## **Supporting Information**

Lembong et al. 10.1073/pnas.0810728106

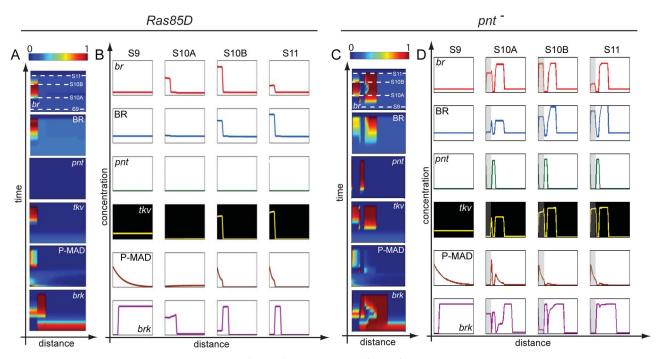
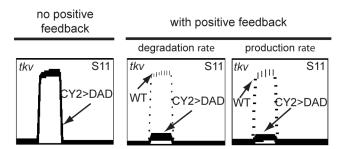


Fig. S1. Detailed modeling results in mutant backgrounds. (A and C) Simulation results for all of the network components in the Ras hypomorph and  $pnt^-$  clone backgrounds as shown by the color plots of the concentration of each component as a function of space and time. The concentrations are normalized so that their values range from 0 to 1. (B and D) The corresponding predicted concentration profiles of each of the network components in the Ras hypomorph and  $pnt^-$  clone backgrounds. Gray boxes mark the locations of the  $pnt^-$  clones.



**Fig. 52.** Modeling the DPP signaling positive feedback loop. (*Left*) Simulations without the positive feedback loop predict that *tkv* expression in the DAD overexpression background is indistinguishable from its wild-type expression pattern. (*Middle* and *Right*) Simulations with positive feedback loop show that *tkv* expression is greatly reduced in CY2-DAD background. (*Middle*) The degradation rate of *tkv* is enhanced in the absence of P-MAD. (*Right*) The production rate of *tkv* is reduced in the absence of P-MAD.

Table S1. Experimental evidence for the regulatory interactions included in the model

No.	Interaction	Evidence	Refs.
1	EGFR signaling induces PNT	Loss of <i>pnt</i> in <i>grk</i> -null background	4
2	EGFR signaling induces <i>br</i>	Down-regulation of dorsolateral BR in <i>Ras</i> clones Lateral shift of <i>br</i> in 2P{GRK} background  br expression is fused ventrally in K10 background, etc.*	5–7
3	EGFR signaling induces BRK	Loss of brk-lacZ in EGFR <sup>-</sup> clones brk-lacZ expression is shifted posteriorly in grk mutant	8
4	PNT represses br	Down-regulation of <i>br</i> in <i>pnt</i> overexpression Ectopic BR in anterior <i>pnt</i> clones	6, this paper
5	BR induces TKV	Loss of tkv in br clones  Overexpression of br leads to ectopic tkv	5
6	P-MAD represses BRK	Complementary expression patterns of P-MAD and <i>brk</i> Ectopic <i>brk-lacZ</i> in <i>mad</i> <sup>-</sup> and <i>put</i> <sup>-</sup> clones <sup>†</sup>	8
7	P-MAD represses <i>br</i>	<ul> <li>dpp overexpression shifts br and BR expression posteriorly</li> <li>br is ectopically expressed in the anterior in dpp mutant</li> <li>Loss of br in dpp clones</li> </ul>	5, 6, 9
8	BRK antagonizes P-MAD repression on br	BR pattern is similar in $dpp$ overexpression and in large $brk^-$ clones BR is down-regulated in $brk^-$ clones	8

References are given in *SI Text*.
\*In K10 (*fs*(1)*K10*) background, GRK production lost its dorsal ventral polarity.
†PUT is the type II receptor of DPP.

