Pharmacological inhibition of LSD1 triggers myeloid differentiation by targeting GSE1, a novel oncogene in AML

Luciano Nicosia¹, Francesca Ludovica Boffo¹⁺, Elena Ceccacci¹⁺, Isabella Pallavicini¹, Fabio Bedin¹, Enrico Massignani¹, Roberto Ravasio¹, Saverio Minucci^{1,2} and Tiziana Bonaldi^{1*}

¹ Department of Experimental Oncology, IEO, European Institute of Oncology IRCCS, Milan 20139, Italy.

² Department of Biosciences, University of Milan, Milan 20133, Italy.

⁺ These authors contributed equally to this work.

* Corresponding author: tiziana.bonaldi@ieo.it

Abstract

The histone de-methylase LSD1 is over-expressed in haematological tumours and has emerged as a promising target for anti-cancer treatment, so that several LSD1 inhibitors are under development and testing, in pre-clinical and clinical settings. However, the complete understanding of their complex mechanism of action is still unreached. Here, we unravelled a novel mode of action of the LSD1 inhibitors MC2580 and DDP-38003, showing that they can induce differentiation of AML cells through the down-regulation of the chromatin protein GSE1. Analysis of the phenotypic effects of GSE1 depletion in NB4 cells showed a strong decrease of cell viability *in vitro* and of tumour growth *in vivo*. Mechanistically, we found that a set of genes associated with immune response and cytokine signalling pathways are up-regulated by LSD1 inhibitors through GSE1 protein reduction and that LSD1 and GSE1 co-localise at promoters of a subset of these genes at the basal state, enforcing their transcriptional silencing. Moreover, we show that LSD1 inhibitors lead

24 to the reduced binding of GSE1 to these promoters, activating transcriptional programs that trigger
25 myeloid differentiation. Our study offers new insights on GSE1 as a novel therapeutic target for
26 AML.

27

28 Introduction

29 By catalyzing the removal of methyl-groups from mono- and di- methylated forms of lysine 4 and
 30 lysine 9 of histone H3 (H3K4me1/me2 and H3K9me1/me2), the epigenetic enzyme Lysine-Specific
 31 histone Demethylase 1A (LSD1/KDM1A) has emerged as a major player in gene expression
 32 modulation in eukaryotes ^{1,2}. In different cell types, this enzyme can act as either a transcriptional
 33 co-repressor or co-activator, depending on the distinct set of interactions established ³. More
 34 frequently, LSD1 is embedded in transcriptional repressive complexes, such as CoREST and NuRD
 35 ⁴⁻⁶, where different subunits modulate LSD1 activity: CoREST confers to LSD1 the ability to bind
 36 nucleosomes, directs/channels its demethylase activity towards H3K4me1/me2 and protects it from
 37 proteasomal degradation; HDACs create an hypo-acetylated chromatin environment that stimulates
 38 LSD1 catalytic activity ⁶⁻⁸. Less often, such as in the context of androgen (AR)- and oestrogen
 39 (ER)- receptor dependent transcription, LSD1 acts as transcriptional co-activator by demethylating
 40 H3K9me1/me2 and thus promoting the downstream expression of AR- and ER- target genes ^{2,9}.
 41 Some proteins, such as the protein kinase C beta I (PKCbeta I) and PELP1 helps directing the LSD1
 42 demethylase activity towards H3K9 more than H3K4 ^{10,11}.

43 LSD1 is overexpressed in various solid and haematological tumours, where its increased levels are
 44 linked to poor prognosis ¹²⁻¹⁵. Various studies demonstrated LSD1 contribution to the onset and
 45 progression of Acute Myeloid Leukemia (AML), indicating that this enzyme can be a therapeutic
 46 target for treatment of different AML subtypes ¹⁶⁻¹⁹. In particular, LSD1 has been shown to
 47 stimulate the clonogenic activity of Leukemic Stem Cells (LSCs), trigger their oncogenic
 48 transcriptional programs ¹⁶ and also inhibit myeloid differentiation, as confirmed by the fact that
 49 LSD1 inhibition induces the activation of myeloid lineage genes, such as CD11b and CD86 ^{19,20}.
 50 All these evidence have prompted the drug discovery field to develop LSD1 inhibitors as epigenetic
 51 anti-cancer drugs ²¹⁻²⁴.

52 Because of the structural similarities of LSD1 with the monoamine oxidases (MAOs) MAO-A and
 53 MAO-B, already known inhibitors targeting MAOs have been chosen as starting scaffolds for the
 54 development of small molecules more specific/selective towards LSD1. In particular, the
 55 nonselective MAO inhibitor tranylcypromine (TCP) -which was the first compound described to
 56 efficiently inhibit LSD1 catalytic activity²⁵ - was the starting point for the design of MC2580²⁶ and
 57 DDP-38003²⁷, two probes that present higher potency and selectivity towards LSD1 than LSD2,
 58 MAO-A and MAO-B in *in-vitro* assays and, moreover, were shown to inhibit tumor growth and
 59 induce differentiation, when tested in murine AML blasts^{26,27}.

60 Various studies have helped dissecting the mechanisms of action (MoA) of these inhibitors in AML
 61 and solid tumours²⁸. Recently, a number of publications have surprisingly shown that they can
 62 trigger AML differentiation not through the expected inhibition of its catalytic activity, but by
 63 altering LSD1 binding to some of its interactors. In particular, LSD1 interaction to the transcription
 64 factors GFI1^{19,29} and GFI1b³⁰ was found to be strongly affected by these drugs, with the
 65 consequent effects of cell proliferation reduction and induction of myeloid differentiation in AML.
 66 These findings are particularly interesting because highlighted for the first time the role of LSD1 for
 67 the assembly of multi-protein complexes on chromatin and suggested that small molecules
 68 originally developed to target LSD1 catalytic activity can physically inhibit this scaffolding
 69 function, with therapeutic implications.

70 In this study, we further elaborated on our recent results on the dynamic LSD1 interactome upon its
 71 pharmacological inhibition²⁹ and focused on GSE1, whose binding to LSD1 is reduced upon cell
 72 treatment with MC2580 and DDP-38003 inhibitors, as a consequence of its diminished protein
 73 expression. Few studies have investigated the molecular and cellular function of GSE1 in cancer, so
 74 far. GSE1 has been described as an oncogene overexpressed in solid tumours, such as breast and
 75 gastric cancers, and its increased level has linked with enhanced cell proliferation, colony
 76 formation, cell migration, and invasion^{31,32}. Recently, a tumour suppressor role has been ascribed to

77 GSE1 in neuro-epithelial stem (NES) cells³³. GSE1 effect in haematological malignancies and
 78 AML in particular has not been investigated yet, especially in the context of its physical and
 79 functional interaction with LSD1^{34–37}.

80 Through the molecular and phenotypical characterization of the effect of these drugs on GSE1
 81 expression and activity on chromatin, we provide evidence of an oncogenic role of GSE1 in AML
 82 cells and demonstrate that its drug-induced reduction enforces myeloid differentiation in AML, with
 83 relevant therapeutic implications.

