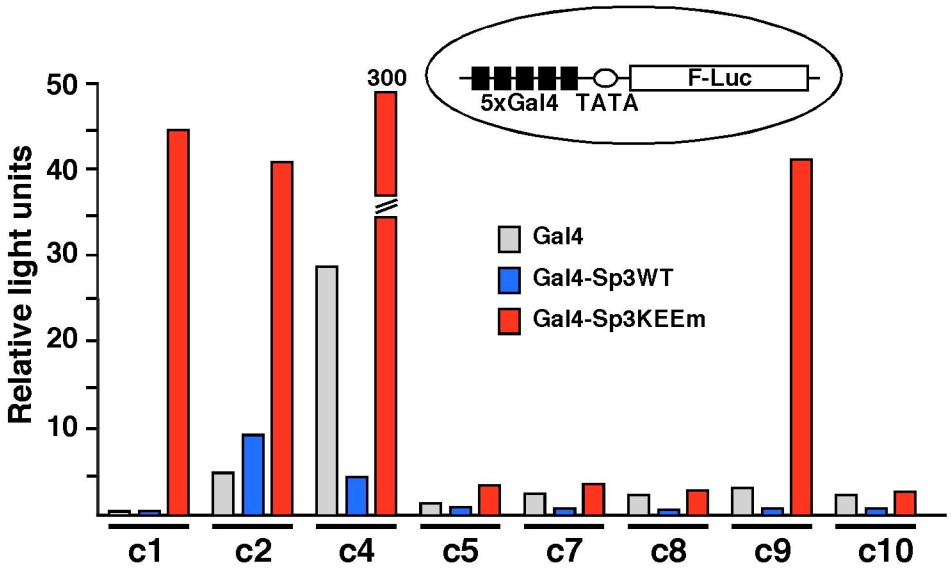
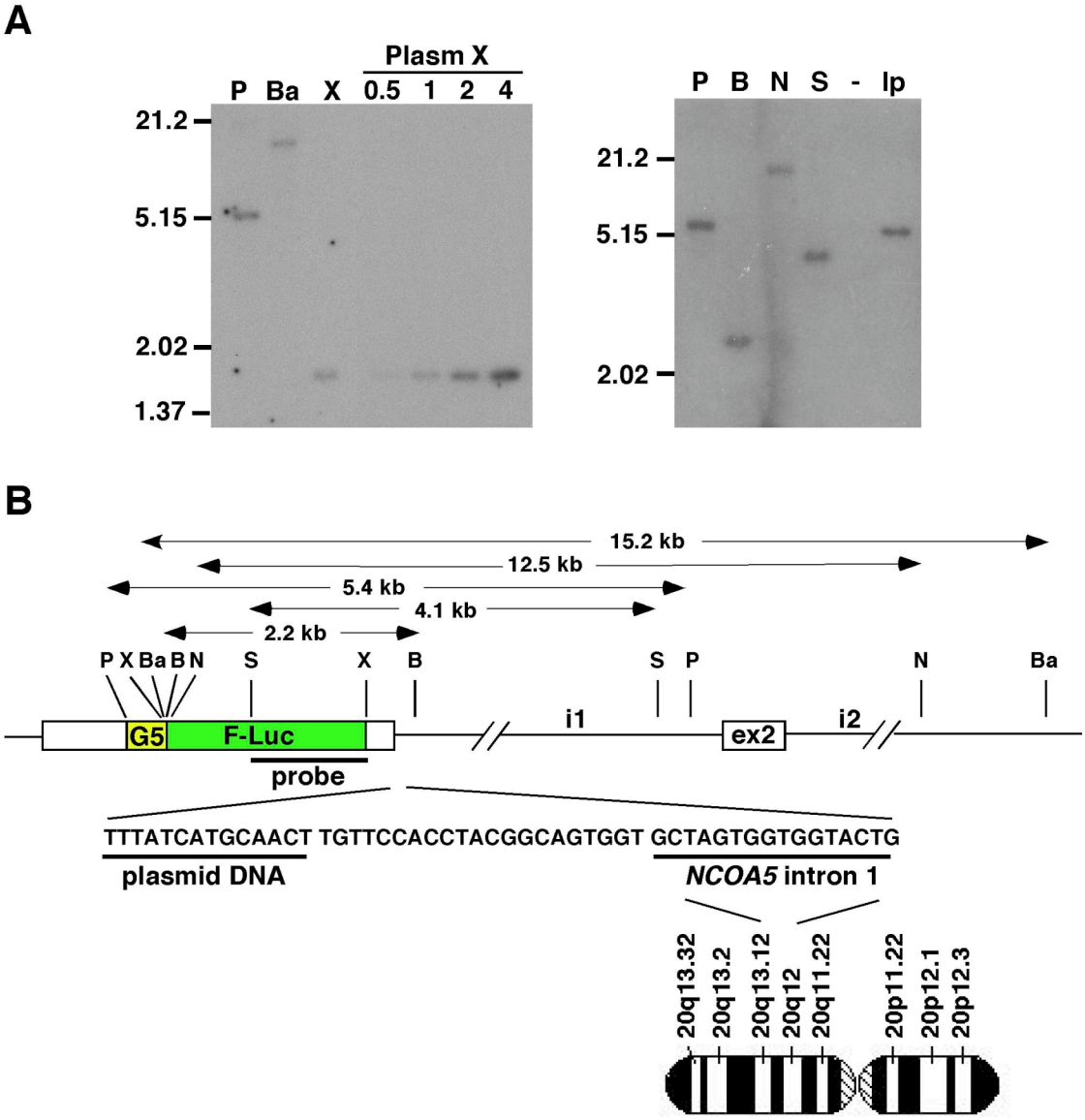
Stielow et al: Supplementary Information

