## SUPPLEMENTARY MATERIALS

# Deciphering the role of Wnt and Rho signaling pathway in iPSC-derived ARVC cardiomyocytes by *in silico* mathematical modeling

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**Content**

**Supplementary Data**

In this section we provide an in-depth description of the ODE-based mathematical model and of all its species, as well as the differential reactions in which each species is involved.

**Model of Wnt Canonical Pathway (WCP)**

In **Table S1** are reported all the species introduced in the extended Lee's model [1] . In particular, **Table S1** provides information about the full name, abbreviation used in the ODE system, and the initial conditions set in the model. **Table S2** shown all the reaction of the extended WCP model.

| **Species** | **Compartment** | **Type** | **Concentration [nmol/l]** |
|---|---|---|---|
| Dshi | cytoplasm (c) | reactions | 100 |
| Dsha | cytoplasm (c) | reactions | 0 |
| APC_Axin_GSK3β | cytoplasm (c) | reactions | 0 |
| APC_Axin | cytoplasm (c) | reactions | 0 |
| GSK3β | cytoplasm (c) | reactions | 50 |
| APC | cytoplasm (c) | reactions | 100 |
| Axin | cytoplasm (c) | reactions | 0 |
| *f*APC_*f*Axin_ GSK3β | cytoplasm (c) | reactions | 0 |
| β-catenin{c} | cytoplasm (c) | reactions | 0 |
| β-catenin_*f*APC_ *f*Axin_ GSK3β | cytoplasm (c) | reactions | 0 |
| *f*β-catenin_*f*APC_ *f*Axin_ GSK3β | cytoplasm (c) | reactions | 0 |
| β-catenin{n} | nucleus (n) | reactions | 0 |
| *f*β-catenin | cytoplasm (c) | reactions | 0 |
| TCF | nucleus (n) | reactions | 0 |
| β-catenin_TCF | nucleus (n) | reactions | 0 |
| β-catenin_APC | cytoplasm (c) | reactions | 0 |
| plakoglobin_TCF | nucleus (n) | reactions | 0 |
| *g*PPAR | nucleus (n) | reactions | 0 |
| *g*TCF | nucleus (n) | reactions | 0 |

| | | | |
|---|---|---|---|
| PPAR | nucleus (n) | reactions | 0 |
| *g*Axin | nucleus (n) | reactions | 0 |
| Wnt | cytoplasm (c) | fixed | 0 |
| plakoglobin | nucleus (n) | reactions | 0÷500 |

**Table S1. Species and conditions.** Overview of the dependent variables defined in the extended WCP model. The complex between two species is indicated with "_". The prefix "g" refers to the mRNA relative to each species, transcribed from the specific gene. The prefix "f" refers to the phosphorylated species. The "type" of reaction indicates the concentration relative to a given species determined either though a set of reactions, or by a fixed value. Same species present in two different compartments is considered mathematically distinct ({c} for cytoplasmatic species and {n} for nuclear ones). Concentrations are reported as [nmol/l].

| **Reaction** | **Function** |
|---|---|
| Dshi → Dsha; Wnt | Dsh Activation (canonical Wnt) |
| Dsha → Dshi | Dsh Inactivation |
| APC_Axin_ GSK3β -> APC_Axin + GSK3β; Dsha | Destruction core inactivation via Dsha |
| APC_Axin_ GSK3β = *f*APC_*f*Axin_ GSK3β | (De) Phosphorilation Destruction core |
| APC_Axin + GSK3β = APC_Axin_ GSK3β | Destruction core Formation |
| Axin + APC = APC_Axin | Destruction core Formation |
| β-catenin{c} + *f*APC_*f*Axin_ GSK3β = β-catenin_*f*APC_*f*Axin_ GSK3β | β-catenin binding destruction core |
| β-catenin_*f*APC_*f*Axin_ GSK3β → = *f*β-catenin_*f*APC_*f*Axin_ GSK3β | Phosphorilation β-catenin |
| *f*β-catenin_*f*APC_*f*Axin_ GSK3β → *f*β-catenin + *f*APC_*f*Axin_ GSK3β | Release of phosphorylated β-catenin |
| *f*β-catenin → | Phosphorylated β-catenin degradation |
| → β-catenin{c} | Synthesis β-catenin |
| β-catenin{c} → | Indipendent Wnt β-catenin degradation |
| **→ *g*Axin; β-catenin_TCF plakoglobin_TCF** | **gAxin expression** |
| Axin → APC | Axin degradation |
| **β-catenin{c} = β-catenin{n}** | **β-catenin Transport** |
| β-catenin{n} + TCF = β-catenin_TCF | β-catenin binding TCF |
| β-catenin{c} + APC = β-catenin_APC | β-catenin binding APC |
| **→ *g*TCF; β-catenin_TCF plakoglobin_TCF** | **gTCF expression** |

