Figure S1

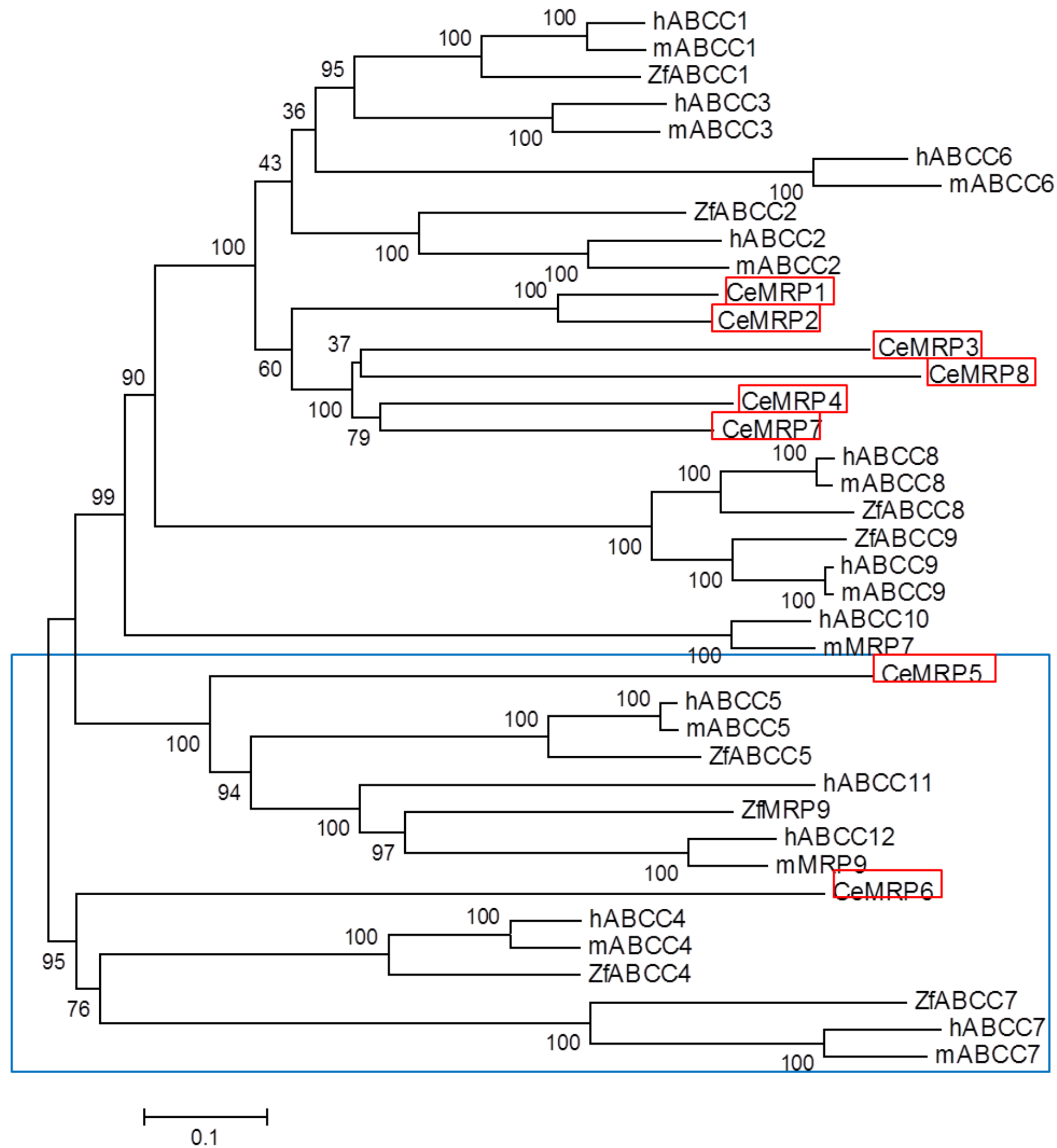


Figure S1: Related to Figure 1 - Phylogenetic tree of the ABCC/MRP subfamily. Phylogenetic analysis of the MRP/ABCC protein family members in *C. elegans*, zebrafish, mice, and humans. Sequences were aligned using ClustalW and a phylogenetic tree was generated using the Neighbor-Joining method in MEGA5. *C. elegans mrp* genes are boxed in red, ABCC/MRP proteins lacking the N-terminal MSD0 domain are boxed in blue.

