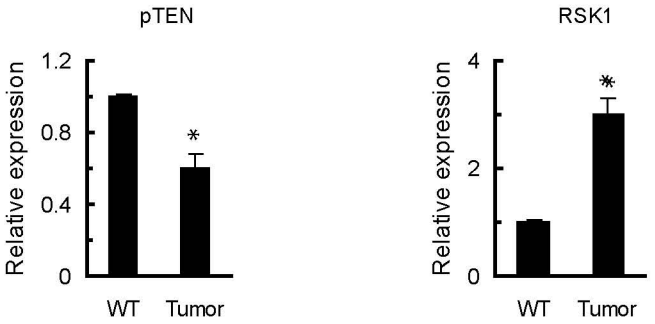
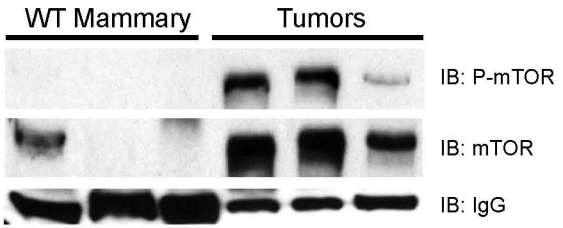
Supplemental Figure 1, Mosley et. al.

A



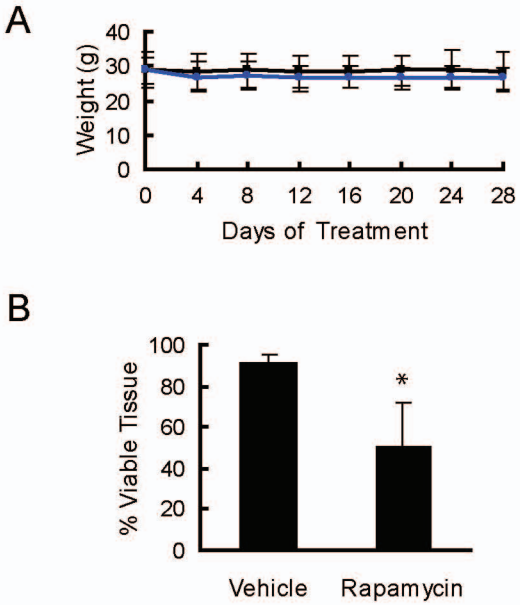
B



Supplemental Figure 1: *Mammary tumors in MMTV-c-Neu mice have activated mTOR signaling.*

A. Age-matched wild type glands (WT, n=3 pools of 5 mice each) were collected and compared to primary tumors (n=7). Mean signal intensities from microarrays were normalized to wild-type levels, and the normalized values are displayed on the y-axis with tissue type on the x-axis. The graphs show relative mRNA levels for pTEN and RSK1. Error bars represent standard errors, (*)=$p<0.01$.

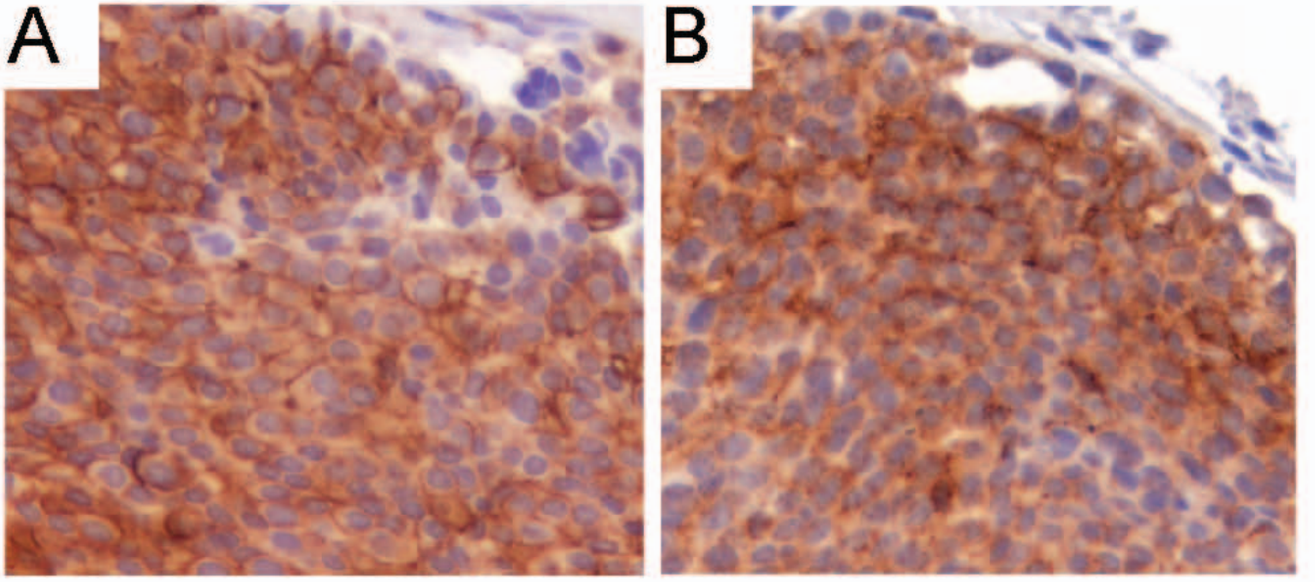
B. Immunoblot analysis shows increased total and phosphorylated mTOR in MMTV-c-Neu tumors. Whole cell lysates (100 µg) from wild-type mammary glands were evaluated by western blotting using an antibody directed against phosphorylated mTOR (Serine 2448) and total mTOR. An IgG band is shown as a loading reference.



