Supplemental Figure legends

Yadav et al., “Reengineering the signaling properties of a Src family kinase”

Supplemental Figure 1. Phosphorylation of cellular proteins by PDZ-kinases and an activated form of Hck. A mutant form of Hck was generated in which the inhibitory C-terminal tyrosine (Y527, Src numbering) was mutated to phenylalanine. SYF cells were transfected with the YF mutant or with the indicated constructs, and analyzed as in the legend to Figure 2.

Supplemental Figure 2. Phosphorylation of nNOS. SYF cells were transiently transfected with the nNOS PDZ domain together with WT Hck or the engineered kinases ZK or KV. The cells were harvested 40 hours post-transfection and lysates were subjected to immunoprecipitation using anti-HA antibody. The immunoprecipitates were separated by 10% SDS-PAGE, and Western blotting was carried out with anti-pTyr antibody. Kinase expression was measured in whole cell lysates using anti-Flag antibody.

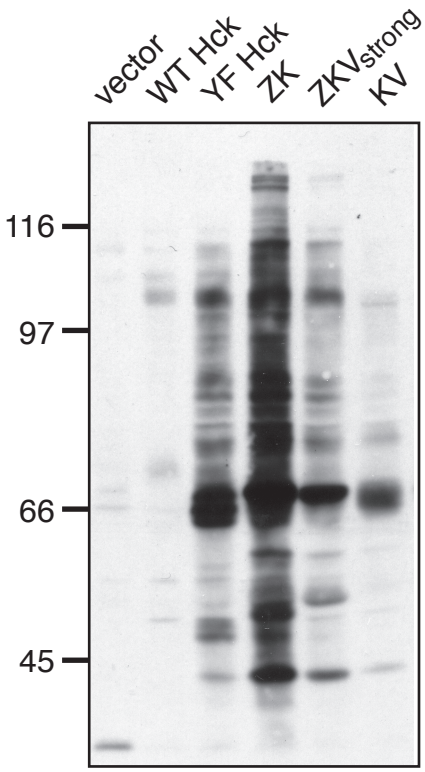
Supplemental Figure 3. PDZ-kinase and reengineered Cas restore migration defect of Cas-deficient cells. Cas deficient cells were transfected with Cas-VKESLV-PPX and the indicated kinase constructs. Migration assays were carried out in a 96 well transwell migration assay chamber, as described in the legend to Figure 6.

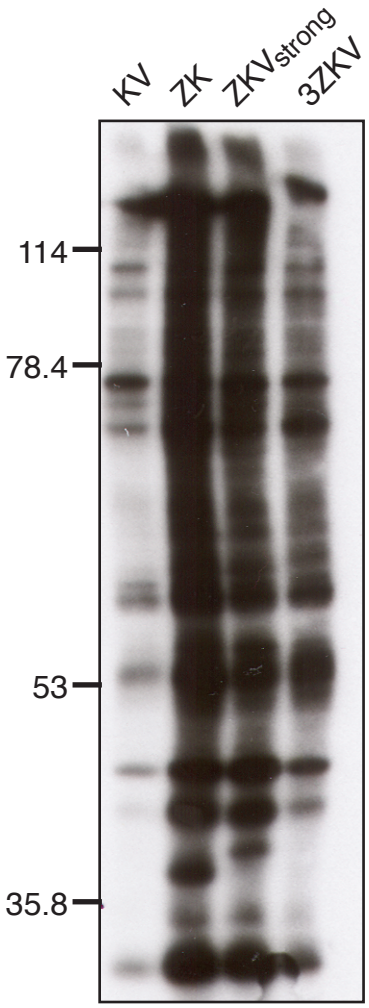
Supplemental Figure 4. The expression of kinases and Cas constructs used for migration experiments (Figure 6) was confirmed by Western blotting using anti-Cas and anti-Flag antibodies. Panel A shows Cas-deficient cells expressing Cas-VKESLV, and Panel B shows Cas-deficient cells expressing Cas-VKESLV-PPX.

Supplemental Figure 5. Rewiring of Ras-MAPK signaling. The expression of ErbB2 and kinases used in the Ras-MAPK experiments (Figure 7) was confirmed by Western blotting.

Supplemental Video 1. Wound healing assays of $Cas^{-/-}$ cells alone.

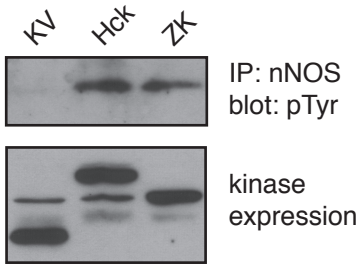
Supplemental Video 2. Wound healing assays of $Cas^{-/-}$ cells expressing ZK and Cas-VKESLV-PPX.



