SUPPLEMENTAL MATERIAL

Alexandre et al., http://www.jem.org/cgi/content/full/jem.20142350/DC1

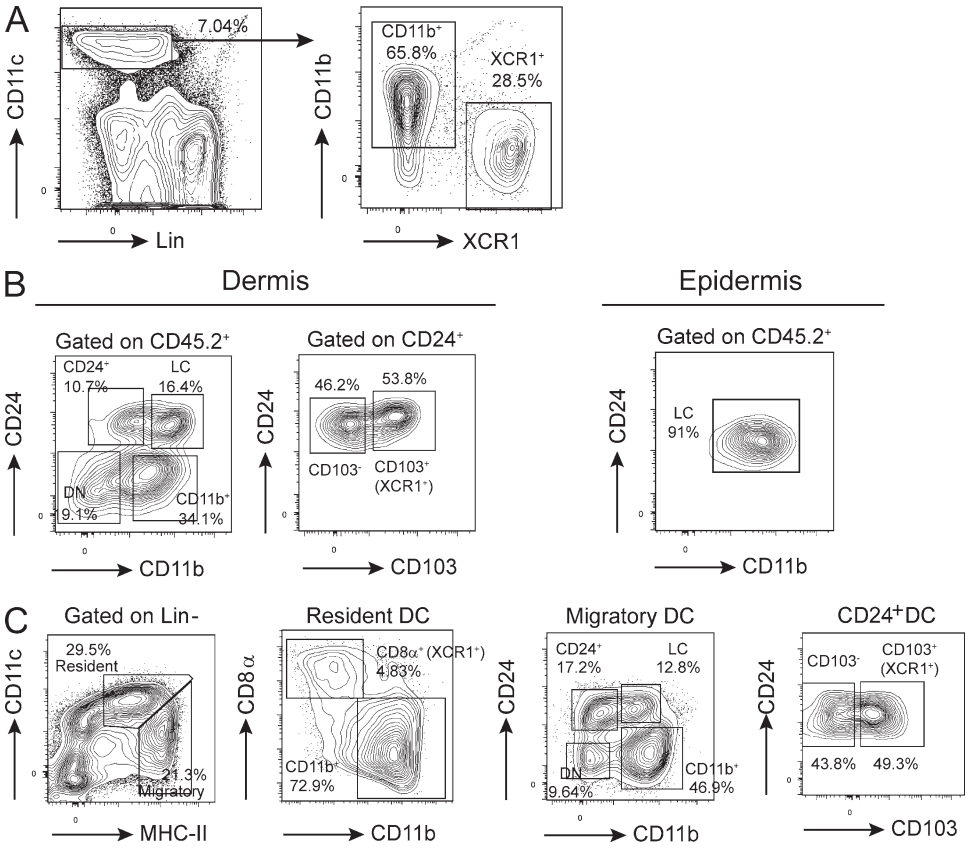

Figure S1. **Gating strategies used to examine the tdTomato expression in DCs of *Karma* mice.** (A) Gating strategy applied to examine the tdTomato expression by DCs in spleen of *Karma* mice. In spleen, $Lin^{-}CD11c^{hi}$ cells are divided into $CD11b^{+}XCR1^{-}$ DCs and $CD11b^{-}XCR1^{+}$ or $XCR1^{+}$ DCs. One experiment representative of five, each with three mice per group, is shown. (B) Gating strategy applied to examine the tdTomato expression by DCs in the skin of *Karma* mice. Immune cells extracted from the skin are defined as $CD45.2^{+}$ cells. The epidermis harbors only Langerhans cells (LC), whereas the dermis includes four distinct DC subsets besides LC en route to the CLNs. (C) Gating strategy applied to examine the tdTomato expression by the lymphoid-resident conventional and migrating DCs subsets in the CLNs of *Karma* mice. This gating was applied on Lin^{-} ($CD3e^{-}CD19^{-}NK1.1^{-}$) cells. For skin and CLN, one experiment representative of three with three mice per group is shown.

