beta-transducin repeat containing E3 ubiquitin protein ligase or F-box/WD repeat-containing protein 1A (BTRC/FBXW1A) : Time behavioural study of 3rd order combinations in WNT3A stimulated HEK 293 cells

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Abstract

βTrCP (BTRC/FBXW1A) encodes a member of the F-box protein family, characterized by an approximately 40 residue structural motif, the F-box. The F-box proteins form one of the four subunits of ubiquitin protein ligase complex called Skp1-Cul1-F-box (SCF) protein, which often, but not always, recognize substrates in a phosphorylation-dependent manner. BTRC associates with phosphorylated β-catenin destruction motifs, probably functioning in multiple transcriptional programs by regulating the WNT pathways. 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 BTRC related 3rd order combinations in a forest of ${}^{71}C_3$ combinations using four different sensitivity methods; • show the conserved rankings for BTRC-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

[☆] Time behavioural study of 3-odr BTRC comb. in WNT3A stimulated cells

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further wet lab analysis.

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

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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 BTRC 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. beta-transducin repeat containing E3 ubiquitin protein ligase (BTRC)

Linfoot et al. [4] measured cell-cycle, phase-specific cell kill caused by carmustine (BCNU), in 4 cell lines with different sensitivities to the drug. Enriched subpopulations in various phases of the cell cycle were obtained and assayed for cell survival, after the cells were treated with BCNU for 1 hour. They measured levels of activity of guanine O6-alkyltransferase for each line and and found that only BTRC-19, a clone of the 9L line, had significant levels of alkyltransferase activity and exhibited a relatively flat age-response curve to BCNU. Spevak et al. [5] constructed a Xenopus oocyte cDNA library in a Saccharomyces cerevisiae expression vector and used it to isolate genes that could function in yeast cells to suppress the temperature sensitive (corrected) defect of the CDC15 mutation. Two maternally expressed Xenopus cDNAs which fulfilled these conditions were isolated. One of these clones encoded Xenopus N-ras. They observed that overexpression of Xenopus N-ras in S. cerevisiae did not activate the RAS-cyclic AMP (cAMP) pathway, but resulted in decreased levels of intracellular cAMP in both mutant CDC15 and wild-type cells. Their results suggested that a key step of the cell cycle was dependent upon a phosphorylation event catalyzed by cAMP-dependent protein kinase. Additionally, they observed that the second clone, beta TrCP (BTRC), encoded a protein of 518 amino acids that showed significant homology to the beta subunits of G proteins in its C-terminal half. In this region, BTRC was composed of seven beta-transducin repeats.

HIV-1 Vpu interacts with CD4 in the endoplasmic reticulum and triggers CD4 degradation, presumably by proteasomes. Human BTRC identified by interaction with Vpu connected CD4 to this proteolytic machinery, and Margottin et al. [6] via coimmunoprecipitation detected CD4-Vpu-beta TrCP (BTRC) ternary complexes. They observed that BTRC binding to Vpu and its recruitment to membranes required two phosphoserine residues in Vpu essential for CD4 degradation. In BTRC, WD repeats at the C terminus mediated binding to Vpu, and an F-box near the N terminus was involved in

interaction with Skp1p (a targeting factor for ubiquitin-mediated proteolysis). Further, an F-box deletion mutant of BTRC had a dominant-negative effect on Vpu-mediated CD4 degradation. Their data suggested that BTRC and Skp1p represented components of a novel ER-associated protein degradation pathway that mediated CD4 proteolysis.

Members of the WNT/Wingless (Wg) families of secreted proteins control many aspects of growth and patterning during animal development. Wg signal transduction causes increased stability of Armadillo (Arm/β-catenin), a possible co-factor for the transcriptional regulator LEF1/TCF. Jiang and Struhl [7] described a new gene, slimb (for supernumerary limbs), which negatively regulated the Wg pathway along with the Hedgehog (Hh) pathway. They found that loss of function of slimb resulted in a cell-autonomous accumulation of high levels of both Ci and Arm, and the ectopic expression of both Hh- and Wg- responsive genes. They observed that the slimb gene encoded a conserved F-box/WD40-repeat protein related to CDC4P (a protein in budding yeast) that targets cell-cycle regulators for degradation by the ubiquitin/proteasome pathway. They further proposed that slimb protein targeted Ci and Arm/β-catenin for processing or degradation by the ubiquitin/proteasome pathway, and that Wg regulate gene expression at least in part by inducing changes in Ci and Arm, which protect them from slimb-mediated proteolysis.

Based on the above findings of Jiang and Struhl [7], Marikawa and Elinson [8] examined the role of vertebrate homolog betaTrCp (BTRC) in the WNT/β-catenin signaling and dorsal axis formation in Xenopus embryos. They observed that co-injection of BTRC mRNA diminished Xwnt8 mRNA-induced axis formation and expression of Siamois and Xnr3, thus suggesting that BTRC was a negative regulator of the WNT/β-catenin signaling pathway. An mRNA for a BTRC mutant construct (DeltaF), which lacked the F-box domain, was found to induce an ectopic axis and expression of Siamois and Xnr3. Because this activity of DeltaF was suppressed by co-injection of DeltaF TrCP mRNA, DeltaF was thought to act in a dominant negative fashion. Further, the activity of DeltaF was diminished by C-cadherin, GSK3 and AXIN, but not by a dominant negative dishevelled (DSH/DVL) . Their results pointed that BTRC could act as a negative regulator of dorsal axis formation in Xenopus embryos.

While screening a maternal Xenopus expression library for activities that synergize with low levels of injected β-catenin, Lagna et al. [9] isolated a clone encoding the C-terminal end of x-beta TrCP-2, a highly conserved protein belonging to the F-box/WD40 family of ubiquitin-ligase specificity factors. They showed that x-beta TrCP-2 expression reduced dorsal axis formation in Xenopus embryos, while a dominant negative mutant lacking the F-box triggered the opposite effect, thus inducing secondary axes and activation of the expression of WNT responsive genes in ectodermal explants. In light of the existence of BTRC transcripts associated with the vegetal cortex, they proposed that BTRC played an important role in the establishment of the dorsal determinants during cortical rotation in Xenopus.

I present 3rd order combinations of BTRC 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 [10]. 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 [10].

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 BTRC-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 [11]) and Sobol indicies (with 2002 implementation in Saltelli [12] and martinez implementation in Martinez [13] and Baudin et al. [14]).

