

The Wnt co-receptor Arrow-LRP5/6 is required for Planar Cell Polarity establishment in *Drosophila*

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Supplementary Figures 1 - 6

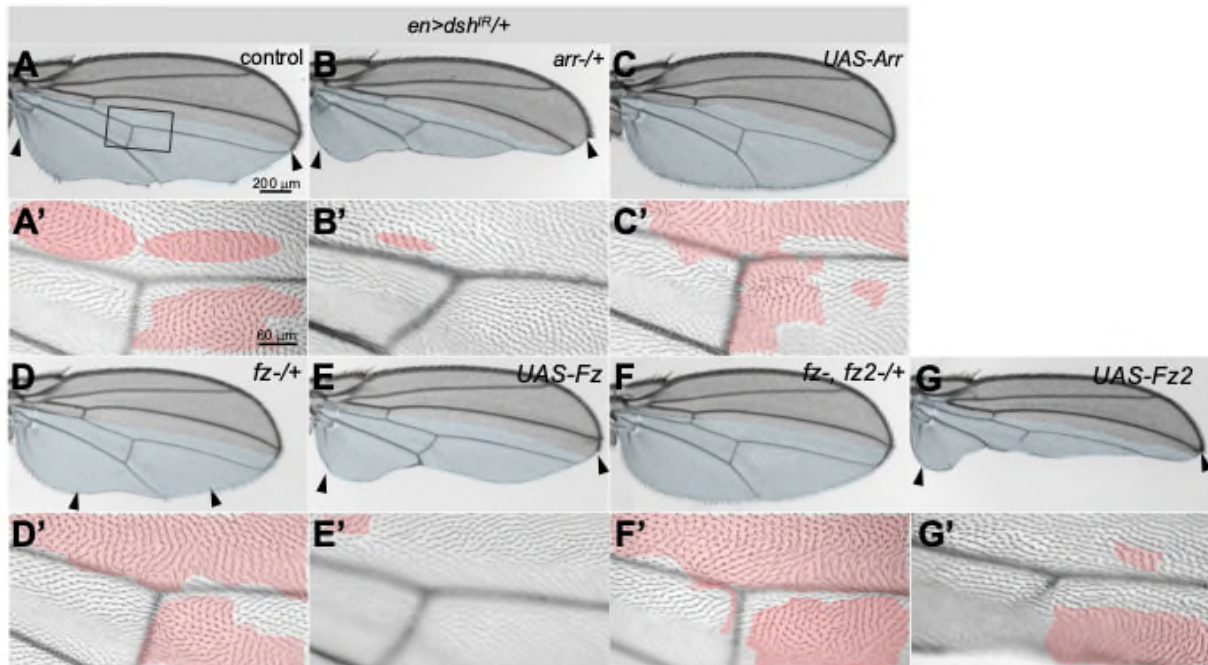
Supplementary Tables 1, 2

Supplementary Methods:

Genotypes, schematics and experimental details for Supplementary and Main Figures

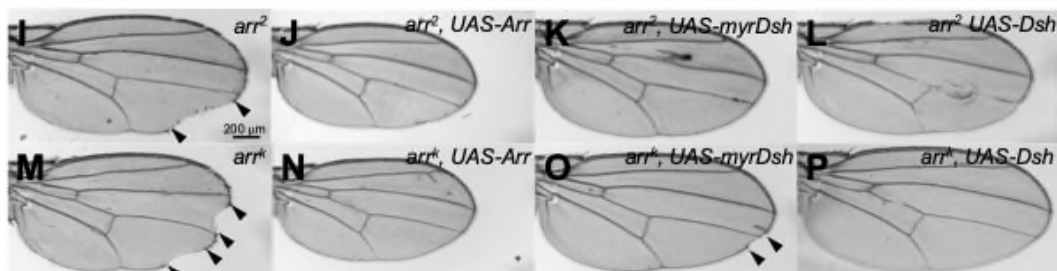
Statistics and Reproducibility Table

References



H Summary of components of both Wnt signaling branches and their interaction with *dsh* knockdown

Gene	Function/feature	Tool used	Interaction with <i>en>dsh^{IR/+}</i>
<i>wg</i>	ligand	LOF allele	less notching, more whorls
<i>wg</i>	"	<i>UAS-Wg</i>	more notching, less whorls
<i>Wnt4</i>	ligand	IR	lethally interacting
<i>Wnt4</i>	"	<i>UAS-Wnt4</i>	less notching, less whorls
<i>wg, Wnt4</i>	ligands	LOF alleles	less notching, more whorls
<i>fz</i>	receptor for Wnt/PCP	LOF alleles	less notching, more whorls
<i>fz</i>	"	<i>UAS-Fz</i>	more notching, less whorls
<i>fz, fz2</i>	receptors for wg/ β -catenin	LOF	less notching, more whorls
<i>fz2</i>	receptor for wg/ β -catenin	<i>UAS-Fz2</i>	more notching, less whorls
<i>arr</i>	co-receptor	LOF alleles, IRs	more notching, less whorls
<i>arr</i>	"	<i>UAS-Arr</i>	less notching, more whorls
<i>axin, sgg(GSK3), APC</i>	negative regulators, destruction complex	LOF alleles, IRs	less notching (mild), no effects
<i>arm/βcatenin</i>	downstream effector	LOF alleles, IR	lethally interacting/no effects
<i>naked cuticle/Nkd</i>	neg. feedback inhibitor	LOF alleles	no effects
<i>pangolin/TCF</i>	repressor/activator	LOF alleles, IR	lethally interacting/no effects
<i>fmi, stbm, pk, dgo</i>	Wnt/core PCP	LOF alleles, <i>UAS-Gene</i>	no effects on notching, interaction with whorls



Q Rescue of *arr* notching phenotype via Arr or Dsh

Genotype	Wings with clones (n)	Notched wings (n)	% Notched wings
<i>arr[2]</i>	39	26	66.7%
<i>arr[2], UAS-Arr</i>	35	4	11.4%
<i>arr[2], UAS-myrDsh</i>	71	1	1.4%
<i>arr[2], UAS-Dsh</i>	82	7	8.5%
<i>arr[k]</i>	65	31	47.7%
<i>arr[k], UAS-Arr</i>	23	10	43.5%
<i>arr[k], UAS-myrDsh</i>	26	15	57.7%
<i>arr[k], UASDsh</i>	35	6	17.1%
<i>ALZ, UAS-myrDsh</i>	22	1	4.5%
<i>ALZ, UAS-Dsh</i>	47	3	6.4%



T Rescue of *arr* knockdown notching phenotype via Arr

Genotype	Total wings (n)	% Notched wings
<i>arr-IRHMS</i>	29	58.6%
<i>arr-IRHMS, UAS-Arr</i>	71	7.0%

Supplementary Figure 1

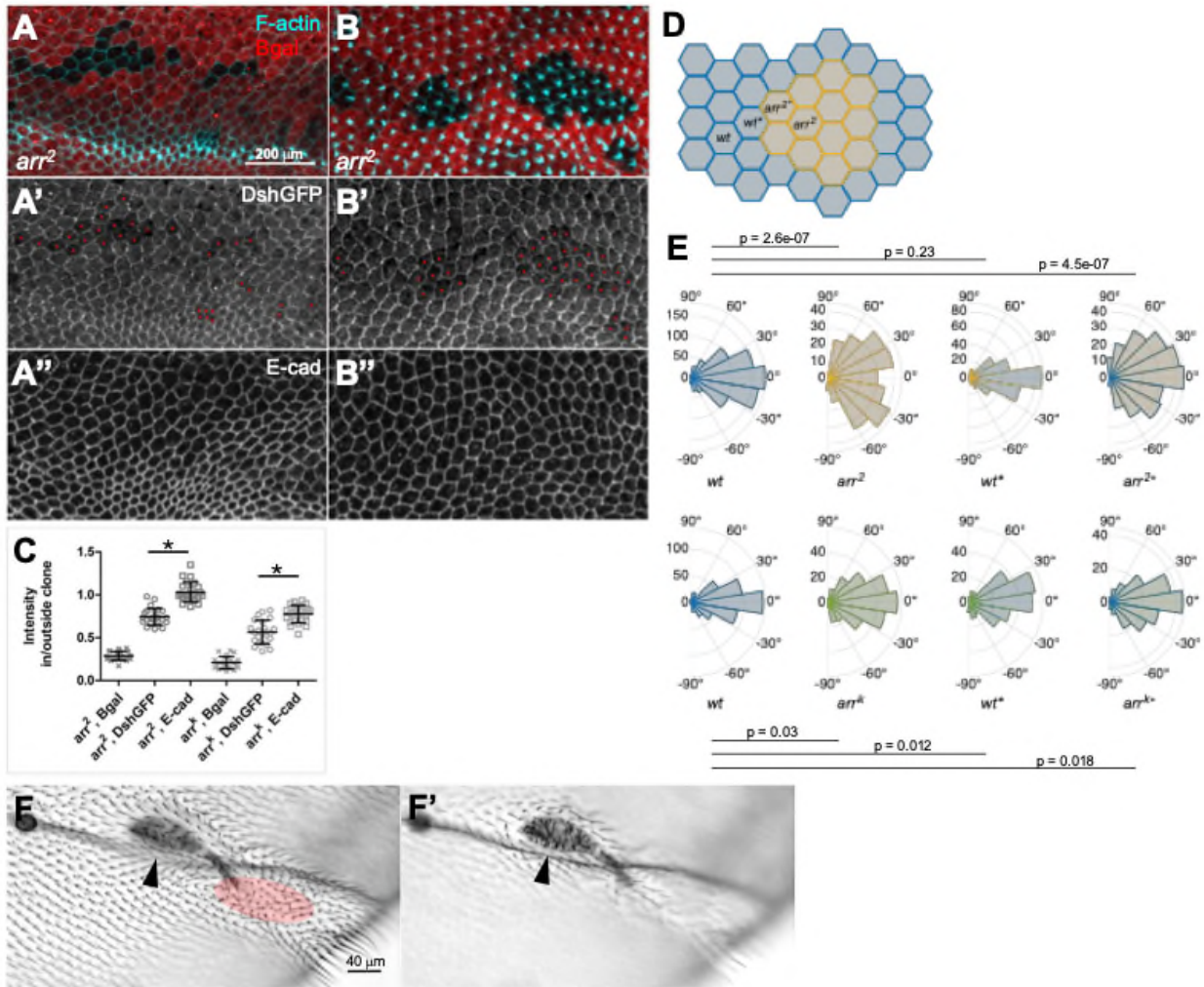
Changing *Arrow* levels shifts *dsh* knockdown induced phenotypes from Wnt/ β -catenin to Wnt/PCP, and rescue of *arr* mutant defects.

