

Supplemental Data

A Combinatorial Code for Pattern

Formation in *Drosophila* Oogenesis

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Supplemental Experimental Procedures

RNA Extraction

Total RNA was extracted with the RNeasy kit (Qiagen) and RNA qualification was performed in a Gene Chip RNA 6000 Nano Assay (Agilent Technologies, Palo Alto, CA). RNA quantification was performed on 1 μ l total RNA sample in a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Based on our estimates, this procedure yields 10-15 μ g of total RNA per replicate, which corresponds to 0.1-0.15 μ g/egg chamber. RNA samples were stored at -80°C.

Microarray Hybridization

The following backgrounds were used in microarray experiments: *CY2> λ -top 4.2*, *CY2>dnEgfr*, *CY2>tkv**, *CY2>Dad*, and *OreR* as a control. 3 μ g of total RNA was used in One Cycle Target Labeling for 1st and 2nd strand cDNA synthesis to obtain biotinylated labeled cRNA (Affymetrix GeneChip protocol, Santa Clara, CA). 20 μ g of fragmented cRNA from each sample was hybridized for 16 hr at 45°C to an Affymetrix *Drosophila* GeneChip 1 array. After hybridization, each array was stained with a streptavidin-phycoerythrin conjugate (Molecular Probes, Eugene, Oregon), washed and visualized with a Genearray™ Scanner (Agilent Technologies, Palo Alto, CA). Images were inspected visually for hybridization artifacts. In addition, quality assessment metrics were generated for each scanned image and evaluated based on empirical data from previous hybridizations and on the signal intensity of internal standards that were present in the hybridization cocktail.

Microarray data analysis was done using the probe sets from the *Drosophila* genome annotations v4.3 (www.flybase.org). Data analysis was performed on each of the genetic backgrounds compared to the wild type using the Golden Spike method [1]. The codes implementing the method compute eight sets of probe summaries for each of the arrays, and then normalize across the entire set of arrays using a Loess normalization [2]. For each probe set summary, the comparison of the genetic background to the wild type controls was performed using Cyber-T, an extension of a Student's t-test that assumes the standard deviation of the measurements is a function of signal intensity [3]. Finally, the probe summaries were combined and summarized as a single value and to account for the testing of multiple hypotheses, a false-discovery rate (q-value) is estimated using a permutation approach [1]. The final output is a fold-change and a q-value, which characterizes the significance of the observed change.

To generate a list of candidate targets of the EGFR and DPP in the follicle cells, we first set a threshold, based on the fold change and q-value. Next, we selected those genes that responded to perturbations of both pathways. The change in response to a pathway

perturbation implies that a transcript changed in abundance in response to either gain or loss-of-function perturbation. The number of potential targets depends on the values for the fold change and q-value (Figure S1). Using a fold-change cutoff of 1.75 and a q-value of 0.1, we obtained a list of 193 genes. Two genes from this list (*Vm32E*, *Dad*) have been previously shown to respond to both EGFR and BMP signaling [4, 5]. Three genes (*yellow-g2* [6], *CG4009* [7], *18w* [8]) were shown to be expressed in non-uniform spatial expression patterns. Finally, one gene (*Cp36*) was a previously validated target of EGFR [9].

Additional Filters and qRT-PCR Validation

All targets from the generated list of genes were validated by qRT-PCR (using MX-3000P, Stratagene). 1 µg of each of the total RNA sample was used for first strand cDNA synthesis using TaqMan Reverse Transcription Kit (Roche, Branchburg, NJ) according to the manufacturer's protocol. For RT-PCR, the reaction consisted of calculated 25 ng first strand cDNA template, primer mix, ROX and SYBR Green PCR mix (Stratagene, La Jolla, CA), in a total volume of 25 µl, in 96-well plates. In most cases, PCR primers were designed to span an intron (except for genes with no introns), to generate a product amplicon at the size range of 90-120 bp. Primers that did not give any amplified product were redesigned one more time, and in most cases (80%) we were able to obtain the correct amplicon. A Beckman Coulter Biomek FX provided a high-throughput liquid handling system for the large-scale qRT-PCR screen.

The design of the qRT-PCR matches the array design with at least three biological replicates and a comparison of the mutant background to the wild type. A comparison of the fold changes for the two different methods shows a high correlation ($R^2 = 0.87$; Figure S2, Tables S4 and S5).

For 39 genes we failed (after two attempts) to design primers that would amplify the gene from an OreR cDNA library, suggesting that these genes are not expressed in the wild type. These genes were excluded from the following analysis, which reduced the list of targets to 154 (Table S6). A significant number of genes showed coordinated response in all backgrounds (the direction of change, increase or decrease, was similar in all four backgrounds). This suggests that these changes might be due to stress, and/or induced by high levels of ectopic gene expression. To reduce false positive changes due to stress, as an additional filter, we tested whether the remaining 154 targets respond to overexpression of the GFP, driven by the same CY2-GAL4 driver. From 154 genes, 107 did not change in the CY2>GFP background and were included in the *in situ* hybridization step of our screen.

Table S1. List of the Flybase Gene IDs, Gene Symbols, and Pattern Annotations from Literature and This Work

