

Supplementary Information

**The paxillin-plectin-EPLIN complex promotes apical elimination
of RasV12-transformed cells by modulating HDAC6-regulated
tubulin acetylation**

Nobuhiro Kasai, Ailijiang Kadeer, Mihoko Kajita, Sayaka Saitoh, Susumu

Ishikawa, Takeshi Maruyama and Yasuyuki Fujita

Supplementary Figure S1-3

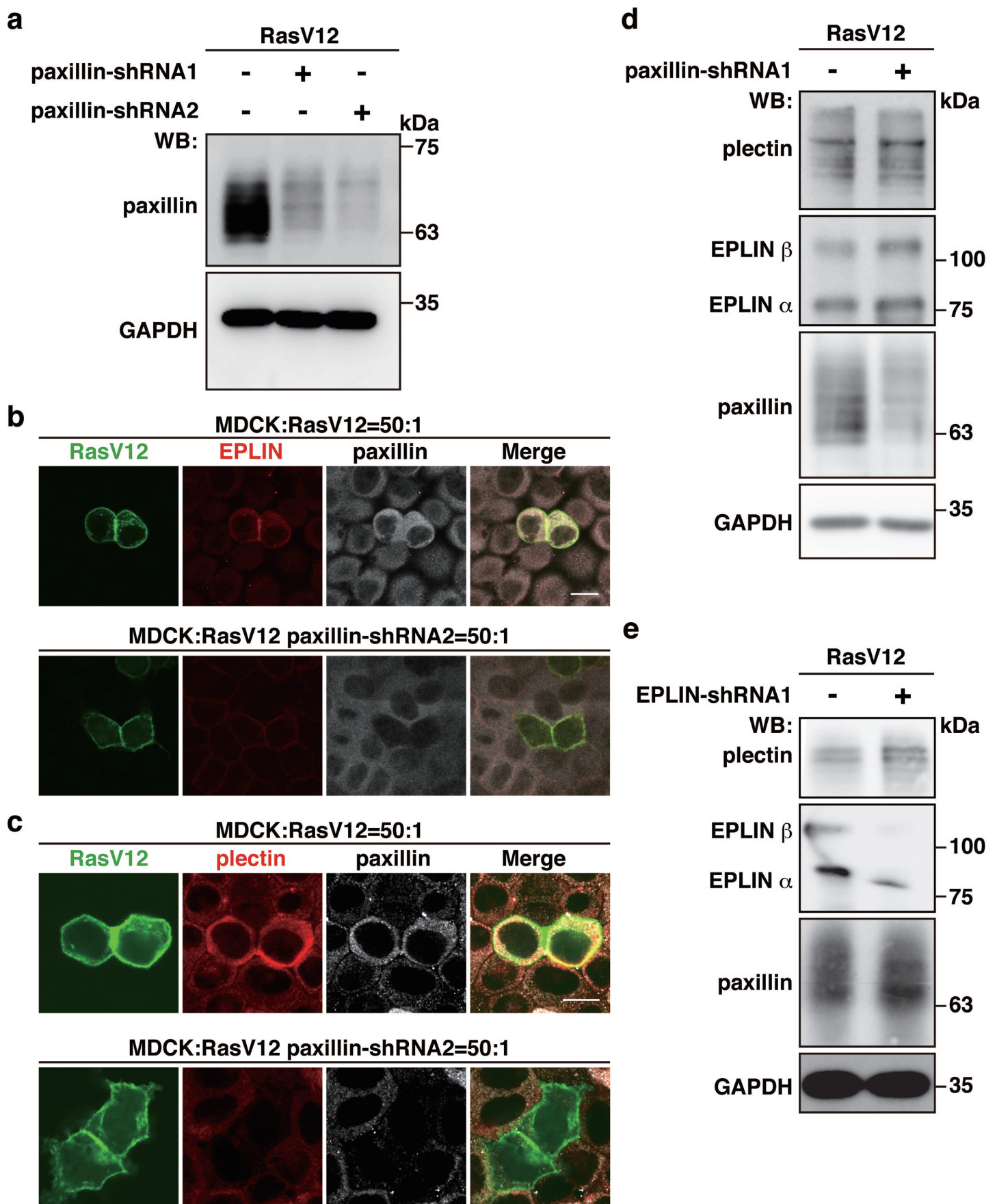


Figure S1. Paxillin regulates accumulation of EPLIN and plectin in RasV12-transformed cells that are surrounded by normal epithelial cells. (a) Establishment of MDCK-pTR GFP-RasV12 cells stably expressing paxillin-shRNA1 or -shRNA2. Expression of GFP-RasV12 was induced with tetracycline treatment, followed by western blotting with the indicated antibodies. (b,c) Effect of paxillin-knockdown on EPLIN accumulation (b) or plectin accumulation (c). MDCK-pTR GFP-RasV12 cells or MDCK-pTR GFP-RasV12 paxillin-shRNA2 cells were mixed with normal MDCK cells on collagen gels. Cells were fixed after 16 h incubation with tetracycline and stained with anti-EPLIN (b) or anti-plectin (c) (red), and anti-paxillin (grey) antibodies and Hoechst (blue). Scale bars, 10 μ m. (d, e) Effect of paxillin- or EPLIN-knockdown on expression of paxillin, plectin and EPLIN in MDCK-pTR GFP-RasV12 cells cultured alone. Cell lysates of the parental or knockdown cells were analysed by western blotting with the indicated antibodies.

