

SUPPLEMENTARY MATERIAL

In vitro effects of *Streptococcus oralis* biofilm on peri-implant soft-tissue cells

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Supplementary Materials and Methods

The quality and quantity of isolated total RNA was determined using the Agilent 2100 Bioanalyzer according to the manufacturer's protocol

Gene lists generated after analysis with the Qlucore Omics Explorer, were submitted to Pathview (pathway based data integration and visualization) (Luo W, Pant G, Bhavnasi YK, Blanchard SG, Brouwer C. Pathview Web: user friendly pathway visualization and data integration. Nucleic Acids Res, 2017, Web Server issue, doi: 10.1093/nar/gkx372; Luo W, Brouwer C. Pathview: an R/Bioconductor package for pathway-based data integration and visualization. Bioinformatics, 2013, 29(14):1830-1831, doi: 10.1093/bioinformatics/btt285) in order to present gene expression data on graphs of the enriched pathways. The gene lists together with the values of difference in expression from HGF and HGEp were submitted separately. In addition, the lists of enriched pathways, which resulted from analysis using DAVID (ref), were also submitted to Pathview for each cell type.

Supplementary Results

Quality of isolated RNA from tissue cells in control and biofilm groups

Table S1. Quality of RNA isolated from human gingival fibroblasts after 2, 4, or 6 hours of co-culture with the *S. oralis* biofilm.

Co-culture time	RNA integrity number (RIN)	
	Control	Biofilm
2 hours	9	8.5
4 hours	9.9	6.1
6 hours	8.4	5.8

Table S2. Quality of RNA isolated from human gingival fibroblasts and epithelial cells after 2 hours of co-culture with the *S. oralis* biofilm. Samples used for gene expression analysis.

Human gingival fibroblasts		Human gingival epithelial cells	
Sample	RIN	Sample	RIN
Control 1.1	8.6	Control 1.1	10
Biofilm 1.1	8.2	Control 1.2	9
Biofilm 1.2	7.5	Biofilm 1.1	9.4
Control 2.1	9.6	Biofilm 1.2	9.5
Control 2.2	7.8	Control 2.1	9.8
Biofilm 2.1	8.1	Control 2.2	10
Biofilm 2.2	8.8	Biofilm 2.1	9.
Control 3.1	9.7	Biofilm 2.2	8.7
Control 3.2	9.7	Control 3.1	9.9
Biofilm 3.1	7.7	Control 3.2	9.7
Biofilm 3.2	7.8	Biofilm 3.1	10
Biofilm 3.3	7.7	Biofilm 3.2	10

Lists of differentially regulated genes in HGFs after S. oralis biofilm challenge

Table S3. List of upregulated genes in HGFs after 2 hours *S. oralis* biofilm challenge.

Gene name	Systematic name	p	Fold change	log2 difference
HAS2	NM_005328	9.88E+11	0.382783	1.385401913
FOS	NM_005252	5.41E+09	0.021441	5.543471366
DUSP1	NM_004417	2.98E+09	0.204405	2.290499958
RGS2	NM_002923	1.95E+11	0.441866	1.178319526
NGF	NM_002506	0.002184	0.403271	1.310178257
KLF2	NM_016270	2.09E+09	0.275347	1.860675518
PTX3	NM_002852	2.76E+11	0.490559	1.027501925
C5orf30	NM_033211	4.74E+10	0.454952	1.13621441
S100A14	NM_020672	2.61E+11	0.359811	1.474688529
AQP10	NM_080429	0.000124	0.337651	1.566396987
ADM	NM_001124	5.59E+10	0.294003	1.766096438
SNAI1	NM_005985	0.000259	0.45946	1.12198867
SIK1	NM_173354	0.007908	0.251556	1.991050834
CSF2	NM_000758	4.93E+10	0.151731	2.720409818
ID3	NM_002167	1.25E+11	0.354185	1.497425404
KRTAP1-1	NM_030967	0.000236	0.423329	1.240147974
EPHA2	NM_004431	3.03E+11	0.425327	1.233354735
DUSP5	NM_004419	0.001637	0.380686	1.393325057
AMOTL2	NM_016201	1.26E+09	0.314793	1.667525496

RBM24	NM_153020	0.000114	0.415854	1.265851891
MAFB	NM_005461	0.001049	0.475464	1.072591301
CTGF	NM_001901	0.000454	0.273796	1.868825508
SGK1	NM_005627	0.000173	0.38038	1.394487813
SOCS3	NM_003955	3.58E+09	0.320188	1.643007711
FGF5	NM_004464	1.77E+11	0.476314	1.070015568
EGR1	NM_001964	0.004469	0.213632	2.226797291
EDN1	NM_001955	0.000304	0.207265	2.270450306
CITED2	NM_006079	9.60E+10	0.397261	1.33184084
MYC	NM_002467	6.02E+09	0.163676	2.611084438
RGS16	NM_002928	1.65E+11	0.090682	3.463041047
DKK1	NM_012242	7.85E+08	0.212383	2.235259083
PLK2	NM_006622	8.02E+09	0.277368	1.850127626
CXCL2	NM_002089	0.002368	0.18088	2.46689369
RGS16	NM_002928	5.93E+10	0.088819	3.492988393
ARC	NM_015193	0.000259	0.328544	1.605841407
HIST4H4	NM_175054	5.79E+11	0.384887	1.377493898
ZFP36	NM_003407	2.50E+11	0.432125	1.210480084
HIST1H4E	NM_003545	0.000217	0.194982	2.358585551
MT1E	XM_005255956	2.46E+10	0.246194	2.022133858
HIST1H4A	NM_003538	0.001586	0.413369	1.27449883
CYR61	NM_001554	2.46E+11	0.073352	3.769029088
EGR2	NM_000399	0.005119	0.320845	1.640050488
HHEX	NM_002729	0.00091	0.393783	1.344526928
KCTD4	NM_198404	1.68E+11	0.269334	1.892529718
PLK3	NM_004073	7.96E+10	0.443934	1.171584169
BAMBI	NM_012342	0.000654	0.473096	1.079795455
YRDC	NM_024640	1.35E+10	0.497683	1.00669981
IL6	NM_000600	5.55E+10	0.06041	4.049065147
CXCL1	NM_001511	2.82E+11	0.24464	2.031265011
CH25H	NM_003956	0.005659	0.47507	1.073789259
CCL2	NM_002982	9.02E+11	0.302539	1.724804732
IL12A	NM_000882	1.67E+10	0.340828	1.552882459
CAMK1G	NM_020439	0.004734	0.41882	1.255599012
HBEGF	NM_001945	7.75E+09	0.137862	2.858702659
BU963192	BU963192	0.000195	0.451813	1.146202843
CXCL3	NM_002090	4.83E+11	0.182483	2.454167179
RNU12	NR_029422	1.55E+11	0.105308	3.247310659
ANKRD37	NM_181726	0.000559	0.437629	1.1922199
GADD45B	NM_015675	1.07E+10	0.193766	2.367610894
JUNB	NM_002229	0.002233	0.305398	1.71123543

TRIB1	NM_025195	0.004115	0.499263	1.002127015
CXCL2	NM_002089	7.55E+11	0.233587	2.09796693
SNHG7	NR_003672	9.36E+10	0.409629	1.287609235
DUSP2	NM_004418	0.000216	0.170917	2.548635935
lnc-EIF2D-1	lnc-EIF2D-1:1	1.37E+09	0.24567	2.025205843
IER2	NM_004907	4.43E+11	0.400609	1.319735027
SCARNA17	NR_003003	3.28E+10	0.183274	2.44792748
CITED2	NM_006079	0.001596	0.420152	1.251015998
A_33_P3216635	N/A	0.001576	0.434635	1.202124387
MAFB	NM_005461	0.003991	0.461305	1.116207469
A_33_P3223860	N/A	0.000422	0.481083	1.055641186
SNORD3B-1	NR_003271	7.77E+08	0.052537	4.250529791
F3	NM_001993	9.95E+11	0.337466	1.567185169
A_33_P3227225	N/A	4.70E+11	0.365531	1.451932693
BF515046	BF515046	3.89E+10	0.318425	1.650972597
LOC100131831	AK129685	7.25E+11	0.445451	1.166661864
SNORD3B-1	NR_003271	2.85E+09	0.082341	3.602252784
GADD45G	NM_006705	0.005489	0.496825	1.009191247
AK127417	AK127417	6.24E+10	0.374447	1.417168101
HIST2H3A	NM_001005464	0.000342	0.3083	1.697593959
EDN1	NM_001955	0.000558	0.248585	2.00818798
PTGES2-AS1	NR_024425	4.44E+10	0.395364	1.338746196
WDR74	ENST00000538098	9.63E+11	0.27061	1.885710372
RNVU1-18	NR_004400	1.23E+10	0.133612	2.903883908
A_33_P3269528	N/A	0.002235	0.466229	1.100889836
SCARNA2	NR_003023	6.96E+08	0.22407	2.157976402
LINC01347	NR_029401	0.000492	0.478987	1.061942106
RNU2-1	NR_002716	7.02E+09	0.161047	2.634449677
SNORD116-19	NR_001290	1.44E+11	0.341702	1.549191231
lnc-ATG2B-2	lnc-ATG2B-2:1	2.40E+09	0.273891	1.868327478
HOXA10	NM_018951	0.000302	0.462853	1.111373514
RHOB	ENST00000272233	0.000105	0.492857	1.020758776
KPRP	NM_001025231	0.001408	0.335608	1.575152119
A_33_P3317460	N/A	7.01E+10	0.299464	1.739547153
JUN	NM_002228	0.000272	0.348336	1.521447851
THC2492293	THC2492293	8.65E+11	0.406263	1.29951287
lnc-ZNF33A-2	lnc-ZNF33A-2:1	5.48E+10	0.266121	1.909844445
CXCL1	NM_001511	4.54E+10	0.300343	1.735316877
LINC00936	NR_028138	0.001191	0.496884	1.009018869
RNA28S5	NR_003287	1.62E+11	0.466794	1.099141942
NUAK2	NM_030952	1.78E+11	0.316795	1.658379112

