

**Supplementary figures and tables**

**Sulforaphane delays fibroblast senescence by curbing cellular glucose uptake, increased glycolysis and oxidative damage**

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TABLE S1: Transcriptomic analysis of genes with changed expression in senescence of MRC-5 fibroblasts *in vitro*.

No	Gene ID	Name	P-value	$\Delta\text{Log}_2$ expression
1.	NR5A2	Nuclear receptor subfamily 5 group A member 2	0.043	6.83
2.	NPY	Pro-neuropeptide Y	0.022	6.50
3.	DNAJC6	Putative tyrosine-protein phosphatase auxilin	0.002	6.41
4.	APBB1IP	Amyloid beta A4 precursor protein-binding family B member 1-interacting protein	0.007	5.91
5.	ATCAY	Caytaxin	0.019	5.91
6.	ACKR4	Atypical chemokine receptor 4	0.003	5.64
7.	TRIM63	E3 ubiquitin-protein ligase	0.012	5.26
8.	PDE11A	Dual 3',5'-cyclic-AMP and -GMP phosphodiesterase 11A	0.044	5.18
9.	FOXL2	Forkhead box protein L2	0.029	5.01
10.	HSD17B6	17-beta-hydroxysteroid dehydrogenase type 6	0.044	4.68
11.	LYPD5	Ly6/PLAUR domain-containing protein 5	0.020	4.43
12.	TMEM176A	Transmembrane protein 176A	0.020	4.39
13.	C11orf70	Uncharacterized protein C11orf70	0.000	3.87
14.	MMP3	Matrix metalloproteinase-3	0.010	3.77
15.	SELENOP	Selenoprotein P	0.037	3.63
16.	BANK1	B-cell scaffold protein with ankyrin repeats	0.003	3.53
17.	RGS16	Regulator of G-protein signaling 16	0.011	3.23
18.	CPPED1	Serine/threonine-protein phosphatase CPPED1	0.003	3.10
19.	LRRC69	Leucine-rich repeat-containing protein 69	0.005	2.97
20.	SYPL2	Synaptophysin-like protein 2	0.034	2.92
21.	PPP1R3G	Protein phosphatase 1 regulatory subunit 3G	0.013	2.78
22.	TMEM47	Transmembrane protein 47	0.044	2.69
23.	SLC14A1	Urea transporter 1	0.009	2.57
24.	DAP	Death-associated protein 1	0.048	2.49
25.	STYK1	Aspartyl aminopeptidase	0.017	2.39
26.	D4S234E	Tyrosine-protein kinase STYK1	0.022	2.33
27.	INA	Alpha-internexin	0.031	2.18
28.	GMFG	Glia maturation factor gamma	0.007	2.08
29.	MATN3	Matrilin-3	0.037	1.97
30.	PSG1	Pregnancy-specific beta-1-glycoprotein 1	0.040	1.96
31.	SHC4	SHC-transforming protein 4	0.040	1.95
32.	DAAM2	Disheveled-associated activator of morphogenesis 2	0.028	1.95
33.	MOB4	MOB-like protein phocein	0.000	1.81
34.	PDK2	Pyruvate dehydrogenase kinase, isoenzyme 2	0.038	1.73
35.	SORT1	Sortilin 1	0.035	1.69
36.	PSG9	Pregnancy-specific beta-1-glycoprotein 9	0.048	1.68

TABLE S1: Transcriptomic analysis of genes with changed expression in senescence of MRC-5 fibroblasts *in vitro* (cont'd).

