

CXCL13 is essential for lymph node initiation and is induced by retinoic acid and neuronal stimulation.

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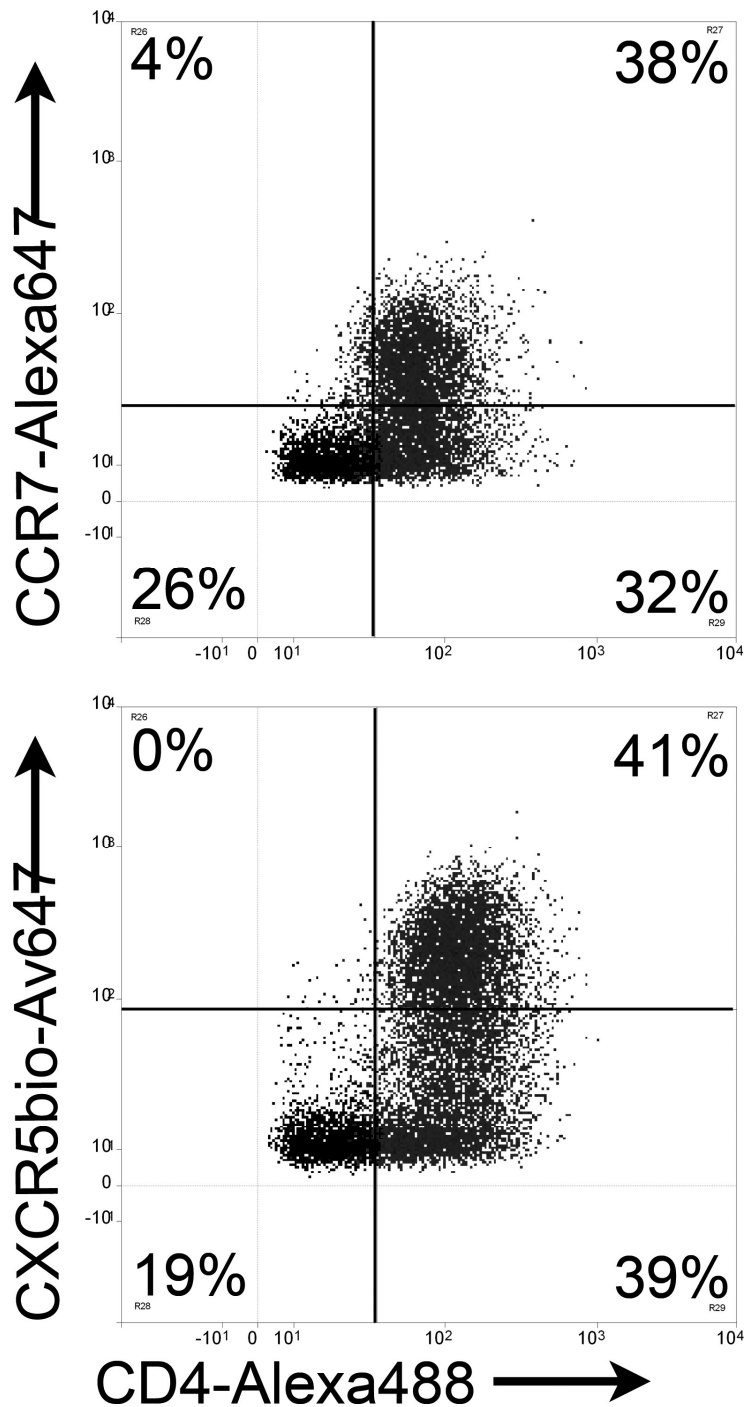
Supplementary Information

Factor	Model	Begin	Length	Sequence
_00000 RAR-beta	I00402 (RAR-beta)	-1671	6	tgacc
_00000 RAR-beta	I00402 (RAR-beta)	-70	6	gggtca
_00000 RAR-beta	I00402 (RAR-beta)	+001	6	tgacc
_00000 RAR-beta	I00402 (RAR-beta)	+255	6	tgacc

