Non-self RNA rewires IFNβ signaling: A model of the innate immune response

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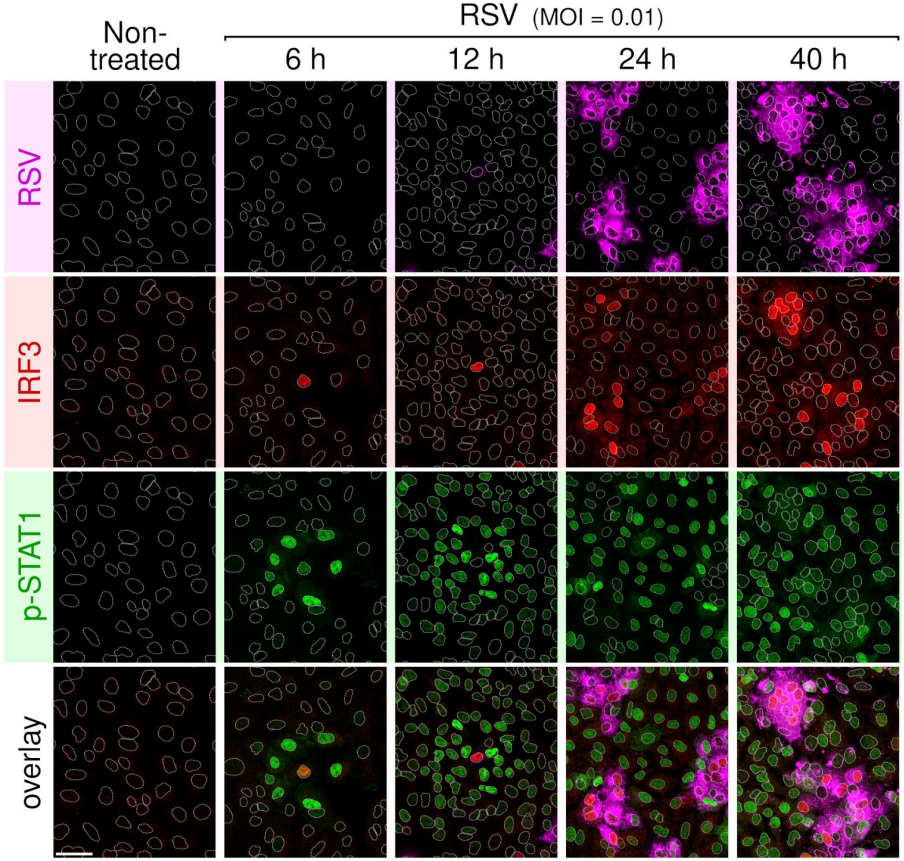
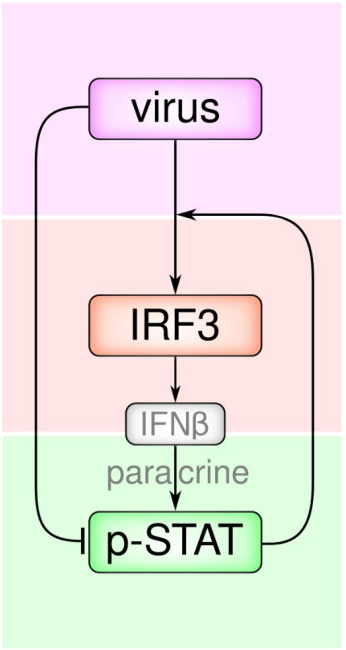
Motivation

→ “Antagonism between viral infection and innate immunity at the single-cell level”

