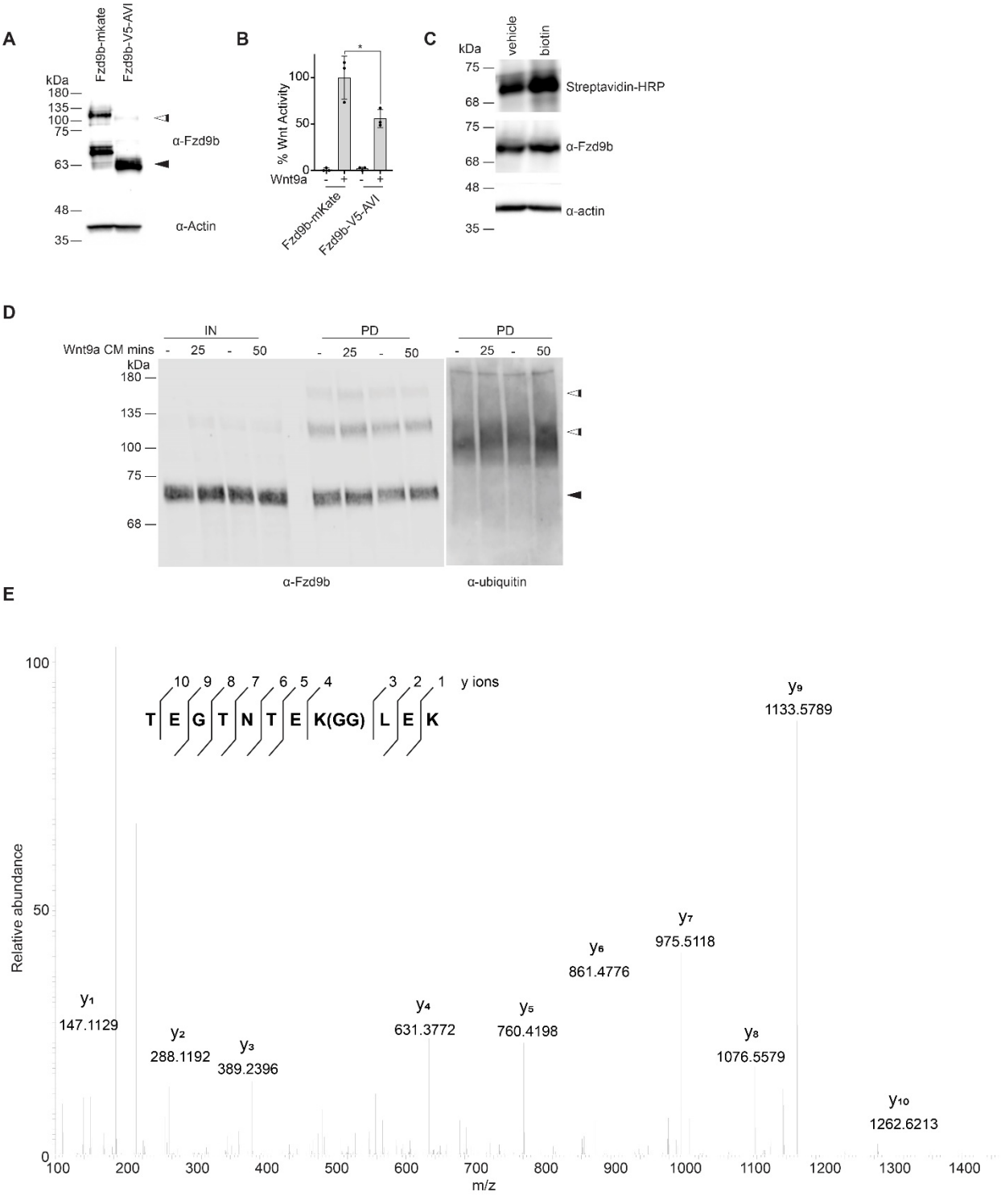
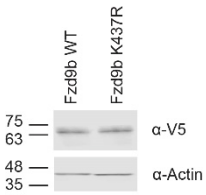
Supplementary Figures

