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Supplemental information

Integration of transcriptomes of senescent cell models with multi-tissue patient samples reveals reduced COL6A3 as an inducer of senescence

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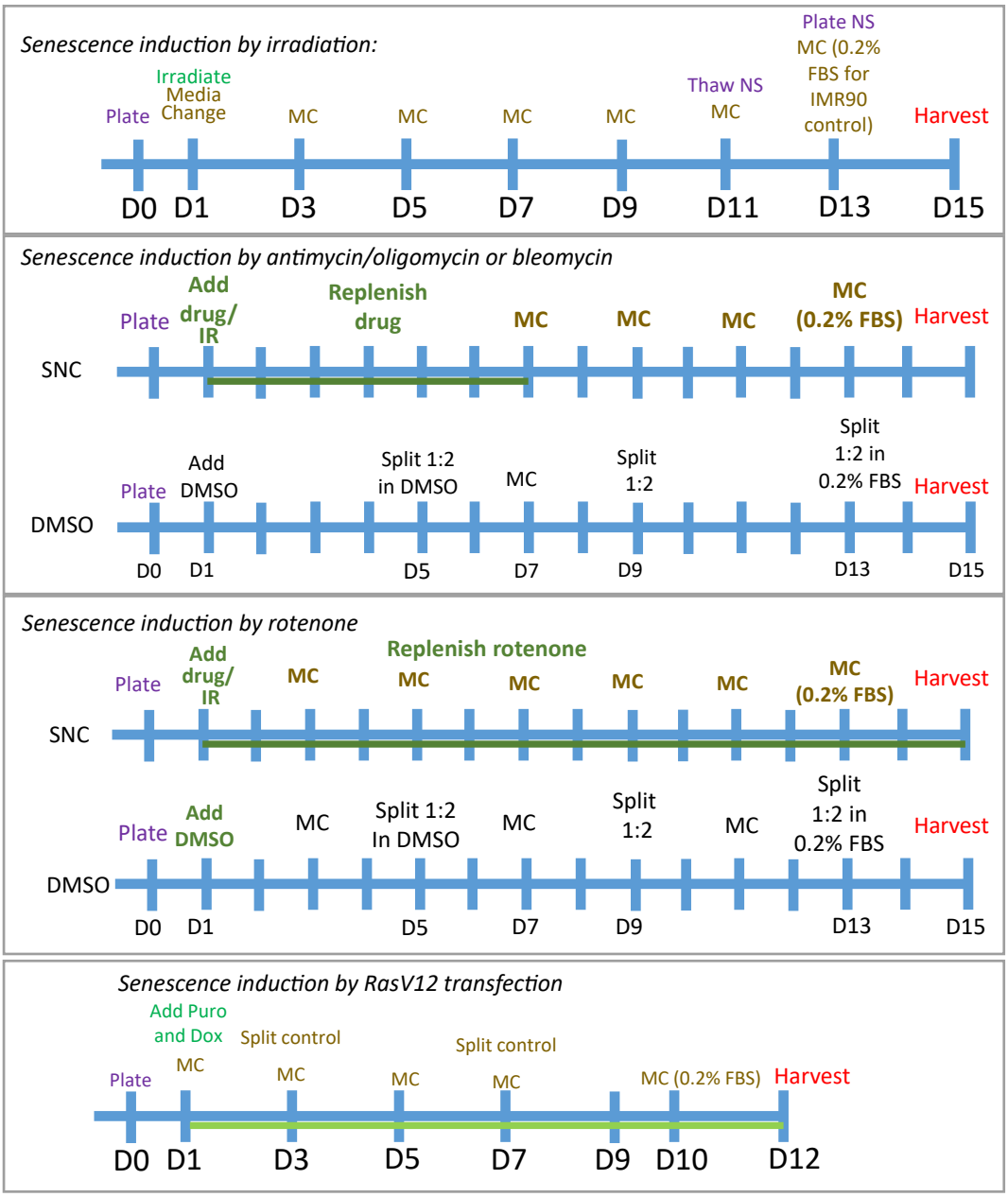
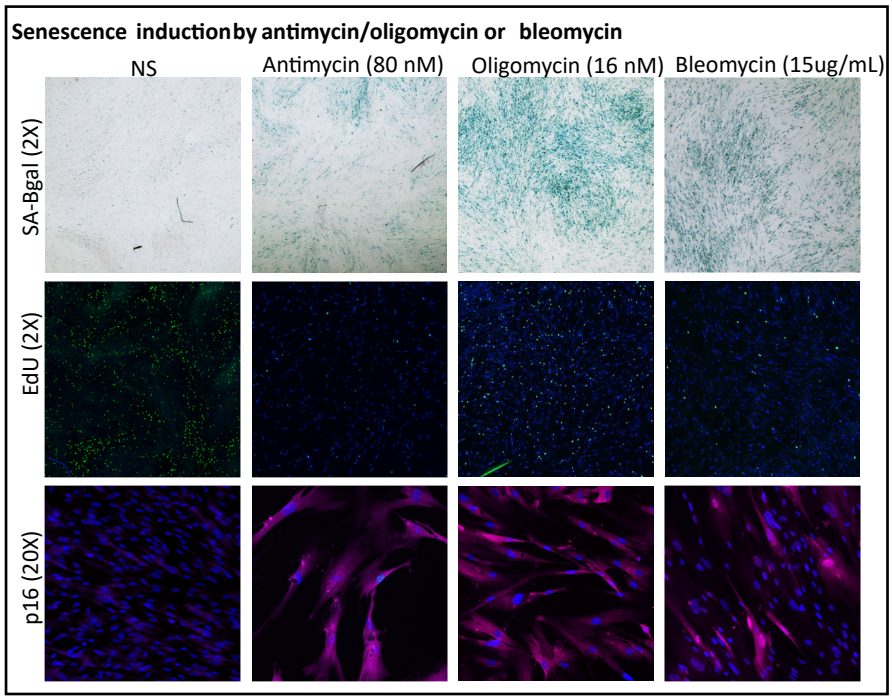
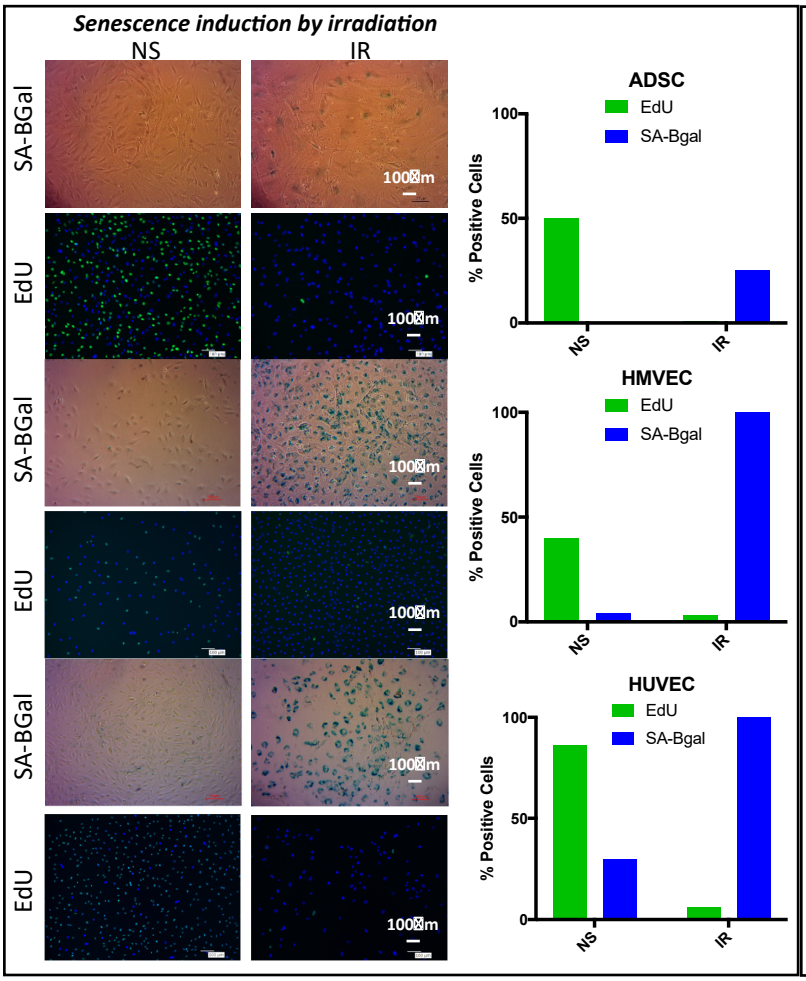
Supplementary Figure 1:

