Data S1

Materials and methods

Transmission electron microscopy (TEM). Chinese oral mucosal melanoma (COMM) with adhesive morphology (COMM-AD) and grown in suspension (COMM-SUS) cell pellets were fixed overnight at 4˚C with 2.5% glutaraldehyde/4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), then post-fixed for 90 min with 1% osmium tetroxide dissolved in distilled water, dehydrated in a graded series of ethanol solutions (30, 50, 70, 80 and 95%) for 10 min each and anhydrous acetone for 20 min, and embedded in Epon. Ultra-thin sections were cut with an RMC MT-7000 ultramicrotome and stained with 4% uranyl acetate and lead citrate for examination under a 120 kV transmission electron microscope (JEM1400, JEOL Ltd.).

Survival analysis. RNA-sequencing expression (level 3) profiles and corresponding clinical information for melanoma were downloaded from the TCGA dataset (https://portal.gdc.com). Log-rank test was used to compare differences in survival between these groups. The timeROC (v 0.4) analysis was used to compare the predictive accuracy of SLC16A1 mRNA. For Kaplan-Meier curves, P-values and hazard ratio (HR) with 95% confidence intervals (CI) were generated using log-rank tests and univariate cox proportional hazards regression. All the analysis methods and R packages were implemented by R (foundation for statistical computing 2020) version 4.0.3. $P<0.05$ was considered to indicate a statistically significant difference.

Figure S1. (A) STR analyses of the cells and corresponding tissues in COMM-1 and COMM-2. (B) Image analyses by transmission electron microscope in COMM-1 and COMM-2 cells. (C) GFP was efficiently overexpressed in COMM-1 cells. Green light represents GFP expression. (D) Immunodeficient mice model of lung metastases were harvested after tail intravenous injection of COMM-AD and COMM-SUS cells with eGFP respectively at 4 weeks. Immunohistochemical positive staining of GFP indicated the COMM cells in seriallung sections. Scale bar, 100.0 μm. COMM, Chinese oral mucosal melanoma; COMM-AD, cells with adhesive morphology; COMM-SUS, cells grown in suspension.

