

Figure W1. Migration of MDA-MB-231 cells in response to increasing concentrations of organ-CM. Cells (5×10^4 per well) were plated in triplicate ($n = 3$) on top of gelatin-coated transwells ($8\text{-}\mu\text{m}$ pore size) before placement into either basal media (DMEM/F12 + MITO+) or increasing concentrations (0.0156, 0.0312, 0.0468, and 0.0624 g of tissue per milliliter) of organ-CM: (A) bone, (B) brain, (C) liver, (D) LN, or (E) lung. Migration was allowed to occur for more than 18 hours at 37°C (5% CO_2). Transwells were then fixed with glutaraldehyde, stained with Harris' hematoxylin, and developed with ammonium hydroxide. Five HPFs of view were captured per transwell, and migrated cells were enumerated using ImageJ software (NIH). Data are presented as means \pm SEM ($N = 3$; fold change from negative control of basal media). No significant differences between CM concentrations were noted. Therefore, we carried out the remainder of our migration experiments with the lowest dose, 0.0156 g/ml, which allowed us to see physiologically relevant differences in migration and still use the media in a conservative fashion, thus reducing the number of mice needed to generate enough media for all experiments.

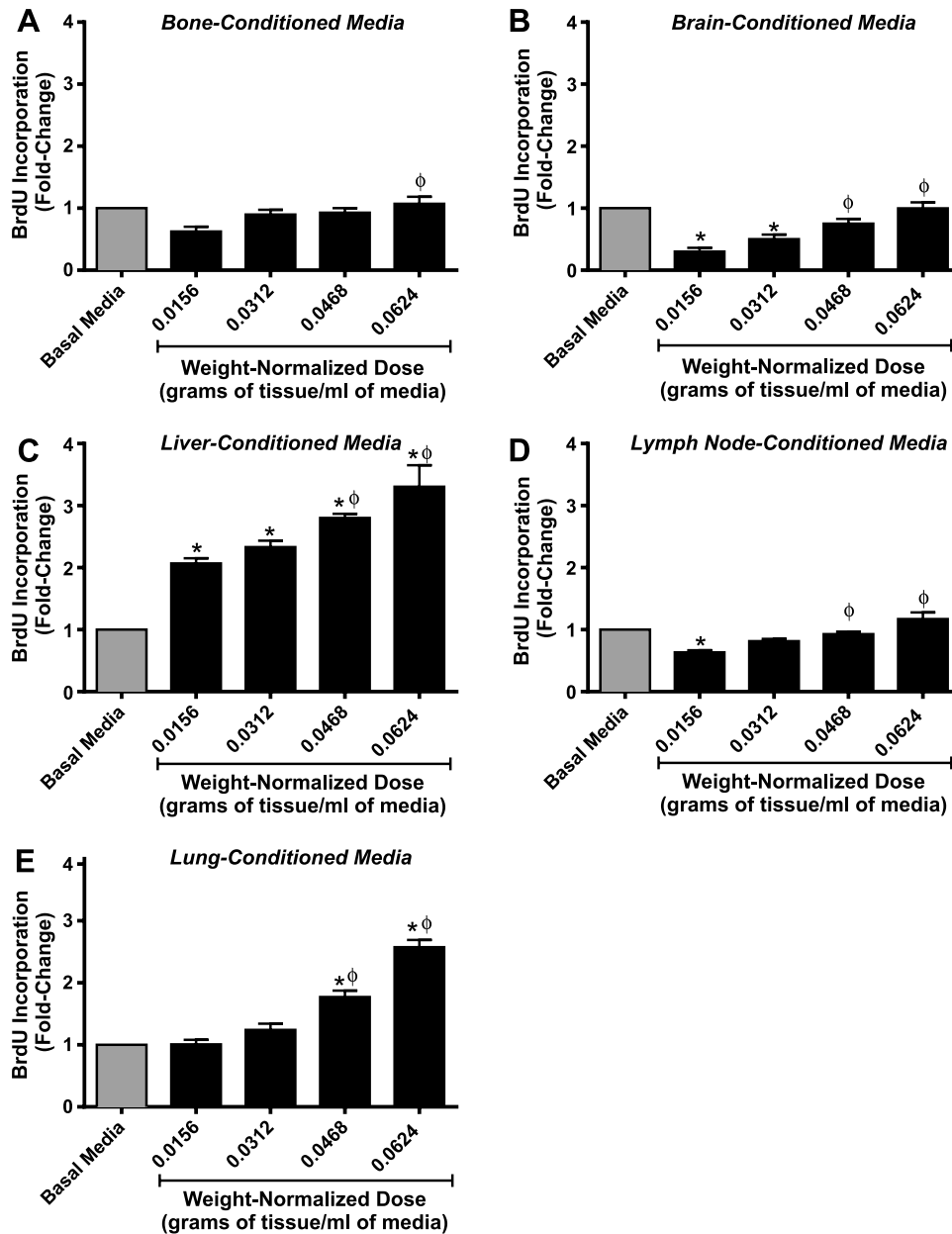


