Expanded View Figures

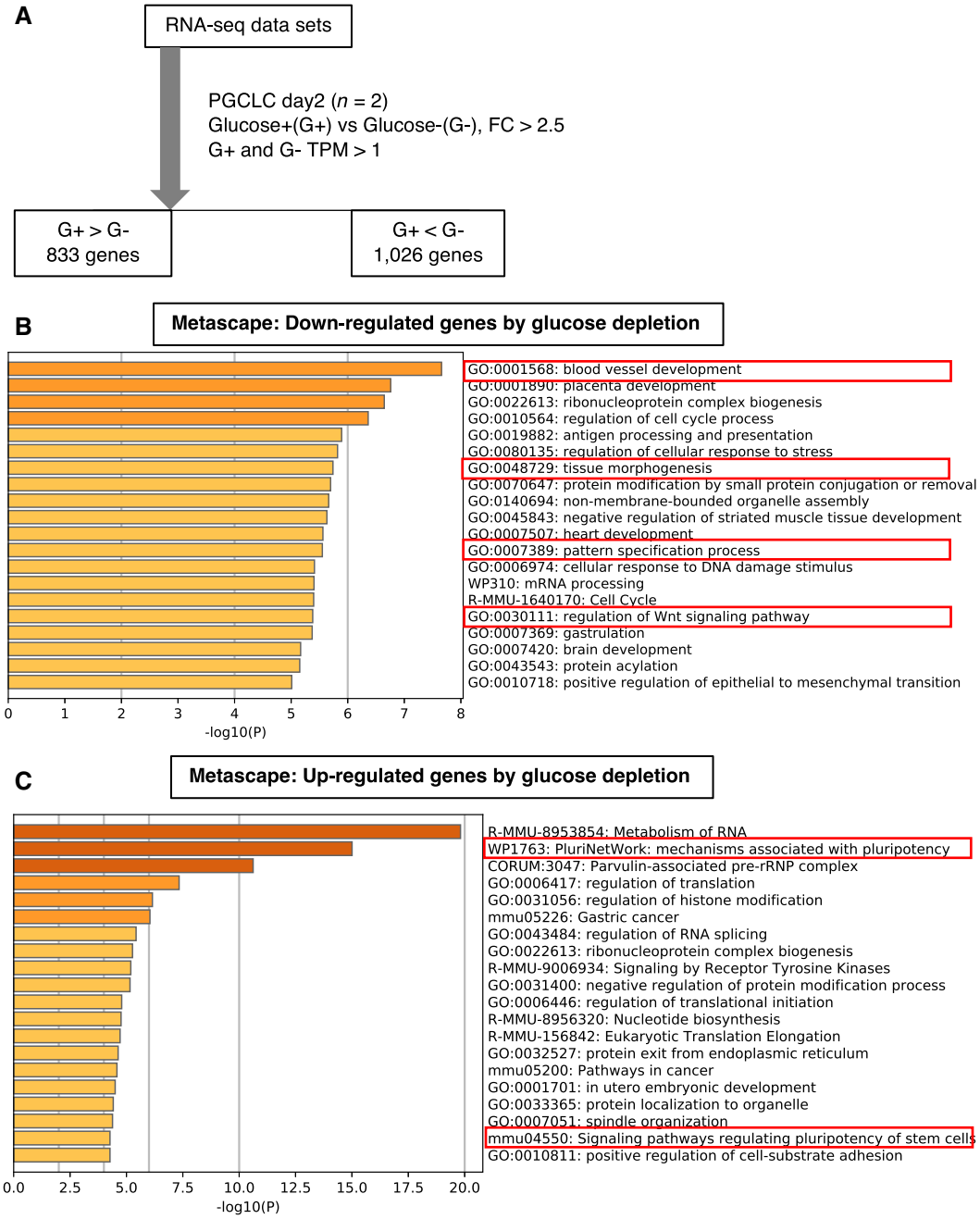


Figure EV1. RNA-seq analysis of PGCLC-containing aggregates cultured with or without glucose.

A A scheme of RNA-seq analysis of the PGCLC-containing aggregates at day 2 cultured with and without glucose.

B, C Functional annotation analysis of differentially expressed genes (DEGs). Metascape analysis of down- (B) and up-regulated (C) genes by glucose depletion. Focused terms are highlighted by red.

