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ARTICLE TYPE

Hilbert-Schmidt and Sobol sensitivity indices for static and time series Wnt signaling measurements in colorectal cancer† [work in progress]

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Ever since the accidental discovery of Wingless [Sharma *R.P., Drosophila information service*, 1973, **50**, p 134], research in the field of Wnt signaling pathway has taken significant strides in wet lab experiments and various cancer clinical trials augmented by recent developments in advanced computational modeling of the pathway. Information rich gene expression profiles reveal various aspects of the signaling pathway at work and help in studying different issues simultaneously. Hitherto, not many computational studies exist which incorporate the simultaneous study of these issues. This manuscript is an endeavour to • explore the strength of contributing factors in the signaling pathway, • analyze the existing causal relations among the inter/extracellular factors effecting the pathway based on prior biological knowledge and • investigate the recently found prevalence of psychophysical laws working in the pathway in a multi-parameter setting. To achieve this goal, local and global sensitivity analysis is conducted on the (non)linear responses between the factors, obtained from static and time series expression profiles, using the density (Hilbert-Schmidt Information Criterion) and variance (Sobol) based sensitivity indicies. The results in the manuscript show the superiority of the density based indices in comparison to the use of variance based indices mainly due to the former's employment of distance measures using the kernel trick via Reproducing kernel Hilbert space (RKHS) that capture nonlinear relations among various intra/extracellular factors of the pathway in a higher dimensional space. In time series data, using these indices it is now possible to observe where in time, which factors get influenced as well as contribute to the pathway as changes in concentration of the other factors are made. This synergy of prior biological knowledge, sensitivity analysis indices and representations in higher dimensional spaces facilitates the above study to reveal a rich amount of hidden biological information within the data from colorectal cancer samples.

1 Introduction

1.1 A short review

The accidental discovery of the Wingless (Sharma[1]) played a pioneering role in the emergence of a widely expanding research field of the Wnt signaling pathway. A majority of the work has focused on issues related to • the discovery of genetic and epigenetic factors affecting the pathway (Thorstensen *et al.*[2] & Baron and Kneissel[3]), • implications of mutations in the pathway and its dominant role on cancer and other diseases (Clevers[4]), • investigation into the pathway's contribution towards embryo development (Sokol[5]), homeostasis (Pinto *et al.*[6], Zhong *et al.*[7]) and apoptosis (Pećina-Šlaus[8]) and • safety and feasibility of drug design for the wnt pathway (Kahn[9], Garber[10], Voronkov and Krauss[11], Blagodatski *et al.*[12] & Curtin and Lorenzi[13]). Approximately forty years after the discovery, important strides have been made in the research work involving several wet lab experiments and cancer clinical trials (Kahn[9], Curtin and Lorenzi[13]) which have been augmented by the recent developments in the various advanced computational modeling techniques of the pathway.

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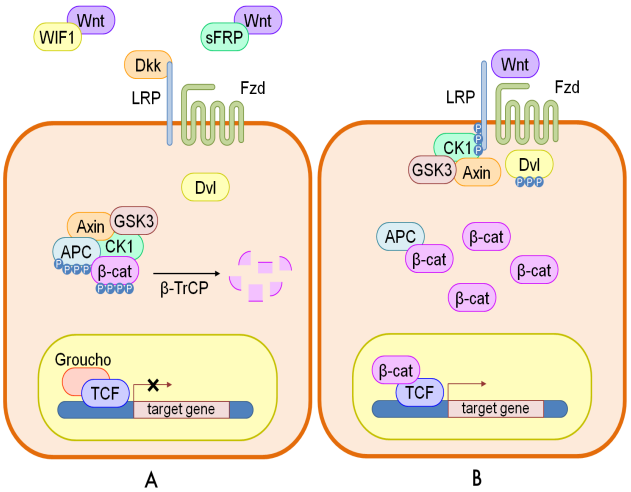

Fig. 1 A cartoon of wnt signaling pathway contributed by Verhaegh *et al.*[18]. Part (A) represents the destruction of β-*catenin* leading to the inactivation of the wnt target gene. Part (B) represents activation of wnt target gene.

More recent informative reviews have touched on various issues related to the different types of the Wnt signaling pathway and have stressed not only the activation of the Wnt signaling pathway via the Wnt proteins (Rao and Kühl[14]) but also the on the secretion mechanism that plays a major role in the initiation of the Wnt activity as a prelude (Yu and Virshup[15]).

The work in this paper investigates some of the current aspects of research regarding the pathway via sensitivity analysis while using static (Jiang *et al.*[16]) and time series (Gujral and MacBeath[17]) gene expression data retrieved from colorectal cancer samples.

1.2 Canonical Wnt signaling pathway

Before delving into the problem statement, a brief introduction to the Wnt pathway is given here. From the recent work of Sinha[19], the canonical Wnt signaling pathway is a transduction mechanism that contributes to embryo development and controls homeostatic self renewal in several tissues (Clevers[4]). Somatic mutations in the pathway are known to be associated with cancer in different parts of the human body. Prominent among them is the colorectal cancer case (Gregorieff and Clevers[20]). In a succinct overview, the Wnt signaling pathway works when the Wnt ligand gets attached to the Frizzled(FZD)/LRP coreceptor complex. FZD may interact with the Dishevelled (DVL) causing phosphorylation. It is also thought that Wnts cause phosphorylation of the LRP via casein kinase 1 ($CK1$) and kinase $GSK3$. These developments further lead to attraction of Axin which causes inhibition of the formation of the degradation complex. The degradation complex constitutes of $AXIN$, the β-*catenin* transportation complex APC, $CK1$ and $GSK3$. When the pathway is active the dissolution of the degradation complex leads to stabilization in the concentration of β-*catenin* in the cytoplasm. As β-*catenin* enters into the nucleus it displaces the $GROUCHO$ and binds with transcription cell factor TCF thus instigating transcription of Wnt target genes. $GROUCHO$ acts as lock on TCF and prevents the transcription of target genes which may induce cancer. In cases when the Wnt ligands are not captured by the coreceptor at the cell membrane, $AXIN$ helps in formation of the degradation complex. The degradation complex phosphorylates β-*catenin* which is then recognized by $FBOX/WD$ repeat protein β-$TRCP$. β-$TRCP$ is a component of ubiquitin ligase complex that helps in ubiquitination of β-*catenin* thus marking it for degradation via the proteasome. Cartoons depicting the phenomena of Wnt being inactive and active are shown in figures 1(A) and 1(B), respectively.

2 Problem statement & sensitivity analysis

Succinctly, the endeavour is to address the following issues - • explore the strength of contributing factors in the signaling pathway, • analyse the existing causal relations among the inter/extracellular factors effecting the pathway based on prior biological knowledge and • investigate the recently found prevalence of psychophysical laws working in the pathway in a multi-parameter setting. The issues related to • inference of hidden biological relations among the factors, that are yet to be discovered and • discovery of new causal relations using hypothesis testing, will be addressed in a subsequent manuscript.

In order to address the above issues, sensitivity analysis (SA) is performed on either the datasets or results obtained from biologically inspired causal models. The reason for using these tools of sensitivity analysis is that they help in observing the behaviour of the output and the importance of the contributing input factors via a robust and an easy mathematical framework. In this manuscript both local and global SA methods are used. Where appropriate, a description of the biologically inspired causal models ensues before the analysis of results from these models. The approach taken here is that first a problem will be addressed and then the analysis of results and discussion ensues before working with the next issue.

2.1 Sensitivity analysis

Seminal work by Russian mathematician Sobol'[21] lead to development as well as employment of SA methods to study various complex systems where it was tough to measure the contribution of various input parameters in the behaviour of the output. A recent unpublished review on the global SA methods by Iooss and Lemaître[22] categorically delineates these methods with the fol-

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lowing functionality • screening for sorting influential measures (Morris[23] method, Group screening in Moon *et al.*[24] & Dean and Lewis[25], Iterated factorial design in Andres and Hajas[26], Sequential bifurcation in Bettonvil and Kleijnen[27] and Cotter[28] design), • quatitative indicies for measuring the importance of contributing input factors in linear models (Christensen[29], Saltelli *et al.*[30], Helton and Davis[31] and McKay *et al.*[32]) and nonlinear models (Homma and Saltelli[33], Sobol[34], Saltelli[35], Saltelli *et al.*[36], Saltelli *et al.*[37], Cukier *et al.*[38], Saltelli *et al.*[39], & Tarantola *et al.*[40] Saltelli *et al.*[41], Janon *et al.*[42], Owen[43], Tissot and Prieur[44], Da Veiga and Gamboa[45], Archer *et al.*[46], Tarantola *et al.*[47], Saltelli *et al.*[41] and Jansen[48]) and • exploring the model behaviour over a range on input values (Storlie and Helton[49] and Da Veiga *et al.*[50], Li *et al.*[51] and Hajikolaei and Wang[52]). Iooss and Lemaître[22] also provide various criteria in a flowchart for adapting a method or a combination of the methods for sensitivity analysis.

Besides the above Sobol'[21]'s variance based indicies, more recent developments regarding new indicies based on density, derivative and goal-oriented can be found in Borgonovo[53], Sobol and Kucherenko[54] and Fort *et al.*[55], respectively. In a more recent development, Da Veiga[56] propose new class of indicies based on density ratio estimation (Borgonovo[53]) that are special cases of dependence measures. This in turn helps in exploiting measures like distance correlation (Székely *et al.*[57]) and Hilbert-Schmidt independence criterion (Gretton *et al.*[58]) as new sensitivity indicies. The basic framework of these indicies is based on use of Csiszár *et al.*[59] f-divergence, concept of dissimilarity measure and kernel trick Aizerman *et al.*[60]. Finally, Da Veiga[56] propose feature selection as an alternative to screening methods in sensitivity analysis. The main issue with variance based indicies (Sobol'[21]) is that even though they capture importance information regarding the contribution of the input factors, they • do not handle multivariate random variables easily and • are only invariant under linear transformations. In comparison to these variance methods, the newly proposed indicies based on density estimations (Borgonovo[53]) and dependence measures are more robust.

2.2 Relevance in systems biology

Recent efforts in systems biology to understand the importance of various factors apropos output behaviours has gained prominence. Sumner *et al.*[61] compares the use of Sobol'[21] variance based indices versus Morris[23] screening method which uses a One-at-a-time (OAT) approach to analyse the sensitivity of *GSK*3 dynamics to uncertainty in an insulin signaling model. Similar efforts, but on different pathways can be found in Zheng and Rundell[62] and Marino *et al.*[63].

