S100A4 and its Role in Metastasis - Simulations of Knockout and Amplification of Epithelial Growth Factor Receptor and Matrix Metalloproteinases

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Supplementary Material

Contents

1 Text ESI 1

1.1 Principal Component Analysis

Principal component analysis (PCA) is included in our simulation program and is applied to detect co-variation patterns at the level of steady-state activities of the network components (equivalent to experimental co-expression patterns) as well as to co-variation of sensitivities with respect to the applied varying conditions (reflecting co-regulatory patterns). Representations in Figures ESI 5-8 do not display the network components that were systematically varied during the simulation procedure. These varied components are however considered in the PCA computation. Consequently, the dependent variables that are represented in Figures ESI 5-8 are scaled to the ranges of variation of the independent variables applied in the simulation. The names of the variables used in the PCA are found in the scheme of Figure 1.

1.2 PCA: S100A4 Knockout

Steady-state values appear not to be influenced by variation in EGFR. Groups associating *CapGrowth* with *Plasmin*, *EphrA1*, and a cluster comprising *EGFR*, *NFKB* and *cytoskeletal proteins* maintain stable distance to *CellDiss*. A cluster composed of *CellDiss*, *CapGrowth*, *OPN*, *Plasmin*, *uPA_uPAR* is observed in the sensitivity PCA in which single variables conserve their distances. With increasing EGFR, this cluster moves towards a group formed by *BCat*, *EGFR*, *NKFB* and *Myo9*. *ECadh* and *EphrA1* remain distant from the other variables. (See Figure ESI 5).

1.3 PCA: Inhibition of MMPs

Differently from the situation when knocking out S100A4, MMPs inhibition appears to create barriers between groups of variables. These boundaries increase the separation at higher activity of EGFR and lead to the formation of groups associating *CapGrowth*, *Plasmin*, *NKFB*, with *EphrA1* and *OPN* together moving towards the stable variables *MMPs* and *cytoskeletal proteins*. In addition, *CellDiss* and *EGFR* move towards each other and swap their relative position at high EGFR. In the sensitivity PCA three groups are observed. *ECadh* and *Myo9*, *BCat* and *MMPs* maintain their positions as EGFR's activity increases. A third compact group comprising the remaining variables moves towards the latter one with increasing EGFR. (See Figure ESI 6).

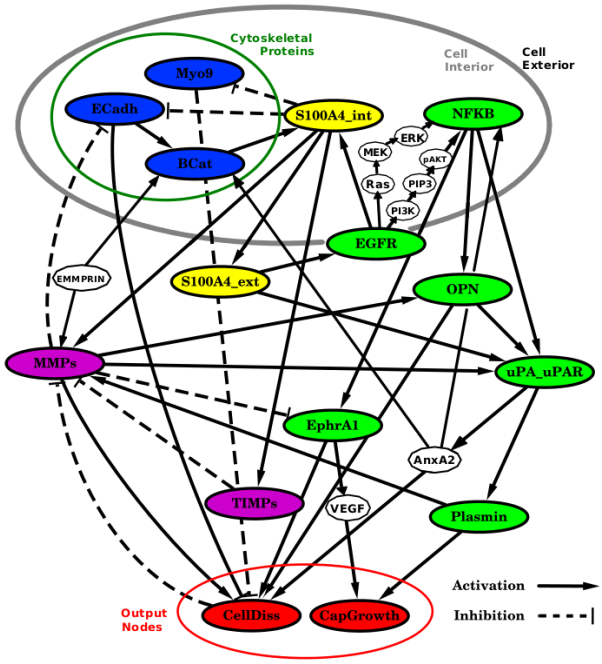
1.4 PCA: S100A4 Knockout and Inhibition of MMPs

The variables' steady-state is unchanged by increasing NF-κB's activity from low to medium with exception of *CellDiss* and *NFKB* whose relative distance increases. At high NF-κB the relative distance of the latter variables increases further and the other variables assume different pattern with respect to lower levels of NF-κB. Boundaries increasing separation between groups of variables are observed in the PCA of sensitivity values. *CapGrowth*, *CellDiss* and *Plasmin* constitute a group closely positioned to another compact cluster formed by *OPN*, *EphrA1* and *uPA_uPAR* in the vicinity of *NFKB*. Relative distances between these groups increase proportionally to NF-κB activity which makes them segregate them apart form each other. (See Figure ESI 7).

