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Supplemental Information

Glycogen Dynamics Drives Lipid Droplet Biogenesis during Brown Adipocyte Differentiation

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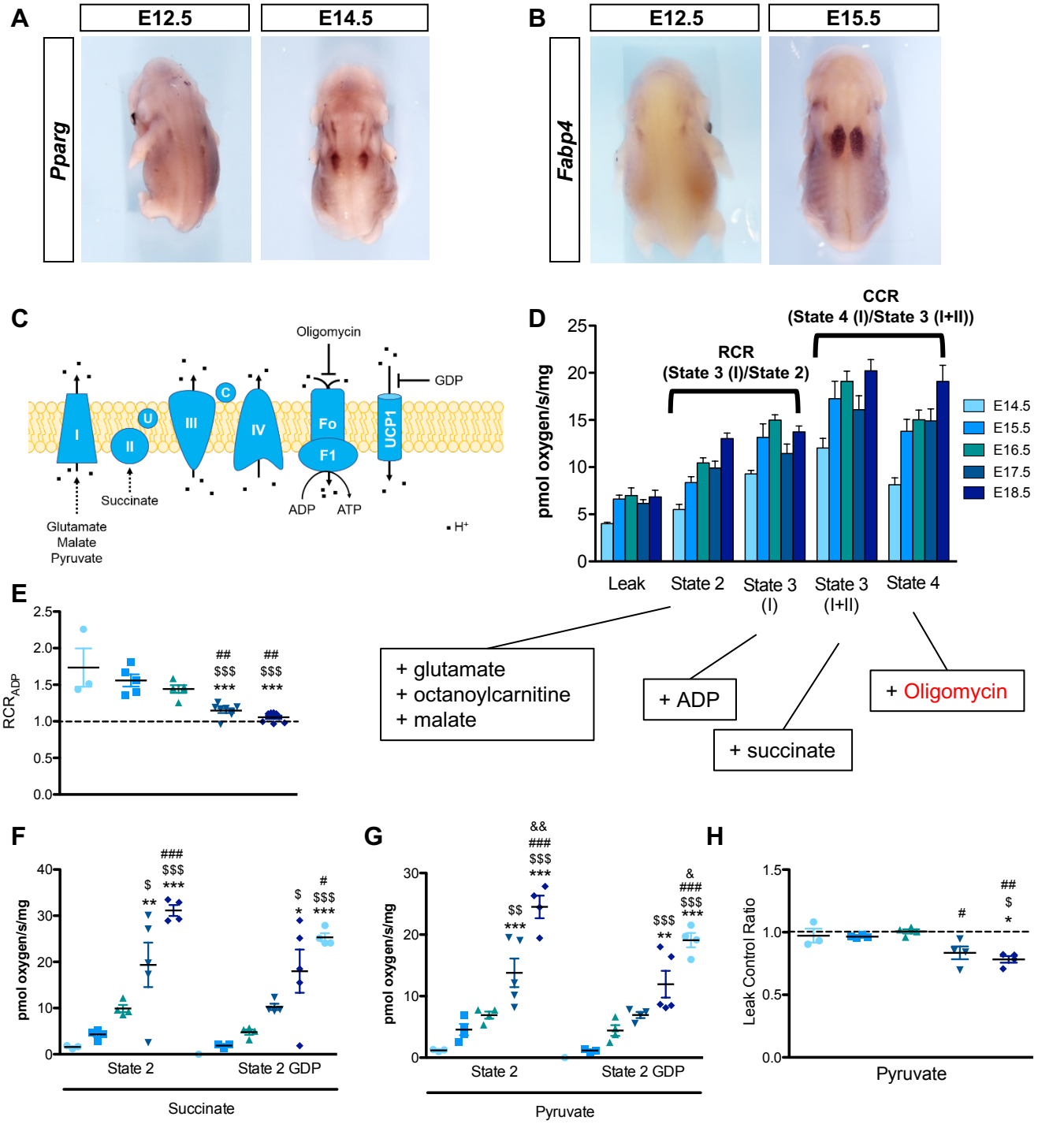

Figure S1. Identification of embryonic BAT with uncoupled mitochondria from E17.5. Related to Figure 1. A-B, Whole mount *in situ* hybridization of embryos with *Pparg* (A) and *Fabp4* (B) probes. **C**, Schematic representation of the mitochondrial respiratory chain. Oligomycin inhibits ATP synthase activity while GDP inhibits UCP1. **D**, Comparison of respiratory states in BAT isolated from E14.5 to E18.5 mouse embryos. Boxes indicate the corresponding added substrates for the different states. The Coupling Control Ratio (CCR) is calculated by dividing State 4 (I) by State 3 (I+II). When inferior to 1, this indicates that mitochondria is sensitive to oligomycin and thus is coupled to ATP synthase. **E**, The Respiratory Control Ratio (RCR) for ADP calculated by dividing State 3 (I) by State 2. When close to 1, this indicates that mitochondria are not coupled, as shown from E17.5. **F-G**, Oxygen consumption after addition of succinate (F) or pyruvate (G) (State 2) for BAT isolated from different embryonic stages. Addition of GDP, an UCP1 inhibitor, indicates that mitochondria respiration depends on the UCP1 activity. GDP has no effects before E17.5 indicating that mitochondria are not sensitive to GDP, and therefore not uncoupled before this stage. **H**, The Leak Control Ratio (LCR) for BAT was calculated from respiration data (G) by dividing state 2_{GDP} respiration by state 2. All the results are expressed as means ± s.e.m; *p < 0.05, **p < 0.01, ***p<0.001 by One-way ANOVA with Bonferroni post-hoc analysis, *referred to E14.5, $ to E15.5, # to E16.5 and & to E17.5.

