SUPPLEMENTAL MATERIAL

# Increased RTN3 leads to obesity and hypertriglyceridemia by interacting with HSPA5

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## Supplemental Methods

*Experimental animals:*

All experiments involving mice were approved by the Review Board of the Central South University in China. RTN3-null mice were generated and genotyping was performed as described previously[1.2.3].

*C. elegans* germline transgene experiments were performed as described previously[4]. Transgene mixtures contained 10 ng/μl pPD95.79::ret-1p::ret-1 and 20 ng/μl pPD95.86::GFP (*myo-3p*::GFP) plasmid (which expresses GFP in body-wall muscles) as a co-injection marker.

*Real-time PCR*

Total RNA was extracted by the PureLink® RNA Mini (Thermo Fisher Scientific, #12183025) from mice liver tissues. cDNA was synthesized from a total of 1 μg of RNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, #K1621) with oligo (dT) primers. Real-time PCR reactions were carried out in Fast 7500 Real-Time PCR Systems (Applied Biosystems) using Maxima SYBR Green/ROX qPCR Master Mix (2×) (Thermo Fisher Scientific, #K0221). The primers are supplied in Table S1.

*RNAi screening*

Young adult *C. elegans* were fed with HT115 (DE3) bacteria containing plasmids expressing dsRNAs targeting *ret-1* on NGM plates with 1 mM IPTG and 0.1 mg/ml Ampicillin for 2 days.

*Co-IP*

3T3L1 cells were first grown in DMEM for 24 h in 60-mm plates to ~ 80% confluence and then transfected with the indicated expression constructs. After being cultured for 48 h, cells were lysed and equal amounts of lysates (500 μg in 1 ml) were used for immunoprecipitation with Myc or Flag-conjugated beads overnight. The extensively-washed immunoprecipitates were resolved on a 4-12% NuPage Bis-Tris gel, followed by standard Western blotting with the antibodies specified above. Chemiluminescent signals were scanned and integrated density values were calculated with a

chemiluminescent imaging system (Alpha Innotech).

*Hematoxylin-eosin (H&E) staining*

Paraformaldehyde-fixed fat tissue was embedded in paraffin and sliced into 6 μm sections. The sections were stained with H&E and examined by routine light microscopy (Olympus Corporation, Tokyo, Japan). Briefly, sections were dried, followed by xylene dewaxing and rehydration with decreasing concentrations of alcohol. The slides were then stained with hematoxylin for 15 min and differentiated with hydrochloric acid alcohol for 30 sec. Following staining with 1% ammonia, sections were stained with 1% eosin for 2 min, followed by dehydration in alcohol and mounting.

*Statistical analysis*

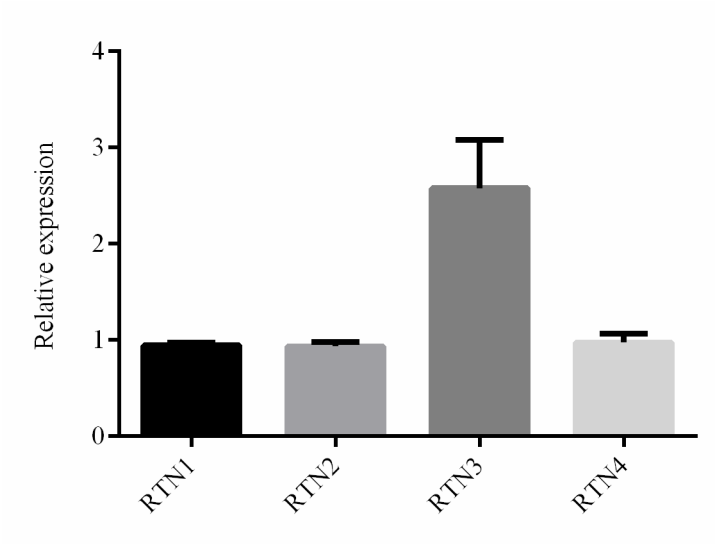
Data were subjected to statistical analysis with Graph-Pad Prism 5 (GraphPad Software) and were plotted by AI Illustrator (Adobe). Results represent the mean ± SEM of at least 3 independent experiments as indicated in the figure legends. Two-tailed Student's t-tests based on ANOVA were used for two-group comparisons. For multiple comparisons such as Figure S2, we conducted one-way ANOVAs with Dunnett's correction to analyze differences among the control group and one or more independent treatment groups. Differences were considered statistically significant at $P < 0.05$, with significance indicated in figures as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. NS represents no significant difference.