84

Results

LSD1 inhibitors reduce the protein expression of GSE1 in AML

Using the Differential Enrichment analysis of Proteomics data (DEP) R software package³⁸, we re-interrogated the recently published dynamic LSD1 interactome upon treatment of NB4 Acute Promyelocytic Leukemia (APL) cells with the LSD1 inhibitor MC2580 (Fig. 1A)²⁹ and we found that, in addition to the already described GFI1, also the binding to the GSE1 protein was significantly downregulated after drug treatment (Fig 1B and 1C). We confirmed the MS results by western blot (WB) analysis, profiling GSE1 level in the LSD1 co-IP and LSD1 in the reciprocal GSE1 co-IP, in control and MC2580-treated NB4 cells. While validating the reduced interaction between LSD1 and GSE1, inspection of both experiments led to the observation that GSE1 protein was already decreased in the nuclear input of both co-IPs after LSD1 inhibition (Fig. 1D).

Hence, differently from GFI1, we hypothesized that the reduced presence of GSE1 in the LSD1 co-IP was not due to the physical interference of their interaction by the drug, but to GSE1 diminished expression. We validated this hypothesis performing an *in vitro* interaction assay in which NB4 nuclear extract was incubated with increasing concentrations of MC2580 or DMSO as control for 6 hours, prior to carrying out the LSD1 co-IP. In line with our model, GSE1 was co-immunoprecipitated with LSD1 with the same efficiency in control- and MC2580-treated cells, while GFI1 was evicted by LSD1 after pharmacological inhibition, as previously reported^{19,29} (Fig. 1E).

We next assessed whether the reduction of GSE1 upon LSD1 inhibition occurred only at the protein or also at the transcript level. We treated NB4 cells with MC2580 and DDP-38003 for 12 and 24 hours and measured both GSE1 mRNA and protein by real time quantitative PCR (RT-qPCR) and WB analysis, respectively: while the reduction of GSE1 protein was confirmed, peaking at 24 hours upon drug treatment (Fig. 1F), GSE1 transcript did not changed significantly (Fig. 1G), ruling out the transcriptional regulation of GSE1 upon LSD1 inhibition. This finding was corroborated by the observation that an exogenous V5-tagged form of GSE1 -cloned in two different lentiviral vectors

110 and transduced in NB4 cells- also displayed a reduction 24 hours post-treatment with MC2580.
 111 Since the expression of the exogenous GSE1 was under the control of different promoters compared
 112 to that of the endogenous gene, this result further excludes the possibility of transcriptional
 113 inhibition of GSE1 gene by LSD1 inhibitors (Fig. S1). By assessing GSE1 levels also in other non-
 114 APL AML cell lines, such as THP-1, SKNO-1 and OCI-AML2 (Fig. S2), we confirmed that the
 115 protein down-regulation upon LSD1 inhibitors was consistent and independent from their different
 116 cytogenetic features.

117 In light of recent experimental evidence supporting a role of LSD1 in modulating the stability of
 118 target proteins independently from its catalytic activity³⁹⁻⁴², we next asked whether GSE1 reduction
 119 was the consequence of post-translational mechanisms associated with protein destabilization. First,
 120 we assessed GSE1 protein level at different time points upon treatment with cycloheximide (CHX),
 121 a translational elongation inhibitor used to monitor protein stability, and observed a strong reduction
 122 12 hours post-treatment, while c-MYC was efficiently degraded 3 hours post CHX treatment, as
 123 already described⁴³ (Fig. S3). Then, we profiled GSE1 protein level at 3,6, 9 and 12 hours upon
 124 CHX treatment in the presence or absence of MC2580 and observed that GSE1 protein stability was
 125 not altered when LSD1 was pharmacologically inhibited, suggesting that the diminished level of
 126 GSE1 is not due to the decreased stability or enhanced degradation of the protein, but likely to
 127 translation impairment (Fig. 1H, Fig. S4).

128 Pharmacological data were corroborated by the analysis of LSD1 knock-out (KO) cells, which
 129 showed diminished GSE1 protein compared to NB4 wild-type (WT) cells (Fig. 1I). Last, when we
 130 transduced LSD1 KO cells with a vector re-expressing an exogenous WT form of LSD1²⁹, we
 131 observed GSE1 protein re-established to a level significantly higher than in the cells transduced
 132 with an empty vector (EV) (Fig. 1I). This result specifically links GSE1 protein reduction to the
 133 inhibition or depletion of LSD1, excluding off-targets effects induced by the drugs.

134

GSE1 is an oncogene important for viability and *in vivo* tumour growth of NB4 cells

We then set to explore the phenotypic effects of GSE1 down-regulation in AML. To do it, we silenced GSE1 in NB4 cells by RNA interference, using two short hairpin RNAs (shRNAs) that displayed different efficiency in depleting GSE1, with shB2 being stronger than shA1 (Fig. 2A). We observed a robust reduction of cell viability that appeared dependent on GSE1 level. Alongside with reduced cell growth, cell death measured by trypan blue staining also increased with time and correlated positively with silencing efficiency (Fig. 2B and 2C). This result was corroborated by the detection of the cleaved caspase-3, a marker of apoptosis, in both shA1- and shB2- transduced cells 72 hours post-infection (Fig. 2D). The link between GSE1 KD and apoptosis was further confirmed in the shB2-transduced cells by measuring the reduction of total (not cleaved) caspase-3, which indicated that, in these cells, the majority of the enzyme was in its active form already 72 hours post-infection (Fig. 2D). Together, these experiments demonstrated that GSE1 depletion impairs NB4 cell growth and induces apoptosis.

We also assessed the effect of GSE1 downregulation on cell cycle progression by measuring the DNA content of cells with Propidium Iodide (PI), at early time points upon infection: we observed a 10%-20% increase of the proportion of cells in G1-phase, mirrored by a corresponding decrease in the percentage of cells in S-phase, in GSE1 KD cells compared to control EV-transduced ones (Fig. S5).

Based on these results *in vitro*, we tested the effect of GSE1 depletion on tumor growth *in vivo*: 24 hours post transduction with shA1, shB2 and control EV (Fig. S6), NB4 cells were injected subcutaneously in NSG mice and tumor growth was measured at time intervals, until control EV-injected mice were sacrificed when the tumor reached a maximal diameter of about 15 mm. GSE1 KD strongly affected tumor growth, as indicated by the fact that mice injected with GSE1 KD cells presented palpable tumors only at day 22 after transplantation (Fig. 2E), when almost all control mice had already been sacrificed. Furthermore, the survival curve showed that mice transplanted

160 with EV-transduced NB4 cells died between day 20 and day 22, whereas mice transplanted with
161 GSE1-depleted cells had a prolonged lifespan, with median survival time of 38 days (Fig. 2F).
162 These *in vivo* results confirm the *in vitro* data and indicate that GSE1 is a relevant oncogene in
163 AML.

