SUPPORTING INFORMATION

Inhibition of nucleotide synthesis mediates replicative senescence of human mammary epithelial cells

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Running title: *Nucleotide synthesis regulates epithelial cell senescence*

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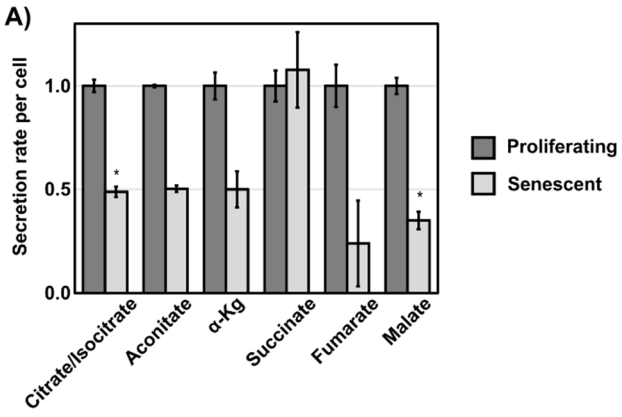
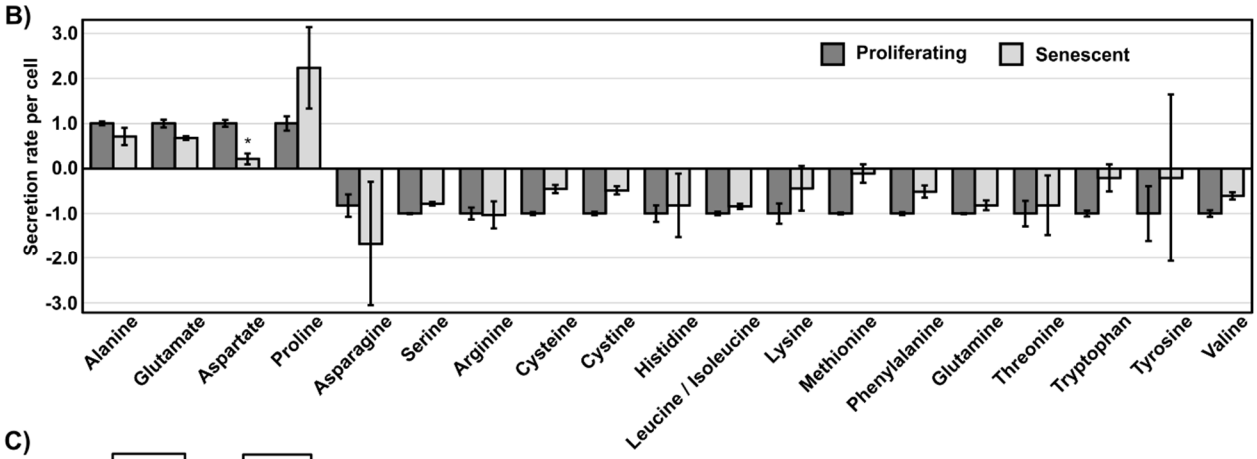
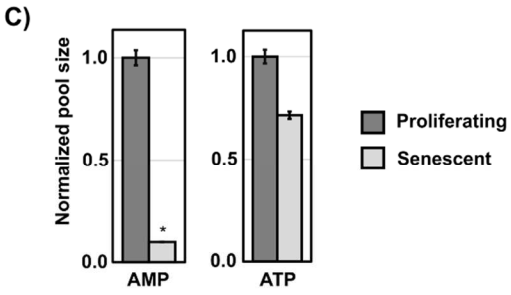
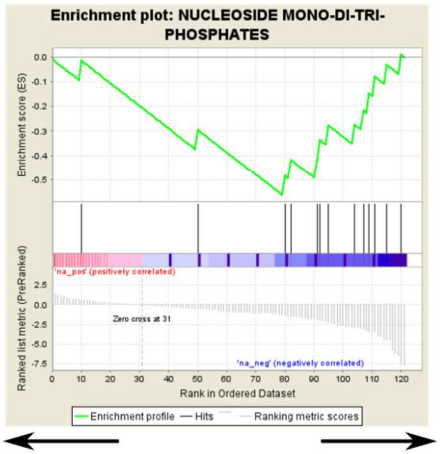






