SUPPLEMENTARY APPENDIX

Disruption of the MBD2-NuRD complex but not MBD3-NuRD induces high level HbF expression in human adult erythroid cells

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SUPPLEMENTARY METHODS

Antibodies and reagents. Antibodies for MBD2 (D-15), KLF1 (F-8), GAPDH (FL-355), β-globin (Hemoglobin β 37-8), and γ-globin (Hemoglobin γ 51-7) used in immunoblotting studies, were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). LRF (13097S) and BCL11a (D4E3P) antibodies were purchased from Cell Signaling. MTA2 (ab66051), HDAC2 (ab7029) and GATAD2A (ab87663) antibodies were purchased from Abcam. CHD4 (06-1306) antibody was purchased from Millipore. FLAG (M2) antibody was purchased from Sigma. For antibodies used in in flow cytometric analysis, PE-conjugated CD235a (H1R2) and APC-conjugated CD71 (OKT9) were purchased from eBioscience (San Diego, CA). APC-conjugated HbF antibody (MHFF05) was purchased from Thermo Fisher (Pittsburgh, PA). StemSpan SFEM II, recombinant human stem cell factor (SCF), StemSpan CC100 (100X), StemSpan Erythroid Expansion Supplement (100X) cytokine cocktails were obtained from (StemCell Technologies, Inc.) Doxycycline (DOX) was from Takara Bio USA, Inc. (Mountain View, CA). Dexamethasone (DEX), recombinant human insulin, heparin and human AB serum and human AB plasma were purchased from Sigma-Aldrich (St. Louis, MO). Recombinant Erythropoietin (EPO) was purchased from Amgen (Thousand Oaks, CA). Holo-human transferrin was purchased from Prospec (East Brunswick, NJ).

Human CD34+ cell culture and differentiation. After isolation, human CD34+ cells were initially cultured for expansion in StemSpan SFEM II supplemented with 1x CC100 cytokine cocktail and 2% penicillin/streptomycin. Cells were induced to erythroid differentiation by culturing in 1x EES cytokine cocktail in Iscove's Modified Dulbecco's Media (IMDM) supplemented with 3% human AB serum, 3IU/mL EPO and 2% penicillin/streptomycin for 5-10 days. The cells were cultured at 37°C in the presence of 5% CO2.

HUDEP-2 cell culture and differentiation. HUDEP-2 cells were maintained and expanded in StemSpan SFEM II supplemented with 50ng/mL SCF, 3IU/mL EPO, 10^{-6}M of DEX, 1ug/mL of DOX, 1% L-Glutamine and 2% penicillin/streptomycin (expansion medium). To induce erythroid

differentiation, HUDEP-2 cells were cultured for 3 days in Iscove's Modified Dulbecco's Media (IMDM) supplemented with 3IU/mL EPO, 1ug/mL DOX, 10ug/mL human recombinant insulin, 3IU/mL heparin, 0.5mg/mL of holo-transferrin, 5% human AB serum, 2% penicillin/streptomycin, and 1% L-Glutamine (differentiation medium). For immunoblotting of Hemoglobin β and Hemoglobin γ protein, cells were cultured for 2 more days in differentiation medium without DOX to induce the final differentiation. The cells were cultured at 37°C in the presence of 5% CO2.

Generation of LentiCRISPR-AcGFP vectors targeting MBD2 and MBD3. LentiCRISPR v2 plasmid was purchased from Addgene (plasmid # 52961) [1]. Two sgRNA sequences targeting *MBD2* gene (sgRNA-1: CCCCGGTGAGCGGCGTGCGC, sgRNA-2: GTGCGCAGGGAAGGCGCTCG) and two targeting the MBD3 gene (sgRNA-4: GATGGACGCCGTCTGGCGCA, sgRNA-9: CGTGGTGTTGAGCCATACGC) were selected using an online CRISPR design tool (http://crispr.mit.edu/) [2]. A scrambled control sgRNA sequence was also generated: ACAAGCCGCACGATAATCCT. The lentiviral vector expressing sgRNA and AcGFP1 was cloned by inserting the annealed sgRNA oligonucleotides into the BsmB I restriction enzyme cutting site and replacing the Puromycin resistance gene with the AcGFP1 gene.

Lentiviral preparation. 5 x 10^6 293T cells were seeded in 10cm culture dishes with 10ml DMEM supplemented with 10%FBS, 1%HEPES, and 1%NEAA. 24 hours later, the cells were given fresh media and co-transfected with 8ug of a lentiviral expression plasmid (LentiCRISPR-sgRNA-AcGFP, pRRL.H1.shRNA, or pLV203 expression vector) together with 6ug psPAX2 (plasmid #12260) and 4ug pMD2.G (plasmid #12259) using polyethylenimine (PEI). 18 hours post transfection, the media was replaced with 5ml DMEM supplemented with 5%FBS, 1%HEPES, and 1%NEAA. Viral supernatants were collected 48 and 72 hours post transfection, centrifuged at 3000 x g for 15 min at 4°C, and filtered through 0.45 um filters. Viral

supernatants were either used fresh to infect target cells or concentrated by ultracentrifugation, snap frozen, stored at -80°C, and titered.

Lentiviral transduction of HUDEP-2 cells. HUDEP-2 cells were transduced by centrifugation with lentivirus at 2,800 rpm, 32°C for 90 min with 10ug/mL polybrene in HUDEP-2 expansion medium. The cells were incubated for 3 hours with virus at 37°C and then transferred to fresh medium.