THC2570839	THC2570839	0.0017	0.479285	1.061044209
A_33_P3351276	N/A	3.01E+11	0.448869	1.155634621
THC2605028	THC2605028	0.004947	0.496012	1.011553411
NCF1	NM_000265	9.78E+10	0.458247	1.125803389
RPPH1	NR_002312	5.62E+09	0.1254	2.995393588
RNA18S5	NR_003286	0.002138	0.477303	1.067022697
TNFSF9	NM_003811	0.004402	0.446402	1.163584271
RHOB	ENST00000272233	0.003034	0.495559	1.012870897
RNA5-8S5	NR_003285	2.08E+09	0.154435	2.694928119
RMRP	NR_003051	0.000522	0.445248	1.167317781
THC2610631	THC2610631	0.000777	0.46705	1.098352328
LOC51145	XR_108997	2.73E+11	0.328416	1.60640451
AK075182	AK075182	0.008814	0.473871	1.077433834
LOC729040	ENST00000440769	1.32E+11	0.150754	2.729733191
RNA28S5	NR_003287	6.08E+09	0.235089	2.088721489
PMAIP1	NM_021127	0.001366	0.452891	1.142764913
TTC34	ENST00000579787	0.000274	0.374122	1.418419429
LOC644656	NR_036539	1.11E+11	0.323966	1.626086895
RNU4ATAC	DW419002	2.13E+07	0.012498	6.322121567

Table S4. List of downregulated genes in human gingival fibroblasts after 2 hours *S. oralis* biofilm challenge.

Gene name	Systematic name	p	Fold change	log2 difference
RPL23AP32	NR_002229	0.000954	2.15889525	-1.110293245
SUN1	ENST00000340926	0.002126	5.05566359	-2.337900468
CYP1A1	NM_000499	3.81E+11	3.47593546	-1.797401295
TP53INP1	NM_033285	0.001116	2.26221585	-1.177736591
ZNF608	NM_020747	0.001641	2.07679629	-1.054359711
ACRC	NM_052957	1.47E+11	2.28180909	-1.190178092
NANOG	NM_024865	1.70E+11	2.26603317	-1.180168979
CYP1B1	NM_000104	0.001446	2.44686651	-1.290935397
AKAP12	NM_144497	0.000732	2.32048917	-1.214428964
DDX17	NM_006386	0.000825	2.1100769	-1.077295578
SMG1	NM_015092	0.001303	2.19501948	-1.134233743
ND6	ENST00000361681	0.006892	2.1047225	-1.073630032
MNT	NM_020310	0.003126	2.12345171	-1.0864113
IRF9	ENST00000558468	6.51E+11	2.05145907	-1.03665037
ZNF518A	NM_014803	0.000534	2.13881731	-1.096813258
UBXN7	NM_015562	0.0003	2.05791044	-1.041180198
ZMYND8	ENST00000468376	0.002043	2.48970103	-1.31597251
CIRBP	ENST00000621399	0.000579	2.052737	-1.037548799
BMF	NM_001003940	0.000581	4.60692549	-2.203804265

BCOR	ENST00000615339	0.007912	2.9262135	-1.549035034
PER2	NM_022817	0.000643	2.18363047	-1.126728733
ARSG	ENST00000448504	0.000929	2.00072145	-1.000520322
NKD2	NM_033120	0.001831	2.03036332	-1.021737911
CCDC149	ENST00000324309	0.000623	2.51190472	-1.328781742
LOC646652	XR_427693	0.005487	2.29977345	-1.201491749
KRTAP4-2	NM_033062	0.004488	2.19721675	-1.135677195
ZNF652	NM_014897	0.00079	2.54058075	-1.34515832
AGPAT9	NM_032717	0.000264	2.34585047	-1.230111056
AGPAT4-IT1	NR_024277	0.000167	2.26200008	-1.17759898
HIST1H1A	NM_005325	0.001465	2.1732018	-1.119822147
DPM3	NM_018973	0.000159	2.04108787	-1.029338293
PLEKHF1	NM_024310	0.006258	2.01473904	-1.010592985
RIPK4	NM_020639	0.003928	3.08336568	-1.624506
NPLOC4	ENST00000374747	5.82E+11	3.15629387	-1.658231535
SCAPER	ENST00000303521	0.002297	2.25812101	-1.1751228
KIAA1731NL	NM_001243541	0.002869	2.56277609	-1.357707436
ANKRD12	NM_015208	0.000135	2.10337424	-1.072705562
GHRLOS	NR_073566	0.000938	2.31651187	-1.211954075
ZMYM2	ENST00000382881	0.005484	2.0298245	-1.021354996
LRRC37A2	NM_001006607	0.000378	2.0118742	-1.008540098
WNT11	NM_004626	0.001102	2.22566485	-1.154236362
ETS2	NM_005239	0.000208	2.13565636	-1.094679527
KIAA0319L	ENST00000485551	0.007486	2.75236797	-1.46067336
SLC7A5	NM_003486	0.003406	2.0060339	-1.004345986
SLC1A2	NM_004171	0.000428	2.04170227	-1.029772501
ENST00000579793	ENST00000579793	8.79E+10	2.04316831	-1.030808054
DUSP5P1	NR_002834	0.000789	4.41938829	-2.143846693
PROSER2	NM_153256	0.001035	2.16247177	-1.112681299
AVIL	NM_006576	4.60E+11	2.43493295	-1.283882046
PROSER3	NM_001039887	0.001828	2.17051482	-1.118037273
RHOU	NM_021205	0.000603	2.01941586	-1.013938037
EPM2AIP1	NM_014805	1.03E+11	2.20756054	-1.142453003
FAM217B	NM_022106	0.002532	2.10387897	-1.073051713
RUNX1-IT1	NR_026812	0.002015	2.67922306	-1.421814698
MGEA5	AF307332	7.44E+11	3.89478946	-1.961545343
LOC344887	NR_033752	0.008974	2.08120322	-1.057417845
LOC100190986	NR_024456	0.000848	4.2426796	-2.084975733
SMA4	NR_024054	0.000705	2.0562644	-1.040025782
KIDINS220	NM_020738	7.47E+11	2.2173295	-1.148823174
AGAP9	NM_001190810	0.000878	2.43373466	-1.283171886

BC104430	BC104430	9.31E+11	2.87922478	-1.525680424
WDFY3	ENST00000426414	0.001613	2.0461092	-1.032883143
MALAT1	NR_002819	0.00306	2.00173068	-1.001247882
KIAA0754	NM_015038	7.55E+10	3.21638417	-1.685439735
UBR4	ENST00000375218	0.00025	2.30601835	-1.205403993
HUWE1	NM_031407	0.001036	2.54597998	-1.348221075
LOC100131541	AY358248	0.000259	4.09708786	-2.034598831
RUFY2	NM_001042417	0.000855	2.95314741	-1.562253372
PHACTR1	ENST00000379350	0.000835	2.7942214	-1.482446337
DIDO1	NM_080797	2.90E+11	2.21032119	-1.144256028
AP5B1	NM_138368	0.000762	2.15955639	-1.110734988
SNX29	BC029857	0.001585	2.82935047	-1.500470894
ACVR2B	NM_001106	0.000774	2.13936472	-1.097182455
BMS1P5	NR_003611	0.000931	2.01643753	-1.011808711
SPDYE5	NM_001099435	0.000811	2.02685356	-1.019241858
E2F7	NM_203394	0.000304	2.37126875	-1.245659181
SLC30A4	NM_013309	0.003707	2.12850642	-1.089841442
PLGLB1	NM_001032392	0.000633	2.11657405	-1.081730963
LINC01000	NR_024368	6.46E+11	2.03459978	-1.024745034
IRF2BPL	NM_024496	0.001498	2.0574522	-1.040858913
HIVEP3	NM_024503	0.000186	2.16847992	-1.116684084
PAG1	NM_018440	0.000411	2.19593239	-1.134833636
NBPF10	NM_001039703	0.000329	2.0280571	-1.020098272
LOC728061	AK025151	0.004528	2.2925241	-1.1969369
IBA57	NM_001010867	0.001085	2.2745564	-1.185585208
PAG1	NM_018440	8.53E+09	3.44843411	-1.785941401
THC2591311	THC2591311	0.006165	2.07261372	-1.051451262
A_33_P3220994	N/A	0.002091	6.00716686	-2.586684738
LOC101927270	XR_432672	0.004032	2.32764244	-1.218869456
LPP	NM_005578	0.003646	2.40796924	-1.267816963
OFD1	NM_003611	7.09E+11	2.07574224	-1.053627305
A_33_P3225983	N/A	2.99E+11	2.21899414	-1.149905858
CD7	NM_006137	0.000498	2.00708461	-1.005101436
SLC25A34	NM_207348	0.000816	2.32178283	-1.215233035
TDRD6	NM_001010870	8.50E+10	2.13658547	-1.09530703
MBTD1	NM_017643	0.000317	2.29673624	-1.199585185
GOLGA6L9	NM_198181	0.008294	2.11158109	-1.078323651
NF1	NM_000267	0.006159	2.14273286	-1.099451997
SLMAP	ENST00000467901	0.002237	2.12118769	-1.084872281
FAM71F2	NM_001290254	0.0007	2.30533075	-1.204973752
C2CD2	AK129520	0.001895	3.80056643	-1.926214451

