Current Biology, Volume 30

## **Supplemental Information**

## **Two MicroRNAs Are Sufficient**

## for Embryonic Patterning in *C. elegans*

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**Figure S1. miRNAs are essential for morphogenesis and organogenesis. Related to Figure 1. A**) Western Blot against endogenous, AID-Myc-tagged PASH-1 and DRSH-1 in gravid adults, 24h post Auxin treatment. **B**) mRNA levels measured by RT-qPCR in 2-cell embryos, 24h post pash-1 RNAi treatment compared to wild type under standard conditions. **C**) Quantification of the arrest phenotype of various strains bearing AID-tagged PASH-1 and/or DRSH-1 and expressing the TIR1 ubiquitin ligase, or TIR1 only as control, upon Auxin treatment. **D**, **E**) Images and quantification of arrest phenotype of RNAiD, *pash-1(ts)*, miR-35<sup>tam</sup>, and miR-51<sup>tam</sup> mutant embryos at 20°C and 25°C. **F**) Hatching assay of embryos bearing the pash-1(ts) temperature-sensitive allele mj100. Penetrant embryonic lethality at the restrictive temperature 25°C is rescued by expression of wild-type pash-1 from an extra-chromosomal array. **G**) miRNA-profile of pash-1(ts) embryos at the 2-cell stage and at the end of gastrulation as determined by small RNA sequencing using spike-in oligos for quantitative measure of absolute miRNA levels across samples, plotted as in Figure 1B. Note that the samples analyzed here are the same as in Figures S2G, 4A, S4A.



Figure S2. Mirtron design and expression. Related to Figure 2. A) Consensus sequence at the 3' end of all 12 annotated C. elegans mirtrons (S1). B) Sequence of miR-35 and miR-51 family miRNAs and the corresponding designed mirtrons. C) Pre-miRNA hairpin structure for endogenous miRNA and mirtron counterpart as predicted by the UnaFold TwoState folding algorithm for RNA at 20°C (S2); the basis for the design of mirtron-51 was the family member miR-54 as it required relatively few changes to be turned into a mirtron. Residues that have been changed in the mirtron hairpins are highlighted in red. D) Schematic design of mirtron-expressing transgenes. The endogenous promoter sequence upstream of pre-mir-35 (2.0 kb) or pre-mir-52 (2.5 kb) drives expression of a GFP transcript bearing the mirtron in its middle intron, followed by an inert tbb-2 (tubulin) 3' UTR. E) Hatching rates of embryos bearing deletions for the miR-35 family (nDf49, nDf50), or the miR-51 family (nDf58, nDf67, n4100), respectively. Shown are multiple independent transgenic lines expressing the respective mirtron from an extra-chromosomal array or the integrated transgenes that were used for subsequent experiments. F) Mirtron-read abundance per nucleotide derived from sRNA-seg data from mirtron-expressing RNAiD embryos at gastrulation (mirtron read-profiles were highly similar for other time points). Mirtron-35 had an untemplated single-nucleotide addition in 25-45% of the reads, which was a U in >90% of cases, consistent with observations in Drosophila (S3, S4). Mirtron-51 was trimmed by one nucleotide in 90-95% of cases. G) Comparison of the sum of the abundances of all endogenous miR-NA family members in wild-type embryos (dark shades) and the respective mirtron abundance in Microprocessor depleted animals (light shades) throughout embryogenesis, derived from quantitative sRNA-seq analysis. Mirtrons are not detected at the 2-cell stage, consistent with commonly observed silencing of multicopy transgenes in the C. elegans germline. Thus, germline-related phenotypes such as the loss in fertility are not rescued by the mirtron transgenes.



Figure S3. Two miRNAs are sufficient for embryonic patterning in the absence of Drosha and Pasha. Related to Figure 3. A) Stage of arrest in the ~50% of RNAiD embryos with mirtron-35 and -51 that fail to hatch, compared to embryos without mirtrons. For comparison data of RNAiD embryos is replotted from Figure S1E. B) Proportion of hatching of Microprocessor-deficient embryos expressing either mirtron-35 or mirtron-51 individually. C) Stage of arrest of RNAiD embryos expressing either mirtron individually, or mirtron-51 and the seed-mutant version of mirtron-35. For the seed-mutant mirt-35, three independent lines behaved identically of which one is shown. D) Representative images of animals scored in C. E-G) Body measurements of mirtron-rescued RNAiD or *pash-1(ts)* animals compared to wild type under standard conditions. Data is derived from micrographs using ImageJ. Pharynx length was measured using the expression area of a myo-2prom::mCherry reporter. Number of intestinal cells was scored by expression of a nuclear *elt-2prom::dsRed* reporter. Mean and range are plotted for each genotype; statistical significance was determined by an unpaired t-test, significance levels are: P>0.05 = n.s.; P<0.05 = \*; P<0.01 = \*\*; P<0.001 = \*\*\*. H) *pash-1(ts)* backshift experiment: miR-NA-depleted embryos that developed at the restrictive temperature of 25°C and expressed mirt-35/51 individually or in combination, were transferred to the permissive temperature 16°C directly after hatching. Survival to adulthood was scored within the next 7 days.



**Figure S4. miRNA levels in microprocessor-depleted embryos. Related to Figure 4 and Table S2. A)** For each timepoint profiled, shown are (top) the sum of the absolute abundances of canonical miRNAs grouped by families as measured by quantitative sRNA-seq (related to Figure 4A), and (bottom) the relative abundance of each miRNA family under RNAiD or pash-1(ts)-mediated depletion, in comparison to wild type. The horizontal dotted line indicates 10% of wild type. Depletion at the level of families is very strong except as noted also for individual miRNAs at the 2-cell stage in *pash-1(ts)* and at the L1 stage in RNAiD. There is no consistent leftover of other families over both depletion conditions that suggests that other miRNAs could contribute to early development. Note that the outliers in relative abundance at gastrulation and 3-fold stages correspond to families with very low absolute abundance. **B**) miRNA abundance at gastrulation in *pash-1(ts)* embryos with or without mirtrons. Mirtron-expression has no systematic effect on levels of left-over miRNAs. **C**) Embryonic expression time course of transcripts bearing conserved miR-35/51 family miRNA binding sites as predicted by TargetScan Worm 6.2 (S5, S6). Shown are median expression and 95% confidence intervals. For miR-35 family all 89 predicted targets are shown. For miR-51 family targets the top 71 expressing transcripts (out of 264 called targets in the dataset) are shown, selected based on an average FPKM value > 1 across all timepoints. The most abundant predicted miR-51<sup>fam</sup> target *rps-20* (ribosomal protein, small subunit) has been removed as an outlier due to extreme abundance. Data derived from (S7).

