Supporting Information

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SI Materials and Methods

Abs. ***Abs used for Western blot.*** From Santa Cruz Biotechnology, we used anti-$TF_{II}Hp89$ (sc-293), anti-Med1(sc-8998), anti-Med12 (sc-5372), anti-Med17 (sc-12453), anti-Med26 (sc-137196), anti-Sin3a (sc-767), anti-DNMT3a (sc-20703), and anti-DNMT3b (sc-20704). From Perseus Proteomics, we used anti-G9a (PP-A8620A-00) and anti-GLP (PP-B0422-00). From Abcam, we used anti-Med12 (ab70842), anti-Med14 (ab72141), anti-DNMT1 (ab87656), and anti-Suz12 (ab12073). From Active Motif, we used anti-Ezh2 (39103). From Bethyl, we used anti-Jarid1a (a300-8974). From Covance, we used Abs against nonphosphorylated Pol II (8WG16). From Upstate/Millipore, we used anti-His tag (05-531) to detect Ubc4 and anti-HDAC1 (06-720). From ProSci, Inc., we used anti-Wiz (6117). The Ash2L Ab was described previously (1).

Abs used for immunoprecipitation. The Abs used for immunoprecipitations (IPs) were the same as described above. In addition, for mock IPs, we used normal rabbit (sc-2027) and mouse (sc-2025) IgG from Santa Cruz Biotechnology.

Abs used for ChIP. The Abs used for ChIP were the same as described above, with the following exceptions. From Abcam, we used anti-Jarid1a (ab65769). From Santa Cruz Biotechnology, we used anti-Med12 (sc-5372) and anti-Med17 (sc-12453). In addition, from Abcam, we used anti-trimethylated histone H3 lysine 4 histone mark (H3K4me3; ab8580) and anti-dimethylated H3K9 (H3K9me2; ab1220). For mock ChIPs, we used normal rabbit (sc-2027), mouse (sc-2025), and goat (sc-2028) IgG from Santa Cruz Biotechnology.

Real-Time RT-Quantitative PCR Analysis. Total RNA was isolated using the RNA STAT-60 (TEL-TEST, Inc.) according to the manufacturer's instructions. One-step real-time RT-quantitative PCR (qPCR) was done on a Rotorgene instrument 6000 (Corbett Research) using TaqMan probes and primers. Statistical significance was determined using a Student *t* test.

Primers and Probes Used for Real-Time RT-qPCR. For *G9a*, the following primers and probes were used:

Forward primer: AAAACCATGTCCAAACCTAGCAA
Reverse primer: GCGGAAATGCTGGACTTCAG
TaqMan probe: FAM-ACAGCCTCCAATCCCTGAGAAGC-GG-BHQ1, which incorporates a fluorophore (FAM) and blackhole quencher (BHQ1)

For *Jarid1a*, the following primers and probes were used:

Forward primer: CATGGGCTCTAGTCTCTATGTGGA
Reverse primer: GCCTGCTGGAGCTCTTGCT
TaqMan probe: FAM-TACCTGAATTGCCCCGACT-BHQ1

For *Ey*, the following primers and probes were used:

Forward primer: GCAAGAAGGTGCTGACTGCTT
Reverse primer: GTAGCTTGTCACAGTGCAGTTCACT
TaqMan probe: FAM-TGGAGAGTCCATTAAGAACCTA-GACAACCTCAAGTC-MGBNFQ

For β^{maj}, the following primers and probes were used:

Forward primer: GAAGGCCCATGGCAAGAAG
Reverse primer: GCCCTTGAGGCTGTCCAA
TaqMan probe: FAM-TGATAACTGCCTTTAACGATGG-CCTGAATCA-MGBNFQ, which incorporates a minor groove binder (MGB) and nonfluorescent quencher (NFQ)

For *GAPDH*, the following primers and probes were used:

Forward primer: TTGTGGAAGGGCTCATGA
Reverse primer: CATCACGCCACAGCTTT
TaqMan probe: FAM-CATGCCATCACTGCCACCCA-BHQ1

Primers and Probes Used for Real-Time qPCR. For HS2, the following primers and probes were used:

Forward primer: CAGAGGAGGTTAGCTGGGCC
Reverse primer: CAAGGCTGAACACACCCACA
TaqMan probe: FAM-AGGCGGAGTCAATTCTCTACTCCC-CACC-BHQ1

For E^y, the following primers and probes were used:

Forward primer: CTTCAAAGAATAATGCAGAACAAAGG
Reverse primer: CAGGAGTGTCAGAAGCAAGTACGT
TaqMan probe: FAM-ATTGTCTGCGAAGAATAAAAGG-CCACCACTT-BHQ1

For β^{maj}, the following primers and probes were used:

Forward primer: CTGCTCACACAGGATAGAGAGGG
Reverse primer: GCAAATGTGAGGAGCAACTGATC
TaqMan probe: FAM-AGCCAGGGCAGAGCATATAAG-GTGAGGT-BHQ1

G9a-Jarid1a Direct Interaction. Recombinant purified G9a was a kind gift from S. Pradhan (New England Biolabs). Recombinant purified Jarid1a was purchased from BPS Bioscience (catalog no. 50110). His-tagged Ubc4 was produced in bacteria and purified by Ni^{2+} chromatography. The G9a-Jarid1 direct interaction experiment was performed as previously described (2).

Cell Culture. Murine erythroid (MEL) cells (clone 745) were cultured in RPMI containing 10% (vol/vol) FBS, 1% glutamine, and antibiotics, and they were differentiated toward the erythroid lineage by adding 2% (vol/vol) DMSO for 4 d as previously described (3). E14 murine embryonic stem (mES) cells (4) were cultured on 0.1% gelatin-coated tissue culture plastic in DMEM supplemented with 15% (vol/vol) FBS, 10^3 U/mL leukemia inhibitory factor, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 50 μg/mL penicillin/streptomycin, and 10^{-4} M β-mercaptoethanol. Cells were routinely passaged using 0.05% trypsin every 2–3 d. mES cells were grown to ∼70% confluency and harvested using TrypLE cell dissociation reagent (Gibco).

Establishment of MEL Clones with Inducible Knockdown of Jarid1a and G9a. To obtain stable MEL clones with a single knockdown (KD) of Jarid1a, we first modified the pGJ10 doxycycline (Dox)-inducible shRNA expression vector (described in 1) to replace the neomycin (Neo)-resistance gene by a puromycin (Puro)-resistance gene. For this purpose, the pGJ10 expression vector was restriction-digested at ClaI/SmaI sites to remove the Neo gene. The Puro DNA fragment was obtained by amplification using gene-specific forward (5′ AAAGAATCGATCAACCATGACCGAGTACAAGC 3′) and reverse (5′ AACCCGGGTCAGGCACCGGGCTTG 3′) primers that inserted ClaI/SmaI restriction sites at the 5′ and 3′ ends of the gene, respectively. The Puro fragment was then inserted into the ClaI/SmaI site of the digested pGJ10 vector. This vector is named pGJ10-puro. Two different shRNA sequences targeting Jarid1a

(shRNA1: 5′ GATCCCGAGGCAATGACTAGAGTAATTCA-AGAGATTACTCTAGTCATTGCCTCTTTTTGGAAA 3′ and shRNA2: 5′ GATCCCGCCAAACTCGACA AGTAAATTCAA-GAGATTTACTTGTCGAGTTTGGCTTTTTGGAAA 3′) were then cloned into pGJ10 and/or pGJ10-puro vector at Bgl II/Not I restriction sites to obtain the pGJ10-Jarid1a-sh1, pGJ10-puro-Jarid1a-sh1, and the pGJ10-puro-Jarid1a-sh2 vectors. These vectors were then transfected separately into the MEL/tetracycline repressor (TR) cell line (1) that expresses high levels of the TR. After screening for G418- and/or Puro-resistant cells, stable clones that display a Dox-inducible KD of Jarid1a were selected by Western blot and RT-qPCR. In addition, we verified that these clones are able to efficiently differentiate toward the erythroid lineage in the absence of Dox.

