Supplementary Information

Supplementary Figure Legends

Figure S1. Glis2 directly interacts with PIAS4. (A) F.Glis2, V5.PIAS4 and V5.CD2AP were expressed using cell free wheat germ extracts. Immuno-precipitation of V5.PIAS4 but not V5.CD2AP immobilized F.Glis2 (B) SUMO-3 fusion failed to inhibit the ubiquitylation of Glis2. HEK 293T cells were transfected with the plasmids as indicated. After 24 hours of transfection, F.Glis2 and F.SUMO-3.Glis2 were precipitated using FLAG-M2 beads; Glis2 ubiquitin species were detected in F.Glis2 as well as in F.SUMO-3.Glis2 immunoprecipitates (C) No change in protein stability was observed for the SUMO-3.Glis2 fusion protein. HEK 293T cells transfected with V5.Glis2 and V5.SUMO-3.Glis2 were treated with cycloheximide as indicated. (D) The graph demonstrates the fraction of Glis2 and SUMO-3.Glis2, remaining after 0 to 10 hours of cycloheximide treatment.
Figure. S1

A

F.Glis2+V5.CD2AP  
F.Glis2+V5.PIAS4  

Lysates: anti-Flag

IP: anti-V5, WB: anti-FLAG

F.Glis2

100 -  
75 -  
kDa

F.Glis2

100 -  
75 -  
kDa

Lysates: anti-V5

IP: anti-V5, WB: anti-V5

B

Vector  
HA.ubi

HA.ubi+F. SUMO-3.Glis2  
HA.ubi+F.Glis2

IP: anti-Flag, WB: anti-HA

Ubi-Glis2

150 -  
100 -  
75 -  
kDa

IP: anti-Flag, WB: anti-Flag

F.Glis2

75 -  
kDa

F.SUMO-3.Glis2

100 -  
75 -  
kDa

F.Glis2

100 -  
75 -  
kDa

Lysates: anti-FLAG

C

V5.Glis2

Lysates: anti-V5

V5.SUMO3-Glis2

Lysates: anti-V5

0hr  2hr  4hr  6hr  8hr  10hr  
(Hrs of treatment-cycloheximide)

D

Relative protein stability

Hours of CHX treatment

- Glis2
- SUMO-3.Glis2

n = 3