Supplemental Data

Supplementary Figure 1. R-cadherin cytosolic expression occurs in a subset of invasive breast cancer and is reminiscent of the pattern of R-cad expression in MDA-MB-231. (A) An example of a poorly differentiated invasive duct carcinomas (IDC) expressing a more cytosolic form of R-cad, compared to (B) a tumor with membranous R-cad localization.

Supplementary Figure 2. R-cadherin is downregulated during TGFβ-induced EMT in normal MCF10A breast cells. MCF10A cells were treated with 5 ng/ml TGFβ for 1-4 days. (A) At each time point, a cell lysate was analyzed by immunoblotting for endogenous levels of N-cad, E-cad, R-cad and fibronectin. (B) Immunoblots were quantified by densitometry and results are shown as bar graphs representing SEM ± SD.

Supplementary Figure 3. R-cadherin knockdown in MDA-MB-231 cells does not affect the invasive phenotype. (A) MDA-MB-231 cells were treated with control siRNA (lane1) or a pool of 3 R-cad siRNA (lane 2) and controlled for efficiency of knockdown by immunoblotting with anti-R-cad antibody relative to α-tubulin loading controls. (B) After control and R-cad knockdown cells were applied to Matrigel-coated transwells, the number of transmigrating cells were determined in triplicate wells and plotted as the mean ± SEM.

Supplementary Figure 4. R-cadherin inhibits invasion of the highly invasive HS578T breast cancer cell line and induces morphogenesis. (A) HS578T cells transfected with either control vector (left panel) or R-cadmyc (right panel) were grown in Matrigel. (B) The cells were also applied to Matrigel-coated transwell chambers for testing invasion. The number of invading cells was counted in 3 independent experiments. Average values were plotted as mean ± SEM. (C) Western blots of control and HS578T-R-cad cell lysates, were reacted with antibodites to R-cad, cad-11, N-cad, or tubulin. Endogenous N-cad levels were slightly reduced

in HS578T-R-cad cells relative to controls (lanes 2 and 1, right panel, top). In contrast, no difference in cad-11 expression was observed between R-cad and vector expressing cells (right panel, bottom).

Supplementary Figure 5. R-cadherin expression reduces endogenous cadherin-11 expression and stabilizes catenin expression in MDA-MB-231 cells. (A) GFP control (lanes 1-3) and MDA-MB-231/R-cad-GFP cells (lanes 4-6) were plated on 6-well dishes at low, intermediate, and high density (0.3, 0.7 and $3X10^5$ cells/well). Western blots analyzed each sample for cad-11, α-cat, β-cat, p120, and α-tubulin as control. (B) Immunofluorescent staining with anti β-cat, or anti-p120 antibody followed by TRITC secondary detection, shows stabilization of β-cat and p120 at cell-cell junctions in R-cad-GFP expressing cells. In contrast, the localizations were more diffuse in GFP-expressing controls.

Supplementary Figure 6. R-cadherin expression into a cadherin-null MDA-MB-231 cells suppresses invasion, induces acini and Rac1 activation. (A) A variant clone of MDA-MB-231 cells devoid of endogenous R-cad and cad-11 expression (231v) was transfected with either GFP (left panel) or R-cad-GFP (right panel). Cells were applied onto Matrigel-coated transwell chambers and tested for invasion (top panels) or cultured in Matrigel to test for acinus formation (bottom panels). Note the suppressive effect of R-cad on invasion and stimulatory effect on acini. (B) The number of invading cells was counted in 3 independent experiments. Results are plotted as mean ± SEM. (C) Western blots of 231v or 231 wild type cell lysates show absence of cad-11 and R-cad in the parental 231v cell line, and induced expression of R-cad after retroviral infection (right panel). (D) Lysates from 231v-GFP and 231v-R-cad-GFP cells were grown either in Matrigel (3D) or on plastic (2D) and assayed for Rac1 activation. In this assay,

proteins binding to GST-CRIB were detected on Western blots with antibody to Rac1 (top panels). A lysate from each sample was analyzed for total Rac1 levels.