| | |
|---|---|
| **$g$TCF →** | **gTCF degradation** |
| **Plakoglobin + TCF = plakoglobin_TCF** | **Plakoglobin binding TCF** |
| **→TCF; $g$TCF** | **TCF synthesis** |
| **TCF →** | **TCF degradation** |
| **→ $g$PPAR; β-catenin_TCF plakoglobin_TCF** | **gPPAR expression** |
| **$g$PPAR →** | **gPPAR degradation** |
| **$g$Axin →** | **gAxin degradation** |
| **→ Axin; $g$Axin** | **Axin synthesis** |
| **→PPAR; $g$PPAR** | **PPAR synthesis** |
| **PPAR →** | **PPAR degradation** |

**Table S2. Reactions in the extended WCP model. In bold are reported the reactions add to the original Lee's model.**

| Reaction | Parameter | Value |
|---|---|---|
| Dsh activation | $k_1$ | 0.182 [l nmol$^{-1}$ min$^{-1}$] |
| Dsh inactivation | $k_2$ | 0.0182 [min$^{-1}$] |
| Desctruction core inactivation via Dsha | $k_3$ | 0.05 [l nmol$^{-1}$ min$^{-1}$] |
| Destruction core formation | $k_4$ | 0.0909 [l nmol$^{-1}$ min$^{-1}$] |
| | $k_5$ | 0.909 [min$^{-1}$] |
| Destruction core formation – APC/Axin | $k_6$ | 1 [l nmol$^{-1}$ min$^{-1}$] |
| | $k_7$ | 50 [min$^{-1}$] |
| Destruction core (De) phosphorylation | $k_8$ | 0.267 [min$^{-1}$] |
| | $k_9$ | 0.133 [min$^{-1}$] |
| $\beta - catenin$ binding destruction core | $k_{10}$ | 1 [l nmol$^{-1}$ min$^{-1}$] |
| | $k_{11}$ | 120 [min$^{-1}$] |
| $\beta - catenin$ phosphorylation | $k_{12}$ | 206 [min$^{-1}$] |
| $\beta - catenin$ binding APC | $k_{13}$ | 1 [l nmol$^{-1}$ min$^{-1}$] |
| | $k_{14}$ | 1200 [min$^{-1}$] |
| Axin degradation | $k_{15}$ | 0.33 [min$^{-1}$] |
| | $k_{16}$ | 98 [nmol l$^{-1}$] |
| $\beta - catenin$ synthesis | v | 0.423 [nmol min$^{-1}$ l$^{-1}$] |
| Indipendent Wnt β-catenin degradation | $k_{17}$ | 0.000257 |
| $\beta - catenin$ transport | $k_{18}$ | 0.182 [min$^{-1}$] |
| | $k_{19}$ | 0.055 [min$^{-1}$] |
| Phosphorylated β-catenin degradation | $k_{20}$ | 0.417 [min$^{-1}$] |
| Release of phosphorylated $\beta - catenin$ | $k_{21}$ | 206 [min$^{-1}$] |
| β-catenin binding TCF | $k_{22}$ | 1 [l nmol$^{-1}$ min$^{-1}$ ] |
| | $k_{23}$ | 30 [min$^{-1}$] |
| Plakoglobin binding TCF | $k_{24}$ | 1 [l nmol$^{-1}$ min$^{-1}$ ] |
| | $k_{25}$ | 30 [min$^{-1}$] |

