S 1 EXAMPLE STEP BY STEP WALK-THROUGH OF THE ALGORITHM

We consider the network given in Figure S1 for demonstrating the algorithm. Let us assume that in the beginning only TCF is present. In the first step (see Figure 7 of the paper), at first, all inhibiting reactions (only R2) are checked. This reaction is not active as neither the destruction complex nor β-catenin are present. The model does not contain propagating reactions, hence, as next all other reactions are checked. Only R0 can be active as all its substrates are present (R0 does not have any substrates but that is valid as well). Hence, its product β-catenin becomes present.

In the second step, the same procedure is performed. The inhibiting reaction R2 is not active as the destruction complex is not active. Subsequently, R1 is found to be active as both substrates, TCF and β-catenin are present. As a consequence, the TCF/β-catenin complex becomes present which is the final step of the algorithm.

In case that the destruction complex is present from the beginning, the algorithm proceeds differently. In the first step, β-catenin is produced as before. In the second step, the inhibiting reaction R2 is found to be active as the destruction complex and β-catenin are present. This leaves β-catenin as depleted. Subsequently, the β-catenin/destruction complex complex is set as present. Reaction R1 is not found to be active as β-catenin is depleted. As a consequence, the TCF/β-catenin will not become present. This is the final step of the algorithm.

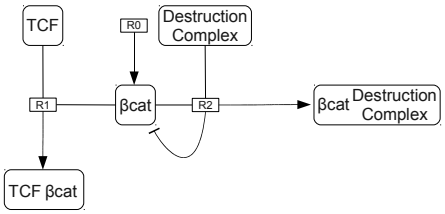

Fig. S1. An example network for a step by step walk-through of the algorithm. Rounded boxes represent compounds, rectangles reactions. Substrates and products of the reactions are indicated by arrow types (arrow head points to products; no heads for substrates; tee head for inhibited substrates).

Figure S2 gives an example for inihibition propagation. We assume that the destruction complex and the E3 ubiquitin ligase are present. In the first step, again β-catenin is produced. In the second step R2 produces the β-catenin/destruction complex complex. The propagating function of R2 is ignored, as its product is not depleted. In the third step, the inhibiting reaction R3 is found to be active leading to a depletion of the β-catenin/destruction complex complex. In the end, β-catenin is degraded (i.e. "degraded β-catenin" is set to present) and the destruction complex is freed. In the fourth step, the propagating reaction R3 is found to be active as its product is now depleted. Hence, the depletion is also assigned to β-catenin, which is the final step of the algorithm. In contrast to the last example, here β-catenin won't be depleted if the E3 ligase is not present. Also, β-catenin inhibition is not immidiate.

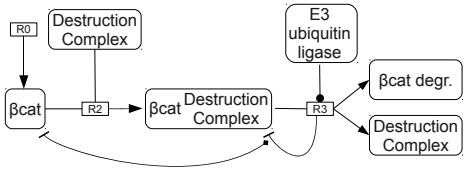

Fig. S2. An example network for inihibition propagation. Here it is assumed that the inhibitory effect is actually controlled by the degradation of β-catenin and not already by its binding to the destruction complex. R3 is a inhibiting reaction while R2 is a depletion propagating reaction which depletes its substrate β-catenin if its product, the β-catenin/destruction complex complex, is depleted. This is represented by an tee-shaped arrow pointing from the product to the substrate. The catalytic effect of the E3 ubiquitin ligase is represented by a circle arrow head.

S 2 CLASSICAL BOOLEAN NETWORK REPRESENTATION OF THE SCOPES APPROACH

The proposed approach can be converted into a classical Boolean network. The dificulty lies within the treatment of inhibition, in particular the discussed indirect inhibition as occuring in the fully mechanistic view. The simple approach of inhibiting a compound through the activity of its depleting reaction (see Figure S3) does not work, as it leads to artificial oscillations.

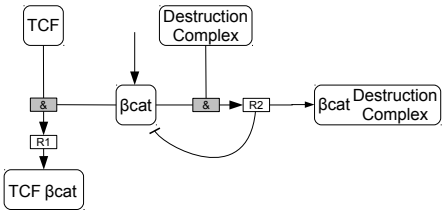

Fig. S3. A possibility of including indirect inhibition into a Boolean network which leads to artificial oscillations. Compounds and reaction are equitable nodes in the Boolean network. The gray shaded nodes in the graph are the Boolean functions which combine the inputs.

