

Inactivation of the E3 ubiquitin ligase IDOL protects mice from diet-induced obesity and metabolic dysfunction

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Supplemental material

Supplementary figure legends

Supplementary figure I. Growth curves and plasma lipids after 21 weeks of WTD.

Wildtype (N = 11) and Idol-KO (N = 9) male mice were fed a 60% fat 0.2% cholesterol diet for 21 weeks. (A) Body weight was measured weekly and differed significantly from 11 weeks of WTD. At the end of the experiment, mice were fasted for 4 hours and the plasma level of (B) triglycerides and (C) total cholesterol determined. Each bar or point represents the mean \pm SEM. * $p < 0.01$

Supplementary figure II. Phenotype of chow-fed Idol-KO mice.

Female Idol (wildtype, N = 6/ heterozygous, N = 5/ knockout, N = 5) and male Idol (wildtype, N = 6 / heterozygous, N = 6 / knockout, N = 6) were fed chow diet *ad libitum*. (A) Bodyweight was evaluated biweekly, and (B) Blood glucose levels were measured in the indicated mice after 4 hour fasting at the age of 28 week. * $p < 0.01$. Each bar or point represents the mean \pm SEM.

Supplementary figure III. Oral glucose tolerance test in Idol-KO mice fed a WTD.

Wildtype (N = 12) and Idol-KO (N = 9) male mice were fed a 60% fat 0.2% cholesterol diet for (A) 8 weeks or (B) 20 weeks. Following a 4 hour fast mice were loaded with 2.0 g glucose / kg body weight by oral gavage and blood glucose measured at the indicated time. Each point represents the mean \pm SEM.

Supplementary figure IV. AST and ALT levels in WTD-fed wildtype and Idol-KO mice.

Wildtype (N = 12) and Idol-KO (N = 9) male mice were fed a 60% fat 0.2% diet for 21 weeks. Plasma was collected at sacrifice and AST and ALT levels measured. Each bar represents the mean \pm SEM

Supplementary figure V. Quantification of UCP1 and TH in BAT of WTD-fed mice.

Wildtype (N = 11) and Idol-KO (N = 9) male mice were fed a WTD-diet for 21 weeks as described in [Figure 4](#). Uncoupling protein 1 (UCP1) and Tyrosine hydroxylase (TH) staining in BAT of these mice was quantified as detailed in the “materials and methods” section. ** $p < 0.01$, *** $p < 0.001$. Each bar represents the mean \pm SEM.