To obtain stable MEL clones with a double KD of G9a plus Jarid1a, the previously described Neo-resistant G9a KD MEL cell line, which expresses anti-G9a shRNA (5′ CCCTGATCTTT-GAGTGTAA 3′) in a Dox-inducible manner (2), was transfected with the pGJ10-puro-Jarid1a-sh1 and the pGJ10-puro-Jarid1a-sh2 vectors, separately. MEL cells that are resistant to both G418 and Puro were selected and screened by Western blot for efficient Dox-inducible double KD of G9a plus Jarid1a.

1. Demers C, et al. (2007) Activator-mediated recruitment of the MLL2 methyltransferase complex to the beta-globin locus. *Mol Cell* 27(4):573–584.
2. Chaturvedi CP, et al. (2009) Dual role for the methyltransferase G9a in the maintenance of beta-globin gene transcription in adult erythroid cells. *Proc Natl Acad Sci USA* 106(43): 18303–18308.
3. Friend C, Scher W, Holland JG, Sato T (1971) Hemoglobin synthesis in murine virus-induced leukemic cells in vitro: Stimulation of erythroid differentiation by dimethyl sulfoxide. *Proc Natl Acad Sci USA* 68(2):378–382.
4. Hooper M, Hardy K, Handyside A, Hunter S, Monk M (1987) HPRT-deficient (Lesch-Nyhan) mouse embryos derived from germline colonization by cultured cells. *Nature* 326(6110):292–295.

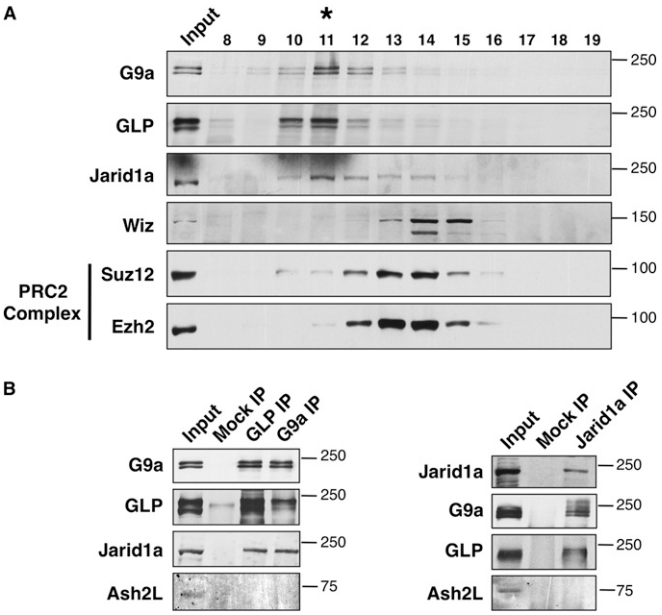

Fig. S1. Jarid1a associates with the G9a-GLP heterodimer in mES cells. (*A*) G9a, GLP, and Jarid1a coelute by size exclusion chromatography. A mES cell nuclear extract was separated using a Superose-6 column, and the presence of specific proteins in different fractions was revealed by immunoblotting with Abs indicated on the left. Molecular masses (in kilodaltons) are indicated on the right. (*B*) G9a and GLP associate with Jarid1a in mES cell nuclear extracts. Proteins immunoprecipitated via Abs against endogenous G9a, GLP, and Jarid1a were analyzed by Western blot. Mock IPs with normal IgG were used as negative controls. Abs used for Western blot (*Left*) and molecular masses (*Right*; in kilodaltons) are indicated.

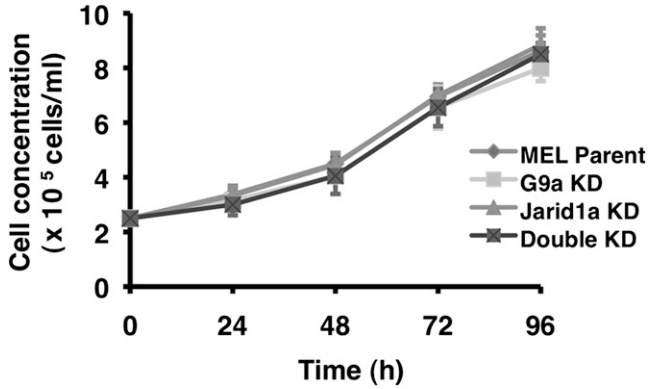

Fig. S2. KDs of Jarid1a and G9a and double KD of Jarid1a plus G9a have no effect on cell growth during erythroid differentiation. Dox-treated MEL cells were counted every 24 h after induction of differentiation (0 h).

