underlined*), was inserted into the "6120" version of an RSV full length cDNA plasmid [49], using a unique StuI restriction site located in the RSV G/F intergenic region [50]; aa, amino acids. See Supplemental Methods for further details. **b** Viral titer of RSV-ova or RSV-wt from the lung of infected mice at 4 DPI measured by plaque assays (see Methods for details). **c** Kinetics of RSV-ova viral titer as measured by plaque assay in neonatal and adult mice from 0-8 DPI. Dashed line indicates limit of detection. **d** Numbers of epitope-specific $CD8^+$ T cells identified by tetramer staining from the lungs of adult and neonatal mice 7 days post RSV-wt or RSV-ova infection. **e** Naïve splenocytes were pulsed *in vitro* with different conditions: H-$2K^{b/d}$ haplotype splenocytes with $M2_{82\text{-}90}$ peptide (matched haplotype/mismatched peptide); K^d splenocytes with $OVA_{257\text{-}264}$ peptide (mismatched haplotype/matched peptide); $K^{b/d}$ splenocytes with $OVA_{257\text{-}264}$ (matched haplotype/matched peptide), and stained with the monoclonal antibody 25-D1.16 (anti-K^b-OVA) to show specificity of the antibody. **f** Splenocytes with H-$2K^b$ haplotype were pulsed with varying concentrations of $OVA_{257\text{-}264}$ peptide (SIINFEKL) to show the dose dependency of the binding of the monoclonal antibody 25-D1.16 (anti-K^b-OVA). **g** Flow cytometric analysis and gating strategy of murine respiratory DC subsets in the dLN. Cell gate is based on forward versus side scatter to eliminate debris, followed by a singlet gate with forward scatter on both axes comparing height and area, followed by a live gate with side scatter versus the fluorescence of the viability dye to eliminate dead cells. **h, i** DC subsets were analyzed for K^b-OVA expression by staining with two K^b-OVA antibodies with the same specificity, but labeled with two distinct fluorochromes to improve accuracy of identifying rare cell populations. Resulting diagonal staining events indicate cells that equally bound both antibodies and therefore were likely to be presenting K^b-OVA, while those binding a single antibody represent background staining. **h** Analysis of K^b-OVA expression on $CD11c^-$ cells from the dLN that typically have limited ability to cross-present demonstrates absence of double staining. Gating of $CD11c^+$ $CD3^-$ cells demonstrates that nearly all cells double positive for K^b-OVA staining are within this population. **i** Mice infected with RSV-wt were used as a negative control to confirm K^b-OVA staining in every experiment. **j** Cell count of K^b-OVA positive cells from the dLN in RSV-ova or RSV-wt infected mice at 2 DPI. **k** Mean fluorescence intensity (MFI) of K^b-OVA expression on DC subsets from the dLN 2 DPI represented as histograms and as a bar graph. **l** Expression of costimulatory molecules (by MFI) on K^b-OVA expressing or total cDC1s from the dLN 1 DPI. **b, c** Four mice per group, indicated as individually plotted points or as a single average point. **j, k, l** 3-6 mice pooled per sample, 4-5 samples

