

# NKD inhibitor of WNT signaling pathway 1 / naked cuticle homolog 1 (NKD1) : Time behavioural study of 3rd order combinations in WNT3A stimulated HEK 293 cells

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## Abstract

NKD1 is a member of naked cuticle (NKD) family that inhibits the WNT signaling pathway, by binding to Dishevelled (DVL) family of proteins. 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 NKD1 related 3rd order combinations in a forest of  $^{71}C_3$  combinations using four different sensitivity methods; • show the conserved rankings for NKD1-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

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<sup>☆</sup>Time behavioural study of 3-odr NKD1 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 NKD1 related combinations using individual gene expressions measured in time, in WNT3A stimulated HEK 293 cells.

## 2. Introduction

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

### 2.1. A combinatorial problem

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

### 2.2. A possible solution

In this work, after estimating the individual effects of factors for a higher order combination, the individual indices are considered as discriminative features. A combination,

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

### **2.3. NKD inhibitor of WNT signaling pathway 1 / naked cuticle homolog 1 (NKD1)**

Naked cuticle (NKD) is a conserved family of intracellular proteins encoded in most animal genomes, the original mutants of which were discovered by 1995 Nobel laureates Christiane Nusslein-Volhard and Eric F. Wieschaus and colleagues in their genetic screens for pattern-formation mutants in *Drosophila melanogaster* Jurgens et al. [4]. During animal development, cells have to respond appropriately to localized secreted signals and WNT signals have been found to be crucial in development and neoplasia. Zeng et al. [5] show that NKD, a *Drosophila* segment-polarity gene, encodes an inducible antagonist for the WNT signal Wingless (WG). In fly embryos and imaginal discs *nkd* transcription is induced by WG while, overproduction of NKD in *Drosophila* and misexpression of NKD in the vertebrate *Xenopus laevis*, result in phenotypes resembling those of loss of WG/Wnt function. Using ectopic expression, Rousset et al. [6] found that NKD affected, in a cell-autonomous manner, a transduction step between the WNT signaling components *Drosophila* Dishevelled (DSH) and Zeste-white 3 kinase (ZW3). Their results showed that NKD acted directly through DSH to limit WG activity and determined how efficiently WNT signals stabilized Armadillo (Arm)/ $\beta$ -catenin and activated downstream genes.

In the fruit fly *Drosophila*, embryos defective in signaling mediated by the WNT protein Wingless (WG) exhibit severe segmentation defects. The *Drosophila* segment polarity gene NKD encodes an EF hand protein that regulates early WG activity by acting as an inducible antagonist. NKD antagonizes WG via a direct interaction with the Wnt signaling component *Drosophila* Dishevelled (DSH). Wharton Jr et al. [7] describe two mouse and human proteins, NKD-1/2, related to fly NKD. They observe that the most conserved region among the fly and vertebrate proteins, the EFX domain, includes the putative EF hand and flanking sequences where EFX corresponds to a minimal domain required for fly or vertebrate NKD to interact with the basic/PDZ domains of fly DSH or vertebrate DVL proteins in the yeast two-hybrid assay. Katoh [8] cloned and characterized human NKD-1/2. Both were predicted to encode 470- and 451- amino-acid polypeptide, respectively. NKD-1/2, showed 43.8% total amino-acid identity, were more homologous in the NH1, NH2, NH3, and NH4 domains. The conserved sequence blocks, from N-terminal to C-terminal, are as follows: • a N-terminal membrane anchoring motif, which in mammals is subject to myristoylation; Li et al. [9] demonstrate that a member of the NKD family is myristoylated. Both NKD1 and

NKD2 contain the consensus sequence for N-myristoyl transferase (Met-Gly-X-X-X-Ser/Thr), along with adjacent basic residues and a polyhistidine tract at their C termini. Their study identified an unexpected function of NKD2 that is not shared by NKD1, that is, myristoylation-dependent escorting of TGF $\alpha$  to the basolateral surface of polarized epithelial cells. Mammalian NKD homologs have N-terminal consensus sequences that direct the post-translational addition of a lipophilic myristoyl moiety, but fly and mosquito NKD, while sharing N-terminal sequence homology, lack a myristoylation consensus sequence. Chan et al. [10] provide evidence that fly NKD acts cell-autonomously in the embryo, with its N-terminus able to confer unique functional properties and membrane association that cannot be mimicked in vivo by heterologous myristoylation consensus sequences. • a single, extended EF hand motif (called "EFX" or "NH2") that binds DSH/DVL proteins; in Wharton Jr et al. [7] as mentioned earlier and Yan et al. [11] identified an interacting protein, mNKD, that is 30% identical to Drosophila NKD and both mNKD and Drosophila NKD contain a single EF-hand (common helix-loop-helix calcium-binding motif) calcium-binding motif which has the most similarity to the EF-hand found in the Recoverin family of calcium-binding proteins. The domain of mNKD that interacts with mDVL in the yeast two-hybrid experiments is located between amino acids 107 and 230 and includes the EF-hand. • a thirty amino acid motif in the fly NKD protein mediates nuclear translocation; Nkd can bind and inhibit the WNT signal transducer Dishevelled (DSH), but the mechanism by which NKD limits Wnt signaling in the fly embryo is not understood. Waldrop et al. [12] show that NKD mutants exhibit elevated levels of the beta-catenin homolog Armadillo but no alteration in DSH abundance or distribution. While DSH-binding regions of NKD contribute to its activity, they identified a conserved 30-amino-acid motif, separable from DSH-binding regions, that is essential for NKD function and nuclear localization. Replacement of the 30-amino-acid motif with a conventional nuclear localization sequence rescued a small fraction of NKD mutant animals to adulthood. And, Guo et al. [13] show that one function of the vertebrate NKD 30-amino-acid motif is to oppose NKD1-EFX/DVL interactions, which is itself apparently opposed by further C-terminal sequence that is deleted in their MSI-CRC tumors. • a C-terminal Histidine-rich sequence of unknown function.

Wnt signaling controls a wide range of developmental processes and its aberrant regulation can lead to disease. To better understand the regulation of this pathway, Van Raay et al. [14] identified zebrafish homologues of NKD, i.e NKD-1/2, which have previously been shown to inhibit canonical WNT/ $\beta$ -catenin signaling. They observed that zebrafish NKD1 expression increased substantially after the mid-blastula transition in a pattern mirroring that of activated canonical WNT/ $\beta$ -catenin signaling, being expressed in both the ventrolateral blastoderm margin and also in the axial mesendoderm. In contrast, zebrafish NKD2 was maternally and ubiquitously expressed. Overexpression of NKD-1/2A suppressed canonical WNT/ $\beta$ -catenin signaling at multiple stages of early zebrafish development and also exacerbated the cyclopia and axial mesendoderm convergence and extension (C&E) defect in the non-canonical WNT/PCP mutant *silberblick* (SLB/WNT11). Further, the establishment of the left-right (LR) axis in zebrafish embryos relies on signals from the dorsal forerunner cells (DFC) and the Kupffer's vesicle (KV). Schneider et al. [15] analyzed the expression patterns of three zebrafish NKD homologs and found enriched expression of NKD1 in DFCs and KV.

