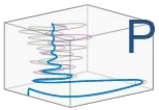
viral infection ↔ innate immunity at the single-cell level

Marek Kochańczyk, Frederic Grabowski, Zbigniew Kowek,
Maciej Czerkies, Wiktor Prus, & Tomasz Lipniacki



Laboratory of Modeling
in Biology and Medicine
(**Lipniacki lab**)

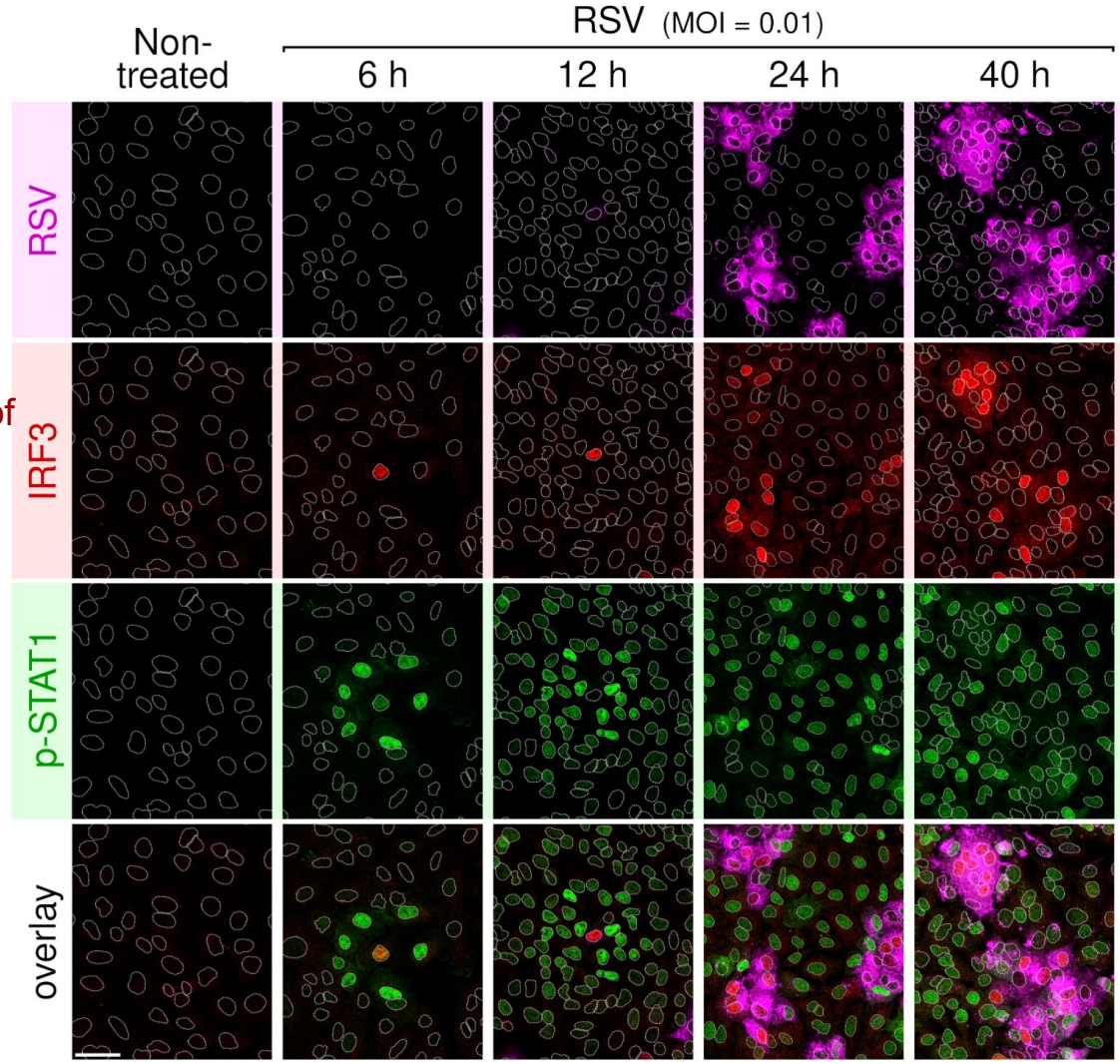
Institute of Fundamental
Technological Research
of the…

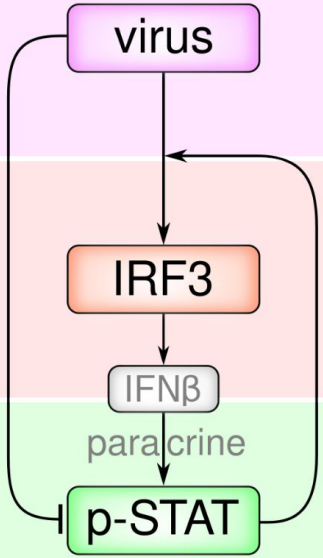
…Polish Academy of Sciences

Recognition of viral RNA in some A549 cells

→ signaling cascade culminating in activation of transcription factors IRF3 and NF-κB that jointly trigger synthesis of type I (IFNβ) and type III (IFNλ) interferons.

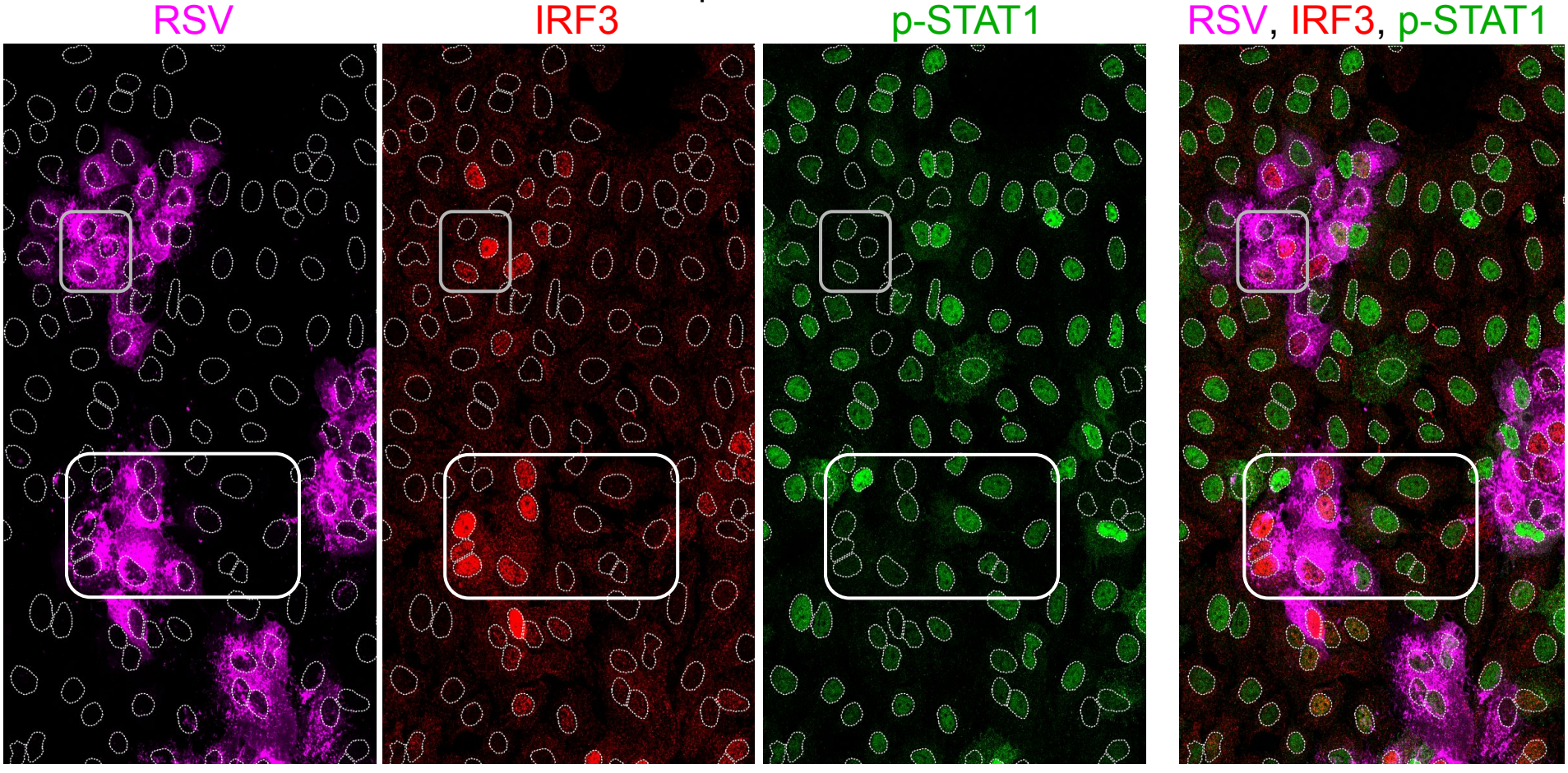
Secreted IFNs prompt non-infected bystander cells to prepare for infection before they encounter a virus.





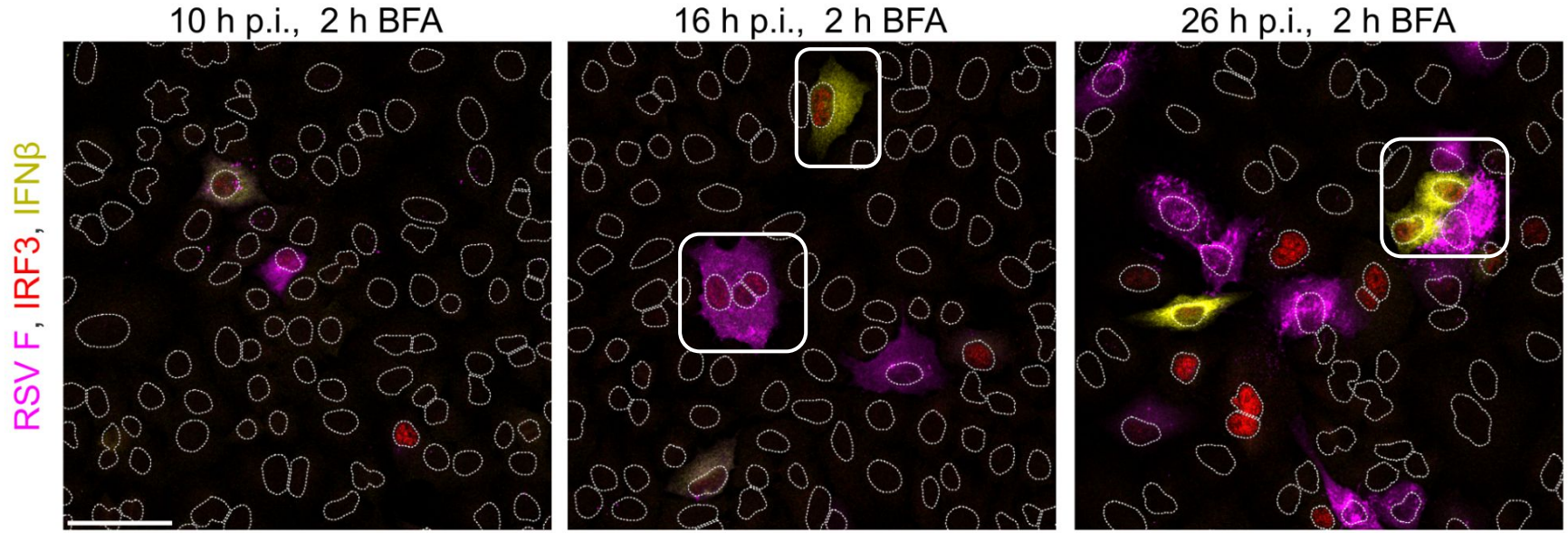
Infection with RSV

24 h p.i. at MOI 0.01



Infection with RSV

MOI 0.01



Motivation

population level	single-cell level
• IRF3 is activated by virus infection,	**but** IRF3 in not active in all infected cells.
• STAT1 is activated by viral infection,	**but** p-STAT1 is present preferentially in cells that are not infected.
• IFNβ produced upon viral infection,	**but** IFNβ does not accumulate in cells that express viral proteins (mutual exclusion).

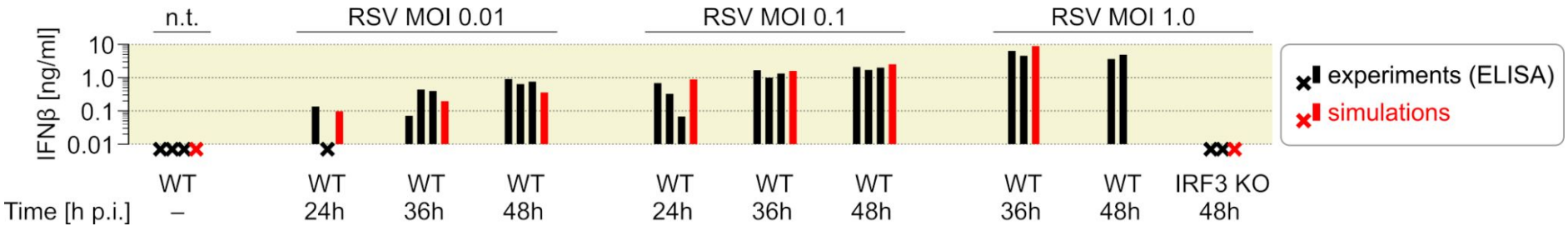
OBJECTIVE: Investigate the **mutual exclusion** between virus proliferation and innate immune signaling within a mechanistic **computational model**.
(The model needs to be stochastic and spatial.)

METHOD: We performed experiments using:

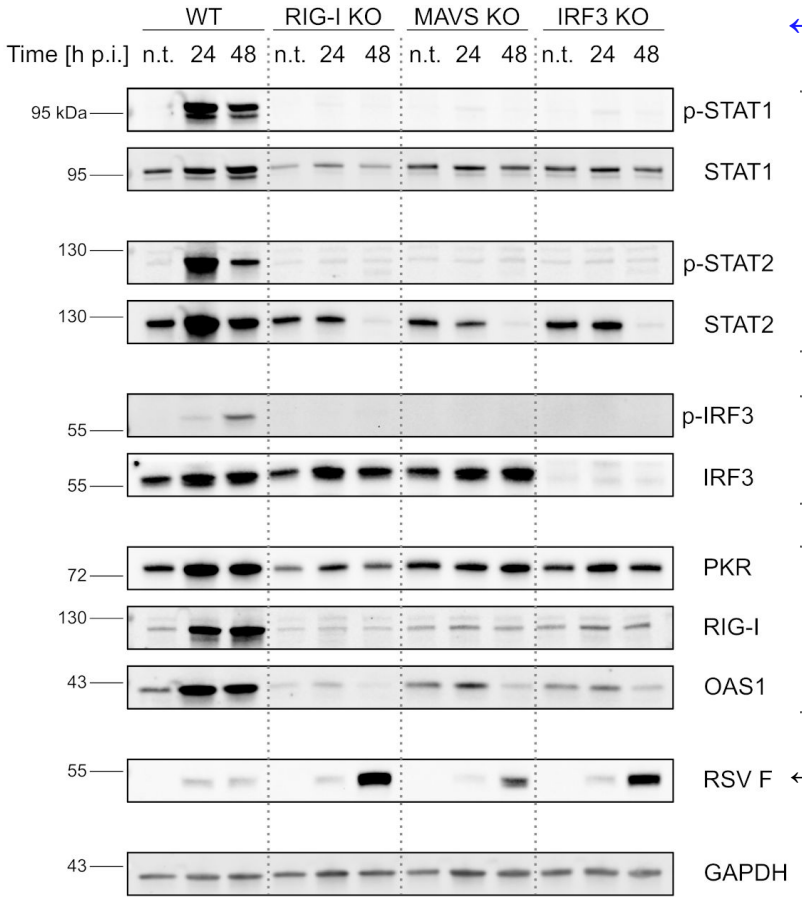
cell-population level techniques (ELISA, Western blotting, dPCR)
to calibrate the rates of virus–cell (and interferon–cell) interactions

and **single-cell techniques** (multi-channel immunostaining)
to capture interactions between (neighboring) cells,
that give rise rise to the observed spatiotemporal organization.