MAP3K8	ENST00000375322	0.008473	2.27255154	-1.184313014
A_33_P3247678	N/A	0.003823	2.289891	-1.195278927
A_33_P3255677	N/A	0.001873	2.69003177	-1.427623211
ENST00000362058	ENST00000362058	0.00167	2.17138314	-1.118614312
58				
CNTRL	ENST00000373851	0.000105	3.62975621	-1.859872654
SERP2	ENST00000474333	0.000335	2.37016487	-1.244987417
ENST00000501122	ENST00000501122	0.001848	3.39659214	-1.764087991
22				
ENST00000413484	ENST00000413484	0.001824	2.64272356	-1.402025521
84				
LRRC66	NM_001024611	2.30E+10	2.62119579	-1.39022512
THC2524986	THC2524986	0.001182	2.09768033	-1.068794839
GREB1L	NM_001142966	0.000865	2.39037967	-1.257239783
A_33_P3275163	N/A	0.005939	2.9778266	-1.574259748
ENST00000413484	ENST00000413484	0.004556	2.15155911	-1.105382476
84				
LINC01000	NR_024368	0.000112	2.46771288	-1.303174546
A_33_P3280502	N/A	0.000701	2.37380552	-1.247201743
ENST00000479369	ENST00000479369	0.003569	2.13835478	-1.096501234
69				
IL6R	NM_000565	0.000403	3.40214157	-1.766443176
COX11	ENST00000572088	0.000106	2.94779086	-1.559634172
AAK1	NM_014911	5.84E+11	2.15656686	-1.108736445
CYP1B1	NM_000104	0.001384	3.04727221	-1.60751838
lnc-GAPDH-3	lnc-GAPDH-3:2	0.007685	2.86324286	-1.517650045
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FAM160B1	ENST00000369246	0.000298	2.45033383	-1.292978313
MAN1B1	ENST00000536349	0.007928	2.59901404	-1.377964427
PARP4	NM_006437	0.000253	2.78609753	-1.478245762
RPS6KA5	ENST00000614987	6.08E+10	3.11494994	-1.639208978
ZNF507	ENST00000587084	0.000413	2.40289903	-1.264776028
LINC01530	NR_034159	0.00994	2.18186927	-1.125564663
PROSER2	NM_153256	0.003125	2.02263665	-1.016237175
SH3BP2	NM_001145855	0.000928	2.38270521	-1.252600471
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ZNF345	NM_003419	0.000376	2.18423462	-1.127127832
LOC100506191	AF289610	0.000239	2.12926841	-1.090357824
ATRX	NM_000489	0.001162	2.07207704	-1.051077644
BQ934349	BQ934349	0.002371	3.54020691	-1.823833682
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STARD9	ENST00000564158	0.001977	2.11518884	-1.08078647

LOC100130463	AK124300	0.001838	2.5452354	-1.347799092
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ANKRD36	NM_001164315	1.90E+11	2.44074607	-1.287322208
ENST00000424493	ENST00000424493	0.000129	2.16586709	-1.114944714
ENST00000479369	ENST00000479369	0.002676	2.1030972	-1.072515529
ZNF480	NM_144684	0.00384	2.02456117	-1.017609233
SNX29	BC029857	0.006084	2.1325314	-1.092566984
UBN2	NM_173569	6.46E+11	2.10754228	-1.075561574
GPAM	ENST00000369425	0.009722	2.04457808	-1.031803159
PPTC7	NM_139283	0.000816	2.09126496	-1.064375861
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LOC101929115	XR_246488	0.00636	2.07574582	-1.053629793
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TTC9C	ENST00000294161	7.46E+10	2.78477788	-1.47756226
FLJ45482	AK127393	0.000383	3.09512901	-1.629999545
DENND2C	NM_001256404	0.000667	3.11176372	-1.637732519
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A_33_P3362601	N/A	0.000192	3.42218709	-1.774918634
AW571659	AW571659	0.00019	3.58901381	-1.843587474
VAPB	NM_004738	0.00443	2.02027059	-1.014548537
NBPF12	NM_001278141	0.000935	2.03257871	-1.023311221
ARHGAP23	NM_001199417	0.002019	2.06454468	-1.045823641
SZT2	ENST00000470139	0.000636	2.40900993	-1.26844034
MKI67	NM_002417	0.002725	2.2814362	-1.18994231
HNRNPU-AS1	NR_026778	0.001191	2.16117501	-1.111815905
RPRD2	ENST00000369067	0.000144	2.13067055	-1.091307537
DMTF1	NR_024549	0.001745	2.04241896	-1.030278836
FAM215A	NR_026770	0.000601	2.41895247	-1.274382422
C5orf56	AK025221	0.001358	2.30438709	-1.20438308
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THC2560329	THC2560329	3.66E+11	2.04745913	-1.033834654
AKAP11	NM_016248	0.004092	2.01967621	-1.014124022
FAM43A	NM_153690	0.001658	2.34842801	-1.231695369
ENST000004786	ENST00000478672	0.002599	2.11921573	-1.083530457

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LOC285178	AK091571	0.008305	2.04253817	-1.030363039
LOC284581	AK094426	0.004792	2.38852954	-1.256122719
MGC12488	BC005372	1.31E+11	2.58524823	-1.370302812
LOC284219	AK094436	0.004335	2.23474121	-1.160107772
LARS	NM_020117	0.000859	2.28395677	-1.191535344
PRO2852	XR_158845	0.005598	2.30767536	-1.206440282
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4.1-1				
ENST000005656	ENST00000565617	1.78E+11	2.49083066	-1.316626943
17				
LINC01004	NR_039981	0.002012	2.05579877	-1.039699055
C8orf60	AK022255	0.00565	2.14602637	-1.101667804
MGC24103	BC020879	0.000403	2.07136106	-1.050579052
BC015643	BC015643	0.001401	2.22087598	-1.151128831
LOC340335	AK074459	0.001859	2.05849171	-1.041587639
LOC10096405	XM_003846604	0.000296	4.82268286	-2.269835941
ENST000004484	ENST00000448494	3.60E+11	2.04559708	-1.032522006
94				

Lists of differentially regulated genes in HGEps after S. oralis biofilm challenge

Table S5. List of upregulated genes in HGEps after 2 hours *S. oralis* biofilm challenge.

Gene name	Systematic name	p	Fold change	log2 difference
EDN2	NM_001956	0.001493	0.316414	1.660116703
CXCL2	NM_002089	0.007035	0.474041	1.076916568
TNF	NM_000594	0.00174	0.273871	1.868432034
IL6	NM_000600	0.003107	0.348669	1.520069903
PDE4B	NM_001037341	0.006957	0.474189	1.076466039
ENST00000390597	ENST00000390597	0.009541	0.295235	1.760066624
CXCL8	NM_000584	0.007087	0.27371	1.869281458
FRY	NM_023037	0.008224	0.273557	1.870084855
CXCL1	NM_001511	0.003937	0.49058	1.027440573
PRDM1	NM_001198	0.008165	0.412511	1.277494805
ARHGAP8	NM_001198726	0.002551	0.30301	1.722563179

Table S6. List of downregulated genes in human gingival epithelial cells after 2 hours *S. oralis* biofilm challenge.

Gene name	Systematic name	p	Fold change	log2 difference
ZNF518A	NM_014803	0.001126	2.05710673	-1.040616648
TRMT13	ENST00000370139	0.009544	2.52427936	-1.335871581
ZNF772	NM_001024596	0.002298	2.46420121	-1.301120062
XRCC5	ENST00000392133	0.008775	2.077034	-1.054524833

Enriched pathways of upregulated genes in tissue cells after *S. oralis* biofilm challengeTable S7. List of all enriched pathways in HGFs and HGEps after 2 hours *S. oralis* biofilm challenge.

Human gingival fibroblasts		Human gingival epithelial cells	
Pathway	p	Genes	p
African trypanosomiasis			0.0237
Amoebiasis			0.0023
Amphetamine addiction	0.0793	FOS, ARC, JUN	
Chagas disease (American trypanosomiasis)	0.0061	FOS, IL6, CCL2, JUN, IL12A	0.0022
Chemokine signaling pathway	0.0421	CXCL1, CCL2, NCF1, CXCL3, CXCL2	0.0068
Colorectal cancer	0.0712	FOS, JUN, MYC	
Cytokine-cytokine receptor interaction	0.0791	CSF2, IL6, CCL2, IL12A, TNFSF9	0.0103
FoxO signaling pathway	0.0003	SGK1, IL6, PLK3, PLK2, GADD45G, GADD45B, KLF2	
Graft-versus-host disease			0.0237
Hematopoietic cell lineage			0.0600
Hepatitis B	0.0190	FOS, IL6, EGR2, JUN, MYC	0.0042
Hepatitis C			0.0926
Herpes simplex infection	0.0092	FOS, IL6, CCL2, SOCS3, JUN, IL12A	
HTLV-I infection	0.0020	ZFP36, EGR1, CSF2, FOS, IL6, EGR2, JUN, MYC	
Hypertrophic cardiomyopathy (HCM)			0.0552
Inflammatory bowel disease (IBD)	0.0752	IL6, JUN, IL12A	0.0455
Influenza A	0.0342	IL6, CCL2, SOCS3, JUN, IL12A	0.0060
Insulin resistance			0.0758
Jak-STAT signaling pathway	0.0190	CSF2, IL6, SOCS3, IL12A, MYC	
Legionellosis	0.0005	CXCL1, IL6, CXCL3, CXCL2, IL12A	0.0000
Leishmaniasis	0.0137	FOS, NCF1, JUN, IL12A	
Malaria	0.0469	IL6, CCL2, IL12A	0.0005
MAPK signaling pathway	0.0001	DUSP5, FGF5, FOS, DUSP2, DUSP1, JUN, GADD45G, GADD45B, MYC, NGF	
NF-kappa B signaling pathway			0.0614
NOD-like receptor signaling pathway			0.0006
Non-alcoholic fatty liver disease (NAFLD)			0.0045
Osteoclast differentiation	0.0135	FOS, SOCS3, NCF1, JUN, JUNB	
p53 signaling pathway	0.0814	GADD45G, PMAIP1,	

