C. elegans Multidrug Resistance Protein 5 (MRP-5) Transports Vitamin B12 from the Intestine to the Gonad to Support Embryonic Development

Huimin Na, Olga Ponomarova, Gabrielle E. Giese, and Albertha J.M. Walhout*

Program in Systems Biology and Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605, USA

*Correspondence: marian.walhout@umassmed.edu

SUMMARY

Vitamin B12 functions as a cofactor for methionine synthase to produce the anabolic methyl donor S-adenosylmethionine (SAM) and for methylmalonyl-CoA mutase to catabolize the short chain fatty acid propionate. In the nematode *Caenorhabditis elegans*, maternally supplied vitamin B12 is required for the development of her offspring. However, the mechanism for transporting vitamin B12 from the mother's intestine into her gonad is not yet known. Here, we use RNAi of more than 200 transporters with a vitamin B12-sensor transgene to identify the ABC transporter MRP-5 as a candidate vitamin B12 exporter. We show that injection of vitamin B12 into the gonad of *mrp-5* knockdown mothers rescues embryonic lethality in her offspring. Altogether, our findings identify a maternal mechanism for the transit of an essential vitamin to support the development of the next generation.

INTRODUCTION

Maternal micronutrient status during pregnancy greatly affects embryonic development and fetal health (Dror and Allen, 2008; Fall et al., 2003; Owens and Fall, 2008; Skjaerven et al., 2016). For instance, the intake of folate supplements by pregnant women reduces the occurrence of neural tube defects in newborns (Czeizel and Dudas, 1992; Viswanathan et al., 2017). Folate functions together with vitamin B12, or cobalamin, in the one carbon cycle to recycle methionine from homocysteine (Moreno-Garcia et al., 2013)(**Figure 1A**). One of the main functions of the one-carbon cycle is to produce S-adenosylmethionine (SAM), a major methyl donor required to synthesize phosphatidylcholine, an important component of cellular membranes, and to modify histones and DNA to ensure proper genomic functions (Rosenblatt and Whitehead, 1999). The other function of vitamin B12 is as a cofactor in the breakdown of the short chain fatty acid propionate (Carrillo-Carrasco and Venditti, 1993)(**Figure 1A**). Humans obtain vitamin B12 through ingestion of animal products in which this stable micronutrient is efficiently stored (Martens et al., 2002). Deficiency of vitamin B12 in newborns is mostly caused either by maternal depletion or malabsorption, although rare recessive inborn errors of vitamin B12 processing or utilization have also been identified (Rosenblatt and Cooper, 1990; Rosenblatt and Whitehead, 1999).

Recently, the nematode *Caenorhabditis elegans* has been established as a

powerful model for studying vitamin B12-dependent processes (Yilmaz and Walhout, 2014). *C. elegans* is a bacterivore that can be fed individual bacterial species and strains that can elicit different effects on the animal's life history traits and gene expression. For instance, when fed *Comamonas aquatica* DA1877 bacteria, the animals develop faster, have reduced fecundity, and shorter lifespan relative to animals fed the standard laboratory diet of *Escherichia coli* OP50 (MacNeil et al., 2013; Watson et al., 2013). These dietary differences are due, at least in part, to the fact that *Comamonas* synthesizes vitamin B12, whereas *E. coli* does not (Watson et al., 2014). Dietary vitamin B12 is required to support *C. elegans* development, as the animal itself cannot synthesize this cofactor (Bito et al., 2013; Watson et al., 2014). Vitamin B12 is ingested by the mother and needs to be transported from the intestine into the gonad to support the development of her offspring. However, the mechanism by which this occurs is not yet known. Here, we report that the ABC transporter *mrp-5* controls vitamin B12 transport from the intestine into the gonad to support *C. elegans* embryonic development.

RESULTS

An RNAi Screen Identifies the ABC Transporter MRP-5 as a Candidate to Transport Vitamin B12 from the *C. elegans* Intestine into the Gonad

We have previously established the *Pacdh-1::GFP* transgenic *C. elegans* strain

as a powerful reporter of dietary vitamin B12 status: in this strain GFP is highly expressed when vitamin B12 is low and repressed when levels of this micronutrient are high (Arda et al., 2010; MacNeil et al., 2013; Watson et al., 2014; Watson et al., 2016). GFP expression in this strain is under the control of the promoter of the acyl-CoA dehydrogenase *acdh-1*. This gene encodes the first enzyme of an alternate propionate breakdown pathway, or propionate shunt, which does not require vitamin B12 (Watson et al., 2016)(**Figure 1A**).

In *Pacdh-1::GFP* transgenic animals GFP expression in embryos is usually similar as in the mother. For instance, when fed a bacterial diet high in vitamin B12 (*e.g., Comamonas*), GFP levels are low in mothers and embryos whereas GFP levels are high in both when mothers are fed a diet low in vitamin B12 (*e.g., E. coli* OP50) (MacNeil et al., 2013; Watson et al., 2014)(**Figure 1B**). However, we noticed that this correlation does not hold when animals are fed a diet of *E. coli* HT115, a strain used for RNAi by feeding experiments (Conte et al., 2015). With this strain, GFP levels are intermediate in the mother but low in her offspring (**Figure 1B**). This difference may be due to differences in vitamin B12 uptake from the media between these *E. coli* strains, since neither strain is capable of synthesizing vitamin B12. Importantly, this difference presented a convenient opportunity to perform an RNAi screen for transporters that deposit vitamin B12 into developing embryos as we could screen for genes that, when knocked down, led to reduced GFP in the mothers in the presence of vitamin B12, but increased

levels of GFP in their offspring. This phenotype would indicate that vitamin B12 is present in the intestine of the mother, but is lacking in her embryos, which would point to a failure of transport between the mother's intestine and gonad (**Figure 1C**).

We performed RNAi knockdown of 215 predicted *C. elegans* transporters of the ABC transporter and solute carrier families by feeding animals *E. coli* HT115 bacteria expressing double-stranded RNA in the presence of 64 nM vitamin B12 (**Figure 1C**). We identified a single gene, *mrp-5,* that, when knocked down, resulted in mothers with low levels of GFP expression and dead embryos with high GFP expression (**Figure 1D**). *mrp-5* encodes an ABC transporter belonging to the multi-drug resistance protein (MRP) family, which consists of nine members in *C. elegans* (Korolnek et al., 2014). We retested eight of the nine members of this family for which RNAi clones were available, and found that only *mrp-5* knockdown caused activation of GFP expression in the F_1 generation (**Figure 1E**). We then titrated vitamin B12 from 6.4 nM to 6.4 μM and found that GFP expression remains activated in *mrp-5* knockdown embryos (**Figure S1**).

MRP-5 is expressed in the intestine (Korolnek et al., 2014). If *mrp-5* indeed encodes a transporter that pumps vitamin B12 from the *C. elegans* intestine into the gonad, this would lead to the prediction that higher levels of this cofactor should be retained in the intestine when the transporter is perturbed. To test this prediction, we generated a single copy insertion strain that expresses GFP fused

to the histone H2B protein under the control of the *acdh-1* promoter (*Pacdh-1::GFP::H2B*), so that GFP fluorescence would be localized to the nucleus, and differences in GFP levels could be more easily monitored. In addition, we used an RNAi-compatible strain of *E. coli* OP50 (Xiao et al., 2015), because embryos from mother animals fed this diet express much higher levels of GFP than embryos from animals fed *E. coli* HT115, the parental RNAi strain (**Figure 1B**). We found that 0.32 nM vitamin B12 was sufficient to repress intestinal GFP in *mrp-5* (RNAi) animals whereas 3.2 nM was needed to repress GFP in vector control animals (**Figure 1F**). This finding suggests that vitamin B12 is retained in the intestine when *mrp-5* is knocked down. Together these findings suggest that *mrp-5* encodes a transporter that exports vitamin B12 from the intestine into the gonad of the mother to support embryonic development of the offspring.

