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Supplemental information

Toll receptors remodel epithelia by directing planar-polarized Src and PI3K activity

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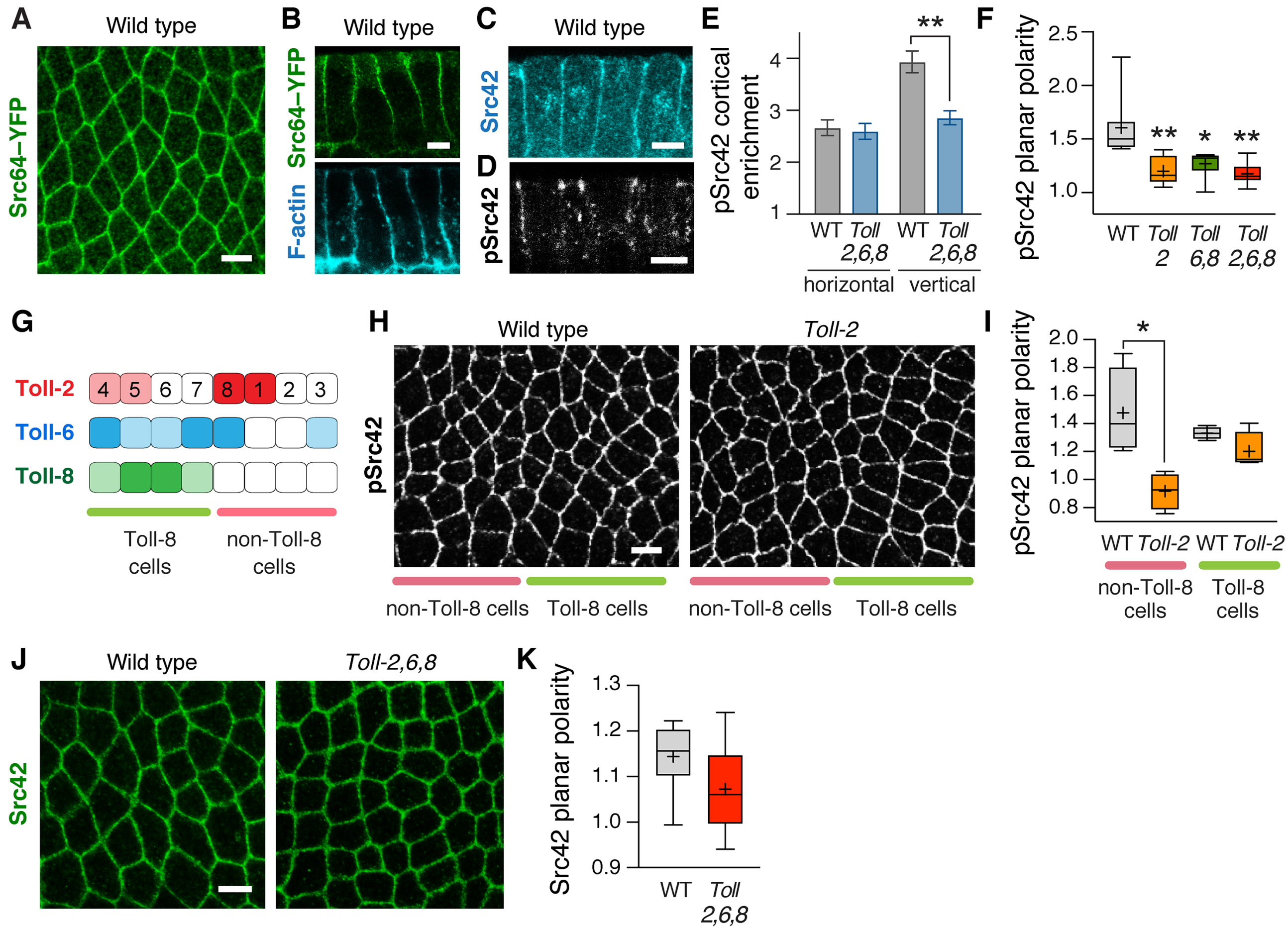


Figure S1. Src localization in wild-type embryos and Toll receptor mutants. Related to Figure 1. (**A**) Src64–YFP localization. (**B-D**) Cross sections of Src64–YFP and F-actin (B), total Src42 (C), and phosphorylated Src42 (pSrc42) (D). (**E**) pSrc42 enrichment at horizontal (0-15°) and vertical (75-90°) edges normalized to the mean image intensity. Mean±SEM. Angular ranges are relative to the anterior-posterior (AP) axis. (**F**) pSrc42 planar polarity (vertical-to-horizontal edge intensity ratio) is reduced in *Toll-2* single mutants, *Toll-6,8* double mutants, and *Toll-2,6,8* triple mutants. (**G**) *Drosophila* Toll receptors are expressed in striped patterns along the anterior-posterior (AP) axis that repeat every two compartments (parasegments). Cells 1-4, even compartments. Cells 5-8, odd compartments. (**H**) pSrc42 localization in wild type and *Toll-2* mutant embryos. (**I**) pSrc42 planar polarity is significantly reduced in *Toll-2* mutant cells that do not express Toll-8 (non-Toll-8 cells). (**J,K**) Total Src42 localization is not significantly altered in *Toll-2,6,8* mutants. Boxes, 2nd and 3rd quartiles; whiskers, min to max; horizontal line, median; +, mean, 3-5 fixed stage 7 embryos/genotype, $**p<0.005$, $*p\leq0.03$, Welch's t-test. Anterior left, ventral down (A, H, and J). Cross sections, apical up (B-D). Bars, 5 µm.

