

SUPPORTING INFORMATION

Rapid and synchronous chemical induction of replicative-like senescence via a small molecule inhibitor

Spiros Palikyras, *et al.*

Contents

This sections contains supplementary **Figures S1-S8** and **Tables S1-S7**.

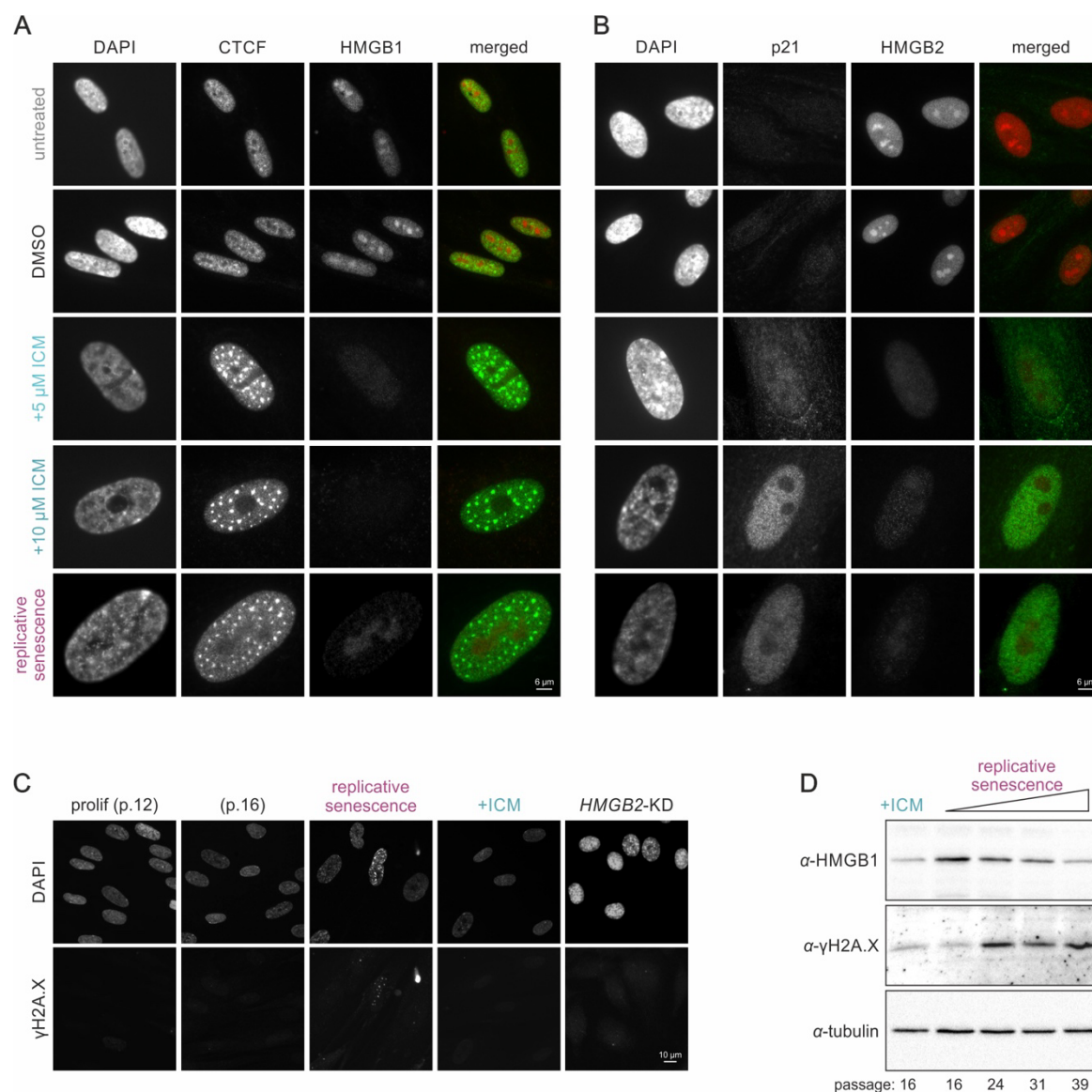


FIGURE S1 ICM treatment depletes HMGB1 and -B2 from IMR90 cell nuclei.

(A) Representative widefield images of proliferating, DMSO-, and 3- or 6-day ICM-treated IMR90 immunostained for CTCF and HMGB1, and counterstained with DAPI. Bar: 6 μ m. (B) As in panel A, but immunostained for p21 and HMGB2. Bar: 6 μ m. (C) As in panel A, but immunostained for phospho- γ -H2A.X. (D) Western blots showing changing HMGB1 and phospho- γ -H2A.X levels in ICM-treated or progressively passaged IMR90. Tubulin levels provide a loading control.