SA provides a way of analyzing various factors taking part in a biological phenomena and deals with the effects of these factors on the output of the biological system under consideration. Usually, the model equations are differential in nature with a set of inputs and the associated set of parameters that guide the output. SA helps in observing how the variance in these parameters and inputs leads to changes in the output behaviour. The goal of this manuscript is not to analyse differential equations and the parameters associated with it. Rather, the aim is to observe which input genotypic factors have greater contribution to observed phenotypic behaviour like a sample being normal or cancerous in both static and time series data. In this process, the effect of fold changes in time is also considered for analysis in the light of the recently observed psychophysical laws acting downstream of the Wnt pathway (Goentoro and Kirschner[64]).

2.3 Sensitivity indices

Given the range of estimators available for testing the sensitivity, it might be useful to list a few which are going to be employed in this research study. Also, a brief introduction into the fundamentals of the derivation of the three main indicies has been provided.

2.3.1 Variance based indices

The variance based indices as proposed by Sobol'[21] prove a theorem that an integrable function can be decomposed into summands of different dimensions. Also, a Monte Carlo algorithm is used to estimate the sensitivity of a function apropos arbitrary group of variables. It is assumed that a model denoted by function $u = f(x)$, $x = (x_1, x_2, ..., x_n)$, is defined in a unit n-dimensional cube $\mathscr{K}^n$ with u as the scalar output. The requirement of the problem is to find the sensitivity of function $f(x)$ with respect to different variables. If $u^* = f(x^*)$ is the required solution, then the sensitivity of u^* apropos x_k is estimated via the partial derivative $(\partial u/\partial x_k)_{x=x^*}$. This approach is the local sensitivity. In global sensitivity, the input $x = x^*$ is not specified. This implies that the model $f(x)$ lies inside the cube and the sensitivity indices are regarded as tools for studying the model instead of the solution. Detailed technical aspects with examples can be found in Homma and Saltelli[33] and Sobol[65].

Let a group of indices $i_1, i_2, ..., i_s$ exist, where $1 \leq i_1 < ... < i_s \leq n$ and $1 \leq s \leq n$. Then the notation for sum over all different groups of indices is -

$$\widehat{\Sigma} T_{i_1,i_2,...,i_s} = \Sigma_{i=1}^{n} T_i + \Sigma_{s=1}^{n} \Sigma_{1 \leq i < j \leq n} T_{i,j} + ... + T_{1,2,...,n} \qquad (1)$$

Then the representation of $f(x)$ using equation 1 in the form -

$$\begin{aligned} f(x) &= f_0 + \widehat{\Sigma} f_{i_1,i_2,...,i_s} \qquad (2) \\ &= f_0 + \Sigma_i f_i(x_i) + \Sigma_{i<j} f_{i,j}(x_i, x_j) + ... + f_{1,2,...,n}(x_1, x_2, ..., x_n) \end{aligned}$$

is called ANOVA-decomposition from Archer *et al.*[46] or expan-

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sion into summands of different dimensions, if f_0 is a constant and integrals of the summands $f_{i_1,i_2,...,i_s}$ with respect to their own variables are zero, i.e,

$$f_0 = \int_{\mathscr{K}^n} f(x)dx \tag{3}$$

$$\int_0^1 f_{i_1,i_2,...,i_s}(x_{i_1},x_{i_2},...,x_{i_s})dx_{i_k} = 0, 1 \leq k \leq s \tag{4}$$

It follows from equation 3 that all summands on the right hand side are orthogonal, i.e if at least one of the indices in $i_1,i_2,...,i_s$ and $j_1,j_2,...,j_l$ is not repeated i.e

$$\int_0^1 f_{i_1,i_2,...,i_s}(x_{i_1},x_{i_2},...,x_{i_s})f_{j_1,j_2,...,j_l}(x_{j_1},x_{j_2},...,x_{j_s})dx = 0 \tag{5}$$

Sobol'[21] proves a theorem stating that there is an existence of a unique expansion of equation 3 for any $f(x)$ integrable in $\mathscr{K}^n$. In brief, this implies that for each of the indices as well as a group of indices, integrating equation 3 yields the following -

$$\int_0^1 ... \int_0^1 f(x)dx/dx_i = f_0 + f_i(x_i) \tag{6}$$

$$\int_0^1 ... \int_0^1 f(x)dx/dx_i dx_j = f_0 + f_i(x_i) + f_j(x_j) + f_{i,j}(x_i,x_j) \tag{7}$$

were, dx/dx_i is $\prod_{\forall k \in \{1,..,n\}; i \notin k} dx_k$ and $dx/dx_i dx_j$ is $\prod_{\forall k \in \{1,..,n\}; i,j \notin k} dx_k$. For higher orders of grouped indices, similar computations follow. The computation of any summand $f_{i_1,i_2,...,i_s}(x_{i_1},x_{i_2},...,x_{i_s})$ is reduced to an integral in the cube $\mathscr{K}^n$. The last summand $f_{1,2,...,n}(x_1,x_2,...,x_n)$ is $f(x) - f_0$ from equation 3. Homma and Saltelli[33] stresses that use of Sobol sensitivity indices does not require evaluation of any $f_{i_1,i_2,...,i_s}(x_{i_1},x_{i_2},...,x_{i_s})$ nor the knowledge of the form of $f(x)$ which might well be represented by a computational model i.e a function whose value is only obtained as the output of a computer program.

Finally, assuming that $f(x)$ is square integrable, i.e $f(x) \in \mathscr{L}_2$, then all of $f_{i_1,i_2,...,i_s}(x_{i_1},x_{i_2},...,x_{i_s}) \in \mathscr{L}_2$. Then the following constants

$$\int_{\mathscr{K}^n} f^2(x)dx - f_0^2 = D \tag{8}$$

$$\int_0^1 ... \int_0^1 f^2_{i_1,i_2,...,i_s}(x_{i_1},x_{i_2},...,x_{i_s})dx_{i_1}dx_{i_2}...dx_{i_s} = D_{i_1,i_2,...,i_s} \tag{9}$$

are termed as variances. Squaring equation 3, integrating over $\mathscr{K}^n$ and using the orthogonality property in equation 5, D evaluates to -

$$D = \widehat{\Sigma} D_{i_1,i_2,...,i_s} \tag{10}$$

Then the global sensitivity estimates is defined as -

$$S_{i_1,i_2,...,i_s} = \frac{D_{i_1,i_2,...,i_s}}{D} \tag{11}$$

It follows from equations 10 and 11 that

$$\widehat{\Sigma} S_{i_1,i_2,...,i_s} = 1 \tag{12}$$

Clearly, all sensitivity indices are non-negative, i.e an index $S_{i_1,i_2,...,i_s} = 0$ if and only if $f_{i_1,i_2,...,i_s} \equiv 0$. The true potential of Sobol indices is observed when variables $x_1,x_2,...,x_n$ are divided into m different groups with $y_1,y_2,...,y_m$ such that $m < n$. Then $f(x) \equiv f(y_1,y_2,...,y_m)$. All properties remain the same for the computation of sensitivity indices with the fact that integration with respect to y_k means integration with respect to all the x_i's in y_k. Details of these computations with examples can be found in Sobol[65]. Variations and improvements over Sobol indices have already been stated in section 2.1.

2.3.2 Density based indices

As discussed before, the issue with variance based methods is the high computational cost incurred due to the number of interactions among the variables. This further requires the use of screening methods to filter out redundant or unwanted factors that might not have significant impact on the output. Recent work by Da Veiga[56] proposes a new class of sensitivity indicies which are a special case of density based indicies Borgonovo[53]. These indicies can handle multivariate variables easily and relies on density ratio estimation. Key points from Da Veiga[56] are mentioned below.

Considering the similar notation in previous section, $f : \mathscr{R}^n \rightarrow \mathscr{R}$ $(u = f(x))$ is assumed to be continuous. It is also assumed that X_k has a known distribution and are independent. Baucells and Borgonovo[66] state that a function which measures the similarity between the distribution of U and that of $U|X_k$ can define the impact of X_k on U. Thus the impact is defined as -

$$S_{X_k} = \mathscr{E}(d(U, U|X_k)) \tag{13}$$

were $d(\cdot,\cdot)$ is a dissimilarity measure between two random variables. Here d can take various forms as long as it satisfies the criteria of a dissimilarity measure. Csiszár *et al.*[59]'s f-divergence between U and $U|X_k$ when all input random variables are considered to be absolutely continuous with respect to Lebesgue measure on $\mathscr{R}$ is formulated as -

$$d_F(U||U|X_k) = \int_{\mathscr{R}} F\left(\frac{p_U(u)}{p_{U|X_k}(u)}\right) p_{U|X_k}(u)du \tag{14}$$

were F is a convex function such that $F(1) = 0$ and p_U and $p_{U|X_k}$ are the probability distribution functions of U and $U|X_k$. Standard choices of F include Kullback-Leibler divergence $F(t) = -\log_e(t)$, Hellinger distance $(\sqrt{t}-1)^2$, Total variation distance $F(t) = |t-1|$, Pearson χ^2 divergence $F(t) = t^2 - 1$ and Neyman χ^2 divergence $F(t) = (1-t^2)/t$. Substituting equation 14 in equation 13, gives

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the following sensitivity index -

$$\begin{aligned} S^F_{X_k} &= \int_{\mathscr{R}} d_F(U||U|X_k)p_{X_k}(x)dx \\ &= \int_{\mathscr{R}}\int_{\mathscr{R}} F(\frac{p_U(u)}{p_{U|X_k}(u)})p_{U|X_k}(u)p_{X_k}(x)dxdu \\ &= \int_{\mathscr{R}^2} F(\frac{p_U(u)p_{X_k}(x)}{p_{U|X_k}(u)p_{X_k}(x)})p_{U|X_k}(u)p_{X_k}(x)dxdu \\ &= \int_{\mathscr{R}^2} F(\frac{p_U(u)p_{X_k}(x)}{p_{X_k,U}(x,u)})p_{X_k,U}(x,u)dxdu \end{aligned} \quad (15)$$

were p_{X_k} and $p_{X_k,Y}$ are the probability distribution functions of X_k and (X_k,U), respectively. Csiszár *et al.*[59] f-divergences imply that these indices are positive and equate to 0 when U and X_k are independent. Also, given the formulation of $S^F_{X_k}$, it is invariant under any smooth and uniquely invertible transformation of the variables X_k and U (Kraskov *et al.*[67]). This has an advantage over Sobol sensitivity indices which are invariant under linear transformations.

By substituting the different formulations of F in equation 15, Da Veiga[56]'s work claims to be the first in establishing the link that previously proposed sensitivity indices are actually special cases of more general indices defined through Csiszár *et al.*[59]'s f-divergence. Then equation 15 changes to estimation of ratio between the joint density of (X_k,U) and the marginals, i.e -

$$S^F_{X_k} = \int_{\mathscr{R}^2} F(\frac{1}{r(x,u)})p_{X_k,U}(x,u)dxdu = \mathscr{E}_{(X_k,U)}F(\frac{1}{r(X_k,U)}) \quad (16)$$

were, $r(x,y) = (p_{X_k,U}(x,u))/(p_U(u)p_{X_k}(x))$. Multivariate extensions of the same are also possible under the same formulation.

