

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

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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 H₂ (PGH₂). PGH₂ is a common precursor for prostanoid biosynthesis such as PGD₂, PGE₂, PGF₂ α , PGI₂, and thromboxane A₂. 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 κ B (NF- κ B). NF- κ B 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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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% CO₂ 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 IC₅₀ 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	75 μ M	1.77 μ M	20 μ M
	Celecoxib	Accelerated	15 μ M	0.04 μ M	0.5 μ M
Non-selective	Aspirin	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 $(I_{NS-398} - I_{DMSO})$ for the comparison of

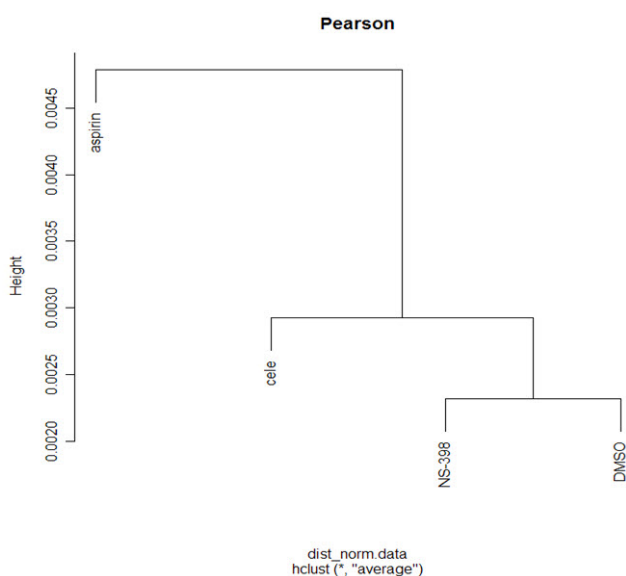


Fig. 1. Segregation between the drug-treated HDFs. Unsupervised hierarchical cluster analysis was done between four drug-treated HDFs using 20,271 probe sets with the detection p -value < 0.05 .

NS-398 versus DMSO; the 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).

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.

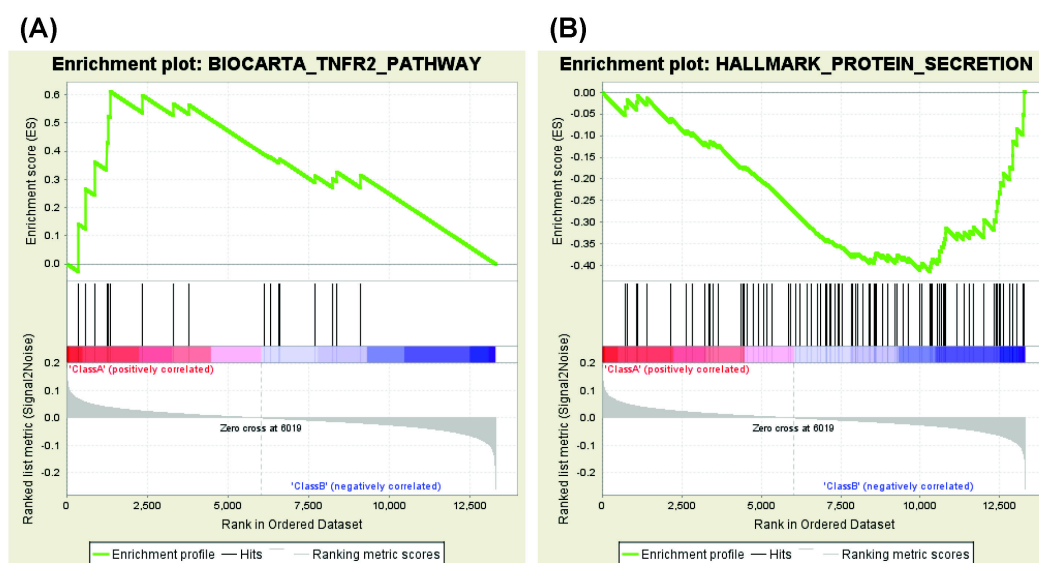


Fig. 2. Enrichment plots (NS-398 vs. DMSO). (A) A representative enriched gene set in NS-398-treated HDFs. (B) A representative enriched gene set in DMSO-treated HDFs. DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast.

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

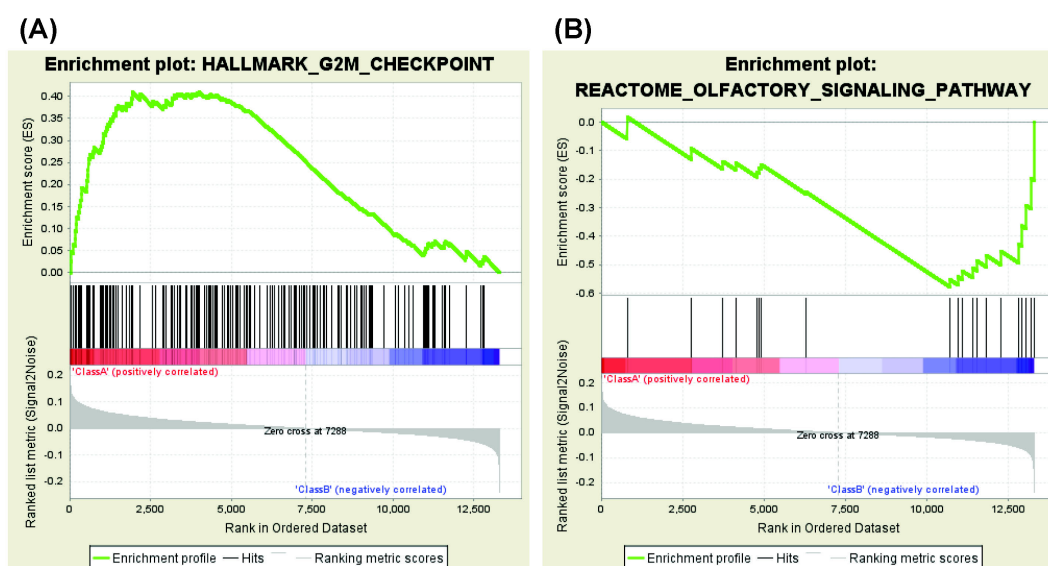


Fig. 3. Enrichment plots (celecoxib vs. DMSO). (A) A representative enriched gene set in celecoxib-treated HDFs. (B) The representative enriched gene set in DMSO-treated HDFs. DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast.

