# Supporting Information

## Hu et al. 10.1073/pnas.0900437106

### SI Text

**Transfection.** A GAL4-thymidine kinase-luciferase reporter (GAL4-TK-Luc) was introduced into HeLa cells along with the indicated amounts of plasmids by using LipofectAmine LTX reagent (Invitrogen). The total mass of DNA transfected was normalized by the addition of the appropriate vector plasmid. The extracts were prepared 40–48 h after transfection. A renilla luciferase expression vector (Dual-Luciferase Reporter Assay System; Promega) was cotransfected to adjust for variation in transfection efficiency, and luciferase assays were performed following the manufacturer's instructions (Promega).

**Oligonucleotides for Mouse *Lsd1* shRNA.** The top strand was TCGAGAAAAA*CACAAGGAAAGCTAGAAGAT*CTCTTGA*ATCTTCTAGCTTTCCTTGTG*GGG; the bottom strand was GATCCCCC*CACAAGGAAAGCTAGAAGAT*TCAAGAG*ATCTTCTAGCTTTCCTTGTG*TTTTTC. They were designed by using the Oligoengine program and cloned into the pSUPER-retro vector (Oligoengine). The retrovirus generation and infection were performed as described (1). Briefly, $5 \times 10^5$ exponentially-growing MEL or ES cells were cocultured with virus particles in the presence of 4 $\mu$g of Polybrene per mL followed by puromycin selection (1 $\mu$g/mL) for 2 weeks.

**Plasmid Constructs.** LSD1 cDNA was a kind gift from Yang Shi. The C-terminal deleted (amino acids 1–427) LSD1 cDNA were cloned as described (2). A variety of pGEX-TAL1 constructs, pcDNA-TAL1, pGAL4-TAL1, and GAL4-TK-lucferase reporter plasmids were as described (1). Plasmid expressing GST-TAL1$^{142–185}$ was constructed by subcloning a TAL1 PCR fragment that corresponded to amino acids 142–185 of TAL1 protein into the pGEX-5X-1 vector (GE Healthcare).

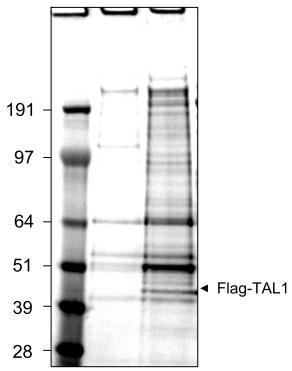
**Primers for Quantitative RT-PCR.** The primers were: *P4.2* gene, forward TCCCAAACAACCCTCAACCGTC, reverse TGGTATGAAACATCTGAACACCCC; *β-globin* gene, forward CACCTTTGCCAGCCTCAGTG, reverse GGTTTAGTGGTACTTGTGAGCC; *GATA-1* gene, forward CAGGGATCCCATGGATTTTCCTGGTC, reverse TCCACAGTTCACACACTCTCTGGC; *GATA-2* gene, forward TGCAACACACCACCCGATACC, reverse CAATTTGCACAACAGGTGCCC; *β-actin* gene, forward GTGGGCCGCTCTAGGCACCA, reverse: TGGCCTTAGGGTGCCAGGGGG.

**The Primers for Quantitative ChIP Assay.** The primers were: P4.2 gene locus, −6 Kb, forward CTAAGCGGCATCATGAGAGA, reverse CTCCCTCCCTGCTGCTATCT; Promoter, forward ACAGATGGTTTCCTGACATGC, reverse ACTCTGAACCTCCTGCCTCA; 3′ UTR, forward AGAACCTGACCGGCTACAGA, reverse: AGACGGTTGAGGGTTGTTTG.


1. Huang S, Brandt SJ (2000) mSin3A regulates murine erythroleukemia cell differentiation through association with the TAL1 (or SCL) transcription factor. *Mol Cell Biol* 20:2248–2259.

2. Shi Y, et al. (2004) Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 119:941–953.

A




B **LC-MS/MS Identification from TAL1 expressed Jurkat cells:**

| Protein ID | No. of Peptide |
|---|---|
| CHD4 | 28 |
| CHD3 | 8 |
| RB | 17 |
| BC11B | 14 |
| LSD1 | 11 |
| HTF4 (HEB) | 19 |
| TFE2 (E12/E47) | 11 |
| ETO-2 | 2 |
| HDAC1 | 16 |
| HDAC2 | 2 |
| CoREST | 10 |
| TAL1/SCL | 6 |
| RUNX1 | 3 |
| LDB1 | 2 |
| Lyl1 | 1 |
| SSBP3 | 3 |
| SSBP2 | 1 |

**Fig. S1.** Identification of the TAL1 interacting proteins in T-ALL cell line Jurkat cells. (*A*) Polypeptides purified by the Flag antibody affinity column from the Flag-TAL1 expressing Jurkat cells were resolved by SDS/PAGE. (*B*) A partial list of proteins identified by MS analysis is indicated.

