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Porcupine O-Acyltransferase (PORCN) : Time Behavioural Study of 3rd Order Combinations in WNT3A Stimulated HEK 293 Cells

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Abstract: PORCN belongs to the family of membrane bound O-acyl transferase (MBOAT). MBOAT members contain multiple transmembrane domains and carry two conserved residues, a conserved histidine (His) embedded in a hydrophobic stretch of residues and an asparagine (Asn) or histidine within a more hydrophilic region some 30-50 residues upstream. It can add palmitoleate groups to WNT proteins that is necessary for WNT ligand secretion. 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 PORCN related 3rd order combinations in a forest of ${}^{71}C_3$ combinations using four different sensitivity methods; • show the conserved rankings for PORCN-X-X combinations, which point to existence of biological synergy of some of these combinations across the different sensitivity methods; and • study the behaviour of some of these combinations related to WNT3A response genes that are ranked by the machine learning search engine (Sinha [2]) in time. Patterns of combinations emerge, some of which have been tested in wet lab, while others require further wet lab analysis.

Keywords: sensitivity analysis; support vector ranking; Hilbert Schmidt Independence Criterion indices (HSIC) and Sobol indicies; WNT3A

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 PORCN related combinations using individual gene expressions measured in time, in WNT3A stimulated HEK 293 cells.

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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. Porcupine (PORCN)

The Drosophila segment polarity gene product Porcupine (Porc) was first identified as being necessary for processing Wingless (Wg), a Drosophila Wnt (Wnt) family member. Tanaka et al. [4] identified Mouse (Mporc) and Xenopus (Xporc) homologs of porc and found that they encode endoplasmic reticulum (ER) proteins with multiple transmembrane domains. Further, Mporc mRNA was differentially expressed during embryogenesis and in various adult tissues, demonstrating that the alternative splicing is regulated to synthesize the specific types of Mporc. In transfected mammalian cells, they found all types of Mporc affected the processing of mouse WNT1, WNT3A, WNT4, WNT6, and WNT7B but not WNT5A. Lastly, they also found that all types of Mporc co-immunoprecipitated with various WNT proteins. Their results suggested that Mporc may function as a chaperone-like molecule for WNT.

Caricasole et al. [5] report that the human Porcupine locus (MG61/PORC) spans 15 exons over approximately 12 kb of genomic sequence on Xp11.23. Like its mouse and Xenopus homologues, MG61/PORC encodes four protein isoforms (A–D) generated through alternative splicing and expressed in a tissue-specific fashion. They present evidence indicating that MG61/PORC can influence the activity of a human WNT7A expression construct in a T-cell factor-responsive reporter assay.

Liu et al. [6] indicate that post-translational modification of WNTs includes lipid modification and glycosylation. The former is performed by PORCN. PORCN is a membrane-bound O-acyltransferase located in the endoplasmic reticulum and can add palmitoleate groups to WNT proteins that is necessary for WNT ligand secretion, and it is a member of the membrane-bound O-acyltransferases

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(MBOATs). Lipid modification is necessary for Wnt activity, and the opposite is true for glycosylation as observed by Willert et al. [7]. Liu et al. [8] developed a screen for small molecules that blocked WNT secretion and discovered LGK974, a potent and specific small-molecule PORCN inhibitor. They show that LGK974 inhibits WNT signaling, including reduction of the WNT-dependent LRP6 phosphorylation and the expression of WNT target genes, like as AXIN2. The inhibitor is effective in multiple tumor models at well-tolerated doses. Together, their findings provide a strategy and and a tool for targeting WNT-driven cancers through the inhibition of PORCN. Further down the line, Madan et al. [9] developed a novel potent, orally available PORCN inhibitor, ETC-1922159 that blocked the secretion and activity of all WNTs. ETC-1922159 is remarkably effective in treating RSPO-translocation bearing colorectal cancer (CRC) patient-derived xenografts. This is the first example of effective targeted therapy for this subset of CRC. By this demonstration they show that inhibition of WNT signaling by PORCN inhibition holds promise as differentiation therapy in genetically defined human cancers.

Sutton [10], conver a range of PORCN related developmental disorders. Till now, most research work has focused on the role of PORCN along with WNTs. In this research work, I present 3rd order combinations of PORCN with other genes, apart from the WNTs, 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 [11]. 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 [11].

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

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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

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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 PORCN-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 [12]) and Sobol indicies (with 2002 implementation in Saltelli [13] and martinez implementation in Martinez [14] and Baudin et al. [15]).

6.3. Conserved Machine Learning Rankings for Tested PORCN-X-X Combinations

A total of 2415, 3rd order combinations involving PORCN 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.

