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Article

Supplementary Materials: PRC1 Prevents Replication Stress during Chondrogenic Transit Amplification

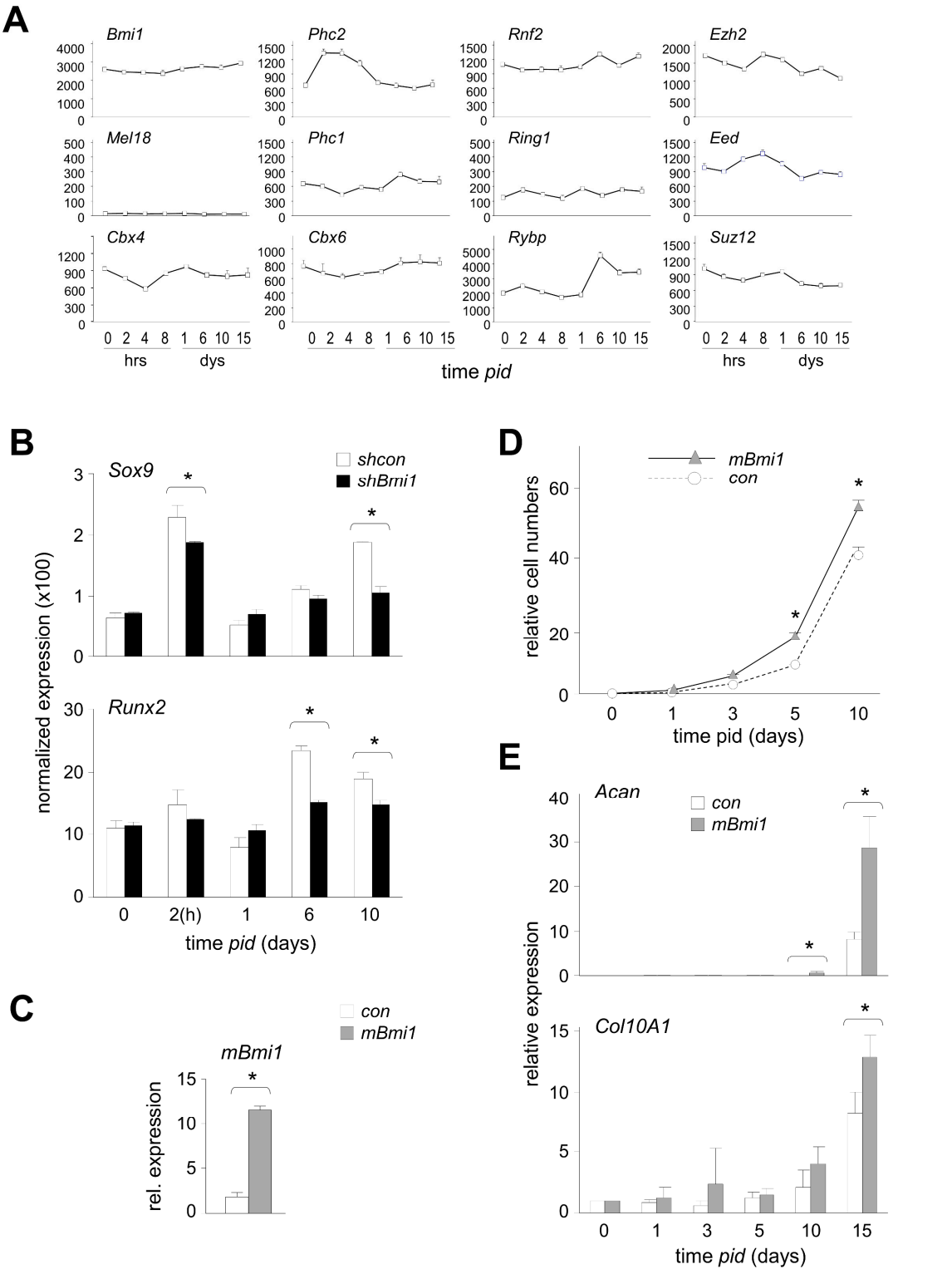


Figure S1. BMI1 is required for chondrogenic differentiation. (**A**) Array expression analysis of PRC1 genes during chondrogenesis; *Bmi1*, *Mel18*, *Phc* (polyhomeotic homologs 1 and 2), *Ring1* (*Rnf2*, *Ring1*),

Rybp (RING1 and YY1 binding protein), *Cbx* (chromobox homologs 4 and 6) and of PRC2 genes *Ezh2*, *Eed* and *Suz12*; values in all panels represent mean of triplicates ± s.d. (**B**) Expression analysis of *Sox9* and *Runx2* mRNA in *shcon* and *shBmi1* cells (qRT-PCR; triplicates). (**C**) Confirmation of murine BMI1 mRNA (*mBmi1*) overexpression in ATDC5 cells; empty vector was used as control (*con;* qRT-PCR; triplicates). (**D**) Proliferation curves of ATDC5 cells overexpressing murine BMI1 (*mBmi1*) vs control cells (*con*). (**E**) *Acan* and *Col10A1* expression during differentiation in ATDC5 cells overexpressing murine *Bmi1* (*mBmi1*) cDNA *versus* control (*con*) cells (qRT-PCR; triplicates); asterisks (*; C-E): p<0.05.

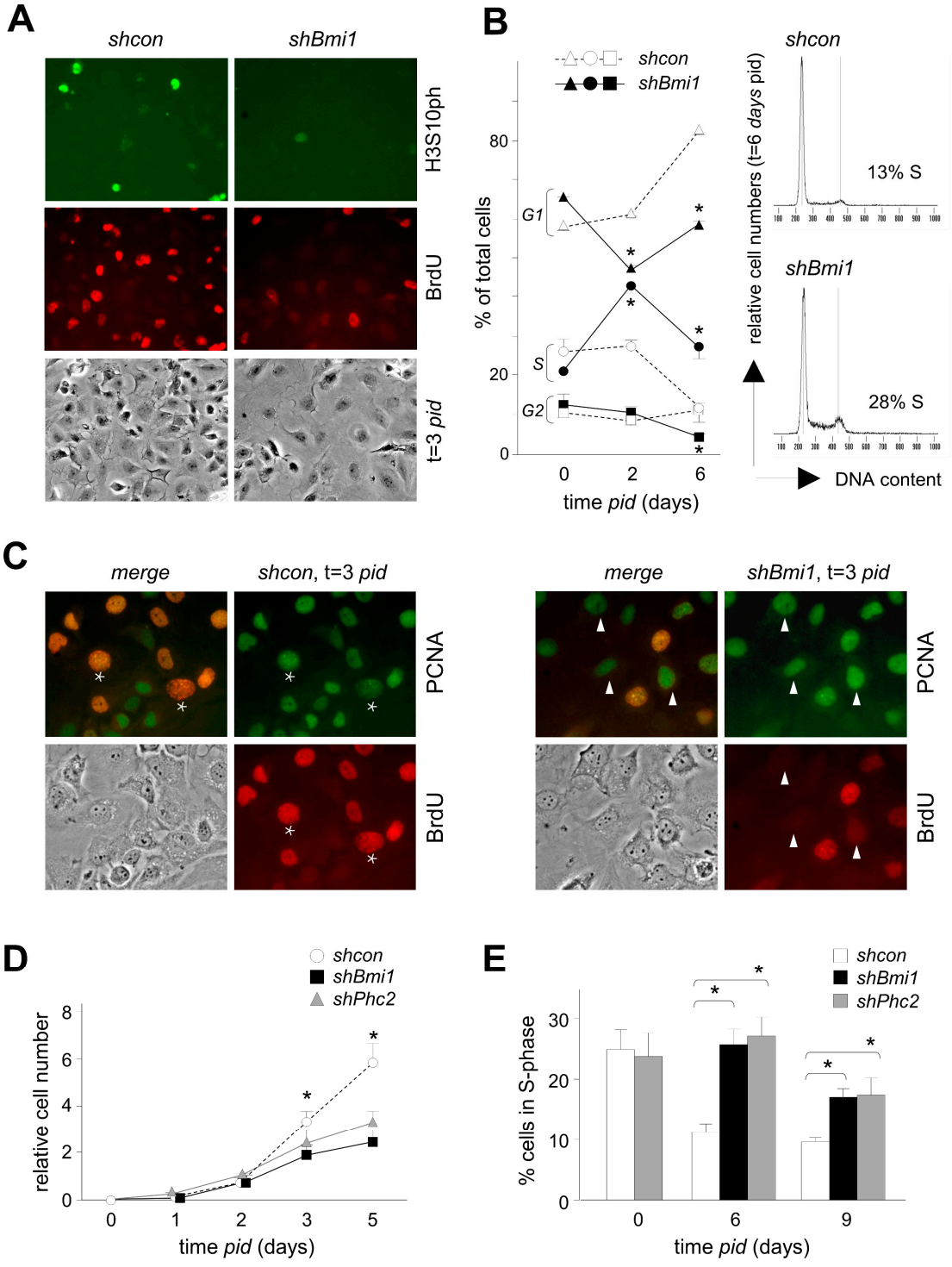
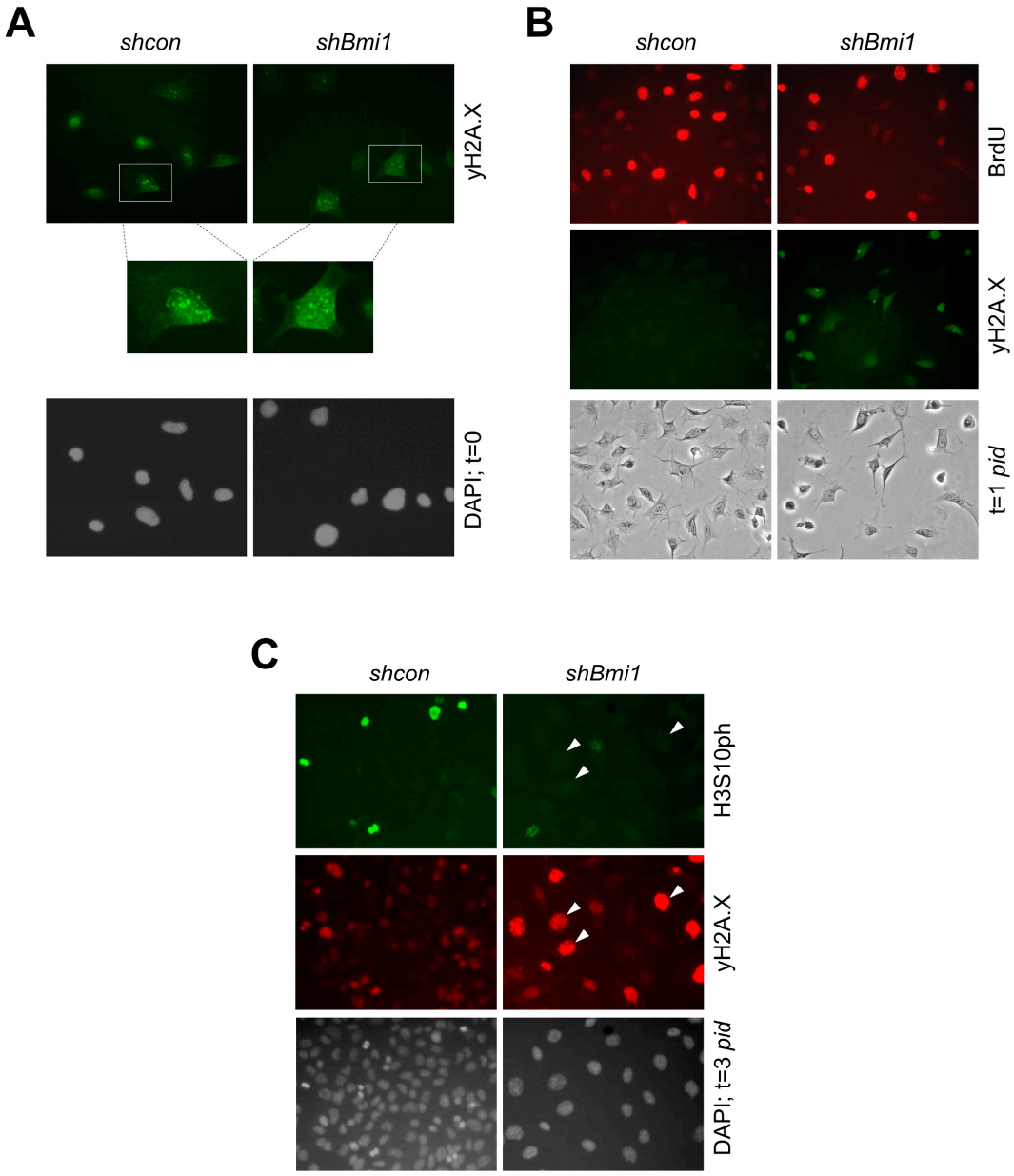