Injection of Vitamin B12 into the Gonad Rescues Embryonic Lethality Caused by Loss of *mrp-5*

We predicted that supplementation of vitamin B12 into the gonad should bypass the requirement for vitamin B12 transport from the intestine and rescue embryonic lethality caused by perturbation of *mrp-5*. To test this prediction, we injected either vitamin B12 or water (vehicle) as a control into the gonad of *Pacdh-1::GFP* animals that were exposed to *mrp-5* or vector control RNAi. If the vitamin B12 injection into the gonad bypassed MRP-5-dependent transport, we expected to

observe live embryos with low GFP expression, while injecting water would lead to dead embryos with high GFP expression (**Figure 2A**). Indeed, injection of vitamin B12 into the gonad fully and specifically rescued embryonic viability in *mrp-5* knockdown animals (**Figure 2B**). In addition, in *Pacdh-1::GFP* transgenic animals exposed to *mrp-5* RNAi, injecting vitamin B12 into the gonad resulted in live larvae with low levels of GFP, while injection of water resulted in dead embryos with high GFP expression (**Figure 2B**). We recapitulated these findings with *mrp-5(ok2067)* deletion mutant animals. Since *mrp-5* is located on the X chromosome and mothers homozygous for the *mrp-5* deletion display embryonic lethality with a small percentage hatching but arresting at the L1-L2 stage, the *mrp-5* deletion is maintained genetically balanced with +/svT1[*lon-2*(e678)] (**Figure 2C**). As above, heterozygous mothers injected with vitamin B12 into their gonad produced offspring that survived to the adult stage (**Figure 2D**, lower left). Importantly, injection of the vehicle control showed a few F_1 *mrp-5* null embryos that hatched and survived to the L1-L2 stage, while *mrp-5* deficient animals injected with vitamin B12 survived to the adult stage. Genotyping the offspring from mothers injected with vitamin B12 confirmed that viable adult F_1 animals homozygous for the *mrp-5* deletion were obtained (**Figure 2D**, lower right). Further, offspring of the F_1 *mrp-5* deficient animals are mostly embryonic lethal, suggesting that in the absence of *mrp-5* vitamin B12 cannot be passed on to the next (F_2) generation. Altogether, these findings show that vitamin B12 injected into

the gonad of *mrp-5* deficient mothers can rescue embryonic lethality in her offspring, further supporting the finding that *mrp-5* is involved in transporting vitamin B12 from the intestine to the gonad.

Heme Rescues Embryonic Lethality in *mrp-5* (RNAi) Animals

A previous study proposed that *mrp-5* encodes a heme transporter (Korolnek et al., 2014). This finding was supported by the observation that embryonic lethality in *mrp-5* knockdown animals could be rescued by feeding the animals high doses of heme (Korolnek et al., 2014). How could either feeding of heme or injection of vitamin B12 rescue embryonic lethality of *mrp-5* knockdown or mutant animals? ABC transporters are known to be capable of transporting numerous similar types of molecules (Lee and Rosenbaum, 2017; Locher, 2016; Slot et al., 2011). Heme and vitamin B12 share some structural similarities; heme is an iron-containing porphyrin, while vitamin B12 is related to porphyrins and contains cobalt as a metal ion (**Figure 3A**). Thus, it is possible that MRP-5 transports both molecules. However, if lack of heme *and* vitamin B12 explains embryonic lethality of *mrp-5* knockdown animals, one would expect that neither cofactor alone would be sufficient for rescue. Our observation that embryonic lethality can be rescued by injection of vitamin B12 into the gonad indicates that lack of vitamin B12 rather than heme may be the cause of embryonic lethality when MRP-5 function is perturbed. To further examine the effects of heme on animal development, we

repeated the experiment published in the previous study (Korolnek et al., 2014) and confirmed that feeding 500 μM heme can rescue embryonic lethality in the offspring of *mrp-5(RNAi)* animals (**Figure 3B**). Interestingly, however, such a high dose of heme was toxic to vector control animals (**Figure 3B**). Importantly, one can argue that feeding heme does not actually test for transport because the cofactor would be stuck in the intestine when *mrp-5* is perturbed, which would thus not result in the rescue of embryonic development in the offspring.

One explanation for the rescue of embryonic development by feeding heme is that this actually increases vitamin B12 passage into the gonad. Therefore, we next asked whether heme affects the vitamin B12-responsive *Pacdh-1* reporter. We supplemented increasing concentrations of heme and found that the reporter was unaffected by heme in adult animals (**Figure 3D**). As noted above, embryos from mothers treated with *mrp-5* RNAi without supplemented heme expressed high levels of GFP, suggesting that vitamin B12 cannot enter the embryos and repress the reporter. Importantly, however, GFP levels were greatly reduced in embryos from *mrp-5* knockdown animals supplemented with 500 μM heme (**Figures 3C**). These findings suggest that supplying a high dose of heme facilitates transport of vitamin B12 into the gonad and, therefore, that the cause for embryonic lethality is lack of vitamin B12 and not heme. Indeed, we found that while injection of vitamin B12 into the gonad rescues embryonic development in *mrp-5* knockdown animals, direct injection of 200 μM

heme poorly rescued, with some L1-L2 larvae expressing high levels of GFP (**Figure 3E**).

Methionine and S-Adenosyl Methionine Content is Reduced in Offspring from *mrp-5* (RNAi) Mothers

Although vitamin B12 is a cofactor for two different metabolic enzymes (**Figure 1A**), our previous studies demonstrated that the developmental delay caused by vitamin B12 deficiency is mainly due to its function as a cofactor for the methionine synthase enzyme METR-1, which generates the methyl donor SAM from methionine (Watson et al., 2014). To determine if the lack of vitamin B12 or the lack of heme in *mrp-5* deficient animals results in defects in the SAM cycle, we measured the levels of methionine and SAM in embryos from mothers treated with *mrp-5* or vector control RNAi. We reasoned that differences in methionine and SAM content would be most clear in the presence of supplemented vitamin B12 because the *E. coli* diet is naturally low in this cofactor. Indeed, we detected a reduced methionine and SAM content in embryos from mothers treated with *mrp-5* RNAi relative to control animals in the presence of vitamin B12 (**Figures 4A and 4B**). Further, SAM levels could be restored to wild type levels by treating the animals with 500 µM heme. These results suggest that a defect in the SAM cycle caused by the lack of vitamin B12 in *mrp-5* deficient embryos contributes to embryonic lethality.

