ras homolog family member U / Wnt-1 responsive Cdc42 homolog (RHOU/WRCH1) : Time behavioural study of 3rd order combinations in WNT3A stimulated HEK 293 cells

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Abstract

RHOU (orWRCH1) is a small 21 kDa signaling guanine nucleotide-binding protein (G protein), belonging to the class of GTPase (hydrolase enzymes that bind to the nucleotide guanosine triphosphate (GTP) and hydrolyze it to guanosine diphosphate (GDP)), and is a member of the Rho family of GTPases (a subfamily of Rat sarcoma virus or Ras superfamily). Gujral and MacBeath [1] provides a quantitative, and dynamic study of WNT3A-mediated stimulation of HEK 293 cells, where they record time based expression profiles of several response genes which correlated significantly with proliferation and migration. By monitoring the dynamics of gene expression using self-organizing maps, they identified clusters of genes that exhibit similar expression dynamics and uncovered previously unrecognized positive and negative feedback loops. However, their study depicts/uses singular measurements of individual gene expression at different time snapshots/points to infer the system wide analysis of the pathway. At any particular time point, it is often the case that genes are working synergistically in combinations, even though their expression measurements are singular in nature. Here, I • enumerate and rank all 2415 RHOU related 3rd order combinations in a forest of ${}^{71}C_3$ combinations using four different sensitivity methods; • show the conserved rankings for RHOU-X-X combinations, which point to existence of biological synergy of some of these combinations across the different sensitivity methods; and • study the behaviour of some of these combinations related to WNT3A response genes that are ranked by the machine learning search engine (Sinha [2]) in time. Patterns of combinations emerge, some of which have been tested in wet lab, while others require further wet lab analysis.

Keywords: Sensitivity analysis, Support vector ranking, Hilbert Schmidt Independence Criterion indices (HSIC) and Sobol indicies, WNT3A

☆Time behavioural study of 3-odr RHOU/WRCH1 comb. in WNT3A stimulated cells

[1] Aspects of unpublished work were presented in a poster session at Cell Symposia: Technology. Biology. Data Science, 9-11 October 2016, Berkeley, California, USA.

1. Significance

Sinha [2] recently demonstrated the use of machine learning based search engine to rank/reveal gene combinations at 2nd order for the time series data by Gujral and MacBeath [1] and showed how it is possible to locate combinations of priority that might be working synergistically, using sensitivity methods and powerful support vector ranking algorithm. However, the problem explodes combinatorially with even a small set of 71 recorded genes in the study by Gujral and MacBeath [1], when one steps to explore 3rd order combinations. With the total number of $^{71}C_3$ (= 57155) combinations, it becomes nearly impossible for any biologist to study the system wide dynamics of any pathway. Also, the amount of time usually needed to search for and test a combination is far more than the search down by the machine learning based search engine. Here, I extend the research work by Sinha [2] to conduct a behavioral study of 3rd order RHOU related combinations using individual gene expressions measured in time, in WNT3A stimulated HEK 293 cells.

2. Introduction

The details of the machine learning based search engine has been recently published in Sinha [2] and deployed to explore the 2nd order combinations of genes in the data set provided by Gujral and MacBeath [1]. Nevertheless, here, I point to the fundamentals of the published work for completeness.

2.1. A combinatorial problem

Sensitivity analysis plays a major role in computing the strength of the influence of involved factors in any phenomena under investigation. When applied to expression profiles of various intra/extracellular factors that form an integral part of a signaling pathway, the variance and density based analysis yields a range of sensitivity indices for individual as well as various combinations of factors. These combinations denote the higher order interactions among the involved factors. Computation of higher order interactions is often time consuming but it gives a chance to explore the various combinations that might be of interest in the working mechanism of the pathway. For example, in a range of fourth order combinations among the various factors of the Wnt pathway, it would be easy to assess the influence of the destruction complex formed by APC, AXIN, CSKI and GSK3 interaction. But the effect of these combinations vary over time as measurements of fold changes and deviations in fold changes vary. So it is imperative to know how an interaction or a combination of the involved factors behave in time and Sinha [2] develops a procedure to track the behaviour by exploiting the influences of these involved factors.

2.2. A possible solution

In this work, after estimating the individual effects of factors for a higher order combination, the individual indices are considered as discriminative features. A combination, then, is a feature set in higher order ($\geq$2 ,i.e multivariate). With an excessively large number of factors involved in the pathway, it is difficult to search for important combinations in a wide search space over different orders. Exploiting the analogy with the issues of prioritizing webpages using ranking algorithms, for a particular order, a full set of combinations of interactions can then be prioritized based on these features using a powerful ranking algorithm via support vectors Joachims [3]. Recording the changing rankings of the combinations over time reveals how higher order interactions behave within the pathway and when an intervention might be necessary to influence the interaction within the pathway.

2.3. ras homolog family member U / Wnt-1 responsive Cdc42 homolog (RHOU/WRCH1)

RHOU (orWRCH1) is a small 21 kDa signaling guanine nucleotide-binding protein (G protein), belonging to the class of GTPase (hydrolase enzymes that bind to the nucleotide guanosine triphosphate (GTP) and hydrolyze it to guanosine diphosphate (GDP)), and is a member of the Rho family of GTPases (a subfamily of Rat sarcoma virus or Ras superfamily).

The transition of guanine nucleotide binding proteins between the 'on' (GTP-bound) and 'off' (GDP-bound) states is a paradigm of molecular switching after a chemical reaction. Stouten et al. [4] try to provide a picture of the process of the mechanism by which the switch signal is transmitted to the downstream recipients in the intracellular signal pathway, though it has been extensively studied by biochemical, biophysical and genetic methods. Based on the similarities of ras-p21 and elongation factor Tu, they proposed a model of the GDP state of ras-p21 that was in agreement with all relevant experimental evidence. Their model provided important clues about: (1) a possible molecular mechanism for signal transmission from the site of GTP hydrolysis to downstream effectors; (2) a major conformational change during signal generation and a key residue involved in this process i.e Tyr-64; and (3) regions in ras-p21 that could be differentially recognized by binding to external partners in a GTP/GDP state dependent fashion, most notably residues D69, Q70, R73, T74, R102, K104, D105 at the end of the α-helices 2 and 3.

GTPases of the Rho family play important roles in converting and amplifying external signals into cellular effects. Not only do they control the dynamics of the F-actin cytoskeleton, they have also been implicated in many basic cellular processes that influence cell proliferation, differentiation, motility, adhesion, survival, or secretion. To elucidate the evolutionary history of the Rho family, Boureux et al. [5] analyzed over 20 species covering major eukaryotic clades from unicellular organisms to mammals, and reconstructed the ontogeny and the chronology of emergence of the different subfamilies. Their data established that the 20 mammalian Rho members are structured into 8 subfamilies, among which Rac is the founder of the whole family. Rho, Cdc42, RhoUV, and RhoBTB subfamilies appeared before Coelomates and RhoJQ, Cdc42 isoforms, RhoDF, and Rnd emerged in chordates. Their analysis of Rho mRNA expression patterns in mouse tissues showed that subfamilies had tissue-specific and low-level expression that supported their implication only in narrow time windows or in differentiated metabolic functions.

