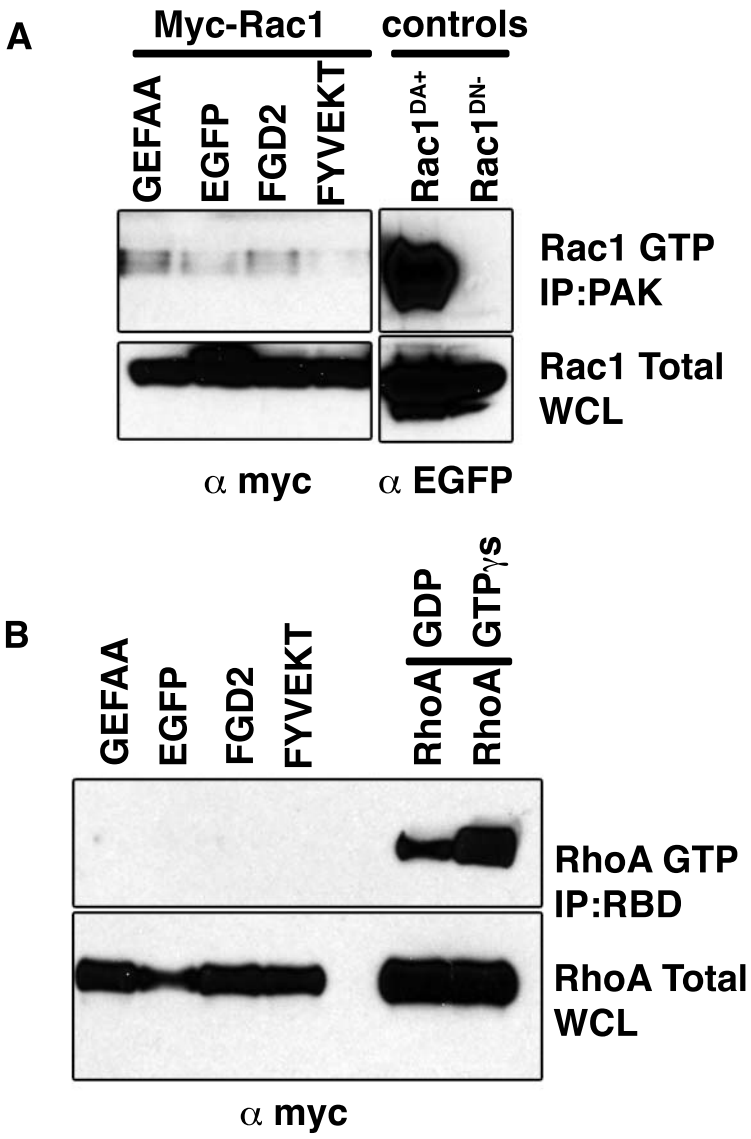
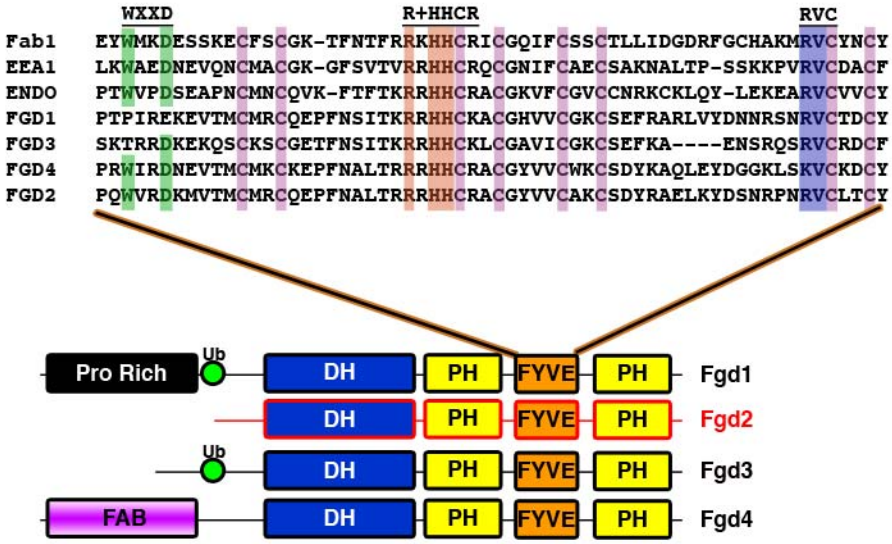
Supplementary Figure legends

Supplementary Figure 1: FGD2 fails to activate Rac1 and RhoA.
A, Lysates from COS-7 cells transfected with myc-Rac1 and EGFP, EGFP-tagged FGD2, $FGD2^{GEFAA}$ mutant (GEFAA), or $FGD2^{FYVEKT}$ mutant (FYVEKT) were immunoprecipitated with PAK1-crib-GST beads. Immunoprecipitated beads (IP: PAK, upper panels) or whole cell lysates (WCL, lower panels) were electrophoresed and immunoblotted with anti-Myc (left panels) or anti-EGFP (right panels). Lysates from transfected COS-7 cells expressing dominant active ($Rac1^{DA+}$) and negative ($Rac1^{DN-}$) EGFP tagged Rac1 mutants were used as controls (right panel). This experiment was performed twice with reproducible results.
B, Lysates from COS-7 cells transfected with myc-RhoA and EGFP, EGFP-tagged FGD2, $FGD2^{GEFAA}$ mutant (GEFAA), or $FGD2^{FYVEKT}$ mutant (FYVEKT) were immunoprecipitated with Rhotekin-RBD-GST beads. Immunoprecipitated beads (IP: RBD, upper panels) or whole cell lysates (WCL, lower panels) were electrophoresed and immunoblotted with anti-Myc. Lysates from transfected COS-7 cells expressing Myc-RhoA were loaded with either GDP or GTPγS and used as negative and positive controls respectively. This experiment was performed twice with reproducible results.

Supplementary Figure 2: Amino acid alignment of FYVE domains of various proteins (upper panel) and domain layout of Fgd family members (lower panel). Note the tryptophan in the WxxD motif is not conserved in FGD1 and FGD3, but is canonical in FGD2. Pro Rich; Proline rich domain; Ub; ubiquitinylation site, DH; Dbl Homology domain, PH; Pleckstrin homology domain, FYVE; Fab1, YotB, Vac1 and EEA1 domain, FAB; F actin binding domain.



Supplementary Figure 1



SUPPLEMENTARY FIGURE 2