Supplemental Materials and Methods

Construction of TetR-EYFP-LSD1 Fusion Constructs

The coding sequence for full-length human LSD1 (NCBI accession BC048134) in pBluescriptR was purchased from SourceBioScience / GeneService and amplified with oligonucleotide primers LSD1_F (5’-AAAAAAGATCTGAGATGTTATCTGGGAAGAAGGC-3’) and LSD1_R (5’-AAAAAAGATCTATGCATCTGTCTCACATGCTTGG-3’) containing BglII restriction sites. The PCR product was digested with BglII and cloned into the BamHI restriction site of tYIP (Cardinale et al., 2009) to generate tYIP-LSD1, expressing tetR-EYFP-LSD1WT from a CMV promoter and conferring resistance to puromycin through an internal ribosome entry site. To generate the K661A mutant, the LSD1 ORF in pBluescriptR was subjected to site-directed mutagenesis, and a construct expressing tetR-EYFP-LSD1K661A was created analogously to tYIP-LSD1.

Generation of 1C7-LSD1 Cell Lines

1C7 cells stably expressing tetR-EYFP-LSD1WT or tetR-EYFP-LSD1K661A were generated by transfection with tYIP-LSD1 or tYIP-LSD1(K661A), respectively, using Fugene HD (Roche) as described previously (Cardinale et al., 2009). Clonal cell lines were isolated by limiting dilution and grown in RPMI (Gibco) containing 10% FBS (Gibco), in the presence of 4µg/ml blasticidin S (Invitrogen), 1µg/ml doxycycline (Sigma) and 1-2µg/ml puromycin (Sigma). Expression levels of the fusion construct were measured on a FACSCalibur flow cytometer (BD BioScience). Nuclear localization and targeting to the HAC after doxycycline wash out were confirmed by fluorescence microscopy. Doxycycline
wash out experiments where conducted as previously described (Cardinale et al., 2009).

**Immunostaining and Fluorescence Signal Quantification**

Indirect immunofluorescence staining of cells fixed in 4% PFA/PBS was performed using standard protocols. Preparation and staining of unfixed mitotic chromosomes was essentially performed as described in Keohane et al (Keohane et al., 1996). In brief, cells were blocked in 100ng/ml colcemid (KaryoMax, Gibco) for two hours, and mitotic cells collected by shake-off. Cells were subject to hypotonic treatment, cytopspun on glass slides and incubated in KCM buffer (10mM Tris pH8.0; 120mM KCl; 20mM NaCl; 0.5mM EDTA; 0.1% Triton X-100) for 10 minutes prior to labelling with antibodies in KCM buffer, fixation in 4% PFA/KCM and counter-staining in DAPI. Cells were subsequently mounted in VectaShield (VectorLabs). Antibodies used were mouse anti CENP-A (AN1), rabbit anti CENP-A (Valdivia et al., 1998), rabbit anti CENP-C (R554) and mouse anti H3K36me2 (2C3). Fluorophore-conjugated secondary antibodies were purchased from Jackson Labs.

**Chromatin Immunoprecipitation Experiments**

Exponentially growing cells where washed in D-PBS (Gibco), harvested with TrypLE Express (Gibco), resuspended in D-PBS to a final concentration of 1x10⁶/ml and crosslinked in a final 1% formaldehyde (Fischer Scientific) for 5 minutes at room temperature, followed by quenching in 0.5M glycine for an additional 5 minutes. Cells where washed in TBS and 5x10⁶ cells where lysed in lysis buffer (10mM Tris pH8.0; 10mM NaCl; 0.5% NP-40) containing protease inhibitors (1µg/ml CLAP; 0.5µg/ml aprotinin; 1mM PMSF) for 10 minutes on ice. Nuclei where briefly washed
in lysis buffer containing protease inhibitors and resuspended in 300µl Dilution Buffer 1 (50mM Tris pH8.0; 2mM EDTA; 0.2% SDS; 134mM NaCl; 0.88% Triton X-100; 0.088% Na-Deoxycholate) containing protease inhibitors. Chromatin was sheared by sonication in a Bioruptor sonicator (Diagenode) for 14 cycles of 30sec on / 30sec off at a high setting at 4°C. Supernatants where diluted with 300µl Dilution Buffer 1, 500µl Dilution Buffer 2 (50mM Tris pH8.0; 167mM NaCl; 1.1% Triton X-100; 0.11% Na-Deoxycholate) and 500µl RIPA buffer containing 150mM NaCl (RIPA-150) and protease inhibitors. Anti-mouse IgG Dynabeads M-280 (Invitrogen) where pre-blocked with BSA and subsequently coupled with the relevant antibodies for 4-6 hours in RIPA-150 / 0.5% BSA at 4°C, washed twice in RIPA-150 / 0.5% BSA, and 500µl of the sheared chromatin was incubated with the beads over night at 4°C. Beads where then washed twice with RIPA-150 containing protease inhibitors, followed by two washes in RIPA-500 and a final wash in TE pH8.0. Antibody / chromatin complexes where eluted at 65°C in TE / 1% SDS. An equal volume of Post-Elution Buffer (10mM Tris pH8.0; 9mM EDTA; 600mM NaCl) was added and crosslinks reversed at 65°C over night. Samples where treated with RNAse A and proteinase K followed by phenol / chloroform extraction, and DNA was finally resuspended in TE.