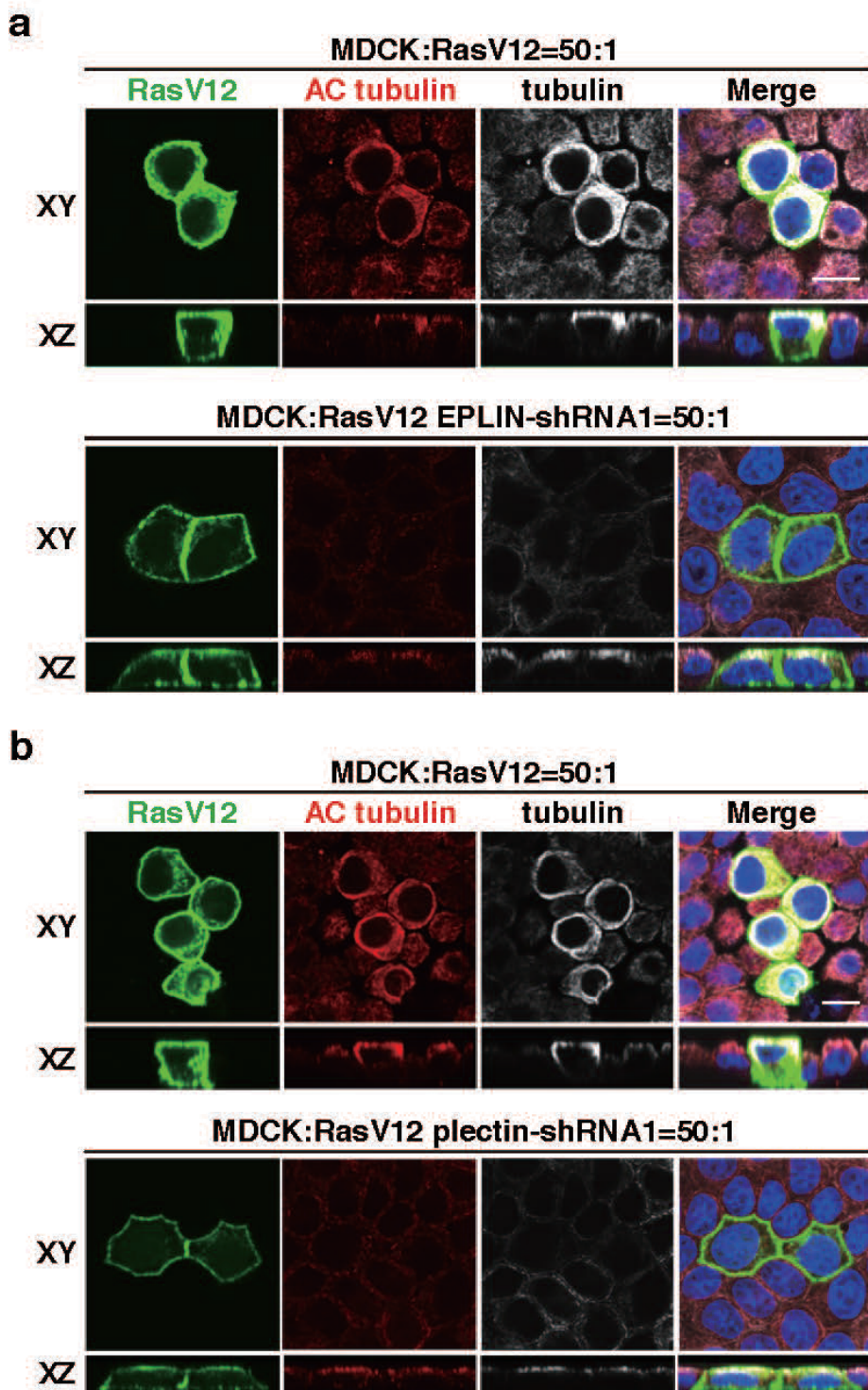
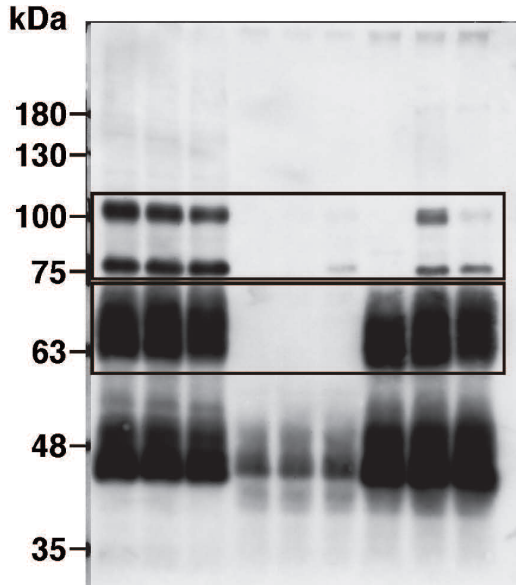
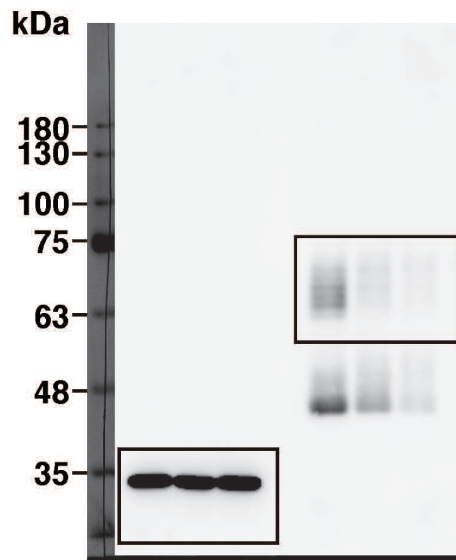
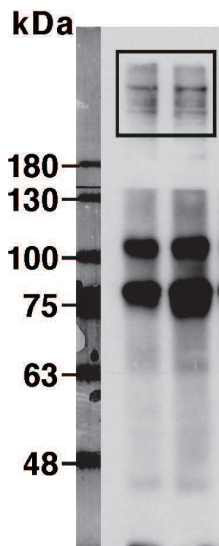
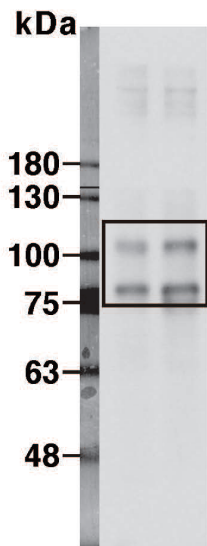
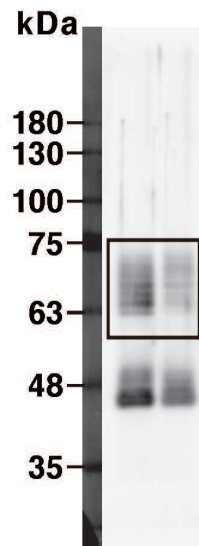
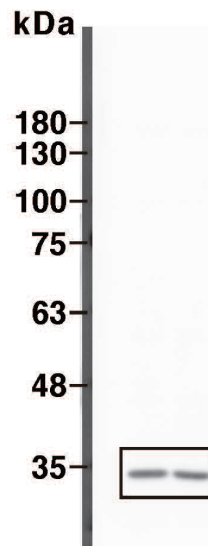
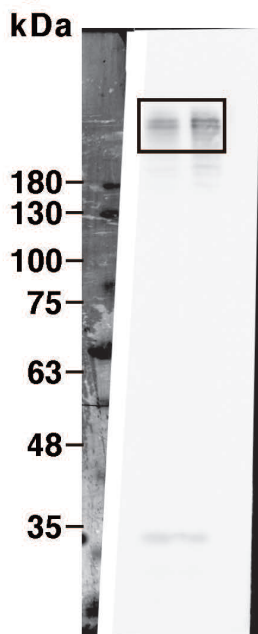
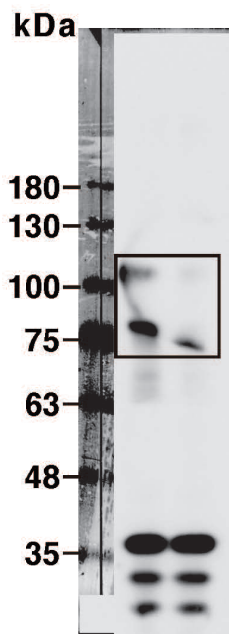
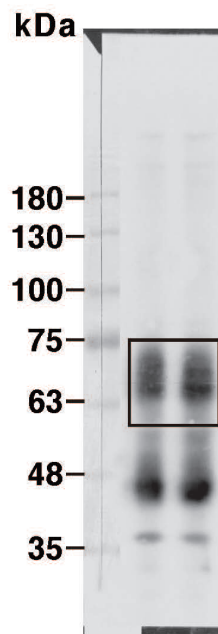