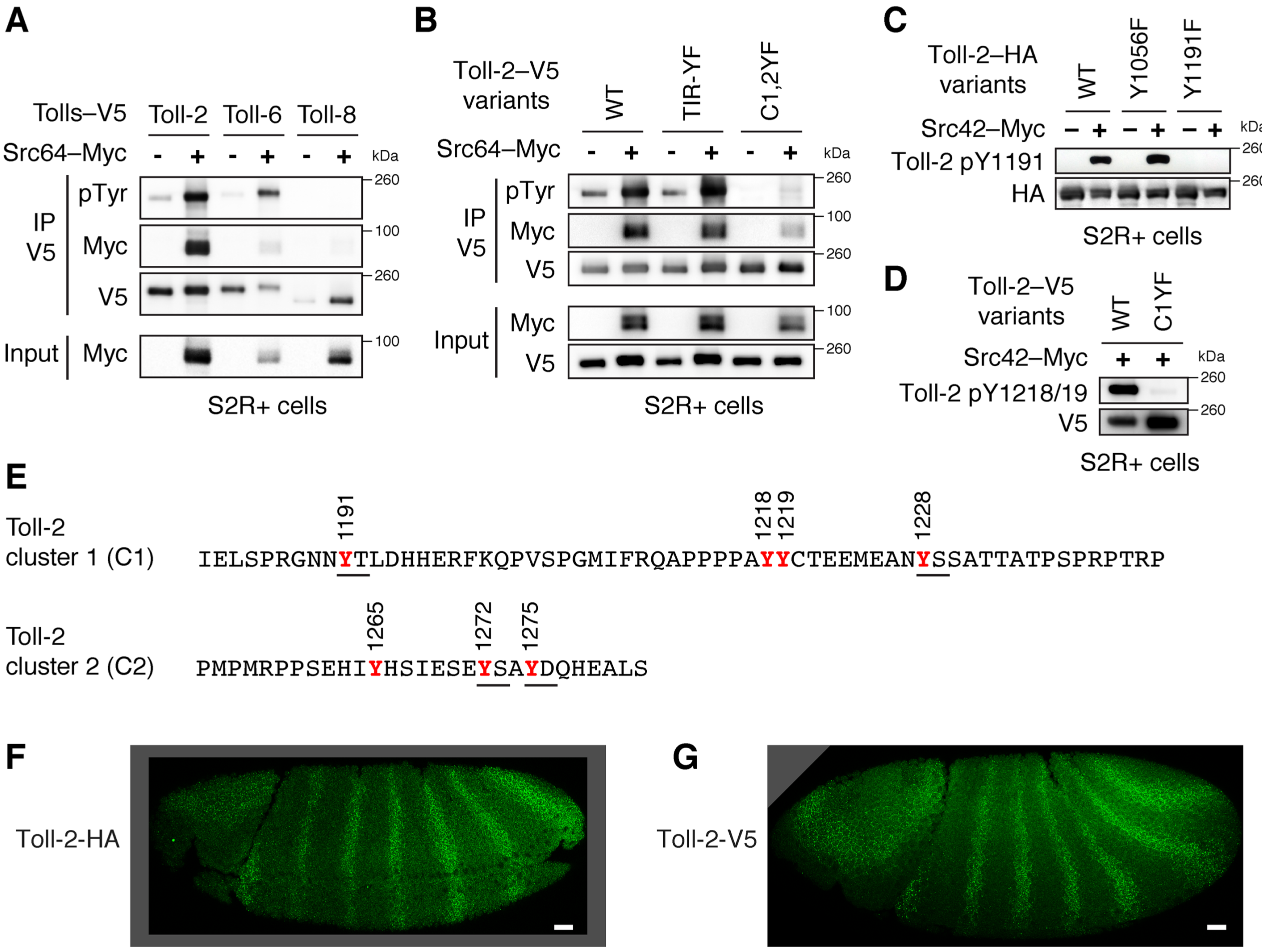
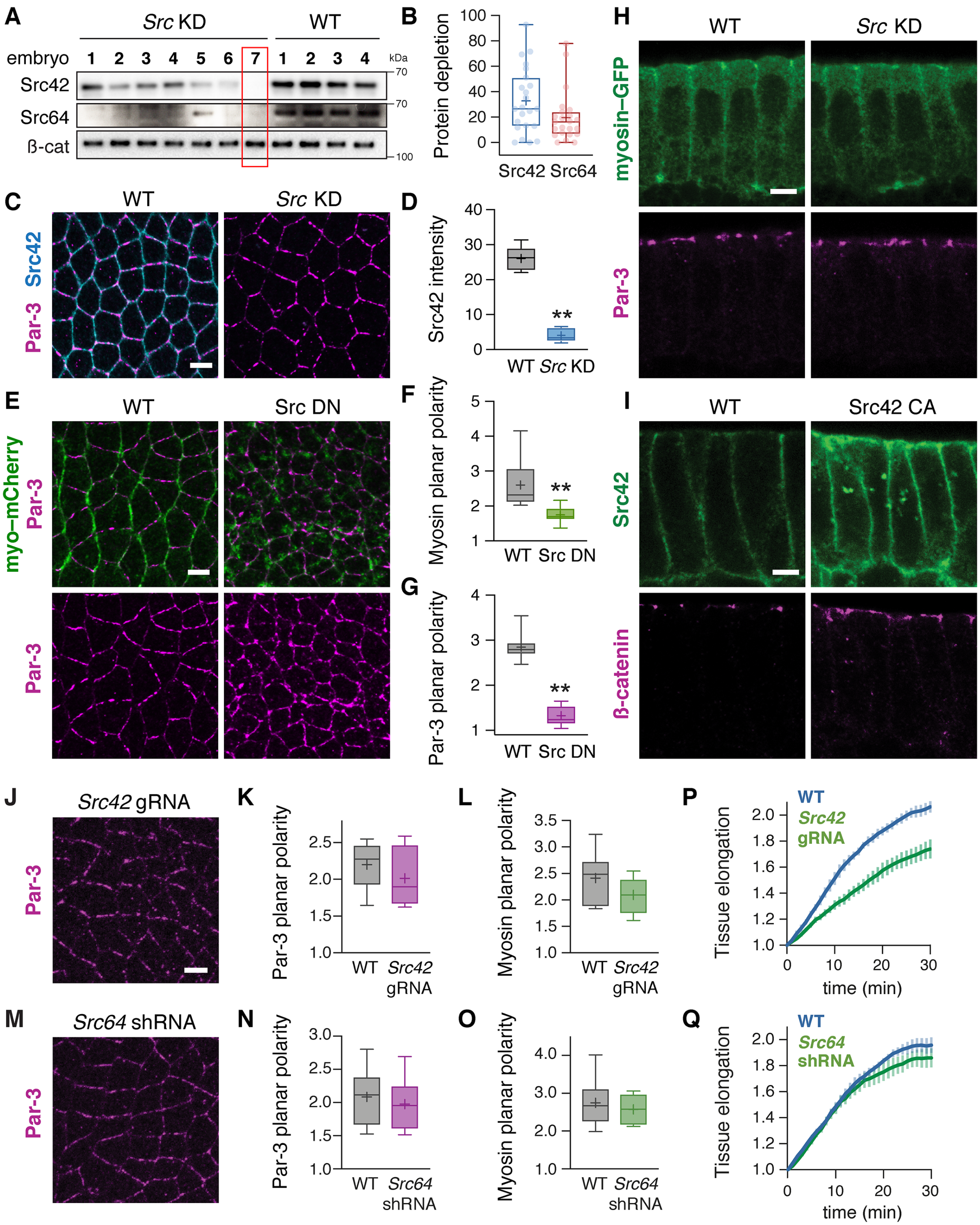


Figure S2. Toll-2 tyrosine phosphorylation is enhanced by Src42 and Src64. Related to Figure 1. (**A**) Src64 promotes the tyrosine phosphorylation of Toll-2 and Toll-6, but not Toll-8, in *Drosophila* S2R$^+$ cells. Src64 associates strongly with Toll-2 and weakly with Toll-6. (**B**) Src64 associates with and promotes the tyrosine phosphorylation of wild-type Toll-2 (WT) and Toll-2$^{TIR\text{-}YF}$ but not Toll-2^{C1,2YF} in S2R$^+$ cells. (**C**) Src42 promotes the phosphorylation of Toll-2–HA in S2R$^+$ cells, detected with a phosphospecific antibody to Toll-2 Y1191. No signal is detected in cells expressing Toll-2^{Y1191F}, confirming antibody specificity. (**D**) Src42 promotes the phosphorylation of Toll-2–V5 in S2R$^+$ cells, detected with a phosphospecific antibody to Toll-2 Y1218/Y1219. No signal is detected in cells expressing Toll-2^{C1YF}, confirming antibody specificity. Toll-2 variants were immunoprecipitated (IP) from S2R$^+$ cells. (**E**) Sequences of Toll-2 tyrosine clusters C1 and C2. Predicted Src consensus sites are underlined (based on c-Src motifs in Schwartz and Gygi, 2005). (**F,G**) Toll-2–HA (F) and Toll-2–V5 (G) are expressed in the wild-type striped pattern in fixed stage 7 embryos. Anterior left, ventral down. Bars, 20 µm.



A
Src KD
WT
embryo 1 2 3 4 5 6 7 1 2 3 4
kDa
70
Src42
70
Src64
β-cat
100
B
Protein depletion
100
80
60
40
20
0
Src42 Src64
H
WT
Src KD
myosin–GFP
Par-3
C
WT
Src KD
Par-3 Src42
D
Src42 intensity
40
30
20
10
0
**
WT Src KD
E
WT
Src DN
myo-mCherry Par-3
Par-3
F
Myosin planar polarity
5
4
3
2
1
**
WT Src DN
G
Par-3 planar polarity
4
3
2
1
**
WT Src DN
I
WT
Src42 CA
Src42
β-catenin
J
Src42 gRNA
Par-3
K
Par-3 planar polarity
2.5
2.0
1.5
1.0
WT Src42 gRNA
L
Myosin planar polarity
3.5
3.0
2.5
2.0
1.5
1.0
WT Src42 gRNA
P
Tissue elongation
WT
Src42 gRNA
2.0
1.8
1.6
1.4
1.2
1.0
0 10 20 30
time (min)
M
Src64 shRNA
Par-3
N
Par-3 planar polarity
3.0
2.5
2.0
1.5
1.0
WT Src64 shRNA
O
Myosin planar polarity
4.0
3.0
2.0
1.0
WT Src64 shRNA
Q
Tissue elongation
WT
Src64 shRNA
2.0
1.8
1.6
1.4
1.2
1.0
0 10 20 30
time (min)

Figure S3. Src kinases regulate planar polarity, but not apical-basal polarity, in the *Drosophila* embryo. Related to Figure 2. (**A**) Western blot of wild-type (WT) and *Src* KD embryos recovered after time-lapse imaging (1 embryo/lane). Embryo 7 was selected for further analysis. (**B**) Src42 and Src64 levels in single-embryo western blots of *Src* KD embryos. Protein levels are shown as a percentage of the mean WT value (23 *Src* KD and 11 WT embryos). (**C,D**) Total Src42 (shown in both panels in C) is strongly reduced at the membrane in *Src* KD embryos. The ratio of the mean intensity of all edges to the mean image intensity is shown in D. (**E-G**) Myosin–mCherry (green) and Par-3 (magenta) planar polarity are disrupted in Src DN embryos. (**H,I**) Apical-basal polarity occurs normally in *Src* KD (H) and Src42 CA (I) embryos. (**J-O**) Par-3 and myosin–GFP planar polarity were not significantly affected in embryos expressing a maternal gRNA targeting *Src42* (J-L) or a maternal shRNA targeting *Src64* (M-O) ($p>0.2$, Welch's t-test). (**P,Q**) Axis elongation (tissue AP length relative to t=0 min) in WT vs. *Src42* gRNA embryos ($p<0.009$ at t=30 min) (P) and in WT vs. *Src64* shRNA embryos ($p=0.3$ at t=30 min) (Q). Mean±SEM in (P and Q). Boxes, 2nd and 3rd quartiles; whiskers, min to max; horizontal line, median; +, mean, 3-7 fixed stage 7 embryos/genotype in (C-O) and 3-4 living stage 7-8 embryos/ genotype in (P and Q), $^{**}p<0.009$, Welch's t-test. Anterior left, ventral down (C, E, J, and M). Cross sections, apical up (H and I). Bars, 5 µm.

