



Title	Plectin is a novel regulator for apical extrusion of RasV12-transformed cells
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## **Supplementary Information**

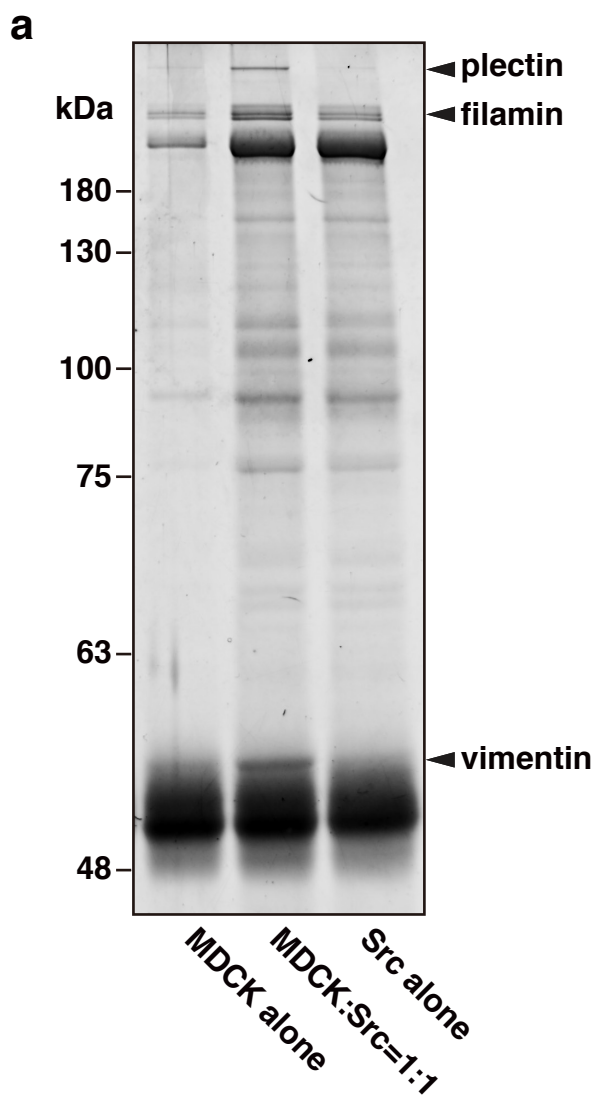
### **Plectin is a novel regulator for apical extrusion of RasV12-transformed cells**

Ailijiang Kadeer, Takeshi Maruyama, Mihoko Kajita, Tomoko Morita, Ayana

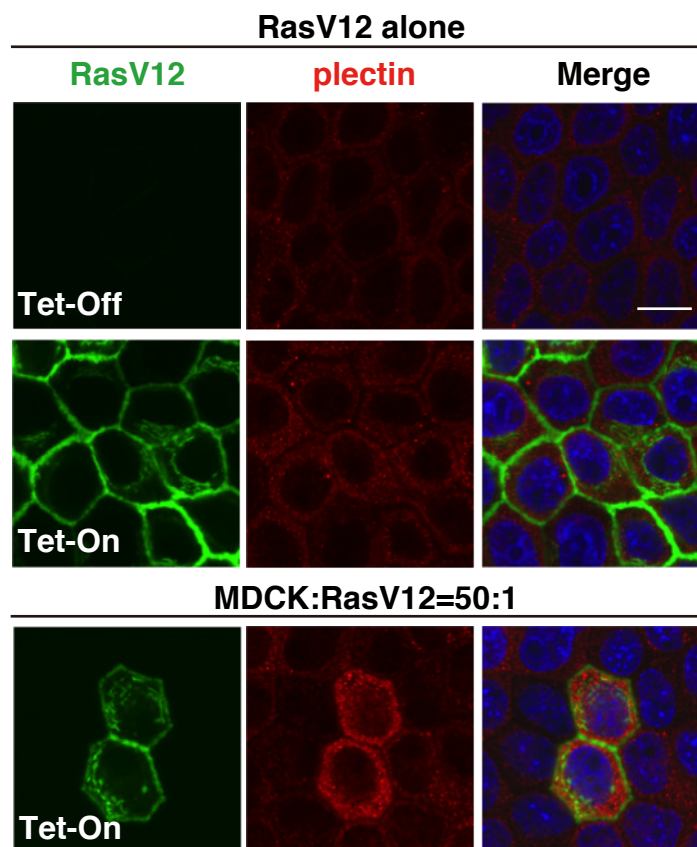
Sasaki, Atsuko Ohoka, Susumu Ishikawa, Masaya Ikegawa, Takashi Shimada,

