Supplementary Materials

Figure S1: Full simulation of TGFβ vs forskolin treatment

Figure S2: Other validated relationships

Figure S3: Robustness of validation accuracy to changes in baseline input levels

Figure S4: Full sensitivity analysis

Figure S5: Clustering analysis

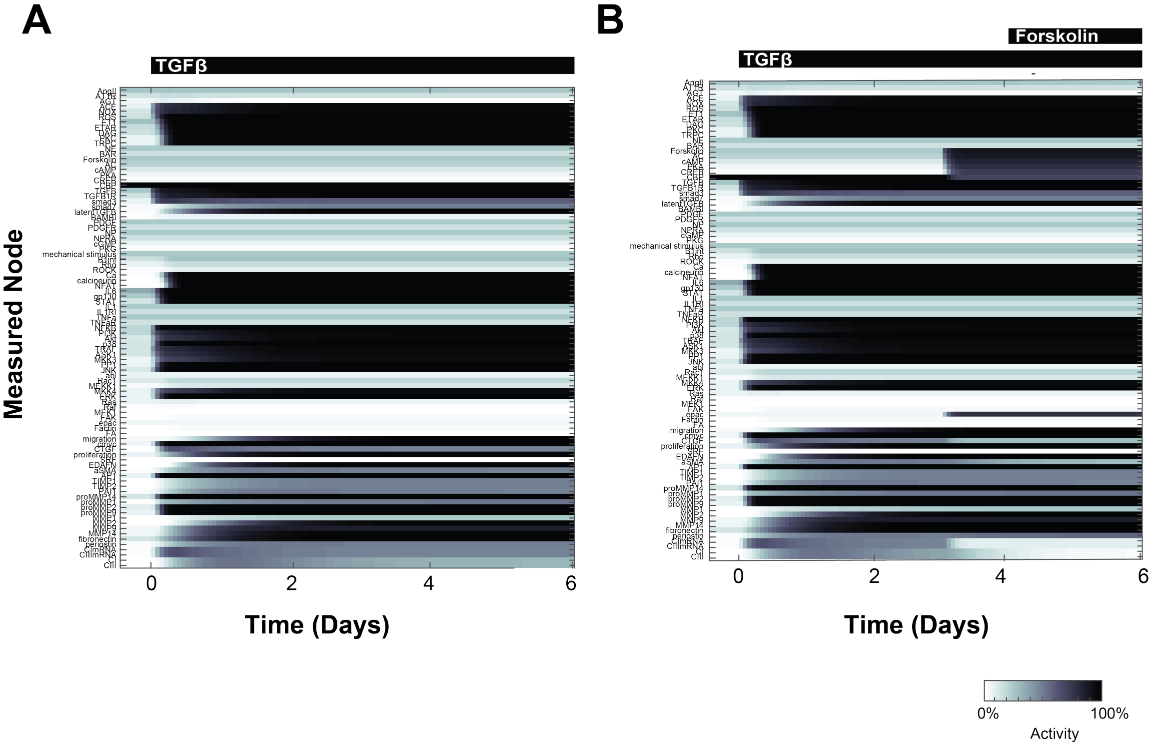
Figure S6: Full comparison of topological features vs influence

Table S1: Definitions of network topology metrics

Database S1: Network model

Database S2: Validation relationships

Supplementary Figure 1: Full simulation of TGF-β versus forskolin treatment. Predicted dynamic activity of all nodes for 6 days of TGF-β alone (A) or for 4 days of TGF-β followed by 2 days of TGF-β + forskolin. The difference in activation of each node with and without forskolin stimulation is plotted on the network to map the specific pathways altered in these two conditions (C).



