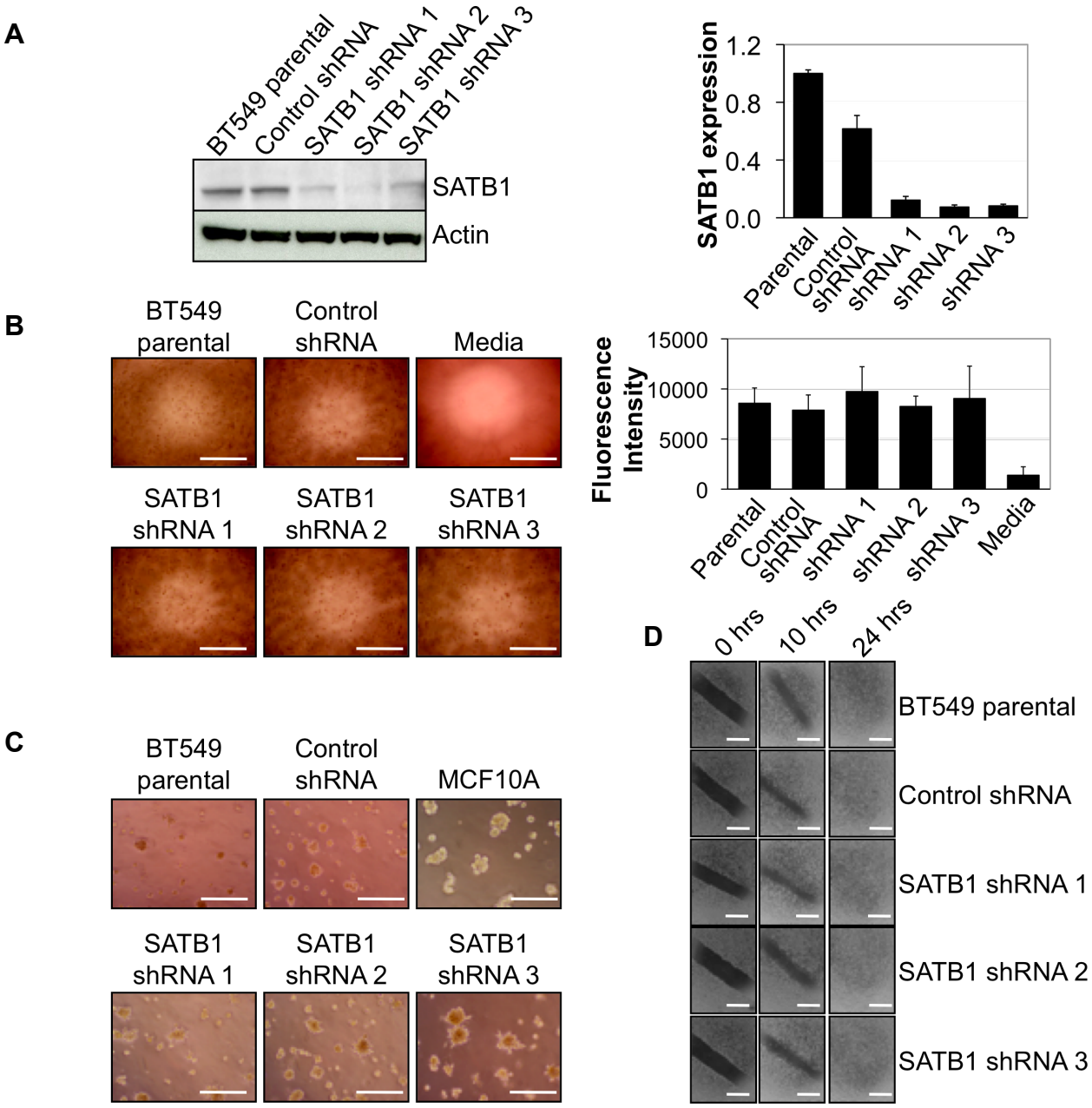
SUPPLEMENTARY FIGURE 1



Supplementary Figure 1. Effect of SATB1 depletion on the aggressive cancer cell phenotype in vitro. For these experiments, we used SATB1 short hairpin RNA (shRNA) 1, 2, and 3 BT549 cells, control shRNA BT549 cells, and parental BT549 cells. **A)** SATB1 mRNA and protein expression. **Left)** SATB1 protein expression by immunoblot analysis. Actin protein expression was the loading control. **Right)** SATB1 mRNA expression by quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) analysis. Data are expressed as normalized SATB1 expression, calculated relative to expression of endogenous GAPDH mRNA control and adjusted relative to the expression of GAPDH normalized SATB1 in parental BT549 cells. SATB1 mRNA experiments were repeated three times, with each point in triplicate. Error bars = 95% confidence intervals (CIs). **B)** Colony formation in soft agar after 8 days of culture. **Left)** Representative micrographs of colonies. Scale bars = 1 mm. **Right)** Quantification of colony formation in soft agar after 8 days of culture. To measure colony formation, agar was solubilized, cells were lysed, and the number of cells in the colonies was assessed by use of CyQuant® GR Dye with a fluorescence plate reader. Fluorescence intensity is directly proportional to cell number. The experiment was performed twice, with triplicate samples. Error bars = 95% CIs. **C)** Acinar morphology after 5 days of culture on matrigel. MCF10A cells were used as a control for normal mammary epithelial cell acinar formation. Invasive protrusions from acinar demonstrate aggressiveness. The experiment was performed twice, with triplicate samples. Representative micrographs are shown. Scale bars = 1 mm. **D)** Cell migration as assessed by wound healing. Confluent cell cultures, as indicated, were wounded by scratching a single wound through the middle of the cell monolayer with a 200-µL pipette tip and wounds were photographed 0, 10, and 24 hours after wounding. Rates of cell migration are shown by closing of the wounds. Representative micrographs are shown. The experiment was performed twice, with triplicate samples. Scale bars = 0.5 mm.