Finally, given two random vectors $X \in \mathscr{R}^p$ and $Y \in \mathscr{R}^q$, the dependence measure quantifies the dependence between X and Y with the property that the measure equates to 0 if and only if X and Y are independent. These measures carry deep links (Sejdinovic *et al.*[68]) with distances between embeddings of distributions to reproducing kernel Hilbert spaces (RHKS) and here the related Hilbert-Schmidt independence criterion (HSIC by Gretton *et al.*[58]) is explained.

In a very brief manner from an extremely simple introduction by Daumé III[69] - "We first defined a field, which is a space that supports the usual operations of addition, subtraction, multiplication and division. We imposed an ordering on the field and described what it means for a field to be complete. We then defined vector spaces over fields, which are spaces that interact in a friendly way with their associated fields. We defined complete vector spaces and extended them to Banach spaces by adding a norm. Banach spaces were then extended to Hilbert spaces with the addition of a dot product." Mathematically, a Hilbert space $\mathscr{H}$ with elements $r,s \in \mathscr{H}$ has dot product $\langle r,s\rangle_{\mathscr{H}}$ and $r \cdot s$. When $\mathscr{H}$ is a vector space over a field $\mathscr{F}$, then the dot product is an element in $\mathscr{F}$. The product $\langle r,s\rangle_{\mathscr{H}}$ follows the below mentioned properties when $r,s,t \in \mathscr{H}$ and for all $a \in \mathscr{F}$ -

- Associative : $(ar)\cdot s = a(r\cdot s)$
- Commutative : $r\cdot s = s\cdot r$
- Distributive : $r\cdot(s+t) = r\cdot s + r\cdot t$

Given a complete vector space $\mathscr{V}$ with a dot product $\langle\cdot,\cdot\rangle$, the norm on $\mathscr{V}$ defined by $||r||_{\mathscr{V}} = \sqrt{(\langle r,r\rangle)}$ makes this space into a Banach space and therefore into a full Hilbert space.

A reproducing kernel Hilbert space (RKHS) builds on a Hilbert space $\mathscr{H}$ and requires all Dirac evaluation functionals in $\mathscr{H}$ are bounded and continuous (on implies the other). Assuming $\mathscr{H}$ is the $\mathscr{L}_2$ space of functions from X to $\mathscr{R}$ for some measurable X. For an element $x \in X$, a Dirac evaluation functional at x is a functional $\delta_x \in \mathscr{H}$ such that $\delta_x(g) = g(x)$. For the case of real numbers, x is a vector and g a function which maps from this vector space to $\mathscr{R}$. Then δ_x is simply a function which maps g to the value g has at x. Thus, δ_x is a function from $(\mathscr{R}^n \mapsto \mathscr{R})$ into $\mathscr{R}$.

The requirement of Dirac evaluation functions basically means via the Riesz[70] representation theorem, if ϕ is a bounded linear functional (conditions satisfied by the Dirac evaluation functionals) on a Hilbert space $\mathscr{H}$, then there is a unique vector ℓ in $\mathscr{H}$ such that $\phi g = \langle g,\ell\rangle_{\mathscr{H}}$ for all $\ell \in \mathscr{H}$. Translating this theorem back into Dirac evaluation functionals, for each δ_x there is a unique vector k_x in $\mathscr{H}$ such that $\delta_x g = g(x) = \langle g,k_x\rangle_{\mathscr{H}}$. The reproducing kernel K for $\mathscr{H}$ is then defined as : $K(x,x') = \langle k_x,k_{x'}\rangle$, were k_x and $k_{x'}$ are unique representatives of δ_x and $\delta_{x'}$. The main property of interest is $\langle g,K(x,x')\rangle_{\mathscr{H}} = g(x')$. Furthermore, k_x is defined to be a function $y \mapsto K(x,y)$ and thus the reproducibility is given by $\langle K(x,\cdot),K(y,\cdot)\rangle_{\mathscr{H}} = K(x,y)$.

The Hilbert-Schmidt independence criterion (HSIC) proposed by Gretton *et al.*[58] is based on kernel approach for finding dependences and on cross-covariance operators in RKHS. Let $X \in \mathscr{X}$ have a distribution P_X and consider a RKHS $\mathscr{A}$ of functions $\mathscr{X} \to \mathscr{R}$ with kernel $k_{\mathscr{X}}$ and dot product $\langle\cdot,\cdot\rangle_{\mathscr{A}}$. Similarly, Let $U \in \mathscr{Y}$ have a distribution P_Y and consider a RKHS $\mathscr{B}$ of functions $\mathscr{U} \to \mathscr{R}$ with kernel $k_{\mathscr{B}}$ and dot product $\langle\cdot,\cdot\rangle_{\mathscr{B}}$. Then the cross-covariance operator $C_{X,U}$ associated with the joint distribution P_{XU} of (X,U) is the linear operator $\mathscr{B} \to \mathscr{A}$ defined for every $a \in \mathscr{A}$ and $b \in \mathscr{B}$ as -

$$\langle a,C_{XU}b\rangle_{\mathscr{A}} = \mathscr{E}_{XU}[a(X),b(U)] - \mathscr{E}_X a(X)\mathscr{E}_U b(U) \quad (17)$$

The cross-covariance operator generalizes the covariance matrix by representing higher order correlations between X and U through nonlinear kernels. For every linear operator $C : \mathscr{B} \to \mathscr{A}$ and provided the sum converges, the Hilbert-Schmidt norm of C is given by -

$$||C||^2_{HS} = \Sigma_{k,l}\langle a_k,Cb_l\rangle_{\mathscr{A}} \quad (18)$$

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were a_k and b_l are orthonormal bases of $\mathscr{A}$ and $\mathscr{B}$, respectively. The HSIC criterion is then defined as the Hilbert-Schmidt norm of cross-covariance operator -

$$HSIC(X,U)_{\mathscr{A},\mathscr{B}} = \begin{cases} ||C_{XU}||^2_{HS} = \\ \mathscr{E}_{X,X',U,U'} k_{\mathscr{X}}(X,X') k_{\mathscr{U}}(U,U') + \\ \mathscr{E}_{X,X'} k_{\mathscr{X}}(X,X') \mathscr{E}_{U,U'} k_{\mathscr{U}}(U,U') - \\ 2\mathscr{E}_{X,Y}[\mathscr{E}_{X'} k_{\mathscr{X}}(X,X') \mathscr{E}_{U'} k_{\mathscr{U}}(U,U')] \end{cases} \tag{19}$$

were the equality in terms of kernels is proved in Gretton *et al.*[58]. Finally, assuming (X_i,U_i) $(i = 1,2,...,n)$ is a sample of the random vector (X,U) and denote $K_{\mathscr{X}}$ and $K_{\mathscr{U}}$ the Gram matrices with entries $K_{\mathscr{X}}(i,j) = k_{\mathscr{X}}(X_i,X_j)$ and $K_{\mathscr{U}}(i,j) = k_{\mathscr{U}}(U_i,U_j)$. Gretton *et al.*[58] proposes the following estimator for $HSIC_n(X,U)_{\mathscr{A},\mathscr{B}}$ -

$$HSIC_n(X,U)_{\mathscr{A},\mathscr{B}} = \frac{1}{n^2} Tr(K_{\mathscr{X}} H K_{\mathscr{U}} H) \tag{20}$$

were H is the centering matrix such that $H(i,j) = \delta_{i,j} - \frac{1}{n}$. Then $HSIC_n(X,U)_{\mathscr{A},\mathscr{B}}$ can be expressed as -

$$HSIC(X,U)_{\mathscr{A},\mathscr{B}} = \begin{cases} \frac{1}{n^2}\Sigma^n_{i,j=1} k_{\mathscr{X}}(X_i,X_j) k_{\mathscr{U}}(U_i,U_j) \\ +\frac{1}{n^2}\Sigma^n_{i,j=1} k_{\mathscr{X}}(X_i,X_j) \frac{1}{n^2}\Sigma^n_{i,j=1} k_{\mathscr{U}}(U_i,U_j) \\ -\frac{2}{n}\Sigma^n_{i=1}[\frac{1}{n}\Sigma^n_{j=1} k_{\mathscr{X}}(X_i,X_j) \frac{1}{n}\Sigma^n_{j=1} k_{\mathscr{U}}(U_i,U_j)] \end{cases} \tag{21}$$

Finally, Da Veiga[56] proposes the sensitivity index based on distance correlation as -

$$S^{HSIC_{\mathscr{A},\mathscr{B}}}_{X_k} = R(X_k,U)_{\mathscr{A},\mathscr{B}} \tag{22}$$

were the kernel based distance correlation is given by -

$$R^2(X,U)_{\mathscr{A},\mathscr{B}} = \frac{HSIC(X,U)_{\mathscr{A},\mathscr{B}}}{\sqrt{(HSIC(X,X)_{\mathscr{A},\mathscr{A}} HSIC(U,U)_{\mathscr{B},\mathscr{B}})}} \tag{23}$$

were kernels inducing $\mathscr{A}$ and $\mathscr{B}$ are to be chosen within a universal class of kernels. Similar multivariate formulation for equation 20 are possible.

2.3.3 Choice of sensitivity indices

The SENSITIVITY PACKAGE (Faivre *et al.*[71] and Iooss and Lemaître[22]) in R langauge provides a range of functions to compute the indices and the following indices will be taken into account for addressing the posed questions in this manuscript.

1. sensiFdiv - conducts a density-based sensitivity analysis where the impact of an input variable is defined in terms of dissimilarity between the original output density function and the output density function when the input variable is fixed. The dissimilarity between density functions is measured with Csiszar f-divergences. Estimation is performed through kernel density estimation and the function kde of the package ks. (Borgonovo[53], Da Veiga[56])

2. sensiHSIC - conducts a sensitivity analysis where the impact of an input variable is defined in terms of the distance between the input/output joint probability distribution and the product of their marginals when they are embedded in a Reproducing Kernel Hilbert Space (RKHS). This distance corresponds to HSIC proposed by Gretton *et al.*[58] and serves as a dependence measure between random variables.

3. soboljansen - implements the Monte Carlo estimation of the Sobol indices for both first-order and total indices at the same time (all together 2p indices), at a total cost of (p+2) $\times$ n model evaluations. These are called the Jansen estimators. (Jansen[48] and Saltelli *et al.*[41])

4. sobol2002 - implements the Monte Carlo estimation of the Sobol indices for both first-order and total indices at the same time (all together 2p indices), at a total cost of (p+2) $\times$n model evaluations. These are called the Saltelli estimators. This estimator suffers from a conditioning problem when estimating the variances behind the indices computations. This can seriously affect the Sobol indices estimates in case of largely non-centered output. To avoid this effect, you have to center the model output before applying "sobol2002". Functions "soboljansen" and "sobolmartinez" do not suffer from this problem. (Saltelli[35])

5. sobol2007 - implements the Monte Carlo estimation of the Sobol indices for both first-order and total indices at the same time (all together 2p indices), at a total cost of (p+2) $\times$ n model evaluations. These are called the Mauntz estimators. (Saltelli *et al.*[41])

6. sobolmartinez - implements the Monte Carlo estimation of the Sobol indices for both first-order and total indices using correlation coefficients-based formulas, at a total cost of (p + 2) $\times$ n model evaluations. These are called the Martinez estimators.