Lentiviral knockdown of MBD2 in HUDEP-2 and CD34+ cells. shRNA sequences targeting the human *MBD2* gene (GGGTAAACCAGACTTGAA) or a scrambled control shRNA were cloned into a pRRL.H1.shRNA vector and used to infect CD34+ or HUDEP-2 cells as described previously [3, 4].

NuRD Co-Immunoprecipitation with WT and mutant MBD2. 2 x 10^7 293T cells were infected with fresh MBD2sgR, IDRmutsgR, or CCmutsgR lentivirus. Cells were lysed 72h later in 1ml micrococcal nuclease (MNase) digestion buffer (25mM HEPES-KOH pH 7.6, 100mM NaCl, 5mM MgCl2, 3mM CaCl2, 0.2% NP-40, 10% glycerol and 1X EDTA-free protease inhibitor cocktail (Roche)) and MNase digestion was performed as described previously [3]. 2ug of anti-FLAG M2 antibody (Sigma) or normal mouse IgG (Santa Cruz) was incubated with Dynabeads Protein G (ThermoFischer) and immunoprecipitation was performed using the manufacturer's protocol, substituting MNase digestion buffer for the wash buffer. Precipitates were probed for MTA2, HDAC2, CHD4, GATAD2A, and FLAG by western blotting.

RNA-Seq experiments. Total RNA was extracted from 10^7 MBD2KO or sgSCR HUDEP-2 cells before and after 3 days of erythroid differentiation using the mirVana miRNA Isolation Kit (Invitrogen) according to the manufacturer's protocol, followed by DNaseI treatment. Library preparation was performed by the Brigham Young University DNA Sequencing Center using the TruSeq Stranded Total RNA with Ribo-Zero Globin Kit (Illumina). All samples were sequenced on the Illumina HiSeq 2500 according to Illumina's sequencing-by-synthesis protocol. Samples were sequenced at ~21 million 125bp paired-end reads. Sequencing adapters was removed

using Trimmomatic [5]. Quality control at each processing step was performed using the FastQC tool (quality base calls, CG content distribution, duplicate levels, complexity level) (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

Paired-end reads were aligned to the latest assembly of the human genome (GRCh38/hg38) using the subread v.1.6.2 aligner [6]. We obtained gene counts for each sample based on the last hg38 version of Ensembl transcriptome (v.87) using the featureCounts v.1.2.6 software [7]. RNA-seq counts were preprocessed and analyzed for differential expression using the edgeR v.3.18.1 R package [8]. P-values for differentially expressed genes were corrected using a False Discovery Rate (FDR) multiple testing correction method [9].

qRT-PCR. Real time PCR was performed using TaqMan primers and carboxyfluorescein (FAM) – labeled probe sets from Thermo Fisher (Pittsburgh, PA) or custom probe-primer sets as described previously [10]. Primer and probe sequences are listed in Table S1. Target gene expression was normalized to cyclophilin A (PPIA) and analyzed using the $2^{-\Delta\Delta CT}$ relative quantification method.

SUPPLEMENTARY FIGURES AND TABLES

Target	Vendor	Identifier	Forward Primer	Reverse Primer	Probe
human PPIA	ThermoFischer	Hs99999904_m1			
human GAPDH	ThermoFischer	Hs99999905_m1			
human GYPA	ThermoFischer	Hs00266777_m1			
human MBD3	ThermoFischer	Hs00922219_m1			
human MBD2			5'-TTA ACA CAT CTC AAC CCC TCT G	5'-TGT CTG CCA TCA GTG CTT C	/56-FAM/TTG CTG TAC /ZEN/ TCG CTC TTC CTG TTT CC /3IABkFQ/
human γ-globin			5'-GTG GAA GAT GCT GGA GGA GAA A	5'-TGC CAT GTG CCT TGA CTT TG	FAM/AGG CTC CTG GTT GTC TAC CCA TGG ACC /BHQ
human β-globin			5'-GCA AGG TGA ACG TGG ATG AAG T	5'-TAA CAG CAT CAG GAG TGG ACA GA	FAM/CA GGC TGC TGG TGG TCT ACC CTT GGA CCC /BHQ

Table S1. qRT-PCR probe and primer sets used for all experiments described in the methods section.

