Supporting Information

Proteomic analysis via standard-flow liquid chromatography and thermal focusing electrospray ionization elucidates altered liver proteins in late stage Niemann-Pick, type C1 disease

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Primary Antibody	MW (kDa)	Dilution	Host	Vender	Product Number
Anti-Gapdh	37	1:2500 WB	Goat	R&D Systems	AF5718
Anti-NPC1	143	1:1000 WB	Rabbit	Abcam	Ab134113
Anti-Fabp5	15	1:1000 WB	Rabbit	Cell Signaling	D1A7T
Anti-Fabp7	15	1:1000 WB	Rabbit	Abcam	Ab32423
Anti-Limp2	85	1:1000 WB	Rabbit	Abcam	Ab176317
Anti-Lamp1	120	1:1000 WB 1:600 IF	Rat	Santa Cruz Biotechnology	SC-19992
Anti-Rab5b	23	1:300 WB, 1:300 IF	Rabbit	Santa Cruz Biotechnology	SC-598
Anti-Rab7a	24	1:500 WB, 1:100 IF	Rabbit	Abcam	Ab126712
Secondary Antibody	**Color**	**Dilution**	**Host**	**Vender**	**Product Number**
Anti-Goat IgG HRP conjugated	-	1:1000	Rabbit	R and D Systems	HAF109
Anti-Rabbit IgG HRP conjugated	-	1:1000	Goat	R and D Systems	HAF008
Anti- Rat IgG HRP conjugated	-	1:5000	Goat	Abcam	Ab97057
Alexa Flour Anti-Rabbit 488	Green	1:1000	Goat	Thermo Scientific	A-11008
Alexa Flour Anti-Rat 546	Red	1:1000	Goat	Thermo Scientific	A-11081

Table S1. List of antibodies used for Western blot and immunofluorescence studies.