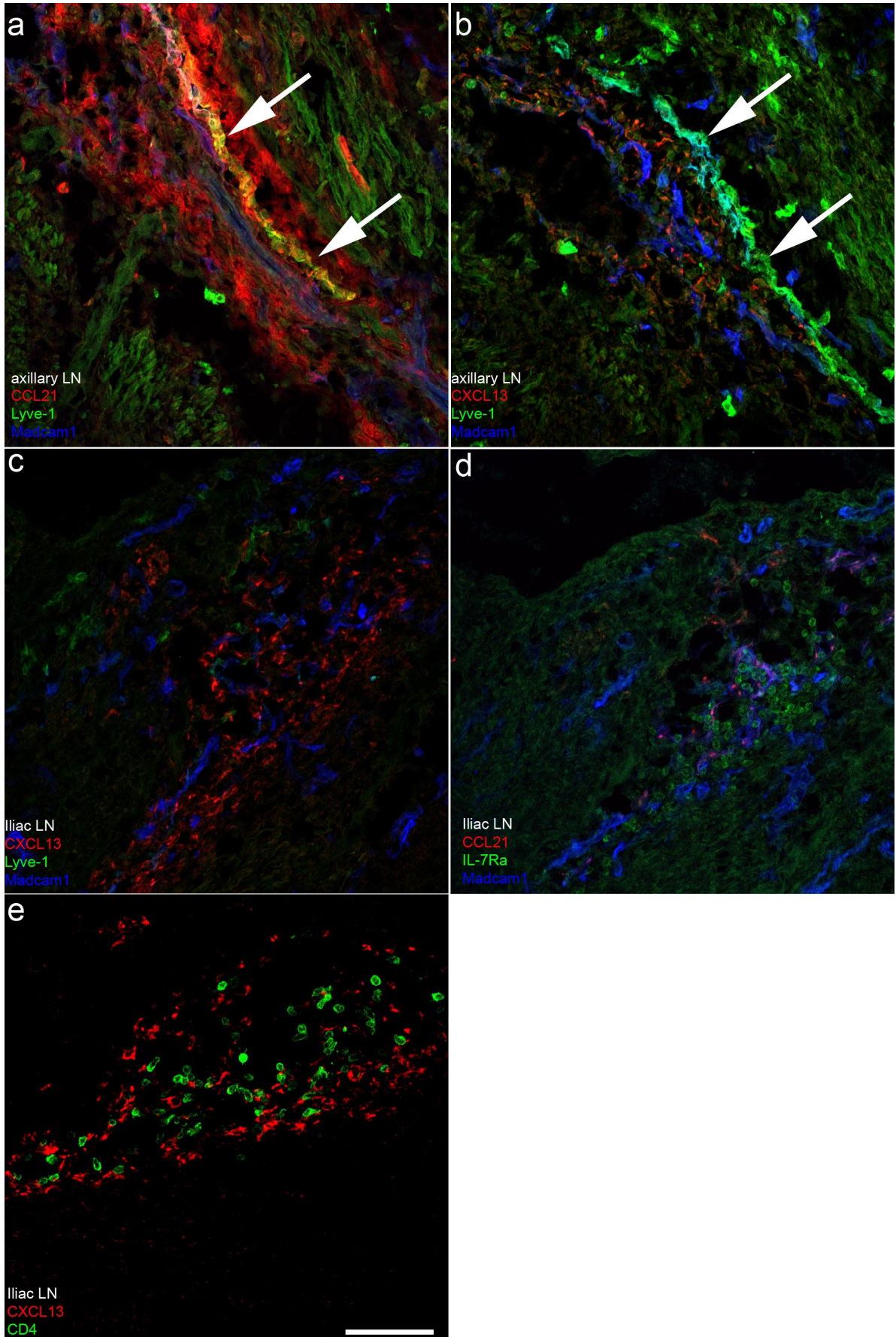
Supplementary Table 1: The 3000 bp upstream and 2000 bp downstream genomic region of *Cxcl13* contains RARE sites for only RAR β (<http://www.cbil.upenn.edu/cgi-bin/tess/tess>).

SUPPLEMENTARY TABLE 2. Oligonucleotides used for Real Time Quantitative PCR as described previously¹