Table S1

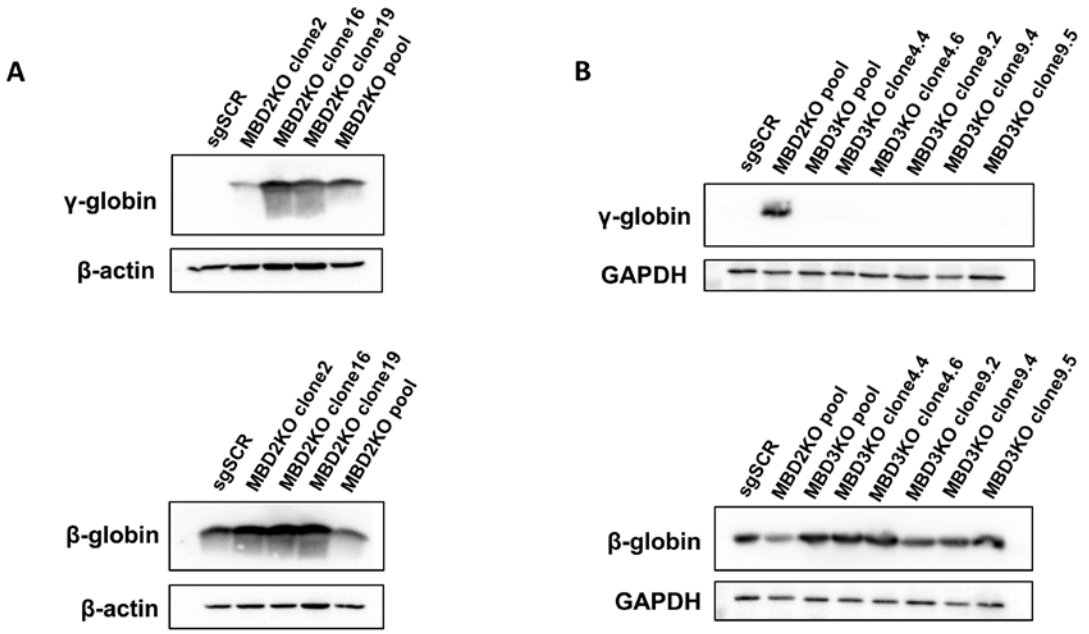

Figure S1. Knockout of MBD2 in HUDEP-2 cells results in a large increase in γ-globin protein expression while knockout of MBD3 shows no detectable increase in γ-globin protein by western blot. (A) W.B. depicting γ-globin and β-globin protein levels in 3 independent MBD2KO clonal populations and the pooled line compared to scramble. (B) W.B. depicting γ-globin and β-globin protein levels in 5 independent MBD3KO clonal populations and the pooled line compared to scramble and the MBD2KO pooled line. All cells were harvested after 5 days of erythroid differentiation.

Figure S1

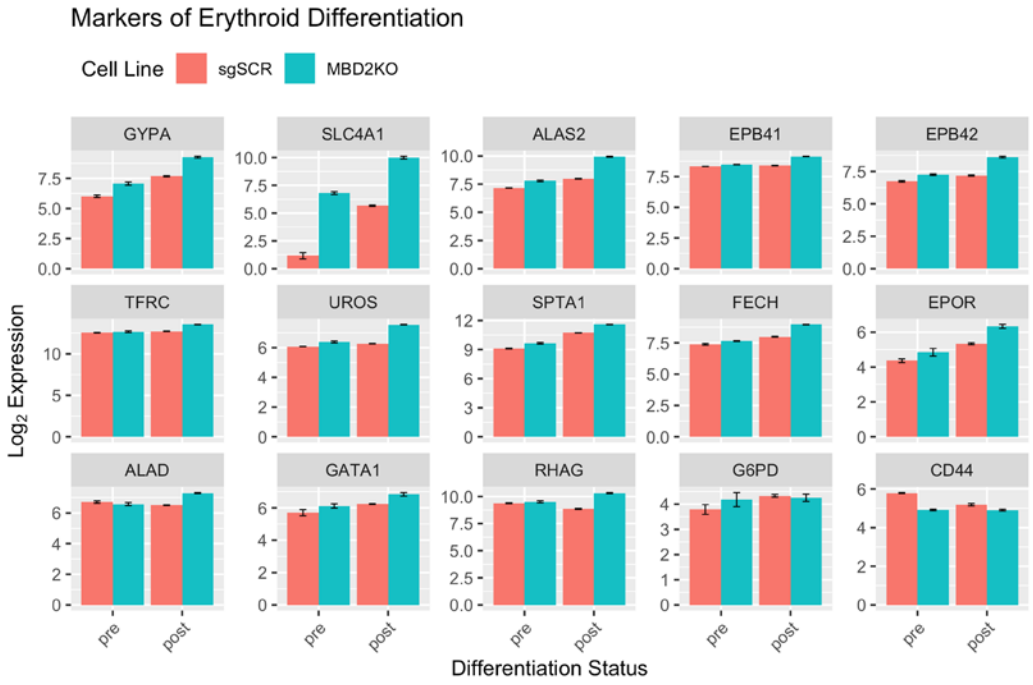

Figure S2. Knockout of MBD2 results in a pattern of gene expression consistent with a later stage of erythroid differentiation compared to scramble controls in HUDEP-2 cells. The plot above depicts log_2 transformed mRNA expression levels of 15 markers of erythroid differentiation in MBD2KO and sgSCR HUDEP-2 cells both before and after erythroid differentiation by RNA-sequencing.

Figure S2

Symbol	Ensgene	log_2FC	log_2CPM	LR	p-value	FDR
GYPA	ENSG00000170180	1.0620076	7.985159	76.465967	2.24E-18	1.56E-16
SLC4A1	ENSG00000004939	5.592872	8.207515	938.885664	3.46E-206	2.03E-202
ALAS2	ENSG00000158578	0.6340214	8.63505	56.769983	4.90E-14	2.39E-12
EPB41	ENSG00000159023	0.1556173	8.624551	4.544195	3.30E-02	1.29E-01
EPB42	ENSG00000166947	0.5103589	7.62879	30.391823	3.53E-08	8.30E-07
TFRC	ENSG00000072274	0.1348737	12.928807	1.489583	2.22E-01	4.64E-01
UROS	ENSG00000188690	0.3050217	6.688873	15.236213	9.49E-05	1.11E-03
SPTA1	ENSG00000163554	0.5486681	10.567754	33.393441	7.53E-09	1.93E-07
FECH	ENSG00000066926	0.2528552	8.125686	9.486308	2.07E-03	1.53E-02
EPOR	ENSG00000187266	0.507156	5.401932	10.351431	1.29E-03	1.05E-02
ALAD	ENSG00000148218	-0.132147	6.815137	2.036136	1.54E-01	3.72E-01
GATA1	ENSG00000102145	0.4068422	6.288815	7.902786	4.94E-03	3.08E-02
RHAG	ENSG00000112077	0.1349955	9.617272	1.900205	1.68E-01	3.93E-01
G6PD	ENSG00000160211	0.4227055	4.186387	4.00109	4.55E-02	1.64E-01
CD44	ENSG00000026508	-0.8751699	5.263431	98.298453	3.60E-23	3.57E-21

Table S2. Differential gene expression of 15 markers of erythroid differentiation between MBD2KO and sgSCR HUDEP-2 cells **before erythroid differentiation** by RNA-seq. Positive log_2FC indicates higher levels of expression in the MBD2KO cell population.