	Flybase ID	Symbol	Stage 10A	Stage 10B	Stage 11	Stage 12	Source of information
1	FBgn0004364	<i>l8w</i>	A+U	$A \cup (D \setminus R)$	$A \cup (D \setminus R)$	$A \cup M$	2006[8]*
2	FBgn0004569	<i>argos</i>		$A \cap D$	M	M	1997[10]*
3	FBgn0023407	<i>B4</i>	A				2005[5]
4	FBgn0001090	<i>bnb</i>				$A \cap D$	2005[5]
5	FBgn0000210	<i>br</i>	$U \setminus M$	$(A \setminus D) \cup R$			1997[11]*
6	FBgn0024250	<i>brk</i>	$U \setminus A$	$U \setminus A$			2006[12]*
7	FBgn0031150	<i>bves</i>	$U \setminus D$	$U \setminus D$			2007[13]
8	FBgn0039709	<i>Cad99C</i>	U	A	$A \cup (D \setminus R)$	$A \setminus D$	2005[14]*
9	FBgn0041342	<i>Cct1</i>	A	A		R	2003[15]
10	FBgn0034709	<i>CG3074</i>	A	$A \cup M$	F	F	2004[6]
11	FBgn0038469	<i>CG4009</i>		$D \setminus M$	$D \setminus M$	$D+U$	2006[7]*
12	FBgn0033631	<i>CG9027</i>				$A \cap D$	2004[6]
13	FBgn0032126	<i>CG13113</i>			F		2006[7]*
14	FBgn0032120	<i>CG33298</i>		D	D	A	2005[5]
15	FBgn0000359	<i>Cp36</i>			A+D	A+D	1993[9]
16	FBgn0020493	<i>Dad</i>	A	A			2005[5]*
17	FBgn0000490	<i>dpp</i>	A	A	A	A	1996[16]
18	FBgn0000568	<i>Eip75B</i>			D		1999[17]
19	FBgn0004865	<i>Eip78C</i>				D	1999[18]
20	FBgn0000575	<i>emc</i>	A	A	$A \cup R$	$A \cup R$	2005[19]
21	FBgn0010470	<i>Fkbp13</i>	A+U	$(A \cup D)+U$	$(A \cup D)+U$	$(A \cup D)+U$	1999[18]*
22	FBgn0011591	<i>fnf</i>	$U \setminus D$	$U \setminus D$			2000[20]
23	FBgn0001987	<i>Gli</i>			F		2005[5]
24	FBgn0001257	<i>ImpL2</i>	A	M	$F \cup R$	F	2005[5]*
25	FBgn0011225	<i>jar</i>	U	A+U	F	$F \cup R$	1999[21]*
26	FBgn0027339	<i>jim-2</i>	$U \setminus (A \cup D)$	$U \setminus D$			1999[22]
27	FBgn0001297	<i>kay-RB</i>	U		F	$A \setminus D$	2001[23]
28	FBgn0015399	<i>kekl</i>	M	M	F		1997[10]*
29	FBgn0020278	<i>loco-c2</i>	D	D	D		2001[24]
30	FBgn0024211	<i>mfas</i>	$U \setminus D$				2005[5]
31	FBgn0014343	<i>mirr</i>	$A \cup D$	$A \cup R$	F		2000[20]*
32	FBgn0002778	<i>mnd</i>		$A \cup (U \setminus D)$			2005[5]
33	FBgn0003089	<i>pip-RA</i>	$A \cup (U \setminus D)$	$U \setminus D$			1998[25]*
34	FBgn0003118	<i>pnt-P1</i>	$A \cup M$	$A \cup M$	F		1996[26]*
35	FBgn0003118	<i>pnt-P2</i>	M	R	R		1996[26]*
36	FBgn0243512	<i>puc</i>		A	$A \cup D$		2001[27]
37	FBgn0004635	<i>rho</i>	$D \setminus M$	F	F		1993[28]*
38	FBgn0010851	<i>sgl</i>		F	F	M	2005[5]*
39	FBgn0003388	<i>shd</i>		A			2003[29]
40	FBgn0005638	<i>slbo</i>	A	A			1992[30]
41	FBgn0023423	<i>slmb</i>		F	R	R	2005[31]*
42	FBgn0085450	<i>Snoo</i>	$(A \cap D) \cup (D \setminus D)$	$D \setminus D$			2007[32]*
43	FBgn0003463	<i>sog</i>	$A \cap D$	$A \cap D$			2000[33]*
44	FBgn0014388	<i>sty</i>	M	$A \cup M$	$(A \setminus D) \cup F$		1999[34]*
45	FBgn0003716	<i>tkv</i>	$(A \setminus D) \cup R$	$(A \setminus D) \cup R$			1999[35]*
46	FBgn0014076	<i>Vm32E</i>	$U \setminus (A \cup D)$	$U \setminus A$			2000[36]
47	FBgn0003984	<i>vn</i>		F			1998[37]
48	FBgn0041709	<i>yellow-g</i>			$D \setminus R$		2004[6]
49	FBgn0035328	<i>yellow-g2</i>		$A \setminus D$	$M \cup (U \setminus D)$	$M \cup (U \setminus D)$	2004[6]*
50	FBgn0011746	<i>ana</i>	$A \cap D$	$A \cap D$			this study
51	FBgn0036715	<i>Cad74A</i>		$U \setminus R$	$U \setminus R$	$U \setminus R$	this study
52	FBgn0039905	<i>CG2052</i>	$U \setminus D$	$U \setminus D$			this study
53	FBgn0036612	<i>CG4998</i>	A	$A \cup (D \setminus (M \cup R))$	$A \cup (D \setminus (M \cup R))$	$A \cup (D \setminus (M \cup R))$	this study

54	FBgn0029568	<i>CG11381</i>	$M \cup (U \setminus D)$	$M \cup (U \setminus D)$	$M \cup (U \setminus D)$	$M \cup (U \setminus D)$	this study
55	FBgn0039637	<i>CG11880</i>	A	A		$A \cup D$	this study
56	FBgn0039695	<i>CG12068</i>	U	$(A \cup D) \setminus M$	$A \cup (D \setminus F)$	$(A \cup D) \setminus M$	this study
57	FBgn0029966	<i>CG15324</i>		$U \setminus M$	U	U	this study
58	FBgn0051522	<i>CG31522</i>	U	A+U	A	A	this study
59	FBgn0051900	<i>CG31900</i>			$A \cup (U \setminus D)$	$A \cup (U \setminus D)$	this study
60	FBgn0052774	<i>CG32774</i>			$D \setminus R$	$D \setminus R$	this study
61	FBgn0053099	<i>CG33099</i>	U	$(A \setminus D) \cup (U \setminus A)$			this study
62	FBgn0085446	<i>CG34417</i>	$U \setminus M$	$(U \setminus M) + R$	$(U \setminus M) + R$	R	this study
63	FBgn0000355	<i>Cp15</i>	U	A+U	U	U	this study
64	FBgn0000360	<i>Cp38</i>		$D \setminus M$	$(A+U) \setminus M$	U	this study
65	FBgn0014465	<i>Cp7Fb</i>	$A \cup D$	$A \cup R$	$U \setminus F$	$U \setminus R$	this study
66	FBgn0014466	<i>Cp7Fc</i>		$(A \cup D) \setminus M$	$(A+U) \setminus M$	$(A+U) \setminus M$	this study
67	FBgn0011577	<i>dally</i>	A+U	A+U	$A \setminus D$		this study
68	FBgn0004638	<i>drk/Grb2</i>	U	U	M	M	this study
69	FBgn0034335	<i>GstE1</i>	$U \setminus A$	$U \setminus M$	R	F	this study
70	FBgn0001230	<i>Hsp68</i>	A	A+U		$D \setminus R$	this study
71	FBgn0023001	<i>melt</i>			D	$D \setminus M$	this study
72	FBgn0014342	<i>mia</i>		$A \cap D$	A+U	A	this study
73	FBgn0011754	<i>PhKγ</i>	A	$A \setminus D$			this study
74	FBgn0003205	<i>Ras85D</i>	D	F	F	F	this study
75	FBgn0033033	<i>scarface</i>		F	F	F	this study
76	FBgn0015296	<i>Shc</i>	U	F	F	F	this study
77	FBgn0003507	<i>srp</i>			$D \setminus M$	D	this study
78	FBgn0003865	<i>tsg</i>		$A \cap D$			this study
79	FBgn0004397	<i>Vinc</i>		F	$F \cup R$	$F \cup R$	this study
80	FBgn0016075	<i>vkg</i>		A	A	D	this study
81	FBgn0024179	<i>wit</i>	A	$A \setminus D$			this study

*Denotes previously published patterns of genes that were repeated for this study.

Table S2. Genes Arranged by the Number of Stages in which They Are Expressed

One stage (13)	Two stages (23)	Three stages (26)	Four stages (19)
<i>B4</i>	<i>ana</i>	<i>argos</i>	<i>I8w</i>
<i>bnb</i>	<i>br</i>	<i>Cad74A</i>	<i>Cad99C</i>
<i>CG9027</i>	<i>brk</i>	<i>Cct1</i>	<i>CG3074</i>
<i>CG13113</i>	<i>bves</i>	<i>CG4009</i>	<i>CG4998</i>
<i>Eip75B</i>	<i>CG2052</i>	<i>CG11880</i>	<i>CG11381</i>
<i>Eip78C</i>	<i>CG31900</i>	<i>CG15324</i>	<i>CG12068</i>
<i>Gli</i>	<i>CG32774</i>	<i>CG33298</i>	<i>CG31522</i>
<i>mfas</i>	<i>CG33099</i>	<i>Cp38</i>	<i>CG34417</i>
<i>mnd</i>	<i>Cp36</i>	<i>Cp7Fc</i>	<i>Cp15</i>
<i>shd</i>	<i>Dad</i>	<i>dally</i>	<i>Cp7Fb</i>
<i>vn</i>	<i>fng</i>	<i>Hsp68</i>	<i>dpp</i>
<i>yellow-g</i>	<i>jim-2</i>	<i>kay-RB</i>	<i>drk</i>
<i>tsg</i>	<i>melt</i>	<i>kek1</i>	<i>emc</i>
	<i>PhKy</i>	<i>loco-c2</i>	<i>Fkbp13</i>
	<i>pip-RA</i>	<i>mia</i>	<i>GstE1</i>
	<i>puc</i>	<i>mirr</i>	<i>ImpL2</i>
	<i>slbo</i>	<i>pnt-P1</i>	<i>jar</i>
	<i>Snoo</i>	<i>pnt-P2</i>	<i>Ras85D</i>
	<i>sog</i>	<i>rho</i>	<i>Shc</i>
	<i>srp</i>	<i>scarface</i>	
	<i>tkv</i>	<i>sgl</i>	
	<i>Vm32E</i>	<i>slmb</i>	
	<i>wit</i>	<i>sty</i>	
		<i>Vinc</i>	
		<i>vkg</i>	
		<i>yellow-g2</i>	

Corresponds to Figure 5B.

