

Supplemental information

Niche-expressed Galectin-1 is involved in pre-B acute lymphoblastic leukemia relapse through pre-B cell receptor activation

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Supplemental data

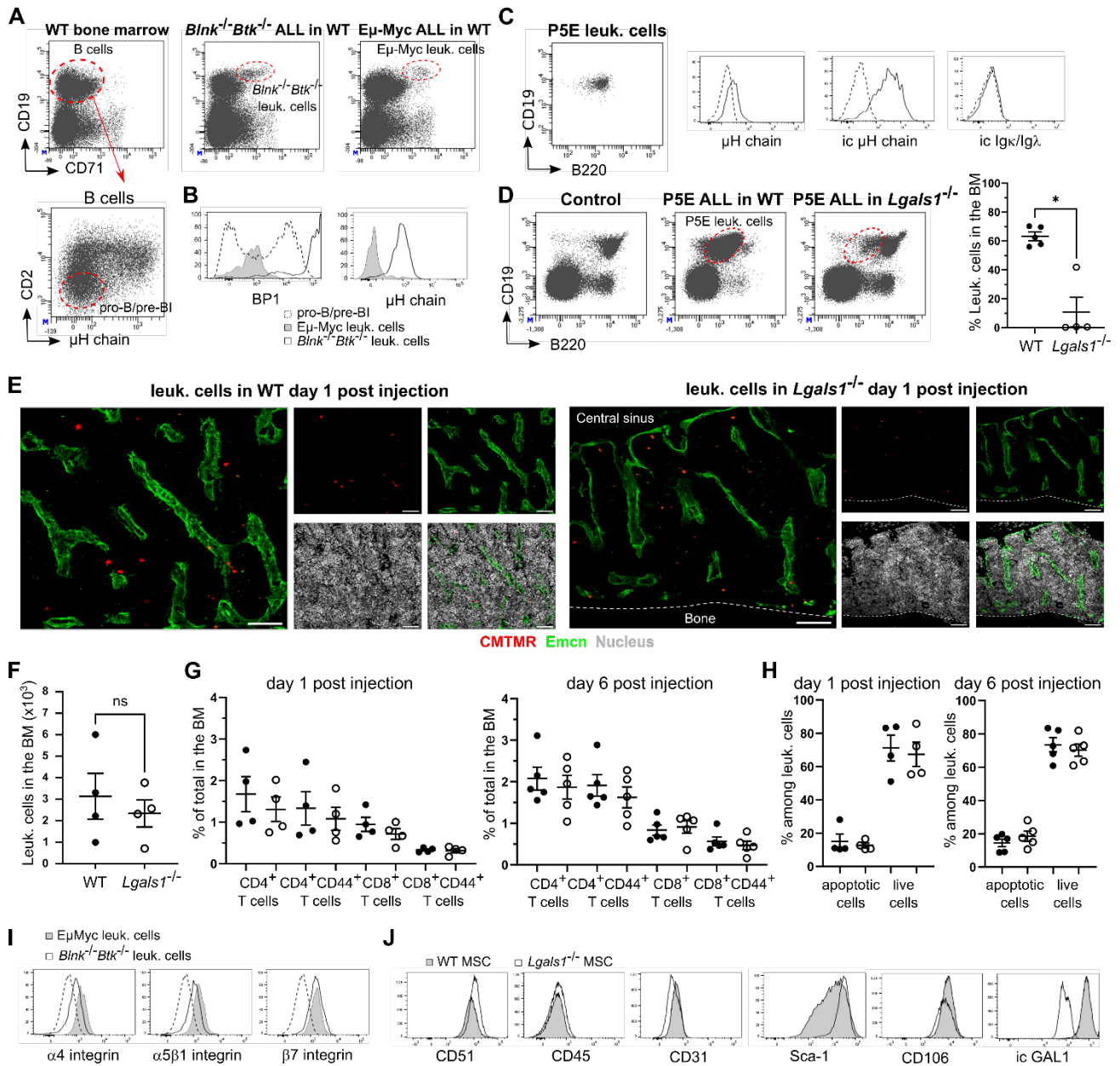


Figure S1. Influence of GAL1 on murine pre-B and common B-ALL. Related to Figure 1 and Figure 2. (A) WT mice were injected with PBS or with leukemic cells obtained from *Blmk^{-/-}Btk^{-/-}* or μ -Myc mice. Bone marrow was prepared 5 days after injection for flow cytometry analysis. Normal pro-B/pre-BI cells from the host were gated as CD19⁺CD2⁺IgM⁺. Injected leukemic cells were identified as CD19⁺CD71⁺ cells. (B) *Blmk^{-/-}Btk^{-/-}* pre-B ALL were identified as BP1^{hi} μ H chain⁺ and μ -Myc B-ALL as BP1^{lo} μ H chain⁺. (C) Flow cytometry phenotyping of pre-B leukemic cells obtained from PAX5-ELN transgenic mice. Leukemic cells are B220^{lo}CD19⁺, intracellular (ic) Ig κ /Ig λ ⁺, ic and surface μ H chain⁺. The dotted histogram corresponds to background controls. (D) P5E leukemic cells injected to WT and *Lgals1^{-/-}* mice were analyzed 12 days after injection. Representative dot plots are shown on the left and the proportion of leukemic cells for all samples in the right panel. Statistical significance was calculated using a Mann-Whitney