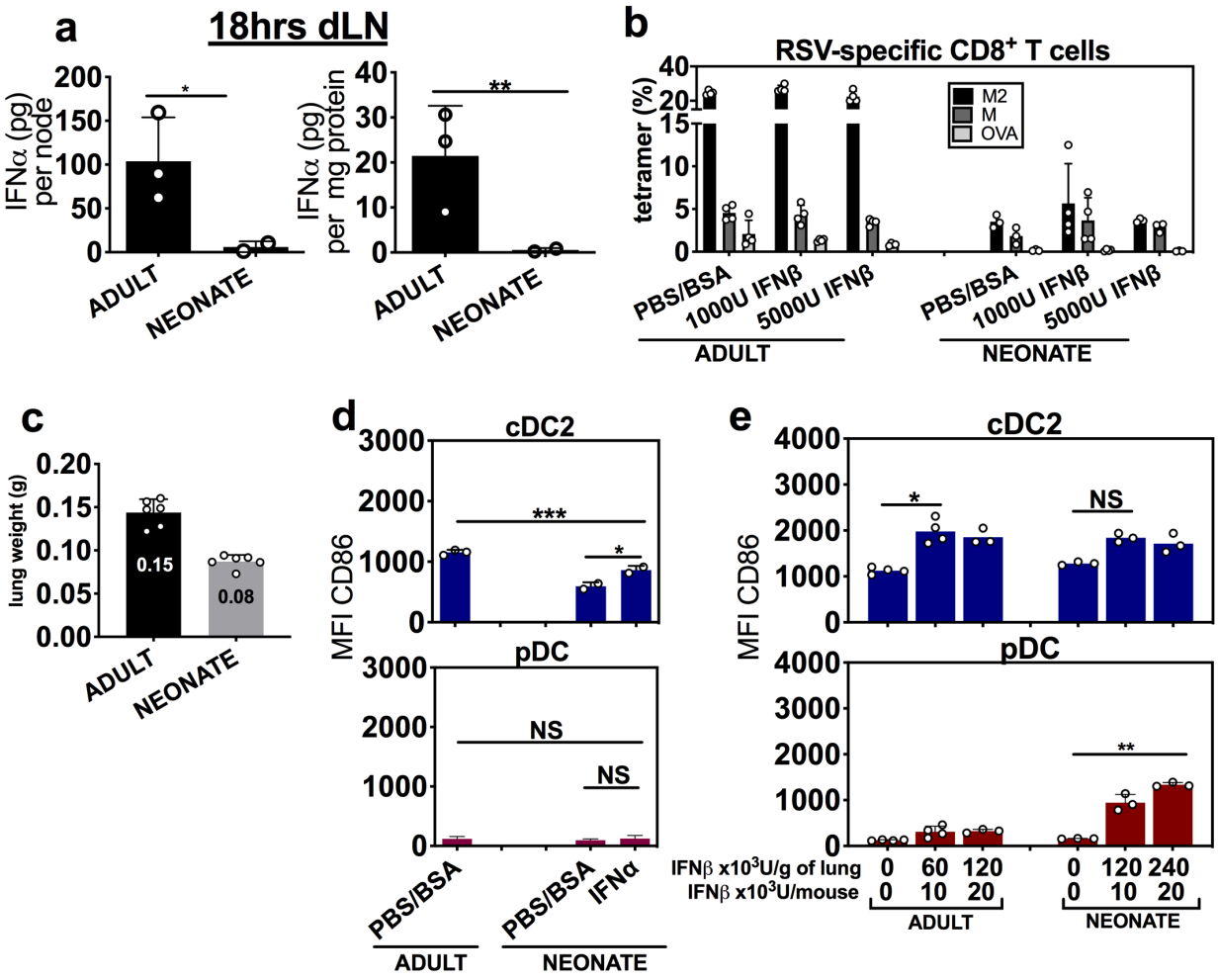
per group. Data are representative of two or three experiments. Data were analyzed using multiple *t*-tests (**k**), or two-way ANOVA with Sidak's multiple comparisons test (**l**). ****p<0.00001, **p<0.001, NS=not significant