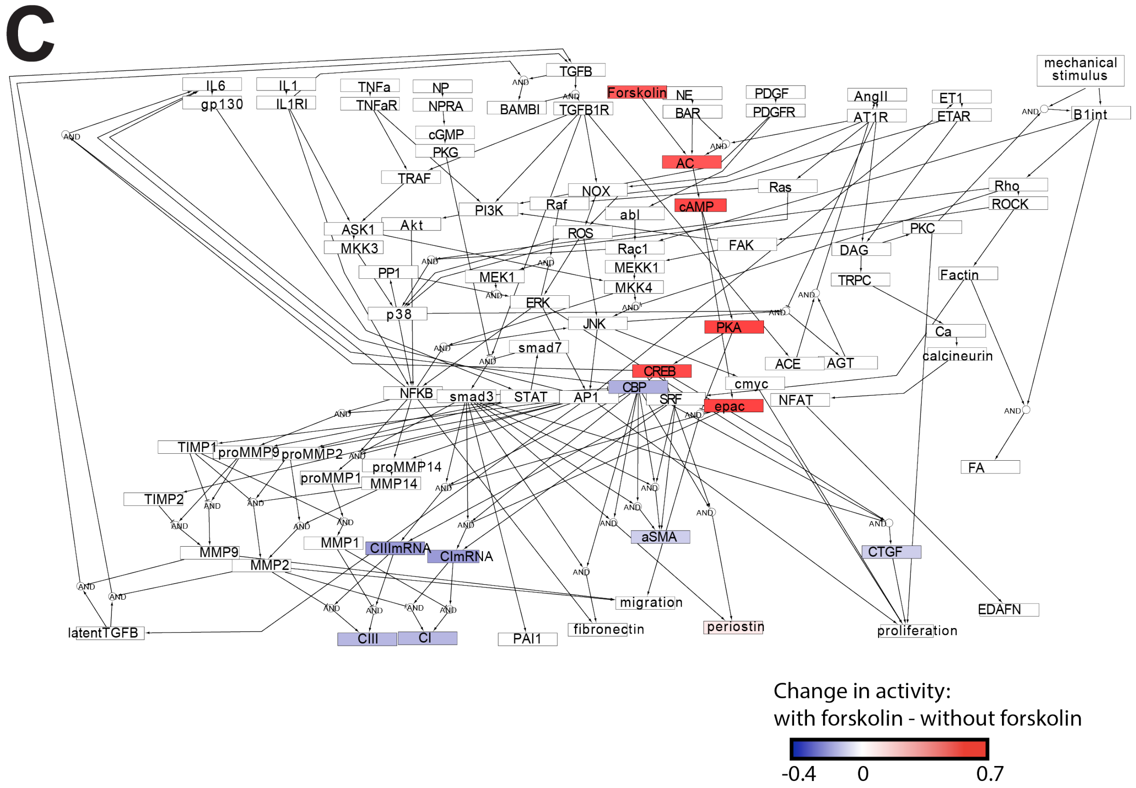

C
TGFB
IL6
gp130
IL1
IL1RI
TNFa
TNFaR
NP
NPRA
cGMP
PKG
BAMBI
TGFB1R
Forskolin
NE
BAR
PDGF
PDGFR
AngII
AT1R
ET1
ETAR
mechanical stimulus
B1int
AC
cAMP
TRAF
PI3K
Raf
NOX
abl
Ras
Rho
ROCK
ASK1
Akt
MKK3
ROS
Rac1
FAK
PKC
DAG
TRPC
Factin
PP1
MEK1
MEKK1
MKK4
ERK
p38
JNK
PKA
Ca
calcineurin
smad7
ACE
AGT
CREB
CBP
cmyc
NFKB
smad3
STAT
AP1
SRF
epac
NFAT
FA
TIMP1
proMMP9
proMMP2
proMMP14
proMMP1
MMP14
TIMP2
MMP1
MMP9
MMP2
CIIImRNA
CImRNA
aSMA
CTGF
migration
latentTGFB
CIII
CI
PAI1
fibronectin
periostin
proliferation
EDAFN
AND
Change in activity:
with forskolin - without forskolin
-0.4
0
0.7

Figure S2: Other validated relationships.
Model prediction and comparison to experimental validation data for all relationships not included in the subset shown in Figure 3. Color indicates model prediction (blue for decrease, white for no change, red for increase) and the check indicates agreement with literature where an x indicates disagreement. Inputs are shown on the vertical axis and outputs are shown on the horizontal axis. Relationships are grouped by input.

