

Supplemental Figure 1

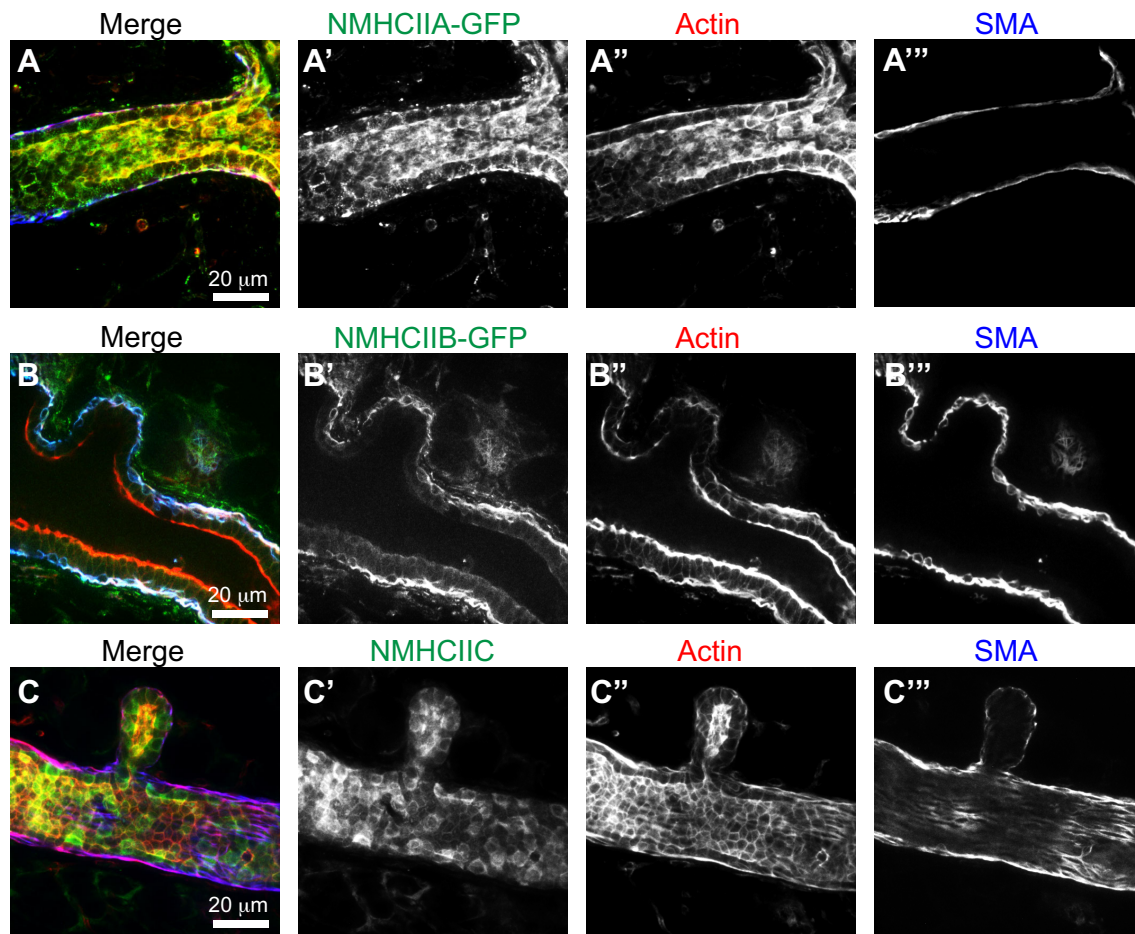


Figure S1. Expression and localization of NMII isoforms in the mammary epithelium. Mammary epithelial ducts were imaged in multicolor (A-C) to reveal localization of (A') NMHIIA-GFP (located in both luminal and myoepithelial cells), (B') NMHIIIB-GFP (located in myoepithelial and in luminal cells at the apical membrane), and (C') stained for NMHIIIC (located predominantly in luminal epithelial cells), (A''-C'') F-actin, (A'''-C''') smooth muscle actin (SMA). Scale bars: 20μm.

