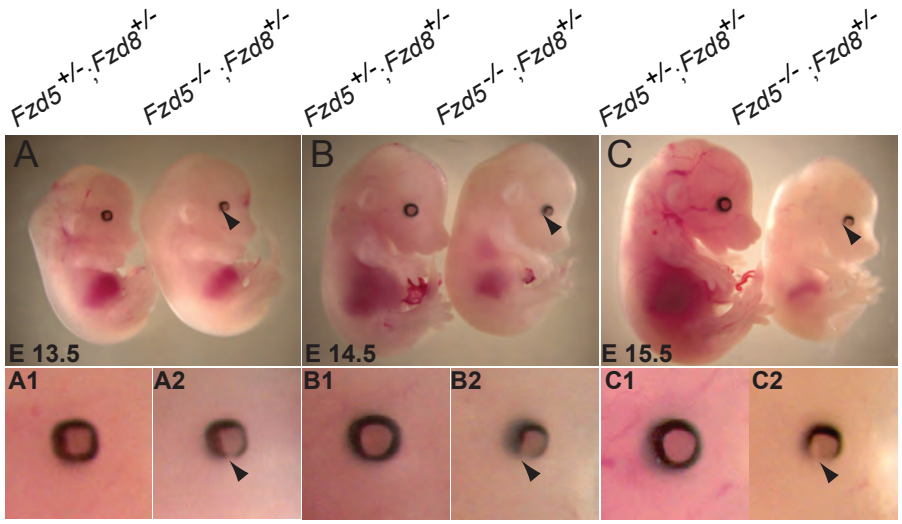
Supplementary table 1: Rare *FZD5* variant identified in coloboma patients

Patient id	cDNA change	Protein change	dbSNP id	1000G/EVS MAF	Polyphen prediction	SIFT prediction
49	c.A290T	*D97V	NA	NA	Possibly damaging	Damaging

* D97V variant has not been detected in control genomes, SNP database and 1000 Genome Project. Primary tests show that this variant increases FZD5-mediated canonical Wnt activity.

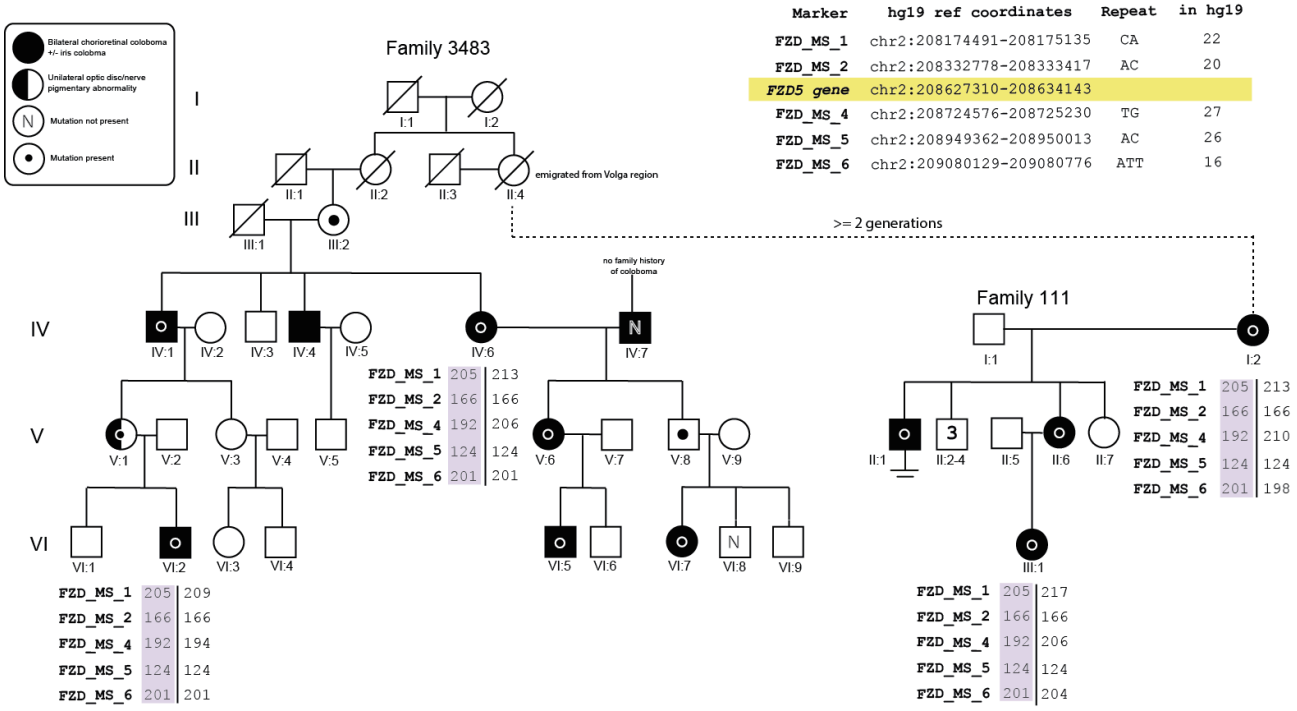
Supplementary table 2: *FZD5* gene amplification primers (5'>3')

FZD5 Primer1F	tgccaggcgcgctcgccctcc
FZD5 Primer2F	taaccgtctctccccagccctatc
FZD5 Primer3F	cgcgacgccgaggtcctctgcatg
FZD5 Primer4F	gcagtacttccacctggctgcgt
FZD5 Primer5R	cacccactacctctcaggcac
FZD5 Primer6R	agaaacgcaaaatagaatacac
FZD5 Primer7R	cgtcttggtgccgccctgcttg
FZD5 Primer8R	ggtagcagggtaccgcgcag
*FZD5*Primer9R	gatgggtcaggccgagccatc
*FZD5*_ex2_1F	gtagcgcgacggccagtgagatttggagacagctcgc
*FZD5*_ex2_2F	gtagcgcgacggccagtctggaggtgcaccagttct
*FZD5*_ex2_3F	gtagcgcgacggccagtgtccttcagtgccgacgag
*FZD5*_ex2_4F	gtagcgcgacggccagtgcaaccagaacctgaactcg
*FZD5*_ex2_1R	cagggcgcagcgatgacgcagacagatgggcgtgta
*FZD5*_ex2_2R	cagggcgcagcgatgactccatgtcgatgaggaaggt
*FZD5*_ex2_3R	cagggcgcagcgatgacgaagagcgacacgaagcc
*FZD5*_ex2_4R	cagggcgcagcgatgactaaacggaagtgaccttggc



Supplementary Figure 1