7. sobol - implements the Monte Carlo estimation of the Sobol sensitivity indices. Allows the estimation of the indices of the variance decomposition up to a given order, at a total cost of (N + 1) $\times$ n where N is the number of indices to estimate. (Sobol'[21])

3 Description of the dataset & design of experiments

STATIC DATA - A simple static dataset containing expression values measured for a few genes known to have important role in human colorectal cancer cases has been taken from Jiang *et al.*[16]. Most of the expression values recorded are for genes that play a role in Wnt signaling pathway at an extracellular level and are known to

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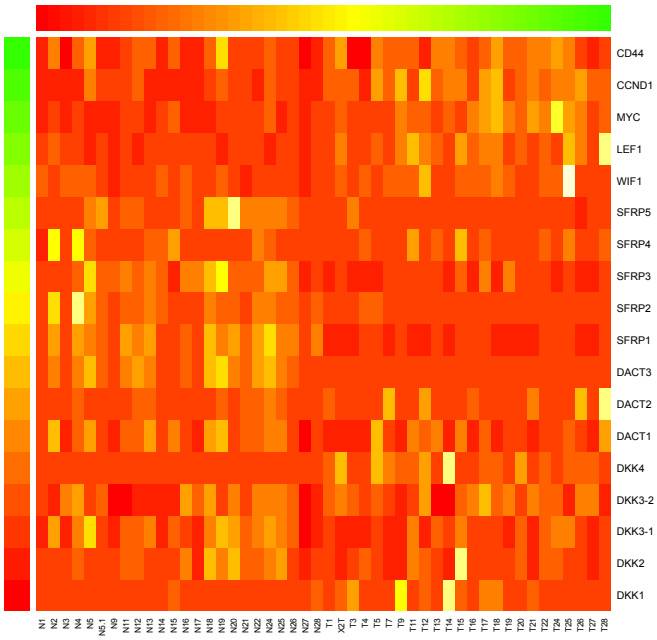

Fig. 2 Heat map for gene expression values for each of the 24 normal mucosa and 24 human colorectal tumor cases from Jiang *et al.*[16]

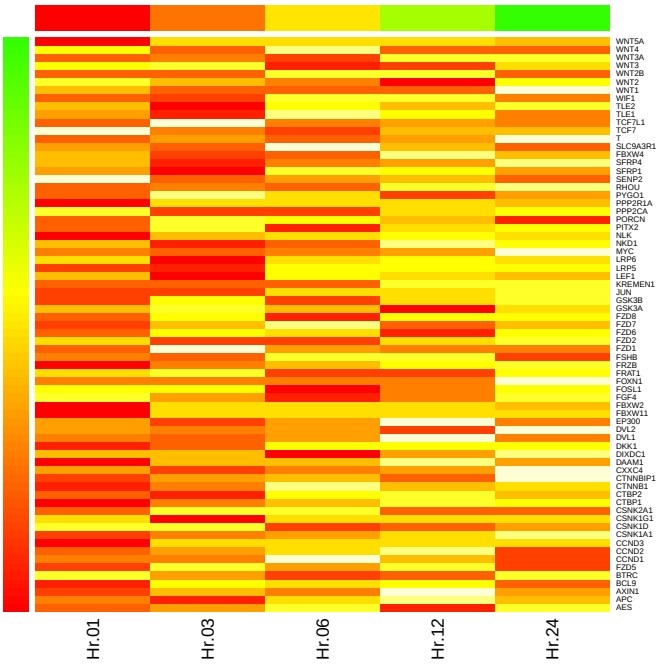

Fig. 3 Heat map for gene expression values for 6 time points from Gujral and MacBeath[17]

have inhibitory affect on the Wnt pathway due to epigenetic factors. For each of the 24 normal mucosa and 24 human colorectal tumor cases, gene expression values were recorded for 14 genes belonging to the family of *SFRP*, *DKK*, *WIF*1 and *DACT*. Also, expression values of established Wnt pathway target genes like *LEF*1, *MYC*, *CD*44 and *CCND*1 were recorded per sample.

TIME SERIES DATA - Contrary to the static data described above, Gujral and MacBeath[17] presents a bigger 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 *WNT*3*A*-stimulated HEK 293 cells in time relative to their levels in unstimulated, serum-starved cells at 0-hour. The 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 were considered significant. Positive (negative) numbers represent up (down) -regulation.

Note that green (red) represents activation (repression) in the heat maps of data in Jiang *et al.*[16] and Gujral and MacBeath[17]. Figures 2 and 3 represent the heat maps for the static and time series data respectively.

DESIGN OF EXPERIMENTS - The reported results will be based on scaled as well as unscaled datasets. For the static data, only the scaled results are reported. This is mainly due to the fact that the measurements vary in a wide range and due to this there is often an error in the computed estimated of these indices. The data for time series does not vary in a wide range and thus the results are reported for both the scaled and the non scaled versions. Total sensitivity indices and 1st order indices will be used for sensitivity analysis. For addressing a biological question with known prior knowledge, the order of indices might be increased. While studying the interaction among the various genetic factors using static data, tumor samples are considered separated from normal samples. Bootstrapping without replicates on a smaller sample number is employed to generate estimates of indices which are then averaged. This takes into account the variance in the data and generates confidence bands for the indices. For the case of time series data, interactions among the contributing factors are studied by comparing (1) pairs of fold-changes at single time points and (2) pairs of deviations in fold changes between pairs of time points. Generation of distribution around measurements at single time points with added noise is done to estimate the indices.

4 Static data

To measure the strength of the contributing factors in the static dataset by Jiang *et al.*[16], 1st order and total sensitivity indices were generated. For each of the expression values of the genes recorded in the normal and tumor cases, the computation of the indices was done using bootstrapped samples in three different experiments each with a sample size of 8, 16 and 24, respectively.

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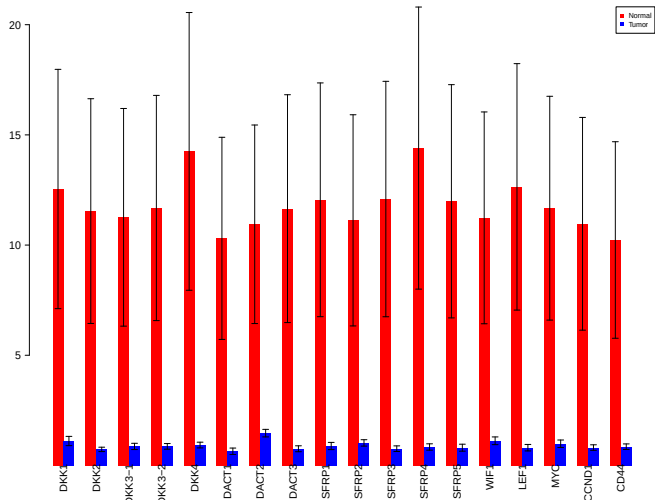

Fig. 4 sensiFdiv indices using Total Variation distance. Red - indices for normal. Blue - indices for tumor.

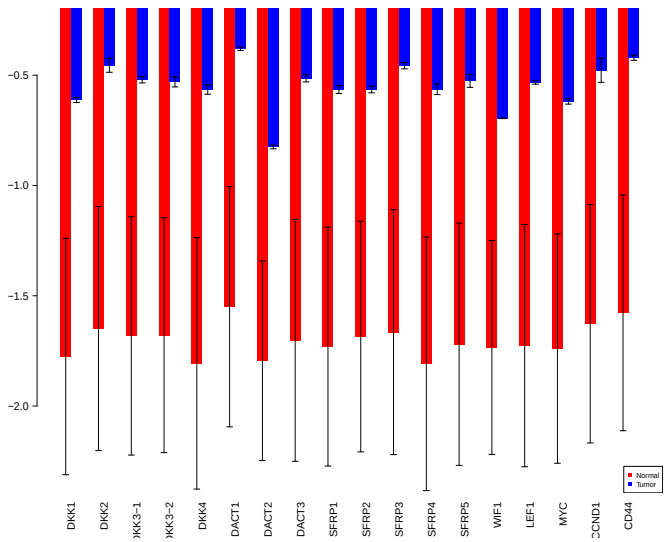

Fig. 5 sensiFdiv indices for Kullback-Leibler divergence. Red - indices for normal. Blue - indices for tumor.

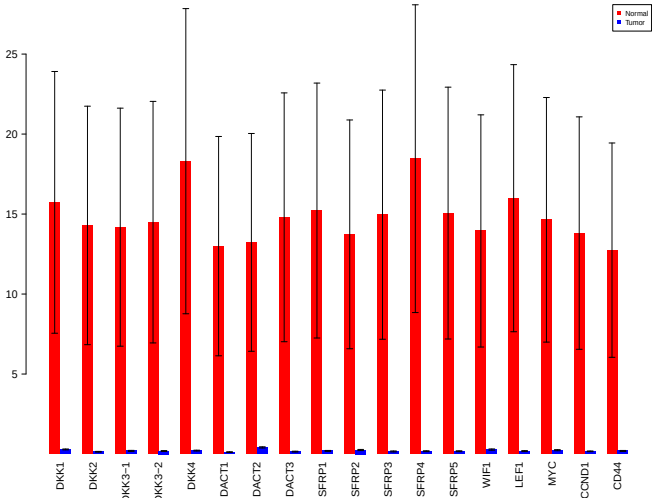

Fig. 6 sensiFdiv indices for Hellinger distance. Red - indices for normal. Blue - indices for tumor.

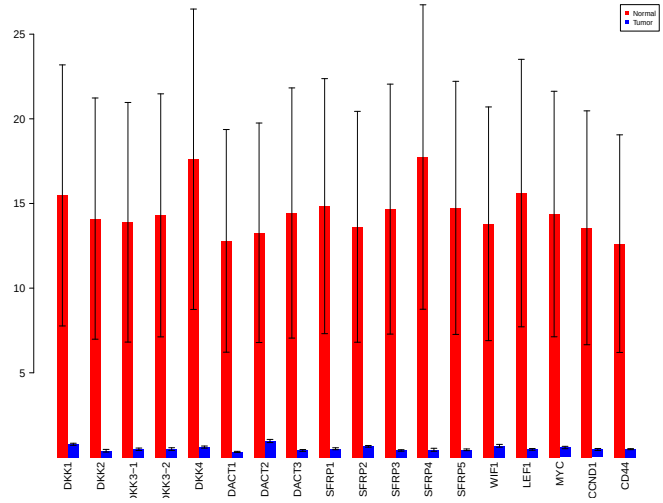

Fig. 7 sensiFdiv indices for Pearson χ^2 distance. Red - indices for normal. Blue - indices for tumor.