The Rho family of GTPases is a subfamily of the Rat sarcoma virus (RAS) superfamily. In the course of routine passage of Moloney's leukemogenic virus (MLV), Harvey [6] collected plasma from a leukemic rat (Chester Beatty Institute outbred albino strain) which had been inoculated with MLV-containing mouse plasma when newborn. After storage at 70° C for three months, they diluted the rat plasma 1 in 30 with Hanks's saline and passed through a 'Selas 02' filter, tested and found it impervious to Esch. coli. The filtrate was injected into 15 new-born BALB/c mice, for potency test and they reported that only 6 survived to weaning and on the 32nd day, 5 had tumours at or near the injection site, and all had grossly enlarged spleens. This was identified as the beginning of RAS research. Three additional retroviruses were identified, i.e • Kirsten and Mayer [7] passed mouse erythroblastosis virus (MEV) to newborn W/Fu rats. They observed that the rats developed generalized malignant lymphomas after 6-7 months. Two separate serial cell-free passage sedes were initiated. Rat lymphomas induced erythroblastosis in less than 20% of the rats that died within 4 weeks after inoculation. The surviving rats remained apparently healthy for months until they died from lymphomas. Inoculation of newborn C3Hf/Gs mice with the same filtrates caused only lymphomas in 3550% of the mice after 38 months. • Peters et al. [8] identified naturally occurring sarcoma virus of the BALB/cCr mouse; and • Rasheed et al. [9] observed that a Sprague-Dawley (SD-1) rat embryo culture, at low passage level, released an endogenous ecotropic type C virus (SD-RaLV) and after about 20 further passages it underwent spontaneous transformation. The SD-RaLV, released from the transformed cells, did not cause rapid transformation of other rat embryo cells, but, when the transformed cells were repeatedly cocultivated with three different chemically transformed and serially transplanted rat tumor cell lines (sarcoma, carcinoma, and hepatoma), rapidly fibroblast-transforming "sarcoma" viruses (RaSV) were recovered after each attempt. The productive clones were found to be positive for rat specific p30 antigen and the RaSVs released were serially transmitted to other rat embryo cells. RaSV genome was rescued from the nonproductive clones by superinfection with SD-RaLV, wild rat type C virus, and several heterologous type C viruses. Their findings pointed to naturally occurring transformation-specific (src) genes being recovered in vitro in the form of stable "sarcoma" viruses. Malumbres and Barbacid [10] sumarise the developments related to the first 30 years of research regarding RAS. A survey of RAS mutations in cancer can be found in Prior et al. [11].

Tao et al. [12] reported the isolation and cloning of the WNT-1 responsive Cdc42 homolog (WRCH1) cDNA, whose mRNA level increased in response to WNT-1 signaling in WNT-1 transformed cells, WNT-1 transgene induced mouse mammary tumors, and WNT-1 retrovirus infected cells. To confirm that WRCH1 was regulated by WNT-1 signaling, they infected C57MG cells with either an empty retroviral vector or a WNT-1-expressing retrovirus. WRCH1 mRNA was found to be up-regulated more than fourfold in C57MG/WNT-1 cells versus C57MG/vector cells. Further, overexpression of WRCH1 phenocopied WNT-1 in morphological transformation of mouse mammary epithelial cells. To identify if WRCH1 was a homolog of the Rho family ofGTPases, they cloned full-length mouse and human WRCH1 cDNAs, based on the partial sequence of mouse WRCH1 cDNA isolated from PCR-select cDNA subtraction. They obserevd that the cDNAs of human and mouse WRCH1 encoded proteins of 258 and 261 amino acid residues, respectively, which are 92% identical. Their analysis of the deduced amino acid sequence of WRCH1 identified it as a homolog of the Rho family of GTPases. The human WRCH1 protein shared 57% identity and 70% similarity with Cdc42, and 30%-55% similarity with other members of the Rho family. It was observed that both human and mouse WRCH1 contained GTP and GDP binding domains, the effector domain, and a CAAX lipid modification signal at their C termini, all of which were conserved among most members of the Rho family, as documented in Ridley [13]. Like Cdc42, they found that WRCH1 could activate PAK-1 and JNK-1, and induce filopodium formation and stress fiber dissolution.

I present 3rd order combinations of RHOU with other genes, that the machine learning based search engine points to, as possible synergistic combinations that might be working in time.

3. Methods

Please refer to sections of Sinha [2] for methods, design of study and analysis of data for 2nd order combinations. The same method and design of study is used to generate results for 3rd order combinations presented in this study.

4. Time series data

Gujral and MacBeath [1] present a set of 71 WNT-related gene expression values for 6 different times points over a range of 24-hour period using qPCR. The changes represent the fold-change in the expression levels of genes in 200 ng/mL WNT3A-stimulated HEK 293 cells in time relative to their levels in unstimulated, serum-starved cells at 0-hour. Gujral and MacBeath [1] state that qPCR data are the means of three biological replicates. Only genes whose mean transcript levels changed by more than two-fold at one or more time points during the 24-hour time course were considered significant. Positive (negative) numbers represent up (down) -regulation. We have already covered the issues related to these data sets in detail in Sinha [14]. Readers are requested to go through them in the pointed reference. The tools of study which are used here have been published in another foundational work in Sinha [14].

5. Design of experiment

5.1. Pipeline for time series data

For the case of time series data, interactions among the contributing factors are studied by comparing triplets of fold-changes at single time points. The prodecure begins with the generation of distribution around measurements at single time points with added noise is done to estimate the indices. A distribution is generated for the fold changes at single time points. Then for every gene, there is a vector of values representing fold changes as well as deviations in fold changes for different time points and durations between time points, respectively. Next a listing of all C_k^n combinations for k number of genes from a total of n genes is generated. k is ≥ 2 and $\leq (n-1)$. Each of the combination of order k represents a unique set of interaction between the involved genetic factors. After this, the datasets are combined in a specifed format which go as input as per the requirement of a particular sensitivity analysis method. Thus for each p^{th} combination in C_k^n combinations, the dataset is prepared in the required format from the distributions for two separate cases which have been discussed above. (See .R code in mainScript-1-1.R). After the data has been transformed, vectorized programming is employed for density based sensitivity analysis and looping is employed for variance based sensitivity analysis to compute the required sensitivity indices for each of the p combinations. This procedure is done for different kinds of sensitivity analysis methods.

After the above sensitivity indices have been stored for each of the p^{th} combination, the next step in the design of experiment is conducted. Since there is only one recording of sensitivity index per combination, each combination forms a training example which is alloted a training index and the sensitivity indices of the individual genetic factors form the training example. Thus there are C_k^n training examples for k^{th} order interaction. Using this training set SVM_{learn}^{Rank} Joachims [3] is used to generate a model on default value C value of 20. In the current experiment on toy model C value has not been tunned. The training set helps in the generation of the model as the different gene combinations are numbered in order which are used as rank indices. The model is then used to generate score on the observations in the testing set using the $SVM_{classify}^{Rank}$ Joachims [3]. Note that due to availability of only one example per combination, after the model has been built, the same training data is used as test data to generates the scores. This procedure is executed for each and every sensitivity analysis method. This is followed by sorting of these scores along with the rank indices (i.e the training indices) already assigned to the gene combinations. The end result is a sorted order of the gene combinations based on the ranking score learned by the SVM^{Rank} algorithm. Finally, this entire procedure is computed for sensitivity indices generated for each and every fold change at time point and deviations in fold change at different durations. Observing the changing rank of a particular combination at different times and different time periods will reveal how a combination is behaving.

Note that the following is the order in which the files should be executed in R, in order, for obtaining the desired results (Note that the code will not be explained here) - • use source("mainScript-1-1.R") with arguments for Dynamic data • source("SVMRank-Results-D.R"), to rank the interactions (again this needs to be done separately for different kinds of SA methods), • use source("Combine-Time-files.R"), if computing indices separately via previous file, • source("Sort-n-Plot-D.R") to sort the interactions. Note that the sorting is chages the interaction ranking in time. Thus • use source("Interaction-Priority-Intime.R") to find the prioritized ranking of each and every interaction over the different time points and finally • use source("Print-Ranking-AND-Interaction-Rank.R") to print individual ranking of the required input factor with other interaction factors.

6. Results & Discussion

6.1. Time series data by Gujral and MacBeath [1]

NOTE - Ranking was assigned on scores that were sorted in DECREASING values. So, 1 was assigned to highest score and vice versa.

Results for the 3^{rd} order interactions are presented here. The results first discuss the behaviour of interactions across the snapshots of time using the computed sensitivities on fold change measurements per time snapshot. The analysis was done using 4 different sensitivity indices. Out of the ${}^{71}C_3$ combinations, I consider/present only those combinations that show a ranking within first 10,000 out of 57,155. This choice is liberal and biologists/oncologists can have a more stricter choice as per need. Two observations are made, • the ranking of a particular combination is conserved (i.e within the 10,000 range) in a particular time point or in the early phase or late phase of WNT3A stimulation, across the majority of the four sensitivity methods, which is a strict criteria of assessment or • the ranking of a particular combination is conserved across time points/phase (i.e they are within the 10,000 range) and the majority of the four sensitivity methods, which is relaxed criteria of assessment. Applying this filter helps reveal important combinations of interest that might be working synergistically at a higher order level in the cell.