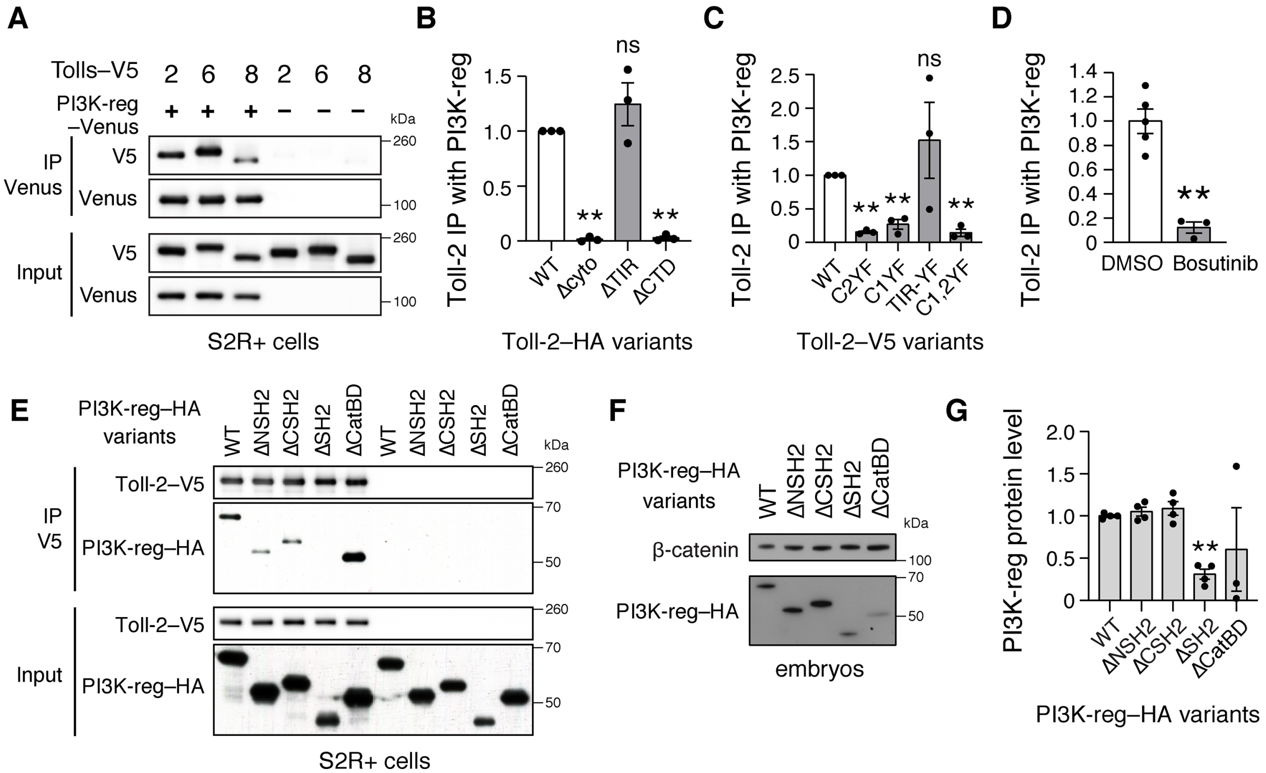
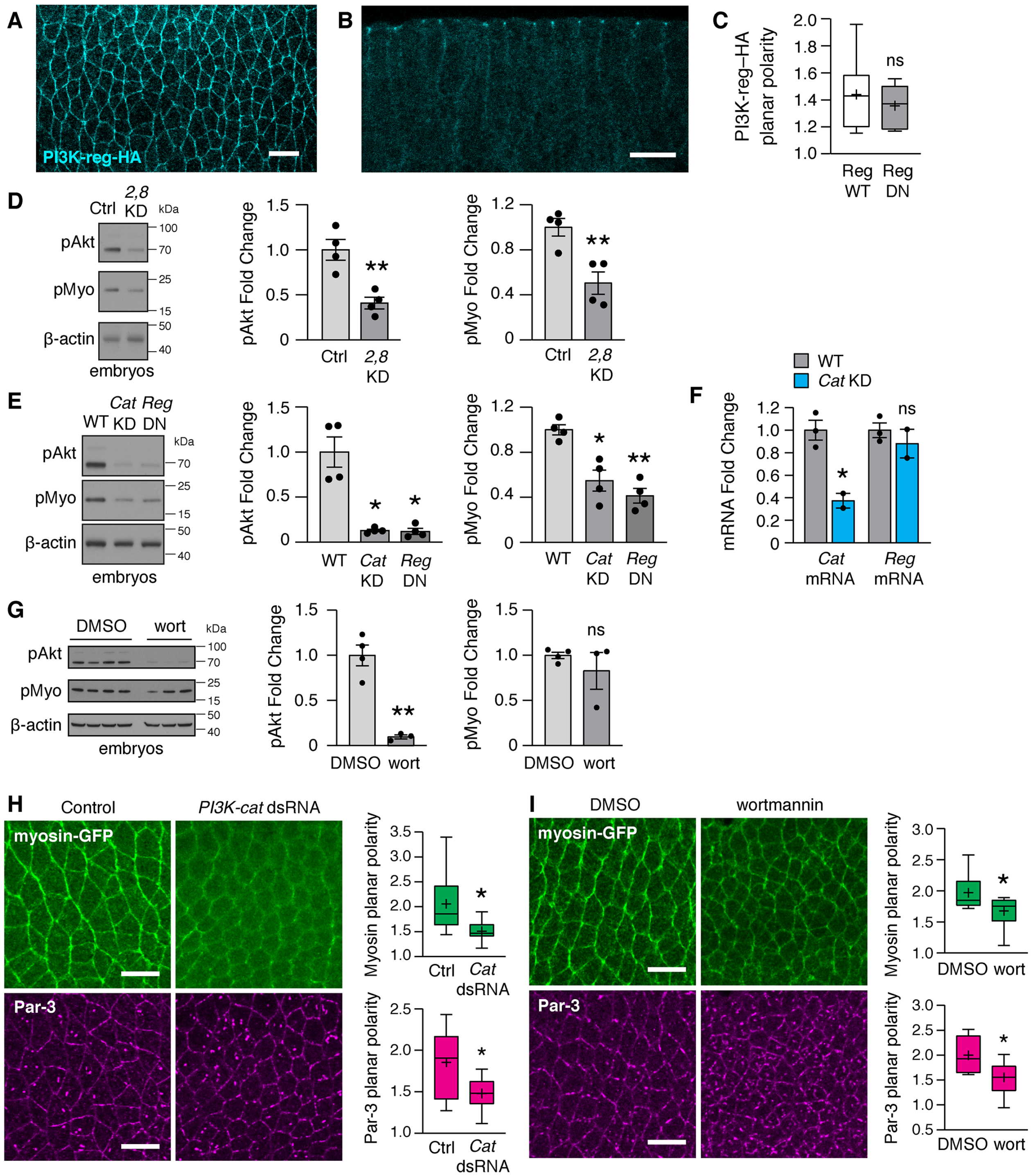


Figure S4. Biochemical analysis of the association between Toll-2 and PI3K-reg. Related to Figure 3. (**A**) Immunoprecipitation (IP) of PI3K-reg–Venus pulled down Toll-2, Toll-6, and Toll-8 C-terminally tagged with V5 in *Drosophila* S2R$^+$ cells. (**B**) Quantification of Toll-2–HA variants immunoprecipitated with PI3K-reg–Venus in S2R$^+$ cells (example in Figure 3B). Toll-2–HA IP with PI3K-reg–Venus = $HA_{eluted}/HA_{input}/Venus_{eluted}$. (**C**) Quantification of Toll-2–V5 variants immunoprecipitated with PI3K-reg–Venus in S2R$^+$ cells (example in Figure 3C). Toll-2–V5 IP with PI3K-reg–Venus = $V5_{eluted}/V5_{input}/Venus_{eluted}$. (**D**) Quantification of Toll-2–V5 immunoprecipitated with PI3K-reg–Venus in the presence or absence of Bosutinib in S2R$^+$ cells (example in Figure 3D). (**E**) PI3K-reg–HA variants immunoprecipitated with Toll-2–V5 in S2R$^+$ cells. (**F,G**) Western blot (F) and quantification (G) of expression levels of PI3K-reg–HA variants in stage 7 embryos. Mean±SEM, each dot is a biological replicate, **$p<0.01$, Welch's t-test.



