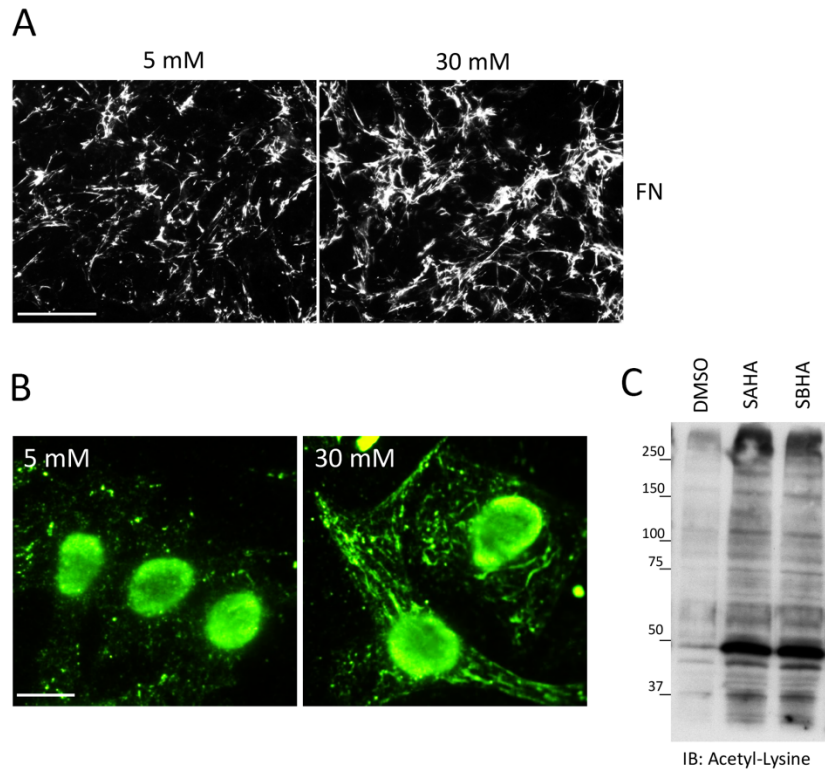


## **Supplemental Materials**

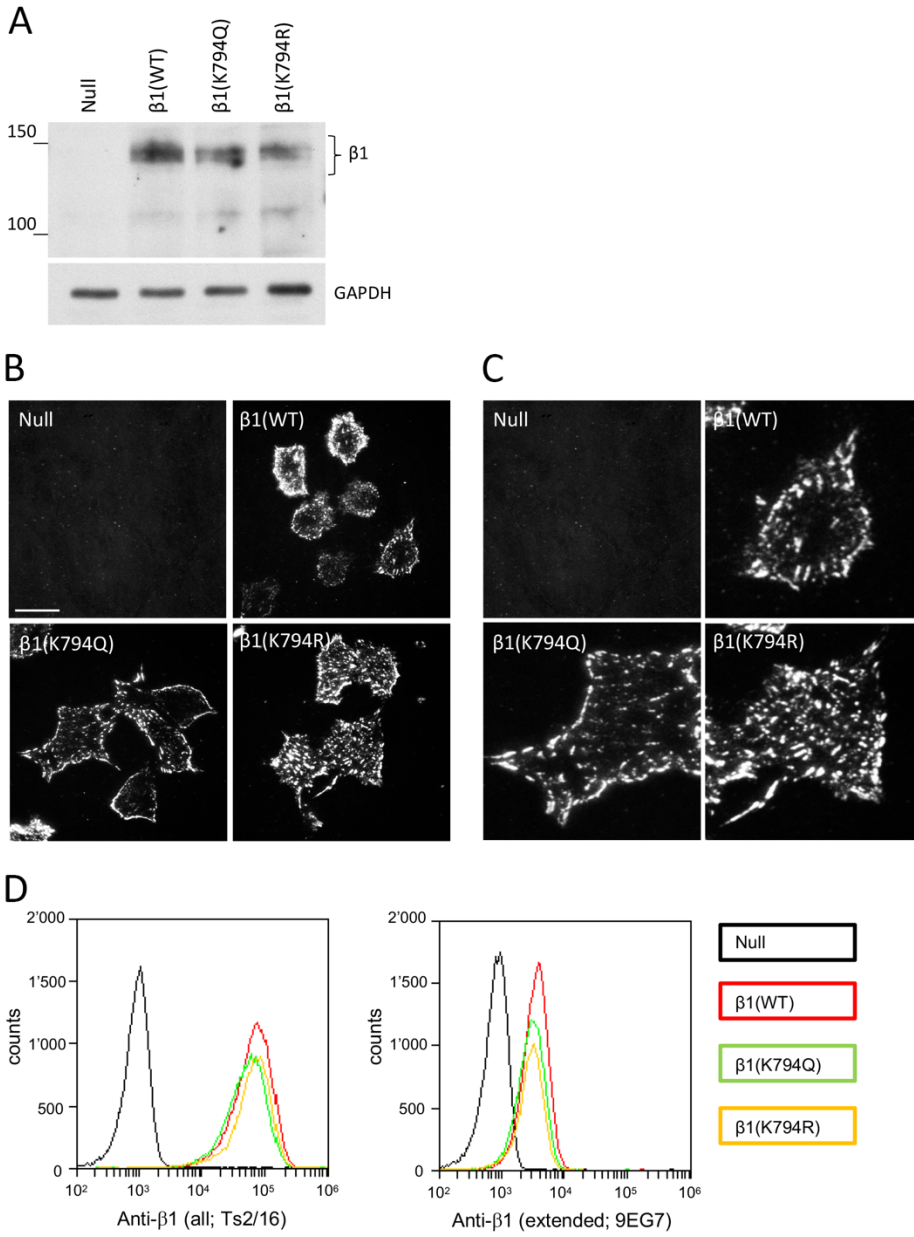
*Cells*

Stimulation of fibronectin matrix assembly by lysine acetylation

Maria E. Vega, et al. 2020

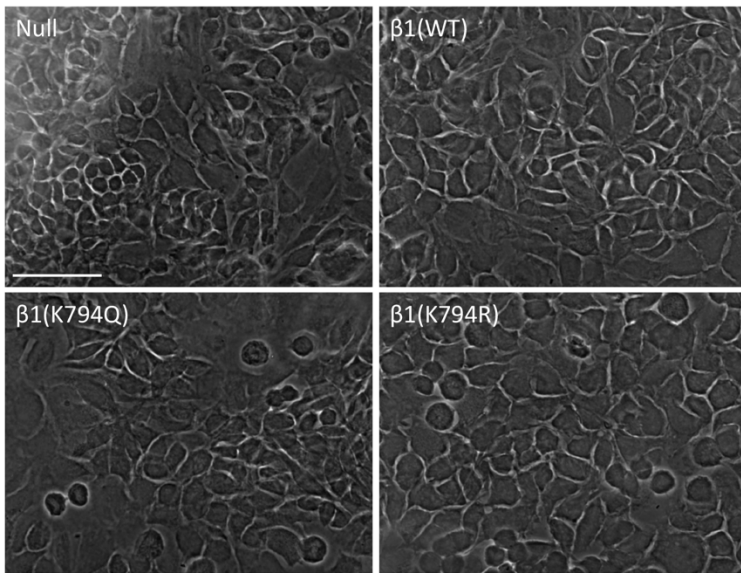


**Supplemental Figure 1.** Increased FN matrix and acetylation in mesangial cells grown in elevated glucose or treated with deacetylase inhibitor. (A) Mesangial cells were grown in 5 mM or 30 mM glucose with 10  $\mu\text{g}/\text{mL}$  human plasma FN for 48 h and stained with anti-FN monoclonal antibody HFN7.1. Scale bar = 50  $\mu\text{m}$ . (B) Mesangial cells grown in 5 mM or 30 mM glucose for 24 h were seeded on 10  $\mu\text{g}/\text{mL}$  plasma FN coated glass-bottom dish for 2 h before staining with anti-acetyl-lysine antibody and imaging by TIRF microscopy. Scale bar = 10  $\mu\text{m}$ . (C) Mesangial cells grown in 30 mM glucose were treated with 5  $\mu\text{M}$  SAHA or SBHA or vehicle (DMSO) for 24 h before lysis in DOC buffer. Equal amounts of protein in DOC-soluble fractions were immunoblotted with anti-acetyl-lysine antibodies.