Secretion of IFNβ upon RSV infection



Viral RNA triggers an innate immune signaling cascade



← **interrupting signal propagation at various steps**

response to IFNβ (**2**)
virus inhibits STAT1/2 signaling

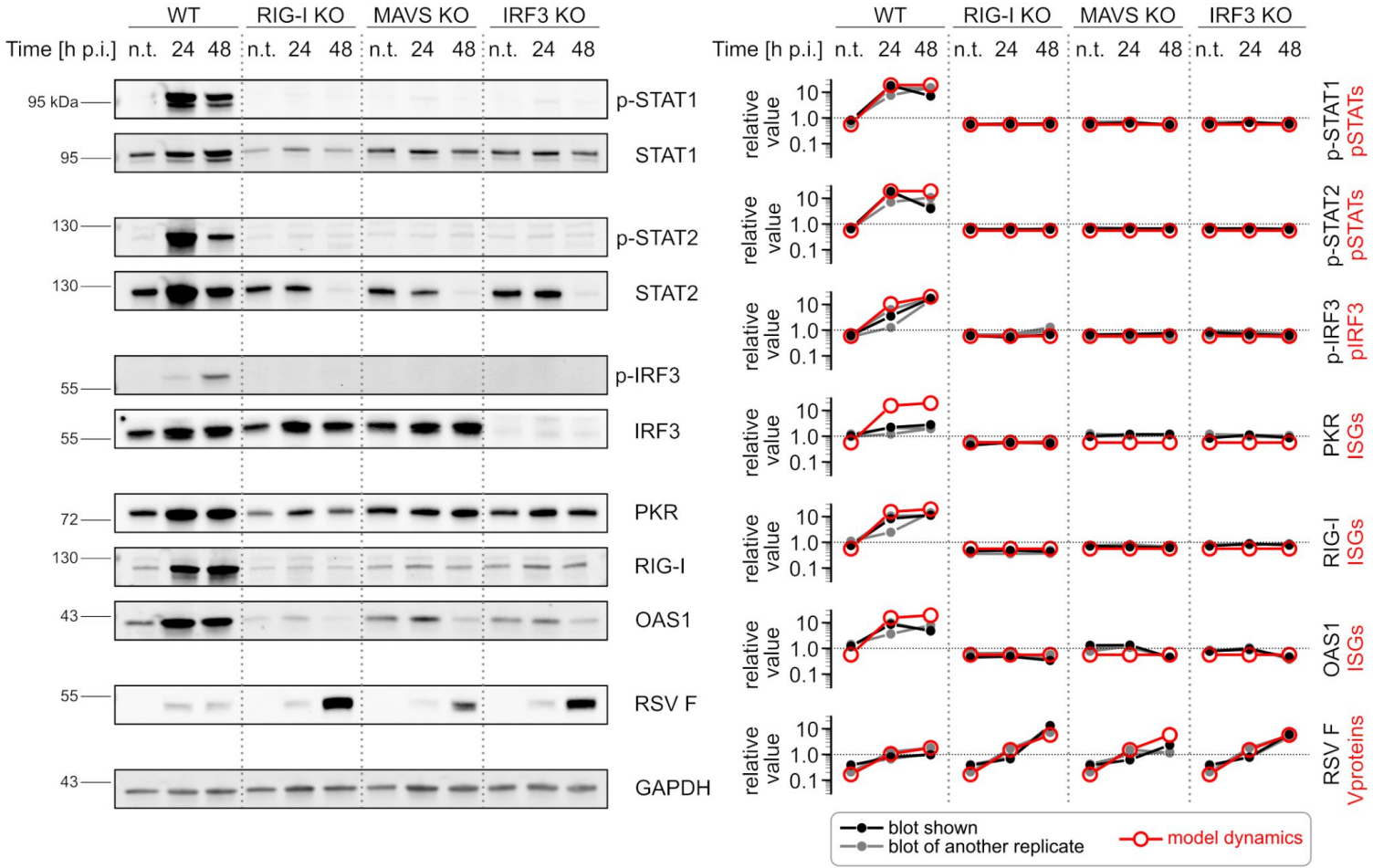
transcription factor of IFNβ (**1**)

IFN-stimulated genes (ISGs) (**3**)

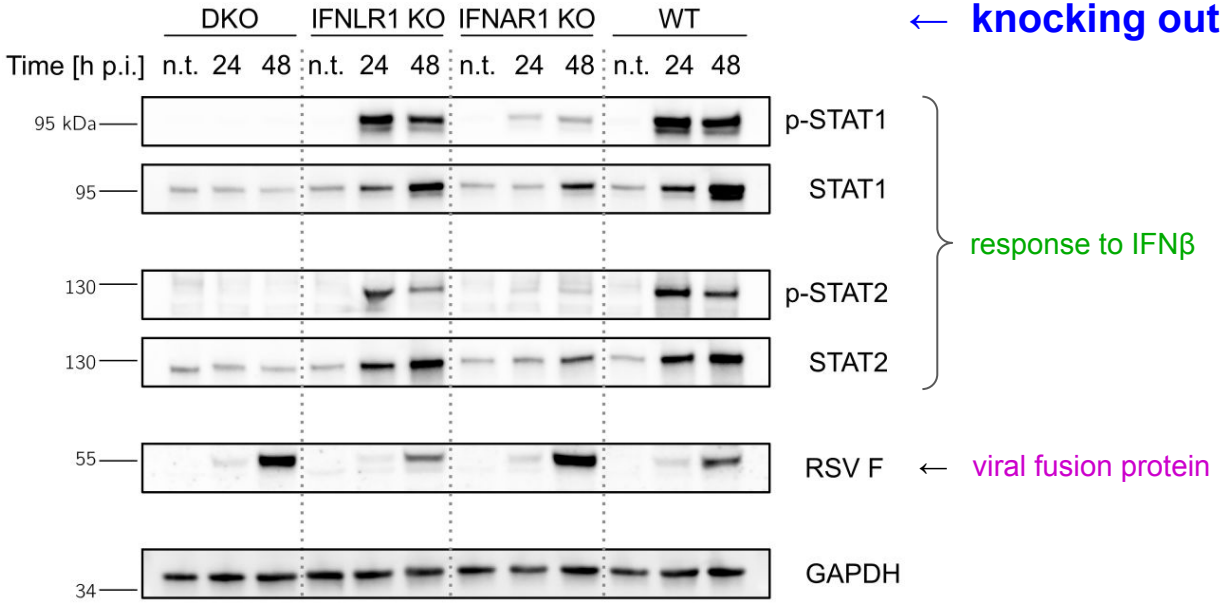
← viral fusion protein

innate immune signaling inhibits virus replication

Viral RNA triggers an innate immune signaling cascade



IFNβ/λ-induced STAT1/2 signaling attenuates viral infection

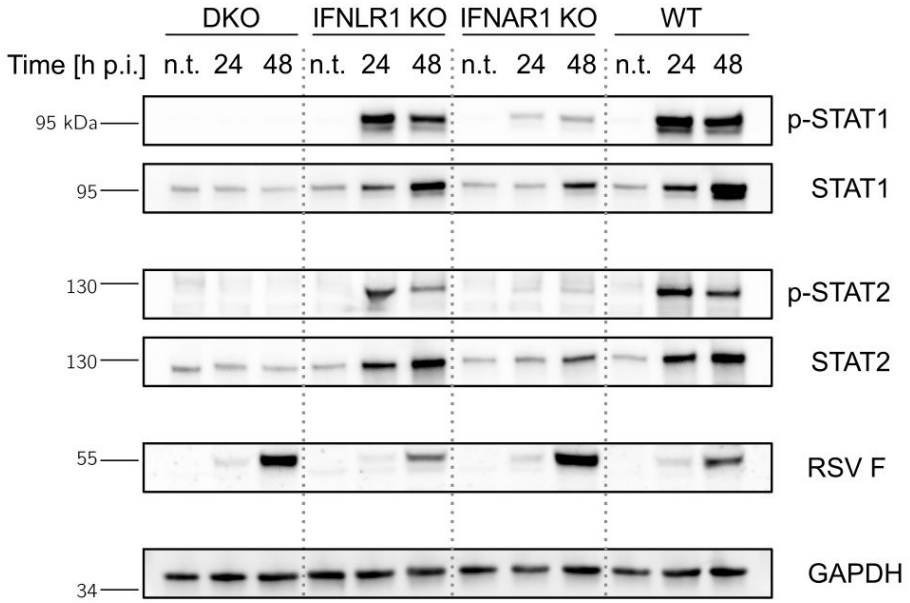


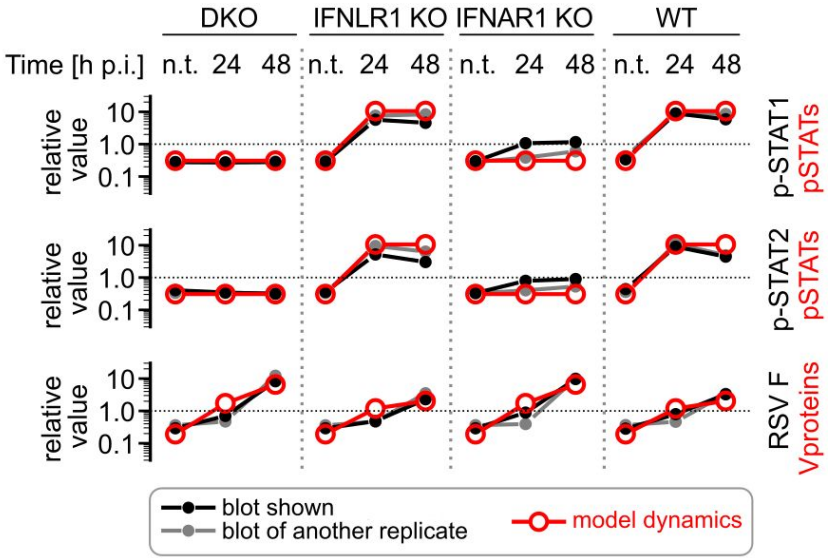
← **knocking out IFNλ/β receptors**

response to IFNβ

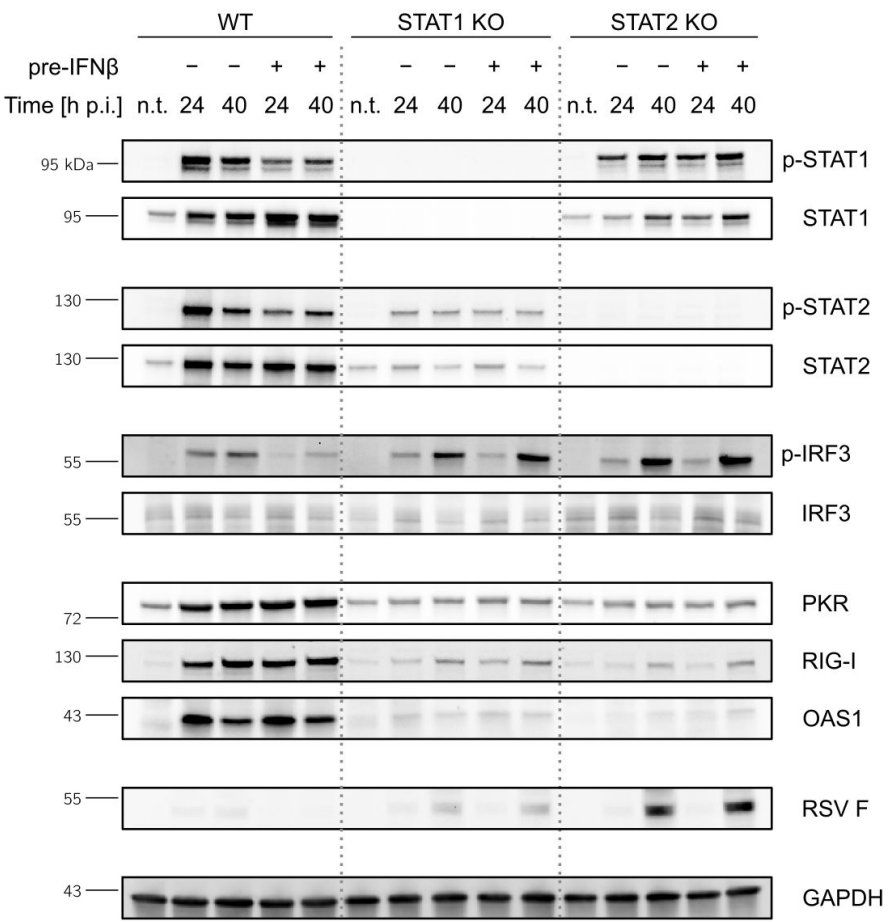
← viral fusion protein

IFNβ/λ-induced STAT1/2 signaling attenuates viral infection





Activity of STAT1/2 attenuates the spread of RSV infection



← **disrupting STAT1/2 expression**

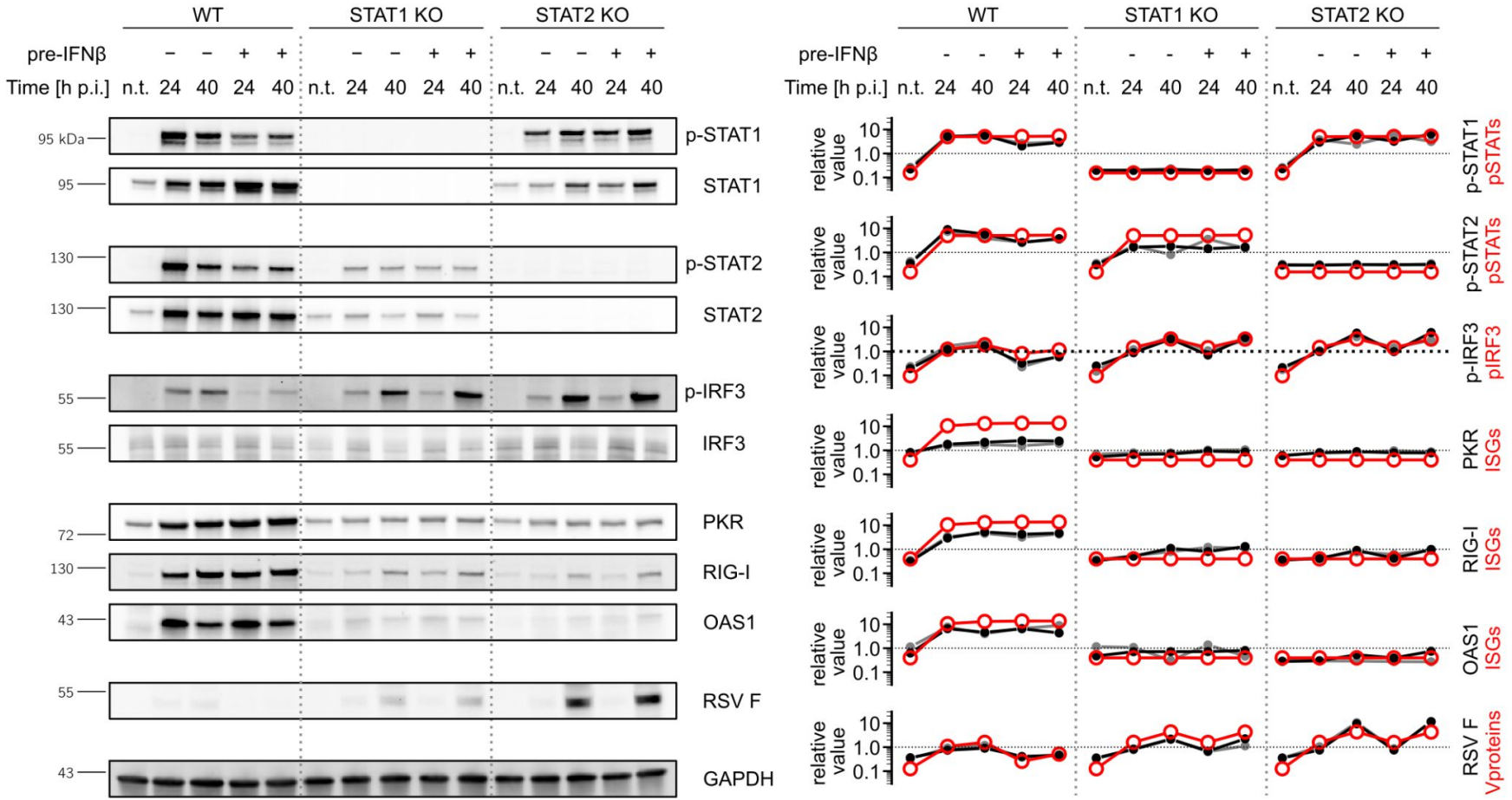
response to IFNβ

transcription factor of IFNβ

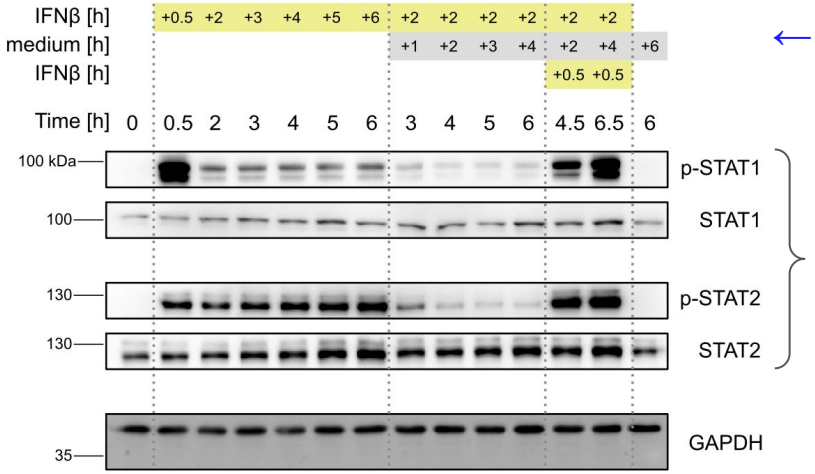
IFN-stimulated genes (RIG-I is a viral RNA sensor)

