

Transcriptomic Landscape of Sodium Butyrate-Induced Growth Inhibition on Human Colorectal Cancer Organoids

Supplementary methods, figures, and tables

Materials and Methods

Cryopreservation and Resuscitation of Organoids

Cryopreservation was undertaken after the growth status of organoid was stable. To stock organoids, organoids were harvested using cold PBS and then centrifuged at 1700 rpm for 3 min at 4 °C. Supernatant was removed and organoid pellet was digested with TrypLE™ Express Enzyme (Thermo, USA) for 45 min at 37 °C. Then, organoid pellet was resuspended in Cell Culture Freezing Medium (Beyotime, China, C0210). The stock vials were stored at -80°C for 24 h and then transferred to the liquid nitrogen tank for long-term storage. For the first 2 weeks after thawing, the culture medium was supplemented with Y-27632 (10 μM) so as to ensure organoid growth.

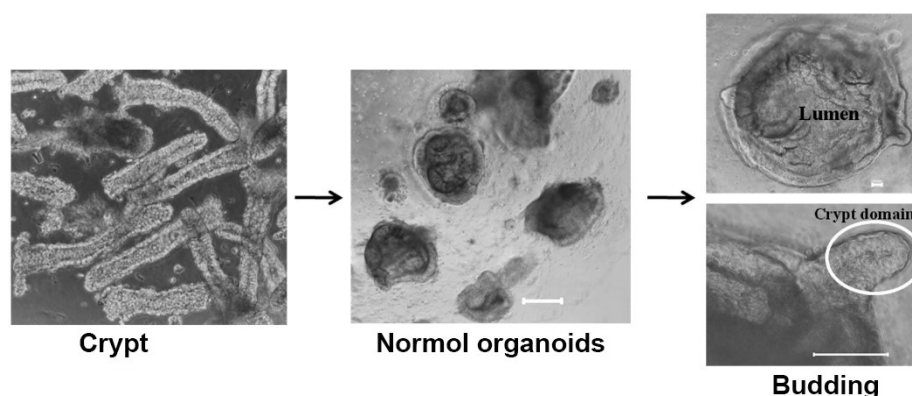


Figure S1. Representative images of human normal colon organoids development from crypts. After a few days of cultivation, normal organoids show budding state, consisting of a central lumen lined by villus-like epithelium and several surrounding crypt-like domains. Scale bar, 100 μM.

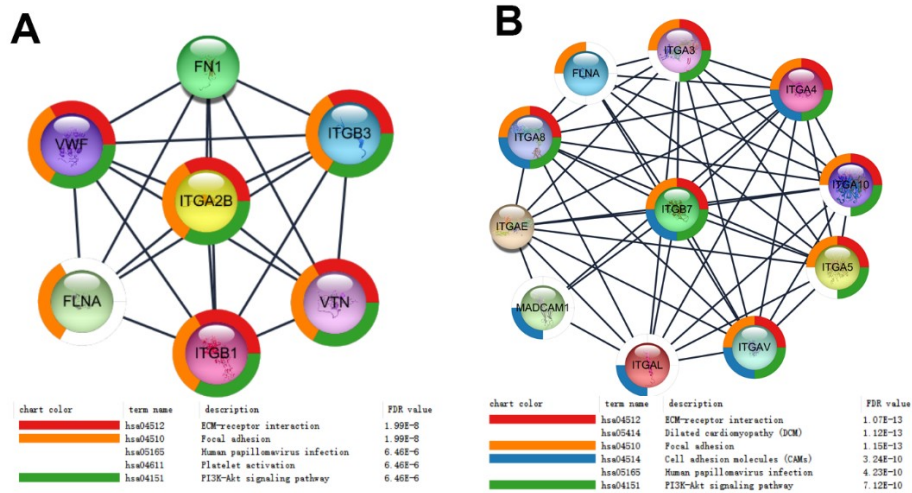


Figure S2. Subnetworks based on *ITGA2B* (A) and *ITGB7* (B). Significantly candidate genes including *ITGA2B* and *ITGB7* play central roles in ECM-integrin pathway.

