Supplementary Materials

Radiation induced senescence reprograms secretory and metabolic pathways in colon cancer HCT-116 cells

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Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijms22094835/s1
Figure S1: Radiation induced senescence in tumor cell lines. **Figure S2:** Photomicrographs of HCT-116 cells 7 days post-IR (20 Gy). **Figure S3:** Levels of adherent senescent HCT-116 cells following 20 Gy post-IR. **Figure S4:** Effects of radiation dose (Gy) on the expression of senescent marker proteins. **Figure S5.** Levels of PRPP synthetase pathway metabolites in control and senescent HCT-116 cells. **Figure S6.** Schematic presentation of complete pathway analysis of the relative levels of metabolites in IR-induced senescent HCT-116 cells compared to proliferating cells (control) studied in this report**. Table S1.** Proteins secreted by proliferating and senescent HCT116 cells. **Table S2.** Metabolite Concentrations in Proliferating and Senescent HCT116 cells

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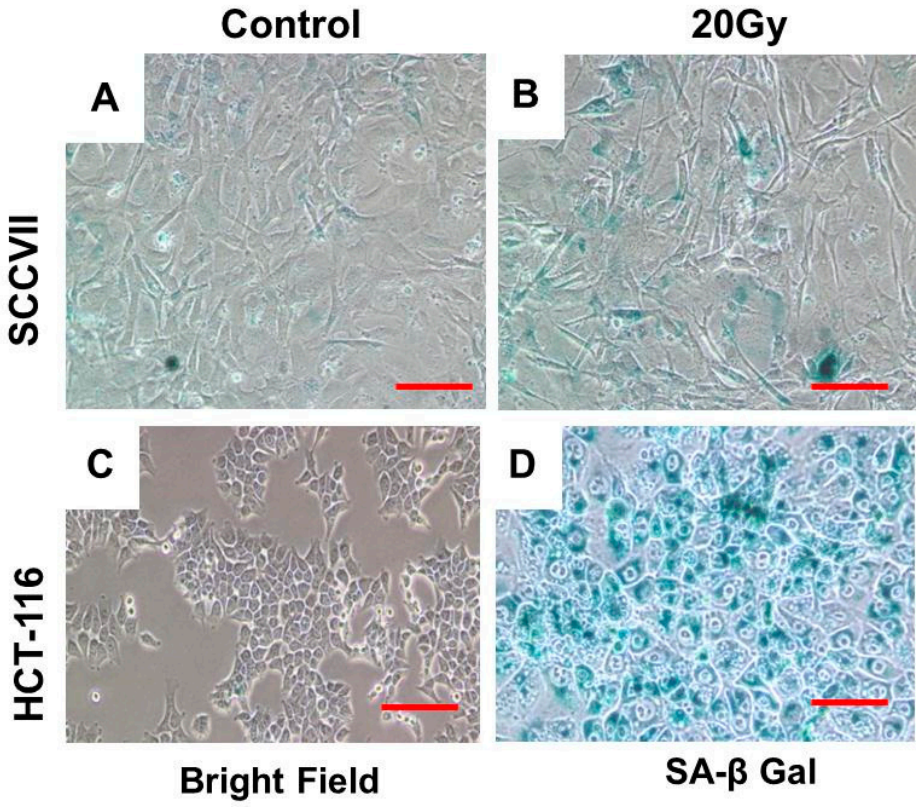


Figure S1: Radiation induced senescence in tumor cell lines. (**A-B**) SCCVII (Murine squamous cell carcinoma) and (**C-D**) HCT116 (human colon cancer) cells were exposed to the indicated dose of IR (Gy). Senescent cells were observed by SA-β gal staining 4 days post IR (**B** and **D**). **A** and **C** represents the phase-contrast microphotographs of control unexposed cells. Scale bar is 100 μm. Magnification is 200X.