Column descriptions: “Symbol” – gene symbol. “Ensgene” – Ensembl ID. "log_2FC" – log_2 fold change. "log_2CPM" – average Counts Per Million, log_2. "LR" – log-likelihood. ”p-value” – non-FDR-adjusted p-value. “FDR” – false discovery rate adjusted p-value.

Symbol	Ensgene	log_2FC	log_2CPM	LR	p-value	FDR
GYPA	ENSG00000170180	1.5764357	7.985159	174.660147	7.10E-40	6.73E-38
SLC4A1	ENSG00000004939	4.3301183	8.207515	891.6513823	6.41E-196	2.83E-192
ALAS2	ENSG00000158578	1.9592634	8.63505	538.6068931	3.79E-119	5.14E-116
EPB41	ENSG00000159023	0.7493377	8.624551	104.6900857	1.43E-24	6.44E-23
EPB42	ENSG00000166947	1.4143786	7.62879	235.1938457	4.39E-53	7.17E-51
TFRC	ENSG00000072274	0.7921578	12.928807	50.7822152	1.03E-12	1.93E-11
UROS	ENSG00000188690	1.2769091	6.688873	277.5484094	2.57E-62	5.96E-60
SPTA1	ENSG00000163554	0.8581858	10.567754	82.0551503	1.32E-19	4.31E-18
FECH	ENSG00000066926	0.9608852	8.125686	139.7353577	3.04E-32	2.08E-30
EPOR	ENSG00000187266	1.004389	5.401932	44.1116043	3.10E-11	4.93E-10
ALAD	ENSG00000148218	0.7919507	6.815137	72.1193186	2.03E-17	5.66E-16
GATA1	ENSG00000102145	0.6081449	6.288815	17.9279694	2.29E-05	1.55E-04
RHAG	ENSG00000112077	1.4659566	9.617272	214.870203	1.19E-48	1.72E-46
G6PD	ENSG00000160211	-0.0717798	4.186387	0.1150112	7.35E-01	8.34E-01
CD44	ENSG00000026508	-0.2798369	5.263431	8.3843267	3.78E-03	1.43E-02

Table S3. Differential gene expression of 15 markers of erythroid differentiation between MBD2KO and sgSCR HUDEP-2 cells **after erythroid differentiation** by RNA-seq. Positive log_2FC indicates higher levels of expression in the MBD2KO cell population.

Column descriptions: “Symbol” – gene symbol. “Ensgene” – Ensembl ID. "log_2FC" – log_2 fold change. "log_2CPM" – average Counts Per Million, log_2. "LR" – log-likelihood. ”p-value” – non-FDR-adjusted p-value. “FDR” – false discovery rate adjusted p-value

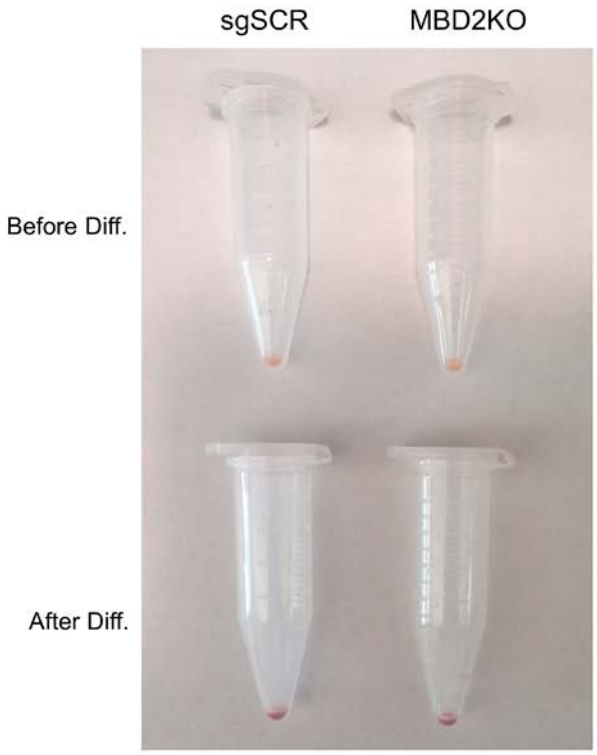

Figure S3. Gross appearance of scrambled sgRNA and MBD2KO HUDEP-2 cells before and after erythroid differentiation depicting no difference in the packing and hemoglobinization of cells during differentiation upon depletion of MBD2.