D)

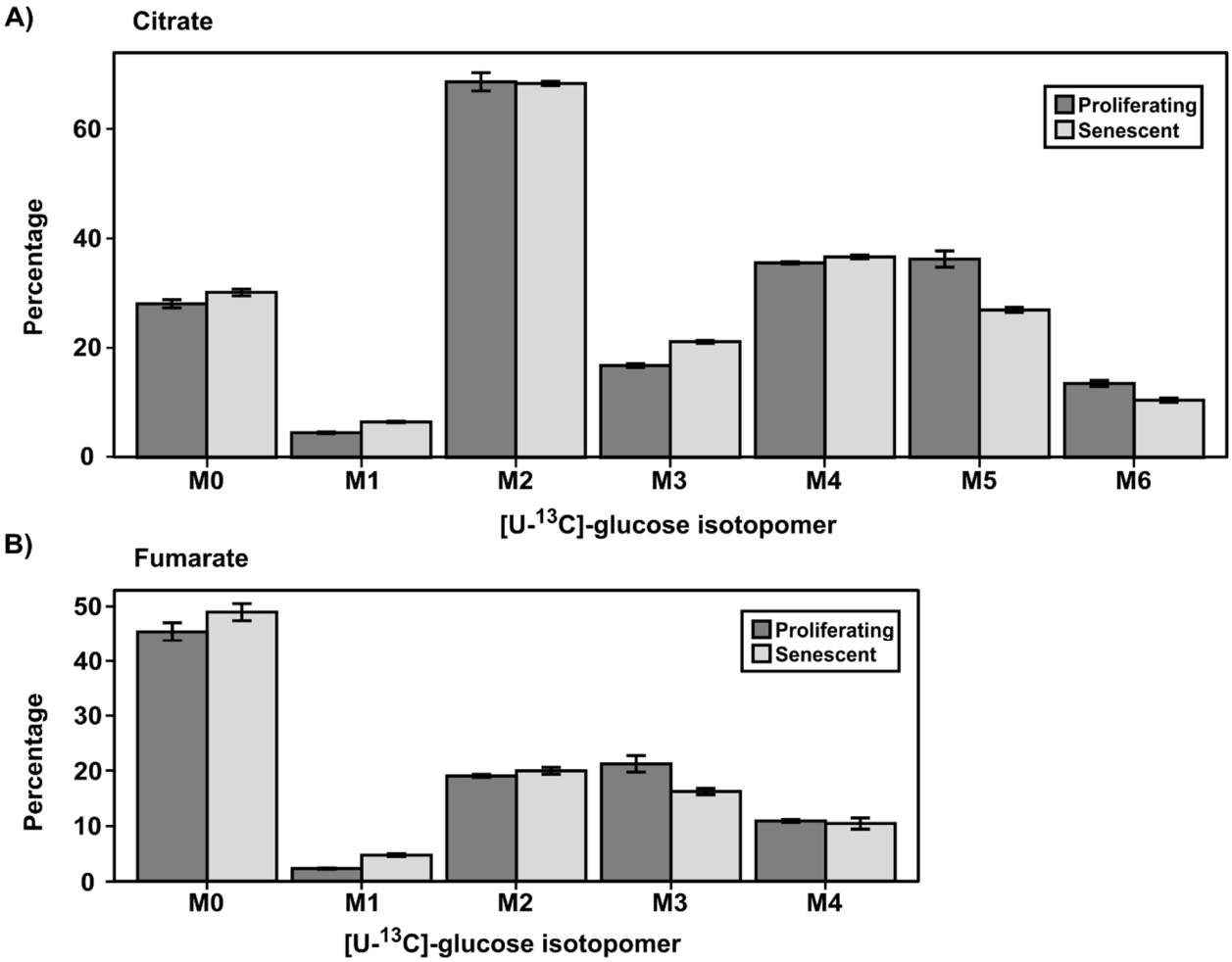


Higher concentration in senescent HMEC

Higher concentration in proliferating HMEC

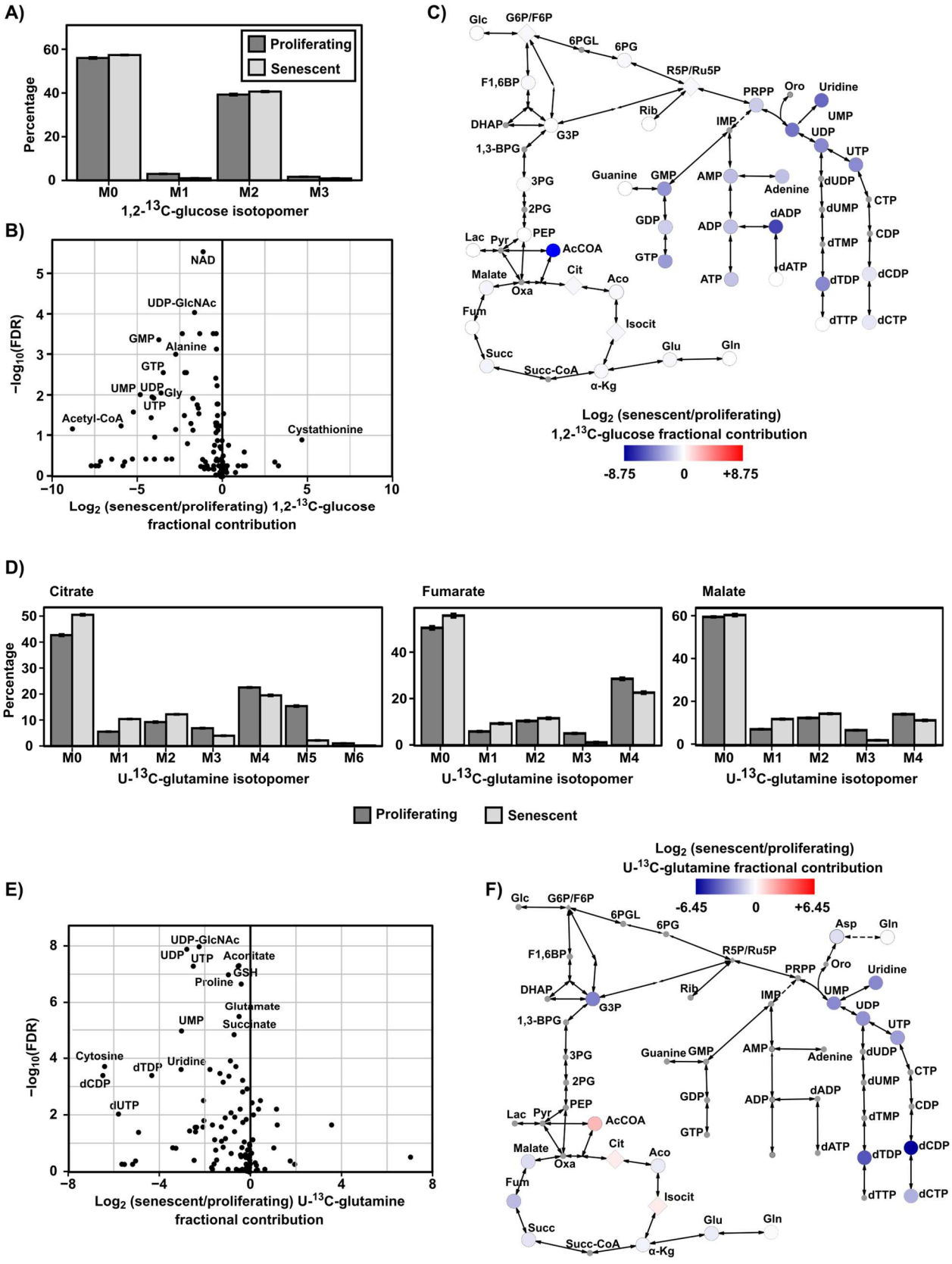
Replicative senescence pool sizes				
COMPOUND CLASS	SIZE	NES	NOM p-val	FDR q-val
AMINO ACIDS	16	1.89	0.01	0.05
HYDROPHOBIC AMINO ACIDS	5	1.87	0.01	0.03
POLAR AMINO ACIDS	8	1.49	0.07	0.15
NUCLEOBASES	6	1.36	0.14	0.20
NUCLEOSIDES	5	1.04	0.40	0.48
URACIL DERIVATIVES	5	1.03	0.40	0.41
PENTOSE PHOSPHATE PATHWAY	6	-0.69	0.85	0.86
GUANINE DERIVATIVES	5	-0.75	0.77	0.86
GLYCOLYSIS	6	-0.84	0.65	0.82
HYPOXANTHINE DERIVATIVES	8	-0.94	0.51	0.75
THYMINE DERIVATIVES	5	-1.05	0.37	0.64
DEOXYNUCLEOSIDE MONO-DI-TRI-PHOSPHATE	10	-1.52	0.06	0.13
CYTOSINE DERIVATIVES	9	-1.55	0.06	0.15
TCA CYCLE	7	-1.72	0.02	0.08
NUCLEOSIDE MONOPHOSPHATES	5	-1.88	0.01	0.05
NUCLEOSIDE MONO-DI-TRI-PHOSPHATES	13	-2.35	0.0004	0.003

