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Supplemental Information

A Family of Argonaute-Interacting Proteins Gates Nuclear RNAi

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LAP::NRDE-3 - wild-type background				LAP::NRDE-3 - *enri-1(tag1601)* background			
Sequence name	Protein	# datasets detected	Peptide coverage	Sequence name	Protein	# datasets detected	Peptide coverage
F43C11.9	ENRI-1	5/5	26-50%	T24C2.2	ENRI-2	2/2	46.3-46.8%
T08B2.9	FARS-1	4/5	4-16%	K08E7.2	HSB-1	2/2	35%
F16A11.3	PPFR-1	4/5	1-3%	Y56A3A.21	TRAP-4	2/2	21.4-44%
K02A11.1	GFI-2	3/5	9-14%	F26F4.13	KBP-2	2/2	18.5-26.6%
C02C6.1	DYN-1	3/5	4-7%	Y37E3.9	PHB-1	2/2	20-31.3%
Y54H5A.1	Y54H5A.1	3/5	8-9%	T05G5.3	CDK-1	2/2	21.1-39.8%
C27A12.8	ARI-1	3/5	6-14%	K04G7.4a	NUO-4	2/2	11-30.3%
Y57B8A.30	PPH-4.1	3/5	8-22%	R05F9.10	SGT-1	2/2	18.4%
C33G3.3	LGC-21	3/5	7-12%	Y116A8C.35a	UAF-2	2/2	10.2-15.1%
F46A9.5	SKR-1	2/5	31-33%	C44B7.10	ACER-1	2/2	17.8-18.7%
T24C2.2	ENRI-2	2/5	14-31%	C09H10.3	NUO-1	2/2	9-10.6%
C25B8.4	CLEC-266	2/5	13-22%	K12D12.1	TOP-2	2/2	10.9-12.8%
T12D8.8	HIP-1	2/5	10-28%	Y71G10AL.1	Y71G10AL.1	2/2	8.3-12%
F42E11.4	TNI-1	2/5	23%	C25A1.10b	DAO-5	2/2	3.2-10.4%
B0303.15	B0303.15	2/5	20%	W01G7.3	RPB-11	2/2	17.2-27%
F30F8.9	F30F8.9	2/5	17-18%	F44E5.4	F44E5.4	2/2	13.8-14%
Y54E10A.6	Y54E10A.6	2/5	13-15%	Y49E10.1	RPT-6	2/2	15.6-26.9%
W02D3.2	DHOD-1	2/5	13-15%	F31E3.3	RFC-4	2/2	23.4-37.1%
H06O01.1	PDI-3	2/5	11-13%	R04F11.2	R04F11.2	2/2	10.3-40.2%
B0361.5	PSD-1	2/5	14%	F58F12.1	F58F12.1	2/2	16-25.2%
C01B10.11	C01B10.11	2/5	14%	Y110A2AL.13	PINN-1	2/2	12.4-26.7%
C06G3.9	C06G3.9	2/5	7-13%	B0250.5	B0250.5	2/2	16.1-41.1%
B0511.1	FKB-7	2/5	8-10%	F56F11.4	F56F11.4	2/2	12.1-24%

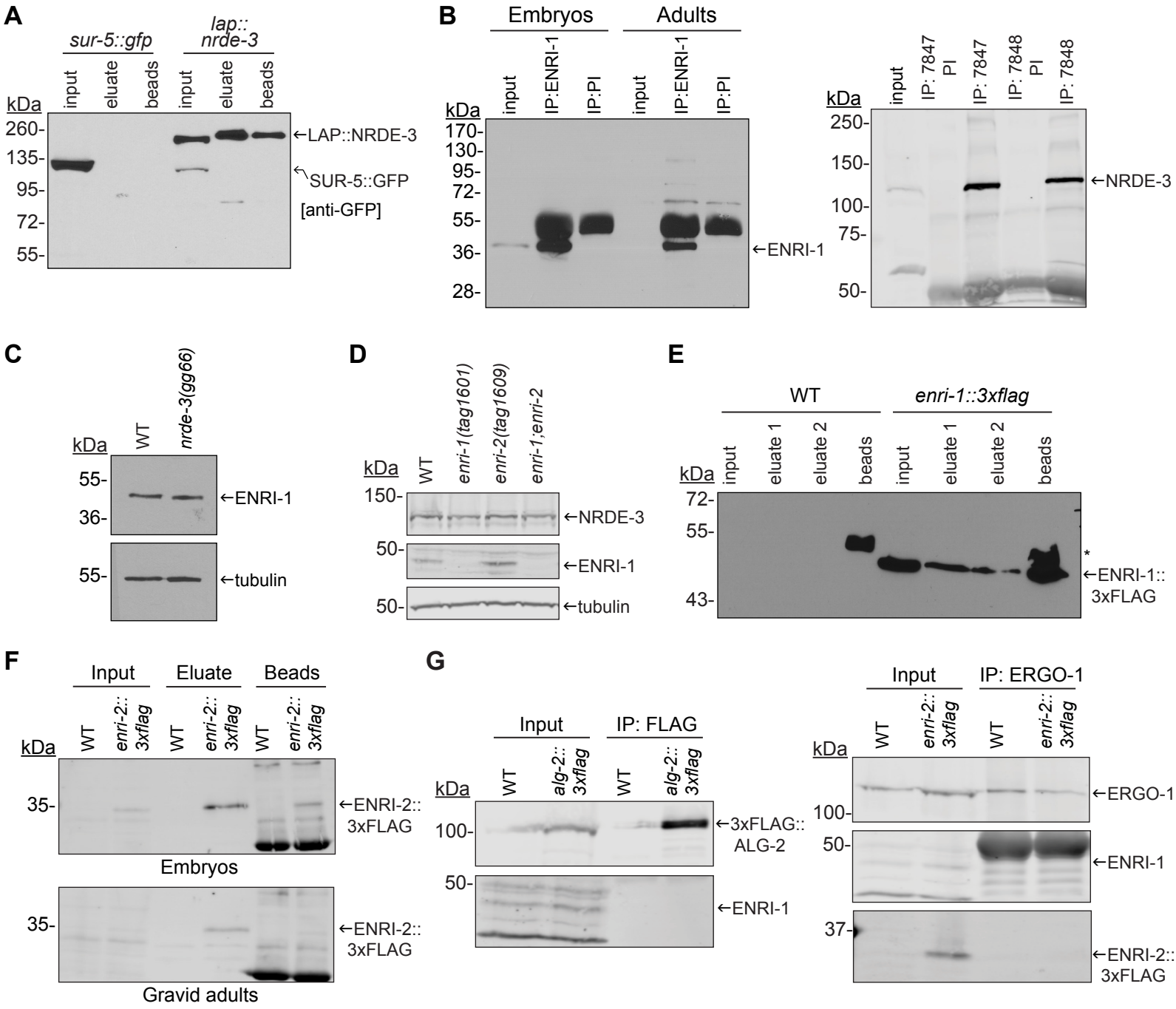
F37C4.5	F37C4.5	2/5	4-17%	C52D10.9	SKR-8	2/2	10.8-23.7%
C06A5.6	C06A5.6	2/5	5-11%	Y105C5B.13	SKR-10	2/2	19.8-21.4%
C07E3.1	STIP-1	2/5	4-11%	C04C3.3	PDHB-1	2/2	16.2-16.5%
Y37D8A.9	MRG-1	2/5	9%	W02D3.1	CYTB-5.2	2/2	15.6-22%
B0495.8	B0495.8	2/5	8-9%	Y53F4B.22a	ARP-1	2/2	8.6-21.4%
F33D4.6	F33D4.6	2/5	6-8%	H34C03.2a	H34C03.2a	2/2	14.2-21.9%
Y108G3AL.2	Y108G3AL.2	2/5	7%	C36B1.3	RPB-3	2/2	14.2%
T21D12.4	PAT-6	2/5	7%	Y71H2AM.23	TUFM-1	2/2	4.8-19.6%
K02F2.2	AHCY-1	2/5	7%	C26E6.4	RPB-2	2/2	14.8-21%
Y55F3BR.1	Y55F3BR.1	2/5	6-7%	F37C4.5a	F37C4.5a	2/2	9-21.1%
Y47D3A.16	RSKS-1	2/5	5-7%	T01G9.6a	KIN-10	2/2	12-26.5%
F22B7.5	DNJ-10	2/5	5-7%	D2085.1	PYR-1	2/2	11.1-12.6%
C25H3.4	C25H3.4	2/5	3-8%	R07E5.3	SNFC-5	2/2	16.8-21.8%
ZK1053.4	ZK1053.4	2/5	5%	W08F4.3	W08F4.3	2/2	17.1-22.8%
T10F2.3	ULP-1	2/5	4%	F56G4.2	PES-2.1	2/2	9.4-10.3%
C33G3.6	C33G3.6	2/5	3-4%	Y44E3A.6	Y44E3A.6	2/2	9.5-20.5%
F58G1.1	WAGO-4	2/5	2-3%	C15H11.7	PAS-1	2/2	9.3-15.4%
Y45F10D.7	Y45F10D.7	2/5	2%	K10C3.6	NHR-49	2/2	10.2-12.4%
ZK688.5	ZK688.5	2/5	2%	Y60A3A.9	Y60A3A.9	2/2	8.5-13.3%
F02E9.4	SIN-3	2/5	2%	Y47D3A.26	SMC-3	2/2	10.7-11.3%

