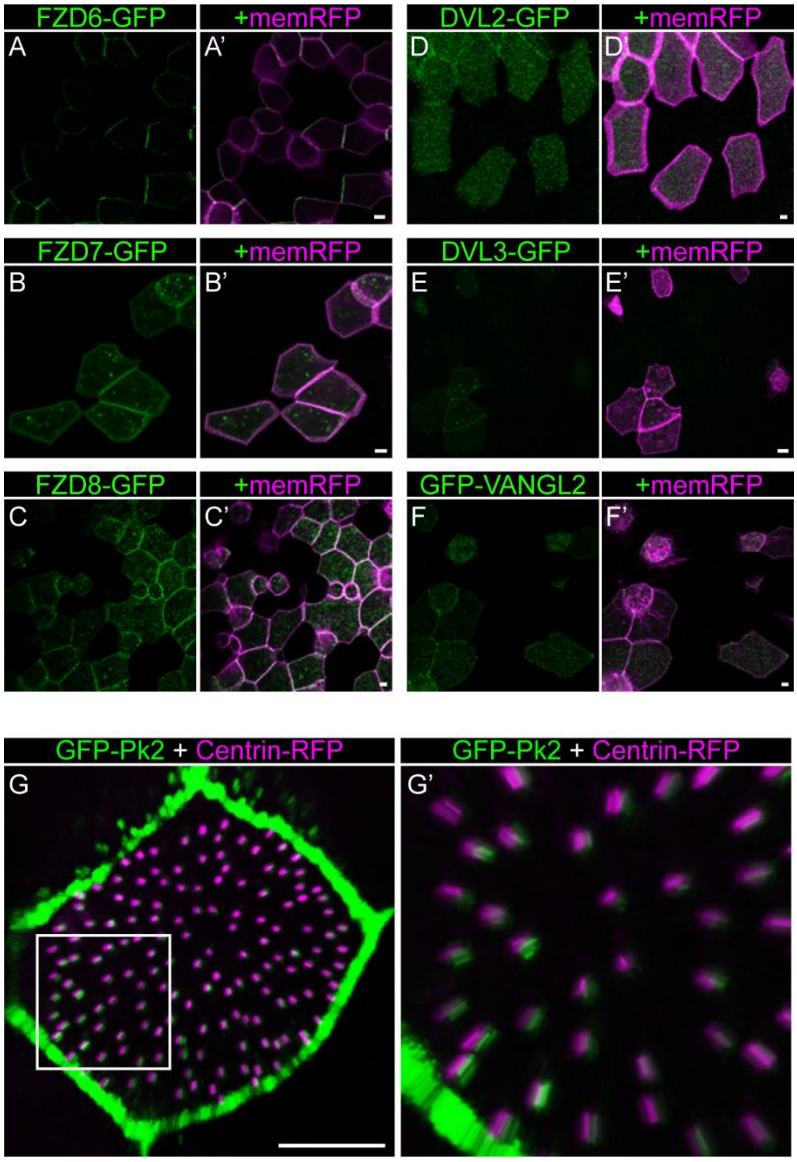


Fig. S1. Alternate Core PCP family members differentially localize in the *Xenopus* epidermis. (A-F) Mosaically-labeled cells in stage 31 embryos labeled with PCP proteins fused to GFP and membraneRFP and surrounded by unlabeled neighbors. Frizzled-6 (A) displays asymmetric localization while Frizzled-7 (B), Frizzled-8 (C), Dishevelled-2 (D), Dishevelled-3 (E), and Vangl2 (F) all decorate the cortex in a symmetrical fashion. Scale represents 10μm. (G) GFP-Pk2 assumes a polarized localization near basal bodies labeled with Centrin-RFP after the finalization of basal orientation refinement (St.35 shown). Note that this cell is surrounded by similarly labeled cells, making the Pk2 cortical asymmetry unapparent. Box in (G) demarcates the area magnified in (G′). Scale bar represents 10μm.

