LEGEND TO SUPPLEMENTAL FIGURES

Fig. S1 Expression of LRP1 in fibroblasts and breast cancer cells

LRP1β protein expression is shown in panel a. Whole cell extracts were subjected to SDS-PAGE and immunoblotting with the anti-LRP1β hybridoma. LRP1β transfected COS lysate was used as a positive control, PEA13 (LRP1-deficient MEF) cells as a negative control, and β–actin as a loading control. LRP1 mRNA was quantified by real-time quantitative RT-PCR (panel b). Mean +/- s.d. of 3 independent experiments is shown.

Fig. S2. Co-purification of endogenous LRP1 with ^{D231N}cath-D

D231N cath-D-transfected MEFs (CD55-/-D231N) grown to 90% confluence without medium change for 3 days were lysed in PLC buffer and loaded on an anti-cath-D M1G8 affinity column that binds to 52-, 48-, and 34-kDa forms of cath-D. Eluted fractions were subjected to SDS-PAGE and immunoblotting with the anti-cath-D antibody (top panel) and anti-LRP1β hybridoma (bottom panel).

Figure S3. Silencing LRP1 in pro-cath-D secreting MEFs inhibits outgrowth

CD55-/-cath-D cells transfected with LRP1 siRNA4 or Luc siRNA were embedded in Matrigel 24 h post-transfection (panel a), and analyzed as described in the legend to Figure 7B. LRP1β expression was monitored 24 h post-transfection of CD55-/-cath-D cells with Luc siRNA or LRP1 siRNA4 (panel b). Bars; - - -, 75 µm; ─ , 750 µm.

Figure S4. LRP1 silencing in HMF fibroblasts prevents pro-cath-D-induced outgrowth

Phase contrast optical photomicrographs of HMF fibroblasts after 3 days of co-culture are shown in panel a. HMF fibroblasts transfected with Luc siRNA (panel a, top) or LRP1 siRNA2 (panel a,

bottom) were embedded 48h post-transfection in the presence of a bottom layer of 3Y1-Ad12 cancer cell lines secreting no cath-D or human cath-D. LRP1β expression was monitored 48 h post-transfection before the co-culture assays (panel b). Pro-cath-D secretion was analyzed after 3 days of co-culture of Luc or LRP1 siRNA2 HMFs with 3Y1Ad12/control or 3Y1Ad12/cath-D cells by immunoblotting. *, non-specific contaminant protein. Bars, 75 µm.

Figure S5. LRP1 expression is required for fibroblastic morphology in three-dimensional matrices

HMF fibroblasts transfected with Luc siRNA, LRP1 siRNA1 or LRP1 siRNA2 were embedded in Matrigel 48h post-transfection (panels a and b). Phase contrast images taken after culturing for 3 days are shown (panels a and b, top) and p-nitrotetrazolium violet cell staining after culturing for 5 days is also given (panel a, bottom). Insets illustrate fibroblast morphology. Data from one representative experiment out of 3 is shown. Cells transiently expressing Luc siRNA, LRP1 siRNA1 or LRP1 siRNA2 were analyzed by immunoblotting 48h post-transfection (panels a and b, right). Bars (---, 75 µm; ⁻, 750 µm).

SUPPLEMENTAL MATERIALS AND METHODS

RNA extraction, RT-PCR and Q-PCR. RNA extraction and reverse transcription were performed, and Q-PCR was carried out using a LightCycler and the DNA double-strand specific SYBR Green I dye for detection (Roche). Q-PCR was performed using gene-specific oligonucleotides, and results were normalized to RS9 levels.