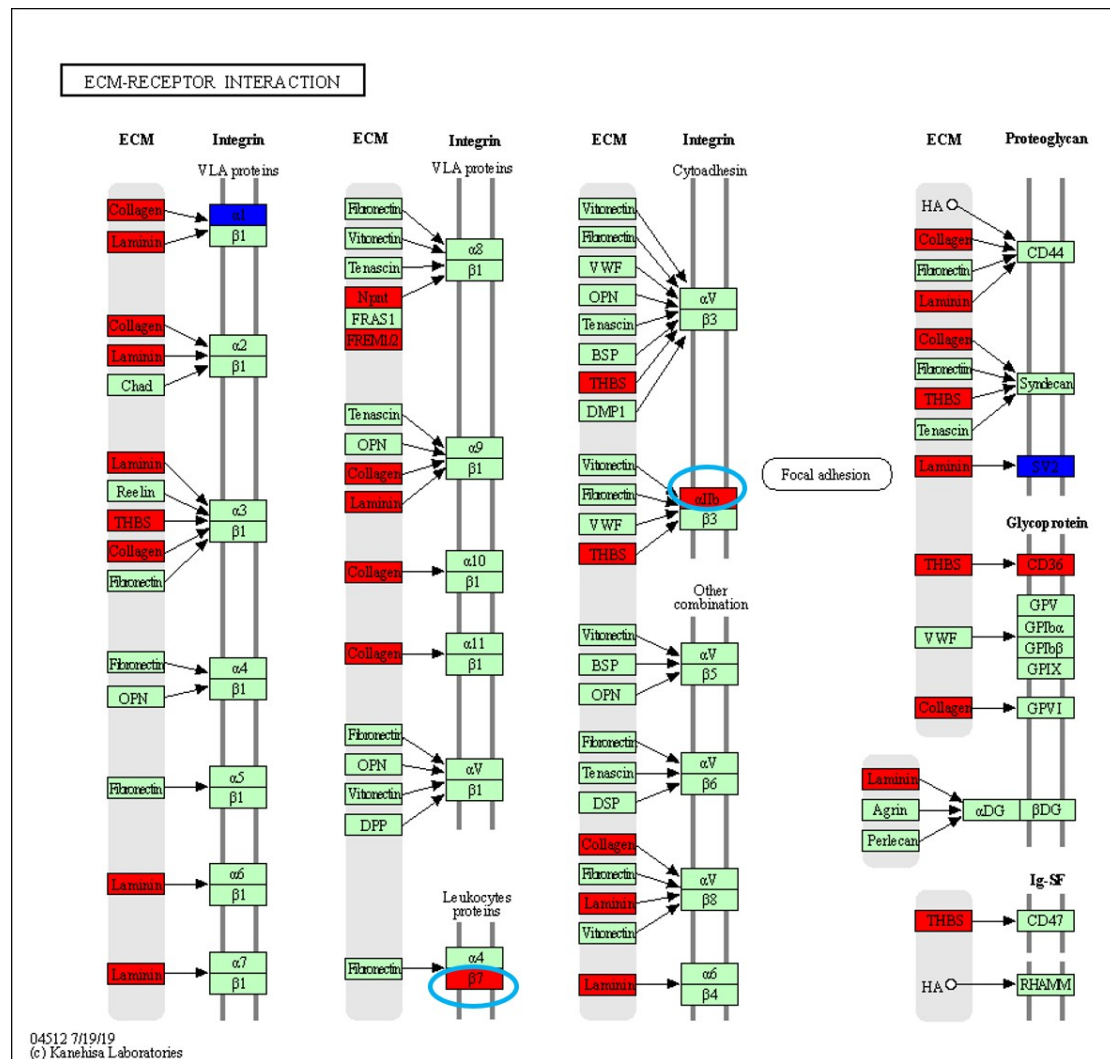


Figure S3. Pathway analysis of ECM-receptor interaction. Red represents up-regulated proteins and blue for down-regulated proteins.