A
PI3K-reg-HA
B
C
PI3K-reg–HA planar polarity
2.0
1.8
1.6
1.4
1.2
1.0
ns
Reg WT
Reg DN
D
Ctrl
2,8 KD
kDa
pAkt
pMyo
β-actin
embryos
pAkt Fold Change
pMyo Fold Change
**
E
Cat KD
Reg DN
WT
F
WT
Cat KD
mRNA Fold Change
Cat mRNA
Reg mRNA
G
DMSO
wort
H
Control
PI3K-cat dsRNA
myosin-GFP
Par-3
Myosin planar polarity
Par-3 planar polarity
Ctrl
Cat dsRNA
I
DMSO
wortmannin

Figure S5. Loss of PI3K disrupts myosin II and Par-3 planar polarity. Related to Figure 4. (**A,B**) PI3K-reg–HA is enriched at adherens junctions in a planar polarized fashion. (**C**) Vertical-to-horizontal edge intensity ratio. Wild type PI3K-reg–HA and dominant negative PI3K-reg-ΔCatBD–HA (Reg DN) are both planar polarized ($p>0.4$). (**D**) Embryos injected with *Toll-2* and *Toll-8* dsRNAs show reduced levels of phosphorylated Akt (pAkt) (serine 505, corresponding to serine 473 in mammals) and phosphorylated myosin (pMyo) (serine 19 of the regulatory light chain) compared with *Toll-3* dsRNA-injected controls. (**E**) *PI3K-cat* KD and PI3K-reg DN embryos show reduced levels of pAkt and pMyo compared with wild type (WT). (**F**) Analysis of PI3K-reg and PI3K-cat mRNA levels in *PI3K-cat* KD embryos by quantitative RT-PCR. (**G**) Embryos injected with wortmannin (wort) show reduced levels of pAkt compared with control embryos injected with DMSO. (**H,I**) Localization of myosin II (visualized with myosin–GFP, top panels) and Par-3 (bottom panels) in embryos injected with control *Toll-3* (control), *PI3K-cat* dsRNA, DMSO (0.05% DMSO), or wortmannin (1 μM wortmannin in 0.05% DMSO) (final concentrations). Myosin–GFP planar polarity (vertical-to-horizontal edge intensity ratio) and Par-3 planar polarity (horizontal-to-vertical edge intensity ratio) were significantly reduced in *PI3K-cat* dsRNA-injected embryos compared with *Toll-3* dsRNA-injected controls (H) and in wortmannin-injected embryos compared with DMSO-injected controls (I). (C,H,I) Boxes, 2nd and 3rd quartiles; whiskers, min to max; horizontal line, median; +, mean, 6-12 fixed stage 7 embryos/genotype. (D-G) Mean±SEM, each dot is a biological replicate. $*p<0.05$, $**p<0.009$, Welch's t-test. Anterior left, ventral down. Bars, 10 μm.

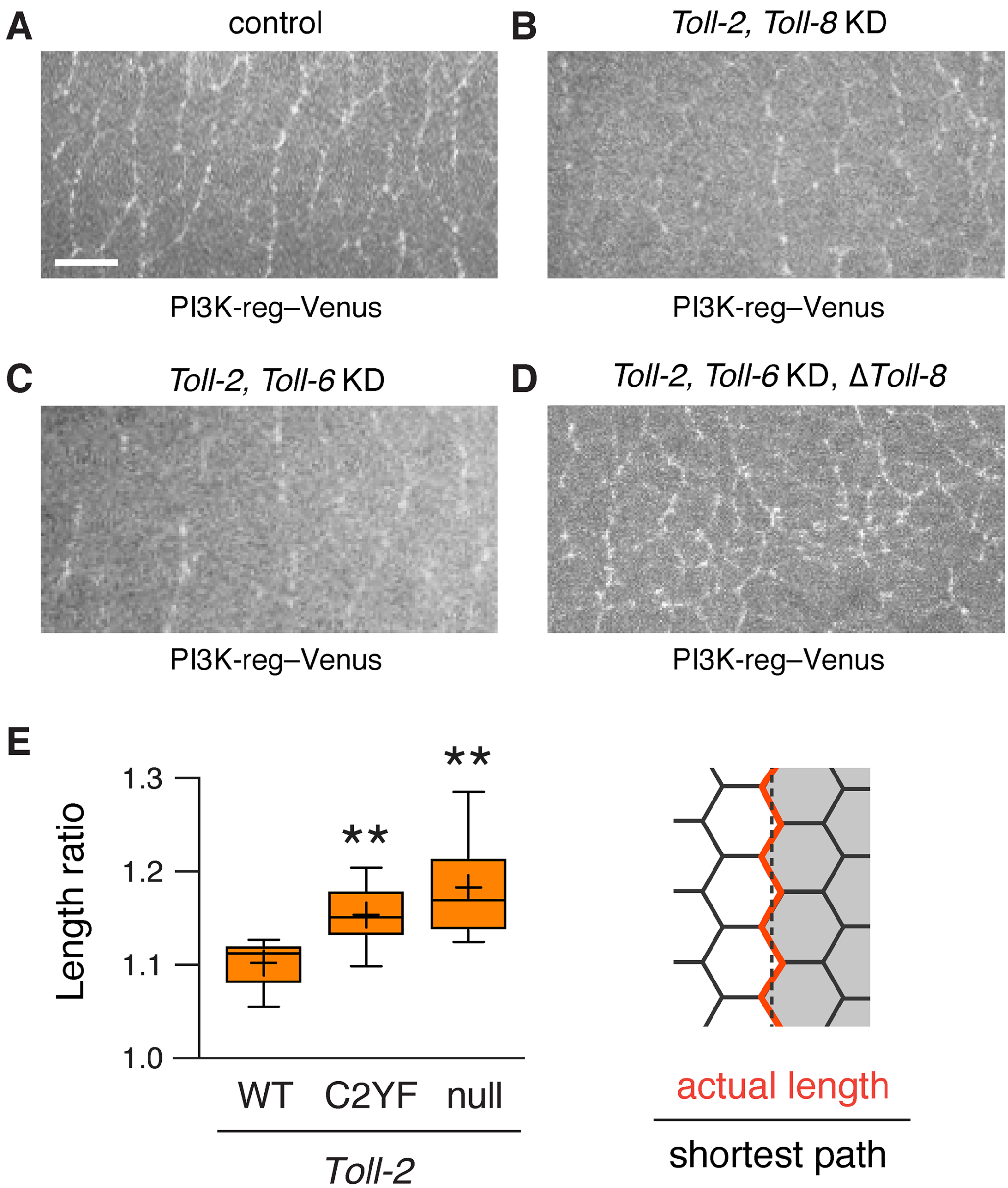


Figure S6. Toll receptors are required for PI3K–reg planar polarity and cell shape. Related to Figures 4 and 6. (**A-D**) PI3K-reg–Venus is enriched at vertical edges in control embryos injected with water (A). PI3K-reg–Venus planar polarity is reduced in embryos injected with dsRNAs targeting *Toll-2* and *Toll-8* (*Toll-2, Toll-8* KD) (B), embryos injected with dsRNAs targeting *Toll-2* and *Toll-6* (*Toll-2, Toll-6* KD) (C), and in *Toll-8* mutants injected with dsRNAs targeting *Toll-2* and *Toll-6* (*Toll-2, Toll-6* KD, Δ*Toll-8*) (D). (**E**) Border length ratio (actual length divided by the shortest path) is increased at Toll-2 stripe borders in *Toll-2*^{C2YF} and *Toll-2*^{null} embryos compared to *Toll-2*^{WT}, 7-8 embryos/genotype, **p≤0.007, Welch's t-test. Living embryos are shown in (A-D). Anterior left, ventral down. Bar, 10 µm.

Table S1. Summary of n and p values. Related to Figures 1-7 and S1-S6.				
Figure	**n value (emb = embryos)**	**Mean ± SEM**	**P value**	**Statistical test**
1 A	total Src42: 9 emb, 2410 edges, 12 images pSrc42: 4 emb, 699 edges, 7 images	1.174 ± 0.02 1.69 ± 0.12	n/a n/a	n/a n/a
1 B	WT: 3 emb, 1841 edges, 6 images *Toll-2,6,8*: 4 emb, 2410 edges, 7 images	1.64 ± 0.13 1.14 ± 0.03	p = 0.0123	Welch's t-test
2 A	WT: 4 emb, 1848 edges, 8 images *Src* KD: 4 emb, 1596 edges, 5 images	3.07 ± 0.32 1.90 ± 0.22	p = 0.0112	Welch's t-test
2 B	WT: 4 emb, 1877 edges, 8 images *Src* KD: 5 emb, 2187 edges, 10 images	3.30 ± 0.19 2.13 ± 0.14	p = 0.0002	Welch's t-test
2 C	control: 6 emb, 1081 edges, 6 images Src CA: 6 emb, 1199 edges, 6 images	1.49 ± 0.02 1.12 ± 0.03	p < 0.0001	Welch's t-test
2 D	control: 6 emb, 1081 edges, 6 images Src CA: 6 emb, 1199 edges, 6 images	2.28 ± 0.14 1.25 ± 0.07	p < 0.0001	Welch's t-test
2 G	WT: 4 emb *Src* KD: 4 emb	1.87 ± 0.06 1.50 ± 0.09	p = 0.0145	Welch's t-test (at t=30 min)
2 H	WT: 4 emb, 140 edges *Src* KD: 4 emb, 134 edges	0.62 ± 0.04 µm/min 0.33 ± 0.02 µm/min	p = 0.004	Welch's t-test
2 I	WT: 4 emb, 212 edges *Src* KD: 4 emb, 231 edges	22 ± 3% errors 48 ± 7% errors	p = 0.0218	Welch's t-test
4 D left	*Toll-3* control: 4 emb, 1751 edges, 5 images *Toll-2*, *Toll-8* KD: 3 emb, 846 edges, 4 images	1.81 ± 0.13 1.13 ± 0.19	p = 0.024	Welch's t-test
4 D middle	DMSO: 5 emb, 715 edges, 7 images Bosutinib: 4 emb, 585 edges, 6 images	1.71 ± 0.20 1.07 ± 0.09	p = 0.0173	Welch's t-test
4 D right	WT: 5 emb, 784 edges, 8 images Src CA: 5 emb, 789 edges, 6 images	1.63 ± 0.18 1.00 ± 0.02	p = 0.0091	Welch's t-test
4 G left	WT: 14 emb, 3824 edges *Cat* KD: 11 emb, 2991 edges Reg DN: 8 emb, 1402 edges	1.90 ± 0.10 1.37 ± 0.10 1.15 ± 0.07	WT vs. *Cat* KD: p = 0.0009 WT vs. Reg DN: p < 0.0001 *Cat* KD vs. Reg DN: p = 0.0911	Welch's t-test