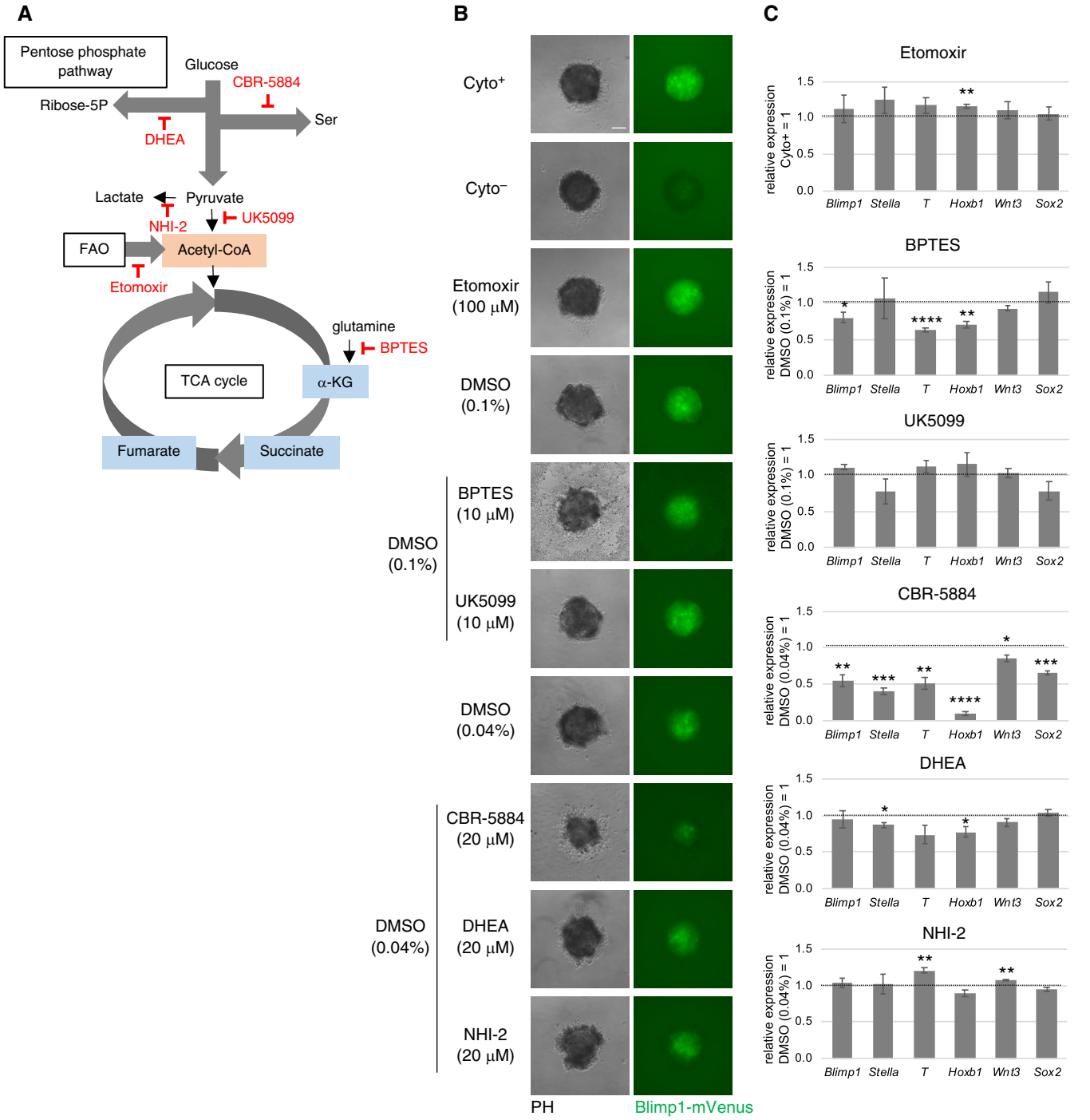


Figure EV2. The effects of inhibition of glycolysis-related metabolic pathways during PGCLC induction.

A A schematic representation of glycolysis-related metabolic pathways. Inhibitors used in this study are highlighted by red.

B, C The effects of various inhibitors for glycolysis-related pathways on BV fluorescence (B) and gene expression (C) in the PGCLC-containing aggregates at Day 2. Scale bar: 100 μm. Values are plotted as mean ± SE of three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Student's t-test).