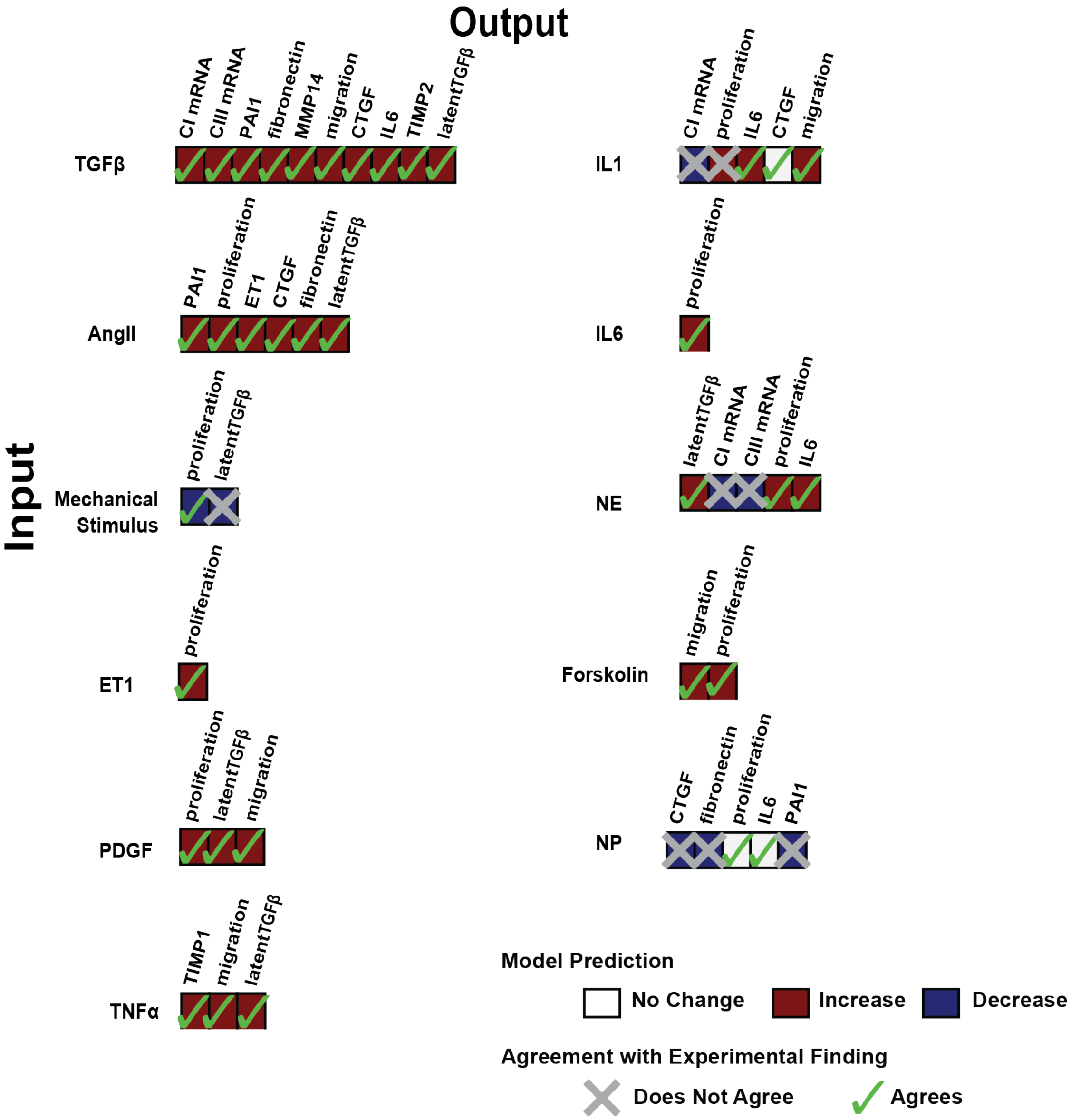


Figure S3: Robustness of validation accuracy to changes in baseline input levels. The baseline input levels were varied within a uniform distribution of width 5%-50% of the original value (25% activity). This uniform distribution was sampled 100 times, and the validation accuracy was calculated for each random sampling represented at a dot in the plot. The validation accuracy is robust to variation within 12-37% activity.