Table S1. List of proteins found in NRDE-3 purifications. Refers to Figure 1. All proteins found in at least 2 biological replicates (2 independent purifications) are listed with their sequence name, protein name, number of datasets they were found in, and peptide coverage.

Oligonucleotide sequence	Source
enri-1 crRNA (*qe16*): TACGACAGCAAAACTATATT	This study
enri-2 crRNA (*qe21*): CCGTCAAGAAAGTCACCGATAGC	This study
enri-2 crRNA (*qe21*): ACGATTACAATCCATTCAAGTGG	This study
enri-3 crRNA (*qe17*): ttttacagattaatagac	This study
enri-3 crRNA (*qe19, qe20*): CTGACAGAAATGCCCGATGG	This study
enri-1 crRNA 5' *(tag1600, tag1601):* GGATCCGGAATATGATTCGG	This study
enri-1 crRNA 3' (*tag1600, tag1601)*: GTAAAATTGCGGAGTCGTTG	This study
enri-2 crRNA 5' (*tag1609)*: TTTTTCAATGGTTTTTATGG	This study
enri-2 crRNA 3' (*tag1609)*: TTTTTTGGGCTCCGCAACGG	This study
enri-2::3xflag crRNA: GTTGGGGTTTTAAAGAGTTG	This study
gfp::enri-1 crRNA: ATTGGATGTTTGGTAATTTA	This study
enri-2::gfp crRNA: ACAATCCATTCAAGTGGTTG	This study
enri-3::gfp crRNA: CTTTGGGTTTGGGAAATTAT	This study
enri-1(qe16) repair template*:* CGGCAATGGCAGCTCTGCTAACACTCTACGACAGCAAAACTATtagctgactaagcttcgc ATTCGGAACGTTTTCGGAATATCTAATAACAAATGAC	This study
enri-2::3xflag repair template: GATGACGACGATTACAATCCATTCAAGTGGTTGGGGTTTgactacaaggaccacgacggtg actacaaggaccacgaTatcgactacaaggacgacgacgacaagTAAagagttgtggagatttgttttaaattgatat cacg	This study
2X TY1 *enri-1* GFP primer F: AATATGATTCGGAGGAAGAGTATAATTGGATGTATAAATTGGATGTTTGGGAAGTG CATACCAATCAGGACCCGC	This study
2X TY1 *enri-1* GFP primer R: TTCTTGGGTTCAACGTTGTCATGCAAGAAAAAGAAGAGATCCATAAATTACTTGTA GAGCTCGTCCATTCCGTGG	This study
2X TY1 *enri-2* GFP primer F: AGCGCTGAGGATGATGAATATGATTCCGAATATGATGACGACGACTACAACCCTT TTAAATGGTTGGGGTTTGAAGTGCATACCAATCAGGACCCGC	This study
2X TY1 *enri-2* GFP primer R: CTAGAAAATAAACGTGATATCAATTTAAAACAAATCTCCACAACTCTTTACTTGTAG AGCTCGTCCATTCCGTGG	This study
2X TY1 *enri-3* GFP primer F: ATGCTGCGCGTTACGATGATGGTTACGACGAAGGCCACGAATACTGCCAAGGAG GCGGGGGTTCTGGGGGAGGTG	This study
2X TY1 enri-3 GFP primer R: CGGGGAACCGTAAAAATGGGGATAAAATGTGCTTTGGGTTTGGGAAATTACTTGT AGAGCTCGTCCATTCCGTGG	This study
pBlueScript Gibson *pbli-1::enri-2* primer F: GGGTTTTAAGAGCTCCAGCTTTTGTTCCCTT	This study
pBlueScript Gibson *pbli-1::enri-2* primer R: acgacgtGAGCTCCAGCTTTTGTTCCCTT	This study
nip-2 Gibson *pbli-1::enri-2* primer F: taacctcatcATGTTTAGCGTTTACGCCCACC	This study
nip-2 Gibson *pbli-1::enri-2* primer R: TGGAGCTCacgtcgtttattgaaatgaactcaacgaac	This study
bli-1 Gibson *pbli-1::enri-2* primer F: CGAATTCttcccgcggactcccaggacc	This study
bli-1 Gibson *pbli-1::enri-2* primer R: CGCTAAACATgatgaggttagatcacactactctccg	This study
pBlueScript Gibson *pbli-1::enri-1* primer F: cgcgggaaGATATCAAGCTTATCGATACCGTCGACCT	This study
pBlueScript Gibson *pbli-1::enri-1* primer R: tacaagactaGGATCCACTAGTTCTAGAGCGGC	This study
bli-1 Gibson *pbli-1::enri-1* primer F: TTGATATCttcccgcggactcccaggacc	This study