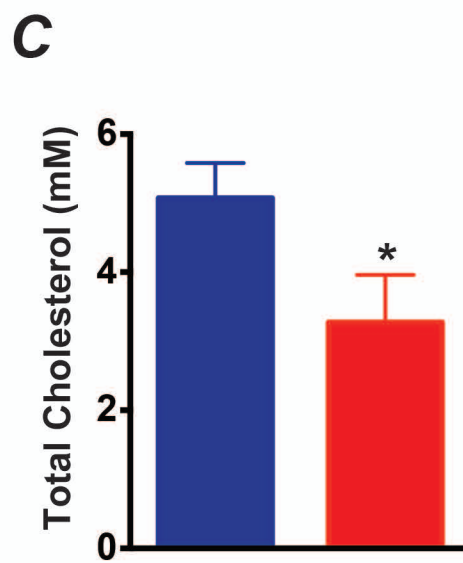
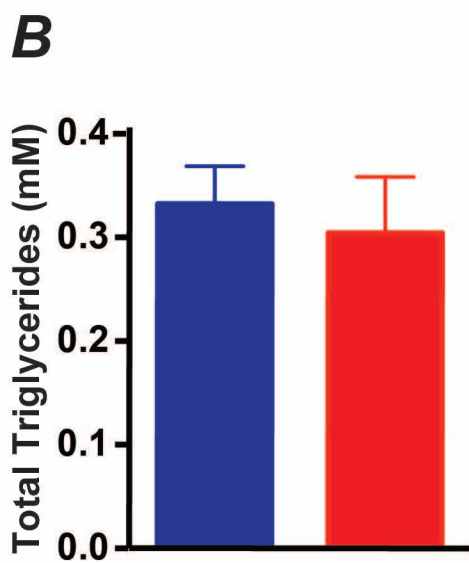
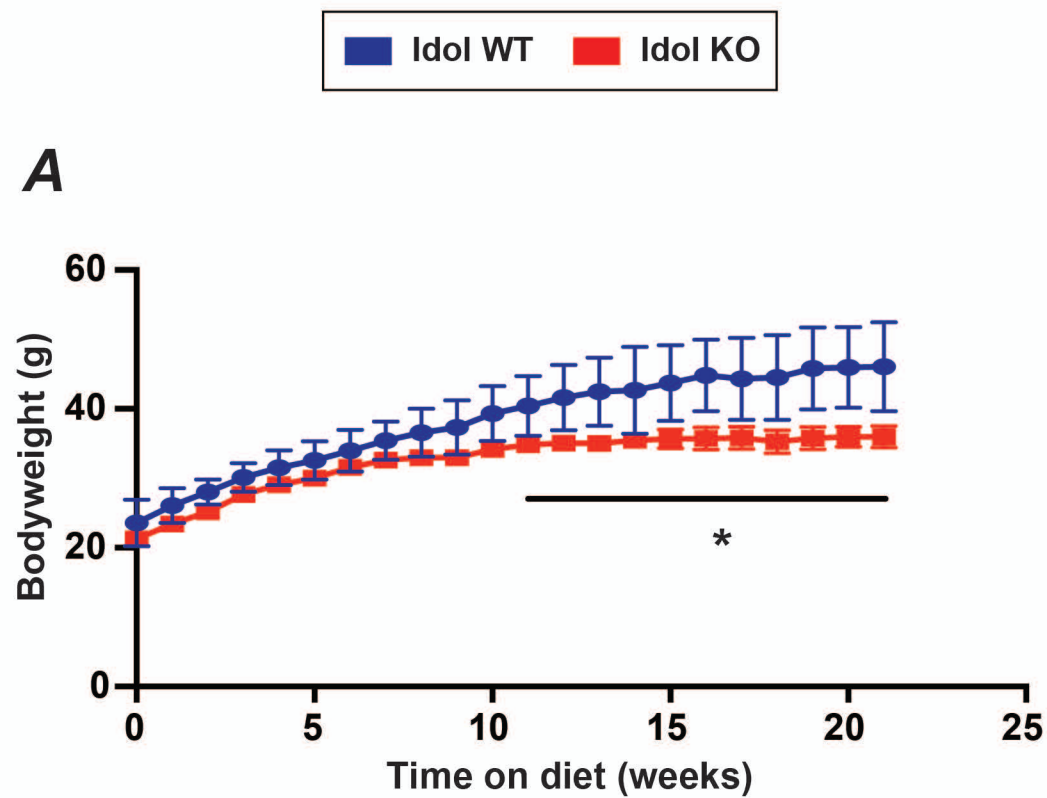
Supplementary figure VI. Intestinal fat uptake and VLDL-TG production is unaltered in Idol-KO mice.