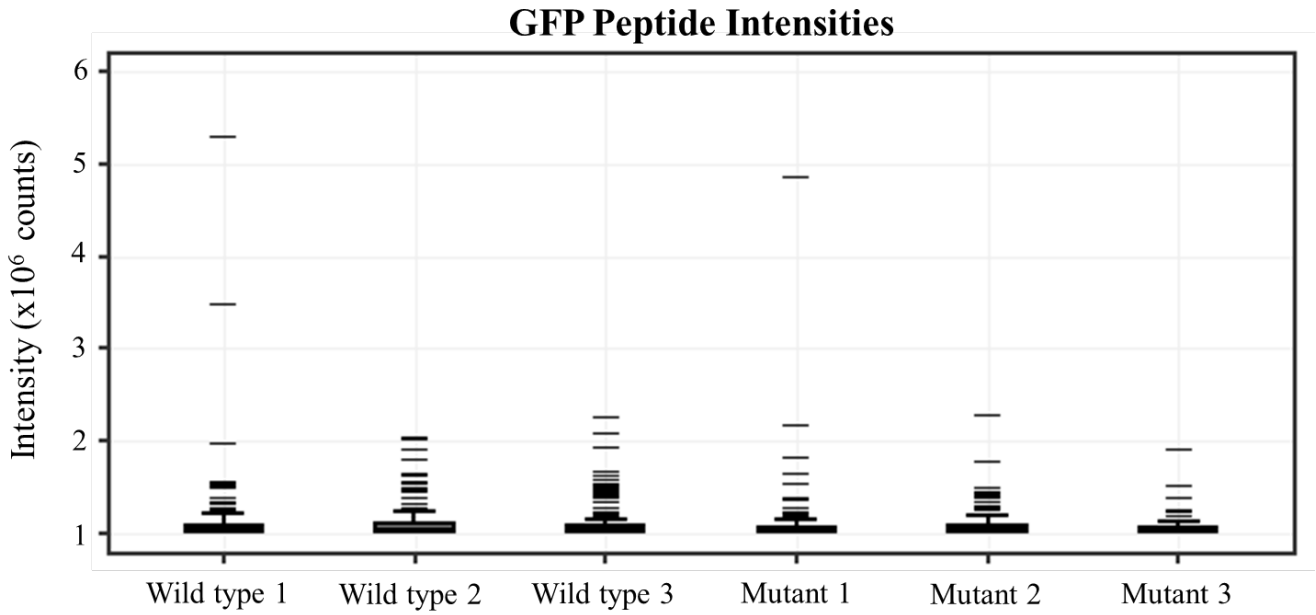

Figure S1. Intensity of peptides measured for the internal standard (GFP protein). LC-MS/MS data for each of the wild type and mutant technical replicates was reviewed in order to determine whether intensity normalization was needed between replicates. Here, the intensity for the measured GFP peptides were plotted and it was determined that normalization between samples was not needed as the median intensity was comparable between all replicates.

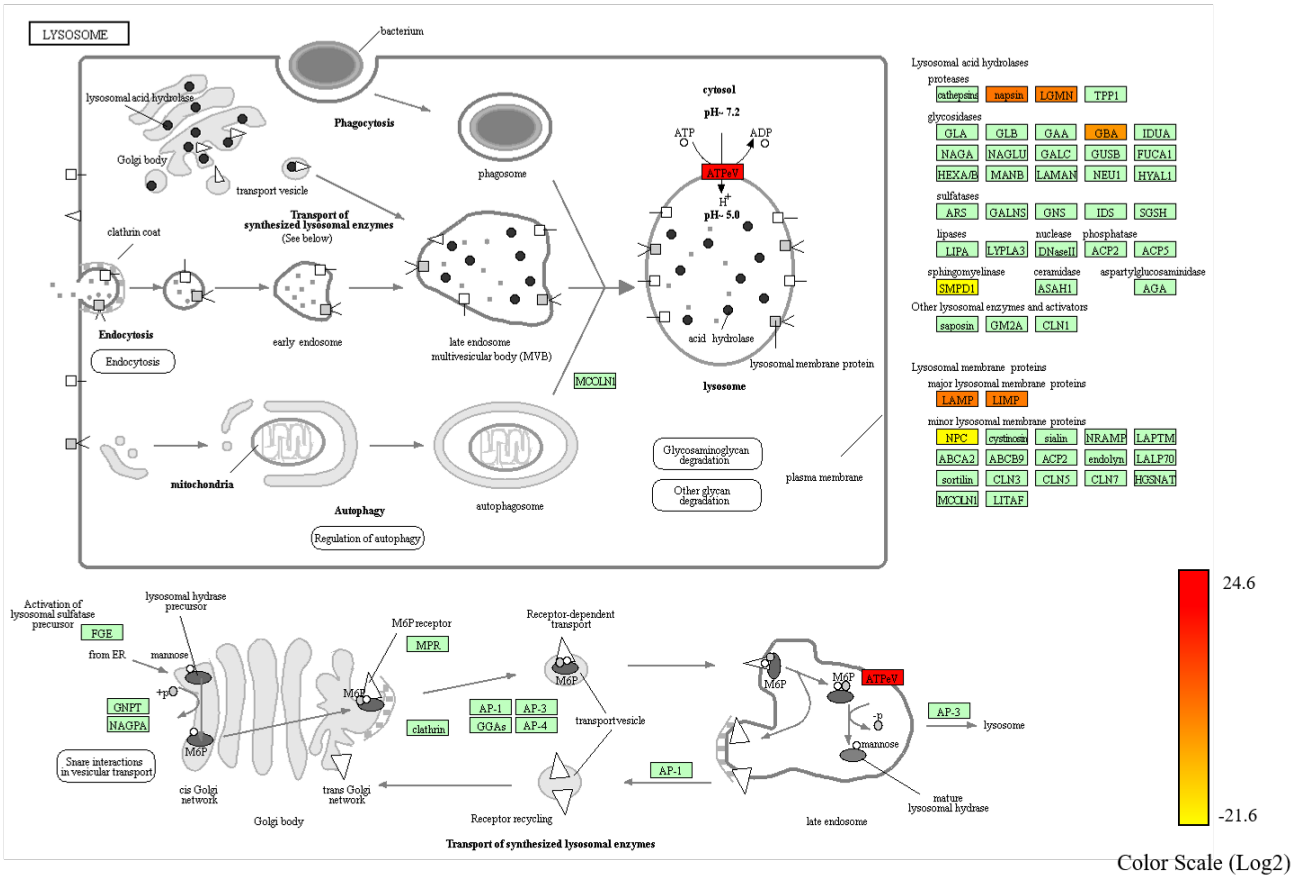

Figure S2. KEGG pathway of analysis of lysosome-related proteins. Differential proteins from the liver proteome were overlapped with the Lysosome KEGG pathway (04142).

