

Supporting Information – Figures

S1 Fig. Immunoblot examining Pvr knockdown efficiency and cell cycle progression in Kc and Kcp35 cell lines.

Immunoblot examining Pvr, phospho-histone H3, and total histone H3 after two days with no treatment or two days of Pvr or GFP dsRNA treatment. Samples of equal numbers of cells were loaded.

doi:10.1371/journal.pgen.1005056.s001

(PDF)

S2 Fig. RNAi knockdown efficiencies of amplicons.

Summary of dsRNA-mediated knockdown efficiencies assessed by quantitative real time PCR (qRT-PCR) or immunoblot.

doi:10.1371/journal.pgen.1005056.s002

(PDF)

S3 Fig. Expression of Pvf2 following Akt knockdown.

Plotted (y-axis) is the level of Pvf2 transcript remaining in Kc cells treated with dsRNA targeting Akt relative to a dsRNA targeting GFP. For normalization, Ribosomal protein L32 was used as a reference gene.

doi:10.1371/journal.pgen.1005056.s003

(PDF)

S4 Fig. Verification screen cell counts of 22 Pvr Suppressors.

Live/dead cell counting performed after silencing of 22 Pvr Suppressors or insulin stimulation in combination with Pvr, and compared to Pvr and GFP (control) knockdown.

doi:10.1371/journal.pgen.1005056.s004

(PDF)

S5 Fig. 20HE has no effect on Pvr levels.

Immunoblot examining Pvr after treatment of Kc and Kcp35 cells with 0.01 ug/ml 20HE for three days.

doi:10.1371/journal.pgen.1005056.s005

(PDF)

S6 Fig. Expression of rpr and E93 following Pvr and EcR knockdown.

Plotted (y-axis) is the level of rpr or E93 transcript remaining in Kc cells treated with dsRNA targeting Pvr or EcR relative to a dsRNA targeting GFP. For normalization, Ribosomal protein L32 was used as a reference gene. Two non-overlapping qPCR primers for each gene were used. No significant changes were observed.

doi:10.1371/journal.pgen.1005056.s006

(PDF)

S7 Fig. Hemocyte numbers during the embryo-larva transition.

Live hemocyte counts of embryos and larvae at the indicated times after egg laying (AEL), grown at 25C. UAS-Stinger; Pxn-GAL4 was crossed to w1118 (control), or UAS-EcRA dn, respectively. Note that around the time of hatching (grey bar), hemocyte numbers have dropped to about 60% of embryonic counts. Hemocyte-specific expression of UAS-EcRA dn does not significantly protect hemocytes from the decline, as indicated for 22h AEL.

doi:10.1371/journal.pgen.1005056.s007

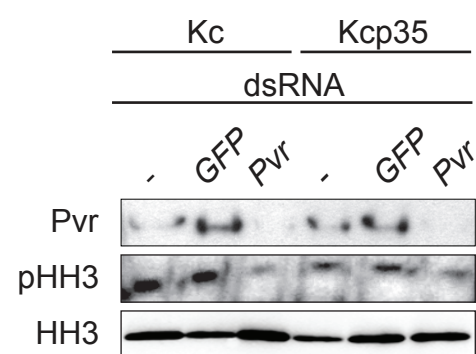
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S8 Fig. Pvr and EcR knockdown efficiency in mass spectrometry samples.

Immunoblot confirming knockdown of Pvr and EcR (top panels) after two days dsRNA treatment in Kc cells used for phosphoproteomic analysis by mass spectrometry.