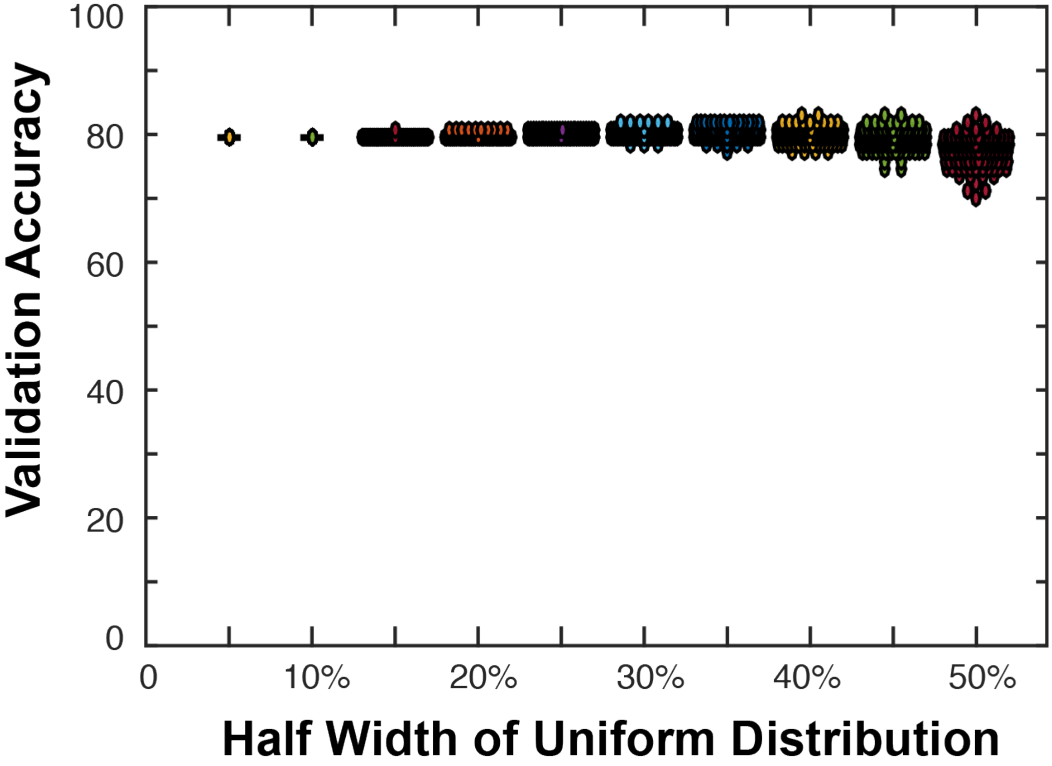
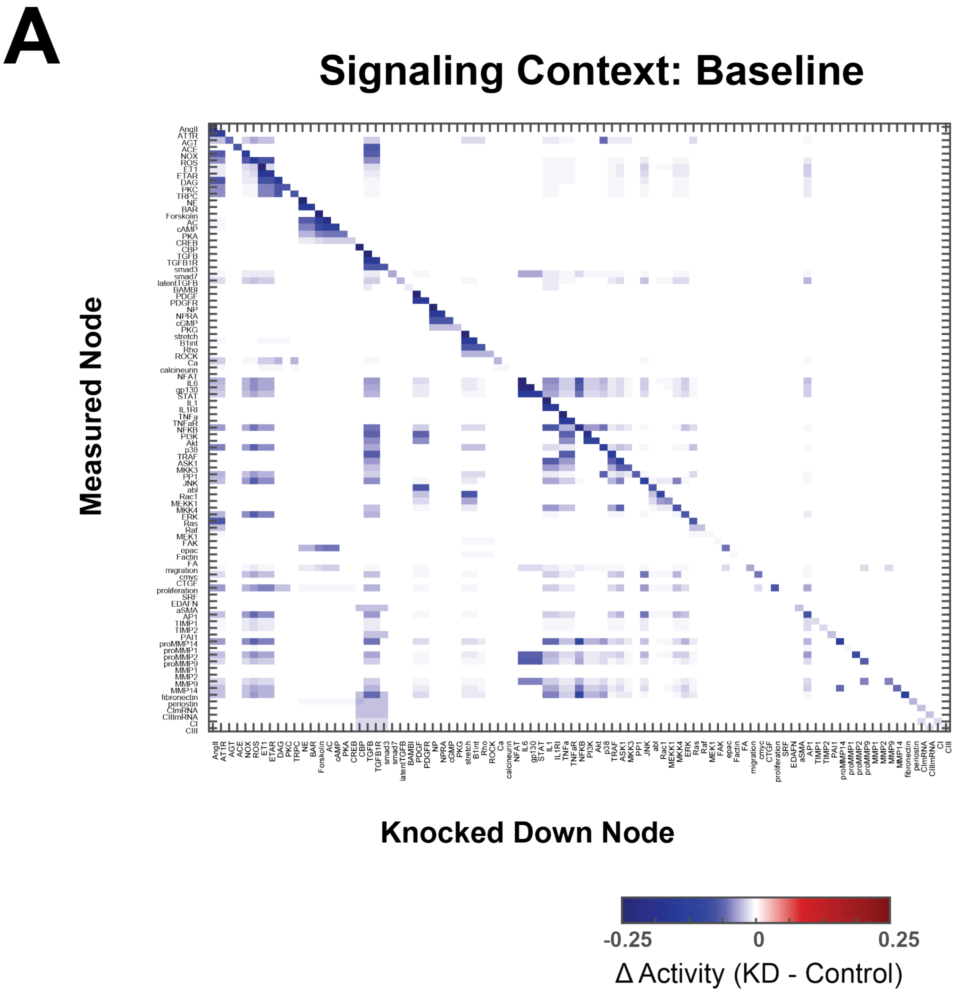