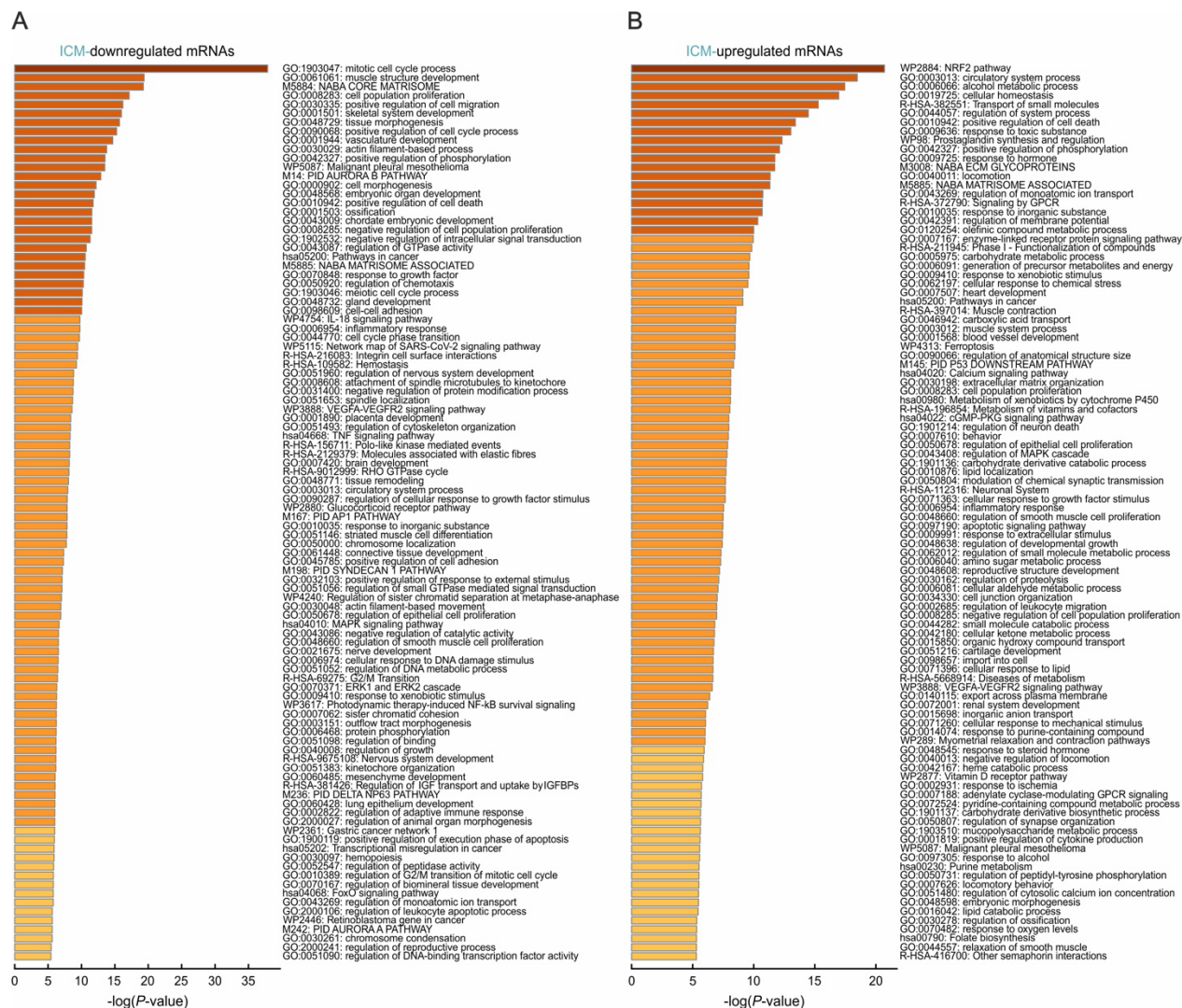


FIGURE S2 Effects of ICM treatment at the level of mRNA in IMR90.

(A) Top 100 GO terms/pathways associated with mRNAs downregulated following 6 days of 10 μ M ICM treatment.

(B) As in panel A, but for ICM-upregulated mRNAs.