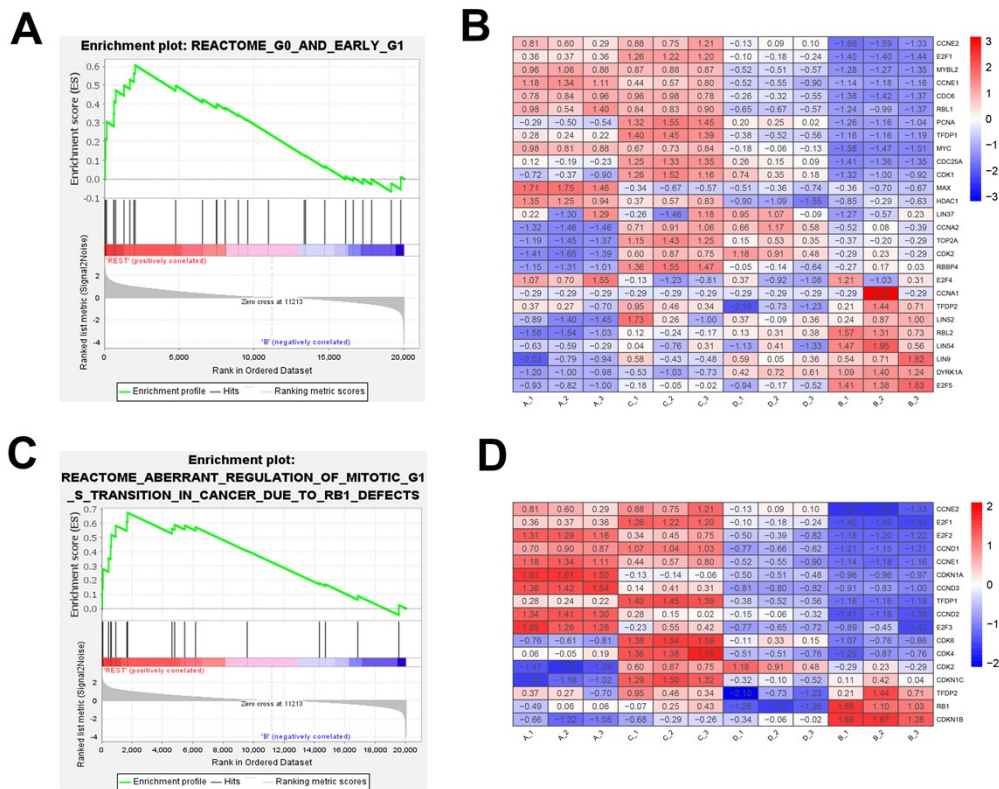


Figure S4. Result of gene set enrichment analysis (GSEA). (A) Enrichment plot of “G0 and early G1” between NaB-treated and control samples. (B) Heatmap of get sets in “G0 and early G1” pathway based on transcripts expression. (C) Enrichment plot of “aberrant regulation of mitotic G1/S transition in cancer due to RB1 defects” between NaB-treated and control samples. (D) Heatmap of get sets in “aberrant regulation of mitotic G1/S transition in cancer due to RB1 defects” based on transcript expression.

