Supplemental Experimental Procedures

CRISPR/Cas9 Genome Editing: Sequences targeting murine c-Src, Fyn, and Lyn were determined by the CRISPR Design Tool (http://crispr.mit.edu): c-Src 5'-GAGCCCTCGGAAAACGTGCACGG-3', Fyn 5'-GGGACCCTACGCACGAGAGGAGG-3', Lyn 5'-GTTCGGTCAGTATTACGTACTGG-3'. The gBlocks (Integrated DNA Technologies) containing U6 promoter, the designed target sequence, gRNA scaffold and termination signal were subcloned into the pQCXIB expression vector. CRISPR guide sequences were stably transfected into NMuMG-NeuNT Flag-tagged ShcA and ShcA mutant expressing cells and selected with 8µg/ml Blasticidin. Loss of c-Src, Fyn, or Lyn expression was verified by immunoblot analysis. Cell lines represent pools of 3-4 clonal populations.

Immunoblot Analysis: Whole-cell lysates were generated from cell lines in PLCγ cell lysis buffer (50mM HEPES [pH 7.5], 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM EGTA, 1.5 mM $MgCl_2$, 10mM sodium orthovanadate, 10 mM NaF, and 1 µg/ml Chymostatin, 2 µg/ml Antipain, 2 µg/ml Leupeptin, 1µg/ml Pepstatin, 2 µg/ml Aprotinin) as previously described [1]. Lysates were separated by SDS-PAGE, transferred onto polyvinylidene difluoride (PVDF) membranes, blocked in 3% Bovine Serum Albumin (BSA; Bioshop Cat: ALB001.500), and probed with antibodies listed in Supplementary Table 2. For studies involving inhibitors, cell lines were seeded into full culture media and allowed to attached overnight. The following day, media was changed to 2.5% FBS DMEM for a minimum of 12 hours. Cell were then treated with 2.5% FBS DMEM containing inhibitors. Whole-cell lysates were collected seven-hours post treatment. Densitometric analysis was conducted using Image J software.

Immunoprecipitation: Cell lines were lysed with cytoplasmic extraction lysis buffer (10 mM Tris [pH 8.4], 140 mM NaCl, 1.5 mM $MgCl_2$, 0.5% NP40, 1 mM DTT, 10mM sodium orthovanadate, 10 mM NaF, and 1 µg/ml Chymostatin, 2 µg/ml Antipain, 2 µg/ml Leupeptin, 1 µg/ml Pepstatin, 2 µg/ml Aprotinin). Cytoplasmic extracts were mixed with 30 µl bed volume of a 1:1 ratio of protein G-Sepharose 4 Fast Flow (GE Health-care Cat: 17-0618-02)–cytoplasmic extraction lysis buffer solution. Flag-tagged ShcA was immunoprecipitated with FLAG-specific antibodies (1:250; Sigma Aldrich Cat: F1804) at 4°C overnight. Beads were subsequently washed three times with cytoplasmic extraction lysis buffer (without protease and phosphatase inhibitors). Complexes were eluted with SDS Page Loading Buffer (4X Tris-HCL/SDS [pH 6.8], 3% Glycerol, 10%SDS, 12 mg bromophenol blue) at 95°C for 8 minutes.

BioID: Flag-tagged ShcA and ShcA mutant cDNA was subcloned into pcDNA3.1(-) MycBirA-R118G (a gift from Dr. Marc Fabian) and subsequently subcloned into the pQCXIB expression vector. N-terminal MycBirA-tagged ShcA wild-type, ShcA mutants, and MycBirA-only vector control was expressed in MMTV Middle T antigen mammary epithelial cells and selected with Blasticidin. Cells were cultured in 5% FBS DMEM supplemented with MEGS. Cells were seeded into complete media. The following day, media was changed to 1% FBS DMEM supplemented with MEGS for a minimum of 12 hours and then pulsed with 50 µM Biotin (Sigma-Aldrich Cat: B4501-1G) in 5% FBS DMEM supplemented with MEGS for 24 hours. Cells were harvested by trypsinization and collected by centrifugation at 250 x g for 5 minutes. Whole cell lysates were generated using RIPA buffer (1% Triton X-100, 50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.1% SDS) supplemented with 50U Benzonase (Millipore Cat: 70664-3), 0.5% Sodium Deoxycholate (Sigma Aldrich Cat: D6750), and phosphatase and protease inhibitors. Lysates were then sonicated two times at 50% amplitude for 10 seconds on ice and subsequently centrifuged at 16 000 xg for 30 minutes at 4°C. Biotinylated proteins were pulled down using 30 µl bed volume of 1:1 Avidin Agarose beads (Thermo Scientific Cat: 20219)-RIPA

buffer solution, rotating for 3-4 hours at 4C. Beads were collected by centrifugation at 3000 rpm for 1 minute at 4°C and then washed three times with RIPA Buffer (without protease and phosphatase inhibitors). Proteins were eluted using 2x Laemmli buffer (4% SDS, 20% Glycerol, 120mM Tris-HCl [pH 6.8]) at 95°C for 8 minutes. Eluates were characterized by immunoblot analysis.

Immunohistochemistry: Paraffin or OCT embedded breast tumor sections were stained with primary antibodies and staining conditions listed in Supplementary Table 3. Slides were scanned using a Aperio ScanScope XT Digital Slide Scanner and data was analyzed using positive pixel count or nuclear algorithms (Aperio ImageScope).

Bioinformatics: All gene signatures were projected across 1218 human breast cancers from TCGA dataset using single sample gene set enrichment analysis (ssGSEA) as described before [2]. Briefly, a score is defined to represent the degree of enrichment of a given gene set in a sample: gene expression values for each sample are rank-normalized, and an enrichment score is produced using the Empirical Cumulative Distribution Functions (ECDF) of genes, with the final score computed by integrating the difference between a weighted ECDF of genes in the signature and the ECDF of the remaining genes [2]. This calculation is repeated for each signature and each sample in the dataset. To compute ssGSEA scores, we used the GenePattern software implementation from the Broad Institute, ssGSEAProjection (v6) [3]. Spearman correlations between each signature and expression values of specific genes (*Src, Fyn and Lyn*) were then computed. For visualization purposes, patients were ranked-ordered and stratified in quartiles, and the mean expression value for each gene and each quartile was computed. By keeping the patients in the same quartiles, we then interrogated relative pY317-ShcA, pY416-Src and pS235/6-rS6 levels in these human breast tumors using the TCGA RPPA breast cancer datasets (n=747). Finally, for tumors in which we had both RNA-Seq and RPPA data, we also interrogated

77 copy number levels for *Shc1* (ShcA), *PTPN12* (PTP-PEST) and *INPPL1* (SHIP2) using the TCGA

78 copy number breast cancer dataset.

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References: Supplemental Experimental Procedures

1. Ursini-Siegel, J., et al., *ShcA signalling is essential for tumour progression in mouse models of human breast cancer.* EMBO J, 2008. **27**(6): p. 910-20.
2. Barbie, D.A., et al., *Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1.* Nature, 2009. **462**(7269): p. 108-12.
3. Reich, M., et al., *GenePattern 2.0.* Nat Genet, 2006. **38**(5): p. 500-1.