A



B

Marker	hg19 ref coordinates	Repeat	Repeats in hg19
FZD_MS_1	chr2:208174491-208175135	CA	22
FZD_MS_2	chr2:208332778-208333417	AC	20
FZD5 gene	chr2:208627310-208634143		
FZD_MS_4	chr2:208724576-208725230	TG	27
FZD_MS_5	chr2:208949362-208950013	AC	26
FZD_MS_6	chr2:209080129-209080776	ATT	16

Supplementary Figure 2

Frizzled 5 protein N-terminal alignment across species

```
                Protein Domains  [---------signal peptide(aa1-26)------]
Human WT              MARPD----PSAPPS--------LLLLLLAQLVGRAAAASKAPVCQEITVPMCRGIGYNL 48
cow                   MARPD----PSAPPS--------LLLLLLAQLAGRAAAASKAPACQEITVPMCRGIGYNL 48
pig                   MARPD----PSAPPS--------LLLLLLAQLAGRATAASKAPVCQEITVPMCRGIGYNL 48
dog                   MARPD----PCAPPS--------LLLLLLAQLLGRAAAASKAPVCQEITVPMCRGIGYNL 48
elephant              MARLD----PSAPPP--------LLLLLLAQLVGRAAAASKAPVCQEITVPMCRGIGYNL 48
gorilla               MARPD----PSAPPS--------LLLLLLAQLVGRAAAASKAPVCQEITVPMCRGIGYNL 48
chimp                 MARPD----PSAPPS--------LLLLLLAQLVGRAAAASKAPVCQEITVPMCRGIGYNL 48
mouse                 MARPD----PSAPPS--------LLLLLLAQLVGRAAAASKAPVCQEITVPMCRGIGYNL 48
zebrafish             MRKPADEHHFTMETSGMHLVGFWLHVLLLFQLSGLGDSASKDIVCEPITVPMCKGIGYNH 60
                      * :          ..        * :*** ** * . :***  .*: ******:*****.