and Yasuyuki Fujita

Supplementary Figure S1-6



**b**

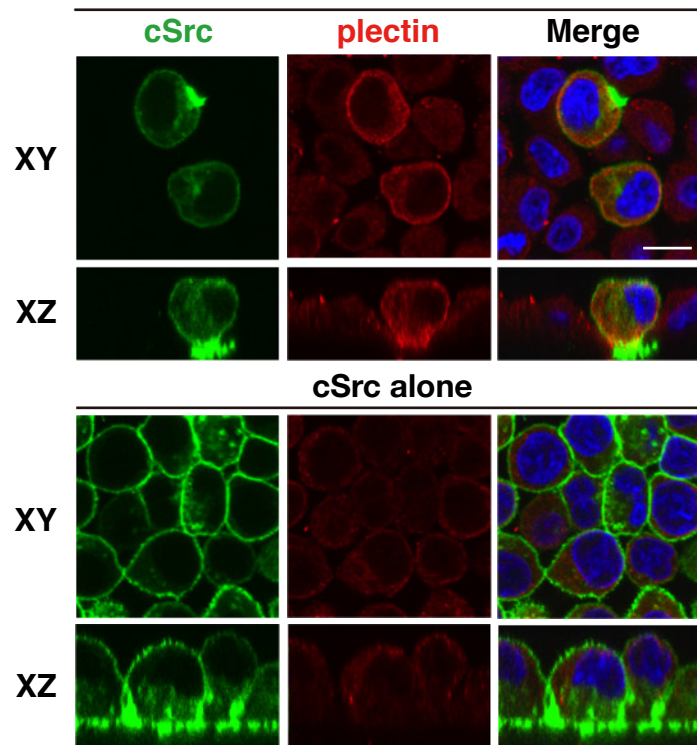


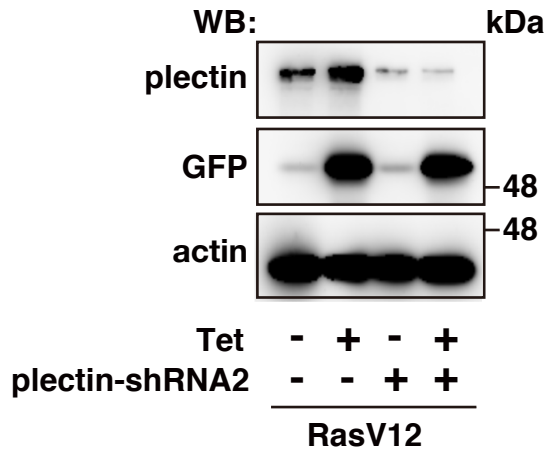
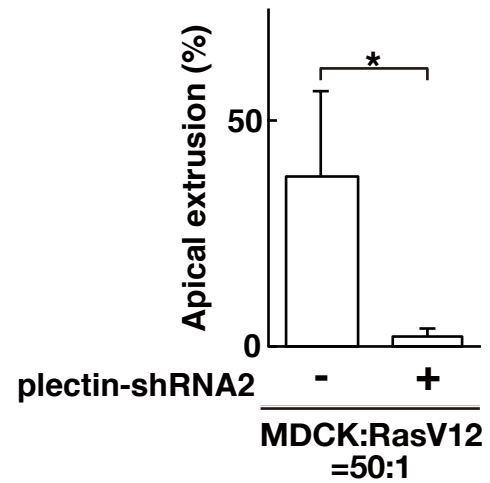
**Figure S1. Plectin is accumulated in Src-transformed cells that are surrounded by normal epithelial cells.**