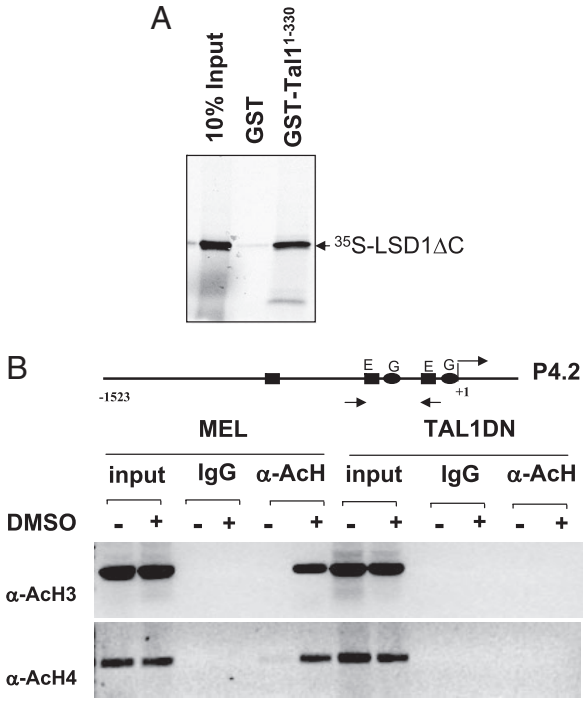



**Fig. S2.** TAL1 is important for establishment of local chromatin modification and p4.2 promoter activity. (*A*) The N-terminal domain (amino acids 1- 421) of LSD1 that lacks the demethylase domain directly interacts with TAL1 protein. $^{35}$S-labeled LSD1ΔC was incubated with GST and GST-TAL1 fusion protein preabsorbed to glutathione-Sepharose beads. Bound LSD1 was visualized by fluorography after SDS/PAGE. (*B*) Histone acetylation is enriched at the p4.2 promoter upon erythroid differentiation. Soluble chromatin prepared from WT MEL or TAL1 dominant-negative mutant (TAL1DN) MEL cells in the presence (+) or absence (−) of 1.5% DMSO was precipitated with antibodies against acetylated H3 (α-AcH3), acetylated H4 (α-AcH4), or rabbit IgG as control, then analyzed by PCR using primers probing the promoter region.

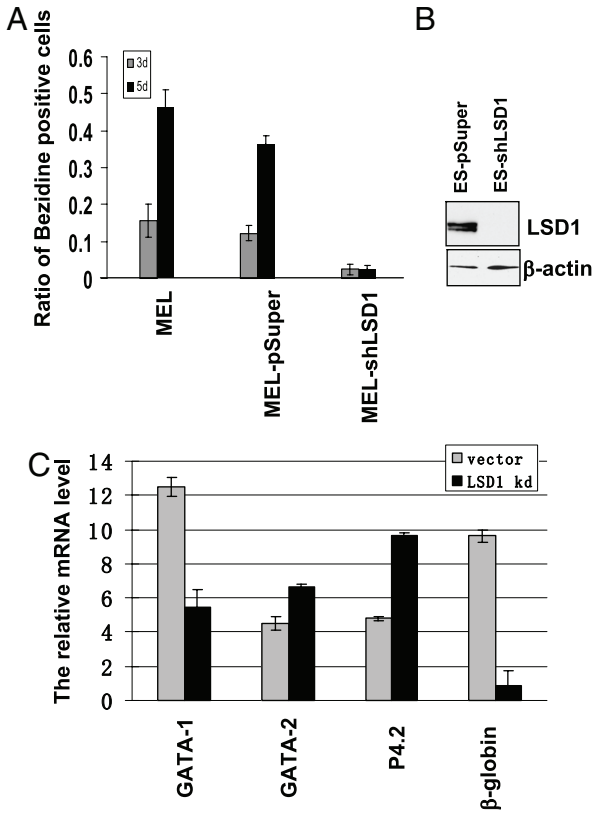



**Fig. S3.** shRNA-mediated KD of LSD1 results in a blockage of erythroid differentiation. (*A*) The MEL cell clones stably transduced with retrovirus-encoded pSuper vector or LSD1 shRNA (shLSD1) were treated with 1.5% DMSO, and hemoglobinization was assayed by benzidine staining at day 5. (*B*) shRNA-mediated KD of LSD1 expression in mouse ES cells. WB analysis of whole-cell extracts from the pSuper control or shLSD1 ES cells stimulated with 2 units/mL EPO for 9 days is shown. (*C*) Loss of LSD1 affects the expression of erythroid-specific genes in murine ES cells. The RNA from murine ES cell clones stably expressed the pSuper vector control and the shRNA specific for LSD1 in the presence of EPO for 9 days was isolated, and quantitative RT-PCR was performed by using primers specific to p4.2, $\beta$-globin, GATA-1, GATA-2, and $\beta$-actin.

