

WNT inhibitory factor 1 (WIF1) : Time behavioural study of 3rd order combinations in WNT3A stimulated HEK 293 cells

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Abstract

WNT inhibitory factor 1 (WIF1), a secreted protein that binds to WNT proteins and inhibits their activities. 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 WIF1 related 3rd order combinations in a forest of $^{71}C_3$ combinations using four different sensitivity methods; • show the conserved rankings for WIF1-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 indices, WNT3A

[☆]Time behavioural study of 3-odr WIF1 comb. in WNT3A stimulated cells

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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 WIF1 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 (≥ 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. WNT inhibitory factor 1 (WIF1)

Hsieh et al. [4] describe that WIF1 binds to WNT proteins and inhibits their activities. Using *Xenopus* embryos they show that WIF1 overexpression affects somitogenesis (the generation of trunk mesoderm segments), in agreement with its normal expression in paraxial mesoderm and further, in vitro, WIF1 binds to *Drosophila* Wingless and *Xenopus* WNT8 produced by *Drosophila* S2 cells. Their results indicate that WNT proteins interact with structurally diverse extracellular inhibitors to fine-tune the spatial and temporal patterns of WNT activity. Reguart et al. [5] report the identification of the 5' promoter region (1.5 kb) of the human WIF1 gene, functional analysis of which shows that a whole fragment displays high basal promoter activity in different cell lines, while the truncated forms do not, indicating that integrity of the WIF1 promoter region may be important for WIF1 activity. Liepinsh et al. [6] and Malinauskas et al. [7], study the crystal structure of WIF1 and the molecular mechanisms of WNT inhibition via WIF1.

Malinauskas et al. [7] report that WIF1 is present across vertebrate families and consists of an N-terminal secretion signal sequence, the WIF domain (WD, 143 amino acid residues), five EGF-like domains (3133 residues each) and a hydrophilic C terminus. The WIF domain of WIF1 is sufficient to inhibit Wnt signalling⁷, but the function of the five EGF-like domains (EGFs IV) has remained obscure. They used a series of WIF1 constructs in a WNT response assay, to investigate the roles of the domains of WIF1 in inhibition of WNT3A. WNT3A signaling was inhibited in a dose-dependent manner (33500 nM) by WIF1 Δ C. WIF1_{WD} and WIF1_{WD-EGF-I} were also inhibitory, consistent with Hsieh et al. [4] report that the WIF domain in isolation can inhibit WNT signaling, but both these constructs were notably less effective than the molecules that contained the WIF domain and EGFs IV.

In this research work, I present 3rd order combinations of WIF1 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 [8]. 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 [8].

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 procedure 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 specified 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 allotted 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 tuned. 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 changes 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 3rd 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 7C_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** function 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 WIF1-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 vary 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 [9]) and Sobol indices (with 2002 implementation in Saltelli [10] and martinez implementation in Martinez [11] and Baudin et al. [12]).

6.3. Conserved machine learning rankings for tested WIF1-X-X combinations

A total of 2415, 3rd order combinations involving WIF1 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-WIF1-X combinations

Gudjonsson et al. [13] subjected 58 paired biopsies from lesional and uninvolved psoriatic skin and 64 biopsies from normal skin to global gene expression profiling. They found WNT5A transcripts upregulated fivefold in lesional skin, accompanied by increased WNT5A protein levels. In contrast, expression of WIF1 mRNA was downregulated ~ 10 -fold in lesional skin, along with decreased WIF1 immunostaining. These results suggested a shift away from canonical Wnt signaling toward noncanonical pathways driven by interactions between WNT5A and its cognate receptors in psoriasis, accompanied by impaired homeostatic inhibition of Wnt signaling by WIF1 and dickkopf. In early osteochondrosis (OC) cartilage canal chondrocytes compared to controls, Kinsley et al. [14] found significantly decreased WNT11 and increased β -catenin, WNT5B, DKK1, LRP6, WIF1, AXIN1, and SC-PEP gene expression.