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

A total of 2415, 3rd order combinations involving BTRC 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 FBXW-BTRC-X combinations

Suzuki et al. [15] had found that overexpression of an F-box protein βTrCP1 (BTRC) and the structurally related βTrCP2 (FBXW11) augmented ubiquitination of phosphorylated IκBα (pIκBα) induced by tumor necrosis factor-α (TNF-α), but the relationship of the two homologous βTrCP proteins remained unknown. Suzuki et al. [15] revealed that deletion mutants of βTrCP-1/2 lacking the F-box domain suppressed

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}
BTRC-GSK3A-FBXW4	399	54756	38346	27654	17743	BTRC-GSK3A-WNT4	427	51118	43719	18596	24127
BTRC-GSK3A-T	433	50464	41129	26608	19994	BTRC-GSK3A-NLK	685	15173	52709	30778	8565
APC-BTRC-WNT2	692	20019	7270	10948	40790	BTRC-FOXN1-FZD8	853	10860	9974	20227	10455
APC-BTRC-DAAM1	945	6005	22642	3587	1629	BTRC-GSK3A-SENP2	1269	31571	50158	24473	28342
BTRC-FOXN1-WNT4	1304	7824	14183	18230	50189	BTRC-PPP2CA-WNT4	1638	35087	32587	22025	51750
BTRC-GSK3A-SLC9A3R1	1828	47182	56469	39060	32535	BTRC-GSK3A-WNT2	1829	55217	56594	37274	29011
APC-BTRC-PPP2CA	1858	9257	27876	20401	45249	BTRC-GSK3A-WNT2B	1933	56634	52638	55432	37508
BTRC-GSK3A-WNT3A	1946	45085	56552	39291	36207	APC-BTRC-FRAT1	1993	4234	3760	19668	20988
BTRC-GSK3A-LRP5	2279	50637	43268	46359	31488	BTRC-GSK3A-GSK3B	2331	27348	55816	23870	24273
BTRC-GSK3A-LEF1	2442	51793	56318	34877	43742	APC-BTRC-TCF7	2478	22925	18323	15264	35151
AXIN1-BTRC-DVL2	2500	11477	16610	53250	27050	APC-BTRC-FBXW2	2597	1236	21415	35908	27898
BTRC-GSK3A-PITX2	2613	49655	52881	6241	50065	APC-BTRC-FBXW11	2628	10595	8869	37962	22016
APC-BTRC-FZD5	2646	23572	8780	23130	34875	BTRC-PPP2CA-SFRP4	2649	45450	55216	41081	48220
APC-BTRC-KREMEN1	2657	22730	7427	25126	52825	BTRC-CCND3-WNT4	2710	41552	34711	18962	45323
BTRC-FOXN1-KREMEN1	2723	18292	12669	9692	56435	APC-BTRC-SENP2	2742	2914	7275	11614	33253
BTRC-GSK3A-PYGO1	2771	52085	53096	35408	47985	BTRC-FOXN1-SENP2	2819	7789	14297	7017	45881
BTRC-GSK3A-KREMEN1	3010	54393	56679	45265	52103	BTRC-WNT1-WNT4	3062	33354	28305	33341	21189
APC-BTRC-FZD1	3143	5688	12315	18344	2535	BTRC-RHOU-TCF7	3204	41293	40925	4745	45576
BTRC-RHOU-FBXW4	3368	55758	50831	743	47356	BTRC-GSK3A-SFRP4	3507	52295	54637	26019	28662
APC-BTRC-SFRP4	3513	8753	5458	12692	40484	BTRC-GSK3A-MYC	3555	32516	35803	30274	45481
BTRC-FOXN1-LRP5	3607	18421	14993	15012	46079	BTRC-WNT2-WNT3A	3667	53111	52839	51977	37208
BTRC-FOXN1-FRAT1	3675	26618	6170	28752	42168	BTRC-FOXN1-WNT5A	3687	8784	26520	30828	49695
BTRC-PPP2CA-T	3795	43011	19216	31904	44253	BTRC-FOXN1-SLC9A3R1	3990	7530	9458	10316	38446
AXIN1-BTRC-WNT2	4161	12843	11806	22233	22893	BTRC-NKD1-WIF1	4240	14000	50136	49384	38469
BTRC-GSK3A-TCF7L1	4309	38628	56805	38238	43590	BTRC-PPP2CA-TCF7L1	4339	35835	48253	44827	55748
BTRC-NKD1-WNT2B	4358	53865	52313	44893	36090	BTRC-CCND3-CXXC4	4501	45355	32569	38665	55920
BTRC-GSK3A-TCF7	4603	42589	43515	30573	20648	AES-BTRC-FZD5	4783	25177	12006	24403	11782
BTRC-GSK3A-WNT5A	4818	34238	56433	32265	47742	BTRC-GSK3A-TLE2	4865	53235	52059	36282	26238
