SUPPLEMENTAL INFORMATION

Sumoylation at Chromatin Governs Coordinated Repression of a Transcriptional Program Essential for Cell Growth and Proliferation

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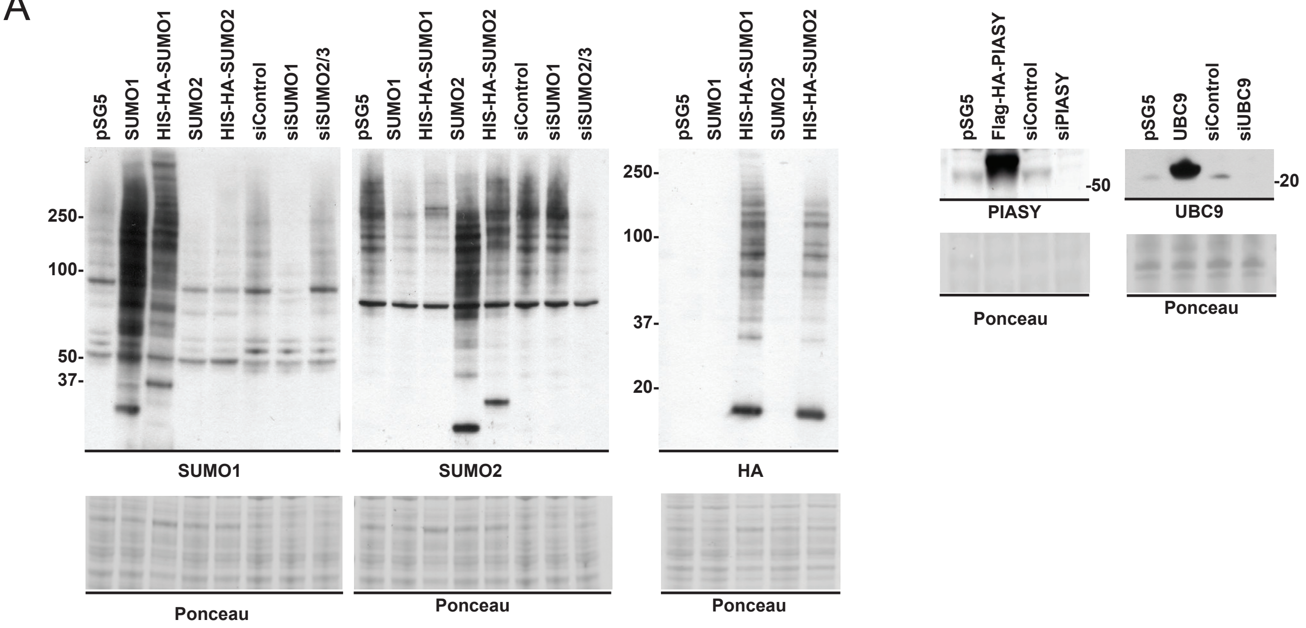
SUPPLEMENTAL FIGURES AND LEGENDS

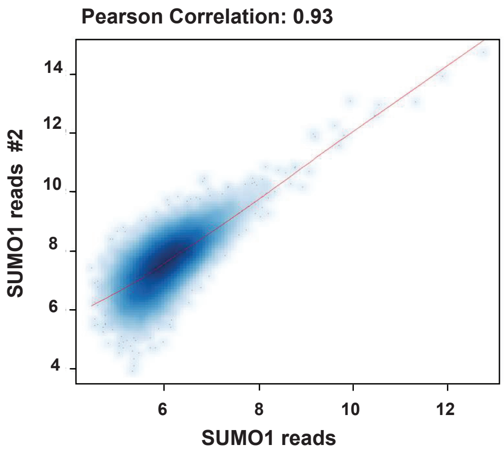
A



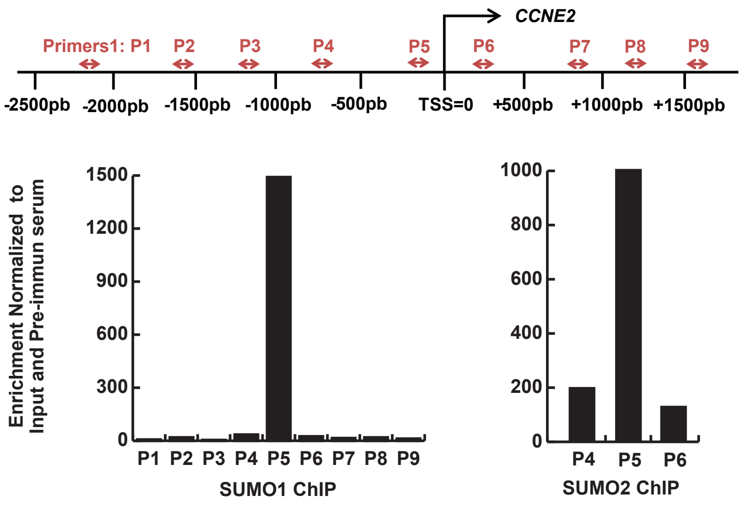
B

	Proliferation			Senescence		
ChIP-seq	Filtered reads	Unique reads	Peaks	Filtered reads	Unique reads	Peaks
Input	20 487 028	13 246 084		25 656 523	16 404 803	
SUMO-1	35 109 455	11 245 087	25 268	30 194 132	14 136 615	4 691
SUMO-2	30 957 206	14 475 774	46 768	33 714 090	15 505 727	23 786
PIASY	33 938 262	14 106 411	3 520	36 666 431	10 098 865	2 421
UBC9	36 478 620	13 364 644	7 486	24 695 680	1 461 932	4 698
Pol II	31 538 009	5 454 737	74460	26 808 908	7 395 769	79192
H3K4me3	32 333 231	11 385 193	22824	28 481 353	13 058 853	22913
H3K27me3	20 245 466	8 282 708	108743	25 720 227	13 998 058	61183
H3K9me3	35 040 040	13 740 738	125804	25 815 411	7 930 690	161547