They found DVL degraded upon NKD1 overexpression in zebrafish. Their findings showed that NKD1 acted as a  $\beta$ -catenin antagonist in the DFCs necessary for LR patterning.

I present 3rd order combinations of NKD1 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 [16]. 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 [16].

### 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  $\geq 2$  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 generate 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 3<sup>rd</sup> order interactions are presented here. The results first discuss the behaviour of interactions across the snapshots of time using the computed sensi-

tivities on fold change measurements per time snapshot. The analysis was done using 4 different sensitivity indices. Out of the  $^{71}\text{C}_3$  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  $\mu$  and population standard deviation  $\sigma$ . 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 NKD1-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 [17]) and Sobol indices (with 2002 implementation in Saltelli [18] and martinez implementation in Martinez [19] and Baudin et al. [20]).

## 6.3. Conserved machine learning rankings for tested NKD1-X-X combinations

A total of 2415, 3rd order combinations involving NKD1 were obtained from a full set of  $^{71}\text{C}_3 = 57155$  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.

RANKING @ $t_i$ USING HSIC - LINEAR												
3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	
FZD7-NKD1-SEN2	78	10179	4761	28020	28474	FSHB-NKD1-PPP2R1A	134	11978	48147	4744	11658	
DIXDC1-NKD1-SEN2	139	26878	5252	13422	20937	FZD7-NKD1-WNT2B	140	22580	30435	30959	23507	
DIXDC1-NKD1-TLE2	211	181	25309	23711	748	DIXDC1-NKD1-TCF7L1	239	17174	2661	14197	5839	
FZD7-NKD1-PPP2CA	369	8802	35791	35537	25608	FZD7-NKD1-SFRP4	384	21227	4937	21897	25651	
FSHB-NKD1-TLE2	425	30274	24343	8644	12849	DIXDC1-NKD1-PPP2CA	440	13162	40123	35144	6197	
FZD5-NKD1-WNT2B	450	18227	31166	30382	5811	FSHB-NKD1-SEN2	574	7025	14205	7566	35992	
DKK1-NKD1-SEN2	575	5975	43246	35996	27099	FBXW11-NKD1-SEN2	618	34583	6281	9193	28276	
FZD7-NKD1-TCF7L1	657	23179	2067	50336	28795	FSHB-NKD1-WNT2B	699	45757	26960	8191	17214	
FRZB-NKD1-SEN2	705	6025	7511	12942	30682	FZD7-NKD1-FBXW4	729	9858	18476	41281	18997	
FZD5-NKD1-TLE2	785	4126	18422	33090	3434	FZD2-NKD1-WNT2	792	33006	2260	28026	532	
LEF1-NKD1-PPP2R1A	793	3084	55944	23057	25903	FRZB-NKD1-WNT2B	861	8764	29474	26264	14733	
FZD1-NKD1-SEN2	865	3117	15529	25963	26289	FOSL1-NKD1-SFRP4	875	160	5276	11851	11033	
DIXDC1-NKD1-WNT2	883	442	1751	18827	1642	DKK1-NKD1-WNT2B	912	10412	49963	52787	8425	
CSNK1G1-NKD1-PPP2CA	926	17356	35232	35369	12912	FSHB-NKD1-RHOU	974	40540	14067	9128	35039	
FSHB-NKD1-SFRP1	1011	27987	21471	7065	1983	FBXW11-NKD1-WNT2B	1027	51970	24642	25865	43366	
CCND3-NKD1-WNT2B	1067	19542	49729	33902	7166	FZD7-NKD1-WNT2	1099	15793	1721	33173	28181	
FZD7-NKD1-TLE1	1127	6936	12734	50934	40716	FZD5-NKD1-SFRP4	1137	15039	9065	28525	21751	
FSHB-NKD1-WNT5A	1146	8921	29165	19619	2206	CSNK1G1-NKD1-TCF7L1	1194	9892	6186	28656	13545	
FZD1-NKD1-SFRP4	1200	13902	31686	31642	11656	CSNK1G1-NKD1-SEN2	1257	16631	15972	26700	19860	
FZD1-NKD1-WNT2B	1274	10870	41498	34943	5517	FRZB-NKD1-SFRP4	1296	647	21775	12472	26405	
FZD7-NKD1-RHOU	1307	26801	3329	51286	46329	FZD2-NKD1-RHOU	1310	19610	4807	33843	1134	
FSHB-NKD1-TCF7L1	1387	37613	10650	8338	29279	KREMEN1-NKD1-WNT2B	1400	34496	34759	43275	20181	
LRP5-NKD1-SEN2	1447	1797	8631	4376	40581	KREMEN1-NKD1-SEN2	1463	18745	6060	20808	42111	