Figure S2. IntraS-phase accumulation during TA in the absence of PRC1. (**A**) Representative IF images showing co-staining for BdrU-incorporation and H3S10 phosphorylation (H3S10ph) in *shcon* and

shBmi1 ATDC5 cells at t=3 days *pid*; 19 of 100 *shcon* cells were positive for H3S10ph, of which 7 were brightly stained (G2/M); less than 3% of *shBmi1* cells was weakly positive (late S/G2). BrdU pulse: 45 min. (**B**) Cell cycle distribution of *shcon* and *shBmi1* ATDC5 cells throughout differentiation (left panel). SubG1 fractions (t=6 days *pid*) *shcon:* 0.47% ±0.035, *shBmi1*: 0.43% ±0.041; asterisks (*): $p<0.05$; representative cell cycle profiles (of triplicates) of ATDC5 *shcon* and *shBmi1* at 6 days *pid* (right panels); DNA content was measured by propidium-iodide (PI) staining; values represent percentages S-phase cells of total cells analysed. (**C**) Representative IF images showing co-staining for PCNA-/chromatin association (green) and BrdU-incorporation (red). Note: methanol-fixation (throughout) ensures detection of only chromatin-associated proteins. The most PCNA-dim nuclei (*shcon*) represent G1/early S or G2-phase cells; S-phase cells gain PCNA-brightness as they progress through S-phase; in mid-/late S-phase the appearance of conspicuously bright foci signals late nucleolar DNA replication (asterisks; *shcon*). Of PCNA-positive nuclei (64% and 68%, *shcon* and *shBmi1*, respectively) 81% and 43% was also positive for BrdU in *shcon* and *shBmi1* cells, respectively. Arrowheads mark examples of low BrdU-incorporation (green) in PCNA-bright *shBmi1* cells, indicative of intraS-phase arrest. (**D**) Analysis of proliferation rate (Crystal-violet extraction) and (**E**) cell numbers in S-phase (DNA-profiling; right) of ATDC5 cells expressing *shBmi1* and *shPhc2* at indicated time-points during differentiation (in days *pid*). Asterisks (*; D, E): $p<0.05$.


A
shcon
shBmi1
γH2A.X
DAPI; t=0
B
shcon
shBmi1
BrdU
γH2A.X
t=1 pid
C
shcon
shBmi1
H3S10ph
γH2A.X
DAPI; t=3 pid

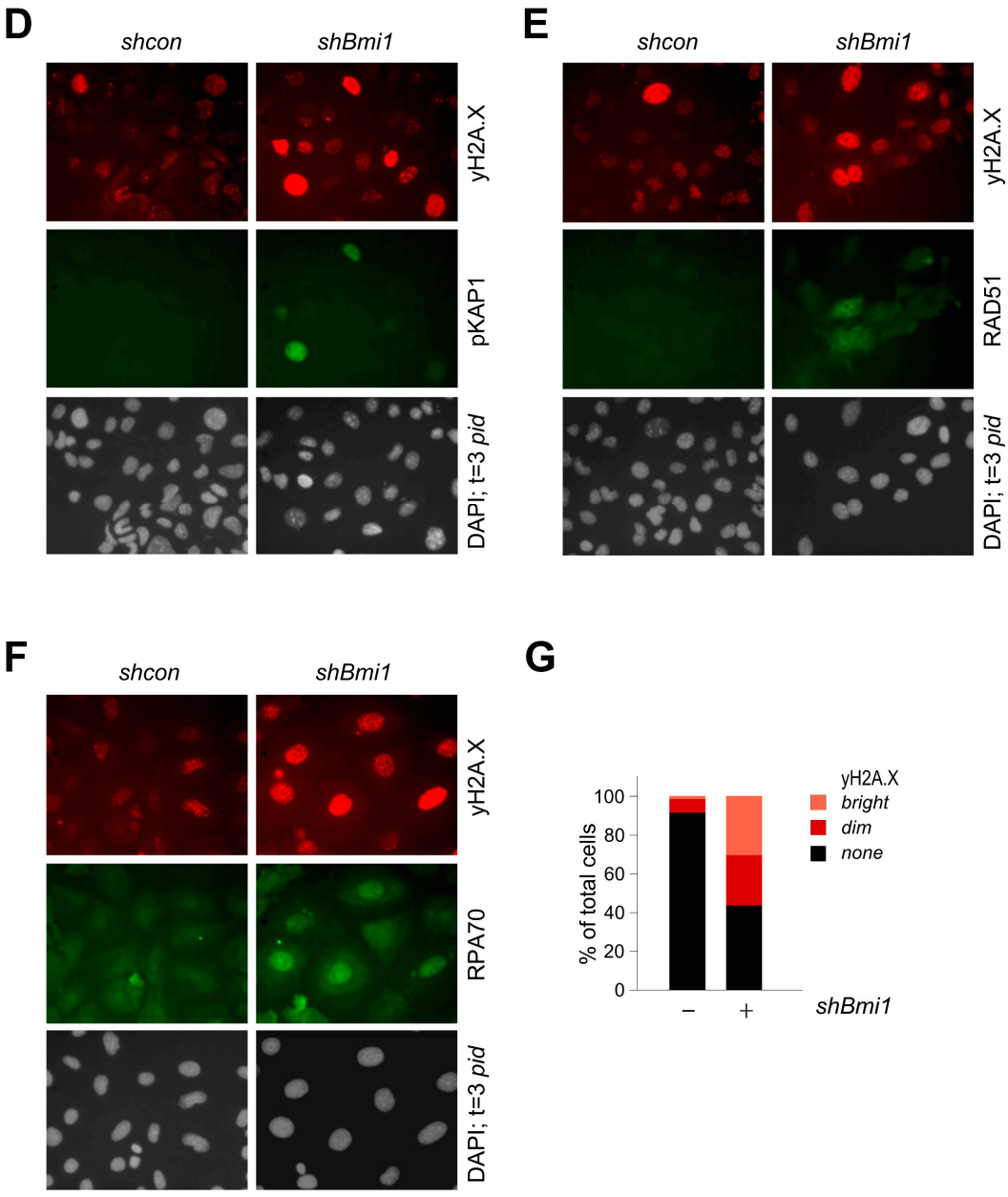
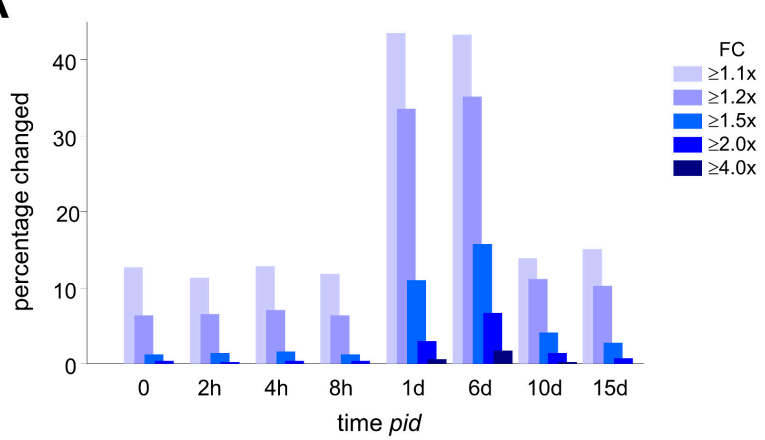


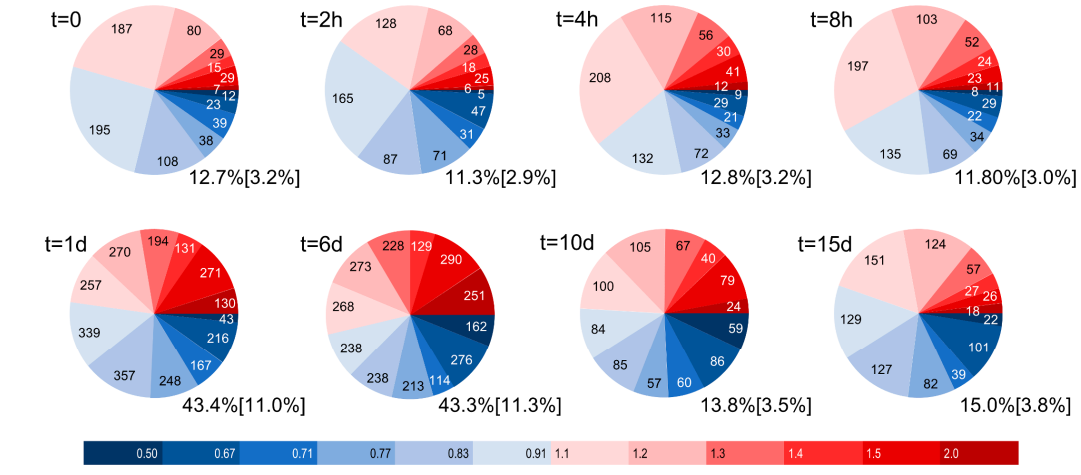
Figure S3. Increased DDR during TA in the absence of PRC1. (**A**) Representative IF images showing basal, low levels of replication-associated DNA damage in non-differentiating *shcon* and *shBmi1* ATDC5 cells; insets are overexposed to visualize γH2A.X foci. (**B**) Representative IF images showing co-staining active DNA synthesis (BrdU) and enhanced DDR (γH2A.X) in ATDC5 *shcon* and *shBmi1* cells at t=1 day *pid*. (**C**) IF analysis of γH2A.X and H3S10ph; arrowheads indicate examples of large, γH2A.X-bright/H3S10ph-dim nuclei in representative image. Of note: approximately 50% of shBmi1 cells were γH2A.X-positive; 4% of *shBMi1* cells were double bright, late S/G2-phase cells; all double bright *shcon* cells (7% of total cells) were late G2/M-phase cells, during which H2A.X is also phosphorylated. Representative co-staining images for (**D**) γH2A.X and phospho-KAP1, (**E**) γH2A.X and RAD51, and (**F**) γH2A.X and RPA70 in *shBmi1* (*vs shcon*) cultures at t=3 days *pid*. Less than 2% of *shcon* cells were positive for any of the damage markers. (**G**) Quantification of γH2A.X staining in human HAC cells (*cf.* Figure 3D).