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}
CTNNBIP1-WIF1-WNT2B	5	716	18217	51222	29536	TLE1-WIF1-WNT2B	65	13326	20278	36821	11947
CCND1-WIF1-WNT2B	86	32096	35653	38353	29538	CTNNBIP1-WIF1-WNT5A	128	2117	50325	55816	30661
TLE1-WIF1-WNT2	152	12574	27880	35684	24762	TLE1-WIF1-WNT2B	162	206	11704	50552	55877
CTNNBIP1-WIF1-WNT4	380	415	11991	33905	19908	NLK-WIF1-WNT2	431	13840	49796	35119	6813
CCND1-WIF1-WNT4	439	23377	41096	38262	34292	TLE1-WIF1-WNT3A	449	13164	8253	51282	7381
CTNNBIP1-WIF1-WNT3A	603	57	12000	57039	19928	FOSL1-WIF1-WNT2B	727	2770	23114	37617	17980
DVL2-JUN-WIF1	788	37918	18319	11932	34672	NLK-WIF1-WNT2B	877	15574	29869	50706	14922
FBXW11-WIF1-WNT2B	980	2151	19852	53626	35239	CTNNBIP1-WIF1-WNT3	1062	2046	28129	43847	22398
TLE1-WIF1-WNT4	1157	30379	12684	11265	1014	TLE1-WIF1-WNT5A	1188	23457	43932	51409	21021
FZD1-WIF1-WNT2B	1329	23	38111	30027	18511	CCND1-WIF1-WNT2	1543	11595	53523	46295	23076
TLE2-WIF1-WNT3A	1645	195	4085	52703	19685	CXXC4-WIF1-WNT3A	1745	353	12465	51440	34488
DKK1-JUN-WIF1	1820	23431	35933	34937	52606	FBXW11-JUN-WIF1	1901	11991	20361	28288	29058
TCF7L1-WIF1-WNT3A	2015	7414	38348	38153	24687	NKD1-WIF1-WNT3A	2028	3129	43733	48011	30914
CCND1-WIF1-WNT3A	2203	19821	39265	34450	38713	CCND1-WIF1-WNT5A	2206	17920	56231	50097	15244
TCF7L1-WIF1-WNT4	2218	10007	38673	37345	36797	SLC9A3R1-TLE1-WIF1	2489	8000	13237	31531	3962
CSNK1G1-WIF1-WNT2B	2561	1995	24404	34807	53934	CSNK1G1-WIF1-WNT4	2582	628	24563	33380	53761
CTNNB1-FRAT1-WIF1	2625	18691	37238	24416	4230	KREMEN1-WIF1-WNT3A	2762	721	11586	56871	31145
CXXC4-WIF1-WNT2	2794	318	38171	33660	55940	CXXC4-WIF1-WNT2B	2798	244	27556	44507	52219
BCL9-FGF4-WIF1	2803	15565	47332	49359	43280	FBXW2-WIF1-WNT4	2836	2416	50951	26493	30178
CTBP2-JUN-WIF1	2868	17637	22151	3690	5044	GSK3B-JUN-WIF1	2874	57004	14204	24719	995
LRP6-TCF7L1-WIF1	2890	24748	56683	45634	14436	NKD1-WIF1-WNT4	2997	2461	45561	55000	34857
AES-FOXN1-WIF1	3020	27902	1754	12067	18564	DAAM1-WIF1-WNT2B	3043	759	50931	27606	47145
FRAT1-WIF1-WNT4	3157	3802	25184	32202	1523	CSNK1D-FGF4-WIF1	3202	18099	34798	40988	22019
EP300-JUN-WIF1	3213	26743	1259	1082	53260	PYGO1-WIF1-WNT4	3238	2703	55318	32252	32949
FZD8-WIF1-WNT2B	3269	243	27377	19078	48272	FZD7-WIF1-WNT3A	3300	22512	13048	31811	10684
LRP5-WIF1-WNT2	3309	8027	54170	21981	39712	FRAT1-WIF1-WNT3A	3328	15377	26382	55074	7682
FBXW4-WIF1-WNT2B	3407	2879	36747	43254	40889	FZD1-WIF1-WNT4	3425	32	39696	18088	22320
FZD2-NKD1-WIF1	3430	39091	22109	26358	3	FZD1-WIF1-WNT2	3541	469	55529	20037	32149
FRZB-WIF1-WNT2B	3820	12763	27144	38688	14118	DIXDC1-NKD1-WIF1	3909	12873	22358	12920	23739
FBXW2-JUN-WIF1	3982	6153	53985	45810	46608	TCF7L1-WIF1-WNT5A	4007	25712	49240	53231	50932
FZD5-CTNNBIP1-WIF1	4022	52675	7175	11477	29088	FZD5-WIF1-WNT5A	4070	24066	49908	53334	34000
SFRP4-WIF1-WNT3A	4077	1667	15047	43673	14284	LEF1-WIF1-WNT3	4224	9702	36696	52931	22759
BTRC-NKD1-WIF1	4240	14000	50136	49384	38469	LRP6-PYGO1-WIF1	4293	17363	23754	6785	4390
PPP2R1A-TLE1-WIF1	4307	33741	44977	32847	32390	TCF7L1-WIF1-WNT2	4345	20158	51362	40340	48389
NLK-WIF1-WNT4	4406	21046	38575	49983	13952	KREMEN1-NKD1-WIF1	4459	12175	15117	38662	27477
AES-JUN-WIF1	4472	28415	49294	40746	21208	FRZB-WIF1-WNT4	4481	3483	22127	37869	5037
KREMEN1-WIF1-WNT4	4505	6	32895	41843	42797	CSNK1G1-WIF1-WNT3A	4524	651	24433	41940	24409
DKK1-WIF1-WNT4	4532	13090	48760	30623	55584	FRAT1-WIF1-WNT2	4559	16950	52343	25199	28909
FSHB-NKD1-WIF1	4621	8153	24750	5910	428	CCND3-JUN-WIF1	4630	5154	35719	23637	33462
CSNK1G1-JUN-WIF1	4634	18256	33517	5537	47380	FBXW11-FGF4-WIF1	4650	8304	41904	29881	15466
NLK-WIF1-WNT3A	4697	36349	45660	34357	1641	CCND3-NKD1-WIF1	4703	15466	48536	38259	5001
CTNNB1-NLK-WIF1	4707	9899	6192	11981	1923	JUN-WIF1-WNT5A	4730	23210	50885	51206	36664
FOSL1-TLE1-WIF1	4735	9994	4780	43075	2516	CSNK1A1-NLK-WIF1	4795	14710	6213	20851	31609
FZD8-JUN-WIF1	4797	14662	20872	250	14885	FZD7-NKD1-WIF1	4840	43438	23817	39330	17629
AXIN1-FZD2-WIF1	4880	56931	43233	17761	6844	GSK3B-NLK-WIF1	4909	52847	4703	44869	2761
PYGO1-WIF1-WNT5A	4948	8911	30428	21376	24898	SEN2-WIF1-WNT2	4997	18610	47165	55988	55683
PPP2R1A-WIF1-WNT4	5019	37728	49097	41364	45936	CSNK1G1-NLK-WIF1	5054	9876	3023	50217	40547
FBXW11-TLE1-WIF1	5151	21116	13884	43214	2581	CXXC4-NLK-WIF1	5268	10350	6512	1160	15363
SEN2-WIF1-WNT4	5283	14344	21051	43416	45904	BCL9-NLK-WIF1	5369	11251	10475	3676	22747
FBXW11-PYGO1-WIF1	5380	8837	55777	3899	37175	FOSL1-WIF1-WNT2	5404	15643	45844	47156	39486
CTNNB1-T-WIF1	5406	20960	19739	11635	21009	CXXC4-WIF1-WNT4	5410	19	16924	45160	54529
TCF7L1-WIF1-WNT3	5469	28902	52401	25619	27868	DVL1-JUN-WIF1	5674	37144	16904	4823	37727
FRZB-WIF1-WNT2	5709	5485	51972	46418	31405	DKK1-FGF4-WIF1	5714	42039	36616	21416	49824
FZD6-PORCN-WIF1	5717	34355	48055	274	39658	DKK1-WIF1-WNT2	5738	10295	54604	36927	57021
PPP2CA-WIF1-WNT4	5751	1807	53735	25829	47191	DAAM1-JUN-WIF1	5830	12099	53131	3838	44361
FBXW11-FOXN1-WIF1	5840	10050	554	10106	13305	EP300-FGF4-WIF1	5848	31277	41493	38422	55833
PPP2R1A-WIF1-WNT3A	5919	34346	48174	40443	24235	EP300-TLE1-WIF1	5972	16128	8443	34895	42825
LRP6-NKD1-WIF1	5998	19435	35302	21912	36590	DVL2-WIF1-WNT4	6024	27351	28400	29068	46173
FBXW2-PYGO1-WIF1	6116	9988	36252	7568	54910	PPP2R1A-WIF1-WNT2B	6135	34753	36857	36017	55555
CSNK1D-JUN-WIF1	6149	20727	36070	15822	2807	CXXC4-FRAT1-WIF1	6172	16667	52879	168	44602
SFRP1-WIF1-WNT2	6253	42208	44024	23110	51839	FOSL1-WIF1-WNT5A	6286	2387	48506	51990	25900
DAAM1-PYGO1-WIF1	6296	13073	20827	9619	26318	AXIN1-FOXN1-WIF1	6413	26633	589	11640	3200
LRP5-TLE1-WIF1	6476	8699	27950	38670	3372	FSHB-WIF1-WNT4	6540	764	36551	30104	9874

