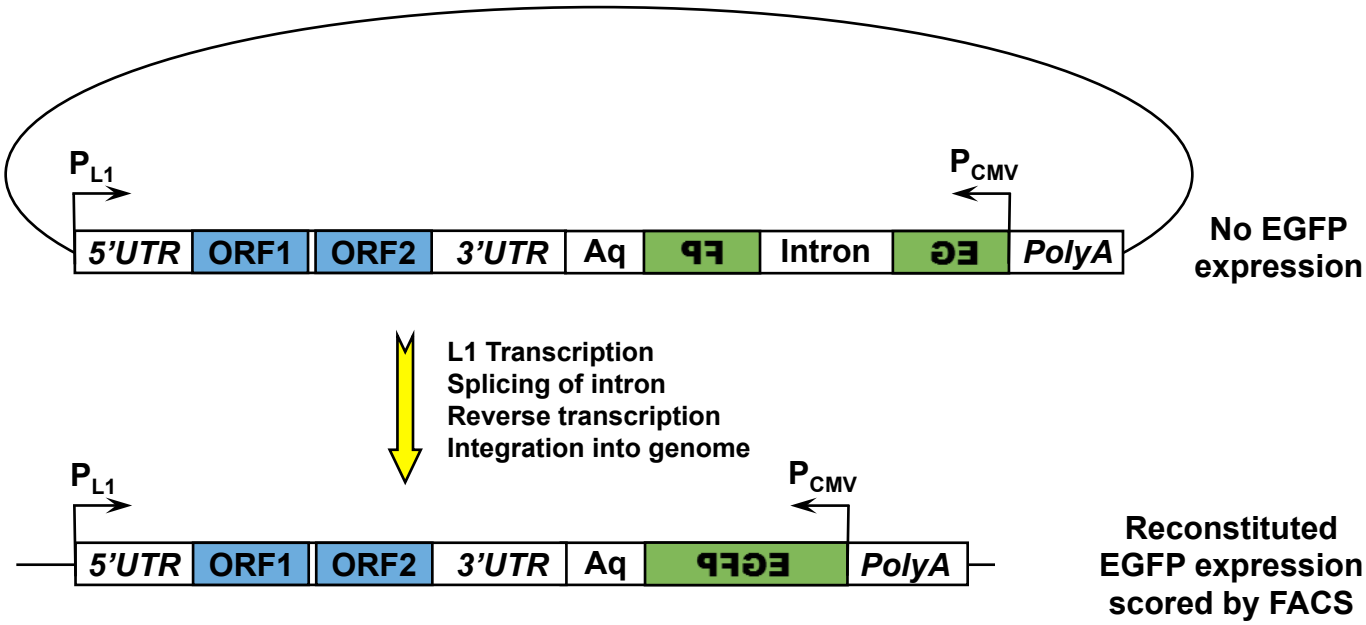
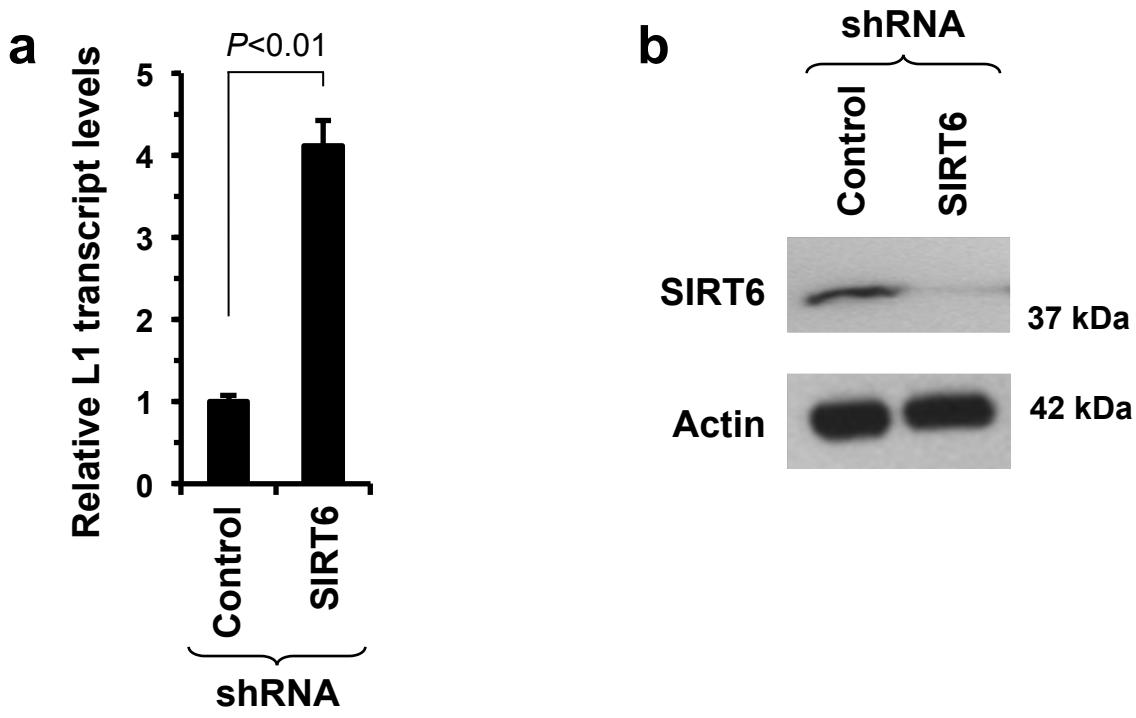
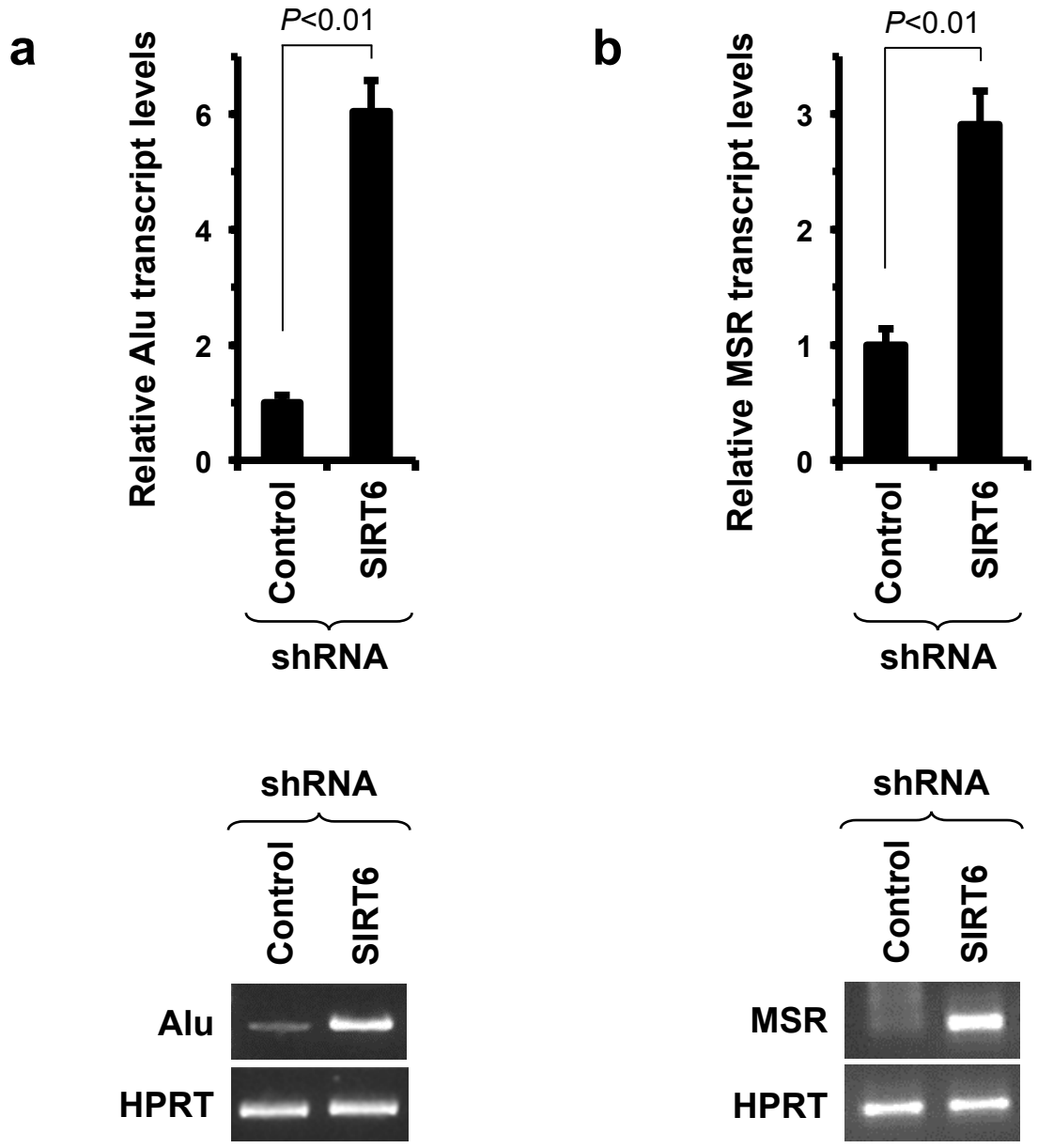