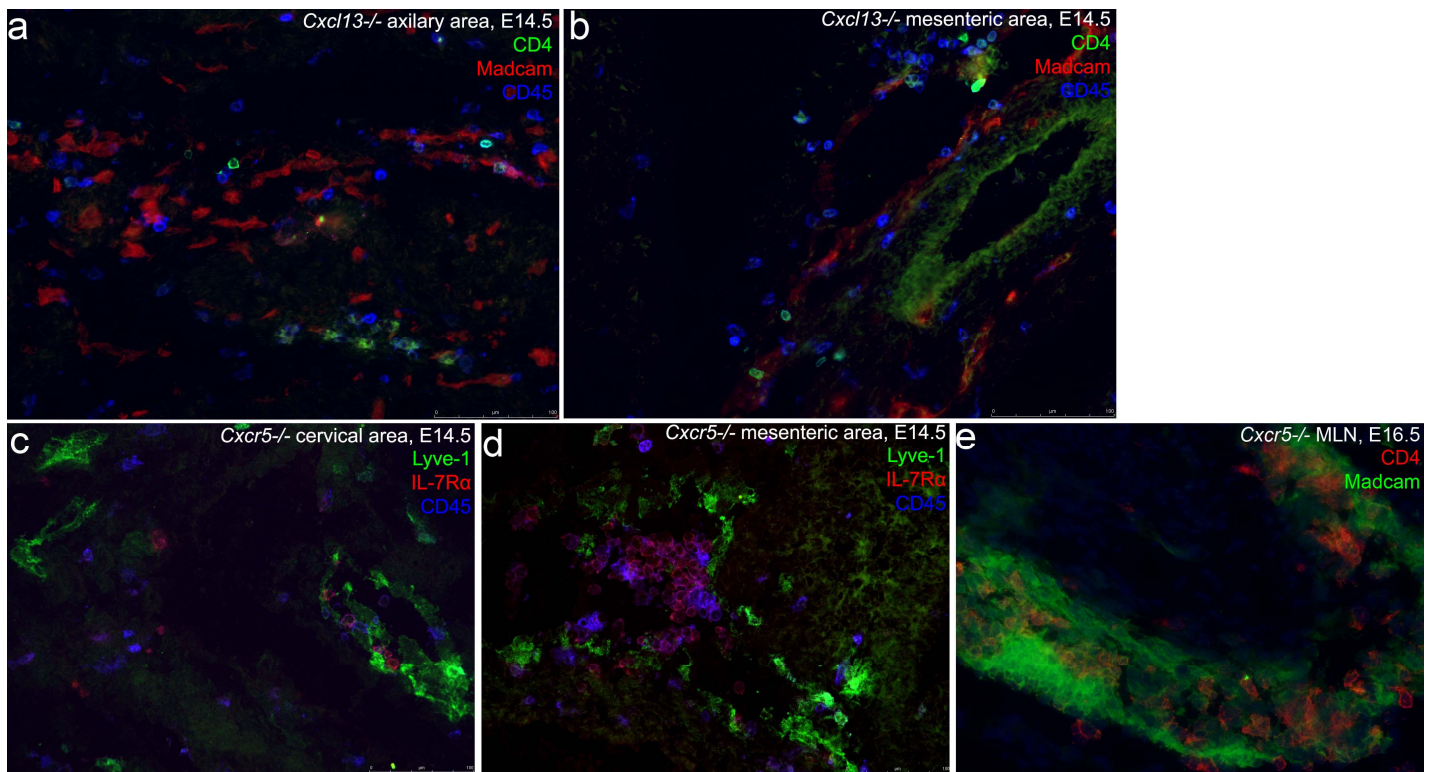
<i>Cyclo</i> forward	5'- ACCCATCAAACCATTTCCTTCTGTA -3'
Reverse	5'- TGAGGAAAATATGGAACCCAAAGA -3'
<i>Hprt</i> forward	5'- CCTAAGATGAGCGCAAGTTGAA -3'
Reverse	5'- CCACAGGACTAGAACACCTGCTAA -3'
<i>Vcam-1</i> forward	5'- ACTACAAGTCTACATCTCTCCCAGGAAT -3'
Reverse	5'- CCTCGCTGGAACAGGTCATT -3'
<i>Madcam-1</i> forward	5'- CTGGTGCTGACCCATAGAAAGG-3'
reverse	5'- GGCTCAGCAGAGGTCGTGTT-3'
<i>Prox-1</i> forward	5'- ATGTCCGACATCTCACCTTATTCA-3'
Reverse	5'- GCGGGTGTAAGAAGAACATGAGTT-3'
<i>Cxcl13</i> forward	5'- CATAGATCGGATTCAAGTTACGCC -3'
Reverse	5'- TCTTGGTCCAGATCACAATTCA -3'
<i>Ccl21</i> forward	5'- GCTGCAAGAGAACTGAACAGACA -3'
Reverse	5'- CGTGAACCACCCAGCTTGA -3'



