**Supplementary Table 1**

**Strains**

|  |  |  |
| --- | --- | --- |
| **Strain** | **Genotype** | **Used in Figures** |
| LP399 | *rap-1(cp151*[mNeonGreen^3xFlag::*rap-1*]*)* IV | Fig. 2,S4 |
| FZ222 | *rap-1(tm861) IV* |  |
| DV3152 | *rap-1(tm861)* IV (Outcrossed 12x) | Fig. 3,4,S5 |
| FZ181 | *rap-1(pk2082)* IV | Fig. 3 |
| GS5231 | *arIs116[Plst-5*::*2xNLS*::*tag-RFP*, P*ttx-3::GFP*, pha-1(+)] X | Fig. 3 |
| DV3595 | *rap-1(tm861)* IV; *arIs116[Plst-5*::*2xNLS*::*tag-RFP*, P*ttx-3::GFP*, pha-1(+)] X | Fig. 3 |
| PS1524 | *unc-4(e120) let-23(sa62*gf*)/mnC1 dpy-10(e128) unc-52(e444)* II | Fig. 4 |
| DV2901 | *unc-4(e120) let-23(sa62*gf*)* II; *rap-1(tm861)* IV | Fig. 4 |
| DV2968 | *unc-4(e120) let-23(sa62*gf*)* II; *mfIs70*[P*lin-31::rde-1(+),* P*myo-2::GFP*] IV; *rde-1(ne219)* V | Fig. 4,8,S8 |
| DV3313 | *rap-1(re180*gf*)* IV (Outcrossed 3x) | Fig. 5 |
| MT8675 | *ksr-1(n2509)* X | Fig. 4,S5 |
| DV3593 | *rap-1(tm861)* IV; *ksr-1(n2509)* X | Fig. 4,S5 |
| MT2124 | *let-60(n1046*gf*)* IV | Fig. S5 |
| DV3512 | *let-60(n1046*gf*) rap-1(tm861)* IV | Fig. S5 |
| MH37 | *mpk-1(ku1) unc-32(e189) III* | Fig. S5 |
| DV3592 | *mpk-1(ku1) unc-32(e189) III*; *rap-1(tm861)* IV | Fig. S5 |
| MH2209 | *kuIs57*[P*col-10*::*lin-45*(gf), *sur-5*::GFP] | Fig. S5 |
| DV3591 | *kuIs57*[P*col-10*::*lin-45*(gf), *sur-5*::GFP]; *rap-1(tm861)* IV | Fig. S5 |
| GS4892 | *arIs131*[P*lag-2::YFP*, P*ceh-22::GFP*, *pha-1(+)*] III | Fig. 6 |
| DV3392 | *arIs131*[P*lag-2::YFP*, P*ceh-22::GFP*, *pha-1(+)*] III; *rap-1(re180*gf*)* IV | Fig. 6 |
| CM117 | *unc-119(e2498)* III; *saIs14*[P*lin-48::GFP*, *unc-119(+)*] | Fig. 7 |
| DV2353 | *unc-119(e2498)* III; *saIs14*[P*lin-48::GFP*, *unc-119(+)*];*let-60(n1046*gf*)* IV | Fig. 7 |
| DV3393 | *unc-119(e2498)* III; *saIs14*[P*lin-48::GFP*, *unc-119(+)*]; *rap-1(re180*gf*)* IV | Fig. 7 |
| FZ117 | *bjIs40*[P*pxf-1::GFP*] | Fig. 8 |
| DV2261 | *arIs92*[P*egl-17::NLS-CFP-LacZ*, P*ttx-3*::GFP, *unc-4(+)*] *mrt-(re58)* V | Fig. S7 |
| DV3391 | *rap-1(re180*gf*)* IV; *arIs92*[P*egl-17::NLS-CFP-LacZ*, P*ttx-3::GFP*, *unc-4(+)*] *mrt-(re58)* V | Fig. S7 |
| GS5090 | *arIs222[Plag-2*::*2xNLS*::*t*ag*-RFP*, P*ttx-3::GFP*, pha-1(+)*] V* | Fig. S6 |
| DV3594 | *rap-1(tm861)* IV; *arIs222[*P*lag-2*::*2xNLS*::*tag-RFP*, P*ttx-3::GFP*, pha-1(+)*] V* | Fig. S6 |
| CB2065 | *dpy-11(e224) unc-76(e911)* V |  |
| JK2958 | *nT1[qIs51]* (IV;V)/*dpy-11(e224) unc-42(e270)* V |  |

