Expanded View Figures

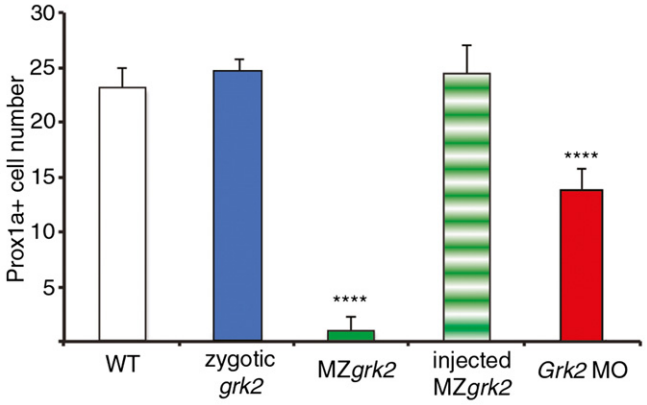

Figure EV1. MZ*grk2* shows severe Hh defects.
Numbers of Prox1a^{+ve} cells in wild-type (WT), zygotic *grk2* mutants, MZ*grk2*, MZ*grk2* injected with Grk2-GFP and Grk2 ATG morphant embryos at 30hpf (n = 8). The error bars indicate SD. Asterisks indicate statistically significant differences based on unpaired Student's t-test. ****$P < 0.0001$.

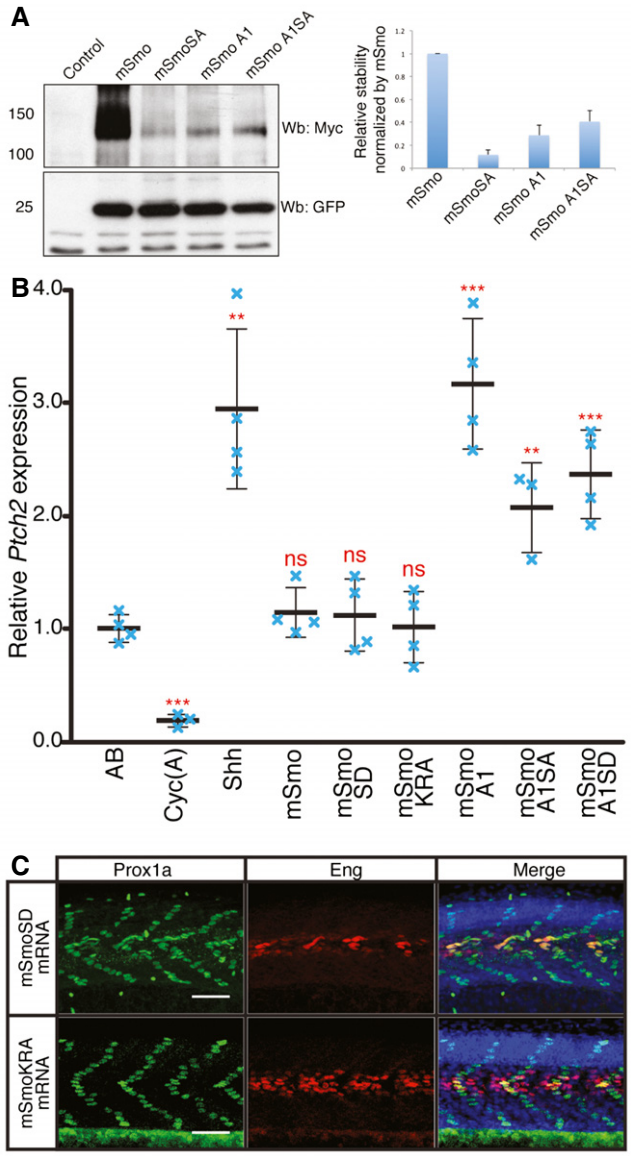

Figure EV2. Phosphorylation and dimerisation of Smo does not impair or enhance Hh activity.