164

165 **Reducing GSE1 protein level in AML, LSD1 inhibitors promote the activation of cytokine-** 166 **mediated signaling and immune response pathways**

167 Next, we set to investigate the molecular mechanisms underpinning the phenotypic effects
168 observed. Since GSE1 is a subunit of different transcriptional regulatory complexes, we decided to
169 assess the impact of GSE1 depletion on the NB4 transcriptome, carrying out RNA-sequencing
170 (RNA-seq) analysis of cells infected with either the two GSE1- shRNAs, or the EV as control. The
171 analysis was carried out at 48 hours post-infection, a time point when cell death is still negligible
172 (Fig. 2C). Upon GSE1 KD, 720 genes were up-regulated and 131 down-regulated in shA1- infected
173 cells, and 999 were up-regulated and 521 down-regulated genes in shB2- transduced NB4 (Fig. 3A,
174 Table S1). This is in line with the evidence that GSE1 is mainly associated with co-repressor
175 complexes, such as the HDAC2 and BHC complexes^{35,37}. Intersecting the differentially expressed
176 genes (DEG) in common between the two KD conditions, we obtained 422 common up-regulated
177 and 47 common down-regulated genes (Fig. 3B). Gene ontology (GO) analysis of the up-regulated
178 gene group revealed an enrichment of terms related to immune and inflammatory responses and cell
179 proliferation, while no significant enrichment of specific biological process occurred in the down-
180 regulated gene group (Table S2). In particular, using the Revigo Web server⁴⁴, we found the
181 enrichment of the biological process (BP) terms “cytokine mediated signalling”, “negative
182 regulation of viral genome replication”, “regulation of cell migration”, “extracellular matrix
183 organization” and “regulation of cell proliferation”. Within the term “negative regulation of viral
184 genome replication” we found biological processes associated with both immune response - like

185 “neutrophil mediated immunity” and “negative regulation of leukocyte mediated cytotoxicity”- and
 186 myeloid differentiation, such as “regulation of monocyte differentiation”. Instead, the term
 187 “cytokine mediated signalling” included several BP sub-terms linked to inflammation, like
 188 “regulation of I-kappaB kinase/NF-kappaB signalling” (Fig. 3C). Similar results were obtained
 189 when we analysed the upregulated gene sets with Reactome, to highlight the significant enriched
 190 biological pathways ⁴⁵ (Fig. S7).

191 The transcriptomic data on the one hand confirmed the phenotypic results obtained by the cellular
 192 assays carried out upon GSE1 depletion, such as the activation of genes involved in the regulation
 193 of cell proliferation, on the other hand highlighted the possible role of this protein in other
 194 processes, like myeloid differentiation and inflammatory response.

195 In light of the observed link between LSD1 pharmacological inhibition and GSE1 down-regulation,
 196 we then compared the transcriptomic changes observed upon GSE1 depletion with the RNA-seq
 197 data of NB4 cells treated with MC2580, in order to unravel possible overlapping transcriptional
 198 programs that may suggest the presence of molecular pathways activated by LSD1 inhibitors, in
 199 dependence of GSE1. Compared to GSE1 KD, MC2580 induced significantly lower transcriptional
 200 changes, with only 109 significantly up-regulated and 3 down-regulated genes, respectively (Fig.
 201 S8, Table S3). This is in line with the stronger cellular effects observed upon GSE1 down-
 202 regulation compared to those elicited by LSD1 pharmacological inhibition. Under the hypothesis
 203 that a subset of genes may be transcriptionally induced by the drug through GSE1 downregulation,
 204 we first assessed the expression levels of the 109 genes up-regulated by MC2580 within the GSE1
 205 KD cell transcriptome. Most of the genes modulated by MC2580 also showed an increasing trend
 206 upon GSE1 depletion; in particular, 40% of them were significantly up-regulated after infection
 207 with both shRNAs (Fig. 3D). Given this overlap, we assessed through Ingenuity Pathway Analysis
 208 (IPA) whether the molecular pathways activated by LSD1 inhibition were the same as those
 209 stimulated by GSE1 depletion: 55% of the pathways triggered by the drug were also significantly

210 activated by GSE1 depletion and the majority were associated with “cytokine-mediated signaling”
 211 and “immune response” GO terms (Fig. 3E, Table S4). We confirmed the up-regulation of some of
 212 the genes belonging to these biological processes by RT-qPCR upon both MC2580/DDP-38003
 213 treatment (Fig. S9A) and GSE1 KD (Fig. S9B).

214 These results suggest that LSD1 inhibitors upregulate genes belonging to cytokine-mediated
 215 signalling and immune response pathways via GSE1 down-regulation. This hypothesis was
 216 confirmed by profiling the expression of a panel of genes involved in these processes in NB4 cells
 217 upon MC2580 treatment, in combination or not with GSE1 over-expression. Observing that the up-
 218 regulation of these genes upon LSD1 inhibition was significantly reduced when GSE1 was over-
 219 expressed (Fig. 3F), we propose a novel function of GSE1 as an important co-repressor of genes
 220 linked to inflammatory- and immune- response pathways and suggest the use of these inhibitors to
 221 unlock them through GSE1 targeting.

222

223 **LSD1 pharmacological inhibition reduces GSE1 association to LSD1-bound promoters of** 224 **genes involved in immune response and inflammatory pathways**

225 Transcriptomic analysis suggested that the pharmacological inhibition of LSD1 leads to the GSE1-
 226 dependent activation of specific transcriptional programs. Since GSE1 is a chromatin-associated
 227 factor, we decided to profile the effect of its reduction on chromatin by performing Chromatin
 228 Immuno-Precipitation (ChIP). ChIP experiments were carried out in NB4 cells upon expression of
 229 the V5-tagged form of GSE1, whereby the V5-tag was used as bait for affinity enrichment of bound
 230 chromatin, to overcome the problem of the unavailability of ChIP-grade antibodies against GSE1.
 231 As the drug induces down-regulation of the exogenous V5-GSE1 to a similar extent to the
 232 endogenous protein (Fig. S1), we reasoned that ChIP-seq analysis of the V5-tagged isoform could
 233 be a good proxy to assess the effects of LSD1 inhibitors on GSE1 genomic localization.

ChIP-seq analysis proved that GSE1 binding to chromatin was globally reduced upon drug treatment, with the number and overall intensity of assigned peaks almost halved in treated cells (Fig. 4A and 4B). By inspection of the genomic distribution of the assigned peaks in both functional states, we found that at basal state GSE1 mainly localizes at distal intergenic (~ 25%), intronic (~ 35%) and promoter (~ 25%) regions, while upon LSD1 inhibition its binding slightly increases at distal intergenic and intronic regions and decreases at the promoters (Fig. 4C).

