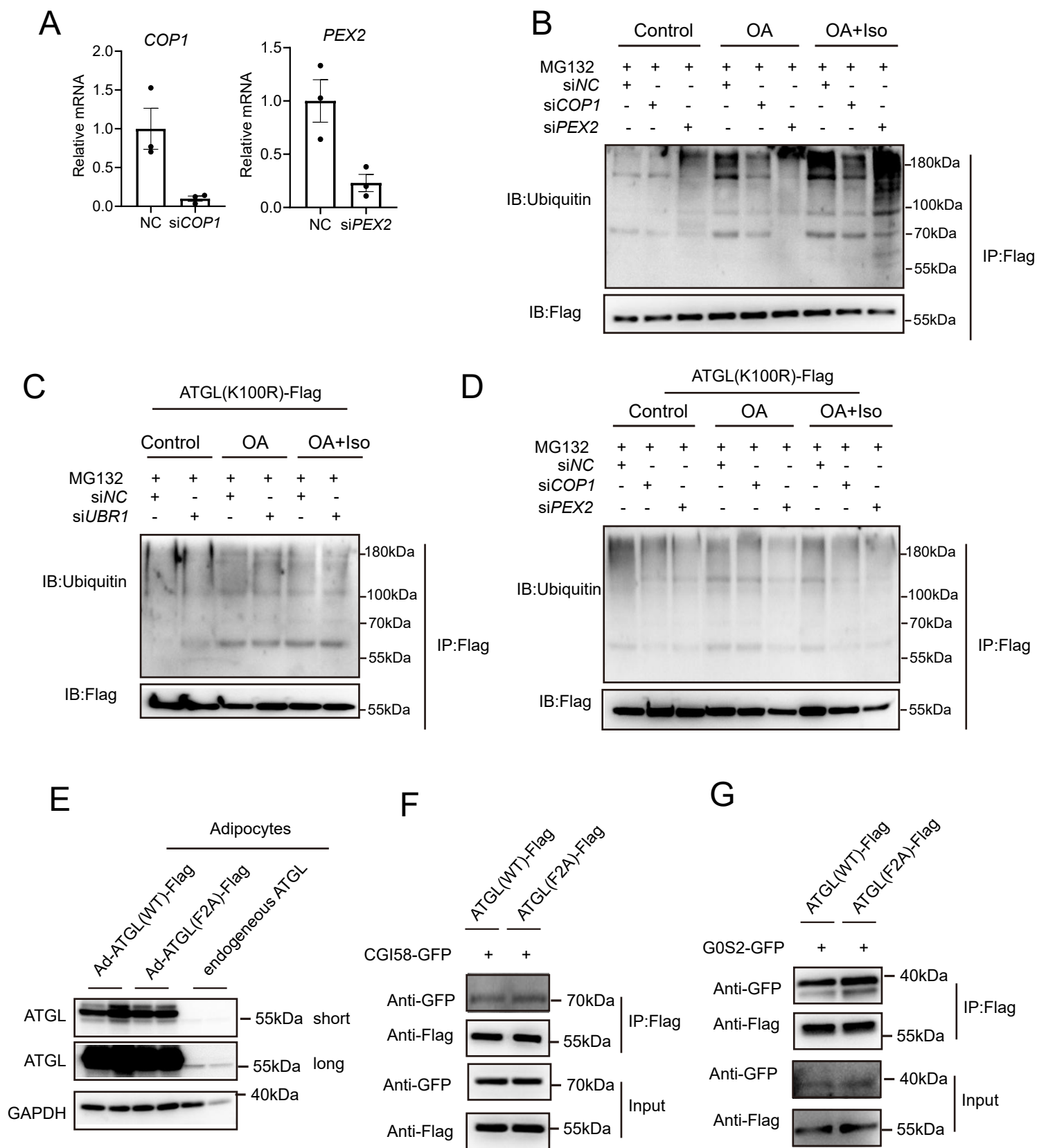


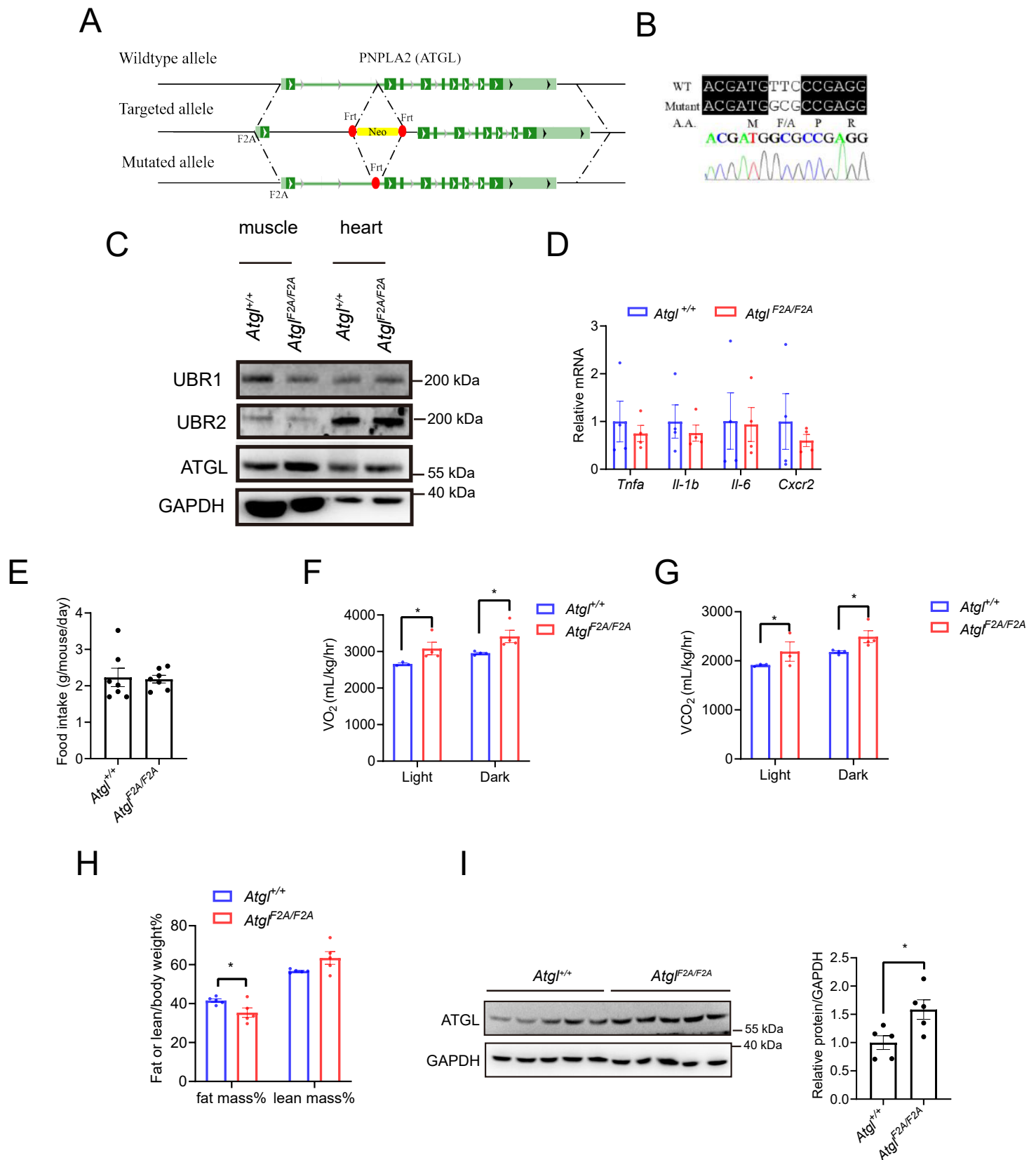
Supplemental Fig. 1 N-end rule regulation of ATGL

(A) Oil-Red-O staining for lipid droplets in *C. elegans* treated by *pas-5* RNAi compared to the N2 negative control (scale bar represents 40  $\mu$ m). (B) DIC images of lipid droplets in the 3<sup>rd</sup> instar salivary gland in fruit fly. *ppl>mdy*: overexpression of DGAT1 in salivary gland; *ppl>mdy, rpn2*: overexpression of DGAT1 and proteasome subunit Rpn2 in salivary gland (arrows indicate lipid droplets, scale bar represents 25  $\mu$ m). (C) Western blot analysis of proteins in HeLa cells transfected with indicated siRNAs. (D) Immunoprecipitation of ATGL(1-160aa)-Flag truncated protein and western blot analysis of ubiquitin in HeLa cells transfected with ATGL(1-160aa)-Flag and the indicated siRNAs. Cells were treated with MG132 for 12 hours. (E) The lysine residues in the N-terminal region of the ATGL protein. (F-G) Western blot analysis of proteins in HeLa cells transfected with ATGL(mut)-Flag vector and siRNAs. Cells were treated with MG132 for 12 hours.