4 G right	WT: 14 emb, 3824 edges *Cat* KD: 11 emb, 2991 edges Reg DN: 8 emb, 1402 edges	2.00 ± 0.10 1.26 ± 0.06 1.41 ± 0.07	WT vs. *Cat* KD: $p < 0.0001$ WT vs. Reg DN: $p = 0.0001$ *Cat* KD vs. Reg DN: $p = 0.1274$	Welch's t-test
5 D	WT: 3 emb *Cat* KD: 3 emb Reg DN: 3 emb control: 3 emb *Cat* dsRNA: 5 emb	2.13 ± 0.01 1.31 ± 0.05 1.46 ± 0.12 2.00 ± 0.08 1.57 ± 0.06	WT vs. *Cat* KD: $p = 0.0034$ WT vs. Reg DN: $p = 0.0278$ $p = 0.0109$	Welch's t-test (at t=30 min)
5 E	WT: 3 emb, 135 edges *Cat* KD: 3 emb, 156 edges Reg DN: 3 emb, 140 edges control: 3 emb, 164 edges *Cat* dsRNA: 5 emb, 257 edges	13 ± 3% errors 42 ± 2% errors 31 ± 4% errors 13 ± 2% errors 32 ± 6% errors	WT vs. *Cat* KD: $p = 0.0022$ WT vs. Reg DN: $p = 0.0241$ *Cat* KD vs. Reg DN: $p = 0.0876$ $p = 0.0336$	Welch's t-test
5 F	WT: 3 emb, 115 edges *Cat* KD: 3 emb, 104 edges Reg DN: 3 emb, 107 edges control: 3 emb, 130 edges *Cat* dsRNA: 5 emb, 186 edges	0.95 ± 0.02 μm/min 0.65 ± 0.07 μm/min 0.69 ± 0.04 μm/min 0.96 ± 0.06 μm/min 0.73 ± 0.04 μm/min	WT vs. *Cat* KD: $p = 0.0468$ WT vs. Reg DN: $p = 0.0114$ *Cat* KD vs. Reg DN: $p = 0.7182$ $p = 0.0436$	Welch's t-test
6 C	*Toll-2*^{WT} left: 8 emb, 192 edges *Toll-2*^{null} left: 7 emb, 207 edges *Toll-2*^{WT} int: 8 emb, 202 edges *Toll-2*^{null} int: 7 emb, 166 edges *Toll-2*^{WT} right: 8 emb, 249 edges *Toll-2*^{null} right: 7 emb, 251 edges	3.32 ± 0.27 1.74 ± 0.19 5.26 ± 0.4 4.08 ± 0.62 2.73 ± 0.12 1.74 ± 0.17	left border: $p = 0.0004$ internal: $p = 0.1390$ right border: $p = 0.0006$	Welch's t-test
6 H and I	*Toll-2*^{WT}: 6 emb, 362 edges *Toll-2*^{C1,2YF}: 6 emb, 336 edges *Toll-2*^{WT}: 10 emb, 432 edges *Toll-2*^{C2YF}: 8 emb, 357 edges *Toll-2*^{null}: 7 emb, 385 edges	2.16 ± 0.16 1.38 ± 0.07 2.25 ± 0.19 1.52 ± 0.15 1.30 ± 0.15	$p = 0.0032$ *Toll-2*^{WT} vs. *Toll-2*^{C2YF}: $p = 0.008$ *Toll-2*^{WT} vs. *Toll-2*^{null}: $p = 0.0013$ *Toll-2*^{C2YF} vs. *Toll-2*^{null}: $p = 0.3141$	Welch's t-test
6 J and K	*Toll-2*^{WT}: 6 emb, 362 edges *Toll-2*^{C1,2YF}: 6 emb, 336 edges *Toll-2*^{WT}: 8 emb, 448 edges *Toll-2*^{C2YF}: 7 emb, 321 edges *Toll-2*^{null}: 7 emb, 458 edges	2.48 ± 0.14 1.85 ± 0.16 3.00 ± 0.18 2.27 ± 0.16 1.72 ± 0.16	$p = 0.0162$ *Toll-2*^{WT} vs. *Toll-2*^{C2YF}: $p = 0.0099$ *Toll-2*^{WT} vs. *Toll-2*^{null}: $p = 0.0001$ *Toll-2*^{C2YF} vs. *Toll-2*^{null}: $p = 0.0304$	Welch's t-test

6 L	*Toll-2*^{WT}: 16 emb, 432 edges, 17 images	1.24 ± 0.02	*Toll-2*^{WT} vs. *Toll-2*^{C2YF}: p = 0.0001	Welch's t-test
	Toll-2^{C2YF}: 7 emb, 140 edges, 7 images	1.07 ± 0.02	*Toll-2*^{WT} vs. *Toll-2*^{C1,2YF}: p = 0.0285	
	Toll-2^{C1,2YF}: 6 emb, 148 edges, 6 images	1.14 ± 0.03	*Toll-2*^{C2YF} vs. *Toll-2*^{C1,2YF}: p = 0.1173	
7 B	*Toll-2*^{WT}*,6,8*: 4 emb	1.91 ± 0.02	*Toll-2*^{WT} vs. *Toll-2*^{C2YF}: p = 0.0265	Welch's t-test (at t=30 min)
	Toll-2^{C2YF}*,6,8*: 6 emb	1.68 ± 0.08	*Toll-2*^{WT} vs. *Toll-2*^{null}: p = 0.0006	
	Toll-2^{null}*,6,8*: 5 emb	1.67 ± 0.03	*Toll-2*^{C2YF} vs. *Toll-2*^{null}: p = 0.9317	
7 C	*Toll-2*^{WT}*,6,8*: 4 emb, 200 edges	27 ± 2% errors	*Toll-2*^{WT} vs. *Toll-2*^{C2YF}: p = 0.0069	Welch's t-test
	Toll-2^{C2YF}*,6,8*: 6 emb, 300 edges	44 ± 4% errors	*Toll-2*^{WT} vs. *Toll-2*^{null}: p = 0.0002	
	Toll-2^{null}: 9 emb, 650 edges	48 ± 3% errors	*Toll-2*^{C2YF} vs. *Toll-2*^{null}: p = 0.4305	
S1E	WT horiz: 3 emb, 196 edges	2.67 ± 0.15	WT horiz vs. *Toll-2,6,8* horiz: p = 0.7551	Welch's t-test
	Toll-2,6,8 horiz: 4 emb, 301 edges	2.60 ± 0.15		
	WT vertical: 3 emb, 395 edges	3.94 ± 0.21	WT vertical vs. *Toll-2,6,8* vertical: p = 0.0022	
	Toll-2,6,8 vertical: 4 emb, 343 edges	2.86 ± 0.13		
S1F	WT: 4 emb, 2407 edges, 8 images	1.606 ± 0.1		Welch's t-test
	Toll-2: 4 emb, 2551 edges, 8 images	1.2 ± 0.05	WT vs. *Toll-2*: p = 0.004	
	Toll-6,8: 3 emb, 1855 edges, 6 images	1.27 ± 0.05	WT vs. *Toll-6,8*: p = 0.014	
	Toll-2,6,8: 5 emb, 3474 edges, 10 images	1.18 ± 0.03	WT vs. *Toll-2,6,8*: p = 0.003	
S1I	WT non-8: 4 emb, 862 edges	1.476 ± 0.15	WT vs. *Toll-2* (non-Toll-8 cells): p = 0.03	Welch's t-test
	Toll-2 non-8: 4 emb, 647 edges	0.917 ± 0.07		
	WT 8: 4 emb, 1137 edges	1.33 ± 0.02	WT vs. *Toll-2* (Toll-8 cells): p = 0.1502	
	Toll-2 8: 4 emb, 1007 edges	1.2 ± 0.07		
S1K	WT: 3 emb, 1362 edges, 6 images	1.14 ± 0.03	p = 0.2056	Welch's t-test
	Toll-2,6,8: 3 emb, 1751 edges, 6 images	1.07 ± 0.04		
S3B	Src42: 23 emb	33 ± 5%	n/a	n/a
	Src64: 23 emb	20 ± 4%	n/a	n/a
S3D	WT: 4 emb, 1877 edges, 8 images	26.05 ± 1.202	p < 0.0001	Welch's t-test
	Src KD: 5 emb, 2187 edges, 10 images	4.040 ± 0.5815		
S3F	WT: 5 emb, 2236 edges, 9 images	2.60 ± 0.25	p = 0.0085	Welch's t-test
	Src DN: 4 emb, 2439 edges, 9 images	1.75 ± 0.08		
S3G	WT: 5 emb, 2236 edges, 9 images	2.85 ± 0.10	p < 0.0001	Welch's t-test
	Src DN: 4 emb, 2439 edges, 9 images	1.32 ± 0.07		