Figure EV3. Gene expression changes in PGCLC-containing aggregates with OGT inhibition by OSMI-1 or siRNA.

A A scheme of the RNA-seq analysis.

B, C Functional annotation analysis of DEGs. Metascape analysis of down-regulated (B) and up-regulated (C) genes by OGT inhibition. Focused terms are highlighted by red.

D, E Venn diagram showing the overlap between genes down- (D) or up-regulated (E) by glucose depletion and those down- or up-regulated, by OSMI-1, respectively (left). Metascape analysis of the overlapped genes (right). Focused terms are highlighted by red.

F, G The effect of *Ogt* knockdown using siRNAs (siOgt3 and 5) on BV fluorescence (F), and gene expression (G) in the PGCLC-containing aggregates at day 2. AS: AllStars negative control siRNA.

H *Ogt* knockdown efficiency and influence on *Oga* expression in the aggregates at day 1.

Data information: Values are plotted as mean ± SE of three biological replicates (three technical replicates for each biological replicate sample in G and H). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ (Student's *t*-test). $P < 0.1$ is also indicated by actual values to show difference tendency. Scale bar: 100 μm (F).
Source data are available online for this figure.

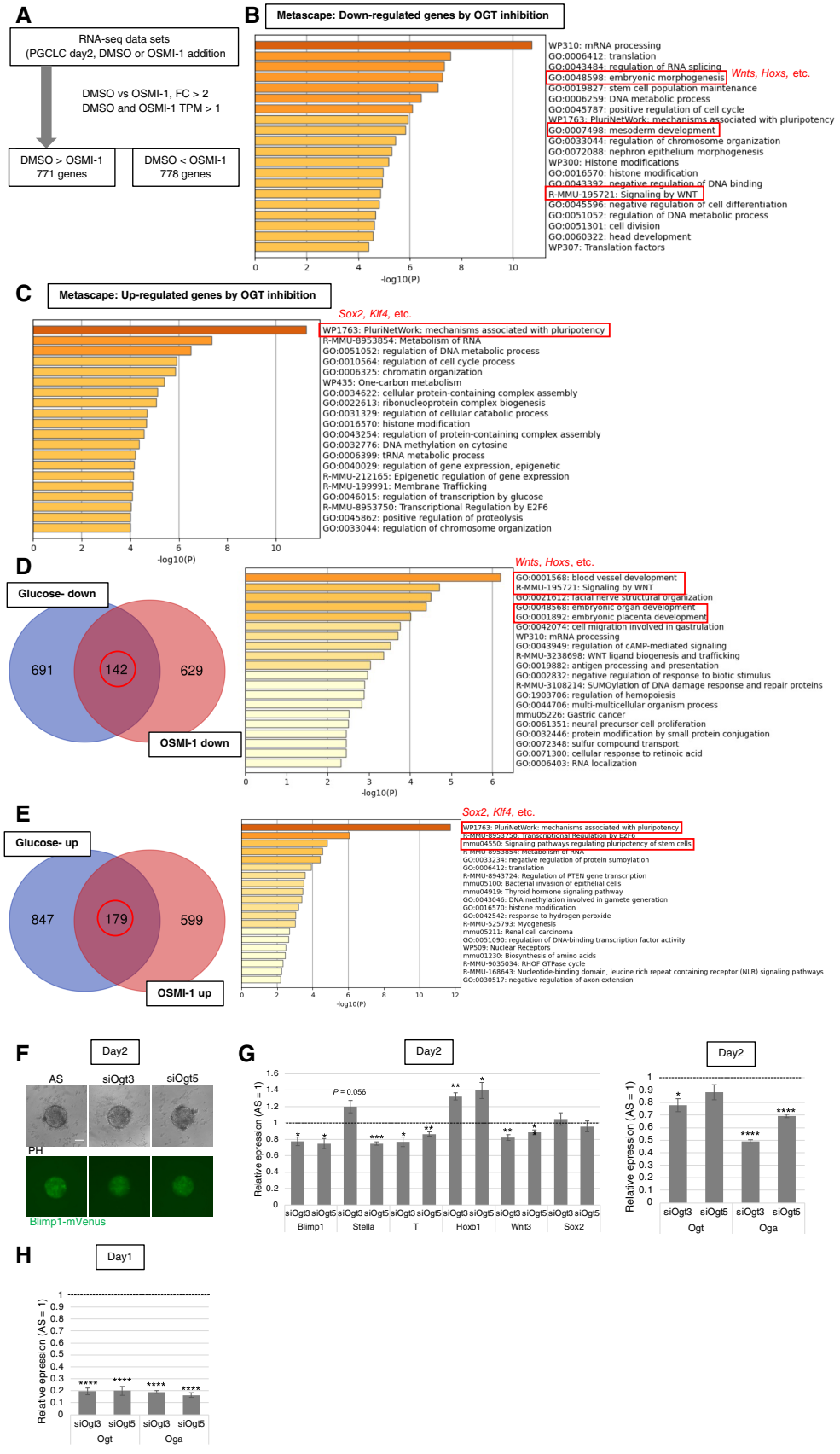
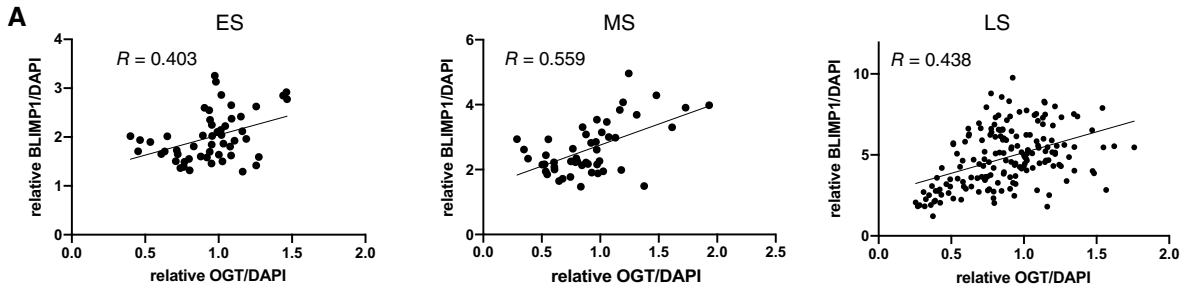
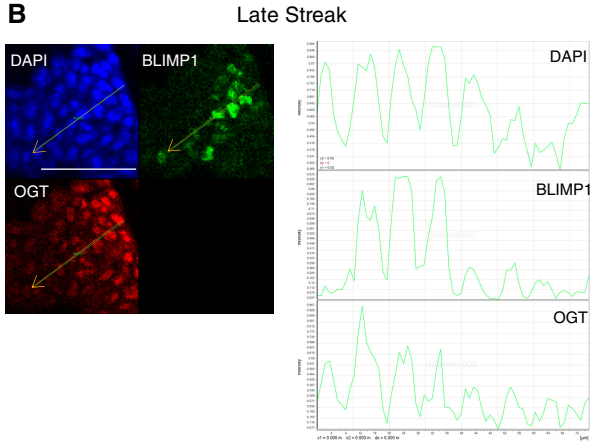
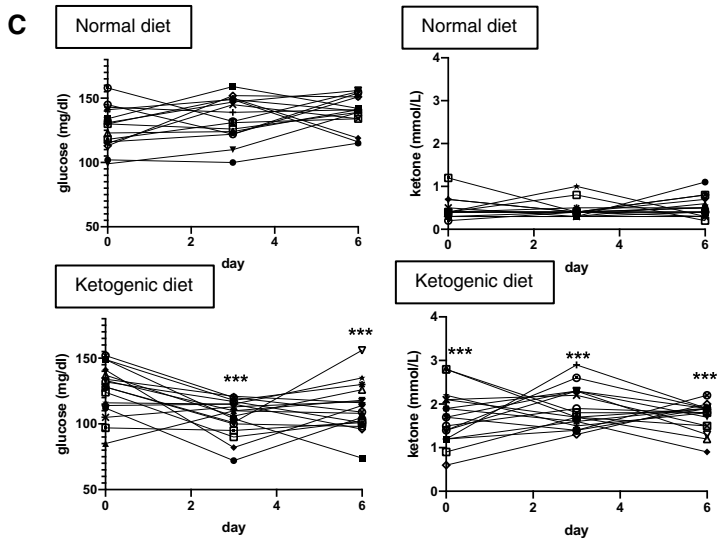

