**Supplemental file 1: *Drosophila* recombinants and crosses**

The *Drosophila* stocks are labelled numerically in table 1. The stock numbers in rows 20-29 were made with standard genetic crosses. The F2 embryos for live imaging or fixed staining were obtained from crosses shown in Table 2 at the given temperature.

**Table 1: *Drosophila* stocks and recombinants**

|  |  |  |
| --- | --- | --- |
| **Stock** | **Genotype** | **Source/Reference** |
| 1 | Canton-S | Lab stock originally obtained Bloomington *Drosophila* Stock Center, BDSC #1 |
| 2 | *nanos*-Gal4 | Lab stock ([Mavrakis et al., 2009, 2008)](https://paperpile.com/c/YxeQem/W0z6Y+fAXN) |
| 3 | w; *mat67*-Gal4; *mat15*-Gal4 | Girish Ratnaparkhi, IISER, Pune, India |
| 4 | w; P{Sqh-mCherry.M}3 | Bloomington *Drosophila* Stock Center (BDSC), Indiana, USA |
| 5 | y[1] v[1]; P{TRiP.HMC03427}attP40 (GRAF shRNA1) | BDSC, #51853 |
| 6 | y[1] v[1]; P{TRiP.GL01207}attP40 (myosin binding subunit, MBS shRNA) | BDSC, #41625 |
| 7 | y[1] sc[\*] v[1] sev[21]; P{TRiP.HMS01118}attP2 (RhoGEF2 shRNA) | BDSC, #34643 |
| 8 | y[1] w[\*]; P{UASpT7.RhoGEF2}5 (RhoGEF2 overexpression) | BDSC, #9386 |
| 9 | *ubi-*GFP::AnillinRBD/TM3 | Munjal et al., 2015, Thomas Lecuit, France |
| 10 | w-FM7a/w-FM7a(white eye) | Girish Ratnaparkhi, IISER, Pune, India |
| 11 | UASp-rok-shRNA (*rok*i) | Zhang et al., 2018 |
| 12 | y[1] w[67]c[23] P{EPgy2}Graf[EY09461] (GRAF overexpression) | BDSC, #17571 |
| 13 | w;*mat67* Spider-GFP-Sqh-mcherry/TM3ser | Martin et al., 2009 |
| 14 | y[1] sc[\*] v[1] Graf[CR57]/FM7a (*Graf*CR57) | Crispr mutant of *Graf* generated in this study |
| 15 | y[1] sc[\*] v[1] sev[21]; P{TKO.GS00762}attP40 | BDSC, #76993 |
| 16 | P{KK102763}VIE-260B (GRAF shRNA2, *Graf*2i) | Vienna *Drosophila* Stock Center, #v110812 |
| 17 | y[1] sc[\*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=nos-Cas9.R}attP40 | BDSC #78781 |
| 18 | [w]\*;p[UASp-GRAF-EGFPG1] attp40/cyo | Full length GRAF-GFP generated in this study |
| 19 | [w]\*;p[UASp-GRAFΔRhoGAP-GFP] attp40/cyo | GRAF lacking the RhoGAP domain generated in this study |
| 20 | y[1] w[67]c[23] P{EPgy2}Graf[EY09461]/FM7a; P{UASpT7.RhoGEF2}5/Tb (GRAF-OE;RhoGEF2-OE) | Recombinant containing overexpression of GRAF and RhoGEF2 generated for this study |
| 21 | y[1] sc[\*] v[1] sev[21]; P{TRiP.HMC03427}attP40/cyo;P{TRiP.HMS01118}attP2/Tb (*Graf*i;*RhoGEF2*i) | Recombinant containing GRAF RNAi1 and RhoGEF2 RNAi generated in this study |
| 22 | mat67-Sqh-mCherry; *ubi-*AnillinRBD-GFP-NG4/Tb | Recombinant containing mat67-Gal4, Sqh-mCherry and *ubi*-AnillinRBD-GFP generated for this study |
| 23 | y[1] sc[\*] v[1] GRAF[CR57]/FM7a; P{TRiP.HMS01118}attP2/Tb (*Graf*CR57;*RhoGEF2*i) | Recombinant containing the Crispr mutant of *Graf* and RhoGEF2 RNAi generated for this study |
| 24 | y[1] sc[\*] v[1] GRAF[CR57]/FM7a; UASp-rok-shRNA/Tb(*Graf*CR57;roki) | Recombinant between the Crispr mutant of *Graf* and Rok RNAi generated for this study |
| 25 | y[1] w[67]c[23] P{EPgy2}Graf[EY09461]/FM7a; P{TRiP.GL01207}attP40/cyo (GRAF-OE;*mbs*i) | Recombinant between overexpression of GRAF and RNAi for *mbs* generated for this study |
| 26 | y[1] sc[\*] v[1] GRAF[CR57]/FM7a;mat67, Sqh-mCherry/CyO | Recombinant between the Crispr mutant of *Graf*, mat67-Gal4 and Sqh-mCherry generated for this study |
| 27 | y[1] sc[\*] v[1] GRAF[CR57]/FM7a; p[pUASp GRAF EGFP G1] attp40/cyo | Recombinant between Crispr mutant of *Graf* and GRAF-GFP generated for this study |
| 28 | y[1] sc[\*] v[1] GRAF[CR57]/FM7a; p[pUASp GRAF RhoGAP del EGFP] attp40/cyo | Recombinant between Crispr mutant of *Graf* and GRAF lacking the RhoGAP domain with GFP tag generated for this study |
| 29 | y[1] sc[\*] v[1] GRAF[CR57]/FM7a; *ubi*-AnillinRBD-GFP/cyo | Recombinant between the Crispr mutant of *Graf* and *ubi*-AnillinRBD-GFP generated for this study |