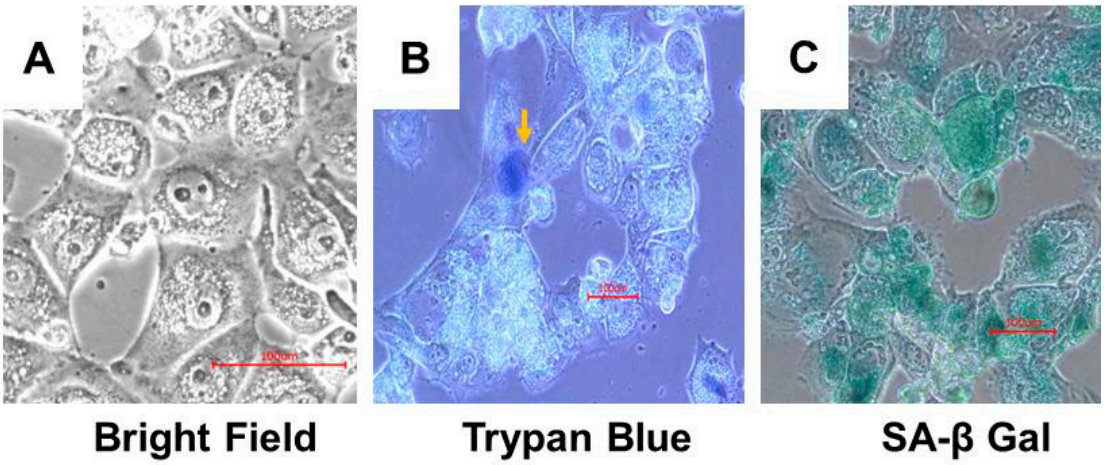


Figure S2: Photomicrographs of HCT-116 cells 7 days post-IR (20 Gy). (**A**) Representative phase-contrast photomicrographs of SCC. (**B**) Trypan blue stained cells. Yellow arrow represents the trypan blue positive dead cells. (**C**) Representative senescent cells observed by SA-β gal staining 7 days post IR (20 Gy). Scale bar is 100 µm. Magnification is 200X.