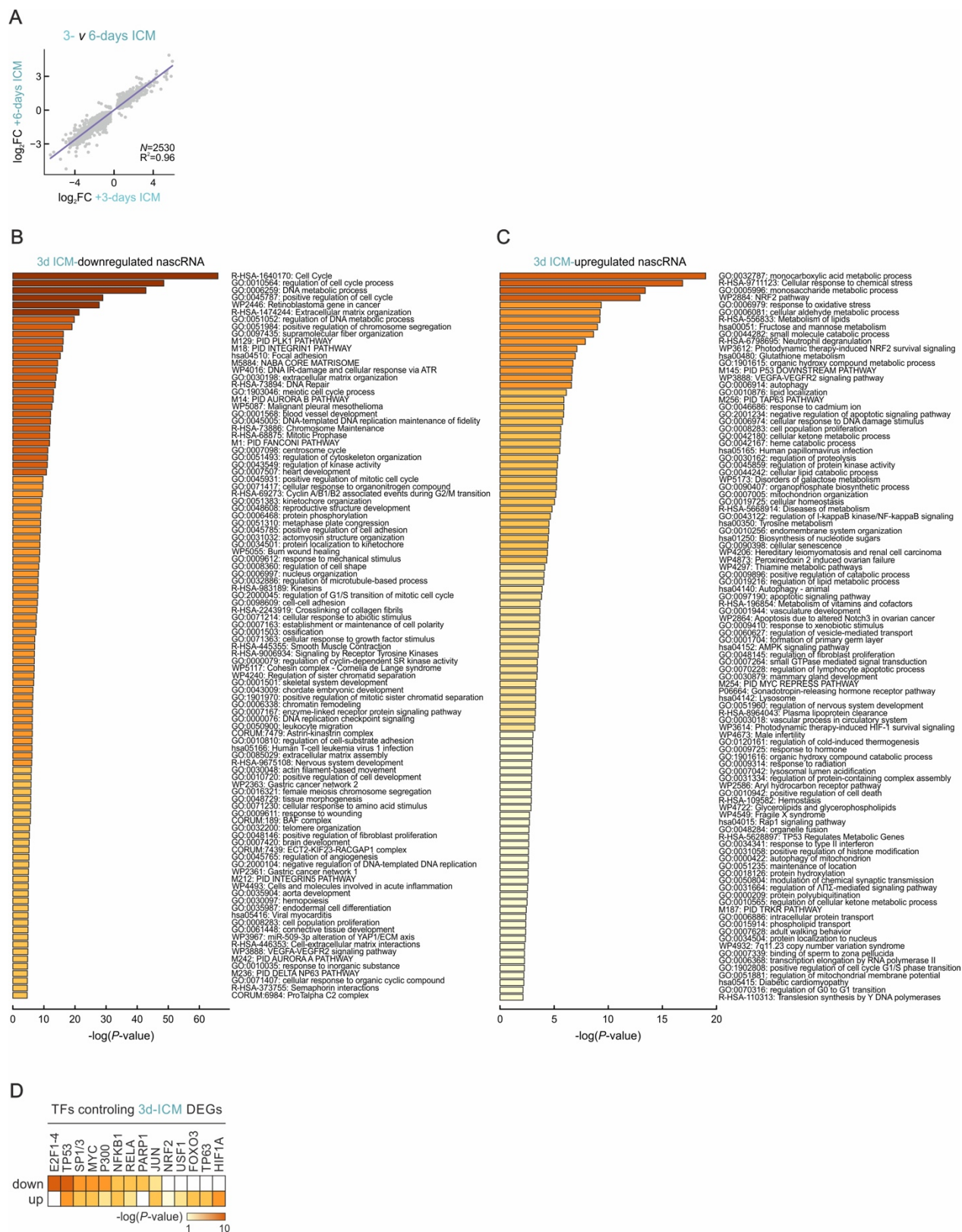


FIGURE S3 Effects of 3-day ICM treatment at the level of nascent RNA production in IMR90.

(A) Plot correlating changes ($\log_2FC$) in nascent RNA levels of genes regulated after 3 and 6 days of ICM treatment. The number of the queried genes (N) and the deduced Spearman's correlation coefficient (R^2) are shown. (B) Top 100 GO

terms/pathways associated with nascent RNAs downregulated after 3 days of 10 μ M ICM treatment. **(C)** As in panel B, but for 3-day ICM-upregulated nascent RNAs. **(D)** Heatmap showing transcription factors (TFs) predicted to bind ICM-regulated genes from panels A and B based on TTRUST motif enrichment.

