Supplementary Data for:

D-2-Hydroxyglutarate is Necessary and Sufficient for Isocitrate Dehydrogenase 1 Mutant-induced *MIR148A* Promoter Methylation

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Supplementary Figure S1: Treatment with octyl-D-2-HG-induces *MIR148A* methylation. Bis-Seq chromatogram images show octyl-D-2-HG-induced *MIR148*A methylation at passage 32**.** Top panel: untreated 293T cells. Middle panel: octyl-D-2-HG treated 293T cells. Bottom panel: methylation positive control of Sss I (methyltransferase)-treated DNA. Black arrows indicate methylated CpG sites (n=1).

Supplementary Figure S2: Octyl-D-2-HG-induced *MIR148A* methylation status remains constant at late passages. Bis-Seq chromatogram images show octyl-D-2-HG-induced *MIR148a* methylation at passages 35, 40 and 45. Octyl-D-2-HG treatment beyond passage 35 does not result in a further increase in *MIR148A* methylation levels. Black arrowheads indicate methylated CpG sites (n=1).

Supplementary Figure S3: Octyl-D-2-HG induces CpG island methylation of the *RBP1* promoter in 293T cells. Chromatogram images of *RBP1* promoter methylation status determined by Bis-Seq in control and octyl-D-2-HG treated 293T cells (passage 40 of treatment). Black arrowheads indicate methylated CpG sites (n=1).

Supplementary Figure S4: Octyl-D-2-HG induces CpG island methylation of the *MIR148A* promoter in normal human astrocytes (NHA). A: Octyl-D-2-HG elevates D-2-HG levels in NHA cells (passage 2-4). Figure shows an increase in intracellular levels after 2 days of treatment (0.5 mM) (n=2, bars show SEM). **B:** Chromatogram images of *MIR148A* promoter methylation status determined by Bis-Seq in control (passage 4) and octyl-D-2-HG treated NHA cells (passage 0, 2 and 4). Black arrowheads indicate methylated CpG sites at passage 2 (with very weak C peaks at CpG sites 5, 7 and 8) and passage 4 (with stronger C peaks from CpG site 5 through 12). Site 6 (open arrowhead) remains unmethylated. **C:** Chromatogram images of *MIR148A* methylation induced by octyl-α-KG and octyl-L-2-HG in NHA cells. Statistical analysis for bar graph performed using two-tailed student t-test. *** indicates $p<0.001$, ** indicates $p<0.01$ and * indicates $p<0.05$ compared with control. Black arrowheads in chromatogram images indicate methylated CpG sites. Site 6 (open arrowhead) remains unmethylated (n=1).

Supplementary Figure S5: *MIR148A* promoter CpG methylation in IDH1^{MUT} -transfected 293T cells (passage 27). Chromatogram in top panel shows the Bis-Seq result. Bottom panel shows the T-A cloning result in a lollipop diagram. Black arrowheads indicate methylated CpG sites (n=1).

Supplementary Figure S6: C227 prevents *IDH1* mutant-induced *MIR148A* promoter methylation. Chromatogram images of miR148a Bis-Seq in empty vector (top), and IDH1^{MUT}-293T cells without (middle) and with (bottom) C227 treatment at passage 40. C227 prevents the development of *MIR148A* promoter methylation in IDH1^{MUT}-293T cells. Black arrowheads indicate methylated CpG sites (n=1).

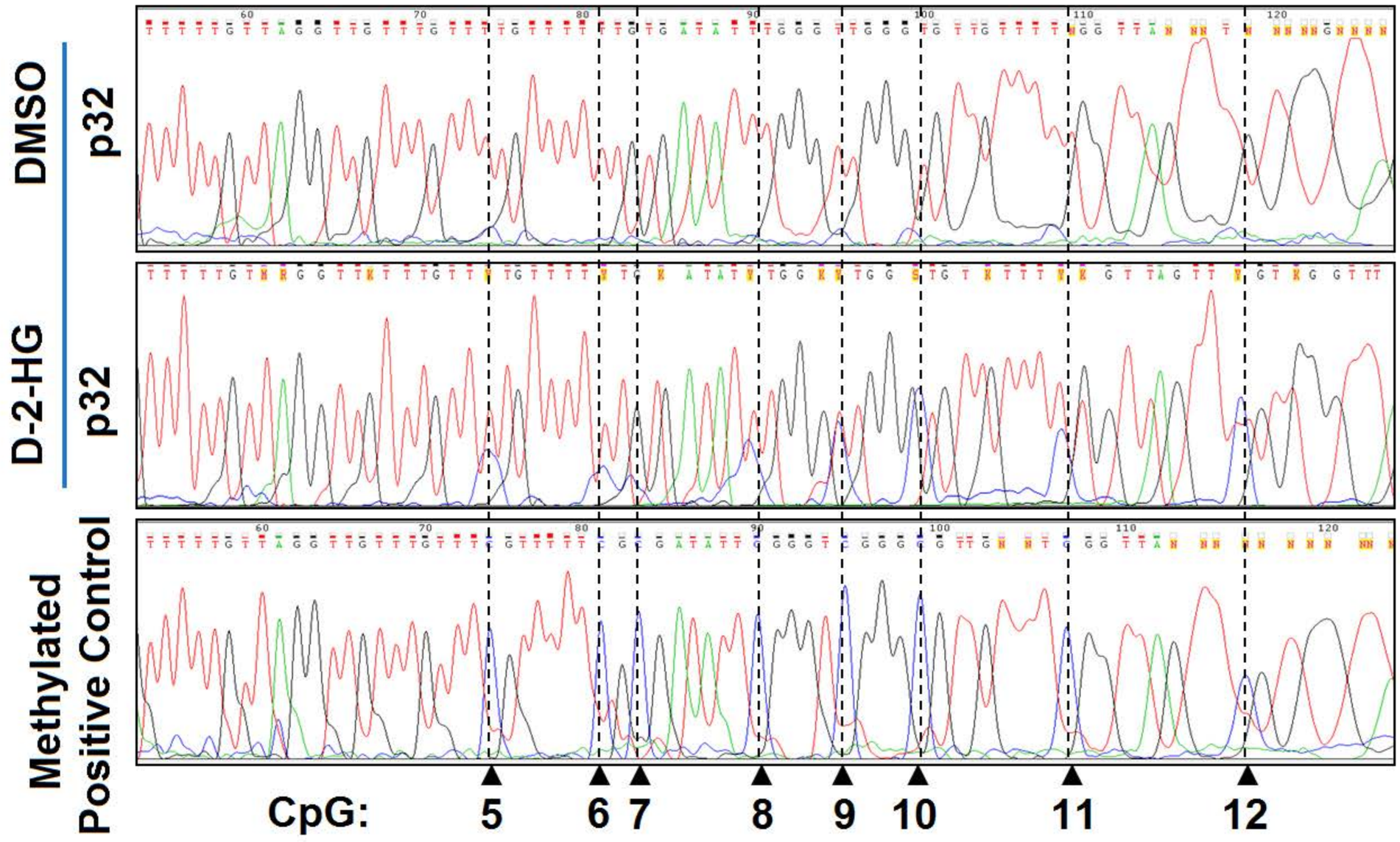
Supplementary Figure S7: C227 prevents *IDH1* mutant-induced *RBP1* promoter methylation. Chromatogram images of *RBP1* Bis-Seq in IDH1^{MUT}-293T cells without (top) and with (bottom) C227 treatment (41 passages). C227 prevents the development of *RBP1* promoter methylation in IDH1^{MUT}-293T cells. Black arrowheads indicate methylated CpG sites (n=1).

