

Supplementary File S1 For "SNAIL driven by a Feed Forward Loop Motif Promotes TGF β Induced Epithelial to Mesenchymal Transition"

Table S1. Initial Conditions and Updating rules for Logical Modelling Simulations

Table S1(a) Epithelial initial conditions

SMURF = True	EXTGFB = False
PPM1A = True	ENTGFB = False
TCF1 = True	SNAIL = False
TCF2 = True	MIR200 = True
MDM2 = False	SMAD7 = True
DNADAMAGE = True	RSCYT = False
P53 = True	RSNUC = False
MIR34A = True	RSPCYT = False
ZEB = False	RSPNUC = False
EC = True	COCYT = True
NC = False	CONUC = False
TBR = False	RSPCYTCOCYT = False
TBRP = False	RSPNUCCONUC = False
EMT = False	

Table S1(b) updating rules for the E/M transition

ENTGFB* = SNAIL and not MIR200
TBR* = EXTGFB or (TBRP and SMAD7) or ENTGFB
TBRP* = (TBR and ENTGFB) or (TBR and EXTGFB)
RSCYT* = TBRP or RSNUC
RSPCYT* = (TBRP and RSCYT) or (MDM2 and RSCYT) or RSPCYTCOCYT
COCYT* = COCYT or CONUC or RSPCYTCOCYT
RSPCYTCOCYT* = RSPCYT and COCYT
RSNUC* = RSCYT or (RSPNUC and PPM1A)
RSPNUC* = RSPNUCCONUC or RSPCYT and not SMURF
RSPNUCCONUC* = RSPCYTCOCYT or (RSPNUC and CONUC)
CONUC* = RSPNUCCONUC or COCYT
MDM2* = (TCF1 and RSPNUCCONUC) or P53
P53* = DNADAMAGE and not MDM2
SNAIL* = (TCF2 and RSPNUCCONUC) or MDM2 and not MIR34A
MIR34A* = P53 and not SNAIL and not ZEB
ZEB* = SNAIL and not MIR200
MIR200* = P53 and not SNAIL and not ZEB
EC* = not SNAIL and not ZEB
NC* = SNAIL and ZEB
EMT* = NC and not EC

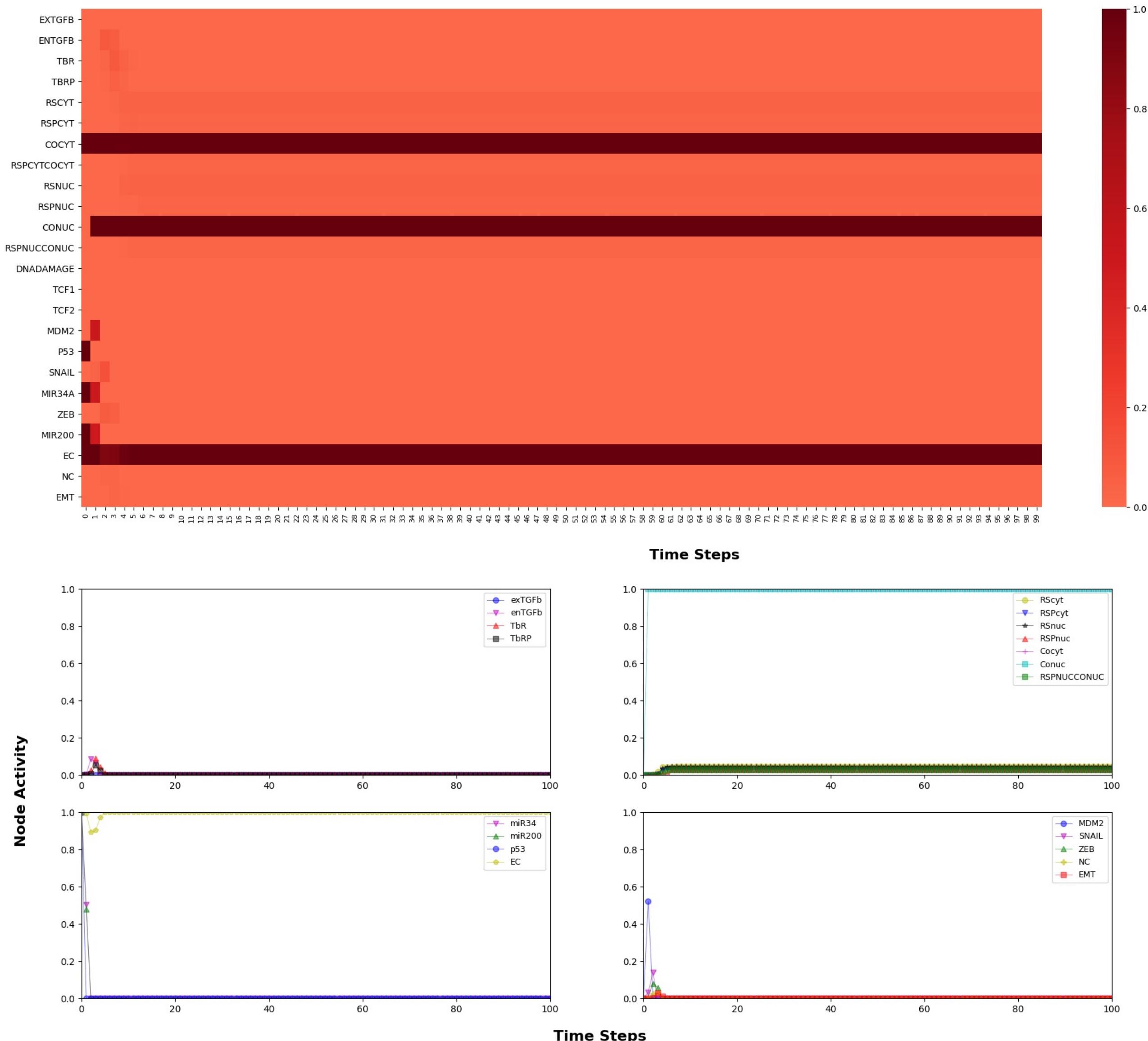


Figure S2. Simulation result of Logical modelling showing Transition from E to M phenotype for initial epithelial conditions. In absence of any dysregulations (DNA Damage) No E to M transition was observed. Both the mesenchymal markers (SNAIL, ZEB, N-Cadherin(NC)) and epithelial markers (p53, miR-34, miR-200, E-Cadherin(EC)) do not change their states

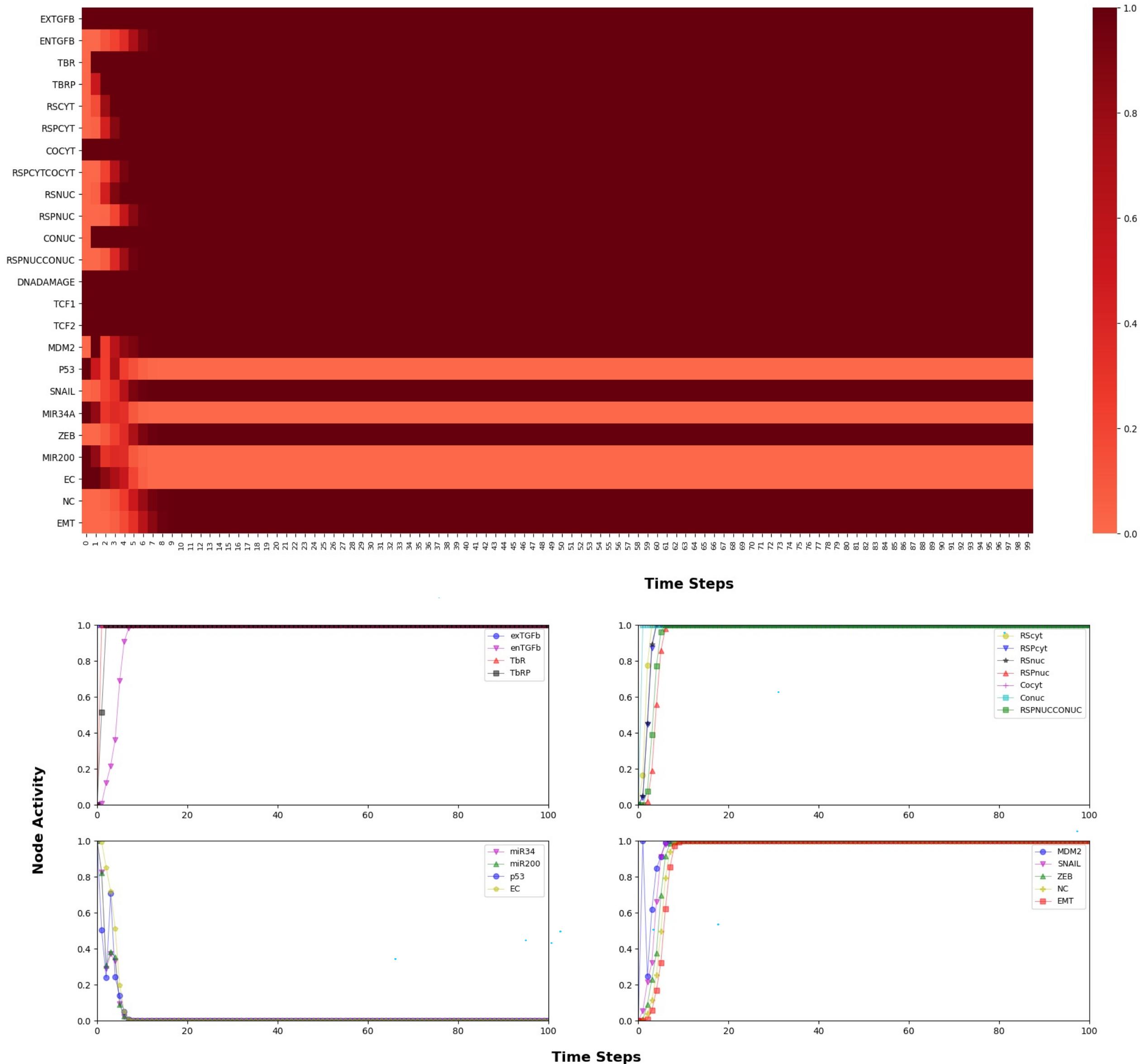


Figure S3. Simulation result of Logical modelling showing Transition from E to M phenotype when the $TCF1 = TCF2 = \text{True}$; $DNADamage = \text{True}$; $exTGFb = \text{True}$. Simulation was run for 5000 steps, 500 Iterations in asynchronous mode and complete transition was observed with in 10 timesteps. With the increase in total time steps the mesenchymal markers (SNAIL, ZEB, N-Cadherin(NC)) are transcriptionally activated supressing the epithelial markers (p53, miR-34, miR-200, E-Cadherin(EC)). Prescence of external stress causes the system to make a swift change from epithelial to mesenchymal phenotype with no hybrid phenotype

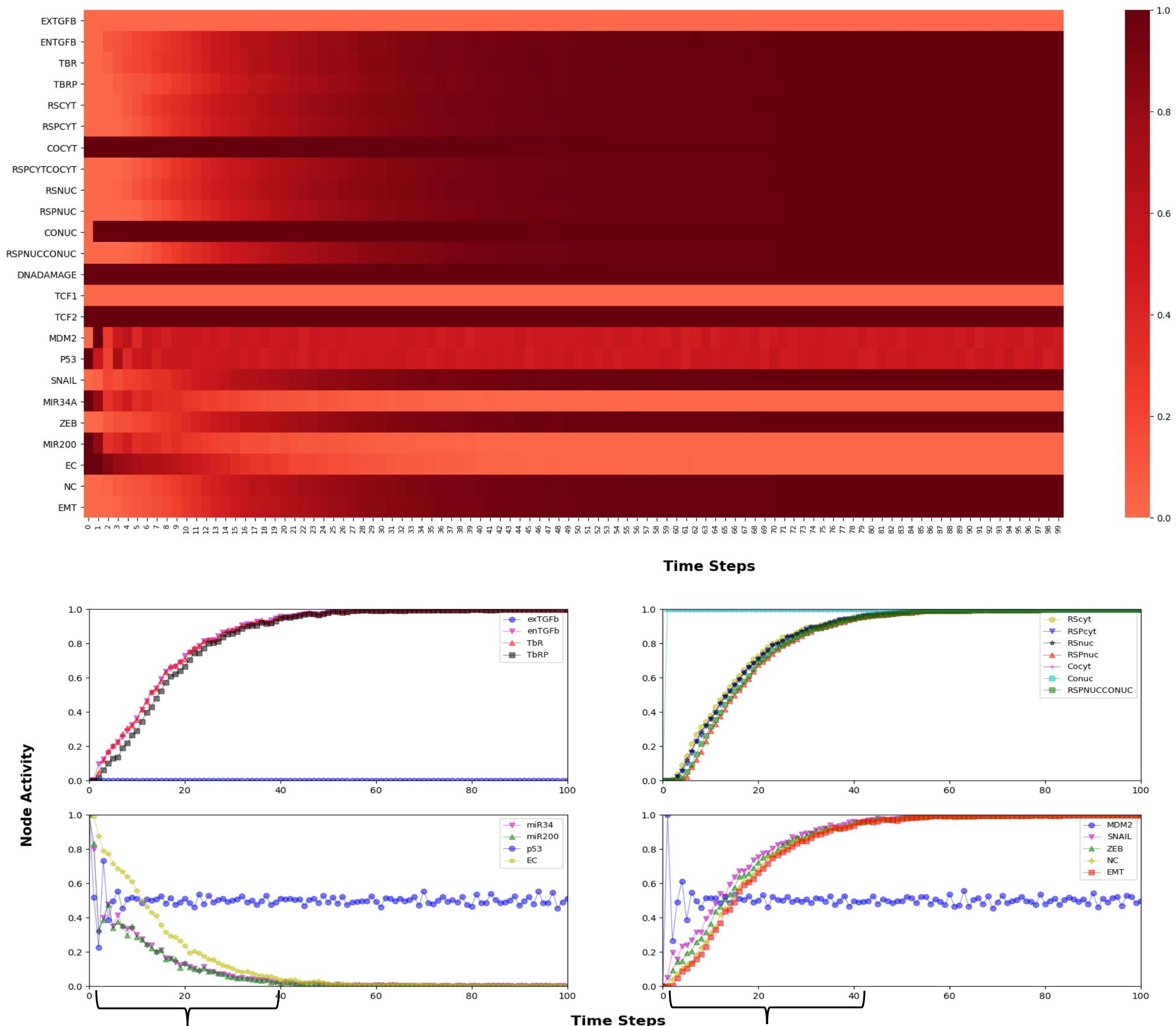


Figure S4. Simulation result of Logical modeling showing Transition from E to M phenotype when the co transcription factors for MDM2 TCF1 = False and SNAIL TCF2 = True; DNA Damage = True. Simulation was run for 5000 steps, 500 iterations in asynchronous mode and complete transition was observed with in 70 timesteps. With the increase in total time steps the mesenchymal markers (SNAIL, ZEB, N-Cadherin(NC)) are transcriptionally activated supressing the epithelial markers (p53, miR-34, miR-200, E-Cadherin(EC)). With the absence of input to MDM2 activation, MDM2 do not reach to its maximum to inhibit p53 thus p53-MDM2 arm of network oscillates

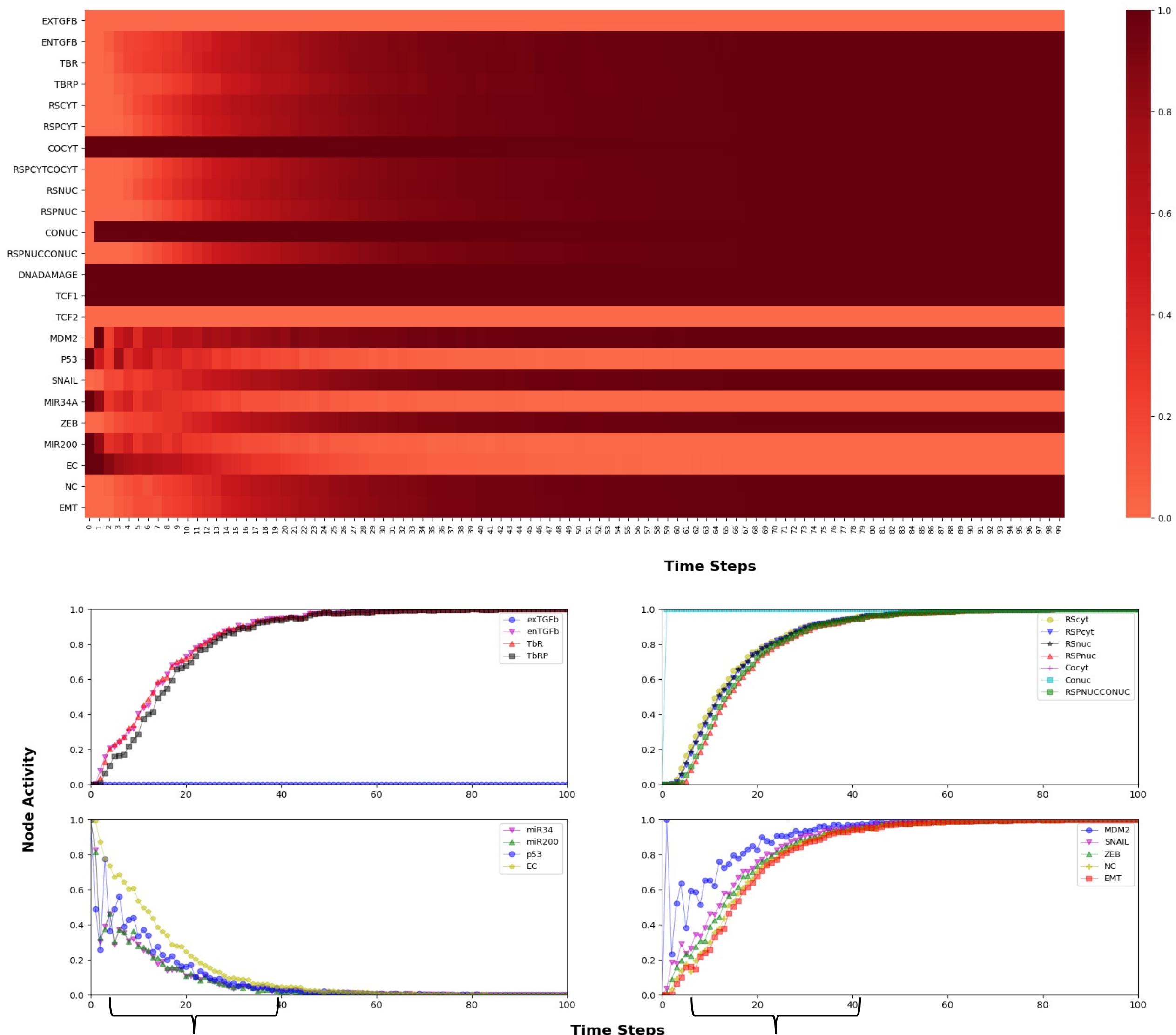


Figure S5. Simulation result of Logical modeling showing Transition from E to M phenotype when the co transcription factors for MDM2 TCF1 = True and SNAIL TCF2 = False; DNA Damage = True. Simulation was run for 5000 steps , 500 Iterations in asynchronous mode and complete transition was observed with in 70 timesteps. With the increase in total time steps the mesenchymal markers (SNAIL, ZEB, N-Cadherin(NC)) are transcriptionally activated supressing the epithelial markers (p53, miR-34, miR-200, E-Cadherin(EC)). With the absence of TCF2, SNAIL receives input only through MDM2 that further activates the enTGFb for SNAIL upregulation

