

SUPPLEMENTARY FIGURE LEGENDS

Suppl. Figure 1: Impaired LN development in $Clec1b^{-/-}$ embryos

A-D, Flow-cytometric analysis showing the gating of Ter119⁺CD41⁺ platelets (**A**) and the expression of CLEC-2 on platelets in the blood of E14.5 $Clec1b^{+/+}$ and $Clec1b^{-/-}$ littermates (**B**), E14.5 (**C**) and newborns (**D**) $Clec1b^{fl/fl}$ and $Clec1b^{fl/fl}PF4-Cre$ littermates.

Suppl. Figure 2: Increased spleen/body ratio weight in $Clec1b^{fl/fl}PF4-Cre$ mice

A, Chart showing the weight ratio spleen/body of $Clec1b^{fl/fl}$ and $Clec1b^{fl/fl}PF4-Cre$ adult littermates. **B**, Immunofluorescence analysis of section from adult spleen stained with B220 (green), Gp38/Podoplanin (red) and CD4 (blue) antibodies (first column) and with CD31 (green), IgM (red) and Lyve-1 (blue) antibodies (second column).

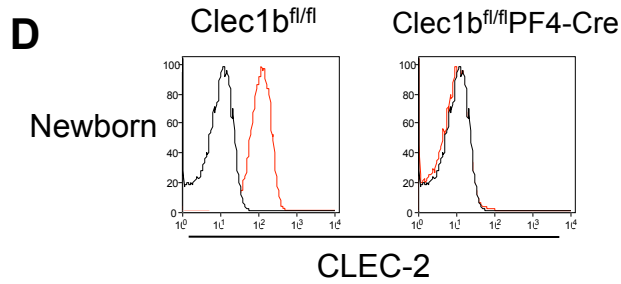
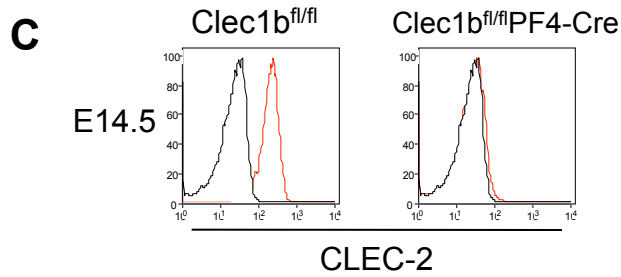
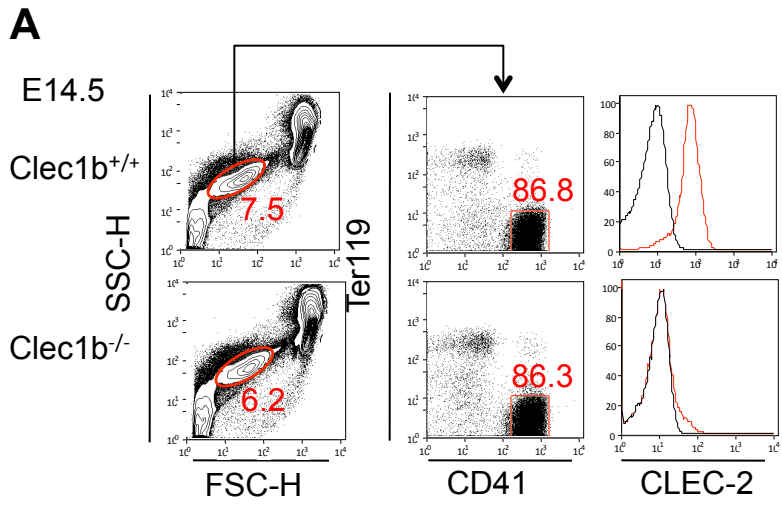
Suppl. Figure 3: Normal haematopoietic composition of the LNs in $Clec1b^{fl/fl}PF4-Cre$ mice

