Supplemental Figure Legends

Figure S1: CERT transcript levels in prostate cancer microarray datasets. CERT expression data was extracted from different normalized gene expression microarray datasets. Box blots with CERT mRNA levels were generated for the different subtypes described in the studies and student’s T-test was performed. A) GSE3325/GDS1439 (Varambally et al., 2005): 13 prostate tumors, 6 benign tissues; * p=6.7E-7, ** p=4.6E-8. (B) GSE6919/GDS2547 (Chandran et al., 2007): 17 normal prostate tissues, 58 normal prostate tissues adjacent to the tumor, 64 primary and 25 metastatic prostate tumor samples; * p=3.2E-5, **p=7.0E-13, ***p=2. 7E-5.

Figure S2: Lipid analyses by MALDI-TOF mass spectrometry. Total lipids were extracted by the method of Bligh and Dyer. Positive ion mass spectra were recorded with DHB (0.5 M in methanol) and negative ion mass spectra with 9-AA (10 mg/ml in isopropanol/acetonitrile (60:40, v/v). Samples were diluted 1:1 (v/v) with the corresponding matrix solutions and directly applied onto a gold-coated MALDI target. Mass spectra were acquired on a Bruker Daltonics Autoflex system, utilizing a pulsed nitrogen laser emitting at 337 nm. The extraction voltage was 20 kV, and the ‘low mass gate’ was turned on to prevent the saturation of the detector by ions resulting from the matrix. For each mass spectrum, > 100 single laser shots were averaged. The laser strength was kept about 10% above threshold to obtain the best signal-to-noise ratio. To enhance the spectral resolution, all spectra were measured in the reflector mode. Peaks are labeled according to their m/z ratios and lipid species were assigned. Matrix peaks and peaks of impurities (plasticers) are marked by asterisks. Compared with the control, the intensity of the cholesterol peak (red) in the positive ion spectra is increased relative to the individual PC peaks (green) in the sample from CERT knockdown cells.
**Figure S3:** A second CERT-specific siRNA confirms the impact on EGF-induced Akt activation, cell migration and focal adhesion clustering. MCF7 cells were transiently transfected with siCERT#2 or siLacZ as a control. (A) Two days post transfection, cells were serum-starved overnight, stimulated with 10 ng/ml EGF for the indicated times and lysed. Equal amounts of proteins were separated by SDS-PAGE and analyzed by immunoblotting using pAkt(T308)-specific antibody (top panel). Expression of CERT and total Akt were verified with CERT- and Akt-specific antibodies (bottom panels). The membrane was reprobed with a tubulin-specific antibody. (B) Cells were harvested two days post transfection and subjected to a Transwell migration assay with 50 ng/ml EGF as a chemotactic stimulus. (C) Two days post transfection cells were replated onto collagen-coated coverslips, starved overnight and then fixed and stained with primary antibodies directed against the indicated proteins, followed by Alexa Fluor-conjugated secondary antibodies. The images shown are stacks of several confocal sections. Scale bars, 20 μm.

**Figure S4:** Downregulation of SM synthases recapitulates the effects of CERT depletion on EGF-induced Akt activation and cell migration. (A) MCF7 cells were transiently transfected with SMS1- or SMS2-specific siRNAs and, three days post transfection, SMS expression was evaluated by Western blotting using SMS-specific antibodies (top panels). Cells transfected with a LacZ-specific siRNA were used as a negative control. Equal loading was verified by reprobing the membrane with tubulin-specific antibody (bottom panels). (B) Akt activation was quantified by In-Cell-Western analysis. One day post siRNA transfection cells were replated into 96 well plates. The next day cells were starved overnight and then treated with 10 ng/ml EGF for the indicated times. Cells were fixed and permeabilized and then incubated with rabbit anti-phospho-T308-Akt and mouse anti-Akt 40D4 (pan) mAbs, followed by secondary antibodies labeled with IRDye 680/800. After scanning with the Odyssey system (Li-Cor), the pAkt signal was normalized to that of total Akt. Mean values of duplicate samples are plotted (fold induction of the untreated control). (C)
Cells were harvested two days post transfection and subjected to a Transwell migration assay with 50 ng/ml EGF as a chemotactic stimulus.

**Figure S5: CERT downregulation enhances HRG-induced ErbB2/ErbB3 signaling.** MCF7 cells were transfected with siCERT or siLacZ. Two days post transfection, cells were serum-starved overnight, left untreated or stimulated with 25 ng/ml HRG for the indicated times. (A) Cells were harvested and equal amounts of total protein were subjected to SDS-PAGE and transferred to membrane. Akt activation was analyzed by immunoblotting using a pAkt(T308)-specific antibody (top panel). Expression of CERT and total Akt were verified by immunoblotting with CERT- and Akt-specific antibodies (bottom panels). (B) Cells were lysed and ErbB receptors were immunoprecipitated with specific mouse monoclonal antibodies. Immunoprecipitated proteins were subjected to SDS-PAGE followed by immunoblotting with phosphotyrosine antibody (pY; top panels). Total amounts of immunoprecipitated receptor were determined by reprobing the membranes with respective ErbB receptor-specific rabbit polyclonal antibodies. (C) Cells were collected, resuspended in PBS with 0.02% azide and incubated with 2 µg/ml FITC-labeled anti-ErbB2 (Santa Cruz Biotechnology) and PE-labeled anti-ErbB3 (Biolegend) antibodies, respectively, for 1 h at 4 °C. Cells were then washed and fluorescence intensity was measured with a FACSCalibur Flow Cytometer (BD Bioscience). FITC-labeled mouse IgG1 (Santa Cruz Biotechnology) and PE-labeled mouse IgG2A (Immunotools) were used as controls (shaded area).

**Figure S6: A second PLD2-specific siRNA confirms the effects on ErbB1 and Akt activation.** MCF7 cells were transiently transfected with siCERT, siPLD2#4 or a combination of both. LacZ-specific siRNA was used as a control and to adjust the siRNA amount in each transfection mix. Two days post transfection, cells were serum-starved overnight. Cells were stimulated with 10 ng/ml EGF for 15 min. Lysates were analyzed by immunoblotting using pErbB1(Y1068)- and pAkt(T308)-
specific antibodies (top panels). Expression of CERT and total Akt were determined by immunoblotting with CERT- and Akt-specific antibodies (middle panels). The membrane was reprobed with a tubulin-specific antibody (bottom panel). The panels shown are from the same gel.