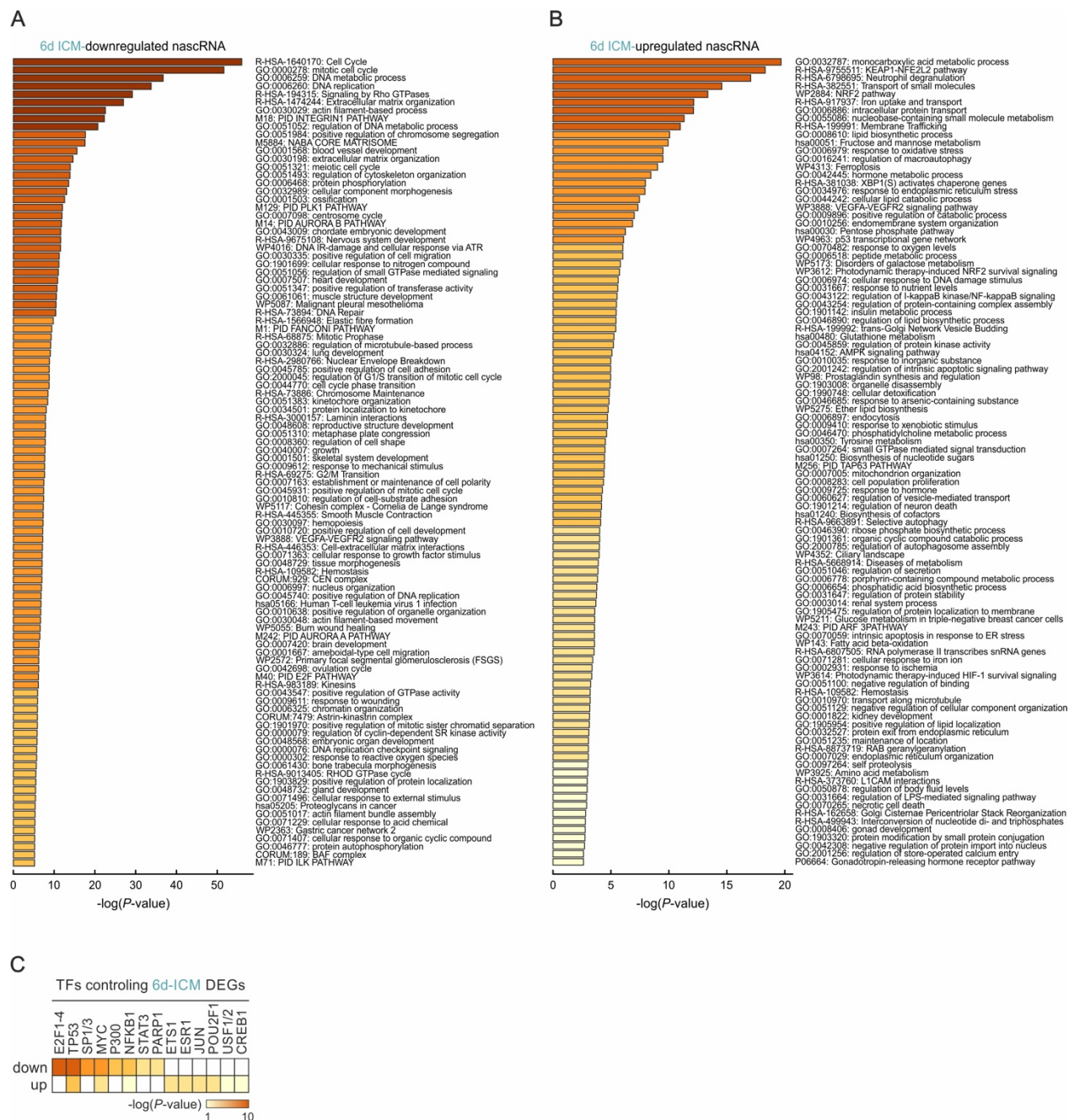


FIGURE S4 Effects of 6-dayICM treatment at the level of nascent RNA production in IMR90.

(A) Top 100 GO terms/pathways associated with nascent RNAs downregulated after 6 days of 10 μ M ICM treatment.

(B) As in panel A, but for 6-day ICM-upregulated nascent RNAs. (C) Heatmap showing transcription factors (TFs) predicted to bind ICM-regulated genes from panels A and B based on TTRUST motif enrichment.

