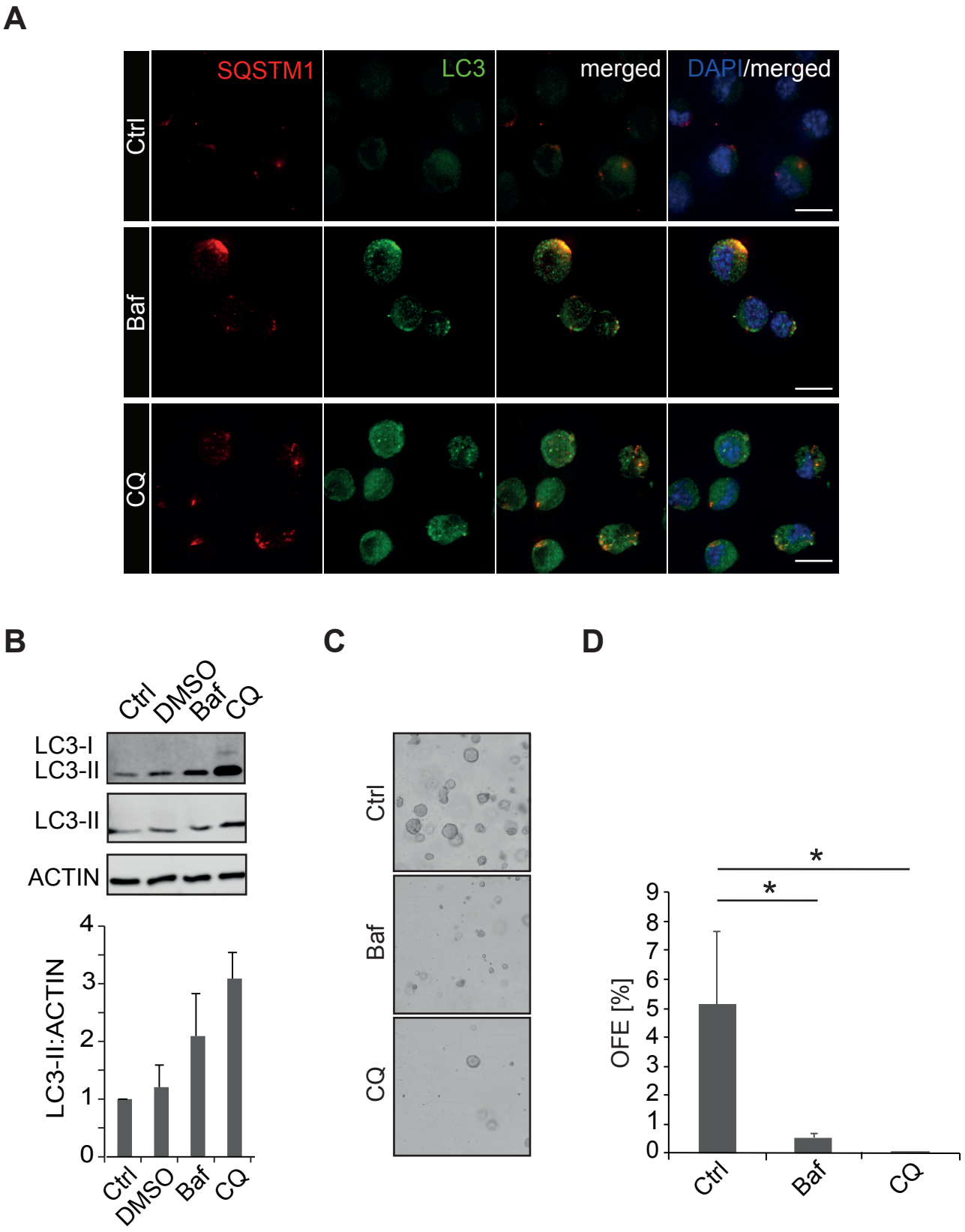

Figure S1. Autophagy inhibition blocks SG organoid formation. (**A**) Single SGSCs obtained by trypsinization from primary 3D cultures were treated with 100 nm Baf, 100 µM CQ or DMSO for 5h in the MM medium, before to be processed for immunofluorescence with anti-LC3 and anti-SQSTM1 antibodies. Nuclei were stained with Hoechst. Z-stacks of images separated by 0.2 µm were collected, deconvolved and analyzed as described in Material and Methods. A representative Z-projection of combined focal planes is shown. Scale bars: 5 µm. (**B**) Single SGSCs isolated as in panel A were lysed and proteins examined by western blot using antibodies against LC3. ACTIN was used as the loading control. The upper LC3 blot has been overexposed to observe the bands corresponding to LC3-I. The bars