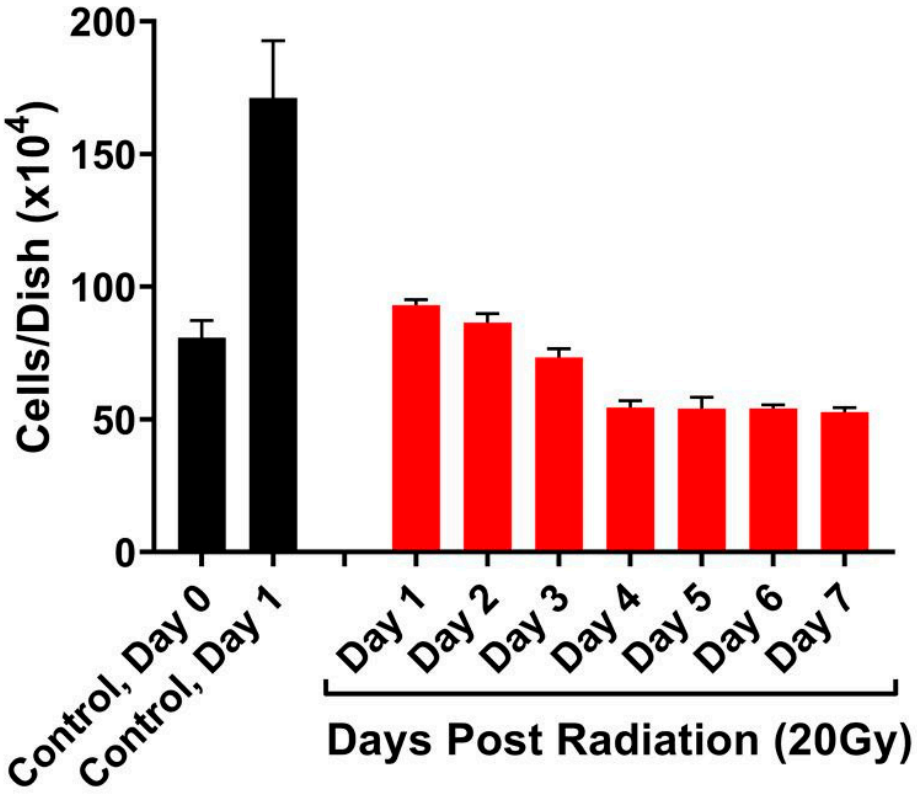


Figure S3. Levels of adherent senescent HCT-116 cells following 20 Gy post-IR. 50k cells were plated in 60 mm culture dishes. After three days media were replaced with fresh medium and exposed to 20 Gy ionizing radiation. On various days (day 0 to 7), cultures were washed with PBS to remove dead and floating cells. Attached cells were dissociated with trypsin-EDTA solution and counted using Beckman Coulter Counter. Day 0 was the day cells were exposed to IR. Triplicate dishes were used for each treatment. Results are from one typical experiment representing two other experiments.

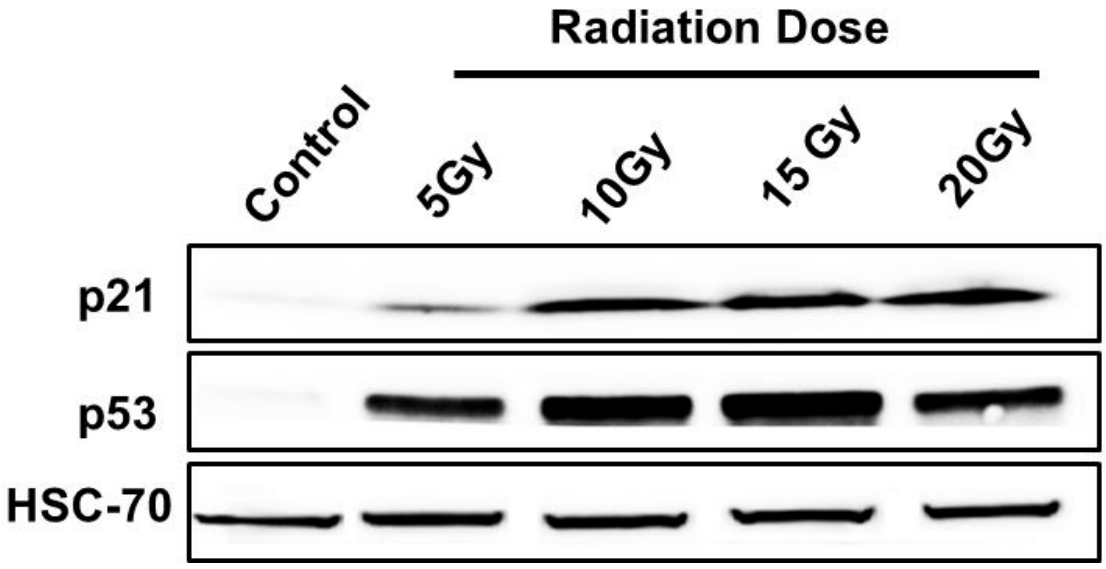


Figure S4. Effects of radiation dose (Gy) on the expression of senescent marker proteins. HCT116 cells were exposed to the indicated dose of radiation. Protein lysates were collected 4 days post radiation. Immunoblot shows the change in the expression of key senescent markers proteins, p53 and p21.

