

# forkhead box N1 (FOXN1) : Time behavioural study of 3rd order combinations in WNT3A stimulated HEK 293 cells

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## Abstract

FOXN1 belongs to the family of FOX (forkhead box) proteins that act as transcription factors which play important roles in regulating the expression of genes involved in cell growth, proliferation, differentiation, and longevity. 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 FOXN1 related 3rd order combinations in a forest of  $^{71}C_3$  combinations using four different sensitivity methods; • show the conserved rankings for FOXN1-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 FOXN1 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 FOXN1 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. forkhead box N1 (FOXN1)

Franke et al. [4] calculated the nuclear pore flow rate (NPFR) for ribosomal and transfer RNA from the steady-state values (mean nuclear pore number and RNA synthesis rates) of the differentiated rat liver cells. They compared their hepatocyte values with the corresponding RNA transport performance of the nuclear pore complexes of other cell types.

The region-specific homeotic gene fork head (*fkf*) is known to promote terminal as opposed to segmental development in the *Drosophila* embryo. Weigel et al. [5] cloned the *fkf* region by chromosomal walking. P element-mediated germ-line transformation and sequence comparison of wild-type and mutant alleles identified the *fkf* gene within the cloned region. They observed that *fkf* was expressed in the early embryo in the two terminal domains that were homeotically transformed in *fkf* mutant embryos. The nuclear localization of the *fkf* protein suggested that *fkf* regulated the transcription of other, subordinate, genes. The *fkf* gene product, however, did not contain a known protein motif, such as the homeodomain or the zinc fingers, nor was it similar in sequence to any other known protein. Forkhead genes are a subgroup of the helix-turn-helix class of proteins (Brennan and Matthews [6]).

The hepatocyte nuclear factor 3 (HNF3) gene family is composed of three proteins ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) that are transcription factors involved in the coordinate expression of several liver genes. Pani et al. [7] focused on the HNF3 $\beta$  protein, and reported the localization of two transcriptional activation domains with a cotransfection assay with HNF3 $\beta$  reporter and expression plasmids. More specifically, they developed a cotransfection assay in Hep-G2 cells to define amino acid residues responsible for HNF3 $\beta$  transcriptional activation. They defined a position-independent activation domain at the HNF3 $\beta$  carboxyl terminus (361-458) which could potentiate the expression of a TATA box-CAT reporter construct containing multimeric DNA recognition sites for the HNF3 protein. They found that this HNF3 $\beta$  activation domain required region II and III sequences which were conserved with the HNF3 family and the *Drosophila* fork head protein.

Since their discovery, the conserved family of fork head/HNF3-related transcription factors gained increasing importance for the analysis of gene regulatory mechanisms during embryonic development and in differentiated cells. Different members of this family, which were defined by a conserved 110 amino acid residues encompassing DNA binding domain of winged helix structure (that has four helices and a two-strand beta-sheet), served as regulatory keys in embryogenesis, in tumorigenesis or in the

maintenance of differentiated cell states. The review by Kaufmann and Knochel [8] summarized the accumulating amount of data on structure, expression and function of fork head/HNF3-related transcription factors.

FOXN1 belongs to the family of FOX (forkhead box) proteins. It is known that mutations at the nude locus of mice and rats disrupt normal hair growth and thymus development, thus causing nude mice and rats to be immune-deficient. Nehls et al. [9] showed that one of the genes from the mouse nude locus which had been localized on chromosome 11 (within a region of <1 megabase), designated winged helix nude (WHN / FOXN1), encoded a new member of the winged-helix domain family of transcription factors and that it was disrupted on mouse *nu* and rat *rnuN* alleles. Further, mutant transcripts did not encode the characteristic DNA-binding domain, thus pointing to the fact that WHN gene was the nude gene.

The differentiation of primitive epithelial precursor cells in the thymic primordium into subcapsular, cortical, and medullary epithelial cells of the mature thymus required the activity of WHN. It was also required for proper keratinization of the hair shaft. Schorpp et al. [10] determined the nucleotide sequence of a 58 kilobase region on mouse chromosome 11 that encompassed the mouse WHN and part of the two neighboring genes. Using cross-hybridization, they isolated the human orthologue of the mouse WHN. They observed that the human WHN protein also consisted of 648 amino acids, 85% of which was identical to the mouse WHN protein. They found that like the mouse gene, the human gene consisted of eight coding exons and utilized two alternative first exons in a tissue-specific fashion.

I present 3rd order combinations of FOXN1 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 [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 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 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  $\mu$  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 FOXN1-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 [12]) and Sobol indices (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 FOXN1-X-X combinations**

A total of 2415, 3rd order combinations involving FOXN1 were obtained from a full set of  ${}^{71}C_3 = 57155$  combinations. Further, from this selected set, using the above criteria for conserved rankings, I report/tabulate the meaningful combinations that might be working synergistically. Tables 2, 3 and 4 show the rankings for the same combinations as in table 1, but using rbf kernel for HSIC, 2002 implementation for SOBOL and martinez implementation for SOBOL, respectively. As one tallies the rankings of across these tables for a particular combination, one finds that the role of the combination of interest is conserved. This conservation points to the existence of the biological synergy, whether the combination has been tested or unexplored/untested.

### **6.3.1. Examining the behaviour of KREMEN1-FOXN1-X combinations**

Fully mature and diverse epithelial microenvironment of the thymus is required for T cell development and selection. The acquisition of these characteristics is dependent on expression of FOXN1, as a lack of functional FOXN1 results in aberrant epithelial morphogenesis and an inability to attract lymphoid precursors to the thymus primordium. Balciunaite et al. [16] report that secreted WNT glycoproteins, expressed by thymic epithelial cells and thymocytes, regulate epithelial FOXN1 expression in both autocrine and paracrine fashions. Thus, WNT signaling has been reported to regulate thymocyte proliferation and selection at several stages during T cell ontogeny, as well as the expression of FOXN1 in thymic epithelial cells (TECs). KREMEN1 (KRM1) is a negative regulator of the canonical WNT signaling pathway, and functions together with the secreted WNT inhibitor Dickkopf (DKK) by competing for the lipoprotein receptor-related protein (LRP6) co-receptor for WNTs. Osada et al. [17] used KRM1 knockout mice to examine KRM1 expression in the thymus and its function in thymocyte and TEC development. They detected KRM1 expression in both cortical and medullary TEC subsets, as well as in immature thymocyte subsets, beginning at the CD25+CD44+ (DN2) stage and continuing until the, CD4+CD8+(DP) stage. They observed that neonatal mice showed elevated expression of KRM1 in all TEC subsets, while KRM1<sup>-/-</sup> mice exhibited a severe defect in thymic cortical architecture, including large epithelial free regions. Further, a TOPFlash assay revealed a 2-fold increase in

