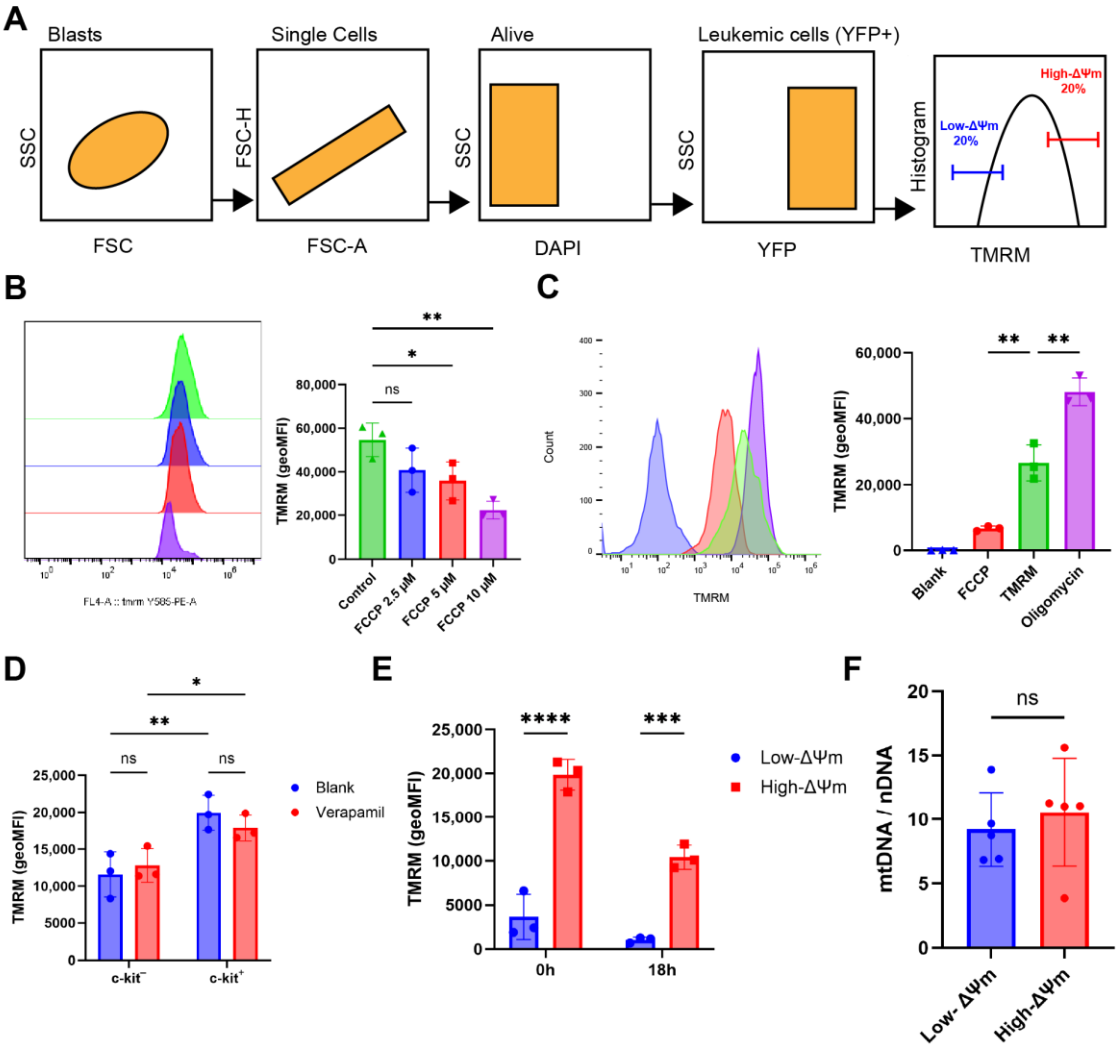

Figure S1. Gating strategy and validation of the ΔΨm readout. (**A**) Diagram of gating strategy. (**B**) FCCP dose–response (0–10 µM). (**C**) TMRM geoMFI of unstained (Blank), FCCP-treated (10 µM), untreated (TMRM) and oligomycin-treated (2 µg/mL) cells, overlaid on a common baseline. (**D**) Efflux control: TMRM geoMFI of c-Kit⁻ and c-Kit⁺ cells with or without verapamil (50 µM, 30 min). (**E**) TMRM geoMFI of the low- and high-ΔΨm fractions at 0 h and after 18 h in culture. (**F**) Relative mitochondrial DNA content (mt-Co1/Ndufv1) of the sorted fractions (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns not significant).

