Supplementary Materials and Methods

Generation of the ErbB3Δ85 allele.

The targeting strategy for generation of ErbB3Δ85 mice was adopted from the one used previously (15). Briefly, the 5’- homologous region was constructed from a HindIII-BamHI fragment containing exons 8 and 9 and a BamHI fragment containing exon 10. The mutated human cDNA including the 3’-untranslated region was ligated in frame into exon 10 using a conserved BclI site (position 1569 in NM_001982). The neomycin-resistance-cassette was flanked by LoxP-sites, and a 7.5kb BamHI genomic fragment, which contained exon 15, functions as 3’-homologous region. Homologous recombination was performed in ES14.1 cells and successfully recombined ES cell clones were identified by Southern blot using 5’- and 3’- external probes. ES cells were injected into blastocysts, chimaeric mice were mated with C57Bl/6 mice. F1 offspring were mated with Cre-Deleter mice to remove the neo-cassette and to generate the ErbB3Δ85 allele in the mouse germ line. The ErbB3Δ85 strain was then backcrossed to FVB/N genetic background (approximately 10 generations).

Immunohistochemical analysis of tissue sections

For immunohistochemistry, tissue sections were deparaffinized in xylene, endogenous peroxidase activity was blocked with 3% peroxide hydrogen in methanol, and antigen retrieval was accomplished in 10 mmol/L sodium citrate (pH 6) using a pressure cooker (Cuisinart). Sections were then blocked with Power Block Universal Blocking Agent (Biogenex) and incubated in primary antibody. Rabbit polyclonal Ki67 (ab15580) and cleaved Caspase-3 (9661) were purchased respectively from Abcam and Cell Signaling Technology. E-Cadherin (610182)
mouse monoclonal antibody was obtained from BD Transduction Laboratories, SMA (A5228) from Sigma. For immunohistofluorescence analyses, sections were incubated with rabbit polyclonal β-casein (250558, Abbiotec), rabbit polyclonal WAP (sc-25526, Santa Cruz Biotechnology), guinea pig polyclonal cytokeratin-8/18 (RDI-PR0GP11, Fitzgerald) and rabbit polyclonal cytokeratin-14 (PRB-155P, Covance) primary antibodies. Samples were then incubated with the appropriate Alexa Fluor secondary antibodies (Molecular Probe) for 1h at room temperature followed by incubation with DAPI (Sigma) for 10 min. They were visualized using a Zeiss LSM 510 META confocal microscope.

**Western blotting and immunoprecipitations primary antibodies**

Antibodies for western blots include Sigma β-actin (clone AC-15, A5441), Millipore phosphotyrosine (clone 4G10, 05-321) and Src (clone GD11, 05-184), and BD Transduction Laboratories Shc (610081), Bad (610392), and EGFR (610017). Neu (sc-284), ErbB3 (sc-285), ErbB4 (sc-283), cytokeratin-8 (sc-101459), PCNA (sc-56), CyclinD1 (sc-450) and PI-3K p85α (sc-1637) antibodies were from Santa Cruz Biotechnology. Phospho-EGFR (3777), phospho-ErbB2 (2249), phospho-ErbB3 (4791), phospho-Shc (2431), phospho-Cleaved caspase-3 (9661), phospho-Bad (5286), p70 S6 Kinase (9202), phospho-p70 S6 Kinase (9205), 4E-BP1 (9452), GSK-3β (9315), phospho-GSK-3α/β (9331), PDK1 (3062), phospho-PDK1 (3061), AKT (9272), phospho-AKT (9271), PTEN (9559), phospho-PTEN (9554), mTOR (2972), phospho-mTOR (2971), ERK1/2 (9102), phospho-ERK1/2 (9106) and phospho-Src (2101) antibodies were purchased from Cell Signaling Technology. The mouse monoclonal antibody used to immunoprecipitate ErbB2/Neu (Ab-4) was purchased from EMD Chemicals. ErbB3 and EGFR were immunoprecipitated with the same antibodies used for western blotting.
Supplemental Figure legends

Supplemental Figure S1. Southern blot analysis of genomic DNA digested with SacI and SpeI/SphI. SacI- and SpeI/SphI-digested genomic DNA from mice with the indicated genotypes were hybridized with respectively 5’- and 3’-external probes. Sizes in kb of the hybridizing bands are indicated.