DISCUSSION

We identified MRP-5 as a candidate transporter that exports vitamin B12 from the *C. elegans* intestine into the gonad in the mother to support the development of her offspring. MRP-5 is a member of the *C. elegans* ABC transporter family and these transporters are known to function as exporters of metabolites and drugs (Locher, 2016; Sheps et al., 2004; Slot et al., 2011). MRP-5 has previously been reported to export heme in *C. elegans* (Korolnek et al., 2014). *C. elegans* can synthesize neither vitamin B12 nor heme and depends on dietary supplementation of both cofactors. Loss or reduction of *mrp-5* by RNAi knockdown or by genetic mutation results in embryonic or larval lethality. As reported previously, we confirm that this lethality can be rescued by feeding the mother high concentrations of heme. However, it can also be rescued by injecting vitamin B12 directly into the gonad, bypassing the need for import from the intestine. Wild type *C. elegans* require only low doses of heme (20 μM) (Rao et al., 2005) and high doses of heme are toxic to many systems (Chiabrando et al., 2014). Indeed, supplementing wild type animals with a dose of 500 μM heme was toxic to *C. elegans* as well (**Figure 3B**). One mechanism of heme toxicity is by enhanced membrane permeability (Chiabrando et al., 2014). Therefore, we propose that supplementing *mrp-5* knockdown animals with high concentrations of heme leads to increased intestinal membrane permeability, enabling both heme

and vitamin B12 to pass through without the need for a dedicated transporter (**Figure 4B**).

SUPPLEMENTAL INFORMATION

Supplemental information includes one figure.

AUTHOR CONTRIBUTIONS

H.N. and A.J.M.W. conceived the study. H.N. performed all the experiments. G.G. generated the single copy *Pacdh-1::H2B::GFP* strain. O.P. performed the GC-MS analysis. H.N. and A.J.M.W. wrote the manuscript.

ACKNOWLEDGEMENTS

We thank members of the Walhout lab and Amy Walker for discussion and critical reading of the manuscript. We thank Amy Holdorf for manuscript editing and Shawn Xu for the *E. coli* OP50 RNAi strain. This work was supported by a grant from the National Institutes of Health (DK068429) to A.J.M.W. Some bacterial and nematode strains used in this work were provided by the CGC, which is funded by the NIH Office or Research Infrastructure Programs (P40 OD010440).

REFERENCES

FIGURE LEGENDS

Figure 1. A *C. elegans* Transporter RNAi Screen Identifies *mrp-5* as a Potential Vitamin B12 Transporter

(A) Cartoon of the two vitamin B12-dependent metabolic pathways. Gene names are from *C. elegans*. 3HP – 3-hydroxypropionate; 5-methyltetrahydrofolate; 5, 10-meTHF - 5,10-methylenetetrahydrofolate; D-MM-CoA – D-methylmalonyl-CoA; Gly – glycine; HCy – homocysteine; L-MM-CoA – L-methylmalonyl-CoA; Met – methionine; MSA – malonic semialdehyde; SAH – S-adenosyl homocysteine; SAM – S-adenosyl methionine; THF – tetrahydrofolate. The *acdh-1* branch indicates the vitamin B12-independent propionate shunt.

(B) Fluorescence and DIC microscopy images of *Pacdh-1::GFP* animals fed *E. coli* OP50, *E. coli* HT115 or *C. aquatica* DA1877 at P_0 (first) and F_1 (second) generations. Scale bars, 50 µm for embryos and 100 µm for adults.

(C) Diagram of *C. elegans* transporter RNAi library screen. RNAi-treated animals that displayed GFP fluorescence in the F_1 but not the P_0 generation in the presence of 64 nM vitamin B12 were considered hits.

(D) Fluorescence and DIC microscopy images of *Pacdh-1*::GFP animals subjected to RNAi of *mrp-5* in *E. coli* HT115 compared to vector control at P_0 and F_1 generations in the presence or absence of 64 nM vitamin B12. Scale bars, 50 µm for embryos and 100 µm for adults.

(E) Fluorescence and DIC microscopy images of *Pacdh-1::GFP* animals subjected to RNAi of *mrp* family members in *E. coli* HT115 compared to control knockdown in the F_1 generation. An RNAi clone for *mrp-7* was not available. Scale bar, 50 μm.

(F) Fluorescence microscopy images of single copy insertion *Pacdh-1::GFP::H2B* animals subjected to *mrp-5* RNAi in *E. coli* OP50 compared to control RNAi with low dose titration of vitamin B12. 5X indicates a five-times longer exposure. Scale bar, 100 μm.

Figure 2. **Injection of Vitamin B12 into the Gonad of *mrp-5* deficient Mothers Rescues Lethality in her Offspring**

(A) Experimental design. Briefly, mothers were injected either 3.2 mM vitamin B12 or with water (vehicle) control into the gonad of *Pacdh-1*::GFP animals that were exposed to *mrp-5* or vector control RNAi. If the vitamin B12 injection rescues embryonic lethality, live offspring will show low GFP expression, while water injected animals will show dead embryos with high GFP expression.

(B) Top: DIC and fluorescence microscopy images of embryos from *Pacdh-1::GFP* mothers subjected to *mrp-5* RNAi in *E. coli* OP50 injected into the gonad with water (left panel) or with 3.2 mM vitamin B12 (middle panel). Animals treated with vector control RNAi without injection are shown as a control (right panel). Scale bars, 50 μm for embryos and 100 μm for adults. Bottom:

Quantification of lethality and GFP expression in embryos from *Pacdh-1::GFP* animals described above.

(C) Diagram of genotypes and phenotypes of the offspring of genetically balanced *mrp-5* (+/-) mutant animals. All P_0 adult animals are heterozygous for *mrp-5.* F_1 larval arrest or embryonic lethality occurs in *mrp-5* (-/-) or szT1 aneuploid animals.

(D) Top: Diagram of genotypes and phenotypes of viable offspring from genetically balanced *mrp-5* (+/-) mutant animals after vitamin B12 injection. All P_0 adult animals are heterozygous for *mrp-5.* Live animals can either be heterozygous or homozygous for the *mrp-5*(ok2067) deletion allele. F_2 larval arrest or embryonic lethality occurs in all offspring of the F_2 generation of *mrp-5* (-/-) animals. Bottom left: DIC microscopy images of *mrp-5* (+/-) and *mrp-5* (-/-) animals with or without vitamin B12 injection. Scale bar, 100 μm. Bottom right: Agarose gel image of the genotyping results of heterozygous and homozygous *mrp-5* adult animals with and without injection of vitamin B12.

Figure 3. High Concentrations of Heme Rescue Embryonic Lethality in *mrp-5(RNAi)* Animals but Do Not Affect *acdh-1* Promoter Activity

(A) Heme and vitamin B12 are porphyrin or porphyrin-like molecules with structural similarities.

(B) Bright field images of feeding 500 μM heme to *mrp-5* RNAi-treated mothers on *E. coli* HT115 rescues viability in her offspring, but is toxic to offspring of

vector-treated mothers. Scale bar, 500 μm.

(C) Fluorescence and DIC microscopy images show that feeding 500 μM heme to *mrp-5* RNAi-treated mothers on *E. coli* HT115 reduces *Pacdh-1::GFP* reporter activity in her offspring, but not in the offspring of vector control mothers. Scale bar, 50 μm.

(D) Fluorescence and DIC microscopy images demonstrate that feeding heme to mothers with *E. coli* OP50 does not affect *Pacdh-1::GFP* reporter activity. Scale bar, 100 μm.

(E) Bright field (top) and fluorescence and DIC microscopy images (bottom) shows injection of 200 μM heme into *mrp-5* RNAi-treated mothers with *E. coli* HT115 does not rescue embryonic lethality in her offspring, while injection of vitamin B12 leads to viable offspring and repression of the *acdh-1* promoter. Scale bar, 500 μm for bright field and 100 μm for fluorescence microscopy.

Figure 4. Methionine and S-Adenosyl Methionine Content is Reduced in Offspring from Mothers Treated with *mrp-5* RNAi

(A) SAM content was measured in the embryos of vector or *mrp-5* RNAi-treated mothers on *E. coli* HT115. SAM content was greatly reduced in *mrp-5* deficient embryos compared to vector control. *** $p < 0.0001$, ** $p < 0.001$, * $p < 0.05$ relative to untreated embryos.