A



t	n	1.1	1.2	1.5	2.0	4.0
0h	6000	762	380	71	19	0
2h	5991	679	386	83	11	0
4h	5931	758	418	91	21	0
8h	5986	707	375	71	19	0
1d	6044	2623	2027	660	173	31
6d	6194	2680	2174	979	413	104
10d	5985	826	662	248	83	12
15d	6027	903	623	167	40	5

B



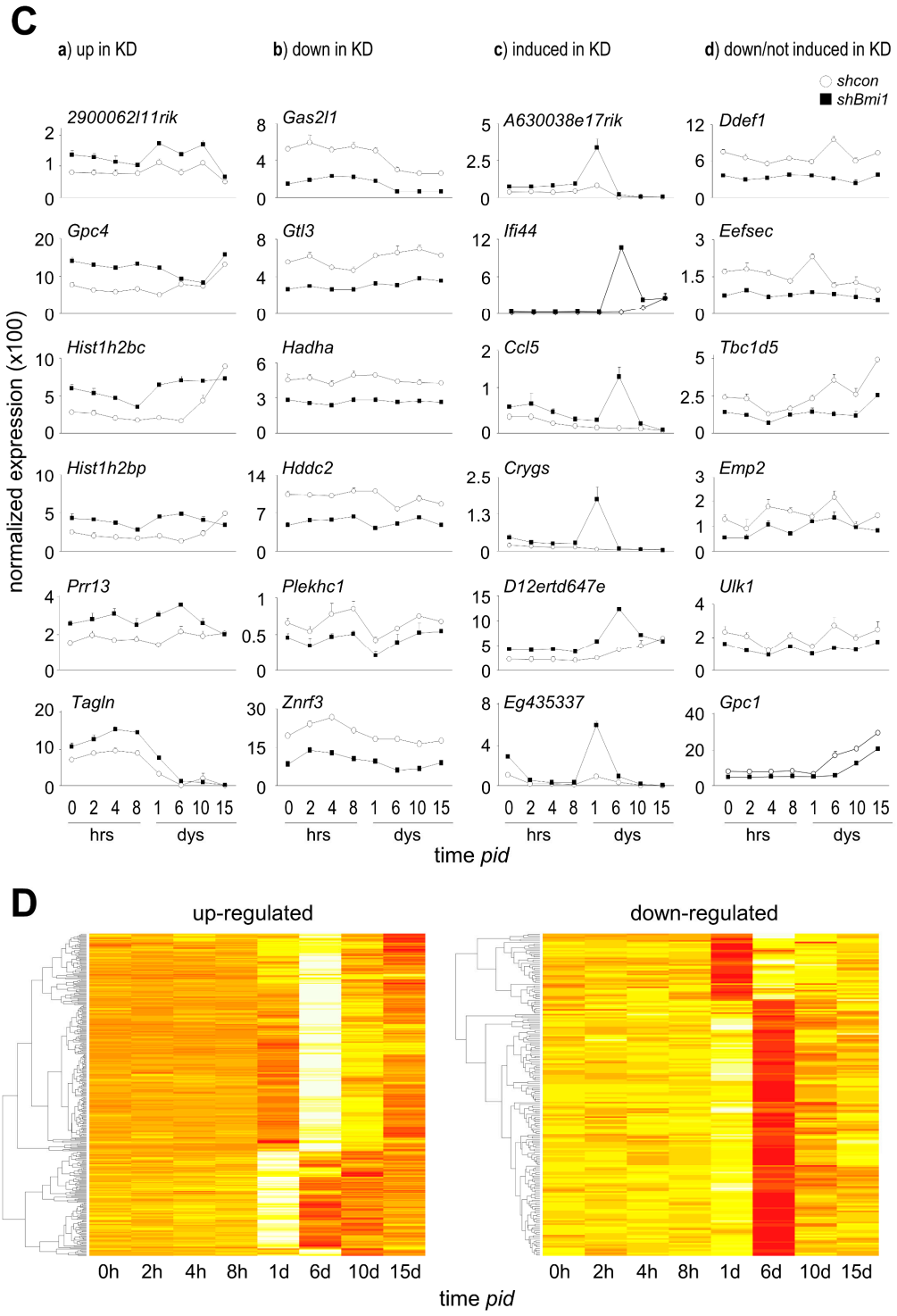
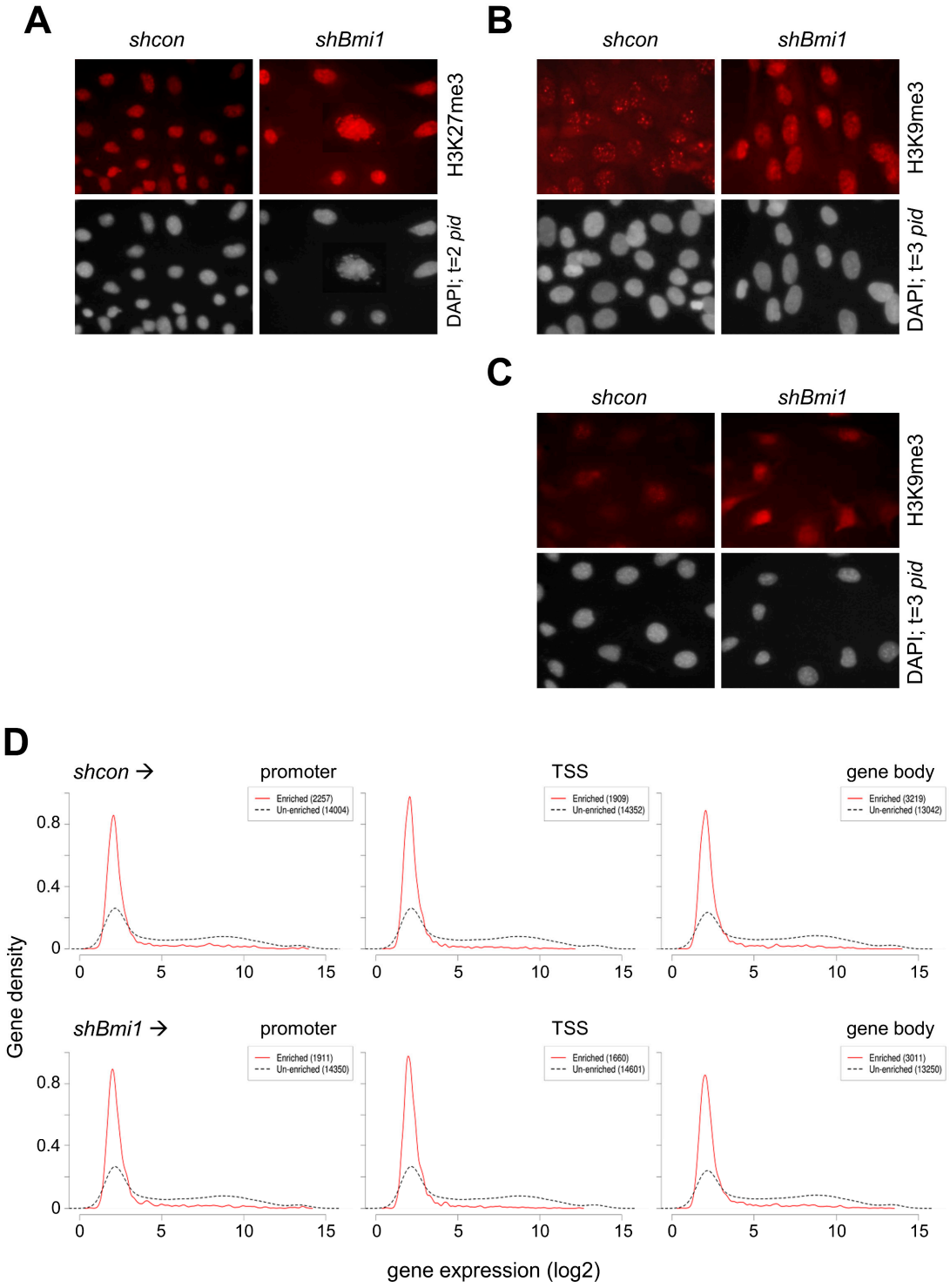
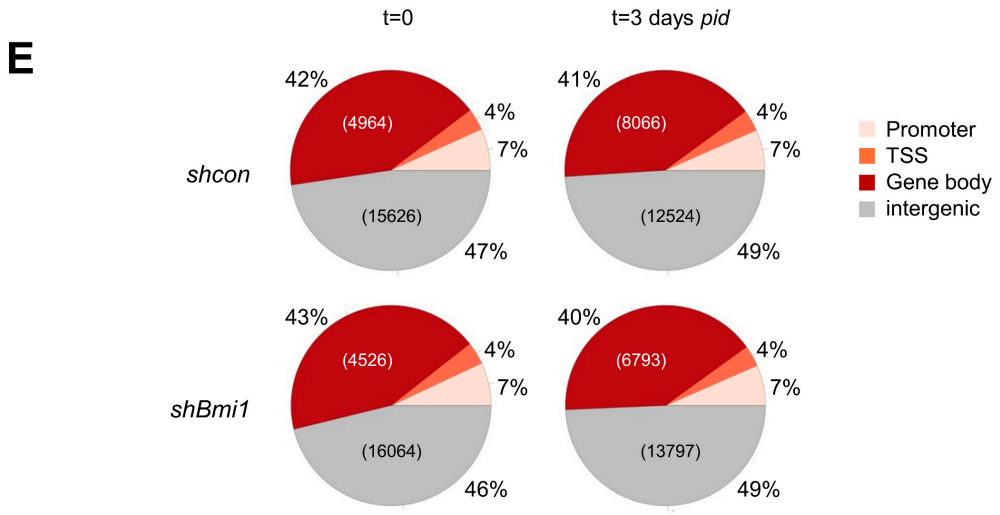
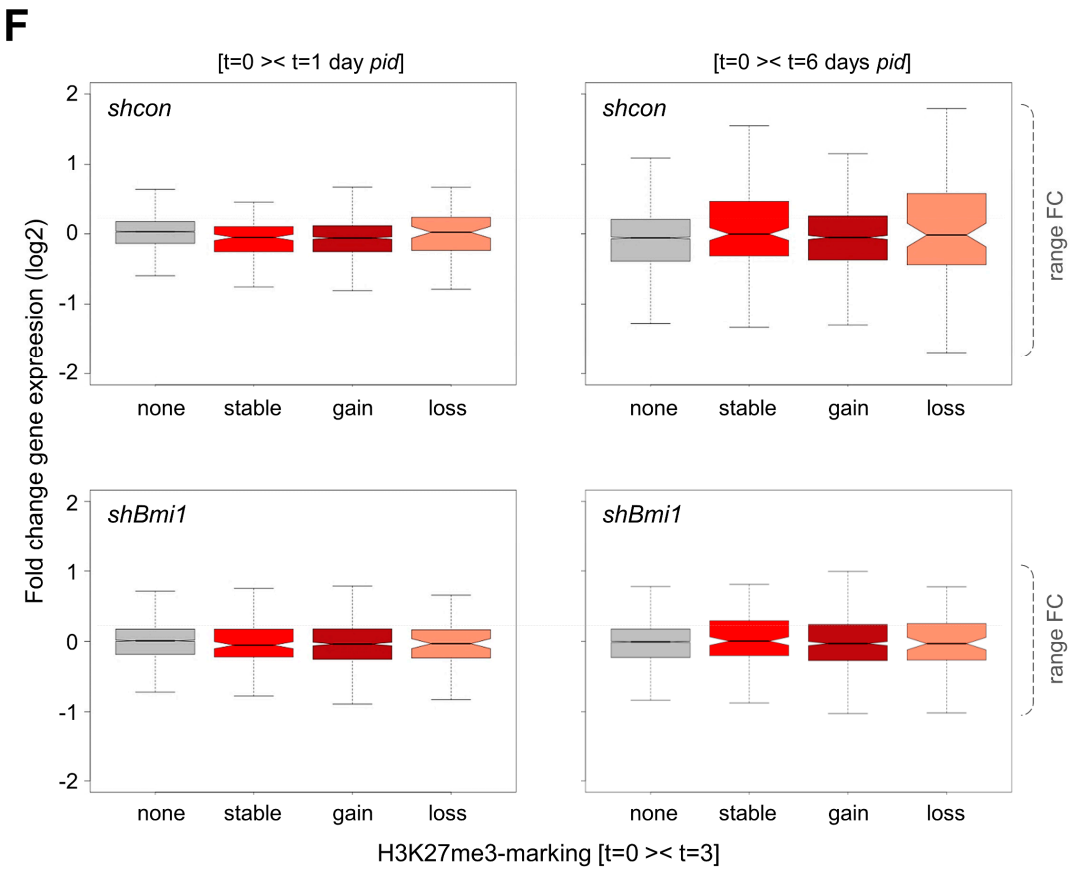