Table 1: Rankings of WIF1-X-X. A list of approximately first 125 combinations with rankings below 10,000 out of 57,155. SA - HSIC; Kernel - linear

Looking at the tables above, one finds the following combinations for WNT3A along with WIF1, to be prominent at 3rd order level - CTNNBIP1-WIF1-WNT3A,

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}
CTNNB1P1-WIF1-WNT2B	38092	3733	8392	37231	50461	TLE1-WIF1-WNT2B	39953	13690	3178	37473	11178
CCND1-WIF1-WNT2B	36761	20542	11221	38644	7317	CTNNB1P1-WIF1-WNT5A	23143	5998	4289	37385	52573
TLE1-WIF1-WNT2	43453	5975	28430	42450	32889	TLE2-WIF1-WNT2B	39741	6829	13257	43555	31091
CTNNB1P1-WIF1-WNT4	28408	15354	16297	16001	53872	NLK-WIF1-WNT2	40307	10226	11647	34273	29809
CCND1-WIF1-WNT4	29821	25068	12712	33994	6600	TLE1-WIF1-WNT3A	50523	24823	48636	56307	10235
CTNNB1P1-WIF1-WNT3A	28101	8157	26911	47091	41515	FOSL1-WIF1-WNT2B	41773	13018	2276	54404	49689
DVL2-JUN-WIF1	6060	40504	15256	30934	48673	NLK-WIF1-WNT2B	20533	18090	8143	25594	25892
FBXW11-WIF1-WNT2B	38776	30910	903	8803	21329	CTNNB1P1-WIF1-WNT3	44721	9765	12738	26593	56684
TLE1-WIF1-WNT4	40180	37225	30537	13365	9063	TLE1-WIF1-WNT5A	40747	33211	3589	52410	46132
FZD1-WIF1-WNT2B	46438	3992	11382	45997	45602	CCND1-WIF1-WNT2	37082	11406	11541	14342	16511
TLE2-WIF1-WNT3A	40072	5491	24360	49488	34844	CXXC4-WIF1-WNT3A	11746	12153	2343	56809	35373
DKK1-JUN-WIF1	14470	30176	11543	7858	42039	FBXW11-JUN-WIF1	13667	34474	37922	11980	35650
TCF7L1-WIF1-WNT3A	2909	25918	43790	53902	35000	NKD1-WIF1-WNT3A	48793	3970	17300	56778	21427
CCND1-WIF1-WNT3A	22745	5551	30083	51087	4790	CCND1-WIF1-WNT5A	37414	8236	3639	32526	9840
TCF7L1-WIF1-WNT4	13995	23515	42960	42008	36503	SLC9A3R1-TLE1-WIF1	31186	3969	23029	44406	46868
CSNK1G1-WIF1-WNT2B	38910	5666	25272	47015	36127	CSNK1G1-WIF1-WNT4	16896	27273	7830	48279	26589
CTNNB1-FRAT1-WIF1	47615	22068	25131	13309	23212	KREMEN1-WIF1-WNT3A	38760	4243	33739	39807	7207
CXXC4-WIF1-WNT2	51580	27076	41572	21992	55327	CXXC4-WIF1-WNT2B	39278	29517	3444	42367	49205
BCL9-FGF4-WIF1	11263	34919	2796	10943	15683	FBXW2-WIF1-WNT4	20440	27918	8101	14377	2991
CTBP2-JUN-WIF1	8251	10577	39311	24911	56633	GSK3B-JUN-WIF1	8724	56927	50822	35202	56101
LRP6-TCF7L1-WIF1	48592	41414	27227	22690	3657	NKD1-WIF1-WNT4	20047	23652	20170	17000	48569
AES-FOXP1-WIF1	1100	27353	38089	31438	12357	DAAM1-WIF1-WNT2B	45979	2760	20066	47459	10609
FRAT1-WIF1-WNT4	11715	10008	40620	23391	16427	CSNK1D-FGF4-WIF1	2570	27850	35517	16644	26870
EP300-JUN-WIF1	11523	21819	31185	9903	31253	PYGO1-WIF1-WNT4	30659	11937	27320	45398	3025
FZD8-WIF1-WNT2B	15532	7478	16375	18773	20549	FZD7-WIF1-WNT3A	3521	32340	43606	53450	22727
LRP5-WIF1-WNT2	27791	17603	14834	26995	40760	FRAT1-WIF1-WNT3A	9771	4923	24250	54851	24596
FBXW4-WIF1-WNT2B	27391	4792	20325	52634	39190	FZD1-WIF1-WNT4	42556	11976	32015	44929	41241
FZD2-NKD1-WIF1	45250	38648	56326	24018	7601	FZD1-WIF1-WNT2	38666	2790	24054	10334	47807
FRZB-WIF1-WNT2B	12032	17926	1240	36454	45817	DIXDC1-NKD1-WIF1	44180	9421	57087	48747	10766
FBXW2-JUN-WIF1	46353	25273	18325	14967	6352	TCF7L1-WIF1-WNT5A	19561	51039	4589	44974	51657
FZD5-CTNNB1P1-WIF1	14151	52702	31378	42083	18375	FZD5-WIF1-WNT5A	8783	34105	21011	56941	51881
SFRP4-WIF1-WNT3A	43798	5901	48178	45486	27167	LEF1-WIF1-WNT3	36462	29053	27342	16772	47153
BTRC-NKD1-WIF1	40459	4738	29145	25508	9960	LRP6-PYGO1-WIF1	40589	27373	31407	1058	42246
PPP2R1A-TLE1-WIF1	18734	28938	477	39566	970	TCF7L1-WIF1-WNT2	18637	24345	47332	21762	47245
NLK-WIF1-WNT4	27894	29051	3153	6969	779	KREMEN1-NKD1-WIF1	11200	6413	56333	14493	14792
AES-JUN-WIF1	10120	24212	19751	10267	44412	FRZB-WIF1-WNT4	7084	20245	38285	19795	37188
KREMEN1-WIF1-WNT4	15263	25410	14854	30920	27336	CSNK1G1-WIF1-WNT3A	39224	8471	4045	39840	22187
DKK1-WIF1-WNT4	31737	32517	6740	28018	30494	FRAT1-WIF1-WNT2	9313	6116	22848	25187	25230
FSHB-NKD1-WIF1	24095	15522	18330	7234	28767	CCND3-JUN-WIF1	13838	6148	31656	30680	20733
CSNK1G1-JUN-WIF1	4351	22508	8866	26497	52893	FBXW11-FGF4-WIF1	5724	14852	19141	15691	14573
NLK-WIF1-WNT3A	15025	31663	634	53620	7624	CCND3-NKD1-WIF1	36891	17882	28735	49474	2688
CTNNB1-NLK-WIF1	36869	4645	37317	37303	40718	JUN-WIF1-WNT5A	17879	25683	7574	50125	47128
FOSL1-TLE1-WIF1	31047	3632	40704	50017	52759	CSNK1A1-NLK-WIF1	11282	8274	56968	38447	50254
FZD8-JUN-WIF1	21742	21278	37642	8182	16093	FZD7-NKD1-WIF1	50953	34059	55345	47706	386
AXIN1-FZD2-WIF1	7321	57022	1548	25380	17152	GSK3B-NLK-WIF1	54258	51883	54492	6800	41476
PYGO1-WIF1-WNT5A	42194	2788	21830	11541	35248	SEN2-WIF1-WNT2	32421	15895	24851	26629	35264
PPP2R1A-WIF1-WNT4	26629	52985	27249	6249	3150	CSNK1G1-NLK-WIF1	47859	22704	35817	25257	48817
FBXW11-TLE1-WIF1	14107	27186	19295	24017	3290	CXXC4-NLK-WIF1	37250	20017	55603	45686	50949
SEN2-PYGO1-WNT4	21321	27321	19436	29566	8098	BCL9-NLK-WIF1	42377	8645	50877	47640	35234
FBXW11-PYGO1-WIF1	2569	13645	24651	1922	27374	FOSL1-WIF1-WNT2	45340	16844	53312	9456	53004
CTNNB1-T-WIF1	7778	26191	45773	17171	20512	CXXC4-WIF1-WNT4	42891	41168	17267	19696	42795
TCF7L1-WIF1-WNT3	34046	41878	23724	48456	52552	DVL1-JUN-WIF1	26119	22108	34781	31590	19241
FRZB-WIF1-WNT2	36167	6734	39162	17055	44934	DKK1-FGF4-WIF1	3492	49887	7655	5221	44988
FZD6-PORCN-WIF1	480	48176	21990	7324	56972	DKK1-WIF1-WNT2	16362	15213	7425	4997	43739
PPP2CA-WIF1-WNT4	17433	21585	17502	30799	13227	DAAM1-JUN-WIF1	26905	6840	9993	8788	19186
FBXW11-FOXP1-WIF1	419	2385	47172	11148	12509	EP300-FGF4-WIF1	1112	29993	9169	25908	27395
PPP2R1A-WIF1-WNT3A	17742	45653	30117	54996	1436	EP300-TLE1-WIF1	44472	4388	23964	55747	29783
LRP6-NKD1-WIF1	30969	16249	36176	44255	21690	DVL2-WIF1-WNT4	52812	33588	6800	19576	11848
FBXW2-PYGO1-WIF1	7912	12588	25947	9153	9982	PPP2R1A-WIF1-WNT2B	32326	45372	32478	24409	8115
CSNK1D-JUN-WIF1	31036	23659	55667	9066	28938	CXXC4-FRAT1-WIF1	6873	35270	49661	19416	37560
SFRP1-WIF1-WNT2	9718	49056	22258	22488	18176	FOSL1-WIF1-WNT5A	32757	9493	2822	49605	45745
DAAM1-PYGO1-WIF1	3980	697	11295	592	10924	AXIN1-FOXP1-WIF1	2440	40095	51373	27135	13422
LRP5-TLE1-WIF1	34546	9992	6916	37075	13965	FSHB-WIF1-WNT4	55011	29325	3556	5035	26747

