

Expanded View Figures

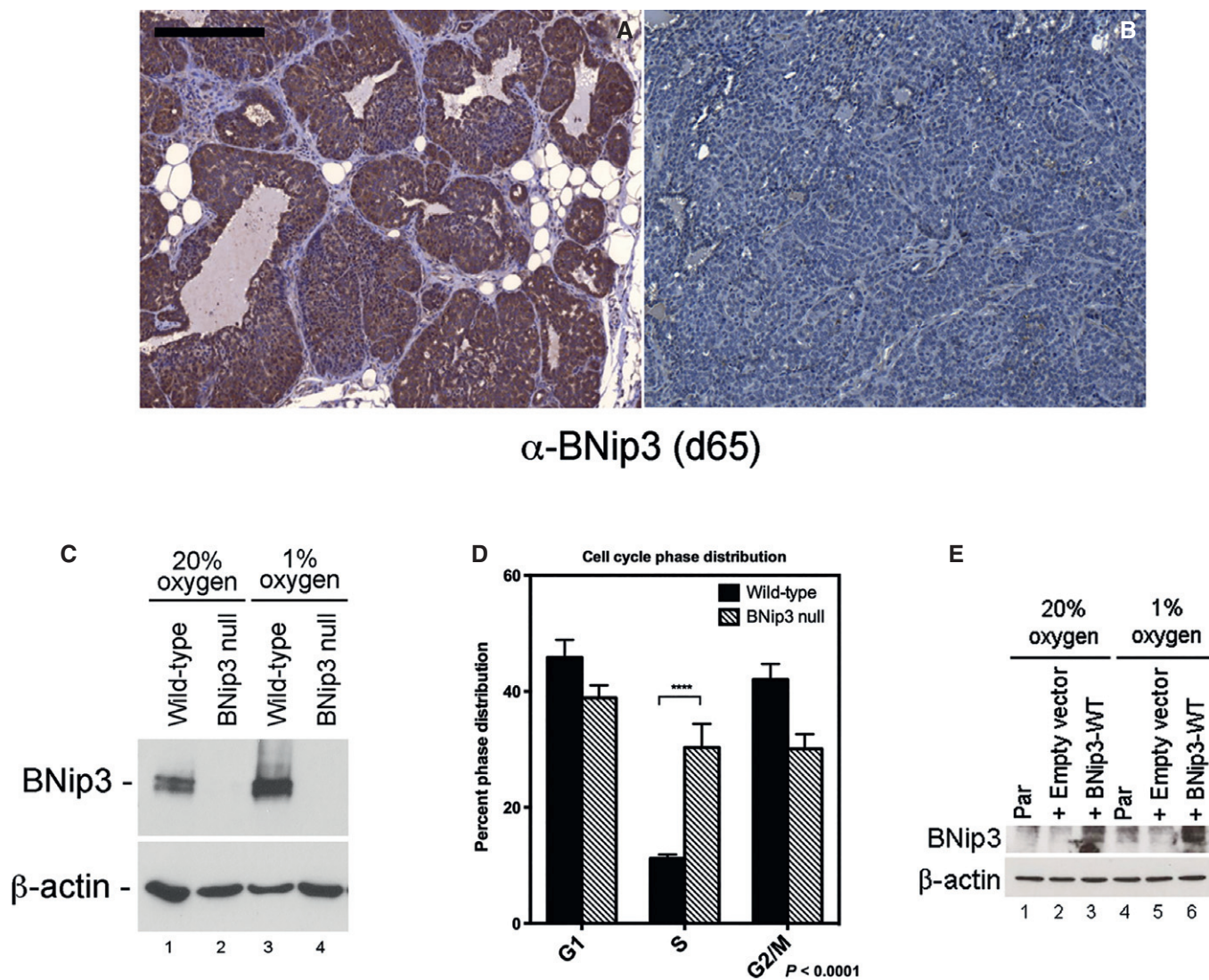


Figure EV1. Verification of loss of BNip3 expression and re-expression.