Supplementary Figure S8: Schematic graph of *MIR148A* genomic location, CpG island, and promoter-reporter construct. Black arrows indicate Bis-Seq regions, and white arrows indicate the region cloned into the pGL vector. Location within the UCSC Genome Browser is shown. Expanded from **Figure 3B.**

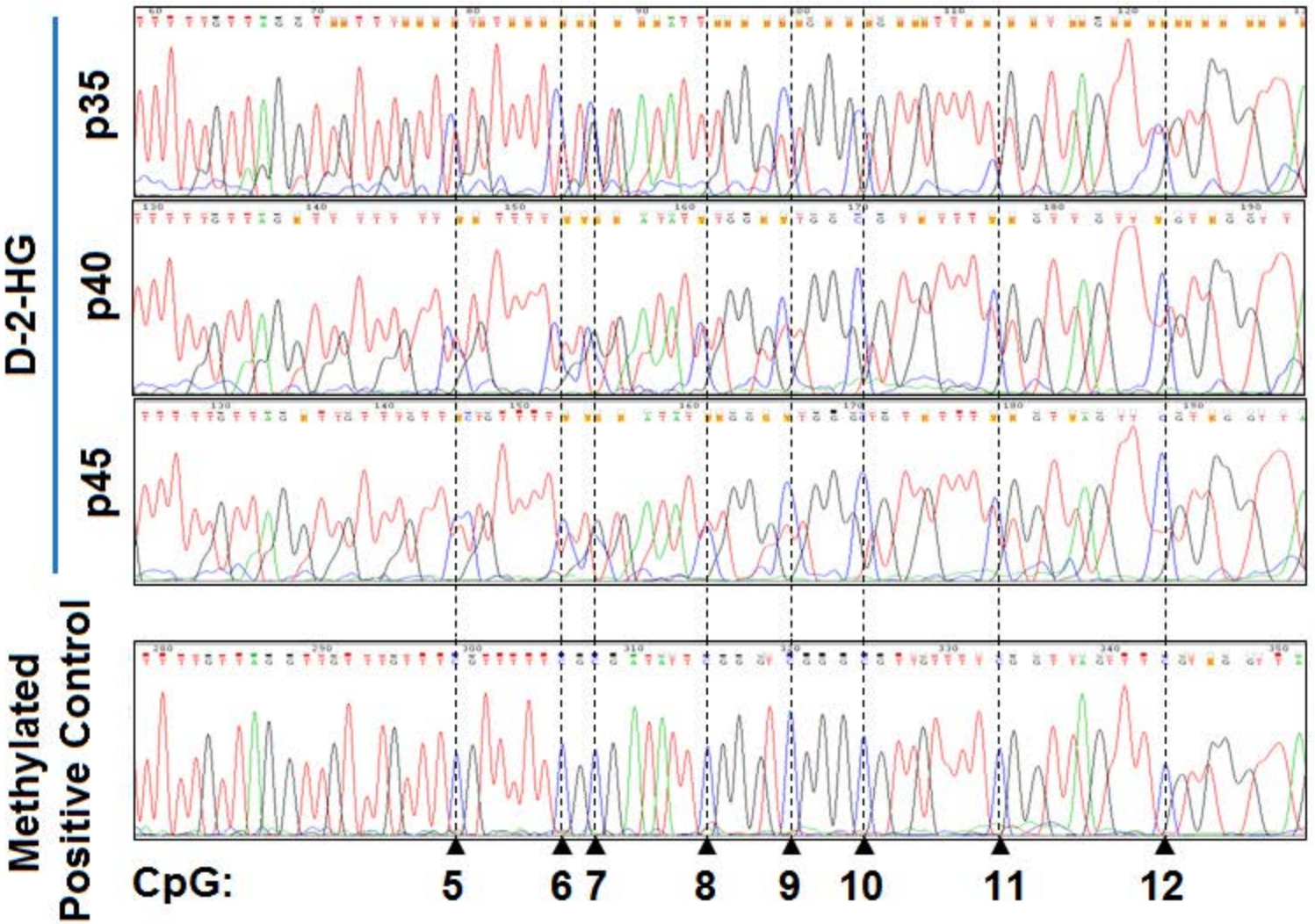
Supplementary Figure S9: MIR148a expression is unchanged by octyl-D-2-HG treatment in early passages in 293T and NHA cells. A: MIR148a expression in early passages (p10-p15) of D-2-HG-treated 293T cells. Analysis by RT-qPCR showed no significant difference in 293T cells treated with D-2-HG versus vehicle (average of 10 and 15 passages of treatment). **B:** MIR148a expression in early passages (p2) of NHA cells treated with octyl-α-KG, -D-2-HG and -L-2-HG. Analysis by RT-qPCR showed no significant difference in NHA cells treated with octyl-α-KG, -D-2-HG and -L-2-HG versus vehicle for 2 passages. Bars show SEM (n=2). Statistical analysis in graphs performed using two-tailed student t-test. ns indicates no significance.

