Supplementary Figure 1. Generation of MMTV-Akt2-DD transgenic mice. A, Schematic representation of the injection fragment. An HA-tagged Akt2-DD (Akt2 T309D/S474D) cDNA was cloned between the MMTV promoter and the SV40 Poly Adenylation sequence. B, Total RNA was isolated from 8 week old virgin mammary glands and analyzed by quantitative RT-PCR. Transgene expression was detected by amplification of a sequence corresponding to the SV40 Poly A region. Transgene expression was normalized to GAPDH and is the mean of triplicate runs performed for each RNA sample (+/- SD). C, Protein was extracted from the mammary gland of 8 week virgin mice and analyzed by Western blot. The Akt2-DD protein was detected using an antibody to the HA epitope (upper panel). Grb2 protein was detected as a control for protein loading (lower panel).

Supplementary Figure 2. Expression of Akt2 delays mammary gland involution. A, Hematoxylin and eosin stained paraffin-embedded sections (i-iv) of mammary glands removed at days 1 and 3 post-parturition from FVB (i,iii) and Akt2 (ii,iv) mice. Bar, 0.5 mm. Wholomount analyses of mammary glands excised at day 3 post-parturition from FVB (v) and Akt2 (vi) mice. Bar, 1 mm. B, TUNEL stained paraffin-embedded sections of mammary glands removed at days 3 and 7 post-parturition from FVB (i,iii) and Akt2 transgenic (ii,iv) mice. Bar, 0.1mm.

Supplementary Figure 3: RNA expression levels of the erbB2 transgene are similar in NDL, NDL/Akt1 and NDL/Akt2 mammary tumors. Total mammary tumor RNA was isolated and analyzed by quantitative RT-PCR for the transcript levels of the activated
erbB2 transgene. The expression levels were normalized to GAPDH and represent the mean of triplicate runs performed for 5 independent RNA samples for each genotype (+/- SD).

Supplementary Figure 4: Activated Akt2 expression increases invasion. TM15 clones 6, 13, 7 and 10 (A, B, C and D respectively) stably expressing activated Akt1 and Akt2 were generated and assayed for invasion through Matrigel in transwell assays. Pixel count analyses of Crystal Violet stained membranes were performed and relative invasion is displayed with the empty vector (EV) control set to 1. Values represent the mean of assays performed in triplicate (+/- SD) and statistical analyses were performed using a student’s t-test. Immunoblots were performed on cell lysates for the HA-tagged activated Akt proteins, total Akt1, total Akt2. Grb2 was detected as a control for loading.

Supplementary Figure 5. Akt2 downregulation impairs invasion. Akt2 expression was knocked down in the highly metastatic TM15 clone 7 (A) and Akt1 expression knocked down in the low metastatic TM15 clones 6 and 13 (B and C, respectively) using siRNA duplexes. Invasion through Matrigel was performed for mock, red negative control, and Akt1- or Akt2-specific siRNA transfected cells using transwell assays. Pixel count analyses of Crystal Violet stained membranes were performed and relative invasion is displayed with the mock transfection (mock) control set to 1. Values represent the mean of assays performed in triplicate (+/- SD) and statistical analyses were performed using a student’s t-test. Immunoblots were performed on cell lysates for Akt1 and Akt2, with Grb2 detected as a control for protein loading.