1.5 PCA: Inhibition of EGFR-mediated Feedback of S100A4

A group constituted by *S100A4_int*, *S100A4_ext*, *EGFR*, *NFKB*, *BCat*, *ECadh*, *Myo9*, *EphrA1* appears in a S100A4-independent pattern of steady-state values. Three groups formed by *ECadh*, *EphrA1*; *BCat*, *S100A4_int*, *S100A4_ext*, *EGFR*, *NFKB*, *Myo9*; and *CapGrowth*, *CellDiss*, *Plasmin*, *uPA_uPAR* can be distinguished in the PCA of sensitivities. The latter group moving to the second one by increasing S100A4. (See Figure ESI 8).

2 Supplementary Figures



Node ID	Full Name
S100A4_int	Intracellular S100A4
S100A4_ext	Extracellular S100A4
MMPs	Matrix metalloproteinases
TIMPs	Tissue inhibitors of metalloproteinases
OPN	Osteopontin
ECadh	E-Cadherin
NFKB	NF-κB
Myo9	Myosin 9 (MYH9)
BCat	β-catenin
EphrA1	Ephrin-A1 (EFNA1)
EGFR	Epidermal growth factor receptor
uPA_uPAR	Urokinase plasminogen activator (receptor)
Plasmin	Plasmin
CapGrowth	Capillary growth
CellDiss	Cell dissociation
EMMPRIN	Extracellular matrix metalloproteinase inducer
VEGF	Vascular endothelial growth factor
AnxA2	Annexin A2
Ras	Ras GTPase
MEK	Mitogen-activated protein kinase kinase
ERK	Extracellular-signal-regulated kinases
PI3K	Phosphatidylinositide 3-kinase
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
pAKT	Protein kinase B

Figure ESI 1: S100A4 extended scheme. The nodes added to the S100A4 network shown in Figure 1 are represented in white.

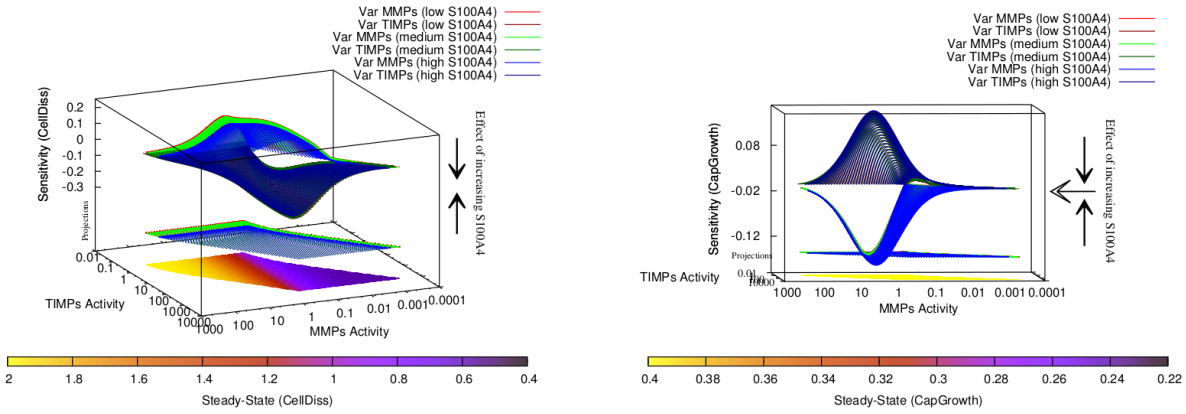


Figure ESI 2: Sensitivity landscapes of the S100A4 extended scheme. Sensitivity of cell dissociation (left) and capillary growth (right). The colour code corresponds to the sensitivity surfaces in Figure 5.

