Growth Factor Independence (GFI) 1B-mediated transcriptional repression and lineage allocation require Lysine Specific Demethylase (LSD)1-dependent recruitment of the BHC complex

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Running Title

GFI1B function requires BHC complex binding via LSD1

ABSTRACT

Growth Factor Independence (GFI)1B coordinates assembly of transcriptional repressor complexes comprised of co-repressors and histone modifying enzymes to control gene expression programs governing lineage allocation in hematopoiesis. Enforced expression of GFI1B in K562 erythroleukemia cells favors erythroid over megakaryocytic differentiation, providing a platform to define molecular determinants of binary fate decisions triggered by GFI1B. We deployed proteome-wide proximity labeling to identify factors whose inclusion in GFI1B complexes depends upon GFI1B's obligate effector, Lysine Specific Demethylase (LSD)1. We show that GFI1B preferentially recruits core and putative elements of the BRAF-histone deacetylase (HDAC) (BHC) chromatin remodeling complex (LSD1, RCOR1, HMG20A, HMG20B, HDAC1, HDAC2, PHF21A, GSE1, ZMYM2 and ZNF217) in an LSD1-dependent manner to control erythroid fate specification. Among these, depletion of both HMG20A and HMG20B, or GSE1 block GFI1B-mediated erythroid differentiation, phenocopying impaired differentiation brought on by LSD1 depletion or disruption of GFI1B—LSD1 binding. These findings demonstrate the central role of the GFI1B—LSD1 interaction as a determinant of BHC complex recruitment to enable cell fate decisions driven by GFI1B.

INTRODUCTION

Transcriptional regulation is critical for cell growth, homeostasis, and fate determination in multicellular organisms. In hematopoiesis, cell surface receptors, signaling networks, transcription factors and their effectors cooperate to influence chromatin state and gene expression, directing hematopoietic progenitors toward specific fates, and once achieved, ensuring proper function for each cell type. Failure to properly control chromatin structural dynamics governing stage-specific gene expression programs can cause qualitative and quantitative hematopoietic disorders, including malignancies. Failures can arise from altered expression of transcription factors or from malfunction or misdirection of their partners (1-3). Thus, knowing the composition of protein complexes that regulate gene expression by cell fate specifying transcription factors is essential to understanding normal and malignant hematopoiesis, and may have therapeutic relevance (4).

Growth Factor Independence (GFI) family transcriptional repressors, GFI1 and GFI1B, are master regulators of developmental hematopoiesis (5, 6). GFI1 controls hematopoietic stem cell self-renewal, B- and T-lymphopoiesis, and directs the granulocyte versus monocyte fate decision during late stages of myeloid differentiation (7-9). Notably, *Gfi1*-null mice are viable, but are characterized by severe neutropenia with compensatory monocytosis and sensorineural hearing loss (10-12). Altered *GFI1* expression is found in multiple hematologic malignancies, most notably acute myeloid leukemia (AML) and T-cell acute lymphoblastic leukemia (T-ALL) (13-17). Moreover, loss of function mutations in *GFI1* cause severe congenital neutropenia (SCN) type 2 and nonimmune chronic idiopathic neutropenia (CIN) of adults, both of which predispose to AML (18, 19). GFI1B is required for definitive erythropoiesis and megakaryopoiesis, and

like GFI1, also helps to maintain hematopoietic stem cell quiescence (20-23). In mice, *Gfi1b* nullizygosity is lethal at embryonic day (E) 15 due to erythropoietic failure (24). Mutations in *GFI1B* are associated with both qualitative and quantitative disorders of platelets and elevated *GFI1B* expression is found in AML (25-28). However, the role of GFI1B in AML is uncertain. Despite *GFI1B* often being increased in AML patients (29-32), a recent study suggests low *GFI1B* expression correlates with poor patient outcome and that in mice, *Gfi1b* depletion accelerates progression to AML from myelodysplasia driven by a NUP98-HOXD13 fusion protein (33). Collectively, these observations suggest widespread contributions by GFI family proteins to lineage allocation during normal hematopoiesis and both oncogenic and oncosuppressor roles in hematologic malignancies. A clear understanding of GFI1/1B contributions to hematopoiesis and malignancy requires comprehensive accounting of their protein partners and their dependency relationships during cell fate decision making.

GFI family proteins feature a shared structural organization (5, 34). Both GFI1 and GFI1B are comprised of a Snail/Slug/Gfi1 (SNAG) domain and a highly conserved concatemer of six C₂H₂-type zinc-fingers (ZnF) at their N- and C-termini, respectively. Linker regions with limited conservation separate the SNAG and ZnF domains. Sequence-specific DNA binding by both GFI1 and GFI1B is coordinated by ZnFs 3, 4 and 5 (32, 35-37), while ZnFs 1, 2, and 6 enable protein—protein interactions with an incompletely defined subset of transcriptional partners (38-41). Linker regions provide a platform for protein binding and post-translational modifications that regulate innate functions of GFI proteins (40, 42). GFI1 and GFI1B SNAG domains differ only by a conservative S→T substitution at amino acid 14, but are otherwise identical. Moreover,

the SNAG domain is near invariant among orthologs from human to zebrafish, underscoring the critical role of the SNAG domain and its binding partners. Prominent among these is Lysine Specific Demethylase (LSD)1 (KDM1A, AOF2, BHC110, NPAO, KIAA0601), which is required for transcriptional repression by GFI proteins (10, 43). The SNAG domain is necessary and sufficient for LSD1 binding by GFI1 and GFI1B (44). SNAG domain deletion, alanine substitution at proline 2 (P2A) or leucine substitution at lysine 8 (K8L) of the SNAG domain abolishes LSD1 binding to GFI family proteins and profoundly impairs their activity in multiple functional assays (43, 45-47).

LSD1 is a flavin-dependent monoamine oxidase that demethylates mono- and dimethylated histone 3 lysines 4 and 9 (H3K4me1/2 and H3K9me1/2) to impact chromatin structure and gene expression (48, 49). LSD1 also demethylates non-histone targets including p53, STAT3, ER α , MYPT1, HIF1 α , DNMT1, E2F and SOX2, suggesting broad influence over cellular homeostasis (50-57). LSD1 operates within multiple assemblies that regulate transcription, including Nucleosome Remodeling and Deacetylase (NuRD), C-terminal Binding Protein (CtBP), Mixed Lineage Leukemia (MLL) coactivator and BRAF-Histone deacetylase (BHC) complexes (58-62). Its inclusion in distinct multiprotein assemblies suggests both enzymatic and structural contributions to transcriptional control, yet affiliations between LSD1-containing complexes and the DNA binding proteins that recruit them are poorly understood.

We leveraged GFI1B-driven erythroid differentiation in K562 cells, GFI1B fusion proteins with a promiscuous biotin ligase (BirA*) (63, 64) and unbiased mass-spectral quantitation to define proximity relationships between GFI1B and partners that depend upon GFI1B—LSD1 binding. We show that GFI1B—LSD1 binding is necessary but not

sufficient for GFI1B-mediated erythroid differentiation in K562 cells. We also show that recruitment of core (65) (LSD1, RCOR1, HDAC1, HDAC2, HMG20B and PHF21A) and putative (66, 67) (HMG20A, GSE1, ZMYM2, and ZNF217) BHC complex components by GFI1B depends upon GFI1B—LSD1 binding and that depletion of candidate BHC complex proteins HMG20A and HMG20B, as well as GSE1 impairs GFI1B-mediated erythroid differentiation even though GFI1B—LSD1 interaction potential remains. Our results show that GFI1B-mediated lineage allocation depends upon LSD1’s function as a scaffolding component for BHC complex assembly and highlight the need to consider protein assemblies as modules which act in concert as determinants of cell fate.

RESULTS

LSD1 depletion impairs GFI1B-mediated erythroid differentiation

The human erythroleukemia cell line, K562, displays erythroid differentiation with enforced GFI1B expression (68), reflected by hemoglobinization and benzidine positive staining (Fig. 1A). Likewise, K562 cells adopt megakaryocytic features, including CD61 expression, with tetradecanoyl-1-phorbol-13-acetate (TPA) treatment (Fig. 1B). These dual fates are mutually exclusive, enabling GFI1B dependency relationships relevant to fate specification to be discerned. Notably, deletion of the GFI1B SNAG domain or placement of an epitope tag at the GFI1B N-terminus abolishes GFI1B-driven erythroid differentiation, suggesting primacy of the SNAG domain for this phenotype (68-70). Being necessary and sufficient for LSD1 binding, we surmised that disrupting or blocking access to the SNAG domain would impair LSD1 recruitment, and by extension, that LSD1 function would be required for GFI1B-driven erythroid differentiation. To confirm this, we

depleted K562 cells of LSD1 and tested GFI1B's ability to induce erythroid differentiation and for TPA to trigger cell surface expression of CD61. Immunoblots show depletion of LSD1 by two distinct shRNA constructs relative to a scramble control sequence, and equivalent expression of GFI1B (Fig. 1C). LSD1 depletion almost completely eliminates the detection of benzidine positive cells in response to enforced expression of GFI1B compared to controls yet had limited impact on CD61 expression at the cell surface following TPA treatment (Fig. 1D and E). LSD1 depletion also blocked expression of alpha (*HBA1/2*) and beta (*HBB*) globins and alpha hemoglobin stabilizing protein (*AHSP*), as well as 5-aminolevulinic acid synthase (*ALAS2*) and ferrochelatase (*FECH*), which are rate limiting enzymes required for heme biosynthesis. Expression of the erythroid fate marker glycophorin A (*GYPA*) was also significantly impaired (Fig. 1F and G). To confirm contributions to GFI1B-mediated transcriptional repression, we examined mRNA levels for GFI1B direct targets *CDKN1A*, *GFI1B* and *MYB* in the context of LSD1 depletion. Repression of each gene was reversed by LSD1 depletion (Fig. 1H). Together these data show that LSD1 enables transcriptional repression by GFI1B to trigger an erythroid fate specifying program in K562 cells and that LSD1 is dispensable for TPA-driven megakaryocytic differentiation.

GFI1B—LSD1 binding is required for GFI1B-mediated erythroid differentiation

LSD1 cooperates with an extensive roster of DNA binding proteins to influence gene expression (71), and therefore could influence this binary fate decision through partnerships with factors other than GFI1B. To test whether an interaction between GFI1B and LSD1 is needed for GFI1B-mediated erythroid differentiation, we compared K562

cells with enforced expression of GFI1B-WT or its -P2A derivative. The P2A substitution abolishes GFI1B—LSD1 binding, as well as transcriptional repression by fusion proteins between Gal4 and either GFI1B or the SNAG domain in isolation (Fig. 2A and B). While GFI1B-WT triggers an erythroid differentiation program in K562 cells, as reflected by hemoglobinization and erythroid-specific gene expression, GFI1B-P2A fails to do so (Fig. 2C-F). GFI1B-WT expression also blocks TPA-induced megakaryocytic differentiation in K562 cells, yet GFI1B-P2A displays no such antagonism (Fig. 2G). Additionally, failure by GFI1B-P2A either to trigger erythroid differentiation or to block CD61 expression in response to TPA correlates with impaired transcriptional repression of direct GFI1B targets (Fig. 2H). These findings indicate LSD1 recruitment by GFI1B is necessary to direct the erythroid fate-specifying program and is not required for TPA-induced megakaryocytic differentiation.

GFI1B partners identified by proximity-dependent biotinylation

GFI1B—LSD1 binding is required for GFI1B-mediated transcriptional control and fate specifying functions in hematopoiesis (43, 45). However, LSD1 is a constituent of multiple transcriptional regulatory complexes. Which among these governs GFI1B function to direct erythroid differentiation is unknown. To more comprehensively catalogue GFI1B partners, we engineered a chimeric protein, GFI1B-BirA*:HA, comprised of the complete GFI1B primary structure fused in frame with a promiscuous variant of the biotin ligase BirA (BirA*) from *Aquifex aeolicus* (63, 64, 72) and a C-terminal HA epitope tag under the control of a doxycycline-inducible promoter. Proteins with a proximity relationship to BirA*:HA or GFI1B-BirA*:HA are biotinylated spontaneously and

can be further characterized (Fig. 3A). GFI1B-BirA*:HA or BirA*:HA were inducibly expressed in K562 cells and proteome-wide biotinylation was compared to K562 cells transduced with vector only. GFI1B-BirA*:HA and BirA*:HA were equivalently expressed and produced a broad array of biotinylated protein substrates as reflected by streptavidin (SAv):HRP-mediated detection in whole cell extracts (Fig. 3B). Both qualitative and quantitative differences in the pattern of biotinylated proteins are apparent, suggesting preferential labeling of targets by BirA*:HA when anchored to GFI1B. In the vector control, a single 72kD band was seen, likely representing propionyl-CoA carboxylase expression in K562 cells (73). To further validate the proximity labeling technique, we confirmed enrichment for established GFI1B associated proteins, LSD1 and RCOR1, among biotinylated proteins from cells expressing GFI1B-BirA*:HA relative to those expressing non-targeted BirA*:HA. LSD1 binds directly to the GFI1/1B SNAG domain while RCOR1 engages GFI1B through LSD1 (43). LSD1 and RCOR1 were readily identified among biotinylated proteins from GFI1B-BirA*:HA-expressing cells collected on SAv-Sepharose beads, but only minimally so from cells expressing BirA*:HA or empty vector (Fig. 3C). Serving as an internal control for equal target biotinylation potential, GFI1B-BirA*:HA and BirA*:HA were comparably modified with biotin, purified on SAv-Sepharose and detected by α -HA western blot (Fig. 3C). These findings indicate preferential biotinylation of factors known to have a proximity relationship with GFI1B. Moreover, they confirm detection of 1° (intrinsic), 2° (direct binding) and 3° (indirect binding) labeling events and validate this platform for identifying GFI1B partners proteome-wide.

To comprehensively catalog GFI1B proximity partners, biotinylated proteins were collected and purified on SAv-Sepharose beads from whole cell extracts of K562 cells

inducibly expressing BirA*:HA or GFI1B-BirA*:HA. Assays were performed in triplicate. Purified proteins were denatured, fractionated over acrylamide gels, excised, subjected to trypsin digestion *in situ*, then analyzed by LC-MS-MS to unequivocally establish their identities and extrapolate their abundance from sum read intensities (Supplementary Data S1). Fold enrichment (GFI1B-BirA*:HA vs. BirA*:HA only) was determined using average sum read intensities from triplicate analysis for proteins purified from GFI1B-BirA*:HA-expressing cells compared to those expressing BirA*:HA only. *P*-values were determined with a two-tailed Student's *t*-test using variances of the 20 closest ranked proteins to account for differences in protein abundance across the data set. Fold enrichment (x-axis) for each protein is shown relative to *p*-value (y-axis) in a volcano plot with logarithmic axes, and statistically significant outliers are labeled (on the plot, and inset) (Fig. 3D). Notably, we observed hemoglobinization of cells expressing GFI1B-BirA*:HA comparable to those expressing GFI1B only, but not for cells expressing BirA*:HA (data not shown).

GFI1B is best known as a transcriptional repressor, and its contributions to multiple functional assays can be traced to this role (20, 74-79). Therefore, we focused first on partnerships between GFI1B and determinants of chromatin structure and transcriptional control. In the GFI1B-BirA*:HA proximitytome, we identified elements of multiple complexes highly relevant to regulated gene expression (Fig. 4). Components of protein complexes enriched in the GFI1B-BirA*:HA proximitytome relative to BirA*:HA control are shaded black. Those present, but not significantly above control are shaded gray and those not found in the GFI1B-BirA*:HA proximitytome are shown on white background. Prominent among those proteins present and enriched in the GFI1B-BirA*:HA proximitytome are

members of the BHC, CtBP and BAF complexes, from which we discovered 10/10 (core + putative), 7/8 and 13/15 complex members, respectively. Similarly, we observed each component of the NuRD complex in the GFI1B-BirA*:HA proximitome, yet only a subset of these was found enriched relative to the BirA*:HA control. Components from several other complexes (NuRF, CHRAC, canonical and non-canonical PRC1, PRC2, SIN3A and NCoR) were found, and for each, some elements enriched, suggesting they also contribute to GFI1B-mediated transcriptional control and lineage allocation. Noteworthy is identification of GFI1B, being physically tethered to BirA*, as the most highly enriched protein found in this unbiased screen, further validating the strategy from a quantitative perspective.