represent the levels of LC3 normalized to ACTIN, and relative to the untreated, control single SGSCs. Bars represent the means of 3 experiments +/-SD. Asterisks annotate significant differences of $p<0.05$. (**C**) Brightfield microscope images of secondary organoids obtained from single SGSCs treated with 100 nm Baf, 100 µM CQ or DMSO for 5 h, after 7 days in Matrigel®. (**D**) The OFE of the samples analyzed in panel C was determined at the end of p1. Bars represent the means of 3 experiments +/-SD. Asterisks annotate significant differences of $p<0.05$.

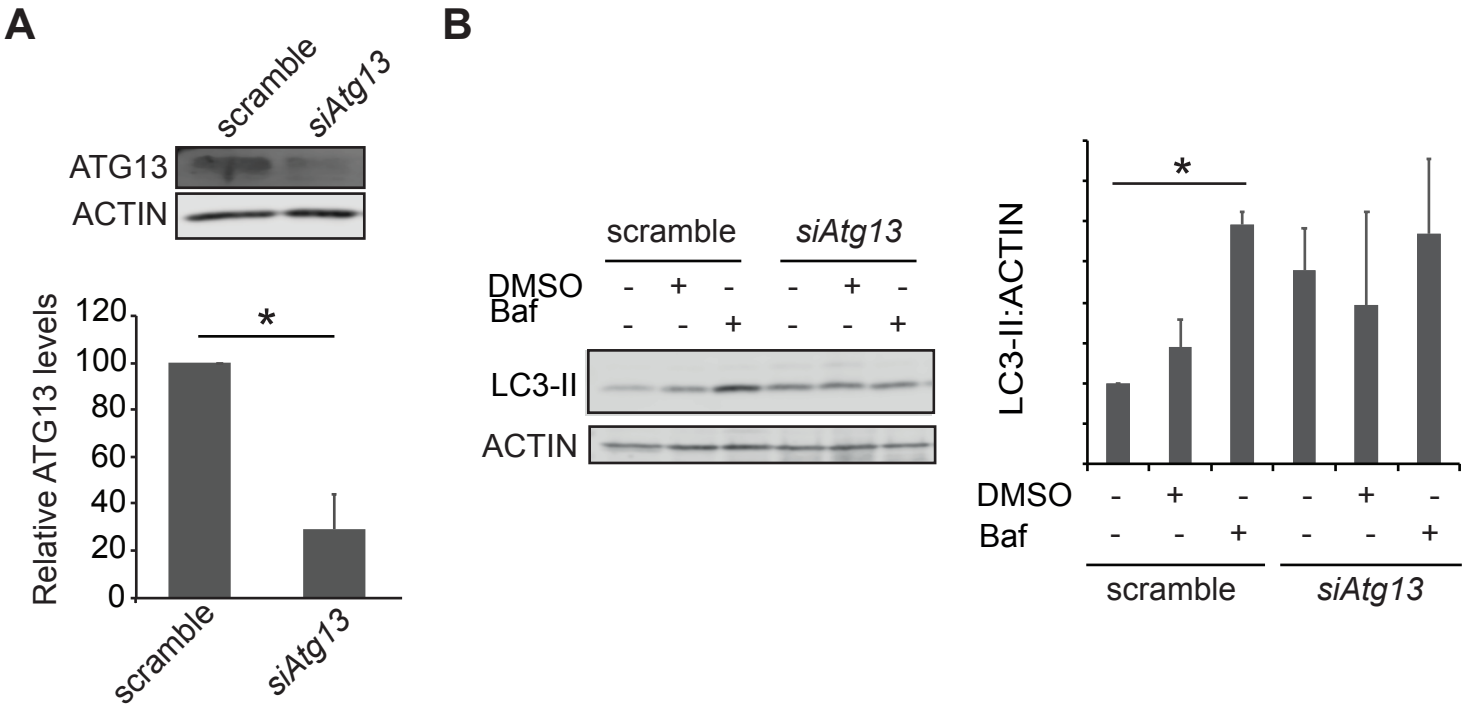

Figure S2. ATG13 depletion blocks SG organoid formation. (**A**) SGSCs transfected with either scrambled siRNAs (scramble) or siRNAs targeting Atg13 (siAtg13) for 48 h, were subjected to western blot analysis with anti-ATG13 