LETTER TO THE EDITOR

IL1RAP is expressed in several subtypes of pediatric acute lymphoblastic leukemia and can be used as a target to eliminate *ETV6::RUNX1*-positive leukemia cells in preclinical models

Helena Ågerstam,[1,2] Henrik Lilljebjörn,[1] Marianne Rissler,[1] Carl Sandén[1] and Thoas Fioretos[1,2]

[1]Division of Clinical Genetics, Department of Laboratory Medicine, Lund University, Lund and [2]Department of Clinical Genetics and Pathology, Office for Medical Services, Region Skåne, Lund, Sweden

Correspondence:
H. ÅGERSTAM - helena.agerstam@med.lu.se
T. FIORETOS - thoas.fioretos@med.lu.se

https://doi.org/10.3324/haematol.2022.281059

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Helena Ågerstam[1, 2*], Henrik Lilljebjörn[1], Marianne Rissler[1], Carl Sandén[1], Thoas Fioretos[1, 2*].

[1]Division of Clinical genetics, Department of Laboratory Medicine, Lund University, Lund, Sweden.

[2]Department of Clinical Genetics and Pathology, Office for Medical Services, Region Skåne, Lund, Sweden

* Corresponding authors

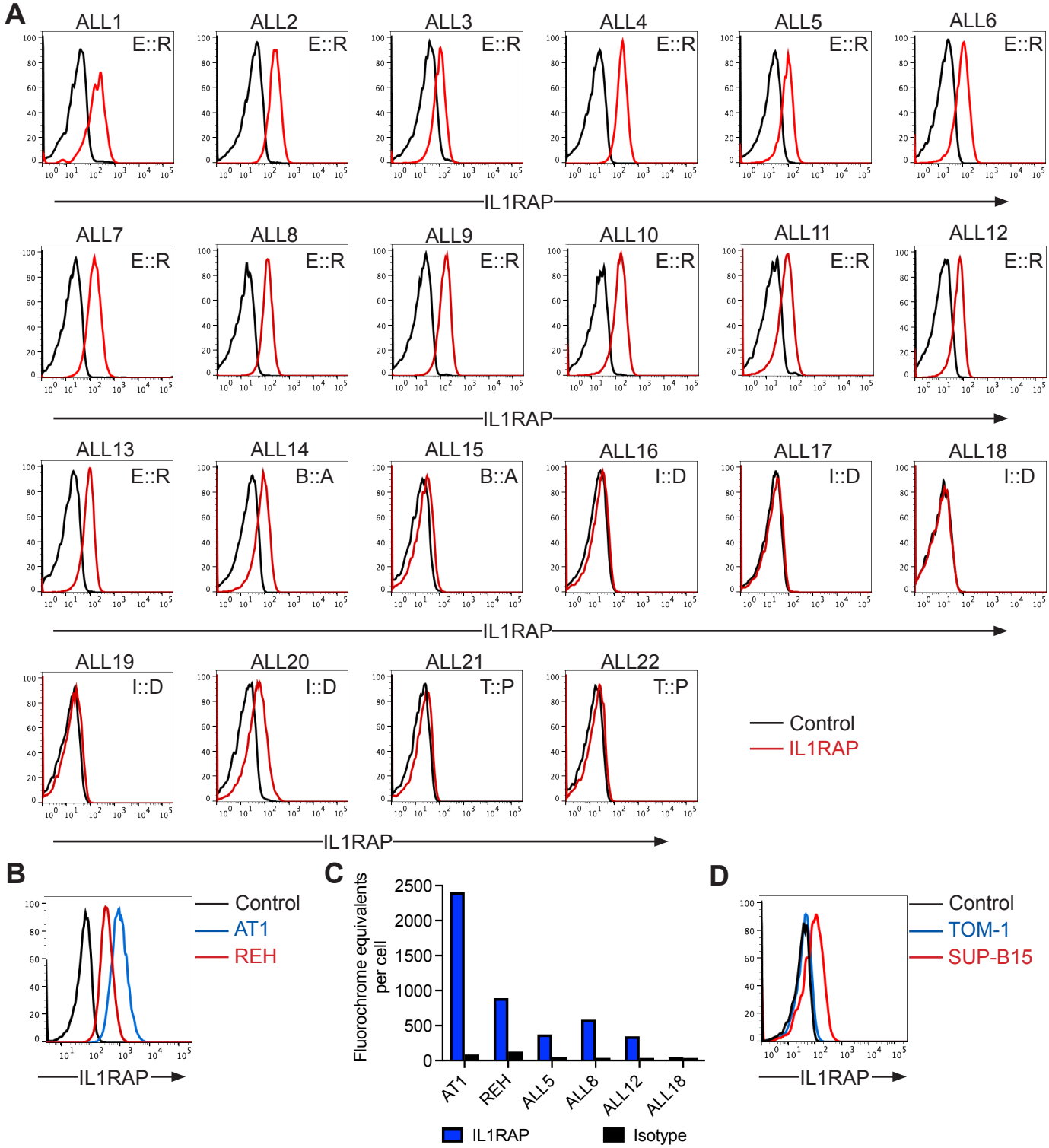
Details corresponding authors

