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Supplemental information

Natural killer cells and dendritic epidermal $\gamma\delta$ T cells orchestrate type 1 conventional DC spatiotemporal repositioning toward $CD8^+$ T cells

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Supplementary material:

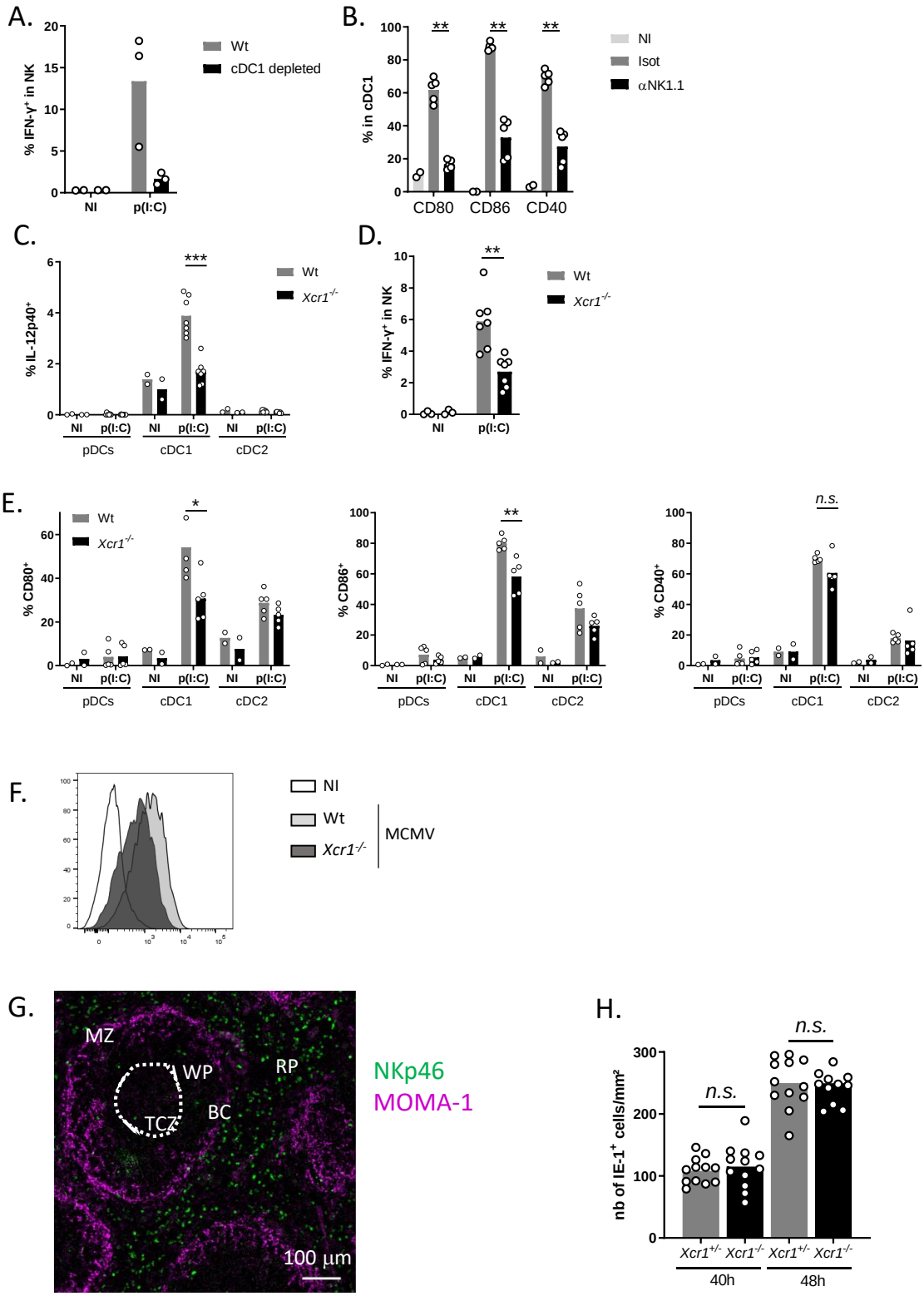


Figure S1. XCR1 promotes mutual activation of cDC1 and NK cells upon poly(I:C) administration, Related to Figure 1: A) IFN-g production by NK cells in cDC1-depleted mice 3h post-poly(I:C) (p(I:C)) injection. *Karma-tdTomato-hDTR* (cDC1 depleted) and Wt controls were injected with 32ng/g of body weight of DT 24h prior poly(I:C) administration. One experiment representative of two independent ones with 3 mice per injected group is shown. NI, non-injected. **B)** cDC1 maturation in the spleen of $NK1.1^+$ cell-depleted animals 20h post poly(I:C) administration. Two independent experiments with 2-3 mice per group were pooled. Isot, Isotype; αNK1.1, anti-NK1.1; **, $p<0.01$. **C-D)** IL-12p40 induction in pDCs, cDC1 and cDC2 **(C)** and IFN-g production by NK cells **(D)** in Wt vs *Xcr1*-deficient spleens 3h post-poly(I:C) administration. Splenic DCs ($CD19^-TCR\beta^-NK1.1^-CD11c^{lo/hi}$) were gated to define pDCs ($CD11c^{lo}SiglecH^+$), cDC1 ($CD11c^{hi}SiglecH^-CD8a^+CD11b^-$) and cDC2 ($CD11c^{hi}SiglecH^-CD8a^-CD11b^+$). Two independent experiments with at least 3 mice per injected group were pooled. NI, non-injected. ***, $p<0.001$; **, $p<0.01$. **E)** Analysis of DC maturation in Wt vs *Xcr1*-deficient spleens 20h post poly(I:C) administration. Two independent experiments with three mice per injected group were pooled. NI, non-injected. *, $p<0.05$; **, $p<0.01$, *n.s.*, non-significant. **F**) Representative histograms of CD69 expression in splenic NK cells of non-infected (NI), or of $Xcr1^{-/-}$ and Wt mice 40h post-MCMV infection. **G**) Spleen sections of steady state $Karma^{Cre};Rosa26^{tdRFP}$ were stained for MOMA-1/CD169 (purple) and NKp46 (green). RP, red pulp; MZ, marginal zone; WP, white pulp; BC, bridging channel; TCZ, T cell zone. Scale bar: 100mm. One image representative of 12 analyzed mice from 6 independent experiments is shown. **H**) Quantification of IE-1-expressing cells on whole spleen sections of XCR1-deficient mice and their littermate controls. For 40h, three independent experiments with 4 mice per group were pooled. For 48h, two independent experiments with 6 mice per group were pooled. n.s., non-significant.

