Supplemental data

Supplemental experiment procedures:

Construction of siRNA vector- The pU6pro vector was used to construct pU6pro-si-mock (si-mock) and pU6pro-si-Ezrin (si-Ezrin) following the recommended protocol. For the si-mock and si-Ezrin, we synthesized primers for the si-mock (general scramble: sense, 5'-TTTGACTACCGTTGTTAT AGGTGTTCAAGAGACACCTATAACAACGGTAGTTTTTT-3', and antisense, 5'-CTAGAAA AAACTACCGTTGTTATAGGTGTCTCTTGAACACCTA TAACAACGGTAGT-3') and for si-Ezrin [Set1 5'-CCCCAAAGAUUGGCUUUCC-3' (position in the ORF 704–722)', and Set 2 5'-UCCACUAUGUGGAUAAUAA-3'(ORF 140–158)']. All constructs were confirmed by restriction enzyme mapping and DNA sequencing.

Gene transfection and stable-transfected cell lines- 5-8F cells (5.0×10^5) were seeded in 100 mm tissue culture dish. After culturing at 37°C for 16-24 h, the cells were respectively transfected with 4 μg of pU6pro-si-mock or pU6pro-si-Ezrin using LipofectAMINE2000 reagent (Life Technologies, Inc.) following the manufacture's suggested protocol. The stably-transfected cell lines, 5-8F-si-Ezrin cell and 5-8F-si-mock cell, were obtained by selection for G418 resistance (400 μg/ml) and further confirmed by assessing Ezrin expression.

Cell motility and invasive assay- The invasiveness of 5-8F-si-Ezrin and 5-8F-si-mock cell was tested by the Boden chamber invasion assay in vitro. Matrigel (Collaborattiv Biomedical Products, Bedford, MA) was diluted to 25 mg / 50ml with cold filtered distilled water and applied to 8 mm pore size polycarbonate membrane filters and then cells were seeded to Boden chamber (Neuro Probe, cabin John, MD) at the upper part at a density of 1.5×10^4 cells / well in 50 μl of serum-free-medium and then incubated for 12 h at 37ºC. The bottom chamber also contained standard medium with 20% feta bovine serum (FBS). The cells invaded to the lower surface of the membrane were fixed with methanol and stained with hematoxylin and eosin. To determine their motility, cells were seed into Boyden Champer on membrane filters, which not coated with Matrigel. Migration of cells was measured as described in the invasion assay. Random field was counted for invaded or motility cells under a light microscope.

Metastasis evaluation in nude mice- 100 μl aliquots of 5-8F-si-Ezrin and 5-8F-si-mock cell suspensions (1×10^4 cells) mixed with Matrigel (Collaborative Biochemical Products Inc., MA) were respectively injected into the tail vein of nude mice, 10 mice per group. The metastasis was evaluated by measuring the weight of metastasized tumor at mediastinal lymph nodes on day 30 after the injection.

Supplemental figure legends

Supplemental Fig. A, 5-8F cells were transfected respectively with pU6pro-si-mock or pU6pro-si-Ezrin. The stably-transfected cell lines were determined by G418 selection. Ezrin expression in these stable-transfected cell lines was detected by immunoblotting. B, motility and invasion of 5-8F-si-Ezrin and 5-8F-si-mock cells. These cells were subjected to analyses for motility and invasion as described in Supplemental experimental procedures. Results were statistically analyzed by one-way ANOVA with post hoc Dunnett's *test* (* $p<0.05$). C, lymph node metastasis. Mice were injected with 5-8F-si-Ezrin and 5-8F-si-mock cell suspensions

containing Matrigel through trail vein. The weight of metastatic tumors at the mediastinal lymph node was weighed on day 30 after implantation. Tow-sided Welch's *t-test* (* p<0.01).

Supplemental figures