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ORIGINAL ARTICLE

Analysis of Gene Expression in Human Dermal Fibroblasts Treated with Senescence-Modulating COX Inhibitors

Jeong A. Han[1]*, Jong-Il Kim[2,3,4]**

[1]Department of Biochemistry and Molecular Biology, Kangwon National University School of Medicine, Chuncheon 24341, Korea,
[2]Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, Seoul 03080, Korea,
[3]Cancer Research Institute, Seoul National University College of Medicine, Seoul 03080, Korea,
[4]Department of Biomedical Sciences, Seoul National University Graduate School, Seoul 03080, Korea

We have previously reported that NS-398, a cyclooxygenase-2 (COX-2)–selective inhibitor, inhibited replicative cellular senescence in human dermal fibroblasts and skin aging in hairless mice. In contrast, celecoxib, another COX-2–selective inhibitor, and aspirin, a non-selective COX inhibitor, accelerated the senescence and aging. To figure out causal factors for the senescence-modulating effect of the inhibitors, we here performed cDNA microarray experiment and subsequent Gene Set Enrichment Analysis. The data showed that several senescence-related gene sets were regulated by the inhibitor treatment. NS-398 up-regulated gene sets involved in the tumor necrosis factor β receptor pathway and the fructose and mannose metabolism, whereas it down-regulated a gene set involved in protein secretion. Celecoxib up-regulated gene sets involved in G2M checkpoint and E2F targets. Aspirin up-regulated the gene set involved in protein secretion, and down-regulated gene sets involved in RNA transcription. These results suggest that COX inhibitors modulate cellular senescence by different mechanisms and will provide useful information to understand senescence-modulating mechanisms of COX inhibitors.

Keywords: cyclooxygenase 2, fibroblast, gene set enrichment analysis, inhibitor, senescence

Introduction

Prostaglandin endoperoxide synthase, also called as cyclooxygenase (COX), is an enzyme converting arachidonic acid to prostaglandin H_2 (PGH_2). PGH_2 is a common precursor for prostanoid biosynthesis such as PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2. These prostanoids are known to be important chemical mediators for inflammation as well as other biological processes [1]. There are two isoforms of COX. COX-1 (*PTGS1*) is expressed constitutively in most cells and responsible for basal level of prostanoid biosynthesis. COX-2 (*PTGS2*) is induced by various stimuli such as bacterial endotoxins, cytokines, genotoxic agents, growth factors, or oncogene products [2, 3].

Most non-steroidal anti-inflammatory drugs are COX inhibitors. These drugs inhibit the COX catalytic activity by occupying the active site of COX. Aspirin, ibuprofen, or flurbiprofen is a non-selective COX inhibitor, which inhibits both COX-1 and COX-2 catalytic activity. In contrast, NS-398, celecoxib, or nimesulide is a selective COX-2 inhibitor, which inhibits COX-2 catalytic activity specifically [3, 4].

The mechanism of aging has not been fully understood. However, it has been proposed that the pro-inflammatory catalytic activity of COX-2 is a causal factor for aging. The hypothesis proposes that reactive oxygen species (ROS) generated in the process of normal metabolism or inflammation activate the transcription factor nuclear factor κB (NF-κB). NF-κB increases the transcription of pro-inflammatory target genes such as COX-2, which in turn stabilizes a chronic inflammatory circuit by generating ROS. This chronic inflammation causes tissue damage and aging [5].

If the pro-inflammatory catalytic activity of COX-2 is a causal factor for aging, COX-2 inhibitors should conceivably

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***Corresponding author:** Tel: +82-33-250-8832, Fax: +82-33-250-8807, E-mail: gshja@kangwon.ac.kr
****Corresponding author:** Tel: +82-2-740-8251, Fax: +82-2-744-4534, E-mail: jongil@snu.ac.kr

inhibit aging. In this context, we have previously examined the effect of COX-2 inhibitors on aging both in the replicative cellular senescence model of human dermal fibroblasts (HDFs) and in the intrinsic skin aging model of hairless mice. We observed that among three selective COX-2 inhibitors studied, only NS-398 inhibited the cellular senescence whereas celecoxib and nimesulide accelerated the senescence. In addition, three non-selective COX inhibitors including aspirin, ibuprofen, and flurbiprofen accelerated the senescence [6]. Also, we observed that only NS-398 inhibited the skin aging while celecoxib and aspirin accelerated the skin aging in hairless mice [3]. These studies strongly suggest that the pro-inflammatory catalytic activity of COX-2 is not a causal factor for aging and that the aging-modulating effect of COX inhibitors is attributable to a catalytic activity–independent mechanism.

In an attempt to figure out underlying mechanisms by which COX inhibitors modulate aging, we here performed cDNA microarray experiment and subsequent Gene Set Enrichment Analysis (GSEA) in HDFs treated with three COX inhibitors, NS-398, celecoxib, and aspirin.

Methods

Materials and cell culture

NS-398 and aspirin were purchased from Cayman Chemicals (Ann Arbor, MI, USA). Celecoxib was a generous gift from Dr. S.V. Yim (Kyung Hee University, Seoul, Korea). HDFs, isolated from foreskin [7], were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Life Technologies, Carlsbad, CA, USA), penicillin (100 units/mL) and streptomycin (100 units/mL) in a 5% CO_2 incubator [6].

RNA isolation

Total RNA was extracted from HDFs with Trizol (Life Technologies), purified with the addition of chloroform, and precipitated with the addition of isopropanol. The RNA concentration was determined by spectrophotometer and the quality of RNA was evaluated by OD 260/280 ratio and gel electrophoresis [8].

cDNA microarray experiment

The following procedures were carried out by Macrogen Co. (Seoul, Korea). Five hundred fifty nanograms of total RNA was reverse-transcribed to cDNA using a T7 oligo(dT) primer. Second-strand cDNA was synthesized, *in vitro* transcribed, and labeled with biotin-NTP. After purification, 750 ng of labeled cRNA was hybridized to Illumina Human HT12 v.4 bead array (Illumina, San Diego, CA, USA) for 16-18 h at 58°C. The array signal was detected by using Amersham fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences, Little Chalfont, UK). Arrays were scanned with an Illumina bead array Reader confocal scanner. Array data were filtered by detection p-value < 0.05 (similar to signal to noise). The average signal values of filtered genes were transformed by logarithm and normalized by the quantile method [8].

Gene Set Enrichment Analysis (GSEA)

The beta version of GSEA software and MSigDB 5.2 were downloaded from the Broad Institute (http://software.broadinstitute.org/gsea/index.jsp). GSEA was carried out as described previously [9]. Enrichment of gene sets was considered statistically significant if the normalized p-value was < 0.01 and the false discovery rate (FDR) was < 0.20.

Results

Treatment of HDFs with COX inhibitors

We have previously shown that among COX inhibitors studied, NS-398, a COX-2-selective inhibitor, inhibited replicative cellular senescence in HDFs as well as skin aging in hairless mice, whereas celecoxib, another COX-2-selective inhibitor, and aspirin, a non-selective COX inhibitor, accelerated the senescence and aging. At that time, we treated cells or skin with inhibitors every day for more than a month (Table 1) [3, 6].

To figure out causal factors for the senescence-modulating effect of the inhibitors, we treated HDFs with NS-398, celecoxib, aspirin, or dimethyl sulfoxide (DMSO) (the vehicle) every day for only 3 days in this study. The IC_{50} values have been reported for recombinant human COX-1 and COX-2 of NS-398 and celecoxib [10, 11], and for recombinant ovine COX-1 and COX-2 of aspirin [12]. In the

Table 1. Summary of senescence-modulating effect of COX inhibitors and used doses

Inhibitors		Effect on HDF senescence [6]	Effect on skin aging [3]	IC_{50} for COX-1 [6]	IC_{50} for COX-2 [6]	Used doses in this study
COX-2–selective	NS-398	Delayed	Delayed	75 μM	1.77 μM	20 μM
	Celecoxib	Accelerated	Accelerated	15 μM	0.04 μM	0.5 μM
Non-selective	Aspirin	Accelerated	Accelerated	0.75 mM	1.25 mM	1 mM

case of NS-398 and celecoxib, we used approximately 10-fold higher concentration of IC_{50} to inhibit COX-2 catalytic activity sufficiently. NS-398 and celecoxib showed no acute cellular toxicity at this concentration. In the case of aspirin, however, we used IC_{50} because 10-fold higher concentration caused acute cellular toxicity (Table 1) [6].

DNA microarray and GSEA

We performed cDNA microarray experiment using RNA extracted from the drug-treated HDFs. Among 47,319 probe sets, 20,271 probe sets passed the criteria of the detection p-value < 0.05. Unsupervised hierarchical cluster analysis showed that drug-treated cells were well segregated in the order of DMSO, NS-398, celecoxib, and aspirin (Fig. 1).

To figure out underlying mechanisms by which COX inhibitors modulate senescence, we performed GSEA using 17,777 probe sets having all information including gene symbols and gene descriptions. We sorted the data sets based on the value of ($I_{NS\text{-}398} - I_{DMSO}$) for the comparison of NS-398 versus DMSO; the value of ($I_{Celecoxib} - I_{DMSO}$) for the comparison of celecoxib versus DMSO; and the value of ($I_{Aspirin} - I_{DMSO}$) for the comparison of aspirin versus DMSO to rank the data sets as described previously [9].

We then tested (1) the Hallmark gene sets (H); (2) gene sets regulating canonical pathways—i.e., Biocarta gene sets (C2:CP:BIOCARTA), Kyoto Encyclopedia of Genes and Genomes (KEGG) gene sets (C2:CP:KEGG), and Reactome gene sets (C2:CP:REACTOME); and (3) gene ontology gene sets—i.e., biological process gene sets (G5:BP), cellular component gene sets (G5:CC), and molecular function gene sets (G5:MF).