Helena Ågerstam, e-mail: helena.agerstam@med.lu.se

Thoas Fioretos, e-mail: thoas.fioretos@med.lu.se

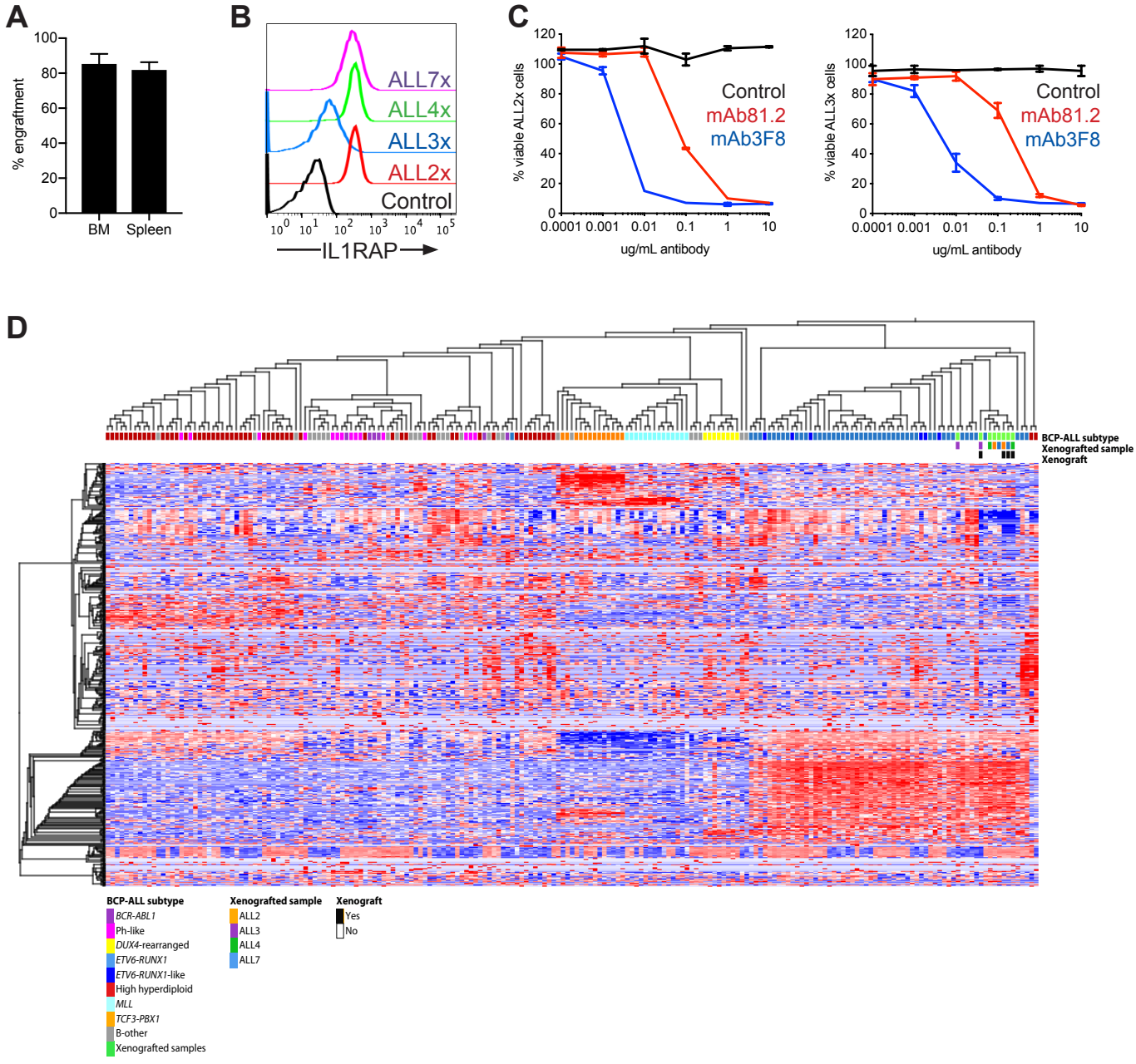
Supplementary material

Supplementary Figure S1



Supplementary Figure S1. Cell surface expression of IL1RAP on primary BM samples of different BCP-ALL genetic subtypes.
A. Flow cytometric analysis of primary leukemic BM cells from 13 patients with *ETV6::RUNX1*-positive (E::R; ALL1-ALL13), two patients with *BCR::ABL1*-positive (B::A; ALL14-ALL15), five patients with *IGH::DUX4*-positive (I::D; ALL16-ALL20) and two patients with *TCF3::PBX1*-positive (T::P; ALL21-ALL22) BCP-ALL. **B.** Flow cytometric analysis of IL1RAP expression on *ETV6::RUNX1*-positive REH and AT1 cells. **C.** Using the BD Quantibrite assay, an estimate of the total number of IL1RAP molecules on the cell surface of AT1, REH, and the four primary samples ALL5, ALL8, ALL12 and ALL18 was made based on the assumption of a 1:1 flourochrome to antibody ratio. **D.** Flow cytometric analysis of IL1RAP expression on the two BCR::ABL1-expressing cell lines TOM-1 and SUP-B15. In all cases, isotype antibody-stained cells were used as control.