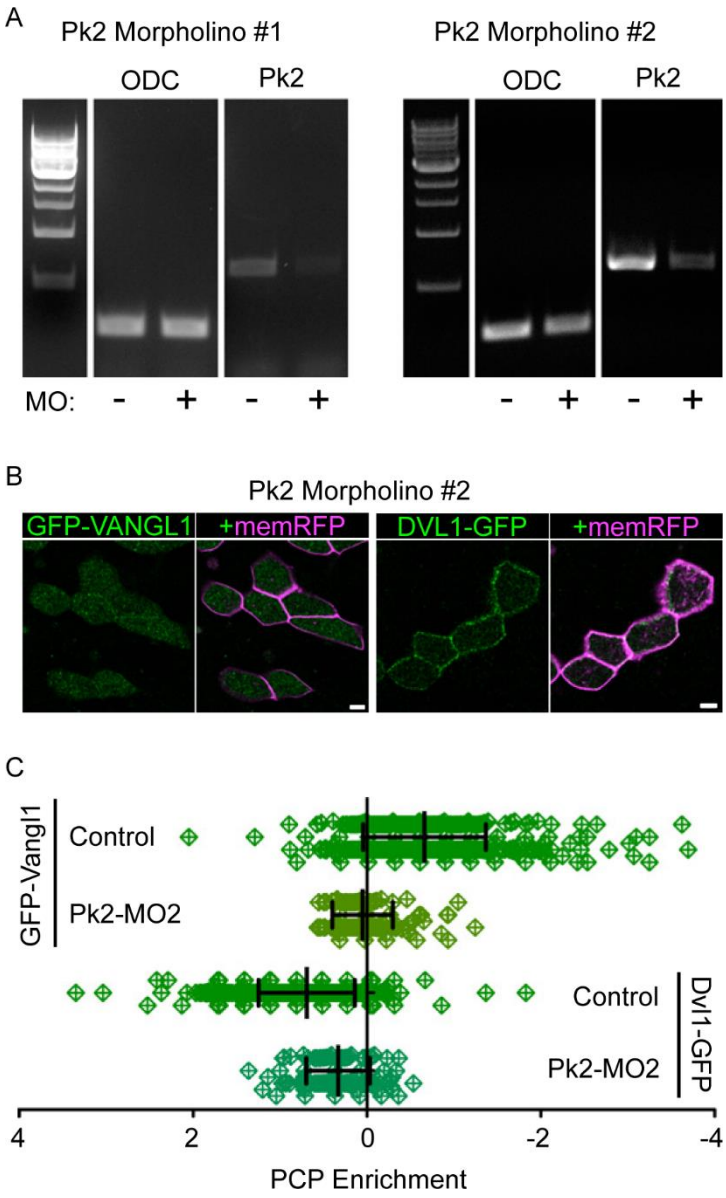


Fig. S2. Pk2 morpholinos reduce Pk2 mRNA levels, resulting in a reduction of Dvl1 and Vang1 asymmetry. (A) RT-PCR results for the amplification of a control, ornithine decarboxylase (ODC), and morpholino-targeted Pk2 sequence from cDNA of embryos that were either uninjected or injected with Pk2 morpholino #1 or #2 into 4 of 4 cells, demonstrating a significant reduction of only the Pk2 PCR product in morpholino-injected embryos. (B) Cells in stage 31 embryos labeled with PCP proteins fused to GFP and membraneRFP with an included dose of Pk2 morpholino #2, showing similar effects to Pk2 morpholino #1 shown in Fig.s 2 and 4. Scale bars represent 10μm. (C) Graph depicting changes in localization of Vangl1 and Dvl1 caused by Pk2 knockdown with a second Pk2 morpholino (Pk2-MO#2) targeting an alternative splicing site from the first. PCP enrichment is significantly reduced for both GFP-Vangl1 ($p<0.0001$***) and Dvl1-GFP ($p<0.0001$***) in morphant cells (n= 137 for Vangl1 and 131 for Dvl1) in comparison to controls (n= 519 cells for Vangl1 and 508 cells for Dvl1). Error bars indicate standard deviation of the mean.