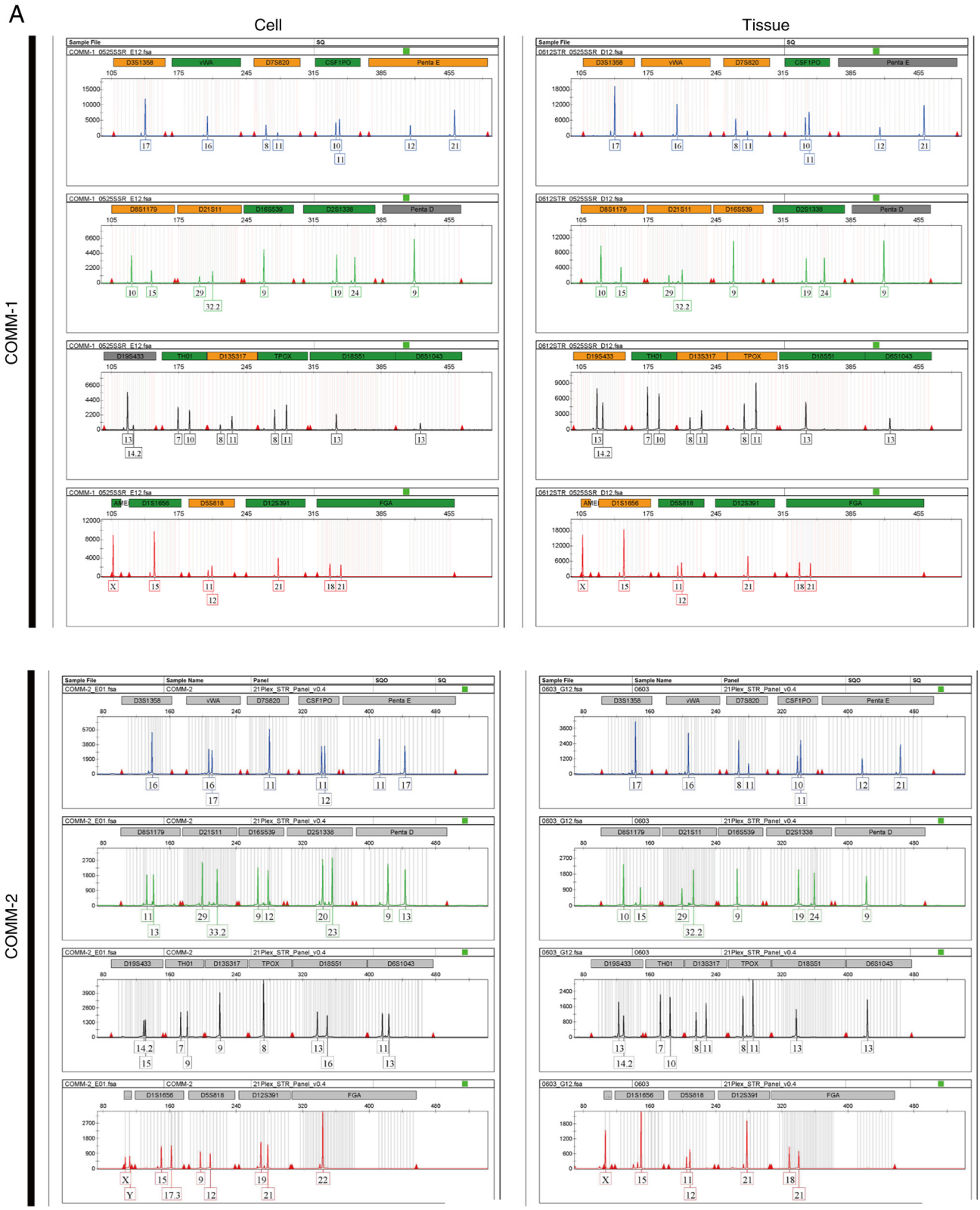

Figure S1. Continued.

