## **Expanded View Figures**



# α-BNip3 (d65)



#### Figure EV1. Verification of loss of BNip3 expression and re-expression.

A, B BNip3 protein expression determined by immunohistochemistry at early carcinoma stage of tumorigenesis (65–80 days of age) in wild-type (A) and BNip3 null (B). Scale bar is 200 μm.

C Western blot analysis for expression of BNip3 in extracts from wild-type and BNip3 null MECs grown at 20% and 1% oxygen.

D Cell cycle phase distribution of wild-type and BNip3 null MECs measured in triplicate experiments by BrdU labeling over 5 h in culture followed by labeling of fixed cells with  $\alpha$ -BrdU and Pl. Results are expressed as the mean  $\pm$  SEM. \*\*\*\*P < 0.0001.

E Western blot analysis for expression of exogenous BNip3 in extracts from parental BNip3 null MECs (Par), BNip3 null MECs expressing empty control vector (+ Empty vector) or BNip3 null MECs expressing exogenous BNip3 (+ BNip3-WT) grown at 20% and 1% oxygen.



TUNEL staining for cell death



## Figure EV2. Measurement of cell death in wild**type and BNip3 null tumors.** A–D TUNEL staining on wild-type and BNip3 null

- tumors at d65 and d80.
- Е Quantification of TUNEL staining from (A–D). Results are expressed as the mean  $\pm$  SEM.
- Flow cytometric analysis of PI exclusion as a measure of cell death on wild-type and BNip3 null MECs grown at 20% or 1% oxygen. Results are expressed as the mean  $\pm$  SEM.



Figure EV3. Loss of BNip3 has cell-autonomous effects on tumor cell growth in vivo.

- A Weights of tumors forming from wild-type or BNip3 null MECs transplanted into the mammary fat pad of wild-type or BNip3 null host mice. Results are expressed as the mean  $\pm$  SEM. \**P* < 0.05.
- B Histological analysis of tumors formed from wild-type or BNip3 null MECs following transplant into the mammary fat pad of wild-type mice. White scale bar is 50  $\mu$ m. Black scale bar is 100  $\mu$ m.
- C Quantification of lung metastases forming from wild-type or BNip3 null MECs transplanted into the mammary fat pad of wild-type or BNip3 null host mice. Results are expressed as the mean  $\pm$  SEM.



### Figure EV4. Additional effects of BNip3 loss on metabolism.

A Mass spectrometric measurement of total levels of glycerol-3-phosphate and serine plus the glycolytic intermediates from which they are generated.

- B, C Oxygen consumption by wild-type and BNip3 null MECs grown in the presence of glutamine (B) or glucose plus glutamine (C).
- D Oxygen consumption by BNip3 null MECs following expression of either empty vector (hashed line) or BNip3-expressing vector (solid line) (6 experimental replicates per point), performed in triplicate experiments.
- E Mass spectrometric measurement of TCA cycle intermediates after 24 h of growth in [U-<sup>13</sup>C]-glutamine.
- F Mass spectrometric measurement of total levels of amino acids in wild-type and BNip3 null MECs grown on glucose.

Data information: Results are expressed as the mean  $\pm$  SEM. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001.



### Figure EV5. Additional controls and effects of quenching ROS for tumorigenesis.

- A, B Staining with dihydroethidine for superoxide levels *in situ* on BNip3 null tumor sections at d80 following supplementation of mouse diet with BHA (B) or not (A). Scale bars are 50 μm.
- C, D Immunohistochemical staining for 8-hydroxyguanine to assess effects of BHA diet (D) on levels of ROS in tumors from BNip3 null mice compared to untreated BNip3 null mice (C). Scale bars are 50  $\mu$ m.
- E, F Immunohistochemical staining for TOM20 to assess effects of BHA diet (F) on mitochondrial mass in tumors from BNip3 null mice compared to untreated BNip3 null mice (E). Scale bars are 100 μm.
- G, H Immunohistochemical staining for Ki67 to assess the effects of BHA diet (H) on tumor cell proliferation in tumors from BNip3 null mice compared to untreated BNip3 null mice (G). Scale bars are 500 μm.
- Metastasis numbers in serial sections of lungs from wild-type or BNip3 null mice sacrificed at d80 either untreated or treated with BHA diet from d65–d80. Wild-type/UT, n = 21; BNip3 null/UT, n = 24; Wild-type/BHA, n = 11; BNip3 null/BHA, n = 14.
- J RNAseq-derived mRNA expression levels of BNip3 correlated with chromosomal copy number variation of BNip3 in non-TNBC samples of TCGA breast cancer cohort (*n* = 576).
- K RNAseq-derived mRNA expression levels of BNip3 in TNBC correlated with chromosomal copy number variation of BNip3 in TNBC samples of the TCGA breast cancer cohort (*n* = 113).