Supplementary Data

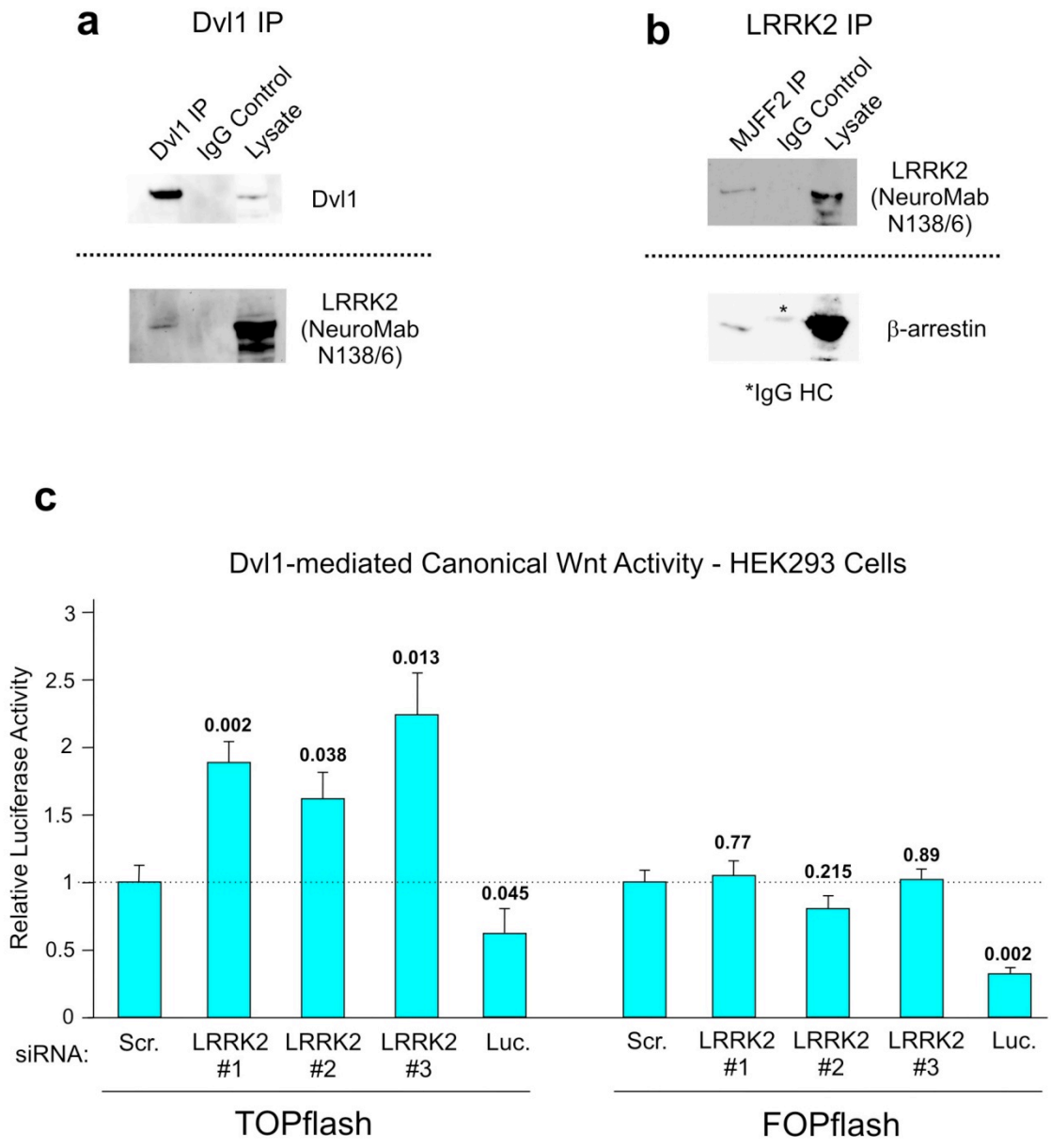

Figure S1. Further evidence for a role for endogenous LRRK2 in Wnt signaling. A) DVL1 immunoprecipitates from mouse brain cytosol show immunoreactivity for LRRK2. B) LRRK2 interacts with β-arrestin in mouse brain. Experiments performed in parallel with those shown in Figure 1a. IgG heavy chain present in the control IgG lane is indicated by an asterisk. C) Cells were transfected with DVL1 and the indicated siRNA to LRRK2 or a scrambled siRNA control and resultant canonical Wnt activity was assayed 24 hrs later. siRNA to luciferase was used as a positive control for knockdown. The figure shows that three independent LRRK2 siRNAs enhance DVL1-mediated TOPflash activity but have no significant effects on the control FOPflash reporter plasmid.

