SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: miRNA expression profile

miRNA expression profiles of TAP::ALG-1 transgenic and wild-type animals were determined by one-color arrays. The average $\log_{10}$ transformed expression values of all the detected miRNAs are shown (see Supplemental Table 4). The miRNA expression profile correlates strongly (Pearson's square correlation fractor (R^2) = 0.92). Bantam/miR-58 is indicated by the red dot in the graph. Total extracts from the same mixed-stage TAP::ALG-1 transgenic and wild-type animals were used as for the RIP-chip experiments. 3 independent biological replicate measurements for TAP::ALG-1 transgenic and wild-type animals were performed.

Supplemental Figure 2: TAP::ALG-1 RIP-chip enriches for mRNAs with seed binding sites for abundant miRNAs

miRNA seeds were arranged in groups of five based on their miRNA microarray expression values (see Supplemental Table 4). TOP 1 includes the 5 most highly expressed miRNA seeds (including bantam/miR-58), TOP 2 the next 5 most highly expressed seeds, etc. The CTL 1, CTL 2 and CTL 3 groups each contained 5 miRNA seeds that were below detection limit on the miRNA microarray (all seed groups are listed in Supplemental Table 13). Relative seed binding site enrichment in the 3'UTRs of TAP::ALG-1-associated mRNAs compared to non-associated mRNAs was determined for each group as described in "Materials and Methods". Five different *P*-values and four different signal to noise ratios (SNR) cutoffs were applied to define the TAP::ALG-1 associated mRNAs.

Supplemental Figure 3: mRNA changes confirm that a substantial fraction of the RIP-chip candidates are indirect targets

Cumulative fraction plot of the $\log_2$ mRNA changes ("*mir-58*" to "wild-type") of potential bantam/miR-58 targets identified by RIP-chip or TargetScan prediction, and of a random control group. The RIP-chip candidates (blue dashed line) were further subdivided into "RIP-chip (Protein up)" (dark blue line) and "RIP-chip (Protein down)" (light blue line) groups, based on whether their protein levels were elevated ($\log_2$ protein$_{mir\text{-}58/\text{wt}} > 0$) or reduced ($\log_2$ protein$_{mir\text{-}58/\text{wt}} < 0$) in "*mir-58*" mutants,

respectively.

SUPPLEMENTAL TABLE LEGENDS

Supplemental Table 1: Expression table of all mRNAs detected in the RIP-chip experiments of mixed-stage TAP::ALG-1 transgenic and wild-type animals

RNA isolated from mixed-stage TAP::ALG-1 transgenic and wild-type animals was analyzed for each IP sample and for each total worm extract sample. The $\log_2$ ratios of RIP mRNA (Cy5 labeled) versus total mRNA (Cy3 labeled) are given for each independent experiment. The *P*-values have been determined by a two-sample Student's t-test. Three independent biological replicates have been performed.

Supplemental Table 2: mRNA species that were significantly changed in TAP::ALG-1 RIP-chips when compared to wild-type RIP-chips

RNA isolated from mixed-stage TAP::ALG-1 transgenic and wild-type animals was analyzed for each IP sample and for each total worm extract sample (see Supplemental Table 1). All mRNA species that were significantly changed in TAP::ALG-1 transgenic animals when compared to wild-type animals (*P*-value < 0.05; two-sample Student's t-test, equal variances) are shown. In addition the mock normalized average $\log_2$ ratios (average TAP::ALG-1 transgenic / average wild type) are listed. The table also indicates which oligo probe signals passed a certain SNR cutoff in the Cy5 channel (RIP mRNA).

Supplemental Table 3: TAP::ALG-1 associated mRNAs overlap significantly with a previously published list of ALG-1 associated mRNAs

The set of 3750 TAP::ALG-1-associated mRNAs identified in this study overlaps strongly (*P*-value < $3*10^{-59}$; hypergeometric distribution) with a previously published set of ALG-1-associated mRNAs identified by Clip-seq (Zisoulis et al. 2010). Only Clip-seq mRNAs that had also been detected on our microarrays (2560 out of 3093) were included in the analysis. The total number of expressed genes (12890) on our microarray was used for the statistical analysis.