RANKING @ $t_1$ USING HSIC - LINEAR												
3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	
FOXNI-KREMENI-WNT2B	170	28253	38493	15670	30910	FOXNI-KREMENI-PPP2R1A	187	38390	45372	22596	7296	
FOXNI-KREMENI-WNT3	217	53012	56130	3088	18960	CSNK1G1-FOXNI-TLE2	226	3994	5316	18517	42187	
FOXNI-KREMENI-LRP5	260	52346	51858	34049	42979	FBXW11-FOXNI-KREMENI	302	14589	4660	6330	19835	
FZD5-FOXNI-LRP5	307	845	9386	28797	54200	DIXDC1-FOXNI-FBXW4	330	3748	17556	12016	30746	
AES-FOXNI-FZD7	338	21947	6320	11489	39355	DKK1-FOXNI-SEN2	342	12351	29242	3585	34803	
DKK1-FOXNI-FRZB	357	16995	47883	4088	8042	CSNK1G1-FOXNI-KREMENI	390	10815	10745	12917	12924	
FBXW11-FOXNI-SEN2	392	5743	6882	5378	5741	CSNK1G1-FOXNI-SLC9A3R1	395	1314	2366	9985	14166	
AES-FOXNI-WNT2	402	20097	14407	14326	3071	AXIN1-FOXNI-WNT2	423	119	6856	11943	1929	
FOSL1-FOXNI-FRZB	426	18495	3914	3925	50337	CSNK1D-FOXNI-LEF1	437	1005	10226	23334	20989	
CTNNB1-FOXNI-FRZB	452	1997	7527	5341	50936	FZD5-FOXNI-SEN2	473	7569	6670	5785	7868	
CTNNB1-FOXNI-RHOU	475	856	5984	14676	48295	DKK1-FOXNI-KREMENI	480	13941	53110	3917	4043	
CTNNB1-FOXNI-KREMENI	484	1965	14838	7676	28339	AES-FOXNI-PPP2R1A	486	23646	9985	11397	37399	
DAAMI-FOXNI-SFRP4	509	41264	31480	8686	35677	FOXNI-KREMENI-SFRP4	510	51802	54641	8830	39747	
FZD5-FOXNI-KREMENI	511	1786	8577	7989	16170	AES-FOXNI-SEN2	518	22315	8527	7631	2724	
FBXW11-FOXNI-SFRP4	561	2766	4849	11457	25387	FBXW11-FOXNI-FZD1	563	3393	3457	5989	33677	
CTBP2-FOXNI-SEN2	566	19815	6401	2342	17983	DIXDC1-FOXNI-TLE2	581	740	1891	12323	28293	
DIXDC1-FOXNI-FZD8	584	8409	1800	17070	5733	FZD5-FOXNI-SLC9A3R1	611	409	1127	6472	13971	
DVL1-FOXNI-FRAT1	613	42124	2382	9631	35079	CSNK1D-FOXNI-SEN2	624	4830	5783	7469	36423	
DVL1-FOXNI-FZD1	635	46799	7174	3229	38528	FOSL1-FOXNI-SFRP4	680	23049	4982	9425	19314	
DAAMI-FOXNI-FZD6	681	45572	41066	6876	38373	CTNNB1-FOXNI-FZD6	691	3712	21540	10206	42092	
FBXW11-FOXNI-FRZB	718	6154	5240	5636	29172	CSNK2A1-FOXNI-TCF7	736	11246	6655	11945	13187	
FOSL1-FOXNI-KREMENI	742	14009	6113	8523	21187	CSNK2A1-FOXNI-KREMENI	775	7975	18477	6845	18012	
AXIN1-FOXNI-FBXW4	790	11390	15320	10702	18907	DVL1-FOXNI-SFRP4	845	41274	6107	5709	48162	
BTRC-FOXNI-FZD8	853	10860	9974	20227	10455	DKK1-FOXNI-WNT2B	864	31296	16351	4633	30734	
DAAMI-FOXNI-FRZB	896	43246	33591	4294	17015	DVL1-FOXNI-SEN2	907	37314	6190	2465	34563	
FBXW11-FOXNI-TCF7L1	916	13133	4109	10602	35818	CTNNB1-FOXNI-SLC9A3R1	917	2286	2153	7068	20569	
FBXW11-FOXNI-LRP5	928	10686	10354	28842	25833	DIXDC1-FOXNI-KREMENI	939	687	5376	7847	13303	
CSNK1G1-FOXNI-PPP2R1A	960	4562	8931	10045	26824	FBXW11-FOXNI-FZD8	965	10160	4020	13865	51185	
CTNNB1-FOXNI-PPP2R1A	966	1729	7371	11775	25639	DVL1-FOXNI-PPP2CA	977	25908	15392	4810	48741	
AES-FOXNI-FZD8	981	7828	6668	16769	40810	FOSL1-FOXNI-GSK3B	983	14647	1776	17080	41173	
FOXNI-KREMENI-SFRP1	985	42401	54457	12806	414	CTNNB1-FOXNI-TLE2	988	5543	9432	15644	20859	
FBXW11-FOXNI-WNT2B	989	38275	4532	5886	43707	AXIN1-FOXNI-FRAT1	990	1738	4384	15268	22220	
FBXW11-FOXNI-TLE2	1004	10423	4208	12987	45489	DAAMI-FOXNI-LRP5	1009	45625	30080	34466	47125	
DAAMI-FOXNI-PPP2CA	1014	50289	35190	8828	44977	FZD5-FOXNI-GSK3B	1022	3638	2875	20381	28710	
CSNK2A1-FOXNI-WNT2B	1037	3175	7624	2538	6838	FOSL1-FOXNI-PPP2CA	1072	22257	16994	9632	25457	
AES-FOXNI-GSK3A	1074	9734	18353	13099	38607	DKK1-FOXNI-TLE2	1080	18592	26323	11091	53163	
CXXC4-FOXNI-SLC9A3R1	1089	2458	1605	6818	34700	CSNK1D-FOXNI-KREMENI	1122	3656	11331	13933	38262	
AXIN1-FOXNI-TCF7	1145	4915	2559	14562	18591	CSNK2A1-FOXNI-FZD8	1170	17750	11184	10347	9970	
CCND3-FOXNI-FZD8	1183	34917	5116	11771	42093	CSNK1G1-FOXNI-FBXW4	1201	11491	20301	17590	26677	
AES-FOXNI-SFRP4	1211	25834	8687	12229	21429	CSNK2A1-FOXNI-SEN2	1233	22059	16672	4525	3145	
CTNNB1-FOXNI-TCF7L1	1237	6273	7647	12061	42159	AES-FOXNI-TLE1	1240	18162	8419	8417	9300	
AES-FOXNI-FRZB	1247	23681	6537	2125	30062	CSNK1D-FOXNI-PPP2CA	1252	17427	16752	21509	34632	
CXXC4-FOXNI-RHOU	1258	949	4951	19719	52102	DAAMI-FOXNI-FZD1	1262	38972	34040	4743	31791	
FOSL1-FOXNI-SLC9A3R1	1275	28011	885	4881	51370	CSNK1G1-FOXNI-TCF7L1	1281	8663	5550	16139	11644	
CSNK2A1-FOXNI-FZD6	1295	29245	25754	7254	28379	DAAMI-FOXNI-SLC9A3R1	1302	35893	19727	4671	23335	
BTRC-FOXNI-WNT4	1304	7824	14183	18230	50189	FOSL1-FOXNI-FZD8	1314	19509	2343	16192	19984	
DVL1-FOXNI-PPP2R1A	1316	40931	10069	3721	36477	FBXW11-FOXNI-FZD7	1326	9731	6528	14525	29594	
CSNK1D-FOXNI-WNT2B	1328	17615	2986	7052	5219	EP300-FOXNI-TLE2	1334	6564	2241	419	8940	
DKK1-FOXNI-WNT5A	1346	18310	46663	16625	23963	FBXW11-FOXNI-PPP2CA	1357	8568	16917	13742	17017	
CSNK1D-FOXNI-FZD8	1359	10239	4066	27563	3347	DKK1-FOXNI-SFRP4	1382	19069	48285	11895	39000	
DKK1-FOXNI-SLC9A3R1	1384	38790	31886	5342	8410	FOSL1-FOXNI-LRP5	1396	12463	8896	42948	43152	
DVL1-FOXNI-SFRP1	1402	41345	3878	6051	10938	FBXW11-FOXNI-WNT4	1404	854	7156	11739	28714	
CSNK2A1-FOXNI-PPP2CA	1415	18676	25465	10950	9597	APC-FOXNI-FRAT1	1418	596	2065	13524	33264	
DVL1-FOXNI-RHOU	1438	38257	4549	11910	29038	AXIN1-FOXNI-TLE2	1439	321	2295	11236	23819	
DIXDC1-FOXNI-FRZB	1442	1877	4968	4749	2791	AES-FOXNI-SLC9A3R1	1445	27866	3775	6327	34957	
CTNNB1-FOXNI-FBXW4	1456	9113	25436	11398	551	AXIN1-FOXNI-FZD8	1457	7791	1656	16700	31078	
CSNK2A1-FOXNI-SFRP4	1460	20356	19876	8892	40	FBXW11-FOXNI-PPP2R1A	1485	2012	6552	10843	25217	
DIXDC1-FOXNI-SEN2	1491	2077	4322	6058	1110	CTNNB1-FOXNI-TCF7	1494	2724	3212	16430	18802	
DVL1-FOXNI-FZD7	1495	41164	4874	7923	37918	CXXC4-FOXNI-FRZB	1502	99	9554	17642	46105	
DAAMI-FOXNI-WNT2B	1503	50616	30398	2836	38247	FOSL1-FOXNI-TLE2	1506	19146	2341	7352	15489	
CTNNB1-FOXNI-SFRP4	1509	2040	14820	13619	31097	AXIN1-FOXNI-RHOU	1511	738	4253	15602	21213	
AXIN1-FOXNI-FZD7	1535	435	3620	13698	37972	AXIN1-FOXNI-SLC9A3R1	1537	5364	1010	5913	21906	
FBXW11-FOXNI-FRAT1	1538	7360	6561	15328	24410	CSNK1G1-FOXNI-FRZB	1549	4678	4300	19697	7337	