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With only 24 samples in total, 20 bootstraps were generated for each set and the results were generated. From these replicates, the mean of the indices is reported along with the 0.95% confidence bands.

Using the sensiFdiv, all indices are computed as positive and those nearing to zero indicate the contribution of a factor as independent from the behaviour under consideration. Here, while comparing the indices of the gene expression values for normal and tumor cases, it was found that most of the involved intra/extracellular factors had some degree of contribution in the normal case and almost negligible contribution in the tumor case (see figures 4, 6 and 7). Apart from the negative reading for the KL divergence 5 the interpretations remain the same. This implies that the basic Csiszár *et al.*[59] f-divergence based indices might not capture the intrinsic genotypic effects for the normal and the tumorous cases. From the biological perspective, these graphs do not help in interpreting the strength of the contributions in normal and tumor cases. One might rank the indices for relative contributions, but this might not shed enough light on the how the factors are behaving in normal and tumor cases.

A more powerful way to analyse the contributions is the newly proposed HSIC based measures by Da Veiga[56]. These distances use the kernel trick which can capture intrinsic properties inherent in the recorded measurements by transforming the data into a higher dimensional space. Using these distances in sensiHSIC, it was found that the contributions of the various factors in the normal and the tumor cases vary drastically. This is shown in figures 8, 9 and 10. The laplace and the rbf kernels give more reliable sensitivity estimates for the involved factors than the linear kernel. Studying the results in figures 6 and 7 of Sinha[19] based on prior biological knowledge encoded in the Bayesian network models along with the indices of aforementioned figures, it can be found that indices of the family of $DACT-1/2/3$ show higher (lower) sensitivity in the normal (tumor) case where the activation (repression) happens. Again, of the $DACT$ family, $DACT-1$ has greater influence than $DACT-3$ (than $DACT-2$) based on the values of the sensitivity indices. These indices indicate the dependence of a factor on the output of the model characterized by the signaling being active (passive) in the normal (tumor) cases. 0(1) mean no (full) dependence of the output on the input factor. The laplace and the rbf kernels were found to give more consistent results than the linear kernel and the following description discusses the results from these kernels. For the $SFRP$ family $SFRP-1/2/5$ show higher (lower) sensitivity in normal (tumor) case where the activation (repression) happens (see figures 9 and 10). For $SFRP-3/4$ the influence is higher (lower) in the tumor (normal) case. In all the three types of kernels, $WIF1$, MYC and $CCND1$ show stronger (weaker) influence of repression (activation) in the normal (tumor) case (see figures 9 and 10). $CD44$ showed variable influence while observing the normal and tumor cases. Sinha[19] could not derive proper inferences for $LEF1$ but the sensitivity indices indicate that the influence of $LEF1$ in tumor samples to be higher than in normal samples. This points to the $LEF1$'s major role in tumor cases. Finally, for the family of DKK, $DKK1$ and $DKK3-2$ show similar behaviour of expression (repression) in normal (tumor cases) (see Sinha[19]). For the former, the prominence of the influence is shown in the higher (lower) sensitivity for tumor (normal) case. For the latter higher (lower) sensitivity was recorded for normal (tumor) case. This implies that the latter has more influential role in normal while the former has more influential role in tumor case. $DKK3-1$ was found to be expressed (repressed) in normal (tumor) and its dominant role is prominent from the higher bar sensitivity bar for normal than the tumor. Similar behavior of $DKK2$ was inferred by Sinha[19] but the sensitivity indices point to varied results and thus a conclusion cannot be drawn. Note that greater the value of the sensitivity index, greater is an input factor's contribution to the model.

The first order indices generated by sobol functions implemented in sobol2002 (figure 11), sobol2007 (figure 12), soboljansen (figure 13), sobolmartinez (figure 14) and sobol (figure 15) do not point to significant dependencies of the input factors. This can be attributed to the fact that there are less number of samples that help in the estimation of the sensitivity indicies. Finally, the total order indices need to be investigated in the context of the first order indices. It can be observed , sobol2002 (figure 16) and sobol2007 (figure 16) give much better estimates than soboljansen (figure 18) and sobolmartinez (figure 19). Most importantly, it is the former two that closely match with the sensitivity indices estimated using the HSIC distance measures. Interpretations from sobol2002 (figure 16) and sobol2007 (figure 17) are the same as those described above using the laplace and the rbf kernels from density based HSIC measure.

In summary, the sensitivity indices confirm the inferred results in Sinha[19] but do not help in inferring the causal relations using the static data. In combination with the results obtained from the Bayesian network models in Sinha[19] it is possible to study the effect of the input factors for the pathway in both normal and tumor cases. The results of sensitivity indices indicate how much these factors influence the pathway in normal and tumor cases. Again, not all indices reveal important information. So users must be cautious of results and see which measures reveal information that are close to already established or computationally estimated biological facts. Here the density based sensitivity indices captured information more precisely than the variance based indices (except for the total order indices from sobol2002/7 which gave similar results as sensiHSIC). This is attributed to the analytical strength provided by the distance measures using the kernel trick via RKHS that capture nonlinear relations in higher dimensional space, more precisely.

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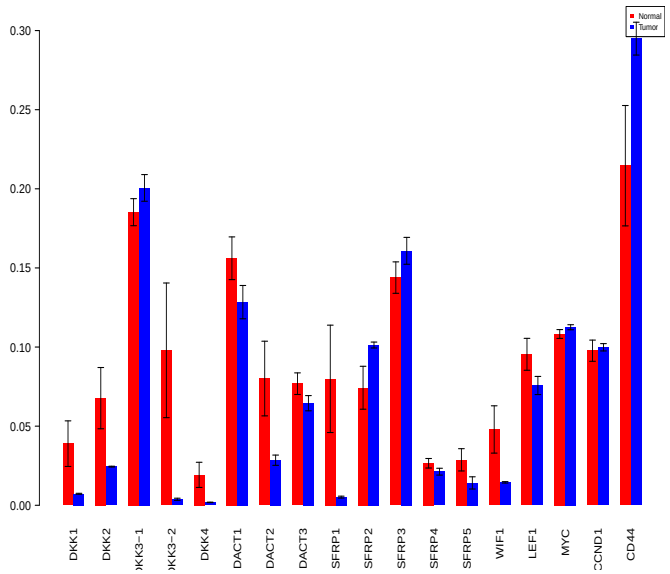

Fig. 8 sensiHSIC indices for linear kernel. Red - indices for normal. Blue - indices for tumor.

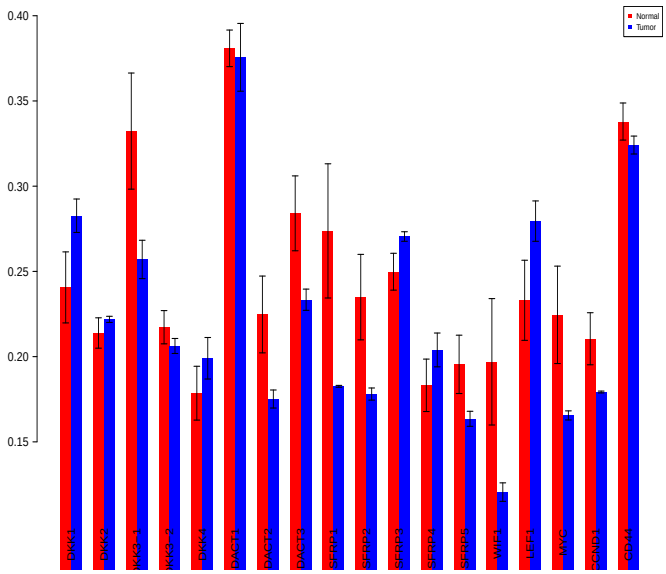

Fig. 9 sensiHSIC indices for laplace kernel. Red - indices for normal. Blue - indices for tumor.

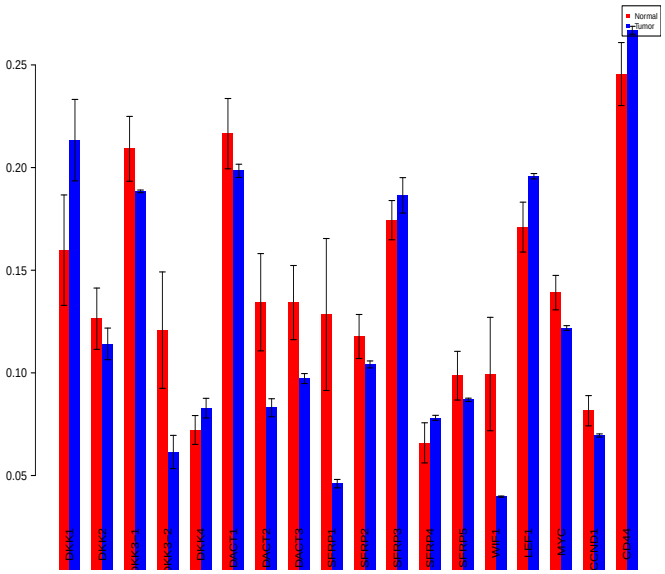

Fig. 10 sensiHSIC indices for rbf kernel. Red - indices for normal. Blue - indices for tumor.

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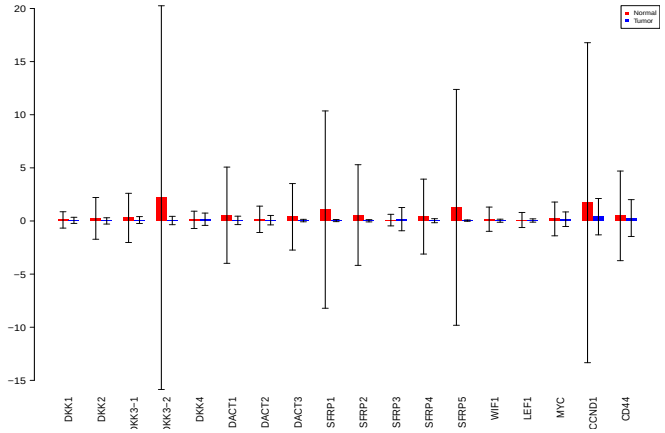

Fig. 11 Sobol 2002 first order indices. Red - indices for normal. Blue - indices for tumor.