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- κ B signaling that mediates cell proliferation, anti-apoptosis, inflammation, differentiation, or development (Supplementary Fig. 1) [13]. NF- κ B, 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- κ B1 knockout mice showed

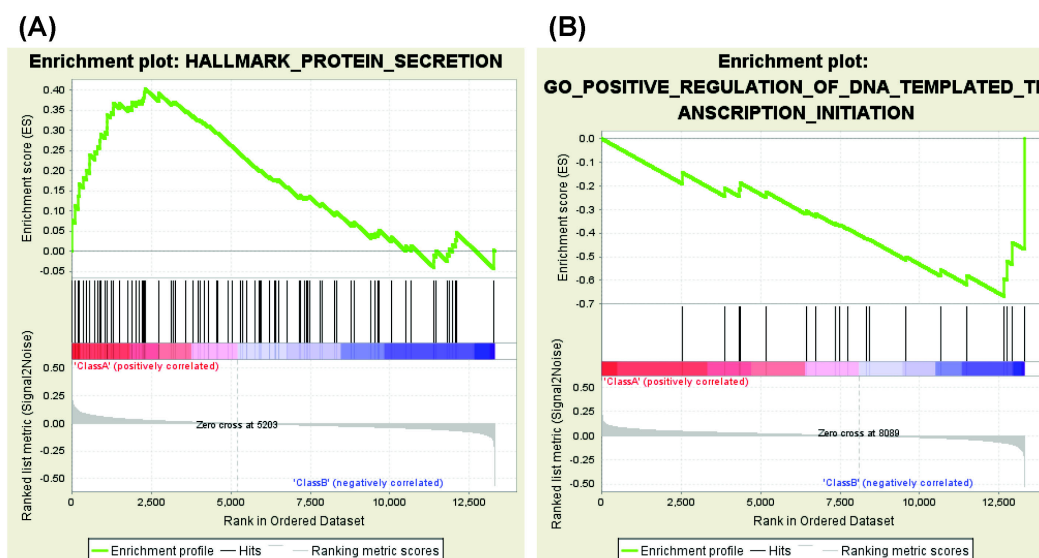


Fig. 4. Enrichment plots (aspirin vs. DMSO). (A) A representative enriched gene set in aspirin-treated HDFs. (B) A representative enriched gene set in DMSO-treated HDFs. DMSO, dimethyl sulfoxide; HDF, human dermal fibroblast.

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- κ B2 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- κ B 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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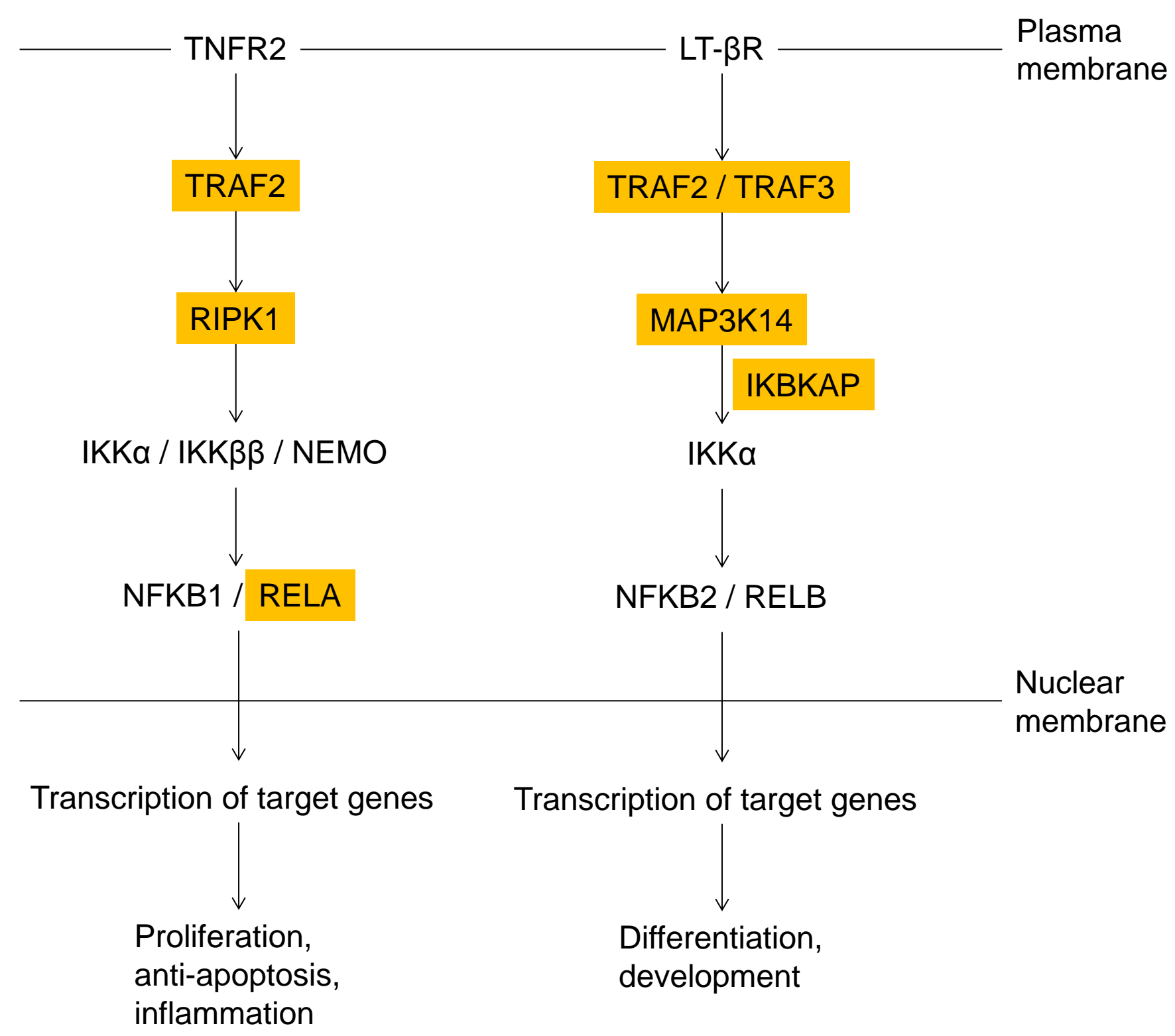
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SUPPLEMENTARY INFORMATION