ChIP’ed and Input DNA was subject to real-time PCR analysis using a SYBR Green Mastermix (Sigma) on a LightCycler480 system (Roche). For each primer pair, a standard curve was prepared from the input material and included on every plate to calculate the % of precipitated DNA relative to the input material. Oligonucleotide primer pairs were described previously (Cardinale et al., 2009).
Histone antibodies used for ChIP were described previously (Kimura et al., 2008). Other antibodies were mouse anti CENP-A (AN1) and mouse anti RNA polymerase II [8WG16] (Abcam). Normal mouse IgG was used as control.

**RNA Immunoprecipitation**

Crosslinking of cells, preparation of nuclei, sonication and immunoprecipitation were essentially performed as in ChIP experiments above, except that buffers were adjusted to a pH of 7.6. Sonicated nuclear material corresponding to 3x10⁶ cells was used in each IP. IP’ed material was eluted off the beads in the presence of RNAse inhibitor (RNAsin, Promega) at 42°C. Proteinase K and NaCl were added to final concentrations of 0.5mg/ml and 300mM, respectively. Samples were subsequently incubated for one hour at 42°C and transferred to 65°C for an additional two hours to reverse crosslinks. RNA was subsequently extracted using TRizol (Invitrogen), precipitated with isopropanol in the presence of 15µg GlycoBlue (Ambion) and resuspended in RNAse-free water.

Samples were treated using DNaseI Turbo (Ambion) according to the manufacturer’s instructions, and 2µl DNase-treated RNA were directly used for real-time RT-PCR using the iScript one-step real-time RT-PCR kit (BioRad). For each sample, control reactions in the absence of reverse transcriptase (-RT) were performed in parallel to determine potential contamination with genomic DNA.

**CENP-A-SNAP Quench-Pulse-Chase Experiments**

A plasmid containing the coding sequence for CENP-A-SNAP-3xHA (pLJ184, Jansen et al., 2007) was digested with BglII and NotI, and the 1.1kb
fragment containing the coding sequence of the construct was ligated into pIRES-Puro2 (Clontech) digested with BamHI and NotI to yield pCENP-A-SNAP-IP.

1C7 cells were seeded on coverslips and transiently co-transfected with 0.8µg of either tetR-EYFP fusion construct and 0.2µg pCENP-A-SNAP-IP using 3µl Fugene 6 (Roche) in a transfection mix of 100µl OptiMEM, essentially according to the manufacturer’s instructions. The next day, thymidine (Sigma) was added to a final concentration of 2mM, and transfected cells were enriched for by selection in the presence of 1µg/ml puromycin. 10 hours after addition of thymidine, existing SNAP-tag was quenched for 20 minutes in medium containing 10µM non-fluorescent bromothienylpteridine (SNAP-Cell Block, NEB), and cells were subsequently released from thymidine arrest. 18 hours after release, newly-synthesized SNAP-tagged CENP-A was fluorescently labelled in medium containing 3µM TMR-Star (NEB) for 30 minutes. Cells were fixed and processed for fluorescence microscopy one hour after labelling.

Quantification of HAC-associated TMR-Star signal was performed on maximum intensity projections of deconvolved image stacks. For each cell displaying a single HAC, the mean fluorescence was calculated within a 9 pixel diameter circular region of interest (ROI) and was normalized to the mean fluorescence intensity measured at endogenous centromeres within the image stack using a ROI of the same size.
Supplemental Figure Legends

Figure S1| Active centromeres share a transcriptionally permissive chromatin environment (related to Fig. 1). A) ChIP analysis of AB2.2.18.21 cells as in Fig. 1. Note the different scaling of individual panels. B) Real-time RT-PCR analysis of transcripts from the synthetic alphoid\textsuperscript{tetO} (tetO) and endogenous chromosome 21 (chr.21) centromeres as well as the blasticidin resistance (Bsr) marker in AB2.2.18.21 16 hours after treatment with or without 100 ng/ml actinomycin D. Transcript levels are expressed over background (no RT). Note the log scale. C) RNA immunoprecipitation analysis using either unspecific IgG or an antibody against the transcription elongation mark H3K36me2. Real-time RT-PCR was performed on IP’ed material in the presence (+) or absence (-) of reverse transcriptase (RT). Specific enrichment of alphoid transcripts from the HAC (tetO) and chromosome 21 (chr. 21) centromere, but not the 5’ region of the Bsr marker, was reproducibly obtained in H3K36me2 pull downs.