		wild type control strain (Brenner, 1974)	1B-D, S1B, S1F, 2B, S2E, S2G, 4A, S4A
mik-35 family relate	d		
MT14533	nDf49, nDf50/mIn1 [mIs14 dpy-10(e128)] II	balanced mir-35 family deletion (Alvarez-Saavedra and Horvitz, 2010)	1C, S1D, S1E, 2B, S2E
MIC812	nDf49, nDf50, 11;	mir-35 family deletion,	52F
WICCOTZ	lucEx521 (mir35p::GFP_mirtron35::tbb2, myo2::mCherry)	generated by microin stor So, micro generated by microin into MT14533 micro 5 formits delation	522
MLC825	nDf49, nDf50, l1; lucEx522 (mir35p::GFP_mirtron35::tbb2, myo2::mCherry)	rescued by mirtron-35, line 2	S2E
MIC826	nDf49, nDf50, II;	mir-35 family deletion, rescued by mirron-35 line 3	\$2F
integeo	lucEx523 (mir35p::GFP_mirtron35::tbb2, myo2::mCherry)	generated by microining of the Singer	
MLC1016	nDf49, nDf50, l1; lucls20 (mir35p::GFP_mirtron35::tbb2, myo2::mCherry)	rescued by mitron-35, line 1	2B, S2E
MIC1017	nDf49, nDf50, II;	mir-35 family deletion, rescued by mitron-35 line 2	52F
	lucls21 (mir35p::GFP_mirtron35::tbb2, myo2::mCherry)	integrated via irradiation of MLC826	
MLC2353	nDf49, nDf50 / mIn1, II lucEx1240 (mir35p::GFP_mirtron35(seed-mutant)::tbb2, myo2::mCherry)	expressing a seed-mutant version of mirt-35 that does not rescue	S2E
	nDf49, nDf50 / min1, 1i	balanced mir-35 family deletion,	
MLC2354	lucEx1241 (mir35p::GFP_mirtron35(seed-mutant)::tbb2, myo2::mCherry)	expressing a seed-mutant version of mirt-35 that does not rescue generated by microinjection into MT14533	SZE
MLC2355	nDf49, nDf50 / mIn1, II luc5v1242 (mir25p::GEP, mittron35/seed.mutant)::thb2 mvo2::mCherny)	balanced mir-35 family deletion, expressing a seed-mutant version of mirt-35 that does not rescue	S2E
miR-51 family relate	d	generated by microinjection into MT14533	
Thirt-S1 family relate		mir-51 family deletion	
MLC1800	n4114, nDf67, IV; nDf58, X;	rescued by a partially penetrant extrachromosomal array expressing mir-54-56	1C, S1D, S1E, 2B, S2E
		generated by 50 Gravity delation	
MLC836	n4114, nDf67, IV/nT1 [qIS51] (IV,V); nDf58, X;	expressing mirtron-51, line 1,	S2E
	lucEx525 (mir52p::GFP_mirtron54.1::tbb2, myo2::mCherry)	homozygous mutant line can be isolated, generated by microinjection into MT17143	
	n4114 nDf67 IV/nT1 [nIS51] (IV V): nDf58 X:	balanced mir-51 family deletion, expressing mirtron-51 line 2	
MLC839	lucEx528 (mir52p::GFP_mirtron54.1::tbb2, myo2::mCherry)	homozygous mutant line can be isolated,	S2E
		generated by microinjection into MT17143 balanced mir-51 family deletion,	
MLC903	n4114, nDf67, IV/nT1 [qIS51] (IV,V); nDf58, X;	expressing mirtron-51, line 3 (stop codon in GFP exon 1)	2B, S2E
	luusz4 (miszp. Grrstop_mintolis4.1.,tobz, myözmicheny)	generated by microinjection into MT17143, spontaneous integrant	
pash-1(ts)		pash-1(ts), strain SX1359 (Lehrbach et al., 2012).	
MLC860	mj100, l	outcrossed four times, lacking mjEx331	S1D-G, S2G, 3A, S3B, 4A, 4B, S4A, S4B
MLC881	mj100, i; mjEx331 (eft-3p::pash-1::GFP::unc-54, myo-2p::mCherry::unc-54 3'UTR)	pash-1(ts), strains SX1359 (Lenroach et al., 2012), outcrossed four times	\$1F
MLC1104	mj100, I; lucls20 (m35p::GEP_mittron-35::tbb2_myo2::mCherry)	pash-1(ts) expressing mirtron-35.	S3B, S3H
MLC1105	mj100, I;	pash-1(ts),	S3B, S3H
	lucis24 (mr52p::GFPstop_mrtron54.1::tbb-2, ett2::dsRed, myo2::mCherry) mj100, l;	expressing mirtron-51	
MLC1795	lucls20 (m35p::GFP_mirtron-35::tbb2, myo2::mCherry), lucls24 (mir52p::GFPstop_mirtron54.1::tbb-2, elt2::dsRed, myo2::mCherry)	expressing mirtron-35 and mirtron-51	S1G, S2G, 3A, 3B, S3E-H, 4A, S4A, S4B
Auxin-inducible-degr			
MLC1040	iesis7 [eft-3p::11R1::mRuby::unc-343 01R + Cbr-unc-119(+)], ii; ieSi38 [sun-1p::TIR1::mRuby::sun-1 3'UTR + Cbr-unc-119(+)], IV	control strain expressing TIR-1 in soma & germline	1A, S1C, 3A
	ieSi57 [eft-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], II; ieSi38 [sun-1p::TIR1::mRuby::sun-1 3'UTR + Cbr-unc-119(+)], IV;		
MLC1077		control strain expressing TIK-1 in soma & germline	
	lucls20 (m35p::GFP_mirtron-35::tbb2, myo2::mCherry);	expressing mirtron-35 + mirtron-51	3B, S3E-G
	lucls20 (m35p::GFP_mirton-35::tbb2, wpo2::mCherry) lucls24 (mir52p::GFP_stop_mirton-54.1::tbb-2, el2::dsRed, myo2::mCherry) luc71 (pash1::PASH-1::2xGGSG::3xFLAG::AID::myc), I;	control strain expressing 114-1 in soma & germline expressing mirtron-35 + mirtron-51 endogenous nash-1 bearing C-terminal AID-tag = nash-1:AID	3B, \$3E-G
MLC1065	Luds20 (m35p::GFP_mitron-35::tbb2, myo2::mCherny): Luds24 (mir52p::GFPstop_mitron54.1::tbb-2, elt2::dsRed, myo2::mCherny) Luds24 (mir52p::GFPstop_mitron54.1::tbb-2, elt2::d	control strain expressing 114-1 in soma & germline expressing mirtron-35 + mirtron-51 endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID expressing TIR-1 in soma & germline	38, S3E-G S1A, S1C