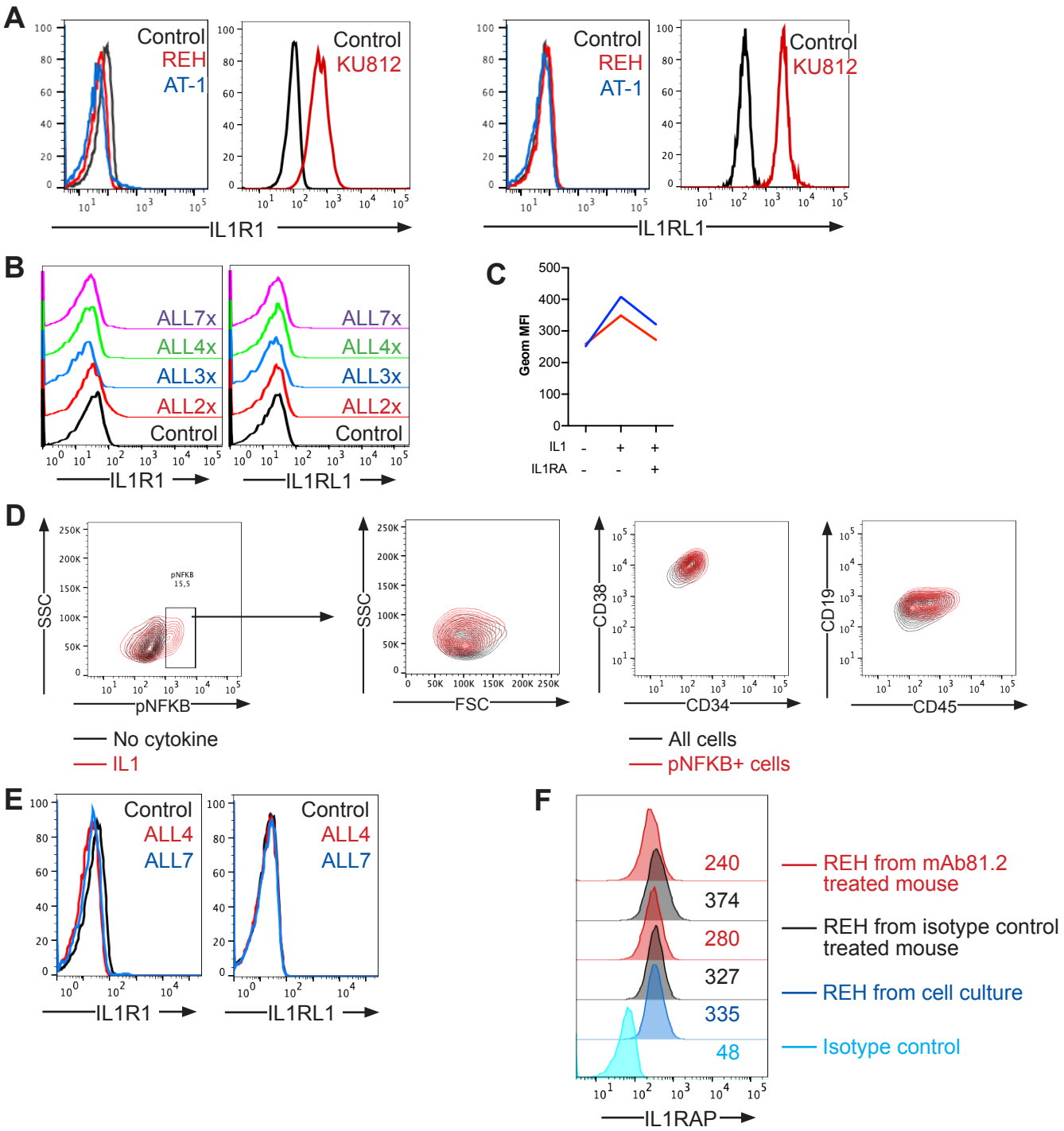
Supplementary Figure S2



Supplementary Figure S2. Creation and characterization of *ETV6::RUNX1*-postive xenografts

A. One to two million MNCs isolated from BM samples of *ETV6::RUNX1*-postive BCP-ALL patients (ALL2, ALL3, ALL4 and ALL7) were transplanted by tail vein injections into sublethally irradiated NSG mice (n=7). Around seven months after transplantation the mice were sacrificed due to disease and displayed a mean of 85% and 82% BCP-ALL cells in BM and spleens, respectively. Error bars represents SEM. **B.** The xenografted BCP-ALL cells display cell surface expression of IL1RAP as shown by flow cytometry. Isotype antibody-stained cells were used as control. **C.** Two of the xenografted samples (ALL2x and ALL3x) were incubated overnight with the IL1RAP antibodies mAb81.2 or mAb3F8 or a corresponding hIgG1 isotype control antibody and human NK cells. The result is presented as the number of viable target cells in wells with antibody divided by the number of viable target cells in wells without antibody. The ADCC-assay was performed with NK cells from two different donors, and the data is presented as mean with error bars representing range. **D.** RNA-sequencing of BCP-ALL cells confirmed a retained global gene expression profile in the xenografted BCP-ALL cells ALL2x, ALL3x, ALL4x and ALL7x. The PDX-samples cluster with the primary *ETV6::RUNX1*-positive samples in the dataset of 195 pediatric BCP-ALL cases by Lilljebjörn et al. Dim-blue and green boxes represents the *ETV6::RUNX1*-positive samples in the BCP-ALL subtype row. ALL2 is represented by orange boxes, ALL3 by purple boxes, ALL4 by green boxes and ALL7 by blue boxes in the Xenografted samples row. The xenografted samples are marked by black boxes in the Xenograft row.