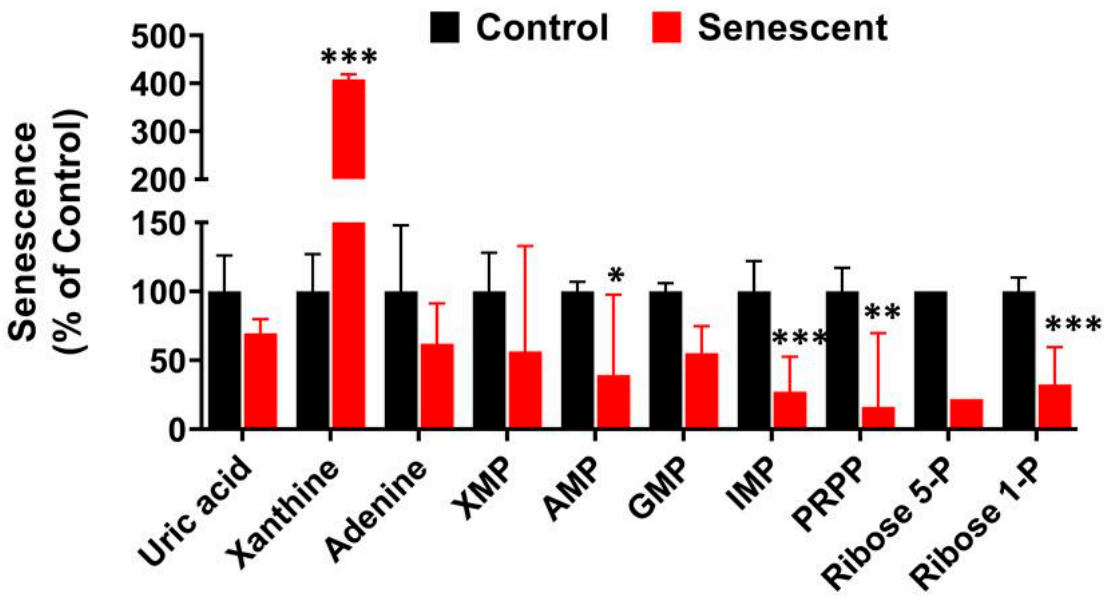


Figure S5. Levels of PRPP synthetase pathway metabolites in control and senescent HCT-116 cells. Differential expression of metabolites in the PRPP synthetase pathway is shown between the control (*black bar*) and senescent cells (*red bar*). The levels of each of the indicated metabolites is normalized to the control and represented as percentage of the control. Each bar represents mean ± standard deviation. The results are representative of samples analyzed in triplicates by the Welch's t-test * $p<0.05$, **<0.001 and ***$p<0.0001$.