		GADD45B		
Pertussis	0.0158	FOS, IL6, JUN, IL12A	0.0011	IL6, TNF, CXCL8
PI3K-Akt signaling pathway	0.0959	FGF5, SGK1, IL6, MYC, EPHA2, NGF		
Rheumatoid arthritis	0.0034	CSF2, FOS, IL6, CCL2, JUN	0.0016	IL6, TNF, CXCL8
RIG-I-like receptor signaling pathway			0.0496	TNF, CXCL8
Salmonella infection	0.0000	CXCL1, CSF2, FOS, IL6, CXCL3, JUN, CXCL2	0.0000	CXCL1, IL6, CXCL2, CXCL8
Systemic lupus erythematosus	0.0689	HIST2H3A, HIST4H4, HIST1H4A, HIST1H4E		
TNF signaling pathway	0.0000	CXCL1, CSF2, FOS, IL6, CCL2, SOCS3, CXCL3, JUN, EDN1, CXCL2, JUNB	0.0000	CXCL1, IL6, TNF, CXCL2
Toll-like receptor signaling pathway	0.0389	FOS, IL6, JUN, IL12A	0.0023	IL6, TNF, CXCL8
Transcriptional misregulation in cancer	0.0306	HIST2H3A, CSF2, HHEX, IL6, MYC		
Viral carcinogenesis	0.0145	HIST4H4, EGR2, HIST1H4A, JUN, HIST1H4E, PMAIP1		
Wnt signaling pathway	0.0739	DKK1, JUN, BAMBI, MYC		

Cell nuclei of gingival tissue cells on titanium disks after S. oralis biofilm challenge

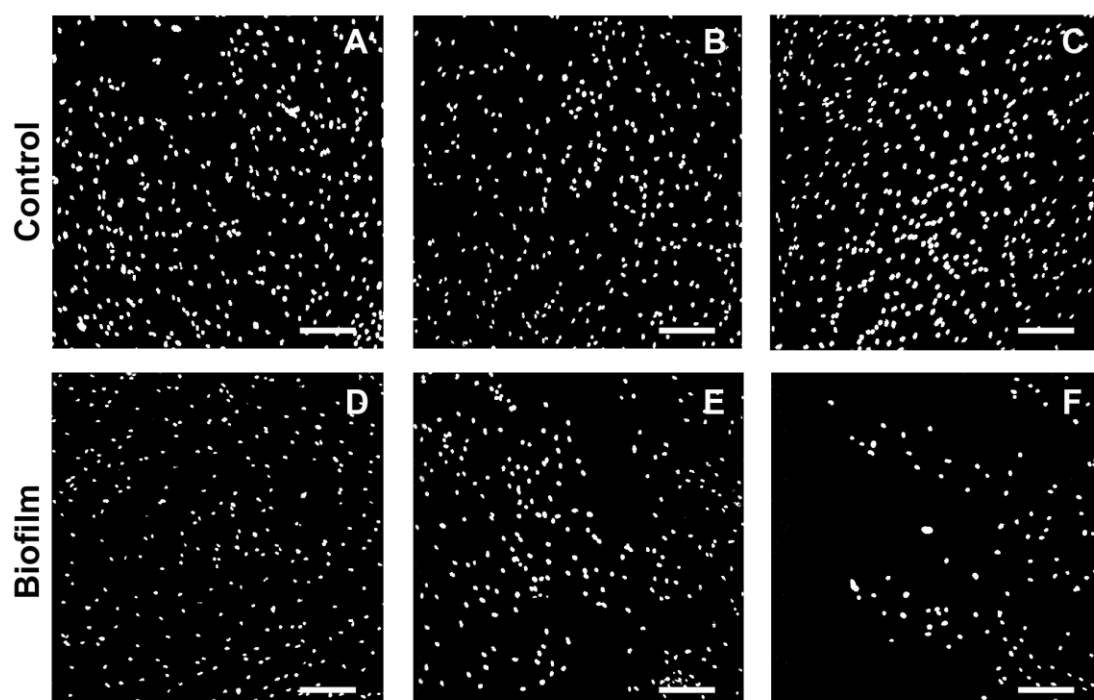


Figure S1. Cell nuclei of HGFs on titanium disks after *S. oralis* biofilm challenge. HGFs grown on titanium disks were co-cultured with the *S. oralis* biofilm or a control membrane for up to 6 h. Subsequently, the cells were fixed after examination of the LIVE/DEAD staining and their nuclei were stained with DAPI (white). The distribution of cell nuclei remained similar in the control samples after 2, 4 or 6 h - Figures (A), (B), and (C), respectively. Also, after 2 h of co-culture with the *S. oralis* biofilm, the distribution of cell nuclei was similar to that of the control (D). Less cell nuclei were detected after 4 h (E) and much less after 6 h (F). Typical pictures from two independent experiments. Scale bars: 200 μ m

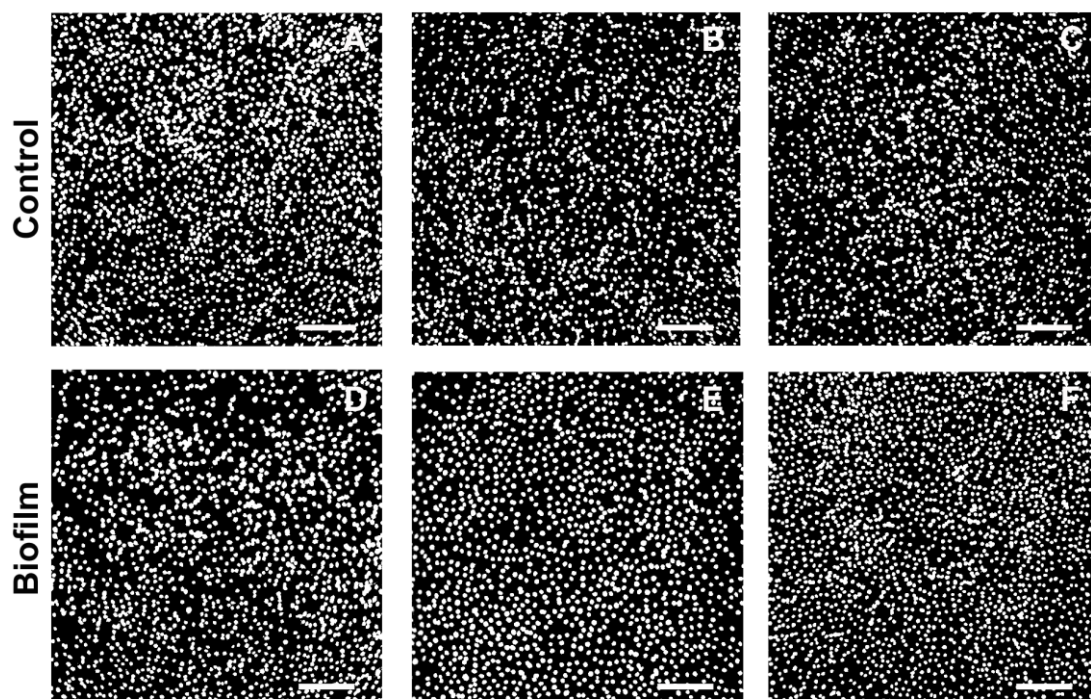


Figure S2. Cell nuclei of HGEps on titanium disks after *S. oralis* biofilm challenge. HGEps grown on titanium disks were co-cultured with the *S. oralis* biofilm or a control membrane for up to 6 h. Subsequently, the cells were fixed after examination of the LIVE/DEAD staining and their nuclei were stained with DAPI (white). The distribution of cell nuclei remained similar in the control samples after 2, 4 or 6 h - Figures (A), (B), and (C), respectively. Also, after co-culture with the *S. oralis* biofilm for 2, 4 or 6 h - Figures (D), (E), and (F), respectively, the distribution of cell nuclei was similar to that of the controls. Typical pictures from two independent experiments. Scale bars: 200 μm

Effect of tissue cell culture medium on S. oralis biofilm

In order to determine the effects of the tissue cell culture media on the volume and vitality of the *S. oralis* biofilm, they were cultured for 2 hours in the same setting as for the co-cultures without tissue cells on titanium disks. After incubation, the biofilms were washed once with PBS and stained with the LIVE/DEAD®BacLight™ Bacterial Viability Kit (Life Technologies, Darmstadt, Germany). Briefly, the biofilms were fluorescently stained with SYTO9 and propidium iodide as a 1:1,000 dilution in PBS for 30 min. Then, they were washed with PBS and fixed for 5 min with 2.5% glutardialdehyde in PBS. The stained biofilms were scanned at three random positions with a 40-fold magnification by CLSM (Leica TCS SP2). The acquired z-plane images were reconstructed into 3D images by the Imaris® x 64 6.2.1 software package (Bitplane). Subsequently, the biofilm volume (μm^3) was calculated applying the surpass mode. The live and dead percentages were calculated after setting the total biofilm volume to 100%.