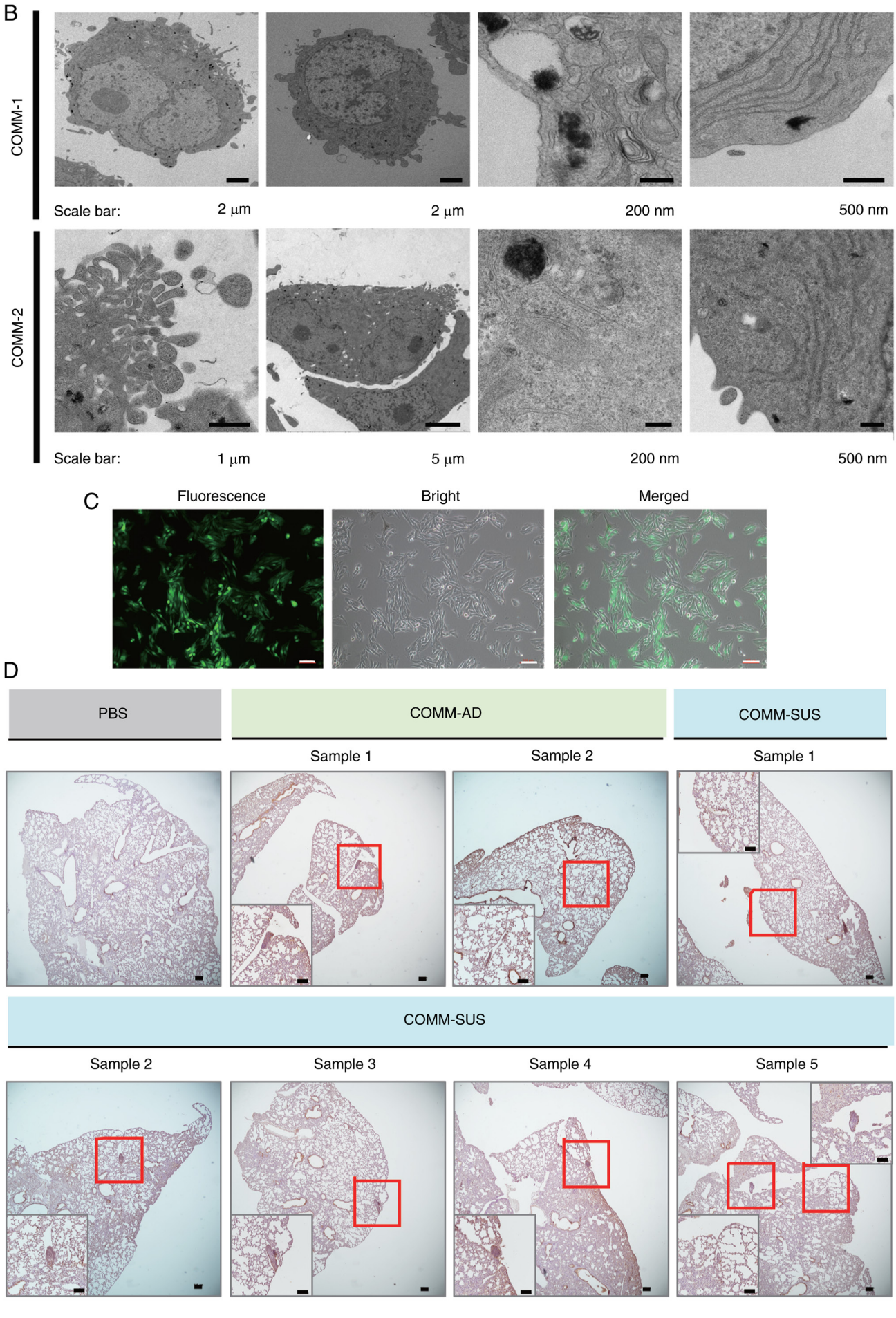

Figure S2. (A) Viability of COMM-2 AD and COMM-2 SUS cells measured following culturing in HHS for 24 h. (B) COMM-2 AD cells were treated with different inhibitors of death for 24 h. (C) Cell viability of COMM-1 SUS cells was measured followingtreatmentwith erastin or DMSO (as the control) for 24 h. (D) RT-qPCR analysis of the expression of GPX4, FSP1, GSR and IRS1 in COMM-2 AD and COMM-2 SUS cells. (E) The amount of intracellular NADH and NADPH in COMM-2 AD and COMM-2 SUS cells. (F) The total intracellular lactate levels of COMM-2 AD and COMM-2 SUS cells were measured by Lactate Assay Kit-WST. (G) RT-qPCR analyses of MCT1, MCT4 and LDHB expression in COMM-2 cells. (H) The number of viable COMM-2 SUS cells was determined in 72 h following treatment with serial concentrations of sodium lactate. (I) Quantification of immunofluorescence staining for template DNA co-segregate asymmetrically after COMM-2 cells were treated with different concentrations of sodium lactate. Significance was determined using a Student' s t-test or one-way ANOVA or two-way ANOVA (*P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001; ns, not significant, P>0.05). COMM, Chinese oral mucosal melanoma; COMM-AD, cells with adhesive morphology; COMM-SUS, cells grown in suspension; HHS, healthy human serum.