Figure S2

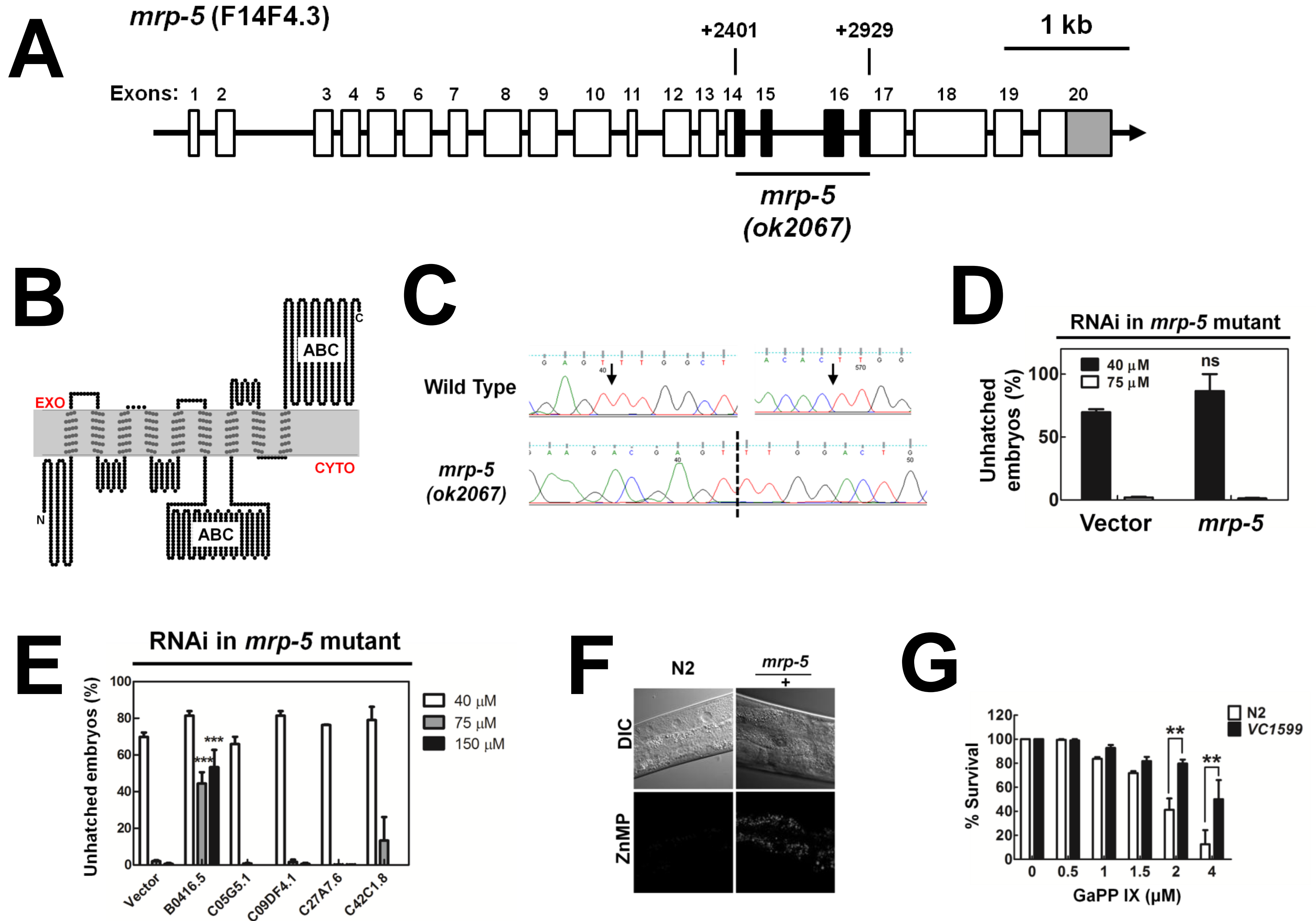
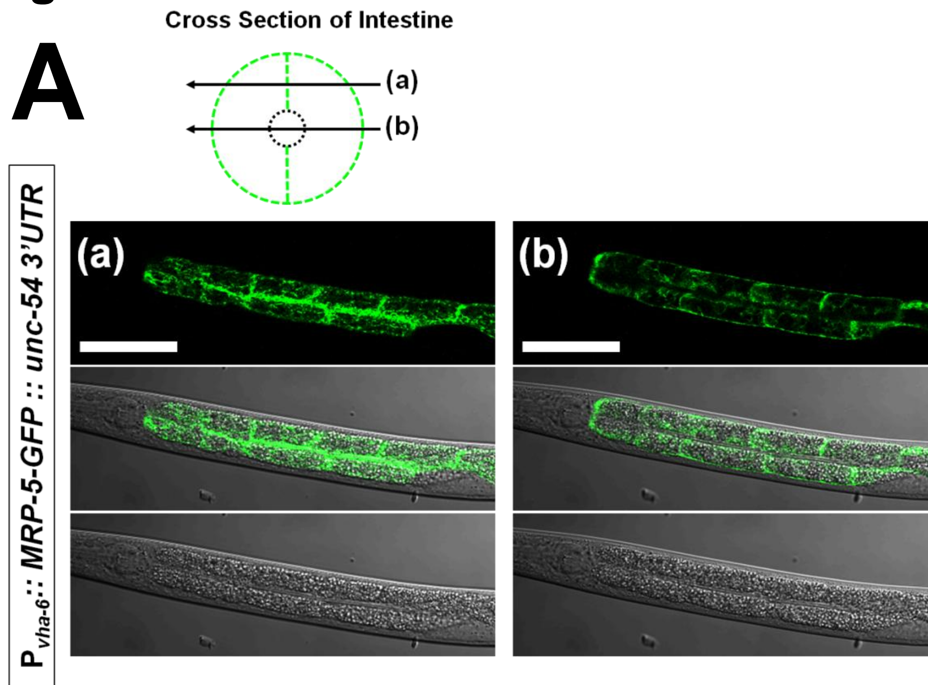
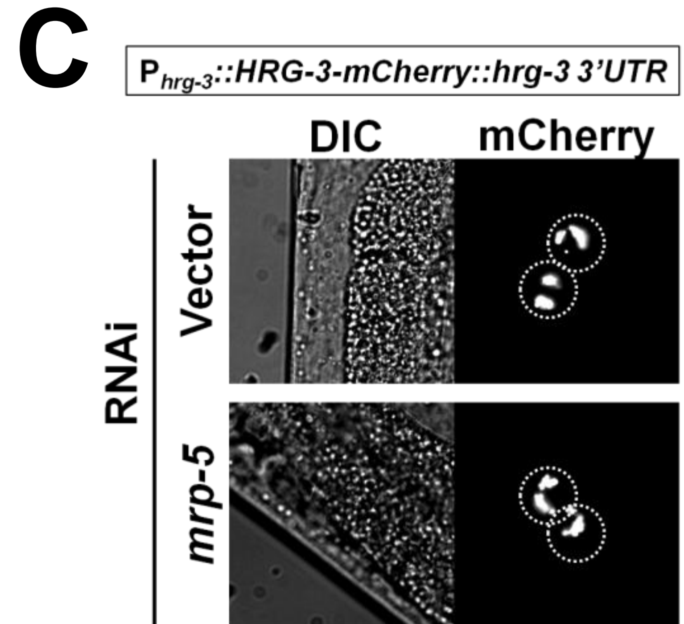