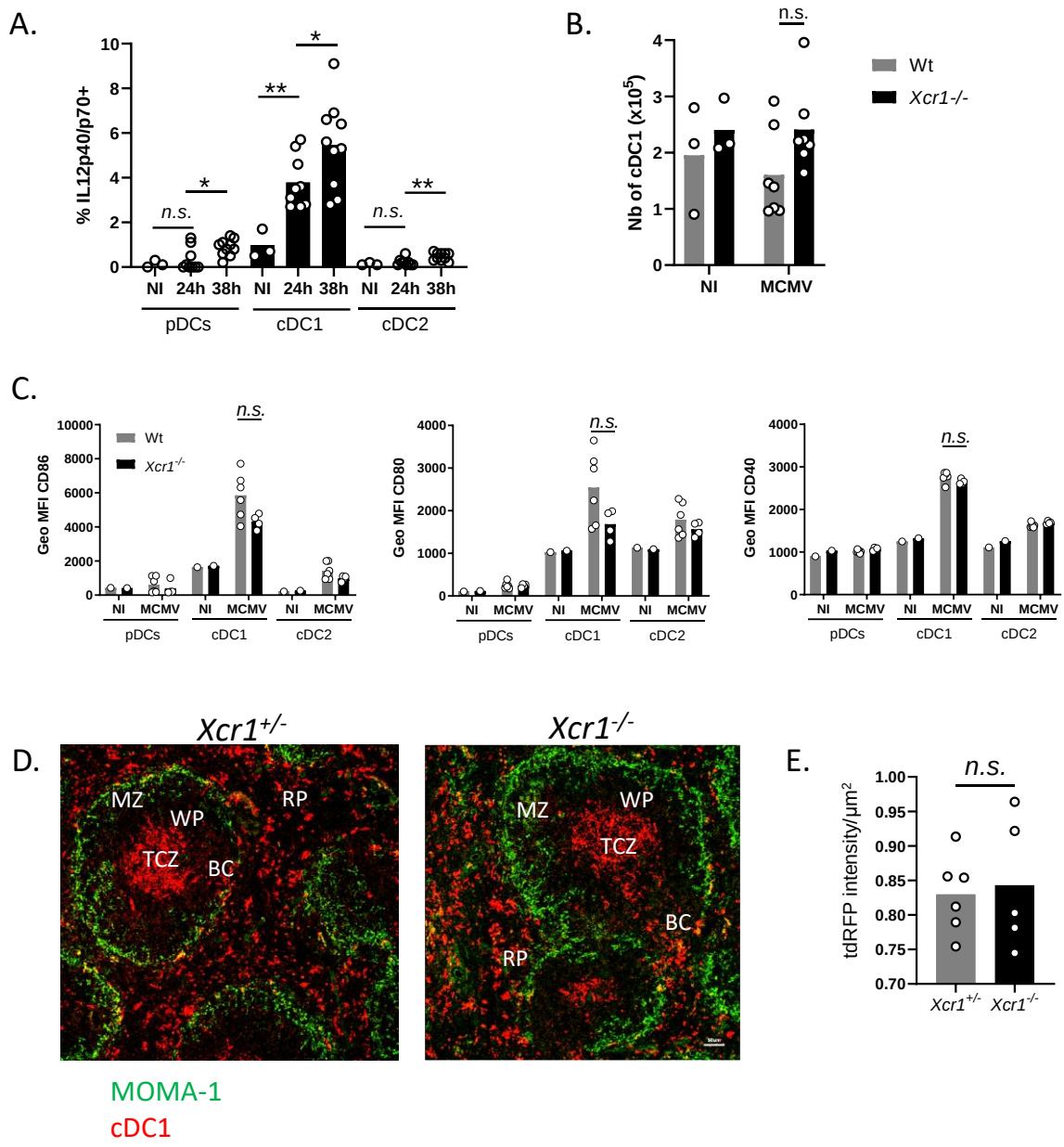


Figure S2. Analysis of IL-12 production by cDC1 upon MCMV and of cDC1 localization in the spleen at steady state, Related to Figure 2: A) Spleens were harvested at 24 and 38h post MCMV infection to analyze IL-12p40/p70 production by DCs in Wt animals. Three independent experiments with at least 3 mice per infected group were pooled. NI, non-infected; *n.s.*, non-significant; *, $p<0.05$; **, $p<0.01$**. B**) Absolute number of cDC1 in the spleen of Wt and *Xcr1*$^{-/-}$ mice at steady state and 40h post-MCMV infection. **C**) Analysis of CD86, CD80 and CD40 expression by splenic DCs of *Xcr1*$^{-/-}$ and Wt animals 48h post-infection. One

experiment representative of at least 4 independent ones with at least 4 mice per infected group is shown. NI, non-infected; *n.s.*, non-significant; *, $p<0.05$. **D**) Analysis of cDC1 distribution in the spleen of *Xcr1*$^{-/-}$ and littermate controls at steady state. *Karma*^{Cre}*;Rosa26*^{tdRFP} spleen sections were stained for MOMA-1/CD169 (green) and tdRFP (red). RP, red pulp; MZ, marginal zone; WP, white pulp; BC, bridging channel; TCZ, T cell zone. One image representative of 12 analyzed mice from 6 independent experiments is shown. **E**) Mean fluorescent intensity of tdRFP per μm^2 quantified on whole spleen sections analyzed in Fig. S2D (steady state). Two independent experiments with 2-3 mice per infected group were pooled. *n.s.*, non-significant.