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

	RANKING @ t_i USING HSIC - 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}
CXXC4-PORCN-SENP2	10	33446	17033	17387	32253	CXXC4-PORCN-WNT4	49	6186	19448	20672	51388
CXXC4-PORCN-WNT2B	60	17097	46765	26682	32940	CXXC4-PORCN-SFRP4	80	28448	27819	28835	44315
CCND3-PORCN-WNT4	91	42809	21302	8841	38191	CCND3-PORCN-FBXW4	143	52831	15807	7893	14041
CXXC4-PORCN-TCF7	147	56042	37081	25343	32394	CXXC4-PORCN-FBXW4	168	26465	19028	12201	29004
CSNK1D-PORCN-SENP2	254	26488	43944	21480	22511	PITX2-PORCN-SENP2	270	20159	27718	25665	18827
FOSL1-PORCN-SFRP4	292	44876	11834	15903	36394	APC-PORCN-WNT4	310	33562	14239	15555	28291
FBXW2-PORCN-SFRP4	311	28602	1442	40221	38787	APC-PORCN-TCF7L1	320	33618	6586	15674	22420
FZD7-PORCN-FBXW4	331	24784	14647	7522	19704	NLK-PORCN-PPP2CA	339	30605	43435	41734	4166
FOSL1-PORCN-SENP2	349	31659	11861	10245	21849	NLK-PORCN-SFRP4	368	53128	52278	37793	4619
FZD5-PORCN-SENP2	377	32693	14759	8273	55426	FZD6-PORCN-WNT2B	379	19259	55786	24330	16739
FZD1-PORCN-SENP2	382	25494	17903	13765	16485	FZD6-PORCN-WNT4	394	39861	46523	785	6046
DKK1-PORCN-SENP2	400	29700	53693	2734	52517	CSNK1G1-PORCN-SENP2	410	36322	35831	12245	17913
BCL9-PORCN-PPP2CA	415	15959	34237	38189	34019	CXXC4-PORCN-PPP2R1A	424	27798	37533	50501	37618
FOSL1-PORCN-WNT4	455	37570	20729	10105	38487	PITX2-PORCN-WNT5A	460	36097	17787	53210	23094
APC-PORCN-SENP2	466	12387	7261	13497	19215	FRZB-PORCN-SENP2	467	13159	11733	10122	22264
LRP5-PORCN-SENP2	476	41616	8750	51278	21981	CXXC4-PORCN-PPP2CA	481	22027	38709	38555	36190
FZD6-PORCN-TLE2	482	39422	46346	2340	25087	FZD6-PORCN-SENP2	497	45212	45263	687	14394
BCL9-PORCN-SFRP4	498	29468	3887	31671	36411	PITX2-PORCN-FBXW4	499	12554	20396	26439	17842
FBXW2-PORCN-WNT4	531	21565	4445	36629	31422	CXXC4-PORCN-WNT5A	535	15961	16253	55679	46631
FZD8-PORCN-SFRP1	556	26745	7918	11285	32679	PITX2-PORCN-TCF7L1	572	42893	22702	25919	13429
CSNK1D-PORCN-WNT2B	573	51683	44617	47981	16208	FZD6-PORCN-SFRP1	615	42793	48666	4863	20350
PITX2-PORCN-WNT2B	630	42884	44910	50269	15186	FZD8-PORCN-SENP2	639	19701	11231	9109	12830
FOSL1-PORCN-WNT2B	670	19667	46545	40946	19709	KREMEN1-PORCN-WNT4	693	6753	23243	1864	44869
DKK1-PORCN-WNT4	724	36114	54617	3866	55268	NLK-PORCN-SENP2	751	51440	56864	21216	6122
FZD7-PORCN-WNT3A	780	35610	948	25632	12174	FZD8-PORCN-PPP2R1A	807	27524	31540	26322	40650
PITX2-PORCN-TLE1	813	43605	21513	22845	15786	FRAT1-PORCN-SFRP4	816	23261	18955	23358	33807
DKK1-PORCN-SFRP4	824	35293	52036	5440	53719	LRP5-PORCN-SFRP4	872	1618	3850	57012	38203
FRAT1-PORCN-SENP2	915	19198	9986	14436	18176	APC-PORCN-SFRP1	973	22402	11999	21396	30025
LRP5-PORCN-WNT2	975	5747	5037	56461	37999	CCND3-PORCN-SFRP1	984	46966	24689	11920	26237
CCND3-PORCN-TLE2	1002	48876	25152	13843	25724	PITX2-PORCN-PPP2R1A	1003	8071	39783	44256	5481
NLK-PORCN-WNT2B	1006	26349	37659	35498	4512	FBXW11-PORCN-WNT2B	1048	54134	36167	45459	15114
CSNK1D-PORCN-TLE2	1050	41629	52347	39317	20436	KREMEN1-PORCN-SENP2	1055	20253	16697	487	37387
FRAT1-PORCN-RHOU	1077	31453	10861	21530	17749	FZD1-PORCN-RHOU	1095	21176	12741	16941	18130
NLK-PORCN-SLC9A3R1	1108	35858	56545	26507	3768	FZD7-PORCN-TLE2	1111	50028	20809	22737	16490
CSNK1D-PORCN-TCF7	1115	16160	38935	28820	29405	LEF1-PORCN-PPP2R1A	1139	34004	43790	26178	5620
NLK-PORCN-TCF7	1154	48490	41769	34836	12221	FZD6-PORCN-TCF7	1180	33602	45874	5530	14682
FZD1-PORCN-WNT2B	1214	23191	49164	44140	14132	PITX2-PORCN-RHOU	1220	38816	23756	29958	13324

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Table 1. *Cont.*

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}
DKK1-PORCN-WNT2B	1222	26601	56978	25305	51044	FZD1-PORCN-SFRP4	1272	20986	27308	23520	32421
BCL9-PORCN-RHOU	1284	46597	6968	29490	21809	CSNK1G1-PORCN-TLE2	1291	31155	47884	19907	37757
CSNK1G1-PORCN-RHOU	1299	27191	33972	15284	20376	FOSL1-PORCN-RHOU	1333	44749	10281	23020	23848
APC-PORCN-WNT2B	1336	19495	29454	32718	22662	KREMEN1-PORCN-WNT3A	1377	25809	2588	12830	26801
BCL9-PORCN-WNT2B	1394	28608	33398	47599	21197	KREMEN1-PORCN-WNT2B	1401	36444	47192	17886	28451
CCND3-PORCN-TCF7L1	1408	36413	14249	8914	16004	FZD8-PORCN-WNT4	1416	27491	15836	9789	36903
FBXW11-PORCN-WNT4	1417	38911	18306	23726	35310	FZD8-PORCN-TCF7	1423	29081	35097	18363	11878
FBXW11-PORCN-SFRP4	1424	40575	3082	26157	24346	BCL9-PORCN-TCF7L1	1434	34720	6524	28798	16832
FOSL1-PORCN-TLE2	1446	44769	21226	13796	30066	DKK1-PORCN-TLE2	1461	36221	53569	8435	54639
FZD6-PORCN-SFRP4	1468	43415	46173	1426	23003	FZD7-PORCN-SFRP1	1470	54865	4185	15633	25384
DIXDC1-PORCN-SENP2	1529	21963	6981	10680	50015	FZD7-PORCN-WNT5A	1546	23763	6363	31855	40603
NLK-PORCN-WNT2	1557	46116	57071	36752	14418	FZD1-PORCN-TCF7	1572	38315	38185	25247	19741
CCND3-PORCN-TLE1	1580	36600	16976	3464	18298	CSNK1D-PORCN-SLC9A3R1	1596	41508	51420	34746	16992
PITX2-PORCN-SFRP4	1607	32189	32121	34063	21972	APC-PORCN-TCF7	1608	44043	39040	24555	20881
NLK-PORCN-TLE2	1657	49377	56092	39434	12430	FZD1-PORCN-SLC9A3R1	1698	18363	16380	21532	14659
FZD8-PORCN-PPP2CA	1713	51044	29439	20913	20485	FZD1-PORCN-FBXW4	1723	25108	19068	11515	19611
NLK-PORCN-WNT4	1742	49908	50644	30880	6042	FOSL1-PORCN-SFRP1	1757	36317	8057	17969	48972
FRZB-PORCN-SFRP4	1758	2975	26008	22234	42068	FRZB-PORCN-WNT4	1770	28522	21808	15299	35164
FRAT1-PORCN-WNT2B	1796	26246	45830	36444	11708	CSNK1G1-PORCN-SLC9A3R1	1815	10503	40046	19851	14743
FRZB-PORCN-PPP2R1A	1881	14863	41848	44601	12655	FZD7-PORCN-WNT2B	1892	48697	42677	33490	16364
GSK3B-PORCN-WNT4	1893	29701	28162	8296	19073	FOSL1-PORCN-FBXW4	1896	35711	18446	8569	19117
KREMEN1-PORCN-TCF7L1	1902	38888	15726	2313	30252	PITX2-PORCN-SFRP1	1905	39236	31192	37387	9110
CSNK1D-PORCN-SFRP1	1945	41776	54231	29005	26519	FZD1-PORCN-TLE2	1962	28422	26300	32913	23948
FZD5-PORCN-SFRP1	1980	55503	9426	16493	55566	FRAT1-PORCN-WNT4	1985	2835	18017	16053	23730
DKK1-PORCN-TCF7L1	1996	8327	51626	5019	50393	FRZB-PORCN-WNT2	2018	14494	10314	22043	37904
FZD6-PORCN-WNT2	2024	30877	41431	1558	29140	LRP5-PORCN-RHOU	2053	8242	9590	48746	20172

