Supplementary Figures 1-6

for

Group 2 innate lymphoid cells are an essential source of interleukin-5 required for development and function of murine B1 cells

Running title: Essential functions of ILC2s for B1 cell development

Karoline F. Troch[1,*], Manuel O. Jakob[1,*], Patrycja M. Forster[1], Katja J. Jarick[1], Jonathan Schreiber[2], Alexandra Preusser[1], Gabriela M. Guerra[3], Pawel Durek[3], Caroline Tizian[1], Nele Sterczyk[1], Sofia Helfrich[1], Claudia U. Duerr[1], David Voehringer[4], Mario Witkowski[1,5], David Artis[6,7,8,9,10], Tim Rollenske[2], Andrey A. Kruglov[3], Mir-Farzin Mashreghi[3,11], and Christoph S.N. Klose[1,#]

Authors' affiliations:
[1] Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Department of Microbiology, Infectious Diseases and Immunology, Hindenburgdamm 30, 12203 Berlin, Germany.
[2] Institute of Molecular Medicine and Experimental Immunology, University Hospital Bonn, Bonn, Germany.
[3] Deutsches Rheuma-Forschungszentrum (DRFZ), an Institute of the Leibniz Association, Berlin, Germany.
[4] Department of Infection Biology, University Hospital Erlangen and FAU Profile Center Immunomedicine (FAU I-MED), Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany
[5] The Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[6] Jill Roberts Institute for Research in Inflammatory Bowel Disease, Weill Cornell Medicine, Cornell University, New York, NY 10021, USA
[7] Friedman Center for Nutrition and Inflammation, Weill Cornell Medicine, Cornell University, New York, NY 10021, USA.
[8] Joan and Sanford I. Weill Department of Medicine, Weill Cornell Medicine, Cornell University, New York, NY 10021, USA.
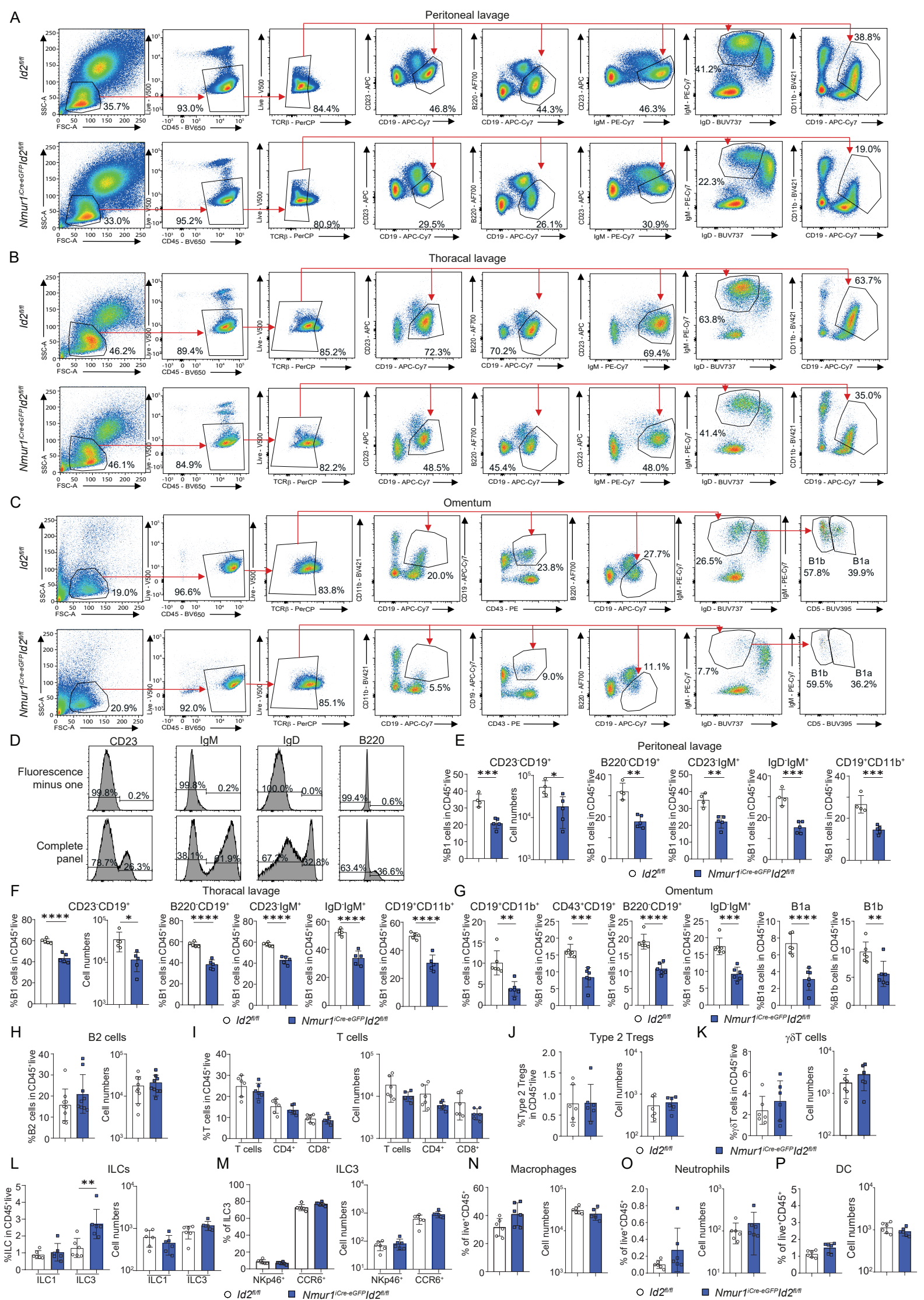[9] Department of Microbiology and Immunology, Weill Cornell Medicine, Cornell University, New York, NY 10021, USA.
[10] Allen Discovery Center for Neuroimmune Interactions, Weill Cornell Medicine, Cornell University, New York, NY 10021, USA.
[11] German Center for Child and Adolescent Health (DZKJ), Partner Site Berlin, Berlin, Germany
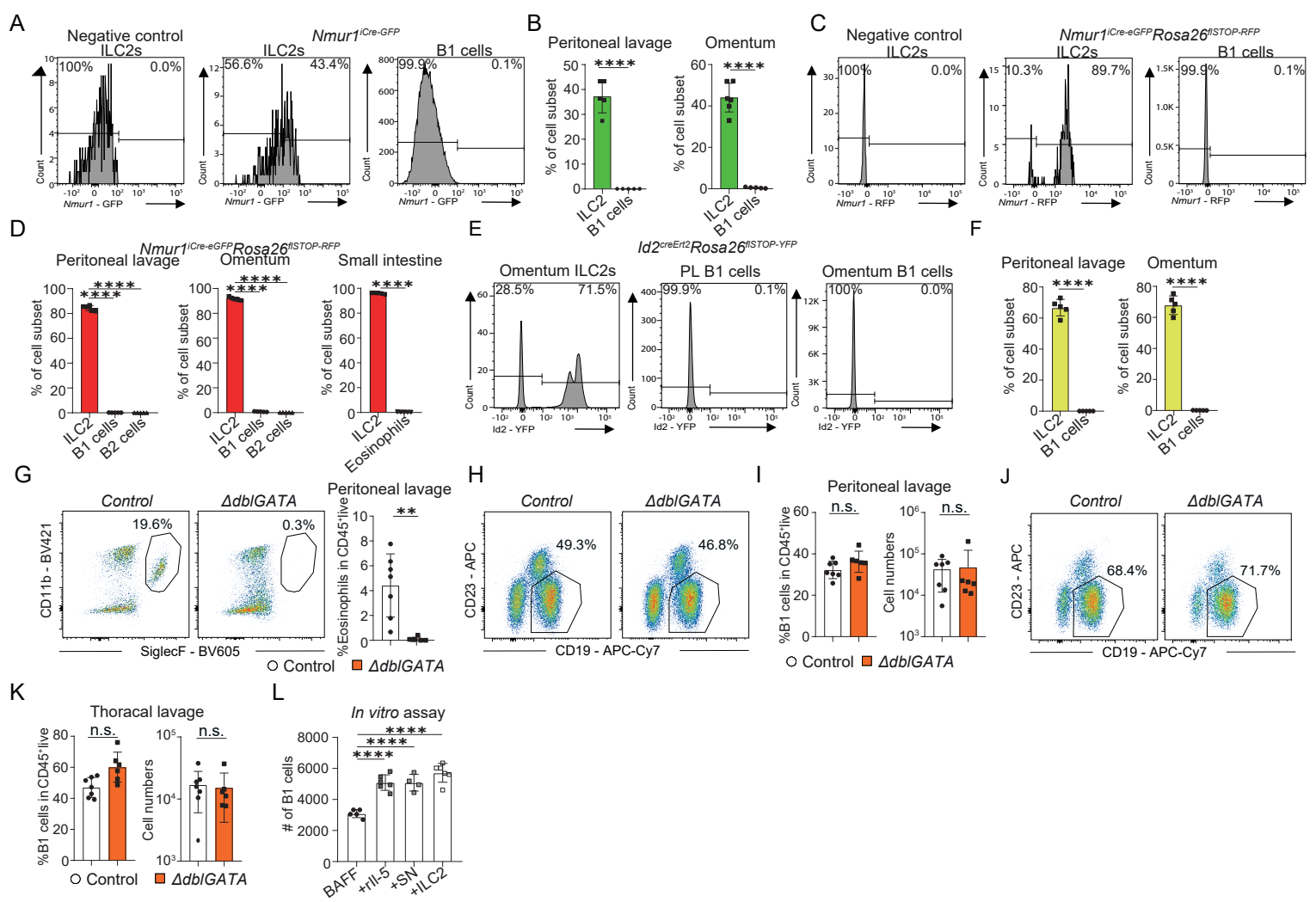
* These authors contributed equally to the work

Corresponding author: Christoph S.N. Klose. Department of Microbiology, Infectious Diseases and Immunology Charité - Universitätsmedizin Berlin: Campus Benjamin Franklin
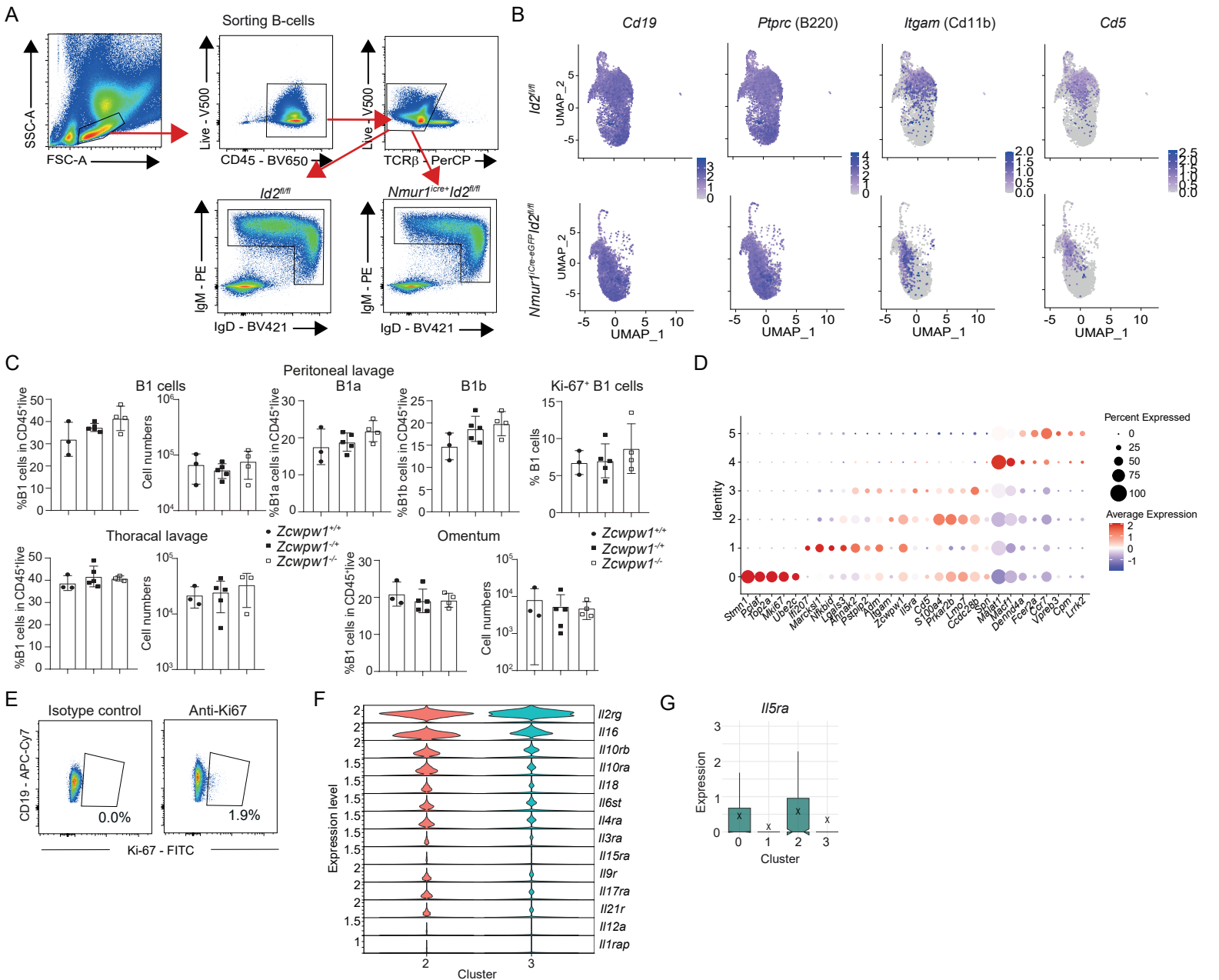Hindenburgdamm 30 12203 Berlin, Germany. Email: christoph.klose@charite.de

