Expanded View Figures

Figure EV1. Results of modifier screen and identification of AMAN-2/Golgi Alpha-mannosidase II.

A Table of alleles isolated in the *lect-2(gk864764)* hypomorph modifier screen.

B Graph of single nucleotide polymorphism (SNP) results after whole genome sequencing of the *dz261* allele (Doitsidou *et al*, 2016). Axes are denoted above. The green box shows the genomic position of *aman-2*.

C Table showing the candidate genes tested as a result of the SNP data in (B). Pools of fosmids covering the regions of indicated transcripts were injected into the *lect-2(hyp); dz261* double mutant. Only the pool containing a fosmid including *aman-2* showed rescue. *pyc-1* was eliminated because a null allele *(gk689405)* failed to enhance the *lect-2(hyp)*. Numbers indicate number of biological replicates that showed rescue. 25 animals were scored for each line.

D Quantification of the number of secondary branches (indicated in schematic) in the genotypes indicated. Data are represented as mean ± SEM. Statistical significance was calculated using the Kruskal–Wallis test, ns: no significance. $n = 15$ for all genotypes and are biological replicates.

E Viability of *aman-2* mutant animals assessed via brood size comparison in the number of eggs that hatched and produced L1 larvae in wild-type control and *aman-2 (tm1078)* animals. Data are represented as mean ± SEM. Statistical significance was calculated using the Mann–Whitney test, ns: no significance. $n = 5$ for all genotypes and are biological replicates.

F Fluorescent images of the *dma-1* null allele, *dma-1(tm5159)*, alone (top) and in a double null mutant *aman-2(gk248486)* background (bottom). Asterisk indicates location of the cell body. Scale bars represent 20 μm.

Source data are available online for this figure.

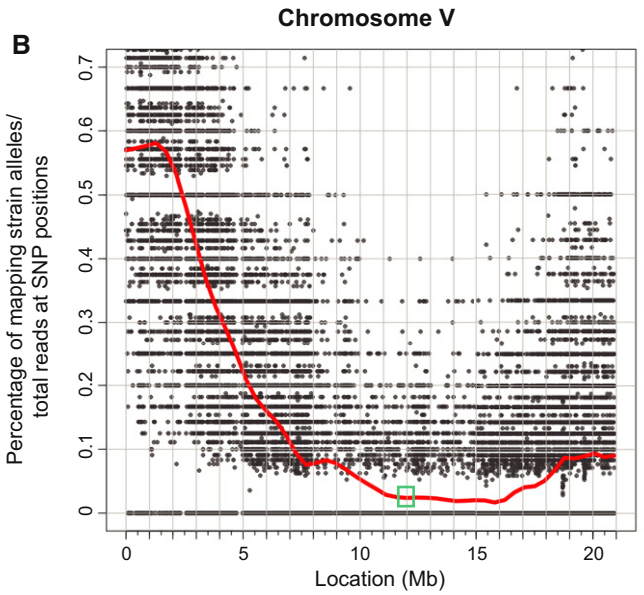
Figure EV1.

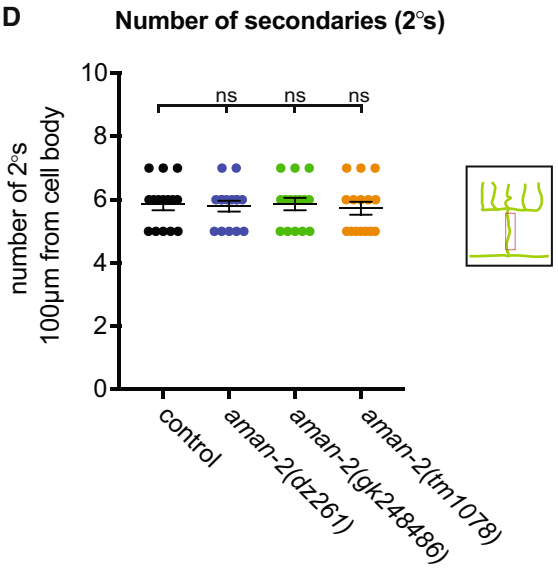
A Novel alleles isolated in *lect-2(hyp)* modifier screen