Regarding technical points of implementation, the rankings were generated without scaling/normalizing the time series data provided by Gujral and MacBeath [1]. For estimating the sensitivity indices, a small gaussian distribution using the function **rnorm** that generates a vector of normally distributed random variables given a vector length n (here 9, the 10th one is the mean/recorded gene regulation itself), a population mean μ and population standard deviation σ. The syntax for using rnorm is as follows: **rnorm(n, mean, sd)**. Further, I use the **jitter** funtion to add a little bit of noise to the data. This helps to see if the generated rankings are robust or not.

6.2. Enumeration and ranking of 2415 RHOU-X-X combinations from Gujral and MacBeath [1]

In the supplementary section, I present four files, each containing the rankings of 3rd order combinations, that wary in time (shown for 5 time points). Each file represents the rankings computed using a particular sensitivity method. The changing rankings in time for a particular combination represents the importance of contribution/role that combination plays in the cell stimulated with WNT3A. The sensitivity methods used are Hilbert Schmidt Independence Criterion indices (HSIC) indices (with rbf and linear kernel in Da Veiga [15]) and Sobol indicies (with 2002 implementation in Saltelli [16] and martinez implementation in Martinez [17] and Baudin et al. [18]).

6.3. Conserved machine learning rankings for tested RHOU-X-X combinations

A total of 2415, 3rd order combinations involving RHOU were obtained from a full set of ${}^{71}C_3$ = 57155 combinations. Further, from this selected set, using the above criteria for conserved rankings, I report/tabulate the meaningful combinations that might be working synergistically. Tables 2, 3 and 4 show the rankings for the same combinations as in table 1, but using rbf kernel for HSIC, 2002 implementation for SOBOL and martinez implementation for SOBOL, respectively. As one tallies the rankings of across these tables for a particular combination, one finds that the role of the combination of interest is conserved. This conservation points to the existence of the biological synergy, whether the combination has been tested or unexplored/untested.

6.3.1. Examining the behaviour of WNT-RHOU-X combinations

We know from the above literature that WNT1 up-regulates WRCH1/RHOU. Looking at the tables above, one finds the following combinations for members of WNT family along with RHOU, to be prominent at 3rd order level - LRP6-RHOU-WNT2B, FBXW11-RHOU-WNT2B, RHOU-SLC9A3R1-WNT4, LRP6-RHOU-WNT2, EP300-RHOU-WNT2, RHOU-WNT1-WNT4, LRP6-RHOU-WNT5A, RHOU-SFRP1-WNT4 and GSK3B-RHOU-WNT2. All these combinations indicate the existence of a possible synergy when they take a higher rank in the list of combinations.

6.3.2. Examining the behaviour of FZD-RHOU-X combinations

It is also known that the WNT signaling pathways are activated by the binding of a WNT-protein ligand to a Frizzled family receptor (FZD), which passes the biological signal to the Dishevelled (DVL) protein inside the cell. Looking at the tables above, one finds the following combinations for members of FZD family along with RHOU, to be prominent at 3rd order level - FZD5-JUN-RHOU, FZD5-CCND2-RHOU, FZD1-PORCN-RHOU, FZD2-NKD1-RHOU, APC-FZD6-RHOU, FZD5-GSK3A-RHOU, FZD1-NKD1-RHOU, FSHB-FZD2-RHOU, CXXC4-FZD2-RHOU, FZD8-GSK3A-RHOU, FZD1-FZD2-RHOU, FZD8-LRP6-RHOU, FZD7-NKD1-RHOU, CXXC4-FZD7-RHOU, FRZB-FZD2-RHOU, FZD5-NKD1-RHOU, CTNNBIP1-FZD2-RHOU, FZD7-PORCN-RHOU, FZD5-LRP6-RHOU, AXIN1-FZD2-RHOU and FZD1-GSK3A-RHOU. All these combinations indicate the existence of a possible synergy when they take a higher rank in the list of combinations.

6.3.3. Examining the behaviour of DVL-RHOU-X combinations

Again, it is also known that the WNT signaling pathways are activated by the binding of a WNT-protein ligand to a Frizzled family receptor (FZD), which passes the biological signal to the Dishevelled (DVL) protein inside the cell. Looking at the tables above, one finds the following combinations for members of DVL family along with RHOU, to be prominent at 3rd order level - AXIN1-DVL1-RHOU, DVL1-FBXW11-RHOU, DKK1-DVL2-RHOU, DVL2-FGF4-RHOU, DVL1-LRP6-RHOU, CXXC4-DVL2-RHOU, DVL1-FOXN1-RHOU, DVL1-RHOU-TCF7 and CTNNBIP1-DVL2-RHOU. All these combinations indicate the existence of a possible synergy when they take a higher rank in the list of combinations.