Supplemental Table 4: miRNA expression in mixed-stage TAP::ALG-1 transgenic and wild-type animals

The miRNA expression was determined by one-color microarray. Only intensity expression values are shown that passed the lower limit of detection (intensity > 350). Total extracts from the same mixed-stage TAP::ALG-1 transgenic and wild-type (mock) animals were used as for the RIP-chip experiments. 3 independent biological replicate measurements for TAP::ALG-1 transgenic and wild-type animals were performed.

Supplemental Table 5: Expression table of all mRNAs detected in the RIP-chip experiments of synchronized L4-stage TAP::ALG-1 transgenic "wild-type" and "*mir-58*" animals

RNA isolated from synchronized L4-stage TAP::ALG-1 transgenic "wild-type" and "*mir-58*" animals was analyzed for each IP sample by one-color Affymetrix arrays. The table lists the gene name (CDS), the associated Affymetrix spot name, the average $\log_2$ ratio of the mRNA ("wild-type" / "*mir-58*") and the average as well as the single replicate $\log_2$ transformed signal intensity value for the mRNA isolated from "wild-type" and "mir-58" animals. FDRs have been determined by Significance Analysis of Microarrays (SAM; paired) (Tusher et al. 2001). Three independent biological replicates have been performed.

Supplemental Table 6: RIP-chip candidates selected for protein quantification by SRM

The table lists all potential bantam/miR-58 targets that have been identified by the RIP-chip assays and selected for further validation by determining their protein abundances in "wild-type" and "*mir-58*" animals by SRM. The selected candidates had either an FDR < 6 % (SAM, paired, one-sided) or and FDR < 10 % (SAM, paired, one-sided) plus being predicted to be a bantam/miR-58 target (Lall et al. 2006; Ruby et al. 2006; Lewis et al. 2005; Griffiths-Jones et al. 2007; Stark et al. 2005; Watanabe et al. 2006). In order to develop SRM assays for each protein, 1 to 5 synthetic tryptic peptides, representing each protein, have been ordered (JPT technologies, Germany). The last column indicates if the protein had tryptic peptides that were compatible for synthetic peptide synthesis and SRM measurements. Proteins that did not have any compatible peptides (indicated in red) were excluded from SRM measurements.

Supplemental Table 7: Relative protein abundances of potential bantam/miR-58 targets and randomly selected candidates

Protein quantification results for the 87 proteins that could be analyzed by SRM. The table lists the gene name, the mean $\log_2$ ratio of the protein ("*mir-58*" / "wild type"; averaged over all measurements), the Standard-deviation of the $\log_2$ ratio of the protein ("*mir-58*" / "wild type"), the number of biological replicates where the protein could be quantified by at least one peptide, the *P*-value for the protein change (one sample Student's t-test), and their group affiliation from the candidate list. Four independent biological replicate measurements have been performed.

Supplemental Table 8: Relative total mRNA levels ("*mir-58*" / "wild type") of all genes detected by microarray analysis

RNA was isolated from total worm extracts and mRNA expression analysis was performed by microarray experiments. The spot identifier, the corresponding gene name, the average $\log_2$ value of the signal intensity (A), the average $\log_2$ ratio (M, "*mir-58*" / "wild type"), the *P*-value (one sample Student's t-test) and the False Discovery Rate (FDR, Benjamini Hochberg) are listed. All identifiers that passed the quality control filter (average signal intensity > 250 (A ($\log_2$ of signal intensity) > 7.966)) are listed. Three independent biological replicate measurements have been performed.