We then grouped the genomic regions bound by GSE1 in four different categories: “Regions bound by GSE1 similarly in both DMSO- and MC2580-treated cells”, “GSE1- bound regions that display reduced binding upon MC2580”, “regions bound by GSE1 only in DMSO condition” and “GSE1- bound regions only upon MC2580” (Table S5). We profiled by ChIP-seq the localisation of LSD1 and different histone post-translational modifications (PTMs) at these four genomic regions and found that LSD1 and all histone PTMs analysed co-localized with V5-GSE1 in all the genomic regions, except from those bound exclusively by GSE1 upon drug treatment. Furthermore, LSD1 binding at GSE1 regions was not affected by the inhibitor, while histone PTMs associated with active transcription (mainly H3K4me3 and slightly H3K4me2) were enriched, particularly at those loci where GSE1 binding was reduced/abolished by the compound (“GSE1- bound regions that display reduced binding upon MC2580” and “regions bound by GSE1 only in DMSO condition”) (Fig. 4D, Fig. S10). This suggests that the decreased association of GSE1 to chromatin may be linked to a chromatin state more prone to transcriptional activation. Moreover, in light of the reduced localization of GSE1 at promoters upon MC2580 and of the increased H3K4me3 level observed at the same regions (Fig. 4D, Fig. S11), we hypothesized that the decreased GSE1 association could directly mediate the transcriptional changes measured upon LSD1 inhibition. To confirm this hypothesis, we assessed the presence of promoters of the genes up-regulated by MC2580 within the subsets of genomic regions where GSE1 binding was diminished by the drug and found several promoters of genes associated with cytokine-mediated signalling and immune

response, like IFI16, IL31RA, CD86, FOS, CD48. LSD1 co-localized with GSE1 at these promoters and its binding was not altered by the drug, while the H3K4me3 level increased (Fig. 4E). We validated the ChIP-seq results by ChIP-qPCR for some of these promoters. With ChIP-qPCR we also detected a reduced binding of GSE1 at the promoters of ICAM-1 and ICAM-4 upon MC2580, other two interesting genes involved in cytokine signalling pathways that were up-regulated by LSD1 inhibition (Fig. 4F). The ChIP data corroborate the transcriptomic results and provide a mechanistic insight about the effect of LSD1 inhibitors on GSE1 localisation and activity at chromatin regulatory regions.

GSE1 down-regulation induced by LSD1 inhibition triggers myeloid differentiation

Pharmacological inhibition of LSD1 represents a promising epigenetic approach for AML treatment through the release of the differentiation block and the induction of differentiation processes in leukemic blast cells^{17,19}. In line with this, our transcriptomic data demonstrated that the majority of the DEGs induced both by MC2580 and GSE1 depletion were involved in haematological system development and haematopoiesis (Fig. S12). Thus, we hypothesized that the LSD1-dependent reduction of GSE1 on chromatin might be phenotypically linked to the induction of myeloid differentiation. To test this hypothesis, first we profiled by flow cytometry the expression of the cell surface differentiation marker CD11b in GSE1 KD NB4 cells, observing 4- and 8- fold increase in the percentage of cells expressing this marker 48 hours post transduction with shB2 and shA1 constructs, respectively (Fig. 5A). Induction of CD11b upon GSE1 KD was detected also in THP-1 monocytic cells, where we could also assess CD14, the monocyte differentiation antigen typically up-regulated upon a differentiation stimulus: 4- to 6- fold increase of CD14 was observed in GSE1 KD cells compared to the control. Furthermore, the majority of CD14- positive cells were also positive to CD11b (Fig. 5B).

Then, we asked whether GSE1 reduction induced by LSD1 inhibition was important to trigger

284 myeloid differentiation by measuring with flow cytometry CD11b expression at 24 hours post
 285 MC2580 treatment, in wild type (EV-infected) NB4 cells and in the presence of GSE1 over-
 286 expression (OE). The observation that the increased CD11b level upon LSD1 inhibition was
 287 significantly reduced when GSE1 was overexpressed suggests that the differentiation process
 288 elicited by MC2580 also depends on GSE1 protein level (Fig. 5C). We also analysed the expression
 289 of CD11b and CD14 in control (EV) THP-1 cells and in GSE1 overexpressing ones and confirmed
 290 the same results observed in NB4 (Fig. 5D). Interestingly, the overexpression of GSE1 in NB4 also
 291 led to the increase of the subpopulation of cells expressing the stemness marker cKIT. This
 292 indicates that GSE1 not only prevents myeloid differentiation but also favours the maintenance of a
 293 stem phenotype, at least in this cell line (Fig. S13 and S14).

294 Altogether the data collected led to the elaboration of a model whereby the inhibition of LSD1 with
 295 MC2580 and DDP-38003 reduces GSE1 protein level, leading to its reduced binding to chromatin
 296 and in particular to LSD1-target promoter regions regulating the expression of genes linked with
 297 cytokine-signalling and immune response pathways. The consequent up-regulation of these
 298 transcriptional programs enforces myeloid differentiation in leukemic cells (Fig. 5E).

299

300

301 Discussion

302 In this study, we show for the first time that the LSD1 inhibitors MC2580 and DDP-38003 can
 303 elicit myeloid differentiation in AML through the down-regulation of GSE1 protein, a poorly
 304 explored LSD1 interactor. Our data add another layer of information on the mechanism of action of
 305 these drugs in leukaemia, beyond the already known inhibitory effects on the lysine histone
 306 demethylase activity ²⁰ and on LSD1-GFI1 interaction ^{19,29}. Furthermore, our results unveil the
 307 oncogenic role of GSE1 in AML.

308 Our data suggest that the drugs in use most likely affect GSE1 translation rather than transcription
 309 or stability, even if the details of the mechanism remain to be elucidated. One possibility is that the
 310 decrease of GSE1 protein level may be caused by the drug-induced upregulation of some miRNA
 311 targeting this gene. This hypothesis is supported by the evidence that various miRNAs can
 312 modulate GSE1 expression ^{31,32,46}. Alternatively, GSE1 downregulation could be caused by the
 313 block of its translational initiation ⁴⁷, in line with recent data showing that -in some cell lines- LSD1
 314 inhibitors activate the mTOR signalling cascade ^{48,49}, which directly affects protein synthesis ⁵⁰.
 315 Future investigations will allow dissecting mechanistically how LSD1 inhibitors modulate GSE1
 316 translation.

317 The RNA-seq data show that in NB4 cells GSE1 depletion induces more extensive transcriptional
 318 changes than those caused by LSD1 inhibitors. In line with this, also the phenotypic effects elicited
 319 upon GSE1 KD are more pronounced than those observed upon treatment with LSD1 inhibitors,
 320 which consist in a mild reduction of cell viability and colony-forming ability in liquid and semi-
 321 solid culture, respectively ²⁹. These molecular and phenotypic differences can be explained in light
 322 of the significantly different reduction of GSE1 protein level caused by these two perturbations,
 323 with MC2580/DDP-38003 leading to a milder GSE1 down-regulation (around 40%) than the shA1
 324 and shB2 constructs (around 80%-90%). The detected dose-dependency of the phenotypic effects to

325 GSE1 reduction induced by the two shRNA constructs used in this study corroborates this
326 hypothesis.

327 The evidence that cellular levels of GSE1 are critical for NB4 cell viability is particularly intriguing
328 as it suggests that this oncogene can represent a new target for AML treatment.