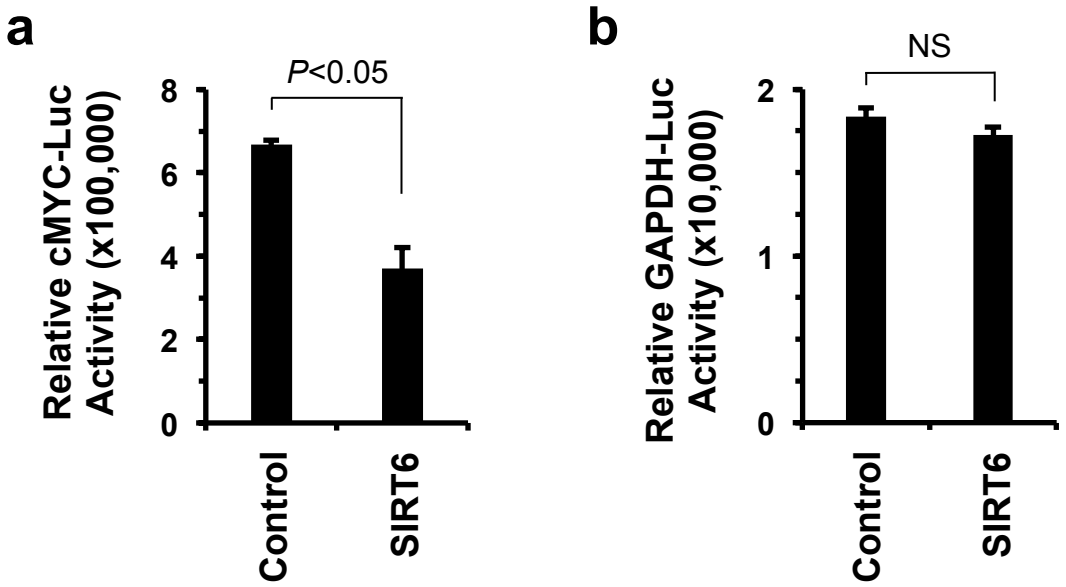
Supplementary Figure 1. Diagram of L1 reporter assay. In this assay, cells are transfected with the L1 reporter, allowed to grow for 72 hours and then harvested and sorted by FACS; GFP+ cells represent cells in which a de novo retrotransposition event has occurred. The L1 reporter consists of a full length L1 element (comprised of the L1 5'UTR, two open reading frames, ORF1 and ORF2, and a 3'UTR capped by a poly(A) tail) which lies in the inverse orientation of a EGFP gene, which is interrupted by a γ-globin intron, driven by a cytomegalovirus promoter. In the initial configuration, the γ-globin intron prevents EGFP from being expressed. Successful retrotransposition of the reporter, however, will remove the intron and activate the EGFP gene, allowing new retrotransposition events to be quantified[25].



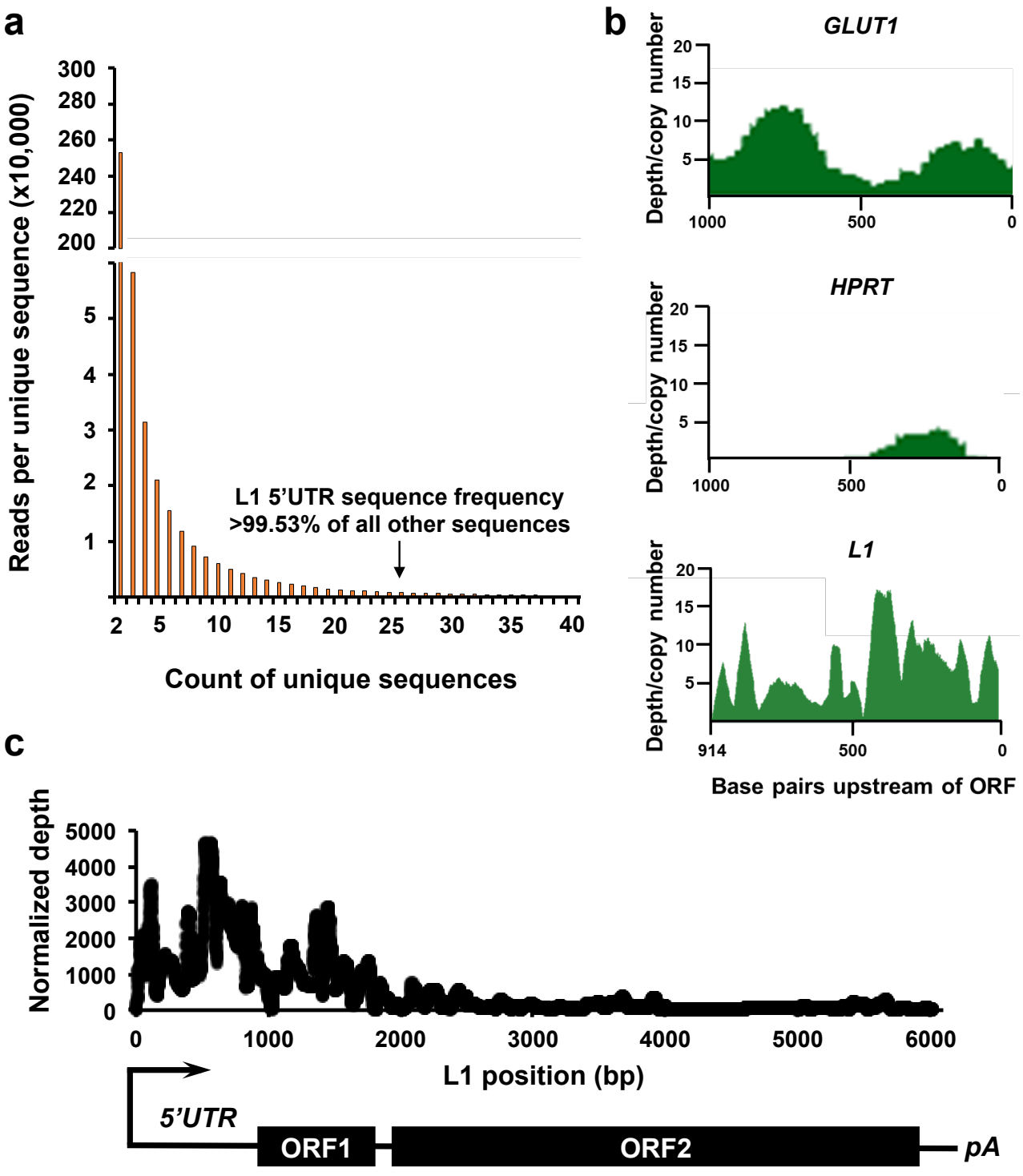
Supplementary Figure 2. Depletion of SIRT6 leads to an increase in L1 transcription in human cells. (a) Human dermal fibroblast cells were stably transfected with either scrambled shRNAs (Control) or SIRT6-targeting shRNAs. L1 expression was quantified using qRT-PCR; n=3, error bars indicate s.d. **(b)** Western blot demonstrating efficiency of SIRT6 knock-down in human dermal fibroblasts. HDF cells were stably transfected with a pool of the indicated shRNAs. Representative immunoblot is shown; n=3.



Supplementary Figure 3. Alu and MSR repetitive elements are also transcribed more abundantly in the absence of SIRT6. (a) Alu mRNA is more abundant in SIRT6 knock-down cells than control (scrambled shRNA) cells. Total cellular RNA was extracted from control and SIRT6 knock-down human dermal fibroblasts. Alu mRNA was quantified by qRT-PCR; quantification was normalized to actin mRNA levels; n=4, error bars indicate s.d. **(b)** Minor satellite repeat (MSR) mRNA is more abundant in SIRT6 knock-down cells than control (scrambled shRNA) cells. Total cellular RNA was extracted from control and SIRT6 knock-down human dermal fibroblasts. MSR mRNA was quantified by qRT-PCR; quantification was normalized to actin mRNA levels; n=4, error bars indicate s.d.

