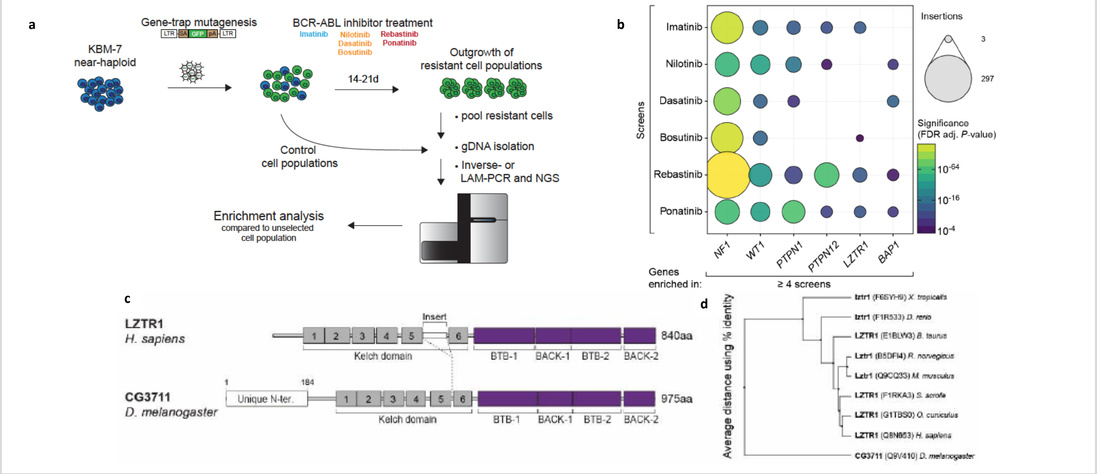
Lztr1 is a conserved regulator of Ras/MAPK activity

Giovanna M. Collu[1], Jeremy Baidoo[2], Marek Mlodzik[1]
1) Cell, Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY;
2) Center for Excellence in Youth Education, Icahn School of Medicine at Mount Sinai, New York, NY.

LZTR1 was recently identified in a screen of human chronic myeloid leukaemia cells aimed at discovering the genetic basis of drug resistance mechanisms [Bigenzahn, Collu *et al.*]. Specifically, LZTR1 was shown to regulate RAS ubiquitination and RAS/MAPK pathway activation in cell culture models. Here we demonstrate a conserved function for the fly orthologue Lztr1/CG3711 in regulating Ras activity *in vivo*. Knockdown of *CG3711* leads to Ras gain-of-function phenotypes in the wing, which can be rescued by loss of one copy of *Ras*. Further, through epistasis experiments we show that CG3711 acts in the Egrf/Ras/MAPK cascade to control wing vein patterning. We are currently investigating the role of *Lztr1* in the developing eye.

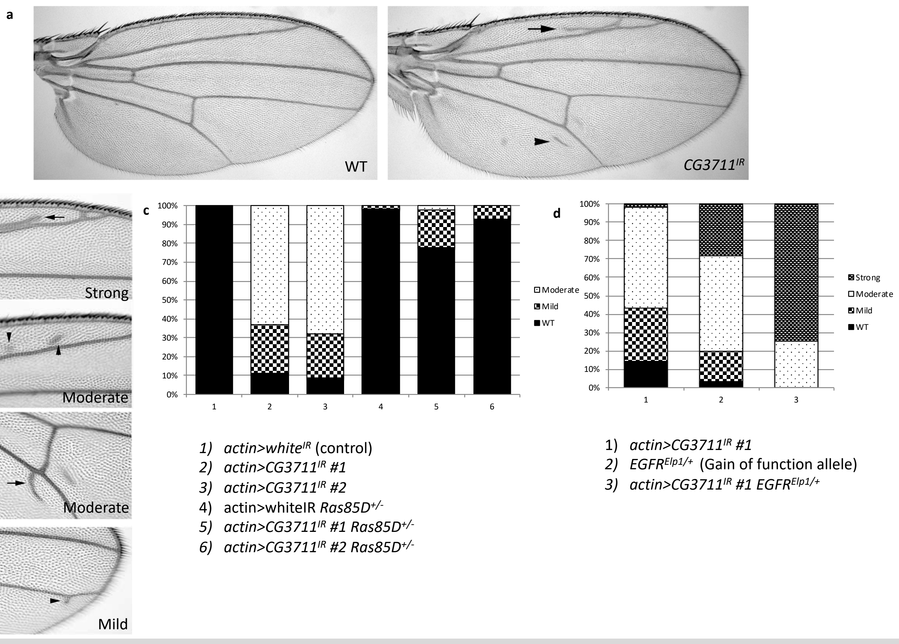
Background



a) Schematic of mutagenesis screen employed by our collaborators in the Superti-Furga laboratory (see Bigenzahn, Collu *et al.* for full details). b) 'TOP SIX' hits from screen – only LZTR1 had no known function in pathways associated with drug-resistance. c) *CG3711* is the *D. melanogaster* homologue of *LZTR1*. d) Conservation of LZTR1 family across species