bli-1 Gibson *pbli-1::enri-1* primer R: GCTATTTTCATgatgaggttagatcacactactctccg	This study
enri-1 Gibson *pbli-1::enri-1* primer F: ctaacctcatcATGAAAATAGCAAAATTCACCCGAATTAATGC	This study
enri-1 Gibson *pbli-1::enri-1* primer F: CTAGTGGATCCtagtcttgtatgtattcttgtagtctatactgtaaatggactat	This study
enri-1 cDNA primer F for pET-28a cloning: ATAATAGAATTCATGAAAATAGCAAAATTCACC	This study
enri-1 cDNA primer R for pET-28a cloning: TATTATGTCGACTTACCAAACATCCAATTTATACA	This study
nrde-3 cDNA (aa 1-383) primer F for pSMT3 cloning: AATTAAGAGCTCGATCTCCTAGACAAAGTAATG	This study
nrde-3 cDNA (aa 1-383) primer R for pSMT3 cloning: AATAAACTCGAGATAATCAATTTCACCATCACGC	This study
T7-*unc-22* RNAi F: TAATACGACTCACTATAGGGGGAAGATGGGTTCCATGCG	Kamath et al., 2003
T7-*unc-22* RNAi R: TAATACGACTCACTATAGGGCCGAGCACAATGACCTCAAC	Kamath et al., 2003
T7-*lir-1* RNAi F: GTAATACGACTCACTATAGGGACTCCGCTCCACCCCTATAC	Kamath et al., 2003
T7-*lir-1* RNAi R: GTAATACGACTCACTATAGGGGCTCACCCTCTCAACTCGTC	Kamath et al., 2003
T7-*dpy-11* RNAi F: TAATACGACTCACTATAGGGAGATCTTCCAAGCTCGTCTTTCT	Kamath et al., 2003
T7-*dpy-11* RNAi R: TAATACGACTCACTATAGGGATGTCGCTTTAATTACGTGTCGT	Kamath et al., 2003
T7-*bli-1* RNAi F: TAATACGACTCACTATAGGGCACACCGACAAACTCCACAC	Kamath et al., 2003
T7-*bli-1* RNAi R: TAATACGACTCACTATAGGGGGGGAAGGGGTGAAAATAAA	Kamath et al., 2003
nrde-3 (aa 1-383) primer F for pGEX-6p-1 cloning: ATAGAATTCATGGATCTCCTAGACAAAGTAATGG	This study
nrde-3 (aa 1-383) primer R for pGEX-6p-1 cloning: AATACTCGAGATAATCAATTTCACCATCACGC	This study
nrde-3 (aa 365-701) primer F for pGEX-6p-1 cloning: ATAGAATTCGGGAACAGTAGAAAATACGATG	This study
nrde-3 (aa 365-701) primer R for pGEX-6p-1 cloning: ATAGTCGACTTAGGTTTGCTGTCCAAGCTTCTC	This study
nrde-3 (aa 683 – 1058) primer F for pGEX-6p-1 cloning: ATAGAATTCCGACCAGATATGCATGACATTCTC	This study
nrde-3 (aa 683 – 1058) primer R for pGEX-6p-1 cloning: ATAGTCGACTTATGCCCAAAAGTTGCGTC	This study
enri-1 primer F for pSMT3 cloning: ATCGGCGGAGCTCATGAAAATAGCAAAATTCACCCG	This study
enri-1 primer R for pSMT3 cloning: ATAATAGCGGCCGCCCAAACATCCAATTTATAC	This study
enri-2 primer F for pSMT3 cloning: AATTAAGAGCTCTTTAGCGTTTACGCCCAC	This study
enri-2 primer R for pSMT3 cloning: AATTAACTCGAGTTAAAACCCCAACCACTTGAATG	This study

Table S3. Related to STAR methods. List of oligonucleotides including primers, crRNAs and repair templates used throughout the manuscript.



I

ENRI-1/NRDE-3 shared interactors	ENRI-1/ENRI-2 shared interactors			ENRI-2/NRDE-3 shared interactors
SKR-1	NRDE-3	DLI-1	PCN-1	C06A5.6
FARS-1	CSN-5	DNC-2	T05F1.2	ARI-1
HIP-1	CSN-1	GST-1	SUP-36	
C33G3.6	CSN-6	IFE-1	UNC-59	
C01B10.11	CDL-1	MRPS-34	UNC-61	

J

	NIP-1::3xFLAG PTM analysis	
	Replicate #1	**Replicate #2**
Number of peptides	25 unique 64 total peptides	27 unique 72 total peptides
Peptide coverage	35.28% peptide coverage	23.61% peptide coverage
Phosphorylated residues	S224 and S283	S224 and S283

Figure S1

Figure S1. **Refers to Figure 1. Purification of NRDE-3 and ENRI-1.** (A) Representative western blot (GFP antibody) from FLAG purifications performed on *lap::nrde-3* embryos and *sur-5::gfp* embryos (negative control). Proteins were eluted with FLAG peptide and 25% of the eluate was analyzed by western blot to confirm efficacy of purifications. (B) Left: Newly generated ENRI-1 antibody was used to IP endogenous ENRI-1 from wild-type adult and embryo extracts using the pre-immune (PI) serum as a negative control. The same antibody was used to perform a western blot to confirm its ability to IP ENRI-1. Right: Newly generated NRDE-3 antibodies from two rabbits (#7847 and #7848) were used to IP endogenous NRDE-3 from wild-type embryo extracts using the pre-immune (PI) serum as a negative control. The serum from rabbit #7848 was used to perform a western blot to confirm its ability to IP and detect NRDE-3. Serum #7848 was used in IPs and western blots for the rest of the experiments. (C) Wild-type and *nrde-3(gg66)* embryo lysates were probed with the ENRI-1 antibody and tubulin antibody. (D) Wild-type, *enri-1(tag1601)*, *enri-2(tag1609), enri-1(tag1601);enri-2(tag1609)* embryo lysates were probed with polyclonal antibodies to NRDE-3 and ENRI-1 and a tubulin antibody. (E) Representative western blot (FLAG antibody) from FLAG purifications performed on transgenic *enri-1::3xflag* embryos. Proteins were eluted with FLAG peptide and 25% of eluates were analyzed by western blot to confirm efficacy of purifications. Beads were incubated twice with the FLAG peptide to maximize elution efficacy. (F) Representative western blot (FLAG antibody) from FLAG purifications performed on *enri-2::3xflag* embryos and gravid adults. Proteins were eluted with 0.5M NH_4OH pH 11 and 25% of the eluate was analyzed by western blot to confirm efficacy of purifications. (G) Left: Wild-type and *alg-2::3xflag* embryos were subjected to FLAG immunoprecipitation and probed for FLAG and ENRI-1. Right: Wild-type and *enri-2::3xflag* embryos were subjected to endogenous ERGO-1 immunoprecipitation and probed for ERGO-1, ENRI-1, and FLAG. (H) Wild-type and *enri-2::3xflag* embryos were subjected to FLAG immunoprecipitation. Input and IPs were probed for FLAG, and endogenous ALG-2 and ERGO-1. In the FLAG and ALG-2 blots, the dotted lines represent the removal of two unrelated lanes. (I) Shared interactors between ENRI-1/NRDE-3, ENRI-1/ENRI-2, and ENRI-2/NRDE-3. (J) NIP-1::3xFLAG was immunoprecipitated from embryos and the band corresponding to NIP-1::3xFLAG was cut out of a Coomassie-stained gel and subsequently sent for mass

spectrometry and post-translational modification analysis. Table depicts the number of peptides recovered in each replicate, peptide coverage and the two confidently assigned phosphorylated residues.