Table 2: Rankings of WIF1-X-X. A list of approximately first 125 combinations with rankings below 10,000 out of 57,155. SA - HSIC; Kernel - rbf

TLE2-WIF1-WNT3A, TCF7L1-WIF1-WNT3A, CCND1-WIF1-WNT3A, SFRP4-WIF1-WNT3A, NLK-WIF1-WNT3A, PPP2R1A-WIF1-WNT3A, TLE1-WIF1-WNT3A, CXXC4-WIF1-WNT3A, NKD1-WIF1-WNT3A, KREMEN1-WIF1-WNT3A, FZD7-WIF1-WNT3A,

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}
CTNNBIP1-WIF1-WNT2B	32269	9564	44982	43273	5808	TLE1-WIF1-WNT2B	42718	23842	52893	55585	8492
CCND1-WIF1-WNT2B	53452	50296	53198	44416	331	CTNNBIP1-WIF1-WNT5A	37517	8726	47830	33901	17853
TLE1-WIF1-WNT2	14466	33261	4260	1575	48694	TLE2-WIF1-WNT2B	35013	18233	37906	28769	3346
CTNNBIP1-WIF1-WNT4	19686	48738	9309	23329	39168	NLK-WIF1-WNT2	8017	43046	20756	8948	54881
CCND1-WIF1-WNT4	4841	13848	2511	11475	50379	TLE1-WIF1-WNT3A	50693	6752	56468	57096	11947
CTNNBIP1-WIF1-WNT3A	41974	54167	46904	51223	14244	FOSL1-WIF1-WNT2B	25173	2114	24567	1335	44580
DVL2-JUN-WIF1	15451	4813	8548	10559	52944	NLK-WIF1-WNT2B	49149	14168	36378	48222	2298
FBXW11-WIF1-WNT2B	29590	51803	34137	33592	22138	CTNNBIP1-WIF1-WNT3	15189	2978	10191	5960	42924
TLE1-WIF1-WNT4	24611	48165	2574	1753	54527	TLE1-WIF1-WNT5A	32505	9057	54581	55409	2636
FZD1-WIF1-WNT2B	41197	35896	29093	49255	31536	CCND1-WIF1-WNT2	3711	6976	3951	12662	56830
TLE2-WIF1-WNT3A	33475	13433	36234	29978	9382	CXXC4-WIF1-WNT3A	54553	44801	48557	52030	1570
DKK1-JUN-WIF1	6674	53375	11978	4656	40657	FBXW11-JUN-WIF1	28563	6852	14244	2120	54094
TCF7L1-WIF1-WNT3A	50706	10131	45130	48883	856	NKD1-WIF1-WNT3A	37352	21808	33337	55217	6162
CCND1-WIF1-WNT3A	30286	43921	47521	55757	2241	CCND1-WIF1-WNT5A	52311	43612	54645	45673	6949
TCF7L1-WIF1-WNT4	11430	37104	6438	4338	54134	SLC9A3R1-TLE1-WIF1	49432	25242	42714	39347	50342
CSNK1G1-WIF1-WNT2B	13733	56852	4680	24169	53252	CSNK1G1-WIF1-WNT4	53996	12035	47320	37468	7141
CTNNB1-FRAT1-WIF1	20069	13878	9022	3674	41411	KREMEN1-WIF1-WNT3A	36608	31863	33558	56397	51574
CXXC4-WIF1-WNT2	15365	18609	6335	8974	52925	CXXC4-WIF1-WNT2B	41811	38799	50795	48219	4194
BCL9-FGF4-WIF1	6521	47584	531	5681	51087	FBXW2-WIF1-WNT4	1570	2869	13912	18127	41471
CTBP2-JUN-WIF1	26632	28197	10200	26952	24504	GSK3B-JUN-WIF1	2820	53362	17047	20670	42347
LRP6-TCF7L1-WIF1	46690	44500	38326	52913	1244	NKD1-WIF1-WNT4	27161	54046	639	3320	54064
AES-FOXN1-WIF1	14926	30887	11020	27362	56513	DAAM1-WIF1-WNT2B	51968	9011	43968	43585	1804
FRAT1-WIF1-WNT4	23572	3207	18683	9445	56168	CSNK1D-FGF4-WIF1	34683	24427	28639	39649	25219
EP300-JUN-WIF1	42039	52746	44541	44510	6591	PYGO1-WIF1-WNT4	35126	29183	31527	51724	48035
FZD8-WIF1-WNT2B	56387	21556	34997	44557	31717	FZD7-WIF1-WNT3A	462	1898	24495	17586	7553
LRP5-WIF1-WNT2	26293	46706	8593	21321	39427	FRAT1-WIF1-WNT3A	38203	56268	35949	38506	8834
FBXW4-WIF1-WNT2B	40094	8029	54106	49256	2718	FZD1-WIF1-WNT4	4277	11332	28577	4077	43256
FZD2-NKD1-WIF1	48304	2115	44826	40847	8209	FZD1-WIF1-WNT2	16007	21304	28054	7826	25984
FRZB-WIF1-WNT2B	40472	54680	38479	42695	53648	DIXDC1-NKD1-WIF1	2783	6193	1919	15666	44738