RANKING @ t_i USING HSIC - LINEAR

3rd order comb.	t_1	t_3	t_6	t_{12}	t_{24}	3rd order comb.	t_1	t_3	t_6	t_{12}	t_{24}
FBXW11-LRP6-RHOU	11	53077	50648	30258	27900	CCND1-FGF4-RHOU	77	55663	35981	27067	10821
FZD5-JUN-RHOU	105	12942	56571	25707	3718	CTNNBIP1-JUN-RHOU	119	11333	54553	37428	52492
CSNK1D-FGF4-RHOU	137	7470	45846	49163	20155	DAAM1-LRP6-RHOU	160	47568	49021	54157	20385
CCND2-LRP6-RHOU	195	23197	17269	56080	2265	DKK1-JUN-RHOU	210	3670	42736	27568	47765
AXIN1-DVL1-RHOU	314	6052	39138	50246	11753	APC-PITX2-RHOU	397	38592	32191	27698	15148
DKK1-LRP6-RHOU	401	7782	55096	29348	20780	FZD8-LRP6-RHOU	468	41362	6887	53473	47929
CTNNB1-FOXN1-RHOU	475	856	5984	14676	48295	CSNK2A1-MYC-RHOU	501	22087	50879	39343	4386
LRP6-RHOU-WNT2B	521	21284	29060	52854	25865	AES-AXIN1-RHOU	524	50360	32314	44626	11228
CXXC4-FGF4-RHOU	583	24551	34633	53319	56248	FBXW2-RHOU-TCF7	658	32560	43438	3752	18218
FZD5-CCND2-RHOU	694	44791	4209	21405	30546	CXXC4-DVL2-RHOU	703	7173	6556	19343	52966
EP300-GSK3B-RHOU	762	29861	9716	10624	2207	FBXW11-RHOU-SENP2	810	39829	54582	1076	13192
FOSL1-PPP2R1A-RHOU	846	41555	5035	55239	46031	CXXC4-JUN-RHOU	881	1789	43156	41625	53423
FBXW11-RHOU-WNT2B	887	56605	51928	47819	44859	GSK3B-RHOU-TLE2	898	47931	45636	21326	13471
FSHB-NKD1-RHOU	974	40540	14067	9128	35039	CCND1-PYGO1-RHOU	1040	56164	34165	35225	53648
GSK3B-LRP6-RHOU	1052	31885	19160	38602	12879	FRAT1-PORCN-RHOU	1077	31453	10861	21530	17749
FZD1-PORCN-RHOU	1095	21176	12741	16941	18130	EP300-RHOU-WNT2	1132	42400	48712	18390	28303
GSK3B-RHOU-TCF7	1149	7136	32592	11483	26116	BCL9-FGF4-RHOU	1153	22570	51717	42895	26298
CTBP1-FGF4-RHOU	1171	15515	48677	31280	24137	RHOU-WNT1-WNT4	1198	602	1780	21941	43967
CTBP2-GSK3B-RHOU	1205	55707	18585	4716	40362	PITX2-PORCN-RHOU	1220	38816	23756	29958	13324
GSK3B-RHOU-SENP2	1230	39467	21376	11806	9365	CXXC4-FOXN1-RHOU	1258	949	4951	19719	52102
BCL9-PORCN-RHOU	1284	46597	6968	29490	21809	CSNK1A1-LRP6-RHOU	1294	10065	15184	54599	10820
CSNK1G1-PORCN-RHOU	1299	27191	33972	15284	20376	FZD7-NKD1-RHOU	1307	26801	3329	51286	46329
FZD2-NKD1-RHOU	1310	19610	4807	33843	1134	AXIN1-EP300-RHOU	1312	1646	32903	47413	5408
FOSL1-PORCN-RHOU	1333	44749	10281	23020	23848	CTNNBIP1-FGF4-RHOU	1375	44903	56902	26308	45676
DVL1-FBXW11-RHOU	1388	30499	7484	11321	46544	DVL1-FOXN1-RHOU	1438	38257	4549	11910	29038
APC-FZD6-RHOU	1482	46230	8189	40700	5636	AXIN1-FOXN1-RHOU	1511	738	4253	15602	21213
FBXW11-RHOU-SFRP4	1516	48741	46254	2195	24642	DKK1-FGF4-RHOU	1553	5607	24412	21107	41674
KREMEN1-RHOU-TCF7	1573	55772	27141	223	46704	CSNK1G1-FOXN1-RHOU	1587	4796	8477	31603	16974
BCL9-JUN-RHOU	1653	6622	56085	44704	6331	FOSL1-FOXN1-RHOU	1660	21880	3384	19296	29104
DKK1-RHOU-SENP2	1744	25698	53164	542	50407	CXXC4-FZD7-RHOU	1774	20795	27044	52150	54907
DIXDC1-NKD1-RHOU	1798	6195	3765	22405	3157	FBXW11-GSK3B-RHOU	1864	39036	24146	19812	16311
DKK1-DVL2-RHOU	1867	1810	33595	42349	53523	FRZB-GSK3B-RHOU	1880	22893	26634	12199	52276
CSNK1G1-JUN-RHOU	1898	1165	55257	34817	23988	FRZB-FZD2-RHOU	1944	892	20805	28505	49730
DAAM1-FOXN1-RHOU	1998	39331	29857	17353	20914	DVL1-RHOU-TCF7	2030	52920	36405	7523	7698
LRP5-PORCN-RHOU	2053	8242	9590	48746	20172	FZD5-NKD1-RHOU	2086	9792	3471	18764	2521
FZD5-GSK3A-RHOU	2110	29982	2718	17049	4051	JUN-PYGO1-RHOU	2122	21105	6824	25993	11386
FZD1-NKD1-RHOU	2176	20088	11316	49737	2429	RHOU-TCF7-TLE2	2188	38946	13808	2888	33725
FSHB-FZD2-RHOU	2193	41442	55751	28895	48790	FRZB-GSK3A-RHOU	2205	2231	3514	26794	25129
CTBP1-GSK3A-RHOU	2252	6219	1718	35013	4228	CXXC4-GSK3A-RHOU	2262	20534	4082	47103	49826
DKK1-PYGO1-RHOU	2300	4971	25047	37502	11114	DKK1-FOXN1-RHOU	2310	27439	38761	12307	33517
CXXC4-FZD2-RHOU	2317	6671	22273	26084	55079	CSNK1G1-NKD1-RHOU	2328	3565	14141	34128	1880
FZD8-GSK3A-RHOU	2345	48981	3671	53392	35952	LRP6-RHOU-WNT5A	2371	52871	42173	56381	27277
DKK1-GSK3A-RHOU	2372	2731	45043	25134	42183	CTNNBIP1-FZD2-RHOU	2379	32067	25451	19277	52045
DVL2-FGF4-RHOU	2435	40706	45840	38213	42586	FZD7-PORCN-RHOU	2438	54301	6262	19661	38352
RHOU-SLC9A3R1-WNT4	2468	14726	19963	23342	24539	DAAM1-GSK3A-RHOU	2493	51743	28750	45637	28520
FRZB-LRP6-RHOU	2562	9229	2215	54525	26580	CTNNBIP1-DVL2-RHOU	2585	40458	19092	23270	50147
FBXW11-FOXN1-RHOU	2589	20834	7791	16907	24143	RHOU-SFRP1-WNT4	2662	39129	39941	42257	16102
DVL1-LRP6-RHOU	2680	50479	18313	55087	36829	FSHB-GSK3A-RHOU	2698	43462	34330	18933	10698
FZD1-FZD2-RHOU	2705	42439	38341	30031	28378	DKK1-PORCN-RHOU	2715	27097	49203	3892	51823
CCND3-PORCN-RHOU	2736	51197	20602	16051	28331	GSK3B-RHOU-WNT2	2741	33291	48297	6678	23276
FRZB-PORCN-RHOU	2754	2260	14728	21581	34283	FBXW2-RHOU-SENP2	2810	46432	53903	6485	38309
CTNNBIP1-LEF1-RHOU	2851	53935	15418	28550	56338	CSNK1D-FOXN1-RHOU	2891	4517	7734	29359	18640
CTNNBIP1-FRAT1-RHOU	2910	38029	54971	19935	51723	CTBP2-LRP6-RHOU	3030	56710	12170	56649	31539
FBXW11-FGF4-RHOU	3056	50852	55649	31582	13375	FZD5-LRP6-RHOU	3179	36603	10552	23856	22468
CSNK1G1-GSK3B-RHOU	3190	3405	4726	1433	8406	CTNNBIP1-NKD1-RHOU	3194	27112	11412	35287	15481
BTRC-RHOU-TCF7	3204	41293	40925	4745	45576	AXIN1-FZD2-RHOU	3216	31244	38793	27939	11583
CSNK1G1-DIXDC1-RHOU	3247	13611	55894	17887	29476	CSNK1G1-GSK3A-RHOU	3261	12094	4223	43874	25027
DAAM1-JUN-RHOU	3292	50276	41716	52344	40041	FBXW2-RHOU-SFRP4	3317	44736	54923	11444	31855
CTNNBIP1-NLK-RHOU	3322	501	7291	43407	48933	DIXDC1-JUN-RHOU	3325	2215	36161	42538	1991
BTRC-RHOU-FBXW4	3368	55758	50831	743	47356	CTNNBIP1-PORCN-RHOU	3494	46942	8439	8140	23879
FRZB-NKD1-RHOU	3499	2171	5014	35449	27330	FZD1-GSK3A-RHOU	3616	49363	7865	50000	3642
LRP6-RHOU-WNT2	3651	45818	48676	55006	6351	APC-GSK3A-RHOU	3658	24361	6768	42843	11981
DAAM1-FGF4-RHOU	3669	42155	55076	53871	14927	DIXDC1-GSK3A-RHOU	3688	25193	1738	30131	17732

Table 1: Rankings of RHOU-X-X. A list of approximately first 125 combinations with rankings below 10,000 out of 57,155. SA - HSIC; Kernel - linear