Supplemental Table S1

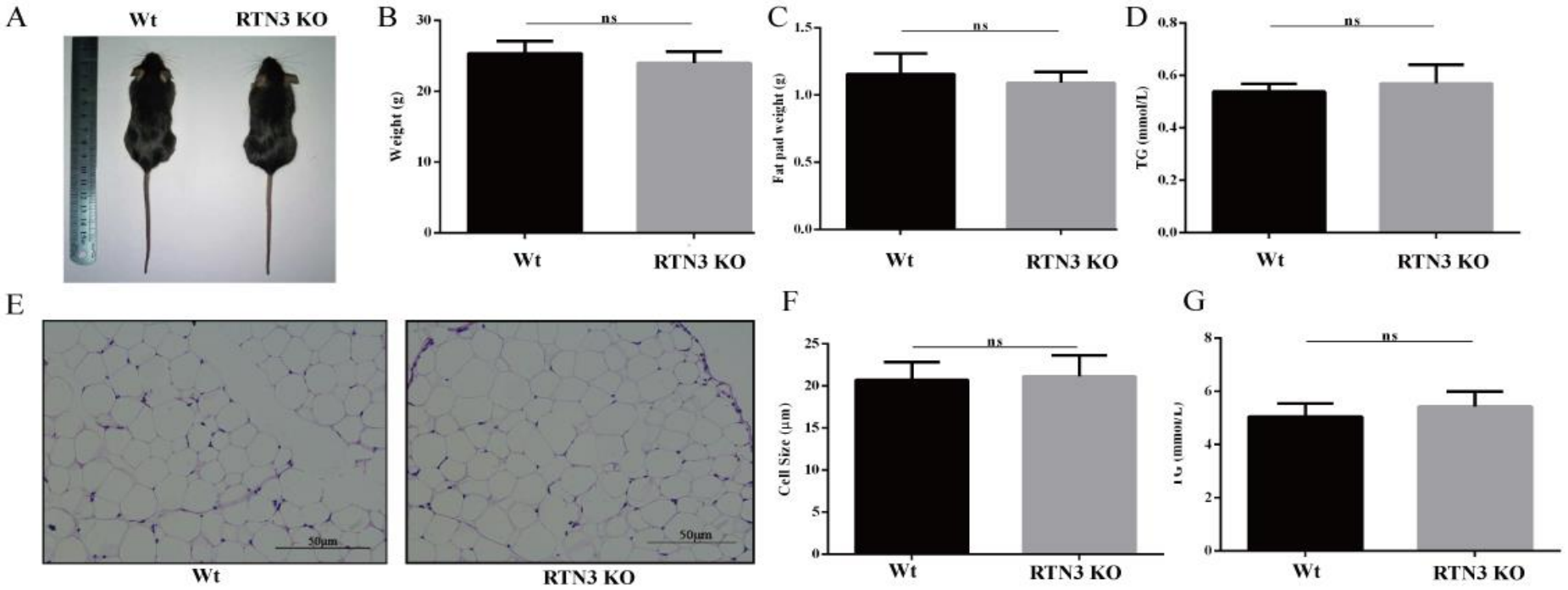
Table S1. The primers of Real-time PCR.

| Gene name | Forward | Reverse |
|---|---|---|
| Rtn1 (mouse) | GAAGCAAGACCTGTGTCTGAA | GGTTCTGTTCCAGAAGATGGAG |
| Rtn2 (mouse) | CCTCTGCTGAATGTGGTAGAA | CAACAGGTCTGCCACTAGAA |
| Rtn3 (mouse) | GGTAGAAGACTTGGTTGACTCC | GGCGAGAATCAGAAGGGTAAT |
| Rtn4 (mouse) | TGCCATATCAGAGGAATTGGTT | CGTAAGTAAATACCCACATCAACAC |
| Gapdh (housekeeping mouse) | GGCTGCCCAGAACATCAT | CGGACACATTGGGGGTAG |
| ret1 (C. elegans) | AGTCGTCACATATTCGCTTCTT | TGCTCAGAATCGGTCTTCTTG |
| tba1 (housekeeping C. elegans) | GAACTATGCCATCAGACCAACAAGC | CCAGTGCGGATCTCATCAACAACA |