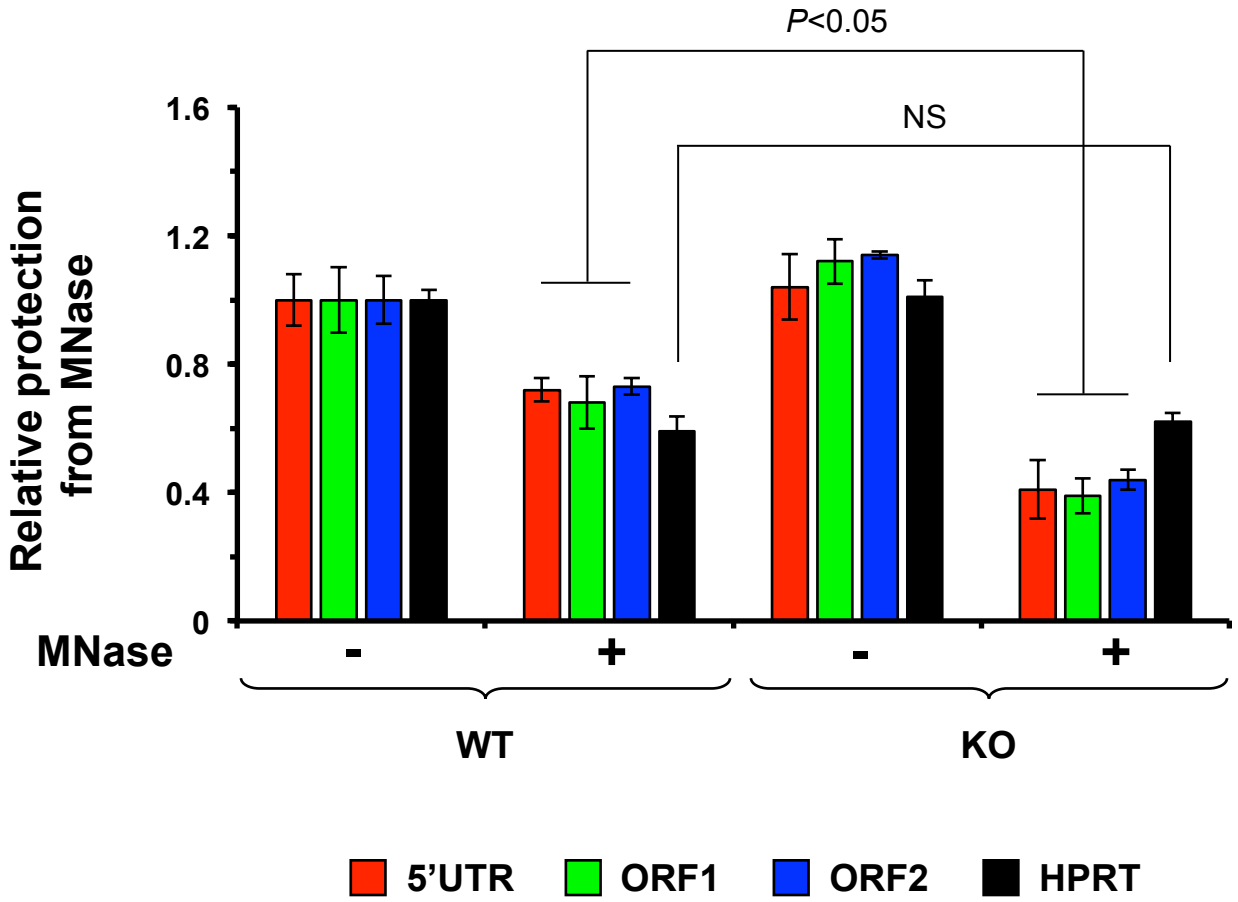


Supplementary Figure 4. SIRT6 represses L1 similarly to other SIRT6 regulated genes. HeLa cells were transfected with 3µg of the indicated luciferase reporter and 5µg of the indicated expression vector. **(a)** SIRT6 overexpression repressed c-MYC (a SIRT6 regulated gene) transcriptional activity by approximately 60%. **(b)** Overexpression of SIRT6 did not significantly repress GAPDH (a gene that is not regulated by SIRT6) transcriptional activity. N=5, error bars indicate s.d.

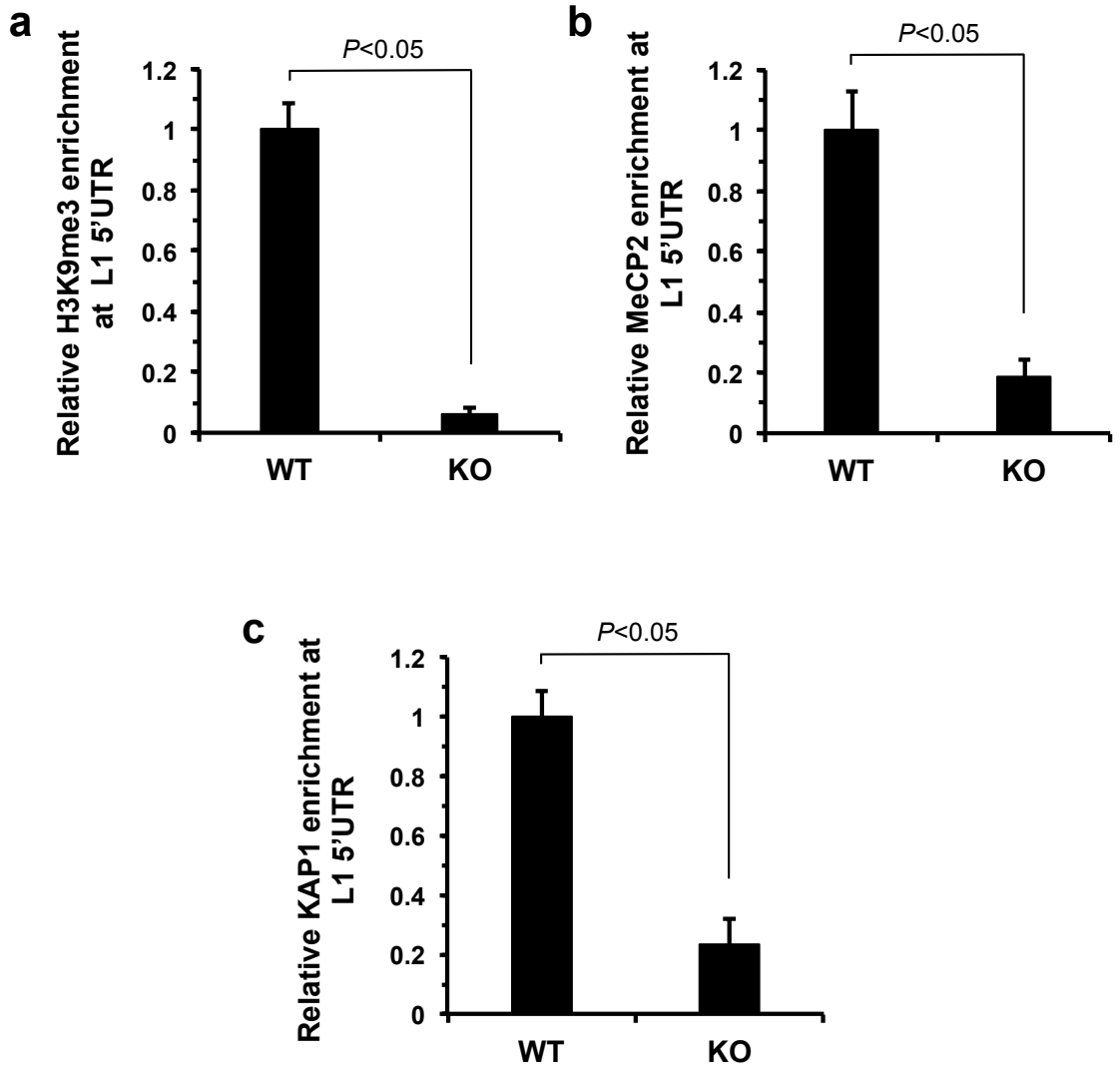


Supplementary Figure 5. SIRT6 is enriched at L1 loci. (a) L1 5'UTR sequences are heavily represented in the SIRT6 ChIP-seq dataset. Analysis of SIRT6 ChIP-seq in H1-hESCs revealed 2,548,326 reads that occurred more than once in the dataset. Bins were created containing reads that occurred the indicated number of times (x-axis), and scored for the number of reads that segregated into that bin (y-axis). The resultant histograph indicates the frequency with which reads are enriched in the dataset. *(Continued on next page)*

