

Figure S1 Two interacting DrosDel deficiencies identified in the initial screen covered two known factors, *ds* and *Dl*, acting in parallel to or downstream of the Fz/PCP signaling pathway. Graphs show average rotation and chirality defects as determined by the *Rh1-GFP* assay for indicated genotypes for both *sev-Gal4*, *UAS-dgo* (G) and *sev-Gal4*, *UAS-pk* (G2). (A) *Df*(*2R*)*ED62*, subdividing deficiencies *Df*(*2R*)*ED49* and *Df*(*2R*)*Exel8003* enhanced rotation defects of *sev-Gal4*, *UAS-dgo* significantly (*=P<0.03). *Notchless* (*Nle*) might be the candidate gene responsible for that interaction. Subdividing deficiencies *Df*(*2R*)*ED49*, *Df*(*2R*)*ED94* and *ds*^{UA071} enhanced chirality defects of *sev-Gal4*, *UAS-dgo* significantly (**=P<0.02), confirming the initial chirality interaction (***=P<0.1) and identifying *dachsous*(*ds*) as the gene responsible for it. No effects were seen with *sev-Gal4*, *UAS-gk*. (B) *Df*(*2R*)*ED94* enhanced rotation defects of *sev-Gal4*, *UAS-pk*. (B) *Df*(*3R*)*ED5942*, subdividing deficiencies *Df*(*3R*)*Cha9* and *DI*^{*RF*} enhanced rotation defects of *sev-Gal4*, *UAS-dgo* significantly (*=P<0.1), confirming the initial interaction and identifying *Delta*(*Dl*) as the gene responsible for it. Deficiency *Df*(*3R*)*Cha9* also enhanced chirality defects of *sev-Gal4*, *UAS-dgo*. 4 eyes were analyzed each and 90-150 ommatidia were evaluated per genotype.



Figure S2 Graph summarizing suppression of *sev-dsh* by two DrosDel deficiencies. Eye sections of 4 eyes were analyzed for ommatidial chirality of indicated genotypes. *Df*(*2L*)*ED793* and *Df*(*3R*)*ED5644* significantly suppressed *sev-dsh* induced PCP defects of symmetrical photoreceptor arrangement (*=P<0.003). For comparison, the strength of suppression by *misshapen* (*msn*), an established downstream effector of Fz/Dsh signaling in the eye, is shown (**=P<0.03) (Paricio et al. 1999). 2-3 eyes and 200-350 ommatidia were evaluated per genotype.



Figure S3 (A) Independent assay confirming *dgo* and *CG15283* loss-of-function interaction. Graph shows average wing hair defects as observed in *en-GAL4*, *UAS-dgo-IR* and enhancement by *CG15283-IR* knockdown. 20 misoriented wing hairs at 45-180 degrees compared to wild-type were recorded as a value of 1 and n was 24-26 wings analyzed for each genotype. (B) Quantification of the rotation defects associated with the *sevGAL4*, *UAS-Nmo*, *CG69633-IR* and *sev-Stan/Fmi* genotypes. Note that CG6963/CK1g knock down enhances Nmo GOF rotation defects, whereas *nmo-/+* suppresses the CG6963-IR defects, indicating an antagonistic relationship between these genes. In addition, rotation defects associated with *sev-Fmi/Stan* is enhanced by CG6963-IR knock down. P values are *<0.03, **<0.001, and ***<0.0001, with the number of ommatidia analyzed being n=422-633 in 3 eyes for each genotype.

