Supplementary Information

Legends

Supplementary Figure 1: Size-fractionation profiles by Superose 6 column of GATA-1 (panel A), members of the MeCP1 and ACF/WCRF complexes (panel B) and transcription factors (panel C). Molecular mass markers are indicated on the top. V_0: void volume. N.E.: input nuclear extract.

GATA-1 displays a broad fractionation profile with several peaks. The profile of tagged GATA-1 closely follows that of endogenous GATA-1, is stable in salt concentrations up to 1M and is not dependent on the presence of DNA (data not shown). Members of the MeCP1 complex showed overlapping peaks around fractions 20-22 (e.g. MTA2, RbAp46/48, Mbd2/3) in contrast to Sin3A which peaked around fractions 18-20. SNF2h and ACF1 peaked around fractions 26-28 and are distinctly different from those of the MeCP1 complex. p17 elutes in fractions 34-36, further suggesting that GATA-1 interactions with SNF2h and ACF1 occur in the context of the ACF/WCRF complex. These observations also suggest that GATA-1 interactions with MeCP1 and ACF/WCRF occur in distinct complexes. Fractionation profiles of transcription factors FOG-1, Ldb1, TAL-1 and Gfi-1b are largely coincident within the higher molecular weight fractions 16-28, though peaks vary between them. For most of these factors little or no protein is detected in the free protein fractions (i.e. fractions 32-38), in contrast to Sp1 which elutes as free protein.

Supplementary Figure 2: DNase I treatment of nuclear extracts shows the indirect co-purification of abundant chromatin associated proteins with GATA-1. Nuclear extracts from MEL cells treated or not treated with DNAse I were immunoprecipitated with GATA-1 antibodies. Co-immunoprecipitation of abundant chromatin associated proteins such as topoisomerase I orPARP, identified by mass spectroscopy as co-purifying with GATA-1, was lost upon DNAse I treatment. By contrast, co-immunoprecipitation of MTA2, a member of the MeCP1 complex co-purified with GATA-1, was unaffected by DNase I treatment thus showing a direct interaction with GATA-1.

Supplementary Figure 3: Histone deacetylase (HDAC) assays. (A) HDAC activity associated with proteins immunoprecipitated by the indicated antibodies. Protein G beads and rat and goat immunoglobulins (IgG) were used as background controls. Three different nuclear extracts were

used in the GATA-1 immunoprecipitations (extract 1: non-transfected MEL cells; extracts 2 and 3: biotinylated GATA-1). (B) HDAC activity immunoprecipitated by HDAC2, FOG-1 and GATA-1antibodies is sensitive to the Class I HDAC inhibitors TSA and OSI-2040. (C) Antibodies against the Mi2/NuRD-associated protein MTA2 deplete a considerable part of the HDAC activity associated with FOG-1 and GATA-1 immunoprecipitates. Immunoglobulins (IgG) were used as background control.

Supplementary Figure 4: ChIP assay to show GATA-1, Mbd2 and Mi-2β binding to the GATA-1 (panel A) and MBP (panel B) loci in induced MEL cells. It is clear that the binding pattern observed for GATA-1 and Mbd2 in these gene loci in Figure 5A and 5C is reproduced by the binding pattern of Mi-2β. Mi-2β antibody: as in (Fujita et al., 2003).

Supplementary Figure 5: DNA methylation assay by restriction enzyme digestion at the –2.8kb element of the GATA-2 locus. Genomic DNA (5-10μg) from non-induced (left panel) and induced (right panel) MEL cells was first digested with Eco RI, which releases a 3.9kb fragment, followed by digestion with Eag I (methylation sensitive, indicated by asterisk), or Xmn I as control for complete digestion of the genomic DNA samples. Eag I maps close to the GATA-1 binding sites in the –2.8kb element of the GATA-2 locus and within the PCR fragment (grey box, not to scale) amplified in the ChIP assays shown in Figures 5 and 6. Digested DNA was blotted and probed with a 1.2kb Apa I fragment (solid line) which detects 1kb and 2.1kb fragments on the Eag I digests and 1.7kb and 2.2kb fragments on the Xmn I digests. For both digests, the larger fragments are weaker due to their limited overlap with the probe. It can be seen that Eag I digests completely in DNA of both induced and non-induced MEL cells, thus suggesting that its recognition site is not affected by DNA methylation.

Materials and Methods

ChIP PCR primer sequences:

EKLF upstream enhancer forward PCR primer: 5'-CTGGCCCCCCTACCTGAT-3'

EKLF upstream enhancer reverse PCR primer: 5'-GGCTCCCTTTCAGGCATTATC-3'

EKLF -1.35kb forward PCR primer: 5'-TGCTCCCCACTATGATAATGGA-3'

EKLF -1.35kb reverse PCR primer: 5'-GCCACAACCAAAGAAGACATTTT-3'

MBP -1.2kb forward PCR primer: 5'-GGGTCTAATTCCGAGGGTGAGT-3'

MBP -1.2kb reverse PCR primer: 5'-GGCCTGGAAATCACTGAGCTA-3'

MBP promoter forward PCR primer: 5'-CCGCCAAGGTGTCTATAAATGC-3'

MBP promoter reverse PCR primer: 5'-TGGGTCTTGTCAAGTTTGCAAA-3'

MBP +0.6kb forward PCR primer: 5'-GAAGTAGAGGCAGGATAATCAGGAA-3'

MBP +0.6kb reverse PCR primer: 5'-AGGATGAACCAGGGCTAATGC-3'

MBP +1.8kb forward PCR primer: 5'-TGTGACAGACGTGGACCTTCA-3'

MBP +1.8kb reverse PCR primer: 5'-TGCATCCAGAGTCACCCATAAG-3'