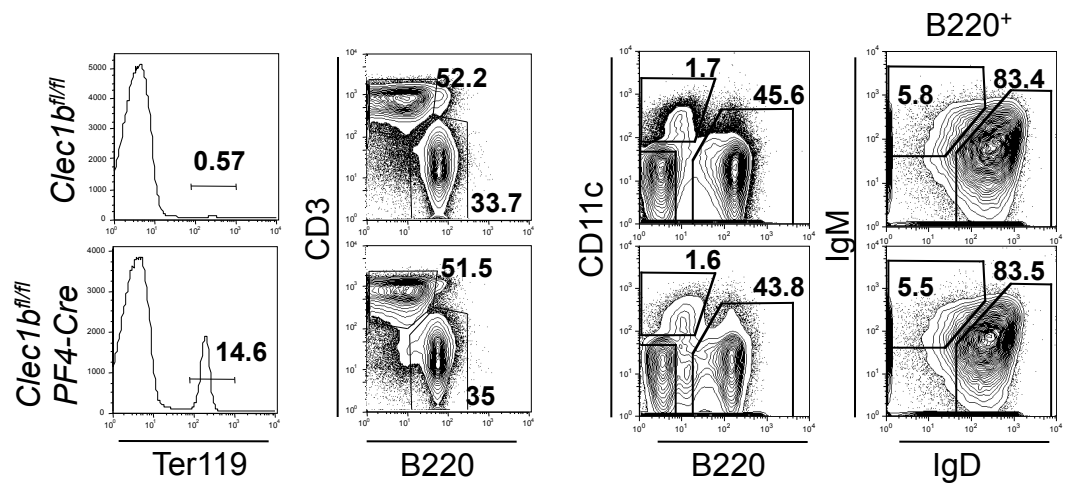
A, Flow-cytometric analysis of mixed inguinal and mesenteric LNs single cell suspensions from 6-7 week old $Clec1b^{fl/fl}$ and $Clec1b^{fl/fl}PF4-Cre$ mice stained with Ter119, CD3 B220, CD11c, IgM and IgD. Percentages of Ter119⁺ red blood cells are shown in the histograms (first column). Percentages of CD3⁺ T cells and B220⁺ B cells (second column), CD11c⁺B220^{-low} dendritic cells (third column), IgM^{high}IgD^{-low} immature and IgD^{High}IgM^{-Low} mature B lymphocytes (forth column) are shown in the dot plots.

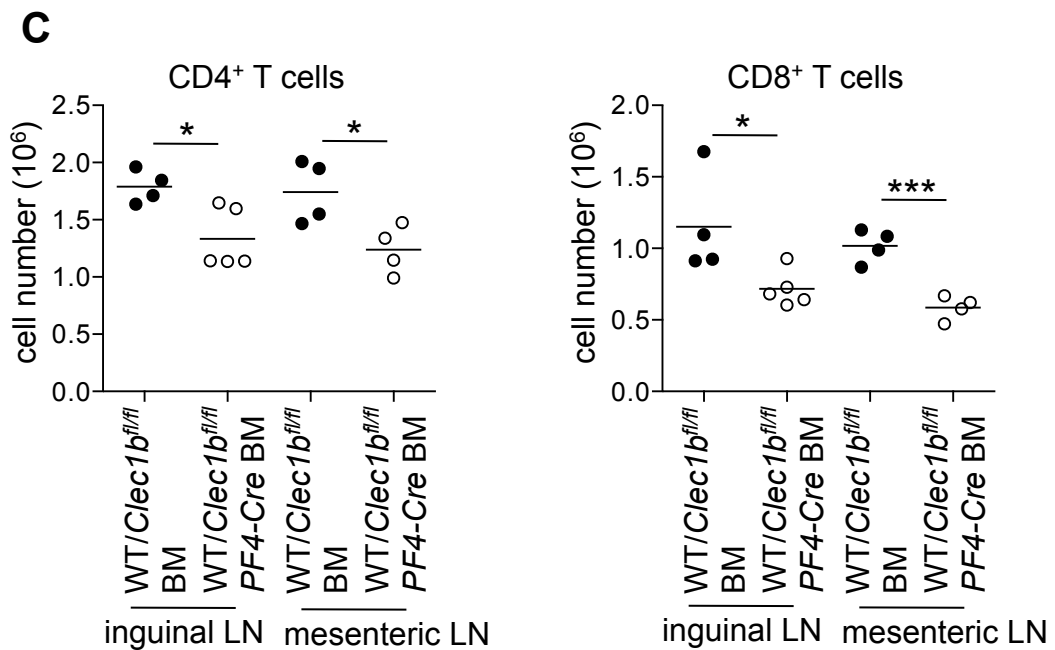
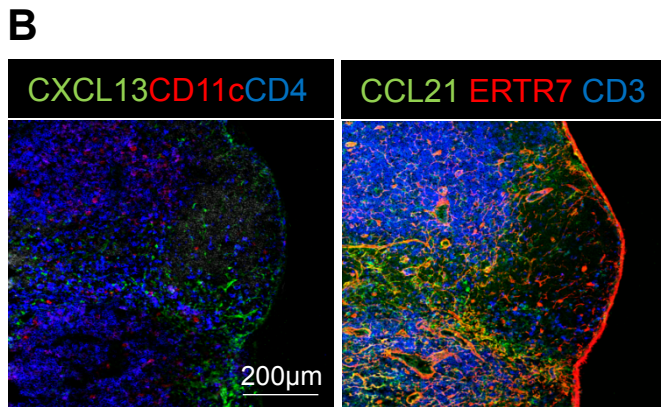
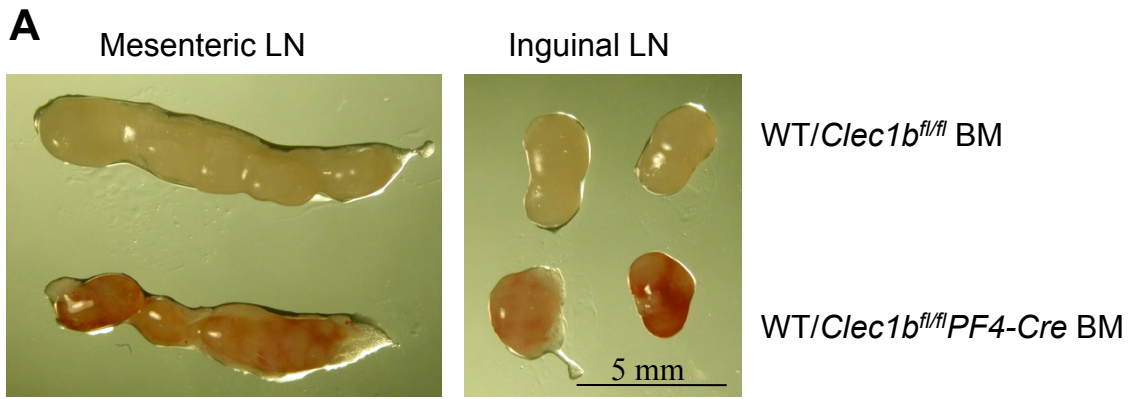
Suppl. Figure 4: Clec1b^{fl/fl}/PF4-cre bone marrow reconstitution led to the LN blood filled phenotype