RANKING @ t_i USING HSIC - RBF

3rd order comb.	t_1	t_3	t_6	t_{12}	t_{24}	3rd order comb.	t_1	t_3	t_6	t_{12}	t_{24}
FBXW11-LRP6-RHOU	1176	49283	31761	47601	19209	CCND1-FGF4-RHOU	22773	55407	6866	49316	21295
FZD5-JUN-RHOU	21830	6512	2365	50002	36411	CTNNBIP1-JUN-RHOU	47402	8172	34623	51918	36687
CSNK1D-FGF4-RHOU	3836	6390	11977	25185	40250	DAAM1-LRP6-RHOU	5359	47314	26024	52506	4274
CCND2-LRP6-RHOU	8558	8419	29663	54868	11672	DKK1-JUN-RHOU	4232	243	27543	37520	46403
AXIN1-DVL1-RHOU	1499	2414	34585	56937	39976	APC-PITX2-RHOU	4699	39874	24475	1820	49384
DKK1-LRP6-RHOU	1841	6184	26589	8672	17929	FZD8-LRP6-RHOU	154	40298	40911	29348	20618
CTNNB1-FOXN1-RHOU	3224	1823	49426	36371	35760	CSNK2A1-MYC-RHOU	476	15315	41082	55249	19587
LRP6-RHOU-WNT2B	29361	19040	30535	21669	39906	AES-AXIN1-RHOU	36330	41468	23777	16934	20955
CXXC4-FGF4-RHOU	2335	25378	25285	54463	41595	FBXW2-RHOU-TCF7	19093	37638	45479	3884	6816
FZD5-CCND2-RHOU	891	46126	19611	43690	49150	CXXC4-DVL2-RHOU	24302	4880	47885	35622	25708
EP300-GSK3B-RHOU	18018	15875	23808	17448	24731	FBXW11-RHOU-SENP2	2811	29045	28581	338	17622
FOSL1-PPP2R1A-RHOU	19254	49825	54278	50968	41085	CXXC4-JUN-RHOU	7270	2533	37155	46380	45935
FBXW11-RHOU-WNT2B	34750	57120	1020	11008	24856	GSK3B-RHOU-TLE2	42470	52597	41214	4926	48118
FSHB-NKD1-RHOU	13620	39126	15440	38560	46234	CCND1-PYGO1-RHOU	5175	54648	29737	46772	20403
GSK3B-LRP6-RHOU	5657	25041	55512	23603	11713	FRAT1-PORCN-RHOU	3460	15685	47256	50127	56007
FZD1-PORCN-RHOU	5740	25675	39166	40916	55717	EP300-RHOU-WNT2	9914	43007	45594	678	52515
GSK3B-RHOU-TCF7	10503	9196	27827	12420	49609	BCL9-FGF4-RHOU	19089	23759	6627	49760	39772
CTBP1-FGF4-RHOU	3119	21207	4713	56266	47439	RHOU-WNT1-WNT4	751	1923	30562	18071	6917
CTBP2-GSK3B-RHOU	5160	52647	1434	32350	32837	PITX2-PORCN-RHOU	11795	30595	6426	8297	54722
GSK3B-RHOU-SENP2	5807	32711	51623	1013	46591	CXXC4-FOXN1-RHOU	4808	4398	51558	29746	40158
BCL9-PORCN-RHOU	13417	51514	7531	35538	56157	CSNK1A1-LRP6-RHOU	38981	447	43109	54028	16718
CSNK1G1-PORCN-RHOU	18456	21677	26712	34529	54262	FZD7-NKD1-RHOU	29725	14170	51670	56277	8735
FZD2-NKD1-RHOU	50910	12898	54711	9572	46912	AXIN1-EP300-RHOU	31074	6355	22262	56648	46901
FOSL1-PORCN-RHOU	1550	39845	36998	42942	56120	CTNNBIP1-FGF4-RHOU	7927	47621	10362	41933	34043
DVL1-FBXW11-RHOU	7780	32624	51645	52838	8534	DVL1-FOXN1-RHOU	5953	29822	43726	42040	18510
APC-FZD6-RHOU	10657	31789	52810	33715	52051	AXIN1-FOXN1-RHOU	7032	3608	53224	33577	37035
FBXW11-RHOU-SFRP4	40361	48871	15908	47467	16716	DKK1-FGF4-RHOU	883	7024	16320	38746	36189
KREMEN1-RHOU-TCF7	13632	56061	32578	2640	36177	CSNK1G1-FOXN1-RHOU	5126	2277	43717	34280	32087
BCL9-JUN-RHOU	27668	3483	7713	42147	31193	FOSL1-FOXN1-RHOU	1989	10797	48585	37026	31459
DKK1-RHOU-SENP2	39831	24828	19766	14059	39096	CXXC4-FZD7-RHOU	8290	22007	17701	54867	39963
DIXDC1-NKD1-RHOU	22817	19488	48855	52946	7640	FBXW11-GSK3B-RHOU	17964	26461	18881	28302	5948
DKK1-DVL2-RHOU	20039	4039	29167	28694	11572	FRZB-GSK3B-RHOU	3735	18295	18902	35166	35991
CSNK1G1-JUN-RHOU	2915	263	26104	42711	34946	FRZB-FZD2-RHOU	2451	4112	11617	7347	43123
DAAM1-FOXN1-RHOU	691	35634	35461	25867	3242	DVL1-RHOU-TCF7	9816	51952	24100	5347	31176
LRP5-PORCN-RHOU	12060	10648	7628	45521	55586	FZD5-NKD1-RHOU	17419	5033	39813	31860	48308
FZD5-GSK3A-RHOU	1006	22102	46550	19816	44067	JUN-PYGO1-RHOU	3507	2626	38630	48273	44356
FZD1-NKD1-RHOU	9230	9523	50610	53836	44264	RHOU-TCF7-TLE2	47781	46765	48664	12439	119
FSHB-FZD2-RHOU	1282	37769	4155	7339	22181	FRZB-GSK3A-RHOU	708	9715	56920	36267	43978
CTBP1-GSK3A-RHOU	734	7381	54552	51414	46017	CXXC4-GSK3A-RHOU	258	9516	53313	35938	49928
DKK1-PYGO1-RHOU	594	8755	12396	40458	43195	DKK1-FOXN1-RHOU	929	29221	40033	12951	25807
CXXC4-FZD2-RHOU	10828	29932	33193	7959	25644	CSNK1G1-NKD1-RHOU	7968	13163	45029	45281	48042
FZD8-GSK3A-RHOU	1931	48518	53137	7904	32484	LRP6-RHOU-WNT5A	25925	53449	10404	18899	8677
DKK1-GSK3A-RHOU	30	5789	37034	29066	45155	CTNNBIP1-FZD2-RHOU	17899	36787	3912	13028	28143
DVL2-FGF4-RHOU	3479	42689	7256	39244	42604	FZD7-PORCN-RHOU	14697	51974	29922	46046	47907
RHOU-SLC9A3R1-WNT4	4823	12213	14742	31515	1411	DAAM1-GSK3A-RHOU	1647	53750	46676	44691	24041
FRZB-LRP6-RHOU	1167	20884	39823	46466	18930	CTNNBIP1-DVL2-RHOU	48236	37356	39527	26133	20575
FBXW11-FOXN1-RHOU	568	22151	44152	28260	12999	RHOU-SFRP1-WNT4	1469	25767	11493	33127	20094
DVL1-LRP6-RHOU	25617	45110	50306	50323	5672	FSHB-GSK3A-RHOU	10474	45128	43164	20106	41469
FZD1-FZD2-RHOU	9932	35208	38235	46112	36204	DKK1-PORCN-RHOU	4479	18174	16557	35661	56399
CCND3-PORCN-RHOU	16278	51731	25103	52905	45857	GSK3B-RHOU-WNT2	474	36759	31507	9286	55538
FRZB-PORCN-RHOU	4743	6437	6011	42453	56180	FBXW2-RHOU-SENP2	26954	54904	2525	1257	9766
CTNNBIP1-LEF1-RHOU	16942	55537	37065	39562	3888	CSNK1D-FOXN1-RHOU	3693	1529	28226	32793	21419
CTNNBIP1-FRAT1-RHOU	31950	44062	33709	39051	21440	CTBP2-LRP6-RHOU	8404	56974	38406	55177	18701
FBXW11-FGF4-RHOU	4214	45954	23791	46231	36711	FZD5-LRP6-RHOU	150	40847	44742	38863	36654
CSNK1G1-GSK3B-RHOU	31434	1254	24292	12304	14579	CTNNBIP1-NKD1-RHOU	49378	32634	47683	47402	32347
BTRC-RHOU-TCF7	35804	52449	25786	16725	22733	AXIN1-FZD2-RHOU	14092	34494	24481	12625	27554
CSNK1G1-DIXDC1-RHOU	49459	11518	16506	41618	49774	CSNK1G1-GSK3A-RHOU	19387	9576	34864	29016	31935
DAAM1-JUN-RHOU	28495	49515	10993	53564	13265	FBXW2-RHOU-SFRP4	26341	43636	30972	48503	7352
CTNNBIP1-NLK-RHOU	20208	392	56468	42238	24576	DIXDC1-JUN-RHOU	18846	1396	22896	56027	5582
BTRC-RHOU-FBXW4	46779	55127	25525	2995	35190	CTNNBIP1-PORCN-RHOU	7681	36925	10950	40241	52218
FRZB-NKD1-RHOU	9972	3765	49769	37400	50607	FZD1-GSK3A-RHOU	2943	41915	53501	47405	45432
LRP6-RHOU-WNT2	15756	54204	37433	20505	38601	APC-GSK3A-RHOU	222	19791	51035	30443	41078
DAAM1-FGF4-RHOU	777	43380	11272	42444	19413	DIXDC1-GSK3A-RHOU	1189	22635	55336	44689	20684