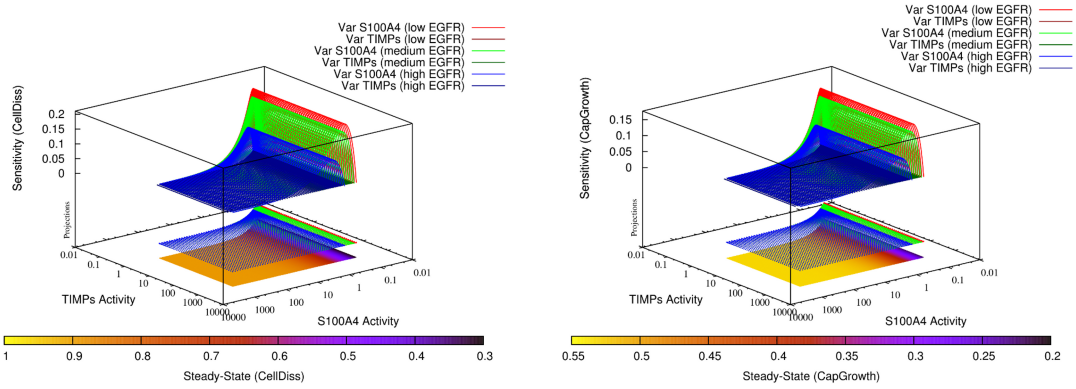


Figure ESI 3: Inhibition of MMPs. Sensitivity of cell dissociation (left) and capillary growth (right). Upper, sensitivity surfaces are calculated in response to variation of S100A4 activity levels ($\varepsilon^{CellDiss}_{S100A4} = \frac{\Delta[ln(CellDiss)]}{\Delta[ln(S100A4)]}$ and $\varepsilon^{CapGrowth}_{S100A4} = \frac{\Delta[ln(CapGrowth)]}{\Delta[ln(S100A4)]}$) and are shown in bright colours. The lower surfaces are calculated in response to variation of TIMPs activity levels ($\varepsilon^{CellDiss}_{TIMPs} = \frac{\Delta[ln(CellDiss)]}{\Delta[ln(TIMPs)]}$ and $\varepsilon^{CapGrowth}_{TIMPs} = \frac{\Delta[ln(CapGrowth)]}{\Delta[ln(TIMPs)]}$) and are shown in dark colours overlapping at the zero level of the z-axis (note that the system is independent on TIMPs). EGFR levels correspond to basal activity values set to $low = 0.001$, $medium = 0.01$ and $high = 0.1$.