BTRC-JUN-TCF7L1	4943	27837	47631	38349	49359	APC-BTRC-CTBP1	4950	9391	8017	9007	18060
BTRC-CCND2-WNT4	4953	52895	11943	23405	44507	BTRC-PPP2CA-TCF7	4981	40531	13651	43079	34410
AES-BTRC-DVL2	5030	12391	15984	50914	7881	APC-BTRC-FZD7	5075	17371	3368	9728	6810
BTRC-FBXW2-WNT3A	5104	43223	55035	20534	48314	BTRC-FGF4-NLK	5183	27874	56631	56082	16933
BTRC-LRP6-FBXW4	5274	56436	32556	47321	9771	BTRC-GSK3A-PPP2R1A	5299	26077	46257	26921	27177
BTRC-GSK3A-RHOU	5363	54749	48852	32167	37017	BTRC-RHOU-SFRP1	5398	50476	50062	18513	5471
APC-BTRC-CSNK1D	5442	10883	12646	22647	6204	BTRC-CCND3-FZD7	5447	42687	17440	28743	32509
BTRC-NKD1-WNT4	5567	46251	34634	38788	50581	APC-BTRC-WNT5A	5579	11328	15422	26429	56417
BTRC-FOXN1-FZD1	5639	18624	18170	16160	42888	BTRC-GSK3A-LRP6	5724	52749	52308	52281	23121
BTRC-JUN-LRP5	5888	37770	52457	34858	25652	APC-BTRC-WNT4	5911	19764	3320	4428	21935
BTRC-JUN-WNT3A	5912	40253	46236	41851	33745	BTRC-FOXN1-TLE2	6027	19449	11379	20626	38897
BTRC-GSK3A-SFRP1	6145	52112	56467	35900	15415	APC-BTRC-SLC9A3R1	6177	4157	5216	19065	21916
AXIN1-BTRC-FOSL1	6468	1244	4919	15349	38579	BTRC-CCND3-WNT2B	6518	55220	31850	31279	47240
BTRC-NKD1-TCF7L1	6683	39081	40177	56129	51053	BTRC-FOXN1-T	6737	27226	10335	37112	39462
BTRC-PPP2CA-SENP2	6802	36289	41584	41333	55323	APC-BTRC-LRP6	6911	28737	4615	26743	4691
AES-BTRC-DKK1	6960	18725	17933	22340	4842	BTRC-CCND3-FOSL1	6965	37686	28713	22894	44780
BTRC-RHOU-SENP2	6988	41062	38132	3821	50210	BTRC-JUN-WNT4	7215	48917	43726	14501	33278
BTRC-NKD1-SENP2	7336	41049	43342	47787	47593	BTRC-NKD1-SFRP1	7350	47032	49057	53468	51
BTRC-CCND3-EP300	7453	26437	27976	19877	47477	BTRC-PPP2CA-RHOU	7476	44384	28579	43147	51079
BTRC-RHOU-WNT4	7503	46563	30678	9407	48820	AES-BTRC-GSK3A	7511	649	23146	16630	18979
BTRC-GSK3A-WIF1	7516	12519	55092	29987	46937	BTRC-CCND3-DKK1	7699	42444	50739	46484	28100
BTRC-CCND3-TLE1	7893	37196	27722	17214	40980	BTRC-WNT1-WNT3A	7906	35272	32813	57093	19625
BTRC-PPP2CA-WNT5A	7995	31259	42194	31725	49589	AES-BTRC-PPP2CA	8064	4470	33612	23597	26264
BTRC-FOXN1-FSHB	8118	14636	21198	33671	46291	AXIN1-BTRC-SENP2	8132	4244	7993	9640	29213
AES-BTRC-RHOU	8185	12590	5280	21149	27171	BTRC-JUN-PYGO1	8263	45096	50540	41901	54741
BTRC-JUN-SENP2	8343	24938	49553	30325	39253	AES-BTRC-JUN	8360	1344	7172	18393	10377
BTRC-CCND3-FZD2	8456	51566	34885	26667	37022	BTRC-PYGO1-WNT3A	8510	43352	44095	22599	55396
BTRC-CCND3-TCF7	8538	37690	31426	32017	39147	APC-BTRC-PYGO1	8570	7302	8530	26749	53032
AES-BTRC-FOSL1	8607	22428	10525	19327	9562	AXIN1-BTRC-FZD5	8616	22820	8767	21827	26979
BTRC-KREMEN1-WNT4	8701	48514	48223	13062	56268	AXIN1-BTRC-CTBP1	8711	1472	9482	8596	26250
BTRC-LRP6-SLC9A3R1	8815	46754	26428	52824	19745	AXIN1-BTRC-CCND3	8836	8488	11500	25054	29424
BTRC-NKD1-PPP2CA	8947	49640	54091	53329	49700	BTRC-JUN-LEF1	8950	36031	39447	23517	51208
BTRC-NKD1-SFRP4	8970	49955	44941	50705	38441	BTRC-NKD1-TLE2	9050	49909	47985	49811	37145
AXIN1-BTRC-PPP2CA	9123	8661	42188	25227	33335	BTRC-FGF4-WNT4	9178	41675	44825	23499	35812
BTRC-JUN-WNT2	9191	44964	44172	23594	37421	AES-BTRC-WIF1	9239	22292	6516	14211	26584

