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Supplemental data

Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis

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Supplemental experimental procedures

Time-lapse microscopy

Cells were plated in T25 tissue culture flasks equipped with tubing to allow cell culture in ischemic gas environment and placed in a time-lapse environmental chamber. The time-lapse microscopy system consisted of an Olympus IX71 inverted microscope fitted with temperature, humidity and CO_2 controlled environmental chamber (Solent Scientific, UK) and a Coolsnap ES cooled CCD camera. Image capturing and analysis were performed using ImagePro Plus software (Media Cybernetics, USA). Phase contrast images (100X) at 10 different fields were obtained at 10-minute intervals for up to 5 days. Time-lapse images were converted to movies using ImagePro Plus and a workstation equipped for graphic data processing (Dell Precision 670). Movie files are available from the author upon request.

Tumor growth and analysis

All tumor growth was performed in 6-week old nude mice with IACUC approved protocols. H&E, immunohistochemistry (IHC) for active caspase-3 and P-H3 were preformed on tumors at approximately 3 weeks (D3 with H-RAS and AKT), 8 weeks (W2 AKT and D3) or 12 weeks (W2 3.1.5) post injection. This corresponds to the maximal tumor volume indicated on the tumor volume graphs ($1000mm^3$). Hypoxyprobe staining was performed on D3 and D3 AKT tumors after growth for 35 and 21 days, respectively, when the tumors were of equal size ($500mm^3$). Each cell line was injected into five mice and tumor samples from each mouse were analyzed by H&E, IHC, and hypoxyprobe, representative sections are shown.

Figure S1. Histology, apoptosis, and proliferation in different tumor genotypes

Histology (H&E), active caspase-3 (apoptosis marker), and phospho-histone H-3 (mitotic marker) IHC in W2, W2 AKT, W2 RAS, D3, D3 AKT, and D3 RAS tumors.

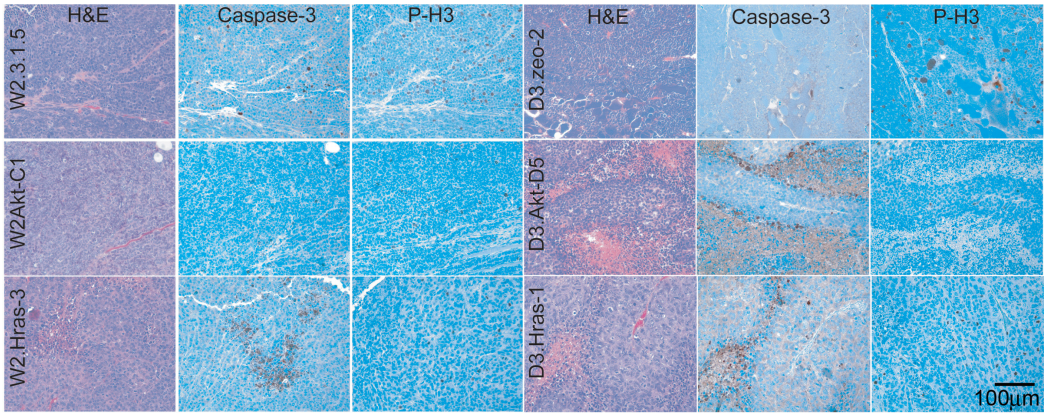

Figure S2. RNAi for either of two essential autophagy genes *beclin1* or *atg5* impairs survival of apoptosis-defective cells to metabolic stress

To test if autophagy is the mechanism used by D3 cells to survive ischemia, the expression of two essential autophagy genes (*atg5* and *beclin1*) was knocked down using RNAi, and the impact on cell viability under ischemic conditions was determined. (A) Western blot showing knockdown of Beclin1 or ATG5 in D3 cells under normal and ischemic conditions relative to the LaminA/C control. At 24 hours post transfection D3 cells were either maintained under normal growth conditions (control) or placed in ischemic chambers (ischemia) and examined by Western blotting for Beclin1 and ATG5 at 24 and 48 hours (48 and 72 hours post transfection). RNAi targeting a sequence different from that shown in Fig. 3C and D also reduced Beclin1 protein levels relative to the LaminA/C knockdown control at 24 and 48 hours under both normal and ischemic conditions, as were ATG5, albeit to a lesser extent. (B), Knockdown of Beclin1 and ATG5 expression in D3 cells under ischemic conditions induces cell death. Viability of D3 cells with RNAi for *beclin1*, *atg5*, and non-specific control *laminA/C* oligonucleotides. At 24 hours post transfection D3 cells were subjected to ischemia and viability was monitored at 24 and 48 hours. The values represent the mean ± standard deviation for n≥3. Error bars represent ± one standard deviation.