Figure S4. Abnormal transcriptional responses in PRC1-deficient cells. (**A**) Fold change (FC) increases during the differentiation-associated proliferative amplification. Table (right panel) indicates for each time point *t* (hours (h) or days (d) *pid*) the number *n* of reporters reaching the expression limit of ^{2}log(100) in either group (*shcon* or *shBmi1*); additional columns: the number fulfilling significance ($p<0.05$), fold change (FC) ≥1.1, 1.2, 1.5, 2.0 and ≥4.0x (up or down) and expression above limit; bars (left) represent the relative values in columns. (**B**) Distribution of fold change (FC): pie charts of all reporters fulfilling ≥1.1 FC (up (red) or down (blue); expression >^{2}log(100), $p<0.05$) in *shcon vs. shBmi1* cultures for all time-points *pid*; numbers in pie sections correspond to gene numbers; *%[%]*: percentage of reporters fulfilling all criteria relative to reporters above expression limit [percentage of reporters fulfilling all criteria relative to the total number of reporters]. (**C**) Profile clustering

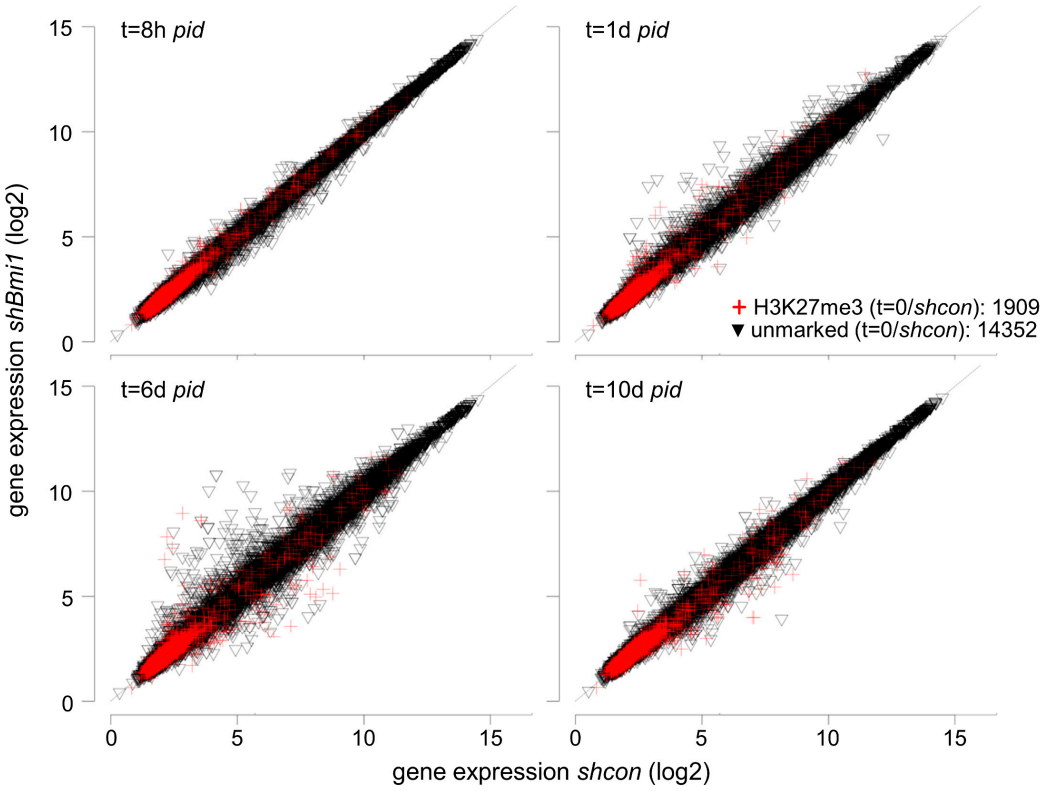
analysis of the most prominently de-regulated genes identified 4 distinct clusters: a) overall higher (*up in KD*) or, b) lower (*down in KD*) in BMI1-KD cells, c) not regulated during chondrogenesis in control cells, but dramatically deregulated in BMI1-KD cultures (*induced in KD*) during differentiation-associated proliferation, and d) induced at hyperproliferation in control but not in BMI1-KD cells (*down/not induced in KD*). Representative genes are shown for each cluster; values in all panels: mean of triplicates ± S.D. (**D**) Heatmap of the log-ratios of all reporters fulfilling $p<0.05$, FC>2 (left) or FC<0.5 (right), expression >2log(100) in either group, at t=1 or 6 days *pid*. Euclidean distance and complete linkage hierarchical clustering were used to cluster and reorder the reporters.




E
t=0
t=3 days *pid*
shcon
shBmi1
42%
(4964)
4%
7%
(15626)
47%
41%
(8066)
4%
7%
(12524)
49%
43%
(4526)
4%
7%
(16064)
46%
40%
(6793)
4%
7%
(13797)
49%
Promoter
TSS
Gene body
intergenic


F
[t=0 >< t=1 day *pid*]
[t=0 >< t=6 days *pid*]
shcon
shBmi1
Fold change gene expreesion (log2)
2
1
0
-1
-2
none
stable
gain
loss
range FC
H3K27me3-marking [t=0 >< t=3]

G


t=8h *pid*
t=1d *pid*
t=6d *pid*
t=10d *pid*
+ H3K27me3 (t=0/*shcon*): 1909
▼ unmarked (t=0/*shcon*): 14352
gene expression *shBmi1* (log2)
gene expression *shcon* (log2)
0
5
10
15

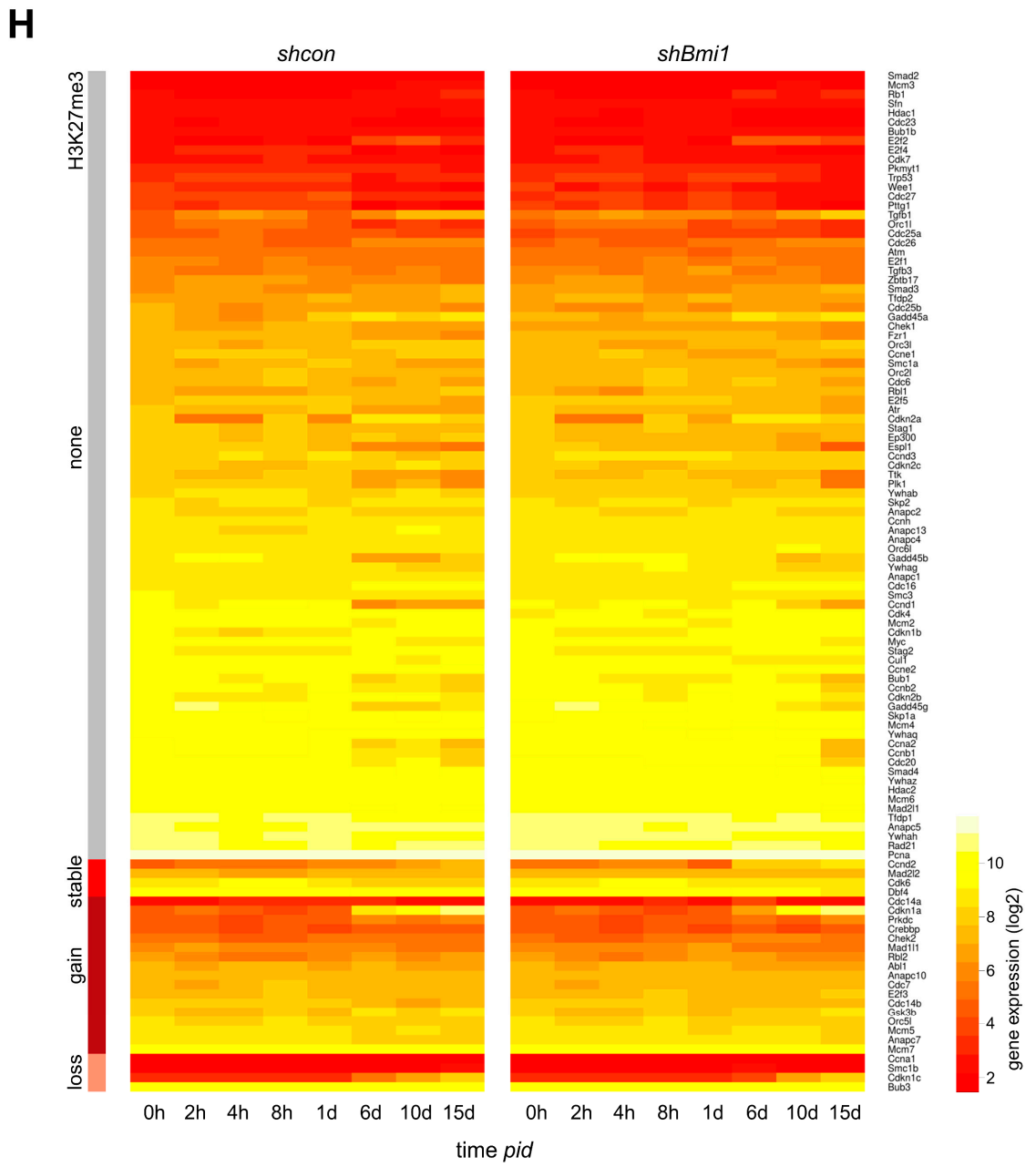


Figure S5. Gene expression changes independently of altered H3K27me3-occupation. Comparative analysis in representative IF images of nuclear (**A**) H3K27me3, (**B**) H3K9me3 in *shcon* and *shBmi1* ATDC5 cells and of (**C**) H3K9me3 in *shBmi1* HAC cells, at t=3 days *pid*. (**D**) Pre-chondrogenic gene expression status depends solely on H3K27me3-enrichment status, independent of *shcon*/*shBmi1* status or specific genomic location. Density plots of gene expression of all genes (16261) at t=0, for H3K27me3-enriched (solid red lines) and H3K27me3 unmarked genes (dashed black lines). H3K27me3-enrichment of promoter (-3000/-100 base pairs (bp) relative to the TSS; left panels), TSS (-100/+1000 bp; middle panels) and gene body (+1000 bp to end of last exon; right panels) regions are considered separately. Top graphs: *shcon*, bottom graphs: *shBmi1*. (**E**) Comparison of distribution of H3K27me3-occupation between *shcon* and *shBmi1* cultures at any time-point *pid*, in genic and intergenic regions. Genic regions were divided into three regions (*cf.* D): the promoter, the TSS region and the gene body region. The total enrichment for these regions was summarized for all genes and compared to the total enrichment in non-genic regions; numbers in pie sections correspond to gene numbers displaying region-specific enrichment. (**F**) Gene expression boxplots of H3K27me3-enriched and unmarked genes in *shcon* and *shBmi1* cells, each comparing two time-points: t=0 *vs* 1 day *pid* (left