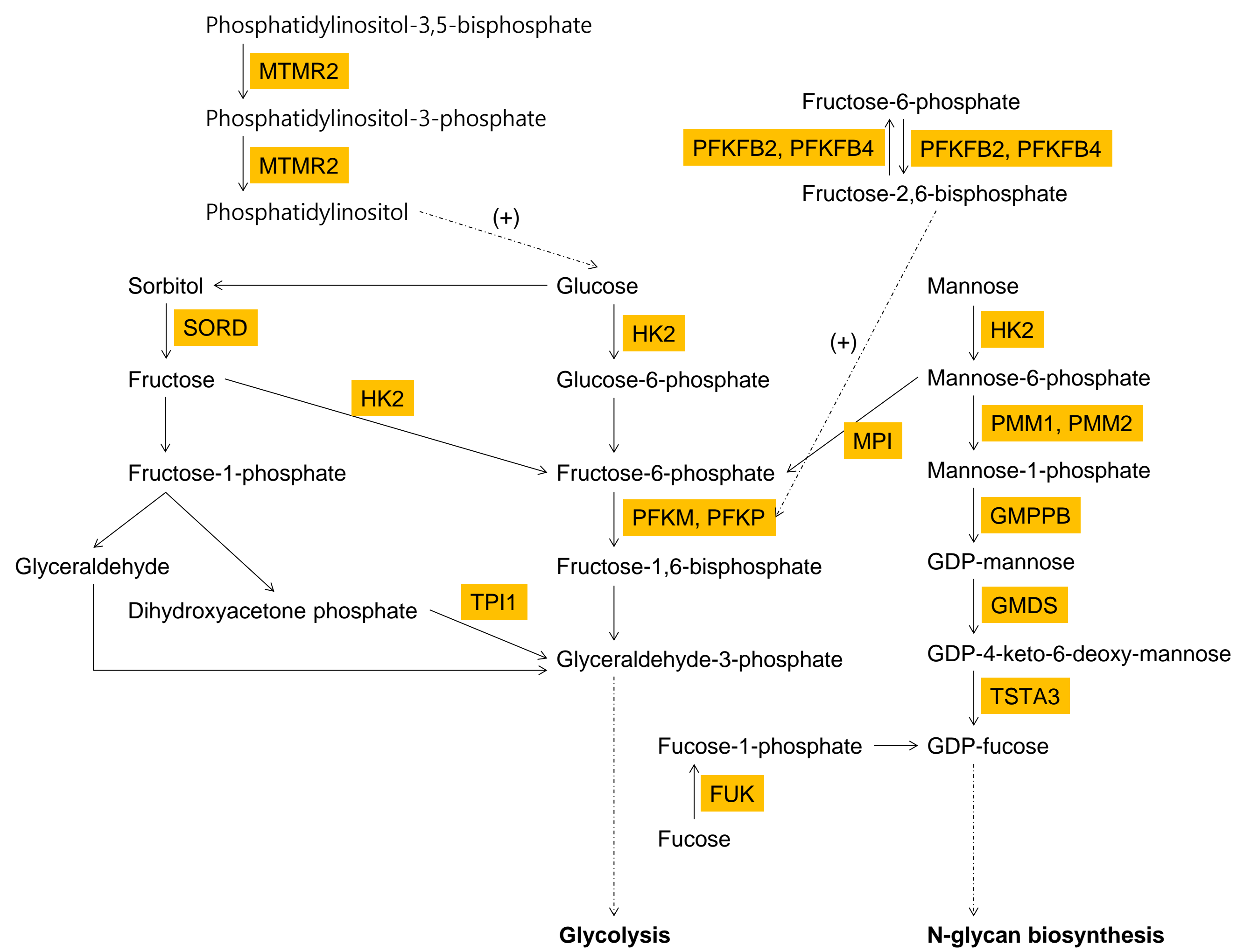
Analysis of Gene Expression in Human Dermal Fibroblasts Treated with Senescence-Modulating COX Inhibitors

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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
<i>TRAF3</i>	TNF receptor-associated factor 3	0.612
<i>IKBKAP</i>	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	0.522
<i>TRAF2</i>	TNF receptor-associated factor 2	0.430
<i>RIPK1</i>	Receptor (TNFRSF)-interacting serine-threonine kinase 1	0.361
<i>RELA</i>	v-rel avian reticuloendotheliosis viral oncogene homolog A	0.266
<i>MAP3K14</i>	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_MANNANOSE_METABOLISM in NS-398–treated HDFs (NS-398 vs.

DMSO)

Symbol	Description	Running ES
<i>MTMR2</i>	Myotubularin related protein 2	0.535
<i>PMM1</i>	Phosphomannomutase 1	0.534
<i>PMM2</i>	Phosphomannomutase 2	0.521
<i>SORD</i>	Sorbitol dehydrogenase	0.518
<i>TPI1</i>	Triosephosphate isomerase 1	0.518
<i>GMDS</i>	GDP-mannose 4,6-dehydratase	0.515
<i>PFKFB4</i>	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	0.502
<i>GMPPB</i>	GDP-mannose pyrophosphorylase B	0.484
<i>PFKFB2</i>	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 2	0.443
<i>FUK</i>	Fucokinase	0.400
<i>HK2</i>	Hexokinase 2	0.362
<i>MPI</i>	Mannose phosphate isomerase	0.290
<i>PFKM</i>	Phosphofructokinase, muscle	0.225
<i>PFKP</i>	Phosphofructokinase, platelet	0.143
<i>TSTA3</i>	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
<i>BCKDHB</i>	Branched chain keto acid dehydrogenase E1, beta polypeptide	-0.405
<i>MAP1B</i>	Microtubule-associated protein 1B	-0.405
<i>HAS2</i>	Hyaluronan synthase 2	-0.401
<i>F3</i>	Coagulation factor III (thromboplastin, tissue factor)	-0.396
<i>SRI</i>	Sorcin	-0.393
<i>ATP2B4</i>	ATPase, Ca ⁺⁺ transporting, plasma membrane 4	-0.390
<i>VLDLR</i>	Very low density lipoprotein receptor	-0.387
<i>NRPI</i>	Neuropilin 1	-0.386
<i>IGF1R</i>	PREDICTED: Homo sapiens hypothetical protein MGC18216 (MGC18216), mRNA.	-0.385
<i>FBLN5</i>	Fibulin 5	-0.384
<i>SMAD3</i>	SMAD family member 3	-0.384
<i>SLC7A1</i>	Solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 1	-0.382
<i>PTPRM</i>	Protein tyrosine phosphatase, receptor type, M	-0.381
<i>INSIG1</i>	Insulin induced gene 1	-0.380
<i>DDAHI</i>	Dimethylarginine dimethylaminohydrolase 1	-0.378
<i>DAB2</i>	Dab, mitogen-responsive phosphoprotein, homolog 2 (Drosophila)	-0.378
<i>CDC42BPA</i>	CDC42 binding protein kinase alpha (DMPK-like)	-0.378
<i>ABCCI</i>	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	-0.377
<i>YTHDC1</i>	YTH domain containing 1	-0.376
<i>SNAI2</i>	Snail family zinc finger 2	-0.373
<i>MMP16</i>	Matrix metalloproteinase 16 (membrane-inserted)	-0.371
<i>ICAI</i>	Islet cell autoantigen 1, 69kDa	-0.368
<i>EFEMP1</i>	EGF containing fibulin-like extracellular matrix protein 1	-0.364
<i>PMP22</i>	Peripheral myelin protein 22	-0.357

