Figure S1

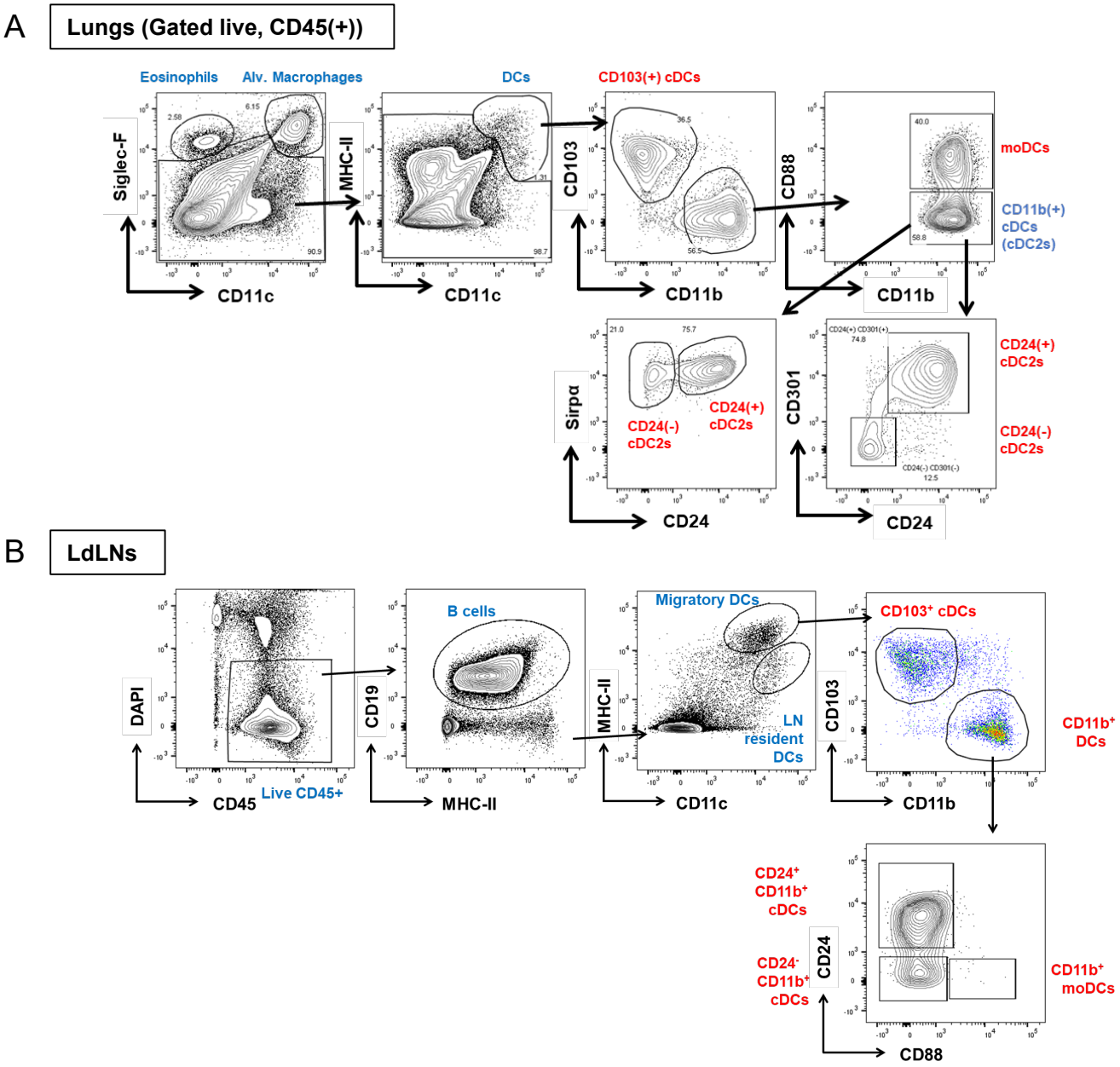

Figure S1, related to Materials & Methods and Introduction: Lung (A) and lung-draining lymph node (B) DC gating. After exclusion of Siglec-F(+) eosinophils and alveolar macrophages, lung DCs are defined as CD11c(+) and MHC-II high. This excludes interstitial macrophages, which are CD11c(-). DCs in the lung either express CD103 or CD11b, apart from a small population of pDCs expressing neither. The CD103(+) population are cDC1s. The CD11b(+) DCs include populations of CD88(+) monocyte-derived DCs, CD24(-) conventional DC2s, and CD24(+) conventional DC2s. As MoDC are not known to be migratory, very few are found in the LdLN.