Table S3. Lists of Genes Expressed in Stages 10A-12 of Oogenesis

Stage 10A(50)	Stage S10B(66)	Stage S11(52)	Stage 12(45)
<i>I8w</i>	<i>I8w</i>	<i>I8w</i>	<i>I8w</i>
<i>ana</i>	<i>ana</i>	<i>argos</i>	<i>argos</i>
<i>B4</i>	<i>argos</i>	<i>Cad74A</i>	<i>bnb</i>
<i>br</i>	<i>br</i>	<i>Cad99C</i>	<i>Cad74A</i>
<i>brk</i>	<i>brk</i>	<i>CG3074</i>	<i>Cad99C</i>
<i>bves</i>	<i>bves</i>	<i>CG4009</i>	<i>Cct1</i>
<i>Cad99C</i>	<i>Cad74A</i>	<i>CG4998</i>	<i>CG11381</i>
<i>Cct1</i>	<i>Cad99C</i>	<i>CG11381</i>	<i>CG11880</i>
<i>CG2052</i>	<i>Cct1</i>	<i>CG12068</i>	<i>CG12068</i>
<i>CG3074</i>	<i>CG2052</i>	<i>CG13113</i>	<i>CG15324</i>
<i>CG4998</i>	<i>CG3074</i>	<i>CG15324</i>	<i>CG3074</i>
<i>CG11381</i>	<i>CG4009</i>	<i>CG31522</i>	<i>CG31522</i>
<i>CG11880</i>	<i>CG4998</i>	<i>CG31900</i>	<i>CG31900</i>
<i>CG12068</i>	<i>CG11381</i>	<i>CG32774</i>	<i>CG32774</i>
<i>CG31522</i>	<i>CG11880</i>	<i>CG33298</i>	<i>CG33298</i>
<i>CG33099</i>	<i>CG12068</i>	<i>CG34417</i>	<i>CG34417</i>
<i>CG34417</i>	<i>CG15324</i>	<i>Cp15</i>	<i>CG4009</i>
<i>Cp15</i>	<i>CG31522</i>	<i>Cp36</i>	<i>CG4998</i>
<i>Cp7Fb</i>	<i>CG33099</i>	<i>Cp38</i>	<i>CG9027</i>
<i>Dad</i>	<i>CG33298</i>	<i>Cp7Fb</i>	<i>Cp15</i>
<i>dally</i>	<i>CG34417</i>	<i>Cp7Fc</i>	<i>Cp36</i>
<i>dpp</i>	<i>Cp15</i>	<i>dally</i>	<i>Cp38</i>
<i>drk</i>	<i>Cp38</i>	<i>dpp</i>	<i>Cp7Fb</i>
<i>emc</i>	<i>Cp7Fb</i>	<i>drk</i>	<i>Cp7Fc</i>
<i>Fkbp13</i>	<i>Cp7Fc</i>	<i>Eip75B</i>	<i>dpp</i>
<i>fng</i>	<i>Dad</i>	<i>emc</i>	<i>drk</i>
<i>GstE1</i>	<i>dally</i>	<i>Fkbp13</i>	<i>Eip78C</i>
<i>Hsp68</i>	<i>dpp</i>	<i>Gli</i>	<i>emc</i>
<i>ImpL2</i>	<i>drk</i>	<i>GstE1</i>	<i>Fkbp13</i>
<i>jar</i>	<i>emc</i>	<i>ImpL2</i>	<i>GstE1</i>
<i>jim-2</i>	<i>Fkbp13</i>	<i>jar</i>	<i>Hsp68</i>
<i>kay-RB</i>	<i>fng</i>	<i>kay-RB</i>	<i>ImpL2</i>
<i>kek1</i>	<i>GstE1</i>	<i>kek1</i>	<i>jar</i>
<i>loco-c2</i>	<i>Hsp68</i>	<i>loco-c2</i>	<i>kay-RB</i>
<i>mfas</i>	<i>ImpL2</i>	<i>melt</i>	<i>melt</i>
<i>mirr</i>	<i>jar</i>	<i>mia</i>	<i>mia</i>
<i>PhKy</i>	<i>jim-2</i>	<i>mirr</i>	<i>Ras85D</i>
<i>pip-RA</i>	<i>kek1</i>	<i>pnt-P1</i>	<i>scarface</i>
<i>pnt-P1</i>	<i>loco-c2</i>	<i>pnt-P2</i>	<i>sgl</i>
<i>pnt-P2</i>	<i>mia</i>	<i>puc</i>	<i>Shc</i>
<i>Ras85D</i>	<i>mirr</i>	<i>Ras85D</i>	<i>slmb</i>
<i>rho</i>	<i>mnd</i>	<i>rho</i>	<i>srp</i>
<i>Shc</i>	<i>PhKy</i>	<i>scarface</i>	<i>Vinc</i>
<i>slbo</i>	<i>pip-RA</i>	<i>sgl</i>	<i>vkg</i>
<i>Snoo</i>	<i>pnt-P1</i>	<i>Shc</i>	<i>yellow-g2</i>
<i>sog</i>	<i>pnt-P2</i>	<i>slmb</i>	
<i>sty</i>	<i>puc</i>	<i>srp</i>	
<i>tkv</i>	<i>Ras85D</i>	<i>sty</i>	
<i>Vm32E</i>	<i>rho</i>	<i>Vinc</i>	
<i>wit</i>	<i>scarface</i>	<i>vkg</i>	
	<i>sgl</i>	<i>yellow-g</i>	
	<i>Shc</i>	<i>yellow-g2</i>	
	<i>shd</i>		
	<i>slbo</i>		
	<i>slmb</i>		
	<i>Snoo</i>		
	<i>sog</i>		
	<i>sty</i>		
	<i>tkv</i>		
	<i>tsg</i>		
	<i>Vinc</i>		
	<i>vkg</i>		
	<i>Vm32E</i>		
	<i>vn</i>		
	<i>wit</i>		
	<i>yellow-g2</i>		

Corresponds to Figure 5B.

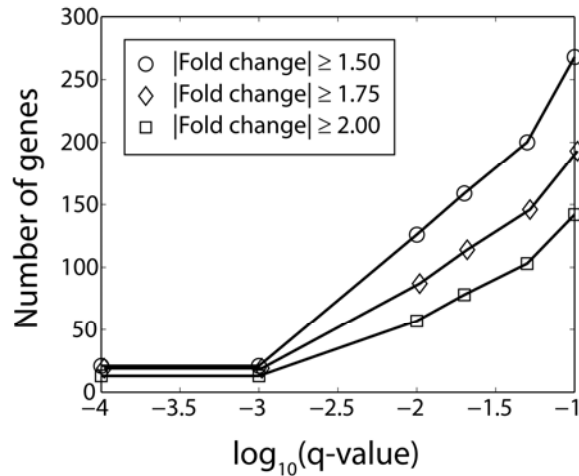


Figure S1. The Number of Candidate Genes as a Function of Statistical Filter Parameters

See Experimental Procedures for more details.

