Supplementary Figure 1: *mina-1(If) phenotypes*

(a, b) Synchronized control(RNAi) and *mina-1(RNAi)* young adults were exposed to IR (60 Gy) and germline apoptosis were analyzed by DIC microscopy 24h post-treatment. Arrows indicate germ cell corpses. Scale bars, 10 µm.

(c) Representative DIC image of a germ line of *mina-1(ok1521)* mutants. Arrows point to germ cell corpses. Scale bars, 10 µm.

(d) Quantification of germline apoptosis after control and *mina-1* RNAi in wild type and *ced-9(n1950)* animals 48 h post-L4 stage. Data shown represent the average of three independent experiments ± s.d. (n > 20 animals for each experiment)

(e) Quantification of germline apoptosis in wild type, *mina-1(ok1521)*, and *opls408( P_{mina-1::mina-1::gfp::mina-1(3'UTR)}: mina-1(ok1521)* animals 36 h post-L4 stage. Data shown represent the average n = 20 animals ± s.d.

(f) Synchronized L1 wild type and *rad-5(mn159)* were raised on RNAi empty vector control or *mina-1(RNAi)* plates and exposed to IR as young adults. Germline apoptosis was quantified by DIC microscopy at the indicated time points. Data shown represent the average ± s.d. of n > 20 animals for each time point.

(g-j) Transcriptional *mina-1* reporter *opls338(P_{mina-1::gfp::let-858(3'UTR)})* is expressed in the mitotic zone (g, h), pachytene as well as in oocytes (i, j). Scale bar, 10 µm.

(k) Representative DIC image showing additional developmental germline defects in *mina-1(ok1521)* mutant animals such as appearance of distal oocytes (gogo phenotype: germ cell, oocyte, germ cell, oocyte; white arrow). Scale bars, 10 µm.

(l, m) Confocal microscopy images of *mina-1(ok1521)* germ lines stained with DAPI (blue). Arrowheads: Distal oocytes undergoing endoreplication and becoming polyploid. Scale bar, 15 µm (l), 10 µm (m).

(n) *mina-1* mutants contain often regions with a swollen appearance and a reduced density of germ cell nuclei in the distal germ line (DIC). Scale bar, 20 µm.

(o) TEM image of a *mina-1* mutant germ line. Arrows point to a low-density region that likely correspond to the swollen region observed by DIC. Scale bar, 4 µm.

(p, q) Quantification of number of embryos laid per hour per animal and embryonic lethality in wild type and *mina-1(ok1521)* animals. Data shown are average of three independent experiments ± s.d. (n=20 animals / experiment).
Supplementary Figure 2

**a**

- KH1: ELIPQOKHAKFAWPGQCALVAALEGQFQCIVIWDEHELASSADGVAVDINGIEKILDVWRK
- KH2: VQHREALNASCYTHICHTLLPRAYCAVULFSSLDQRRSRCTDI1IDQFTGFVNTHFGETQAVNHKAREHMMIECLTEH
- KH3: DXXKQVPTTEVGKILQDAAAANKHERQFNCVTIVRTETVSFGATPEIVAQNEKGCQREAD
- Consensus: BDXXXXAAXGXXXEAAXA

**b**

- NOE scatter plot

**c**

- Residue vs. NOE

**d**

- Fraction of top mRNAs vs. number of sites per 3'UTR

**e**

- Fraction of top CLIPed sites vs. relative position in 3'UTR

**f**

- Fraction of top CLIPed reads vs. position in 3'UTR (nucleotides)
Supplementary Figure 2: MINA-1 KH domain characterization and RNA binding sites distribution

(a) Sequence and secondary structure elements of the three putative KH domains of MINA-1. Red letters indicate residues that deviate from the KH domain consensus sequence.

(b) \[^{1}H,^{15}N\]-HSQC spectrum with NMR assignments of the uniformly \[^{13}C,^{15}N\]-labeled KH3 construct comprising residues 254–334 of MINA-1. BMRB deposition ID: 34220.

(c) \[^{15}N\}{^{1}H\}-NOE experiment of MINA-1(254–334) recorded at 600 MHz and 20°C in NMR buffer (20 mM sodium phosphate at pH 5.0, 2 mM DTT, 0.5 mM EDTA, 95% (v/v) H\(_2\)O / 5% (v/v) D\(_2\)O).