MLC1065	Luds20 (m35p::GFP_mitron-35::bb2, myo2::mCherny) Luds24 (mis5p::GFP_mitron-35::bb2, myo2::mCherny) Luds24 (mis5p::GFP_stop_mitron54.1::bb2, el2::dsRed, myo2::mCherny) Lud51 (pash1::PASH-1::2xGGSG::3xFLAG::ALD::m(y), I; leSi57 [ef-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], II; leSi38 [sun-1p::TIR1::mRuby::sun-13'UTR + Cbr-unc-119(+)], II; Lud528 [um2::TIR1::mRuby::sun-13'UTR + Cbr-unc-119(+)], IV Lud528 [um2::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], IV Lud528 [um2::TIR1::mRuby:unc-54 3'UTR + Cbr-unc-119(+)], IV Lud528 [um2::TIR1::TI	control strain expressing 11F-1 in soma & germinne expressing mittron-35 + mittron-51 endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID expressing TIR-1 in soma & germline endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID	38, S3E-G S1A, S1C
MLC1065 MLC1245	Luds20 (m35p::GFP_mitron-35::bb2, myo2::mCherry) Luds24 (mis3p::GFP_mitron-35::bb2, myo2::mCherry) Luds24 (mis3p::GFP_mitron-34:1:tbb2,el2::dsRed, myo2::mCherry) Luc71 (pash1::PASH-1::2xGGSG::3xFLAG::ALG::MD;:myc), l; leSi57 [eft-3p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], ll; leSi57 [eft-3p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], ll; leSi57 [eft-3p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], ll; leSi57 [eft-3p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], ll; leSi38 [sun-1p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], ll; leSi38 [sun-1p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], ll;	control strain expressing ITF-1 in Soma & germinne expressing mirtron-35 + mirtron-51 endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID expressing TIR-1 in soma & germline endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID Expressing TIR-1 in soma & germline	38, S3E-G S1A, S1C S1A, S1C
MLC1065 MLC1245	Luds20 (m35p::GFP_mitron-35::bb2,myo2::mCherry): Luds24 (mi52p::GFP_mitron-35::bb2,myo2::mCherry): Luds24 (mi52p::GFP_stop_mitron54.1::bb2,el2::dsRed,myo2::mCherry) Luc71 (pash1::PASH-1::2xGGSG::3xFLAG::AL0::myc), I; leSi57 [eft-3p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], II; leSi57 [eft-3p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], II; leSi57 [eft-3p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], II; leSi58 [sun-1p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], II; leSi38 [sun-1p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], II; leSi38 [sun-1p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], IV; luc21 (pash1::PASH-1::2xGGSG::3xFLAG::AL0::mVc), Luc22 (my::Al0::3XFLAG::AKGSG::3xFLAG::AL0::mVc), I; leSi29 [sun-1p::TIR1::mRuby::un-13'UTR + Cbr-unc-119(+)], IV; luc21 (pash1::PASH-1::2xGGSG::3xFLAG::AL0::mVc), Luc22 (my::Al0::3XFLAG::AKGSG::3xFLAG::AL0::mVc), I; luc22 (my::Al0::3XFLAG::AKGSGS::dxFLAG::AL0::mVc), I; luc22 (my::Al0::3XFLAG::AKGSGS::dxFLAG::AL0::mVc), I; luc22 (my::Al0::3XFLAG::AKGSGS::dxFLAG::AL0::mVc), I; luc22 (my::Al0::3XFLAG::AKGSGS::dxFLAG::AL0::mVc), I; luc22 (my::Al0::3XFLAG::AL0::M2GSG::dxFLAG::AL0::mVc), I; luc22 (my::Al0::Al0::Al0::Al0::M2GSG::dxFLAG::AL0::mVc), I; luc22 (my::Al0::Al0::Al0::Al0::Al0::M2GSG::AxFLAGSGS::Al0::M2GSGS::Al0::Al0::mVc), I; luc22 (my::Al0::Al0::Al0::Al0::M2GSG::AxFLAGSGS::Al0::M2GSGS::AxFLAGSGSE::AxFLAGSGSE::AxFLAGSGSE::AxFLAGSGSE::AxFLAGSGSE::AxFLAGSGSE::AxFLAGSGSE::AXFLAGSGSE::AXFLAGSGSE::AXFLAGSGSE::AXFLAGSGSE::AXFLAGSGSE::	control strain expressing 11K-1 in soma & germline expressing mirtron-35 + mirtron-51 endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID expressing TIR-1 in soma & germline endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID Expressing TIR-1 in soma & germline Endogenous drsh-1 and pash-1 bearing AID-tag	38, S3E-G S1A, S1C S1A, S1C
MLC1065 MLC1245 MLC1726	Luds20 (m35p::GFP_mitron-35::bb2, myo2::mCherny): Luds21 (mis5p::GFP_mitron-35::bb2, myo2::mCherny): Luds21 (mis5p::GFP_mitron-34::L4G::AID::myc), l; lesi57 [eft-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], II; lesi58 [unc-1p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], IV Luc82 (myc::AID::3XFLAG::4xGGSG::drsh-1::4xGGSG::3xFLAG::AID::myc), l; lesi58 [unc-1p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], IV Luc82 [myc::AID::3XFLAG::4xGGSG::3xFLAG::AID::myc), Luc71 (pash1::PASH-1::2xGGSG::3xFLAG::AUC:myc), Luc82 (myc::AID::3XFLAG::4xGGSG::3xFLAG::AID::myc), Luc82 [myc::AID::3XFLAG::4xGGSG::3xFLAG::AID::myc), Luc71 [unsh1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], IV Luc82 [myc::AID::3XFLAG::4xGGSG::3xFLAG::AID::myc), Luc82 [myc::AID::3XFLAG::4xGGSG::AID::myc], Luc82 [myc::AID::3XFLAG::4xGGSG::AID::myc], Luc82 [myc::AID::3XFLAG::4xGGSG::3xFLAG::AID::myc], Luc82 [myc::AID::3XFLAG::4xGGSG::AID::myc], Luc82	control strain expressing TIF-1 in Soma & germline         expressing mitron-35 + mitron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline	38, S3E-G S1A, S1C S1A, S1C 1A, S1C-E, 3A, S3A, S3B