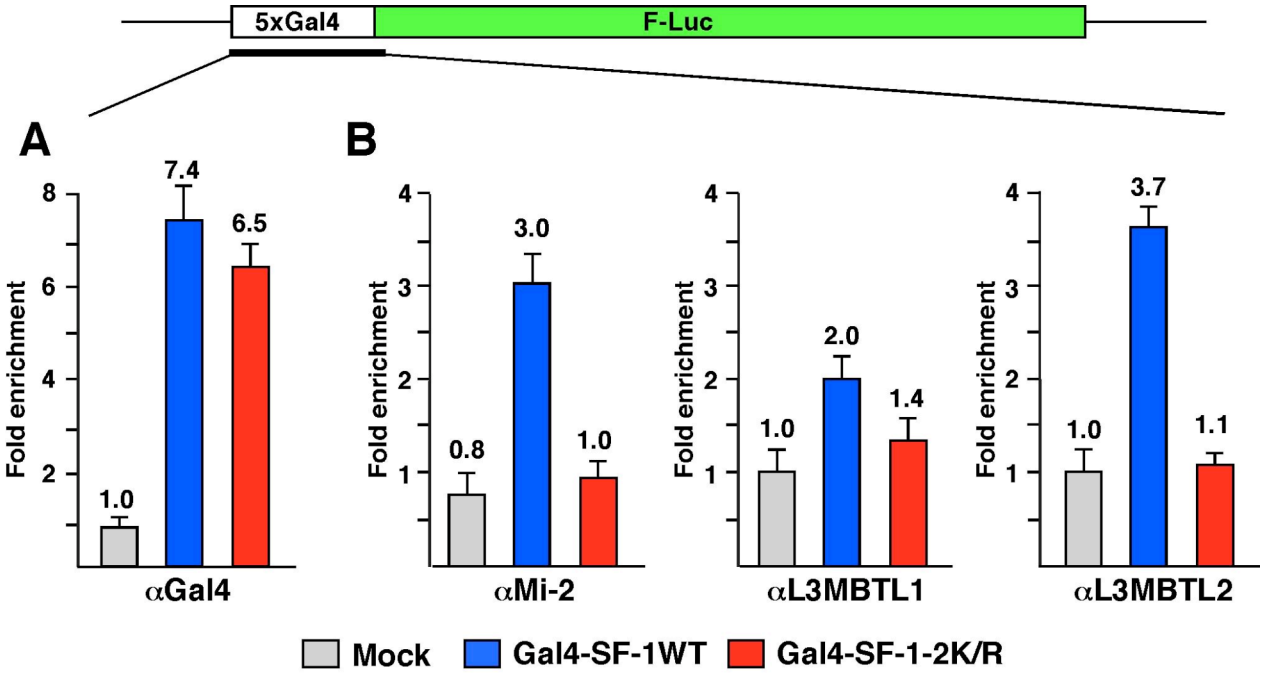
Supplementary Figures



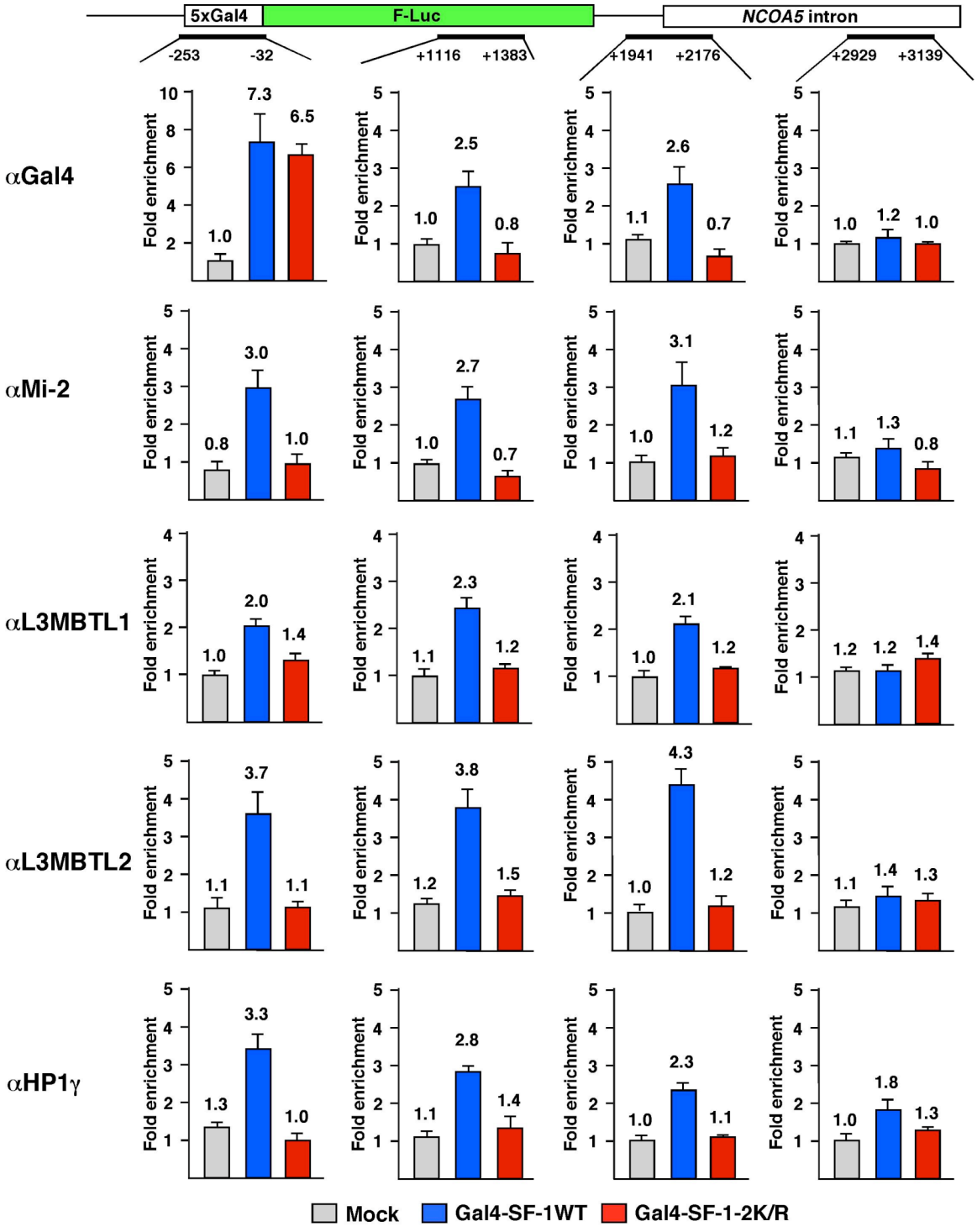
Supplementary Fig 1. Generation of a stable cell line to study SUMO-dependent gene silencing on a chromatinized transgene. Individual HEK 293 cell clones stably transfected with a 5xGal4-luciferase reporter were transiently transfected with expression plasmids for the Gal4 DNA binding domain (Gal4), a Gal4-Sp3WT fusion (Gal4-Sp3WT) or a SUMOylation-deficient Gal4-Sp3 mutant (Gal4-Sp3KEEm) along with a ß-galactosidase control reporter. Cells were lysed 48 hours posttransfection and luciferase activities determined.



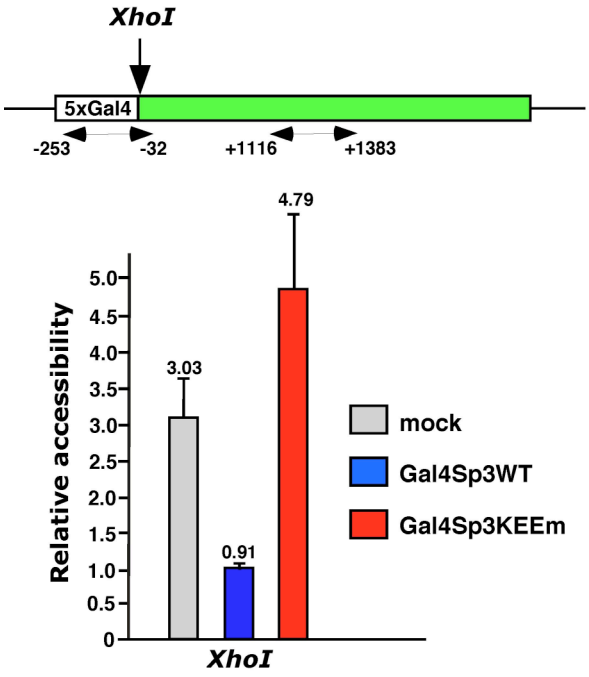
Supplementary Fig 2. Southern Blot analyses and integration site mapping of the 5xGal4 luciferase transgene in clone c1. **(A)** Southern blot analyses of the integrated 5xGal4-luciferase transgene in clone c1. Abbreviations are: P, PstI; Ba, BamHI; X, XbaI; B, BglII; N, NcoI; S, SphI; lp, linearized plasmid control). The gel on the left contains 0.5, 1, 2 and 4 copies of the XbaI-linearized 5xGal4-luc plasmid (Plasm X). **(B)** Schematic illustration of the transgene integration site in the first intron of the NCOA5 gene on chromosome 20. The probe used for the Southern blots shown in (A) is indicated. The integration site of the transgene within the first intron of the *NCOA5* gene was determined by inverse PCR using religated BglII-digested genomic DNA. The nucleotide sequence depicts the transition between the plasmid DNA and the genomic *NCOA5* sequence on chromosome 20.