The filter first sets a fold change and q-value threshold for each of the four genetic backgrounds (*CY2>caTKV*, *CY2>Dad*, *CY2>caEGFR*, *CY2>dnEGFR* compared to OreR). If a least one background per pathway is activated or repressed, the gene is considered a candidate for future investigation. The number of targets for a range of options is shown. A q-value = 0.1 and an absolute fold change = 1.75 was selected, resulting in 193 candidate genes.

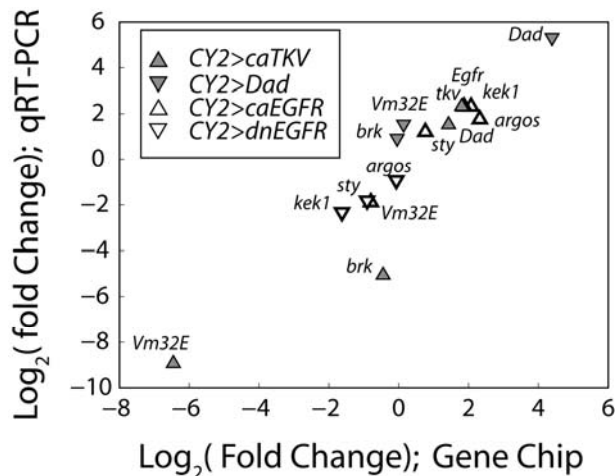


Figure S2. Comparison of qRT-PCR and Affymetrix Gene Chip Fold Changes

The mRNA abundance measurements in the four used pathway perturbations compared to wild type using both qRT-PCR and Affymetrix Gene Chip assays. There is a linear correlation with a slope of 1.3 and Pearson correlation coefficient equal to 0.87.

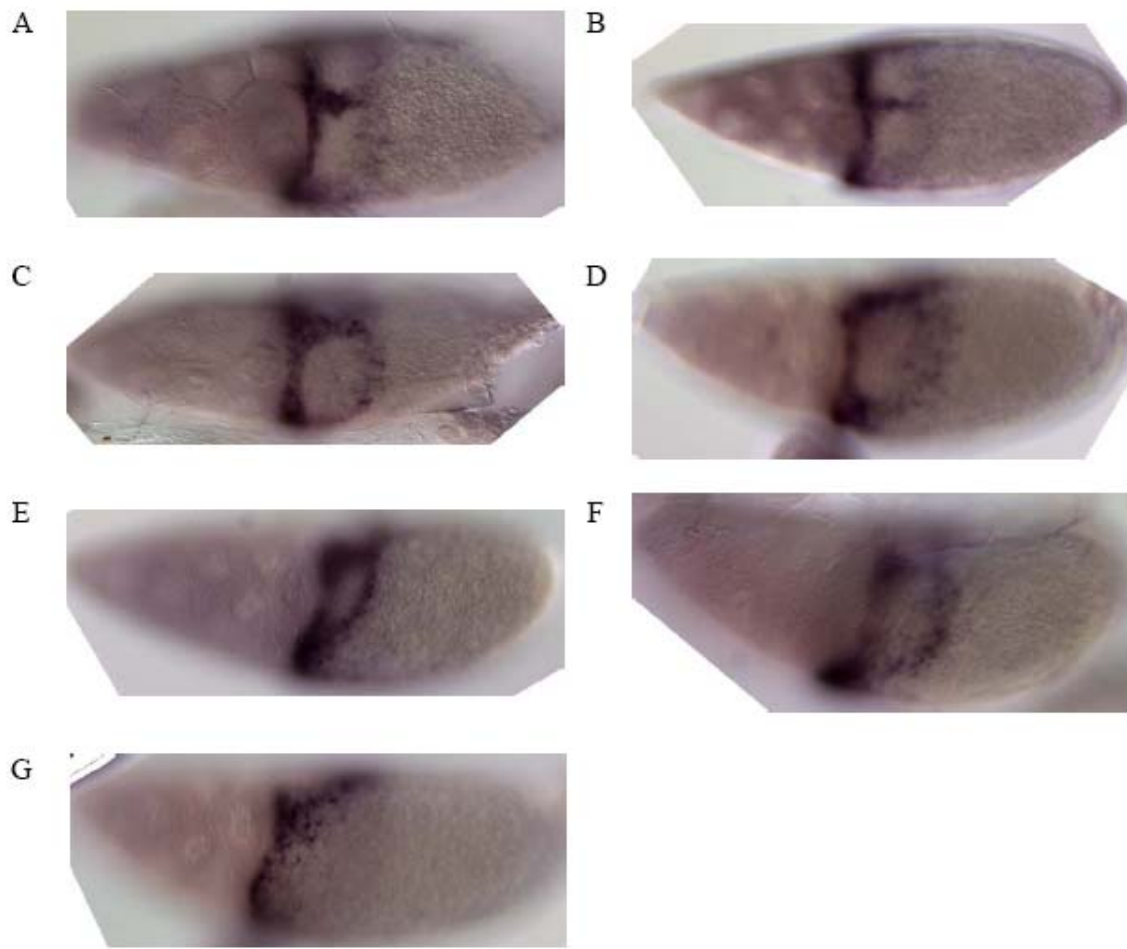


Figure S3. Examples of Images of the Wild-Type Expression Pattern of 18w
Images are collected from egg chambers in different stages of development and dorsoventral orientations on the microscope slide. Note how the egg chamber in (F, with the lateral view of expression pattern) is deformed as a result of its connection to other egg chamber within the ovariole.