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

ubiquitination and destruction of pIκBα as well as transcriptional activation of NF-κB. They observed that the ectopically expressed βTrCP-1/2 formed both homodimer

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}
BTRC-GSK3A-FBXW4	8681	52442	37486	14095	29234	BTRC-GSK3A-WNT4	12669	46498	44112	7495	6959
BTRC-GSK3A-T	6821	52908	40648	46892	3130	BTRC-GSK3A-NLK	8965	2718	45185	45189	49869
APC-BTRC-WNT2	9163	27280	43155	9369	48381	BTRC-FOXN1-FZD8	2072	28008	41249	39653	29423
APC-BTRC-DAAM1	3743	41091	17783	36176	30576	BTRC-GSK3A-SENP2	9345	20665	52714	11947	10821
BTRC-FOXN1-WNT4	5283	22892	41659	11506	604	BTRC-PPP2CA-WNT4	13782	33555	41012	5416	5243
BTRC-GSK3A-SLC9A3R1	6194	38474	43825	24758	45073	BTRC-GSK3A-WNT2	14070	55767	42437	51240	22931
APC-BTRC-PPP2CA	4261	9237	29395	1410	54704	BTRC-GSK3A-WNT2B	9987	55898	47839	49215	4144
BTRC-GSK3A-WNT3A	12858	35286	25253	43232	19671	APC-BTRC-FRAT1	3582	6272	14221	27977	50407
BTRC-GSK3A-LRP5	11198	46675	45351	47368	14161	BTRC-GSK3A-GSK3B	4319	5498	47667	46132	13660
BTRC-GSK3A-LEF1	12346	48661	30805	40415	35765	APC-BTRC-TCF7	4705	13188	32095	8871	53791
AXIN1-BTRC-DVL2	29410	5833	29596	43603	19764	APC-BTRC-FBXW2	4038	1370	25809	43009	39049
BTRC-GSK3A-PITX2	2406	44730	43962	54217	10925	APC-BTRC-FBXW11	3206	17373	31138	32376	35753
APC-BTRC-FZD5	5256	25681	42284	19958	53619	BTRC-PPP2CA-SFRP4	23701	47316	54163	28713	3631
APC-BTRC-KREMEN1	8265	27604	37492	52851	54717	BTRC-CCND3-WNT4	19497	48624	44461	7332	36428
BTRC-FOXN1-KREMEN1	2135	23342	46688	49929	2379	APC-BTRC-SENP2	2108	12223	33937	28608	53523
BTRC-GSK3A-PYGO1	16077	51303	28870	23885	34429	BTRC-FOXN1-SENP2	1453	25844	25117	25651	4578
BTRC-GSK3A-KREMEN1	5140	53781	54895	53606	19024	BTRC-WNT1-WNT4	32427	25452	32871	49	5853
APC-BTRC-FZD1	2781	7110	14462	30867	34404	BTRC-RHOU-TCF7	35804	52449	25786	16725	22733
BTRC-RHOU-FBXW4	46779	55127	25525	2995	35190	BTRC-GSK3A-SFRP4	1874	50406	40267	35344	14342
APC-BTRC-SFRP4	9796	14815	39313	5822	33503	BTRC-GSK3A-MYC	14888	25284	36568	2248	10669
BTRC-FOXN1-LRP5	3273	30198	36284	23532	241	BTRC-WNT2-WNT3A	31077	52852	5122	22613	1175
BTRC-FOXN1-FRAT1	4169	16478	20795	41841	6870	BTRC-FOXN1-WNT5A	4629	21553	49380	16835	38205
BTRC-PPP2CA-T	42753	54274	45558	42965	26218	BTRC-FOXN1-SLC9A3R1	987	5633	38486	32921	3284
AXIN1-BTRC-WNT2	19940	34779	51998	35294	51645	BTRC-NKD1-WIF1	40459	4738	29145	25508	9960
BTRC-GSK3A-TCF7L1	5048	35224	47809	21415	23988	BTRC-PPP2CA-TCF7L1	12141	39746	46412	24183	1736
BTRC-NKD1-WNT2B	34862	55446	41413	44594	7779	BTRC-CCND3-CXXC4	28171	55841	38665	6723	12780
BTRC-GSK3A-TCF7	2679	48529	45973	20262	37169	AES-BTRC-FZD5	23300	36671	28933	1416	44834
BTRC-GSK3A-WNT5A	1331	34656	55842	14294	39235	BTRC-GSK3A-TLE2	7469	51695	40008	8656	21337
BTRC-JUN-TCF7L1	24959	20714	12947	17672	1488	APC-BTRC-CTBP1	22415	7521	32949	1572	36489
BTRC-CCND2-WNT4	37807	46204	36799	15309	3667	BTRC-PPP2CA-TCF7	21166	51448	42519	19099	5073
AES-BTRC-DVL2	35795	3432	49151	31669	27699	APC-BTRC-FZD7	1697	7624	29404	31973	44902
BTRC-FBXW2-WNT3A	39006	39760	21198	32505	45697	BTRC-FGF4-NLK	3757	10635	33258	31931	37107
BTRC-LRP6-FBXW4	48827	55300	26952	16212	26925	BTRC-GSK3A-PPP2R1A	8962	30689	29658	9241	43973
BTRC-GSK3A-RHOU	7245	54116	45531	48716	24019	BTRC-RHOU-SFRP1	54868	48925	28543	13342	47372
APC-BTRC-CSNK1D	2236	12178	36083	5029	45903	BTRC-CCND3-FZD7	20781	46050	30736	40377	49178
BTRC-NKD1-WNT4	42581	46731	36629	5396	4774	APC-BTRC-WNT5A	7886	8328	47613	21423	25207
BTRC-FOXN1-FZD1	4693	25266	25295	4116	67	BTRC-GSK3A-LRP6	13109	51610	41523	24298	23690
BTRC-JUN-LRP5	47907	24894	17380	45629	3061	APC-BTRC-WNT4	2113	8450	42844	16	50560
BTRC-JUN-WNT3A	37538	33079	9131	18895	7570	BTRC-FOXN1-TLE2	5417	15877	5904	34425	5486
BTRC-GSK3A-SFRP1	1664	48238	36146	6688	23640	APC-BTRC-SLC9A3R1	37704	1044	33896	21821	50112
AXIN1-BTRC-FOSL1	28079	9720	56293	17570	50701	BTRC-CCND3-WNT2B	24254	53442	21428	39052	47106
BTRC-NKD1-TCF7L1	35223	44544	35152	5915	7887	BTRC-FOXN1-T	2442	42400	34919	11494	2235
BTRC-PPP2CA-SENP2	8723	30185	52002	10650	31985	APC-BTRC-LRP6	49993	33786	30995	2710	30279
AES-BTRC-DKK1	29312	29441	54503	3484	32873	BTRC-CCND3-FOSL1	29635	39122	40121	26979	11246
BTRC-RHOU-SENP2	22351	33999	30105	8174	21370	BTRC-JUN-WNT4	35141	42866	20608	11140	4126
BTRC-NKD1-SENP2	37667	27919	25558	3108	6916	BTRC-NKD1-SFRP1	33217	47597	38470	24028	21787
BTRC-CCND3-EP300	16838	27677	31497	24519	27349	BTRC-PPP2CA-RHOU	21096	51135	35466	34465	21156
BTRC-RHOU-WNT4	17430	46737	39943	11925	27423	AES-BTRC-GSK3A	27622	3812	52880	9059	31196
BTRC-GSK3A-WIF1	8879	9323	38999	19148	14457	BTRC-CCND3-DKK1	16607	38588	8857	2264	31589
BTRC-CCND3-TLE1	34230	30664	34418	21643	23378	BTRC-WNT1-WNT3A	35227	22898	11695	12947	7878
BTRC-PPP2CA-WNT5A	32549	27398	48062	6477	24654	AES-BTRC-PPP2CA	30718	1100	13573	25518	52217
BTRC-FOXN1-FSHB	6670	20222	33570	45284	22	AXIN1-BTRC-SENP2	18487	9499	51352	5160	50244
AES-BTRC-RHOU	31407	10383	39972	41558	56068	BTRC-JUN-PYGO1	37910	44163	1594	29208	42451
BTRC-JUN-SENP2	29065	17777	6958	20628	6213	AES-BTRC-JUN	35333	7469	37870	8738	37322
BTRC-CCND3-FZD2	34361	45890	35141	11003	14788	BTRC-PYGO1-WNT3A	30112	36444	28501	12063	23745
BTRC-CCND3-TCF7	23555	47574	37247	26531	11513	APC-BTRC-PYGO1	51355	9058	6097	3990	45511
AES-BTRC-FOSL1	33302	22693	50032	1890	51445	AXIN1-BTRC-FZD5	13481	19478	52911	4874	45918
BTRC-KREMEN1-WNT4	47643	49959	34801	36178	5914	AXIN1-BTRC-CTBP1	33060	3616	54070	3	28263
BTRC-LRP6-SLC9A3R1	44418	44933	17317	32739	29397	AXIN1-BTRC-CCND3	14456	12461	46193	30484	34903
BTRC-NKD1-PPP2CA	29398	53369	45941	27262	4730	BTRC-JUN-LEF1	30590	27148	1645	43584	10953
BTRC-NKD1-SFRP4	43680	50931	6721	32883	11764	BTRC-NKD1-TLE2	39341	53201	13833	15359	18076
AXIN1-BTRC-PPP2CA	18230	23560	32745	6358	50799	BTRC-FGF4-WNT4	3320	45404	24731	7888	11796
BTRC-JUN-WNT2	41795	46313	18106	41395	6164	AES-BTRC-WIF1	31157	16922	12256	24179	30618