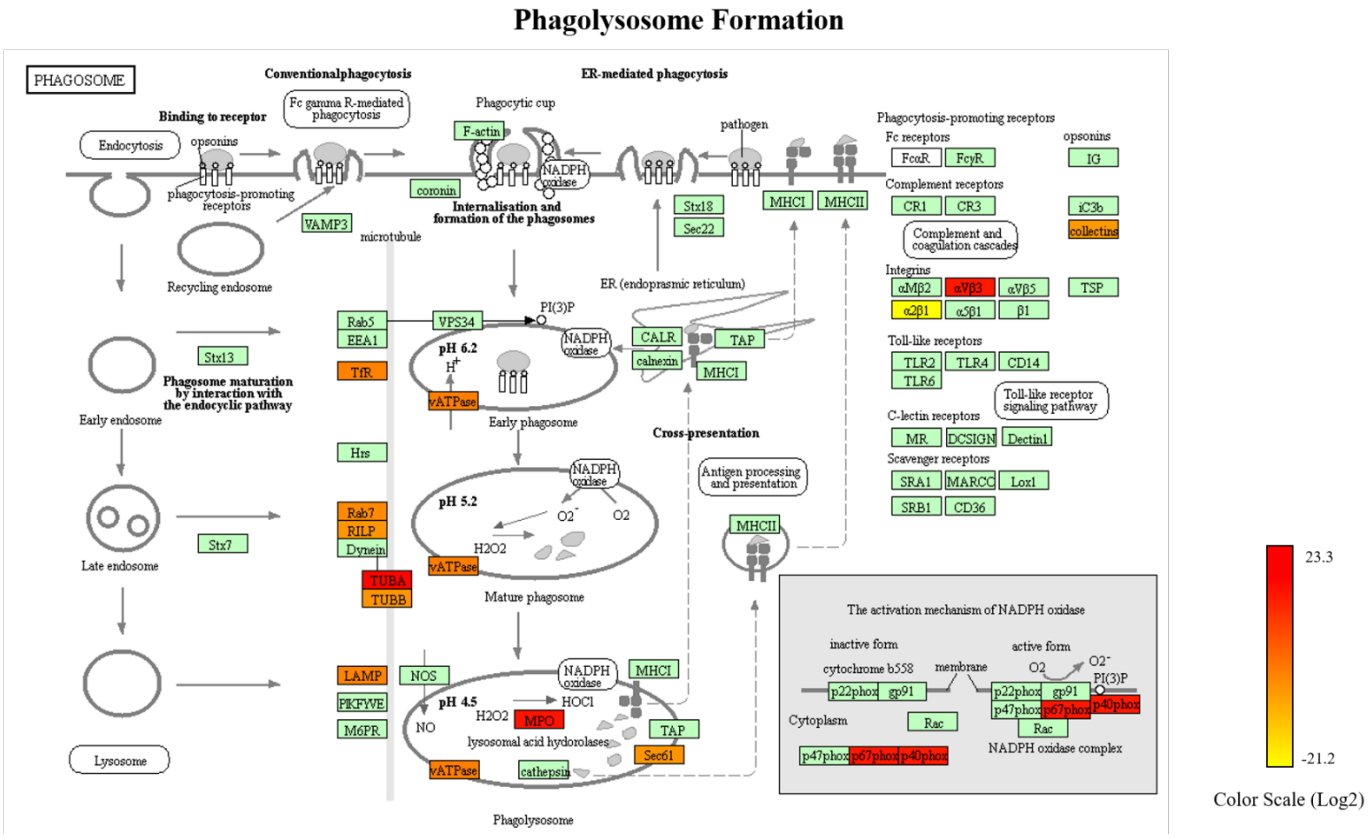

Figure S3. KEGG pathway of analysis of phagosome biosynthesis proteins. Differential proteins from the liver proteome were overlapped with the Phagosome Biosynthesis KEGG pathway (04146).