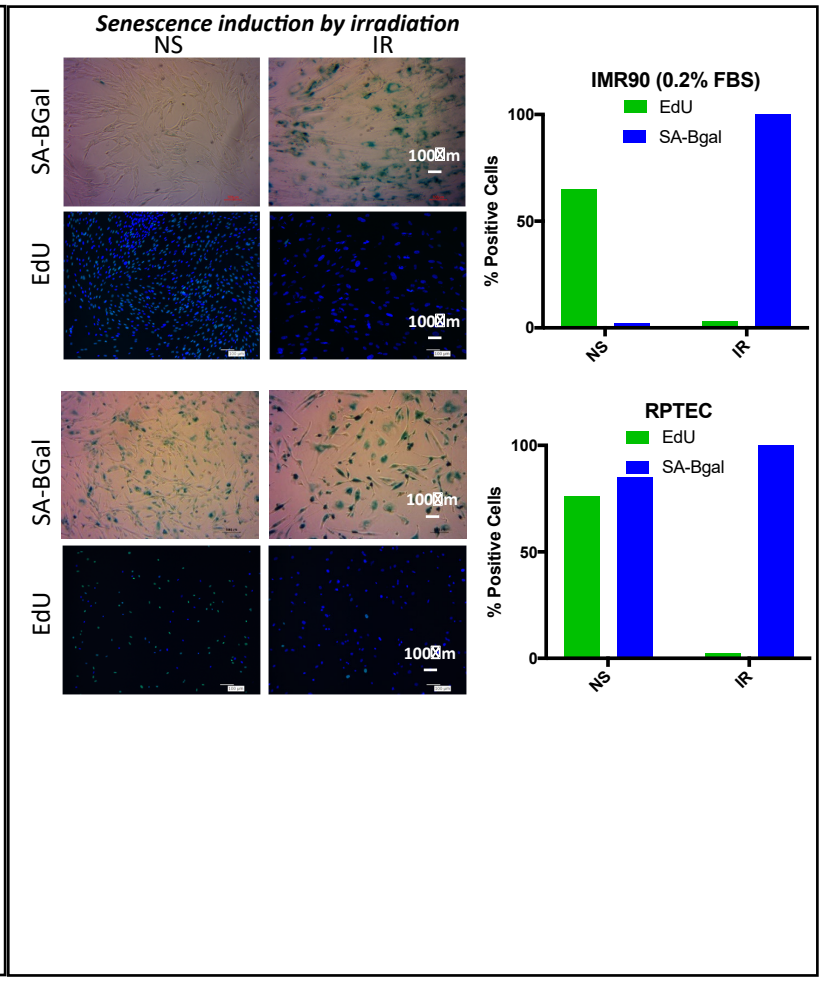
Protocols:

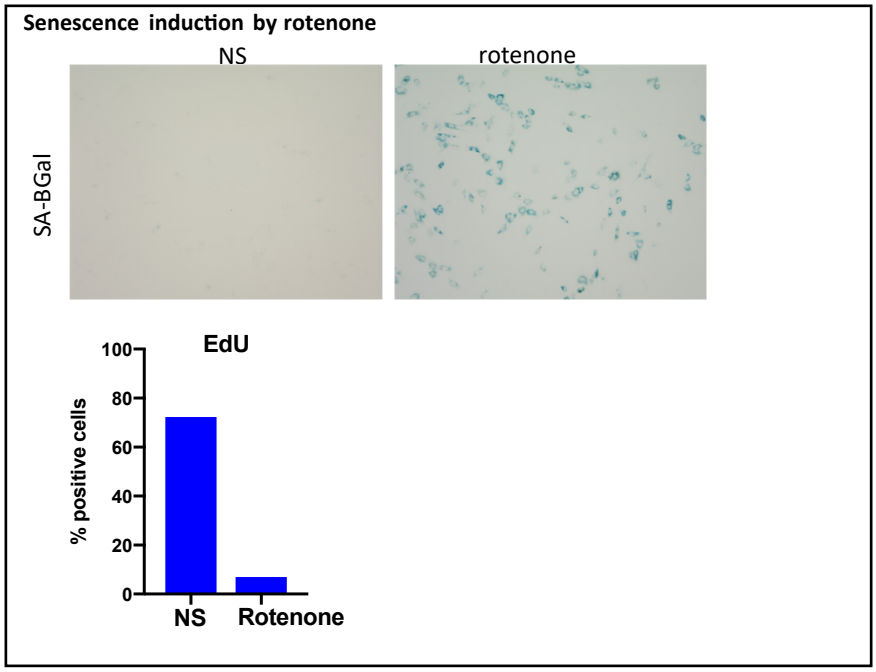


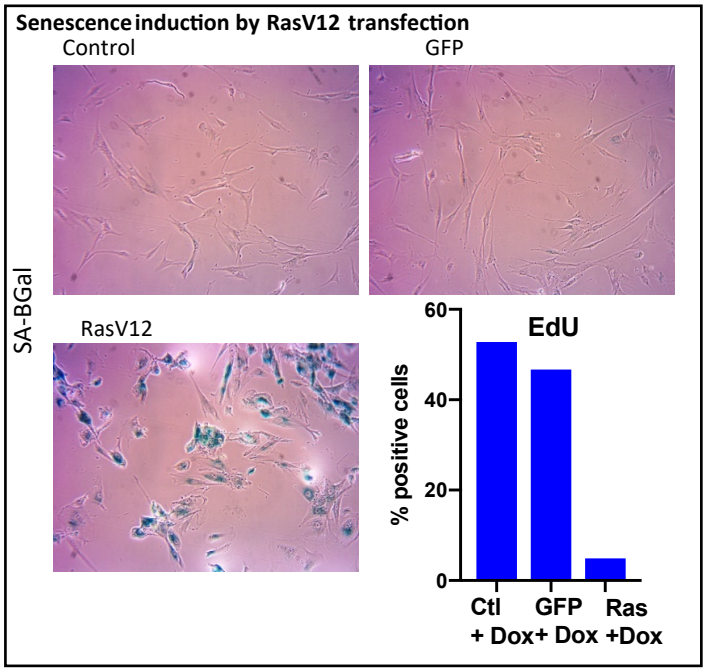


Supplementary Figure 1



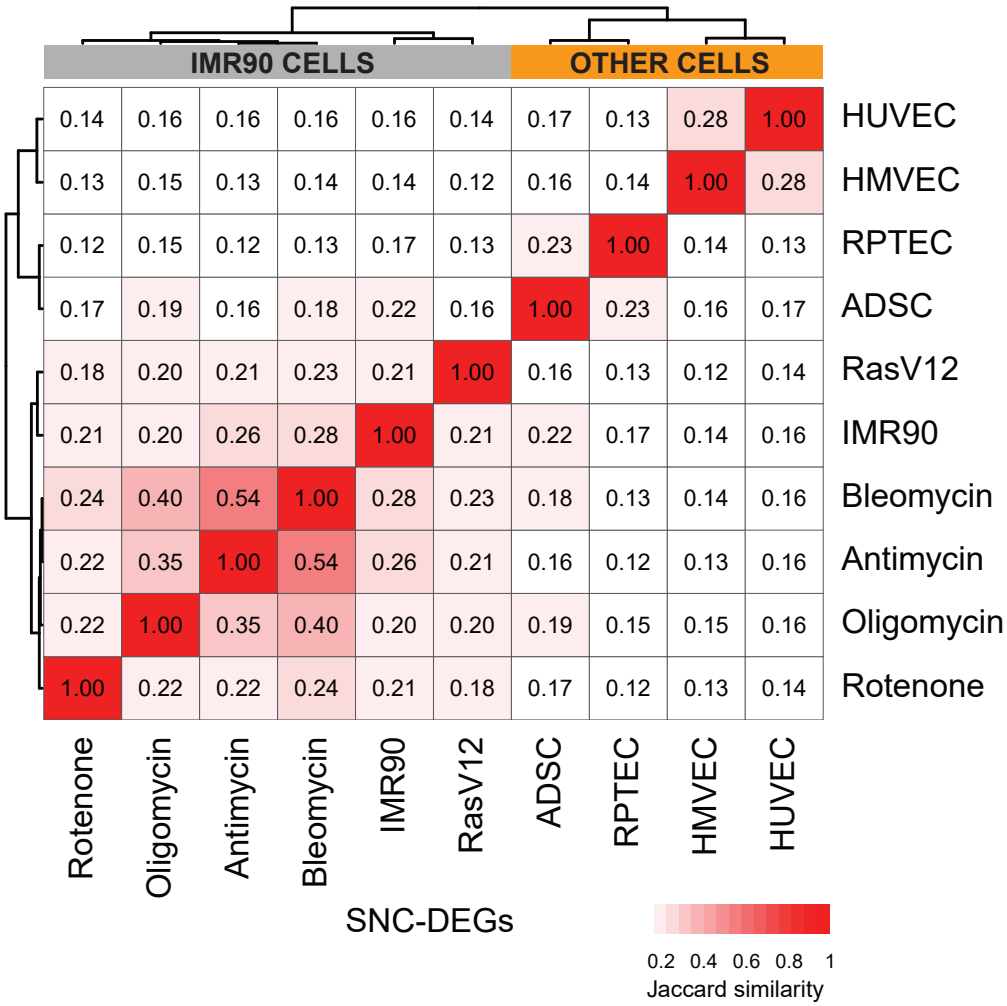






Supplementary Fig. 1 (Related to Figure 1): Confirmation of increased SA-β-galactosidase staining, and decreased EdU staining in the various in vitro senescent cell model systems. a Schematic overview of the experimental details for senescence induction. EdU and SA-β-Gal staining and quantification of b irradiated ADSC, HMVEC, HUVEC, RPTEC and IMR90 cells; Antimycin, oligomycin, bleomycin, rotenone and RasV12 transfected IMR90 cells. NS = not senescent; SNC = senescent; DMSO = dimethyl sulfoxide; D = day; MC = media changed; Plate = cells seeded for experiments; Harvest = cells collected for analyses; EdU = 5-ethynyl-2'-deoxyuridine; SA-β-Gal = senescence associated beta galactosidase.