← viral fusion protein

Activity of STAT1/2 attenuates the spread of RSV infection

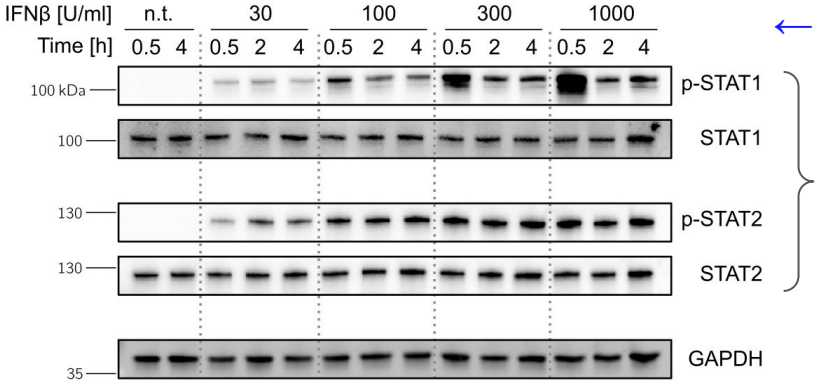


STAT1/2 are activated quickly; IFNβ is required to sustain STAT1/2 activity



← **IFNβ protocols:** give, give→take, give→take→give

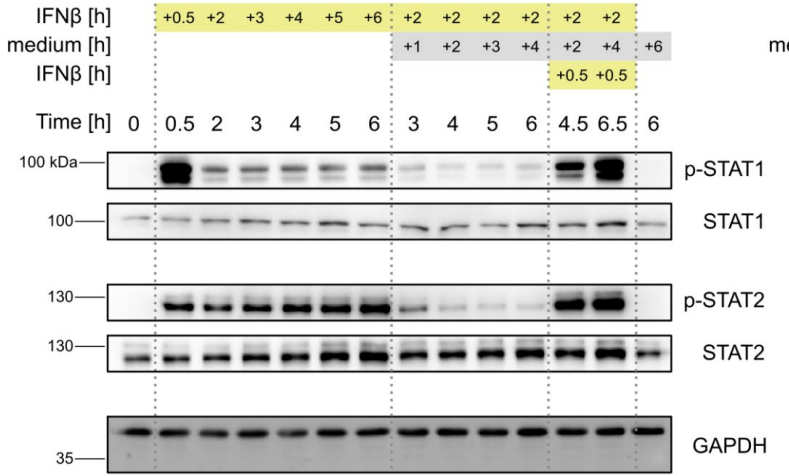
response to IFNβ

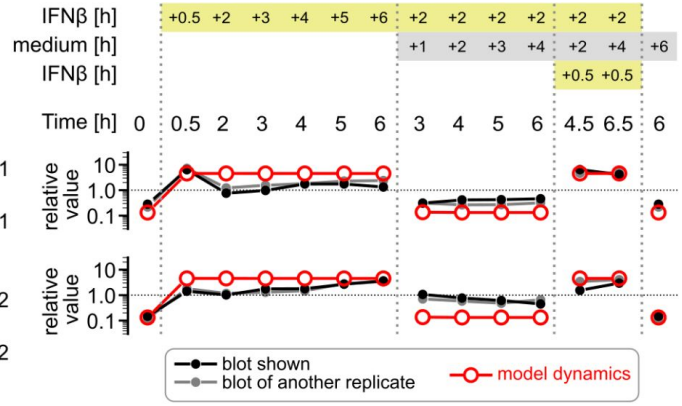


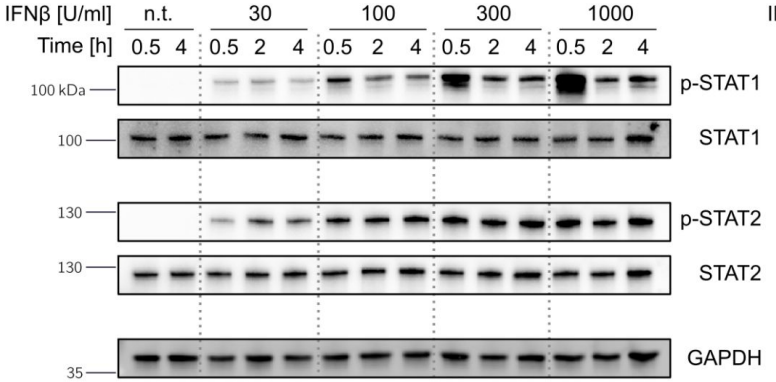
← **IFNβ "titration"**

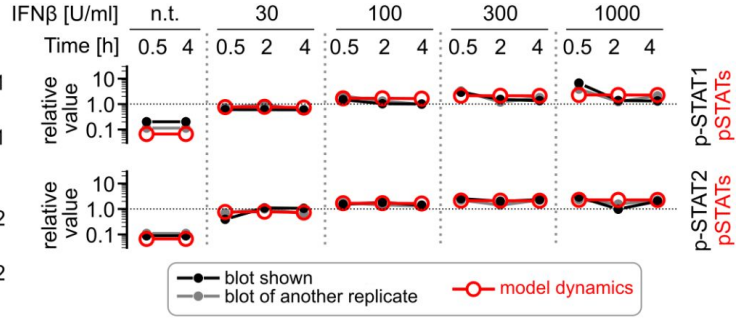
response to IFNβ

STAT1/2 are activated quickly; IFNβ is required to sustain STAT1/2 activity

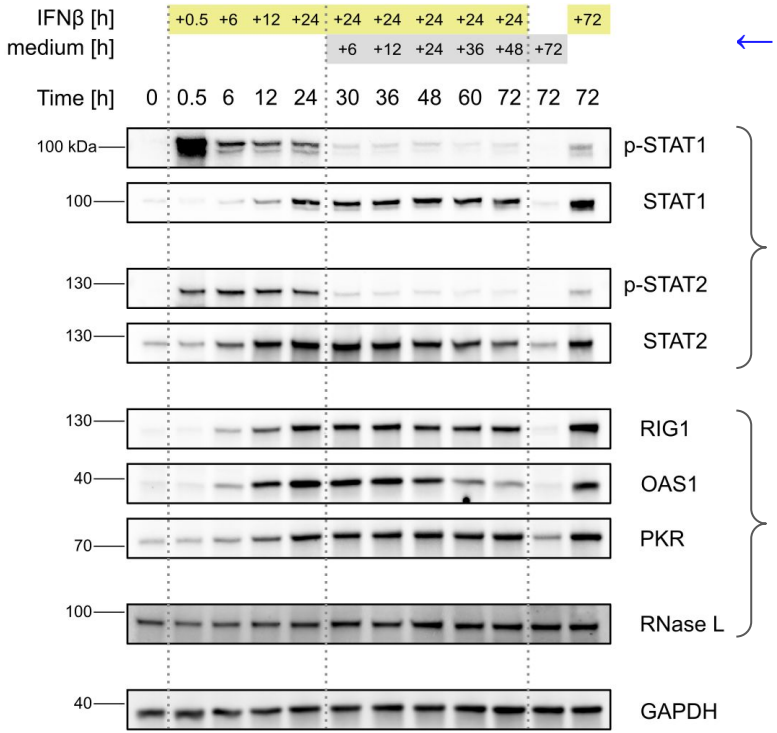








Proteins of IFN-stimulated genes accumulate & degrade slowly

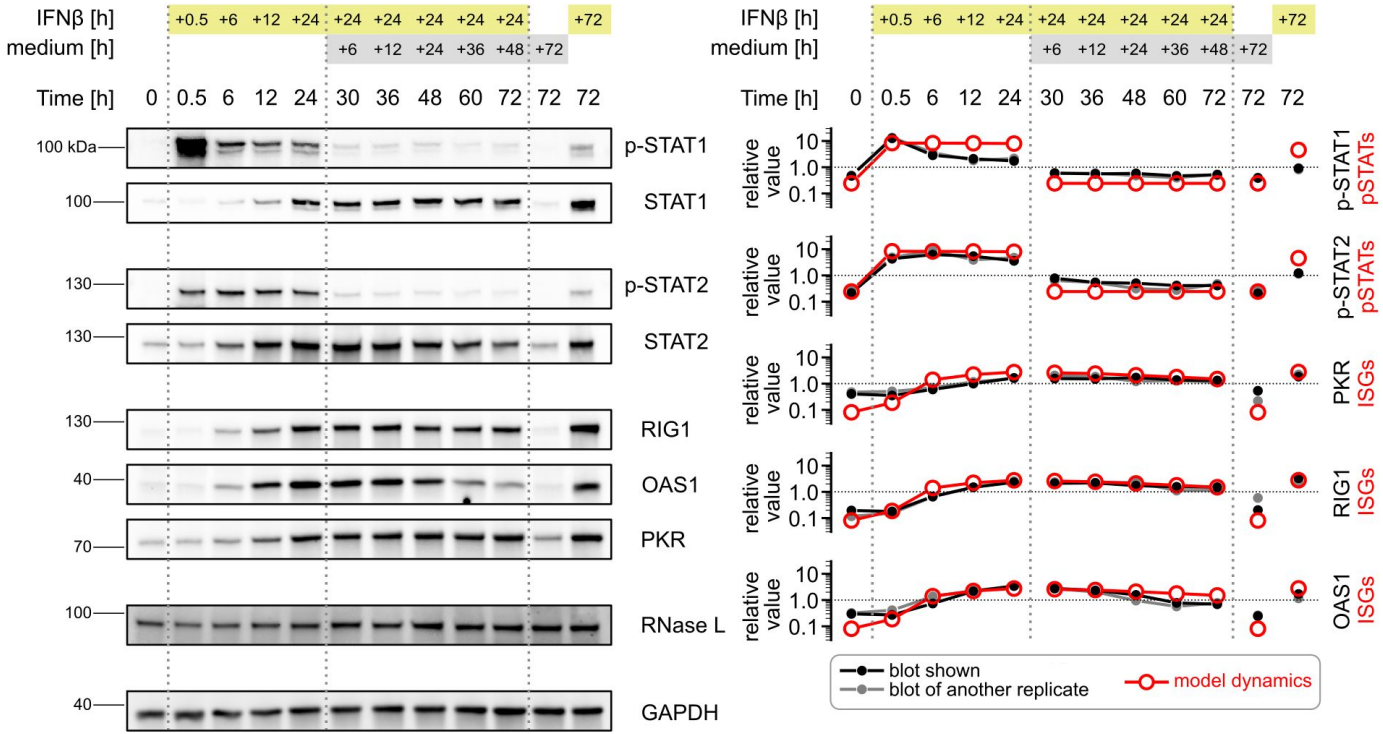


← **IFNβ protocols:** give, give→take

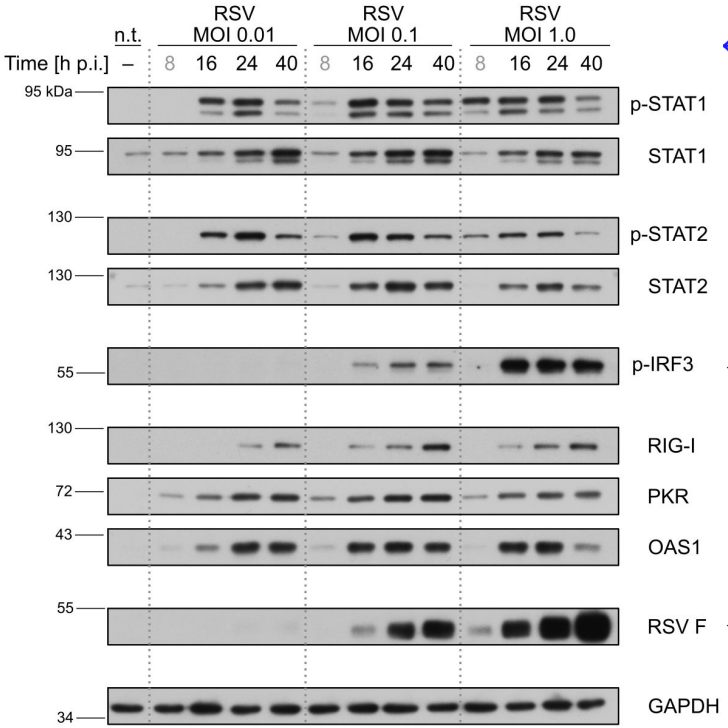
response to IFNβ

IFN-stimulated genes

Proteins of IFN-stimulated genes accumulate & degrade slowly



Pre-stimulation with IFNβ impedes virus spread



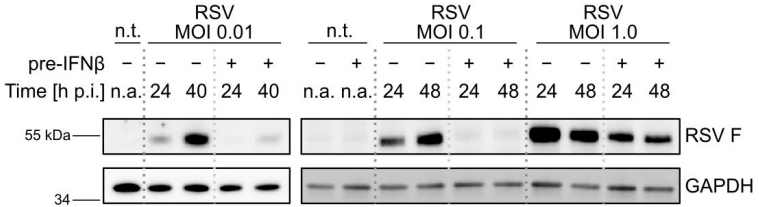
← **"Titration" of virus MOI**

response to IFNβ

← transcription factor of IFNβ

IFN-stimulated genes

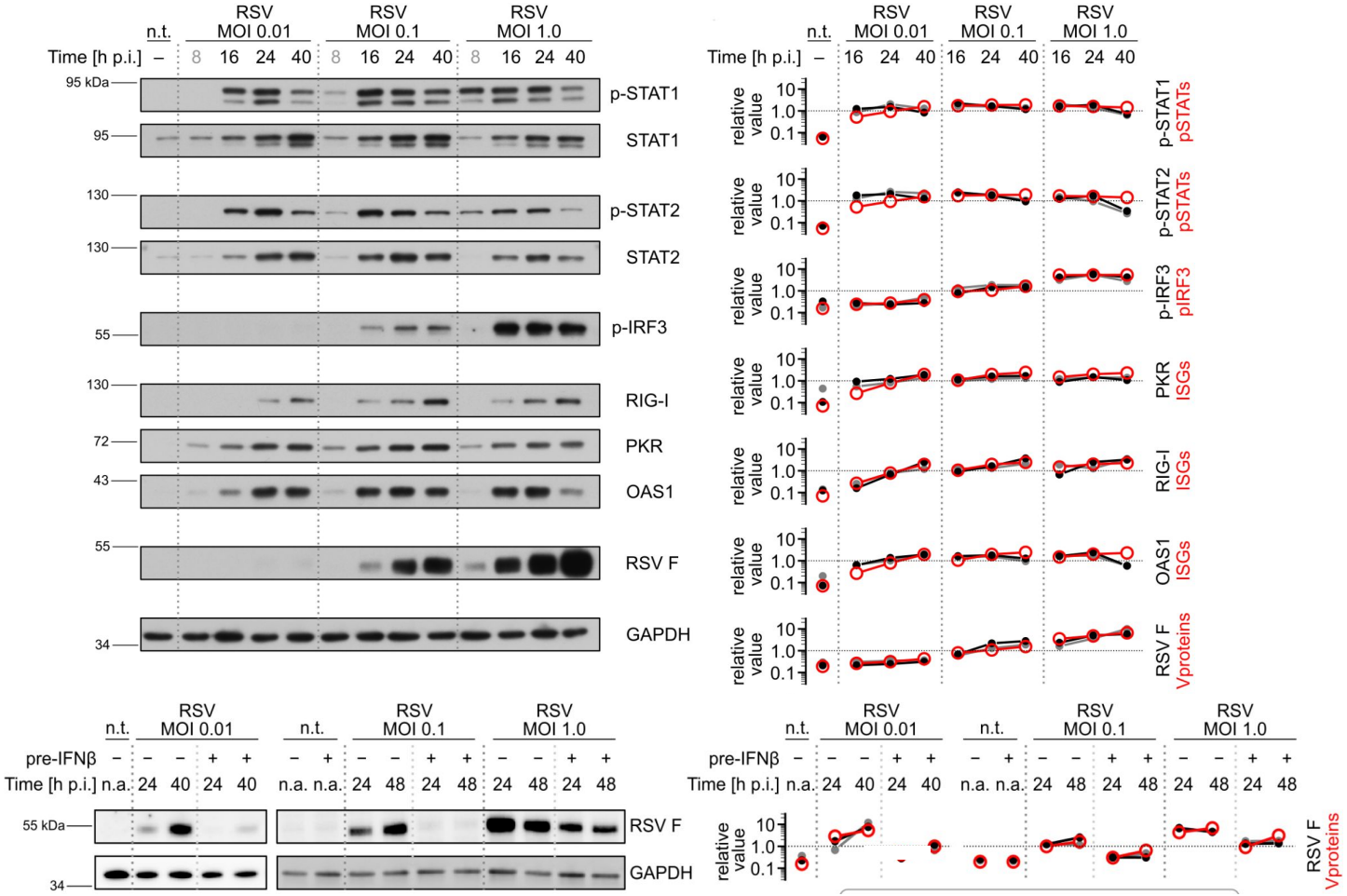
← viral fusion protein



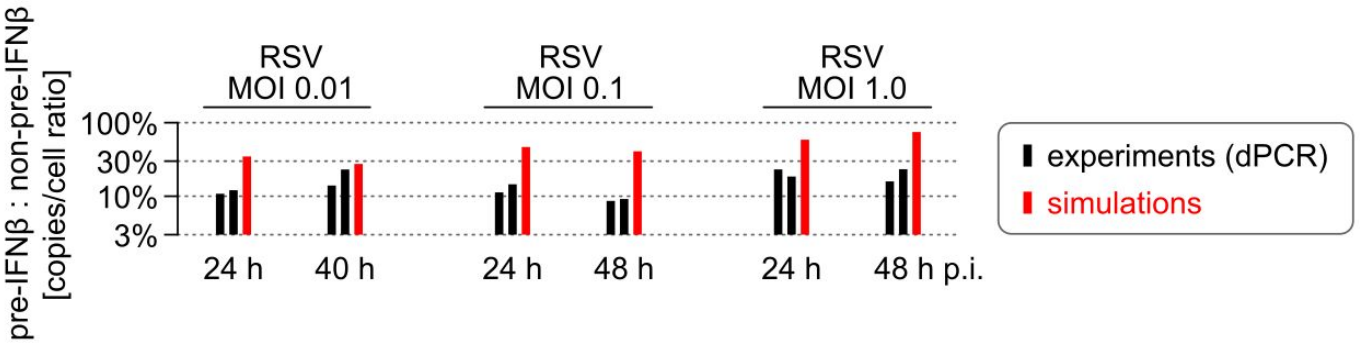
← **"Titration" of virus MOI, +/− IFNβ pre-stimulation**