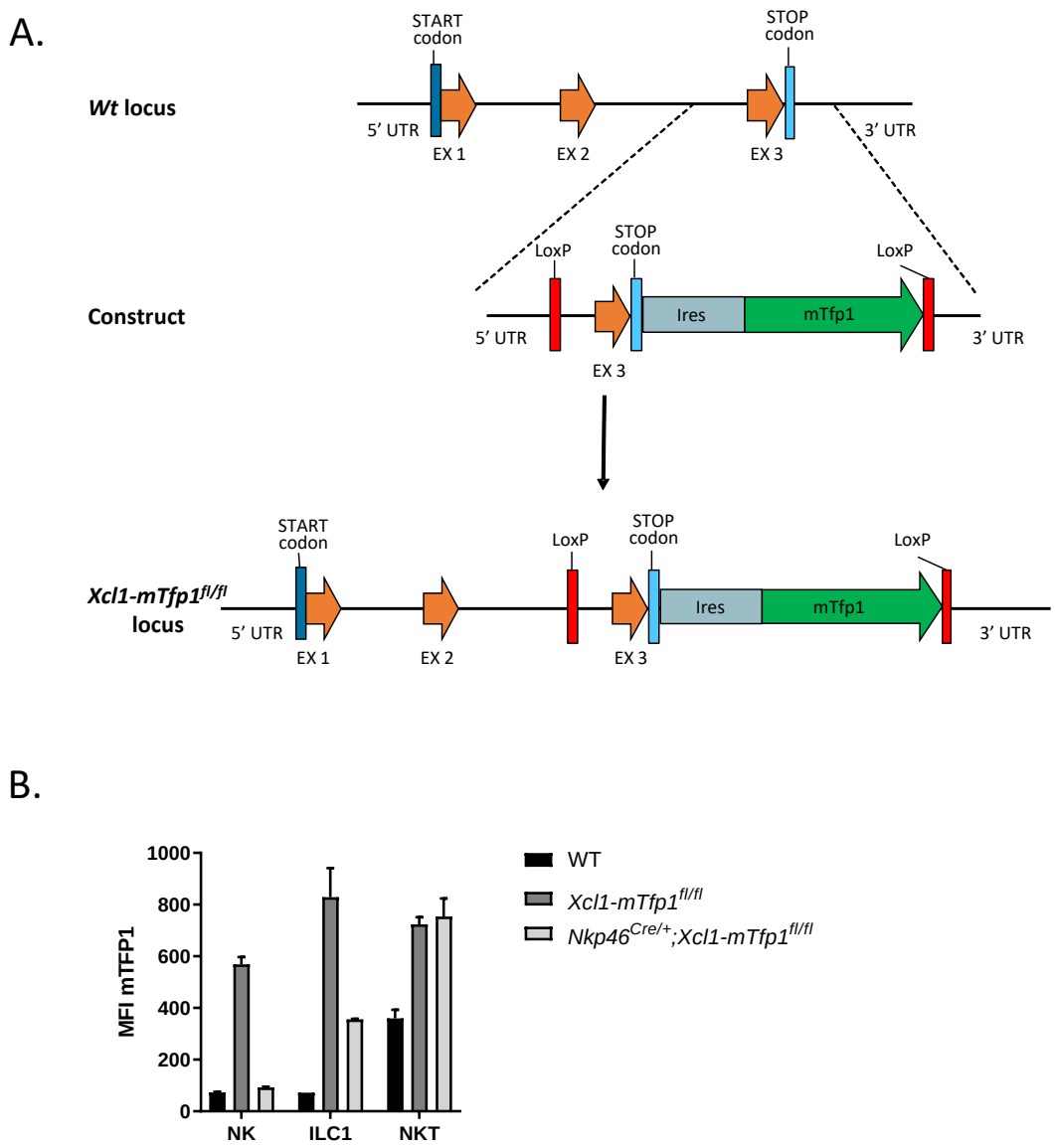