CSNK1G1-NKD1-SFRP4	1474	513	13103	24557	6931	LRP5-NKD1-SFRP4	1486	21467	6314	10196	31377	
FZD2-NKD1-TLE2	1505	29421	21252	27865	2130	FZD1-NKD1-TLE2	1523	3791	21524	33601	4582	
FSHB-NKD1-TCF7	1528	46050	40792	9733	32939	DIXDC1-NKD1-WNT4	1536	13509	3143	4994	28564	
FBXW2-NKD1-WNT2B	1547	22973	29232	13160	53054	FZD7-NKD1-TCF7	1550	5115	43100	44210	33757	
FZD7-NKD1-WNT3A	1664	5491	2056	28279	37124	LRP5-NKD1-WNT2B	1686	21363	28403	16564	24813	
KREMEN1-NKD1-TCF7L1	1699	28241	2070	41242	30439	DIXDC1-NKD1-RHOU	1798	6195	3765	22405	3157	
LRP5-NKD1-TCF7L1	1804	29713	4980	13354	42899	DIXDC1-NKD1-WNT5A	1830	5103	33835	49403	2232	
FOSL1-NKD1-PPP2CA	1845	14335	40783	24581	23211	FZD5-NKD1-TCF7L1	1914	20430	1935	26746	22679	
FRZB-NKD1-PPP2CA	1923	12714	42040	19751	23511	APC-NKD1-TLE2	1939	24	20649	13050	16733	
CCND3-NKD1-WNT3A	1995	32953	30709	40159	23393	NKD1-WIF1-WNT3A	2028	3129	43733	48011	30914	
FZD8-NKD1-WNT2B	2038	34027	33288	19731	46263	FZD5-NKD1-FBXW4	2042	6515	20216	24601	16637	
CTBP1-NKD1-SFRP4	2057	1716	14512	24063	41784	FZD7-NKD1-SLC9A3R1	2065	23275	9662	24185	39745	
FZD5-NKD1-RHOU	2086	9792	3471	18764	2521	DIXDC1-NKD1-PPP2R1A	2140	5484	54992	25998	568	
DKK1-NKD1-PPP2CA	2158	21388	52610	49493	9273	FZD1-NKD1-RHOU	2176	20088	11316	49737	2429	
CXXC4-NKD1-WNT2B	2217	9878	36698	23848	1631	FRZB-NKD1-TLE2	2272	121	33128	21637	12907	
CSNK1G1-NKD1-RHOU	2328	3565	14141	34128	1880	FOSL1-NKD1-SEN2	2466	6869	5781	10026	30259	
FZD2-NKD1-SEN2	2523	36940	7846	15207	17515	FSHB-NKD1-WNT2	2558	23961	6398	3570	16109	
KREMEN1-NKD1-TLE2	2619	18016	16139	44657	37610	FZD5-NKD1-WNT4	2642	3099	2732	32040	47167	
KREMEN1-NKD1-WNT3A	2675	23678	2816	47552	40285	DIXDC1-NKD1-TCF7	2704	16324	46726	24262	6288	
FZD5-NKD1-TCF7	2753	4697	38384	26960	22996	FBXW2-NKD1-PPP2CA	2839	23721	34814	16169	56279	
CTBP1-NKD1-TCF7L1	2845	7922	3120	35646	35460	CTBP2-FZD7-NKD1	2976	52126	40799	40259	46777	
GSK3B-NKD1-TLE2	2977	22800	23954	42499	38689	FBXW2-NKD1-SEN2	2988	37184	10657	11033	56876	
NKD1-WIF1-WNT4	2997	2461	45561	55000	34857	FZD5-NKD1-WNT5A	3021	6206	35575	52940	2092	
CXXC4-NKD1-SEN2	3069	14633	9774	13334	17352	CCND3-NKD1-SFRP4	3074	31551	41849	39607	12267	
DVL1-NKD1-WNT2B	3089	2831	24130	36326	51502	KREMEN1-NKD1-FBXW4	3108	22301	16832	29059	23025	
CSNK2A1-NKD1-WNT2B	3148	4519	40339	22219	23905	CXXC4-NKD1-TLE2	3160	26	22413	13930	94	
NKD1-PPP2CA-SFRP4	3169	46409	13326	29656	43339	CTNNBIP1-NKD1-RHOU	3194	27112	11412	35287	15481	
CCND3-NKD1-PPP2CA	3285	34449	42225	49257	26308	NKD1-WNT2B-WNT4	3336	43254	28275	6099	51615	
CCND1-NKD1-PPP2CA	3363	11274	37598	54652	44854	FZD7-NKD1-WNT5A	3371	2073	37837	34079	40424	
FZD2-NKD1-WIF1	3430	39091	22109	26358	3	FZD2-NKD1-WNT2B	3444	30135	33767	29289	1990	
NKD1-WNT2B-WNT3A	3477	51633	33941	7298	45111	FRZB-NKD1-RHOU	3499	2171	5014	35449	27330	
CSNK1G1-NKD1-WNT2	3527	1109	9218	26094	482	FZD5-NKD1-PPP2R1A	3621	21515	54524	36048	1557	
CCND3-NKD1-WNT2	3637	32192	36071	49096	2809	FSHB-NKD1-FBXW4	3657	26327	16817	8776	23172	
FZD8-NKD1-PPP2CA	3681	49836	33279	27543	15965	FZD7-NKD1-WNT4	3704	14766	3963	41558	50663	
FBXW11-NKD1-TCF7L1	3783	32677	2358	19136	41733	LEF1-NKD1-WNT3	3813	14659	40690	18495	25196	
FBXW2-NKD1-WNT4	3822	22628	8291	3237	57029	KREMEN1-NKD1-WNT4	3871	11426	4535	41285	47213	
DIXDC1-NKD1-WIF1	3909	12873	22358	12920	23739	DAAM1-NKD1-WNT2B	3944	45573	30236	23673	33353	
CXXC4-NKD1-RHOU	3957	5011	6876	36465	23781	LEF1-NKD1-SFRP1	3999	1638	28264	15491	1219	
GSK3B-NKD1-TCF7L1	4012	30306	1476	36252	37466	CSNK2A1-NKD1-PPP2CA	4025	15782	45180	27270	33423	
FBXW2-NKD1-TLE2	4091	22177	18065	12184	55575	CSNK2A1-NKD1-PPP2R1A	4118	2001	56920	13318	8736	

