SUPPLEMENTAL INFORMATION

Constructs and Reagents-The vectors pBlueLZRS-1, -2, and LZBOBneo/pac/hygro were the kind gift of Dr. Keith R. Johnson (University of Nebraska Medical Center, Omaha, NE) and derived from vectors described by Johnson et al., 2004 and Maeda et al., 2006. CA RhoGTPases used by Johnson et al., 2004 were subcloned in LZBOBneo1. To obtain clones of the p120 isoforms, total RNA was isolated from the MMTV-c-Neu mouse mammary tumor cell line EN064 using TRIzol® reagent according to the manufacturer's instructions (Gibco BRL). Samples were DNAse treated to eliminate contaminating genomic DNA with the DNA-free kit according to the manufacturer's specifications (Ambion) and reversed transcribed using Superscript™ II RT (Invitrogen). The cDNA was used to PCR amplify full length mouse p120 isoform using the forward primer 5'-CGAACCTCGCTGGATTTGTCTTT-'3 and reverse primer 5'-GGCGCTAAATCTTCTGCAT-'3. PCR products were directionally cloned (NotI/EcoRI) into pBlueLZRS-1 and sequenced. Upon confirmation, cDNAs were subcloned into the retroviral expression vectors LZBOBpac1/LZBOBneo1. The shRNA lentiviral vectors used were modified versions of Lentilox 3.7 purchased from ATCC, with GFP replaced by the neomycin resistance gene to produce the LentiNeo vector (59). The p120 shRNA were adapted from sequences first reported in (Davis et al., 2003) and directionally subcloned into the LentiNeo vector. The pharmacological inhibitors PP2, JNK Inhibitor II and AG825 were purchased from Calbiochem. PD98059, LY294002, SB203580 and PD168393 were purchased from Promega. Iressa was obtained from AstraZeneca and NRG1-β1/HRG1-β1was purchased from R&D Systems. The mouse siRNA oligodeoxynucleotide (CCAGAAGUGGUGCGAAUAU) for CTNND1 was purchased from Dharmacon, Inc

FIGURE LEGENDS

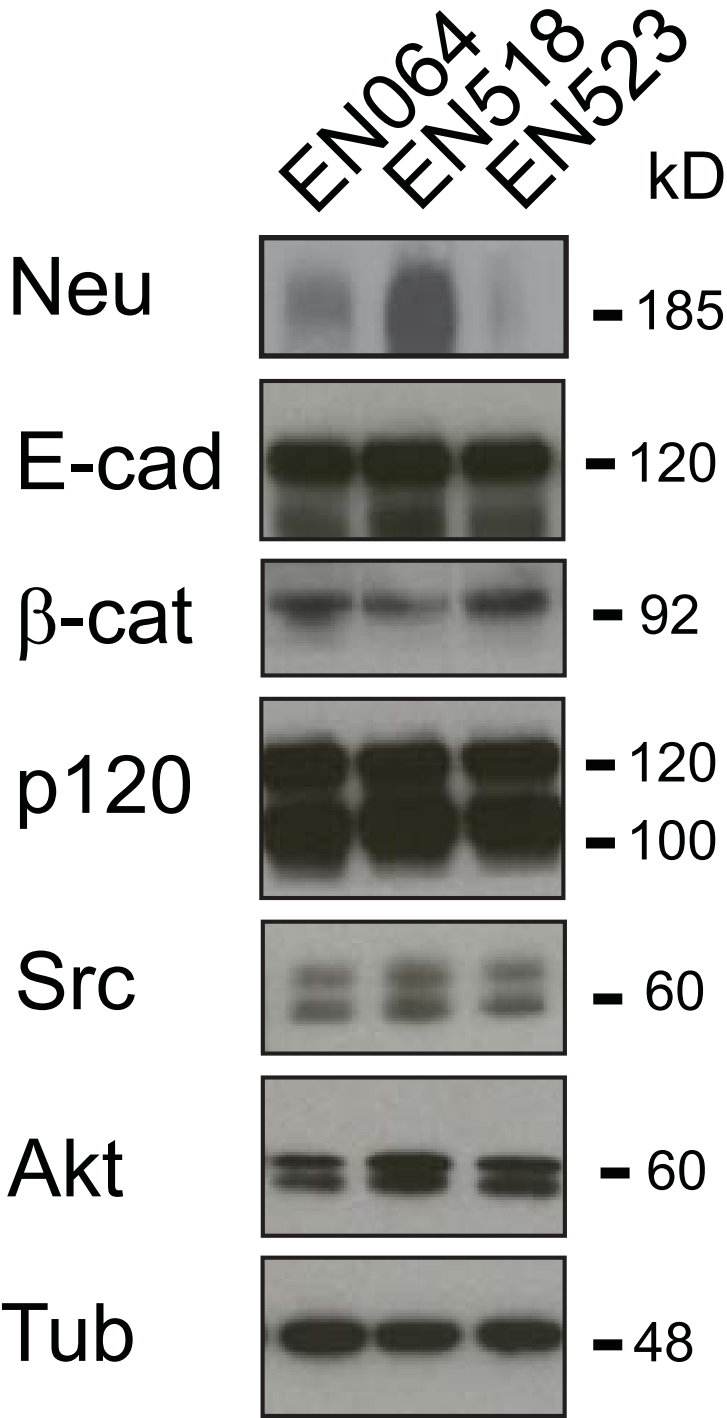
Supplemental Figure 1. Characterization of NeuN cell lines. Murine MMTV-c-Neu cell lines were generated by explant culturing of tumor fragments. Protein lysates were prepared with RIPA buffer and probed various protein expression markers related to ErbB2 amplification.