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 μ m.

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 α -BrdU and PI. 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.

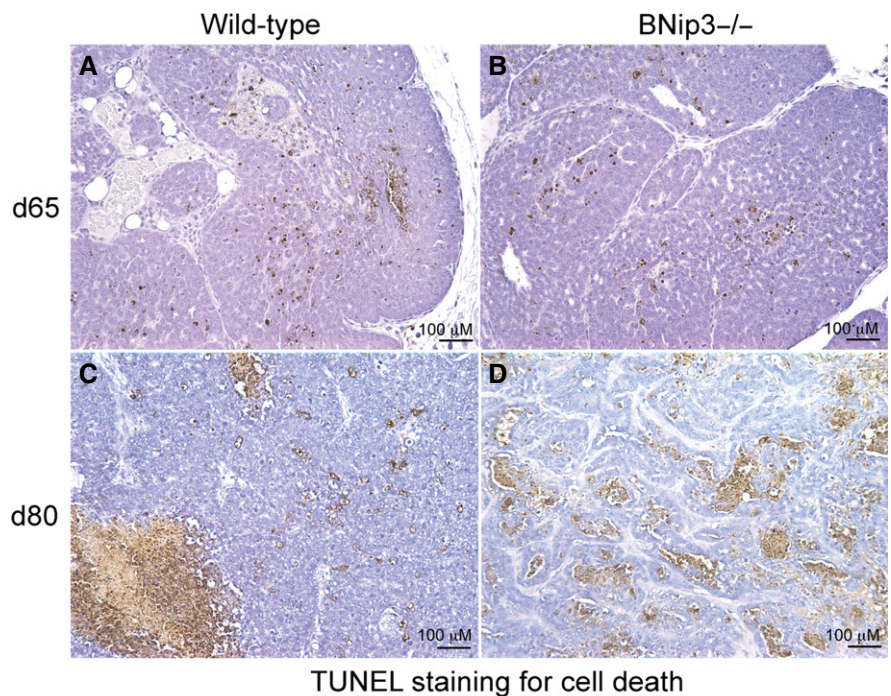
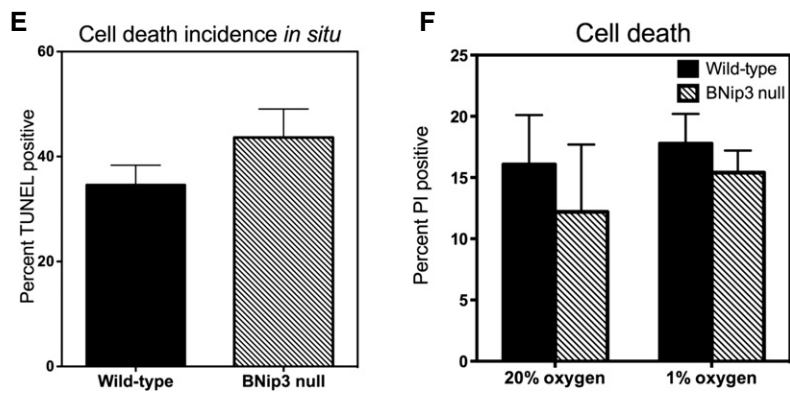


Figure EV2. Measurement of cell death in wild-type and BNip3 null tumors.
 A–D TUNEL staining on wild-type and BNip3 null tumors at d65 and d80.
 E Quantification of TUNEL staining from (A–D). Results are expressed as the mean \pm SEM.
 F Flow cytometric analysis of PI exclusion as a measure of cell death on wild-type and BNip3 null MECs grown at 20% or 1% oxygen. Results are expressed as the mean \pm SEM.