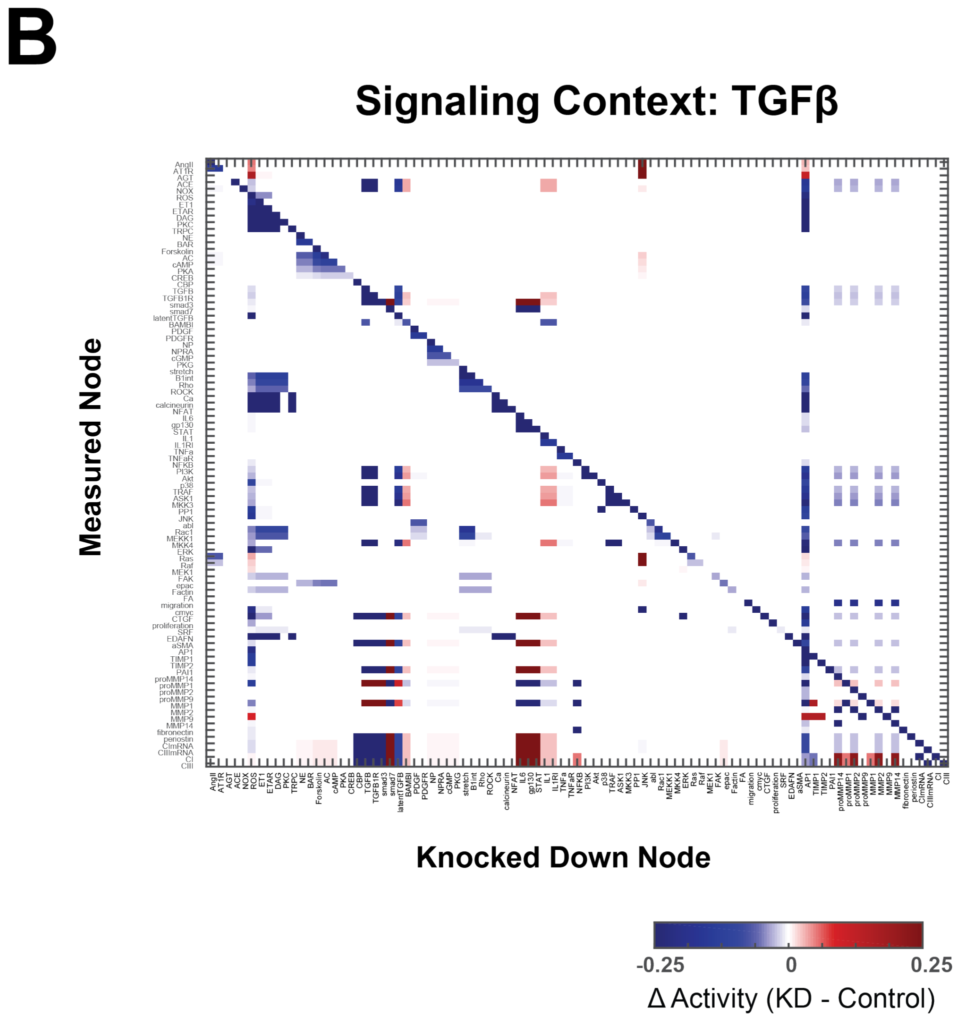