← viral fusion protein

Pre-stimulation with IFNβ impedes virus proliferation

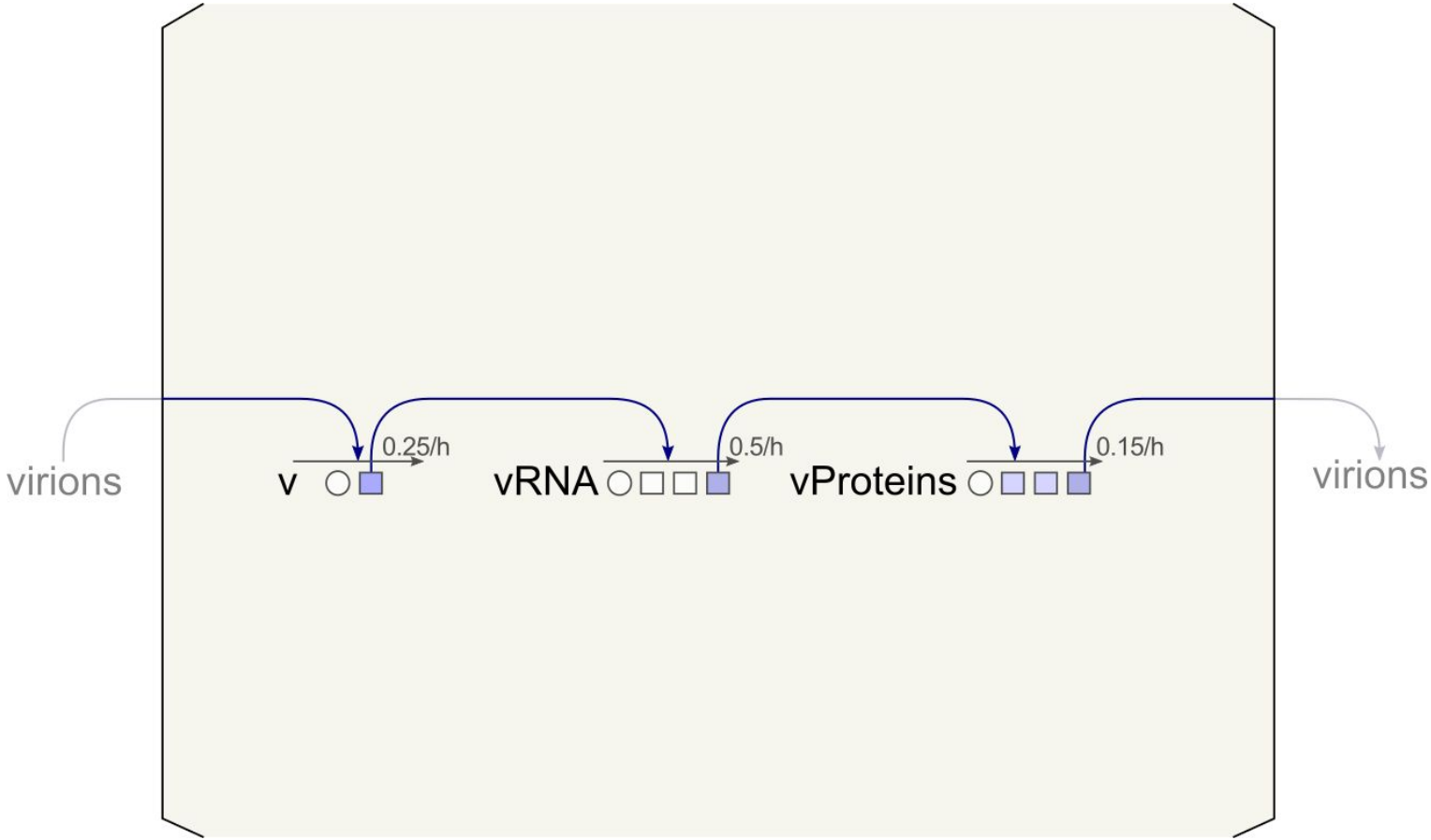


Pre-stimulation with IFNβ impedes virus proliferation

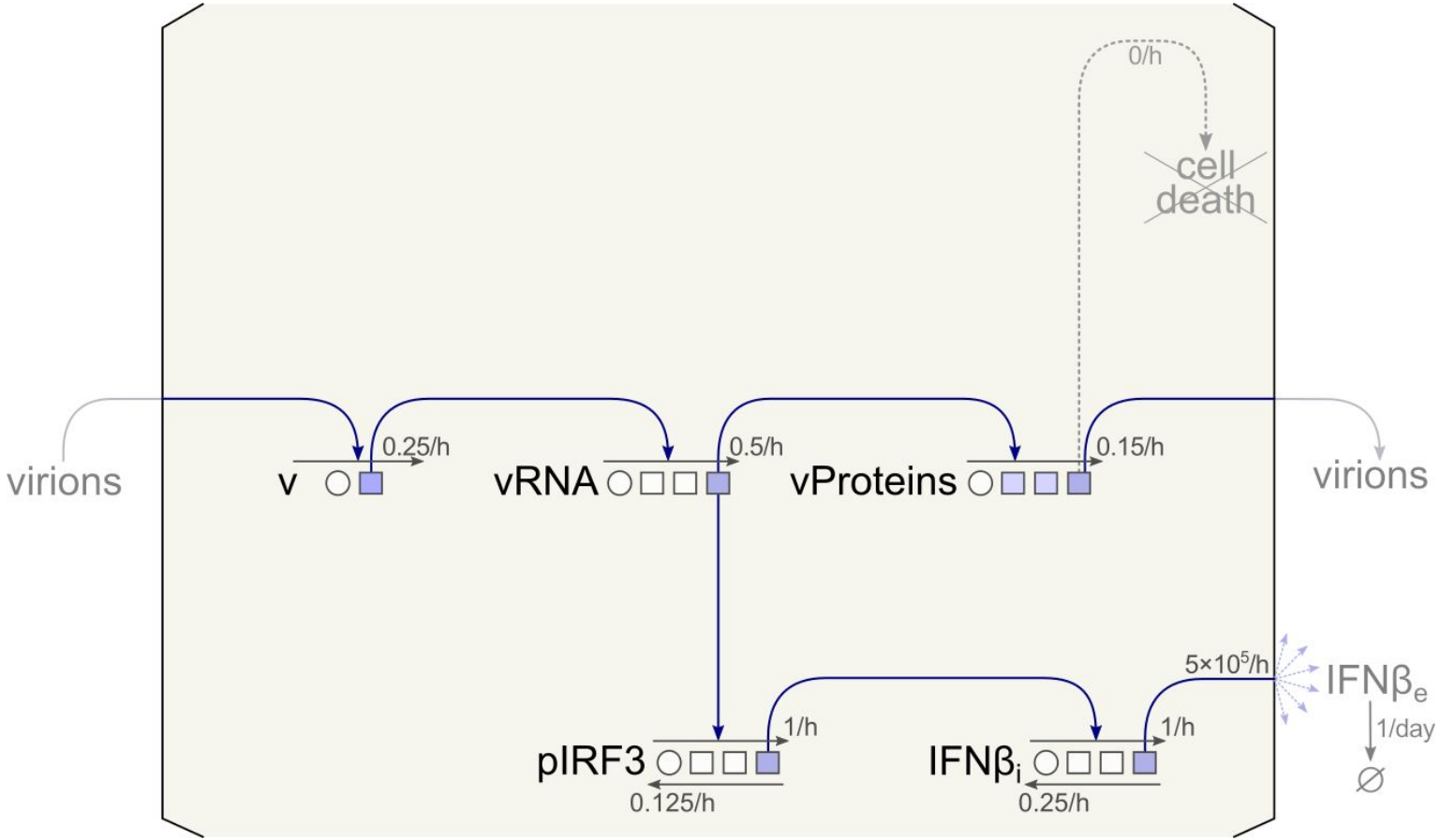


↼ **+/− IFNβ pre-stimulation**

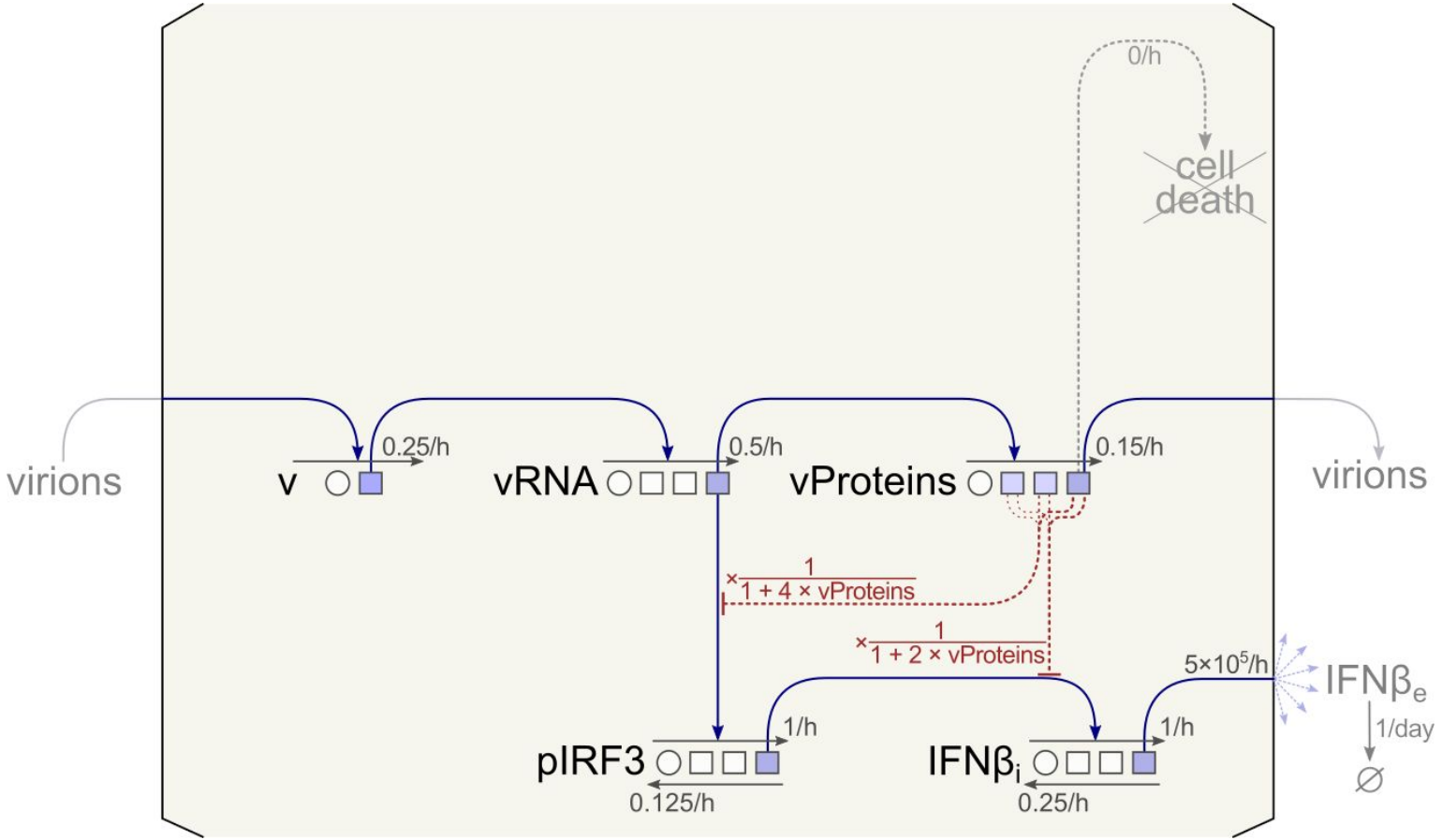
Model: virus–host interactions in a single cell



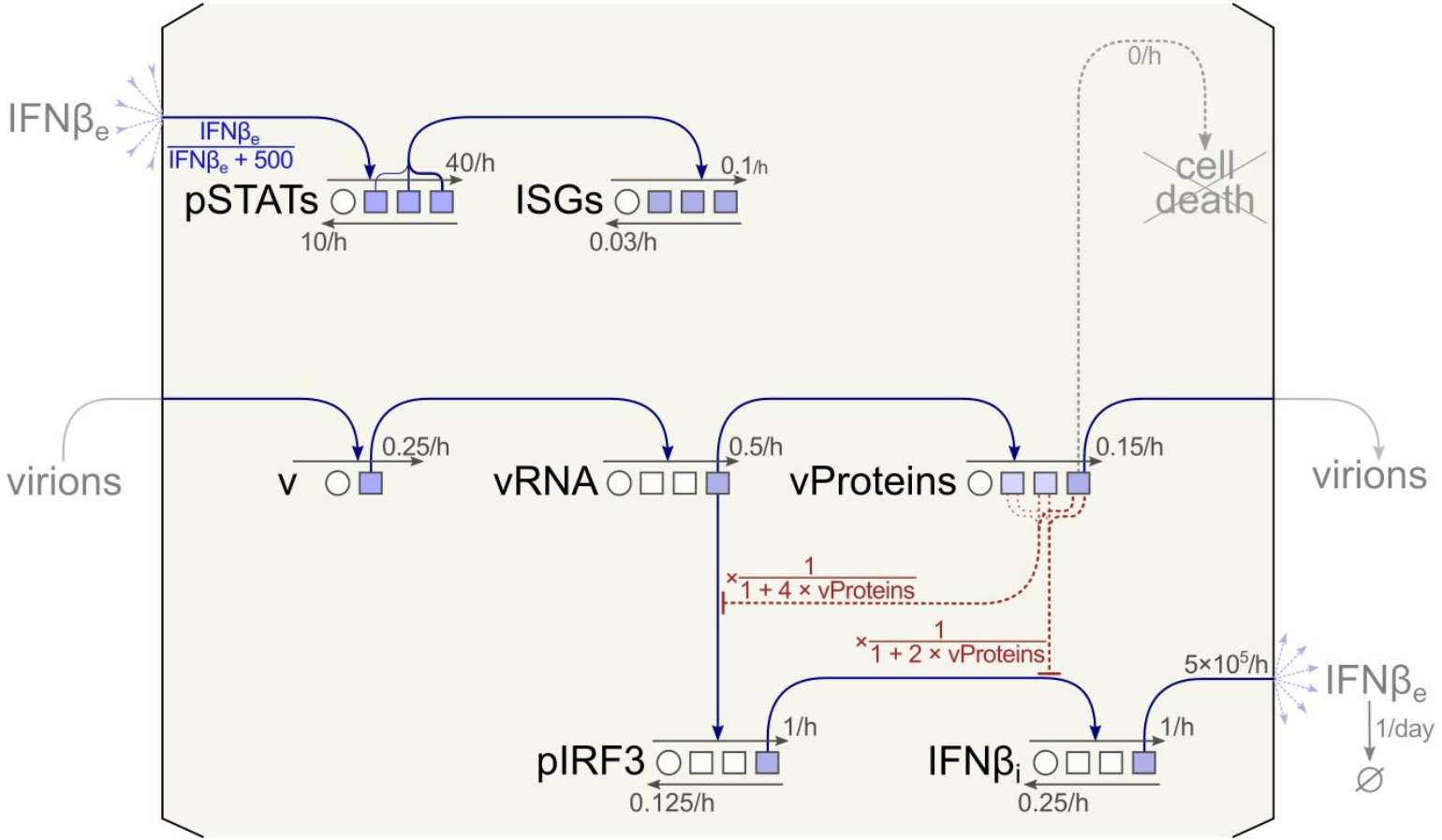
Model: virus–host interactions in a single cell