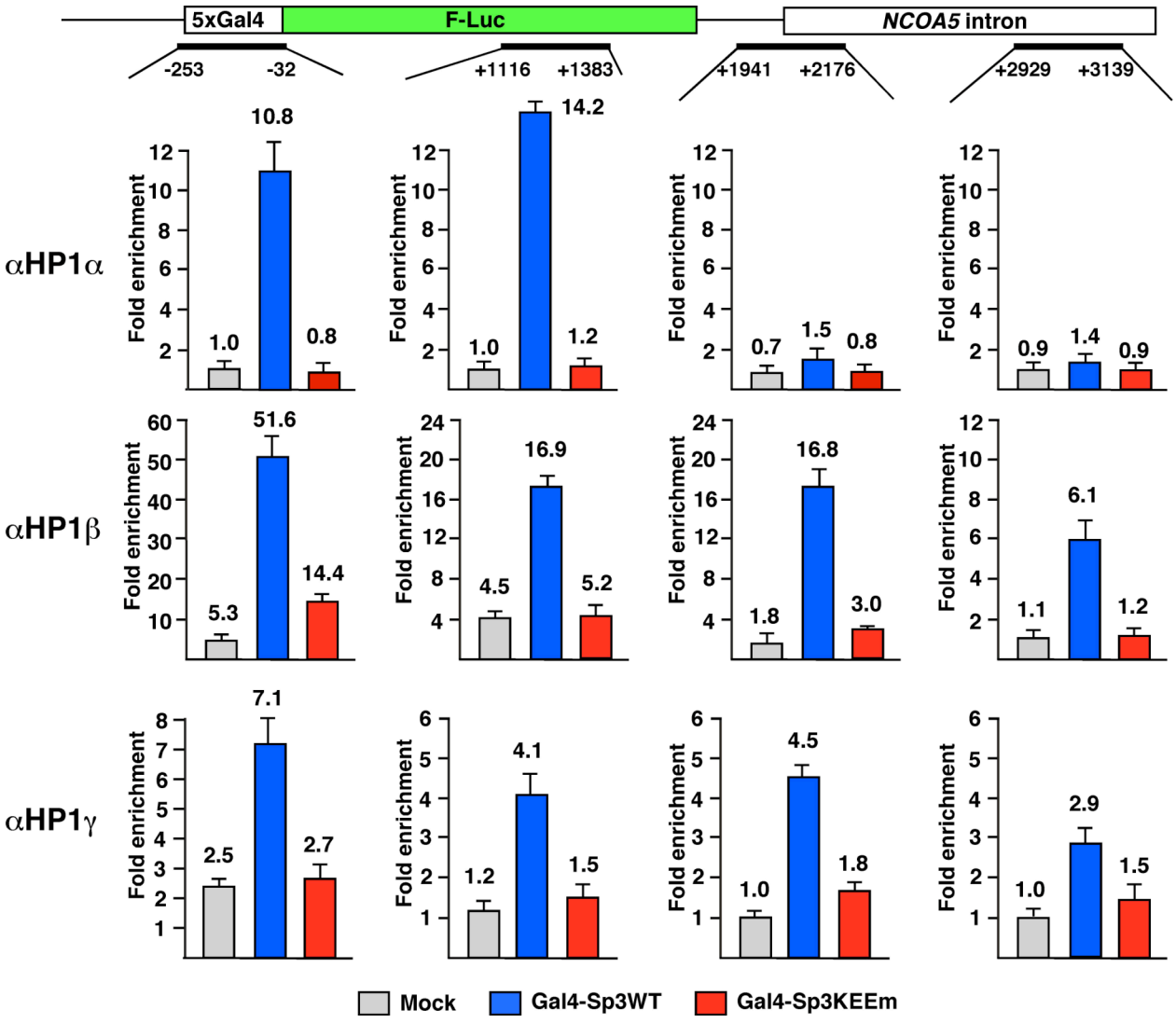
Supplementary Fig 3. SUMOylated Gal4-SF-1WT recruits Mi-2, L3MBTL1 and L3MBTL2 to the 5xGal4 promoter. ChIP-qPCR. ChIPs were performed with antibodies to **(A)** Gal4 and **(B)** Mi-2, L3MBTL1 and L3MBTL2 as indicated. Recoveries are expressed as fold enrichment relative to the control antibody (mean +/-SD).