MLC1065 MLC1245 MLC1726	Luds20 (m35p::GFP_mitron-35::tbb2, myo2::mCherry);           Luds24 (mir52p::GFPstop_mitron-34::tbb2, vip2::mCherry);           Luds25 (my::ADD::MyCl_112; vip2::mCherry);           Luds26 (my::ADD::MyCl_112; vip2::mCherry);           Luds27 (my::ADD::MyCl_112; vip2::mCherry);           Luds28 (my::ADH::MyCl_122; vip2::mCherry);           Luds28 (my::ADH::MyCl_112; vip2::mCherry);           Luds28 (my::ADH::MyCl_112; vip2::mCherry);           Luds28 (my::ADH::MyCl_112; vip2::mCherry);           Luds28 (my::ADH::MyCl_122; vip2::mCherry);           Luds28 (myCl_122; vip2::mCherry);           Luds28 (myCl_122; vip2::mCherr	control strain expressing TIR-1 in Soma & germline         expressing mirtron-35 + mirtron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline	38, S3E-G S1A, S1C S1A, S1C 1A, S1C-E, 3A, S3A, S38
MLC1065 MLC1245 MLC1726 MLC1727	Luds20 (m35p::GFP_mitron-35::bb2, myo2::mCherny): Luds24 (mis5p::GFP_mitron-35::bb2, myo2::mCherny): Luds24 (mis5p::GFP_mitron-34:1::bb2, el2::dsRed, myo2::mCherny) Luds24 (mis5p::GFP_mitron-34:1::bb2, el2::dsRed, myo2::mCherny) Luds24 (mis5p::GFP_mitron-34::Ds2, el2::ds2, el2	control strain expressing ITF-1 in soma & germline expressing mirtron-35 + mirtron-51 endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID expressing TIR-1 in soma & germline endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID Expressing TIR-1 in soma & germline Endogenous drsh-1 and pash-1 bearing AID-tag expressing TIR-1 in soma & germline endogenous drsh-1 and pash-1 bearing AID-tag expressing TIR-1 in soma & germline	38, S3E-G S1A, S1C S1A, S1C 1A, S1C-E, 3A, S3A, S3B S3B-D
MLC1065 MLC1245 MLC1726 MLC1727	Luds20 (m35p::GFP_mitron-35::bb2_mvo2::mCherny): Luds24 (mis5p::GFP_mitron-35::bb2_mvo2::mCherny): Luds24 (mis5p::GFP_mitron-35::bb2_mvo2::mCherny) LuC71 (pash1::PASH-1::2xGGSG::3xFLAG::AlD::myc), 1; leSi57 [df-3p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], 11; leSi57 [df-3p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], 11; leSi58 [sun-1p::TIR1::mRuby::un-54 3'UTR + Cbr-unc-119(+)], 12; leSi58 [sun-1p::TIR1::mRuby::	control strain expressing ITF-1 in soma & germline         expressing mitron-35 + mitron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline	38, S3E-G       S1A, S1C         S1A, S1C       IA, S1C         S1A, S1C       S3B-D
MLC1065 MLC1245 MLC1726 MLC1727	Luds20 (m35p::GFP_mitron-35::bb2, myo2::mCherry): Luds24 (mis3p::GFP_mitron-35::bb2, myo2::mCherry): Luds24 (mis3p::GFP_mitron-34::tbb2,el2::dsRed, myo2::mCherry) Luc71 (pash1::PASH-1::2xGGSG::3xFLAG::AlD::myc), 1; le5157 [eff-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; le5157 [eff-3p::TIR1::mRuby::unc-54 3'UTR + C	control strain expressing ITF-1 in soma & germline         expressing mitron-35 + mitron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing TIR-1 in soma & germline         expressing TIR-1 and pash-1 bearing AID-tag         expressing mitron-35	38, 53E-G 51A, 51C 51A, 51C 1A, 51C-E, 3A, 53A, 53B 53B-D
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728	Luds20 (m35p::GFP_mitron-35::bb2, myo2::mCherry): Luds24 (mis3p::GFP_mitron-35::bb2, myo2::mCherry): Luds24 (mis3p::GFP_mitron-35::bb2, myo2::mCherry) Luc71 (pash1::PASH-1::2xGGSG::3xFLAG::AlD::myc), 1; leiS157 [eff-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leiS157 [eff-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leiS18 [un-1p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leiS18 [eff-4p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leiS17 [eff-4p::TIR1::mRuby::unc-54 3'UTR +	control strain expressing ITF-1 in soma & germline         expressing mitron-35 + mitron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         expressing mitron-35         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-35         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing mitron-35	38, S3E-G 51A, S1C 51A, S1C 1A, S1C-E, 3A, S3A, S3B S3B-D S3B-D
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728	Luds20 (mis3p::GFP_mitron-35::bb2, myo2::mCherry): Luds24 (mis3p::GFP_mitron-35::bb2, myo2::mCherry): Luds24 (mis3p::GFP_mitron-35::bb2, myo2::mCherry) Luc71 (pash1::PASH-1::2xGGSG::3xFLAG::AlD::myc), 1; leSiS7 [eft-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leSiS7 [eft-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leSiS8 [sun-1p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leSiS8 [sun-1p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leSiS7 [eft-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leSiS8 [sun-1p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leSiS8 [sun-2p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leSiS8 [sun-2p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leSiS8 [sun-2p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leSiS7 [eft-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leSiS8 [sun-2p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leSiS7 [eft-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leSiS7 [eft-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+)], 11; leSiS7 [eft-3p::TIR1::mRuby::unc-54 3'UTR + Cbr-unc-119(	control strain expressing ITF-1 in soma & germline         expressing mitron-35 + mitron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-35         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-35         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-35	38, S3E-G         S1A, S1C         S1A, S1C         1A, S1C-E, 3A, S3A, S3B         S3B-D         S3B-D