Results

Knockdown of *CG3711* causes Ras gain-of-function phenotypes in the wing

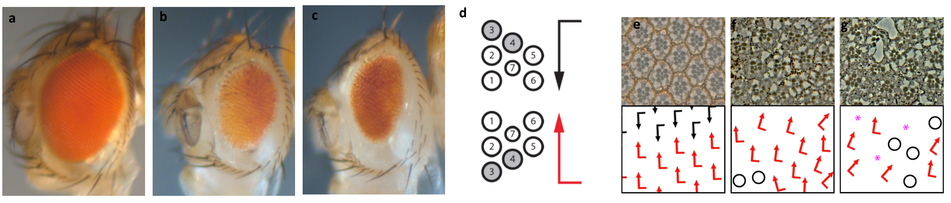


1) *actin>white^{IR}* (control)
2) *actin>CG3711^{IR} #1*
3) *actin>CG3711^{IR} #2*
4) actin>whiteIR *Ras85D$^{+/-}$*
5) *actin>CG3711^{IR} #1 Ras85D$^{+/-}$*
6) *actin>CG3711^{IR} #2 Ras85D$^{+/-}$*

1) *actin>CG3711^{IR} #1*
2) *EGFR$^{Elp1/+}$* (Gain of function allele)
3) *actin>CG3711^{IR} #1 EGFR$^{Elp1/+}$*

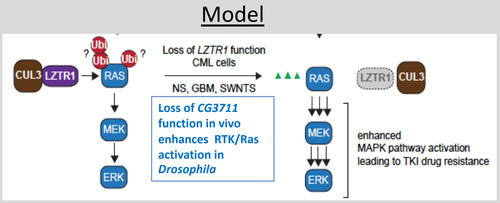
a) *actin-GAL4* driven knockdown of *CG3711* causes ectopic wing vein formation – a hallmark of enhanced Ras/MAPK signalling. b) Examples of ectopic wing vein formation can be grouped into classes of phenotypic severity, as indicated. c) Loss of one copy of *Ras85D* can suppress the *CG3711^{IR}* phenotype from two independent RNAi lines. d) Knockdown of *CG3711* enhances the *EGFR^{Elp1}* gain of function phenotype.

Knockdown of *CG3711* enhances EGFR- and Sevenless gain-of-function phenotypes in the eye



a-c) *actin-GAL4* driven knockdown of *CG3711* enhances *EGFR* gain of function phenotype in the eye. a) WT eye. b) EGFR$^{Elp1/+}$ causes a small-eye phenotype (here with control *white^{IR}*) c) *CG3711^{IR}* knockdown enhances the small-eye phenotype. d) Schematic of WT adult eye photoreceptor arrangement. e) Tangential eye section of adult wild type eye. f) *EGFR$^{Elp1/+}$* does not substantially alter photoreceptor patterning (circles indicate clusters where one or more photoreceptor has failed to be specified). g) Knockdown of *CG3711* in addition to EGFR gain of function, causes conversion of outer photoreceptors 1/6 into an R7 inner photoreceptor fate (marked by magenta asterisk). Conversion to R7 fate is a hallmark of Sevenless/Ras gain of function signalling.

Model



References & Acknowledgements

Background results were published: **Bigenzahn JW, Collu GM, Kartnig F, et al. LZTR1 is a regulator of RAS ubiquitination and signaling. *Science*. 2018;362(6419):1171–1177. doi:10.1126/science.aap8210**

Ongoing Drosophila *in vivo* experiments are being carried out in the Mlodzik lab, with support from the CEYE Sherman Scholars Program.

We thank the Bloomington Stock Center and the VDRC and for generating and sharing Drosophila strains and reagents. We are grateful to the entire Mlodzik lab for helpful discussions. This work was supported by grants from the NEI and NIGMS of the National Institutes of Health to MM.