(A-G) Wings with *dsh* knockdown in posterior compartment (*en-GAL4*) highlighted in blue. Genotypes as indicated, control wings were *arm*^{-/+}, *nkd*^{-/+} or *ry*⁵⁰⁶^{-/+}. Extent of margin loss is indicated by arrowheads. Note that depth of notching increases with length of notch. (A'-C') Area at posterior crossvein, boxed in (A), showing trichome misorientations in dorsal knockdown domain highlighted in red. Reduction of *arr* copy number deepens and extends notching while suppressing trichome misorientation (B,B'), *Arr* overexpression caused the opposite effects for both phenotypes and induced ectopic margin bristles (C,C'). (D-G) Similar interactions were observed for the Wnt/PCP receptor *fz*, where copy number reduction (D,D') versus *Fz* overexpression (E,E'), and for Wnt/ β -catenin receptors *fz* and *fz2*, copy number reduction for each (F,F') versus *Fz2* overexpression (G,G') had opposite effects. Representative images were selected from 5-7 wings analyzed for each genotype in the genetic screen, with all wings of a given genotype showing the same type of interaction. More detailed quantifications will be published elsewhere. (H) Table summarizing *en>dshIR* pilot screen results for genes in both Wnt signaling branches (β -catenin and PCP). First column lists genes assayed, second column their function, third column which kind of assay was used, and last column summarizes phenotypic effects seen with respect to Wnt/ β -catenin signaling (notching) or Wnt/PCP pathway (trichome whorls) defects in *en>dshIR* wings. Note that ligands (*wg*, *Wnt4*) and receptors (*fz*, *fz2*) are able to tilt the phenotypic balance of the *dsh* knockdown and *arr* can do so as well (highlighted in green). Noteworthy, the direction of the interaction with the receptors is not predictable based on naïve, current expectations. For example, the direction of the interactions with *Fz2* GOF and *fz*, *fz2* double mutant LOF backgrounds is opposite to the expected: adding more *Fz2* enhanced the notching defects (Wg/ β -catenin defects) (G), while it would have been expected to rescue these.

Similarly, removing one copy of both Fz-receptors suppresses notching, but it would be expected to enhance these. The interactions with *arr* LOF (B) suppresses trichome orientation, while the opposite effect would be expected based on all other data presented in this study.

Please also note that Wnt/ β -catenin signaling specific downstream genes, for example *axin*, *sgg* and *APC*, show no significant effects on Wnt/PCP phenotypes (trichome orientation/whorls), whereas Wnt/core PCP factors only affect trichome orientation/whorls (highlighted in orange).

(I-T) Rescue of *arr* LOF wing phenotypes. (I-P) Wings containing clones and clonal rescue as indicated, summarized in (Q). Control is *arm-lacZ* (ALZ). *arr*² and *arr*^k clones induce notches and ectopic margin bristles, indicated by arrowheads, and trichome orientation defects (I,M). *Arr*, *myrDsh* and *Dsh* rescue margin defects (J-L,N-P,Q), whereas only *Arr* rescues PCP/trichome whorl defects, see Fig. 1G-I. Table summarizes extent of wing margin rescue (Q). *arr*² is rescued in all assays with reduced occurrence of notches, *arr*^k was rescued with *UAS-Dsh*, other rescues only reduced notch size. (R-T) *arr* knockdown in wing margin via *C96-GAL4* induces loss of wing margin (notching), indicated by arrowheads (R). *Arr* overexpression rescues *arr*^{IR} notching phenotype from 58.6% to 7% (S,T), validating *IR* and overexpression tools.



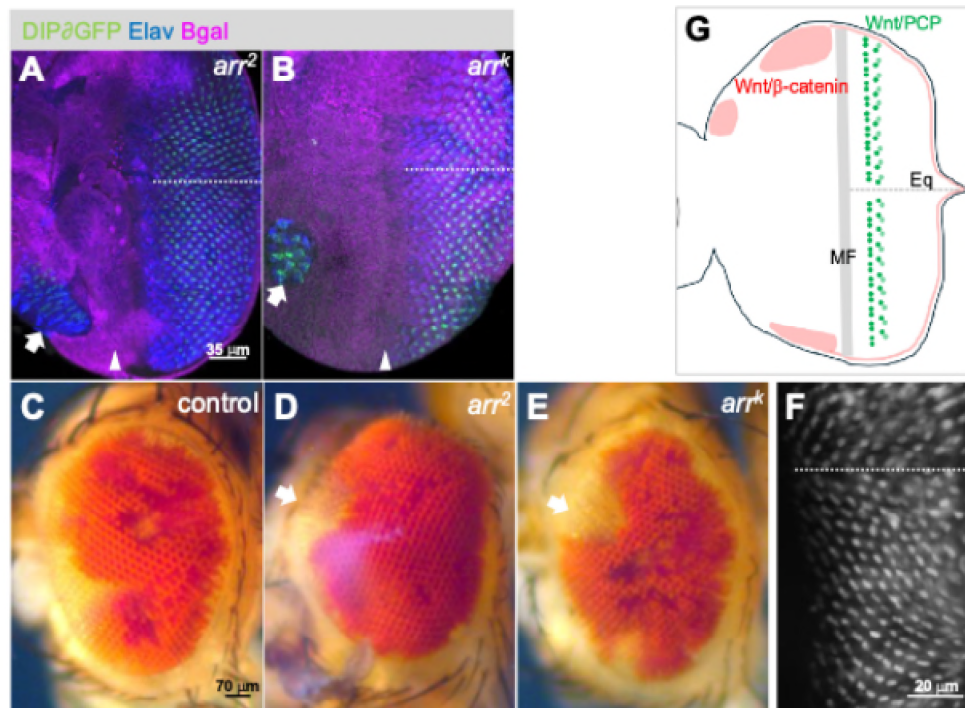
Supplementary Figure 2

***arr* LOF cells show reduced levels, loss of membrane association and asymmetric localization of Dsh.**

(A,B) DshGFP staining in *arr*² clones, marked by loss of βgal in red, and stained for F-actin in cyan. Note reduced DshGFP membrane association (monochrome in A',B'). (C) Quantification of DshGFP levels relative to controls for both alleles. (A-A'') Prior to prehair formation, DshGFP is reduced and asymmetric localization is lost in *arr* mutant cells, indicated by red dots (A'). DshGFP tends to accumulate at membranes shared between mutant and *wild-type* cells (A',B'). (B-B'') At time of prehair formation, DshGFP asymmetric localization (B') and membrane levels are reduced in *arr* mutant cells. E-cad levels are not affected in clones (A'',B''). (C) Quantification of staining

intensities in mutant vs *wild-type* cells, indicated as relative intensity in/outside clone for clonal marker Bgal, DshGFP (reduced), and E-cad (as control), of 24 clones each, from 6 or 9 wings for *arr^k* or *arr²*, respectively from 5 independent experiments each. Student's two-tailed t-test, * $P < 0.0001$ for DshGFP compared to E-cad for both alleles, mean and standard deviation (SD) are shown.

(D) Schematic displaying cell distribution of how cellular nematic order was analyzed in distinct cell populations. (E) Rosette plot quantifications of angle distribution in *arr²* and *arr^k* mutant cells and the respective cells at clonal boundaries. Note that angle distribution is overall randomized in mutant cells as compared to *wt* control tissue (angle distributions can vary between individual mutant clones, and the sum of mutant clones shows a randomized distribution), and also that cells touching clonal borders display different distributions than their respective fully mutant or fully *wt* counterparts, see Methods for details. (F,F') Wing with *arr²* clone marked by *shn*, indicated in red. Mutant tissue has induced overgrowth, which is mostly located wing internally, between dorsal and ventral surface (F', compare Fig. 2C-C").



Supplementary Figure 3

***arr* clones induce ectopic eye fields in polar and anterior region leading to misshapen eyes.**

Developing (A-B) and adult eyes (C-E) with *arr* mutant tissue, genotypes as indicated. (A,B) Confocal images of clones marked by loss of B-gal (magenta), photoreceptors labelled by Elav (blue) and R3 fate (*DIPδGFP*, green) in eye imaginal discs. Equators indicated with white dotted line. Both *arr* alleles induce ectopic eye fields, examples marked by white arrows near the poles and anterior to the morphogenetic furrow, indicated by white arrowhead (A-B). Such ectopic eye fields cause bulging areas in adult eyes (D-E), as eye fields merge during development (examples indicated by white arrows) (D,E). These defects are caused by loss in Wg/β-catenin signaling, as mediated by Arrow. (C) Control clones and (D,E) marked by loss of pigment marker, *white*. (F) *sep-GAL4, UAS-GFP/+* eye disc, imaged with an epifluorescence microscope. *Sevenless* promoter-enhancer *GAL4* construct (*sep>*, see Methods) drives in photoreceptors R3 and R4 at

the time of PCP establishment (^{1,2,3}) and has tighter promoter control than *sev-GAL4* (heatshock promoter–*sev* enhancer *GAL4* construct by Konrad Basler, e.g ref ⁴). (G) Eye disc schematic after ⁵ generated in Powerpoint. Morphogenetic furrow indicated by grey bar (MF), behind which photoreceptors are being recruited sequentially into ommatidial preclusters. Some photoreceptors R3 and R4 indicated by green dots and by dark or light green dots, respectively, as they acquire their cell identity via Wnt/PCP signaling and start to rotate (45° stage shown) in opposite directions on dorsal versus ventral side of disc, forming equator (Eq), and acquiring mirror symmetric chiral adult forms on dorsal versus ventral side of eye (^{6,7,8}). Red marked areas indicate Wnt/β-catenin signaling activity inhibiting eye field and equator formation (^{9,10,5}). Note that Wnt/β-catenin and Wnt/PCP signaling are temporally and spatially separated in this tissue.

Of note, while Wnt/β-catenin signaling regulates the graded expression of *ds* and *four-jointed (fj)*, which affect PCP in the eye via the Fat-Ds system (see main text), the phenotype of either *ds* or *fj* mutant clones is very different with pure chirality inversions (Fig 3K) and non-autonomous effects at clonal borders close or on the equator (^{11, 12}). These phenotypes are very different from those observed in *arr*- clones in the equatorial region (Fig 3A,B) and thus we can exclude that the phenotypes we are describing are related to Wg/β-catenin signaling function of Arr. In particular, clones at or near the equator of neither *arr* allele show effects comparable to *fj* or *ds* mutant clones (for *arr^k* – 11 eyes, and for *arr²* – 13 eyes, from 4 experiments each were analyzed). Only polar *arr*- clones, close to the disc margins, show non-autonomous effects (see also Supplementary Fig. 3A,B,D,E and associated text).

Supplementary Figure 4

Fz, as Fz2, overexpression increases Arr levels and induces Wnt/PCP or Wnt/ β -catenin signaling defects, respectively. Arr expression in developing eyes.

(A-D) Adult wings from *dppGAL4* overexpression studies in main Figure 4C-G, induce wing defects, as expected, genotype as indicated. Dpp domain is pseudo-colored in blue (A,A'). Vang/Stbm causes weaker effects in this assay (D,D'). Trichome misorientations (Wnt/PCP) and ectopic margin bristles (Wnt/ β -catenin) are highlighted with arrows or circled, respectively (A',C',B').

