Supplemental Methods

Supplementary Figure 2.

For competition experiments, COS-7 cells were transfected with Xp-Ras wild-type. Cell extracts were treated in vitro with GTPγS or GDP to activate or inactivate Ras, following manufactory's instructions (Ras activation kit, Upstate Biothenology). Cell lysates-GDP-loaded were incubated with GST-GILZ fusion protein in binding buffer (250 mM NaCl, 50 mM HEPES, pH 7.5, 0.5 mM EDTA, 0.1% (v/v) NP-40, 0.2 mM PMSF, 1 mM DTT, 100 μg/ml bovine serum albumin) for 1 h at 4° C. The GST-GILZ was purified by adsorption to glutathione Sepharose beads, washed and resuspended in PBS. Cell lysates-GTP loaded were then added. After 1 h a 4° C, the GST-GILZ beads were washed and examined for associated Ras by Western blotting with anti-Xp Ab. To control Ras activation, cell lysates were also incubated with GST-Raf fusion protein corresponding to the human Ras Binding Domain (RBD, residues 1-149) of Raf-1 (GST-Raf-RBD), following manufactory's instructions (Ras activation kit, Upstate Biothenology).

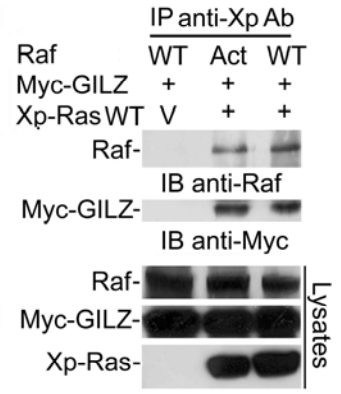
Supplemental Figure Legends

Supplemental Figure 1

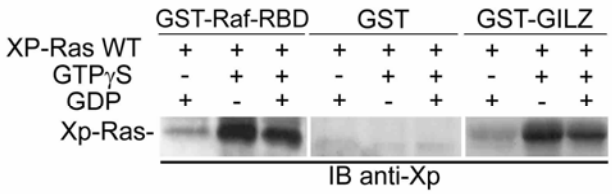
GILZ/Ras binding is independent on activation status of Raf. COS-7 cells were cotransfected with either wild-type or constitutively active pUSEamp-Raf-1, along with myc-GILZ and wild-type Xp-Ras vectors. Immunoprecipitation was performed with anti-Xp Ab and immunoreactive proteins were revealed with anti-Raf-1 or anti-myc antibodies. Whole-cell lysates were loaded to control GILZ, Raf-1 and Ras expression.

Supplemental Figure 2.

GILZ binds GTP Ras with more affinity than GDP Ras. Xp-Ras wild-type was expressed in COS-7 cells and cellular lysates (200μg), preloaded with GDP or GTPγS, were incubated for 1 h with GST-GILZ fusion protein. The complex was purified by adsorption to glutathione Sepharose beads, washed and resuspended in PBS. Cellular extracts from Xp-Ras-transfected COS-7 cells (200μg), loaded with GTPγS, were added to GST-GILZ/Ras-GDP beads. Following 1h at 4° C, the GST-GILZ beads were washed and examined for associated proteins by Western blot with anti-Xp Ab (right). To control Ras activation, the same groups were precipitated with GST-Raf-RBD fusion protein (left). Control GST alone (middle).



Supplemental Figure 1



Supplemental Figure 2