FBXW2-JUN-WIF1	13125	27968	19762	1412	50668	TCF7L1-WIF1-WNT5A	45712	19992	50710	52827	3045
FZD5-CTNNBIP1-WIF1	3612	9589	10637	15392	7584	FZD5-WIF1-WNT5A	46018	34226	43880	48456	34278
SFRP4-WIF1-WNT3A	11286	51732	2804	2874	51088	LEF1-WIF1-WNT3	6586	23760	7555	20182	48604
BTRC-NKD1-WIF1	48449	54705	56221	55648	12202	LRP6-PYGO1-WIF1	44508	17042	34865	29362	9716
PPP2R1A-TLE1-WIF1	45090	23493	53498	33748	24410	TCF7L1-WIF1-WNT2	10773	36458	5086	1330	38184
NLK-WIF1-WNT4	8622	27260	19165	3572	56997	KREMEN1-NKD1-WIF1	41242	46530	54613	56937	13792
AES-JUN-WIF1	15247	17980	7285	26738	54994	FRZB-WIF1-WNT4	19577	3033	23872	23782	7044
KREMEN1-WIF1-WNT4	9057	21375	20526	13352	5900	CSNK1G1-WIF1-WNT3A	3166	45406	9828	19653	50304
DKK1-WIF1-WNT4	11135	42111	15998	24015	43039	FRAT1-WIF1-WNT2	15701	80	23990	2644	43140
FSHB-NKD1-WIF1	8501	17679	24446	9809	47619	CCND3-JUN-WIF1	24199	9395	27874	18876	36923
CSNK1G1-JUN-WIF1	50325	56104	52234	45553	1152	FBXW11-FGF4-WIF1	35293	36422	33789	54132	26098
NLK-WIF1-WNT3A	38872	26733	39808	47467	11370	CCND3-NKD1-WIF1	36042	23837	33038	45243	36980
CTNNB1-NLK-WIF1	27962	27173	4445	7873	33696	JUN-WIF1-WNT5A	11573	40788	5006	2485	37662
FOSL1-TLE1-WIF1	23465	12761	28282	7112	46249	CSNK1A1-NLK-WIF1	32459	19376	28696	36711	12615
FZD8-JUN-WIF1	9234	4498	18346	7363	52993	FZD7-NKD1-WIF1	20117	52838	9275	5598	51615
AXIN1-FZD2-WIF1	52063	20768	50568	49372	8948	GSK3B-NLK-WIF1	48655	42899	46413	40079	20496
PYGO1-WIF1-WNT5A	9422	32695	26276	12872	6210	SEN2-WIF1-WNT2	21618	32250	3233	7633	26426
PPP2R1A-WIF1-WNT4	25703	38532	8212	14678	55797	CSNK1G1-NLK-WIF1	11567	4092	1250	25403	55481
FBXW11-TLE1-WIF1	30644	52535	45101	43945	12232	CXXC4-NLK-WIF1	47523	52787	49697	39912	8695
SEN2-WIF1-WNT4	26805	33663	4825	21788	43902	BCL9-NLK-WIF1	8901	32070	18405	1896	47629
FBXW11-PYGO1-WIF1	25061	37723	9816	10733	38354	FOSL1-WIF1-WNT2	31773	4678	36771	49261	17929
CTNNB1-T-WIF1	21499	29614	11202	15378	31898	CXXC4-WIF1-WNT4	9049	33567	635	7452	53825
TCF7L1-WIF1-WNT3	6465	46841	11999	8295	56290	DVL1-JUN-WIF1	133	39629	17853	21639	41516
FRZB-WIF1-WNT2	16627	2487	18655	14490	3420	DKK1-FGF4-WIF1	46668	45941	37440	39369	52245
FZD6-PORCN-WIF1	158	1040	22006	19159	51608	DKK1-WIF1-WNT2	15360	23242	18889	22471	39072
PPP2CA-WIF1-WNT4	15333	32299	8636	19834	50303	DAAM1-JUN-WIF1	17770	14297	8451	520	24284
FBXW11-FOXN1-WIF1	22524	9008	6699	14880	46642	EP300-FGF4-WIF1	21091	2394	11152	17151	51131
PPP2R1A-WIF1-WNT3A	46290	12066	50238	34915	12432	EP300-TLE1-WIF1	24274	39167	8540	1490	50475
LRP6-NKD1-WIF1	2949	22887	2144	9835	53060	DVL2-WIF1-WNT4	21201	2424	24042	11008	43966
FBXW2-PYGO1-WIF1	23813	40455	7171	7535	53909	PPP2R1A-WIF1-WNT2B	40634	31244	45244	35349	8785
CSNK1D-JUN-WIF1	15080	11320	26129	19806	34275	CXXC4-FRAT1-WIF1	48970	56362	46322	38709	39988
SFRP1-WIF1-WNT2	15158	45628	2995	27504	34635	FOSL1-WIF1-WNT5A	22573	9683	23381	1031	51508
DAAM1-PYGO1-WIF1	14907	56052	14575	20848	37739	AXIN1-FOXN1-WIF1	5961	53853	25598	20239	32508
LRP5-TLE1-WIF1	36205	52063	56337	55035	4521	FSHB-WIF1-WNT4	31734	34663	50353	51787	17129