Indeed, in order to obtain the same function as provided by the scopes method, one has to introduce two nodes per compound, denoted by *PRE* and *ACTIVE*. Reactions producing a compound actually set the *PRE* node to *TRUE*. Only depleting reactions actually use the *PRE* node as a substrate. The *PRE* node itself activates the *ACTIVE* node iff no depleting reaction is active. All other reactions using the compound as substrate or catalyst connect to the *ACTIVE* node only. See Figure S4 for an example.

Following the biological interpretation of the two Boolean variables of the scopes method, here, the two nodes may represent two concentrations of the compound, a low concentration (*PRE* node) which unsufficient to activate further reactions, and a high concentration (*ACTIVE* node) which does activate downstream targets.

There are in fact a few differences in the two approaches because of which we prefer our algorithm over the classical Boolean network formulation. First, each compound in the classical representation is represented by two nodes in the network which may make it a

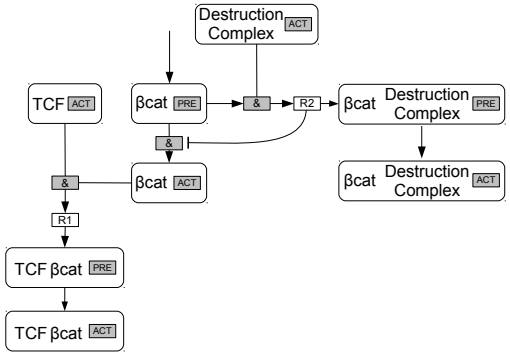

Fig. S4. Equivalent formulation of the scopes approach as Boolean network. Reactions and Compounds are nodes (white boxes). Boolean functions are represented by grey boxes

bit more complicated to handle, even though one should note, that our approach has two variables per compound. Second, due to the two nodes per compound, the classical representation needs two simulation steps in order to activate a compound. Further, inhibition by inhibiting reactions is not immidiate. If the *PRE* node is set, the *ACTIVE* node and the inhibiting reactions get activated in the next step. Only in the following step, the inhibiting reactions will deactivate the *ACTIVE* node.

S 3 ATTRACTOR SETS OF THE CLASSICAL AND THE SCOPES APPROACH

We compared the attractor sets of the two approaches. Therefore we exported the mechanistic network as used by the scopes approach to a boolean network as described in section S 2. We used the R package "Boolnet" (2) for a exhaustive attractor scan in case of the classical approach. As the converted mechanistic model contains 107 nodes altogether, a monte-carlo has been used to identify attractors here.

Both model qualitatively show the same behavior. As expected, both models reach a stable attractor in presence of WNT, EGF or both which is consistent with the observations in (1). For the "off-state", i.e. in case of the absence of EGF and WNT, oscillations occur as already reported in the main text. These oscillations are due to a positive feedback loop in the model (ERK $\dashv$ GSK3 $\dashv$ bcat $\rightarrow$ X $\rightarrow$ RAF $\rightarrow$ ERK). Depending on the exact initial conditions on this cycle, different oscilatory attractor are reached. In case of the classical model, 36 attractors exist, of which 2 are stationary, one representing the real off state (i.e. all nodes were initially *FALSE* and one being a activated state (i.e. all nodes were initially *TRUE*. Figure S5 shows all attractors for the classical model.

In case of the mechanistic model, 1000 random initial conditions have been chosen. Each of them lead to a unique oscialltory attractor. This is due to the fact that the mentioned feedback cycle consists of 43 nodes, and hence bears 2^43 possible initial states. Although it is unfeasable to exhaustively determine all attractors in this case, the qualitative behavior of the two models appears to be identical.