Supplemental Figure 2



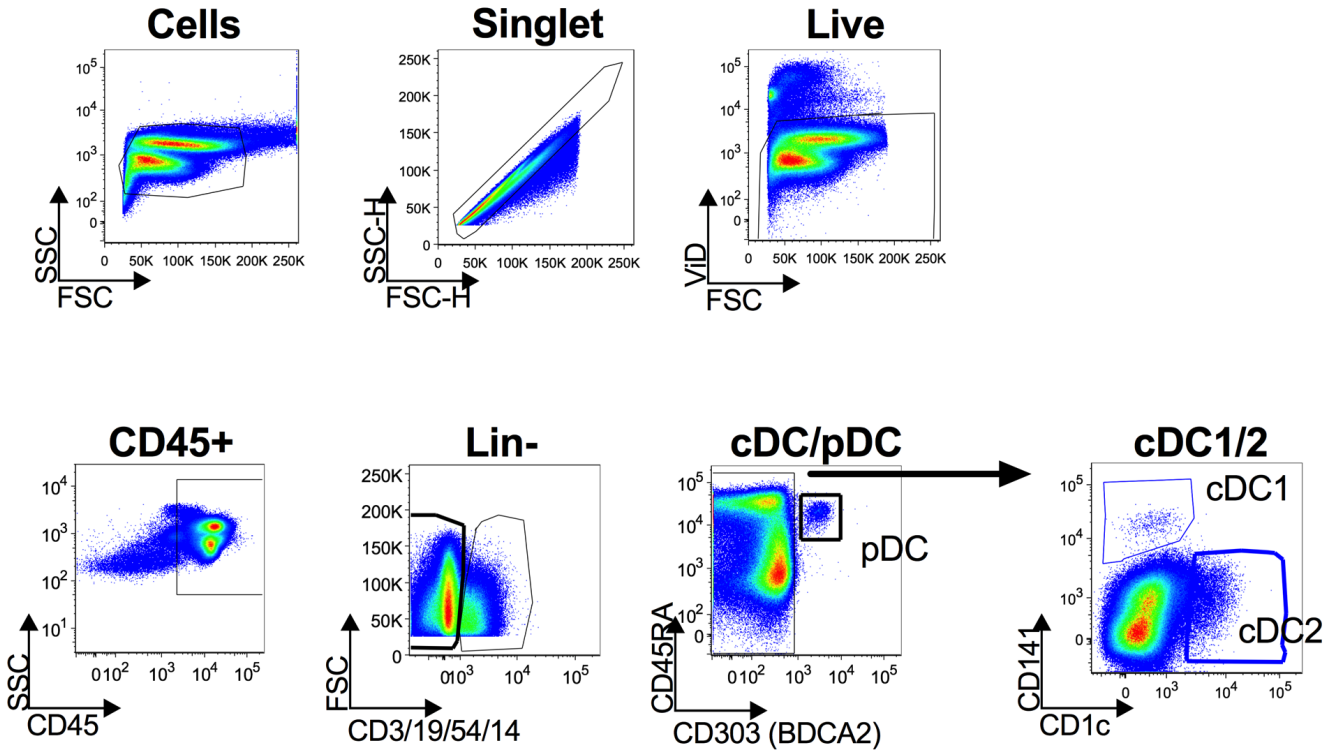
Supplemental Fig. 2 RNA-seq analysis of K^b-OVA-enriched cDC1s. **a** Pathway analysis of DEG from adult compared to neonatal K^b-OVA-enriched cDC1s (isolated from the dLN 2DPI) using QIAGEN Ingenuity Pathway Analysis software. The bars represent the ratio of the number of genes in the data set analyzed versus the total number of genes associated with the pathway. The point shows the – log of the p value for each individual pathway. **b, c** Heatmaps are based on RNA-seq data as described in **a**. Data are shown as log fold-change (Log_{FC}) of adult to neonatal genes expressed during dendritic cell maturation (**b**) and required for antigen processing and presentation (**c**). Genes marked in grey were identified by IPA analysis. Genes not shaded in grey were identified from the literature to expand upon the pathway highlight by IPA (**b**). Antigen presentation genes were not differentially expressed between neonatal and adult K^b-OVA^+ cDC1s and was not a pathway identified by IPA supporting the similar expression of K^b-OVA between the two age groups (**c**).

Supplemental Figure 3



Supplemental Fig. 3 IFN-I production during RSV-ova infection and responsiveness of immune cells to exogenous IFN-I administration. a IFNα in the dLN was quantified by ELISA at 18 hours post RSV-ova infection. **b** Effect of exogenous IFNβ given intranasally 8 and 24 hours post RSV-ova infection on $M2_{82-90}$, $M_{187-195}$ and $OVA_{257-264}$ specific $CD8^+$ T cells isolated from adult and neonatal murine lung 7 DPI. Epitope-specific responses were measure with tetramer by flow cytometry. **c** Weights of naïve lungs were measured in order to calculate the IFNβ dosage used in Figure 4 and Supplemental Fig. 3d and e. **d** Effect of exogenous intranasal IFNα on the expression of CD86 on cDC2 and pDC subsets isolated from the dLN during RSV-ova infection **e** Effect of exogenous intranasal IFNβ on CD86 expression by cDC2 and pDC subsets isolated from the lung of uninfected neonatal and adult mice 18-20 hours after the intranasal administration of IFNβ at doses indicated. **p<0.001, *p<0.05, NS=not significant

Supplemental Figure 4



Supplemental Fig. 4 Gating strategy of human dendritic cell (DC) subsets. Flow cytometric gating strategy for human DC subsets from mononuclear cells isolated from human adult peripheral blood or umbilical cord blood.