Supplemental Figure 2. Transient siRNA-mediated knockdown of p120 in EN cells yields the same outcomes as observed in the shRNA stable knockdown cells. (A) Protein lysates were prepared at 10 and 24 hrs post-transfection of EN064 cells with 100nM of siRNA against mouse p120 (KD) or a non-silencing siRNA (NS). (B) Cell migration was assessed after transfection with mouse p120 or non-silencing siRNA in EN064 cells. Bars represent means ± S.D. (n=3). (C) Both EN064 and EN518 cells were transfected with 100nm of siRNA or the non-silencing siRNA and the impact on chemotactic cell migration toward 10% FBS or 20 µg/ml Heregulin was ascertained by transwell migration assays. Bars represent means ± S.D. (n=3).

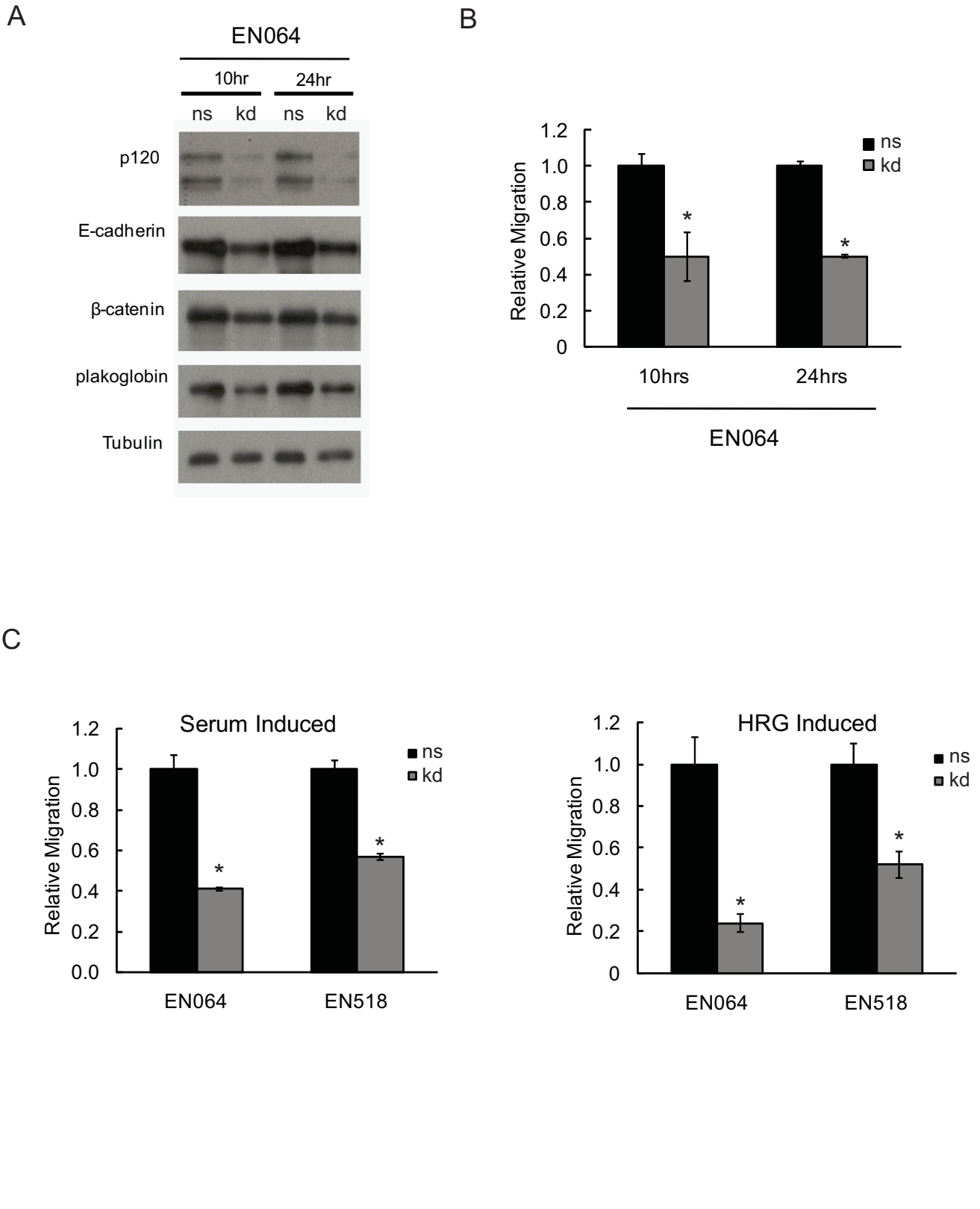
Supplemental Figure 3. Src regulates Heregulin induced p120 expression. (A) SkBr3 cells were stimulated with Heregulin (5µg/ml) over a 2hr time course and lysates were probed for alterations in expression or activity of p120, Akt and MAPK. (B) Blockade of ErbB2, EGFR and the downstream effector Src prevent induction of p120 expression. Heregulin (5µg/ml) induction of p120 expression can be attenuated by pretreatment with Iressa (Ir) 1µM, PD168393 (PD16), and PP2 10µM but not by PD98059 20µM. Total and phosphorylated forms of ErbB2 downstream signaling molecules were assessed to confirm inhibitor efficacy (n = minimum of 3 replicates for each experiment).

Supplemental Figure 1

A



Supplemental Figure 2



Supplemental Figure 3