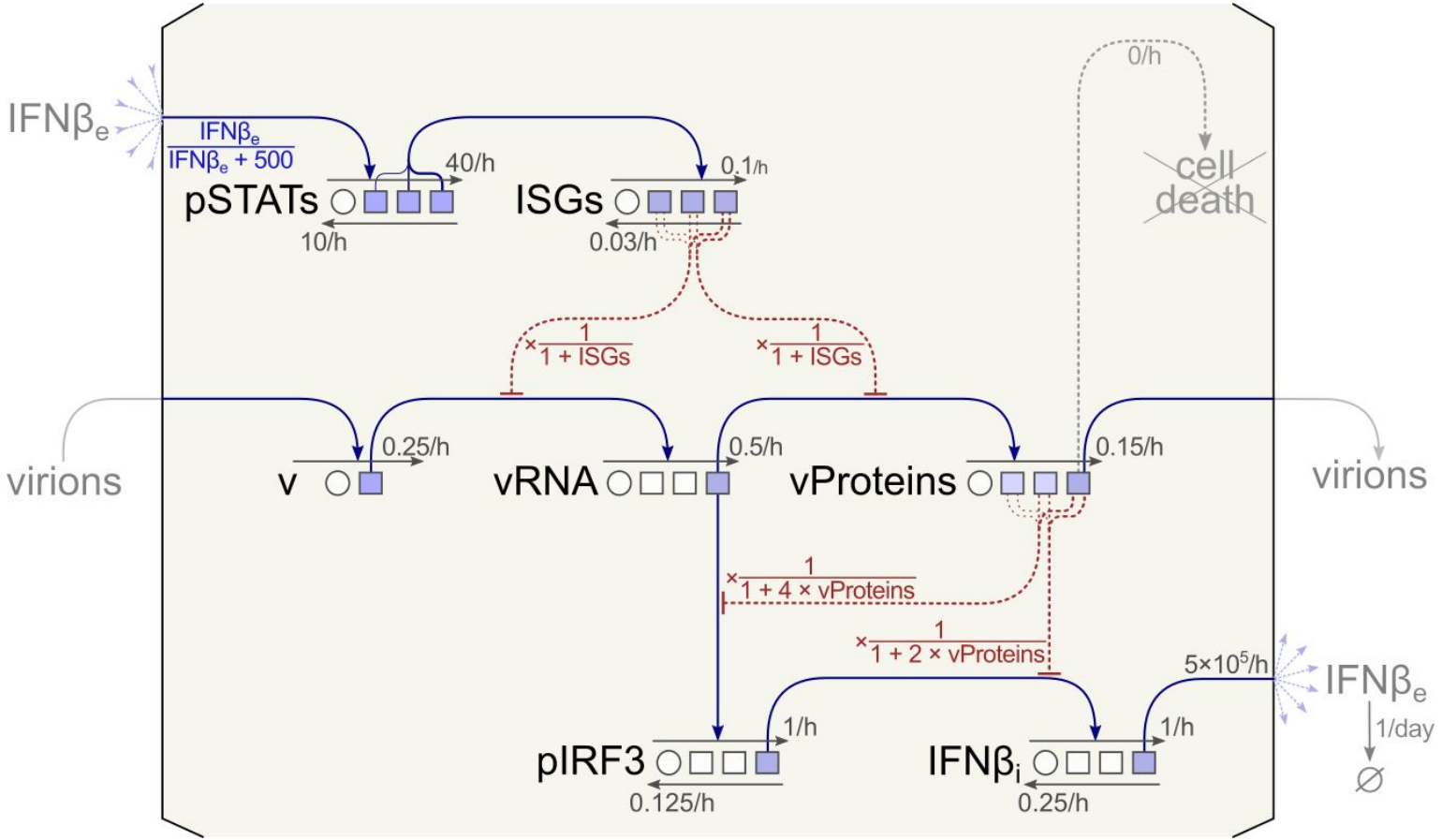
Model: virus–host interactions in a single cell



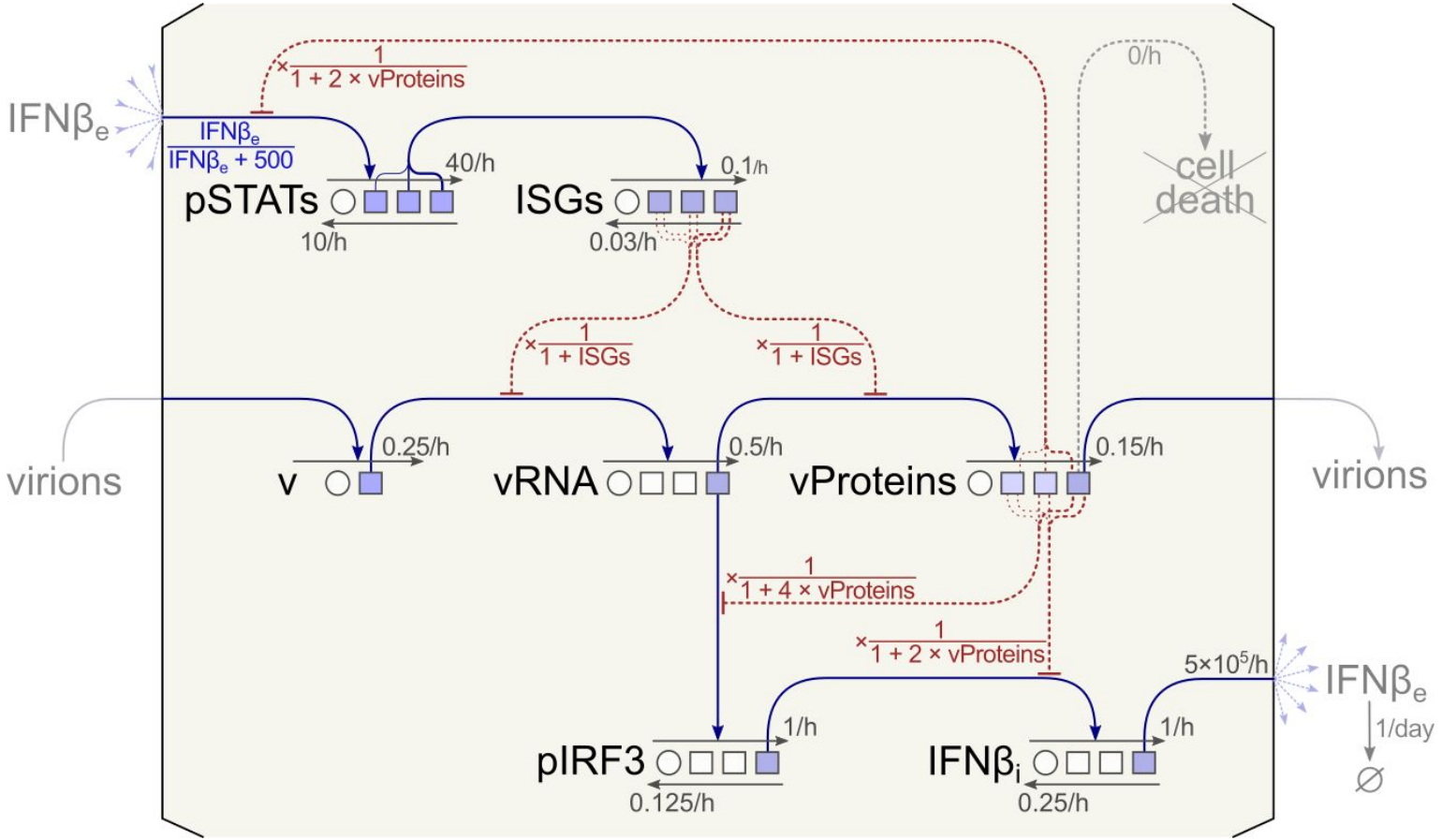
Model: virus–host interactions in a single cell



Model: virus–host interactions in a single cell

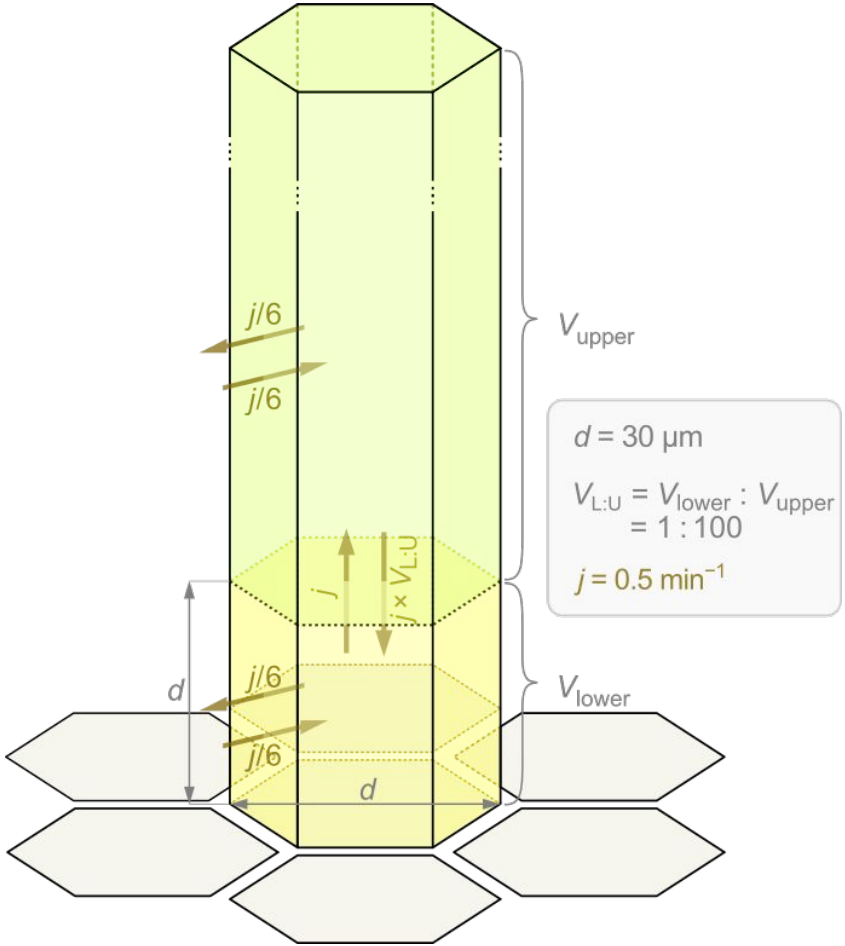


Model: virus–host interactions in a single cell



Transport of extracellular IFNβ

Two-storey compartments with $IFN\beta_e$ above each cell



Example simulation

MOI: 0.01

Time: 0h

Vinf 0:	99%	(#=4058)
Vinf 1:	**1%**	**(#=38)**
VRNA 0:	100%	(#=4096)
VRNA 1:	0%	(#=0)
VRNA 2:	0%	(#=0)
VRNA 3:	**0%**	**(#=0)**
Vprot 0:	100%	(#=4096)
Vprot 1:	0%	(#=0)
Vprot 2:	0%	(#=0)
Vprot 3:	**0%**	**(#=0)**
pIRF3 0:	100%	(#=4096)
pIRF3 1:	0%	(#=0)
pIRF3 2:	0%	(#=0)
pIRF3 3:	**0%**	**(#=0)**
IFNi 0:	100%	(#=4096)
IFNi 1:	0%	(#=0)
IFNi 2:	0%	(#=0)
IFNi 3:	**0%**	**(#=0)**
pSTAT 0:	100%	(#=4096)
pSTAT 1:	**0%**	**(#=0)**
pSTAT 2:	**0%**	**(#=0)**
pSTAT 3:	**0%**	**(#=0)**
ISG 0:	100%	(#=4096)
ISG 1:	**0%**	**(#=0)**
ISG 2:	**0%**	**(#=0)**
ISG 3:	**0%**	**(#=0)**
fields:	100%	(#=4096)
dead:	0%	(#=0)

IFNeU/node:	0
IFNeL/node:	0

Example simulation

Time: 2h

Vinf 0:	99%	(#=4058)
Vinf 1:	**1%**	**(#=38)**
VRNA 0:	99%	(#=4075)
VRNA 1:	0%	(#=10)
VRNA 2:	0%	(#=8)
VRNA 3:	**0%**	**(#=3)**
Vprot 0:	100%	(#=4096)
Vprot 1:	0%	(#=0)
Vprot 2:	0%	(#=0)
Vprot 3:	**0%**	**(#=0)**
pIRF3 0:	100%	(#=4096)
pIRF3 1:	0%	(#=0)
pIRF3 2:	0%	(#=0)
pIRF3 3:	**0%**	**(#=0)**
IFNi 0:	100%	(#=4096)
IFNi 1:	0%	(#=0)
IFNi 2:	0%	(#=0)
IFNi 3:	**0%**	**(#=0)**
pSTAT 0:	100%	(#=4096)
pSTAT 1:	**0%**	**(#=0)**
pSTAT 2:	**0%**	**(#=0)**
pSTAT 3:	**0%**	**(#=0)**
ISG 0:	100%	(#=4096)
ISG 1:	**0%**	**(#=0)**
ISG 2:	**0%**	**(#=0)**
ISG 3:	**0%**	**(#=0)**
fields:	100%	(#=4096)
dead:	0%	(#=0)

IFNeU/node:	0
IFNeL/node:	0

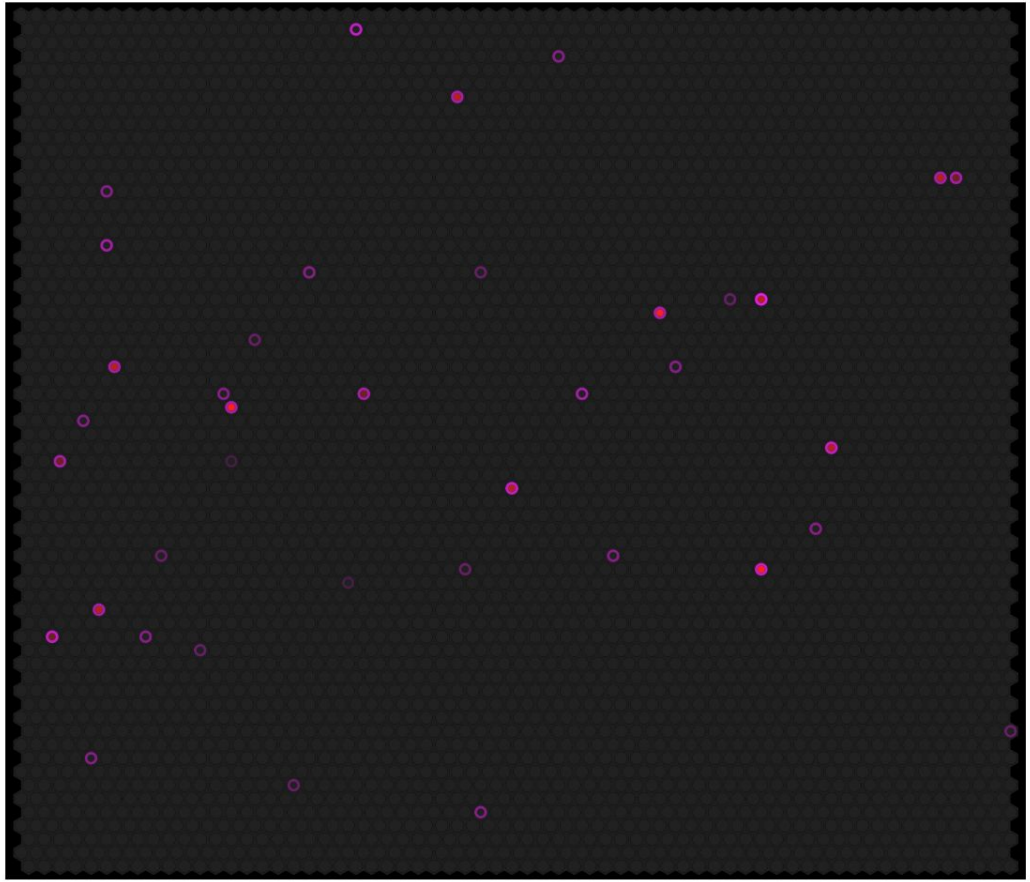
Example simulation

Time: 4h

Vinf 0:	99%	(#=4058)
Vinf 1:	**1%**	**(#=38)**
VRNA 0:	99%	(#=4065)
VRNA 1:	0%	(#=14)
VRNA 2:	0%	(#=7)
VRNA 3:	**0%**	**(#=10)**
Vprot 0:	100%	(#=4095)
Vprot 1:	0%	(#=0)
Vprot 2:	0%	(#=1)
Vprot 3:	**0%**	**(#=0)**
pIRF3 0:	100%	(#=4086)
pIRF3 1:	0%	(#=7)
pIRF3 2:	0%	(#=2)
pIRF3 3:	**0%**	**(#=1)**
IFNi 0:	100%	(#=4095)
IFNi 1:	0%	(#=1)
IFNi 2:	0%	(#=0)
IFNi 3:	**0%**	**(#=0)**
pSTAT 0:	100%	(#=4096)
pSTAT 1:	**0%**	**(#=0)**
pSTAT 2:	**0%**	**(#=0)**
pSTAT 3:	**0%**	**(#=0)**
ISG 0:	100%	(#=4096)
ISG 1:	**0%**	**(#=0)**
ISG 2:	**0%**	**(#=0)**
ISG 3:	**0%**	**(#=0)**
fields:	100%	(#=4096)
dead:	0%	(#=0)