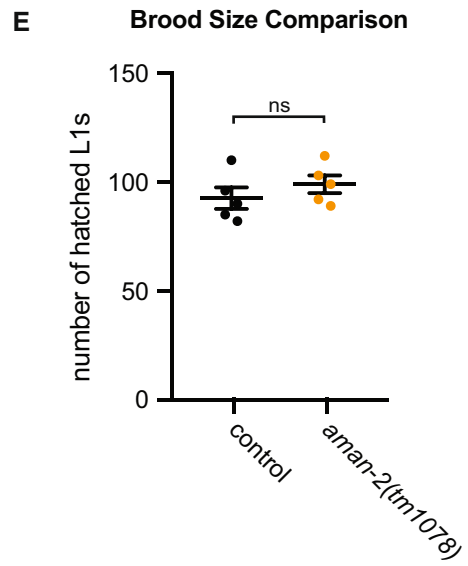
allele	gene	mutation
dz252	*mnr-1*	*R513H (R459H) in MNR-1L (MNR-1S)*
dz253	*sax-7*	*loss of splice donor site exon 4*
dz254	*kpc-1*	*L535F*
dz261	*aman-2*	*W237Opal*

C Fosmid rescue of PVD defects in *lect-2(hyp); dz261* mutants

gene/transcript ID	fosmid number	rescued lines
pyc-1	WRM0640aD10	**3/5**
aman-2	WRM0617dH10	
mpst-7	WRM0624aG04	0/3
srh-24	WRM0624cC12	
T26E4.4	WRM0638dB04	0/5
sqst-4	WRM0628aH05	
F59A1.11	WRM0637bA11	







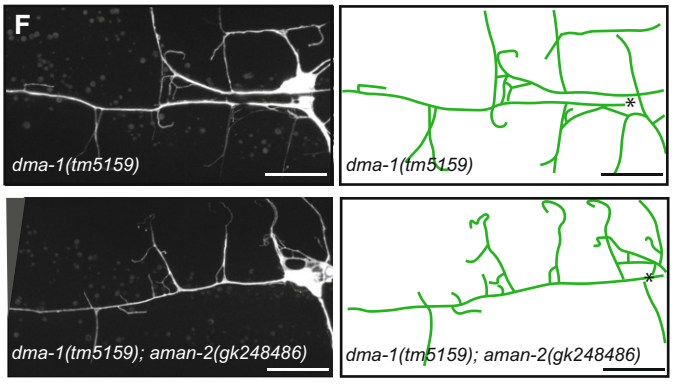


Figure EV1.

Figure EV2. The requirement of AMAN-2 in PVD may be specific rather than global.

A Quantification of "Os" (overlapping branches as shown in schematic) of denoted genotypes at different temperatures. Data are presented as box and whisker plots, with the median and 25th and 75th percentile indicated. Whiskers show minimum and maximum. Statistical comparisons were performed using two-way ANOVA tests, *$P \leq 0.05$, ***$P \leq 0.001$, ****$P \leq 0.0001$, ns: not significant. $n > 11$ for all groups.

B Quantification of full tertiaries of denoted genotypes at different temperatures. Data are presented as box and whisker plots, with the median and 25th and 75th percentile indicated. Whiskers show minimum and maximum. Statistical comparisons were performed using two-way ANOVA tests, ***$P \leq 0.001$, ns: not significant. $n > 11$ for all groups.

C Quantification of DMA-1::GFP fluorescence in control and *aman-2(gk248486)* animals. GFP intensity is quantified in arbitrary fluorescent units by dividing the fluorescent area of the soma by the background. Data are represented as mean ± SEM. Statistical comparisons were performed using the Mann–Whitney test, ns: not significant. $n = 12$ for all genotypes and are biological replicates.

D Quantification of DMA-1::GFP fluorescence in control and *aman-2(gk248486)* animals. GFP intensity is quantified in arbitrary fluorescent units by measuring the fluorescence along the primary dendrite, up to 60 μm anterior from the cell body. Data are represented as mean ± SEM. Statistical comparisons were performed using the Mann–Whitney test, ns: not significant. $n = 12$ for all genotypes and are biological replicates.