| | | |
|---|---|---|
| TCF synthesis | $k_{26}$ | 1 [$min^{-1}$] |
| TCF degradation | $k_{27}$ | 0.084 [$min^{-1}$] |
| gPPAR synthesis | $v_{0s}$ | 0.00884 [nmol $min^{-1}$ $l^{-1}$] |
| | $k_{1s}$ | 0 |
| | $k_{as}$ | 23 |
| | $n_s$ | 3 |
| | $k_{2s}$ | 0.00884 |
| | $k_{bs}$ | 23 |
| | $k_{3s}$ | 0 |
| | $k_{4s}$ | 1 |
| | $k_{5s}$ | 1 |
| | $k_{6s}$ | 0 |
| gPPAR degradation | $k_{28}$ | 0.01 [$min^{-1}$] |
| gTCF synthesis | $v_{0s}$ | 0.0061 [nmol $min^{-1}$ $l^{-1}$] |
| | $k_{1s}$ | 0.0361 |
| | $k_{as}$ | 23 |
| | $n_s$ | 3 |
| | $k_{2s}$ | 0 |
| | $k_{bs}$ | 23 |
| | $k_{3s}$ | 0 |
| | $k_{4s}$ | 1 |
| | $k_{5s}$ | 1 |
| | $k_{6s}$ | 0 |
| gTCF degradation | $k_{29}$ | 0.01 [$min^{-1}$] |
| gAxin synthesis | $v_{0s}$ | 0.0061 [nmol $min^{-1}$ $l^{-1}$] |
| | $k_{1s}$ | 0.0361 |
| | $k_{as}$ | 23 |
| | $n_s$ | 3 |
| | $k_{2s}$ | 0 |
| | $k_{bs}$ | 23 |
| | $k_{3s}$ | 0 |
| | $k_{4s}$ | 1 |
| | $k_{5s}$ | 1 |
| | $k_{6s}$ | 0 |
| gAxin degradation | $k_{30}$ | 0.01 [$min^{-1}$] |
| PPAR synthesis | $k_{31}$ | 1 [$min^{-1}$] |
| PPAR degradation | $k_{32}$ | 0.084 [$min^{-1}$] |

**Table S3**. **Overview of all the parameters and their values, for the extended WCP model.**

Below are reported all the equations for the WCP extended model. Especially, each ODE describes the variation, over time, of the species concentrations [S] reported in **Table S1**. All the parameter values are reported in **Table S3**.

$$[\dot{Dsha}]V_c = V_c(k_1[Dshi][Wnt]) - V_c(k_2[Dsha])$$

$$[\dot{Dshi}]V_c = -V_c(k_1[Dshi][Wnt]) + V_c(k_2[Dsha])$$

$$[\dot{APC\_Axin}]V_c = V_c(k_3[APC\_Axin\_GSK3\beta][Dsha]) - V_c(k_4[APC\_Axin][GSK3\beta] - k_5[APC\_Axin\_GSK3\beta]) + V_c(k_6[Axin][APC] - k_7[APC\_Axin])$$

$$[\dot{GSK3\beta}]V_c = V_c(k_3[APC\_Axin\_GSK3\beta][Dsha]) - V_c(k_4[APC_{Axin}][GSK3\beta] - k_5[APC\_Axin\_GSK3\beta])$$

$$[\dot{APC\_Axin\_GSK3\beta}]V_c = V_c(k_4[APC\_Axin][GSK3\beta] - k_5[APC\_Axin\_GSK3\beta]) - V_c(k_8[APC\_Axin\_GSK3\beta] - k_9[fAPC\_fAxin\_GSK3\beta]) + V_c(k_3[APC\_Axin\_GSK3\beta][Dsha])$$

$$[\dot{fAPC\_fAxin\_GSK3\beta}]V_c = V_c(k_8[APC\_Axin\_GSK3\beta] - k_9[fAPC\_fAxin\_GSK3\beta]) - V_c(k_{10}[\beta - catenin\{C\}][fAPC\_fAxin\_GSK3\beta] - k_{11}[\beta - catenin\_fAPC\_fAxin\_GSK3\beta]) + V_c(k_{12}[f\beta - catenin\_fAPC\_fAxin\_GSK3\beta])$$

$$[\dot{APC}]V_c = -V_c(k_{13}[\beta - catenin\{c\}][APC] - k_{14}[\beta - cateni\_APC]) - V_c(k_6[Axin][APC] - k_7[APC\_Axin])$$

$$[\dot{Axin}]V_c = -V_c\left(\frac{k_{15}[Axin][APC]}{k_{16}+[APC]}\right) + V_c(([gAxin], 1)) - V_c(k_6[Axin][APC] - k_7[APC\_Axin])$$

