

Figure S2

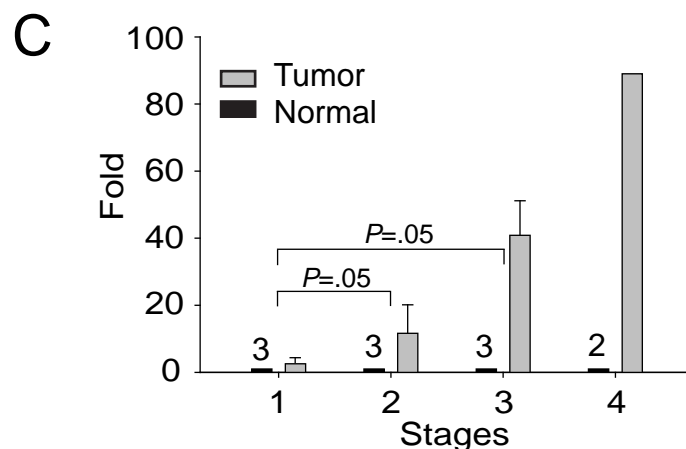
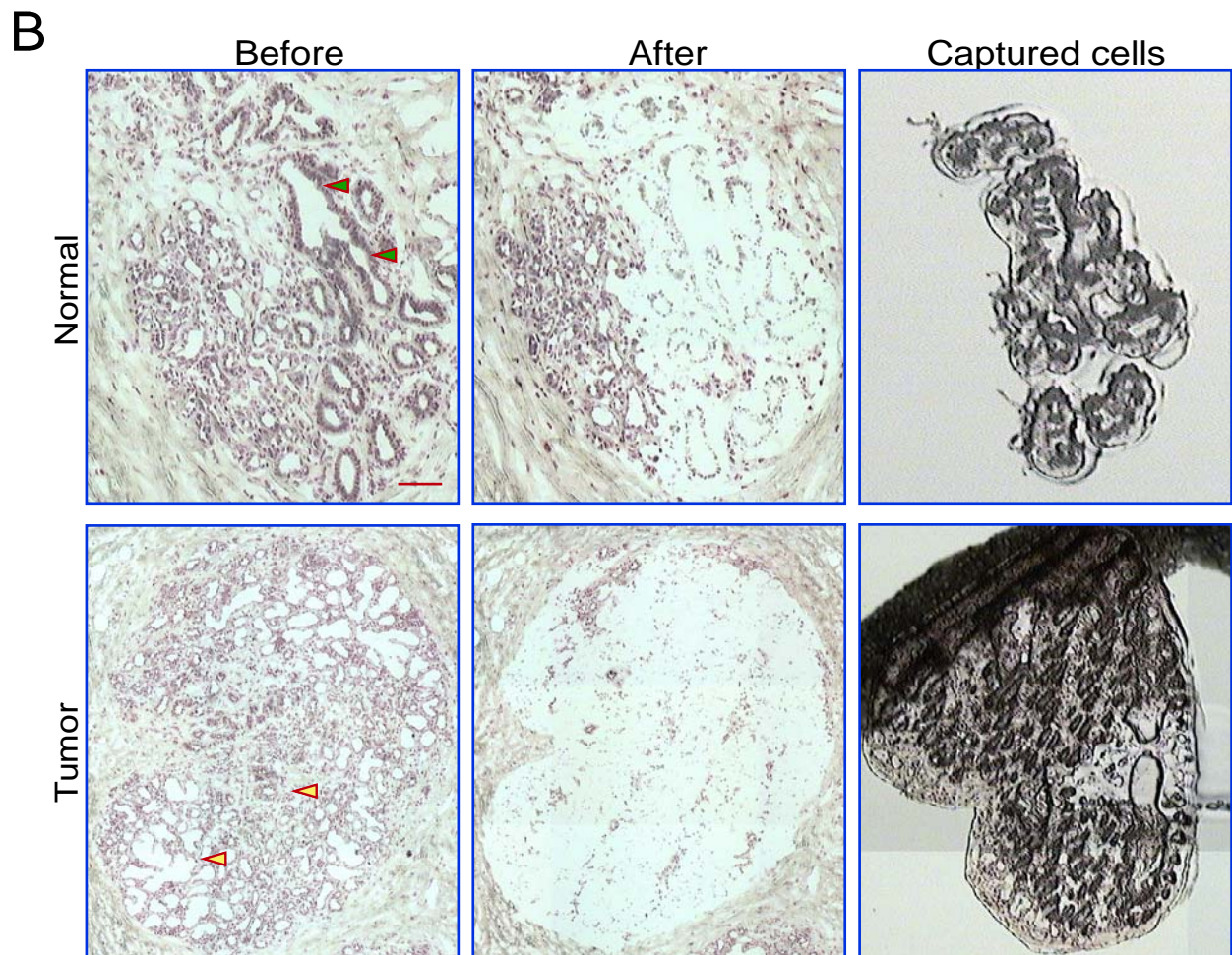
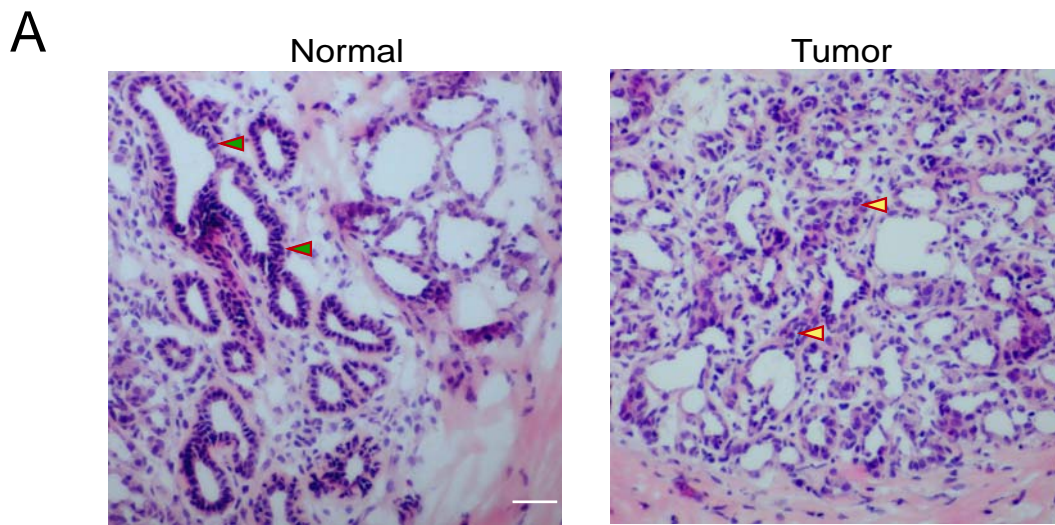