E Quantification of DMA-1::GFP fluorescent puncta in control and *aman-2(gk248486)* animals. Puncta in the tertiary branches 60 μm anterior to the cell body were counted. Data are represented as mean ± SEM. Statistical comparisons were performed using the Mann–Whitney test, ns: not significant. $n = 12$ for all genotypes and are biological replicates.

F Localization of LECT-2::mNeonGreen (*dz249* endogenous reporter) in wild-type and *aman-2(dz261)* null mutant animals. No obvious differences in general neuronal or hypodermal staining were observed. Vesicular gut autofluorescence is visible as white circular staining. Scale bars represent 20 μm. 15 animals were assessed per genotype.

G Localization of SAX-7::GFP (*ddIs290* fosmid reporter) in wild type and *aman-2(dz261)* null mutant animals. No obvious differences in general neuronal or hypodermal staining were observed. Vesicular gut autofluorescence is visible as white circular staining. Scale bars represent 20 μm. 15 animals were assessed per genotype.

Source data are available online for this figure.

Figure EV3.

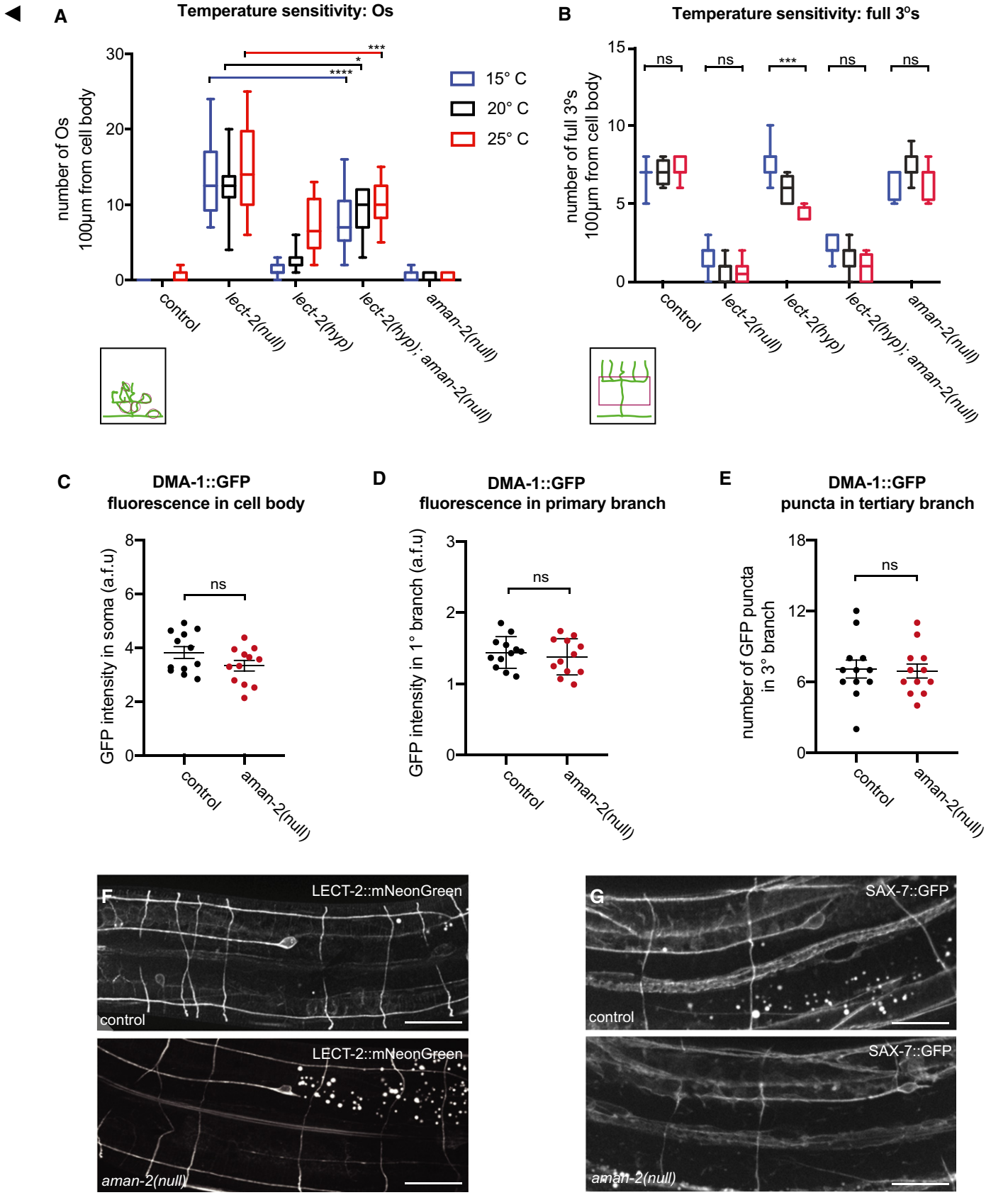


Figure EV2.