Supplementary Figure S10: Octyl-D-2-HG withdrawal restores *MIR148A* promoter to an unmethylated state. Bis-Seq chromatogram images show *MIR148A* with continued octyl-D-2-HG treatment versus withdrawal. Top panel shows octyl-D-2-HG-treated 293T cells (passage 48). Middle panel shows demethylation following octyl-D-2-HG withdrawal (passage 48; 8 passages post-withdrawal). Bottom panel shows methylation positive control of Sss I (methyltransferase)-treated DNA. Black arrowheads indicate methylated CpG sites (n=1).

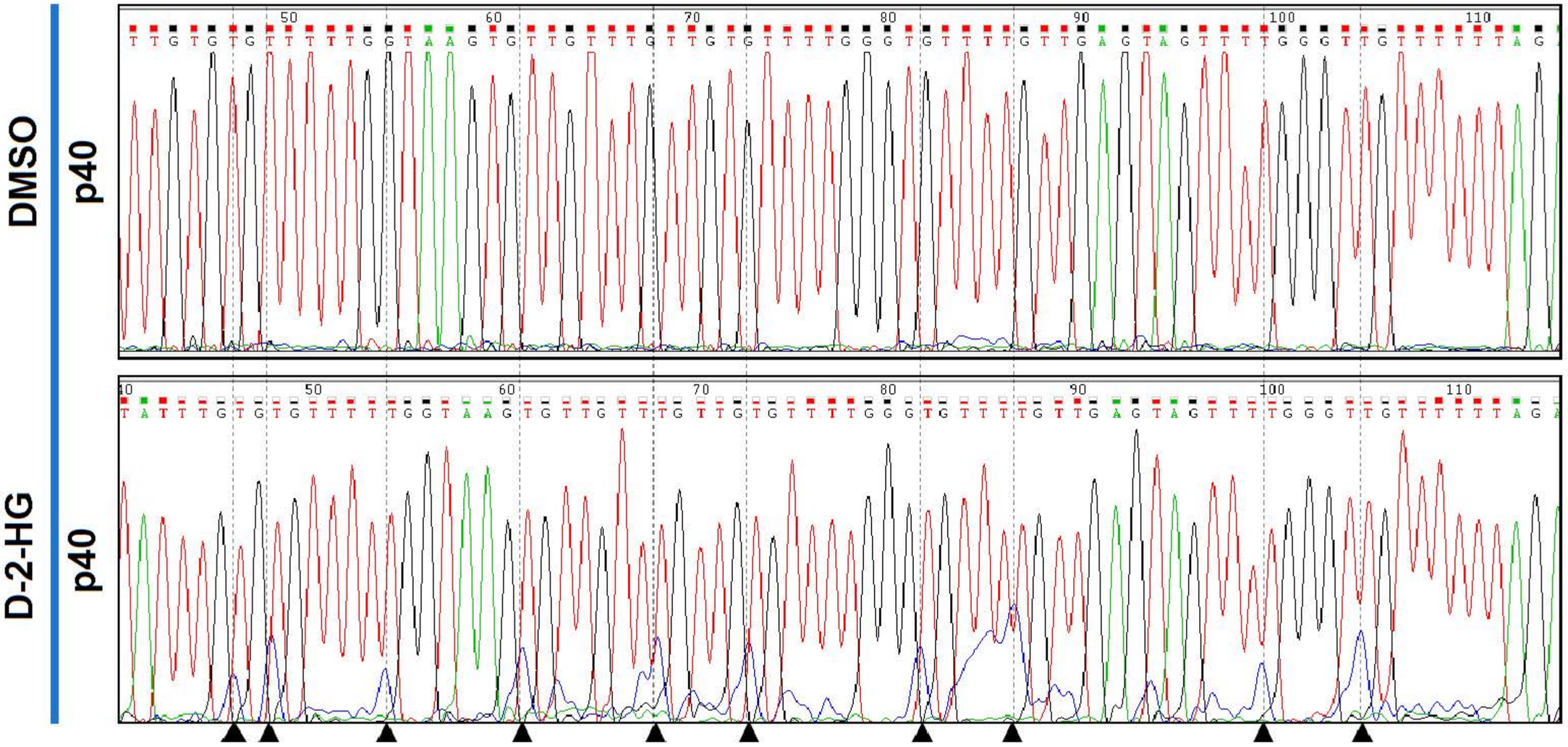
Supplementary Figure S1