329 From a molecular standpoint, the transcriptomic data collected in this study indicate that the
330 induction of genes involved in cytokine-signalling and immune response pathways by LSD1
331 inhibitors is GSE1-dependent. Specifically, we propose that this transcriptional response is
332 mediated by the reduction of GSE1 on chromatin and in particular, among the different genomic
333 regions bound by GSE1, we focused on promoters -most of them bound by also LSD1- because of
334 the possibility to directly link the effects of GSE1 binding variation to the expression of the
335 associated genes, by the intersection of ChIP-seq and RNA-seq data. It shall be noted, however, that
336 the ChIP-seq data also showed the binding of GSE1 to distal intergenic and intronic regions and the
337 overall co-localization with H3K4me1 and H3K27ac, two histone modifications traditionally
338 associated with enhancers. This observation points towards the presence of GSE1 at other *cis*-
339 regulatory elements, so that its reduced association to chromatin after drug treatment could cause
340 more global transcriptional effects, still to be described.

341 In summary, by describing for the first time the molecular and cellular implications of GSE1
342 modulation in AML, this study paves the way to the thorough assessment of the role of this
343 chromatin factor in haematological malignancies and of the possibility of targeting it with LSD1
344 inhibitors to trigger myeloid differentiation for AML treatment.

345

346 **Materials and Methods**

347 **Cell culture**

348 NB4 and THP-1 cell lines were grown in RPMI plus 10% of foetal bovine serum (FBS), 2 mM
349 glutamine (Glu) and 1% Penicillin/Streptomycin (P/S). SKNO-1 cells were cultured in the same
350 conditions, with the addition of 10 ng/ml GM-CSF. OCI-AML2 cell line was grown in 80% alpha-
351 MEM supplemented with 20% FBS, 2 mM Glu and 1% P/S. Cultures were maintained in a
352 humidified tissue culture incubator at 37°C in 5% CO₂.

353

354 **Compounds**

355 The LSD1 inhibitors MC2580 and DDP-38003 were provided by the Department of Drug
356 Chemistry and Technologies of the Sapienza University of Rome (Italy) and the Experimental
357 Therapeutic Unit of the IFOM-IEO Campus, respectively ^{26,27}. Cycloheximide was purchased from
358 Sigma Aldrich (C7698).

359

360 **RNA-sequencing (RNA-seq) and data analysis**

361 mRNA-sequencing (mRNA-seq) libraries were prepared with the TruSeq RNA Sample Preparation
362 v2 kit (RS-122-2002, Illumina) according to the manufacturer's protocol, starting from 500 ng of
363 total RNA per sample. Sequencing was performed using the NovaSeq 6000 (Illumina) instrument.
364 Raw reads were mapped to the human reference genome hg38 using STAR aligner ⁵¹ and quantified
365 through the *rsem-calculate-expression* function of the RSEM package ⁵². Differentially expressed
366 genes (DEG) were determined with the DEseq2 package ⁵³, as follows: genes with an adjusted p-
367 value lower than 0.01 and a log₂ Fold Change (FC) greater than 1 and smaller than -1 (log₂ FC < -1
368 and > 1) were considered as upregulated and downregulated, respectively. GO analysis of the
369 enriched biological processes (BP) was carried out with the EnrichR software ⁵⁴, while biological

370 pathway analysis was executed using the Reactome database ⁴⁵ contained within EnrichR software
 371 and the Ingenuity Pathway Analysis (IPA) (QIAGEN Inc.,
 372 <https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis>). Significant BP and
 373 Reactome terms had an adjusted p-value < 0.05, while significant IPA pathways had a -log(p-
 374 value) > 2. Voronoi plot of the GO was generated using the R-package voronoiTreemap
 375 (<https://github.com/uRosConf/voronoiTreemap>).

376

377 **Lentiviral and retroviral constructs for exogenous protein expression**

378 NB4 LSD1 KO cells and NB4 LSD1 KO cells transduced with the LSD1 N-terminal truncated
 379 (172-
 380 833) form or the empty PINCO vector were generated as previously described in ²⁹. To knock-down
 381 (KD) GSE1, short hairpin RNA (shRNA) constructs targeting the protein were cloned into the
 382 pLKO.1 puro expression vector. The primers used for shRNA production and cloning were the
 383 following:

384 1. shA1:

385 a. FW 5'-
 386 CCGGGAACCTCACCTTGACGTCAATGCTCGAGCATTGACGTCAAGGTGAG
 387 TTCTTTTGTG-3'

388 b. REV 5'-
 389 AATTCAAAAAGAACTCACCTTGACGTCAATGCTCGAGCATTGACGTCAA
 390 GGTGAGTTC-3'

391 2. shB2:

392 a. FW 5'-
 393 CCGGCTGAGCATGCTTCACTATATCCTCGAGGATATAGTGAAGCATGCTC
 394 AGTTTTTGTG-3'

three times per week with a linear caliper and the volume was calculated using the formula $V = (a \times b^2)/2$, where a and b are the longest and the shortest diameters of the tumor, respectively. Results are reported as tumor volume (mm³). Mice were sacrificed when the longest diameter of the tumor reached a size of approximately 15 mm.

425

426 **Flow cytometry analysis of cell surface markers**

About 1×10^6 cells were harvested, re-suspended in 300 μ l of 5% BSA dissolved in PBS and blocked for 30 minutes at room temperature. Then, cells were re-pelleted and re-suspended in 100 μ l primary antibody diluted in 1% BSA in PBS and let for 1h at room temperature in the dark. At this point, cells were washed with 1 ml of 1% BSA in PBS, centrifuged and re-suspended in 250 μ l cold PBS. Transduced cells were, then, fixed by the addition of 250 μ l 2% formaldehyde in PBS and incubated for 20 minutes in ice. After a further spinning, cell pellets were re-suspended in 500 μ l PBS and analyzed by the FACS Celesta flow cytometer (BD Biosciences). Data analysis was performed using the FlowJo software. The antibodies used for the flow cytometry analysis of the cell surface markers were: anti-human CD11b (740965, BD OptiBuild™, 1:100), anti-human CD14 (11-0149-42, Thermo Fisher Scientific, 1:100), anti-human CD117 known also as cKIT (12-1178-42, Thermo Fisher Scientific, 1:100).