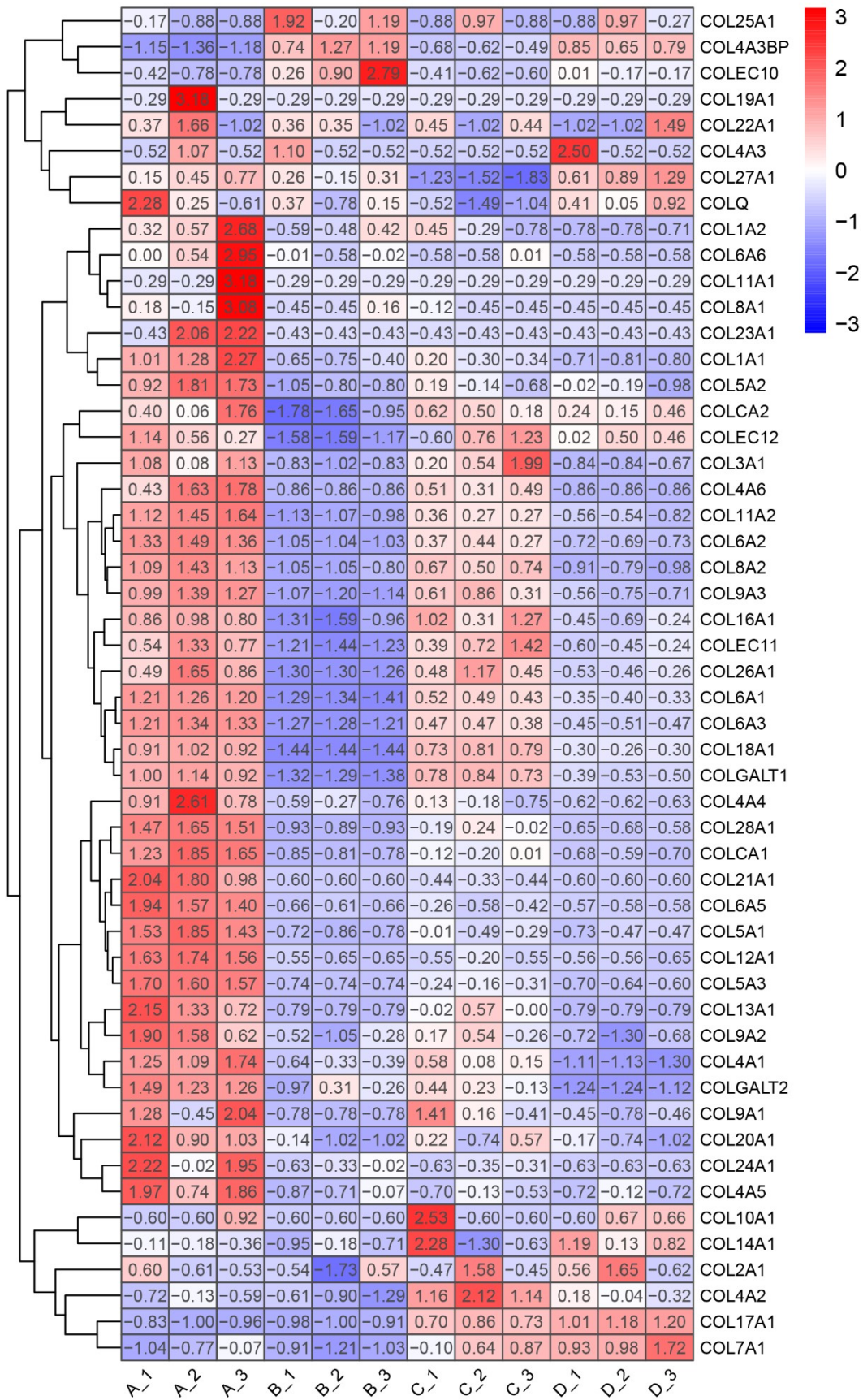


Figure S5. The expression levels of all collagen family genes in the samples.

