

Figure S1 Level of Fz expression from the *Act5C-fz* transgene. Western blot of 28 hr pupal wing extracts from w^{1118} , fz^{P21} and w; *Act5C-fz/+;* fz^{P21} animals, probed for Fz protein (upper panel) or Actin (lower panel). One copy of the *Act5C-fz* transgene gives about five-fold higher expression of Fz than two copies of the endogenous gene as seen in the w^{1118} control strain.

29 30 31 32 35 Y Y R S G G G tat tat cgc agc ggc ggg gga C<u>H</u> <u>N</u> <u>SD</u> RE RE G = S G T E tct ggg acc gaa F RE= I= K ccc acc ctg SL= I= = = \underline{P} LS= V<u>T</u>= -<u>S</u>= R= VT - = S= VT _____ Α 54 55 E D 56 57 52 $v_{\underline{T}}$ atc acc $v_{\underline{T}} = v_{\underline{T}}$ C = Etgt gaa $\underline{X} = K$ $\underline{R} = \underline{G} =$ sL= I SL= = cat ttt <u>*</u> = <u>R</u> <u>LS</u>= L C S L Y V P V C T I L E R P I P P C R S L C E S A R V C E K L M to tot stt tot sty tot sty tate sty tot sign sty start sty star start sty sage sign so at tot sty so construction of the start sty sage start at sty and start sty sage start sty s aaa acc tac aac ttt ER= A HC DS LS= DS R 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 H G G E D L C V A E N T T S S A S T A A T P T R S V A K V T T R K H Cat gap ago cargo car ER= LS= 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 $\begin{array}{cccc} g_{Ca} & ctc & tac & ttc & tgt \\ TV & F & = & = & \underline{Y} \\ & = & P & \underline{HC} & \underline{LS} & \underline{R} & = \end{array}$ gcc TV= LS \mathbf{E} \mathbf{E} 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 T D K L E R L M L R I G F F S G L F I L P A V G L L G C L F Y E Y Y N F D ga and a cas dt gaar got cas dt gaa 100 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 \mathbb{R}^{+} W L Y S S K T M V S W R N P V E R L Q G K E P R T R A Q A Y V \mathbb{R}^{+} tg ctg tat tc cs ago asag ago tag ctg cg ago tac tc ct cg gag ago tag ago tag ago cag ago cag ago tac cg to ct \mathbb{R}^{+} \mathbb{R}^{+} \mathbb{R}^{-} \mathbb{R}^{+} $\mathbb{R}^{$

Figure S2 Amino acid changes in the Fz coding sequence caused by GC:AT and AT:GC base pair changes. First line indicates the codon number, the second line is the wild-type amino acid in one letter code, the third line shows the wild-type codon, the fourth line shows the amino acid change produced by a GC:AT change of the nucleotide indicated immediately above, the fifth line shows the amino acid change produced by an AT:GC change of the nucleotide indicated immediately above. Amino acid changes underlined are Stop codons and those predicted by the SIFT method (Ng and Henikoff 2001) to alter protein activity (i.e. those with a SIFT value of ≤ 0.05). GC:AT transpositions at 50 nucleotide positions result in Stop codons (nonsense mutations), and at 187 nucleotide positions result in amino acid changes (missense mutations) predicted to be deleterious by the SIFT method.



Figure S3 Chart showing relative planar polarity and canonical allele strengths. For each missense and nonsense allele subjected to detailed analysis, the strength of the planar polarity defect is plotted in terms of the number of extra tarsal segments (blue bars). The canonical defect is show in arbitrary units with a large red bar indicating a "strong" phenotype with anterior wing margin notching of more than 10%, and a medium sized red bar indicating an "intermediate" phenotype with notching between 0-10% and fewer than 10% *yellow* bristles present. Red arrows indicate alleles where Fz protein is not stably localize to junctions. Turquoise arrows indicate alleles showing phenotypes consistent with similar defects in canonical and planar polarity activity and magenta arrows indicates alleles with a strong defect in planar polarity activity but normal canonical activity (magenta).

Amino acid change and Allele	Regio n of protei n	Planar polarit y phenot ype (extra tarsal joints)	Canonical phenotype (wing margin): Category	Canonical phenotype (wing margin): Proportion of anterior wing margin notched	Canonical phenotype (wing margin): Proportion of anterior wing margin with yellow bristles
C53F	CRD	2.7	Intermediate	0.07	0.01
C53Y	CRD	3.5	Strong	0.16	0.00
C107Y	CRD	2.9	Strong	0.40	0.01
P112L	CRD	2.3	Intermediate	0.02	0.05
R274C	ICL1	1.6	Rescued	0.00	0.37
R274H	ICL1	0.8	Rescued	0.00	0.44
R274H	ICL1	1.1	Rescued	0.00	0.52
P278L	ICL1	1.8	Intermediate	0.03	0.03
P278L	ICL1	2.3	Intermediate	0.08	0.03
P278S	ICL1	1	Rescued	0.00	0.45
P278S	ICL1	1	Rescued	0.00	0.38
E279K	ICL1	3.3	Rescued	0.00	0.19
E373K	ICL2	1	Rescued	0.00	0.29
E373K	ICL2	0.9	Rescued	0.00	0.47
H383L	TM4	1.3	Rescued	0.00	0.44
H383Y	TM4	1.7	Rescued	0.00	0.45
P390L	TM4	2.4	Intermediate	0.02	0.03
P390L	TM4	2.3	Intermediate	0.06	0.02
G444R	TM5	1.9	Strong	0.22	0.00
I451F	ICL3	2.1	Rescued	0.00	0.47
M469K	ICL3	2.3	Strong	0.16	0.00
M469T/G	ICL3/T	1	Rescued	0.00	0.36
I472K	TM6	1.8	Rescued	0.00	0.36
G545R	TM7	2.6	Strong	0.25	0.00
Y553X	C-	2.3	Intermediate	0.05	0.04
\$554F	C-	2.3	Strong	0.19	0.03
W561X	C-	2.4	Rescued	0.00	0.25
W561X	C-	2.2	Rescued	0.00	0.24
W561X	C-	2.9	Intermediate	0.03	0.03
W561R	C-	2.1	Rescued	0.00	0.22

Table S1 Quantitative scoring of missense alleles