Figure S4: Full sensitivity analysis.

Results of a full sensitivity analysis where all possible knock downs (x axis) are performed under baseline (A), high TGFβ (B), and high mechanical stimulus (C) signaling context and the change in activity (knock down - control) for each output node (y axis) is measured as a change in color. The top 10 most influential nodes (columns) and top 10 most sensitive nodes (rows) for the baseline and high TGFβ are shown in Fig. 4A-B. Blue indicates a decrease in activity with knock down of the perturbed node and red indicates an increase in activity with knock down of the perturbed node.




B
Signaling Context: TGFβ
Measured Node
AngII
AT1R
AGT
ACE
NOX
ROS
ET1
ETAR
DAG
PKC
TRPC
NE
BAR
Forskolin
AC
cAMP
PKA
CREB
CBP
TGFB
TGFB1R
smad3
smad7
latentTGFB
BAMBI
PDGF
PDGFR
NP
NPRA
cGMP
PKG
stretch
B1int
Rho
ROCK
Ca
calcineurin
NFAT
IL6
gp130
STAT
IL1
IL1RI
TNFa
TNFaR
NFKB
PI3K
Akt
p38
TRAF
ASK1
MKK3
PP1
JNK
abl
Rac1
MEKK1
MKK4
ERK
Ras
Raf
MEK1
FAK
epac
Factin
FA
migration
cmyc
CTGF
proliferation
SRF
EDAFN
aSMA
AP1
TIMP1
TIMP2
PAI1
proMMP14
proMMP1
proMMP2
proMMP9
MMP1
MMP2
MMP9
MMP14
fibronectin
periostin
CImRNA
CIIImRNA
CI
CIII
AngII
AT1R
AGT
ACE
NOX
ROS
ET1
ETAR
DAG
PKC
TRPC
NE
BAR
Forskolin
AC
cAMP
PKA
CREB
CBP
TGFB
TGFB1R
smad3
smad7
latentTGFB
BAMBI
PDGF
PDGFR
NP
NPRA
cGMP
PKG
stretch
B1int
Rho
ROCK
Ca
calcineurin
NFAT
IL6
gp130
STAT
IL1
IL1RI
TNFa
TNFaR
NFKB
PI3K
Akt
p38
TRAF
ASK1
MKK3
PP1
JNK
abl
Rac1
MEKK1
MKK4
ERK
Ras
Raf
MEK1
FAK
epac
Factin
FA
migration
cmyc
CTGF
proliferation
SRF
EDAFN
aSMA
AP1
TIMP1
TIMP2
PAI1
proMMP14
proMMP1
proMMP2
proMMP9
MMP1
MMP2
MMP9
MMP14
fibronectin
periostin
CImRNA
CIIImRNA
CI
CIII
Knocked Down Node
-0.25
0
0.25
Δ Activity (KD - Control)

C

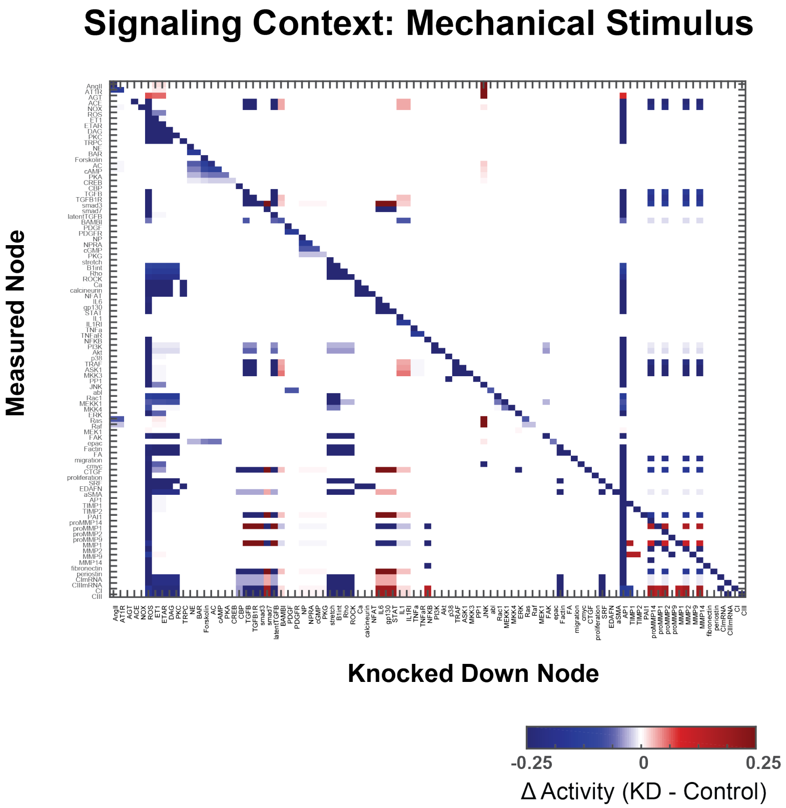

Signaling Context: Mechanical Stimulus
Measured Node
AngII
AT1R
AGT
ACE
NOX
ROS
ET1
ETAR
DAG
PKC
TRPC
NE
BAR
Forskolin
AC
cAMP
PKA
CREB
CBP
TGFB
TGFB1R
smad3
smad7
latentTGFB
BAMBI
PDGF
PDGFR
NP
NPRA
cGMP
PKG
stretch
B1int
Rho
ROCK
Ca
calcineurin
NFAT
IL6
gp130
STAT
IL1
IL1RI
TNFa
TNFaR
NFKB
PI3K
Akt
p38
TRAF
ASK1
MKK3
PP1
JNK
abl
Rac1
MEKK1
MKK4
ERK
Ras
Raf
MEK1
FAK
epac
Factin
FA
migration
cmyc
CTGF
proliferation
SRF
EDAFN
aSMA
AP1
TIMP1
TIMP2
PAI1
proMMP14
proMMP1
proMMP2
proMMP9
MMP1
MMP2
MMP9
MMP14
fibronectin
periostin
CImRNA
CIIImRNA
CI
CIII
Knocked Down Node
-0.25
0
0.25
Δ Activity (KD - Control)

Figure S5: Clustering analysis

(A) The full sensitivity analysis under high TGFβ signaling context ordered by cluster on both axes. (B) The full network with each node colored by module as labeled in A.