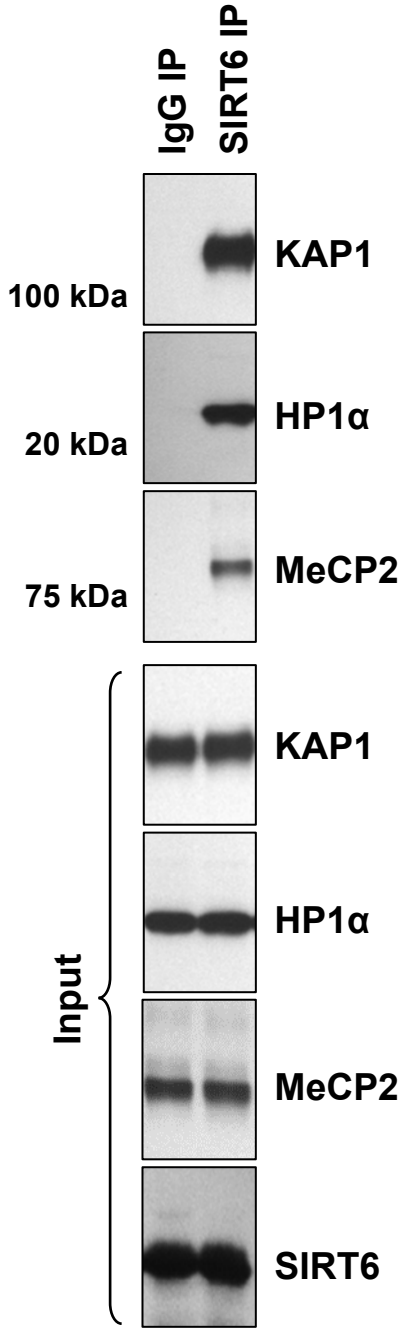
The vast majority of reads occurred twice in the dataset. Reads that mapped to the L1 5'UTR reads occurred on average 25 or more times in the dataset, meaning that they occur more frequently than 99.53% of all other reads. **(b)** SIRT6 is specifically enriched at L1 loci. To gain insight into the enrichment of SIRT6 at L1 loci, relative to other loci, we compared read depth/copy number (so as to normalize for the increased number of L1 loci, relative to protein coding loci) at the promoters of a SIRT6-regulated gene, *GLUT1*, a gene not regulated by SIRT6, *HPRT*, and across the L1 5'UTR. SIRT6 was strongly enriched at both L1 5'UTR sequences and the GLUT1 promoter, relative to the HPRT promoter. Read depth for *GLUT1* and *HPRT* was obtained from the UCSC genome browser, using the SIRT6 ChIP-seq dataset in H1-hESC cells; the same dataset was used to generate read depth for L1s. **(c)** To examine the distribution of read depth across L1 elements, we performed a reference alignment of SIRT6 ChIP-seq reads to a full-length L1 sequence. Initial analysis revealed heavy enrichment of SIRT6 across the entire L1 locus, with a bias toward the 3' end. However, since over 90% of L1s in the human genome are 5'truncated, we reasoned that this 3' bias was an artifact. The frequency of L1s of any given length in the genome is roughly known. We corrected for this 5' bias in the dataset by normalizing the read depth at each position to the fraction of truncated L1s that contain that position, allowing us to gain insight into SIRT6 occupancy on full-length L1s. Once normalized, the read depth alignment indicated that SIRT6 is preferentially enriched at the 5'UTR of full-length L1s.



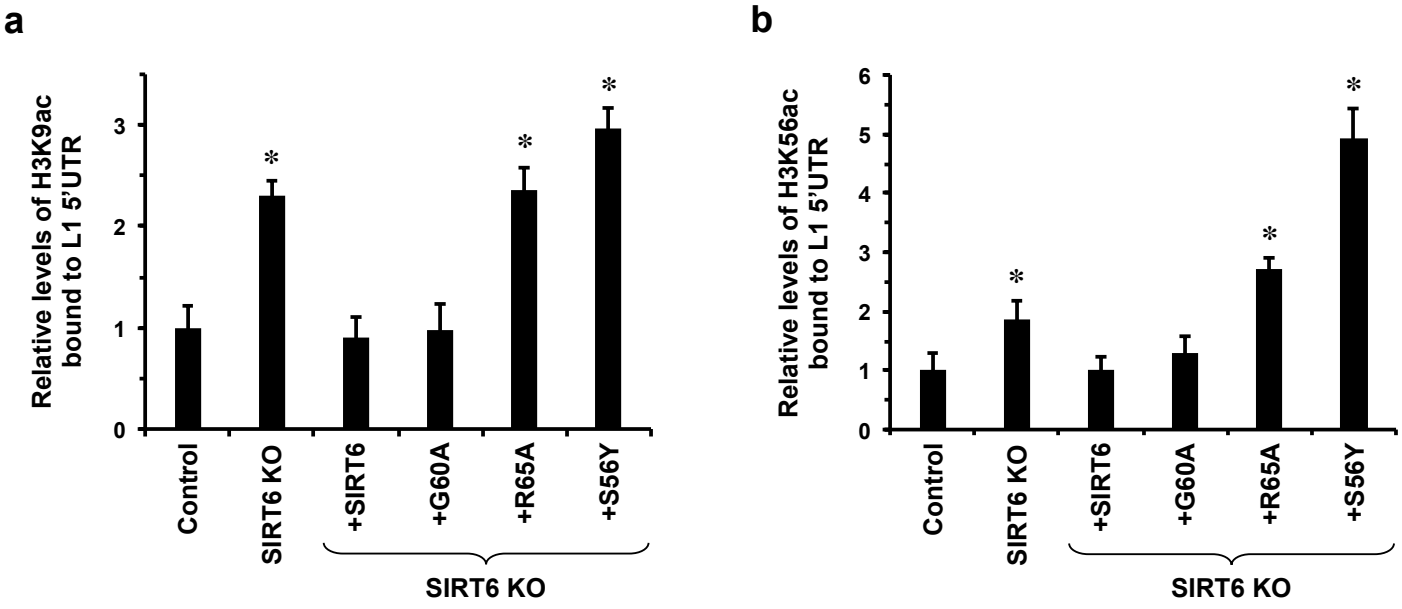
Supplementary Figure 6. Higher order heterochromatin is perturbed more at L1 loci than other loci in SIRT6 KO cells. Total genomic DNA was isolated from WT and SIRT6 KO cells and then digested with 0.5 units of micrococcal nuclease (MNase). Relative protection from MNase was quantified by qRT-PCR, based on amplification efficiency across the L1 locus, using primers that spanned the L1 5'UTR, L1 ORF1, L1 ORF2 or a non-L1 locus (data for the HPRT promoter is shown). Higher amplification intensity by qRT-PCR indicated less digestion by MNase and therefore more heterochromatinization. We observed that the L1 locus, but not the HPRT promoter, is susceptible to digestion by MNase in SIRT6 KO cells indicating that SIRT6 specifically regulates heterochromatin structure at the L1 locus.



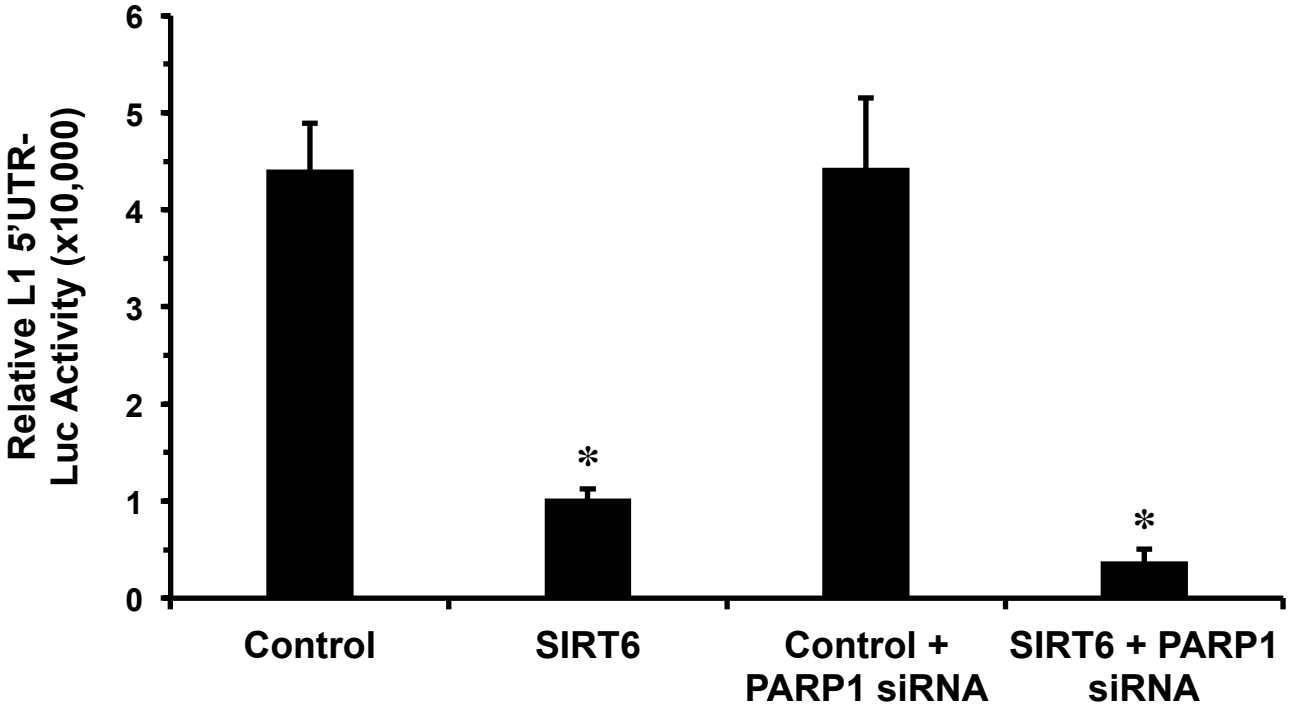
Supplementary Figure 7. Quantification of enrichment of heterochromatin proteins at L1 5'UTR in WT and SIRT6 KO cells. Representative images are shown in Figure 2c. **(a)** ChIP was performed using antibodies against the H3K9me3 in WT and SIRT6 KO MEFs. Primers spanning the L1 5'UTR were used to quantify the enrichment of H3K9me3 at this region of the L1 locus. IgG immunoprecipitation was used as a negative control. All values are normalized to input and H3 IP. This experiment was performed 3 times, error bars indicate s.d. **(b)** ChIP was performed using antibodies against the MeCP2 in WT and SIRT6 KO MEFs. Primers spanning the L1 5'UTR were used to quantify the enrichment of MeCP2 at this region of the L1 locus. IgG immunoprecipitation was used as a negative control. All values are normalized to input and H3 IP. This experiment was performed 3 times, error bars indicate s.d. **(c)** ChIP was performed using antibodies against the KAP1 in WT and SIRT6 KO MEFs. Primers spanning the L1 5'UTR were used to quantify the enrichment of KAP1 at this region of the L1 locus. IgG immunoprecipitation was used as a negative control. All values are normalized to input and H3 IP. This experiment was performed 3 times, error bars indicate s.d.