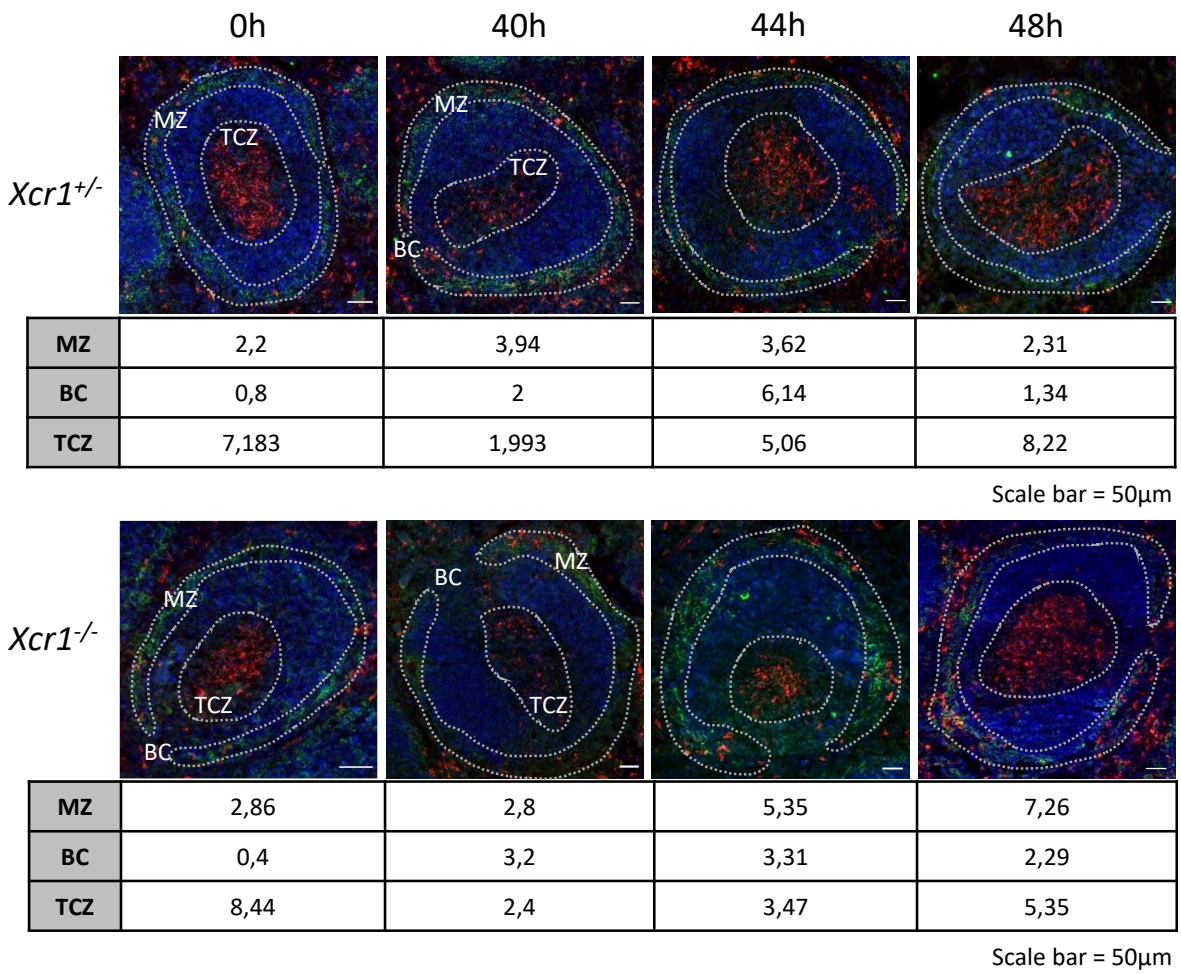