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728	Luds20 (m35p::GFP_mitron-35::bb2_mvo2::mCherny): Luds24 (mi52p::GFPztop_mitron54.1::bb2_el2::dsRed,myo2::mCherny) Luds24 (mi52p::GFPztop_mitron54.1::bb2_el2::dsRed,myo2::mCherny) Luc71 (pash1::PASH-1::2xGGSG::3xFLAG::ADD::myc), 1; le5157 [eft-3p::TIR1::mRuby::sun-13 UTR + Cbr-unc-119(+]), 11; le5157 [eft-3p::TIR1::mRuby::sun-13 UTR + Cbr-unc-119(+]), 11; le5157 [eft-3p::TIR1::mRuby::sun-13 UTR + Cbr-unc-119(+]), 11; le5157 [eft-3p::TIR1::mRuby::sun-13 UTR + Cbr-unc-119(+]), 11; le5158 [sun-1p::TIR1::mRuby::sun-54 3'UTR + Cbr-unc-119(+]), 11; le5157 [eft-3p::TIR1::mRuby::sun-54 3'UTR + Cbr-unc-119(+]), 11; le5158 [sun-1p::TIR1::mRuby::sun-54 3'UTR + Cbr-unc-119(+]), 11; le5158 [sun-1p::TIR1::mRuby::sun-54 3'UTR + Cbr-unc-119(+]), 11; le5158 [sun-1p::TIR1::mRuby::sun-54 3'UTR + Cbr-unc-119(+]), 11; le5158 [sun-1p::TIR1::mRuby::sun-5 4 3'UTR + Cbr-unc-119(+]), 11; le5158 [sun-1p::TIR1::mRuby::sun-3 3'UTR + Cbr-unc-119(+]), 11; le5157 [eft-3p::TIR1::mRuby::sun-3 3'UTR +	control strain expressing ITF-1 in soma & germline         expressing mittron-35 + mittron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mittron-35         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mittron-35         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mittron-35	38, S3E-G       S1A, S1C         S1A, S1C       IA, S1C-E, JA, S3A, S3B         S3B-D       S3B-D
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728	Luds20 (m35p::GFP_mitron-35::bb2_mvo2::mCherny): Luds24 (mis3p::GFP_mitron-35::bb2_mvo2::mCherny): Luds24 (mis3p::GFP_mitron-35::bb2_mvo2::mCherny): Luds24 (mis3p::GFP_mitron-35::bb2_mvo2::mCherny): Luds24 (mis3p::GFP_mitron-35::bb2_mvo2::mCherny): Luds24 (mis3p::GFP_mitron-35::bb2_mvo2::mCherny): Luds25 (myc::Al0::3XFLAG::4XGGSG::drsh-1::4XGGSG::3xFLAG::Al0::myc), 1; Luds25 (myc::Al0::3XFLAG::4XGGSG::drsh-1::4XGGSG::3xFLAG::Al0::myc), 1; Luds25 (myc::Al0::3XFLAG::4XGGSG::drsh-1::4XGGSG::3xFLAG::Al0::myc), 1; Luds26 (myc::Al0::3XFLAG::4XGGSG::drsh-1::4XGGSG::3xFLAG::Al0::myc), 1; Luds27 (msh::mshuby::unc-54 3'UTR + Chr-unc-119(+)], 1V; Luds28 (myc::Al0::3XFLAG::4XGGSG::drsh-1::4XGGSG::3xFLAG::Al0::myc), 1; Luds21 (msh::mshuby::unc-54 3'UTR + Chr-unc-119(+)], 1V; Luds28 (mi:Al0::3XFLAG::4XGGSG::drsh-1::4XGGSG::3xFLAG::Al0::myc), 1; Luds28 (mi:Al0::3XFLAG::4XGGSG::drsh-1::4XGGSG::3xFLAG::Al0::myc), 1; Luds28 (mi:Al0::3XFLAG::4XGGSG::drsh-1::4XGGSG::3xFLAG::Al0::myc), 1; Luds28 (mi:Al0::3XFLAG::4XGGSG::drsh-1::4XGGSG::3xFLAG::Al0::myc), 1; Luds28 (mi:Al0::3XFLAG::4XGGSG::3xFLAG::Al0::myc), 1; Luds28 (mi:Al0::3XFLAG::4XGGSG::3xFLAG::Al0::myc), 1; Luds28 (mi:Al0::3XFLAG::4XGGSG::3xFLAG::Al0::myc), 1; Luds28 (mi:Al0::3XFLAG::4XGGSG::3xFLAG::Al0::myc), 1; Luds28 (mi:Al0::3XFLAG::4X	control strain expressing TIR-1 in soma & germline         expressing mitron-35 + mitron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing mitron-35         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline	38, S3E-G         S1A, S1C         S1A, S1C         1A, S1C-E, 3A, S3A, S3B         S3B-D         S3B-D         1B, S2F, S2G, 3A, 3B, S3A, S3E-G, 4A, S4A
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728 MLC1729	Luck20 (mis3p::GFP_mittron-3::tbb2, myo2::mCherry)         Luck24 (mis3p::GFP_mittron-3::tbb2, myo2::mCherry)	control strain expressing ITF-1 in Softa & germline         expressing mitron-35 + mitron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline	38, S3E-G         S1A, S1C         S1A, S1C         1A, S1C-E, 3A, S3A, S3B         S3B-D         S3B-D         1B, S2F, S2G, 3A, 3B, S3A, S3E-G, AA, S4A
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728 MLC1729	Lucis20 (mis3p::GFP_mitron-35::tbb2, myo2::mCherry)           Lucis24 (mis3p::GFP_mitron-35::tbb2, myo2::mCherry)           Lucis25 (fef-3p::TIR1::mRuby::sun-3 3'UTR + Cbr-unc-119(+)], II;           LeisS7 (fef-3p::TIR1::mRuby::sun-3 3'UTR + Cbr-unc-119(+)], II;           LeisS7 (fef-3p::TIR1::mRuby::sun-3 3'UTR + Cbr-unc-119(+)], II;           Lucis20 (mS5p::GFP_mitron-35::tbb2, myo2::mCherry);           Lucis21 (pash1::PASH-11::2xGGSG::3xFLG::AlD::myc), I;           Lucis21 (pash1::PASH-11::2xGGSGS::3xFLG::AlD::myc), I;           Lucis21 (pash1::PASH-11::2xGGSGS::3xFLG::AlD::myc), I;           Lucis22 (mis5p::GFP_mitron-35::tbb2, myo2::mCherry);           Lucis24 (mis5p::GFP_mitron-54:JUTR + Cbr-	control strain expressing ITF-1 in soma & germline         expressing mittron-35 + mittron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing mittron-51	38, S3E-G       S1A, S1C         S1A, S1C       IA, S1C         S3B-D       S3B-D         18, S2F, S2G, 3A, 3B, S3A, S3E-G, 4A, S4A
