SUPPLEMENTAL MATERIAL Kazmierczak et al.

The CONJUDOR pipeline for multiplexed knockdown of gene pairs identifies RBBP-5 as a germ cell reprogramming barrier in C. elegans

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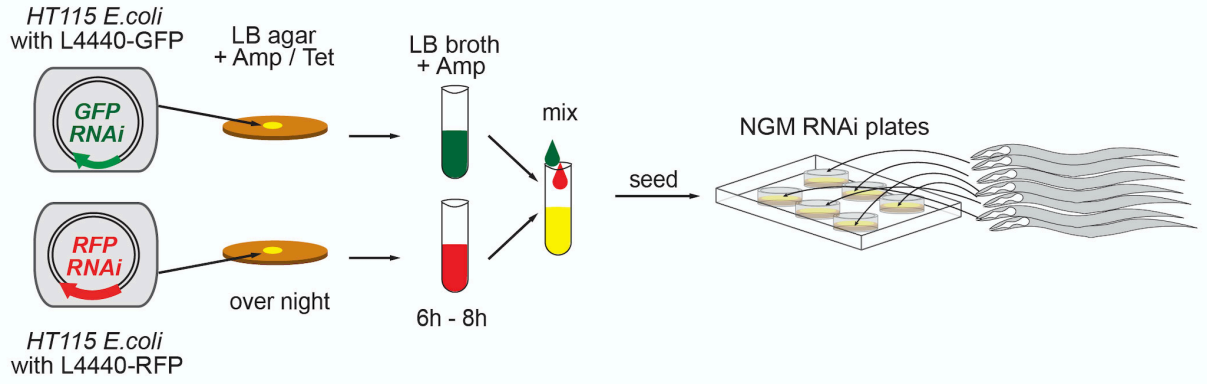
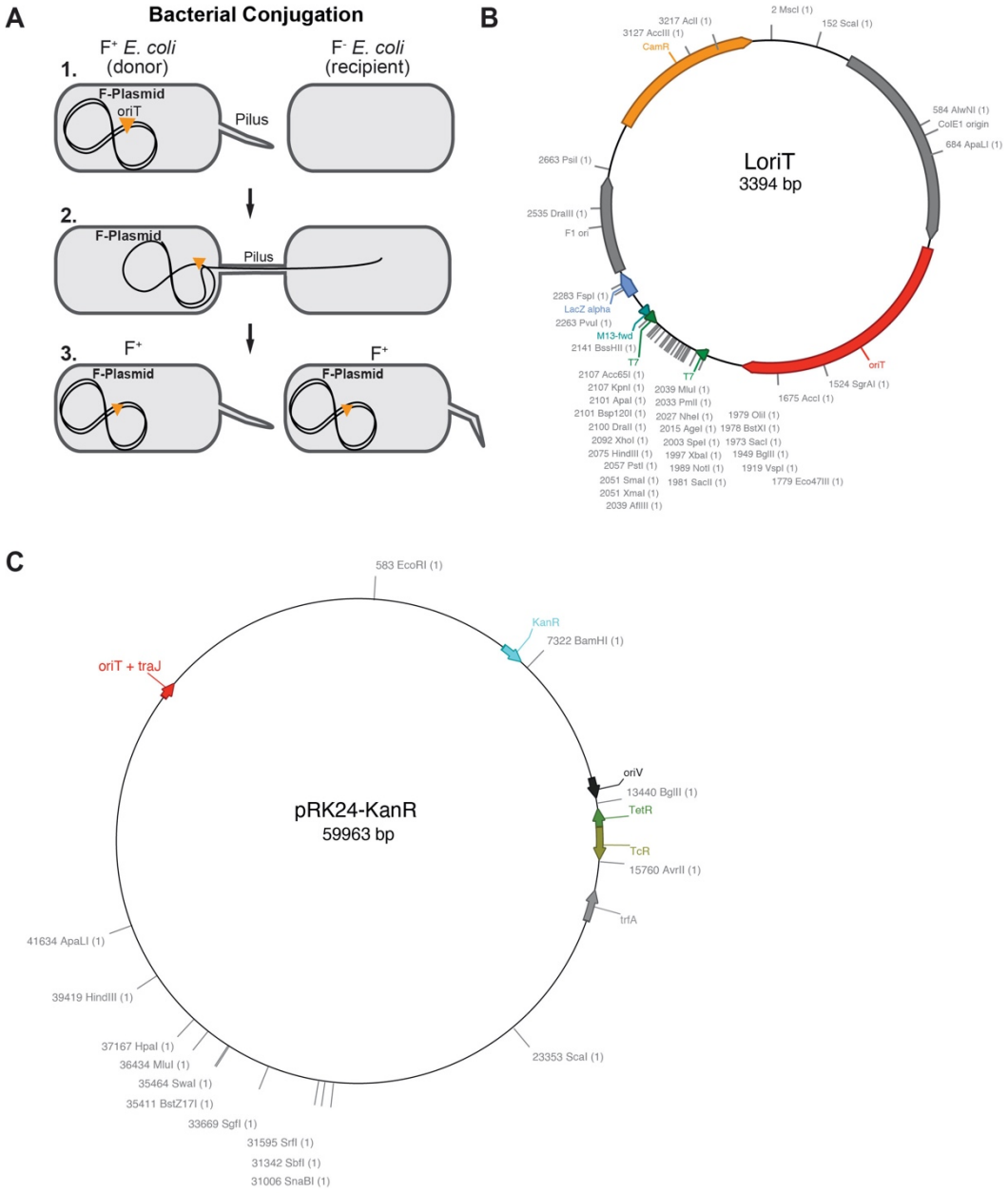
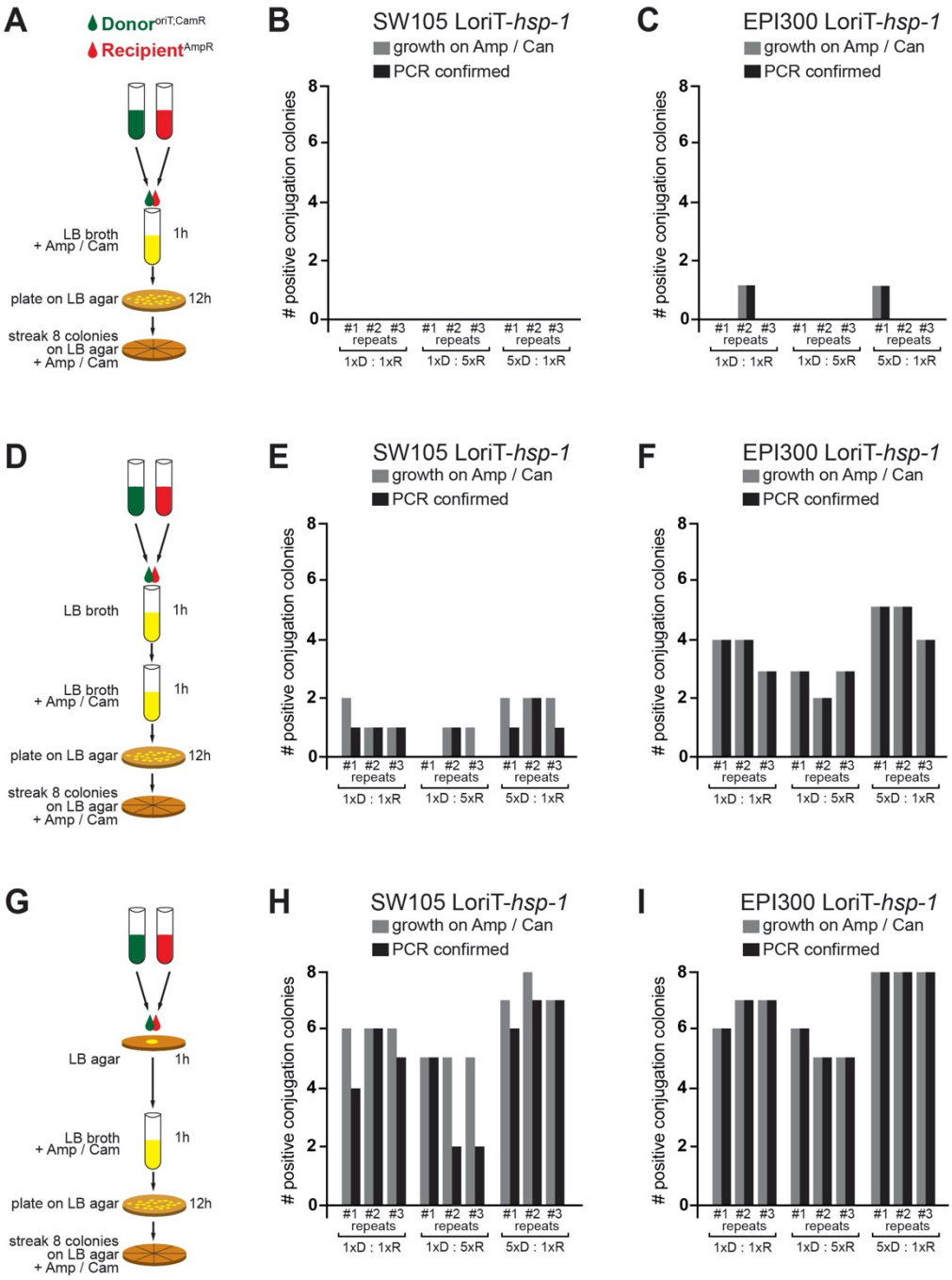
SUPPLEMENTAL FIGURES