Figure W2. Proliferation of MDA-MB-231 cells in response to increasing concentrations of organ-CM. Cells (1.5×10^4 per well) were plated in triplicate ($n = 3$) in eight-well chamber slides and allowed to adhere for 24 hours. Cells were washed with PBS and incubated for 72 hours in serum-free media. The media were then changed to either basal media (DMEM/F12 + MITO⁺) or increasing concentrations (0.0156, 0.0312, 0.0468, and 0.0624 g of tissue per milliliter) of organ-CM: (A) bone, (B) brain, (C) liver, (D) LN, or (E) lung. After 24 hours, cells were incubated with 5 μ l/ml BrdU for 30 minutes before fixation and staining for BrdU incorporation through immunofluorescence. Positive cells were enumerated and determined as a percentage of total cells present (through nuclear staining with DAPI). Data are presented as means \pm SEM ($N = 3$; fold change from negative control of basal media). *, significantly different from basal media; ϕ , significantly different from lowest respective organ-CM concentration (0.0156 g of tissue per milliliter of media) (ANOVA with Dunnett posttest; $P < .05$). Because the highest concentration of organ-CM (0.0624 g of tissue per milliliter of media) gave the most consistent proliferative response across organs, we therefore carried out the remainder of our proliferation assays using this concentration.