antibodies. ACTIN detection served as a loading control. ATG13 protein levels were quantified and normalized to the loading controls and expressed relative to the scramble control. Bars represent the means of 3 independent experiments +/-SD. The asterisk annotates a significant difference of $p<0.05$. (**B**) SGSCs treated as in panel E were incubated with DMSO or 100 nm Baf for 5 h before to be analyzed by western blot using anti-LC3 and anti-ACTIN antibodies. ATG13 protein levels were quantified and normalized to those of ACTIN. A change in the LC3-II:ACTIN ratio upon Baf treatment indicates an autophagic flux, while the absence of a significant difference reveals a block in autophagy [30]. Bars represent the means of 2 independent experiments +/-SD.

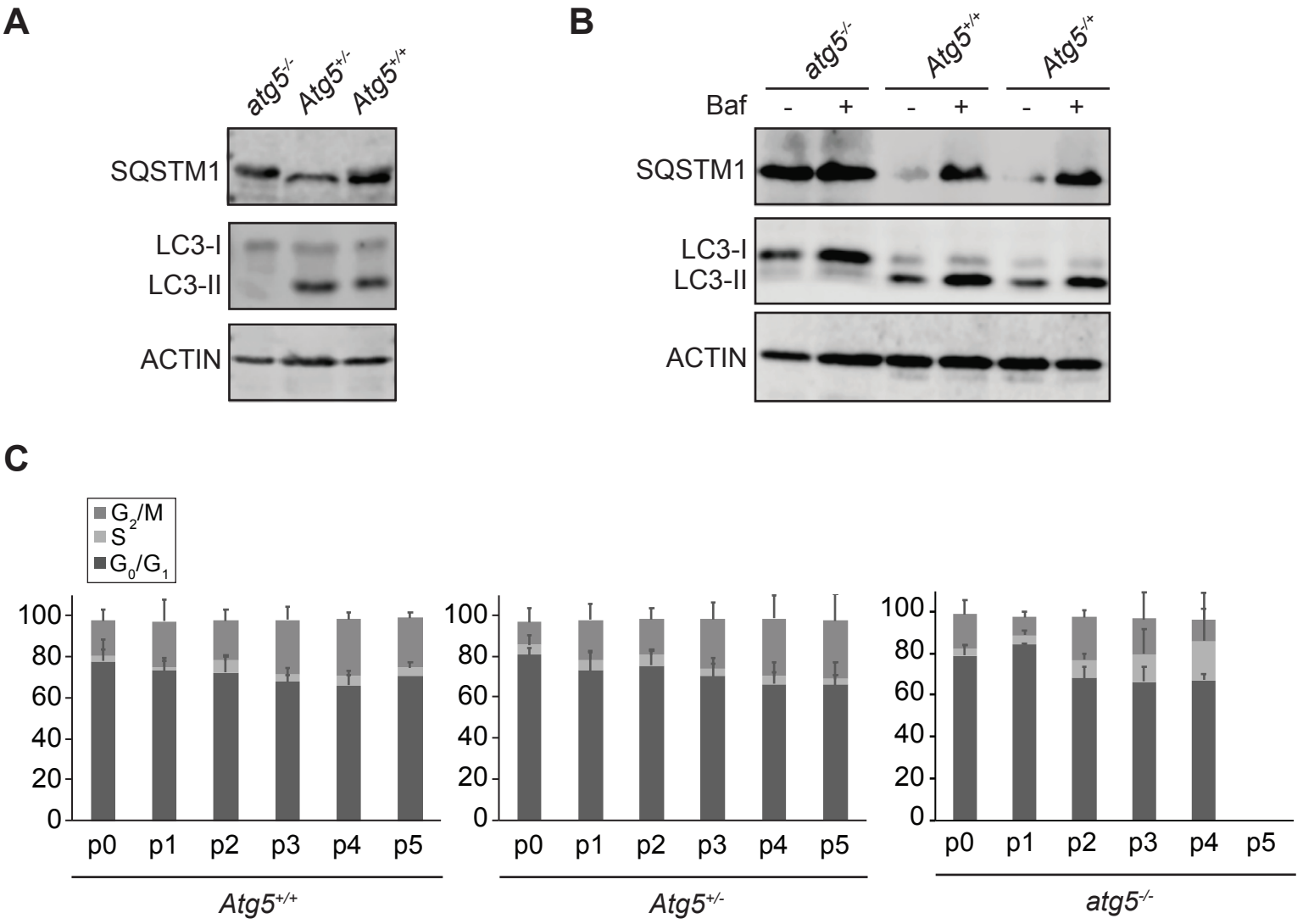

Figure S3. SGs. (**A**) Protein extracts from *Atg5*$^{+/+}$, *Atg5*$^{+/-}$ and *atg5*$^{-/-}$ SGs from embryos at stage e18.5 were analyzed by western blot using anti-SQSTM1 and anti-LC3 antibodies. ACTIN detection served as a loading control. (**B**) Passage 1 organoids obtained from embryonic *Atg5*$^{+/+}$, *Atg5*$^{+/-}$ or *atg5*$^{-/-}$ SG at the embryonal stage E18.5, were treated or not with 100 nm Baf for 5 h, before preparing protein extracts and analyze them by western blot using anti-SQSTM1 and LC3 antibodies. ACTIN detection served as a loading control. (**C**) Cell cycle analysis of *Atg5*$^{+/+}$, *Atg5*$^{+/-}$ and *atg5*$^{-/-}$ SGs from p0 to p5 was carried out using propidium iodide staining at the end of each passage, before determining the number of cells in G_0/G_1, S or G_2/M cell cycle phases by flow cytometry.

