Expanded View Figures

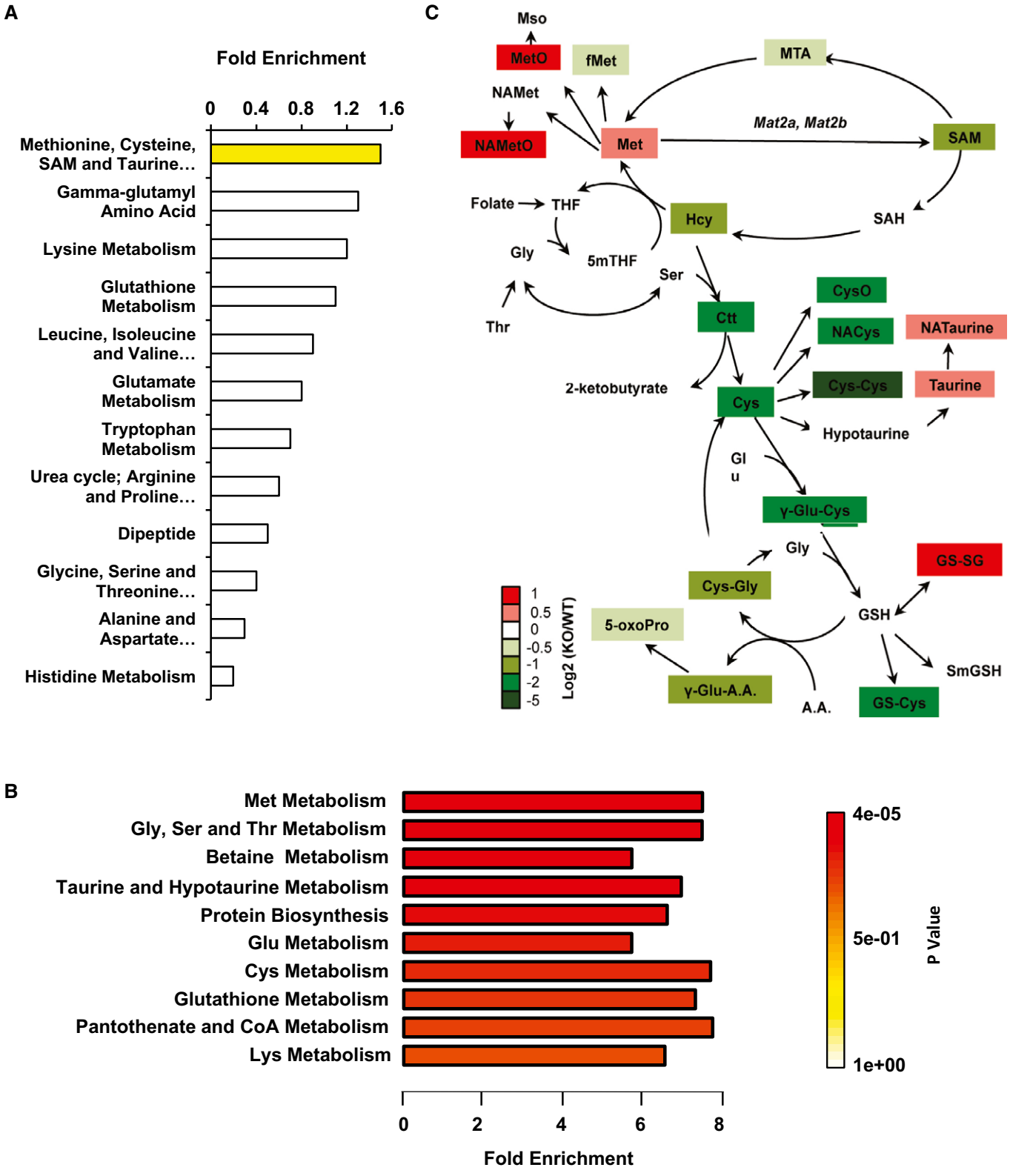

Figure EV1.

