SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Sanger sequencing validation of mutations detected in TSC1, SETD2 and TP53 genes. Sanger sequencing chromatograms showing mutations (black arrows) detected in TSC1 (a), SETD2 (b) and TP53 (c) genes present in the indicated healthy tissue, tumor tissue and cell lines. Reference wild type (WT) and mutated (MUT) sequences for each gene are shown.

Figure S2. Sanger sequencing of IDH1 and IDH2 genes in CDS17 and CDS20 models. Sanger sequencing of mutation hotspots in the IDH2 gene in tumor tissue, primary cell line, and cell lines derived from xenografts of the CDS17 model. Chromatograms show non-synonymous mutations (black arrows) at residue R172 (a) and wild type sequence at residue R140 (b). (c-e) Sequencing of tumor tissue and primary cell line of the CDS23 model showing no motutations in IDH1-R132L (c), IDH2-R172 (d) and IDH2-R140 (e) hotspots.

Figure S3. long-term effect of enasidenib on IDH2 mutant chondrosarcoma cell lines. (a-b) Cell viability (WST-1 assays) measured after the treatment of the indicated IDH mutant cell lines with increasing concentrations of enasidenib (a-b), ivosidenib (c) and vorasidenib (d) for 144 h. Cell medium containing drugs were refreshed after 72 h. IDH2 mutation status of chondrosarcoma cell lines and $IC5_{50}$ values for enasidenib treatments are shown (b).

Figure S4. Dose-dependent effect of IDH1mut inhibitors on the IDH1 mutant chondrosarcoma cell line CDS11. Cell viability (WST-1 assays) measured after the treatment of CDS11 cells with increasing concentrations of AGI-5198 (a) or DS-1001b (b) for 72 h.

Figure S5. Analysis of apoptosis and DNA damage in enasidenib treated chondrosarcoma cells. (a-b) Annexin V / propidium iodide binding assay of T-CDS17#4 cells treated with 60.8 µM enasidenib for 24, 48 and 72 hours. Representative dot plots (a) and summary (mean and SD) of three independent experiments (b) are shown. Asterisks indicate statistically significant differences in the values of accumulated Annexin V+ cells with the control series (**: $p < 0.01$; ***:$p < 0.001$, one-way ANOVA). (c-d) Analysis of γ-H2AX foci formation after treatment of T-CDS-17#4 cells with IC_{50} (17.5 µM) and IC_{90} (60.8 µM) concentrations of enasidenib for 0 h (control), 24 h and 48 h. (c) Representative images of immunostaining experiments (γ-H2AX immunodetection: red fluorescence; DAPI staining: blue fluorescence) for each condition. Scale bars = 25 µm. (d) Quantification of γ-H2AX foci. Means (horizontal bars) and SD of the number of foci of at least 100 cells for each condition are shown.

Figure S6. Intracellular D2HG levels after the targeting of IDH mutations in chondrosarcoma cell lines. (a-b) D2HG levels were determined in SW1353 (a) and L2975 cells (b) treated or not with the indicated concentration of enasidenib ror 48 h. (c-d) D2HG levels after the treatment of CDS11 (c) and T-CDS17#1 (d) cells with the indicated concentration of CB-839 and Metformin for 48 h. Error bars represent the standard deviation of two independent experiments in triplicates. Asterisks indicate statistically significant differences (*:$p<0.05$ **:$p<0.05$; two tailed t-test in a-b and one-way ANOVA in c-d).

Figure S7. Effect of a 120 hours-treatment with enasidenib on the methylome of IDH2-mutant chondrosarcoma cells. T-CDS-17#1 cells were treated in biological triplicates with DMSO (control; CON) or 20 µM enasidenib (ENA) for 120 h prior to be processed for DNA methylation analysis using the Infinium MethylationEPIC v2.0 kit. For a better comparison with data show in figure 4a-b, only

common probes between EPIC v1.0 and EPICv2.0 arrays were selected for the analysis. A CON replicate was discarded from the analysis for failing quality controls. Heat map showing the beta methylation values at the top 5,000 most variable CpG sites in CON and ENA samples. No differentially methylated positions (FDR<0.05, |Δβ|>20%) between ENA and CON samples were found.

Figure S8. (a) Western blotting analysis of the methylation status of H3K4, H3K9, and H3K27 in control (DMSO) and enasidenib-treated TCDS17#1 cells in triplicates. βactin levels were used as loading controls. (b) Quantification of trimethylated histone levels normalized to those of βactin. Western blotting analyses were performed as previously described (DOI: 10.3390/jcm8081157). Primary antibodies used in these analyses were: anti-H3K9me3 [Millipore (17-625), 1:1000 dilution] (AB_916348), H3K4me3 [abcam (ab8580), 1:1000] (AB_306649), H3K27me3 [abcam (ab6002), 1:1000] (AB_305237) and anti-β-Actin [Sigma Aldrich (A5441), 1:10,000] (AB_476744). The IRDye Infrared Fluorescent secondary antibodies anti-Rabbit and anti-Mouse IRDye 800CW and IRDye 680RD (LI-COR Biosciences) were used for detection. Membranes were scanned with the Odyssey Fc Dual-Mode Imaging System (LI-COR Biosciences) using the red (700 nm) and green (800 nm) channels, and signal analysis was performed using Image Studio Lite software (LI-COR, Nebraska).

Figure S9. Correlation between experimental variables measured in mice treated with vehicle solvent (control) enasidenib in figure 7. (A & C) Values of tumor weight, % of PAS-Alcian positive area and % of Ki67 positive cells in each mice from control (A) and enasidenib-treated (C) series. (B & D) correlation between variables in the control (B) and enasidenib-treated (D) series. The r values indicate Pearson's correlation coefficient with corresponding p-value (P) and R square (R2) for correlation.