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

and heterodimer complexes without displaying the trimer complex. Intriguingly, they further observed that the βTrCP homodimer, but not the heterodimer, was selectively recruited to pIκBα induced by TNF-α. Their results indicated that not only βTrCP1

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}
BTRC-GSK3A-FBXW4	14575	36852	4261	5253	41097	BTRC-GSK3A-WNT4	51941	54427	52319	56519	12981
BTRC-GSK3A-T	4078	8649	4154	2512	48496	BTRC-GSK3A-NLK	3999	6285	26940	4495	55882
APC-BTRC-WNT2	13113	21227	5897	19830	42798	BTRC-FOXN1-FZD8	30847	6428	51729	52456	19470
APC-BTRC-DAAM1	33244	43178	42663	37614	3256	BTRC-GSK3A-SENP2	49345	53825	50292	50442	26863
BTRC-FOXN1-WNT4	9396	56292	5649	9293	40497	BTRC-PPP2CA-WNT4	44520	22084	53956	47933	11904
BTRC-GSK3A-SLC9A3R1	42522	20325	52892	51907	16309	BTRC-GSK3A-WNT2	45557	45269	50182	52752	6214
APC-BTRC-PPP2CA	22392	28302	364	23734	44217	BTRC-GSK3A-WNT2B	8517	28470	17221	9308	40220
BTRC-GSK3A-WNT3A	5197	2769	4826	638	44313	APC-BTRC-FRAT1	22788	28000	7246	25437	29647
BTRC-GSK3A-LRP5	9530	48791	9921	9340	22565	BTRC-GSK3A-GSK3B	11033	25387	13335	5339	52121
BTRC-GSK3A-LEF1	48318	6688	35792	54260	23862	APC-BTRC-TCF7	26439	18362	1119	22649	44218
AXIN1-BTRC-DVL2	53714	1563	54008	57138	6502	APC-BTRC-FBXW2	16792	24227	18246	23393	54715
BTRC-GSK3A-PITX2	53128	50908	30242	52659	1281	APC-BTRC-FBXW11	37845	26335	45028	33571	5532
APC-BTRC-FZD5	21924	24690	26149	25838	55405	BTRC-PPP2CA-SFRP4	48696	12060	50892	40797	32684
APC-BTRC-KREMEN1	37985	37331	55282	41112	5158	BTRC-CCND3-WNT4	5431	8557	4996	27798	44486
BTRC-FOXN1-KREMEN1	50834	8273	50274	53098	8493	APC-BTRC-SENP2	10405	18731	1106	24128	24326
BTRC-GSK3A-PYGO1	40306	12238	50108	55258	7556	BTRC-FOXN1-SENP2	4996	4590	11199	1069	45646
BTRC-GSK3A-KREMEN1	8824	50530	21375	2882	33323	BTRC-WNT1-WNT4	16066	29448	23493	10082	11232
APC-BTRC-FZD1	45416	29488	50474	29775	1568	BTRC-RHOU-TCF7	9610	54915	13320	12426	54672
BTRC-RHOU-FBXW4	51026	21328	54104	51465	2296	BTRC-GSK3A-SFRP4	53524	5152	50656	56734	1230
APC-BTRC-SFRP4	19987	21768	18081	23838	31465	BTRC-GSK3A-MYC	16430	50738	15638	14825	35023
BTRC-FOXN1-LRP5	34685	15281	53629	52519	21070	BTRC-WNT2-WNT3A	7977	19933	27864	6975	11980
BTRC-FOXN1-FRAT1	7013	46922	9257	20604	31255	BTRC-FOXN1-WNT5A	47737	862	51520	47865	16688
BTRC-PPP2CA-T	1966	5549	26054	18488	55393	BTRC-FOXN1-SLC9A3R1	6270	50153	425	6823	27089
AXIN1-BTRC-WNT2	4072	41628	6490	7464	49712	BTRC-NKD1-WIF1	48449	54705	56221	55648	12202
BTRC-GSK3A-TCF7L1	10975	36821	21029	662	46632	BTRC-PPP2CA-TCF7L1	8390	10968	5708	13240	27007
BTRC-NKD1-WNT2B	9943	44754	6337	1927	44195	BTRC-CCND3-CXXC4	3788	21413	15712	1544	55222
BTRC-GSK3A-TCF7	53068	48434	52996	54645	8674	AES-BTRC-FZD5	10238	10206	8523	19625	24537
BTRC-GSK3A-WNT5A	10037	4468	5019	1007	49446	BTRC-GSK3A-TLE2	8883	56875	3760	3994	39770
BTRC-JUN-TCF7L1	41892	14418	49628	40739	11763	APC-BTRC-CTBP1	25660	21077	14558	22907	41985
BTRC-CCND2-WNT4	15626	49986	13117	26916	22262	BTRC-PPP2CA-TCF7	55174	51646	31139	38706	1770
AES-BTRC-DVL2	39655	35327	33957	29388	3687	APC-BTRC-FZD7	17467	22287	1183	21284	42434
BTRC-FBXW2-WNT3A	28098	7577	9789	9657	50007	BTRC-FGF4-NLK	21988	11228	10319	2639	38875
BTRC-LRP6-FBXW4	52642	2648	46889	43279	9316	BTRC-GSK3A-PPP2R1A	16790	45091	7079	1887	49809
BTRC-GSK3A-RHOU	7742	3339	6860	6712	30482	BTRC-RHOU-SFRP1	49655	56414	50456	35533	420
APC-BTRC-CSNK1D	42702	43953	47146	33780	8438	BTRC-CCND3-FZD7	12423	16992	7082	1140	51456
BTRC-NKD1-WNT4	36353	45989	52854	55033	2326	APC-BTRC-WNT5A	37292	32960	42572	30117	15615
BTRC-FOXN1-FZD1	51102	48881	31757	48770	7729	BTRC-GSK3A-LRP6	47614	8453	47225	47799	34973
BTRC-JUN-LRP5	42390	28195	45460	33041	3105	APC-BTRC-WNT4	19918	24205	14539	27046	41468
BTRC-JUN-WNT3A	44699	13418	50216	50971	18338	BTRC-FOXN1-TLE2	45634	11348	35123	30845	27786
BTRC-GSK3A-SFRP1	3614	52084	6510	422	55945	APC-BTRC-SLC9A3R1	15052	20024	2662	26363	41585
AXIN1-BTRC-FOSL1	6751	53807	7200	3158	56293	BTRC-CCND3-WNT2B	55114	6599	55230	57083	3485
BTRC-NKD1-TCF7L1	6685	28368	19040	10364	42411	BTRC-FOXN1-T	50874	6954	56730	50341	29882
BTRC-PPP2CA-SENP2	47847	4431	34550	43683	16427	APC-BTRC-LRP6	16076	25528	16889	27004	49775
AES-BTRC-DKK1	35898	49563	40922	38076	20573	BTRC-CCND3-FOSL1	1282	55556	13427	23870	56288
BTRC-RHOU-SENP2	7467	757	6685	21602	56731	BTRC-JUN-WNT4	9804	30684	9862	12047	24264
BTRC-NKD1-SENP2	53038	41656	56014	55851	8779	BTRC-NKD1-SFRP1	10440	33880	22944	2931	46082
BTRC-CCND3-EP300	6939	48431	4177	26002	48669	BTRC-PPP2CA-RHOU	9296	52745	22617	13475	40784
BTRC-RHOU-WNT4	7174	31535	348	11435	53270	AES-BTRC-GSK3A	23426	12754	9616	22979	36138
BTRC-GSK3A-WIF1	48255	286	53394	53169	17461	BTRC-CCND3-DKK1	34260	12203	44913	32851	18355
BTRC-CCND3-TLE1	2706	40691	6639	3949	26032	BTRC-WNT1-WNT3A	42784	23471	34199	53125	44329
BTRC-PPP2CA-WNT5A	4362	14774	3261	22306	51429	AES-BTRC-PPP2CA	22181	16883	11192	25928	54871
BTRC-FOXN1-FSHB	6024	8297	25440	8392	49408	AXIN1-BTRC-SENP2	15319	27556	1874	3997	13152
AES-BTRC-RHOU	41852	35113	36818	36607	6041	BTRC-JUN-PYGO1	15383	31289	4126	2363	52568
BTRC-JUN-SENP2	22386	17626	11963	25584	45829	AES-BTRC-JUN	19935	35868	9194	20939	56808
BTRC-CCND3-FZD2	15288	45158	5674	19316	55871	BTRC-PYGO1-WNT3A	48622	13458	43129	36915	836
BTRC-CCND3-TCF7	9292	48829	7427	26854	2542	APC-BTRC-PYGO1	15480	25155	320	26883	40404
AES-BTRC-FOSL1	24055	17493	11882	19765	26740	AXIN1-BTRC-FZD5	18719	13527	7181	25652	24607
BTRC-KREMEN1-WNT4	25791	40598	8150	136	46292	AXIN1-BTRC-CTBP1	10301	33907	6019	14982	35013
BTRC-LRP6-SLC9A3R1	6764	1717	3748	2757	48576	AXIN1-BTRC-CCND3	50499	7332	51110	46975	33
BTRC-NKD1-PPP2CA	52234	9229	56857	56785	893	BTRC-JUN-LEF1	14780	28735	11648	24148	54027
BTRC-NKD1-SFRP4	46680	23946	34147	54225	11107	BTRC-NKD1-TLE2	8682	2505	923	1510	45059
AXIN1-BTRC-PPP2CA	2761	55013	15678	7494	34645	BTRC-FGF4-WNT4	54278	638	41213	42506	9136
BTRC-JUN-WNT2	20612	43056	7930	3498	55489	AES-BTRC-WIF1	23239	11236	6198	14129	57001