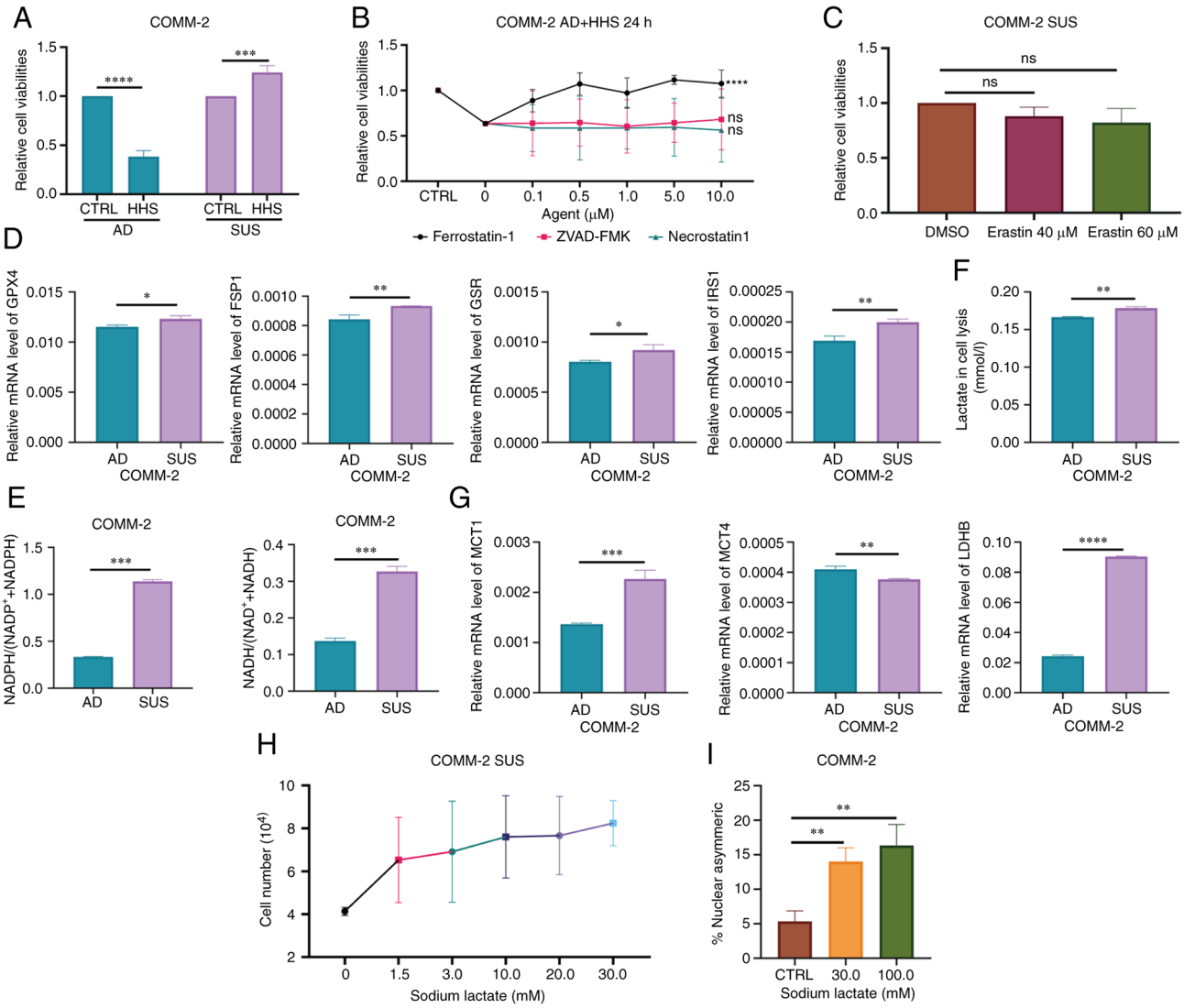

Figure S3. (A) The expression of the main enzymes was measured in COMM-2 cells analyzed using RT-qPCR. (B) Detection of knockdown efficiency of siRNA in COMM cells using RT-qPCR. (C) The amount of intracellular NADH and NADPH were evaluated after suppressing the enzymes expression in COMM-AD cells. Significance was determined using a Student's t-test or one-way ANOVA (*P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001; ns, not significant, P>0.05). COMM, Chinese oral mucosal melanoma; COMM-AD, cells with adhesive morphology; COMM-SUS, cells grown in suspension.