Figure S3

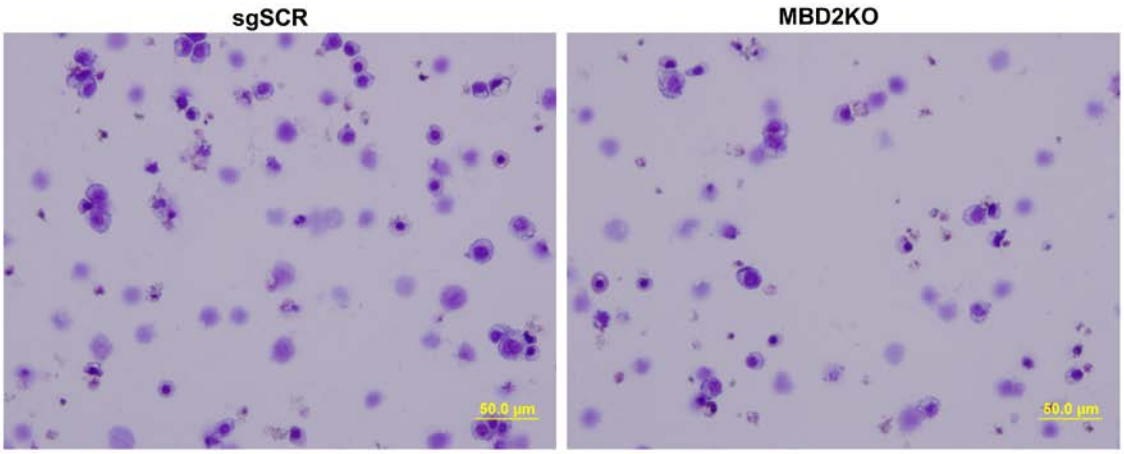

Figure S4. Wright stained histologic appearance of MBD2KO HUDEP-2 cells after 5 days of erythroid differentiation (Wright staining) compared to scrambled gRNA HUDEP-2 cells showing no obvious morphologic differences and similar number and sizes of enucleated cells.

Figure S4

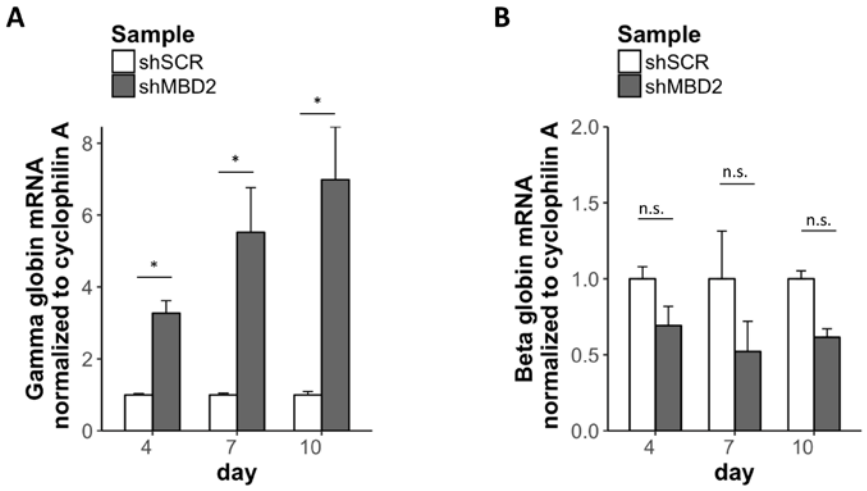

Figure S5. Lentiviral knockdown of MBD2 in HUDEP-2 cells results in progressively increased γ-globin gene expression. See Figure 2 for additional data from this set of experiments. After infection, HUDEP-2 cells were expanded for 4, 7, or 10 days prior to a 3-day differentiation period. (A) Relative γ-globin mRNA expression normalized by cyclophilin A compared to the shSCR sample by qRT-PCR at 4, 7, and 10-day timepoints. (B) Relative β-globin mRNA expression normalized by cyclophilin A compared to the shSCR sample by qRT-PCR at 4, 7, and 10-day timepoints. Error bars represent +/- s.d. of 3 biological repeats. * = $p < 0.05$. ** = $p < 0.01$. n.s. = $p > 0.05$. Statistical testing was performed using the Student's t-test.

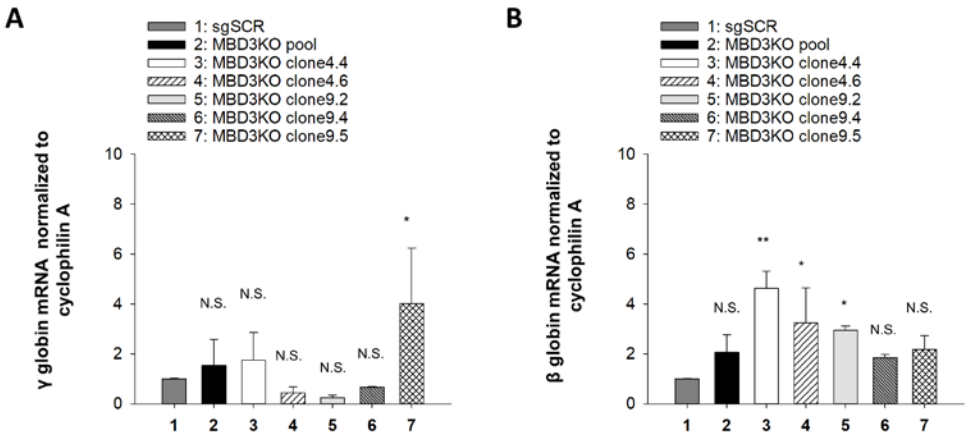

Figure S6. Effects of CRISPR/Cas9 mediated knockout of MBD3 in HUDEP-2 cells on γ-globin and β-globin gene expression. See Figure 3 for additional data from this experiment. (A) Plot of relative γ-globin mRNA by qRT-PCR in the 5 clonal MBD3KO, and pooled MBD3KO HUDEP-2 cell lines compared to scrambled guide RNA (sgSCR) controls. (B) Plot of relative β-globin mRNA by qRT-PCR in the 5 clonal MBD3KO, and pooled MBD3KO HUDEP-2 cell lines compared to scrambled guide RNA (sgSCR) controls. Error bars represent +/- s.d. of 3 biological repeats. * = $p < 0.05$. ** = $p < 0.01$. n.s. = $p > 0.05$. Statistical testing was performed using analysis of variance followed by the Tukey's HSD post-hoc test.