SUPPLEMENTAL METHODS AND MATERIALS

Generation of RSV-ova virus

A 1,313 nucleotide (nt) insert containing a 1,289 nt fragment (nt 57 to 1345 of the chicken ovalbumin mRNA, Genbank Accession number V00383.1), framed by RSV gene start (GGGGCAAATA) and gene end signals (AGTTATAAAAAA), was inserted into the "6120" version of an RSV full length cDNA plasmid,[49] using a unique StuI restriction site located in the RSV G/F intergenic region.[50] The "6120" version contains a 112 nt deletion of the 3' noncoding region of the SH gene, and five translationally silent nucleotide changes in the downstream end of the SH open reading frame, designed to stabilize RSV full-length cDNA plasmids during propagation in bacteria, and were deemed to be phenotypically inconsequential.[49] The recombinant RSV-ova virus was generated from cDNA as described previously,[50] and its genomic sequence was confirmed by sequence analysis. In some experiments (**Fig. 3a**; **Supplemental Fig. 2**), a previous version of RSV-ova was used that contained the complete 3' non-coding region of the RSV SH gene. The genome of this version of RSV-ova was later found to contain a 1,338 transposon sequence derived from *E. coli*, inserted between nucleotides 4,998 and 4,999 of the RSV genome (Genbank Accession number KT992094). Ovalbumin expression was not affected by the presence of the transposon.

RNA-seq

RNA extraction yielded high RNA integrity numbers (RIN=9). Single-end sequencing with 75-80 million generated reads per sample identified 126,006 genes; 20,259 of which were nonredundant. 397 genes were determined to be differentially expressed between adult and neonatal K^b-OVA-cDC1s by Welch's t-test followed by multiple testing corrections with Benjamini-Hochberg False Discover Rate (p value <0.055 and fold change >1.5).

SUPPLEMENTAL TABLE 1

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-mouse CD16/32 (clone 2.4G2)	BD Biosciences	Cat#553142; RRID:AB_394657
anti-mouse CD24, PerCP/Cy5.5 conjugated (clone M1/69)	BioLegend	Cat#101824; RRID:AB_1595491
anti-mouse CD24 Antibody, PE/Cy5 conjugated (clone M1/69)	BioLegend	Cat#: 101812; RRID:AB_439714
anti-mouse CD80, FITC conjugated (clone 16-10A1)	BioLegend	Cat#104706; RRID:AB_313127
anti-mouse CD80, BV421 conjugated (clone 16-10A1)	BioLegend	Cat#: 104726; RRID:AB_2561445
anti-mouse CD45, PE/Cy7 conjugated (clone 30-F11)	BioLegend	Cat#103114; RRID:AB_312979
anti-mouse CD40, PE/Cy7 conjugated (clone 3/23)	BioLegend	Cat#124622; RRID:AB_10897812
anti-mouse CD40, FITC conjugated (clone 3/23)	BioLegend	Cat# 124608 RRID:AB_1134096
anti-mouse CD11b, PE/Cy5 conjugated (clone M1/70)	BioLegend	Cat#101210; RRID:AB_312793
anti-mouse CD11b, BV785 conjugated (clone M1/70)	BioLegend	Cat# 101243; RRID:AB_2561373
anti-mouse CD103, PE conjugated (clone 2E7)	BioLegend	Cat#121406; RRID:AB_1133989
anti-mouse CD86, AlexFluor 700 conjugated (clone GL-1)	BioLegend	Cat#105024; RRID:AB_493721
anti-mouse CD86, BV605 conjugated (clone GL-1)	BioLegend	Cat# 105037 RRID:AB_11204429
anti-mouse H-2K^{b} bound to SIINFEKL, APC conjugated (clone 25-D1.16)	BioLegend	Cat#141606 RRID:AB_11219595
anti-mouse H-2K^{b} bound to SIINFEKL, APC conjugated (clone 25-D1.16)	Thermo Fisher Scientific	Cat# 17-5743-82; RRID:AB_1311286
anti-mouse H-2K^{b} bound to SIINFEKL, biotin conjugated (clone 25-D1.16)	Thermo Fisher Scientific	Cat# 13-5743-82; RRID:AB_1210600
anti-mouse I-A^{b} (Aβ^{b}), biotin conjugated (clone 25-9-17)	BioLegend	Cat#114403; RRID:AB_313578
anti-mouse I-A/I-E, biotin conjugated (clone M5/114.15.2)	BioLegend	Cat# 107643; RRID:AB_2565976
anti-mouse I-A/I-E, BV711 conjugated (clone M5/114.15.2)	BioLegend	Cat# 107643 RRID:AB_2565976
Brilliant Violet 421 Streptavidin	BioLegend	Cat# 405226
anti-mouse CD11c, Brilliant Violet 785 conjugated (clone N418)	BioLegend	Cat#117336; RRID:AB_2565268
anti-mouse CD11c, PE-eFluor 610 conjugated (clone N418)	Thermo Fisher Scientific	Cat# 61-0114-80; RRID:AB_2574529
anti-mouse CD64 (Fc$_{\gamma}$RI), Brilliant Violet 711 conjugated (clone X54-5/7.1)	BioLegend	Cat#139311; RRID:AB_2563846
anti-mouse CD64 (Fc$_{\gamma}$RI), Brilliant Violet 605 conjugated (clone X54-5/7.1)	BioLegend	Cat# 139323; RRID:AB_2629778
anti-mouse CD197 (CCR7), Brilliant Violet 421 conjugated (clone 4B12)	BioLegend	Cat#120120; RRID:AB_2561446