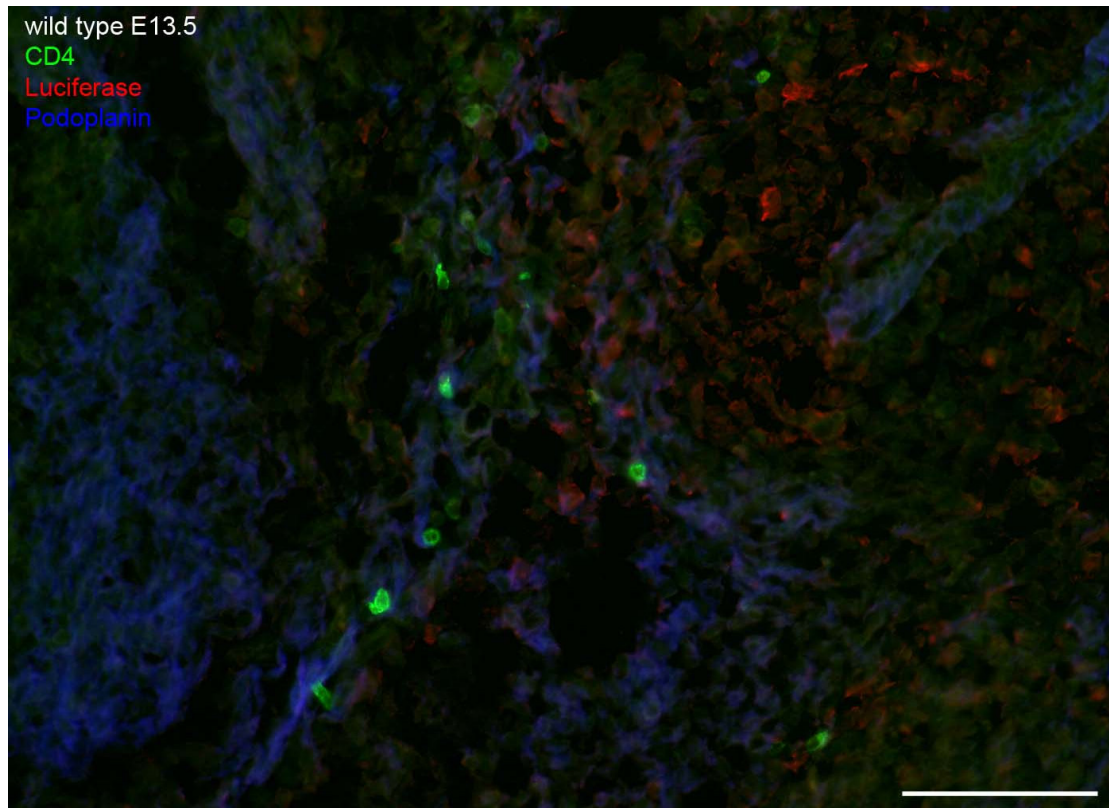
Supplementary Figure 1: CD4⁺CD45⁺ LTI cells express both CCR7 and CXCR5. Embryonic E13.5 cell suspensions, enriched for LNs, were stained for CCR7 (top) and CXCR5 (bottom) in combination with CD4 and CD45. Cell populations were gated for alive events and CD45 expression. As positive control for the individual chemokine receptors adult LNs were used (data not shown). Data shown is a representative figure of 4 individual experiments of 1 litter E13.5 embryos each.



