Supplementary figure 1. Ren et al

A

GFP  EzT567D-GFP

B

<table>
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<tr>
<th></th>
<th>GFP</th>
<th>Ezrin-GFP</th>
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<td>Merlin</td>
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<td>β-actin</td>
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Supplementary figure 1.

a. Overexpression of constitutively active Ezrin (EzrinT567D-GFP) induces cell surface microvilli formation. Cells were fixed in culture plates with 4% formaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate buffer at 4°C overnight. After rinsing thoroughly in cacodylate buffer, cells were post-fixed in 1% osmium tetroxide for 1 hr at room temperature and dehydrated in a series of graded ethanol ended with 100% tetramethylsilane. The cells were mounted on SEM stubs and coated with Pd/Au. The digital images were taken on the Hitachi S-3000N scanning electron microscope operated at 7 kV.

b. Expression of merlin and other ERM family proteins is unchanged following the forced expression of Ezrin mutants in OS cells. Immunoblot detection of Ezrin, Radixin, Moesin, Merlin and phospho-ERM in K7M2 cells expressing GFP, Ezrin-GFP, EzrinT567A-GFP and EzrinT567D-GFP. β-Actin was used as the loading control.
Supplementary figure 2. Ren et al

A

<table>
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B

H & E

Cleaved Caspase 3

EzT567D-GFP

GFP

C

GFP

EzT567A-GFP

EzT567D-GFP

D

Percent survival

0 20 40 60 80 100

Days

GFP

EzrinT567D-GFP
Experimental metastasis of MG63.2/GFP and MG63.2/EzrinT567A-GFP

Percent survival

Time

p=0.0011
**Supplementary Figure 2.**

**a.** Primary tumor tissues from 5 mice bearing K7M2/EzrinT567A-GFP cells and 2 mice bearing K7M2/GFP cells were snap frozen and lysed in T-Per buffer (Pierce Biotechnology) with protease inhibitor cocktail (Roche) according to manufacturer’s recommendation. Total proteins (50 μg) from each sample were loaded on a 4-12% SDS-PAGE. Western blot for Ezrin and GFP was undertaken. Cell lysates (C) from K7M2 expressing GFP and EzrinT567A-GFP were used as controls. Ezrin fusion proteins were detected in all the tumor tissues bearing K7M2/EzrinT567A-GFP cells. Free GFP was detected in the tumor tissues bearing K7M2/GFP cells.

**b.** Tissue samples were collected from mice, 4 days after appendicular orthotopic tumor cell injection. Samples were fixed in neutral buffered 10% formalin (Fisher, Newark, DE, USA) for 24 hours, and then transferred to 80% ethanol. All tissues were embedded in paraffin, sectioned at 5 mm thickness and mounted on glass slides. Slides were deparaffinized and rehydrated as previously described (3). Slides were incubated in preheated target retrieval solution (Dako, Carpinteria, CA, USA), pH6, in a steam cooker for 20 min. Anti-cleaved Caspase 3 antibody (Cell Signaling Technology Inc., Danvers, MA, USA) was used at 1:400 dilution. The samples were counterstained with hematoxylin (Dako) for 30 s and mounted. ★ indicates primary tumors. Bar=200μm.

**c.** Lungs were collected at the end point (metastasis-associated morbidity) of experimental metastasis (tail vein) assay and were inflated by tracheobronchial injection of 1.0 ml neutral buffered 10% formalin. All the tissues were processed the same way as described above for paraffin sections and H&E staining.

**d.** Experimental metastasis assay was performed in SCID mice with injection of K7M2 cells expressing GFP and EzrinT567D-GFP. The mice with K7M2/GFP cells were euthanized due to pulmonary metastasis by day 15, while mice receiving ezrinT567D-GFP expressing cells were alive until the experiment termination (90 days).

**e.** Ezrin dysregulation impairs human osteosarcoma metastasis. MG63.2 cells expressing GFP or EzrinT567A-GFP were intravenously injected into SCID mice (10 mice per group). Mice were followed over time for the development of metastasis-associated morbidity. Necropsy confirmed widespread pulmonary metastasis in all mice at sacrifice or death.
Supplementary figure 3. Ren et al

**Maximum OCR**

- GFP vs EzT567A
- K7M2 vs K12
- MG63.2 vs MG63

**ATP dependent OCR**

- GFP vs EzT567A
- K7M2 vs K12
- MG63.2 vs MG63

Legend:
- High metastatic cells
- Low metastatic cells
Supplementary Figure 3
An XF24 Analyzer (Seahorse Biosciences) was used to measure bioenergetic function in three pairs of low and high metastatic osteosarcoma cells (MG63.2 and MG63, K7M2 and K12, K7M2/GFP and K7M2/ezrinT567A-GFP). Briefly, cells were seeded into culture plates at 25,000 cells/well 12-14h before the assay. The culture medium was replaced with unbuffered DMEM (Dulbecco's modified Eagle's medium, pH 7.4) supplemented with 4 mM L-glutamine (Gibco) one hour prior to the assay. Basal oxygen consumption was measured for 6-8 minutes before injecting a series of compounds or inhibitors to measure various indices of mitochondrial function. Oligomycin, FCCP and Rotenone were injected sequentially through ports to final concentrations of 2 μg/ml, 0.3 μM and 1 μM respectively. This allowed determination of the basal level of oxygen consumption, the amount of oxygen consumption linked to ATP production, the maximal respiration capacity and the non-mitochondrial oxygen consumption. Maximum oxygen consumption rate is plotted as percent basal oxygen consumption rate as the cells were treated with FCCP, an uncoupler of respiration. ATP linked respiration is represented as percent change of basal oxygen consumption inhibited by Oligomycin. * p<0.05.