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

canonical WNT, signaling in TEC lines derived from  $KRM1^{-/-}$  mice, when compared with  $KRM^{+/+}$  derived TEC lines. Fluorescence activated cell sorting (FACS) analysis of dissociated thymus revealed a reduced frequency of both cortical ( $BP1^{+}EpCAM^{+}$ ) and medullary ( $UEA-1^{+}EpCAM^{hi}$ ) epithelial subsets, within the  $KRM1^{-/-}$  thymus.



RANKING @ $t_i$ USING HSIC - RBF												
3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	
FOXN1-KREMEN1-WNT2B	20404	27973	36396	884	30778	FOXN1-KREMEN1-PPP2R1A	21547	15130	581	7154	55001	
FOXN1-KREMEN1-WNT3	13156	54946	25548	2636	21279	CSNK1G1-FOXN1-TLE2	3512	4562	37294	11299	13219	
FOXN1-KREMEN1-LRP5	11126	50667	3123	17750	44144	FBXW11-FOXN1-KREMEN1	332	11091	39492	5306	3719	
FZD5-FOXN1-LRP5	6222	3631	43311	16286	12181	DIXDC1-FOXN1-FBXW4	3530	9085	27138	3878	80	
AES-FOXN1-FZD7	1606	10949	51740	1419	32162	DKK1-FOXN1-SEN2	1097	45908	33686	6660	22662	
DKK1-FOXN1-FRZB	2424	22948	17226	12105	45671	CSNK1G1-FOXN1-KREMEN1	7103	1710	43024	36832	31611	
FBXW11-FOXN1-SEN2	128	6559	40566	11817	4361	CSNK1G1-FOXN1-SLC9A3R	4227	955	39936	15459	33468	
AES-FOXN1-WNT2	1377	9293	42185	20047	814	AXIN1-FOXN1-WNT2	7365	9601	53234	18	663	
FOSL1-FOXN1-FRZB	1736	15846	50466	9743	17620	CSNK1D-FOXN1-LEF1	6150	3755	45826	20298	28446	
CTNNB1-FOXN1-FRZB	5276	5613	54024	3609	15356	FZD5-FOXN1-SEN2	755	12834	53767	11567	34539	
CTNNB1-FOXN1-RHOU	3224	1823	49426	36371	35760	DKK1-FOXN1-KREMEN1	881	21837	24344	36049	9457	
CTNNB1-FOXN1-KREMEN1	3133	5700	50426	39968	6394	AES-FOXN1-PPP2R1A	723	21691	22365	18788	29319	
DAAMI-FOXN1-SFRP4	3365	36634	40858	22210	87	FOXN1-KREMEN1-SFRP4	21541	47835	13452	25040	38289	
FZD5-FOXN1-KREMEN1	2473	6345	54537	47495	8093	AES-FOXN1-SEN2	1218	24082	52757	25404	14040	
FBXW11-FOXN1-SFRP4	1186	2565	33626	28405	852	FBXW11-FOXN1-FZD1	808	5651	40991	17020	5225	
CTBP2-FOXN1-SEN2	7635	19129	49786	125	42056	DIXDC1-FOXN1-TLE2	1845	16001	48828	19868	1211	
DIXDC1-FOXN1-FZD8	2700	34651	54623	33036	17361	FZD5-FOXN1-SLC9A3R1	7530	2172	55354	30145	48570	
DVL1-FOXN1-FRAT1	5430	52202	38249	34820	8736	CSNK1D-FOXN1-SEN2	1289	8520	39455	7070	25852	
DVL1-FOXN1-FZD1	12403	42347	27218	6716	3146	FOSL1-FOXN1-SFRP4	3581	13413	50450	24051	14731	
DAAMI-FOXN1-FZD6	1927	40856	30711	15954	7578	CTNNB1-FOXN1-FZD6	11515	4963	34761	21621	26869	
FBXW11-FOXN1-FRZB	399	8848	44432	12716	405	CSNK2A1-FOXN1-TCF7	215	23416	42414	29565	28676	
FOSL1-FOXN1-KREMEN1	2901	4767	41791	45598	13163	CSNK2A1-FOXN1-KREMEN1	393	11453	41930	49554	21564	
AXIN1-FOXN1-FBXW4	10622	8925	10482	6312	6744	DVL1-FOXN1-SFRP4	7106	43765	40948	42922	1021	
BTRC-FOXN1-FZD8	2072	28008	41249	39653	29423	DKK1-FOXN1-WNT2B	1531	23991	43537	22407	32006	
DAAMI-FOXN1-FRZB	941	43695	29607	4270	188	DVL1-FOXN1-SEN2	3061	30960	37499	23295	15142	
FBXW11-FOXN1-TCF7L1	214	35323	46275	16010	3890	CTNNB1-FOXN1-SLC9A3R1	5536	1236	55646	25818	55006	
FBXW11-FOXN1-LRP5	3329	14741	47735	14948	367	DIXDC1-FOXN1-KREMEN1	3184	5794	52693	37317	689	
CSNK1G1-FOXN1-PPP2R1A	3415	1125	22606	474	39586	FBXW11-FOXN1-FZD8	898	23493	49484	30381	27534	
CTNNB1-FOXN1-PPP2R1A	6266	597	36708	568	27942	DVL1-FOXN1-PPP2CA	3346	22402	46063	15109	11834	
AES-FOXN1-FZD8	952	20530	45343	38349	44784	FOSL1-FOXN1-GSK3B	1043	18255	47348	52130	53425	