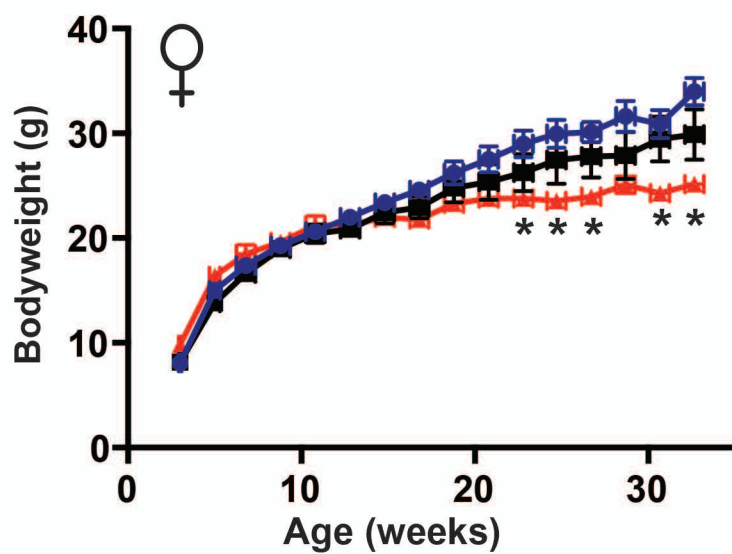
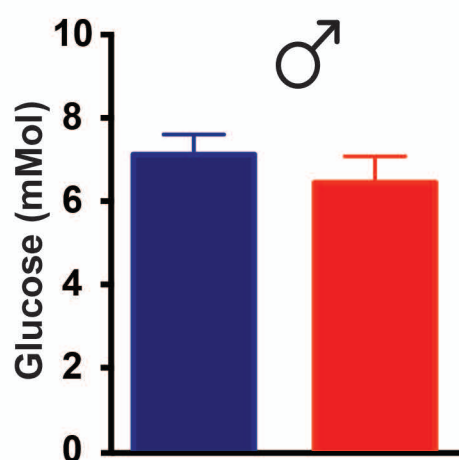
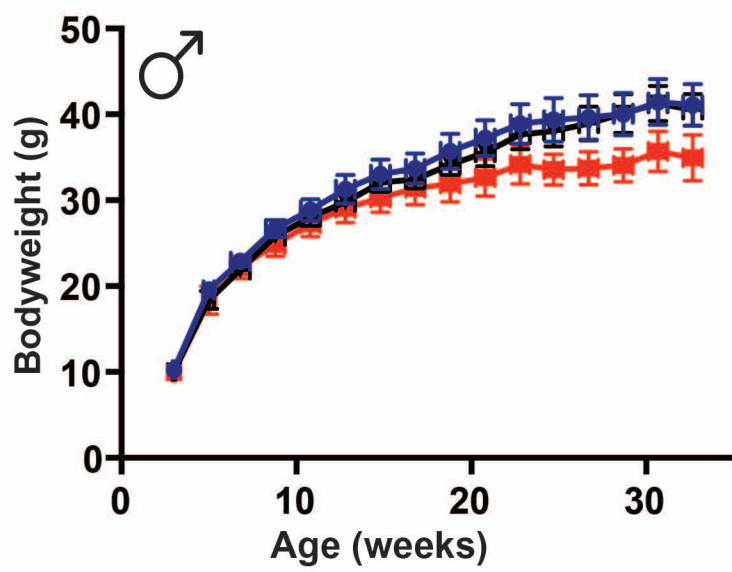
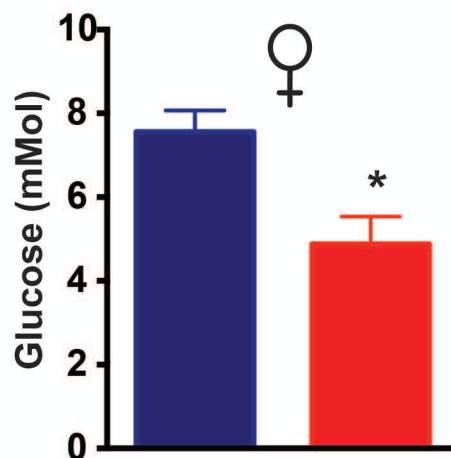
(A) Wildtype and IDOL-KO male mice (N = 6 / group ; 10-12 weeks old) were fasted for 4 hours, after which they received an oral gavage of 400 μ L olive oil. Subsequently, blood samples were drawn from the tail vein at the indicated times and TG levels determined. (B) Wildtype and IDOL-KO male mice (N = 3 / group ; 18 weeks old) were fasted for 4 hours, after which they received an intraperitoneal injection of 1gr/kg Poloxamer 470. Subsequently, blood samples were drawn from the tail vein at the indicated times and TG levels determined. *Inset*: area under the curve comparison of wildtype and Idol-KO mice. Each point represents the mean \pm SEM.