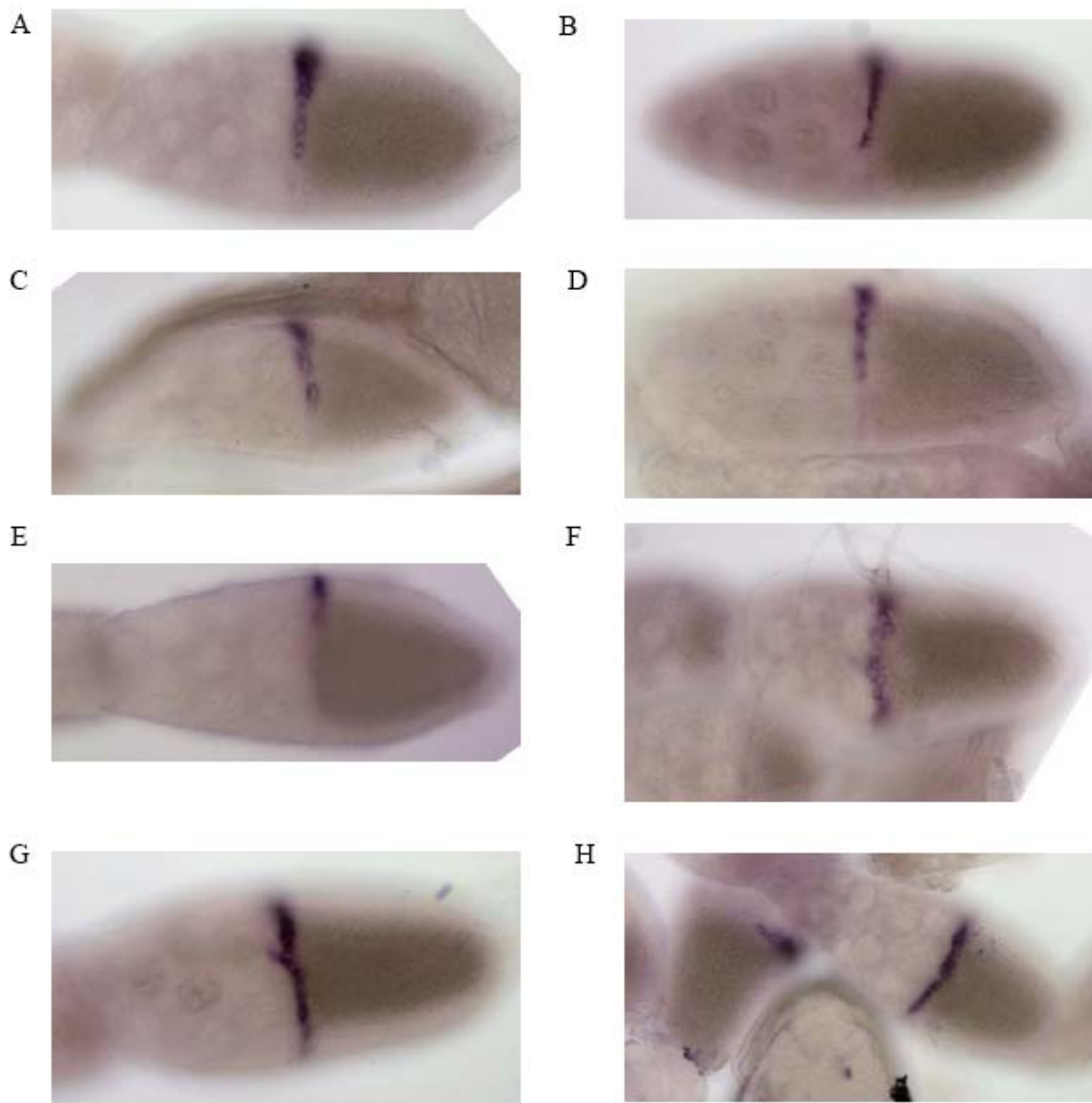


Figure S4. Examples of Images of the Wild-Type Expression Pattern of ana
Images F and H show that useful information about the expression pattern can be extracted from images collected from clusters of connected egg chambers, a situation that presents a major challenge for a purely computational approach.

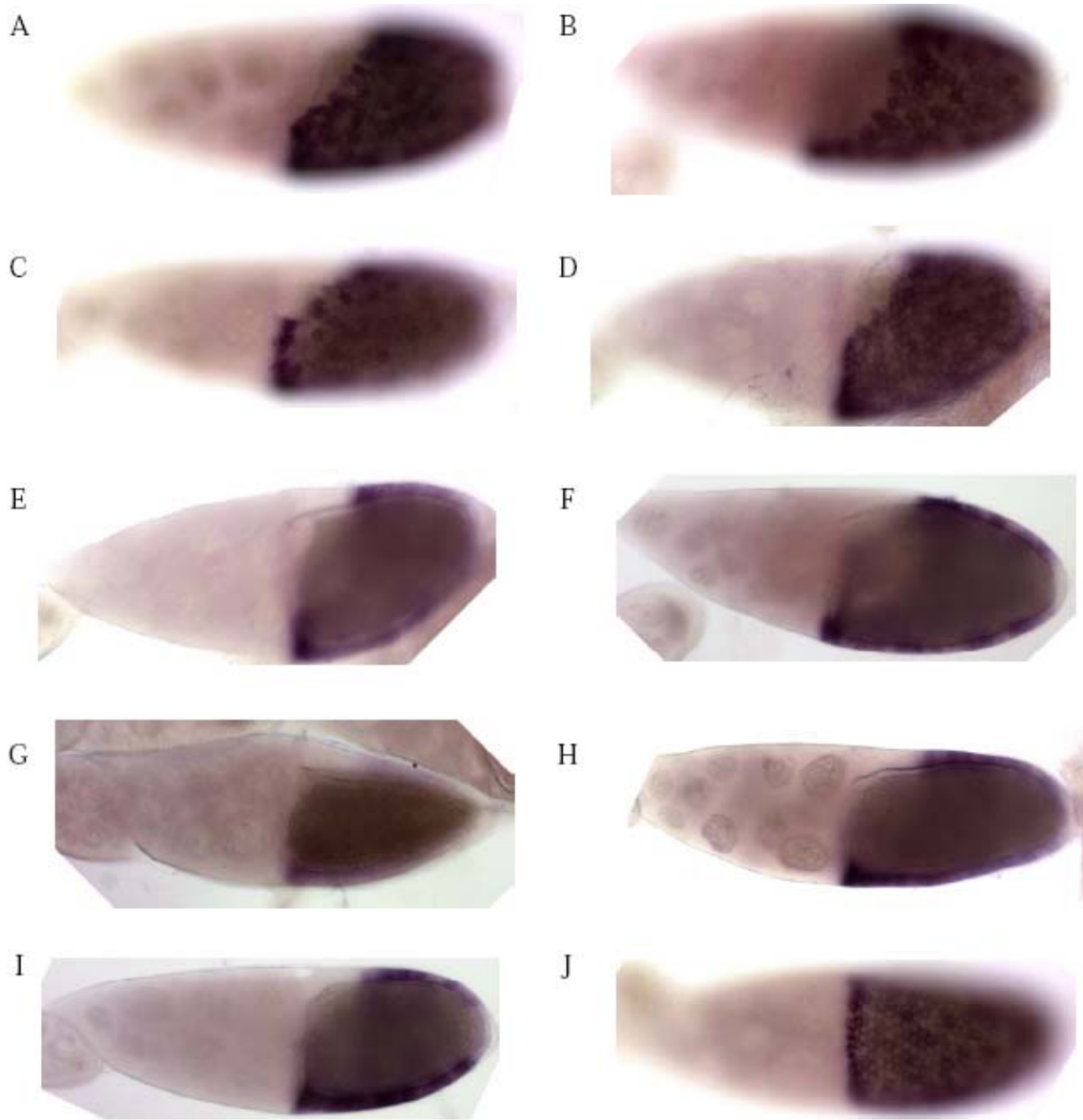


Figure S5. Examples of Images Collected for the Wild-Type Expression Pattern of CG2025

As in Figures S3 and S4, images are collected from egg chambers in different orientations and different locations of the focal plane.

Table S4. qRT-PCR Data for Method Validation and Platform Comparison

Gene	Genetic Background	Fold Change (log 2)	P-value
<i>Egfr</i>	<i>CY2>caEGFR</i>	2.33	1.39E-05
<i>argos</i>	<i>CY2>caEGFR</i>	3.40	1.56E-06
<i>kek1</i>	<i>CY2>caEGFR</i>	5.01	2.92E-03
<i>sty</i>	<i>CY2>caEGFR</i>	2.32	1.35E-02
<i>Vm32E</i>	<i>CY2>caEGFR</i>	-1.89	2.00E-04
<i>argos</i>	<i>CY2>dnEGFR</i>	-0.93	4.81E-02
<i>kek1</i>	<i>CY2>dnEGFR</i>	-2.33	1.38E-03
<i>sty</i>	<i>CY2>dnEGFR</i>	-1.82	9.74E-03
<i>Dad</i>	<i>CY2>Dad</i>	5.32	1.59E-07
<i>brk</i>	<i>CY2>Dad</i>	0.93	4.97E-02
<i>Vm32E</i>	<i>CY2>Dad</i>	1.55	5.31E-02
<i>tkv</i>	<i>CY2>caTKV</i>	2.33	2.17E-05
<i>brk</i>	<i>CY2>caTKV</i>	-5.06	1.00E-04
<i>Dad</i>	<i>CY2>caTKV</i>	1.53	8.58E-04
<i>Vm32E</i>	<i>CY2>caTKV</i>	-8.94	3.75E-07

The fold change was calculated using the Delta-delta Ct method and p-value is based on a student t-test (n = 3).