S3K	WT: 3 emb, 1186 edges, 6 images *Src42 gRNA*: 7 emb, 1683 edges, 9 images	2.20 ± 0.13 2.01 ± 0.13	p = 0.3382	Welch's t-test
S3L	WT: 3 emb, 1186 edges, 6 images *Src42 gRNA*: 7 emb, 1683 edges, 9 images	2.41 ± 0.21 2.10 ± 0.11	p = 0.2215	Welch's t-test
S3N	WT: 3 emb, 1444 edges, 6 images *Src64 shRNA*: 3 emb, 1555 edges, 6 images	2.09 ± 0.18 1.98 ± 0.17	p = 0.672	Welch's t-test
S3O	WT: 3 emb, 1444 edges, 6 images *Src64 shRNA*: 3 emb, 1555 edges, 6 images	2.75 ± 0.28 2.58 ± 0.18	p = 0.6222	Welch's t-test
S3P	WT: 3 emb *Src42 gRNA*: 4 emb	2.06 ± 0.03 1.74 ± 0.06	p = 0.0085	Welch's t-test (at t=30 min)
S3Q	WT: 3 emb *Src64 shRNA*: 3 emb	1.96 ± 0.05 1.86 ± 0.06	p = 0.2994	Welch's t-test (at t=30 min)
S4B	WT: n = 3 Δcyto: n = 3 ΔTIR: n = 3 ΔCTD: n = 3	1.00 ± 0.00 0.01 ± 0.01 1.24 ± 0.19 0.03 ± 0.02	WT vs. Δcyto: p = 0.0002 WT vs. ΔTIR: p = 0.3361 WT vs. ΔCTD: p = 0.0003	Welch's t-test
S4C	WT: n = 3 C2YF: n = 3 C1YF: n = 3 TIR-YF: n = 3 C1,2YF: n = 3	1.00 ± 0.00 0.16 ± 0.02 0.27 ± 0.07 1.52 ± 0.57 0.14 ± 0.05	WT vs. C2YF: p = 0.0005 WT vs. C1YF: p = 0.0099 WT vs. TIR-YF: p = 0.4542 WT vs. C1,2YF: p = 0.0041	Welch's t-test
S4D	DMSO: n = 5 Bosutinib: n = 3	1.00 ± 0.10 0.12 ± 0.05	p = 0.0004	Welch's t-test
S4G	WT: n = 4 ΔNSH2: n = 4 ΔCSH2: n = 4 ΔSH2: n = 4 ΔCatBD: n = 3	1.00 ± 0.02 1.05 ± 0.05 1.09 ± 0.08 0.31 ± 0.06 0.60 ± 0.50	WT vs. ΔNSH2: p = 0.4045 WT vs. ΔCSH2: p = 0.3501 WT vs. ΔSH2: p = 0.0009 WT vs. ΔCatBD: p = 0.5080	Welch's t-test
S5C	Reg WT: 8 emb, 4035 edges, 8 images Reg DN: 6 emb, 3011 edges, 6 images	1.44 ± 0.09 1.36 ± 0.06	p = 0.4728	Welch's t-test
S5D left	*Toll-3* dsRNA control: n = 4 *Toll-2*, *Toll-8* KD: n = 4	1.00 ± 0.11 0.41 ± 0.06	p = 0.0075	Welch's t-test
S5D right	*Toll-3* dsRNA control: n = 4 *Toll-2*, *Toll-8* KD: n = 4	1.00 ± 0.08 0.50 ± 0.10	p = 0.0087	Welch's t-test

S5E left	WT: n = 4 *Cat* KD: n = 4 Reg DN: n = 4	1.00 ± 0.17 0.13 ± 0.01 0.12 ± 0.03	WT vs. *Cat* KD: p = 0.0135 WT vs. Reg DN: p = 0.0116	Welch's t-test
S5E right	WT: n = 4 *Cat* KD: n = 4 Reg DN: n = 4	1.00 ± 0.05 0.55 ± 0.09 0.41 ± 0.07	WT vs. *Cat* KD: p = 0.0099 WT vs. Reg DN: p = 0.0005	Welch's t-test
S5F	WT: n = 3 *Cat* KD: n = 2	1.00 ± 0.09 (*Cat* mRNA) 1.00 ± 0.07 (*Reg* mRNA) 0.37 ± 0.07 (*Cat* mRNA) 0.88 ± 0.13 (*Reg* mRNA)	WT vs. *Cat* KD: p = 0.0107 (*Cat* mRNA) WT vs. *Cat* KD: p = 0.5118 (*Reg* mRNA)	Welch's t-test
S5G left	DMSO: n = 4 wortmannin: n = 3	1.00 ± 0.12 0.10 ± 0.02	p = 0.0035	Welch's t-test
S5G right	DMSO: n = 4 wortmannin: n = 3	1.00 ± 0.04 0.83 ± 0.20	p = 0.4909	Welch's t-test
S5H top	*Toll-3* dsRNA control: 8 emb, 2699 edges *Cat* dsRNA: 10 emb, 3350 edges	2.06 ± 0.23 1.51 ± 0.06	p = 0.0481	Welch's t-test
S5H bottom	*Toll-3* dsRNA control: 8 emb, 2699 edges *Cat* dsRNA: 10 emb, 3350 edges	1.86 ± 0.15 1.48 ± 0.06	p = 0.0429	Welch's t-test
S5I top	DMSO: 8 emb, 2841 edges wortmannin: 12 emb, 4314 edges	1.97 ± 0.10 1.68 ± 0.07	p = 0.0331	Welch's t-test
S5I bottom	DMSO: 8 emb, 2841 edges wortmannin: 12 emb, 4314 edges	2.00 ± 0.13 1.55 ± 0.09	p = 0.0151	Welch's t-test
S6E	*Toll-2*^{WT}: 8 emb, 2-4 borders/emb *Toll-2*^{C2YF}: 8 emb, 2 borders/emb *Toll-2*^{null}: 7 emb, 2-4 borders/emb	1.10 ± 0.01 1.15 ± 0.01 1.18 ± 0.02	*Toll-2*^{WT} vs. *Toll-2*^{C2YF}: p = 0.0043 *Toll-2*^{WT} vs. *Toll-2*^{null}: p = 0.0071 *Toll-2*^{C2YF} vs. *Toll-2*^{null}: p = 0.2521	Welch's t-test