Table 3: Rankings of WIF1-X-X. A list of approximately first 125 combinations with rankings below 10,000 out of 57,155. SA - SOBOL; Implementation - 2002

FRAT1-WIF1-WNT3A and CSNK1G1-WIF1-WNT3A. Looking at the tables above, one finds the following combinations for other members of WNT family, along with WIF1, to be prominent at 3rd order level - CTNNBIP1-WIF1-WNT2B, CCND1-WIF1-

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}
CTNNBIP1-WIF1-WNT2B	11399	24367	25017	23530	21484	TLE1-WIF1-WNT2B	22858	4824	44491	36814	47038
CCND1-WIF1-WNT2B	41667	49854	1695	14733	9964	CTNNBIP1-WIF1-WNT5A	28881	38782	26985	15415	14875
TLE1-WIF1-WNT2	38422	53440	6240	13522	5226	TLE1-WIF1-WNT2B	32197	2739	53514	46829	47415
CTNNBIP1-WIF1-WNT4	2693	38494	35470	17508	51665	NLK-WIF1-WNT2	8814	45087	57018	56594	46968
CCND1-WIF1-WNT4	19090	34087	44892	9619	637	TLE1-WIF1-WNT3A	54823	52	43313	24834	36086
CTNNBIP1-WIF1-WNT3A	53630	42083	719	36277	23420	FOSL1-WIF1-WNT2B	12903	16845	50910	16896	7421
DVL2-JUN-WIF1	37056	9990	21687	38184	36567	NLK-WIF1-WNT2B	27488	14739	50681	54964	10671
FBXW11-WIF1-WNT2B	36968	5122	16875	36018	40185	CTNNBIP1-WIF1-WNT3	18434	14042	23556	23987	43254
TLE1-WIF1-WNT4	13417	53175	11985	6640	5468	TLE1-WIF1-WNT5A	13523	477	24572	24146	33247
FZD1-WIF1-WNT2B	45148	33096	22965	3267	28017	CCND1-WIF1-WNT2	33290	51561	24288	24264	1827
TLE2-WIF1-WNT3A	30043	1060	49500	49207	34907	CXXC4-WIF1-WNT3A	52245	44345	37185	10089	25395
DKK1-JUN-WIF1	36399	2586	32258	23564	50068	FBXW11-JUN-WIF1	3422	55009	23844	21787	1674
TCF7L1-WIF1-WNT3A	25144	47879	2878	6546	54575	NKD1-WIF1-WNT3A	37743	54707	30419	4236	24113
CCND1-WIF1-WNT3A	9447	44577	2811	36770	20781	CCND1-WIF1-WNT5A	44698	46968	24492	10707	12993
TCF7L1-WIF1-WNT4	16671	56895	12326	3087	5863	SLC9A3R1-TLE1-WIF1	47566	28683	41491	4402	20065
CSNK1G1-WIF1-WNT2B	2279	53121	35371	56846	2011	CSNK1G1-WIF1-WNT4	56789	43140	52845	54585	19132
CTNNB1-FRAT1-WIF1	53443	45241	16624	35157	4135	KREMEN1-WIF1-WNT3A	6067	35361	30834	25461	23532
CXXC4-WIF1-WNT2	4105	33661	27901	37024	52946	CXXC4-WIF1-WNT2B	15613	42383	30854	40430	36422
BCL9-FGF4-WIF1	34771	21431	7326	10911	50551	FBXW2-WIF1-WNT4	29533	55498	12217	29219	42911
CTBP2-JUN-WIF1	42458	15347	28164	12030	5215	GSK3B-JUN-WIF1	20269	4104	20323	54326	13962
LRP6-TCF7L1-WIF1	9668	56487	4795	1025	31970	NKD1-WIF1-WNT4	29254	43808	28256	8965	5464
AES-FOXN1-WIF1	32037	29367	4179	8858	6115	DAAM1-WIF1-WNT2B	11234	9732	7813	35044	39060
FRAT1-WIF1-WNT4	30251	45228	4311	5672	47777	CSNK1D-FGF4-WIF1	43363	48941	1472	39959	56626
EP300-JUN-WIF1	41696	318	254	3586	37500	PYGO1-WIF1-WNT4	26212	511	30152	3118	21443
FZD8-WIF1-WNT2B	53936	26449	85	10354	36808	FZD7-WIF1-WNT3A	28792	30157	25705	48713	7016
LRP5-WIF1-WNT2	42496	51817	11651	8318	6182	FRAT1-WIF1-WNT3A	41904	51607	5191	8446	11262
FBXW4-WIF1-WNT2B	50696	1449	53701	55522	19561	FZD1-WIF1-WNT4	5594	10548	13709	51263	7368
FZD2-NKD1-WIF1	49375	42309	3775	2699	9393	FZD1-WIF1-WNT2	2368	8118	25811	23710	6852
FRZB-WIF1-WNT2B	15693	14332	2861	28763	27414	DIXDC1-NKD1-WIF1	25261	12692	13277	18270	29788
FBXW2-JUN-WIF1	38459	7510	55611	27017	45332	TCF7L1-WIF1-WNT5A	47869	53134	38896	13270	55762
FZD5-CTNNBIP1-WIF1	16780	18324	14851	12015	5083	FZD5-WIF1-WNT5A	32810	14319	40457	910	16551