IFNeU/node:	0
IFNeL/node:	0

Example simulation

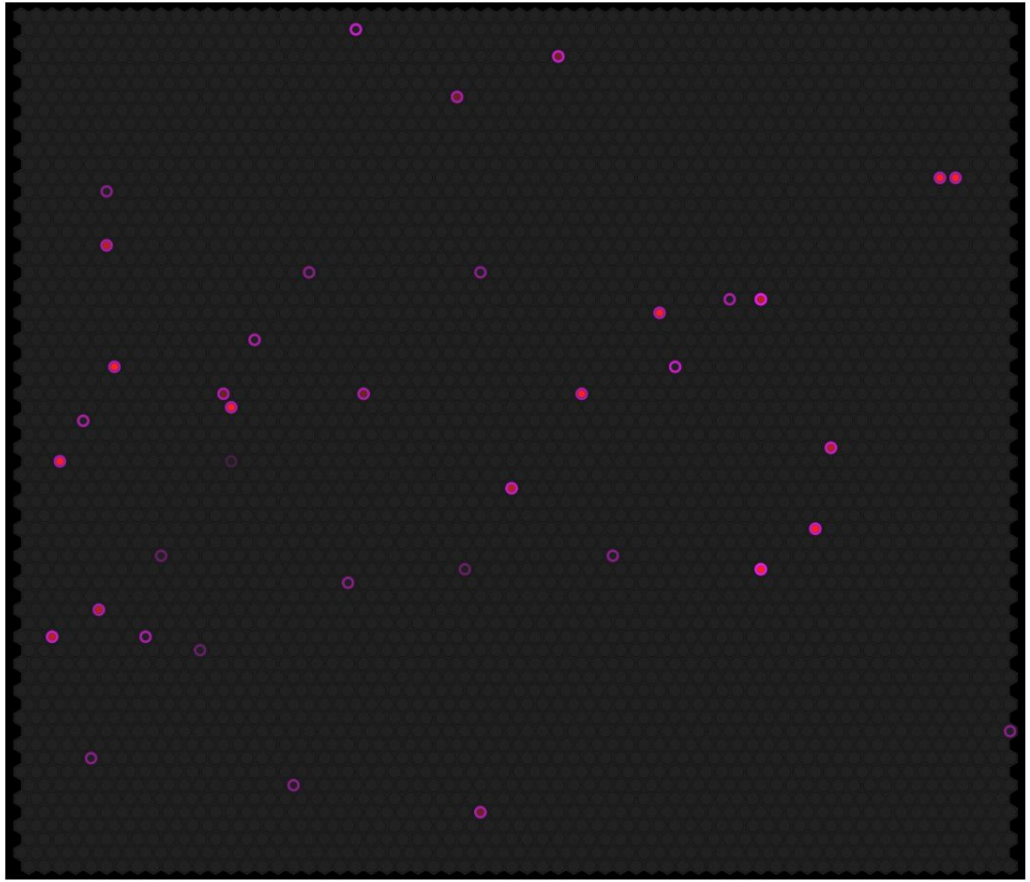


Time: 6h

Vinf 0:	99%	(#=4058)
Vinf 1:	**1%**	**(#=38)**
VRNA 0:	99%	(#=4060)
VRNA 1:	0%	(#=8)
VRNA 2:	0%	(#=11)
VRNA 3:	**0%**	**(#=17)**
Vprot 0:	100%	(#=4090)
Vprot 1:	0%	(#=5)
Vprot 2:	0%	(#=1)
Vprot 3:	**0%**	**(#=0)**
pIRF3 0:	100%	(#=4082)
pIRF3 1:	0%	(#=4)
pIRF3 2:	0%	(#=7)
pIRF3 3:	**0%**	**(#=3)**
IFNi 0:	100%	(#=4095)
IFNi 1:	0%	(#=1)
IFNi 2:	0%	(#=0)
IFNi 3:	**0%**	**(#=0)**
pSTAT 0:	100%	(#=4096)
pSTAT 1:	**0%**	**(#=0)**
pSTAT 2:	**0%**	**(#=0)**
pSTAT 3:	**0%**	**(#=0)**
ISG 0:	100%	(#=4096)
ISG 1:	**0%**	**(#=0)**
ISG 2:	**0%**	**(#=0)**
ISG 3:	**0%**	**(#=0)**
fields:	100%	(#=4096)
dead:	0%	(#=0)

IFNeU/node:	0
IFNeL/node:	0

Example simulation

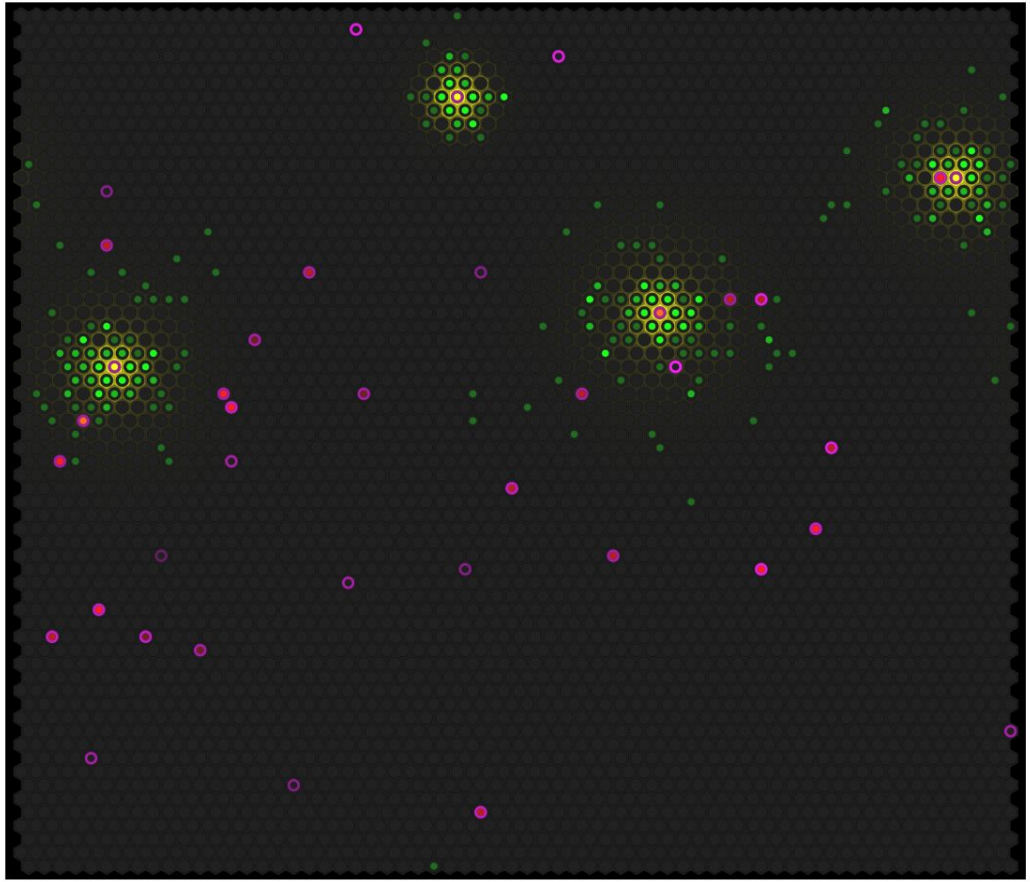


Time: 8h

Vinf 0:	99%	(#=4058)
Vinf 1:	**1%**	**(#=38)**
VRNA 0:	99%	(#=4059)
VRNA 1:	0%	(#=3)
VRNA 2:	0%	(#=8)
VRNA 3:	**1%**	**(#=26)**
Vprot 0:	100%	(#=4087)
Vprot 1:	0%	(#=7)
Vprot 2:	0%	(#=2)
Vprot 3:	**0%**	**(#=0)**
pIRF3 0:	100%	(#=4076)
pIRF3 1:	0%	(#=5)
pIRF3 2:	0%	(#=6)
pIRF3 3:	**0%**	**(#=9)**
IFNi 0:	100%	(#=4091)
IFNi 1:	0%	(#=2)
IFNi 2:	0%	(#=3)
IFNi 3:	**0%**	**(#=0)**
pSTAT 0:	100%	(#=4096)
pSTAT 1:	**0%**	**(#=0)**
pSTAT 2:	**0%**	**(#=0)**
pSTAT 3:	**0%**	**(#=0)**
ISG 0:	100%	(#=4096)
ISG 1:	**0%**	**(#=0)**
ISG 2:	**0%**	**(#=0)**
ISG 3:	**0%**	**(#=0)**
fields:	100%	(#=4096)
dead:	0%	(#=0)

IFNeU/node:	0
IFNeL/node:	0

Example simulation

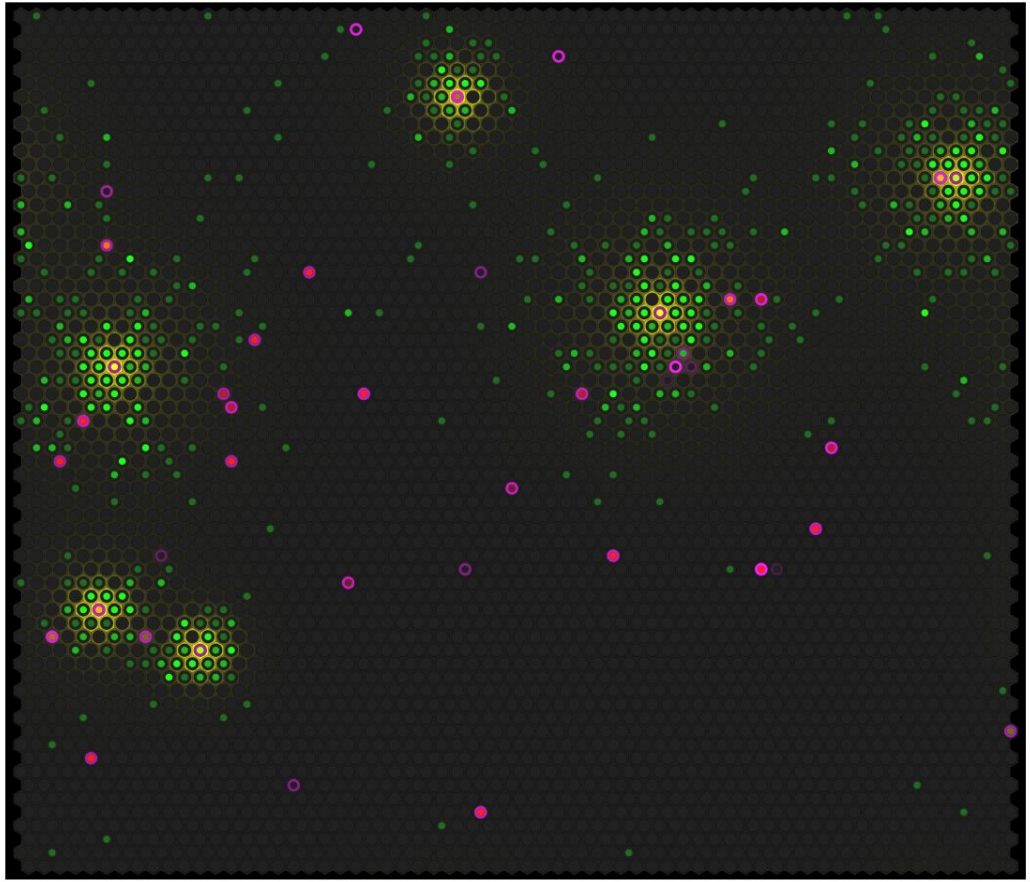


Time: 10h

Vinf 0:	99%	(#=4058)
Vinf 1:	**1%**	**(#=38)**
VRNA 0:	99%	(#=4058)
VRNA 1:	0%	(#=1)
VRNA 2:	0%	(#=4)
VRNA 3:	**1%**	**(#=33)**
Vprot 0:	100%	(#=4082)
Vprot 1:	0%	(#=8)
Vprot 2:	0%	(#=5)
Vprot 3:	**0%**	**(#=1)**
pIRF3 0:	99%	(#=4070)
pIRF3 1:	0%	(#=4)
pIRF3 2:	0%	(#=10)
pIRF3 3:	**0%**	**(#=12)**
IFNi 0:	100%	(#=4086)
IFNi 1:	0%	(#=5)
IFNi 2:	0%	(#=1)
IFNi 3:	**0%**	**(#=4)**
pSTAT 0:	95%	(#=3902)
pSTAT 1:	**3%**	**(#=116)**
pSTAT 2:	**1%**	**(#=40)**
pSTAT 3:	**1%**	**(#=38)**
ISG 0:	100%	(#=4079)
ISG 1:	**0%**	**(#=16)**
ISG 2:	**0%**	**(#=1)**
ISG 3:	**0%**	**(#=0)**
fields:	100%	(#=4096)
dead:	0%	(#=0)

IFNeU/node:	593
IFNeL/node:	22

Example simulation

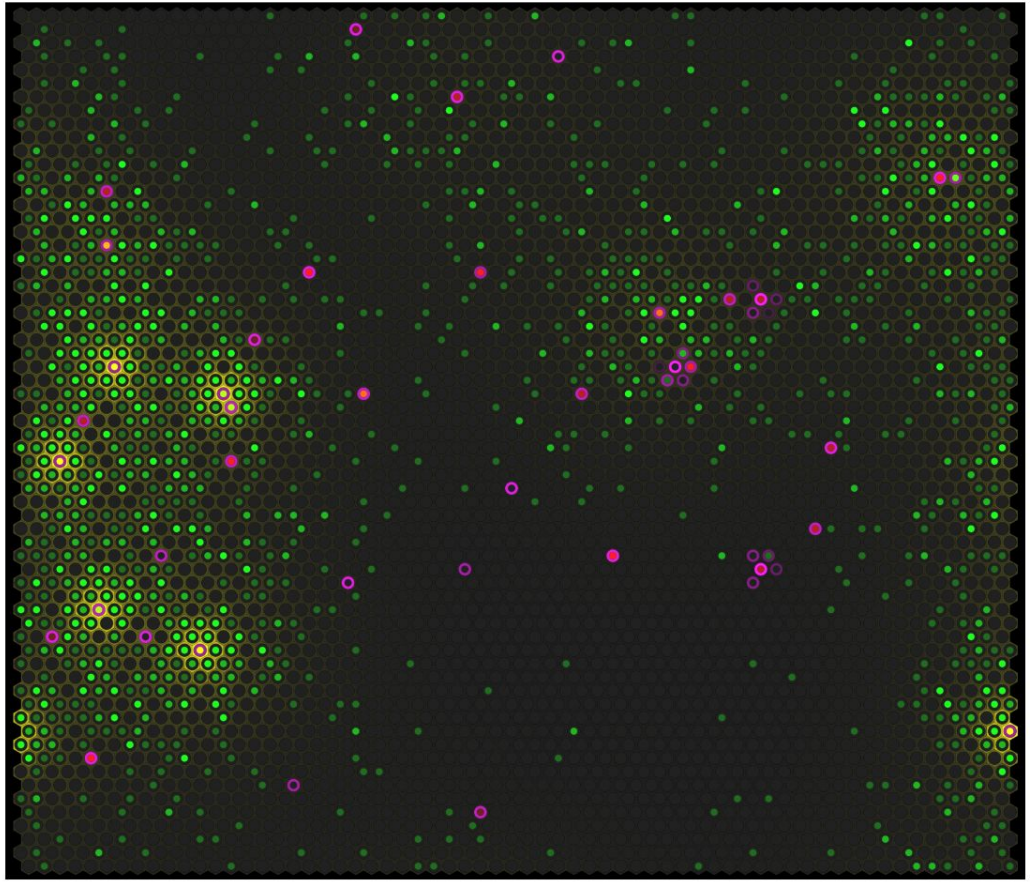


Time: 12h

Vinf 0:	99%	(#=4054)
Vinf 1:	**1%**	**(#=42)**
VRNA 0:	99%	(#=4056)
VRNA 1:	0%	(#=2)
VRNA 2:	0%	(#=4)
VRNA 3:	**1%**	**(#=34)**
Vprot 0:	99%	(#=4075)
Vprot 1:	0%	(#=12)
Vprot 2:	0%	(#=7)
Vprot 3:	**0%**	**(#=2)**
pIRF3 0:	99%	(#=4066)
pIRF3 1:	0%	(#=4)
pIRF3 2:	0%	(#=9)
pIRF3 3:	**0%**	**(#=17)**
IFNi 0:	100%	(#=4082)
IFNi 1:	0%	(#=8)
IFNi 2:	0%	(#=0)
IFNi 3:	**0%**	**(#=6)**
pSTAT 0:	89%	(#=3652)
pSTAT 1:	**7%**	**(#=273)**
pSTAT 2:	**2%**	**(#=85)**
pSTAT 3:	**2%**	**(#=86)**
ISG 0:	97%	(#=3987)
ISG 1:	**2%**	**(#=101)**
ISG 2:	**0%**	**(#=5)**
ISG 3:	**0%**	**(#=3)**
fields:	100%	(#=4096)
dead:	0%	(#=0)