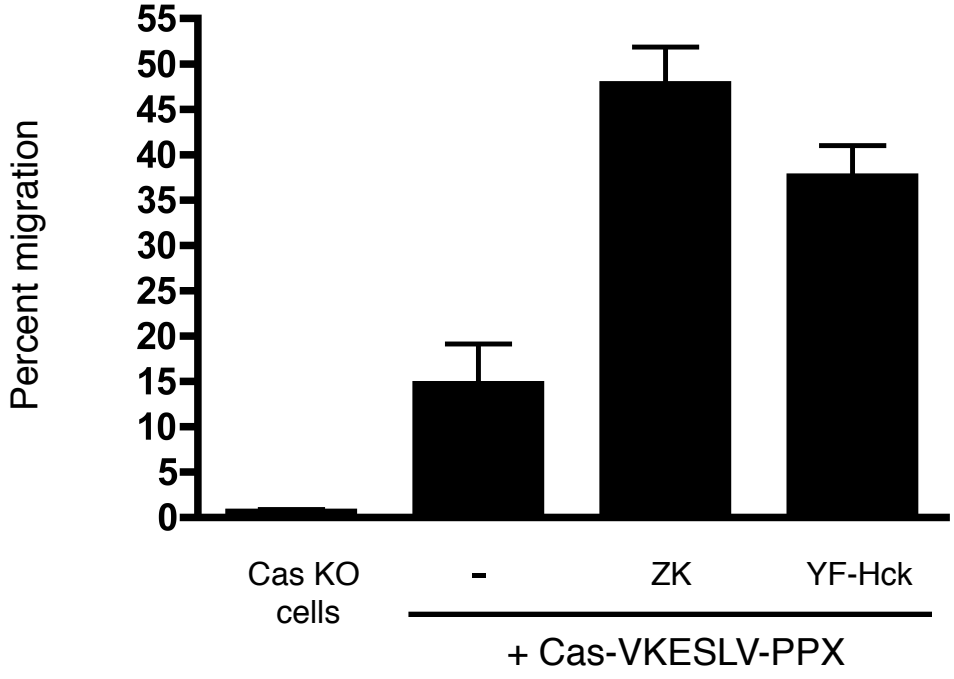


SYF whole cell lysates
Blot: anti-pTyr

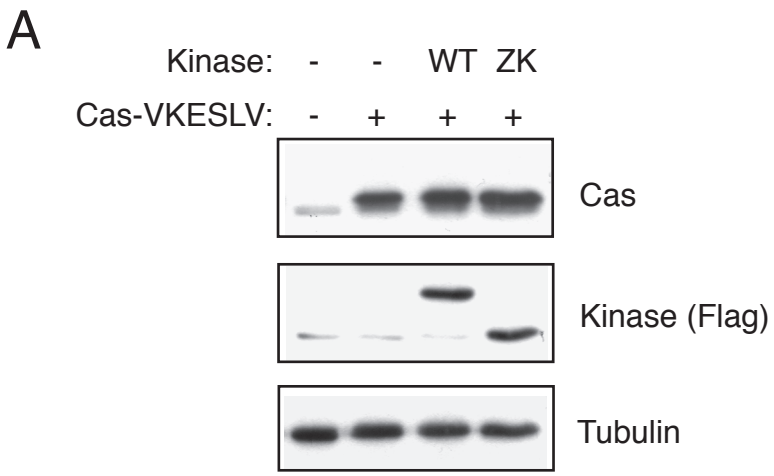
Supplemental Figure 1

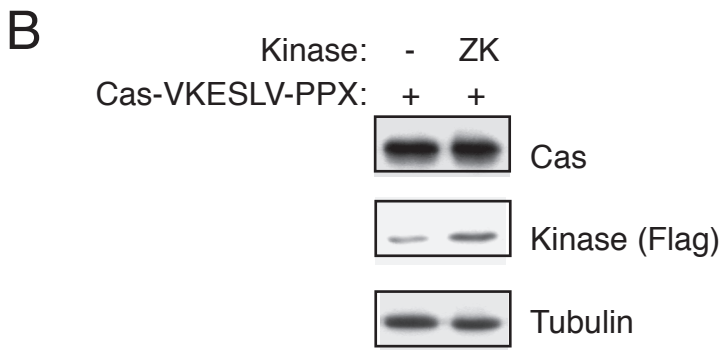


Supplemental Figure 2

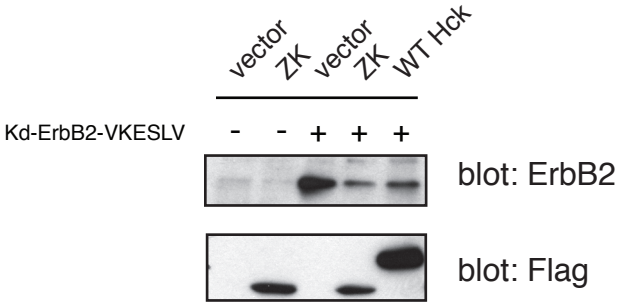


Supplemental Figure 3





Supplemental Figure 4



Supplemental Figure 5