Figure S3. Construction of *Xcl1-mTfp1*$^{fl/fl}$ mice and analysis of *Xcl1* inactivation in ILCs, Related to Figure 3: A) The *Xcl1-mTfp1*$^{fl/fl}$ mouse model was generated by inserting a LoxP-exon3-IRES-mTfp1-LoxP cassette in frame of the *Xcl1* gene. The *mTfp1* gene codes for the monomeric Teal fluorescent protein mTFP1 (Ex:462; Em:492). In this mouse, the expression of the *Xcl1* locus resulted in an *Xcl1-Ires-mTfp1* transcript, which led to the production of two distinct proteins: XCL1 and mTFP1. **B**) Analysis of the geometric mean of the mTFP1 fluorescence in ILCs of Wt, *Xcl1-mTfp1*$^{fl/fl}$ and *Nkp46*$^{Cre/+}$*;Xcl1-mTfp1*$^{fl/fl}$ mice. One experiment representative of two independent ones with at least 2 mice per group is shown. Error bars represent standard deviations.



	0h	40h	44h	48h
MZ	2,2	3,94	3,62	2,31
BC	0,8	2	6,14	1,34
TCZ	7,183	1,993	5,06	8,22

Scale bar = 50µm

	0h	40h	44h	48h
MZ	2,86	2,8	5,35	7,26
BC	0,4	3,2	3,31	2,29
TCZ	8,44	2,4	3,47	5,35

Scale bar = 50µm

Figure S4. tdRFP (cDC1) quantification in different regions of the spleen during MCMV infection, Related to Figure 4: Whole spleen sections of *Karma^{Cre};Rosa26^{tdRFP};Xcr1$^{-/-}$* mice and *Karma^{Cre};Rosa26^{tdRFP};Xcr1$^{+/-}$* controls were prepared at indicated time after MCMV infection, and stained for MOMA-1/CD169 (green), B220 (dark blue), tdRFP (red) and nuclei (not shown), before being scanned with Pannoramic Scan slide scanner. The tdRFP intensity per pixel^2 quantified in each sample shown here has been reported in a table below each respective image. The quantification method has been detailed in the material and methods. MZ, marginal zone; TCZ, T cell zone; BC, bridging channel.