C

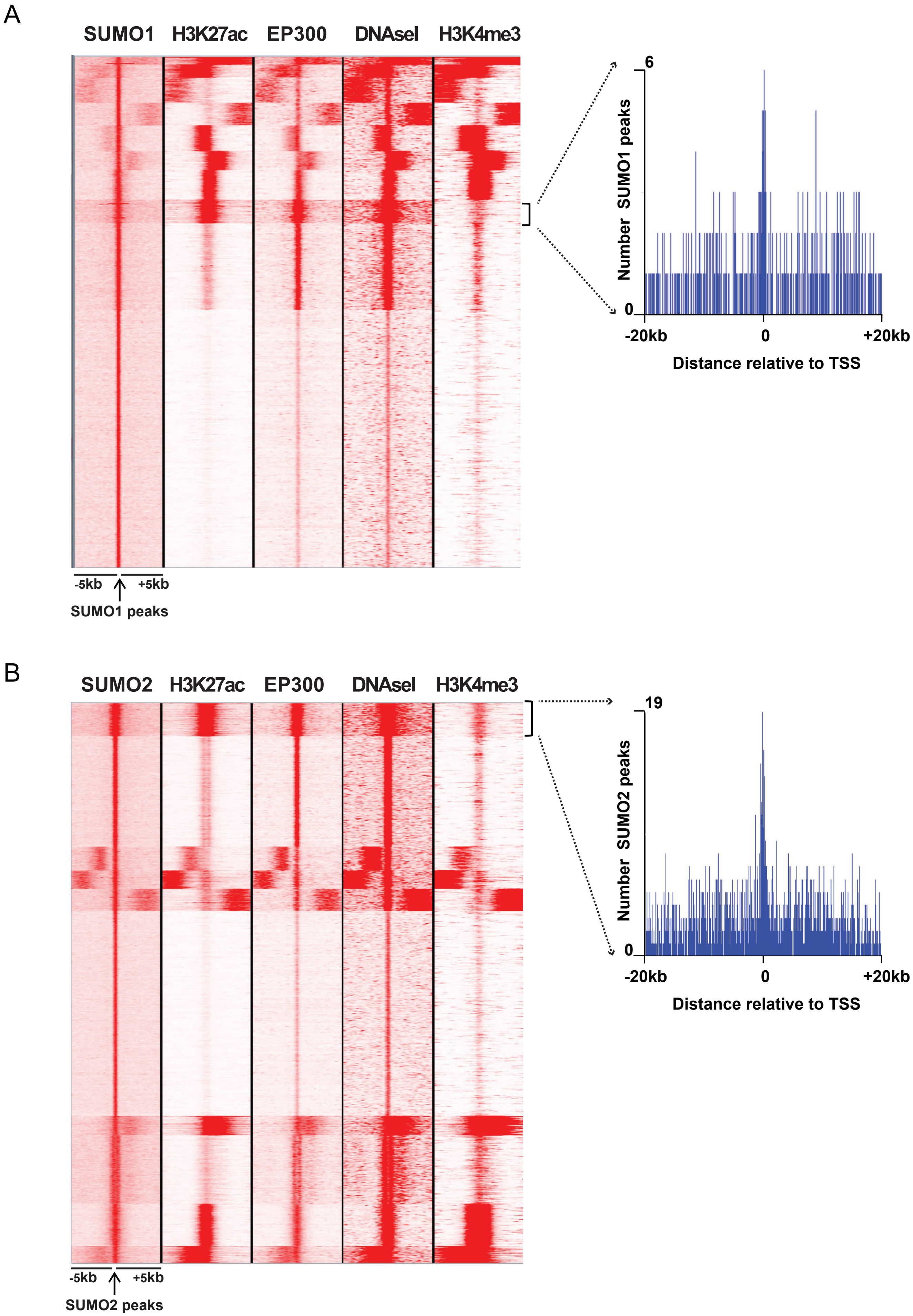


D

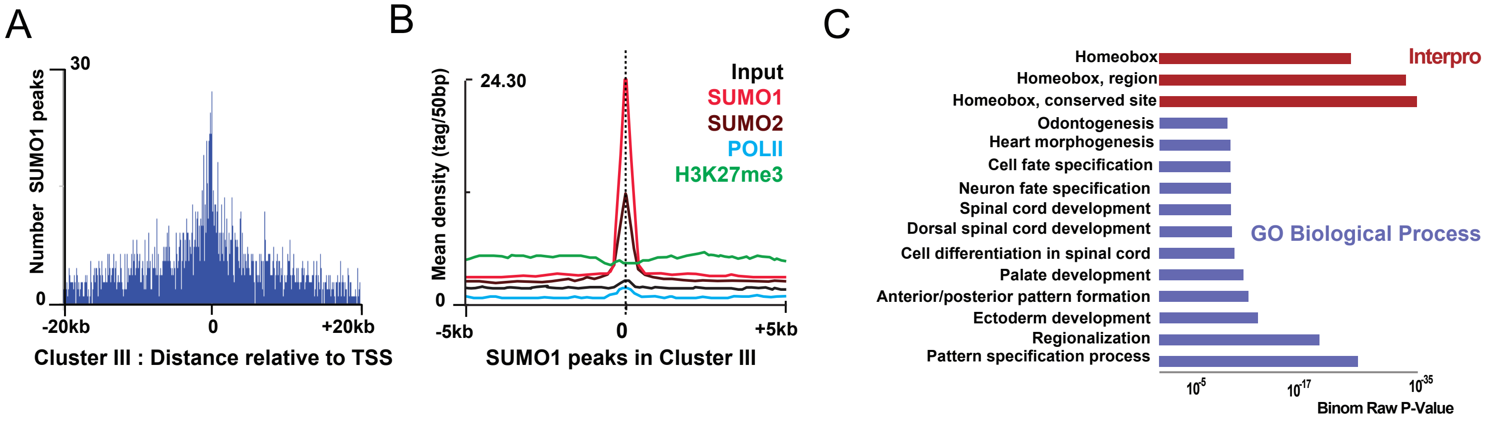


Supplemental Figure S1. Antibody specificity and ChIP-seq statistics. **A.** Western blot analysis of extracts of HeLa cells where the indicated genes (SUMO1, SUMO2, SUMO3, PIASY, UBC9) have been overexpressed or knocked-down by siRNA where corresponding increase and loss of signal is observed. The antibodies used to reveal Western blots are those used in the ChIP-seq experiments. **B.** Summary of sequencing statistics for the indicated ChIP-seq data sets. The numbers filtered and unique reads are indicated with the number of MACS detected peaks in proliferating and oncogenic HRAS^{G12V}-induced senescent WI38 fibroblasts. **C.** Scatter plot correlation showing SUMO1 ChIP-seq signals of the two independent replicates in proliferating WI38 cells. **D.** ChIP-qPCR for SUMO1 and SUMO2 on several regions of the *CCNE2* locus amplified with specific primers pairs (P1 to P9) in proliferating WI38 cells.