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

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}
CXXC4-PORCN-SENP2	9869	47028	41747	45009	41195	CXXC4-PORCN-WNT4	12492	2278	24920	46755	55842
CXXC4-PORCN-WNT2B	11272	21120	2288	41634	56160	CXXC4-PORCN-SFRP4	9369	30775	45417	32220	54090
CCND3-PORCN-WNT4	32692	49112	4162	52578	21791	CCND3-PORCN-FBXW4	14645	48562	10056	3289	18987
CXXC4-PORCN-TCF7	8174	50291	19771	4132	42869	CXXC4-PORCN-FBXW4	19853	43651	1014	10933	51164
CSNK1D-PORCN-SENP2	5664	26748	24402	26200	37885	PITX2-PORCN-SENP2	9499	29209	4986	29421	35908
FOSL1-PORCN-SFRP4	1126	41952	37755	38422	51768	APC-PORCN-WNT4	3592	12533	35169	40131	56018
FBXW2-PORCN-SFRP4	6999	27618	7405	7672	31324	APC-PORCN-TCF7L1	2882	26995	28827	39506	53073
FZD7-PORCN-FBXW4	8121	22895	5486	11733	22661	NLK-PORCN-PPP2CA	6640	37879	19229	30844	45007
FOSL1-PORCN-SENP2	314	29835	38646	3075	34319	NLK-PORCN-SFRP4	26548	54453	3595	31340	34837
FZD5-PORCN-SENP2	13359	46673	16639	12543	32648	FZD6-PORCN-WNT2B	47	9685	16997	36922	48852
FZD1-PORCN-SENP2	5513	34056	9744	10153	31014	FZD6-PORCN-WNT4	49	30454	13795	53236	50505
DKK1-PORCN-SENP2	2598	41263	6134	19723	13188	CSNK1G1-PORCN-SENP2	13585	41454	10606	1967	17443
BCL9-PORCN-PPP2CA	19443	12204	3989	2860	44188	CXXC4-PORCN-PPP2R1A	20998	30567	5511	22186	56640
FOSL1-PORCN-WNT4	1429	25705	30547	51837	52319	PITX2-PORCN-WNT5A	11335	32674	6548	15458	54809
APC-PORCN-SENP2	311	33317	27533	12463	51663	FRZB-PORCN-SENP2	2427	25944	18926	35699	48606
LRP5-PORCN-SENP2	11545	45801	9754	1937	37320	CXXC4-PORCN-PPP2CA	9663	20715	7491	12348	51537
FZD6-PORCN-TLE2	1065	45384	18117	3169	43333	FZD6-PORCN-SENP2	3	53444	8379	12707	32509
BCL9-PORCN-SFRP4	18390	13860	4099	36393	45375	PITX2-PORCN-FBXW4	2782	19946	19676	35972	50951
FBXW2-PORCN-WNT4	26557	1122	13989	31473	19951	CXXC4-PORCN-WNT5A	24704	5412	10695	26556	50085
FZD8-PORCN-SFRP1	11923	6846	34413	24802	45677	PITX2-PORCN-TCF7L1	13222	48332	9570	21227	51753
CSNK1D-PORCN-WNT2B	8595	53305	9816	26691	42121	FZD6-PORCN-SFRP1	1350	53618	15134	15153	53828
PITX2-PORCN-WNT2B	6802	25276	16938	13140	54357	FZD8-PORCN-SENP2	1042	32328	3606	28410	19273
FOSL1-PORCN-WNT2B	4296	5489	1279	29512	55035	KREMEN1-PORCN-WNT4	4572	591	18551	4045	46258
DKK1-PORCN-WNT4	9761	29610	8470	23618	51513	NLK-PORCN-SENP2	25839	52725	11882	9881	37272
FZD7-PORCN-WNT3A	7046	46922	42358	23913	36667	FZD8-PORCN-PPP2R1A	3136	21194	9602	10973	55135
PITX2-PORCN-TLE1	13872	39019	23792	7146	28106	FRAT1-PORCN-SFRP4	3261	12036	26076	48387	48335
DKK1-PORCN-SFRP4	10967	45207	3433	23697	54991	LRP5-PORCN-SFRP4	7636	690	3149	40164	40704
FRAT1-PORCN-SENP2	1688	40659	28876	5431	52097	APC-PORCN-SFRP1	7111	14469	39969	4242	53927
LRP5-PORCN-WNT2	19897	6038	17781	5354	56782	CCND3-PORCN-SFRP1	33184	51464	6743	7129	29071
CCND3-PORCN-TLE2	27878	51557	10879	4451	14924	PITX2-PORCN-PPP2R1A	11355	50277	26063	28070	55628
NLK-PORCN-WNT2B	30300	3466	8133	31316	44880	FBXW11-PORCN-WNT2B	4588	54697	1382	3672	49591
CSNK1D-PORCN-TLE2	12595	39793	26325	7235	43651	KREMEN1-PORCN-SENP2	2897	40815	14540	5841	26554
FRAT1-PORCN-RHOU	3460	15685	47256	50127	56007	FZD1-PORCN-RHOU	5740	25675	39166	40916	55717
NLK-PORCN-SLC9A3R1	39803	20122	9428	9998	42527	FZD7-PORCN-TLE2	38186	53192	36658	6999	30131
CSNK1D-PORCN-TCF7	38186	53192	36658	6999	30131	LEF1-PORCN-PPP2R1A	9650	37275	29706	14200	48587
NLK-PORCN-TCF7	14350	41604	26400	15610	37827	FZD6-PORCN-TCF7	9	22463	16490	29529	32076
FZD1-PORCN-WNT2B	12628	31292	8901	34846	55210	PITX2-PORCN-RHOU	11795	30595	6426	8297	54722
DKK1-PORCN-WNT2B	6263	7902	41853	840	49894	FZD1-PORCN-SFRP4	11010	1938	293	36645	52104
BCL9-PORCN-RHOU	13417	51514	7531	35538	56157	CSNK1G1-PORCN-TLE2	25416	24850	21439	2281	53031
CSNK1G1-PORCN-RHOU	18456	21677	26712	34529	54262	FOSL1-PORCN-RHOU	1550	39845	36998	42942	56120
APC-PORCN-WNT2B	3093	4702	5177	37025	54684	KREMEN1-PORCN-WNT3A	8946	29482	33980	6745	37128