No	Gene ID	Name	P-value	$\Delta\text{Log}_2$ expression
37.	MAP7D3	MAP7 domain-containing protein 3	0.049	1.66
38.	GPC4	Glypican-4	0.016	1.60
39.	KCTD12	Pfetin	0.016	1.58
40.	UHMK1	Serine/threonine-protein kinase Kist	0.007	1.50
41.	MANBAL	Protein MANBAL	0.016	1.45
42.	RCAN3	Calcipressin-3	0.039	1.44
43.	PDP2	Mitochondrial pyruvate dehydrogenase-phosphatase 2	0.031	1.34
44.	MALSU1	Mitochondrial assembly of ribosomal large subunit protein 1	0.049	1.33
45.	UFD1L	Ubiquitin recognition factor in ER-associated degradation protein 1	0.038	1.25
46.	HLA-E	HLA class I histocompatibility antigen, $\alpha$ -chain E	0.002	1.25
47.	NDST2	Bifunctional heparan sulfate N-deacetylase/N-sulfotransferase 2	0.016	1.23
48.	ZMAT2	Zinc finger matrin-type protein 2	0.001	1.09
49.	MUC1	Mucin-1	0.015	1.06
50.	CTBS	Di-N-acetylchitobiase (EC 3.2.1.-)	0.037	1.04
51.	ABTB2	Ankyrin repeat and BTB/POZ domain-containing protein 2	0.002	0.99
52.	LYNX1	Ly-6/neurotoxin-like protein 1	0.024	0.85
53.	DNAJC19	Mitochondrial import inner membrane translocase subunit TIM14	0.008	0.82
54.	COL4A3BP	Collagen type IV alpha-3-binding protein	0.001	0.81
55.	TMEM165	Transmembrane protein 165	0.014	0.80
56.	TAX1BP1	Tax1-binding protein 1	0.011	0.76
57.	HSD11B1L	Hydroxysteroid 11-beta-dehydrogenase 1-like protein	0.004	0.75
58.	TMX2	Thioredoxin-related transmembrane protein 2	0.045	0.66
59.	ZNF525	Zinc finger protein 525	0.045	0.65
60.	RAI1	Retinoic acid-induced protein 1	0.048	-0.29
61.	ACAD10	Acyl-CoA dehydrogenase family member 10	0.031	-0.64
62.	MTRF1	Peptide chain release factor 1, mitochondrial	0.009	-1.24
63.	OXR1	Oxidation resistance protein 1	0.009	-1.47
64.	COLEC11	Collectin-11	0.003	-1.78
65.	ABCC3	Canalicular multispecific organic anion transporter 2	0.019	-1.80
66.	PDE4DIP	Myomegalin	0.001	-1.86
67.	MT-ND1	Mitochondrially encoded NADH:Ubiquinone Oxidoreductase Core Subunit 1	0.022	-1.86
68.	PRPF39	Pre-mRNA-processing factor 39 (PRP39 homolog)	0.021	-1.88
69.	TRIM45	Tripartite motif-containing protein	0.014	-1.94
70.	ZNF169	Zinc finger protein 169	0.035	-1.95

TABLE S1: Transcriptomic analysis of genes with changed expression in senescence of MRC-5 fibroblasts *in vitro* (cont'd).