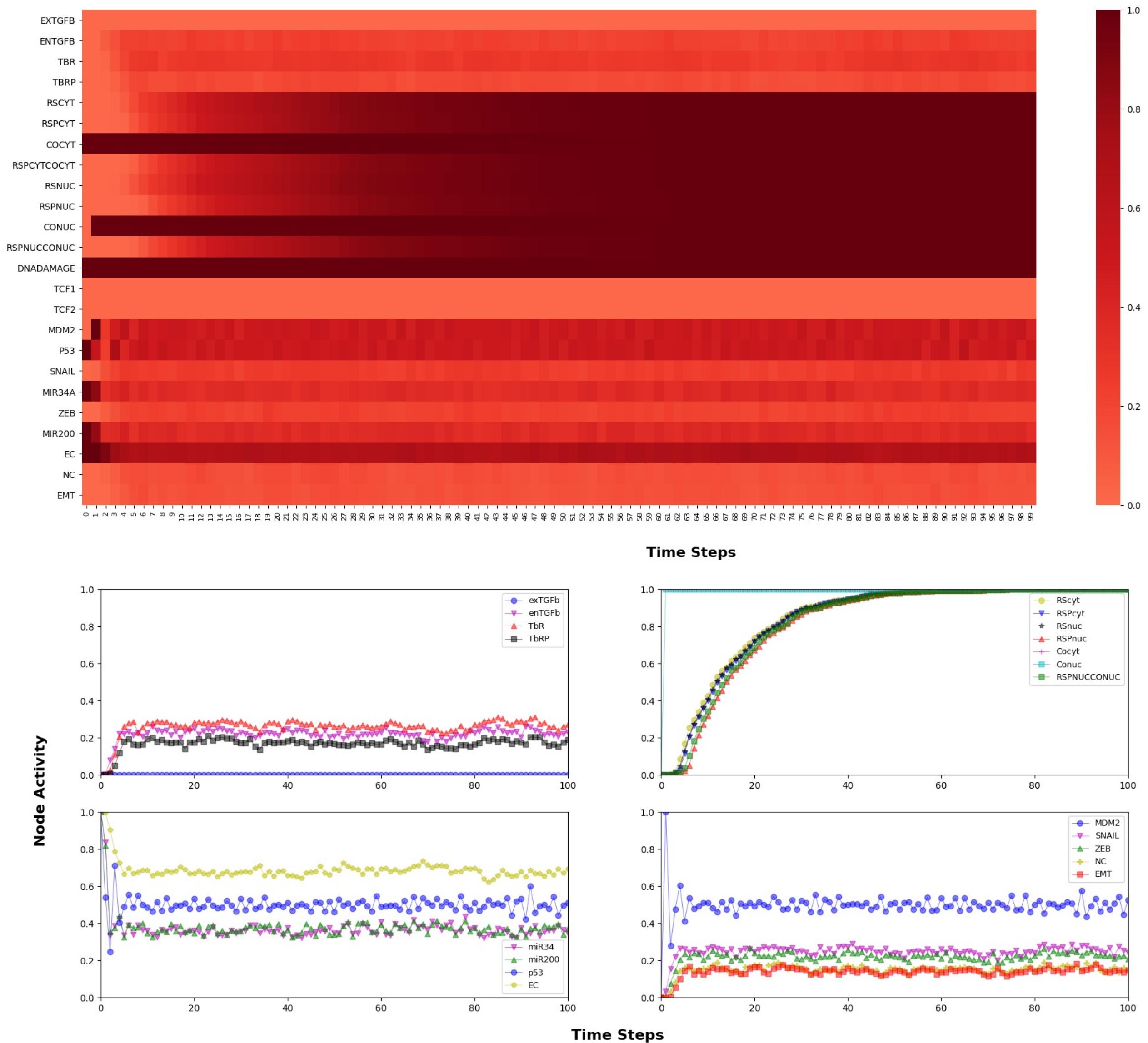


Figure S6. Simulation result of Logical modeling showing Transition from E to M phenotype when the co transcription factors for MDM2 TCF1 = False and SNAIL TCF2 = False; DNA Damage = True. Simulation was run for 5000 steps, 500 Iterations in asynchronous mode and a hybrid thenotype was observed through out the process. With the increase in total time steps the mesenchymal markers (SNAIL, ZEB, N-Cadherin(NC)) are transcriptionally activated supressing the epithelial markers (p53, miR-34, miR-200, E-Cadherin(EC)). With the absence of input to both SNAIL and MDM2, the nodes do not reach its maximum activity for the transition to happen

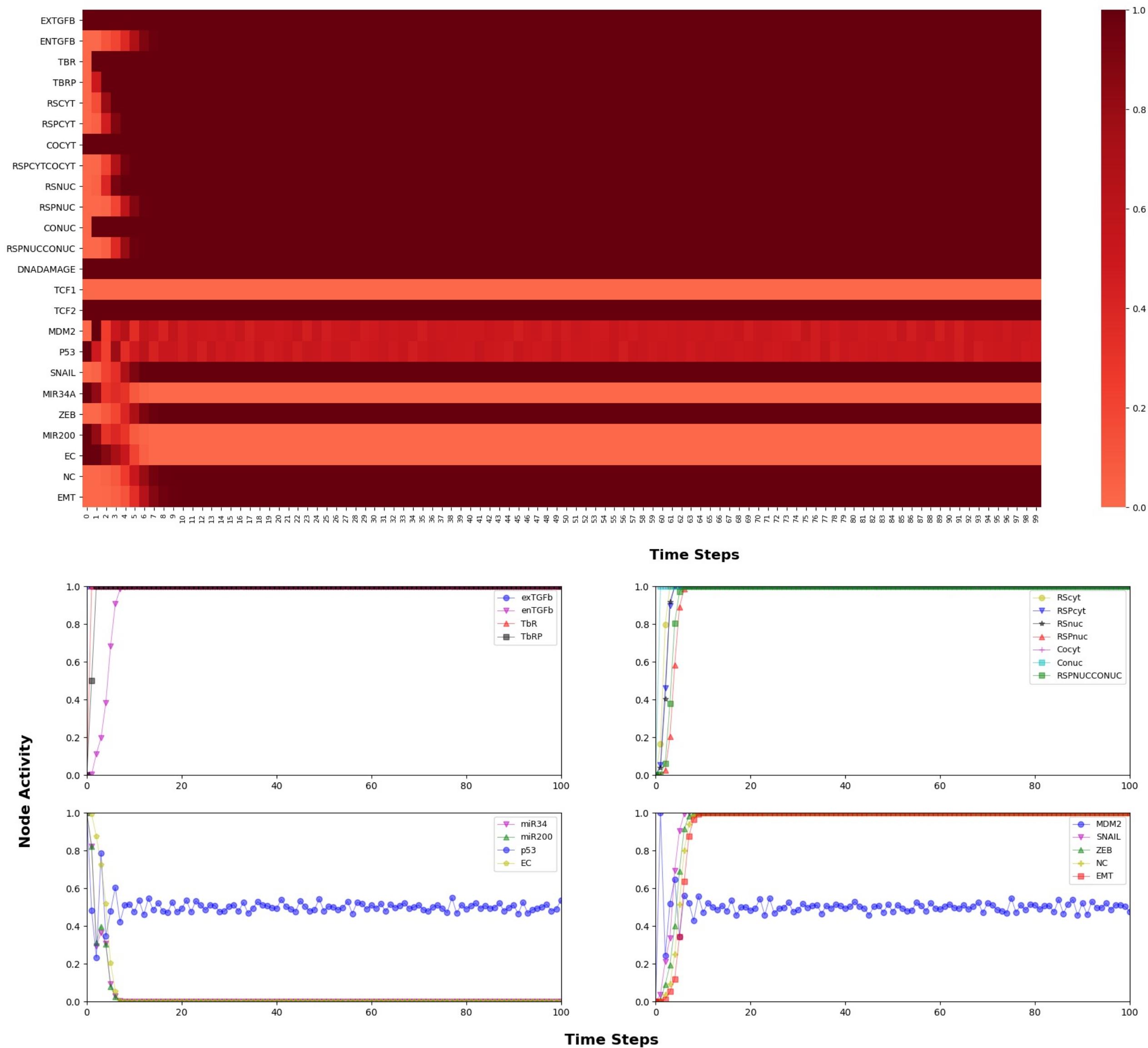


Figure S7. Simulation result of Logical modeling showing Transition from E to M phenotype when the TCF1 = False; TCF2 = True; DNADamage = True; exTGFb = True. Simulation was run for 5000 steps, 500 Iterations in asynchronous mode and complete transition was observed with in 10 timesteps. With the increase in total time steps the mesenchymal markers (SNAIL, ZEB, N-Cadherin(NC)) are transcriptionally activated supressing the epithelial markers (p53, miR-34, miR-200, E-Cadherin(EC)). Prescence of external stress causes the system to make a swift change from epithelial to mesenchymal phenotype with no hybrid phenotype But the lack of input to the MDM2 causes the p53-MDM2 feedback loop to fluctuate.

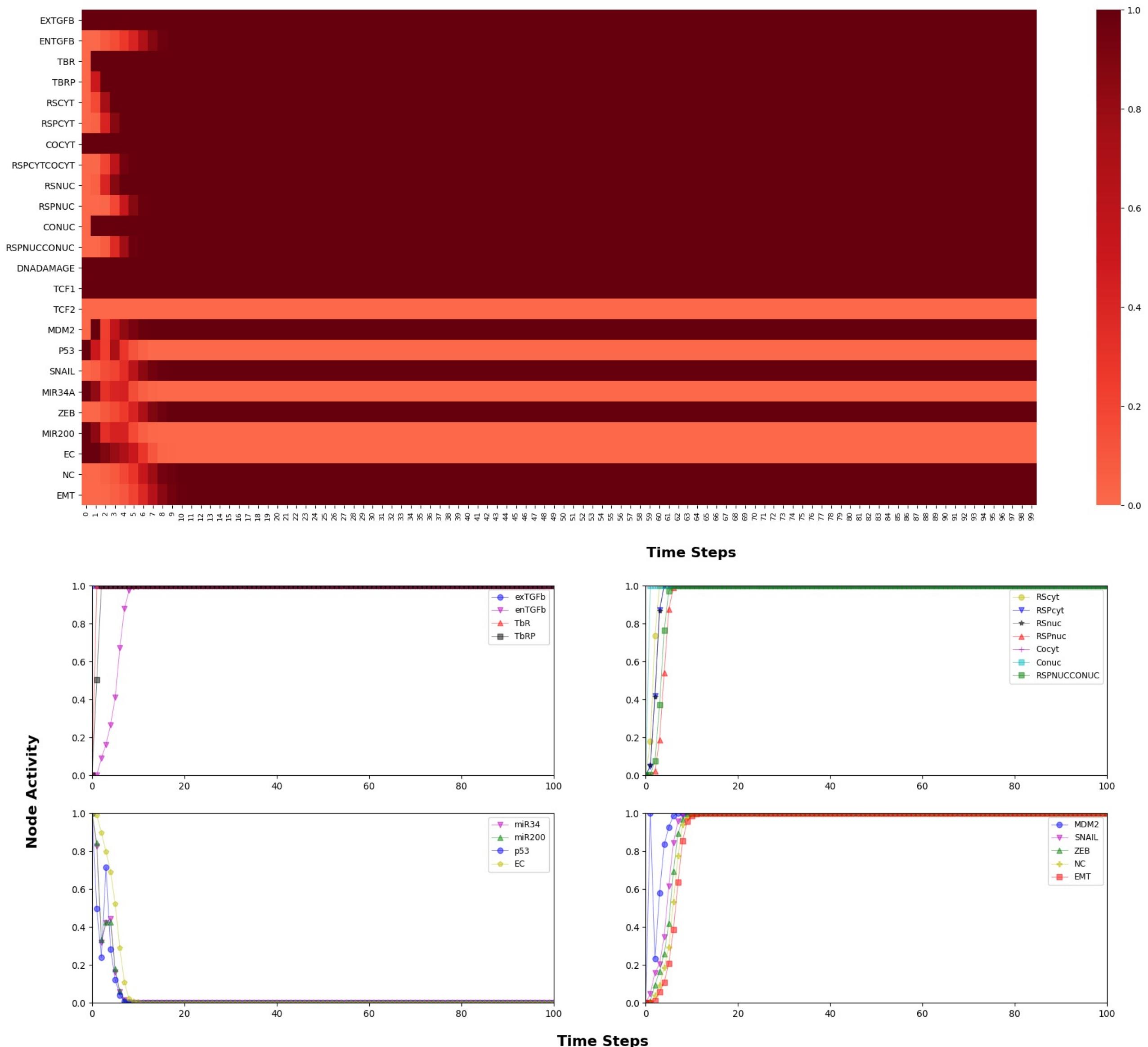


Figure S8. Simulation result of Logical modeling showing Transition from E to M phenotype when the TCF1 = True; TCF2 = False; DNADamage = True; exTGFb = True. Simulation was run for 5000 steps, 500 Iterations in asynchronous mode and complete transition was observed with in 10 timesteps. With the increase in total time steps the mesenchymal markers (SNAIL, ZEB, N-Cadherin(NC)) are transcriptionally activated suppressing the epithelial markers (p53, miR-34, miR-200, E-Cadherin(EC)). Prescence of external stress causes the system to make a swift change from epithelial to mesenchymal phenotype with no hybrid phenotype. With the absence of input to SNAIL activation through SMAD complex the only input SNAIL receives is through MDM2 that further activates the exTGFb for its upregulation