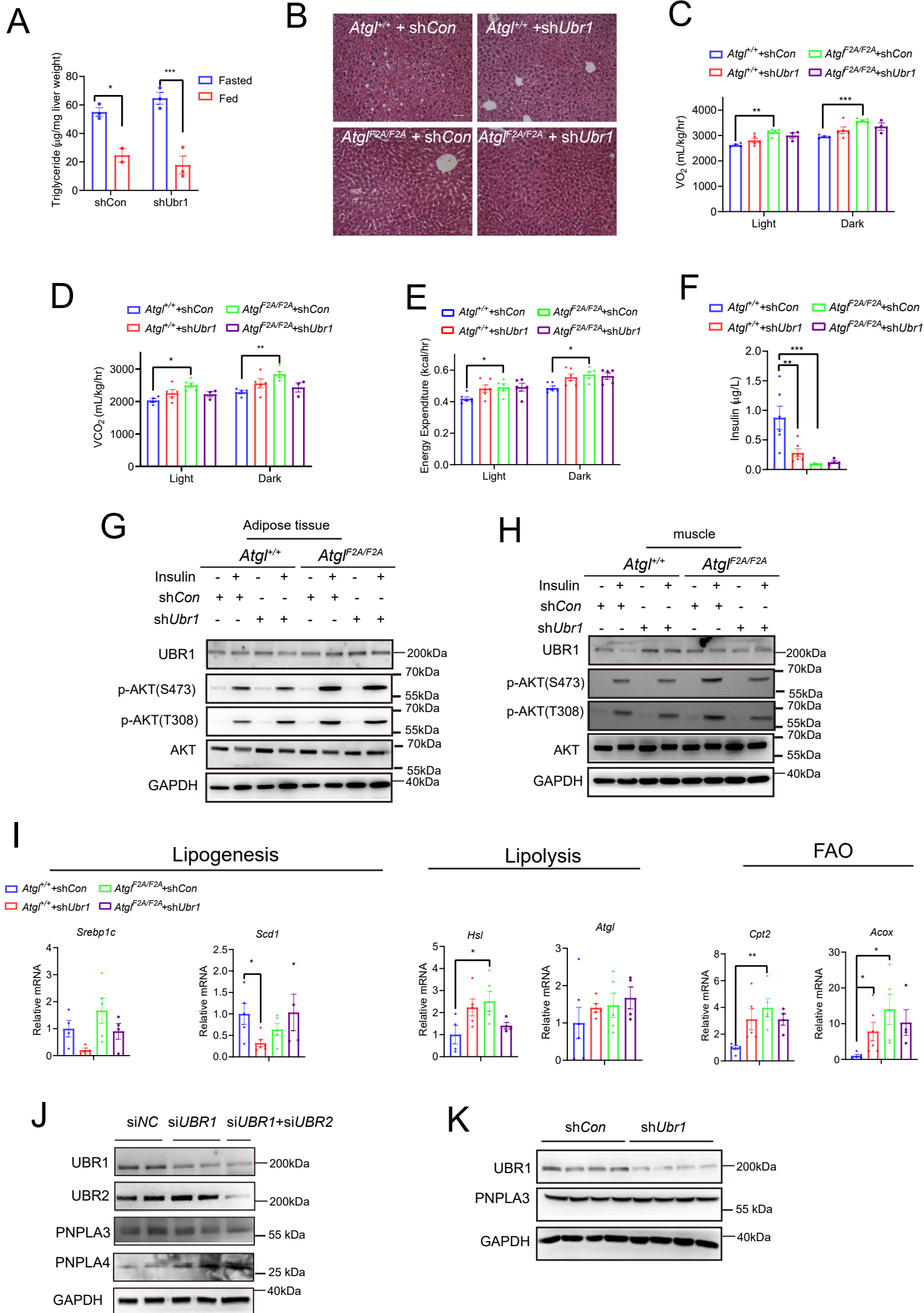
Supplemental Fig. 2 Regulation of the polyubiquitination levels of ATGL

(A) mRNA levels of *COP1* and *PEX2* in HeLa cells transfected with indicated siRNAs. (B) Immunoprecipitation of ATGL(WT)-Flag and western blot analysis of the polyubiquitination levels of ATGL in HeLa cells transfected with indicated siRNAs and treated with or without 100  $\mu$ M OA for 16 hours or with 100  $\mu$ M OA for 16 hours followed by ISO (0.25 mM IBMX/1  $\mu$ M isoproterenol) for 8 hours. (C-D) Immunoprecipitation of ATGL(K100R)-Flag and western blot analysis of the polyubiquitination levels of ATGL(K100R) in HeLa cells transfected with indicated siRNAs and treated with or without 100  $\mu$ M OA for 16 hours or with 100  $\mu$ M OA for 16 hours followed by ISO (0.25 mM IBMX/1  $\mu$ M isoproterenol) for 8 hours. (E) Western blot analysis of proteins in differentiated 3T3L1 adipocytes infected with equal amounts of Ad-ATGL(WT)-Flag or Ad-ATGL(F2A)-Flag. (F-G) Immunoprecipitation of ATGL(WT)-Flag or ATGL(F2A)-Flag and western blot analysis of associated proteins. All data are presented as mean $\pm$ SEM.



