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Supplemental information

Cryosectioning and immunofluorescence of *C. elegans* reveals endogenous polyphosphate in intestinal endo-lysosomal organelles

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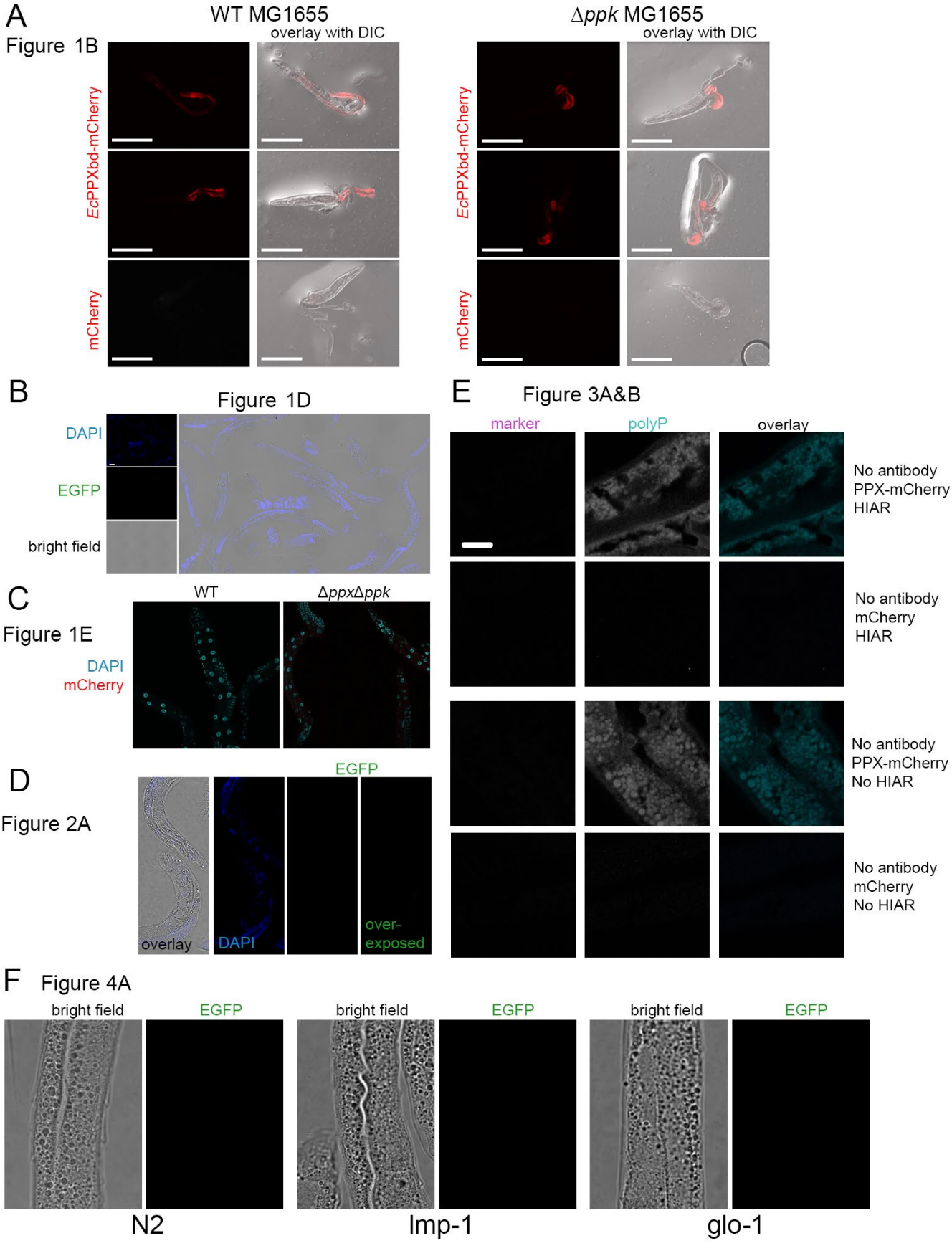
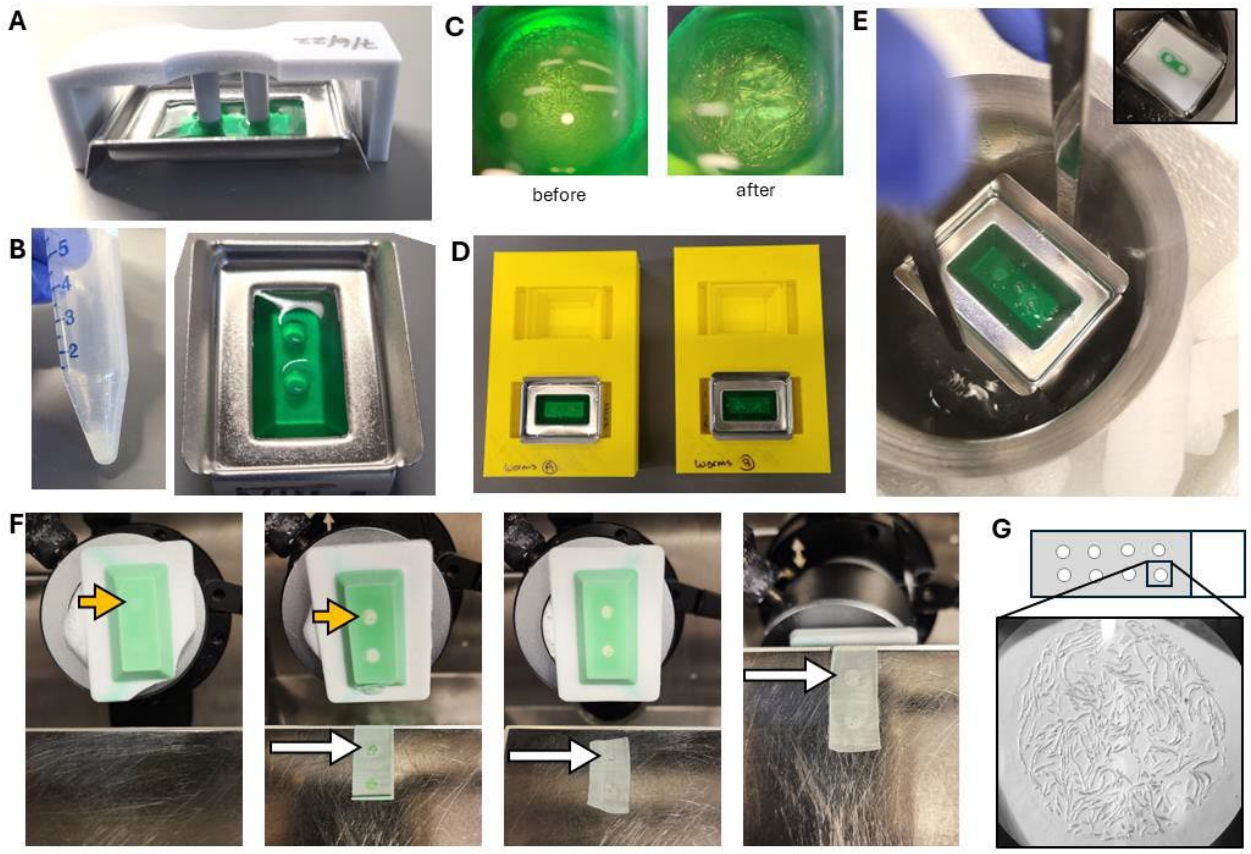
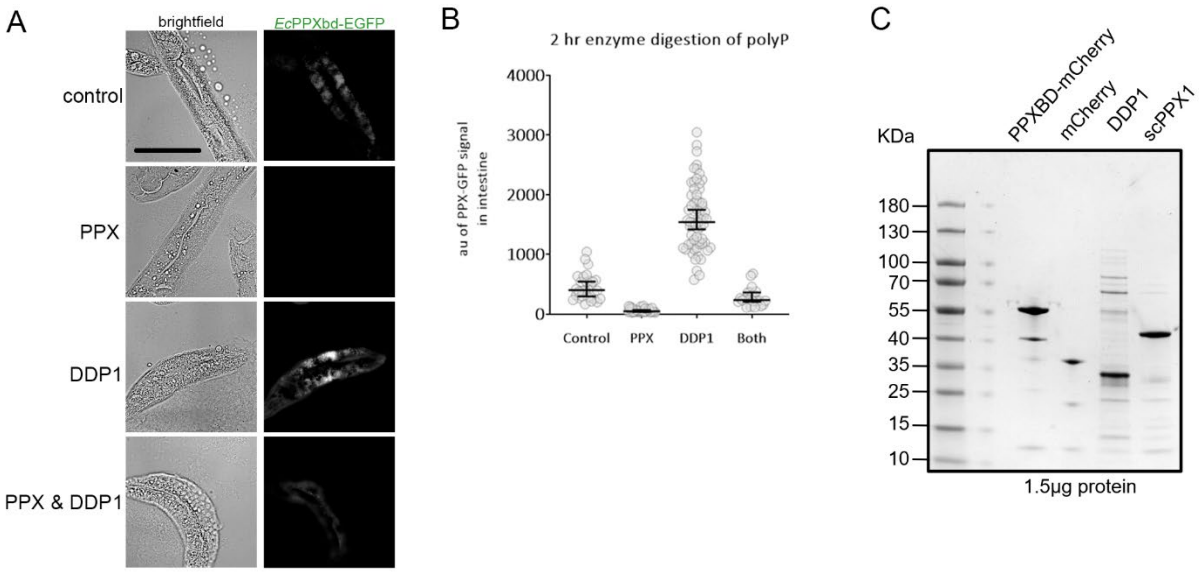
SUPPORTING FIGURES