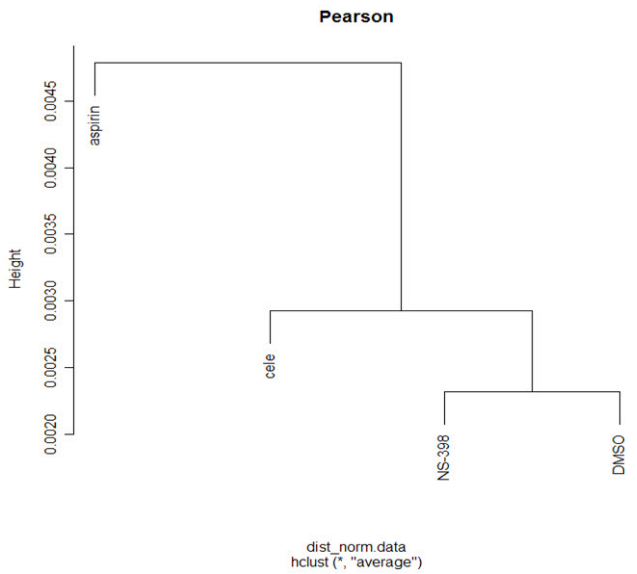

Fig. 1. Segregation between the drug-treated HDFs. Unsupervised hierarchical cluster analysis was done between four drug-treated HDFs using 20,271 probe sets with the detection p-value < 0.05.

NS-398 versus DMSO

The analysis of NS-398 versus DMSO showed that two gene sets are enriched in NS-398–treated HDFs as compared with DMSO-treated HDFs. These gene sets consist of genes regulating the tumor necrosis factor beta receptor (TNFR2) pathway and the fructose and mannose metabolism (Table 2, Fig. 2A). Enriched genes in each pathway were shown in Supplementary Tables 1 and 2, and Supplementary Figs. 1 and 2.

On the other hand, four gene sets were enriched in DMSO-treated HDFs as compared with NS-398–treated HDFs: genes down-regulated in response to ultraviolet (UV) radiation, and genes regulating the protein secretion, the trefoil factor pathway and the receptor-regulated Smads (R-SMAD) binding (Table 3, Fig. 2B). Enriched genes in each gene set were shown in Supplementary Tables 3–6.

Celecoxib versus DMSO

The analysis of celecoxib versus DMSO showed that four gene sets were enriched in celecoxib-treated HDFs as compared with DMSO-treated HDFs. These gene sets consist of genes involved in the G2M checkpoint, E2F targets, γ tubulin complex and the four way junction (Holliday junction) DNA binding (Table 4, Fig. 3A). Enriched genes in each gene set were shown in Supplementary Tables 7–10.

On the other hand, one gene set was enriched in DMSO-treated HDFs as compared with celecoxib-treated

Table 2. Enriched gene sets in NS-398–treated HDFs (NS-398 vs. DMSO)

Name	NES	Normalized p-value	FDR q-value
C2:CP:Biocarta			
BIOCARTA_TNFR2_PATHWAY	1.808	0.000	0.044
C2:CP:KEGG			
KEGG_FRUCTOSE_AND_MANNOSE_METABOLISM	1.721	0.009	0.159

HDF, human dermal fibroblast; DMSO, dimethyl sulfoxide; NES, normalized enrichment score; FDR, false discovery rate.

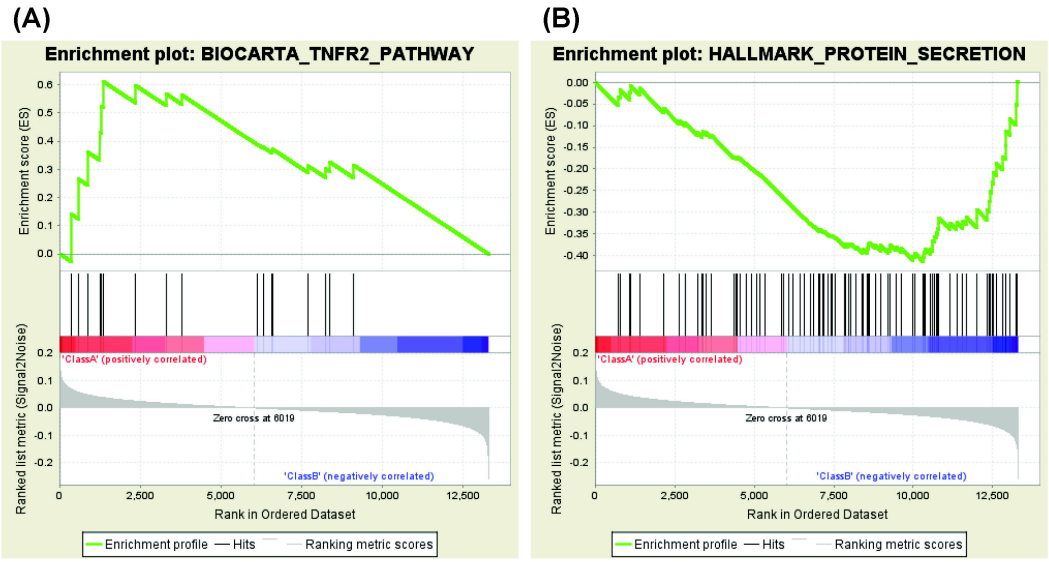

Fig. 2. Enrichment plots (NS-398 vs. DMSO). (A) A representative enriched gene set in NS-398–treated HDFs. (B) A representative enriched gene set in DMSO-treated HDFs. DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast.

Table 3. Enriched gene sets in DMSO-treated HDFs (NS-398 vs. DMSO)

Name	NES	Normalized p-value	FDR q-value
H			
HALLMARK_UV_RESPONSE_DN	−1.625	0.001	0.110
HALLMARK_PROTEIN_SECRETION	−1.545	0.004	0.140
C2:CP:Biocarta			
BIOCARTA_TFF_PATHWAY	−1.799	0.000	0.160
C5:MF			
GO_R_SMAD_BINDING	−1.823	0.000	0.166

DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast; NES, normalized enrichment score; FDR, false discovery rate.

Table 4. Enriched gene sets in celecoxib-treated HDFs (celecoxib vs. DMSO)

Name	NES	Normalized p-value	FDR q-value
H			
HALLMARK_G2M_CHECKPOINT	1.642	0.000	0.074
HALLMARK_E2F_TARGETS	1.427	0.007	0.164
C5:CC			
GO_GAMMA_TUBULIN_COMPLEX	1.830	0.001	0.154
C5:MF			
GO_FOUR_WAY_JUNCTION_DNA_BINDING	1.884	0.002	0.104

HDF, human dermal fibroblast; DMSO, dimethyl sulfoxide; NES, normalized enrichment score; FDR, false discovery rate.

HDFs. This gene set consists of genes regulating olfactory signaling pathway (Table 5, Fig. 3B). The list of enriched genes in this pathway was shown in Supplementary Table 11.

Aspirin versus DMSO

In the case of aspirin versus DMSO, four gene sets were enriched in aspirin-treated HDFs as compared with DMSO-treated HDFs. These gene sets consist of genes involved in the protein secretion, keratin filament and intermediate filament, and genes down-regulated in response to UV radiation (Table 6, Fig. 4A). Enriched genes in each gene set were shown in Supplementary Tables 12–15.

On the other hand, three gene sets of C2:CP were enriched in DMSO-treated HDFs as compared with aspirin-treated

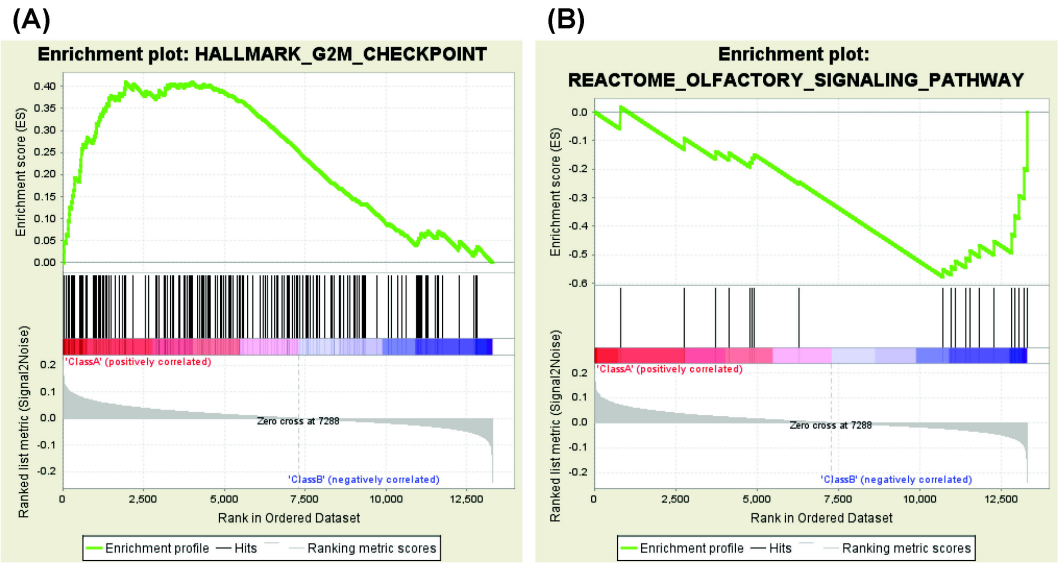

Fig. 3. Enrichment plots (celecoxib vs. DMSO). (A) A representative enriched gene set in celecoxib–treated HDFs. (B) The representative enriched gene set in DMSO-treated HDFs. DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast.

Table 5. Enriched gene sets in DMSO-treated HDFs (celecoxib vs. DMSO) (C2:Reactome)

Name	NES	Normalized p-value	FDR q-value
REACTOME_OLFACTORY_SIGNALING_PATHWAY	−1.853	0.000	0.146

DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast; NES, normalized enrichment score; FDR, false discovery rate.

Table 6. Enriched gene sets in aspirin-treated HDFs (aspirin vs. DMSO)

Name	NES	Normalized p-value	FDR q-value
H			
HALLMARK_PROTEIN_SECRETION	1.764	0.000	0.037
HALLMARK_UV_RESPONSE_DN	1.495	0.000	0.123
C5:CC			
GO_KERATIN_FILAMENT	2.204	0.000	0.000
GO_INTERMEDIATE_FILAMENT	1.960	0.000	0.008

HDF, human dermal fibroblast; DMSO, dimethyl sulfoxide; NES, normalized enrichment score; FDR, false discovery rate.

HDFs: genes regulating prostate cancer, colorectal cancer, and cardiomyopathy (Table 7). In addition, 34 gene sets of C5:BP, three gene sets of C5:CC and five gene sets of C5:MF were enriched in DMSO-treated HDFs as compared with aspirin-treated HDFs. These gene sets consist of genes involved in embryonic development, negative regulation of protein localization to plasma membrane, DNA-dependent RNA transcription, cell differentiation, glutamate receptor binding, or Smad binding (Tables 7 and 8, Fig. 4B). Of note, the gene set involved in platelet aggregation was enriched in DMSO-treated HDFs as compared with aspirin-treated HDFs (Table 8, FDR, 0.179). Enriched genes in representative gene sets were shown in Supplementary Tables 16–22.

