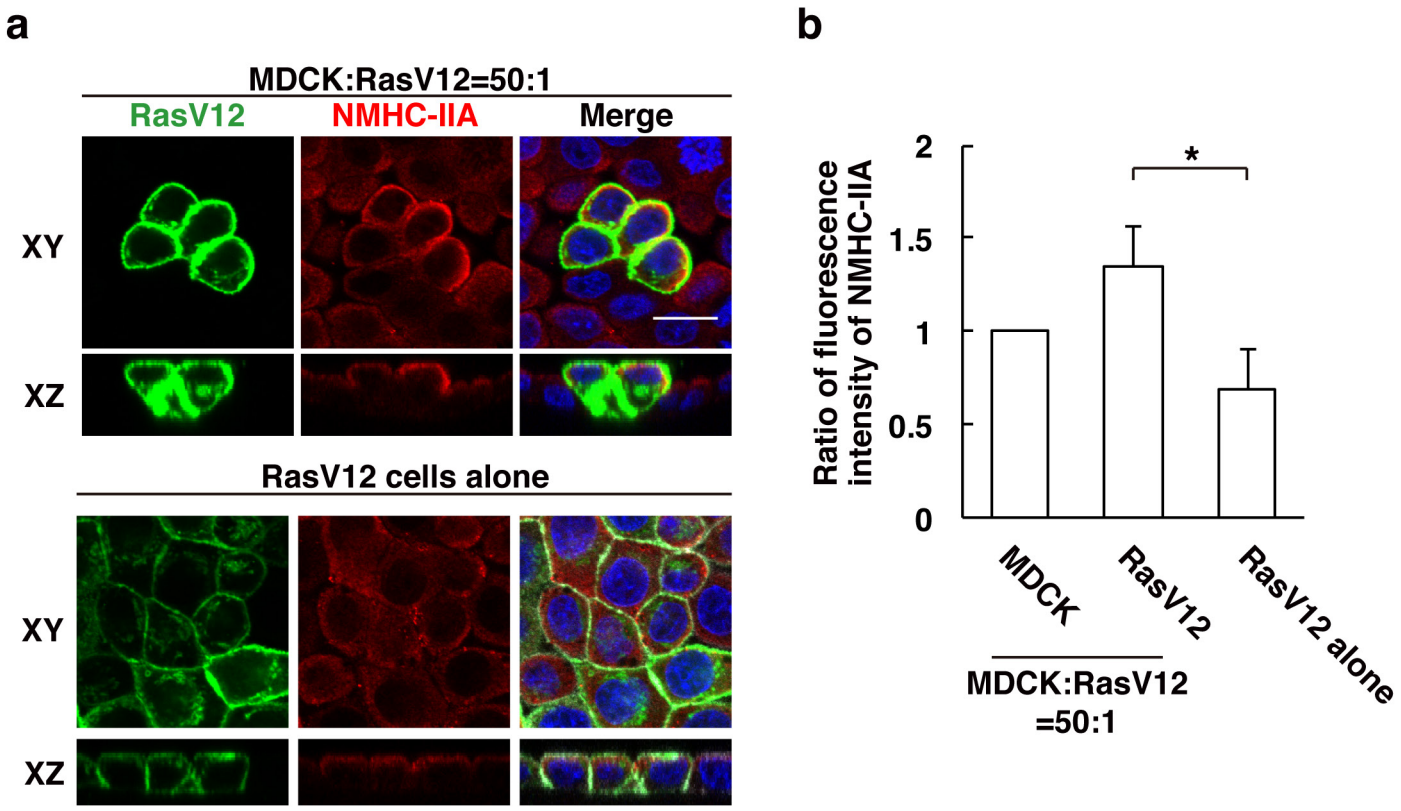
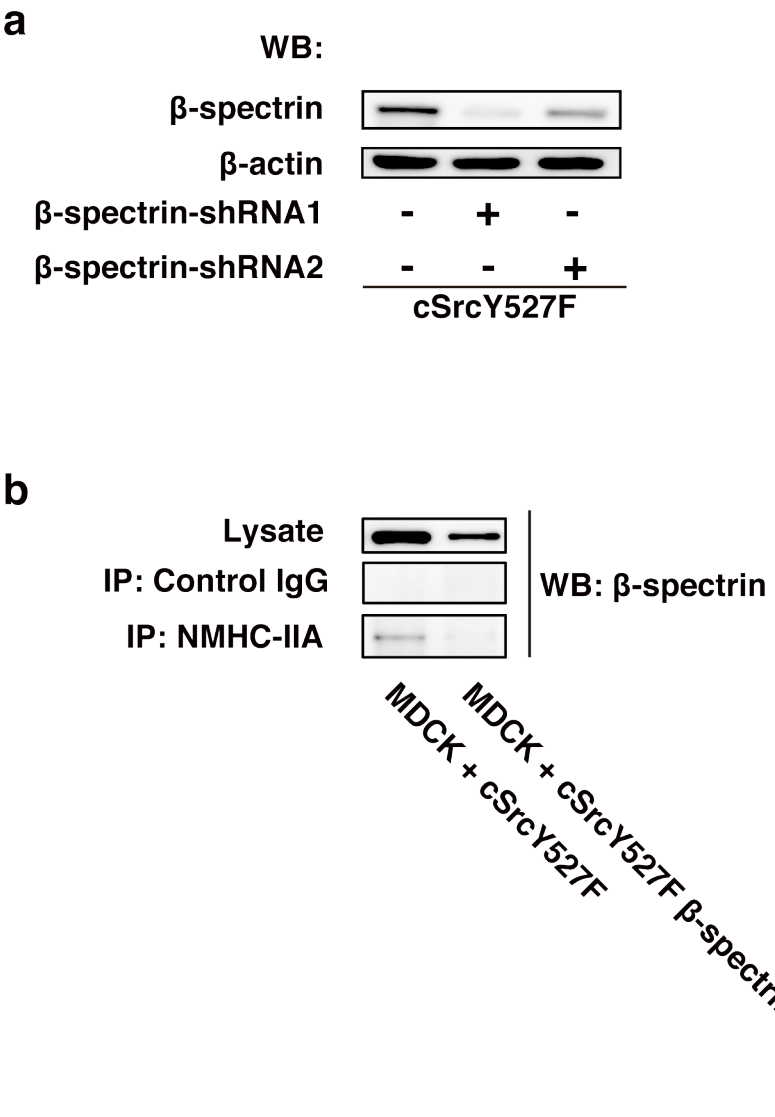
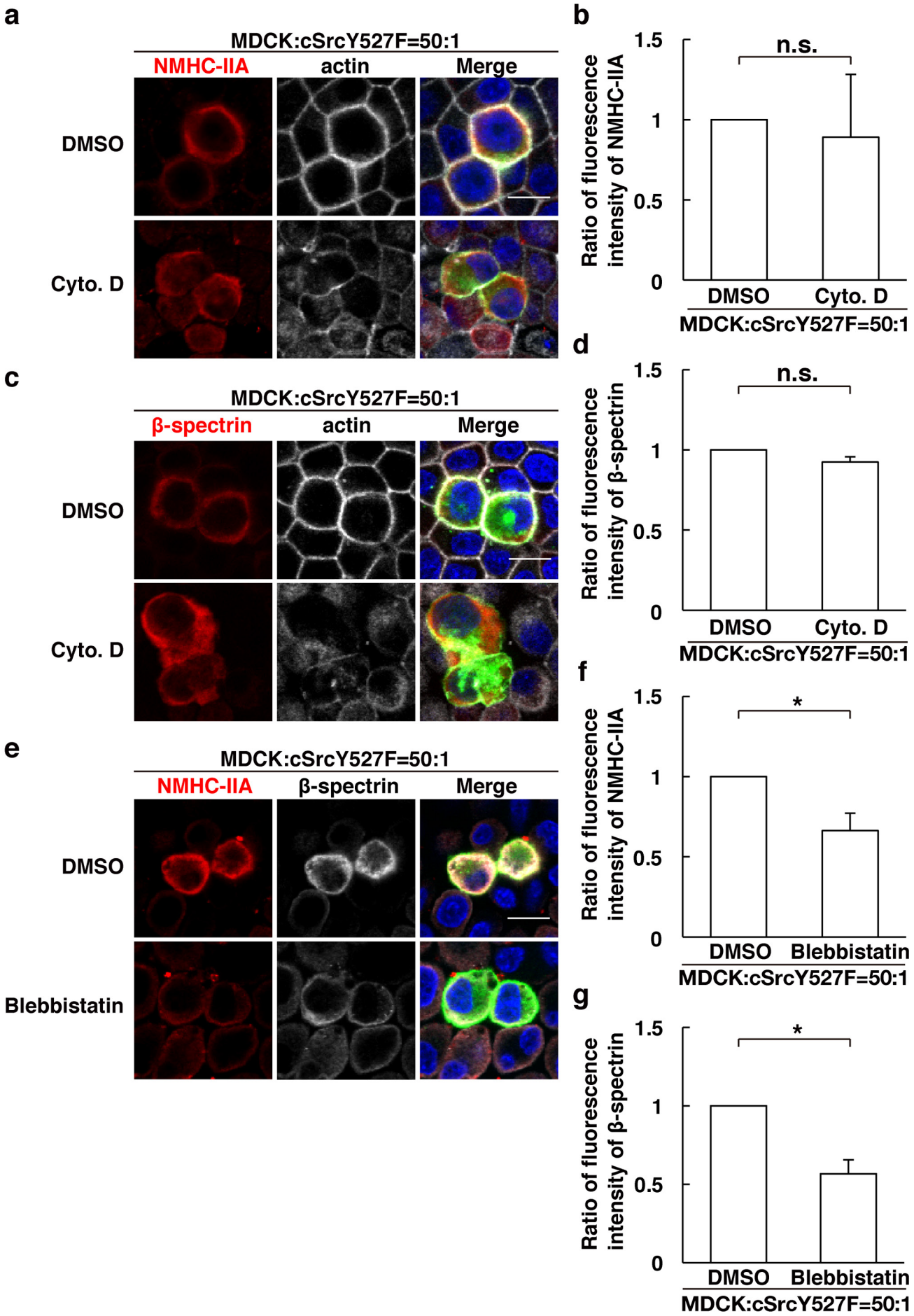


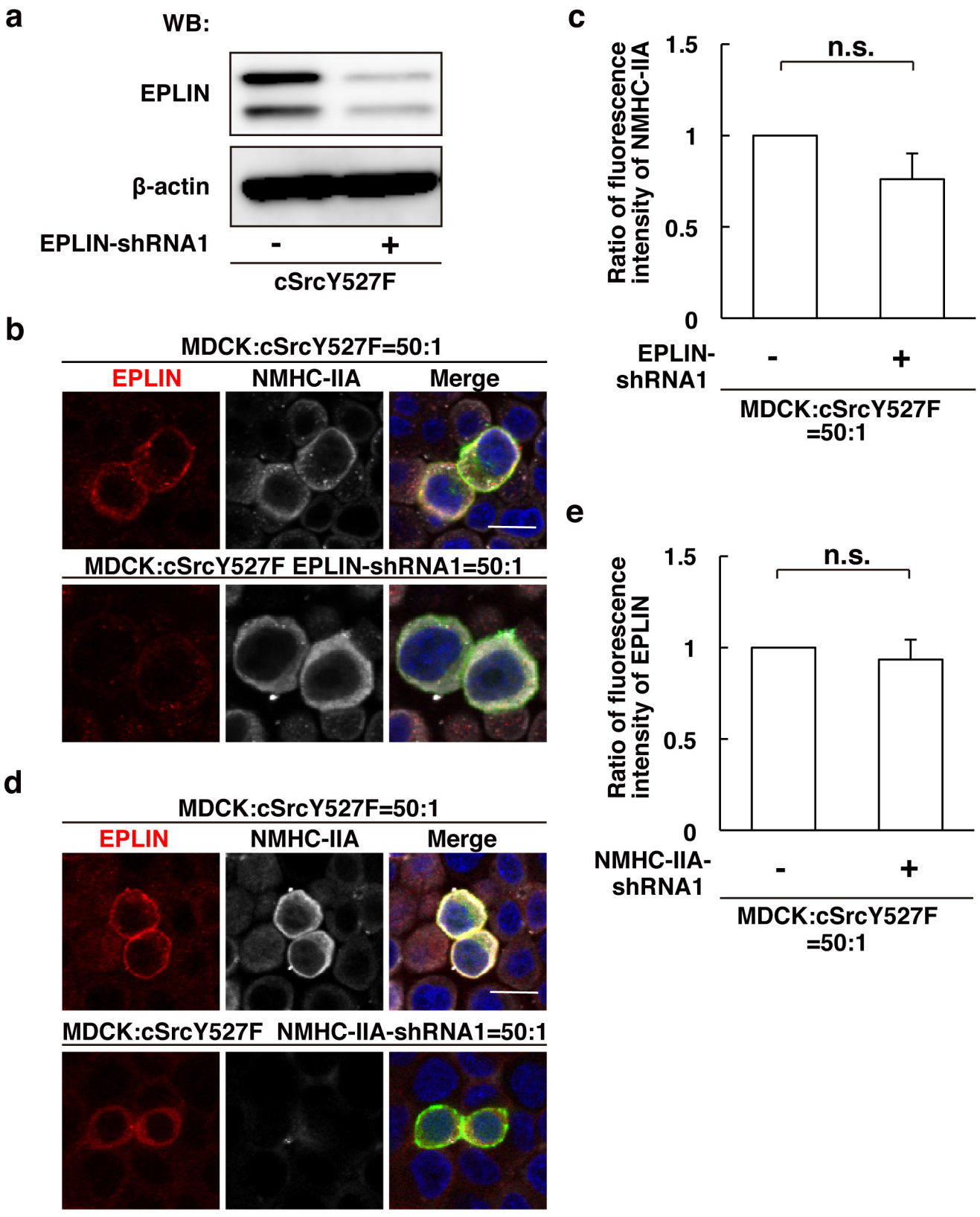
SUPPLEMENTARY FIGURE 1 Myosin-II accumulates in RasV12-transformed cells surrounded by normal cells. (a) Immunofluorescence images of MDCK-pTR GFP-RasV12 cells mixed with normal MDCK cells or cultured alone. Cells were incubated with tetracycline for 16 h and stained with anti-NMHC-IIA antibody (red) and Hoechst (blue). Scale bar, 10 µm. (b) Quantification of the immunofluorescence intensity of NMHC-IIA. Data are mean ± SD from three independent experiments. *P<0.05 (Student t-test). More than 30 cells were analyzed in each experimental condition.