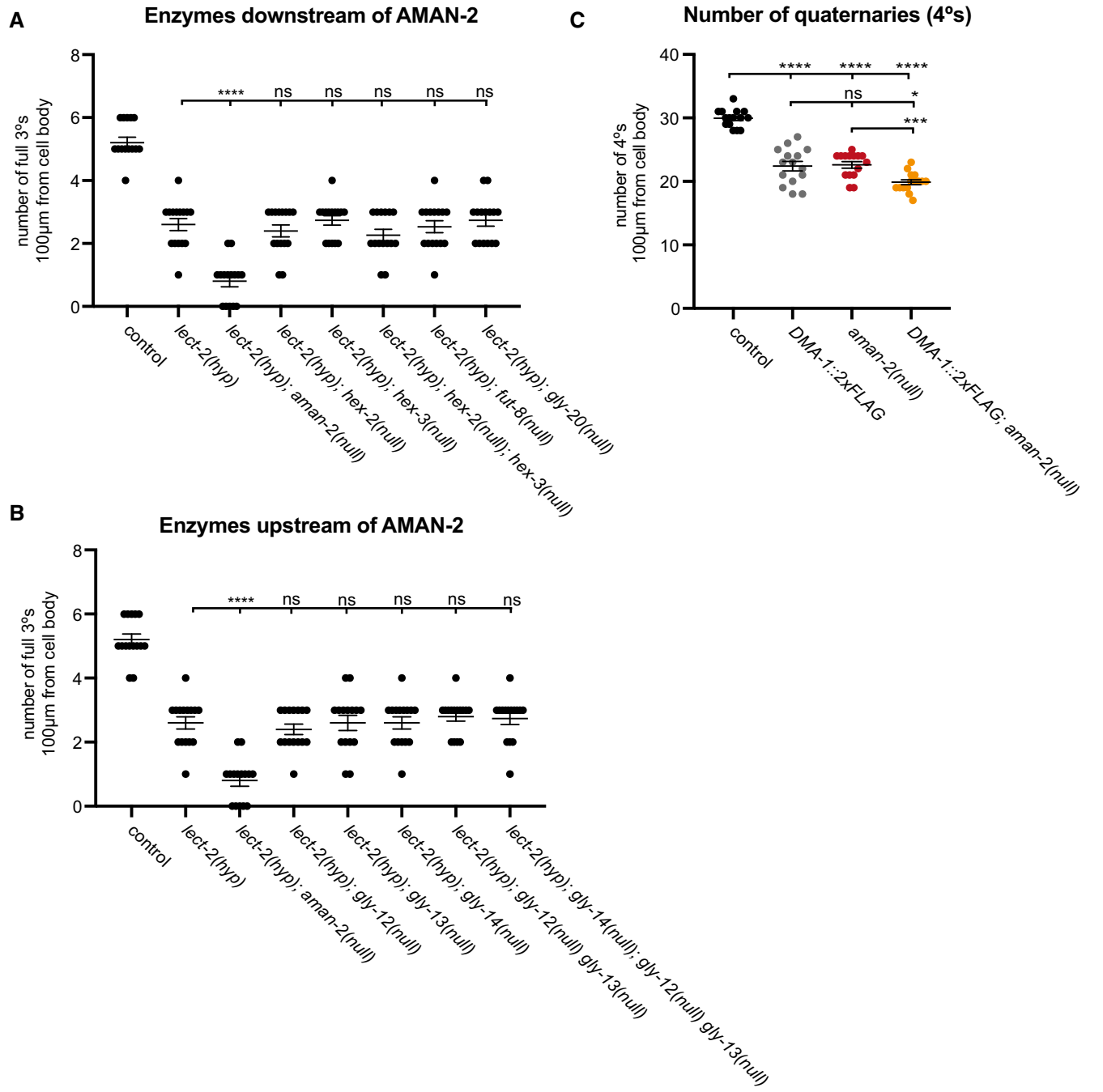


Figure EV3. Quantification of branching in *N*-glycosylation pathway mutants.