Figure S2

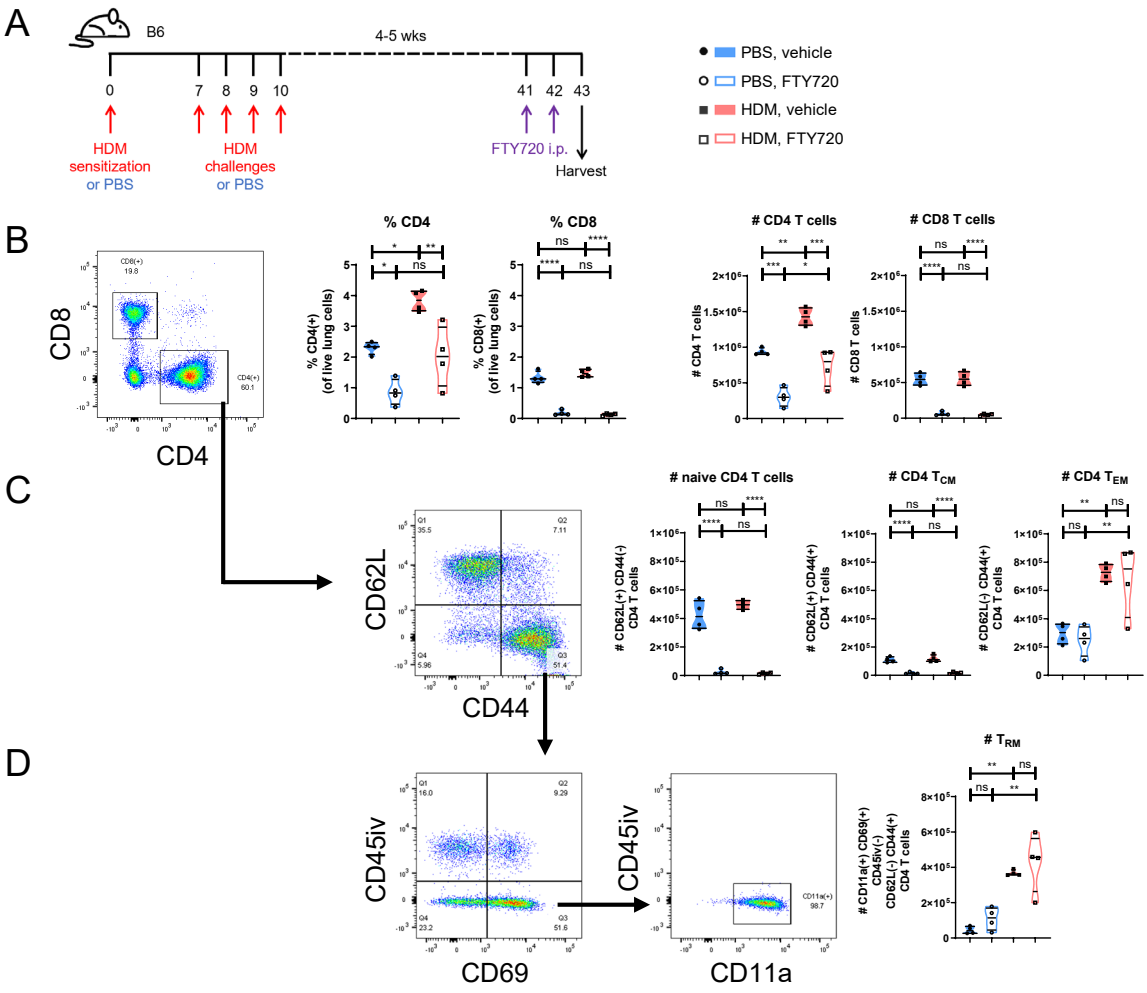

Figure S2, related to Fig. 1: Systemic treatment with FTY720 depletes circulating T cell populations but leaves lung T_{RM} intact. (**A**) Schematic depicting experimental protocol. After sensitization and challenge with HDM and resolution of the acute response, mice underwent treatment with 25 ug FTY720 administered intraperitoneally daily until sacrifice. (**B**) Examination of the lung T cell populations revealed considerable depletion of the CD4 compartment and near-total depletion of the CD8 compartment. (**C**) CD4 T cells were defined as naïve (CD44(low)CD62L(high)), central memory (T_{CM}, CD44(high)CD62L(high)), or effector memory (TEM, CD44(high)CD62L(low)). FTY720 depleted the naïve and T_{CM}, but not T_{EM}. (**D**) CD4 T_{RM} were defined as CD4 T_{EM} which expressed CD69 and CD11a, but did not display intravascular CD45 staining. CD4 T_{RM} were not depleted by FTY720 treatment. Data represent one experiment with n = 4 mice per group, and statistics (ordinary one-way ANOVA with Tukey's multiple comparisons test) were performed in GraphPad Prism. Bar represents the mean ± SEM (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; **** $p < 0.0001$; ns, not significant).