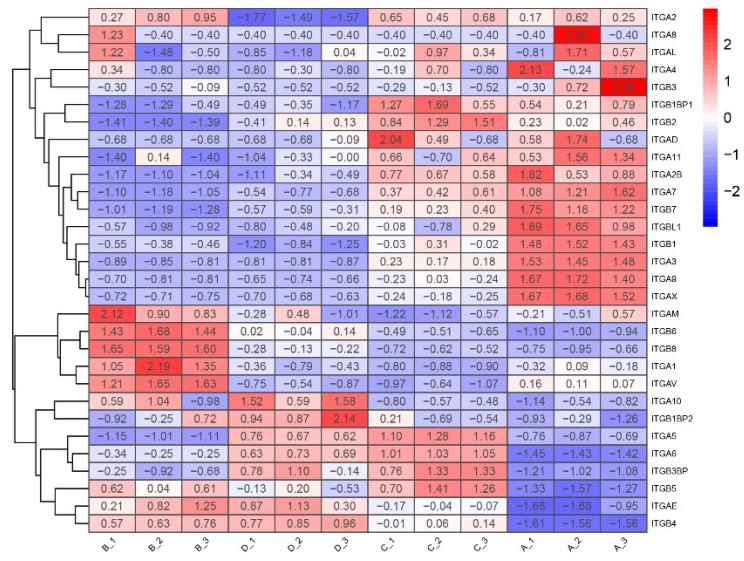


Figure S6. The expression levels of all integrin family genes in the samples.

Table S1 The KEGG terms enriched in DEGs

Term	Gene count	p value	Significant genes
Complement and coagulation cascades	11	4.69556E-05	CFD, C3, CR2, C6G, CFH, PLA1, PROS1, SERPING1, C4BPB, A2M, KNG1
Hematopoietic cell lineage	12	7.16522E-05	CR2, HLA-DRB5, CD8B, IL1R1, ANPEP, ITGA1, ITGA2B, CD7, CD1D, CD36, CD33, HLA-DRB1
Lishmaniasis	10	0.0003215	C3, MAPK11, HLA-DMA, HLA-DRB5, NOS2, TGFβ3, ITGB2, TLR4, HLA-DRB1, HLA-DPA1
FCM-receptor interaction	11	0.000339118	COMP, LAMA2, SV2B, LAMA1, COL6A2, COL11A2, ITGA1, ITGA2B, COL6A3, ITGB7, CD36
Phagosome	14	0.000754856	COLEC12, COLEC11, HLA-DRB5, ITGB2, TUBB4A, HLA-G, C3, COMP, HLA-DMA, CTS1, CD36, TLR4, HLA-DRB1, HLA-DPA1
Staphylococcus aureus infection	8	0.001305503	CFD, C3, HLA-DMA, HLA-DRB5, CFH, ITGB2, HLA-DRB1, HLA-DPA1
Legionellosis	8	0.001305503	C3, ITGB2, EFF1A2, IL18, CXCL1, CXCL3, CXCL2, TLR4
Cell adhesion molecules (CAMs)	13	0.001493909	HLA-DRB5, ITGB2, HLA-G, CLDN11, HLA-DMA, NFASC, PTPRC, CD8B, CLDN18, ITGB7, TIGIT, HLA-DRB1, HLA-DPA1
Rheumatoid arthritis	10	0.001562851	HLA-DMA, HLA-DRB5, TGFβ3, MMP1, CTS1, ITGB2, IL18, TLR4, HLA-DRB1, HLA-DPA1
Inflammatory bowel disease (IBD)	8	0.003523692	HLA-DMA, HLA-DRB5, TGFβ3, RORC, IL18, TLR4, HLA-DRB1, HLA-DPA1
Malaria	7	0.003866474	COMP, TGFβ3, ITGB2, IL18, CD36, HBA1, TLR4
Amoebiasis	10	0.005554102	C6G, LAMA2, NOS2, IL1R1, TGFβ3, PRKCB, LAMA1, COL11A2, ITGB2, TLR4
