## Supplemental files

Fig. S1 Transwell permeable support 8 µm pores, 6.5 mm inserts.

Fig. S2 EGFP-talin and pEGFP-N2 were transfected into CHO cells, and the

proteins EGFP-talin (WT) and EGFP were analyzed by western blots with

monoclonal anti-phospho-threonine and anti-phospho-serine antibodies. EGFP

exhibited very low phospho-threonine or phospho-serine levels.

Fig. S3 Calpain 2 siRNA was co-transfected into CHO cells with talin<sup>T144A+T150A</sup>.

The cells were lysed, and the protein was analyzed by western blots with an anti-

calpain2 antibody (top), or immunoprecipitated and analyzed by western blots with

a monoclonal anti-GFP antibody (bottom).

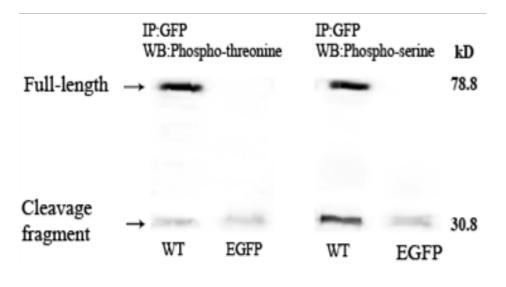
Fig. S4 No focal adhesions were observed in cells expressing pEGFP-N2. Scale bars, 20µm.

Fig. S5 Cells uniformly expressing talin<sup>T144A+T150A</sup> and talin<sup>S446A</sup>, which were obtained by flow cytometry and observed in bright field and fluorescent field, varied greatly in cell adhesion and growth after the cells were plated on fibronectin-coated MatTek dishes for 4 h. Cells expressing talin<sup>S446A</sup> adhered well and were larger and elongated, whereas most of the cells expressing talin<sup>T144A+T150A</sup> did not adhere during this period and were smaller and round. Scale bars, 20µm.

## Supplemental files



Fig. S1





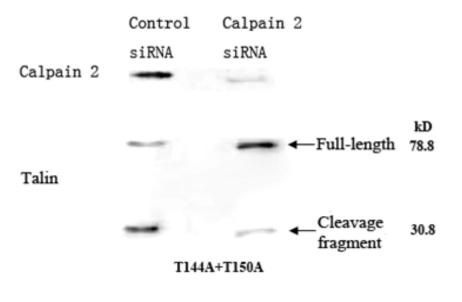


Fig. S3

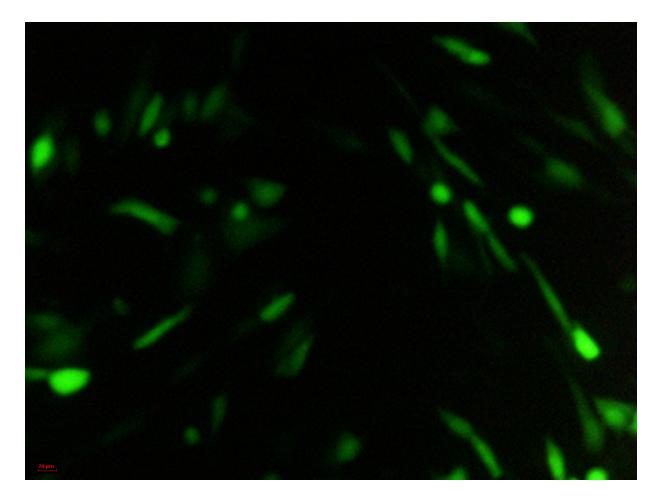


Fig. S4

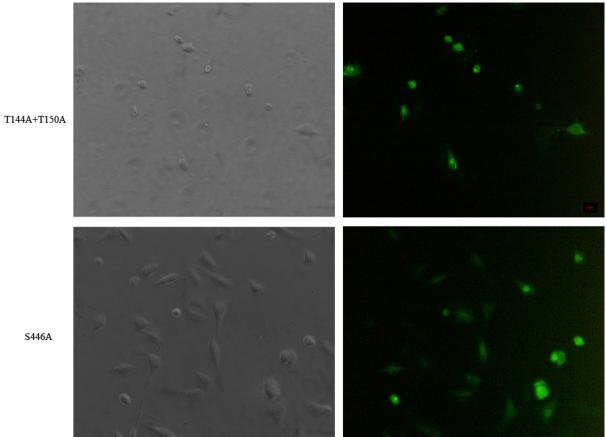


Fig. S5