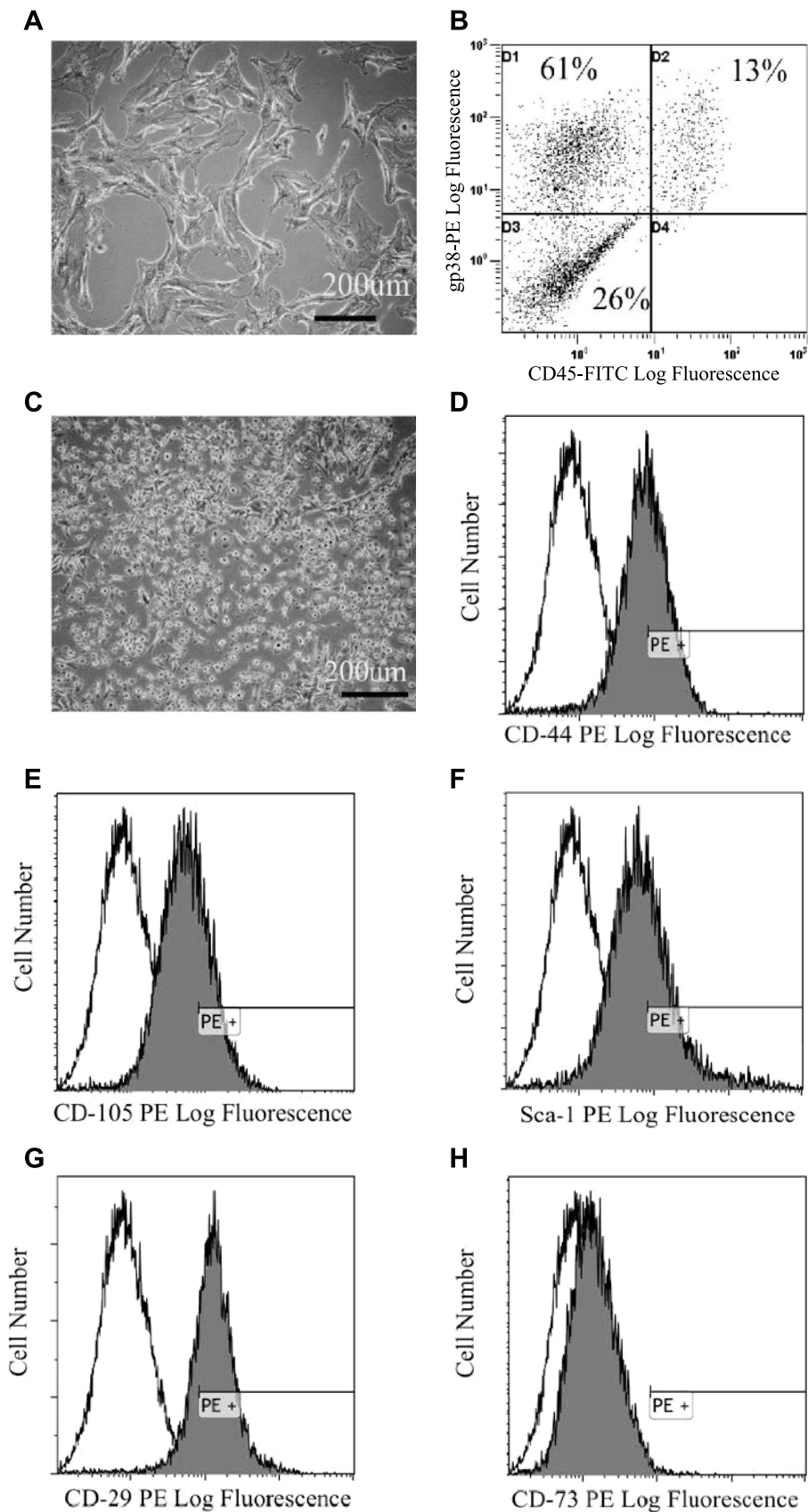


Figure W3. Characterization of LNSCs and BMSCs. (A) Bright-field microscopy image showing the morphology of LNSCs. (B) Representative flow cytometry analysis of LNSCs using a PE-conjugated gp38 antibody and a FITC-conjugated CD45 antibody. (C) Bright-field microscopy image showing the morphology of BMSCs. (D–H) Representative flow cytometry analysis of BMSCs using PE-conjugated (black profiles) antibodies against (D) CD44, (E) CD106, (F) Sca-1, (G) CD29, and (H) CD73 antibodies relative to the isotype control (white profiles). A minimum of 10,000 viable events were collected per sample.