Supplemental Figure 1: Double RNAi in *C. elegans* by mixing bacteria. RNAi in *C. elegans* is straightforward and can be applied by feeding worms with bacteria that produce dsRNA against the target gene. The standard procedure to perform simultaneous knockdown of two genes is to mix two bacterial strains each producing specific dsRNAs. The illustration shows mixing of bacteria that produce dsRNA against *GFP* or *RFP*. The dsRNA is produced by *HT115 E. coli* bacteria that contain the RNAi plasmid L4440 plasmid. The gene of interest is cloned into L4440, which allows IPTG-induced dsRNA production.

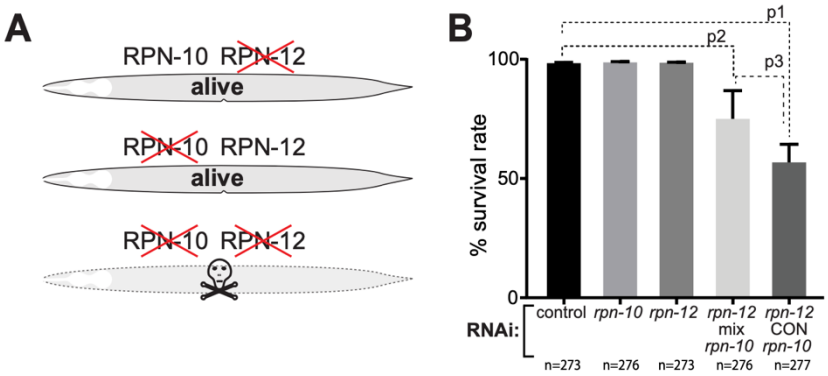


Supplemental Figure 2: Generating a bacterial conjugation system to combine RNAi plasmids. (A) Competence for bacterial conjugation requires presence of the fertility factor, also termed F-plasmid, which contains several genes of the *tra* locus for the formation of a pilus appendage. Bacteria with the F-plasmid are denoted as F^+ (donor) connect via the pilus to F^- bacteria (recipient) and transfer plasmids or other genetic material containing an *oriT* to the recipient. (B) Generating the selectable 'donor' RNAi plasmid based on *L4440*, which can be transferred by conjugation, needed the addition of the *oriT* and replacement of AmpR with Chloramphenicol (CamR) resistance. This allows selection for presence of the transferred RNAi plasmid together with the resident AmpR-containing *L4440* RNAi plasmid after conjugation. We termed the newly generated donor plasmid '*LoriT*', which is basically L4440 carrying *oriT* and CamR instead AmpR. (C) To adopt bacterial conjugation for combining RNAi plasmids, we made the F-plasmid *pRK24* (1) we replaced the Ampicillin resistance (AmpR) of *pRK24* with Kanamycin resistance (KanR) since *L4440* used in the standard 'Ahringer' *C. elegans* RNAi library (2, 3) already carries AmpR. To exchange AmpR with KanR we used recombineering, as previously described (4) due to the extensive size of *pRK24*.

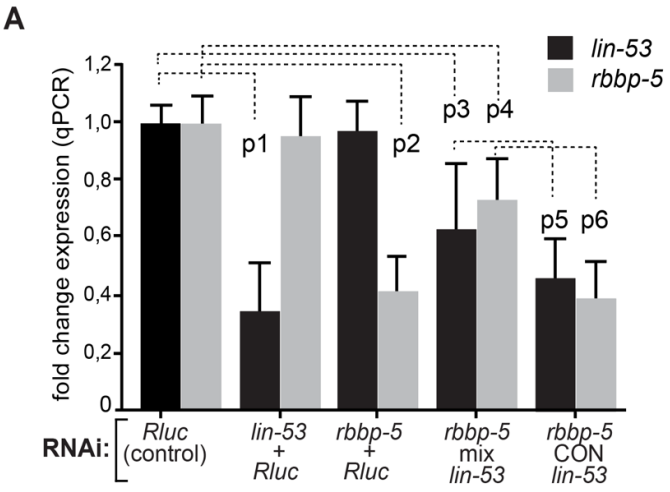


Supplemental Figure 3: Assessment of conjugation procedures for efficient transfer. (A) Conjugation in liquid culture by combining F^+ donor bacteria (*SW105* or *EPI300* containing *pRK24-Kan*) and recipient *HT115*. Incubation of donor and recipient bacteria in liquid LB media containing Amp/Cam for 1h and subsequent plating on LB-Agar plates for 12h. The last step of streaking 8 colonies (if any grown) was to test for colony PCR to verify presence of donor (*LoriT-hsp-1*, CamR) and recipient (*L4440-ogt-1*, AmpR). (B and C) The two *E.coli* strains *SW105* or *EPI300* were used previously to handle large DNA constructs such as fosmids (4) and therefore chosen as the host strains for the *pRK24-KanR* episome (F-plasmid for conjugation competence). We aimed for testing 8 colonies from each procedure of conjugation either combing a ratio of 1:1, 1:5, or 5:1 of donor D and recipient R bacteria. In some cases no colonies were obtained. Obtained colonies were tested by PCR to confirm successful conjugation. The procedure as shown in (A) performed overall poorly. (D) Conjugation by combining donor and recipient bacteria in liquid LB media without antibiotics for 1h, and then with Amp/Cam for 1h

with subsequent plating on LB-agar plates to select at least 8 colonies (if any grown) for examining by PCR. (E and F) As for (B and C) but with more obtained colonies. Still the yield is low and *SW105* F^+ donor bacteria appeared to perform very poorly. As before, we could not even obtain 8 colonies for this procedure as shown in (D) to test. (G) Conjugation on solid LB-agar without antibiotics by combining donor and recipient bacteria for 1h. Afterwards, incubation in liquid LB broth with Amp/Cam for 1h (either directly adding liquid LB, if performed in 96-well or transferring colony to culture tube) with subsequent plating on LB-Agar plates (Amp/Cam) to select at least 8 colonies for examining by PCR. (H and I) this procedure yielded the most efficient conjugations; however the use of *SW105*-based donor bacteria showed less robustness. The use of 5:1 (donor D : recipient R) yielded highly efficient conjugation with correct conjugation in all tested cases.