<i>SERPINE1</i>	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	-0.349
<i>MGLL</i>	Monoglyceride lipase	-0.343
<i>WDR37</i>	WD repeat domain 37	-0.341
<i>BDNF</i>	Brain-derived neurotrophic factor	-0.338
<i>SIPAIL1</i>	Signal-induced proliferation-associated 1 like 1	-0.335
<i>ADORA2B</i>	Adenosine A2b receptor	-0.333
<i>GCNT1</i>	Glucosaminyl (N-acetyl) transferase 1, core 2	-0.333
<i>CITED2</i>	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	-0.330
<i>KCNMA1</i>	Potassium channel, calcium activated large conductance subfamily M alpha, member 1	-0.325
<i>NR1D2</i>	Nuclear receptor subfamily 1, group D, member 2	-0.321
<i>PTGFR</i>	Prostaglandin F receptor (FP)	-0.316
<i>TFPI</i>	Tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	-0.307
<i>BHLHE40</i>	Basic helix-loop-helix family, member e40	-0.304
<i>ATXN1</i>	Ataxin 1	-0.304
<i>ADD3</i>	Adducin 3 (gamma)	-0.299
<i>DYRK1A</i>	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	-0.293
<i>RND3</i>	Rho family GTPase 3	-0.289
<i>ANXA4</i>	Annexin A4	-0.281
<i>PPARG</i>	Peroxisome proliferator-activated receptor gamma	-0.271
<i>MAGI2</i>	Membrane associated guanylate kinase, WW and PDZ domain containing 2	-0.258
<i>GRK5</i>	G protein-coupled receptor kinase 5	-0.246
<i>LPHN2</i>	Latrophilin 2	-0.236
<i>LPAR1</i>	Lysophosphatidic acid receptor 1	-0.236
<i>ATP2B1</i>	ATPase, Ca ⁺⁺ transporting, plasma membrane 1	-0.225
<i>PEX14</i>	Peroxisomal biogenesis factor 14	-0.223
<i>ACVR2A</i>	Activin A receptor, type IIA	-0.209
<i>AGGF1</i>	Angiogenic factor with G patch and FHA domains 1	-0.194

<i>ATP5S</i>	ATP synthase, H ⁺ transporting, mitochondrial Fo complex, subunit s (factor B)	-0.179
<i>ATRN</i>	Attractin	-0.166
<i>TJP1</i>	Tight junction protein 1	-0.151
<i>RASA2</i>	RAS p21 protein activator 2	-0.143
<i>NEK7</i>	NIMA-related kinase 7	-0.128
<i>MAPK14</i>	Mitogen-activated protein kinase 14	-0.111
<i>NIPBL</i>	Nipped-B homolog (Drosophila)	-0.098
<i>PRDM2</i>	PR domain containing 2, with ZNF domain	-0.081
<i>COL11A1</i>	Collagen, type XI, alpha 1	-0.061
<i>PLCB4</i>	Phospholipase C, beta 4	-0.037
<i>ATP2C1</i>	ATPase, Ca ⁺⁺ transporting, type 2C, member 1	-0.016
<i>CDKN1B</i>	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
<i>YIPF6</i>	Yip1 domain family, member 6	-0.402
<i>ICAI</i>	Islet cell autoantigen 1, 69kDa	-0.394
<i>IGF2R</i>	Insulin-like growth factor 2 receptor	-0.385
<i>ARFGAP3</i>	ADP-ribosylation factor GTPase activating protein 3	-0.384
<i>ERGIC3</i>	ERGIC and golgi 3	-0.377
<i>TMED2</i>	Transmembrane emp24 domain trafficking protein 2	-0.365
<i>GALC</i>	Galactosylceramidase	-0.357
<i>TOML1</i>	Target of myb1 (chicken)-like 1	-0.347
<i>SNAP23</i>	Synaptosomal-associated protein, 23kDa	-0.338
<i>STX7</i>	Syntaxin 7	-0.326
<i>MON2</i>	MON2 homolog (<i>S. cerevisiae</i>)	-0.325
<i>KIF1B</i>	Kinesin family member 1B	-0.324
<i>ARFGEF1</i>	ADP-ribosylation factor guanine nucleotide-exchange factor 1 (brefeldin A-inhibited)	-0.321
<i>RER1</i>	Retention in endoplasmic reticulum sorting receptor 1	-0.314
<i>RAB5A</i>	RAB5A, member RAS oncogene family	-0.313
<i>CLTC</i>	Clathrin, heavy chain (Hc)	-0.311
<i>SGMS1</i>	Sphingomyelin synthase 1	-0.294
<i>VAMP4</i>	Vesicle-associated membrane protein 4	-0.294
<i>TMED10</i>	Transmembrane emp24-like trafficking protein 10 (yeast)	-0.274
<i>CTSC</i>	Cathepsin C	-0.252
<i>DST</i>	Dystonin	-0.231
<i>LAMP2</i>	Lysosomal-associated membrane protein 2	-0.208
<i>DOPEY1</i>	Dopey family member 1	-0.187
<i>ADAM10</i>	ADAM metallopeptidase domain 10	-0.171