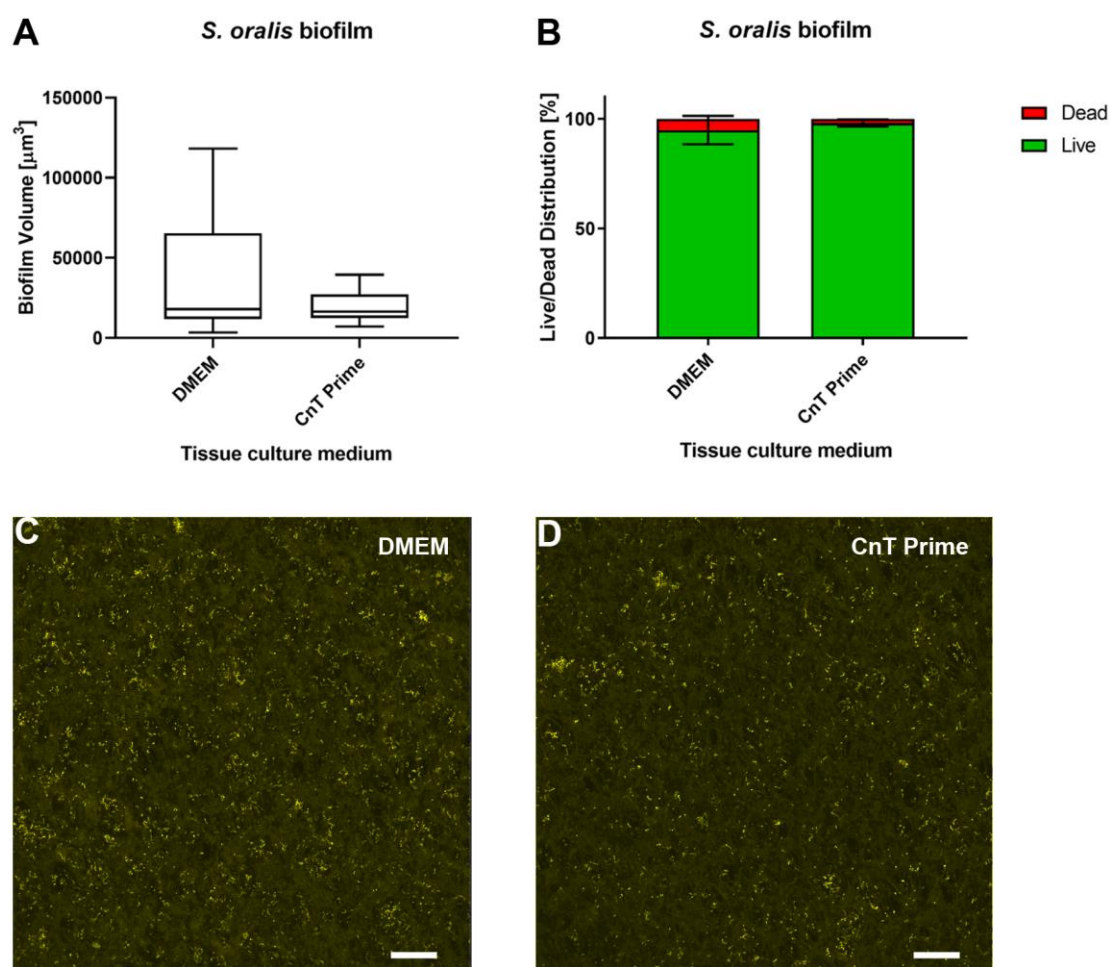


Figure S3. Effect of cell culture media on *S. oralis* biofilm volume and vitality. The biofilms were placed for 2 hours in the DMEM or CnT Prime medium, which were used for the co-cultures with human gingival fibroblasts and epithelial cells, respectively. Bacterial LIVE/DEAD staining was performed to calculate the biofilm volume as well as live and dead proportions of three independent experiments and nine samples in total. The biofilm volume is presented as Box & Whiskers with Tukey error bars (A). Live and dead proportions are depicted in graph bars with error bars indicating the standard deviation (B). Microscopic pictures show *S. oralis* biofilms after culture in DMEM (C) or CnT Prime (D). Live bacteria are depicted in yellow and dead in blue. Error bars: 30 μm .

Role of differentially regulated genes in HGF and HGEp within common enriched pathways

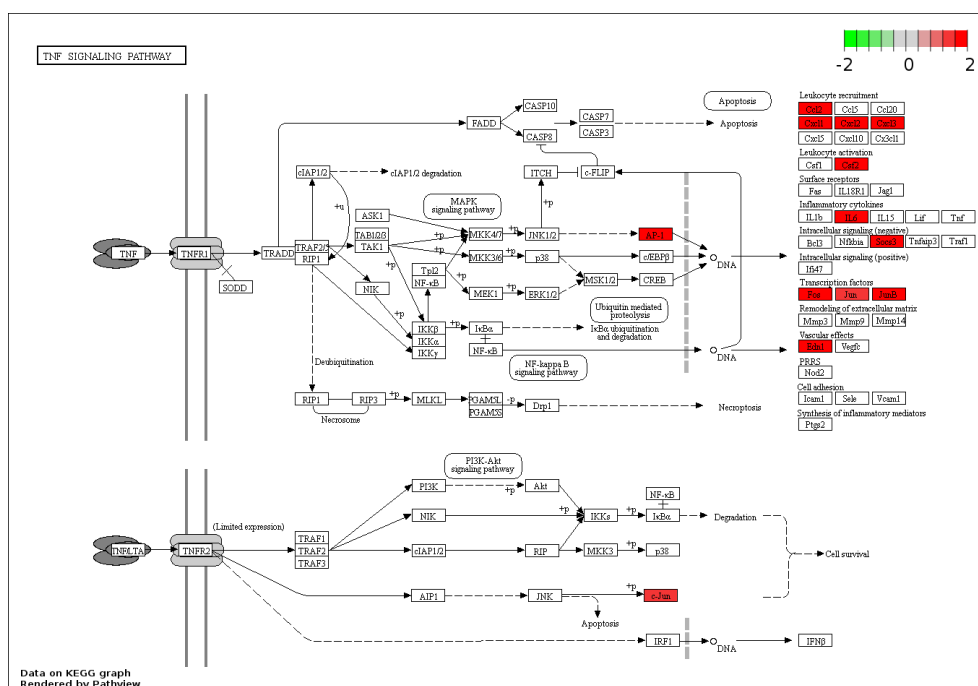


Figure S4. Differentially regulated genes in HGF within the TNF signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

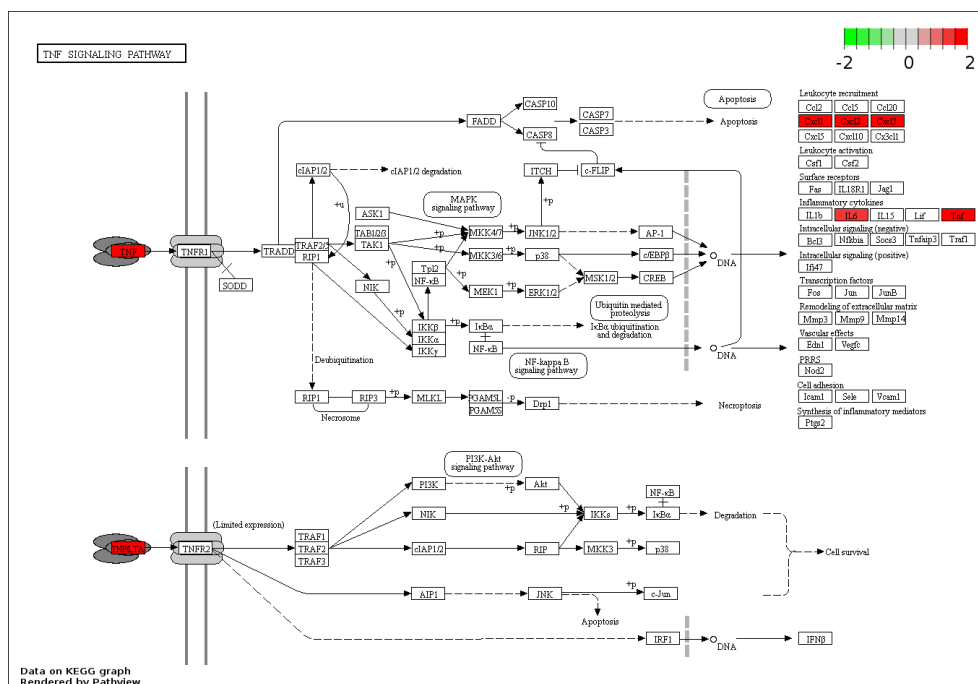


Figure S5. Differentially regulated genes in HGEp within the TNF signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a

molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

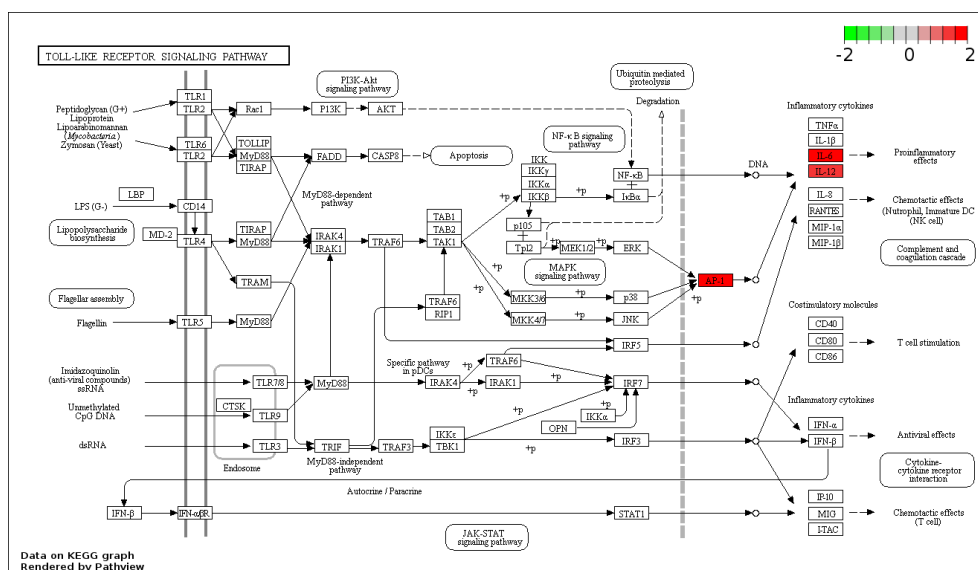


Figure S6. Differentially regulated genes in HGF within the toll-like receptor signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