Supplemental Fig. 3 Characterization of *Atgl*<sup>F2A/F2A</sup> mice.

(A) Schematic illustration of the *Atgl*<sup>+/+</sup> and *Atgl*<sup>F2A/F2A</sup> alleles. (B) Comparison of the *Atgl*<sup>+/+</sup> and *Atgl*<sup>F2A/F2A</sup> sequences. In the mutant allele, the second codon is changed from F (TTC) to A (GCG). (C) Western blot analysis of proteins in the muscle and heart from *Atgl*<sup>+/+</sup> and *Atgl*<sup>F2A/F2A</sup> mice. (D) mRNA levels of inflammation genes in the adipose tissues from *Atgl*<sup>+/+</sup> and *Atgl*<sup>F2A/F2A</sup> mice which were fed an HFD for 8 weeks (N=4 mice per group). (E) Food intake in 8-week-old *Atgl*<sup>+/+</sup> and *Atgl*<sup>F2A/F2A</sup> mice which were pair-fed an HFD for 16 weeks (N=7 mice per group). (F-G) Oxygen consumption (VO<sub>2</sub>) (F) and carbon dioxide production (VCO<sub>2</sub>) (G) in HFD-fed *Atgl*<sup>+/+</sup> and *Atgl*<sup>F2A/F2A</sup> mice (N=3-4 mice per group). (H) Body composition of *Atgl*<sup>+/+</sup> and *Atgl*<sup>F2A/F2A</sup> mice which were fed an HFD for 8 weeks (N=5 mice per group). (I) Western blot analysis of ATGL in the liver of chow-fed mice (N=5 mice per group). All data are presented as mean±SEM. \* p < 0.05.



Supplemental Fig. 4 Knockdown of *Ubr1* suppresses HFD-induced hepatic steatosis dependent on the activity of ATGL.

(A) 8-week-old C57BL/6 mice were injected with AAV-TBG-sh*Con* or AAV-TBG-sh*Ubr1* and fed a chow diet for 1 month. Hepatic TAG levels were measured in fed or 16-hr fasted mice (N=2-3 mice per group). (B) H&E staining of liver sections from HFD-fed *Atgl*<sup>+/+</sup> and *Atgl*<sup>F2A/F2A</sup> mice injected with AAV-TBG-sh*Con* or AAV-TBG-sh*Ubr1*. (C-E) The oxygen consumption (C), CO<sub>2</sub> production (D), and energy expenditure (E) in 8-week-old *Atgl*<sup>+/+</sup> and *Atgl*<sup>F2A/F2A</sup> mice injected with AAV-TBG-sh*Con* or AAV-TBG-sh*Ubr1* and fed an HFD for 8 weeks (N=3-6 mice per group). (F) Plasma insulin levels in 8-week-old *Atgl*<sup>+/+</sup> and *Atgl*<sup>F2A/F2A</sup> mice injected with AAV-TBG-sh*Con* or AAV-TBG-sh*Ubr1* and fed an HFD for 8 weeks (N=4-6 mice per group). (G, H) Western blot analysis of proteins from the adipose tissue or muscle of HFD-fed mice which were fasted for 12 hours and injected with 1 U/kg insulin (i.p.). (I) mRNA levels of genes in lipogenesis, lipolysis, and FAO in 8-week-old *Atgl*<sup>+/+</sup> and *Atgl*<sup>F2A/F2A</sup> mice injected with AAV-TBG-sh*Con* or AAV-TBG-sh*Ubr1* and fed an HFD for 8 weeks (N=4-6 mice per group). (J) Western blot analysis of proteins in HeLa cells transfected with indicated siRNAs. (K) Western blot analysis of proteins in *Atgl*<sup>+/+</sup> mice injected with AAV-TBG-sh*Con* or AAV-TBG-sh*Ubr1* and fed an HFD for 8 weeks. \* p < 0.05, \*\* p < 0.01, \*\*\*p < 0.001.