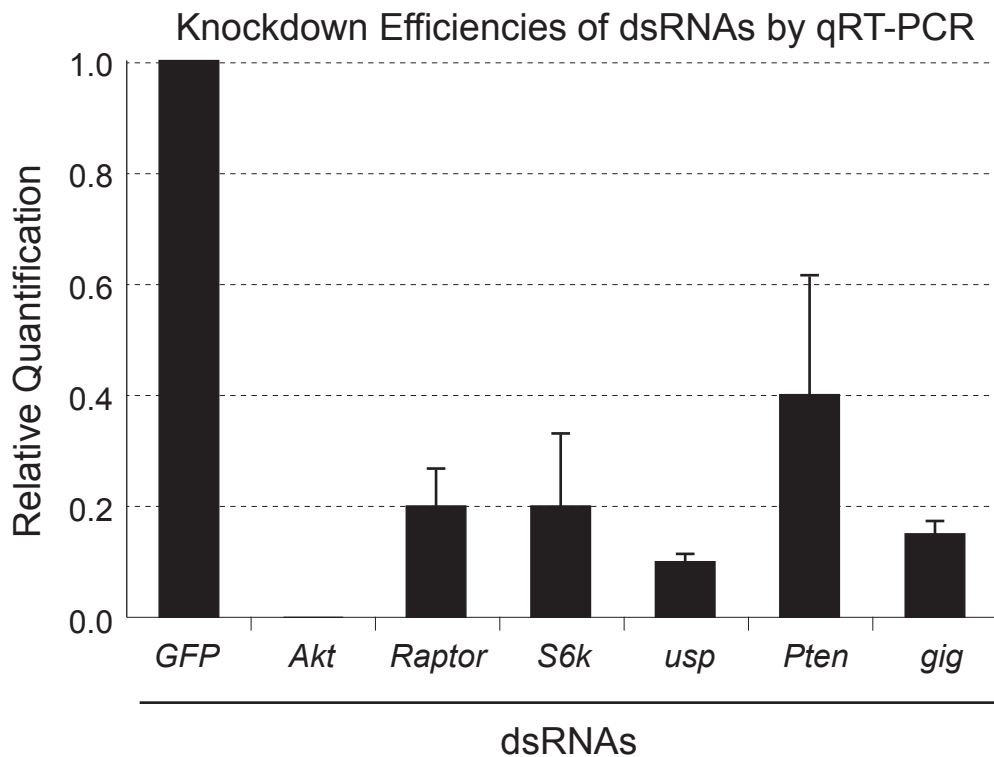
doi:10.1371/journal.pgen.1005056.s008

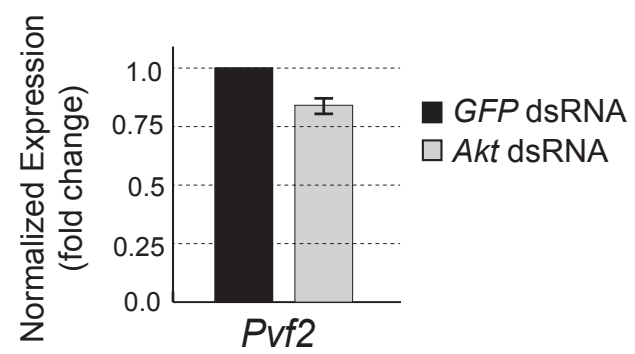
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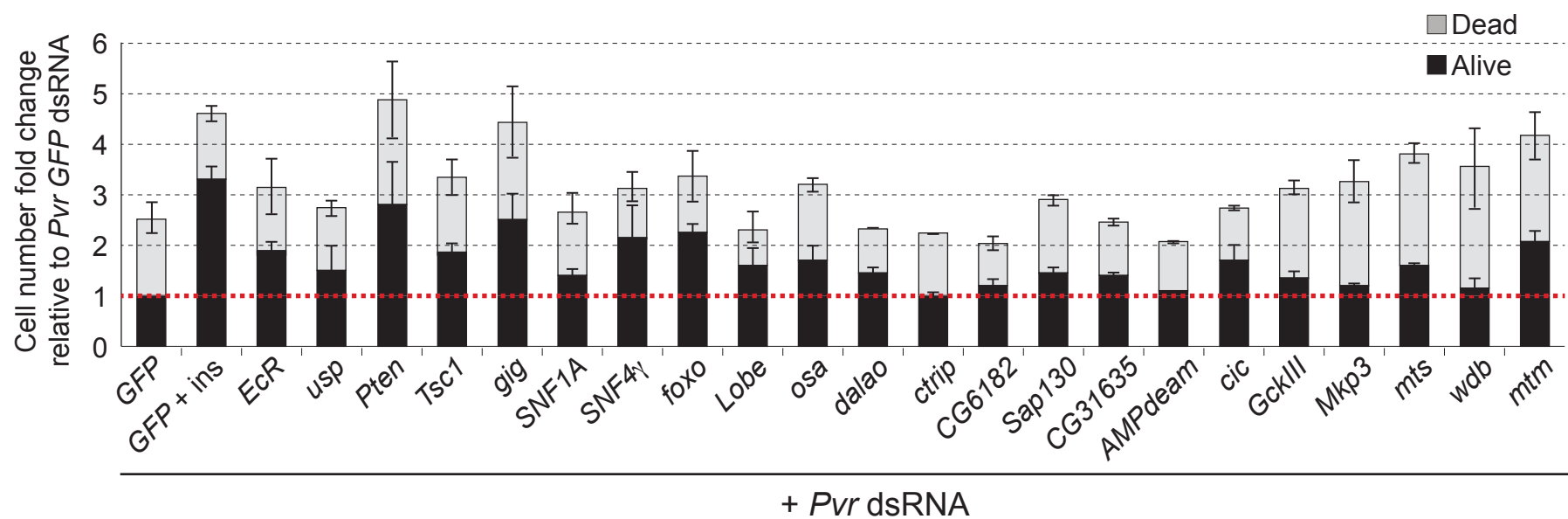
Sopko et al. Suppl. Figure 1