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728 MLC1729	Luds20 (m35p::GFP_mitron-35::bb2, myo2::mCherry)         Luds24 (mis52p::GFP_mitron-35::bb2, myo2::mCherry)         Luds24 (my::AD::MyCherry)         Luds25 (my::AD::MyCherry)         Luds26 (my::AD::MyCherry)         Luds26 (my::AD::MyCherry)         Luds26 (my::AD::MyCherry)         Luds27 (my::AD::MyCherry)         Luds28 (my::AD::MyCherry)         Luds24 (mi:Sp::GFP mitron-35::bD2, myO2::mCherry) <td>control strain expressing ITF-1 in Soma &amp; germline         expressing mittron-35 + mittron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma &amp; germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma &amp; germline         Endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma &amp; germline         expressing TIR-1 in soma &amp; germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma &amp; germline         expressing mittron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mittron-551</td> <td>38, S3E-G         S1A, S1C         S1A, S1C         1A, S1C-E, 3A, S3A, S3B         S3B-D         S3B-D         18, S2F, S2G, 3A, 3B, S3A, S3E-G, 4A, S4A</td>	control strain expressing ITF-1 in Soma & germline         expressing mittron-35 + mittron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing mittron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mittron-551	38, S3E-G         S1A, S1C         S1A, S1C         1A, S1C-E, 3A, S3A, S3B         S3B-D         S3B-D         18, S2F, S2G, 3A, 3B, S3A, S3E-G, 4A, S4A
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728 MLC1729 MLC2345	Luds20 (m35p::GFP_mittron-35::bb2, myo2::mCherry)         Luds24 (mi52p::GFPztop_mittron54.1::bb2,el2::dsRed,myo2::mCherry)         Luds24 (mi52p::GFPztop_mittron54.1::bb2,el2::dsRed,myo2::mCherry)         Luds24 (mi52p::GFPztop_mittron54.1::bb2,el2::dsRed,myo2::mCherry)         Luds24 (mi52p::GFPztop_mittron54.1::bb2,el2::dsRed,myo2::mCherry)         Luds2 (myc::AlD::3xFLAG::AlG::AlD::myc), l;         Le5157 (Ef-3p::TIR1::mRuby::sun-13'UTR + Cbr-unc-119(+)], l1;         Le5157 (Ef-3p	control strain expressing ITF-1 in soma & germline         expressing mittron-35 + mittron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         expressing mittron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mittron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mittron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mittron-51	38, S3E-G         S1A, S1C         S1A, S1C         1A, S1C-E, 3A, S3A, S3B         S3B-D         S3B-D         18, S2F, S2G, 3A, 3B, S3A, S3E-G, 4A, S4A         S3C, S3D
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728 MLC1729 MLC2345	Luds20 (m35p::GFP_mitron-35::bb2, myo2::mCherry)           Luds24 (mi52p::GFPztop_mitron54.1::bb2,el2::dsRed,myo2::mCherry)           Luds24 (mi52p::GFPztop_mitron54.1::bb2,el2::dsRed,myo2::mCherry)           Luds24 (mi52p::GFPztop_mitron54.1::bb2,el2::dsRed,myo2::mCherry)           Luds24 (mi52p::GFPztop_mitron54.1::bb2,el2::dsRed,myo2::mCherry)           Luds2 (myc::AlD::3XFLAG::AlG::GSG::dsFLAG::AlD::myc),l;           Le5S7 (eff-3p::TIR1::mRuby::sun-13'UTR + Cbr-unc-119(+)],l1;           Le5S38 [sun-1p::TIR1::mRuby::sun-54'UTR + Cbr-unc-119(+)],l1;           Le5S38 [sun-1p::TIR1::mRuby::sun-13'UTR + Cbr-unc-119(+)],l1;           Le5S38 [sun-1p::TIR1::mRuby::sun-3'UTR + Cbr-unc-119(+)],l1;           Le5S38 [sun-1p::TIR1::mRuby::sun-3'UTR + Cbr-unc-119(+)],l1;           Le5S38 [sun-2p::TIR1::mRuby::sun-3'UTR + Cbr-unc-119(+)],l1;           Le5S38 [sun-3p::TIR1::mRuby::sun-3'UTR + Cbr-unc-119(+)],l1;           Le5S38 [sun-1p::TIR1::mRuby::sun-3'UTR + Cbr-unc-119(+)],l1;           Le5S38 [sun-1p::TIR1::mRuby::sun-3'UTR + Cbr-unc-119(+)],l1;           Le5S38 [sun-1p::TIR1::mRuby::sun-13'UTR + Cbr-unc-119(+)],l1;           Le5S38 [sun-1p::TIR1::mRuby::sun-13'UTR + Cbr-unc-119(+)],l1;	control strain expressing ITF-1 in Soma & germline         expressing mitron-35 + mitron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing mitron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline	38, S3E-G         S1A, S1C         S1A, S1C         IA, S1C-E, 3A, S3A, S3B         S3B-D         S3B-D         18, S2F, S2G, 3A, 3B, S3A, S3E-G, 4A, S4A         S3C, S3D