IFNeU/node:	1559
IFNeL/node:	39

Example simulation

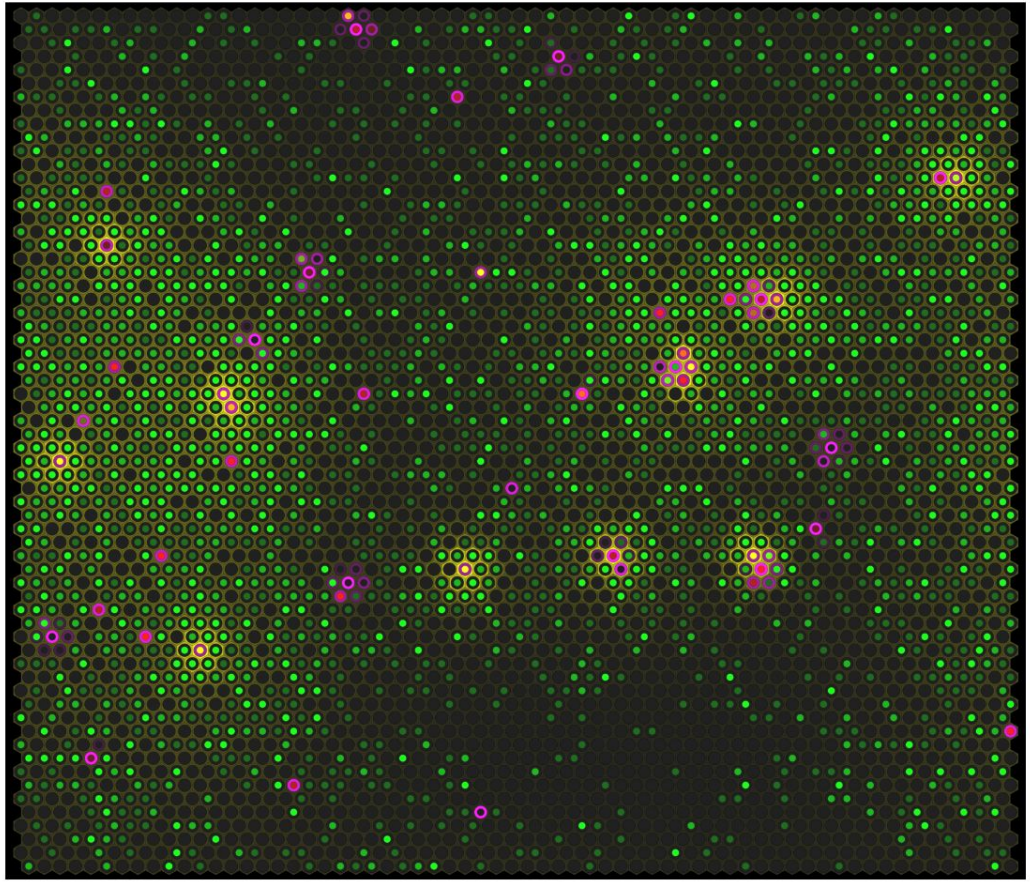


Time: 16h

Vinf 0:	99%	(#=4045)
Vinf 1:	**1%**	**(#=51)**
VRNA 0:	99%	(#=4047)
VRNA 1:	0%	(#=4)
VRNA 2:	0%	(#=5)
VRNA 3:	**1%**	**(#=40)**
Vprot 0:	99%	(#=4069)
Vprot 1:	0%	(#=11)
Vprot 2:	0%	(#=13)
Vprot 3:	**0%**	**(#=3)**
pIRF3 0:	99%	(#=4065)
pIRF3 1:	0%	(#=5)
pIRF3 2:	0%	(#=12)
pIRF3 3:	**0%**	**(#=14)**
IFNi 0:	100%	(#=4076)
IFNi 1:	0%	(#=9)
IFNi 2:	0%	(#=5)
IFNi 3:	**0%**	**(#=6)**
pSTAT 0:	72%	(#=2948)
pSTAT 1:	**16%**	**(#=670)**
pSTAT 2:	**7%**	**(#=271)**
pSTAT 3:	**5%**	**(#=207)**
ISG 0:	89%	(#=3645)
ISG 1:	**9%**	**(#=355)**
ISG 2:	**2%**	**(#=71)**
ISG 3:	**1%**	**(#=25)**
fields:	100%	(#=4096)
dead:	0%	(#=0)

IFNeU/node:	4493
IFNeL/node:	69

Example simulation

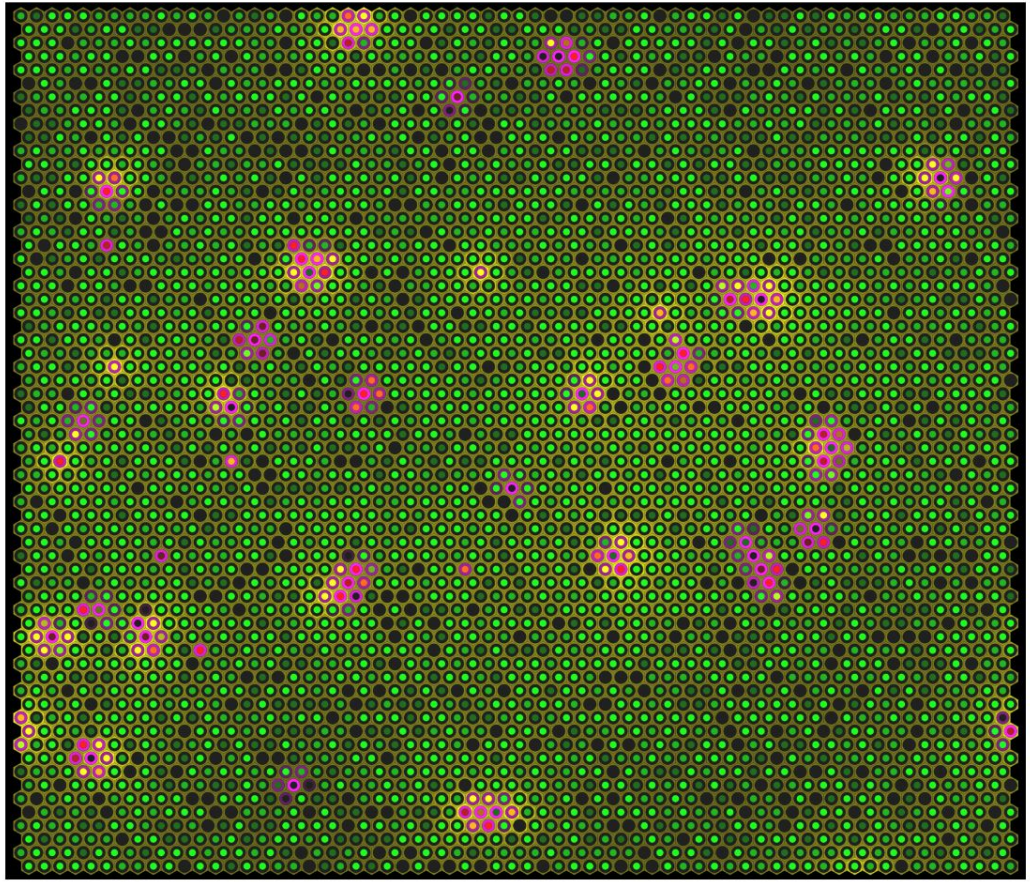


Time: 24h

Vinf 0:	98%	(#=3997)
Vinf 1:	**2%**	**(#=99)**
VRNA 0:	98%	(#=4015)
VRNA 1:	0%	(#=15)
VRNA 2:	0%	(#=8)
VRNA 3:	**1%**	**(#=58)**
Vprot 0:	99%	(#=4058)
Vprot 1:	0%	(#=11)
Vprot 2:	0%	(#=11)
Vprot 3:	**0%**	**(#=16)**
pIRF3 0:	99%	(#=4051)
pIRF3 1:	0%	(#=10)
pIRF3 2:	0%	(#=13)
pIRF3 3:	**1%**	**(#=22)**
IFNi 0:	100%	(#=4076)
IFNi 1:	0%	(#=2)
IFNi 2:	0%	(#=6)
IFNi 3:	**0%**	**(#=12)**
pSTAT 0:	46%	(#=1893)
pSTAT 1:	**24%**	**(#=963)**
pSTAT 2:	**16%**	**(#=644)**
pSTAT 3:	**15%**	**(#=596)**
ISG 0:	60%	(#=2456)
ISG 1:	**25%**	**(#=1018)**
ISG 2:	**9%**	**(#=378)**
ISG 3:	**6%**	**(#=244)**
fields:	100%	(#=4096)
dead:	0%	(#=0)

IFNeU/node:	10925
IFNeL/node:	154

Example simulation

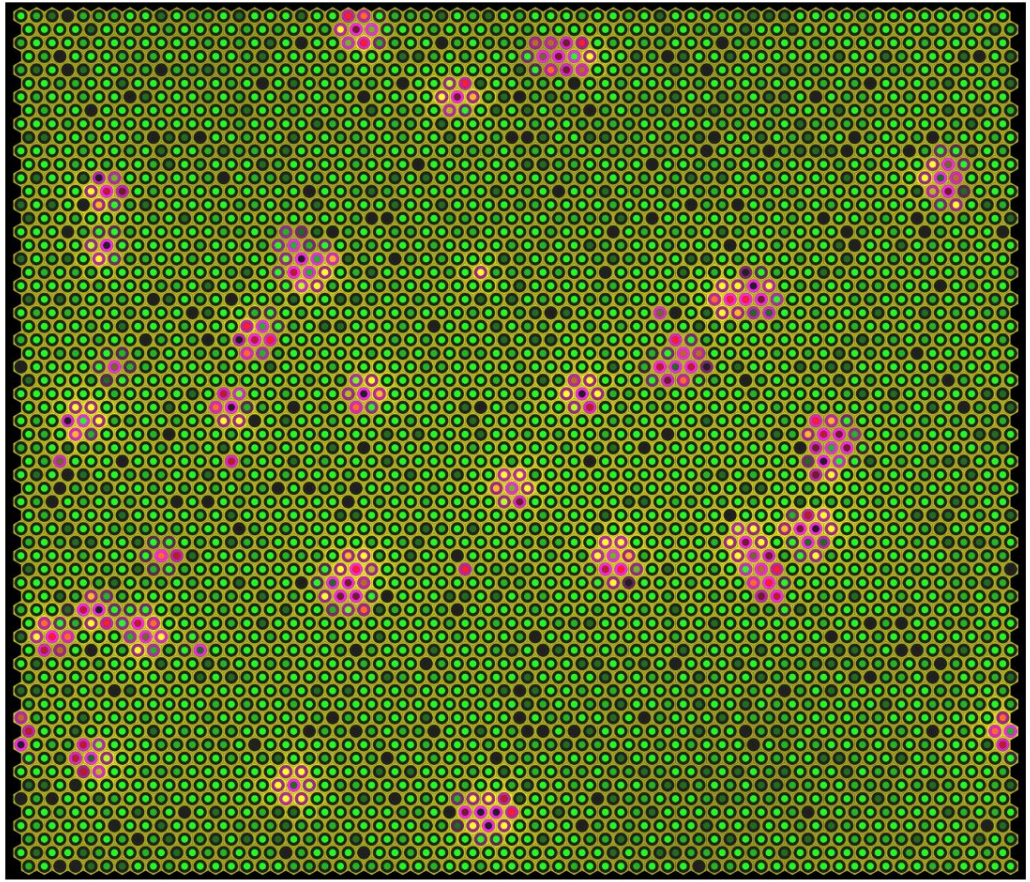


Time: 48h

Vinf 0:	94%	(#=3863)
Vinf 1:	**6%**	**(#=233)**
VRNA 0:	95%	(#=3883)
VRNA 1:	1%	(#=26)
VRNA 2:	1%	(#=34)
VRNA 3:	**4%**	**(#=153)**
Vprot 0:	98%	(#=3996)
Vprot 1:	1%	(#=35)
Vprot 2:	1%	(#=24)
Vprot 3:	**1%**	**(#=41)**
pIRF3 0:	97%	(#=3966)
pIRF3 1:	1%	(#=22)
pIRF3 2:	1%	(#=26)
pIRF3 3:	**2%**	**(#=82)**
IFNi 0:	98%	(#=4033)
IFNi 1:	0%	(#=15)
IFNi 2:	0%	(#=19)
IFNi 3:	**1%**	**(#=29)**
pSTAT 0:	11%	(#=436)
pSTAT 1:	**16%**	**(#=643)**
pSTAT 2:	**27%**	**(#=1099)**
pSTAT 3:	**47%**	**(#=1918)**
ISG 0:	4%	(#=165)
ISG 1:	**11%**	**(#=432)**
ISG 2:	**21%**	**(#=843)**
ISG 3:	**65%**	**(#=2656)**
fields:	100%	(#=4096)
dead:	0%	(#=0)

IFNeU/node:	38569
IFNeL/node:	502

Example simulation

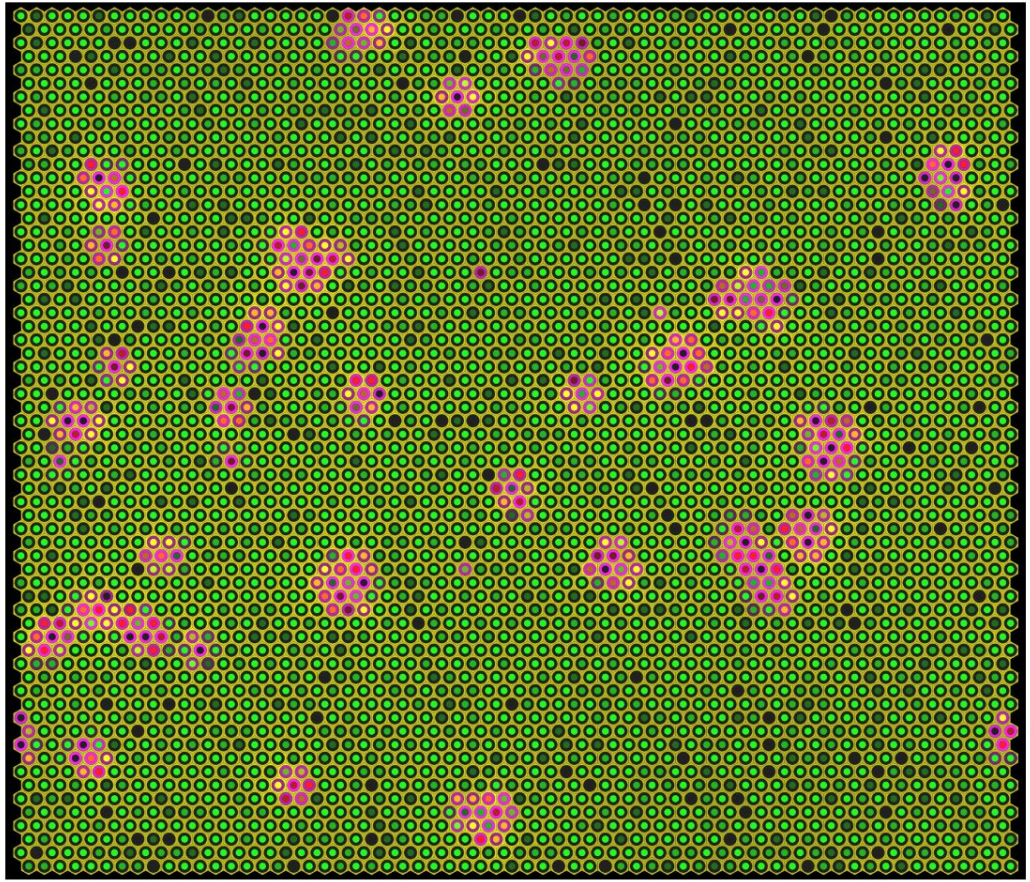


Time: 72h

Vinf 0:	92%	(#=3787)
Vinf 1:	**8%**	**(#=309)**
VRNA 0:	93%	(#=3808)
VRNA 1:	1%	(#=27)
VRNA 2:	1%	(#=29)
VRNA 3:	**6%**	**(#=232)**
Vprot 0:	96%	(#=3938)
Vprot 1:	1%	(#=52)
Vprot 2:	1%	(#=38)
Vprot 3:	**2%**	**(#=68)**
pIRF3 0:	95%	(#=3909)
pIRF3 1:	1%	(#=40)
pIRF3 2:	1%	(#=46)
pIRF3 3:	**2%**	**(#=101)**
IFNi 0:	97%	(#=3985)
IFNi 1:	1%	(#=25)
IFNi 2:	1%	(#=29)
IFNi 3:	**1%**	**(#=57)**
pSTAT 0:	5%	(#=224)
pSTAT 1:	**10%**	**(#=409)**
pSTAT 2:	**23%**	**(#=933)**
pSTAT 3:	**62%**	**(#=2530)**
ISG 0:	0%	(#=19)
ISG 1:	**2%**	**(#=87)**
ISG 2:	**11%**	**(#=471)**
ISG 3:	**86%**	**(#=3519)**
fields:	100%	(#=4096)
dead:	0%	(#=0)