non-parametric test (* $p < 0.05$). (E) *Blmk^{-/-}Btk^{-/-}* pre-B ALL were stained with CMTMR orange and injected to WT or *Lgals1^{-/-}* mice. One day after injection, mice were sacrificed. BM sections were analyzed by confocal microscopy. Representative images of CMTMR⁺ leukemic cells (red), Emcn⁺ vessels (green) and nucleus (white) are shown. Scale bars: 50 μ m. (F) CMTMR⁺ leukemic cells were analyzed by flow cytometry in the BM one day after injection to WT or *Lgals1^{-/-}* mice. (G) The proportion of total CD4⁺ and CD8⁺ T cells, and effector T cells (CD44⁺CD4⁺ and CD44⁺CD8⁺) were determined by flow cytometry 1 day and 6 days after injection of *Blmk^{-/-}Btk^{-/-}* pre-B ALL to WT (black dots) or *Lgals1^{-/-}*

(white dots) mice. **(H)** Proportion of apoptotic (Sytox AAD⁻ Caspase3/7 Green⁺) and live (Sytox AAD⁻ Caspase3/7 Green⁻) *Blnk*^{-/-}*Btk*^{-/-} pre-B ALL cells 1 day and 6 days after injection to WT or *Lgals1*^{-/-} mice. **(I)** Expression of integrins by *Blnk*^{-/-}*Btk*^{-/-} or Eμ-Myc leukemic cells. The dotted histogram corresponds to background controls. **(J)** Flow cytometry phenotyping of MSCs obtained from WT and *Lgals1*^{-/-} mice. The MSCs are as expected CD31⁻CD45⁻CD51⁺Sca1⁺. They also express CD106, ligand of the α4β1 integrin involved in pre-BCR clustering. WT MSC are also GAL1⁺.