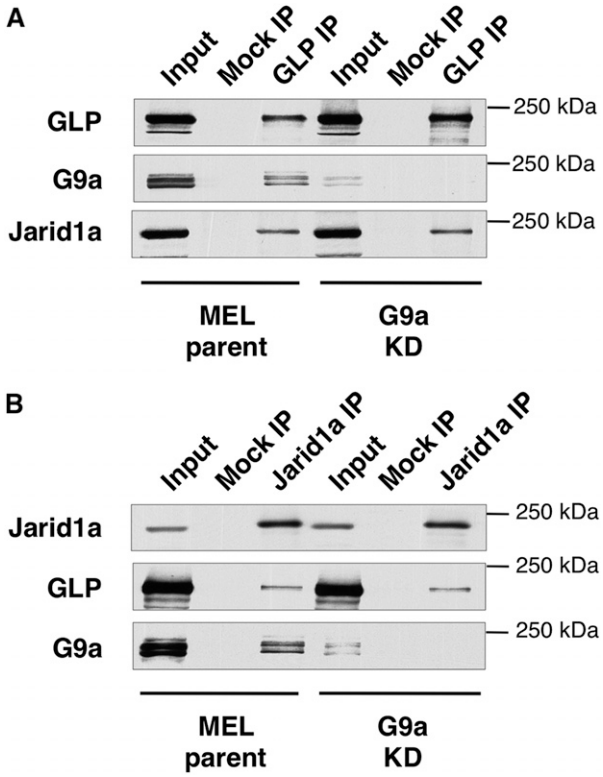

Fig. S3. Jarid1a interacts with GLP in the absence of G9a. (*A*) GLP and associated proteins were immunoprecipitated from a MEL nuclear extract containing a normal (MEL parent) or reduced (G9a KD) amount of G9a. (*B*) Jarid1a and associated proteins were immunoprecipitated from a MEL nuclear extract containing a normal (MEL parent) or reduced (G9a KD) amount of G9a. Mock IPs with normal IgG were used as negative controls. Immunoprecipitated proteins were analyzed by Western blot. Abs used for Western blot (*Left*) and molecular masses (*Right*; in kilodaltons) are indicated.