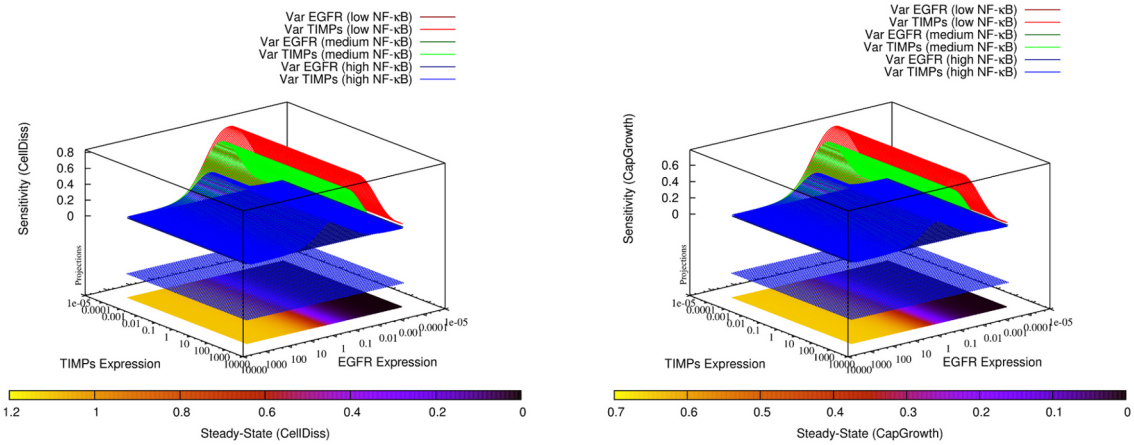


Figure ESI 4: Combination of S100A4 knockout and inhibition of MMPs. Sensitivity of cell dissociation (left) and capillary growth (right). Upper, sensitivity surfaces are calculated in response to variation of EGFR activity levels ($\varepsilon^{CellDiss}_{EGFR} = \frac{\Delta[ln(CellDiss)]}{\Delta[ln(EGFR)]}$ and $\varepsilon^{CapGrowth}_{EGFR} = \frac{\Delta[ln(CapGrowth)]}{\Delta[ln(EGFR)]}$) and are shown in bright colours. Lower surfaces are calculated in response to variation of TIMPs activity levels ($\varepsilon^{CellDiss}_{TIMPs} = \frac{\Delta[ln(CellDiss)]}{\Delta[ln(TIMPs)]}$ and $\varepsilon^{CapGrowth}_{TIMPs} = \frac{\Delta[ln(CapGrowth)]}{\Delta[ln(TIMPs)]}$) and are shown in dark colours overlapping at the zero level of the z-axis (note that the system is independent on TIMPs). NF-κB levels correspond to basal activity values set to $low = 0.001$, $medium = 0.01$ and $high = 0.1$.

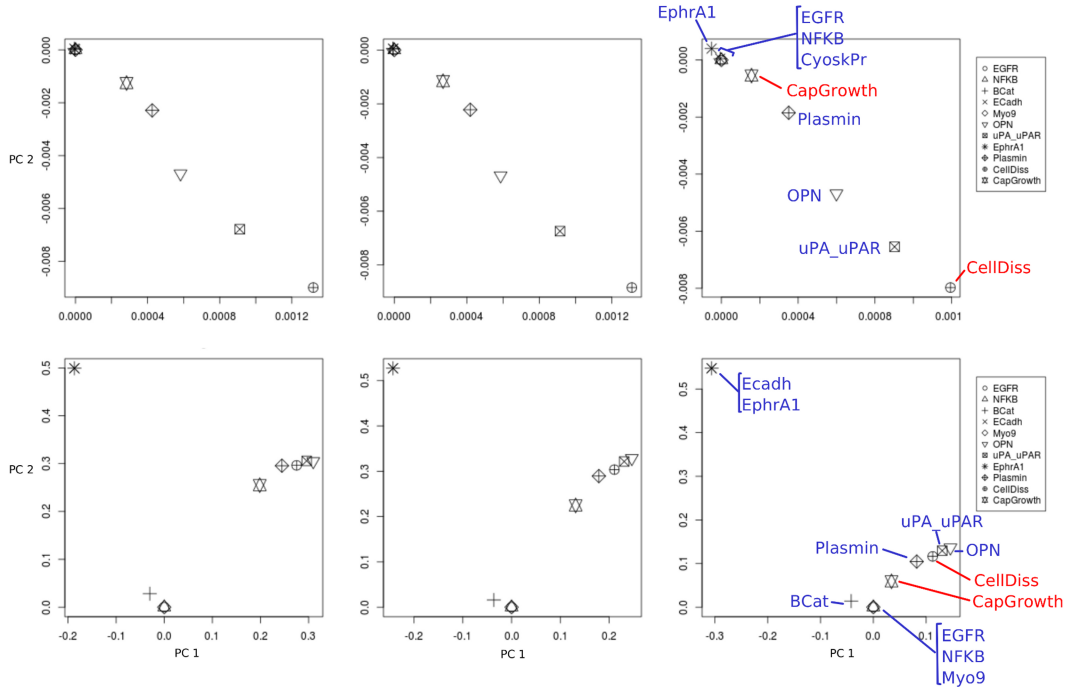


Figure ESI 5: S100A4 knockout. Loading plots of MMPs and TIMPs variation. Low (left), medium (middle), high (right) EGFR; upper row: steady-state, lower row: sensitivity.

