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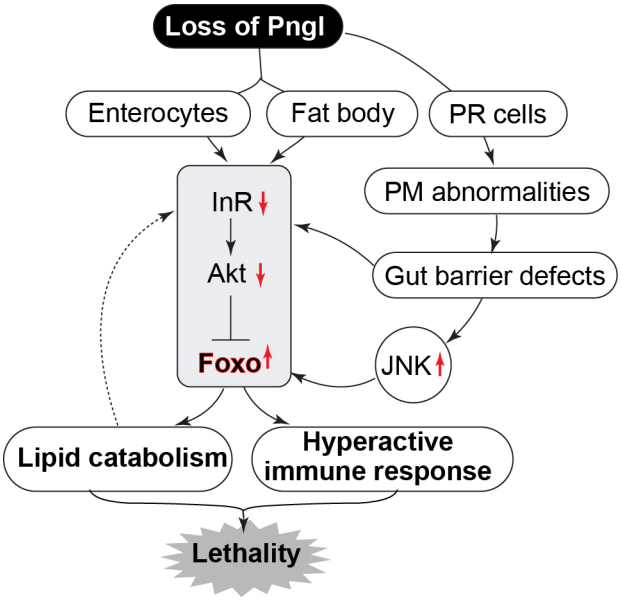
Grant Title: Role of deglycosylation at the interface of metabolism and innate immunity

ABSTRACT

1. Objectives. Pathogenic variants in *N*-glycanase 1 (*NGLY1*) lead to a rare, multisystem disorder with global developmental delay, seizures, liver abnormalities, chronic constipation, and a host of other symptoms. NGLY1 is a cytosolic enzyme responsible for removing *N*-glycans (i.e., de-*N*-glycosylation) of misfolded glycoproteins during the endoplasmic reticulum-associated degradation (ERAD). We have previously shown that loss of *Drosophila Pngl* leads to tissue-specific defects in BMP and AMPK signaling. However, defects in these two pathways only partially explain the lethality and developmental delay in $Pngl^{-/-}$ animals. The goal of the current study was to identify other critical processes that are affected by the loss of *Pngl*.

2. Methods used. The techniques used in this project include *Drosophila* genetics, real-time quantitative RT-PCR, western blotting, generation of germ-free animals, Nile red staining, triacylglycerol and free fatty acid measurement, dextran feeding assay for gut barrier defect quantification, lectin staining, and imaging.

3. Results. Loss of *Pngl* led to gut barrier defects in *Drosophila* larvae, causing starvation and JNK overactivation. These abnormalities, along with reduced Akt phosphorylation, led to overactivation of Foxo. Increased Foxo activity resulted in a dramatic increase in the expression of innate immune response genes in the gut and in the fat body as well as enhancement of lipid catabolism, which together contributed to the lethality of *Pngl*-mutant animals. Loss of *Pngl* resulted in the accumulation of $ConA^{+}$ and WGA^{+} puncta in specific secretory cells called PR cells, which are responsible for the secretion of the peritrophic matrix (equivalent to the mammalian gut mucus layer). This observation suggested that *Pngl* is required for the trafficking and/or secretion of some *N*-glycoproteins involved in gut barrier formation. Germ-free rearing of *Pngl* mutants rescued their developmental delay but not lethality. However, raising *Pngl* mutants on isocaloric, fat-rich diets partially rescued lethality. Our data indicate that Pngl functions in *Drosophila* larvae to establish the gut barrier, and that the lethality caused by loss of *Pngl* is in part mediated through induction of immune and metabolic abnormalities. For details, please see Pandey et al, 2023, *Nature Communications* 14(1):5667 (PMID: 37704604).



Schematic model showing that loss of Pngl in several cell types of the Drosophila larvae results in Foxo overactivation and subsequent innate immune gene expression and lipid catabolism, leading to lethality. PR cells secrete the peritrophic matrix (PM). InR, insulin receptor. From PMID 37704604.