Figure EV1. SIRT1 KO mESCs have altered methionine metabolism and SAM production when cultured in the M10 maintenance medium.

A Significant enrichment of the methionine metabolism pathway in SIRT1 KO versus WT mESCs cultured in the M10 medium. WT and SIRT1 KO mESCs cultured in the complete medium were harvested and subjected to global metabolomic analysis by Metabolon. The fold enrichment of amino acid metabolism pathways was analyzed by Cytoscape 2.8.3 (n = 5 independent experiments). Yellow, P = 0.03.

B Significant enrichment of the methionine metabolism pathway in SIRT1 KO versus WT mESCs cultured in the complete medium. WT and SIRT1 KO mESCs were cultured and analyzed by Metabolon as in (A). The fold enrichment of amino acid metabolism pathways was analyzed by the metabolite set enrichment analysis (MSEA) of MetaboAnalyst 3.0 (n = 5 independent experiments).

C Schematic of one-carbon metabolism and glutathione homeostasis in SIRT1 KO mESCs relative to WT mESCs in the complete M10 medium. WT and SIRT1 KO mESCs cultured as in (A). The log ratios of the relative abundance of metabolites in indicated pathways in KO/WT mESCs were presented by color scale (n = 5 independent experiments). All colored metabolites were significantly changed in KO mESCs compared to WT mESCs with $P < 0.05$.