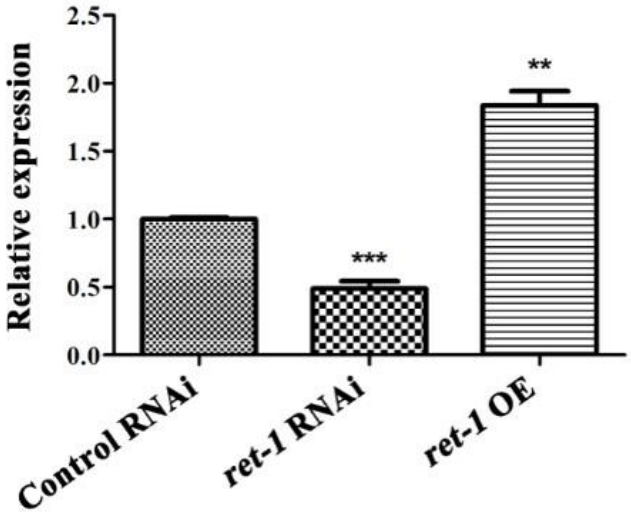
Supplemental Figure S1



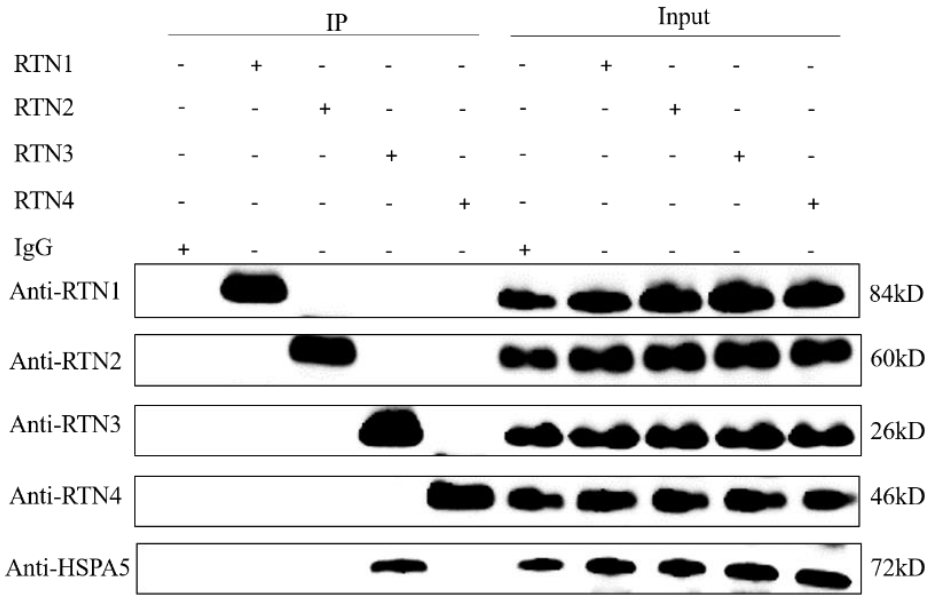
Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4



Figures legends for Supplemental Figures

Figure S1: Real-time PCR identified that only RTN3 mRNA levels have increased in Tg-RTN3 mice.

Figure S2: In normal condition, there are no obvious different between Wt and RTN3 KO mice. (A, B) The bodily shape and weight of Wt mice (n=7) and RTN3 KO mice (n=7) at six months with normal diet (C) The weight of epididymal fat tissues in Wt and RTN3 KO mice at six months. (D) Peripheral blood TG levels of Wt (n=7) and RTN3 KO mice (n=7). (EF) H&E analysis showing the size of fat cells in Wt and RTN3 KO mice. (G) Fat tissues TG levels of Wt mice (n=7) and RTN3 KO mice (n=7). The ns represents no not significant.

Figure S3: Real-time PCR detected the ret1 mRNA levels in different C.elegans. ** represents $p<0.01$, *** represents $p<0.001$.

Figure S4: Co-IP confirmed that HSPA5 can only interact with RTN3.

## References for Supplemental Methods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