$$[\dot{\beta - catenin\{c\}}]V_c = -V_c(k_{10}[\beta - catenin\{c\}][fAPC\_fAxin\_GSK3\beta] - k_{11}[\beta - catenin\_fAPC\_fAxin\_GSK3\beta]) + V_c v - V_c(k_{17}[\beta - catenin\{c\}]) - (k_{18}V_c[\beta - catenin\{c\}] - k_{19}V_n[\beta - catenin\{n\}]) - V_c(k_{13}[\beta - catenin\{c\}][APC] - k_{14}[\beta - catenin\_APC])$$

$$[\dot{\beta - catenin\_fAPC\_fAxin\_GSK3\beta}]V_c = V_c(k_{10}[\beta - catenin\{c\}][fAPC\_fAxin\_GSK3\beta] - k_{11}[\beta - catenin\_fAPC\_fAxin\_GSK3\beta]) - V_c(k_{12}[\beta - catenin\_fAPC\_fAxin\_GSK3\beta])$$

$$[\dot{f\beta - catenin}]V_c = V_c(k_{12}[\beta - catenin\_fAPC\_fAxin\_GSK3\beta]) - V_c(k_{20}[f\beta - catenin])$$

$$[\dot{f\beta - catenin\_fAPC\_fAxin\_GSK3\beta}]V_c = V_c(k_{11}[\beta - catenin\_fAPC\_fAxin\_GSK3\beta]) - V_c(k_{21}[f\beta - catenin\_fAPC\_fAxin\_GSK3\beta])$$

$$[\dot{\beta - catenin\_TCF}]V_n = V_n(k_{22}[\beta - catenin\{n\}][TCF]) - k_{23}[\beta - catenin\_TCF])$$

$$[\dot{\beta - catenin\_APC}]V_c = V_c(k_{13}[\beta - catenin\{c\}][APC]) - k_{14}[\beta - catenin\_APC])$$

$$[\dot{\beta - catenin\{n\}}]V_n = (k_{18}V_c[\beta - catenin\{c\}] - k_{19}V_n[\beta - catenin\{n\}]) - V_c(k_{22}[\beta - catenin\{n\}][TCF] - k_{23}[\beta - catenin\_TCF])$$

$$[\dot{TCF}]V_n = -V_n(k_{22}[\beta - catenin\{n\}][TCF] - k_{23}[\beta - catenin_{TCF}]) - V_n(k_{24}[plokoglobin][TCF] - k_{25}[plakoglobin\_TCF]) + V_n(k_{26}[gTCF]) - V_n(k_{27}[TCF])$$

$$[\dot{gPPAR}]V_n = V_c\left(\frac{vos*\left(1+\left(\frac{[plakoglobin\_TCF]}{kbs}\right)^{ns}\right)}{1+\left(\frac{[plakoglobin\_TCF]}{kbs}\right)^{ns}+\left(\frac{[\beta-catenin\_TCF]}{kas}\right)^{ns}}\right) - V_n(k_{28}[gPPAR])$$

$$[\dot{plakoglobin\_TCF}]V_n = V_n(k_{24}[plakoglobin][TCF] - k_{25}[plakoglobin\_TCF])$$

$$[\dot{plakoglobin}]V_n = -V_n(k_{24}[plakoglobin][TCF] - k_{25}[plakoglobin\_TCF])$$

$$[\dot{gTCF}]V_n = V_n\left(\frac{\left(v_{0s}+k_{1s}\left(\frac{[\beta-catenin\_TCF]}{k_{as}}\right)^{n_s}\right)}{1+\left(\frac{[plakoglobin\_TCF]}{k_{bs}}\right)^{n_s}+\left(\frac{[\beta-catenin\_TCF]}{k_{as}}\right)^{n_s}}\right) - V_n(k_{29}[gTCF])$$

$$[\dot{gAxin}]V_n = V_n\left(\frac{\left(v_{0s}+k_{1s}\left(\frac{[\beta-catenin\_TCF]}{k_{as}}\right)^{n_s}\right)}{1+\left(\frac{[plakoglobin\_TCF]}{k_{bs}}\right)^{n_s}+\left(\frac{[\beta-catenin\_TCF]}{k_{bs}}\right)^{n_s}}\right) - V_n(k_{30}[gAxin])$$

$$[\dot{PPAR}]V_n = V_n(k_{31}[gPPAR]) - V_n(k_{32}[PPAR])$$

**System a.** ODE equations in WCP extended model.

## Rho-kinase pathway model (RKP)

Since no model for Rho-kinase pathway is available, we started with the identification of the reaction scheme to be included in the model definition phase.