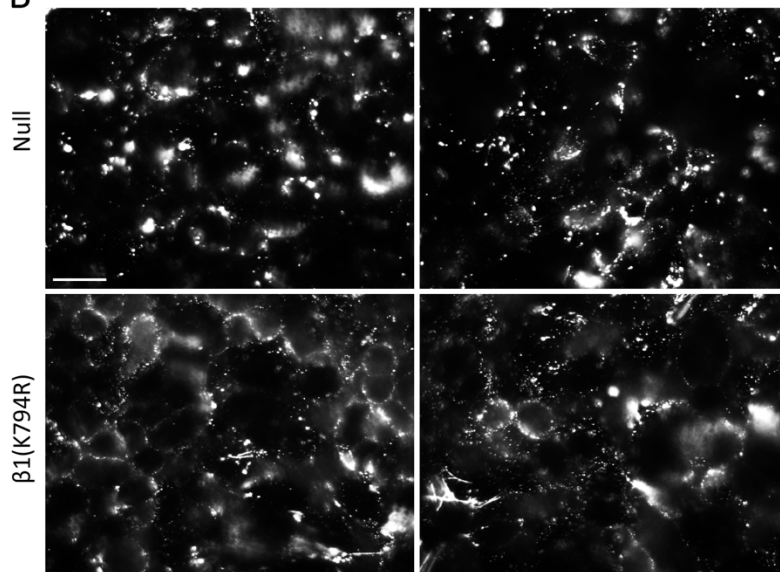


**Supplemental Figure 2.** Integrin  $\beta 1$  mimetic expression in GD25 integrin  $\beta 1$  null fibroblasts. (A) Whole cell lysates from GD25  $\beta 1$ (null),  $\beta 1$ (WT) and mutants  $\beta 1$ (K794Q) and  $\beta 1$ (K794R) were separated in a 6% polyacrylamide-SDS gel and immunoblotted with antibodies against integrin  $\beta 1$  (top). The GAPDH control immunoblot is shown below. (B) TIRF microscopy of GFP-tagged integrins shows localization to focal adhesions. Scale bar = 10  $\mu$ m. (C) Enlarged images of individual cells from panel B are shown. (D) Cell surface expression analysis by flow cytometry of  $\beta 1$  integrins in stably transfected GD25 cells labelled with mouse anti-human  $\beta 1$ -integrin mAb Ts2/16, recognizing all surface expressed integrins (left panel), or with rat anti- $\beta 1$ -integrin mAb 9EG7 recognizing extended or activated forms (right panel). Curves represent total cell counts,  $\beta 1$ (null) in black,  $\beta 1$ (WT) in red,  $\beta 1$ (K794Q) in green and  $\beta 1$ (K794R) in yellow.