Figure S2. Laser capture-microdissection of human breast tissue sections and $\alpha 9$ -nicotinic acetylcholine receptor ($\alpha 9$ -nAChR) gene expression in stage-associated tumor cell clusters. **A)** Hematoxylin and eosin (H.E.)-stained tumor tissue sections from one representative patient sample that had normal (left) and tumor (right) cells prior to microdissection. Scale bar = 100 μ m. **B)** Laser-capture microdissection of matched tumor and normal cell specimens. Sections stained with hematoxylin and eosin were subjected to laser-capture microdissection using a PixCell Iie system (Arcturus Engineering, Mountain View, CA) [34]. The parameters used for laser-capture microdissection included a laser diameter of 8 μ m and a laser power of 48–65 mW. For each specimen, 15,000 laser pulse discharges were used to capture 10,000 morphologically normal epithelial cells or malignant cells. Each population was visualized under a microscope to ensure that the captured cells were homogeneous. After the cells were captured (right panel), total RNA was isolated, according to the manufacturer's protocols. From this cohort, 11 tumor cell samples were obtained. **Green arrowheads:** laser imprint with captured normal cells; **yellow arrowheads:** tumor cells. We were able to obtain individually matched, tumor-adjacent normal cells. Scale bar = 200 μ m. **C)** Fold differences in $\alpha 9$ -nAChR mRNA expression levels in laser-capture microdissected tumor vs normal paired samples as detected by real-time-polymerase chain reaction [59]. Data represent the mean fold ratios from laser capture microdissection-dissected samples with different clinical staging criteria from stage 1-4; error bars indicate 95% confidence intervals. Comparisons were performed for stage 1 vs stage 2 ($P < .001$) and stage 1 vs stage 3 ($P < .001$). Data were analyzed using an overall nonparametric test (Kruskal Wallis test), and multiple comparisons were made with the

Scheffe test. All P -values are two-sided.