<i>SCAMPI</i>	Secretory carrier membrane protein 1	-0.145
<i>ARFIP1</i>	ADP-ribosylation factor interacting protein 1	-0.113
<i>SEC31A</i>	SEC31 homolog A (<i>S. cerevisiae</i>)	-0.085
<i>ARF1</i>	ADP-ribosylation factor 1	-0.050
<i>SEC22B</i>	SEC22 vesicle trafficking protein homolog B (<i>S. cerevisiae</i>) (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
<i>APAF1</i>	Apoptotic peptidase activating factor 1	-0.582
<i>CASP9</i>	Caspase 9, apoptosis-related cysteine peptidase	-0.522
<i>SOS1</i>	Son of sevenless homolog 1 (Drosophila)	-0.432
<i>CYCS</i>	Cytochrome c, somatic	-0.251
<i>CTNNB1</i>	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
<i>PPM1A</i>	Protein phosphatase, Mg ²⁺ /Mn ²⁺ dependent, 1A	-0.558
<i>ZEB2</i>	Zinc finger E-box binding homeobox 2	-0.420
<i>RANBP3</i>	RAN binding protein 3	-0.266
<i>CTNNB1</i>	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	Description	Running ES
<i>BCL3</i>	B-cell CLL/lymphoma 3	0.410
<i>CDKN1B</i>	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	0.410
<i>WHSC1</i>	Wolf-Hirschhorn syndrome candidate 1	0.408
<i>SUV39H1</i>	Suppressor of variegation 3-9 homolog 1 (Drosophila)	0.407
<i>PDS5B</i>	PDS5 cohesin associated factor B	0.406
<i>CENPE</i>	Centromere protein E, 312kDa	0.406
<i>CDC45</i>	Cell division cycle 45	0.406
<i>CENPF</i>	Centromere protein F, 350/400kDa	0.406
<i>KIF15</i>	Kinesin family member 15	0.405
<i>TACC3</i>	Transforming, acidic coiled-coil containing protein 3	0.405
<i>TOP2A</i>	Topoisomerase (DNA) II alpha 170kDa	0.405
<i>KPNB1</i>	Karyopherin (importin) beta 1	0.405
<i>DDX39A</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39A	0.404
<i>UCK2</i>	Uridine-cytidine kinase 2	0.403
<i>SYNCRIP</i>	Synaptotagmin binding, cytoplasmic RNA interacting protein	0.403
<i>NEK2</i>	NIMA-related kinase 2	0.402
<i>TRAF1</i>	TRAF interacting protein	0.401
<i>RAD23B</i>	RAD23 homolog B (<i>S. cerevisiae</i>)	0.400
<i>ORC5</i>	Origin recognition complex, subunit 5	0.400
<i>PBK</i>	PDZ binding kinase	0.398
<i>TMPO</i>	Thymopoietin	0.398
<i>SLC7A1</i>	Solute carrier family 7 (cationic amino acid transporter, y ⁺ system), member 1	0.397
<i>CUL5</i>	Cullin 5	0.396
<i>KIF5B</i>	Kinesin family member 5B	0.392
<i>HNRNPU</i>	Heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A)	0.390

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

<i>RASAL2</i>	RAS protein activator like 2	0.288
<i>MYC</i>	v-myc avian myelocytomatosis viral oncogene homolog	0.284
<i>MNAT1</i>	MNAT CDK-activating kinase assembly factor 1	0.281
<i>CDKN2C</i>	Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	0.274
<i>HMGN2</i>	High mobility group nucleosomal binding domain 2	0.268
<i>RAD21</i>	RAD21 homolog (<i>S. pombe</i>)	0.259
<i>G3BP1</i>	GTPase activating protein (SH3 domain) binding protein 1	0.246
<i>MTF2</i>	Metal response element binding transcription factor 2	0.233
<i>YTHDC1</i>	YTH domain containing 1	0.221
<i>BUB1</i>	BUB1 mitotic checkpoint serine/threonine kinase	0.209
<i>FBXO5</i>	F-box protein 5	0.196
<i>TTK</i>	TTK protein kinase	0.193
<i>KPNA2</i>	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	0.179
<i>SMC2</i>	Structural maintenance of chromosomes 2	0.165
<i>HIF1A</i>	Hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	0.152
<i>ODF2</i>	Outer dense fiber of sperm tails 2	0.137
<i>SLC12A2</i>	Solute carrier family 12 (sodium/potassium/chloride transporter), member 2	0.125
<i>RACGAP1</i>	Rac GTPase activating protein 1	0.109
<i>MAD2L1</i>	MAD2 mitotic arrest deficient-like 1 (yeast)	0.095
<i>XPO1</i>	Exportin 1	0.077
<i>HMMR</i>	Hyaluronan-mediated motility receptor (RHAMM)	0.063
<i>BUB3</i>	BUB3 mitotic checkpoint protein	0.047
<i>CDC25B</i>	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
<i>SUV39HI</i>	Suppressor of variegation 3-9 homolog 1 (Drosophila)	0.357
<i>PCNA</i>	Proliferating cell nuclear antigen	0.353
<i>TACC3</i>	Transforming, acidic coiled-coil containing protein 3	0.350
<i>TOP2A</i>	Topoisomerase (DNA) II alpha 170kDa	0.350
<i>PDS5B</i>	PDS5 cohesin associated factor B	0.347
<i>SPC25</i>	SPC25, NDC80 kinetochore complex component	0.346
<i>CENPM</i>	Centromere protein M	0.343
<i>HELLS</i>	Helicase, lymphoid-specific	0.343
<i>GIN5A</i>	GIN5 complex subunit 4 (Sld5 homolog)	0.342
<i>TMPO</i>	Thymopoietin	0.339
<i>SYNCRIP</i>	Synaptotagmin binding, cytoplasmic RNA interacting protein	0.339
<i>RAD51C</i>	RAD51 paralogue C	0.339
<i>NUP205</i>	Nucleoporin 205kDa	0.338
<i>TBRG4</i>	Transforming growth factor beta regulator 4	0.338
<i>CENPE</i>	Centromere protein E, 312kDa	0.337
<i>CHEK2</i>	Checkpoint kinase 2	0.336
<i>SLBP</i>	Stem-loop binding protein	0.336
<i>DLGAP5</i>	Disks, large (Drosophila) homolog-associated protein 5	0.334
<i>PMS2</i>	PMS2 postmeiotic segregation increased 2 (S. cerevisiae)	0.334
<i>DDX39A</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39A	0.331
<i>H2AFZ</i>	H2A histone family, member Z	0.330
<i>PLK1</i>	Polo-like kinase 1	0.326
<i>CBX5</i>	Chromobox homolog 5	0.326

