Genetics_302891_R1_Supplemtnal Information 1

Genome-wide RNAi screen for context-dependent tumor suppressors identified using *in vivo* models for neoplasia in *Drosophila*

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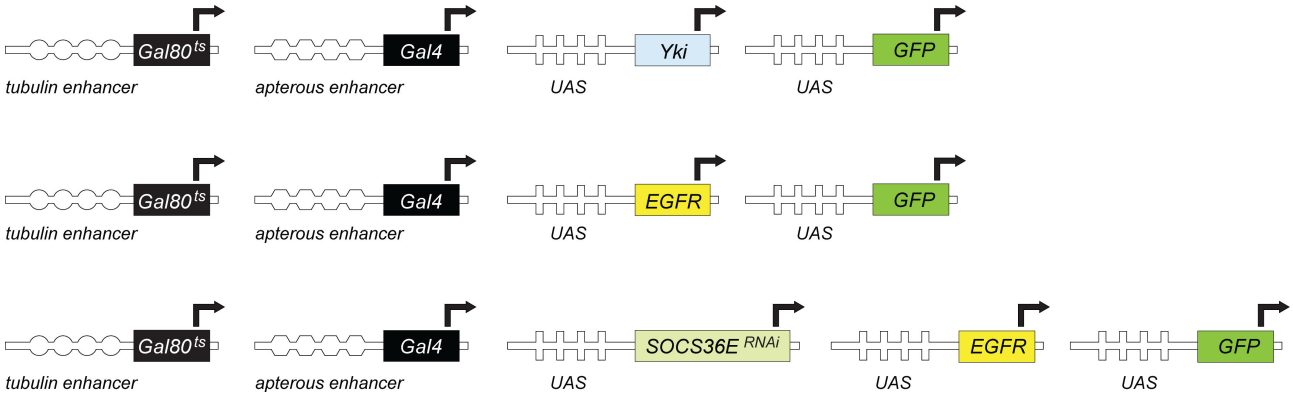
Supplemental data

Figure S1: screen design and controls

(A) Transgenes used in the three screens: In Yki and EGFR screens, there were 5 transgenes involved including RNAi in the final larvae that were screened for phenotypes. In case of EGFR-SOCS screen, there were 6 transgenes in the final larvae that were screened for phenotypes. To faciliate fast, reliable and reproducible testing, we combined all transgenes (as shown below), except the VDRC KK RNAi line being tested, to produce stable stocks. These stocks were then crossed to the KK library of UAS-RNAi stocks (see methods for genotypes). The integrity of these stocks was verified weekly.



(B) Screen workflow:

The figure illustrates how the screen was done. To ensure that the screen (which involved close to 30,000 crosses) is fast, reliable and reproducible, we strictly adhered to a predtermined schedule for all steps and temperature regimes. The schematic below shows when the virgins were collected, when the crosses were set up, when the larvae were shifted to higher temperature to induce GAL4 expression and when the larvae were scored for the phenotype. Each cycle took 21 days to complete.

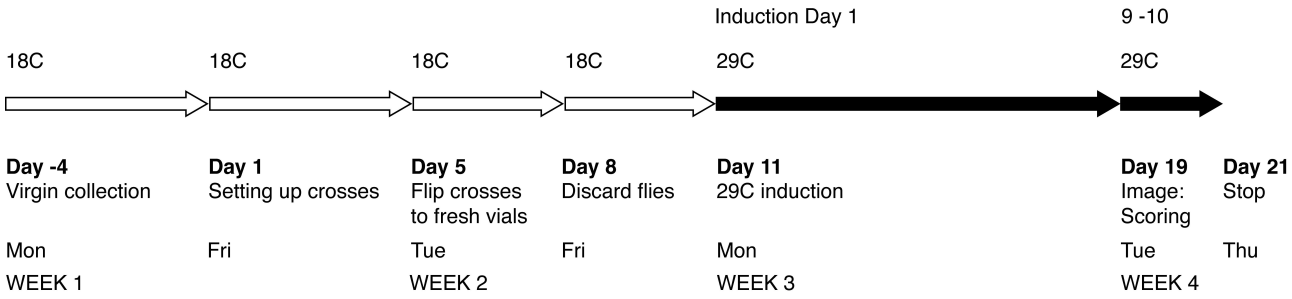
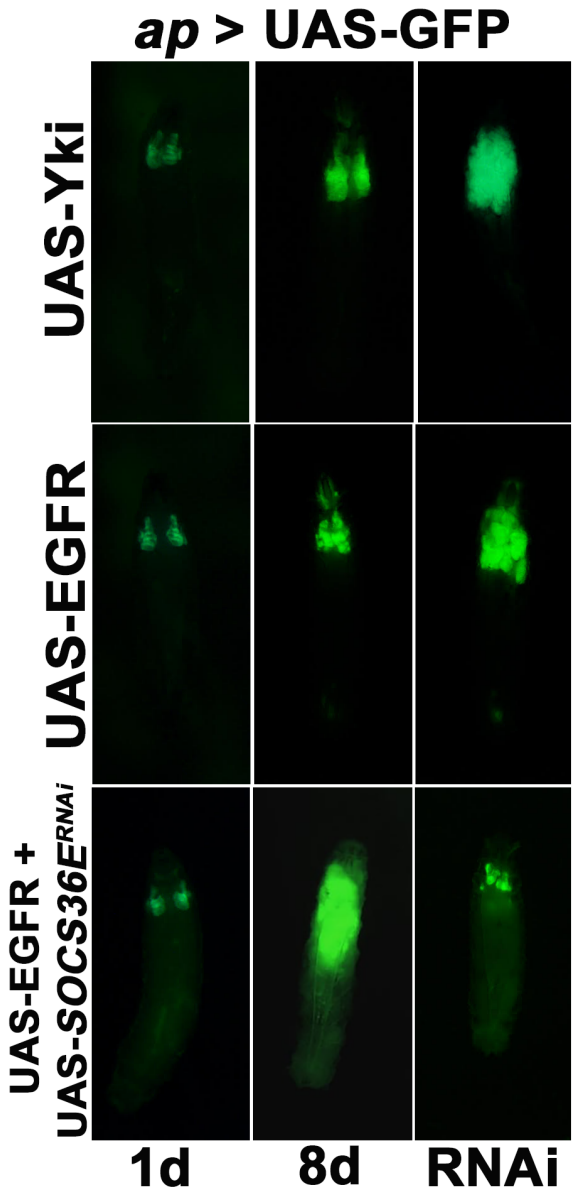