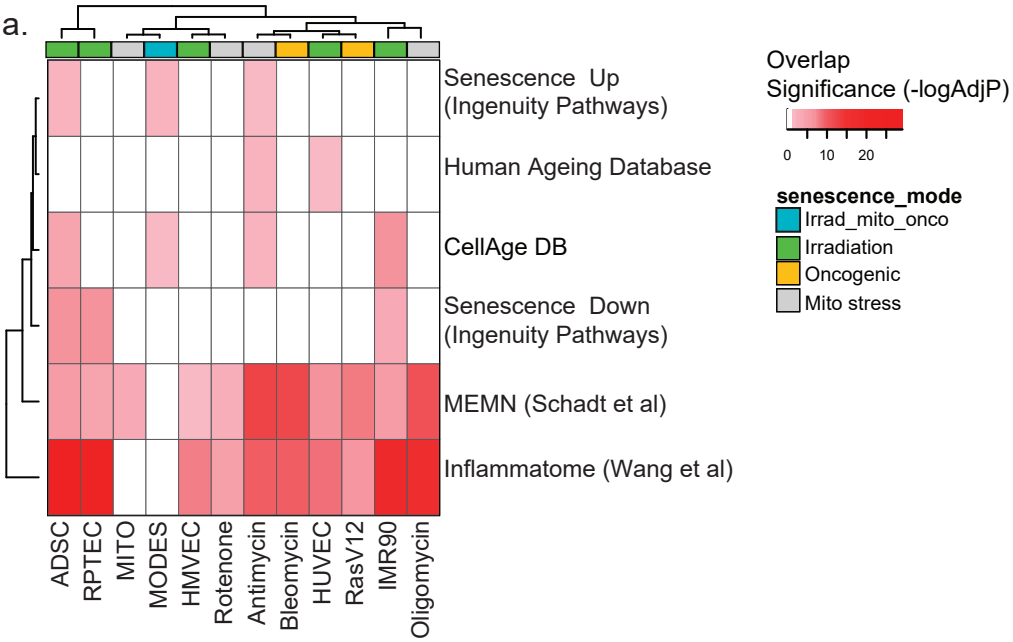
Supplementary Figure 2



Supplementary Fig. 2 (Related to Figure 1): A heatmap summarizing the Jaccard indices reflecting gene signature similarity across the 10 senescence DEGs.

Supplementary Figure 3

a.

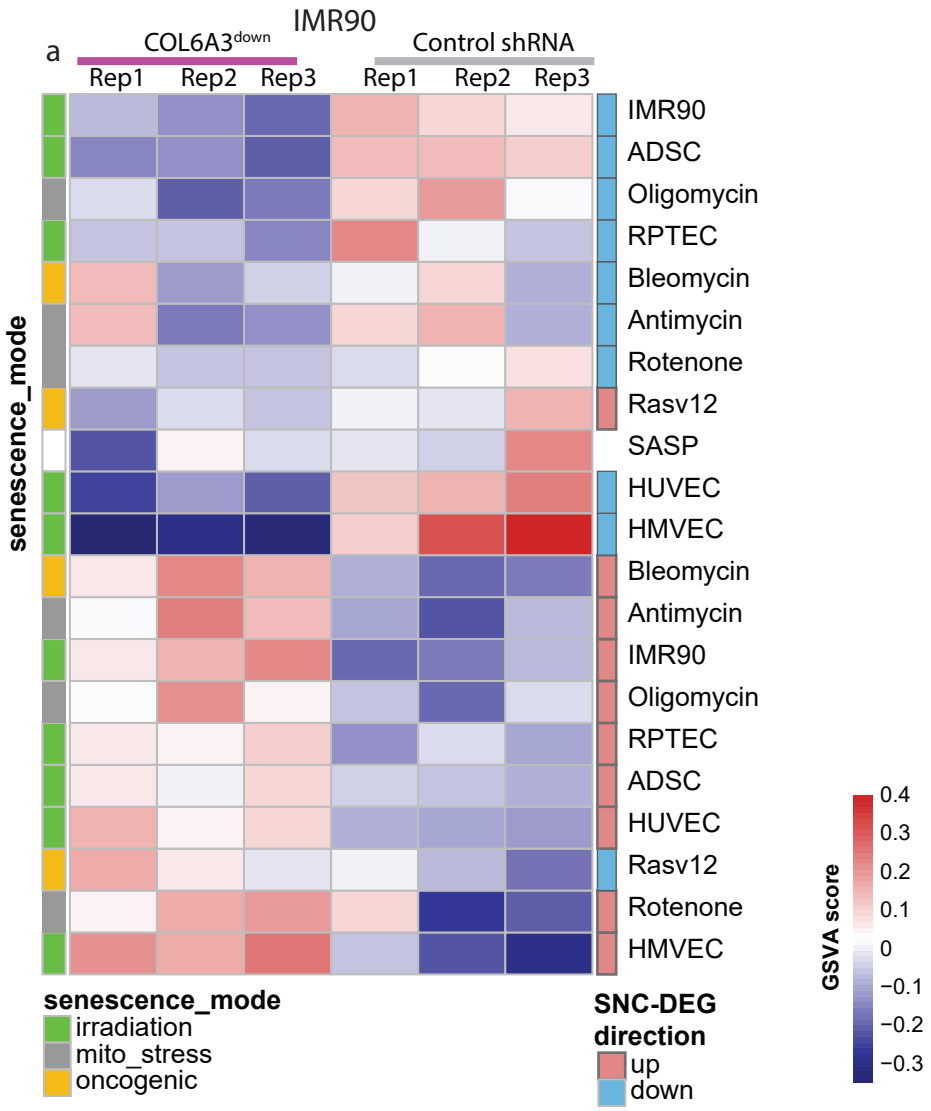


b.

Module	Enriched cell types	FE	Adj P
224.TS_LIV	Cycling_SAMac_fromMPclusterS8	14.2	3.92E-38
	Cycling_cDC2_fromMPcluster S8	23.5	9.08E-31
	Cycling_NK_cell	16.0	3.96E-28
	Cycling_T_cell	24.2	1.47E-24
	Cycling_cDC1_fromMPclusterS8	8.2	4.90E-19
	Cycling_KC_fromMPclusterS8	14.0	1.75E-17
	Gamma delta T cells	42.4	3.77E-17
28.CT_LIV	Fibroblasts	7.7	1.02E-26
	SAMes	7.6	4.60E-23
	Smooth muscle cells	9.6	5.58E-18
	Mesenchyme _VSMC	12.9	3.56E-07
	Hepa c stellate cells	8.2	2.26E-06
	Endothelial cells	3.9	8.21E-06
	Peritubular myoid cells	10.3	2.39E-04
	Myofibroblasts	16.5	6.00E-03
	Cardiomyocytes	4.4	2.24E-02
	Pancrea c stellate cells	7.7	2.47E-02
	SAEndo_1	3.1	3.77E-02
37.TS_SF	Macrophages	15.4	2.61E-14
	TM_3_fromMPclusterS8	17.7	6.26E-08
	SAMac_2	4.0	7.15E-07
	Microglia	14.9	2.44E-06
	Kupffer cells	18.5	1.38E-04
	Dendri c cells	9.1	5.33E-04
	SAMac_2_fromMPclusterS8	18.2	1.83E-02
	KC_1_fromMPclusterS8	5.9	2.05E-02
219.TS BLOOD:	Neutrophils	7.4	8.69E-07

Supplementary Fig. 3 (Related to Figure 3): a Heatmap of the significance of enrichment across the 10 senescence DEGs in curated gene sets (Chen et al., 2008; Wang et al., 2012) or Ingenuity Pathways CellAge or HumanAgeing databases (SupplementaryData5-6). b. Summary of the fold enrichment (FE) and significance (Bonferroni adj p value) of the Fisher's Exact test for the enrichment of genes in 224.TS_LIV, 28CT_LIV, 37.TS_SF and 219.TS_BLOOD SAMS according to cell type enrichment gene sets (see methods).

