Supplementary material

Table S1. List of strains used in this study.

Table S2. Mass spectrometry data of spatially restricted enzymatic tagging in *C. elegans*.

Figure S1. Localization of APX to the intestinal cytoplasm of *C. elegans.*

Figure S2. Use of triplex reductive dimethylated samples to quantitatively compare GFP-APX-NES, GFP-APX-NLS, and GFP only samples.

Figure S3. Reproducibility of the number of proteins identified from each location.

Figure S4. Identification of cytoplasm or nuclear localized proteins.

Figure S5. Quantitative mass spectrometry ratios of proteins selected for validation.

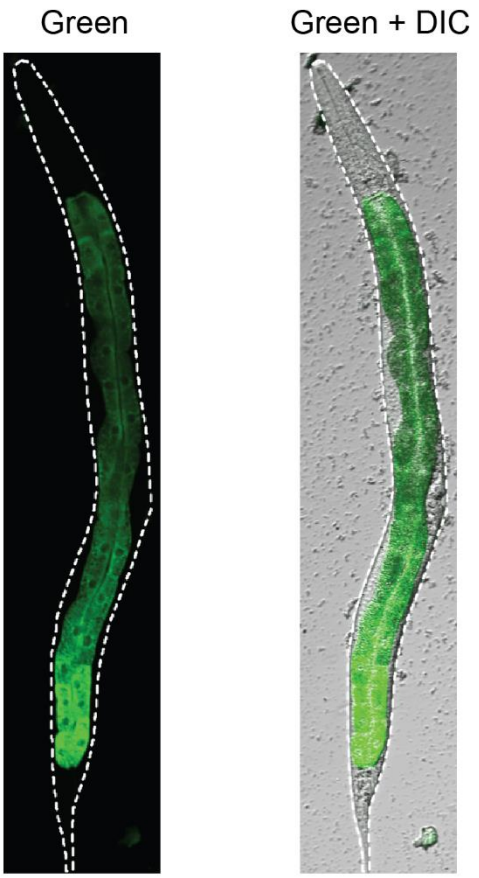

Figure S1. Localization of APX to the intestinal cytoplasm of *C. elegans.*

Microscopy of animals expressing GFP-APX-NES under the *spp-5* promoter. This strain of *C. elegans* expresses the APX enzyme fused to GFP in the intestinal cytoplasm. Green is the fluorescence channel and green + DIC (differential interference contrast) is the merged fluorescence and DIC channel.

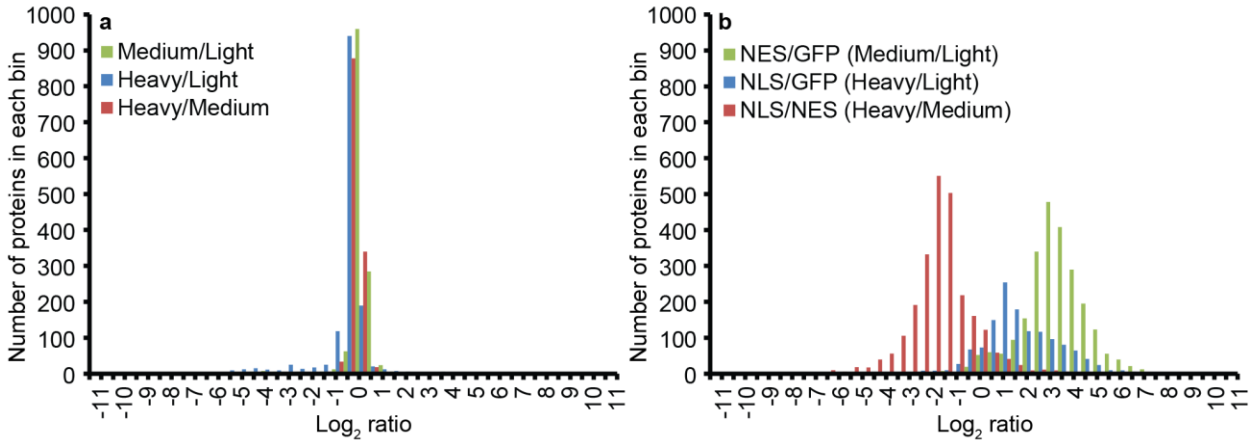

Figure S2. Use of triplex reductive dimethylated samples to quantitatively compare no APX, APX-NES, and APX-NLS samples. Animals expressing intestinal APX-NES, APX-NLS, and the GFP-only control were used to perform spatially restricted enzymatic tagging. Biotinylated proteins were isolated and digested into peptides. **a.** Peptides from the three samples were mixed together, split into thirds, with each portion being labeled with a different isotopic label. All the isotopically labeled samples were then mixed back together. Shown is the Log2 transformed protein abundance ratio. **b.** Peptides from the three samples were each labeled with a different isotopic label. All the isotopically labeled samples were then mixed back together.