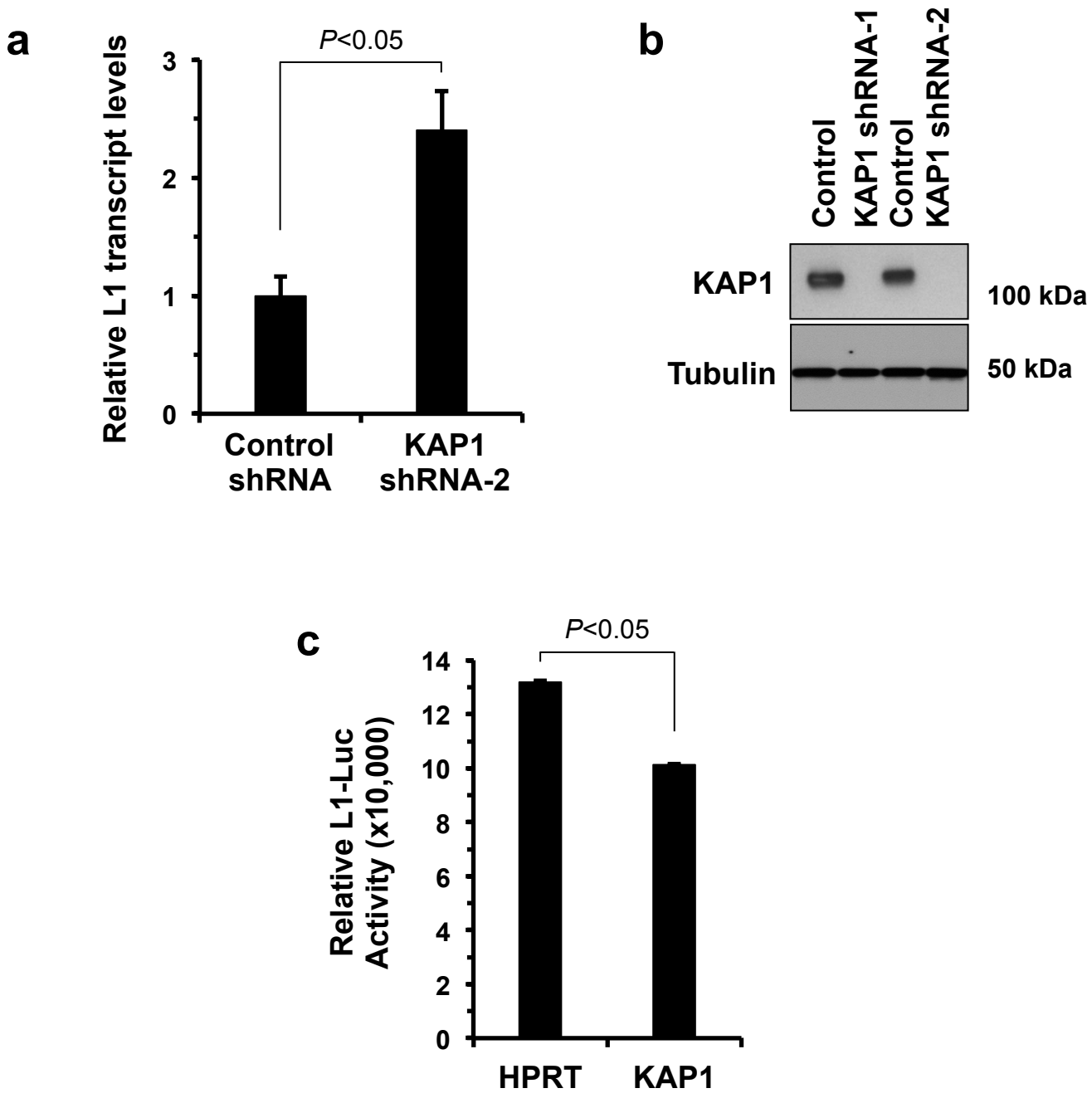
Supplementary Figure 8. DNA is not required to mediate interactions between SIRT6 and KAP1 and HP1α. Five μg of SIRT6 was incubated with 5 μg of either KAP1, HP1α or MeCP2 for two hours. SIRT6 was then immunoprecipitated from the mixture, and interactions with the indicated protein were probed for by immunoblot. We observed interactions between SIRT6 and each KAP1, HP1α and MeCP2 under these in vitro conditions.



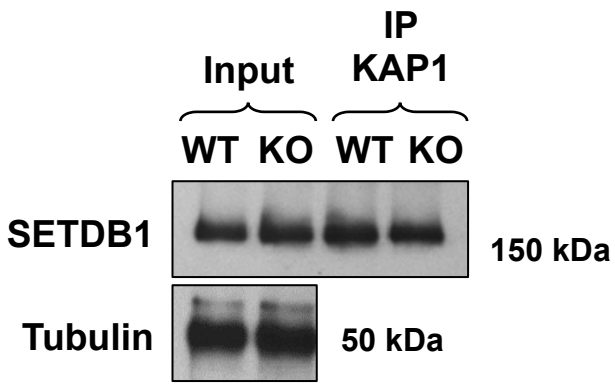
Supplementary Figure 9. Enrichment of acetylated H3K9 and H3K56 at L1 loci increases modestly in the absence of SIRT6. (a) ChIP was performed using H3K9ac antibodies and primers spanning the L1 promoter to quantify H3K9ac enrichment at the L1 5'UTR in WT and SIRT6 KO cells. H3K9ac enrichment at the locus was elevated approximately 2-fold in the SIRT6 KO cells, relative to wild type. When the same ChIP experiment was performed in SIRT6 KO cells overexpressing wild type SIRT6 or SIRT6 functional mutants, we observed that overexpression of either wild type SIRT6 or SIRT6 G60A (deacetylase activity only mutant) was sufficient to restore H3K9ac enrichment in SIRT6 KO cells to wild type levels. **(b)** ChIP was performed using H3K9ac antibodies and primers spanning the L1 promoter to quantify H3K56ac enrichment at the L1 5'UTR in WT and SIRT6 KO cells. H3K56ac enrichment at the locus was elevated approximately 1.8-fold in the SIRT6 KO cells, relative to wild type. When the same ChIP experiment was performed in SIRT6 KO cells overexpressing wild type SIRT6 or SIRT6 functional mutants, we observed that overexpression of either wild type SIRT6 or SIRT6 G60A (deacetylase activity only mutant) was sufficient to restore H3K56ac enrichment in SIRT6 KO cells to wild type levels. N=3; error bars indicate s.d.