A
RNA-seq data sets
(PGCLC day2, DMSO or OSMI-1 addition
DMSO vs OSMI-1, FC > 2
DMSO and OSMI-1 TPM > 1
DMSO > OSMI-1
771 genes
DMSO < OSMI-1
778 genes
B
Metascape: Down-regulated genes by OGT inhibition
WP310: mRNA processing
GO:0006412: translation
GO:0043484: regulation of RNA splicing
GO:0048598: embryonic morphogenesis
Wnts, Hoxs, etc.
GO:0019827: stem cell population maintenance
GO:0006259: DNA metabolic process
GO:0045787: positive regulation of cell cycle
WP1763: PluriNetWork: mechanisms associated with pluripotency
GO:0007498: mesoderm development
GO:0033044: regulation of chromosome organization
GO:0072088: nephron epithelium morphogenesis
WP300: Histone modifications
GO:0016570: histone modification
GO:0043392: negative regulation of DNA binding
R-MMU-195721: Signaling by WNT
GO:0045596: negative regulation of cell differentiation
GO:0051052: regulation of DNA metabolic process
GO:0051301: cell division
GO:0060322: head development
WP307: Translation factors
-log10(P)
C
Metascape: Up-regulated genes by OGT inhibition
Sox2, Klf4, etc.
WP1763: PluriNetWork: mechanisms associated with pluripotency
R-MMU-8953854: Metabolism of RNA
GO:0051052: regulation of DNA metabolic process
GO:0010564: regulation of cell cycle process
GO:0006325: chromatin organization
WP435: One-carbon metabolism
GO:0034622: cellular protein-containing complex assembly
GO:0022613: ribonucleoprotein complex biogenesis
GO:0031329: regulation of cellular catabolic process
GO:0016570: histone modification
GO:0043254: regulation of protein-containing complex assembly
GO:0032776: DNA methylation on cytosine
GO:0006399: tRNA metabolic process
GO:0040029: regulation of gene expression, epigenetic
R-MMU-212165: Epigenetic regulation of gene expression
R-MMU-199991: Membrane Trafficking
GO:0046015: regulation of transcription by glucose
R-MMU-8953750: Transcriptional Regulation by E2F6
GO:0045862: positive regulation of proteolysis
GO:0033044: regulation of chromosome organization
-log10(P)
D
Glucose- down
691
142
629
OSMI-1 down
Wnts, Hoxs, etc.
GO:0001568: blood vessel development
R-MMU-195721: Signaling by WNT
GO:0021612: facial nerve structural organization
GO:0048568: embryonic organ development
GO:0001892: embryonic placenta development
GO:0042074: cell migration involved in gastrulation
WP310: mRNA processing
GO:0043949: regulation of cAMP-mediated signaling
R-MMU-3238698: WNT ligand biogenesis and trafficking
GO:0019882: antigen processing and presentation
GO:0002832: negative regulation of response to biotic stimulus
R-MMU-3108214: SUMOylation of DNA damage response and repair proteins
GO:1903706: regulation of hemopoiesis
GO:0044706: multi-multicellular organism process
mmu05226: Gastric cancer
GO:0061351: neural precursor cell proliferation
GO:0032446: protein modification by small protein conjugation
GO:0072348: sulfur compound transport
GO:0071300: cellular response to retinoic acid
GO:0006403: RNA localization
-log10(P)
E
Glucose- up
847
179
599
OSMI-1 up
Sox2, Klf4, etc.
WP1763: PluriNetWork: mechanisms associated with pluripotency
R-MMU-8953750: Transcriptional Regulation by E2F6
mmu04550: Signaling pathways regulating pluripotency of stem cells
R-MMU-8953854: Metabolism of RNA
GO:0033234: negative regulation of protein sumoylation
GO:0006412: translation
R-MMU-8943724: Regulation of PTEN gene transcription
mmu05100: Bacterial invasion of epithelial cells
mmu04919: Thyroid hormone signaling pathway
GO:0043046: DNA methylation involved in gamete generation
GO:0016570: histone modification
GO:0042542: response to hydrogen peroxide
R-MMU-525793: Myogenesis
mmu05211: Renal cell carcinoma
GO:0051090: regulation of DNA-binding transcription factor activity
WP509: Nuclear Receptors
mmu01230: Biosynthesis of amino acids
R-MMU-9035034: RHOF GTPase cycle
R-MMU-168643: Nucleotide-binding domain, leucine rich repeat containing receptor (NLR) signaling pathways
GO:0030517: negative regulation of axon extension
-log10(P)
F
Day2
AS
siOgt3
siOgt5
PH
Blimp1-mVenus
G
Day2
Relative epression (AS = 1)
P = 0.056
siOgt3 siOgt5
Blimp1
Stella
T
Hoxb1
Wnt3
Sox2
Day2
Relative epression (AS = 1)
siOgt3 siOgt5 siOgt3 siOgt5
Ogt
Oga
H
Day1
Relative epression (AS = 1)
siOgt3 siOgt5 siOgt3 siOgt5
Ogt
Oga