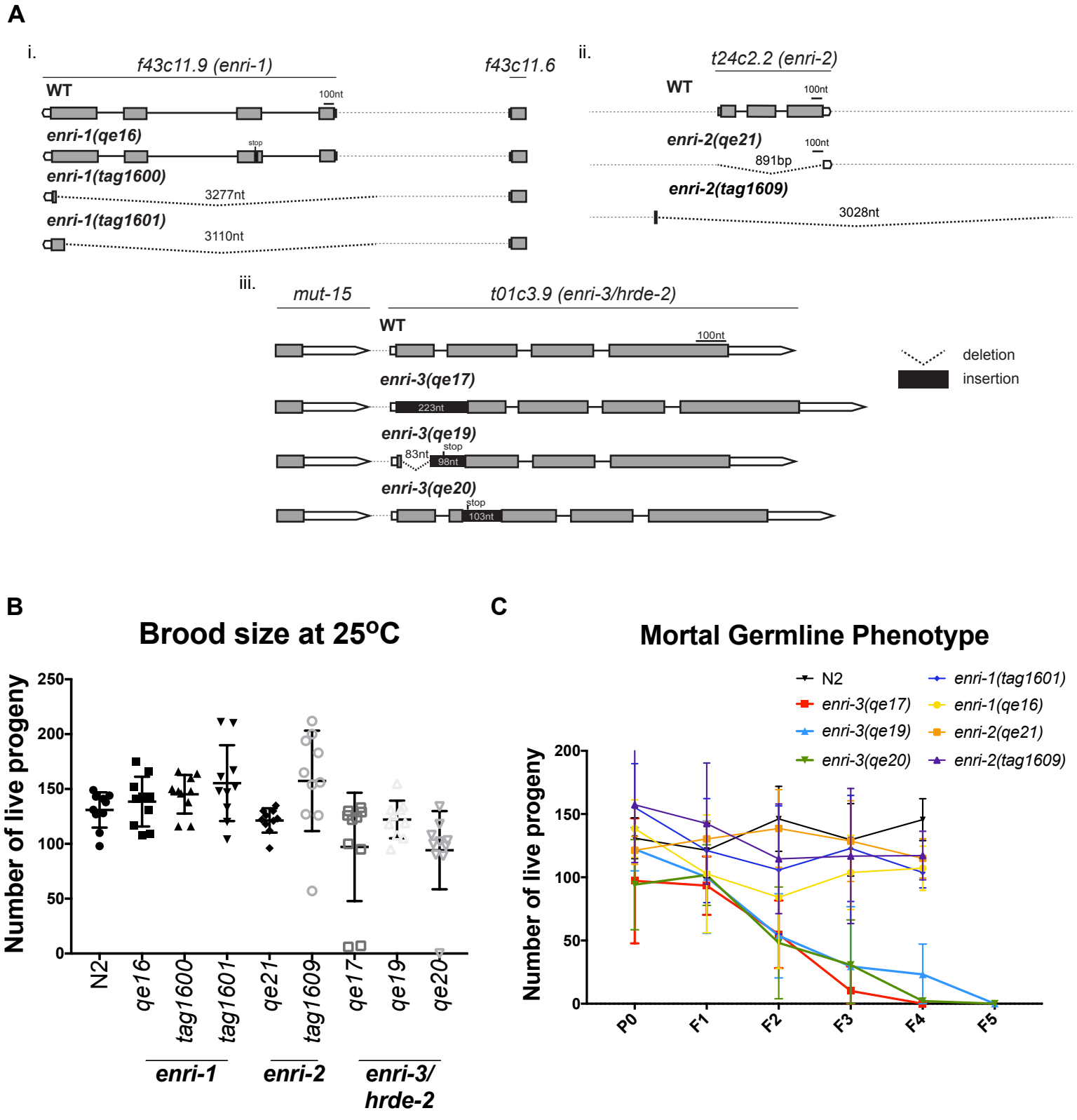

A
i.
f43c11.9 (enri-1)
f43c11.6
WT
100nt
enri-1(qe16)
stop
enri-1(tag1600)
3277nt
enri-1(tag1601)
3110nt
ii.
t24c2.2 (enri-2)
WT
100nt
enri-2(qe21)
100nt
891bp
enri-2(tag1609)
3028nt
iii.
mut-15
t01c3.9 (enri-3/hrde-2)
WT
100nt
deletion
insertion
enri-3(qe17)
223nt
enri-3(qe19)
83nt
stop
98nt
enri-3(qe20)
stop
103nt
B
Brood size at 25°C
Number of live progeny
250
200
150
100
50
0
N2
qe16
tag1600
tag1601
qe21
tag1609
qe17
qe19
qe20
enri-1
enri-2
enri-3/
hrde-2
C
Mortal Germline Phenotype
N2
enri-3(qe17)
enri-3(qe19)
enri-3(qe20)
enri-1(tag1601)
enri-1(qe16)
enri-2(qe21)
enri-2(tag1609)
Number of live progeny
200
150
100
50
0
P0
F1
F2
F3
F4
F5

Figure S2

Figure S2. **Refers to Figure 2. Generation and characterization of *enri* mutants using CRISPR/Cas9.** (A) Null alleles were generated for *enri-1*, *enri-2* and *enri-3* using the CRISPR/Cas9 gene editing system. Deletions and insertions are to scale. (B) Brood sizes of n > 10 worms were scored at 25°C. Data is depicted as mean ± SD. (C) Animals were switched to 25°C after being grown at 16°C for at least 3 generations. Brood size of n > 10 worms were monitored at each generation for up to 5 generations to monitor for the mortal germline phenotype. Data is depicted as mean ± SD.