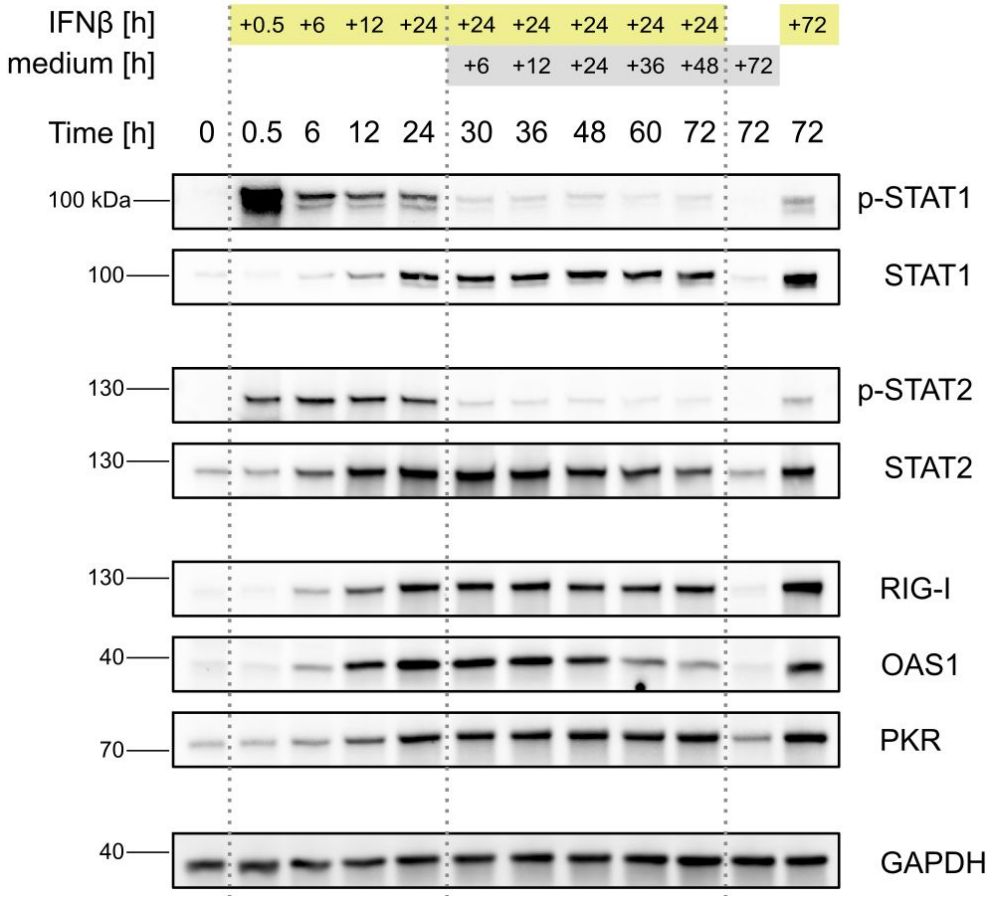
by Marek Kochańczyk
Friday, 2:20–2:40pm

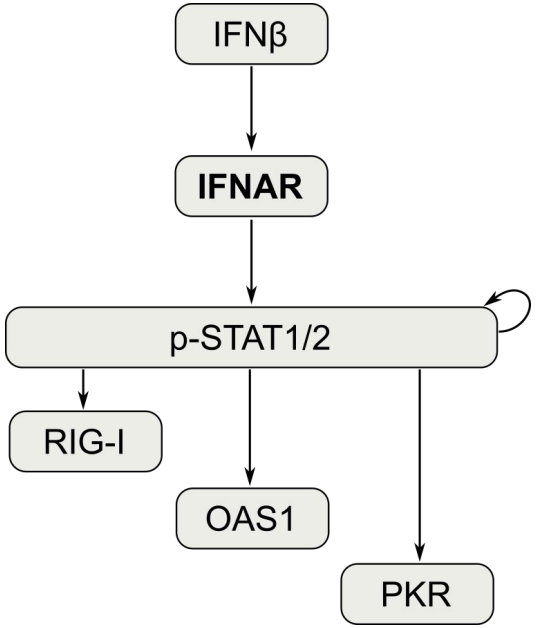