Figure EV3.







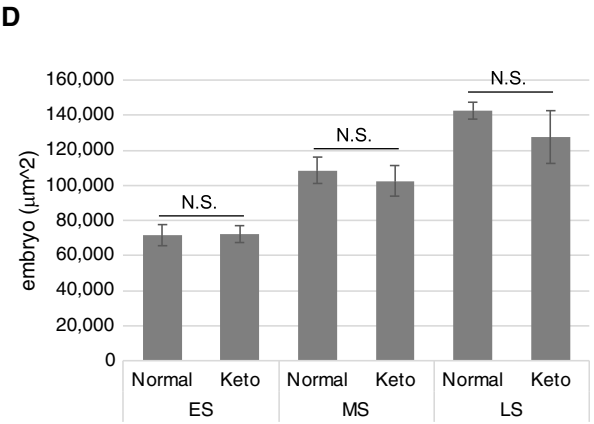


Figure EV4. Characterization of *Ogt*-conditional knockout mice and ketogenic mice.

A, B Correlation between DAPI-normalized BLIMP1 and OGT signal intensity in regions existing PGCs in the embryos at early to late streak stages (n = 3 each). Plotted fluorescent intensity of BLIMP1 and OGT (A), and line-scan histograms of the regions (B). The orange dotted lines shown in (B) for the line-scan. Scale bar: 75 μm.

C Blood glucose and BHB levels in the pregnant mice fed with normal and ketogenic diets. Measurement of blood glucose (left) and BHB (right) levels in the pregnant mice fed with normal (top) and ketogenic (bottom) diets on days 0, 3, and 6 after mating. Each line shows individual pregnant mice. normal, n = 15; ketogenic, n = 17. Significance of differences between normal and ketogenic diets were estimated by Student's t-test. ***$P < 0.001$.

D The size of embryos used for immunostaining, calculated by the area of DAPI-stained images of the largest part of embryos. Values are plotted as mean ± SE. ES (normal, n = 9; ketogenic, n = 7), MS (normal, n = 6; ketogenic, n = 6), LS (normal, n = 6; ketogenic, n = 5). N.S.: not significant (Student's t-test).