panels; *[t=0 >< t=1 day pid]*), and t=0 *vs* 6 days *pid* (right panels; *[t=0 >< t=6 days pid]*). H3K27me3-marking status was consistently determined between t=0 and 3 days *pid ([t=0 >< t=3])* per condition (*i.e. shcon, shBmi1*). Definition epigenic categories: unmarked (*none*), stably marked (*stable*) at both t=0 and 3 days *pid*, or loci that acquired (*gain*) or lost (*loss*) H3K27me3-marks (t=0 *vs* t=3 days *pid*). (see Methods section for further definition of the marker set H3K27me3). Notches in box-plots indicate confidence intervals (5-95%) of the median; non-overlapping notches are an indication of significant differences ($p<0.05$). (**G**) Matrix display of scatter-plots of log2 gene expression values at indicated time points between *shcon* and *shBmi1* cells, shows deregulation of transcription between t=0 and 10 days *pid*. Symbols: red crosses represent H3K27me3-decorated loci (*H3K27me3*; n=1909), black triangles H3K27me3-free loci (*unmarked*; n=14352) at t=0 in *shcon* cultures (*t=0*/*shcon*). Analyses was based on reporters with expression >log2(100). (**H**) Heatmap of representative marker expression for '*Cell cycle*' (*cf* Table S4) for *shcon* (left panel) and *shBmi1* (right panel) ATDC5 cells. H3K27me3-enrichment status was defined based on ChIP-seq data comparison between t=0 and 3 days *pid* in the *shcon* experiment: unmarked (*none*), stably marked (*stable*) or loci that had acquired (*gain*) or lost (*loss*) H3K27me3-marking (see Methods section for further definition of the marker set H3K27me3).

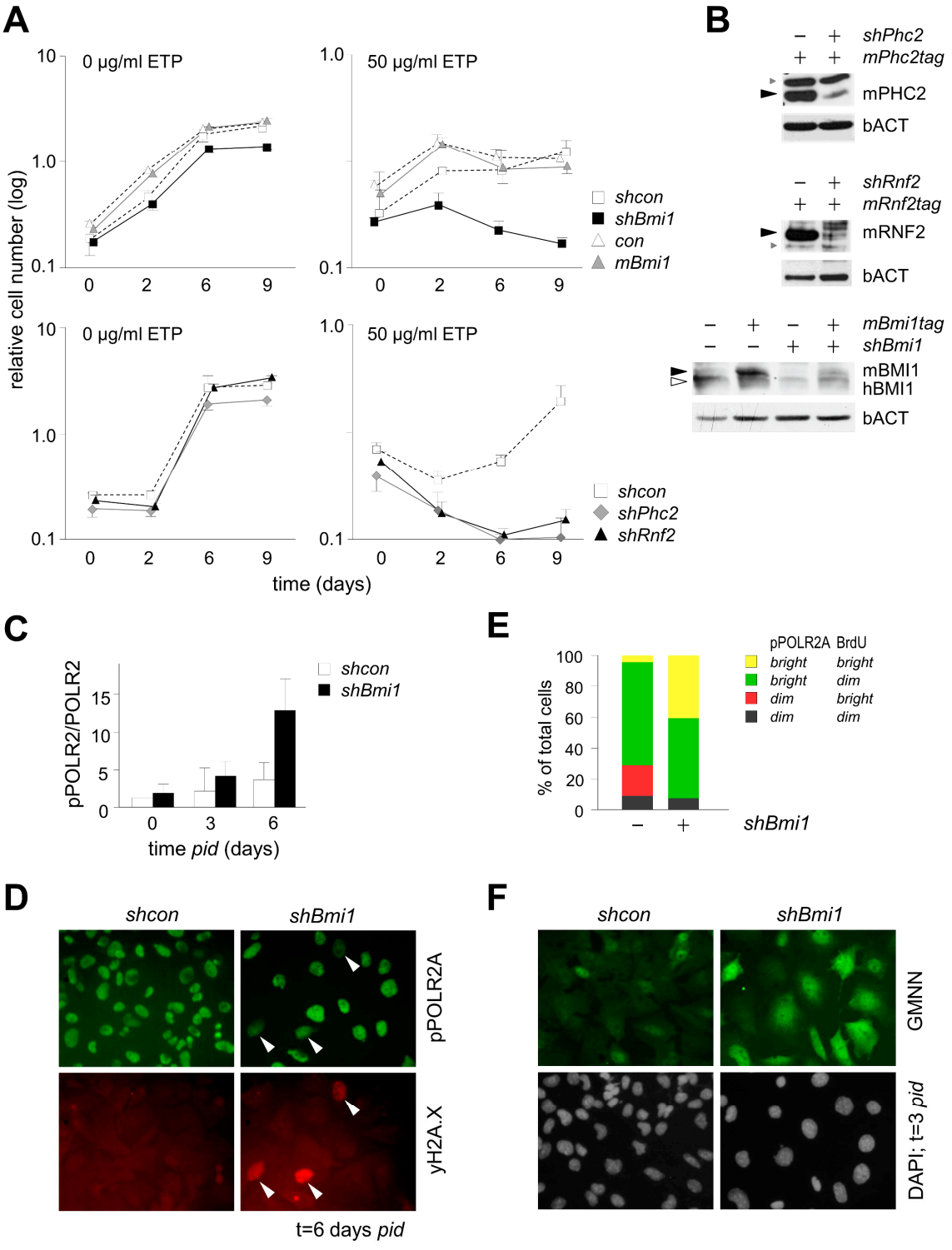
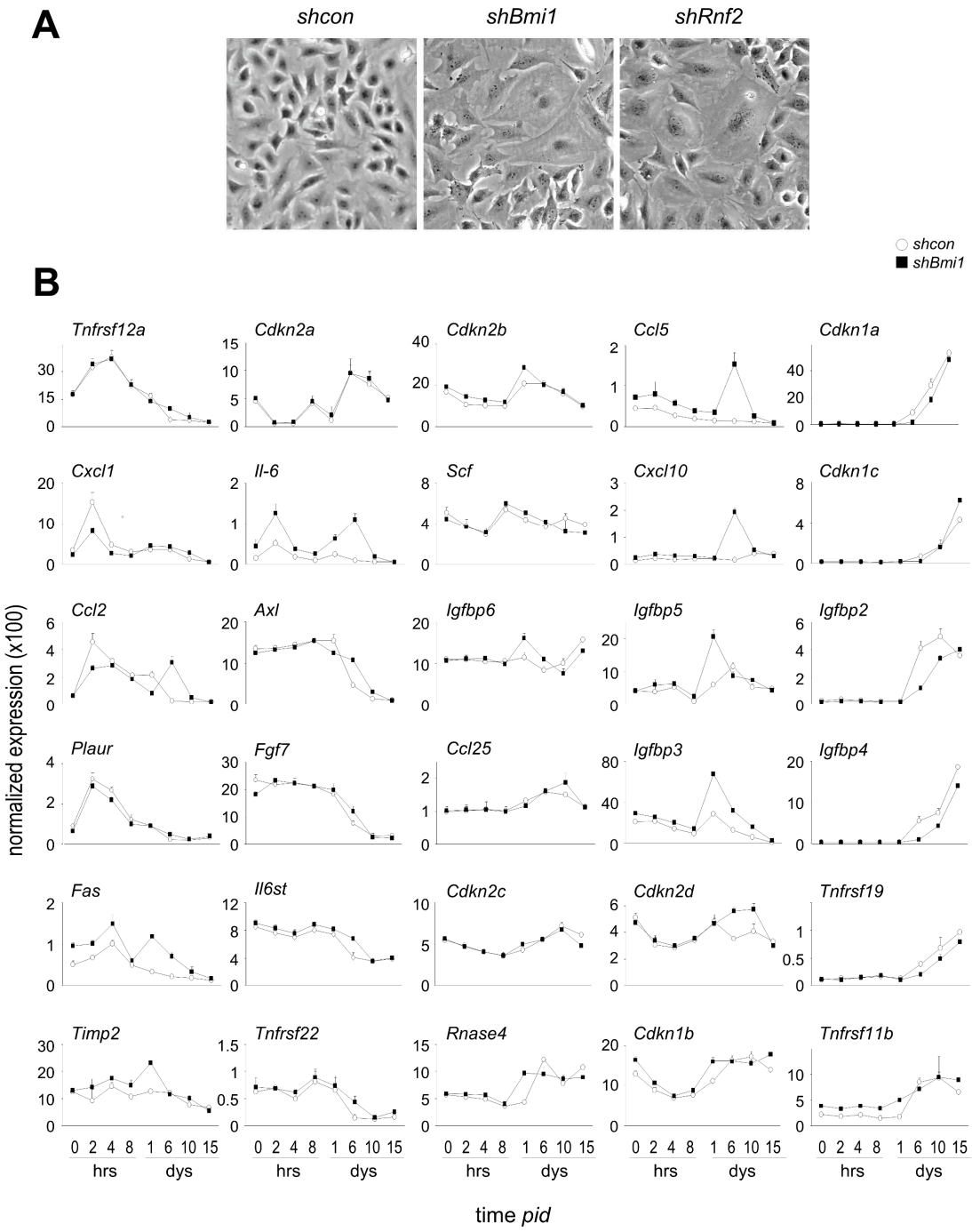


Figure S6. Deregulated nuclear TOP2A and pPOLR2A in the absence of BMI1. (**A**) Sensitivity proliferation assays showing the sensitivity of gain or loss-of-PRC1 function ATDC5 cells to the TOP2A poison etoposide (ETP): effect of loss (*shBmi1*) or gain (*mBmi1*) of BMI1 (top right panel) and (bottom right panel; left panels no ETP controls) of loss of PHC2 or RNF2 on cell proliferation in the presence of 50 µg/ml ETP. (**B**) Immunoblot (IB) analysis of RNAi-mediated knock-down efficiency for indicated PRC1 proteins (*i.e.* BMI1, PHC2, RNF2): shRNAi vectors targeting the indicated murine PRC1 proteins were tested using tagged murine cDNA constructs (*mBmi1-2PY*, *HA-mPhc2*, *HA-mRnf2*) expressed in human U2-OS cells; large black arrowheads indicate expected murine PRC1 protein sizes (including tag); BMI1-panel: open arrowheads correspond to the size of human BMI1;

PHC2 and RNF2-panels: small grey arrowheads indicate background bands. (**C**) Quantification of pPOLR2A:tPOLR2A ratios at t=0, 3, and 6 days *pid;* data corresponding to Figure 5C. All proteins levels were normalised to aTUB levels. (**D**) Representative IF images of nuclear co-staining for pPOLR2A and yH2A.X in *shcon* and *shBmi1* ATDC5 cells (arrowheads: examples of yH2A.X-bright/pPOLR2A-dim nuclei; less than 2 percent of *shcon* cells were positive for yH2A.X. (**E**) Quantification of IF staining for pPOLR2A and BrdU; data corresponding to Figure 5D. (**F**) Representative IF images of nuclear staining for GMNN in *shcon* and *shBmi1* ATDC5 cells; less than 5% and more than 90% of *shcon* and *shBmi1* cells, respectively, were positive for GMNN.