Supplemental Figure 2

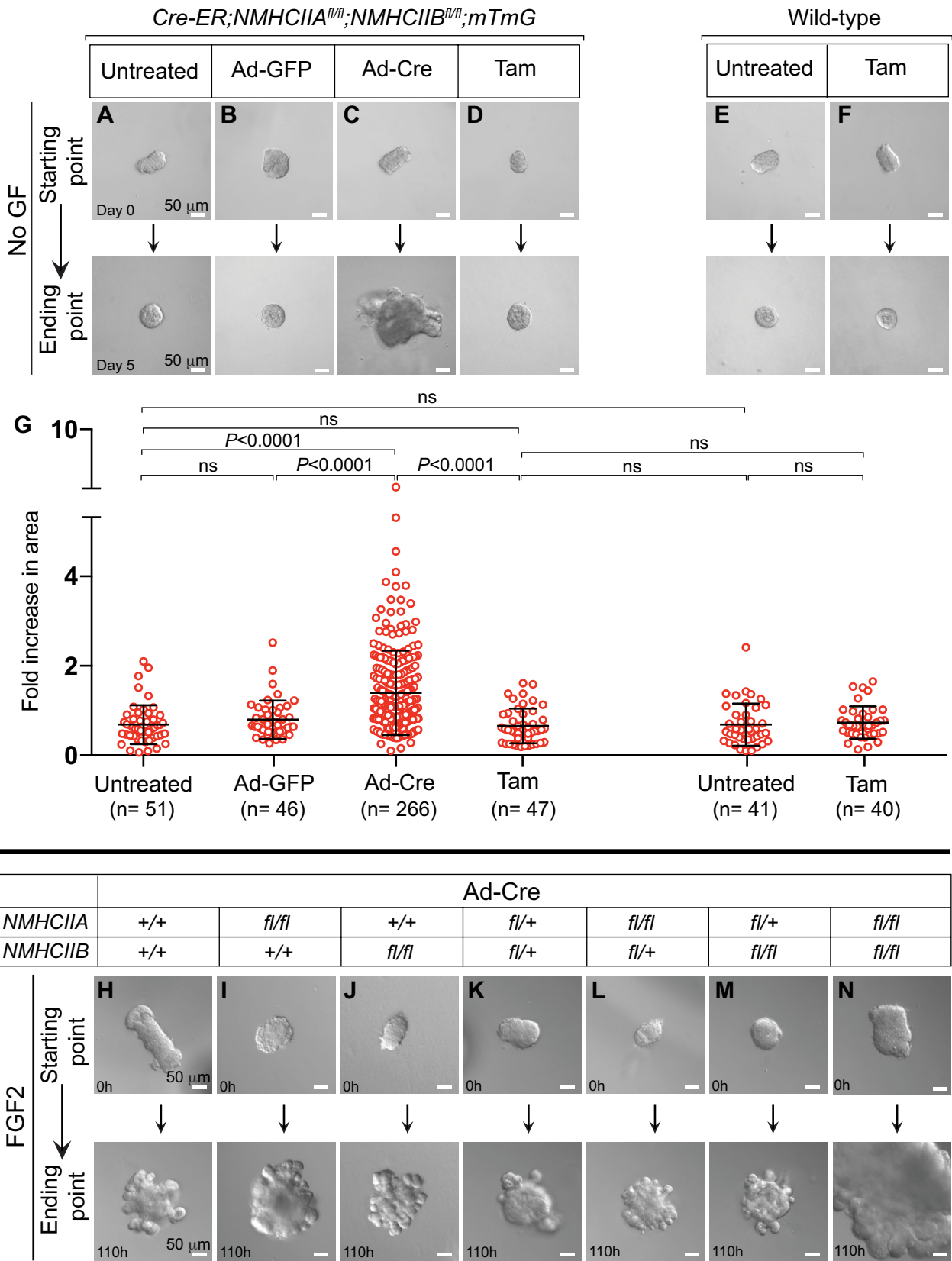


Figure S2. Mosaic deletion of both alleles of *NMIIA* and *NMIIB* induces proliferation in organoids cultured in basal medium or with FGF2 supplementation. (A-D) Representative still images of mammary organoids from *Cre-ER;NMIIA^{fl/fl};NMIIB^{fl/fl}* mice for control (Untreated), adenoviral GFP (Ad-GFP, adenoviral control), adenoviral Cre recombinase (Ad-Cre) and tamoxifen (Tam) treatments. (E-F) Representative still images of mammary epithelial organoids from wild-type mice not carrying loxP-flanked *NMHCIIA* and *NMHCIIIB* alleles for control (Untreated) and Tam treatment. (G) Mammary organoid growth in basal medium was evaluated by fold increase in projected surface area of organoids at day 5 divided day 0 in culture. Images and data correspond to figure 1D. Data are presented as mean+/-s.d., ns, non-significant. n, total number of organoids for 3 mice ($P<0.0001$, one-way ANOVA). The data for *Cre-ER;NMIIA^{fl/fl};NMIIB^{fl/fl}* organoids in the Untreated, Ad-Cre, and Tam conditions are duplicated from Figure 1D for ease of comparison. (H-N) Still images from time-lapse movies of organoids carrying different genetic variants of *NMHCIIA* and *NMHCIIIB* alleles treated with Ad-Cre in media supplemented with FGF2. Organoids were selected to illustrate the most branched representatives of each genotype. Scale bars: 50 μ m.

Supplemental Figure 3

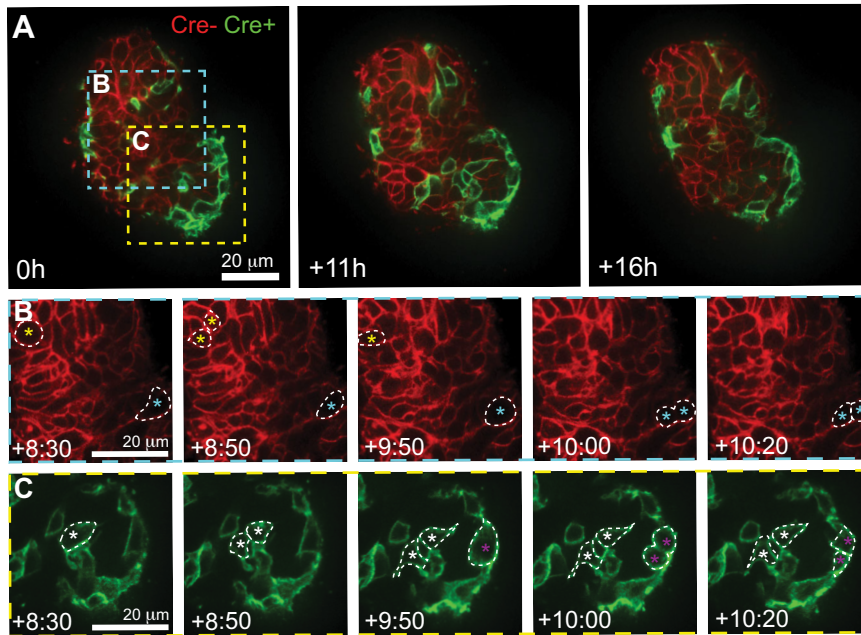


Figure S3. NMIIA and NMIIB double deletion induces proliferation of both wild-type and NMIIA,B null cells in mosaic organoids. (A) Frames from a representative confocal time-lapse movie of a mosaic NMIIA,B-null organoid in basal medium conditions. (B) Insets of the red channel to depict cell division in wild-type cells. (C) Insets of the green channel to depict cell division in NMIIA,B-null cells. White dash lines marked changes in cell shape throughout cell division. Scale bars: 20µm.

