# Supplemental Figure Legends

**Figure S1.** Integrin expression in breast tumors and breast cancer cell lines. **A,** Heat map of the mean-centered gene expression values of individual genes that comprise the hypoxia score using patient data from the TCGA Breast Cancer Data Set. Red = greater than median expression, Green = less than median expression. Each bar represents data from 1 of the 597 patients, which are rank-ordered according to the HIF signature score. **B**, RT-qPCR analysis of ITGA2, ITGA3, ITGA4, ITGA6, and P4HA1 levels in MCF10A, MCF-7, HCC1954, MDA-MB-231, or MDA-MB-435 cells exposed to 20% or 1% O2 conditions. Student’s t-test was utilized to test for significant difference in expression under 20% versus 1% O2 conditions. mean ± SEM, n = 3, \*\*\**P* < 0.001, vs. NTC (one-way ANOVA).

**Figure S2.** MCF-7 cells require HIF-1 and HIF-2 for cell surface expression of ITGA5. **A,** mRNA expression of ITGA5 in MCF-7 cells expressing an shRNA against NTC or HIF-1α (sh1α) or HIF-2α (sh2α) under 20% or 1% O2 conditions for 24 h. **B,** Flow cytometric analysis of MCF-7 subclones for cell surface expression of ITGA5.

**Figure S3.** Characterization of ITGA5 expression in MDA-MB-231 subclones. **A,** ITGA5 mRNA expression in control (NTC) or ITGA5 knockdown (shA5-1 and shA5-2) MDA-MB-231 subclones exposed to 20% or 1% O2 for 24 h. **B,** Immunoblot assay of lysates from control (NTC) or ITGA5 knockdown (shA5-1 and shA5-2) MDA-MB-231 subclones exposed to 20% or 1% O2 for 48 h. **C,** Flow cytometric analysis of cell surface expression of ITGA5.

**Figure S4.** Characterization of fibronectin-collagen gels. **A,** Reflection confocal microscopy was utilized to examine the microstructure of 3D collagen gels incorporated with 0, 10, or 50 µg of fibronectin per mg of collagen. **B,** The area between fibers was quantified using image analysis in order to compare the pore size of the indicated 3D matrices. **C,** MDA-MB-231 NTC or shITGA5-1 spheroids imaged at day 3 to assess invasion into the surrounding collagen-fibronectin matrix.

**Figure S5.** Growth and metastasis characteristics of MDA-MB-231 subclones. **A,** Growth kinetics of control (NTC) or ITGA5 knockdown (shA5-1 and shA5-2) subclone in vitro measured by a presto blue assay. **B,** Quantification of lymph node sections that were subjected to immunohistochemistry using an antibody specific for human vimentin in Figure 6d (mean ± SEM, n = 5, \*\*P < 0.01, vs. NTC, one-way ANOVA).

**Figure S6. A,** Survival analysis based on ITGB1 expression. Kaplan-Meier analysis of disease-specific survival of 1,881 breast cancer patients stratified by ITGB1 mRNA expression above the median level (High) or below the median level (Low) was performed using patient data obtained from the GOBO database. **B,** The 1,881 breast cancer patients in the GOBO database were divided by estrogen receptor (ER) status (top) and LN status (bottom) and then stratified by ITGA5 mRNA expression above the median level (High) or below the median level (Low) for Kaplan Meier Analysis (n = number of patients in each group).