Supplemental Methods

Akt3 isoform CRISPR-Cas9 design and construction. To silence Akt3/+S472 expression, we performed CRISPR-Cas9 (GeCKO) of human PKB gamma (hAkt3-WT) by targeting exon 13. Using the MIT CRISPR server (http://crispr.mit.edu/), three gRNA sequences specific for exon 13 (without PAM sequence) were identified. Guide #1: TCTGTCTCAGATGTTACTTG, Guide #2: GATGTTACTTGAGGTTTAAA, Guide #3:GTTACTTGAGGTTTAAAAGG. Zhang Lab protocol was used to clone guide # 1 RNA using oligo1 (hAkt3-E13-5) 5'-CACCGTCTGTCTCAGATGTTACTTG-3' and oligo2 (hAkt3-E13-3) 5'-AAACCAAGTAACATCTGAGACAGAC-3' (Integrated DNA Technology). Oligos pair for guide #1 was phosphorylated, annealed, and diluted in nuclease free water. LentiCRISPRv2 vector was linearised with BsmB1, dephosphoylated, purified and ligated with diluted oligoduplex using Quick Ligase. Ligation product was electroporated in to ElectroMAX Stbl4 cells using BioRad GenePulserII for transformation. After transformation, bacterial cells were plated on Agar/Ampicillin plates overnight at 37^{o}C. Individual colonies were picked and grown in LB medium with ampicillin. Plasmid was purified using Mini Prep kit and the presence and orientation of gRNA sequence was checked by sequencing using the primers hAkt3-E13-5, hAkt3-E13-3 and LKO.1(5'-GACTATCATATGCTTACCGT-3',U6 promoter region. Similarly, for GeCKO of Akt3/-S472, humanPKB gamma v1(hAkt3-v1) exon 14, that is absent from Akt3/+S472, was targeted. The most effective sequence without PAM of targeting single guide RNA: GCAATCAGATTGTGGCATGC. Oligos1(hAkt3-E14-5):5'-CACCGGCAATCAGATTGTGGCATGC-3' and oligo 2(hAkt3-E14-3): 5'-AAACGCATGCCACAATCTGATTGCC-3' were used to make the CRISPR construct (CRISPRv2-hAkt3-v1) using the above protocol. As control, a non targeting sequence, NT1, 5'-GCGAGGTATTCGGCTCCGCG-3' , derived from a negative control sequence pulled from the GeCKOv2 Mouse Library Pool A was used to contruct the CRISPRv2-NT1 lentiviral vector (Sanjana et al; 2014. Improved vectors and genome-wide libraries for CRISPR screening; Nat Methods 11, 783-784).

Genomic validation of CRISPR-Cas9 mediated Akt3 knockout. To identify the deletion or insertion events around the gRNA recognition site of Akt3 genomic locus and thereby to validate the genomic deletion by CRISPR, we designed primers flanking the gRNA recognition site. Initially, Sanger sequencing was employed

to locate the indel events. Following primers: Akt3/+S472; forward CCT TAC CCA GCG ACT CAG CAT TGT AG reverse GGA ACT CAA TAT TCT TAA ATT CAA TTC, and for Akt3/-S472 forward CCA GCT CCA GCC CGA CTG CGT G reverse CAT ATG TGA AGG GCT TTG ATG TGG were obtained from IDT. Genomic DNA from 3475 or MDA-MB-231 cell cultures or tumors expressing control non-targeting vector, CRISPR-Akt3/+S472, or CRIPSR-Akt3/-S472 was isolated using Qiagen DNeasy Blood and Tissue kit. For PCR, genomic DNA was denatured for 10 minutes at 94^{o}C and then subjected to 40 cycles consisting of : gradual decrement of annealing temperature from 60^{o}C to 52^{o}C covering 45 sec at 94^{o}C, 45 sec at each annealing temperature (60^{o}C to 52^{o}C), 45 sec at 72^{o}C, with final extension at 72^{o}C for 10 minutes. Amplicon was run on 2.5% Agarose gel, excised from the gel and purified using Qiagen Gel Extraction kit. Purified PCR product was sequenced by Sanger's method using sequencing primers.

Deep Sequencing analysis. To precisely look at the indel events at target site and evaluate the percentage of targeting efficiency, we sought to perform the Next Generation Sequencing (Deep Sequencing) of amplicon obtained around the gRNA binding site. Following primers were used to amplify and generate 200bp amplicon: Akt3/+S472 Forward GAA TTG AAA TCA GTT AAT AAA AC Reverse CAG ATG AGT TTT TAA AAG ACT G and for Akt3/-S472 Forward CAT ATT AAG TCT AAA CCA GAT G Reverse CAT TTT TAG CAA CAG GTT TTT TTC. The size of amplicon was chosen as 200bp to ensure the complete sequencing of PCR product. Same temperature profile was followed to amplify the target using 250ng genomic DNA. Genomic DNA obtained from 3475 tumor cells and mammary xenografts were used to amplify the genomic locus. Multiple PCR reaction was carried out to enrich the PCR product and loaded onto 2.5% agarose gel and band size was identified, excised and gel purified as mentioned above. For Deep sequencing NGS sequence adapter and Barcode sequences were added to the finished PCR product before placing the sample in HiSeq Sequencer. Multiple sequence alignment was done with Clustal and relative abundance of each variants was calculated with Illumina Pipeline.