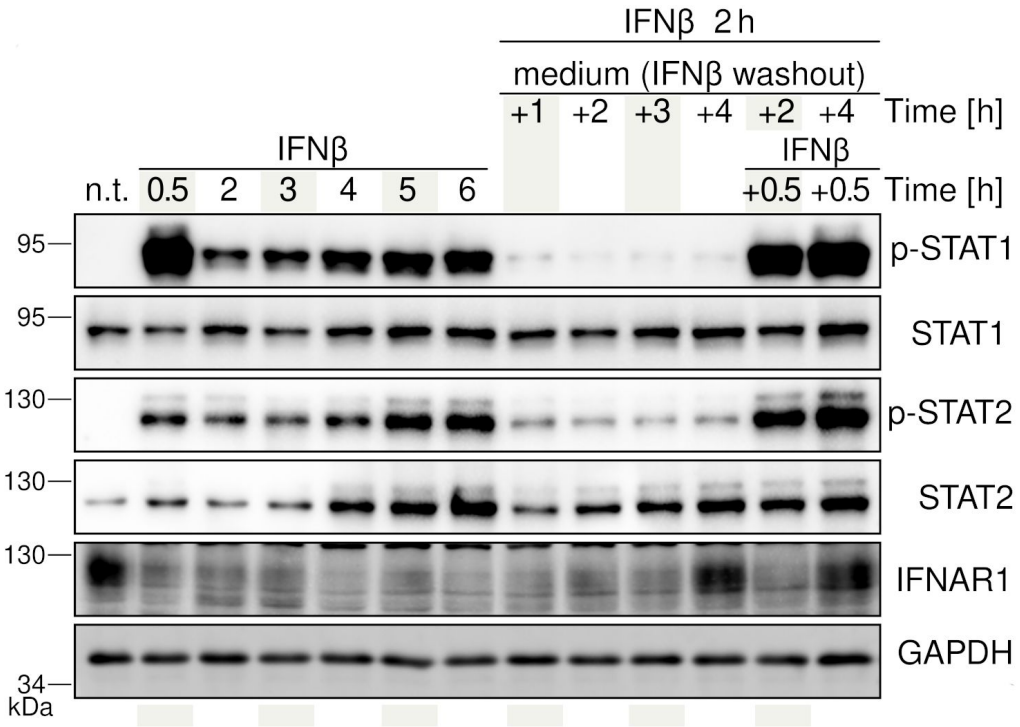
RESTING —IFNβ→ **ANTI-VIRAL** —non-self RNA / poly(I:C)→ **SIGNALING** → **IFNβ**, IL6, IL8