Figure S3

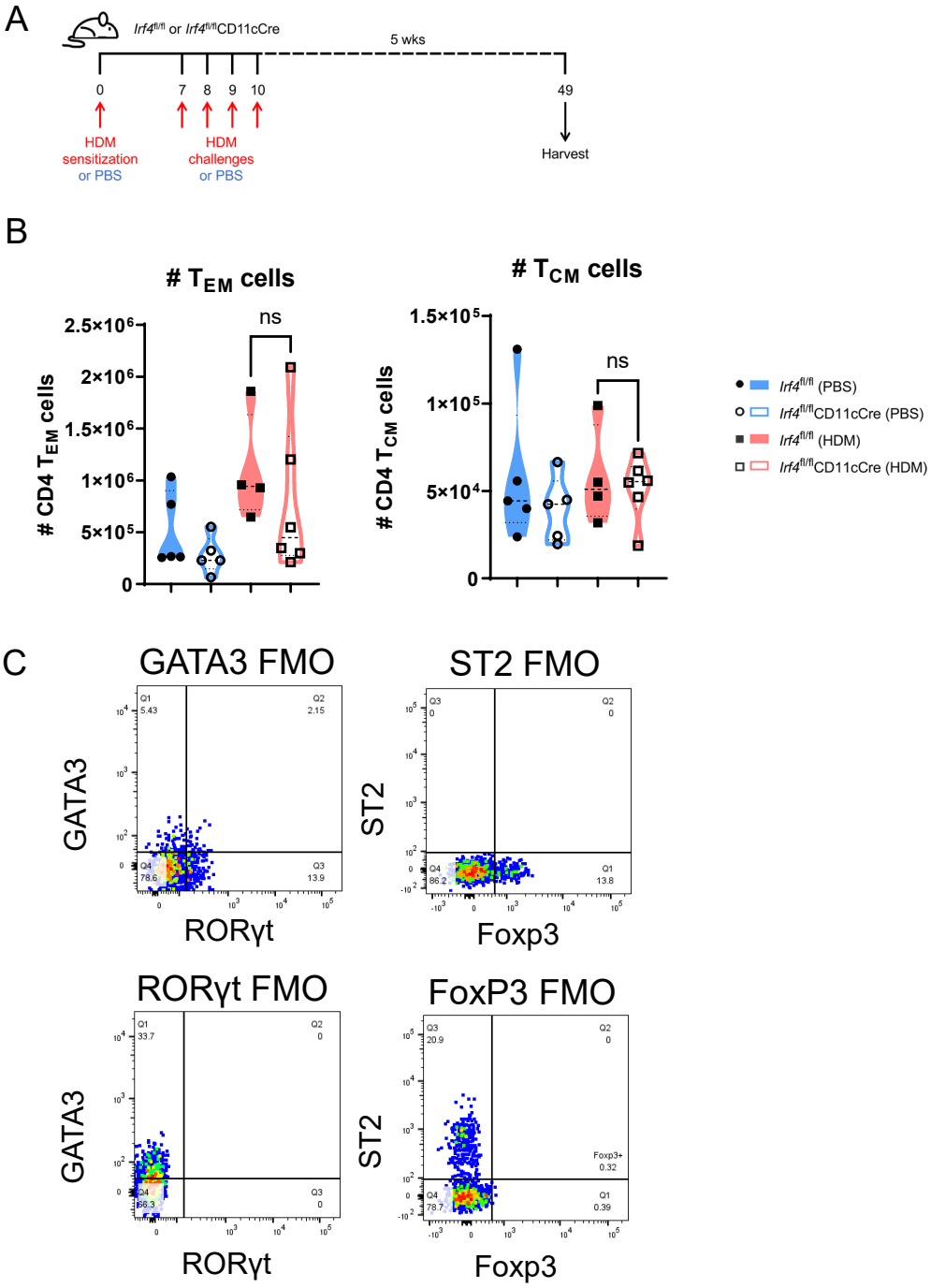

Figure S3, related to Fig. 2: Lungs of mice with IRF4-deficient DCs contain equal numbers of effector memory and central memory T cells during the memory phase. (**A**) Schematic of experimental protocol for resting memory lung analysis (**B**) Quantification of total CD4 effector memory (T_{EM}) and central memory (T_{CM}) cells in the lungs of mice with resting memory to HDM. (**C**) Flow plots demonstrating fluorescence-minus-one controls (FMOs) for the transcription factors GATA3, FoxP3, RORγt, and the IL33 receptor, ST2. Cells were pre-gated on live, CD3+CD4+CD44+CD62L-. Data represent two experiments with n = 4-6 mice per group, and statistics (ordinary one-way ANOVA with Tukey's multiple comparisons test) were performed in GraphPad Prism. Bar represents the mean ± SEM (ns, not significant).