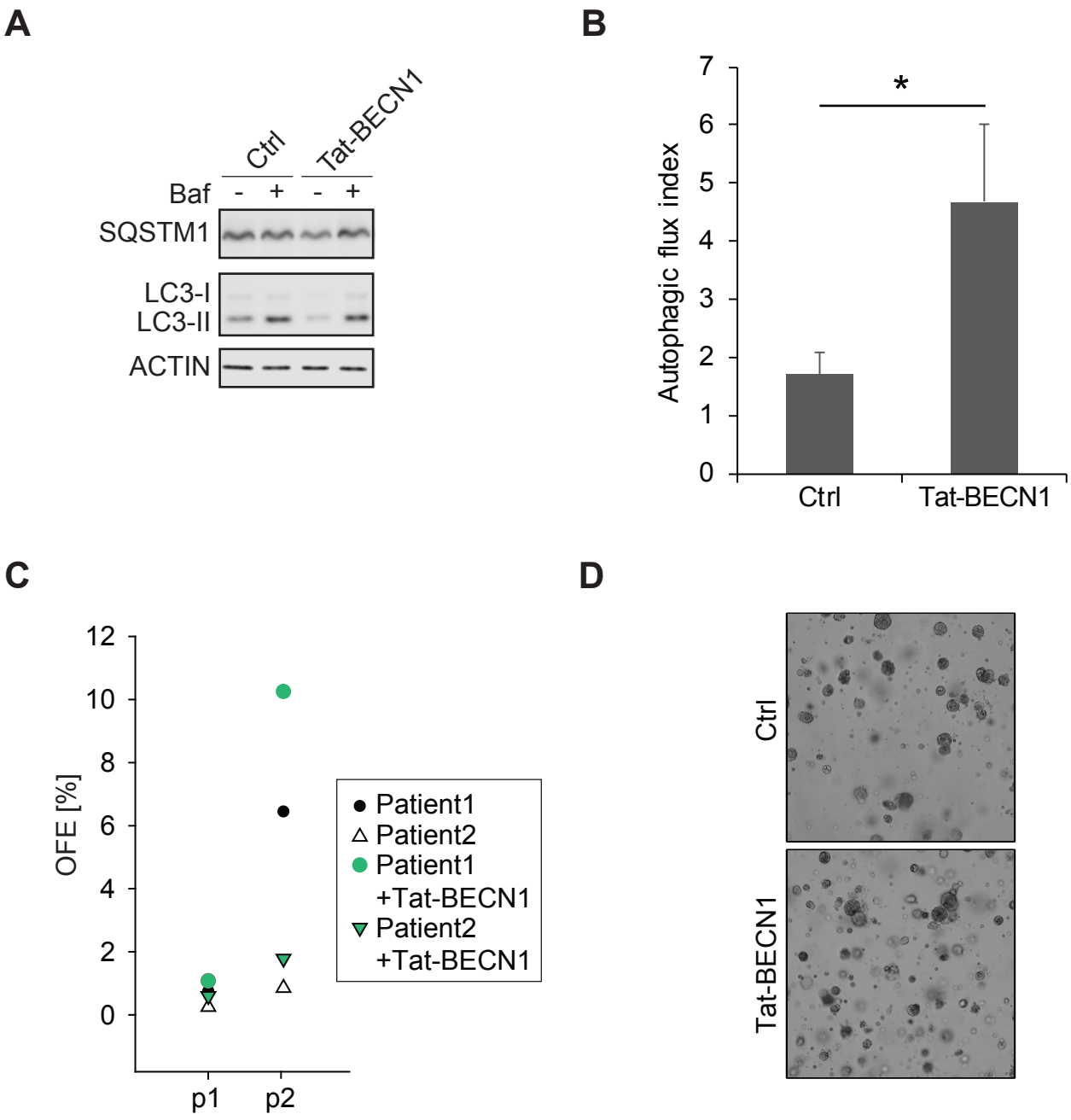

Figure S4. Tat-BECN1 induces autophagic flux in SGSCs. (**A**) Protein extracts from SGSCs grown in the absence (Ctrl) or the presence of 50 nM Tat-BECN1 and incubated with or without 100 nM Baf for 5 h, were examined by western blot using antibodies against SQSTM1 and LC3. ACTIN was used as the loading control. (**B**) The autophagy flux index in each sample of panel A was determined by dividing the amounts of LC3-II in Baf-treated cells with that in cells not exposed to Baf. Protein levels were normalized to ACTIN, which was used as the loading control. Means of 3 independent experiments are normalized to the autophagy flux of control cells +/-SD. The asterisk annotates the significant differences of $p<0.05$. (**C**) The OFE of human 3D SG cultures over 2 passages (p1 to p2), treated or not with 50 nM Tat-BECN1 as in Figure 3A. Each data point represents the measurement of one sample. (**D**) Representative brightfield microscope images of primary organoids from human SGSCs after 7 days in Matrigel®, treated or not with 50 nM Tat-BECN1.

