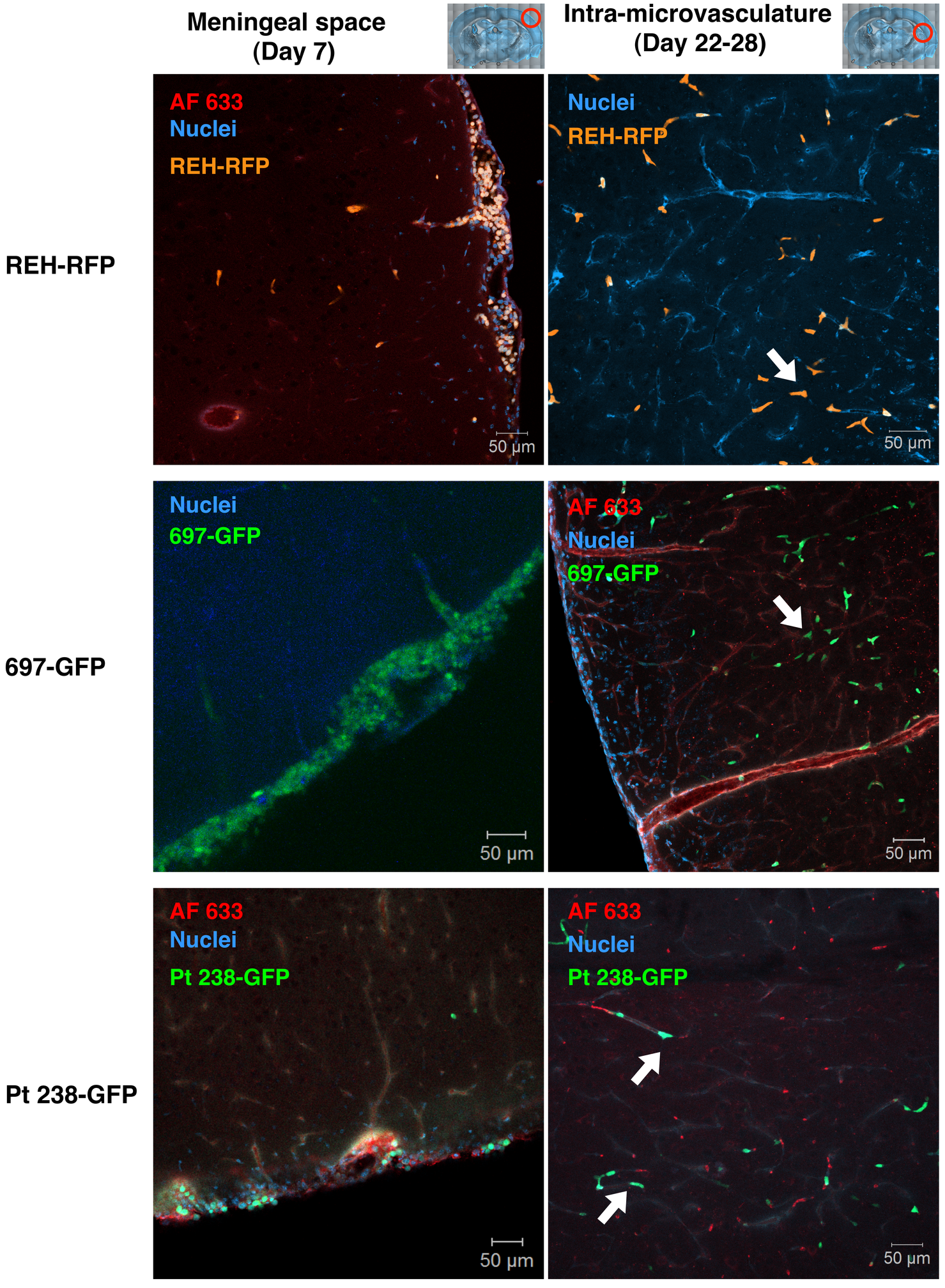

Supplemental Figure 1. Brain section images capture patterns of meningeal and brain microvasculature invasion in mice engrafted with 3 additional BCP-ALL cell sources. REH-RFP, 697-GFP and patient-derived Pt238-GFP blasts were injected into NSG mice. Brains of engrafted mice were harvested, fixed, and sections incubated with DAPI (blue) or vascular dye (Alexa Fluor 633 Hydrazide), followed by confocal imaging.