(d) Analysis of sites that were repeatedly CLIPed in at least 2 out of 3 CLIP data sets. Among the top 100, top 1000 and top 10000 sites with the most reads, there were 87, 886 and 6960 sites located in 3'UTRs. Number of sites per 3'UTR was analyzed.

(e) Position of the sites (in nucleotides) within the 3'UTR among the top 100, top 1000 and top 10000 sites.

(f) Relative position of the CLIPed sites within the 3'UTR among the top 100, top 1'000 and top 10'000 sites.
Supplementary Figure 3

a) MINA1(254-334) with CUGUGAAUA-RNA

b) MINA1(254-334) with GAAUA-RNA

Residue

KTCVWEK I KWPTTEVGK I LNGRAAVVHHK ERDFNCV IY T E V DG S F PGAPVE I VAQNEKSEQE A R N W E L QGSDDDK

Free MINA1(254-334)

+ 0.5 eq. RNA

+ 1.0 eq. RNA

+ 2.0 eq. RNA

+ 3.0 eq. RNA

ω2 (1H) [ppm]

ω1 (15N) [ppm]

Free "GDDA"-MINA1

+ 0.5 eq. RNA

+ 1.0 eq. RNA

+ 1.5 eq. RNA

+ 2.0 eq. RNA

+ 2.5 eq. RNA

ω1 (15N) [ppm]
Supplementary Figure 3: RNA binding surface analysis of MINA-1 wt and MINA-1 GDDA mutant

(a, b) Superposition of 2D $[^1\text{H},^{15}\text{N}]$HSQC spectra of the RNA titration experiments of 250 μM MINA-1(254–334) with (a) CUGUGAAUA-RNA and (b) GAAUA-RNA. The RNA was stepwise incremented up to a molar ratio of 3 equivalents RNA to 1 equivalent MINA-1(254–334). The individual spectra at various ratios are color-coded according to the indicated scheme in the figure. The assignments of signals with enhanced chemical shift perturbations upon RNA binding are indicated. All titrations were conducted at 20°C and 500 MHz in NMR buffer at pH 5.

(c) Combined chemical shift perturbation (CCSP) of the amide resonances of 250 μM MINA-1(254–334) upon titration with CUGUGAAUA (black bars), CUGUG (red bars) and GAAUA (green bars) RNA to a molar ratio of 3 equivalents RNA to 1 equivalent protein. As compared to the 5-mer RNAs, titration of MINA-1(254–334) with the longer 9-mer RNA induces significantly increased perturbations throughout the whole domain, indicating that this atypical KH domain preferentially accommodates longer target RNA.

(d, e) Superposition of 2D $[^1\text{H},^{15}\text{N}]$HSQC spectra of the RNA titration experiments of 200 μM MINA-1(254–334) “GDDA” mutant with (d) GAAUA and (e) CUGUGAAUA RNA. The RNA was stepwise incremented to a final molar ratio of 1.5 equivalents GAAUA RNA (d) and 2.5 equivalents CUGUGAAUA RNA (e) to 1 equivalent of the MINA-1(254–334) “GDDA” variant. The individual spectra at various ratios are color-coded according to the indicated schemes in the figure. All titrations were conducted at 20°C and 700 MHz in NMR buffer at pH 7.

(f) Combined chemical shift perturbation (CCSP) of the amide resonances of wild-type MINA-1(254–334) (black bars) and the “GDDA” mutant (red bars) upon titration with CUGUGAAUA RNA to a final molar ratio of 2.5 equivalents RNA to 1 equivalent protein. Titrations were conducted at 20°C and 500 MHz in NMR buffer at pH 5 (wild-type MINA-1) and pH 7 (“GDDA” mutant).