FOXN1-KREMEN1-SFRP1	27973	46263	7809	24618	51572	CTNNB1-FOXN1-TLE2	6280	17739	53356	13450	21250	
FBXW11-FOXN1-WNT2B	3637	18134	11920	13018	6771	AXIN1-FOXN1-FRAT1	11810	7964	53217	15423	27549	
FBXW11-FOXN1-TLE2	703	4758	34636	12462	1117	DAAMI-FOXN1-LRP5	3170	48457	41942	33504	19	
DAAMI-FOXN1-PPP2CA	609	47634	41711	1552	16742	FZD5-FOXN1-GSK3B	2002	13824	55143	46713	44426	
CSNK2A1-FOXN1-WNT2B	1203	7766	40805	5216	17738	FOSL1-FOXN1-PPP2CA	2050	20570	56654	7068	31115	
AES-FOXN1-GSK3A	5614	14376	46340	29843	24805	DKK1-FOXN1-TLE2	2263	27657	20761	18942	14648	
CXXC4-FOXN1-SLC9A3R1	5835	4242	55138	22461	50612	CSNK1D-FOXN1-KREMEN1	3060	1922	41865	30003	8137	
AXIN1-FOXN1-TCF7	13244	8054	54509	20647	45016	CSNK2A1-FOXN1-FZD8	255	29912	45362	18779	48225	
CCND3-FOXN1-FZD8	1397	31287	48789	38612	24901	CSNK1G1-FOXN1-FBXW4	6456	4971	6852	14306	22075	
AES-FOXN1-SFRP4	2089	18334	55582	19404	4752	CSNK2A1-FOXN1-SEN2	142	13206	48212	17145	40808	
CTNNB1-FOXN1-TCF7L1	2168	10793	56115	29127	14142	AES-FOXN1-TLE1	4311	39109	52117	30044	17018	
AES-FOXN1-FRZB	1605	12489	50016	38983	5419	CSNK1D-FOXN1-PPP2CA	677	25144	54449	22625	44186	
CXXC4-FOXN1-RHOU	4808	4398	51558	29746	40158	DAAMI-FOXN1-FZD1	988	42509	43886	1406	307	
FOSL1-FOXN1-SLC9A3R1	3153	36795	52930	36241	48302	CSNK1G1-FOXN1-TCF7L1	2258	13561	45680	37954	10050	
CSNK2A1-FOXN1-FZD6	138	14978	35128	24657	38128	DAAMI-FOXN1-SLC9A3R1	1344	32331	48311	28919	3119	
BTRC-FOXN1-WNT4	5283	22892	41659	11506	604	FOSL1-FOXN1-FZD8	2757	28761	55000	20964	54956	
DVL1-FOXN1-PPP2R1A	7337	28739	5315	11310	23091	FBXW11-FOXN1-FZD7	145	35268	39903	13575	28607	
CSNK1D-FOXN1-WNT2B	2477	5135	39962	11954	23525	EP300-FOXN1-TLE2	1483	17209	53526	19752	11793	
DKK1-FOXN1-WNT5A	6288	34859	41852	39340	54131	FBXW11-FOXN1-PPP2CA	551	13483	50027	13549	5121	
CSNK1D-FOXN1-FZD8	484	13507	45944	36021	49258	DKK1-FOXN1-SFRP4	6549	20275	30679	15932	4932	
DKK1-FOXN1-SLC9A3R1	4065	38156	35913	28567	35213	FOSL1-FOXN1-LRP5	6494	8694	40579	24357	5973	
DVL1-FOXN1-SFRP1	4030	42573	44105	866	23391	FBXW11-FOXN1-WNT4	740	5464	41361	27962	1025	
CSNK2A1-FOXN1-PPP2CA	278	35287	48412	13705	23060	APC-FOXN1-FRAT1	3161	1596	53917	10232	43563	
DVL1-FOXN1-RHOU	5953	29822	43726	42040	18510	AXIN1-FOXN1-TLE2	5475	3810	51423	13930	10241	
DIXDC1-FOXN1-FRZB	2525	19420	51785	5266	224	AES-FOXN1-SLC9A3R1	1732	22096	50779	28472	22844	
CTNNB1-FOXN1-FBXW4	11108	9146	31589	18968	25721	AXIN1-FOXN1-FZD8	1586	21758	56745	32193	53352	
CSNK2A1-FOXN1-SFRP4	363	18135	45540	43080	8388	FBXW11-FOXN1-PPP2R1A	848	15861	49514	22629	20720	
DIXDC1-FOXN1-SEN2	1565	39282	50555	4197	2183	CTNNB1-FOXN1-TCF7	5722	8679	50274	17038	47411	
DVL1-FOXN1-FZD7	3648	31690	36198	26788	18813	CXXC4-FOXN1-FRZB	2132	6567	49876	23824	32081	
DAAMI-FOXN1-WNT2B	1209	51245	29982	6041	1467	FOSL1-FOXN1-TLE2	2685	18230	45559	25782	35344	
CTNNB1-FOXN1-SFRP4	3275	1822	55437	22387	4399	AXIN1-FOXN1-RHOU	7032	3608	53224	33577	37035	
AXIN1-FOXN1-FZD7	2665	2108	55426	10290	29770	AXIN1-FOXN1-SLC9A3R1	19889	17956	54483	21472	34039	
FBXW11-FOXN1-FRAT1	1470	1272	34561	8385	2950	CSNK1G1-FOXN1-FRZB	4205	5593	40227	150	17600	

Table 2: Rankings of FOXN1-X-X. A list of approximately first 125 combinations with rankings below 10,000 out of 57,155. SA - HSIC; Kernel - rbf

However, their data suggested that a loss of KRM1 led to a severe defect in thymic architecture.