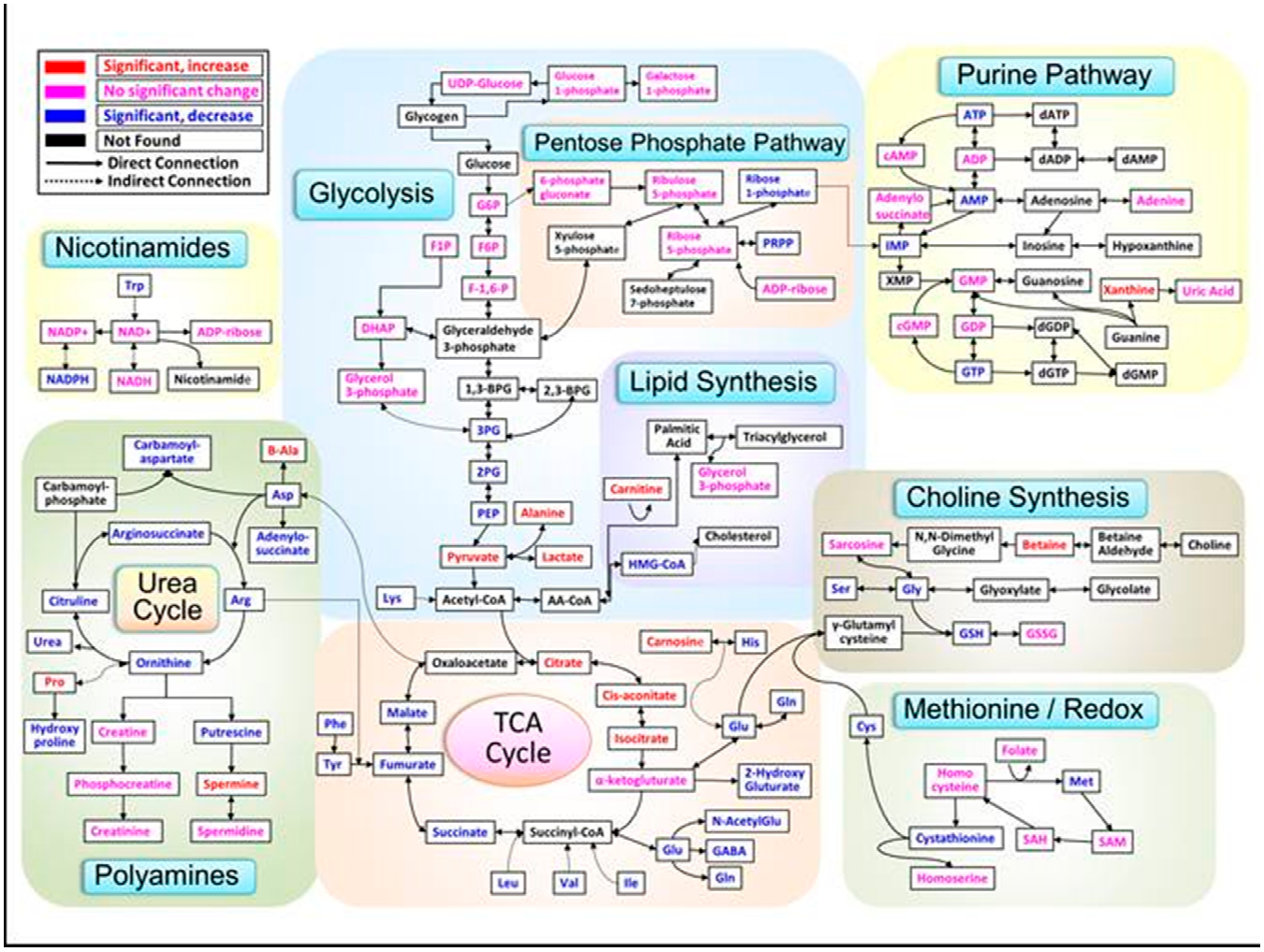


Figure S6. Schematic presentation of complete pathway analysis of the relative levels of metabolites in IR-induced senescent HCT-116 cells compared to proliferating cells (control) studied in this report. Included in the figure are metabolites which were not measured in this report, but which make up part of the various pathways listed. Of the metabolites shown in Table S2, 9 were increased, 37 were decreased, and 29 showed no change. The boxes with **red letters** (Statistically significant increases), **blue letters** (statistically significant decreases) and **pink letters** (no significant changes) indicate metabolite levels in senescent cells in comparison to proliferating cells. White boxes with **black letters** indicate metabolites not detected.

Table S1. Proteins secreted by proliferating and senescent HCT116 cells.

