



Supplementary Figure S3. Mutant IDH1 in the absence of wild type IDH1 is not sufficient to efficiently produce D-2HG in HCT116 cells. Gene-targeting technology was used to introduce the *IDH1*^{R132H} allele to HCT116 to generate *IDH1*^{R132H/WT} clones. In one of the knockin clones, wild type IDH1 and mutant IDH1 were expressed at approximately equal levels based on cDNA sequencing, and these clones were associated with 100-fold increased cellular D-2HG levels. Another clone, which was derivative from HCT116-ATCC and had a confirmed *IDH1*^{R132H} knockin, was noted during our initial characterization to only express the *IDH1*^{R132H} allele, without expression of the wild type IDH1 allele. This allelic variation occurred for unexplained reasons. *A*, Representative sequencing chromatograms for IDH1 codon 132 in gDNA and cDNA of parental HCT116 and *IDH1*^{R132H} knockin cell lines. *B*, Western blot verification of *IDH1*^{R132H} expression in *IDH1*^{R132H} knockin cell lines. *C*, D-2HG levels in lysates of HCT116 parental and *IDH1*^{R132H} knockin cell lines.