Supplementary information

Supplementary methods

Drug Preparation and Administration. ORY-3001 was obtained from Oryzon Genomics S.A (Barcelona, Spain). The drug was stored at 4°C and freshly dissolved in deionized water prior to administration. Serial dilutions were made and the drug was administered orally to mice at 0.25, 0.5 and 1.0 mg/kg/d for four days. For baboon studies oral administration was performed via gavage on anesthetized animals. The range of doses tested in the baboons was between 10 and 200 μg/Kg (dose volume per unit body = 2 mL/Kg; formulation strength = 5-100 μg/mL).

RNA purification and analysis. RNA was purified from mouse peripheral blood using a Mouse RiboPure Blood RNA Isolation kit (Life Technologies, Waltham, MA.) and samples were treated with the DNA-free DNase Treatment and removal kit. cDNA was synthesized using the RevertAid first strand cDNA synthesis kit (Thermo Scientific, Waltham, MA.) according to the manufacturer's instructions. Levels of human β- (Hs00361131_g1), and γ-globin (Hs00747223_g1) mRNA and mouse were measured by real time PCR using the indicated Taqman primer-probe combinations (Life Technologies, Waltham, MA.). [1]

RNA was isolated from directly from the peripheral blood (50 μL) of anemic baboons or from normal baboon reticulocytes enriched from the peripheral blood by Percoll (GE Healthcare, Upsalla, Sweden) gradient sedimentation[2] using RNAeasy Mini Kits (Qiagen, Hilden, Germany) The transcript levels of both β and γ hemoglobin chains were quantified by real-time PCR using primers amplifying the baboon γ- and β-globin transcripts using Taqman primer-probe combinations.[3] The sequence of the primers and probes are listed below:

β forward GCT GGT GGT CTA CCC TTG GA
β reverse AGG AGA GGA CAG ATC CCC AAA
β probe 6FAMCCA GAG GTT CTT TGA TTC MGBNFQ
γ forward CGG CAA GAA GGT GCT CAC TT
γ reverse GCC CTT GAG ATC ATC CAG GTT
γ probe 6FAMCTT GGG AGA TGC CG MGBNFQ

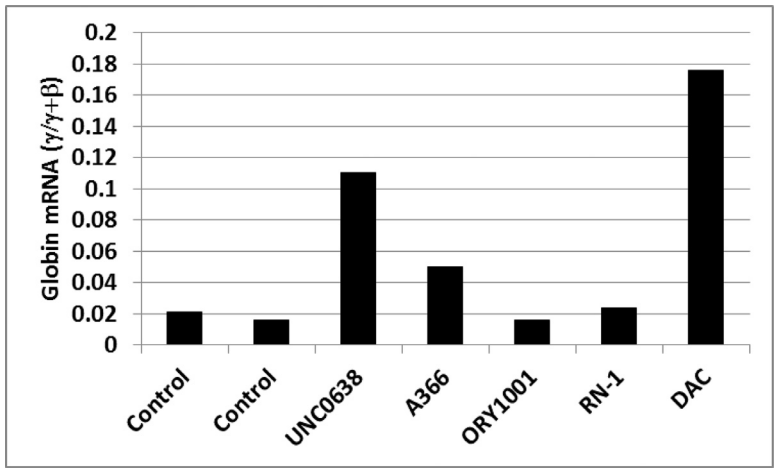
F cell and F retic analysis. F-cells and F-retics in samples were analyzed by flow cytometry using the Cytomics FC500 (Beckman Coulter, Brea, Ca.) system after staining with thiazole orange and PE-conjugated anti- HbF (BD Bioscience, San Jose, Ca.).

Analysis of Globin chain synthesis. Biosynthetic radiolabeling of globin chain synthesis was performed by incubation of peripheral blood in the presence of [^{3}H] leucine (Perkin Elmer, Shelton, CT.). Incubations were performed with 50uL peripheral blood from anemic baboons or reticulocyte-enriched Percoll fractions of peripheral blood form normal baboons. Incubations were performed in media consisting of leucine-free α-MEM (Life Technologies), 20% v/v extensively dialyzed fetal bovine serum, glutamine,1mg/mL holotransferrin (Sigma, St. Louis, MO), 1.25 mg/mL ferrous ammonium chloride hexahydrate and 50 μCi [^{3}H] leucine at 37°C for 3-4 hours. Following incubations, cells were washed three times in phosphate-buffered saline and pellets stored at -80°C until samples were processed.

Following the addition of 0.5 mL H_2O, the pellets were subjected to 3 freeze-thaw cycles in a dry ice-methanol bath to lyse the cells. Lysates were centrifuged 10,000 RPM, 15 minutes at 4°C. The supernatant was removed and then re-centrifuged prior to filtration using a Nalgene 0.2 μm syringe filter (Thermo Fisher). Separation of globin chains was achieved by high-performance liquid chromatography using a Thermo Fisher Spectra high performance liquid chromatography system.[4] A volume of 100 uL of filtered lysate was injected into a LiChrospher RP8 Column (EMD chemicals, Germany) separation of globin chains was performed using gradients of Buffer A (68:4:28 ratio of acetonitrile: methanol: 0.155 M NaCl) and Buffer B (26:33:41 ratio of acetonitrile: methanol: 0.077 M NaCl, pH 2.7) Fraction (0.5 mL) were collected directly into 7 ml scintillation vials using a fraction collector. 3 mL of Biosafe II scintillation fluid was added (RPI, Mt. Prospect, Il) and radioactive content quantitated using a TriCARB 3110TR scintillation counter (Perkin Elmer, Shelton, CT.).

Supplementary Table 1. Comparison of inhibitory activities (EC_{50}) of TCP (tranylcypromine, parnate), RN-1, and ORY-3001 for LSD1, MAO-A, and MAO-B.

Compound	LSD1 EC_{50} (μM)	MAO-A EC_{50} (μM)	MAO-B EC_{50}(μM)
TCP (parnate)	15 - 35	2	0.6
RN-1	0.03	11	9
ORY-3001	0.018	>100	>100



Supplemental Figure 1. Effect of drugs targeting the epigenetic-modifying enzymes G9A, LSD1 and DNMT1 on γ-globin expression in HUDEP-2 cell line. Exponentially growing HuDEP-2 cells were incubated for 72 hours in the presence of the G9A inhibitors UNC0638 and A366 (3 × 10-6M each), the LSD1 inhibitors ORY-1001 and RN-1 (3 × 10-6 m each), the DNMT1 inhibitor decitabine (3 × 10-7M). Drug was not added to control cultures. Effect on γ-globin mRNA (γ/γ+β) was measured by RT-PCR.

Supplementary Data

Supplementary Table 1

Supplemental Figure 1

Supplemental References.

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