

Supplemental information

Single-cell analysis of autophagy activity in normal and *de novo* transformed human mammary cells

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The authors declare no conflict of interest.

Running title: Autophagy in human mammary progenitors

Keywords: human mammary cells, progenitor heterogeneity, tumor initiation, tandem RFP-GFP-LC3B, breast cancer.

Figure S1. ATG-related proteins levels in human primary mammary sorted or cultured cells. (A) Quantification of the ratios of human ATG4B to H3 protein levels shown in Figure 2A (WBs) for matched cells from 3 normal individuals. (B) Quantification of the ratios of ATG7/ACTIN and ATG4B/ACTIN levels shown in Figure 2E. P values were calculated using the Student t-test.

Figure S2. Maintenance of high autophagy status *in vitro*. RFP and GFP intensities in human BCs and LPs transduced with lenti-*RFP-GFP-LC3B* virus after being sorted 4 days later according to their R⁺ or G⁺ status and then re-analyzed for their R⁺ or G⁺ status after another 3 days *in vitro*. P-values were calculated using the Student t-test.

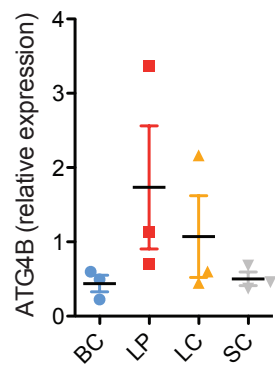
Figure S3. Inducible shATG7 efficiency. (A) Levels of ACTIN and LC3B determined in WBs of FACS-purified primary mammary cells from two different normal donors after incubation at 37°C for two hours in SF7 medium containing either DMSO (0.5%), 100 or 200 nM BafA₁, 2 or 20 μM CQ, or starved in EBSS media. (B) Demonstration by WB of doxycycline-induced ATG7 expression in HeLa cells transduced with pTRIPZ-shScr or shATG7 (1) or shATG7 (2). After puromycin selection, cells were treated for 48h with doxycyclin before harvesting. (C) Photomicrographs of representative colonies produced by human BCs and LPs transduced with pTRIPZ-shScr or shATG7 (1) or shATG7 (2). Scale bar represents 200μm.

Figure S4. Low autophagy status enhances tumor initiation from cells transduced with *KRAS*^{G12D}. Related to Figure 4. (A) Representative views of IgG staining of normal human breast tissue (negative control) and LC3B immunostaining of MDA-MB-231 cells. (B) High efficiency of *KRAS*^{G12D}-YFP transduction of R⁺ and G⁺ BC cells (blue symbols) and LPs (red symbols) detected in terms of %YFP+ cells measured after another 3 days *in vitro*. (C) Representative pictures of *de novo* tumors generated from R⁺ or G⁺ cells 4 weeks after transplantation into mice.

Figure S5. Uncropped images for which boxed areas are shown in the published panels of Figures 1, 2 and 4. (A) Corresponding to Figure 1E. (B) Corresponding to Figure 2A. (C) Corresponding to Figure 2C. (D) Corresponding to Figure 2E. (E) Corresponding to Figure 4A.