Table 1: Rankings of NKD1-X-X. A list of approximately first 125 combinations with rankings below 10,000 out of 57,155. SA - HSIC; Kernel - linear



RANKING @ $t_i$ USING HSIC - RBF												
3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	
FZD7-NKD1-SEN2	22248	3166	47262	13293	2567	FSHB-NKD1-PPP2R1A	25236	3206	25555	29398	54336	
DIXDC1-NKD1-SEN2	24325	22750	54264	38554	4248	FZD7-NKD1-WNT2B	47917	26281	13366	54412	1630	
DIXDC1-NKD1-TLE2	22103	8452	55942	25492	11873	DIXDC1-NKD1-TCF7L1	32308	45794	57018	24322	3679	
FZD7-NKD1-PPP2CA	15127	35957	31673	37533	1778	FZD7-NKD1-SFRP4	22923	15141	38823	43517	2354	
FSHB-NKD1-TLE2	17403	24824	7167	5397	44661	DIXDC1-NKD1-PPP2CA	9807	43231	37477	30170	1078	
FZD5-NKD1-WNT2B	18497	19475	22567	24649	24247	FSHB-NKD1-SEN2	14879	5325	16207	3306	6877	
DKK1-NKD1-SEN2	7012	22034	30235	20793	14207	FBXW11-NKD1-SEN2	3968	23882	33729	15929	5877	
FZD7-NKD1-TCF7L1	40741	32897	48058	29669	1354	FSHB-NKD1-WNT2B	24792	50507	25909	42911	36692	
FRZB-NKD1-SEN2	3644	10458	50187	29431	36573	FZD7-NKD1-FBXW4	39942	9295	41002	45990	5647	
FZD5-NKD1-TLE2	3831	2363	53067	35683	47894	FZD2-NKD1-WNT2	54548	26947	57006	32814	25385	
LEF1-NKD1-PPP2R1A	24944	9286	39491	44583	37109	FRZB-NKD1-WNT2B	26947	18964	19602	32120	37731	
FZD1-NKD1-SEN2	16283	248	42563	17609	26499	FOSL1-NKD1-SFRP4	38557	1693	50390	24818	21820	
DIXDC1-NKD1-WNT2	30452	9192	55932	6605	10010	DKK1-NKD1-WNT2B	33321	46336	43515	12948	3783	
CSNK1G1-NKD1-PPP2CA	8799	37693	42013	33688	15900	FSHB-NKD1-RHOU	13620	39126	15440	38560	46234	
FSHB-NKD1-SFRP1	28217	31095	6105	12696	47885	FBXW11-NKD1-WNT2B	42436	56089	5208	5851	6025	
CCND3-NKD1-WNT2B	31750	9905	43256	46510	4045	FZD7-NKD1-WNT2	38995	9386	56253	3799	2844	
FZD7-NKD1-TLE1	27415	7679	53298	43858	894	FZD5-NKD1-SFRP4	15328	9399	47362	16033	40262	
FSHB-NKD1-WNT5A	45339	3228	1592	43013	28219	CSNK1G1-NKD1-TCF7L1	6006	22577	49200	31622	30401	
FZD1-NKD1-SFRP4	43048	8656	45237	21687	17892	CSNK1G1-NKD1-SEN2	4079	7354	54740	12528	13804	
FZD1-NKD1-WNT2B	35381	16131	32653	43586	18237	FRZB-NKD1-SFRP4	30663	4537	52953	18729	25710	
FZD7-NKD1-RHOU	29725	14170	51670	56277	8735	FZD2-NKD1-RHOU	50910	12898	54711	9572	46912	
FSHB-NKD1-TCF7L1	29187	51071	22006	21070	31900	KREMEN1-NKD1-WNT2B	10715	45017	15095	25349	17172	
LRP5-NKD1-SEN2	14990	3717	34426	11779	16566	KREMEN1-NKD1-SEN2	730	3287	51067	20876	22424	
CSNK1G1-NKD1-SFRP4	10332	8994	35937	45547	19919	LRP5-NKD1-SFRP4	26254	21185	21350	26108	17079	
FZD2-NKD1-TLE2	24712	20087	50718	44876	38227	FZD1-NKD1-TLE2	24945	3728	49365	3491	46221	
FSHB-NKD1-TCF7	16167	43862	3127	18402	9845	DIXDC1-NKD1-WNT4	28297	20930	51455	19765	821	
FBXW2-NKD1-WNT2B	33781	33859	11109	13486	210	FZD7-NKD1-TCF7	37444	15437	35879	47781	305	
FZD7-NKD1-WNT3A	16651	467	45031	55399	13378	LRP5-NKD1-WNT2B	32145	41013	38068	34089	24010	
KREMEN1-NKD1-TCF7L1	6544	33167	55781	7544	9258	DIXDC1-NKD1-RHOU	22817	19488	48855	52946	7640	
LRP5-NKD1-TCF7L1	17394	31853	35078	16573	14922	DIXDC1-NKD1-WNT5A	50477	15744	20410	52735	35648	
FOSL1-NKD1-PPP2CA	21934	29546	42661	35675	17456	FZD5-NKD1-TCF7L1	18061	23794	54846	25790	41167	
FRZB-NKD1-PPP2CA	1942	36043	22753	20309	35265	APC-NKD1-TLE2	23869	4453	50209	22108	50421	
CCND3-NKD1-WNT3A	19177	14820	26493	56175	64	NKD1-WIF1-WNT3A	48793	3970	17300	56778	21427	
FZD8-NKD1-WNT2B	49791	35392	25199	27885	3968	FZD5-NKD1-FBXW4	9460	12253	43946	48270	55667	
CTBP1-NKD1-SFRP4	34262	7238	53046	16240	32125	FZD7-NKD1-SLC9A3R1	40405	7627	50823	42113	3058	
FZD5-NKD1-RHOU	17419	5033	39813	31860	48308	DIXDC1-NKD1-PPP2R1A	41282	2633	37452	52221	22216	
DKK1-NKD1-PPP2CA	845	43108	38644	15851	21492	FZD1-NKD1-RHOU	9230	9523	50610	53836	44264	
CXXC4-NKD1-WNT2B	33112	30020	8852	27851	43373	FRZB-NKD1-TLE2	14829	3484	49881	39277	47223	
CSNK1G1-NKD1-RHOU	7968	13163	45029	45281	48042	FOSL1-NKD1-SEN2	15372	3697	55084	25337	9352	
FZD2-NKD1-SEN2	25897	35442	55987	51047	12409	FSHB-NKD1-WNT2	18619	15105	23935	22810	37921	
KREMEN1-NKD1-TLE2	2919	14915	47965	33551	33612	FZD5-NKD1-WNT4	12678	521	57060	25537	26670	
KREMEN1-NKD1-WNT3A	9700	7837	52991	45907	10853	DIXDC1-NKD1-TCF7	22420	28291	44348	38473	4381	
FZD5-NKD1-TCF7	16271	1612	53778	20615	31084	FBXW2-NKD1-PPP2CA	15124	44885	17926	12733	2217	
CTBP1-NKD1-TCF7L1	22297	13060	56917	8766	27600	CTBP2-FZD7-NKD1	31868	50353	6812	34202	44114	
GSK3B-NKD1-TLE2	7424	17188	52854	26876	49828	FBXW2-NKD1-SEN2	11695	33380	11958	18807	1265	
NKD1-WIF1-WNT4	20047	23652	20170	17000	48569	FZD5-NKD1-WNT5A	33749	1909	44565	56409	39882	
CXXC4-NKD1-SEN2	23566	21575	56539	18663	8724	CCND3-NKD1-SFRP4	21810	15481	41109	37803	2086	
DVL1-NKD1-WNT2B	38080	13959	43055	47296	2221	KREMEN1-NKD1-FBXW4	16975	10848	31634	30290	26950	
CSNK2A1-NKD1-WNT2B	17855	5115	19163	36411	21386	CXXC4-NKD1-TLE2	14475	5894	51388	28114	51127	
NKD1-PPP2CA-SFRP4	40324	48641	51898	9183	19710	CTNNBIP1-NKD1-RHOU	49378	32634	47683	47402	32347	
CCND3-NKD1-PPP2CA	19772	35706	47585	36014	3731	NKD1-WNT2B-WNT4	31109	52225	48686	5473	15716	
CCND1-NKD1-PPP2CA	31094	31919	45839	10804	5523	FZD7-NKD1-WNT5A	53120	6594	44749	54824	2305	
FZD2-NKD1-WIF1	45250	38648	56326	24018	7601	FZD2-NKD1-WNT2B	53572	28271	33551	10732	5520	
NKD1-WNT2B-WNT3A	50905	55634	51652	46273	6139	FRZB-NKD1-RHOU	9972	3765	49769	37400	50607	
CSNK1G1-NKD1-WNT2	6387	6428	51082	8449	39348	FZD5-NKD1-PPP2R1A	23335	9105	47323	31744	56453	
CCND3-NKD1-WNT2	36776	17718	45542	2330	4094	FSHB-NKD1-FBXW4	37869	19703	30672	38958	40988	
FZD8-NKD1-PPP2CA	31727	55399	17443	4434	10950	FZD7-NKD1-WNT4	19019	5699	47235	25398	489	
FBXW11-NKD1-TCF7L1	8257	42198	35700	9053	14548	LEF1-NKD1-WNT3	33634	12910	24295	24445	28299	
FBXW2-NKD1-WNT4	15630	22910	34768	11469	618	KREMEN1-NKD1-WNT4	1146	12492	44039	43786	11253	
DIXDC1-NKD1-WIF1	44180	9421	57087	48747	10766	DAAM1-NKD1-WNT2B	21385	46690	24575	48240	1108	
CXXC4-NKD1-RHOU	49373	16254	50519	35698	48470	LEF1-NKD1-SFRP1	33942	6111	31628	47136	38942	
GSK3B-NKD1-TCF7L1	43296	37741	56267	44754	33094	CSNK2A1-NKD1-PPP2CA	6422	22743	30647	42195	19402	
FBXW2-NKD1-TLE2	16737	27286	16157	17903	3635	CSNK2A1-NKD1-PPP2R1A	19188	9240	34119	49629	44582	