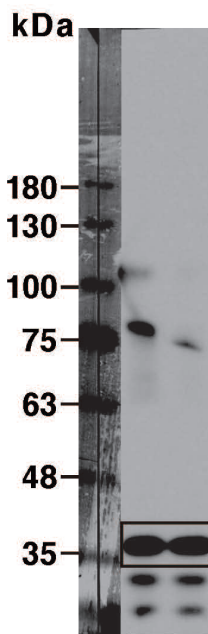


Figure S2. EPLIN and plectin regulate accumulation of acetylated α -tubulin in RasV12-transformed cells surrounded by normal epithelial cells. (a,b) Effect of EPLIN-knockdown (a) or plectin-knockdown (b) on accumulation of acetylated tubulin. MDCK-pTR GFP-RasV12 cells, MDCK-pTR GFP-RasV12 EPLIN-shRNA1 cells or MDCK-pTR GFP-RasV12 plectin-shRNA1 cells were mixed with normal MDCK cells on collagen gels. Cells were fixed after 16 h incubation with tetracycline and stained with anti-acetylated α -tubulin (red) and anti- α -tubulin (grey) antibodies and Hoechst (blue). Scale bars, 10 μ m.

1a)**EPLIN, paxillin****S1a)****GAPDH, paxillin****S1d)****plectin****EPLIN****paxillin****GAPDH****S1e)****plectin****EPLIN****paxillin****GAPDH****Figure S3. Full-length gels and blots.**