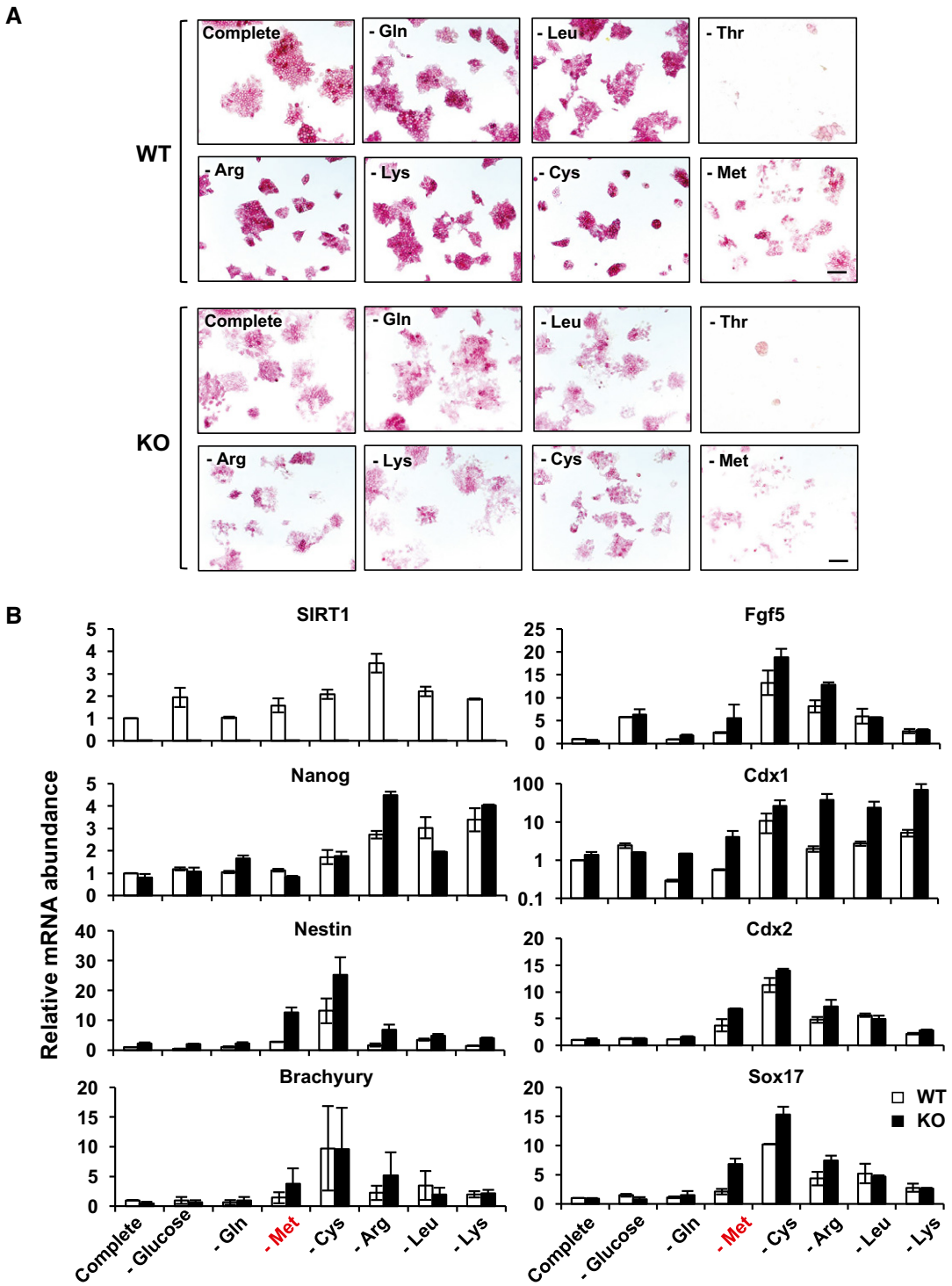

Figure EV2. SIRT1 KO mESCs are sensitive to methionine depletion-induced differentiation.

A SIRT1 KO mESCs display enhanced differentiation phenotypes in a methionine-free M10 medium. WT and KO mESCs were cultured in the complete M10 medium or modified M10 media depleted the indicated nutrients for 48 h. Cells were stained for the AP activities. Scale bars, 100 μm.

B Relative mRNA expression of stem cell marker and differentiation markers in WT and KO mESCs cultured in indicated nutrient-deprived medium. WT and KO mESCs cultured in indicated media for 48 h were harvested, and the relative mRNA levels of different markers were analyzed by qPCR. Please note that methionine depletion induced higher expression levels of all tested differentiation markers in KO than in WT mESCs (n = 3 independent experiments). Data are presented as mean ± SEM.

Figure EV3. Deletion of SIRT1 in mESCs alters global gene expression profiles.

A The mRNA expression profiles of WT and SIRT1 KO mESCs cultured in the complete medium or methionine-restricted medium for 6, 24, or 72 h. The mRNAs were analyzed by the mouse whole-genome microarrays as described in Materials and Methods (n = 3 independent experiments, adjusted $P < 0.05$, cutoff fold changes: 1.5).

B Venn-diagram representation of the genes that were significantly altered by more than 1.5-fold upon methionine restriction for indicated times in WT and SIRT1 KO mESCs.

C WT and SIRT1 KO mESCs have distinct transcriptional responses to methionine restriction. WT and SIRT1 KO mESCs cultured in the complete medium or methionine-restricted medium for 6 h. Unique gene lists from WT (1,638) and SIRT1 iKO (1,348) mESCs were analyzed by the IPA software, and the top five canonical pathways in each gene list were listed (n = 3 independent experiments, adjusted $P < 0.05$, cutoff fold changes: 1.5).

D The numbers of differentially expressed gene probes between WT and SIRT1 KO mESCs cultured in the complete medium or methionine-restricted medium for 24 h (n = 3 independent experiments, adjusted $P < 0.05$, cutoff fold changes: 1.5).

E The mRNA levels of 2,704 overlapped genes were presented by heatmaps. Please note that 1,614 out of these overlapped genes were altered in the same direction by SIRT1 deletion or methionine restriction in mESCs (genes inside the blue boxes).