Table S5. Affymetrix GeneChip Data for Method Validation and Platform Comparison

Gene sym	Affyprobe ID	Genetic Background	Fold Change (log 2)	q-value
<i>Egfr</i>	142966_at	<i>CY2>caEGFR</i>	1.87	2.81E-03
<i>argos</i>	143483_at	<i>CY2>caEGFR</i>	2.32	5.57E-02
<i>kek1</i>	141473_at	<i>CY2>caEGFR</i>	2.08	2.34E-03
<i>sty</i>	143757_at	<i>CY2>caEGFR</i>	0.76	3.78E-02
<i>Vm32E</i>	143749_at	<i>CY2>caEGFR</i>	-0.84	0.00E+00
<i>argos</i>	143483_at	<i>CY2>dnEGFR</i>	-0.07	9.92E-01
<i>kek1</i>	141473_at	<i>CY2>dnEGFR</i>	-1.62	6.83E-02
<i>sty</i>	143757_at	<i>CY2>dnEGFR</i>	-0.86	2.43E-01
<i>Dad</i>	152910_at	<i>CY2>Dad</i>	4.39	0.00E+00
<i>brk</i>	141498_at	<i>CY2>Dad</i>	-0.05	9.04E-01
<i>Vm32E</i>	143749_at	<i>CY2>Dad</i>	0.14	1.90E-01
<i>tkv</i>	143377_at	<i>CY2>caTKV</i>	1.81	0.00E+00
<i>brk</i>	141498_at	<i>CY2>caTKV</i>	-0.45	7.57E-02
<i>Dad</i>	152910_at	<i>CY2>caTKV</i>	1.43	2.55E-02
<i>Vm32E</i>	143749_at	<i>CY2>caTKV</i>	-6.45	0.00E+00

The fold change values are calculated using the Golden Spike script.

Table S6. qRT-PCR Transcriptional Profiling of Stage 9-10 Egg Chambers in which the UAS-GFP Was Expressed using the CY2-Gal4 Driver

Affyprobe ID	CG id	FBgn ID	Fold change (log2)	P-value
148885_at	CG4998	FBgn0036612	-1.34	5.93E-02
150043_at	CG4009	FBgn0038469	-1.32	5.58E-02
144376_at	CG11381	FBgn0029568	-1.08	7.47E-02
142610_at	CG17270	FBgn0038828	-1.07	5.99E-02
142851_at	CG8891	FBgn0031663	-0.99	1.29E-02
147662_at	CG11275	FBgn0034706	-0.95	2.40E-04
146008_at	CG31900	FBgn0051900	-0.94	1.00E-01
153734_at	CG7137	FBgn0034422	-0.90	1.10E-01
151850_at	CG3960	FBgn0029876	-0.88	5.48E-02
153930_at	CG5224	FBgn0034354	-0.81	1.68E-01
147990_at	CG13926	FBgn0035243	-0.78	2.68E-03
149494_at	CG7352	FBgn0037581	-0.78	1.48E-03
145026_at	CG11158	FBgn0030511	-0.76	1.87E-01
152113_at	CG8084	FBgn0011746	-0.75	1.39E-01
142726_at	CG10390	FBgn0037337	-0.73	5.52E-03
144023_at	CG8357	FBgn0024732	-0.67	3.13E-02
151192_at	CG15386	FBgn0040715	-0.63	3.15E-02
146217_at	CG16834	FBgn0040096	-0.58	2.34E-02
144705_at	CG15351	FBgn0030045	-0.55	3.52E-01
142672_at	CG10157	FBgn0039099	-0.48	2.63E-02
142350_at	CG8063	FBgn0038105	-0.45	2.41E-01
152281_at	CG3654	FBgn0036004	-0.44	3.63E-01
142446_at	CG13344	FBgn0033884	-0.44	5.24E-02
146264_s_at	CG31864	FBgn0051864	-0.42	5.33E-02
141687_at	CG3992	FBgn0003507	-0.41	4.13E-01
146878_at	CG8799	FBgn0010549	-0.39	8.02E-02
150058_at	CG5285	FBgn0038490	-0.39	7.83E-02
144704_at	CG15350	FBgn0030044	-0.30	4.84E-01
152907_at	CG8896	FBgn0004364	-0.27	8.95E-02
148945_at	CG6445	FBgn0036715	-0.26	6.29E-01
151838_at	CG2789	FBgn0031263	-0.26	2.78E-01
150837_at	CG7592	FBgn0039685	-0.25	7.16E-01
143790_at	CG2849	FBgn0015286	-0.22	7.05E-02
145488_at	CG17600	FBgn0031195	-0.17	3.33E-01
142117_at	CG9754	FBgn0034617	-0.15	5.24E-01
146832_at	CG14745	FBgn0043575	-0.15	8.21E-01
144058_at	CG14796	FBgn0025390	-0.14	8.48E-01
151591_at	CG3606	FBgn0011571	-0.13	3.37E-01
144406_at	CG13758	FBgn0040378	-0.13	5.53E-01
151208_at	CG15068	FBgn0040733	-0.13	8.57E-01
143459_at	CG3299	FBgn0004397	-0.08	6.69E-01
152598_at	CG5164	FBgn0034335	-0.06	8.46E-01
150110_at	CG31243	FBgn0000363	-0.02	7.16E-01
147507_s_at	CG18367	FBgn0034460	-0.01	9.85E-01
148775_at	CG10006	FBgn0036461	-0.01	9.72E-01
143472_at	CG5723	FBgn0004449	0.01	9.83E-01
142587_at	CG33099	FBgn0053099	0.03	9.05E-01
143601_at	CG17603	FBgn0010355	0.03	9.18E-01
142181_at	CG30079	FBgn0050079	0.05	9.06E-01
141361_at	CG11880	FBgn0039637	0.05	8.47E-01
145269_at	CG6835	FBgn0030882	0.05	7.33E-01
152132_at	CG14956	FBgn0035403	0.07	7.25E-01
143934_at	CG8085	FBgn0020620	0.07	2.13E-01
152313_at	CG10241	FBgn0015714	0.07	6.27E-01
145610_at	CG15356	FBgn0031377	0.09	8.82E-01
149053_at	CG9451	FBgn0036876	0.09	7.19E-01
143317_at	CG9375	FBgn0003205	0.09	4.91E-01
146696_at	CG18584	FBgn0033107	0.11	6.48E-01