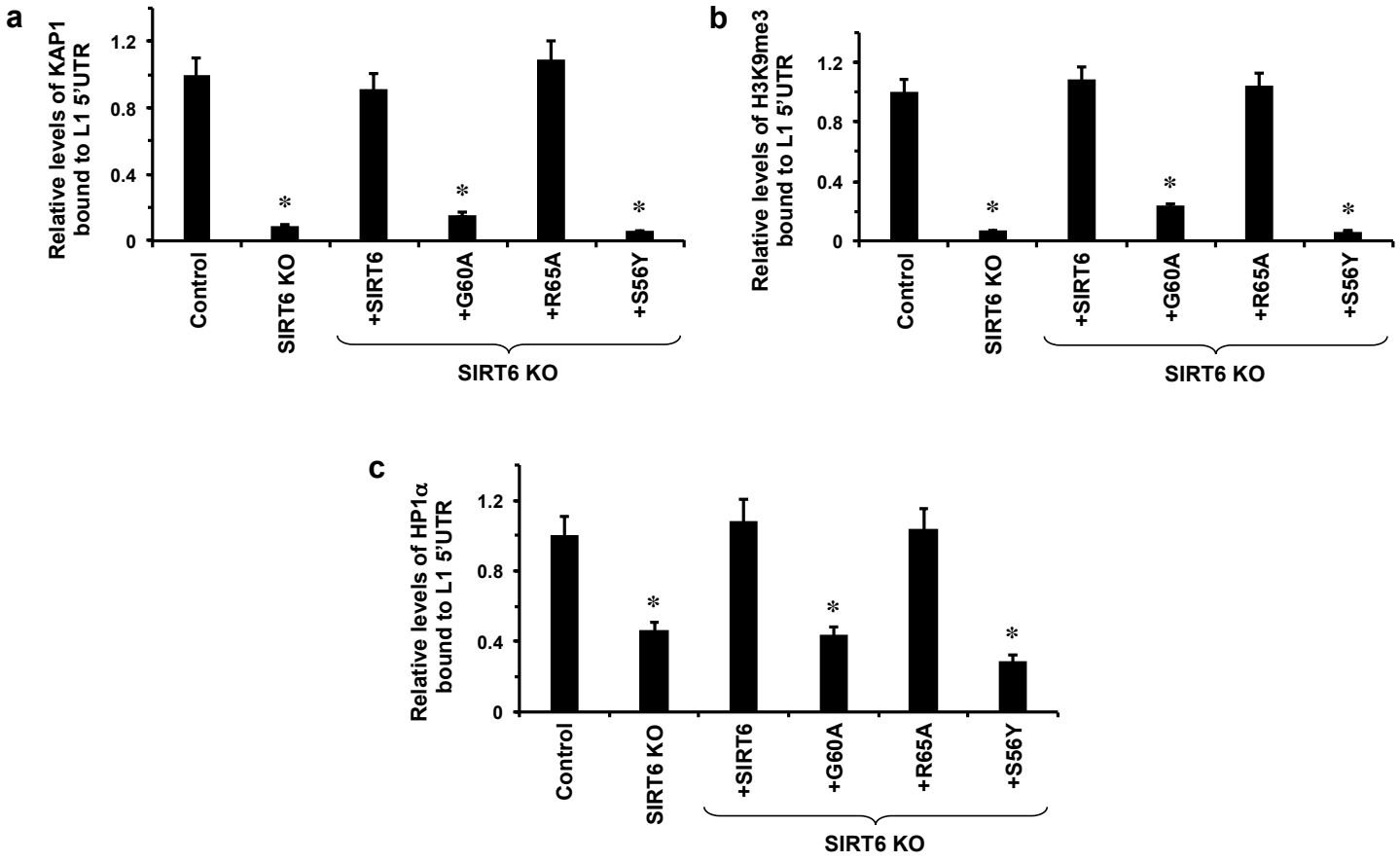
Supplementary Figure 10. PARP1 is not required for SIRT6 mediated silencing of L1s. HDF cells were co-transfected with 5μg of either a Control expression vector (HPRT) or a SIRT6 expression vector and 3μg of a L1 5'UTR-Luciferase reporter in the presence or absence of PARP1 (depleted by siRNA). Luciferase activity is a measure of L1 activity. PARP1 was not required for SIRT6 mediated silencing of L1 transcription. N=5; error bars indicate s.d.



Supplementary Figure 11. KAP1 represses L1 transcriptional activity. (a) Human dermal fibroblast cells were stably transfected with either scrambled shRNAs (Control) or KAP1-targeting shRNAs (shRNA-1 is shown in Figures 3c, d; shRNA-2 is shown here). L1 expression was quantified using qRT-PCR; n=4, error bars indicate s.d. **(b)** Western blot demonstrating efficiency of KAP1 knock-down in human dermal fibroblasts; representative result is shown, n=3. **(c)** Human dermal fibroblasts were transfected with the L1 5'UTR-Luciferase construct and either a Control (HPRT) or KAP1 expression vector. Overexpression of KAP1 was sufficient to reduce L1 transcription by approximately 20%; n=5, error bars indicate s.d.

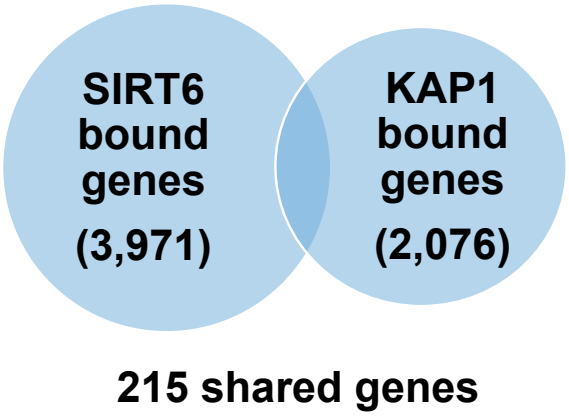


Supplementary Figure 12. SIRT6 does not modulate the stability of KAP1-SETDB1 interactions *in vivo*. KAP1 was immunoprecipitated from WT and SIRT6 KO cells, and then the precipitate was probed with HP1α antibodies via immunoblotting. We did not observe a significant difference between the amount of SETDB1 co-immunoprecipitated in WT or SIRT6 KO cells. Representative result is shown, n=3.



Supplementary Figure 13. Overexpression of SIRT6 rescues loss of KAP1, HP1α and H3K9me3 enrichment at L1 loci in SIRT6 KO cells. (a) ChIP was performed using KAP1 antibodies and primers spanning the L1 promoter to quantify KAP1 enrichment at the L1 5'UTR in WT and SIRT6 KO cells. KAP was depleted from the L1 locus by approximately 10-fold in the SIRT6 KO cells, relative to wild type. When the same ChIP experiment was performed in SIRT6 KO cells overexpressing wild type SIRT6 or SIRT6 functional mutants, we observed that overexpression of either wild type SIRT6 or SIRT6 R65A (mono-ADP ribosyltransferase activity only mutant) was sufficient to restore KAP1 enrichment in SIRT6 KO cells to wild type levels. **(b)** ChIP was performed using H3K9me3 antibodies and primers spanning the L1 promoter to quantify H3K9me3 enrichment at the L1 5'UTR in WT and SIRT6 KO cells. H3K9me3 was depleted from the L1 locus by approximately 11-fold in the SIRT6 KO cells, relative to wild type. When the same ChIP experiment was performed in SIRT6 KO cells overexpressing wild type SIRT6 or SIRT6 functional mutants, we observed that overexpression of either wild type SIRT6 or SIRT6 R65A (mono-ADP ribosyltransferase activity only mutant) was sufficient to restore H3K9me3 enrichment in SIRT6 KO cells to wild type levels. **(c)** ChIP was performed using HP1a antibodies and primers spanning the L1 promoter to quantify HP1a enrichment at the L1 5'UTR in WT and SIRT6 KO cells. HP1a was depleted from the L1 locus by approximately 2.5-fold in the SIRT6 KO cells, relative to wild type. When the same ChIP experiment was performed in SIRT6 KO cells overexpressing wild type SIRT6 or SIRT6 functional mutants, we observed that overexpression of either wild type SIRT6 or SIRT6 R65A (mono-ADP ribosyltransferase activity only mutant) was sufficient to restore HP1a enrichment in SIRT6 KO cells to wild type levels. N=3; error bars indicate s.d.

