**Supplemental Materials and Methods**

**Cell lines:** NIH3T3 cells (immortalized Swiss mouse embryonic fibroblasts) were from the European Collection of Animal Cell Cultures (Salisbury, UK). The human melanoma RPM-MC cells, negative for CD44, were kindly provided by Ivan Stamenkovic (University of Lausanne, Switzerland). Mouse embryonic fibroblasts (MEFs) were isolated frommice with floxed *cd44 alleles* (*cd44*fl/fl; GT(Rosa)26-Cre (B6/129). HEKNE cells are HEK293T cells stably infected with pB-FLAG-NRG1-EGFP and also with Angiotensin II receptor 1 (Dang et al. 2013), MDA-MB-231 are human triple negative breast cancer cells obtained from ATCC. All cells were grown in Dulbecco's modified Eagle's medium (DMEM, PAA Laboratories, GE Healthcare) supplemented with 10% FBS (PAA Laboratories, GE Healthcare). Cells were maintained in a humidified atmosphere with 5% CO2 at 37°C.

**Transfections:** All DNA and siRNA transfections were performed in 6-well and 12-well plates or 10-cm and 15-cm plates (for biotinylation and immunoprecipitation experiments) using the liposomal transfection reagent Lipofectamine 2000 (Invitrogen).

**Precipitation of Proteins by TCA-DOC (Trichloro Acetic Acid - Na Deoxycholate):** For detection of soluble CD44 ectodomain or neuregulin the cells were cultured in serum-free medium. Cell culture supernatants were pre-cleared by centrifugation at 10,000 rpm for 10 min to pellet cell debris. The pre-cleared supernatant was mixed with 1/100 volume of 2% DOC, vortexed and kept on ice for 30 min. Then 1/10 volume of 100% TCA was added, and the samples were kept at 4°C overnight. The precipitate was recovered by centrifugation at 15,000 g for 15 min, rinsed twice with acetone and re-dissolved in RIPA buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS).

**Co-immunoprecipitation (Co-IP) from Cell Lysates**: For co-immunoprecipitation of CD44 and merlin transfected NIH3T3 cells were grown in 15 cm-plates at high cell density. Cells were washed once in ice-cold 1x PBS and harvested in IP buffer containing 1x complete protease inhibitor cocktail (Roche, Basel, Switzerland). The following IP buffer was used: 10mM Tris HCl pH 7.5, 0.5% Triton X-100, 0.5% NP-40, 2 mM EDTA. After shearing DNA the lysate was pre-cleared. Endogenous merlin was immunoprecipitated using 30 µl of Protein G plus (Santa Cruz Biotechnology, Inc, Santa Cruz, CA) gel beads, pre-conjugated with 2 μg of merlin antibody. Immuno-complexes were recovered by centrifugation and washed four times with cold IP buffer and eluted with 2x Laemmli sample buffer.

**Preparation of Cell Lysates for Western Blot Analysis**: For Western blot analysis, cells were plated on 6-well tissue culture plates and cultured initially in DMEM supplemented with 10% FBS, then changed to low serum medium overnight prior to treatments. Cells were subsequently pretreated, for 30 min, with control (DMSO) or 10 µM batimastat (BB94) (Calbiochem, Billerica, MA) or Latrunculin B (Calbiochem, Billerica, MA), and in the presence or absence of 10 M DAPT (Sigma-Aldrich, St. Louis, MO) to avoid further proteolysis by γ secretase (see Inhibition of Cleavage Conditions above).Cells were then either control treated (DMSO) or stimulated with 100 ng/ml of TPA, or growth factors as outlined in the figure legends. Cells were subsequently lysed in RIPA buffer (50 mM Tris-HCl pH 8, 150 mM NaCl, 1 % NP-40, 0.5 % sodium deoxycholate, 0.1 % SDS) containing a cocktail mix of protease and phosphatase inhibitors (Sigma-Aldrich, St. Louis, MO). Samples were then centrifuged and the supernatants without insoluble proteins were collected for protein concentration determination (Bio-Rad Protein Assay, 500-0006). For immunoblot samples were mixed with 2x Laemmli sample buffer. Prior to loading, samples were heated to 96 °C for 5-10 min.