No	Gene ID	Name	P-value	$\Delta\text{Log}_2$ expression
71.	LAT	Linker for activation of T-cells family member 1	0.037	-1.95
72.	PPM1N	Probable protein phosphatase 1N (EC 3.1.3.16)	0.010	-2.09
73.	AMPD3	AMP deaminase 3	0.017	-2.10
74.	NKTR	NK-tumor recognition protein	0.024	-2.33
75.	HIST2H2AC	Histone H2A type 2-C	0.006	-2.36
76.	COL23A1	Collagen alpha-1(XXIII) chain	0.014	-2.41
77.	GOLGA6L9	Golgin subfamily A member 6-like protein 9	0.028	-2.41
78.	L2HGDH	L-2-hydroxyglutarate dehydrogenase, mitochondrial	0.042	-2.44
79.	NUTM2G	NUT family member 2G	0.047	-2.47
80.	TBC1D3G	TBC1 domain family member 3G	0.031	-2.86
81.	ABCC10	Multidrug resistance-associated protein 7	0.026	-2.89
82.	ADHFE1	Hydroxyacid-oxoacid transhydrogenase, mitochondrial	0.022	-2.92
83.	BCOR	BCL-6 corepressor	0.014	-2.95
84.	FBXL19-AS1	Long non-coding RNA FBXL19-AS1	0.035	-2.96
85.	SCARNA13	Small Cajal Body-Specific RNA 13	0.002	-2.98
86.	CXCL2	C-X-C motif chemokine 2	0.000	-3.04
87.	SLC25A16	Solute carrier family 25 member 16	0.038	-3.11
88.	HIST1H4A	Histone H4	0.022	-3.16
89.	SNORD114-26	Small Nucleolar RNA, C/D Box 11426	0.016	-3.23
90.	FBXL17	F-box/LRR-repeat protein 17	0.021	-3.32
91.	SAP25	Histone deacetylase complex subunit SAP25	0.036	-3.41
92.	CTSL	Cathepsin L1	0.044	-3.46
93.	SNORD114-17	Small Nucleolar RNA, C/D Box 114-17	0.035	-3.51
94.	SNORD114-3	Small Nucleolar RNA, C/D Box 1143	0.009	-3.54
95.	MALAT1	Long non-coding RNA 47	0.036	-3.78
96.	FOSB	Protein fosB (G0/G1 switch regulatory protein 3)	0.016	-3.92
97.	CCDC144A	Coiled-coil domain-containing protein 144A	0.003	-3.93
98.	SMA4	$\beta$ -Glucuronidase-like protein SMA4	0.033	-4.06
99.	CASKIN2	Caskin-2 (CASK-interacting protein 2)	0.030	-4.39
100.	RPL23AP32	Ribosomal Protein L23a Pseudogene 32	0.043	-4.43
101.	ANKRD36	Ankyrin repeat domain-containing protein 36A	0.046	-4.62
102.	CISH	Cytokine-inducible SH2-containing protein	0.047	-5.36
103.	MIAT	Long non-coding RNA 66	0.050	-5.71
104.	RNU6ATAC	U6atac Small Nuclear RNA	0.038	-5.82
105.	SNORD12C	Small Nucleolar RNA, C/D Box 12C	0.007	-6.02
106.	NAMPT	Nicotinamide phosphoribosyltransferase (Visfatin)	0.028	-6.27

TABLE S1: Transcriptomic analysis of genes with changed expression in senescence of MRC-5 fibroblasts *in vitro* (cont'd).

Genes listed had expression changes between passages 3 and 11 in MRC-5 fibroblasts. Gene expression changes are rank ordered by  $\log_2$  expression change (highest to lowest). P-values are with Bonferroni-correction applied.

## Legends to supplementary figures

FIGURE S1: Changes in gene expression induced by sulforaphane in early passage, non-senescent MRC-5 cells *in vitro*. Key: + 1  $\mu$ M SFN ( $\circ$ — $\circ$ ) and control, + 0.002% DMSO ( $\bullet$ — $\bullet$ ). (a) CAT ( $P = 0.002$ ), (b) SOD1 ( $P = 0.028$ ), (c) SOD2 ( $P = 1 \times 10^{-4}$ ), (d) FTH1 ( $P = 0.005$ ,  $P' = 0.021$ ), (e) GPX1 ( $P = 5 \times 10^{-5}$ ,  $P'' = 0.024$ ), (f) PRDX1 ( $P' = 0.028$ ), (g) GCLC ( $P = 0.008$ ,  $P' = 0.023$ ), (h) GSTP1 ( $P = 0.006$ ), (i) MRP2 ( $P' = 0.010$ ), (j) FASN ( $P = 0.002$ ), (k) CCL2 ( $P = 0.013$ ), (l) ( $P = 5 \times 10^{-4}$ ), (m) IL1B, (n) IL15, (o) TLR4, (p) NFKB1, (q) NFKB3 ( $P = 0.003$ ,  $P' = 0.015$ ), (r) MMP3 ( $P = 2 \times 10^{-4}$ ), (s) MMP13, (t) SERPINE1 ( $P = 0.002$ ), (u) AKR1C1 ( $P' = 0.013$ ), (v) CDKN1A, (w) ( $P = 1 \times 10^{-5}$ ), (x) ( $P = 0.033$ ), (y) GLO1 ( $P = 0.002$ ), (z) PFKFP4 ( $P = 0.003$ ), (aa) SERPINB2 ( $P = 2 \times 10^{-4}$ ), (ab) ACTB ( $P = 0.002$ ,  $P' = 5 \times 10^{-4}$ ,  $P'' = 0.042$ ), (ac) CLTC ( $P = 0.004$ ) and (ad) GUSB ( $P = 0.012$ ). Data are mean  $\pm$  SD,  $N = 3$ . Significance:  $P$ ,  $P'$  and  $P''$ , significance values for time, SFN treatment and time x SFN treatment effects, respectively.