a



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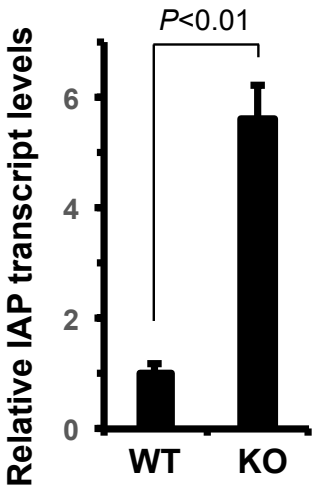
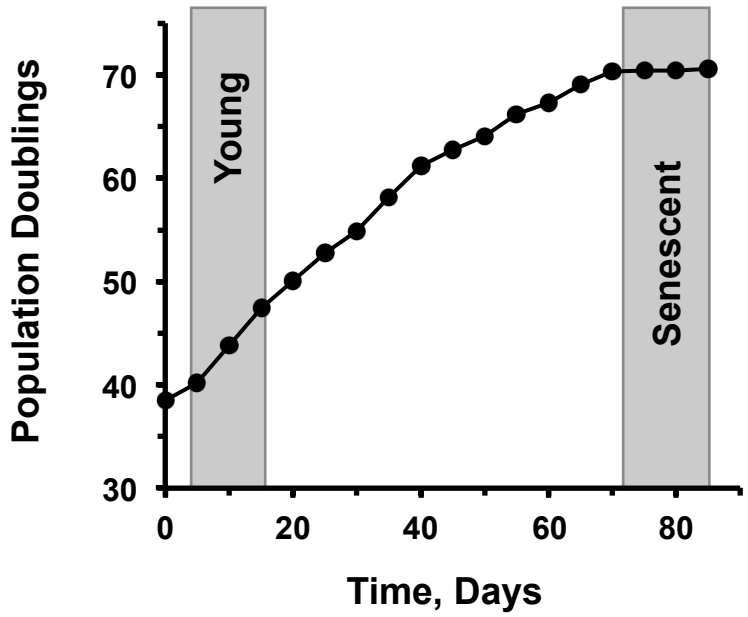


b

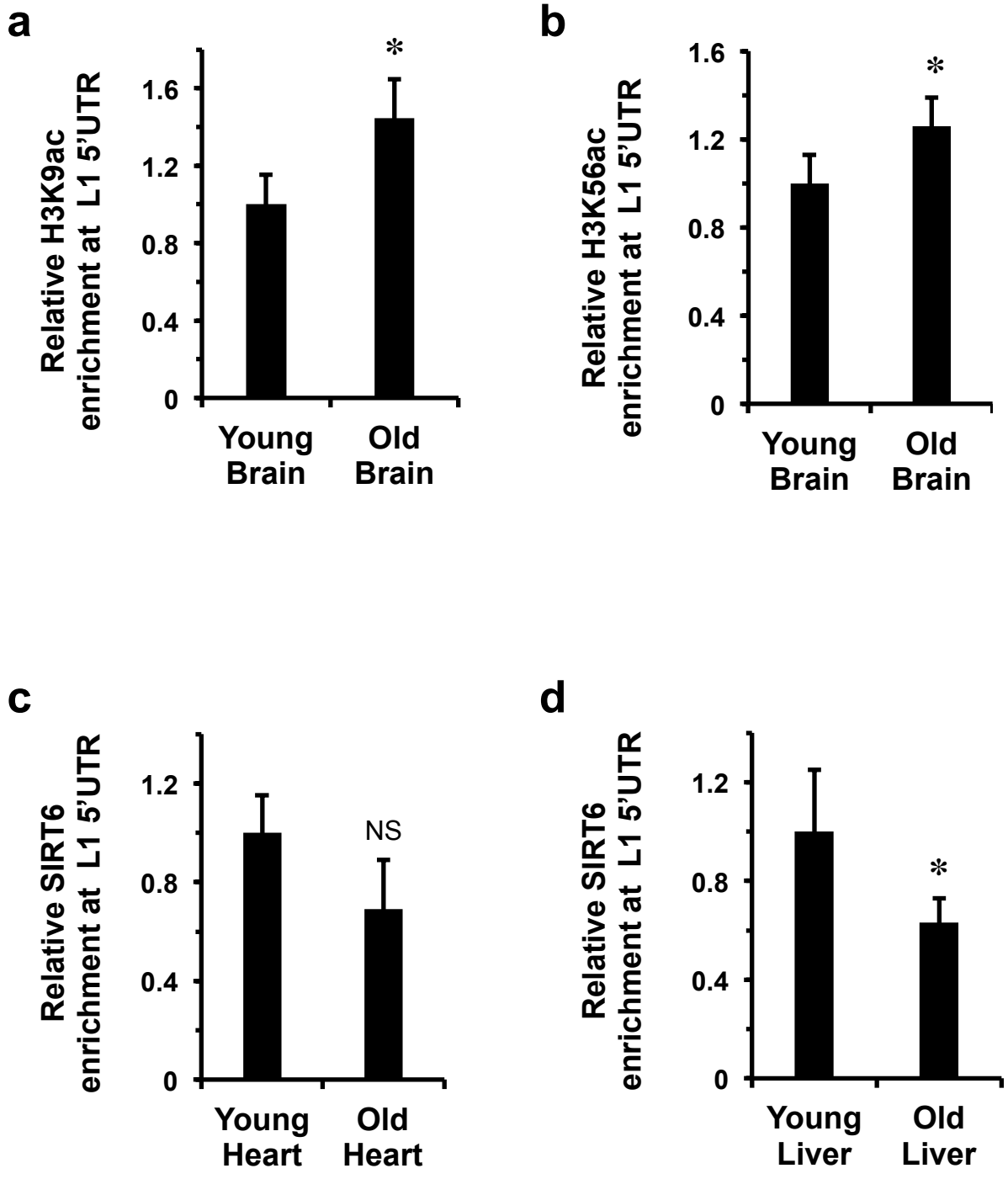
List of promoters co-occupied by both SIRT6 and KAP1

ACADM	BMPR2	DAPK1	ENC1	GFRA1	IL12B	LTBP2	NSBP1	PUM1	SNTG1	THOC1
ACTN1	BRD1	DDEF1	EOMES	GHR	IL1R1	LY96	NUP50	RAB17	SNX8	THRB
ACTN4	BRD2	DDEF2	EPHA4	GLI3	IMPA2	LYN	NXPH1	RAB21	SOX17	TIAM1
ADA	BSND	DDR2	ERBB3	GPC3	ING5	LZTS1	OLIG2	RAB30	SP4	TMCC3
ADCY5	CABP2	DEK	ERCC6	GPC4	INHBA	MEIS1	OTX2	RAB35	SPG20	TRHDE
AGGF1	CAP1	DHRS3	EREG	GPC6	IRS1	MFAP1	PAK2	RAB7	SSBP2	TSN
AHR	CARD4	DLX1	ETFB	GPR39	ISL1	MGAT1	PARVA	RAP2A	SSBP3	UPP1
AKAP2	CAV3	DMAP1	ETV6	GRID1	KCNA4	MPP6	PAX6	RAP2B	STAG2	UPP2
AKAP3	CD44	DPF3	FGF9	GSC	KCNC1	MSRA	PAX9	RASA3	STAG3	VAPB
ALS2	CDK6	DRD2	FHIT	GSK3B	KCND2	MSX1	PDE3A	RIPK1	STX11	VRK2
ANXA2	CETN3	DSCAM	FLRT2	HAND2	KCNK1	MYCL1	PDK1	RNH1	SVIL	WEE1
AOAH	CHST3	DTNB	FOXL1	HIF1A	KLF5	MYH7	PEX5	SALL1	SYN3	WNT11
AP4M1	CHST7	DYRK2	FRG1	HLX1	KLF6	MYO10	PFTK1	SAMD4	SYNPR	XPOT
ARL4A	CNGA3	EDG1	FSTL1	HPCA	LARGE	MYO5C	POLS	SASH1	SYT12	XRN2
ARRB1	CRADD	EFNA5	FZD7	HSPB1	LASS2	MYST3	POMT1	SEPT9	SYT3	ZFP1
ATN1	CREB1	EGLN3	FZD8	HTRA1	LECT1	NCF4	PRKX	SFRS6	TAL1	
ATP4A	CSMD2	ELK3	GAS7	HUNK	LHX4	NCOA1	PTN	SFRS8	TBL1X	
BAMBI	CSRP2	EMP1	GDF2	ID2	LHX5	NEK7	PTPN2	SIX6	TBR1	
BCAT1	CTGF	EMP2	GDNF	ID4	LIMK1	NFIA	PTPRG	SMOC2	TBX3	
BMP4	DAD1	EMX2	GFI1	IGBP1	LRFN5	NR4A3	PTPRU	SMYD2	TERF2	