A

Figure EV4.

◀ B Quantification of the number of full tertiaries in wild-type controls, *lect-2(gk864764)* hypomorphs, and *lect-2(gk864764)* in combination with mutants of enzymes acting downstream of *aman-2*. See strain list for alleles. Data are represented as mean ± SEM. Statistical significance was calculated using the Kruskal–Wallis test, ****$P \leq 0.0001$, ns: not significant). $n = 15$ for all genotypes and are biological replicates.

C Quantification of the number of quaternary branches in wild-type control animals, DMA-1::2XFLAG (denoted as DMA-1 wild type in Fig 4), and in combination with *aman-2(gk248486)*. In the DMA-1::2XFLAG (*wy1041)* background, there is a baseline decrease in the number of branches, possibly due to the insertion of the tag (Dong *et al*, 2016). While equivalent to the phenotype of *aman-2(gk248486)*, combining the two backgrounds results in a further decrease in quaternary branches, providing an additional example of how hypomorphic alleles in the Menorin pathway can be enhanced by the loss of *aman-2*. Data are represented as mean ± SEM. Statistical significance was calculated using the Kruskal–Wallis test, *$P \leq 0.05$, ***$P \leq 0.001$, ****$P \leq 0.0001$. $n = 15$ for all genotypes and are biological replicates.

Source data are available online for this figure.

