

Supplementary Information

A Quantitative Model of Cellular Decision Making in Direct Neuronal Reprogramming

Adriaan Merlevede¹, Emilie M. Legault³, Viktor Drugge¹, Roger A. Barker², Janelle Drouin-Ouellet^{3,*}, Victor Olariu^{1,*}

¹Computational Biology and Biological Physics, Department of Astronomy and Theoretical Physics, Lund University, Lund, 223 63, Sweden

² Cambridge Centre for Brain Repair, University of Cambridge, Forvie Site, Robinson Way, Cambridge CB2 2PY, UK.

³Faculté de Pharmacie, Université de Montréal, Montréal, Québec, H3T 1J4, Canada

*Correspondence: victor.olariu@thep.lu.se, janelle.drouin-ouellet@umontreal.ca

qRT-PCR analysis

See main Methods section for details.

Supplementary Table 1: List of primers used for qRT-PCR analysis

Gene	Direction	Sequence (5' → 3')
<i>ACTB</i>	Forward	CCTTGACATGCCGGAG
	Reverse	GCACAGAGCCTCGCCTT
<i>GAPDH</i>	Forward	TTGAGGTCAATGAAGGGGTC
	Reverse	GAAGGTGAAGGTCGGAGTCA
YWHAZ	Forward	GAAGCATTGGGGATCAAGAA
	Reverse	AGCAGATGGCTCGAGAATACA
<i>REST</i>	Forward	AAATGTGGCCTTAAGTGGGAA
	Reverse	TCTGTCTTTCTCACCACCAG
<i>PTB</i>	Forward	GGGTCGGTTCCTGCTATTCC
	Reverse	AAAAGCTCGTCAGATCCCCG
nPTB	Forward	TGGTTACGCCCCAAAGTCTG
	Reverse	TGGTTTCCATCAGCCATCTGT
<i>Endogenous Ascl1</i>	Forward	CTAAAGATGCAGGTTGTGCG
	Reverse	GGAGCTTCTCGACTTCACCA
<i>Viral Ascl1</i>	Forward	CATCTCCCCCAACTACTCCA
	Reverse	TCTGGGCTAAGAGGGTCGTA

	Literature	nPTB \rightarrow PTB
<i>resti</i>	1.25	3.17
<i>viral</i>	0.793	0.813
α_P	0.0889	18.8
$k_{M,P}$	0.000387	0.0306
$h_{M,P}$	0.154	1.63
α_N	179.	18.1
$k_{M,N}$	7.88e6	1.44e4
$h_{M,N}$	2.65	3.99
$k_{P,N}$	0.181	0.203
$h_{P,N}$	4.	4.
α_M	0.384	6.05
$k_{R,M}$	2.14e-17	0.529
$h_{R,M}$	0.0000195	2.63
β_M	0.0555	0.402
α_R	0.0934	9.68
$k_{P,R}$	3.81e7	0.561
$h_{P,R}$	0.0000825	4.
$k_{M,R}$	4.4	3.5
$h_{M,R}$	4.	4.
β_R	3.53	9.54
α_A	612.	5.28
$k_{R,A}$	0.0711	0.123
$h_{R,A}$	4.	4.
δ_P	0.0185	0.138
δ_N	0.0797	0.0177
δ_M	0.0102	0.374
δ_R	0.0765	9.32

	Literature	nPTB \rightarrow PTB
δ_A	0.245	0.0042
$k_{N,P}$	-	1.63
$h_{N,P}$	-	4.
β_P	-	2.02

Supplementary Table 2: Parameter values for the literature and PTB \rightarrow nPTB model, see equations 5 and 6. Parameters and equations for other models are provided in online supplement.

Supplementary Table 3: Initial values of simulations in the three stages of the experiment. The values are normalized so that a value of 1 is equal to the fibroblast level, or to the detection limit in the case of Ascl1, which was not observed in fibroblasts.