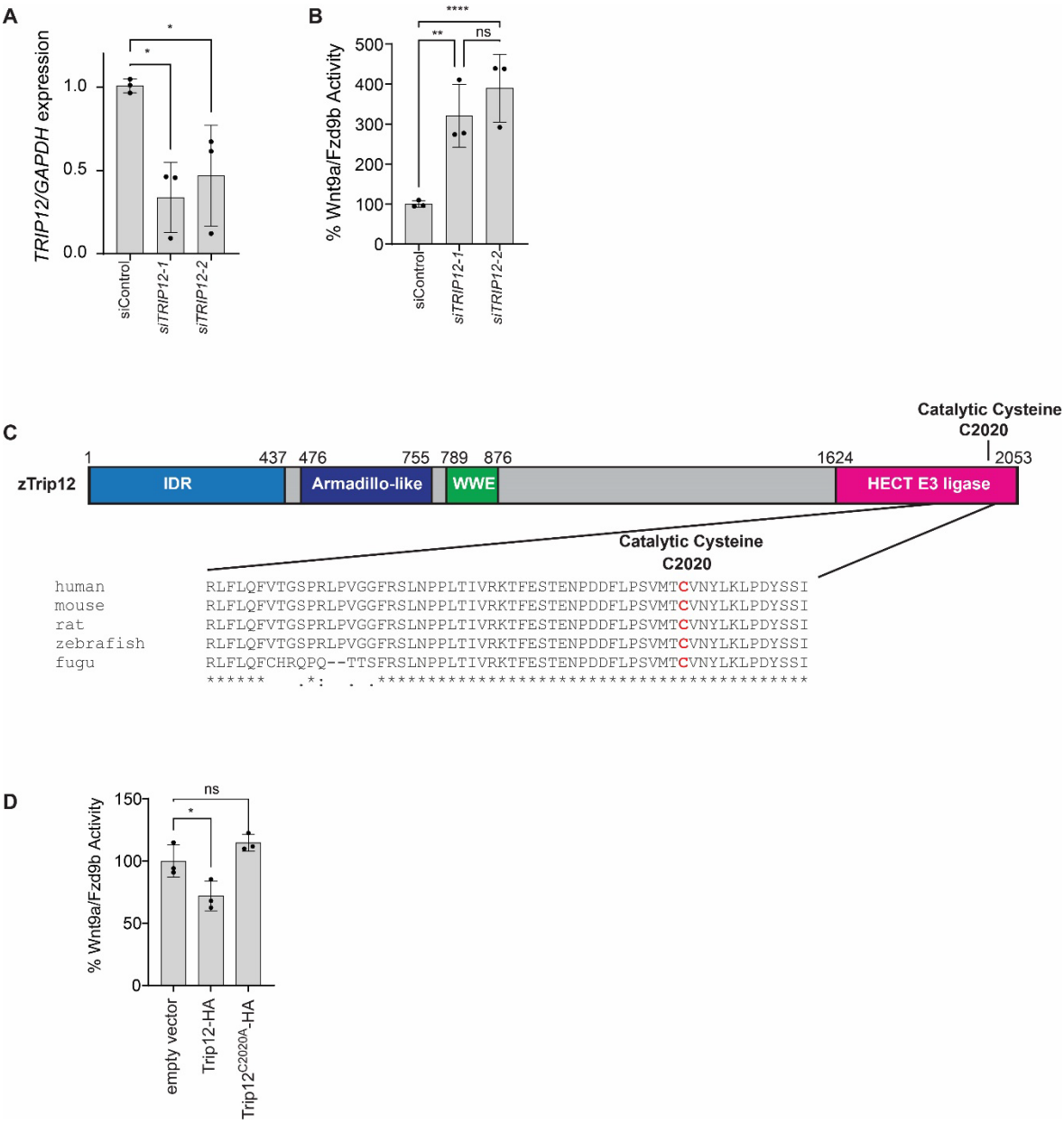


Supplementary Figure 1: Fzd9b is ubiquitinated. A. Representative immunoblot of protein extracted from HEK293 Fzd9b-mKate or Fzd9b-V5-AVI cells. Black arrowhead points to the