FIGURE S2: Effect of sulforaphane on end-of-passage gene expression in young and senescent MRC-5 fibroblasts *in vitro*. Key: Solid bars, control; unfilled bars + 1  $\mu$ M SFN. (a) MMP1 ( $P = 0.002$ ,  $P' = 0.002$ ,  $P'' = 2 \times 10^{-4}$ ), (b) COL1A1 ( $P'' = 0.011$ ), (c) MMP3 ( $P = 0.001$ ), (d) MMP13, (e) CDKN1A, (f) SERPINB2 ( $P = 0.002$ ), (g) AKR1C1 ( $P = 2 \times 10^{-4}$ ,  $P' = 0.002$ ,  $P'' = 0.003$ ), (h) HIF1A ( $P'' = 2 \times 10^{-4}$ ), (i) SOD1 ( $P = 0.011$ ), (j) GCLC, (k) GPX1 ( $P = 0.023$ ), (l) MRP2/UGT1A1 ( $P = 0.017$ ), (m) GLO1, (n) MAFG ( $P = 0.007$ ), (o) ELN ( $P = 0.014$ ), (p) TLR4 ( $P = 0.031$ ), (q) NFKB1 ( $P = 0.009$ ,  $P'' = 0.041$ ), (r) NFKB3, (s) GLB1 ( $P = 0.023$ ) and (t) CDKN1A ( $P = 0.022$ ,  $P'' = 0.012$ ). Significance:  $P$ , passage no effect;  $P'$ , +SFN effect; and  $P''$ , passage no x SFN treatment effect.