Figure S2: Related to Figure 2 - The *mrp-5(ok2067)* allele is an in-frame deletion that results in a dysfunctional protein. **(A)** Detailed exonic structure of *mrp-5*, including the *ok2067* deletion. Boxes indicate exons, with open boxes showing protein coding regions, and shaded boxes indicating untranslated regions. The region deleted in *ok2067* is shown in black, with the start and end positions of the mutation in the spliced transcript indicated above the deleted region. **(B)** Schematic of mutant MRP-5 protein produced from *mrp-5(ok2067)* deletion allele. The deletion removes three transmembrane domains, causing the second ATP-binding cassette (ABC) domain to be located on the extracellular side of the membrane. **(C)** Sequencing chromatogram showing deletion boundaries (arrows) and deleted region (dotted line), respectively, in the wild type (top) and *mrp-5(ok2067)* (bottom) transcripts after RT-PCR. **(D)** RNAi of *mrp-5* in *mrp-5(ok2067)* mutant worms causes no added embryonic lethal phenotype. Mutant worms were grown at 500 µM heme, and their synchronized L1 progeny were grown on vector or *mrp-5(RNAi)* plates and their subsequent progeny were scored for hatching. Error bars represent standard error of the mean; ns, not significant (two-way ANOVA, Bonferroni post-test). **(E)** RNAi of FLVCR homologs in *mrp-5(ok2067)* background results in no added heme-specific phenotypes. Experiment was performed as in Figure S2D. ***$P<0.001$ when compared to vector control under identical conditions (two-way ANOVA, Bonferroni post-test). **(F)** Resistance to a toxic heme analog, gallium protoporphyrin IX (GaPPIX), was measured in wild type N2 and VC1599 (*mrp-5/+)* worms. Resistance was assessed after L4 worms were exposed to varying concentrations of GaPPIX for 48 hours. Worms were considered dead when unresponsive to a physical stimulus. Each data point represents the mean of three separate experiments and is depicted as percentage of survival compared to control plates with no GaPPIX. **$P<0.01$ when compared to wild type worms on identical plates. **(C)** ZnMP staining in N2 control or VC1599 (*mrp-5/+)* worms. Worms were exposed to RNAi from L1 to L4 stage, pulsed with 60 µM ZnMP for 3 hr, and imaged on an inverted microscope. Representative images of intestinal granules containing ZnMP in control and mutant worms are shown.