Neyret-Kahn_Supplemental Figure S2

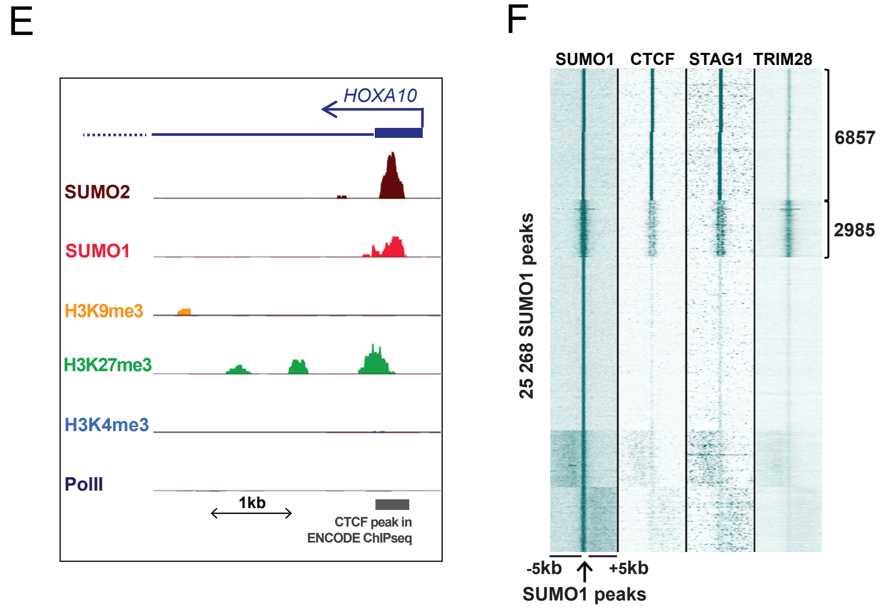


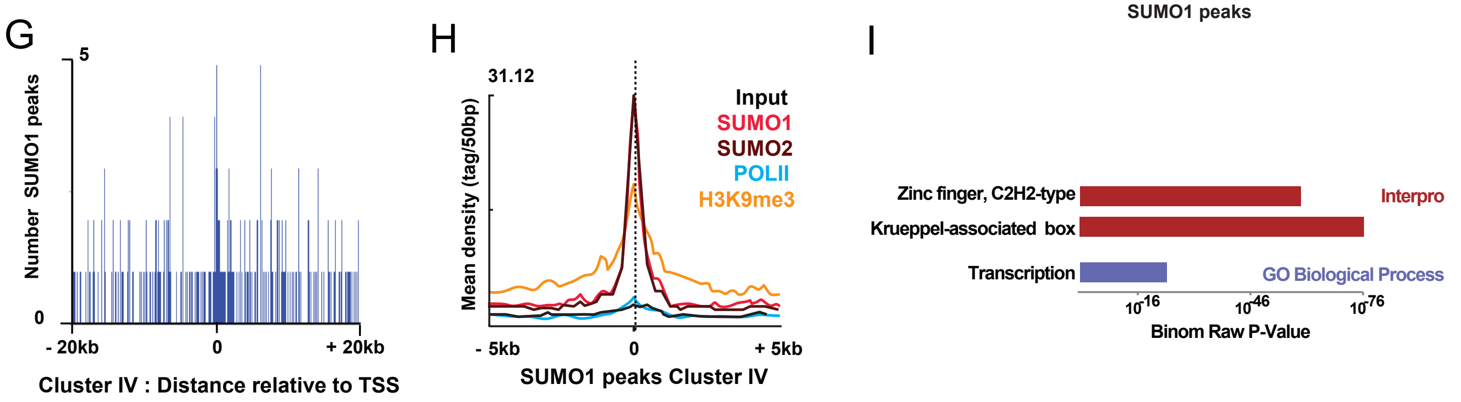
Supplemental Figure S2. SUMO1 and SUMO2 are present at enhancers. **A**. Association of SUMO1 with H3K27ac, EP300, DNaseI hypersensitivity, and H3K4me3. Comparison of tag density in the region of +/-5 kb around the SUMO1-occupied loci. The frequency of SUMO1 peaks with respect to the distance from the TSS in the indicated cluster corresponding to regions with enhancer marks is shown on the right. **B**. Association of SUMO2 with the indicated marks as described in panel A. In all cases, comparison was made between the SUMO1, SUMO2 and H3K4me3 data sets described in this paper and the ENCODE data sets from NHLF (normal human lung fibroblasts): H3K27ac (GSM733646), EP300 (GSM929091), DNaseI hypersensitivity (GSM736612).

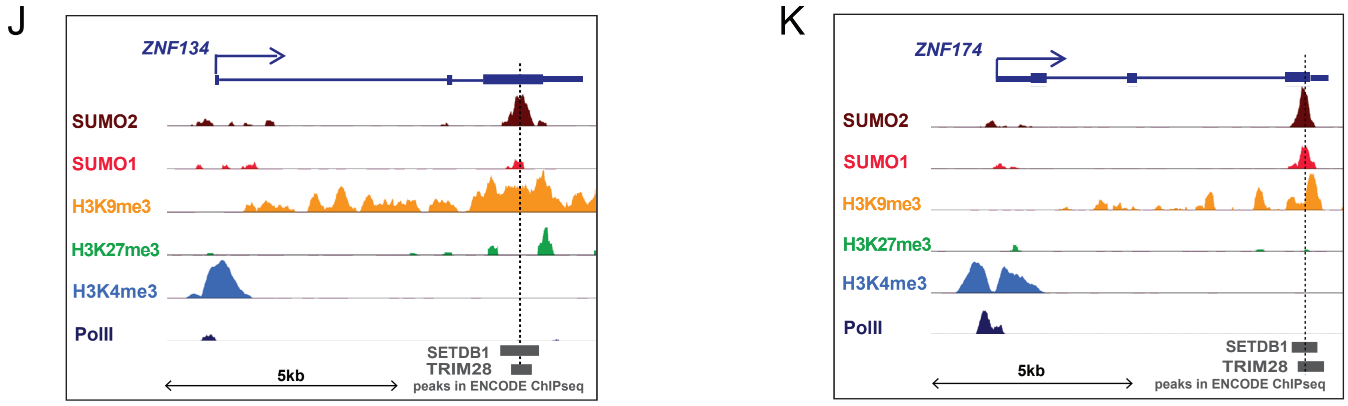


D

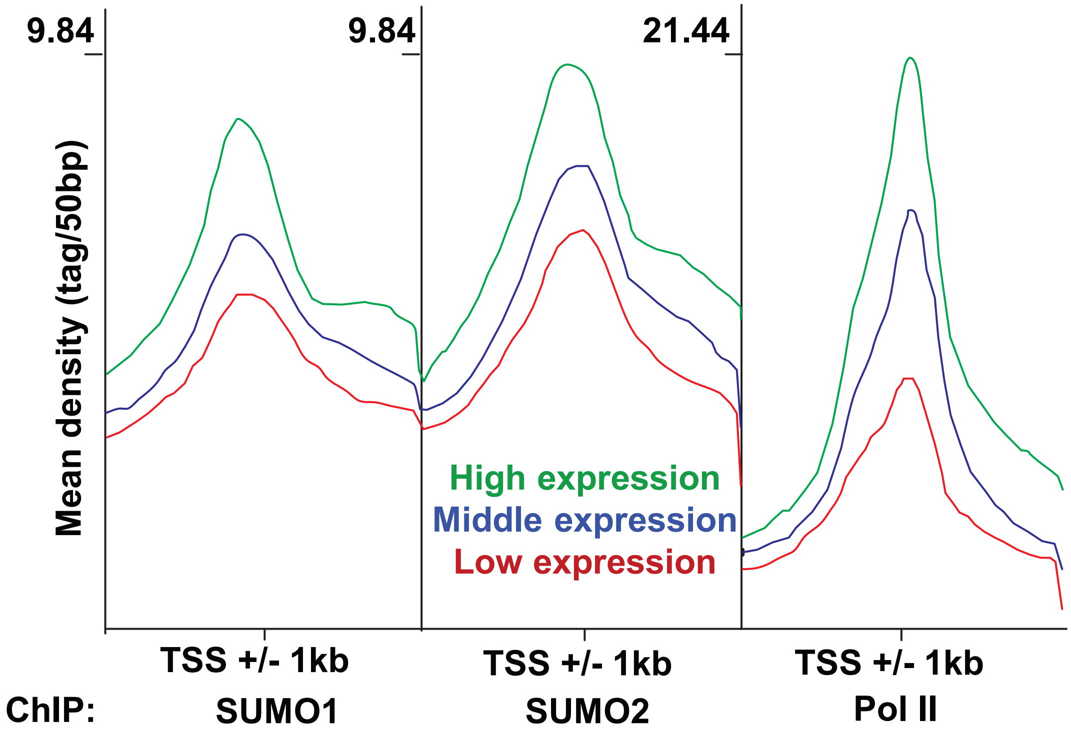
Cluster	Number of SUMO1 peaks	Number of SUMO1 peaks with a CTCF motif	Percentage
III	3316	1846	55.7
IV	406	34	8.4
V	9562	3456	36.1
I-II	5119	1342	26.2