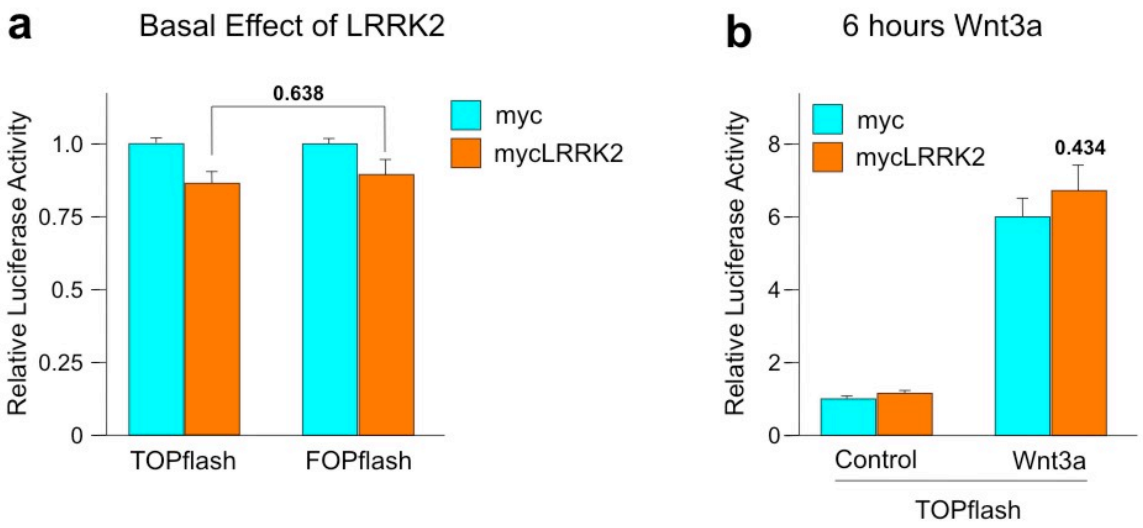

Figure S2. No effect of LRRK2 over-expression on basal and Wnt3a-stimulated β-catenin activation in SH-SY5Y cells. A) Combined data from all experiments investigating the basal effect of LRRK2 over-expression on TOPflash or FOPflash activity relative to vector-transfected controls. TOPflash + vector, n=43; TOPflash + mycLRRK2, n=44; FOPflash + vector, n=27; FOPflash + LRRK2, n=27. LRRK2 over-expression induces a weak but near-identical suppression of both reporters, and thus any apparent inhibition of canonical Wnt activity elicited by ectopic LRRK2 is most likely non-specific. B) LRRK2 over-expression also has no significant effect on TOPflash activity elicited by 6 hrs 100 ng/ml Wnt3a treatment.

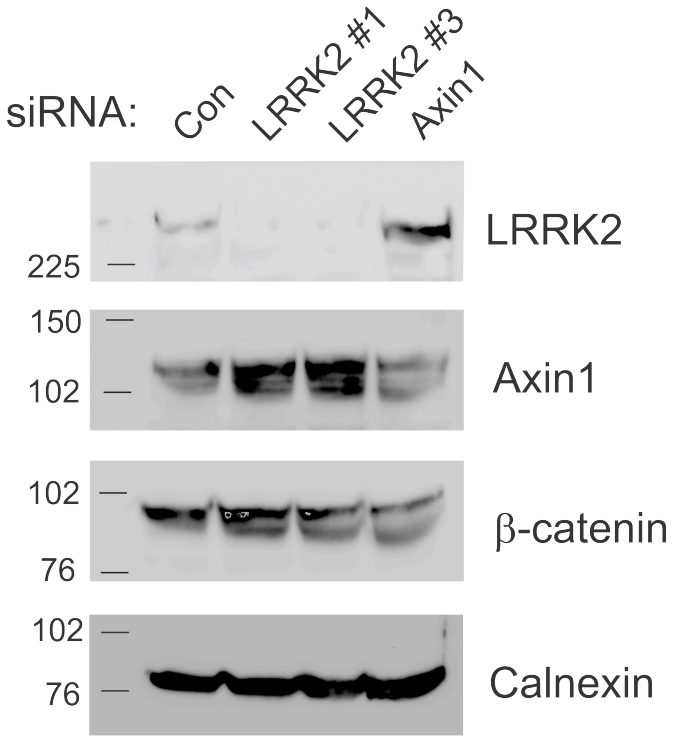

Figure S3. Specificity of siRNAs used in Figure 1c.
HEK293 cells grown in 60mm dishes were transfected with 500 pmol of each of the indicated siRNAs. After 48 hours cells were extracted into buffer containing 20mM Tris, pH 7.5, 50 mM NaCl, 1% Triton X-100 1x complete protease inhibitor cocktail (Roche) and 1x Halt phosphatase inhibitor cocktail (Pierce). Lysates were clarified by centrifugation and resolved by SDS-PAGE prior to transfer to PVDF membrane. Membranes were probed with MJFF2 LRRK2 antibody (Epitomics; upper panel) to show efficient knockdown of endogenous LRRK2 with LRRK2 siRNAs #1 and #3, and Axin1 antibody (Cell Signaling; second panel) to use knockdown of endogenous Axin. Samples were reprobed with antibody to b-catenin (Cell Signaling; third panel) to show increased electrophoretic mobility of b-catenin following knockdown of LRRK2 or Axin1. Reprobes for calnexin (Abcam; lower panel) are included as loading controls.

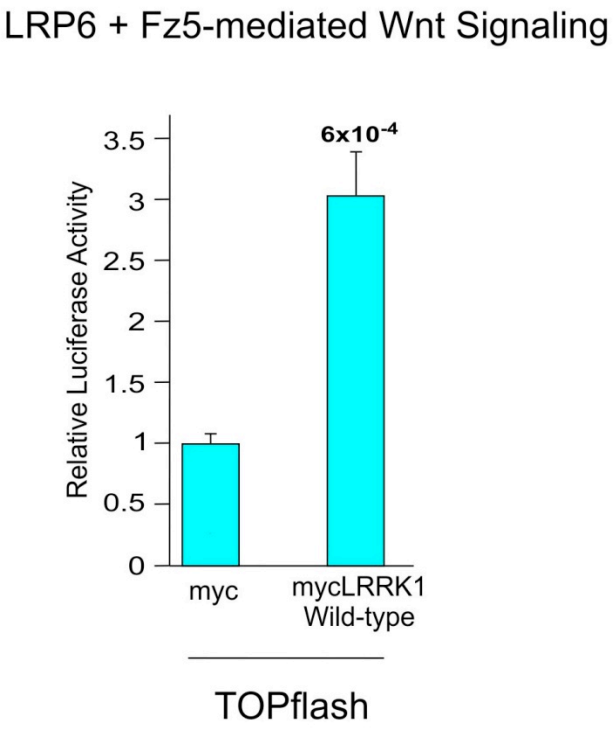

Figure S4. LRRK1 enhances LRP6 and Fz5-driven β-catenin activation. Co-transfected mycLRRK1 increases the TOPflash activation elicited by the combination of EGFP-Fz5 and LRP6-HA compared to myc-epitope control.

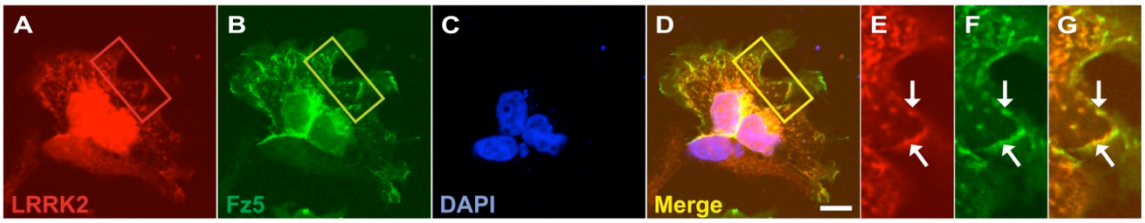

Figure S5. Partial co-localization of LRRK2 with Fz receptors. Co-expression of myc-tagged LRRK2 (red) and EGFP-Fz5 (green) led to partial co-localization of the two proteins (A-G). Comparable results were obtained using two further EGFP-tagged Frizzled Receptors, EGFP-Fz1 and EGFP-Fz4 (data not shown). DNA staining with DAPI (blue). Please note that the image for LRRK2 is calibrated to show the residual amount of LRRK2 present at the cell membrane under basal conditions. LRRK2 is not expressed in the nuclei of the cells shown. Scale bar: 10 μm.

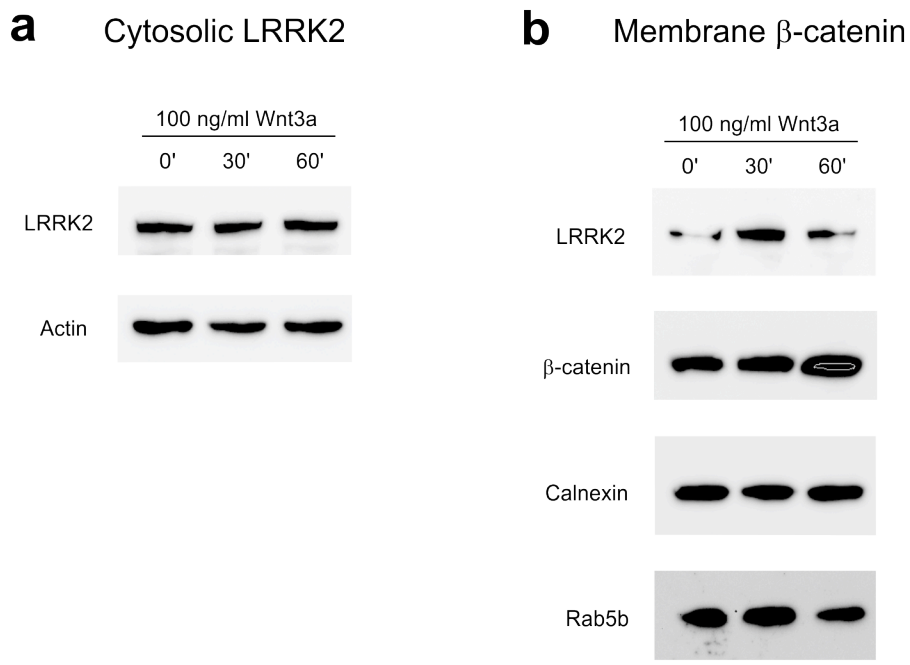

Figure S6. LRRK2 and β-catenin are recruited to membranes by Wnt3a. Acute treatment of HEK293 cells with recombinant Wnt3a increases the amount of endogenous LRRK2 and β-catenin present in crude membrane fractions. a) LRRK2 protein levels in the cytosolic fraction and b) LRRK2 and β-catenin levels in the membrane fraction after Wnt3a treatment.

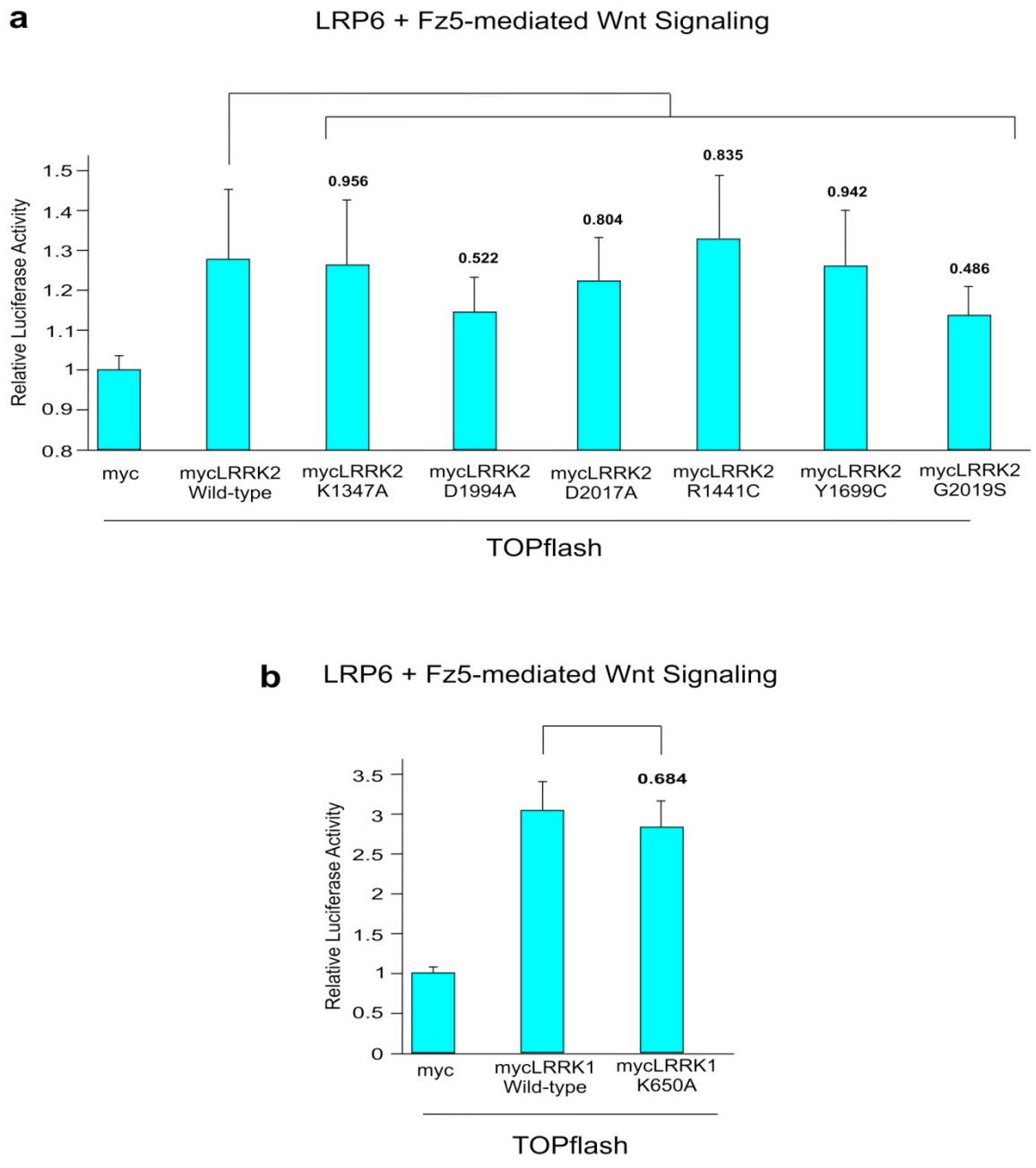

Figure S7. β-catenin activation induced by LRP6 and Fz5 over-expression is unaffected by pathological *PARK8* mutations, kinase-dead mutations or GTP-non-binding mutations. A) Artificial mutations preventing the binding of ATP to the LRRK2 kinase domain (D1994A, D2017A) or GTP/GDP to the LRRK2 Roc domain (K1347A) have no effect on the increase in LRP6 and Fz5-induced TOPflash activity elicited by LRRK2 over-expression. Similarly, the pathogenic *PARK8* mutations, R1441C, Y1699C and G2019S also enhance LRP6 and Fz5-driven Wnt signaling to a similar extent to wild-type LRRK2. B) Introduction of the analogous GTP/GDP-non-binding mutation into LRRK1 (K650A) has no effect on the capacity of this protein to enhance LRP6 and Fz5-induced TOPflash activity.