(B) Methionine content was measured by GC-MS of vector control or *mrp-5* RNAi-treated mothers fed *E. coli* HT115 with and without 64 nM vitamin B12 supplementation. Each dot represents a biological replicate, and values are relative to vector RNAi without vitamin B12 supplementation.

(C) Model of MRP-5 function. In wild type animals both vitamin B12 and heme are transported by MRP-5 from the intestine to the gonad. Vitamin B12 can enter the embryo and repress the *acdh-1* reporter (top). In the absence of MRP-5 neither vitamin B12 nor heme can cross the intestinal membrane into the gonad, the embryos arrest, and the *acdh-1* reporter remains active (middle). The presence of 500 µM heme causes membrane permeability in *mrp-5*-deficient mothers leading to vitamin B12 crossing the membrane into the gonad, entering the embryo to rescue lethality, and repressing the *acdh-1* promoter (bottom).

STAR ★ METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals and Commercial Assays		
Adenosyl Cobalamin	Sigma Aldrich	Cat#: C0884
Hemin	Sigma Aldrich	Cat#: H9039
Glass beads	Sigma Aldrich	Cat#: G1277
Methoxyamine hydrochloride	Sigma Aldrich	Cat#: 226904
N-Methyl-N-(trimethylsilyl)trifluoroacetamide	Sigma Aldrich	Cat#: 69479
Isopropyl β-D-1 thiogalactopyranoside (IPTG)	US Biological	Cat#: I8500
Bridge-It® S-Adenosyl Methionine (SAM) Fluorescence Assay Kit	Mediomics, LLC	Cat#:SKU: 1-1-1003

Experimental Models: Organisms/Strains		
Caenorhabditis elegans N2	Caenorhabditis Genetics Center (CGC); http://cgc.umn.edu/	N/A
C. elegans, wwIs24 [*acdh-1*p::GFP + *unc-119*(+)]	Arda *et al*., 2010	Strain VL749
C. elegans, wwSi1 [*Pacdh-1*::GFP::H2B + *unc-119*(+)] II;*avr-14*(ad1302) I;*unc-119*(ed3) III;*avr-15*(ad1051); *glc-1*(pk54) V	This study	Strain VL1168
C. elegans, +/szT1 [*lon-2*(e678)] I; *mrp-5*(ok2067)/szT1 X	CGC	Strain VL1599
Escherichia coli OP50	CGC	N/A
Escherichia coli HT115	CGC	N/A
Escherichia coli OP50 RNAi compatible	Xiao *et al*., 2015	N/A
Comamonas aquatic DA1877	CGC	N/A
Escherichia coli HT115 Ahringer RNAi Library	Kamath *et al*., 2003	N/A
Escherichia coli HT115 ORFeome RNAi Library	Reboul *et al*., 2001	N/A
Software, Algorithms and Instruments		
Eclipse Ti Inverted Microscope	Nikon	N/A
Eclipse 90i Inverted Microscope	Nikon	N/A
FastPrep-24 bead beater	MP Biomedicals	N/A
SpeedVac concentrator SPD111V	Thermo Fisher	N/A
DS Ri1 Camera	Nikon	N/A
EVOS FL Auto Imaging System	Invitrogen	N/A
FemtoJet® 4i electronic microinjector	Eppendorf	N/A
Microinjection Arm	Narishige	N/A

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to

and will be fulfilled by the Lead Contact, Marian Walhout (Marian.Walhout@umassmed.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

C. elegans Strains

All *C. elegans* strains were maintained at standard laboratory conditions as described (Brenner, 1974), and strain N2 was used as wild type. Construction of VL749 (wwIs24 [*Pacdh-1::GFP* + unc-119(+)]) strain has been previously described (Arda et al., 2010). Strain VL1168 (*wwSi1[Pacdh-1::GFP::H2B unc-119(+)] II;avr-14(ad1302) I; unc-119(ed3) III; avr-15(ad1051); glc-1(pk54) V*) was generated by *mos1*-mediated single copy insertion (Frokjaer-Jensen et al., 2008). The *mrp-5* mutant strain VC1599 (+/szT1 [*lon-2*(e678)] I; *mrp-5*(ok2067)/szT1 X) was obtained from the *C. elegans* Genetics Center (CGC). Bacterial strains *E. coli* OP50, HT115 and *Comamonas aquatica* DA1877 were obtained from CGC. The *E. coli* OP50 RNAi compatible bacterial strain was generously provided by the Xu lab (Xiao et al., 2015).

RNAi Screen

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to predict *C. elegans* ABC transporters and solute carrier transporters (Kanehisa et al., 2015). The transporter RNAi mini-library was generated by selecting 215 clones from both the ORFome and Ahringer RNAi libraries (Kamath et al., 2003; Rual et al., 2004). RNAi screening was carried out on 24-well Nematode Growth Medium (NGM) agar plates containing 2 mM isopropyl β-D-1-thiogalactopyranoside (IPTG), 50 µg/ml ampicillin with or without 64 nM Ado-Cbl (vitamin B12). *Pacdh-1*::GFP animals were grown on regular NGM

plates with *E. coli* OP50 without Ado-Cbl supplementation for several generations before harvesting embryos. Embryos were incubated in M9 buffer overnight to obtain synchronized L1 animals. Approximately 15 L1 animals were added to each well containing individual RNAi clones. Animal phenotypes were observed after 80 hours of incubation, when the F_1 generation was at the young adult stage.

Vitamin B12 Injection

Animals were treated with *mrp-5* RNAi generated in an *E. coli* OP50 RNAi compatible strain. Vitamin B12 (3.2 mM) was injected into the gonad of L4 animals using a Narishige microinjection arm attached to the body of a Nikon Eclipse Ti inverted microscope. Sterile water was injected into the control group. After injection, animals were singled and treated with the same RNAi (*mrp-5* or vector). Phenotypes were scored after two days. *mrp-5*(ok2067) heterozygotes were singled at the L4 stage. Vitamin B12 was injected as above. After injection, animals were singled and the phenotype was scored after five days.

S-Adenosyl Methionine (SAM) Measurement

SAM was measured using a Bridge-It S-Adenosyl Methionine Fluorescence assay kit purchased from Mediomics (SKU: 1-1-1004) following the manufacturer's recommendations.

Heme Solution Preparation

10 mM Hemin solution was prepared as described (Hickman and Winston, 2007). 6.5 mg/ml Hemin (Sigma-Aldrich) was dissolved in 0.1 M NaOH and incubated at 37°C for 1 hour. 1 M Tris, pH 7.5, was then added to a final concentration of 0.1 M. The pH was adjust using HCl, and the hemin solution was stored at 4°C, protected from light and used within two days.

Relative Quantification of Methionine using GC-MS

Approximately 150,000 embryos were homogenized with 0.5 ml of 200-300 µm acid-washed glass beads (Sigma-Aldrich) in 1 ml 80% methanol using FastPrep-24 bead beater (MP Biomedicals), with intermittent cooling in dry ice / ethanol bath. Samples were then extracted on dry ice for 15 min, centrifuged for 10 min at 20,000 g and 250 µl of supernatant were dried under vacuum. Derivatization of dried samples was performed first with 20 µl of 20 mg/ml methoxyamine hydrochloride (Sigma-Aldrich) in pyridine at 37°C for 90 minutes, followed by addition of 50 µl of N-Methyl-N-(trimethylsilyl)trifluoroacetamide (Sigma-Aldrich) and incubation for 3 hours at 37°C. Reaction was completed during another 5 hours at room temperature. Measurements were performed on an Agilent 7890B/5977B quadrupole GC-MS equipped with 30 m x 0.25 mm x 0.25 µm HP-5MS UI capillary column. The inlet temperature was set to 230°C; transfer line was at 280°C, MS source and quadrupole were at 230°C and 150°C respectively. Trimethylsilyl derivative of methionine was quantified as a 176 m/z ion with two qualifier ions 128 and 293 m/z. Relative quantification of peak areas was done using samples within a linear response range, after mean normalization to total metabolites and blank subtraction.