Protein Name	Total Con*** (pg/mg)	Total Sen *** (pg/mg)	Ratio (Sen/Pro)	background control § (pg/mg)
Angiogenesis Mediators				
Endothelim-1	15.8(1.8)**	127(6.3)	8	16.7
HB-EGF	31.7(4.3)*	279(8.5)	8.8	2.1
IL-8	216(20)*	3,161(161)	14.6	8.2
PLGF	62(6)*	572(24)	9.3	0.94
VEGF-A	1,766(308)*	675(42)	0.38	2.5
Interleukins				
IL-4	4.01(1.1)*	1.55(0.2)	0.39	1.1
IL-6	1.06(0.1)**	2.65(0.2)	2.5	0.94
IL-15	5.09(0.5)**	8.75(1.1)	1.7	4.7
IL-18	1.04(0.1)**	1.48(0.1)	1.4	0.94
IL-27	15.5(13)*	182(13)	11.7	15.9
IL-8 (CXCL8)	216(20)*	3,161(161)	14.6	8.2
Chemokines				
CCL-2 (MCP-1)	6.41(0.5)**	13.2(1)	2	5.8
CCL-22 (MDC)	0.92(0.1)**	11.4(1)	12.5	0.94
CXCL-1 (GROα)	18.4(0.9)*	379(20)	20.6	2.1
CXCL-10 (IP-10)	4.1(0.3)**	109(6)	26	4.6
CCL-5 (RANTES)	2.73(0.4)**	500(20)	183	2.1
MMPs/TIMPs				
MMP-1	59.1(4.1)**	619(133)	10.5	58.9
TIMP-1	21,098(3,090)*	75,824(6,731)	3.6	1178
TIMP-2	63,730(5,848)*	230,269(14,724)	3.6	1178
TIMP-3	1,419(100)**	36,919(4,801)	26	1178
TIMP-4	55(66)*	65(74)	1.1	24.6
Growth Factors				
M-CSF	71.3(4.2)**	319(42)	4.47	61.4
GM-CSF	0.32(0.1)**	46.6(5.5)	144	0.24
TGF-α	153(17)*	980(191)	6.4	2.4
PDGF-αα	778(48)*	2,592(270)	3.3	20
PDGF-ββ/αβ	115(19)*	1,917(219)	16.7	9.1
Inflammatory mediators				
IFN-α	15(1.8)**	10.4(0.4)**	0.69	21
IFN-γ	2(0.1)**	1.4(0.1)**	0.71	1.8
TNF-α	11.9(1.5)**	10.2(0.1)	0.86	10.1
TNF-β	36.1(7.7)*	14.6(1.3)	0.4	2.1
IL-1α	7.5(0.3)**	27.4(9)	3.6	7.3
IL-1β	3.6(1.8)**	2.1(0.4)**	0.58	3.1

Concentrations corrected for background medium levels. ** Not different from background medium levels. Background was not subtracted from either control or Senescence cells. *** Values in () are standard deviations. Con -- Control, Sen – Senescence. (pg/mg) – picogram chemokine/mg protein. § Background of medium blank using control cell numbers for calculation. Essentially, medium blank (with serum) had similar levels as medium taken from control cells indicating that control cells were not actively secreting these proteins.

Table S2. Metabolite Concentrations in Proliferating and Senescent HCT116 cells

	nmol metabolite/gm protein					
Metabolite	**Mean (Con)**	**sd (Con)**	**Mean (Sen)**	**sd (Sen)**	**ratio (Sen/Con)**	**pvalue**
AMP	1,065	70	419	244	0.393	0.036
ADP	10,094	4,085	5,599	2,878	0.495	NS
ATP	180,699	5,954	123,393	939	0.683	0.003
total Adenylate	191,857	7,993	129,411	2,214	0.727	0.015
Adenylate Energy Charge	0.968	0.010	0.975	0.013	1.007	NS
GMP	266	16	147	29	0.551	NS
GDP	1,999	873	1,133	556	0.567	NS
GTP	46,839	3,226	31,362	1,382	0.670	0.007
total Guanylate	49,105	4,015	32,593	812	0.664	0.016
Guanylate Energy Charge	0.975	0.007	0.979	0.011	1.005	NS
Phosphocreatine	63,229	7,367	48,780	1,767	0.771	NS
creatine	218,131	8,345	229,766	10,593	1.100	NS
Phosphocreatine/creatine	0.290	N.A.	0.212	N.A.	0.73	N.A.
Glutathione (GSH)	567,379	48,943	347,769	6,803	0.613	0.015
Glutathione (GSSG)	67,921	37,946	23,976	1,588	0.353	NS
Total Glutathione	703,220	26,965	395,720	4,727	0.563	0.002
GSH/GSSG ratio	10.154	4.835	14.556	1.164	1.433	NS
NADPH	608	88	305	103	0.502	0.019
NADP+	984	139	814	199	0.827	NS
NADPH/NADP+ ratio	0.622	0.095	0.407	0.198	0.655	NS
NADH	1,727	287	2,040	45	1.182	NS
NAD+	22,041	1,985	19,518	1,051	0.886	NS
NADH/NAD+	0.078	0.006	0.105	0.004	1.343	0.005
Phosphoenolpyruvic acid	304	55	50	5	0.164	0.015
3-Phosphoglyceric acid	1,668	167	485	147	0.291	0.001
2,3-Diphosphoglyceric acid	280	N.A.	44	5	0.157	N.A.
2-Phosphoglyceric acid	171	22	54	23	0.313	0.003
Dihydroxyacetone phosphate	3,738	2,041	875	239	0.234	NS
Fructose 1,6-diphosphate	7,841	1,983	3,860	609	0.492	NS
Fructose 6-phosphate	465	84	396	37	0.852	NS
Fructose 1-phosphate	976	150	1,633	340	1.674	NS
Glucose 6-phosphate	2,079	342	1,729	52	0.831	NS
Glucose 1-phosphate	190	49	179	87	0.941	NS
UDP-glucose	10,759	218	10,780	550	1.002	NS
Lactic acid	314,791	31,783	642,905	49,994	2.042	0.001
Malic acid	40,308	3,843	23,510	379	0.583	0.016
Fumaric acid	7,480	710	3,827	453	0.512	0.003