(E-H) Confocal Z-line scans through wing discs as indicated in Figure 4C' of experiment shown in Figure 4C-G, for the two Fz receptors (Fz and Fz2) or transmembrane core PCP factors (Vang and Fmi) as marked. Arr antibody staining in magenta, *dpp* domain in green and E-cad in blue, monochromes in panels below (E',E'',E'''-H',H'',H'''). Note that *Fz* overexpression can recruit/stabilize Arr (E,E'), similar to *Fz2* (F,F') indicated by white arrowheads, while *Fmi* and *Vang* overexpression had no effects on Arr levels (G,G', H,H'). 10 individual scans of 3-4 wing discs were acquired each.

(I-K) Clonal overexpression of Fz increases Arr protein levels. Confocal images of peripodial eye disc cells, anterior to the left, mosaic for either *FzMyc* (I) or *Fz2Myc* (J), with expressing cells marked by GFP, stained for Arr in magenta and monochrome (I',J') and Myc in blue and monochrome (I'',J''). (I',J') Overexpressing clones are outlined with orange line and show elevated Arr levels. (E''',F''') E-cad expression serves as control. (K) Quantification summarizing Arr level increase in Fz and Fz2 overexpression clones, as compared to neighboring *wild-type* tissue, see Methods for specifics. E-cad levels are not affected, Student's two-tailed t-test, *P=0.0003 for both *Fz* and *Fz2* compared to E-cad, from 5 (>Fz) or 7 (>Fz2) discs, 12 clones each, 2 independent experiments each.

(L-N) Adult wings with overexpression clones of *lacZ* (control) or as indicated, from experiment shown in I,J. (L'-N') Enlarged view of anterior crossvein area or tip of wing. Fz overexpression induces trichome misorientations and multiple trichomes (Wnt/PCP defects) (M,M'), and Fz2 induces ectopic margin bristles in wing blade (Wnt/ β -catenin defects) (N,N') compared to control, confirming their known activities in these assays.

(O-Q) Arr expression and localization in eye discs via antibody staining. Note that *arr* clones reduce Arr levels and affect ommatidial cluster orientation, anterior to the left. (O,P) Clones of genotype as indicated, marked by the absence of Bgal expression in blue. E-cad highlighting ommatidial clusters behind the furrow in green, and monochrome in O'',P'' and Arr in magenta, and monochrome in O',P'. Arr localization is punctate in *wt* regions and reduced in clones (O',P',Q). Note Arr being present in peripheral regions of wild-type developing ommatidial clusters as seen in (O',P'). (Insets P-P'') High magnification images of the first six rows of ommatidial clusters emerging from furrow, apical projections for Arr, E-cad and Fmi localizations as marked, in a similar *wild-type* area as indicated with white box in O. E-cad and Fmi highlight outlines of photoreceptor precursors R3 and R4 or R2, R8 and R5, respectively. (Inset P) Arr in red, E-cad in green and Fmi in white. Fmi is starting to get enriched in R4 in the last ommatidial row shown. (Insets P,P') Note Arr does not localize in R3 and R4 as compared to Fmi, nor in more basal levels of these photoreceptors. (Q) Quantification summarizing Arr and E-cad level changes in clones for both *arr* alleles. Student's two-tailed t-test, *P < 0.0001, **P = 0.0004, ***P = 0.0033 compared to 1, from 3 (*arr^h*) or 7 (*arr²*) discs, 14 clones each from 4 independent experiments.

List of genetic assays, phenotypes, genotypes and reference to figures			
Genetic manipulation	Wnt/PCP phenotype	Wnt/ β -catenin phenotype	Assay
arr, PCP context			tissue, figure
<i>dsh</i> k.d.	+++	+++	<i>en>dsh RT</i>
<i>dsh</i> k.d., less <i>arr</i>	+	+++++	wing
<i>dsh</i> k.d., more <i>arr</i>	+++++	+	Sup. fig. 1
<i>Dsh</i> o.e.	+++	+++	<i>sep>Dsh</i>
<i>Dsh</i> o.e., less <i>arr</i>	+	+	eye
<i>Dsh</i> o.e., more <i>arr</i>	+++++	+++++	Fig. 5
<i>Fz</i> o.e.	+++	n.a.	<i>sev>, pnr>Fz</i>
<i>Fz</i> o.e., less <i>arr</i>	n.e./+	n.a.	eye/notum
<i>Fz</i> o.e., more <i>arr</i>	+++++	n.a.	Fig. 5, Sup. fig. 5
<i>arr</i> k.d.	n.e./*	+++	<i>en>arr-IR</i>
<i>arr</i> k.d., less <i>fz</i>	n.d.	+	wing
<i>arr</i> k.d., more <i>fz</i>	n.d.	+++++	Sup. fig. 5
<i>Fmi</i> o.e.	++++	n.e.	<i>sev>Fmi</i>
<i>Fmi</i> o.e., less <i>dsh</i>	++++	n.e.	eye
<i>Fmi</i> o.e., less <i>arr</i>	++++	n.e.	Sup. fig. 5
<i>Fmi</i> o.e., more <i>arr</i>	++++	n.e.	
<i>Vang</i> o.e.	+++	n.e.	<i>sev>Vang</i>
<i>Vang</i> o.e., less <i>dsh</i>	+	n.e.	eye
<i>Vang</i> o.e., less <i>arr</i>	++++	n.e.	Sup. fig. 5
<i>Vang</i> o.e., more <i>arr</i>	++	n.e.	
<i>arr</i> LOF	+++	+++	MARCM clones
<i>arr</i> LOF, more <i>arr</i>	+	+	wing
<i>arr</i> LOF, more (<i>myr</i>) <i>dsh</i>	+++++	+	Fig. 1, Sup. fig. 1
<i>dsh</i> , <i>arr</i> , <i>fz</i> LOF	+++	n.e.	triple heterozygous <i>dsh</i> , <i>arr</i> , <i>fz</i>
<i>dsh</i> , <i>arr</i> LOF	++	n.e.	wing
<i>dsh</i> , <i>fz</i> LOF	+	n.e.	Fig. 6, Sup. fig 6
<i>arr</i> , <i>fz</i> LOF	++	n.e.	
<i>wg</i> , <i>Wnt4</i> , <i>arr</i> , <i>fz</i> LOF	+++	n.e.	quadruple heterozygous <i>wg</i> , <i>Wnt4</i> , <i>arr</i> , <i>fz</i>
<i>wg</i> , <i>Wnt4</i> , <i>arr</i> LOF	n.e.	n.e.	wing
<i>wg</i> , <i>Wnt4</i> , <i>fz</i> LOF	n.e.	n.e.	Fig. 6, Sup. fig 6
<i>arr</i> , <i>fz</i> LOF	+	n.e.	
<i>wg</i> , <i>arr</i> , <i>fz</i> LOF	+	n.e.	
arr, β-catenin context			
<i>arm</i> , <i>arr</i> , <i>fz</i> LOF	+	n.e.	triple heterozygous <i>arm</i> , <i>arr</i> , <i>fz</i>
<i>arm</i> , <i>arr</i> LOF	n.e.	n.e.	wing
<i>arm</i> , <i>fz</i> LOF	n.e.	n.e.	Sup. fig 6
<i>arr</i> , <i>fz</i> LOF	++	n.e.	
<i>arm</i> , <i>wg</i> , <i>Wnt4</i> , <i>arr</i> LOF	n.e.	n.e.	quadruple heterozygous <i>arm</i> , <i>arr</i> , <i>wg</i> , <i>Wnt4</i>
<i>arm</i> , <i>wg</i> , <i>Wnt4</i> LOF	n.e.	n.e.	wing
<i>arm</i> , <i>arr</i> LOF	n.e.	n.e.	Sup. fig 6
<i>wg</i> , <i>Wnt4</i> , <i>arr</i> LOF	n.e.	n.e.	

Supplementary Table 1

Summary of functional genetic interactions between *arr* and Wnt/PCP or Wnt/ β -catenin pathway components.

Left column lists genetic manipulations via LOF alleles, knockdown (k.d.), overexpression (o.e.) or LOF clones (LOF) and decrease (less) or increase (more) of gene copy number or function. Second and third columns indicate phenotypic effects caused and how their relative strength changes, marked by + for either signaling pathway, indicated in header. Fourth column lists genotypes used, which tissue was examined and reference to specific figures. Genetic interactions for *arr* in Wnt/PCP signaling with Wg, Wnt4 ligands, Fz receptor, Dsh adaptor, Fmi/Celsr or Vang/Stbm or in Wnt/ β -catenin signaling with Arm. Note *arr*^{-/+} suppresses the *arr*^{-/+}, *fz*^{-/+} PCP phenotype.

No effects (n.e.), * = *arr* clones induce PCP defects, full genotypes see Methods.

List of <i>arr</i> knockdown assays, phenotypes and genotypes			
Genetic manipulation	Wnt/PCP phenotype	Wnt/ β -catenin phenotype	Assay
<i>arr</i> k.d.	n.e./*	+++	<i>en>arr-IR</i>
<i>arr</i> k.d., <i>Wnt6</i> k.d.	none	+++	wing
<i>arr</i> k.d., <i>Wnt10</i> k.d.	none	+++	
<i>arr</i> k.d., <i>Wnt4</i> k.d.	none	+++++	
<i>arr</i> k.d., <i>wg</i> k.d.	none	+++++	
<i>arr</i> k.d., less <i>wg</i> , <i>Wnt4</i>	none	+++++	
<i>arr</i> k.d., less <i>fz</i> , <i>fz2</i>	none	+++++	
<i>arr</i> k.d., <i>fz2</i> k.d.	none	+++++	
<i>arr</i> k.d.	n.e./*	+++	<i>en>arr-IR</i>
<i>arr</i> k.d., less <i>dsh</i>	none	+++	wing
<i>arr</i> k.d., less <i>fmi/Celsr</i>	none	+++	
<i>arr</i> k.d., less <i>stbm/Vang</i>	none	+++	