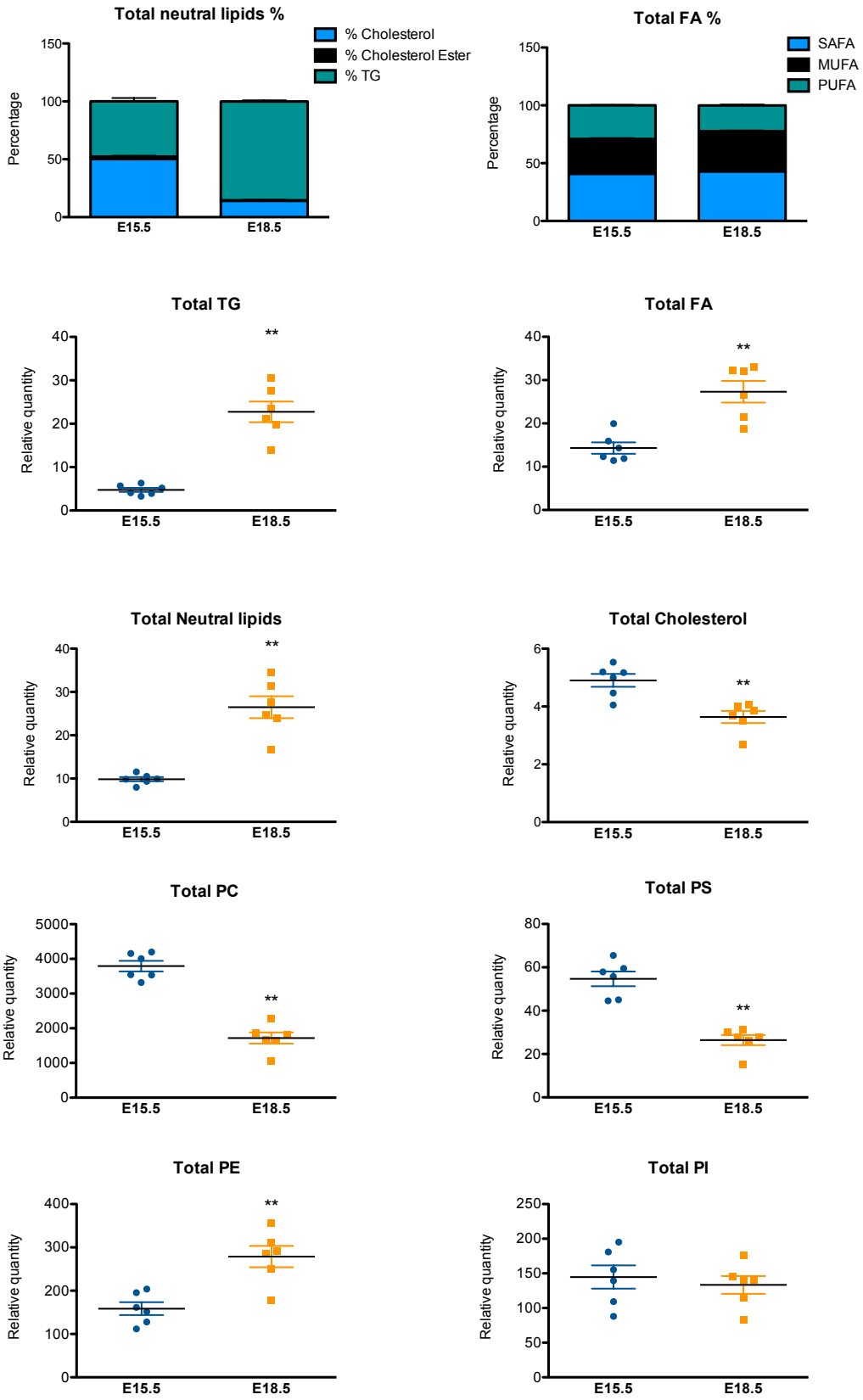

Figure S2. Lipidomic analysis of BAT at E15.5 and E18.5. Related to Figure 2. Lipidomic analysis of BAT at E15.5 and E18.5 (MUFA, MonoUnsaturated Fatty Acids; SAFA, Saturated Fatty Acids; PUFA, Poly Unsaturated Fatty Acids; PE, Phosphatidylethanolamine; PC, Phosphatidylcholine; PS, Phosphatidylserine; PI, Phosphatidylinositol; TG, Triglyceride; FA, Fatty acid). Relative quantity corresponds to the ratio between the area of the molecule of interest and the internal standard. Results are expressed as means ± s.e.m; **p < 0.01, by Mann and Whitney test).