Kinetics of IFNβ signaling and accumulation of ISGs





Kinetics of IFNβ signaling, continued

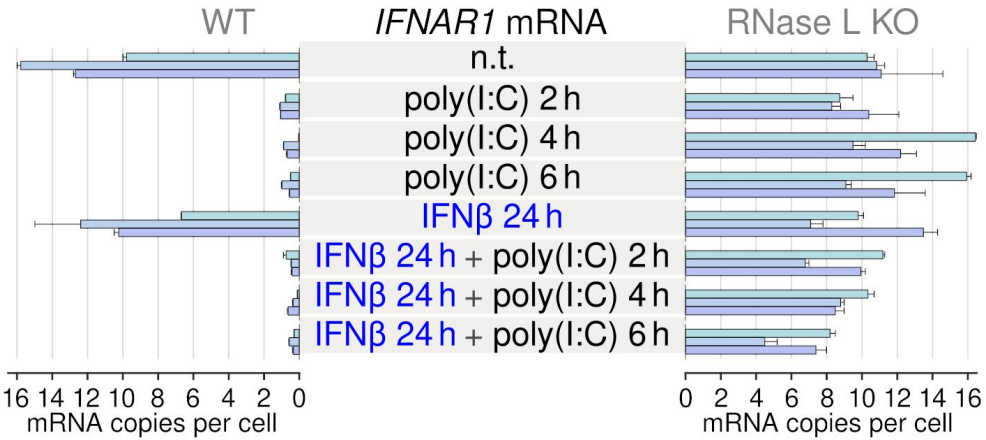


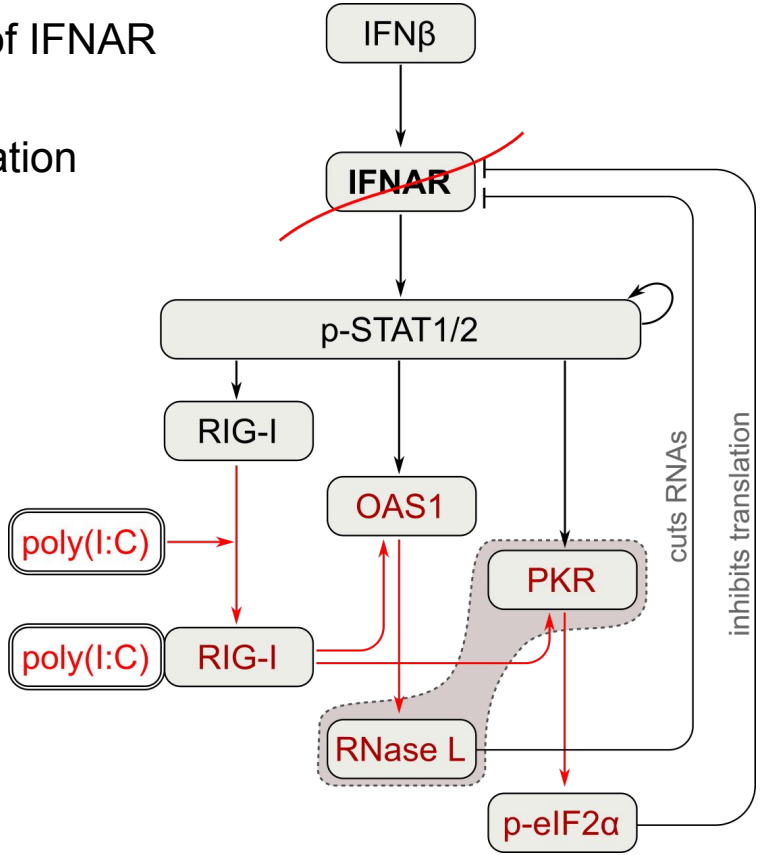
- rapid STAT1/2 dephosphorylation after IFNβ washout
- partial STAT1/2 dephosphorylation due to IFNAR endocytosis
- recovery of IFNAR after 4 h-long break and strong response to the second pulse of IFNβ

Stimulation with poly(I:C)