Figure S3





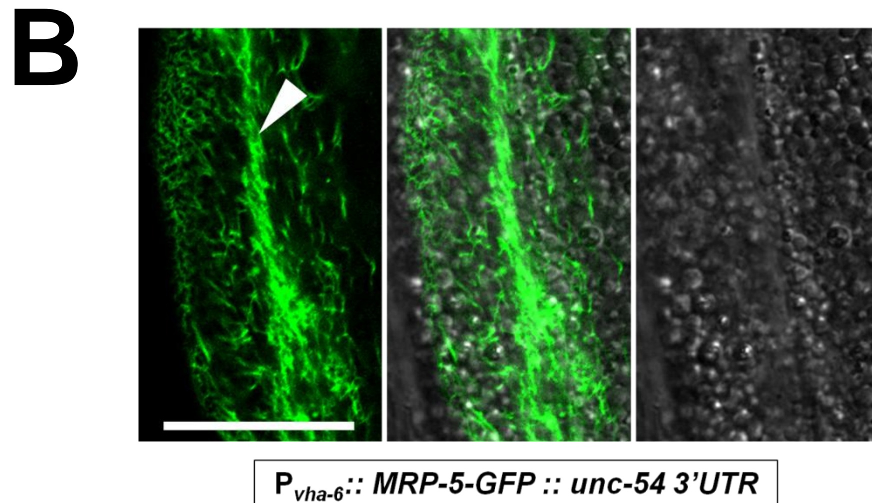


Figure S3: Related to Figure 3 - MRP-5 is a putative intestinal heme exporter in *C. elegans*. **(A)** Images of a worm expressing the *MRP-5::GFP* translational fusion protein. Images were taken at two different planes along the dorsal-ventral axis. Localization of *MRP-5::GFP* to lateral membranes of adjacent intestinal cells can be seen in (a), while localization of MRP-5::GFP to the basolateral membranes and not the apical membrane surrounding the intestinal lumen can be seen in (b). The anterior of the animal is to the left. Scale bar, 50 μM. **(B)** Magnified image of *MRP-5::GFP* localization to basolateral sorting vesicles. Image is taken in the same plane as (a) in Figure S3A. Arrowhead indicates lateral membranes of adjacent intestinal cells. Scale bar, 20 μM. **(C)** RNAi depletion of *mrp-5* does not inhibit the secretion of *hrg-3* from the intestine. Transgenic worms containing the *Phrg-3::HRG-3::mCherry* translational fusion were fed vector control or *mrp-5* RNAi bacteria from L1 to L4 stages. HRG-3::mCherry in extraintestinal cells (coelomocytes) was imaged on an inverted microscope. Dotted circles indicate location of coelomocytes.