Supplementary Figure 1 (refers to Figure 1): Different gating strategies for B1 cells show similar results and lack of ILC2s does not further alter peritoneal cellularity

a-c, Flow cytometric gating strategy of B1 cells in *Nmur1^iCre-eGFP^Id2^fl/fl^* and littermate *Id2^fl/fl^* mice with gating via CD23- / CD19+, B220low / CD19+, CD23- / IgM+, IgDlow / IgM+, CD11b+ / CD19+ in the **a**, peritoneal cavity **b**, thoracal lavage. **c**, omentum with the adjusted gating strategy by using CD11b+ / CD19+, CD43+ / CD19+, B220low / CD19+, IgM+ / IgDlow B1 cells. B1a and B1b cells further subdivided using the marker CD5. **d**, Fluorescence minus one controls of indicated markers are shown, **e,** quantification of a (*Id2^fl/fl^* $n=4$, *Nmur1^iCre-eGFP^Id2^fl/fl^* $n=5$), **f**, quantification of b (*Id2^fl/fl^* $n=5$, *Nmur1^iCre-eGFP^Id2^fl/fl^* $n=5$) **g,** quantification of c (*Id2^fl/fl^* $n=6$, *Nmur1^iCre-eGFP^Id2^fl/fl^* $n=6$). **h-p**, Flow cytometric quantification of indicated cell subsets in the peritoneal lavage in *Nmur1^iCre-eGFP^Id2^fl/fl^* (blue bars) and littermate *Id2^fl/fl^* (white bars) mice. All cells were pre-gated on live CD45+. **h**, B2 