438

439 **Chromatin Immuno-Precipitation-sequencing (ChIP-seq)**

ChIP-seq analysis was carried out in NB4 cells transduced with the pLEX_307 GSE1 and treated for 24 hours with either MC2580 or DMSO. As negative ChIP control we used NB4 cells transduced with the empty vector pLEX_307 (pLEX_307 EV). About 1×10^8 cells for each condition were cross-linked by formaldehyde at 1% final concentration, which was added to the culture medium and incubated for 10 minutes with shaking. The reaction was stopped with 0.125 M Glycine and then samples were left shaking for 5 minutes at room temperature. Cells were then washed twice with PBS and lysed in SDS buffer (50 mM Tris-HCl pH 8.0, 0.5% SDS, 100 mM

NaCl, 5 mM EDTA, 0.02% NaN₃), supplemented with protease inhibitors (Roche, 04693116001) and 0.5 mM PMSF. At this point, we added the Triton Dilution buffer (100 mM NaCl, 100 mM Tris-HCl pH 8.5, 5 mM EDTA, 5% Triton X-100, 0.02% NaN₃) supplemented with protease inhibitors (Roche, 04693116001) and 0.5 mM PMSF to the whole cell extracts to obtain the IP buffer conditions (100 mM NaCl, 33 mM Tris-HCl pH 8.0, 5 mM EDTA, 0.02% NaN₃, 0.33% SDS, 1.7% Triton X-100, 33 mM Tris-HCl pH 8.5). Chromatin was then subjected to 35 cycles of sonication (30 seconds each) using a Branson Sonifier 250 to obtain DNA fragments of 300-bp average length. Subsequently, sheared chromatin was pre-cleared by incubation with 100 µl of Dynabeads protein G (Invitrogen, 10004D) for 2 hours on a rotating wheel at 4 °C. After preclearing, chromatin was used as input in the immuno-precipitation experiment, carried out overnight on a rotating wheel at 4 °C in the presence of 20 µg of anti-V5 antibody (Abcam, ab9116). 2.5% of the input sample was collected just before addition of the antibody and stored at -20 °C, for subsequent tests. After the overnight incubation, 200 µl of Dynabeads protein G were added to the antibody-chromatin reaction tube and incubated for 3 hours on a rotating wheel at 4 °C. Later, beads were washed thrice with Washing buffer A (1% Triton X-100, 0.1% SDS, 150mM NaCl, 2mM EDTA pH 8, 20mM Tris-HCl pH 8) and once with Washing buffer B (1% Triton X-100, 0.1% SDS, 500mM NaCl, 2mM EDTA pH 8, 20mM Tris-HCl pH 8) supplemented with protease inhibitors (Roche, 04693116001), followed by a final washing step with TE 1X buffer. At each wash, beads were incubated for 5 minutes on a rotating wheel at 4 °C. De-crosslinking/elution step of the ChIP samples were carried out by the addition of 300 µl de-crosslinking buffer (2% SDS, 0.25 mg/ml Proteinase K in TE 1X), followed by overnight incubation at 65 °C. The de-crosslinking step was also carried out for the input sample, by adding 3 volumes of de-crosslinking buffer to 1 volume of input. The day after, DNA from ChIPs and Input samples were purified using the DNA purification kit (QIAquick PCR Purification Kit, Qiagen, 28106) according to the manufacturer's protocol. DNA libraries were prepared with 10 ng of DNA through an in-house protocol⁵⁸ by the IEO genomic facility and sequenced on a NovaSeq 6000 (Illumina) instrument.

473

474 ChIP-seq data analysis

475 Short reads obtained from Illumina Genome Analyzer II were quality-filtered according to the
 476 ENCODE pipeline ⁵⁹. Reads were aligned to the hg38 reference genome using Bowtie (v 4.8.2) ⁶⁰.
 477 MACS (v 1.4.2) ⁶¹ was used as peakcaller to identify regions of ChIP-seq enrichment of the V5-
 478 GSE1 in DMSO and MC2580-treated cells, using as background both the respective input and the
 479 V5-ChIP performed in NB4 cells transduced with the pLEX_307 EV. Resulting enriched regions
 480 were annotated as specifically bound by GSE1 in each condition. Only reads with a unique match to
 481 the genome and with two or fewer mismatches (-m 1 -v 2) were retained. MACS p-value threshold
 482 was set to 10^{-5} for all the data sets. The four genomic region categories displayed in the Fig. 4D and
 483 the Fig. S10-S11 were obtained as follows: genomic regions bound by GSE1 in both DMSO- and
 484 MC2580-treated cells were defined as regions with peaks in both conditions and at least 1 bp of
 485 overlap between the two samples. These loci were further divided in “common same intensity”, that
 486 displayed a $\log_2FC$ RPKM of DMSO vs MC2580 < 0.5 and “common MC2580 reduced intensity”
 487 showing a $\log_2FC$ RPKM of DMSO vs MC2580 > 0.5 . Regions commonly detected in both
 488 conditions and showing a $\log_2FC$ RPKM of MC2580 vs DMSO > 0.5 were only 32 (“common
 489 DMSO reduced intensity”) and were not shown in the figures. “DMSO only” and “MC2580 only”
 490 categories instead contained genomic regions with identified peaks in DMSO or MC2580-treated
 491 cells, respectively. Within these regions, read counts were calculated with bedtools suite and only
 492 regions with a $\log_2FC$ RPKM of DMSO vs MC2580 > 1 for “DMSO only” and $\log_2FC$ RPKM of
 493 MC2580 vs DMSO > 1 for “MC2580 only” were kept. Annotation of the genomic regions in the
 494 different conditions was achieved using the R package ChIPseeker ⁶² and ChIPpeakAnno ⁶³. The
 495 bigwig files for UCSC browser visualization of genome profiles were normalized with the
 496 deepToos suite. ChIP-seq data of LSD1, H3K4me1, H3K4me2, H3K4me3 and H3K27ac used for
 497 comparative analysis with V5-GSE1 ChIP-seq are described and present in ²⁹.

498

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512 (SEMM).

513

514 **Data and code availability**

515 The accession number for all the RNA-seq data and the V5-GSE1 ChIP-seq data reported in this
516 paper is GEO database: GSE164560.

517 The accession number for the LSD1, H3K4me1, H3K4me2, H3K4me3 and H3K27ac ChIP-seq data
518 reported in ²⁹ is GEO database: GSE128530.

519 The accession number for the MS-proteomics data reported in ²⁹ is ProteomeXchange database:
520 PXD012954.

521

522 **Conflict of interest**

523 The authors declare that they have no conflict of interest.

524

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711

712 Figure Legends

713 **Figure 1: LSD1 inhibitors decrease translation of GSE1 in AML.** A) Experimental design of the
 714 SILAC/LSD1 co-IP strategy setup to study the changes in LSD1 interactome upon treatment with 2
 715 μ M of MC2580, as described in ²⁹. B) Volcano plot displaying the alteration in the LSD1
 716 interactome upon MC2580 treatment. Significantly evicted and recruited LSD1 interactors are
 717 marked as red dots, while the proteins previously defined as specific LSD1 binders ²⁹ are indicated
 718 by red triangles. The blue dashed line indicates the p-value threshold used to define the interactors
 719 modulated by the LSD1 inhibitors (p value < 0.05 calculated with limma ⁶⁴). Analysis of the evicted
 720 and recruited LSD1 interactors after pharmacological treatment is performed using the Differential
 721 Enrichment analysis of Proteomics data (DEP) R software package ³⁸. C) *Upper panel*: MS1
 722 spectrum of the SILAC peak doublet for the peptide 897 -ALSAAVADSLTNSPR- 911 of GSE1
 723 protein in the SILAC-LSD1 co-immuno-precipitated (co-IP) sample from NB4 cells treated for 24
 724 hours with 2 μ M of MC2580 (heavy peak) and DMSO-treated control cells (light peak). *Lower*
 725 *panel*: Table displaying the H/L (forward replicate) and L/H (reverse replicate) ratios for GSE1 in
 726 SILAC-LSD1 co-IPs of NB4 cells treated with the compound MC2580 or DMSO. The table also
 727 shows the Ratio Count (RC) of each replicate, which corresponds to the number of peptides used
 728 for SILAC-based protein quantitation. The inhibitor was added to the heavy channel in the forward
 729 replicate and the light channel in the reverse one. D) Western Blot analysis of GSE1, LSD1 and H3
 730 in LSD1 co-IPs (*left panel*) and GSE1 co-IPs (*right panel*), both in control and MC2580-treated
 731 cells (2 μ M) for 24 hours. Rabbit IgG was used as negative control co-IP. E) Western Blot analysis
 732 of GSE1, LSD1, GF11 and H3 in *in vitro* LSD1 co-IPs using as input NB4 nuclear cell extracts co-
 733 incubated for 6 hours with increasing doses of MC2580 (2, 10 and 20 μ M) and DMSO as control.
 734 F) *Left panel*: Western Blot analysis of GSE1 in NB4 cells treated with MC2580 (2 μ M), DDP-
 735 38003 (2 μ M) and control DMSO for 12 and 24 hours. Vinculin was used as loading control. *Right*
 736 *panel*: Bar-graph displaying the quantitation results of GSE1 protein, normalized over the Vinculin,