                    -----frizzled domain(~aa30-152)(aka cysteine rich domain)---
Human WT                THMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTPICLPDYHKPLPPCRSV 108
cow                     THMPNQFNHDTQDEAGLEVHQFWPLVEIHCSPDLRFFLCSMYTPICLPDYHKPLPPCRSV 108
pig                     THMPNQFNHDTQDEAGLEVHQFWPLVEIHCSPDLRFFLCSMYTPICLPDYHKPLPPCRSV 108
dog                     THMPNQFNHDTQDEAGLEVHQFWPLVEIHCSPDLRFFLCSMYTPICLPDYHKPLPPCRSV 108
elephant                THMPNQFNHDTQDEAGLEVHQFWPLVEIHCSPDLRFFLCSMYTPICLEDYHKPLPPCRSV 108
gorilla                 THMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTPICLPDYHKPLPPCRSV 108
chimp                   THMPNQFNHDTQDEAGLEVHQFWPLVEIQCSPDLRFFLCSMYTPICLPDYHKPLPPCRSV 108
mouse                   THMPNQFNHDTQDEAGLEVHQFWPLVEIHCSPDLRFFLCSMYTPICLPDYHKPLPPCRSV 108
zebrafish               TYMPNQFNHDNQDEVGLEVHQFWPLVRIHCSPDLLFFLCSMYTPICLQDYKKPLPPCRSV 120
                        *:********.***.***********.*:***** ************ **:*********