(a) SYPRO ruby staining (8% SDS-PAGE) of immunoprecipitated proteins with a mixture of anti-phospho-tyrosine antibodies. Cells were cultured under three different conditions: i) normal MDCK cells alone, ii) 1:1 mix culture of normal and Src-transformed MDCK cells, and iii) Src-transformed MDCK cells alone. Cell lysates were collected after 16 h incubation with the temperature shift, followed by immunoprecipitation. The arrowheads indicate the band for plectin, filamin, and vimentin. (b) Immunofluorescence images of plectin in RasV12-transformed MDCK cells that are cultured alone or co-cultured with normal MDCK cells in the presence or absence of tetracycline. MDCK-pTR GFP-RasV12 cells were mixed with normal MDCK cells or cultured alone on collagen gels. Cells were fixed after 16 h incubation with tetracycline and stained with anti-plectin antibody (red) and Hoechst (blue). Scale bar, 10  $\mu$ m. (c) Immunofluorescence images of plectin in the mix culture of normal and Src-transformed MDCK cells. MDCK-pTR GFP-cSrcY527F cells were mixed with normal MDCK cells or cultured alone on collagen gels. Cells were fixed after 16 h incubation with tetracycline and stained with anti-plectin antibody (red) and Hoechst (blue). Scale bar, 10  $\mu$ m.