Identification of LSD1-dependent GFI1B binding partners

LSD1 binding is necessary for GFI1B function (10, 43). However, discovering elements from functionally distinct transcriptional regulatory complexes within the GFI1B proximitome suggests an important interplay between these elements and LSD1. Moreover, at least three of these complexes (BHC, NuRD and CtBP) contain LSD1 as a core component (58, 60, 80). Given the importance of LSD1 for GFI1B function, we hypothesized one or more transcriptional regulatory complexes represented in the GFI1B proximitome would be recruited in an LSD1-dependent manner, and if its components were depleted, would impair GFI1B function even when GFI1B—LSD1 binding potential is preserved. To address this hypothesis, we engineered expression constructs encoding fusion proteins comprised of BirA* and either LSD1 binding-competent forms of GFI1B (GFI1B-BirA*:HA and SNAG-BirA*:HA) or GFI1B variants with impaired LSD1 binding

(GFI1B-P2A-BirA*:HA and GFI1B- Δ SNAG-BirA*:HA). A BirA*:HA construct served as an additional negative control (Fig. 5A). Fusion proteins were inducibly expressed in K562 cells and their biotinylating ability compared in whole cell lysates by SDS-PAGE, transblotting and SAv:HRP detection (Fig. 5B). Equivalent expression and robust, proteome-wide biotinylation were observed for each construct induced with doxycycline. We then tested the ability of each fusion protein to establish proximity relationships with known SNAG domain binding proteins, LSD1 and RCOR1 (43, 45). As expected, GFI1B-BirA*:HA and SNAG-BirA*:HA display clear enrichment for biotinylated LSD1 and RCOR1 in SAv-Sepharose pull downs, while LSD1 non-binding variants (P2A and Δ SNAG) show biotinylation comparable to background seen for the BirA*:HA negative control (Fig. 5C). Equally important, we observed BirA*:HA biotinylation comparable to each of its fusion proteins, suggesting equal capacity for spontaneous biotinylation and mitigating concerns that the fusion partner might adversely impact BirA* enzyme activity. Collectively, these results support use of GFI1B variants to unambiguously define the LSD1-dependent elements of GFI1B complexes governing transcriptional control and cell fate specification.

In pursuit of this goal, we deployed these tools for quantitative proximitome analyses to compare partnerships between GFI1B forms which are either capable of (GFI1B-BirA*:HA and SNAG-BirA*:HA) , or deficient in (GFI1B-P2A-BirA*:HA, GFI1B- Δ SNAG-BirA*:HA and BirA*:HA) LSD1 binding. Fusion protein expression was induced by addition of doxycycline and biotinylated proteins collected on SAv-Sepharose beads as above. After high stringency washing, affinity purified proteins were analyzed by LC-MS-MS as described above. Triplicate read intensities were used to calculate an average sum read intensity for each protein identified in the mass spectral screen. Proteins were

ranked according to average sum read intensities in the LSD1 non-binding control and fold enrichment for each protein was calculated as a ratio of average sum read intensities between LSD1 binding and non-binding variants. *P*-values were calculated from variation among the twenty nearest ranked neighbors for each protein in the data set. Compared to LSD1 non-binding variants GFI1B- Δ SNAG-BirA*:HA (Fig. 6A) and GFI1B-P2A-BirA*:HA (Fig. 6B), we observed enrichment for nine of the ten core (LSD1, RCOR1, PHF21A, HMG20B, HDAC1 and HDAC2) and putative (GSE1, HMG20A, and ZMYM2) components of the BHC complex in the GFI1B-BirA*:HA proximitome. These results indicate BHC complex recruitment is lost with impaired GFI1B—LSD1 binding. To focus specifically on the SNAG—LSD1 interaction as a determinant of BHC complex recruitment, we determined enrichment for BHC complex components in the SNAG-BirA*:HA proximitome relative to BirA*:HA only. Again, we find enrichment for these nine BHC complex components in the SNAG-BirA*:HA proximitome.

LSD1, HDAC1, and HDAC2 are shared among the BHC, CtBP and NuRD complexes, while RCOR1 is found in both BHC and CtBP complexes. To confirm the LSD1-dependence of BHC complex recruitment specifically, we surveyed the top 100 enriched proteins in each binary proximitome comparison (GFI1B-BirA*:HA vs. GFI1B- Δ SNAG-BirA*:HA, GFI1B-BirA*:HA vs. GFI1B-P2A-BirA*:HA, and SNAG-BirA*:HA vs. BirA*:HA) for the unique components of BHC, CtBP and NuRD complexes (Table 1 and Table S1). While unique components of the BHC complex were found in each binary proximitome comparison performed, none of those unique to the CtBP or NuRD complexes were observed. These findings indicate that among LSD1-containing

complexes detected in the GFI1B proximitome, only the BHC complex displays LSD1-dependence.

GFI1B-mediated transcriptional repression and cell fate require the BHC complex

To confirm findings from mass spectral screens, we first assessed associations between GFI1B and candidate BHC complex components and tested their dependence upon GFI1B—LSD1 binding. We focused on unique elements of the BHC complex. GFI1B-BirA*:HA, GFI1B-P2A-BirA*:HA, GFI1B-ΔSNAG-BirA*:HA or SNAG-BirA*:HA were inducibly expressed in K562 cells and biotinylated proteins purified on SA-Sepharose beads. HMG20A, HMG20B, and PHF21A were then quantified among purified proteins by western blot. Each was enriched among proteins purified from cells expressing GFI1B-BirA* fusions capable of LSD1 binding relative to non-binding controls (Fig. 7A). Notably, no such enrichment was observed for SMARCB1 or SMARCC1, members of the SWI/SNF complex that showed enrichment but no LSD1 dependence in GFI1B proximitome analyses. Nor did SMARCB1 or SMARCC1 show a proximity relationship when SNAG-BirA*:HA was employed, confirming a distinct and LSD1-independent means of associating with GFI1B. Using flag-tagged wild type and -P2A forms of GFI1B, we extended findings from proximity labeling studies to traditional co-precipitation assays. For HMG20A, HMG20B, and PHF21A, binding to GFI1B-P2A was significantly impaired relative to wild type GFI1B, confirming dependence upon LSD1 recruitment (Fig. 7B-D). This observation is further supported by co-precipitation assays showing interactions with LSD1 for both HMG20A and HMG20B (Fig. 7E).

LSD1 is required for both GFI1B function and BHC complex recruitment, but whether LSD1 exerts its impact through BHC complex recruitment is not known. To address this question, we targeted unique BHC complex components for depletion in K562 cells then tested GFI1B's ability to direct changes in cell fate and gene expression (Fig. 8). HMG20A and HMG20B are known to bind structured elements in DNA in a sequence non-specific manner (81-83). They have been shown to have an oppositional role in neuronal differentiation via modulation of REST-responsive genes (80, 84). Their contributions to hematopoietic differentiation have not been described. HMG20A and HMG20B were depleted alone or together using inducible shRNA constructs (Fig. 8A), then effects of enforced GFI1B expression on K562 cell differentiation and gene expression were assessed. While neither HMG20A nor HMG20B depletion alone had a notable impact on K562 cell hemoglobinization, simultaneous depletion of both factors prevented this GFI1B-driven phenotype (Fig. 8B). Likewise, impaired TPA-induced megakaryocytic differentiation brought on by enforced GFI1B expression was not significantly affected by depletion of either HMG20A or HMG20B alone. Yet, their concurrent depletion enabled accumulation of CD61 on the K562 cell surface despite GFI1B overexpression (Fig. 8C). Erythroid differentiation blockade brought on by concurrent depletion of HMG20A and HMG20B correlated with reduced expression of genes required for heme and globin chain synthesis and with loss of repression of GFI1B target genes (Fig. 8D-F). Notably, LSD1 expression is unaffected by HMG20A or HMG20B depletion (Fig. 8A). Thus, HMG20A and HMG20B make functional contributions to LSD1-dependent outcomes driven by GFI1B. Their concurrent absence impairs GFI1B-driven erythroid fate in K562 cells and enables alternate, TPA-driven megakaryocytic

differentiation in the context of enforced GFI1B expression that would normally block it. Moreover, unlike the oppositional relationship between HMG20A and HMG20B observed in REST-regulated neural differentiation, these findings intimate functional redundancy between them in GFI1B-mediated transcription and its control of cellular identity.

To extend observations made with HMG20A and HMG20B, we tested the effects of GSE1 depletion on GFI1B functions in transcriptional repression and erythroid fate specification in K562 cells. GSE1 is a proline-rich coiled-coil domain protein implicated in pathogenesis of breast and gastric cancers (85, 86). It is widely expressed in human cells, but its role in cell fate decisions in hematopoiesis have not been studied. As for HMG20 proteins, reduced GSE1 expression blocks erythroid differentiation and gene expression changes in K562 cells which depend upon the GFI1B—LSD1 binding relationship (Fig. 9). Together with results from LSD1, HMG20A and HMG20B depletion, these data highlight that LSD1 is necessary but not sufficient for GFI1B-driven outcomes and showcase a critical role for the BHC complex as an LSD1-dependent determinant of GFI1B-mediated transcriptional repression and cell fate decisions in hematopoiesis.

DISCUSSION

The control of gene expression involves DNA binding proteins that recognize discrete sequence elements in regulatory regions of genes, directing site-selective assembly of multiprotein complexes that modulate chromatin structure, and ultimately, access to coding regions by the basal transcription machinery (87, 88). By deploying individual elements in a modular fashion, cells insure flexibility and economy as they orchestrate transcriptional programs to control the specification of cell fate. Proper

function of these complexes is layered upon a spatial and temporal order of events that combine to create a flow of information needed for a specific outcome among the myriad of possibilities. Yet, this strategy necessarily creates dependency relationships between factors cooperating to accomplish functions required for an outcome to be realized. Malfunction of just one complex component, or disordered operation of a complex in space or time may impair functional outputs and thus mask the underlying molecular complexities required to achieve them.

The relationship between GFI family proteins and LSD1 exemplifies this theme. Both GFI1 and GFI1B require LSD1 binding for transcriptional repression and point mutations in their SNAG domains completely abolish activity in multiple functional assays (43, 45, 46, 89). It is not, however, immediately apparent that other factors might enable LSD1's central role, and identifying these collaborators is technically challenging. We deployed an unbiased, proteome-wide proximity labeling strategy based upon a promiscuous variant of BirA (BirA*) to prospectively and systematically label with biotin factors that dwell in the vicinity of GFI1B and then to determine functionally relevant partners which are recruited in a manner requiring LSD1, its obligate effector. In so doing, we show that while LSD1 recruitment is critical to GFI1B-driven erythroid differentiation, it does not operate alone as its singular depletion in K562 cells may suggest. Rather, LSD1 is responsible for selective recruitment of the BHC complex, implying that its contributions to GFI1B-mediated outcomes may reflect both its intrinsic demethylase activity and its role as a scaffolding component for this multiprotein complex. A model summarizing the collaborative relationship between LSD1, BHC complex components and other partners in GFI1/1B-mediated transcriptional repression is shown (Fig. 10).

LSD1 displays *in vitro* demethylase activity toward peptides containing mono- and di-methylated lysines (K), best exemplified by demethylation of H3K4me1/me2, and toward purified histones (48, 90). However, in a chromatinized context, methylated histones are poor substrates for LSD1 (91). Efficient demethylation of chromatinized histones by LSD1 requires concurrent RCOR1 binding, and this activity can be further stimulated by an interaction between RCOR1 and SUMO2/3 (91, 92). Likewise, LSD1's demethylase activity is favored by HDAC1-mediated deacetylation of LSD1-K374ac (93). Observations such as these support the notion that LSD1's demethylase activity may hinge upon its inclusion in multiprotein complexes to enable its proper post-translational modifications and allosteric modulation. Additionally, by serving as a scaffold, LSD1 may enable regulation of other components of the BHC complex, or perhaps discrete regulatory modules recruited to operationalize GFI1B activity as a transcriptional repressor. Absent these cooperative relationships, LSD1 may fail to execute its obligate effector role for GFI1B despite the means of its recruitment by GFI1B being intact. Investigating these possibilities presents a daunting challenge, but one that can now be tackled using the situational capabilities of proximity labeling approaches. Moreover, a holistic view of LSD1 contributions to GFI1B's repressor and cell fate specifying activities is consistent with both its pivotal role and its dependence upon partnerships.

To establish evidence supporting this view, we focused on BHC complex components HMG20A and HMG20B as LSD1 partners. This focus was stimulated by studies in *Schizosaccharomyces pombe* concerning the role of Lsd1/2 in replication fork pausing associated with mating type class switching (94). Lsd1/2 is required for growth and viability in *S. pombe*. Notably, *S. pombe* strains rendered catalytically inactive at *lsd1/*

412 *lsd2* are viable and do not phenocopy *lsd1/lsd2* deletion, suggesting they have a non-
413 enzymatic role. Moreover, Lsd1 contains a C-terminal HMG domain that is lacking in
414 mammalian LSD1, and HMG domain mutations impair mating type class switching
415 through defective imprinting at the *mat1* locus brought on by dysfunctional replication fork
416 pausing (48, 94). The importance of the Lsd1 HMG domain to a phenotype dependent
417 upon structural change in DNA, the absence of the HMG domain in mammalian LSD1,
418 the suggestion of a non-enzymatic role for Lsd1 and the inclusion of HMG20 proteins in
419 the GFI1B proximitome made HMG20A and HMG20B attractive candidates in GFI1B—
420 LSD1- dependent outputs in K562 cells. Our finding that concurrent HMG20A/B depletion
421 blocks LSD1-dependent functions of GFI1B suggests that their inclusion in the BHC
422 complex is essential to enable LSD1 actions. Similar observations made by depleting
423 GSE1 reinforce this notion. Notably, HMG20A and HMG20B are ubiquitously expressed
424 (95). They contain N-terminal HMG boxes which bind DNA in a sequence non-specific
425 manner and C-terminal coiled-coil domains required for dimerization and incorporation
426 into multiprotein complexes (67, 84). In neural development, HMG20A and HMG20B
427 display an oppositional relationship at REST-regulated genes (65, 80, 84). Yet, our data
428 suggest these proteins may also compensate for deficiencies in one another and that
429 perhaps the relative abundance of each may fine tune transcriptional activity of the BHC
430 complex at sites to which it has been recruited. Thus, with site-selective DNA binding
431 proteins (e.g., GFI1B), epigenetic modifiers (e.g., LSD1, HDAC1/2), their cofactors (e.g.,
432 RCOR1), and readers of epigenetic state (e.g., PHF21A) as partners, a network of
433 interdependent proteins may cooperate to direct the quality, quantity and timing of
434 transcriptional programs that govern the specification of cell fate. Our findings provide a

framework for exploring the interdependent and situational relationships among these classes of proteins.