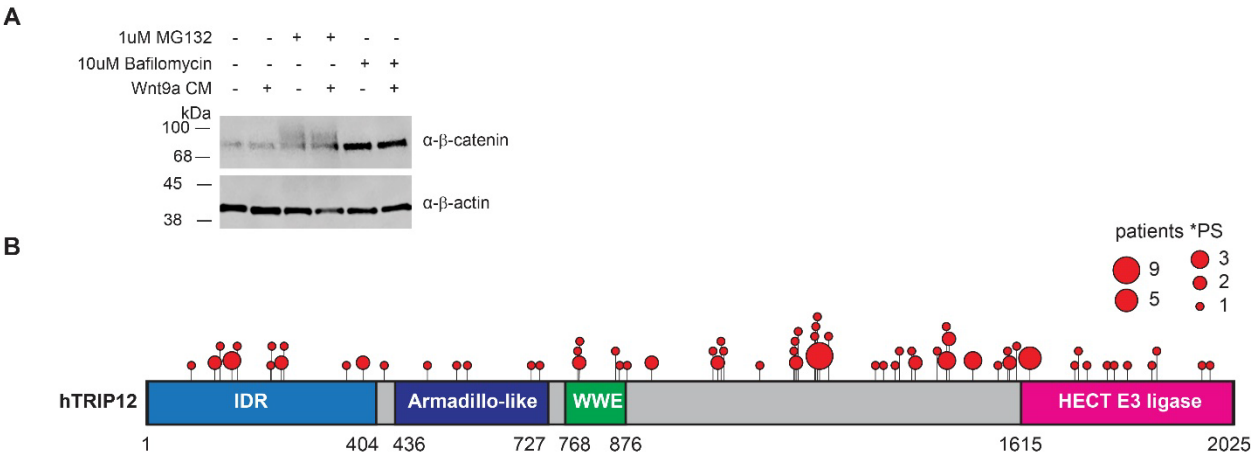
expected size for Fzd9b; white arrowhead points to additional sized bands detected by the Fzd9b antibody. **B.** Super TOP Flash assays to measure Wnt responses in Fzd9b-mKate or Fzd9b-V5-AVI HEK293 cells treated with Wnt9a conditioned medium as indicated. All data presented are from N=3 biological replicates, with different experiments conducted on different days a total of 3 times with similar results. *P<0.05 by ANOVA with Tukey post-hoc comparison. **C**. Representative immunoblot of protein extracted from HEK293 Fzd9b-V5-AVI cells treated with vehicle or biotin. **D**. Immunoblot of input (5%, IN) and pulldown (PD) of protein extracted from HEK293 Fzd9b-V5-AVI cells treated with mock or Wnt9a conditioned medium (CM) for 25 or 50 minutes as indicated. Blots for Fzd9b and ubiquitin antibodies are shown. Black arrowhead points to the expected size for Fzd9b; white arrowheads point to additional sized bands detected by the Fzd9b antibody. **E**. MS2 spectra of the synthetic ubiquitinated peptide (TEGTNTEK(GG)LEK) of Fzb9b, displaying the relative abundance of the fragment ions. All fragment ions were manually assigned. GG denotes the di-glycyl remnant produced on ubiquitinated lysine residues (K-ε-GG) following trypsin digestion.



Supplementary Figure 2: Fzd9b K437R does not impact on Fzd9b expression. Immunoblot of WT and K437R Fzd9b protein lysates.



Supplementary Figure 3: TRIP12 regulates Wnt9a/Fzd9b signaling. A. Relative quantification of human *TRIP12* transcript following transfection with control, or 2 different *TRIP12* siRNAs. **B.** Super TOP Flash assays to measure Wnt responses in Fzd9b-V5-AVI HEK293 cells treated with siRNAs and Wnt9a conditioned medium as indicated. **C.** Diagram of zebrafish Trip12 with protein domains noted. Below, alignment of several species of Trip12 showing conserved catalytic cysteine. **D.** Super TOP Flash assays to measure Wnt responses in Fzd9b-V5-AVI HEK293 cells transfected with empty vector, Trip12, or Trip12^{C2020A}, and treated Wnt9a conditioned medium as indicated. All data presented are from N=3 biological replicates, with different experiments conducted on different days a total of 3 times with similar results. ns-not significant, *P<0.05, **P<0.01, ****P<0.0001 by ANOVA with Tukey post-hoc comparison.