Supplemental Figure 4: Synthetic lethality induced upon co-depletion of proteasomal subunits. (A) We targeted the 26S-Proteasome subunit genes *rpn-10* and *rpn-12,* which cause synthetic lethality when co-depleted (5). (B) CONJUDOR-mediated simultaneous knockdown of *rpn-10* and *rpn-12* reduced survival by around 50%. In contrast, 25% of the animals fed with mixed *rpn-10* and *rpn-12* RNAi bacteria died indicating that CONJUDOR is more efficiently depleting *rpn-10* and *rpn-12* simultaneously. Control: Rluc RNAi. Statistics: t-test with two-tailed distribution. p1 = 0.0001; p2 = 0.007; p3 = 0.007; Total analyzed animals (triplicate) n= 1375; Error bars represent SEM. For detailed scoring numbers see Suppl. Table 4.



Supplemental Figure 5: Quantification of mRNA levels by qPCR upon *lin-53* and *rbbp-5* knockdown. RNAi was performed as described before with Rluc as control, which was also used to mix 1:1 for single RNAi experiments. Quantitative PCR (qPCR) was performed and quantified by comparative $2^{-\Delta\Delta CT}$ method as described previously (6, 7) and in material and methods. As reference genes *cdc-42* and *pmp-3* were used. 4 biological repeats each with triplicate measurements were performed. Statistics: Student's t-Test with two-tailed distribution with homoscedastic variance. p1= < 0.0001; p2= < 0.0001; p3= 0,024; p4= 0,012; p5= 0.039; p6= 0.004 Error bars represent SEM. Primer sequences are provided in Suppl. Table 2.

SUPPLEMENTAL TABLES

Supplemental Table 1: RNAi clones used in the study

Target Gene	Function	Source
lin-53	histone-chaperone LIN-53ortholog of human RBBP4	Chromatin RNAi library; Hajduskjova et al., 2019
rpn-10	proteasome subunit	Chromatin RNAi library; Hajduskjova et al., 2019
rpn-12	proteasome subunit	Chromatin RNAi library; Hajduskjova et al., 2019
rbbp-5	Retino blastoma protein binding Protein; Set1/MLL methyltransferase complex member	Chromatin RNAi library; Hajduskjova et al., 2019
oma-1	Oocyte Maturation defective; Zn-Finger	Ahringer RNAi library; Kamath et al., 2003
oma-2	Oocyte Maturation defective; Zn-Finger	Ahringer RNAi library; Kamath et al., 2003
gld-1	Translational regular; ortholog of human QKI - KH domain containing RNA binding	Ahringer RNAi library; Kamath et al., 2003
mex-3	ortholog of human MEX3A RNA binding family member	Ahringer RNAi library; Kamath et al., 2003

RNAi clones used for CONJUDOR screen in combination with *LoriT-lin-53* (Figure 6) were derived from the Chromatin RNAi library described in Hajduskova et al., 2019 (see Table S1 from Hajduskova et al., 2019; Genetics; doi: 10.1534/genetics.118.301674) (8).

Supplemental Table 2: Primers used in the study

cloning rpn-10	
oMK03 FWD	tgg atc cac cgg ttc cat ggT GGA ATT CTG TCA ATG GCA AAG
oMK04 REV	gg atc cac gcg tca cgt ggG AGC TCC ATC CAC ATC CAT TTG
cloning rpn-12	
oMK05 FWD	tgg atc cac cgg ttc cat ggA AAT CTT CTG GCT GTG TG
oMK06 REV	ggg atc cac gcg tca cgt ggT GCT AAA ACA ATG CAT CG
cloning oma-1	
oMK33 FWD	tgg atc cac cgg ttc cat ggC CGA ATG CAG AAA CCA GAA TC
oMK34 REV	ggg atc cac gcg tca cgt ggG GCC AAG TTT CTA TGG GAC
cloning oma-2	
oMK35 FWD	tgg atc cac cgg ttc cat ggC CGA ATG CAG AAA CCA GAA TC
oMK36 REV	ggg atc cac gcg tca cgt ggA AAC GGA CTG ATT GGA CG
cloning Cam	
oBT1135 FWD	taa act tgg tct gac agT TAC GCC CCG CCC TGC CA
oBT1137 REV	ttg ttt att ttt cta aat aca ACG TAA GAG GTT CCA ACT TTC ACC ATA ATG AAA TAA GAT CAC
cloning Kan	
oBT1263 FWD	ttc gag ctc cac cgc CCT GTG ACG GAA GAT CAC TTC
oBT1264 REV	gag ctc aaa atc ccg cAG CGC TTT TCC GCT GCA T
oBT1414 FWD	GAA GTT TTA AAT CAA TCT AAA GTA TAT ATG AGT AA ACT TGG TCT GAC AGt tat tag aaa aat tca tcc agc aga cg
oBT1415 REV	TGT ATT TAG AAA AAT AAA CAA ATA GG GGT TCC GCG CAC ATT TCC CCG AAA AGc gcg gaa ccc cta ttt gt tta ttt ttc