                        ----------------------------------------]
Human WT                CERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATTAPPRPFPAKPT 168
cow                     CERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATTAPPRPFPVKPT 168
pig                     CERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATTAPPRPFPAKPT 168
dog                     CERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATTAPPRPFPAKPT 168
elephant                CERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATTAPPRPFTAKPT 168
gorilla                 CERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATTAPPRPFPAKPT 168
chimp                   CERAKAGCSPLMRQYGFAWPERMSCDRLPVLGRDAEVLCMDYNRSEATTAPPRPFPAKPT 168
mouse                   CERAKAGCSPLMRQYGFAWPERMSCDRLPVLGGDAEVLCMDYNRSEATTASPKSFPAKPT 168
zebrafish               CERAKRGCSPLMIQYGFEWPERMSCEQLPMLG-DTDRLCMDRNSSETTTLSP-PFP-KPT 177
                        ***** ****** **** *******::**:** *:: **** * **:** .* .*. ***

                            First altered aa (A219X) in frameshift
Human WT                LPGPPGAPASGGECPAG-GPFVCKCREPFVPILKESHPLYNKVRTGQVPNCAVPCYQP-- 227
cow                     LSGLPGSPASGNDCAAG-GPSVCKCREPFVPILKESHPLYNKVRTGQVPNCAVPCYQP-- 227
pig                     LSGPPGAPASGSDCVAG-GPSVCKCREPFVPILKESHPLYNKVRTGQVPNCAVPCYQP-- 227
dog                     LPGPAGGPASGAECAAG-APSVCKCREPFVPILKESHPLYNKVRTGQVPNCAVPCYQP-- 227
elephant                HPGLPGAPASGVECAAG-GPSVCKCREPFVPILKESHPLYNKVRTGQVPNCAVPCYQP-- 227
gorilla                 LPGPPGAPASGGECPAG-GPFVCKCREPFVPILKESHPLYNKVRTGQVPNCAVPCYQP-- 227
chimp                   LPGPPGAPASGGECPAG-GPFVCKCREPFVPILKESHPLYNKVRTGQVPNCAVPCYQP-- 227
mouse                   LPGPPGAPSSGGECPSG-GPSVCTCREPFVPILKESHPLYNKVRTGQVPNCAVPCYQP-- 227
zebrafish               PKGTPRHRATAKSAPPQKCDRECHCRGPLVPIKKEAHPLHNRVNTGSLPNCALPCHQP-- 237