Table 2: Rankings of NKD1-X-X. A list of approximately first 125 combinations with rankings below 10,000 out of 57,155. SA - HSIC; Kernel - rbf

### 6.3.1. Examining the behaviour of DIXDC1-NKD1-X combinations

Dishevelled (DVL-1/2/3) are DIX-domain proteins implicated in the WNT signalling pathway. NKD1 is a member of naked cuticle (NKD) family that inhibits the

RANKING @ $t_i$ USING SOBOL - 2002											
3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$
FZD7-NKD1-SEN2	1050	48745	26187	16335	39423	FSHB-NKD1-PPP2R1A	46297	55865	50849	48169	20237
DIXDC1-NKD1-SEN2	12200	40063	24628	25782	18022	FZD7-NKD1-WNT2B	56201	48723	45825	38106	22510
DIXDC1-NKD1-TLE2	43632	33173	40815	48692	18613	DIXDC1-NKD1-TCF7L1	55683	3768	38514	52231	4444
FZD7-NKD1-PPP2CA	9005	18778	1246	2338	53443	FZD7-NKD1-SFRP4	1652	49835	17828	19974	25039
FSHB-NKD1-TLE2	44409	50330	45264	42198	2365	DIXDC1-NKD1-PPP2CA	13275	33550	23785	28183	10803
FZD5-NKD1-WNT2B	1021	7682	6037	13559	34507	FSHB-NKD1-SEN2	15201	10828	10232	10064	44517
DKK1-NKD1-SEN2	52464	1370	44052	49768	19418	FBXW11-NKD1-SEN2	43774	38481	39045	45242	3193
FZD7-NKD1-TCF7L1	55972	47406	30315	33918	1994	FSHB-NKD1-WNT2B	44401	54732	42873	52925	7821
FRZB-NKD1-SEN2	45346	338	32222	51005	1360	FZD7-NKD1-FBXW4	55515	7176	39286	37121	32527
FZD5-NKD1-TLE2	4927	12650	8066	13258	28908	FZD2-NKD1-WNT2	45791	28382	44993	52942	2234
LEF1-NKD1-PPP2R1A	9769	22805	4489	9080	56436	FRZB-NKD1-WNT2B	16138	5567	23173	23193	50096
FZD1-NKD1-SEN2	43989	55534	37195	51478	14369	FOSL1-NKD1-SFRP4	26523	39016	6871	18225	44405
DIXDC1-NKD1-WNT2	2739	39551	16389	12555	40067	DKK1-NKD1-WNT2B	16505	52805	21482	25919	33616
CSNK1G1-NKD1-PPP2CA	2818	12720	1066	16639	39238	FSHB-NKD1-RHOU	45675	10162	48275	54199	600
FSHB-NKD1-SFRP1	41925	46232	46892	47087	12907	FBXW11-NKD1-WNT2B	2665	20622	20959	28247	43772
CCND3-NKD1-WNT2B	2665	20622	20959	28247	43772	FZD7-NKD1-WNT2	960	8333	11286	19016	34814
FZD7-NKD1-TLE1	4766	12625	13408	20647	22594	FZD5-NKD1-SFRP4	54899	3865	34153	50519	15164
FSHB-NKD1-WNT5A	37314	18350	48369	53041	3700	CSNK1G1-NKD1-TCF7L1	44067	52737	54968	47023	15857
FZD1-NKD1-SFRP4	51271	55211	39032	48850	401	CSNK1G1-NKD1-SEN2	8054	22379	116	14940	56704
FZD1-NKD1-WNT2B	14960	396	11807	807	36198	FRZB-NKD1-SFRP4	40918	30306	34533	32518	5522
FZD7-NKD1-RHOU	54154	29225	44144	41641	766	FZD2-NKD1-RHOU	21681	13556	14938	2459	47881
FSHB-NKD1-TCF7L1	46622	3458	47720	55073	7419	KREMEN1-NKD1-WNT2B	3720	31186	24394	8640	52443
LRP5-NKD1-SEN2	36325	43295	47491	56731	21232	KREMEN1-NKD1-SEN2	56836	50251	32521	51554	1744
CSNK1G1-NKD1-SFRP4	10766	6807	490	470	32258	LRP5-NKD1-SFRP4	55207	46779	55016	42654	8506
FZD2-NKD1-TLE2	8880	55035	12321	16298	48987	FZD1-NKD1-TLE2	12264	6284	17866	9330	48663
FSHB-NKD1-TCF7	10577	53765	9421	2083	49699	DIXDC1-NKD1-WNT4	4313	56293	22786	21278	56181
FBXW2-NKD1-WNT2B	6500	43410	325	13681	45117	FZD7-NKD1-TCF7	1197	9793	26864	23174	55177
FZD7-NKD1-WNT3A	56393	1785	42882	38968	851	LRP5-NKD1-WNT2B	6733	21320	13120	11007	47150
KREMEN1-NKD1-TCF7L1	584	45730	97	3743	56440	DIXDC1-NKD1-RHOU	51754	30025	31933	35781	48497
LRP5-NKD1-TCF7L1	18179	24091	8776	4357	46354	DIXDC1-NKD1-WNT5A	52842	853	34383	35927	975
FOSL1-NKD1-PPP2CA	2142	38096	5085	20731	35691	FZD5-NKD1-TCF7L1	375	6072	26396	9687	46953
FRZB-NKD1-PPP2CA	40960	47308	36156	34176	2451	APC-NKD1-TLE2	24708	34239	9544	22200	54054
CCND3-NKD1-WNT3A	17921	19886	26294	22858	34282	NKD1-WIF1-WNT3A	37352	21808	33337	55217	6162
FZD8-NKD1-WNT2B	7879	40127	24943	28578	45650	FZD5-NKD1-FBXW4	1999	46844	9338	12423	26882
CTBP1-NKD1-SFRP4	44098	40610	36176	52087	23553	FZD7-NKD1-SLC9A3R1	9571	55800	18296	26725	33995
FZD5-NKD1-RHOU	5628	29397	12428	11897	46956	DIXDC1-NKD1-PPP2R1A	43958	23448	33394	28989	46225
DKK1-NKD1-PPP2CA	56475	3125	43099	41619	4718	FZD1-NKD1-RHOU	13199	1642	19944	5713	42741
CXXC4-NKD1-WNT2B	15436	10525	10086	1323	45129	FRZB-NKD1-TLE2	6902	11605	22453	24380	21223
CSNK1G1-NKD1-RHOU	40596	5950	54719	48495	7475	FOSL1-NKD1-SEN2	16870	1004	11411	25457	43247
FZD2-NKD1-SEN2	35446	44507	42211	54707	9424	FSHB-NKD1-WNT2	12767	2441	14264	4232	49363
KREMEN1-NKD1-TLE2	15875	10503	2532	217	43597	FZD5-NKD1-WNT4	56303	4227	51100	48739	7738
KREMEN1-NKD1-WNT3A	3850	55089	612	11917	55918	DIXDC1-NKD1-TCF7	1474	53318	18628	4936	52772
FZD5-NKD1-TCF7	41668	6794	49395	38003	19355	FBXW2-NKD1-PPP2CA	48789	9344	52507	46333	3977
CTBP1-NKD1-TCF7L1	9556	37066	25397	25632	40919	CTBP2-FZD7-NKD1	18838	33237	3879	25058	35439
GSK3B-NKD1-TLE2	3305	49506	15390	28096	54349	FBXW2-NKD1-SEN2	52029	55516	53508	42787	13989
NKD1-WIF1-WNT4	27161	54046	639	3320	54064	FZD5-NKD1-WNT5A	7463	43025	2190	5552	51516
CXXC4-NKD1-SEN2	53247	30638	52373	44913	3619	CCND3-NKD1-SFRP4	38147	26441	33850	41412	22456
DVL1-NKD1-WNT2B	19138	50971	6751	18628	46290	KREMEN1-NKD1-FBXW4	15922	39691	1142	12162	54069
CSNK2A1-NKD1-WNT2B	25433	37	1485	5548	41572	CXXC4-NKD1-TLE2	667	11124	4405	10259	37949
NKD1-PPP2CA-SFRP4	36127	4970	52255	56608	11433	CTNNBIP1-NKD1-RHOU	26327	12981	6330	1732	44989
CCND3-NKD1-PPP2CA	42363	24532	31404	38395	21255	NKD1-WNT2B-WNT4	52604	6381	47217	35940	20660
CCND1-NKD1-PPP2CA	45411	49544	40077	40095	7701	FZD7-NKD1-WNT5A	44945	5271	38820	34295	16856
FZD2-NKD1-WIF1	48304	2115	44826	40847	8209	FZD2-NKD1-WNT2B	13761	1602	14480	12232	32670
NKD1-WNT2B-WNT3A	4519	50837	9924	21240	36721	FRZB-NKD1-RHOU	11852	56820	24882	6154	55783
CSNK1G1-NKD1-WNT2	15676	18894	5930	17029	46372	FZD5-NKD1-PPP2R1A	8457	17431	20808	3578	44409
CCND3-NKD1-WNT2	35215	22812	32133	34875	9400	FSHB-NKD1-FBXW4	50332	51183	48187	53103	1776
FZD8-NKD1-PPP2CA	44458	15474	35698	28867	8497	FZD7-NKD1-WNT4	12248	51694	18286	22845	40524
FBXW11-NKD1-TCF7L1	25004	18804	13085	18091	43725	LEF1-NKD1-WNT3	55855	55317	33628	55268	3949
FBXW2-NKD1-WNT4	34271	50552	56691	52020	14412	KREMEN1-NKD1-WNT4	53306	2099	56540	45244	1241
DIXDC1-NKD1-WIF1	2783	6193	1919	15666	44738	DAAM1-NKD1-WNT2B	16864	51727	8394	24436	37248
CXXC4-NKD1-RHOU	3913	27370	4774	12281	53733	LEF1-NKD1-SFRP1	3039	2584	11321	3258	51239
GSK3B-NKD1-TCF7L1	643	5561	18709	6000	55933	CSNK2A1-NKD1-PPP2CA	35993	44148	33793	48908	1488
FBXW2-NKD1-TLE2	4815	3719	8065	7672	53473	CSNK2A1-NKD1-PPP2R1A	22699	6006	1296	8582	18490