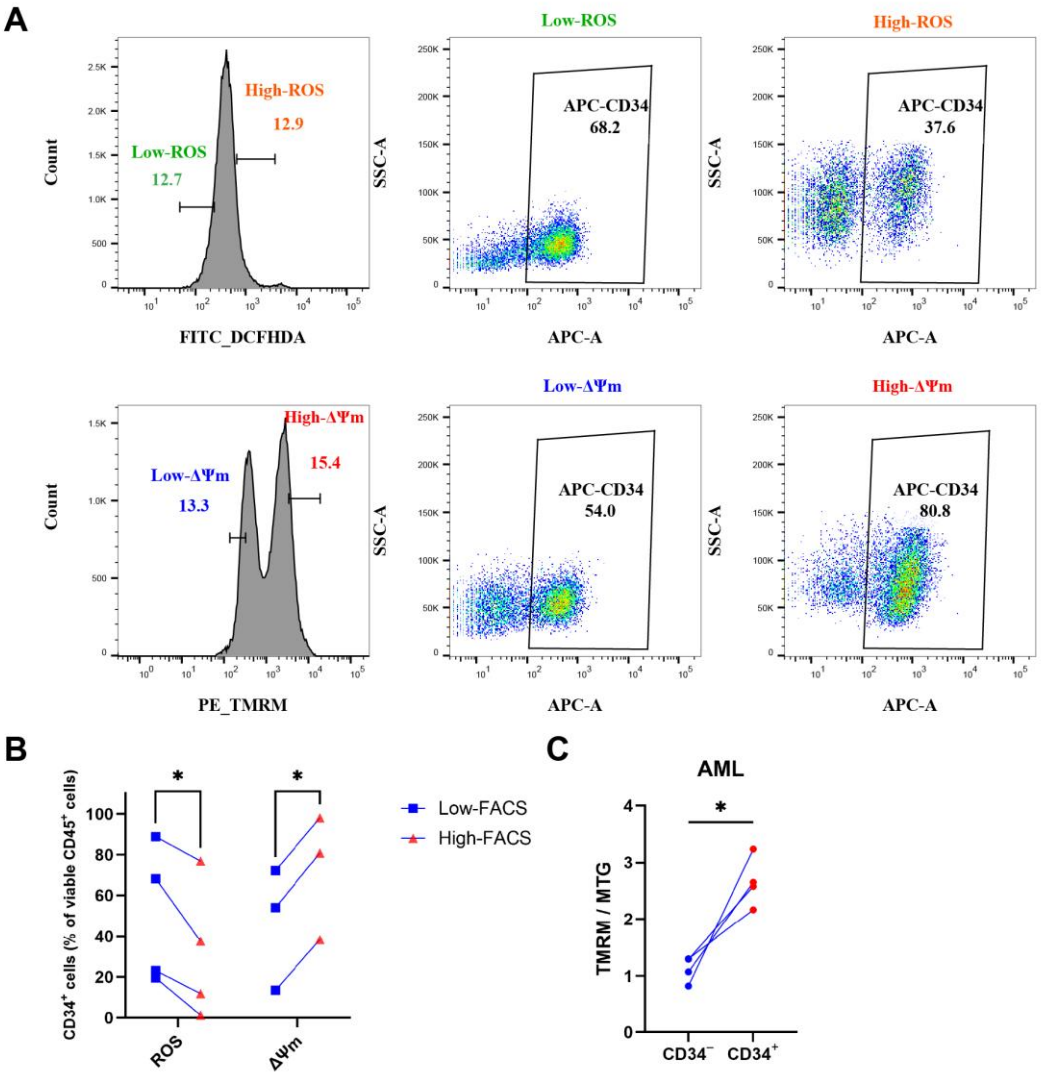

Figure S2. ΔΨm- versus ROS-based fractionation in primary human AML. (**A**) Representative flow cytometric analysis of fractionation by ROS (DCFH-DA) and by ΔΨm (TMRM), with CD34 expression in each fraction. (**B**) $CD34^+$ cells (% of viable $CD45^+$ cells) in the low- and high-ROS (paired t test, $n = 4$) and low- and high-ΔΨm (paired t test, $n = 3$) fractions. (**C**) TMRM/MitoTracker Green (MTG) ratio of $CD34^-$ and $CD34^+$ cells ($n = 4$, * $p < 0.05$).