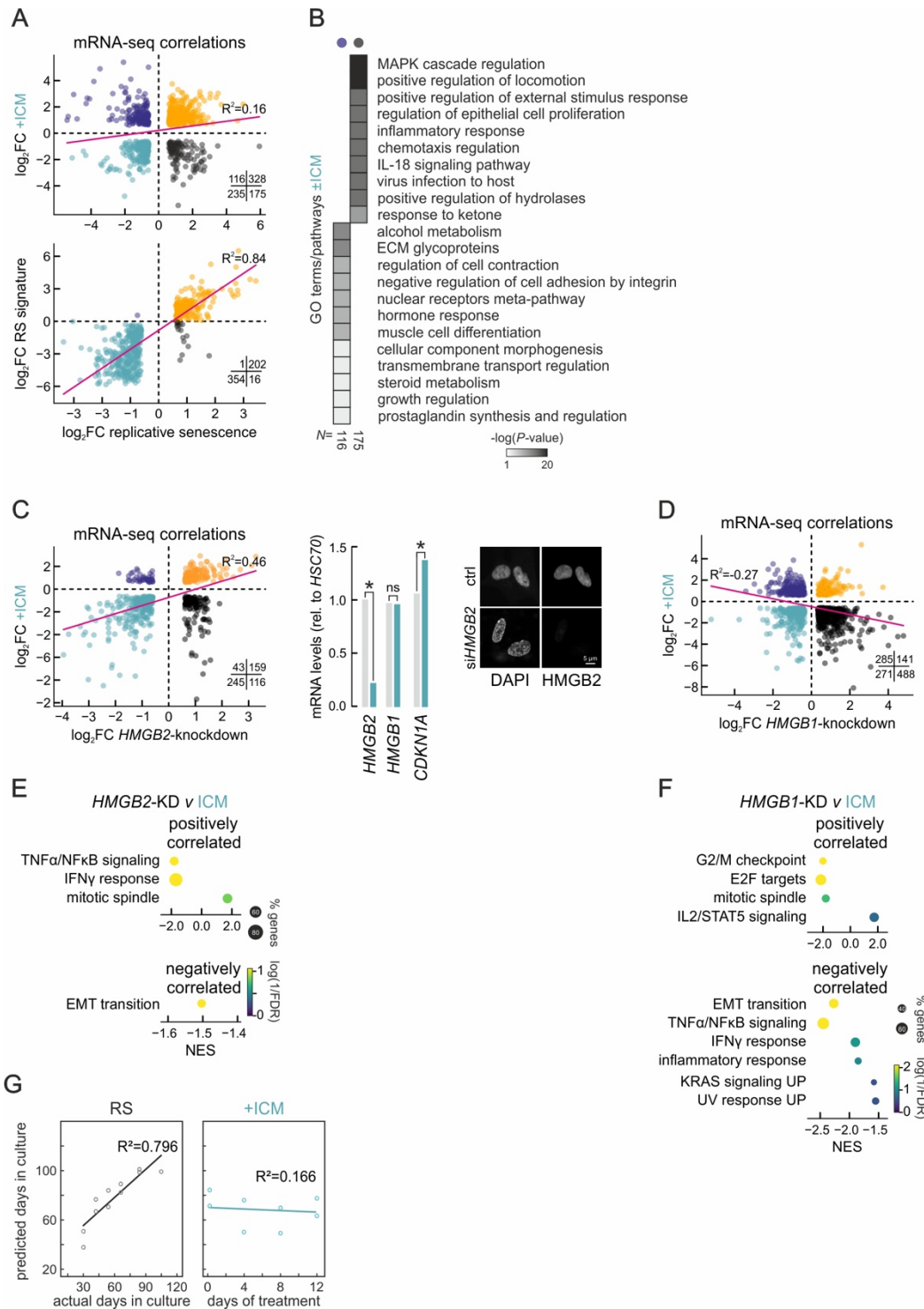


FIGURE S5 Transcriptional changes and methylation clock in 6-day ICM-treated IMR90.

(A) Top: Scatter plots showing correlation of differentially expressed mRNAs from ICM-treated and replicatively senescent IMR90. Bottom: As above, but for mRNAs from ICM-treated IMR90 and a consensus senescence signature. Spearman's correlation coefficients (R^2) and the number of genes in each quadrant (N) are shown. (B) Heatmap of GO terms/pathways associated with the two diverging gene subsets from panel A (up in ICM and down in RS – purple; down in ICM and up in RS – black). The number of genes in each subset (N) is indicated. (C) Left: As in panel A, but correlating differentially expressed mRNAs from ICM-treated and *HMGB2*-knockdown IMR90. Middle: Bar plot

showing relative mRNA levels (mean of two RT-qPCR replicates) of *HMGB2*, *-B1*, and *CDKN1A* upon 72 h of *HMGB2* knockdown. * $P < 0.05$, unpaired two-tailed Student's t-test. Right: Representative IMR90 immunofluorescence imaging showing HMGB2 nuclear depletion upon 72 h of *HMGB2* knockdown. Bar: 5 μ M. **(D)** As in panel A, but correlating differentially expressed mRNAs from ICM-treated and *HMGB1*-knockdown IMR90. **(E)** Plots showing GSEA results for mRNAs correlating positively (green/orange data points) or negatively (blue/black data points) in panel C. **(F)** As in panel E, but using positively/negatively correlated mRNAs from panel D. **(G)** Plots correlating IMR90 passage predicted by methylation changes at six senescence-associated CpGs with actual passage (left) or days of ICM treatment (right). Spearman's correlation coefficients (R^2) are shown.