MATERIALS AND METHODS

Cell culture and viral transduction

COS7L, HEK293T (American Type Culture Collection), MEL and 293-T-Rex-5xGal-luciferase cells (generously provided by Raphael Margueron) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). K562 cells were maintained in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% FBS and 25 μ M HEPES. All cell culture media was supplemented with 2 μ M L-Glutamine, 50 units/ml penicillin, and 50 μ g/ml streptomycin. All cell media and additives were obtained from Thermo-Fisher. All cell lines were verified using the Geneprint 10 system (Promega). For viral production, HEK293T cells were transfected with a viral vector containing the transgene along with the respective lenti- (psPAX2 and pMD2G) or retroviral (gag/pol and VSVG) packaging plasmids using polyethylenimine (PEI) (3 μ g PEI:1 μ g DNA). Viral supernatant was collected 48- and 72-hours post-transfection and clarified by filtration (Corning, 45 μ m). K562 cells were transduced with viral particles in the presence of 8 μ g/ml polybrene for 16 hours. Stable cells were obtained by selecting with 5 μ g/ml puromycin for 4 days, 400 μ g/ml G418/neomycin or hygromycin for 10 days. For LSD1 and HMG20B small hairpin (shRNA) experiments, K562 cells were transduced with Tet-pLKO-neo (a gift from Dmitri Wiederschain, Addgene plasmid #21916) containing either LSD1-, HMG20B-targeted shRNA or scrambled control and selected in neomycin to establish stable isolates. For HMG20A

and GSE1 shRNA experiments, K562 cells were transduced with EZ-Tet-pLKO-Hygro (a gift from Cindy Miranti, Addgene plasmid #85972) containing either HMG20A-, GSE1-targeted shRNA or scrambled control and selected in hygromycin. To obtain double HMG20A and B shRNA knockdown, K562 cells were first transduced with either HMG20B or scrambled shRNA vector, selected with neomycin, and subsequently transduced with either HMG20A or scrambled shRNA vector and selected with hygromycin. shRNA expression was achieved by treating transduced cells with 500 ng/ml doxycycline every 24 hours. shRNA sequences are listed in Table S2.

RNA extraction and gene expression analysis

Total RNA was prepared using RNeasy (Qiagen), as per the manufacturer's instructions, including on-column DNase I digestion to avoid potential gDNA contamination. The cDNA was generated from 100-300 ng of total RNA using iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad). cDNA was diluted 2X with water, and 1 µl of cDNA was used as a template for real-time PCR (qPCR). Quantitation was performed by qPCR with the CFX96 Real Time System (Bio-Rad), using 5 µl SsoAdvanced Universal SYBR Green Supermix (Bio-Rad), and 330 nM of the indicated forward and reverse primers in a 10 µl reaction. Relative mRNA expression was determined by the $\Delta\Delta C_t$ method and normalized to a *GUS* reference gene. Each experiment was performed in triplicate. Statistical significance was determined by two-sided unpaired *t*-tests using GraphPad Prism 7.0. Sequences of all oligonucleotides used are listed in Table S2.

Constructs and subcloning

481 GFI1B (NM_008114.3), PHF21A (NM_008114.3), HMG20A (NM_025812.3), and
 482 HMG20B (NM_010440.3) were amplified by PCR (Platinum SuperFi DNA Polymerase,
 483 Invitrogen) from cDNA obtained from MEL cells and subcloned into pcDNA3.1(-)
 484 (Invitrogen) with either a 3x-flag, HA, or V5 tag on the 3' end of the gene. The GFI1B-P2A
 485 mutation was made by site-directed mutagenesis. Retroviral GFI1B:3x-flag vectors were
 486 produced by subcloning GFI1B into the pMSCV (murine stem cell virus)-IRES-Puro vector
 487 using EcoR1. GFI1B fusion proteins to Gal4 were generated by PCR amplification of the
 488 GFI1B SNAG domain (amino acid 1-20) or GFI1B- Δ 1C (amino acids 1-244 only—
 489 eliminating ZnF 4, 5 and 6, which remove intrinsic DNA binding via GFI1B sequence) and
 490 inserted into pcDNA3.1 N-terminally and in frame with the Gal4 DNA-binding domain
 491 (amino acids 1-147) by EcoR1/Xba1. GFI1B fusion proteins with BirA* were made by
 492 subcloning GFI1B-WT, -P2A, - Δ SNAG (amino acids 21-330), or the SNAG domain (amino
 493 acids 1-20) into EcoR1/BamH1 sites in frame to BirA* in the MCS-BioID2-HA vector, a
 494 gift from Kyle Roux (Addgene plasmid #74224). Subsequently, GFI1B-BirA*:HA forms
 495 were expressed from PCR amplified sequences subcloned into pLVX-Tight-Puro
 496 (Clontech) with Not1/Mlu1 for doxycycline inducible expression with the Tet-On Advanced
 497 (Clontech) system. BirA*:HA only vector was created by inserting a start codon and an
 498 EcoR1 site into Age1/BamH1 (5' of BirA*:HA) in pcDNA3.1 and subsequently put into
 499 pLVX-Tight-Puro by EcoR1. Primers used for cloning are listed in Table S2. The LSD1
 500 construct was a generous gift from Sunil Sharma. Complete open-reading frame
 501 sequences for all constructs were confirmed by automated di-deoxy sequencing in the
 502 University of Utah DNA sequencing HSC Core Facility.

503

Reagents and antibodies

Mouse monoclonal α -Flag (M2) and α -tubulin (clone B-5-1-2) were obtained from Sigma. Rabbit polyclonal α -HA (ab9110) and α -HMG20B (ab167415) were obtained from Abcam. Rabbit polyclonal α -LSD1 (C69G12) and α -RCOR1 (D6I2U) were obtained from Cell Signaling Technology. Mouse monoclonal α -HMG20A (D-5) and α -GFP (B-2) were obtained from Santa Cruz Biotechnology. Rabbit polyclonal α -SMARCB1 (A301-087A) and α -PHF21A (A303-603A) were obtained from Bethyl Laboratories and α -SMARCC1 (17722-1-AP) was obtained from Proteintech. Mouse monoclonal α -V5 (46-0705) was obtained from Invitrogen. Horse Radish Peroxidase (HRP) conjugated α -mouse (715-035-150) and α -rabbit (711-035-152) antibodies were obtained from Jackson ImmunoResearch. HRP-conjugated streptavidin (RPN1231) (SAv) was obtained from GE Healthcare UK Limited. APC α -human CD61 (VI-PI.2), and 4',6-diamidino-2-phenylindole, dilactate (DAPI) (422801) were obtained from BioLegend. Restriction endonucleases and ligases were obtained from New England Biosciences. Puromycin (puro), hygromycin (hygro), and G418/Geneticin (Neo) were obtained from Invitrogen. Protein-G Sepharose 4 Fast Flow and SAv-Sepharose beads were obtained from GE Healthcare Bio-Sciences AB. Biotin was obtained from TCI Chemicals and doxycycline (doxy) was obtained from Fisher Scientific. Benzidine di-hydrochloride, 12-O-Tetradecanoylphorbol-13-acetate (TPA), and polybrene were obtained Sigma. Polyethylenimine (PEI) 25kD Linear Form was obtained from Polysciences (#23966-2). All other materials were of reagent grade.

Transient transfection and co-immunoprecipitation

Transient transfections in COS7L cells were performed using expression plasmids in the indicated combination and Lipofectamine 2000 per the manufacturer's instructions. Monolayers were washed twice with phosphate-buffered saline (PBS), scraped into ice-cold lysis buffer (50 mM Tris, 150 mM NaCl, 10% glycerol, 1 mM EDTA, 1 mM dithiothreitol [DTT], 1% Triton X-100, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride [PMSF], 10 µg/ml aprotinin [pH 8.0]), disrupted by sonication (10 1-second pulses at 5W using Virtis Virsonic sonicator), rocked 30 min at 4°C, and clarified by centrifugation. As indicated by the figures, antibodies were added to clarified extracts for 90 min, then the immune complexes were captured by Protein-G-Sepharose beads that were washed in lysis buffer. Immune complexes were washed 4X with lysis buffer and boiled in SDS-PAGE sample buffer supplemented with DTT.

Immunoblotting

After separation by SDS-PAGE, cleared lysates or immune complexes were examined by immunoblot following standard methods. Briefly, separated protein products were transferred to nitrocellulose filters (0.45 µm, Genesee Scientific) using 25 mM Tris, 192 mM glycine (pH 8.35) for 1 hour at 4°C. Nitrocellulose filters were blocked with PBS-T (1X PBS, 0.05% Tween-20) supplemented with 0.25% gelatin and 0.2% NaN₃ (GBB) and incubated with primary antibodies overnight at 4°C as indicated in the figures. Filters were then washed successively with PBS-T and incubated with species-appropriate HRP-conjugated antibody for 1 hour at room temperature. After PBS-T washes, proteins were visualized by chemiluminescence detection (ECL; 30 mM Tris [pH 8.5], 375 µM Luminol, 67.5 µM p-Coumaric acid, 0.02% H₂O₂) using autoradiography film (Genesee Scientific).

549

550 **Luciferase reporter assays**

551 Luciferase assays were performed using the Dual-Luciferase Reporter Assay System
 552 (Promega) as per manufacturer's instructions. Briefly, 3.25×10^5 293-T-Rex-5xGal-
 553 luciferase cells were plated per well in 6-well plates. Transient transfection of the
 554 respective GFI1B:Gal4 expression constructs was performed with Lipofectamine 2000 as
 555 per manufacturer's instructions. Reporter activity was measured in cell lysates collected
 556 48 hours post transfection using a Modulus Microplate reader (Turner BioSystems).
 557 Firefly luciferase activity was normalized to constitutively expressed, co-transfected
 558 *Renilla* luciferase control. Statistical significance was determined by two-sided unpaired
 559 *t*-tests using GraphPad Prism 7.0.

560

561 **Chemical differentiation and phenotypic assays**

562 4×10^5 K562 cells were treated with 30 nM TPA. Cells were harvested 4 days post-
 563 induction, washed and incubated with α -CD61 antibody diluted 1:100 in Hanks Buffered
 564 Saline Solution (HBSS, Gibco) supplemented with 0.5% BSA (RPI Corp). Fluorescence
 565 signal from DAPI-negative cells was detected by flow cytometry (Fortessa, BD
 566 Biosciences) and analyzed using FlowJo analysis software (TreeStar). Heme/porphyrin
 567 analysis was analyzed by UPLC with an Acquity UPLC BEH C18, 1.7 μ M, 2.1 x 100 mm
 568 column performed at the Iron and Heme Core facility of the University of Utah. Benzidine
 569 staining was performed by collecting 1×10^6 K562 cells, washing with PBS, and staining
 570 with 0.2% benzidine dihydrochloride in 0.5% acetic acid for 15 minutes at room
 571 temperature. Benzidine positive cells were quantified by hemocytometer using bright-field

microscopy. Experiments were performed in triplicate with 200 cells or more counted from randomly selected fields for each condition. Statistical significance was determined by two-sided unpaired *t*-tests using GraphPad Prism 7.0.

Streptavidin affinity capture of biotin-modified proteins

To induce GFI1B-BirA*:HA in K562 cells, 6×10^7 cells were treated with 1 $\mu\text{g/ml}$ doxycycline for 48 hours. 30 μM biotin was added 16 hours before harvesting. Cells were collected, washed 2X with ice cold PBS, lysed with ice cold lysis buffer (50mM Tris pH7.5, 500 mM NaCl, 0.5 mM EDTA, 1% Triton X-100), sonicated and cleared by centrifugation. Biotinylated complexes were captured by SAv-Sepharose High Performance Beads and rocked at 4°C overnight. Beads were washed 5X with lysis buffer supplemented with SDS, boiled in DTT-containing sample buffer and fractionated by SDS-PAGE (10% T/2.67% C, 4% Stack). The gel was stained with Colloidal Coomassie Blue (Invitrogen). Lanes were divided into two equal sections for downstream LC-MS-MS analysis.

Protein identification by mass spectrometry

Mass spectrometry was performed by the Taplin Mass Spectrometry Facility, Cell Biology Department, Harvard Medical School. Briefly, samples were reduced using 1 mM DTT in 50 mM ammonium bicarbonate for 30 minutes at 60°C, cooled and 5 mM iodoacetamide in 50 mM ammonium bicarbonate added for 15 minutes in the dark at ambient. 5 mM DTT was added to quench iodoacetamide derivatization. 5 ng/ μl of sequencing-grad trypsin (Promega) was added and proteins digested overnight at 37°C. Samples were then desalted and reconstituted in 5-10 μl of HPLC solvent A (2.5% acetonitrile, 0.1% formic

acid). A nano-scale reverse-phase HPLC capillary column was created by packing 2.6 μm C18 spherical silica beads into a fused silica capillary (100 μm inner diameter x ~30 cm length) with a flame-drawn tip. After column equilibration, each sample was loaded via a Famos auto sampler (LC Packings) onto the column. A linear gradient was formed to elute peptides with increasing concentrations of solvent B (97.5% acetonitrile, 0.1% formic acid). Eluted peptides were subjected to electrospray ionization coupled to an LTQ Orbitrap Velos Pro ion-trap mass spectrometer (Thermo-Fisher Scientific) to produce tandem mass spectra whose *b*- and *y*-ion series patterns were compared to theoretical digests of the proteome in Sequest (Thermo-Fisher Scientific) to establish protein identity. Data were filtered to a 1-2% peptide false discovery rate.

Statistical analysis of mass spectrometry data

The read intensities were averaged across the biological triplicates for each sample. To establish the GFI1B proximitome (assessment of GFI1B-BirA* vs. BirA* only), sum read intensities among detected proteins were ranked from 0 to 100 percent. A total of 2751 proteins were detected in the BirA* only sample and were utilized to establish a background intensity ranking. The intensity fold change for each ranked protein was determined by dividing the GFI1B-BirA*:HA average read intensity by the BirA*:HA only average read intensity. This calculated fold change of the intensity was compared to the average intensity rank and was observed to follow a strong diminution of variance with increasing values as expected. To identify proteins with aberrantly increased detection, z-scores were calculated for each of the 2751 proteins as the deviance in rank from the localized (across the background intensity axis) mean rank compared to the localized

standard deviation of the ranks after the manner of Eubank (96). The corresponding p -value from a t -distribution of a 20-protein smoothing selection was determined and utilized to rank prioritize proteins showcasing the most consistent and increased abundance compared to expectation. Proteins with a p -value of <0.05 were considered hits. This approach was deemed appropriate as background intensity of pertinent proteins varied and detecting relative abundance compared to background was paramount. A volcano plot was created from the $-\log_{10}$ of the p -value and the $\log_2$ of the intensity fold change. Read intensities detected in GFI1B-BirA*:HA but not in the BirA*:HA only sample (500 proteins) were analyzed separately considering only the top 50-100 percentile of ranked GFI1B-BirA*:HA proximity partners as hits (a total of 35 proteins). Likewise, assessment of the GFI1B-BirA*:HA vs. GFI1B- Δ SNAG-BirA*:HA, GFI1B-BirA*:HA vs. GFI1B-P2A-BirA*:HA, and SNAG-BirA*:HA vs. BirA*:HA only was done by establishing a background percentile ranking of GFI1B- Δ SNAG-BirA*:HA (2897 proteins), GFI1B-P2A-BirA*:HA (2938 proteins), and BirA*:HA (2751 proteins). Proteins were then plotted according to background rank score and fold change. Statistical analysis was performed as described above for GFI1B-BirA*:HA vs. BirA*:HA using nonparametric regression and spline smoothing (97). SAS version 9.4 and Microsoft Excel were used for statistical calculations and data comparisons.

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REFERENCES

1. Feinberg AP, Koldobskiy MA, Göndör A. 2016. Epigenetic modulators, modifiers and mediators in cancer aetiology and progression. *Nat Rev Genet* 17:284–299.
2. Avgustinova A, Benitah SA. 2016. Epigenetic control of adult stem cell function. *Nat Rev Mol Cell Biol* 17:643–658.
3. Ntziachristos P, Abdel-Wahab O, Aifantis I. 2016. Emerging concepts of epigenetic dysregulation in hematological malignancies. *Nature Immunology* 17:1016–1024.