SFRP4-WIF1-WNT3A	44582	27162	7862	12468	48512	LEF1-WIF1-WNT3	19929	19003	53805	55548	7589
BTRC-NKD1-WIF1	51933	43038	33017	1515	47873	LRP6-PYGO1-WIF1	27947	43528	52323	46450	25041
PPP2R1A-TLE1-WIF1	41681	6027	12229	35646	49978	TCF7L1-WIF1-WNT2	15131	18161	17131	5323	8597
NLK-WIF1-WNT4	1987	42325	56959	36622	45848	KREMEN1-NKD1-WIF1	7530	48074	39498	38667	8927
AES-JUN-WIF1	31213	14062	3877	24619	5796	FRZB-WIF1-WNT4	1396	24594	18388	52092	7572
KREMEN1-WIF1-WNT4	3031	53892	55038	12626	48465	CSNK1G1-WIF1-WNT3A	27586	15015	56427	56385	23797
DKK1-WIF1-WNT4	10251	40779	18783	50875	2991	FRAT1-WIF1-WNT2	1696	21214	4854	2010	25832
FSHB-NKD1-WIF1	14017	26546	32668	27446	43796	CCND3-JUN-WIF1	13233	7003	23234	45316	44744
CSNK1G1-JUN-WIF1	42178	37089	30107	26394	28967	FBXW11-FGF4-WIF1	43259	29979	53257	1320	30533
NLK-WIF1-WNT3A	9557	16952	33543	54081	7414	CCND3-NKD1-WIF1	39810	27228	3481	16856	38586
CTNNB1-NLK-WIF1	50485	2227	39887	31399	31013	JUN-WIF1-WNT5A	32685	30228	10807	3557	2870
FOSL1-TLE1-WIF1	23051	21670	15028	26223	26495	CSNK1A1-NLK-WIF1	30129	8991	3923	16574	38964
FZD8-JUN-WIF1	42368	27017	4330	12175	24739	FZD7-NKD1-WIF1	1974	15753	44114	56522	24777
AXIN1-FZD2-WIF1	45117	37402	35068	7303	42377	GSK3B-NLK-WIF1	34339	35642	7661	48067	38058
PYGO1-WIF1-WNT5A	23700	55503	49268	17706	33207	SEN2-WIF1-WNT2	19857	44113	39376	5151	49899
PPP2R1A-WIF1-WNT4	21743	39446	45990	24538	5034	CSNK1G1-NLK-WIF1	1030	52920	26945	19201	23448
FBXW11-TLE1-WIF1	39152	9088	31660	45997	30784	CXXC4-NLK-WIF1	56734	30187	12246	43838	9761
SEN2-WIF1-WNT4	5692	52447	28551	4522	42500	BCL9-NLK-WIF1	2980	22079	8164	47737	1113
FBXW11-PYGO1-WIF1	11667	41784	16432	56976	14544	FOSL1-WIF1-WNT2	37979	15711	49818	29240	55234
CTNNB1-T-WIF1	46182	2228	18095	53931	44591	CXXC4-WIF1-WNT4	2001	20311	7825	52825	47541
TCF7L1-WIF1-WNT3	9801	54254	34821	7083	33754	DVL1-JUN-WIF1	10134	5753	23658	36888	5991
FRZB-WIF1-WNT2	4109	41064	11388	50563	2583	DKK1-FGF4-WIF1	8223	26574	5833	38880	12008
FZD6-PORCN-WIF1	4293	27898	18139	1661	9549	DKK1-WIF1-WNT2	43803	15008	5463	52567	12347
PPP2CA-WIF1-WNT4	33983	42503	40723	2972	2678	DAAM1-JUN-WIF1	6089	33996	21243	29467	54995
FBXW11-FOXN1-WIF1	6714	13156	40224	10084	55777	EP300-FGF4-WIF1	22898	53742	55336	56572	51051
PPP2R1A-WIF1-WNT3A	53684	439	34255	43002	54800	EP300-TLE1-WIF1	35859	50879	32535	11209	9617
LRP6-NKD1-WIF1	1964	56311	30931	3714	149	DVL2-WIF1-WNT4	15823	25440	56333	53782	1274
FBXW2-PYGO1-WIF1	47574	20956	5995	16395	15149	PPP2R1A-WIF1-WNT2B	27820	454	43603	46634	49248
CSNK1D-JUN-WIF1	25882	6915	22330	29491	5103	CXXC4-FRAT1-WIF1	55643	35185	8505	32879	28658
SFRP1-WIF1-WNT2	21242	36645	56881	55285	1587	FOSL1-WIF1-WNT5A	15143	4122	42644	17755	37731
DAAM1-PYGO1-WIF1	10787	16967	53500	32091	1557	AXIN1-FOXN1-WIF1	48998	38604	49387	37043	2187
LRP5-TLE1-WIF1	6420	53920	42057	32197	10298	FSHB-WIF1-WNT4	13365	49004	45468	2035	27872

Table 4: Rankings of WIF1-X-X. A list of approximately first 125 combinations with rankings below 10,000 out of 57,155. SA - SOBOL; Implementation - martinez

WNT2B, TLE1-WIF1-WNT2, CTNNBIP1-WIF1-WNT4, CCND1-WIF1-WNT4, FBXW11-WIF1-WNT2B, TLE1-WIF1-WNT4, FZD1-WIF1-WNT2B, TCF7L1-WIF1-WNT4, CSNK1G1-WIF1-WNT2B, CXXC4-WIF1-WNT2, FRAT1-WIF1-WNT4, FZD8-WIF1-