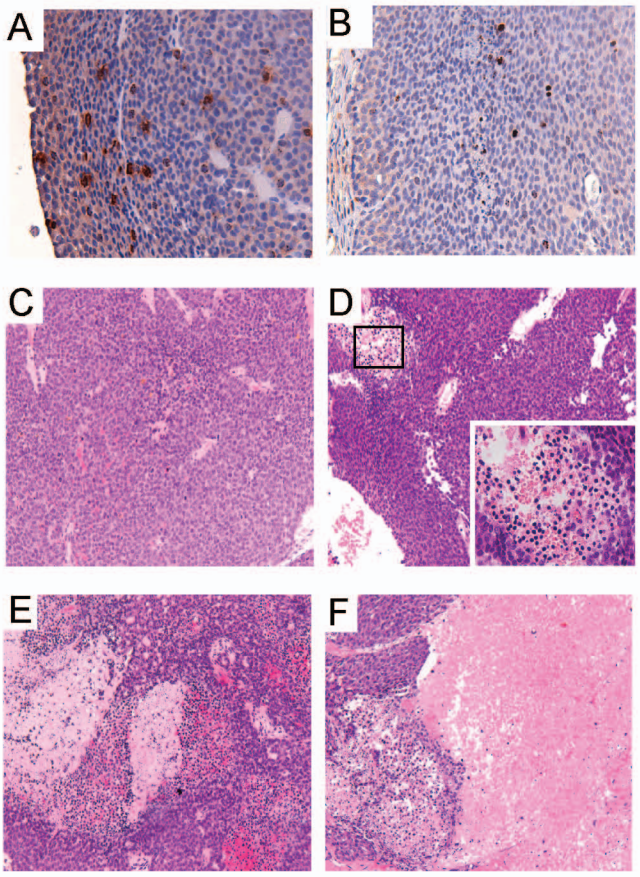
Supplemental Figure 2: *Rapamycin induces regression of primary tumors without causing weight loss.*

A. Rapamycin treatment does not induce weight loss. Mice bearing mammary tumors were treated with 150 µg of rapamycin (n=5) or vehicle (n=5) every other day for 32 days. Weights were measured every four days. The graph depicts the average weights of vehicle (black line) and rapamycin (blue line) treated mice. Error bars represent standard deviations. B. The graph represents the average proportion of the total tumor area that was not comprised of necrotic tissue in vehicle (n=4) and rapamycin (n=4) treated tumors. Error bars represent standard deviations, * = $p<0.05$.

Supplemental Figure 3, Mosley et. al.



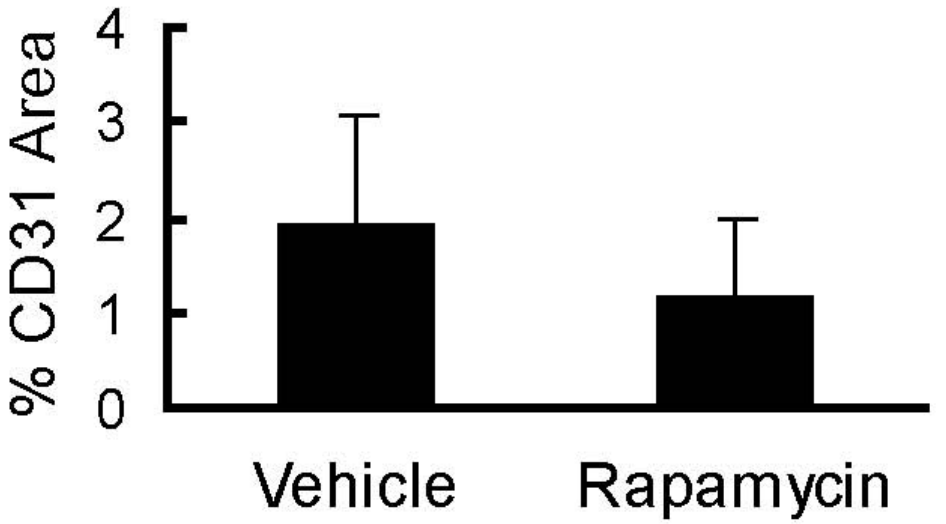
Supplemental Figure 3: *Rapamycin does not alter c-Neu/ErbB2 activity in tumors.*
Primary MMTV-c-Neu tumors were collected from vehicle (A) or rapamycin (B) treated mice after 32 days of treatment. Representative sections (200X) immunostained for phosphorylated HER2/c-Neu (Tyr-877) are shown. Note the membrane-associated staining that is characteristic of phosphorylated ErbB2.