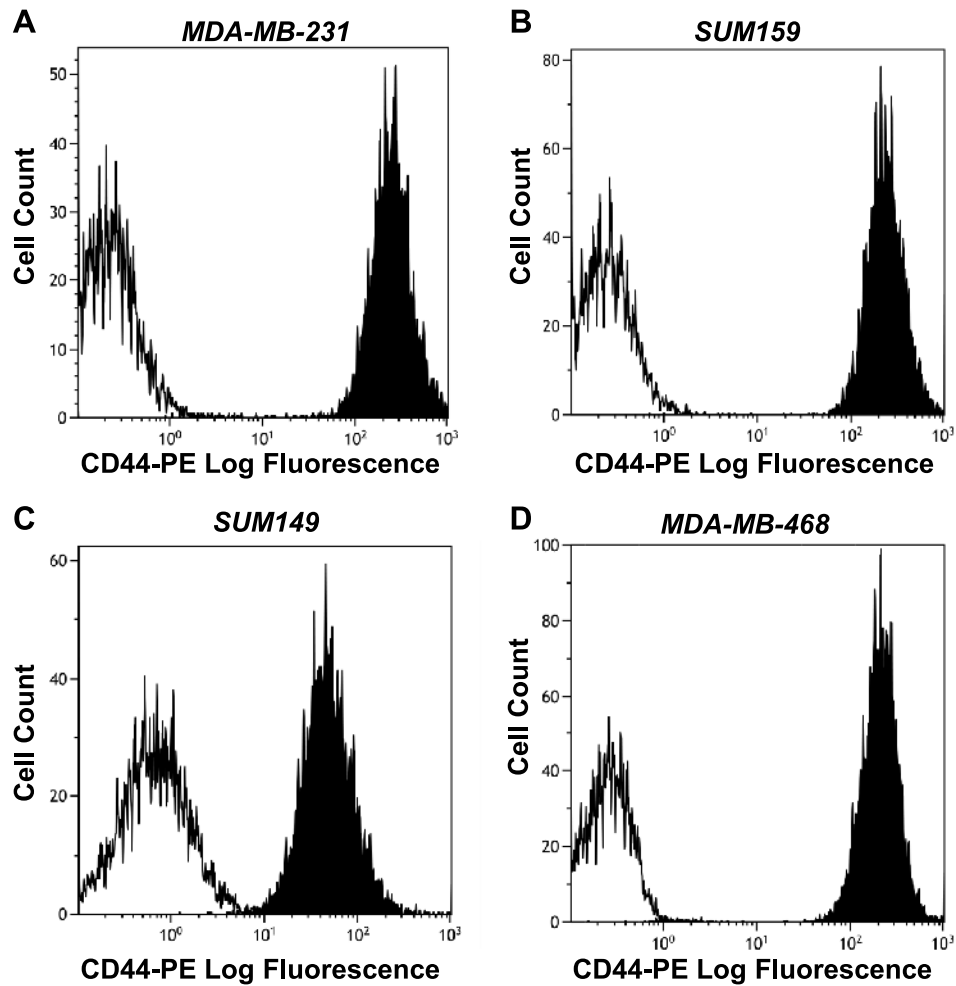


Figure W4. Human breast cancer cell lines express CD44. (A–D) Flow cytometry analysis of CD44 in (A) MDA-MB-231, (B) SUM 159, (C) SUM 149, or (D) MDA-MB-468 human breast cancer cells. Cells (1×10^5) were labeled with an anti-CD44 antibody (clone IM7) conjugated to PE or an IgG-PE isotype control. Representative histograms of CD44 expression (black profiles) relative to the IgG isotype control (white profiles) are shown. A minimum of 10,000 viable events were collected per sample.

