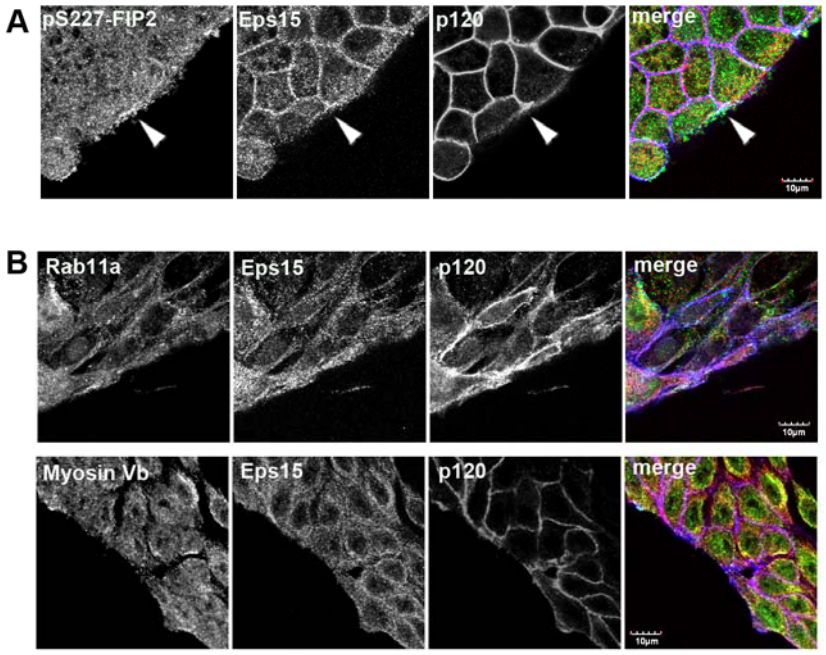
Supplemental Materials

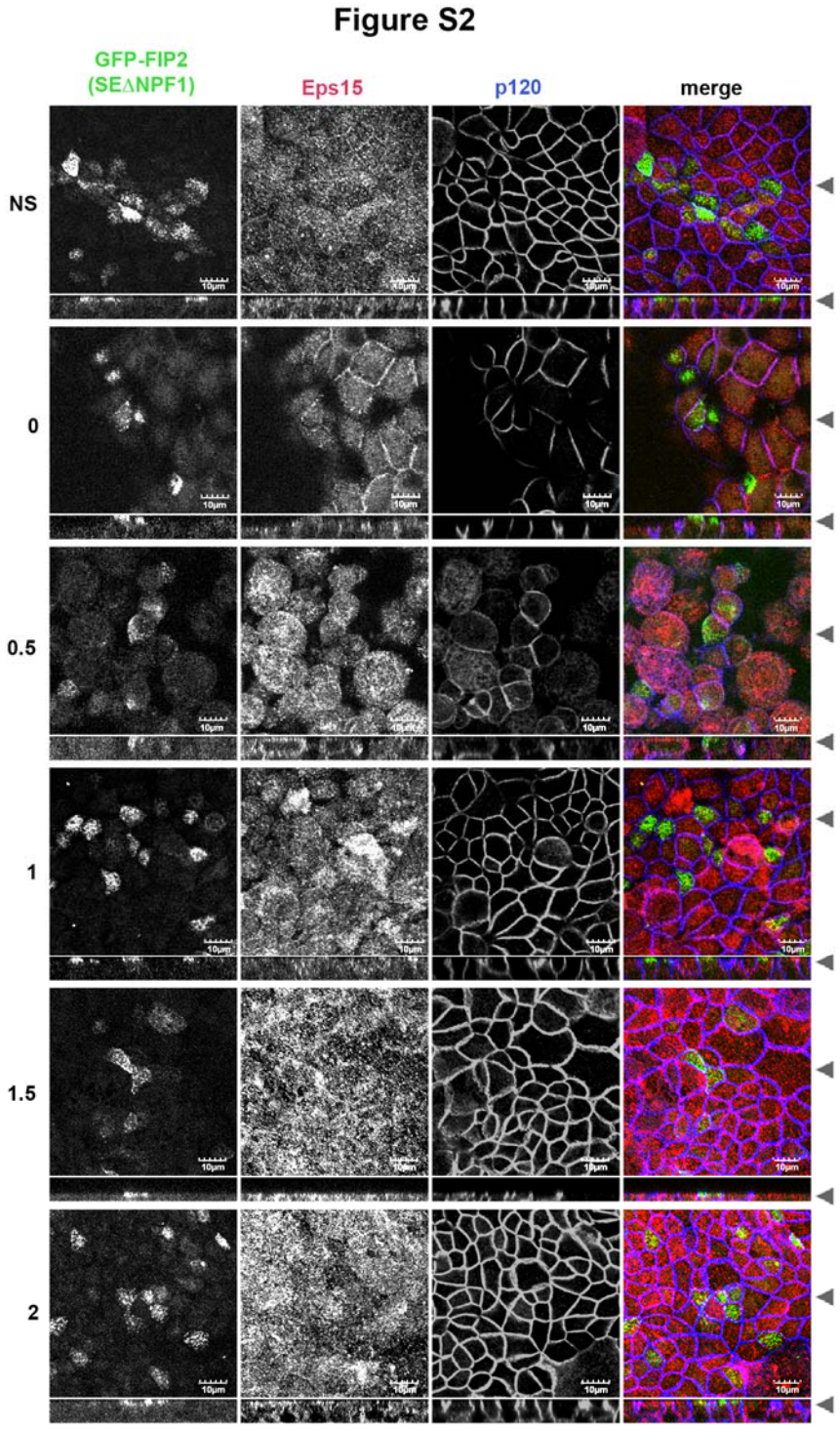
Molecular Biology of the Cell

Lapierre et al.

Figure S1



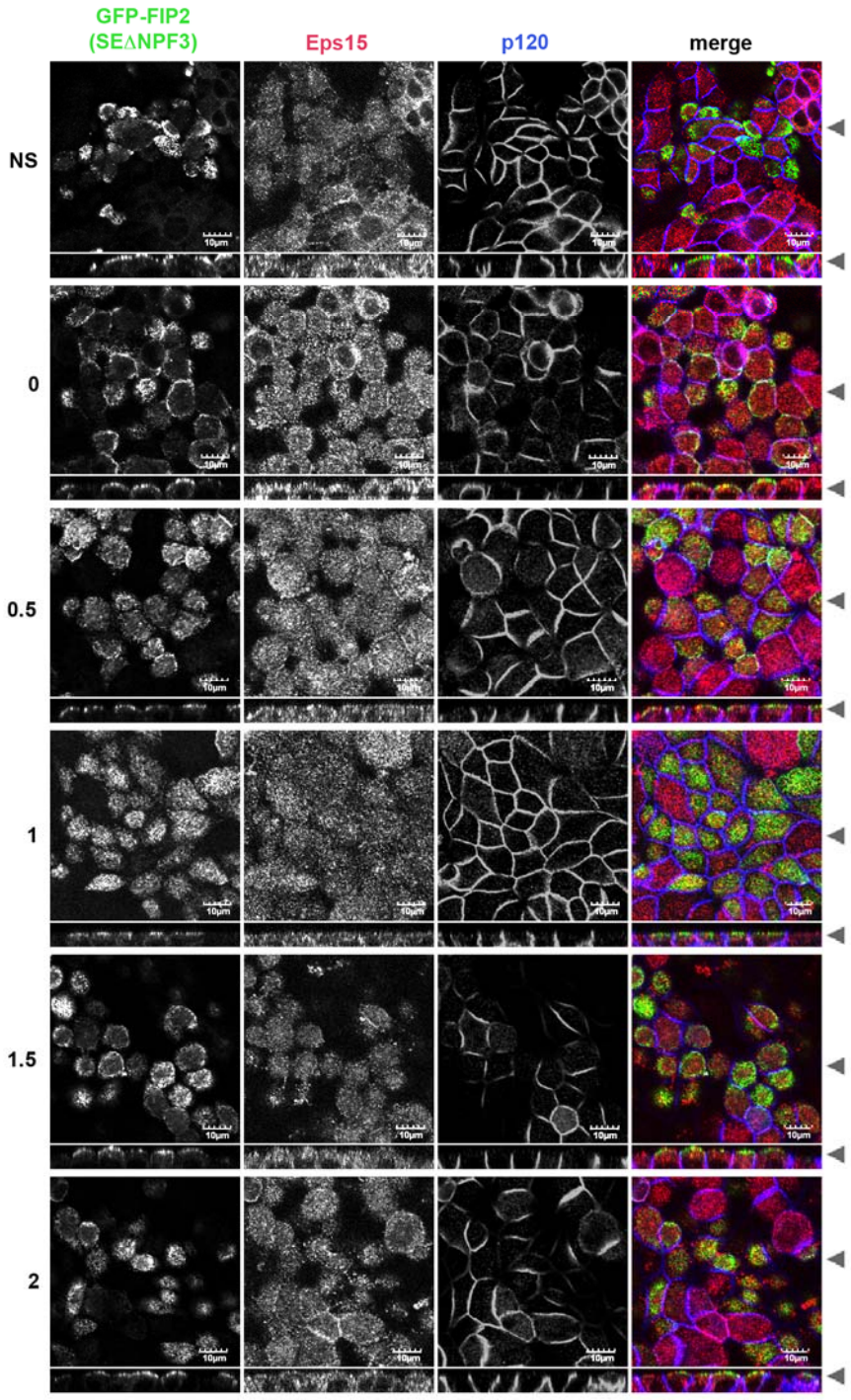
Supplemental Figure 1: Eps15 co-localizes with pS227-FIP2 at the leading edge of a wounded monolayer, but not with Rab11a or Myosin Vb. MDCK cells were plated onto coverslips allowed to reach confluence. The monolayer was scratched and allowed to regrow for 24 hours, fixed with methanol (A) or 4% paraformaldehyde (B) and stained for Eps15 (red in merge), p120 (blue in merge) and either (A) pS227-FIP2, or (B) Rab11a, or Myosin Vb (green in merge). White arrowhead in (A) indicates area of co-localization of pS227-FIP2, Eps15 and p120. Bar =10 μm.