Looking at the tables above, one finds the following combinations for KREMEN1 along with FOXN1, to be prominent at 3rd order level - FOXN1-KREMEN1-WNT2B, FOXN1-KREMEN1-WNT3, FOXN1-KREMEN1-LRP5, CTNNB1-FOXN1-KREMEN1,

RANKING @ $t_1$ USING SOBOL - 2002												
3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	
FOXNI-KREMEN1-WNT2B	48078	14775	45903	47094	15768	FOXNI-KREMEN1-PPP2R1A	42868	43393	35692	37283	25597	
FOXNI-KREMEN1-WNT3	22912	27457	7524	8198	32025	CSNK1G1-FOXNI-TLE2	21537	42757	2336	15141	32041	
FOXNI-KREMEN1-LRP5	48531	10599	34009	31625	7563	FBXW11-FOXNI-KREMEN1	36681	54148	38407	41684	18205	
FZD5-FOXNI-LRP5	50939	54347	48592	45940	3145	DIXDC1-FOXNI-FBXW4	3908	42858	13043	142	51587	
AES-FOXNI-FZD7	19062	27345	15364	27396	51338	DKK1-FOXNI-SEN2	15411	46643	24807	26053	15345	
DKK1-FOXNI-FRZB	56159	54281	37322	38536	39284	CSNK1G1-FOXNI-KREMEN1	12568	9848	1206	5693	56612	
FBXW11-FOXNI-SEN2	18268	562	25898	8742	43081	CSNK1G1-FOXNI-SLC9A3R1	29604	29598	56041	56960	9679	
AES-FOXNI-WNT2	22557	13207	2984	22391	15990	AXIN1-FOXNI-WNT2	2868	6422	26686	13307	56795	
FOSL1-FOXNI-FRZB	4845	4217	9346	8710	50240	CSNK1D-FOXNI-LEF1	18416	22419	26392	15687	40976	
CTNNB1-FOXNI-FRZB	28573	30528	6394	8512	31823	FZD5-FOXNI-SEN2	844	38547	5609	13856	38976	
CTNNB1-FOXNI-RHOU	27653	16770	5400	1038	34726	DKK1-FOXNI-KREMEN1	49863	2268	35179	34070	52801	
CTNNB1-FOXNI-KREMEN1	28350	28602	22304	4596	30984	AES-FOXNI-PPP2R1A	41646	39241	36628	36288	5132	
DAAMI-FOXNI-SFRP4	7880	7330	20179	6203	43724	FOXNI-KREMEN1-SFRP4	7110	32958	11953	11793	25861	
FZD5-FOXNI-KREMEN1	40550	9655	41021	31346	25618	AES-FOXNI-SEN2	15326	21444	1720	26394	49021	
FBXW11-FOXNI-SFRP4	23022	11706	11276	14639	29327	FBXW11-FOXNI-FZD1	43295	56184	48236	32827	13322	
CTBP2-FOXNI-SEN2	18763	34976	4397	1891	34643	DIXDC1-FOXNI-TLE2	862	48313	19636	847	30685	
DIXDC1-FOXNI-FZD8	1805	11167	25446	13189	38746	FZD5-FOXNI-SLC9A3R1	1697	45660	2641	5040	45318	
DVL1-FOXNI-FRAT1	8406	42213	25214	3249	49498	CSNK1D-FOXNI-SEN2	22145	41275	26967	5135	47598	
DVL1-FOXNI-FZD1	42452	4219	43963	54332	17477	FOSL1-FOXNI-SFRP4	45107	48753	50599	52560	3992	
DAAMI-FOXNI-FZD6	37054	18577	34216	48915	5647	CTNNB1-FOXNI-FZD6	27972	37688	21082	2927	32408	
FBXW11-FOXNI-FRZB	35831	56202	37041	46497	19251	CSNK2A1-FOXNI-TCF7	8332	7205	8216	24521	24469	
FOSL1-FOXNI-KREMEN1	5566	2122	4725	4323	49299	CSNK2A1-FOXNI-KREMEN1	57030	55282	44745	35369	8120	
AXIN1-FOXNI-FBXW4	45257	35139	37267	33334	379	DVL1-FOXNI-SFRP4	14743	49762	8514	7812	45294	
BTRC-FOXNI-FZD8	30847	6428	51729	52456	19470	DKK1-FOXNI-WNT2B	55818	51616	29843	30059	41899	
DAAMI-FOXNI-FRZB	38384	5521	41219	53808	10246	DVL1-FOXNI-SEN2	16471	55562	16915	23813	45857	
FBXW11-FOXNI-TCF7L1	36868	54253	50665	49153	24407	CTNNB1-FOXNI-SLC9A3R1	28707	39717	56562	50180	26501	
FBXW11-FOXNI-LRP5	35853	55696	42397	46070	23566	DIXDC1-FOXNI-KREMEN1	21482	50446	17603	1664	40258	
CSNK1G1-FOXNI-PPP2R1A	26102	33906	21109	71	51037	FBXW11-FOXNI-FZD8	42310	48440	49621	56601	17969	
CTNNB1-FOXNI-PPP2R1A	25582	48808	11814	14904	35610	DVL1-FOXNI-PPP2CA	5338	52223	20041	27389	38036	
AES-FOXNI-FZD8	38220	29623	41762	29743	5846	FOSL1-FOXNI-GSK3B	284	8167	4904	8619	50083	
FOXNI-KREMEN1-SFRP1	46908	27207	43387	43980	36486	CTNNB1-FOXNI-TLE2	28469	21955	8815	15870	30584	
FBXW11-FOXNI-WNT2B	38643	50360	51216	56044	10628	AXIN1-FOXNI-FRAT1	2352	55910	24610	26180	53343	
FBXW11-FOXNI-TLE2	43255	17569	50698	53129	8571	DAAMI-FOXNI-LRP5	49155	51746	40759	50206	6924	
DAAMI-FOXNI-PPP2CA	21001	49584	22517	19252	54625	FZD5-FOXNI-GSK3B	42472	51735	50523	46783	4543	
CSNK2A1-FOXNI-WNT2B	53238	9771	44983	35149	2439	FOSL1-FOXNI-PPP2CA	51329	15073	48971	51617	23899	
AES-FOXNI-GSK3A	22002	20917	10608	19647	56904	DKK1-FOXNI-TLE2	50424	1630	33165	29699	44189	
CXXC4-FOXNI-SLC9A3R1	3959	7438	19286	25956	7951	CSNK1D-FOXNI-KREMEN1	31821	16389	28671	30403	11306	
AXIN1-FOXNI-TCF7	6178	48880	26554	15511	40506	CSNK2A1-FOXNI-FZD8	49203	56401	44801	39472	19441	
CCND3-FOXNI-FZD8	56524	3238	46054	36414	2475	CSNK1G1-FOXNI-FBXW4	27569	27578	1117	197	47485	
AES-FOXNI-SFRP4	27040	19598	1668	15848	33804	CSNK2A1-FOXNI-SEN2	9019	3852	10133	23930	23783	
CTNNB1-FOXNI-TCF7L1	28405	31378	6237	4800	32835	AES-FOXNI-TLE1	24068	30660	5191	23803	22607	
AES-FOXNI-FRZB	34642	43005	47089	35277	742	CSNK1D-FOXNI-PPP2CA	18148	46116	27668	8407	45131	
CXXC4-FOXNI-RHOU	49343	54575	54627	33981	50794	DAAMI-FOXNI-FZD1	50207	13696	41541	54869	2724	
FOSL1-FOXNI-SLC9A3R1	34742	13119	54271	41553	7238	CSNK1G1-FOXNI-TCF7L1	24011	53828	1796	3138	42955	
CSNK2A1-FOXNI-FZD6	37168	52306	41314	39825	20513	DAAMI-FOXNI-SLC9A3R1	15713	57120	17680	21465	44986	
BTRC-FOXNI-WNT4	9396	56292	5649	9293	40497	FOSL1-FOXNI-FZD8	18145	45592	11421	12903	51267	
DVL1-FOXNI-PPP2R1A	51816	4887	37165	29807	19052	FBXW11-FOXNI-FZD7	14846	8663	7520	555	39082	
CSNK1D-FOXNI-WNT2B	34971	36541	30580	44435	4295	EP300-FOXNI-TLE2	25264	3588	9029	20821	56127	
DKK1-FOXNI-WNT5A	36811	44788	36126	29818	15014	FBXW11-FOXNI-PPP2CA	19429	5233	11810	11471	27138	
CSNK1D-FOXNI-FZD8	31892	7178	33756	49005	20364	DKK1-FOXNI-SFRP4	1117	8285	22947	16604	8982	
DKK1-FOXNI-SLC9A3R1	10979	44117	21947	28433	8295	FOSL1-FOXNI-LRP5	19194	9612	13710	10552	49252	
DVL1-FOXNI-SFRP1	40691	1566	40271	33337	11288	FBXW11-FOXNI-WNT4	17360	35949	3866	7278	42454	
CSNK2A1-FOXNI-PPP2CA	9022	16430	12781	23632	52199	APC-FOXNI-FRAT1	27393	16291	3934	10975	21998	
DVL1-FOXNI-RHOU	42063	37467	42621	45694	17029	AXIN1-FOXNI-TLE2	56962	694	33915	38186	32161	
DIXDC1-FOXNI-FRZB	7469	45639	21161	7194	29894	AES-FOXNI-SLC9A3R1	20022	9823	13434	19989	45042	
CTNNB1-FOXNI-FBXW4	28447	17064	589	6990	30612	AXIN1-FOXNI-FZD8	54876	1120	32554	46287	1002	
CSNK2A1-FOXNI-SFRP4	14008	32657	7345	20701	33835	FBXW11-FOXNI-PPP2R1A	37757	51830	45317	45654	30175	
DIXDC1-FOXNI-SEN2	49092	3697	41057	55136	27309	CTNNB1-FOXNI-TCF7	28828	13488	55490	37242	20556	
DVL1-FOXNI-FZD7	6530	40771	21238	937	46641	CXXC4-FOXNI-FRZB	47727	56222	50026	44836	51771	
DAAMI-FOXNI-WNT2B	49677	19444	42826	45397	19848	FOSL1-FOXNI-TLE2	10111	34166	2997	1459	46410	
CTNNB1-FOXNI-SFRP4	29143	23020	37799	31356	27341	AXIN1-FOXNI-RHOU	52036	21355	34481	35981	2174	
AXIN1-FOXNI-FZD7	2307	56032	24600	10882	56159	AXIN1-FOXNI-SLC9A3R1	19	31167	22460	20513	55141	
FBXW11-FOXNI-FRAT1	21263	930	20115	10646	37562	CSNK1G1-FOXNI-FRZB	16921	42333	17206	26037	35243	