Table S2. Oligonucleotides used in this study. Related to Figures 1-7 and S1-S6.		
Project	**Identifier**	**Sequence**
pUASp-W-HA (insert)	1xHA C-term Fwd	AAAGCAGGCTCCGCGGCCGCCCCCTTCACCACGCGTTACCCATACGATGTT
	1xHA C-term Rev	CAAGAAAGCTGGGTCGGCGCGCCCACCCTTTTAAGCGTAATCTGGAACAT
pUASp-W-V5 (insert)	V5 C-term Fwd	GATCCGGTAAGCCTATCCCTAACCCTCTCCTCGGTCTCGATTCTACGTAAA
	V5 C-term Rev	CGCGTTTACGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACCG
pUASp-PI3K-reg–Venus	UASp-Pi3K21B-C-msVenus Fwd	AAAGCAGGCTCCGCGGCCGCCCCCTTCACCATGCGGCCACAGGCGCCCGGATCGCTGGTC
	UASp-Pi3K21B-C-msVenus Rev	GCCTGAAGAACCGCTGGACCCCGAACTTCCCTGCGATGTGCTGAAGTTGGAGGGCG
pUASp-PI3K-reg–HA	21B_nterm_F	CCGCGGCCGCCCCCTTCACCGATGCGGCCACAGGCGC
	21B_cterm_cHA_R	CTGGAACATCGTATGGGTACTGCGATGTGCTGAAGTTGGAGGGCG
	21B_cterm_cHA_F	GCCCTCCAACTTCAGCACATCGCAGTACCCATACGATGTTCCAGATTAC
	UASp-W-cHA_R	CTGGGTCGGCGCGCCCACCCTTATTAAGCGTAATCTGGAACATCGTATGG
pUASp-PI3K-reg-ΔNSH2–HA	21B_nterm_F	CCGCGGCCGCCCCCTTCACCGATGCGGCCACAGGCGC
	21B_no_NSH2_R	GCTGCGACTCCTCATCCTCCATGCGTAGCTCGTCCTCGTTG
	21B_no_NSH2_F	CAACGAGGACGAGCTACGCATGGAGGATGAGGAGTCGCAGC
	21B_cterm_cHA_R	CTGGAACATCGTATGGGTACTGCGATGTGCTGAAGTTGGAGGGCG
	21B_cterm_cHA_F	GCCCTCCAACTTCAGCACATCGCAGTACCCATACGATGTTCCAGATTAC
	UASp-W-cHA_R	CTGGGTCGGCGCGCCCACCCTTATTAAGCGTAATCTGGAACATCGTATGG
pUASp-PI3K-reg-ΔCSH2–HA	21B_nterm_F	CCGCGGCCGCCCCCTTCACCGATGCGGCCACAGGCGC
	21B_no_CSH2_R	CAGCGTGTCGTTATGCTCTTCGTTGCTGTGCGGCTG
	21B_no_CSH2_F	CAGCCGCACAGCAACGAAGAGCATAACGACACGCTG
	21B_cterm_cHA_R	CTGGAACATCGTATGGGTACTGCGATGTGCTGAAGTTGGAGGGCG
	21B_cterm_cHA_F	GCCCTCCAACTTCAGCACATCGCAGTACCCATACGATGTTCCAGATTAC
	UASp-W-cHA_R	CTGGGTCGGCGCGCCCACCCTTATTAAGCGTAATCTGGAACATCGTATGG
pUASp-PI3K-reg-ΔSH2–HA	21B_nterm_F	CCGCGGCCGCCCCCTTCACCGATGCGGCCACAGGCGC
	21B_noSH2_nterm_R	GCGGCTGCGACTCCTCATCCTCCATGCGTAGCTCGTCCTC
	21B_noSH2_midterm_F	GAGGACGAGCTACGCATGGAGGATGAGGAGTCGCAGCCGC

	21B_noSH2_midterm_R	GGTCAGCGTGTCGTTATGCTCTTCGTTGCTGTGCGGCTGG
	21B_noSH2_cterm_F	CCAGCCGCACAGCAACGAAGAGCATAACGACACGCTGACC
	21B_cterm_cHA_R	CTGGAACATCGTATGGGTACTGCGATGTGCTGAAGTTGGAGGGCG
	21B_cterm_cHA_F	GCCCTCCAACTTCAGCACATCGCAGTACCCATACGATGTTCCAGATTAC
	UASp-W-cHA_R	CTGGGTCGGCGCGCCCACCCTTATTAAGCGTAATCTGGAACATCGTATGG
pUASp-PI3K-reg-ΔCatBD–HA (DN)	21B_nterm_F	CCGCGGCCGCCCCCTTCACCGATGCGGCCACAGGCGC
	21B_iSH2nfrag_to_linker_R	CCGCTGGACCCCGAACTTCCGCGGAATATCTTCTCTGTATTCCCG
	21B_iSH2nfrag_to_linker_F	CGGGAATACAGAGAAGATATTCCGCGGAAGTTCGGGGTCCAGCGG
	21B_linker_to_iSH2cfrag_R	GTACTTGTCCTTGCGCAACTGGGAACTGCCTGAAGAACCGC
	21B_linker_to_iSH2cfrag_F	GCGGTTCTTCAGGCAGTTCCCAGTTGCGCAAGGACAAGTAC
	21B_cterm_cHA_R	CTGGAACATCGTATGGGTACTGCGATGTGCTGAAGTTGGAGGGCG
	21B_cterm_cHA_F	GCCCTCCAACTTCAGCACATCGCAGTACCCATACGATGTTCCAGATTAC
	UASp-W-cHA_R	CTGGGTCGGCGCGCCCACCCTTATTAAGCGTAATCTGGAACATCGTATGG
	GSS_Linker_sense	GGAAGTTCGGGGTCCAGCGGTTCTTCAGGCAGTTCC
	GSS_Linker_antisense	GGAACTGCCTGAAGAACCGCTGGACCCCGAACTTCC
pCS2-PI3K-reg–HA	pcs2-21bcHA-f	GGATCCCATCGATTCGATGCGGCCACAGGCG
	pcs2-21bcHA-r	GAGGCTCGAGAGGCCTTGTTAAGCGTAATCTGGAACATCG
pUASp-Toll-2–V5	UASp-Toll-2-V5_f	AAGCAGGCTCCGCGGCCGCCCCCTTCACCGATGCCAGCCACATCTTCCA
	UASp-Toll-2-V5_r	CCGAGGAGAGGGTTAGGGATAGGCTTACCGACCAGGAAAGCTTGGCCGTTC
pUASp-Toll-2^{C1YF}–V5	KSB047 (UASp_F_forSDM)	AAAGCAGGCTCCGCGGCCGCCCCCTTCACC
	KSB056 (2_Y1228F_SDM_r)	CGTGGTGGCTGAGCTAAAGTTCGCCTCCATTTCCTCGGTG
	KSB055 (2_Y1228F_SDM_f)	CACCGAGGAAATGGAGGCGAACTTTAGCTCAGCCACCACG
	KSB048 (UASp_R_forSDM)	CAAGAAAGCTGGGTCGGCGCGCCCACCCTT
pUASp-Toll-2^{C2YF}–V5	MT001	CGCGGCCGCCCCCTTCACCGAT
	MT002	CGATGCTGTGGAAGATGTGCTCTGAGGGC
	MT003	GAGCACATCTTCCACAGCATCG
	MT004	GGTTAGGGATAGGCTTACCGAC

pUASp-Toll-2^{C1,2YF}*–V5*	KSB047 (UASp_F_forSDM)	AAAGCAGGCTCCGCGGCCGCCCCCTTCACC
	KSB014 (Toll2_R7)	CAGCTCAATTGGGATGGCGT
	gBlock GB004	ACGCCATCCCAATTGAGCTGTCGCCCCGTGGAAACAACTTCACATTGGACCATCATGAGCGCTTCAAGCAGCCAGTGTCGCCGGGCATGATCTTCCGCCAGGCGCCACCACCGCCAGCCTTCTTCTGCACCGAGGAAATGGAGGCGAACTTTAGCTCAGCCACCACGGCCACGCCATCACCACGCCCAACGAGACCAGGAGGAGCCGCTAGGATCGTGGACTCTATGCCCATGCCGATGCGTCCGCCCTCAGAGCACATCTTCCACAGCATCGAGTCGGAGTTCAGTGCGTTTGATCAGCATGAGGCGCTGTCGATGATTCCCACGGGCCTAATGCATCAGCACCAGCAGCAGCAGTTGCGTCTGCATCAGCAACAACAACAGCAGCAGCAACAGCGCTTGCTGCAACCGCAGTTCCGGGCGATGCCGCAGCAGGCGATTCCCGCTCCTTCCGCGCCGGTGCACCTGCGCAGCGGCAGTGGTTTGAGCCAGGCCAG
	KSB006 (Toll2-F8)	GTGGTTTGAGCCAGGCCAG
	KSB048 (UASp_R_forSDM)	CAAGAAAGCTGGGTCGGCGCGCCCACCCTT
pUASp-Toll-2^{TIR-YF}*–V5*	KSB033 (UASp-Toll2-GA-F)	AAAGCAGGCTCCGCGGCCGCCCCCTTCACCATGCCAGCCACATCTTCCATC
	KSB035 (Toll2_R10)	GATGGCGTCGAACAGTTTGCCGGCATC
	gBlock GB010	CTGTTCGACGCCATCATCCTGCACTCGGAGAAGGACTTCGAGTTTGTGTGCCGCAACATCGCCGCCGAACTGGAGCATGGTCGACCACCCTTCCGGCTCTGCATTCAGCAGCGAGATCTGCCTCCCCAGGCATCCCACCTTCAGCTGGTGGAGGGAGCAAGGGCGTCGAGGAAGATCATCCTGGTGCTGACCCGCAATCTCTTGGCCACCGAATGGAATCGCATTGAGTTCCGTAATGCTTTCCACGAGTCCCTGAGGGGCTTGGCCCAGAAGCTGGTGATCATCGAGGAGACAAGTGTTTCCGCCGAGGCCGAGGACGTTGCCGAGTTGTCGCCGTTCTTGAAATCGGTACCCTCCAACCGACTGCTGACCTGCGACAGATTCTTCTGGGAGAAGCTGCGCTTC
	KSB036 (Toll2_F9)	GAAGCTGCGCTTCGCCATCCCAATTG
	UASp-Toll-2-V5_r	CCGAGGAGAGGGTTAGGGATAGGCTTACCGACCAGGAAAGCTTGGCCGTTC
pUASp-Toll-6–V5	UASp-Toll-6_f	AAAGCAGGCTCCGCGGCCGCCCCCTTCACCATGATCTACTATATGCTACTC
	UASp-Toll-6-V5_r	CCGAGGAGAGGGTTAGGGATAGGCTTACCCGCCCACAGGTTCTTCTGCTGATCG
pUASp-Toll-8–V5	UASp-Toll-8_f	AAAGCAGGCTCCGCGGCCGCCCCCTTCACCATGCTGGCCACCAC
	UASp-Toll-8-V5_r	CCGAGGAGAGGGTTAGGGATAGGCTTACCCATGTGCAGATTTCTAGACGCC
pUASp-Toll-2-Δcyto–HA	Toll2:HA 1xHA Fwd	AAAGCAGGCTCCGCGGCCGCCCCCTTCACCATGCCAGCCACATCTTCCATC
	Toll2:HADEL Cyto Rev	TTAAGCGTAATCTGGAACATCGTATGGGTACTCGAAGCGGGGCTCGCACACC
pUASp-Toll-2-ΔTIR–HA	Toll2:HA 1xHA Fwd	AAAGCAGGCTCCGCGGCCGCCCCCTTCACCATGCCAGCCACATCTTCCATC
	Toll2:HADEL TIR R1	GGCGACAGCTCAATTGGGATGGCGTTTTGCCGGCATCCTCGAAGCGGGGC