A
shcon
shBmi1
shRnf2
shcon
shBmi1
B
Tnfrsf12a
Cdkn2a
Cdkn2b
Ccl5
Cdkn1a
Cxcl1
Il-6
Scf
Cxcl10
Cdkn1c
Ccl2
Axl
Igfbp6
Igfbp5
Igfbp2
Plaur
Fgf7
Ccl25
Igfbp3
Igfbp4
Fas
Il6st
Cdkn2c
Cdkn2d
Tnfrsf19
Timp2
Tnfrsf22
Rnase4
Cdkn1b
Tnfrsf11b
normalized expression (x100)
0 2 4 8 1 6 10 15
hrs dys
time pid

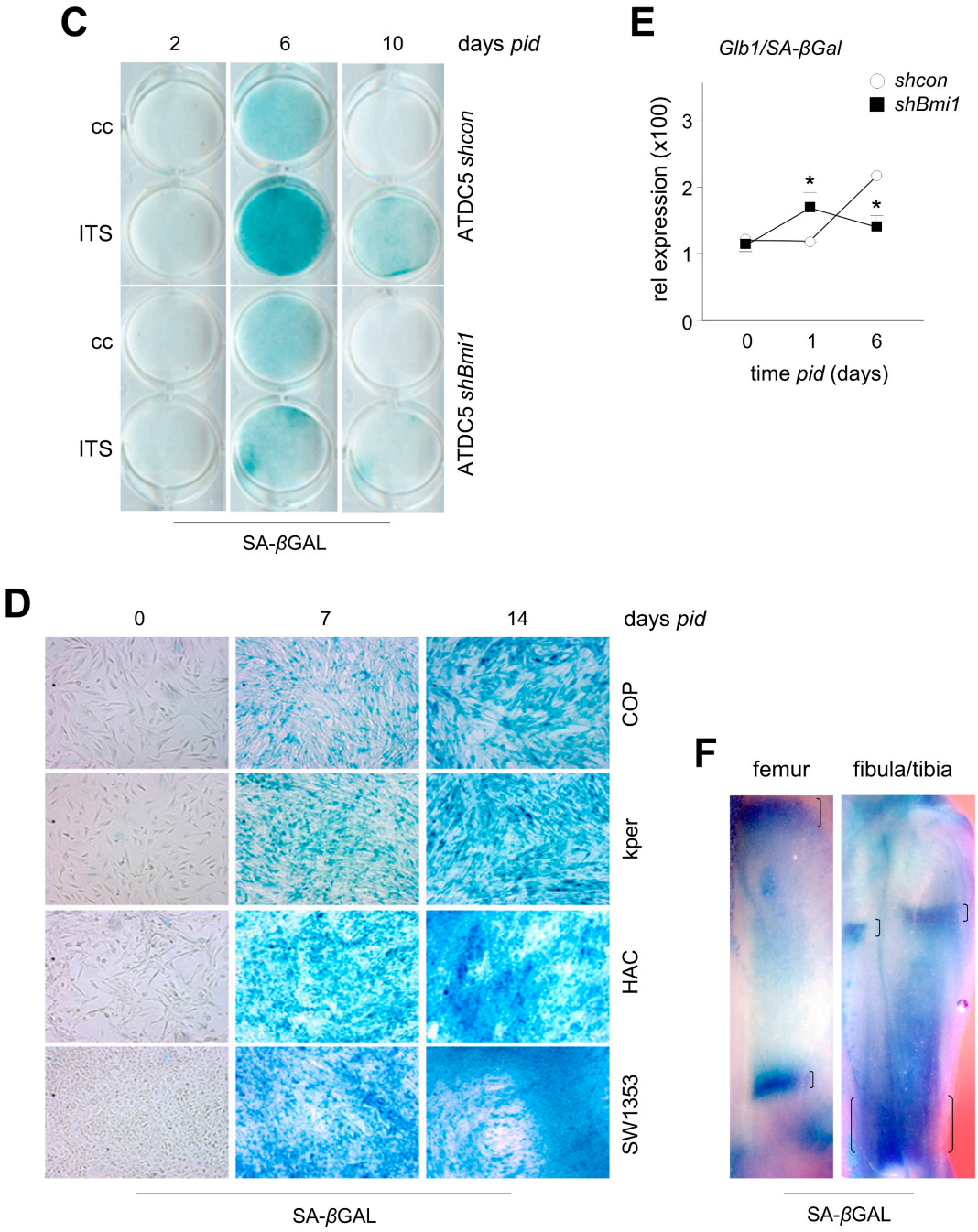


Figure S7. Normal and abnormal senescence signalling in chondrogenesis. (**A**) Phase contrast images of *shcon*, *shBmi1* or *shRnf2* human U2-OS cells. (**B**) Global expression analysis of senescence-associated genes in ATDC5 control and BMI1-KD (*shcon*; *shBmi1*) cultures during differentiation. Microarrays; values in all panels represent mean of triplicates ± S.D. (**C**) Induction of senescence-associated β-Galactosidase (SA-βGAL/GLB1) activity in ATDC5 cultures under differentiating (*ITS*) *versus* non-differentiating conditions (*cc*). (**D**) Representative microscopic images (one of three repeat experiments) of SA-βGAL activation in differentiating rabbit knee cartilage-derived chondrogenic cells (COP), rabbit periost-derived chondrogenic progenitors (kper), human HAC and SW1353 (chondrosarcoma) cells. (**E**) microarray-based analysis of *Glb1*-expression in control *versus*. BMI1-KD cultures. Asterisks (*): $p<0.05$. (**F**) SA-βGAL activity in localizes to growth plates of mouse femur and fibula/tibia; brackets mark PZ/HZ zones.

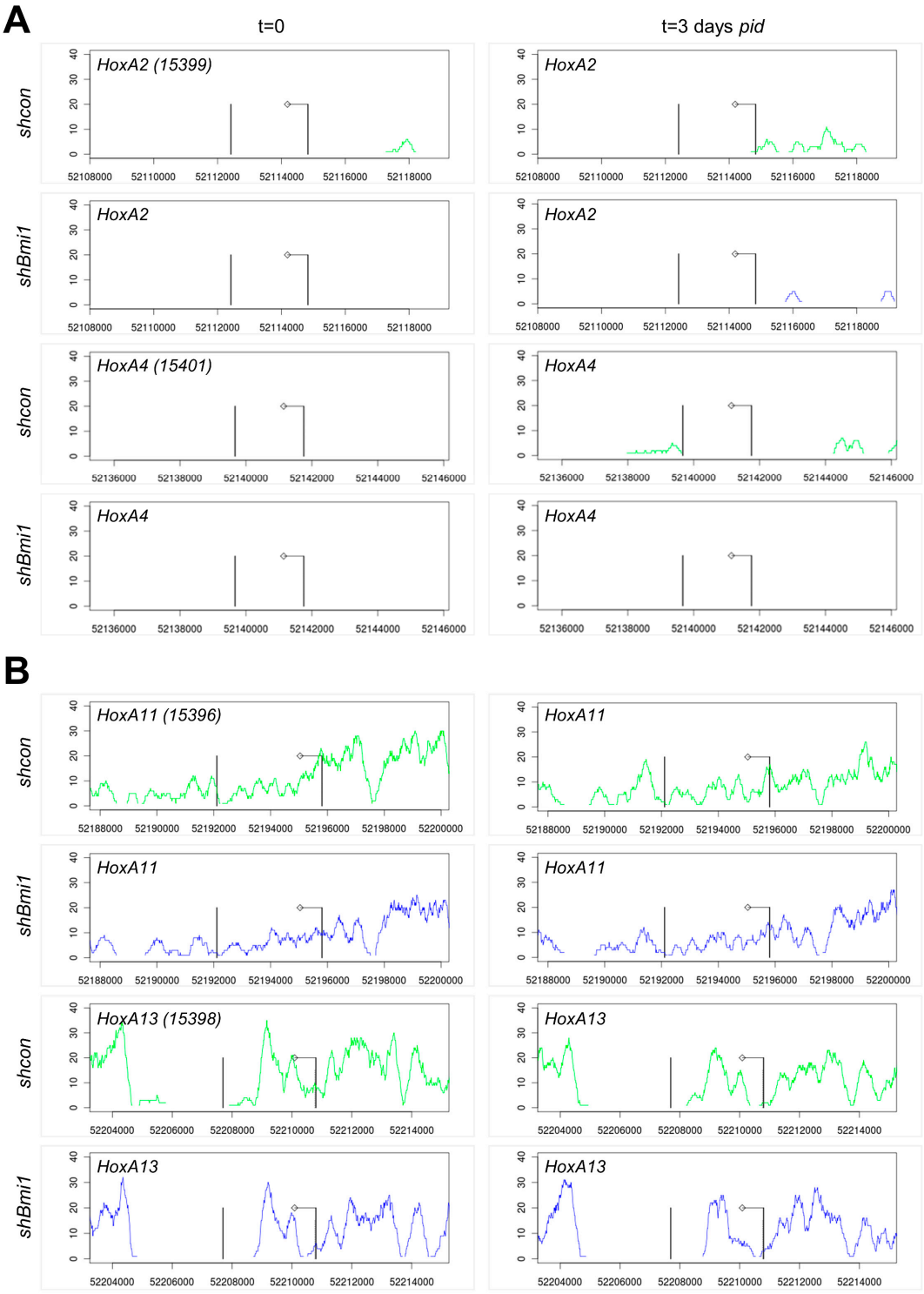


Figure S8. Genome tracks for the (**A**) non-PRC1 target loci *HoxA2*, *HoxA4* and (**B**) PRC1-target genes *HoxA11* and *HoxA13* loci. The number between brackets is the corresponding Entrez gene ID. In each panel the H3K27me3 enrichment is visualized as peaks at time point t=0 (maintenance conditions; left panels) or t=3 days *pid* (differentiation conditions; right panels). The top tracks for each locus shows data of the *shcon* samples (green); the bottom tracks of the *shBmi1* samples (blue). The solid line with diamond arrow represents the transcription start site (TSS); the second solid line represents the end of the last exonic region of the gene (3'-prime of coding region). All coordinates are given with respect to the forward strand.

Table S1. Activation of DNA repair pathways in *shBmi1* cultures. Upregulation of DNA damage response/repair genes at t=6 days *pid* presented as log fold change; based on GenMAPP analysis.