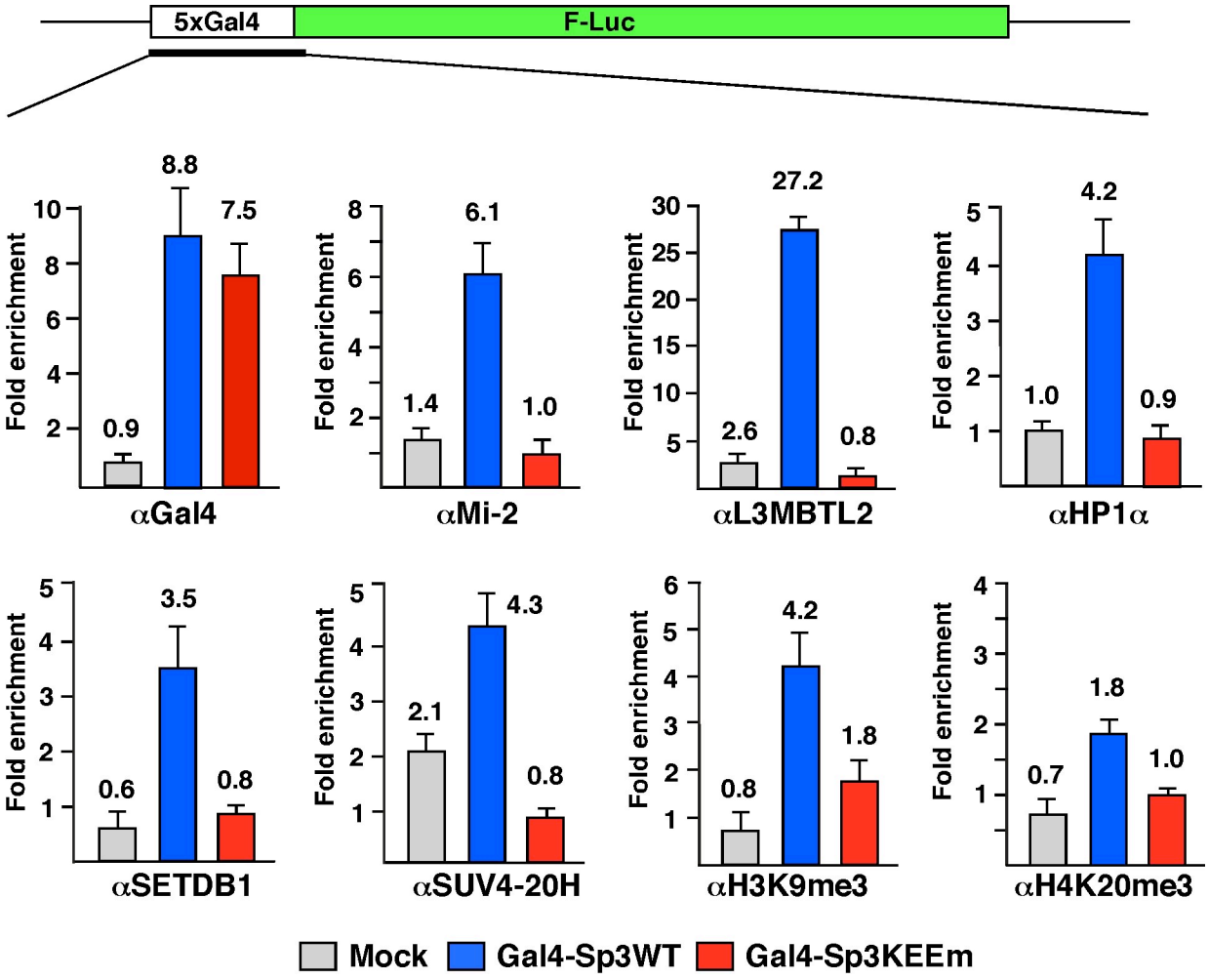
Supplementary Fig 4. SUMO-dependent association of Gal4-SF-1, Mi-2, L3MBTL1, L3MBTL2 and HP1γ with regions remote from the promoter. ChIP-qPCR. ChIPs were performed with antibodies to Gal4, Mi-2, L3MBTL1, L3MBTL2 and HP1γ as indicated. Precipitated material was amplified by qPCR with primers specific for the indicated regions (mean +/-SD).



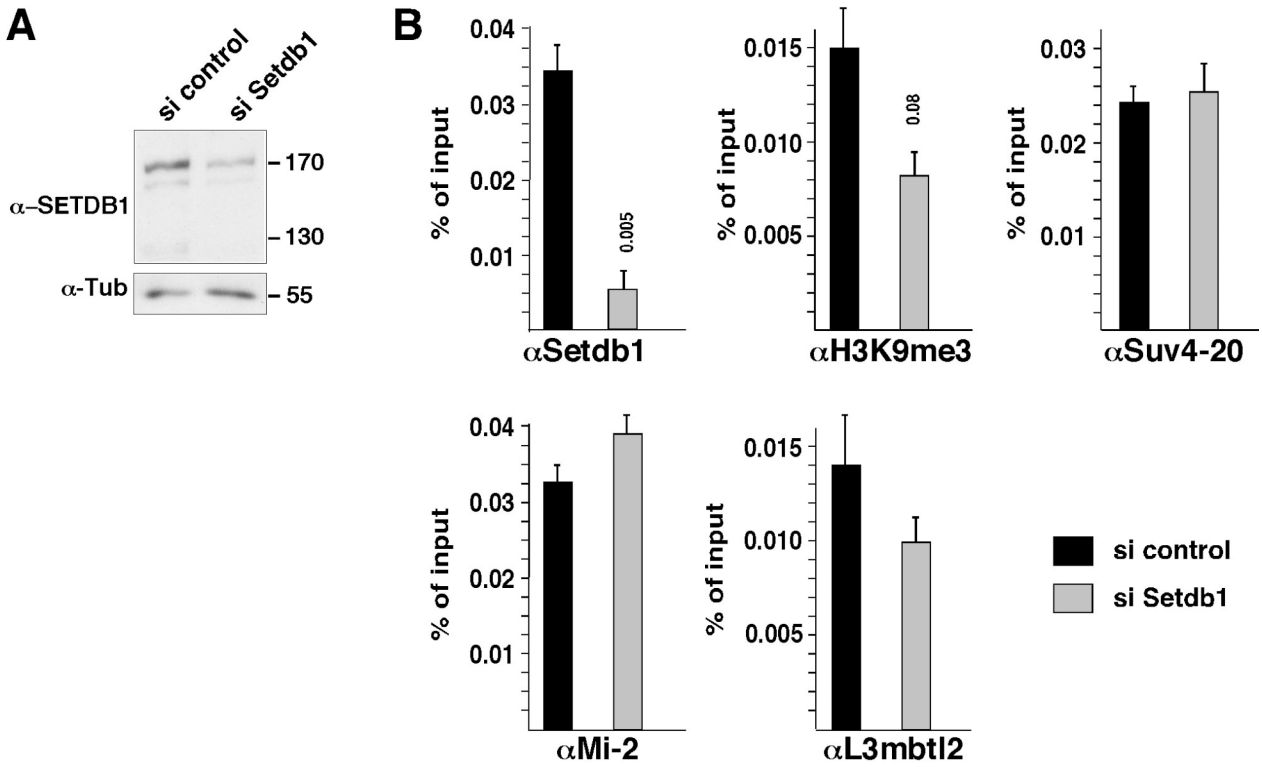
Supplementary Fig 5. Restriction enzyme accessibility assay. Nuclei from clone 1 expressing Gal4-Sp3WT or Gal4-Sp3KEEm were digested with *XhoI*, and extracted genomic DNA was amplified by qPCR with primers specific for the 5xGal4 promoter. To compensate for variation of template concentration, qPCRs were normalized to the CT values obtained with primers specific for the luciferase gene. Relative accessibility was calculated according to the $2^{-\Delta CT}$ method (Livak and Schmittgen 2001): $2^{-(\text{normalized CT undigested - normalized CT } \textit{XhoI digested})}$. The Gal4 promoter is less accessible for *XhoI* in the presence of repressive Gal4Sp3WT as compared to activating Gal4Sp3KEEm. This is consistent with compaction of chromatin in the presence of SUMOylated Gal4Sp3WT.