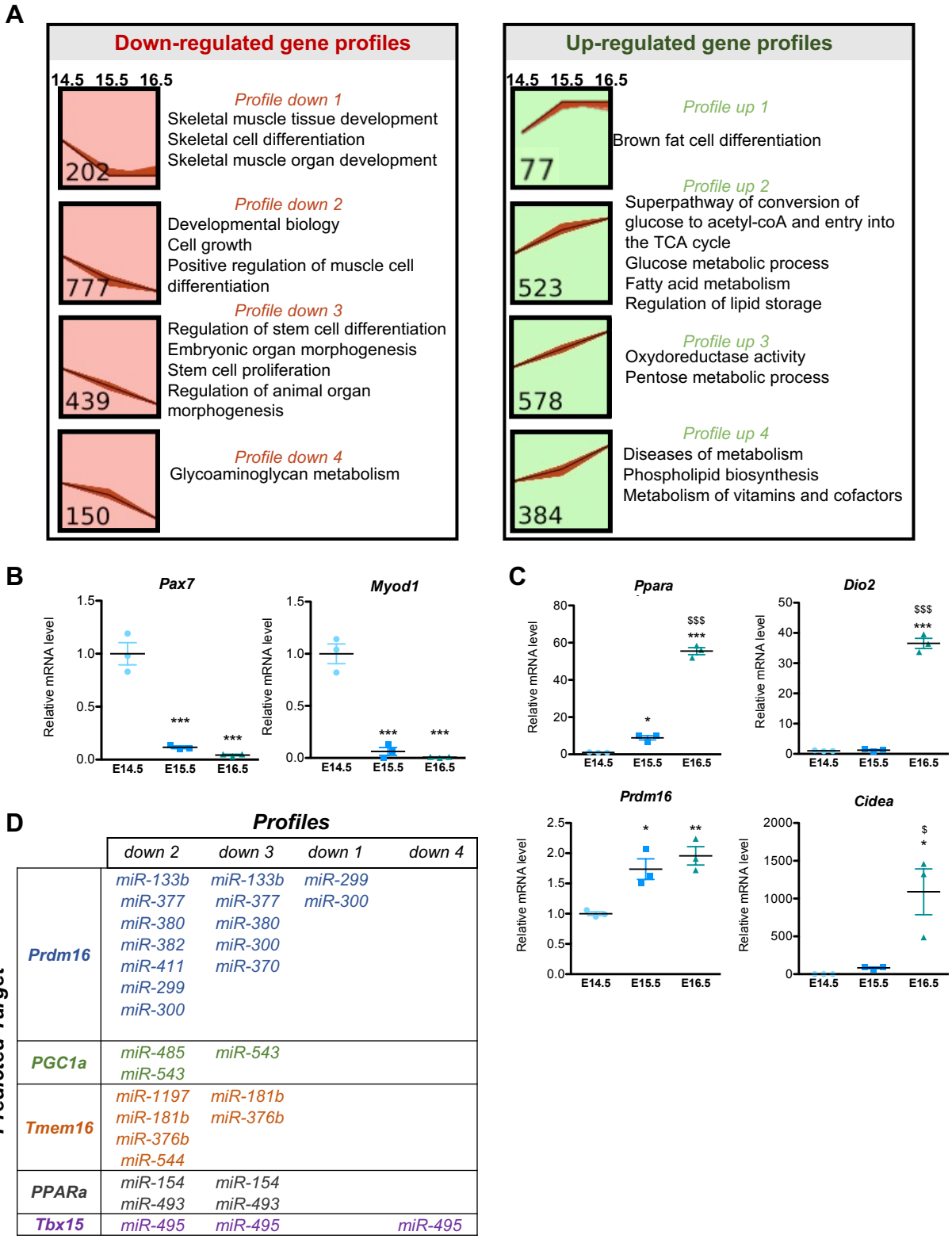

Figure S3. Transcriptomic analysis of BAT between E14.5 and E16.5. Related to Figure 3. A. Identification of genes with different temporal expression profiles using the Short Time-series Expression Miner. The top 4 (based on their p-values) expression patterns of down-regulated genes are represented in red and the top 4 expression patterns of up-regulated genes in green. The number of genes associated with each pattern is written at the bottom left of each panel. Selection of GO-term corresponding to each pattern is indicated. The list of genes for each profile is given in the Supplementary Table 1. **B**, The myogenic factors *Pax7* and *Myod1* are downregulated between E14.5 and E15.5, E16.5 in BAT as shown by RT-qPCR analysis (reference gene: *36B4*). **C**, Upregulation of genes of the brown adipocyte differentiation program between E14.5 and E16.5 as shown by RT-qPCR analysis. Results are expressed as means ± s.e.m; *p < 0.05, **p < 0.01, ***p<0.001 by One-way ANOVA with Bonferroni post-hoc analysis, *referred to E14.5, $ to E15.5. **D**, Table of miRNA (miR), with their respective predicted targets, and their expression profile identified by Short time-series expression Miner (STEM) analysis.