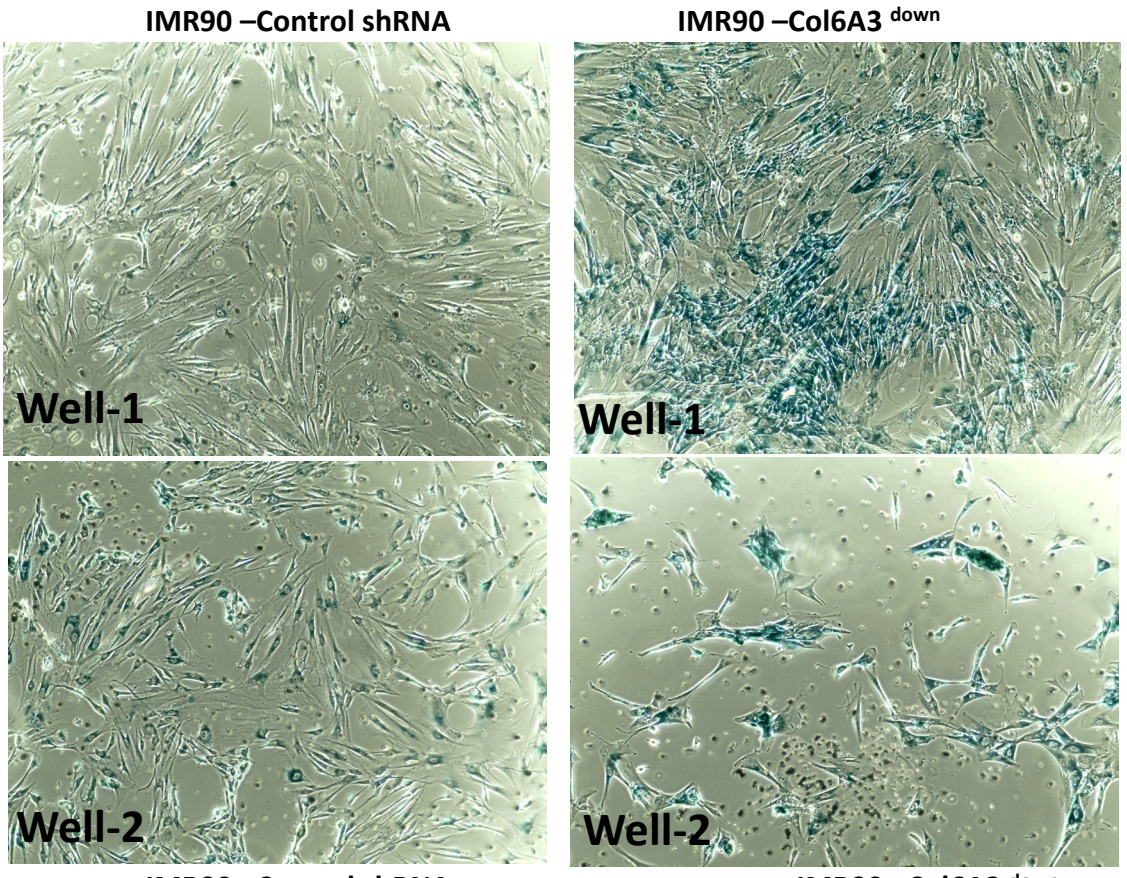
Supplementary Figure 4



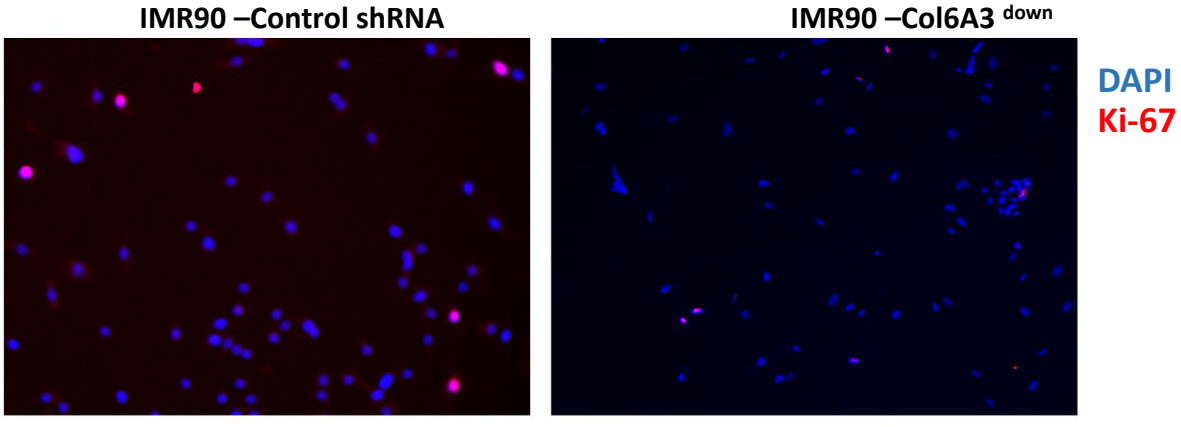
Supplementary Fig. 4 (Related to Figure 4): Heatmap summarizing the activity of the 10 senescence DEGs in the IMR90 COL6A3 knockdown and control shRNA transcriptomes using gene set variance analysis (GSVA). Generally the scores of genes down-regulated with senescence were lower expressed in IMR90 cells with reduced expression of COL6A3.