Table S2. Summary of the model's parameters

No.	Symbol	Description	
1	$D_{GRK}$	Diffusivity of GRK	
2	$D_{DPP}$	Diffusivity of DPP	
3	$k_{on,GRK}$	GRK-EGFR binding constant	
4	K <sub>off,GRK</sub>	GRK-EGFR unbinding constant	
5	$k_{e,GRK}$	GRK-EGFR complex endocytosis/degradation rate constant	
6	$k_{on,DPP}$	DPP-TKV binding constant	
7	K <sub>off,DPP</sub>	DPP-TKV unbinding constant	
8	$k_{e,DPP}$	DPP-TKV complex endocytosis rate constant	
9	k <sub>d,PNT</sub>	Degradation rate constant of PNT	
10	$k_{d,br}$	Degradation rate constant of br	
11	$k_{d,BR}$	Degradation rate constant of BR	
12	$k_{d,TKV}$	Ligand independent degradation rate constant of TKV	
13	k <sub>d,BRK</sub>	Degradation rate constant of BRK	
14	$k_{d,C_{DPP-TKV}}$	Degradation rate constant of internalized DPP-TKV complex	
15	$V_{GRK}$	Rate of production of GRK	
16	$V_{DPP}$	Rate of production of DPP	
17	$\theta_{[S_{FGFR}],[PNT]}$	Threshold of EGFR signaling above which PNT is activated	
18	$\theta_{[S_{FGFR}],[br]}$	Threshold of EGFR signaling above which br is activated	
19	$\theta_{[S_{EGFR}],[BRK]}$	Threshold of EGFR signaling above which BRK is activated	
20	$\theta_{[PNT],[br]}$	Threshold of PNT concentration above which br is repressed	
21	$\theta_{[BR],[TKV]}$	Threshold of BR concentration above which TKV is activated	
22	$\theta_{[S_{DPP}],[BRK]}$	Threshold of DPP signaling above which BRK is repressed	
23	$\theta_{[S_{DPP}],[br]}$	Threshold of DPP signaling above which br is repressed	
24	$ heta_{[BRK],[br]}$	Threshold of BRK concentration above which P-MAD repression on br is eliminated	
25	γbr	br production rate constant	
26	ΎΡΝΤ	PNT production rate constant	
27	<i>γτκν</i>	TKV production rate constant	
28	γBRK	BRK production rate constant	
29	ΎBR	BR translation rate constant	
30	L	Length of the quasi-one-dimensional subsystem	
31	$f_{br,g,i.}$	GRK independent production rate of br	
32	$lpha_{ extit{EGFR}}$	Signal transduction constant for EGFR signaling	
33	$lpha_{DPP}$	Signal transduction constant for DPP signaling	
34	$X_{GRK}$	Lateral boundary of GRK source	
35	$lpha_{br}$	Uniform concentration of br at the beginning of stage 9	
36	$lpha_{ extsf{TKV}}$	Uniform concentration of TKV at the beginning of stage 9	
37	$\alpha_{BRK}$	Uniform concentration of BRK at the beginning of stage 9	

Table S3. Summary of model-based analysis of mutant backgrounds

No.	Background	Genotype	Eggshell phenotype	Patterning defects*	Refs.
1	Wild type	OreR	N/A	N/A	
2	Ras <sup>-</sup> (anterior)	<i>FRT</i> <sup>82B</sup> ras <sup>∆C40b</sup> /FRT <sup>82B</sup> ubi- <i>GFP</i>	Not measured	Ectopic anterior BR	10, 11
3	Ras <sup>-</sup> (roof)	<i>FRT</i> <sup>82B</sup> ras <sup>∆C40b</sup> /FRT <sup>82B</sup> ubi- <i>GFP</i>	Deformed appendages	Loss of roof BR	10, 11
4	<i>grk</i> -null	grkHF	No appendages	Loss of BR	12
5	Ras hypomorph	Ras85D <sup>E62K</sup> /Ras85D <sup>05703</sup>	A single appendage	Anterior, merged <i>br</i> patches Anterior, merged BR patches Anterior, merged <i>tkv</i> patches Anterior shift of P-MAD	13, this paper
6	2P{GRK}	X7;28.20	Appendages further apart	Lateral shift in tkv Lateral shift in br Lateral shift in BR Lateral shift in P-MAD	6, 10, 14
7	QY1	Egfr <sup>QY1</sup>	A single appendage	Anterior shift of tkv	12
8	$\lambda$ -top overexpression	CY2-λ top	No appendages	Loss of br	15
9	dad overexpression	CY2-dad	Short flat appendages, small operculum	<i>br</i> is expressed longer	5, this paper
				Loss of tkv	
10	tkv RNAi	tkv- RNAi (vdrc#3059)	Multiple, flat appendages	Loss of P-MAD	5
11	pnt RNAi	pnt- RNAi (vdrc#7171)	Appendages fused at the base	Ectopic BR	This paper
12	mae overexpression	CY2-UAS mae (edl)	Wide single appendage	Anterior ectopic <i>tkv</i> Anterior shift in P-MAD Anterior shift in BR	16, this paper
13	brk <sup>-</sup>	brk <sup>CA54</sup> FRT <sup>19A</sup>	Loss of appendage, large operculum when clone is large	Loss of BR Loss of P-MAD	8, this paper
14	br <sup>-</sup>	br <sup>CJ89</sup> FRT <sup>19A</sup>	Loss of appendage, large operculum when clone is large	Loss of tkv Loss of P-MAD	8
15	<i>br</i> overexpression	CY2Gal80-BrZ1	Single deformed appendage	Anterior ectopic <i>tkv</i> Anterior shift in P-MAD	5
16	tkv <sup>-</sup>	FRT <sup>40A</sup> tkv <sup>12</sup>	Not measured	Loss of P-MAD	5
17	pnt <sup>-</sup>	pnt $^{\Delta 88}$ FRT $^{82B}$	Fused appendages	Ectopic BR	This
	-			-	paper
18	Mad <sup>-</sup> (roof)	FRT <sup>40A</sup> mad <sup>12</sup> /FRT <sup>40A</sup> ubi-GFP	Not measured	No effect on BR	5
19	Med <sup>-</sup> (roof)	FRT <sup>82B</sup> med <sup>1</sup> /FRT <sup>82B</sup> ubi-GFP	Not measured	No effect on BR	5