Discussion

Our data showed that NS-398 treatment up-regulated the gene set involved in the TNFR2 pathway (Table 2, Fig. 2A, Supplementary Table 1). This pathway is well known to activate the NF-κB signaling that mediates cell proliferation, anti-apoptosis, inflammation, differentiation, or development (Supplementary Fig. 1) [13]. NF-κB, a transcription factor, has been reported to regulate cellular senescence though its role in the senescence is controversial. Overexpression of c-Rel resulted in premature senescence in normal human keratinocytes [14]. On the contrary, mouse embryonic fibroblasts from NF-κB1 knockout mice showed

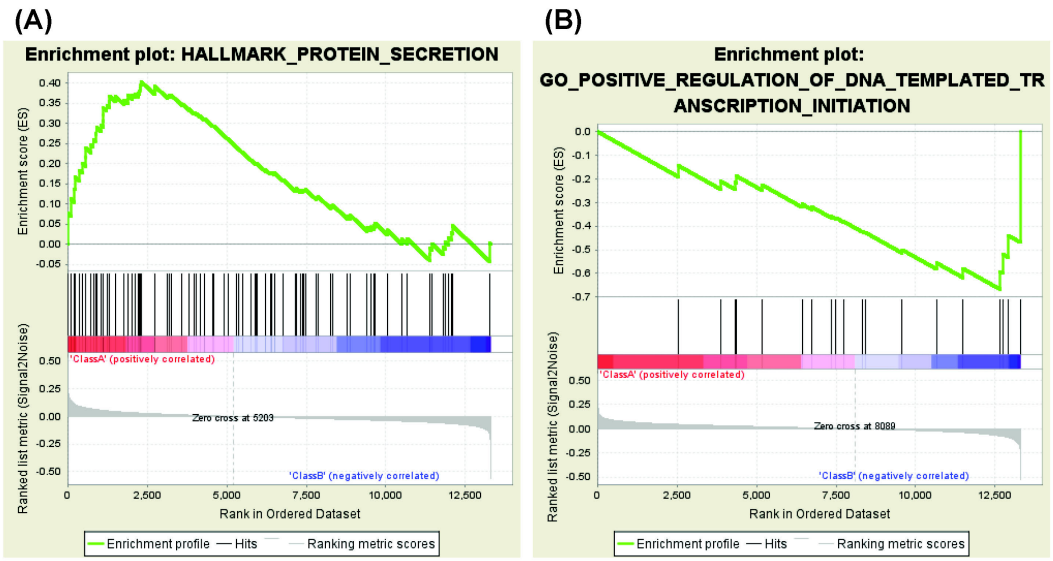

Fig. 4. Enrichment plots (aspirin vs. DMSO). (A) A representative enriched gene set in aspirin-treated HDFs. (B) A representative enriched gene set in DMSO-treated HDFs. DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast.

Table 7. Enriched gene sets in DMSO-treated HDFs (aspirin vs. DMSO)

Name	NES	Normalized p-value	FDR q-value
C2:CP:KEGG			
KEGG_PROSTATE_CANCER	−1.826	0.000	0.171
KEGG_COLORECTAL_CANCER	−1.742	0.000	0.172
KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC	−1.635	0.006	0.199
C5:CC			
GO_INTERCALATED_DISC	−1.930	0.000	0.077
GO_EUCHROMATIN	−1.963	0.000	0.120
GO_DENDRITIC_SHAFT	−1.757	0.008	0.199
C5:MF			
GO_IONOTROPIC_GLUTAMATE_RECEPTOR_BINDING	−1.947	0.003	0.062
GO_RNA_POLYMERASE_II_ACTIVATING_TRANSCRIPTION_FACTOR_BINDING	−1.922	0.003	0.062
GO_GLUTAMATE_RECEPTOR_BINDING	−1.952	0.000	0.077
GO_SMAD_BINDING	−1.981	0.000	0.085
GO_ACTIVATING_TRANSCRIPTION_FACTOR_BINDING	−2.032	0.000	0.088

DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast; NES, normalized enrichment score; FDR, false discovery rate.

enhanced cellular senescence [15]. In addition, siRNA against NF-κB2 or RelB induced premature senescence in HDFs in a p53-dependent manner [16]. These studies suggest that the anti-senescent effect of NS-398 might be attributable to a regulation of NF-κB signaling.

NS-398 treatment also up-regulated the gene set involved in the fructose and mannose metabolism (Table 2, Supplementary Table 2). This metabolic pathway leads to enhanced glycolysis and N-glycan biosynthesis (Supplementary Fig. 2). Alterations of glucose metabolism have been reported in cellular senescence though the data is conflicting. In human mammary epithelial cells, B-Raf–induced premature senescence was associated with a reduction of glucose uptake, and overexpression of hexokinase 2 prevented the oncogene-induced senescence [17]. On the contrary, glucose consumption and hexokinase activity were increased in senescent HDFs as compared to young HDFs [18]. These studies suggest that NS-398 might delay cellular senescence via regulation of glycolysis.

It is intriguing that the gene set involved in protein secretion is down-regulated by NS-398 treatment but is up-regulated by aspirin treatment (Tables 3 and 6, Figs. 2B and 4A, Supplementary Tables 4 and 12). It has been reported that cellular senescence is accompanied by an increase in the secretion of intercellular signaling molecules including interleukins, chemokines, growth factors, pro-

Table 8. Enriched gene sets in DMSO-treated HDFs (aspirin vs. DMSO) (C5:BP)

Name	NES	Normalized p-value	FDR q-value
GO_GENITALIA_DEVELOPMENT	−2.072	0.000	0.036
GO_NEGATIVE_REGULATION_OF_PROTEIN_LOCALIZATION_TO_PLASMA_MEMBRANE	−2.076	0.000	0.041
GO_NEGATIVE_REGULATION_OF_PROTEIN_LOCALIZATION_TO_CELL_PERIPHERY	−2.077	0.000	0.049
GO_ENDOTHELIAL_CELL_DEVELOPMENT	−2.144	0.000	0.057
GO_ORGAN_FORMATION	−2.011	0.005	0.058
GO_BETA_CATENIN_TCF_COMPLEX_ASSEMBLY	−2.021	0.000	0.059
GO_REGULATION_OF_SISTER_CHROMATID_COHESION	−2.079	0.000	0.059
GO_POSITIVE_REGULATION_OF_DNA_TEMPLATED_TRANSCRIPTION_INITIATION	−2.101	0.000	0.061
GO_ESTABLISHMENT_OF_ENDOTHELIAL_BARRIER	−2.154	0.000	0.098
GO_REGULATION_OF_HISTONE_METHYLATION	−1.954	0.003	0.103
GO_CEREBRAL_CORTEX_CELL_MIGRATION	−1.926	0.000	0.130
GO_REGULATION_OF_CHROMATIN_BINDING	−1.909	0.000	0.138
GO_HOMOTYPIC_CELL_CELL_ADHESION	−1.852	0.000	0.140
GO_REGULATION_OF_CHONDROCYTE_DIFFERENTIATION	−1.856	0.000	0.142
GO_REGULATION_OF_KERATINOCYTE_PROLIFERATION	−1.859	0.000	0.146
GO_EMBRYONIC_PATTERN_SPECIFICATION	−1.869	0.003	0.148
GO_EMBRYONIC_DIGIT_MORPHOGENESIS	−1.861	0.000	0.150
GO_EMBRYONIC_AXIS_SPECIFICATION	−1.870	0.000	0.156
GO_REGULATION_OF_HISTONE_H3_K4_METHYLATION	−1.825	0.008	0.162
GO_REGULATION_OF_OSTEOCLAST_DIFFERENTIATION	−1.830	0.003	0.163
GO_ENDOTHELIAL_CELL_DIFFERENTIATION	−1.870	0.000	0.166
GO_PALATE_DEVELOPMENT	−1.876	0.000	0.167
GO_POSITIVE_REGULATION_OF_EPITHELIAL_TO_MESENCHYMAL_TRANSITION	−1.814	0.003	0.171
GO_FOREBRAIN_CELL_MIGRATION	−1.809	0.003	0.172
GO_REGULATION_OF_CARTILAGE_DEVELOPMENT	−1.804	0.003	0.174
GO_POSITIVE_REGULATION_OF_MUSCLE_TISSUE_DEVELOPMENT	−1.783	0.008	0.174
GO_ANTERIOR_POSTERIOR_AXIS_SPECIFICATION	−1.879	0.000	0.175
GO_POSITIVE_REGULATION_OF_TELOMERE_MAINTENANCE_VIA_TELOMERE_LENGTHENING	−1.786	0.000	0.176
GO_NEGATIVE_REGULATION_OF_EPITHELIAL_CELL_DIFFERENTIATION	−1.796	0.008	0.179
GO_PLATELET_AGGREGATION	−1.788	0.008	0.179
GO_MYOBLAST_DIFFERENTIATION	−1.792	0.006	0.179
GO_ODONTOGENESIS	−1.772	0.000	0.185
GO_ODONTOGENESIS_OF_DENTIN_CONTAINING_TOOTH	−1.762	0.000	0.196
GO_REGULATION_OF_DNA_TEMPLATED_TRANSCRIPTION_INITIATION	−1.750	0.000	0.197

DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast; NES, normalized enrichment score; FDR, false discovery rate.

teases, and extracellular matrix proteins [19, 20]. For example, production of interleukin-1, -6, chemokine (C-C motif) ligand-1, -2, -3, -7, -8, -12, -13, -16, -20, -26, chemokine (C-X-C motif) ligand-1, -2, -4, -5, -6, -8, insulin-like growth factor binding protein-2, -3, -4, -5, -6, -7, connective tissue growth factor, granulocyte-macrophage colony-stimulating factor, granulocyte colony stimulating factor, matrix metalloproteinase-1, -3, -10, plasminogen activator inhibitor 1, or fibronectin increased in senescent HDFs as compared to in young HDFs [21]. Ectopic expression of chemokine receptors such as CXCR1 or CXCR2 induced premature senescence in HDFs [22]. Extracellular matrix from young HDFs restored senescent HDFs to an apparently youthful state [23]. In addition, there is a report that p16-induced senescence is accompanied by an increase in the glucose-stimulated insulin secretion in mouse and human pancreatic beta cells [24]. These studies suggest that regulation of protein secretion might be an important common mechanism by which NS-398 delays but aspirin accelerates cellular senescence.