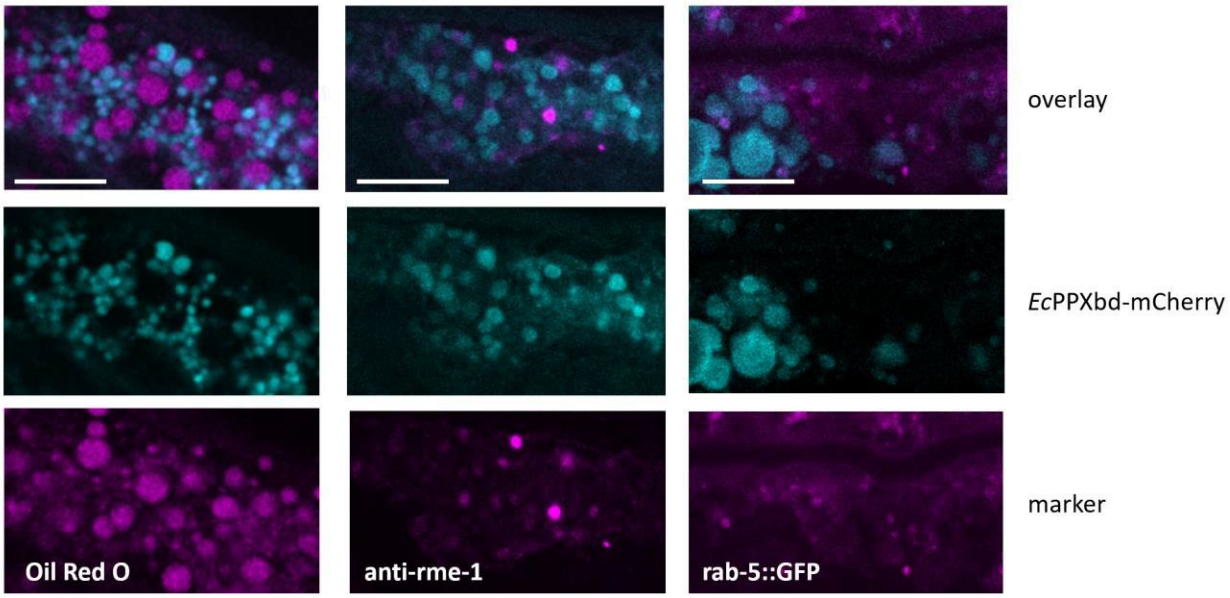
Supporting Figure 1. Example images of control conditions for all main text Figures. A) Higher resolution images of Figure 1B: Dissected adult N2 worms, stained with *Ec*PPXbd-mCherry, and the DIC image, shown in overlay. Scale bars are 200 µm. The worms were raised on either wild-type MG1655 *E. coli*, or a *ppk* deletion mutant. Bottom row of each bacteria type: worms stained with mCherry alone. B) *Ec*PPXbd-EGFP control stained sample for Figure 1D. C) mCherry control stained samples for Figure 1E. D) EGFP control stained sample for Figure 2A. E) Four staining-control conditions shown from top to bottom for Figure 3A&B (No antibody used on N2 wildtype sample, +/- PPX-mCherry vs mCherry staining, +/- Heat Induced Antigen Retrieval which was used for lmp-1 antibody testing). Scale bar is 8.5 microns. Images are zoomed out to show clear lack of polyP-related signal. F) Bright field and EGFP channels for N2, lmp-1KO, and glo-1KO samples serving as controls for Figure 4A. All images are brightness corrected to the same level as the polyP-channels in the figures in the main text.