Supplementary Table S1: Lipid storage phenotypes caused by RNAi knockdown of genes encoding proteasome components in *C. elegans*

Gene function		Gene name	phenotype
Proteasome core subunit	Alpha	<i>pas-1</i>	nd
		<i>pas-2</i>	nd
		<i>pas-3</i>	nd
		<i>pas-4</i>	---
		<i>pas-5</i>	---
		<i>pas-6</i>	-
		<i>pas-7</i>	nd
	Beta	<i>pbs-1</i>	---
		<i>pbs-2</i>	---
		<i>pbs-3</i>	nd
		<i>pbs-4</i>	--
		<i>pbs-5</i>	-
		<i>pbs-6</i>	---
		<i>pbs-7</i>	-
Proteasome regulatory particle	Non ATPase-like	<i>rpn-1</i>	-
		<i>rpn-2</i>	nd
		<i>rpn-3</i>	--
		<i>rpn-4</i>	-
		<i>rpn-5</i>	normal
		<i>rpn-6.1</i>	---
		<i>rpn-6.2</i>	normal
		<i>rpn-7</i>	--
		<i>rpn-8</i>	--
		<i>rpn-9</i>	nd
		<i>rpn-10</i>	normal
		<i>rpn-11</i>	-
		<i>rpn-12</i>	nd
	ATPase-like	<i>rpt-1</i>	--
		<i>rpt-2</i>	-
		<i>rpt-3</i>	normal
		<i>rpt-4</i>	normal
		<i>rpt-5</i>	--
		<i>rpt-6</i>	nd

Lipid storage phenotypes were evaluated using PLIN-1::GFP (Liu et al., 2014). ---, -- and - stand

for severe, medium and mild reduction, respectively, in lipid storage compared to control worms.  
nd, not determined.

Supplementary Table S2: Metabolic profile of chow-diet fed *Atgl*<sup>+/+</sup> and *Atgl*<sup>F2A/F2A</sup> mice.

	<i>Atgl</i> <sup>+/+</sup>	<i>Atgl</i> <sup>F2A/F2A</sup>
Body weight (g)	23.45±0.56	23.77±0.78
Plasma triglyceride (mg/dL)	60.19±4.84	72.69±4.81
Plasma cholesterol (mg/dL)	83.08±2.92	72.98±4.82
Plasma glucose (mM)	9.06±1.67	7.57±0.75
Plasma insulin (μg/L)	0.19±0.033	0.13±0.027
Plasma FFA (fed) (mM)	0.12±0.0098	0.15±0.019
Plasma FFA (fasted overnight) (mM)	1.38±0.13	1.90±0.13 *
Hepatic triglyceride (μg/mg liver weight)	9.92±1.28	9.62±0.77
Hepatic cholesterol (μg/mg liver weight)	5.08±0.64	4.93±0.36

\* p<0.05

Supplementary Table S3: Plasma parameters in HFD-fed *Atgl*<sup>+/+</sup> and *Atgl*<sup>F2A/F2A</sup> mice.

	<i>Atgl</i> <sup>+/+</sup>	<i>Atgl</i> <sup>F2A/F2A</sup>
Plasma triglyceride (mg/dL)	50.25±5.13	55.83±5.97
Plasma cholesterol (mg/dL)	170.51±23.06	189.79±18.28
Plasma glucose (mM)	8.11±1.35	7.27±0.56
Plasma insulin (μg/L)	2.60±0.76	0.87±0.098*
Plasma FFA (mM)	0.46±0.0088	0.56±0.034*
Adiponectin (ng/mL)	10.39±1.48	9.48±0.99
Leptin (fold change)	1±0.37	0.78±0.17

\* p<0.05

Supplementary Table S4: siRNA sequences

siRNA	sense (5'-3')	antisense (5'-3')
human UBR1	5' - GGCGUUGAGUCUUCGAUUATT- 3'	5'- UAAUCGAAGACUCAACGCCTT- 3'
human UBR2	5' - GCCGCUUUGAACUUAUCATT- 3'	5'- UGAUAAAGUCAAAGCGGCTT- 3'
human ATGL	5' - CGGCGAGAAUGUCAUUAUATT- 3'	5'- UAUAAUGACAUUCUCGCCGTT- 3'
human PEX2	5'- GCUAGUUUGGUCCCAGUUUTT- 3'	5'- AAACUGGGACCAAACUAGCTT- 3'
human COP1	5'- GCUGUGGUCUACCAAUCUATT- 3'	5'- UAGAUUGGUAGACCACAGCTT- 3'