Supplemental Figure 2: Mutation of the first NPF domains in GFP-FIP2(S227E) released Eps15 from the GFP-FIP2(S227E) compartment, allowing Eps15 to localize at the lateral membrane. The MDCK cell lines expressing GFP-FIP2(S227E NPF1) was grown on transwells, switched into low calcium media then allowed to recover for the hours listed to the left of the panels. Cells were fixed in 4% paraformaldehyde and

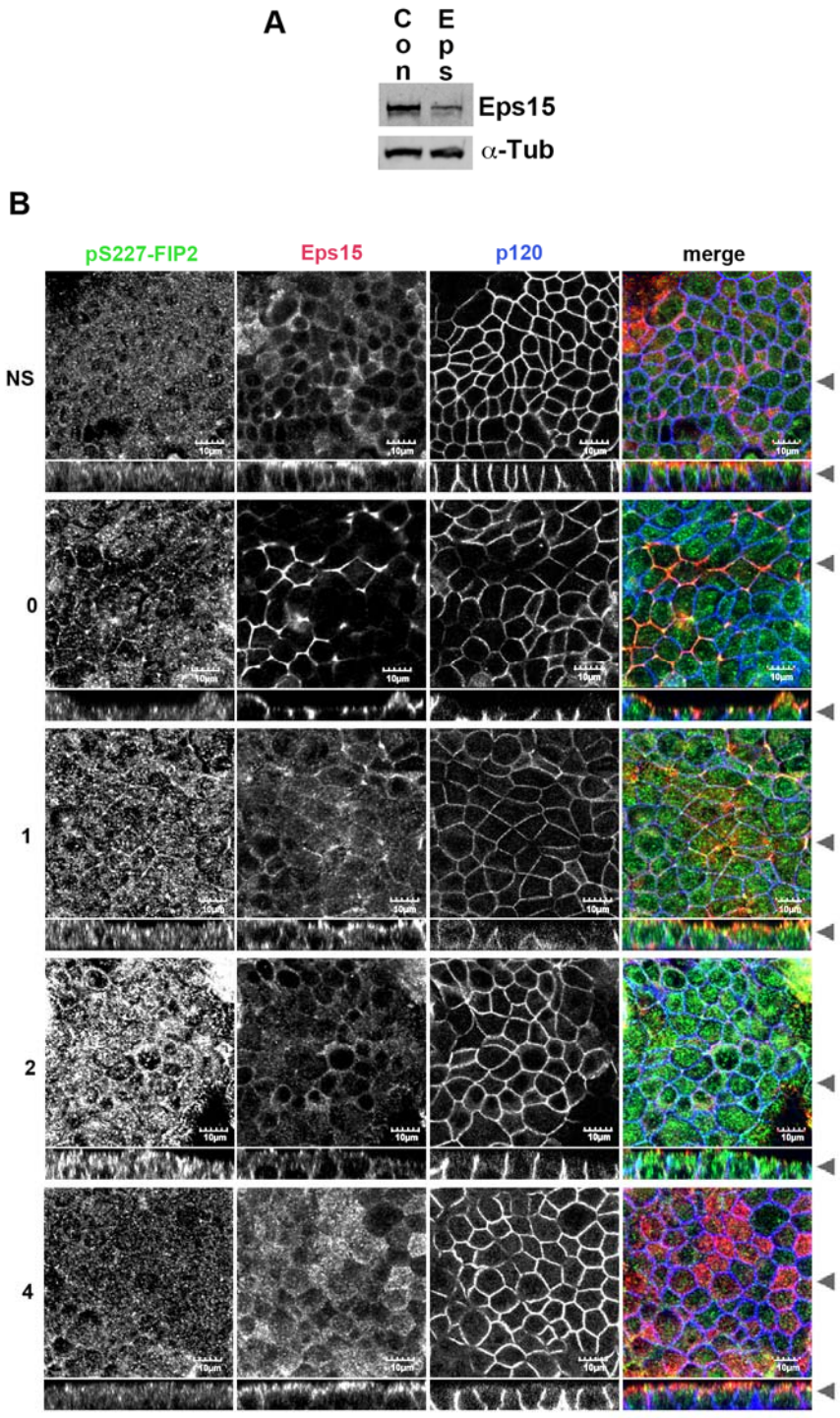
stained for Eps15 (red in merge) and p120 (blue in merge). Black arrowheads indicate where the xy and xz slices were taken. Bar = 10 μm.

Figure S3



Supplemental Figure 3: Mutation of the third NPF domains in GFP-FIP2(S227E) released Eps15 from the GFP-FIP2(S227E) compartment, allowing Eps15 to localize at the lateral membrane. The MDCK cell lines expressing GFP-FIP2(S227E NPF3) was grown on transwells, switched into low calcium media then allowed to recover for the hours listed on the left of the panels. Cells were fixed in 4% paraformaldehyde and stained for Eps15 (red in merge) and p120 (blue in merge). Black arrowheads indicate where the xy and xz slices were taken. Bar = 10 μm.

Figure S4



Supplemental Figure 4: The temporal and spatial timing of pS227-FIP2 in control shRNA knockdown was similar to parental MDCK ce**lls.** (A) Representative western demonstrating the knockdown of Eps15 in the Eps15 knockdown MDCK cell line, but not in the control knockdown MDCK cell line, quantitation of three similar westerns

indicated an average of 63% knockdown of Eps15. (B) MDCK cell line expressing control shRNA was grown on transwells, allowed to recover from low calcium for the indicted hours, fixed in methanol and stained for pS227-FIP2 (green in merge), Eps15 (red in merge) and p120 (blue in merge). pS227-FIP2 was observed in almost all of the time points with the least seen at 1 hour. Black arrowheads indicate where the xy and xz slices were taken. Bar = 10 μm.

Supplemental Table 1: Proteomics results of pS227-FIP2 immunoprecipitatedfrom CHAPS lysed MDCK cells. Identified proteins were only included if they were observed in at least three of four separate experiments. Number of peptides indicates the number of unique peptides identified in all experimental runs.