Figure S2| tetR-EYFP-LSD1 efficiently reduces H3K4me2 levels at the HAC centromere. A) IF analysis of interphase 1C7 cells two days after transiently expressing either tetR-EYFP, tetR-EYFP-LSD1\textsuperscript{WT} or tetR-EYFP-LSD1\textsuperscript{K661A} stained for H3K4me2 (green) and CENP-A (red). The presence of LSD1 catalytic activity at the HAC (arrowheads) reduces H3K4me2 staining to nuclear background levels. B) PFA-fixed mitotic spreads transfected and stained as in (A). Scale bars represent 5\textmu m.

Figure S3| Tethering of LSD1 does not alter H3K9 and H3K27 methylation patterns. A) ChIP analysis in 1C7-LSD1\textsuperscript{WT} cells as in Fig. 3, three days after Dox wash-out. Data represent the mean of two or more independent ChIP experiments. %
of input values were normalized to the 5S rDNA locus. Error bars represent S.E.M. Changes in alphoid<sup>tetO</sup>-associated histone methylation levels are not significant. B) Complete time course of the CENP-A IF quantification in 1C7-LSD1<sup>WT</sup> cells shown in Fig. 3E and 4A.

**Figure S4** LSD1 catalytic activity interferes with kinetochore structure. A) IF analysis of interphase 1C7 cells transiently expressing tetR-EYFP, tetR-EYFP-LSD1<sup>WT</sup> or tetR-EYFP-LSD1<sup>K661A</sup> four days after transfection with the corresponding plasmids. CENP-C staining at the LSD1-targeted HAC appears to be reduced compared to HACs targeted by tetR-EYFP alone. Displayed cells represent the median values of the quantification in (B). Arrowheads depict the HAC. Scale bars: 5µm. B) CENP-C IF signal quantification of interphase HACs transiently targeted by either of the three constructs. Cells with similar expression levels (as judged by the perceived EYFP signal) where chosen. Arbitrary fluorescence units (AFU) are plotted and the median indicated by the solid bar. The difference in CENP-C staining between the two constructs was statistically significant (p<0.001).

**Figure S5** Fusion construct expression level in 1C7-derived clones. (A) Flowcytometric analysis of tetR-EFYP fusion levels in cell lines stably expressing the wild-type LSD1 construct at low (1C7-LSD1<sup>low</sup>) or high (1C7-LSD1<sup>WT</sup>) levels, or that express the catalytically inactive mutant LSD1<sup>K661A</sup> fusion. For reference, maternal 1C7 cells not expressing any fluorescent construct are shown. Average fluorescence values are indicated. (B) Quantification of EYFP fluorescence signals associated with the HAC in three 1C7-LSD1 clones expressing tetR-EYFP-LSD1<sup>WT</sup> at different levels. Solid bars and associated numbers indicate median values. The same exposure
time was used for each cell line. Note that for 1C7-LSD1WT cells, a 50% neutral density (N.D.) filter was used. Expression level determined by flow cytometry as in (A) is indicated under the graph.

**Figure S6| RNA polymerase II ChIP analysis.** Analysis of 1C7-LSD1WT cells as in Fig. 3C. Compared to the actively transcribed Bsr gene, extremely low enrichment of RNA polymerase II relative to unspecific IgG is reproducibly detected at the synthetic HAC centromere before Dox wash-out (+Dox). 24 hours after Dox wash-out, the HAC centromere lacks detectable RNA polymerase II levels. At neither time point is RNA polymerase II detected at the endogenous chromosome 21 centromere, which is likely to be a too large genomic region to allow sufficiently sensitive detection of low polymerase molecule numbers. Data represented corresponds to the mean and standard deviation of two independent ChIP experiments.
Supplementary Figure 3

(A) Bar plot showing the percentage of input [5s rDNA] for H3K9me3, H3K27me2, and H3K27me3 across different conditions and time points.

(B) Scatter plot showing the HAC-associated CENP-A signal normalized AFU over time with exposure to 1C7-LSD1WT.
A tetR-EYFP

CENP-C

4dpt

tetR-EYFP-

LSD1<sub>WT</sub>

CENP-C

4dpt

tetR-EYFP-

LSD1<sub>K661A</sub>

CENP-C

4dpt

B HAC-associated CENP-C signal

AFU

*** p<0.001

Bergmann et al, Supp Fig. 4
Bergmann et al, Supp Fig. 5

A

average: 3 7 47 90

relative cell number

1C7
1C7-LSD1WT-low
1C7-LSD1WT
1C7-LSD1K661A

EYFP

B

HAC-bound tetR-EYFP-LSD1WT

clone: 1C7-LSD1WT-low 1C7-LSD1med 1C7-LSD1WT

expression level: 7 9 47

(AFU)

(50% N.D.)

4.4x10^5
7.5x10^5
15.4x10^5