Figure S4

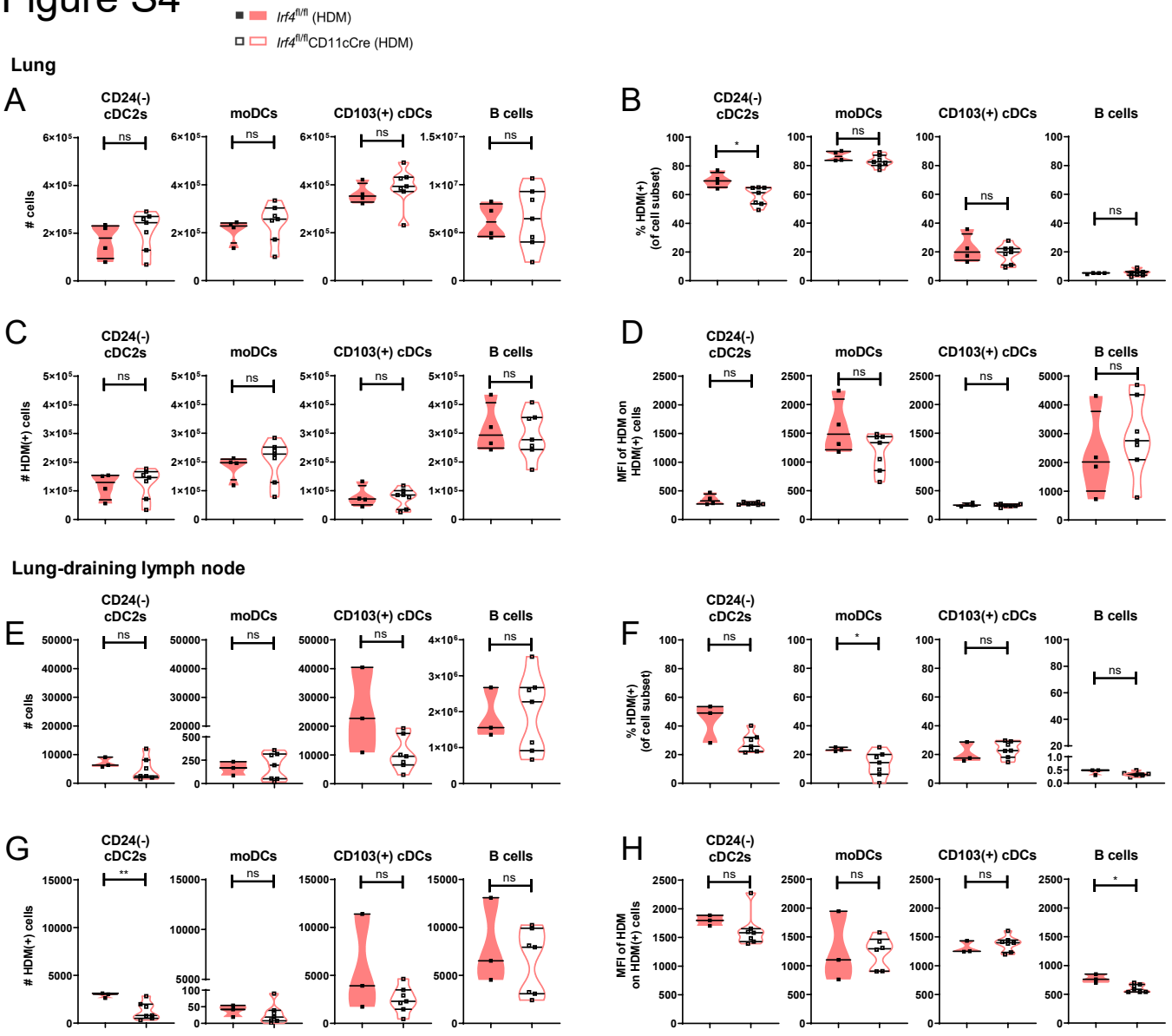

Figure S4, related to Fig. 4: (**A**) Number of lung CD24(-) cDC2s, moDCs, CD103(+) cDCs, and B cells (**B**) HDM uptake by lung CD24(-) cDC2s, moDCs, CD103(+) cDCs, and B cells, (**C**) Number of HDM-bearing lung CD24(-) cDC2s, moDCs, CD103(+) cDCs, and B cells (**D**) MFI of HDM on HDM-bearing lung CD24(-) cDC2s, moDCs, CD103(+) cDCs, and B cells (**E**) Number of LN CD24(-) cDC2s, moDCs, CD103(+) cDCs, and B cells (**F**) HDM uptake by LN CD24(-) cDC2s, moDCs, CD103(+) cDCs, and B cells, (**G**) Number of HDM-bearing LN CD24(-) cDC2s, moDCs, CD103(+) cDCs, and B cells (**H**) MFI of HDM on HDM-bearing LN CD24(-) cDC2s, moDCs, CD103(+) cDCs, and B cells. Data are representative of at least 2 independent experiments with n ≥ 4 mice per group, and statistics (unpaired t test with Welch's correction) were performed in GraphPad Prism. Bar represents the mean ± SEM (*, $p < 0.05$; **, $p < 0.01$; ns, not significant).