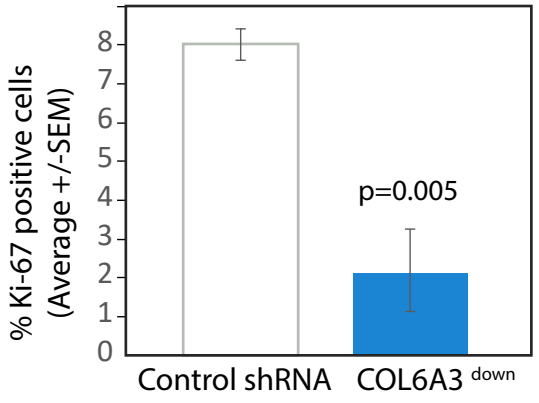
Supplementary Figure 5

a **SA-BGal Staining:**



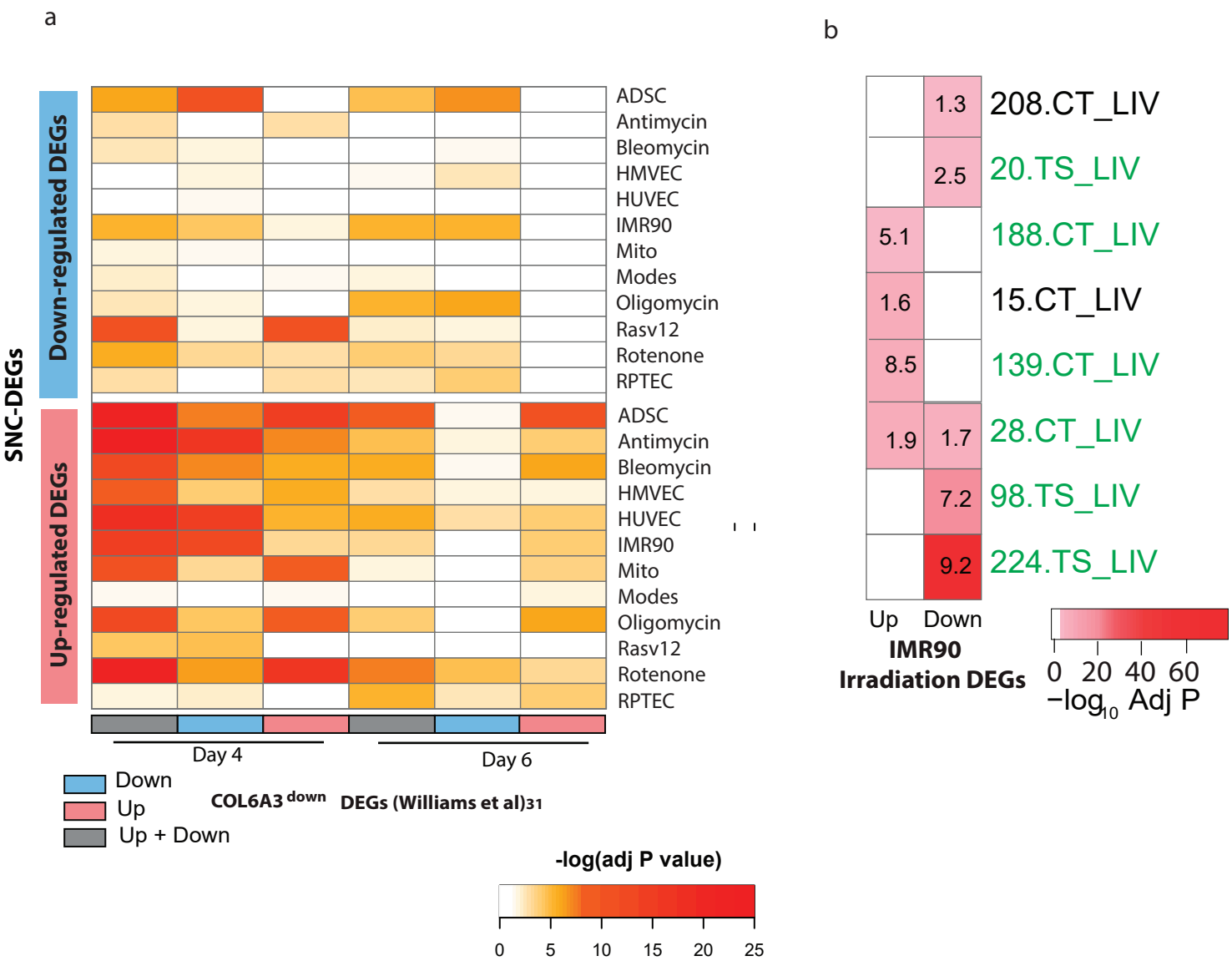
b





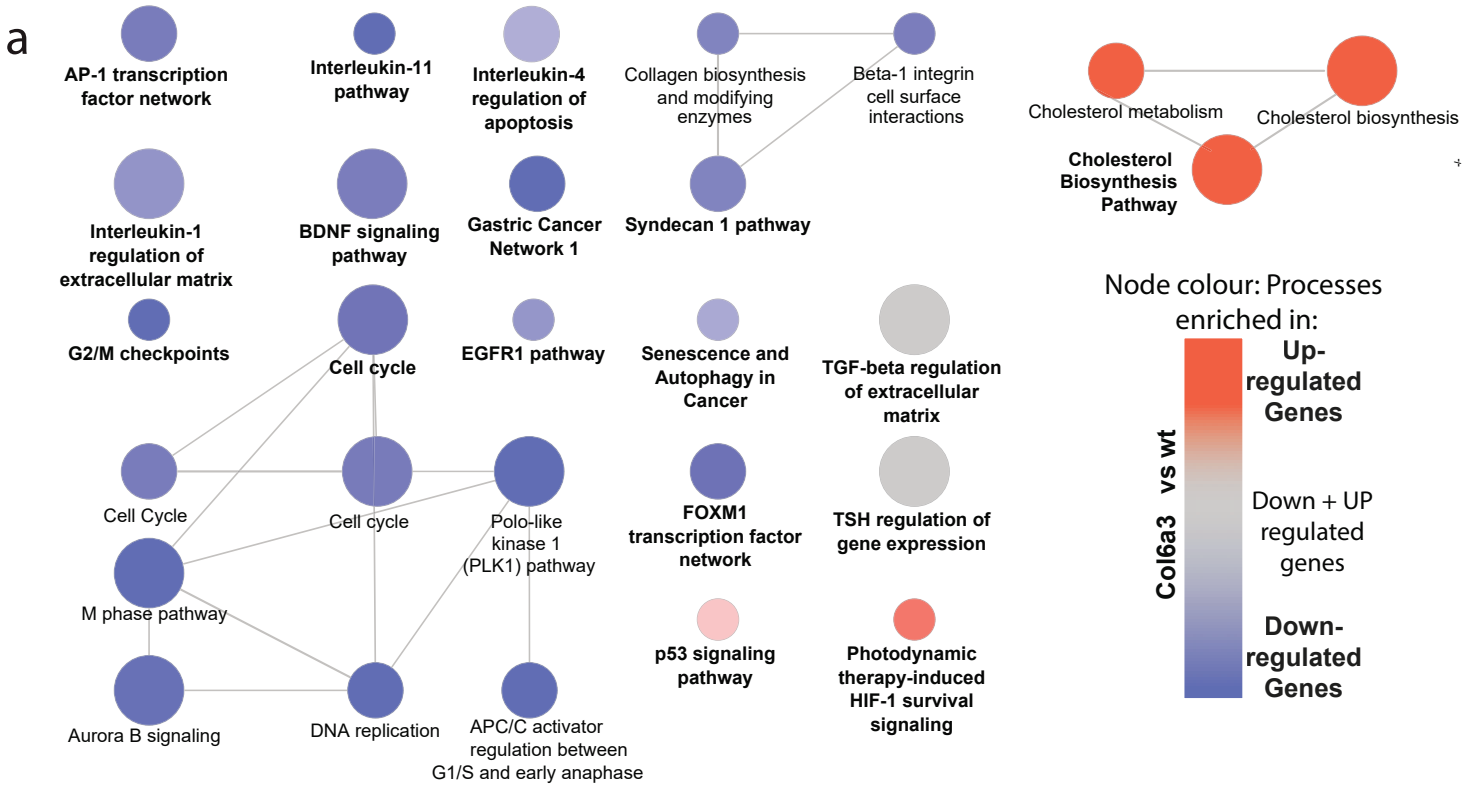
Supplementary Fig. 5 (Related to Figure 4 and 5) Validation of COL6A3 in senescence processes. a. Images of IMR90 control shRNA and COL6A3 knockdown cells following staining for senescence-associated beta-galactosidase activity (see methods). Two images are shown from two separate culture wells for each condition. IMR90 cells with reduced COL6A3 show visibly greater levels of beta-galactosidase activity supporting an increase in senescence cells. b. Representative images of IMR90 cells treated with control shRNA or COL6A3 shRNA, fixed and stained with the cell cycle marker protein, Ki67. Cellular nuclei were marked with DAPI. The percent of Ki67 positive cells for each