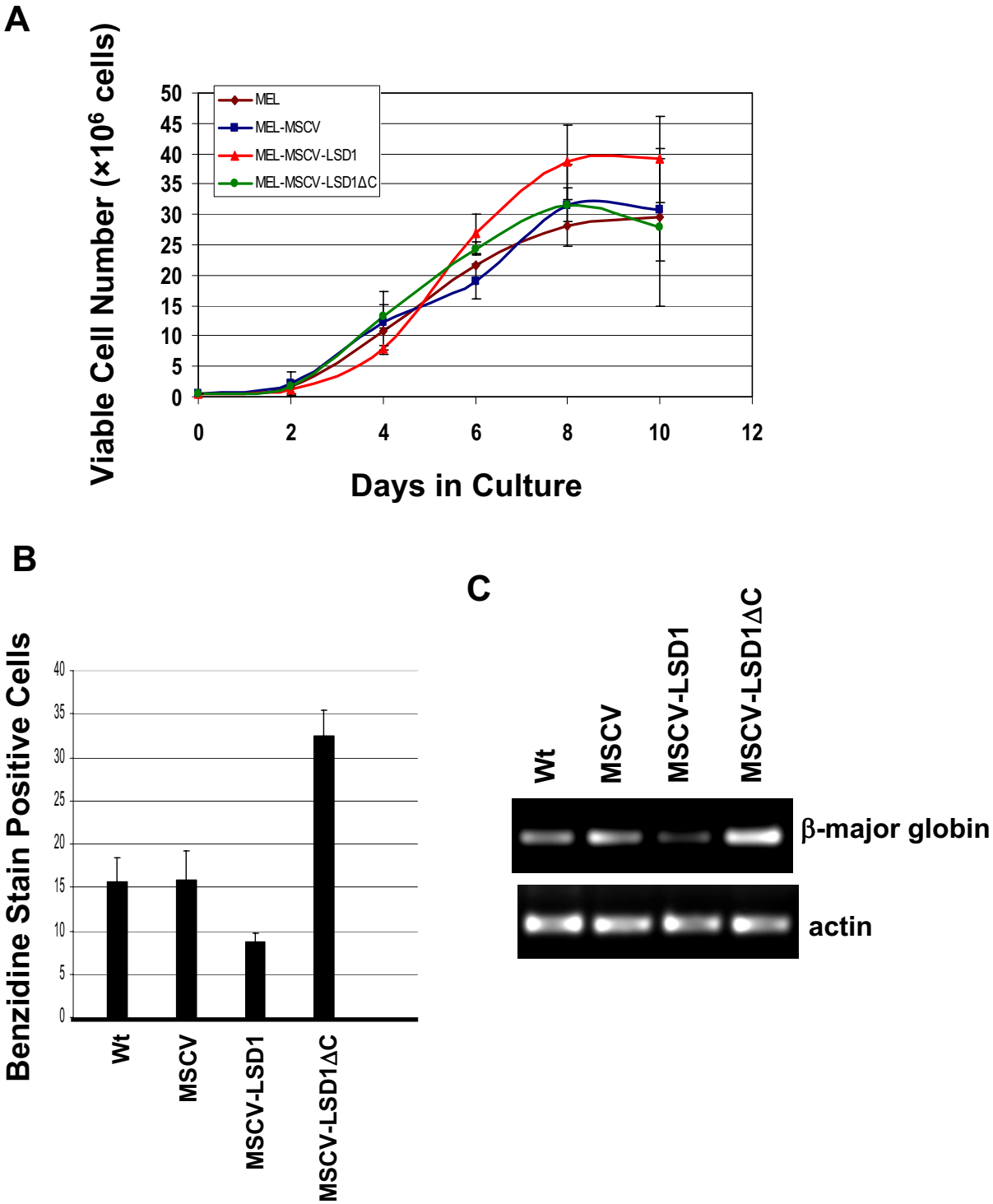



**Fig. S4.** The role of LSD1 in DMSO-induced MEL cell differentiation. (*A*) MEL cells were stably infected with retrovirus encoding the vector control, LSD1, and LAS1ΔC mutant. A total of $5 \times 10^6$ cells were inoculated into medium. The number of viable cells was counted in 24-h intervals for 10 days. The data were collected from 3 independent experiments. (*B*) After 3-day DMSO induction, the cells were collected for analysis of hemoglobin expression by using benzidine staining. (*C*) Total RNAs from transduced cells incubated with DMSO for 3 days were prepared and analyzed for RT-PCR.