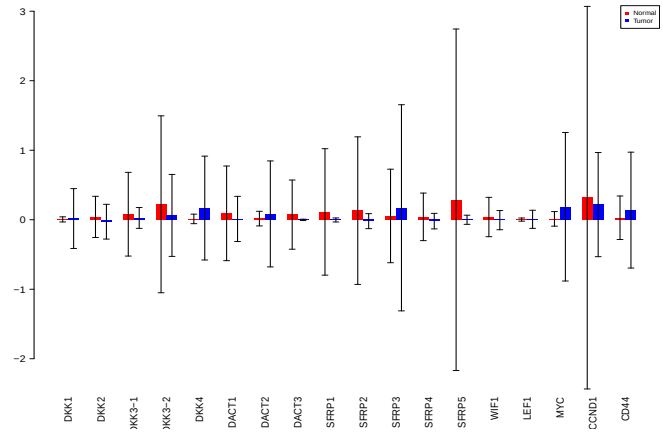

Fig. 12 Sobol 2007 first order indices. Red - indices for normal. Blue - indices for tumor.

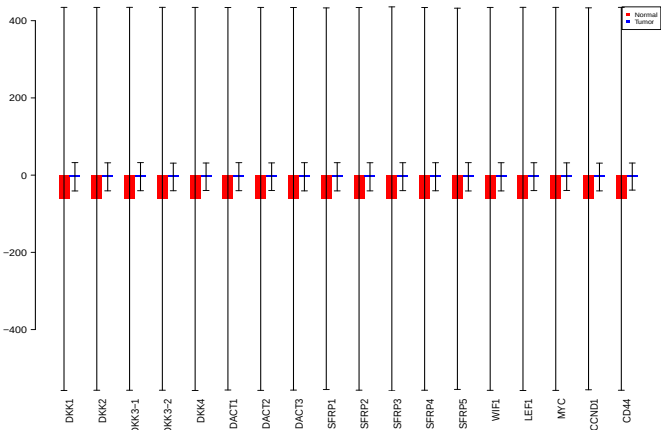

Fig. 13 Sobol jansen first order indices. Red - indices for normal. Blue - indices for tumor.

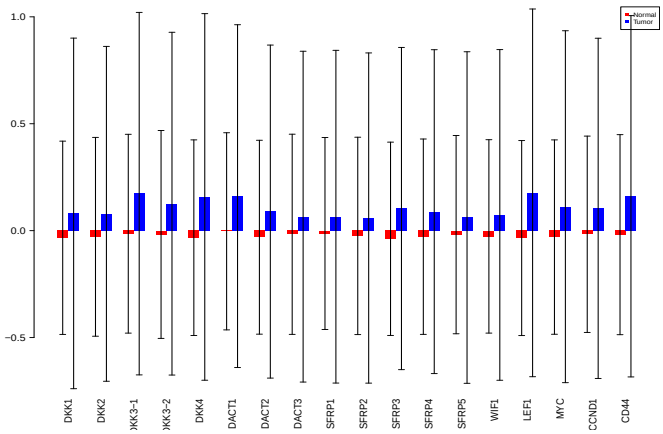

Fig. 14 Sobol martinez first order indices. Red - indices for normal. Blue - indices for tumor.

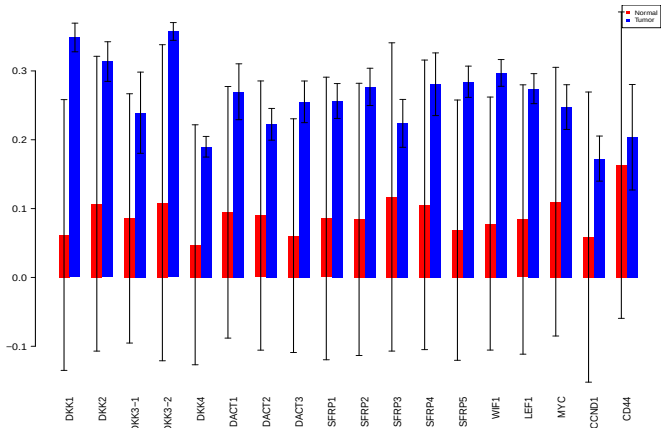

Fig. 15 Sobol first order indices. Red - indices for normal. Blue - indices for tumor.

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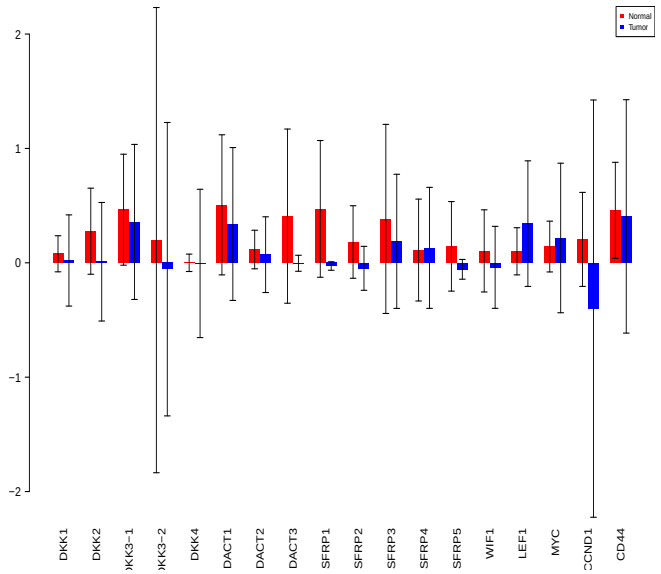

Fig. 16 Sobol 2002 total order indices. Red - indices for normal. Blue - indices for tumor.

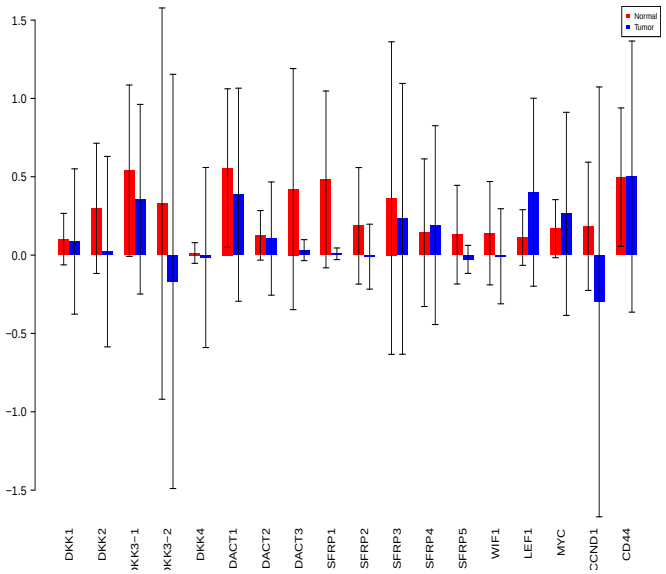

Fig. 17 Sobol 2007 total order indices. Red - indices for normal. Blue - indices for tumor.

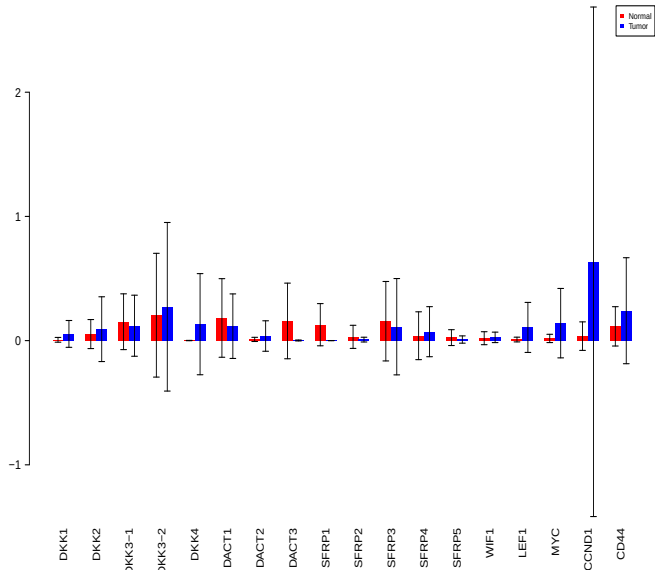

Fig. 18 Sobol jansen total order indices. Red - indices for normal. Blue - indices for tumor.

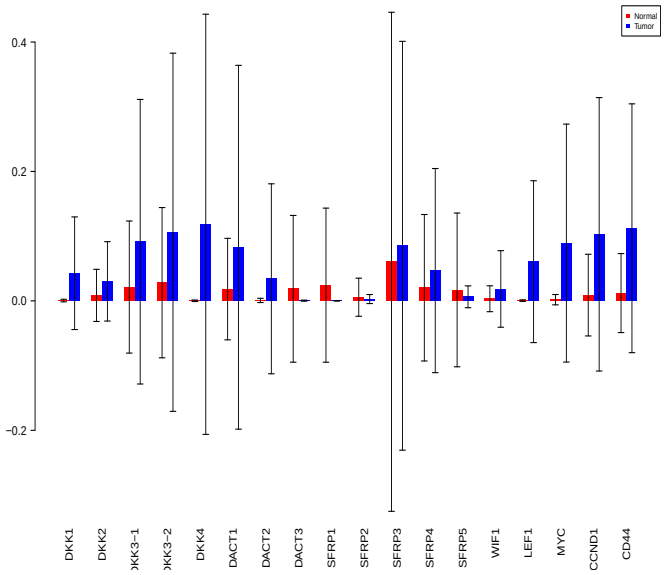

Fig. 19 Sobol martinez total order indices. Red - indices for normal. Blue - indices for tumor.

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5 Time series data

Next, the analysis of the time series data is addressed using the sensitivity indices. There are two experiments that have been performed. First is related to the analysis of a pair of the fold changes recorded at to different consecutive time points i.e t_i & t_{i+1}. The second is related to the analysis of a pair of deviations in fold changes recorded at t_i & t_{i+1} and t_{i+1} & t_{i+2}. The former compares the measurements in time while the latter takes into account the deviations that happens in time. For each measurement at a time point a normal distribution was generated with original recorded value as the mean, a standard deviation of 0.005 and an added noise in the form of jitter (see function jitter in R langauge). For the time measurements of each of the genes recorded in Gujral and MacBeath[17] an analysis of the sensitivity indices for both the scaled and the non-scaled data was done. Here the analysis for non-scaled data is presented. The reason for not presenting the scaled data is that the sample measurements did not vary drastically as found in the case of static data which caused troubles in the estimation of indices earlier. Another reason for not reporting the results on the scaled data is that the non-scaled ones present raw sensitive information which might be lost in scaling via normalization. Note that the authors use self organizing maps (SOM) to cluster data and use correlational analysis to derive their conclusions. In this work, the idea of clustering is abandoned and sensitivity indices are estimated for recorded factors participating in the pathway. Also the correlational analysis is dropped in favour of highly analytical kernel based distance measures.