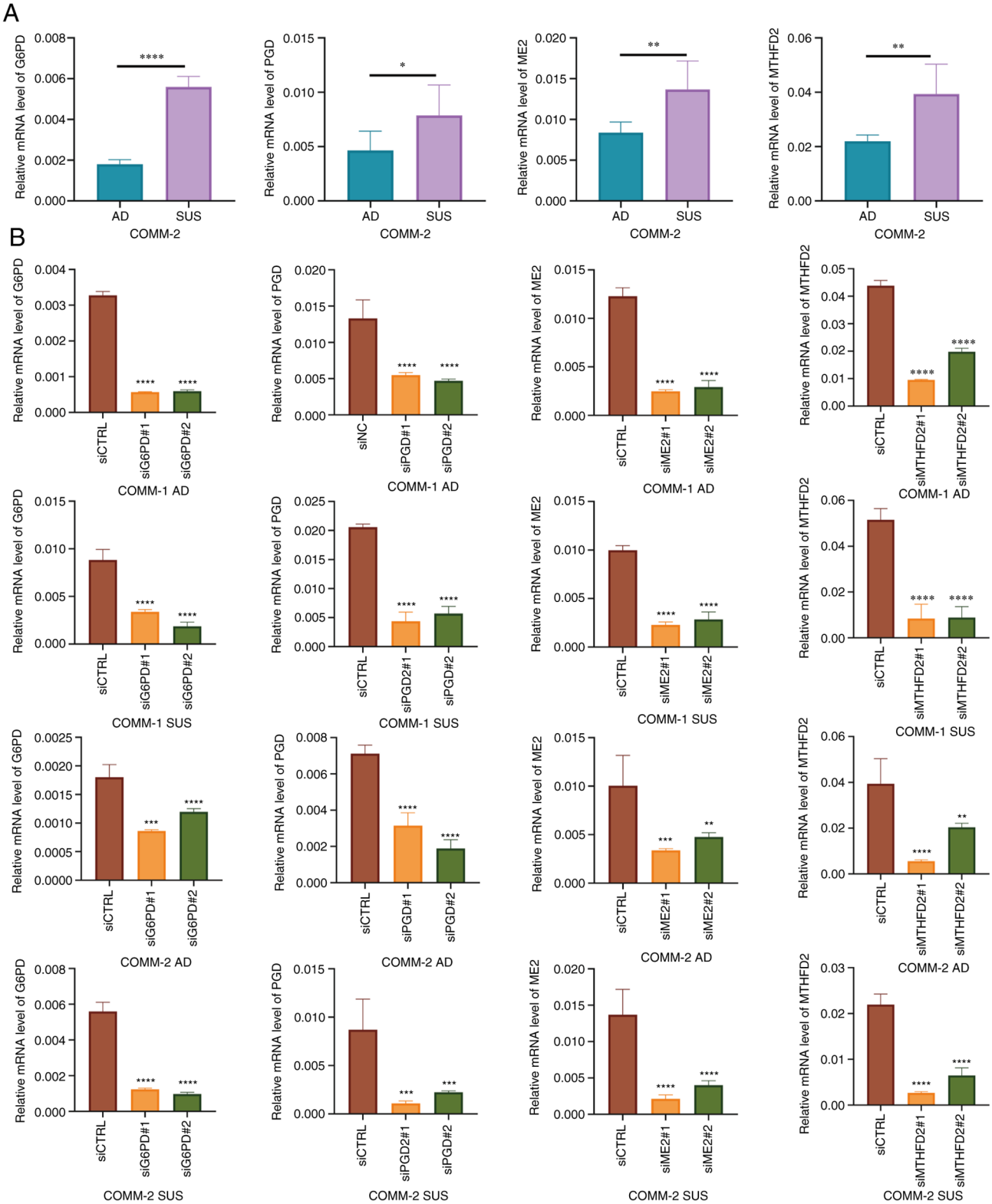

Figure S3. Continued.