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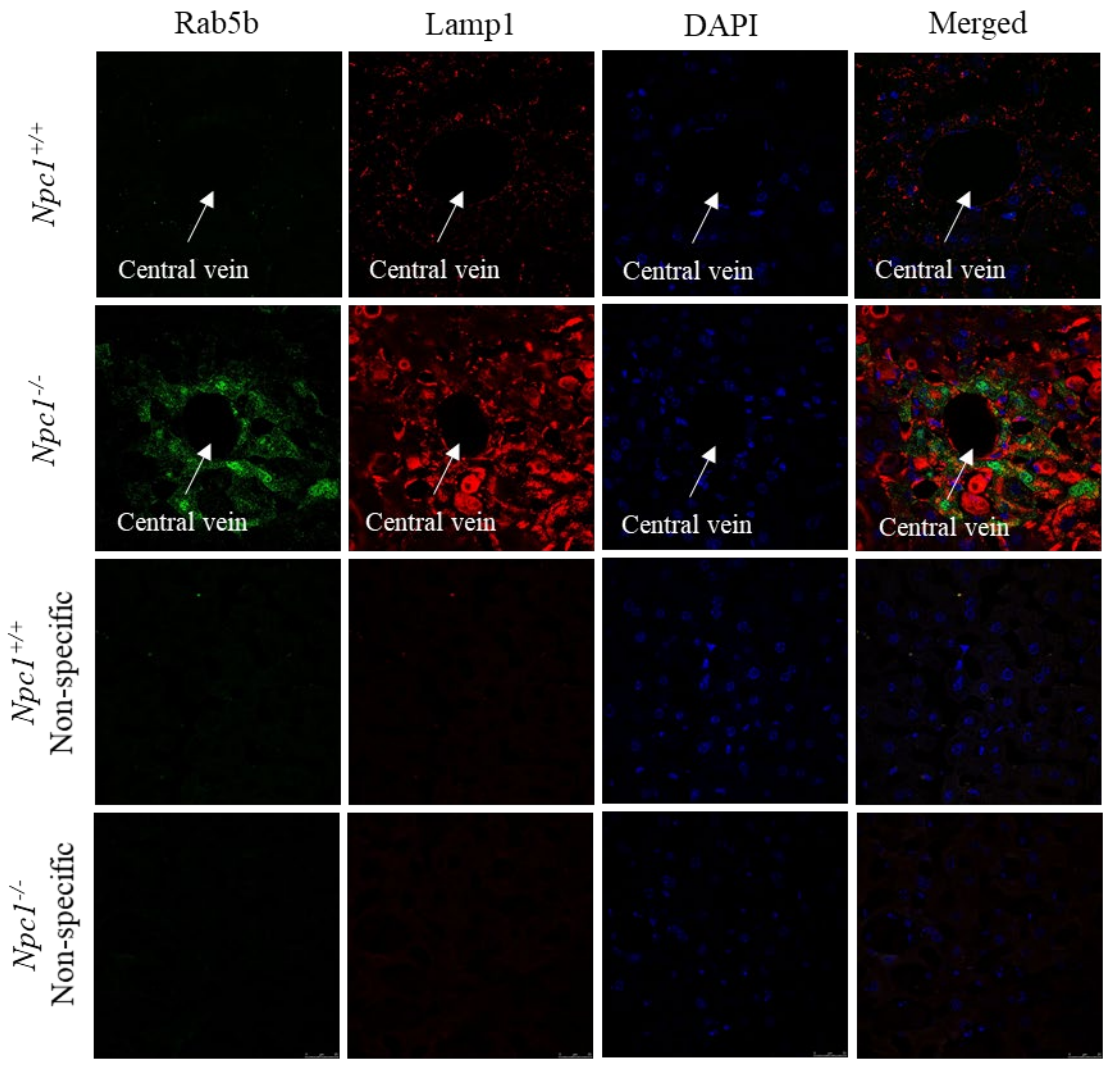
Rab5b (+)
Rab5b
Lamp1
DAPI
Merged
Npc1+/+
Npc1-/-
Npc1+/+ Non-specific
Npc1-/- Non-specific
Central vein