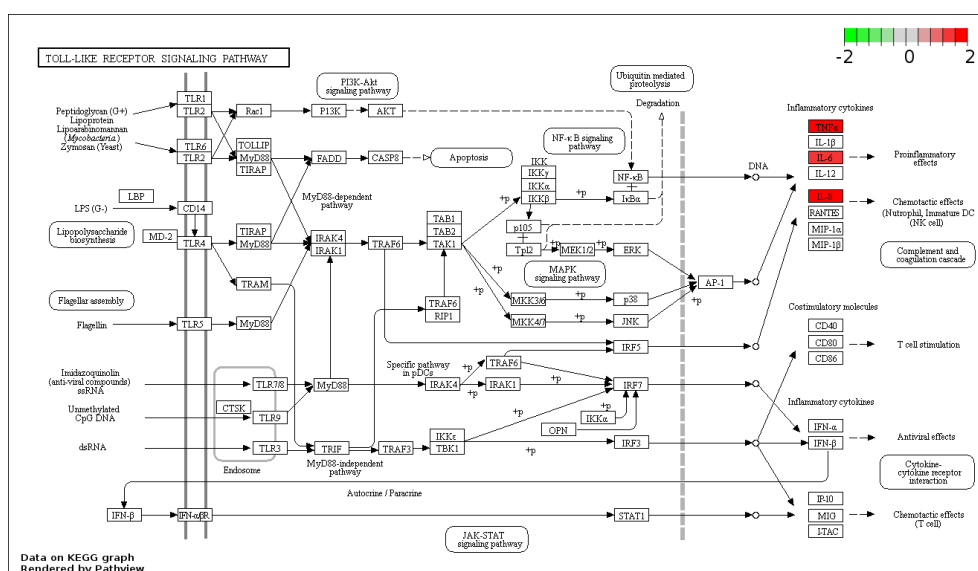


Figure S7. Differentially regulated genes in HGEp within the toll-like receptor signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

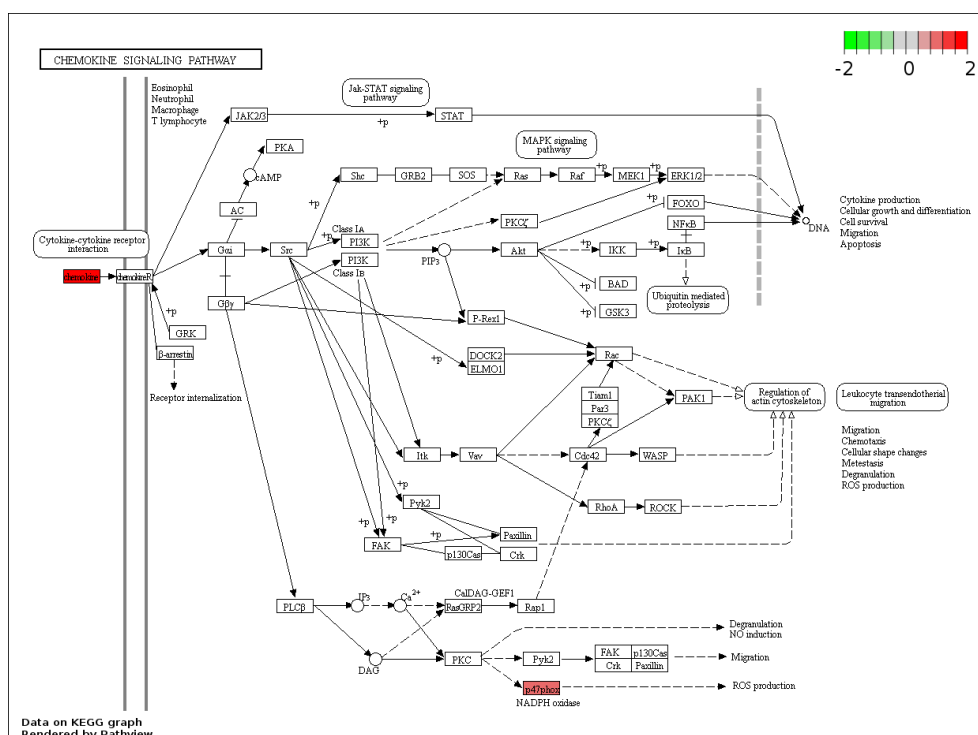


Figure S8. Differentially regulated genes in HGF within the chemokine signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

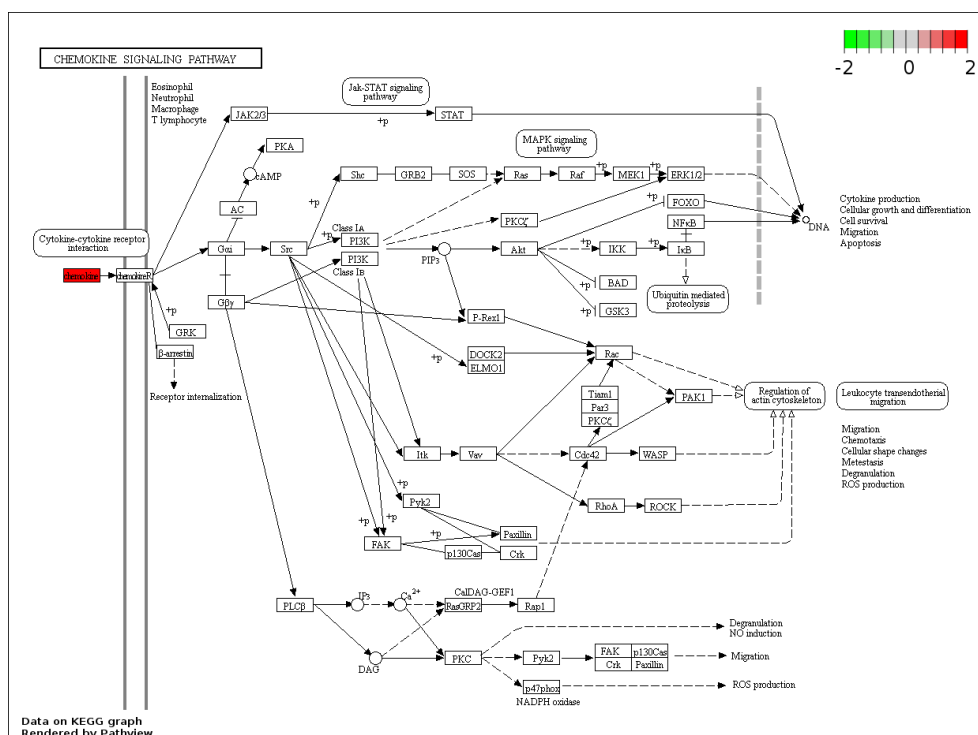


Figure S9. Differentially regulated genes in HGEp within the chemokine signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow

indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

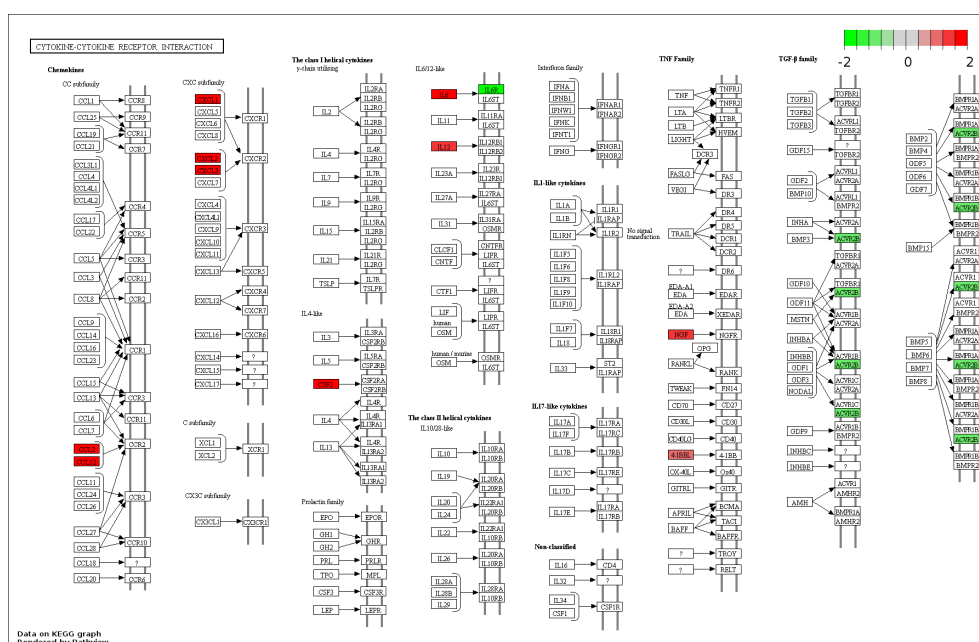


Figure S10. Differentially regulated genes in HGF within the cytokine-cytokine receptor interaction, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