Supplemental Figure 4

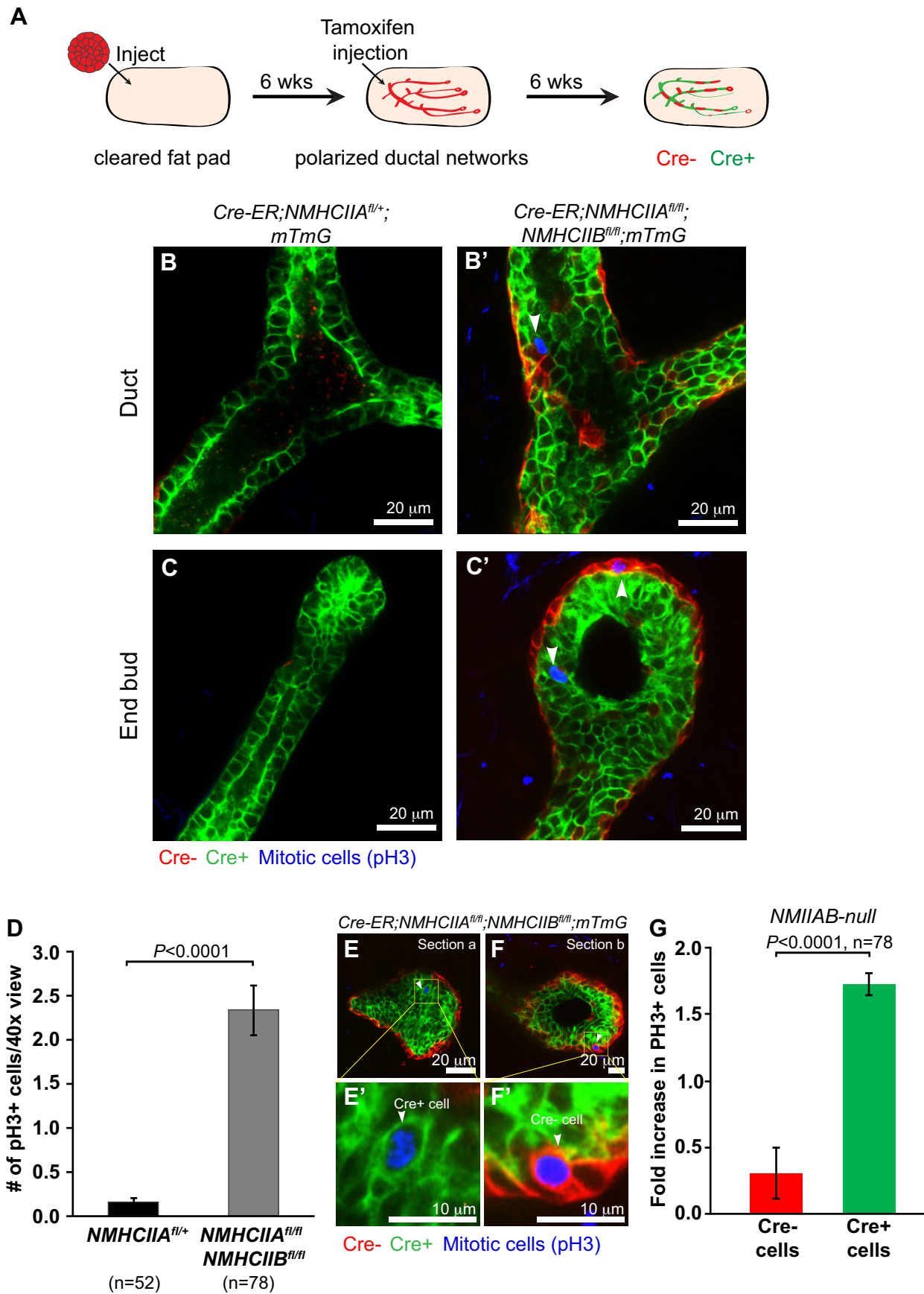
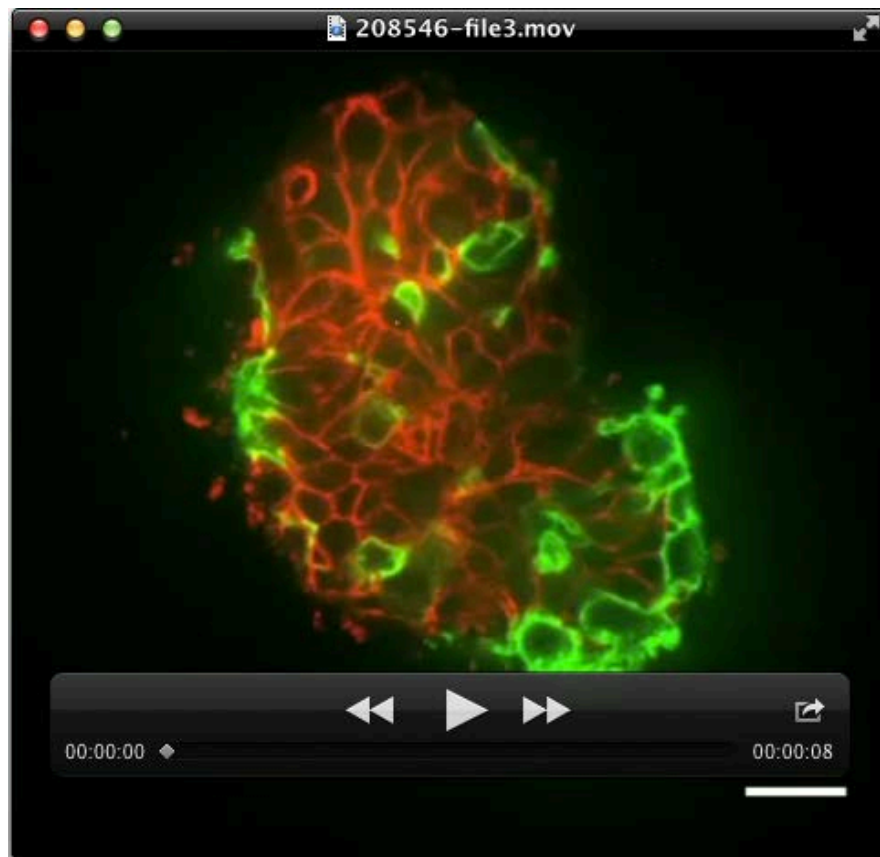