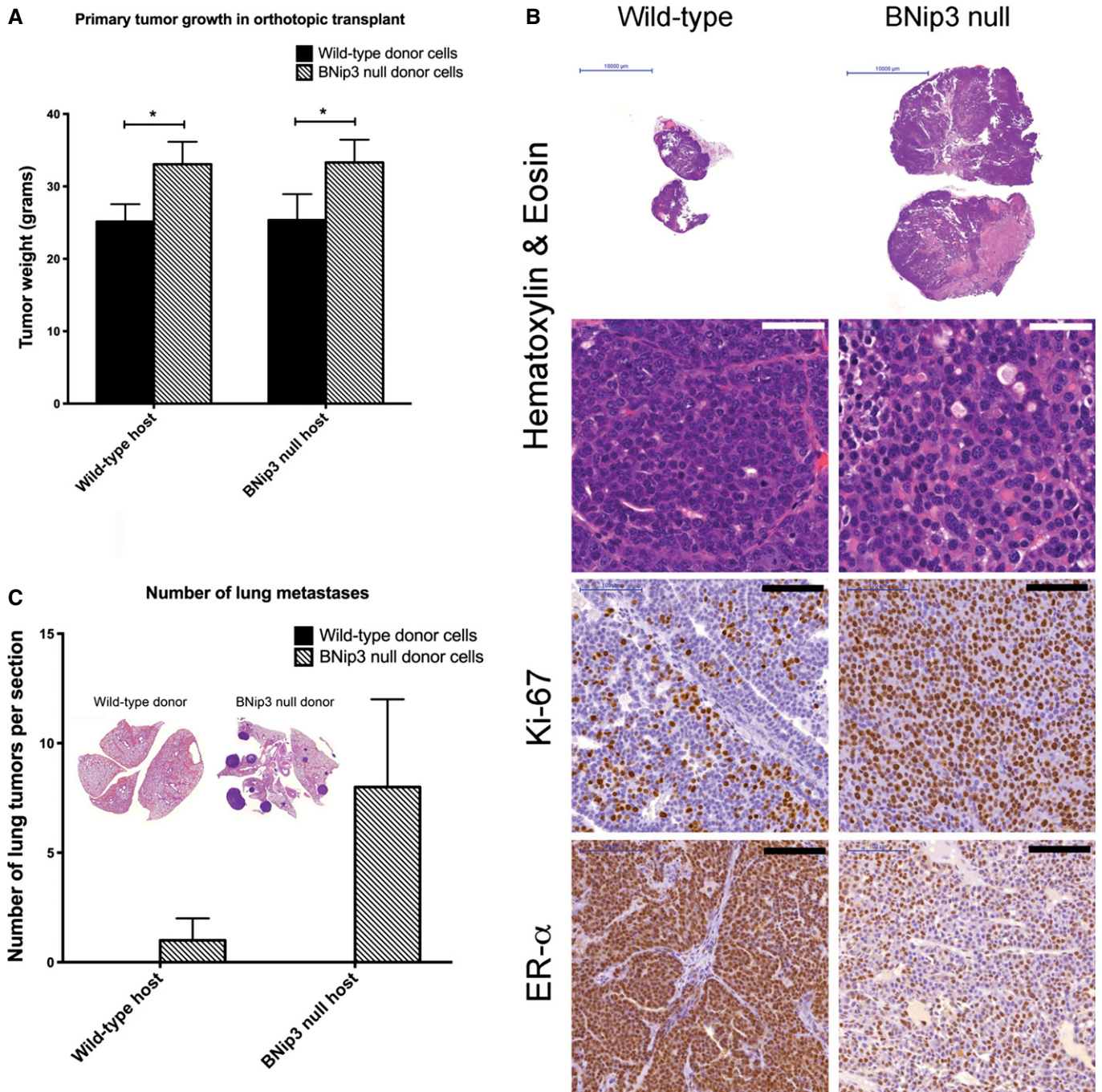
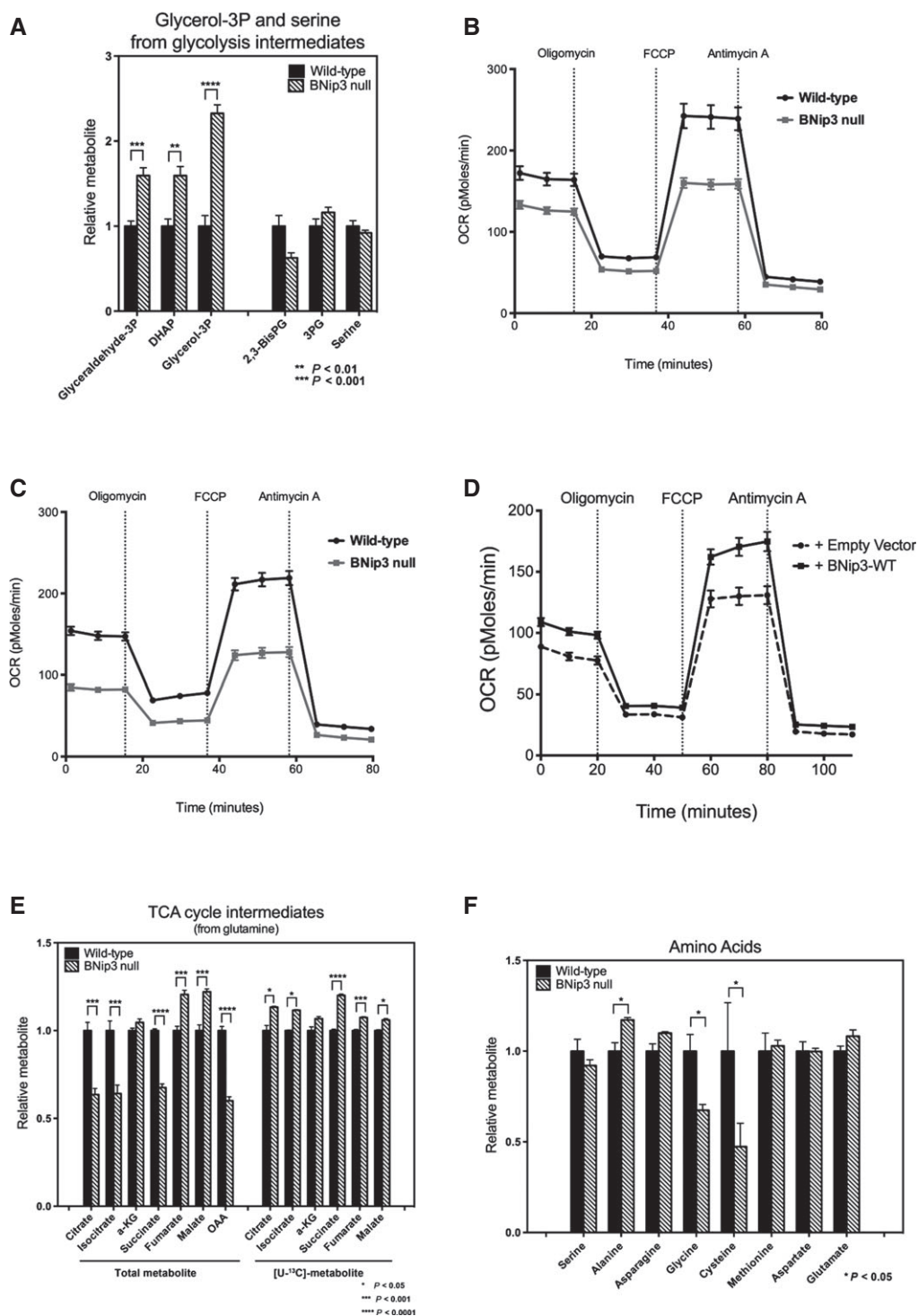


Figure EV3. Loss of BNip3 has cell-autonomous effects on tumor cell growth *in vivo*.

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 μ m. Black scale bar is 100 μ 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-¹³C]-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 ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