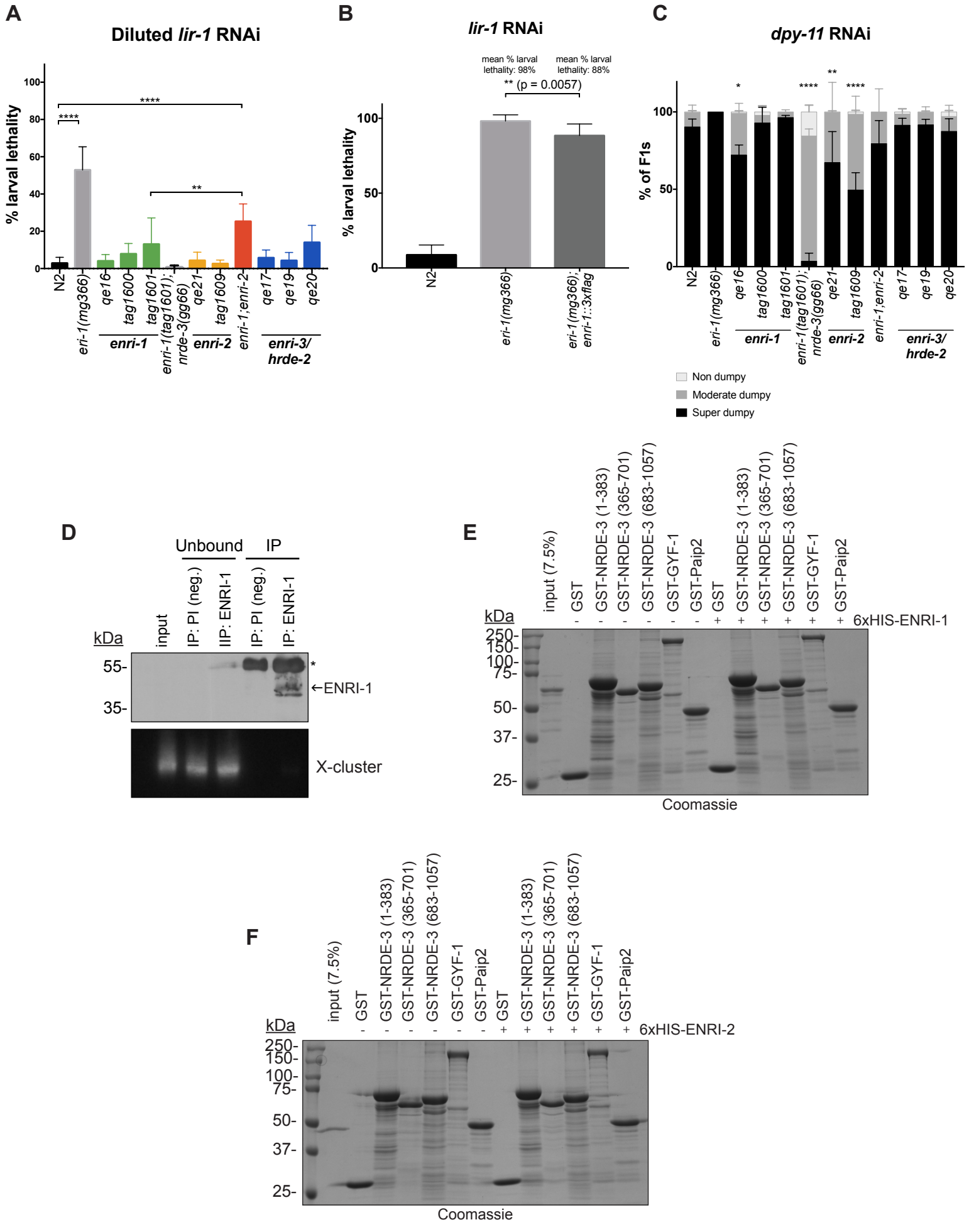

A
Diluted lir-1 RNAi
% larval lethality
N2
eri-1(mg366)
qe16
tag1600
tag1601
enri-1
enri-1(tag1601); nrde-3(gg66)
qe21
tag1609
enri-2
enri-1;enri-2
qe17
qe19
qe20
enri-3/ hrde-2
B
lir-1 RNAi
mean % larval lethality: 98%
mean % larval lethality: 88%
** (p = 0.0057)
% larval lethality
eri-1(mg366)
eri-1(mg366); enri-1::3xflag
C
dpy-11 RNAi
% of F1s
Non dumpy
Moderate dumpy
Super dumpy
D
Unbound
IP
input
IP: PI (neg.)
IIP: ENRI-1
IP: PI (neg.)
IP: ENRI-1
kDa
55-
35-
←ENRI-1
X-cluster
E
input (7.5%)
GST
GST-NRDE-3 (1-383)
GST-NRDE-3 (365-701)
GST-NRDE-3 (683-1057)
GST-GYF-1
GST-Paip2
6xHIS-ENRI-1
kDa
250-
150-
100-
75-
50-
37-
25-
Coomassie
F
input (7.5%)
GST
GST-NRDE-3 (1-383)
GST-NRDE-3 (365-701)
GST-NRDE-3 (683-1057)
GST-GYF-1
GST-Paip2
6xHIS-ENRI-2
kDa
250-
150-
100-
75-
50-
37-
25-
Coomassie

Figure S3

Figure S3. **Refers to Figure 2 and 3*.* ENRI-1 and ENRI-2 bind unloaded NRDE-3 and suppress nuclear RNAi.** (A) *lir-1* RNAi bacteria was diluted 3-fold in M9 before seeding on plates. Tukey's multiple comparisons test was performed to determine statistical significance. Data is depicted as mean ± SD. (B) Worms overexpressing transgenic ENRI-1::3xFLAG in the *eri-1(mg366)* background, as well as wild-type and *eri-1(mg366)* worms were exposed to *lir-1* feeding RNAi at the L1 stage and 3 days later the number of animals that had survived to the gravid adult stage were quantified (n > 3 biological replicates). Tukey's multiple comparisons test was performed to determine statistical significance. Data is depicted as mean ± SD. (C) Worms were exposed to *dpy-11* RNAi at the L4 stage and their progeny was scored for the dumpy phenotype at the gravid adult stage. Worms were scored as being non-dumpy, moderately dumpy, or super dumpy. Asterisks indicate differences in the number of super dumpy worms compared to N2 (WT). Data is depicted as mean ± SD. For all RNAi assays: p-value: 0.05 – 0.0332 (*), 0.0333 – 0.0021 (**), 0.0022 – 0.0002 (***), < 0.0001 (****). (D) RNA extracted from an endogenous ENRI-1 IP (pre-immune (PI) serum used as a negative control) was probed for the X-cluster. (E + F) Coomassie stained gel of the GST pull-down between 6xHIS-ENRI-1 (E) or 6xHIS-ENRI-2 (F) and three fragments of GST-NRDE-3, and GST, GST-GYF-1, and GST-Paip2 as negative controls. 50% of the pull-down was loaded onto a 10% SDS-PAGE gel for Coomassie staining. Each pull-down was performed at least 4 times and a representative blot for each is shown.