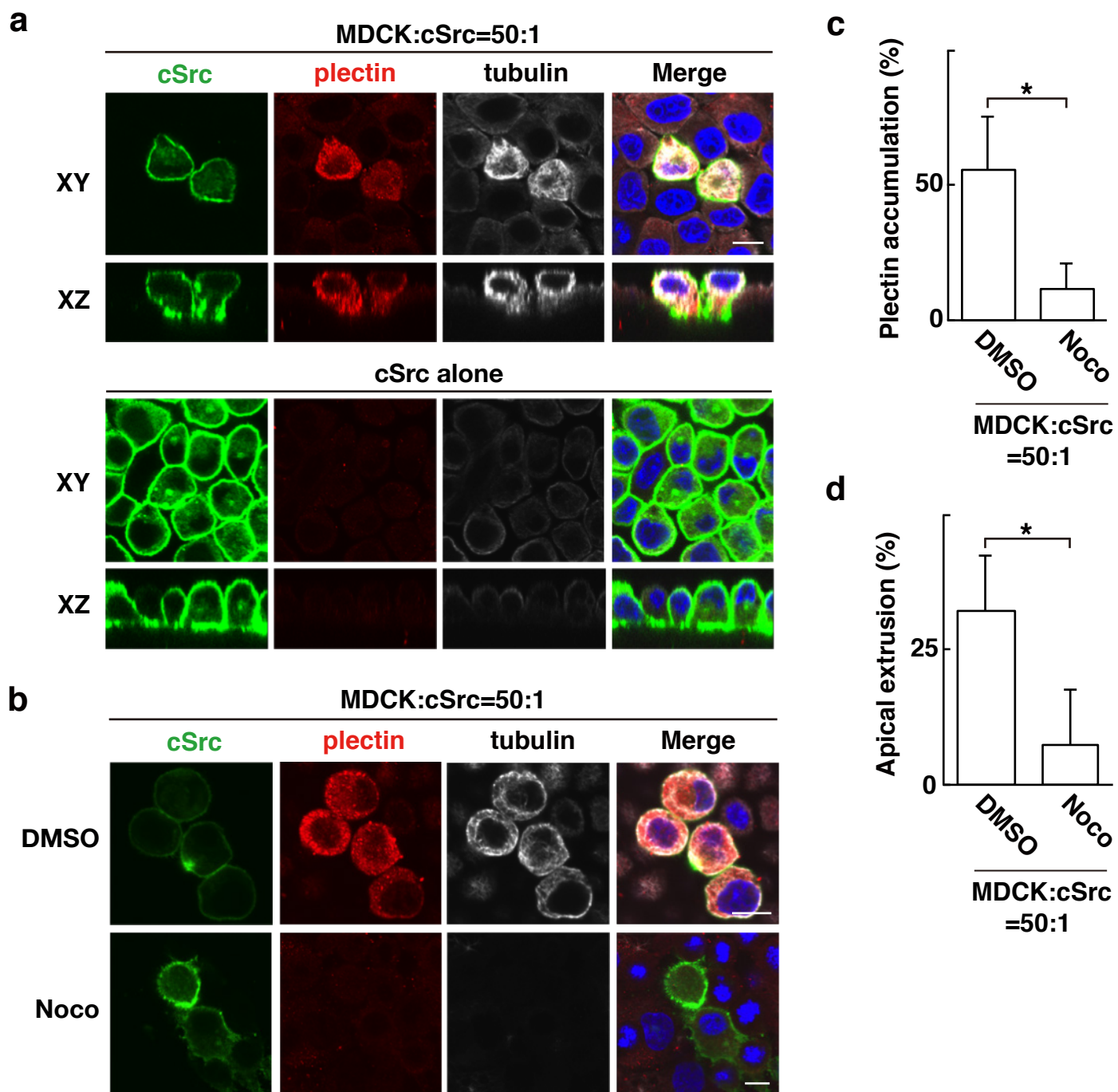
**c**

MDCK:cSrc=50:1

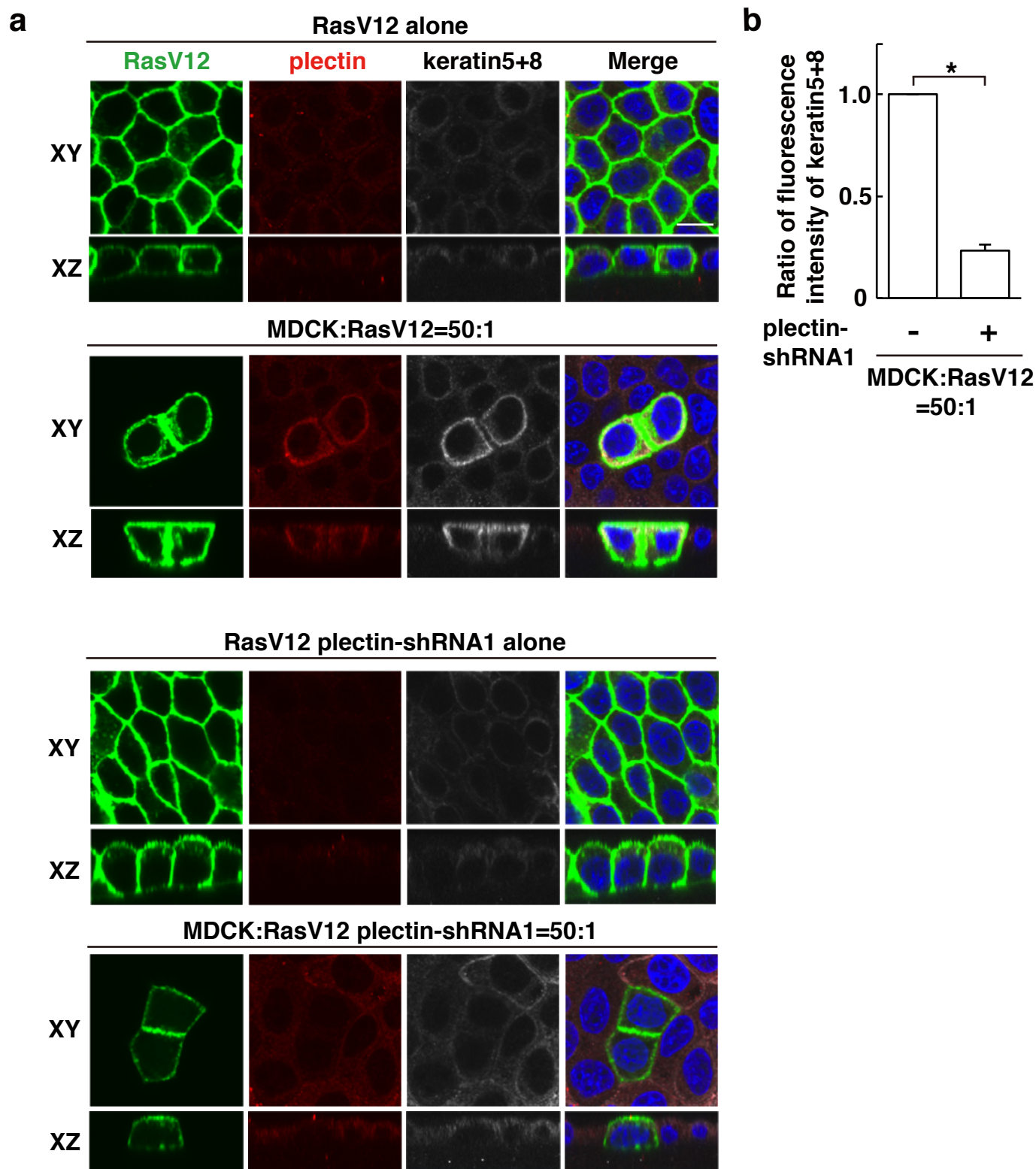


**a****b**

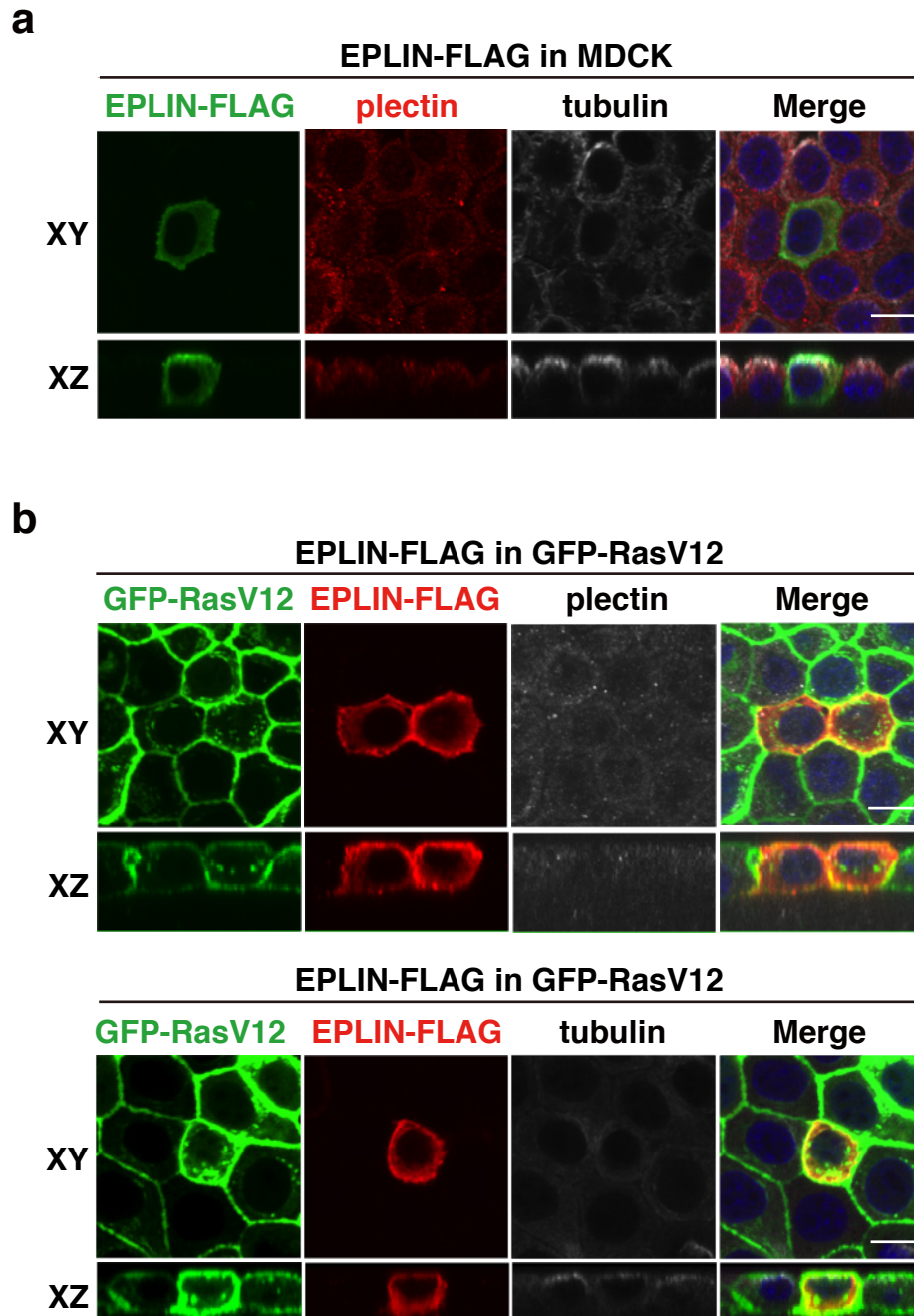
**Figure S2. Plectin plays a positive role in apical extrusion of RasV12-transformed cells.** (a) Establishment of MDCK-pTR GFP-RasV12 cells stably expressing plectin-shRNA2. Expression of GFP-RasV12 was induced with tetracycline treatment, followed by western blotting with the indicated antibodies. (b) Quantification of the effect of plectin-knockdown on apical extrusion of RasV12 cells. Data are mean  $\pm$  SD from seven and three independent experiments. \* $P < 0.05$ ;  $n=100-290$  cells for each experimental condition.