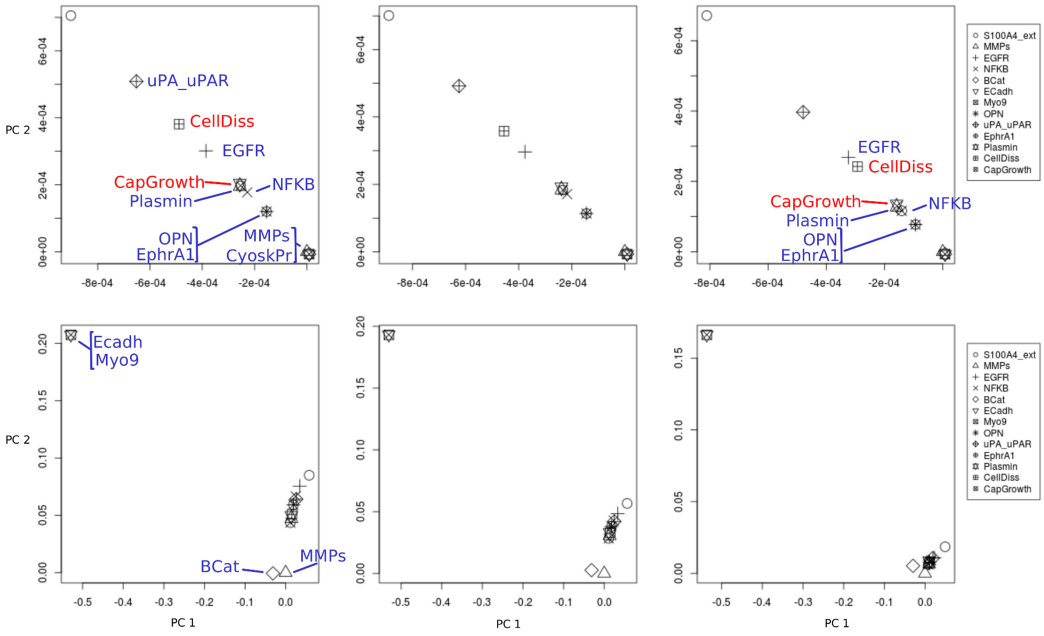


Figure ESI 6: Inhibition of MMPs. S100A4 and TIMPs variation (loading plots); low (left), medium (middle), high (right) EGFR; upper row: steady-state, lower row: sensitivity.