<i>POLD1</i>	Polymerase (DNA directed), delta 1, catalytic subunit	0.323
<i>PSIP1</i>	PC4 and SFRS1 interacting protein 1	0.322
<i>LMNB1</i>	Lamin B1	0.320
<i>DUT</i>	Deoxyuridine triphosphatase	0.319
<i>NUDT21</i>	Nudix (nucleoside diphosphate linked moiety X)-type motif 21	0.318
<i>PRPS1</i>	Phosphoribosyl pyrophosphate synthetase 1	0.318
<i>RP43</i>	Replication protein A3, 14kDa	0.315
<i>PPM1D</i>	Protein phosphatase, Mg ²⁺ /Mn ²⁺ dependent, 1D	0.313
<i>DCK</i>	Deoxycytidine kinase	0.309
<i>ING3</i>	Inhibitor of growth family, member 3	0.309
<i>TIPIN</i>	TIMELESS interacting protein	0.308
<i>NME1</i>	NME/NM23 nucleoside diphosphate kinase 1	0.307
<i>DCLRE1B</i>	DNA cross-link repair 1B	0.306
<i>CDKI</i>	Cyclin-dependent kinase 1	0.304
<i>DEK</i>	DEK proto-oncogene	0.304
<i>LYAR</i>	Ly1 antibody reactive	0.303
<i>CDKN1B</i>	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	0.299
<i>RAD1</i>	RAD1 checkpoint DNA exonuclease	0.298
<i>MCM4</i>	Minichromosome maintenance complex component 4	0.293
<i>USP1</i>	Ubiquitin specific peptidase 1	0.290
<i>DEPDC1</i>	DEP domain containing 1	0.290
<i>EZH2</i>	Enhancer of zeste 2 polycomb repressive complex 2 subunit	0.290
<i>CKS2</i>	CDC28 protein kinase regulatory subunit 2	0.285
<i>BRMS1L</i>	Breast cancer metastasis-suppressor 1-like	0.282
<i>PSMC3IP</i>	PSMC3 interacting protein	0.281
<i>SMC6</i>	Structural maintenance of chromosomes 6	0.275
<i>EED</i>	Embryonic ectoderm development	0.265

<i>HMGB2</i>	High mobility group box 2	0.259
<i>TUBG1</i>	Tubulin, gamma 1	0.252
<i>MTHFD2</i>	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase	0.248
<i>CCP110</i>	Centriolar coiled coil protein 110kDa	0.236
<i>NBN</i>	Nibrin	0.226
<i>MYC</i>	v-myc avian myelocytomatosis viral oncogene homolog	0.215
<i>BRCA1</i>	Breast cancer 1, early onset	0.204
<i>CDKN2C</i>	Cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	0.193
<i>SMC3</i>	Structural maintenance of chromosomes 3	0.182
<i>RAD21</i>	RAD21 homolog (<i>S. pombe</i>)	0.177
<i>MMS22L</i>	MMS22-like, DNA repair protein	0.167
<i>DIAPH3</i>	Diaphanous-related formin 3	0.154
<i>LUC7L3</i>	LUC7-like 3 (<i>S. cerevisiae</i>)	0.141
<i>MSH2</i>	mutS homolog 2	0.128
<i>NAP1L1</i>	Nucleosome assembly protein 1-like 1	0.114
<i>TCF19</i>	Transcription factor 19	0.108
<i>KPNA2</i>	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	0.093
<i>RACGAP1</i>	Rac GTPase activating protein 1	0.088
<i>MAD2L1</i>	MAD2 mitotic arrest deficient-like 1 (yeast)	0.074
<i>XPO1</i>	Exportin 1	0.055
<i>HMMR</i>	Hyaluronan-mediated motility receptor (RHAMM)	0.041
<i>CDC25B</i>	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
<i>TUBG1</i>	Tubulin, gamma 1	0.685
<i>MZTI</i>	Mitotic spindle organizing protein 1	0.595
<i>BRCAl</i>	Breast cancer 1, early onset	0.490
<i>CEP290</i>	Centrosomal protein 290kDa	0.391
<i>ZNF365</i>	Zinc finger protein 365	0.293
<i>TOPORS</i>	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
<i>MEN1</i>	Multiple endocrine neoplasia I	0.723
<i>DMC1</i>	DNA meiotic recombinase 1	0.640
<i>HMGB1</i>	High mobility group box 1	0.534
<i>HMGB2</i>	High mobility group box 2	0.434
<i>RAD51D</i>	RAD51 paralog D	0.330
<i>YY1</i>	YY1 transcription factor	0.209
<i>MSH2</i>	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
<i>GNBI</i>	Guanine nucleotide binding protein (G protein), beta polypeptide 1	-0.553
<i>OR1L8</i>	Olfactory receptor, family 1, subfamily L, member 8	-0.543
<i>OR10W1</i>	Olfactory receptor, family 10, subfamily W, member 1	-0.523
<i>OR4C15</i>	Olfactory receptor, family 4, subfamily C, member 15	-0.513
<i>OR5A5I</i>	Olfactory receptor, family 5, subfamily AS, member 1	-0.486
<i>OR9A4</i>	Olfactory receptor, family 9, subfamily A, member 4	-0.468
<i>OR8B12</i>	Olfactory receptor, family 8, subfamily B, member 12	-0.452
<i>OR4C12</i>	Olfactory receptor, family 4, subfamily C, member 12	-0.429
<i>OR4A16</i>	Olfactory receptor, family 4, subfamily A, member 16	-0.364
<i>OR1D4</i>	Homo sapiens olfactory receptor, family 1, subfamily D, member 4 (OR1D4), mRNA.	-0.293
<i>OR3A2</i>	Olfactory receptor, family 3, subfamily A, member 2	-0.199
<i>OR10H4</i>	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
<i>ARFGAP3</i>	ADP-ribosylation factor GTPase activating protein 3	0.403
<i>CTSC</i>	Cathepsin C	0.395
<i>YIPF6</i>	Yip1 domain family, member 6	0.389
<i>STX7</i>	Syntaxin 7	0.381
<i>PAM</i>	Peptidylglycine alpha-amidating monooxygenase	0.375
<i>VAMP7</i>	Vesicle-associated membrane protein 7	0.373
<i>SCAMP1</i>	Secretory carrier membrane protein 1	0.370
<i>CLTC</i>	Clathrin, heavy chain (Hc)	0.369
<i>IGF2R</i>	Insulin-like growth factor 2 receptor	0.368
<i>GLA</i>	Galactosidase, alpha	0.367
<i>SNX2</i>	Sorting nexin 2	0.360
<i>AP3S1</i>	Adaptor-related protein complex 3, sigma 1 subunit	0.358
<i>SGMS1</i>	Sphingomyelin synthase 1	0.355
<i>DOPEY1</i>	Dopey family member 1	0.346
<i>TMED10</i>	Transmembrane emp24-like trafficking protein 10 (yeast)	0.338
<i>APIG1</i>	Adaptor-related protein complex 1, gamma 1 subunit	0.322
<i>TMED2</i>	Transmembrane emp24 domain trafficking protein 2	0.307
<i>M6PR</i>	Mannose-6-phosphate receptor (cation dependent)	0.295
<i>ARFGEF1</i>	ADP-ribosylation factor guanine nucleotide-exchange factor 1 (brefeldin A-inhibited)	0.290
<i>STAM</i>	Signal transducing adaptor molecule (SH3 domain and ITAM motif) 1	0.274
<i>MON2</i>	MON2 homolog (<i>S. cerevisiae</i>)	0.260
<i>ADAM10</i>	ADAM metallopeptidase domain 10	0.246
<i>NAPG</i>	N-Ethylmaleimide-sensitive factor attachment protein, gamma	0.238
<i>RAB5A</i>	RAB5A, member RAS oncogene family	0.216