leads to activation of RNase L and PKR and depletion of IFNAR

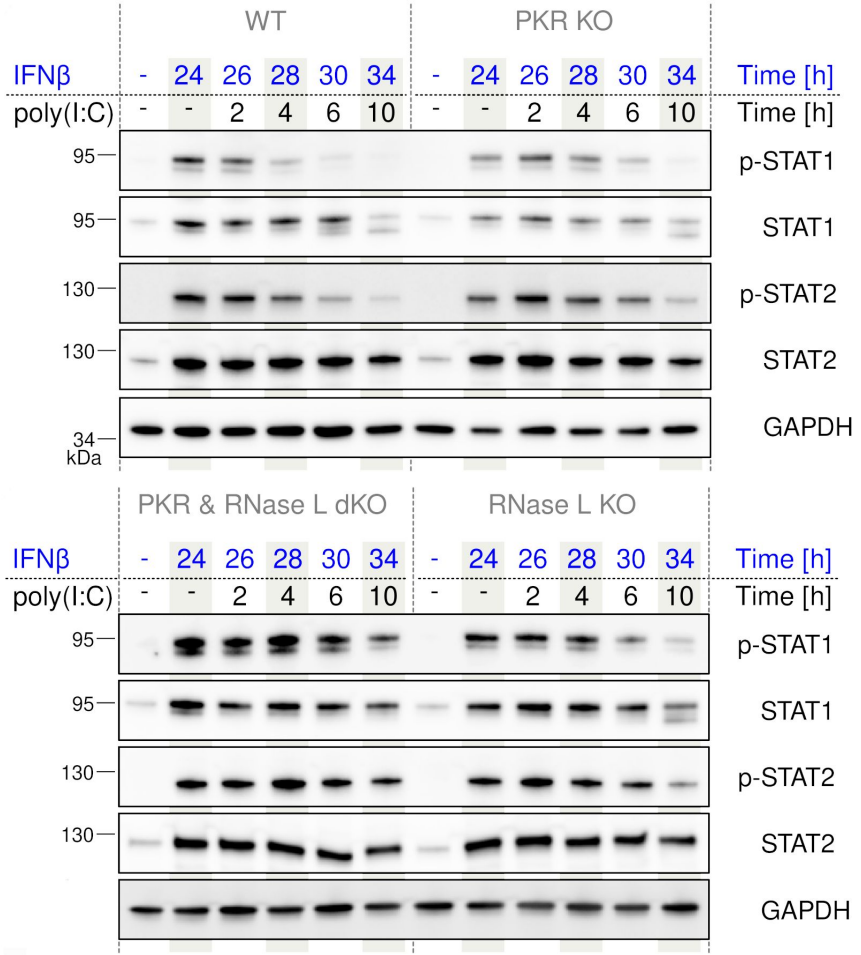
by degradation of IFNAR mRNA and inhibition of translation