**Supplementary Table 2**

**RNAi**

|  |  |  |
| --- | --- | --- |
| **RNAi target** | **Source BioScience Library Position** | **Used in Figures** |
| *luciferase* | pREW2 | Fig. 3, 4, 7, S7 |
| *rap-1 (sjj\_C27B7.8)* | IV-4F17 | Fig. 3 |
| *lip-1 (sjj\_C05B10.1)* | IV-3I16 | Fig. 3, 4 |
| *gap-1 (sjj\_T24C12.2)* | X-1D10 | Fig. 3 |
| *pxf-1 (sjj\_T14G10.2)* | IV-5I14 | Fig. 7 |
| *“Rap1GEF”(sjj2\_Y34B4A.4)* | X-8D15 | Fig. S7 |
| *pop-1 (sjj\_W10C8.2)* | I-1K04 | Used as RNAi control |

**Supplementary Table 3**

**Primers**

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Sequence**  | **Ta** | **Used for** |
| oNR001 | TTCGTGCAGGGAATTTTCGT | 50°C | *rap-1(tm861) genotyping* |
| oNR004 | GAGCAATTCACAGCGATGC | 50°C | *rap-1(tm861) genotyping* |
| oNR003 | TGTTAGCCTCCTTTTCATTGAGA | 50°C | *rap-1(tm861) genotyping* |
| oNR122 | TGTGTCATCTGGTCTGTACTTGG | 50°C | *rap-1(re180*gf*) genotyping* |
| oNR123 | TCCCCTGCACGAATTGTACC | 50°C | *rap-1(re180*gf*) genotyping* |
| oNR114 | ATTGTTGTGCTCGGATCTGGGTTTAAGAGCTATGCTGGAAACAG | 53°C | *rap-1*(G12V)sgRNA mutagenesis - FW |
| oNR024 | CAAGACATCTCGCAATAGG | 53°C | sgRNA mutagenesis - RV |

**Supplementary Table 4**

**Plasmids**

|  |  |  |
| --- | --- | --- |
| **Name** | **Description**  | **Used for** |
| pNR21 | pJW1236 *rap-1(G12V)* sgRNA + Cas9 | *rap-1(G12V)* CRISPR knock-in |
| pJA58 | *dpy-10(cn64)* sgRNA | *dpy-10(cn64)* co-CRISPR knock-in |
| pPD118.33 | P*myo-2::GFP* | Co-injection marker |

**Supplementary Table 5**

**sgRNA sequences and PAMs**

|  |  |
| --- | --- |
| **sgRNA sequence and PAM** | **Used for**  |
| ATTGTTGTGCTCGGATCTGG**AGG** | *rap-1* |
| GCTACCATAGGCACCACGAG**CGG** | *dpy-10* |

**Supplementary Table 6**

**Repair ssODN**

|  |  |
| --- | --- |
| **ssODN sequence** | **Description** |
| 5’-GAATGCGGGAGTATAAGATTGTTGTGCTCGGATCCGTAGGAGTAGGAAAATCCGCACTGGTATAG- 3’ | Repair ssODN for *rap-1*(G12V) (65 bp) |
| 5’–CACTTGAACTTCAATACGGCAAGATGAGAATGACTGGAAACCGTACCGCATGCGGTGCCTATGGTAGCGGAGCTTCACATGGCTTCAGACCAACAGCCTAT- 3’ | Repair ssODN for *dpy-10(cn64)* (101 bp) |