In addition to the up-regulation of protein secretion, aspirin down-regulated gene sets involved in DNA-dependent RNA transcription (Table 7, FDR, 0.062 and 0.088; Table 8, FDR, 0.061 and 0.197; Fig. 4B). Compatible with these results, a cDNA microarray study reported that genes involved in transcription were down-regulated specifically during senescence in HDFs [25]. In addition, there is a report that RNA transcription was decreased in aged rat

brain as compared to in young rat brain [26]. Therefore, aspirin might accelerate cellular senescence by down-regulation of DNA-dependent RNA transcription.

It is well known that aspirin inhibits platelet aggregation and thereby thrombus formation [27]. Consistent with this, our data showed that the gene set involved in platelet aggregation was down-regulated by aspirin treatment (Table 8, FDR, 0.179).

Cyclin-dependent kinase inhibitors (CKIs) are categorized into two families, that is, the Ink4 family including p15, p16, p18, and p19, and the Cip/Kip family including p21, p27, and p57. It has been reported that these CKIs are actively involved in cellular senescence. For example, ectopic expression of p15, p16, p19, p21, or p27 was reported to induce premature senescence in HDFs [28, 29]. According to our data, celecoxib treatment up-regulated gene sets relating G2M checkpoint and E2F targets (Table 4, Fig. 3A). In addition, *CDKN1B* encoding p27 and *CDKN2C* encoding p18 were enriched in both gene sets (Supplementary Table 7, Running enrichment score [ES], 0.410 and 0.274; Supplementary Table 8, Running ES, 0.299 and 0.193). These data suggest that celecoxib might accelerate cellular senescence through up-regulation of CKIs.

Collectively, our results suggest that COX inhibitors modulate cellular senescence by different mechanisms though they have the anti-catalytic activity commonly. We believe that our study will provide useful information to understand senescence-modulating mechanisms of COX inhibitors.

Supplementary materials

Supplementary data including 22 tables and two figures can be found with this article online http://www.genominfo.org/src/sm/gni-15-56-s001.pdf.

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References

1. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. *Arterioscler Thromb Vasc Biol* 2011;31:986-1000.
2. Han JA, Kim JI, Ongusaha PP, Hwang DH, Ballou LR, Mahale A, *et al*. P53-mediated induction of Cox-2 counteracts p53- or genotoxic stress-induced apoptosis. *EMBO J* 2002;21:5635-5644.
3. Lee ME, Kim SR, Lee S, Jung YJ, Choi SS, Kim WJ, *et al*. Cyclooxygenase-2 inhibitors modulate skin aging in a catalytic activity-independent manner. *Exp Mol Med* 2012;44:536-544.
4. Flower RJ. The development of COX2 inhibitors. *Nat Rev Drug Discov* 2003;2:179-191.
5. Chung HY, Cesari M, Anton S, Marzetti E, Giovannini S, Seo AY, *et al*. Molecular inflammation: underpinnings of aging and age-related diseases. *Ageing Res Rev* 2009;8:18-30.
6. Kim SR, Park JH, Lee ME, Park JS, Park SC, Han JA. Selective COX-2 inhibitors modulate cellular senescence in human dermal fibroblasts in a catalytic activity-independent manner. *Mech Ageing Dev* 2008;129:706-713.
7. Yeo EJ, Hwang YC, Kang CM, Kim IH, Kim DI, Parka JS, *et al*. Senescence-like changes induced by hydroxyurea in human diploid fibroblasts. *Exp Gerontol* 2000;35:553-571.
8. Han JA, Kim JY, Kim JI. Analysis of gene expression in cyclooxygenase-2-overexpressed human osteosarcoma cell lines. *Genomics Inform* 2014;12:247-253.
9. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, *et al*. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005;102:15545-15550.
10. Barnett J, Chow J, Ives D, Chiou M, Mackenzie R, Osen E, *et al*. Purification, characterization and selective inhibition of human prostaglandin G/H synthase 1 and 2 expressed in the baculovirus system. *Biochim Biophys Acta* 1994;1209:130-139.
11. Penning TD, Talley JJ, Bertenshaw SR, Carter JS, Collins PW, Docter S, *et al*. Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benze nesulfonamide (SC-58635, celecoxib). *J Med Chem* 1997;40:1347-1365.
12. Johnson JL, Wimsatt J, Buckel SD, Dyer RD, Maddipati KR. Purification and characterization of prostaglandin H synthase-2 from sheep placental cotyledons. *Arch Biochem Biophys* 1995;324:26-34.
13. Gerondakis S, Fulford TS, Messina NL, Grumont RJ. NF-κB control of T cell development. *Nat Immunol* 2014;15:15-25.
14. Bernard D, Gosselin K, Monte D, Vercamer C, Bouali F, Pourtier A, *et al*. Involvement of Rel/nuclear factor-kappaB transcription factors in keratinocyte senescence. *Cancer Res* 2004;64:472-481.
15. Bernal GM, Wahlstrom JS, Crawley CD, Cahill KE, Pytel P, Liang H, *et al*. Loss of Nfkb1 leads to early onset aging. *Aging (Albany NY)* 2014;6:931-943.
16. Iannetti A, Ledoux AC, Tudhope SJ, Sellier H, Zhao B, Mowla S, *et al*. Regulation of p53 and Rb links the alternative NF-κB pathway to EZH2 expression and cell senescence. *PLoS Genet* 2014;10:e1004642.
17. Gitenay D, Wiel C, Lallet-Daher H, Vindrieux D, Aubert S, Payen L, *et al*. Glucose metabolism and hexosamine pathway regulate oncogene-induced senescence. *Cell Death Dis* 2014;5:e1089.
18. Zwerschke W, Mazurek S, Stöckl P, Hütter E, Eigenbrodt E, Jansen-Dürr P. Metabolic analysis of senescent human fibroblasts reveals a role for AMP in cellular senescence. *Biochem J* 2003;376(Pt 2):403-411.
19. Coppe JP, Patil CK, Rodier F, Krtolica A, Beauséjour CM,

Parrinello S, *et al*. A human-like senescence-associated secretory phenotype is conserved in mouse cells dependent on physiological oxygen. *PLoS One* 2010;5:e9188.
20. Freund A, Orjalo AV, Desprez PY, Campisi J. Inflammatory networks during cellular senescence: causes and consequences. *Trends Mol Med* 2010;16:238-246.
21. Coppé JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol* 2010;5:99-118.
22. Acosta JC, O'Loghlen A, Banito A, Guijarro MV, Augert A, Raguz S, *et al*. Chemokine signaling via the CXCR2 receptor reinforces senescence. *Cell* 2008;133:1006-1018.
23. Choi HR, Cho KA, Kang HT, Lee JB, Kaeberlein M, Suh Y, *et al*. Restoration of senescent human diploid fibroblasts by modulation of the extracellular matrix. *Aging Cell* 2011;10:148-157.
24. Helman A, Klochendler A, Azazmeh N, Gabai Y, Horwitz E, Anzi S, *et al*. p16(Ink4a)-induced senescence of pancreatic beta cells enhances insulin secretion. *Nat Med* 2016;22:412-420.
25. Zhang H, Pan KH, Cohen SN. Senescence-specific gene expression fingerprints reveal cell-type-dependent physical clustering of up-regulated chromosomal loci. *Proc Natl Acad Sci U S A* 2003;100:3251-3256.
26. Lindholm DB. Decreased transcription of neuronal polyadenylated RNA during senescence in nuclei from rat brain cortex. *J Neurochem* 1986;47:1503-1506.
27. Smith JB, Willis AL. Aspirin selectively inhibits prostaglandin production in human platelets. *Nat New Biol* 1971;231:235-237.
28. McConnell BB, Starborg M, Brookes S, Peters G. Inhibitors of cyclin-dependent kinases induce features of replicative senescence in early passage human diploid fibroblasts. *Curr Biol* 1998;8:351-354.
29. Wei W, Hemmer RM, Sedivy JM. Role of p14(ARF) in replicative and induced senescence of human fibroblasts. *Mol Cell Biol* 2001;21:6748-6757.

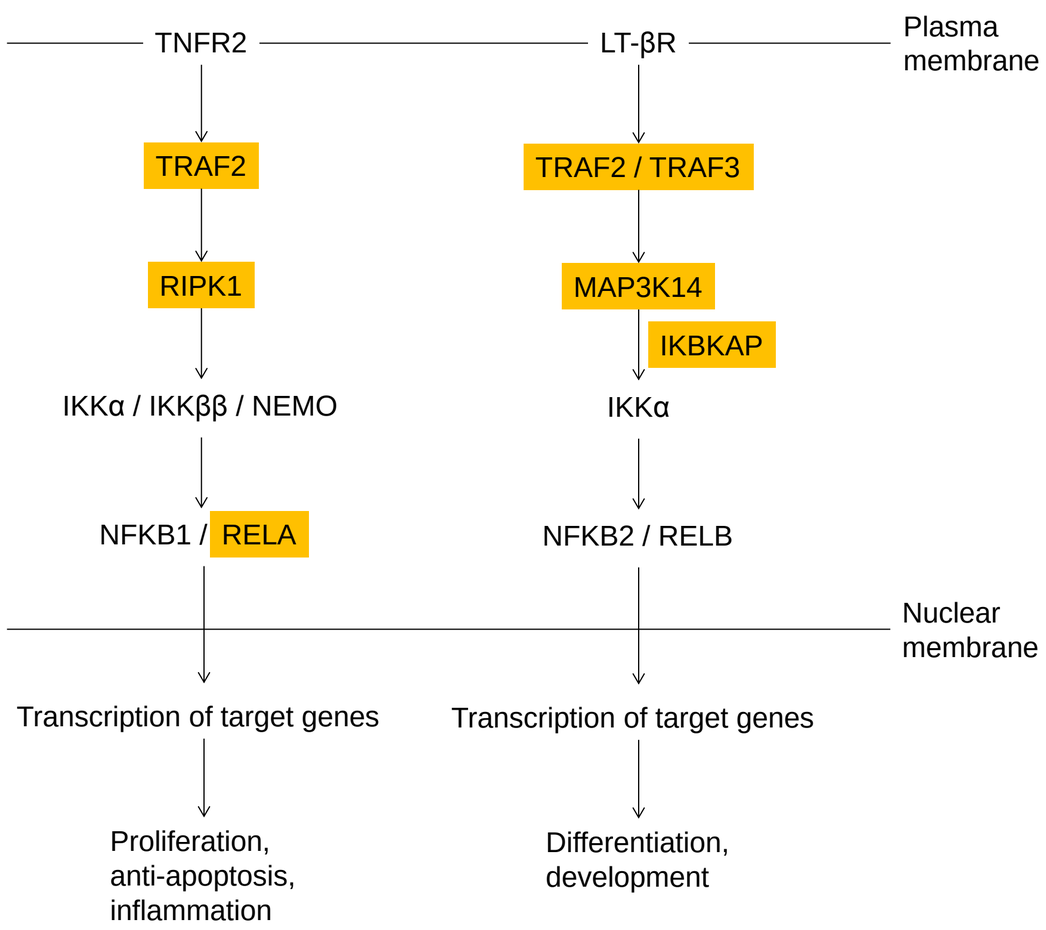
SUPPLEMENTARY INFORMATION

Analysis of Gene Expression in Human Dermal Fibroblasts Treated with Senescence-Modulating COX Inhibitors