**Table 2: *Drosophila* crosses**

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| --- | --- | --- |
| **F2 embryos were imaged from these crosses, using above stock numbers/genotypes. Non-balancer females were used for cages.**  Stocks # (Virgins x males) | **Figure** | **Temperature (0C)** |
| 2 x 1 control | 1C,D and 1-figure supplement 2A | 28 |
| 2 x 5 *Graf*i using *nanos*-Gal4 | 1C,D and 8B | 28 |
| 3 x 16 *Graf*2i using *mat*-Gal4 | 1-figure supplement 2A | 28 |
| 17X15 *Gra*fgRNA using *nanos*-Cas9 | 1-figure supplement 2A | 28 |
| 14x14 *Graf*CR57 | 1C,D; 2A; 6A | 25 |
| 26X27 *Graf*CR57;GRAF-GFP/*mat*67-Gal4, Sqh-mCherry | 2D; 4F; 4-figure supplement 1A | 18 |
| 22X1 mat67 Sqh-mCherry/+; *ubi*-AnillinRBD-GFP NG4/+ | 4A | 25 |
| 26X29 *Graf*CR57;*ubi*-AnillinRBD-GFP /*mat*67-Gal4, Sqh-mCherry | 4C | 25 |
| 4X4 Sqh-mCherry | 3A,E | 25 |
| 13x1 *mat67-*Gal4 Spider-GFP-Sqh-mcherry/+ | 4F:5D; 6C; 7A; 8A; 9A | 25 |
| 26X26 *Graf*CR57;*mat67*-Gal4, Sqh-mCherry | 3A,E; 4F; 6C; 8A; 9A; 6-figure supplement 1A,B | 25 |
| 26x28 *Graf*CR57;GRAFΔRhoGAP-GFP/*mat67*-Gal4, Sqh- mCherry | 4F; 4-figure supplement 1A,B | 18 |
| 3x12 GRAF-OE using *mat*-Gal4 | 5B; 7B | 28 |
| 13X12 GRAF-OE; *mat67* Spider-GFP-Sqh-mCherry | 5D; 6C; 7A; 6-figure supplement 1A,B | 25 |
| 3X8 RhoGEF2-OE using *mat*-Gal4 | 7,B; 7-figure supplement 1A | 25 |
| 13X8 RhoGEF2-OE;*mat67* Spider-GFP-Sqh-mCherry | 7A; 7-figure supplement 1B | 25 |
| 2X7 *RhoGEF2*i using *nanos*-Gal4 | 8B | 28 |
| 3X7 *RhoGEF2*i using *mat*-Gal4 | 8-figure supplement 1 | 25 |
| 13X7 *RhoGEF2*i ;*mat67*-Gal4 Spider-GFP-Sqh-mCherry | 8A; 7-figure supplement 1B | 25 |
| 13X20 GRAF-OE;RhoGEF2-OE/*mat67*-Gal4 Spider-GFP, Sqh-mCherry | 7A | 25 |
| 3X20 GRAF-OE;RhoGEF2-OE using *mat*-Gal4 | 7B | 28 |
| 2X21 *Graf*i;*RhoGEF2*i using *nanos*-Gal4 | 8B | 28 |
| 3X6 *mbs*i using *mat*-Gal4 | 9-figure supplement 1 | 25 |
| 3X25 GRAF-OE;*mbs*i using *mat*-Gal4 | 9-figure supplement 1 | 25 |
| 13x11 *rok*i/*mat67*-Gal4 Spider-GFP-Sqh-mCherry | 9A | 25 |
| 13X24 *Graf*CR57;*rok*i/*mat67-*Gal4 Spider-GFP-Sqh-mCherry | 9A | 25 |