                        * .   ::. .. .      * ** *:*** **:***:*:*.**.:****:**:** *
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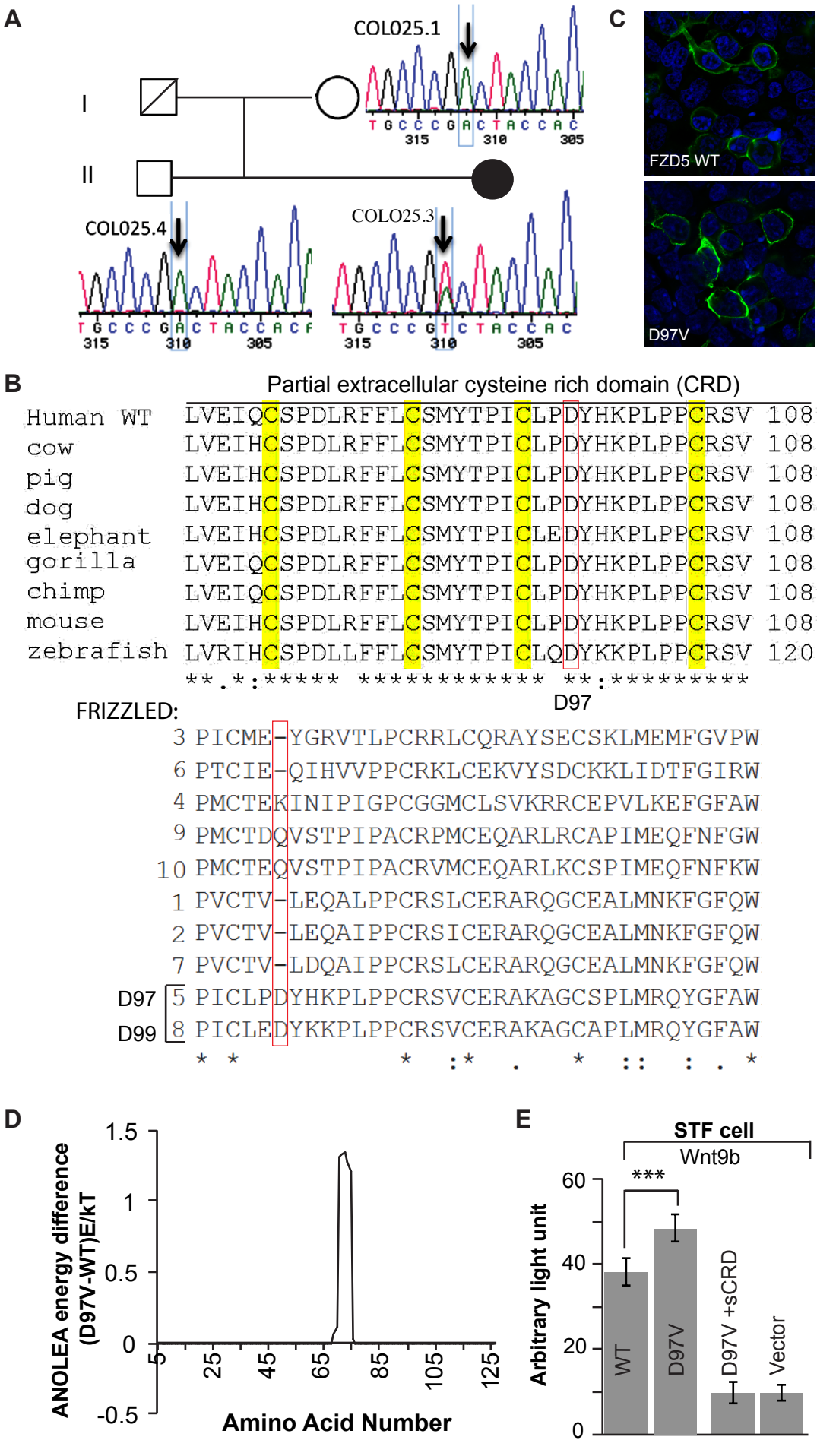
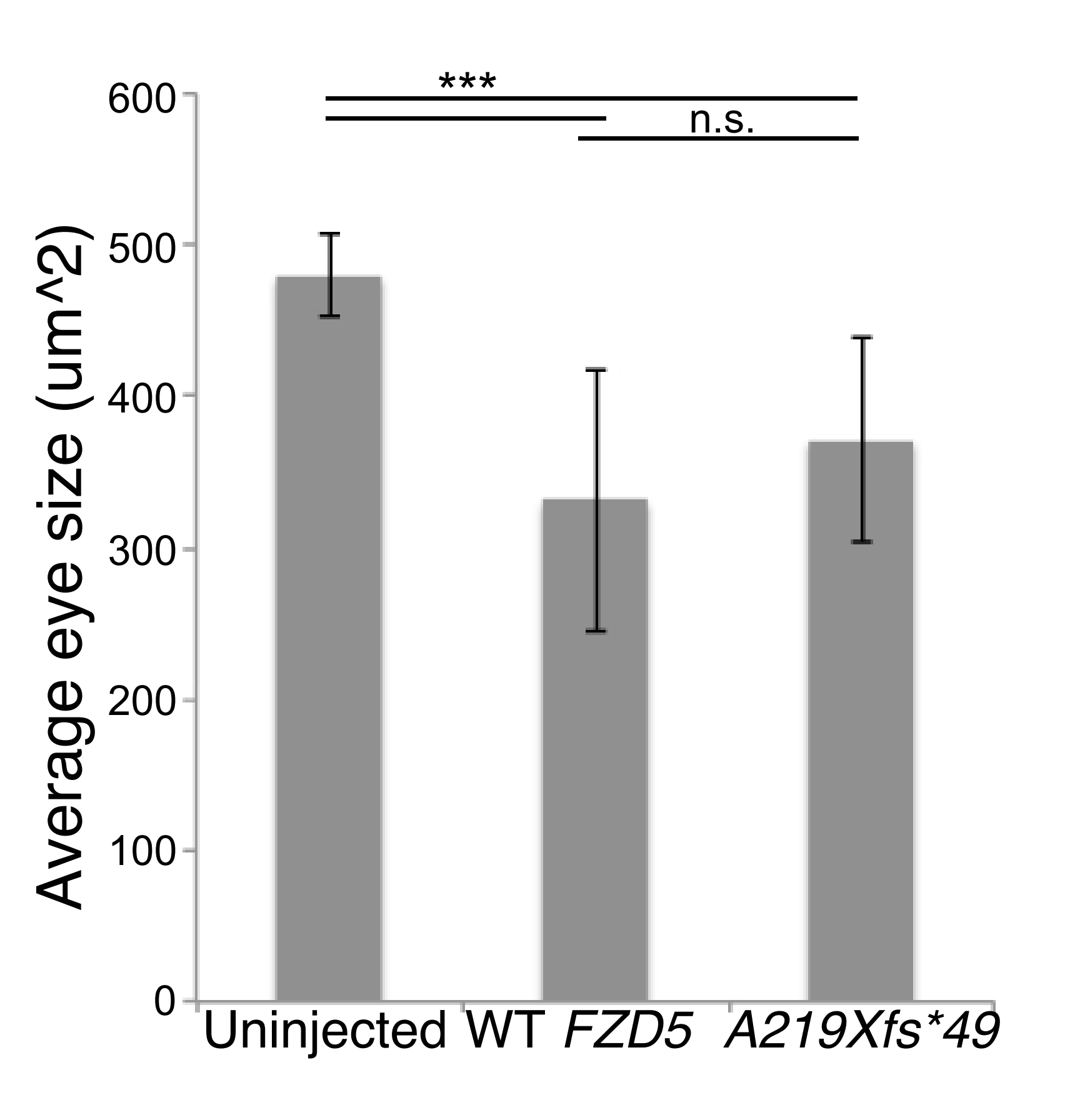
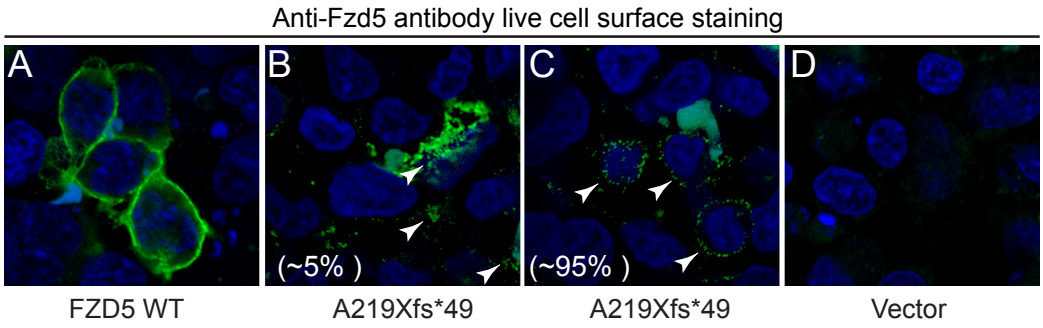
Supplementary Figure 3

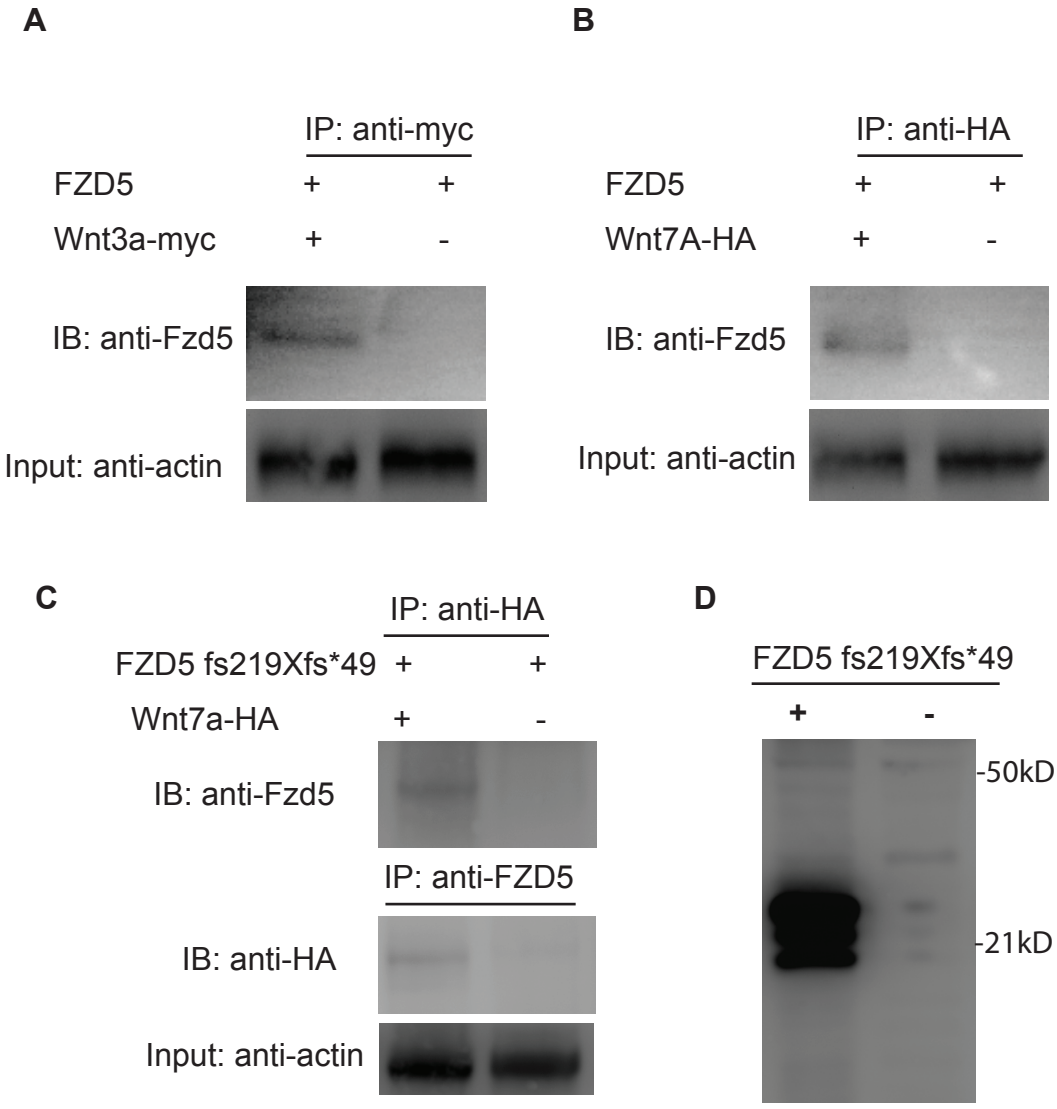


Supplementary Figure 4

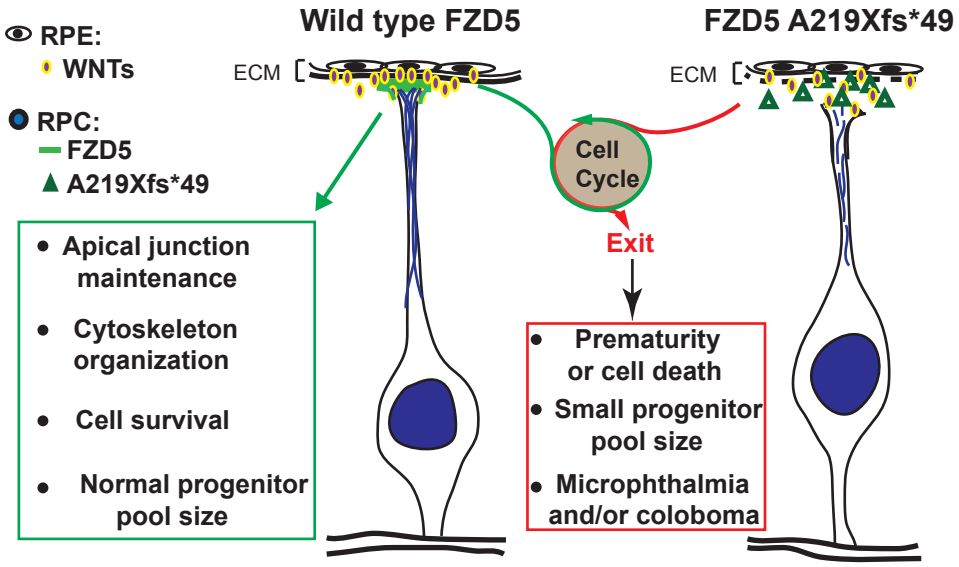

***
n.s.
600
500
400
300
200
100
0
Average eye size (um^2)
Uninjected
WT
FZD5
A219Xfs*49



Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8

Supplementary Figure Legends

Supplementary Figure 1. Microphthalmia and retinal coloboma manifested in the compound mutants of homozygous *Fzd5* conditional knockout (*Fzd5*$^{-/-}$) with one allele of *Fzd8* mutation (Fzd8$^{+/-}$). Lower panels are larger views of developing eyes corresponding to upper panels. Arrowheads indicate open optic fissure/coloboma. Transgenic *Sox2*-Cre was used to excise loxP sites. A, A1, A2, E13.5; B, B1, B2, E14.5; C, C1, C2, E15.5.

Supplementary Figure 2. Microsatellite markers and Haplotype analysis of Families 3484 and 111. A. Shows the same pedigree as Figure 1. **B**. Gives the absolute genomic coordinates of the five microsatellite markers used in this analysis and shows their position relative to the *FZD5* gene (yellow highlight). Under four affected individuals (Family 3483 IV:6 & VI:2 and Family 111 1:2 & III:1) are shown the most plausible locus haplotypes for each individual. The purple highlight indicates the identical haplotype shared by each of the affected individual suggesting recent common ancestry. It should be noted that markers FZD_MS_2 and FZD_MS_5 are not informative for haplotype construction.

Supplementary Figure 3. Conservation of FZD5 Wnt-binding cysteine rich domain (CRD) across species. Yellow bars highlighted 10 conserved cystines in FZD5 CRD. The purple bar indicates mutant protein insertion/deletion point (p219A).