Supporting Fig. S1: Extracellular medium analysis of amino acid and TCA cycle metabolites, intracellular AMP/ATP levels, and MSEA analysis of senescent HMEC intracellular metabolite pools
A) Extracellular metabolite secretion data for TCA cycle metabolites in proliferating and senescent HMEC. Metabolite extracts from blank and conditioned media were analyzed by LC-MS. Secretion or uptake values were normalized to integrated cell number. Secreted metabolites have positive values, and consumed metabolites have negative values. * denotes p-value less than 0.05 by FDR-corrected Student's t-test. See Supporting Table S1 for all measured metabolites.
B) Same as in A for amino acids. * denotes p-value less than 0.05 by FDR-corrected Student's t-test. See Supporting Table S1 for all measured metabolites.
C) Intracellular pool sizes of AMP and ATP of proliferating and senescent HMEC. * denotes p-value less than 0.05 by FDR-corrected Student's t-test. See Supporting Table S2 for all measured metabolites.
D) Metabolite set enrichment analysis (MSEA) analysis for intracellular metabolites pool sizes of proliferating and senescent HMEC. Metabolites were ranked based on $\log_2$ fold change of senescent/proliferating. Shown are the mountain plot of nucleoside mono/di/tri-phosphates (left), and table of all tested metabolic pathways (right).



Supporting Fig. S2: [U-^{13}C]-labeled glucose isotopomer distributions are not altered in senescent HMEC.

A-B) [U-^{13}C]-labeled glucose isotopomer distributions of citrate and fumarate. Proliferating and senescent HMEC show similar labeling patterns for TCA cycle metabolites. See Supporting Table S3 for all measured metabolite isotopomer distributions.


A)
Proliferating
Senescent
Percentage
M0
M1
M2
M3
1,2-13C-glucose isotopomer
B)
−log10(FDR)
NAD
UDP-GlcNAc
GMP
Alanine
GTP
UMP
UDP
Gly
UTP
Acetyl-CoA
Cystathionine
Log2 (senescent/proliferating) 1,2-13C-glucose fractional contribution
C)
Glc
G6P/F6P
6PGL
6PG
R5P/Ru5P
F1,6BP
PRPP
Oro
Uridine
Rib
DHAP
G3P
IMP
UMP
UDP
UTP
1,3-BPG
3PG
Guanine
GMP
AMP
Adenine
dUDP
CTP
2PG
GDP
ADP
dADP
dUMP
Lac
Pyr
PEP
AcCOA
GTP
dTMP
CDP
Malate
Cit
Aco
ATP
dATP
dTDP
dCDP
Oxa
Fum
Isocit
dTTP
dCTP
Succ
Glu
Gln
Succ-CoA
α-Kg
Log2 (senescent/proliferating)
1,2-13C-glucose fractional contribution
-8.75
0
+8.75
D)
Citrate
Fumarate
Malate
Percentage
M0
M1
M2
M3
M4
M5
M6
U-13C-glutamine isotopomer
Proliferating
Senescent
E)
−log10(FDR)
UDP-GlcNAc
UDP
UTP
Aconitate
GSH
Proline
Glutamate
UMP
Succinate
Cytosine
dTDP
Uridine
dCDP
dUTP
Log2 (senescent/proliferating) U-13C-glutamine fractional contribution
F)
Log2 (senescent/proliferating)
U-13C-glutamine fractional contribution
-6.45
0
+6.45
Glc
G6P/F6P
6PGL
6PG
Asp
Gln
F1,6BP
R5P/Ru5P
PRPP
Oro
Uridine
DHAP
Rib
G3P
IMP
UMP
UDP
UTP
1,3-BPG
3PG
Guanine
GMP
AMP
Adenine
dUDP
CTP
2PG
GDP
ADP
dADP
dUMP
PEP
Lac
Pyr
AcCOA
GTP
dTMP
CDP
Malate
Cit
Aco
dATP
dTDP
dCDP
Oxa
Fum
Isocit
dTTP
dCTP
Succ
Glu
Gln
Succ-CoA
α-Kg