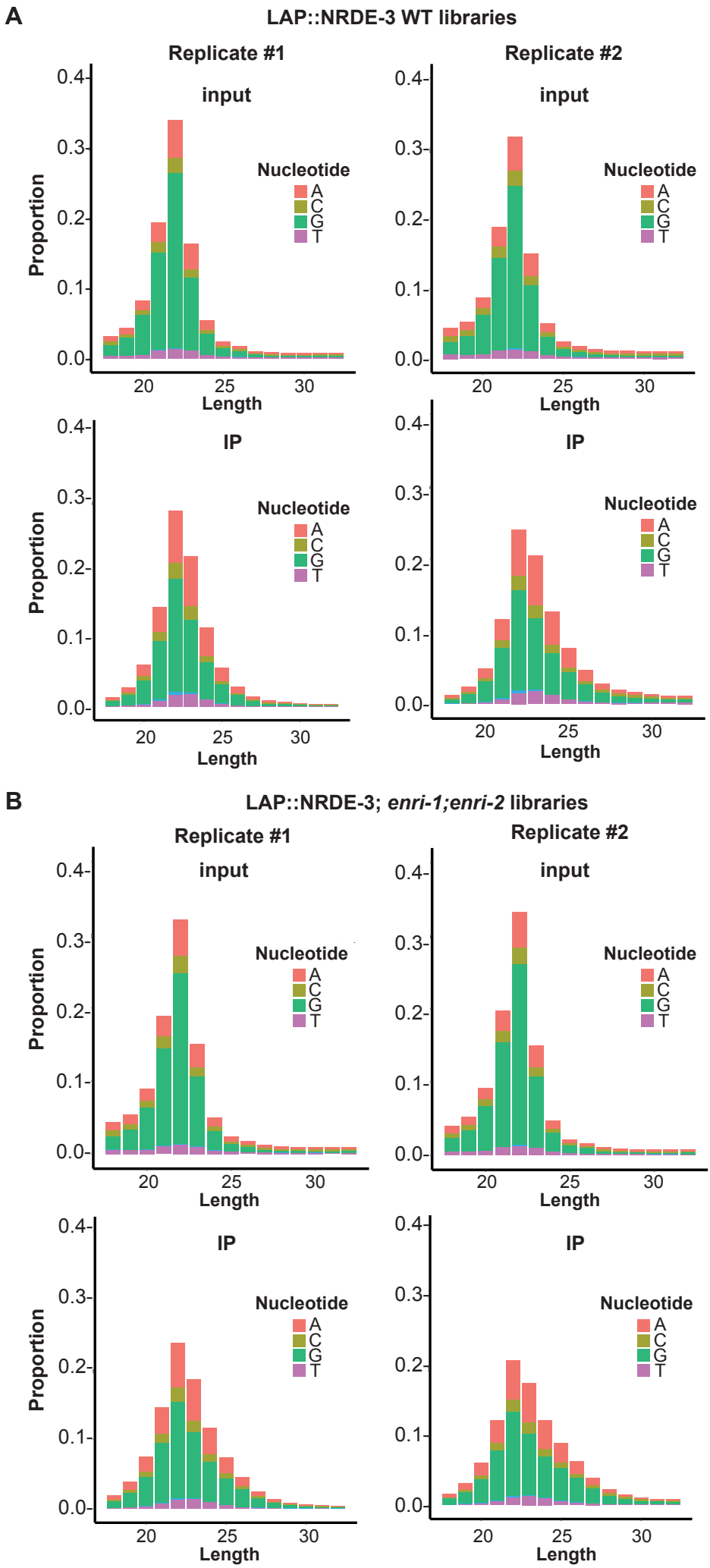

A
LAP::NRDE-3 WT libraries
Replicate #1
Replicate #2
input
IP
Proportion
Length
Nucleotide
A
C
G
T
B
LAP::NRDE-3; enri-1;enri-2 libraries

Figure S4

***Figure S4*. Refers to Figure 5. 22G-RNAs are enriched in libraries.** (A) Enrichment of 22G-RNAs after 5' independent library preparation from the input (top) and LAP::NRDE-3 IP (bottom) fractions in wild-type embryos. (B) Enrichment of 22G-RNAs after 5' independent library preparation from the input (top) and LAP::NRDE-3 IP (bottom) fractions in *enri-1(tag1601);enri-2(tag1609)* embryos.

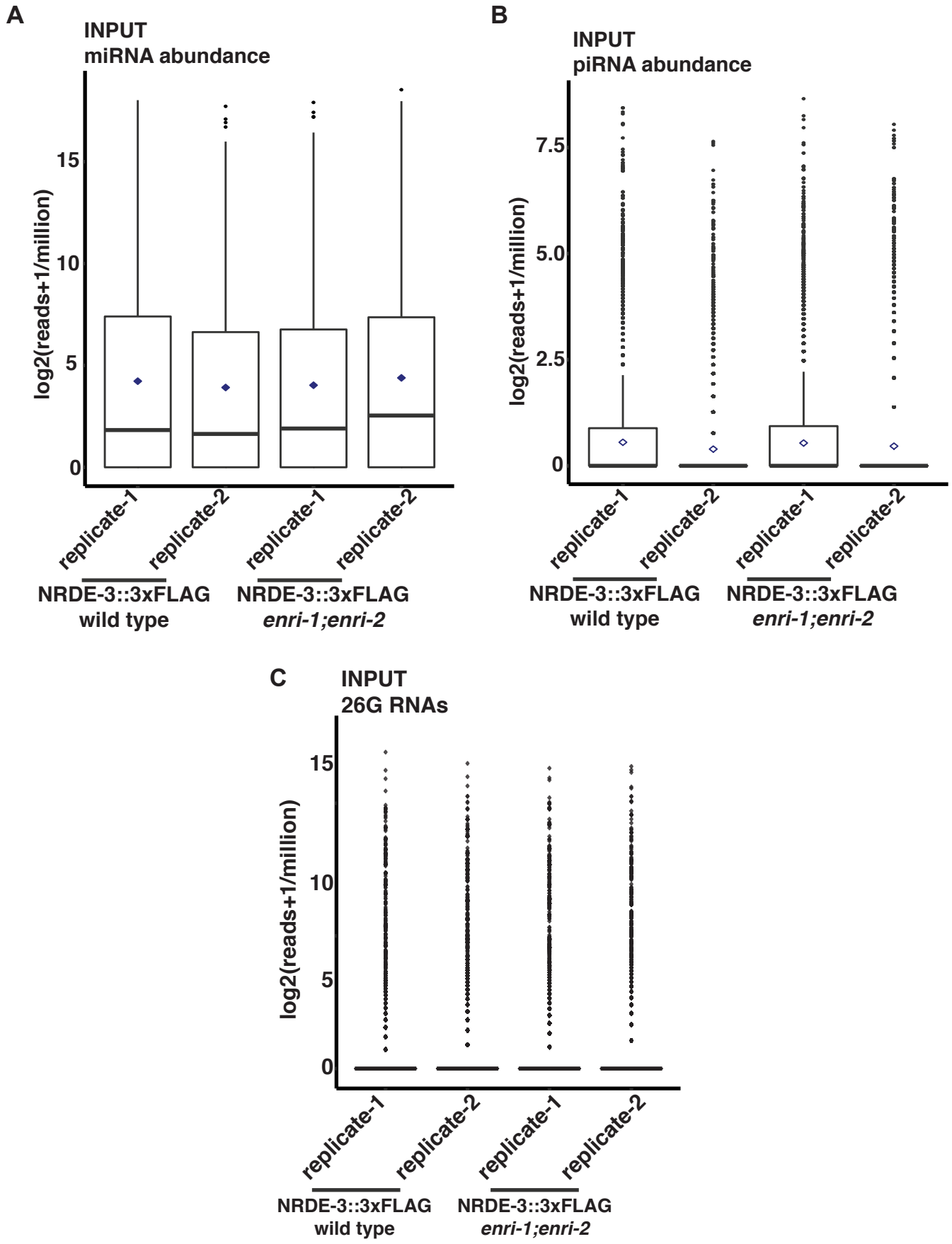

A
INPUT
miRNA abundance
log2(reads+1/million)
15
10
5
0
replicate-1
replicate-2
replicate-1
replicate-2
NRDE-3::3xFLAG
wild type
NRDE-3::3xFLAG
enri-1;enri-2
B
INPUT
piRNA abundance
log2(reads+1/million)
7.5
5.0
2.5
0
replicate-1
replicate-2
replicate-1
replicate-2
NRDE-3::3xFLAG
wild type
NRDE-3::3xFLAG
enri-1;enri-2
C
INPUT
26G RNAs
log2(reads+1/million)
15
10
5
0
replicate-1
replicate-2
replicate-1
replicate-2
NRDE-3::3xFLAG
wild type
NRDE-3::3xFLAG
enri-1;enri-2

Figure S5

Figure S5. **Refers to Figure 5. Abundance of other small RNAs species in the libraries**. (A) Abundance of miRNAs in the input fraction of each replicate from wild-type and *enri-1;enri-2* embryos. (B) Abundance of piRNAs in the input fraction of each replicate from wild-type and *enri-1;enri-2* embryos. (C) Abundance of 26G-RNAs in the input fraction of each replicate from wild-type and *enri-1;enri-2* embryos.