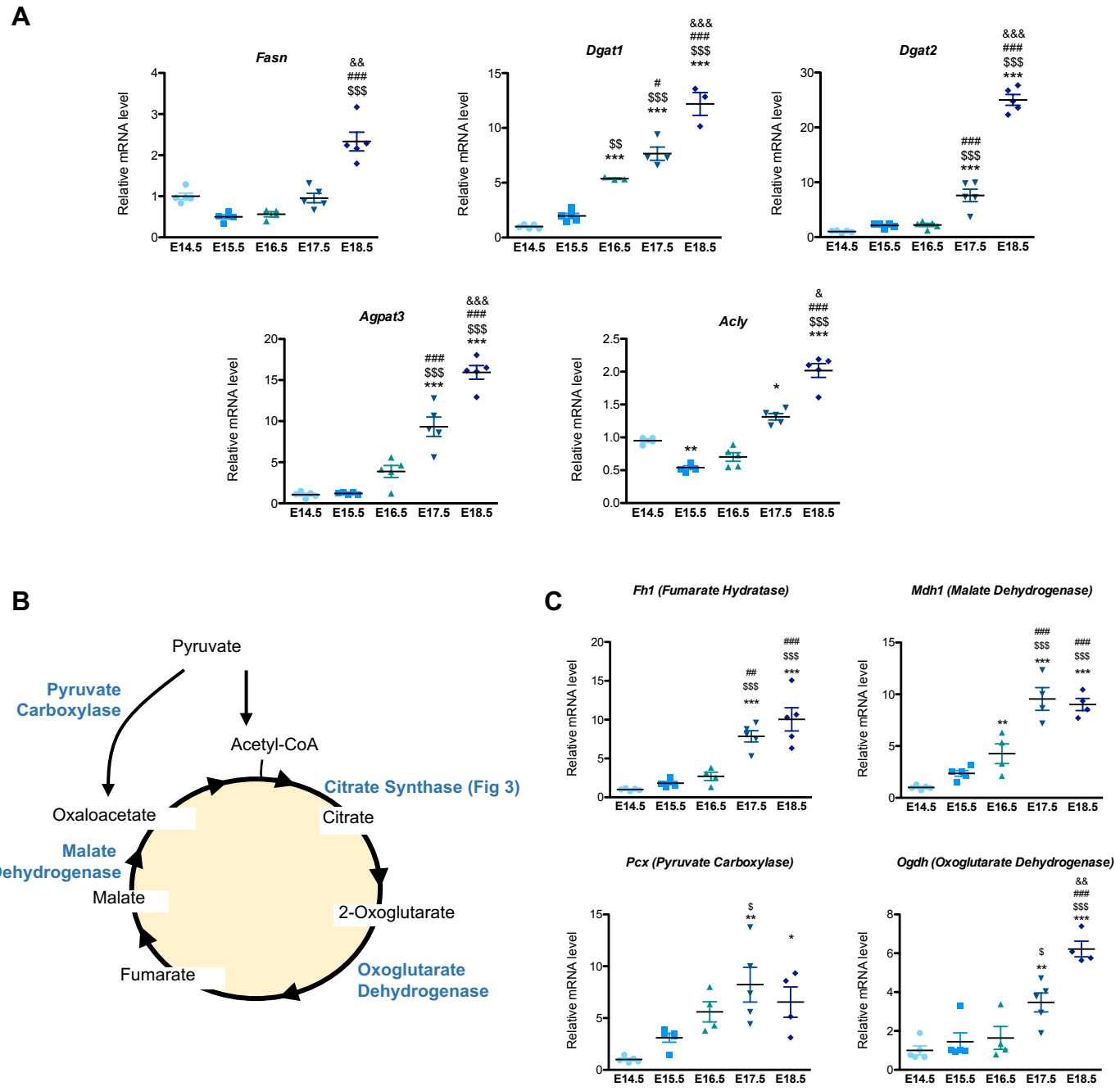

Figure S4. Upregulation of lipogenic- and TCA cycle- related genes is associated with close proximity between mitochondria and LD during BAT differentiation. Related to Figure 3. A. Genes involved in lipogenesis are upregulated between E14.5 and E18.5, as shown by RTqPCR analysis (reference gene: *Ppia*). **B**, Schematic representation of the TCA cycle. **C**, Genes involved in the TCA cycle are upregulated between E14.5 and E18.5, as shown by RTqPCR analysis (reference gene: *Ppia*). Results are expressed as means ± s.e.m; *p < 0.05, **p < 0.01, ***p<0.001 by One-way ANOVA with Bonferroni post-hoc analysis, *referred to E14.5, $ to E15.5, # to E16.5 and & to E17.5. **D**, TEM pictures show a strong proximity between lipid droplets (LD) and mitochondria (yellow stars) between E15.5 and E18.5 (G, Glycogen, SB 2µm).