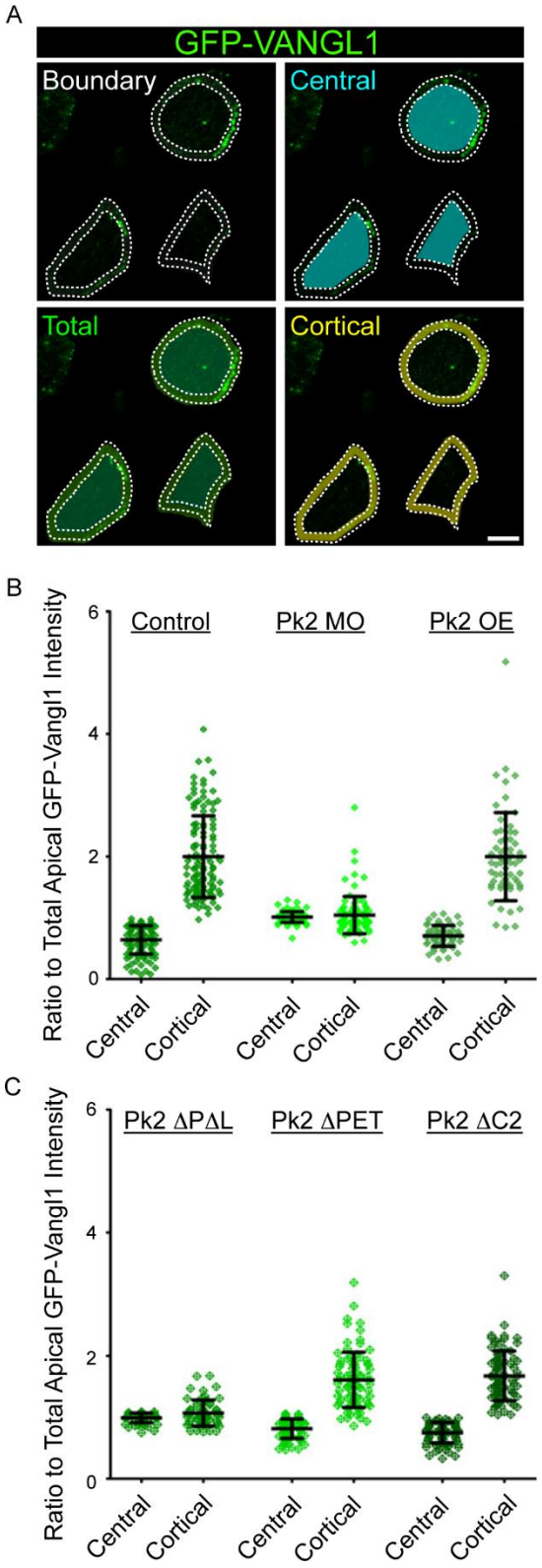


Fig. S3. GFP-Vangl1 Enrichment is abolished upon Pk2 MO knockdown and Pk2-ΔPETΔLIM overexpression. (A) Schematic of quantification method of data presented in (B,C). Either the average cortical fluorescence intensity or the average intensity of the remaining central area within each cell was

divided by the average fluorescence intensity of the total apical surface area (both regions combined). Scale represents 10μm. (B) Quantification data showing a significant loss of cortical enrichment of GFP-Vangl1 upon Pk2-MO knockdown. Each dot represents one cell, and each cell has both a Central and a Cortical quotient presented with the total apical fluorescence measure as the divisor. Combined, these two measurements are highly significant from another in the control (n= 123) and Pk2-OE (n=63) conditions ($p<0.0001$***) but not significant for Pk2-MO knockdown (n=94) ($p=0.2854$). Error bars indicate Standard deviation of the mean. (C) Quantification data showing a significant loss of cortical enrichment of GFP-Vangl1 upon overexpression of Pk2-ΔPETΔLIM. Each dot represents one cell, and each cell has both a Central and a Cortical measurement as presented in (B). Combined, these two measurements are highly significant from another in the Pk2-ΔPET (n= 85) and Pk2-ΔC2 (n= 84) conditions ($p<0.0001$***) but not significant for Pk2-ΔPETΔLIM (n=58) ($p=0.1519$). Error bars indicate standard deviation of the mean.