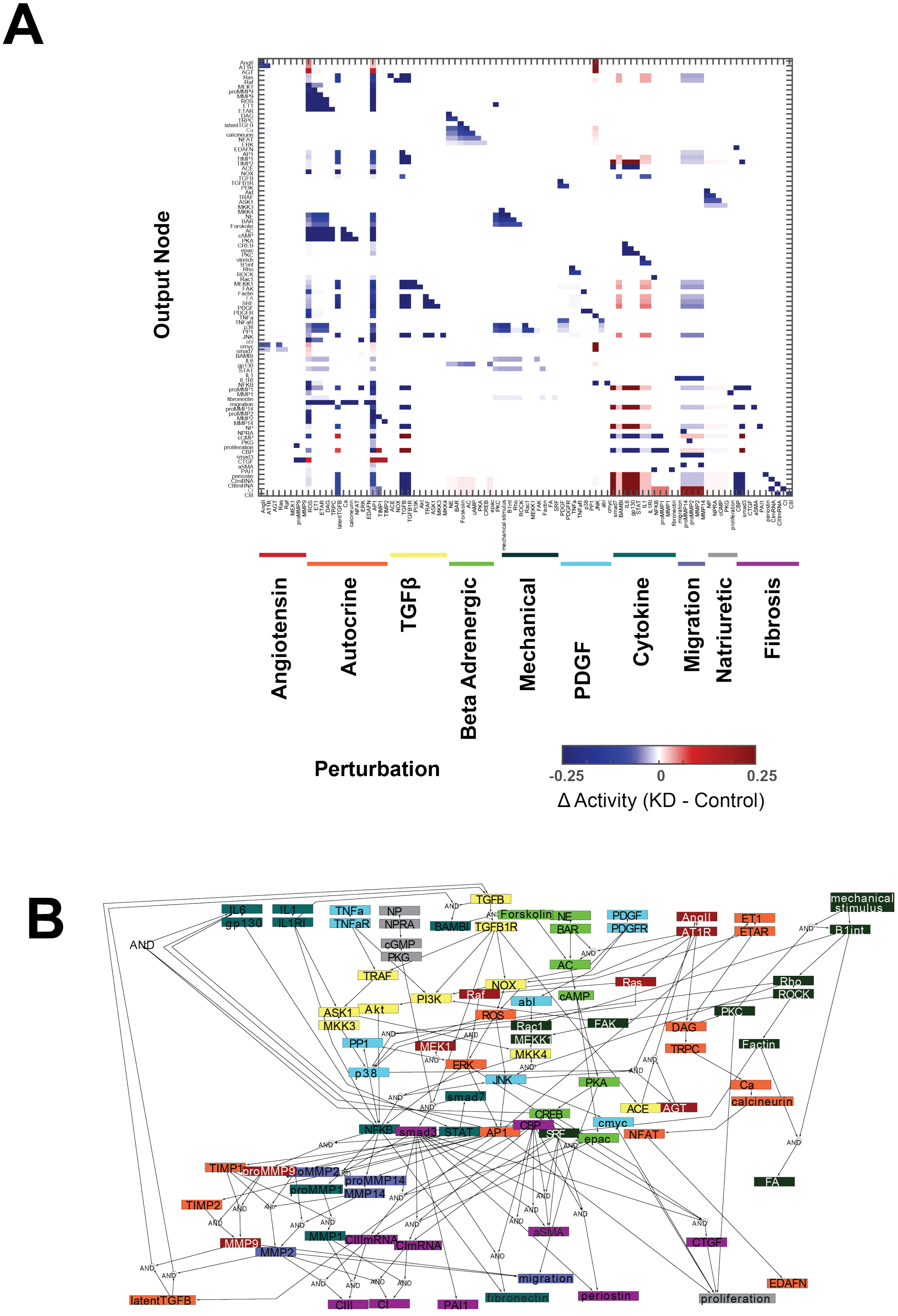


Figure S6: Full comparison of topological features versus influence

Each topological feature is plotted against influence. R2 values are shown.