737 in 3 independent replicates of NB4 cells treated with MC2580 (2 μ M), DDP-38003 (2 μ M) and
738 DMSO. The results are plotted as fold change (FC) of GSE1 protein level in the treated samples
739 over control (DMSO). The chart represents mean + standard deviation (SD) (n=3 biological
740 replicates; one sample T-test, **p value<0.01, *p-value<0.05). G) RT-qPCR analysis on GSE1
741 transcript in NB4 cells treated for 12 and 24 hours with either MC2580 (2 μ M), DDP-38003 (2 μ M)
742 and DMSO as control. GSE1 ct values are normalized against GAPDH. The results are plotted as
743 FC of GSE1 mRNA levels in LSD1 inhibitors-treated conditions over DMSO. Chart represents
744 mean + SD of three (n=3) biological replicates. H) *Upper panel*: Representative Western Blot
745 analysis of GSE1 and c-MYC in NB4 cells treated with cycloheximide (0.1 mg/ml) in combination
746 with MC2580 (2 μ M) and DMSO as control for 3, 6, 9 and 12 hours. Vinculin was used as loading
747 control. *Lower panel*: Line-plot displaying the percentage of GSE1 protein level normalized over
748 Vinculin in cycloheximide-treated NB4 cells, together with 2 μ M of MC2580 or DMSO as control.
749 Chart represents mean $\pm$ SD (n=2 biological replicates). I) *Left panel*: Western blot analysis of
750 GSE1 and LSD1 in NB4 WT, NB4 LSD1-KO and LSD1-KO cells transduced with either a
751 retroviral empty PINCO vector (EV) or a PINCO vector containing an exogenous LSD1 N-terminal
752 truncated (172-833) form ²⁹. Vinculin was used as loading control. Arrows indicate the endogenous
753 and the exogenous LSD1. *Right panel*: Bar-graph displaying the quantitation of GSE1 protein level,
754 normalized over Vinculin level in 3 replicates of NB4 WT, NB4 LSD1-KO and LSD1-KO cells,
755 transduced with either an empty PINCO vector (EV) or a PINCO vector containing an exogenous
756 LSD1 N-terminal truncated (172-833) form ²⁹. In all samples the data are plotted as FC of GSE1
757 protein level compared to the NB4 WT, with mean + SD from n=3 biological replicates (one sample
758 T-test, **p value<0.01, ***p-value<0.001).

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Figure 2: GSE1 knock-down (KD) affects NB4 cell viability and *in vivo* tumour growth in NOD/SCID gamma (NSG) mice. A) *Left panel:* Western blot analysis of GSE1 protein in NB4 cells transduced with either an empty lentiviral pLKO.1 puro vector (EV) or pLKO.1 puro containing the shRNA constructs targeting GSE1 (shA1 and shB2) for 72 hours. Vinculin was used as loading control. *Right panel:* Bar-graph displaying the quantitation of GSE1 protein level normalized over the Vinculin in three independent replicates (n=3), whereby the results are plotted as fold change (FC) of GSE1 level in the knock-down (KD) samples over the control EV. Chart represents mean + standard deviation (SD) (n=3 biological replicates; one sample T-test, *p value<0.05). B) Growth curve of NB4 cells transduced with either an empty pLKO.1 puro vector (EV) or pLKO.1 puro containing the shA1 and shB2 targeting GSE1. Graph represents mean $\pm$ SD from two biological replicates (n=2). C) Bar-graph displaying the percentage of dead cells in control EV and GSE1 KD cells. The analysis is performed after 48, 72, 96, 120 and 144 hours of infection. Cell viability was measured by trypan blue staining. Chart represents mean + SD from two (n=2) biological replicates. D) Western Blot analysis of GSE1, cleaved caspase-3 and total caspase-3 in cells transduced with either the control pLKO.1 puro (EV) or pLKO.1 containing the two shRNAs targeting GSE1 (shA1 and shB2) for 72 and 96 hours. Vinculin was used as loading control. The arrow indicates the cleaved form of the caspase-3. E) Tumour growth curve in NSG mice transplanted subcutaneously with NB4 cells transduced 24 hours earlier with either an empty pLKO.1 puro or pLKO.1 puro with the insert of the shRNAs (shA1 and shB2) targeting GSE1. Graph represents mean $\pm$ SD of the tumour volume in each condition (n=8, 8 distinct mice for each group). F) Kaplan-Meier survival curve of mice transplanted with NB4 cells previously transduced with either an empty pLKO.1 vector or pLKO.1 containing shA1 or shB2 targeting GSE1 (n=8 for each group; Log-rank Mantel-Cox test, ****p-value<0.0001).