IFNeU/node:	103712
IFNeL/node:	1267

Example simulation

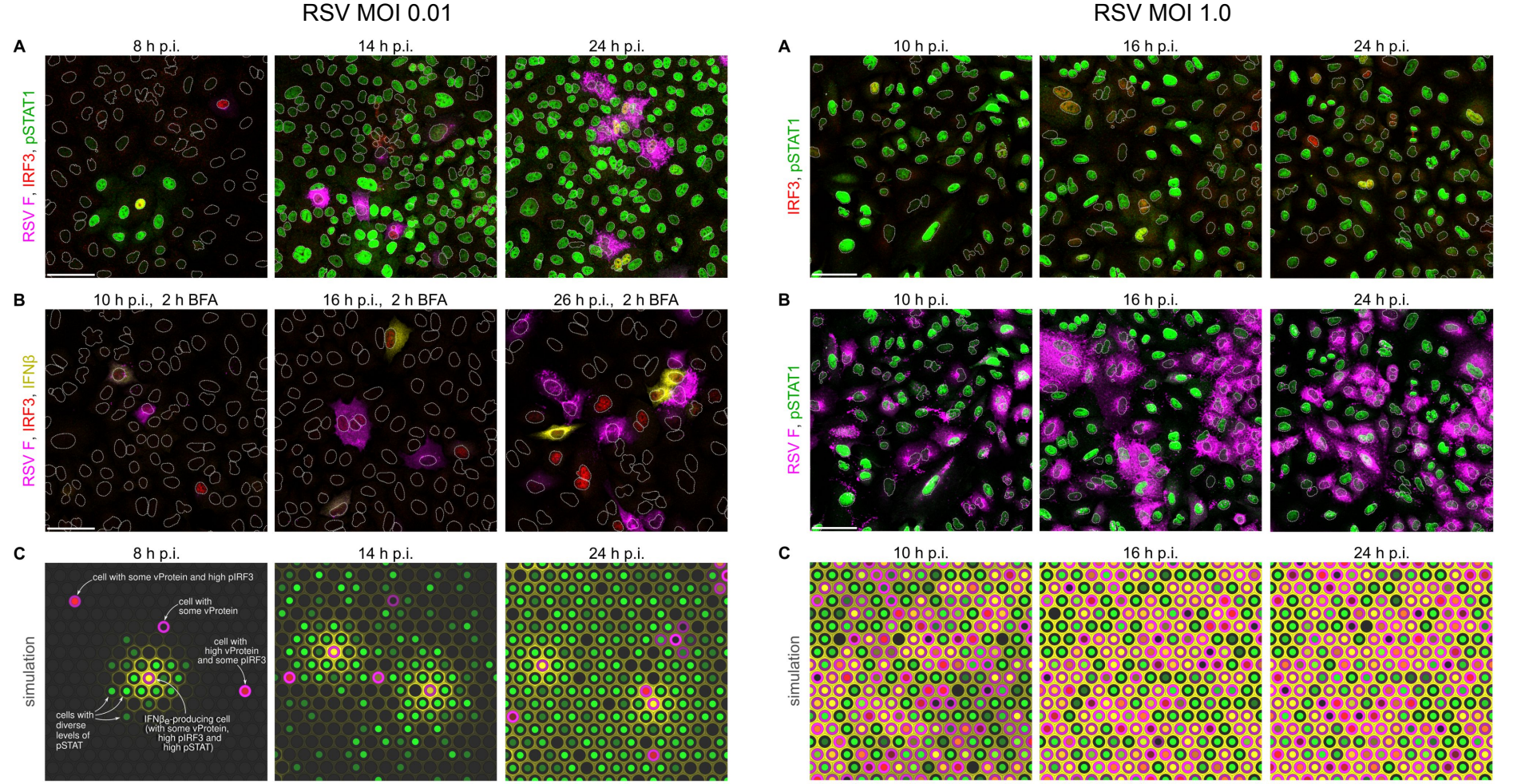


Time: 96h

Vinf 0:	90%	(#=3695)
Vinf 1:	**10%**	**(#=401)**
VRNA 0:	91%	(#=3729)
VRNA 1:	1%	(#=35)
VRNA 2:	0%	(#=17)
VRNA 3:	**8%**	**(#=315)**
Vprot 0:	94%	(#=3853)
Vprot 1:	2%	(#=80)
Vprot 2:	1%	(#=52)
Vprot 3:	**3%**	**(#=111)**
pIRF3 0:	94%	(#=3852)
pIRF3 1:	1%	(#=49)
pIRF3 2:	1%	(#=57)
pIRF3 3:	**3%**	**(#=138)**
IFNi 0:	97%	(#=3957)
IFNi 1:	1%	(#=39)
IFNi 2:	1%	(#=39)
IFNi 3:	**1%**	**(#=61)**
pSTAT 0:	5%	(#=200)
pSTAT 1:	**9%**	**(#=354)**
pSTAT 2:	**22%**	**(#=911)**
pSTAT 3:	**64%**	**(#=2631)**
ISG 0:	0%	(#=14)
ISG 1:	**2%**	**(#=71)**
ISG 2:	**11%**	**(#=455)**
ISG 3:	**87%**	**(#=3556)**
fields:	100%	(#=4096)
dead:	0%	(#=0)

IFNeU/node:	149236
IFNeL/node:	1742

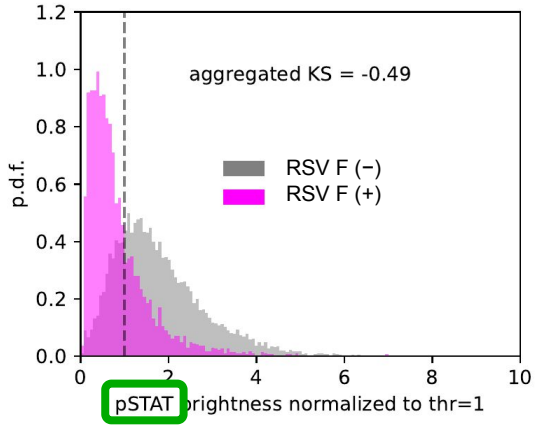
Experiment *vs.* simulation – spatially

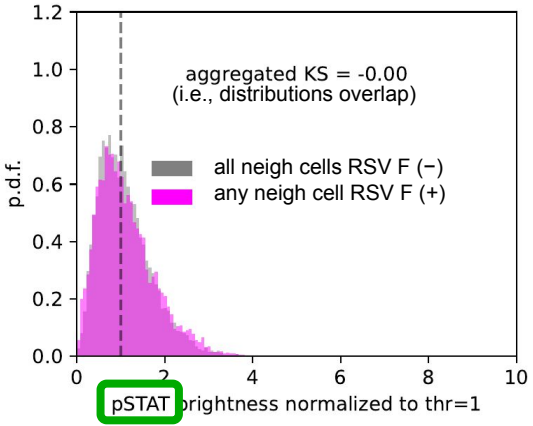


Experiment *vs.* simulation: signaling versus responding cells

one EXPERIMENT

24 h post-infection with RSV at MOI 0.1

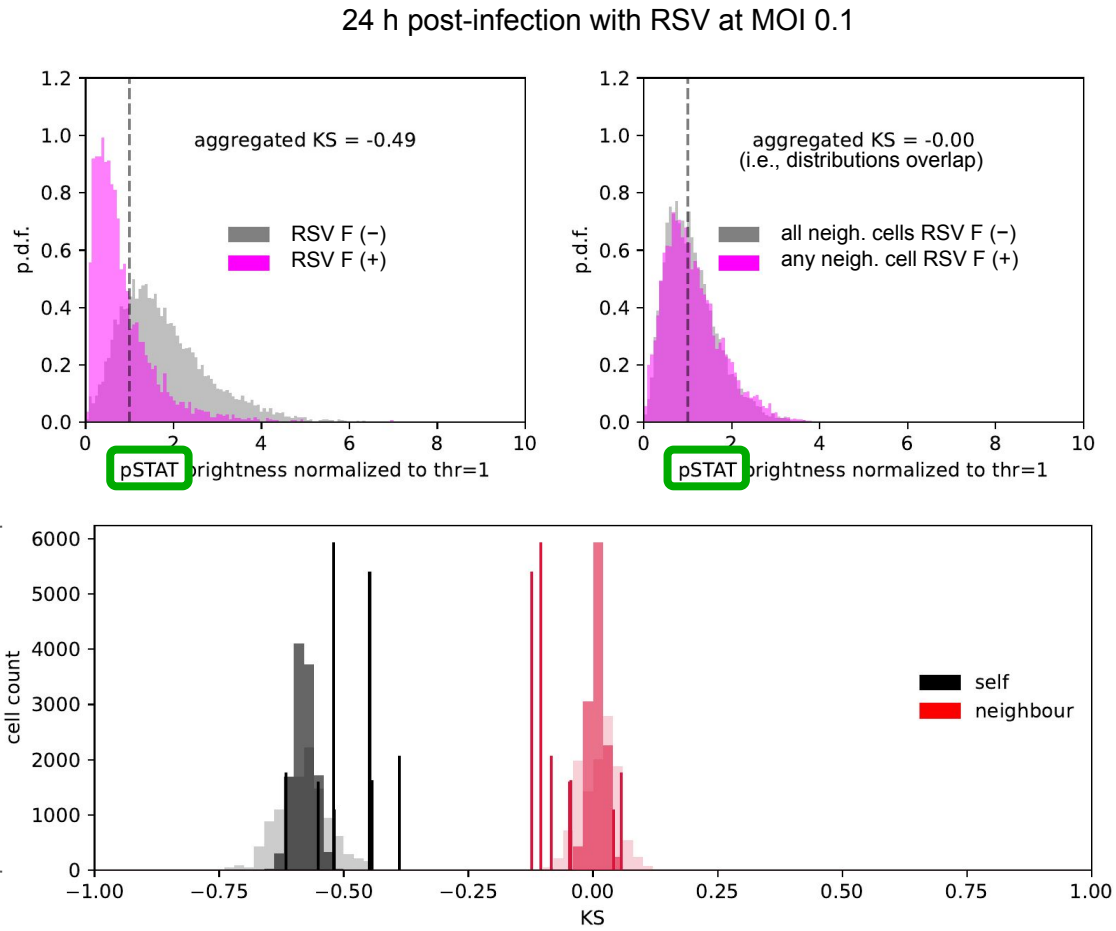




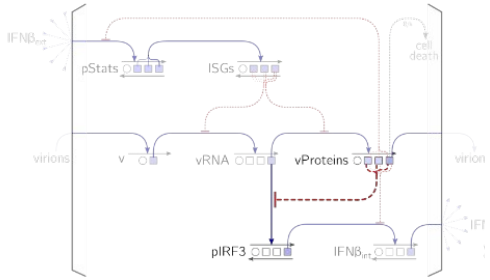
Experiment *vs.* simulation: signaling versus responding cells

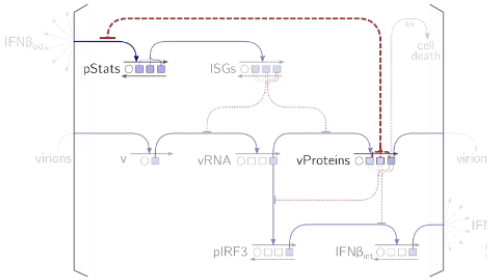
one EXPERIMENT

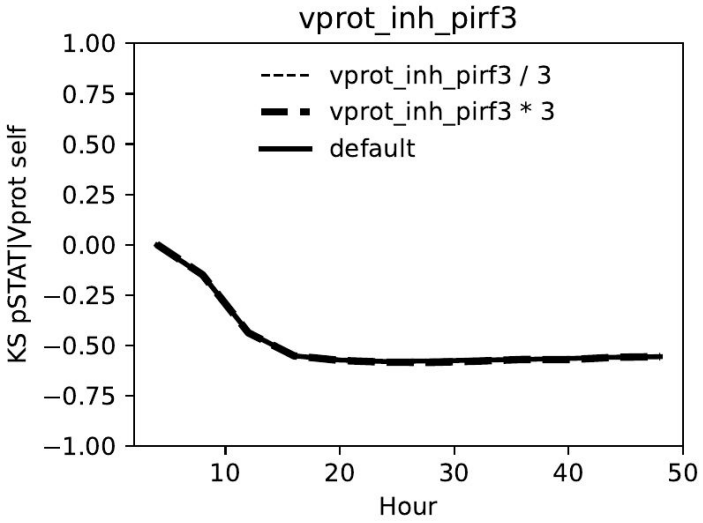
SIMULATION (bars)
EXPERIMENTs (spikes)

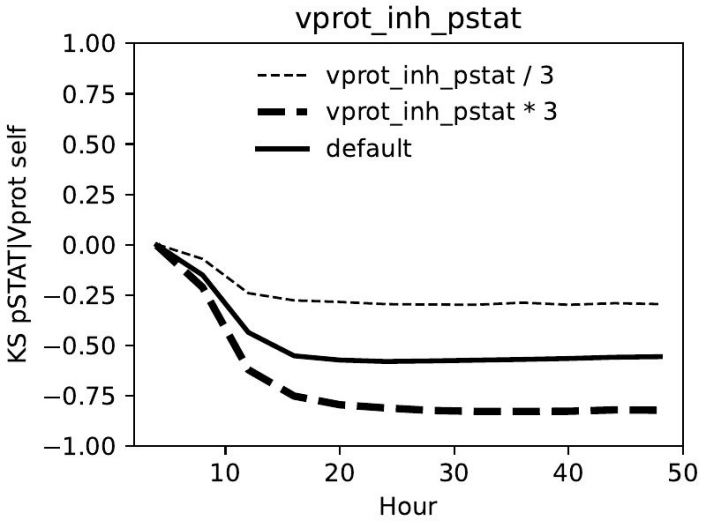


Experiment *vs.* simulation: signaling versus responding cells

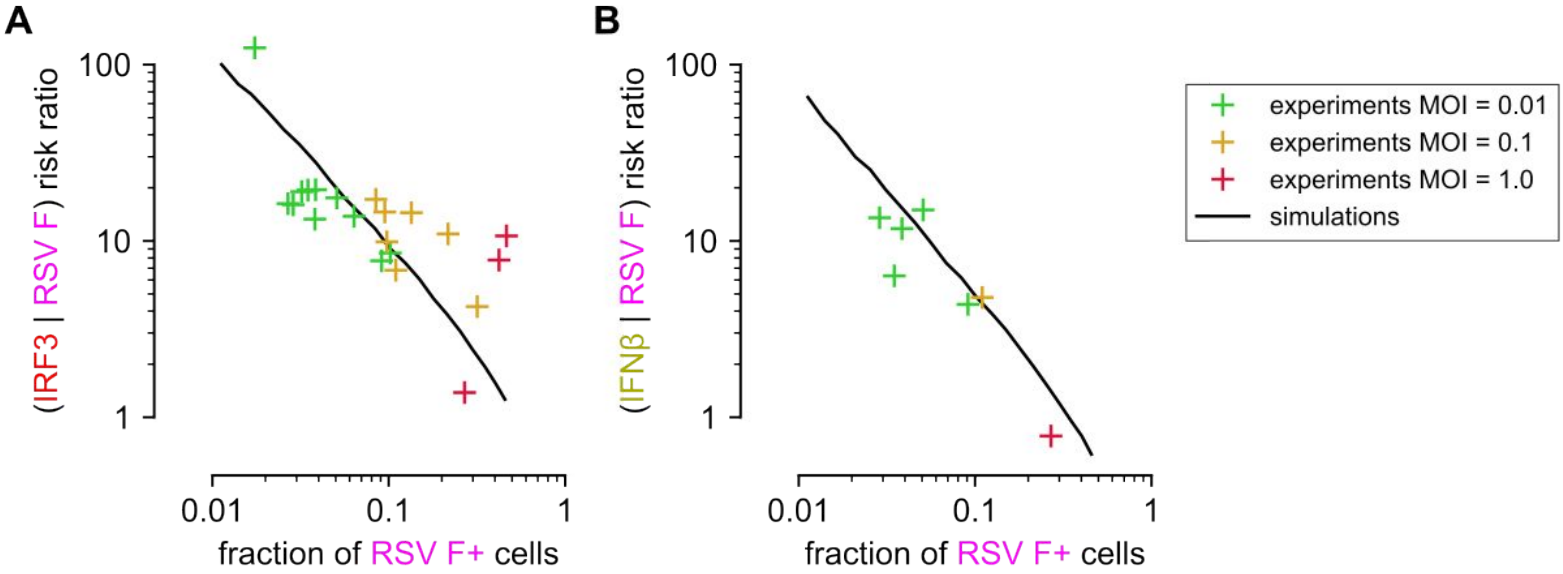








Experiment *vs.* simulation: antagonism of virus & immune response



24 h post-infection with RSV at indicated MOIs

Conclusion

Data from **cell-population** level experiments
and **single-cell** imaging data

model of: virus ↔ innate immunity

that explains **spatial** organization and **bistable** cell responses
at the single-cell level.

Thank you

EXPERIMENT: Zbigniew Kowek, Maciej Czerkies, Wiktor Prus
MODELING: Marek Kochańczyk, Frederic Grabowski, Tomasz Lipniacki

This research is funded by the **Norwegian Financial Mechanism GRIEG-1** (grant 2019/34/H/NZ6/00699, operated by the National Science Centre, Poland).