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Table 2. *Cont.*

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}
BCL9-PORCN-WNT2B	7648	28196	20793	10962	34371	KREMEN1-PORCN-WNT2B	4060	34624	13586	33545	46659
CCND3-PORCN-TCF7L1	23178	26953	5435	27259	35443	FZD8-PORCN-WNT4	3177	7307	28634	31520	31714
FBXW11-PORCN-WNT4	6702	42970	13553	51464	33227	FZD8-PORCN-TCF7	3913	45328	27883	32520	22942
FBXW11-PORCN-SFRP4	4481	17010	1946	40382	32523	BCL9-PORCN-TCF7L1	23603	38078	31638	19229	41402
FOSL1-PORCN-TLE2	11375	42064	44489	3406	53873	DKK1-PORCN-TLE2	15980	45554	11547	10317	52314
FZD6-PORCN-SFRP4	58	47582	15962	31907	46706	FZD7-PORCN-SFRP1	46709	56734	39833	5383	34239
DIXDC1-PORCN-SENP2	16346	39480	19973	18502	14705	FZD7-PORCN-WNT5A	20174	37185	18191	15667	34821
NLK-PORCN-WNT2	44692	38504	8829	1995	53685	FZD1-PORCN-TCF7	2001	52600	33800	5805	49403
CCND3-PORCN-TLE1	13616	41666	12307	4061	29372	CSNK1D-PORCN-SLC9A3R1	26693	34397	22670	453	31614
PITX2-PORCN-SFRP4	7652	26224	19994	9630	41059	APC-PORCN-TCF7	671	37810	35108	9656	51194
NLK-PORCN-TLE2	41691	54802	745	25237	46608	FZD1-PORCN-SLC9A3R1	18304	25091	21402	3214	52196
FZD8-PORCN-PPP2CA	4402	55143	2005	12002	37055	FZD1-PORCN-FBXW4	15874	28222	17086	4292	47953
NLK-PORCN-WNT4	33383	45823	7542	34942	49961	FOSL1-PORCN-SFRP1	10522	38362	29766	6077	55724
FRZB-PORCN-SFRP4	6537	11064	22288	34580	50227	FRZB-PORCN-WNT4	9103	50017	36210	40277	54561
FRAT1-PORCN-WNT2B	5506	22998	20346	48317	52835	CSNK1G1-PORCN-SLC9A3R1	33423	29599	9114	6260	50642
FRZB-PORCN-PPP2R1A1	3914	23845	13959	7815	56301	FZD7-PORCN-WNT2B	35742	42851	5084	32980	11716
GSK3B-PORCN-WNT4	7755	14164	10378	27077	53726	FOSL1-PORCN-FBXW4	996	45964	6696	11811	50330
KREMEN1-PORCN-TCF7L1	5105	42564	13754	28956	27851	PITX2-PORCN-SFRP1	20244	35446	37738	24843	55456
CSNK1D-PORCN-SFRP1	36889	38723	2444	15209	50430	FZD1-PORCN-TLE2	29353	42139	4457	3385	52832
FZD5-PORCN-SFRP1	19216	56452	45353	3271	51709	FRAT1-PORCN-WNT4	6345	412	40450	45568	49626
DKK1-PORCN-TCF7L1	10025	8033	19471	39600	52807	FRZB-PORCN-WNT2	7253	15397	42587	3591	56438
FZD6-PORCN-WNT2	586	40987	4888	4733	51712	LRP5-PORCN-RHOU	12060	10648	7628	45521	55586