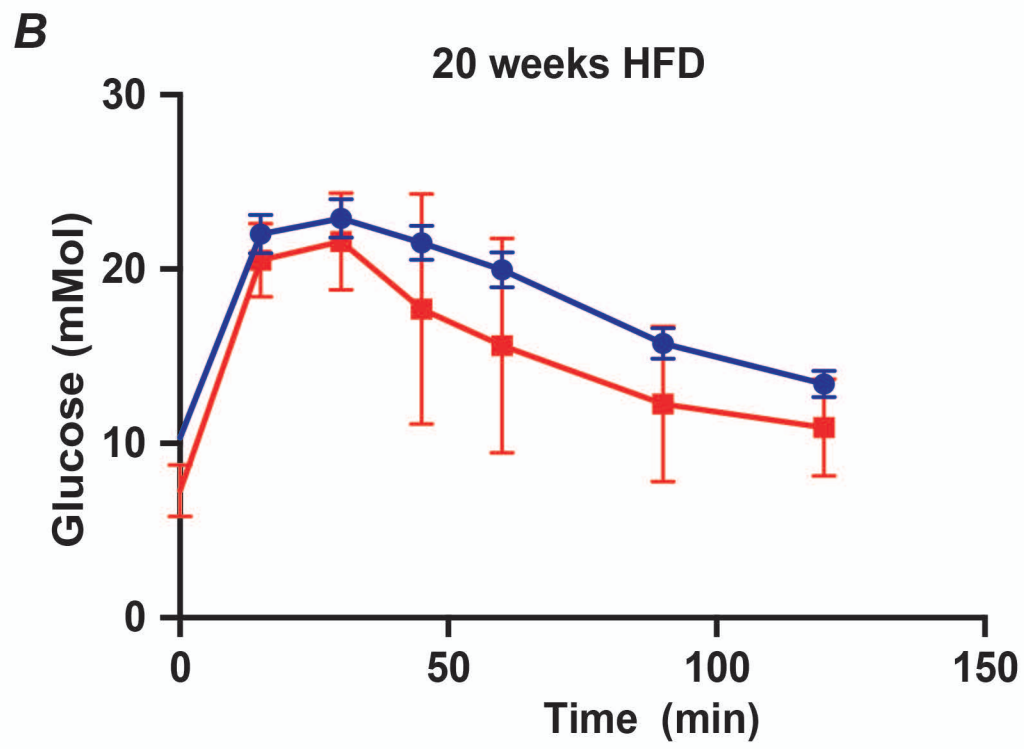
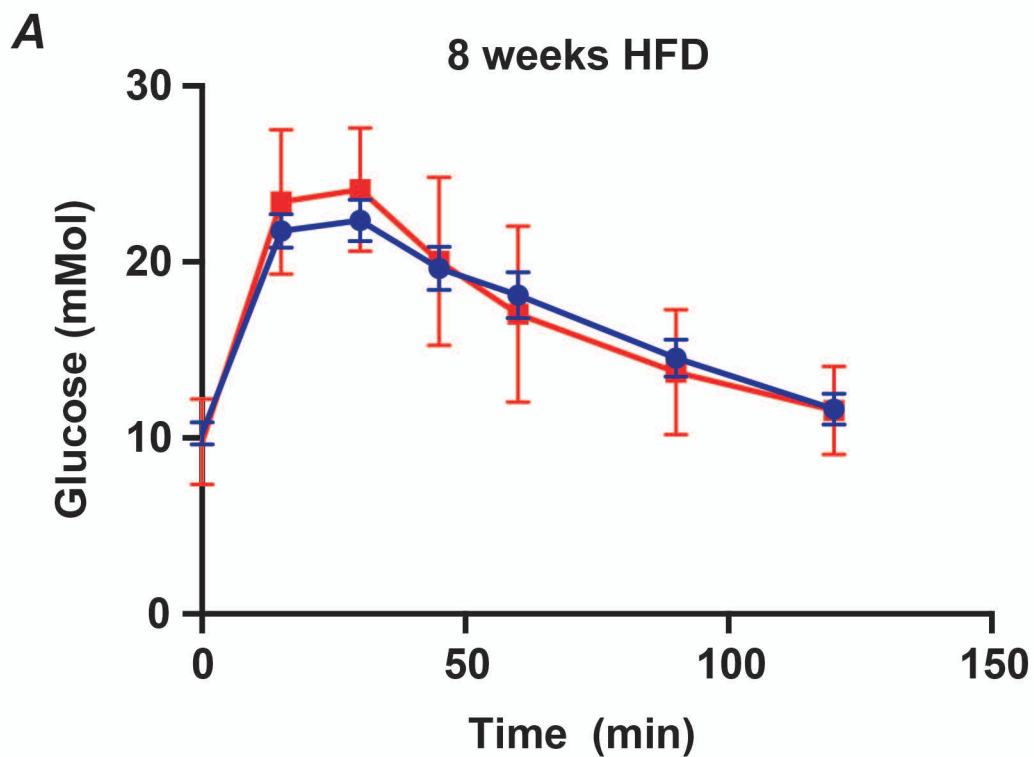
Supplementary figure VII. Expression of *Anptl4* in gWAT and iBAT.