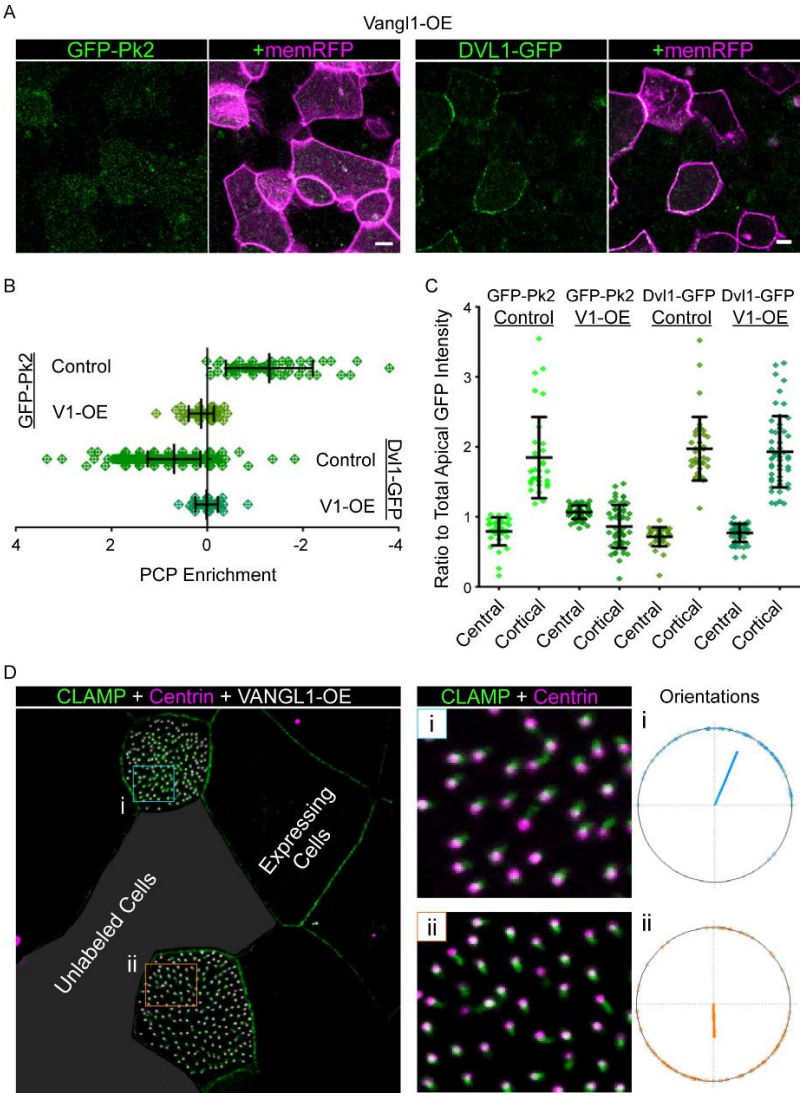


Fig. S4. Vangl1 Overexpression leads to PCP patterning and nonautonomous tissue-level polarity defects. (A) Cells in stage 31 embryos labeled with PCP proteins fused to GFP and membraneRFP as well as overexpressing Vangl1 display loss of Dvl1-GFP asymmetry and reduction in GFP-Pk2 cortical enrichment. Scale bars represent 10μm. (B) Graph depicting changes in PCP Enrichment of Pk2 and Dvl1 caused by Vangl1 overexpression (V1-OE). PCP enrichment is significantly reduced in cells overexpressing Vangl1 (n= 60 for Pk2, n= 55 for Dvl1) in comparison to controls for both GFP-Pk2 (n= 64) ($p<0.0001$***) and Dvl1-GFP (n=243) ($p<0.0001$***). Error bars indicate standard deviation of the mean. (C) Quantification data showing a significant loss of cortical enrichment of GFP-Pk2 but not Dvl1-GFP upon Vangl1 overexpression (V1-OE). Each dot represents one cell, and each cell has both a Central and a Cortical measurement presented as divided by the total apical fluorescence (See Fig. S3A for quantification schematic). These