Table 3: Rankings of NKD1-X-X. A list of approximately first 125 combinations with rankings below 10,000 out of 57,155. SA - SOBOL; Implementation - 2002

WNT signaling pathway, by binding to Dishevelled (DVL) family of proteins, as has been mentioned in the foregoing literature. DIX domain containing 1 (DIXDC1) also belongs to Dishevelled segment polarity proteins. Looking at the tables above, one finds the following combinations for DIXDC1 along with NKD1, to be promi-

RANKING @ $t_i$ USING SOBOL - MARTINEZ												
3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	
FZD7-NKD1-SEN2	35427	30303	49552	55744	274	FSHB-NKD1-PPP2R1A	53109	51767	230	25045	9445	
DIXDC1-NKD1-SEN2	6022	11818	20358	22850	47306	FZD7-NKD1-WNT2B	56222	35279	4725	54614	43292	
DIXDC1-NKD1-TLE2	6700	20026	2140	33064	56018	DIXDC1-NKD1-TCF7L1	29646	5112	10423	27152	45909	
FZD7-NKD1-PPP2CA	41077	46695	30291	56451	41703	FZD7-NKD1-SFRP4	18058	19466	26447	53480	5794	
FSHB-NKD1-TLE2	45128	54511	27034	495	9751	DIXDC1-NKD1-PPP2CA	5108	14247	10722	14891	52539	
FZD5-NKD1-WNT2B	27769	32100	5018	27110	12151	FSHB-NKD1-SEN2	26326	50101	3632	18950	54806	
DKK1-NKD1-SEN2	11947	15501	1575	31335	38725	FBXW11-NKD1-SEN2	55745	36584	9866	51299	52063	
FZD7-NKD1-TCF7L1	51873	43708	36994	50628	22614	FSHB-NKD1-WNT2B	55721	42851	2627	5107	7553	
FRZB-NKD1-SEN2	55847	31620	30451	51922	16894	FZD7-NKD1-FBXW4	41536	27883	22504	53165	22672	
FZD5-NKD1-TLE2	9448	17741	9119	46085	26061	FZD2-NKD1-WNT2	53419	19878	8108	23571	7764	
LEF1-NKD1-PPP2R1A	2716	16678	11945	8557	27909	FRZB-NKD1-WNT2B	3508	55779	21413	56958	54884	
FZD1-NKD1-SEN2	49139	38431	23193	31391	8701	FOSL1-NKD1-SFRP4	46318	21183	12035	36344	30342	
DIXDC1-NKD1-WNT2	41110	34517	15046	10924	44951	DKK1-NKD1-WNT2B	2	21468	4661	31962	47604	
CSNK1G1-NKD1-PPP2CA	19784	21076	9213	14040	31099	FSHB-NKD1-RHOU	55892	43953	469	18180	10414	
FSHB-NKD1-SFRP1	28002	16077	279	14622	6815	FBXW11-NKD1-WNT2B	46316	49834	31463	34942	54972	
CCND3-NKD1-WNT2B	29468	17702	29050	41716	43213	FZD7-NKD1-WNT2	23577	23624	26052	52649	4506	
FZD7-NKD1-TLE1	5046	8910	20408	56472	4025	FZD5-NKD1-SFRP4	26852	51362	10357	39721	29152	
FSHB-NKD1-WNT5A	54779	40235	14719	3551	9105	CSNK1G1-NKD1-TCF7L1	25433	42313	19013	1420	12490	
FZD1-NKD1-SFRP4	50515	31728	30075	46148	11768	CSNK1G1-NKD1-SEN2	5658	19388	11502	16684	22979	
FZD1-NKD1-WNT2B	20807	52131	38823	55834	199	FRZB-NKD1-SFRP4	55472	49543	31547	55964	12016	
FZD7-NKD1-RHOU	25956	55118	38253	54733	33656	FZD2-NKD1-RHOU	15517	54256	22224	17209	52842	
FSHB-NKD1-TCF7L1	46897	40396	1839	679	7727	KREMEN1-NKD1-WNT2B	41860	50698	42188	13420	1100	
LRP5-NKD1-SEN2	5008	10901	23301	1343	19759	KREMEN1-NKD1-SEN2	36876	47876	34322	22045	9642	
CSNK1G1-NKD1-SFRP4	3477	44788	33518	12420	52257	LRP5-NKD1-SFRP4	20279	51061	45027	1459	10569	
FZD2-NKD1-TLE2	31121	27914	7868	7728	49947	FZD1-NKD1-TLE2	12654	19657	19161	47099	36753	
FSHB-NKD1-TCF7	32538	48825	14879	5232	4084	DIXDC1-NKD1-WNT4	46229	42232	4694	26610	37481	
FBXW2-NKD1-WNT2B	51198	7198	8275	25665	44537	FZD7-NKD1-TCF7	43352	19438	6560	56891	35938	
FZD7-NKD1-WNT3A	55002	31493	37129	54525	25394	LRP5-NKD1-WNT2B	6506	56675	30108	35596	51172	
KREMEN1-NKD1-TCF7L1	47281	23426	24140	15351	37941	DIXDC1-NKD1-RHOU	35301	33949	301	25928	32354	