Toxoplasmosis	10	0.00706023	MAPK11, HLA-DMA, HLA-DRB5, LAMA2, NOS2, TGFβ3, LAMA1, TLR4, HLA-DRB1, HLA-DPA1
Tuberculosis	13	0.008951785	HLA-DRB5, NOS2, TGFβ3, ITGB2, IL18, C3, MAPK11, HLA-DMA, ITGAX, LBP, TLR4, HLA-DRB1, HLA-DPA1
Mineral absorption	6	0.011257196	MT2A, ATP1A3, MTIG, MTIH, MTIE
Arachidonic acid metabolism	7	0.011288206	GPX3, CYP4F2, GPX8, PLA2G3, CYP4F8, ALOX15B, PTGS1
Salmonella infection	8	0.014363393	MAPK11, NOS2, IL18, CXCL1, LBP, CXCL3, CXCL2, TLR4
Graft-versus-host disease	5	0.018380837	HLA-DMA, HLA-DRB5, HLA-G, HLA-DRB1, HLA-DPA1
Protein digestion and absorption	8	0.01929757	COL18A1, PRSS1, MEP1B, MEP1A, COL6A2, COL11A2, COL6A3, ATP1A3
Allograft rejection	5	0.026955161	HLA-DMA, HLA-DRB5, HLA-G, HLA-DRB1, HLA-DPA1
Pertussis	7	0.028681117	C3, MAPK11, NOS2, ITGB2, SERPING1, C4BPB, TLR4
Antigen processing and presentation	7	0.030362124	HLA-DMA, HLA-DRB5, CD8B, CTS1, HLA-G, HLA-DRB1, HLA-DPA1
Viral myocarditis	6	0.03143227	HLA-DMA, HLA-DRB5, ITGB2, HLA-G, HLA-DRB1, HLA-DPA1
Steroid hormone biosynthesis	6	0.033559443	SULT1E1, HSD3B1, CYP11A2, UGT2A3, UGT2B7, UGT2A1, SULT1A2
Chemical carcinogenesis	7	0.037726826	ALDH3A1, ALDH3B2, CYP11A2, UGT2A3, UGT2B7, SULT2A1, SULT1A2
Cytokine-cytokine receptor interaction	14	0.039269633	CCR1, CNTR, IL1R1, TGFβ3, CD70, TNFRSF18, IL18, CXCL1, LIFR, CXCL3, CXCL2, BMP2, TNFRSF8, CCL26
Type 1 diabetes mellitus	5	0.040550025	HLA-DMA, HLA-DRB5, HLA-G, HLA-DRB1, HLA-DPA1
TGF-beta signaling pathway	7	0.046146221	CDKN2B, BMP2, LEFTY1, TGFβ3, BAMBI, LTBP1, SMAD7

