Supplementary Figures

Supplementary Figure 1. Cells cultured on coverslips were fixed with 4% paraformaldehyde for 10-15 min and permeabilized for 30 min in PBS 0.1% saponin. Cells were sequentially incubated with 1/100 rAb ERM/ETV5 (Sta. Cruz Biotechnology, Santa Cruz, CA), and with anti-rabbit secondary antibodies conjugated with TRITC (Sigma, Saint Louis, MO) for 1 hr at RT. Coverslips were mounted using the Vectashield mounting medium (Vector Laboratories, Inc., CA), and fluorescence was visualized and imaged on a DM-IRBE inverted fluorescence microscope (Leica, Wetzlar, Germany) coupled to a TCS-NT argon/krypton confocal laser (Leica, Wetzlar, Germany).

As expected, immunofluorescence showed positive GFP staining in the Hec-1A GFP cells, localized in both the nucleus and the cytoplasm, and low nuclear ERM/ETV5 staining corresponding to the endogenous protein, similar to the non-transfected Hec-1A cells (Suppl. Fig. 1). In contrast, Hec-1A GFP-ERM/ETV5 cells showed mainly nuclear GFP fluorescence co-localizing with increased ERM/ETV5 signal that corresponded to both the transfected construct and the endogenous proteins (Suppl. Fig. 1).

Supplementary Figure 2. Two different populations from the Hec-1A GFP-ERM/ETV5 cell line expressing low and high levels of the fusion protein GFP-ERM/ETV5, were selected by flow cytometry and cell sorting depending on the GFP fluorescence. Flow cytometry was performed using a Cell Sorter (BD FacsAria, Becton and Dickinson) equipped with a blue laser tuned to 488 nm. Data acquisition and analysis was carried out using CELLQuest software (Becton Dickinson). Forward angle and side-scatter light gating was used to exclude dead cells and debris. A 502 nm filter with a band-pass of 530/30 nm was used to collect the EGFP signal.

The procedure allowed us to collect GFP positive cells in two tubes depending on its GFP levels, one fraction with high levels of GFP-ERM/ETV5 and another one with low levels of fusion protein. These populations were further cultivated and processed for cell migration assay by video-microscopy.

Western blotting (upper panel; Suppl. Fig. 2) and RT-Q-PCR (middle panel; Suppl. Fig. 2) demonstrated the different levels of expression of the selected populations, without any change on the levels of the endogenous ERM/ETV5

protein. Cell migration assays demonstrated a gradation in the promotion of migration by the expression of the fusion protein, as shown by the MRDO quantification of 100 cells tracked from each population (lower panel; Suppl. Fig. 2).

Supplementary Figure 3. Endometrial tissue-array for ERM/ETV5 and MMP-2 protein expression. For the global analysis of ERM/ETV5 and MMP-2 expression, 74 endometrial carcinomas from patients of the Hospital del Mar in Barcelona (Spain) were evaluated on a TMA described elsewhere (6).

Representative examples of corresponding immunohistochemical intensities in different sections from the same cores in the TMA are shown, stained for MMP-2 (left panels) and ERM/ETV5 (right panels). Both ERM/ETV5 and MMP-2 proteins showed specific staining in the epithelial glands of the endometrial cancer tissues, while they showed negative labelling in the stroma.

High-levels of MMP-2 correlated to strong ERM/ETV5 up-regulation (Suppl. Fig. 3*A*-*B*); intermediate MMP-2 labeling coincided with medium ERM/ETV5 expression (Suppl. Fig. 3*C*-*D*); and, MMP-2 and ERM/ETV5 showed corresponding levels of low intensity staining (Suppl. Fig. 3*E*-*F*).Images were taken at 20x.