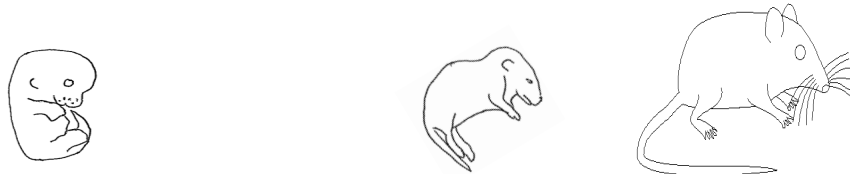
A, Macroscopic appearance of mesenteric (left) and inguinal (right) LNs from adult WT mice reconstituted with *Clec1b^{fl/fl}* and *Clec1b^{fl/fl}PF4-Cre* BM. **B**, Immunofluorescence analysis of LN sections of WT mice reconstituted with *Clec1b^{fl/fl}PF4-Cre* BM stained for CXCL13 (green), CD11c (red), and CD4 (blue) (left) and CCL21 (green), ERTR-7 (red) and CD3 (blue) (right). **C**, Chart showing the absolute number of CD4⁺ T cells and CD8⁺ T cells per inguinal and mesenteric LNs from WT mice reconstituted with *Clec1b^{fl/fl}* (black circle) or *Clec1b^{fl/fl}PF4-Cre* (open circles) BM. Unpaired Student's t test, *p<0.05, *** p<0.005.

Suppl. Figure 5: Model showing the role of CLEC-2 in LNs formation and function









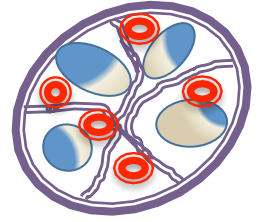
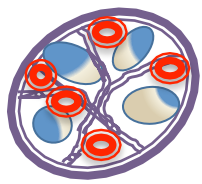
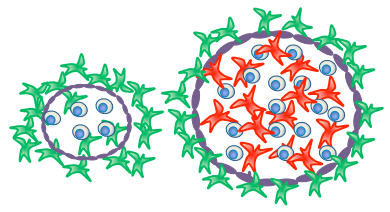
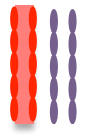
Formation of the lymphatic vasculature

Formation of LNs

Primary immune response

Re-immunisation

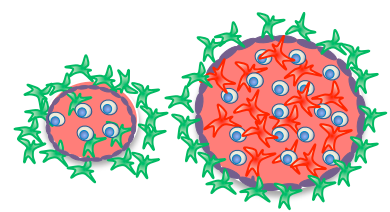
WT



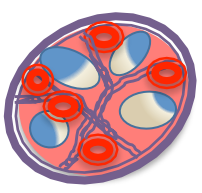
Clec1b^{fl/fl} PF4-Cre
(loss of CLEC-2 on platelets)



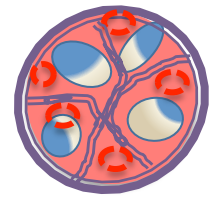
blood-lymphatic mixing



Development of bloody LNs



Normal primary immune responses

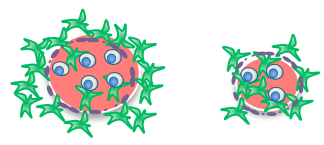


Defect in subsequent recruitment of naïve T cells and B cells (leaky HEV?)

Clec1b^{-/-}



blood-lymphatic mixing
Impaired lymphatic growth (defect in LEC proliferation)



Defect in lymphatic growth leads to abortion of LN formation

- BEC
- LEC
- Mesenchymal cells
- LTi cells
- LTo cells
- HEV