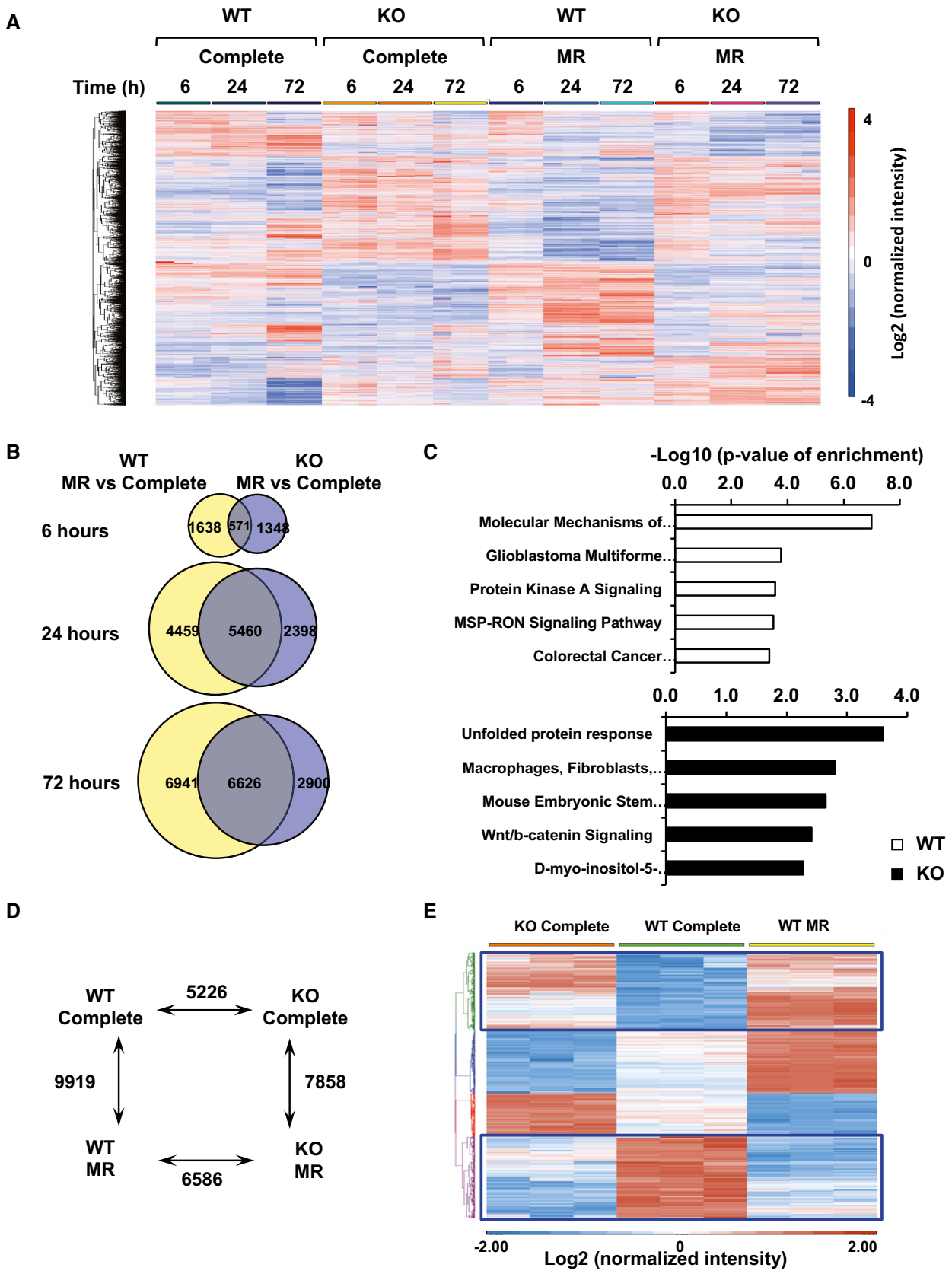

Figure EV3.