Supplemental Table 9: Relative total mRNA levels ("*mir-58*" / "wild type") of the SRM quantified candidates

Total mRNA quantification results for 71 candidates (out of a total of 87, see Supplemental Table 7) whose protein levels could be determined by SRM. The relative total mRNA levels ("*mir-58*" / "wild type"; averaged over all measurements) were determined by microarray analysis (see Supplemental Table 8). The table lists the gene name, the average $\log_2$ ratio of the mRNA ("*mir-58*" / "wild type"; averaged over all measurements), the $\log_2$ ratio of the protein ("*mir-58*" / "wild type"; averaged over all measurements, see also Supplemental Table 7) and the group affiliation. Three independent biological replicate measurements have been performed for the mRNA measurements.

Supplemental Table 10: Bantam/miR-58 seed binding site analysis for all SRM quantified candidates

3'UTR sequences for *C. elegans* were obtained from Table S2, (Mangone et al. 2010). For all 3'UTRs, the occurrences of either the 7mer "CGATCTC" or "GATCTCA" (complementary miR-58 seed binding sites) were counted, with either a perfect match or one mismatch being allowed. The gene name (CDS), the group affiliation, the 3'UTR length, the 7mer matches (if a mismatch is present, it is indicated by a capital letter) and the 3'UTR sequence are listed.

Supplemental Table 11: Bantam/miR-58 seed binding site analysis for the *C. briggsae* orthologs of all SRM quantified candidates

C. briggsae 3'UTR sequences, defined as the first 250bp downstream of the stop codon, were downloaded from WormMart (wormbase.org, release 215). Orthology information was also downloaded from WormMart (wormbase.org, release 215). For all 3'UTRs, the occurrences of either the 7mer "CGATCTC" or "GATCTCA" (complementary miR-58 seed binding sites) were counted, with either a perfect match or one mismatch being allowed. The gene name (CDS), the corresponding *C. elegans* ortholog, the group affiliation, the 3'UTR length, the 7mer matches (if a mismatch is present, it is indicated by a capital letter) and the 3'UTR sequence are listed.

Supplemental Table 12: GO term assignment for the upregulated RIP-chip-SRM candidates

The "Gene Ontology" website (http://www.geneontology.org/) was used for GO term assignments for all upregulated RIP-chip-SRM targets as well as for background distributions (the whole *C. elegans* genome). Several GO terms that might be relevant to the mutant phenotype shown by the quadruple bantam/mir-58 knockout strain are presented. The table lists the GO term, the associated *P*-value (hypergeometric distribution) and corrected *P*-value (Bonferroni correction) of potential overrepresentation of that term among the upregulated RIP-chip-SRM targets, the number and percentage of upregulated RIP-chip-SRM targets associated with that GO term, the total number of RIP-chip-SRM targets with any GO term assignment, the number and percentage of all genes in the *C. elegans* genome assigned to that GO term (= background distribution), the total number of all *C. elegans* genes with any GO term assignment and the gene names of the RIP-chip-SRM targets associated with that GO term.

Supplemental Table 13: miRNA seeds grouping according to their expression values

The seeds contained in each group used for the miRNA seed enrichment analysis are listed. miRNAs containing the same seeds (miRNA families) were collapsed to one seed. The expression values for the seeds of families was adjusted to match the average of all family members and grouped according to that expression value.

Supplemental Table 14: Peptides that have been synthesized for SRM assay development

The table lists all 761 synthetic peptides representing 262 proteins, which have been ordered and used for the development of SRM assays for all candidate proteins. Next to the peptide sequence, the gene name and the group affiliation of the corresponding protein are listed.

Supplemental Table 15: Transitions and input parameters for the SRM quantifications of predicted miR-58 targets and random negative controls

All transitions used for the quantification of the 471 unique peptides representing 215 unique proteins are listed. Each peptide was measured with ten transitions, five corresponding to the light and five to the heavy labeled peptide. The expected retention times were experimentally determined on the Q-Trap using the synthetic peptides. The collision energies were calculated as described in (Jovanovic et al. 2010). Transitions marked with 1 in the "decoy" column were used as negative controls for data analysis.

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