Supplementary information for

Analysis of the Effect of LRP-1 Silencing on the Invasive Potential of Cancer Cells by Nanomechanical Probing and Adhesion Force Measurements using Atomic Force Microscopy

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SUPPLEMENTARY FIGURES

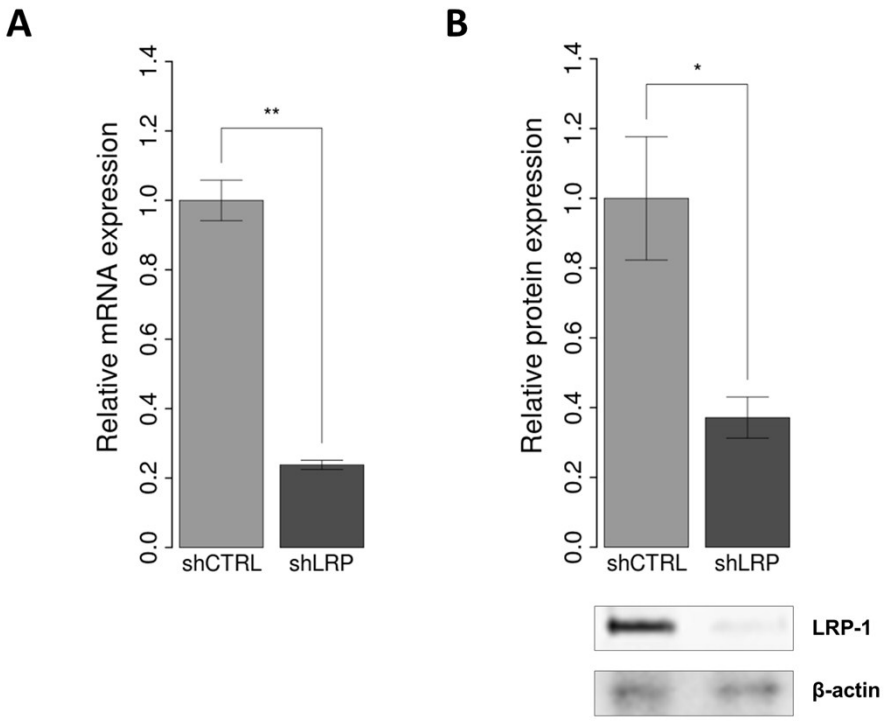

Figure S1. Stable transfection of an shRNA-encoding plasmid into FTC-133 cells leads to LRP-1 silencing. (A) The total RNA from the FTC-133 control cells (shCTRL) and the FTC-133 cells that stably overexpress shRNAs against LRP-1 (shLRP) was purified. The level of the LRP-1 transcript was then assessed by RT-qPCR, and the results show a 77% ($p \leq 0.01$) decrease in expression in the transfected cells compared with the control cells. (B) Whole-cell extracts from the shCTRL cells and shLRP cells were analyzed by immunoblotting with an anti-LRP-1 antibody. The densitometry analysis revealed a 63% ($p \leq 0.05$) decrease in protein expression in the transfected cells relative to the level observed in the control cells. The inserts show representative western blot bands for LRP-1 and β actin, which was used for normalization.

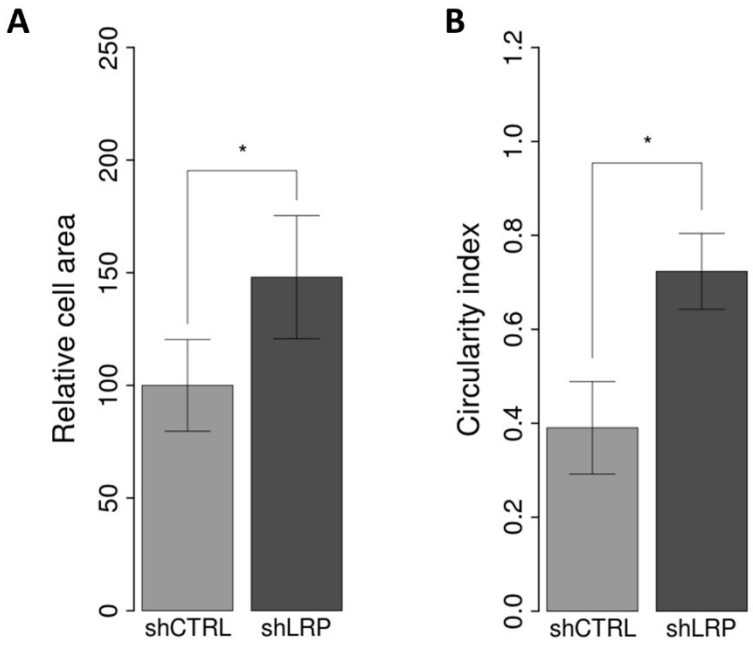

Figure S2. Statistical analysis of phenotypic parameters confirms the alteration in FTC-133 cells' morphology observed by light microscopy and AFM after LRP-1 silencing (see Fig. 1 in main material). (A) Statistical analysis of the relative cell area of control cells (shCTRL) and LRP-1-silenced cells (shLRP) shows that the cell area is increased by 50% after LRP-1 silencing. (B) Statistical analysis of the circularity index of shCTRL cells and shLRP cells (shLRP) shows that the cell circularity index is increased from 0.4 to 0.7, showing a more rounded shape ($p \leq 0.05$ in both experiments).