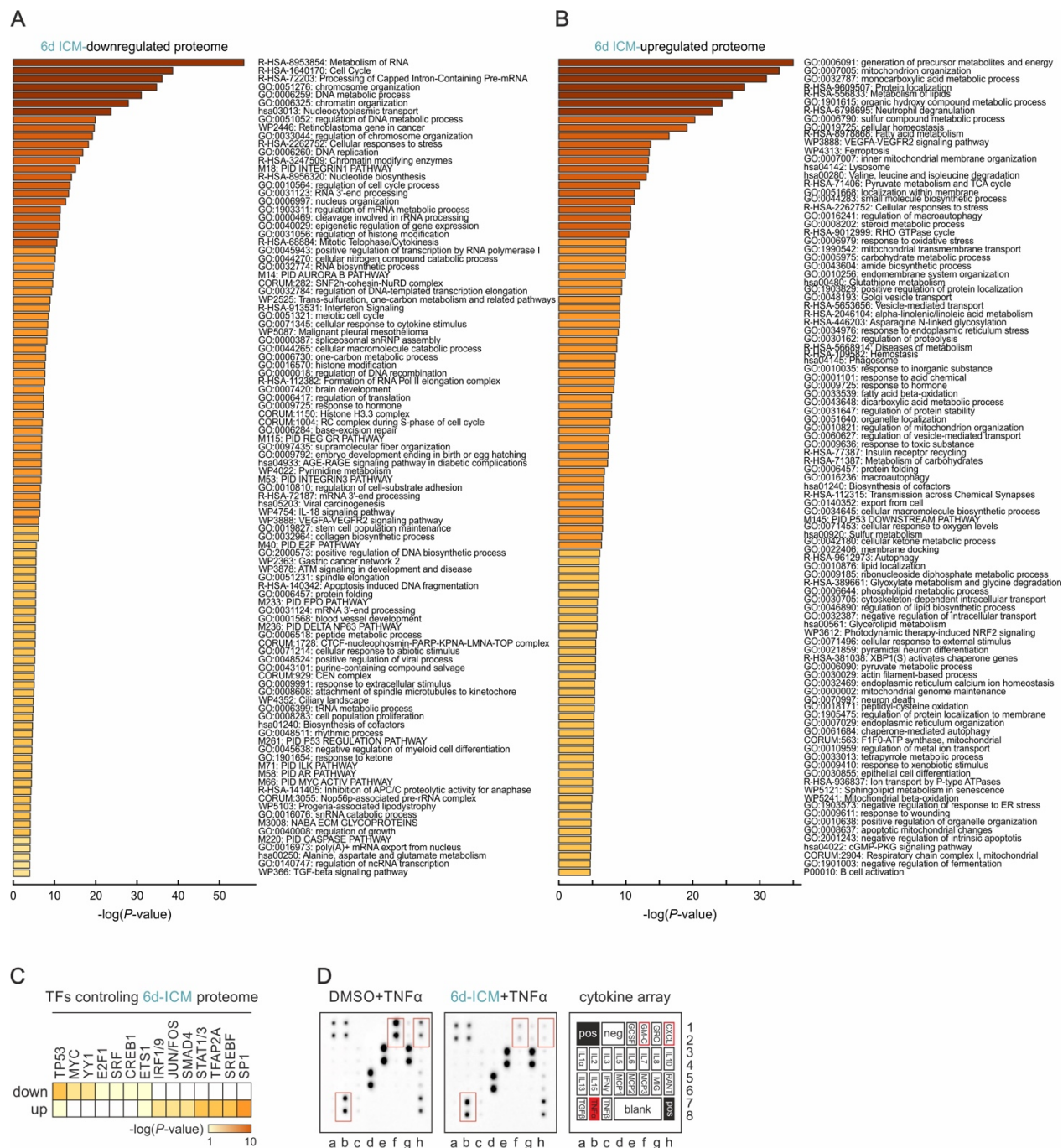


FIGURE S6 Effects of ICM treatment at the level of IMR90 whole-cell proteome and secretome.

(A) Top 100 GO terms/pathways associated with proteins downregulated after 6 days of 10 μ M ICM treatment. (B) As in panel A, but for ICM-upregulated proteins. (C) Heatmap showing transcription factors (TFs) predicted to bind the genes encoding the ICM-regulated genes from panels A and B based on TTRUST motif enrichment. (D) Cytokine profiling of the supernatant from TNF α -treated proliferating (DMSO) or ICM-induced (for 6 days) IMR90 cultures.