Supplementary Table 2

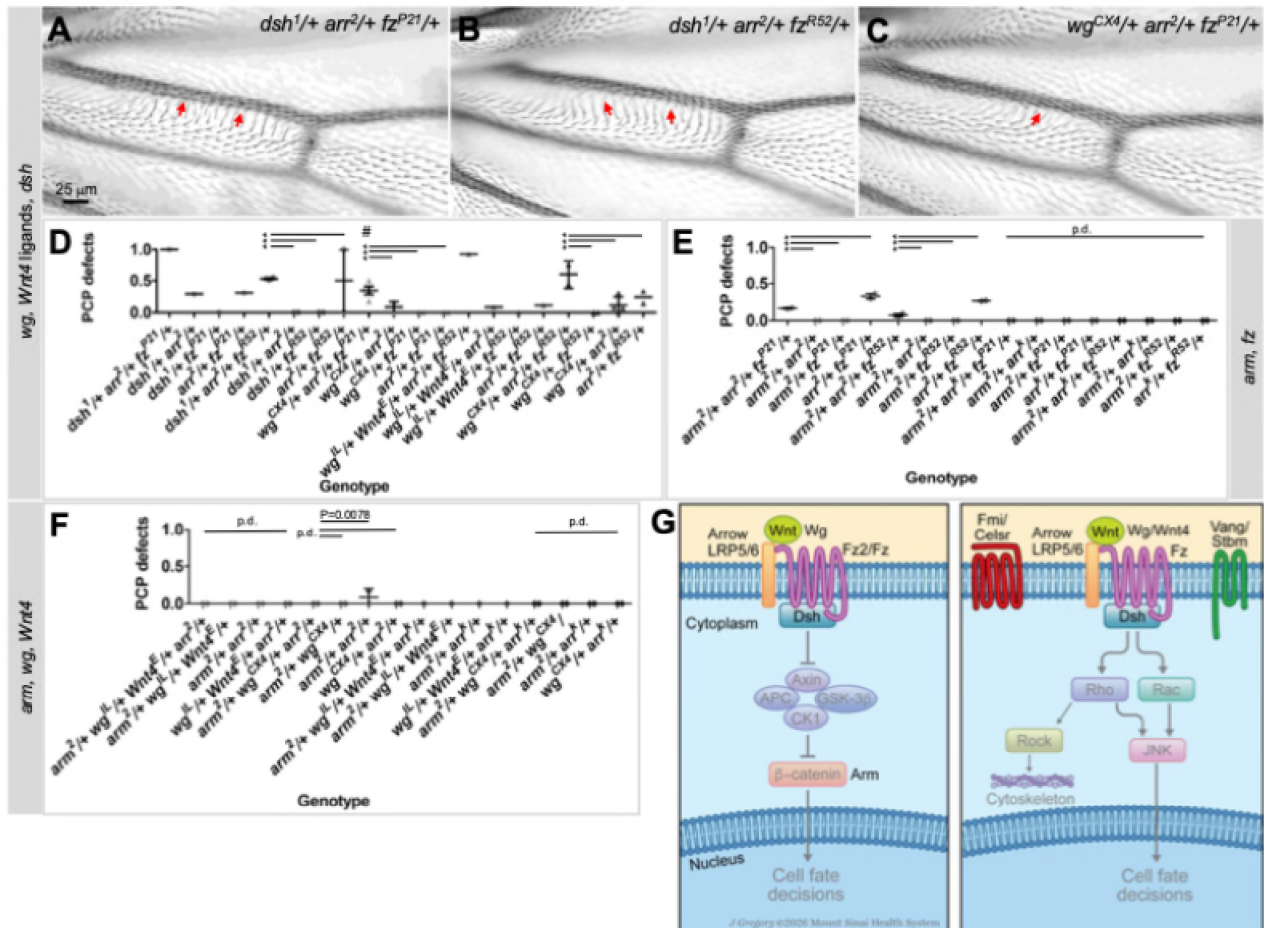
Summary of functional genetic interactions between *arr* knockdown and Wnts, *fz*, *fz2* or core PCP genes.

Left column in table lists genetic manipulations via LOF alleles, knockdown (k.d.) or decrease (less) of gene copy number or function. Second and third columns indicate phenotypic effects caused and how their relative strength changes, marked by + for either signaling pathway, indicated in header. Fourth column lists genotypes used, which tissue was examined. Summary of *arr* knockdown experiments testing several Wnts and Fz, Fz2 as control or Dsh, Fmi or Vang/Stbm for interactions. Only *Wnt4*, *wg*, and *fz2* enhanced notching phenotype.

No effects (n.e.), * = *arr* clones induce PCP defects, full genotypes see Methods.

details see Methods). Reducing *fz* copy number dominantly suppresses this phenotype (E,G,H), while Fz overexpression enhances it comparably to a *fz2* knockdown (F,H). (H) Quantification of relative margin loss (notching) compared to length of L3 in *en>arr^{IR}* wings. Student's two-tailed t-test: *P<0.0001 compared to *lacZ*.

(I,K) *sev>Fmi* overexpression visualized with *Rh1GFP* is not modified by *dsh^{-/+}*, *arr^{-/+}*, *arr* knockdown or *UAS-Arr/+* overexpression, quantified in (I). (J,L) *sev>Vang* overexpression phenotype visualized with *Rh1GFP* is suppressed by *dsh^{-/+}* and *UAS-Arr/+* and enhanced by *arr^{2/+}* compared to *lacZ*. The effects of gain and reduction of *arr* are opposite to effects seen in *sep>Dsh* and *sev>Fz* backgrounds (Fig. 5E,F, Supplementary Fig. 5B,C). Student's two-tailed t-test, for all n and P values see table of statistics and reproducibility in Methods.



Supplementary Figure 6

***wg*, *Wnt4*, *arr*, *fz*, *dsh* multi-heterozygous conditions result in PCP defects, and *arm* containing heterozygotes show no PCP defects.**

(A-C) Wings multiply heterozygous for *dsh*, *arr*, *fz* or *wg*, *Wnt4* with focus on the *dsh¹* allele or *wg^{CX4}* alone. Genotypes as indicated. Proximal area to anterior crossvein between vein L3 and L4 is shown, which is sensitive to PCP perturbations. Note trichome misorientations, indicated by red arrows (A-C) compared to control wings (main Fig. 6A). Quantification summarizing % of PCP defects observed (penetrance of phenotype) (D). Triple heterozygous *dsh^{1/+}*, *arr^{2/+}*, *fz^{P21/+}* wings show 100% PCP defects (A,D), compared to *dsh^{1/+}*, *arr^{2/+}* (0%) or *arr^{2/+}*, *fz^{P21/+}* (0%) and *dsh^{1/+}*, *fz^{P21/+}* (30%). A similar effect is seen with *fz^{R52/+}* heterozygous wings (B,D). Note that combinations with the hypomorphic allele *dsh¹* or *fz^{R52}* yield generally a lower phenotypic

frequency than the *dsh* null allele, *dsh*^{V26} or *fz*^{P21} (main Fig. 6). The single *wg*^{CX4} heterozygous combination phenotypes are weaker than the double heterozygous *wg*, *Wnt4* defects (Fig. 6 and Supplementary Fig. 6D), Student's two-tailed t-test, #P<0.0001. *wg*^{IL}/+, *Wnt4*^{EMS}/+, *arr*²/+, *fz*^{R52}/+ shows 92.9% PCP defects compared to *wg*^{CX4}/+, *arr*²/+, *fz*^{R52}/+ (44.4%). (E,F) *arm* heterozygotes with *arr*, *fz* (E) or *wg*, *Wnt4*, *arr* (F). *arm* heterozygosity does not affect PCP establishment in the context of *arr*/+, *wg*/+, *Wnt4*/+ but suppresses in *arr*/+, *fz*/+. Phenotypic penetrance at level of *arr*, *fz* alone are seen, as observed in previous experiments (Supplementary Fig. 6D). (G) Summary panels of Wnt/β-catenin and Wnt/PCP factors tested in multiple heterozygote assays for *arr* in this study (drawn by Jill Gregory), used with permission from Mount Sinai Health System, licensed under CC BY license: <https://creativecommons.org/licenses/by/4.0/deed.en>.

Supplementary Methods

Genotypes, schematics and experimental details by Supplementary figure

Supplementary Figure	Genotype	Experimental details
1A-C	<i>w; en-GAL4, UAS-dsh^{IRHMC}/+, arr²/+,</i> see next column	<i>en-GAL4, UAS-dsh^{IRHMC}</i> combination stock generated and tested with <i>UAS-Dsh</i> rescue, crosses grown at 23°C for <i>arr²/+, arr^k/+, UAS-ArrHA/+</i> , controls were <i>arm-/+, ry⁵⁰⁶/+, nkd-/+</i> , alleles used were <i>arm¹, arm², nkd², nkd⁴</i>
1D-L	<i>y w hs FLP, tub-GAL4, UAS-GFP/+; FRT42D^{neo} GAL80 /FRT42D^{neo} arr; UAS-Arr/+</i>	MARCM rescue, heatshock 1x 1 hour at 37°C, 24-48 hours after egg laying, then 18°C for entire development
1M-O	<i>w UAS-Dcr2; UAS-arr^{IRHMS}/+; C96-GAL4/+; UAS-Arr/+</i>	<i>w; UAS-Arr; UAS-arr^{IRHMS}</i> combinant made, crosses seeded at 25°C, shifted to 29°C at second to early third instar stage for <i>UAS-arr^{IRHMS}, UAS-Arr</i> and combination stock
1P-R	as in Supplementary Fig. 1D-L	<i>arr²</i> clones were identified by the <i>shn</i> marker, live examined on microscope, adjusting focus, while individual trichome orientation was recorded based on basal trichome shape, counted and individual clones indicated on wing schematics
2A-C	<i>w ubxFLP; FRT42D^{neo} arr⁻ /FRT42D^{neo} armlacZ; dshDsh-GFP/+</i>	Pupal wing preparation and quantification as above
2D,E	<i>w ubxFLP; FRT42D^{neo} arr⁻ /FRT42D^{neo} armlacZ</i>	More detailed packing analyzer segmentation results
3A,B	<i>eyFLP; FRT42D^{neo} arr⁻; DIPδGFP/FRT42D^{neo} armLacZ</i>	Antibody stainings as above
3C-E	<i>w eyFLP; FRT42D^{neo} arr⁻ /FRT42D^{neo} P[w+]</i>	adult eye images taken of frozen, needle immobilized and 70% ethanol submerged flies
3G	Wnt/b-catenin and Wnt/PCP signaling domains within the developing eye	Designed and drawn in PowerPoint by U.W.
4A-F	<i>w hsFLP/+ ; act>y+>GAL4, UAS-GFP, UAS-fzMyc/+ or UAS-fz2Myc/+</i>	heatshock 2x 7-8 minutes at 37°C on day 3 or 4 AEL, rest as above
4G-I	<i>w eyFLP; FRT42D^{neo} arr⁻ /FRT42D^{neo} armLacZ</i> for <i>arr²</i> and <i>arr^k</i>	as above
4J-M	<i>w; dppGAL4, UAS-FzMyc or UAS-Fz2Myc or UAS-Fmi or UAS-Vang</i>	Adult wings from figure 4C-G
Table 1	See individual figures	See individual figures