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

but also βTrCP2 participated in the ubiquitination-dependent destruction of IκBα by forming SCF$^{\beta TrCP1-\beta TrCP1}$ and SCF$^{\beta TrCP2-\beta TrCP2}$, ubiquitin-ligase complexes.

Looking at the tables above, one finds the following combinations for members of

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}
BTRC-GSK3A-FBXW4	28375	49111	16408	11381	55691	BTRC-GSK3A-WNT4	52752	44376	35197	6343	52255
BTRC-GSK3A-T	7002	38859	9221	19534	40999	BTRC-GSK3A-NLK	8078	21590	4643	7767	37219
APC-BTRC-WNT2	22116	2364	16726	9851	55084	BTRC-FOXN1-FZD8	7699	34343	29979	33524	35444
APC-BTRC-DAAM1	45408	5054	36815	49175	19654	BTRC-GSK3A-SENP2	39429	55220	2991	34867	49837
BTRC-FOXN1-WNT4	46136	7354	21239	41736	23521	BTRC-PPP2CA-WNT4	18544	53592	12219	41275	6887
BTRC-GSK3A-SLC9A3R1	13221	55805	17668	23216	7713	BTRC-GSK3A-WNT2	27001	49909	36775	533	23386
APC-BTRC-PPP2CA	27066	7976	28250	12749	21392	BTRC-GSK3A-WNT2B	3582	20261	29185	8102	27659
BTRC-GSK3A-WNT3A	2575	13354	10047	4920	37543	APC-BTRC-FRAT1	23852	12515	51125	11345	29980
BTRC-GSK3A-LRP5	2709	54807	2422	3048	23404	BTRC-GSK3A-GSK3B	14771	57052	5169	8439	48888
BTRC-GSK3A-LEF1	25986	52372	2128	975	33011	APC-BTRC-TCF7	6747	19012	4503	14570	49087
AXIN1-BTRC-DVL2	32728	50795	47353	26946	46735	APC-BTRC-FBXW2	17961	5806	28596	56835	53138
BTRC-GSK3A-PITX2	47166	48343	22973	655	24369	APC-BTRC-FBXW11	54702	28111	38754	51173	9700
APC-BTRC-FZD5	42227	6423	32578	55391	19082	BTRC-PPP2CA-SFRP4	36763	46459	15211	1307	23454
APC-BTRC-KREMEN1	51514	22765	34066	39078	26946	BTRC-CCND3-WNT4	40306	33905	50218	23253	472
BTRC-FOXN1-KREMEN1	28736	20728	33589	46458	19066	APC-BTRC-SENP2	21318	8579	26257	7717	41847
BTRC-GSK3A-PYGO1	11357	49908	16814	7464	44597	BTRC-FOXN1-SENP2	32482	5031	15405	26720	32412
BTRC-GSK3A-KREMEN1	1807	43605	24019	2592	3552	BTRC-WNT1-WNT4	2996	53539	2844	6885	23127
APC-BTRC-FZD1	52678	43636	29078	40467	15828	BTRC-RHOU-TCF7	42008	56452	22053	19872	3883
BTRC-RHOU-FBXW4	36812	53994	7853	42141	37734	BTRC-GSK3A-SFRP4	44624	42308	34442	9450	27386
APC-BTRC-SFRP4	4458	1606	16344	7932	48764	BTRC-GSK3A-MYC	11177	54100	14322	3527	4139
BTRC-FOXN1-LRP5	8761	41389	31789	41906	9136	BTRC-WNT2-WNT3A	8706	18897	11483	4932	41262
BTRC-FOXN1-FRAT1	9062	46060	27336	34652	4476	BTRC-FOXN1-WNT5A	19210	37398	35493	33336	33577
BTRC-PPP2CA-T	6893	28574	13996	11972	37713	BTRC-FOXN1-SLC9A3R1	26301	37879	9848	36959	11796
AXIN1-BTRC-WNT2	5649	56873	47108	18042	26618	BTRC-NKD1-WIF1	51933	43038	33017	1515	47873
BTRC-GSK3A-TCF7L1	5380	52774	5061	6424	27128	BTRC-PPP2CA-TCF7L1	22895	12454	9404	29802	21543
BTRC-NKD1-WNT2B	4171	29758	15542	13390	2157	BTRC-CCND3-CXXC4	2425	56224	7557	25800	4714
BTRC-GSK3A-TCF7	46275	21990	28421	23242	45496	AES-BTRC-FZD5	22479	9954	5806	11000	34596
BTRC-GSK3A-WNT5A	30595	22112	8606	29405	28103	BTRC-GSK3A-TLE2	42257	22430	3376	3593	4941
BTRC-JUN-TCF7L1	47717	21760	7480	7190	52336	APC-BTRC-CTBP1	1942	2070	44398	54551	5391