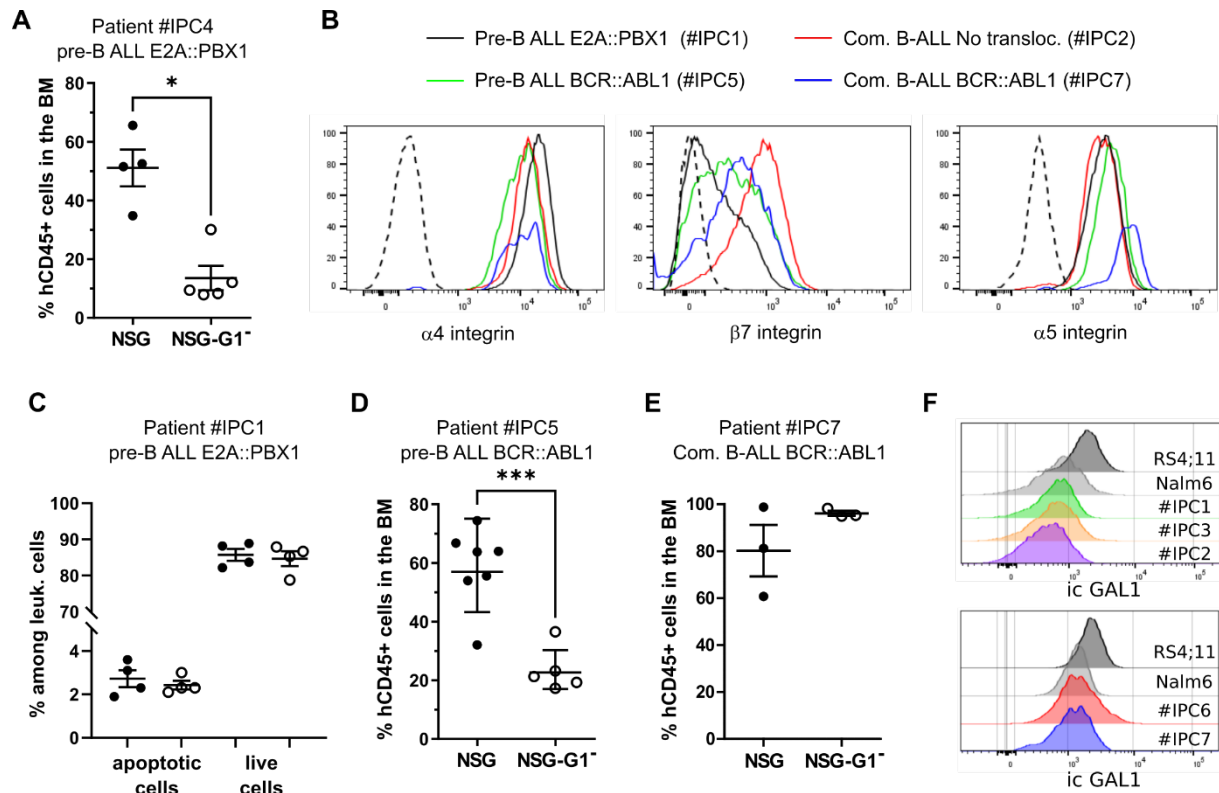


Figure S2. Influence of GAL1 on human pre-B and common B-ALL. Related to Figure 3.

(A) Human E2A::PBX1⁺ pre-B ALL were xenografted to NSG and NSG-*Lgals1*^{-/-} mice. The proportion of CD45⁺ human leukemic cells in the bone marrow was determined by flow cytometry 10 weeks after transplantation. (B) Expression of integrins by pre-B and common B-ALL (patients #IPC1, #IPC2, #IPC5 and #IPC7). The dotted histogram corresponds to background controls. (C) Proportion of apoptotic (Sytox AAD⁻ Caspase3/7 Green⁺) and live (Sytox AAD⁻ Caspase3/7 Green⁻) leukemic cells 10 weeks after injection of E2A::PBX1⁺ pre-B ALL to WT or *Lgals1*^{-/-} mice. (D) BCR::ABL1⁺ pre-B ALL and (E) BCR::ABL1⁺ common B-ALL samples separately xenografted to NSG and NSG-*Lgals1*^{-/-} mice were analyzed as in (A). Error bars represent SEM. Statistical significance was calculated using Mann-Whitney non-parametric test (A) or unpaired t test without Welch correction (D). *p < 0.05; ***p < 0.001. (F) The expression of Gal1 from patient samples used in the study was determined by flow cytometry. The RS4;11 MLL-rearranged cell line and the Nalm6 cell line were used as positive and negative controls respectively.