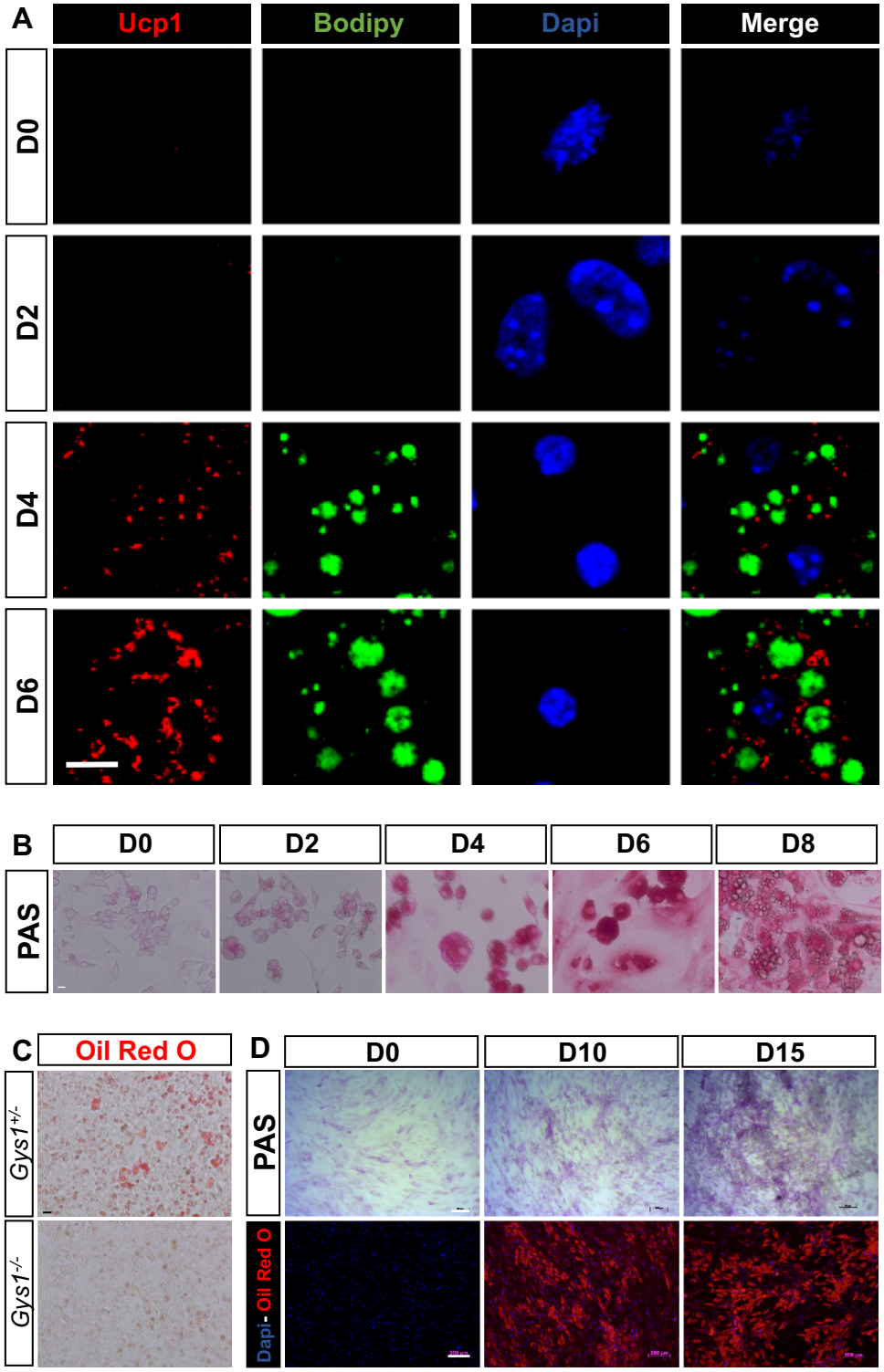

Figure S5. Glycogen accumulates in primary embryonic brown adipocytes and hMADS cells during differentiation, as *in vivo* in embryonic BAT. Related to Figure 3. A, Immunostaining with Ucp1 antibody, bodipy and dapi on primary brown adipocytes isolated from the BAT of the mouse embryo, at E14.5 and differentiated *ex vivo* during 6 days (SB 10µm). **B**, PAS staining on primary brown preadipocytes during *ex vivo* differentiation into brown adipocytes (SB 10µm). **C,** Representative picture of Oil Red O staining of BAT at E18.5 in *Gys1*$^{+/-}$ and *Gys1*$^{-/-}$ embryos showing a decrease of LD in the BAT from *Gys1* KO mice at E18.5. (n>4, SB=10µm). **D**, PAS (SB 20µm) and Oil Red O (SB 200µm) staining on hMADS cells during brown adipocyte differentiation. (D, day after induction of differentiation).