cloning oriT	
oBT1285 FWD	cca ccg gtt cca tgg GGC GCT CGG TCT TGC CTT
oBT1286 REV	cca cgc gtc acg tgg AGC GCT TTT CCG CTG CAT AAC
cloning lin-53	
oBT2241 FWD	tgg atc cac cgg ttc cat ggC TCG TAA TGA CAC ATG CG
oBT2242 REV	tga tat cga att cct gca gcG AGA AAT CGC TGA TCT TGG
oBT2391 FWD	tgg atc cac cgg ttc cat ggC TCG TAA TGA CAC ATG CG
oBT2392 REV	tga tat cga att cct gca gcG AGA AAT CGC TGA TCT TG
qPCR *lin-53*	
oBT4235 FWD	ATGGAACCTCCGAAGATCGC
oBT4236 REV	CGCTGTTATCCTTCGCAACG
qPCR rbbp-5	
oBT4239 FWD	TGATGGCAGGGTGCTGATTT
oBT4240 REV	TGTCGTTTGCAAGAACTGTTTGA
qPCR cdc-42	(reference gene for normalization)
oBT4231 FWD	CGACAATTACGCCGTCACAG
oBT4232 REV	AAACACGTCGGTCTGTGGAT
qPCR pmp-3	(reference gene for normalization)
oBT873 FWD	GTT CCC GTG TTC ATC ACT CAT
oBT874 REV	ACA CCG TCG AGA AGC TGT AGA

Supplemental Table 3: Media recipes used in the study

LB (Luria Bertani), (liquid medium) (1L): 25 g LB broth (Carl Roth GmbH + Co. KG), ddH2O

LB/Amp medium (1 L): 25 g LB broth (Carl Roth GmbH + Co. KG), ddH2O, Ampicillin (100 µg/ml final concentration)

LB/Amp plates (1 L): 25 g LB broth (Carl Roth GmbH + Co. KG), 15 g Agar (Carl Roth GmbH + Co. KG), ddH2O, Ampicillin (100 µg/ml final concentration)

LB/Amp+Tet plates (1 L): 25 g LB broth (Carl Roth GmbH + Co. KG), 15 g Agar (Carl Roth GmbH + Co. KG), ddH42O, Ampicillin (100 µg/ml final concentration), Tetracycline (12.5 µg/ml final concentration)

LB/Amp+Tet+Cam plates: 25 g LB broth (Carl Roth GmbH + Co. KG), 15 g Agar (Carl Roth GmbH + Co. KG), ddH2O, Ampicillin (100 µg/ml final concentration)

LB/Amp+Tet plates (1 L): 25 g LB broth (Carl Roth GmbH + Co. KG), 15 g Agar (Carl Roth GmbH + Co. KG), ddH42O, Ampicillin (100 µg/ml final concentration), Tetracycline (12.5 µg/ml final concentration), Chloramphenicol (20 µg/mL final concentration)

LB/Cam plates: 25 g LB broth (Carl Roth GmbH + Co. KG), 15 g Agar (Carl Roth GmbH + Co. KG), ddH42O, Chloramphenicol (20 µg/mL final concentration)

NGM (1 L): 3 g NaCl, 20 g Agar (CarlRothGmbH+Co.KG), 2,5g Peptone (Becton, Dickinson and Company), ddH2O, after autoclaving add: 1 ml Cholesterol (5 mg/ml in 95% EtOH stock solution), 1 ml 1 M MgSO4, 1 ml 1 M CaCl2, 25 ml 1 M K2PO4, 1 ml fungizone (Amphotericin B 2.5 mg/ml stock)

NGM for RNAi (1 L): 3 g NaCl, 20 g Agar (CarlRothGmbH+Co.KG), 2,5g Peptone (Becton, Dickinson and Company), ddH2O after autoclaving add: 1 ml Cholesterol (5 mg/ml in 95% EtOH), 1 ml 1 M MgSO4, 1 ml 1 M CaCl2, 25 ml 1 M K2PO4, 1 ml fungizone (Amphotericin B 2.5 mg/ml stock), add 50 µg/ml ampicillin and 1 mM (final) IPTG

Supplemental Table 4: Scoring data of RNAi phenotypes

BAT1616: myo-3prom::GFP::NLS; myo-3prom::RFP::NLS

RNAi control (Rluc)

	exp1		exp2		exp3	
animal	# GFP-positive muscle	# RFP-positive muscle	# GFP-positive muscle	# RFP-positive muscle	# GFP-positive muscle nuclei	# RFP-positive muscle
1	90,000	94,000	94,000	95,000	95,000	92,000
2	90,000	91,000	92,000	89,000	95,000	93,000
3	94,000	96,000	91,000	87,000	93,000	95,000
4	93,000	94,000	93,000	88,000	88,000	94,000
5	93,000	91,000	94,000	90,000	93,000	89,000
6	92,000	94,000	93,000	93,000	88,000	93,000
7	93,000	94,000	92,000	95,000	90,000	90,000
8	94,000	93,000	93,000	95,000	90,000	90,000
9	95,000	92,000	91,000	91,000	87,000	94,000
10	94,000	92,000	91,000	90,000	90,000	95,000
AVERAGE	92,800	93,100	92,400	91,300	90,900	92,500
STDEV	1,687	1,595	1,174	3,020	2,923	2,173
SEM	0,533	0,504	0,371	0,955	0,924	0,687

Multiple T.Test using GraphPad Prism7: control vs mixed for GFP: <0.00001

Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%.

Each row was analyzed individually, without assuming a consistent SD.