As already discussed, the information regarding the reaction and the respective parameters for some mechanisms, such as phosphorylation and de-phosphorylation of RhoA protein and Rho-Kinase activation, were taken from literature. For all other reactions, for which no quantitative reference was found in literature, were described through simple mass-action kinetics, while their parameters were obtained through a fitting phase, keep in the physiological ranges reported in **Table S4**.

| Parameter | Description | Realistic Range | Applied Range |
|---|---|---|---|
| k | Half-maximal activation coefficient | $10^{-3}$-10 | $10^{-3}$-10 |
| H | Half-life in intracellular environment | 1-$10^4$ min (for mRNA or protein) | 5-100 min |
| n | Hill-coefficient | 1-50 (highest measured is 35) | 1-10 |
| $\alpha$ | Saturability coefficient for an enhancer | 1-10 | 1-10 |
| transfer rates | How much reactions occurs per unit time | $10^{-3}$-10 | $10^{-3}$-10 |
| transform rates | For cleavage, phosphorylation, etc…. | $10^{-3}$-10 | $10^{-3}$-10 |

**Table S4. Physiological range of kinetic parameters [2, 3].**

Species and reactions considered for the model of Rho pathway are listed in**Tables S5** and **S6**, respectively.

| **Species** | **Compartment** | **Type** | **Concentration [nmol/l]** |
|---|---|---|---|
| RhoaGDP | cytoplasm (c) | reactions | 120 |
| RhoaGTP | cytoplasm (c) | reactions | 0 |
| RhoGEF | cytoplasm (c) | reactions | 100 |
| RhoGAP | cytoplasm (c) | reactions | 0 |
| ROCK | cytoplasm (c) | reactions | 680 |
| pROCK | cytoplasm (c) | reactions | 0 |
| ncDsha | cytoplasm (c) | reactions | 0 |
| ncDshi | cytoplasm (c) | reactions | 100 |
| Wnt5b | cytoplasm (c) | fixed | 0 |
| Daam1a | cytoplasm (c) | reactions | 0 |
| Daam1i | cytoplasm (c) | reactions | 50 |
| *f*Actin | cytoplasm (c) | reactions | 100 |
| *g*Actin | cytoplasm (c) | reactions | 0 |
| MKL1{c} | cytoplasm (c) | reactions | 1 |
| *g*Actin_MKL1 | cytoplasm (c) | reactions | 0 |
| MKL1{n} | nucleus (n) | reactions | 0 |
| *g*PPAR | nucleus (n) | reactions | 0 |
| PPAR | nucleus (n) | reactions | 0 |

**Table S5**. **Overview of the dependent variables defined in the RKP model**. The complex between two species is indicated with "_". The prefix "g" refers to the mRNA relative to each species, transcribed from the specific gene. The prefix "f" refers to the phosphorylated species. The "type" of reaction indicates the concentration relative to a given species determined either though a set of reactions, or by a fixed value. Same species present in two different compartments is considered mathematically distinct ({c} for cytoplasmatic species and {n} for nuclear ones). Concentrations are reported as [nmol/l].

| **Reaction** | **Function** |
|---|---|
| RhoaGDP → RhoaGTP; RhoGEF | (De) Phosphorilation |
| RhoaGTP → RhoaGDP; RhoGAP | (De) Phosphorilation |
| ROCK → pROCK; RhoaGTP | (De) Phosphorilation |
| pROCK → ROCK | Henri-Michaelis-Menten (irreversible) |
| ncDshi + Wnt5b → ncDsha | Mass action (irreversible) |

| | |
|---|---|
| ncDsha → ncDshi | Mass action (irreversible) |
| Daam1i → Daam1a; ncDsha | Rate law for *Daam1 Activation* |
| Daam1a → Daam1i | Mass action (irreversible) |
| RhoaGDP → RhoaGTP; Daam1a | (De) Phosphorilation |
| RhoaGTP → RhoaGDP | Mass action (irreversible) |
| *f*Actin → *g*Actin | Mass action (irreversible) |
| *g*Actin → *f*Actin; pROCK | Rate law for *Actin filaments Formation* |
| *g*Actin + MKL1{c} = *g*Actin | Mass action (reversible) |
| MKL1{c} = MKL1{n} | Transport |
| → *g*PPAR; MKL1{n} | Gene regulation |
| *g*PPAR → | Mass action (irreversible) |
| → PPAR; *g*PPAR | Translation |
| PPAR → | Mass action (irreversible) |