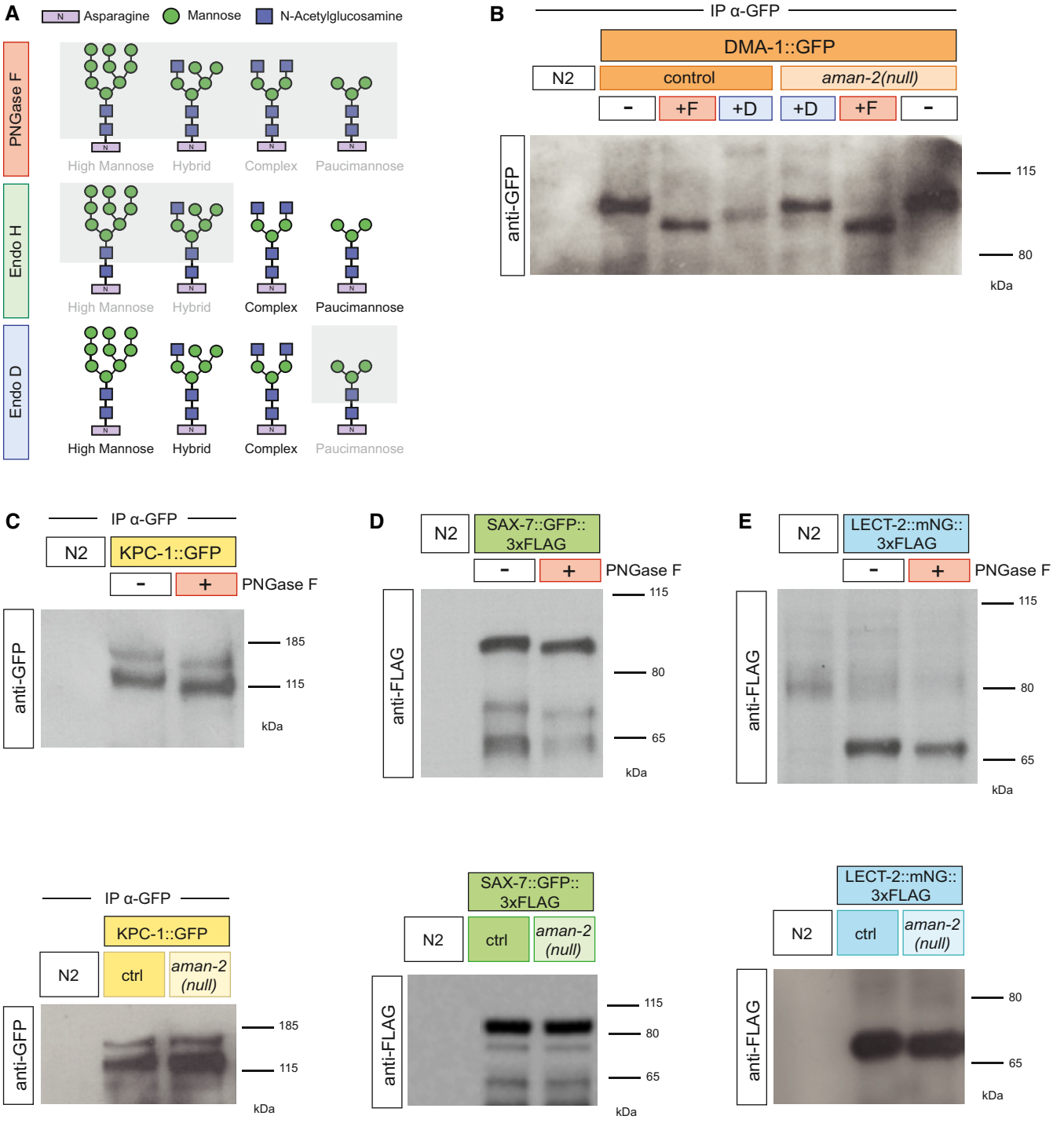


Figure EV4. Members of the Menorin pathway are *N*-glycosylated.

A Schematic of endoglycosidase activity on *N*-glycan chains. The gray shading represents parts of the chain that are cleaved by each respective endoglycosidase. Glycan residues are consistent with Fig 2A, and colors of endoglycosidases correspond to Fig 3E and G.

B Western blot against GFP in *C. elegans* lysate DMA-1::GFP (*qyIs369)*, after precipitating with anti-GFP antibody. Control indicates an otherwise wild-type background as opposed to an *aman-2(gk248486)* background. The red boxed +F indicates that the lysate is treated with the PNGase F glycosidase, while the green boxed +D corresponds to the Endo D glycosidase, which cleaves paucimannose type *N*-glycans. Size shifts indicate that some paucimannose structures are present on DMA-1 (left), and that the *aman-2* mutant results in the loss of paucimannose structures on DMA-1 (right). Ladder is marked in kilodaltons (kDa). The experiment was repeated four times with biological replicates.

C Western blot against GFP in *C. elegans* lysate expressing no transgenes (N2) and expressing KPC-1::GFP (*dzEx1865)*, after precipitating with anti-GFP antibody. The molecular weights of wild-type lysate are 130 and 150 kDa. The red boxed plus sign indicates that the lysate is treated with the PNGase F glycosidase. The downward size shift reveals that *N*-glycan structures are present on KPC-1. In the bottom blot, control indicates an otherwise wild-type background as opposed to an *aman-2 (gk248486)* background. No visible size shift is observed, and the experiment was repeated three times with unique samples.

D Western blot against FLAG in *C. elegans* lysate expressing no transgenes (N2) and expressing SAX-7::GFP::3XFLAG (*ddIs290)*. Robust expression precludes the need for immunoprecipitation. The molecular weights of wild-type lysate are 100, 70, and 65 kDa. The red boxed plus sign indicates that the lysate is treated with the PNGase F glycosidase. The downward size shift reveals that *N*-glycan structures are present on SAX-7. In the bottom blot, control indicates an otherwise wild-type background as opposed to an *aman-2(gk248486)* background. No visible size shift is observed, and the experiment was repeated three times with unique samples. The FLAG epitope contains no *N*-glycosylation sites.

E Western blot against FLAG in *C. elegans* lysate expressing no transgenes (N2) and expressing endogenous LECT-2::mNeonGreen::3XFLAG (*dz249)*. Robust expression precludes the need for immunoprecipitation. The molecular weight of wild-type lysate is 70 kDa. The red boxed plus sign indicates that the lysate is treated with the PNGase F glycosidase. The small downward size shift reveals that *N*-glycan structures are present on LECT-2. In the bottom blot, control indicates an otherwise wild-type background as opposed to an *aman-2(gk248486)* background. No visible size shift is observed, and the experiment was repeated three times with unique samples.