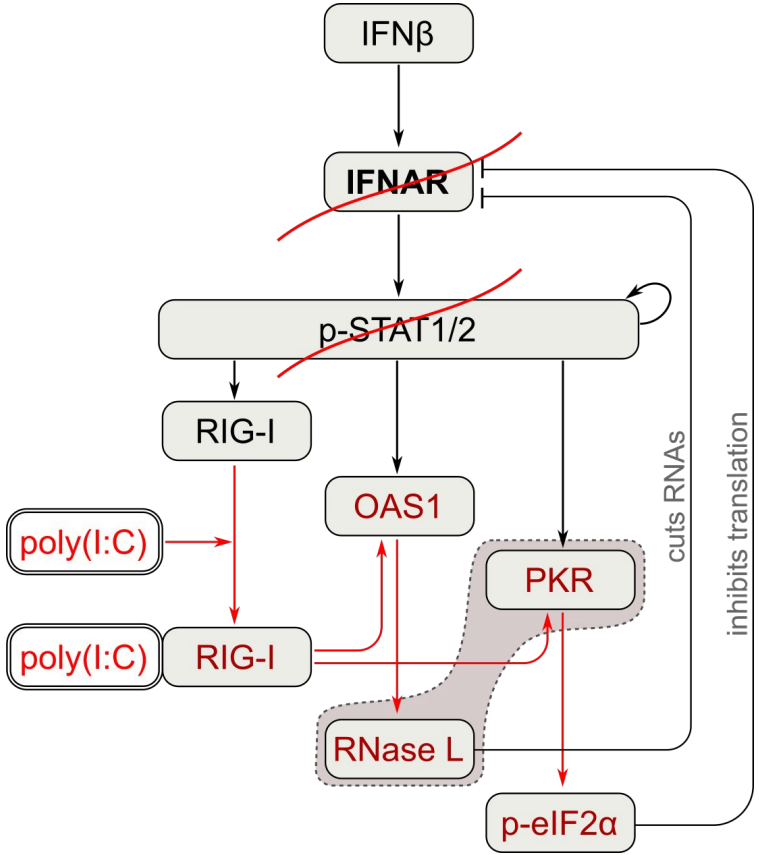




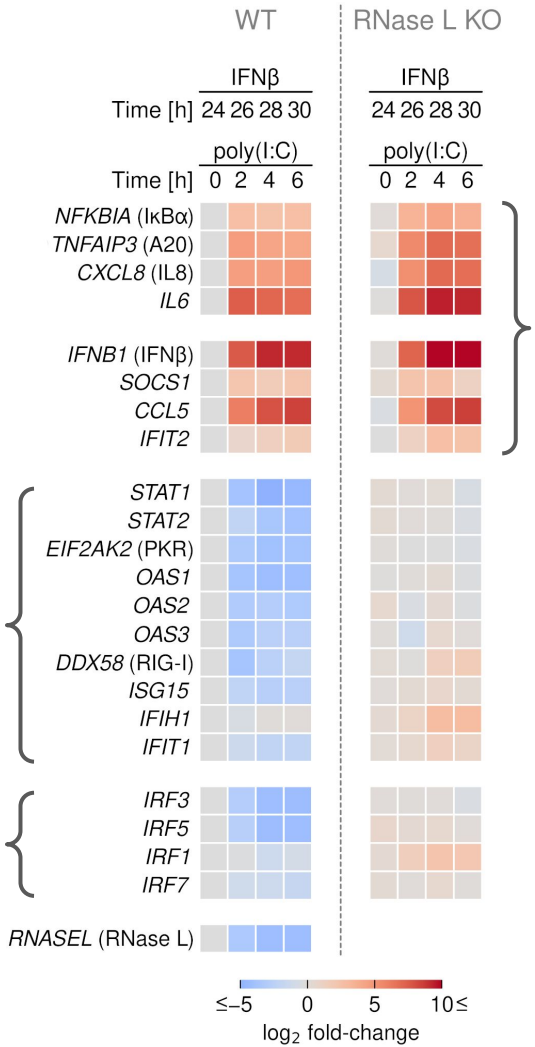
and…

termination of STAT1/2 activity





poly(I:C)-activated RNase L:



- but leaves almost intact mRNAs of NF-κB- and IRF3-regulated genes, including IFNβ (*will come back to this*)