SUPPLEMENTAL INFORMATION

Figure S1. Fluorescence and DIC microscopy images of embryos from *Pacdh-1::GFP* mothers subjected to vector control RNAi or *mrp-5* RNAi and supplemented with increasing concentrations of vitamin B12. In the upper row, the

inset box in the lower right indicates 15 times the exposure time. Scale bar, 50 µm.

References

Arda, H.E., Taubert, S., Conine, C., Tsuda, B., Van Gilst, M.R., Sequerra, R., Doucette-Stam, L., Yamamoto, K.R., and Walhout, A.J.M. (2010). Functional modularity of nuclear hormone receptors in a *C. elegans* gene regulatory network. Molecular Systems Biology *6*, 367.

Bito, T., Matsunaga, Y., Yabuta, Y., Kawano, T., and Watanabe, F. (2013). Vitamin B12 deficiency in *Caenorhabditis elegans* results in loss of fertility, extended life cycle, and reduced lifespan. FEBS Open Bio *3*, 112-117.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans.* Genetics *77*, 71-94.

Carrillo-Carrasco, N., and Venditti, C. (1993). Propionic Acidemia. In GeneReviews(R), R.A. Pagon, M.P. Adam, H.H. Ardinger, T.D. Bird, C.R. Dolan, C.T. Fong, R.J.H. Smith, and K. Stephens, eds. (Seattle (WA)).

Chiabrando, D., Vinchi, F., Fiorito, V., Mercurio, S., and Tolosano, E. (2014). Heme in pathophysiology: a matter of scavenging, metabolism and trafficking across cell membranes. Front Pharmacol *5*, 61.

Conte, D., Jr., MacNeil, L.T., Walhout, A.J., and Mello, C.C. (2015). RNA Interference in *Caenorhabditis elegans*. Curr Protoc Mol Biol *109*, 26 23 21-26 23 30.

Czeizel, A.E., and Dudas, I. (1992). Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med *327*, 1832-1835.

Dror, D.K., and Allen, L.H. (2008). Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. Nutr Rev *66*, 250-255.

Fall, C.H., Yajnik, C.S., Rao, S., Davies, A.A., Brown, N., and Farrant, H.J. (2003). Micronutrients and fetal growth. J Nutr *133*, 1747S-1756S.

Frokjaer-Jensen, C., Davis, M.W., Hopkins, C.E., Newman, B.J., Thummel, J.M., Olesen, S.P., Grunnet, M., and Jorgensen, E.M. (2008). Single-copy insertion of transgenes in *Caenorhabditis elegans*. Nat Genet *40*, 1375-1383.

Hickman, M.J., and Winston, F. (2007). Heme levels switch the function of Hap1 of *Saccharomyces cerevisiae* between transcriptional activator and transcriptional repressor. Mol Cell Biol *27*, 7414-7424.

Kamath, R.S., Fraser, A.G., Dong, Y., Poulin, G., Durbin, R., Gotta, M., Kanapin, A., Le Bot, N., Moreno, S., Sohrmann, M.*, et al.* (2003). Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. Nature *421*, 231-237.

Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M., and Tanabe, M. (2015). KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res.

Korolnek, T., Zhang, J., Beardsley, S., Scheffer, G.L., and Hamza, I. (2014). Control of metazoan heme homeostasis by a conserved multidrug resistance protein. Cell Metab *19*, 1008-1019.

Lee, J.Y., and Rosenbaum, D.M. (2017). Transporters Revealed. Cell *168*, 951-953.

Locher, K.P. (2016). Mechanistic diversity in ATP-binding cassette (ABC) transporters. Nat Struct Mol Biol *23*, 487-493.

MacNeil, L.T., Watson, E., Arda, H.E., Zhu, L.J., and Walhout, A.J.M. (2013). Diet-induced developmental acceleration independent of TOR and insulin in *C. elegans*. Cell *153*, 240-252.

Martens, J.H., Barg, H., Warren, M.J., and Jahn, D. (2002). Microbial production of vitamin B12. Appl Microbiol Biotechnol *58*, 275-285.

Moreno-Garcia, M.A., Rosenblatt, D.S., and Jerome-Majewska, L.A. (2013). Vitamin B(12) metabolism during pregnancy and in embryonic mouse models. Nutrients *5*, 3531-3550.

Owens, S., and Fall, C.H. (2008). Consequences of poor maternal micronutrition before and during early pregnancy. Trans R Soc Trop Med Hyg *102*, 103-104.

Rao, A.U., Carta, L.K., Lesuisse, E., and Hamza, I. (2005). Lack of heme synthesis in a free-living eukaryote. Proc Natl Acad Sci U S A *102*, 4270-4275.

Rosenblatt, D.S., and Cooper, B.A. (1990). Inherited disorders of vitamin B12 utilization. Bioessays *12*, 331-334.

Rosenblatt, D.S., and Whitehead, V.M. (1999). Cobalamin and folate deficiency: acquired and hereditary disorders in children. Semin Hematol *36*, 19-34.

Rual, J.-F., Ceron, J., Koreth, J., Hao, T., Nicot, A.-S., Hirozane-Kishikawa, T., Vandenhaute, J., Orkin, S.H., Hill, D.E., van den Heuvel, S.*, et al.* (2004). Toward improving *Caenorhabditis elegans* phenome mapping with an ORFeome-based RNAi library. Genome Res *14*, 2162-2168.

Sheps, J.A., Ralph, S., Zhao, Z., Baillie, D.L., and Ling, V. (2004). The ABC transporter gene family of *Caenorhabditis elegans* has implications for the evolutionary dynamics of multidrug resistance in eukaryotes. Genome Biol *5*, R15.

Skjaerven, K.H., Jakt, L.M., Dahl, J.A., Espe, M., Aanes, H., Hamre, K., and Fernandes, J.M. (2016). Parental vitamin deficiency affects the embryonic gene expression of immune-, lipid transport- and apolipoprotein genes. Sci Rep *6*, 34535.

Slot, A.J., Molinski, S.V., and Cole, S.P. (2011). Mammalian multidrug-resistance proteins (MRPs). Essays Biochem *50*, 179-207.

Viswanathan, M., Treiman, K.A., Kish-Doto, J., Middleton, J.C., Coker-Schwimmer, E.J., and Nicholson, W.K. (2017). Folic Acid Supplementation for the Prevention of Neural Tube Defects: An Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA *317*, 190-203.