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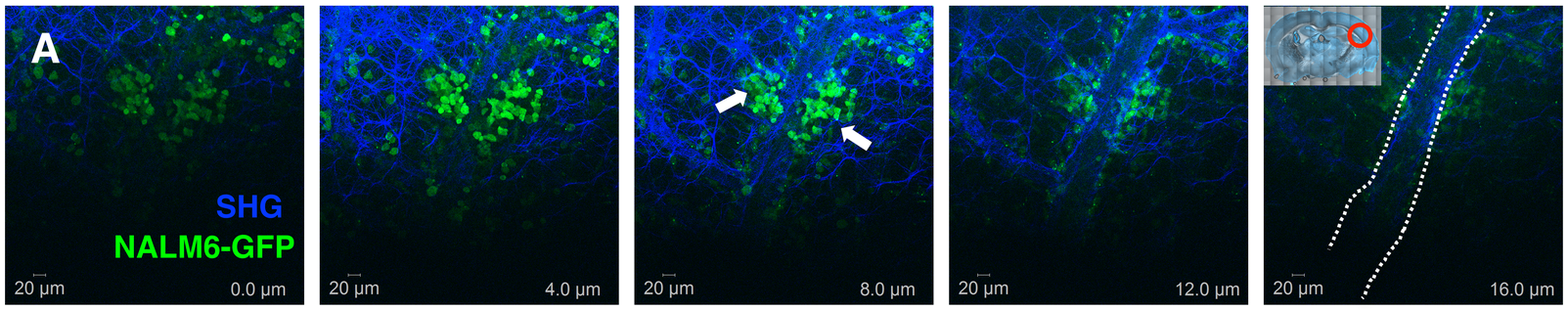
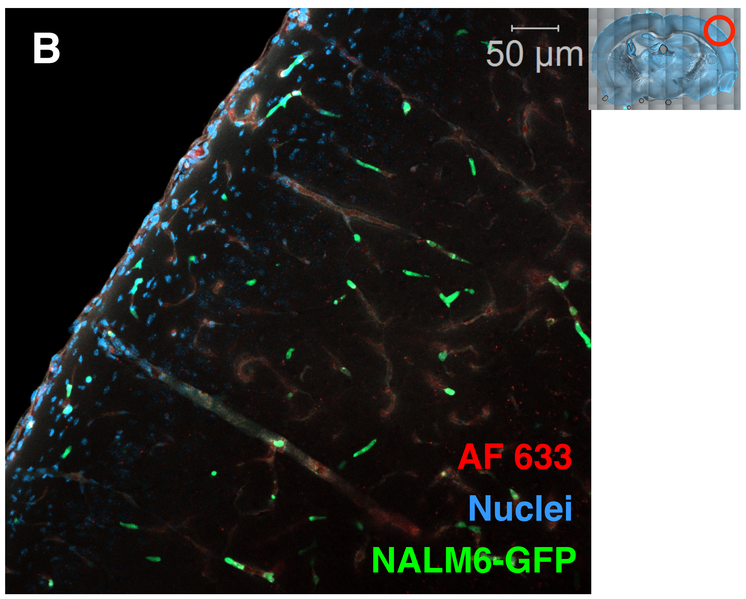
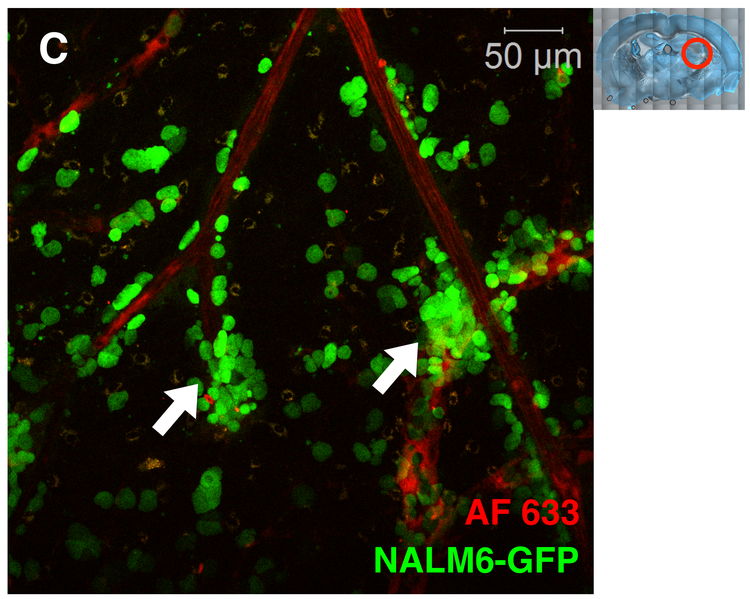
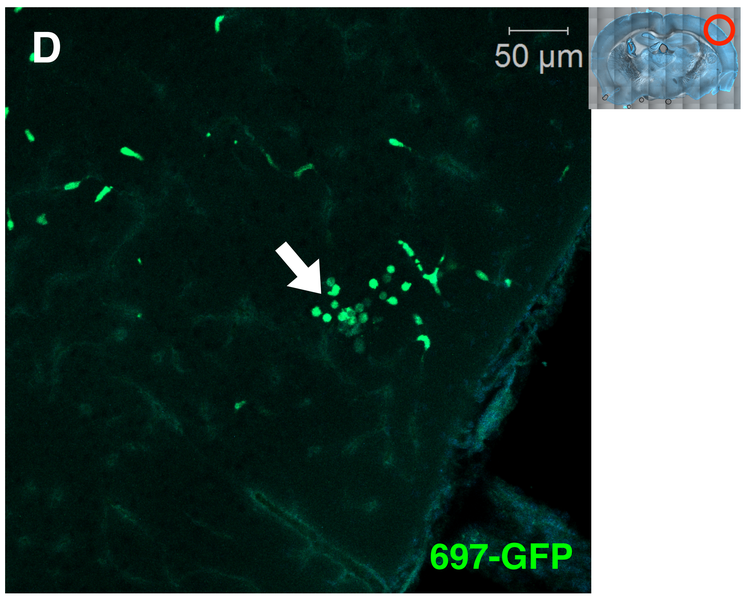
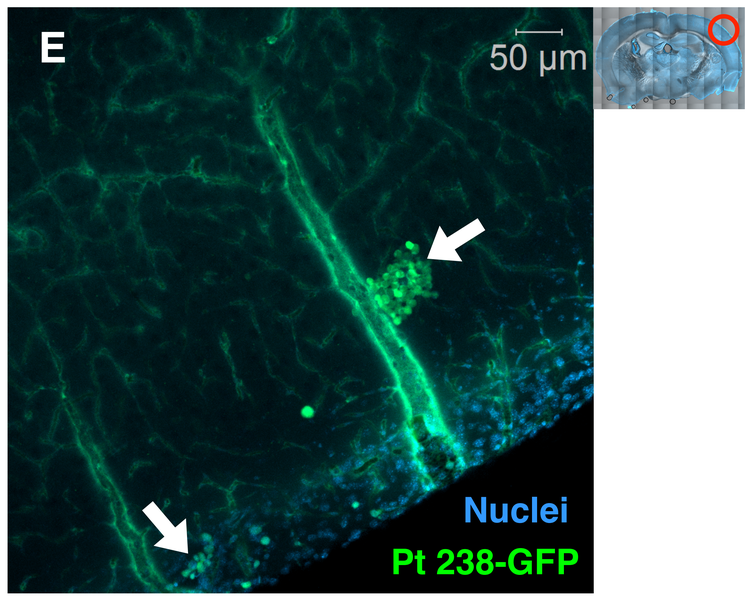
Figure 2











Supplemental Figure 2. Representative images of colonized leukemia cells at brain parenchyma. **(A)** NALM6-GFP cells engrafted mouse brain was imaged by a two-photon microscope on day 24. GFP^+ cells were found close to arteries with visible vascular wall by second-harmonic generation (outlined by white dot lines). **(B,C)** 24 days after NALM6-GFP cells engraftment, GFP^+ cells were found inside the brain microvasculature (B) and perivascular lesion (C). **(D,E)** BCP-ALL cells derived 697-GFP cell line (D; day 25), as well as Pt 238-GFP blasts (E; day 27), colonize brain parenchyma of engrafted mice at low frequency. Vessels are visible with their high auto-fluorescence. White arrows indicate the colonized BCP-ALL colonies.