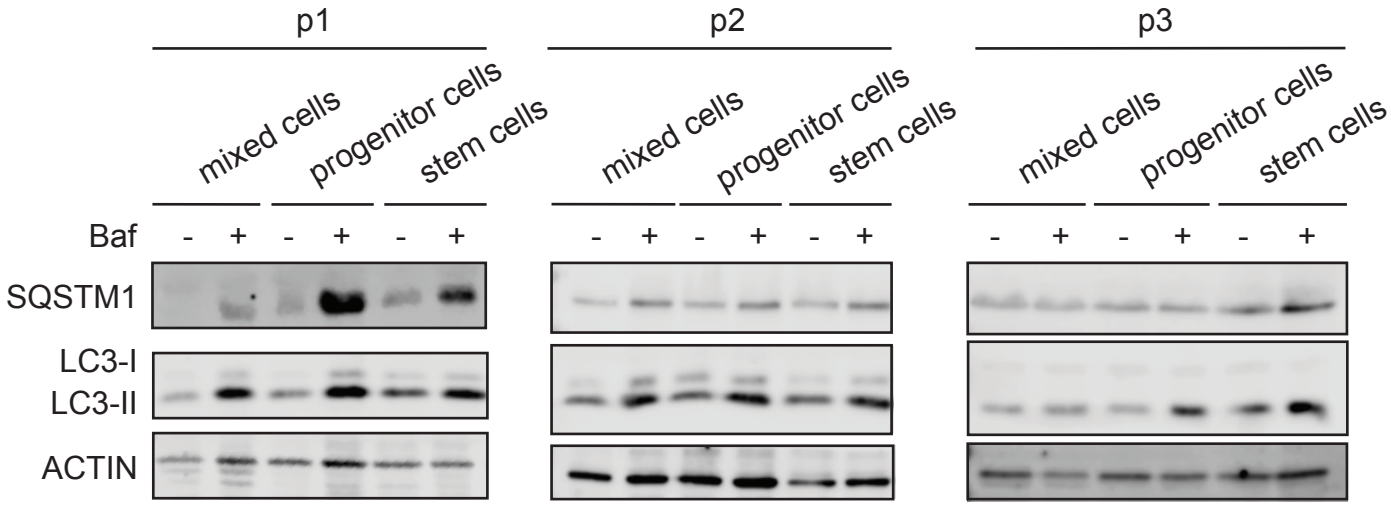

Figure S5. Autophagy flux induction correlates with self-renewal. Tat-BECN1 induces autophagic flux in SGSCs. Mixed cell, SGSCs progenitor (ITGB1$^{medium/hi}$ and CD24$^{medium/hi}$) and SGSCs (ITGB1$^{hi/hi}$ and CD24$^{hi/hi}$) populations from passage 1 to 3 (p1-p3) isolated by FACS, were treated or not with 100 nM Baf for 5 h before to be lysed. Protein extracts were subsequently examined by western blot using antibodies against SQSTM1 and LC3. ACTIN was used as the loading control.