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

FZD5-FOXNI-KREMEN1, FOSL1-FOXNI-KREMEN1, FOXNI-KREMEN1-SFRP1, FOXNI-KREMEN1-PPP2R1A, FBXW11-FOXNI-KREMEN1, CSNK1G1-FOXNI-KREMEN1, DKK1-FOXNI-KREMEN1, FOXNI-KREMEN1-SFRP4, CSNK2A1-FOXNI-KREMEN1, DIXDC1-FOXNI-KREMEN1 and CSNK1D-FOXNI-KREMEN1. All these combinations indicate the existence of a possible synergy when they take a higher

RANKING @ $t_1$ USING SOBOL - MARTINEZ												
3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	3rd order comb.	$t_1$	$t_3$	$t_6$	$t_{12}$	$t_{24}$	
FOXNI-KREMENI-WNT2B	21687	4778	50738	46118	18804	FOXNI-KREMENI-PPP2R1A	9818	15093	45644	39058	38490	
FOXNI-KREMENI-WNT3	36925	40473	11710	51127	46925	CSNK1G1-FOXNI-TLE2	4073	26473	32009	15332	6483	
FOXNI-KREMENI-LRP5	18720	14285	27556	23995	33506	FBXW11-FOXNI-KREMENI	45193	35978	856	882	52991	
FZD5-FOXNI-LRP5	44576	48060	53289	52324	29903	DIXDC1-FOXNI-FBXW4	268	9366	30340	20615	51974	
AES-FOXNI-FZD7	23629	15808	9753	38379	53960	DKK1-FOXNI-SEN2	168	13005	4602	14511	33848	
DKK1-FOXNI-FRZB	24937	21524	7118	32367	18602	CSNK1G1-FOXNI-KREMENI	4187	16379	10411	17376	49738	
FBXW11-FOXNI-SEN2	15519	50540	42098	37233	6768	CSNK1G1-FOXNI-SLC9A3R1	11281	33656	17132	33702	26731	
AES-FOXNI-WNT2	50321	6279	9461	8038	52099	AXIN1-FOXNI-WNT2	19083	12759	41582	50880	12205	
FOSL1-FOXNI-FRZB	36987	50279	6665	29743	28829	CSNK1D-FOXNI-LEF1	32374	24326	23110	46046	3210	
CTNNB1-FOXNI-FRZB	43659	3886	37710	7055	13577	FZD5-FOXNI-SEN2	40428	20162	38623	7744	42912	
CTNNB1-FOXNI-RHOU	45065	1679	7941	10083	48532	DKK1-FOXNI-KREMENI	10750	27873	12499	26591	19395	
CTNNB1-FOXNI-KREMENI	44655	43789	50547	9976	47585	AES-FOXNI-PPP2R1A	41046	36730	9801	33717	13773	
DAAMI-FOXNI-SFRP4	7751	18344	55424	50782	3197	FOXNI-KREMENI-SFRP4	7114	42866	55853	44346	3242	
FZD5-FOXNI-KREMENI	9688	45351	53521	1084	18548	AES-FOXNI-SEN2	39680	6172	11814	7048	33251	
FBXW11-FOXNI-SFRP4	27065	10077	8466	15392	30219	FBXW11-FOXNI-FZD1	52024	33086	18979	1753	48074	
CTBP2-FOXNI-SEN2	32469	35593	13053	16587	40403	DIXDC1-FOXNI-TLE2	36166	9638	12945	39505	51979	
DIXDC1-FOXNI-FZD8	1511	18357	6077	28359	49589	FZD5-FOXNI-SLC9A3R1	47206	6283	15496	40681	47082	
DVL1-FOXNI-FRAT1	8925	23233	8529	30875	33079	CSNK1D-FOXNI-SEN2	38843	9650	25689	48746	11032	
DVL1-FOXNI-FZD8	34693	13868	26030	2645	56499	FOSL1-FOXNI-SFRP4	11238	13543	43498	33908	10953	
DAAMI-FOXNI-FZD6	43127	42151	39069	52241	40214	CTNNB1-FOXNI-FZD6	44233	2851	9365	12079	47134	
FBXW11-FOXNI-FRZB	29823	38826	14329	2782	20352	CSNK2A1-FOXNI-TCF7	11863	6115	56630	47612	7264	
FOSL1-FOXNI-KREMENI	6535	4193	12656	15365	2682	CSNK2A1-FOXNI-KREMENI	57068	22013	50507	44487	22506	
AXIN1-FOXNI-FBXW4	17414	54376	47352	14043	54975	DVL1-FOXNI-SFRP4	12720	4595	12161	10625	46908	
BTRC-FOXNI-FZD8	7699	34343	29979	33524	35444	DKK1-FOXNI-WNT2B	22219	29187	3624	37031	31136	
DAAMI-FOXNI-FRZB	32032	15236	37075	49721	19011	DVL1-FOXNI-SEN2	6587	3940	24129	7079	19484	
FBXW11-FOXNI-TCF7L1	52608	36304	3744	46495	28797	CTNNB1-FOXNI-SLC9A3R1	18072	1206	35157	27756	40997	
FBXW11-FOXNI-LRP5	53130	32983	1512	7731	33974	DIXDC1-FOXNI-KREMENI	11078	11553	5133	32051	21036	
CSNK1G1-FOXNI-PPP2R1A	2180	53719	33104	7285	38261	FBXW11-FOXNI-FZD8	56662	40498	3800	2651	29521	
CTNNB1-FOXNI-PPP2R1A	42625	3532	15726	6439	5650	DVL1-FOXNI-PPP2CA	9766	3745	17308	8852	45843	