<i>TOMIL1</i>	Target of myb1 (chicken)-like 1	0.201
<i>GBF1</i>	Golgi brefeldin A resistant guanine nucleotide exchange factor 1	0.183
<i>DST</i>	Dystonin	0.167
<i>LMAN1</i>	Lectin, mannose-binding, 1	0.137
<i>ABCA1</i>	ATP-binding cassette, sub-family A (ABC1), member 1	0.112
<i>SEC22B</i>	SEC22 vesicle trafficking protein homolog B (<i>S. cerevisiae</i>) (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
<i>PLCB4</i>	Phospholipase C, beta 4	0.318
<i>CDKN1B</i>	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	0.318
<i>PTGFR</i>	Prostaglandin F receptor (FP)	0.316
<i>COL11A1</i>	Collagen, type XI, alpha 1	0.315
<i>ZMIZ1</i>	Zinc finger, MIZ-type containing 1	0.313
<i>SYNE1</i>	Spectrin repeat containing, nuclear envelope 1	0.312
<i>PPARG</i>	Peroxisome proliferator-activated receptor gamma	0.311
<i>VLDLR</i>	Very low density lipoprotein receptor	0.309
<i>MTA1</i>	Metastasis associated 1	0.308
<i>SNAI2</i>	Snail family zinc finger 2	0.306
<i>SDC2</i>	Syndecan 2	0.304
<i>SIPAIL1</i>	Signal-induced proliferation-associated 1 like 1	0.303
<i>LDLR</i>	Low density lipoprotein receptor	0.302
<i>DDAHI</i>	Dimethylarginine dimethylaminohydrolase 1	0.302
<i>APBB2</i>	Amyloid beta (A4) precursor protein-binding, family B, member 2	0.302
<i>RBPMS</i>	RNA binding protein with multiple splicing	0.301
<i>FAM179B</i>	Family with sequence similarity 179, member B	0.299
<i>PDLIM5</i>	PDZ and LIM domain 5	0.297
<i>SERPINE1</i>	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	0.297
<i>MAGI2</i>	Membrane associated guanylate kinase, WW and PDZ domain containing 2	0.297
<i>PRDM2</i>	PR domain containing 2, with ZNF domain	0.296
<i>TGFBR3</i>	Transforming growth factor, beta receptor III	0.290
<i>SPOP</i>	Speckle-type POZ protein	0.279
<i>KALRN</i>	Kalirin, RhoGEF kinase	0.269

<i>IRS1</i>	Insulin receptor substrate 1	0.258
<i>DYRK1A</i>	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	0.248
<i>AGGF1</i>	Angiogenic factor with G patch and FHA domains 1	0.246
<i>PEX14</i>	Peroxisomal biogenesis factor 14	0.237
<i>RND3</i>	Rho family GTPase 3	0.230
<i>LPHN2</i>	Latrophilin 2	0.216
<i>BHLHE40</i>	Basic helix-loop-helix family, member e40	0.208
<i>ATRX</i>	Alpha thalassaemia/mental retardation syndrome X-linked	0.197
<i>BMPRIA</i>	Bone morphogenetic protein receptor, type IA	0.180
<i>YTHDC1</i>	YTH domain containing 1	0.164
<i>MAP1B</i>	Microtubule-associated protein 1B	0.149
<i>TJP1</i>	Tight junction protein 1	0.133
<i>ITGB3</i>	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	0.122
<i>NR1D2</i>	Nuclear receptor subfamily 1, group D, member 2	0.112
<i>ABCC1</i>	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	0.098
<i>INSIG1</i>	Insulin induced gene 1	0.076
<i>NEK7</i>	NIMA-related kinase 7	0.049
<i>RASA2</i>	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
<i>KRTAP9-4</i>	Keratin associated protein 9-4	0.788
<i>KRT80</i>	Keratin 80, type II	0.761
<i>KRTAPI-4</i>	PREDICTED: Homo sapiens similar to keratin associated protein 1.6 (LOC730743), mRNA.	0.732
<i>KRT7</i>	Keratin 7, type II	0.690
<i>CSNK1A1</i>	Casein kinase 1, alpha 1	0.667
<i>KRT14</i>	Keratin 14, type I	0.605
<i>KRTAP4-8</i>	PREDICTED: Homo sapiens keratin associated protein 4-8, transcript variant 2 (KRTAP4-8), mRNA.	0.544
<i>KRT86</i>	Keratin 86, type II	0.481
<i>KRTAP2-1</i>	Keratin associated protein 2-1	0.428
<i>KRT81</i>	Keratin 81, type II	0.331
<i>KRTAP4-12</i>	Keratin associated protein 4-12	0.227
<i>KRT8</i>	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
<i>KRTAP9-4</i>	Keratin associated protein 9-4	0.542
<i>NME1</i>	NME/NM23 nucleoside diphosphate kinase 1	0.527
<i>KRT80</i>	Keratin 80, type II	0.514
<i>KRTAP1-4</i>	PREDICTED: Homo sapiens similar to keratin associated protein 1.6 (LOC730743), mRNA.	0.508
<i>NEFH</i>	Neurofilament, heavy polypeptide	0.491
<i>KRT7</i>	Keratin 7, type II	0.464
<i>INA</i>	Internexin neuronal intermediate filament protein, alpha	0.446
<i>CSNK1A1</i>	Casein kinase 1, alpha 1	0.437
<i>KRT14</i>	Keratin 14, type I	0.408
<i>SLC1A4</i>	Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	0.374
<i>KRTAP4-8</i>	PREDICTED: Homo sapiens keratin associated protein 4-8, transcript variant 2 (KRTAP4-8), mRNA.	0.345
<i>KRT86</i>	Keratin 86, type II	0.316
<i>KRT17</i>	Keratin 17, type I	0.287
<i>NARF</i>	Nuclear prelamin A recognition factor	0.248
<i>KRTAP2-1</i>	Keratin associated protein 2-1	0.215
<i>KRT81</i>	Keratin 81, type II	0.167
<i>KRTAP4-12</i>	Keratin associated protein 4-12	0.114
<i>KRT8</i>	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
<i>MAP2K1</i>	Mitogen-activated protein kinase kinase 1	-0.418
<i>RBI</i>	Retinoblastoma 1	-0.410
<i>CDK2</i>	Cyclin-dependent kinase 2	-0.406
<i>PDGFD</i>	Platelet derived growth factor D	-0.394
<i>MDM2</i>	MDM2 proto-oncogene, E3 ubiquitin protein ligase	-0.381
<i>TP53</i>	Tumor protein p53	-0.361
<i>PDGFC</i>	Platelet derived growth factor C	-0.349
<i>CREB5</i>	cAMP responsive element binding protein 5	-0.326
<i>NFKB1A</i>	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	-0.306
<i>TCF7L2</i>	Transcription factor 7-like 2 (T-cell specific, HMG-box)	-0.282
<i>CCNE2</i>	Cyclin E2	-0.261
<i>FGFR1</i>	Fibroblast growth factor receptor 1	-0.225
<i>PIK3CB</i>	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit beta	-0.181
<i>EP300</i>	E1A binding protein p300	-0.134
<i>CTNNB1</i>	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
<i>ITGAI</i>	Integrin, alpha 1	-0.415
<i>DSC2</i>	Desmocollin 2	-0.400
<i>ACTG1</i>	Actin gamma 1	-0.389
<i>LEF1</i>	Lymphoid enhancer-binding factor 1	-0.376
<i>CACNBI</i>	Calcium channel, voltage-dependent, beta 1 subunit	-0.367
<i>ITGAI0</i>	Integrin, alpha 10	-0.363
<i>ITGAV</i>	Integrin, alpha V	-0.349
<i>SLC8A1</i>	Solute carrier family 8 (sodium/calcium exchanger), member 1	-0.346
<i>ITGB3</i>	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	-0.322
<i>TCF7L2</i>	Transcription factor 7-like 2 (T-cell specific, HMG-box)	-0.282
<i>ITGA9</i>	Integrin, alpha 9	-0.243
<i>ITGA4</i>	Integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	-0.197
<i>CTNNB1</i>	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_GENTALIA_DEVELOPMENT in DMSO-treated HDFs (aspirin vs. DMSO)