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

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}
CXXC4-PORCN-SENP2	4981	10546	9375	17394	34482	CXXC4-PORCN-WNT4	9600	4879	1294	6463	37839
CXXC4-PORCN-WNT2B	39582	44080	51571	43634	12796	CXXC4-PORCN-SFRP4	3428	54623	4989	9629	43028
CCND3-PORCN-WNT4	18880	48655	24219	6338	25351	CCND3-PORCN-FBXW4	38267	39026	29866	43538	34752
CXXC4-PORCN-TCF7	2527	11671	4576	9976	25876	CXXC4-PORCN-FBXW4	53747	2497	52161	47571	14085
CSNK1D-PORCN-SENP2	26180	49740	24174	6964	30394	PITX2-PORCN-SENP2	49351	13297	36355	31422	8016
FOSL1-PORCN-SFRP4	44543	52571	34231	46029	13871	APC-PORCN-WNT4	14017	20902	10996	23651	3554
FBXW2-PORCN-SFRP4	20379	42106	5481	2200	45859	APC-PORCN-TCF7L1	44663	25485	50099	29372	8460
FZD7-PORCN-FBXW4	5102	26281	21241	14546	50046	NLK-PORCN-PPP2CA	2376	11307	5767	13480	29665
FOSL1-PORCN-SENP2	54760	25670	47342	32899	26575	NLK-PORCN-SFRP4	1207	52071	4041	14592	51831
FZD5-PORCN-SENP2	27413	45632	5424	7312	44530	FZD6-PORCN-WNT2B	31884	54376	34870	43757	175
FZD1-PORCN-SENP2	21577	45473	27023	26028	44572	FZD6-PORCN-WNT4	12889	17968	25060	5439	42744
DKK1-PORCN-SENP2	7496	43787	13993	24495	52737	CSNK1G1-PORCN-SENP2	43281	37454	56745	49095	6460
BCL9-PORCN-PPP2CA	50172	47074	50265	53138	6075	CXXC4-PORCN-PPP2R1A	44156	3317	53822	38916	15985
FOSL1-PORCN-WNT4	47380	31804	36756	47696	19151	PITX2-PORCN-WNT5A	4366	18564	14041	19978	22471
APC-PORCN-SENP2	19313	15156	9308	25386	44309	FRZB-PORCN-SENP2	13854	51961	26520	17468	45862
LRP5-PORCN-SENP2	14420	8910	7971	16494	35454	CXXC4-PORCN-PPP2CA	13026	53831	3318	18245	41235
FZD6-PORCN-TLE2	34987	46334	34368	44469	14201	FZD6-PORCN-SENP2	2457	23664	19666	22956	54834
BCL9-PORCN-SFRP4	36442	6519	42650	32000	10740	PITX2-PORCN-FBXW4	8808	13705	22864	22147	31748
FBXW2-PORCN-WNT4	22459	33611	12168	3597	56281	CXXC4-PORCN-WNT5A	47497	52019	55859	50726	19154
FZD8-PORCN-SFRP1	57034	872	39503	50676	3226	PITX2-PORCN-TCF7L1	7523	29695	12381	16214	18408
CSNK1D-PORCN-WNT2B	39627	20751	32331	36708	28715	FZD6-PORCN-SFRP1	54700	33984	37489	34160	2322
PITX2-PORCN-WNT2B	9536	5794	12824	18961	17911	FZD8-PORCN-SENP2	124	56299	17625	6462	53950
FOSL1-PORCN-WNT2B	9686	2290	2189	14059	39340	KREMEN1-PORCN-WNT4	2994	1634	5445	11732	52786
DKK1-PORCN-WNT4	10378	26852	12688	21000	45686	NLK-PORCN-SENP2	700	5829	4828	22993	34644
FZD7-PORCN-WNT3A	20917	23923	24750	6039	49403	FZD8-PORCN-PPP2R1A	38026	11841	38929	49830	12841
PITX2-PORCN-TLE1	49615	27631	44758	40897	38885	FRAT1-PORCN-SFRP4	22000	49118	15018	7354	55844
DKK1-PORCN-SFRP4	6590	38173	25437	13461	54299	LRP5-PORCN-SFRP4	1137	1730	7697	15694	49072
FRAT1-PORCN-SENP2	8802	23948	15600	4011	48205	APC-PORCN-SFRP1	37874	41908	47833	31775	12763
LRP5-PORCN-WNT2	4115	8874	8590	8682	48251	CCND3-PORCN-SFRP1	43819	39418	33323	37198	21161
CCND3-PORCN-TLE2	40133	14147	33853	48391	12066	PITX2-PORCN-PPP2R1A	10644	13926	20214	8562	45301
NLK-PORCN-WNT2B	53778	43223	50000	50320	693	FBXW11-PORCN-WNT2B	33797	43559	32346	51083	11772
CSNK1D-PORCN-TLE2	40721	9512	29575	32383	21204	KREMEN1-PORCN-SENP2	1460	11304	1074	20122	52679
FRAT1-PORCN-RHOU	39358	56828	38942	45808	6638	FZD1-PORCN-RHOU	30838	53067	37641	51753	3889
NLK-PORCN-SLC9A3R1	30838	53067	37641	51753	3889	FZD7-PORCN-TLE2	516	10377	22833	25279	42930
CSNK1D-PORCN-TCF7	10132	38975	27387	12057	30165	LEF1-PORCN-PPP2R1A	55205	5382	39482	30816	5128
NLK-PORCN-TCF7	1320	10309	1882	15974	55425	FZD6-PORCN-TCF7	650	4470	21051	13771	35494
FZD1-PORCN-WNT2B	53071	43558	43509	40293	16263	PITX2-PORCN-RHOU	7818	43749	20769	25705	49200
DKK1-PORCN-WNT2B	41383	41457	40170	35729	7578	FZD1-PORCN-SFRP4	26299	2303	16361	5585	37104
BCL9-PORCN-RHOU	10051	9401	3406	8081	39145	CSNK1G1-PORCN-TLE2	7372	47733	19657	27924	17147
CSNK1G1-PORCN-RHOU	13901	19785	413	8056	50675	FOSL1-PORCN-RHOU	2397	31652	9884	24315	30810
APC-PORCN-WNT2B	42479	39207	52650	38804	46078	KREMEN1-PORCN-WNT3A	36088	42706	41171	37942	19003
BCL9-PORCN-WNT2B	619	12023	16698	20050	36808	KREMEN1-PORCN-WNT2B	52764	52181	54179	35890	32504
CCND3-PORCN-TCF7L1	35354	38098	31141	40678	30232	FZD8-PORCN-WNT4	4950	13451	24888	5374	42530
FBXW11-PORCN-WNT4	16540	28739	25012	3970	46428	FZD8-PORCN-TCF7	878	38866	27534	4182	38998
FBXW11-PORCN-SFRP4	24695	13868	22467	19792	21432	BCL9-PORCN-TCF7L1	620	5955	3602	13422	37724
FOSL1-PORCN-TLE2	11344	44887	618	8380	48021	DKK1-PORCN-TLE2	47698	6306	40244	42456	1071

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Table 3. *Cont.*

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}
FZD6-PORCN-SFRP4	4229	54312	23210	6977	52780	FZD7-PORCN-SFRP1	9509	38539	24258	7006	48857
DIXDC1-PORCN-SENP2	9509	38539	24258	7006	48857	FZD7-PORCN-WNT5A	4325	13687	23959	15423	50273
NLK-PORCN-WNT2	3371	14108	7182	6820	56445	FZD1-PORCN-TCF7	26285	21179	19395	7189	51548
CCND3-PORCN-TLE1	16999	43087	23309	8763	45240	CSNK1D-PORCN-SLC9A3R1	22475	41256	24969	21096	28384
PITX2-PORCN-SFRP4	52035	24948	36616	49778	2115	APC-PORCN-TCF7	12536	31467	7048	27774	48666
NLK-PORCN-TLE2	56416	24825	38132	40938	7611	FZD1-PORCN-SLC9A3R1	26566	53321	20018	23847	47064
FZD8-PORCN-PPP2CA	19196	45384	18240	7276	44603	FZD1-PORCN-FBXW4	30836	54852	40824	51594	19986
NLK-PORCN-WNT4	3277	2846	7185	12509	42889	FOSL1-PORCN-SFRP1	12651	4591	23008	11171	43358
FRZB-PORCN-SFRP4	18403	6827	17409	23455	37645	FRZB-PORCN-WNT4	10841	20110	18202	7735	48889
FRAT1-PORCN-WNT2B	37208	46905	41078	48022	2109	CSNK1G1-PORCN-SLC9A3R1	37208	46905	41078	48022	2109
FRZB-PORCN-PPP2R1A	37208	46905	41078	48022	2109	FZD7-PORCN-WNT2B	5088	17699	18559	1094	44896
GSK3B-PORCN-WNT4	1508	45949	15059	14068	43601	FOSL1-PORCN-FBXW4	19295	6262	4610	7514	13534
KREMEN1-PORCN-TCF7L1	55719	32088	53306	30161	3431	PITX2-PORCN-SFRP1	5118	32289	20516	7388	55055
CSNK1D-PORCN-SFRP1	30977	7376	32969	50220	26759	FZD1-PORCN-TLE2	32064	54282	35983	38833	14916
FZD5-PORCN-SFRP1	29712	11650	51767	49816	12685	FRAT1-PORCN-WNT4	26095	16695	23293	12549	25899
DKK1-PORCN-TCF7L1	52047	33682	42689	38257	6489	FRZB-PORCN-WNT2	8741	36235	19647	20574	56315
FZD6-PORCN-WNT2	25276	2810	22247	13377	56981	LRP5-PORCN-RHOU	56073	12080	38788	31516	4758

