SUPPORTING INFORMATION FOR:

Functional dissection of the N-terminal extracellular domains of Frizzled 6 reveals their roles for receptor localization and Dishevelled recruitment.

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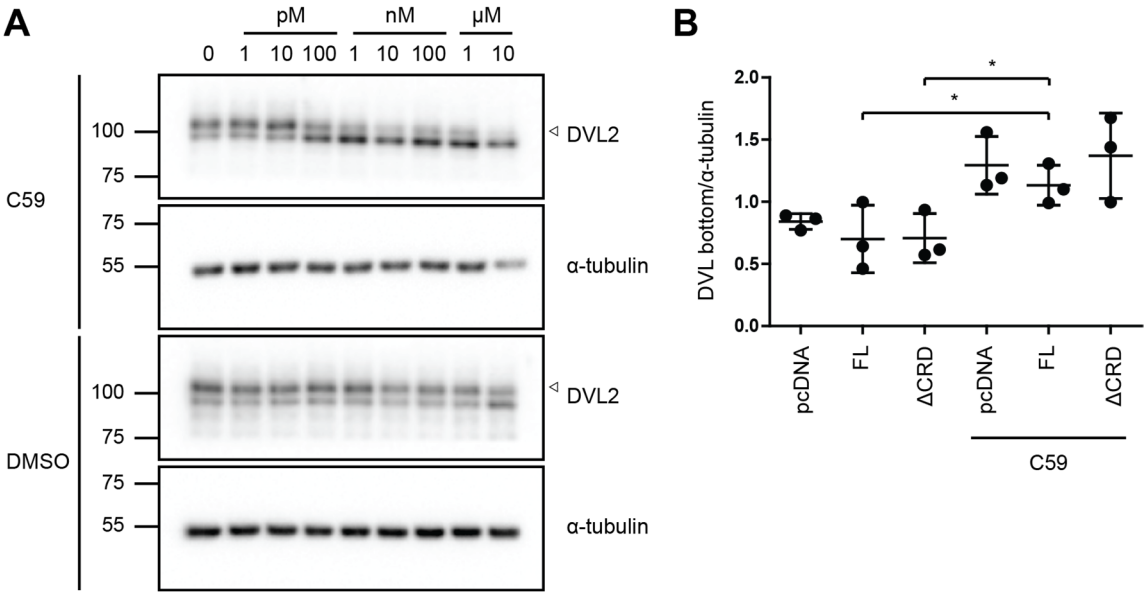
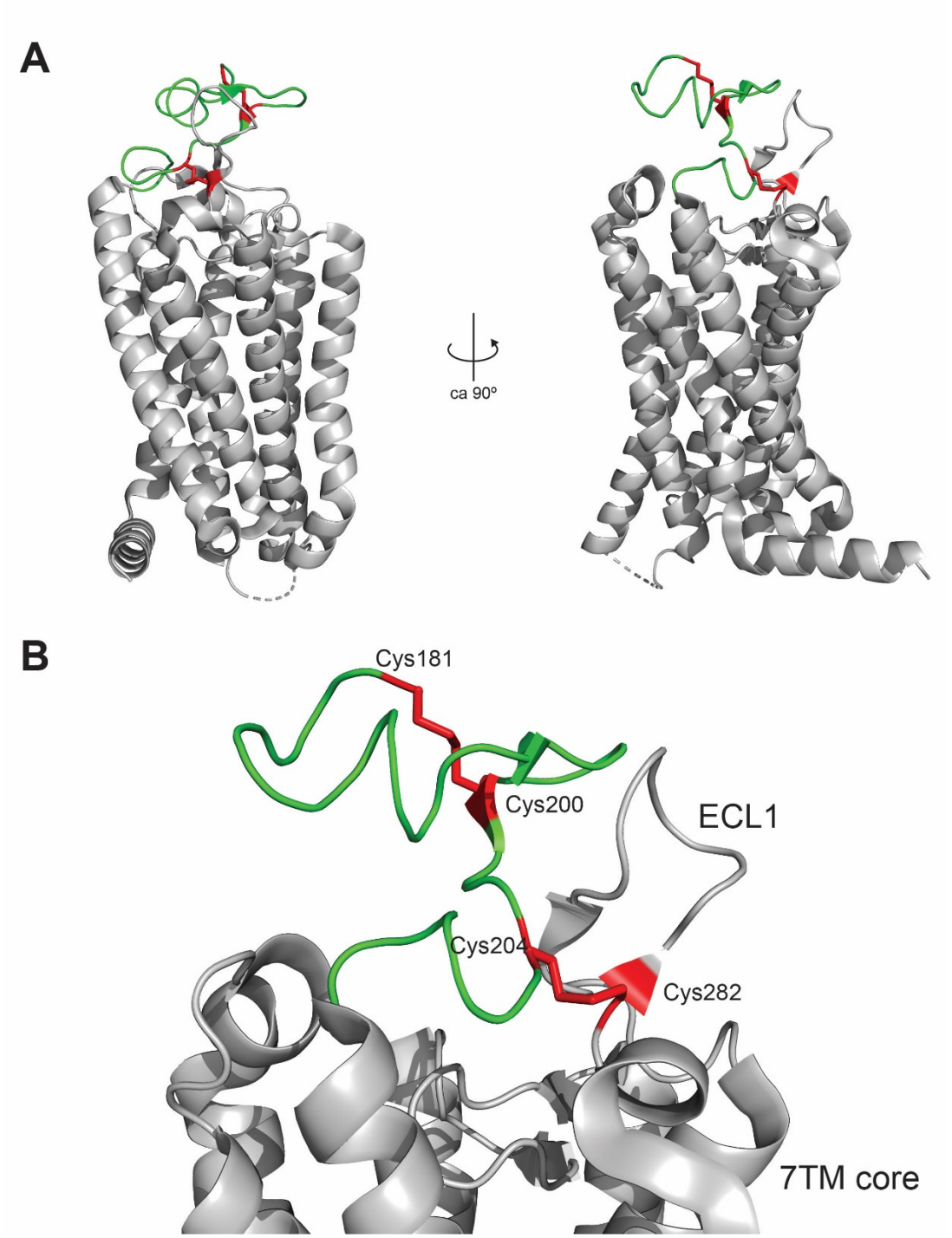
Supporting Figures



Supporting Figure 1: The porcupine inhibitor C59 efficiently reduces the basal electrophoretic mobility shift of endogenously expressed DVL2.

(**A**) HEK293T cells were treated with increasing concentrations of C59 or DMSO overnight. Cell lysates were analysed by immunoblotting using anti-DVL2 and anti-α-tubulin (loading control) for detection. Increasing concentrations of C59 but not DMSO reduced the phosphorylated and shifted form of DVL2 (open triangle) compared to the unshifted form. (**B**) The scatter dot plot summarizes immunoblotting experiments and densitometry analysis of three independent experiments, where HEK293 cells were transfected with control vector (pcDNA), full-length (FL) and ΔCRD FZD_6 and left untreated or were treated with C59 overnight. A representative immunoblot is shown in Fig 3B. The quantification in this figure shows the ratio of the DVL's bottom band over the α-tubulin band. The ratios were normalized by the average of the ratio values from each experiment. Data in the scatter dot plot are shown as mean with SD. The statistical analysis was done by one-way ANOVA and Tukey's multiple comparison post-test. Significance levels given as *P<0,05.



Supporting Figure 2: The extracellular linker domain in the FZD_4 crystal structure contains the conserved triad of cysteines engaged in disulphide bonds stabilizing an antiparallel β sheet.

(**A**) Presentation of the FZD_4 structure (PDB 6BD4; (1)) highlighting the linker domain (green) and the conserved cysteine triade (Cys181, 200, 204) and the cystein in the ECL1 (Cys282) in FZD_4. Numbers refer to amino acid numbering in the human FZD_4 (www.gpcrdb.org). (**B**) Zoom-in to the linker domain to visualize the presence of the antiparallel β-sheet in the linker domain. The structured linker domain of the crystallized FZD_4 protein (ΔCRD-FZD_4; aa 178-517) was resolved starting with Cys181. Structures were rendered using PyMol software (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC).

Supporting Figures

References

1. Yang, S., Wu, Y., Xu, T. H., de Waal, P. W., He, Y., Pu, M., Chen, Y., DeBruine, Z. J., Zhang, B., Zaidi, S. A., Popov, P., Guo, Y., Han, G. W., Lu, Y., Suino-Powell, K., Dong, S., Harikumar, K. G., Miller, L. J., Katritch, V., Xu, H. E., Shui, W., Stevens, R. C., Melcher, K., Zhao, S., and Xu, F. (2018) Crystal structure of the Frizzled 4 receptor in a ligand-free state. *Nature*