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728 MLC1729 MLC2345 Other	Luds20 (m35p::GFP_mittron-35::bb2, myo2::mCherry)         Luds24 (mi52p::GFPztop_mittron54.1::bb2,el2::dsRed,myo2::mCherry)         Luds24 (mi52p::GFPztop_mittron54.1::bb2,el2::dsRed,myo2::mCherry)         Luds24 (mi52p::GFPztop_mittron54.1::bb2,el2::dsRed,myo2::mCherry)         Luds24 (mi52p::GFPztop_mittron54.1::bb2,el2::dsRed,myo2::mCherry)         Luds24 (mi52p::GFPztop_mittron54.1::bb2,el2::dsRed,myo2::mCherry)         Luds2 (myc:AlD::3XFLAG::AlG::GSG::dsFLAG::AlD::myc), l;         Le5157 (eff-3p::TIR1::mRuby::sun-1.3'UTR + Cbr-unc-119(+)], ll;         Le5157 (eff-3p::TIR1::mRuby::sun-1.3'UTR + Cbr-unc-119(+)], ll;         Luds2 (myc:AlD::3XFLAG::AlG::GSG::sh:LAG::AlD::myc),         Luds2 (m3:S::GFP mittron-35::bb2,mv2::mCherry);         Luds2 (m3:S::GFP mittron54::UtFb2,el2::dsRed,my2::mChery)         Luds2 (m3:S::GFP::mittron54::UtFb2	control strain expressing ITF-1 in Soma & germline         expressing mitron-35 + mitron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing TIR-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing TIR-1 in soma & germline         expressing mitron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing mitron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-51	38, S3E-G         S1A, S1C         S1A, S1C         1A, S1C-E, 3A, S3A, S3B         S3B-D         S3B-D         18, S2F, S2G, 3A, 3B, S3A, S3E-G, 4A, S4A         S3C, S3D
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728 MLC1729 MLC2345 Other MLC1867	Luds20 (m35p::GFP_mitron-35::bb2, myo2::mCherry) Luds24 (mi52p::GFPztop_mitron54.1:tbb2,et82::dsRed, myo2::mCherry) Luds24 (mi52p::GFPztop_mitron54.1:tbb2,et82::dsRed, myo2::mCherry) Luds24 (mi52p::GFPztop_mitron54.1:tbb2,et82::dsRed, myo2::mCherry) Luds24 (mi52p::GFPztop_mitron54.1:tbb2,et82::dsRed, myo2::mCherry) Luds24 (myc:AlD::3XFLAG::4XGGSG::dsFLAG::AlD::myc), 1; leiSi38 [sun-1p::TR1::mRuby::sun-1.3UTR + Cbr-unc-119(+]], 11; leiSi38 [sun-1p::TR1::mRuby::sun-1.3UTR + Cbr-unc-119(+]], 11; leiSi38 [sun-1p::TR1::mRuby::sun-1.3UTR + Cbr-unc-119(+]], 11; leiSi37 [eft-3p::TR1::mRuby::sun-1.3UTR + Cbr-unc-119(+]], 11; leiSi37 [eft-3p::TR1::mRuby::sun-1.3UTR + Cbr-unc-119(+]], 11; leiSi37 [eft-3p::TR1::mRuby::sun-1.3UTR + Cbr-unc-119(+]], 11; leiSi37 [eft-3p::TR1::mRuby::sun-1.3UTR + Cbr-unc-119(+]], 11; leiSi38 [sun-1p::TR1::mRuby::sun-1.3UTR + Cbr-unc-119(+]], 11; leiSi38 [sun-1p::TR1::mRuby::sun-1.3UTR + Cbr-unc-119(+]], 11; leiSi38 [sun-1p::TR1::mRuby::sun-1.3UTR + Cbr-unc-119(+]], 11; leiSi37 [eft-3p::TR1::mRuby::sun-3.3UTR + Cbr-unc-119(+]], 11; leiSi38 [sun-1p::TR1::mRuby::sun-3.3UTR + Cbr-unc-119(+]], 11; leiSi38 [eft-3p::TR1::mRuby::sun-3.3UTR + Cbr-unc-119(+]], 11; leiSi37 [eft-3p::TR1::mRuby::sun-3.3UTR + Cbr-unc-119(+]], 11; leiSi37 [eft-3p::TR1::mRuby::sun-3.3UTR + Cbr-unc-119(+]], 11; leiSi37 [eft-3p::TR1::mRuby::sun-3.3UTR + Cbr-unc-119(+]], 11; leiSi37 [eft-3p::TR1::mRuby::sun-3.3UTR + Cbr-unc-119(+]], 11; leiSi38 [sun-1p::TR1::mRuby::sun-3.3UTR + Cbr-unc-119(+]], 11; leiS	control strain expressing ITF-1 in soma & germline         expressing mitron-35 + mitron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-35         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing TIR-1 in soma & germline         expressing mitron-35         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-35 + mitron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-35 + mitron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-35 + mitron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-51      <	38, S3E-G         S1A, S1C         S1A, S1C         1A, S1C-E, 3A, S3A, S3B         S3B-D         S3B-D         1B, S2F, S2G, 3A, 3B, S3A, S3E-G, 4A, S4A         S3C, S3D         48
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728 MLC1729 MLC2345 Other MLC1867 MLC1907	Luds20 (m35p::GFP_mittron-35::bb2_myo2::mCherry)           Luds24 (mi52p::GFPstop_mittron54.1::bb2_elt2::dsRed,myo2::mCherry)           Luds24 (mi52p::GFPstop_mittron54.1::bb2_elt2::dsRed,myo2::mCherry)           Luds24 (mi52p::GFPstop_mittron54.1::bb2_elt2::dsRed,myo2::mCherry)           Luds24 (mi52p::GFPstop_mittron54.1::bb2_elt2::dsRed,myo2::mCherry)           Luds24 (mi52p::GFPstop_mittron54.1::bb2_elt2::dsRed,myo2::mCherry)           Luds2 (myc:AlD::3XFLAG::4XGGSG::dsFL::4XGGSG::3xFLAG::AlD::myc), 1;           LeSIS7 (left-3p::TR1::mRuby::sun-13 UTR + Cbr-unc-119(+]), 11;           LeSIS7 (left-3p::TR1::mRuby::sun-23 UTR + Cbr-unc-119(+]), 11;           LeSIS7 (left-3p::TR1::mRuby::sun-23 UTR + Cbr-unc-119(+]), 11;           LeSIS7 (left-3p::TR1::mRuby::sun-3 UTR + Cbr-unc-119(+]), 11;           LeSIS8 [sun-1p::TR1::mRuby::sun-3 UTR + Cbr-unc-119(+]), 11;           LeSIS8 [sun-1p::TR1::mRuby::sun-3 UTR + Cbr-unc-119(+]), 11;           LeSIS8 [sun-1p::TR1::mRuby::sun-3 UTR + Cbr-unc-119(+]), 11;           LeSIS9 [eft-3p::TR1::mRuby::sun-3 UTR + Cbr-unc-119	control strain expressing ITF-1 in soma & germline         expressing mittron-35 + mittron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing mittron-35         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing TIR-1 in soma & germline         expressing mittron-35 + mittron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing seed-mutant version of mittron-35         control strain expressing goy-5::GFP reporter         mi-35 famil	38, S3E-G         S1A, S1C         S1A, S1C         1A, S1C-E, 3A, S3A, S3B         S3B-D         S3B-D         1B, S2F, S2G, 3A, 3B, S3A, S3E-G, AA, S4A         S3C, S3D         4B