4. Egger G, Liang G, Aparicio A, Jones PA. 2004. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 429:457–463.
5. van der Meer LT, Jansen JH, van der Reijden BA. 2010. Gfi1 and Gfi1b: key regulators of hematopoiesis. *Leukemia* 24:1834–1843.
6. Lancrin C, Mazan M, Stefanska M, Patel R, Lichtinger M, Costa G, Vargel O, Wilson NK, Möröy T, Bonifer C, Göttgens B, Kouskoff V, Lacaud G. 2012. GFI1 and GFI1B control the loss of endothelial identity of hemogenic endothelium during hematopoietic commitment. *Blood* 120:314–322.
7. Zeng H, Yücel R, Kosan C, Klein-Hitpass L, Möröy T. 2004. Transcription factor Gfi1 regulates self-renewal and engraftment of hematopoietic stem cells. *EMBO J* 23:4116–4125.
8. Hock H, Hamblen MJ, Rooke HM, Schindler JW, Saleque S, Fujiwara Y, Orkin SH. 2004. Gfi-1 restricts proliferation and preserves functional integrity of haematopoietic stem cells. *Nature* 431:1002–1007.
9. Yücel R, Karsunky H, Klein-Hitpass L, Möröy T. 2003. The transcriptional repressor Gfi1 affects development of early, uncommitted c-Kit⁺ T cell progenitors and CD4/CD8 lineage decision in the thymus. *J Exp Med* 197:831–844.
10. Fiolka K, Hertzano R, Vassen L, Zeng H, Hermesh O, Avraham KB, Dührsen U, Möröy T. 2006. Gfi1 and Gfi1b act equivalently in haematopoiesis, but have distinct, non-overlapping functions in inner ear development. *EMBO reports* 7:326–333.
11. Karsunky H, Zeng H, Schmidt T, Zevnik B, Kluge R, Schmid KW, Dührsen U, Möröy T. 2002. Inflammatory reactions and severe neutropenia in mice lacking the transcriptional repressor Gfi1. *Nat Genet* 30:295–300.
12. Hock H, Hamblen MJ, Rooke HM, Traver D, Bronson RT, Cameron S, Orkin SH. 2003. Intrinsic requirement for zinc finger transcription factor Gfi-1 in neutrophil differentiation. *Immunity* 18:109–120.

13. Zörnig M, Schmidt T, Karsunky H, Grzeschiczek A, Moroy T. 1996. Zinc finger protein GFI-1 cooperates with myc and pim-1 in T-cell lymphomagenesis by reducing the requirements for IL-2. *Oncogene* 12:1789–1801.
14. Khandanpour C, Thiede C, Valk PJM, Sharif-Askari E, Nüchel H, Lohmann D, Horsthemke B, Siffert W, Neubauer A, Grzeschik K-H, Bloomfield CD, Marcucci G, Maharry K, Slovak ML, Van der Reijden BA, Jansen JH, Schackert HK, Afshar K, Schnittger S, Peeters JK, Kroschinsky F, Ehninger G, Lowenberg B, Dührsen U, Möry T. 2010. A variant allele of Growth Factor Independence 1 (GFI1) is associated with acute myeloid leukemia. *Blood* 115:2462–2472.
15. Khandanpour C. 2017. Growth factor independence 1 (Gfi1) regulates the AML supporting function of mesenchymal stromal cells. *Experimental Hematology* 53:S90.
16. Möry T. 2014. The zinc finger protein Gfi1 maintains development and progression of lymphoid leukemia by blocking the activation of the tumor suppressor p53. *Experimental Hematology* 42:S7.
17. Volpe G, Walton DS, Grainger DE, Ward C, Cauchy P, Blakemore D, Coleman DJL, Cockerill PN, Garcia P, Frampton J. 2017. Prognostic significance of high GFI1 expression in AML of normal karyotype and its association with a FLT3-ITD signature. *Sci Rep* 7:11148.
18. Xia J, Bolyard AA, Rodger E, Stein S, Aprikyan AA, Dale DC, Link DC. 2009. Prevalence of mutations in ELANE, GFI1, HAX1, SBDS, WAS and G6PC3 in patients with severe congenital neutropenia. *Br J Haematol* 147:535–542.
19. Person RE, Li F-Q, Duan Z, Benson KF, Wechsler J, Papadaki HA, Eliopoulos G, Kaufman C, Bertolone SJ, Nakamoto B, Papayannopoulou T, Grimes HL, Horwitz M. 2003. Mutations in proto-oncogene GFI1 cause human neutropenia and target ELA2. *Nat Genet* 34:308–312.
20. Randrianarison-Huetz V, Laurent B, Bardet V, Blobe GC, Huetz F, Dumenil D. 2010. Gfi-1B controls human erythroid and megakaryocytic differentiation by regulating TGF- signaling at the bipotent erythro-megakaryocytic progenitor stage. *Blood* 115:2784–2795.

21. Vassen L, Beauchemin H, Lemsaddek W, Krongold J, Trudel M, Mörröy T. 2014. Growth factor independence 1b (gfi1b) is important for the maturation of erythroid cells and the regulation of embryonic globin expression. *PLoS ONE* 9:e96636.
22. Singh D, Upadhyay G, Sengupta A, Biplob MA, Chakyayil S, George T, Saleque S. 2016. Cooperative Stimulation of Megakaryocytic Differentiation by Gfi1b Gene Targets Kindlin3 and Talin1. *PLoS ONE* 11:e0164506.
23. Beauchemin H, Shooshtarizadeh P, Vadrnais C, Vassen L, Pastore YD, Mörröy T. 2017. Gfi1b controls integrin signaling-dependent cytoskeleton dynamics and organization in megakaryocytes. *Haematologica* 102:484–497.
24. Saleque S, Cameron S, Orkin SH. 2002. The zinc-finger proto-oncogene Gfi-1b is essential for development of the erythroid and megakaryocytic lineages. *Genes Dev* 16:301–306.
25. Stevenson WS, Morel-Kopp M-C, Chen Q, Liang HP, Bromhead CJ, Wright S, Turakulov R, Ng AP, Roberts AW, Bahlo M, Ward CM. 2013. GFI1B mutation causes a bleeding disorder with abnormal platelet function. *J Thromb Haemost* 11:2039–2047.
26. Monteferrario D, Bolar NA, Marneth AE, Hebeda KM, Bergevoet SM, Veenstra H, Laros-van Gorkom BAP, MacKenzie MA, Khandanpour C, Botezatu L, Fransen E, Van Camp G, Duijnhouwer AL, Salemink S, Willemsen B, Huls G, Preijers F, Van Heerde W, Jansen JH, Kempers MJE, Loeys BL, Van Laer L, Van der Reijden BA. 2014. A dominant-negative GFI1B mutation in the gray platelet syndrome. *N Engl J Med* 370:245–253.
27. Uchiyama Y, Ogawa Y, Kunishima S, Shiina M, Nakashima M, Yanagisawa K, Yokohama A, Imagawa E, Miyatake S, Mizuguchi T, Takata A, Miyake N, Ogata K, Handa H, Matsumoto N. 2018. A novel GFI1B mutation at the first zinc finger domain causes congenital macrothrombocytopenia. *Br J Haematol* 181:843–847.
28. Kitamura K, Okuno Y, Yoshida K, Sanada M, Shiraishi Y, Muramatsu H, Kobayashi R, Furukawa K, Miyano S, Kojima S, Ogawa S, Kunishima S. 2016. Functional characterization of a novel GFI1B mutation causing congenital macrothrombocytopenia. *J Thromb Haemost*.

29. Vassen L, Khandanpour C, Ebeling P, Van der Reijden BA, Jansen JH, Mahlmann S, Dührsen U, Mörröy T. 2009. Growth factor independent 1b (Gfi1b) and a new splice variant of Gfi1b are highly expressed in patients with acute and chronic leukemia. *Int J Hematol* 89:422–430.
30. Elmaagacli AH, Koldehoff M, Zakrzewski JL, Steckel NK, Ottinger H, Beelen DW. 2007. Growth factor-independent 1B gene (GFI1B) is overexpressed in erythropoietic and megakaryocytic malignancies and increases their proliferation rate. *Br J Haematol* 136:212–219.
31. Hernández A, Villegas A, Anguita E. 2010. Human promoter mutations unveil Oct-1 and GATA-1 opposite action on Gfi1b regulation. *Annals of Hematology* 89:759–765.
32. Tong B, Grimes HL, Yang TY, Bear SE, Qin Z, Du K, El-Deiry WS, Tschlis PN. 1998. The Gfi-1B proto-oncoprotein represses p21WAF1 and inhibits myeloid cell differentiation. *Mol Cell Biol* 18:2462–2473.
33. Thivakaran A, Botezatu L, Hönes JM, Schütte J, Vassen L, Al-Matary YS, Patnana P, Zeller A, Heuser M, Thol F, Gabdoulline R, Olberding N, Frank D, Suslo M, Köster R, Lennartz K, Görgens A, Giebel B, Opalka B, Dührsen U, Khandanpour C. 2018. Gfi1b: a key player in the genesis and maintenance of acute myeloid leukemia and myelodysplastic syndrome. *Haematologica* 103:614–625.
34. Mörröy T, Vassen L, Wilkes B, Khandanpour C. 2015. From cytopenia to leukemia: the role of Gfi1 and Gfi1b in blood formation. *Blood* 126:2561–2569.
35. Zweidler-McKay PA, Grimes HL, Flubacher MM, Tschlis PN. 1996. Gfi-1 encodes a nuclear zinc finger protein that binds DNA and functions as a transcriptional repressor. *Mol Cell Biol* 16:4024–4034.
36. Lee S, Doddapaneni K, Hogue A, McGhee L, Meyers S, Wu Z. 2010. Solution structure of Gfi-1 zinc domain bound to consensus DNA. *J Mol Biol* 397:1055–1066.
37. Anguita E, Villegas A, Iborra F, Hernández A. 2010. GFI1B controls its own expression binding to multiple sites. *Haematologica* 95:36–46.

38. Nakazawa Y, Suzuki M, Manabe N, Yamada T, Kihara-Negishi F, Sakurai T, Tenen DG, Iwama A, Mochizuki M, Oikawa T. 2007. Cooperative interaction between ETS1 and GFI1 transcription factors in the repression of Bax gene expression. *Oncogene* 26:3541–3550.
39. Huang D-Y, Kuo Y-Y, Chang Z-F. 2005. GATA-1 mediates auto-regulation of Gfi-1B transcription in K562 cells. *Nucleic Acids Research* 33:5331–5342.
40. Marteiijn JAF, van der Meer LT, van Emst L, van Reijmersdal S, Wissink W, de Witte T, Jansen JH, Van der Reijden BA. 2007. Gfi1 ubiquitination and proteasomal degradation is inhibited by the ubiquitin ligase Triad1. *Blood* 110:3128–3135.
41. Dahl R, Iyer SR, Owens KS, Cuylear DD, Simon MC. 2007. The transcriptional repressor GFI-1 antagonizes PU.1 activity through protein-protein interaction. *J Biol Chem* 282:6473–6483.
42. Andrade D, Velinder M, Singer J, Maese L, Bareyan D, Nguyen H, Chandrasekharan MB, Lucente H, McClellan D, Jones D, Sharma S, Liu F, Engel ME. 2016. SUMOylation Regulates Growth Factor Independence 1 in Transcriptional Control and Hematopoiesis. *Mol Cell Biol* 36:1438–1450.
43. Saleque S, Kim J, Rooke HM, Orkin SH. 2007. Epigenetic regulation of hematopoietic differentiation by Gfi-1 and Gfi-1b is mediated by the cofactors CoREST and LSD1. *Mol Cell* 27:562–572.
44. Lin Y, Wu Y, Li J, Dong C, Ye X, Chi Y-I, Evers BM, Zhou BP. 2010. The SNAG domain of Snail1 functions as a molecular hook for recruiting lysine-specific demethylase 1. *EMBO J* 29:1803–1816.
45. Laurent B, Randrianarison-Huetz V, Frisan E, Andrieu-Soler C, Soler E, Fontenay M, Dusanter-Fourt I, Duménil D. 2012. A short Gfi-1B isoform controls erythroid differentiation by recruiting the LSD1-CoREST complex through the dimethylation of its SNAG domain. *J Cell Sci* 125:993–1002.
46. Velinder M, Singer J, Bareyan D, Mezmarich J, Tracy CM, Fulcher JM, McClellan D, Lucente H, Franklin S, Sharma S, Engel ME. 2017. GFI1 functions in transcriptional control and cell

fate determination require SNAG domain methylation to recruit LSD1. *Biochem J* 474:2951–2951.

47. Upadhyay G, Chowdhury AH, Vaidyanathan B, Kim D, Saleque S. 2014. Antagonistic actions of Rcor proteins regulate LSD1 activity and cellular differentiation. *Proc Natl Acad Sci USA* 111:8071–8076.

48. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, Shi Y. 2004. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 119:941–953.

49. Metzger E, Wissmann M, Yin N, Müller JM, Schneider R, Peters AHFM, Günther T, Buettner R, Schüle R. 2005. LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature* 437:436–439.

50. Huang J, Sengupta R, Espejo AB, Lee MG, Dorsey JA, Richter M, Opravil S, Shiekhataar R, Bedford MT, Jenuwein T, Berger SL. 2007. p53 is regulated by the lysine demethylase LSD1. *Nature* 449:105–108.

51. Yang J, Huang J, Dasgupta M, Sears N, Miyagi M, Wang B, Chance MR, Chen X, Du Y, Wang Y, An L, Wang Q, Lu T, Zhang X, Wang Z, Stark GR. 2010. Reversible methylation of promoter-bound STAT3 by histone-modifying enzymes. *Proc Natl Acad Sci USA* 107:21499–21504.

52. Zhang X, Tanaka K, Yan J, Li J, Peng D, Jiang Y, Yang Z, Barton MC, Wen H, Shi X. 2013. Regulation of estrogen receptor α by histone methyltransferase SMYD2-mediated protein methylation. *Proc Natl Acad Sci USA* 110:17284–17289.

53. Cho H-S, Suzuki T, Dohmae N, Hayami S, Unoki M, Yoshimatsu M, Toyokawa G, Takawa M, Chen T, Kurash JK, Field HI, Ponder BAJ, Nakamura Y, Hamamoto R. 2011. Demethylation of RB regulator MYPT1 by histone demethylase LSD1 promotes cell cycle progression in cancer cells. *Cancer Res* 71:655–660.

54. Lee J-Y, Park J-H, Choi H-J, Won H-Y, Joo H-S, Shin D-H, Park MK, Han B, Kim KP, Lee TJ, Croce CM, Kong G. 2017. LSD1 demethylates HIF1 α to inhibit hydroxylation and ubiquitin-mediated degradation in tumor angiogenesis. *Oncogene* 36:5512–5521.
55. Wang J, Hevi S, Kurash JK, Lei H, Gay F, Bajko J, Su H, Sun W, Chang H, Xu G, Gaudet F, Li E, Chen T. 2009. The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. *Nat Genet* 41:125–129.
56. Kontaki H, Talianidis I. 2010. Lysine methylation regulates E2F1-induced cell death. *Mol Cell* 39:152–160.
57. Zhang C, Hoang N, Leng F, Saxena L, Lee L, Alejo S, Qi D, Khal A, Sun H, Lu F, Zhang H. 2018. LSD1 demethylase and the methyl-binding protein PHF20L1 prevent SET7 methyltransferase-dependent proteolysis of the stem-cell protein SOX2. *J Biol Chem* 293:3663–3674.
58. Wang Y, Zhang H, Chen Y, Sun Y, Yang F, Yu W, Liang J, Sun L, Yang X, Shi L, Li R, Li Y, Zhang Y, Li Q, Yi X, Shang Y. 2009. LSD1 is a subunit of the NuRD complex and targets the metastasis programs in breast cancer. *Cell* 138:660–672.
59. Yang Y, Huang W, Qiu R, Liu R, Zeng Y, Gao J, Zheng Y, Hou Y, Wang S, Yu W, Leng S, Feng D, Wang Y. 2018. LSD1 coordinates with the SIN3A/HDAC complex and maintains sensitivity to chemotherapy in breast cancer. *J Mol Cell Biol* 387:49.
60. Ray SK, Li HJ, Metzger E, Schüle R, Leiter AB. 2014. CtBP and associated LSD1 are required for transcriptional activation by NeuroD1 in gastrointestinal endocrine cells. *Mol Cell Biol* 34:2308–2317.
61. Tsai M-C, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY. 2010. Long noncoding RNA as modular scaffold of histone modification complexes. *Science* 329:689–693.
62. Shi Y-J, Matson C, Lan F, Iwase S, Baba T, Shi Y. 2005. Regulation of LSD1 histone demethylase activity by its associated factors. *Mol Cell* 19:857–864.