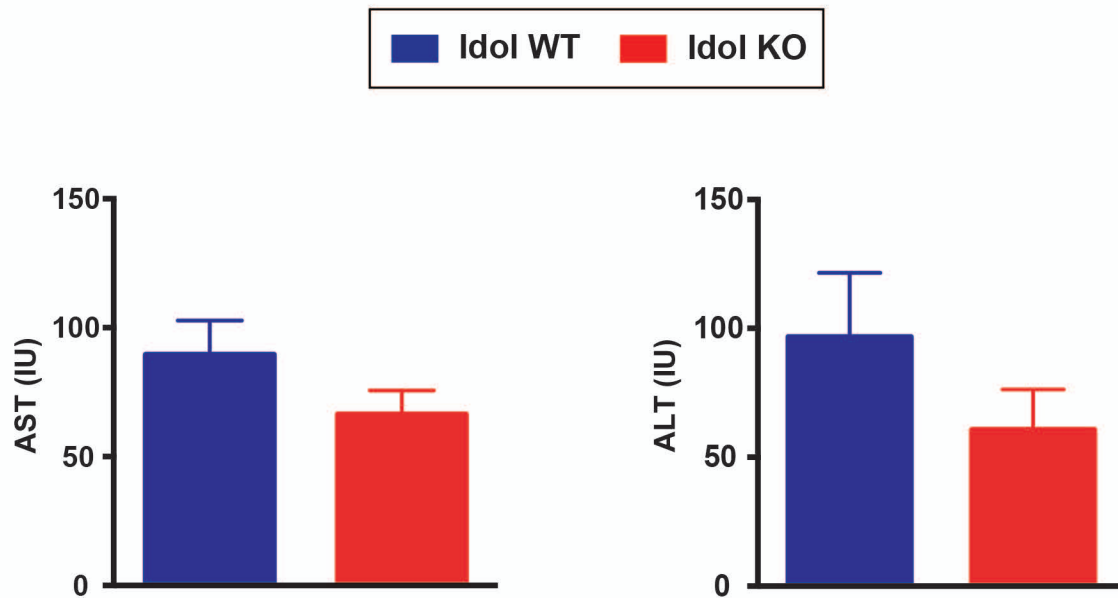
Expression of *Angptl4* and *Lpl* in wildtype (N = 12) and Idol-KO (N = 9) male mice in gonadal WAT (gWAT) and intrascapular BAT (iBAT). Each bar represents the mean \pm SEM.

