Choi et al., http://www.jcb.org/cgi/content/full/jcb.201302131/DC1

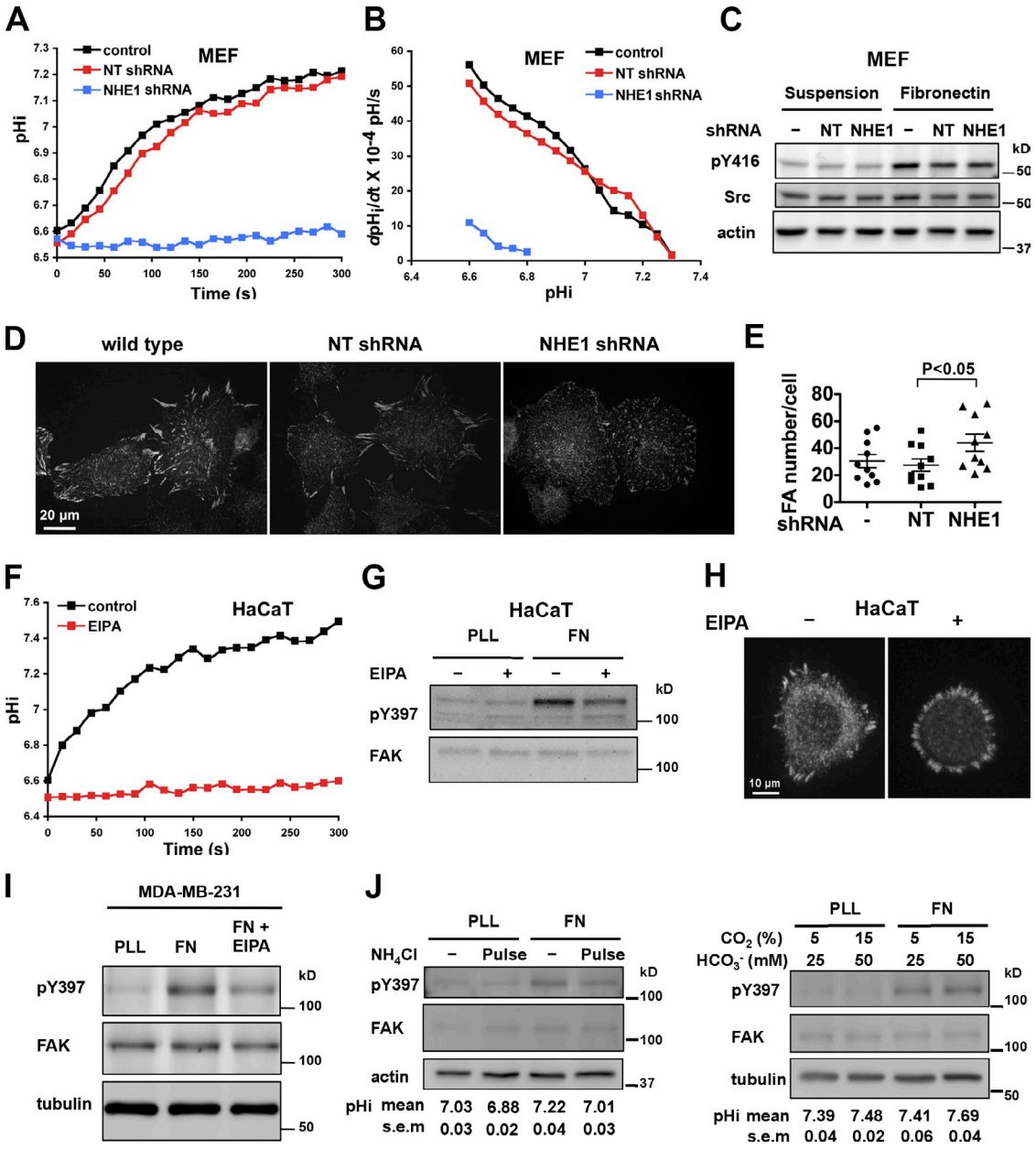

Figure S1. **NHE1-dependent autophosphorylation of FAK and cell spreading.** (A and B) NHE1 activity in MEFs is abolished by expression of NHE1 shRNA, as indicated by the time-dependent pHi recovery from an NH_4Cl-induced acid load in a Hepes buffer (A) and the pH-dependent recovery (dpHi/dt) (B). Data represent means ± SEM of three cell preparations. (C) Immunoblot shows fibronectin-induced increase in Src-pY416 in control (−) MEFs is similar with NT shRNA and NHE1 shRNA. (D) Immunolabeling of vinculin, a marker for mature focal adhesions, shows impaired focal adhesion formation of NHE1 shRNA MEF cells. Confocal images were taken after 60 min of replating on fibronectin. (E) Quantification data for numbers of focal adhesions per cell. (F) EIPA blocks NHE1 activity in HaCaT keratinocytes as determined by the time-dependent pHi recovery from an acid load in a Hepes buffer. Data represent means ± SEM of three cell preparations. (G) Immunoblot of lysates from HaCaT cells shows FAK-pY397 induced by fibronectin (FN) but not poly-L-lysine (PLL) is blocked by EIPA. Cells were pretreated with 10 µM EIPA in suspension for 30 min and were lysed 2 h after plating on PLL or FN. (H) Paxillin immunolabeling shows EIPA inhibits spreading of HaCaT cells on FN. Cells were fixed and stained after 2 h of plating on FN. (I) Immunoblot shows EIPA attenuated FAK-pY397 in MDA-MB-231 human mammary adenocarcinoma cells. Cells were lysed after 2 h of replating on PLL or FN. (J) In NHE1-deficient PS120 cells, an NH_4Cl-induced acid load in a Hepes buffer decreased pHi and attenuated FAK-pY397. In contrast, incubating cells at 15% CO_2 in medium containing 50 mM HCO_3^- (compared with 5% CO_2 and 25 mM HCO_3^-) increased pHi and FAK-pY397. Immunoblots are representative of two independent cell preparations. Below immunoblots are the pH values for each condition, expressed as means ± SEM of triplicate measurements in two independent cell preparations.