**Figure S3. Microtubules and plectin co-regulate their accumulation and promote apical extrusion of Src-transformed cells.** (a) Accumulation of plectin and tubulin in Src-transformed cells surrounded by normal epithelial cells. MDCK-pTR GFP-cSrcY527F cells were mixed with normal MDCK cells or cultured alone on collagen gels. Cells were fixed after 16 h incubation with tetracycline and stained with anti-plectin (red) and anti-tubulin (white) antibodies and Hoechst (blue). (b,c) Effect of nocodazole on accumulation of plectin and tubulin. (c) Data are mean  $\pm$  SD from three independent experiments. \* $P < 0.05$ ;  $n = 150-180$  cells for each experimental condition. (a,b) Scale bars, 10  $\mu\text{m}$ . (d) Effect of nocodazole on apical extrusion of Src-transformed cells. Data are mean  $\pm$  SD from three independent experiments. \* $P < 0.05$ ;  $n = 60-120$  cells for each experimental condition.



**Figure S4. Plectin regulates accumulation of keratin intermediate filaments in RasV12-transformed cells surrounded by normal epithelial cells.** (a) Accumulation of plectin and keratin5+8 in RasV12-transformed cells surrounded by normal epithelial cells. MDCK-pTR GFP-RasV12 cells or MDCK-pTR GFP-RasV12 plectin-shRNA1 cells were mixed with normal MDCK cells or cultured alone on collagen gels. Cells were fixed after 16 h incubation with tetracycline and stained with anti-plectin (red) and anti-keratin5+8 (white) antibodies and Hoechst (blue). (b) Effect of plectin-knockdown on accumulation of keratin5+8. Data are mean  $\pm$  SD from three independent experiments. \*P<0.001; n=30 cells for each experimental condition. Values are expressed as a ratio relative to plectin-shRNA1 (-).



**Figure S5. Overexpression of EPLIN alone is not sufficient to cause accumulation of plectin or microtubules.** EPLIN-FLAG was transiently expressed within a monolayer of MDCK cells (**a**) or MDCK-pTR GFP-RasV12 cells (**b**) in a mosaic manner. Cells were fixed after 24 h of transfection and stained with anti-FLAG, anti-plectin, and/or anti-tubulin antibodies and Hoechst. Scale bars, 10  $\mu$ m.