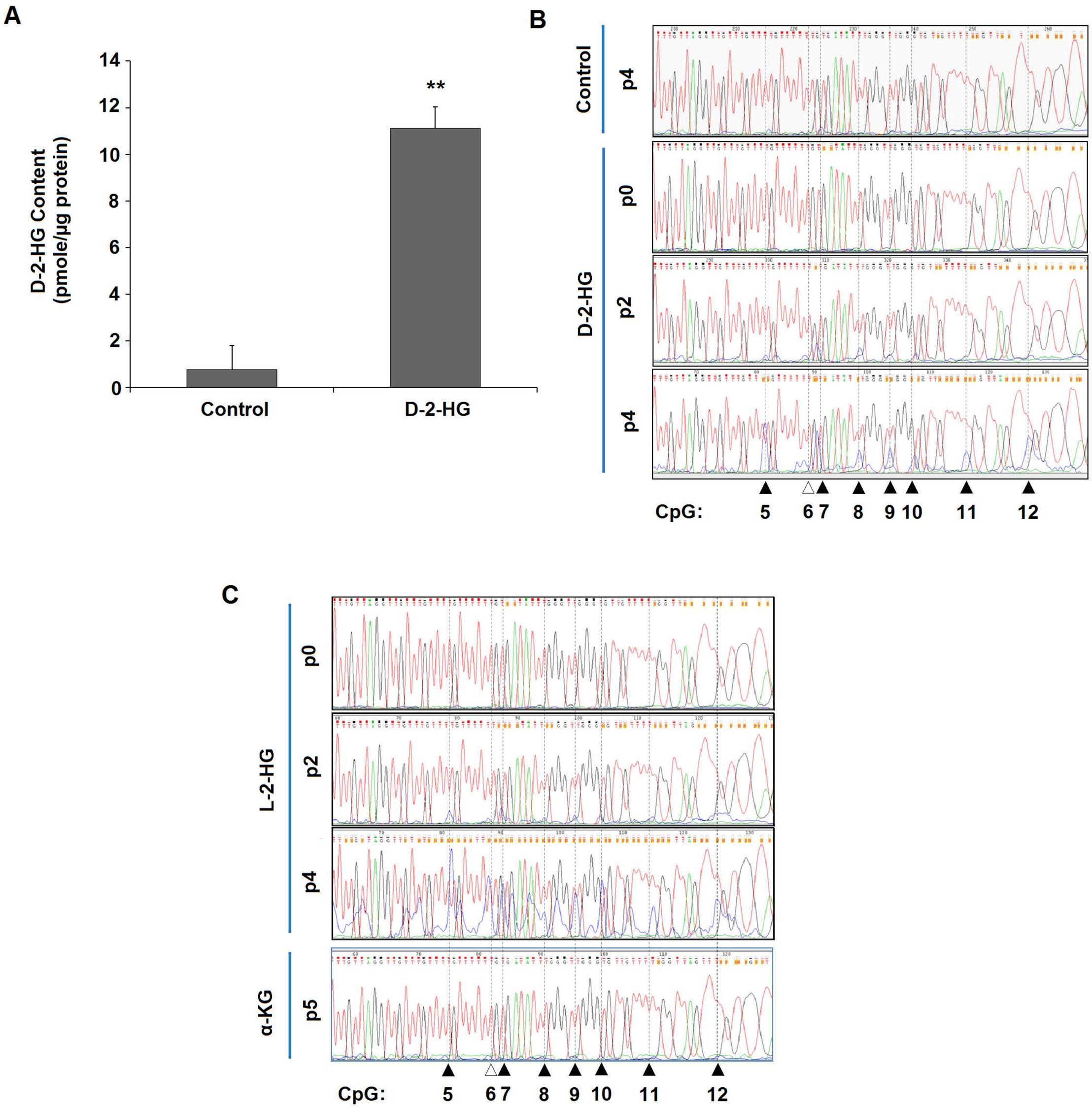
Supplementary Figure S2



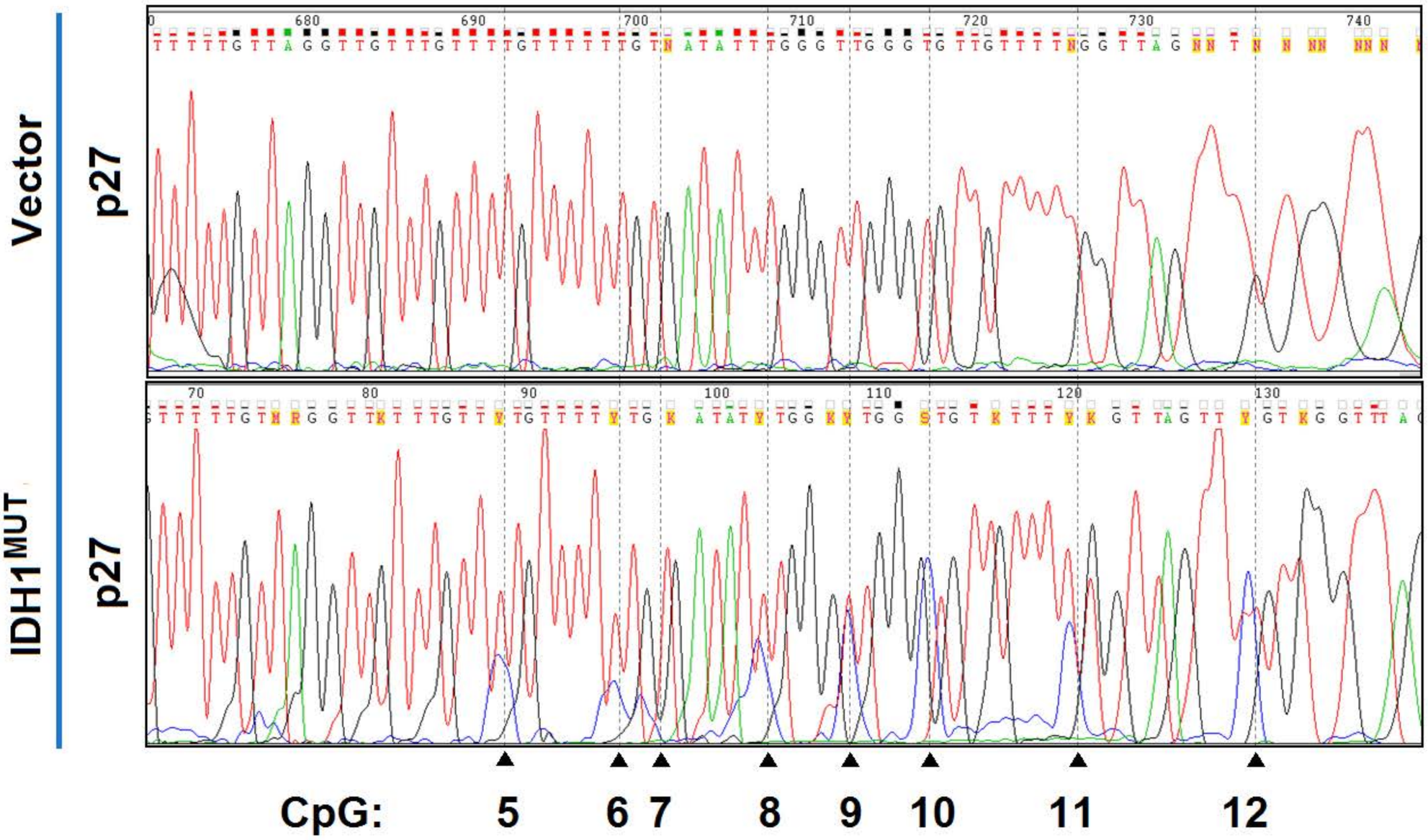
Supplementary Figure S3

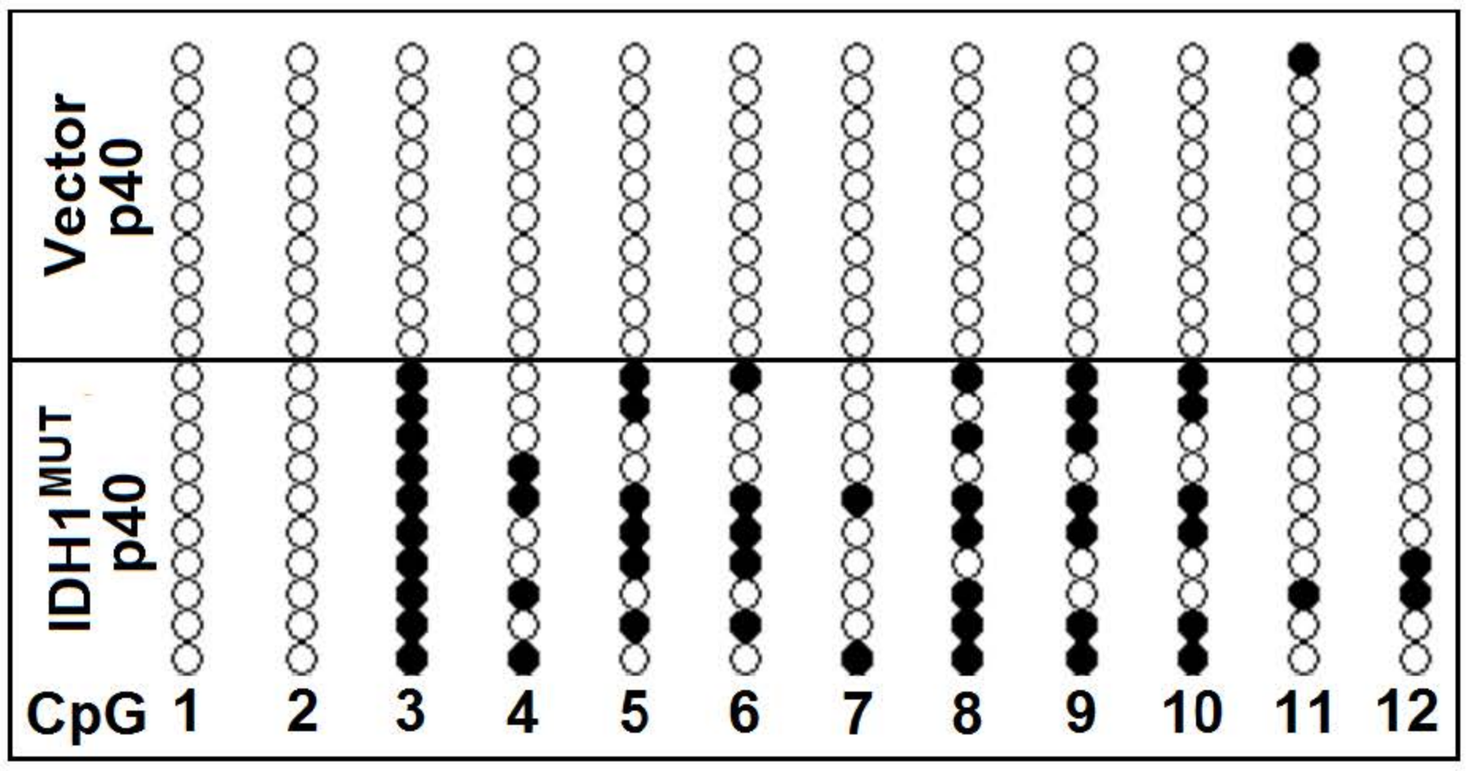