anti-mouse CD197 (CCR7), PE/Cy5 conjugated (clone 4B12)	BioLegend	Cat# 120113 RRID:AB_493571
anti-mouse BST2, PE-eFluor 610 conjugated (clone eBio927)	Thermo Fisher Scientific	Cat#61-3172; RRID:AB_2574603
anti-mouse BST2, PE-Cy7 conjugated (clone eBio927)	Thermo Fisher Scientific	Cat# 25-3172-82 RRID:AB_2573440
anti-mouse CD3e, APC-Cy7 conjugated (clone 145-2C11)	BD Biosciences	Cat#557596; RRID:AB_396759
anti-mouse CD3e, BUV395 conjugated (clone 145-2C11)	BD Biosciences	Cat# 563565 RRID:AB_2738278
anti-mouse CD8a, PE-CF594 conjugated (clone 53-6.7)	BD Biosciences	Cat#562283; RRID:AB_11152075
Tetramer- H-2 Kb OVA (SIINFEKL), BV421 conjugated	MBL International	Cat#TB-5001-4
iTAg Tetramer- H-2 Kd RSV M2 (SYIGSINNI), PE conjugated	MBL International	Cat# TB-M506-1
iTAg Tetramer- H-2 Db RSV Tetramer-NAITNAKII, APC conjugated	MBL International	Cat# TB-5018-2
anti-mouse CD80 (B7-1) (clone 16-10A1)	Bio X Cell	Cat#BE0024; RRID:AB_1107676
anti-mouse CD86 (B7-2) (clone GL-1)	Bio X Cell	Cat#BE0025; RRID:AB_1107678
anti-mouse CD40 (clone FGK4.5)	Bio X Cell	Cat#BE0016-2 RRID:AB_1107647
Armenian Hamster polyclonal IgG	Bio X Cell	Cat#BE0091 RRID:AB_1107773
anti-mouse Rat IgG2a (clone 2A3)	Bio X Cell	Cat#BE0089 RRID:AB_1107769
anti-human CD14, Al700 conjugated (clone TuK4)	Thermo Fisher Scientific	Cat# MHCD1429 RRID:AB_10373535
anti-human HLA-DR, APC conjugated (clone LN3)	Thermo Fisher Scientific	Cat# 17-9956-42 RRID:AB_10670347
anti-human CD86, PE/Cy5 conjugated (clone IT2.2)	BioLegend	Cat# 305408 RRID:AB_314528
anti-human CD80, PE conjugated (clone 2D10)	BioLegend	Cat# 305208 RRID:AB_314504
anti-human CD1c, BV605 conjugated (clone L161)	BioLegend	Cat# 331538 RRID:AB_2629762
anti-human CD19, Al700 conjugated (clone HIB19)	BioLegend	Cat# 302226 RRID:AB_493751
anti-human CD56, Al700 conjugated (clone HCD56)	BioLegend	Cat# 318316 RRID:AB_604104
anti-human CD141, BV785 conjugated (clone M80)	BioLegend	Cat# 344116 RRID:AB_2572195
anti-human CD11c, PE/Dazzle 594 conjugated (clone Bu15)	BioLegend	Cat# 337228 RRID:AB_2564549
anti-human CD45, FITC conjugated (clone HI30)	BioLegend	Cat# 304038 RRID:AB_2562050
anti-human CD3, Al700 conjugated (clone OKT3)	BioLegend	Cat# 317340 RRID:AB_2563408
anti-human CD45RA, BV711 conjugated (clone HI100)	BioLegend	Cat# 304138 RRID:AB_2563815
anti-human CD303/BDCA2, BV421 conjugated (clone 201A)	BioLegend	Cat# 354212 RRID:AB_2563871