Supplemental Figure 4: *Rapamycin inhibits primary tumor cell proliferation and promotes focal cell death.*

A/B. Rapamycin decreases the number of cells expressing phosphorylated histone-H3. Representative sections (20X) of immunohistochemical staining for phosphorylated histone-H3 in primary tumors collected from vehicle (A) and rapamycin (B) treated mice. C. Representative H&E stained tumor section from a mouse treated with vehicle for 32 days. D/E/F. Progression of rapamycin-induced tumor cavitation. Representative H&E stained tumor sections (100X) from mice treated with rapamycin for 5, 10 or 32 days. D) At 5 days small foci of pyknotic nuclei are seen. A higher magnification of the focus is shown in the inset (400X). E) By day 10, broad bands of dying cells are present throughout the tumor. F) After 32 days, much of the central portions of rapamycin-treated tumors are comprised of cells with features of ischemic necrosis.

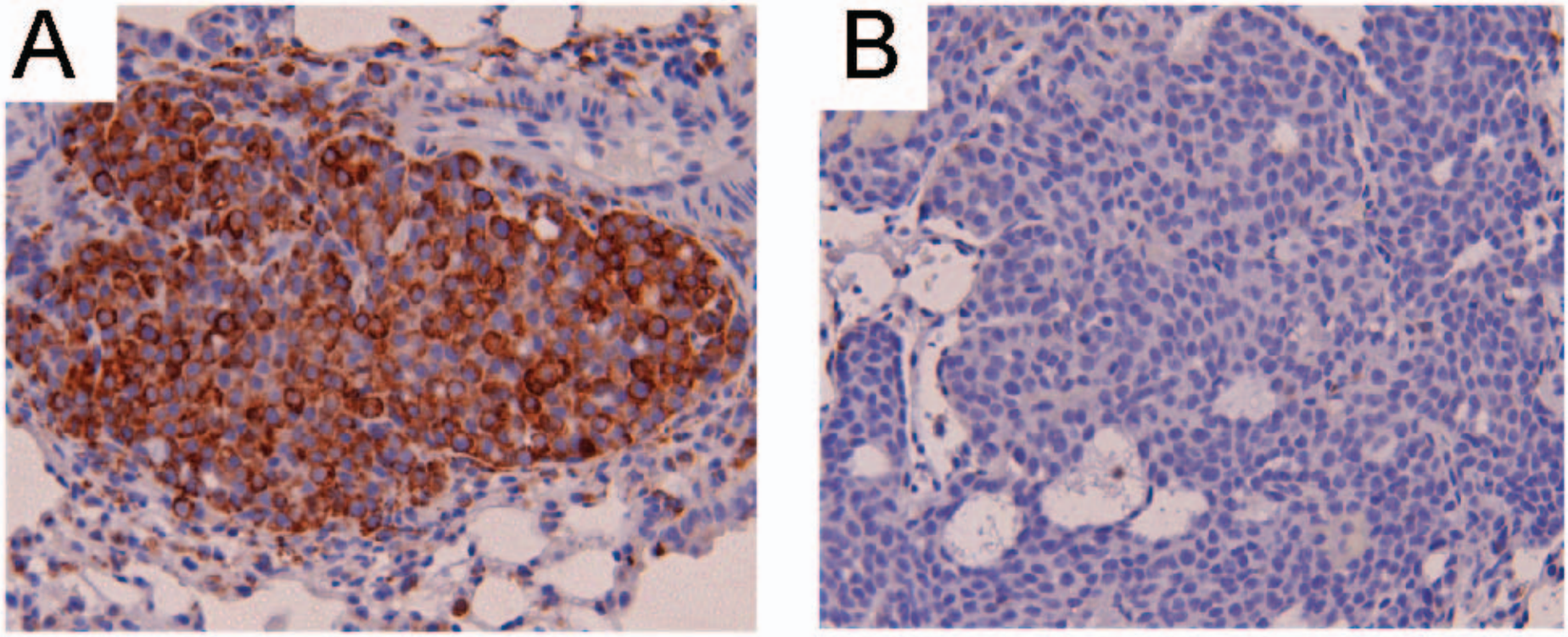
Supplemental Figure 5, Mosley et. al.



Supplemental Figure 5: *Rapamycin does not alter microvessel density.*

The graph depicts the average percentage of tumor area positively stained for CD31 in vehicle (n=5 tumors) and rapamycin (n=6 tumors) treated mice (32 days of treatment). Error bars represent standard deviations. Similar results were observed for tumors treated with rapamycin for 5 days (n=4 vehicle, n=6 rapamycin).

Supplemental Figure 6, Mosley et. al.



Supplemental Figure 6: *Rapamycin inhibits S6 phosphorylation in lung metastases.*
A/B. Representative sections (400X) of immunohistochemical staining of phosphorylated Ribosomal Protein S6 (serine 235/236) in lung metastases collected from mice treated with either A) vehicle or B) rapamycin for 32 days.