63. Roux KJ, Kim DI, Raida M, Burke B. 2012. A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. *J Cell Biol* 196:801–810.
64. Kim DI, Jensen SC, Noble KA, Kc B, Roux KH, Motamedchaboki K, Roux KJ. 2016. An improved smaller biotin ligase for BioID proximity labeling. *Mol Biol Cell* 27:1188–1196.
65. Hakimi M-A, Bochar DA, Chenoweth J, Lane WS, Mandel G, Shiekhata R. 2002. A core-BRAF35 complex containing histone deacetylase mediates repression of neuronal-specific genes. *Proc Natl Acad Sci USA* 99:7420–7425.
66. Gocke CB, Yu H. 2008. ZNF198 stabilizes the LSD1-CoREST-HDAC1 complex on chromatin through its MYM-type zinc fingers. *PLoS ONE* 3:e3255.
67. Rivero S, Ceballos-Chávez M, Bhattacharya SS, Reyes JC. 2015. HMG20A is required for SNAI1-mediated epithelial to mesenchymal transition. *Oncogene* 34:5264–5276.
68. Garçon L, Lacout C, Svinartchouk F, Le Couédic J-P, Villeval J-L, Vainchenker W, Duménil D. 2005. Gfi-1B plays a critical role in terminal differentiation of normal and transformed erythroid progenitor cells. *Blood* 105:1448–1455.
69. Lam LT, Ronchini C, Norton J, Capobianco AJ, Bresnick EH. 2000. Suppression of erythroid but not megakaryocytic differentiation of human K562 erythroleukemic cells by notch-1. *J Biol Chem* 275:19676–19684.
70. Meng Y-S, Khoury H, Dick JE, Minden MD. 2005. Oncogenic potential of the transcription factor LYL1 in acute myeloblastic leukemia. *Leukemia* 19:1941–1947.
71. Maiques-Diaz A, Somervaille TC. 2016. LSD1: biologic roles and therapeutic targeting. *Epigenomics* 8:1103–1116.
72. Kim DI, Roux KJ. 2016. Filling the Void: Proximity-Based Labeling of Proteins in Living Cells. *Trends Cell Biol* 26:804–817.
73. Leon-Del-Rio A, Gravel RA. 1994. Sequence requirements for the biotinylation of carboxyl-terminal fragments of human propionyl-CoA carboxylase alpha subunit expressed in *Escherichia coli*. *J Biol Chem* 269:22964–22968.

74. Vassen L. 2005. Direct transcriptional repression of the genes encoding the zinc-finger proteins Gfi1b and Gfi1 by Gfi1b. *Nucleic Acids Research* 33:987–998.
75. Rodriguez P, Bonte E, Krijgsveld J, Kolodziej KE, Guyot B, Heck AJR, Vyas P, de Boer E, Grosveld F, Strouboulis J. 2005. GATA-1 forms distinct activating and repressive complexes in erythroid cells. *EMBO J* 24:2354–2366.
76. Kuo Y-Y, Chang Z-F. 2007. GATA-1 and Gfi-1B interplay to regulate Bcl-xL transcription. *Mol Cell Biol* 27:4261–4272.
77. Jegalian AG, Wu H. 2002. Regulation of Socs gene expression by the proto-oncoprotein GFI-1B: two routes for STAT5 target gene induction by erythropoietin. *J Biol Chem* 277:2345–2352.
78. Chowdhury AH, Ramroop JR, Upadhyay G, Sengupta A, Andrzejczyk A, Saleque S. 2013. Differential transcriptional regulation of meis1 by Gfi1b and its co-factors LSD1 and CoREST. *PLoS ONE* 8:e53666.
79. Vassen L, Fiolka K, Möröy T. 2006. Gfi1b alters histone methylation at target gene promoters and sites of gamma-satellite containing heterochromatin. *EMBO J* 25:2409–2419.
80. Ceballos-Chávez M, Rivero S, García-Gutiérrez P, Rodríguez-Paredes M, García-Domínguez M, Bhattacharya S, Reyes JC. 2012. Control of neuronal differentiation by sumoylation of BRAF35, a subunit of the LSD1-CoREST histone demethylase complex. *Proc Natl Acad Sci USA* 109:8085–8090.
81. Esteghamat F, van Dijk TB, Braun H, Dekker S, van der Linden R, Hou J, Fanis P, Demmers J, van IJcken W, Ozgür Z, Horos R, Pourfarzad F, Lindern von M, Philipsen S. 2011. The DNA binding factor Hmg20b is a repressor of erythroid differentiation. *Haematologica* 96:1252–1260.
82. Hock R, Furusawa T, Ueda T, Bustin M. 2007. HMG chromosomal proteins in development and disease. *Trends Cell Biol* 17:72–79.

83. Malarkey CS, Churchill MEA. 2012. The high mobility group box: the ultimate utility player of a cell. *Trends Biochem Sci* 37:553–562.
84. Wynder C, Hakimi M-A, Epstein JA, Shilatfard A, Shiekhattar R. 2005. Recruitment of MLL by HMG-domain protein iBRAF promotes neural differentiation. *Nat Cell Biol* 7:1113–1117.
85. Chai P, Tian J, Zhao D, Zhang H, Cui J, Ding K, Liu B. 2016. GSE1 negative regulation by miR-489-5p promotes breast cancer cell proliferation and invasion. *Biochem Biophys Res Commun* 471:123–128.
86. Ding K, Tan S, Huang X, Wang X, Li X, Fan R, Zhu Y, Lobie PE, Wang W, Wu Z. 2018. GSE1 predicts poor survival outcome in gastric cancer patients by SLC7A5 enhancement of tumor growth and metastasis. *J Biol Chem* 293:3949–3964.
87. Lambert SA, Jolma A, Campitelli LF, Das PK, Yin Y, Albu M, Chen X, Taipale J, Hughes TR, Weirauch MT. 2018. The Human Transcription Factors. *Cell* 172:650–665.
88. Clapier CR, Cairns BR. 2009. The biology of chromatin remodeling complexes. *Annu Rev Biochem* 78:273–304.
89. Grimes HL, Chan TO, Zweidler-McKay PA, Tong B, Tsichlis PN. 1996. The Gfi-1 proto-oncoprotein contains a novel transcriptional repressor domain, SNAG, and inhibits G1 arrest induced by interleukin-2 withdrawal. *Mol Cell Biol* 16:6263–6272.
90. Chen Y, Yang Y, Wang F, Wan K, Yamane K, Zhang Y, Lei M. 2006. Crystal structure of human histone lysine-specific demethylase 1 (LSD1). *Proc Natl Acad Sci USA* 103:13956–13961.
91. Yang M, Gocke CB, Luo X, Borek D, Tomchick DR, Machius M, Otwinowski Z, Yu H. 2006. Structural basis for CoREST-dependent demethylation of nucleosomes by the human LSD1 histone demethylase. *Mol Cell* 23:377–387.
92. Ouyang J, Shi Y, Valin A, Xuan Y, Gill G. 2009. Direct binding of CoREST1 to SUMO-2/3 contributes to gene-specific repression by the LSD1/CoREST1/HDAC complex. *Mol Cell* 34:145–154.

93. Nalawansha DA, Pflum MKH. 2017. LSD1 Substrate Binding and Gene Expression Are Affected by HDAC1-Mediated Deacetylation. *ACS Chem Biol* 12:254–264.
94. Holmes A, Roseaulin L, Schurra C, Waxin H, Lambert S, Zaratiegui M, Martienssen RA, Arcangioli B. 2012. Lsd1 and lsd2 control programmed replication fork pauses and imprinting in fission yeast. *Cell Rep* 2:1513–1520.
95. Sumoy L, Carim L, Escarceller M, Nadal M, Gratacòs M, Pujana MA, Estivill X, Peral B. 2000. HMG20A and HMG20B map to human chromosomes 15q24 and 19p13.3 and constitute a distinct class of HMG-box genes with ubiquitous expression. *Cytogenet Cell Genet* 88:62–67.
96. Eubank RL. 1999. *Nonparametric Regression and Spline Smoothing*, Second Edition. CRC Press.
97. Ke C, Wang Y. 2004. Smoothing Spline Nonlinear Nonparametric Regression Models. *Journal of the American Statistical Association* 99:1166–1175.

FIGURE AND TABLE LEGENDS

Figure 1. LSD1 depletion impedes GFI1B-mediated erythroid differentiation. (A) GFI1B overexpression promotes K562 cell hemoglobinization. Cells were infected with empty vector (EV) or virus expressing GFI1B. GFI1B-expressing cells become red and are benzidine positive. Benzidine positive cells are quantified as mean $\pm$ SD from four biological replicates. (B) TPA triggers cell surface expression of CD61 in K562 cells. CD61 expression was quantified in vehicle- vs. TPA-treated viable cells by flow cytometry. (C) LSD1 depletion and GFI1B overexpression in K562 cells. Small hairpin RNAs targeting *LSD1* (LSD1 shRNA) or content-matched scramble control (Scram shRNA) were inducibly transcribed in K562 cells followed by infection at day +3 with GFI1B:flag-expressing virus or EV. LSD1 and GFI1B were quantified by western blot of whole cell lysates (WCL). Tubulin served as a loading control. (D) LSD1 depletion blocks GFI1B-

mediated K562 cell hemoglobinization. (E) LSD1 depletion enables TPA-induced CD61 expression in the context of GFI1B overexpression. (F-G) LSD1 depletion impairs expression of erythroid fate genes in response to GFI1B overexpression. Alpha- and Beta-globins (*HBA1/2* and *HBB*), alpha globin stabilizing protein (*AHSP*), aminolevulinic acid synthase (*ALAS2*), ferrochelatase (*FECH*) and glycophorin (*GYP*)A expression are shown. (H) LSD1 depletion restores expression of GFI1B-repressed target genes. *CDKN1A*, *GFI1B* and *MYB* expression are shown. Gene expression is quantified by RT-qPCR normalized to *GUS*. Results are expressed as mean $\pm$ 2SD from two independent experiments performed in triplicate. * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$, **** $p < 0.00005$.

Figure 2. LSD1 binding is required for GFI1B-regulated cell fate decisions in K562 cells. (A) P2A substitution in GFI1B impairs GFI1B—LSD1 binding. K562 cells were transduced with constructs expressing wild type GFI1B, its P2A variant or empty vector (EV) as shown. GFI1B forms were immune purified with α -flag (M2) antibody and Protein-G Sepharose. LSD1 was quantified in immune complexes (IC) by western blot. Equal expression (LSD1 and GFI1B) and precipitation (GFI1B) were evaluated by western blot of whole cell lysates (WCL) and ICs, respectively. Tubulin was used to confirm equal gel loading. (B) P2A substitution abolishes transcriptional repression by GFI1B. 293-T-Rex-5xGal-luciferase cells were transfected with WT or P2A variants of GFI1B- Δ 1C:Gal4 (left) or SNAG:Gal4 (right) fusion proteins. Firefly luciferase was measured and normalized to constitutively expressed, co-transfected *Renilla* luciferase. Reporter activity is expressed as mean $\pm$ SD from two experiments performed in triplicate. (C) P2A substitution impairs GFI1B-mediated hemoglobinization. Benzidine staining was quantified as in 1A.

Protoporphyrin IX (PPIX) and heme were quantified by UPLC in K562 cells transduced with EV, GFI1B-WT or GFI1B-P2A. (D-F) P2A substitution impairs expression of erythroid fate genes in K562 cells. Expression of β -globin cluster genes, *AHSP*, *ALAS2*, *FECH*, *GYPA* and Kruppel-like factor (*KLF*)1 are shown. (G) P2A substitution abolishes GFI1B-mediated suppression of TPA-induced CD61 expression. Cell surface expression of CD61 in TPA treated cells expressing wild type GFI1B (WT) or its P2A variant was determined by flow cytometry. (H) P2A substitution reverses repression of GFI1B target genes *GFI1B*, *CDKN1A* and *MYB*. Results expressed as mean $\pm$ 2SD. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$; ****, $p < 0.00005$.

Figure 3. GFIB-BirA* defines a GFI1B proximitome. (A) Schematic representation of strategy for biotin modification of GFI1B partners. GFI1B is fused to the promiscuous biotin ligase with an HA epitope tag, BirA*:HA to form GFI1B-BirA*:HA. GFI1B partners are spontaneously biotinylated due to their proximity to BirA*:HA anchored to GFI1B in *cis*. Biotinylated products are captured on streptavidin (SAv)-conjugated beads for downstream analysis via candidate-based or screening approaches. (B) BirA*:HA and GFI1B-BirA*:HA fusion biotinylate diverse targets in K562 cells. Cells were transduced with constructs inducibly expressing GFI1B-BirA*:HA, BirA*:HA or EV, treated with doxycycline and harvested. GFI1B-BirA*:HA and BirA*:HA expression was confirmed by α -HA western blot and biotinylated proteins detected by SAv:HRP. Tubulin served as a loading control. (C) GFI1B interacting protein, LSD1 and RCOR1, are enriched among proteins biotinylated by GFI1B-BirA*:HA compared to BirA* or EV., Biotin-modified proteins were collected on SAv-Sepharose. LSD1, RCOR1 and the BirA* fusion proteins

themselves were quantified among purified proteins (SAv P) by western blot. Equal expression was confirmed by western blot in whole cell lysates (input). (D) GFI1B-BirA*:HA identifies members of a GFI1B proximitome. Factors enriched among biotinylated and SAv-Sepharose purified proteins modified by GFI1-BirA*:HA (test) compared to BirA*:HA (control). Fold enrichment is plotted logarithmically as a ratio of average sum read intensities between test and control samples against their *p*-values calculated from their 20 closest ranked neighbors in the data set as described in Materials and Methods. Individual proteins are labeled with their respective gene names. Inset shows an expanded view of the data contained in the boxed area.

Figure 4. Components of transcriptional repression and chromatin remodeling complexes identified by GFI1B proximity labeling. A roster of transcriptionally relevant complexes and their component members are shown. Proteins are listed according to HUGO gene nomenclature. With respect to the GFI1B proximitome (GFI1B-BirA*:HA vs. BirA*:HA), proteins present and enriched—white letters on black background. Those present by not significantly enriched ($p < 0.05$)—black letters on gray background. Not present (average sum read intensity below that for EV)—black letters on white background. Where appropriate, complexes are subdivided into core and putative members, and/or with canonical vs. non-canonical designations.

Figure 5. LSD1 non-binding variants of GFI1B-BirA* fail to biotinylate LSD1 and RCOR1. (A) Graphical representation of GFI1B-BirA*:HA fusion proteins employed in experiments. Each fusion protein is comprised of the GFI1B regions shown in frame with

the BirA* expression cassette and a C-terminal HA epitope tag to insure equivalent expression of all forms. The BirA*:HA construct lacks GFI1B structures and instead begins with an ATG. Wild type (WT) GFI1B is shown. The SNAG and zinc finger (ZnF) domains (1-6) of GFI1B are shown as black boxes. A thin line represents the GFI1B linker and connections between ZnFs. The proline to alanine substitution (P2A) in the GFI1B SNAG domain is indicated with an *. Δ SNAG represents deletion of the SNAG domain but preservation of the remaining GFI1B primary structure. (B) Expression of GFI1B-BirA*:HA forms and proteome-wide biotin modification in K562 cells. K562 cells were transduced with lentivirus to inducibly express GFI1B-BirA*:HA forms shown and stable isolates selected as polyclonal populations. Doxycycline-inducible expression of each fusion protein was confirmed by western blot against the common HA epitope tag and biotinylating activity confirmed proteome-wide by SAV:HRP detection of transblotted total cellular protein. Tubulin served as a loading control. (C) LSD1 and RCOR1 are enriched among biotinylated proteins generated by BirA*:HA fusions competent for LSD1 binding. Biotin-modified proteins from K562 cells transduced with BirA*:HA fusion proteins shown were purified from whole cell extracts (SAV P), fractionated by SDS-PAGE and subjected to western blot with α -LSD1, α -RCOR1 and α -HA antibodies. Equivalent expression of each protein in whole cell lysates (Input) was confirmed by western blot with these same antibodies.