Supporting Fig. S3: [1,2-^{13}C]-glucose and [U-^{13}C]-glutamine stable isotope tracing

A) [1,2-^{13}C]-labeled glucose isotopomer distribution. The ratio of M1 to M2 lactate does not show a significant change between proliferating and senescent HMEC.

B) Fractional contribution of [1,2-^{13}C]-glucose to purines and pyrimidines is downregulated in senescent HMEC. Volcano plot represents $\log_2$ fold change (senescent/proliferating) for fractional contribution of [1,2-^{13}C]-glucose and FDR-corrected p-value.

C) Metabolic pathway map depicting the average $\log_2$ fold change (senescent/proliferating) of fractional contribution of [1,2-^{13}C]-glucose using the indicated color scale. Metabolites that were not measured are shown as small grey colored shapes.

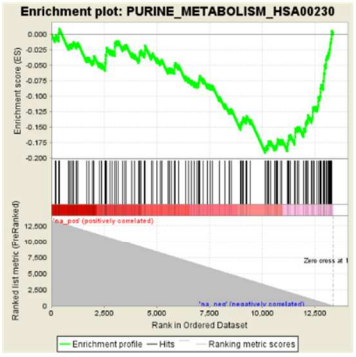
D) [U-^{13}C]-labeled glutamine isotopomer distributions for TCA cycle metabolites. The ratio of reductive to oxidative TCA cycle is slightly decreased in senescent HMEC.

E) Fractional contribution of [U-^{13}C]-glutamine to pyrimidines is downregulated in senescent HMEC. Volcano plot represents average $\log_2$ fold change (senescent/proliferating) for fractional contribution of [U-^{13}C]-labeled glutamine and FDR-corrected combined Fisher's combined p-value from two independent experiments.

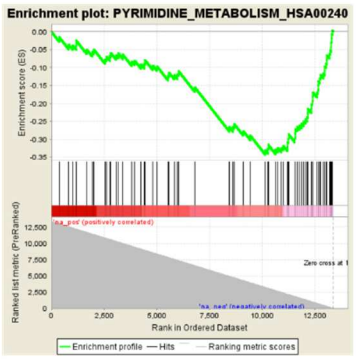
F) Metabolic pathway map depicting the average $\log_2$ fold change (senescent/proliferating) of fractional contribution of [U-^{13}C]-glutamine using the indicated color scale. Metabolites that were not measured or had less than 3% fractional contribution are shown as small grey colored shapes.

A)

HMEC



Normalized enrichment score	-2.59
Nominal p-value	< 0.001
FDR q-value	0.001

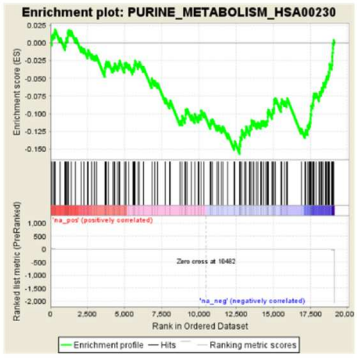


Normalized enrichment score	-3.51
Nominal p-value	< 0.001
FDR q-value	< 0.001

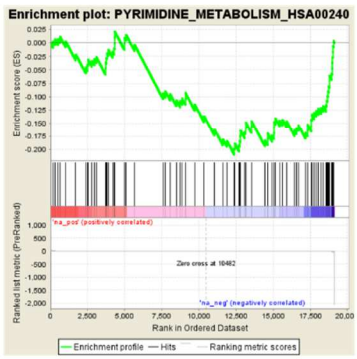
Higher abundance in senescent HMEC ← → Higher abundance in proliferating HMEC