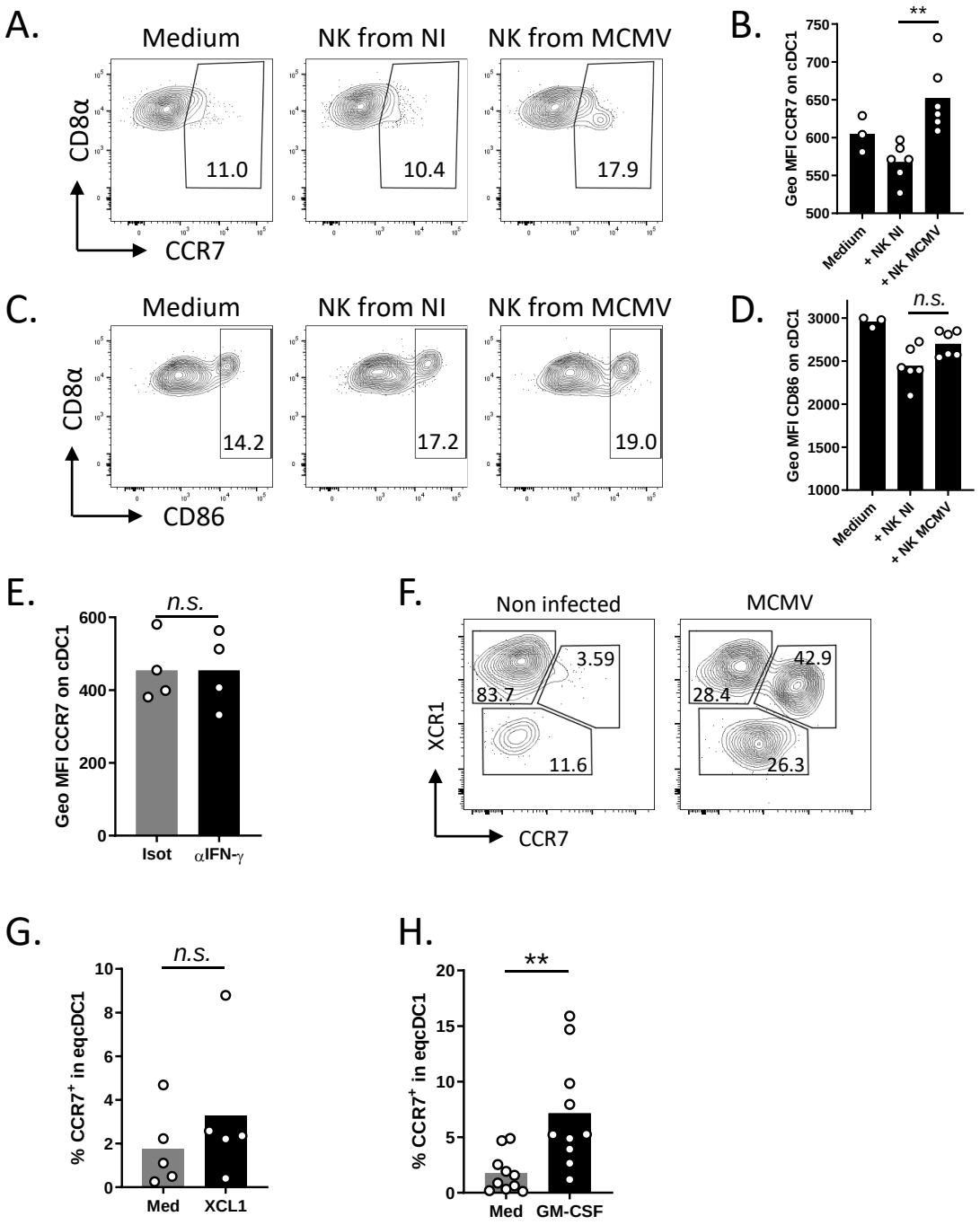


Figure S5. Activated NK cells and GM-CSF upregulate CCR7 expression on cDC, Related to Figure 5: (A-D) Analysis of CCR7 (**A-B**) and CD86 (**C-D**) expression on cDC1 after 7h of co-culture with splenic NK cells from mice infected for 40h with MCMV. One representative experiment of two independent ones with at least four mice per group is shown. NI, non-infected; *n.s.*, non-significant; **, p<0.01. **E**) CCR7 expression on cDC1 after *in vivo* blocking of IFN-g, 48h post MCMV. One experiment representative of two independent ones with 4

mice per group is shown. Isot, Isotype. *n.s.*, non-significant. **F**) CCR7 and XCR1 expression analysis on splenic cDC1 48h after MCMV infection. One experiment representative of 3 independent ones with at least 3 mice per infected group is shown. **G**) CCR7 expression on eqcDC1 differentiated from FLT3-L-cultured BM cells 6h after treatment with recombinant XCL1. Two experiments each with 2-3 mice per group were pooled. *n.s.*, non-significant. **H**) Analysis of CCR7 induction on eqcDC1 differentiated from FLT3-L-cultured BM cells 6h after treatment with recombinant GM-CSF. Three experiments each with 3-4 mice per group were pooled. **, $p<0.01$. Error bars represent standard deviations.