Figure S5

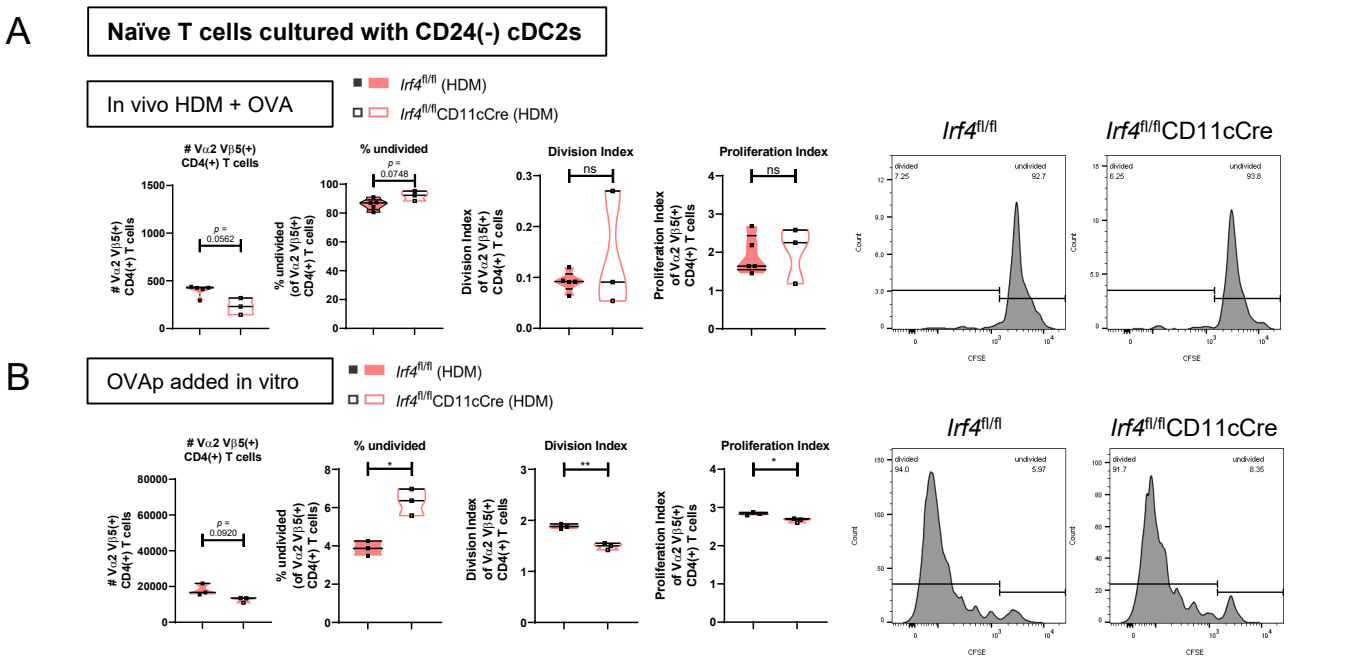

Figure S5, related to Fig. 5: CD24(-) cDC2s are poor stimulators of naïve T cells in response to HDM. CD24(-) cDC2s were sorted from the lungs on day 1 following in vivo sensitization to HDM + OVA, then were cocultured with CFSE-labeled T cells from naïve OTII mice. Depicted are the number of OTII cells after culture, % undivided, division index, proliferation index, and CFSE dilution histograms for (**A**) in vivo HDM + OVA sensitized CD24(-) cDC2s or (**B**) with $OVA_{323-339}$ peptide added. Data are representative of at least 2 independent experiments with n ≥ 3 wells per group, and statistics (unpaired t test with Welch's correction) were performed in GraphPad Prism. Bar represents the mean ± SEM (*, $p < 0.05$; **, $p < 0.01$; ns, not significant).