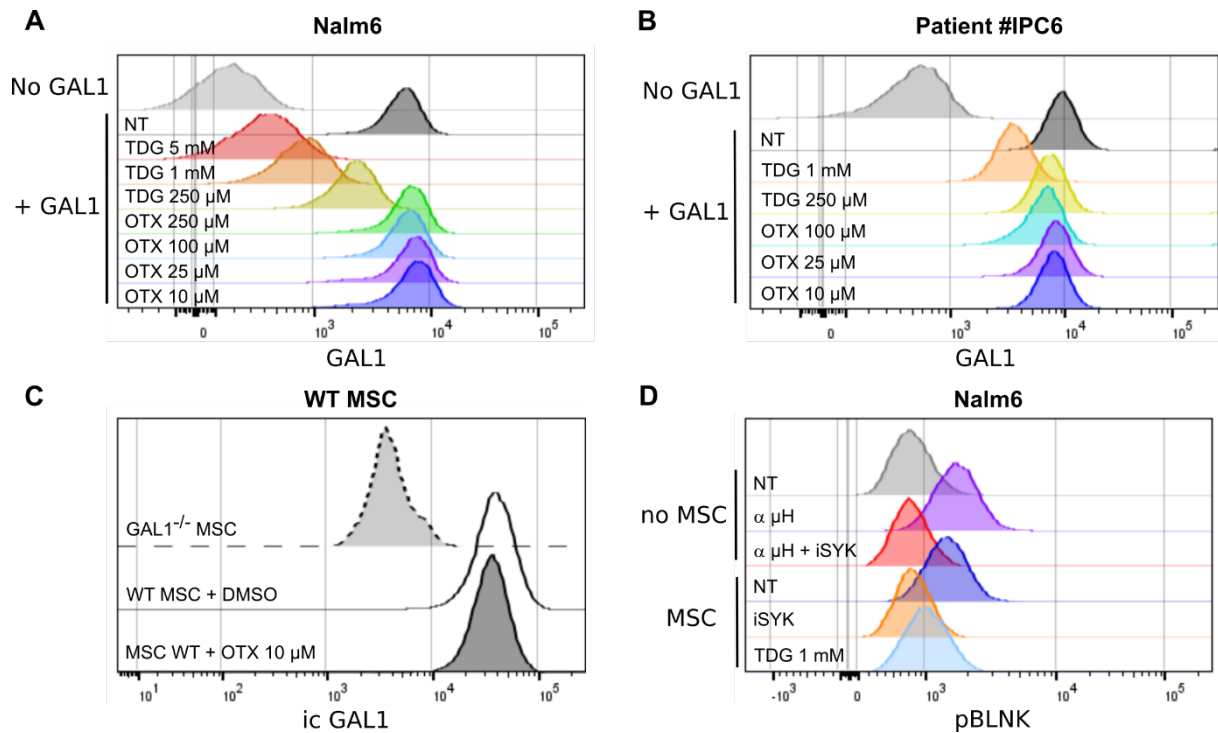


Figure S3. Assessment of GAL1 inhibition with thiodigalactoside and OTX008. Related to Figure 4. (A) Nalm6 cells and (B) pre-B ALL from patient #6 were incubated with recombinant human GAL1 in presence of increasing concentrations of thiodigalactoside (TDG) or OTX008 (OTX) or in their absence (NT). GAL1 expression was determined by flow cytometry using an anti human GAL1 antibody. (C) murine WT MSCs were cultivated 48h with or without 10 μ M OTX008. Intracellular and surface GAL1 expression was determined using an anti murine GAL1 antibody after permeabilization, as compared to *Lgals1*^{-/-} MSCs as a negative control. (D) Nalm6 cells were starved in low serum (3% instead of 10%) and cultivated with or without murine WT MSCs. Cells were left untreated (NT) or treated with an anti μ H chain, the SYK inhibitor AB8779 (iSYK) or TDG as indicated in the panel.

Table S1. Patient samples used in the study. related to STAR Methods.

Patient number	gender	age	karyotype	Pre-BCR immunophenotype*	Classification**
IPC1	Male	31	t(1 ;19) E2A::PBX1	cCD79a ⁺ cμ ⁺ Igκ/λ ⁻	pre-B ALL (BIII-ALL)
IPC2	Male	23	normal	cCD79a ⁺ cμ ⁻ Igκ/λ ⁻	common B-ALL (BII-ALL)
IPC3	Male	26	t(9 ;22) BCR::ABL1	cCD79a ⁺ cμ ⁺ Igκ/λ ⁻	pre-B ALL (BIII-ALL)
IPC4	Female	59	t(1 ;19) E2A::PBX1	cCD79a ⁺ cμ ⁺ Igκ/λ ⁻	pre-B ALL (BIII-ALL)
IPC5	Female	48	t(9 ;22) BCR::ABL1	cCD79a ⁺ cμ ⁺ Igκ/λ ⁻	pre-B ALL (BIII-ALL)
IPC6	Male	40	normal	cCD79a ⁺ cμ ⁺ Igκ/λ ⁻	pre-B ALL (BIII-ALL)
IPC7	Female	57	t(9 ;22) BCR::ABL1	cCD79a ⁺ cμ ⁻ Igκ/λ ⁻	common B-ALL (BII-ALL)

* as determined by flow cytometry (cCD79a and cμ: cytoplasmic CD79a and cytoplasmic μH chain respectively)

** nomenclature used in the manuscript (nomenclature from the EGIL)