A Western blot analysis of wild-type and mutant forms of Smo expressed from bicistronic myc-tagged mSmo and GFP constructs injected into wild-type zebrafish embryos. The histogram shows the quantification of Myc-tagged mSmo vs. GFP (n = 3). The error bars indicate SD.

B Normalised expression of *ptch2* in 18hpf wild-type (AB), Cyc(A)-treated embryos and embryos injected with mRNA encoding Shh or various Smo mutants. Individual data points (blue crosses) are shown; mean is indicated by thick solid line, and SD by thin bars. Unpaired Student's t-test was used to determine the statistical significance between control (AB) and experimental embryos. ***$P < 0.001$; **$P < 0.01$; and n.s. (not significant).

C Prox1a and Eng expression in *smo*^{hi1640} mutant embryos injected with mRNA encoding mSmoSD (n = 7) and mSmoKRA (n = 7). These mutant embryos show full recovery of SSFs and MPs. Embryos shown are at 30hpf. Scale bar, 50 μm.

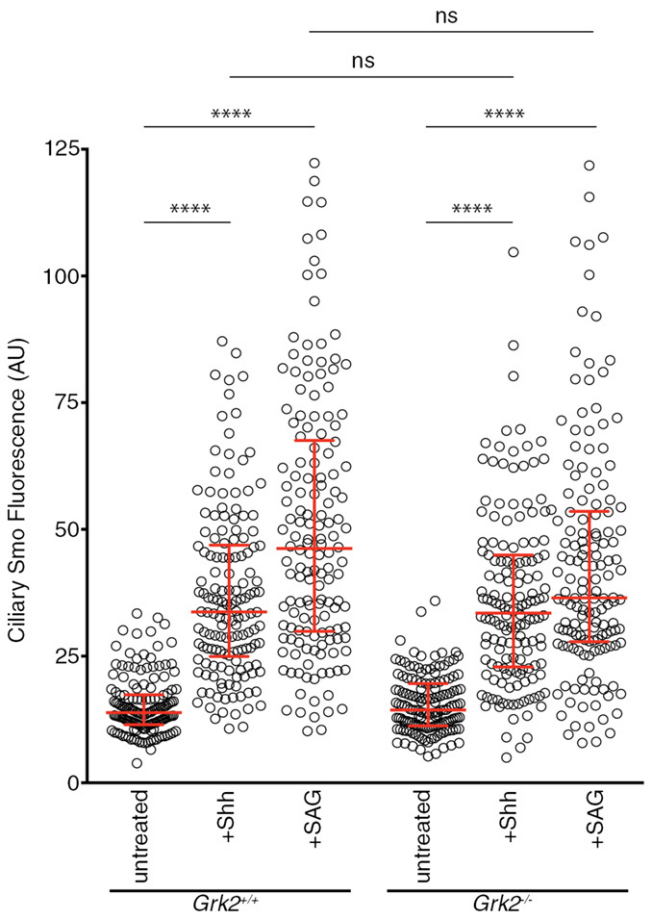

Figure EV3. Smo translocates to the primary cilium independently of Grk2.

Accumulation of endogenous Smo in the PC of wild-type and *Grk2*$^{-/-}$ Flp-In-3T3 cells after 4 h of Shh-N and SAG treatment. The medians with interquartile range of the fluorescence intensities of endogenous Smo are shown, quantified from multiple cilia ($n = 150$). Data were analysed using Kruskal–Wallis non-parametric ANOVA. ****$P < 0.001$ and n.s. (not significant).