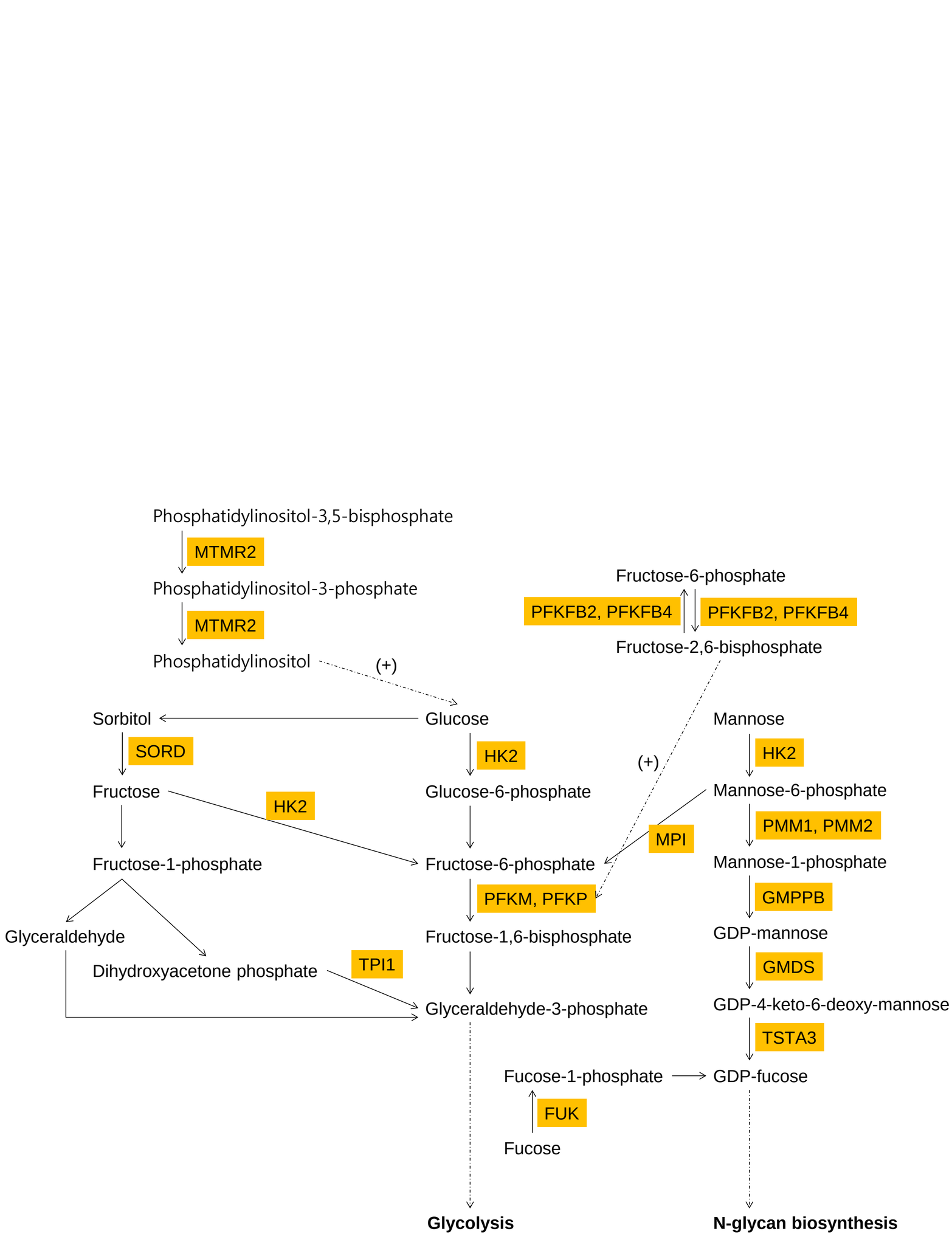
Jeong A. Han[1]*, Jong-Il Kim[2,3,4]**

[1]Department of Biochemistry and Molecular Biology, Kangwon National University School of Medicine, Chuncheon 24341, Korea, [2]Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, Seoul 03080, Korea, [3]Cancer Research Institute, Seoul National University College of Medicine, Seoul 03080, Korea, [4]Department of Biomedical Sciences, Seoul National University Graduate School, Seoul 03080, Korea

http://www.genominfo.org/src/sm/gni-15-56-s001.pdf



Supplementary Fig. 1. BIOCARTA_TNFR2_PATHWAY. Enriched genes were highlighted in orange color.



Supplementary Fig. 2. KEGG_FRUCTOSE_AND_MANNOSE_METABOLISM. Enriched genes were highlighted in orange color.

Supplementary Table 1. Enriched genes of BIOCARTA_TNFR2_PATHWAY in NS-398–treated HDFs (NS-398 vs. DMSO)

Symbol	Description	Running ES
TRAF3	TNF receptor-associated factor 3	0.612
IKBKAP	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	0.522
TRAF2	TNF receptor-associated factor 2	0.430
RIPK1	Receptor (TNFRSF)-interacting serine-threonine kinase 1	0.361
RELA	v-rel avian reticuloendotheliosis viral oncogene homolog A	0.266
MAP3K14	Mitogen-activated protein kinase kinase kinase 14	0.141

HDF, human dermal fibroblast; DMSO, dimethyl sulfoxide; ES, enrichment score.

Supplementary Table 2. Enriched genes of KEGG_FRUCTOSE_AND_MANNOSE_METABOLISM in NS-398–treated HDFs (NS-398 vs. DMSO)

Symbol	Description	Running ES
MTMR2	Myotubularin related protein 2	0.535
PMM1	Phosphomannomutase 1	0.534
PMM2	Phosphomannomutase 2	0.521
SORD	Sorbitol dehydrogenase	0.518
TPI1	Triosephosphate isomerase 1	0.518
GMDS	GDP-mannose 4,6-dehydratase	0.515
PFKFB4	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	0.502
GMPPB	GDP-mannose pyrophosphorylase B	0.484
PFKFB2	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 2	0.443
FUK	Fucokinase	0.400
HK2	Hexokinase 2	0.362
MPI	Mannose phosphate isomerase	0.290
PFKM	Phosphofructokinase, muscle	0.225
PFKP	Phosphofructokinase, platelet	0.143
TSTA3	Tissue specific transplantation antigen P35B	0.063

HDF, human dermal fibroblast; DMSO, dimethyl sulfoxide; ES, enrichment score.

Supplementary Table 3. Enriched genes of HALLMARK_UV_RESPONSE_DN in DMSO-treated HDFs (NS-398 vs. DMSO)

Symbol	Description	Running ES
BCKDHB	Branched chain keto acid dehydrogenase E1, beta polypeptide	–0.405
MAP1B	Microtubule-associated protein 1B	–0.405
HAS2	Hyaluronan synthase 2	–0.401
F3	Coagulation factor III (thromboplastin, tissue factor)	–0.396
SRI	Sorcin	–0.393
ATP2B4	ATPase, Ca++ transporting, plasma membrane 4	–0.390
VLDLR	Very low density lipoprotein receptor	–0.387
NRP1	Neuropilin 1	–0.386
IGF1R	PREDICTED: Homo sapiens hypothetical protein MGC18216 (MGC18216), mRNA.	–0.385
FBLN5	Fibulin 5	–0.384
SMAD3	SMAD family member 3	–0.384
SLC7A1	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 1	–0.382
PTPRM	Protein tyrosine phosphatase, receptor type, M	–0.381
INSIG1	Insulin induced gene 1	–0.380
DDAH1	Dimethylarginine dimethylaminohydrolase 1	–0.378
DAB2	Dab, mitogen-responsive phosphoprotein, homolog 2 (Drosophila)	–0.378
CDC42BPA	CDC42 binding protein kinase alpha (DMPK-like)	–0.378
ABCC1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	–0.377
YTHDC1	YTH domain containing 1	–0.376
SNAI2	Snail family zinc finger 2	–0.373
MMP16	Matrix metallopeptidase 16 (membrane-inserted)	–0.371
ICA1	Islet cell autoantigen 1, 69kDa	–0.368
EFEMP1	EGF containing fibulin-like extracellular matrix protein 1	–0.364
PMP22	Peripheral myelin protein 22	–0.357

SERPINE1	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	–0.349
MGLL	Monoglyceride lipase	–0.343
WDR37	WD repeat domain 37	–0.341
BDNF	Brain-derived neurotrophic factor	–0.338
SIPA1L1	Signal-induced proliferation-associated 1 like 1	–0.335
ADORA2B	Adenosine A2b receptor	–0.333
GCNT1	Glucosaminyl (N-acetyl) transferase 1, core 2	–0.333
CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	–0.330
KCNMA1	Potassium channel, calcium activated large conductance subfamily M alpha, member 1	–0.325
NR1D2	Nuclear receptor subfamily 1, group D, member 2	–0.321
PTGFR	Prostaglandin F receptor (FP)	–0.316
TFPI	Tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	–0.307
BHLHE40	Basic helix-loop-helix family, member e40	–0.304
ATXN1	Ataxin 1	–0.304
ADD3	Adducin 3 (gamma)	–0.299
DYRK1A	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	–0.293
RND3	Rho family GTPase 3	–0.289
ANXA4	Annexin A4	–0.281
PPARG	Peroxisome proliferator-activated receptor gamma	–0.271
MAGI2	Membrane associated guanylate kinase, WW and PDZ domain containing 2	–0.258
GRK5	G protein-coupled receptor kinase 5	–0.246
LPHN2	Latrophilin 2	–0.236
LPAR1	Lysophosphatidic acid receptor 1	–0.236
ATP2B1	ATPase, Ca++ transporting, plasma membrane 1	–0.225
PEX14	Peroxisomal biogenesis factor 14	–0.223
ACVR2A	Activin A receptor, type IIA	–0.209
AGGF1	Angiogenic factor with G patch and FHA domains 1	–0.194

ATP5S	ATP synthase, H+ transporting, mitochondrial Fo complex, subunit s (factor B)	–0.179
ATRN	Attractin	–0.166
TJP1	Tight junction protein 1	–0.151
RASA2	RAS p21 protein activator 2	–0.143
NEK7	NIMA-related kinase 7	–0.128
MAPK14	Mitogen-activated protein kinase 14	–0.111
NIPBL	Nipped-B homolog (Drosophila)	–0.098
PRDM2	PR domain containing 2, with ZNF domain	–0.081
COL11A1	Collagen, type XI, alpha 1	–0.061
PLCB4	Phospholipase C, beta 4	–0.037
ATP2C1	ATPase, Ca++ transporting, type 2C, member 1	–0.016
CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	0.005

DMSO, dimethyl sulfoxide; ES, enrichment score.