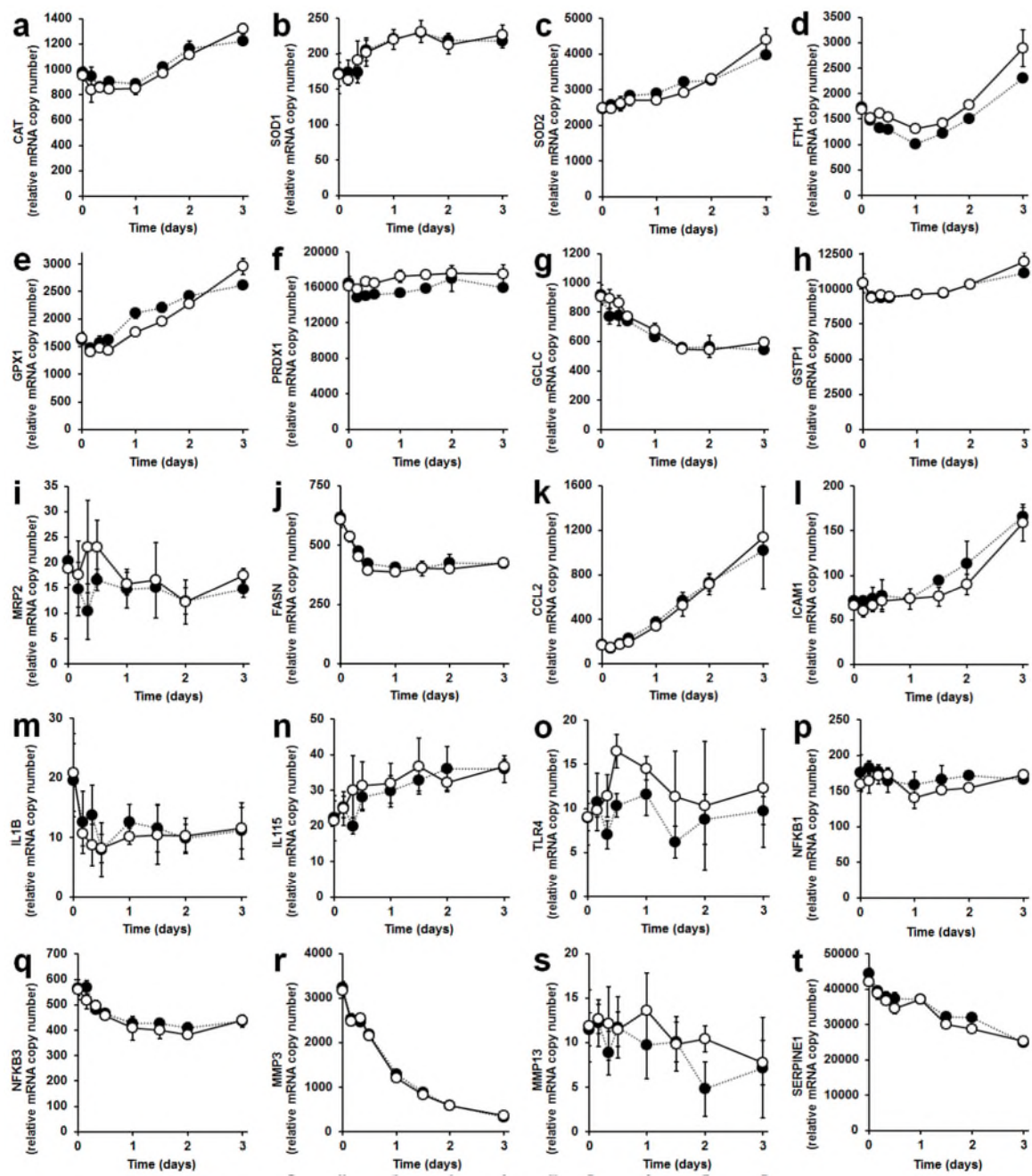


Figure S1

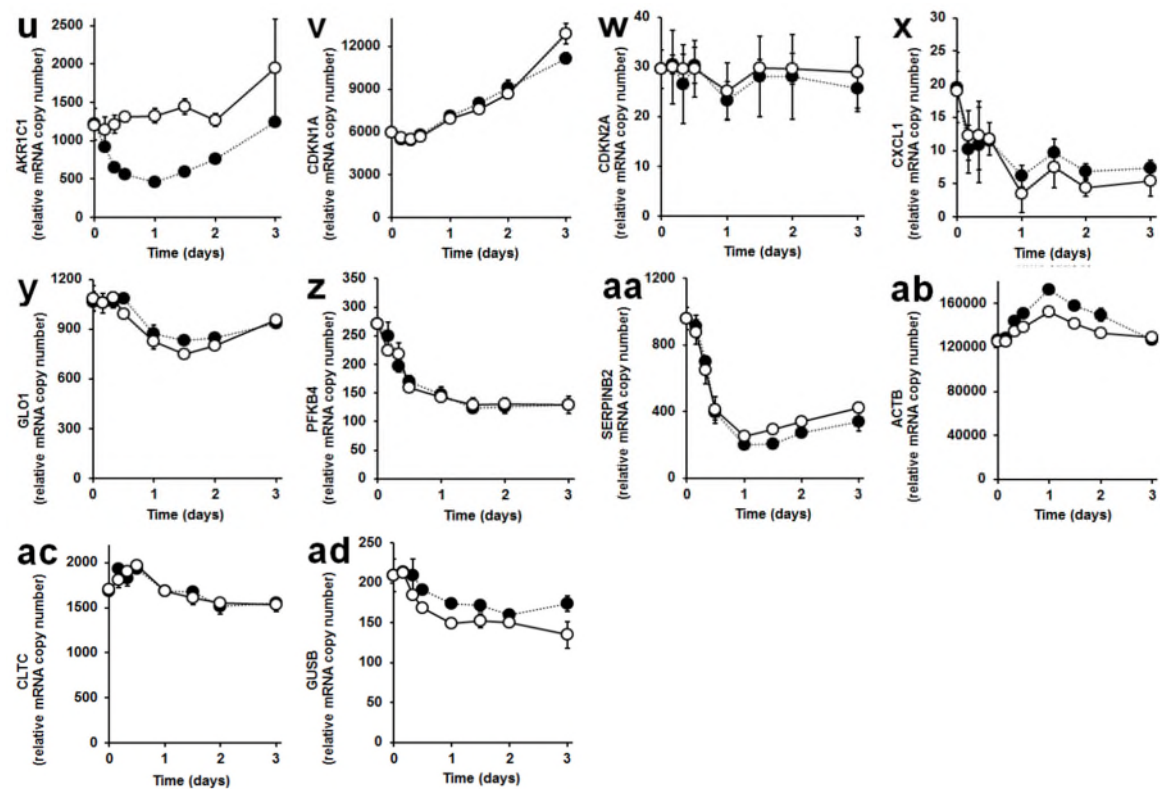


Figure S1 (cont'd)