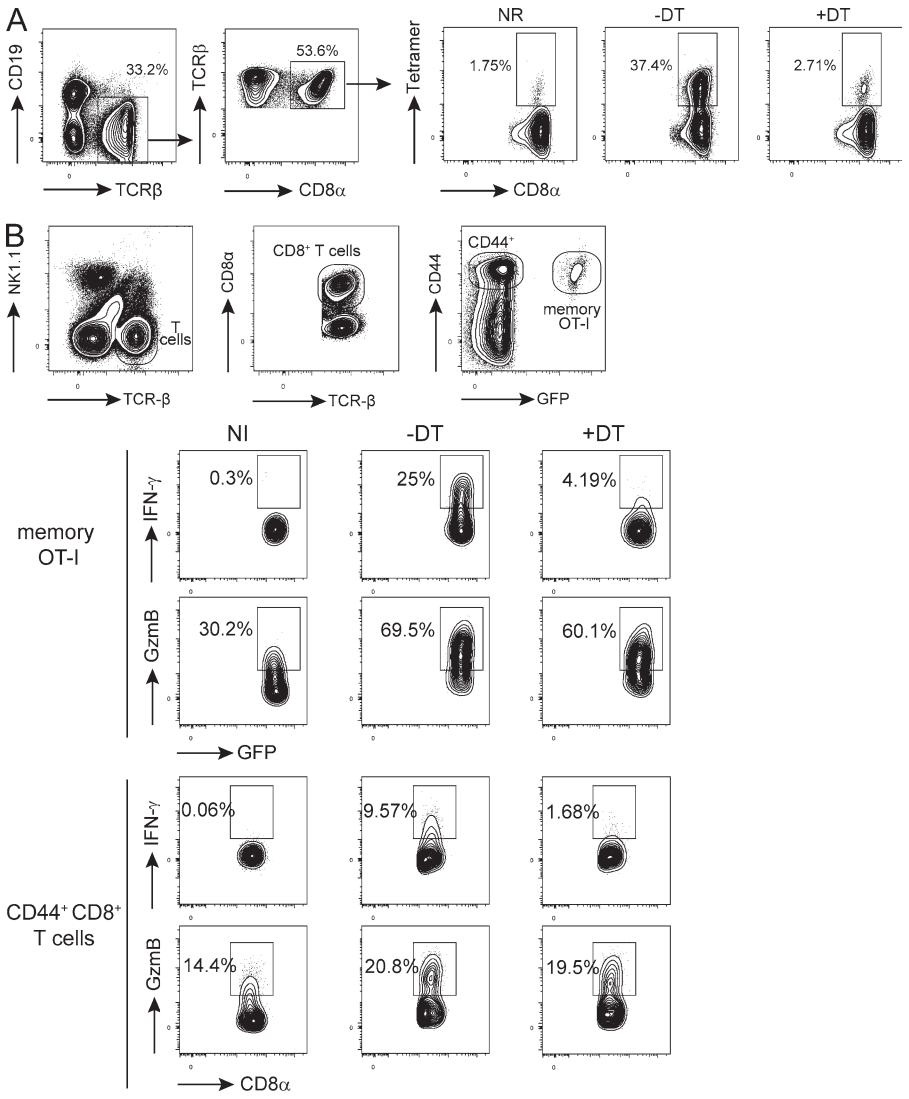

Figure S2. **Analysis of mCTL activation upon secondary challenge with *Lm-OVA*.** (A) Tetramer staining of splenocytes from memory mice 5 d after secondary challenge with *Lm-OVA*. This gating strategy was used for Fig. 3. (B) Analysis of IFN-γ production and granzyme B induction in OT-I mCTLs and polyclonal CD44^{hi} mCTLs 6 h after *Lm-OVA* secondary infection. One representative experiment out of seven, each with at least three mice per group, is shown.