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

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}
CXXC4-PORCN-SENP2	17800	21889	46576	9816	38838	CXXC4-PORCN-WNT4	4276	14596	42774	57022	45130
CXXC4-PORCN-WNT2B	14191	46734	705	50002	13918	CXXC4-PORCN-SFRP4	36656	48495	25105	56369	1896
CCND3-PORCN-WNT4	26806	19860	18309	28843	27352	CCND3-PORCN-FBXW4	37472	26070	4760	10820	54608
CXXC4-PORCN-TCF7	48699	31354	37070	38630	54197	CXXC4-PORCN-FBXW4	56939	49267	33951	53901	13207
CSNK1D-PORCN-SENP2	1456	4258	8869	32822	29157	PITX2-PORCN-SENP2	20617	22563	38695	45455	12377
FOSL1-PORCN-SFRP4	7834	33798	49219	25475	52140	APC-PORCN-WNT4	22457	14933	19953	27502	33252
FBXW2-PORCN-SFRP4	47119	38576	7220	11731	8412	APC-PORCN-TCF7L1	53255	18165	26514	32120	37492
FZD7-PORCN-FBXW4	22000	4081	23973	12350	21278	NLK-PORCN-PPP2CA	37331	14886	22559	45950	6790
FOSL1-PORCN-SENP2	12075	2582	50306	27092	37213	NLK-PORCN-SFRP4	10994	18306	26888	51840	4032
FZD5-PORCN-SENP2	4057	36674	11079	4102	30265	FZD6-PORCN-WNT2B	5774	40911	34822	40189	42079
FZD1-PORCN-SENP2	16152	22375	9599	2134	53565	FZD6-PORCN-WNT4	199	7166	3089	14140	1367
DKK1-PORCN-SENP2	45052	7054	16503	35722	44298	CSNK1G1-PORCN-SENP2	15610	15039	24692	32268	17031
BCL9-PORCN-PPP2CA	36593	35690	46380	55538	25115	CXXC4-PORCN-PPP2R1A	56184	21259	15139	35428	46765
FOSL1-PORCN-WNT4	7917	37593	48481	35640	51574	PITX2-PORCN-WNT5A	23589	48950	34402	37236	3755
APC-PORCN-SENP2	22313	13751	17377	17470	16927	FRZB-PORCN-SENP2	5700	39274	27314	56378	56042
LRP5-PORCN-SENP2	27087	24353	54744	56478	25297	CXXC4-PORCN-PPP2CA	18980	12646	41076	17858	34882
FZD6-PORCN-TLE2	5776	15490	29722	20367	18835	FZD6-PORCN-SENP2	32912	3888	24013	2296	408
BCL9-PORCN-SFRP4	32912	3888	24013	2296	408	PITX2-PORCN-FBXW4	6203	42677	48707	17993	5734
FBXW2-PORCN-WNT4	46672	28991	8289	11846	43257	CXXC4-PORCN-WNT5A	30469	36642	4574	55370	14476
FZD8-PORCN-SFRP1	57127	22976	30094	41223	14197	PITX2-PORCN-TCF7L1	4744	47264	23302	15463	5925
CSNK1D-PORCN-WNT2B	54547	33746	2831	17934	52535	FZD6-PORCN-SFRP1	38472	34746	15469	32897	39560
PITX2-PORCN-WNT2B	32916	28214	19614	42831	17840	FZD8-PORCN-SENP2	51735	28846	18836	25156	31908
FOSL1-PORCN-WNT2B	51756	54848	26032	20042	1751	KREMEN1-PORCN-WNT4	25014	55908	56360	6264	40529
DKK1-PORCN-WNT4	25947	40656	8698	44050	26641	NLK-PORCN-SENP2	9588	21743	36008	30568	4545
FZD7-PORCN-WNT3A	601	7936	6391	14753	43442	FZD8-PORCN-PPP2R1A	8407	43072	773	21094	20949
PITX2-PORCN-TLE1	26366	14552	49163	2938	25505	FRAT1-PORCN-SFRP4	43619	53535	46222	28713	24581
DKK1-PORCN-SFRP4	49382	34289	2711	37701	43300	LRP5-PORCN-SFRP4	11207	53415	55335	54554	14540
FRAT1-PORCN-SENP2	18609	31024	10863	13050	55318	APC-PORCN-SFRP1	44117	3417	44138	40671	30191
LRP5-PORCN-WNT2	35666	56564	52355	52582	3356	CCND3-PORCN-SFRP1	44703	44871	11509	7441	52676
CCND3-PORCN-TLE2	43771	27450	6693	18342	56693	PITX2-PORCN-PPP2R1A	28551	48685	56979	48784	335
NLK-PORCN-WNT2B	23304	40640	40983	7483	30297	FBXW11-PORCN-WNT2B	35745	24457	1321	41380	44484
CSNK1D-PORCN-TLE2	53642	34642	1349	6256	56564	KREMEN1-PORCN-SENP2	53256	34131	37244	42862	23394
FRAT1-PORCN-RHOU	50790	54552	928	2722	13751	FZD1-PORCN-RHOU	35036	50522	2902	5037	14487
NLK-PORCN-SLC9A3R1	17046	52346	6586	54426	44843	FZD7-PORCN-TLE2	1608	32240	31287	2251	50601
CSNK1D-PORCN-TCF7	25748	22359	21805	36766	4140	LEF1-PORCN-PPP2R1A	26478	55431	54500	47281	17795
NLK-PORCN-TCF7	1798	29061	14067	37353	4424	FZD6-PORCN-TCF7	44142	11911	17545	5762	38469
FZD1-PORCN-WNT2B	56201	55890	2978	45069	8402	PITX2-PORCN-RHOU	5900	36986	56261	27068	40618
DKK1-PORCN-WNT2B	13119	7914	33768	34345	53916	FZD1-PORCN-SFRP4	2800	45956	39142	4610	2795
BCL9-PORCN-RHOU	3707	54841	9886	55436	7307	CSNK1G1-PORCN-TLE2	23956	39888	45808	29516	4037
CSNK1G1-PORCN-RHOU	156	28220	17621	19760	27625	FOSL1-PORCN-RHOU	21806	54658	25822	20528	2159
APC-PORCN-WNT2B	43631	26042	25082	33368	9713	KREMEN1-PORCN-WNT3A	5432	49056	32287	41650	15766
BCL9-PORCN-WNT2B	31320	49751	15858	56923	5674	KREMEN1-PORCN-WNT2B	25373	52290	22610	2903	12826
CCND3-PORCN-TCF7L1	38790	32357	2631	15890	53163	FZD8-PORCN-WNT4	16866	18194	19414	12569	12962
FBXW11-PORCN-WNT4	16866	18194	19414	12569	12962	FZD8-PORCN-TCF7	39578	43421	27974	39205	6871
FBXW11-PORCN-SFRP4	39578	43421	27974	39205	6871	BCL9-PORCN-TCF7L1	38738	55691	16260	51286	2241
FOSL1-PORCN-TLE2	54214	44956	26881	18144	5869	DKK1-PORCN-TLE2	13798	31220	1759	16596	51097
FZD6-PORCN-SFRP4	25942	35145	7356	5564	1460	FZD7-PORCN-SFRP1	44619	27080	14061	8409	3502
DIXDC1-PORCN-SENP2	6264	29786	2478	31653	56322	FZD7-PORCN-WNT5A	24607	15441	7643	4565	54720
NLK-PORCN-WNT2	20516	13167	13537	26978	5995	FZD1-PORCN-TCF7	10909	25989	30504	31827	51352
CCND3-PORCN-TLE1	26493	51609	32801	36596	34579	CSNK1D-PORCN-SLC9A3R1	15731	15687	5737	23413	32643
PITX2-PORCN-SFRP4	34090	15340	54473	55067	54878	APC-PORCN-TCF7	10389	12239	3038	22455	44049
NLK-PORCN-TLE2	54185	50313	41574	46224	37007	FZD1-PORCN-SLC9A3R1	5472	7001	20251	1306	47069