cells were gated as TCRβ- CD23+ CD19+ (*Id2^fl/fl^* $n=9$, *Nmur1^iCre-eGFP^Id2^fl/fl^* $n=9$). i-o (*Id2^fl/fl^* $n=6$, *Nmur1^iCre-eGFP^Id2^fl/fl^* $n=6$). **i**, T cells were gated as CD3+ CD5+ +/- CD4+ / CD8+. **j**, Type 2 Tregs were gated as CD3+ CD4+ Foxp3+ Gata3+ KLRG1+ ST2+. k, γδT cells were gated as CD3+ CD5+ TCRγδ+. **l,m**, ILCs cells were pre-gated on Lin- (CD3 CD5 CD19 FCεR1a Ly6G) CD127+. ILC1 were further gated on NK1.1+ NKp46+. ILC3 were gated on NK1.1- c-Kit+ and subclassified in NKp46+ or CCR6+. **n**, Macrophages were gated on Lin- (CD3 CD5 CD19 FCεR1a NK1.1) CD11b+ CD64+. **o**, Neutrophils were gated on Lin- (CD3 CD5 CD19 FCεR1a NK1.1) CD11b+ CD11c+ Ly6G +. **p**, Dendritic cells (DC) were gated on Lin- (CD3 CD5 CD19 FCεR1a NK1.1) Ly6G- CD11b+ CD11c+. Each symbol represents data from one mouse, mean +/- SD, all data are representative of at least two independent experiments. Statistical significance was determined by two-tailed unpaired Student's t-test (e-h,j,k,n-p) or one-way ANOVA (i,l,m). *$p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. PL, peritoneal lavage. Source data, including exact *p*-values, are provided as a Source data file.