**Table S6**. **Reactions used to model the Rho-kinase pathway**. The majority of reactions are expressed as mass action. Daam1 activation and conversion of Actin (from g-Actin to f-Actin and *vice-versa*) are two customized function. ncDsh*a* stands for noncanonical active Dishevelled. The majority of the reactions are expressed as reversible or irreversible mass action laws.

| **Reaction** | **Parameter** | **Value** |
|---|---|---|
| Activation RhoA and ROCK and inactivation RhoA | k | 0.06 [$min^{-1}$] |
| | km | 100 [nmol $l^{-1}$] |
| RhoA-GTP inactivation | $k_{ri}$ | 0.0262 [$min^{-1}$] |
| Inactivation ROCK | $k_2$ | 0.6 [nmol $min^{-1}$ $l^{-1}$] |
| N.C. Dsh activation | $k_3$ | 0.182 [l $nmol^{-1}$ $min^{-1}$] |
| N.C. Dsh inactivation | $k_4$ | 0.0182 [$min^{-1}$] |
| Daam1 activation | $k_5$ | 0.05 [l $nmol^{-1}$ $min^{-1}$] |
| Daam1 inactivation | $k_6$ | 0.262 [$min^{-1}$] |
| Actin filaments degradation | $k_{act}$ | 0.1 [$min^{-1}$] |
| Actin filaments formation | $k_{actf}$ | 0.1 [l $nmol^{-1}$ $min^{-1}$] |
| MKL1 binding | $k_7$ | 0.1 [l $nmol^{-1}$ $min^{-1}$] |
| | $k_8$ | 0.01 [$min^{-1}$] |
| MKL1 transport | $k_9$ | 0.182 [$min^{-1}$] |
| | $k_{10}$ | 0.055 [$min^{-1}$] |
| gPPAR synthesis | $v_0$ | 0.005 [nmol $min^{-1}$ $l^{-1}$] |
| | $k_a$ | 5 [nmol $l^{-1}$] |
| gPPAR degradation | $k_{11}$ | 0.01 [$min^{-1}$] |
| PPAR synthesis | $k_{12}$ | 1 [$min^{-1}$] |
| PPAR degradation | $k_{13}$ | 0.084 [$min^{-1}$] |

**Table S7. Names and values of the parameters in the Rho-kinase pathway.**

Below are reported all the equations used to model the Rho-kinase pathway. All the parameter values are reported in **Table S7**.

$$[\dot{RhoaGDP}]V_c = -V_c\frac{k[RhoGEF][RhoaGDP]}{km+[RhoaGDP]} + V_c\frac{k[RhoGAP][RhoaGTP]}{km+[RhoaGTP]} - V_c\frac{k[Daam1a][RhoaGDP]}{km+[RhoaGDP]} + V_c(k_{ri}[RhoaGTP]) \quad \textbf{(b.1)}$$

$$[\dot{RhoaGTP}]V_c = V_c\frac{k[RhoGEF][RhoaGDP]}{km+[RhoaGDP]} - V_c\frac{k[RhoGAP][RhoaGTP]}{km+[RhoaGTP]} + V_c\frac{k[Daam1a][RhoaGDP]}{km+[RhoaGDP]} - V_c(k_{ri}[RhoaGTP]) \quad \textbf{(b.2)}$$

$$[\dot{ROCK}]V_c = -V_c\frac{k[RhoaGTP][ROCK]}{km+[ROCK]} + V_c\frac{k_2[pROCK]}{km+[pROCK]} \quad \textbf{(b.3)}$$

$$[\dot{pROCK}]V_c = V_c\frac{k[RhoaGTP][ROCK]}{km+[ROCK]} - V_c\frac{k_2[pROCK]}{km+[pROCK]} \quad \textbf{(b.4)}$$

$$[\dot{ncDsha}]V_c = V_c(k_3[ncDshi][Wnt5b]) - V_c(k_4[ncDsha]) \quad \textbf{(b.5)}$$

$$[\dot{ncDshi}]V_c = -V_c(k_3[ncDshi][Wnt5b]) + V_c(k_4[ncDsha]) \quad \textbf{(b.6)}$$

$$[\dot{Daam1a}]V_c = V_c(k_5[Daam1i][ncDsha]) - V_c(k_6[Daam1a]) \quad \textbf{(b.7)}$$