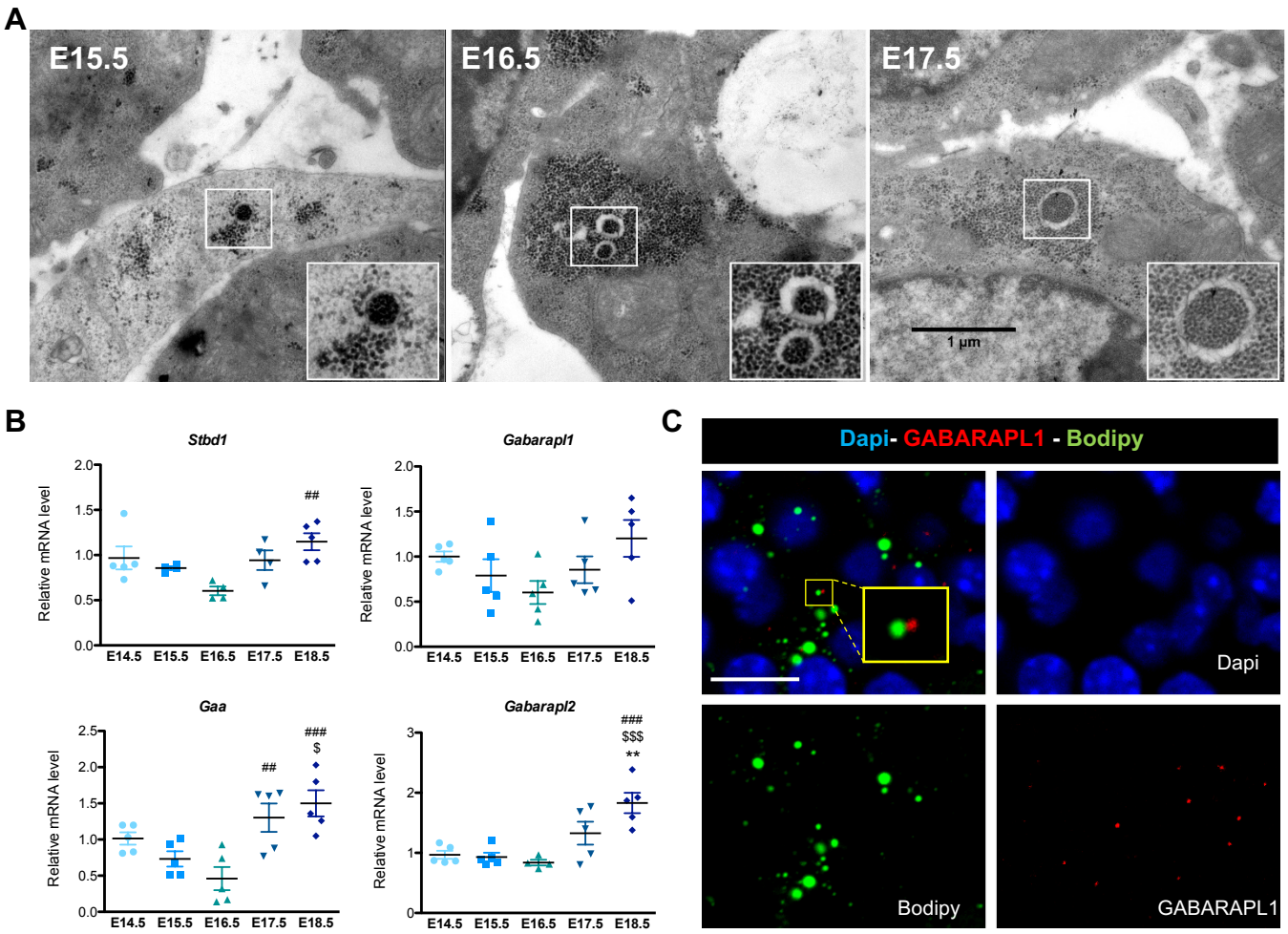

Figure S6. Glycophagy during brown adipocyte differentiation. Related to Figure 4. A, Representative pictures showing glycophagosomes within glycogen granules obtained by TEM on BAT from E15.5 to E17.5 (SB 1µm). **B**, RT-qPCR analysis of transcripts of *Stbd1, Gabarapl1, Gaa, Gabarapl2* normalised to *Ppia* in embryonic BAT. Results are expressed as means ± s.e.m; *p < 0.05, **p < 0.01, ***p<0.001 by One-way ANOVA with Bonferroni post-hoc analysis, *referred to E14.5, $ to E15.5, # to E16.5. **C**, Representative pictures of immunostaining of GABARAPL1 (red), Bodipy (green) and Dapi (blue) on BAT at E16.5 (SB 10µm).