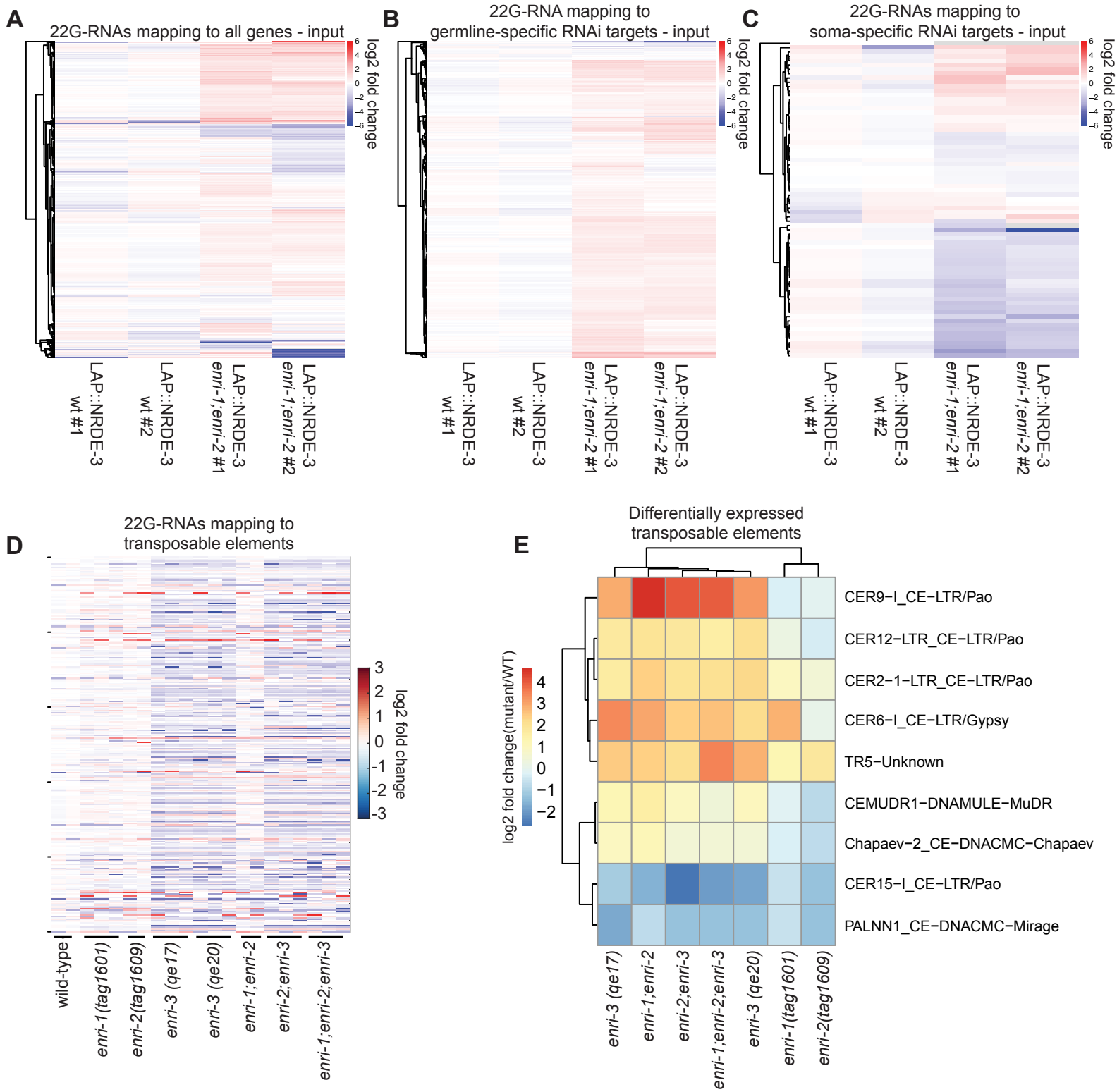
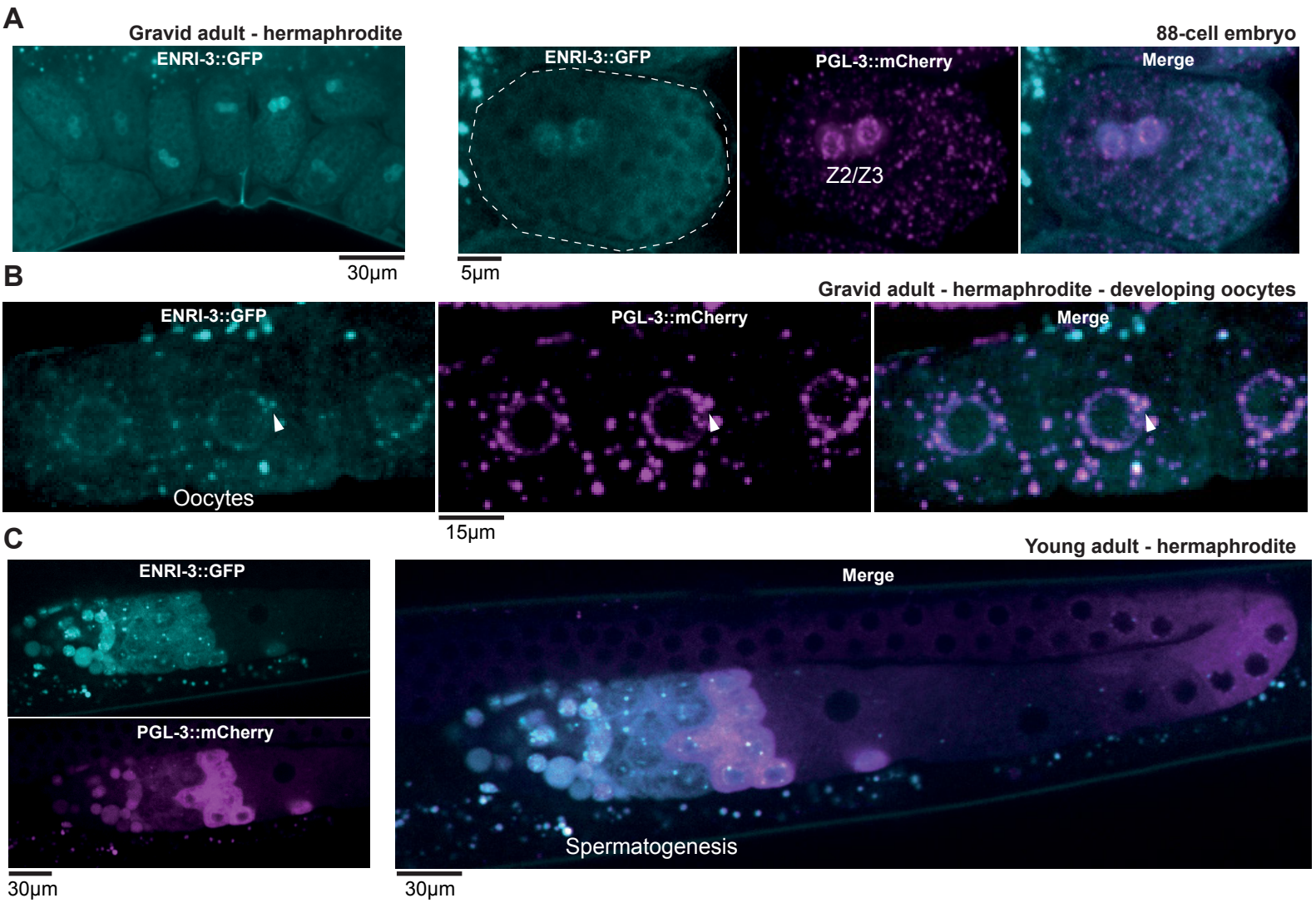
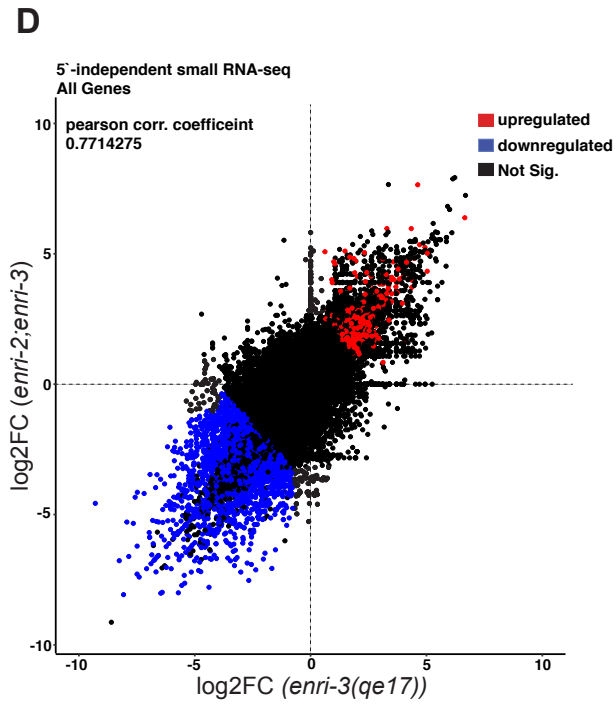