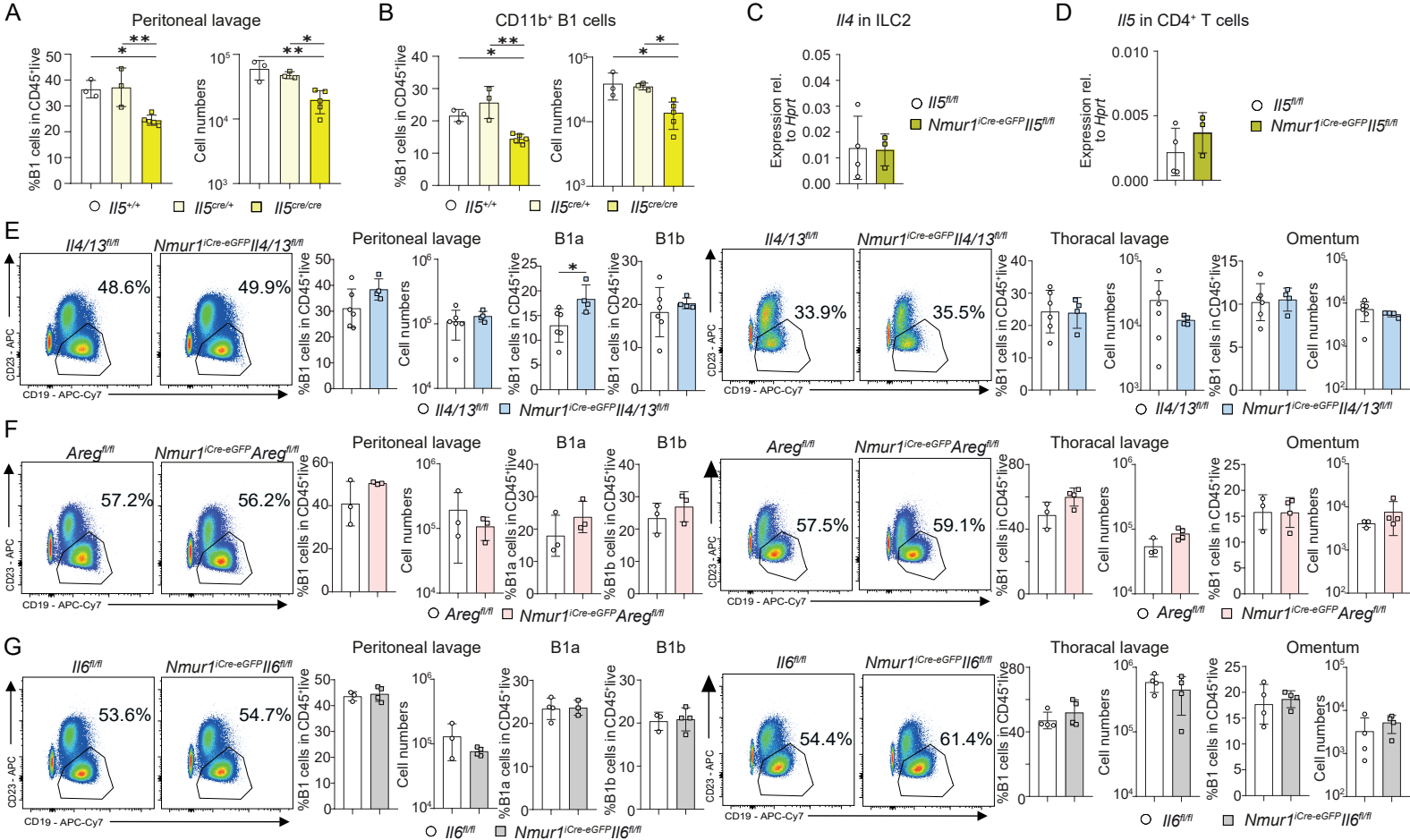
Supplementary Figure 2 (refers to Figure 1): B1 cells and eosinophils do not express Nmur1 and lack of eosino-phils does not alter the B1 cell pool

a, FACS-plots and **b,** quantification of endogenous *Nmur1* (GFP) in B1 cells and ILC2s in *Nmur1*iCre-GFP mice (*n*=6). **c,d,** fate-map experiment identifying the expression of *Nmur1*RFP in B1 cells, B2 cells, eosinophils and ILC2s isolated from *Nmur1*icre-GFP*Rosa26*flSTOP-RFP/+ mice (*n*=5). **e,f,** YFP in B1 cells and ILC2s expression in *Id2*creErt2*Rosa26*flSTOP-YFP/+ mice after 5 weeks of Tamoxifen food administration (*n*=5). **g-k** Control (*4Get*) and *dblGata* (on a *4Get* background) mice were analyzed in steady state (Control *n*=7, *4Get n*=6). **g,** Flow cytometric plots and quantification of eosinophils pre-gated on live CD45+ in the peritoneal cavity. **h-k,** Flow cytometric plots and quantification of B1 cells in the **h,i,** peritoneal cavity and the **j,k,** thoracal lavage. **l,** 10^4 B1 cells (Live CD45+ CD19+ CD23-) from the peritoneal cavity were sort-puri-fied from *n*=6 C57/BL6 wild type mice and cultured for 5 days with the addition of B-cell activating factor (BAFF). Recombinant IL-5 (rIl-5), supernatant (SN) from ILC2s pre-cultured for 3 days in vitro or 10^4 ILC2s were added. B1 cell numbers were analyzed by flow cytometry. Each symbol represents data from one mouse, mean +/- SD, all data are representative of at least two independent experiments. Statistical significance was determined by two-tailed unpaired Student's t-test (b,d,fh,i,k) or one-way ANOVA (d,l). *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. PL, peritoneal lavage. Source data, including exact *p*-values, are provided as a Source data file.