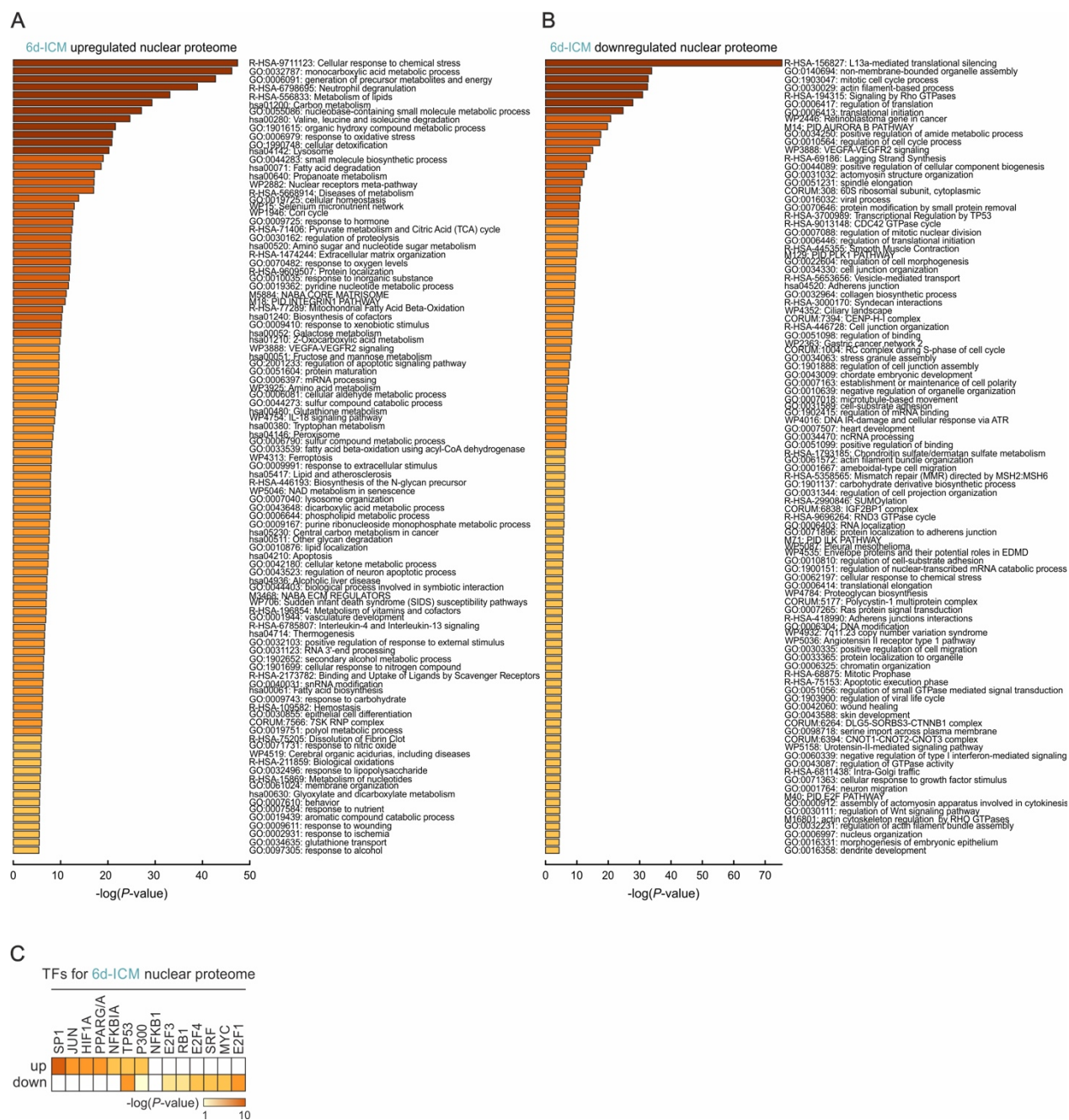


FIGURE S7 Effects of ICM treatment at the level of IMR90 nuclear proteome.

(A) Top 100 GO terms/pathways associated with nuclear proteins upregulated after 6 days of 10 μ M ICM treatment. (B) As in panel A, but for ICM-downregulated nuclear proteins. (C) Heatmap showing TFs predicted to bind the genes encoding the ICM-regulated genes from panels A and B based on TTRUST motif enrichment.