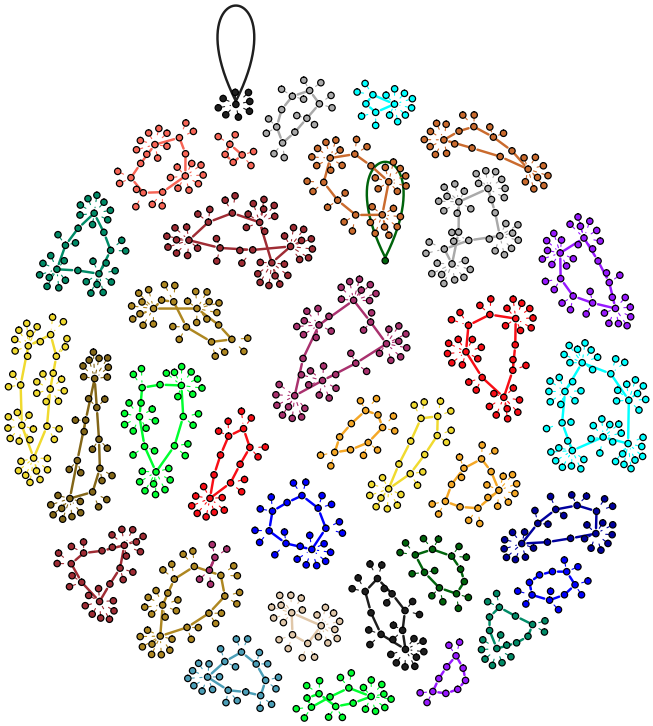

Fig. S5. The 36 attractors of the classical Boolean model. Nodes represent states. Oscillatory attractors show a cyclic structure in the graph (bold line). The two stationary attractors can be identified by a bold curve connecting the attractor node to itself. Nodes connected through a dashed line to any of the attractors are further states which lead to the corresponding attractor.

S 4 RETRIEVING NETWORK DATASETS FROM REACTOME AND THEIR USAGE WITH THE LIBSCOPES PACKAGE

The package libScopes(L1) reads network information from standard SBML(L2) files. At the moment, SBML level 2 version 4 and higher is supported, although only a subset of the SBML standard is acutally imported. There exists a variaty of SBML tools which can be used to create or edit SBML files (see (L2)).

At the time of writing, the Reactome database(L4) provides an export option for SBML. Besides downloading the complete set of human interactions, also the download of particular pathways is supported. The Reactome SBML file does not set the SBO terms for modifier species as required by libScopes. A small perl script(L5) can be used to add this information for the catalysts.

The type of indirect/mechanistic inhibition discussed in this manuscript requires additional information which is not compliant with the SBML standard. Hence, it has to be provided in an additional file (see (L3)).

The obtained sbml file and the inhibition file can used by the libscopes package.

```
testscope sbmlfile inhibition_file
```

will print an extended stoichiometric matrix of the network and can be used for verifying the network information. When including the network files in an experiment description file (see (L3)) the Boolean simulation can be perfomed using the

`runscopes`

executable.

REFERENCES

[1]Kim, D., Rath, O., Kolch, W., and Cho, K.-H. (2007). A hidden oncogenic positive feedback loop caused by crosstalk between wnt and erk pathways. *Oncogene*, **26**(31), 4571–4579.

[2]Müssel, C., Hopfensitz, M., and Kestler, H. A. (2010). Boolnet–an r package for generation, reconstruction and analysis of boolean networks. *Bioinformatics*, **26**(10), 1378–1380.

WEBLINKS

[L1]libScopes: http://code.google.com/p/libscopes/wiki/Paper2011
[L2]SBML: http://sbml.org/
[L3]Documentation of libScopes: http://code.google.com/p/libscopes/wiki/RunnerDocu
[L4]Reactome: http://www.reactome.org/
[L5]perl script for adding SBO terms to Reactome SBML: http://libscopes.googlecode.com/files/reactomeSBO.pl