Experiment stage	PTB	nPTB	REST	miRs	Ascl1
Fibroblast	1	1	1	1	0
REST inhibition	1	1	1	1	0
Conversion	0.831538	1.27675	1.08673	0.645663	0

Supplementary Figures

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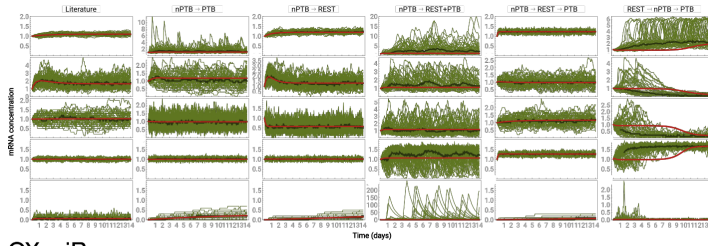
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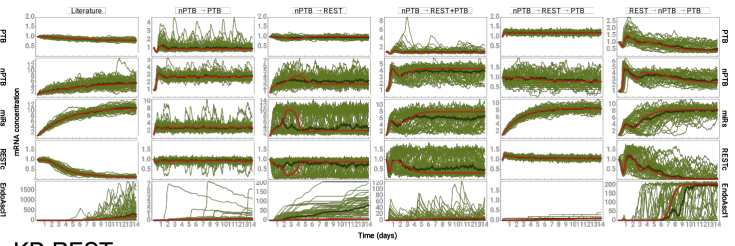
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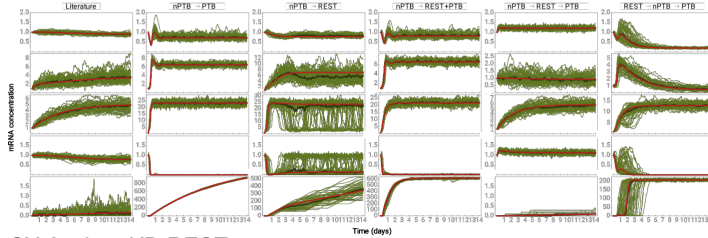
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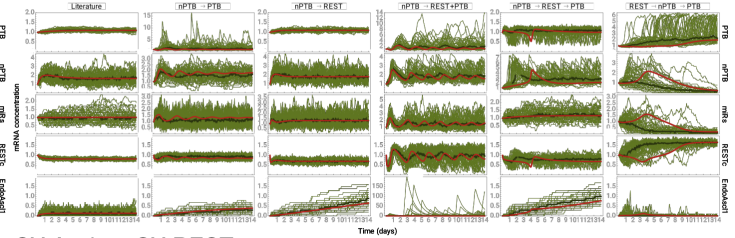
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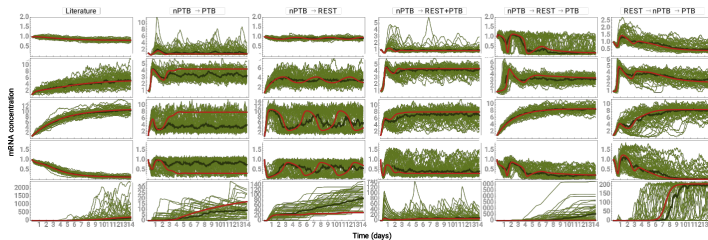
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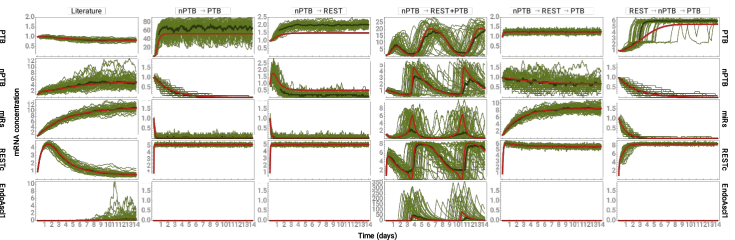
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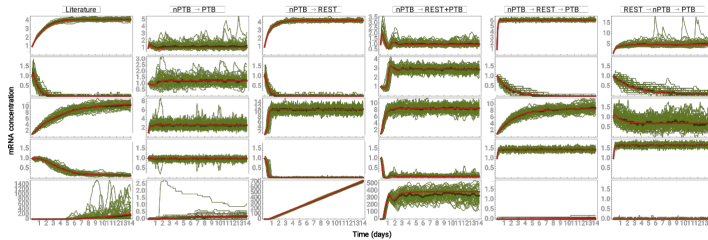
OX Ascl1 + KD REST:



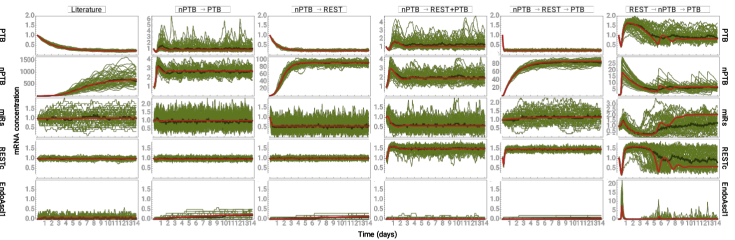
OX Ascl1 + OX REST:



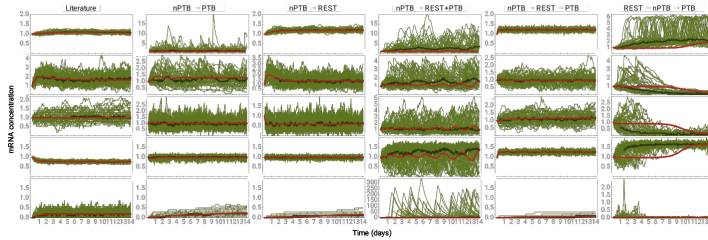
OX Ascl1 + OX PTB:



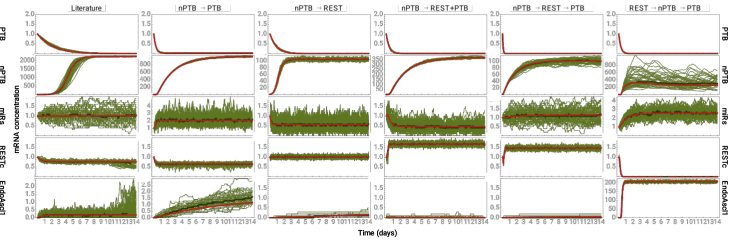
KD PTB:



KDBG REST:

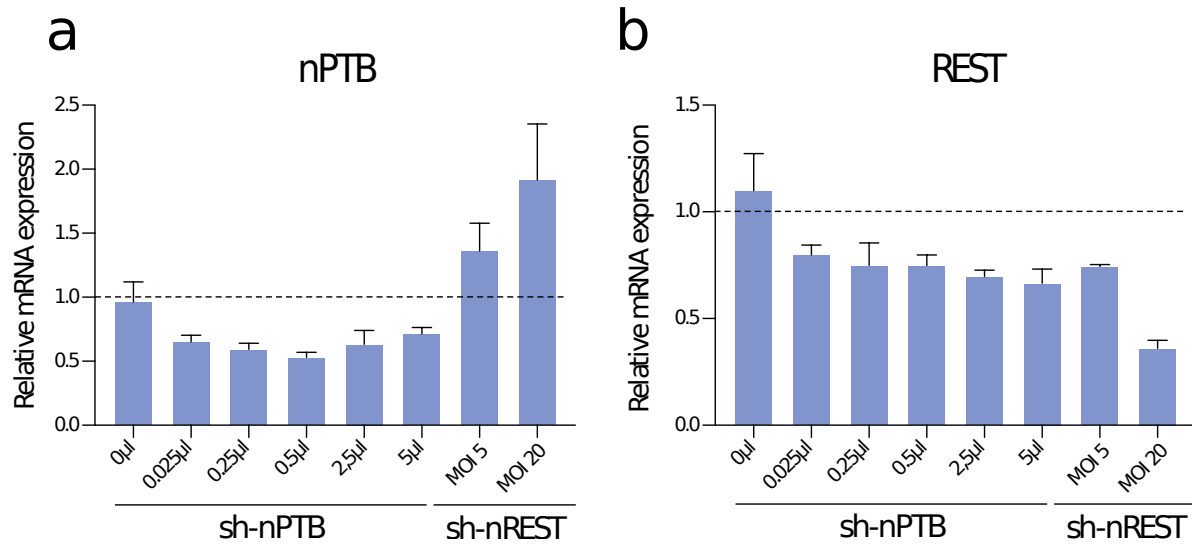


KDBG REST + KD PTB:



— Best fit — Stochastic runs — Median

Supplementary Figure 1: Simulated overexpression and knockdown experiments in several performant models; accompanying Figure 4.



Supplementary Figure 2: **nPTB and REST expression following KD with shRNAs.** (a) Measured transcription levels of nPTB 3 days following infection with different volumes or MOI of lentiviruses containing either shRNAs against nPTB or REST. The measurements are normalized so that a value of 1 is equal to the fibroblast level (represented as a dashed line). Error bars are defined as S.D. (b) Measured transcription levels of REST 3 days following infection with different volumes or MOI of lentiviruses containing either shRNAs against nPTB or REST. The measurements are normalized so that a value of 1 is equal to the fibroblast level (represented as a dashed line). Error bars are defined as S.D.