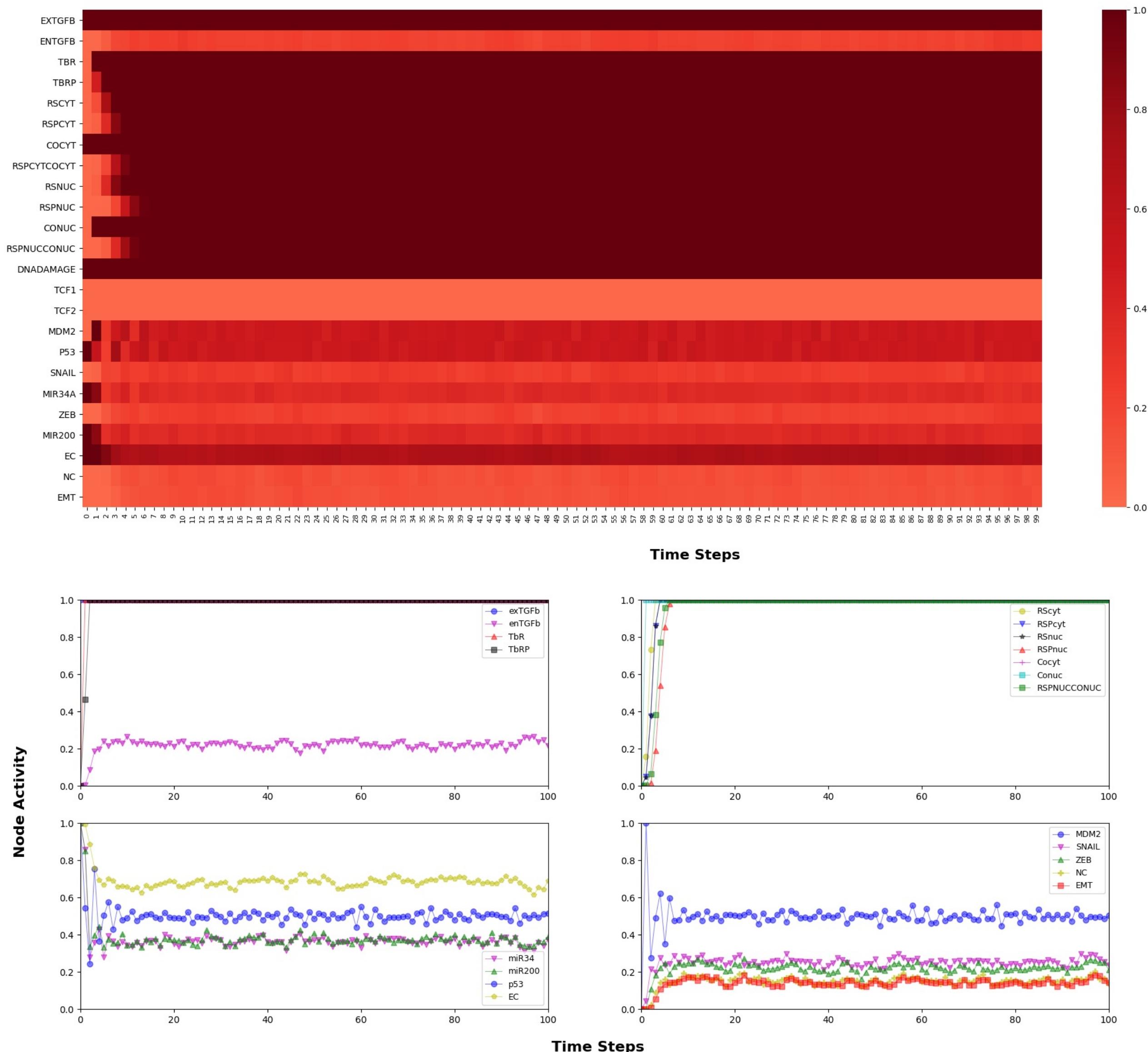
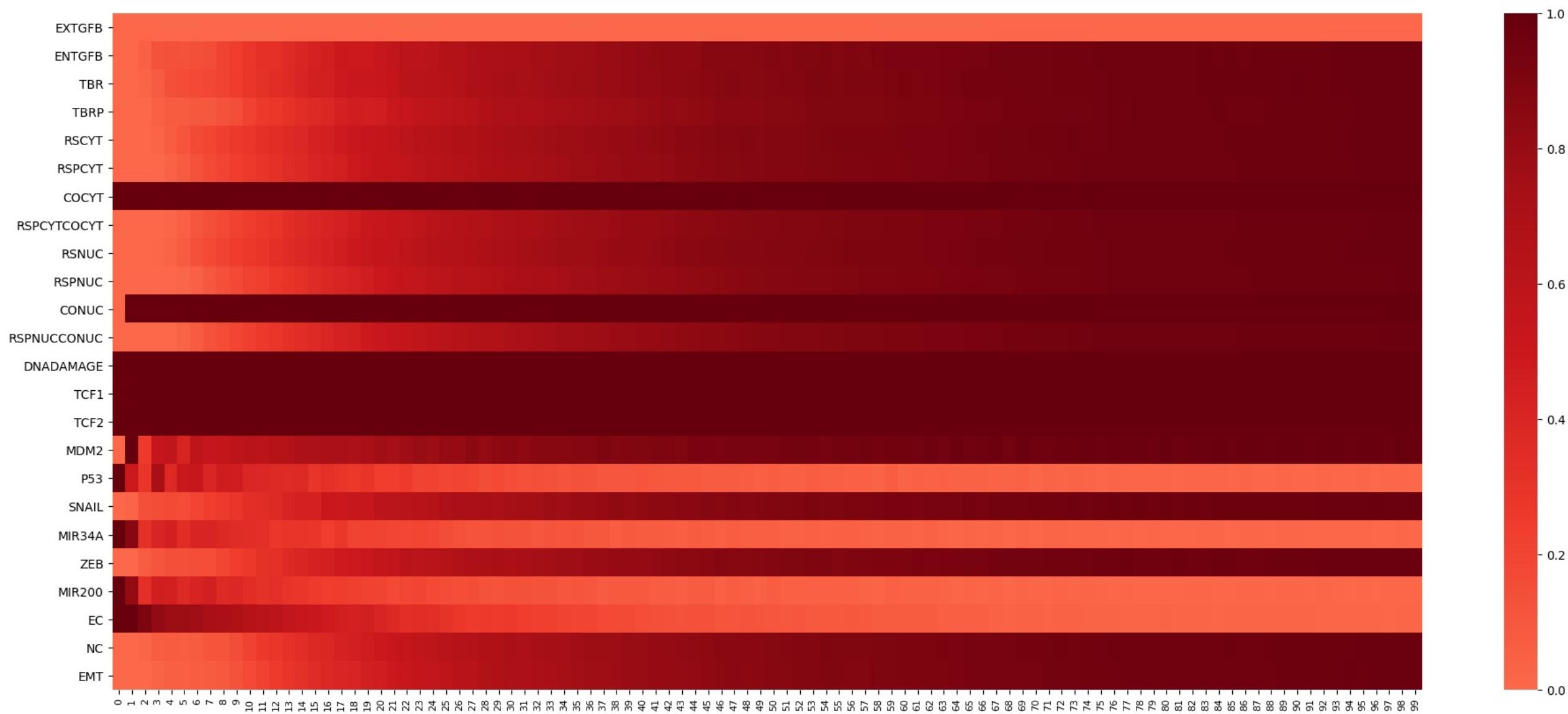


Figure S9. Simulation result of Logical modeling showing Transition from E to M phenotype when the TCF1 = False; TCF2 = False; DNADamage = True; exTGFb = True. Simulation was run for 5000 steps, 500 Iterations in asynchronous mode and hybrid phenotype was observed through the simulation as both the transcription cofactors were turned off



Time Steps

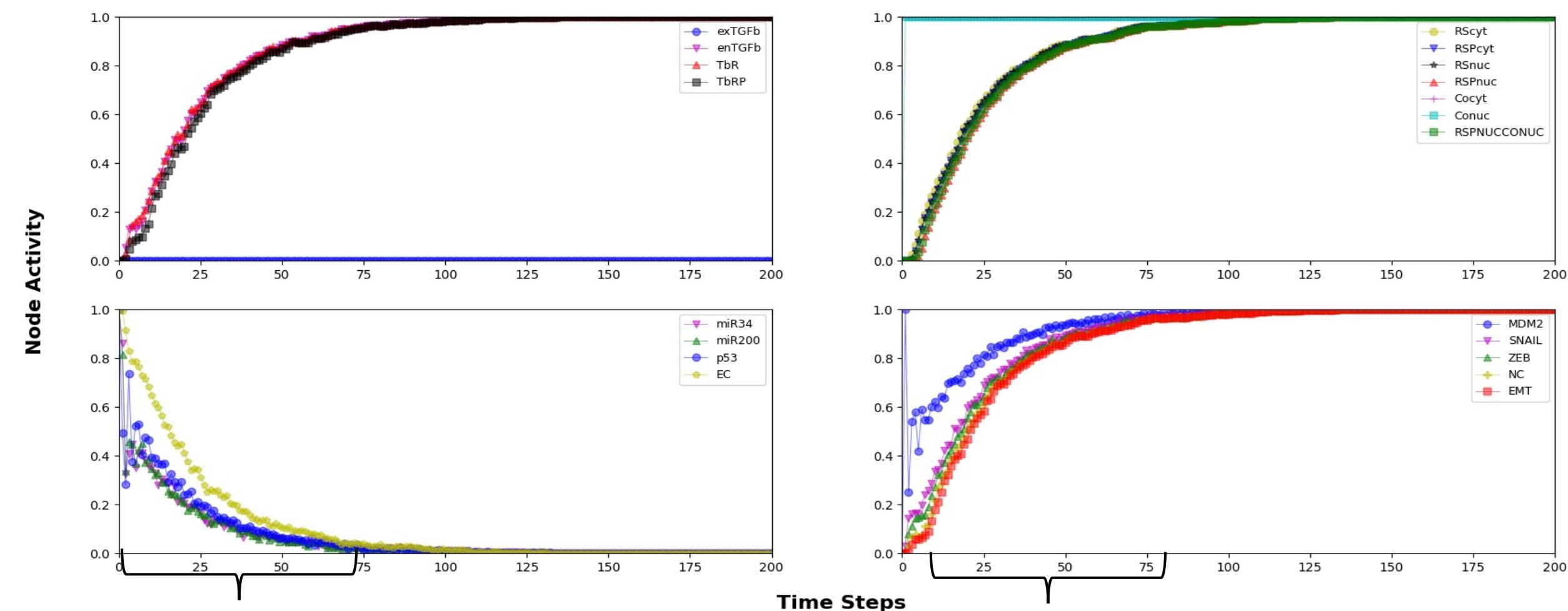


Figure S10. Simulation result of Logical modeling showing Transition from E to M phenotype when the co transcription factors for TCF1 = True and TCF2 = True; DNADamage = True; along with SNAIL Self Inhibition. Simulation was run for 5000 steps, 500 iterations in asynchronous mode and complete transition was observed with in 150 timesteps. . With the increase in total time steps the mesenchymal markers (SNAIL, ZEB, N-Cadherin(NC)) are transcriptionally activated supressing the epithelial markers (p53, miR-34, miR-200, E-Cadherin(EC)). Prescence of SNAIL self inhibition causes the system to stay in hybrid phenotype for longer periods of time (Marked).

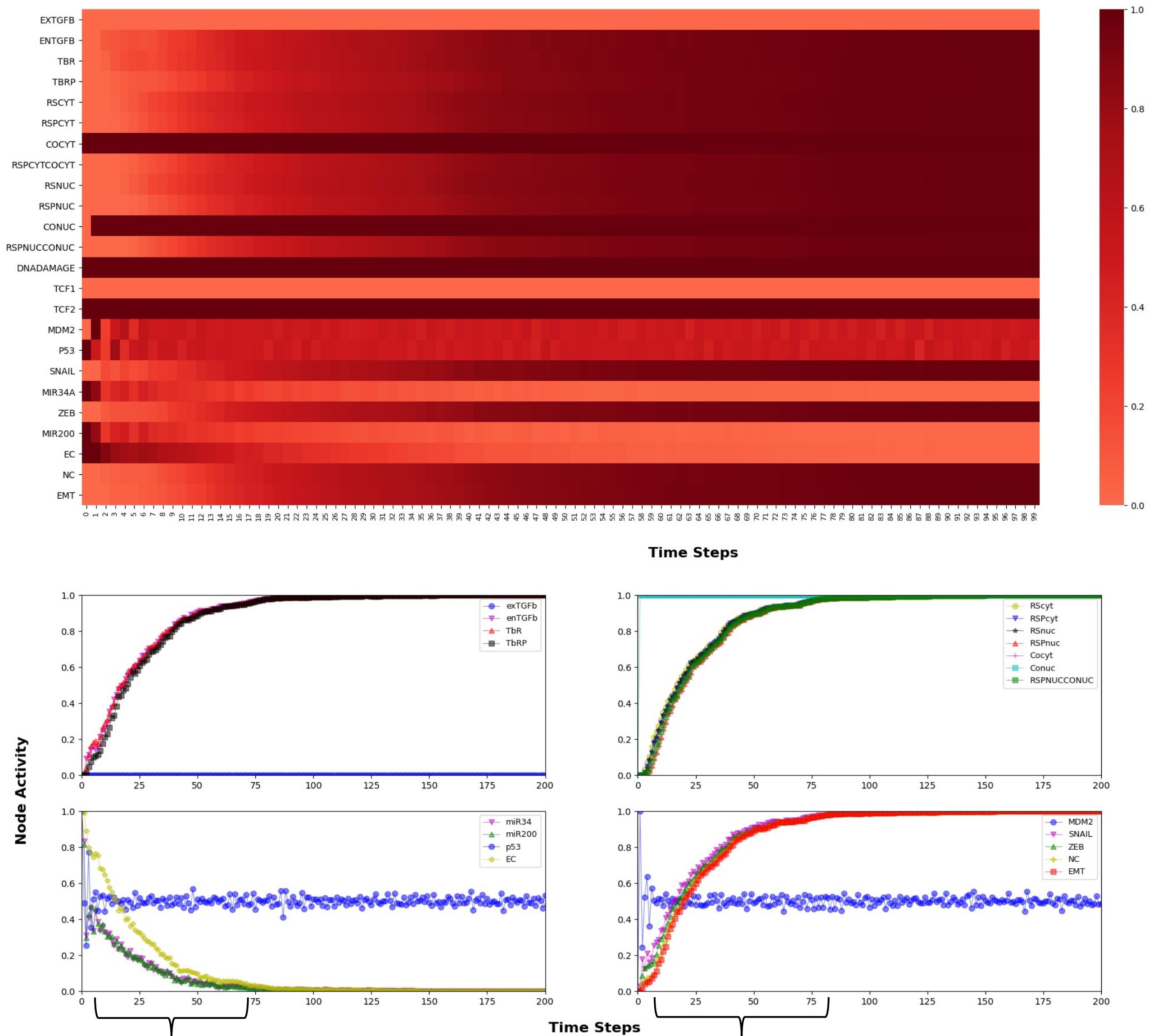
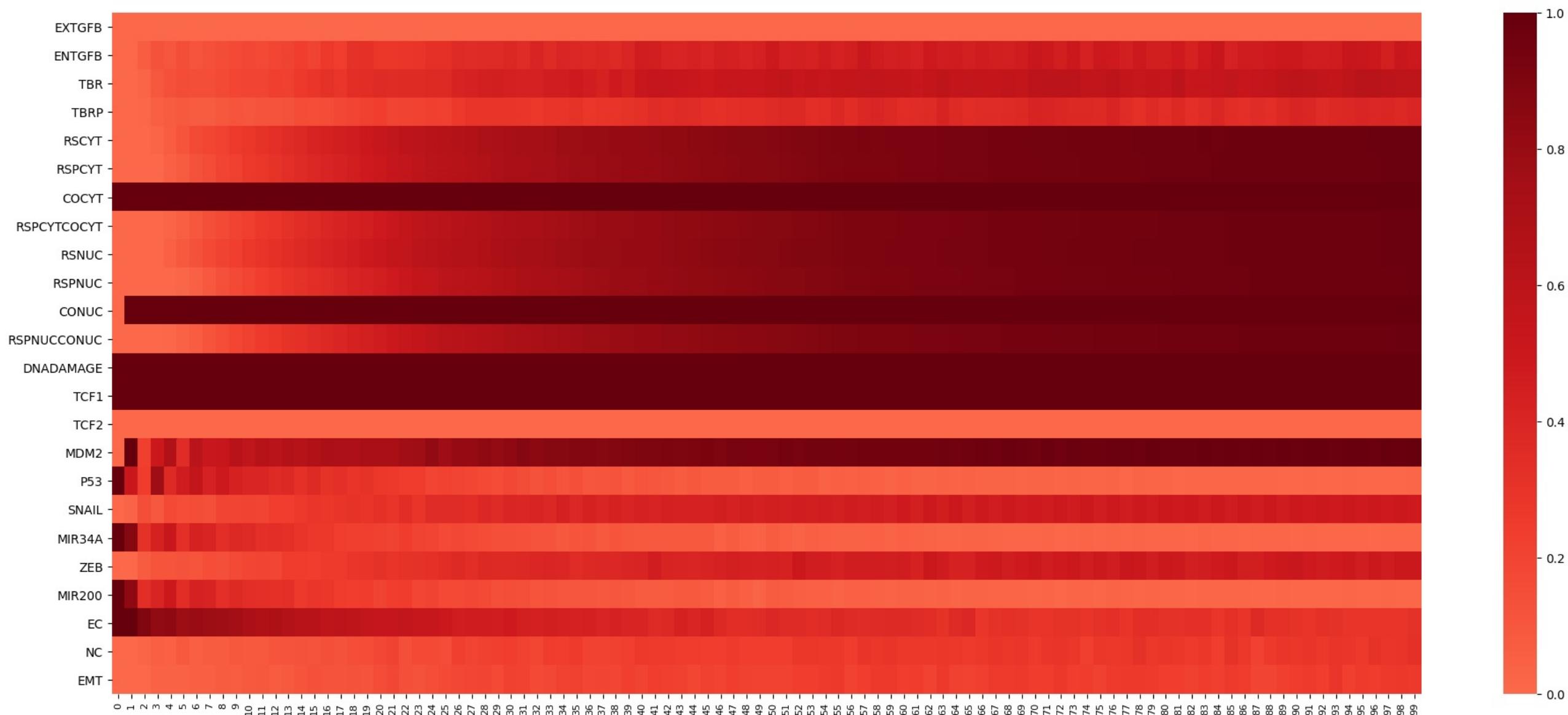
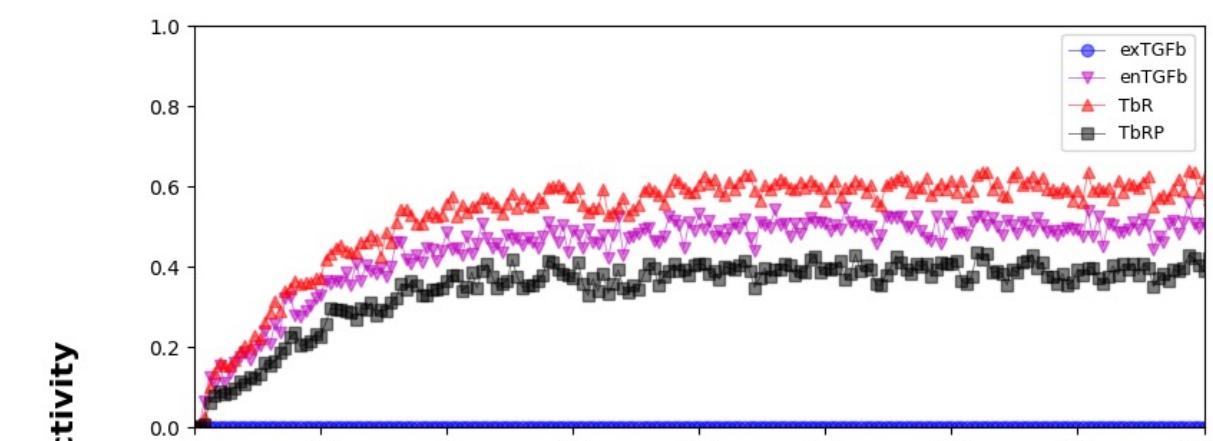