Supplementary Figure S3



Supplementary Figure S3. Response to stimulation with IL1 or IL33

A. REH and AT1 cells does not express IL1R1 or IL1RL1 as shown by flow cytometry. KU812 cells were included as positive control for IL1R1 and IL1RL1 antibody staining. Isotype antibody-stained cells were used as control. **B.** Flow cytometric analysis shows lack of expression of IL1R1 and IL1RL1 on ALL2x, ALL3x, ALL4x and ALL7x. Isotype antibody-stained cells were used as control. **C.** Phosphoflow analysis of NFKB phosphorylation in ALL7x cells following stimulation with IL1 in the absence or presence of 10 ng/mL IL1RA. Two paired samples are shown, presented as blue or red. **D.** Extended flow cytometric analysis showing that the pNFKB-positive ALL7x-cells display essentially the same immunophenotype as the non-responsive cells when stimulated with IL1. **E.** Flow cytometric analysis of IL1R1 and IL1RL1 expression on the two primary ETV6::RUNX1-positive BCP-ALL samples ALL4 and ALL7. Isotype antibody-stained cells were used as control. **F.** Flow cytometric analysis of the REH cells in BM from two mice treated with IL1RAP antibody and two mice treated with control antibody as well as REH from the cell culture. Isotype antibody-stained REH cells from the cell culture were used as control (light blue). The geometric mean fluorescence intensity for each sample is presented along with the histograms.