BAT1616: myo-3prom::GFP::NLS; myo-3prom::RFP::NLS

RNAi L4440-*GFP* in *HT115* mixed with RNAi L4440-*RFP* in *HT115*

	exp1		exp2		exp3	
animal	# GFP-positive muscle	# RFP-positive muscle nuclei	# GFP-positive muscle	# RFP-positive muscle nuclei	# GFP-positive muscle	# RFP-positive muscle
1	38,000	42,000	28,000	26,000	49,000	64,000
2	63,000	60,000	43,000	43,000	72,000	69,000
3	54,000	60,000	55,000	65,000	80,000	79,000
4	27,000	19,000	26,000	33,000	41,000	45,000
5	65,000	82,000	41,000	55,000	76,000	77,000
6	23,000	40,000	60,000	69,000	61,000	60,000
7	38,000	33,000	44,000	61,000	21,000	43,000
8	29,000	31,000	27,000	33,000	39,000	29,000
9	78,000	80,000	13,000	21,000	62,000	71,000
10	77,000	60,000	67,000	78,000	11,000	37,000
AVERAGE	49,200	50,700	40,400	48,400	51,200	57,400
STDEV	20,826	21,066	17,050	19,817	23,275	17,652
SEM	6,586	6,662	5,392	6,267	7,360	5,582

Multiple T.Test using GraphPad Prism7: control vs mixed for RFP: <0.00001

Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%.

Each row was analyzed individually, without assuming a consistent SD.

BAT1616: myo-3prom::GFP::NLS; myo-3prom::RFP::NLS

RNAi LoriT-*GFP* conjugated with L4440-*RFP* in *HT115*

	exp1		exp2		exp3	
animal	# GFP-positive muscle nuclei	# RFP-positive muscle nuclei	# GFP-positive muscle nuclei	# RFP-positive muscle nuclei	# GFP-positive muscle nuclei	# RFP-positive muscle nuclei
1	15,000	5,000	7,000	10,000	15,000	17,000
2	26,000	17,000	18,000	18,000	6,000	20,000
3	7,000	8,000	8,000	17,000	17,000	5,000
4	3,000	11,000	5,000	16,000	15,000	6,000
5	9,000	17,000	15,000	13,000	7,000	16,000
6	8,000	4,000	21,000	7,000	14,000	3,000
7	7,000	15,000	13,000	14,000	7,000	5,000
8	13,000	14,000	20,000	22,000	4,000	24,000
9	10,000	8,000	4,000	6,000	5,000	10,000
10	5,000	18,000	11,000	22,000	11,000	24,000
AVERAGE	10,300	11,700	12,200	14,500	10,100	13,000
STDEV	6,550	5,208	6,197	5,622	4,841	8,179
SEM	2,071	1,647	1,960	1,778	1,531	2,586

Multiple T.Test using GraphPad Prism7: CON vs mixed for GFP: <0.0001

Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%.

Each row was analyzed individually, without assuming a consistent SD.

BAT1616: myo-3prom::GFP::NLS; myo-3prom::RFP::NLS

RNAi L4440-*GFP* in *HT115* mixed with RNAi L4440-*RFP* in *HT115*

	exp1		exp2		exp3	
animal	# GFP-positive muscle nuclei	# RFP-positive muscle nuclei	# GFP-positive muscle nuclei	# RFP-positive muscle nuclei	# GFP-positive muscle nuclei	# RFP-positive muscle nuclei
1	48,000	52,000	31,000	54,000	33,000	56,000
2	52,000	59,000	58,000	70,000	43,000	42,000
3	46,000	59,000	61,000	24,000	55,000	70,000
4	36,000	50,000	27,000	50,000	31,000	31,000
5	27,000	86,000	32,000	25,000	27,000	63,000
6	47,000	57,000	56,000	68,000	75,000	56,000
7	34,000	34,000	41,000	28,000	70,000	34,000
8	63,000	70,000	41,000	49,000	26,000	78,000
9	53,000	54,000	49,000	54,000	39,000	47,000
10	61,000	52,000	45,000	67,000	72,000	37,000
AVERAGE	46,700	57,300	44,100	48,900	47,100	51,400
STDEV	11,567	13,557	11,902	17,698	19,365	15,862
SEM	3,658	4,287	3,764	5,597	6,124	5,016

Multiple T.Test using GraphPad Prism7: CON vs mixed for RFP: <0.0001

Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%.

Each row was analyzed individually, without assuming a consistent SD.

BAT1616: myo-3prom::GFP::NLS; myo-3prom::RFP::NLS

RNAi L4440-*GFP* in *HT115* mixed with control RNAi Rluc in *HT115*

	exp1		exp2		exp3	
animal	# GFP-positive muscle nuclei	# RFP-positive muscle nuclei	# GFP-positive muscle nuclei	# RFP-positive muscle nuclei	# GFP-positive muscle nuclei	# RFP-positive muscle nuclei
1	21,000	91,000	19,000	94,000	14,000	90,000
2	17,000	94,000	12,000	92,000	17,000	94,000
3	16,000	95,000	27,000	93,000	21,000	94,000
4	23,000	95,000	8,000	94,000	21,000	95,000
5	11,000	95,000	23,000	93,000	18,000	92,000
6	17,000	91,000	19,000	92,000	9,000	94,000
7	28,000	93,000	6,000	94,000	13,000	94,000
8	12,000	92,000	14,000	93,000	19,000	92,000
9	19,000	94,000	9,000	94,000	20,000	93,000
10	14,000	93,000	14,000	95,000	11,000	94,000
AVERAGE	17,800	93,300	15,100	93,400	16,300	93,200
STDEV	5,181	1,567	6,806	0,966	4,296	1,476
SEM	1,638	0,496	2,152	0,306	1,359	0,467

Multiple T.Test using GraphPad Prism7: control vs GFP RNAi only for GFP: <0.00001

Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%.