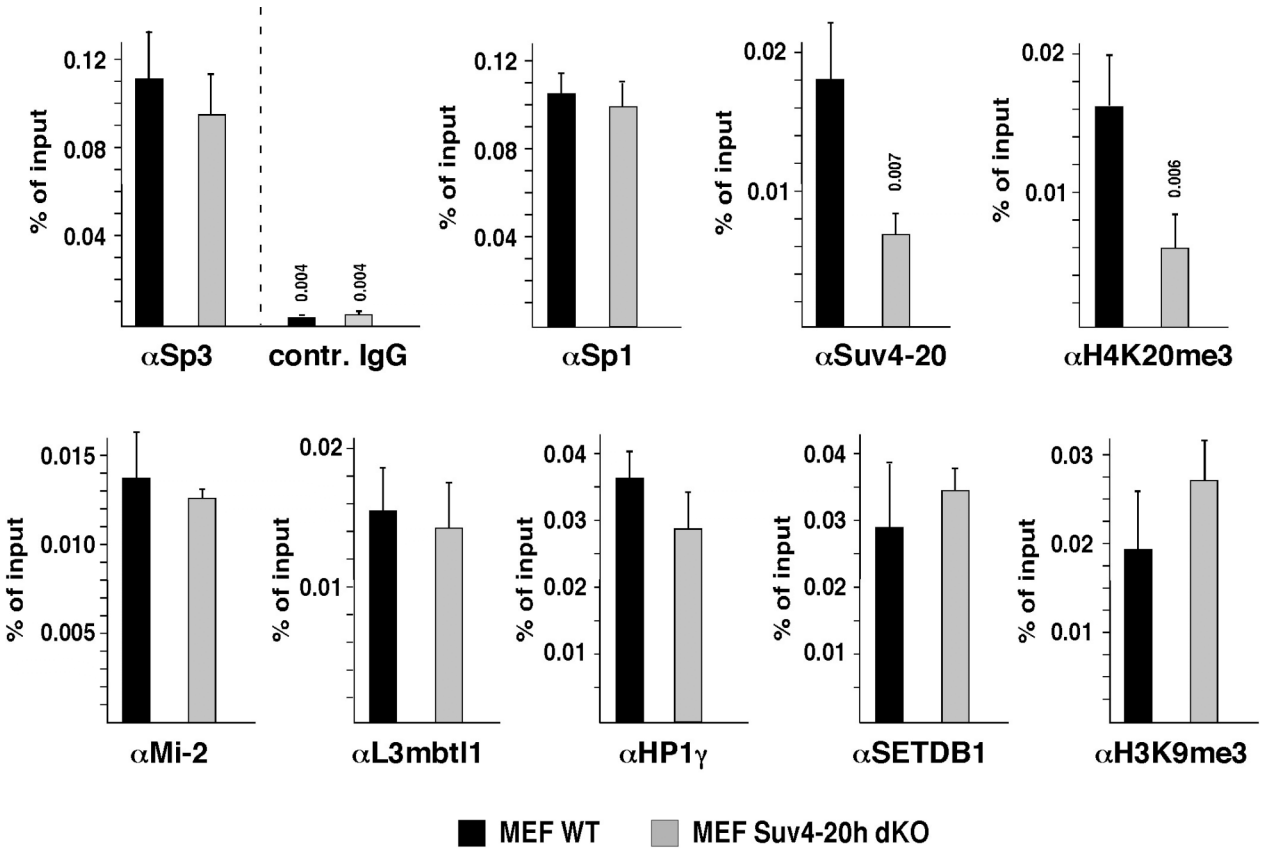
Supplementary Fig 6. SUMO-dependent association of HP1 proteins with regions remote from the promoter. ChIP-qPCR. ChIPs were performed with antibodies to HP1α, HP1ß and HP1γ as indicated. Precipitated material was amplified by qPCR with primers specific for the indicated regions (mean +/-SD).