Table 2	<i>w</i> ; <i>enGAL4</i> , <i>UAS-arr^{IRJF}/+</i>	<i>enGAL4</i> , <i>UAS-arr^{IRJF}</i> combinant stock was generated, <i>arr</i> knockdown interactions at 29°C ts, cross seeding 25°C for 2-3 days, 18°C, 29°C just before 3 rd instar larvae appeared, back to 18°C
5A-C	<i>w</i> ; <i>UAS-Fz/+</i> ; <i>sev-GAL4K25</i> , <i>Rh1GFP/+</i> (<i>sev>Fz</i>), <i>UAS-lacZ/+</i> or see right panel	<i>sev-GAL4K25</i> , <i>UAS-Fz</i> , <i>Rh1GFP</i> stock established, <i>sev>Fz</i> experiment at 22°C with <i>arr^{IRJF}/+</i> , <i>arr^{IRHMS}/+</i> , <i>UAS-Arr/+</i> , imaging as above
5D-H	<i>w</i> ; <i>en-GAL4</i> , <i>UAS-GFP</i> , <i>GAL80ts</i> ; <i>UAS-arr^{IRJF01261}/+</i> (<i>enGGG>arr^{IR}</i>), <i>UAS-lacZ/+</i> or see right panel	<i>en-GAL4</i> , <i>UAS-GAL80ts</i> , <i>UAS-GFP</i> , <i>UAS-arr^{IRJF01261}</i> stock established, <i>enGGG>arr^{IR}</i> with <i>fz^{P21}/+</i> , <i>fz^{R52}/+</i> , <i>UAS-Fz/+</i> , <i>UAS-fz2^{IR}/+</i> , seeding at 25°C for 2 days, 18°C, 30°C from early third instar to early pupae, back to 18°C, Fiji to measure length of notches, divided by vein L3 length
5I,K	<i>w</i> ; <i>sevGAL4</i> , <i>UASFmi</i> , <i>Rh1GFP</i> (<i>sev>Fmi</i>), <i>UAS-LacZ/+</i> or see right panel	<i>sev-GAL4K25</i> , <i>UAS-Fmi</i> , <i>Rh1GFP</i> recombinants generated, genetic interaction crosses at 23°C, <i>sev>Fmi</i> with <i>dsh⁻/+</i> or <i>arr⁻/+</i> or <i>arr^{IR/+}</i> , or <i>UAS-Arr/+</i> , imaged as above
5J-L	<i>w</i> ; <i>sevGAL4</i> , <i>UASVang</i> , <i>Rh1GFP</i> (<i>sev>Vang</i>), <i>UAS-LacZ/+</i> or see right panel	<i>sev-GAL4K25</i> , <i>UAS-Vang</i> , <i>Rh1GFP</i> combinants generated, genetic interaction crosses grown at 23°C, <i>sev>Vang</i> with <i>dsh⁻/+</i> or <i>arr/+</i> or <i>arr^{IR/+}</i> , or <i>UAS-Arr/+</i> , imaged as above
6A-D	<i>dsh¹/+</i> , <i>arr²/+</i> , <i>fz^{P21}/+</i> or <i>dsh¹/+</i> , <i>arr²/+</i> , <i>fz^{R52}/+</i> or <i>wg^{CX4}/+</i> , <i>arr²/+</i> , <i>fz^{P21}/+</i> or <i>wg^{IL}/+</i> , <i>Wnt4^{EMS23}/+</i> , <i>arr²/+</i> , <i>fz^{R52}/+</i> or <i>wg^{CX4}/+</i> , <i>arr²/+</i> , <i>fz^{R52}/+</i>	Triple and quadruple heterozygotes for ligands, <i>arr</i> , <i>fz</i> , <i>dsh</i> and double heterozygote siblings were generated, rest as above
6E	<i>arm²/+</i> , <i>arr²/+</i> , <i>fz^{P21}/+</i> or <i>arm²/+</i> , <i>arr²/+</i> , <i>fz^{R52}/+</i> or <i>arm²/+</i> , <i>arr^k/+</i> , <i>fz^{P21}/+</i> or <i>arm²/+</i> , <i>arr^k/+</i> , <i>fz^{R52}/+</i>	Triple heterozygotes for <i>arm</i> , <i>arr</i> and <i>fz</i> and double heterozygote siblings were generated, rest as above
6F	<i>arm²/+</i> , <i>wg^{IL}/+</i> , <i>Wnt4^{EMS23}/+</i> , <i>arr²/+</i> or <i>arm²/+</i> , <i>wg^{CX4}/+</i> , <i>arr²/+</i> or <i>arm²/+</i> , <i>wg^{IL}/+</i> , <i>Wnt4^{EMS23}/+</i> , <i>arr^k/+</i> or <i>arm²/+</i> , <i>wg^{CX4}/+</i> , <i>arr^k/+</i>	Triple and quadruple heterozygotes for <i>arm</i> , <i>arr</i> and ligands and double heterozygote siblings were generated, rest as above
6G	Wnt signaling branch components tested for β-catenin or PCP	Drawn by Jill Gregory in Adobe Illustrator

Genotypes, schematics and experimental details by main figure

Figure	Genotype	Experimental details
1	<i>w; FRT42D^{neo} arr^k/CyO</i>	<i>arr^k</i> recombination to <i>FRT42D</i> removed <i>white⁺</i> marker, but retained <i>arr</i> lethality which was tested by complementation
1A-C	<i>yw ubxFLP; FRT42D^{neo} shn¹, arr² or FRT42D^{neo} arr^k/ FRT42D^{neo} armlacZ</i>	Crosses seeded at 25°C, for rest of development at 18°C, wings collected and sedimented in overlay of 1xPBS 0,1% triton, 30% glycerol 1xPBS, 80% glycerol 1xPBS, triton and 30% glycerol solution aspirated off, wings mounted and imaged
1F	Wing schematic summarizing nonautonomy effects	Drawn in PowerPoint by U.W. in all layers
1G-I	<i>y w hs FLP, tub-GAL4, UAS-GFP/+; FRT42D^{neo} GAL80 /FRT42D^{neo} arr^k; UAS-Arr/+</i>	MARCM rescue, heatshock 1x 1 hour at 37°C, 24-48 hours after egg laying, then 18°C for entire development <i>arr²</i> clones were identified by the <i>shn</i> marker, live examined on microscope, adjusting focus, while individual trichome orientation was recorded based on basal trichome shape, counted and individual clones indicated on wing schematics
1H,I	Schematics summarizing location of clones analyzed for rescue of PCP defect	Drawn in PowerPoint by U.W. in all layers
2A-G	<i>w ubxFLP; FRT42D^{neo} arr^k /FRT42D^{neo} armlacZ</i>	Wandering third instar larvae were collected and aged at 29°C for 30-32 hours prior to fixation, fixed animals were accumulated at 4°C prior to dissection and staining, antibody staining on fully dissected pupal wings, confocal acquisition on Zeiss lsm880 or 980, Fiji for projections and measuring average grey values for stainings in 15-30 cell ROIs adjacent to each other along A-P axis in mutant versus <i>wild-type</i> tissue, cells at clonal border and of veins were excluded, GraphPad for graph, Packing analyzer for segmentation and nematic order, polarity quantification by Reza Farhadifar
3A-D	<i>yw eyFLP; FRT42D^{neo} P[w⁺]/ FRT42D^{neo} arr^k and FRT19A^{neo}/ w dsh^{V26} FRT19A^{neo}; eyFLP/+</i>	Adult eye sections, Zeiss axioplan phase contrast image acquisition (100x lens), PCP phenotype analysis aligned with mosaic photoreceptor R3-R4 pairs
3D,E,H	n.a., schematics and tables	Generated in Powerpoint by U.W.
3E-J	<i>y w hs FLP; FRT42D^{neo} ubiGFP/ FRT42D^{neo} arr^k; mδlacZ/+ and w; FRT42D^{neo} armlacZ/ FRT42D^{neo} arr^k; eyFLP /+, DIPδGFP/+</i>	Antibody stainings of third larval instar eye imaginal discs and confocal imaging as above

3K	For <i>arr</i> and <i>dsh</i> clones, this paper, see 3A-D, for <i>ft^{fd}</i> , <i>ft⁴²²</i> , <i>ds^{UA071}</i> , <i>ds^{38k}</i> clones/eyes ⁸⁵ , 83, 84	Phenotypic features were counted according to chirality and/or rotation defects observed in adult ommatidia, for <i>ft</i> and <i>ds</i> this was done on published data, see left column
4A,B	<i>w; ptc-GAL4, E-cad::GFP, UAS-ArrHA/+</i>	Pupal wing preparations, stainings, imaging as above
4C-G	<i>w; dppGAL4, UAS-FzMyc or UAS-Fz2Myc or UAS-Fmi or UAS-Vang</i>	Crosses at 25°C, shift to 29°C for 5 or 25 hrs prior to dissections, long ts caused tissue warping in some genotypes, antibody stainings of third larval instar wing discs and confocal imaging as above, quantification with FIJI in 12-24 cell ROIs measuring average grey value on dpp stripe in 1st basal fold proximal to pouch area and in A/P <i>wt</i> area next to it for control, GraphPad for graph
5A-F	<i>w/+; sepGAL4, UAS-Dsh/+; Rh1GFP/+ (sep>Dsh), UASlacZ/+</i> or see right panel	<i>sep-GAL4, UAS-Dsh, Rh1GFP</i> combinant stock established, <i>sep>Dsh</i> at 20°C with <i>arr^{IRHMS}</i> or <i>arr^{IRHMC}</i> or <i>UAS-Arr</i> , concentrically, live imaged eyes ⁽¹²¹⁾ , quantification by manually documenting ommatidial arrangement through stack of images, graph generated in GraphPad
5G-K	<i>w; pnr-GAL4, UAS-Fz/+ (pnr>Fz), w^R/+</i> or see right panel	<i>pnr>UAS-Fz</i> or <i>pnr></i> at 25°C with <i>arr^{IRJF}</i> or <i>UAS-Arr</i> , quantifications by manually counting 45-180° misoriented dorsal bristles between dorsocentrals, graph generated in GraphPad
6A-D	<i>dsh^{V26}/+, arr²/+, fz^{P21}/+</i> or <i>dsh^{V26}/+, arr²/+, fz^{R52}/+</i> or <i>wg^{IL}/+, Wnt4^{EMS23}/+, arr²/+, fz^{P21}/+</i>	triple or quadruple heterozygotes for ligands, <i>arr</i> , <i>fz</i> or <i>dsh</i> and double heterozygote siblings generated by balancer based intercrossing, wing PCP phenotype assessed, graph in GraphPad
6E-H	<i>fmi^{E59}/+, arr²/+, fz^{P21}/+</i> or <i>fmi^{E59}/+, arr²/+, fz^{R52}/+</i> or <i>fmi^{E59}/+, arr^k/+, fz^{P21}/+</i> or <i>fmi^{E59}/+, arr^k/+, fz^{R52}/+</i>	triple heterozygotes for <i>fmi</i> , <i>arr</i> and <i>fz</i> and double heterozygote siblings were generated, rest as above
6I-L	<i>Stbm⁶/+, arr²/+, fz^{P21}/+</i> or <i>Stbm⁶/+, arr^k/+, fz^{P21}/+</i> or <i>Stbm⁶/+, arr^k/+, fz^{R52}/+</i>	Triple heterozygotes for <i>Stbm</i> , <i>arr</i> and <i>fz</i> and double heterozygote siblings were generated, rest as above
7A,B	n.a., model panels of Wnt and intercellular PCP signaling	Designed by Jill Gregory in Adobe Illustrator