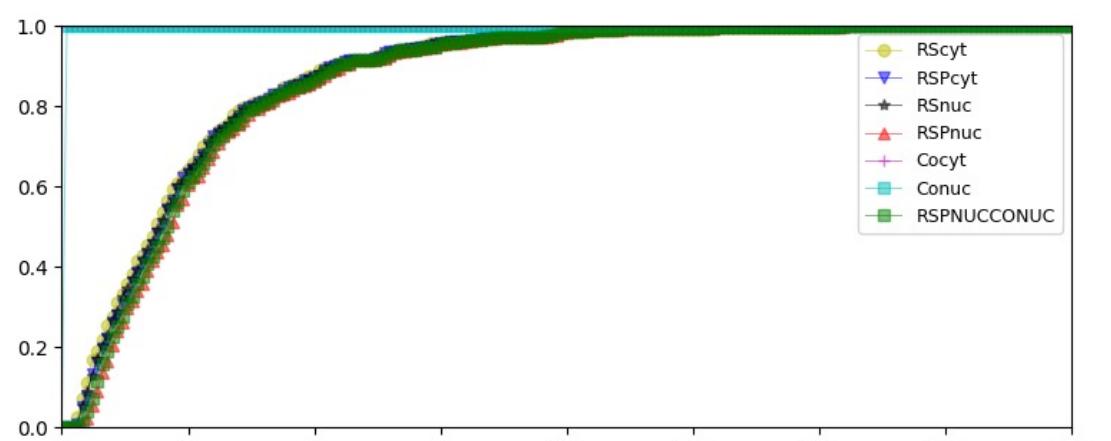
Figure S11. Simulation result of Logical modeling showing Transition from E to M phenotype when the co transcription factors for TCF1 = False and TCF2 = True; DNADamage = True; along with SNAIL Self Inhibition. Simulation was run for 5000 steps, 500 iterations in asynchronous mode and complete transition was observed with in 150 timestep. . With the increase in total time steps the mesenchymal markers (SNAIL, ZEB, N-Cadherin(NC)) are activated supressing the epithelial markers (p53, miR-34, miR-200, E-Cadherin(EC)). Prescence of SNAIL self inhibition causes the system to stay in hybrid phenotype for longer periods of time (Marked). With the absence of input to MDM2 activation, MDM2 do not reach to its maximum to inhibit p53 thus p53-MDM2 arm of network oscillates



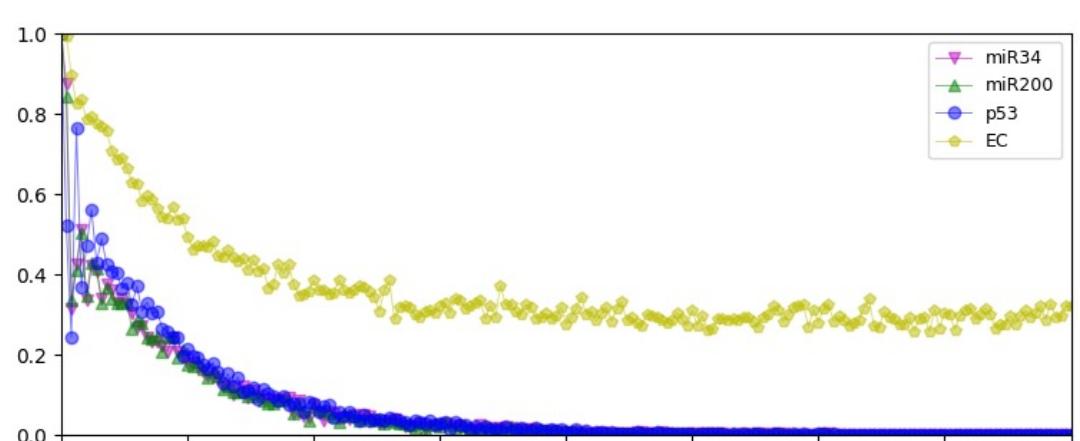
Time Steps



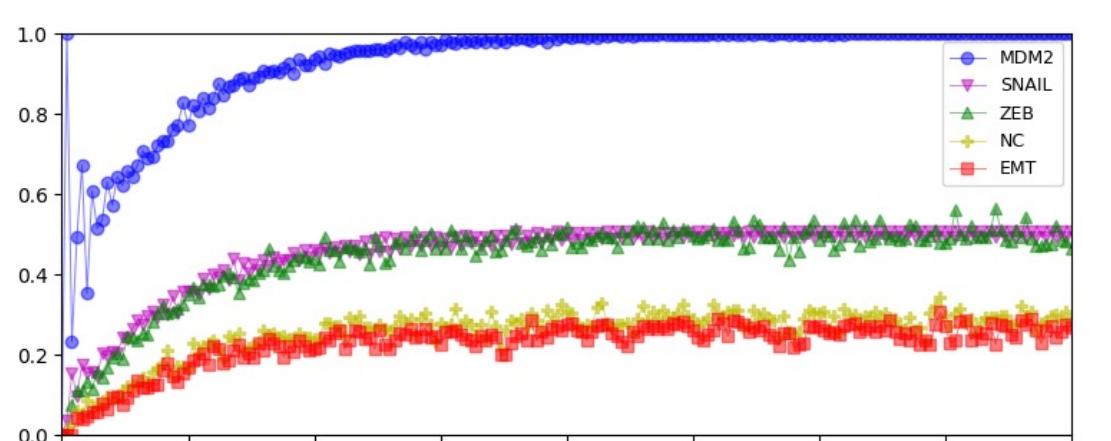
exTGFb
enTGFb
TbR
TbRP



RScyt
RSPcyt
RSnuc
RSPnuc
Cocy
Conuc
RSPNUCCONUC



miR34
miR200
p53
EC



MDM2
SNAIL
ZEB
NC
EMT

Time Steps

Figure S12. Simulation result of Logical modeling showing Transition from E to M phenotype when the co transcription factors for TCF1 = True and TCF2 = False; DNADamage = True; along with SNAIL Self Inhibition. Simulation was run for 5000 steps, 500 iterations in asynchronous mode and a hybrid phenotype was observed. This is because as SNAIL gets activated it controls its own expression through self inhibition thus limiting its own expression causing an hybrid phenotype

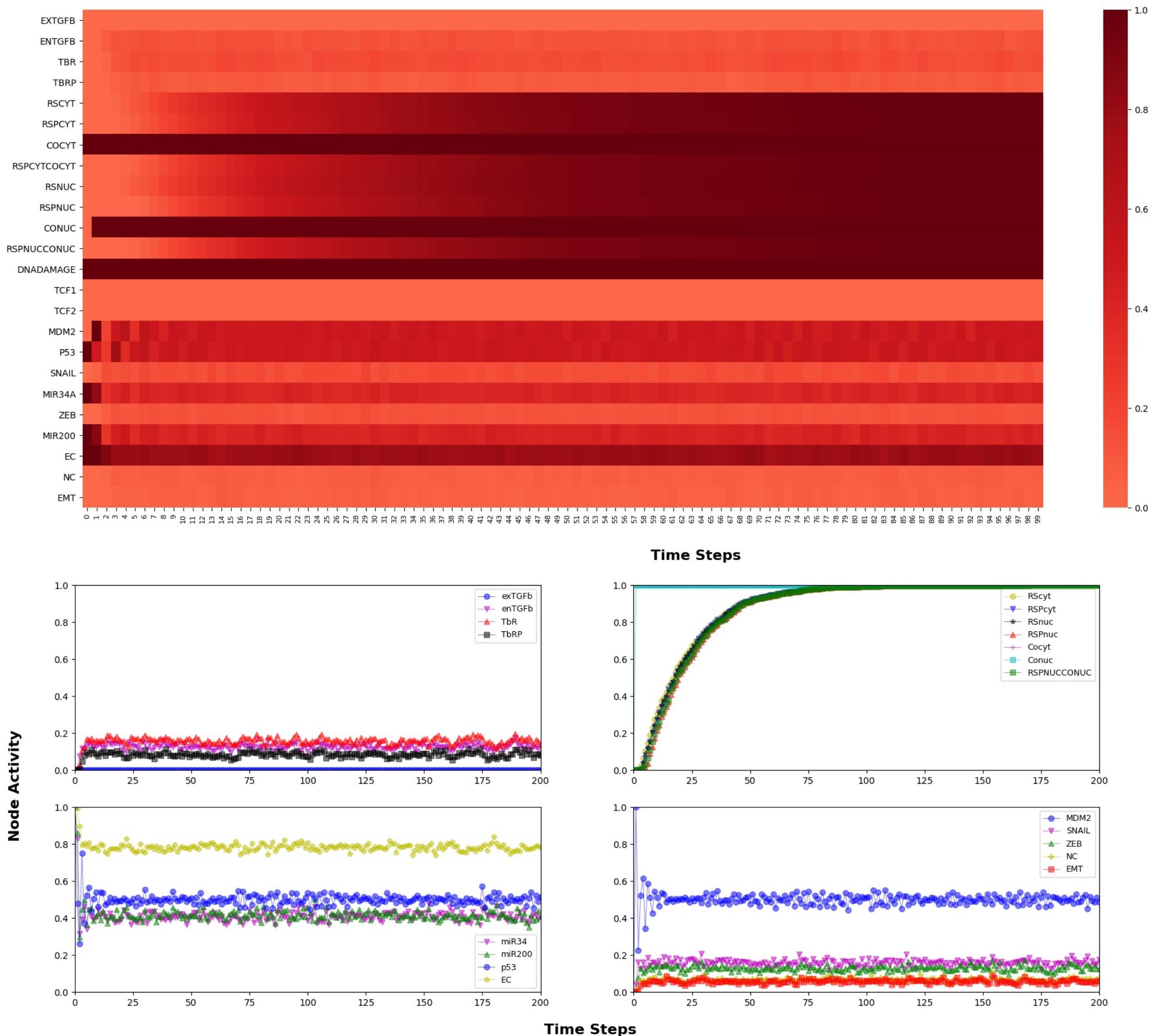


Figure S13. Simulation result of Logical modeling showing Transition from E to M phenotype when the co transcription factors for TCF1 = False and TCF2 = False; DNADamage = True; along with SNAIL Self Inhibition. Simulation was run for 5000 steps, 500 iterations in asynchronous mode and a hybrid phenotype was observed. With the absence of input to both SNAIL and MDM2, and addition of SNAIL self inhibition the nodes do not reach its maximum activity for the transition to happen

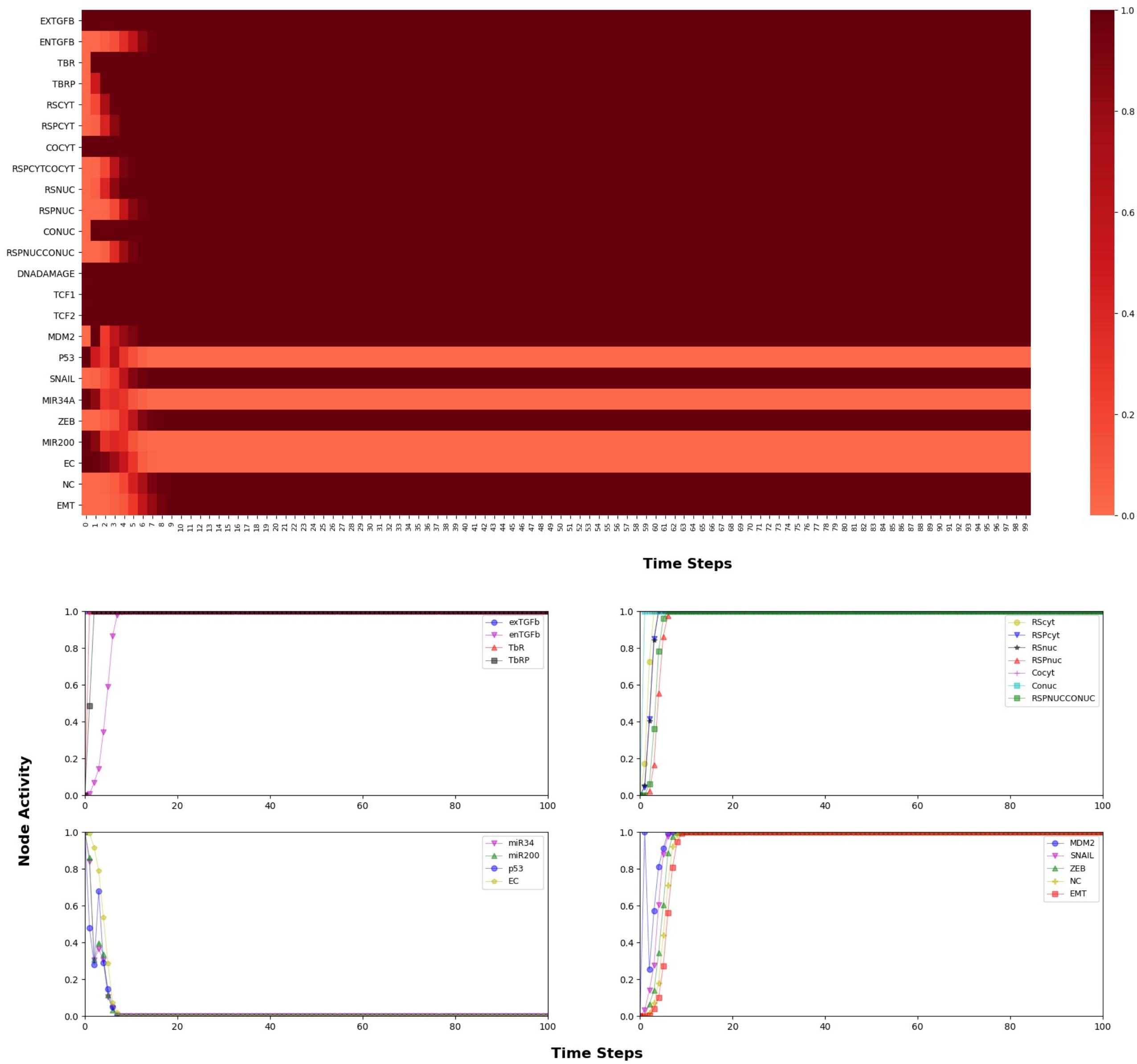
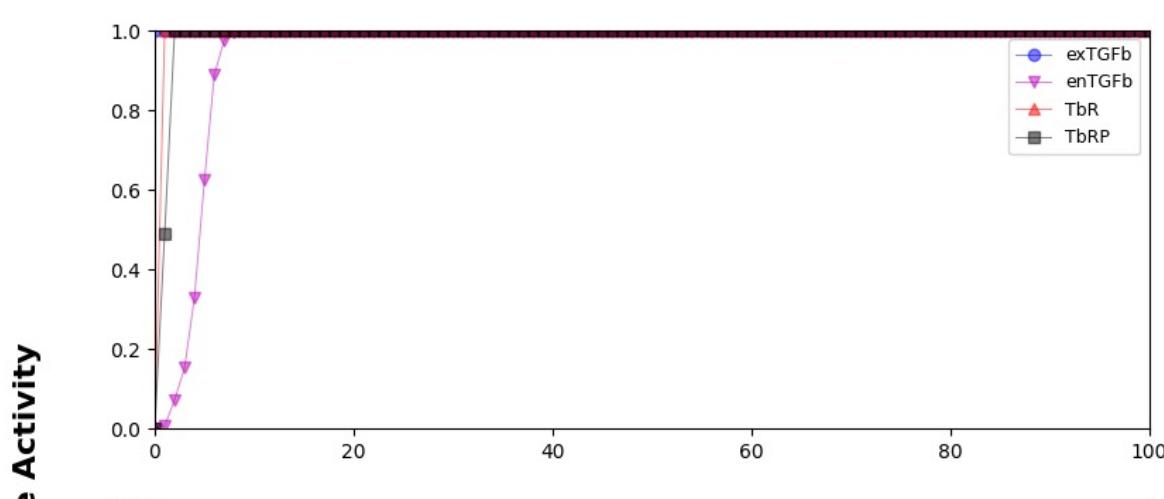


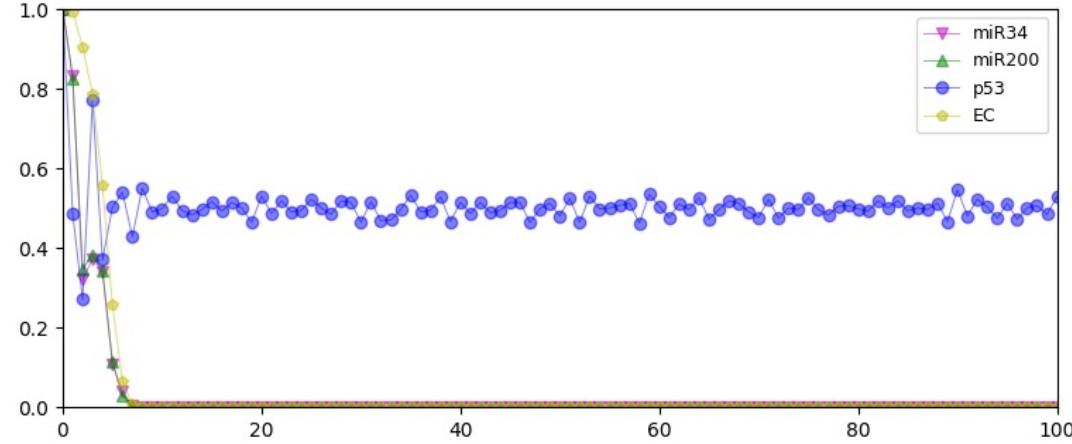
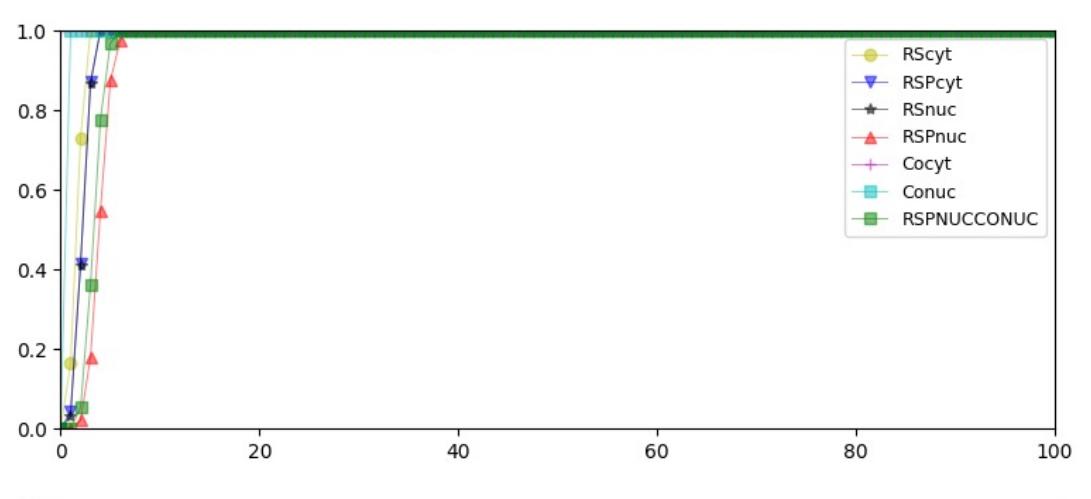
Figure S14. Simulation result of Logical modeling showing Transition from E to M phenotype when the co transcription factors for TCF1 = True and TCF2 = True; DNADamage = True; exTGFb = True; along with SNAIL Self Inhibition. Simulation was run for 5000 steps, 500 iterations in asynchronous mode and complete transition was observed to happen swiftly in 10 time steps. With the increase in total time steps the mesenchymal markers (SNAIL, ZEB, N-Cadherin(NC)) are transcriptionally activated supressing the epithelial markers (p53, miR-34, miR-200, E-Cadherin(EC)). Prescence of external stress causes the system to make a swift change from epithelial to mesenchymal phenotype with no hybrid phenotype