Table S2 Gene set enrichment analysis (GSEA) analysis

REACTOME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
REACTOME_INITIAL_TRIGGERING_OF_COMPLEMENT	22	0.64192	1.70868	0	1	0.245	598 tags=23%	list=3%, signal=23%
REACTOME_METABOLISM_OF_ANGIOTENSINOGEN_TO_ANGIOTENSIN	17	0.81209	1.695	0	1	0.276	1185 tags=41%	list=6%, signal=44%
REACTOME_COMPLEMENT_CASCADE	57	0.59681	1.58431	0	1	0.598	634 tags=23%	list=3%, signal=23%
REACTOME_FORMATION_OF_FIBRIN_CLOT_CLOTTING_CASCADE	39	0.67171	1.55176	0	1	0.656	3520 tags=49%	list=18%, signal=59%
REACTOME_CARGO_CONCENTRATION_IN_THE_ER	33	0.49566	1.54555	0	1	0.66	3390 tags=30%	list=17%, signal=36%
REACTOME_PRE_NOTCH_PROCESSING_N_GOLGI	18	0.56103	1.48347	0	1	0.862	3316 tags=33%	list=17%, signal=40%
REACTOME_PRE_NOTCH_EXPRESSION_AND_PROCESSING	49	0.39499	1.4524	0	1	0.934	3629 tags=31%	list=18%, signal=37%
REACTOME_PHASE_4_RESTING_MEMBRANE_POTENTIAL	19	0.69284	1.44015	0	1	0.949	2692 tags=37%	list=13%, signal=43%
REACTOME_CHAPERONE_MEDIATED_AUTOPHAGY	22	0.49732	1.43005	0	1	0.957	2897 tags=27%	list=14%, signal=32%
REACTOME_ILDIL_CILIFERANCE	19	0.45725	1.42817	0	1	0.957	2155 tags=32%	list=11%, signal=35%
REACTOME_FGFR2_LIGAND_BINDING_AND_ACTIVATION	20	0.57077	1.42695	0	1	0.957	4295 tags=30%	list=21%, signal=38%
REACTOME_COLLAGEN_BIOSYNTHESIS_AND_MODIFYING_ENZYMES	67	0.63973	1.41771	0	1	0.957	2696 tags=42%	list=13%, signal=48%
REACTOME_SEROTONIN_NEUROTRANSMITTER_RELEASE_CYCLE	18	0.68426	1.38208	0	1	0.993	3207 tags=44%	list=16%, signal=53%
REACTOME_COLLAGEN_FORMATION	90	0.58878	1.37823	0	1	0.995	4117 tags=47%	list=21%, signal=58%
REACTOME_NUCLEOTIDE_CATABOLISM	35	0.51596	1.31387	0	1	1	4283 tags=34%	list=21%, signal=44%
REACTOME_ARACHIDONIC_ACID_METABOLISM	58	0.54173	1.42879	0.0055351	1	0.957	3751 tags=45%	list=19%, signal=55%
REACTOME_BINDING_AND_UPTAKE_OF_LIGANDS_BY_SCAVENGER_RECEPTORS	42	0.50038	1.31846	0.0075472	1	1	5741 tags=57%	list=29%, signal=80%
REACTOME_SYNAPTIC_ADHESION_LIKE_MOLECULES	21	0.5754	1.41736	0.0083857	1	0.957	3565 tags=33%	list=18%, signal=41%
REACTOME_REGULATION_OF_INSULIN_LIKE_GROWTH_FACTOR_IGF_TRANSPORT_AND_UPTAKE	123	0.51256	1.40765	0.0092937	1	0.98	5077 tags=42%	list=25%, signal=56%
REACTOME_PHOSPHOLIPASE_C_MEDIATED_CASCADE_FGFR2	18	0.6319	1.49385	0.010453	1	0.836	4295 tags=33%	list=21%, signal=42%
REACTOME_SURFACTANT_METABOLISM	30	0.5678	1.42954	0.0112613	1	0.957	5140 tags=40%	list=26%, signal=54%
REACTOME_IMMUNOREGULATORY_INTERACTIONS_BETWEEN_A_LYMPHOID_AND_A_NON_LYMPHOID_CELL	128	0.54378	1.34093	0.012024	1	1	4976 tags=32%	list=25%, signal=42%
REACTOME_PHASE_1_FUNCTIONALIZATION_OF_COMPOUNDS	102	0.40223	1.3521	0.0136187	0.991342	1	3751 tags=29%	list=19%, signal=36%
REACTOME_PURINERGIC_SIGNALING_IN_LEISHMANIASIS_INFECTION	26	0.48676	1.37069	0.0152964	1	1	2812 tags=27%	list=14%, signal=31%
REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION	301	0.49647	1.3201	0.0197842	1	1	4282 tags=43%	list=21%, signal=53%
REACTOME_BIOLOGICAL_OXIDATIONS	215	0.37674	1.26751	0.0205607	1	1	5664 tags=37%	list=28%, signal=51%
REACTOME_FORMATION_OF_THE_CORNFIELD_ENVELOPE	128	0.45345	1.39602	0.0241449	1	0.993	3973 tags=20%	list=20%, signal=25%
REACTOME_FGFR2_MUTANT_RECEPTOR_ACTIVATION	33	0.42515	1.36033	0.0290102	1	1	4362 tags=30%	list=22%, signal=39%
REACTOME_TNFS BIND THE R PHYSIOLOGICAL RECEPTORS	29	0.51813	1.35804	0.0300885	0.987446	1	3364 tags=38%	list=17%, signal=46%
REACTOME_SYNTHESIS_OF_LEUKOTRIENES_LI1_AND_FOXINS_EX	21	0.69918	1.53915	0.0311284	1	0.687	3751 tags=52%	list=19%, signal=64%
REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS	85	0.56907	1.31316	0.0318949	0.989723	1	2703 tags=41%	list=13%, signal=47%