Symbol	Description	Running ES
<i>WNT5A</i>	Wingless-type MMTV integration site family, member 5A	-0.633
<i>BAK1</i>	BCL2-antagonist/killer 1	-0.611
<i>BAX</i>	BCL2-associated X protein	-0.568
<i>DNAJC19</i>	DnaJ (Hsp40) homolog, subfamily C, member 19	-0.508
<i>LGR4</i>	Leucine-rich repeat containing G protein-coupled receptor 4	-0.449
<i>TBX3</i>	T-box 3	-0.364
<i>CTNNB1</i>	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
<i>NUMB</i>	Numb homolog (Drosophila)	-0.646
<i>CLTC</i>	Clathrin, heavy chain (Hc)	-0.635
<i>TMEM59</i>	Transmembrane protein 59	-0.630
<i>LYPLAI</i>	Lysophospholipase I	-0.590
<i>PIDI</i>	Phosphotyrosine interaction domain containing 1	-0.532
<i>LZTFLI</i>	Leucine zipper transcription factor-like 1	-0.484
<i>GOPC</i>	Golgi-associated PDZ and coiled-coil motif containing	-0.417
<i>GBPI</i>	Guanylate binding protein 1, interferon-inducible	-0.336
<i>RHOQ</i>	ras homolog family member Q	-0.201
<i>TMBIM1</i>	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
<i>ACVRI</i>	Activin A receptor, type I	-0.462
<i>CLIC4</i>	Chloride intracellular channel 4	-0.461
<i>MYADM</i>	Myeloid-associated differentiation marker	-0.454
<i>STC1</i>	Stanniocalcin 1	-0.438
<i>GSTM3</i>	Glutathione S-transferase mu 3 (brain)	-0.437
<i>RAP2B</i>	RAP2B, member of RAS oncogene family	-0.426
<i>RBPJ</i>	Recombination signal binding protein for immunoglobulin kappa J region	-0.410
<i>RAB1B</i>	RAB1B, member RAS oncogene family	-0.387
<i>F2RL1</i>	Coagulation factor II (thrombin) receptor-like 1	-0.361
<i>RDX</i>	Radixin	-0.335
<i>SMAD4</i>	SMAD family member 4	-0.310
<i>TJPI</i>	Tight junction protein 1	-0.293
<i>BMPEP</i>	BMP binding endothelial regulator	-0.259
<i>HEG1</i>	Heart development protein with EGF-like domains 1	-0.227
<i>PDE4D</i>	Phosphodiesterase 4D, cAMP-specific	-0.182
<i>CTNNB1</i>	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
<i>NFE2L2</i>	Nuclear factor, erythroid 2-like 2	-0.567
<i>ATF2</i>	Activating transcription factor 2	-0.548
<i>SIN3A</i>	SIN3 transcription regulator family member A	-0.515
<i>RBI</i>	Retinoblastoma 1	-0.489
<i>BHLHE40</i>	Basic helix-loop-helix family, member e40	-0.458
<i>TBX3</i>	T-box 3	-0.408
<i>EP300</i>	E1A binding protein p300	-0.311
<i>CTNNB1</i>	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
<i>TGFI1</i>	TGFB-induced factor homeobox 1	-0.487
<i>MAGI2</i>	Membrane associated guanylate kinase, WW and PDZ domain containing 2	-0.481
<i>TGFBR3</i>	Transforming growth factor, beta receptor III	-0.469
<i>PPM1A</i>	Protein phosphatase, Mg2+/Mn2+ dependent, 1A	-0.456
<i>USP9Y</i>	Ubiquitin specific peptidase 9, Y-linked	-0.454
<i>SMAD4</i>	SMAD family member 4	-0.437
<i>BMPRIA</i>	Bone morphogenetic protein receptor, type IA	-0.427
<i>AXIN2</i>	Axin 2	-0.417
<i>TOB1</i>	Transducer of ERBB2, 1	-0.383
<i>ZEB2</i>	Zinc finger E-box binding homeobox 2	-0.348
<i>EP300</i>	E1A binding protein p300	-0.308
<i>EID2</i>	EP300 interacting inhibitor of differentiation 2	-0.257
<i>FLNA</i>	Filamin A, alpha	-0.200
<i>MEF2A</i>	Myocyte enhancer factor 2A	-0.139
<i>CTNNB1</i>	Catenin (cadherin-associated protein), beta 1, 88kDa	0.000

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