Time Steps



Node Activity



Time Steps

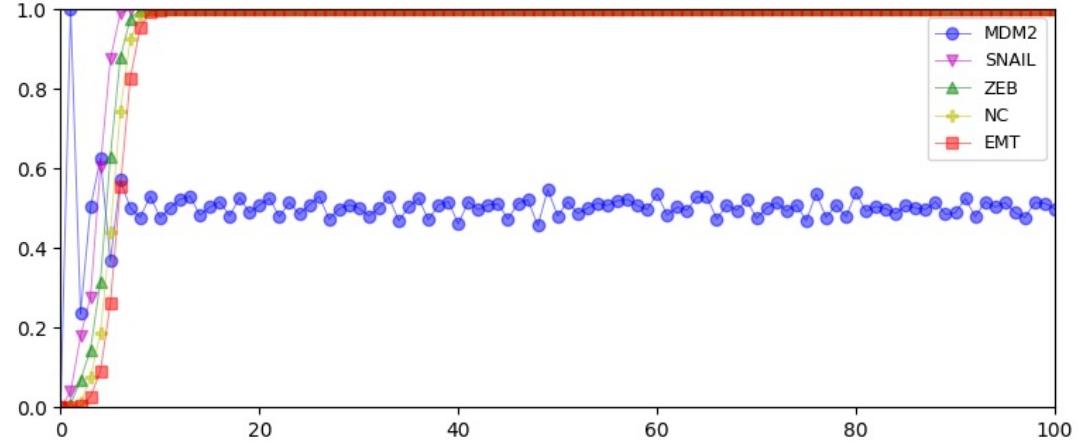


Figure S15. Simulation result of Logical modeling showing Transition from E to M phenotype when the co transcription factors for TCF1 = False and TCF2 = True; DNADamage = True; exTGFb = True; along with SNAIL Self Inhibition. Simulation was run for 5000 steps, 500 iterations in asynchronous mode and complete transition was observed to happen swiftly in 10 time steps. With the increase in total time steps the mesenchymal markers (SNAIL, ZEB, N-Cadherin(NC)) are transcriptionally activated supressing the epithelial markers (p53, miR-34, miR-200, E-Cadherin(EC)). Prescence of external stress causes the system to make a swift change from epithelial to mesenchymal phenotype with no hybrid phenotype. But lack of input to MDM2 causes the p52-MDM2 system to oscillate as MDM2 do not reach its maximum threshold.

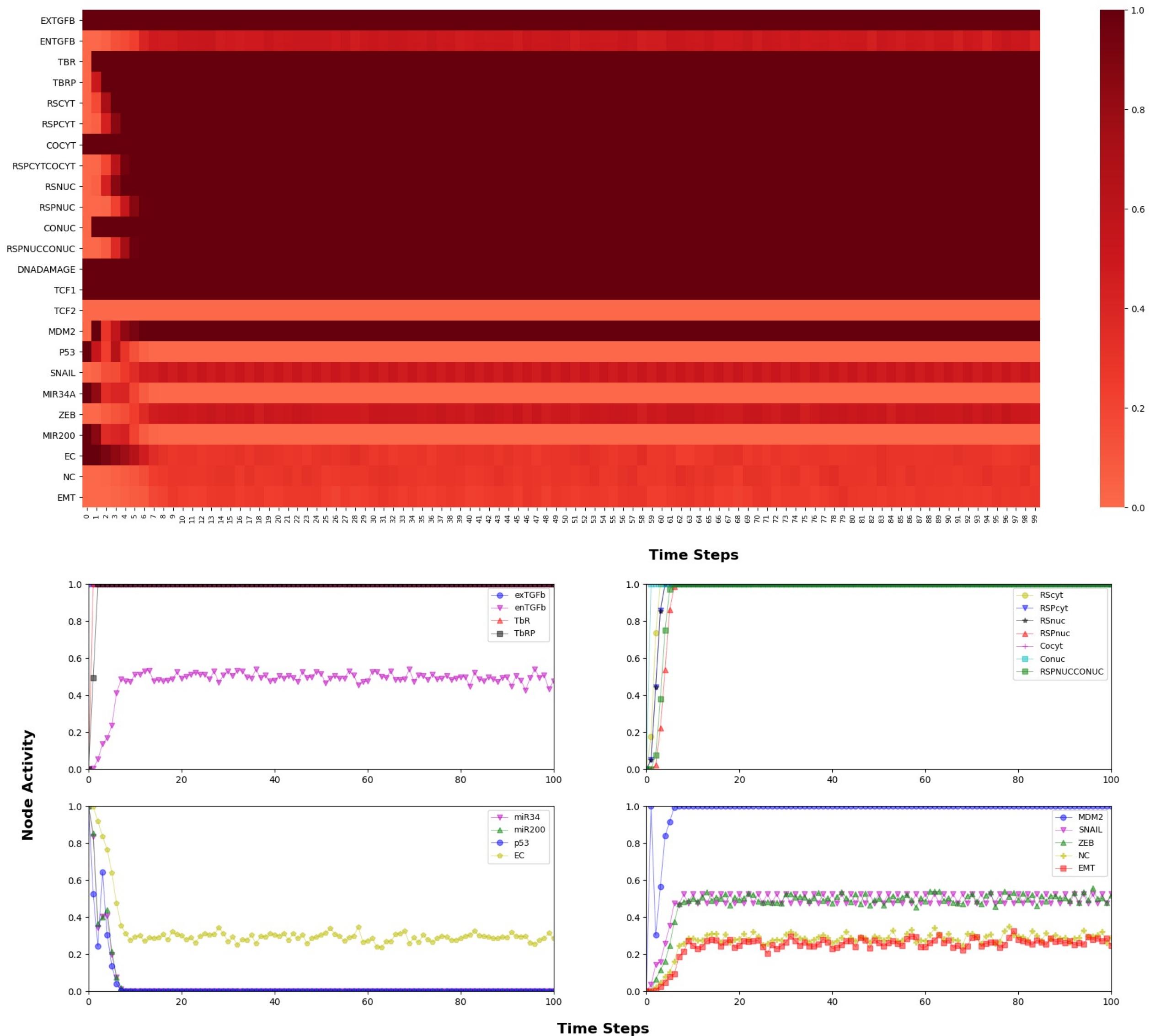


Figure S16. Simulation result of Logical modeling showing Transition from E to M phenotype when the co transcription factors for TCF1 = True and TCF2 = False; DNADamage = True; exTGFb = True; along with SNAIL Self Inhibition. Simulation was run for 5000 steps, 500 iterations in asynchronous mode and a hybrid phenotype was observed. This is because as SNAIL gets activated it controls its own expression through self inhibition thus limiting its own expression causing an hybrid phenotype

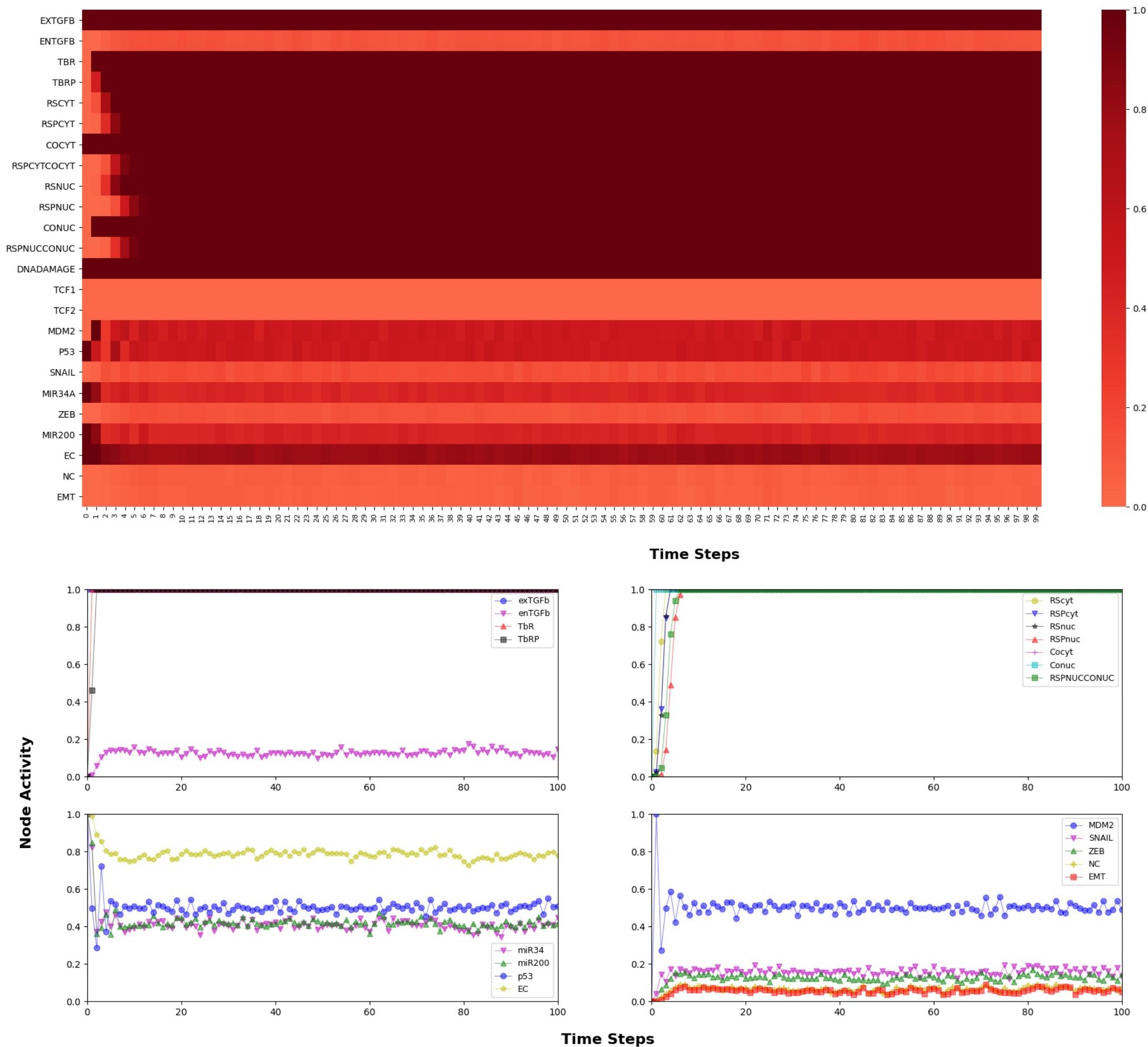
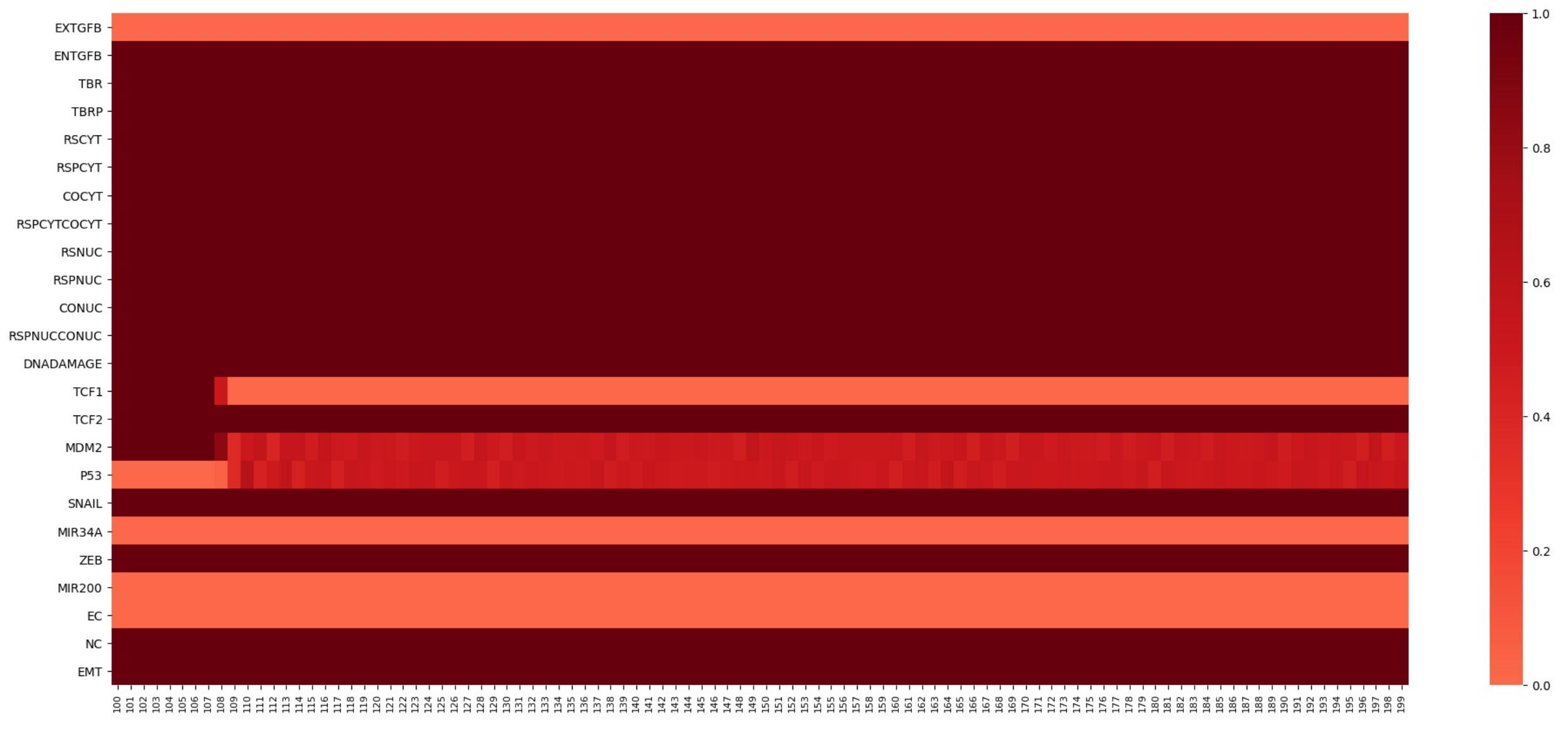


Figure S17. Simulation result of Logical modeling showing Transition from E to M phenotype when the co transcription factors for TCF1 = False and TCF2 = False; DNADamage = True; exTGFb = True; along with SNAIL Self Inhibition. Simulation was run for 5000 steps, 500 iterations in asynchronous mode and a hybrid phenotype was observed through out the process. With the absence of input to both SNAIL and MDM2, and addition of SNAIL self inhibition the nodes do not reach its maximum activity for the transition to happen



Time Steps

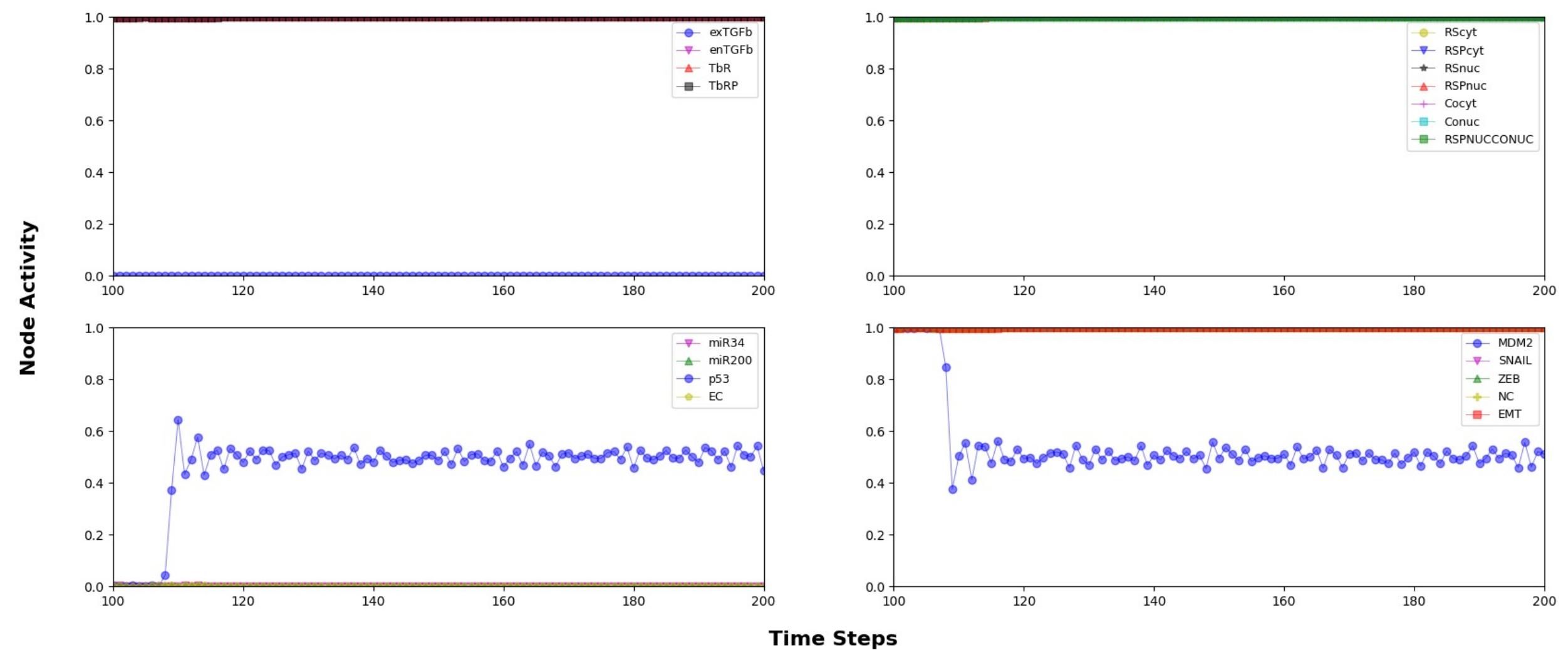


Figure S18. Simulation of logical modelling to access the knockdown of TCF1 on the reversibility of EMT. *i.e.*, TCF1 = False after 110 time steps. Post Knockdown of TCF1 it was observed that mesenchymal phenotype is maintained because of SNAIL activity (memory) due to the initial signal that led to EMT was still persistent