Central and Cortical measurements are highly significant from one another in both Pk (n= 33) and Dvl1 (n=6) control ($p<0.0001$***), Dvl1 with V1-OE (n= 53, $p<0.0001$***), and Pk2 with V1-OE (n= 53, $p=0.0002$***) conditions, but only in the case of GFP-Pk2 in V1-OE embryos is the mean Cortical enrichment less than the Central measure. Error bars indicate standard deviation of the mean. (D) Two multicilated cells situated at the edge of a clone labeled with Centrin-RFP and CLAMP-GFP and overexpressing Vangl1, with cell (i) outlined in blue situated above unlabeled cells and cell (ii) outlined in orange situated below. Magnification of boxed areas and quantification of ciliary orientations and mean polarity vector for each cell demonstrate the tendency for striated rootlets (opposite the effective stroke) to be oriented towards higher Vangl1 levels (towards labeled cells and away from unlabeled cells) (n= 95 orientations for i, n= 124 orientations in ii).

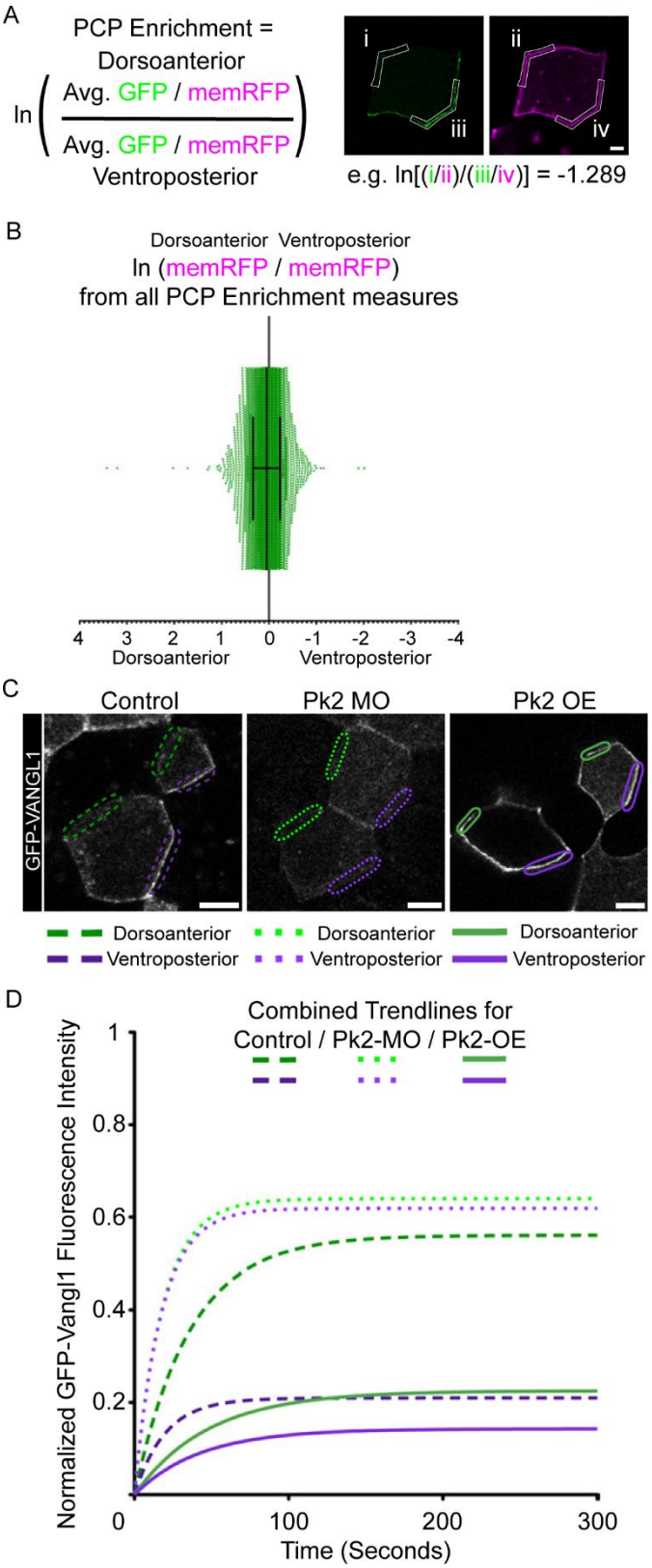
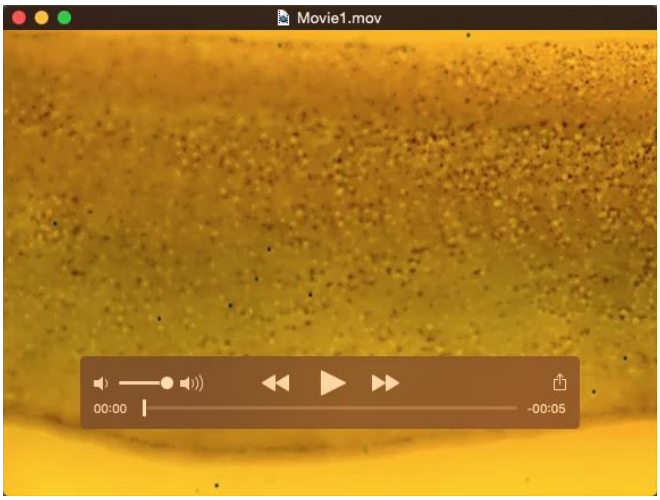