Figure S1

A



B

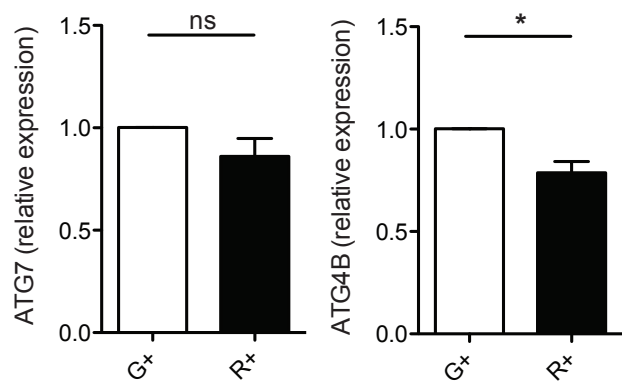


Figure S2

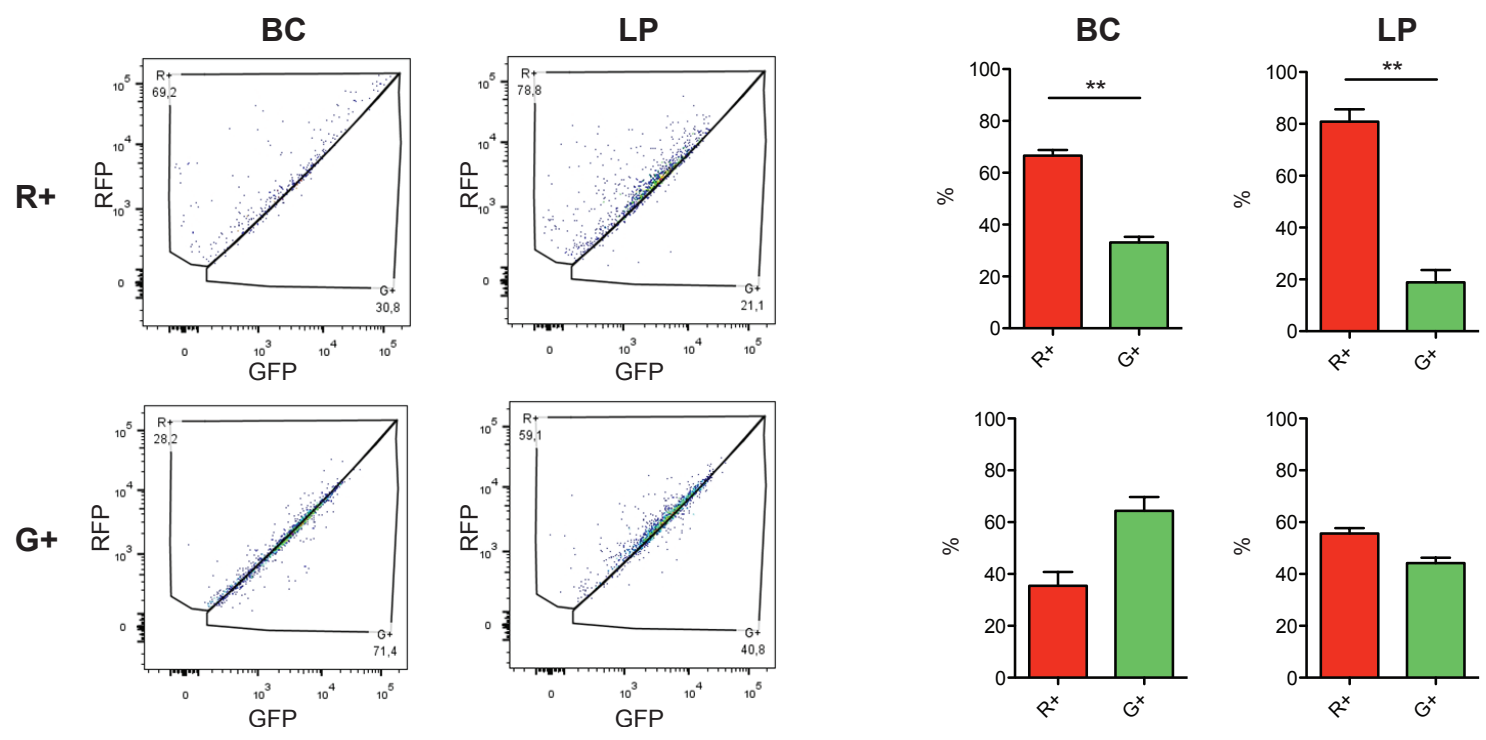
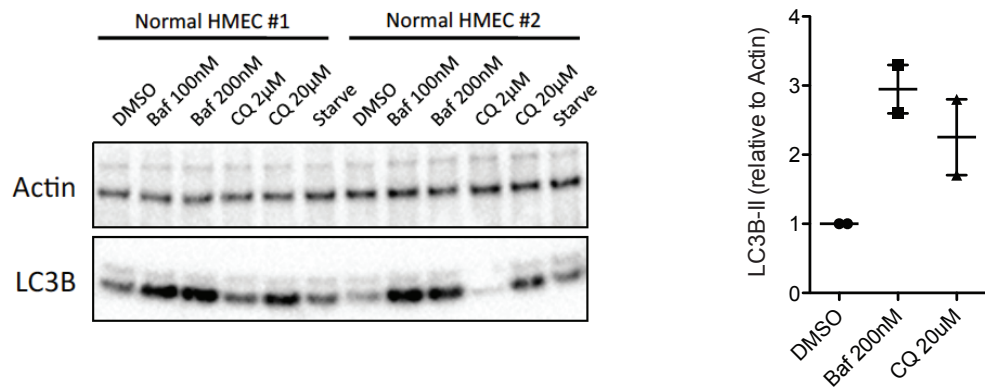
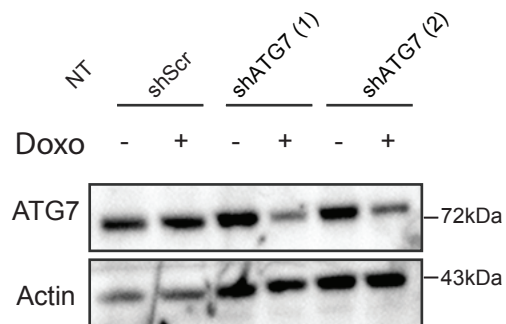