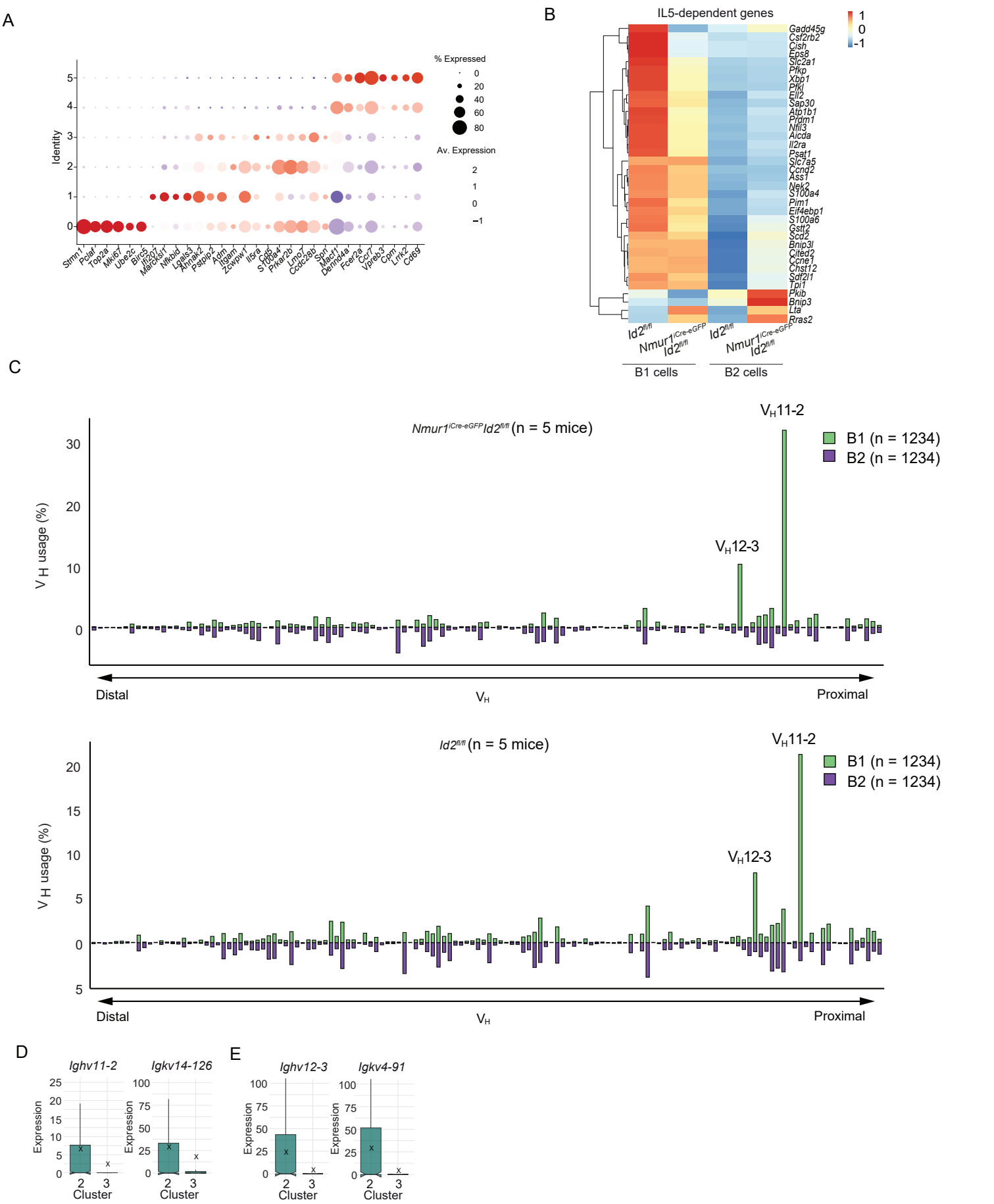
Supplementary Figure 3 (refers to Figure 2): Validation of single-cell sequencing

a, Sorting strategy of B cells from the peritoneal cavity of *Nmur1*[iCre-eGFP]*Id2*^{fl/fl} and littermate *Id2*^{fl/fl} mice for scRNA-seq. 5 mice were pooled for the sequencing. **b,** UMAPs of general B cell surface markers (*CD19*, *Ptprc*) and B1 cell specific surface markers (*Itgam*, *Cd5*). **c,** Flow cytometric quantification of B1 cells in littermate *Zcwpw1*^{-/-}, *Zcwpw1*^{+/-} and *Zcwpw1*^{+/+} in the peritoneal cavity, thoracal lavage and the omentum at steady state (*Zcwpw1*^{+/+} *n*=3, *Zcwpw1*^{+/-} *n*=5, *Zcwpw1*^{-/-} *n*=4). **d,** Dot-plot of the most significant genes in each Cluster from the scRNA-seq. **e,** Ki-67 of B1 cells in comparison to the appropriate isotype control. **f,** Violin plot of expressed cytokine-receptors comparing clusters 2 and 3, **g,** Boxplot of the expression of *Il5ra* in all B1 cell clusters (Clusters 0-3). Each symbol represents data from one mouse, mean +/- SD, all data are representative of at least two independent experiments. Statistical significance was determined by one-way ANOVA (c). *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. PL, peritoneal lavage. Source data, including exact *p*-values, are provided as a Source data file.
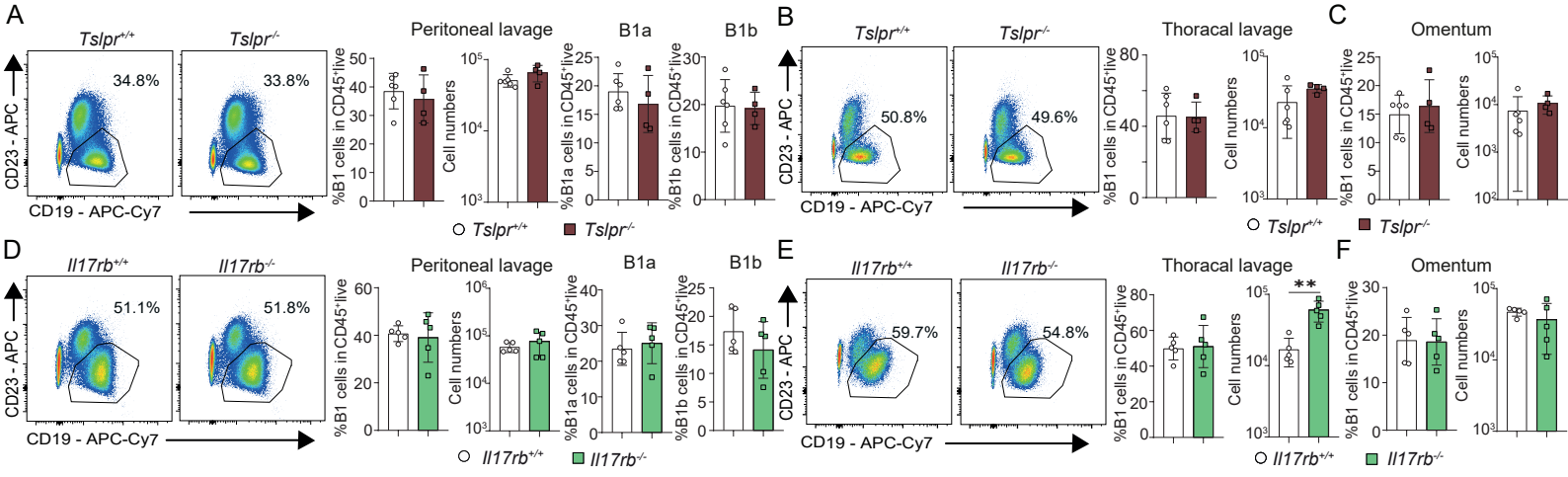
Supplementary Figure 4 (refers to Figure 3): The ILC2-derived factors IL-4/13, Areg and IL-6 do not affect B1 cell development