Table 2: Rankings of RHOU-X-X. A list of approximately first 125 combinations with rankings below 10,000 out of 57,155. SA - HSIC; Kernel - rbf

6.3.4. Examining the behaviour of JUN-RHOU-X combinations

Tao et al. [12] also indicate that like CDC42 and RAC, WRCH1/RHOU can activate PAK1 and JNK1 (were JNK is Jun N-terminal kinase) at least with a constitutively

RANKING @ t_i USING SOBOL - 2002

3rd order comb.	t_1	t_3	t_6	t_{12}	t_{24}	3rd order comb.	t_1	t_3	t_6	t_{12}	t_{24}
FBXW11-LRP6-RHOU	46695	45788	40184	56215	2244	CCND1-FGF4-RHOU	19274	22062	8953	2569	30615
FZD5-JUN-RHOU	34450	36279	38790	52137	4153	CTNNBIP1-JUN-RHOU	42622	37205	47514	55158	1108
CSNK1D-FGF4-RHOU	12115	29224	26900	15818	35002	DAAM1-LRP6-RHOU	43102	55185	43832	43518	5699
CCND2-LRP6-RHOU	8726	11720	8629	609	52286	DKK1-JUN-RHOU	36718	6757	39502	48964	14725
AXIN1-DVL1-RHOU	15595	41560	14225	6623	34284	APC-PITX2-RHOU	39285	21142	46215	32608	9407
DKK1-LRP6-RHOU	53154	5487	51727	45299	12610	FZD8-LRP6-RHOU	36339	20093	33980	28591	53
CTNNB1-FOXN1-RHOU	27653	16770	5400	1038	34726	CSNK2A1-MYC-RHOU	45614	13742	53112	49853	2195
LRP6-RHOU-WNT2B	530	17397	4516	7372	41992	AES-AXIN1-RHOU	9009	14323	930	25290	46325
CXXC4-FGF4-RHOU	252	5480	21939	19031	52845	FBXW2-RHOU-TCF7	18114	12097	2546	6703	52251
FZD5-CCND2-RHOU	48069	41241	52645	47922	10763	CXXC4-DVL2-RHOU	13662	45063	23761	15178	35600
EP300-GSK3B-RHOU	28851	26054	39632	40333	5598	FBXW11-RHOU-SENP2	24228	6117	12293	6320	49693
FOSL1-PPP2R1A-RHOU	40904	5712	55181	51304	16176	CXXC4-JUN-RHOU	43332	26892	49071	46614	1986
FBXW11-RHOU-WNT2B	31931	54618	43300	44261	19228	GSK3B-RHOU-TLE2	41612	18157	29803	30684	22990
FSHB-NKD1-RHOU	45675	10162	48275	54199	600	CCND1-PYGO1-RHOU	43775	56261	50329	56095	7953
GSK3B-LRP6-RHOU	54784	1270	32598	40869	12018	FRAT1-PORCN-RHOU	39358	56828	38942	45808	6638
FZD1-PORCN-RHOU	30838	53067	37641	51753	3889	EP300-RHOU-WNT2	37890	54029	40466	39394	16693
GSK3B-RHOU-TCF7	15961	13802	27303	10637	46184	BCL9-FGF4-RHOU	54634	2712	55587	43437	30521
CTBP1-FGF4-RHOU	10837	4785	12497	26445	40146	RHOU-WNT1-WNT4	11749	31988	13883	25949	25686
CTBP2-GSK3B-RHOU	9771	24076	9219	17692	22809	PITX2-PORCN-RHOU	7818	43749	20769	25705	49200
GSK3B-RHOU-SENP2	374	11610	27039	12714	39384	CXXC4-FOXN1-RHOU	49343	54575	54627	33981	50794
BCL9-PORCN-RHOU	10051	9401	3406	8081	39145	CSNK1A1-LRP6-RHOU	35494	34805	31586	40687	45812
CSNK1G1-PORCN-RHOU	13901	19785	413	8056	50675	FZD7-NKD1-RHOU	54154	29225	44144	41641	766
FZD2-NKD1-RHOU	21681	13556	14938	2459	47881	AXIN1-EP300-RHOU	50173	48448	31004	55578	3620
FOSL1-PORCN-RHOU	2397	31652	9884	24315	30810	CTNNBIP1-FGF4-RHOU	19077	47344	22199	16721	53438
DVL1-FBXW11-RHOU	56593	28308	34489	29327	25312	DVL1-FOXN1-RHOU	42063	37467	42621	45694	17029
APC-FZD6-RHOU	23920	21395	6525	24517	48370	AXIN1-FOXN1-RHOU	52036	21355	34481	35981	2174
FBXW11-RHOU-SFRP4	25136	8225	7949	7228	44418	DKK1-FGF4-RHOU	439	39927	11976	27652	36572
KREMEN1-RHOU-TCF7	4926	18206	668	7937	52639	CSNK1G1-FOXN1-RHOU	22734	36628	1885	6243	38820
BCL9-JUN-RHOU	13091	32869	21205	19079	54663	FOSL1-FOXN1-RHOU	6665	9152	26476	10977	50432
DKK1-RHOU-SENP2	4763	9317	17630	16636	41217	CXXC4-FZD7-RHOU	42809	25100	32087	29550	5695
DIXDC1-NKD1-RHOU	51754	30025	31933	35781	48497	FBXW11-GSK3B-RHOU	28107	1456	18708	11770	47332
DKK1-DVL2-RHOU	5213	55312	6790	18965	45099	FRZB-GSK3B-RHOU	13491	11544	22230	21013	8808
CSNK1G1-JUN-RHOU	7215	6024	2384	13893	54958	FRZB-FZD2-RHOU	19452	32718	25715	1254	12888
DAAM1-FOXN1-RHOU	44514	53618	38243	54996	6695	DVL1-RHOU-TCF7	21272	55223	17360	24124	29947
LRP5-PORCN-RHOU	56073	12080	38788	31516	4758	FZD5-NKD1-RHOU	5628	29397	12428	11897	46956
FZD5-GSK3A-RHOU	6535	53623	2704	307	45441	JUN-PYGO1-RHOU	3266	37143	23419	11201	38259
FZD1-NKD1-RHOU	13199	1642	19944	5713	42741	RHOU-TCF7-TLE2	45357	10782	45537	40557	20723
FSHB-FZD2-RHOU	40361	54590	45165	48229	8430	FRZB-GSK3A-RHOU	25555	46266	17850	12100	55746
CTBP1-GSK3A-RHOU	27075	36364	8939	16061	28224	CXXC4-GSK3A-RHOU	14260	7037	2619	8274	28887
DKK1-PYGO1-RHOU	52268	16276	32787	34998	26390	DKK1-FOXN1-RHOU	49123	44756	34782	37853	53029
CXXC4-FZD2-RHOU	6780	8109	9925	14270	49006	CSNK1G1-NKD1-RHOU	40596	5950	54719	48495	7475
FZD8-GSK3A-RHOU	10274	34225	19444	5897	53061	LRP6-RHOU-WNT5A	1674	54809	6843	11880	53337
DKK1-GSK3A-RHOU	1275	7536	5902	1030	34015	CTNNBIP1-FZD2-RHOU	16877	31312	22151	11301	41497
DVL2-FGF4-RHOU	28536	28767	23698	14543	41950	FZD7-PORCN-RHOU	8023	19659	21409	14466	39661
RHOU-SLC9A3R1-WNT4	49419	19737	53037	53877	5239	DAAM1-GSK3A-RHOU	18311	2881	15876	21195	32789
FRZB-LRP6-RHOU	30078	11551	48420	56545	4606	CTNNBIP1-DVL2-RHOU	22275	7767	15425	8203	36213
FBXW11-FOXN1-RHOU	35000	37964	39076	33976	18467	RHOU-SFRP1-WNT4	37727	26597	31742	40770	45055
DVL1-LRP6-RHOU	52843	3892	45120	55684	20473	FSHB-GSK3A-RHOU	45642	17663	41834	54441	6142
FZD1-FZD2-RHOU	16418	1489	22882	7492	35772	DKK1-PORCN-RHOU	40776	11279	47129	32224	15730
CCND3-PORCN-RHOU	32876	21533	30767	30642	23006	GSK3B-RHOU-WNT2	7376	21228	28242	27908	37197
FRZB-PORCN-RHOU	40327	53535	36935	33068	272	FBXW2-RHOU-SENP2	12503	31040	14232	2525	49748
CTNNBIP1-LEF1-RHOU	24534	13961	4127	21590	42789	CSNK1D-FOXN1-RHOU	31392	16873	29114	44729	3759
CTNNBIP1-FRAT1-RHOU	6678	8213	12006	7433	13473	CTBP2-LRP6-RHOU	37102	31634	47828	39027	19516
FBXW11-FGF4-RHOU	28239	5261	17158	361	19387	FZD5-LRP6-RHOU	34758	42678	33438	43096	72
CSNK1G1-GSK3B-RHOU	41655	54814	54139	46702	874	CTNNBIP1-NKD1-RHOU	26327	12981	6330	1732	44989
BTRC-RHOU-TCF7	9610	54915	13320	12426	54672	AXIN1-FZD2-RHOU	6589	37004	2493	5212	52994
CSNK1G1-DIXDC1-RHOU	16058	1799	7780	18030	48278	CSNK1G1-GSK3A-RHOU	47412	26494	53128	51332	7632
DAAM1-JUN-RHOU	46406	52899	43455	40682	2572	FBXW2-RHOU-SFRP4	4437	15110	2992	8623	37947
CTNNBIP1-NLK-RHOU	1785	9526	2470	12825	45699	DIXDC1-JUN-RHOU	19039	49606	12978	15409	37376
BTRC-RHOU-FBXW4	51026	21328	54104	51465	2296	CTNNBIP1-PORCN-RHOU	47984	51387	52410	50264	8895
FRZB-NKD1-RHOU	11852	56820	24882	6154	55783	FZD1-GSK3A-RHOU	26172	12287	21003	7035	51460
LRP6-RHOU-WNT2	56359	7769	44733	36190	15691	APC-GSK3A-RHOU	25637	1950	6140	15680	14897
DAAM1-FGF4-RHOU	16892	8622	19516	10743	19949	DIXDC1-GSK3A-RHOU	54644	35360	40518	47979	9311