Figure S3

A



B



C

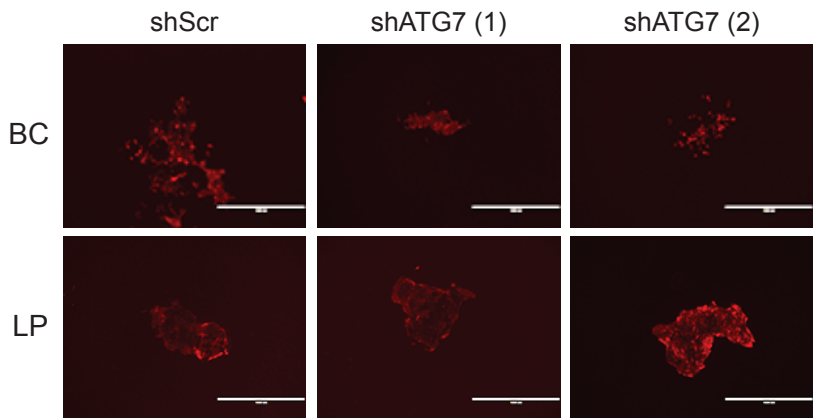


Figure S4

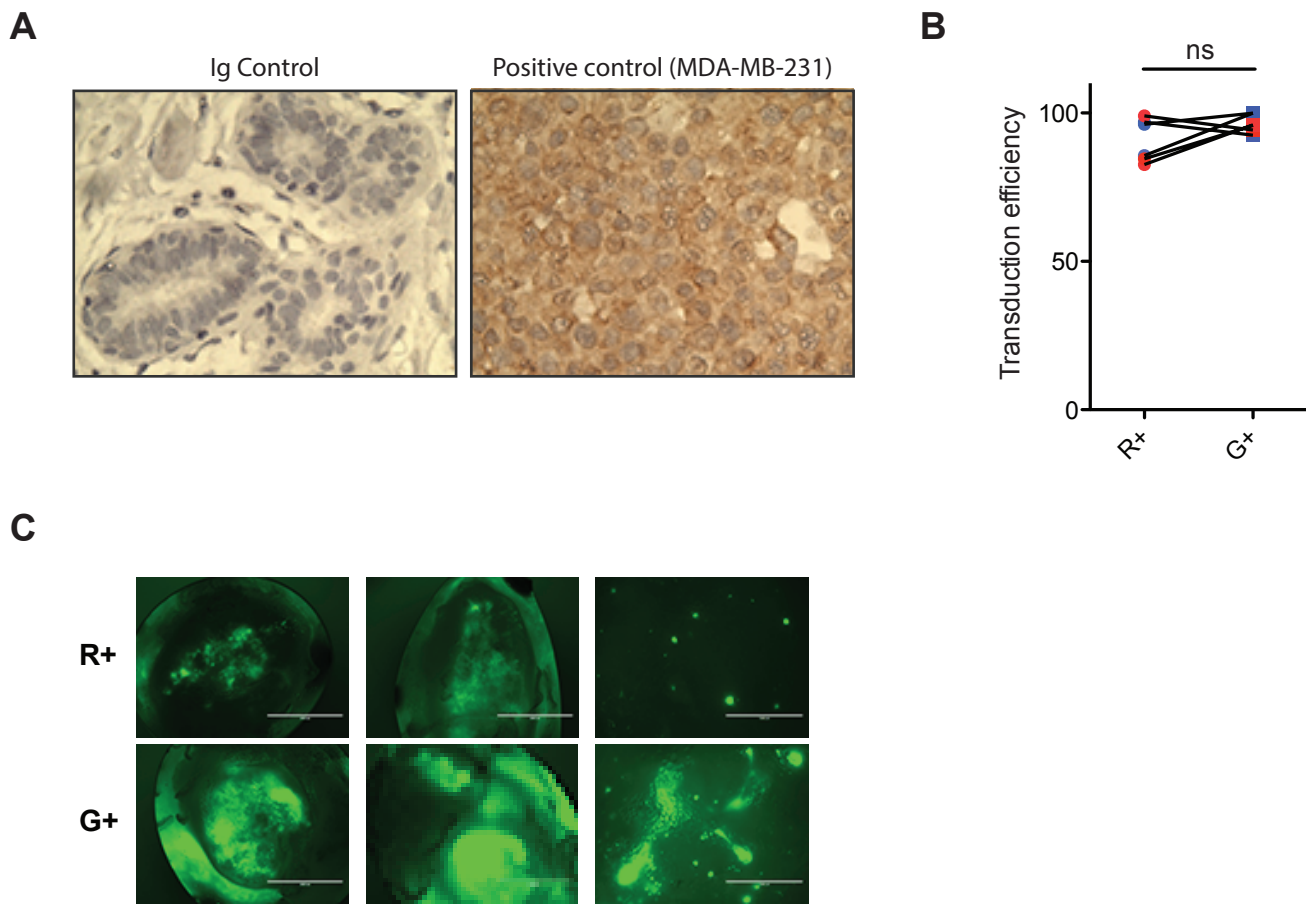
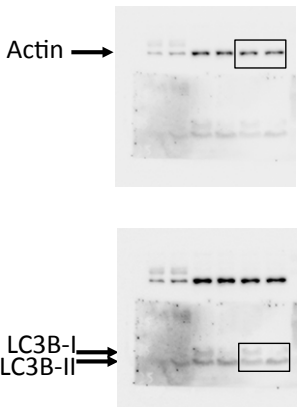