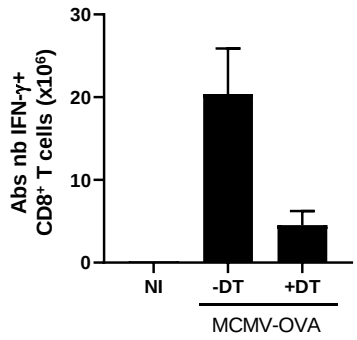


Figure S6. cDC1 are critical for the expansion of virus-specific CD8$^+$ T cells, Related to Figure 6: Analysis of OVA-specific CD8$^+$ T cell expansion in cDC1-depleted (+DT) *vs* non-depleted (-DT) mice 5 days after MCMV-OVA administration (2 x 10^5 PFU/mouse). DT was administrated to *Karma-tdTomato-hDTR* mice 1d prior to infection, and 2.5 d after. Splenocytes were incubated for 4h with SIINFEKL peptide before intracellular IFN-g staining. One representative experiment of two independent ones with at least 3 mice per group is shown. Data are represented as mean (+/- SD). NI, non-infected.

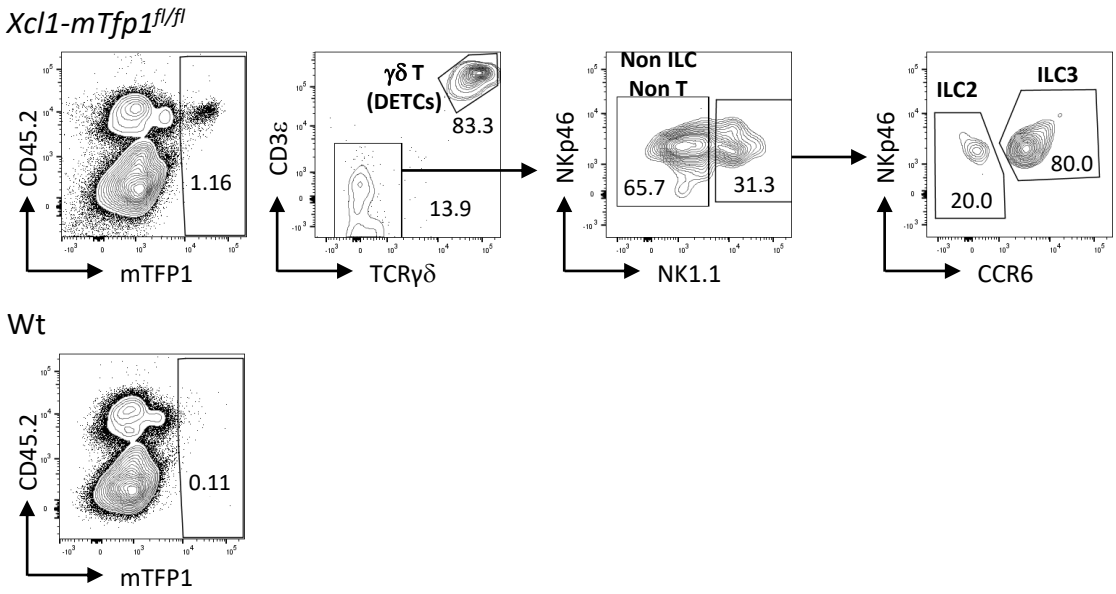


Figure S7. Identification of *Xcl1* expressing cells in skin of *Xcl1-mTfp1*$^{fl/fl}$ mice, Related to Figure 7: One representative experiment of four independent ones with at least 3 mice is shown.