Table 3: Rankings of RHOU-X-X. A list of approximately first 125 combinations with rankings below 10,000 out of 57,155. SA - SOBOL; Implementation - 2002

active WRCH1 mutant. Finally, it is known that JNKs (c-Jun N-terminal kinases) bind and phosphorylate c-JUN on Ser-63 and Ser-73 within its transcriptional activation domain. Looking at the tables above, one finds the following combinations for JUN along

RANKING @ t_i USING SOBOL - MARTINEZ

3rd order comb.	t_1	t_3	t_6	t_{12}	t_{24}	3rd order comb.	t_1	t_3	t_6	t_{12}	t_{24}
FBXW11-LRP6-RHOU	55598	41771	19029	47210	39001	CCND1-FGF4-RHOU	639	6615	47856	16676	45710
FZD5-JUN-RHOU	47751	36706	49382	38988	21511	CTNNBIP1-JUN-RHOU	45818	54814	30791	4085	30728
CSNK1D-FGF4-RHOU	25547	26286	25711	24726	4948	DAAM1-LRP6-RHOU	51129	32808	22103	18327	24629
CCND2-LRP6-RHOU	33893	17990	32731	49142	2616	DKK1-JUN-RHOU	6220	29190	3055	27518	42579
AXIN1-DVL1-RHOU	31366	13680	38817	7772	15180	APC-PITX2-RHOU,	51464	18486	41767	41508	48864
DKK1-LRP6-RHOU	13087	8383	53263	50825	30512	FZD8-LRP6-RHOU	13722	4842	37522	44941	18770
CTNNB1-FOXN1-RHOU	45065	1679	7941	10083	48532	CSNK2A1-MYC-RHOU	32164	4573	41446	52430	8154
LRP6-RHOU-WNT2B	32372	56982	3495	6972	3690	AES-AXIN1-RHOU	17031	6211	8944	33472	24486
CXXC4-FGF4-RHOU	38864	34297	38133	42615	15453	FBXW2-RHOU-TCF7	37264	27346	10823	24477	38569
FZD5-CCND2-RHOU	27412	40036	34314	2246	31592	CXXC4-DVL2-RHOU	28497	27073	51383	56020	325
EP300-GSK3B-RHOU	12504	36745	3027	13814	10645	FBXW11-RHOU-SENP2	10035	17928	5691	56523	4295
FOSL1-PPP2R1A-RHOU	7680	16304	49608	19854	56619	CXXC4-JUN-RHOU	56140	21552	3493	38523	19065
FBXW11-RHOU-WNT2B	40385	21306	912	54428	46020	GSK3B-RHOU-TLE2	5842	15382	18633	32810	27658
FSHB-NKD1-RHOU	55892	43953	469	18180	10414	CCND1-PYGO1-RHOU	31251	50423	11785	10303	41620
GSK3B-LRP6-RHOU	24672	19570	21793	50808	44911	FRAT1-PORCN-RHOU	50790	54552	928	2722	13751
FZD1-PORCN-RHOU	35036	50522	2902	5037	14487	EP300-RHOU-WNT2	48627	4883	16855	50530	27850
GSK3B-RHOU-TCF7	907	35108	41891	46351	398	BCL9-FGF4-RHOU	56509	35178	43145	1080	23535
CTBP1-FGF4-RHOU	25848	26857	45347	49075	4901	RHOU-WNT1-WNT4	14697	29770	55730	10370	1690
CTBP2-GSK3B-RHOU	44497	15286	25405	24851	40005	PITX2-PORCN-RHOU	5900	36986	56261	27068	40618
GSK3B-RHOU-SENP2	45704	3857	31271	32707	133	CXXC4-FOXN1-RHOU	50127	17786	8880	44528	47077
BCL9-PORCN-RHOU	3707	54841	9886	55436	7307	CSNK1A1-LRP6-RHOU	27996	7737	1446	23990	14100
CSNK1G1-PORCN-RHOU	156	28220	17621	19760	27625	FZD7-NKD1-RHOU	25956	55118	38253	54733	33656
FZD2-NKD1-RHOU	15517	54256	22224	17209	52842	AXIN1-EP300-RHOU	16298	47098	45733	28449	24757
FOSL1-PORCN-RHOU	21806	54658	25822	20528	2159	CTNNBIP1-FGF4-RHOU	2594	19553	54926	20241	51564
DVL1-FBXW11-RHOU	36501	8013	7500	46761	42967	DVL1-FOXN1-RHOU	43128	21551	13230	2660	56296
APC-FZD6-RHOU	8489	7918	29741	27450	7093	AXIN1-FOXN1-RHOU	43904	28679	25322	4178	22618
FBXW11-RHOU-SFRP4	21445	17565	3357	57056	6612	DKK1-FGF4-RHOU	586	27600	40479	26497	28863
KREMEN1-RHOU-TCF7	39316	49503	18837	54420	4274	CSNK1G1-FOXN1-RHOU	2715	42081	23816	12990	28784
BCL9-JUN-RHOU	6886	57071	51774	45461	27328	FOSL1-FOXN1-RHOU	52304	13418	6434	16650	5417
DKK1-RHOU-SENP2	38215	6359	24936	17294	7056	CXXC4-FZD7-RHOU	35284	52733	39030	45511	8959
DIXDC1-NKD1-RHOU	35301	33949	301	25928	32354	FBXW11-GSK3B-RHOU	32549	19585	34593	10266	16200
DKK1-DVL2-RHOU	19572	20713	39729	55339	38651	FRZB-GSK3B-RHOU	18071	6844	53909	55385	22104
CSNK1G1-JUN-RHOU	22504	10065	14728	48707	5692	FRZB-FZD2-RHOU	6664	49346	15415	4497	3916
DAAM1-FOXN1-RHOU	56554	41205	14641	51868	41158	DVL1-RHOU-TCF7	39	9466	4154	42409	49475
LRP5-PORCN-RHOU	46666	54394	53539	28346	17280	FZD5-NKD1-RHOU	10311	57096	13196	30493	43591
FZD5-GSK3A-RHOU	40564	19292	4726	35228	48374	JUN-PYGO1-RHOU	21520	15769	56551	7365	30064
FZD1-NKD1-RHOU	2141	38390	13080	52508	46011	RHOU-TCF7-TLE2	37457	3367	31818	26357	16519
FSHB-FZD2-RHOU	48503	50300	18409	3241	10967	FRZB-GSK3A-RHOU	112	5465	51897	16533	53667
CTBP1-GSK3A-RHOU	50560	30807	15883	10443	46849	CXXC4-GSK3A-RHOU	30621	42466	3462	4635	54854
DKK1-PYGO1-RHOU	8459	21111	52092	22523	40765	DKK1-FOXN1-RHOU	7658	27282	6296	36778	12394
CXXC4-FZD2-RHOU	14268	2526	46609	14987	7396	CSNK1G1-NKD1-RHOU	13195	46913	28099	45919	38961
FZD8-GSK3A-RHOU	43294	9979	5025	36863	51502	LRP6-RHOU-WNT5A	29804	56580	34206	18715	801
DKK1-GSK3A-RHOU	47848	6431	15572	19106	41871	CTNNBIP1-FZD2-RHOU	7168	31587	30825	7139	41126
DVL2-FGF4-RHOU	555	11271	1922	23783	44769	FZD7-PORCN-RHOU	34299	4479	26086	3214	53704
RHOU-SLC9A3R1-WNT4	52925	62	40274	23424	10431	DAAM1-GSK3A-RHOU	8955	31931	55618	18923	5393
FRZB-LRP6-RHOU	23911	42173	1038	16565	18051	CTNNBIP1-DVL2-RHOU	5328	27407	44675	6263	36562
FBXW11-FOXN1-RHOU	36346	34941	16730	4310	24210	RHOU-SFRP1-WNT4	37322	9276	39470	33165	21606
DVL1-LRP6-RHOU	10135	194	22978	27381	29350	FSHB-GSK3A-RHOU	56076	30316	9478	10986	7651
FZD1-FZD2-RHOU	1927	9257	9325	14926	50151	DKK1-PORCN-RHOU	9460	2243	4774	24967	22408
CCND3-PORCN-RHOU	33632	33579	7112	3024	46287	GSK3B-RHOU-WNT2	3062	2571	17970	23563	3065
FRZB-PORCN-RHOU	50544	41172	19708	55946	14971	FBXW2-RHOU-SENP2	50804	28940	6819	29035	39766
CTNNBIP1-LEF1-RHOU	12836	10044	6091	51536	33000	CSNK1D-FOXN1-RHOU	34252	18639	2074	27005	35628
CTNNBIP1-FRAT1-RHOU	26033	6207	11613	21857	49407	CTBP2-LRP6-RHOU	21957	6402	35992	4308	20996
FBXW11-FGF4-RHOU	5716	1000	41680	8954	2395	FZD5-LRP6-RHOU	39893	45099	25579	5910	26861
CSNK1G1-GSK3B-RHOU	12634	48456	37726	40555	19173	CTNNBIP1-NKD1-RHOU	2582	14223	25655	7361	44666
BTRC-RHOU-TCF7	42008	56452	22053	19872	3883	AXIN1-FZD2-RHOU	23996	50944	11968	16629	7788
CSNK1G1-DIXDC1-RHOU	37350	50752	24433	17248	3736	CSNK1G1-GSK3A-RHOU	38032	48019	31225	42299	34354
DAAM1-JUN-RHOU	56374	40550	28588	40967	9893	FBXW2-RHOU-SFRP4	54720	29917	17451	24829	49241
CTNNBIP1-NLK-RHOU	28147	3381	3458	1969	48016	DIXDC1-JUN-RHOU	1538	5511	26896	46805	23218
BTRC-RHOU-FBXW4	36812	53994	7853	42141	37734	CTNNBIP1-PORCN-RHOU	54212	30162	2176	35988	26785
FRZB-NKD1-RHOU	15559	52309	37443	46074	49516	FZD1-GSK3A-RHOU	1050	37118	30270	49389	41612
LRP6-RHOU-WNT2	48458	48090	13178	11746	18693	APC-GSK3A-RHOU	2436	36957	24969	16181	4304
DAAM1-FGF4-RHOU	3959	3373	24280	47578	25069	DIXDC1-GSK3A-RHOU	23620	13806	30840	17745	26120