a,b, Flow cytometric quantification of B1 cells in *Il5*^+/+, *Il5*^Cre/+ (light yellow), and *Il5*^Cre/Cre (yellow) from the peritoneal lavage (*Il5*^+/+ *n*=3, *Il5*^Cre/+ *n*=3, *Il5*^Cre/Cre *n*=5). **c,d**, Validation of the newly generated mouse line *Nmur1*^iCre-eGFP*Il5*^fl/fl by qPCR of sort-purified ILC2s gated as live CD45^+ Lin^- (CD3, CD5, CD19, Fcεrl, Ly6G) CD127^+ KLRG1^+ and CD4^+ T cells gated as live CD45^+ CD3^+, CD4^+ from the lung (*Il5*^fl/fl *n*=4, *Nmur1*^iCre-eGFP*Il5*^fl/fl *n*=3). **e-g**, Flow cytometry plots and quantification of total B1, B1a and B1b cells (based on the marker CD5) of peritoneal lavage, thoracal lavage and omentum of **e**, *Nmur1*^iCre-eGFP*Il4/13*^fl/fl and littermate *Il4/13*^fl/fl control mice (*Il4/13*^fl/fl *n*=6, *Nmur1*^iCre-eGFP*Il4/13*^fl/fl *n*=4), **f**, *Nmur1*^iCre-eGFP*Areg*^fl/fl and littermate *Areg*^fl/fl control mice (*Areg*^fl/fl *n*=3, *Nmur1*^iCre-eGFP*Areg*^fl/fl *n*=4), **g**, *Nmur1*^iCre *Il6*^fl/fl and littermate *Il6*^fl/f mice (*Il6*^fl/f *n*=4, *Nmur1*^iCre-eGFP*Il6*^fl/fl *n*=4). Each symbol represents data from one mouse, mean +/- SD, all data are representative of at least two independent experiments. Statistical significance was determined by two-tailed unpaired Student's t-test (c-g) or one-way ANOVA (a,b). n.s. non-significant, *$p<0.05$, ** $p<0.01$. Source data, including exact *p*-values, are provided as a Source data file.

Supplementary Figure 5 (refers to Figure 4): B1 cells mainly rely on V_H11-2 and V_H12-3