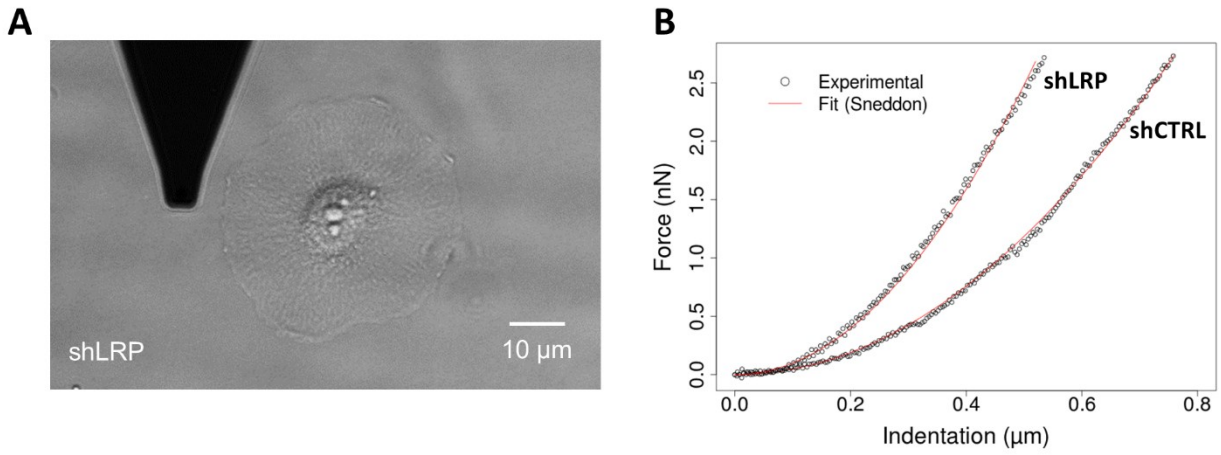

Figure S3. Determination of the Young's modulus of FTC-133 control cells (shCTRL) and LRP-1-silenced cells (shLRP). (A) An AFM cantilever just prior to an indentation measurement is shown in close proximity to a FTC-133 shLRP cell. (B) Typical force-versus-indentation curves obtained after indenting a shCTRL cell (less steep curve) and a shLRP cell (steeper curve). The initial portion (400 nm) of the curve is fitted well by the Sneddon contact model (red line).

SUPPLEMENTARY EXPERIMENTAL

RT-qPCR

RT-qPCR was performed using a Verso SYBR Green 2-Step QRT-PCR Rox kit (Thermo Electron, Courtaboeuf, France) according to the manufacturer's protocols. A mass of up to 250 ng of the isolated RNAs was used for cDNA synthesis in a 20-µl reaction mixture. The mixtures were incubated in a thermocycler at 42°C for 30 min and 95°C for 2 min. Real-time PCR was performed using an Absolute SYBR Green Rox mix (Thermo Electron, Courtaboeuf, France) with the Chromo Four-Color-Real-Time PCR detection system (Bio-Rad, France). The PCR conditions were 15 min at 95°C followed by 40 cycles of 15 s at 95°C (denaturation) and 1 min at 60°C (annealing/extension). The PCR efficiency of the primer sets was controlled by the slope of a standard curve. The results were standardized to the RPL32 and RS18 gene expression levels. The relative gene expression was determined with the following formula:

fold induction = 2 - ΔΔCt, where ΔΔCt = (Ct GI [unknown sample] — Ct GI [reference sample]) — (Ct GR [unknown sample] — Ct GR [reference sample]), GI is the gene of interest (LRP-1), and GR is RPL32 and RS18. The control sample (FTC-133 shCTRL) was chosen to represent a 1x expression of the genes of interest, and the levels in the treated samples are expressed relative to those in the corresponding control sample. The entire experiment, from RNA extraction to real-time PCR, was repeated three times with different sets of samples.

Western blot analysis

The cells were washed with PBS and lysed with TBS-Triton X-100 (10-mM Tris-HCl, pH 7.5, 150-mM NaCl, 5-mM EDTA and 1% (v/v) Triton X-100) containing a protease inhibitor cocktail (Sigma-Aldrich). The cell lysates were harvested, incubated for 30 min on ice with vortexing every 5 min, and centrifuged at 15,000 g and 4°C for 10 min to eliminate the insoluble material. The supernatant containing the solubilized proteins was used immediately or stored at -80°C. The protein concentrations were determined using the BCA protein assay, and 20 μg of the proteins was boiled for 5 min in Laemmli buffer. The proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. The membranes were incubated in blocking buffer (5% (w/v) non-fat dry milk in TBS (50-mM Tris, pH 7.5, and 150-mM NaCl)) for 1 h at room temperature and incubated overnight at 4°C with an anti-LRP-1 (Calbiochem) or an anti-actin (Santa Cruz Biotechnology) primary antibody in blocking buffer with 0.1% (v/v) Tween 20. After five washes with TBS-Tween 20, the membranes were incubated for 1 h at room temperature in the presence of a secondary antibody conjugated with a DyLight 680 fluorophore (Thermo Fisher, used for LRP-1) or horseradish peroxidase (Sigma-Aldrich, used for actin). The immunoreactive bands for actin were first revealed using an ECL Plus chemiluminescence kit (Amersham). All of the bands were detected using an Odyssey Fc imaging system (LI-COR). Actin was used as a

control to ensure equal loading. The bands from the immunoblots were quantified using Image Studio (LI-COR). The experiment was repeated three times with different sets of samples.