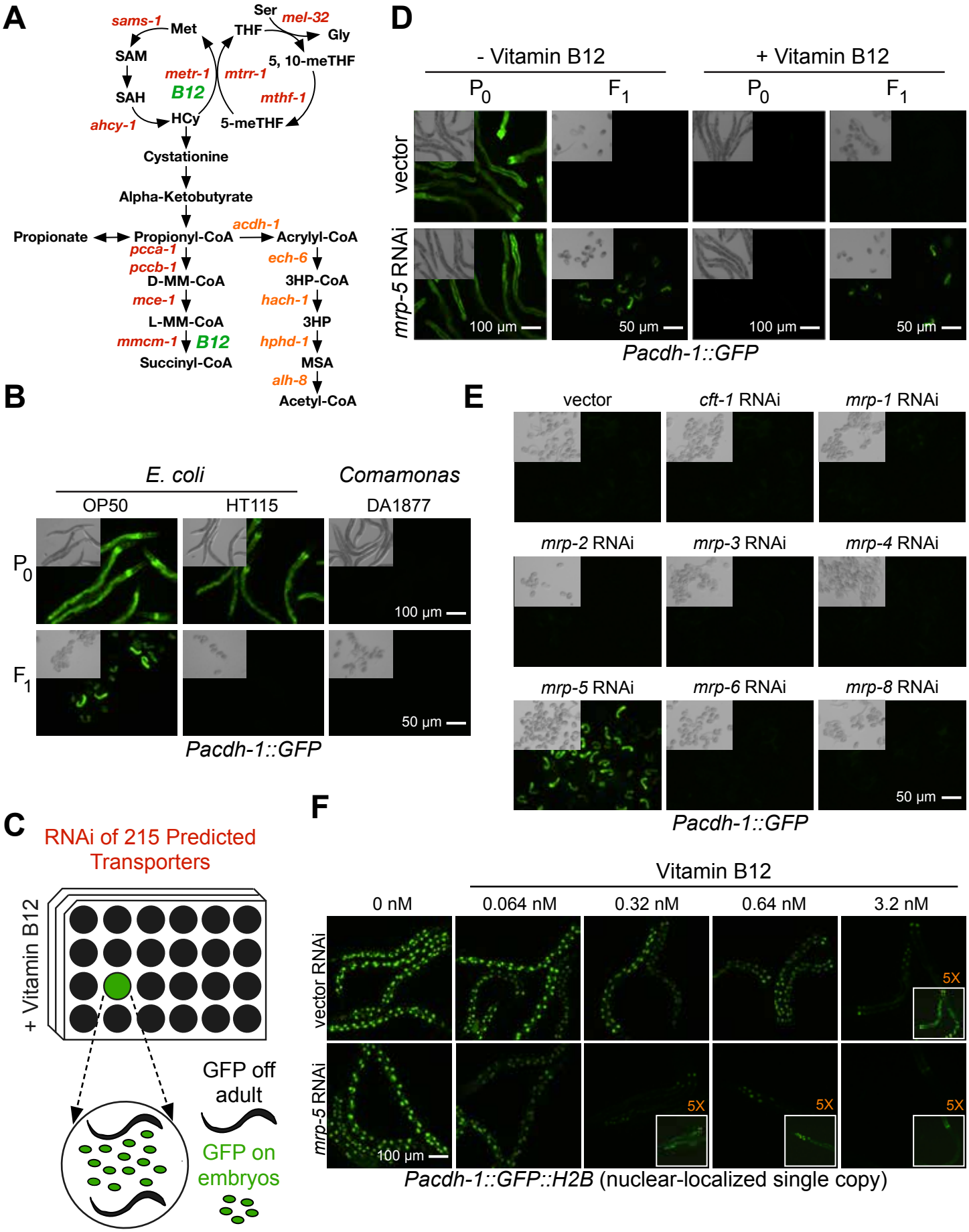
Watson, E., MacNeil, L.T., Arda, H.E., Zhu, L.J., and Walhout, A.J.M. (2013). Integration of metabolic and gene regulatory networks modulates the *C. elegans* dietary response. Cell *153*, 253-266.

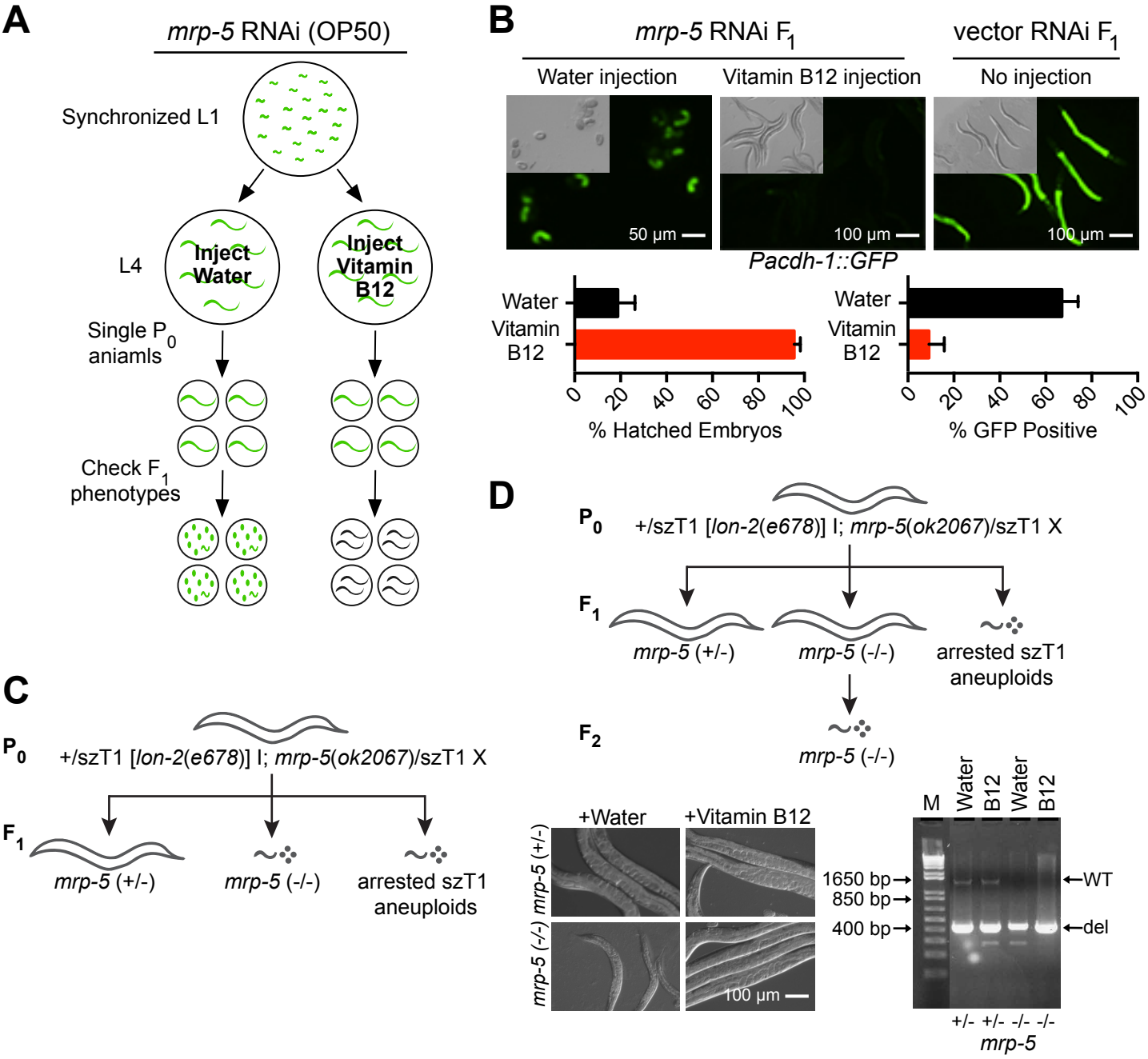
Watson, E., MacNeil, L.T., Ritter, A.D., Yilmaz, L.S., Rosebrock, A.P., Caudy, A.A., and Walhout, A.J.M. (2014). Interspecies systems biology uncovers metabolites affecting *C. elegans* gene expression and life history traits. Cell *156*, 759-770.