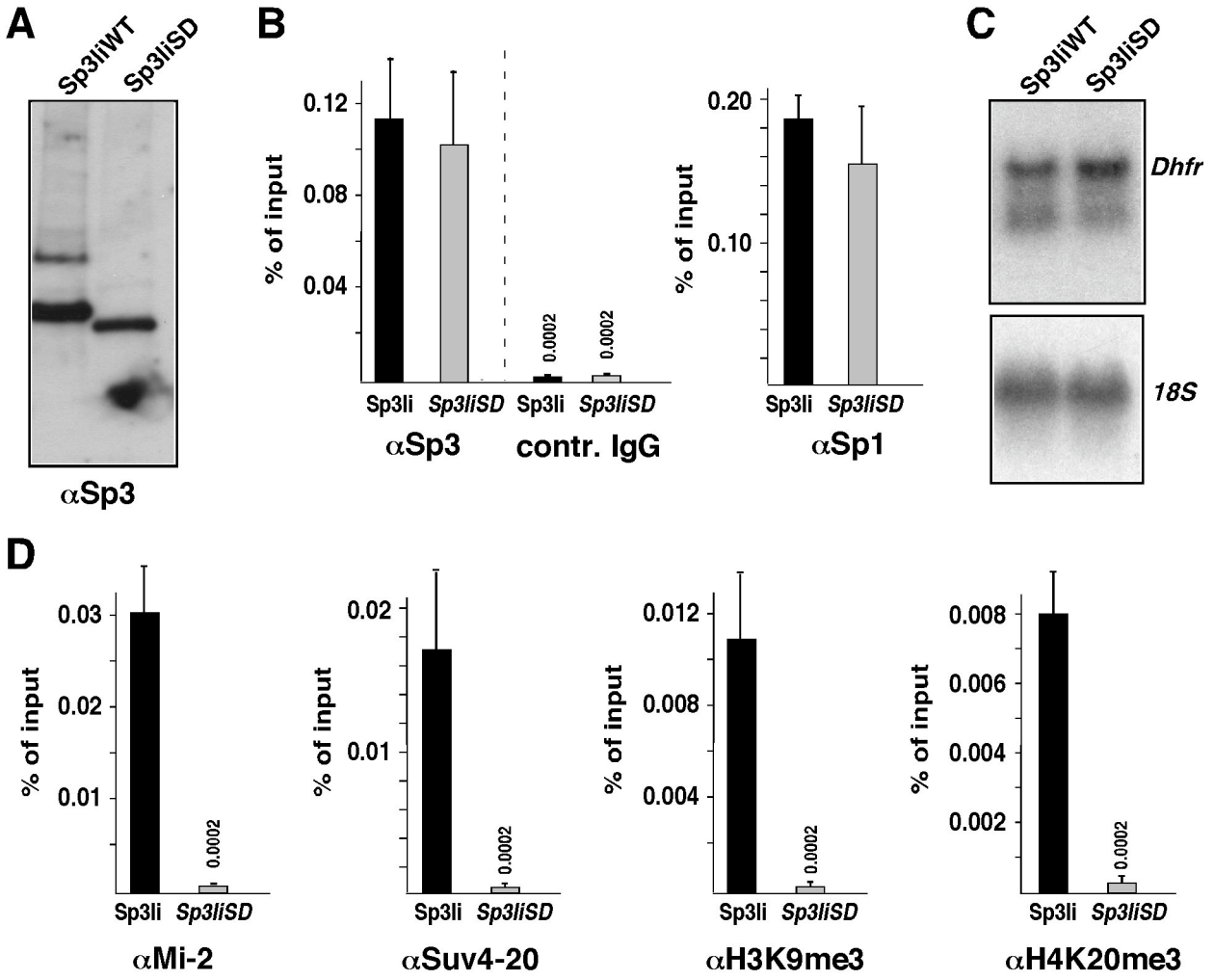
Supplementary Fig 7. SUMO-dependent association of Mi-2, L3MBTL2, HP1α, SETDB1, SUV4-20H, H3K9me3 and H4K20me3 at the 5xGal4 promoter in an alternative cell line. Cell clone no c4 (see Supplementary Fig 1) was transfected with Gal4-Sp3WT or Gal4-Sp3KEEm and subsequently analysed by ChIP-qPCR (mean +/-SD).



Supplementary Fig 8. Reduced H3K9 trimethylation at the mouse *Dhfr* promoter upon knockdown of Setdb1. (A) Immunoblot analysis of Setdb1 after siRNA transfection. (B) ChIP-qPCRs. MEFs were transfected with siRNAs specific for Setdb1 or control siRNAs, and subsequently subjected to ChIP with the indicated antibodies. Precipitates were analysed with primers specific for the mouse *Dhfr* promoter. DNA recoveries are expressed as percentage of input (mean ± SD).



Supplementary Fig 9. Repressive marks at the *Dhfr* promoter in Suv4-20h1/2 double knockout MEFs (MEF Suv4-20h dKO). Suv4-20h1/2 double knockout MEFs (Benetti et al. 2007) and corresponding wild type MEFs were subjected to ChIP-qPCR with the indicated antibodies. Precipitates were analysed with primers specific for the mouse *Dhfr* promoter. DNA recoveries are expressed as percentage of input (mean ± SD).