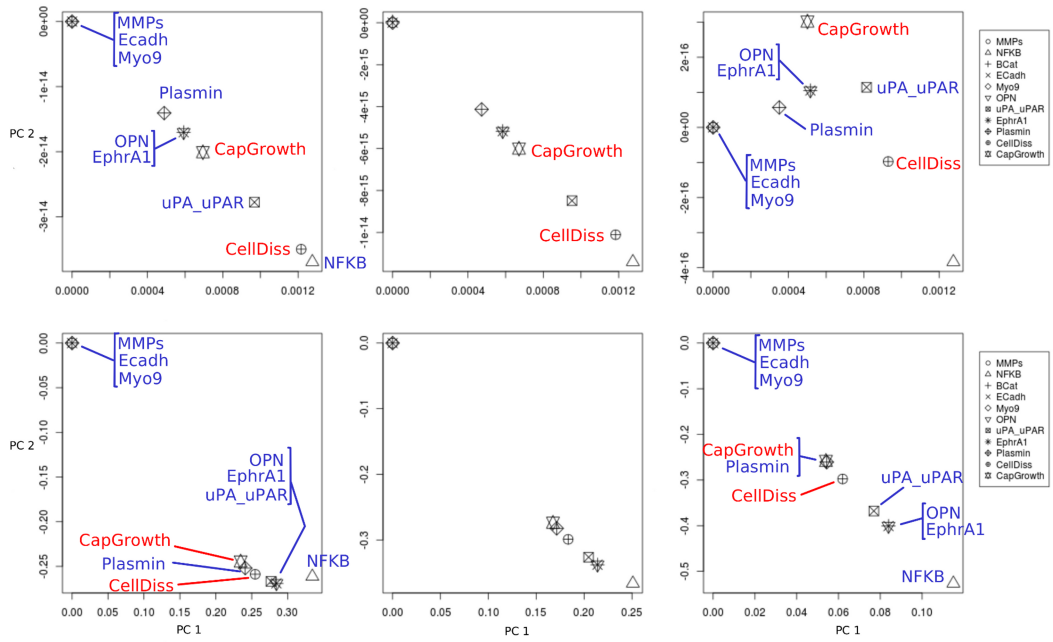


Figure ESI 7: S100A4 knockout and inhibition of MMPs. EGFR and TIMPs variation (loading plots); low (left), medium (middle), high (right) NF-κB; upper row: steady-state, lower row: sensitivity.

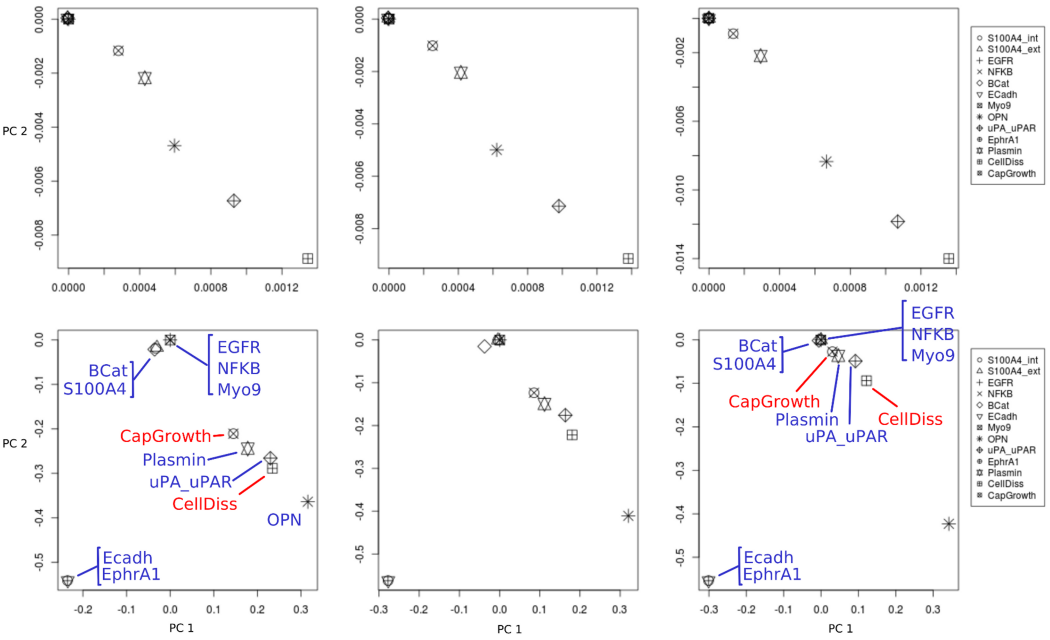


Figure ESI 8: Inhibition of EGFR-mediated feedback of S100A4. MMPs and TIMPs variation (loading plots); low (left), medium (middle), high (right) S100A4; upper row: steady-state, lower row: sensitivity.