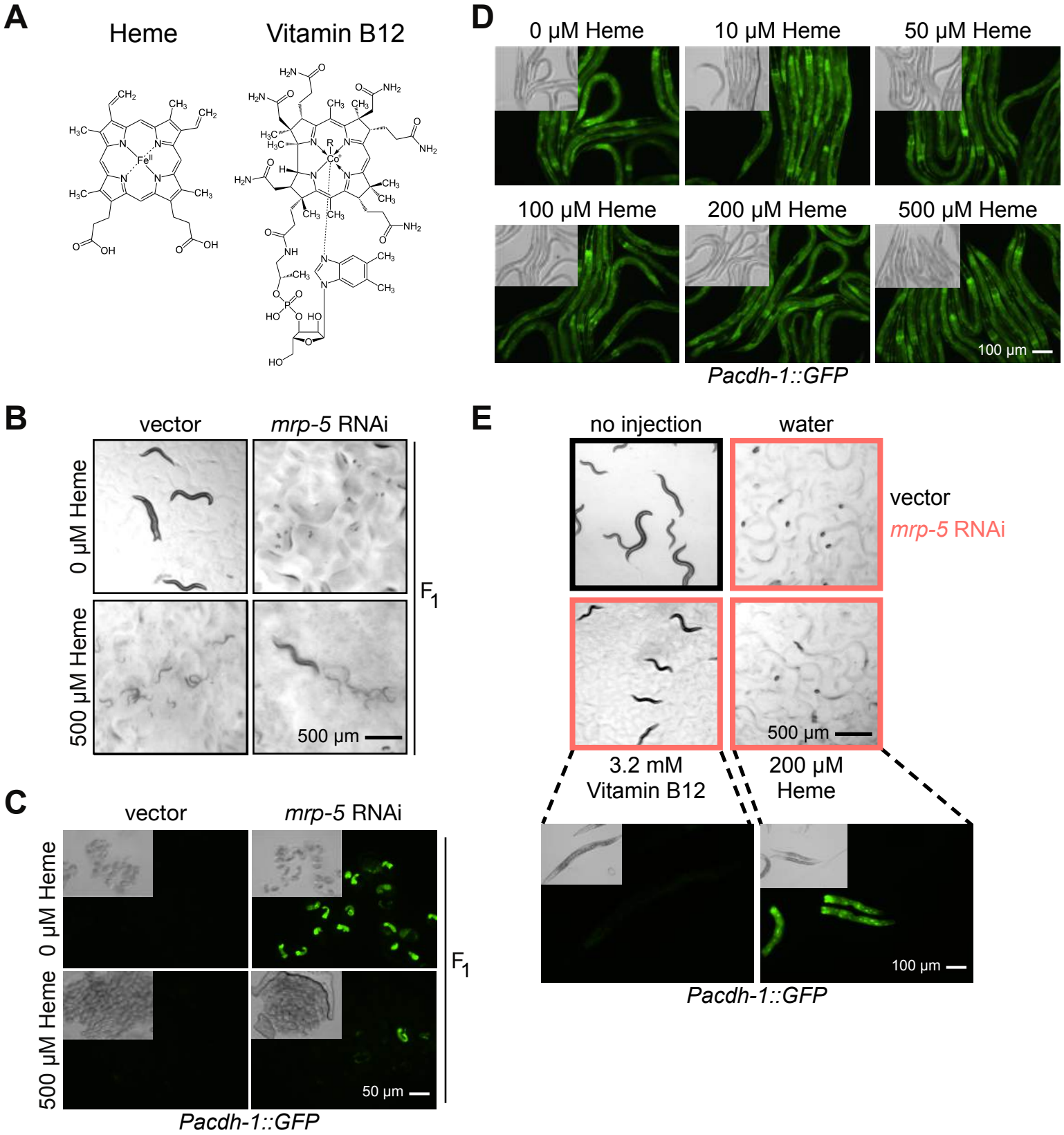
Watson, E., Olin-Sandoval, V., Hoy, M.J., Li, C.-H., Louisse, T., Yao, V., Mori, A., Holdorf, A.D., Troyanskaya, O.G., Ralser, M.*, et al.* (2016). Metabolic network rewiring of propionate flux compensates vitamin B12 deficiency in *C. elegans.* Elife *5*, pii: e17670.

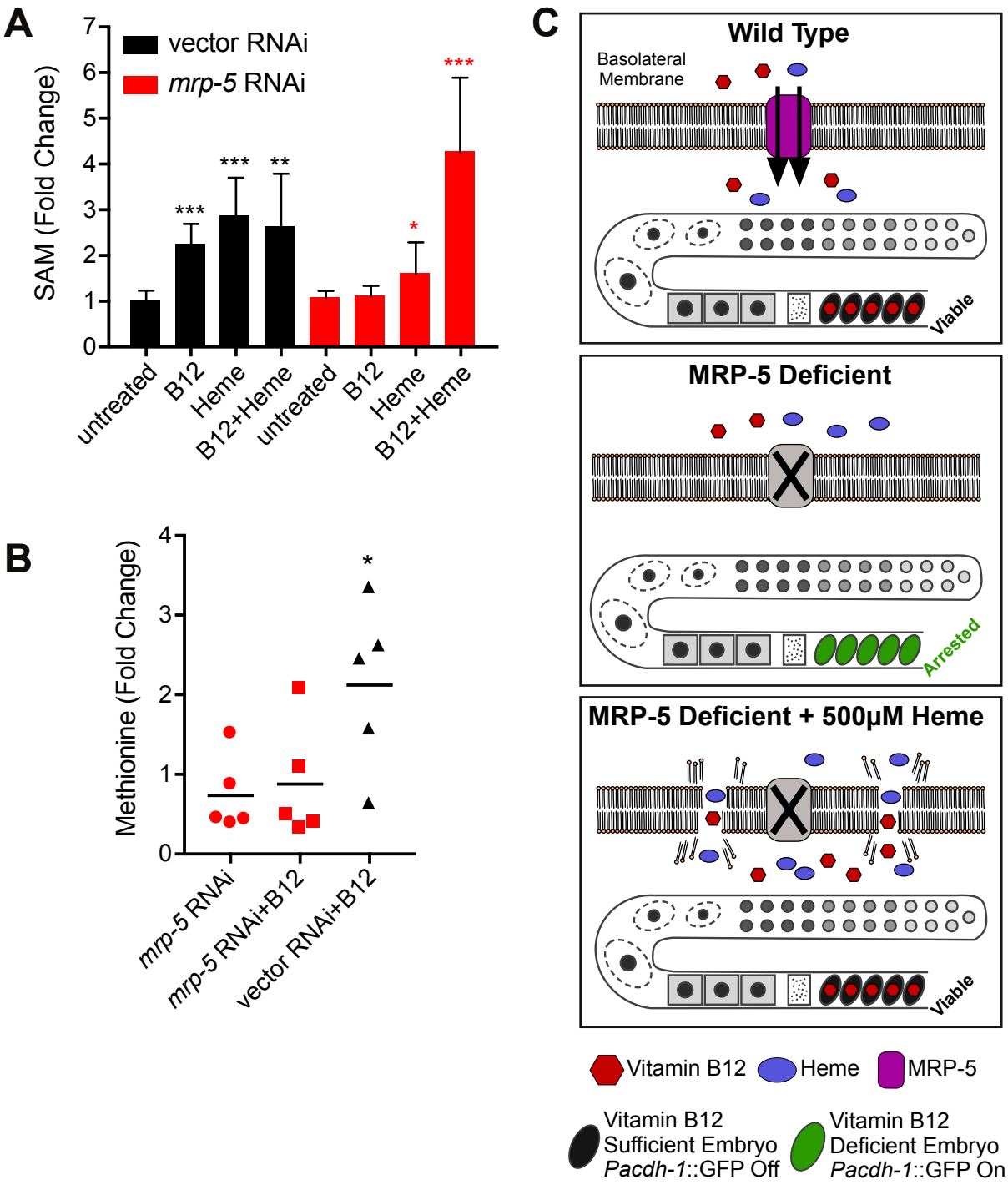
Xiao, R., Chun, L., Ronan, E.A., Friedman, D.I., Liu, J., and Xu, X.Z. (2015). RNAi interrogation of dietary modulation of development, metabolism, behavior, and aging in *C. elegans*. Cell Rep *11*, 1123-1133.