Table S2 Metabolite Concentrations in Proliferating and Senescent HCT116 cells

Succinic acid	**3,535**	**735**	**1,117**	**160**	**0.316**	**0.025**
2-Oxoglutaric acid	11,319	2,181	9,622	823	0.850	NS
Isocitric acid	894	188	1,810	415	2.024	0.045
cis-Aconitic acid	412	50	957	37	2.322	<0.001
Citric acid	26,539	3,172	47,287	2,846	2.289	0.002
Pyruvic acid	3,853	369	12,685	880	3.292	0.001
Coenzyme A	997	217	993	88	0.996	NS
6-phosphoglucoronic acid	670	76	375	227	0.6	0.1
Ribulose 5-phosphate	148	222	19	23	0.131	NS
Ribose 1-phosphate	186	19	60	16	0.325	0.001
Ribose 5-phosphate	148	222	19	23	0.131	NS
PRPP	1,552	269	250	134	0.161	0.005
Glutamine	835,869	24,846	123,183	15,442	0.147	<0.001
Serine	135,454	5,724	22,262	832	0.164	0.001
Methionine	69,023	3,025	20,849	213	0.302	0.001
Histdine	55,560	1,940	16,830	341	0.303	0.001
Tyrosine	128,910	4,396	40,337	684	0.313	0.001
Tryptophan	23,874	903	7,698	169	0.322	0.001
Isoleucine	193,489	6,330	63,596	784	0.329	0.001
Leucine	192,974	5,912	63,921	2,024	0.331	<0.001
Phenylalanine	126,237	3,529	42,429	499	0.336	<0.001
Valine	196,806	4,158	73,645	414	0.374	<0.001
Glutamic acid	1,035,628	18,784	411,642	11,381	0.397	<0.001
Arginine	12,313	610	5,603	189	0.455	0.001
Aspartic acid	56,680	1,799	27,245	834	0.481	<0.001
Lysine	38,086	1,515	21,007	222	0.552	0.002
Threonine	628,246	25,517	405,872	4,832	0.646	0.003
Glycine	746,033	33,526	509,412	5,665	0.683	0.006
Cysteine	780	130	559	N.A.	0.718	N.A.
Asparagine	64,026	2,859	48,163	973	0.752	0.006
Alanine	317,677	13,377	477,591	13,017	1.503	<0.001
Proline	N.A.	N.A.	192,390	9,354	1<	N.A.
Spermine	894	171	2,078	537	2.323	0.051
Spermidine	1,460	106	1,409	250	0.965	NS
Putrescine	2,212	180	298	41	0.135	0.002
Ornithine	1,871	37	743	100	0.397	0.001
5-Adenosylmethionine	1,691	875	816	51	0.483	NS
Citruline	2,142	55	464	34	0.217	<0.001
Putrescine/Spermidine	1.517	0.106	0.213	0.009	0.140	0.002