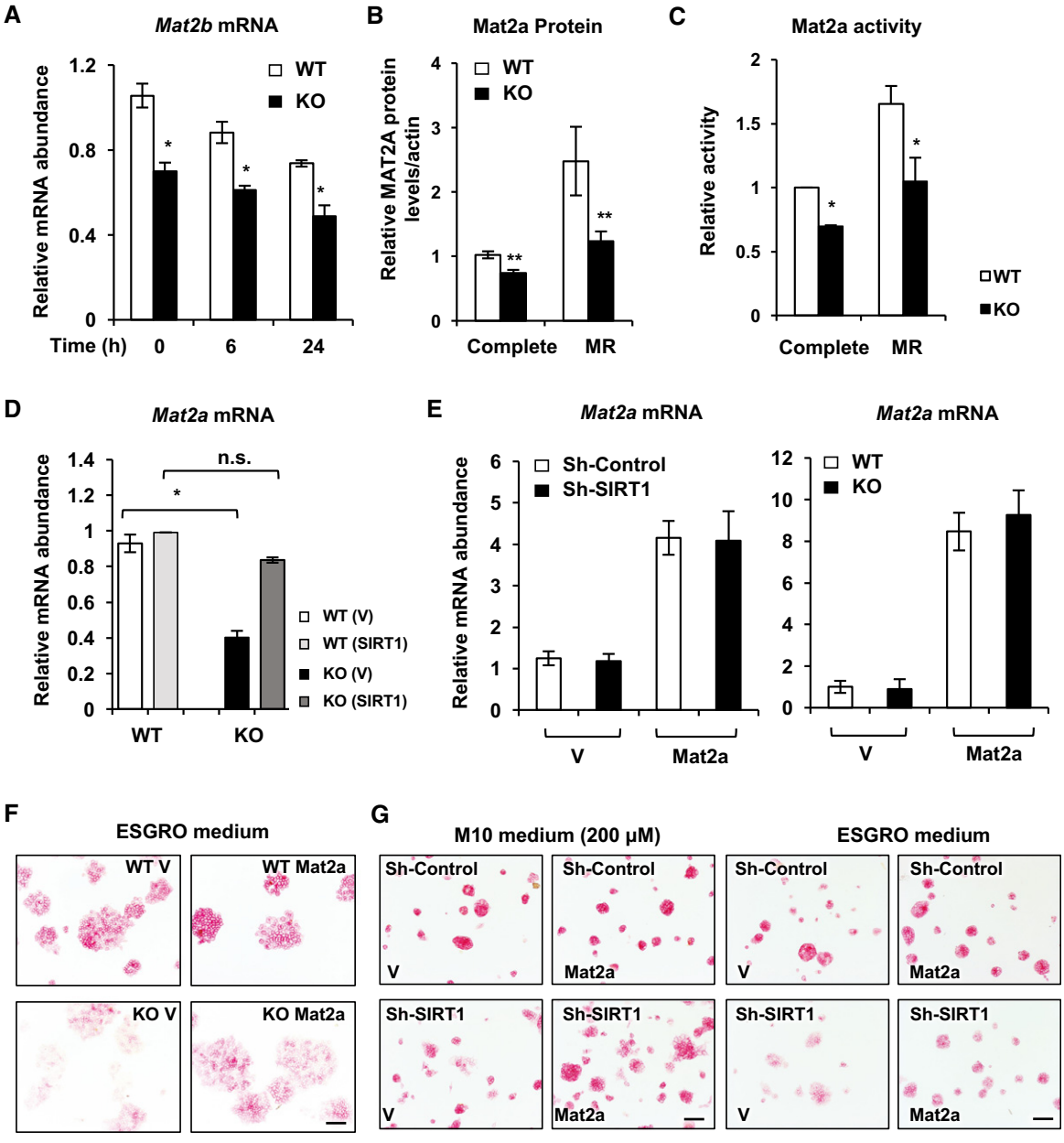

Figure EV4. SIRT1 promotes conversion of methionine to SAM through Mat2a.