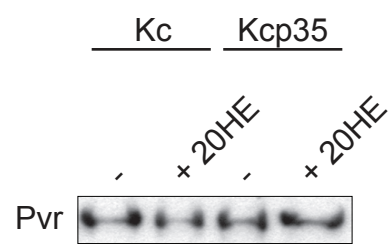
Gene	Knockdown Efficiency	Verified by
<i>Pvr</i>	>99%	immunoblot
<i>Akt</i>	>99%	qRT-PCR
<i>Raptor</i>	~80%	qRT-PCR
<i>S6k</i>	~80%	qRT-PCR
<i>Erk</i>	>99%	immunoblot
<i>Mek</i>	>99%	immunoblot
<i>EcR</i>	>99%	immunoblot
<i>usp</i>	~90%	qRT-PCR
<i>Pten</i>	~60%	qRT-PCR
<i>gig</i>	~85%	qRT-PCR



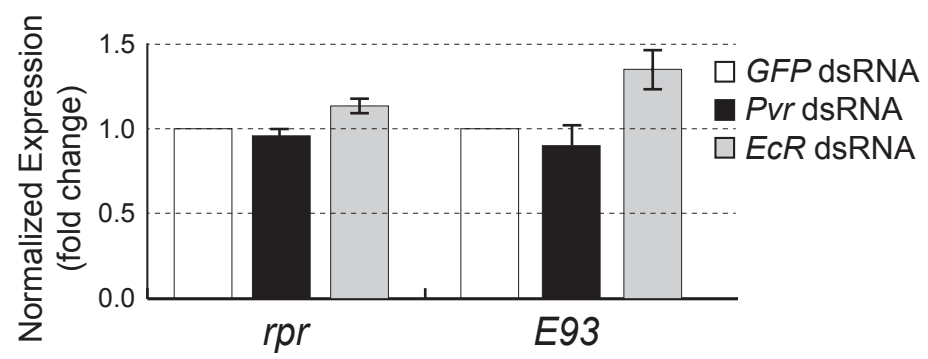


Sopko et al. Suppl. Figure 3

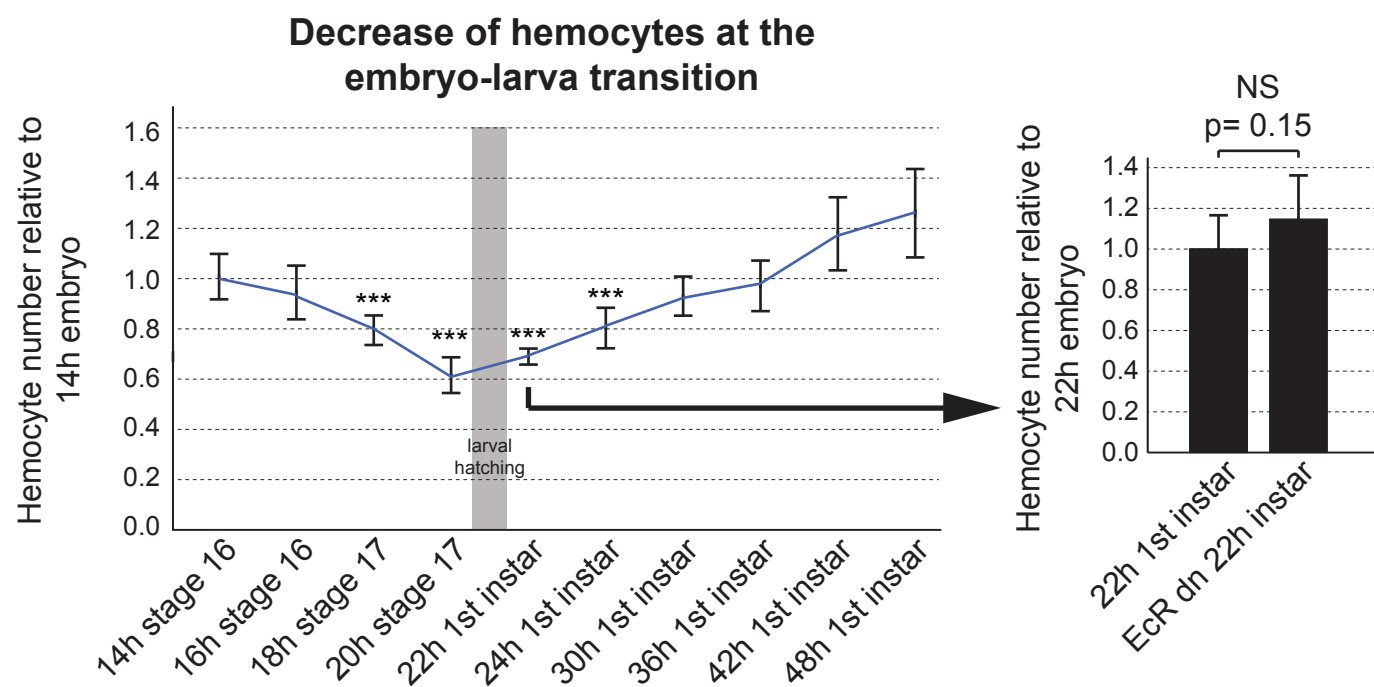




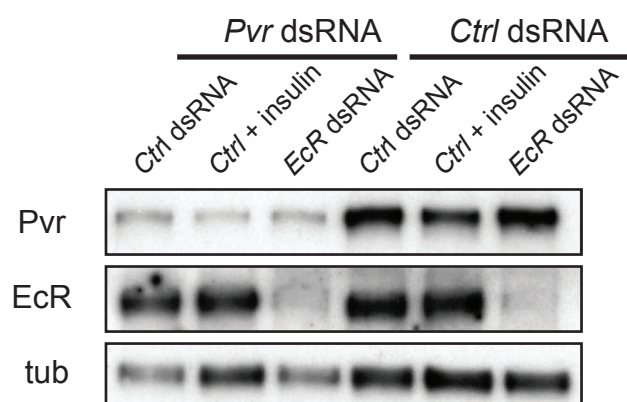
Sopko et al. Suppl. Figure 5



Sopko et al. Suppl. Figure 6



Sopko et al. Suppl. Figure 7



Sopko et al. Suppl. Figure 8