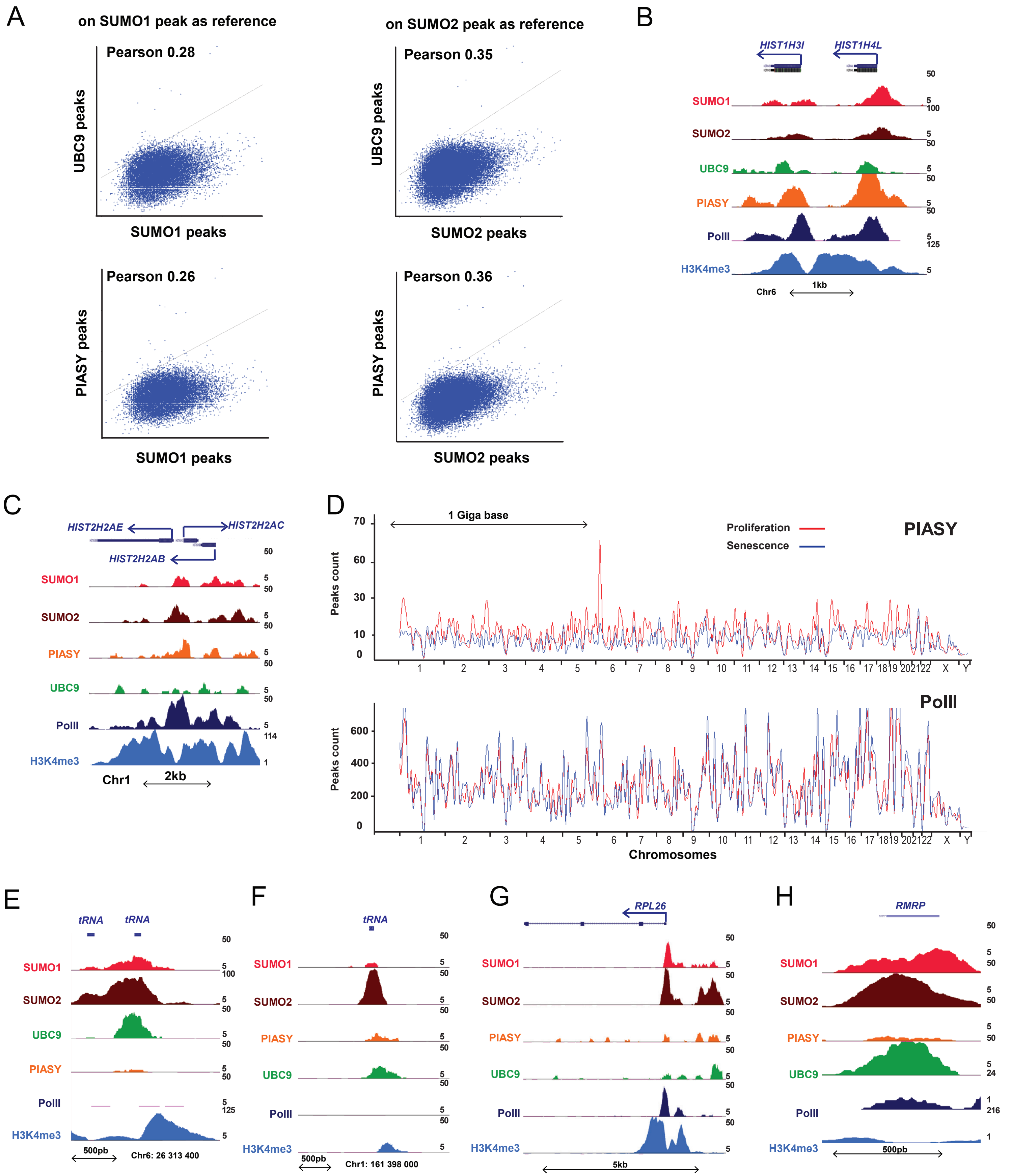




Supplemental Figure S3. Distinct SUMO targets in H3K27me3- and H3K9me3-repressed chromatin. **A.** Frequency of SUMO1 peaks with respect to the distance from the TSS in the H3K27me3 marked cluster III (see Fig. 1A). **B.** Merged profiles of SUMO1, SUMO2, Pol II and H3K27me3 read density around SUMO1-occupied loci in cluster III. **C.** Results of GREAT analysis of genes associated with SUMO1-occupied TSS in cluster III with two indicated ontology classifications. **D.** SUMO1-enriched loci in cluster III are enriched in CTCF motifs compared to SUMO loci in the other clusters. Table summarizing the number of CTCF motifs found at SUMO1-marked sites in the 5 clusters as defined in Fig. 1A. **E.** A genome browser view of ChIP-seq data for the indicated proteins and histone marks at the *HOXA10* locus. **F.** Tag density comparison of SUMO1-enriched loci with ChIP-seq datasets from GEO for CTCF, STAG1 and TRIM28 (GSM614608, GSM651542, GSM700358, respectively). **G.** Frequency of SUMO1 peaks with respect to distance from TSS in H3K9me3-associated cluster IV (Fig. 1A). **H.** Merged profiles of SUMO1, SUMO2, Pol II and H3K9me3 read density around SUMO1-occupied loci in cluster IV. **I.** Results of GREAT analysis of genes associated with SUMO1-enriched TSS in cluster IV with two indicated ontology classifications. **J-K**. Genome browser views of ChIP-seq data for the indicated proteins and histone marks at the *ZNF134* (J) and *ZNF174* (K) loci.