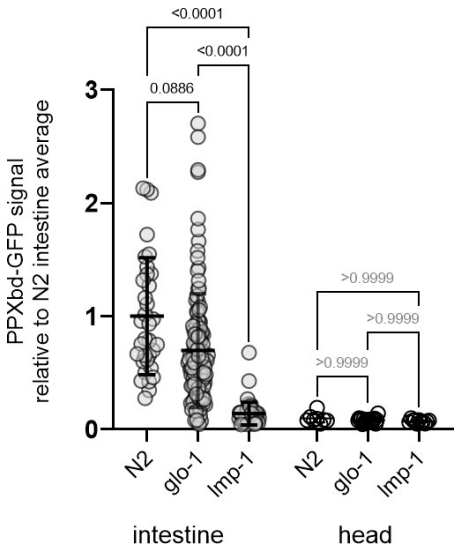
Supporting Figure 2. Images detailing STAR*Methods sections for Cryomold preparation, Freezing worms in cryomolds, and Cryosectioning. A) 3D-printed mold inserted into gelatin-solution in metal cryomold. B) Densely packed worms in PBS, left, to be added to the individual wells left by after 3D printed mold removal from gelatin solution, right. C) After PBS+ worms are added to the wells ("before"), the worms immediately sink to the bottom of the well ("after"). After removing the PBS, the worms are still apparent in the well. D) Metal cryomolds with worms and gelatin solution are filled with O.C.T., placed into centrifuge adaptors, and spun down to ensure worms are aligned with the bottom of the gelatin wells. E) Immediately after removal from the centrifuge, the metal cryomolds are flash frozen by dipping them into an ethanol-dry ice slurry, taking care not to submerge the O.C.T. Insert – the cryomolds are removed from the slurry once most of the volume of O.C.T. has frozen over, as the remainder of the freezer will happen on dry ice. F) Once removed from the metal cryomolds, the frozen blocks are added to a cryostat chuck, keeping the bottom of the wells toward the user. Those wells are only barely visible before any cuts are made (leftmost image) but become highly contrasted from the gelatin when enough of the bottom layers of the block are removed (yellow arrows indicate similar well positions). The slices that come of the block show the well positions more clearly as the user gets closer to the location of the worms, turning from darker-than-surrounding gelatin just below the worm position, to white as the O.C.T. and worm area is entered. Slices with and without worms look the same so long as the O.C.T. is present (white arrows indicate same well position before O.C.T. begins, at the beginning of the worm layer, and well past the worm layer height, from left to right). G) After the slices are mounted on a slide and dried, the O.C.T. and gelatin areas become clear, and the worm material is visible by eye and stereomicroscope.



Supporting Figure 3. *Ec*PPXbd-GFP signal is specific to PPX-digestible material, related to STAR*Methods titled Enzyme Digestion and Purification of Fluorescence Probes. A) Sample images of *Ec*PPXbd-GFP signal and bright field after treating slides with *Sc*PPX1, the endopolyphosphatase DDP1, both heat-inactivated enzymes ("control"), or both active enzymes. Scale bar is 100 µm. B) Quantification of *Ec*PPXbd-GFP signal after two-hour treatment of slides +/- PPX, +/- DDP1. Each circle represents one worm intestine. Lines: mean +/- SEM. C) Protein gel of probes and enzymes used in this study. From left to right, the lanes are protein ladder, empty well, *Sc*PPXbd-mCherry, mCherry, DDP1, and PPX1. Coomassie stain was used to detect total protein. 1.5 µg protein was loaded per well.



Supporting Figure 4. *Ec*PPXbd-mCherry+ puncta are not surrounded by or co-localized with markers of lipid droplets, recycling endosomes, or early endosomes, related to Figure 3. Shown are images of representative D1 worm intestinal cells, expressing the listed markers of lipid droplets (Oil Red O), recycling endosomes (anti-rme-1 with AF488 secondary antibody), and early endosomes (rab-5::GFP), stained with *Ec*PPXbd-mCherry, and overlays. Scale bar is 10 μm. All markers are pseudocolored magenta, and all *Ec*PPXbd-mCherry panels are pseudocolored cyan.



Supporting Figure 5. Knock-out of glo-1 or lmp-1 from the intestine does not change polyP signal in the head, related to Figure 4. Relative fluorescence of *Ec*PPXbd-eGFP signal in intestine and head. Each circle is one worm. Kruskal-Wallis test with Tukey post-hoc. Lines are mean+/-SD. *P*-value is shown for each comparison made.