Statistics and reproducibility table

Figure	Genotype	Statistical details
all	n.a.	Student's two-tailed t-test, graphs display mean and standard deviation (SD)
5C	<i>w; UAS-Fz/+; sev-GAL4K25, Rh1GFP/+ (sev>Fz), UAS-lacZ/+</i>	3 independent experiments, n total ommatidia = 305 from 4 eyes, mean and SD of 4-5 eyes (same for following genotypes)
5C	<i>w/+; sepGAL4, UAS-Dsh/+; Rh1GFP/+, UASlacZ/+, UAS-arr^{IRHMS}/+</i>	3 independent experiments, n total ommatidia = 550 from 5 eyes, P=0.6277 (not significant)
5C	<i>w; UAS-Fz/+; sev-GAL4K25, Rh1GFP/+, UASlacZ/+, UAS-arr^{IRJF}/+</i>	3 independent experiments, n total ommatidia = 694 from 5 eyes, P=0.8259 (not significant)
5C	<i>w; UAS-Fz/+; sev-GAL4K25, Rh1GFP/+, UAS-Arr/+</i>	3 independent experiments, n total ommatidia = 473 from 5 eyes, P=0.0169
5H	<i>w; en-GAL4, UAS-GFP, GAL80ts; UAS-arr^{IRJF01261}/+ (enGGG>arr^{IR}), UAS-lacZ/+</i>	2 experiments, 2 independent fz alleles used, UAS-Fz showed opposite effect, n total wings = 16, mean and SD of 16 wings (same for following genotypes)
5H	<i>w; en-GAL4, UAS-GFP, GAL80ts; UAS-arr^{IRJF01261}/+, fz^{P21}/+</i>	2 experiments, n total wings = 16, P<0.0001
5H	<i>w; en-GAL4, UAS-GFP, GAL80ts; UAS-arr^{IRJF01261}/+, fz^{R52}/+</i>	2 experiments, n total wings = 16, P<0.0001
5H	<i>w; en-GAL4, UAS-GFP, GAL80ts; UAS-arr^{IRJF01261}/+, UAS-Fz/+</i>	2 experiments, n total wings = 16, P<0.0001, UAS-Fz shows the same degree of enhanced notching as UAS-fz2 ^{IR}
5H	<i>w; en-GAL4, UAS-GFP, GAL80ts; UAS-arr^{IRJF01261}/+, UAS-fz2^{IR}/+</i>	2 experiments, n total wings = 16, P<0.0001
5I	<i>w; sevGAL4, UAS-Fmi, Rh1GFP/+ (sev>Fmi), UAS-LacZ /+</i>	2 experiments, n total ommatidia = 391 from 4 eyes, 2 independent alleles per gene (<i>dsh</i> , <i>arr</i>) and 2 independent IRs for <i>arr</i> , mean and SD of 4 eyes (same for following genotypes)
5I	<i>w; sevGAL4, UAS-Fmi, Rh1GFP/+, dsh1/+</i>	2 experiments, n total ommatidia = 460 from 4 eyes, P=0.8665, not significant
5I	<i>w; sevGAL4, UAS-Fmi, Rh1GFP/+, dsh^{V26}/+</i>	2 experiments, n total ommatidia = 393 from 4 eyes, P=0.2009, not significant
5I	<i>w; sevGAL4, UAS-Fmi, Rh1GFP/+, arr²/+</i>	2 experiments, n total ommatidia = 418 from 4 eyes, P=0.7377, not significant
5I	<i>w; sevGAL4, UAS-Fmi, Rh1GFP/+, arr^k/+</i>	2 experiments, n total ommatidia = 374 from 4 eyes, P=0.7442, not significant

5I	<i>w; sevGAL4, UAS-Fmi, Rh1GFP/+ , UAS-arrl^{RHMS}/+</i>	2 experiments, n total ommatidia = from 516 from 4 eyes, P=0.6490, not significant
5I	<i>w; sevGAL4, UAS-Fmi, Rh1GFP/+ , UAS-arrl^{RHMC}/+</i>	2 experiments, n total ommatidia = from 513 from 4 eyes, P=0.6066, not significant
5I	<i>w; sevGAL4, UAS-Fmi, Rh1GFP/+ , UAS-Arr/+</i>	2 experiments, n total ommatidia = from 264 from 4 eyes, P=0.5779, not significant
5J	<i>w; sevGAL4, UAS-Vang, Rh1GFP/+ (sev>Vang) , UAS-LacZ /+</i>	2 experiments, n total ommatidia =474 from 4 eyes, 2 independent alleles per gene (<i>dsh</i> , <i>arr</i>) and 2 independent IRs for <i>arr</i> , mean and SD of 4 eyes (same for following genotypes)
5J	<i>w; sevGAL4, UAS-Vang, Rh1GFP/+ , dsh1/+</i>	2 experiments, n total ommatidia = 504 from 4 eyes, P=0.005
5J	<i>w; sevGAL4, UAS-Vang, Rh1GFP/+ , dsh^{V26}/+</i>	2 experiments, n total ommatidia = 421 from 4 eyes, , P=0.001
5J	<i>w; sevGAL4, UAS-Vang, Rh1GFP/+ , arr²/+</i>	2 experiments, n total ommatidia = 476 from 4 eyes, , P=0.0255
5J	<i>w; sevGAL4, UAS-Vang, Rh1GFP/+ , arr^k/+</i>	2 experiments, n total ommatidia = 508 from 4 eyes, P=0.4226, not significant
5J	<i>w; sevGAL4, UAS-Vang, Rh1GFP/+ , UAS-arrl^{RHMS}/+</i>	2 experiments, n total ommatidia = 566 from 4 eyes, P=0.1125, not significant
5J	<i>w; sevGAL4, UAS-Vang, Rh1GFP/+ , UAS-arrl^{RHMC}/+</i>	2 experiments, n total ommatidia = 745 from 4 eyes, P=0.8488, not significant
5J	<i>w; sevGAL4, UAS-Vang, Rh1GFP/+ , UAS-Arr/+</i>	2 experiments, n total ommatidia = 537 from 4 eyes, , P=0.0358
6D	<i>dsh¹/+ , arr²/+ , fz^{P21}/+</i>	1 experiment, n total wings = 27, we show it to compare to the <i>dsh¹/+ , arr²/+ , fz^{R52}/+</i> experiments
6D	<i>dsh¹/+ , arr²/+</i>	1 experiment, n total wings = 28, n.a.
6D	<i>dsh¹/+ , fz^{P21}/+</i>	1 experiment, n total wings = 14, n.a.
6D	<i>arr²/+ , fz^{P21}/+</i>	1 experiment, n total wings = 23, n.a.
6D	<i>dsh¹/+ , arr²/+ , fz^{R52}/+</i>	2 independent experiments, n total wings = 54, mean and SD of 2 experiments (same for following genotypes)
Supplementary 6D	<i>dsh¹/+ , arr²/+</i>	2 independent experiments, n total wings = 54, P<0.0001
Supplementary 6D	<i>dsh¹/+ , fz^{R52}/+</i>	2 independent experiments, n total wings = 54, P<0.0001
Supplementary 6D	<i>arr²/+ , fz^{R52}/+</i>	2 independent experiments, n total wings = 54, P<0.0001
Supplementary 6D	<i>wg^{CX4}/+ , arr²/+ , fz^{P21}/+</i>	4 independent experiments, n total wings = 35, mean and SD of 4 experiments (same for following genotypes)

Supplementary 6D	<i>wg^{CX4/+}, arr^{2/+}</i>	4 independent experiments, n total wings = 24, mean and SD of 4 experiments, P<0.0001 compared to <i>wg^{CX4/+}, arr^{2/+}, fz^{P21/+}</i>
Supplementary 6D	<i>wg^{CX4/+}, fz^{P21/+}</i>	4 independent experiments, n total wings = 2, mean and SD of 4 experiments, P<0.0001 compared to <i>wg^{CX4/+}, arr^{2/+}, fz^{P21/+}</i>
Supplementary 6D	<i>arr^{2/+}, fz^{P21/+}</i>	4 independent experiments, n total wings = 10, mean and SD of 4 experiments, P<0.0001 compared to <i>wg^{CX4/+}, arr^{2/+}, fz^{P21/+}</i>
Supplementary 6D	<i>wg^{IL/+}, Wnt4^{EMS23/+}, arr^{2/+}, fz^{R52/+}</i>	1 experiment, n total wings = 14, we show it to support the <i>wg^{IL/+}, Wnt4^{EMS23/+}, arr^{2/+}, fz^{P21/+}</i> experiments (Fig.6D), as it shows similar effects
Supplementary 6D	<i>wg^{IL/+}, Wnt4^{EMS23/+}, arr^{2/+}, fz^{R52/+}</i>	1 experiment, n total wings = 12, n.a.
Supplementary 6D	<i>wg^{IL/+}, Wnt4^{EMS23/+}, arr^{2/+}, fz^{R52/+}</i>	1 experiment, n total wings = 18, n.a.
Supplementary 6D	<i>wg^{IL/+}, Wnt4^{EMS23/+}, arr^{2/+}, fz^{R52/+}</i>	1 experiment, n total wings = 18, n.a.
Supplementary 6D	<i>wg^{CX4/+}, arr^{2/+}, fz^{R52/+}</i>	2 independent experiments, n total wings = 22, mean and SD of 2 experiments (same for following genotypes)
Supplementary 6D	<i>wg^{CX4/+}, fz^{R52/+}</i>	2 independent experiments, n total wings = 8, P<0.0001 compared to <i>wg^{CX4/+}, arr^{2/+}, fz^{R52/+}</i>
Supplementary 6D	<i>wg^{CX4/+}, arr^{2/+}</i>	2 independent experiments, n total wings = 24, P<0.0001 compared to <i>wg^{CX4/+}, arr^{2/+}, fz^{R52/+}</i>
Supplementary 6D	<i>arr^{2/+}, fz^{R52/+}</i>	2 independent experiments, n total wings = 20, P<0.0001 compared to <i>wg^{CX4/+}, arr^{2/+}, fz^{R52/+}</i>
Supplementary 6E	<i>arm^{2/+}, arr^{2/+}, fz^{P21/+}</i>	2 independent experiments, n total wings = 36, mean and SD of 2 experiments (same for following genotypes), <i>arm^{2/+}</i> suppresses <i>arr^{2/+}, fz^{P21/+}</i>
Supplementary 6E	<i>arm^{2/+}, arr^{2/+}</i>	2 independent experiments, n total wings = 33, P<0.0001 compared to <i>arm^{2/+}, arr^{2/+}, fz^{P21/+}</i>
Supplementary 6E	<i>arm^{2/+}, fz^{P21/+}</i>	2 independent experiments, n total wings = 31, P<0.0001 compared to <i>arm^{2/+}, arr^{2/+}, fz^{P21}</i>
Supplementary 6E	<i>arr^{2/+}, fz^{P21/+}</i>	2 independent experiments, n total wings = 28, P<0.0001 compared to <i>arm^{2/+}, arr^{2/+}, fz^{P21}</i>
Supplementary 6E	<i>arm^{2/+}, arr^{2/+}, fz^{R52/+}</i>	2 independent experiments, n total wings = 41, mean and SD of 2 experiments (same for following genotypes), <i>arm^{2/+}</i> suppresses <i>arr^{2/+}, fz^{R52/+}</i>
Supplementary 6E	<i>arm^{2/+}, arr^{2/+}</i>	2 independent experiments, n total wings = 31, P<0.0001 compared to <i>arm^{2/+}, arr^{2/+}, fz^{R52/+}</i>
Supplementary 6E	<i>arm^{2/+}, fz^{R52/+}</i>	2 independent experiments, n total wings = 31, <i>arm^{2/+}, arr^{2/+}, fz^{R52/+}</i>