$$[\dot{Daam1i}]V_c = -V_c(k_5[Daam1i][ncDsha]) + V_c(k_6[Daam1a]) \quad \textbf{(b.8)}$$

$$[\dot{fActin}]V_c = -V_c(k_{act}[fActin]) + V_c(k_{actf}[gActin][pROCK]) \quad \textbf{(b.9)}$$

$$[\dot{gActin}]V_c = V_c(k_{act}[fActin]) - V_c\big(k_{actf}[gActin][pROCK]\big) - V_c(k_7[gActin][MKL1\{c\}] - k_8[gActin\_MKL1]) \quad \textbf{(b.10)}$$

$$[\dot{MKL1\{c\}}]V_c = -V_c(k_7[gActin][MKL1\{c\}] - k_8[gActin\_MKL1]) - V_c(k_9[MKL1\{c\}] + V_n\,(k_{10}[MKL1\{n\}])\,) \quad \textbf{(b.11)}$$

$$[\dot{gActin\_MKL1}]V_c = V_c(k_7[gActin][MKL1\{c\}] - k_8[gActin\_MKL1]) \quad \textbf{(b.12)}$$

$$[\dot{MKL1}]V_n = V_c(k_9[MKL1\{c\}]) - V_n(k_{10}[MKL1\{n\}]) \quad \textbf{(b.13)}$$

$$[\dot{gPPAR}]V_n = V_n\frac{v_0}{1+[MKL1\{n\}/k_a]} - V_n(k_{11}[gPPAR]) \quad \textbf{(b.14)}$$

$$[\dot{PPAR}]V_n = V_n(k_{12}[gPPAR]) - V_n(k_{13}[PPAR]) \quad \textbf{(b.15)}$$

**System b.** ODE equations (b.1 - b.15) defined to model the Rho-kinase pathway.

**Integrative model of Wnt/β-catenin and RhoA-ROCK pathways.**

Finally, species and reactions added or changed in the WCP-RKP model are listed in **Tables S8** and **S9**, respectively.

| Species | Compartment | Type | Concentration [nmol/l] |
|---|---|---|---|
| plakoglobin{c} | cytoplasm (c) | reactions | 0 |
| plakoglobin{n} | nucleus (n) | reactions | 0 |
| Dplakoglobin | cytoplasm (c) | reactions | 100 |
| Siah2i | cytoplasm (c) | reactions | 100 |
| Siaha | cytoplasm (c) | reactions | 0 |
| *f*β-catenin | cytoplasm (c) | reactions | 0 |
| *g*TCF | nucleus (n) | reactions | 0 |
| *g*Axin | nucleus (n) | reactions | 0 |

**Table S8. Overview of all the variables that have been added or changed in the WCP-RKP final model**. The complex between two species is indicated with "_". The prefix "g" refers to the mRNA relative to each species, transcribed from the specific gene. The prefix "f" refers to the phosphorylated species. The "type" of reaction indicates the concentration relative to a given species determined either though a set of reactions, or by a fixed value. Same species present in two different compartments is considered mathematically distinct ({c} for cytoplasmatic species and {n} for nuclear ones). *Dplakoglobin* species refers to the desmosomal plakoglobin. Concentrations are reported as [nmol/l].

| Reaction | Function |
|---|---|
| plakoglobin{c} → Dplakoglobin; pROCK | Activation with modifier |
| plakoglobin{c} → plakoglobin{n} | Transport |
| Siah2i → Siah2a; Wnt5b | Activation with modifier |
| Siah2a → Siah2i | Mass action (irreversible) |
| Siah2a + APC = Siah2a_APC | Mass action (reversible) |
| β-catenin{c} → *f*β-catenin; Siah2a_APC | (De) Phosphorilation |
| Dplakoglobin → plakoglobin{c} | Mass action (irreversible) |