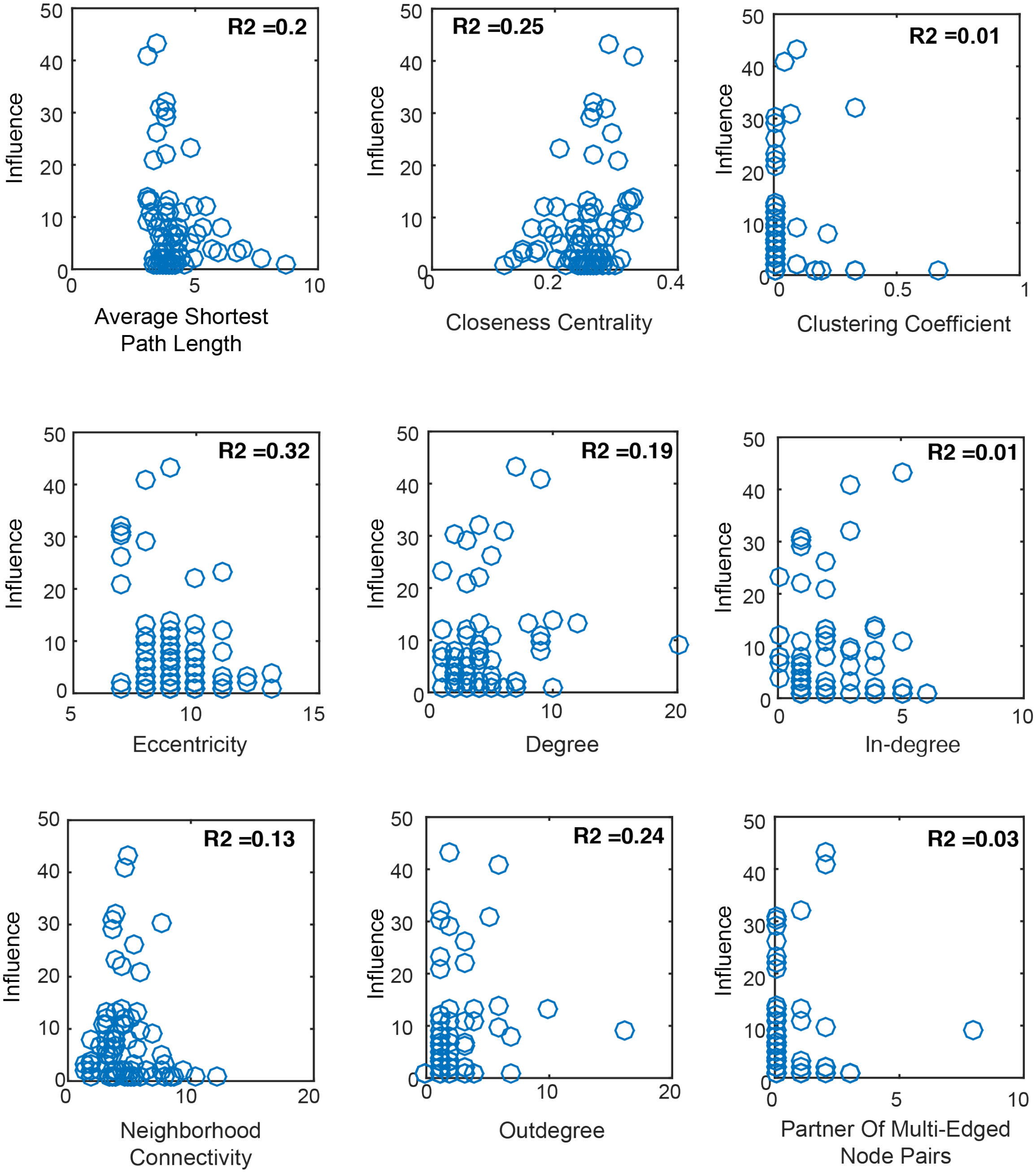


Table S1: Definitions of network topology metrics

Each topological feature as defined by Assenov et al in the NetworkAnalyzer plugin for Cytoscape used for topological analysis of the network [27],[28].

Topology Metric	**Definition**
betweenness centrality	number of shortest paths from all nodes to all others that pass through node *n*
out-degree	number of edges that exit node *n*
in-degree	number of edges that enter node *n*
edge count	total number of edges connected to node *n*
eccentricity	maximal length of a shortest path between node *n* and any other node in the network
neighborhood connectivity	average number of neighbors for all neighbors of node *n*
ave shortest path length	average shortest path between node *n* and any other node
closeness centrality	the reciprocal of the average shortest path length from node *n* (interpreted as a measure of how quickly information spreads from node *n*)
clustering coefficient	measure of the degree to which node *n*'s neighbors form a complete graph
partner of multi-edged node pairs	how many nodes is node *n* a partner of that has multiple edges

Database S1: Network model

This database includes information about each species (node) and each reaction in the cardiac fibroblast signaling network. Included are the names of each species and the genes they represent (when applicable) as well as parameters associated with the nodes or reactions. References for each gene and interaction are also included.

Database S2: Validation relationships

This database includes all input-output relationships validated by the network model as well as references for each relationship and the model prediction compared to the experimental finding.