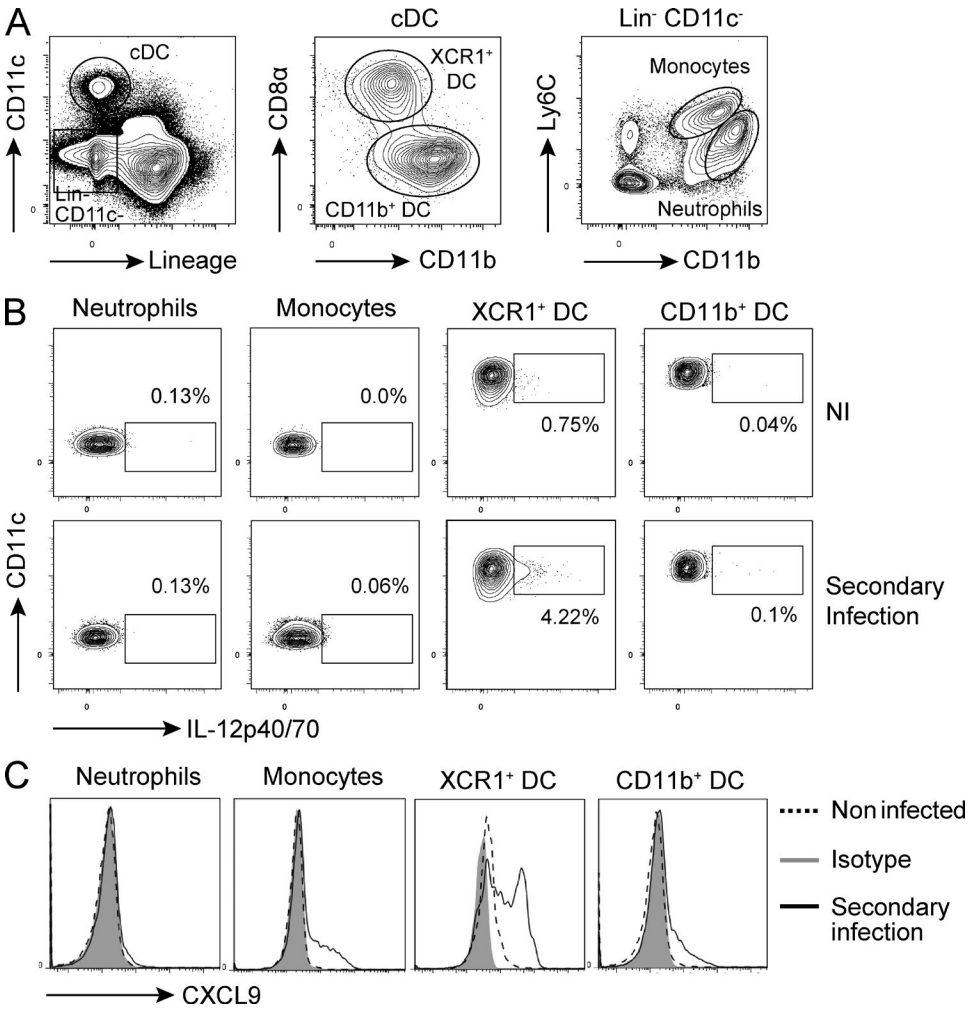

Figure S3. **IL-12 and CXCL9 expression analysis in DCs, monocytes, and neutrophils in spleens of infected animals.** (A) Gating strategy applied to identify splenic XCR1$^+$ DCs, CD11b$^+$ DCs, monocytes and neutrophils in infected animals. One experiment representative of three, each with three mice per group, is shown. (B) Example of IL-12p40/70 staining of splenic DCs, monocytes, and neutrophils of memory mice after *Lm-OVA* secondary infection. One experiment representative of three, with three mice per group is shown. (C) Example of CXCL9 staining of splenic DCs, monocytes, and neutrophils of memory mice 6 h after *Lm-OVA* secondary infection. Histogram overlays are from one experiment representative of two, with three mice per group.