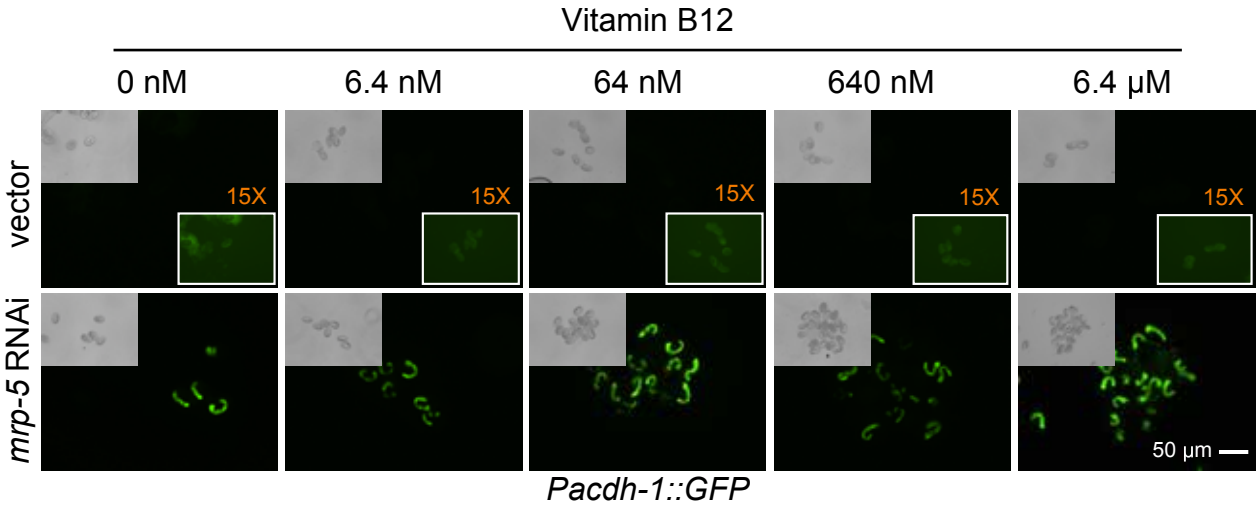
Yilmaz, L.S., and Walhout, A.J.M. (2014). Worms, bacteria and micronutrients: an elegant model of our diet. Trends Genet *30*, 496-503.


A
sams-1
Met
Ser
mel-32
THF
Gly
SAM
5, 10-meTHF
metr-1
mtrr-1
B12
mthf-1
SAH
ahcy-1
HCy
5-meTHF
Cystationine
Alpha-Ketobutyrate
acdh-1
Propionate
Propionyl-CoA
Acrylyl-CoA
pcca-1
pccb-1
ech-6
D-MM-CoA
3HP-CoA
mce-1
hach-1
L-MM-CoA
3HP
mmcm-1
B12
hphd-1
Succinyl-CoA
MSA
alh-8
Acetyl-CoA
B
E. coli
Comamonas
OP50
HT115
DA1877
P0
F1
100 µm
50 µm
Pacdh-1::GFP
C
RNAi of 215 Predicted Transporters
+ Vitamin B12
GFP off adult
GFP on embryos
D
- Vitamin B12
+ Vitamin B12
P0
F1
vector
mrp-5 RNAi
100 µm
50 µm
Pacdh-1::GFP
E
vector
cft-1 RNAi
mrp-1 RNAi
mrp-2 RNAi
mrp-3 RNAi
mrp-4 RNAi
mrp-5 RNAi
mrp-6 RNAi
mrp-8 RNAi
50 µm
Pacdh-1::GFP
F
Vitamin B12
0 nM
0.064 nM
0.32 nM
0.64 nM
3.2 nM
vector RNAi
mrp-5 RNAi
5X
100 µm
Pacdh-1::GFP::H2B (nuclear-localized single copy)


A
mrp-5 RNAi (OP50)
Synchronized L1
L4
Inject Water
Inject Vitamin B12
Single P0 aniamls
Check F1 phenotypes
B
mrp-5 RNAi F1
vector RNAi F1
Water injection
Vitamin B12 injection
No injection
50 µm
100 µm
100 µm
Pacdh-1::GFP
Water
Vitamin B12
0 20 40 60 80 100
% Hatched Embryos
% GFP Positive
C
P0
+/szT1 [*lon-2*(*e678*)] I; *mrp-5*(*ok2067*)/szT1 X
F1
mrp-5 (+/-)
mrp-5 (-/-)
arrested szT1 aneuploids
D
P0
+/szT1 [*lon-2*(*e678*)] I; *mrp-5*(*ok2067*)/szT1 X
F1
mrp-5 (+/-)
mrp-5 (-/-)
arrested szT1 aneuploids
F2
mrp-5 (-/-)
+Water
+Vitamin B12
mrp-5 (+/-)
mrp-5 (-/-)
100 µm
M
Water
B12
Water
B12
1650 bp
850 bp
400 bp
WT
del
+/- +/- -/- -/-
mrp-5


A
Heme
Vitamin B12
B
vector
mrp-5 RNAi
0 μM Heme
500 μM Heme
F1
500 μm
C
vector
mrp-5 RNAi
0 μM Heme
500 μM Heme
F1
50 μm
Pacdh-1::GFP
D
0 μM Heme
10 μM Heme
50 μM Heme
100 μM Heme
200 μM Heme
500 μM Heme
100 μm
Pacdh-1::GFP
E
no injection
water
vector
mrp-5 RNAi
500 μm
3.2 mM Vitamin B12
200 μM Heme
100 μm
Pacdh-1::GFP


A
SAM (Fold Change)
vector RNAi
mrp-5 RNAi
untreated
B12
Heme
B12+Heme
untreated
B12
Heme
B12+Heme
B
Methionine (Fold Change)
mrp-5 RNAi
mrp-5 RNAi+B12
vector RNAi+B12
C
Wild Type
Basolateral Membrane
Viable
MRP-5 Deficient
Arrested
MRP-5 Deficient + 500μM Heme
Viable
Vitamin B12
Heme
MRP-5
Vitamin B12 Sufficient Embryo Pacdh-1::GFP Off
Vitamin B12 Deficient Embryo Pacdh-1::GFP On


Vitamin B12
0 nM
6.4 nM
64 nM
640 nM
6.4 μM
vector
mrp-5 RNAi
15X
50 μm
Pacdh-1::GFP