Each row was analyzed individually, without assuming a consistent SD.

BAT1616: myo-3prom::GFP::NLS; myo-3prom::RFP::NLS

RNAi L4440-*RFP* in *HT115* mixed with control RNAi Rluc in *HT115*

	exp1		exp2		exp3	
animal	# GFP-positive muscle nuclei	# RFP-positive muscle nuclei	# GFP-positive muscle nuclei	# RFP-positive muscle nuclei	# GFP-positive muscle nuclei	# RFP-positive muscle nuclei
1	90,000	15,000	91,000	24,000	93,000	9,000
2	95,000	37,000	93,000	29,000	93,000	21,000
3	95,000	39,000	95,000	24,000	94,000	20,000
4	94,000	19,000	91,000	10,000	94,000	11,000
5	91,000	16,000	95,000	27,000	95,000	31,000
6	95,000	41,000	92,000	16,000	91,000	50,000
7	92,000	10,000	91,000	29,000	94,000	13,000
8	95,000	21,000	92,000	11,000	93,000	29,000
9	94,000	31,000	94,000	12,000	94,000	17,000
10	94,000	25,000	92,000	37,000	95,000	24,000
AVERAGE	93,500	25,400	92,600	21,900	93,600	22,500
STDEV	1,841	10,997	1,578	9,171	1,174	12,095
SEM	0,582	3,478	0,499	2,900	0,371	3,825

Multiple T.Test using GraphPad Prism7: control vs RFP RNAi only for RFP: <0.00001

Two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli, with Q = 1%.

Each row was analyzed individually, without assuming a consistent SD.

N2

RNAi L4440-*oma-1; L4440-oma-2; LoriT-oma-1 conjugated with oma-2*

	exp1			exp2			exp3			Average	STDEV	SEM	T.Test with two-tailed distribution using Excel function	
RNAi	fertile	sterile	sterile %	fertile	sterile	sterile %	fertile	sterile	sterile %	sterile %	sterile	sterile		
control (Rluc)	40,000	0,000	0,000	39,000	1,000	2,500	40,000	0,000	0,000	0,833	1,443	0,833		
oma-1	40,000	0,000	0,000	40,000	0,000	0,000	40,000	0,000	0,000	0,000	0,000	0,000		
oma-2	40,000	0,000	0,000	40,000	0,000	0,000	39,000	1,000	2,500	0,833	1,443	0,833		
oma-1 mix oma-2	24,000	13,000	35,135	35,000	5,000	12,500	29,000	10,000	25,641	24,425	11,366	6,562		
oma-1 CON oma-2 (1)	10,000	30,000	75,000	19,000	20,000	51,282	9,000	29,000	76,316	67,533	14,089	8,134	0,015 vs mix	0,428 vs CON(2)
oma-1 CON oma-2 (3)	17,000	23,000	57,500	10,000	23,000	69,697	21,000	19,000	47,500	58,232	11,117	6,418	0,021 vs mix	0,509 vs CON(3)
oma-1 CON oma-2 (3)	13,000	27,000	67,500	12,000	27,000	69,231	18,000	22,000	55,000	63,910	7,765	4,483	0,008 vs mix	0,725 vs CON(1)
oma-1 stitched oma-2	15,000	56,000	78,873	18,000	61,000	77,215	21,000	37,000	63,793	73,294	8,270	4,774	0,031 vs CON(3)	0,032 vs CON(2)
number of animals	199,000	149,000		213,000	137,000		217,000	118,000				total n:	1033	

otIs355 [rab-3::NLS::TagRFP]

RNAi L4440-*gld-1; L4440-mex-3; LoriT-gld-1 conjugated with mex-3*

	exp1			exp2			exp3			Average	STDEV	SEM	T.Test with two-tailed distribution using Excel function	
RNAi	wt	teratoma	teratoma %	wt	teratoma	teratoma %	wt	teratoma	teratoma %	teratoma	teratoma	teratoma		
control (Rluc)	74,000	0,000	0,000	89,000	0,000	0,000	100,000	0,000	0,000	0,000	0,000	0,000		
gld-1	98,000	2,000	2,000	94,000	6,000	6,000	99,000	1,000	1,000	3,000	2,646	1,528		
mex-3	93,000	0,000	0,000	90,000	0,000	0,000	100,000	0,000	0,000	0,000	0,000	0,000		
gld-1 mix mex-3	87,000	8,000	8,421	93,000	2,000	2,105	79,000	1,000	1,250	3,925	3,917	2,261		
gld-1 CON mex-3	61,000	39,000	39,000	67,000	32,000	32,323	67,000	33,000	33,000	34,774	3,675	2,122	0,001 vs mix	
number of animals	413,000	49,000		433,000	40,000		445,000	35,000				total n:	1415	