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728 MLC1729 MLC1729 MLC2345 Other MLC1867 MLC1907	Luds20 (m35p::GFP_mitron-35::bb2, myo2::mCherry)           Luds24 (mis3p::GFP_mitron-35::bb2, myo2::mCherry)           Luds2 (myc::AlD::3XFLAG::#AGGSG::dsh-1::#AGGSG::3xFLAG::AlD::myc), l;           LeiS17 (left-3p::TR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+]), lV           Lud2 (myc::AlD::3XFLAG::#AGGSG::dsh-1::#AGGSG::3xFLAG::AlD::myc), l;           LeiS17 (left-3p::TR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+]), lV;           Lud2 (my::AlD::3XFLAG::#AGGSG::dsh-1::#AGGSG::3xFLAG::AlD::myc), l;           LeiS17 [left-3p::TR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+]), lV;           Lud2 (my::AlD::3XFLAG::#AGGSG::dsh-1::#AGGSG::3xFLAG::AlD::myc), l;           LeiS17 [left-3p::TR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+]), lV;           Lud2 (myc::AlD::3XFLAG::#AGGSG::dsh-1::#AGGSG::3xFLAG::AlD::myc), l;           LeiS18 [um-1p::TR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+]), lV;           Lud2 (myc::AlD::3XFLAG::#AGGSG::dsh-1::#AGGSG::3xFLAG::AlD::myc), l;           LeiS18 [um-1p::TR1::mRuby::unc-54 3'UTR + Cbr-unc-119(+]), lV;           Lud2 (mis52p::GFP_mitron-3::tbD2, my2::mChery)           Lud2 (mis52p::GFP_mitron-3::tbD2, my2::mChery)           Lud2 (mis52p::GFP_mitron-3	control strain expressing ITF-1 in soma & germline         expressing mitron-35 + mitron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing mitron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing mitron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing seed-mutant version of mitron-35         control strain expressing gcy-5:::GFP r	38, S3E-G         S1A, S1C         S1A, S1C         1A, S1C-E, 3A, S3A, S3B         S3B-D         S3B-D <t< td=""></t<>
MLC1065 MLC1245 MLC1726 MLC1727 MLC1728 MLC1729 MLC1729 MLC2345 Other MLC1867 MLC1907 MLC1926	Indi32 (Indi32)::GFP_mitron-35::tb2_myo2::mCherry)           Indi32 (Indi32)::GFP Extop_mitron54.11:tb2_et2::dSRed_myo2::mCherry)           Indi32 (Indi32)::GFP Extop_mitron54.11:tb2_et2::dSRed_myo2::mCherry)           Indi32 (Indi32)::GFP Extop_mitron54.11:tb2_et2::dSRed_myo2::mCherry)           Indi32 (Indi32)::GFP Extop_mitron54.11:tb2_et2::dSRed_myo2::mCherry)           Indi32 (Indi32)::GFP Extop_mitron54.11:tb2_et2::GSRed_myo2::mCherry)           Indi32 (Indi32)::GFP Extop_mitron54.11:tb2_et2::GSRed_myo2::mCherry)           Indi2 (Indi32)::GSG::GS::GSRFLAG::ALD::myc),           Indi2 (Indi32)::GSG::GS::GSRFLAG::ALD::myc),           Indi2 (Indi32)::GSG::GS::GSRFLAG::ALD::myc),           Indi2 (Indi32)::GSG::GS::GSRFLAG::ALD::myc),           Indi2 (Indi32)::GSG::GSRFLAG::ALD::myc),           Indi2 (Indi32)::GSG::GSRFLAG::ALD::myc),           Indi2 (Indi32)::GFP Entron-S3:::BD2_myd2::mCherry);           Indi2 (Indi32)::GFP Entron-S3:::BD2_myd2::mCherry);           Indi2 (Indi32)::GFP Entron-S3:::BD2_myd2::mCherry);           Indi2 (Indi32)::GFP Entron-S3:::BD2_myd2::mCherry);           Indi32 (Indi32)::GFP Entron-S3:::BD2_myd2::mCherry);           Indi32 (Indi32)::GFP Entron-S3:::BD2_myd2::mCherry);           Indi32 (Indi32)::GFP_mitron-S4:::BCF           Indi32 (Indi32)::GFP_mitron-S4:::BCF           Indi32 (Indi32)::GFP_mitron-S4:::BCF           Indi32 (Indi32):::GFP_mitron54::::BCF           Indi3	control strain expressing ITF-1 in soma & germline         expressing mitron-35 + mitron-51         endogenous pash-1 bearing C-terminal AID-tag = pash-1:AID         expressing TIR-1 in soma & germline         endogenous drsh-1 bearing N- and C-terminal AID-tag = drsh-1:AID         Expressing TIR-1 in soma & germline         Endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         endogenous drsh-1 and pash-1 bearing AID-tag         expressing TIR-1 in soma & germline         expressing mitron-51         endogenous drsh-1 and pash-1 bearing AID-tag         expressing seed-mutant version of mitron-35         control strain expressing gcy-5::GFP reporter         mi-35 family deletion is rescued by mitro	38, S3E-G         S1A, S1C         S1A, S1C         1A, S1C-E, 3A, S3A, S3B         S3B-D         S3B-D         18, S2F, S2G, 3A, 3B, S3A, S3E-G, 4A, S4A         S3C, S3D         48         48         48         48

Table S1. Strains used and generated in this study. Related to STAR Methods. Name, genotype, and origin of strains as well as corresponding figures are denoted in the respective columns. Key strains are deposited with the CGC, other strains are available upon request.

## **Supplemental References**

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