A
22G-RNAs mapping to all genes - input
log2 fold change
LAP::NRDE-3 wt #1
LAP::NRDE-3 wt #2
LAP::NRDE-3 enri-1;enri-2 #1
LAP::NRDE-3 enri-1;enri-2 #2
B
22G-RNA mapping to germline-specific RNAi targets - input
C
22G-RNAs mapping to soma-specific RNAi targets - input
D
22G-RNAs mapping to transposable elements
wild-type
enri-1(tag1601)
enri-2(tag1609)
enri-3 (qe17)
enri-3 (qe20)
enri-1;enri-2
enri-2;enri-3
enri-1;enri-2;enri-3
E
Differentially expressed transposable elements
log2 fold change(mutant/WT)
CER9-I_CE-LTR/Pao
CER12-LTR_CE-LTR/Pao
CER2-1-LTR_CE-LTR/Pao
CER6-I_CE-LTR/Gypsy
TR5-Unknown
CEMUDR1-DNAMULE-MuDR
Chapaev-2_CE-DNACMC-Chapaev
CER15-I_CE-LTR/Pao
PALNN1_CE-DNACMC-Mirage

Figure S6

Figure S6. **Refers to Figures 5 and 6. Mutant *enri-1* and *enri-2* embryos display defects in 22G-RNA accumulation.** (A) Heat map of all 22G-RNAs mapping to individual genes from the input samples (WT and *enri-1;enri-2*). (B) Heat map of 22G-RNAs mapping to all annotated germline-specific RNAi targets from the input samples (WT and *enri-1;enri-2*). (C) Heat map of 22G-RNAs mapping to all annotated soma-specific RNAi targets from the input samples (WT and *enri-1;enri-2*). (D) Heat map of all 22G-RNAs mapping to annotated transposable elements from wild-type, *enri-1(tag1601)*, *enri-2(tag1609)*, *enri-3(qe17), enri-3(qe20), enri-1(tag1601);enri-2(tag1609)*, *enri-2(tag1609);enri-3(qe19)*, *enri-1(tag1601);enri-2(tag1609);enri-3(qe19)*. (E) Heat map of transposable elements (total mRNA) that were differentially expressed in *enri-1(tag1601)*, *enri-2(tag1609)*, *enri-3(qe17), enri-3(qe20), enri-1(tag1601);enri-2(tag1609)*, *enri-2(tag1609);enri-3(qe19)*, and *enri-1(tag1601);enri-2(tag1609);enri-3(qe19)* young adults compared to wild-type.


A
Gravid adult - hermaphrodite
ENRI-3::GFP
30µm
88-cell embryo
ENRI-3::GFP
PGL-3::mCherry
Z2/Z3
Merge
5µm
B
Gravid adult - hermaphrodite - developing oocytes
ENRI-3::GFP
Oocytes
PGL-3::mCherry
Merge
15µm
C
Young adult - hermaphrodite
ENRI-3::GFP
PGL-3::mCherry
30µm
Merge
Spermatogenesis
30µm


D
5`-independent small RNA-seq
All Genes
pearson corr. coefficeint
0.7714275
upregulated
downregulated
Not Sig.
log2FC (enri-2;enri-3)
log2FC (enri-3(qe17))

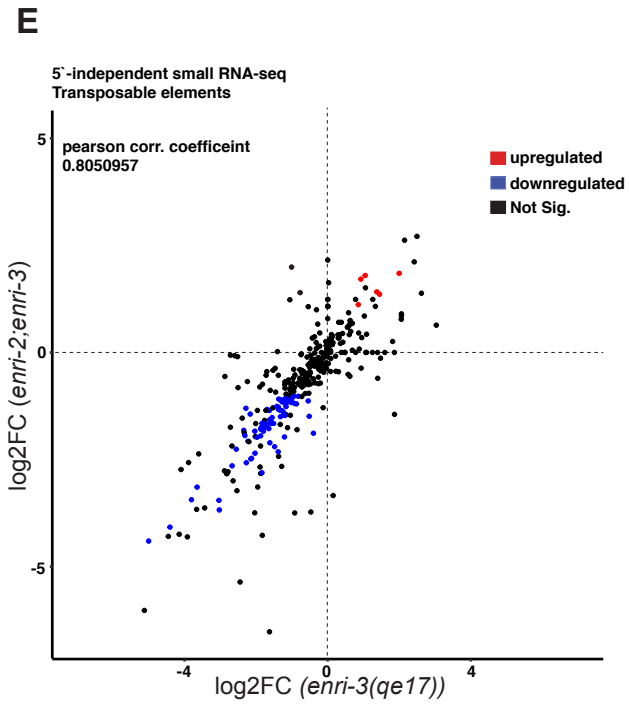

E
5`-independent small RNA-seq
Transposable elements
pearson corr. coefficeint
0.8050957
upregulated
downregulated
Not Sig.
log2FC (enri-2;enri-3)
log2FC (enri-3(qe17))

Figure S7

Figure S7. **Refers to Figure 6. ENRI-3: Small RNA sorting in the male germline.** (A) Left: expression imaging of ENRI-3::GFP in embryos inside gravid adult hermaphrodites. Right: expression imaging of ENRI-3::GFP and PGL-3::mCherry (P-granule marker) in 88-cell embryos. (B) Top: Expression imaging of ENRI-3::GFP and PGL-3::mCherry in oocytes of gravid adult hermaphrodites. Bottom: Expression imaging of ENRI-3::GFP and PGL-3::mCherry in the germline of young adult hermaphrodites. (D) (E) Correlation between 22G-RNA changes in *enri-3(qe17)* and *enri-3(qe19);enri-2(tag1609)* datasets targeting (D) all genes and (E) transposable elements.