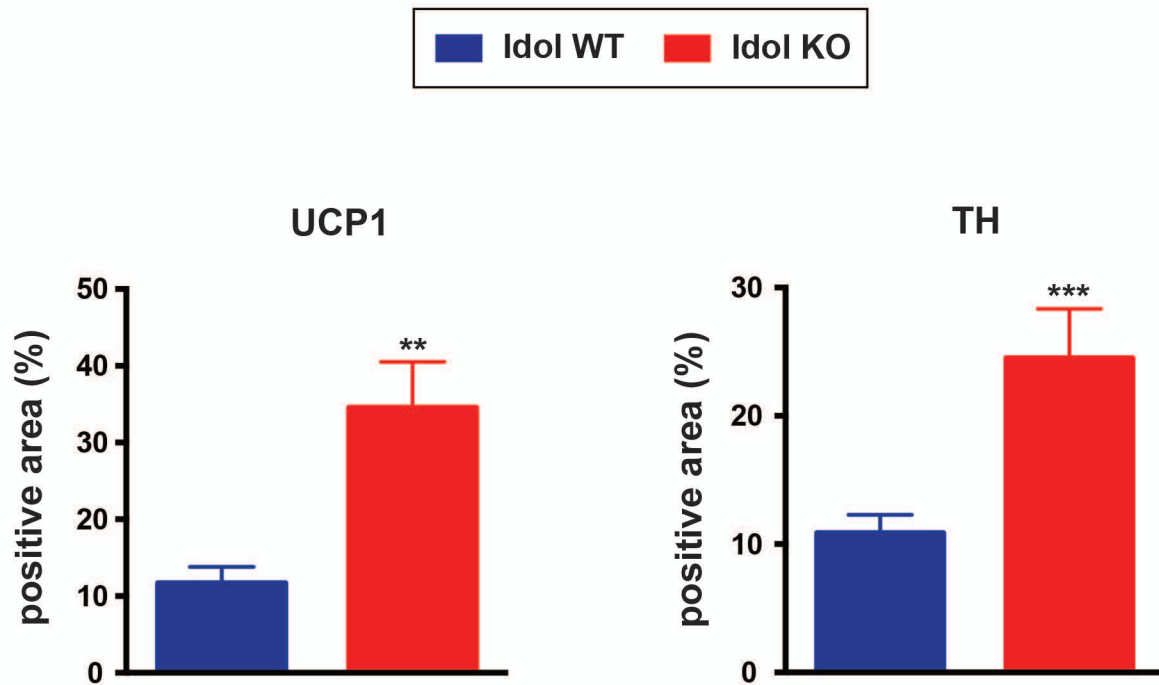
Supplementary figure VIII. Total organ clearance of triglycerides and cholesterol in Idol-KO mice. Wildtype (N = 8) and Idol-KO (N = 11) male mice were fed a WTD for 5 weeks. Mice were fasted for 4 hours and injected with reconstituted lipoproteins that contain glycerol tri³H]oleate and [¹⁴C]cholesteryl oleate. Subsequently, mice were sacrificed, organs were weighed and radioactivity in the indicated tissues was determined. Each bar represents the mean percentage of injected radioactivity per indicated organ \pm SEM. *p<0.01, ** p<0.001, *** p<0.0001 gWAT; gonadal white adipose tissue, vWAT; visceral white adipose tissue, lsWAT; lower subcutaneous white adipose tissue, usWAT; upper subcutaneous white adipose tissue, pVAT; perivascular adipose tissue, iBAT; interscapular brown adipose tissue, sBAT; subscapular brown adipose tissue.