Supplementary Figure 4. Identification of a novel missense mutation -FZD5 D97V. A. Sequences of a two-generation family with the daughter OC patient carrying D97V mutation. **B.** Upper panel: Alignment of Frizzled 5 CRD region from multiple species showing the conservation of D97. Lower panel: Alignment of ten FZD CRDs shows that D97 is variable except for FZD8 and FZD5. **C**. FZD5 D97V protein is correctly localized in transfected cells. **D.** Atomic non-local environment assessment (ANOLEA) predicted that the D97V variant perturbs local interactions. **E.** Slightly increase in Wnt9b induced canonical Wnt activity by D97V can be abolished by FZD5 sCRD. Student t-test was used for statistical analysis. ***, P<0.0001.

Supplementary Figure 5. Eye size analysis of zebrafish embryos injected with *FZD5* mRNA. Zebrafish embryos were injected at the 1-cell stage with 200 pg of either WT *FZD5* or *A219Xfs*49 FZD5* mRNA and imaged at 3 dpf. Ocular area measurements were taken 3 times with ImageJ and averaged for each eye. Measurements for eyes in each injection group were then averaged (Un-injected, N=12 embryos; WT *FZD5*, N=20 embryos; *A219Xfs*49 FZD5*, N=30 embryos). ***, p<0.0003; n.s.=not significant; t-test with Bonferroni correction for multiple comparisons.

Supplementary Figure 6. Cellular localization of wild type and mutant FZD5 protein. Immunofluorescence detection of FZD5 proteins (green) on cell surface.

Images were merged with DAPI indicating the nucleus. **A**, Wild type FZD5 was localized on the cell membrane. **B-C**, A majority of A219Xfs*49 mutant protein was present in extracellular space, presumptively, ECM (arrowheads). The distribution of the mutant proteins appeared to be uneven with about 5% cells showing locally heavy and/or dispersed deposition (**B**, arrowhead), whilst the rest (~95% **C**, arrowhead) showing local (near-membrane) FZD5 distribution. **D**, Negative control with vector transfection was shown in right panel. To avoid the cytoplasm staining, live cells were first incubated with anti-FZD5 antibody in cultured medium, washed with PBS, and then post-fixed with PFA for further immunohistochemistry.

Supplementary Figure 7. FZD5 A219Xfs*49 binds to Wnt. A, FZD5 binds to myc-tagged Wnt3a. HEK293T cells co-transfected with *Wnt3a-myc* and *FZD5*. Cell extracts were immunoprecipitated (IP) with anti-myc antibody, and immunoblotted (IB) with anti-FZD5 antibody. **B,** FZD5 binds to HA-tagged WNT7A. HEK293T cells co-transfected with *WNT7A-HA* and *FZD5*. Cell extracts were immunoprecipitated with anti-HA antibody, and the immunoblot was probed with anti-FZD5 antibody. **C,** FZD5219Xfs*49 protein binds to HA-tagged WNT7A. HEK293 cells were co-transfected with *WNT7A-HA* and *FZD5219Xfs*49* constructs. Cell extracts were immunoprecipitated with anti-HA antibody, and the protein blot was probed with anti-FZD5 antibody. Reverse IP was conducted with anti-FZD5 antibody and the blot was probed with anti-HA antibody. **D,** Multiple bands of FZD5219Xfs*49 were detected under reducing conditions using anti-

FZD5 antibody.

Supplementary Figure 8. A model of coloboma disease mechanism caused by *FZD5 A219Xfs*49*. During development, WNT signaling is crucial for maintenance of neuroblast apical junction, cell polarity, cell survival and proliferation. By competing for Wnt ligands, A219Xfs*49 mutant protein (dark green triangles) may intercept WNTs (yellow circles), which are secreted from RPE at the apical extracellular matrices during development (brackets), preventing FZD5 (from RPC apical membrane, bright green lines) - evoked WNT signaling in the neuroblasts (blue nuclear cells). Consequently, insufficient WNT-FZD5 signaling leads to early cell cycle exit, prematurity and cell death, and reduced progenitor pool size, resulting in microphthalmia and/or coloboma. The model is modified from Liu et al., 2012 (32).