Supplementary Table 4. Enriched genes of HALLMARK_PROTEIN_SECRETION in DMSO-treated HDFs (NS-398 vs. DMSO)

Symbol	Description	Running ES
YIPF6	Yip1 domain family, member 6	–0.402
ICA1	Islet cell autoantigen 1, 69kDa	–0.394
IGF2R	Insulin-like growth factor 2 receptor	–0.385
ARFGAP3	ADP-ribosylation factor GTPase activating protein 3	–0.384
ERGIC3	ERGIC and golgi 3	–0.377
TMED2	Transmembrane emp24 domain trafficking protein 2	–0.365
GALC	Galactosylceramidase	–0.357
TOM1L1	Target of myb1 (chicken)-like 1	–0.347
SNAP23	Synaptosomal-associated protein, 23kDa	–0.338
STX7	Syntaxin 7	–0.326
MON2	MON2 homolog (S. cerevisiae)	–0.325
KIF1B	Kinesin family member 1B	–0.324
ARFGEF1	ADP-ribosylation factor guanine nucleotide-exchange factor 1 (brefeldin A-inhibited)	–0.321
RER1	Retention in endoplasmic reticulum sorting receptor 1	–0.314
RAB5A	RAB5A, member RAS oncogene family	–0.313
CLTC	Clathrin, heavy chain (Hc)	–0.311
SGMS1	Sphingomyelin synthase 1	–0.294
VAMP4	Vesicle-associated membrane protein 4	–0.294
TMED10	Transmembrane emp24-like trafficking protein 10 (yeast)	–0.274
CTSC	Cathepsin C	–0.252
DST	Dystonin	–0.231
LAMP2	Lysosomal-associated membrane protein 2	–0.208
DOPEY1	Dopey family member 1	–0.187
ADAM10	ADAM metallopeptidase domain 10	–0.171

SCAMP1	Secretory carrier membrane protein 1	–0.145
ARFIP1	ADP-ribosylation factor interacting protein 1	–0.113
SEC31A	SEC31 homolog A (S. cerevisiae)	–0.085
ARF1	ADP-ribosylation factor 1	–0.050
SEC22B	SEC22 vesicle trafficking protein homolog B (S. cerevisiae) (gene/pseudogene)	0.002

DMSO, dimethyl sulfoxide; ES, enrichment score.

Supplementary Table 5. Enriched genes of BIOCARTA_TFF_PATHWAY in DMSO-treated HDFs (NS-398 vs. DMSO)

Symbol	Description	Running ES
APAF1	Apoptotic peptidase activating factor 1	–0.582
CASP9	Caspase 9, apoptosis-related cysteine peptidase	–0.522
SOS1	Son of sevenless homolog 1 (Drosophila)	–0.432
CYCS	Cytochrome c, somatic	–0.251
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa	0.001

DMSO, dimethyl sulfoxide; ES, enrichment score.

Supplementary Table 6. Enriched genes of GO_R_SMAD_BINDING in DMSO-treated HDFs (NS-398 vs. DMSO)

Symbol	Description	Running ES
PPM1A	Protein phosphatase, Mg2+/Mn2+ dependent, 1A	–0.558
ZEB2	Zinc finger E-box binding homeobox 2	–0.420
RANBP3	RAN binding protein 3	–0.266
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa	0.001

DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast; ES, enrichment score.

Supplementary Table 7. Enriched genes of HALLMARK_G2M_CHECKPOINT in celecoxib-treated HDFs (celecoxib vs. DMSO)

Symbol	Decription	Running ES
BCL3	B-cell CLL/lymphoma 3	0.410
CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	0.410
WHSC1	Wolf-Hirschhorn syndrome candidate 1	0.408
SUV39H1	Suppressor of variegation 3-9 homolog 1 (Drosophila)	0.407
PDS5B	PDS5 cohesin associated factor B	0.406
CENPE	Centromere protein E, 312kDa	0.406
CDC45	Cell division cycle 45	0.406
CENPF	Centromere protein F, 350/400kDa	0.406
KIF15	Kinesin family member 15	0.405
TACC3	Transforming, acidic coiled-coil containing protein 3	0.405
TOP2A	Topoisomerase (DNA) II alpha 170kDa	0.405
KPNB1	Karyopherin (importin) beta 1	0.405
DDX39A	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39A	0.404
UCK2	Uridine-cytidine kinase 2	0.403
SYNCRIP	Synaptotagmin binding, cytoplasmic RNA interacting protein	0.403
NEK2	NIMA-related kinase 2	0.402
TRAIP	TRAF interacting protein	0.401
RAD23B	RAD23 homolog B (S. cerevisiae)	0.400
ORC5	Origin recognition complex, subunit 5	0.400
PBK	PDZ binding kinase	0.398
TMPO	Thymopoietin	0.398
SLC7A1	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 1	0.397
CUL5	Cullin 5	0.396
KIF5B	Kinesin family member 5B	0.392
HNRNPU	Heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A)	0.390

EWSR1	EWS RNA-binding protein 1	0.388
POLQ	Polymerase (DNA directed), theta	0.388
TFDP1	Transcription factor Dp-1	0.388
SLC38A1	Solute carrier family 38, member 1	0.387
H2AFZ	H2A histone family, member Z	0.385
UBE2C	Ubiquitin-conjugating enzyme E2C	0.385
NUSAP1	Nucleolar and spindle associated protein 1	0.384
ZAK	Sterile alpha motif and leucine zipper containing kinase AZK	0.383
CDK1	Cyclin-dependent kinase 1	0.383
PLK1	Polo-like kinase 1	0.381
LMNB1	Lamin B1	0.380
DR1	Down-regulator of transcription 1, TBP-binding (negative cofactor 2)	0.380
KIF11	Kinesin family member 11	0.377
PTTG3P	Pituitary tumor-transforming 3, pseudogene	0.376
STIL	SCL/TAL1 interrupting locus	0.376
CKS2	CDC28 protein kinase regulatory subunit 2	0.373
DBF4	DBF4 zinc finger	0.366
RBL1	Retinoblastoma-like 1	0.361
CUL4A	Cullin 4A	0.353
SNRPD1	Small nuclear ribonucleoprotein D1 polypeptide 16kDa	0.350
AMD1	Adenosylmethionine decarboxylase 1	0.342
SFPQ	Splicing factor proline/glutamine-rich	0.336
EZH2	Enhancer of zeste 2 polycomb repressive complex 2 subunit	0.330
SAP30	Sin3A-associated protein, 30kDa	0.321
H2AFV	H2A histone family, member V	0.315
CDC6	Cell division cycle 6	0.305
PURA	Purine-rich element binding protein A	0.296

RASAL2	RAS protein activator like 2	0.288
MYC	v-myc avian myelocytomatosis viral oncogene homolog	0.284
MNAT1	MNAT CDK-activating kinase assembly factor 1	0.281
CDKN2C	Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	0.274
HMGN2	High mobility group nucleosomal binding domain 2	0.268
RAD21	RAD21 homolog (S. pombe)	0.259
G3BP1	GTPase activating protein (SH3 domain) binding protein 1	0.246
MTF2	Metal response element binding transcription factor 2	0.233
YTHDC1	YTH domain containing 1	0.221
BUB1	BUB1 mitotic checkpoint serine/threonine kinase	0.209
FBXO5	F-box protein 5	0.196
TTK	TTK protein kinase	0.193
KPNA2	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	0.179
SMC2	Structural maintenance of chromosomes 2	0.165
HIF1A	Hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	0.152
ODF2	Outer dense fiber of sperm tails 2	0.137
SLC12A2	Solute carrier family 12 (sodium/potassium/chloride transporter), member 2	0.125
RACGAP1	Rac GTPase activating protein 1	0.109
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	0.095
XPO1	Exportin 1	0.077
HMMR	Hyaluronan-mediated motility receptor (RHAMM)	0.063
BUB3	BUB3 mitotic checkpoint protein	0.047
CDC25B	Cell division cycle 25B	0.026

HDF, human dermal fibroblast; DMSO, dimethyl sulfoxide; ES, enrichment score.