BTRC-CCND2-WNT4	6498	14117	19723	9627	6446	BTRC-PPP2CA-TCF7	52778	48135	13879	17697	27941
AES-BTRC-DVL2	44367	46447	5336	36574	32959	APC-BTRC-FZD7	20518	3740	7126	9235	52193
BTRC-FBXW2-WNT3A	4016	55886	29890	8595	26581	BTRC-FGF4-NLK	24568	43813	9326	31050	27602
BTRC-LRP6-FBXW4	48119	43718	3825	9143	55856	BTRC-GSK3A-PPP2R1A	44548	29694	10375	6068	32953
BTRC-GSK3A-RHOU	20915	56190	2199	9415	7167	BTRC-RHOU-SFRP1	49498	52154	17727	340	26973
APC-BTRC-CSNK1D	56835	26515	42490	50974	18375	BTRC-CCND3-FZD7	8017	31351	29314	23732	6859
BTRC-NKD1-WNT4	12670	51219	29636	28389	28990	APC-BTRC-WNT5A	39937	31892	3732	33784	19158
BTRC-FOXN1-FZD1	23041	47510	25151	33469	28699	BTRC-GSK3A-LRP6	35892	49020	14530	166	22125
BTRC-JUN-LRP5	48210	39460	23075	242	29736	APC-BTRC-WNT4	16071	24645	10461	9954	55432
BTRC-JUN-WNT3A	47792	35793	38795	8550	45131	BTRC-FOXN1-TLE2	25262	42137	31758	27354	18352
BTRC-GSK3A-SFRP1	19388	16512	6237	52340	49566	APC-BTRC-SLC9A3R1	12889	2140	11433	4336	52818
AXIN1-BTRC-FOSL1	4084	4852	33974	46211	27572	BTRC-CCND3-WNT2B	53437	1296	43213	22666	25373
BTRC-NKD1-TCF7L1	32634	15810	40240	53149	9213	BTRC-FOXN1-T	34747	15473	36866	42495	56275
BTRC-PPP2CA-SENP2	28760	52500	26592	3452	28675	APC-BTRC-LRP6	22668	8057	23730	7870	52038
AES-BTRC-DKK1	47562	27744	9232	32657	13948	BTRC-CCND3-FOSL1	7512	47193	19995	6213	43386
BTRC-RHOU-SENP2	22542	46262	14924	2454	25827	BTRC-JUN-WNT4	50949	24996	8498	3870	34806
BTRC-NKD1-SENP2	51339	35106	26564	44127	41526	BTRC-NKD1-SFRP1	40288	49190	10462	12784	3751
BTRC-CCND3-EP300	2828	40803	14703	22234	53906	BTRC-PPP2CA-RHOU	3345	39798	38749	26501	3409
BTRC-RHOU-WNT4	49565	18990	5657	13466	44759	AES-BTRC-GSK3A	7297	6412	26427	10496	1665
BTRC-GSK3A-WIF1	29499	17485	30066	565	55089	BTRC-CCND3-DKK1	6662	45619	38557	16725	25978
BTRC-CCND3-TLE1	42440	54117	12904	10609	1210	BTRC-WNT1-WNT3A	24400	54764	17080	1551	26411
BTRC-PPP2CA-WNT5A	9239	52216	35606	37583	45967	AES-BTRC-PPP2CA	21402	8147	3584	13743	49944
BTRC-FOXN1-FSHB	48258	20990	2707	27254	48878	AXIN1-BTRC-SENP2	7548	6280	20837	24709	53918
AES-BTRC-RHOU	43845	38563	6089	35894	30345	BTRC-JUN-PYGO1	45930	51391	6008	9068	5060
BTRC-JUN-SENP2	19827	35052	12265	9648	2983	AES-BTRC-JUN	11806	4569	13449	18660	10852
BTRC-CCND3-FZD2	446	27412	22219	6481	42754	BTRC-PYGO1-WNT3A	39315	42033	18410	4002	41326
BTRC-CCND3-TCF7	331	4675	16252	18398	30636	APC-BTRC-PYGO1	29012	4140	7811	19034	42076
AES-BTRC-FOSL1	6523	5371	19537	41784	46784	AXIN1-BTRC-FZD5	28232	19670	11310	9087	41719
BTRC-KREMEN1-WNT4	42056	48795	9111	6165	36983	AXIN1-BTRC-CTBP1	39593	51830	25669	17346	42125
BTRC-LRP6-SLC9A3R1	39483	56867	15151	7559	23676	AXIN1-BTRC-CCND3	36268	35937	25894	1930	32237
BTRC-NKD1-PPP2CA	52166	34020	35956	41777	24056	BTRC-JUN-LEF1	21070	44181	6258	2410	17038
BTRC-NKD1-SFRP4	33293	49938	3273	19859	38201	BTRC-NKD1-TLE2	6660	19060	16887	9281	43420
AXIN1-BTRC-PPP2CA	15297	40533	40185	15929	8948	BTRC-FGF4-WNT4	31181	54250	31131	44026	26304
BTRC-JUN-WNT2	42836	54091	8621	13924	5042	AES-BTRC-WIF1	11413	5508	16472	14151	43113