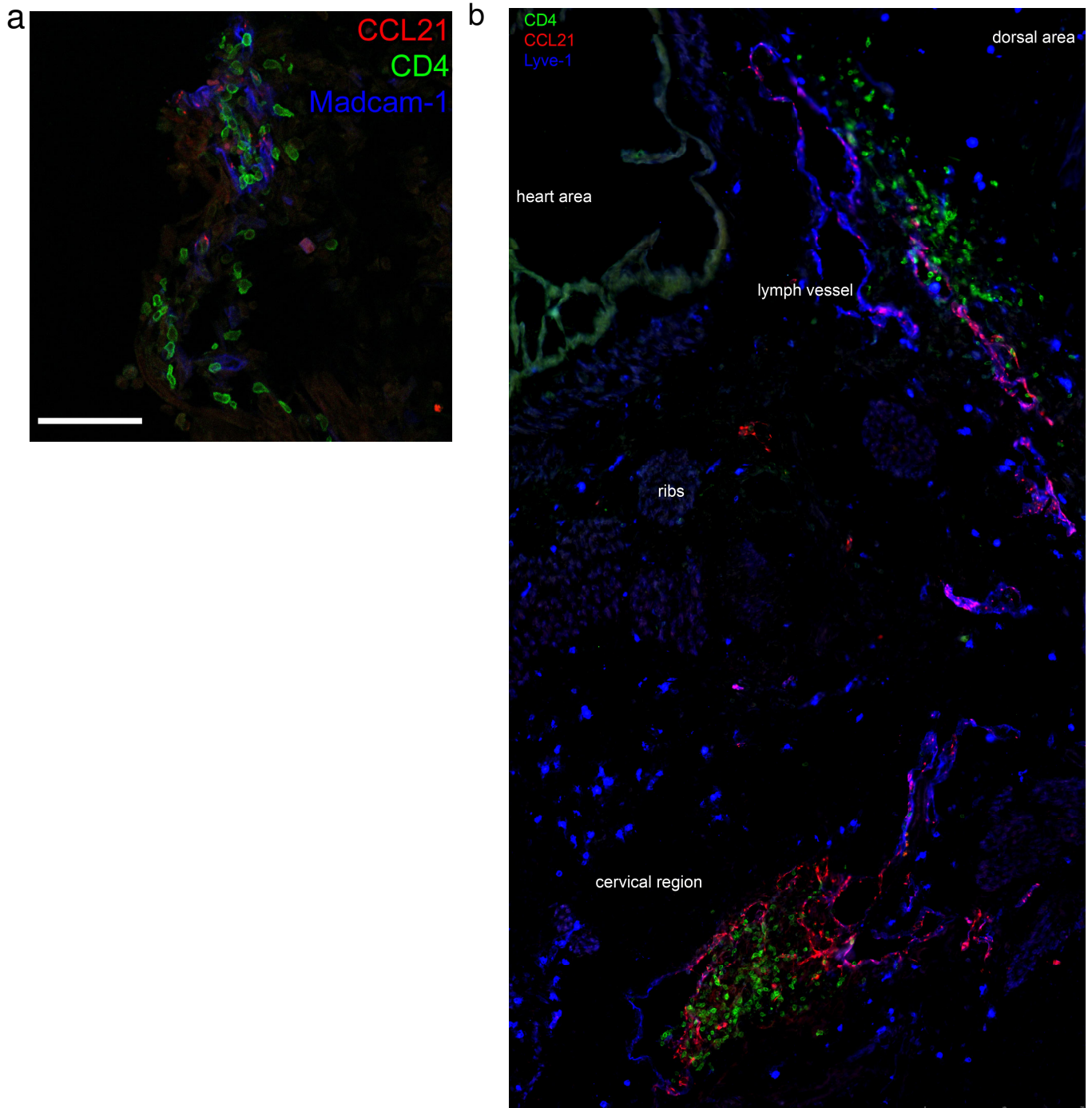
Supplementary Figure 2: CCL21 in LN anlagen at E13.5. (a) Axillary LN anlagen were stained for CCL21, Lyve-1 and Madcam1. Lyve-1 (intense green cells, arrow) are lymphatic endothelium cells. (b) CXCL13 was stained in combination with Lyve-1 (intense green cells, arrow) and Madcam1. (c) In a more posterior located inguinal LN anlagen, Lyve-1 (absent) and CXCL13 were stained. (d) CCL21 was stained in this LN anlagen. (e) The same anlagen was stained for CD4+ and CXCL13. $n = 8$. Bar in e is representative for all figures and is 75 μm .