a-e, Sort-purified B cells underwent single-cell sequencing including sequencing of the B-cell repertoire. Sort-purification analogous to Fig S3. 5 mice were pooled. **a**, dot-plot of the most significant genes in each Cluster excluding BCR sequencing. **b**, differentially-regulated gene expression of reported IL-5-induced B1 cell genes (21) of *Nmur1*[iCre-eGFP]*Id2*^{fl/fl} (blue) and littermate *Id2*^{fl/fl} mice. **c**, *Ighv* usage in B1 and B2 cells from 5 mice from *Nmur1*[iCre-eGFP]*Id2*^{fl/fl} (upper panel) and littermate *Id2*^{fl/fl} mice (lower panel). *Ighv* gene segments are ordered by their relative proximity to the D segments. Number of cells is depicted in the Figure legend. **d,e,** Quantification of the most important phosphatidyl-specific heavy- and corresponding light chains, *Ighv11-2, Igkv14-126,* and *Ighv12-3, Igkv4-91,* comparing clusters 2 and 3.

Supplementary Figure 6 (refers to Figure 6): TSLPR and IL-25 do not shape the B1 cell pool
Flow cytometry plots and quantification of total B1 cells, B1a and B1b cells of **a**, peritoneal lavage, **b**, thoracal lavage and **c**, omentum of *Tslpr*+/+ and *Tslpr*-/- mice and **d**, the peritoneal lavage, **e**, thoracal lavage and **f,** omentum of *Il17rb*+/+ and *Il17rb*-/- mice. Each symbol represents data from one mouse, mean +/- SD, all data are representative of at least two independent experiments. Statistical significance was determined by two-tailed unpaired Student's t-test (a-f). Source data, including exact *p*-values, are provided as a Source data file.