Supplementary 6E	<i>arr</i> ² /+, <i>fz</i> ^{R52} /+	2 independent experiments, n total wings = 30, P<0.0001 compared to <i>arm</i> ² /+, <i>arr</i> ² /+, <i>fz</i> ^{R52} /+
Supplementary 6E	<i>arm</i> ² /+, <i>arr</i> ^k /+, <i>fz</i> ^{P21} /+	2 independent experiments, n total wings = 39, mean=0 and SD=0 of 2 experiments (same for following genotypes)
Supplementary 6E	<i>arm</i> ² /+, <i>arr</i> ^k /+	2 independent experiments, n total wings = 28, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm</i> ² /+, <i>arr</i> ^k /+, <i>fz</i> ^{P21} /+ (no phenotype observed)
Supplementary 6E	<i>arm</i> ² /+, <i>fz</i> ^{P21} /+	2 independent experiments, n total wings = 34, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm</i> ² /+, <i>arr</i> ^k /+, <i>fz</i> ^{P21} /+ (no phenotype observed)
Supplementary 6E	<i>arr</i> ^k /+, <i>fz</i> ^{P21} /+	2 independent experiments, n total wings = 32, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm</i> ² /+, <i>arr</i> ^k /+, <i>fz</i> ^{P21} /+ (no phenotype observed)
Supplementary 6E	<i>arm</i> ² /+, <i>arr</i> ^k /+, <i>fz</i> ^{R52} /+	2 independent experiments, n total wings = 30, mean=0 and SD=0 of 2 experiments (same for following genotypes)
Supplementary 6E	<i>arm</i> ² /+, <i>arr</i> ^k /+	2 independent experiments, n total wings = 28, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm</i> ² /+, <i>arr</i> ^k /+, <i>fz</i> ^{R52} /+ (no phenotype observed)
Supplementary 6E	<i>arm</i> ² /+, <i>fz</i> ^{R52} /+	2 independent experiments, n total wings = 27, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm</i> ² /+, <i>arr</i> ^k /+, <i>fz</i> ^{R52} /+ (no phenotype observed)
Supplementary 6E	<i>arr</i> ^k /+, <i>fz</i> ^{R52} /+	2 independent experiments, n total wings = 32, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm</i> ² /+, <i>arr</i> ^k /+, <i>fz</i> ^{R52} /+ (no phenotype observed)
Supplementary 6F	<i>arm</i> ² /+, <i>wg</i> ^{IL} /+, <i>Wnt4</i> ^{EMS23} /+, <i>arr</i> ² /+	2 independent experiments, n total wings = 38, mean=0 and SD=0 of 2 experiments (same for following genotypes)
Supplementary 6F	<i>arm</i> ² /+, <i>wg</i> ^{IL} /+, <i>Wnt4</i> ^{EMS23} /+	2 independent experiments, n total wings = 27, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm</i> ² /+, <i>wg</i> ^{IL} /+, <i>Wnt4</i> ^{EMS23} /+, <i>arr</i> ² /+ (no phenotype observed)
Supplementary 6F	<i>arm</i> ² /+, <i>arr</i> ² /+	2 independent experiments, n total wings = 6, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm</i> ² /+, <i>wg</i> ^{IL} /+, <i>Wnt4</i> ^{EMS23} /+, <i>arr</i> ² /+ (no phenotype observed)

Supplementary 6F	<i>wg^l/+</i> , <i>Wnt4^{EMS23}/+</i> , <i>arr²/+</i>	2 independent experiments, n total wings = 39, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm²/+</i> , <i>wg^l/+</i> , <i>Wnt4^{EMS23}/+</i> , <i>arr²/+</i> (no phenotype observed)
Supplementary 6F	<i>arm²/+</i> , <i>wg^{CX4}/+</i> , <i>arr²/+</i>	2 independent experiments, n total wings = 16, mean=0 and SD=0 of 2 experiments (for following genotypes)
Supplementary 6F	<i>arm²/+</i> , <i>wg^{CX4}/+</i>	2 independent experiments, n total wings = 24, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm²/+</i> , <i>wg^{CX4}/+</i> , <i>arr²/+</i> (no phenotype observed)
Supplementary 6F	<i>arm²/+</i> , <i>arr²/+</i>	2 independent experiments, n total wings = 22, p=0.0975
Supplementary 6F	<i>wg^{CX4}/+</i> , <i>arr²/+</i>	2 independent experiments, n total wings = 29, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm²/+</i> , <i>wg^{CX4}/+</i> , <i>arr²/+</i> (no phenotype observed)
Supplementary 6F	<i>arm²/+</i> , <i>wg^l/+</i> , <i>Wnt4^{EMS23}/+</i> , <i>arr^k/+</i>	1 experiment, n total wings = 6, we show it to compare to the <i>arm²/+</i> , <i>wg^l/+</i> , <i>Wnt4^{EMS23}/+</i> , <i>arr²/+</i> experiment
Supplementary 6F	<i>arm²/+</i> , <i>wg^l/+</i> , <i>Wnt4^{EMS23}/+</i>	1 experiment, n total wings = 16
Supplementary 6F	<i>arm²/+</i> , <i>arr^k/+</i>	1 experiment, n total wings = 6
Supplementary 6F	<i>wg^l/+</i> , <i>Wnt4^{EMS23}/+</i> , <i>arr^k/+</i>	1 experiment, n total wings = 8
Supplementary 6F	<i>arm²/+</i> , <i>wg^{CX4}/+</i> , <i>arr^k/+</i>	2 independent experiments, n total wings = 36, mean=0 and SD=0 of 2 experiments
Supplementary 6F	<i>arm²/+</i> , <i>wg^{CX4}/+</i>	2 independent experiments, n total wings = 33, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm²/+</i> , <i>wg^{CX4}/+</i> , <i>arr^k/+</i> (no phenotype observed)
Supplementary 6F	<i>arm²/+</i> , <i>arr^k/+</i>	2 independent experiments, n total wings = 32, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm²/+</i> , <i>wg^{CX4}/+</i> , <i>arr^k/+</i> (no phenotype observed)
Supplementary 6F	<i>wg^{CX4}/+</i> , <i>arr^k/+</i>	2 independent experiments, n total wings = 33, mean=0 and SD=0, P not calculatable, perfect data (p.d.), compared to <i>arm²/+</i> , <i>wg^{CX4}/+</i> , <i>arr^k/+</i> (no phenotype observed)

Image quantification and statistical analysis for main Figures 3,5 and 6

Figure	Genotype	Statistical details
all	n.a.	Student's two-tailed t-test, graphs display mean and standard deviation (SD)
3D	<i>arr^k</i> clones	4 independent experiments, n = 22 eyes, 1-5 clones per eye
3D	<i>arr²</i> clones	4 independent experiments, n = 35 eyes, 1-5 clones per eye
3D	<i>dsh^{V26}</i> clones	4 independent experiments, n = 16 eyes, 1-5 clones per eye
3K	<i>arr²</i> clones	4 independent experiments, n = 6 eyes, 5-9 clones per eye, 200 ommatidia
3K	<i>arr^k</i> clones	4 independent experiments, n = 5 eyes, 5-9 clones per eye, 234 ommatidia
3K	<i>dsh^{V26}</i> clones	4 independent experiments, n = 9 eyes, 5-9 clones per eye, 395 ommatidia
3K	<i>ft</i> clones	<i>ft⁴²²</i> and <i>ft^{fd}</i> data pooled, as they show indistinguishable defects, 3 independent experiments from refs 83, 84, 85, n = 6 eyes, 6-7 clones per genotype, 233 ommatidia
3K	<i>ds</i> clones	<i>ds^{UA071}</i> and <i>ds^{38k}</i> data pooled, as they show indistinguishable defects, 3 independent experiments from refs 83, 84, 85, n = 7 eyes, 6-7 clones per genotype, 292 ommatidia
3F	<i>arr^k</i> clones	3 independent experiments, n = 13 eyes, multiple clones per eye
3G	<i>arr²</i> clones	3 independent experiments, n = 29 eyes, multiple clones per eye
3I	<i>arr^k</i> clones	3 independent experiments, n = 6 eyes, multiple clones per eye
3J	<i>arr²</i> clones	3 independent experiments, n = 23 eyes, multiple clones per eye
5F	<i>w/+; sepGAL4, UAS-Dsh/+; Rh1GFP/+ (sep>Dsh), UASlacZ/+</i>	2-5 independent experiments, n total ommatidia = 1384 from 11 eyes, mean and SD of 11 eyes (same for following genotypes)
5F	<i>w/+; sepGAL4, UAS-Dsh/+; Rh1GFP/+, UAS-arr^{IRHMS}/+</i>	2-5 independent experiments, n total ommatidia = 1130 from 11 eyes, P-chirality=0.0064, P-loss=0.0021 compared to <i>w/+; sepGAL4, UAS-Dsh/+; Rh1GFP/+, UASlacZ/+</i>
5F	<i>w/+; sepGAL4, UAS-Dsh/+; Rh1GFP/+, UAS-arr^{IRHMC}/+</i>	2-5 independent experiments, n total ommatidia = 1200 from 10 eyes, P-chirality=0.0424, P-loss=0.0034 compared to <i>w/+; sepGAL4, UAS-Dsh/+; Rh1GFP/+, UASlacZ/+</i>
5F	<i>w/+; sepGAL4, UAS-Dsh/+; Rh1GFP/+, UAS-Arr/+</i>	2-5 independent experiments, n total ommatidia = 1075 from 12 eyes, P-chirality=0.0003, P-loss<0.0001 compared to <i>w/+; sepGAL4, UAS-Dsh/+; Rh1GFP/+, UASlacZ/+</i>
5K	<i>w; pnr-GAL4, UAS-Fz/+ (pnr>Fz), UAS-w^{IR}/+</i>	2 independent experiments, n total cheateas = 378, from 5 thoraxes, mean and SD of 5 thoraxes (same for following genotypes)
5K	<i>w; pnr-GAL4, UAS-Fz/+, UAS-arr^{IRJF}/+</i>	2 independent experiments, n total cheateas = 317 from 5 thoraxes