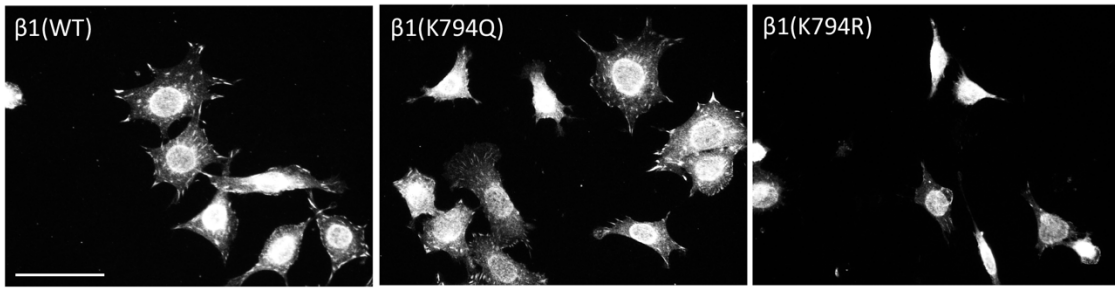
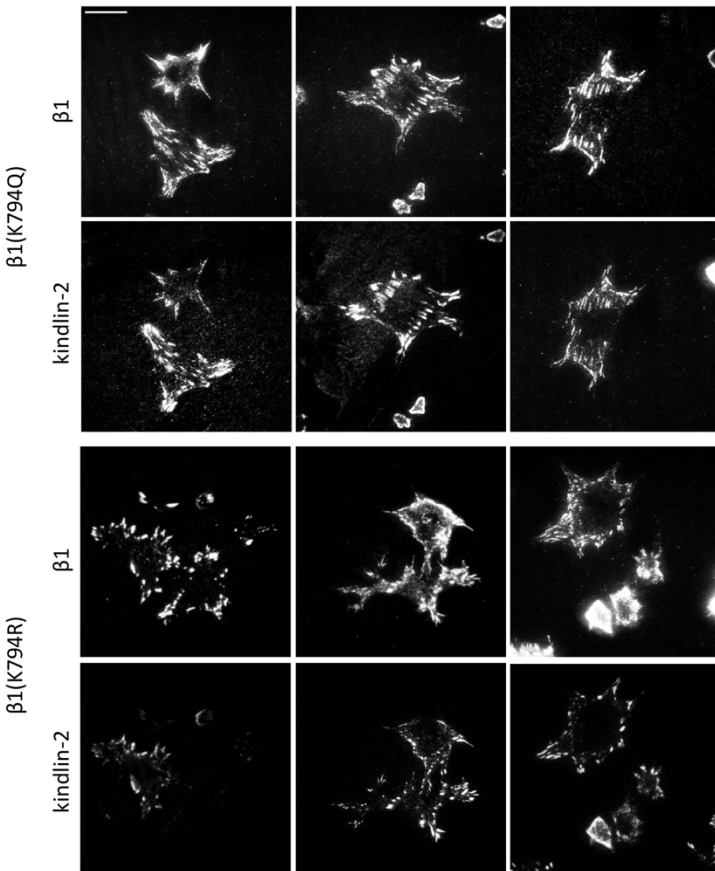
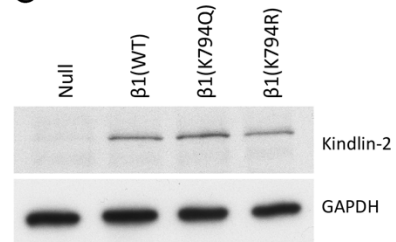
A



B



**Supplemental Figure 3.** Integrin  $\beta 1$ (null) and  $\beta 1$ (K794R) non-acetylated mimetic cells lack fibrillar FN matrix. (A) Phase contrast images of GD25 cell fields from Figure 2C show that cells are at equivalent densities. Scale bar = 50  $\mu$ m (B) Higher magnification images of GD25  $\beta 1$ (null) (top) and  $\beta 1$ (K794R) (bottom) cells stained with anti-FN antibodies (as in Figure 2C) show FN aggregates and the lack of FN fibrils. Scale bar = 10  $\mu$ m.

**A****B****C**

**Supplemental Figure 4.** Kindlin-2 localization at focal adhesions. (A) GD25 cells seeded on a FN-coated surface were allowed to spread for 4 h and then fixed and stained with anti-kindlin-2 antibodies. Kindlin-2 localization was visualized by epifluorescence microscopy. Scale bar = 50  $\mu$ m. (B) TIRF microscopy was used to visualize immunostained kindlin-2 and GFP-tagged  $\beta$ 1 integrin in  $\beta$ 1(K794Q) (top) and  $\beta$ 1(K794R) (bottom) cells on a FN-coated surface. Scale bar = 10  $\mu$ m. (C) Whole cell lysates from GD25  $\beta$ 1(null),  $\beta$ 1(WT) and mutants  $\beta$ 1(K794Q) and  $\beta$ 1(K794R) were immunoblotted with antibodies against kindlin-2 (top) and GAPDH (bottom). The kindlin-2 protein level was lower in GD25  $\beta$ 1(null) cells compared to other GD25  $\beta$ 1 transfected cell lines.