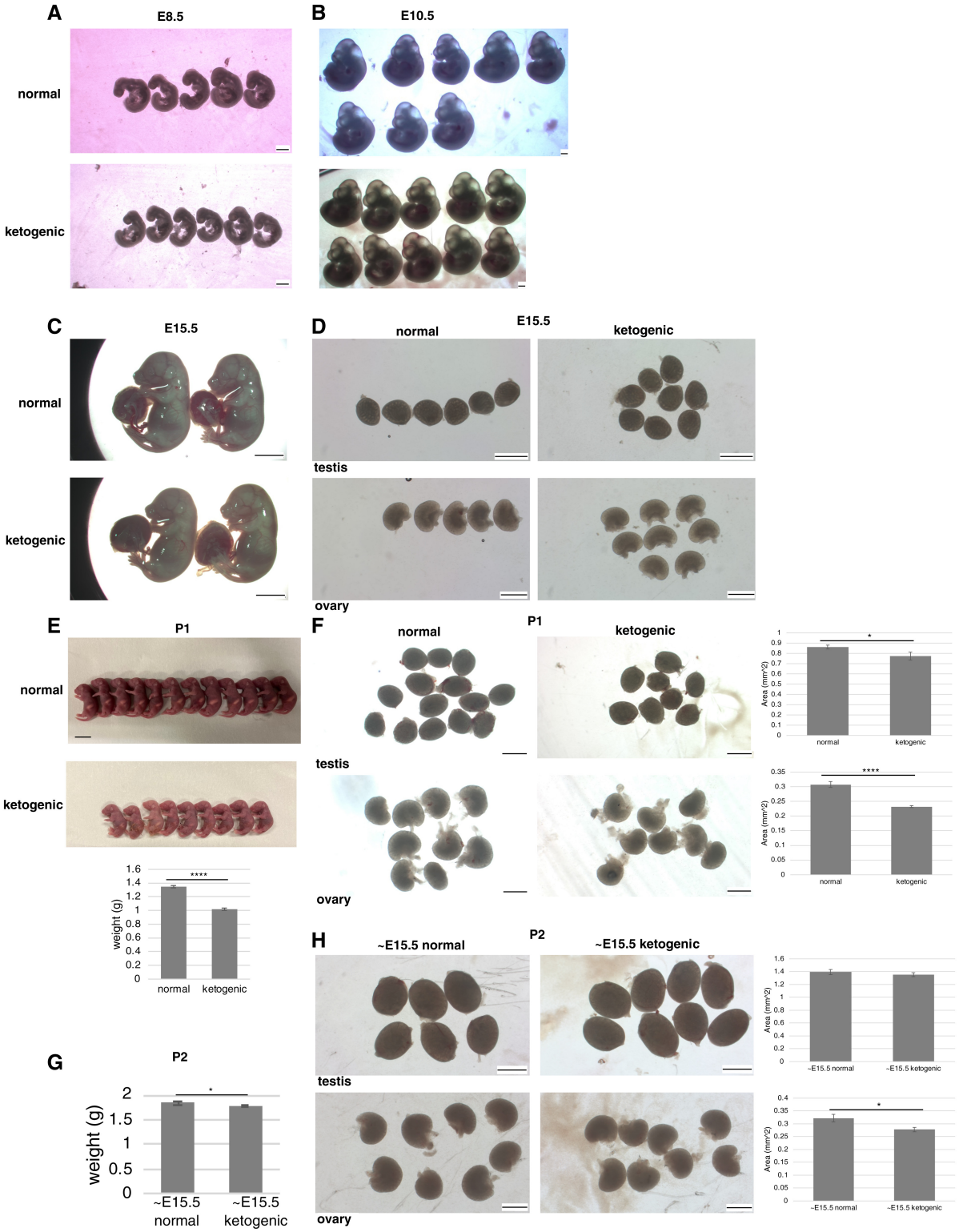

A
E8.5
normal
ketogenic
B
E10.5
C
E15.5
normal
ketogenic
D
E15.5
normal
ketogenic
testis
ovary
E
P1
normal
ketogenic
weight (g)
normal
ketogenic
F
P1
normal
ketogenic
testis
ovary
Area (mm^2)
G
P2
weight (g)
~E15.5 normal
~E15.5 ketogenic
H
P2
~E15.5 normal
~E15.5 ketogenic
testis
ovary
Area (mm^2)

Figure EV5.

Figure EV5. Embryos and newborn pups from mothers fed a normal or ketogenic diet.

A–C E8.5 (A), E10.5 (B), and E15.5 (C) embryos from mothers fed a normal or ketogenic diet.
D Gonads in E15.5 embryos from mothers fed a normal or ketogenic diet.
E P1 pups from mothers fed a normal or ketogenic diet. Weight was measured (normal, $n = 25$; ketogenic, $n = 21$).
F Gonads in P1 pups, testes (normal, $n = 14$; ketogenic, $n = 8$), ovaries (normal, $n = 9$; ketogenic, $n = 8$). Gonad size was measured as the area value of each gonad quantified in Image J.
G Weight of P2 pups from mothers fed a normal or ketogenic diet until E15.5 and normal diet afterwards (normal, $n = 12$; ketogenic, $n = 13$).
H Gonads in P2 pups after switching to a normal diet at E15.5, testes (normal, $n = 6$; ketogenic, $n = 8$), ovaries (normal, $n = 8$; ketogenic, $n = 8$). Gonad size was measured as the area value of each gonad quantified in ImageJ.

Data information: Values are plotted as mean ± SE. *$P < 0.05$, **** $P < 0.0001$ (Student's *t*-test). Scale bar: 500 μm (A, B, ovaries in D, F, and H), 5 mm (C), 1 mm (testes in D, F, and H), 1 cm (E).