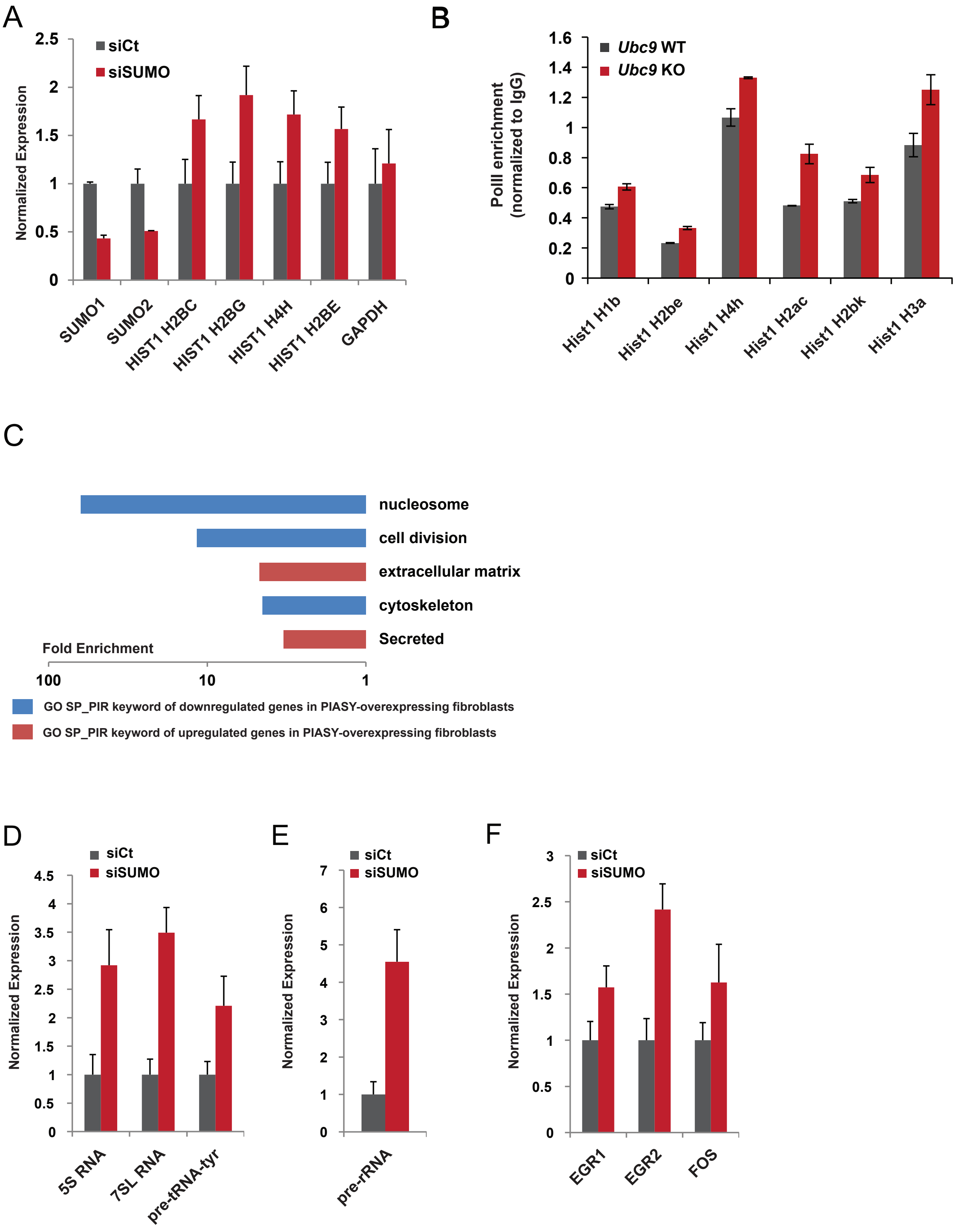

9.84
9.84
21.44
Mean density (tag/50bp)
High expression
Middle expression
Low expression
TSS +/- 1kb
TSS +/- 1kb
TSS +/- 1kb
ChIP:
SUMO1
SUMO2
Pol II

Supplemental Figure S4. Mean profile of SUMO1, SUMO2 and Pol II read density with respect to distance from TSS of all genes except ribosomal and histone genes, showing low, middle and high levels of expression.