References are given in *SI Text*.

 $<sup>{}^*\</sup>text{The model correctly predicts these patterning defects along the 1-dimensional system shown in Fig. 2A}.$ 

Table S4. Summary of the model's parameter groups

No.	Parameter group	Definition
1	$oldsymbol{arepsilon}_{PNT}$	$k_{d,br}$ / $k_{d,PNT}$
2	$oldsymbol{arepsilon}_{TKV}$	$k_{_{d,br}}$ / $k_{_{d,TKV}}$
3	$oldsymbol{\mathcal{E}}_{\mathit{BRK}}$	$k_{_{d,br}}/k_{_{d,BRK}}$
4	$oldsymbol{arepsilon}_{\mathit{BR}}$	$k_{_{d,br}}$ / $k_{_{d,BR}}$
5	$oldsymbol{\mathcal{E}}_{GRK}$	$L^2 \cdot k_{d,br}^{}  /  D_{GRK}^{}$
6	$oldsymbol{\mathcal{E}}_{\mathit{DPP}}$	$L^2 \cdot k_{d,br}$ / $D_{DPP}$
7	$K_{_{d,GRK}}$	$k_{_{e,GRK}} \cdot k_{_{on,GRK}} ig/ ig( k_{_{off,GRK}} + k_{_{e,GRK}} ig)$
8	$K_{_{d,DPP}}$	$k_{\scriptscriptstyle e,DPP} \cdot k_{\scriptscriptstyle on,DPP} / \left( k_{\scriptscriptstyle off,DPP} + k_{\scriptscriptstyle e,DPP}  ight)$
9	$k_{_{d,GRK}}$	$K_{_{d,GRK}} \cdot [EGFR]$
10	$k_{_{d,DPP}}$	$K_{_{d,DPP}}\cdot Tkv_{_{0}}$
11	$oldsymbol{\mathcal{K}}_{d,DPP}$	$K_{_{d,DPP}} \cdot V_{_{DPP}} \cdot L ig/ D_{_{DPP}} \cdot k_{_{TKV}}$
12	${m arphi_{DPP}}^2$	$L^2 \cdot k_{\scriptscriptstyle on,DPP} \cdot k_{\scriptscriptstyle e,DPP} \cdot TKV_{\scriptscriptstyle o} / (D_{\scriptscriptstyle DPP} \cdot (k_{\scriptscriptstyle off,DPP} + k_{\scriptscriptstyle e,DPP}))$
13	${m arphi_{GRK}}^2$	$L^2 \cdot k_{on,GRK} \cdot k_{e,GRK} \cdot [EGFR] / (D_{GRK} \cdot (k_{off,GRK} + k_{e,GRK}))$
14	br	$[br]\!/\!ig(\gamma_{_{br}}/k_{_{d,br}}ig)$
15	PNT	$[PNT] \! \big/ \! \big( \gamma_{\scriptscriptstyle PNT} / k_{\scriptscriptstyle d,PNT} \big)$
16	TKV	$[\mathit{TKV}] / (\gamma_{_{\mathit{TKV}}} / k_{_{d,\mathit{TKV}}})$
17	BRK	$[BRK] / (\gamma_{_{BRK}} / k_{_{d,BRK}})$
18	BR	$[\mathit{BR}] \! / \! \big( \gamma_{_{\mathit{BR}}} \cdot k_{_{d,\mathit{BR}}} \cdot \gamma_{_{\mathit{br}}} \big/ k_{_{d,\mathit{br}}} \big)$
19	GRK	$[\mathit{GRK}] \! / \! (V_{\scriptscriptstyle GRK} / k_{\scriptscriptstyle d,GRK})$
20	DPP	$[\mathit{DPP}] \! \big/ \big( V_{\scriptscriptstyle \mathit{DPP}} \cdot L / D_{\scriptscriptstyle \mathit{DPP}} \big)$
21	$lpha^{'}_{EGFR}$	$(1/S_{EGFR}(S9, x=0)) \cdot \alpha_{EGFR}$
22	$lpha^{'}_{\ DPP}$	$(1/S_{DPP}(S9, x=0)) \cdot \alpha_{DPP}$
23	$ heta_{\scriptscriptstyle S_{EGFR,PNT}}$	$ heta_{_{[S_{GGR}],[PNT]}} / ig(V_{GRK} ig/k_{d,GRK}ig)$
24	$ heta_{_{S_{EGFR}},br}$	$ heta_{_{[S_{EGFR}],[br]}} / (V_{_{GRK}}/k_{_{d,GRK}})$
25	$ heta_{\scriptscriptstyle S_{EGFR},BRK}$	$ heta_{_{[S_{_{CGRR}}],[BRK]}}/\!\left(V_{_{GRK}}/k_{_{d,GRK}} ight)$
26	$ heta_{\scriptscriptstyle S_{DPP},br}$	$\theta_{_{[S_{Dpp}],[br]}} \Big/ \Big( k_{_{e,DPP}} \cdot k_{_{d,DPP}} \cdot V_{_{DPP}} \cdot L \Big/ \Big( k_{_{d,C_{_{DPP-TKV}}}} \cdot D_{_{DPP}} \Big) \Big)$
27	$ heta_{\scriptscriptstyle S_{DPP},BRK}$	$\theta_{\scriptscriptstyle [S_{\tiny DPP}],[BRK]} \Big/ \Big( k_{\scriptscriptstyle e,DPP} \cdot k_{\scriptscriptstyle d,DPP} \cdot V_{\scriptscriptstyle DPP} \cdot L \Big/ \Big( k_{\scriptscriptstyle d,C_{\tiny DPP-TKV}} \cdot D_{\tiny DPP} \Big) \Big)$
28	$ heta_{{\scriptscriptstyle PNT},br}$	$ heta_{_{[PNT],[br]}} / (\gamma_{_{PNT}}/k_{_{d,PNT}})$
29	$ heta_{{}_{BR,TKV}}$	$ heta_{_{[BR],[TKV]}} / ig( \gamma_{_{BR}} \cdot k_{_{d,BR}} \cdot \gamma_{_{br}} / k_{_{d,br}} ig)$
30	$ heta_{_{BRK,br}}$	$ heta_{_{[BRK],[br]}}/\!\left(\gamma_{_{BRK}}/k_{_{d,BRK}} ight)$
31	t	$T/k_{d,br}$
32	x	X/L
33	$x_{_{GRK}}$	$X_{_{Grk}}/L$