GATA2 -4.2kb region forward PCR primer: 5'-GAATTTCCTGCCGGTCCAT-3'

GATA2 -4.2kb region reverse PCR primer: 5'-GACGCGTTGGCTTTGTGTG-3'

GATA-2 -3.4kb forward PCR primer: 5'-TCCATCCAGCAGCTTTAGGAA-3'

GATA-2 -3.4kb region reverse PCR primer: 5'-GGGTTCGAAGCCACTCCAA-3'

GATA-2 -2.8kb region forward PCR primer: 5'-CCGGGCAGATAACGATTGG-3'

GATA-2 -2.8kb region reverse PCR primer: 5'-TTCATCTCGGCCGGCTAAT-3'

GATA-2 -2.2kb region forward PCR primer: 5'-AGGACCCCCTGCTTCTTGTTTC-3'

GATA-2 -2.2kb region reverse PCR primer: 5'-GGCAGTATGAGGCCCAGAATCTT-3'

Myb promoter forward PCR primer: 5'-GGGCGCCAGATTTGG-3'

Myb promoter reverse PCR primer: 5'-GGAGGAAACAGGTTGATATTAAAGT-3'

Myb -0.8kb forward PCR primer: 5'-GTAGGTTTGTCCAGCAAGTGTTTG-3'

Myb -0.8kb reverse PCR primer: 5'-AGGTGCCTACCACGCACTTCT-3'

Myc promoter forward PCR primer: 5'-CCAGACATCGTTTTTCCTGCATA-3'

Myc promoter reverse PCR primer: 5-'CCGCTCAGTGTGTGGAGTGATA-3'

Myc -0.7kb forward PCR primer: 5'-ACACACACATACGAAGGCA-3'

Myc -0.7kb reverse PCR primer: 5'-ACCGTTAACCCCTTCCTCCC-3'

pOVEC exon 2 forward PCR primer: 5'-TCACCTGGACAACCTCAAAGG-3'

pOVEC exon 2 reverse PCR primer: 5'-CAGGATCCACGTGCAGCTT-3'

GAPDH forward PCR primer: 5'-TGAAGGGGAAGCTCAGTCG-3'

GAPDH reverse PCR primer: 5'-TCCACCACCCTGTTGCTGTA-3'

PCR primers for RNA analysis in G1E cells

GATA-2 mRNA primers:

Exon III forward PCR primer: 5'-ACTATGGCAGCAGTCTCTTCCATC-3'

Exon V reverse PCR primer: 5'-AAGGTGGTGGTTGTCGTCTGAC-3'

DNase I treatment of nuclear extracts

Nuclear extracts (50μg) were diluted to 150 mM KCl with HENG buffer and 5 % glycerol. 10 units of DNaseI (Invitrogen) were added and the extract was incubated overnight at 4°C with the addition of 5 mM $CaCl_2$ and 10 mM $MgCl_2$. Extracts were bound to streptavidin beads and analyzed by western blotting. Antibodies used were: Topoisomerase I goat polyclonal (Santa Cruz, sc-5342); PARP rabbit polyclonal (Alexis Biochemicals, ALX-210-302).

HDAC assays. Immunoprecipitations for HDAC assays were carried out using 0.3-0.5mg of nuclear extracts in HENG150 / 0.1% NP-40, as described above. Immunoprecipitates were washed once for 10 minutes in HENG150 / 0.3% NP-40 and three times for 10 minutes each in HENG300 / 0.3%NP-40. Beads were resuspended in HENG50 and HDAC assays were done using approximately 32,000cpm of ^{3}H-labelled core histones per reaction, as previously described (Taunton et al., 1996).

Antibodies. The following antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA): N6 GATA-1 rat monoclonal (sc-265); M20 FOG-1 (sc-9361); SNF2h (sc-8760), Gfi-1b D19 (sc-8559) and TAL-1 (sc-12982) were goat polyclonal antibodies; mouse monoclonal antibodies for mSin3A (sc-5299) and HDAC2 (sc-9959); rabbit polyclonal antibodies for HDAC1 (sc-7872) and Sp1 (sc-59). 15G12 RbAp46/48 mouse monoclonal (ab490), MTA2 goat polyclonal (ab9949) and MBD2a rabbit polyclonal (ab3754) antibodies were purchased from Abcam (Cambridge, UK). p66 mouse monoclonal was purchased from Upstate Biotechnology (07-365; Waltham, MA). Rabbit polyclonal antibodies against TAL-1 were a generous gift by Richard Baer (Columbia University, NY) and by Catherine Porcher (WIMM, Oxford). Rabbit polyclonal antibodies against Ldb1 were kindly donated by Gordon N. Gill (Stanford University, CA). Rabbit polyclonal antibodies against FOG-1 were a generous gift by Stuart H. Orkin (Harvard Medical School, MA). Rabbit polyclonal antibodies against MTA2, Mi2 and Mbd2/3 were kindly donated by Paul A. Wade (NIH/NIEHS, NC). Sheep anti-serum S923 against Mbd2 and rabbit polyclonal antibody R593 against Mbd2/Mbd3 were generously provided by Adrian Bird (University of Edinburgh, UK). Rabbit polyclonal antibodies against ACF1 were a generous gift of Patrick Varga-Weisz (Marie Curie Research Institute, Surrey, UK). Rpd3 rabbit polyclonal antibodies were donated by Alexander Brehm (Adolf Butenandt Institut, Germany). Mouse monoclonal antibody against B23 nucleophosmin was a kind gift by Pui K. Chan (Baylor College of Medicine, TX). Secondary antibodies conjugated to horseradish peroxidase were purchased from Dako (DakoCytomation, Denmark) and Amersham Biosciences and developed by chemiluminescence using the ECL-PLUS kit (Amersham).