Supplemental Figure S2. ErbB3Δ85 lactating mice show altered mammary gland morphology and histology associated with a defect in milk secretion. (A). Top, representative inguinal no.4 mammary gland wholemounts prepared from ErbB3Δ85 and control mice at 3 days of lactation demonstrating extensive development of secretory structures in ErbB3Δ85 mammary glands despite deficient ductal outgrowth. Bottom, higher magnification showing that the ErbB3Δ85 lactating mammary glands contain less expanded alveoli when compared to control glands. (B) Three representative patterns of H&E-stained mammary gland sections from ErbB3Δ85 and control mice at 3 days of lactation are shown. Scale bar: 200µm. (C) Two representative patterns of H&E-stained mammary gland sections at higher magnification are shown. Scale bar: 50µm. (D) β-casein (red, top) and WAP (red, bottom) immunostaining of mammary gland sections from ErbB3Δ85 and control mice at 3 days of lactation. Cytokeratin-8 (green) was used to immunostain the mammary epithelium. Asterisks denote absence of milk protein staining in the alveolar luminal space. Nuclei were stained with DAPI (blue). Scale bar: 20µm. Four mice/genotype were analyzed.
Supplemental Figure S3. Metastatic capacity of \textit{ErbB3}^{Δ85/Δ85}/\textit{NDL2-5} mammary tumors. \textit{ErbB3}^{wt/wt}, \textit{ErbB3}^{Δ85/wt}, and \textit{ErbB3}^{Δ85/Δ85}/\textit{NDL2-5} mice were sacrificed eight weeks following first palpable tumor. (A) Percentage of animals from each group harboring metastatic lesions in the lung is shown (15 animals for \textit{ErbB3}^{wt/wt}, 15 for \textit{ErbB3}^{Δ85/wt} and 18 for \textit{ErbB3}^{Δ85/Δ85}/\textit{NDL2-5} mice). (B) Lung metastases were identified and scored by microscopic analysis of multiple (at least 5) H&E-stained lung step-sections. Quantification of the average number (±SEM) of lesions per lung lobe from animals (11 per group) positive for lung metastases.

Supplemental Figure S4. Tumors from homozygous \textit{ErbB3}^{Δ85/Δ85} show a typical ErbB2/Neu histological pattern. (A) Representative H&E-stained sections of \textit{NDL2-5} late stage mammary tumors derived from virgin females expressing \textit{ErbB3}^{wt/wt} or \textit{ErbB3}^{Δ85/Δ85} are shown. Scale bar: 100µm. (B) Cytokeratin-8 (CK8, green) and cytokeratin-14 (CK14, red) immunostaining of \textit{NDL2-5} mammary tumor sections from \textit{ErbB3}^{wt/wt} or \textit{ErbB3}^{Δ85/Δ85} mice. Nuclei were stained with DAPI (blue). Scale bar: 20µm. (C) \textit{ErbB3}^{wt/wt} and \textit{ErbB3}^{Δ85/Δ85}/\textit{NDL2-5} tumor lysates were subjected to western blot analysis with the indicated specific antibodies. Cytokeratin-8 (CK8) was used as a control for epithelial cell content.

Supplemental Figure S5. Proliferative capacity and apoptotic status of \textit{ErbB3}^{Δ85/Δ85}/\textit{NDL2-5} mammary tumors. (A) Paraffin sections of late stage mammary tumors from \textit{ErbB3}^{wt/wt} and \textit{ErbB3}^{Δ85/Δ85}/\textit{NDL2-5} mice were submitted to immunohistochemistry analysis of Ki67 expression (A) and analyzed by TUNEL assay (B). Quantifications of the Ki67-positive cells and of the TUNEL-positive cells are represented as percent of tumoral cells (±SEM) and were calculated following counting multiple fields from six animals/genotype. Scale bars: 100 µm (A)
and 50 µm (B). (C) Lysates from $ErbB3^{wt/wt}$ and $ErbB3^{Δ85/Δ85}$/NDL2-5 mammary tumors were subjected to western blot analysis with the indicated specific antibodies. β-actin was detected as a control for loading while cytokeratin-8 (CK8) was used as a control for epithelial cell content.

**Supplemental Figure S6. Activation status of Shc, Src and members of the PI-3K signaling pathway.** Lysates from $ErbB3^{wt/wt}$ and $ErbB3^{Δ85/Δ85}$/NDL2-5 mammary tumors were subjected to western blot analysis with the indicated specific antibodies. β-actin was used as a control for loading.