condition were quantified using ImageJ and values are reported in the bar graph and are the result of quantifying ~600 cells per condition.

Supplementary Figure 6



Supplementary Fig. 6 (Related to Figure 4 and 5) Enrichment of senescence DEGs in COL6A3 depleted cell lines: a. Heatmap of the significance of enrichment of the COL6A3 knockdown DEGs in myofibroblasts curated from Williams et al following 4 or 6 days of perturbation in the various senescence DEGs. b Heatmap summarizing the enrichment of liver modules (green text highlighting those that are SAMs) in IMR90-irradiated DEGs, tested as either up- or down-regulated gene sets. Values in cells are the enrichment fold-change.

Supplementary Figure 7



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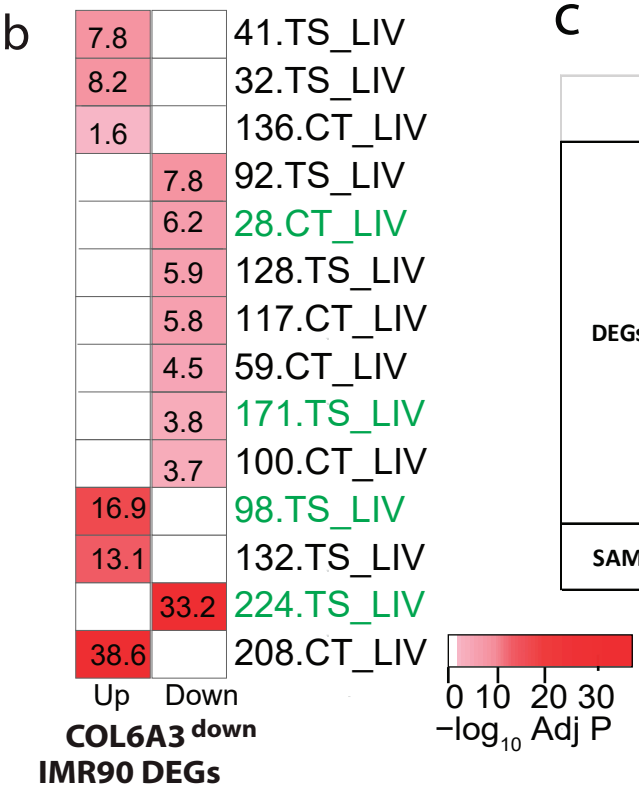
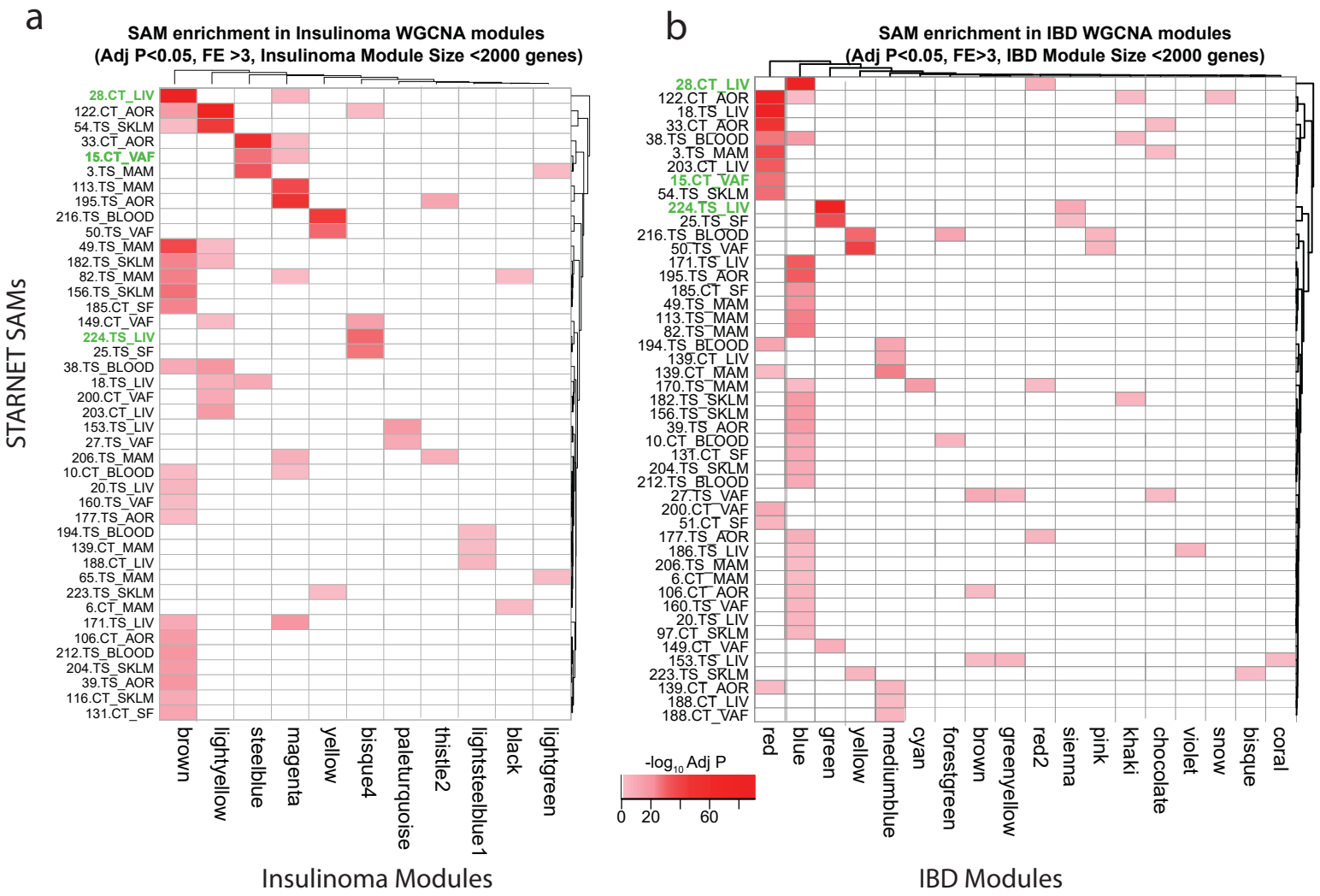