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Table 4. *Cont.*

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}
FZD8-PORCN-PPP2CA	4362	22959	16034	3701	20999	FZD1-PORCN-FBXW4	31466	52694	2101	14678	25905
NLK-PORCN-WNT4	839	35865	37133	52537	3763	FOSL1-PORCN-SFRP1	54293	34318	31561	16647	17560
FRZB-PORCN-SFRP4	10437	53508	50238	56790	885	FRZB-PORCN-WNT4	36039	40997	30705	7328	55361
FRAT1-PORCN-WNT2B	20944	46462	334	27557	17691	CSNK1G1-PORCN-SLC9A3R1	9278	49182	53553	52071	36096
FRZB-PORCN-PPP2R1A	14737	40959	24690	53668	10525	FZD7-PORCN-WNT2B	22739	11163	28241	3088	23003
GSK3B-PORCN-WNT4	22739	11163	28241	3088	23003	FOSL1-PORCN-FBXW4	51245	47518	29410	28956	4946
KREMEN1-PORCN-TCF7L1	55489	51329	54116	43728	32279	PITX2-PORCN-SFRP1	3773	36533	56407	54176	6456
CSNK1D-PORCN-SFRP1	18994	35477	42	21142	50577	FZD1-PORCN-TLE2	39761	45325	7964	5990	9283
FZD5-PORCN-SFRP1	29431	48602	12473	933	27590	FRAT1-PORCN-WNT4	1204	15349	9265	36000	46519
DKK1-PORCN-TCF7L1	16106	156	33693	7046	35333	FRZB-PORCN-WNT2	24065	52945	6943	22842	11082
FZD6-PORCN-WNT2	2617	25245	12529	7270	8966	LRP5-PORCN-RHOU	46666	54394	53539	28346	17280

6.3.1. Examining the Behaviour of CXXC4-PORCN-X Combinations

Ekici et al. [16] identified a homozygous splice site mutation in the CCDC88C gene as a novel cause of a complex hydrocephalic brain malformation, via positional cloning in a consanguineous family with autosomal recessive hydrocephalus. CCDC88C encodes DAPLE (HkRP2), a Hook-related protein with a binding domain for the central WNT signalling pathway protein DVL and DVL is inhibited by CXXC4 and CCDC88C, mediates Wnt signalling via inhibition of GSK3. Looking at the tables above, one finds the following combinations for CXXC4 along with PORCN, to be prominent at 3rd order level - CXXC4-PORCN-SENP2, CXXC4-PORCN-WNT2B, CXXC4-PORCN-TCF7, CXXC4-PORCN-WNT4, CXXC4-PORCN-SFRP4, CXXC4-PORCN-FBXW4, CXXC4-PORCN-PPP2R1A, CXXC4-PORCN-PPP2CA and CXXC4-PORCN-WNT5A. 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 FOSL1-PORCN-X Combinations

The ability of the right ventricle (RV) to adapt to an increased pressure afterload determines survival in patients with pulmonary arterial hypertension. The WNT pathway plays an important role in the development of the RV and may also be implicated in adult cardiac remodeling. Nayakanti et al. [17] show that • WNT/β-catenin signaling molecules are upregulated in RV of patients with pulmonary arterial hypertension and animal models of RV overload (pulmonary artery banding-induced and monocrotaline rat models); • Activation of WNT/β-catenin signaling led to RV remodeling via transcriptional activation of FOSL1 and FOSL2; • Further, the mRNA expression profiling of WNT signaling molecules and immunofluorescence staining of aCTNNB1 (active β-catenin) and PORCN in RV myocardial tissues from human donors (control) and patients with idiopathic PAH (IPAH) demonstrated a significant upregulation of WNT/β-catenin signaling and • finally, pharmacological inhibition of WNT signaling using inhibitor of PORCN, LGK-974 attenuated fibrosis and cardiac hypertrophy leading to improvement in RV function in both, pulmonary artery banding- and monocrotaline-induced RV overload and the blockade using LGK-974 in WNT3A-stimulated cardiac fibroblasts resulted in significant downregulation of mRNA and protein expression of Wnt/β-catenin signaling and its target genes (β-catenin, PORCN, MYC), transcription factors (FOSL1, FOSL2), proliferation marker (CCND1), and fibroblast-to-myofibroblasts transdifferentiation markers (POSTN, CTGF). Looking at the tables above, one finds the following combinations for FOSL1 along with PORCN, to be prominent at 3rd order level - FOSL1-PORCN-SFRP4, FOSL1-PORCN-SENP2, FOSL1-PORCN-WNT4, FOSL1-PORCN-WNT2B, FOSL1-PORCN-TLE2, FOSL1-PORCN-RHOU, FOSL1-PORCN-SFRP1 and FOSL1-PORCN-FBXW4. 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 PITX2-PORCN-X Combinations