Bacterial and Virus Strains		
RSV A2 (RSV-wt)	Barney Graham, NIH	Propagated in-house
RSV A2 -OVA (RSV-ova)	Peter Collins, NIH	Propagated in-house
Biological Samples		
Human peripheral blood from adults	VRC, NIH	
Human cord blood	WRNMMC	
Chemicals, Peptides, and Recombinant Proteins		
Mouse IFNalpha A	PBL Assay Science	Cat#12100-1
Mouse IFN beta	PBL Assay Science	Cat#12405-1
Human IFN beta	PBL Assay Science	Cat# 11415-1
Vybrant® CFDA SE Cell Tracer Kit	Invitrogen	Cat# V12883
Live/dead Fixable Aqua dead cells stain kit	Thermo Fisher Scientific	Cat# L34957
Normal Mouse Serum	Jackson ImmunoResearch	Cat# 015-000-001 RRID:AB_2337186
OVA peptide	Anaspec	Cat# AS-60193
R848	InvivoGen	Cat# tlrl-r848-5
Ficoll-Paque™ PREMIUM	Fisher Scientific	Cat# 45-001-751
Fico/Lite-LM (mouse)	Atlanta Biologicals	Cat# I40650
Critical Commercial Assays		
Mouse IFNalpha ELISA using: Rat monoclonal anti-Mouse IFN Alpha (Clone RMMA-1) Anti-Mouse IFN Alpha Antibody, Rabbit Serum (PAb) Anti-Rabbit IgG (H+L), HRP Conjugate	 PBL Assay Science PBL Assay Science Promega	 Cat#22100-1 RRID:AB_358960 Cat#32100-1 RRID:AB_354242 Cat#W4011 RRID:AB_430833
Mouse IFN-beta DuoSet ELISA	R&D Systems	Cat# DY8234-05
Pierce BCA protein assay kit	Thermo Fisher Scientific	Cat#23227
CD8a+ T Cell Isolation Kit, mouse	Miltenyi Biotec	Cat# 130-104-075
Experimental Models: Cell Lines		
HEp2 cell line	ATCC	Cat# CCL-23 RRID:CVCL_1906
Experimental Models: Organisms/Strains		
Mouse: C57Bl/6J male	The Jackson Laboratory	Strain# 000664 RRID:IMSR_JAX:000664
Mouse: BALB/cJ female	The Jackson Laboratory	Strain# 000651 RRID:IMSR_JAX:000651
Mouse: CB6F1/J female	The Jackson Laboratory	Strain# 100007 RRID:IMSR_JAX:100007
Mouse: OTI mice C56BL/6-Tg(TcraTcrb)1100Mjb/J	The Jackson Laboratory	Stain# 003831 RRID:IMSR_JAX:003831
Software and Algorithms		
BD FACS DiVa (8)	BD Biosciences	http://www.bdbiosciences.com/us/instruments/clinical/software/flow-cytometry-acquisition/bd-facsdiva-software/m/333333/overview

Flowjo V9.9.6	Tree Star, Inc.	https://www.flowjo.com/solutions/flowjo/downloads
Prism v7.0	GraphPad	https://www.graphpad.com/demos
Ingenuity IPA	QIAGEN	https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis