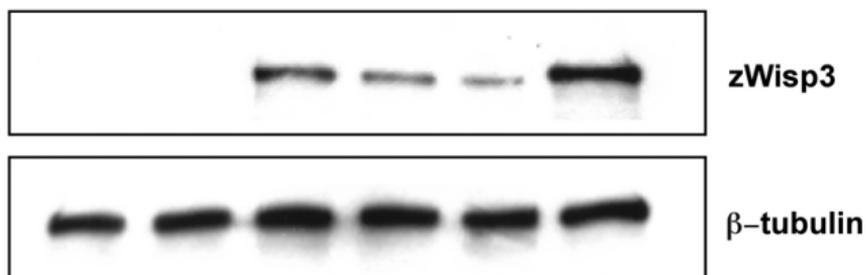
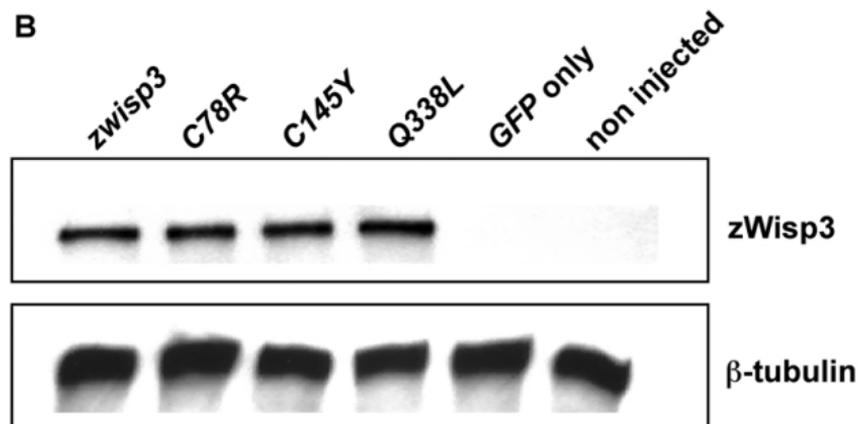


A**B****Figure S1**

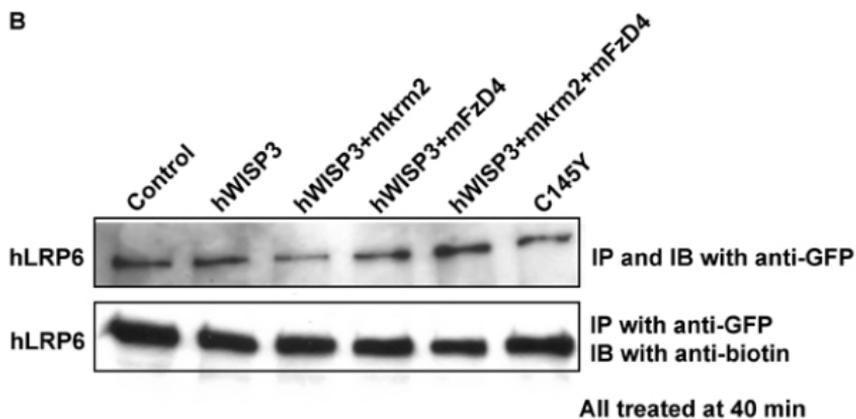
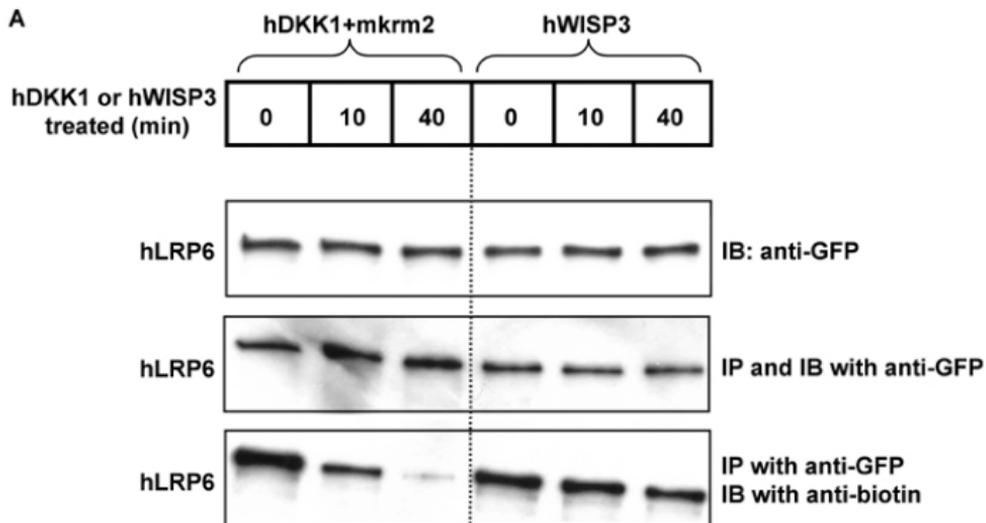


Figure S2

Supplemental Figure 1: WISP3-C antibody specifically detects zWisp3 and wild-type and PPD-associated missense mutants are equally abundant when over-expressed in zebrafish embryos. (A) Western blot of zWisp3 protein extracted from de-yolked embryos (24 hpf) or larvae (120 hpf). Sixty μ g of protein was separated by 10% SDS-PAGE under reducing conditions and immunodetected using WISP3-C antibody (upper panel) or anti- β -tubulin antibody (lower panel) as a control. Note immunodelectable zWisp3 is present at 24 hpf only when over-expressed. See Fig. 2E for temporal pattern of endogenous protein expression. (B) Western blot of protein extracted from de-yolked embryos at 24 hpf that had been injected with RNA encoding wild-type zWisp3 or 3 PPD-associated missense mutants. Sixty μ g of protein was separated by 10% SDS-PAGE under reducing conditions and immunodetected using WISP3-C antibody (upper panel) or anti- β -tubulin antibody (lower panel) as a control. Note comparable intensities of the zWisp3 bands in the wild-type and missense mutant injected embryos. Control 24 hpf embryos (non injected) and embryos injected only with GFP RNA serve as a negative control.

Supplemental Figure 2: hWISP3 does not promote the internalization of hLRP6-EGFP. (A) Cell surface proteins were biotinylated 0, 10, and 40 minutes after addition of CM containing hDDK1 or hWISP3. (top panel) Immunodetection of total hLRP6-EGFP in the cell lysate using anti-GFP antibody (IB: anti-GFP). Note that hDDK1+ mkrm2 and hWISP3 treated cells have comparable total amounts of hLRP6-EGFP. (middle panel) Immunodetection of hLRP6-EGFP following immunoprecipitation of cell lysates with anti-GFP antibody (IP and IB with anti-GFP). Note that comparable

amounts of hLRP6-EGFP are immunoprecipitated in hDKK1+ mkrm2 and hWISP3 treated cells. (lower panel) Immunodetection of biotinylated hLRP6-EGFP following immunoprecipitation of cell lysates using the anti-GFP antibody (IP: anti-GFP, IB: anti-biotin). Note that the amount of biotinylated hLRP6-EGFP decreases in the hDKK1+ mkrm2 treated cells, indicative of receptor internalization. No decrease is observed in the hWISP3 treated cells, suggesting that hWISP3 does not promote internalization of hLRP6-EGFP. **(B)** similar experiment to the 40 minute time point in panel **(A)**, except that hWISP3 CM was given to the cells expressing hLRP6-EGFP, mkrm2 and/or mFzD4 to determine whether these other cell surface proteins were needed to promote hWISP3-mediated hLRP6 internalization. (upper panel) Immunodetection of hLRP6-EGFP following immunoprecipitation of cell lysates with an anti-GFP antibody. Note that comparable amounts of hLRP6-EGFP are immunoprecipitated in all cells. (lower panel) Immunodetection of biotinylated hLRP6-EGFP following immunoprecipitation of cell lysates using the anti-GFP antibody. Note that none of the wild-type hWISP3 including C145Y treated cells have decreased biotinylated hLRP6-EGFP compared to control.