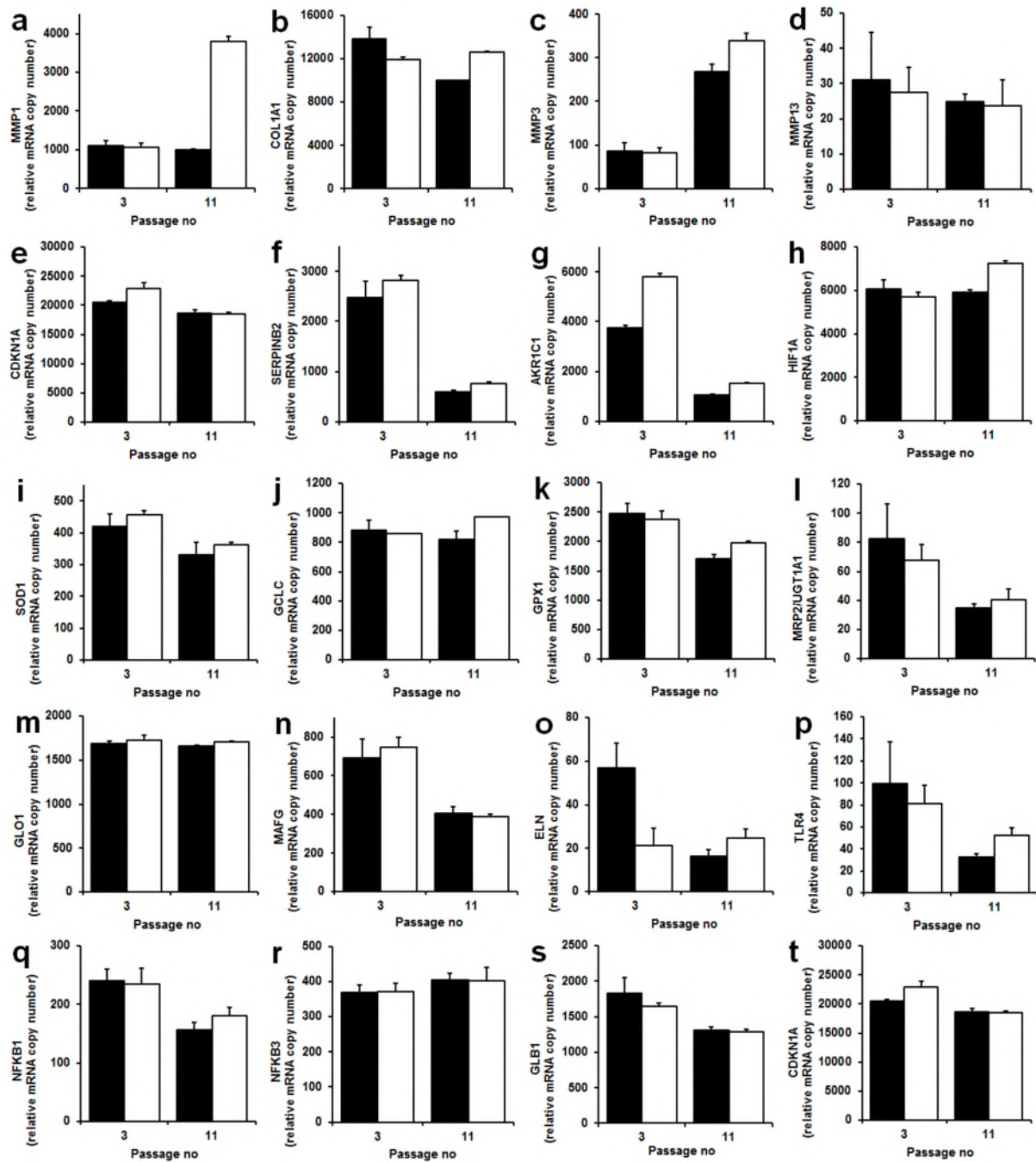


Figure S2