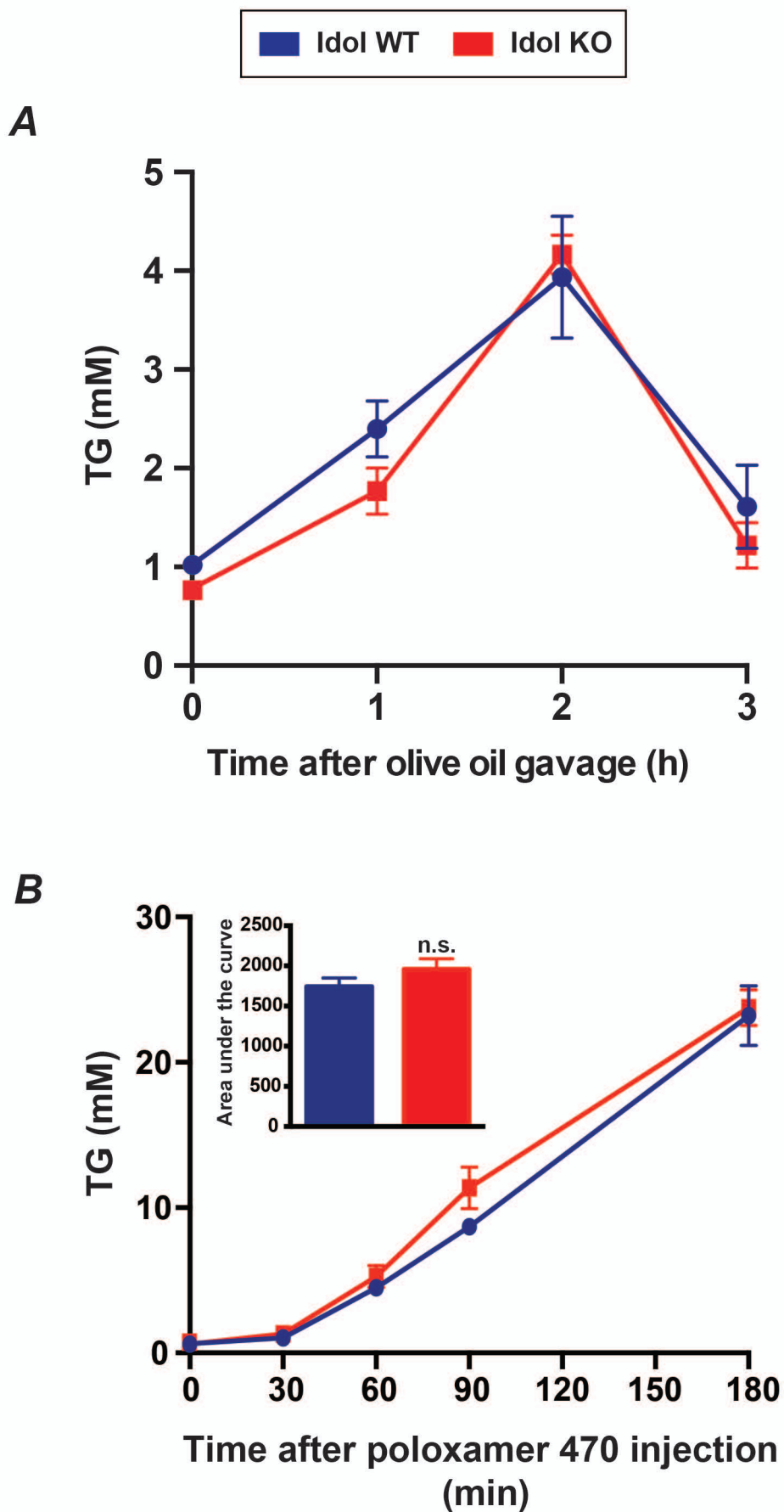


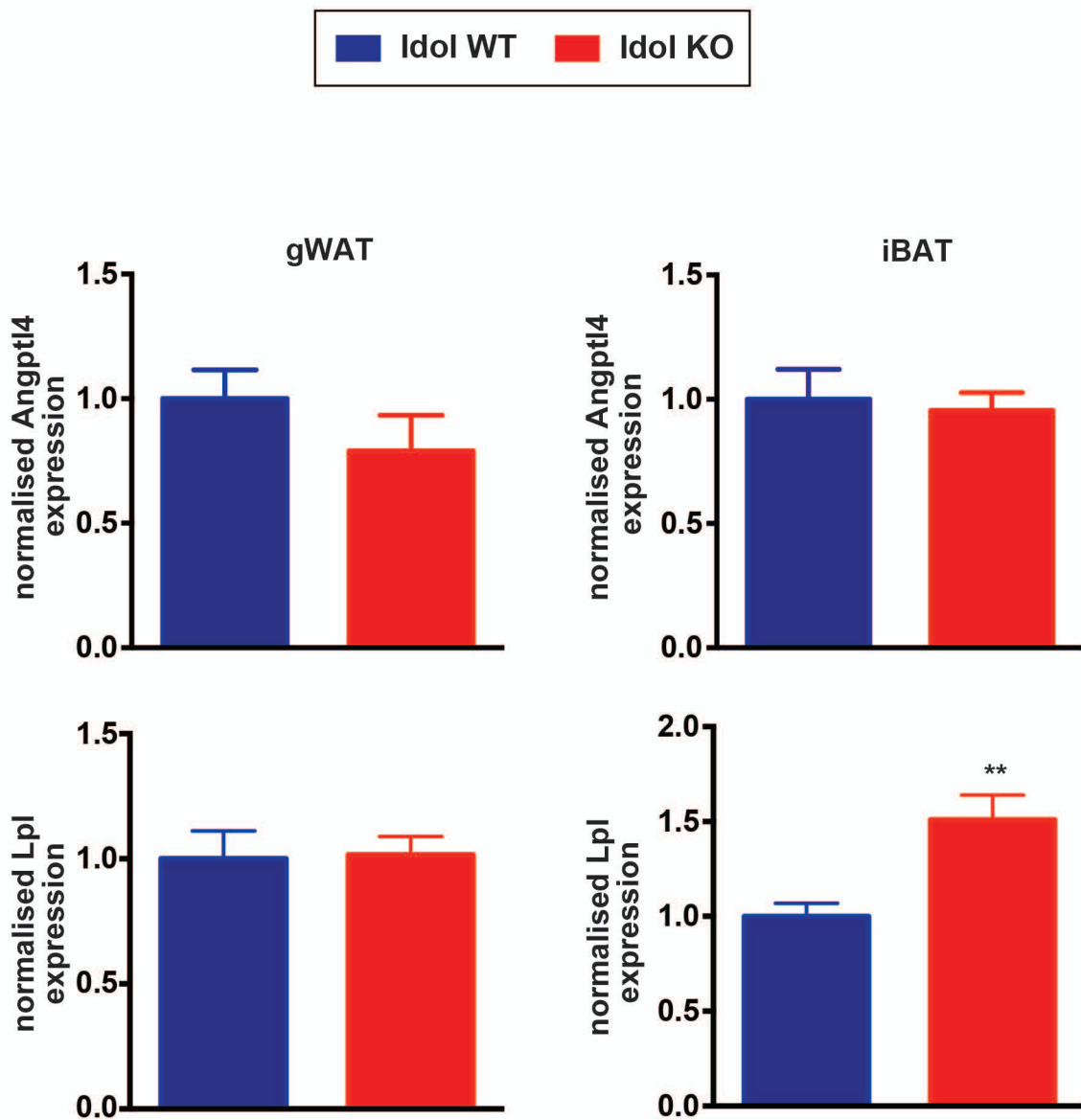
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