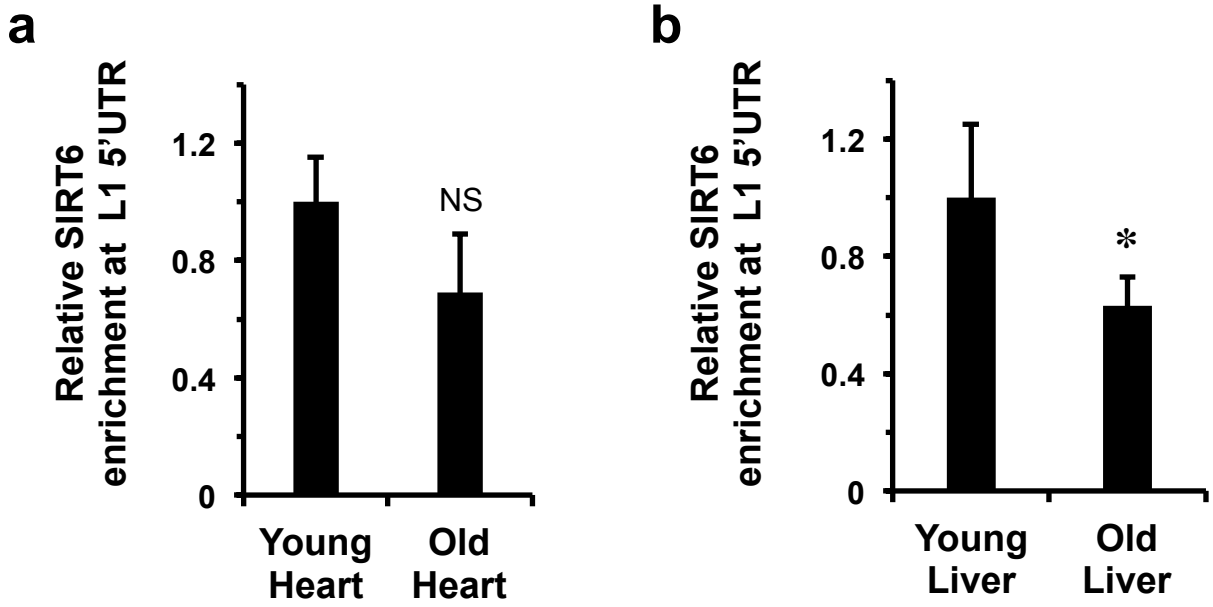
Supplementary Figure 14. SIRT6 and KAP1 participate in silencing retroelements but co-occupy only a small percentage of shared promoters. (a) Promoter binding arrays[41,42] for SIRT6 and KAP1 were compared for well annotated genes. Of these genes, we observed that SIRT6 and KAP1 co-occupy 215 gene promoters, for an approximately 10% overlap. **(b)** List of the 215 genes co-occupied by SIRT6 and KAP1. **(c).** cDNA was made from total cellular RNA extracted from wild type and SIRT6 KO cells. qPCR with primers targeting intracisternal A-type particles (IAP), revealed that IAP transcription is elevated in SIRT6 KO cells (normalized to 18S). N=3; error bars indicate s.d.



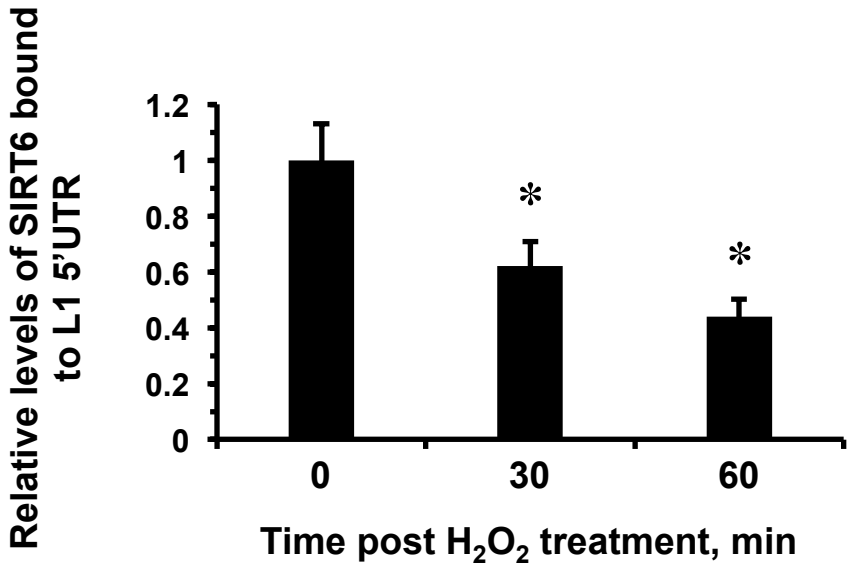
Supplementary Figure 15. Growth curve of human dermal fibroblasts used in experiments. Young cells were sampled during PDs 39-46, senescent cells were sampled at PD 70.



Supplementary Figure 16. Enrichment of H3K9ac and H3K56ac at L1 loci in young and old tissues. (a) Enrichment of H3K9ac was quantified at the L1 promoter in young and old brain tissue by ChIP. H3K9ac was slightly enriched at the L1 locus in old brain tissue, relative to young tissue. **(b)** Enrichment of H3K56ac was quantified at the L1 promoter in young and old brain tissue by ChIP. H3K56ac was slightly enriched at the L1 locus in old brain tissue, relative to young tissue. N=3; error bars indicate s.d.

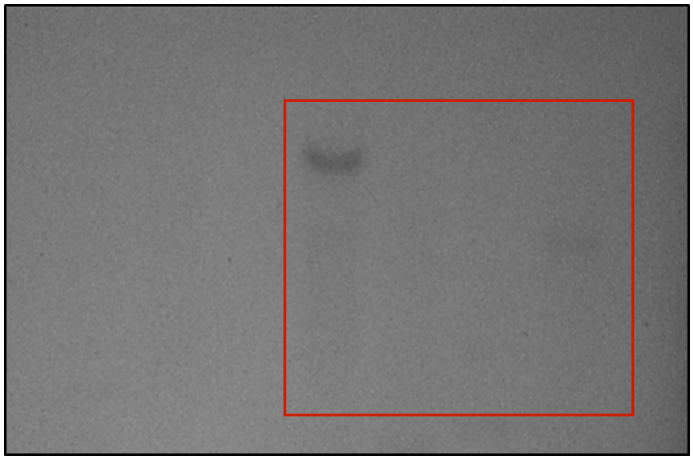


Supplementary Figure 17. SIRT6 depletion from L1 loci in young and old heart and liver tissue (a) Enrichment of SIRT6 was quantified at the L1 promoter in young and old heart tissue by ChIP. There were not statistically significant differences in SIRT6 enrichment at the L1 locus in old heart tissue, relative to young tissue. **(b)** Enrichment of SIRT6 was quantified at the L1 promoter in young and old liver tissue by ChIP. SIRT6 was slightly depleted from the L1 locus in old liver tissue, relative to young tissue. N=3; error bars indicate s.d.

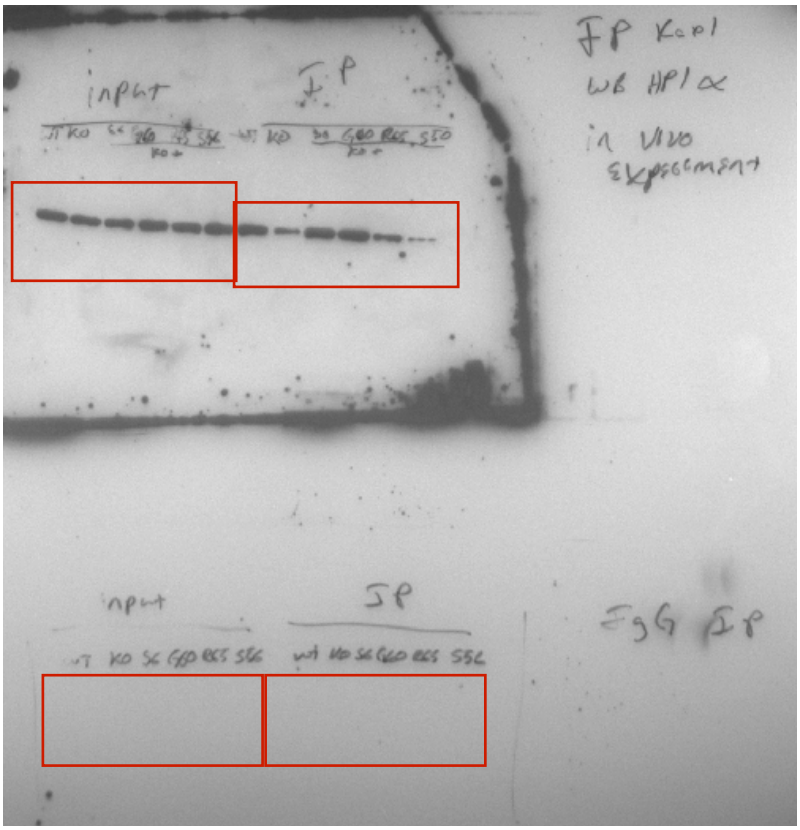


Supplementary Figure 18. Stress induces rapid SIRT6 relocalization. ChIP with SIRT6 antibodies revealed that the protein is rapidly depleted from the L1 5'UTR in response to hydrogen peroxide treatment. Cells were treated with 20 μM hydrogen peroxide for 30 minutes, and ChIP was performed at the indicated time after removal. Relative levels of SIRT6 bound to L1 5' UTR is calculated after normalization to input and H3 IP; IgG IP functioned as a negative control; n=3, error bars indicate s.d, * denotes significant difference from previous time point ($P<0.05$).

For Figure 3b



For Figure 3e



Supplementary Figure 19. Uncropped scans of the important immunoblots.