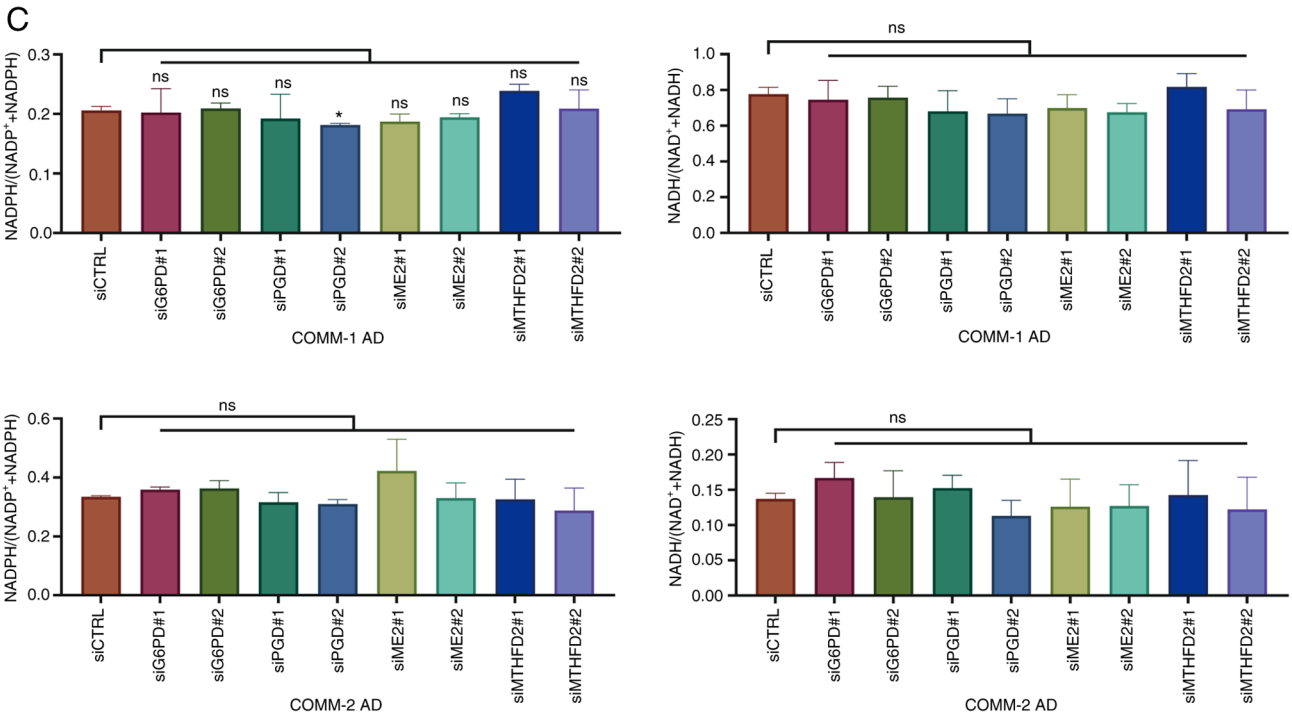

Figure S4. (A) COMM-2 AD cells were treated with AZD3965 and then cultured in HHS for 12, 24 and 48 h. Cell viabilities were analyzed. (B) Immunohistochemical positive staining of GFP indicated COMM-1 cells in serial lungsections. Scale bar, 100.0 μm. (C) Kaplan-Meier survival analysis of the gene signature analysis showed that high MCT1 expression was positively correlated with metastasis and poor overall survival. Significance was determined using one-way ANOVA (*P<0.05, ***P<0.001 and ****P<0.0001; ns, not significant, P>0.05). COMM, Chinese oral mucosal melanoma; COMM-AD, cells with adhesive morphology; COMM-SUS, cells grown in suspension; HHS, healthy human serum; MCT, monocarboxylate transporter.