Also, in a recent development, Goentoro and Kirschner[64] point to two findings namely, • the robust fold changes of β-catenin and • the transcriptional machinery of the Wnt pathway depends on the fold changes in β-catenin instead of absolute levels of the same and some gene transcription networks must respond to fold changes in signals according to the Weber's law in sensory physiology. The second study also carries a weight in the fact that due to the study of the deviations in the fold changes it is possible to check if the recently observed and reported natural psychophysical laws in the signaling pathway hold true or not. Finally, using the sensitivity indicies an effort is made to confirm the existing biological causal relations.

5.1 Analysis of fold changes at different time points

5.1.1 WNT3a

Lets begin with the gene $WNT3a$ as changes in its concentration lead to recording of the measurements of the different genes by Gujral and MacBeath[17]. Of the list of genes recorded, the indices of the those which are influenced by the concentration of $WNT3a$ are analysed. Next based on these confirmations and patterns of indices over time, conclusions for other enlisted genes are drawn. For the former list, the following genes $FZ1$, $FZ2$, $LEF1$, $TCF7$, $TCF7L1$, $LRP6$, $DVL1$, $SFRP1$, $SFRP4$, $CTBP1$, $CTBP2$, $PORCN$, $GSK3\beta$, MYC, APC and $CTNNB1$ are considered.

Figures 20 and 21 represent the indices computed over time. Columns represent the different kinds of indices computed while the rows show the respective genes. Each graph contains the sensitivity index computed at a particular time point (represented by a coloured bar). It should be observed from the aforementioned figures that the variants of the sobol first order (FO) and the total order (TO) indices computed under different formulations were not very informative. This can be seen in graphs were some indices are negative and at some places the behaviour across time and genes remain the same. In contrast to this, the indices generated via the original Sobol function (under the column Sobol-SBL) as well as the sensiHSIC were found to be more reliable. Again, the rbf and laplace kernels under the HSIC formulations showed similar behaviour in comparison to the use of the linear kernel.

Gujral and MacBeath[17] simulate the serum starved HEK293 cells with 200 ng/mL of $Wnt3a$ at different lengths of time. After the first hour (t_1), (under HSIC-rbf/laplace) it was observed that the sensitivity of $WNT3A$ was high (red bar). Due to this there is increased sensitivity of FZ-1/2 as well as $LRP6$. Consistent with the findings of Planutis *et al.*[72], $FZ1$ was found to be expressed. Even though the $FZ2$ was found to be expressed in the first hour, its role is not well studied as it appears to bind to both $WNT3A$ which promotes *Wnt/beta-catenin* signaling and $WNT5A$ which inhibits it as shown by Sato *et al.*[73]. Klapholz-Brown *et al.*[74] and Yokoyama *et al.*[75] show that there is increased *β-catenin* due to $WNT3A$ stimulation which is depicted by the increased sensitivity of $CTNNB1$ expression. The sensitivity of MYC increased monotonically but after the 6th hour it dropped significantly. Probably MYC does not play important role at later stages. $DVL1$ shows a marked increase in sensitivity as the concentration of the $WNT3A$ increases in time. This is supported by the fact that ligand binding at the membrane leads to formation of complex including $DVL1$, FZD and $AXIN$. Negative regulators like $SFRP4$ were found to have lower sensitivity as $WNT3A$ concentration increases, but remained constant for most period. Meanwhile the significance of Wnt antagonist $SFRP1$ (Galli *et al.*[76], Suzuki *et al.*[77] and Caldwell *et al.*[78]) decreases over the period as the concentration of $WNT3A$ increases. As shown by Yokoyama *et al.*[75] $CTBP1$ showed increased sensitivity with increased stimulation of $WNT3A$ in the first hour. This is in line with what Gujral and MacBeath[17] show in their paper. $PORCN$ showed less sensitivity in the initial stages than in final stages indicating its importance in the contribution to Wnt secretion which is necessary for signaling (Willert and Nusse[79]). The sensitivity of $GSK3\beta$ and APC decreased in time indicating the lowering of its significance in time due to no formation of the degradation complex.

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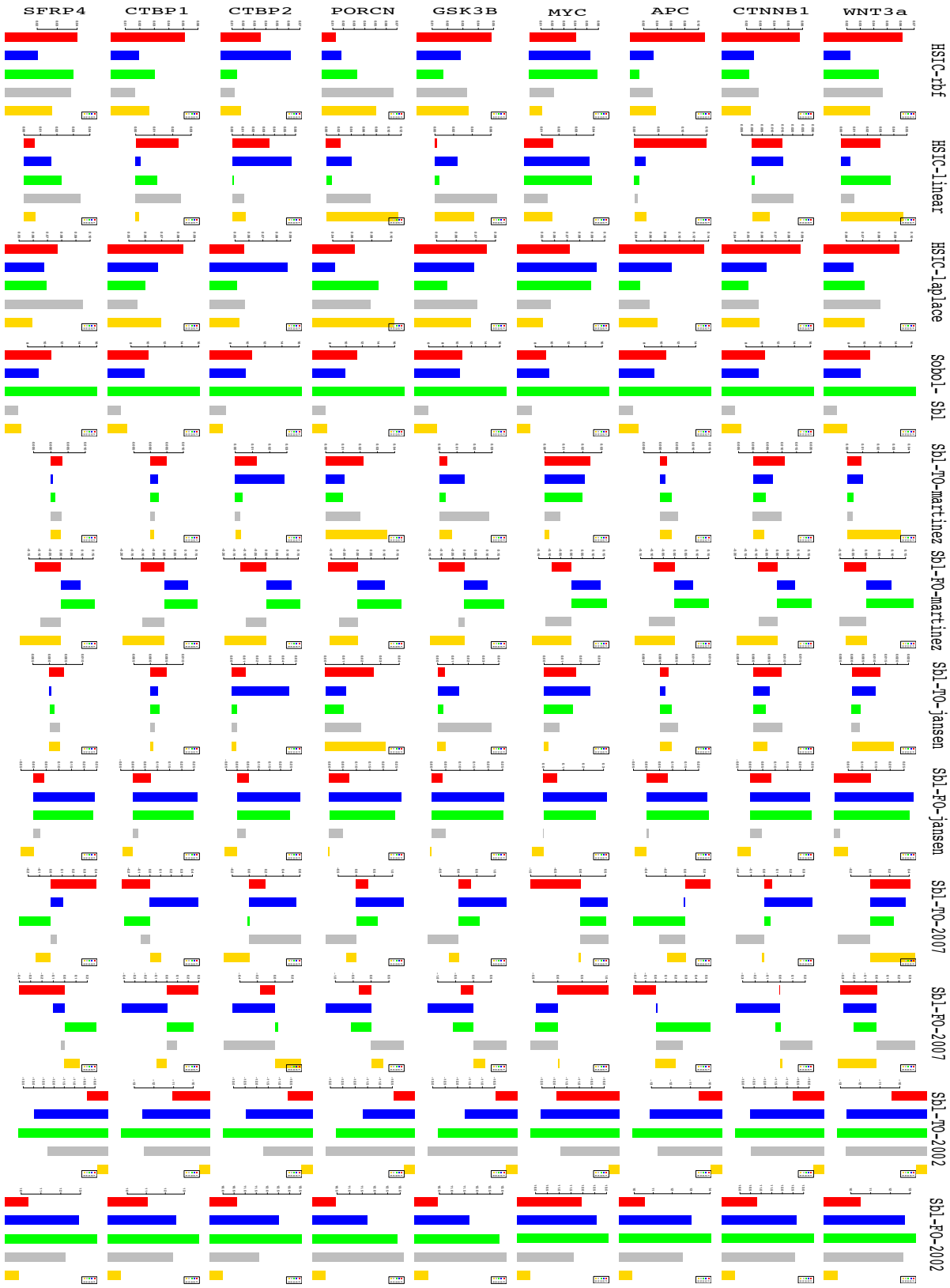

Fig. 20 Column wise - methods to estimate sensitivity indices. Row wise - sensitivity indicies for each gene. For each graph, the bars represent sensitivity indices computed at t1 (red), t2 (blue), t3 (green), t4 (gray) and t5 (yellow). Indices were computed using non scaled time series data. TO - total order; FO - first order; SBL - Sobol

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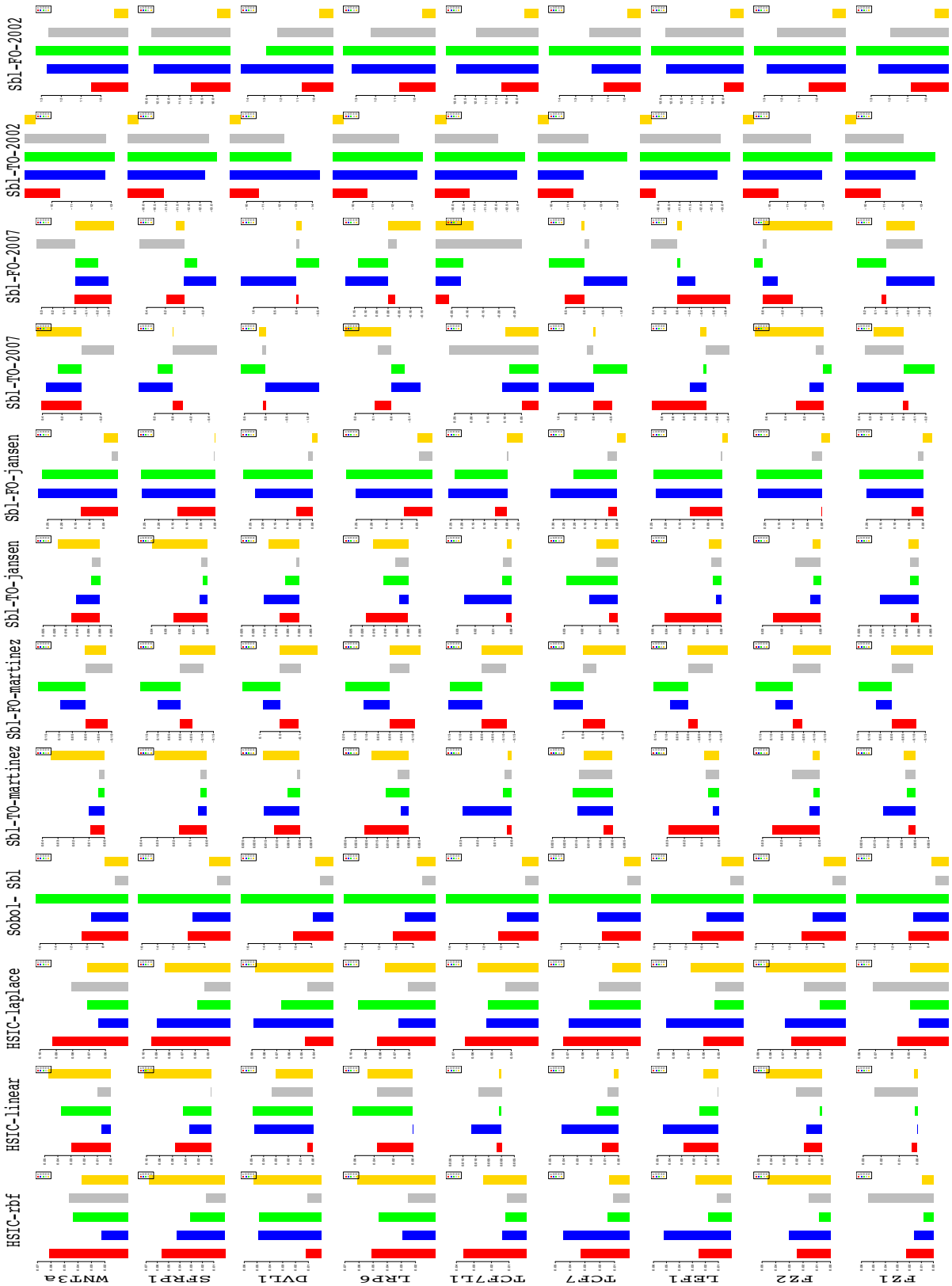

Fig. 21 Column wise - methods to estimate sensitivity indices. Row wise - sensitivity indicies for each gene. For each graph, the bars represent sensitivity indices computed at t1 (red), t2 (blue), t3 (green), t4 (gray) and t5 (yellow). Indices were computed using non scaled time series data. TO - total order; FO - first order; SBL - Sobol

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Now the indicies for other genes are also considered.