A SIRT1 KO mESCs display reduced expression of Mat2b enzymes (n = 3 independent experiments).

B SIRT1 KO mESCs have reduced protein levels of MAT2A in both complete and methionine-restricted medium. WT and SIRT1 KO mESCs were cultured in complete or methionine-restricted medium (MR) for 24 h. The relative intensities of MAT2A were quantified using ImageJ (n = 5 independent experiments).

C SIRT1 KO mESCs have reduced Mat2a activities in both complete and methionine-restricted medium. WT and SIRT1 KO mESCs were cultured in complete or methionine-restricted medium (MR) for 24 h, and the activities were measured as described in Materials and Methods (n = 3 independent experiments).

D SIRT1 re-expression rescues SIRT1 deficiency-induced reduction in Mat2a mRNA. WT and SIRT1 KO mESCs stably infected with lentiviruses containing the empty vector (V) or a construct expressing SIRT1 (SIRT1) were cultured in complete or methionine-restricted medium for 48 h (n = 3 independent experiments).

E Overexpression of *Mat2a* in mESCs. Control and SIRT1-deficient mESCs were infected with lentiviruses containing either empty vector or mouse *Mat2a* gene. Stable lines were selected and analyzed for the mRNA levels of *Mat2a* by qPCR (n = 3 independent experiments).

F Overexpression of exogenous Mat2a rescues SIRT1 deletion-induced mESC differentiation in ESGRO mESC maintenance medium. WT and SIRT1 KO mESCs were stably infected with lentiviruses containing either empty vector or mouse *Mat2a* gene and cultured in the ESGRO maintenance medium. Scale bars, 100 μm.

G Overexpression of exogenous Mat2a rescues SIRT1 deficiency-induced mESC differentiation in complete M10 medium and ESGRO maintenance medium. Sh-Control and sh-SIRT1 mESCs were stably infected with lentiviruses containing either empty vector or mouse *Mat2a* gene and cultured in the complete M10 medium or ESGRO maintenance medium. Scale bars, 100 μm.

Data Information: In (A–E), data are presented as mean ± SEM. *P < 0.05, **P < 0.01 (Mann–Whitney test).

Figure EV5. Maternal methionine restriction increases lethality of SIRT1 KO embryos, whereas maternal methionine supplementation increases the survival of SIRT1 KO newborn mice.

A SIRT1 KO E8.5 embryos have reduced expression levels of *Mat2a* and *Mat2b* (n = 16 control and 8 SIRT1 KO embryos).

B Maternal methionine restriction from E0.5 does not affect litter size. Female SIRT1 heterozygous breeders were fed with either chow diet (n = 7), methionine-restricted diet (MR) from E0.5 (n = 7), or pre-fed with MR diet for 1 week (n = 10). Litter size was analyzed from E8.5–E14.5 embryos.

C WT and SIRT1 KO E9.5 embryos under chow. Scale bars, 200 μm. Black arrows indicate the location of embryo.

D SIRT1 KO E8.5–E14.5 embryos have reduced survival under maternal methionine restriction from E0.5. Arrow heads, SIRT1 KO embryos. The viability of E8.5–E14.5 embryos was analyzed as described in Materials and Methods.

E SIRT1 KO newborn pups have increased survival after maternal methionine supplementation. Arrow heads, SIRT1 KO newborns. The survival of PND1.5–10.5 newborn pups was analyzed as described in Materials and Methods. Scale bar, 1 cm.

Data Information: In (A and B), data are presented as mean ± SEM. $^{**}P < 0.01$, $\#P = 0.1$ (Mann–Whitney test).