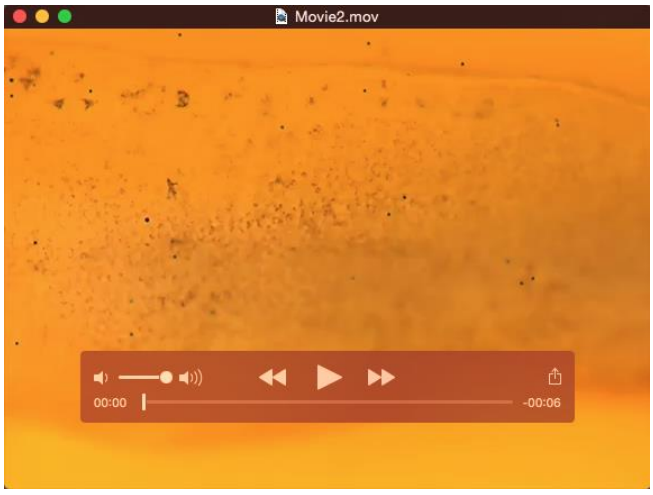
Fig. S5. Supplement for measures and quantifications. (A) Equation used and an example for calculating PCP Enrichment (GFP-Pk2 and memRFP shown). (B) Plot of enrichment values for dorsoanterior and the complementary ventroposterior membraneRFP measurements in consideration

without the associated GFP PCP fusion measurements for all PCP Enrichment measurements in presented in Fig.s 1-5. A value of 0 represents no difference in intensity. n=5,394. Error bars indicate standard deviation of the mean. (C) Demonstrations of typical shape and orientation of regions bleached and measured for intensity recovery quantifications shown in Fig. 4E and key for (D) below. Scale bar represents 10 μm. (D) Combined trendlines for data presented in Fig. 4E for a more direct comparison of recovery trends.