- cuts mRNAs of STAT1/2-regulated genes

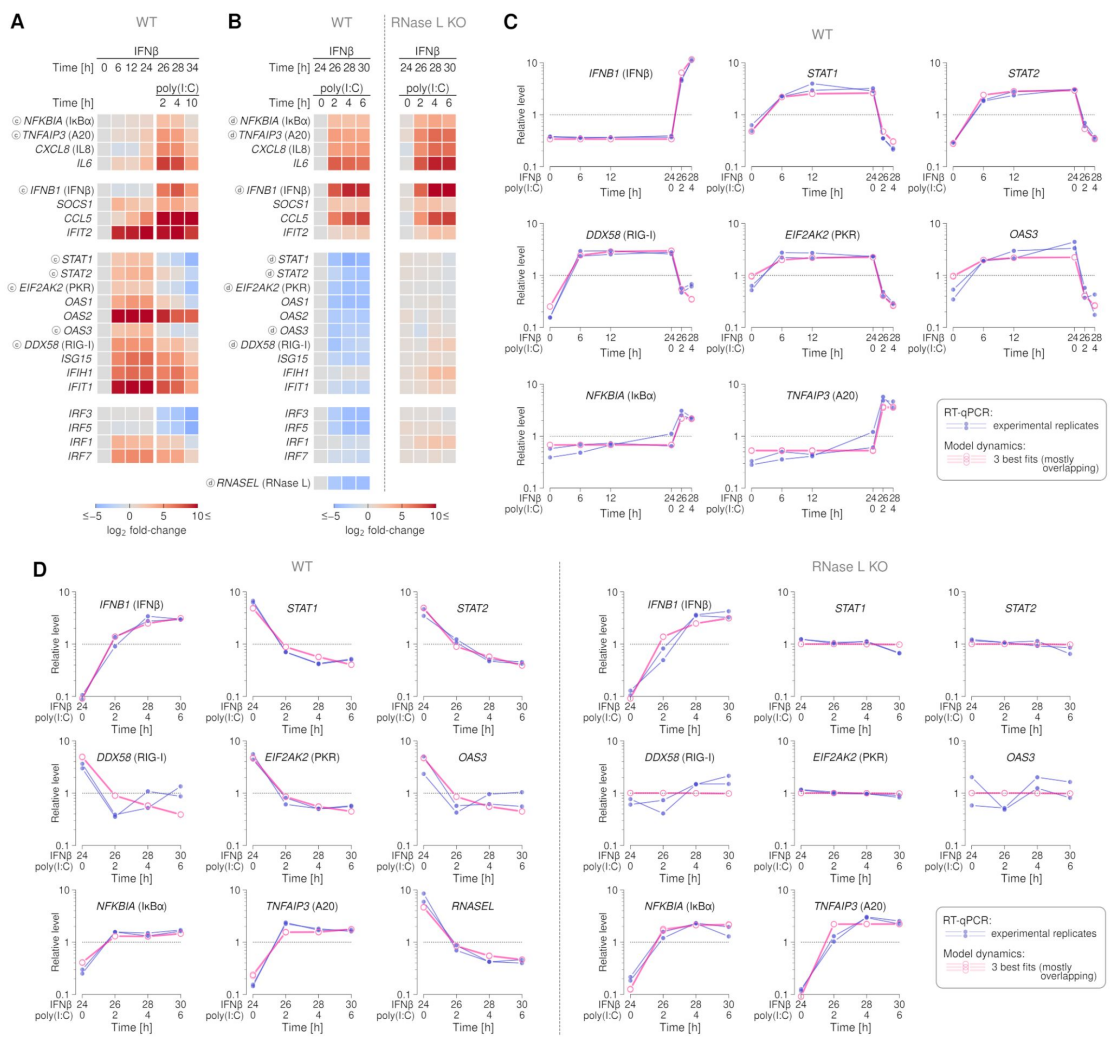
- cuts mRNAs of IRF3/1/5/7

In this way, poly(I:C) with the help of RNase L and PKR fully terminates STAT1/2 signaling.

