Materials and methods

Leukemia cells and reagents. BCP-ALL cell lines (697-GFP and REH-RFP) cells were grown in RPMI medium containing 10% of heat-inactivated fetal bovine serum (Gemini Bio-products), 50 U/ml penicillin-streptomycin, 2 mM L-glutamine and 50 mM β-mercaptoethanol (Invitrogen). Patient-derived leukemia blasts (Pt 238) were obtained after informed consent (HRPO-05-435), according to the Declaration of Helsinki and the ethical review board of institution (University of New Mexico), and characterized in previous work (1, 2). After expansion through serial passages in mice, Pt 238-GFP lines were established by GFP-lentiviral particle transduction (#17-10387; Millipore, Temecula, CA, USA). GFP^+ blasts were sorted and expanded in engrafted mice for following experiments.

Flow cytometry assay.
For IL-15Rα expression analysis, mouse brain endothelial cells (bEnd.3 cell line) were plated and treated with 10 ng/ml of TNF-α. After 24 hr, cells were collected and stained with anti-mIL-15Rα-APC antibody (330209, JM7A4; BioLegend) for 30 min on ice. After two washes, cells were resuspended in 0.5 μg/ml DAPI containing FACS buffer and run on a Fortessa. For exosome uptake analysis, bEnd.3 cells were plated in 6 well plates and added purified DiO-labeled NALM6 derived exosomes next day. After 18 hr incubation, cells were collected and washed with cold-PBS twice. Cells were stained in anti-mCD31-APC antibody (102409, 390; BioLegend) for 30 min on ice. After washes, samples were run on a Fortessa.

Transendothelial migration assay.
bEnd.3 cell monolayers were prepared in 8 well-chambers. After 24 hr incubation with hIL-15 at specified doses or mTNF-α (10 ng/ml), the cell layers were stained with anti-mCD31-APC antibody (102409, 390; BioLegend) for 15 min and gently washed 2x with warmed medium. DiO-labeled WT or IL-15 knockdown-NALM6 cells were overlaid onto the endothelial layers. After additional 2 hr incubation, wells were fixed with 2% PFA and a Z series of confocal images acquired from the top to the bottom of the bEnd.3 cell layer. The number of GFP^+ cells above and below were enumerated.

IL-15 dependent cell growth and survival.
Cell growth of parental and IL-15 or IL-15Rα knockdown-NALM6 cells were in culture medium (2% serum) by counting every 24 hr. The apoptosis rate was determined by annexin V-positive percentage (Annexin V Apoptosis detection kit; 88-8007-74, Thermofisher) by flow cytometry after 48 hr culture in 2 % serum medium.

Statistical analysis. Statistical analyses were performed using Prism software (GraphPad Software). Data were analyzed using a paired or unpaired Student t-test or a one-way ANOVA, as appropriate. Results were considered significant when $p<0.05$.

References

1. Kinjyo, I., Matlawska-Wasowska, K., Chen, X., Monks, N. R., Burke, P., Winter, S. S. & Wilson, B. S. (2017) Characterization of the anti-CD22 targeted therapy, moxetumomab pasudotox, for B-cell precursor acute lymphoblastic leukemia, *Pediatr Blood Cancer.* **64**.
2. Erasmus, F. M., Matlawska-Wasowska K., Kinjyo, I., Mahajan A., Winter, S. S., Xu, L., Horowitz, M., Lidke, D. S., Wilson, B. S. (2016). Dynamic pre-BCR homodimers fine tune autonomous signals for B-cell precursor acute lymphoblastic leukemia survival. Science Signaling 2016, **9**:ra166.