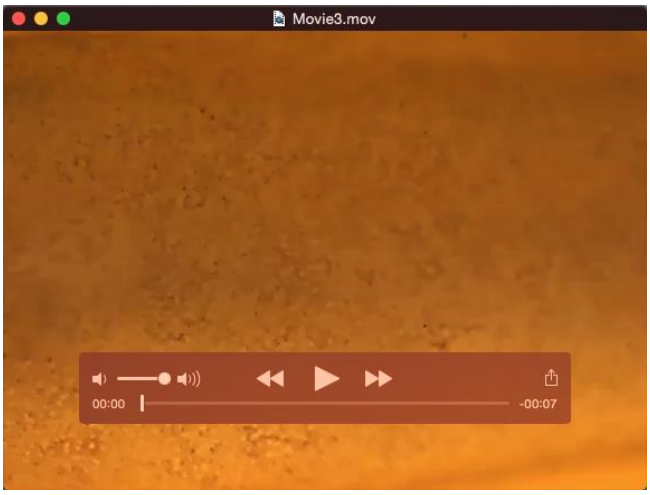
Supplementary Movie 1

Time-lapse movie of latex beads flowing across the epidermis of a control, uninjected stage 31 *Xenopus laevis* embryo.



Supplementary Movie 2

Time-lapse movie of latex beads flowing across the epidermis of a stage 31 *Xenopus laevis* embryo injected with Pk2 morpholino (#1).



Supplementary Movie 3

Time-lapse movie of latex beads flowing across the epidermis of a stage 31 *Xenopus laevis* embryo overexpressing Vangl1.

Table S1. Primers (5′-3′)

Pk2 CS107-GFP

ATAGGCCTATGTTTAACCGGAGCTCTTGGACAAGGGCTTC and

ATGCGGCCGCCTAGGAGATGATGCAATTTTTGCTTTTTCGCCTTT

Pk2 for RFP-Pk2

CACCATGTTTAACCGGAGCTCTTGGACAAGGGCTTCCAGC and

CTAGGAGATGATGCAATTTTTGCTTTTTCGCCTTTTTCTG

Dvl1 (GenBank BC074103.1)

CACCATGGCTGAGACCAAAATCATCTACCATATAGATGAA and

CATGATGTCAACAAAGAATTCACAAGGGTTCCCCA

Dvl3 (NCBI ref NM_001092629.1)

CACCATGGGGGAGACCAAGGTCATCTACCACCTGGATGAA and

AACACCCCAGAATTCTTTGATAACATCCACAAAGAACTCA

Fzd6 (JGIv7b.000063404_134470-165935+)

CACCATGGATCTGATTGGCTGCTGCCTCCAAGCTCCGAGC and

CGCACTTGTCGTATTAATATTAATGTCATTGGCATGG

Vangl1 (JGIv7b.000169011_589431-599179+)

CACCATGGACACGGAATCCAACCACTCGGGATATTCACAT and

TCACAGGTTGGTCTCAGGTTTGCTACTCACAATGAGACGA

ΔPET-1

AGATGGCACCAGTCATGGTTACTGGGAAAAAGTCAAACATCAGCTTGTTC

ΔPET-2

AACTATGAACAAGCTGATGTTTGACTTTTTCCCAGTAACCATGACTGGTG

ΔPETΔLIM-1

AGGCGGAATCTGAAGAGTCCGAGCCATTAAAGTCAAACATCAGCTTGTTC

ΔPETΔLIM-2

AACTATGAACAAGCTGATGTTTGACTTTAATGGCTCGGACTCTTCAGATT

ΔC2-1

CCGTGATAATGAGCAACACTATCGACATTCTTCAGAGTCTGACAATGAAG

ΔC2-2

AGTATCCTTCATTGTCAGACTCTGAAGAATGTCGATAGTGTTGCTCATTA

Dvl1-ΔPDZ$_{partial}$

TGTAGCAGCGGATGGGCGTATTGAACCTATGGGCCCCTCCATGAGCATCATCACAT and

ATGTGATGATGCTCATGGAGGGGCCCATAGGTTCAATACGCCCATCCGCTGCTACA