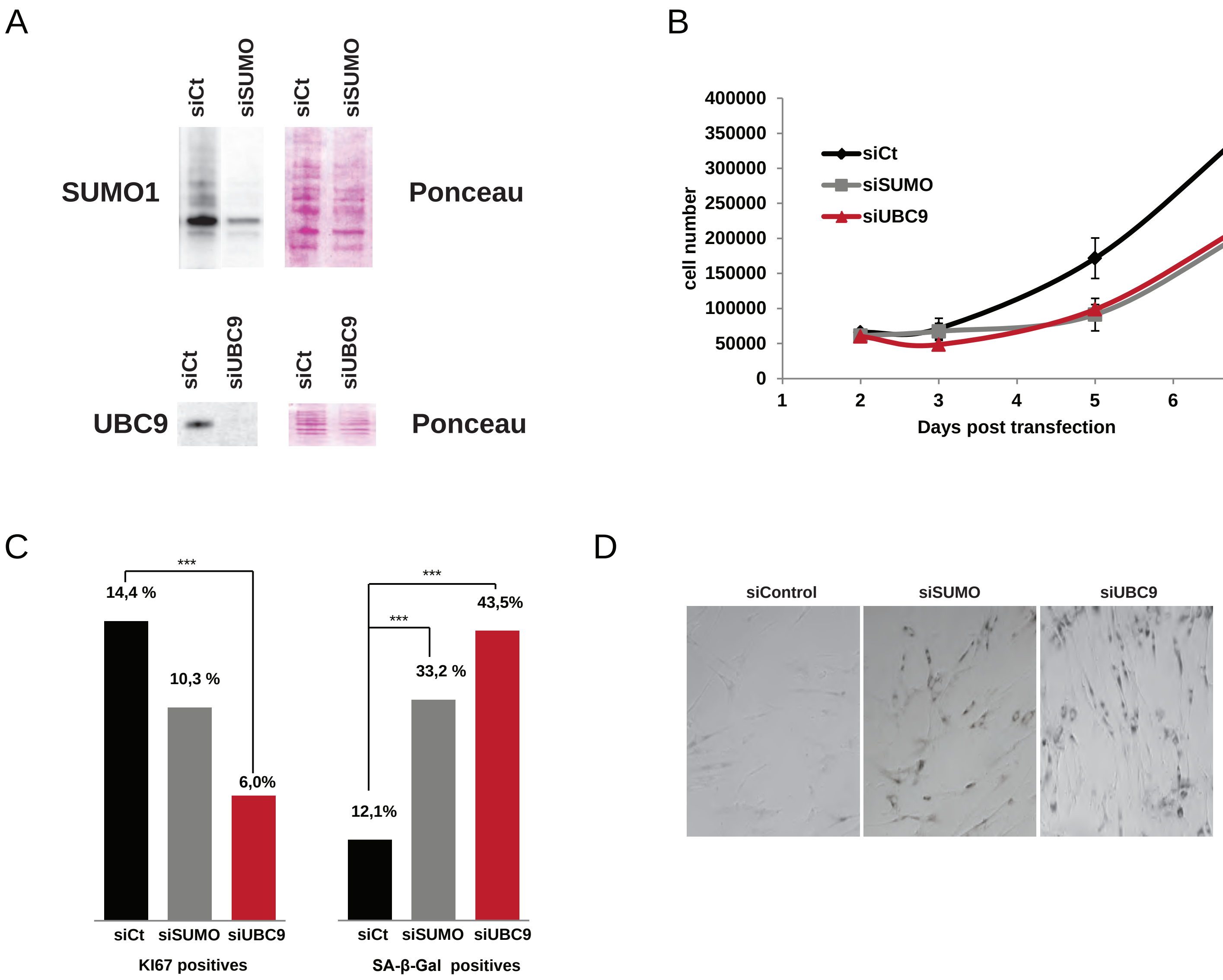

A
on SUMO1 peak as reference
Pearson 0.28
UBC9 peaks
SUMO1 peaks
Pearson 0.26
PIASY peaks
SUMO1 peaks
on SUMO2 peak as reference
Pearson 0.35
UBC9 peaks
SUMO2 peaks
Pearson 0.36
PIASY peaks
SUMO2 peaks
B
HIST1H3I
HIST1H4L
SUMO1
SUMO2
UBC9
PIASY
PolII
H3K4me3
50
5
100
5
50
5
50
5
50
5
125
5
Chr6
1kb
C
HIST2H2AE
HIST2H2AC
HIST2H2AB
SUMO1
SUMO2
PIASY
UBC9
PolII
H3K4me3
50
5
50
5
50
5
50
5
50
5
114
1
Chr1
2kb
D
1 Giga base
Proliferation
Senescence
PIASY
Peaks count
70
60
30
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
PolII
Peaks count
600
400
200
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
Chromosomes
E
tRNA
tRNA
SUMO1
SUMO2
UBC9
PIASY
PolII
H3K4me3
50
5
100
5
50
5
50
5
50
5
125
5
500pb
Chr6: 26 313 400
F
tRNA
SUMO1
SUMO2
PIASY
UBC9
PolII
H3K4me3
50
5
50
5
50
5
50
5
50
5
50
5
500pb
Chr1: 161 398 000
G
RPL26
SUMO1
SUMO2
PIASY
UBC9
PolII
H3K4me3
50
5
50
5
50
5
50
5
50
5
50
5
5kb
H
RMRP
SUMO1
SUMO2
PIASY
UBC9
PolII
H3K4me3
50
5
50
5
50
5
50
5
24
1
216
1
500pb

Supplemental Figure S5. Colocalisation of the SUMO machinery and example of co-occupied genes **A.** Scatter plot comparison of SUMO1-occupied loci (left) and SUMO-2 occupied loci (right) with UBC9 or PIASY ChIP-seq reads showing Pearson correlations. **B, C, E, F, G, H.** Genome browser views of the indicated ChIP-seq data at the following loci: 2 histone loci from Chr:6 (B) and 3 histone loci from Chr:1 (C), *tRNA* from Chr:6 (E), *tRNA* from Chr:1 (F), *RPL26* (G), *RMRP* (H). **D.** Landscape of PIASY or Pol II peak density in proliferating WI38 cells over the genome. Chromosomes are indicated on x axis.