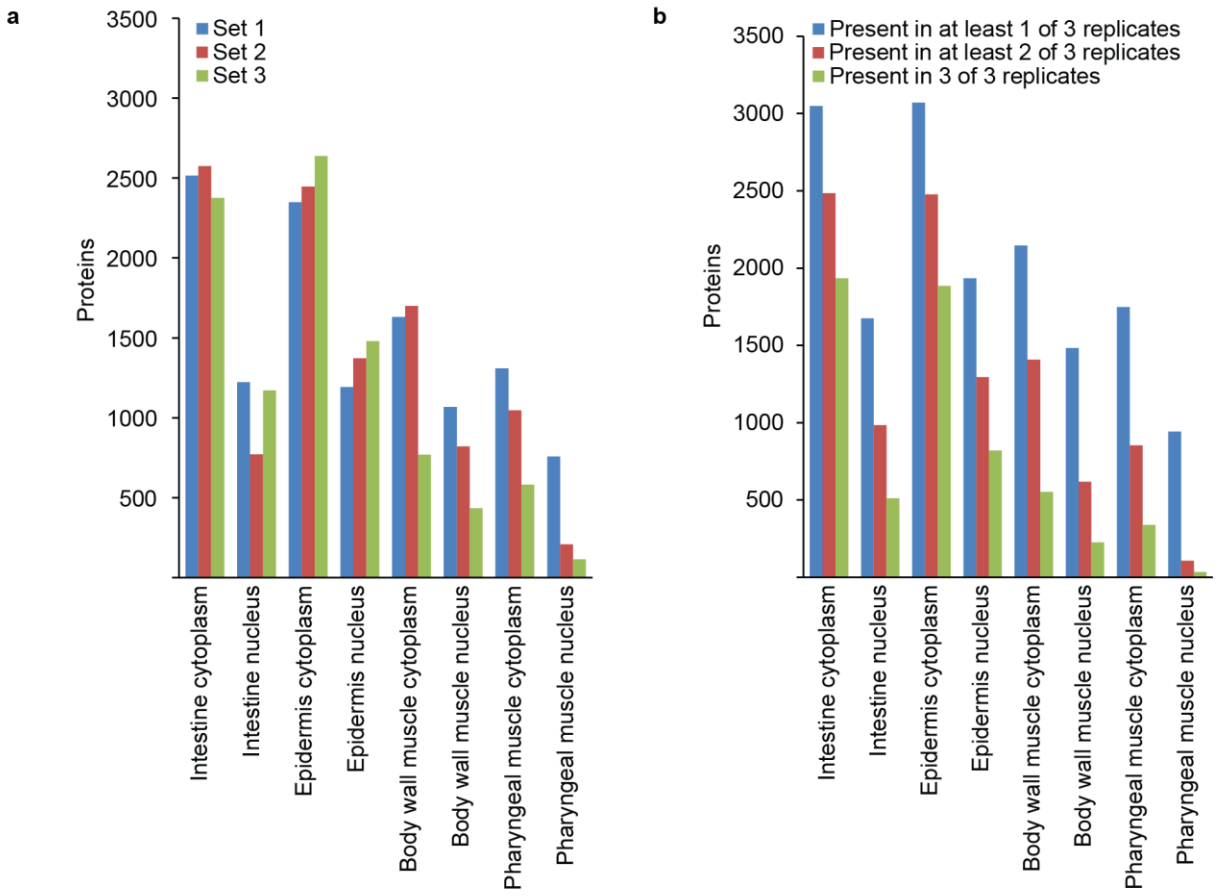

Figure S3. Reproducibility of the number of proteins identified from each location. The number of proteins identified from the tissue or subcellular location for each of the eight locations indicated. **a.** Each location shows the number of proteins identified in each set. **b.** Each location shows the number of protein identified in at least 1 of 3 replicates, at least 2 of 3 replicates, or in all three replicates.