143969_at	CG8624	FBgn0023001	0.12	7.10E-01
150844_at	CG12068	FBgn0039695	0.20	6.50E-01
154786_at	CG18522	FBgn0038347	0.21	4.17E-01
144998_at	CG15745	FBgn0030469	0.22	5.35E-01
152050_at	CG10512	FBgn0037057	0.24	1.76E-01
146672_at	CG1765	FBgn0000546	0.27	2.99E-01
145366_at	CG12199	FBgn0031016	0.28	1.92E-01
152573_at	CG32919	FBgn0052919	0.34	3.46E-01
151626_at	CG31522	FBgn0051522	0.35	6.39E-02
146369_at	CG5996	FBgn0032593	0.36	1.17E-01
152071_at	CG2052	FBgn0039905	0.41	5.95E-02
150265_at	CG10877	FBgn0038804	0.41	1.09E-01
145126_at	CG33206	FBgn0052587	0.42	1.90E-01
146651_at	CG11066	FBgn0033033	0.43	1.01E-01
151528_at	CG3665	FBgn0000635	0.45	3.48E-01
145426_at	CG15457	FBgn0031111	0.46	2.45E-01
145397_at	CG12701	FBgn0031071	0.49	1.36E-03
150459_at	CG31145	FBgn0051145	0.49	2.44E-01
150538_at	CG10845	FBgn0039246	0.50	5.40E-01
148087_at	CG14957	FBgn0035412	0.51	1.51E-01
150834_at	CG7584	FBgn0039682	0.51	2.99E-01
152162_at	CG5888	FBgn0028523	0.53	4.68E-01
150337_at	CG6560	FBgn0038916	0.53	5.58E-01
147986_at	CG12099	FBgn0035232	0.57	9.12E-03
146648_at	CG8245	FBgn0033031	0.62	2.30E-03
145000_at	CG1630	FBgn0030471	0.64	5.03E-02
143105_at	CG1618	FBgn0000346	0.64	3.02E-02
144660_at	CG15324	FBgn0029966	0.66	9.58E-03
154208_at	CG16721	FBgn0029820	0.67	1.20E-03
146317_at	CG15481	FBgn0032487	0.67	3.42E-03
143082_at	CG10422	FBgn0000158	0.67	2.12E-02
141646_at	CG17544	FBgn0032775	0.71	4.78E-02
144233_at	CG15281	FBgn0028536	0.72	2.98E-01
148687_at	CG11263	FBgn0036330	0.72	3.44E-04
153098_at	CG16705	FBgn0039102	0.72	1.43E-01
144007_at	CG10564	FBgn0024150	0.76	1.81E-02
143749_at	CG16874	FBgn0014076	0.82	2.84E-02
146913_at	CG1418	FBgn0033468	0.83	1.36E-03
143112_at	CG11213	FBgn0000360	0.84	7.95E-04
153620_at	CG12342	FBgn0033552	0.85	1.48E-02
150996_at	CG11093	FBgn0039932	0.89	9.14E-03
153182_at	CG17604	FBgn0000246	0.89	2.40E-02
148035_at	CG13804	FBgn0035328	0.92	6.97E-02
152094_at	CG17035	FBgn0036545	0.95	6.77E-04
143107_at	CG6519	FBgn0000355	1.28	5.57E-02
146607_at	CG6691	FBgn0032971	1.31	1.67E-01
148868_at	CG13046	FBgn0036595	2.39	5.44E-02
151558_r_at	CG32737	FBgn0052737	-9.42	5.04E-07
147434_at	CG17522	FBgn0034334	-7.38	2.19E-02
142324_at	CG10618	FBgn0045761	-5.79	1.31E-07
146297_at	CG6167	FBgn0032447	-5.04	1.75E-05
152499_at	CG33008	FBgn0053008	-4.97	1.00E-04
152408_at	CG31714	FBgn0032180	-4.42	9.03E-04
153539_at	CG10287	FBgn0026077	-3.81	5.58E-03
142222_at	CG4486	FBgn0015039	-3.63	1.93E-05
149400_at	CG10284	FBgn0037441	-3.62	4.52E-03
147309_at	CG4398	FBgn0034126	-2.99	3.64E-05
144340_at	CG13375	FBgn0040370	-2.92	2.71E-06
143827_at	CG3801	FBgn0015586	-2.75	2.58E-02
144378_at	CG11382	FBgn0040367	-2.68	2.83E-06
141780_at	CG3616	FBgn0015040	-2.49	4.19E-06

153744_at	CG9650	FBgn0029939	-2.36	6.23E-04
142479_at	CG17533	FBgn0034342	-2.07	1.02E-04
149762_at	CG4421	FBgn0010044	-1.88	2.10E-02
143971_at	CG2457	FBgn0023077	-1.78	1.38E-03
143303_at	CG11205	FBgn0003082	-1.46	1.24E-02
143890_at	CG2187	FBgn0017448	-1.39	1.03E-02
143723_at	CG10246	FBgn0013771	-1.34	8.10E-03
144073_at	CG12467	FBgn0025623	-1.16	6.98E-04
142248_at	CG4604	FBgn0033799	-1.13	9.86E-03
143111_at	CG1478	FBgn0000359	-1.12	3.89E-02
153777_at	CG3027	FBgn0037513	-1.07	7.48E-03
142801_at	CG3217	FBgn0025676	-1.02	1.94E-03
143299_at	CG17725	FBgn0003067	1.26	6.76E-03
154778_at	CG1275	FBgn0035321	1.34	3.72E-03
145061_at	CG32602	FBgn0052602	1.37	4.62E-02
149966_at	CG5302	FBgn0038351	1.44	7.38E-03
144357_at	CG3711	FBgn0040344	1.54	5.90E-03
143868_at	CG16858	FBgn0016075	1.83	4.25E-02
151211_at	CG16844	FBgn0040736	1.91	2.50E-03
143197_at	CG5436	FBgn0001230	2.16	2.27E-02
149757_at	CG4381	FBgn0010039	2.27	1.30E-02
149854_at	CG14367	FBgn0038170	2.36	2.45E-05
148799_at	CG12327	FBgn0036492	2.94	2.40E-02
149756_at	CG4181	FBgn0010038	2.94	6.13E-05
151957_at	CG7985	FBgn0028499	2.96	4.60E-04
146288_at	CG6417	FBgn0032435	3.00	1.60E-03
149755_at	CG10091	FBgn0038020	3.16	6.04E-05
143184_at	CG10045	FBgn0001149	3.59	1.43E-04
153583_at	CG4463	FBgn0001224	3.72	1.02E-06
148908_at	CG13032	FBgn0036652	3.77	3.41E-04
150181_at	CG6042	FBgn0038681	4.66	3.75E-06
152866_at	CG10833	FBgn0031689	7.24	3.28E-06
152645_at	CG7742	FBgn0031690	11.72	3.76E-03

The fold change, with respect to the wild-type was calculated using the Delta-delta Ct method and p-value is a student t-test (n = 3).

Supplemental References

1. Choe, S.E., Boutros, M., Michelson, A.M., Church, G.M., and Halfon, M.S. (2005). Preferred analysis methods for Affymetrix GeneChips revealed by a wholly defined control dataset. *Genome Biology* 6.
2. Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J., et al. (2004). Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.* 5, R80.
3. Baldi, P., and Long, A.D. (2001). A Bayesian framework for the analysis of microarray expression data: regularized t-test and statistical inferences of gene changes. *Bioinformatics* 17, 509-519.
4. Bernardi, F., S. Duchi, V. Cavaliere, A. Donati, D. Andrenacci, and G. Gargiulo, Egfr signaling modulates VM32E gene expression during *Drosophila* oogenesis. *Dev Genes Evol*, 2007. 217(7): p. 529-40.
5. Jordan, K.C., S.D. Hatfield, M. Tworoger, E.J. Ward, K.A. Fischer, S. Bowers, and H. Ruohola-Baker, Genome wide analysis of transcript levels after perturbation of the EGFR pathway in the *Drosophila* ovary. *Dev Dyn*, 2005. 232(3): p. 709-24.
6. Claycomb, J.M., M. Benasutti, G. Bosco, D.D. Fenger, and T.L. Orr-Weaver, Gene amplification as a developmental strategy: Isolation of two developmental amplicons in *Drosophila*. *Developmental Cell*, 2004. 6(1): p. 145-155.
7. Fakhouri, M., M. Elalayli, D. Sherling, J.D. Hall, E. Miller, X. Sun, L. Wells, and E.K. LeMosy, Minor proteins and enzymes of the *Drosophila* eggshell matrix. *Dev Biol*, 2006. 293(1): p. 127-41.
8. Kleve, C.D., D.A. Siler, S.K. Syed, and E.D. Eldon, Expression of 18-wheeler in the follicle cell epithelium affects cell migration and egg morphology in *Drosophila*. *Developmental Dynamics*, 2006. 235(7): p. 1953-1961.
9. Tolia, P.P., M. Konsolaki, M.S. Halfon, N.D. Stroumbakis, and F.C. Kafatos, Elements controlling follicular expression of the s36 chorion gene during *Drosophila* oogenesis. *Mol Cell Biol*, 1993. 13(9): p. 5898-906.
10. Queenan, A.M., A. Ghabrial, and T. Schupbach, Ectopic activation of torpedo/Egfr, a *Drosophila* receptor tyrosine kinase, dorsalizes both the eggshell and the embryo. *Development*, 1997. 124(19): p. 3871-3880.
11. Deng, W.M. and M. Bownes, Two signalling pathways specify localised expression of the Broad-Complex in *Drosophila* eggshell patterning and morphogenesis. *Development*, 1997. 124(22): p. 4639-47.
12. Chen, Y. and T. Schupbach, The role of brinker in eggshell patterning. *Mechanisms of Development*, 2006. 123(5): p. 395-406.
13. Lin, S., D.B. Zhao, and M. Bownes, Blood vessel/epicardial substance (bves) expression, essential for embryonic development, is down regulated by Grk/EFGR signalling. *International Journal of Developmental Biology*, 2007. 51(1): p. 37-44.
14. D'Alterio, C., D.D.D. Tran, M. Yeung, M.S.H. Hwang, M.A. Li, C.J. Arana, V.K. Mulligan, M. Kubesh, P. Sharma, M. Chase, U. Tepass, and D. Godt, *Drosophila melanogaster* Cad99C, the orthologue of human Usher cadherin PCDH15, regulates the length of microvilli. *Journal of Cell Biology*, 2005. 171(3): p. 549-558.
15. Gupta, T. and T. Schupbach, Cct1, a phosphatidylcholine biosynthesis enzyme, is required for *Drosophila* oogenesis and ovarian morphogenesis. *Development*, 2003. 130(24): p. 6075-6087.