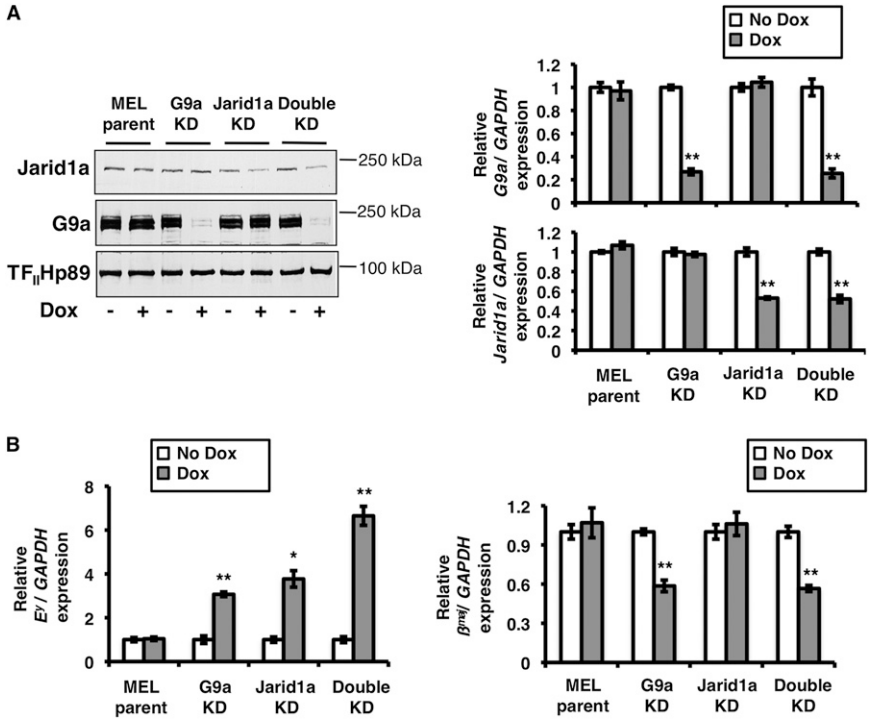

Fig. S4. Jarid1a and G9a cooperate in the maintenance of gene repression. (*A*) Establishment of stable MEL cell clones, where the single KDs of Jarid1a or G9a and the double KD of Jarid1a plus G9a are induced by Dox-mediated expression of specific shRNAs. This figure shows the results using Jarid1a shRNA2 (*SI Materials and Methods*). The levels of Jarid1a and G9a in Dox-treated (Dox) vs. untreated (No Dox) cells were analyzed at the protein level by Western blot of nuclear extracts using indicated Abs (*Left*) and at the mRNA level by real-time RT-qPCR (*Right*). MEL parent represents a control cell line with no induction of shRNA on Dox treatment. (*Left*) Molecular masses of proteins (in kilodaltons) are indicated on the right. (*Right*) Transcript levels are normalized to *GAPDH* with the ratio observed in the absence of Dox set to 1. (*B*) Additive effect of the double Jarid1a plus G9a KD on derepression of the E^y *globin* gene. Transcript levels were measured by real-time RT-qPCR in Dox-treated/untreated cells. Transcript levels are normalized to *GAPDH* with the ratio observed in the absence of Dox set to 1. (*A* and *B*) Average values from triplicate experiments are represented with error bars corresponding to SDs. $*P < 0.05$; $**P < 0.01$.

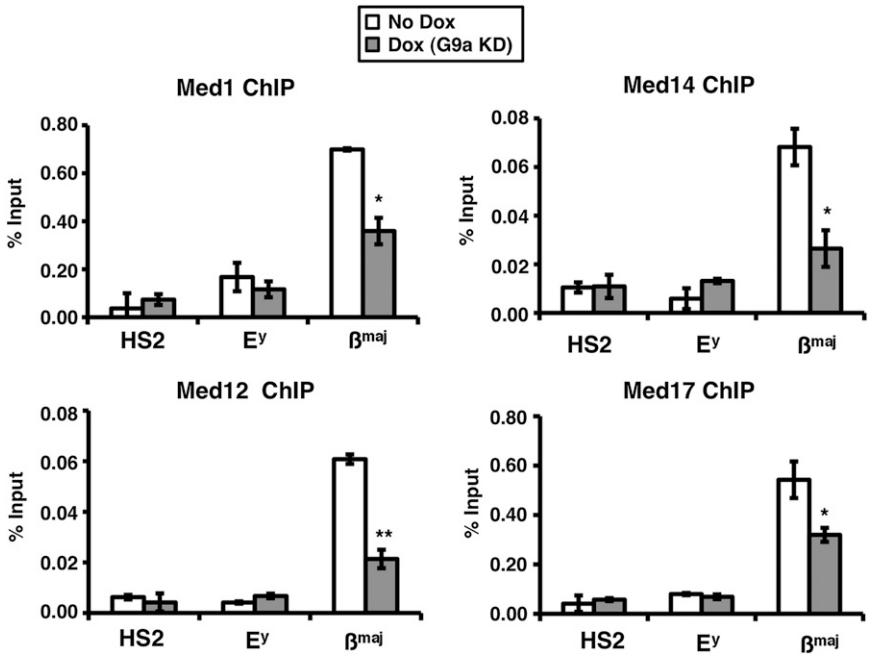

Fig. S5. G9a stabilizes binding of the Mediator complex to the promoter of the β^{maj} *globin* gene in MEL cells. ChIP experiments using Abs against the indicated Mediator complex subunits were performed in the presence of normal (No Dox) or reduced (Dox) levels of G9a. ChIPs were revealed by real-time qPCR using Taqman probes located at the HS2 site of the locus control region and at the promoters of the E^y and β^{maj} genes. Error bars represent SDs calculated from triplicate experiments. $*P < 0.05$; $**P < 0.01$.

Other Supporting Information Files

Dataset S1 (XLSX)