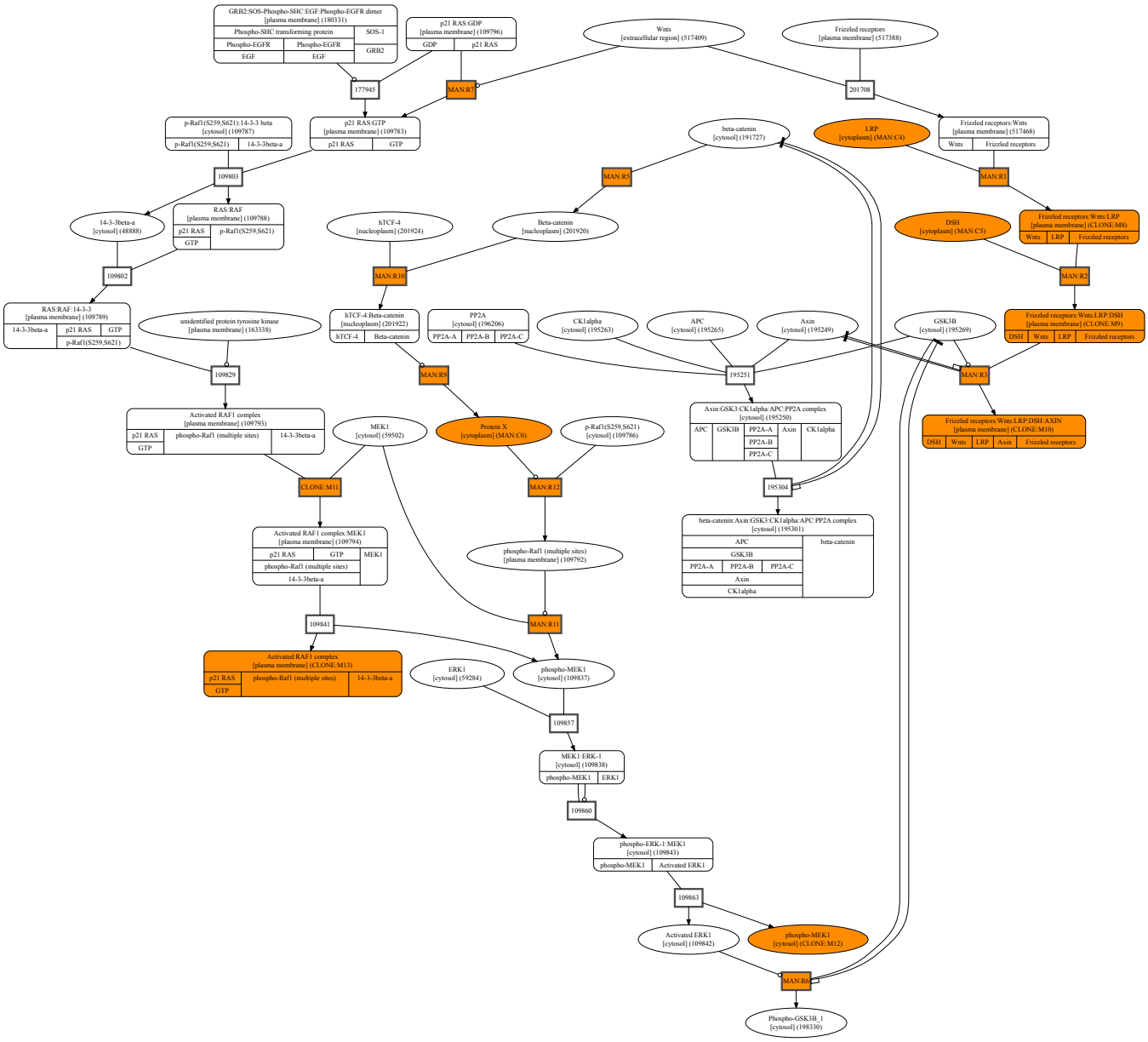

Fig. S6. Mechanistic signaling network describing the crosstalk between the Wnt and MAPK pathways: The reactions are taken form the Reactome database and have been manually corrected according to Kim *et al.*. Dark framed rectangles denote reactions, all other nodes are compounds. Compounds or reactions which have been edited manually are marked in orange. Complexes are shown as boxes composed of their member compounds. The specific marking for the indirect inhibition can be seen by tee-shaped arrows pointing from the reactions to their substrates. Please note that the compound names were taken from the database directly (where applicable) and may differ from the simplified names used for the classical Boolean model in Figure 3 (main document). In the following we list which names from Figure 3 correspond to which compounds in the current figure: EGF/EGFR$\Longleftrightarrow$GRB2:SOS:pSHC:EGF:pEGFR; RAS$\Longleftrightarrow$p21 RAS:GTP; cRAF$\Longleftrightarrow$phosphoRaf-1/Activated Raf-1complex; MEK$\Longleftrightarrow$phospho-MEK1; ERK$\Longleftrightarrow$Activated ERK1; TCF$\Longleftrightarrow$hTCF-4;βcat$\Longleftrightarrow$beta-catenin; Destruction Complex$\Longleftrightarrow$Axin:GSK3:CK1Alpha:APC:PP2A complex; WNT$\Longleftrightarrow$Wnts; AXIN$\Longleftrightarrow$Axin; GSK3$\Longleftrightarrow$GSK3B; APC$\Longleftrightarrow$APC. A SVG version of this graphic is available as additional supplementary file (wnt_mapk.svg)