LRP5-NKD1-TCF7L1	4692	47661	35306	20994	29169	DIXDC1-NKD1-WNT5A	15090	51048	5734	37831	37533	
FOSL1-NKD1-PPP2CA	45879	24082	5855	50930	2635	FZD5-NKD1-TCF7L1	41166	47945	3824	24905	6760	
FRZB-NKD1-PPP2CA	52371	40952	44274	55170	8712	APC-NKD1-TLE2	22717	4449	50188	18631	13063	
CCND3-NKD1-WNT3A	28743	22048	30991	7266	33090	NKD1-WIF1-WNT3A	37743	54707	30419	4236	24113	
FZD8-NKD1-WNT2B	9631	7680	43019	11274	51358	FZD5-NKD1-FBXW4	20216	56291	13242	56676	15298	
CTBP1-NKD1-SFRP4	40663	35868	42585	118	36007	FZD7-NKD1-SLC9A3R1	51974	28295	49824	56907	3877	
FZD5-NKD1-RHOU	10311	57096	13196	30493	43591	DIXDC1-NKD1-PPP2R1A	12774	34350	23086	9498	28407	
DKK1-NKD1-PPP2CA	33743	9918	7761	39827	40352	FZD1-NKD1-RHOU	2141	38390	13080	52508	46011	
CXXC4-NKD1-WNT2B	42290	20950	47826	15060	49390	FRZB-NKD1-TLE2	19078	25853	33372	56599	52124	
CSNK1G1-NKD1-RHOU	13195	46913	28099	45919	38961	FOSL1-NKD1-SEN2	48219	21087	6264	51148	5914	
FZD2-NKD1-SEN2	21431	54344	8812	21950	14042	FSHB-NKD1-WNT2	4151	11327	17224	21348	50813	
KREMEN1-NKD1-TLE2	23720	51881	8553	12334	55039	FZD5-NKD1-WNT4	50883	36657	40818	51578	32742	
KREMEN1-NKD1-WNT3A	47109	46468	16075	12688	6199	DIXDC1-NKD1-TCF7	22606	17292	25852	24237	19475	
FZD5-NKD1-TCF7	17979	50216	18662	45252	33789	FBXW2-NKD1-PPP2CA	11451	1927	41714	38264	27169	
CTBP1-NKD1-TCF7L1	51499	53024	50682	27504	46237	CTBP2-FZD7-NKD1	49733	2135	20015	12598	52930	
GSK3B-NKD1-TLE2	50690	14320	28807	14960	40193	FBXW2-NKD1-SEN2	14858	121	43584	34074	37056	
NKD1-WIF1-WNT4	29254	43808	28256	8965	5464	FZD5-NKD1-WNT5A	40730	52111	18455	48698	51553	
CXXC4-NKD1-SEN2	56237	29199	16253	47019	27127	CCND3-NKD1-SFRP4	37965	38810	5240	26177	55787	
DVL1-NKD1-WNT2B	29360	5337	3854	51297	53084	KREMEN1-NKD1-FBXW4	27950	22905	48525	6087	41176	
CSNK2A1-NKD1-WNT2B	26088	27029	12360	24175	6941	CXXC4-NKD1-TLE2	22761	23290	45133	3186	46183	
NKD1-PPP2CA-SFRP4	7636	40918	29991	8329	13251	CTNNBIP1-NKD1-RHOU	2582	14223	25655	7361	44666	
CCND3-NKD1-PPP2CA	44598	34434	5620	11985	55806	NKD1-WNT2B-WNT4	54071	38498	23678	10568	10595	
CCND1-NKD1-PPP2CA	30462	2994	26006	982	37703	FZD7-NKD1-WNT5A	10258	28094	47222	55721	8269	
FZD2-NKD1-WIF1	49375	42309	3775	2699	9393	FZD2-NKD1-WNT2B	17792	38553	13807	11551	46634	
NKD1-WNT2B-WNT3A	22521	5424	29294	6657	45978	FRZB-NKD1-RHOU	15559	52309	37443	46074	49516	
CSNK1G1-NKD1-WNT2	584	24793	8285	23891	20671	FZD5-NKD1-PPP2R1A	47879	55985	27626	45478	27533	
CCND3-NKD1-WNT2	39396	31534	15371	7194	56769	FSHB-NKD1-FBXW4	56274	37325	2275	13039	8834	
FZD8-NKD1-PPP2CA	23391	14419	3617	33513	22490	FZD7-NKD1-WNT4	2542	5320	32146	56533	50519	
FBXW11-NKD1-TCF7L1	14938	48616	37995	30219	6486	LEF1-NKD1-WNT3	46689	46620	34574	3520	10104	
FBXW2-NKD1-WNT4	6632	41775	44619	33738	31805	KREMEN1-NKD1-WNT4	16754	42641	37982	17345	22299	
DIXDC1-NKD1-WIF1	25261	12692	13277	18270	29788	DAAM1-NKD1-WNT2B	2169	25798	52217	52504	7200	
CXXC4-NKD1-RHOU	25608	2242	45591	46180	54896	LEF1-NKD1-SFRP1	26325	13772	7770	2719	53369	
GSK3B-NKD1-TCF7L1	52990	4868	17906	33552	7233	CSNK2A1-NKD1-PPP2CA	36783	42052	28232	9350	10631	
FBXW2-NKD1-TLE2	44198	2073	6935	10692	41199	CSNK2A1-NKD1-PPP2R1A	32525	9590	31893	25709	13680	