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

FBXW family along with BTRC, to be prominent at 3rd order level - BTRC-GSK3A-FBXW4, BTRC-RHOU-FBXW4, BTRC-FBXW2-WNT3A, BTRC-LRP6-FBXW4, APC-BTRC-FBXW2 and **APC-BTRC-FBXW11**. All these combinations indicate the exis-

tence of a possible synergy when they take a higher rank in the list of combinations.

6.3.2. Examining the behaviour of APC / AXIN / GSK3 complex-BTRC-X combinations

The levels of β-catenin are regulated by the adenomatous polyposis coli (APC) tumor suppressor protein, AXIN, and GSK3β. The APC protein binds to β-catenin (a protein known to interact with TCF and LEF transcription factors). Korinek et al. [16] cloned and characterized, the gene encoding hTCF4 that is expressed in colonic epithelium. They observed that hTCF4 transactivated transcription only when associated with β-catenin and the nuclei of APC-/- colon carcinoma cells were found to contain a stable β-catenin-hTCF4 complex that was constitutively active. Further reintroduction of APC removed β-catenin from hTCF4 and evaded the transcriptional transactivation.

Hart et al. [17] found that human AXIN (hAXIN) bound directly to β-catenin, GSK3β, and APC in vitro. Further, they observed that hAXIN dramatically facilitated the phosphorylation of APC and β-catenin by GSK3β, in vitro. Thus they concluded the AXIN acted as a scaffold upon which APC, β-catenin and GSK3β assembled to coordinate the regulation of β-catenin signaling.

Regulation of β-catenin stability is essential for WNT signal transduction during development and tumorigenesis. It is well known that serine-phosphorylation of β-catenin by the AXIN-GSK3β complex targets β-catenin for ubiquitinationdegradation, and mutations at critical phosphoserine residues stabilize β-catenin and cause human cancers. To define how β-catenin phosphorylation results in its degradation, Liu et al. [18] demonstrated that phosphorylated β-catenin was specifically recognized by β-Trcp (BTRC) that associated with SKP1 (an essential component of the ubiquitination apparatus). Further, β-catenin harboring mutations at the critical phosphoserine residues were observed to escape recognition by BTRC, thus providing a molecular explanation for why these mutations caused β-catenin accumulation that led to cancer. Inhibition of endogenous BTRC function by a dominant negative mutant was observed to stabilize β-catenin, activate WNT/β-catenin signaling, and induce axis formation in Xenopus embryos. Thus their results demostrated that β-Trcp (BTRC) played a central role in recruiting phosphorylated β-catenin for degradation and in dorsoventral patterning of the Xenopus embryo.

Looking at the tables above, one finds the following combinations for APC along with BTRC, to be prominent at 3rd order level - APC-BTRC-WNT2, APC-BTRC-DAAM1, APC-BTRC-PPP2CA, APC-BTRC-FZD5, APC-BTRC-KREMEN1, APC-BTRC-FZD1, APC-BTRC-SFRP4, APC-BTRC-CSNK1D, APC-BTRC-FRAT1, APC-BTRC-TCF7, APC-BTRC-FBXW2, APC-BTRC-FBXW11, APC-BTRC-SENP2, APC-BTRC-CTBP1, APC-BTRC-FZD7, APC-BTRC-WNT5A, APC-BTRC-WNT4, APC-BTRC-SLC9A3R1, APC-BTRC-LRP6 and APC-BTRC-PYGO1. All these combinations indicate the existence of a possible synergy when they take a higher rank in the list of combinations.

Looking at the tables above, one finds the following combinations for members of AXIN family along with BTRC, to be prominent at 3rd order level - AXIN1-BTRC-DVL2, AXIN1-BTRC-WNT2, AXIN1-BTRC-FOSL1, AXIN1-BTRC-PPP2CA, AXIN1-BTRC-SENP2, AXIN1-BTRC-FZD5, AXIN1-BTRC-CTBP1 and AXIN1-BTRC-CCND3.

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

Looking at the tables above, one finds the following combinations for members of GSK3 family along with BTRC, to be prominent at 3rd order level - BTRC-GSK3A-FBXW4, BTRC-GSK3A-T, BTRC-GSK3A-SLC9A3R1, BTRC-GSK3A-WNT3A, BTRC-GSK3A-LRP5, BTRC-GSK3A-LEF1, BTRC-GSK3A-PITX2, BTRC-GSK3A-PYGO1, BTRC-GSK3A-KREMEN1, BTRC-GSK3A-TCF7L1, BTRC-GSK3A-TCF7, BTRC-GSK3A-WNT5A, BTRC-GSK3A-RHOU, BTRC-GSK3A-SFRP1, BTRC-GSK3A-WIF1, BTRC-GSK3A-WNT4, BTRC-GSK3A-NLK, BTRC-GSK3A-SENP2, BTRC-GSK3A-WNT2, BTRC-GSK3A-WNT2B, BTRC-GSK3A-GSK3B, BTRC-GSK3A-SFRP4, BTRC-GSK3A-MYC, BTRC-GSK3A-TLE2, BTRC-GSK3A-PPP2R1A, BTRC-GSK3A-LRP6 and AES-BTRC-GSK3A. 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 BTRC in WNT3A stimulated HEK 293 cells. Based on the extablished 2nd order combinations of the BTRC, 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 BTRC, (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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