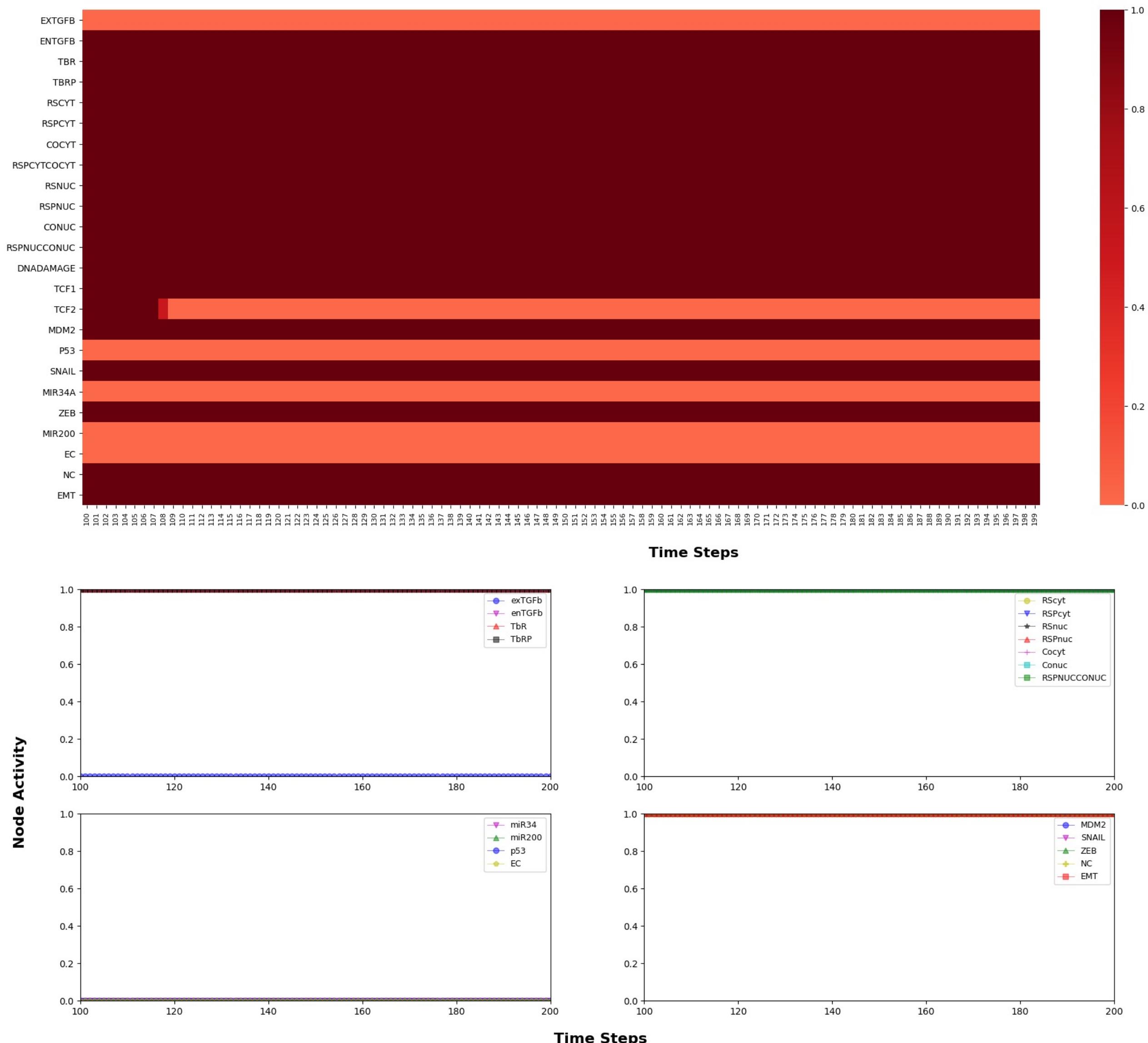


Figure S19. Simulation of logical modelling to access the knockdown of TCF2 on the reversibility of EMT. *i.e.*, TCF2 = False after 110 time steps. Post Knockdown of TCF2 it was observed that mesenchymal phenotype is maintained because of SNAIL activity (memory) due to the initial signal that led to EMT was still persistent

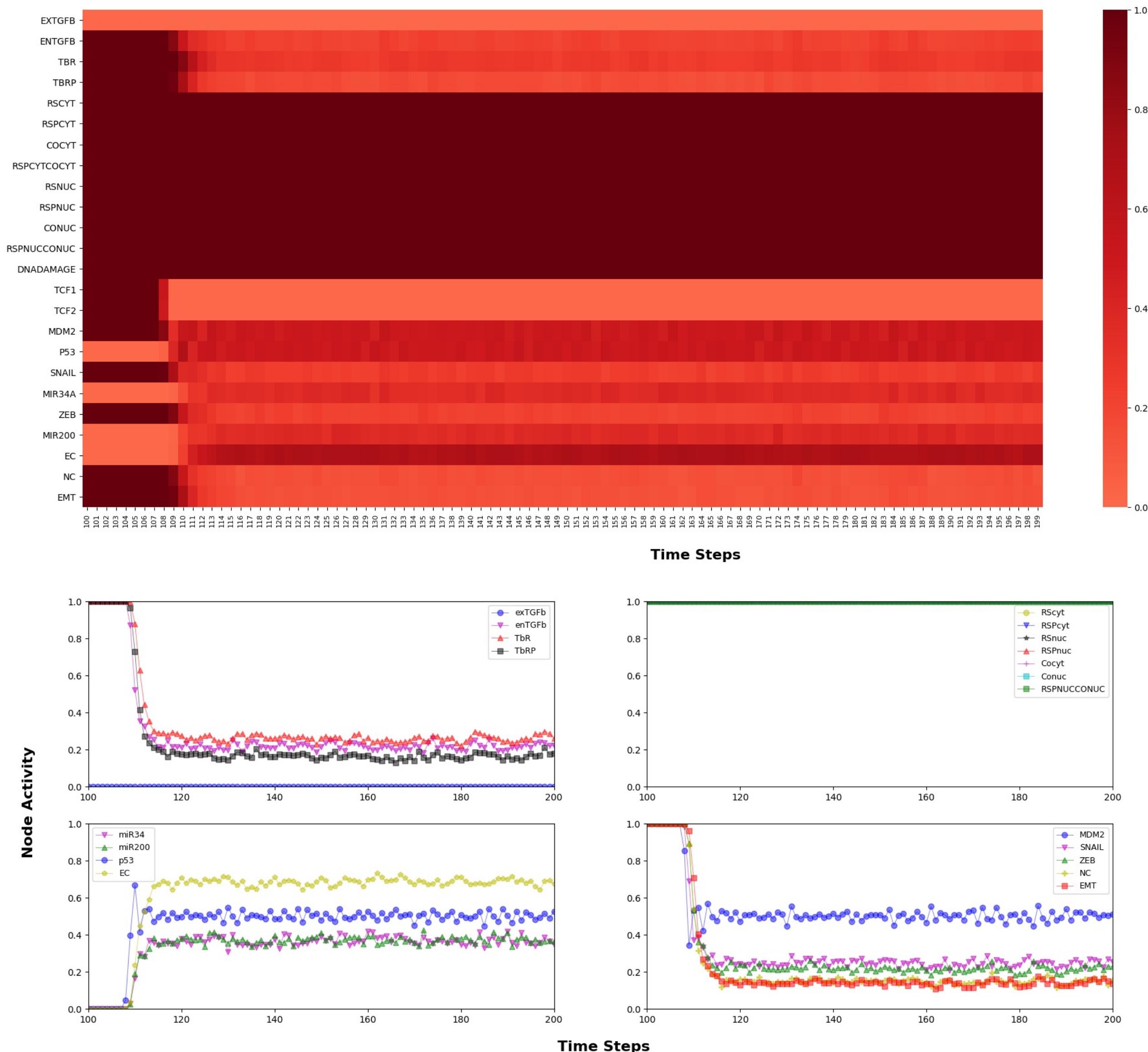


Figure S20. Simulation of logical modelling to access the knockdown of TCF1 and TCF2 on the reversibility of EMT. i.e., TCF1 = TCF2 = False after 110 time steps. knockdown of both TCF1 and TCF2 , i.e SNAIL and MDM2 transcriptional coactivators for SMAD complex has resulted in an hybrid phenotype with E-Cadherin at near maximum activity and N-Cadherin at near minimum activity. Reduced activity of SNAIL also resulted in reduced enTGF- β activation and subsequent reduced activated receptor further maintaining the hybrid phenotype

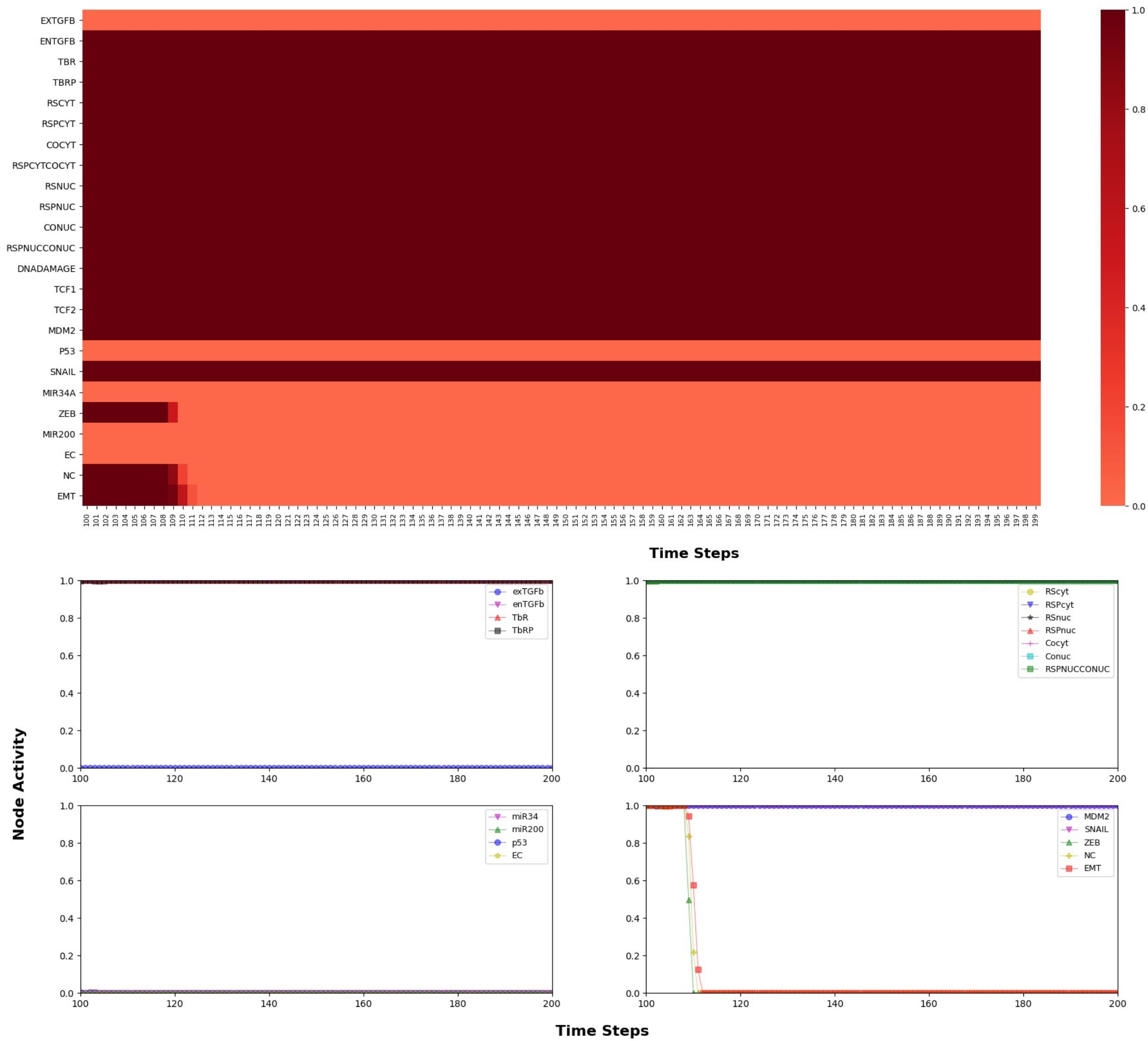


Figure S21. Simulation of logical modelling to access the knockdown of ZEB regulator on the reversibility of EMT. i.e., ZEB = False after 110 time steps. knockdown of ZEB expression inhibited the activity of N-Cadherin and EMT but the Prescence of SNAIL activity (memory) from initial simulation continues suppressing the E-Cadherin resulting in hybrid phenotype

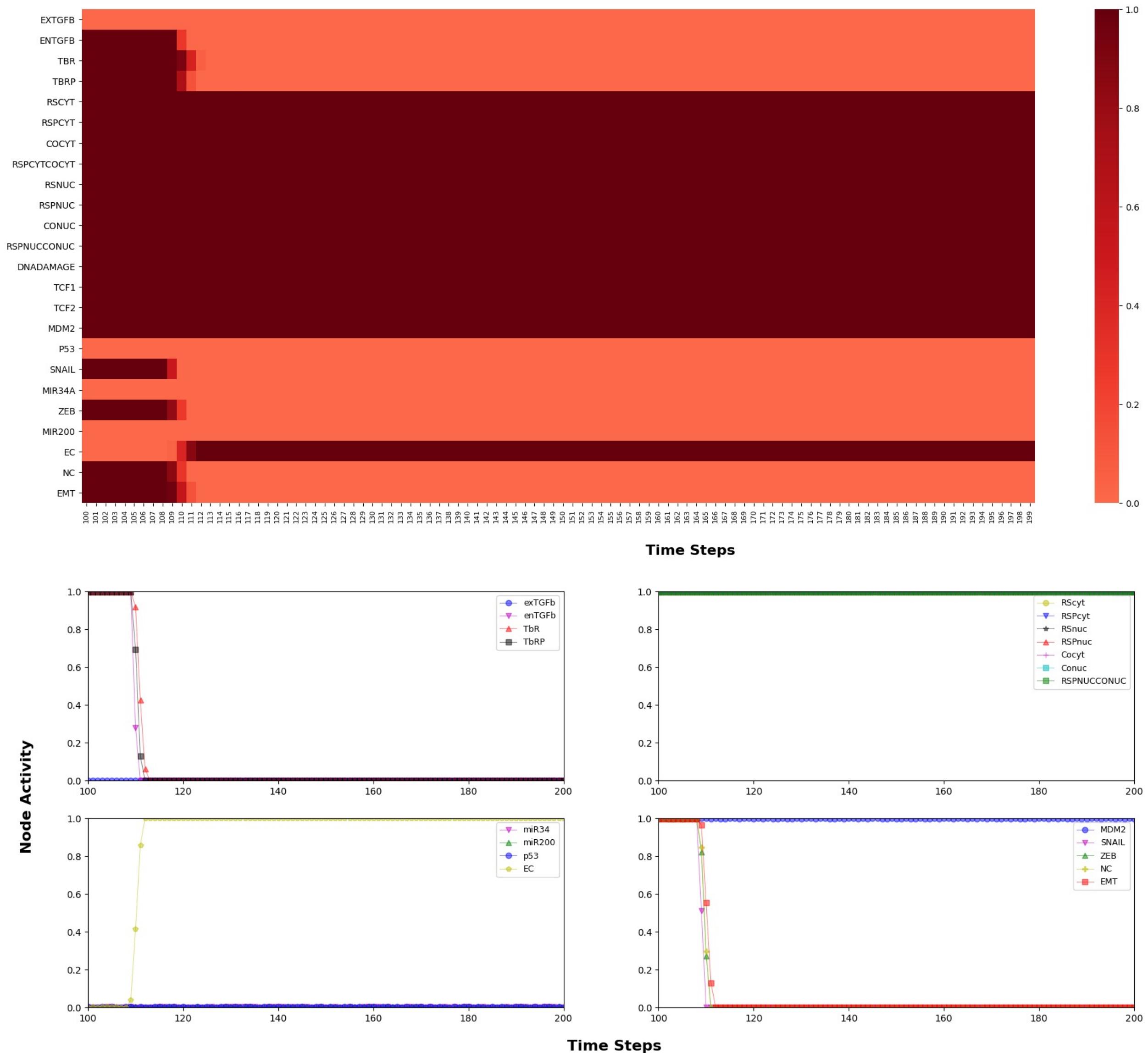


Figure S22. Simulation of logical modelling to access the knockdown of SNAIL regulator on the reversibility of EMT. i.e., SNAIL = False after 110 time steps. knockdown of SNAIL expression alone not only downregulates N-Cadherin (NC) but also upregulates E-Cadherin(EC) reversing the epithelial to mesenchymal transition