Supplementary Fig 10. Rescue of heterochromatic marks at the mouse *Dhfr* promoter by SUMOylated Sp3li. **(A)** Immunoblot analysis of MEFs extracts. **(B and D)** ChIP assays. Immunoprecipitated DNA from Sp3 knockout MEFs rescued with Sp3li or Sp3liSD was amplified by qPCR with primers specific for the *Dhfr* promoter. DNA recoveries are expressed as percentage of input (mean ± SD). **(C)** Northern blot analysis of *Dhfr* expression in Sp3 knockout MEFs rescued with Sp3li and Sp3liSD.

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Plasmids. Expression plasmids for Gal4-Sp3 and Gal4-SF-1 fusions (wild type and SUMOylation-deficient mutants) were described in (Dennig et al. 1996) and (Lee et al. 2005), respectively. The Gal4-driven firefly luciferase reporter plasmid 5xGal4-luc was obtained by recloning the 5xGal4 promoter from G5E1BCAT (Dennig et al. 1996) in pGAW, a self-made derivative of the pGL3-Basic vector (Promega).

Generation of stable cell clones with an integrated Gal4-responsive luciferase reporter. To create mammalian cell lines with a chromatin-integrated Gal4-luciferase reporter, the 5xGal4-luc plasmid was transfected in 293 cells along with a plasmid conferring puromycin resistance (pBabe-Puro) using the calcium phosphate transfection method. Cells were selected with 1 μg/ml of Puromycin (Becton Dickinson) for at least 10 days. Single cell clones were isolated, expanded and analysed in transient reporter assays for the responsiveness of the integrated transgene by transfecting $4x10^5$ cells with 1 μg of expression constructs for Gal4-DBD, Gal4-Sp3WT or Gal4-Sp3KEEm along with 0.5 μg of a ß-galactosidase expression plasmid (RSV-ß-Gal) using the Fugene™ transfection reagent (Roche). 48 hours after transfection cytoplasmatic extracts were prepared and luciferase and ß-galactosidase activities determined.

Generation of Sp3 knockout MEFs expressing Sp3 isoforms. Sp3 knockout MEFs (Bouwman et al. 2000) were transfected with 20 μg of ubiquitin promoter-driven expression plasmids for Sp3siWT, Sp3liWT or corresponding SUMOylation-deficient mutants (Sp3siSD and Sp3liSD) along with 1 μg of a plasmid conferring *hygromycin resistance* (pX343) (Blochlinger and Diggelmann 1984). Cells were selected with 150 μg/ml of hygromycin and subsequently analysed for Sp3 expression by EMSA (Göllner 2002) and Western blotting.

RNAi against Setdb1

$1x10^6$ MEFs were transfected with 300 pmol of the mouse Setdb1 SMARTpool (Dharmacon M-040815) using the Dharmafect transfection reagent. As negative control the Non-Targeting siRNA #1 (Dharmacon; D-001210-01) was used. For ChIP analysis, chromatin was prepared 72 hours post-transfection as described.

Southern blotting and integration site mapping of the transgene. Southern blotting was performed according to standard protocols using restriction enzymes that are single cutters on the transfected 5xGal4 luciferase reporter plasmid (see Supplementary Fig 2). The integration site of the transgene was mapped essentially as described in (Ochman et al. 1988; Ladendorf et al.

2003). In brief, 10 µg of BglII-digested DNA of clone c1 was diluted to 5 ml and religated. Subsequent PCR-amplication was performed with the following primer pair: 5´-CAGTACCGGAATGCCAAGC-3´ and 5´-CTACCGGAAAACTCGACG-3´ resulting in a 600 bp fragment. Sequencing revealed the 3´-breakpoint of the transfected plasmid and the integration site in the first intron of the *NCOA5* gene.

Restriction enzyme accessibility assay. Nuclei were prepared essentially as described in (Mymryk et al. 1997). $3.2x10^4$ nuclei in a volume of 100 μl were then digested with 100 U of *XhoI* (Invitrogen) for 30 minutes at 37 °C. The reaction was terminated by adding 5 μg of proteinase-K in 10 mM EDTA, pH 8.0, 1% SDS for 1 hour at 45 °C, and DNA was phenol-chloroform extracted. QPCR reactions were performed in duplicate with 5 ng of genomic DNA in a total volume of 25 μl containing ImmoMix (Bioline) and SYBRgreen. PCR conditions were 95 °C for 7 min, followed by 40 cycles of 95 °C for 30 s, 68 °C for 1 min and 72 °C for 30 s.

Primers for qPCR. The following primer pairs were used for qPCR reactions: 5xGal4 promoter: 5´-CCAGTGCAAGTGCAGGTG-3´ and 5´-GCTGGTACCGAGCTCTTAC-3´; luciferase coding region (+1200): 5´-GGTTGTGGATCTGGATACCG-3´ and 5´-GGTGTTGGAGCAAGATGGAT-3´; Transgene integration site (+2000): 5´-TGAGAGCCTTCAACCCAGTC-3´ and 5´-AGGTGTTGCCCTATTCTAGAG-3´; NCOA5 intron (+3000): 5´- ACCACCACTTGGACACTATAG-3´ and 5´-AACGGTACTTCTTTCCCCTTC-3´. The primers specific for the mouse D*hfr* promoter were described (Stielow et al. 2008).

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