SUPPLEMENTARY FIGURE 2 β-Spectrin binds to NMHC-IIA in Src-transformed cells that are co-cultured with normal cells. (a) Establishment of MDCK-pTR GFP-cSrcY527F cells stably expressing β-spectrin -shRNA1 or -shRNA2. (b) Effect of knockdown of β-spectrin in Src-transformed cells on co-immunoprecipitation between β-spectrin and NMHC-IIA. Cells were mix-cultured under two different conditions: i) normal MDCK and MDCK-pTR GFP-cSrcY527F cells or ii) normal MDCK and MDCK-pTR GFP-cSrcY527F β-spectrin-shRNA1 cells. The mixture of cells was incubated with tetracycline for 16 h, and the cell lysates were subjected to immunoprecipitation with control IgG or anti-NMHC-IIA antibody, followed by western blotting with anti-β-spectrin antibody.



SUPPLEMENTARY FIGURE 3 Effect of cytochalasin D or blebbistatin on accumulation of NMHC-IIA or β-spectrin in Src-transformed cells surrounded by normal epithelial cells. (a, c, and e) Immunofluorescence images of MDCK-pTR GFP-cSrcY527F cells co-cultured with normal MDCK cells in the absence or presence of cytochalasin D (a, c) or blebbistatin (e). Cells were incubated with tetracycline for 16 h and stained with anti-NMHC-IIA (a, e) and/or anti-β-spectrin (c, e) antibodies, Alexa-Fluor-647-conjugated phalloidin (a, c), and Hoechst. Scale bars, 10 μm. (b, d, f, g) Quantification of the immunofluorescence intensity of NMHC-IIA (b, f) or β-spectrin (d, g). Data are mean ± SD from three independent experiments. More than 30 cells were analyzed in each experimental condition. n.s.: not significant. *P<0.05 (Student t-test).



SUPPLEMENTARY FIGURE 4 Accumulation of myosin-II and EPLIN is likely to be mediated by independent pathways. (a) Establishment of MDCK-pTR GFP-cSrcY527F cells stably expressing EPLIN-shRNA1. Cell lysates were analyzed by western blotting with the indicated antibodies. (b) Immunofluorescence images of MDCK-pTR GFP-cSrcY527F or MDCK-pTR GFP-cSrcY527F EPLIN-shRNA1 cells mixed with normal MDCK cells. Cells were incubated with tetracycline for 16 h and stained with anti-EPLIN (red) and anti-NMHC-IIA (gray) antibodies and Hoechst (blue). (c) Quantification of the immunofluorescence intensity of NMHC-IIA. Data are mean ± SD from three independent experiments. n.s.: not significant. More than 30 cells were analyzed in each experimental condition. (d) Immunofluorescence images of MDCK-pTR GFP-cSrcY527F or MDCK-pTR GFP-cSrcY527F NMHC-IIA-shRNA1 cells mixed with normal MDCK cells. Cells were incubated with tetracycline for 16 h and stained with anti-EPLIN (red) and anti-NMHC-IIA (gray) antibodies and Hoechst (blue). (e) Quantification of the immunofluorescence intensity of EPLIN. Data are mean ± SD from three independent experiments. n.s.: not significant. More than 30 cells were analyzed in each experimental condition.