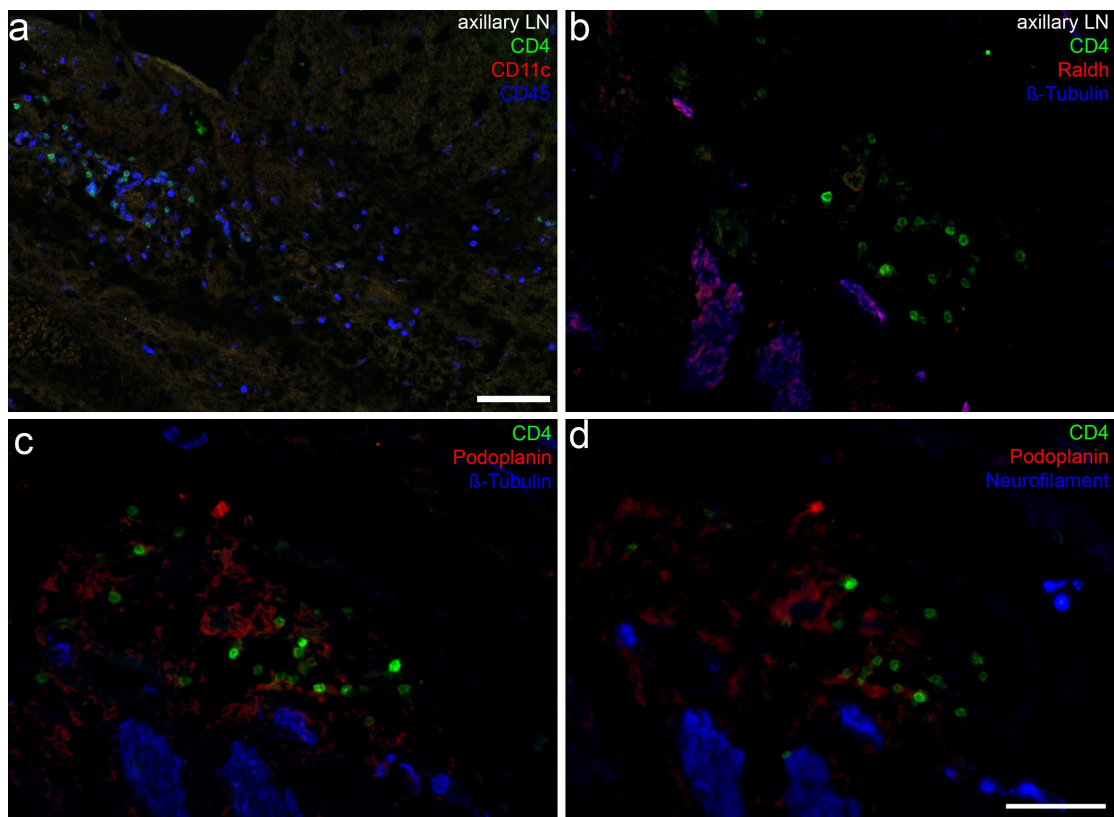
Supplementary Figure 3: Lymph node anlagen are absent in *Cxcl13*^{-/-} and *Cxcr5*^{-/-} embryos. (a-b) *Cxcl13*^{-/-} embryos (E14.5, *n* = 3) were stained for Madcam1, CD4 and CD45. (c) The cervical region of E14.5 *Cxcr5*^{-/-} embryos were stained for IL-7R α , Lyve-1 and CD45 (*n* = 3). (d) The mesenteric region was stained for IL-7R α , Lyve-1 and CD45. (e) In E16.5 *Cxcr5*^{-/-} embryos, the rudimentary MLN was stained for Madcam and CD4 (*n* = 2).



Supplementary Figure 4: anti-Luciferase background staining. Luciferase staining was performed on E13.5 wild type embryos, in combination with Podoplanin and CD4. Shown is a representative figure of a LN anlagen ($n = 4$). Bar represents 75 μm .



Supplementary Figure 5: Residual lymph node anlagen in E14.5 *Raldh2*^{-/-} embryos. Representative pictures of 3 *Raldh2*^{-/-} embryos at E14.5. **(a)** A residual axillary lymph node anlagen characterized with CD4 and Madcam-1 in E14.5 *Raldh2*^{-/-}, stained for CCL21. **(b)** Stich figure of the dorsal area near the heart and the cervical area stained for CD4, CCL21 and Lyve-1. Bars represent 75 μ m.



Supplementary Figure 6: Neurons adjacent to an axillary lymph node anlagen express Raldh-2. (a) An axillary LN anlagen was stained for CD11c, CD4 and CD45. (b-d) Serial sections were stained for Raldh and 2 different neuronal markers at E13.5. Shown is a representative figure of 8 embryos. (b) Raldh-2 was combined with neuronal marker β -III Tubulin and CD4. (c) β -III Tubulin staining was combined with Podoplanin and CD4 staining. (d) Staining for another neuronal marker, Neurofilament, was combined with Podoplanin and CD4 staining. Bar represents 100 μ m in (a) and 75 μ m in (d), representative for (b-d).

Supplementary References

1. Cupedo, T. *et al.* Presumptive lymph node organizers are differentially represented in developing mesenteric and peripheral nodes. *J Immunol* **173**, 2968-2975 (2004).