Figure S2: Quality control. From Left to Right.

Left: w; *ap*-Gal4; UAS-GFP + UAS-Yki, UAS-EGFR or UAS-EGFR+*SOCS36E*^{RNAi} expression after 24h induction at 29°C. Center panel: same combinations after 8d at 29°C. This phenotype was tested on a weekly basis to control for the quality and integrity of the test stocks. Right panel: same combinations crossed to indicated RNAi lines as positive controls.



Supplemental Table 1: Markers of neoplasia.

The table summarizes the results of immunohistochemistry to assess epithelial polarity using antibody to E-Cadherin and Rhodamine-phalloidin to assess apical adherens junctions and actin localization. Antibody to MMP1 was used to assess activation of the JNK pathway during neoplasia, as described (Herranz et al 2012, 10.1016/j.cub.2012.02.050).

CG Number	Trans-ID	Positive in Yki/EGFR Screen	Gene Name	Gene Symbol	IHC status		
					MMP1	Polarity perturbation	
					increased	D-cad	Phalloidin
CG18505	105745	YKI	Acylphosphatase 2	Acyp2	Negative	Negative	Positive
CG32333	100303	YKI	CG32333		Negative	Positive	Positive
CG14071	104291	YKI	CG14071		Positive	Positive	Positive
CG12272	107954	YKI	Strumpellin	Strumpellin	Positive	Positive	Negative
CG42588	100428	YKI	CG42588		Positive	Positive	Negative
CG15824	101232	YKI	CG15824		Negative	Positive	Negative
CG12692	109683	YKI	CG12692		Negative	Positive	Positive
CG5151	102455	YKI	CG5151		Negative	Positive	Positive
CG5125	110702	YKI	neither inactivation nor afterpotential C	ninaC	Positive	Positive	Positive
CG33346	100352	YKI	CG33346		Positive	Positive	Positive
CG14966	100821	YKI	CG14966		Positive	Positive	Positive
CG6947/CG	100401	YKI	CG43896		Positive	Positive	Positive
CG32548	104969	YKI	CG32548		Positive	Negative	Positive
CG30421	103553	YKI	Ubiquitin specific protease 15/31	Usp15-31	Negative	Negative	Positive
CG9241	103568	YKI	Minichromosome maintenance 10	Mcm10	Positive	Positive	Positive
CG4109	107014	YKI	Syntaxin 8	Syx8	Negative	Positive	Positive
CG6751	107563	YKI	No Child Left Behind	NCLB	Positive	Positive	Positive
CG4008	110459	YKI	uninitiated	und	Positive	Positive	Positive
CG8948	110812	YKI	GTPase regulator associated with FAK	Graf	Positive	Positive	Positive
CG5874	106245	YKI	Negative elongation Factor A	Nelf-A	Positive	Positive	Positive
CG10521	100840	YKI	Netrin-B	NetB	Negative	Negative	Positive
CG32816	100838	YKI	CG32816		Negative	Negative	Positive
CG3352	108863	EGFR	fat	ft	Negative	Positive	Positive
CG31926	107437	EGFR	CG31926		Positive	Positive	Positive
CG42857	103539	EGFR	CG42857		Positive	Positive	Positive

Figure S3: Markers of neoplasia. Sample images.

Control and experimental wing imaginal discs of genotypes as shown on the images were stained for F-actin using Phalloidin, E-Cadherin using anti-Cadherin antibodies and MMP1 using anti-MMP1 antibodies. Note disrupted and irregular organization of F-actin and E-Cadherin an indication of loss of apico-basal polarity and cell adhesion properties in neoplastic wing discs. Increased MMP1 in those discs is an indication of epithelial-mesenchymal transition.