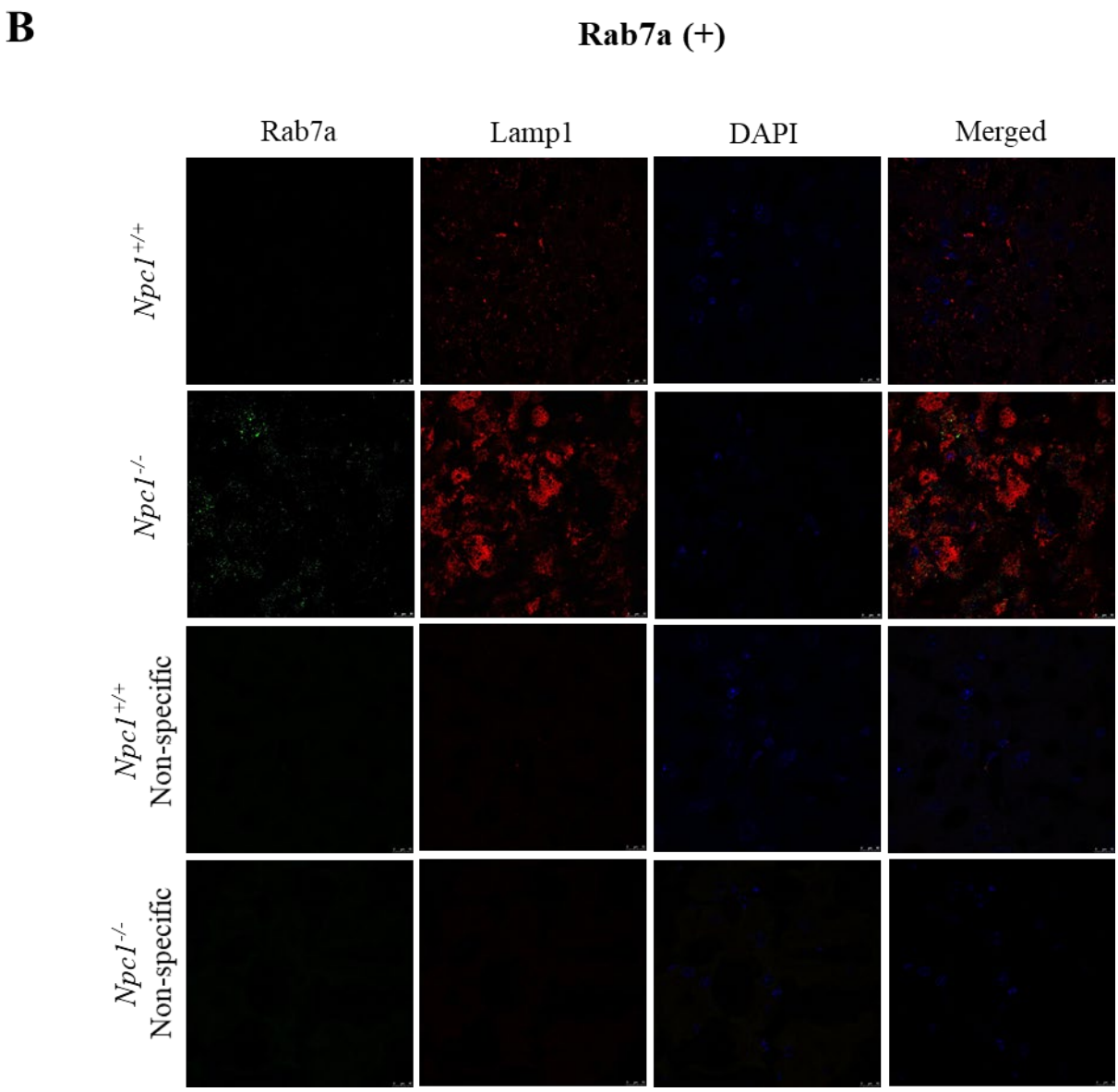

Figure S4. Immunofluorescence images of phagosome/endosome and lysosome markers in the liver of 7-week *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ mice. A) Magnified (63x) regions of the liver from 7-week *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ mice showing Rab5b (green, early endosome), Lamp1 (red, lysosome), and DAPI (blue, nucleus). Confocal fluorescence microscopy shows a strong increase in Lamp1 expression in *Npc1*$^{-/-}$ mice. Merging of the Rab5b and Lamp1 images reveals no colocalization of the two proteins. Visual inspection revealed localization of Rab5b positive vesicles around the bile duct in the *Npc1*$^{-/-}$ tissue. B) Magnified (63x) regions of regions of the liver from 7-week *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ mice showing Rab7a (green, late endosome), Lamp1 (red, lysosome), and DAPI (blue, nucleus). Confocal fluorescence microscopy shows a strong increase in Lamp1 expression in *Npc1*$^{-/-}$ mice. Merging of the Rab7a and Lamp1 images reveals no colocalization of the two proteins.

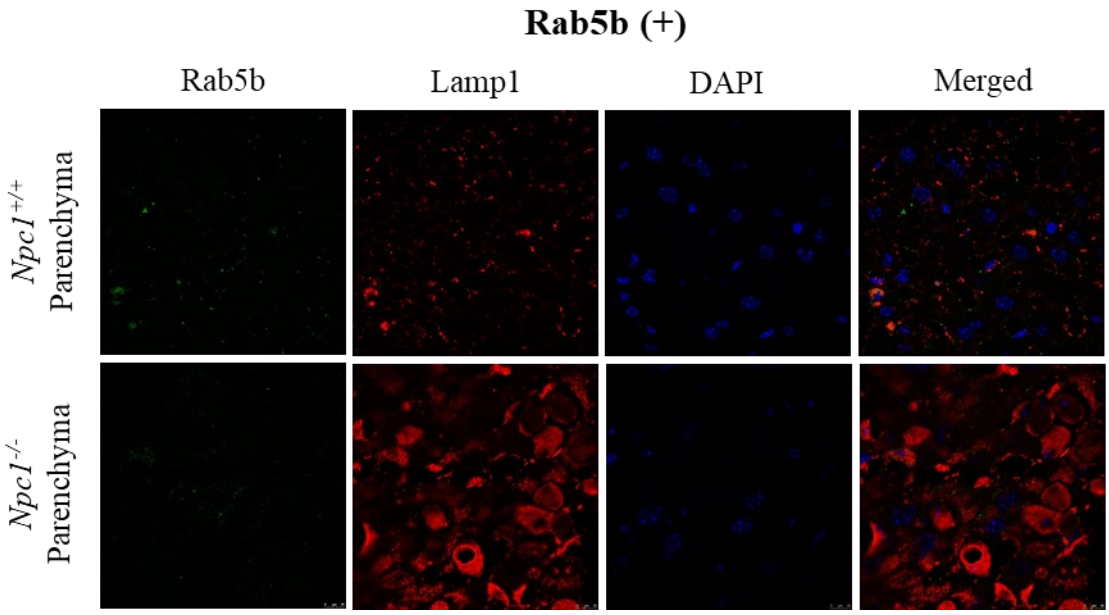

Figure S5. Immunofluorescence images of early endosome (Rab5b) and lysosome (Lamp1) markers in the parenchyma region of the liver of 7-week *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$mice. Magnified (63x) regions of representative parenchyma of the liver from 7-week *Npc1*$^{+/+}$ and *Npc1*$^{-/-}$ mice. Confocal fluorescence microscopy shows a strong increase in Lamp1 (red, lysosome) expression in *Npc1*$^{-/-}$ mice. Merging of the Rab5b (green, early endosome) and Lamp1 images reveals no colocalization of the two proteins.