Figure S6

MBD2 WT cDNA:

ATGCGCGCGCACCCGGGGGGAGGCCGCTGCTGCCCGGAGCAGGAGGAGGGGGAGAGTGCGGCGGGCGGCAGCGG
CGCTGGCGGCGACTCCGCCATAGAGCAGGGGGGCCAGGGCAGCGCGCTCGCCCCGTCCCCGGTGAGCGGCGTGC
GCAGGGAAGGCGCTCGGGGCGGCGGCCGTGGCCGGGGGCGGTGGAAGCAGGCGGGCCGGGGCGGCGGCGTCTGT
GGCCGTGGCCGGGGCCGGGGCCGTGGCCGGGGACGGGGACGGGGCCGGGGCCGGGGCCGCGGCCGTCCCCCGAG
TGGCGGCAGCGGCCTTGGCGGCGACGGCGGCGGCTGCGGCGGCGGCGGCAGCGGTGGCGGCGGCGCCCCCCGGC
GGGAGCCGGTCCCTTTCCCGTCGGGGAGCGCGGGGCCGGGGCCCAGGGGACCCCGGGCCACGGAGAGCGGGAAG
AGGATGGATTGCCCGGCCCTCCCCCCCGGATGGAAGAAGGAGGAAGTGATCCGAAAATCTGGGCTAAGTGCTGG
CAAGAGCGATGTCTACTACTTCAGTCCAAGTGGTAAGAAGTTCAGAAGCAAGCCTCAGTTGGCAAGGTACCTGG
GAAATACTGTTGATCTCAGCAGTTTTGACTTCAGAACTGGAAAGATGATGCCTAGTAAATTACAGAAGAACAAA
CAGAGACTGCGAAACGATCCTCTCAATCAAAATAAGGGTAAACCAGACTTGAATACAACATTGCCAATTAGACA
AACAGCATCAATTTTCAAACAACCGGTAACCAAAGTCACAAATCATCCTAGTAATAAAGTGAAATCAGACCCAC
AACGAATGAATGAACAGCCACGTCAGCTTTTCTGGGAGAAGAGGCTACAAGGACTTAGTGCATCAGATGTAACA
GAACAAATTATAAAAACCATGGAACTACCCAAAGGTCTTCAAGGAGTTGGTCCAGGTAGCAATGATGAGACCCT
TTTATCTGCTGTTGCCAGTGCTTTGCACACAAGCTCTGCGCCAATCACAGGGCAAGTCTCCGCTGCTGTGGAAA
AGAACCCTGCTGTTTGGCTTAACACATCTCAACCCCTCTGCAAAGCTTTTATTGTCACAGATGAAGACATCAGG
AAACAGGAAGAGCGAGTACAGCAAGTACGCAAGAAATTGGAAGAAGCACTGATGGCAGACATCTTGTCGCGAGC
TGCTGATACAGAAGAGATGGATATTGAAATGGACAGTGGAGATGAAGCCTAA

MBD2 WT protein:

MRAHPGGGRCCPEQEEGESAAGGSGAGGDSAIEQGGQGSALAPSPVSGVRREGARGGGRGRGRWKQAGRGGGVC
GRGRGRGRGRGRGRGRGRGRGRPPSGGSGLGGDGGGCGGGGSGGGGAPRREPVPFPSGSAGPGPRGPRATESGK
RMDCPALPPGWKKEEVIRKSGLSAGKSDVYYFSPSGKKFRSKPQLARYLGNTVDLSSFDFRTGKMMPSKLQKNK
QRLRNDPLNQNKGKPDLNTTLPIRQTASIFKQPVTKVTNHPSNKVKSDPQRMNEQPRQLFWEKRLQGLSASDVT
EQIIKTMELPKGLQGVGPGSNDETLLSAVASALHTSSAPITGQVSAAVEKNPAVWLNTSQPLCKAFIVTDEDIR
KQEERVQQVRKKLEEALMADILSRAADTEEMDIEMDSGDEA

■ - Silent Mutation ■ - Substitution Mutation

Figure S7

MBD2sgR cDNA:

ATGCGCGCGCACCCGGGGGGAGGCCGCTGCTGCCCGGAGCAGGAGGAGGGGGAGAGTGCGGCGGGCGGCAGCGG
CGCTGGCGGCGACTCCGCCATAGAGCAGGGGGGCCAGGGCAGCGCGCTCGCCCCGTCCCCGGTGAGTGGAGTAC
GTAGGGAGGGAGCACGTGGCGGCGGCCGTGGCCGGGGGCGGTGGAAGCAGGCGGGCCGGGGCGGCGGCGTCTGT
GGCCGTGGCCGGGGCCGGGGCCGTGGCCGGGGACGGGGACGGGGCCGGGGCCGGGGCCGCGGCCGTCCCCCGAG
TGGCGGCAGCGGCCTTGGCGGCGACGGCGGCGGCTGCGGCGGCGGCGGCAGCGGTGGCGGCGGCGCCCCCCGGC
GGGAGCCGGTCCCTTTCCCGTCGGGGAGCGCGGGGCCGGGGCCCAGGGGACCCCGGGCCACGGAGAGCGGGAAG
AGGATGGATTGCCCGGCCCTCCCCCCCGGATGGAAGAAGGAGGAAGTGATCCGAAAATCTGGGCTAAGTGCTGG
CAAGAGCGATGTCTACTACTTCAGTCCAAGTGGTAAGAAGTTCAGAAGCAAGCCTCAGTTGGCAAGGTACCTGG
GAAATACTGTTGATCTCAGCAGTTTTGACTTCAGAACTGGAAAGATGATGCCTAGTAAATTACAGAAGAACAAA
CAGAGACTGCGAAACGATCCTCTCAATCAAAATAAGGGTAAACCAGACCTGAATACAACATTGCCAATTAGACA
AACAGCATCAATTTTCAAACAACCGGTAACCAAAGTCACAAATCATCCTAGTAATAAAGTGAAATCAGACCCAC
AACGAATGAATGAACAGCCACGTCAGCTTTTCTGGGAGAAGAGGCTACAAGGACTTAGTGCATCAGATGTAACA
GAACAAATTATAAAAACCATGGAACTACCCAAAGGTCTTCAAGGAGTTGGTCCAGGTAGCAATGATGAGACCCT
TTTATCTGCTGTTGCCAGTGCTTTGCACACAAGCTCTGCGCCAATCACAGGGCAAGTCTCCGCTGCTGTGGAAA
AGAACCCTGCTGTTTGGCTTAACACATCTCAACCCCTCTGCAAAGCTTTTATTGTCACAGATGAAGACATCAGG
AAACAGGAAGAGCGAGTACAGCAAGTACGCAAGAAATTGGAAGAAGCACTGATGGCAGACATCTTGTCGCGAGC
TGCTGATACAGAAGAGATGGATATTGAAATGGACAGTGGAGATGAAGCC – 3X FLAG - TAA

MBD2sgR protein:

MRAHPGGGRCCPEQEEGESAAGGSGAGGDSAIEQGGQGSALAPSPVSGVRREGARGGGRGRGRWKQAGRGGGVC
GRGRGRGRGRGRGRGRGRGRGRPPSGGSGLGGDGGGCGGGGSGGGGAPRREPVPFPSGSAGPGPRGPRATESGK
RMDCPALPPGWKKEEVIRKSGLSAGKSDVYYFSPSGKKFRSKPQLARYLGNTVDLSSFDFRTGKMMPSKLQKNK
QRLRNDPLNQNKGKPDLNTTLPIRQTASIFKQPVTKVTNHPSNKVKSDPQRMNEQPRQLFWEKRLQGLSASDVT
EQIIKTMELPKGLQGVGPGSNDETLLSAVASALHTSSAPITGQVSAAVEKNPAVWLNTSQPLCKAFIVTDEDIR
KQEERVQQVRKKLEEALMADILSRAADTEEMDIEMDSGDEA – 3X FLAG

■ - Silent Mutation ■ - Substitution Mutation

Figure S8

CCmutsgR (D366R/R375E/R380E) cDNA:

ATGCGCGCGCACCCGGGGGGAGGCCGCTGCTGCCCGGAGCAGGAGGAGGGGGAGAGTGCGGCGGGCGGCAGCGG
CGCTGGCGGCGACTCCGCCATAGAGCAGGGGGGCCAGGGCAGCGCGCTCGCCCCGTCCCCGGTGAGTGGAGTAC
GTAGGGAGGGAGCACGTGGCGGCGGCCGTGGCCGGGGGCGGTGGAAGCAGGCGGGCCGGGGCGGCGGCGTCTGT
GGCCGTGGCCGGGGCCGGGGCCGTGGCCGGGGACGGGGACGGGGCCGGGGCCGGGGCCGCGGCCGTCCCCCGAG
TGGCGGCAGCGGCCTTGGCGGCGACGGCGGCGGCTGCGGCGGCGGCGGCAGCGGTGGCGGCGGCGCCCCCCGGC
GGGAGCCGGTCCCTTTCCCGTCGGGGAGCGCGGGGCCGGGGCCCAGGGGACCCCGGGCCACGGAGAGCGGGAAG
AGGATGGATTGCCCGGCCCTCCCCCCCGGATGGAAGAAGGAGGAAGTGATCCGAAAATCTGGGCTAAGTGCTGG
CAAGAGCGATGTCTACTACTTCAGTCCAAGTGGTAAGAAGTTCAGAAGCAAGCCTCAGTTGGCAAGGTACCTGG
GAAATACTGTTGATCTCAGCAGTTTTGACTTCAGAACTGGAAAGATGATGCCTAGTAAATTACAGAAGAACAAA
CAGAGACTGCGAAACGATCCTCTCAATCAAAATAAGGGTAAACCAGACCTGAATACAACATTGCCAATTAGACA
AACAGCATCAATTTTCAAACAACCGGTAACCAAAGTCACAAATCATCCTAGTAATAAAGTGAAATCAGACCCAC
AACGAATGAATGAACAGCCACGTCAGCTTTTCTGGGAGAAGAGGCTACAAGGACTTAGTGCATCAGATGTAACA
GAACAAATTATAAAAACCATGGAACTACCCAAAGGTCTTCAAGGAGTTGGTCCAGGTAGCAATGATGAGACCCT
TTTATCTGCTGTTGCCAGTGCTTTGCACACAAGCTCTGCGCCAATCACAGGGCAAGTCTCCGCTGCTGTGGAAA
AGAACCCTGCTGTTTGGCTTAACACATCTCAACCCCTCTGCAAAGCTTTTATTGTCACACGAGAAGACATCAGG
AAACAGGAAGAGGAAGTACAGCAAGTAGAAAAGAAATTGGAAGAAGCACTGATGGCAGACATCTTGTCGCGAGC
TGCTGATACAGAAGAGATGGATATTGAAATGGACAGTGGAGATGAAGCC – 3X FLAG - TAA