5K	<i>w; pnr-GAL4, UAS-Fz/+ , UAS-Arr/+</i>	2 independent experiments, n total cheatea = 373 from 5 thoraxes
5K	<i>w; pnr-GAL4, UAS-arr^{IRJF}/+</i>	2 independent experiments, n total cheatea = 246 from 5 thoraxes, P=0.0029
5K	<i>w; pnr-GAL4, UAS-Arr/+</i>	2 independent experiments, n total cheatea = 360 from 5 thoraxes, P=0.0475
6D	<i>dsh^{V26}/+, arr²/+, fz^{P21}/+</i>	4 independent experiments, n total wings = 40, mean and SD of 4 experiments (same for the following genotypes)
6D	<i>dsh^{V26}/+, arr²/+</i>	4 independent experiments, n total wings = 24 , P<0.0001 compared to <i>dsh^{V26}/+, arr²/+, fz^{P21}/+</i>
6D	<i>dsh^{V26}/+, fz^{P21}/+</i>	4 independent experiments, n total wings = 27, P<0.0001 compared to <i>dsh^{V26}/+, arr²/+, fz^{P21}/+</i>
6D	<i>arr²/+, fz^{P21}/+</i>	4 independent experiments, n total wings = 35, P<0.0001 compared to <i>dsh^{V26}/+, arr²/+, fz^{P21}/+</i>
6D	<i>dsh^{V26}/+, arr²/+, fz^{R52}/+</i>	4 independent experiments, n total wings = 78, mean and SD of 4 experiments (same for the following genotypes)
6D	<i>dsh^{V26}/+, arr²/+</i>	4 independent experiments, n total wings = 28, P<0.0001 compared to <i>dsh^{V26}/+, arr²/+, fz^{R52}/+</i>
6D	<i>dsh^{V26}/+, fz^{R52}/+</i>	4 independent experiments, n total wings = 24, P<0.0001 compared to <i>dsh^{V26}/+, arr²/+, fz^{R52}/+</i>
6D	<i>arr²/+, fz^{R52}/+</i>	4 independent experiments, n total wings = 62, P<0.0001 compared to <i>dsh^{V26}/+, arr²/+, fz^{R52}/+</i>
6D	<i>wg^{IL}/+, Wnt4^{EMS23}/+, arr²/+, fz^{P21}/+</i>	4 independent experiments, n total wings = 15, mean and SD of 4 experiments (same for the following genotypes)
6D	<i>wg^{IL}/+, Wnt4^{EMS23}/+, arr²/+,</i>	4 independent experiments, n total wings = 16, P<0.0001 compared to <i>wg^{IL}/+, Wnt4^{EMS23}/+, arr²/+, fz^{P21}/+</i>
6D	<i>wg^{IL}/+, Wnt4^{EMS23}/+, fz^{P21}/+</i>	4 independent experiments, n total wings = 16, P<0.0001 compared to <i>wg^{IL}/+, Wnt4^{EMS23}/+, arr²/+, fz^{P21}/+</i>
6D	<i>arr²/+, fz^{P21}/+</i>	4 independent experiments, n total wings = 18, P<0.0001 compared to <i>wg^{IL}/+, Wnt4^{EMS23}/+, arr²/+, fz^{P21}/+</i>
6E	<i>fmi^{E59}/+, arr²/+, fz^{P21}/+</i>	2 independent experiments, n total wings = 33, mean=1, SD=0 of 2 experiments, cannot be compared to the other control genotypes, as phenotype of <i>fmi^{E59}/+, arr²/+, fz^{P21}/+</i> is much stronger and spreads to both wing surfaces and beyond veins L3, L4 and anterior cross vein (Fig. 6f,g)
6E	<i>fmi^{E59}/+, arr²/+</i>	2 independent experiments, n total wings = 25, mean and SD of 2 experiments
6E	<i>fmi^{E59}/+, fz^{P21}/+</i>	2 independent experiments, n total wings= 23, P not calculatable because mean=1 and SD=0, perfect data (p.d.), compared to <i>fmi^{E59}/+, arr²/+</i>
6E	<i>arr²/+, fz^{P21}/+</i>	2 independent experiments, n total wings= 20, P<0.0001 compared to <i>fmi^{E59}/+, arr²/+</i>

6E	<i>fmi</i> ^{E59/+} , <i>arr</i> ^{2/+} , <i>fz</i> ^{R52/+}	2 independent experiments, independent <i>fz</i> allele, same effects observed as for <i>fz</i> ^{P21} , n total wings = 32, mean=1, SD=0, cannot be compared to the other control genotypes, as phenotype of <i>fmi</i> ^{E59/+} , <i>arr</i> ^{2/+} , <i>fz</i> ^{R52/+} is much stronger and spreads to both wing surfaces and beyond veins L3, L4 and anterior cross vein
6E	<i>fmi</i> ^{E59/+} , <i>arr</i> ^{2/+}	2 independent experiments, n total wings = 29, mean=1, SD=0
6E	<i>fmi</i> ^{E59/+} , <i>fz</i> ^{R52/+}	2 independent experiments, n total wings = 21, P not calculatable because mean=1 and SD=0, perfect data (p.d.), compared to <i>fmi</i> ^{E59/+} , <i>arr</i> ^{2/+}
6E	<i>arr</i> ^{2/+} , <i>fz</i> ^{R52/+}	2 independent experiments, n total wings = 31, P<0.0001 compared to <i>fmi</i> ^{E59/+} , <i>arr</i> ^{2/+}
6E	<i>fmi</i> ^{E59/+} , <i>arr</i> ^{k/+} , <i>fz</i> ^{P21/+}	2 independent experiments, n total wings = 29, mean=0, SD=0 of 2 experiments
6E	<i>fmi</i> ^{E59/+} , <i>arr</i> ^{k/+}	2 independent experiments, n total wings = 18, P not calculatable because mean=0 and SD=0, perfect data (p.d.), compared to <i>fmi</i> ^{E59/+} , <i>arr</i> ^{k/+} , <i>fz</i> ^{P21/+} , no phenotype
6E	<i>fmi</i> ^{E59/+} , <i>fz</i> ^{P21/+}	2 independent experiments, n total wings = 15, P not calculatable because mean=0 and SD=0, perfect data (p.d.), compared to <i>fmi</i> ^{E59/+} , <i>arr</i> ^{k/+} , <i>fz</i> ^{P21/+} , no phenotype
6E	<i>arr</i> ^{k/+} , <i>fz</i> ^{P21/+}	2 independent experiments, n total wings = 29, P not calculatable because mean=0 and SD=0, perfect data (p.d.), compared to <i>fmi</i> ^{E59/+} , <i>arr</i> ^{k/+} , <i>fz</i> ^{P21/+} , no phenotype
6E	<i>fmi</i> ^{E59/+} , <i>arr</i> ^{k/+} , <i>fz</i> ^{R52/+}	2 independent experiments, n total wings = 26, mean=0, SD=0 of 2 experiments, no phenotype
6E	<i>fmi</i> ^{E59/+} , <i>arr</i> ^{k/+}	2 independent experiments, n total wings = 26, P not calculatable because mean=0 and SD=0, perfect data (p.d.), compared to <i>fmi</i> ^{E59/+} , <i>arr</i> ^{k/+} , <i>fz</i> ^{R52/+} , no phenotype
6E	<i>fmi</i> ^{E59/+} , <i>fz</i> ^{R52/+}	2 independent experiments, n total wings = 16, P not calculatable because mean=0 and SD=0, perfect data (p.d.), compared to <i>fmi</i> ^{E59/+} , <i>arr</i> ^{k/+} , <i>fz</i> ^{R52/+} , no phenotype
6E	<i>arr</i> ^{k/+} , <i>fz</i> ^{R52/+}	2 independent experiments, n total wings = 20, P not calculatable because mean=0 and SD=0, perfect data (p.d.), compared to <i>fmi</i> ^{E59/+} , <i>arr</i> ^{k/+} , <i>fz</i> ^{R52/+} , no phenotype
6I	<i>Stbm</i> ^{6/+} , <i>arr</i> ^{2/+} , <i>fz</i> ^{P21/+}	2 independent experiments, n total wings = 35, mean=1, SD=0 of 2 experiments, cannot be compared to the other control genotypes, as phenotype of <i>Stbm</i> ^{6/+} , <i>arr</i> ^{2/+} , <i>fz</i> ^{P21/+} is much stronger and spreads to both wing surfaces and beyond veins L3, L4 and anterior cross vein (Fig. 6j,k), similar to <i>fmi</i> ^{E59/+} , <i>arr</i> ^{k/+} , <i>fz</i> ^{P21/+} and <i>fmi</i> ^{E59/+} , <i>arr</i> ^{k/+} , <i>fz</i> ^{R52/+} effects
6I	<i>Stbm</i> ^{6/+} , <i>arr</i> ^{2/+}	2 independent experiments, n total wings = 26, mean=1, SD=0
6I	<i>Stbm</i> ^{6/+} , <i>fz</i> ^{P21/+}	2 independent experiments, n total wings = 22, P<0.0001 compared to <i>Stbm</i> ^{6/+} , <i>arr</i> ^{2/+}

6l	<i>arr</i> ² /+, <i>fz</i> ^{P21} /+	2 independent experiments, n total wings = 36, P<0.0001 compared to <i>Stbm</i> ⁶ /+, <i>arr</i> ² /+
6l	<i>Stbm</i> ⁶ /+	2 independent experiments, n total wings = 26, P<0.0001 compared to <i>Stbm</i> ⁶ /+, <i>arr</i> ² /+
6l	<i>Stbm</i> ⁶ /+, <i>arr</i> ^k /+, <i>fz</i> ^{P21} /+	2 independent experiments, n total wings = 33, mean and SD of 2 experiments (same for following genotypes), <i>arr</i> ^k allele is weaker than <i>arr</i> ²
6l	<i>Stbm</i> ⁶ /+, <i>arr</i> ^k /+	2 independent experiments, n total wings = 20, P=0.0249 compared to <i>Stbm</i> ⁶ /+, <i>arr</i> ^k /+, <i>fz</i> ^{P21} /+
6l	<i>Stbm</i> ⁶ /+, <i>fz</i> ^{P21} /+	2 independent experiments, n total wings = 25, P=0.0071 compared to <i>Stbm</i> ⁶ /+, <i>arr</i> ^k /+, <i>fz</i> ^{P21} /+
6l	<i>arr</i> ^k /+, <i>fz</i> ^{P21} /+	2 independent experiments, n total wings = 16, P=0.0008 compared to <i>Stbm</i> ⁶ /+, <i>arr</i> ^k /+, <i>fz</i> ^{P21} /+
6l	<i>Stbm</i> ⁶ /+	2 independent experiments, n total wings = 11, P=0.0027 compared to <i>Stbm</i> ⁶ /+, <i>arr</i> ^k /+, <i>fz</i> ^{P21} /+
6l	<i>Stbm</i> ⁶ /+, <i>arr</i> ^k /+, <i>fz</i> ^{R52} /+	2 independent experiments, n total wings = 34, , mean and SD of 2 experiments (for following genotypes), <i>arr</i> ^k allele is weaker than <i>arr</i> ²
6l	<i>Stbm</i> ⁶ /+, <i>arr</i> ^k /+	2 independent experiments, n total wings = 40, P=0.0183 compared to <i>Stbm</i> ⁶ /+, <i>arr</i> ^k /+, <i>fz</i> ^{R52} /+
6l	<i>Stbm</i> ⁶ /+, <i>fz</i> ^{R52} /+	2 independent experiments, n total wings = 17, P=0.0004 compared to <i>Stbm</i> ⁶ /+, <i>arr</i> ^k /+, <i>fz</i> ^{R52} /+
6l	<i>arr</i> ^k /+, <i>fz</i> ^{R52} /+	2 independent experiments, n total wings = 28, P=0.0056 compared to <i>Stbm</i> ⁶ /+, <i>arr</i> ^k /+, <i>fz</i> ^{R52} /+
6l	<i>Stbm</i> ⁶ /+	2 independent experiments, n total wings = 14, P<0.0001 compared to <i>Stbm</i> ⁶ /+, <i>arr</i> ^k /+, <i>fz</i> ^{R52} /+

For all other Methods please see main text.

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