	Toll2:HADEL TIR F2	GCCCCGCTTCGAGGATGCCGGCAAAACGCCATCCCAATTGAGCTGTCGCC
	Toll2:HADEL TIR R2	TTAAGCGTAATCTGGAACATCGTATGGGTAGACCAGGAAAGCTTGGCCGT
pUASp-Toll-2-ΔCTD–HA	Toll2:HA 1xHA Fwd	AAAGCAGGCTCCGCGGCCGCCCCCTTCACCATGCCAGCCACATCTTCCATC
	Toll2:HADEL C- Rev	CTTTTAAGCGTAATCTGGAACATCGTATGGGTATGGTGATGGCGTGGCCGTGG
pUASp-Toll-2-Y1056F–HA	KSB047 (UASp_F_forSDM)	AAAGCAGGCTCCGCGGCCGCCCCCTTCACC
	KSB039 (SDM_2_1056_R2)	GAGTGCAGGATGATGGCG
	KSB038 (SDM_2_Y1056F_F2)	GGAGAAGGACTTCGAGTTTGTGTG
	KSB048 (UASp_R_forSDM)	CAAGAAAGCTGGGTCGGCGCGCCCACCCTT
pUASp-Toll-2-Y1191F–HA	KSB047 (UASp_F_forSDM)	AAAGCAGGCTCCGCGGCCGCCCCCTTCACC
	KSB042 (SDB_2_Y1191_R2)	CGGGGCGACAGCTCAATT
	KSB041 (SDM_2_Y1191F_F2)	TGGAAACAACTTCACATTGGACCATC
	KSB048 (UASp_R_forSDM)	CAAGAAAGCTGGGTCGGCGCGCCCACCCTT
pCFD3-Src42-gRNA	Src42-gRNA_f	GTCGAAACCTACCAATAGGGTGAC
	Src42-gRNA_r	AAACGTCACCCTATTGGTAGGTTT
pEntr-Src42	pEntr-Src42_f	CACCATGGGTAACTGCCTCACC
	pEntr-Src42_r	GTAGGCCTGCGCCTCTTTGTAGTCG
pEntr-Src64	pEntr-Src64_f	CACCATGGGCAACAAATGCTGCAGC
	pEntr-Src64_r	GTCTTGCACCTCTCGATACG
pEntr-Src42-K276R-msVenus (Src42 DN)	Src42A K276R_f	GGACAATTGGACGAAGGCGGCTTC
	Src42A K276R_r	CCTAAGCTTGGTGTGGCGCAGTTTC
	msVenus_f	GAGGCGCGCCGGAAGTTCGGGGTCCAGCGG
	msVenus_r	CTTACGCGTTTACTTGTACAGCTCGTCCATG
pattB-Toll-2^{WT}–HA	ZKM019 (attB_bb_f)	CTTTTCTACGGGGTCTGACG
	ZKM083 (2_5UTRrevfromORF)	TTTGGTGATTTGCTAGTTGG
	KSB001 (GA_Toll2_cHA_Fwd)	TACCCATACGATGTTCCAGATTACGCTTAAAAACTCCCCCTATGGC
	ZKM020 (attB_bb_r)	CGTCAGACCCCGTAGAAAAG
	ZKM096 (Toll-2_attB_GA_f)	GTTAACTAGCCAACTAGCAAATCACCAAAATGCCAGCCACATCTTCCATCATCACCATC

	KSB002 (GA_Toll2_cHA_Rev)	AGCGTAATCTGGAACATCGTATGGGTAGACCAGGAAAGCTTGGCCG
pattB-Toll-2^{C2YF}–*HA* and *pattB-Toll-2*^{C1,2YF}–*HA*	ZKM019 (attB_bb_f)	CTTTTCTACGGGGTCTGACG
	SS001 (Toll2_2718R)	CTGGAGGCTGTGACGATATTG
	KSB006 (Toll2-F8)	GTGGTTTGAGCCAGGCCAG
	ZKM020 (attB_bb_r)	CGTCAGACCCCGTAGAAAAG
	SS002 (Toll2_2718F)	CAATATCGTCACAGCCTCCAG
	KSB008 (Toll2-R8)	CTGGCCTGGCTCAAACCAC
pattB-Toll-2^{WT}–*V5*, *pattB-Toll-2*^{C1YF}–*V5*, and *pattB-Toll-2*^{C1,2YF}–*V5*	ZKM019 (attB_bb_f)	CTTTTCTACGGGGTCTGACG
	KSB005 (Toll2_1036R)	GTTTTGGATCCAATGCGGGT
	KSB009 (GA_cV5_Toll2_Fwd)	CTCCTCGGTCTCGATTCTACGTAAAAACTCCCCCTATGGCCATATC
	KSB010 (GA_attB_bb_R)	CACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATC
	KSB007 (Toll2_1036F)	ACCCGCATTGGATCCAAAAC
	KSB011 (V5_rev)	CGTAGAATCGAGACCGAGG
pattB-Toll-2^{WT}	ZKM019 (attB_bb_f)	CTTTTCTACGGGGTCTGACG
	ZKM018 (attBf/r)	CATCATGATGGACCAGATGG
	ZKM020 (attB_bb_r)	CGTCAGACCCCGTAGAAAAG
	ZKM085 (2attB5UTR_new)	CCATCTGGTCCATCATGATGACGCAACCCACAACCTAC
	ZKM086 (2_attB3UTR_new)	CCATCTGGTCCATCATGATGGCGAGGCTGAGTGAGATGG
pattB-Toll-2^{C2YF} and *pattB-Toll-2*^{C1,2YF}	ZKM019 (attB_bb_f)	CTTTTCTACGGGGTCTGACG
	KSB005 (Toll2_1036R)	GTTTTGGATCCAATGCGGGT
	KSB006 (Toll2-F8)	GTGGTTTGAGCCAGGCCAG
	ZKM020 (attB_bb_r)	CGTCAGACCCCGTAGAAAAG
	KSB007 (Toll2_1036F)	ACCCGCATTGGATCCAAAAC
	KSB008 (Toll2-R8)	CTGGCCTGGCTCAAACCAC
Toll-2 dsRNA	*Toll-2 #1* 5' primer	TAATACGACTCACTATAGGGAGAAGTTTGAATCGAAACGCGAG
	Toll-2 #1 3' primer	TAATACGACTCACTATAGGGAGAGGACACTGCACCGGATGT

Toll-6 dsRNA	*Toll-6 #1* 5' primer	TAATACGACTCACTATAGGGAGAATCGGCCAAAAAGAGCAGTA
	Toll-6 #1 3' primer	TAATACGACTCACTATAGGGAGAAGCAGCGTGTGCAGATTATT
Toll-8 dsRNA	*Toll-8 #1* 5' primer	TAATACGACTCACTATAGGGAGACAGCTGGAGCGTTTGGAT
	Toll-8 #1 3' primer	TAATACGACTCACTATAGGGAGAAGACGCTTGAGACCCACAAA
PI3K-cat dsRNA	*PI3K92E* 5' primer	TAATACGACTCACTATAGGGGCATCCGACCAGAACCTTT
	PI3K92E 3' primer	TAATACGACTCACTATAGGGGCACGCGTCTTGTCAAAGT
Toll-3 dsRNA	*Toll-3 #1* 5' primer	TAATACGACTCACTATAGGGAGAGAGCCTTGAACATTTGGAGC
	Toll-3 #1 3' primer	TAATACGACTCACTATAGGGAGACAGTTTCGCTGGAAGGTGAT