

Supplemental Figure 1

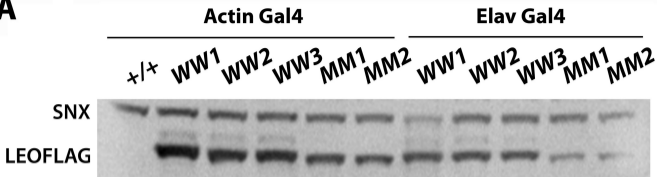
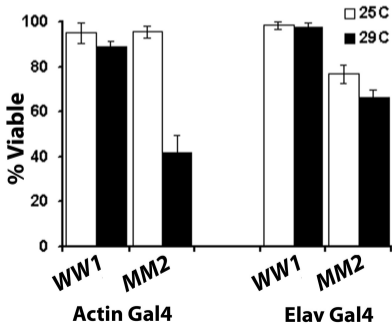
leoIII : MSTVDKEELVQKAKLAEQSERYDDMAQAMKSVTETGVVELSNEERNLLSVAYKNVVGARRSSWRV ISS IEQKTEA--SARKQQLAREYR : 86
 epsilon : --MTERENNVYKAKLAEQAERYDEMVEAMKKVASMDVELTVEERNLLSVAYKNVIGARRASWRIITS IEQKEENKGAEEKLEMITYR : 86
 14-3-3zeta : ---MDKNELVQKAKLAEQAERYDDMAACMKS VTEQGAELSNEERNLLSVAYKNVVGARRSSWRVSS IEQKTEG--AEKKQQMAREYR : 83
 14-3-3epsi : --MDDREDLVYGAKLAEQAERYDEMVESMKKVAGMDVELTVEERNLLSVAYKNVIGARRASWRIISS IEQKEENKGGEDKMKMIREYR : 86

leoIII : ERVEKELREICYEVLGLLDKYLIPKASNPE SKV FYLKMKG DYRYRLAEVATGDARNTVVEDSKKAYQEAFDIAKTKMQPHTPIRLGLA : 174
 epsilon : GQVEKELRDICSDILNVLEKHLIPCATSGESKVFY YKMKGDYHRYLAEFATGSDRDKDAENSLIAYKAASDIAMNDLPPTHTPIRLGLA : 174
 14-3-3zeta : EKIE TELRDICNDVLSLLEKFLIPNASQAESKVFY LKMKG DYRYRLAEVAAGDDKKGIVDQSQQAYQEAFEISKKEMQPHTPIRLGLA : 171
 14-3-3epsi : QMVE TELKLCIDILDVLDKHLIPAANTGESKVFY YKMKGDYHRYLAEFATGNDRKEAAENSLVAYKAASDIAMTELPPHTPIRLGLA : 174

leoIII : LNFSV FY YEIINS PARACHLAKQAFDDAIAELDTLNE DSYKDSTLIMQLLRDNLTLWTS D TQGD-----EAEPQEGGDN----- : 248
 epsilon : LNFSV FY YEILNSPDRACRLAKAAFD DAIAELDTLSEESYKDSTLIMQLLRDNLTLWTS DMQAE EVDPNAGDGEPKEQIQDVEDQDVS : 262
 14-3-3zeta : LNFSV FY YEILNSPEKACSLAKTAFDEAIAELDTLSEESYKDSTLIMQLLRDNLTLWTS D TQGD-----EAEAGEGGEN----- : 245
 14-3-3epsi : LNFSV FY YEILNSPDRACRLAKAAFD DAIAELDTLSEESYKDSTLIMQLLRDNLTLWTS DMQGD-----GEEQNKEALQDVEDENQ- : 255

Supplemental Fig 1.

Sequence alignment of the *Drosophila* 14-3-3 ζ used in this study (LEOIII), D14-3-3 ϵ (epsilon), and their human homologs 14-3-3zeta and 14-3-3epsi respectively. Open arrowheads denote amino acids on D14-3-3 ϵ which can form salt bridges with amino acids in LEO denoted with the black arrow heads. The gray arrow denotes the Serine that is putatively phosphorylated by SDK1.

A**B**

Supplemental Fig 2.

(A) The levels of LEOFLAG^{MM} are suppressed relative to controls irrespective of the tissue where they accumulate. Multiple independent lines of wild type (WW) and doubly mutant LEO (MM) transgenes are represented and driven by the panneuronal driver *Elav-Gal4* and the ubiquitous *Actin-Gal4*. The levels of the transgenic proteins (LEOFLAG) are shown relative to the endogenous syntaxin (SNX), which was used as the loading control. (B) Viability of animals overexpressing the wild type and double mutant transgenes panneuronally under *Elav-Gal4* or ubiquitously under *Actin-Gal4* raised at the two different temperatures as indicated. To calculate viability as represented by the bars in the graph the ratio of non-CyO flies expressing the transgenes to non-expressing CyO flies was determined in a cross between homozygotes for the transgene and *Actin-Gal4/CyO*. Because the *Elav-Gal4* is on the X chromosome, we used males carrying the driver and crossed them to female homozygotes for the transgene, such that the transgenic protein would be present in the female progeny, but not in the males. The ratio of females to males plotted in the graph represents the lethality due to transgene expression