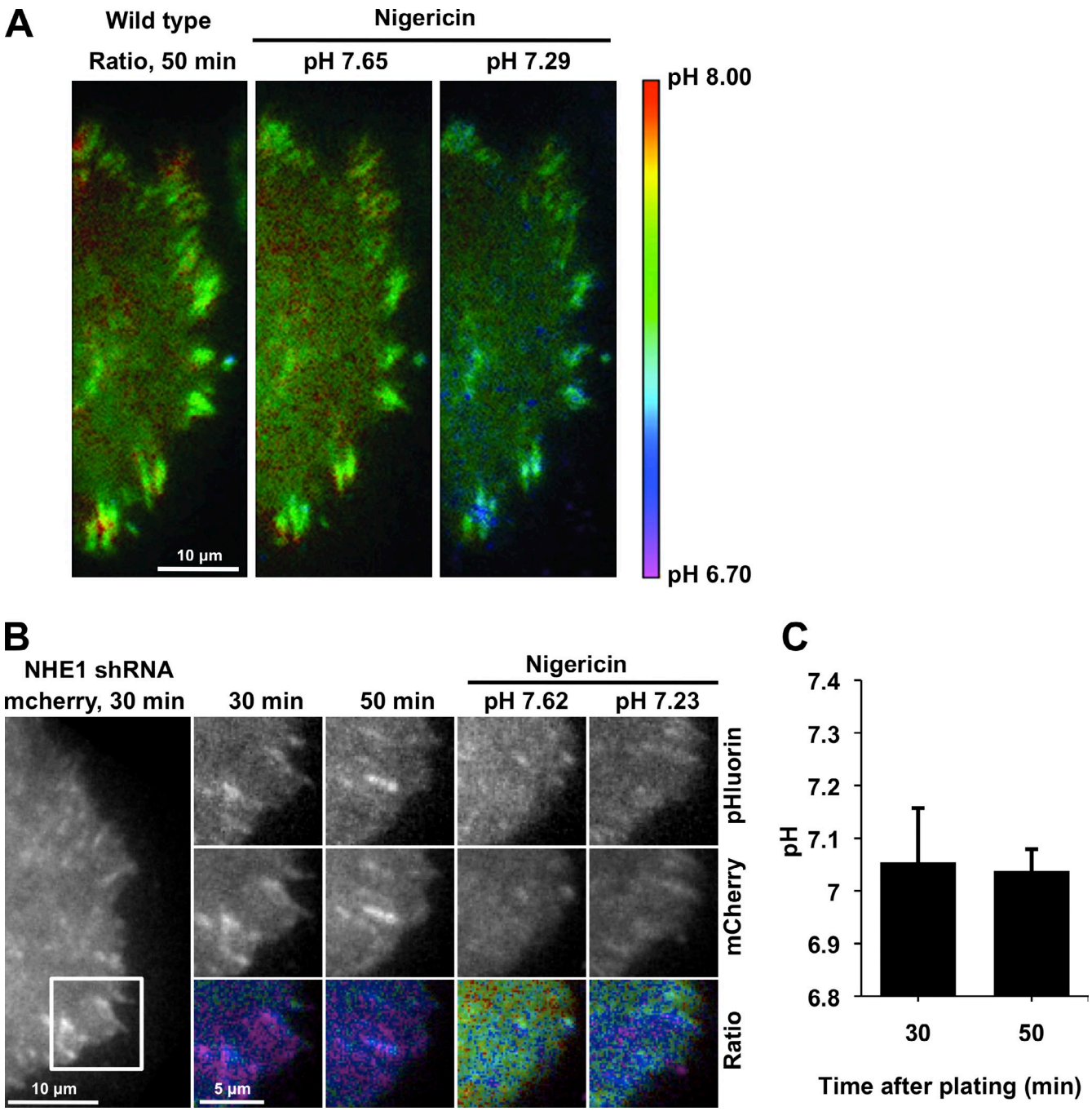

Figure S2. **Calibration of fluorescence ratio to pH.** (A) Ratio images of the wild-type MEF cell shown in Fig. 1 F taken at 50 min (left) and then incubated with a Hepes buffer containing the proton ionophore nigericin at the indicated pH values (middle and right). Nigericin allows equilibration of extracellular and intracellular pH, which allows calibration of the pHluorin-to-mCherry fluorescence ratio to pH units. (B) 10-µm × 10-µm images from NHE1 shRNA cells expressing paxillin-mCherry-pHluorin (marked at the left panel) after 30 and 50 min of replating on FN. pH scale as in A and channels as labeled. (C) Analysis of cytosolic pH in NHE1 shRNA cells using paxillin-mCherry-pHluorin. 5 ROI in 5 cells were analyzed at 30 and 50 min after replating and pH was calculated using nigericin buffers with low and high pH. Columns show mean ± SEM of the average pHi of the five cells. Note that no increase in pH in the NHE1 shRNA cells was observed.

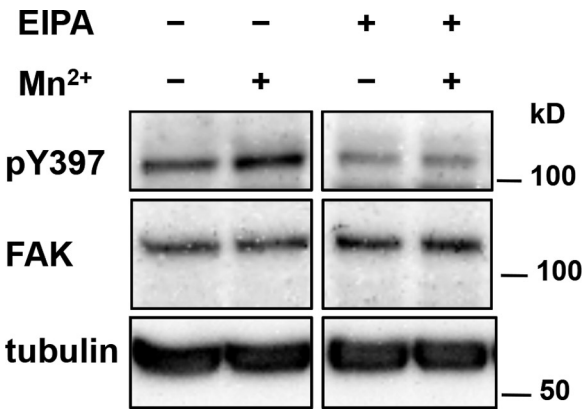

Figure S3. **$MnCl_2$ does not restore attenuated FAK pY397 with EIPA.** Suspensions of HaCaT cells were incubated with 0.25 mM $MnCl_2$ for 10 min, plated on fibronectin, and then lysed after 2 h for immunoblotting.