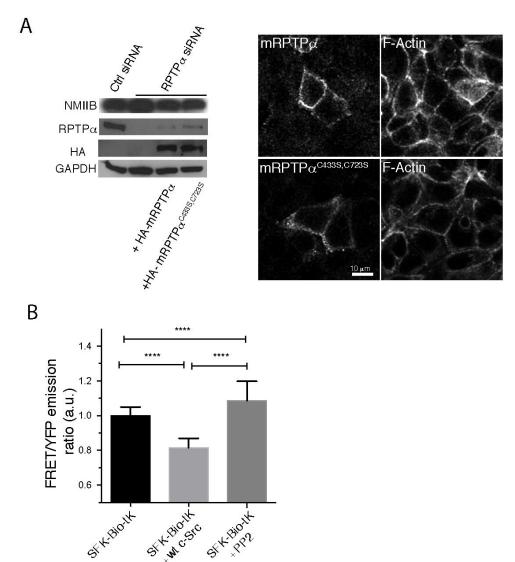
# Supplemental Materials Molecular Biology of the Cell

Gomez et al.

## Supplementary Figure 1



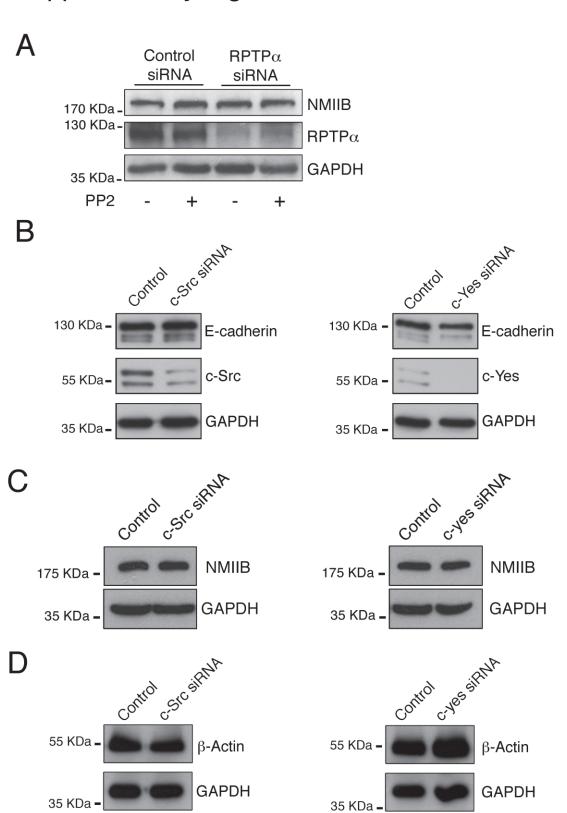
### Supplementary Figure 1.

A) Left, Western blot analysis of NMIIB, RPTP $\alpha$  and HA-tagged RPTP $\alpha$  expression in cells transfected with a control siRNA (Ctrl siRNA); an siRNA against RPTP $\alpha$  alone; and RPTP $\alpha$  siRNA together with either siRNA resistant wild type RPTP $\alpha$  (HA-mRPTP $\alpha$ ) or its phosphatase deficient mutant (HA-mRPTP $\alpha$  C433S,C723S). GAPDH was used as a

loading control. Right, anti-HAimmunofluorescence of RPTP $\alpha$  KD cells transiently expressing either wild type HA-mRPTP $\alpha$ or HA-mRPTP $\alpha^{C433S,C723S}$ . The same cells were co-stained with fluorescently labeled phalloidin (F-actin).

B) Average FRET/YFP emission ratios at the ZA in cells expressing SFK-Bio-tK alone (SFK-Bio-tK), co-expressing SFK-Bio-tK with c-Src-mCherry (SFK-Bio-tK+wt c-Src) or expressing SFK-Bio-tK alone and treated with PP2 (25 μM, 1h). Data are means ±SEM for at least 25 images (~90-100 cells) per condition. \*\*\*\*\*; p<0.0001, One-Way ANOVA.

### Supplementary Figure 2

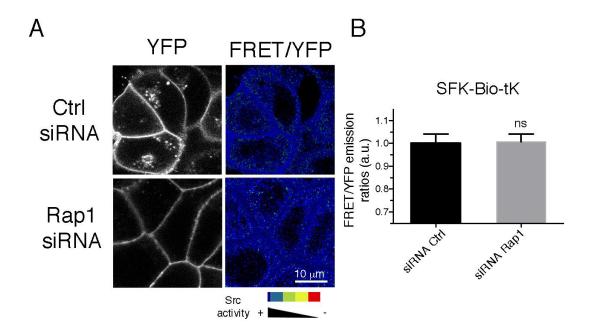


### **Supplementary Figure 2.**

A) Western blot analysis of NMIIB and RPTP $\alpha$  in cells transfected with a control siRNA or a siRNA against RPTP $\alpha$  and treated or not with PP2 (25  $\mu$ M, 1h). GAPDH was used as a loading control.

B-D)Western blot analysis of E-cadherin (B), NMIIB (C) and  $\beta$ -actin (D) expression in Control, c-Src KD and c-Yes KD cells. GAPDH was used as a loading control.

### Supplementary Figure 3



#### **Supplementary Figure 3.**

FRET/YFP emission ratio images (A) and quantitation (B) for the SFK-Bio-tK FRET biosensor in control cells and cells transfected with a Rap1 siRNA. Data are means ±SEM for at least 25 images (~90-100 cells) per condition.ns, not significant. Two-tailed t-test.