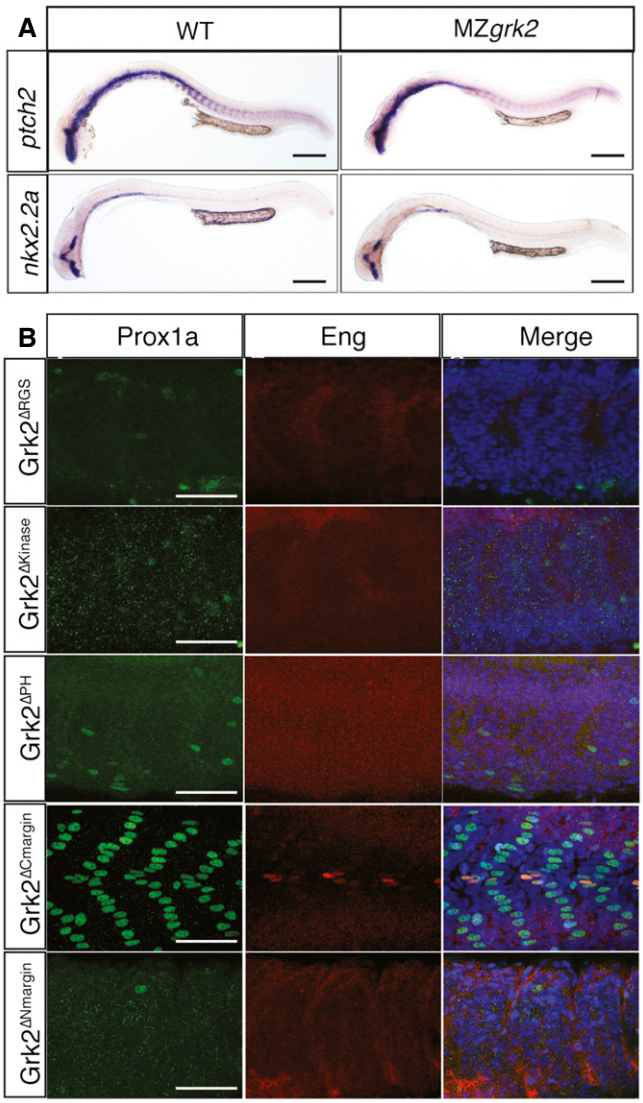

Figure EV4. MZ*grk2* is fully rescued by mRNA encoding Grk2^{K220R} and Grk2 domain swapping assay.

A *In situ* hybridisation of *ptch2* and *nkx2.2a* in 24hpf wild-type (left column) and MZ*grk2* embryos expressing Grk2^{K220R}-GFP (right column) (n = 30 for each sample). Scale bar, 200 μm.

B Prox1a (green) and Eng (red) expression in the myotome of 30hpf MZ*grk2* embryos injected with mRNA encoding Grk2$^{\Delta RGS}$-GFP, Grk2$^{\Delta kinase}$-GFP, Grk2$^{\Delta PH}$-GFP, Grk2$^{\Delta Cmargin}$-GFP and Grk2$^{\Delta Nmargin}$-GFP. Merge images show nuclei labelled with DAPI (blue) (n = 20). Scale bar, 50 μm.

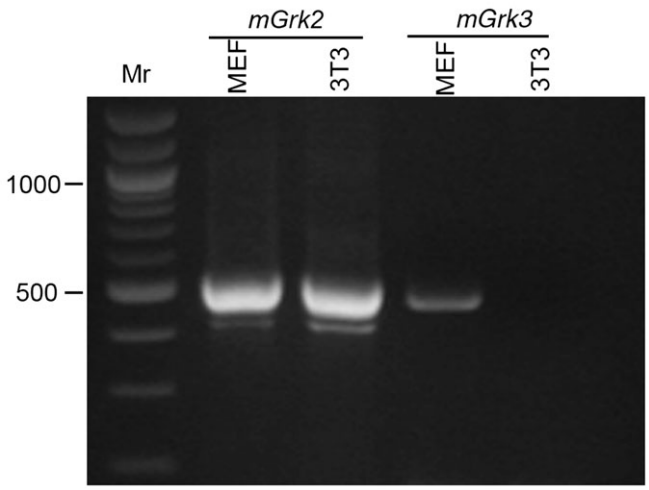

Figure EV5. Grk3 is not expressed in NIH3T3 cells.

RT–PCR assay of Grk2 and Grk3 expression in MEF and NIH 3T3 cells.