Table S5. Parameter values used in the model

No.	Parameter	Value
1	$oldsymbol{\mathcal{E}}_{PNT}$	1
2	$oldsymbol{\mathcal{E}}_{TKV}$	1
3	$oldsymbol{arepsilon}_{\mathit{BRK}}$	1
4	${\cal E}_{BR}$	10
5	$oldsymbol{arepsilon}_{\mathit{GRK}}$	0.1
6	${\cal E}_{\it DPP}$	0.1
7	$oldsymbol{\phi}_{DPP}$	14
8	$oldsymbol{\phi}_{GRK}$	2
9	$f_{br,g.i.}$	0.1
10	$\mathcal{K}_{d,DPP}$	1
11	$ heta_{\scriptscriptstyle S_{EGFR},PNT}$	0.6
12	$ heta_{S_{EGFR},br}$	0.4
13	$ heta_{\scriptscriptstyle S_{EGFR}.BRK}$	0.3
14	$ heta_{\scriptscriptstyle PNT.br}$	0.1
15	$ heta_{{\scriptscriptstyle BR},{\scriptscriptstyle TKV}}$	0.4
16	$ heta_{\scriptscriptstyle S_{DPP},BRK}$	0.06
17	$ heta_{_{S_{\ DPP},br}}$	0.02
18	$ heta_{{\scriptscriptstyle BRK}, br}$	0.05
19	$x_{GRK}$	0.2
20	$lpha_{_{br}}\cdot k_{_{d,br}}/\gamma_{_{br}}$	0.1
21	$lpha_{\scriptscriptstyle TKV} \cdot k_{\scriptscriptstyle d,TKV}/\gamma_{\scriptscriptstyle TKV}$	0.2
22	$lpha_{_{BRK}}k_{_{d,BRK}}/\gamma_{_{_{BRK}}}$	1

## **Other Supporting Information Files**

SI Text