Figure 6. GFI1B recruits the BHC complex in an LSD1-depend manner via the SNAG domain. K562 cells stably and inducibly expressing the BirA*:HA fusion proteins shown in 5A were deployed to define GFI1B proximity partners whose recruitment is LSD1-

dependent. Fusion protein expression was induced by doxycycline addition and biotinylation permitted *in situ*. Biotin-modified proteins were captured on SAv-Sepharose and analyzed by LC-MS-MS as described in Materials and Methods. (A-C) Dot plots are shown comparing fold change in average sum read intensity among fusion proteins with intact SNAG domains and capable of LSD1-binding (GFI1B-BirA*:HA and SNAG-BirA*:HA) to those with disrupted or absent SNAG domains and deficient in LSD1 binding (GFI1B-P2A-BirA*:HA, GFI1B-ΔSNAG-BirA*:HA, and BirA*:HA). For each comparison (A—GFI1B-BirA*:HA vs. GFI1B-ΔSNAG-BirA*:HA, B—GFI1B-BirA* vs. GFI1B-P2A-BirA*:HA, and C—SNAG-BirA*:HA vs. BirA*:HA), average sum read intensities were ranked according to the LSD1-nonbinding sample and plotted on the x-axis. Fold change in average sum read intensities, represented by a ratio between the LSD1-binding and LSD1-nonbinding sample in each comparison, is plotted on the y-axis. Each comparison, A-C, is represented by three panels. On top in each is the entire data set for that comparison, and below are two panels dividing the percentile rank score from 0-50 and from 50-100 with expanded y-axis limits to facilitate visualization of proteins enriched in the data set for the LSD1-binding form. Outlying proteins are labeled with their gene names. A red line indicates equal read intensities, and thus no enrichment, for the LSD1-binding over the LSD1-nonbinding sample.

Figure 7. BHC complex components HMG20A, HMG20B and PHF21A bind GFI1B in an LSD1-dependent manner. (A) BHC complex components are preferentially enriched in proximity labeling assays with fusion proteins competent for LSD1 binding. Biotin-modified proteins were purified from K562 cells inducibly expressing the BirA*:HA fusion

proteins shown. HMG20A, HMG20B, PHF21A, SMARCB1 and SMARCC1, as well as the BirA*:HA fusion proteins, were quantified in SAv-Sepharose pulldowns (SAv P) by western blot. Equivalent input for each protein was confirmed by western blot of whole cell lysates (Input). (B-D) GFI1B binding to HMG20A, HMG20B and PHF21A require GFI1B—LSD1 binding. COS7L cells were transfected with HA-tagged HMG20A (B), HMG20B (C), or PHF21A (D) and wild type (WT) or -P2A variants of GFI1B:flag. HMG20 and PHF21A proteins and their binding partners were purified in α -HA immune complexes. Co-precipitating GFI1B forms were detected in immune complexes by western blot using α -flag antibody. Equivalent expression and precipitation were evaluated in WCLs and ICs, respectively. GFP served as a transfection control. (E) HMG20A and HMG20B bind LSD1. COS7L cells were transfected with HA-tagged HMG20A or HMG20B along with a V5-tagged LSD1. LSD1 and its binding partners were collected in α -V5 immune complexes (IC). Co-precipitating HMG20A and HMG20B were detected in immune complexes by western blot using α -HA antibody. Equivalent expression and precipitation were evaluated in the whole cell lysates (WCL) and ICs, respectively.

Figure 8. HMG20A and HMG20B are required for GFI1B-mediated cell fate changes in K562 cells. (A) HMG20A and HMG20B depletion in K562 cells. RNA (Top) and Protein (Bottom) were collected from K562 cells transduced with shRNA targeting HMG20A, HMG20B, HMG20A and HMG20B (HMG20A+B), or a content-matched scrambled control. HMG20A and HMG20B expression levels were evaluated by RT-qPCR and normalized to *GUS*. HMG20A, HMG20B, LSD1, and GFI1B:flag levels were determined

by western blot. Tubulin served as a loading control. (B-F) HMG20A/B depletion abolishes GFI1B-dependent cell fate determination in K562 cells. K562 cells were transduced with inducible shRNAs targeting HMG20A, HMG20B, HMG20A and B or a scrambled control. The shRNAs were induced with doxycycline, cells were infected with GFI1B:flag-expressing retrovirus and erythroid differentiation allowed to proceed. (B) Benzidine staining was quantified by counting positive cells in bright field microscopy while hemoglobinized cell pellets are shown above each condition shown in the bar graph. (C) GFI1B-dependent inhibition of TPA-induced CD61 expression requires HMG20A/B. K562 cells overexpressing GFI1B and depleted of HMG20A, HMG20B, or HMG20A and B were treated with TPA and cell surface expression of CD61 quantified by flow cytometry. Histograms presented are representative of three independent experiments. (D-F) mRNA for globin genes *HBB* and *HBZ* (D), heme biosynthesis genes *ALAS2* and *FECH* (E) and GFI1B-repressed target genes *GFI1B* and *MYB* (F) were quantified by RT-qPCR normalized to *GUS* reference gene. Results are expressed as mean $\pm$ 2SD. Statistical significance was determined by two-sided unpaired *t*-test; *, $p < 0.05$; **, $p < 0.005$; ****, $p < 0.00005$.

Figure 9. GSE1 is required for GFI1B-mediated cell fate changes in K562 cells. (A) GSE1 depletion in K562 cells. RNA was collected from K562 cells transduced with shRNA targeting GSE1 or a content-matched scrambled control. GSE1 expression levels were evaluated by RT-qPCR and normalized to *GUS*. (B-D) GSE1 depletion abolishes GFI1B-dependent cell fate determination in K562 cells. K562 cells were transduced with inducible shRNAs targeting GSE1 or a scrambled control. The shRNAs were induced with

doxycycline, cells infected with GFI1B:flag expressing retrovirus and erythroid differentiation allowed to proceed. (B) Benzidine staining was quantified by counting positive cells in bright field microscopy. (C-D) mRNA for globin and related genes *AHSP*, *HBA1/2*, *HBZ*, and *HBB*; heme biosynthesis genes *ALAS2* and *FECH* (C); and GFI1B-repressed target genes *GFI1B* and *CDKN1A* (D) were quantified by RT-qPCR and normalized to *GUS* reference gene. Results are expressed as mean $\pm$ 2SD. (E) GFI1B-dependent inhibition of TPA-induced CD61 expression requires GSE1. K562 cells overexpressing GFI1B and depleted of GSE1 were treated with TPA and cell surface expression of CD61 quantified by flow cytometry compared to control cells. Histograms presented are representative of three independent experiments. Statistical significance was determined by two-sided unpaired *t*-test; **, $p < 0.005$; ****, $p < 0.00005$.

Figure 10. A working model for contributions of the BHC complex to GFI1B-mediated repression. LSD1 serves as a bridge between GFI1B and other components of the BHC complex, including HDAC1/2, HMG20A, HMG20B, PHF21A, RCOR1, ZMYM2, ZNF217, and GSE1. Therefore, BHC complex recruitment is rendered LSD1 dependent. A functional GFI1B transcriptional repression complex requires coincident recruitment of these factors, along with other partners brought to the promoter in an LSD1-independent manner by GFI1B or DNA binding proteins with which it collaborates. Dysfunction resulting from (1) an impaired, SNAG-dependent GFI1B—LSD1 recruitment mechanism (2) LSD1 depletion or intrinsic change that disrupts SNAG binding or (3) ineffective formation or operation of BHC complex components could each poison the GFI1B—LSD1 functional axis to create a permissive state for misexpression of GFI1B-

regulated genes. Only when each element is present and functioning properly (4) is the appropriate repressive outcome achieved. *Trans*-regulation by each component toward the others, or toward partners that engage GFI1B in a LSD1-independent manner, may also make important contributions to enabling and fine-tuning target gene expression.

Table 1. LSD1-dependent components of GFI1B proximitomes. Shown are components of LSD1-containing transcription complexes represented in the GFI1B proximitome. The core and unique components of each complex are enumerated (top), and the number of core and unique components from these complexes found in each proximitome comparison is shown.

SUPPLEMENTARY MATERIALS

Supplementary Data S1 File: McClellan et al Mass Spec Data.xlsx (1.6 MB)

Table S1 Top 100 LSD1-dependent proximity partners for each comparison

Table S2 Oligonucleotides used in this study

Figure 1

Figure 1. LSD1 depletion impedes GFI1B-mediated erythroid differentiation. (A) GFI1B overexpression promotes K562 cell hemoglobinization. Cells were infected with empty vector (EV) or virus expressing GFI1B. GFI1B-expressing cells become red and are benzidine positive. Benzidine positive cells are quantified as mean $\pm$ SD from four biological replicates. (B) TPA triggers cell surface expression of CD61 in K562 cells. CD61 expression was quantified in vehicle- vs. TPA-treated viable cells by flow cytometry. (C) LSD1 depletion and GFI1B overexpression in K562 cells. Small hairpin RNAs targeting *LSD1* (LSD1 shRNA) or content-matched scramble control (Scram shRNA) were inducibly transcribed in K562 cells followed by infection at day +3 with GFI1B:flag-expressing virus or EV. LSD1 and GFI1B were quantified by western blot of whole cell lysates (WCL). Tubulin served as a loading control. (D) LSD1 depletion blocks GFI1B-

mediated K562 cell hemoglobinization. (E) LSD1 depletion enables TPA-induced CD61 expression in the context of GFI1B overexpression. (F-G) LSD1 depletion impairs expression of erythroid fate genes in response to GFI1B overexpression. Alpha- and Beta-globins (*HBA1/2* and *HBB*), alpha globin stabilizing protein (*AHSP*), aminolevulinic acid synthase (*ALAS2*), ferrochelatase (*FECH*) and glycophorin (*GYP*)A expression are shown. (H) LSD1 depletion restores expression of GFI1B-repressed target genes. *CDKN1A*, *GFI1B* and *MYB* expression are shown. Gene expression is quantified by RT-qPCR normalized to *GUS*. Results are expressed as mean $\pm$ 2SD from two independent experiments performed in triplicate. * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$, **** $p < 0.00005$.

Figure 2

Figure 2. LSD1 binding is required for GFI1B-regulated cell fate decisions in K562 cells. (A) P2A substitution in GFI1B impairs GFI1B—LSD1 binding. K562 cells were transduced with constructs expressing wild type GFI1B, its P2A variant or empty vector (EV) as shown. GFI1B forms were immune purified with α-flag (M2) antibody and Protein-G Sepharose. LSD1 was quantified in immune complexes (IC) by western blot. Equal expression (LSD1 and GFI1B) and precipitation (GFI1B) were evaluated by western blot of whole cell lysates (WCL) and ICs, respectively. Tubulin was used to confirm equal gel loading. (B) P2A substitution abolishes transcriptional repression by GFI1B. 293-T-Rex-5xGal-luciferase cells were transfected with WT or P2A variants of GFI1B-Δ1C:Gal4 (left) or SNAG:Gal4 (right) fusion proteins. Firefly luciferase was measured and normalized to constitutively expressed, co-transfected *Renilla* luciferase.

Reporter activity is expressed as mean $\pm$ SD from two experiments performed in triplicate. (C) P2A substitution impairs GFI1B-mediated hemoglobinization. Benzidine staining was quantified as in 1A. Protoporphyrin IX (PPIX) and heme were quantified by UPLC in K562 cells transduced with EV, GFI1B-WT or GFI1B-P2A. (D-F) P2A substitution impairs expression of erythroid fate genes in K562 cells. Expression of β -globin cluster genes, *AHSP*, *ALAS2*, *FECH*, *GYP A* and Kruppel-like factor (*KLF*)1 are shown. (G) P2A substitution abolishes GFI1B-mediated suppression of TPA-induced CD61 expression. Cell surface expression of CD61 in TPA treated cells expressing wild type GFI1B (WT) or its P2A variant was determined by flow cytometry. (H) P2A substitution reverses repression of GFI1B target genes *GFI1B*, *CDKN1A* and *MYB*. Results expressed as mean $\pm$ 2SD. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$; ****, $p < 0.00005$.

HA epitope tag, BirA*:HA to form GFI1B-BirA*:HA. GFI1B partners are spontaneously biotinylated due to their proximity to BirA*:HA anchored to GFI1B in *cis*. Biotinylated products are captured on streptavidin (SAv)-conjugated beads for downstream analysis via candidate-based or screening approaches. (B) BirA*:HA and GFI1B-BirA*:HA fusion biotinylate diverse targets in K562 cells. Cells were transduced with constructs inducibly expressing GFI1B-BirA*:HA, BirA*:HA or EV, treated with doxycycline and harvested. GFI1B-BirA*:HA and BirA*:HA expression was confirmed by α -HA western blot and biotinylated proteins detected by SAv:HRP. Tubulin served as a loading control. (C) GFI1B interacting protein, LSD1 and RCOR1, are enriched among proteins biotinylated by GFI1B-BirA*:HA compared to BirA* or EV., Biotin-modified proteins were collected on SAv-Sepharose. LSD1, RCOR1 and the BirA* fusion proteins themselves were quantified among purified proteins (SAv P) by western blot. Equal expression was confirmed by western blot in whole cell lysates (input). (D) GFI1B-BirA*:HA identifies members of a GFI1B proximitome. Factors enriched among biotinylated and SAv-Sepharose purified proteins modified by GFI1-BirA*:HA (test) compared to BirA*:HA (control). Fold enrichment is plotted logarithmically as a ratio of average sum read intensities between test and control samples against their *p*-values calculated from their 20 closest ranked neighbors in the data set as described in Materials and Methods. Individual proteins are labeled with their respective gene names. Inset shows an expanded view of the data contained in the boxed area.

Figure 4

	BHC	CtBP	NuRD	SWI/SNF	ISWI	PRC1	PRC2	SIN3A	NCoR
Core	LSD1	LSD1	LSD1	SMARCC1	NuRF	CHRAC	RING1A	EZH1	SIN3A
	RCOR1	HDAC1	CHD3	SMARCC2	BPTF	SNF2H	RING1B	SAP30	SIN3A
	HMG20B	HDAC2	CHD4	SMARCD1	SNF2L	ACF1	1.2/4	SAP18	HDAC1
	PHF21A	EHMT1	HDAC1	SMARCD2	RBBP7	POLE3	1.3/5	HDAC2	HDAC2
	HDAC1	EHMT2	HDAC2	SMARCD3	RBBP4	CHRAC1	1.1	HDAC3	HDAC3
	HDAC2	RCOR1	MBD2	SMARCB1		PCGF2	1.6	CBFA2T1	TBL1X
	GSE1	CTBP1	MBD3	SMARCE1		PCGF3	Core	CBFA2T2	TBL1XR1
	HMG20A	SIRT1	MTA1	ACTL6A		PCGF4	RBBP7	CBFA2T3	GPS2
	ZMYM2		MTA2	ACTL6B		PCGF5	EED	TET1	CBFA2T1
	ZNF217		RBBP4	β -actin		CBX2	PHF1	TET2	CBFA2T2
Putative			RBBP7	BAF		CBX4	2.1		
				PBAF		CBX6	JARID2		
				SMARCA4		CBX7	AEBP2		
				SMARCA2		CBX8	PHF19		
				ARID1A		PHC1			
				ARID1B		PHC2			
						PHC3			
						SCMH1			

Figure 4. Components of transcriptional repression and chromatin remodeling complexes identified by GFI1B proximity labeling. A roster of transcriptionally relevant complexes and their component members are shown. Proteins are listed according to HUGO gene nomenclature. With respect to the GFI1B proximitome (GFI1B-BirA*:HA vs. BirA*:HA), proteins present and enriched—white letters on black background. Those present by not significantly enriched ($p < 0.05$)—black letters on gray background. Not present (average sum read intensity below that for EV)—black letters on white background. Where appropriate, complexes are subdivided into core and putative members, and/or with canonical vs. non-canonical designations.