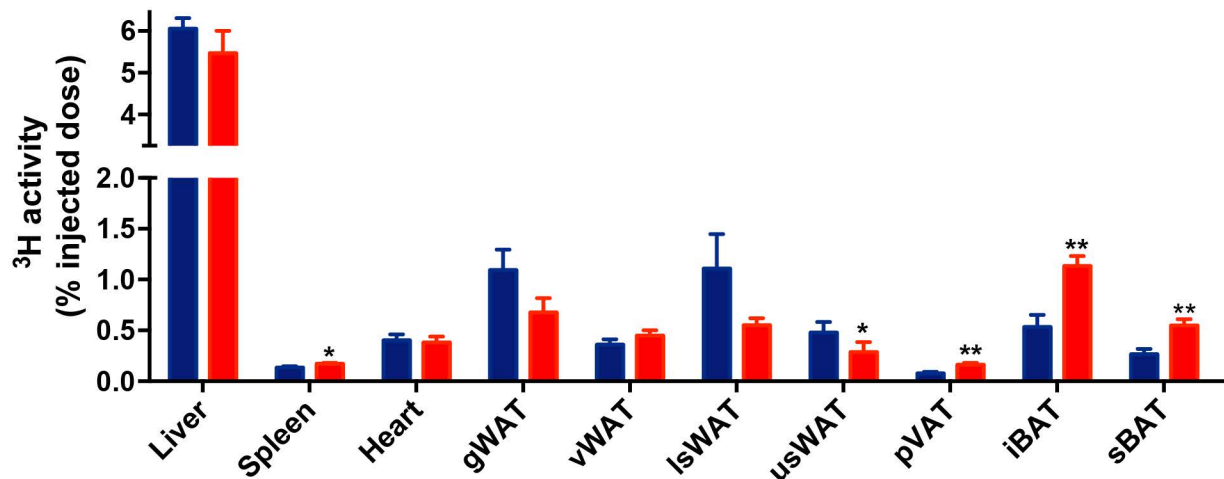










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