Figure S4. NMIIA,B deletion increases spontaneous proliferation *in vivo*. (A) Schematic description of orthotopic transplantation and inducible gene deletion. (B-C) Representative images of different epithelial structures found in the control glands. (B'-C') Representative images of epithelial structures with pH3+ cells found in NMIIA,B deleted glands. (D) The average number of pH3+ cells per epithelial duct imaged at 40x magnification. (E-F) Different section views of the same epithelial duct. (E'-F') Insets of proliferating (pH3) NMIIA,B-null (Cre+) and wild-type (Cre-) cells. (G) Fold increase in proliferating Cre- and Cre+ cells within NMIIA,B-null epithelium. Error bars indicate s.e.m. $P < 0.0001$, Mann-Whitney test. n, number of fields summed across three biologically independent experiments. Scale bars: (B-C' and E-F) 20 μm , (E'-F') 10 μm .



Movie 1. Mosaic deletion of both NMIIA and IIB induces mammary organoid overgrowth in basal medium conditions. Representative DIC time-lapse movies of control (wild-type) and NMIIA,B-null organoid treated with Ad-Cre cultured in 3D Matrigel under basal medium conditions (No GF). Normal epithelium (left) maintained same size. NMIIA,B-null organoid (right) resulted in epithelium tissue overgrowth. Frames were collected every 20 minutes for 100 hours (displayed at 13 frames/s) using a Cell Observer system with an AxioObserver Z1 and an AxioCam MRM camera (Carl Zeiss). Bars, 50 μm.



Movie 2. NMIIA and NMIIIB double deletion induces cell division of both wild-type and NMIIA,B-null cells in mosaic organoids. Representative confocal time-lapse movie of a mosaic NMIIA,B-null organoid (2D slice) showing cell divisions in both Cre- (red) and Cre+ (green) cells under basal medium conditions (No GF). Frames were collected every 20min for 33h (displayed at 15 frames/s) using a spinning-disk confocal microscope (Solamere Technology Group Inc.) with an LD C-Apochromat 40×/1.1 W Korr objective lens (Carl Zeiss). Bars, 20 μ m.