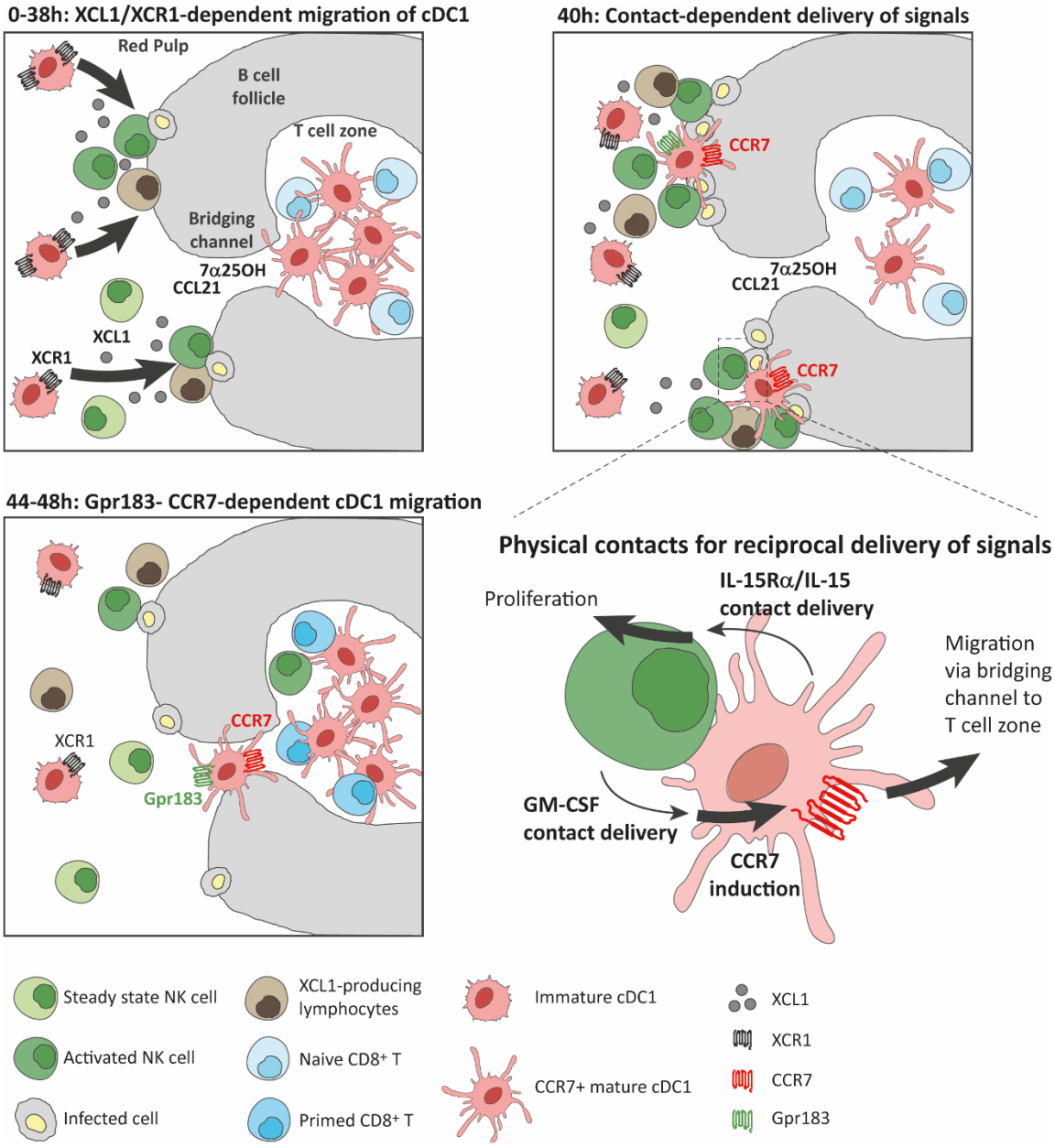


Figure S8. Fine regulation of cDC1 repositioning within the spleen requires physical interactions with NK cells in the marginal zone and reciprocal signal delivery to accelerate the priming of antiviral CD8⁺ T cell responses, Related to Figures 1 to 6. Within 38h following viral infection, NK cells and innate-like lymphocytes, positioned near infected cells in the marginal zone, attract red pulp immature cDC1 through XCL1/XCR1 axes. At 40h, the formation of superclusters of activated NK cells and cDC1 in the marginal zone favors physical interactions between these two cell actors, feeding a reciprocal positive autoregulation loop, wherein cDC1 deliver IL-12 and IL-15/IL-15Rα signals to NK cells, promoting their GM-CSF

production and expansion respectively. In return, NK cell delivery of GM-CSF to cDC1 triggers CCR7 expression, licensing cDC1 to migrate to the T cell zone. Between 44h and 48h post infection, besides CCR7, cDC1 also acquire the expression of the chemokine receptor Gpr183, which recognizes the bridging channel guiding cue 7α,25-dihydroxycholesterol (7α25OH). In T cell zone, $CCR7^{+}$ mature cDC1 prime naïve CD8+ T cells, promoting a rapid and efficient adaptive antiviral immune response.