| Table S1 | DrosDel deficiencies, which showed no dominant external eye or wing modification of sev-GAL4, UAS-dgo and sev- |
|----------|--|
| GAL4, UA | <i>pk</i> phenotype. |

| GAL4, UAS-pk phenotype. | | | |
|-------------------------|--------------|---------------|----------------|
| Df(1)ED404 | Df(1)ED409 | Df(1)ED6574 | Df(1)ED411 |
| Df(1)ED6630 | Df(1)ED6712 | Df(1)ED6802 | Df(1)ED418 |
| Df(1)ED6829 | Df(1)ED6991 | Df(1)ED7005 | Df(1)ED429 |
| Df(1)ED7067 | Df(1)ED7153 | Df(1)ED7217 | Df(1)ED7229 |
| Df(1)ED7294 | Df(1)ED7355 | Df(1)ED7413 | Df(1)ED6906 |
| Df(1)ED7664 | Df(1)ED6849 | Df(2L)ED2809 | Df(2L)ED5878 |
| Df(2L)ED19 | Df(2L)ED87 | Df(2L)ED94 | Df(2L)ED108 |
| Df(2L)ED125 | Df(2L)ED123 | Df(2L)ED136 | Df(2L)ED247 |
| Df(2L)ED284 | Df(2L)ED508 | Df(2L)ED647 | Df(2L)ED678 |
| Df(2L)ED690 | Df(2L)ED701 | Df(2L)ED737 | Df(2L)ED761 |
| Df(2L)ED778 | Df(2L)ED3 | Df(2L)ED1050 | Df(2L)ED1102 |
| Df(2L)ED1109 | Df(2L)ED1158 | Df(2L)ED1165 | Df(2L)ED1186 |
| Df(2L)ED1226 | Df(2L)ED1231 | Df(2L)ED1303 | Df(2L)ED1384 |
| Df(2L)ED1473 | Df(2R)ED1484 | Df(2R)ED1612 | Df(2R)ED1735 |
| Df(2R)ED2155 | Df(2R)ED2219 | Df(2R)ED9045 | Df(2R)ED2354 |
| Df(2R)ED2426 | Df(2R)ED2436 | Df(2R)ED1 | Df(2R)ED3610 |
| Df(2R)ED3923 | Df(2R)ED4061 | Df(2R)ED4071 | Df(2R)Exel6061 |
| Df(2R)ED1770 | Df(2R)ED2098 | Df(3L)ED4079 | Df(3L)ED4256 |
| Df(3L)ED4287 | Df(3L)ED4288 | Df(3L)ED4341 | Df(3L)ED4342 |
| Df(3L)ED210 | Df(3L)ED211 | Df(3L)ED4408 | Df(3L)ED4421 |
| Df(3L)ED4457 | Df(3L)ED4475 | Df(3L)ED215 | Df(3L)ED4486 |
| Df(3L)ED217 | Df(3L)ED218 | Df(3L)ED223 | Df(3L)ED4674 |
| Df(3L)ED4685 | Df(3L)ED4710 | Df(3L)ED224 | Df(3L)ED225 |
| Df(3L)ED4782 | Df(3L)ED4786 | Df(3L)ED228 | Df(3L)ED4799 |
| Df(3L)ED4978 | Df(3L)ED231 | Df(3R)ED4710 | Df(3R)ED5138 |
| Df(3R)ED5147 | Df(3R)ED5156 | Df(3R)ED5177 | Df(3R)ED5196 |
| Df(3R)ED5230 | Df(3R)ED5343 | Df(3R)ED5429 | Df(3R)ED5591 |
| Df(3R)ED5610 | Df(3R)ED5642 | Df(3R)ED10642 | Df(3R)ED5780 |
| Df(3R)ED2 | Df(3R)ED5911 | Df(3R)ED6025 | Df(3R)ED10809 |
| Df(3R)ED10820 | Df(3R)ED6093 | Df(3R)ED6103 | Df(3R)ED6187 |
| Df(3R)ED6235 | Df(3R)ED6255 | Df(3R)ED6265 | Df(3R)ED6310 |
| Df(3R)ED6316 | Df(3R)ED6332 | Df(3R)ED6346 | Df(3R)ED5071 |
| Df(4)ED6364 | Df(4)ED6369 | Df(4)ED6380 | Df(4)ED6382 |
| Df(4)ED6384 | | | |