AES-FOXNI-FZD8	56049	20778	20261	36288	18117	FOSL1-FOXNI-GSK3B	17098	3338	10035	12874	4259	
FOXNI-KREMENI-SFRP1	16869	8888	49106	16150	24856	CTNNB1-FOXNI-TLE2	36111	10469	4743	14510	53577	
FBXW11-FOXNI-WNT2B	55663	32223	6184	10160	38098	AXIN1-FOXNI-FRAT1	7322	33331	52762	5053	1371	
FBXW11-FOXNI-TLE2	55699	33759	11606	36324	18285	DAAMI-FOXNI-LRP5	52143	48228	30635	39939	34172	
DAAMI-FOXNI-PPP2CA	9163	6334	21471	16668	33204	FZD5-FOXNI-GSK3B	13459	53474	52940	50947	29687	
CSNK2A1-FOXNI-WNT2B	56377	38665	54391	50687	12268	FOSL1-FOXNI-PPP2CA	21982	3651	43234	19001	11166	
AES-FOXNI-GSK3A	22465	7506	14344	42996	54652	DKK1-FOXNI-TLE2	11067	30657	7904	29934	15596	
CXXC4-FOXNI-SLC9A3R1	45159	54481	6054	51467	4749	CSNK1D-FOXNI-KREMENI	32146	2230	14527	12211	48112	
AXIN1-FOXNI-TCF7	4373	53527	46577	9826	81	CSNK2A1-FOXNI-FZD8	48413	46315	52935	6291	12792	
CCND3-FOXNI-FZD8	27128	17370	32737	21891	12717	CSNK1G1-FOXNI-FBXW4	2305	4668	32121	14002	38175	
AES-FOXNI-SFRP4	29623	11714	10858	9506	48063	CSNK2A1-FOXNI-SEN2	14555	16853	56191	37447	12743	
CTNNB1-FOXNI-TCF7L1	43229	3941	8947	14778	49336	AES-FOXNI-TLE1	42601	20308	38323	12470	11731	
AES-FOXNI-FRZB	49145	2429	36755	39791	15921	CSNK1D-FOXNI-PPP2CA	35792	53371	27044	46895	15279	
CXXC4-FOXNI-RHOU	50127	17786	8880	44528	47077	DAAMI-FOXNI-FZD1	54222	7558	39440	51624	56191	
FOSL1-FOXNI-SLC9A3R1	6293	20325	34022	31771	12589	CSNK1G1-FOXNI-TCF7L1	20619	35523	15458	17164	22105	
CSNK2A1-FOXNI-FZD6	6588	10061	41823	49185	11365	DAAMI-FOXNI-SLC9A3R1	13007	860	44746	49567	44767	
BTRC-FOXNI-WNT4	46136	7354	21239	41736	23521	FOSL1-FOXNI-FZD8	11298	1385	13720	14297	6541	
DVL1-FOXNI-PPP2R1A	37862	23663	31790	1787	56934	FBXW11-FOXNI-FZD7	17330	13911	29069	3954	8015	
CSNK1D-FOXNI-WNT2B	40727	20657	11342	28759	54605	EP300-FOXNI-TLE2	18282	45030	23829	56381	38000	
DKK1-FOXNI-WNT5A	11041	32783	5504	28116	20846	FBXW11-FOXNI-PPP2CA	11800	8011	18604	2385	6113	
CSNK1D-FOXNI-FZD8	40606	42498	22468	13908	52376	DKK1-FOXNI-SFRP4	34826	33854	19734	41000	39872	
DKK1-FOXNI-SLC9A3R1	41457	13883	15850	11217	40828	FOSL1-FOXNI-LRP5	45040	47246	14065	24765	5919	
DVL1-FOXNI-SFRP1	40981	8756	16949	3369	46641	FBXW11-FOXNI-WNT4	15807	8100	17793	26530	5575	
CSNK2A1-FOXNI-PPP2CA	6777	56192	42475	44384	6122	APC-FOXNI-FRAT1	19229	3828	17383	14340	36160	
DVL1-FOXNI-RHOU	43128	21551	13230	2660	56296	AXIN1-FOXNI-TLE2	56590	40512	50158	3668	27808	
DIXDC1-FOXNI-FRZB	951	47159	3603	6086	38758	AES-FOXNI-SLC9A3R1	32330	8506	13359	4702	44924	
CTNNB1-FOXNI-FBXW4	48030	4096	7626	5571	41224	AXIN1-FOXNI-FZD8	49386	52382	33545	44524	21116	
CSNK2A1-FOXNI-SFRP4	44988	20977	47958	42376	4783	FBXW11-FOXNI-PPP2R1A	48234	30804	26631	857	24851	
DIXDC1-FOXNI-SEN2	12650	15173	6381	13084	40680	CTNNB1-FOXNI-TCF7	20917	523	31969	23796	31090	
DVL1-FOXNI-FZD7	5689	11803	38591	18607	46827	CXXC4-FOXNI-FRZB	47960	19432	1581	42150	17714	
DAAMI-FOXNI-WNT2B	29721	26744	32942	6037	9724	FOSL1-FOXNI-TLE2	48872	8854	6882	23880	54238	
CTNNB1-FOXNI-SFRP4	26222	799	32830	25595	25213	AXIN1-FOXNI-RHOU	43904	28679	25322	4178	22618	
AXIN1-FOXNI-FZD7	10116	48547	37838	39900	93	AXIN1-FOXNI-SLC9A3R1	11668	56684	26626	29427	688	
FBXW11-FOXNI-FRAT1	1254	8187	49001	52657	39237	CSNK1G1-FOXNI-FRZB	757	4827	23287	8957	51220	

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

rank in the list of combinations.