Gene	FC	Description	Processes involved in (GENmapp terms)
Ifi204	4.9	Interferon activated gene 204	Tracri Pol II transport DDR Diff
Rad54b	4.1	RAD54B homolog	Dre DDR
Brip1	3.5	BRCA1 interact protein C terminal helicase1	Nucmet Dre tracri PolII DDR
Fancb	2.5	Fanconi anemia complement group B	Dre DDR
Rad9	2.4	RAD9 homolog (S.pombe)	CC checkpoint DDC Dre DDR RadR apop
Pttg1	2.3	Pituitary tumor transforming 1	Dmet Dre DR CC Cseg mit biog
Rad51	2.2	RAD51 homolog	homR Dre Dmet REP DDR mei meiR
Hspa1b	2.2	Heatshock protein 1B	TELm Dre fold anti-apop UPR hs
Xrcc2	2.1	XRay repair complementing defective repair	Dmet Dre Drec DDR
Rad51ap1	2.1	RAD51 associated protein1	homR Dre Drec DDR
Ddb2	2.0	Damage specific DNA binding protein 2	Dre pyrimidinedimerrepair DDR
Exo1	1.9	Exonuclease 1	Nucmet Dre NER MMR Drec DDR mei
Blm	1.9	Bloom syndrome homolog (human)	REP Dre Drec
Eme1	1.8	Essential meiotic endonuclease 1 homolog 1	Dre Drec DDR
Chaf1a	1.8	Chromatin assembly factor 1 subunit A(p150)	REP Dre tracri fold DDR CC
Sgk	1.8	serum/ glucocorticoid regulated kinase	Kin apop DDR
Hspa1a	1.8	Heatshock protein 1A	TELm Dre fold UPR hs
Brca2	1.8	Breast cancer 2	homR Dre chrom DDR S-CC mitC tracri
Rad51l1	1.8	RAD51 like 1	Dmet Dre Drec DDR
Cdc2a	1.7	cell div cycle 2 homolog A	Kin anti-apop mit CC G2 Cdiv
Trex1	1.7	Three prime repair exonuclease 1	REP Dre MMR Drec DDR
Fen1	1.7	Flap structure specific endonuclease 1	REP DNA repair
Msh3	1.6	mutS homolog 3	Dmet Dre MMR DDR somH somR
Gtf2h1	1.6	General tracri Factor II H polypept 1	Dre tracri DDR
Clspn	1.6	Claspin homolog	DRC Dre DDR CC
Rad50	1.5	RAD50 homolog	Dmet Dre DDR CC mei
Mapk1	1.5	Mitogen activated protein kinase 1	Kin DDR CC ST morf kin cytosine met diff
Topors	1.4	Topoisomerase I binding arginine/serine rich	Ubc apop DDR met prol tracri trapo
Lig1	1.4	Ligase I.DNA.ATP dependent	REP Dre Drec DDR CC div
Gadd45a	1.4	Growth arrest & DNA damage induc 45 α	CCprog Pase DDR CC CC arrest
Rfc5	1.4	Replication factor C (activator1)5	REP DNA repair
Eef1e1	1.4	Eukaryotic tracri. elongation factor 1 ε1	CC Dre trala embr apop DDR
Gtf2h2	1.4	General tracri.factor IIH polypeptide 2	Dre tracri DDR
Xab2	1.3	XPA binding protein 2	Blast Dre TCR tracri R proc
Uvrag	1.3	UV radiation resistance associated gene	Dre
Fanca	1.3	Fancon ianemia complementation group A	Dre DDR male mei Mgon Fgon prol
Bre	1.3	Brain & reprod organ-expressed protein	Ubc apop DDR anti-apop
Pold1	1.3	Polymerase (DNA directed) δ1 cat subunit	S-CC Dre REP BER
Topbp1	1.3	Topoisomerase (DNA) 2b binding protein	Dre DDR meiR
Msh5	1.3	mutS homolog 5	Dmet MMR mei Meil syn Fgam
Hmgb2	1.3	High mobility group box 2	REP Dre BER chrom nucl tracri Pol II
Parp2	1.3	Poly(ADP) ribose polymerase fam memb 2	Dre BER ribos
Smc3	1.2	Structural maintenance of chromosome 3	Dmet DRE DDR CC spin Cseg SCC mit mei STbiog div
Smc5	1.2	Structural maintenance of chromosome 5	Dmet DRE Drec DDR

Table S2. Abnormal transcriptional responses in BMI1-deficient cells. Overrepresented biological pathways based on PathVisio analysis using criteria: p<0.05 *shBmi1 versus shcon*, >2 (upper) or >1.2 FC (bottom) up or down in *shBmi1 versus shcon* cultures, and average group expression ^{2}log(100) in either group at t=1 or 6 days *pid*, as compared to all reporters on the array for which the Affymetrix ID (or Unigene ID) could be mapped to a pathway; for each pathway: (r) number of genes fulfilling criteria, (n) number of genes present in data set.

Pathways all (FC > 2)	**(r)**	**(n)**	**Total**	**%**	**Z Score**
Irinotecan pathway	5	10	13	50	7.59
Endochondral ossification	9	56	68	16.1	4.79
Oxidative stress	5	23	29	21.7	4.47
Cytokines and inflammatory response	4	21	25	19.1	3.63
TGF beta signaling pathway	6	41	52	14.6	3.61
Selenium metabolism/selenoproteins	6	43	49	14	3.46
Osteoblast	2	7	11	28.6	3.4
Prostaglandin synthesis and regulation	4	24	31	16.7	3.27
Adipogenesis	10	108	132	9.3	2.97
Osteoclast	2	13	18	15.4	2.16
Complement activation.classical pathway	2	14	16	14.3	2.03
Pathways down (FC > 2)	**(r)**	**(n)**	**total**	**%**	**Z Score**
Irinotecan pathway	4	10	13	40.0	10.28
Selenium metabolism/selenoproteins	5	43	49	11.6	5.67
Endochondral ossification	5	56	68	8.9	4.77
Oxidative stress	2	23	29	8.7	2.94
Osteoblast	1	7	11	14.3	2.86
GPCRs,class B secretin-like	1	10	13	10.0	2.28
Osteoclast	1	13	18	7.7	1.9
TGF-beta receptor signaling pathway	4	115	149	3.5	1.89
Pathways up (FC > 2)	**(r)**	**(n)**	**Total**	**%**	**Z Score**
Adipogenesis	10	108	132	9.3	4.48
Prostaglandin synthesis and regulation	4	24	31	16.7	4.38
TGFbeta signaling pathway	5	41	52	12.2	3.92
Cytokines and inflammatory response	3	21	25	14.3	3.4
Oxidative stress	3	23	29	13	3.19
Endochondral ossification	9	56	68	8.9	3.04
Notch signaling pathway	1	5	47	20	2.46
Osteoblast	1	7	11	14.3	1.96
Pathways all (FC > 1.2)	**(r)**	**(n)**	**Total**	**%**	**Z Score**
Cholesterol biosynthesis	10	13	15	76.9	4.66
Cell cycle	32	70	88	45.7	4.62
TGF-beta receptor signaling pathway	44	115	149	38.3	4.03
Androgen receptor signaling pathway	33	84	108	39.3	3.65
Irinotecan pathway	7	10	13	70.0	3.56
G1 to S cell cycle control	22	51	64	43.1	3.49
Endochondral ossification	23	56	68	41.1	3.29
TGF beta signaling pathway	18	41	52	43.9	3.24
mRNA processing	102	349	552	29.2	3.06
Selenium metabolism/selenoproteins	18	43	49	41.9	3.00
Apoptosis modulation by HSP70	9	17	18	52.9	2.97
TNF-alpha/NF-kb signaling pathway	47	143	177	32.9	2.94
DNA replication	15	36	41	41.7	2.71
Heme biosynthesis	5	8	9	62.5	2.68
One carbon metabolism	10	23	41	43.5	2.37
Eukaryotic transcription initiation	15	39	41	38.5	2.35
Mitochondrial LC-fatty acid beta-oxidation	6	13	16	46.5	2.01
Apoptosis mechanisms	24	74	86	32.4	2.00

Table S3: Markers in *'Endochondral ossification'* network (WikiPathways). Gene IDs correspond to NCBI and Ensembl gene identifiers (Ensemble Biomart (Mouse genes (GRCm38.p5) database). (www.wikipathways.org/index.php/Pathway:WP474).

Gene	ID (NCBI)	ID (Ensembl)	Description
Acan	11595	ENSMUSG00000030607	aggrecan
Adamts1	11504	ENSMUSG00000022893	a disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 1
Adamts4	240913	ENSMUSG00000006403	a disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 4
Adamts5	23794	ENSMUSG00000022894	a disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 5
Akt1	11651	ENSMUSG00000001729	thymoma viral proto-oncogene 1
Alpl	11647	ENSMUSG00000028766	alkaline phosphatase, liver/bone/kidney
Bmp6	12161	ENSMUSG00000039004	bone morphogenetic protein 6
Bmp7	12162	ENSMUSG00000008999	bone morphogenetic protein 7
Bmpr1a	12166	ENSMUSG00000021796	bone morphogenetic protein receptor, type 1A
Cab39	12283	ENSMUSG00000036707	calcium binding protein 39
Calm1	12313	ENSMUSG00000001175	calmodulin 1
Cdkn1c	12577	ENSMUSG00000037664	cyclin-dependent kinase inhibitor 1C (P57)
Chst11	58250	ENSMUSG00000034612	carbohydrate sulfotransferase 11
Col10a1	12813	ENSMUSG00000039462	collagen, type X, alpha 1
Col2a1	12824	ENSMUSG00000022483	collagen, type II, alpha 1
Cst10	58214	ENSMUSG00000033156	cystatin 10 (chondrocytes)
Ctsl	13039	ENSMUSG00000021477	cathepsin L
Ddr2	18214	ENSMUSG00000026674	discoidin domain receptor family, member 2
Enpp1	18605	ENSMUSG00000037370	ectonucleotide pyrophosphatase/phosphodiesterase 1
Fgf18	14172	ENSMUSG00000057967	fibroblast growth factor 18
Fgf2	14173	ENSMUSG00000037225	fibroblast growth factor 2
Fgfr1	14182	ENSMUSG00000031565	fibroblast growth factor receptor 1
Fgfr3	14184	ENSMUSG00000054252	fibroblast growth factor receptor 3
Frzb	20378	ENSMUSG00000027004	frizzled-related protein
Ghr	14600	ENSMUSG00000055737	growth hormone receptor
Gli3	14634	ENSMUSG00000021318	GLI-Kruppel family member GLI3
Hdac4	208727	ENSMUSG00000026313	histone deacetylase 4
Hmgcs1	208715	ENSMUSG00000093930	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1
Ift88	21821	ENSMUSG00000040040	intraflagellar transport 88
Igf1	16000	ENSMUSG00000020053	insulin-like growth factor 1
Igf1r	16001	ENSMUSG00000005533	insulin-like growth factor I receptor
Igf2	16002	ENSMUSG00000048583	insulin-like growth factor 2
Ihh	16147	ENSMUSG00000006538	Indian hedgehog
Kif3a	16568	ENSMUSG00000018395	kinesin family member 3A
Mef2c	17260	ENSMUSG00000005583	myocyte enhancer factor 2C
Mgp	17313	ENSMUSG00000030218	matrix Gla protein
Mmp13	17386	ENSMUSG00000050578	matrix metallopeptidase 13
Mmp9	17395	ENSMUSG00000017737	matrix metallopeptidase 9
Nkx3-2	12020	ENSMUSG00000049691	NK3 homeobox 2
Plat	18791	ENSMUSG00000031538	plasminogen activator, tissue
Plau	18792	ENSMUSG00000021822	plasminogen activator, urokinase
Prkaca	18747	ENSMUSG00000005469	protein kinase, cAMP dependent, catalytic, alpha
Ptch1	19206	ENSMUSG00000021466	patched 1
Pth	19226	ENSMUSG00000059077	parathyroid hormone
Pth1r	19228	ENSMUSG00000032492	parathyroid hormone 1 receptor
Pthlh	19227	ENSMUSG00000048776	parathyroid hormone-like peptide
Runx2	12393	ENSMUSG00000039153	runt related transcription factor 2
Runx3	12399	ENSMUSG00000070691	runt related transcription factor 3
Scin	20259	ENSMUSG00000002565	scinderin

Table S3 cont.