Figure S4

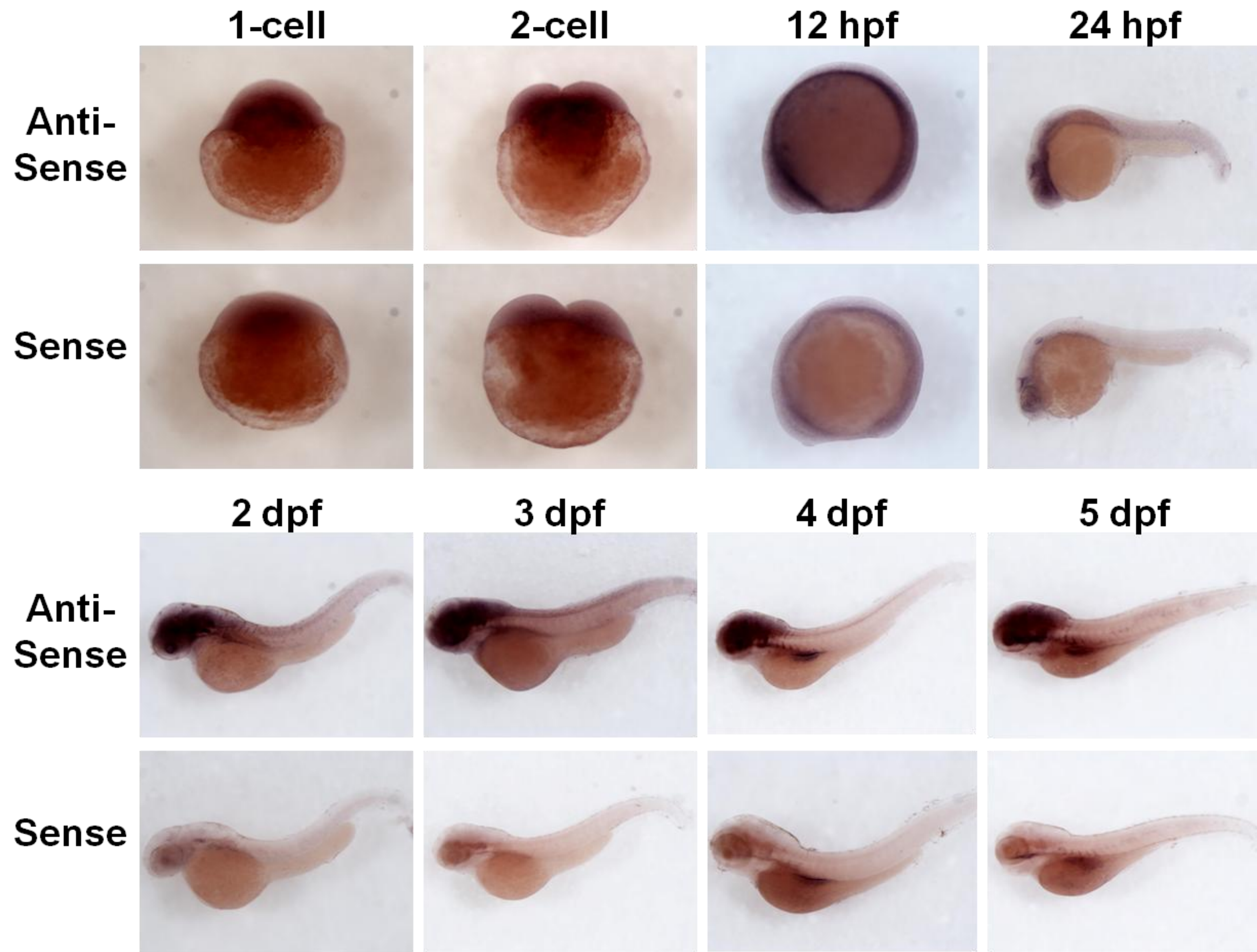


Figure S4: Related to Figure 4 - *In situ* hybridization reveals tissue specific expression of *mrp5*. Zebrafish *mrp5* expression by whole mount *in situ* hybridization using anti-sense probe, at indicated developmental stages. Anterior is to the left. Sense probe image is shown to indicate background staining.