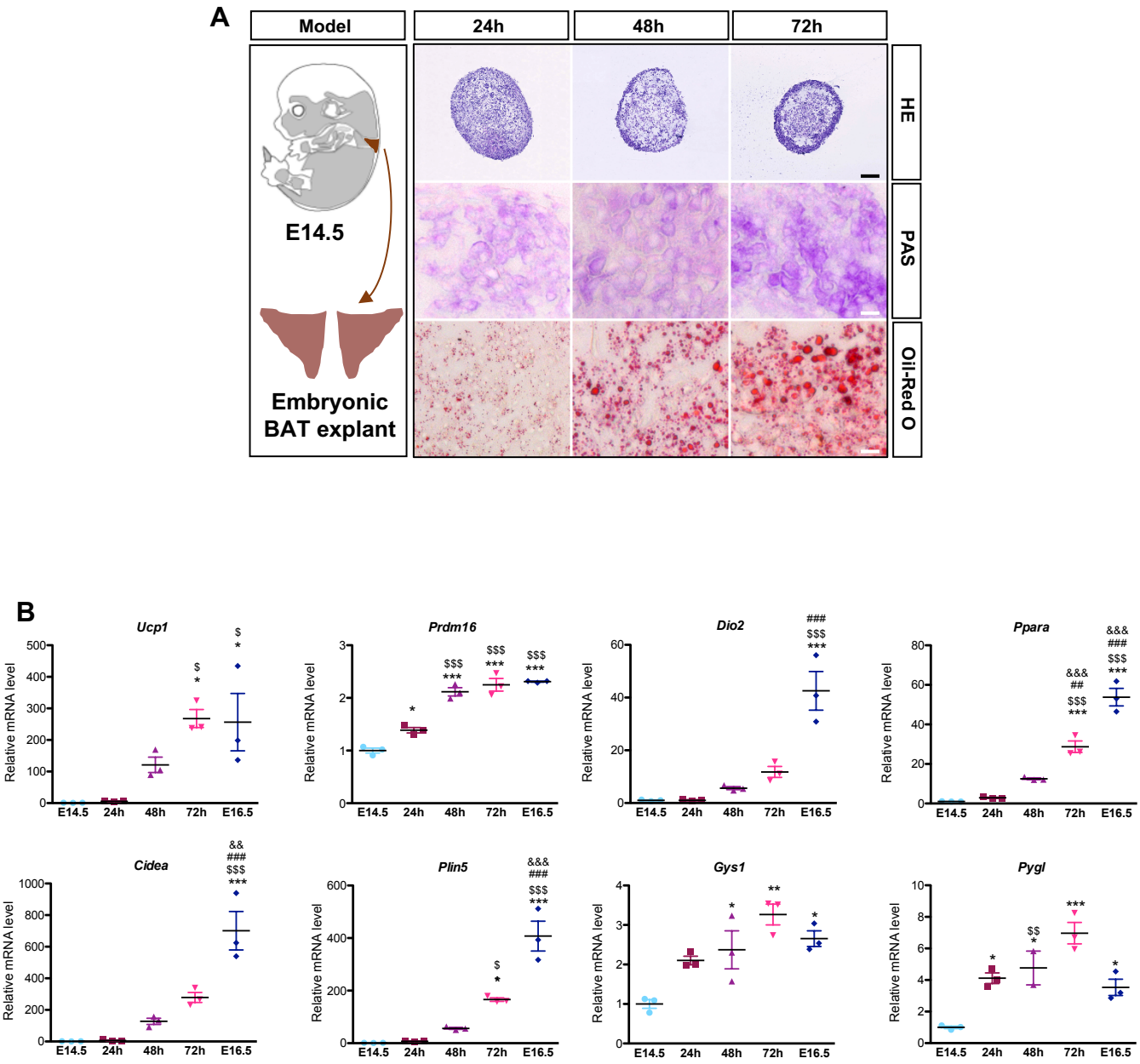

Figure S7. Model of embryonic brown adipose explants. Related to Figure 4. A, Schematic representation of an *ex vivo* BAT explant culture. Two individual deposits of BAT were collected at E14.5 and cultured *ex vivo* in DMEM 10% FBS. Hematoxylin staining (upper panels) (SB 40 µm), PAS staining (middle panels) (SB 10 µm) and Oil-Red O staining (lower panels) (SB 10 µm) of histological sections of embryonic BAT explants after 24h, 48h or 72h culture. **B**, RT-qPCR analysis (reference gene: Ppia) made on explants at 24h, 48h, 72h and compared to *in vivo* isolated BAT at E14.5 and E16.5. Results are expressed as means ± s.e.m; *p< 0.05, **p < 0.01, ***p<0.001 by One-way ANOVA with Bonferroni post-hoc analysis, *referred to E14.5, $ to 24h, # to 48h and & to 72h.