Supplementary Figure S4

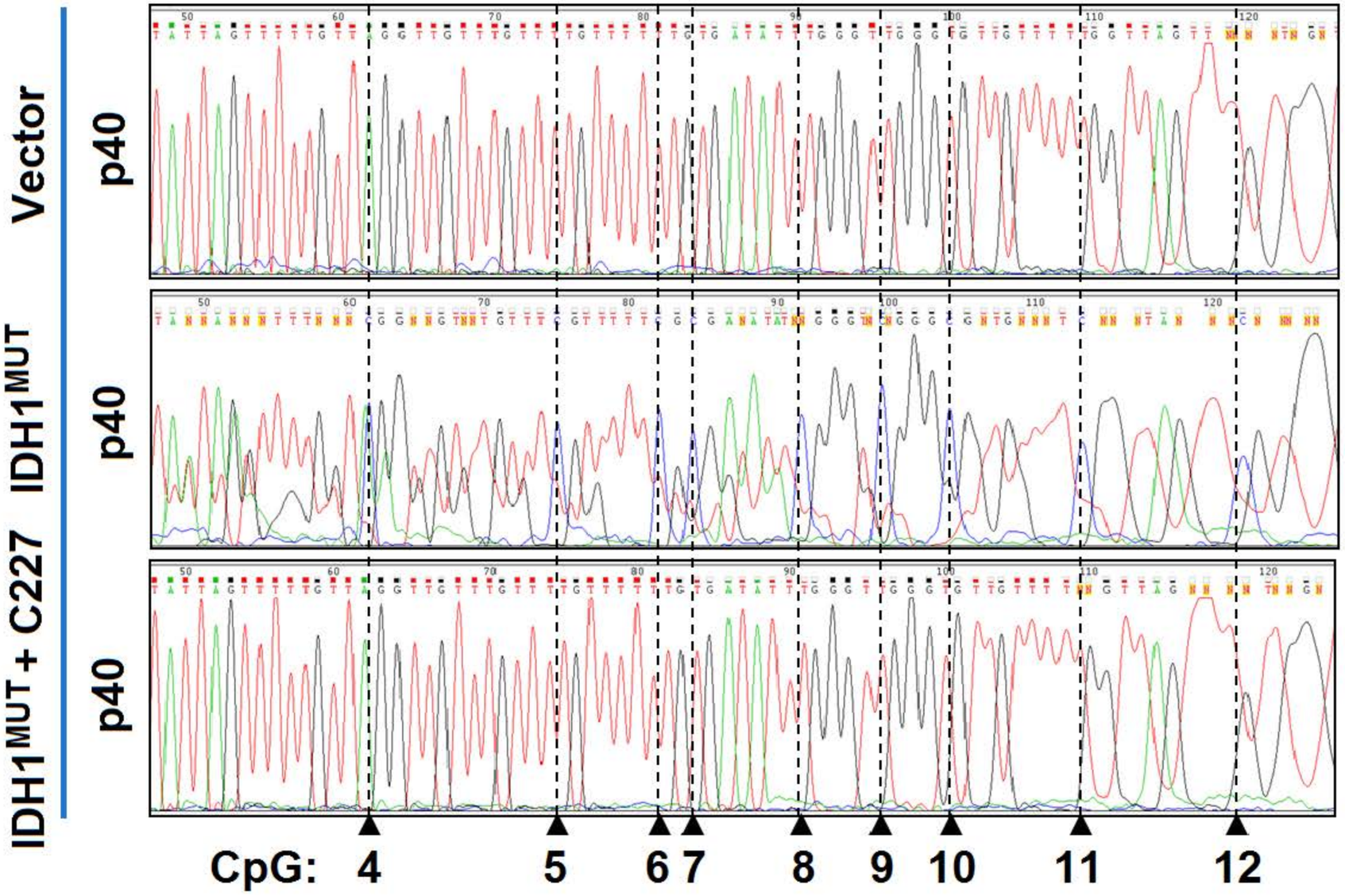


Supplementary Figure S5

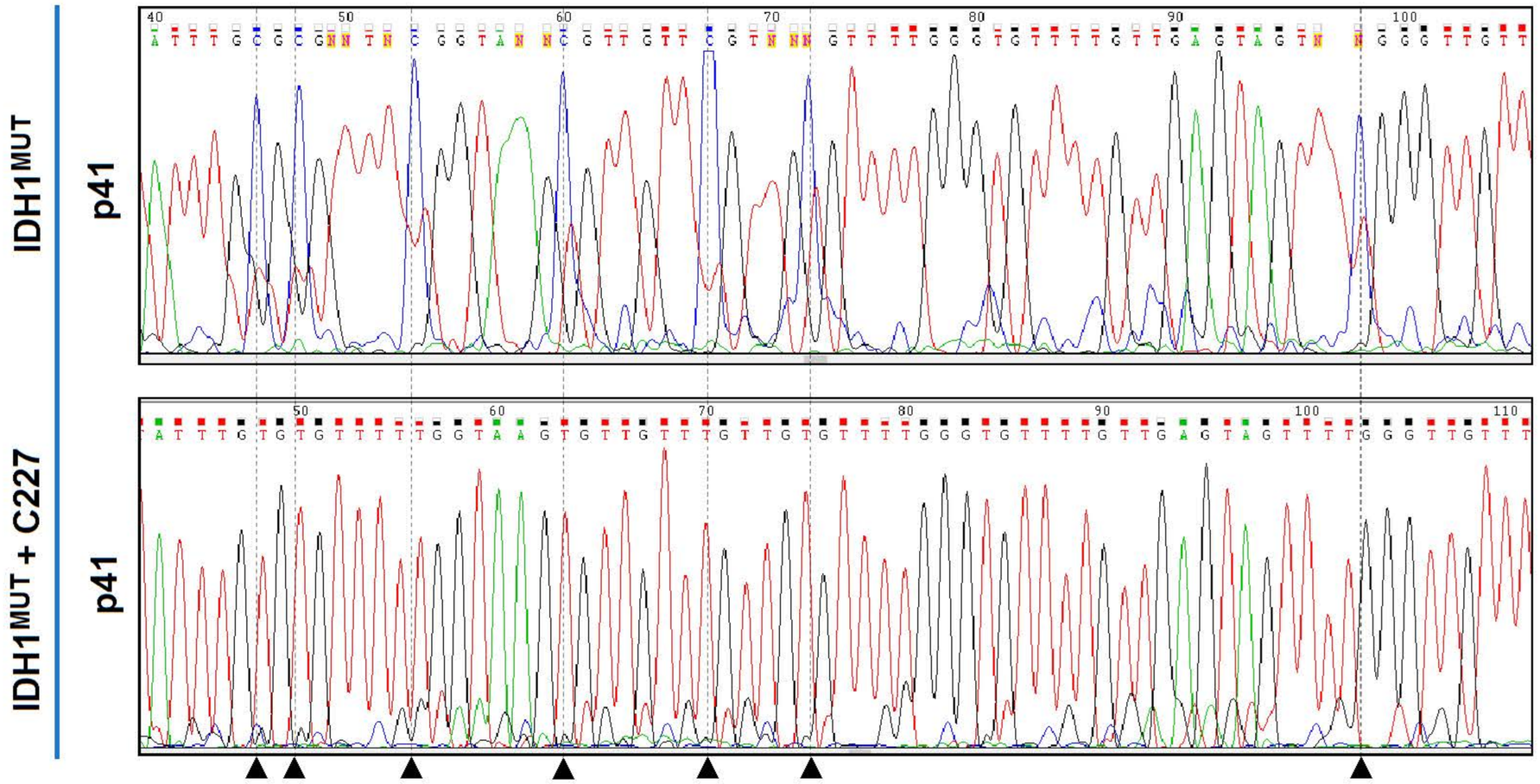




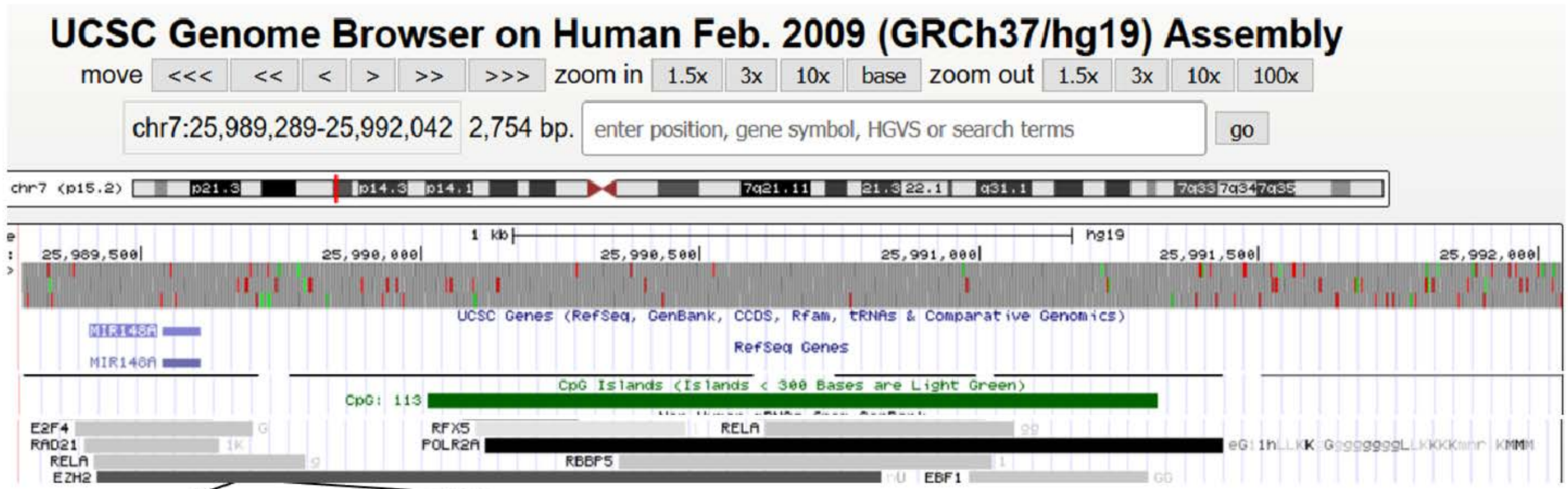
Supplementary Figure S6