**Table S9. Reactions used to model the WCP-RKP cross-talk.**

| Reaction | Parameter | Value |
|---|---|---|
| Activation/inactivation Dsh (c. Wnt) | $k_1$ | 0.182 [l nmol$^{-1}$ min$^{-1}$] |
| | $k_2$ | 0.0182 [min$^{-1}$] |
| Activation/inactivation Dsh (n.c. Wnt) | $k_3$ | 0.182 [l nmol$^{-1}$ min$^{-1}$] |
| | $k_4$ | 0.0182 [min$^{-1}$] |
| pROCK stabilizes the desmosome | $k_5$ | 0.1 |
| Desmosomal plakoglobin | $k_6$ | 0.01 [min$^{-1}$] |
| Plakoglobin transport | $k_7$ | 0.182 [min$^{-1}$] |
| | $k_8$ | 0.055 [min$^{-1}$] |
| Plakoglobin binding TCF | $k_9$ | 1 [l nmol$^{-1}$ min$^{-1}$] |
| | $k_{10}$ | 30 [min$^{-1}$] |
| $\beta - catenin$ binding APC | $k_{11}$ | 1 [l nmol$^{-1}$ min$^{-1}$] |
| | $k_{12}$ | 1200 [min$^{-1}$] |
| Interaction between Siah2 and APC | $k_{13}$ | 1 [l nmol$^{-1}$ min$^{-1}$] |
| | $k_{14}$ | 30 [min$^{-1}$] |
| Destruction core formation | $k_{15}$ | 1 [l nmol$^{-1}$ min$^{-1}$] |
| | $k_{16}$ | 50 [min$^{-1}$] |
| Siah2 activation | $k_{17}$ | 0.1 [l nmol$^{-1}$ min$^{-1}$] |
| Siah2 inactivation | $k_{18}$ | 0.1 [min$^{-1}$] |

**Table S10. Names and values of the parameters in the WCP-RKP cross-talk mechanism.**

The mechanism of crosstalk required the addition of new differential equations in the developed mathematical system, as well as the modification of some previously ones, reported in the following **System c**. All the parameter values are reported in **Table S10**.

$$[\dot{Dshi}]V_c = -V_c(k_1[Dshi][Wnt]) + V_c(k_2[cDsha]) - V_c(k_3[Dshi][Wnt5b]) + V_c(k_4[ncDsha]) \quad \textbf{(c.1)}$$

$$[\dot{Dplakoglobin}]V_c = V_c(k_5[plakoglobin\{c\}][pROCK]) - V_c(k_6[Dplakoglobin]) \quad \textbf{(c.2)}$$

$$[\dot{plakoglobin\{c\}}]V_c = -V_c\left(k_5\left[plakoglobin\{c\}[pROCK]\right]\right) - V_c(k_7[plakoglobin\{c\}]) + V_n(k_8[plakoglobin\{n\}]) + V_c(k_6[Dplakoglobin]) \quad \textbf{(c.3)}$$

$$[\dot{plakoglobin\{n\}}]V_n = -V_n(k_9[plakoglobin\{n\}][TCF]) + V_n(k_{10}[plakoglobin\_TCF]) + V_c(k_7[plakoglobin\{c\}]) - V_n(k_8[plakoglobin\{n\}]) \quad \textbf{(c.4)}$$

$$[\dot{APC}]V_c = -V_c(k_{11}[\beta - catenin\{c\}][APC]) + V_c(k_{12}[\beta - catenin\_APC]) - V_c(k_{13}[Siah2a][APC]) + V_c(k_{14}[Siah2a\_APC]) - V_c(k_{15}[Axin][APC]) + V_c(k_{16}[APC\_Axin]) \quad \textbf{(c.5)}$$

$$[\dot{Siah2a}]V_c = V_c(k_{17}[Siah2i][Wnt5b]) - V_c(k_{18}[Siah2a]) - V_c(k_{13}[Siah2a][APC]) + V_c(k_{14}[Siah2a\_APC]) \quad \textbf{(c.6)}$$

$$[\dot{Siah2i}]V_c = -V_c(k_{17}[Siah2a][Wnt5b]) + V_c(k_{18}[Siah2a]) \quad \textbf{(c.7)}$$

$$[\dot{Siah2a\_APC}]V_c = V_c(k_{13}[Siah2a][APC]) - V_c(k_{14}[Siah2a\_APC]) \quad \textbf{(c.8)}$$

**System c.** ODE equations (c.1 - c.8) added to model the mechanism of crosstalk between the two pathways.

## Table S11. Primers used for qRT-PCR

| Gene | Primer forward | Primer reverse |
|---|---|---|
| *GAPDH* | TCCTCTGACTTCAACAGCGA | GGGTCTTACTCCTTGGAGGC |
| *PPARγ* | TGGCAATTGAATGTCGTGTC | GGAAGAAACCCTTGCATCCT |
| *CEBPα* | ACTTGGGGCTTGGAACCTAA | GACCCACGACCTAGCTTTCT |

## References


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