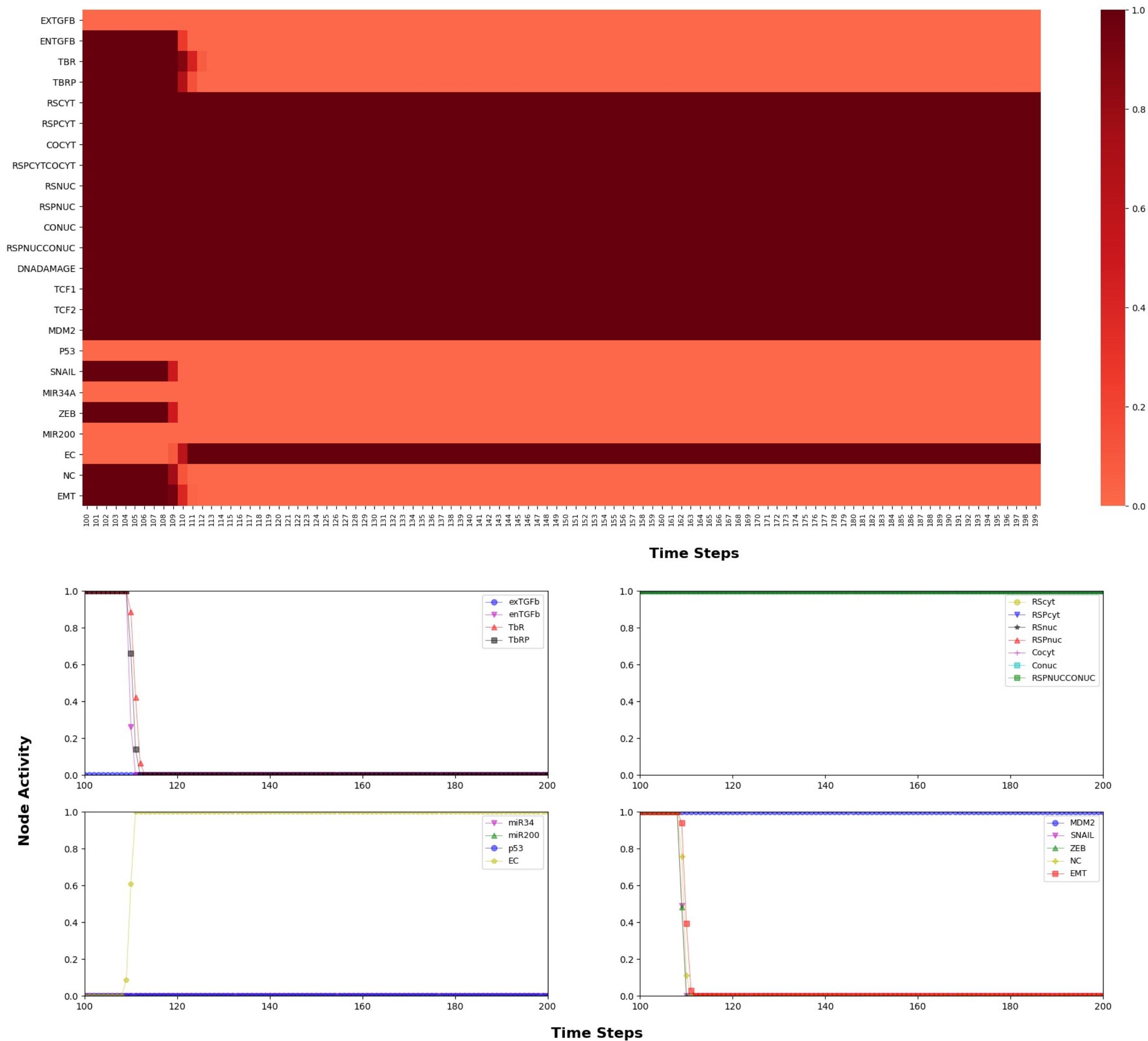


Figure S23. Simulation of logical modelling to access the knockdown of SNAIL and ZEB regulators on the reversibility of EMT. i.e., SNAIL=ZEB = False after 110 time steps. knockdown of SNAIL and ZEB expression together not only downregulates N-Cadherin (NC) but also upregulates E-Cadherin(EC) reversing the epithelial to mesenchymal transition

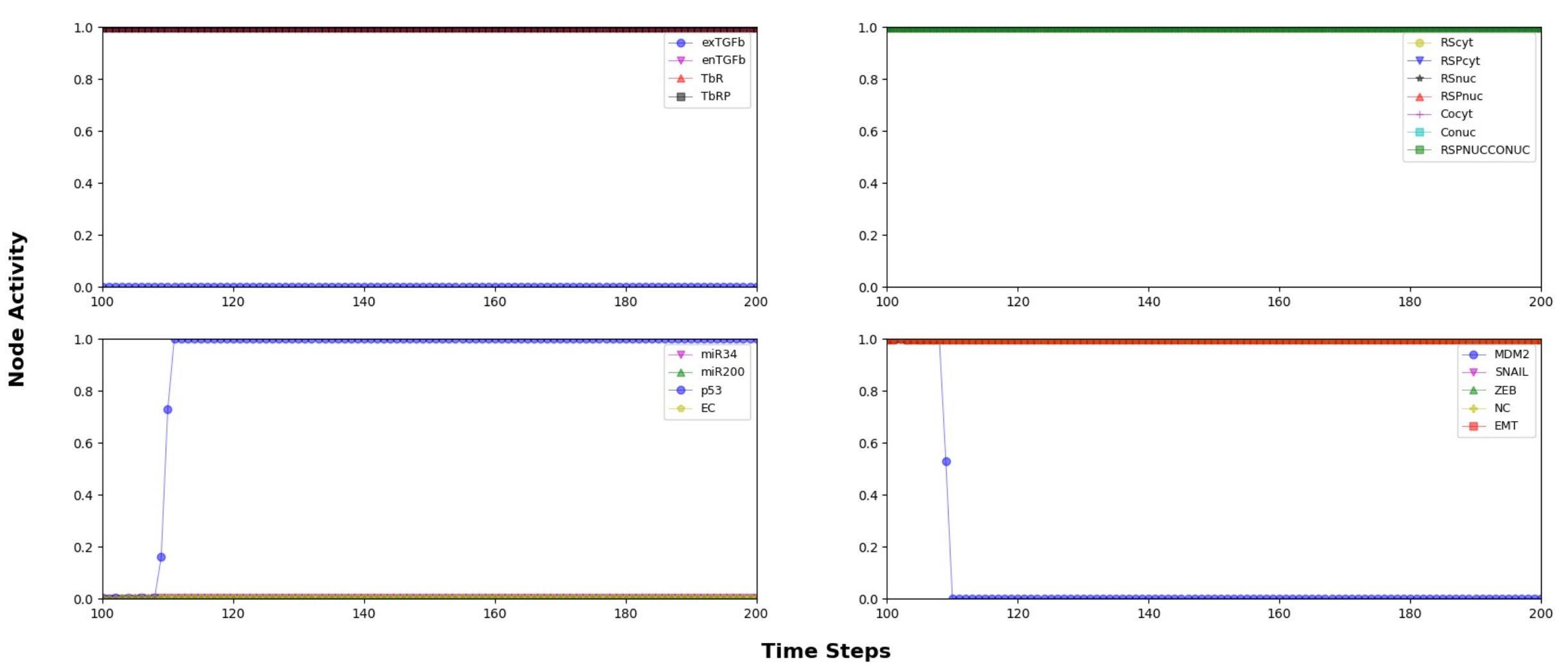
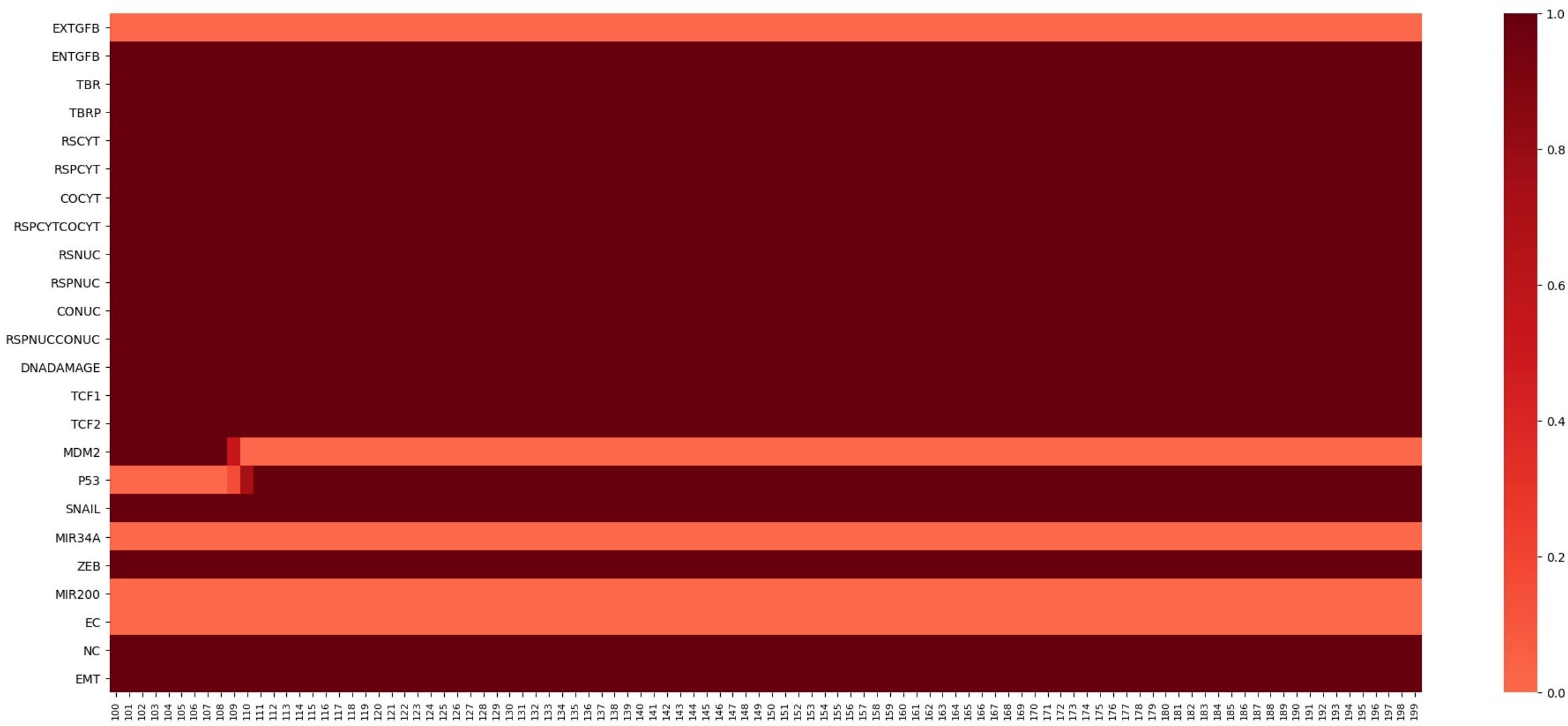


Figure S24. Simulation of logical modelling to access the knockdown of MDM2 on the reversibility of EMT. i.e., MDM2 = False after 110 time steps. knockdown of MDM2 expression upregulated the activity of p53 but did not affect the expressions of miRNAs or the mesenchymal phenotype due to the persistence activity of SNAIL (memory).

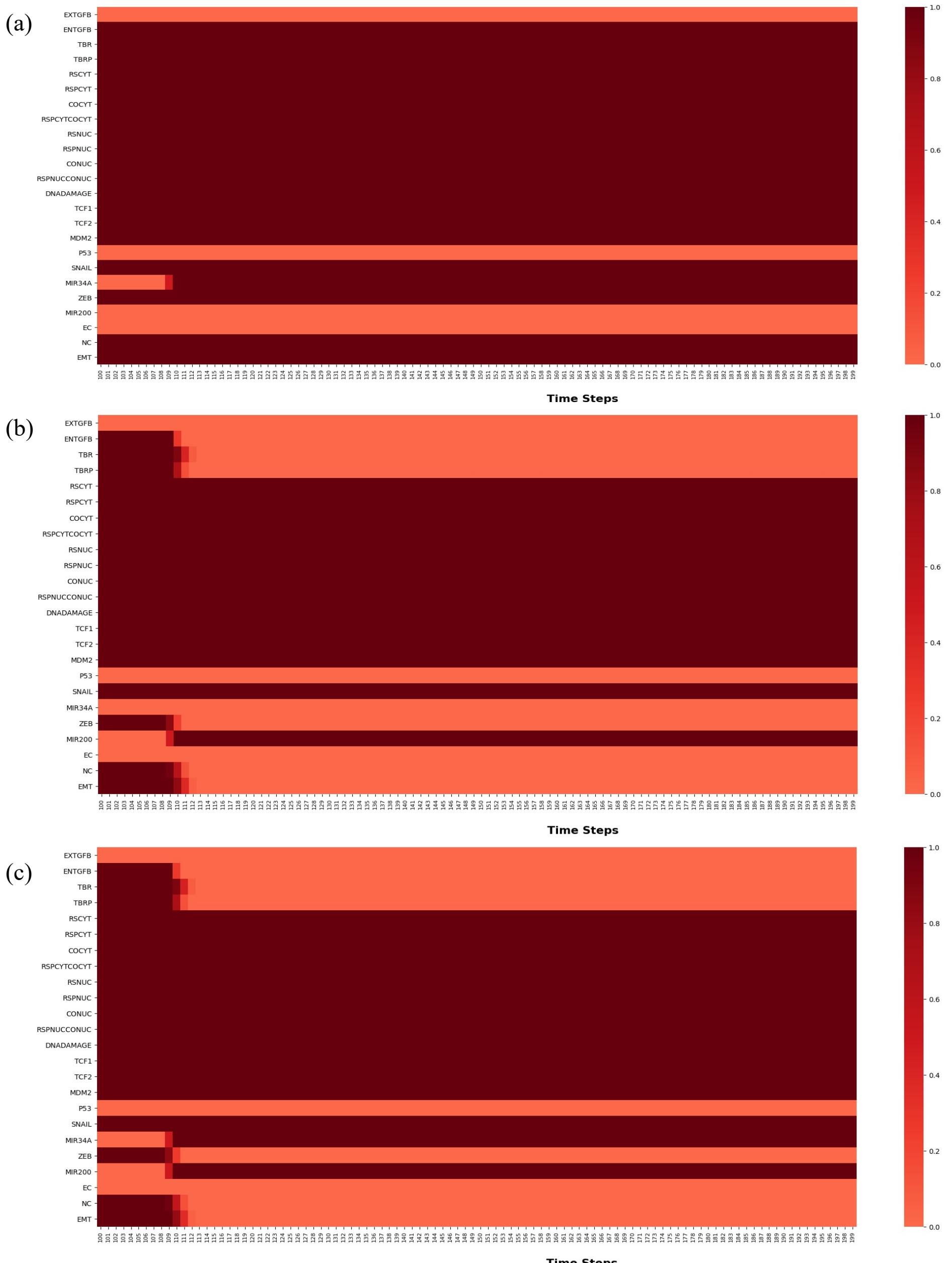


Figure S25. Simulation of logical modelling to access the upregulation of miRNAs after 110 timesteps (a) Expression of miR-34a no change in mesenchymal phenotype (b) Expression of miR-200 downregulates ZEB and further NC but results in hybrid phenotype (c) Expression of both miR-34a and miR-200 downregulates ZEB and further NC but results in hybrid phenotype . This is because the persistent activity of SNAIL (memory) from the initial simulation still persists.

Table S26. Truthtables for AND and OR logic regulation of C1FFL and their associated logical expressions

AND Logic					
TGF β (Sx)	p53 (Sy)	SMAD	MDM2	SNAIL	Delay
1	1	1	1	1	+ (ON Step)
0	1	0	1	0	-
1	0	1	1	1	+ (ON Step)
0	0	0	0	0	-

TGF β (Sx)	p53 (Sy)	SMAD	MDM2	SNAIL	Delay
1	1	1	1	1	+ (OFF Step)
0	1	0	1	1	-
1	0	1	1	1	+ (OFF Step)
0	0	0	0	0	-

SMAD* = Sx
 MDM2* = SMAD or Sy
 SNAIL* = MDM2 and SMAD

SMAD* = Sx
 MDM2* = SMAD or Sy
 SNAIL* = MDM2 or SMAD

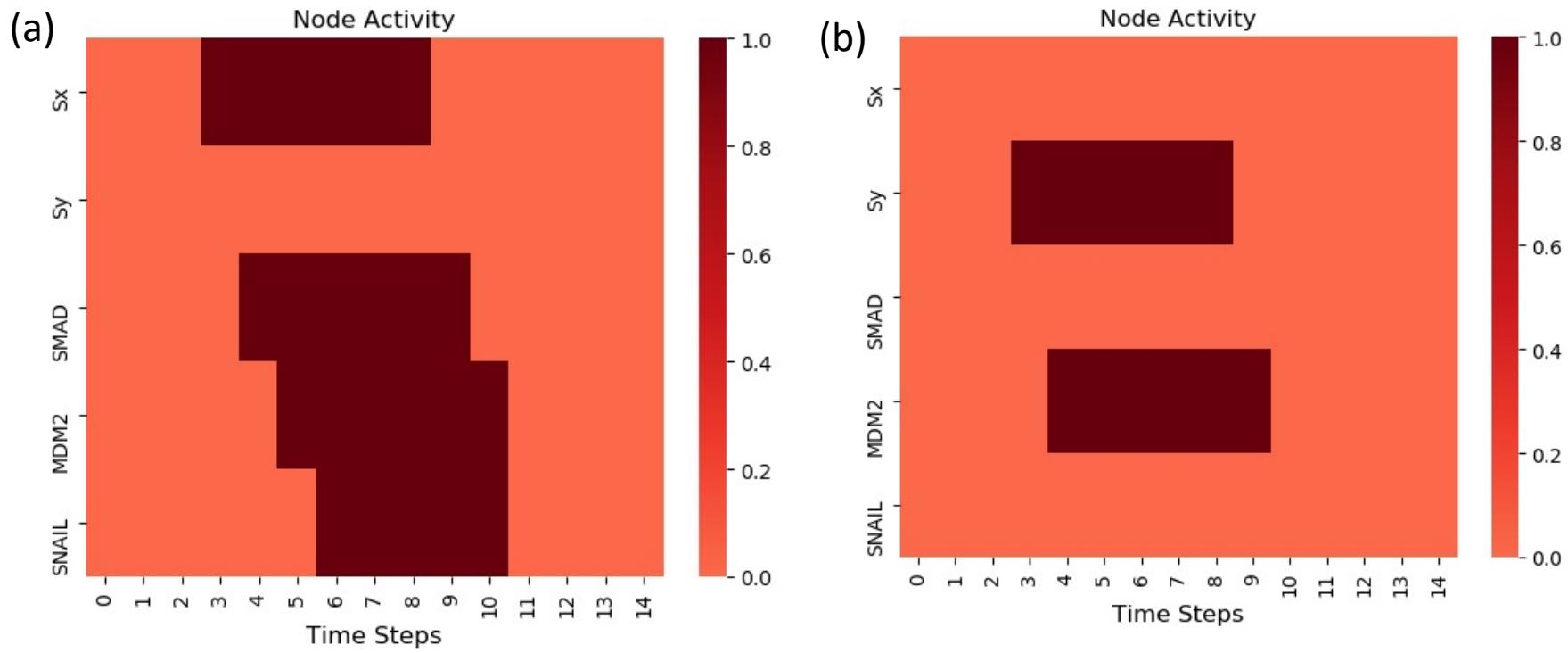


Figure S27. Dynamics of the C1FFL for the AND logic regulation analysed by logical modelling simulation (a) $S_x = \text{ON}$, $S_y = \text{OFF}$; In absence of S_y , SNAIL achieves maximum activity through SMAD that activates MDM2 fulfilling the AND logic regulation (b) $S_x = \text{OFF}$, $S_y = \text{ON}$; the absence of S_x do not alleviate the SNAIL expression as SNAIL activity requires both MDM2 and SMAD signal.