Figure 5

Figure 5. LSD1 non-binding variants of GFI1B-BirA* fail to biotinylate LSD1 and RCOR1.

(A) Graphical representation of GFI1B-BirA*:HA fusion proteins employed in experiments. Each fusion protein is comprised of the GFI1B regions shown in frame with the BirA* expression cassette and a C-terminal HA epitope tag to insure equivalent expression of all forms. The BirA*:HA construct lacks GFI1B structures and instead begins with an ATG. Wild type (WT) GFI1B is shown. The SNAG and zinc finger (ZnF) domains (1-6) of GFI1B are shown as black boxes. A thin line represents the GFI1B linker and connections between ZnFs. The proline to alanine substitution (P2A) in the GFI1B SNAG domain is indicated with an *. ΔSNAG represents deletion of the SNAG domain but preservation of the remaining GFI1B primary structure. (B) Expression of GFI1B-BirA*:HA forms and proteome-wide biotin modification in K562 cells. K562 cells were transduced with lentivirus to inducibly express GFI1B-BirA*:HA forms shown and stable isolates selected as polyclonal populations. Doxycycline-inducible expression of each fusion protein was confirmed by western blot against the common HA epitope tag and biotinylating activity confirmed proteome-wide by SAV:HRP detection of transblotted total cellular protein. Tubulin served as a loading control. (C) LSD1 and RCOR1 are enriched among biotinylated proteins generated by BirA*:HA fusions competent for LSD1 binding. Biotin-modified proteins from K562 cells transduced with BirA*:HA fusion proteins shown were purified from whole cell extracts (SAV P), fractionated by SDS-PAGE and subjected to western blot with α-LSD1, α-RCOR1 and α-HA antibodies. Equivalent expression of each protein in whole cell lysates (Input) was confirmed by western blot with these same antibodies.

Figure 6

Figure 6. GFI1B recruits the BHC complex in an LSD1-depend manner via the SNAG domain. K562 cells stably and inducibly expressing the BirA*:HA fusion proteins shown in 5A were deployed to define GFI1B proximity partners whose recruitment is LSD1-dependent. Fusion protein expression was induced by doxycycline addition and biotinylation permitted *in situ*. Biotin-modified proteins were captured on SA_v-Sepharose and analyzed by LC-MS-MS as described in Materials and Methods. (A-C) Dot plots are shown comparing fold change in average sum read intensity among fusion proteins with intact SNAG domains and capable of LSD1-binding (GFI1B-BirA*:HA and SNAG-BirA*:HA) to those with disrupted or absent SNAG domains and deficient in LSD1 binding (GFI1B-P2A-BirA*:HA, GFI1B-ΔSNAG-BirA*:HA, and BirA*:HA). For each comparison (A—GFI1B-BirA*:HA vs. GFI1B-ΔSNAG-BirA*:HA, B—GFI1B-BirA* vs. GFI1B-P2A-BirA*:HA, and C—SNAG-BirA*:HA vs. BirA*:HA), average sum read intensities were ranked according to the LSD1-nonbinding sample and plotted on the x-axis. Fold change in average sum read intensities, represented by a ratio between the LSD1-binding and LSD1-nonbinding sample in each comparison, is plotted on the y-axis. Each comparison, A-C, is represented by three panels. On top in each is the entire data set for that comparison, and below are two panels dividing the percentile rank score from 0-50 and from 50-100 with expanded y-axis limits to facilitate visualization of proteins enriched in the data set for the LSD1-binding form. Outlying proteins are labeled with their gene names. A red line indicates equal read intensities, and thus no enrichment, for the LSD1-binding over the LSD1-nonbinding sample.

Figure 7

Figure 7. BHC complex components HMG20A, HMG20B and PHF21A bind GFI1B in an LSD1-dependent manner. (A) BHC complex components are preferentially enriched in proximity labeling assays with fusion proteins competent for LSD1 binding. Biotin-modified proteins were purified from K562 cells inducibly expressing the BirA*:HA fusion proteins shown. HMG20A, HMG20B, PHF21A, SMARCB1 and SMARCC1, as well as the BirA*:HA fusion proteins, were quantified in SA_v-Sepharose pulldowns (SA_v P) by western blot. Equivalent input for each protein was confirmed by western blot of whole cell lysates (Input). (B-D) GFI1B binding to HMG20A, HMG20B and PHF21A require GFI1B—LSD1 binding. COS7L cells were transfected with HA-tagged HMG20A (B), HMG20B (C), or PHF21A (D) and wild type (WT) or -P2A variants of GFI1B:flag. HMG20 and PHF21A proteins and their binding partners were purified in α -HA immune complexes. Co-precipitating GFI1B forms were detected in immune complexes by western blot using α -flag antibody. Equivalent expression and precipitation were evaluated in WCLs and ICs, respectively. GFP served as a transfection control. (E) HMG20A and HMG20B bind LSD1. COS7L cells were transfected with HA-tagged HMG20A or HMG20B along with a V5-tagged LSD1. LSD1 and its binding partners were collected in α -V5 immune complexes (IC). Co-precipitating HMG20A and HMG20B were detected in immune complexes by western blot using α -HA antibody. Equivalent expression and precipitation were evaluated in the whole cell lysates (WCL) and ICs, respectively.

Figure 8

Figure 8. HMG20A and HMG20B are required for GFI1B-mediated cell fate changes in K562 cells. (A) HMG20A and HMG20B depletion in K562 cells. RNA (Top) and Protein (Bottom) were collected from K562 cells transduced with shRNA targeting HMG20A, HMG20B, HMG20A and HMG20B (HMG20A+B), or a content-matched scrambled control. HMG20A and HMG20B expression levels were evaluated by RT-qPCR and

normalized to *GUS*. HMG20A, HMG20B, LSD1, and GFI1B:flag levels were determined by western blot. Tubulin served as a loading control. (B-F) HMG20A/B depletion abolishes GFI1B-dependent cell fate determination in K562 cells. K562 cells were transduced with inducible shRNAs targeting HMG20A, HMG20B, HMG20A and B or a scrambled control. The shRNAs were induced with doxycycline, cells were infected with GFI1B:flag-expressing retrovirus and erythroid differentiation allowed to proceed. (B) Benzidine staining was quantified by counting positive cells in bright field microscopy while hemoglobinized cell pellets are shown above each condition shown in the bar graph. (C) GFI1B-dependent inhibition of TPA-induced CD61 expression requires HMG20A/B. K562 cells overexpressing GFI1B and depleted of HMG20A, HMG20B, or HMG20A and B were treated with TPA and cell surface expression of CD61 quantified by flow cytometry. Histograms presented are representative of three independent experiments. (D-F) mRNA for globin genes *HBB* and *HBZ* (D), heme biosynthesis genes *ALAS2* and *FECH* (E) and GFI1B-repressed target genes *GFI1B* and *MYB* (F) were quantified by RT-qPCR normalized to *GUS* reference gene. Results are expressed as mean $\pm$ 2SD. Statistical significance was determined by two-sided unpaired *t*-test; *, $p < 0.05$; **, $p < 0.005$; ****, $p < 0.00005$.

Figure 9

Figure 9. GSE1 is required for GFI1B-mediated cell fate changes in K562 cells. (A) GSE1 depletion in K562 cells. RNA was collected from K562 cells transduced with shRNA targeting GSE1 or a content-matched scrambled control. GSE1 expression levels were evaluated by RT-qPCR and normalized to *GUS*. (B-D) GSE1 depletion abolishes GFI1B-dependent cell fate determination in K562 cells. K562 cells were transduced with inducible shRNAs targeting GSE1 or a scrambled control. The shRNAs were induced with doxycycline, cells infected with GFI1B:flag expressing retrovirus and erythroid differentiation allowed to proceed. (B) Benzidine staining was quantified by counting positive cells in bright field microscopy. (C-D) mRNA for globin and related genes *AHSP*, *HBA1/2*, *HBZ*, and *HBB*; heme biosynthesis genes *ALAS2* and *FECH* (C); and GFI1B-repressed target genes *GFI1B* and *CDKN1A* (D) were quantified by RT-qPCR and normalized to *GUS* reference gene. Results are expressed as mean $\pm$ 2SD. (E) GFI1B-dependent inhibition of TPA-induced CD61 expression requires GSE1. K562 cells overexpressing GFI1B and depleted of GSE1 were treated with TPA and cell surface expression of CD61 quantified by flow cytometry compared to control cells. Histograms presented are representative of three independent experiments. Statistical significance was determined by two-sided unpaired *t*-test; **, $p < 0.005$; ****, $p < 0.00005$.

Figure 10

Figure 10. A working model for contributions of the BHC complex to GF1B-mediated repression. LSD1 serves as a bridge between GF1B and other components of the BHC complex, including HDAC1/2, HMG20A, HMG20B, PHF21A, RCOR1, ZMYM2, ZNF217, and GSE1. Therefore, BHC complex recruitment is rendered LSD1 dependent. A functional GF1B transcriptional repression complex requires coincident recruitment of these factors, along with other partners brought to the promoter in an LSD1-independent manner by GF1B or DNA binding proteins with which it collaborates. Dysfunction resulting from (1) an impaired, SNAG-dependent GF1B—LSD1 recruitment mechanism (2) LSD1 depletion or intrinsic change that disrupts SNAG binding or (3) ineffective formation or operation of BHC complex components could each poison the GF1B—LSD1 functional axis to create a permissive state for misexpression of GF1B-regulated genes. Only when each element is present and functioning properly (4) is the appropriate repressive outcome achieved. *Trans*-regulation by each component toward the others, or toward partners that engage GF1B in a LSD1-independent manner, may also make important contributions to enabling and fine-tuning target gene expression.

Table 1 GFI1B LSD1-dependent proximitomes compared

Complexes & Composition	BHC		CtBP		NuRD	
	Total	Unique	Total	Unique	Total	Unique
	10	6	8	4	11	8
Proximitomes Compared	Number present in top 100 enriched proteins					
WT vs. Δ SNAG	7	4	2	0	2	0
WT vs. P2A	8	5	3	0	2	0
SNAG vs. BirA*	5	3	2	0	1	0

Table 1. LSD1-dependent components of GFI1B proximitomes. Shown are components of LSD1-containing transcription complexes represented in the GFI1B proximitome. The core and unique components of each complex are enumerated (top), and the number of core and unique components from these complexes found in each proximitome comparison is shown.

TABLE S1 Comparison of the top 100 LSD1-dependent protein candidates associated with GFI1B

Rank	GFI1B-WT vs. GFI1B-ΔSNAG		GFI1B-WT vs. GFI1B-P2A		SNAG vs. BirA* Only	
	Gene name	P value ^a	Gene name	P value ^a	Gene name	P value ^a
1	FERMT3	6.13E-22	ZNF451	3.22E-17	LSG1	9.06E-30
2	PRDM10	2.22E-15	RCOR3	4.40E-16	FAM83H	1.89E-26
3	ZNF451	1.34E-13	GSE1	5.28E-14	MARCKSL1	5.14E-13
4	RCOR3	2.28E-12	L3MBTL3	1.62E-13	PHF21A	6.01E-21
5	RCOR1	4.44E-12	RREB1	1.00E-12	RCOR3	7.48E-20
6	KDM1A (LSD1)	1.60E-11	ZNF622	1.98E-12	RREB1	2.20E-17
7	GSE1	6.06E-11	CUL4B	2.51E-12	KDM1A (LSD1)	3.68E-16
8	HMG20B	1.42E-10	PRDM2	2.84E-12	PRKCA	8.17E-16
9	RREB1	1.84E-10	CBX4	1.35E-11	AFTPH	1.70E-14
10	CBX4	2.37E-10	KDM1A (LSD1)	6.68E-11	RCOR1	1.12E-13
11	RAD54L2	3.46E-10	LARP1	7.48E-11	FASTKD1	5.37E-13
12	L3MBTL3	3.51E-10	GFM1	1.53E-10	AP3B1	7.61E-13
13	PTK2B	5.27E-10	SLX4	3.00E-10	NAP1L4	9.14E-13
14	P4HB	1.44E-09	SEPT7	3.73E-10	NAP1L1	2.89E-12
15	NUP188	1.11E-08	ZMYM2	6.66E-10	DNAJB12	4.40E-12
16	EIF2B5	1.73E-08	GIGYF2	1.13E-09	TSR1	7.75E-12
17	HIST1H1A	2.22E-08	SEC23B	3.10E-09	TMED9	2.09E-11
18	FAM83H	2.99E-08	ARVCF	3.85E-09	ROR2	2.61E-11
19	ZNF629	6.54E-08	RCOR1	4.77E-09	MAP2K7	1.12E-10
20	OSBPL3	9.68E-08	PRDM10	5.43E-09	IPO9	1.33E-09
21	ARVCF	1.45E-07	HMG20B	6.28E-09	NOL11	1.08E-08
22	HDAC2	1.58E-07	YARS	1.03E-08	HMG20B	1.51E-08
23	PRDM2	1.77E-07	PHF21A	1.13E-08	WDR43	2.01E-08
24	ZMYM2	2.97E-07	FAM208B	1.20E-08	PPP4R3A	4.59E-08
25	BUB1B	5.45E-07	ZNF609	1.78E-08	UBE2O	8.66E-08
26	HDAC1	5.53E-07	cDNA FLJ51764	4.26E-08	RRP7BP	1.12E-07
27	NCKAP1	6.44E-07	MED14	6.05E-08	CHTF18	1.30E-07
28	TARS2	6.78E-07	MAGEC1	7.59E-08	L3MBTL3	1.45E-07
29	HMG20A	1.28E-06	RAB3GAP1	1.39E-07	MTCH2	2.66E-07
30	EDC4	1.80E-06	NASP	3.22E-07	IPO7	4.73E-07
31	CBSL	1.88E-06	BRD8	9.26E-07	TNRC18	4.94E-07
32	NCOA1	2.34E-06	RAI1	9.74E-07	DDX27	8.64E-07
33	KDM5B	6.37E-06	PIAS2	1.60E-06	NOP14	1.01E-06
34	EHD4	6.58E-06	RSF1	2.66E-06	NOB1	1.61E-06
35	ZFPM1	8.87E-06	GPATCH1	7.60E-06	BYSL	1.62E-06
36	SMARCAL1	1.26E-05	NUP58	8.82E-06	UBE3C	1.83E-06