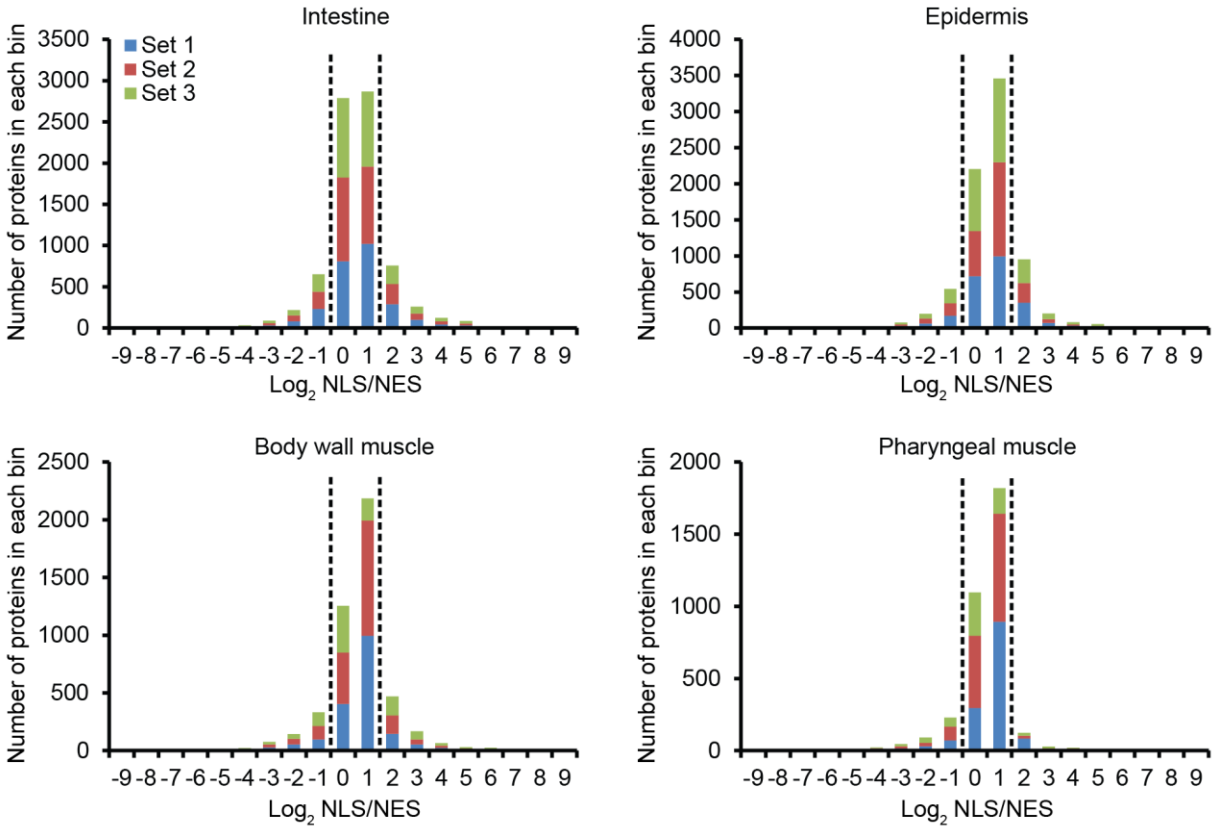

Figure S4. Identification of cytoplasm or nuclear localized proteins. Cytoplasm or nuclear localized proteins were determined by examining the quantitative ratios after APX-mediated proximity labeling in the indicated tissue. Shown is the distribution of the Log_2 NLS/NES ratio for proteins from each biological replicate that display NES/GFP ratios greater than 1 or NLS/GFP ratios less than 1 which filters out proteins identified in the control GFP only labeled samples. Dashed lines demarcate the cutoff for being nucleus specific (greater than 1) or cytoplasm specific (less than -1) for each indicated tissue.