CCmutsgR (D366R/R375E/R380E) protein:

MRAHPGGGRCCPEQEEGESAAGGSGAGGDSAIEQGGQGSALAPSPVSGVRREGARGGGRGRGRWKQAGRGGGVC
GRGRGRGRGRGRGRGRGRGRGRPPSGGSGLGGDGGGCGGGGSGGGGAPRREPVPFPSGSAGPGPRGPRATESGK
RMDCPALPPGWKKEEVIRKSGLSAGKSDVYYFSPSGKKFRSKPQLARYLGNTVDLSSFDFRTGKMMPSKLQKNK
QRLRNDPLNQNKGKPDLNTTLPIRQTASIFKQPVTKVTNHPSNKVKSDPQRMNEQPRQLFWEKRLQGLSASDVT
EQIIKTMELPKGLQGVGPGSNDETLLSAVASALHTSSAPITGQVSAAVEKNPAVWLNTSQPLCKAFIVTREDIR
KQEEEVQQVEKKLEEALMADILSRAADTEEMDIEMDSGDEA – 3X FLAG

■ - Silent Mutation ■ - Substitution Mutation

Figure S9

IDRmutsgR (R286E/L287A) cDNA:

ATGCGCGCGCACCCGGGGGGAGGCCGCTGCTGCCCGGAGCAGGAGGAGGGGGAGAGTGCGGCGGGCGGCAGCGG
CGCTGGCGGCGACTCCGCCATAGAGCAGGGGGGCCAGGGCAGCGCGCTCGCCCCGTCCCCGGTGAGTGGAGTAC
GTAGGGAGGGAGCACGTGGCGGCGGCCGTGGCCGGGGGCGGTGGAAGCAGGCGGGCCGGGGCGGCGGCGTCTGT
GGCCGTGGCCGGGGCCGGGGCCGTGGCCGGGGACGGGGACGGGGCCGGGGCCGGGGCCGCGGCCGTCCCCCGAG
TGGCGGCAGCGGCCTTGGCGGCGACGGCGGCGGCTGCGGCGGCGGCGGCAGCGGTGGCGGCGGCGCCCCCCGGC
GGGAGCCGGTCCCTTTCCCGTCGGGGAGCGCGGGGCCGGGGCCCAGGGGACCCCGGGCCACGGAGAGCGGGAAG
AGGATGGATTGCCCGGCCCTCCCCCCCGGATGGAAGAAGGAGGAAGTGATCCGAAAATCTGGGCTAAGTGCTGG
CAAGAGCGATGTCTACTACTTCAGTCCAAGTGGTAAGAAGTTCAGAAGCAAGCCTCAGTTGGCAAGGTACCTGG
GAAATACTGTTGATCTCAGCAGTTTTGACTTCAGAACTGGAAAGATGATGCCTAGTAAATTACAGAAGAACAAA
CAGAGACTGCGAAACGATCCTCTCAATCAAAATAAGGGTAAACCAGACCTGAATACAACATTGCCAATTAGACA
AACAGCATCAATTTTCAAACAACCGGTAACCAAAGTCACAAATCATCCTAGTAATAAAGTGAAATCAGACCCAC
AACGAATGAATGAACAGCCACGTCAGCTTTTCTGGGAGAAGGAGGCACAAGGACTTAGTGCATCAGATGTAACA
GAACAAATTATAAAAACCATGGAACTACCCAAAGGTCTTCAAGGAGTTGGTCCAGGTAGCAATGATGAGACCCT
TTTATCTGCTGTTGCCAGTGCTTTGCACACAAGCTCTGCGCCAATCACAGGGCAAGTCTCCGCTGCTGTGGAAA
AGAACCCTGCTGTTTGGCTTAACACATCTCAACCCCTCTGCAAAGCTTTTATTGTCACAGATGAAGACATCAGG
AAACAGGAAGAGCGAGTACAGCAAGTACGCAAGAAATTGGAAGAAGCACTGATGGCAGACATCTTGTCGCGAGC
TGCTGATACAGAAGAGATGGATATTGAAATGGACAGTGGAGATGAAGCC – 3X FLAG - TAA

IDRmutsgR (R286E/L287A) protein:

MRAHPGGGRCCPEQEEGESAAGGSGAGGDSAIEQGGQGSALAPSPVSGVRREGARGGGRGRGRWKQAGRGGGVC
GRGRGRGRGRGRGRGRGRGRGRPPSGGSGLGGDGGGCGGGGSGGGGAPRREPVPFPSGSAGPGPRGPRATESGK
RMDCPALPPGWKKEEVIRKSGLSAGKSDVYYFSPSGKKFRSKPQLARYLGNTVDLSSFDFRTGKMMPSKLQKNK
QRLRNDPLNQNKGKPDLNTTLPIRQTASIFKQPVTKVTNHPSNKVKSDPQRMNEQPRQLFWEKEAQGLSASDVT
EQIIKTMELPKGLQGVGPGSNDETLLSAVASALHTSSAPITGQVSAAVEKNPAVWLNTSQPLCKAFIVTDEDIR
KQEERVQQVRKKLEEALMADILSRAADTEEMDIEMDSGDEA – 3X FLAG

■ - Silent Mutation ■ - Substitution Mutation

Figure S10

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