Figure S6

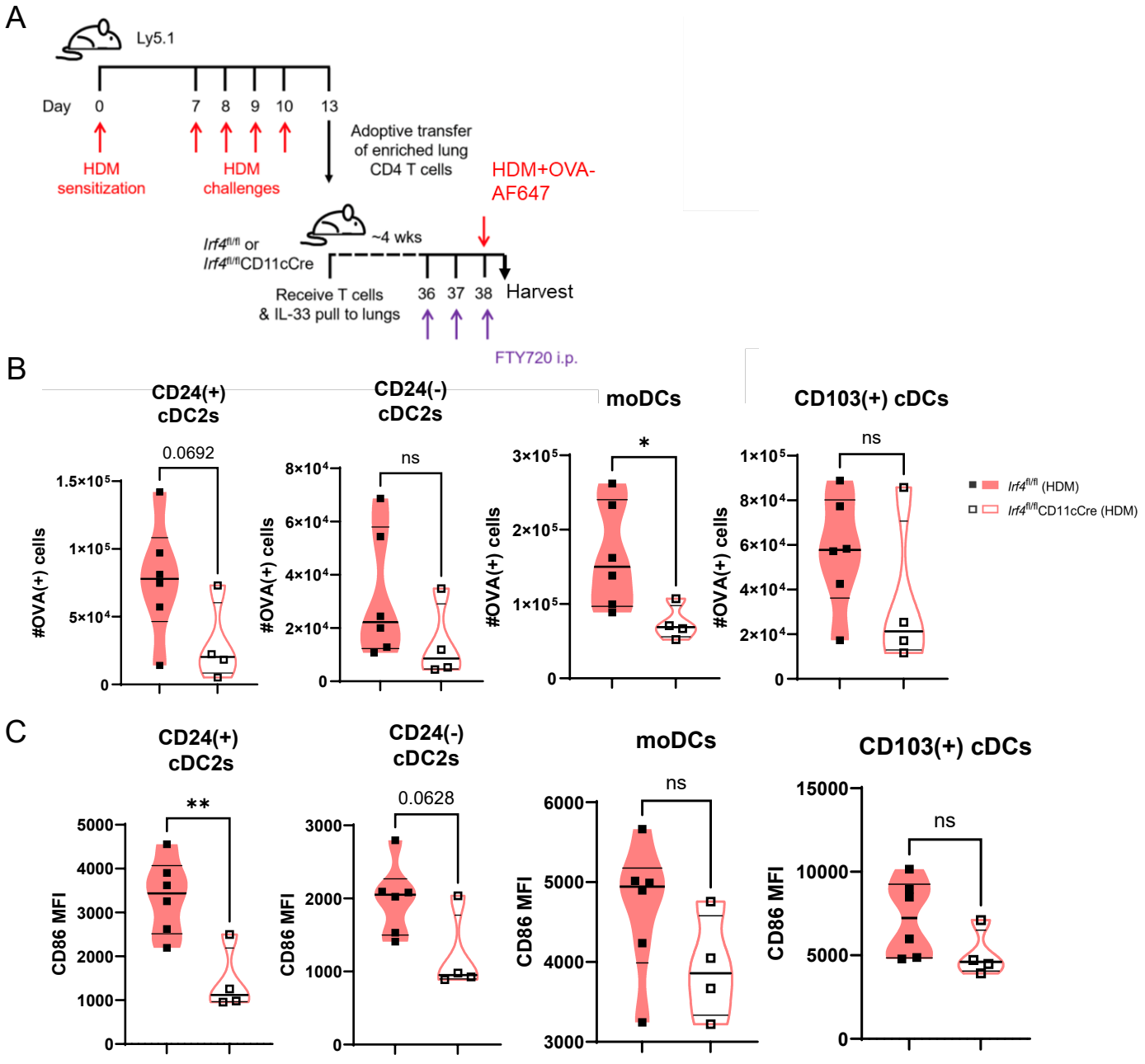

Figure S6, related to Fig. 6: (**A**) Schematic depicting experimental protocol. After sensitization and challenge with HDM, lung CD4 T cells were adoptively transferred into naïve Ly5.1(+) recipients and recruited to the lung via i.t. administration of IL-33. After resting for 4 weeks, mice received three doses of FTY720 before i.t. administration of HDM and OVA-AF647. Mice were sacrificed 18h after HDM+OVA treatment. (**B**) Number of OVA-bearing lung CD24(+) cDC2s, CD24(-) cDC2s, moDCs, and CD103(+) cDCs, (**C**) CD86 expression by lung CD24(+) cDC2s, CD24(-) cDC2s, moDCs, and CD103(+) cDCs. Data are from one experiment with n ≥ 4 mice per group, and statistics (unpaired t test with Welch's correction) were performed in GraphPad Prism. Bar represents the mean ± SEM (*, $p < 0.05$; **, $p < 0.01$; ns, not significant).

Supplementary Table 1

Anti mouse	Clone	Manufacturer(s)
CD3	145-2C11	BD Biosciences, Biolegend
	17A2	Biolegend
CD4	GK1.5	BD Biosciences