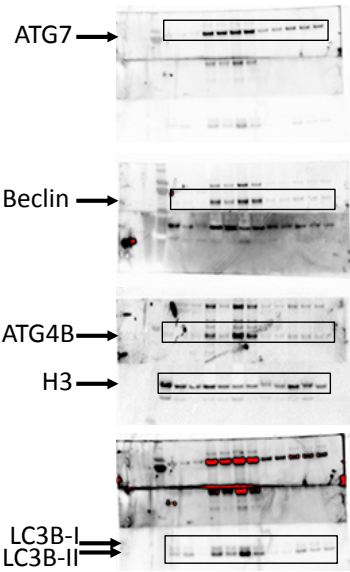


Figure S5

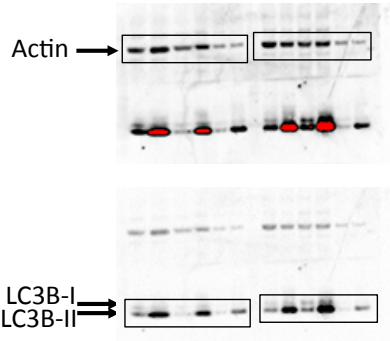
A



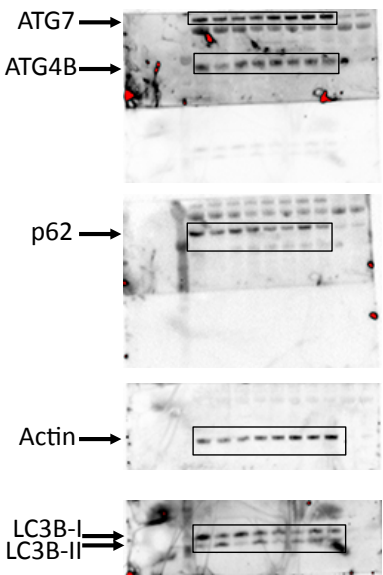
B



C



D



E