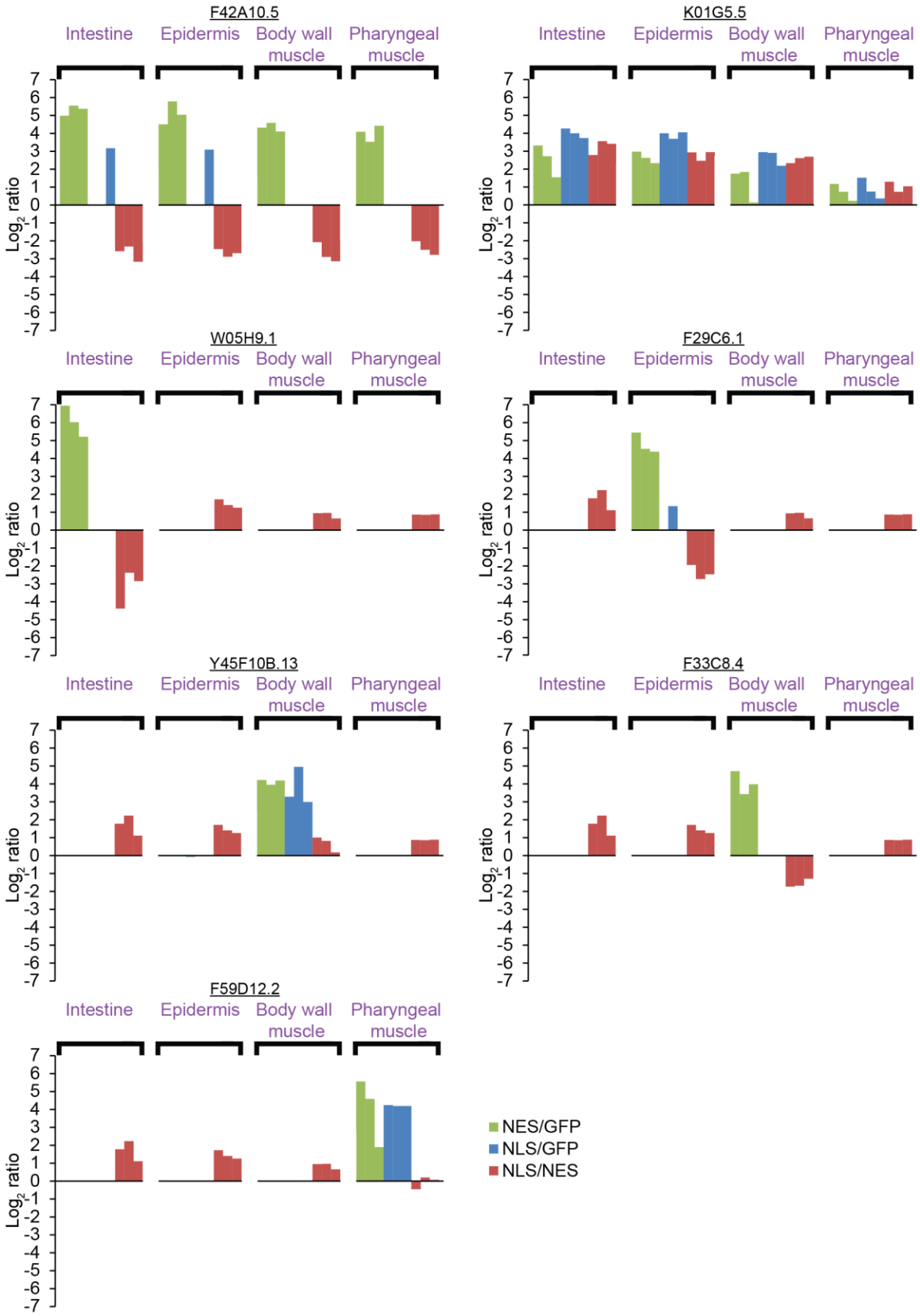
F42A10.5
K01G5.5
W05H9.1
F29C6.1
Y45F10B.13
F33C8.4
F59D12.2
Intestine
Epidermis
Body wall muscle
Pharyngeal muscle
Log2 ratio
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
NES/GFP
NLS/GFP
NLS/NES

Figure S5. Quantitative mass spectrometry ratios of proteins selected for validation. The seven proteins from Figure 5 are shown. For each protein, the NES/GFP, NLS/GFP, and NLS/NES Log_2 ratios from each of the three replicates are shown for each of the four tissues.