	RM4-5	Biolegend
CD8	53-6.7	Biolegend
CD11a	H155-78	Biolegend
CD11b	M1/70	BD Biosciences, Biolegend
CD11c	N418	Biolegend
CD19	6D5	Biolegend
CD24	M1/69	BD Biosciences, Biolegend
CD44	IM7	Biolegend
CD45	30-F11	Biolegend
CD45.1	A20	Biolegend
CD45.2	104	Biolegend
CD62L	MEL-14	eBioscience, Biolegend
CD69	H1.2F3	eBioscience, BD Biosciences, Biolegend
CD80	16-10A1	Biolegend
CD86	GL-1	Biolegend
CD88	20/70	BD Biosciences, Biolegend
CD103	2E7	eBioscience, Biolegend
CCR7	4B12	eBioscience
Foxp3	FJK-16s	eBioscience
GATA3	16E10A23	Biolegend
γ/δ TCR	GL3	Biolegend
IL-4	11B11	eBioscience, Biolegend
IL-5	TRFK5	Biolegend
IL-13	eBio13A	eBioscience
I-A/I-E	M5/114.15.2	Biolegend
RORγt	Q31-378	BD Biosciences
SiglecF	E50-2440	BD Biosciences
ST2	DIH4	Biolegend
Va2 TCR	B20.1	eBioscience, BD Pharmingen
Vβ5.1, 5.2 TCR	MR9-4	eBioscience, BD Pharmingen
PE conjugated streptavidin		eBioscience
Brilliant Violet 711 streptavidin		Biolegend
APC/Cy7 streptavidin		Biolegend

HVTN 405/HPTN 1901 Study Team

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