Serpinh1	12406	ENSMUSG00000070436	serine (or cysteine) peptidase inhibitor, clade H, member 1
Slc38a2	67760	ENSMUSG00000022462	solute carrier family 38, member 2
Sox5	20678	ENSMUSG00000041540	SRY (sex determining region Y)-box 5
Sox6	20679	ENSMUSG00000051910	SRY (sex determining region Y)-box 6
Sox9	20682	ENSMUSG00000000567	SRY (sex determining region Y)-box 9
Spp1	20750	ENSMUSG00000029304	secreted phosphoprotein 1
Stat1	20846	ENSMUSG00000026104	signal transducer and activator of transcription 1
Stat5b	20851	ENSMUSG00000020919	signal transducer and activator of transcription 5B
Tgfb1	21803	ENSMUSG00000002603	transforming growth factor, beta 1
Tgfb2	21808	ENSMUSG00000039239	transforming growth factor, beta 2
Thra	21833	ENSMUSG00000058756	thyroid hormone receptor alpha
Timp3	21859	ENSMUSG00000020044	tissue inhibitor of metalloproteinase 3
Vegfa	22339	ENSMUSG00000023951	vascular endothelial growth factor A

Table S4: Markers in '*Cell cycle*' network (KEGG). Gene-IDs (ID) correspond to NCBI and Ensembl gene identifiers (Ensemble Biomart (Mouse genes (GRCm38.p5) database).(www.genome.jp/kegg-bin/show_pathway?mmu04110).

Gene	ID (NCBI)	ID (Ensemble)	Description
Abl1	11350	ENSMUSG00000026842	c-abl oncogene 1, non-receptor tyrosine kinase
Anapc1	17222	ENSMUSG00000014355	anaphase promoting complex subunit 1
Anapc10	68999	ENSMUSG00000036977	anaphase promoting complex subunit 10
Anapc11	66156	ENSMUSG00000025135	anaphase promoting complex subunit 11
Anapc13	69010	ENSMUSG00000035048	anaphase promoting complex subunit 13
Anapc2	99152	ENSMUSG00000026965	anaphase promoting complex subunit 2
Anapc4	52206	ENSMUSG00000029176	anaphase promoting complex subunit 4
Anapc5	59008	ENSMUSG00000029472	anaphase-promoting complex subunit 5
Anapc7	56317	ENSMUSG00000029466	anaphase promoting complex subunit 7
Atm	11920	ENSMUSG00000034218	ataxia telangiectasia mutated
Atr	245000	ENSMUSG00000032409	ataxia telangiectasia and Rad3 related
Bub1	12235	ENSMUSG00000027379	BUB1, mitotic checkpoint serine/threonine kinase
Bub1b	12236	ENSMUSG00000040084	BUB1B, mitotic checkpoint serine/threonine kinase
Bub3	12237	ENSMUSG00000066979	BUB3 mitotic checkpoint protein
Ccna1	12427	ENSMUSG00000027793	cyclin A1
Ccna2	12428	ENSMUSG00000027715	cyclin A2
Ccnb1	268697	ENSMUSG00000041431	cyclin B1
Ccnb2	12442	ENSMUSG00000032218	cyclin B2
Ccnb3	209091	ENSMUSG00000051592	cyclin B3
Ccnd1	12443	ENSMUSG00000070348	cyclin D1
Ccnd2	12444	ENSMUSG00000000184	cyclin D2
Ccnd3	12445	ENSMUSG00000034165	cyclin D3
Ccne1	12447	ENSMUSG00000002068	cyclin E1
Ccne2	12448	ENSMUSG00000028212	cyclin E2
Ccnh	66671	ENSMUSG00000021548	cyclin H
Cdc14a	229776	ENSMUSG00000033502	CDC14 cell division cycle 14A
Cdc14b	218294	ENSMUSG00000033102	CDC14 cell division cycle 14B
Cdc16	69957	ENSMUSG00000038416	CDC16 cell division cycle 16
Cdc20	107995	ENSMUSG00000006398	cell division cycle 20
Cdc23	52563	ENSMUSG00000024370	CDC23 cell division cycle 23
Cdc25a	12530	ENSMUSG00000032477	cell division cycle 25A
Cdc25b	12531	ENSMUSG00000027330	cell division cycle 25B
Cdc25c	12532	ENSMUSG00000044201	cell division cycle 25C
Cdc26	66440	ENSMUSG00000066149	cell division cycle 26
Cdc27	217232	ENSMUSG00000020687	cell division cycle 27
Cdc45	12544	ENSMUSG00000000028	cell division cycle 45
Cdc6	23834	ENSMUSG00000017499	cell division cycle 6

Table S4cont.

Cdc7	12545	ENSMUSG00000029283	cell division cycle 7 (S. cerevisiae)
Cdk1	12534	ENSMUSG00000019942	cyclin-dependent kinase 1
Cdk2	12566	ENSMUSG00000025358	cyclin-dependent kinase 2
Cdk4	12567	ENSMUSG00000006728	cyclin-dependent kinase 4
Cdk6	12571	ENSMUSG00000040274	cyclin-dependent kinase 6
Cdk7	12572	ENSMUSG00000069089	cyclin-dependent kinase 7
Cdkn1a	12575	ENSMUSG00000023067	cyclin-dependent kinase inhibitor 1A (P21)
Cdkn1b	12576	ENSMUSG00000003031	cyclin-dependent kinase inhibitor 1B
Cdkn1c	12577	ENSMUSG00000037664	cyclin-dependent kinase inhibitor 1C (P57)
Cdkn2a	12578	ENSMUSG00000044303	cyclin-dependent kinase inhibitor 2A
Cdkn2b	12579	ENSMUSG00000073802	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)
Cdkn2c	12580	ENSMUSG00000028551	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)
Cdkn2d	12581	ENSMUSG00000096472	cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)
Chek1	12649	ENSMUSG00000032113	checkpoint kinase 1
Chek2	50883	ENSMUSG00000029521	checkpoint kinase 2
Crebbp	12914	ENSMUSG00000022521	CREB binding protein
Cul1	26965	ENSMUSG00000029686	cullin 1
Dbf4	27214	ENSMUSG00000002297	DBF4 zinc finger
E2f1	13555	ENSMUSG00000027490	E2F transcription factor 1
E2f2	242705	ENSMUSG00000018983	E2F transcription factor 2
E2f3	13557	ENSMUSG00000016477	E2F transcription factor 3
E2f4	104394	ENSMUSG00000014859	E2F transcription factor 4
E2f5	13559	ENSMUSG00000027552	E2F transcription factor 5
Ep300	328572	ENSMUSG00000055024	E1A binding protein p300
Espl1	105988	ENSMUSG00000058290	extra spindle pole bodies 1, separase
Fzr1	56371	ENSMUSG00000020235	fizzy/cell division cycle 20 related 1 (Drosophila)
Gadd45a	13197	ENSMUSG00000036390	growth arrest and DNA-damage-inducible 45 alpha
Gadd45b	17873	ENSMUSG00000015312	growth arrest and DNA-damage-inducible 45 beta
Gadd45g	23882	ENSMUSG00000021453	growth arrest and DNA-damage-inducible 45 gamma
Gsk3b	56637	ENSMUSG00000022812	glycogen synthase kinase 3 beta
Hdac1	433759	ENSMUSG00000028800	histone deacetylase 1
Hdac2	15182	ENSMUSG00000019777	histone deacetylase 2
Mad1l1	17120	ENSMUSG00000029554	MAD1 mitotic arrest deficient 1-like 1
Mad2l1	56150	ENSMUSG00000029910	MAD2 mitotic arrest deficient-like 1
Mad2l2	71890	ENSMUSG00000029003	MAD2 mitotic arrest deficient-like 2
Mcm2	17216	ENSMUSG00000002870	minichromosome maintenance complex component 2
Mcm3	17215	ENSMUSG00000041859	minichromosome maintenance complex component 3
Mcm4	17217	ENSMUSG00000022673	minichromosome maintenance complex component 4
Mcm5	17218	ENSMUSG00000005410	minichromosome maintenance complex component 5
Mcm6	17219	ENSMUSG00000026355	minichromosome maintenance complex component 6
Mcm7	17220	ENSMUSG00000029730	minichromosome maintenance complex component 7
Mdm2	17246	ENSMUSG00000020184	transformed mouse 3T3 cell double minute 2
Myc	17869	ENSMUSG00000022346	myelocytomatosis oncogene
Orc1	18392	ENSMUSG00000028587	origin recognition complex, subunit 1
Orc2	18393	ENSMUSG00000026037	origin recognition complex, subunit 2
Orc3	50793	ENSMUSG00000040044	origin recognition complex, subunit 3
Orc4	26428	ENSMUSG00000026761	origin recognition complex, subunit 4
Orc5	26429	ENSMUSG00000029012	origin recognition complex, subunit 5
Orc6	56452	ENSMUSG00000031697	origin recognition complex, subunit 6
Pcna	18538	ENSMUSG00000027342	proliferating cell nuclear antigen
Pkmyt1	268930	ENSMUSG00000023908	protein kinase, membrane associated tyrosine/threonine 1
Plk1	18817	ENSMUSG00000030867	polo like kinase 1

Table S4cont.

Prkdc	19090	ENSMUSG00000022672	protein kinase, DNA activated, catalytic polypeptide
Pttg1	30939	ENSMUSG00000020415	pituitary tumor-transforming gene 1
Rad21	19357	ENSMUSG00000022314	RAD21 cohesin complex component
Rb1	19645	ENSMUSG00000022105	RB transcriptional corepressor 1
Rbl1	19650	ENSMUSG00000027641	retinoblastoma-like 1 (p107)
Rbl2	19651	ENSMUSG00000031666	RB transcriptional corepressor like 2
Rbx1	56438	ENSMUSG00000022400	ring-box 1
Rbx1-ps	1E+08	ENSMUSG00000049832	ring-box 1, pseudogene
Sfn	55948	ENSMUSG00000047281	stratifin
Skp1a	21402	ENSMUSG00000036309	S-phase kinase-associated protein 1A
Skp2	27401	ENSMUSG00000054115	S-phase kinase-associated protein 2 (p45)
Smad2	17126	ENSMUSG00000024563	SMAD family member 2
Smad3	17127	ENSMUSG00000032402	SMAD family member 3
Smad4	17128	ENSMUSG00000024515	SMAD family member 4
Smc1a	24061	ENSMUSG00000041133	structural maintenance of chromosomes 1A
Smc1b	140557	ENSMUSG00000022432	structural maintenance of chromosomes 1B
Smc3	13006	ENSMUSG00000024974	structural maintenance of chromosomes 3
Stag1	20842	ENSMUSG00000037286	stromal antigen 1
Stag2	20843	ENSMUSG00000025862	stromal antigen 2
Tfdp1	21781	ENSMUSG00000038482	transcription factor Dp 1
Tfdp2	211586	ENSMUSG00000032411	transcription factor Dp 2
Tgfb1	21803	ENSMUSG00000002603	transforming growth factor, beta 1
Tgfb2	21808	ENSMUSG00000039239	transforming growth factor, beta 2
Tgfb3	21809	ENSMUSG00000021253	transforming growth factor, beta 3
Trp53	22059	ENSMUSG00000059552	transformation related protein 53
Ttk	22137	ENSMUSG00000038379	Ttk protein kinase
Wee1	22390	ENSMUSG00000031016	WEE 1 homolog 1 (S. pombe)
Wee2	381759	ENSMUSG00000037159	WEE1 homolog 2 (S. pombe)
Ywhab	54401	ENSMUSG00000018326	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta
Ywhae	22627	ENSMUSG00000020849	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon
Ywhag	22628	ENSMUSG00000051391	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma
Ywhah	22629	ENSMUSG00000018965	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta
Ywhaq	22630	ENSMUSG00000076432	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein theta
Ywhaz	22631	ENSMUSG00000022285	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta
Zbtb17	22642	ENSMUSG00000006215	zinc finger and BTB domain containing 17

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