Supplemental Data

The Nuclear Receptor Coactivator AIB1/SRC-3 is required for *Neu* (ErbB2/HER2) activation, signaling and mammary tumorigenesis in mice

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Supplemental Materials and Methods

***In situ* hybridization.** Mouse *AIB1* cDNA was purchased from Open Biosystems (EMM1003-9333519). A fragment positioned 2127 to 2630 was subcloned into pGEC-T Easy vector (Promega). The following primers were used to amplify the 504 base pair fragment prior to subcloning: 5`-AGA AAC ACC GGA TTT TGC AC-3` and 5`-GAG GAC GAA CAG ACT GCA CA-3`. Two pairs of digoxigenin-labeled riboprobes were generated to correspond to sense and antisense orientations of the mouse *AIB1* mRNA by *in vitro* transcription using T7 and SP6 polymerase with DIG RNA labeling kit (Roche Applied Biosciences) according to manufacturer's instruction.

In situ hybridization was performed as described previously with some modifications (Henke *et al*. Methods. 2006). Briefly, sections from formalin fixed paraffin embedded tissues were deparaffinized and rehydrated by a gradient of ethanol (100%, 90%, and 70%) and finally DEPC treated water. The sections were treated with proteinase K and acetylated in 0.1M triethanolamine hydrochloride and 0.25% acetic anhydride. The DIG-labeled riboprobes (1.5mg probe/1.0µL solution) were hybridized for overnight at 50°C. The sections were washed and digested with RNase A (Roche),

cross-linked with formamide/2X-SSC and subsequently blocked with 2% horse serum. Anti-DIG antibody (1:250, Roche) was added and incubated for overnight at 4°C. Color was developed in nitro blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP). The sections were mounted by clearmount solution (Zymed).

Tumor studies. Tumor formation was monitored on a weekly basis by palpation. Following the detection of a tumor, mice were anesthetized by inhalation of isoflurane and cardiac blood was collected. Mammary tumors were harvested and averaged 1.0 cm in diameter. A portion of the excised tumor was fixed in 10% formalin and embedded in paraffin and sections (5μm) stained with H&E for histological studies. All of the experiments and surgeries performed in this study conform to current federal (National Institutes of Health Guide for the Care and Use of Laboratory Animals) and university standards and were reviewed and approved by Georgetown University Institutional Animal Care and Use Committee.

Supplemental Figure legends

Figure S1. Regulation of SRC-3 mRNA levels in Neu/SRC-3 mice. SRC-3 mRNA expression was detected by *in situ* hybridization with an anti-sense probe specific for mouse *SRC-3* (p/CIP) in representative *Neu*/SRC-3^{wt} and *Neu*/SRC-$3^{+/-}$ tumors. Non-tumorigenic *Neu*/SRC-$3^{-/-}$ mammary gland tissue sections were used to detect possible nonspecific hybridization signals (magnification: 40x). Results represent data from at least 2 mice from each genotype indicated.

Figure S2. Comparison of phenotypic and signaling changes in *Neu* versus *Neu*/SRC-$3^{+/-}$ mice. MMTV-driven *Neu* tumorigenesis induces mammary gland preneoplasia and neoplasia through the activation of kinase signaling and increased cell proliferation. The reduction of *SRC-3* expression in *Neu* overexpressing mice results in a considerable decrease in phosphorylated *Neu* receptor, which most likely leads to a reduction in downstream kinase signaling, cell proliferation and cyclin D1 and E levels. The result is a noticeable absence of growth factor-driven preneoplastic lesions (lateral side-budding) and a significant increase in tumor latency.