N2

RNAi L4440-*rpn-10; L4440-rpn-12;* *LoriT-rpn-10 conjugated with rpn-12*

	exp1			exp2			exp3			Average	STDEV	SEM	T.Test with two-tailed distribution using Excel function
	survival	dead	survival %	survival	dead	dead %	survival	dead	dead %	survival	survival	survival	
RNAi													
control (Rluc)	98,000	0,000	100,000	91,000	0,000	100,000	83,000	1,000	98,810	99,603	0,687	0,397	
rpn-10	87,000	2,000	97,753	87,000	0,000	100,000	100,000	0,000	100,000	99,251	1,297	0,749	
rpn-12	93,000	0,000	100,000	87,000	2,000	97,753	91,000	0,000	100,000	99,251	1,297	0,749	
rpn-12 mix rpn-10	54,000	26,000	67,500	80,000	18,000	81,633	79,000	19,000	80,612	76,582	7,881	4,550	0,007 vs control
rpn-12 CON rpn-10	56,000	37,000	60,215	47,000	48,000	49,474	49,000	40,000	55,056	54,915	5,372	3,102	0,000 vs control 0,01 vs mix
number of animals	388,000	65,000		392,000	68,000		402,000	60,000			total n:		1375,000

BAT28: otIs305[hsp-16.2p::che-1::3xHA], ntIs1[gcy-5p::gfp]

	exp1			exp2			exp3			Average	STDEV	SEM
	no GFP	germline GFP	GFP %	no GFP	germline GFP	GFP %	no GFP	germline GFP	GFP %	GFP	GFP	GFP
RNAi												
control (Rluc)	45	2	4,26	47	0	0	45	0	0,000	1,418	2,457	1,418
lin-53	28	22	44	19	23	54,762	29	27	48,214	48,992	5,423	3,131
number of animals	73	24		66	23		74	27			total n:	287

BAT684: juls8 [unc-25::GFP]; barEx147 [hsp- 16.2/4::unc-30]

	exp1			exp2			exp3			Average	STDEV	SEM
	no GFP	germline GFP	GFP %	no GFP	germline GFP	GFP %	no GFP	germline GFP	GFP %	GFP	GFP	GFP
RNAi												
control (Rluc)	41	9	18	44	6	12	46	3	6,122	12,041	5,939	3,429
lin-53	39	11	22	37	13	26	41	9	18	22	4	2,309
number of animals	80	20		81	19		87	12			total n:	299

LoriT-lin-53 conjugated with:
Chromatin 2.0 library from Hajduskova et al., 2019
https://doi.org/10.1534/genetics.118.301674

				well #1			well #2			Average
clone #	plate #	position	target	no GFP	germline GFP	% GFP+	no GFP	germline GFP	% GFP+	GFP+
27	MH1	C3	*hmg-4*	69	21	23,333	74	9	10,843	17,088
133	MH2	C12	*rbbp-5*	42	29	40,845	58	30	34,091	37,468
166	MH2	F8	*dpl-1*	88	9	9,278	48	7	12,727	11,003
181	MH2	G12	*hda-1*	41	9	18,000	60	11	15,493	16,746
265	MH3	F8	*plk-2*	72	23	24,211	66	4	5,714	14,962
267	MH3	G12	*nhl-1*	48	5	9,434	60	4	6,250	7,842
277	MH3	G11	*hpl-2*	34	2	5,556	41	1	2,381	3,968
397	MH5	A9	*lex-1*	39	13	25,000	47	11	18,966	21,983
435	MH5	D8	*mys-1*	71	16	18,391	70	8	10,256	14,324
582	MH5	H10	*set-30*	36	3	7,692	55	1	1,786	4,739
483	MH5	H11	*set-30*	54	3	5,263	74	5	6,329	5,796
603	MH7	B9	*csp-1*	39	4	9,302	31	1	3,125	6,214
605	MH7	B11	*csp-1*	40	5	11,111	27	2	6,897	9,004
621	MH7	D3	*cbp-1*	64	7	9,859	53	2	3,636	6,748
674	MH8	H8	*prmt-3*	29	7	19,444	44	5	10,204	14,824
675	MH8	H9	*set-31*	66	9	12,000	71	18	20,225	16,112
716	MH8	C10	*ZK337.2*	38	3	7,317	45	5	10,000	8,659

BAT684: juls8 [unc-25::GFP]; barEx147 [hsp- 16.2/4::unc-30]

RNAi L4440-lin-53*; L4440-rbbp-5;* *LoriT-lin-53 conjugated with rbbp-5*

	exp1			exp2			exp3			Average	STDEV	SEM	T.Test with two-tailed distribution using Excel function
	no GFP	germline GFP	GFP %	no GFP	germline GFP	GFP %	no GFP	germline GFP	GFP %	GFP	GFP	GFP	
RNAi													
control (Rluc)	57	6	9,524	33	3	8,333	61	7	10,294	9,384	0,988	0,570	
lin-53	31	9	22,500	40	11	21,569	57	12	17,391	20,487	2,721	1,571	0,612 vs mix
rbbp-5	60	11	15,493	44	20	31,250	50	11	18,033	21,592	8,460	4,884	0,864 vs mix
lin-53 mix rbbp-5	33	9	21,429	65	15	18,750	47	17	26,563	22,247	3,970	2,292	
lin-53 CON rbbp-5	52	39	42,857	29	20	40,816	59	41	41,000	41,558	1,129	0,652	0,001 vs rbbp-5 0,0004 vs lin-53
lin-53 stitch rbbp-5	41	30	42,254	40	39	49,367	45	30	40,000	43,874	4,889	2,823	0,202 vs CON
number of animals	233	74		211	69		274	88			total n:		949,0000

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