5.2 Analysis of deviations in fold changes

5.2.1 WNT3a

Lets begin with the gene $WNT3a$ as changes in its concentration lead to recording of the measurements of the different genes by Gujral and MacBeath[17]. Of the list of genes recorded, the indices in the fold changes of the those which are influenced by the deviations in the concentration of $WNT3a$ are analysed. Next based on these confirmations and patterns of indices over time, conclusions for other enlisted genes. For the former list, the following genes $FZ1$, $FZ2$, $LEF1$, $TCF7$, $TCF7L1$, $LRP6$, $DVL1$, $SFRP1$, $SFRP4$, $CTBP1$, $CTBP2$, $PORCN$, $GSK3\beta$, MYC, APC and $CTNNB1$ are considered.

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6 Conclusions

The conclusions section should come at the end of article. For the reference section, the style file rsc.bst can be used to generate the correct reference style.

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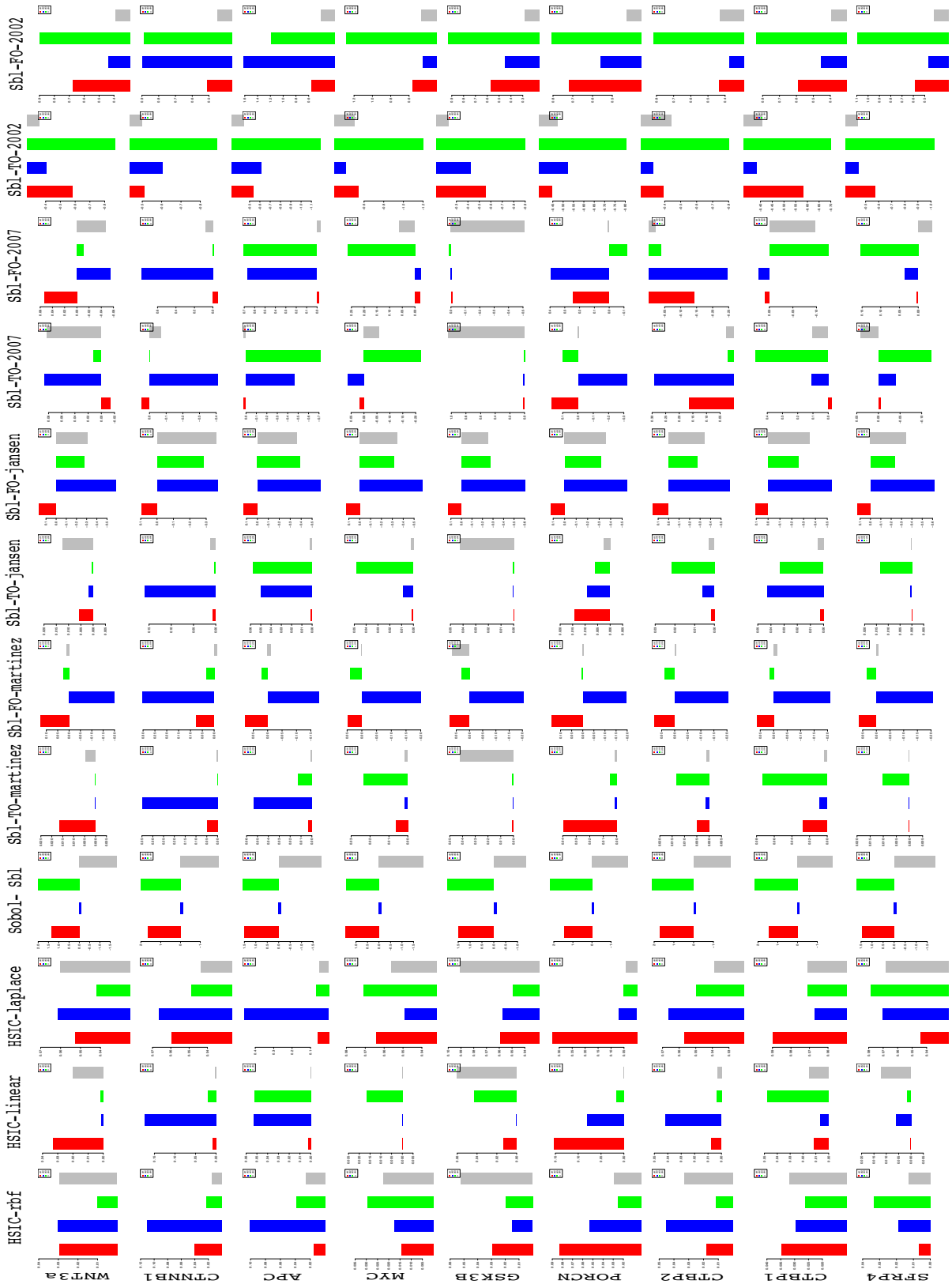

Fig. 22 Column wise - methods to estimate sensitivity indices. Row wise - sensitivity indicies for each gene on deviations in fold change. For each graph, the bars represent sensitivity indices computed at <t1,t2> (red), <t2,t3> (blue), <t3,t4> (green) and <t4,t5> (gray). Indices were computed using non scaled time series data. TO - total order; FO - first order; SBL - Sobol

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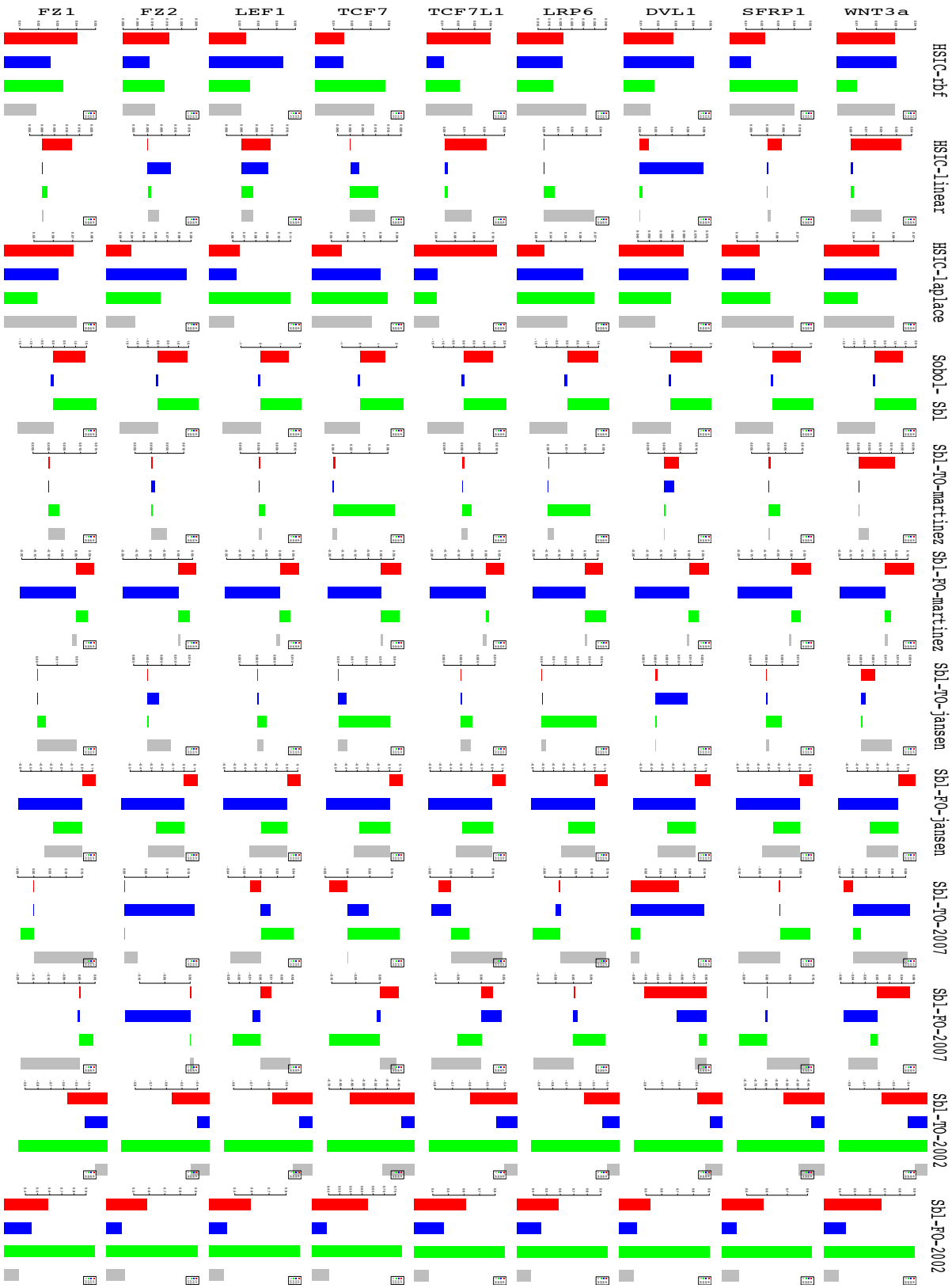

Fig. 23 Column wise - methods to estimate sensitivity indices. Row wise - sensitivity indicies for each gene on deviations in fold change. For each graph, the bars represent sensitivity indices computed at <t1,t2> (red), <t2,t3> (blue), <t3,t4> (green) and <t4,t5> (gray). Indices were computed using non scaled time series data. TO - total order; FO - first order; SBL - Sobol

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