### 6.3.2. Examining the behaviour of KREMEN1-FOXN1-X combinations

$\beta$ -catenin signaling is required for hair follicle development, but it is unknown whether its activation is sufficient to globally program embryonic epidermis to hair follicle fate. To determine whether forced activation of WNT/ $\beta$ -catenin signaling altered the fate of embryonic surface ectoderm, Zhang et al. [18] stimulated  $\beta$ -catenin signaling in vivo by mutating the endogenous CTNNB1 to a dominant active form in epithelial cells. They observed that hair follicle placodes were expanded and induced prematurely in activated  $\beta$ -catenin mutant embryos, but failed to invaginate or form multilayered structures. Further, FOXN1 was found to be upregulated in activated  $\beta$ -catenin mutant epidermis at E15.5.

Looking at the tables above, one finds the following combinations for CTNNB1 along with FOXN1, to be prominent at 3rd order level - CTNNB1-FOXN1-FRZB, CTNNB1-FOXN1-RHOU, CTNNB1-FOXN1-KREMEN1, CTNNB1-FOXN1-PPP2R1A, CTNNB1-FOXN1-TCF7L1, CTNNB1-FOXN1-FBXW4, CTNNB1-FOXN1-SFRP4, CTNNB1-FOXN1-FZD6, CTNNB1-FOXN1-SLC9A3R1, CTNNB1-FOXN1-TLE2 and CTNNB1-FOXN1-TCF7. 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 DKK1-FOXN1-X combinations

Confirmation by Osada et al. [19], that transgenic expression of the WNT inhibitor DKK1 resulted in a decrease in canonical WNT signaling within the Thymic epithelial cells (TECs) was evident from the 3060% decrease in the expression of the WNT target genes AXIN2 and c-MYC observed in TECs sorted from TetO-DKK1 mice, when compared with identical populations sorted from Dox treated ST controls. They also observed similar reductions in FOXN1 expression, thus supporting previous reports of a role of WNT signaling in regulating FOXN1 expression in TECs in vitro.

Looking at the tables above, one finds the following combinations for DKK1 along with FOXN1, to be prominent at 3rd order level - DKK1-FOXN1-FRZB, DKK1-FOXN1-WNT5A, DKK1-FOXN1-SLC9A3R1, DKK1-FOXN1-SEN2, DKK1-FOXN1-KREMEN1, DKK1-FOXN1-WNT2B, DKK1-FOXN1-TLE2 and DKK1-FOXN1-SFRP4. 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 WNT-FOXN1-X combinations

Age-associated thymic involution has considerable physiological impact by inhibiting de novo T-cell selection. This impaired T-cell production led to weakened immune responses. Kvell et al. [20] investigated structural and molecular changes of the murine thymic stroma during aging. They showed that thymic epithelial senescence correlated with significant destruction of epithelial network followed by adipose involution. They also showed in purified thymic epithelial cells the age-related down-regulation of WNT4 (and subsequently FOXN1), and the prominent increase in LAP2 $\alpha$  expression.

Looking at the tables above, one finds the following combinations for members of WNT family along with FOXN1, to be prominent at 3rd order level - FOXN1-

KREMEN1-WNT2B, FOXN1-KREMEN1-WNT3, AES-FOXN1-WNT2, FBXW11-FOXN1-WNT2B, CSNK2A1-FOXN1-WNT2B, BTRC-FOXN1-WNT4, CSNK1D-FOXN1-WNT2B, DKK1-FOXN1-WNT5A, DAAM1-FOXN1-WNT2B, AXIN1-FOXN1-WNT2, DKK1-FOXN1-WNT2B and FBXW11-FOXN1-WNT4. 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 FZD-FOXN1-X combinations

The WNT/ $\beta$ -catenin signaling pathway plays an important role in the commitment and development of thymic epithelial precursors and Kvell et al. [21] documented similarities of thymic epithelial development during embryogenesis in human and mouse by staining for thymic epithelial surface markers (EpCAM1, Ly51, K8) and ligand/receptor pair (WNT4, FZD4). The expression and staining pattern of the secreted WNT4 molecule as well as its ligand FZD4 were tested and both WNT4 and FZD4 were found to be rather ubiquitously expressed in the mouse thymus and both showed significant overlap with EpCAM1. Their results confirmed the relevance of using murine test systems to model human embryonic thymic epithelial cell development. They efficiently transduced murine embryonic epithelial cells using mock (GFP) and WNT/ $\beta$ -catenin-inhibiting (ICAT-encoding) recombinant adenoviral vectors. Their results demonstrated that ICAT and WNT4 had reciprocal effects during embryonic thymic epithelial cell development. It was observed that while WNT4 was capable of increasing the expression level of characteristic intracellular FOXN1, surface MHCII and secreted IL7 molecules, WNT/ $\beta$ -catenin inhibition through ICAT could moderately decrease their expression.

Looking at the tables above, one finds the following combinations for members of FZD family along with FOXN1, to be prominent at 3rd order level - FZD5-FOXN1-LRP5, AES-FOXN1-FZD7, FZD5-FOXN1-KREMEN1, DIXDC1-FOXN1-FZD8, DVL1-FOXN1-FZD1, DAAM1-FOXN1-FZD6, BTRC-FOXN1-FZD8, AES-FOXN1-FZD8, CCND3-FOXN1-FZD8, CSNK2A1-FOXN1-FZD6, CSNK1D-FOXN1-FZD8, DVL1-FOXN1-FZD7, AXIN1-FOXN1-FZD7, FZD5-FOXN1-SEN2, FBXW11-FOXN1-FZD1, FZD5-FOXN1-SLC9A3R1, CTNNB1-FOXN1-FZD6, FBXW11-FOXN1-FZD8, FZD5-FOXN1-GSK3B, CSNK2A1-FOXN1-FZD8, DAAM1-FOXN1-FZD1, FOSL1-FOXN1-FZD8, FBXW11-FOXN1-FZD7 and AXIN1-FOXN1-FZD8. 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 FOXN1 in WNT3A stimulated HEK 293 cells. Based on the established 2nd order combinations of the FOXN1, 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 FOXN1, (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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