Table 4: Rankings of RHOU-X-X. A list of approximately first 125 combinations with rankings below 10,000 out of 57,155. SA - SOBOL; Implementation - martinez

with RHOU, to be prominent at 3rd order level - FZD5-JUN-RHOU, BCL9-JUN-RHOU, CSNK1G1-JUN-RHOU, DAAM1-JUN-RHOU, CTNNBIP1-JUN-RHOU, DKK1-JUN-RHOU, CXXC4-JUN-RHOU, JUN-PYGO1-RHOU and DIXDC1-JUN-RHOU.

All these combinations indicate the existence of a possible synergy when they take a higher rank in the list of combinations.

6.3.5. Examining the behaviour of GSK3-RHOU-X combinations

Coxsackievirus B3 (CVB3) is causes of viral myocarditis and is associated with many other pathological conditions. CVB3 replication relies on host cellular machineries and causes direct damage to host cells. MicroRNAs have been found to regulate viral infections but their roles in CVB3 infection are poorly understood. Ye et al. [19] described a novel mechanism by which miR-126 regulated ERK1/2 and WNT/β-catenin, essential for CVB3 replication. They observed that knockdown of WRCH1 via siRNA, suppressed GSK3β phosphorylation, similar to the function of miR-126. They also found that miR-126 inhibitor successfully repressed cellular miR-126 levels, preserved LRP6 and WRCH1 expression and increased GSK3β phosphorylation, while delaying β-catenin degradation and inhibiting CVB3-induced caspase-3 activation. Looking at the tables above, one finds the following combinations for members of GSK3 along with RHOU, to be prominent at 3rd order level - EP300-GSK3B-RHOU, GSK3B-LRP6-RHOU, GSK3B-RHOU-TCF7, CTBP2-GSK3B-RHOU, GSK3B-RHOU-SENP2, FZD5-GSK3A-RHOU, CTBP1-GSK3A-RHOU, FZD8-GSK3A-RHOU, DKK1-GSK3A-RHOU, CSNK1G1-GSK3B-RHOU, GSK3B-RHOU-TLE2, FBXW11-GSK3B-RHOU, FRZB-GSK3B-RHOU, FRZB-GSK3A-RHOU, CXXC4-GSK3A-RHOU, DAAM1-GSK3A-RHOU, FSHB-GSK3A-RHOU, GSK3B-RHOU-WNT2, CSNK1G1-GSK3A-RHOU, FZD1-GSK3A-RHOU, APC-GSK3A-RHOU, and DIXDC1-GSK3A-RHOU. All these combinations indicate the existence of a possible synergy when they take a higher rank in the list of combinations.

7. Conclusion

This manuscript studies the time behaviour of 3rd order combinations of RHOU in WNT3A stimulated HEK 293 cells. Based on the extablished 2nd order combinations of the RHOU, 3rd order combinations emerge using the machine learning based search engine. These 3rd order combinations might be of interest for further wet lab investigations.

Competing interests

No competing interest is declared.

Author contributions statement

SS conceived and designed the experiments; wrote the code; performed the experiments; analyzed the data; wrote the manuscript.

Availability of code

Code for time series data available at CERN based Zenodo on `https://zenodo.org/records/14637456`.

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Supplementary

The following files (ending with .txt and can be opened in R or in simple text processing program) with these names are made available with this manuscript. For RHOU, (1) **-3-odr-TP-ranking-linear.txt**, (2) **-3-odr-TP-ranking-rbf.txt**, (3) **-3-odr-TP-ranking-2002.txt**, and (4) **-3-odr-TP-ranking-martinez.txt**, contain rankings for 3rd order combinations across each time point for, HSIC (linear kernel), HSIC (rbf kernel), SOBOL (2002 implementation) and SOBOL (martinez implementation), respectively.

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