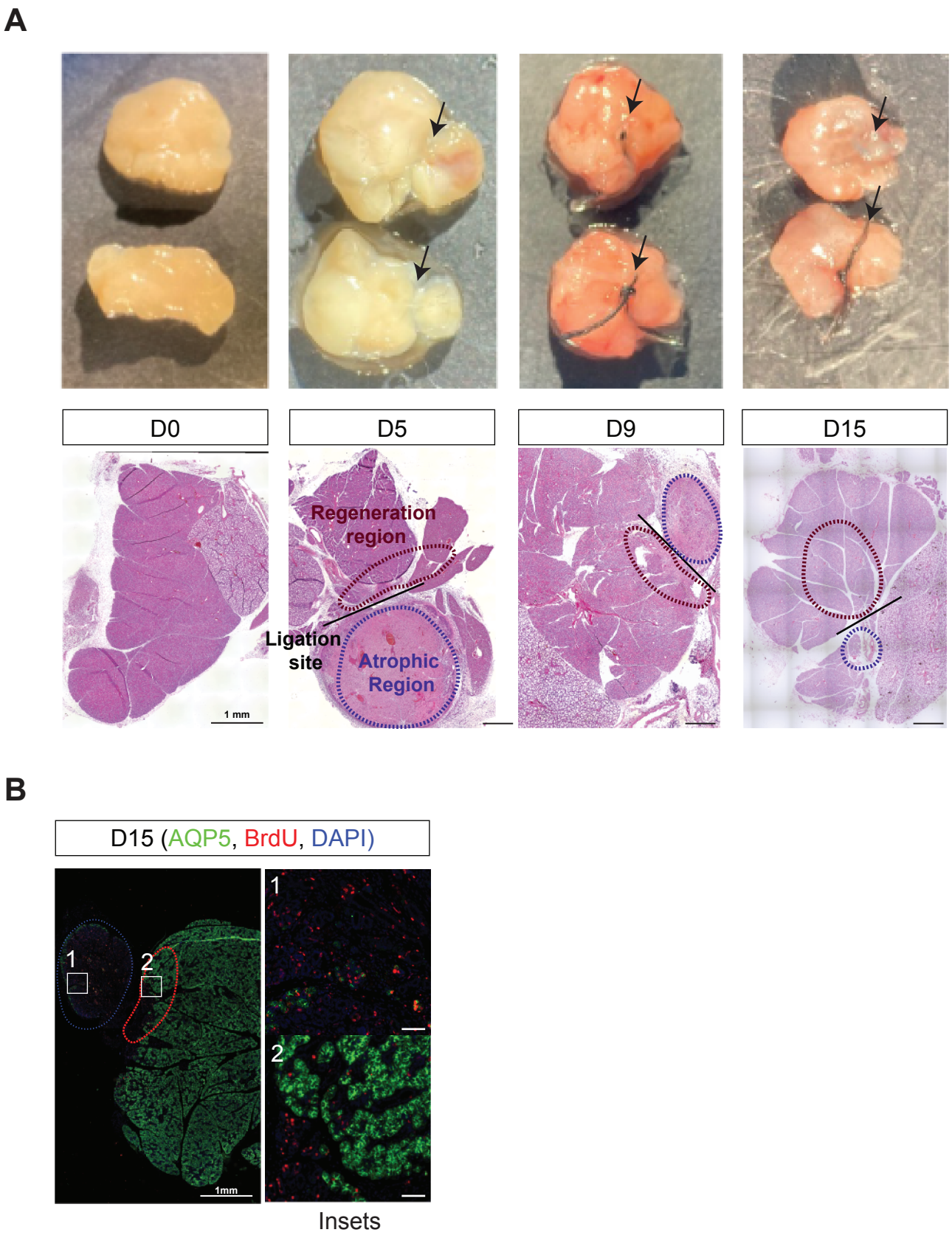

Figure S6. SG ligation induces tissue regeneration. Mouse SGs are ligated to as described in Material and Methods. (**A**) Control and ligated glands are followed over 5, 9 and 15 days post-ligation. Pictures showing examples of removed SGs are shown on the top part of the panel. Ligation sites are indicated by arrowhead. The low part of the panel presents H&E stained immunohistological preparations from the above shown SGs. Atrophic and regenerative regions of each time point are marked with violet and bordau dashed areas. The black lines indicate the ligation sites. Scale bars: 1 mm. (**B**) A representative single plane of regenerative SGs imaged by fluorescence microscopy at 15 days post-ligation is shown. Proliferative regions are labelled with an anti-BrdU antibody, while differentiated acinar regions are labelled with an anti-AQP5 antibody. Insets show a proliferative (1) or a differentiated acinar (2) region at higher magnification. Cell nuclei are stained with DAPI. Scale bars: 10 μm.