c

			E2F TARGETS	G2M CHECKPOINT	MYC TARGETS V1	224.TS LIV
DEGs	COL6A3^{down}	GSEA (NES)	-2.50	-2.18	-2.48	-2.83
	Oligomycin		ns	ns	1.74	-2
	Antimycin		ns	ns	1.76	ns
	Bleomycin		ns	ns	ns	-1.26
	Rotenone		ns	ns	ns	ns
	RasV12		ns	ns	ns	ns
	Irradiation		-2.44	-2.40	-2.19	-2.55
	RPTEC		-2.23	-2.16	-2.09	-2.28
	ADSC		-2.12	-2.07	-2.04	-2.31
	HMVEC		-1.87	-1.52	-2.01	-1.78
	HUVEC		-2.35	-1.87	-2.59	-2.26
SAM	224.TS LIV	FET (FE)	26.4	26.3	7.02	NA

Supplementary Fig. 7 (Related to Figure 4 and 5) Biological enrichment analysis of COL6A3down DEGs (logFC>|0.5|, adjP<0.05). a. According to KEGG, BioPlanet and Wiki Pathway databases by ClueGO. Only pathways significantly enriched are shown (at Bonferroni adj P<0.05). Node coloring represents pathways found more enriched in either up (red) or down (blue) regulated genes or common to both (grey). Node size reflect pathway term adj p value (largest p<0.0005, middle p=0.0005-.005, smallest p=0.005-.05). Network edges connect pathway terms sharing common gene members. b Heatmap summarizing the enrichment of liver modules (green text highlighting those that are SAMs) in I MR90-COL6A3 DEGs. Values in cells are the enrichment fold-change. c Results of a gene set enrichment analysis (GSEA) for various gene sets associated with cell cycle according to Hallmark pathways and liver SAMs. Values shown are the normalized enrichment scores (NES) colored blue to indicate negative or red to indicate positive enrichment values. The ranking metric for gene sets was the direction of logFC * -log10 p-value. The bottom row is the result of a Fisher's Exact Test (FET) in the same pathways as an equivalent ranking was not possible for SAMs. NA = not applicable.

Supplementary Figure 8

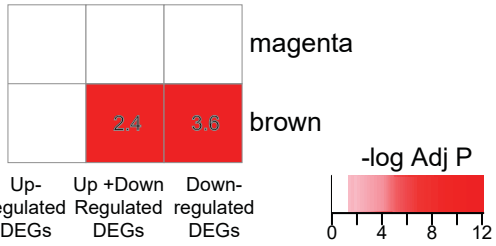


Supplementary Fig. 8 (Related to Figure 2): Enrichment of STARNET SAMs in co-expression networks generated in 3 independent human cohorts. Genes associated with each SAM identified in the STARNET cohort were tested for enrichment in co-expression network modules generated using transcriptome data either from: a) insulinoma tissues from a pancreatic neuroendocrine tumor (PNET) cohort; b) gut biopsies from an inflammatory bowel disease (IBD) cohort; or c) various brain regions of an Alzheimer's disease (AD) cohort. Heatmaps summarize the level of significance (at Bonferroni adj p value <0.05 and in modules with geneset sizes <2000). SAMs listed in green are those that were highlighted in the paper. d) Modules found enriched in the PNET, IBD or AD cohorts in 28.CT_LIV SAM were tested for enrichment in COL6A3down DEGs, either as up-, down- or up + down regulated gene sets. The heatmap summarizes the level of significance of the Fisher's exact test (Bonferroni adj p value) and the values in the cell are the fold enrichment.

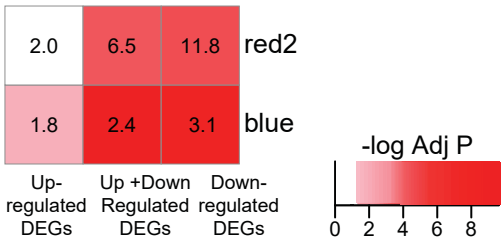
Supplementary Figure 8

C **SAM enrichment in Alzheimer's WGCNA modules (Adj P <0.05, FE>3, Alz Module Size < 2000 genes)**

d **Insulinoma modules enriched in 28.CT_LIV and COL6A3^{down} DEG overlap (FE, Adj P<0.05)**



IBD modules enriched in 28.CT_LIV and COL6A3^{down}DEG overlap (FE, Adj P<0.05)



Alzheimer's modules enriched in 28.CT_LIV and COL6A3^{down} DEG overlap (FE, Adj P<0.05)