B)

IMR90



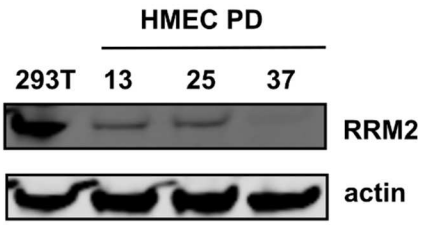
Normalized enrichment score	-2.22
Nominal p-value	0.0012
FDR q-value	0.016



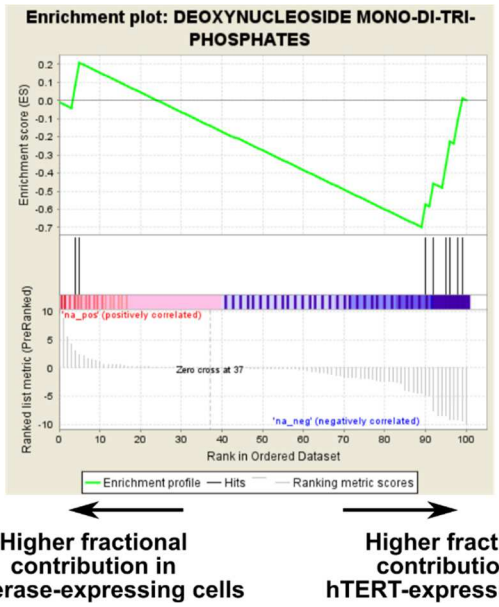
Normalized enrichment score	-2.29
Nominal p-value	0.0004
FDR q-value	0.015

Higher abundance in control IMR90 ← → Higher abundance in senescent IMR90

C)



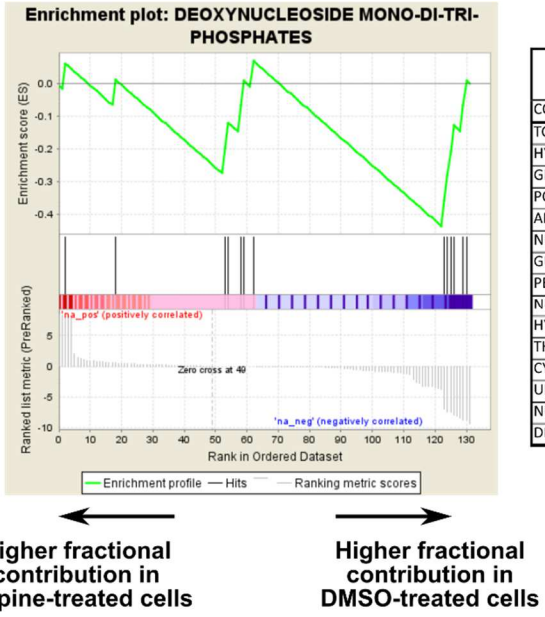
Supporting Fig. S4: RNA expression analysis confirms reduced nucleotide synthesis in senescent cells.
A-B) GSEA analysis of A) microarray data from senescent HMEC (1) and B) RNAseq data from senescent IMR90 cells (2). For HMEC microarray data, genes were compared across pairwise comparisons for stasis and pre-stasis cell cultures. Genes were then ranked by their average rank from individual experiments. For IMR90 RNAseq data, genes were ranked based on signal to noise ratio of senescent/non-senescent. Results show significant suppression of genes in the purine (hsa00230) and pyrimidine (hsa00240) pathways in senescent cells.
C) Western blotting with an RRM2 antibody (Sigma) targeting a distinct epitope from Figure 3E revealed downregulation of RRM2 expression in senescent HMEC. Actin was used as an equal loading control.