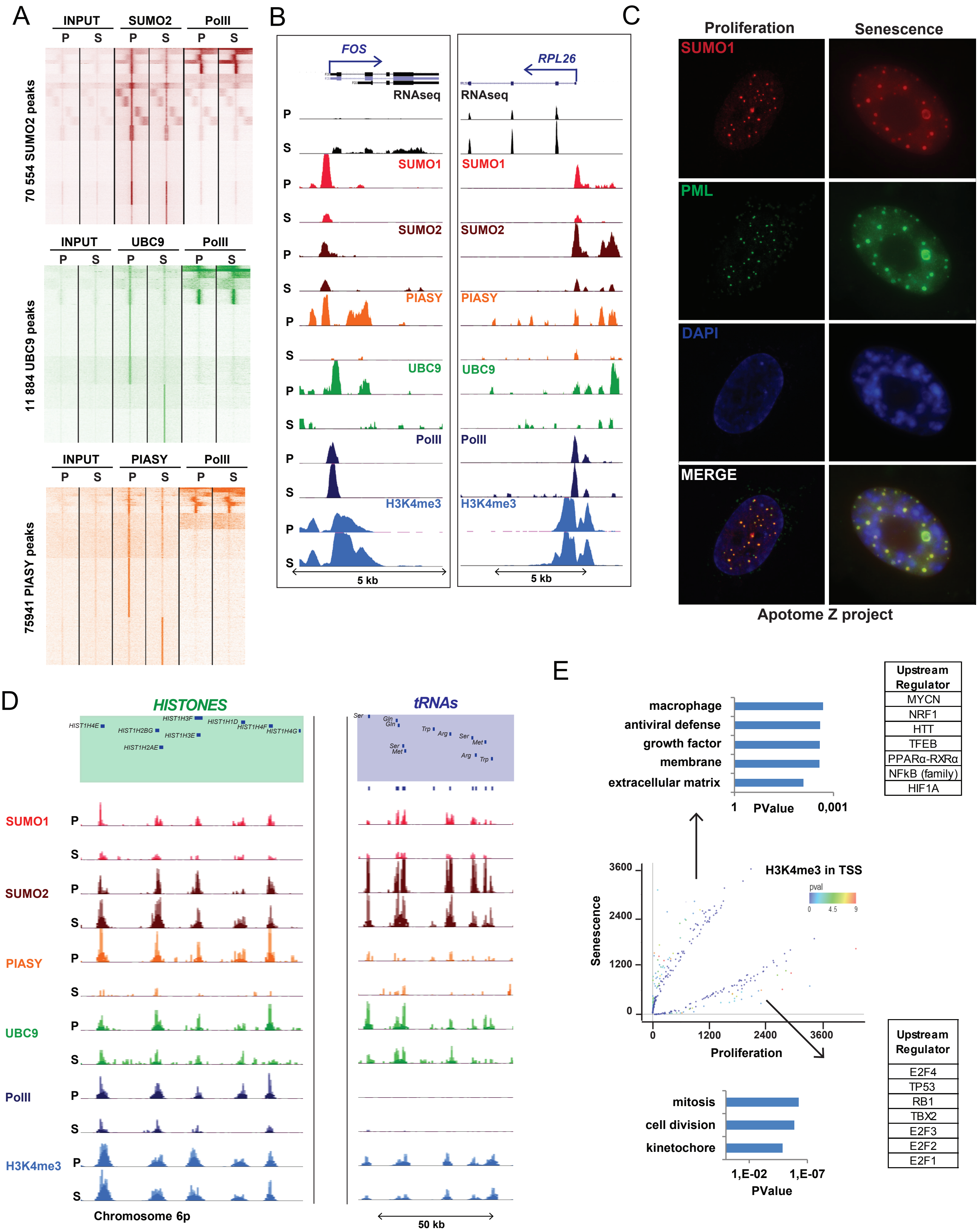
Neyret-Kahn_Supplemental Figure S6



Supplemental Figure S6. Sumoylation regulates expression of histone and growth control genes. **A**. WI38 cells were transfected with a control siCt or siSUMO1/2/3 (siSUMO) and the expression of the indicated genes was analysed by RT-qPCR. **B.** ChIP-qPCR for Pol II in *Ubc9*$^{+/+}$ (WT) and *Ubc9*$^{fl/-;T2}$ (KO) MEFs treated for 6 days by tamoxifen on several histone promoters. **C.** Selected categories from DAVID ontology analysis of genes up-regulated (red) and down-regulated (blue) in WI38 cells stably overexpressing PIASY. **D-F.** Analysis by RT-qPCR of expression of the indicated genes in WI38 transfected with siSUMO *vs* siCt. For all RT-qPCR, experiments were performed in triplicate and data are represented as mean +/− SEM (n=3).


A
siCt
siSUMO
siCt
siSUMO
SUMO1
Ponceau
siCt
siUBC9
siCt
siUBC9
UBC9
Ponceau
B
400000
350000
300000
250000
200000
150000
100000
50000
0
cell number
siCt
siSUMO
siUBC9
1
2
3
4
5
6
7
Days post transfection
C
***
14,4 %
10,3 %
6,0%
siCt
siSUMO
siUBC9
KI67 positives
***
***
43,5%
33,2 %
12,1%
siCt
siSUMO
siUBC9
SA-β-Gal positives
D
siControl
siSUMO
siUBC9

Supplemental Figure S7. Knock down of SUMO induces an atypical senescence phenotype. **A.** Western blot validation of siRNA knockdown efficiency. **B.** Growth curve of WI38 cells transfected with a control siCt or siSUMO1/2/3 (siSUMO) or siUBC9. **C.** Percentage of Ki67 and SA-β-Gal positive WI38 cells 7 days post-siRNA transfection. **D.** Representative micrographs showing SA-β-Gal staining.



Upstream Regulator
MYCN
NRF1
HTT
TFEB
PPARα-RXRα
NFkB (family)
HIF1A

Upstream Regulator
E2F4
TP53
RB1
TBX2
E2F3
E2F2
E2F1

Supplemental Figure S8. Senescence is accompanied by decreased chromatin-association of the SUMO machinery. **A.** Comparison of association of SUMO2, UBC9 and PIASY with Pol II in proliferating (P) and HRAS^{G12V}-induced senescent (S) WI38 cells. **B.** Genome browser views of the indicated ChIP-seq and RNAseq data at the *FOS* and *RPL26* loci in proliferating (P) and senescent (S) cells. **C.** Apotome images of proliferating and senescent cells immunostained for SUMO1 and PML. **D.** A genome browser view of ChIP-seq data across the histone and tRNA gene cluster in chromosome 6p in proliferating and senescent WI38 cells. **E.** Scatter plot relating TSS differentially marked by H3K4me3 in proliferating *vs* senescent cells. Genes associated with TSS showing gain (above) and loss (below) of H3K4me3 were analysed using DAVID functional annotation tool (SP_PIR Keyword) and IPA upstream regulator function.

SUMO1 and SUMO2 common AME output

Motif Name	Species	Sumoylated?
MYC::MAX	Homo sapiens	yes
SPIB	Homo sapiens	
HLF	Homo sapiens	
MEF2A	Homo sapiens	yes
MAX	Homo sapiens	
Fos	Homo sapiens	yes
HIF1A::ARNT	Homo sapiens	yes
AP1	Homo sapiens	yes
TEAD1	Homo sapiens	
FOXA1	Homo sapiens	
FOXF2	Homo sapiens	
FEV	Homo sapiens	
FOXI1	Homo sapiens	
EWSR1-FLI1	Homo sapiens	yes
FOXO3	Homo sapiens	
STAT1	Homo sapiens	yes
NFATC2	Homo sapiens	yes
SP1	Homo sapiens	yes
SPI1	Homo sapiens	
USF1	Homo sapiens	
PBX1	Homo sapiens	
NFYA	Homo sapiens	
RREB1	Homo sapiens	yes
IRF1	Homo sapiens	yes
Myf	Homo sapiens	
MZF1_1-4	Homo sapiens	
NFKB1	Homo sapiens	*yes*
ELK4	Homo sapiens	yes
TAL1::TCF3	Homo sapiens	
NHLH1	Homo sapiens	
REST	Homo sapiens	
Foxa2	Mus musculus	
ELF5	Mus musculus	
GABPA	Mus musculus	
Stat3	Mus musculus	yes
RUNX1	Mus musculus	yes
Klf4	Mus musculus	yes
Arnt	Mus musculus	yes
Mycn	Mus musculus	
CTCF	Mus musculus	yes
Egr1	Mus musculus	yes
Nr2e3	Mus musculus	yes
Zfx	Mus musculus	
Pax4	Mus musculus	
Myc	Mus musculus	yes
Myb	Mus musculus	yes

SUMO2 specific AME output

Motif Name	Species	Sumoylated?
NFE2L2	Homo sapiens	
FOXD1	Homo sapiens	
CREB1	Homo sapiens	yes
RELA	Homo sapiens	yes
FOXL1	Homo sapiens	
IRF2	Homo sapiens	yes
NR1H2::RXRA	Homo sapiens	yes
NR4A2	Homo sapiens	yes
NFIL3	Homo sapiens	
REL	Homo sapiens	
Pax2	Mus musculus	
PPARG::RXRA	Mus musculus	yes
T	Mus musculus	
Nkx2-5	Mus musculus	yes
Evi1	Mus musculus	yes

Transcription factors reported in the litterature to be sumoylated are indicated

Supplemental Table S1. AME output of MEME ChIP analyses of SUMO1 and SUMO2 peaks.