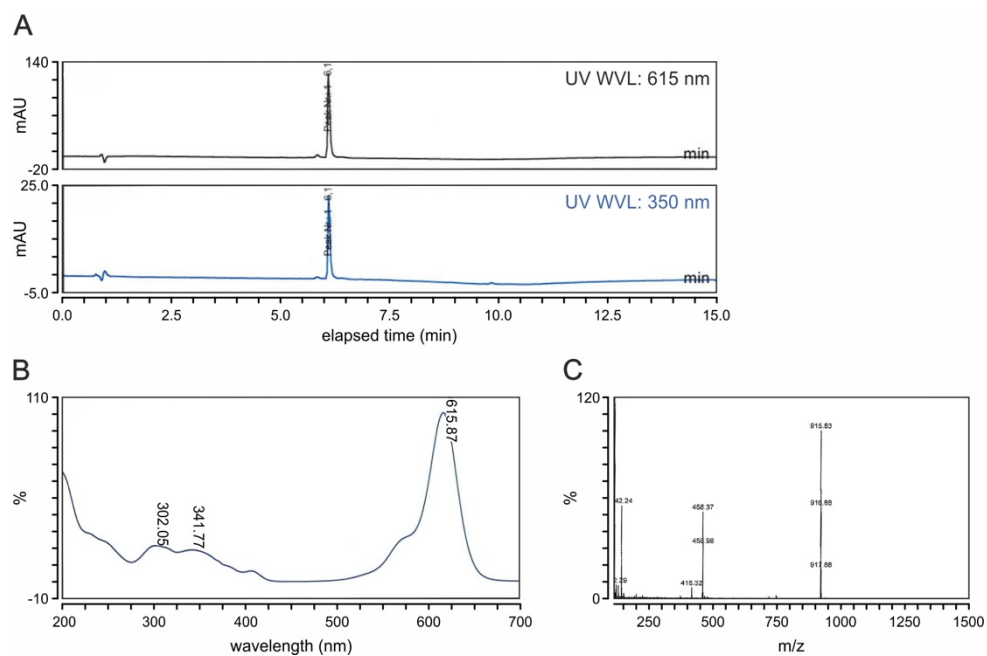


FIGURE S8 Characterization of ICM-C6-610CP.

(A) Analytical LC trace showing high purity of the ICM-C6-610CP compound. (B) Absorbance spectrum of ICM-C6-610CP recorded at 6.1-min retention time of analytical LC. (C) Mass spectrum of ICM-C6-610CP theoretical m/z corresponding to 915.44.

Table S1. Basic mapping statistics of Micro-C experiments.

	proliferating (DMSO-treated)	ICM-treated (6 days)
total reads	1,175,415,686	1,238,470,006
% mapped	82	81
% duplicates	30	23
total valid	614,188,035	711,139,837
total <i>cis</i>	417,456,327	488,155,052
total <i>trans</i>	83,549,563	89,834,674
% long range	46	52

Table S2. List of primers used in RT-qPCR experiments.

gene target	forward primer (5'-3')	reverse primer (5'-3')
<i>HSC70</i>	TTATTGGAGCCAGGCCTACAC	GCGACATAGCTTGGAGTGGT
<i>LMNB1</i>	CTGGCCAAGATGTGAAGGTTA	TCCTCTTCTTCAGGTATGGTTGTT
<i>HMGB1</i>	TGAGCTCCATAGAGACGCG	GATGACATTTTGCCTCTCGG
<i>HMGB2</i>	CCAATGCTCCTAAAAGGCCACC	CCAATGGATAGGCCAGGGTGT
<i>HMGA1</i>	GAAAAGGACGGCACTGAGAA	CCCCGAGGTCTCTTAGGTGT
<i>HDAC9</i>	CATGAGAACTTGACACGGCA	TGCTCCAGTTTCTGCTCCTT
<i>CCND2</i>	TGGCCTCCAACTCAAAGAG	CACTTCAACTTCCCCAGCAC
<i>CDKN1A</i>	TGGAGACTCTCAGGGTCGAA	GGATTAGGGCTTCTCTTGG
<i>SMC1</i>	GGGGAGAAGACAGTGGCAG	TTGGTGTTATCCAAGGCAGC
<i>CTCF</i>	CAGAGGTTAATGCAGAGAAAGTG	AATGCCATGCCACAGAGATG
<i>RBL1</i>	GTATTCCAAGAGAAGTTGTGGCA	GGTCCACTGGAACAGTCAGG

Table S3. List of differentially expressed genes identified by RNA-seq approaches.

(Provided as an .xlsx file.)

Table S4. List of primers used in HMGB2 ChIP-qPCR experiments.

pair	forward primer (5'-3')	reverse primer (5'-3')	location (hg19)
#a	ATGCGGGTTTACCATGCAGA	GGCCGAGAGCCATAAAGACA	chr10:92,671,441-92,671,590
#b	TCAGACTCCGACGAAAGGT	AAGTACGCGCCTTGGTGAG	chr1:205,091,401-205,091,550
#c	CATACAATAAAGGTGGTGCCAG	TTCAGGAGCTTAATACTGGAGGC	chr15: 39,890,983-39,891,451
#d	GACACGCTCAATAGGCTGAGT	GGGCTCTTATCCTTTCCCGA	chr20: 43,229,526-43,229,628
#e	TGAGTGCCATTCACTTAACAGC	GCTGGTAGTGGCTACCTTACG	chr10: 12,085,032-12,085,261
#f	TCAGTGCGAAGCCGATTTC	AACATCTTTCGACTCCGCCC	chr3:197,676,616-197,677,150

Table S5. List of differentially translated/buffered mRNAs identified by Ribo-seq experiments.

(Provided as an .xlsx file.)

Table S6. List of peptides identified by whole-cell proteomics and associated statistics.

(Provided as an .xlsx file.)

Table S7. List of peptides identified by proteomics on fractionated IMR90 and associated statistics.

(Provided as an .xlsx file.)