Supplementary Table 8. Enriched genes of HALLMARK_E2F_TARGETS in celecoxib-treated HDFs (celecoxib vs. DMSO)

Symbol	Description	Running ES
SUV39H1	Suppressor of variegation 3-9 homolog 1 (Drosophila)	0.357
PCNA	Proliferating cell nuclear antigen	0.353
TACC3	Transforming, acidic coiled-coil containing protein 3	0.350
TOP2A	Topoisomerase (DNA) II alpha 170kDa	0.350
PDS5B	PDS5 cohesin associated factor B	0.347
SPC25	SPC25, NDC80 kinetochore complex component	0.346
CENPM	Centromere protein M	0.343
HELLS	Helicase, lymphoid-specific	0.343
GINS4	GINS complex subunit 4 (Sld5 homolog)	0.342
TMPO	Thymopoietin	0.339
SYNCRIP	Synaptotagmin binding, cytoplasmic RNA interacting protein	0.339
RAD51C	RAD51 paralog C	0.339
NUP205	Nucleoporin 205kDa	0.338
TBRG4	Transforming growth factor beta regulator 4	0.338
CENPE	Centromere protein E, 312kDa	0.337
CHEK2	Checkpoint kinase 2	0.336
SLBP	Stem-loop binding protein	0.336
DLGAP5	Discs, large (Drosophila) homolog-associated protein 5	0.334
PMS2	PMS2 postmeiotic segregation increased 2 (S. cerevisiae)	0.334
DDX39A	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39A	0.331
H2AFZ	H2A histone family, member Z	0.330
PLK1	Polo-like kinase 1	0.326
CBX5	Chromobox homolog 5	0.326

POLD1	Polymerase (DNA directed), delta 1, catalytic subunit	0.323
PSIP1	PC4 and SFRS1 interacting protein 1	0.322
LMNB1	Lamin B1	0.320
DUT	Deoxyuridine triphosphatase	0.319
NUDT21	Nudix (nucleoside diphosphate linked moiety X)-type motif 21	0.318
PRPS1	Phosphoribosyl pyrophosphate synthetase 1	0.318
RPA3	Replication protein A3, 14kDa	0.315
PPM1D	Protein phosphatase, Mg2+/Mn2+ dependent, 1D	0.313
DCK	Deoxycytidine kinase	0.309
ING3	Inhibitor of growth family, member 3	0.309
TIPIN	TIMELESS interacting protein	0.308
NME1	NME/NM23 nucleoside diphosphate kinase 1	0.307
DCLRE1B	DNA cross-link repair 1B	0.306
CDK1	Cyclin-dependent kinase 1	0.304
DEK	DEK proto-oncogene	0.304
LYAR	Ly1 antibody reactive	0.303
CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	0.299
RAD1	RAD1 checkpoint DNA exonuclease	0.298
MCM4	Minichromosome maintenance complex component 4	0.293
USP1	Ubiquitin specific peptidase 1	0.290
DEPDC1	DEP domain containing 1	0.290
EZH2	Enhancer of zeste 2 polycomb repressive complex 2 subunit	0.290
CKS2	CDC28 protein kinase regulatory subunit 2	0.285
BRMS1L	Breast cancer metastasis-suppressor 1-like	0.282
PSMC3IP	PSMC3 interacting protein	0.281
SMC6	Structural maintenance of chromosomes 6	0.275
EED	Embryonic ectoderm development	0.265

HMGB2	High mobility group box 2	0.259
TUBG1	Tubulin, gamma 1	0.252
MTHFD2	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase	0.248
CCP110	Centriolar coiled coil protein 110kDa	0.236
NBN	Nibrin	0.226
MYC	v-myc avian myelocytomatosis viral oncogene homolog	0.215
BRCA1	Breast cancer 1, early onset	0.204
CDKN2C	Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	0.193
SMC3	Structural maintenance of chromosomes 3	0.182
RAD21	RAD21 homolog (S. pombe)	0.177
MMS22L	MMS22-like, DNA repair protein	0.167
DIAPH3	Diaphanous-related formin 3	0.154
LUC7L3	LUC7-like 3 (S. cerevisiae)	0.141
MSH2	mutS homolog 2	0.128
NAP1L1	Nucleosome assembly protein 1-like 1	0.114
TCF19	Transcription factor 19	0.108
KPNA2	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	0.093
RACGAP1	Rac GTPase activating protein 1	0.088
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	0.074
XPO1	Exportin 1	0.055
HMMR	Hyaluronan-mediated motility receptor (RHAMM)	0.041
CDC25B	Cell division cycle 25B	0.026

HDF, human dermal fibroblast; DMSO, dimethyl sulfoxide; ES, enrichment score.

Supplementary Table 9. Enriched genes of GO_GAMMA_TUBULIN_COMPLEX in Celecoxib-treated HDFs (celecoxib vs. DMSO)

Symbol	Description	Running ES
TUBG1	Tubulin, gamma 1	0.685
MZT1	Mitotic spindle organizing protein 1	0.595
BRCA1	Breast cancer 1, early onset	0.490
CEP290	Centrosomal protein 290kDa	0.391
ZNF365	Zinc finger protein 365	0.293
TOPORS	Topoisomerase I binding, arginine/serine-rich, E3 ubiquitin protein ligase	0.172

HDF, human dermal fibroblast; DMSO, dimethyl sulfoxide; ES, enrichment score.

Supplementary Table 10. Enriched genes of GO_FOUR_WAY_JUNCTION_DNA_BINDING in celecoxib-treated HDFs (celecoxib vs. DMSO)

Symbol	Description	Running ES
MEN1	Multiple endocrine neoplasia I	0.723
DMC1	DNA meiotic recombinase 1	0.640
HMGB1	High mobility group box 1	0.534
HMGB2	High mobility group box 2	0.434
RAD51D	RAD51 paralog D	0.330
YY1	YY1 transcription factor	0.209
MSH2	mutS homolog 2	0.110

HDF, human dermal fibroblast; DMSO, dimethyl sulfoxide; ES, enrichment score.

Supplementary Table 11. Enriched genes of REACTOME_OLFACTORY_SIGNALING_PATHWAY in DMSO-treated HDFs (celecoxib vs. DMSO)

Symbol	Description	Running ES
GNB1	Guanine nucleotide binding protein (G protein), beta polypeptide 1	–0.553
OR1L8	Olfactory receptor, family 1, subfamily L, member 8	–0.543
OR10W1	Olfactory receptor, family 10, subfamily W, member 1	–0.523
OR4C15	Olfactory receptor, family 4, subfamily C, member 15	–0.513
OR5AS1	Olfactory receptor, family 5, subfamily AS, member 1	–0.486
OR9A4	Olfactory receptor, family 9, subfamily A, member 4	–0.468
OR8B12	Olfactory receptor, family 8, subfamily B, member 12	–0.452
OR4C12	Olfactory receptor, family 4, subfamily C, member 12	–0.429
OR4A16	Olfactory receptor, family 4, subfamily A, member 16	–0.364
OR1D4	Homo sapiens olfactory receptor, family 1, subfamily D, member 4 (OR1D4), mRNA.	–0.293
OR3A2	Olfactory receptor, family 3, subfamily A, member 2	–0.199
OR10H4	Olfactory receptor, family 10, subfamily H, member 4	0.000

DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast; ES, enrichment score.

Supplementary Table 12. Enriched genes of HALLMARK_PROTEIN_SECRETION in aspirin-treated HDFs (aspirin vs. DMSO)

Symbol	Description	Running ES
ARFGAP3	ADP-ribosylation factor GTPase activating protein 3	0.403
CTSC	Cathepsin C	0.395
YIPF6	Yip1 domain family, member 6	0.389
STX7	Syntaxin 7	0.381
PAM	Peptidylglycine alpha-amidating monooxygenase	0.375
VAMP7	Vesicle-associated membrane protein 7	0.373
SCAMP1	Secretory carrier membrane protein 1	0.370
CLTC	Clathrin, heavy chain (Hc)	0.369
IGF2R	Insulin-like growth factor 2 receptor	0.368
GLA	Galactosidase, alpha	0.367
SNX2	Sorting nexin 2	0.360
AP3S1	Adaptor-related protein complex 3, sigma 1 subunit	0.358
SGMS1	Sphingomyelin synthase 1	0.355
DOPEY1	Dopey family member 1	0.346
TMED10	Transmembrane emp24-like trafficking protein 10 (yeast)	0.338
AP1G1	Adaptor-related protein complex 1, gamma 1 subunit	0.322
TMED2	Transmembrane emp24 domain trafficking protein 2	0.307
M6PR	Mannose-6-phosphate receptor (cation dependent)	0.295
ARFGEF1	ADP-ribosylation factor guanine nucleotide-exchange factor 1 (brefeldin A-inhibited)	0.290
STAM	Signal transducing adaptor molecule (SH3 domain and ITAM motif) 1	0.274
MON2	MON2 homolog (S. cerevisiae)	0.260
ADAM10	ADAM metallopeptidase domain 10	0.246
NAPG	N-Ethylmaleimide-sensitive factor attachment protein, gamma	0.238
RAB5A	RAB5A, member RAS oncogene family	0.216

TOM1L1	Target of myb1 (chicken)-like 1	0.201
GBF1	Golgi brefeldin A resistant guanine nucleotide exchange factor 1	0.183
DST	Dystonin	0.167
LMAN1	Lectin, mannose-binding, 1	0.137
ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	0.112
SEC22B	SEC22 vesicle trafficking protein homolog B (S. cerevisiae) (gene/pseudogene)	0.076

HDF, human dermal fibroblast; DMSO, dimethyl sulfoxide; ES, enrichment score.

Supplementary Table 13. Enriched genes of HALLMARK_UV_RESPONSE_DN in aspirin-treated HDFs (aspirin vs. DMSO)

Symbol	Description	Running ES
PLCB4	Phospholipase C, beta 4	0.318
CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	0.318
PTGFR	Prostaglandin F receptor (FP)	0.316
COL11A1	Collagen, type XI, alpha 1	0.315
ZMIZ1	Zinc finger, MIZ-type containing 1	0.313
SYNE1	Spectrin repeat containing, nuclear envelope 1	0.312
PPARG	Peroxisome proliferator-activated receptor gamma	0.311
VLDLR	Very low density lipoprotein receptor	0.309
MTA1	Metastasis associated 1	0.308
SNAI2	Snail family zinc finger 2	0.306
SDC2	Syndecan 2	0.304
SIPA1L1	Signal-induced proliferation-associated 1 like 1	0.303
LDLR	Low density lipoprotein receptor	0.302
DDAH1	Dimethylarginine dimethylaminohydrolase 1	0.302
APBB2	Amyloid beta (A4) precursor protein-binding, family B, member 2	0.302
RBPMS	RNA binding protein with multiple splicing	0.301
FAM179B	Family with sequence similarity 179, member B	0.299
PDLIM5	PDZ and LIM domain 5	0.297
SERPINE1	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	0.297
MAGI2	Membrane associated guanylate kinase, WW and PDZ domain containing 2	0.297
PRDM2	PR domain containing 2, with ZNF domain	0.296
TGFBR3	Transforming growth factor, beta receptor III	0.290
SPOP	Speckle-type POZ protein	0.279
KALRN	Kalirin, RhoGEF kinase	0.269

IRS1	Insulin receptor substrate 1	0.258
DYRK1A	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	0.248
AGGF1	Angiogenic factor with G patch and FHA domains 1	0.246
PEX14	Peroxisomal biogenesis factor 14	0.237
RND3	Rho family GTPase 3	0.230
LPHN2	Latrophilin 2	0.216
BHLHE40	Basic helix-loop-helix family, member e40	0.208
ATRX	Alpha thalassemia/mental retardation syndrome X-linked	0.197
BMPR1A	Bone morphogenetic protein receptor, type IA	0.180
YTHDC1	YTH domain containing 1	0.164
MAP1B	Microtubule-associated protein 1B	0.149
TJP1	Tight junction protein 1	0.133
ITGB3	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	0.122
NR1D2	Nuclear receptor subfamily 1, group D, member 2	0.112
ABCC1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	0.098
INSIG1	Insulin induced gene 1	0.076
NEK7	NIMA-related kinase 7	0.049
RASA2	RAS p21 protein activator 2	0.021

HDF, human dermal fibroblast; DMSO, dimethyl sulfoxide; ES, enrichment score.