PITX2 is activated by canonical WNT signaling, and can also regulate expression of several WNT ligands. Kioussi et al. [18] report that the bicoid-related transcription factor PITX2 is rapidly induced by the WNT/DVL/β-catenin pathway and is required for effective cell-type-specific proliferation by directly activating specific growth-regulating genes. Regulated exchange of HDAC1/β-catenin

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converts PITX2 from repressor to activator (analogous to control of TCF/LEF1), which then serves as a competence factor required for the temporally ordered and growth factor-dependent recruitment of a series of specific coactivator complexes that prove necessary for CCND2 gene induction. Basu and Roy [19] found that PITX2 interacts and regulates WNT2/5A/9A/6/2B genes of the canonical, noncanonical, or other pathways in the human ovarian cancer cell SKOV-3. Looking at the tables above, one finds the following combinations for PITX2 along with PORCN, to be prominent at 3rd order level - PITX2-PORCN-WNT2B, PITX2-PORCN-TLE1, PITX2-PORCN-SFRP4, PITX2-PORCN-SENP2, PITX2-PORCN-WNT5A, PITX2-PORCN-FBXW4, PITX2-PORCN-TCF7L1, PITX2-PORCN-PPP2R1A, PITX2-PORCN-RHOU, and PITX2-PORCN-SFRP1. 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 CSNK1D-PORCN-X Combinations

Zhu et al. [20] show that the circadian gene CSNK1D was significantly upregulated in hepatocellular carcinoma and enhanced malignant behaviors via WNT signaling pathway by stabilizing DVL3. Looking at the tables above, one finds the following combinations for CSNK1D along with PORCN, to be prominent at 3rd order level - CSNK1D-PORCN-SENP2, CSNK1D-PORCN-WNT2B, CSNK1D-PORCN-TLE2, CSNK1D-PORCN-TCF7, CSNK1D-PORCN-SFRP1 and CSNK1D-PORCN-SLC9A3R1. 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 RHOU-PORCN-X Combinations

Having demonstrated that the expression levels of WNT1, PORCN and RSPO2 are downregulated in human Alzheimer's disease (AD) brains, Macyczko et al. [21] examined the potential associations among the expression levels of these genes. It was found that the PORCN mRNA levels were positively correlated not only with the WNT1 mRNA levels, but also with the RSPO2 mRNA levels in human AD brains. Tao et al. [22] demonstrate via three lines of evidence evidence demonstrate that WNT1 responsive Cdc42 homolog (WRCH1 or RHOU) induction is correlated or associated with the expression of WNT1, i.e • After retroviral infection of cells, WRCH1/RHOU is selectively expressed in WNT1 expressing cells but not in control cells infected with an empty viral vector; • WNT1 can induce WRCH1 mRNA levels in cell coculture; and (3) WRCH1 is expressed in WNT1 induced mouse mammary tumors but not in wild-type mouse mammary glands. Also, Schiavone et al. [23] show that WNT1 could induce RHOU promoter transcription at similar levels in the presence or absence of signal transducer and activator of transcription 3 (STAT3). Looking at the tables above, one finds the following combinations for RHOU along with PORCN, to be prominent at 3rd order level - FRAT1-PORCN-RHOU, BCL9-PORCN-RHOU, CSNK1G1-PORCN-RHOU, FZD1-PORCN-RHOU, PITX2-PORCN-RHOU, FOSL1-PORCN-RHOU and LRP5-PORCN-RHOU. All these combinations indicate the existence of a possible synergy when they take a higher rank in the list of combinations.

6.3.6. Examining the Behaviour of FBXW-PORCN-X Combinations

In HEK-293 cells, Xiao et al. [24] identified FBXW7 as the direct downstream gene of miR-367, which consequently released the LIN28 dependent inhibition of suppressive LET7. Through their informatics analysis, miR-367 was predicated to function through WNT signaling, and decreased LET7 played an important role to maintain TCF-4/WNT pathway activity. The reintroduction of FBXW7 abolished the oncogenic stimulation of miR-367 on TCF-4 activity, with WNT signaling factors depression. In conclusion, they demonstrated that a FBXW7-centric signaling that miR-367 function through, and the downstream WNT activation was responsible for miR-367 oncogenic role of self-renewal, in a LIN28B dependent manner. ETC-1922159 (Madan et al. [9]) potently suppressed the FBXW7–wild-type HPAF-II tumor growth and Zhong and Virshup [25] show that in the FBXW7–wild-type HPAF-II xenografts, PORCN inhibition led to decreased phosphorylation of Rb protein and reduced total Rb abundance. Looking at the tables above, one finds the following combinations for members of FBXW family along with PORCN, to be prominent at 3rd order level - FBXW2-PORCN-

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SFRP4, FZD7-PORCN-FBXW4, FBXW2-PORCN-WNT4, FBXW11-PORCN-WNT4, FBXW11-PORCN-SFRP4, CCND3-PORCN-FBXW4, CXXC4-PORCN-FBXW4, PITX2-PORCN-FBXW4, FBXW11-PORCN-WNT2B, FZD1-PORCN-FBXW4 and FOSL1-PORCN-FBXW4. All these combinations indicate the existence of a possible synergy when they take a higher rank in the list of combinations.

6.3.7. Examining the Behaviour of KREMEN1-PORCN-X Combinations

Multiciliated cells contain hundreds of cilia whose directional movement powers the mucociliary clearance of the airways, a vital host defense mechanism and their specification requires canonical WNT signaling, which then must be turned off. Further, ciliogenesis and polarized ciliary orientation are regulated by noncanonical WNT/planar cell polarity (WNT/PCP) signaling. Cooney et al. [26] show that DKK3 and WNT4, act together to facilitate a canonical to noncanonical WNT signaling switch during multiciliated cell formation and demonstrate that DKK3 is contemporaneously expressed with WNT4 by the same population of basal cells, and suggest that it binds to the multiciliated cell surface via KREMEN1. Looking at the tables above, one finds the following combinations for KREMEN1 along with PORCN, to be prominent at 3rd order level - KREMEN1-PORCN-TCF7L1, KREMEN1-PORCN-WNT4, KREMEN1-PORCN-SENP2, KREMEN1-PORCN-WNT3A and KREMEN1-PORCN-WNT2B. 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 PORCN in WNT3A stimulated HEK 293 cells. Based on the extablished 2nd order combinations of the PORCN, 3rd order combinations emerge using the machine learning based search engine. These 3rd order combinations might be of interest for further wet lab investigations.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. 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 every WNT family member, (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.

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

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

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