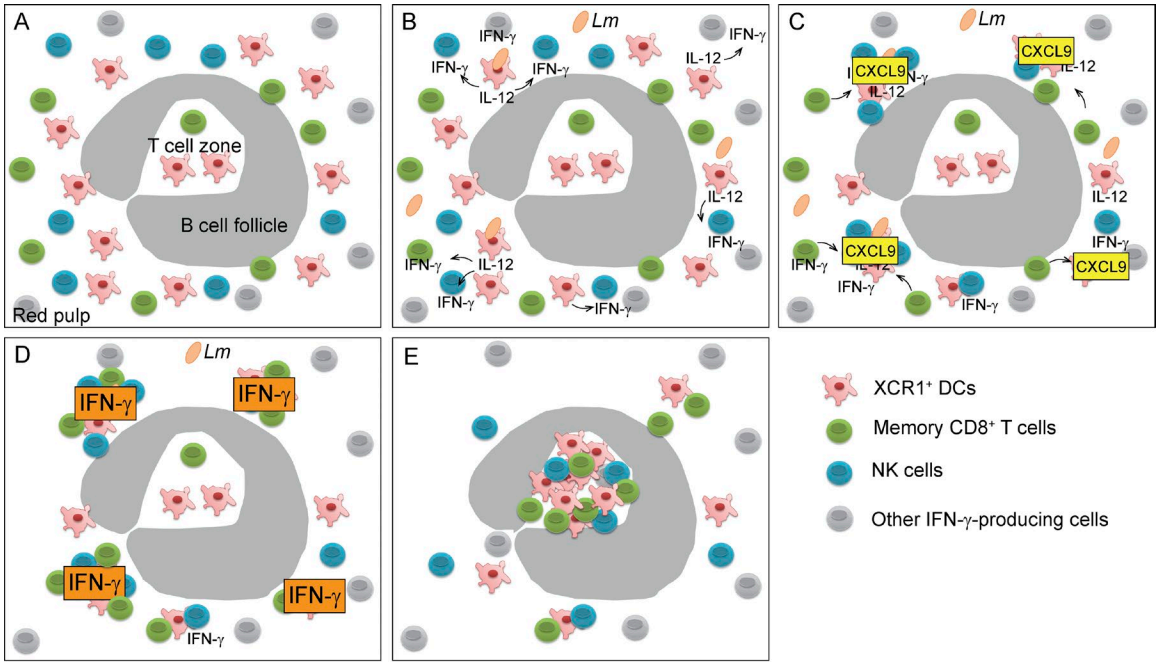

Figure S4. **Schematic model of the recall of mCTLs by XCR1⁺ DCs.** (A) In memory mice, XCR1⁺ DCs reside in the red pulp and the T cell zone whereas NK cells and mCTLs mostly reside in the red pulp. (B) Upon secondary infection with *Lm-OVA*, XCR1⁺ DCs respond swiftly by producing IL-12, which initiates the early production of IFN-γ by NK, mCTLs, and other cells. (C) NK cells and most likely other IFN-γ producers are attracted toward XCR1⁺ DCs, and the early IFN-γ acts on XCR1⁺ DCs amplifying IL-12 and triggering CXCL9 production. The engagement of the receptor for CXCL9, CXCR3, on RP-residing mCTLs further triggers their mobilization toward the edges of the marginal zone into cell clusters encompassing XCR1⁺ DCs and NK cells. (D) The formation of these cell clusters enhances the induction of IFN-γ locally for a few hours, generating a positive feedback loop enhancing the production of IL-12 and CXCL9. (E) By 24 h, both XCR1⁺ DCs, some NK cells and mCTLs migrate into the T cell zone most likely through the bridging channel.

Table S1. **Antibodies used in this study**

Antibody	Clones	Species	Experiment
B220	RA3-6B2	Rat	FC; IF
CCL3	Polyclonal	Goat	FC
CD3ε	145-2c11	Armenian Hamster	FC
CD3ε	500A2	Gold Syrian Hamster	FC
CD4	RM4-5	Rat	FC
CD8α	53-6.7	Rat	FC
CD11b	M1/70	Rat	FC; IF
CD11c	N418	Rat	FC
CD19	1D3	Rat	FC
CD24	M1/69	Rat	FC
CD103	2E7	Armenian Hamster	FC
CXCL9	MIG-2F5.5	Armenian Hamster	FC; IF
CXCR3	CXCR3-173	Armenian Hamster	FC; neutra
F4/80	BM8	Rat	FC
FITC	Polyclonal	Rabbit	IF
GFP	Polyclonal	Rabbit	IF
granzyme B	GB11	Mouse	FC
GR1	RB6-8C5	Rat	FC
IFN-γ	XMG1.2	Rat	FC; IF; neutra
IL-12p40/70	C15.6	Rat	FC
IL-12p40/70	C17.8	Rat	neutra
Isotype CXCR3	Polyclonal	Armenian Hamster	neutra
Isotype IL-12	2A3	Rat	neutra
Isotype IFN-γ	HRPN	Rat	neutra
Ly6C	AL-21	Rat	FC
Ly6G	1A8	Rat	FC
MHC-II	AF6-120.1	Mouse	FC
NK1.1	PK136	Mouse	FC; depl
NKp46	29A1.4	Goat	IF
dsRed/RFP/tdTomato	Polyclonal	Rabbit	IF
SIGLEC-H	551	Rat	FC
SIRPα	P84	Rat	FC
TCR-β	H57-597	Armenian Hamster	FC
XCR1	ZET	Mouse	FC
Rabbit IgG	Secondary	Donkey	IF
Armenian Hamster IgG	Secondary	Goat	IF
Goat IgG	Secondary	Donkey	IF

depl, depletion; FC, flow cytometry; IF, immunofluorescence; neutra, neutralization.