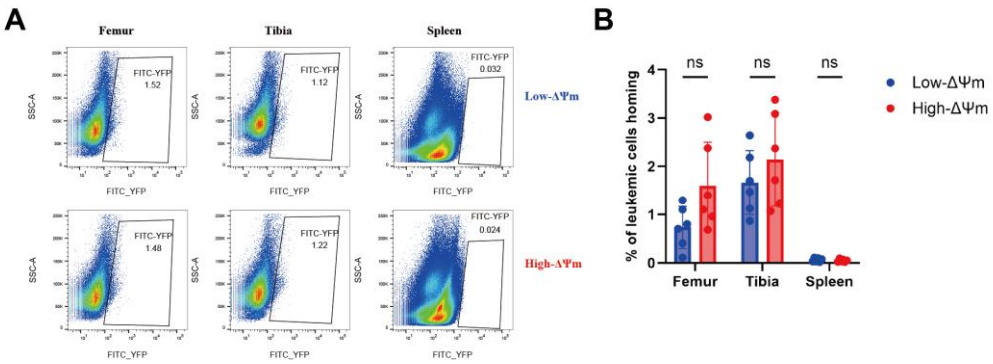

Figure S3. Homing of sorted low- and high-ΔΨm leukemic cells. (**A**) Representative flow cytometric analysis of donor-derived YFP$^+$ cells in femur, tibia and spleen 18 h after transplantation of low- or high-ΔΨm leukemic cells. (**B**) Homing efficiency (n = 6, ns not significant).