TaqMan qRT-PCR. Total RNA was isolated using RNeasy Mini Kit and RNase-free DNase set (Qiagen, Valencia, CA). RNA was extracted from cell lines and mammary tumors. Real-time RT-PCR was carried out using TaqMan RNA-to-Ct 1-Step Kit (Applied Biosystems, Carlsbad, CA) and gene-specific TaqMan probes

(Applied Biosystems, Carlsbad, CA) on a StepOnePlus Real-time PCR system (Applied Biosystems, Carlsbad, CA). QPCR quantitations were normalized to the GAPDH mRNA gene and a specific reference control, within each experiment was used to display the relative mRNA expression levels. For GAPDH, the following Taqman (Applied Biosystems, Carlsbad, CA) gene expression Assay ID was used Mm99999915_g1. QPCR primers for the Akt3/+S472 and Akt3/-S472 isoforms were Taqman custom ordered primers that were designed using the Primer Express 3.0 software (Applied Biosystems). Primers for Akt3/+S472 were selected within exon 13 of AKT3 (forward GCAAGTGGACGAGAATAAGT, reverse CAATTTCATGCAAAAACAAA). Primers for Akt3/-S472 were selected within exons 14,15 (forward CCTGAAAAATGTCAGCAATCAGAT and reverse GGCTGTAGGAAGCCGATTTTTA). Additional Taqman primers include Bim or Bcl2L1 (Hs 00236329_ m1).

Immunoblotting. Cells were solubilized with ice-cold RIPA lysis buffer (25 mM Tris–HCl [pH 7.6], 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor cocktail and phosphatase inhibitor cocktail (Roche, Indianapolis, IN). Blots were probed with indicated antibodies and developed by Pierce chemiluminescence substrate (Thermo scientific, Rockford, IL). Densitometry was performed using Image J gel analyzer.

Immunoprecipitation. Cells were lysed in 0.5% Triton-X-100 lysis buffer including protease and phosphatase inhibitors. For Bax immunoprecipitation, tumors were lysed in CHAPs buffer (150mM NaCL, 10mM HEPES, PH 7.4, 1% CHAPS, protease inhibitors). Total protein (0.5-1.5 mg) was incubated overnight with normal mouse IgG (control), anti-active Bax (6A7) or anti EGFR antibody. Protein A or G beads were added and incubated for 1hr following by 5 times washes in lysis buffer. Pelleted beads were boiled in Laemmli sample buffer and supernatant was analyzed by SDS-PAGE and western blotting using the indicated antibodies.

Immunostaining. Cells were fixed in 3.7% paraformaldehyde for 20 min, permeabilized in PBS/ 0.1% Triton-X-100 for 2 min, blocked in PBS containing 3% bovine serum albumin, 2% goat serum, 0.02% Triton X-100 for 1 h, followed by incubation with primary antibody, and secondary detection using fluorescently labeled antibodies (FITC or TRITC). Slides were mounted in a solution containing DAPI to visualize nuclei. Images were taken using Zeiss Axioskop 2 microscope (Zeiss, Thornwood, NY).

Tumor formation. $1x10^6$ cells were mixed with cold 0.1ml PBS containing 25% Collagen type I, and injected bilaterally into the thoracic mammary fat pads of six 6-8 weeks old athymic Balb/c mice (National Cancer Institute) and repeated twice. Mice were monitored biweekly for tumor growth using calipers and tumor volume (mm^3)= $0.5*a*b^2$ (a and b are the longest and shortest diameters of the tumor respectively) was determined in each mouse.

Lung metastasis and colonization. Metastasis from the primary tumor was determined following tumor growth as described above. Early palpaple tumors were removed under anesthesia by surgical excision. Mice were further incubated for 14 weeks. For lung colonization, $1x10^6$ cells/0.2 ml PBS were injected into the tail vein of athymic Balb/c female mice and incubated for 5 weeks. In either case, mice were sacrificed and lungs perfused by injection of 2.5 ml of 10% formalin and fixed for 24hrs. Paraffin blocks were sectioned at 5 μm thickness in 5 sets of 5 serial sections, at 100 micron intervals to minimize duplicate analysis. Sections were H&E stained, and lung foci counted. Data is displayed as mean number of metastases ± SEM. Statistical analysis was performed using the Wilcoxon Rank Sum Test and significance determined at $p<0.05$.