WNT2B, LRP5-WIF1-WNT2, FBXW4-WIF1-WNT2B, FRZB-WIF1-WNT2B, NLK-WIF1-WNT4, KREMEN1-WIF1-WNT4, DKK1-WIF1-WNT4, PYGO1-WIF1-WNT5A, PPP2R1A-WIF1-WNT4, SENP2-WIF1-WNT4, TCF7L1-WIF1-WNT3, FRZB-WIF1-WNT2, PPP2CA-WIF1-WNT4, SFRP1-WIF1-WNT2, TLE1-WIF1-WNT2B, CTNNBIP1-WIF1-WNT5A, TLE2-WIF1-WNT2B, NLK-WIF1-WNT2, FOSL1-WIF1-WNT2B, NLK-WIF1-WNT2B, CTNNBIP1-WIF1-WNT3, TLE1-WIF1-WNT5A, CCND1-WIF1-WNT2, CCND1-WIF1-WNT5A, CSNK1G1-WIF1-WNT4, CXXC4-WIF1-WNT2B, FBXW2-WIF1-WNT4, NKD1-WIF1-WNT4, DAAM1-WIF1-WNT2B, PYGO1-WIF1-WNT4, FZD1-WIF1-WNT4, FZD1-WIF1-WNT2, TCF7L1-WIF1-WNT5A, FZD5-WIF1-WNT5A, LEF1-WIF1-WNT3, TCF7L1-WIF1-WNT2, FRZB-WIF1-WNT4, FRAT1-WIF1-WNT2, JUN-WIF1-WNT5A, SENP2-WIF1-WNT2, FOSL1-WIF1-WNT2, CXXC4-WIF1-WNT4, DKK1-WIF1-WNT2, DVL2-WIF1-WNT4, PPP2R1A-WIF1-WNT2B, FOSL1-WIF1-WNT5A and FSHB-WIF1-WNT4. 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 DKK / LRP -WIF1-X combinations

In early osteochondrosis (OC) cartilage canal chondrocytes compared to controls, Kinsley et al. [14] found significantly decreased WNT11 and increased β -catenin, WNT5B, DKK1, LRP6, WIF1, AXIN1, and SC-PEP gene expression. Looking at the tables above, one finds the following combinations for DKK1 along with WIF1, to be prominent at 3rd order level - DKK1-JUN-WIF1, DKK1-WIF1-WNT4, DKK1-FGF4-WIF1 and DKK1-WIF1-WNT2. Looking at the tables above, one finds the following combinations for members of LRP family along with WIF1, to be prominent at 3rd order level - LRP6-TCF7L1-WIF1, LRP5-WIF1-WNT2, LRP6-NKD1-WIF1, LRP5-TLE1-WIF1 and LRP6-PYGO1-WIF1. 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 FOX-WIF1-X combinations

FOXF1 decreased expression of WIF1 through direct transcriptional repression. Furthermore, using a global FOXF1 knockout mouse line FOXF1^{-/-}) Ustiyani et al. [15] demonstrated that FOXF1 deficiency disrupts the formation of the lung bud in foregut tissue explants. To identify FOXF1 target genes in lung mesenchyme, they used DERMOL-FOXF1^{-/-} and control FOXF1^{f1/f1} embryos to micro-dissect mesenchyme from E10.5 distal lung tips and perform RNAseq analysis. Expression of genes regulating canonical Wnt signaling pathway such as Wnt ligands WNT-2/5A/11, and known regulators like SNAI2, SIAH2, ZFP 703, BICCL, NOG, RSPOL, IS11, TRPM4 and NKD2 were significantly decreased in DERMOL-FOXF1^{-/-} lung mesenchyme. In contrast, expression of WIF1 was significantly increased in DERMOL-FOXF1^{-/-} lungs. Looking at the tables above, one finds the following combinations for members of FOX family along with WIF1, to be prominent at 3rd order level - AES-FOXN1-WIF1, FBXW11-FOXN1-WIF1 and AXIN1-FOXN1-WIF1. All these combinations indicate the existence of a possible synergy when they take a higher rank in the list of combinations.

6.3.4. Examining the behaviour of NKD1-WIF1-X combinations

Han et al. [16] showed that nobiletin (a natural compound with anticancer activity) significantly inhibited non-small-cell lung cancer (NSCLC) cell colony formation and sphere formation and induced apoptosis, by upregulating negative regulators of WNT/catenin signaling, like NKD1, AXIN2, and WIF1, while inhibiting the expression of WNT6, β -catenin and its downstream genes, including c-MYC, c-JUN, and CCND1. Furthermore, they identified that GN inhibits miR-15-5p expression in NSCLC cells and that NKD1, AXIN2, and WIF1 are the target genes of miR-15-5p. NKD1 and WIF1 might not be working synergistically, but their joint action might be needed in NSCLC. Looking at the tables above, one finds the following combinations for NKD1 along with WIF1, to be prominent at 3rd order level - FZD2-NKD1-WIF1, BTRC-NKD1-WIF1, FSHB-NKD1-WIF1, LRP6-NKD1-WIF1, NKD1-WIF1-WNT3A, NKD1-WIF1-WNT4, DIXDC1-NKD1-WIF1, KREMEN1-NKD1-WIF1, CCND3-NKD1-WIF1 and FZD7-NKD1-WIF1. 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 JUN-WIF1-X combinations

Han et al. [16] showed that nobiletin (a natural compound with anticancer activity) significantly inhibited non-small-cell lung cancer (NSCLC) cell colony formation and sphere formation and induced apoptosis, by upregulating negative regulators of WNT/catenin signaling, like NKD1, AXIN2, and WIF1, while inhibiting the expression of WNT6, β -catenin and its downstream genes, including c-MYC, c-JUN, and CCND1. Furthermore, they identified that GN inhibits miR-15-5p expression in NSCLC cells and that NKD1, AXIN2, and WIF1 are the target genes of miR-15-5p.

Systemic sclerosis (SSc) is an autoimmune disease characterized by extensive visceral organ and skin fibrosis. SSc patients have increased production of autoreactive antibodies and WNT signaling activity and Svegliati et al. [17] found that expression of WIF1 was decreased in fibroblasts from SSc patient biopsies. WIF1 deficiency in SSc patient cells correlated with increased abundance of β -catenin and the production of collagen. Further, the DNA damage checkpoint kinase ataxia telangiectasia mutated (ATM) mediated WIF1 silencing through the phosphorylation of the transcription factor c-JUN, which in turn activated the expression of the gene encoding activating transcription factor 3 (ATF3). ATF3 and c-JUN were recruited together with histone deacetylase 3 (HDAC3) to the WIF1 promoter and inhibited WIF1 expression.

Looking at the tables above, one finds the following combinations for JUN along with WIF1, to be prominent at 3rd order level - DVL2-JUN-WIF1, DKK1-JUN-WIF1, CTBP2-JUN-WIF1, EP300-JUN-WIF1, FBXW2-JUN-WIF1, AES-JUN-WIF1, CSNK1G1-JUN-WIF1, FZD8-JUN-WIF1, CSNK1D-JUN-WIF1, FBXW11-JUN-WIF1, GSK3B-JUN-WIF1, CCND3-JUN-WIF1, JUN-WIF1-WNT5A, DVL1-JUN-WIF1 and DAAM1-JUN-WIF1. 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 WIF1 in WNT3A stimulated HEK 293 cells. Based on the established 2nd order combinations of the WIF1, 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 WIF1, (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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