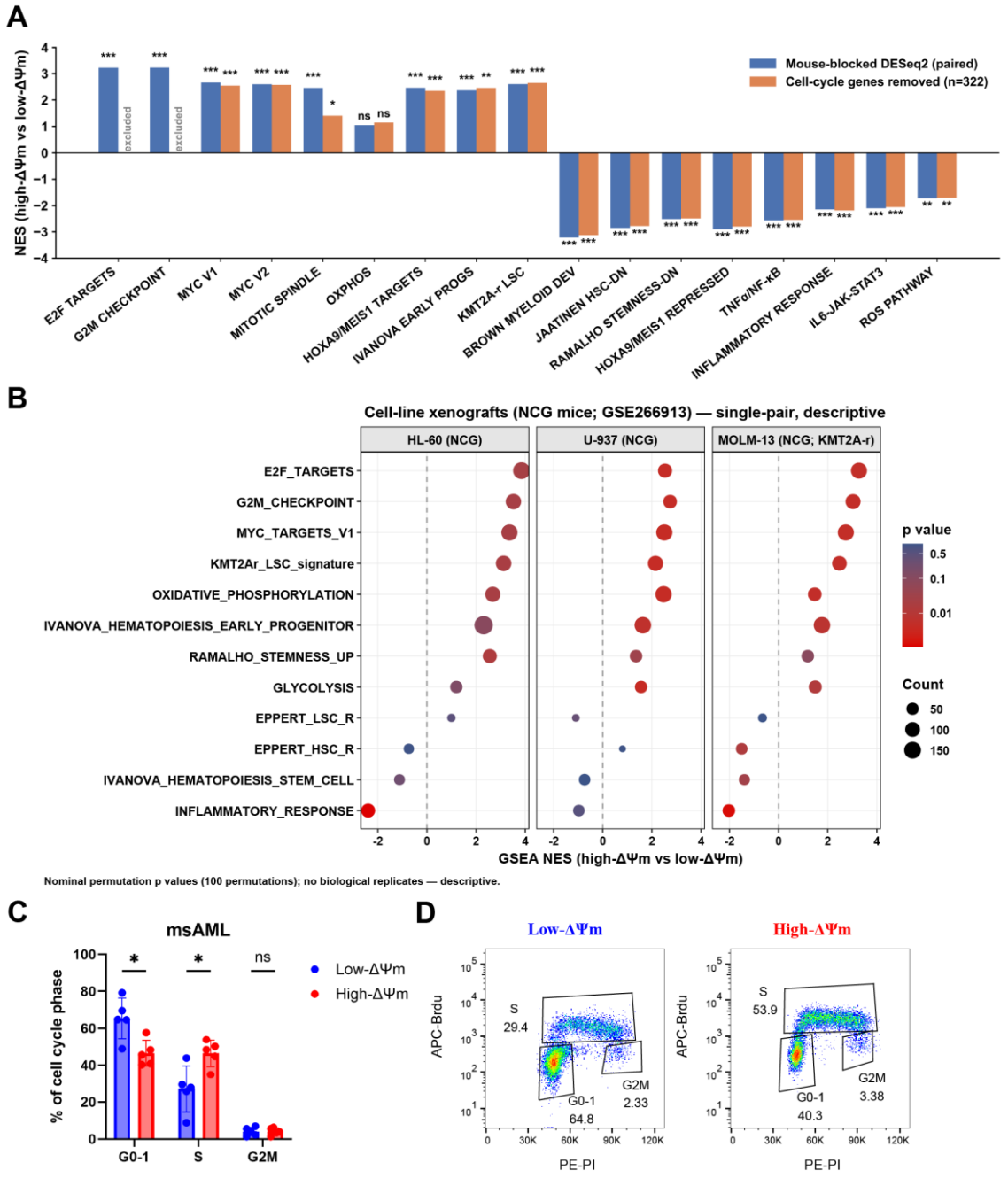

Figure S4. Cell-cycle-corrected gene-set enrichment and cell-cycle distribution. (**A**) Normalised enrichment score (NES) of the indicated gene sets in the paired (mouse-blocked) model (blue) and after removal of cell-cycle genes from the ranked list (orange, n = 322). (**B**) GSEA in cell-line xenografts from GSE266913 (single-pair comparisons). (**C**) Cell-cycle distribution (G0-1/S/G2M) of low- and high-ΔΨm msAML cells (n = 3). (**D**) Representative flow cytometric analysis of BrdU/propidium iodide plots with the G0-1, S and G2M gates (* $p < 0.05$, *** $p < 0.001$, ns not significant).

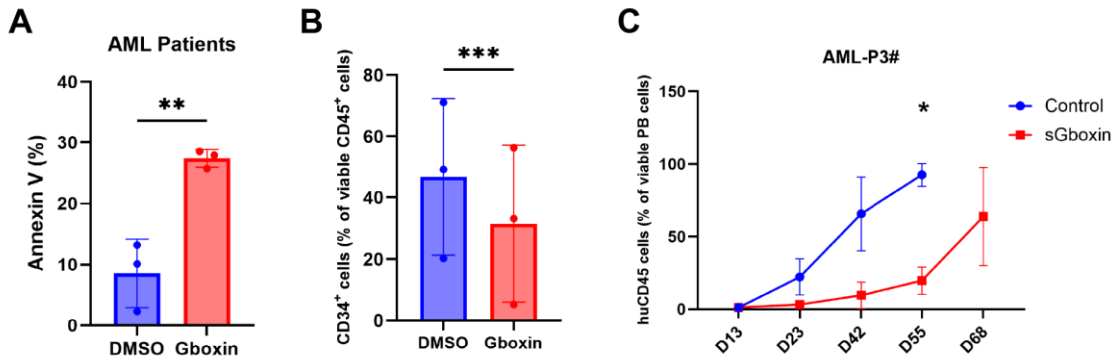

Figure S5. Gboxin response in primary human AML samples and a patient-derived xenograft. (**A**) Annexin V positivity of primary AML cells treated ex vivo with DMSO or Gboxin 5 μM ($n = 3$). (**B**) $CD34^+$ cells (% of viable $CD45^+$ cells) after treatment with DMSO or Gboxin 5 μM ($n = 3$). (**C**) Peripheral-blood $huCD45^+$ cells (% of viable PB cells) in NSG mice engrafted with patient P3# cells and treated with vehicle or sGboxin ($n = 3$) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).