Supplementary Table 14. Enriched genes of GO_KERATIN_FILAMENT in aspirin-treated HDFs (aspirin vs. DMSO)

Symbol	Description	Running ES
KRTAP9-4	Keratin associated protein 9-4	0.788
KRT80	Keratin 80, type II	0.761
KRTAP1-4	PREDICTED: Homo sapiens similar to keratin associated protein 1.6 (LOC730743), mRNA.	0.732
KRT7	Keratin 7, type II	0.690
CSNK1A1	Casein kinase 1, alpha 1	0.667
KRT14	Keratin 14, type I	0.605
KRTAP4-8	PREDICTED: Homo sapiens keratin associated protein 4-8, transcript variant 2 (KRTAP4-8), mRNA.	0.544
KRT86	Keratin 86, type II	0.481
KRTAP2-1	Keratin associated protein 2-1	0.428
KRT81	Keratin 81, type II	0.331
KRTAP4-12	Keratin associated protein 4-12	0.227
KRT8	Keratin 8, type II	0.114

HDF, human dermal fibroblast; DMSO, dimethyl sulfoxide; ES, enrichment score.

Supplementary Table 15. Enriched genes of GO_INTERMEDIATE_FILAMENT in aspirin-treated HDFs (aspirin vs. DMSO)

Symbol	Description	Running ES
KRTAP9-4	Keratin associated protein 9-4	0.542
NME1	NME/NM23 nucleoside diphosphate kinase 1	0.527
KRT80	Keratin 80, type II	0.514
KRTAP1-4	PREDICTED: Homo sapiens similar to keratin associated protein 1.6 (LOC730743), mRNA.	0.508
NEFH	Neurofilament, heavy polypeptide	0.491
KRT7	Keratin 7, type II	0.464
INA	Internexin neuronal intermediate filament protein, alpha	0.446
CSNK1A1	Casein kinase 1, alpha 1	0.437
KRT14	Keratin 14, type I	0.408
SLC1A4	Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	0.374
KRTAP4-8	PREDICTED: Homo sapiens keratin associated protein 4-8, transcript variant 2 (KRTAP4-8), mRNA.	0.345
KRT86	Keratin 86, type II	0.316
KRT17	Keratin 17, type I	0.287
NARF	Nuclear prelamin A recognition factor	0.248
KRTAP2-1	Keratin associated protein 2-1	0.215
KRT81	Keratin 81, type II	0.167
KRTAP4-12	Keratin associated protein 4-12	0.114
KRT8	Keratin 8, type II	0.056

HDF, human dermal fibroblast; DMSO, dimethyl sulfoxide; ES, enrichment score.

Supplementary Table 16. Enriched genes of KEGG_PROSTATE_CANCER in DMSO-treated HDFs (aspirin vs. DMSO)

Symbol	Description	Running ES
MAP2K1	Mitogen-activated protein kinase kinase 1	–0.418
RB1	Retinoblastoma 1	–0.410
CDK2	Cyclin-dependent kinase 2	–0.406
PDGFD	Platelet derived growth factor D	–0.394
MDM2	MDM2 proto-oncogene, E3 ubiquitin protein ligase	–0.381
TP53	Tumor protein p53	–0.361
PDGFC	Platelet derived growth factor C	–0.349
CREB5	cAMP responsive element binding protein 5	–0.326
NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	–0.306
TCF7L2	Transcription factor 7-like 2 (T-cell specific, HMG-box)	–0.282
CCNE2	Cyclin E2	–0.261
FGFR1	Fibroblast growth factor receptor 1	–0.225
PIK3CB	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta	–0.181
EP300	E1A binding protein p300	–0.134
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa	0.000

DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast; ES, enrichment score.

Supplementary Table 17. Enriched genes of KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC in DMSO-treated HDFs (aspirin vs. DMSO)

Symbol	Description	Running ES
ITGA1	Integrin, alpha 1	–0.415
DSC2	Desmocollin 2	–0.400
ACTG1	Actin gamma 1	–0.389
LEF1	Lymphoid enhancer-binding factor 1	–0.376
CACNB1	Calcium channel, voltage-dependent, beta 1 subunit	–0.367
ITGA10	Integrin, alpha 10	–0.363
ITGAV	Integrin, alpha V	–0.349
SLC8A1	Solute carrier family 8 (sodium/calcium exchanger), member 1	–0.346
ITGB3	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	–0.322
TCF7L2	Transcription factor 7-like 2 (T-cell specific, HMG-box)	–0.282
ITGA9	Integrin, alpha 9	–0.243
ITGA4	Integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	–0.197
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa	0.000

DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast; ES, enrichment score.

Supplementary Table 18. Enriched genes of GO_GENITALIA_DEVELOPMENT in DMSO-treated HDFs (aspirin vs. DMSO)

Symbol	Description	Running ES
WNT5A	Wingless-type MMTV integration site family, member 5A	–0.633
BAK1	BCL2-antagonist/killer 1	–0.611
BAX	BCL2-associated X protein	–0.568
DNAJC19	DnaJ (Hsp40) homolog, subfamily C, member 19	–0.508
LGR4	Leucine-rich repeat containing G protein-coupled receptor 4	–0.449
TBX3	T-box 3	–0.364
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa	0.000

DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast; ES, enrichment score.

Supplementary Table 19. Enriched genes of GO_NEGATIVE_REGULATION_OF_PROTEIN_LOCALIZATION_TO_PLASMA_MEMBRANE in DMSO-treated HDFs (aspirin vs. DMSO)

Symbol	Description	Running ES
NUMB	Numb homolog (Drosophila)	–0.646
CLTC	Clathrin, heavy chain (Hc)	–0.635
TMEM59	Transmembrane protein 59	–0.630
LYPLA1	Lysophospholipase I	–0.590
PID1	Phosphotyrosine interaction domain containing 1	–0.532
LZTFL1	Leucine zipper transcription factor-like 1	–0.484
GOPC	Golgi-associated PDZ and coiled-coil motif containing	–0.417
GBP1	Guanylate binding protein 1, interferon-inducible	–0.336
RHOQ	ras homolog family member Q	–0.201
TMBIM1	Transmembrane BAX inhibitor motif containing 1	0.002

DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast; ES, enrichment score.

Supplementary Table 20. Enriched genes GO_ENDOTHELIAL_CELL_DEVELOPMENT in DMSO-treated HDFs (aspirin vs. DMSO)

Symbol	Description	Running ES
ACVR1	Activin A receptor, type I	–0.462
CLIC4	Chloride intracellular channel 4	–0.461
MYADM	Myeloid-associated differentiation marker	–0.454
STC1	Stanniocalcin 1	–0.438
GSTM3	Glutathione S-transferase mu 3 (brain)	–0.437
RAP2B	RAP2B, member of RAS oncogene family	–0.426
RBPJ	Recombination signal binding protein for immunoglobulin kappa J region	–0.410
RAB1B	RAB1B, member RAS oncogene family	–0.387
F2RL1	Coagulation factor II (thrombin) receptor-like 1	–0.361
RDX	Radixin	–0.335
SMAD4	SMAD family member 4	–0.310
TJP1	Tight junction protein 1	–0.293
BMPER	BMP binding endothelial regulator	–0.259
HEG1	Heart development protein with EGF-like domains 1	–0.227
PDE4D	Phosphodiesterase 4D, cAMP-specific	–0.182
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa	0.000

DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast; ES, enrichment score.

Supplementary Table 21. Enriched genes of GO_RNA_POLYMERASE_II_ACTIVATING_TRANSCRIPTION_FACTOR_BINDING in DMSO-treated HDFs (aspirin vs. DMSO)

Symbol	Description	Running ES
NFE2L2	Nuclear factor, erythroid 2-like 2	–0.567
ATF2	Activating transcription factor 2	–0.548
SIN3A	SIN3 transcription regulator family member A	–0.515
RB1	Retinoblastoma 1	–0.489
BHLHE40	Basic helix-loop-helix family, member e40	–0.458
TBX3	T-box 3	–0.408
EP300	E1A binding protein p300	–0.311
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa	0.000

DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast; ES, enrichment score.

Supplementary Table 22. Enriched genes of GO_SMAD_BINDING in DMSO-treated HDFs (aspirin vs. DMSO)

Symbol	Description	Running ES
TGIF1	TGFB-induced factor homeobox 1	–0.487
MAGI2	Membrane associated guanylate kinase, WW and PDZ domain containing 2	–0.481
TGFBR3	Transforming growth factor, beta receptor III	–0.469
PPM1A	Protein phosphatase, Mg2+/Mn2+ dependent, 1A	–0.456
USP9Y	Ubiquitin specific peptidase 9, Y-linked	–0.454
SMAD4	SMAD family member 4	–0.437
BMPR1A	Bone morphogenetic protein receptor, type IA	–0.427
AXIN2	Axin 2	–0.417
TOB1	Transducer of ERBB2, 1	–0.383
ZEB2	Zinc finger E-box binding homeobox 2	–0.348
EP300	E1A binding protein p300	–0.308
EID2	EP300 interacting inhibitor of differentiation 2	–0.257
FLNA	Filamin A, alpha	–0.200
MEF2A	Myocyte enhancer factor 2A	–0.139
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa	0.000

DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast; ES, enrichment score.