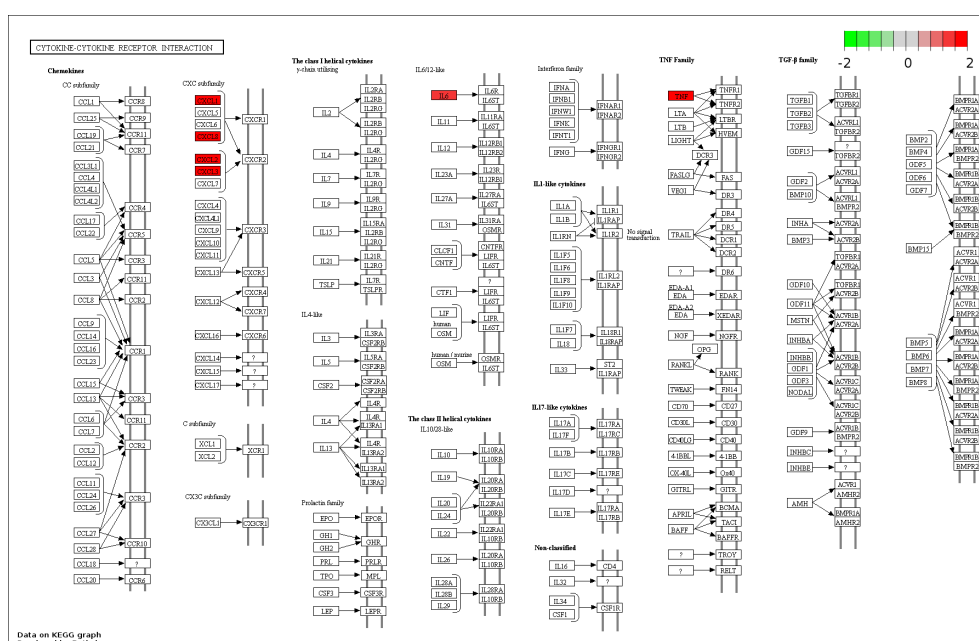


Figure S11. Differentially regulated genes in HGEp within the cytokine-cytokine receptor interaction, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An

arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

Role of differentially regulated genes in HGF within the enriched pathways

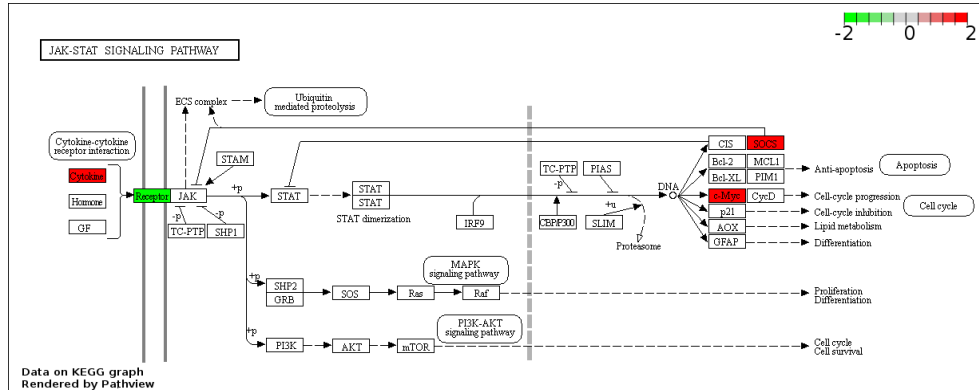


Figure S12. Differentially regulated genes in HGF within the JAK-STAT signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

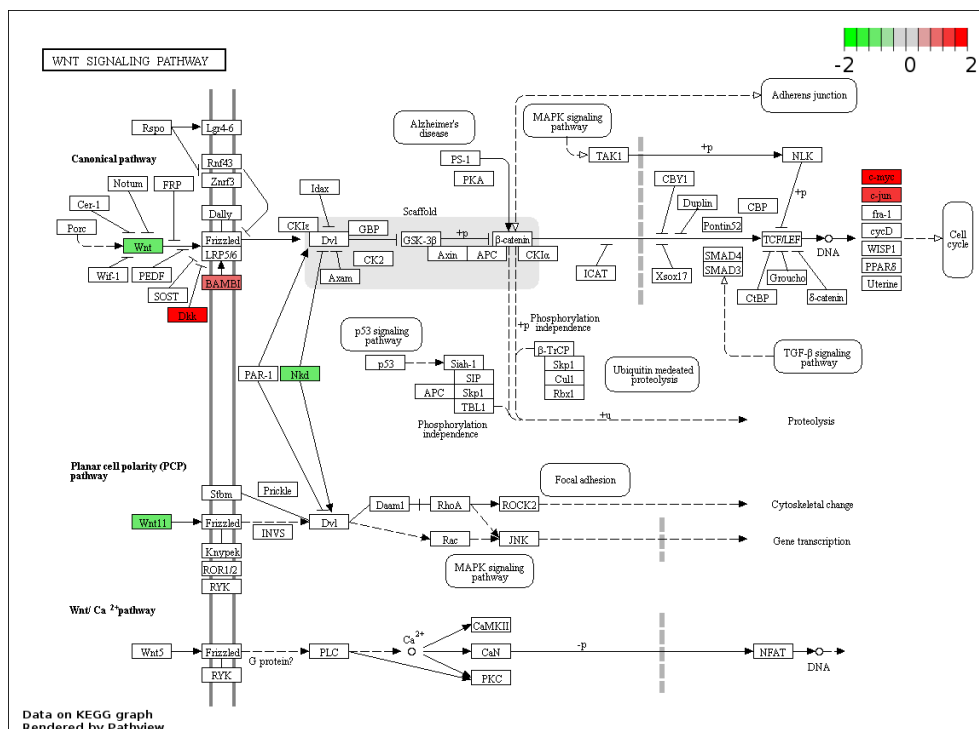


Figure S13. Differentially regulated genes in HGF within the Wnt signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

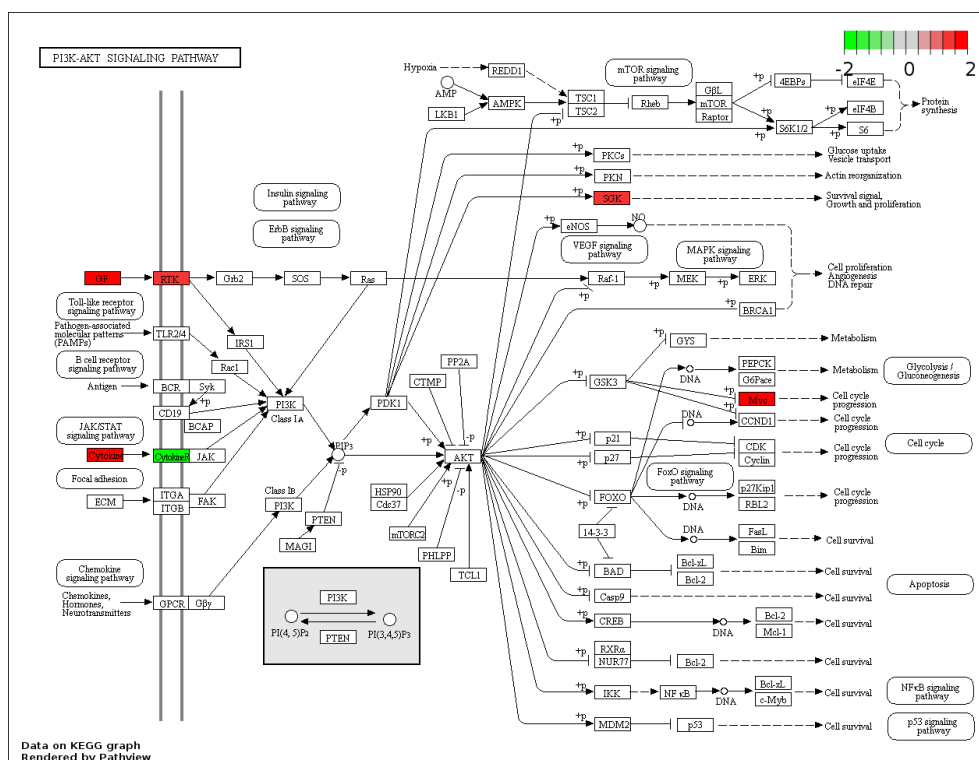


Figure S14. Differentially regulated genes in HGF within the PI3K-Akt signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

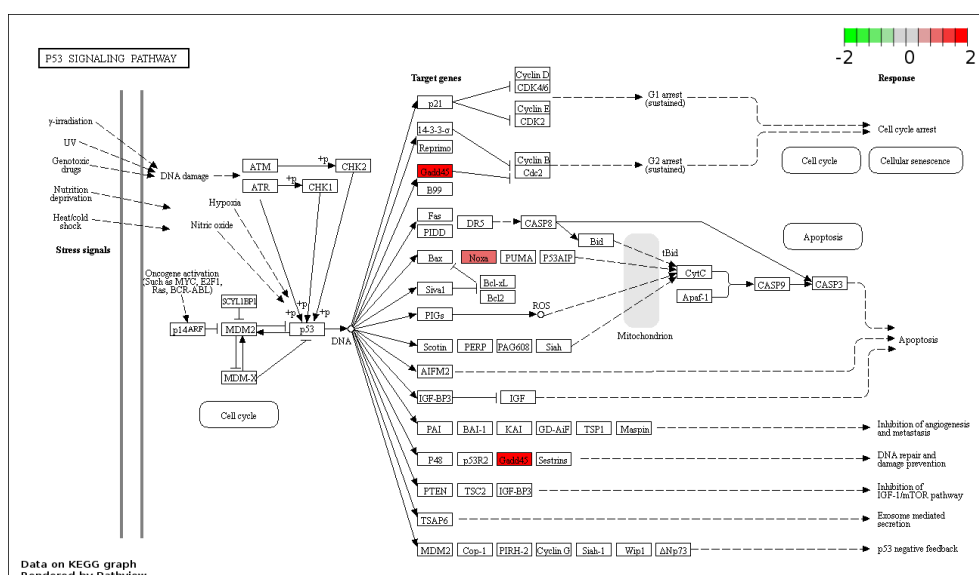


Figure S15. Differentially regulated genes in HGF within the p53 signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in

expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

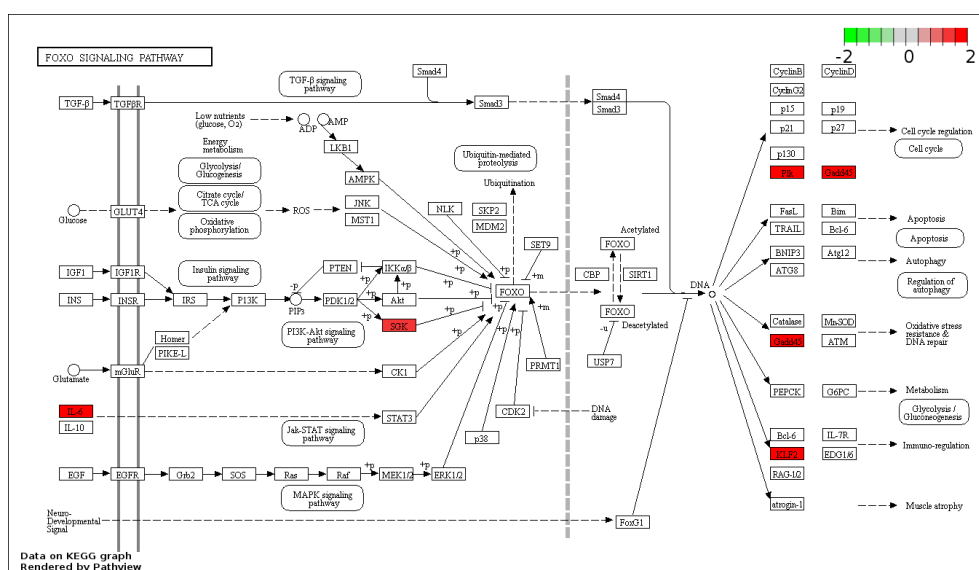


Figure S16. Differentially regulated genes in HGF within the FoxO signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