16. Twombly, V., R.K. Blackman, H. Jin, J.M. Graff, R.W. Padgett, and W.M. Gelbart, The TGF-beta signaling pathway is essential for Drosophila oogenesis. *Development*, 1996. 122(5): p. 1555-65.
17. Buszczak, M., M.R. Freeman, J.R. Carlson, M. Bender, L. Cooley, and W.A. SeGRAves, Ecdysone response genes govern egg chamber development during mid-oogenesis in Drosophila. *Development*, 1999. 126(20): p. 4581-4589.
18. Bryant, Z., L. Subrahmanyam, M. Tworoger, L. LaTray, C.R. Liu, M.J. Li, G. van den Engh, and H. Ruohola-Baker, Characterization of differentially expressed genes in purified Drosophila follicle cells: toward a general strategy for cell type-specific developmental analysis. *Proc Natl Acad Sci*, 1999. 96(10): p. 5559-64.
19. Papadia, S., G. Tzolovsky, D.B. Zhao, K. Leaper, D. Clyde, P. Taylor, E. Asscher, G. Kirk, and M. Bownes, emc has a role in dorsal appendage fate formation in Drosophila oogenesis. *Mechanisms of Development*, 2005. 122(9): p. 961-974.
20. Jordan, K.C., N.J. Clegg, J.A. Blasi, A.M. Morimoto, J. Sen, D. Stein, H. McNeill, W.M. Deng, M. Tworoger, and H. Ruohola-Baker, The homeobox gene mirror links EGF signalling to embryonic dorso-ventral axis formation through Notch activation. *Nature Genetics*, 2000. 24(4): p. 429-433.
21. Deng, W.M., K. Leaper, and M. Bownes, A targeted gene silencing technique shows that Drosophila myosin VI is required for egg chamber and imaginal disc morphogenesis. *Journal of Cell Science*, 1999. 112(21): p. 3677-3690.
22. Doerflinger, H., J.A. Lepesant, and C. Yanicostas, Differential expression of the Drosophila zinc finger gene jim in the follicular epithelium. *Mech Dev*, 1999. 86(1-2): p. 177-82.
23. Dequier, E., S. Soudi, M. Pal, P. Maroy, J.A. Lepesant, and C. Yanicostas, Top-DER- and Dpp-dependent requirements for the Drosophila fos/kayak gene in follicular epithelium morphogenesis. *Mech Dev*, 2001. 106(1-2): p. 47-60.
24. Pathirana, S., D. Zhao, and M. Bownes, The Drosophila RGS protein Loco is required for dorsal/ventral axis formation of the egg and embryo, and nurse cell dumping. *Mechanisms of Development*, 2001. 109(2): p. 137-150.
25. Sen, J., J.S. Goltz, L. Stevens, and D. Stein, Spatially restricted expression of pipe in the Drosophila egg chamber defines embryonic dorsal-ventral polarity. *Cell*, 1998. 95(4): p. 471-481.
26. Morimoto, A.M., K.C. Jordan, K. Tietze, J.S. Britton, E.M. O'Neill, and H. Ruohola-Baker, Pointed, an ETS domain transcription factor, negatively regulates the EGF receptor pathway in Drosophila oogenesis. *Development*, 1996. 122(12): p. 3745-54.
27. Dobens, L.L., E. Martin-Blanco, A. Martinez-Arias, F.C. Kafatos, and L.A. Raftery, Drosophila puckered regulates Fos/Jun levels during follicle cell morphogenesis. *Development*, 2001. 128(10): p. 1845-1856.
28. Ruoholabaker, H., E. Grell, T.B. Chou, D. Baker, L.Y. Jan, and Y.N. Jan, Spatially Localized Rhomboid Is Required for Establishment of the Dorsal-Ventral Axis in Drosophila Oogenesis. *Cell*, 1993. 73(5): p. 953-965.
29. Petryk, A., J.T. Warren, G. Marques, M.P. Jarcho, L.I. Gilbert, J. Kahler, J.P. Parvy, Y.T. Li, C. Dauphin-Villemant, and M.B. O'Connor, Shade is the Drosophila P450 enzyme that mediates the hydroxylation of ecdysone to the steroid insect molting hormone 20-hydroxyecdysone. *Proceedings of the National Academy of Sciences of the United States of America*, 2003. 100(24): p. 13773-13778.
30. Montell, D.J., P. Rorth, and A.C. Spradling, Slow Border Cells, a Locus Required for a Developmentally Regulated Cell-Migration During Oogenesis, Encodes Drosophila C/Ebp. *Cell*, 1992. 71(1): p. 51-62.

31. Muzzopappa, M. and P. Wappner, Multiple roles of the F-box protein Slimb in *Drosophila* egg chamber development. *Development*, 2005. 132(11): p. 2561-2571.
32. Shravage, B.V., G. Altmann, M. Technau, and S. Roth, The role of Dpp and its inhibitors during eggshell patterning in *Drosophila*. *Development*, 2007. 134(12): p. 2261-71.
33. Araujo, H. and E. Bier, sog and dpp exert opposing maternal functions to modify toll signaling and pattern the dorsoventral axis of the *Drosophila* embryo. *Development*, 2000. 127(16): p. 3631-44.
34. Reich, A., A. Sapir, and B.Z. Shilo, Sprouty, a general inhibitor of receptor tyrosine kinase signaling. *Development*, 1999. 126(18): p. 413-47.
35. Mantrova, E.Y., R.A. Schulz, and T. Hsu, Oogenic function of the myogenic factor D-MEF2: Negative regulation of the Decapentaplegic receptor gene thick veins. *Proceedings of the National Academy of Sciences of the United States of America*, 1999. 96(21): p. 11889-11894.
36. Andrenacci, D., V. Cavaliere, F.M. Cernilogar, and G. Gargiulo, Spatial activation and repression of the *drosophila* vitelline membrane gene VM32E are switched by a complex cis-regulatory system. *Dev Dyn*, 2000. 218(3): p. 499-506.
37. Wasserman, J.D. and M. Freeman, An autoregulatory cascade of EGF receptor signaling patterns the *Drosophila* egg. *Cell*, 1998. 95: p. 355-364.