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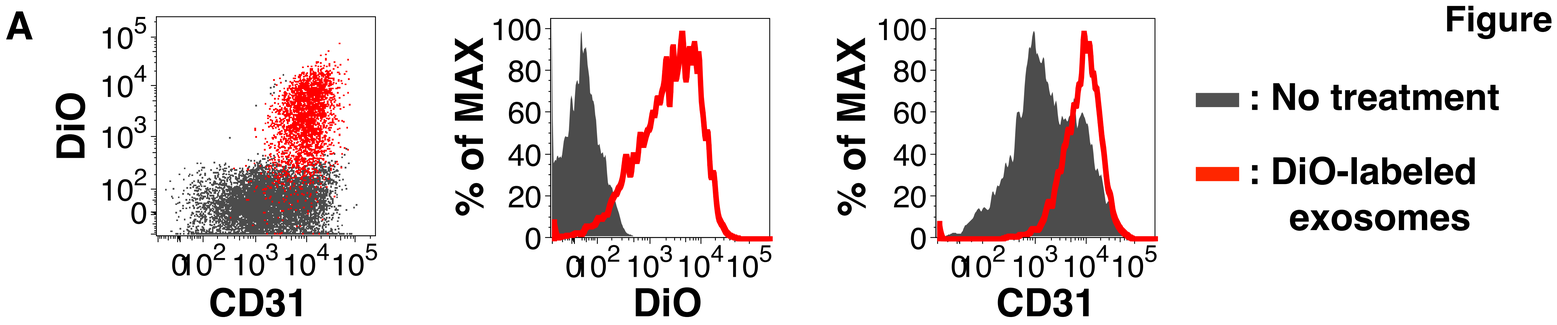
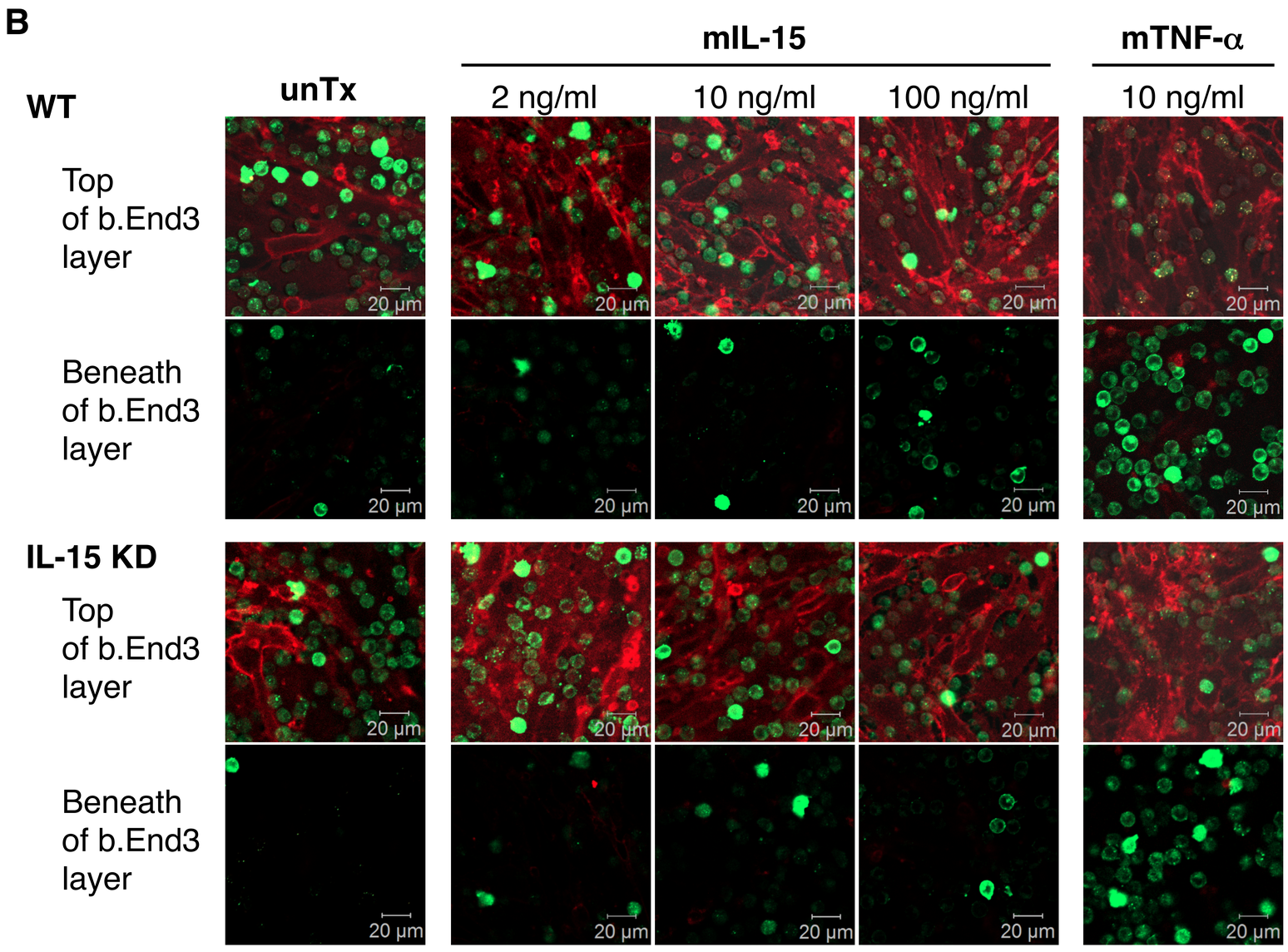
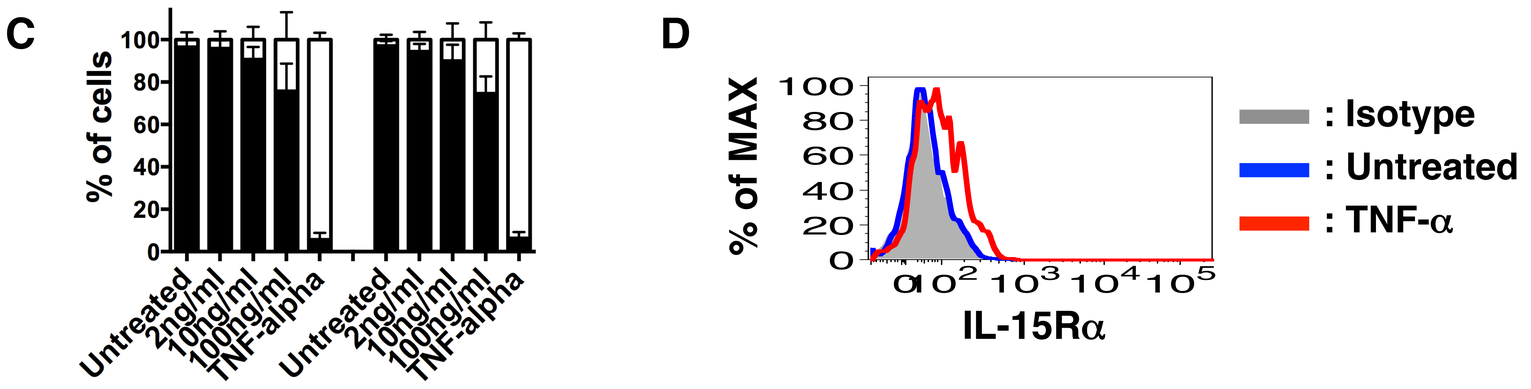
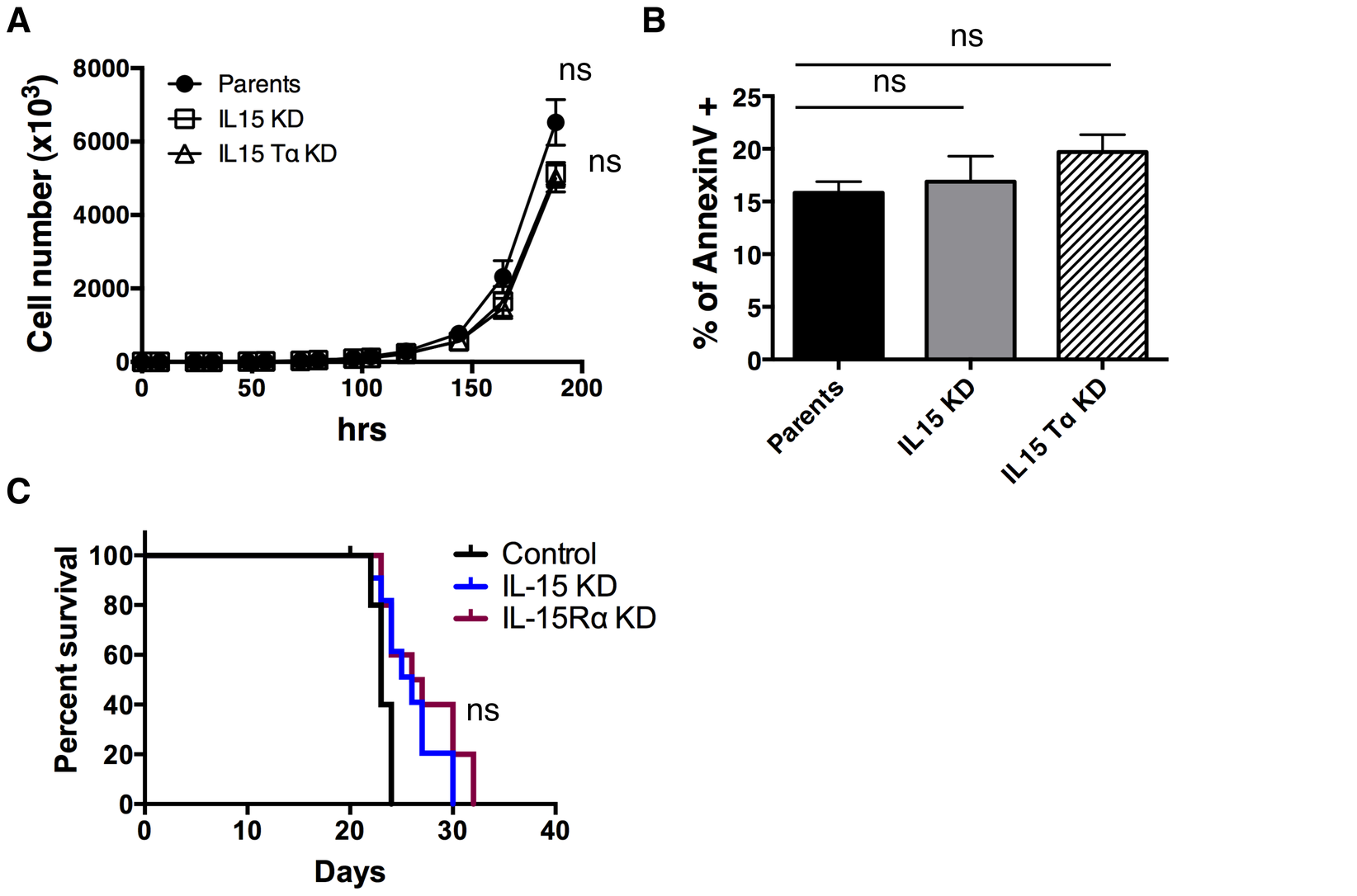
Figure 3







Supplemental Figure 3. Effect of blast-derived factors on brain endothelial cells. **(A)** bEnd.3 cells were incubated with purified DiO-labeled, NALM6-derived exosomes for 4 hrs. After 2 washes, cells were stained with anti-mCD31 and uptake of exosomes measured by flow cytometry. (**B,C)** bEnd.3 monolayers were preincubated with mIL-15 or TNF-α for 16 hr. After washing, monolayers were overlaid with either DiO-labeled WT or IL-15 KD NALM6 cells for 4 hr. Fixed and stained samples were evaluated by confocal imaging to evaluate effects of cytokine treatment on transmigration. Representative z-plane images are shown above and below the endothelial cell layer; the percent transmigrated cells are reported in (C). (**D)** b.End3 cells were stained with anti-mouse IL-15Rα antibodies for flow cytometry analysis, demonstrating modest upregulation of IL-15 receptors after 24 hr TNF-α treatment.



Supplemental Figure 4. IL-15 signaling has little or no effect on BCP-ALL blast growth and apoptosis. **(A)** Proliferation of cultured NALM6 cells, comparing the growth of the parental cell line and cell lines derived after CRISPR-Cas9 gene editing to knock down levels of IL-15 or its receptor.
(B) Percentage of annexin V+ in each of the NALM6 cell lines (mean ± SD; n = 4 from 2 experiments, ns: non significant). **(C)** Survival analysis of IL-15 KD and IL-15 Rα KD engrafted mice against control group. (n = 5-10)