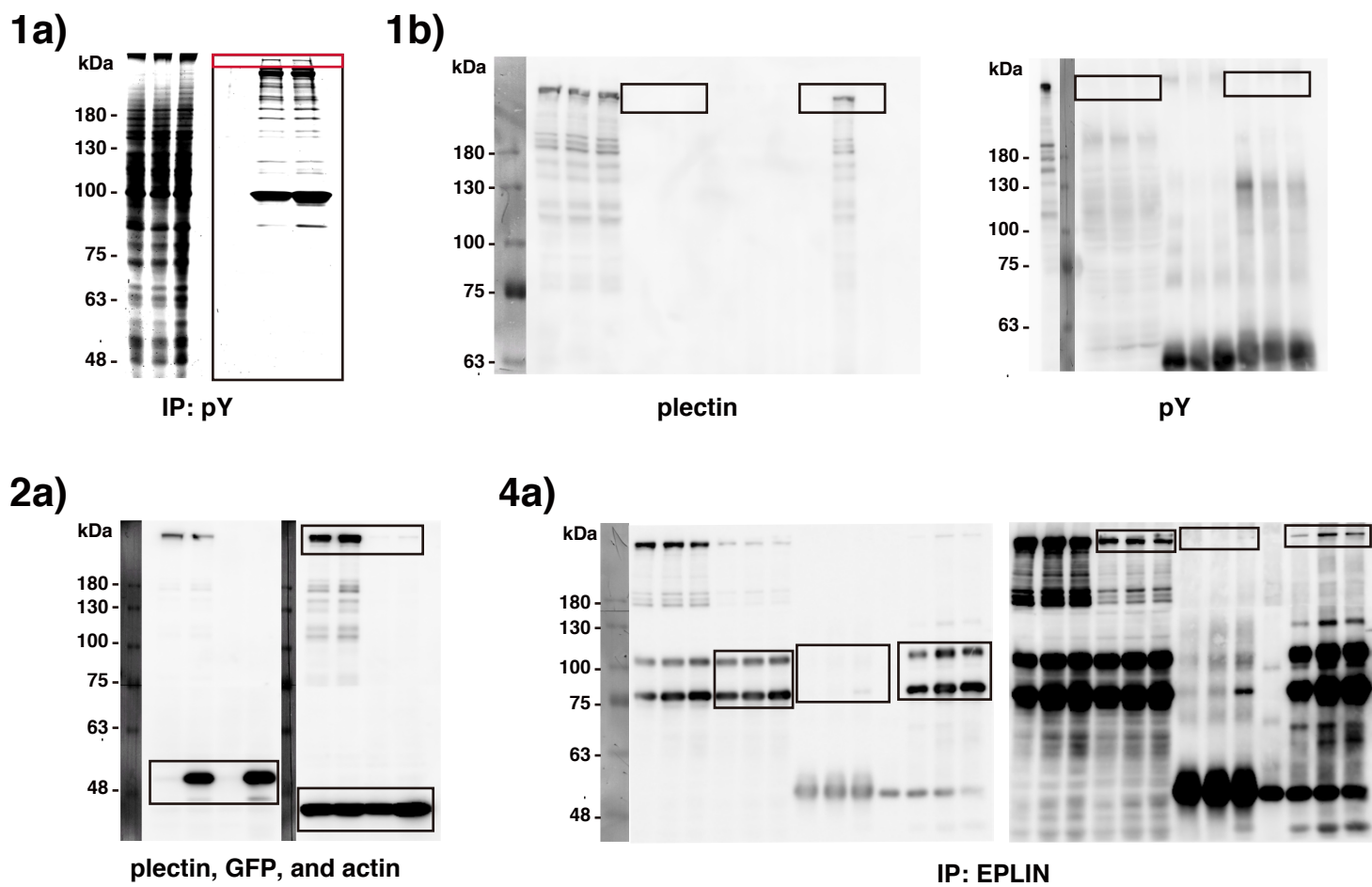


Figure S6. Full-length gels and blots