37	PHF21A	1.53E-05	KIF22	1.06E-05	GSE1	3.06E-06
38	SEC23B	1.75E-05	RUNX1	1.13E-05	PDAP1	4.41E-06
39	ATRX	3.32E-05	HMG20A	1.31E-05	RRP1B	4.42E-06
40	PEX1	4.92E-05	IQSEC1	1.64E-05	RPL15	5.68E-06
41	GRAP2	5.12E-05	MTF2	1.65E-05	PDCD11	1.09E-05
42	ABCB10	6.04E-05	HDAC3	2.74E-05	BUD13	1.40E-05
43	HBZ	6.73E-05	HDAC1	3.06E-05	MRPS27	1.59E-05
44	AMBRA1	6.91E-05	TRIM41	3.10E-05	RRBP1	1.62E-05
45	ANKHD1	9.12E-05	SETD2	3.70E-05	MARS	1.87E-05
46	STAT5B	1.03E-04	TUBB1	4.52E-05	PRDM2	1.92E-05
47	JMJD1C	1.18E-04	PTK2B	4.54E-05	IGF2BP2	3.99E-05
48	CHD9	1.57E-04	WRN	6.36E-05	PGM2	5.05E-05
49	SLX4	2.72E-04	ATRX	8.12E-05	FDPS	6.59E-05
50	MAP1B	2.73E-04	PIAS1	1.17E-04	OPA1	7.06E-05
51	PRR12	3.06E-04	KPNA5	1.33E-04	MYBBP1A	7.87E-05
52	NCOR2	3.12E-04	ABCB10	1.69E-04	GFI1B	8.21E-05
53	BCOR	3.84E-04	NCOR2	1.80E-04	FBRSL1	9.39E-05
54	SLC25A12	4.31E-04	RAD18	1.91E-04	CARM1	1.65E-04
55	ISYNA1	5.51E-04	ZMYND8	1.96E-04	HMG20A	1.70E-04
56	XRCC5	9.24E-04	ZNF384	2.35E-04	NEPRO	1.72E-04
57	PYGB	1.14E-03	PIGT	2.49E-04	COQ5	1.97E-04
58	FLII	1.29E-03	BCOR	2.59E-04	LENG8	2.05E-04
59	TBL1X	1.41E-03	ZFPM1	3.06E-04	ATN1	2.65E-04
60	OTUD4	1.46E-03	TRNAU1AP	3.35E-04	COMMD3	3.37E-04
61	ABCD3	1.52E-03	NR2C2	3.85E-04	LTV1	3.46E-04
62	VPS52	1.62E-03	CKAP5	3.91E-04	RRP12	4.01E-04
63	PIAS1	1.71E-03	SMARCA1	3.95E-04	ORC1	4.56E-04
64	LARP4B	1.79E-03	CTR9	4.23E-04	FERMT3	4.92E-04
65	GALK2	1.98E-03	FUBP3	4.51E-04	PINX1	5.42E-04
66	RUNX1	2.08E-03	cDNA FLJ51028	4.60E-04	GTF2H2C	6.37E-04
67	UBAP2	2.13E-03	CNOT1	6.62E-04	ZNF701	7.84E-04
68	FECH	2.21E-03	SENP6	6.64E-04	NUDT4	8.94E-04
69	PRRC2C	2.35E-03	TAF6L	7.46E-04	KDM5B	9.43E-04
70	EXOC3L2	2.86E-03	ZNF592	7.49E-04	RBFOX1	1.13E-03
71	ASNS	2.88E-03	VPS51	7.89E-04	SLC3A2	1.25E-03
72	AHDC1	2.92E-03	HBZ	8.00E-04	RPP40	1.28E-03
73	CARD6	2.97E-03	CBSL	8.05E-04	LIG3	1.42E-03
74	RICTOR	3.02E-03	PLCD1	9.81E-04	CCDC9	1.78E-03
75	YARS	3.04E-03	SDHA	1.19E-03	SLC2A1	1.99E-03
76	FHOD1	3.36E-03	KDM5B	1.21E-03	NOL6	2.24E-03

77	ZNF672	4.07E-03	SBNO1	1.48E-03	TBC1D14	2.27E-03
78	C16orf58	4.26E-03	TBL1X	1.56E-03	HNRNPH3	2.59E-03
79	HNRNPL	4.49E-03	ATXN2	1.75E-03	IMP4	2.71E-03
80	ALDH18A1	6.11E-03	TBL1XR1	1.79E-03	HIST1H1C	2.99E-03
81	GOLGA2	6.18E-03	RPA1	1.81E-03	MMS19	3.08E-03
82	PLCD1	6.31E-03	KIF5A	1.87E-03	NCOR2	3.19E-03
83	TXNIP	7.04E-03	DGCR14	2.10E-03	RPL7L1	3.39E-03
84	ALDH16A1	7.11E-03	TRMT2A	2.13E-03	BRMS1	3.41E-03
85	ATXN2	7.45E-03	ACACA	2.77E-03	DDX56	3.77E-03
86	PSMB5	7.79E-03	EXOC2	2.96E-03	KIF18A	3.79E-03
87	GFM1	8.53E-03	MSH3	3.04E-03	BAZ1B	4.47E-03
88	CNOT2	1.07E-02	NKTR	3.20E-03	CSPP1	4.54E-03
89	QSER1	1.07E-02	HAUS5	3.50E-03	CBFB	4.57E-03
90	DYRK1A	1.09E-02	DDX51	3.79E-03	SNAP23	4.96E-03
91	RANBP9	1.14E-02	CHD9	4.34E-03	H2AFV	5.23E-03
92	PPA2	1.18E-02	RNPC3	4.60E-03	BRAF	5.27E-03
93	QARS	1.19E-02	ZNF512B	5.33E-03	IQSEC1	5.37E-03
94	BIRC2	1.23E-02	SMG7	5.40E-03	ARAF	5.77E-03
95	SMG7	1.25E-02	BEND3	5.92E-03	EBNA1BP2	5.85E-03
96	CD3EAP	1.27E-02	ZKSCAN4	6.08E-03	USP36	6.04E-03
97	MTFR1L	1.36E-02	RCOR3	6.49E-03	PRR5	6.15E-03
98	UTP14C	1.41E-02	HSD17B10	6.54E-03	PHIP	6.71E-03
99	KDM2B	1.43E-02	HUWE1	6.59E-03	GTPBP4	6.94E-03
100	MAGEC1	1.50E-02	ALDH18A1	6.65E-03	MMTAG2	7.01E-03

^aDetermined by two tailed *t*-distribution of the z-score of the 20 neighboring ranked proteins.

Known BHC complex members in **RED**

Table S2 Oligonucleotides used in this study

Oligo Name	Sequence (5' to 3')	Purpose
hHMG20A qPCR Fwd	GATCTGGCTACCACTGGGT	RT-qPCR
hHMG20A qPCR Rev	CCTCCTCGTTTACTTCGTTGC	RT-qPCR
hHMG20B qPCR2 Fwd	CATGGGGGCTTCGTGGTGA	RT-qPCR
hHMG20B qPCR2 Rev	CGCCAGCATCTTGGTGAT	RT-qPCR
hGSE1 qPCR 1 Fwd	GCCTGCAATGCACTGGCCTA	RT-qPCR
hGSE1 qPCR 1 Rev	GGAGCCTGGAATCCTTTGCTTC	RT-qPCR
hPHF21A qPCR Fwd	CTCCTGCTGCTCTGATTCCC	RT-qPCR
hPHF21A qPCR Rev	GGCCTCTCGCACTTTTAGA	RT-qPCR
hHBA1/2 qPCR Fwd	CCGGTCAACTCAAGCTCCTA	RT-qPCR
hHBA1/2 qPCR Rev	GGTATTGGAGGTGAGCAGCG	RT-qPCR
hHBZ qPCR Fwd	GAGCAGGCCCAACTCCAG	RT-qPCR
hHBZ qPCR Rev	TGTCGTCGATGCTCTTACC	RT-qPCR
hAHSP qPCR Fwd	ATAAGGATCTCATTTCCGACGG	RT-qPCR
hAHSP qPCR Rev	CCTGTCTCGCTCTTGGGG	RT-qPCR
hHBB qPCR Fwd	GTGAACGTGGATGAAGTTGGTGGTGAG	RT-qPCR
hHBB qPCR Rev	TTGGACAGCAAGAAAGCGAGCTTAGTG	RT-qPCR
hHBD qPCR Fwd	GCACGTGGATCCTGAGAACT	RT-qPCR
hHBD qPCR Rev	ACCCTTGAAGTAGGCATTGTGT	RT-qPCR
hHBE1 qPCR Fwd	GGCCAGAACTTCGGCAGTAA	RT-qPCR
hHBE1 qPCR Rev	AAACAACGAGGAGTCTGCCC	RT-qPCR
hHBG1/2 qPCR Fwd	ACTCGCTTCTGGAACGTCTG	RT-qPCR
hHBG1/2 qPCR Rev	CCTCTGGGTCCATGGGTAGA	RT-qPCR
hALAS2 qPCR Fwd	TACCCACCTGTCATTGTTCTG	RT-qPCR
hALAS2 qPCR Rev	GGACCGGAAAATGGCTTCT	RT-qPCR
hFECH qPCR Fwd	ATCCAGCAGCTGGAGGGTCT	RT-qPCR
hFECH qPCR Rev	TGAATCTTGGGGTTCGGCG	RT-qPCR
hGYPA qPCR Fwd	CATTAAGTACCACTGAGGTGGC	RT-qPCR
hGYPA qPCR Rev	TGATGGGCAAGTTGTACCT	RT-qPCR
hKLF1 qPCR Fwd	TGACTTCCTCAAGTGGTGGC	RT-qPCR
hKLF1 qPCR Rev	GGTGAGGAGGAGATCCAGGT	RT-qPCR
hGFI1B qPCR Fwd	GCAGGAAGATGAACCGCTCT	RT-qPCR
hGFI1B qPCR Rev	CCAGGCACTGGTTTGGGAA	RT-qPCR
hMYB qPCR Fwd	GGGCAGAAATCGCAAAGTACT	RT-qPCR
hMYB qPCR Rev	GTTAACAGTGGGTGGCCAG	RT-qPCR
hCDKN1A qPCR Fwd	TTTCTCTGGCTCCCATGT	RT-qPCR
hCDKN1A qPCR Rev	GCTGTATATTCAGCATTGTGGG	RT-qPCR
HMG20A shRNA 1 S	CTAGCAAGGCAAATCTCATAGGCAAGTACTAGTCTTGCTATGAGATTG CCTTTTTTG	shRNA Knockdown (EZ Tet pLKO)

HMG20A shRNA 1 A	AATTCAAAAAAGGCAATCTCATAGGCAAGACTAGTACTGCCTATGAG ATTGCTTG	shRNA Knockdown (EZ Tet pLKO)
HMG20B shRNA 1S	CCGGAGCATCCCTTTAGCTTTCAATCTCGAGATTGAAAGCTAAAGGGATG CTTTTT	shRNA Knockdown (Tet pLKO)
HMG20B shRNA 1A	AATTA AAAAAGCATCCCTTTAGCTTTCAATCTCGAGATTGAAAGCTAAAG GGATGCT	shRNA Knockdown (Tet pLKO)
LSD1 shRNA 2S	CCGGGCTAGACATTAAACTGAATACTCGAGTATTCAGTTTAATGTCTAG GCTTTTT	shRNA Knockdown (Tet pLKO)
LSD1 shRNA 2A	AATTA AAAAAGCCTAGACATTAAACTGAATACTCGAGTATTCAGTTTAATG TCTAGGC	shRNA Knockdown (Tet pLKO)
LSD1 shRNA 3S	CCGGGCTACATCTTACCTTAGTCACTCGAGATGACTAAGGTAAGATGTA GCTTTTT	shRNA Knockdown (Tet pLKO)
LSD1 shRNA 3A	AATTA AAAAAGCTACATCTTACCTTAGTCACTCGAGATGACTAAGGTAAG ATGTAGC	shRNA Knockdown (Tet pLKO)
GSE1 shRNA 2 S	CTAGCGAACTCACCTTGACGTCAATGACTAGTCAATTGACGTCAAGGTGA GTTCTTTTTG	shRNA Knockdown (EZ Tet pLKO)
GSE1 shRNA 2 A	AATTC AAAAAGAACTCACCTTGACGTCAATGACTAGTCAATTGACGTCAA GGTGAGTTCG	shRNA Knockdown (EZ Tet pLKO)
EZ Scramble S	CTAGCCAACAAGATGAAGAGCACCAACTAGTATTGGTGCTCTTCATCTT GTTGTTTTG	shRNA Knockdown (EZ Tet pLKO)
EZ Scramble A	AATTC AAAAACAAGATGAAGAGCACCAACTAGTATTGGTGCTCTTCATCTT ATCTGTTGG	shRNA Knockdown (EZ Tet pLKO)
Scramble shRNA S	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGT TGTTTT	shRNA Knockdown (Tet pLKO)
Scramble shRNA A	AATTA AAAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCAT CTTGTTG	shRNA Knockdown (Tet pLKO)
EcorR1 mHMG20B Fwd	AAAAGAATTCAGCCACCATGTCCACGGTCCCGGCA	Cloning HMG20B into pcDNA3.1
HMG20B no stop BamH1 Rev	AAAAGGATCCCGGTGCTCGTGCTGCTACCC	Cloning HMG20B into pcDNA3.1
Nhe1 mHMG20A Fwd	TTTTGCTAGCGCCACCATGGAGAGCTTGATGGCCAG	Cloning HMG20A into pcDNA3.1
mHMG20A NoStop BamH1 Rev	TTTTGGATCCACGATCAAGCCTGTTCAAC	Cloning HMG20A into pcDNA3.1
Nhe1 mPHF21A Fwd	AAAAGCTAGCGCCACCATGGAGTTGCAGACTCTACA	Cloning PHF21A into pcDNA3.1
mPHF21A no stop Xho1 Rev	AAAACCTCGAGATTAGTCTCTTCCCTGGT	Cloning PHF21A into pcDNA3.1
mGFI1B Nhe1 Fwd	AAAGCTAGCGCCACCATGCCACGGTCTTTCTAGT	Cloning GFI1B into pcDNA3.1
mGFI1B BamH1 NoStop Rev	AAAGGATCCTAAATCCCCCACTTGAGATTGTGTTGAC	Cloning GFI1B into pcDNA3.1
EcoR1 Gfi1b Fwd	CCCCGAATTCGCCACCATGCCACGGTCTTT	Cloning GFI1B forms inframe with Gal4
Gfi1b SNAG Kpn1 Rev	CCCCGGTACCCAGCTCATCACCCTGTGCC	Cloning GFI1B forms inframe with Gal4
Gfi1b D1C Kpn1 Rev	CCCCGGTACCCGAGTGGATGAGCAGGTGGG	Cloning GFI1B forms inframe with Gal4
BamH1-hGFI1B-SNAG-Fwd	AAAAGGATCCGGCTAGCGCCACCATGCCAC	Cloning SNAG-BirA*:HA (pcDNA3.1)
hGFI1B-SNAG-BamH1-Rev	AAAAGGATCCTCCACACGGGGCTGGTGGTAGG	Cloning SNAG-BirA*:HA (pcDNA3.1)
Age1BamH1 with EcoR1 S	CCGGTCGAATTCGCCACCATGGGCGGAGGGTCTGGAGGCGGGAGTG	Cloning BirA*:HA Only (pcDNA3.1)
Age1BamH1 with EcoR1 A	GATCCGACCCGCTCCAGACCTCCGCCCATGGTGGCGAATTCGA	Cloning of BirA*:HA Only (pcDNA3.1)
Not1-d1n-hGFI1B-fwd	AAAAGCGGCCGCGCCACCATGCAGGAAGATGAACCGCTC	Cloning dSNAG-BirA*:HA (pLVX-Tight)
Not1 BioOnly Fwd	AAAAGCGGCCGCTAGGAATTCGCCACCATGG	Cloning BirA*:HA only (pLVX-Tight)
Not1 Gfi1b Bio Fwd	AAAAGCGGCCGGAATTCCTGGCTAGCGCCACCATG	Cloning GFI1B-BirA*:HA (pLVX-Tight)
Ha Mlu1 Rev	AAAAACGCGTACTATGCGTAATCCGGTACATCG	Cloning GFI1B-BirA*:HA (pLVX-Tight)
P2A-Gfi1b-3xFlag-Fwd	CGATCGCCACCATGGCACGGTCTTTCTA	Site-directed mutagenesis
P2A-Gfi1b-3xFlag-Rev	TAGAAAGGACCGTCCATGGTGGCGATCG	Site-directed mutagenesis