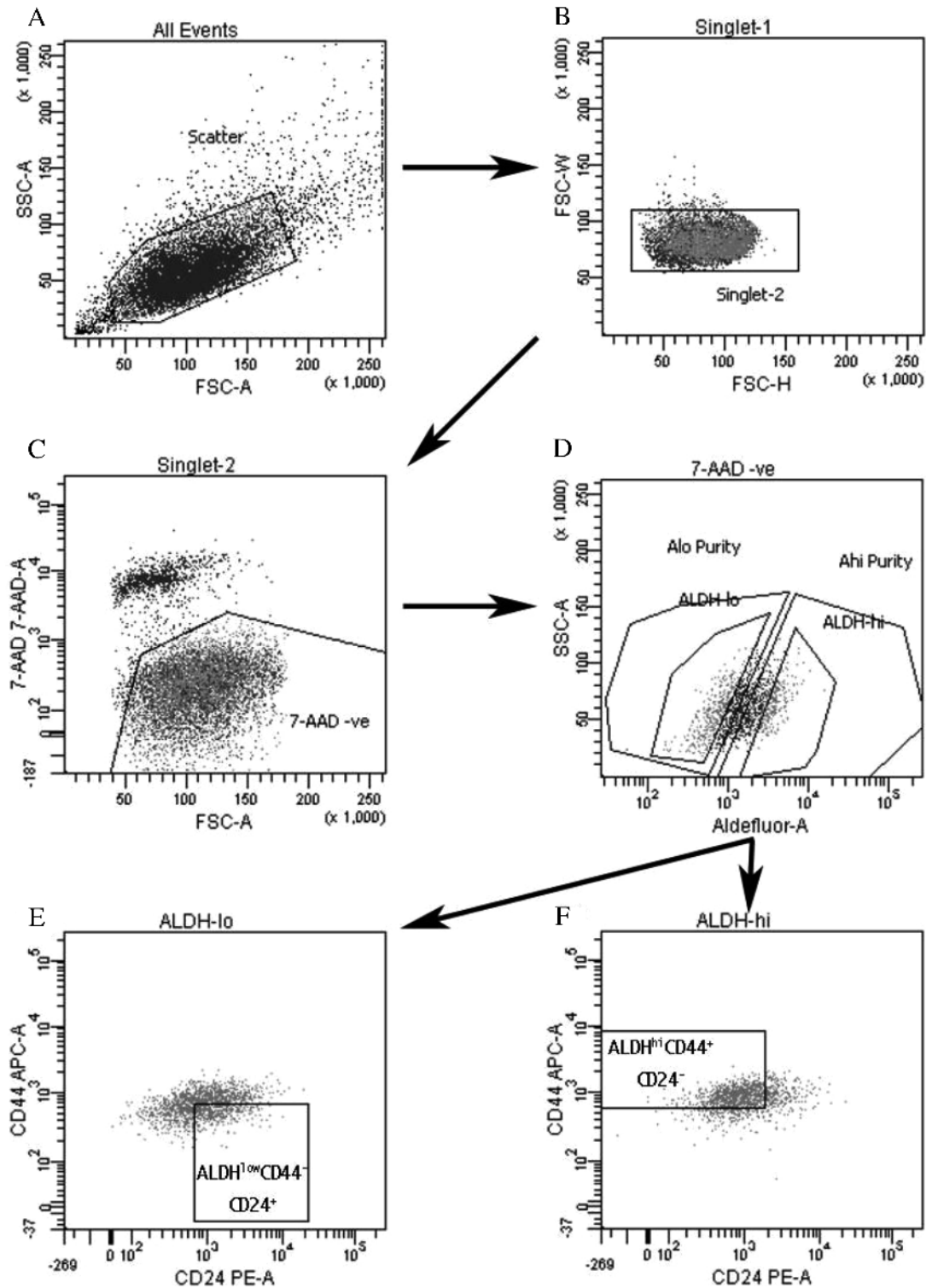


Figure W5. Strategy for FACS isolation of ALDH^{hi}CD44⁺ and ALDH^{low}CD44⁻ cells. MDA-MB-231 cells were concurrently labeled with 7-AAD, CD44-allophycocyanin, CD24-PE, and the ALDEFLUOR assay kit. Cell subsets were isolated using a four-color protocol on a FACSAria III (Becton Dickinson). (A) Cells were selected on the basis of expected light scatter, (B) then for singlets, and (C) viability based on 7-AAD exclusion. (D) Cells were analyzed for ALDH activity, and the top 20% most positive were selected as the ALDH^{hi} population, whereas the bottom 20% of cells with the lowest ALDH activity were deemed to be ALDH^{low}. (E) Finally, 50% of the ALDH^{low} cells were further selected on the basis of a CD44^{low/-}CD24⁺ phenotype, and (F) 50% of the ALDH^{hi} cells were selected on the basis of CD44⁺CD24⁻ phenotype. The isolated cells were used for the functional analyses of migration and growth in the presence of organ-CM *in vitro* (Figure 5, D and E).