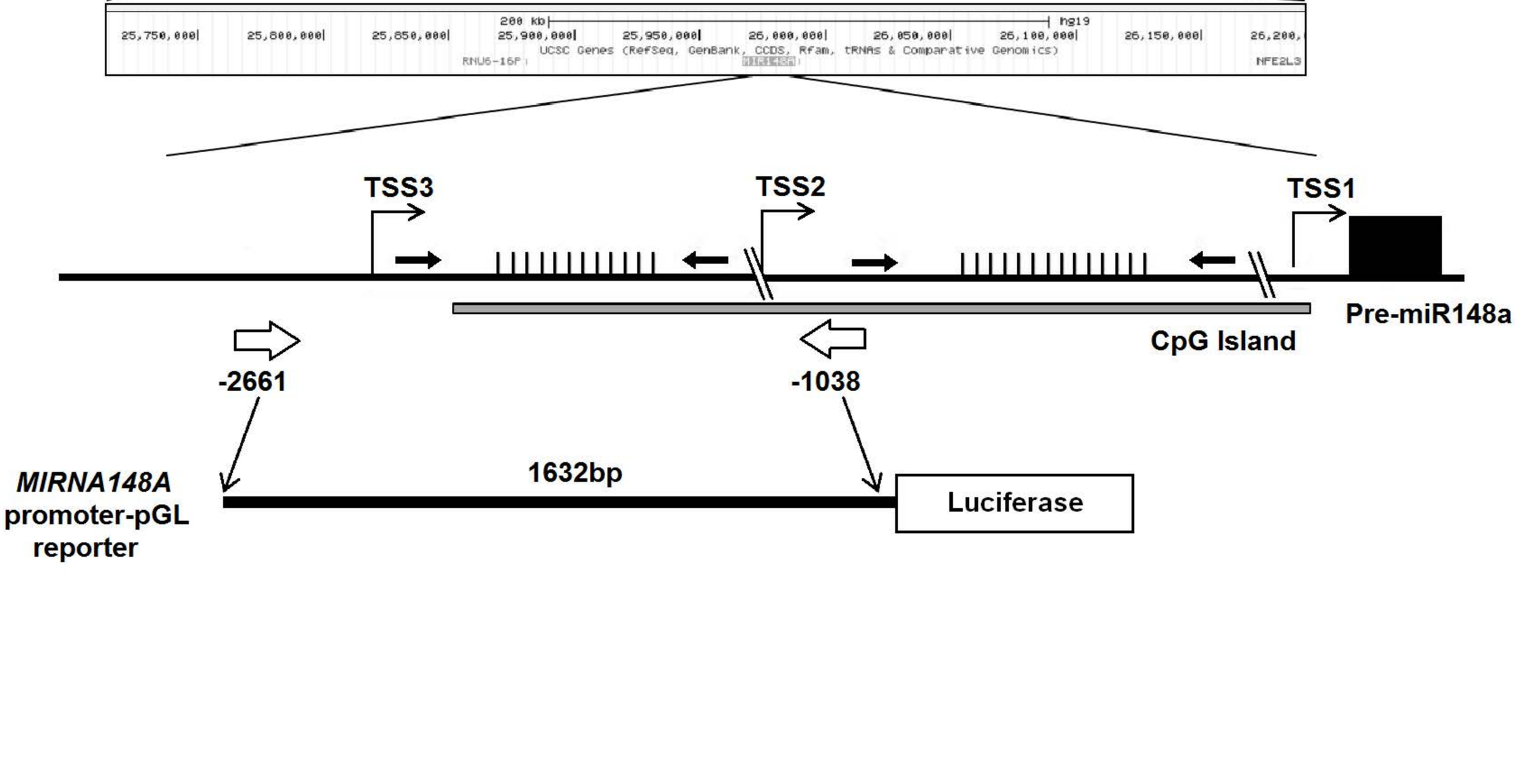


Supplementary Figure S7

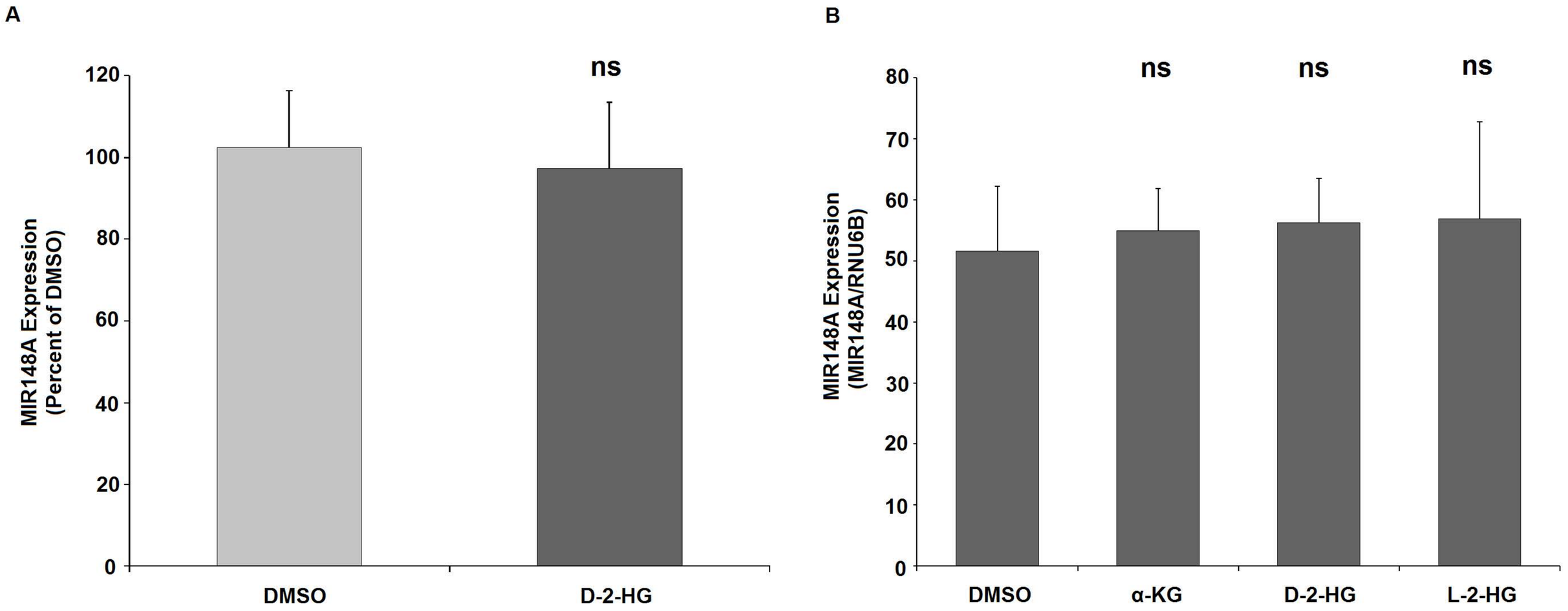


Supplementary Figure S8





Supplementary Figure S9



Supplementary Figure S10