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Figure 3: GSE1 down-regulation elicited by LSD1 inhibitors induces transcription of genes associated with cytokine-mediated signalling and immune response pathways. A) Volcano plot displaying up- and down-regulated genes upon 48 hours transduction with pLKO.1 vector containing shA1 and shB2 inserts. The x-axis shows the $\log_2$ fold change (FC) values of each gene in the shRNAs-transduced cells compared to control empty vector (EV)-transduced, while y-axis displays the $-\log_{10}$ adjusted p-values (p-adj). FC value is calculated with DEseq2 program⁵³, using two biological replicates for each condition (n=2). B) Venn diagrams with number of individual and overlapping up-regulated and down-regulated genes identified 48 hours after transduction with shA1 and shB2 constructs. C) Voronoi tree-map displaying the statistically significant GO biological process (BP) terms associated with the common 422 up-regulated genes upon GSE1 KD with the two shRNA constructs. GO analysis and calculation of the statistically significant BP was performed through EnrichR⁵⁴ (adjusted p-value < 0.05), and then BP were grouped using Revigo⁴⁴. Voronoi plot includes the BP terms mapped to a high hierarchical level. The tassel size corresponds to the $-\log_{10}$ p-value of the enrichment while the colour intensity to the number of genes belonging to each category. D) Unsupervised hierarchical clustering of all significantly up-regulated genes upon MC2580 treatment (p-adj < 0.01, $\log_2$ FC > 1). Heatmap displays the RNA-seq expression z-scores of these genes 48 hours after transduction with pLKO.1 puro containing shA1, shB2 or the EV as control in NB4 cells. Each row in the heatmap represents an individual gene. Z-score values of each gene are plotted as the average of two independent biological replicates, for each condition (n=2). Genes significantly up-regulated by both shA1 and shB2 (p-adj < 0.01, $\log_2$ FC > 1) are flagged in red. Below is shown the pie-chart indicating the percentage of the 109 genes up-regulated by MC2580 that are also significantly induced by GSE1 KD with both shRNAs. E) Bubble-plot displaying the statistically significant IPA pathways ($-\log_{10}$ p-value > 2) obtained from the analysis of the differentially expressed genes (DEG) after MC2580 treatment and comparison with the IPA results achieved from the DEG upon GSE1 KD. The bubble size reflects the number of genes belonging to that pathway, while colours indicate the $-\log_{10}$ p-value associated

with each IPA term. Non-significant IPA pathway terms are coloured in white. IPA pathways are clustered manually, according to specific biological processes. Some IPA pathway terms are not displayed in the current figure panel, but the complete list is in the Table S4. F) RT-qPCR analysis on a panel of genes up-regulated by both LSD1 inhibitors and GSE1 KD, in control and GSE1 over-expressing (OE) NB4 cells treated with 2 μ M of MC2580 treatment. GSE1 OE cells are generated by lentiviral transduction with the pLEX_307 vector containing the GSE1 coding sequence (NM_001134473.3), while control cells are transduced with the empty pLEX_307 (pLEX_307 EV). Ct values for each gene are normalized against GAPDH. The results are plotted as FC of the mRNA levels in the treated conditions relative to the control DMSO. Bar-graph represents mean + standard deviation (SD) from three (n=3) biological replicates; paired T-test, *p-value<0.05).

Figure 4: MC2580 decreases GSE1 localization on the LSD1-bound promoters of genes involved in cytokine-mediated signalling and immune response pathways. A) Heatmap representing the normalized V5-GSE1 ChIP-seq intensities ± 5 kb around the center of the GSE1 target loci, at basal state and after 24 hours treatment with MC2580 (2 μ M). V5-GSE1 ChIP-seq was performed in NB4 GSE1 OE cells produced by lentiviral transduction of the pLEX_307 construct containing the GSE1 coding sequence (NM_001134473.3) (pLEX_307 GSE1), while the negative control ChIP was carried out in cells transduced with the empty pLEX_307 (pLEX_307 EV). B) Bar-graph displaying the number of peaks extrapolated from the V5-GSE1 ChIP-seq in DMSO and MC2580-treated cells. C) Genome annotation of the V5-GSE1 ChIP-seq peaks in control DMSO and LSD1-inhibited NB4 cells. D) Heatmap displaying the normalized V5-GSE1, LSD1 and H3K4me3 ChIP-seq intensities ± 5 kb around the center of the GSE1 target loci after 24 hours treatment with MC2580 or DMSO as control. The heatmap is grouped in different subgroups, according to the presence or absence or differential intensity of the GSE1 binding regions in the two conditions. The subset of “Common GSE1 targets with reduced intensity in DMSO” contains only

32 regions and is not displayed in the figure. The LSD1 and H3K4me3 ChIP-seq used to generate the heatmap were published in ²⁹. E) Heatmap displaying the V5-GSE1, LSD1 and H3K4me3 ChIP-seq intensities at the promoters of genes up-regulated after LSD1 inhibition, which show reduced binding of V5-GSE1 in MC2580 treated-cells. Each row in the heatmap represents an individual gene. For the V5-GSE1 ChIP-seq, reads intensities in regions identified as peaks and annotated to each gene are plotted as log₂ RPKM. Instead for the LSD1 and H3K4me3 ChIP-seq, reads coverage (log₂ RPKM) at promoter (+/- 2.5 kb distance from the TSS) were represented. For each gene its chromatin state is shown as referred in the Fig. 4D. F) ChIP-qPCR profiling of a panel of GSE1-bound promoters of genes significantly up-regulated by LSD1 inhibition in DMSO and MC2580 (2 μM)-treated NB4 GSE1 OE cells using the pLEX_307 GSE1 construct. V5 ChIP in NB4 cells transduced with the pLEX_307 EV was used as negative control ChIP. NEG corresponds to a negative control region. Data are normalized over the respective Input and displayed as fold change (FC) over the control EV. Bar-graph represents mean + standard deviation (SD) from two (n=2) biological replicates.

Figure 5: GSE1 down-regulation contributes to the myeloid differentiation re-activation enforced by LSD1 inhibition. A) Percentage of NB4 CD11b-positive cells measured by flow cytometry 48 hours post transduction with the shA1 and shB2 cloned in pLKO.1 puro, or with the empty pLKO.1 puro vector (EV) used as negative control. Dot plots show the results obtained in one of the two biological replicates of the experiment. Bar-chart represents mean + standard deviation (SD) (n=2 biological replicates). B) Percentage of CD11b- and CD14- positive THP-1 cells, assessed by flow cytometry analysis 48 hours upon transduction with pLKO.1 puro vector containing shA1, shB2 or EV as negative control. Dot plots display the data of one of the two biological replicates of the experiment. Bar-graph shows mean + SD (n=2 biological replicates). C) Percentage of CD11b-positive cells evaluated by flow cytometry in control (EV) and GSE1 OE

860 cells, treated for 24 hours with MC2580 (2 μ M). NB4 GSE1 OE are produced by transducing the
 861 pLEX_307 vector with the GSE1 coding sequence (NM_001134473.3), while control cells are
 862 transduced with the pLEX_307 EV. Chart represents mean + SD (n=4 biological replicates; paired
 863 T-test, **p-value<0.01). D) Flow cytometry analysis of the percentage of CD11b- and CD14-
 864 positive cells in EV and GSE1 OE THP-1 cells treated for 24 hours with MC2580. THP-1 GSE1
 865 OE are generated by lentiviral transduction of the pLEX_307 vector including GSE1 coding
 866 sequence (NM_001134473.3), while control cells are transduced with the pLEX_307 EV. Chart
 867 represents mean + SD (n=3 biological replicates; paired T-test, **p-value<0.01). E) Model of the
 868 molecular effects associated with the down-regulation of GSE1 induced by LSD1 pharmacological
 869 inhibition.

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Figure 1: LSD1 inhibitors decrease translation of GSE1 in AML

Figure 2: GSE1 knock-down (KD) affects NB4 cell viability and *in vivo* tumour growth in NOD/SCID gamma (NSG) mice

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Figure 3: GSE1 down-regulation elicited by LSD1 inhibitors induces transcription of genes associated with cytokine-mediated signalling and immune response pathways

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