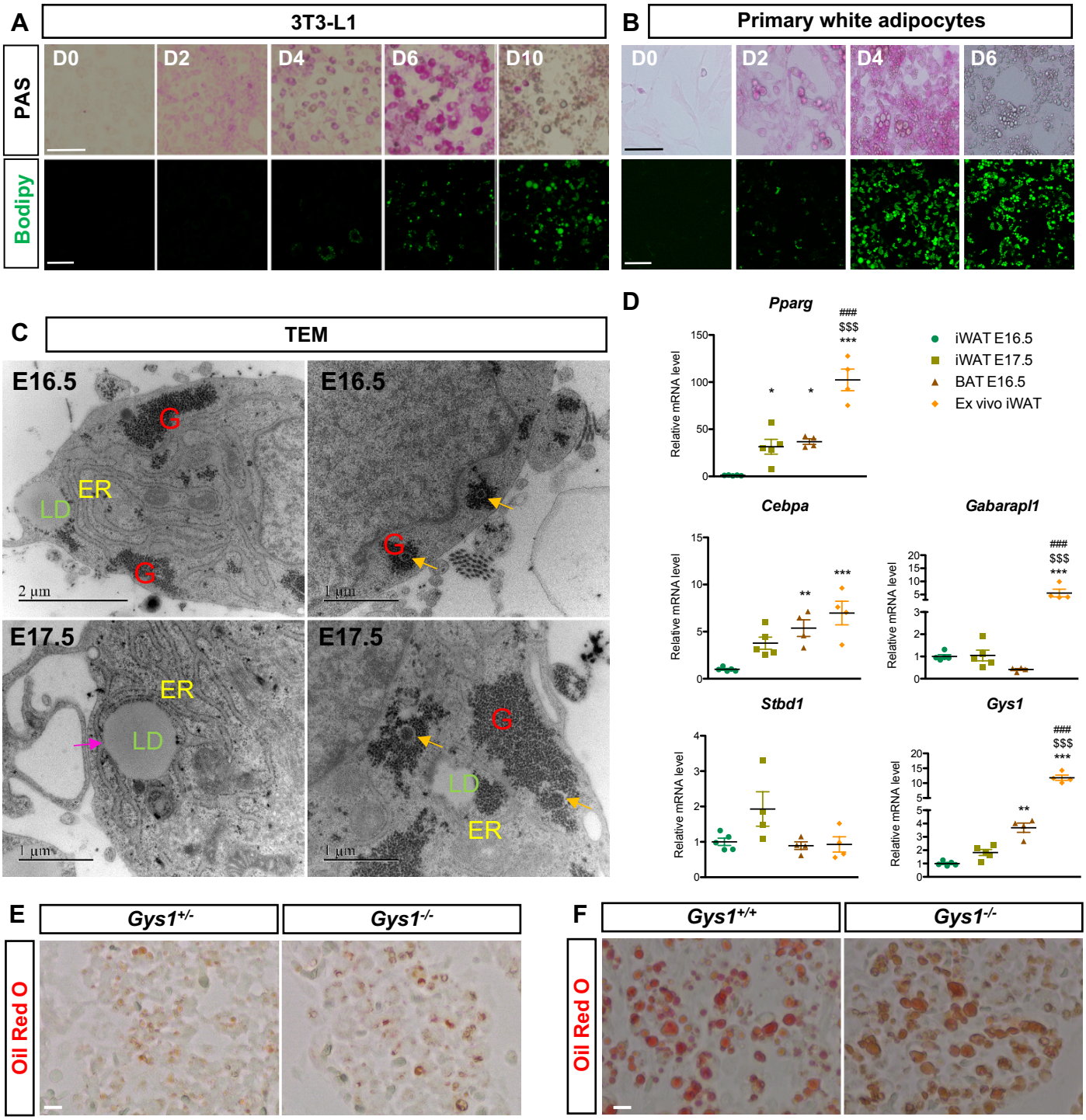

Figure S8. Glycogen dynamics in WAT. Related to Figure 4. A,B, Representative pictures of PAS (SB 100µm) and Bodipy staining (50µm) in 3T3L1 cells (A) and primary white adipocytes (B) at different days of white adipose differentiation (D, Day). **C**, Representative pictures of TEM of iWAT at E16.5 and E17.5 (G, Glycogen, LD, Lipid Droplet, ER, Endoplasmic Reticulum). Orange arrows indicate glycophagosomes. Pink arrows indicate budding of LD from ER. **D**, RTqPCR of genes involved in the adipogenic program and glycophagy in iWAT at E16.5, E17.5, BAT at E16.5 and primary white adipocytes 5 days after induction of differentiation. Glycophagy-related genes are similarly expressed in iWAT than in BAT, as shown by RTqPCR analysis (reference gene: Ppia). Results are expressed as means ± s.e.m; *p < 0.05, **p < 0.01, ***p < 0.001 by One-way ANOVA with Bonferroni post-hoc analysis, *referred to iWAT E16.5, $ to iWAT E17.5, # to BAT E16.5. **E,** Representative picture of Oil Red O staining of iWAT at E18.5 in *Gys1*$^{+/-}$ and *Gys1*$^{-/-}$ embryos. (n>4, SB=10µm). **F,** Representative picture of Oil Red O staining of iWAT at P0 in *Gys1*$^{+/+}$ and *Gys1*$^{-/-}$ embryos. (n=2,3, SB=10µm).

Gene	Forward	Reverse
Acly	*ACTATGCCAAGACCATCCTCTC*	*GGTAATCTCGAATCGCTCTCAC*
Agpat3	*AACTGCCGCTTGGCCTACTCGC*	*AACCGCTCGCACATCGTCCACC*
Cebpa	*GGTGATCAAACAAGAGCCCCG*	*GCG ATC TGG AAC TGC AAG TGG*
Cidea	*TGCTCTTCTGTATCGCCCAGT*	*GCCGTGTTAAGGAATCTGCTG*
Cs	*TCCATCACAGCGGCGAC*	*CAAGGACGAGGCAGGATGAG*
Dio2	*CAGTGTGGTGCACGTCTCCAATC*	*TGAACCAAAGTTGACCACCAG*
Dgat1	*TAGAAGAGGACGAGGTGCGAGA*	*ATGGCACCTCAGATCCCAGTAG*
Dgat2	*GATGCCTCCAGACATCAGGT*	*TCCAGCTGGTGAAGACACAC*
Fasn	*TGATAGCCGGTATGTCGGGGAA*	*TCCATAGAGCCCAGCCTTCCAT*
Fh1	*ACTTTAAGATTGGAGGTGCT*	*CTTTGGATCGAGACCATACT*
Gaa	*GATTGCGCAGGCCTTCAGAAGTA*	*ATGACCCAGGATGACCGCTGTA*
GabarapL1	*ACCTGAGACCTGAGGACGCC*	*TGGTTGTCCTCATACAGCTGGC*
GabarapL2	*AGCCGGCTCCGTCGC*	*CCACGATCACCGGAACTCGG*
Gyg	*CCGGCCACACTATGACAGATCA*	*CCTGTGGGCTGGTGAGTACAAC*
Gys1	*TCTGTGTCCTCGCTTCCAGGAT*	*GTGTAGATGCCACCCACCTTGT*
Mdh1	*CCCAGAGGGAGAGTTCGTGT*	*CGGTCTCCTTTTCCTCGGTC*
Myod1	*TGCAGTCGATCTCTCAAAGCACC*	*GCAGGCTCTGCTGCGCGACC*
Ogdh	*GCTGACATTATCTCATCCAC*	*CCATAGAACCCTCCTACTGT*
Pax7	*TGCCGATATCAGGAGACTGGGTC*	*TTTCTCCACATCCGGAGTCGCC*
Pcx	*GTTGTGGACGTGGCAGTAGA*	*ATGGTAGCCGTGCAATCGAA*
Plin5	*CCATCTCGCCTATGAACACTCTT*	*CAGCTGGGCCAGCATCTC*
Ppara	*ACATGGAGACCTTGTGTATGGC*	*GGGAAATGTCACTGTCATCCAG*
Pparg	*CCGTGATGGAAGACCACTCG*	*AGG CCT GTT GTA GAG CTG GGT C*
Stbd1	*TGCTGAGGTGGTTTGAAGGGC*	*GGCAGCCCATTTGTTGACCC*
Prdm16	*CGTCCACACGGAAGAGCGTGA*	*TGGAGGTTGCTGGGGTCCGT*
Pygl	*CGACAAGTGTCCCAAGAGGGTG*	*TGGTAAATGGCCTCATCGCAGG*
Scd1	*CACACCTTCCCCTTCGACTAC*	*ACAGGAACTCAGAAGCCCAAAG*
Ucp1	*ACTGCCACACCTCCAGTCATT*	*CTTTGCCTCACTCAGGATTGG*

Supplementary Table S2. Primers used for RTqPCR. Related to STAR Methods.