Table 4: Rankings of NKD1-X-X. A list of approximately first 125 combinations with rankings below 10,000 out of 57,155. SA - SOBOL; Implementation - martinez

nent at 3rd order level - DIXDC1-NKD1-SEN2, DIXDC1-NKD1-TLE2, DIXDC1-NKD1-WNT2, DIXDC1-NKD1-WIF1, DIXDC1-NKD1-TCF7L1, DIXDC1-NKD1-PPP2CA, DIXDC1-NKD1-WNT4, DIXDC1-NKD1-RHOU, DIXDC1-NKD1-WNT5A, DIXDC1-NKD1-PPP2R1A and DIXDC1-NKD1-TCF7. The only other interaction

with DVL family was DVL1-NKD1-WNT2B. 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 SFRP-NKD1-X combinations

Secreted Frizzled-related proteins (SFRP) are involved in embryonic development as well as pathological conditions including bone and myocardial disorders and cancer. Because of their sequence homology with the WNT-binding domain of Frizzled (FZD), they have generally been considered antagonists of canonical WNT signaling. Using human embryonic kidney cells (HEK293A), von Marschall and Fisher [21] found that SFRP2 enhanced WNT3A-dependent phosphorylation of LRP6 as well as both cytosolic  $\beta$ -catenin levels and its nuclear translocation. WNT-signaling pathway qPCR arrays showed that SFRP2 enhanced the WNT3A-mediated transcriptional up-regulation of several genes regulated by WNT3A including its antagonists, DKK1, and NKD1. Looking at the tables above, one finds the following combinations for members of SFRP family along with NKD1, to be prominent at 3rd order level - FSHB-NKD1-SFRP1, FZD1-NKD1-SFRP4, CSNK1G1-NKD1-SFRP4, CTBP1-NKD1-SFRP4, NKD1-PPP2CA-SFRP4, FZD7-NKD1-SFRP4, FOSL1-NKD1-SFRP4, FZD5-NKD1-SFRP4, FRZB-NKD1-SFRP4, LRP5-NKD1-SFRP4, CCND3-NKD1-SFRP4 and LEF1-NKD1-SFRP1. The only other interaction with DVL family was DVL1-NKD1-WNT2B. 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 WNT-NKD1-X combinations

Using human embryonic kidney cells (HEK293A), von Marschall and Fisher [21] found that SFRP2 enhanced WNT3A-dependent phosphorylation of LRP6 as well as both cytosolic  $\beta$ -catenin levels and its nuclear translocation. WNT-signaling pathway qPCR arrays showed that SFRP2 enhanced the WNT3A-mediated transcriptional up-regulation of several genes regulated by WNT3A including its antagonists, DKK1, and NKD1. Since NKD inhibits WNT signaling, the NKD genes comprise a negative feedback mechanism. From this and the above known literature of WNT signaling antagonization by NKD1, it would be interesting to see which WNT molecules/combinations might be getting antagonized by NKD1. Looking at the tables above, one finds the following combinations for members of SFRP family along with NKD1, to be prominent at 3rd order level - FZD5-NKD1-WNT2B, DIXDC1-NKD1-WNT2, CCND3-NKD1-WNT2B, FSHB-NKD1-WNT5A, FZD1-NKD1-WNT2B, FBXW2-NKD1-WNT2B, FZD7-NKD1-WNT3A, CCND3-NKD1-WNT3A, FZD8-NKD1-WNT2B, CXXC4-NKD1-WNT2B, KREMEN1-NKD1-WNT3A, NKD1-WIF1-WNT4, DVL1-NKD1-WNT2B, CSNK2A1-NKD1-WNT2B, NKD1-WNT2B-WNT3A, CSNK1G1-NKD1-WNT2, CCND3-NKD1-WNT2, FBXW2-NKD1-WNT4, FZD7-NKD1-WNT2B, FSHB-NKD1-WNT2B, FZD2-NKD1-WNT2, FRZB-NKD1-WNT2B, DKK1-NKD1-WNT2B, FBXW11-NKD1-WNT2B, FZD7-NKD1-WNT2, KREMEN1-NKD1-WNT2B, DIXDC1-NKD1-WNT4, LRP5-NKD1-WNT2B, DIXDC1-NKD1-WNT5A, NKD1-WIF1-WNT3A, FSHB-NKD1-WNT2, FZD5-NKD1-WNT4, FZD5-NKD1-WNT5A, NKD1-WNT2B-WNT4, FZD7-NKD1-WNT5A, FZD2-NKD1-WNT2B, FZD7-NKD1-WNT4, LEF1-NKD1-WNT3, KREMEN1-

NKD1-WNT4 and DAAM1-NKD1-WNT2B. 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 TCF-NKD1-X combinations**

Guo et al. [13] show that in NKD1-mutant CRC, the mutant NKD1 protein no longer binds and promotes DVL turnover, thus stabilizing  $\beta$ -catenin and activating TCF-dependent transcription of target genes. Conversely, activation of NKD1 might have a suppressing effect on TCF-dependent transcription of target genes. Thus it would be interesting to see the combinations of NKD1-TCF that might be getting prominent rankings and which might be playing a role during the WNT3A stimulation. Looking at the tables above, one finds the following combinations for members of TCF family along with NKD1, to be prominent at 3rd order level - FZD7-NKD1-TCF7L1, FSHB-NKD1-TCF7L1, FSHB-NKD1-TCF7, KREMEN1-NKD1-TCF7L1, LRP5-NKD1-TCF7L1, FZD5-NKD1-TCF7, CTBP1-NKD1-TCF7L1, FBXW11-NKD1-TCF7L1, GSK3B-NKD1-TCF7L1, DIXDC1-NKD1-TCF7L1, CSNK1G1-NKD1-TCF7L1, FZD7-NKD1-TCF7, FZD5-NKD1-TCF7L1 and DIXDC1-NKD1-TCF7. 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 SENP2-NKD1-X combinations**

Though no interaction between SENP2 and NKD is known, looking at the tables above, one finds the following combinations for SENP2 along with NKD1, to be prominent at 3rd order level - FZD7-NKD1-SENP2, DIXDC1-NKD1-SENP2, DKK1-NKD1-SENP2, FRZB-NKD1-SENP2, FZD1-NKD1-SENP2, LRP5-NKD1-SENP2, FZD2-NKD1-SENP2, CXXC4-NKD1-SENP2, FSHB-NKD1-SENP2, FBXW11-NKD1-SENP2, CSNK1G1-NKD1-SENP2, KREMEN1-NKD1-SENP2, FOSL1-NKD1-SENP2 and FBXW2-NKD1-SENP2. 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 NKD1 in WNT3A stimulated HEK 293 cells. Based on the established 2nd order combinations of the NKD1, 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 NKD1, (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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