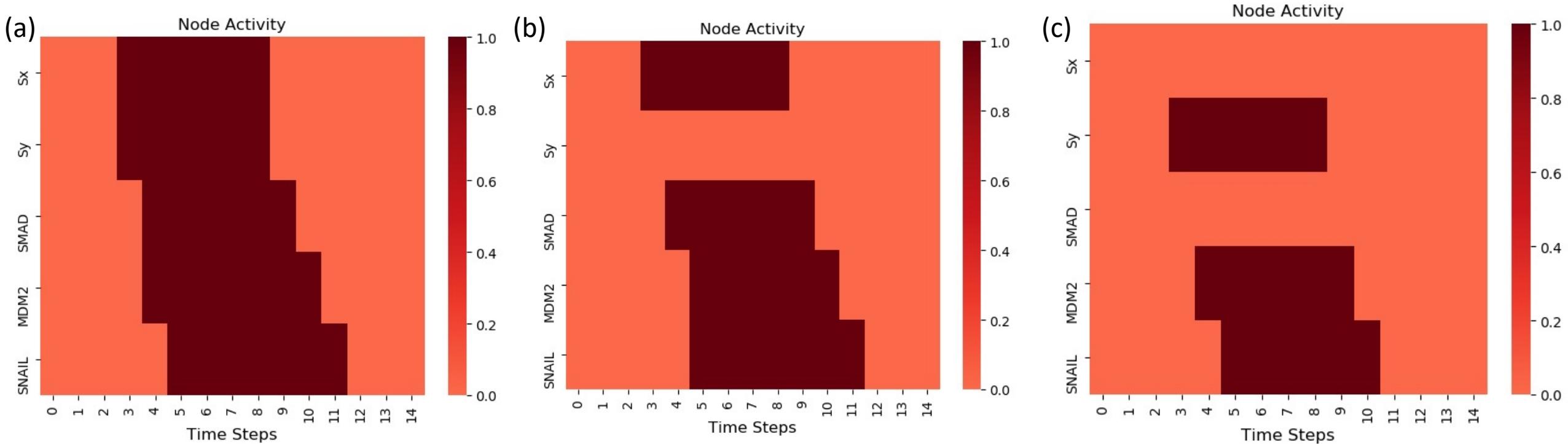


Figure S28. Dynamics of the C1FFL for the OR logic regulation analysed by logical modelling simulation with stimuli (a) both $S_x = S_y = \text{ON}$ (b) $S_x = \text{ON}$, $S_y = \text{OFF}$ (c) $S_x = \text{OFF}$, $S_y = \text{ON}$. SNAIL reaches its maximum activity in all the three cases following a delay in the OFF step.

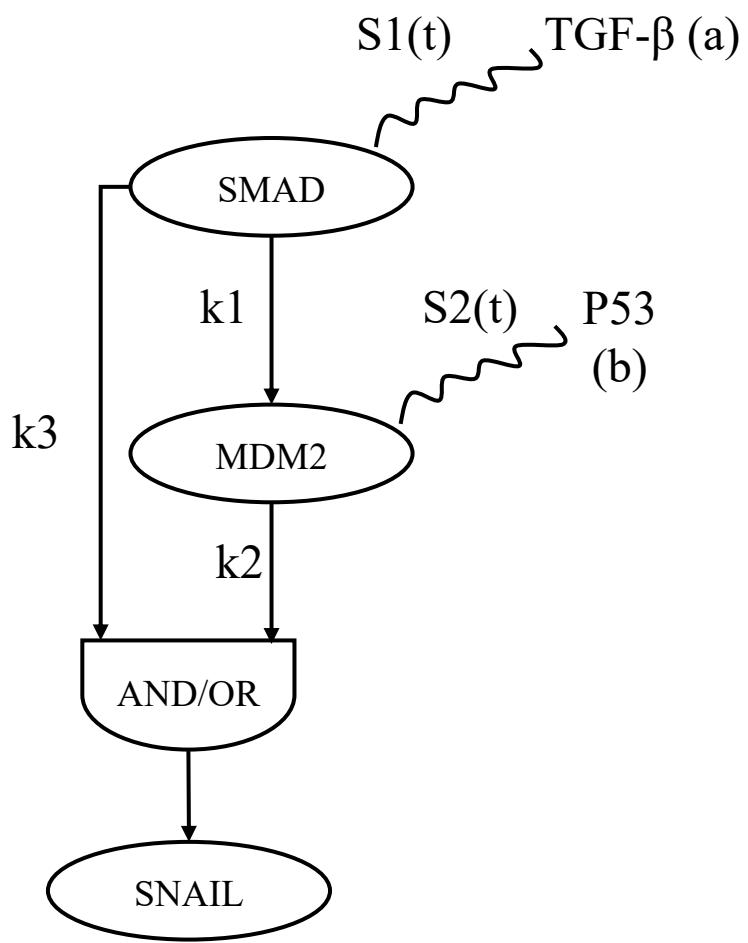


Figure S29. C1FFL with rate constants

Table S29. Parameters for the Dynamic Modelling

Description	AND Logic	OR Logic
TGF-β Dependent Activation Of SMAD Complex (a)	0.02	0.05
P53 Dependent Activation Of MDM2 (b)	0.02	0.05
Production Rate Of SMAD Complex Dependent MDM2 (k1)	1	0.5
Michaelis Menten Constant For SMAD Complex Dependent MDM2 Production (Km1)	0.1	0.1
Production Rate Of MDM2 Dependent SNAIL (k2)	1	0.7
Michaelis Menten Constant For MDM2 Dependent SNAIL Production (Km2)	0.5	1
Production Rate Of SMAD Complex Dependent SNAIL (k3)	0.9	1
Michaelis Menten Constant For SMAD Complex Dependent SNAIL Production (Km3)	0.1	0.1
Degradation Rate Of SMAD Complex (kd1)	0.3	0.15
Degradation Rate Of MDM2 (kd2)	1	1
Degradation Rate Of SNAIL (kd3)	1	1
Hill Coefficient (n)	2	2

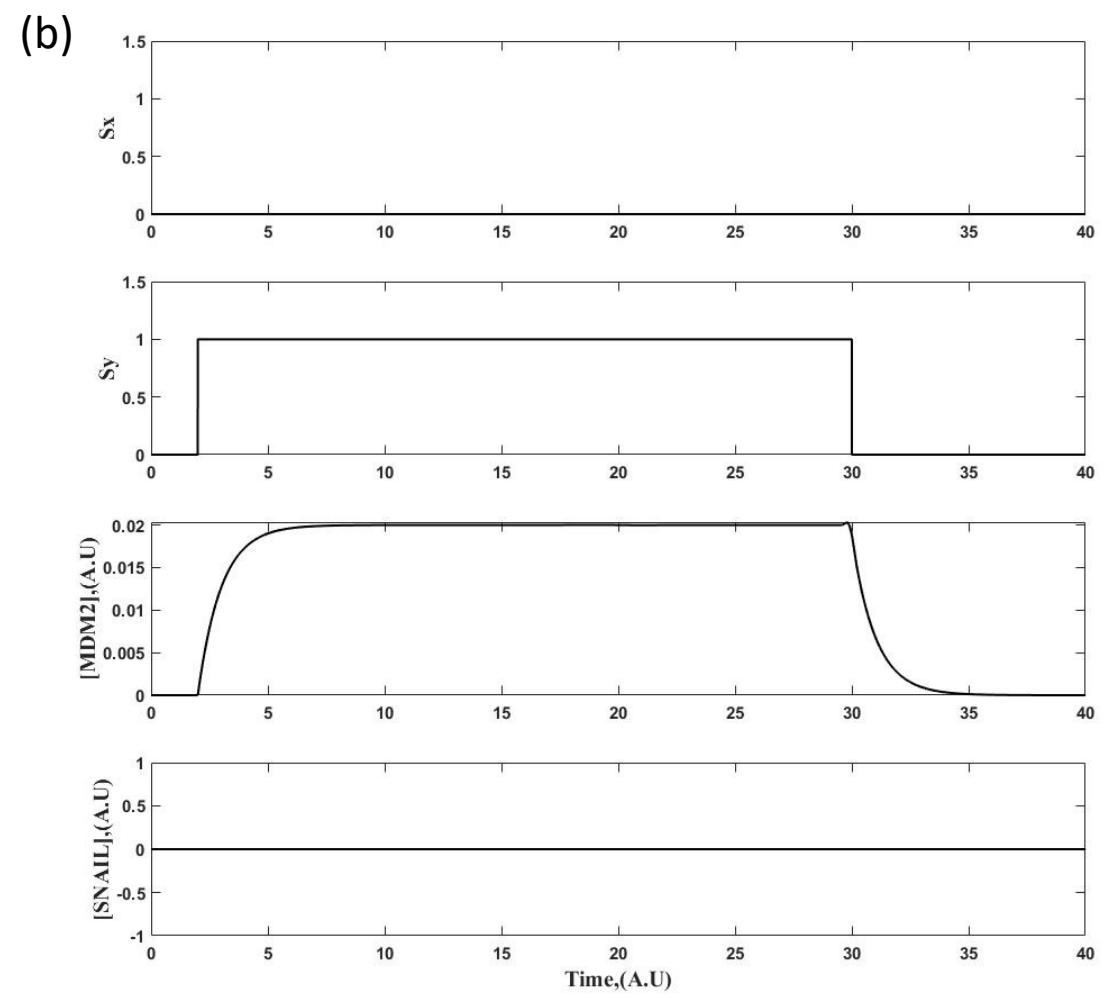
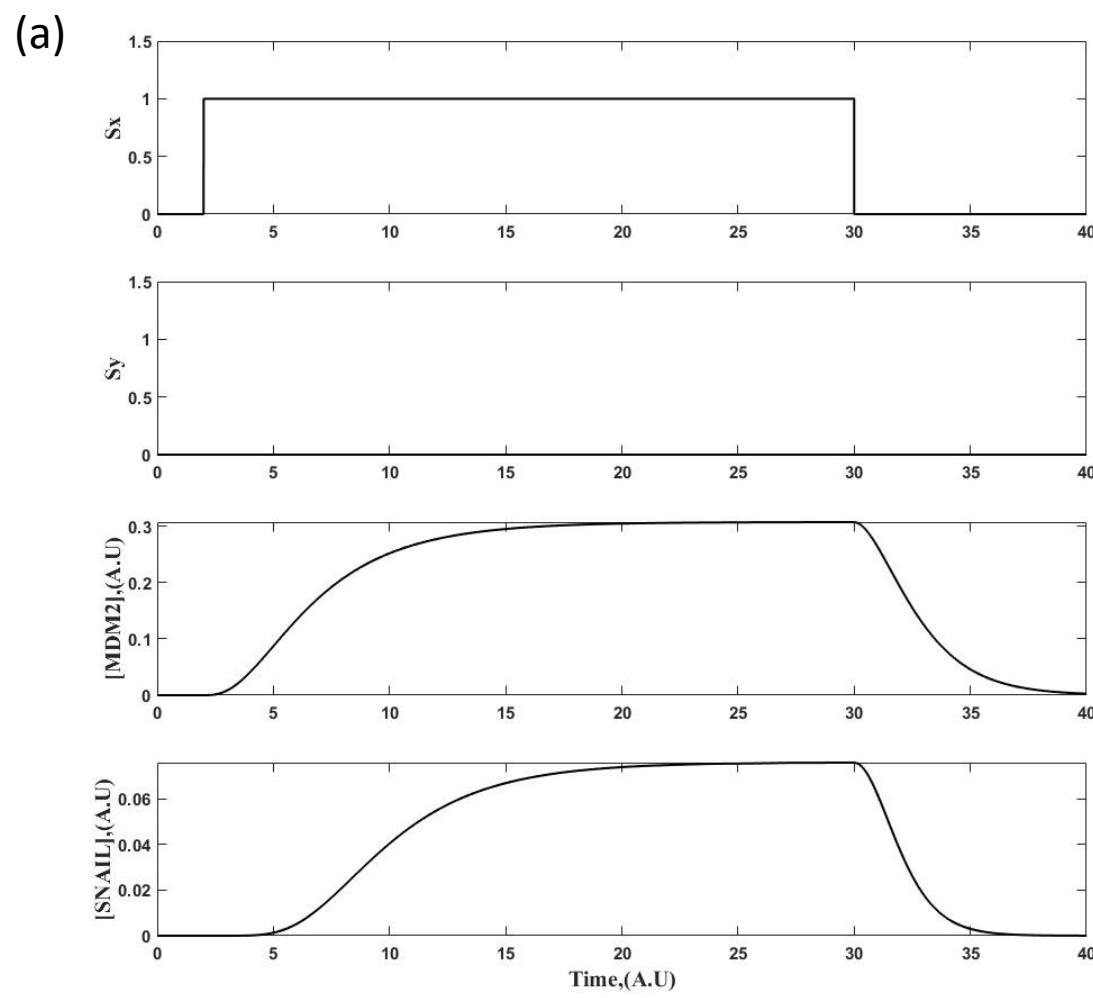


Figure S30. Dynamics of Type 1 CFFL with an AND logic regulation using ODE Modelling (a) $S_x = 1$, $S_y = 0$; (b) $S_x = 0$, $S_y = 1$, Absence in any of the inputs fails to activate the SNAIL expression (to its maximum) for further downstream regulation

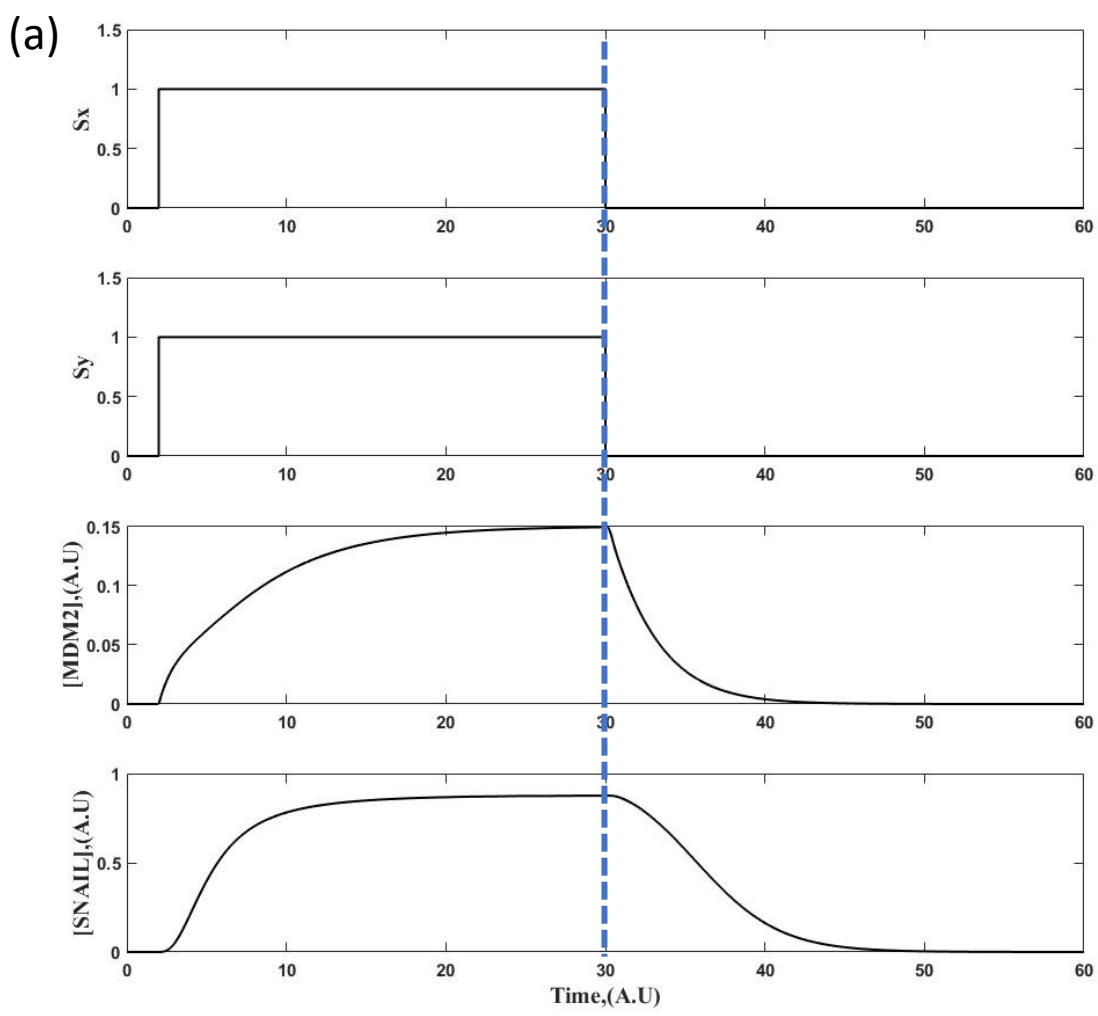


Figure S31. Dynamics of coherent type 1 FFL with respect to OR regulatory logic with the both inputs ON and OFF using ODE modelling (a) $S_x = S_y = 1$; Delay following OFF step was observed (b) & (c) $S_x = 1, S_y = 0$; $S_x = 0, S_y = 1$. Presence of any of both inputs can activate SNAIL expression