hTERT immortalization [U-^{13}C]-glucose fractional label

COMPOUND CLASS	SIZE	NES	NOM p-val	FDR q-val
TCA CYCLE	7	2.45	0.001	0.001
GLYCOLYSIS	6	1.56	0.06	0.19
AMINO ACIDS	16	1.22	0.22	0.52
POLAR AMINO ACIDS	8	1.06	0.36	0.65
HYDROPHOBIC AMINO ACIDS	5	0.74	0.79	1.00
NUCLEOBASES	6	0.71	0.82	0.97
HYPOXANTHINE DERIVATIVES	8	0.65	0.89	0.89
CYTOSINE DERIVATIVES	9	-1.08	0.36	0.35
GUANINE DERIVATIVES	5	-1.10	0.31	0.37
NUCLEOSIDES	5	-1.17	0.25	0.35
PENTOSE PHOSPHATE PATHWAY	6	-1.31	0.15	0.25
THYMINE DERIVATIVES	5	-1.85	0.01	0.03
URACIL DERIVATIVES	5	-2.12	0.00	0.01
NUCLEOSIDE MONO-DI-TRI-PHOSPHATES	13	-2.25	0.002	0.003
DEOXYNUCLEOSIDE MONO-DI-TRI-PHOSPHATES	10	-2.32	0.001	0.004

Supporting Fig. S5: Metabolite set enrichment analysis of hTERT immortalization.
MSEA analysis for fractional contribution of [U-^{13}C]-glucose in hTERT immortalized HMEC. Metabolites were ranked based on $\log_2$ fold change of luciferase/hTERT.



Triapine-induced senescence [U- ^{13}C]-glucose fractional label

COMPOUND CLASS	SIZE	NES	NOM p-val	FDR q-val
TCA CYCLE	7	1.83	0.02	0.11
HYPOXANTHINE DERIVATIVES	8	1.15	0.28	0.97
GLYCOLYSIS	6	1.15	0.28	0.65
POLAR AMINO ACIDS	8	1.04	0.38	0.67
AMINO ACIDS	16	1.04	0.39	0.55
NUCLEOSIDES	5	0.82	0.68	0.68
GUANINE DERIVATIVES	5	-0.78	0.73	0.74
PENTOSE PHOSPHATE PATHWAY	6	-0.85	0.64	0.72
NUCLEOBASES	6	-1.21	0.22	0.28
HYDROPHOBIC AMINO ACIDS	5	-1.22	0.21	0.31
THYMINE DERIVATIVES	5	-1.41	0.11	0.18
CYTOSINE DERIVATIVES	9	-1.74	0.02	0.06
URACIL DERIVATIVES	5	-1.77	0.02	0.07
NUCLEOSIDE MONO-DI-TRI-PHOSPHATES	13	-1.78	0.02	0.09
DEOXYNUCLEOSIDE MONO-DI-TRI-PHOSPHATES	10	-1.82	0.02	0.15

Supporting Fig. S6: Metabolite set enrichment analysis of triapine-induced senescence.
MSEA analysis for fractional contribution of [U-^{13}C]-glucose in triapine-induced senescence. Metabolites were ranked based on $\log_2$ of triapine/DMSO.

REFERENCES

1. Garbe, J. C., Bhattacharya, S., Merchant, B., Bassett, E., Swisshelm, K., Feiler, H. S., Wyrobek, A. J., and Stampfer, M. R. (2009) Molecular Distinctions between Stasis and Telomere Attrition Senescence Barriers Shown by Long-term Culture of Normal Human Mammary Epithelial Cells. *Cancer Res.* **69**, 7557–7568
2. Baar, M. P., Brandt, R. M. C., Putavet, D. A., Klein, J. D. D., Derks, K. W. J., Bourgeois, B. R. M., Stryeck, S., Rijksen, Y., van Willigenburg, H., Feijtel, D. A., van der Pluijm, I., Essers, J., van Cappellen, W. A., van IJcken, W. F., Houtsmuller, A. B., Pothof, J., de Bruin, R. W. F., Madl, T., Hoeijmakers, J. H. J., Campisi, J., and de Keizer, P. L. J. (2017) Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging. *Cell*. **169**, 132-147.e16