C

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Fzd6      326   GIVALNRVRQVIQHDQLNQQKLKRFMI    352
Fzd3a     394   GIAALNRVRMEIPLEKENQDKLVKFMI    420
Fzd3b     404   GIVALNRVRMEIPLEKENQEKLVKFMI    430
Fzd4      393   GLVALFKIRSNLQKDGTKTDKLERLMV    419
Fzd10     415   GFVALFHIRKVMKTEGENTDKLEKLMV    441
Fzd9a     414   GFVALFHIRKIMKTGGTNTEKLEKLMV    440
Fzd9b     416   GFVALFHIRKVMKTEGTNTEKLEKLMV    442
Fzd2      418   GFVSLFRIRTIMKHDGTKTEKLERLMV    444
Fzd1      407   GFVSLFRIRTIMKHDGTKTEKLEKLMV    433
Fzd7a     427   GFVSLFRIRTIMKHDGTKTEKLEKLMV    453
Fzd7b     426   GFVSLFRIRTIMKHDGTKTEKLEKLMV    452
Fzd5      433   GFVSLFRIRSVIKQGGTKTDKLEKLMI    459
Fzd8a     426   GFVSLFRIRSVIKQGGTKTDKLEKLMI    452
Fzd8b     419   GFVSMFRIRSVIKQGGTKTDKLERLMV    445
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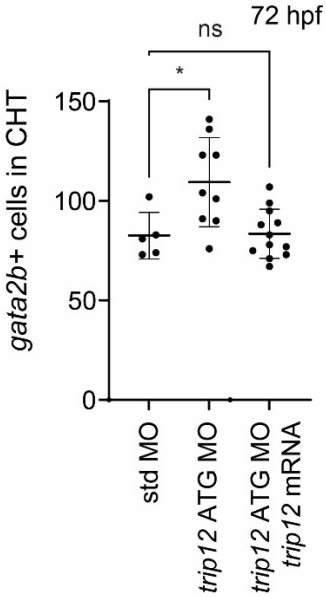
Supplementary Figure 4: Fzd9b is degraded by the lysosome in response to Trip12. A. Immunoblot of protein extracts treated with vehicle, 1 μM MG132 or 10 μM Bafilomycin and Wnt9a conditioned medium (CM) as indicated. **B**. Diagram of mutation frequencies in cancers from data in The Cancer Genome Atlas. Each red spot indicates the location of mutations; the size of red spots indicates the number of patients identified with a mutation. Only nonsense mutations predicted to case premature STOP (PS) codons are shown. **C.** Clustal Omega alignment of third intracellular loop (ICL3) of all zebrafish Fzd proteins showing K437 is conserved.



Supplementary Figure 5: Trip12 is required for hematopoietic stem cells development *in vivo*. Quantification of hematopoietic stem and progenitor cells marked by GFP in Tg(*gata2b:KalTA4;UAS:GFP)* zebrafish caudal hematopoietic tissue at 72 hpf. Each dot represents a biological replicate. *P<0.05 by ANOVA with Tukey post-hoc comparison.

Supplementary Table 1: siRNAs used

target	sequence	catalogue number
siControl	proprietary	invitrogen AM4611
siTRIP12-1	GAUUGAUCUUGUUCCACGATT	invitrogen s17809
siTRIP12-2	GCAAUUUGAUUCGUUCAGATT	invitrogen s17810
siMYCBP2	CAUGAUAUGUUUCACCGAATT	invitrogen s22980
siMSL2	GCAUCCUAGUGAACUGCUATT	invitrogen s30332
siRBBP6	GAAAGAAGAAUAUACUGAUTT	invitrogen s11843
siUBAC2	CAAUUGGAAUCGUCUUUUUTT	invitrogen s223887

Supplementary Table 2: qPCR primers used

target	sequence
zebrafish *trip12*	AGAAGACCACGGGCTCT
	GTTGTTGTCAGAGTCGGCC
zebrafish *gapdh*	CCACCCCCAATGTCTCTGTT
	TACCAGCACCAGCGTCAAAG
zebrafish *cmyb*	GAGGGGCAAGATCTCCACAC
	GGACTTCCTATGGGTCTGCG
human *TRIP12*	TGGTGGTTCGCATGTTTTCTCTG
	CTGACAACTTCCATTGGAGGCAC
human *GAPDH*	CCTGCACCACCAACTGCTTA
	CCATCACGCCACAGTTTCC