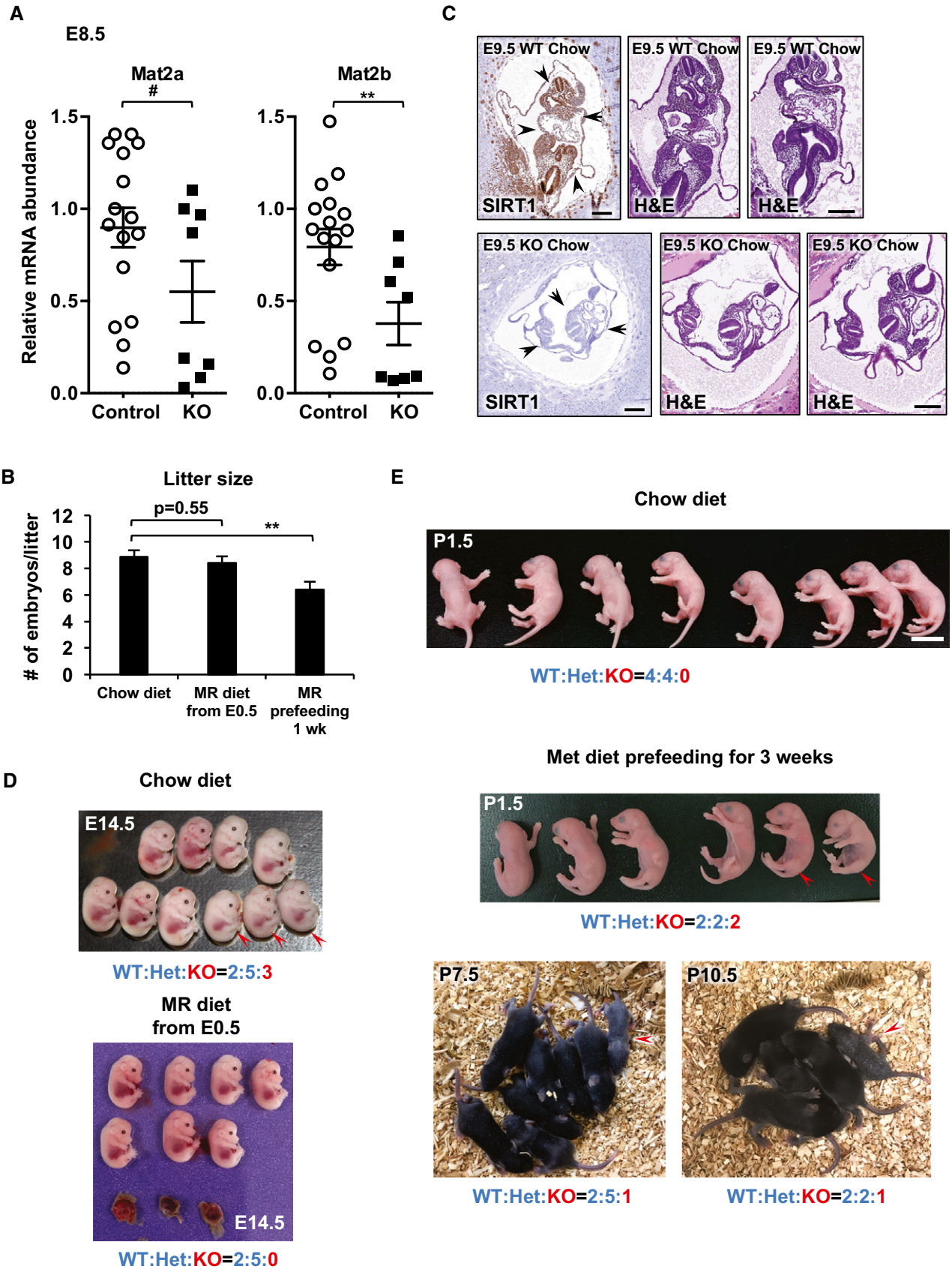

Figure EV5.