(g) The color coding on the cartoon representation correlates to the chemical shift perturbation of the amide resonances of the “GDDA” variant of MINA-1 upon binding to the CUGUGAAUA-RNA. A linear gradient from yellow to red indicates perturbations ranging 0 to ≥ 0.1 ppm, respectively.
Supplementary Figure 4: MINA-1 inhibits translation of its target mRNAs
(a) Illustrated SILAC workflow enabled quantification of 1323 proteins overlapping in two biological replicates between wild-type (WT) and mina-1 mutant worms. Same batch of worms was used to isolate protein and RNA for SILAC and RNA-sequencing experiment.
(b) Cumulative distributions of log$_2$ fold mRNA expression changes of 474 CLIP targets and 12387 non-targets in mina-1 mutants relative to wild-type. P-values were calculated using Kolmogorov-Smirnov (KS) test comparing the fold change distributions (log$_2$) of targets and non-targets (P-value=9.4x10^{-5}).
(c) Cumulative distributions of log$_2$ fold expression changes of the 30 CLIP targets (that matched the list of 563 targets enriched in CLIP and contained MINA-1 binding site) and 1293 non-targets in mina-1 mutants relative to wild-type. P-values were calculated using Kolmogorov-Smirnov (KS) test comparing the fold change distributions (log$_2$) of targets and non-targets (P-value=0.2).
(d) Correlation of number of top clipped sites (top panels) and number of clipped transcripts (bottom panels) with protein (left panels), mRNA fold changes (center panels) and translation efficiency (log$_2$ (fold change protein / fold change mRNA); right panels). Transcripts harboring top clipped sites showed reasonable correlation between CLIP enrichment and protein fold changes (Pearson correlation coefficient > 0.25, considering <= 2000 top clipped sites, located in about 400 transcripts) but little to no correlation between CLIP enrichment and mRNA fold changes (Pearson correlation coefficient < 0.2 when considering <= top 1000 clipped sites, located in about 250 transcripts).
Supplementary Figure 5

(a) gld-1 transcript

(b) fbf-2 transcript

(c) ppw-2 transcript

(d) Germ cell corpses/gonas

(e) gld-1 transcript

(f) wago-3 transcript

(g) wago-4 transcript
Supplementary Figure 5: MINA-1 binds gld-1, fbf-2 and ppw-2; GLD-1 binds wago-4, wago-3 and its own 3′UTR

(a-c) Visualization of MINA-1 HITS-CLIP signal in the 3′UTR (marked in blue) of gld-1 (a), fbf-2 (b), and ppw-2 (c) transcripts. Number of reads per million from the HITS-CLIP experiment along the transcript are shown. Sites where accumulation of reads correspond to one of the two consensus motifs (MBM1 or MBM2) are marked with red boxes.

(d) Synchronized animals were scored for germ cell apoptosis 24 hours post L4 larval stage/adult molt with (grey) and without (black) IR (60 Gy). Data shown are average ± standard deviation of three biological replicates (n=20 animals / experiment). P-values were calculated using Student’s t-test: * P < 0.05, n.s. not significant.

(e-g) GLD-1 CLIP\(^{1,2}\) signal in the 3′UTR of gld-1 (e) and wago-3 (f) and wago-4 (g). Number of reads per million from the respective CLIP experiment along the transcript is indicated. Enrichment representing GLD-1 binding sites are marked with red boxes.
Supplementary Figure 6

(a) DAPI  anti-p-granules  anti-flag  merged

WAGO-4 transgene

WAGO-4 transgene

mina-1(ok1521)

P4

WAGO-4 transgene wild type

2.5x

(b) DAPI  anti-p-granules  anti-flag  merged

wild type

WAGO-4 transgene

(c) 2.5x
Supplementary Figure 6: WAGO-4 localized predominantly in the germline P granules

(a) Embryos of wago-4(tm2401); opls530[3xflag::wago-4] and mina-1(ok1521); wago-4(tm2401); opls530[3xflag::wago-4] stained DNA (DAPI, blue), P granule component (K76, red), and anti-flag(green). Merged channels show co-expression of WAGO-4 and P granules in the P lineage. Enlarged and disorganized P granules can be observed in mina-1 mutant background. Scale bar, 10 µm.

(b) Confocal microscopy images of dissected germ line of wild type and wago-4(tm2401); opls530[3xflag::wago-4] stained DNA (DAPI, blue), P granule component (K76, red), and anti-flag(green). Merged channels show partial co-localization of WAGO-4 and P granules around germ cells of dissected gonads. Scale bar, 2.5 µm.

(c) Magnification (2.5x) of merged channels of wago-4(tm2401); opls530[3xflag::wago-4] dissected gonad.
Supplementary Figure 7. Enlarged P granules phenotype in mina-1(lf) mutant

(a-d) Confocal microscopy images of germ lines of synchronized young adult animals of wild type (a), mina-1(ok1521) (c, d), and mina-1 transgene opIs408 were stained for the P-granule component PGL-1 (red, arrowheads) and DNA (DAPI, blue). mina-1 transgene opIs408 in mina-1 mutant background rescues the phenotype. Scale bars, 3 µm (a, d); 2 µm (b-c, e-g). Scale bars, 2 µm (a, b, d); 3 µm (c).
References Supplementary Figure legends