Supplemental Table S2. Expressed genes (n=13 945) as determined by mRNAseq (≥ 2RPKM in each of the 3 biological replicates).

nucleosome core	**ribosome**	**protein biosynthesis**
histone cluster 1, H2ad	ribosomal protein L35a	tryptophanyl-tRNA synthetase
histone cluster 1, H2ah	ribosomal protein L27	eukaryotic translation elongation factor 1 alpha-like 7
histone cluster 1, H2ai	ribosomal protein L24	eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa
histone cluster 1, H2ak	ribosomal protein L19	eukaryotic translation initiation factor 3, subunit G
histone cluster 1, H2al	ribosomal protein L18	eukaryotic translation initiation factor 5A2
histone cluster 1, H2am	ribosomal protein L11	eukaryotic translation termination factor 1
histone cluster 1, H2bc	ubiquitin C	ribosomal protein L18
histone cluster 1, H2be	ribosomal protein S7	ribosomal protein L19
histone cluster 1, H2bf	ubiquitin B	phenylalanyl-tRNA synthetase 2, mitochondrial
histone cluster 1, H2bg	small nucleolar RNA, H/ACA box 7A	threonyl-tRNA synthetase
histone cluster 1, H3a	ribosomal protein L36	small nucleolar RNA, H/ACA box 7A
histone cluster 1, H3b	ribosomal protein L9	similar to eukaryotic translation initiation factor 4A2
histone cluster 1, H3c	ribosomal protein S14	ribosomal protein S7
histone cluster 1, H3d	ribosomal protein L26	component of oligomeric golgi complex 8
histone cluster 1, H3e	ribosomal protein L21	eukaryotic translation elongation factor 1 alpha 1
histone cluster 1, H3f	ribosomal protein S3A	ribosomal protein S8
histone cluster 1, H3g	ribosomal protein S8	similar to eukaryotic translation elongation factor 1 alpha 1
histone cluster 1, H3h	ribosomal protein S2	eukaryotic translation elongation factor 1 alpha-like 3
histone cluster 1, H3i	ribosomal protein S29	glycyl-tRNA synthetase
histone cluster 1, H4a	small nucleolar RNA, H/ACA box 7B	ribosomal protein L11
histone cluster 1, H4b	ribosomal protein L23	eukaryotic translation initiation factor 4E family member 2
histone cluster 1, H4c	ribosomal protein L5	ubiquitin B
histone cluster 1, H4d	ribosomal protein S18	ubiquitin C
histone cluster 1, H4e	ribosomal protein S4, X-linked	valyl-tRNA synthetase 2, mitochondrial (putative)
histone cluster 1, H4f	ribosomal protein L32	eukaryotic translation initiation factor 5B
histone cluster 1, H4h	ribosomal protein, large, P0	G elongation factor, mitochondrial 2
histone cluster 1, H4i	ribosomal protein L12	PET112-like (yeast)
histone cluster 1, H4j		Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase
histone cluster 1, H4k		SECIS binding protein 2
histone cluster 2, H3a		alanyl-tRNA synthetase 2, mitochondrial (putative)
histone cluster 2, H3c		glutamyl-tRNA synthetase 2, mitochondrial (putative)
histone cluster 2, H3d		mitochondrial translational initiation factor 2
histone cluster 2, H4a		ribosomal protein L9
histone cluster 2, H4b		peptide deformylase (mitochondrial)
histone cluster 4, H4		ribosomal protein L24
H2A histone family, member J		ribosomal protein L27
H2A histone family, member V		eukaryotic translation initiation factor 3, subunit M
histone cluster 1, H2ab		ribosomal protein L35a
histone cluster 1, H2ae		ribosomal protein S14
histone cluster 1, H2ag		ribosomal protein L36
histone cluster 1, H2bb		eukaryotic translation initiation factor 4A, isoform 2
histone cluster 1, H2bd		ribosomal protein L21
histone cluster 1, H2bh		ribosomal protein L26
histone cluster 1, H2bi		ribosomal protein L32
histone cluster 1, H2bj		ribosomal protein L5
histone cluster 1, H2bk		ribosomal protein S18
histone cluster 1, H2bo		ribosomal protein S2
histone cluster 1, H3j		ribosomal protein S29
histone cluster 1, H4l		ribosomal protein S3A
histone cluster 2, H2ab		ribosomal protein S4, X-linked
histone cluster 2, H2ac		ribosomal protein, large, P0
histone cluster 2, H2be		small nucleolar RNA, H/ACA box 7B
histone cluster 3, H2a		
histone cluster 3, H2bb		

Supplemental Table S3. List of genes classified by DAVID in 3 selected categories from Fig. 3A,B corresponding to the highest peaks for SUMO1 and/or SUMO2 (nucleosome core, ribosome, protein biosynthesis).

Gene	Ensembl_Gene
MDM4	ENSG00000198625
RNU5A-8P	ENSG00000200972
RRP15	ENSG00000067533
RP11-145A3.1	ENSG00000227496
RP11-318C2.1	ENSG00000255159
	ENSG00000243172
VPS11	ENSG00000160695
C12orf44	ENSG00000123395
RPS26	ENSG00000197728
RP11-181C3.1	ENSG00000245017
SIRT4	ENSG00000089163
VPS37B	ENSG00000139722
ZFYVE26	ENSG00000072121
CASC4	ENSG00000166734
RAB11A	ENSG00000103769
PML	ENSG00000140464
WWOX	ENSG00000186153
RPL38	ENSG00000172809
	ENSG00000246990
ZNF490	ENSG00000188033
ZNF461	ENSG00000197808
C2orf43	ENSG00000118961
RANBP2	ENSG00000153201
AC005540.3	ENSG00000235852
RHBDD1	ENSG00000144468
C21orf91	ENSG00000154642
ETS2	ENSG00000157557
VTRNA2-1	ENSG00000202030
HIST1H1A	ENSG00000124610
HIST1H4A	ENSG00000196176
HIST1H2BE	ENSG00000197697
HIST1H3E	ENSG00000196966
TRNAI2	ENSG00000238621
HIST1H2AG	ENSG00000196787
HIST1H2BM	ENSG00000196374
ASH2L	ENSG00000129691
DECR1	ENSG00000104325
RP11-122A3.2	ENSG00000253250
DPY19L4	ENSG00000156162
TNFRSF11B	ENSG00000164761
RP11-383J24.1	ENSG00000253227
LOC402191	ENSG00000198000
SLC44A1	ENSG00000070214
NEXN	ENSG00000162614
MTF2	ENSG00000143033
PCBP2	ENSG00000197111
GARNL1	ENSG00000174373
ZSCAN29	ENSG00000140265
U6	ENSG00000207357
JOSD2	ENSG00000161677
CCDC108	ENSG00000181378
PKIB	ENSG00000135549
HTR5A	ENSG00000157219
RP11-1105O14.1	ENSG00000253270

Supplemental Table S4. List of genes whose TSS is marked simultaneously by SUMO1 and/or SUMO2, UBC9 and PIASY.

Supplemental Table S5. List of 187 genes differentially expressed in PIASY-overexpressing WI38 fibroblasts as determined by microarray analysis in 3 biological replicates (pvalue <0.05 Bonferroni).

Supplemental Table S6. List of 1945 genes differentially expressed in UBC9-depleted *vs* control WI38 fibroblasts as determined by Affymetrix analysis of 3 biological replicates (pvalue<0,05 Bonferroni).

Supplemental Table S7. List of 2411 genes differentially expressed in proliferative vs senescent WI38 fibroblasts as determined by RNAseq in 3 biological replicates (adjusted pvalue <0.01, Fold Change ≥2 or ≤ -2)