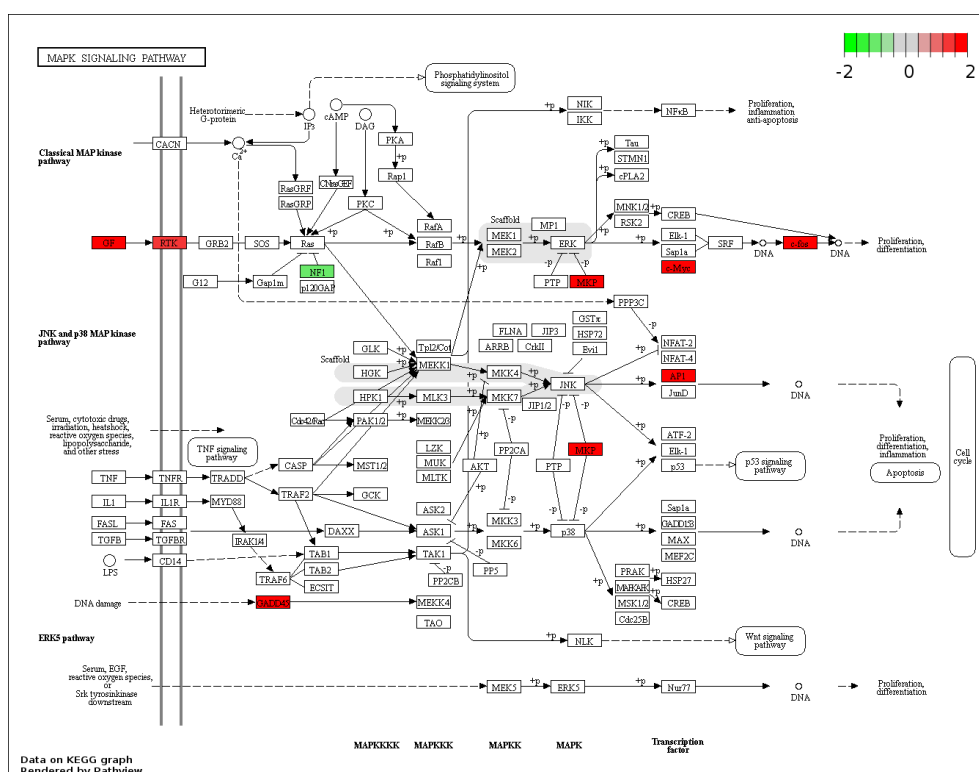


Figure S17. Differentially regulated genes in HGF within the MAPK signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a

molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

Role of differentially regulated genes in HGEp within the enriched pathways

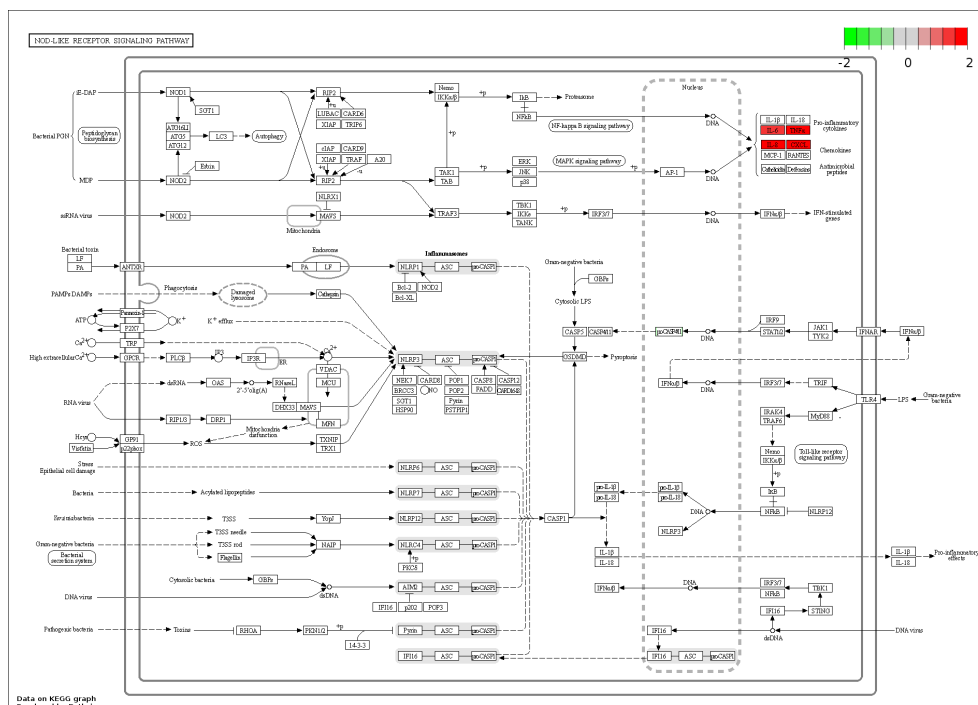


Figure S18. Differentially regulated genes in HGEp within the NOD-like receptor signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

Figure S19. Differentially regulated genes in HGEp within the NF-kappa B signaling pathway, which was adapted from Pathview using the KEGG (Kyoto Encyclopedia of Genes and Genomes) nomenclature. Red color indicates up-regulation and green indicates downregulation. An arrow indicates a molecular interaction resulting in activation, and a line without an arrowhead indicates a molecular interaction resulting in inhibition. The intensity of the color indicates the difference in expression. +p = phosphorylation, -p = dephosphorylation, +u = ubiquitination, -u = deubiquitination, +m = methylation, e = expression.

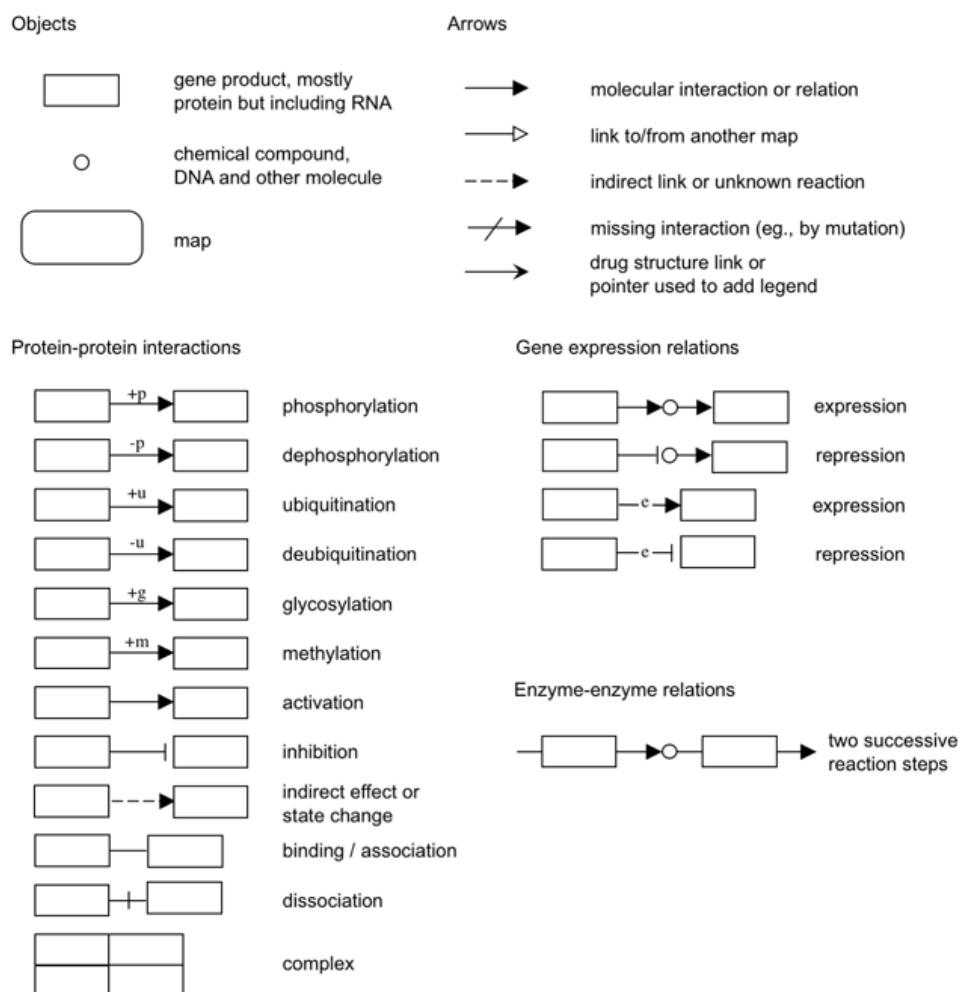


Figure S20. KEGG notation for pathways

https://www.genome.jp/kegg/document/help_pathway.html.