Computational model
(ODEs) advertisement

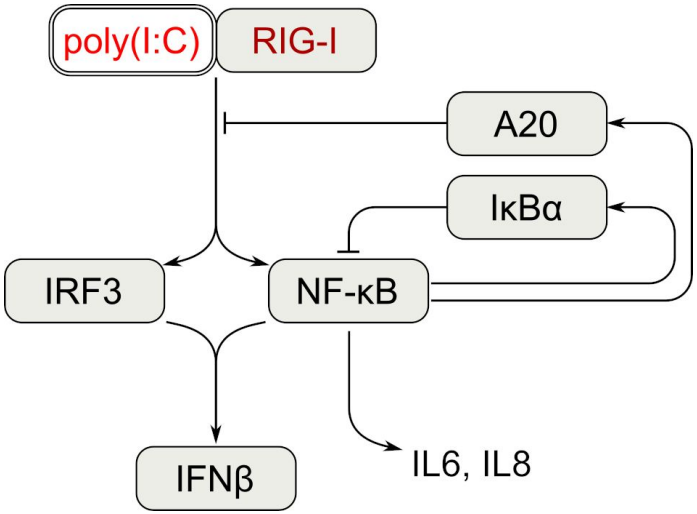
RT-qPCR:
experimental replicates

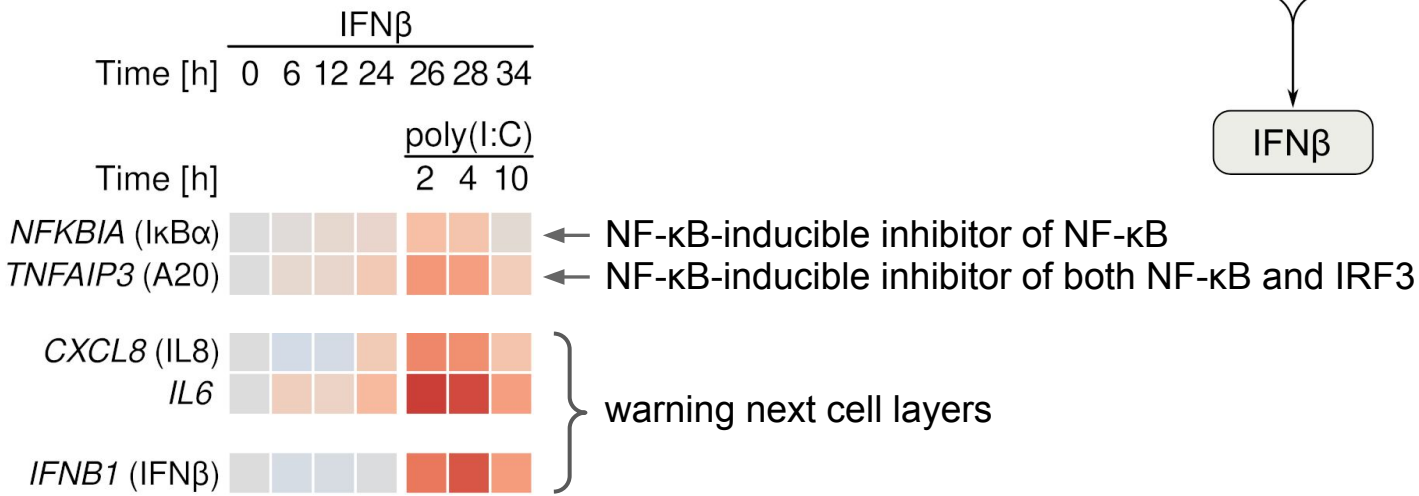
Model dynamics:
3 best fits (mostly overlapping)



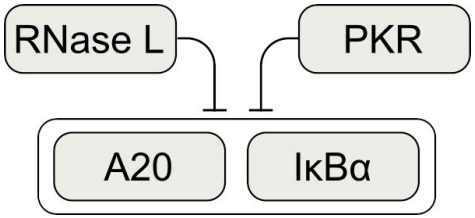
Second poly(I:C) arm

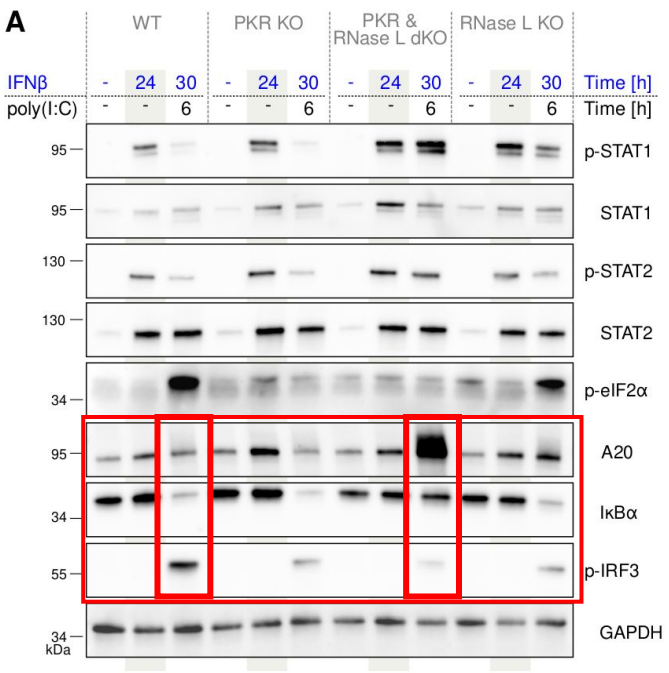
Poly(I:C) activates transcription factors NF-κB and IRF3 leading to synthesis of IFNβ and interleukin-6 and -8.

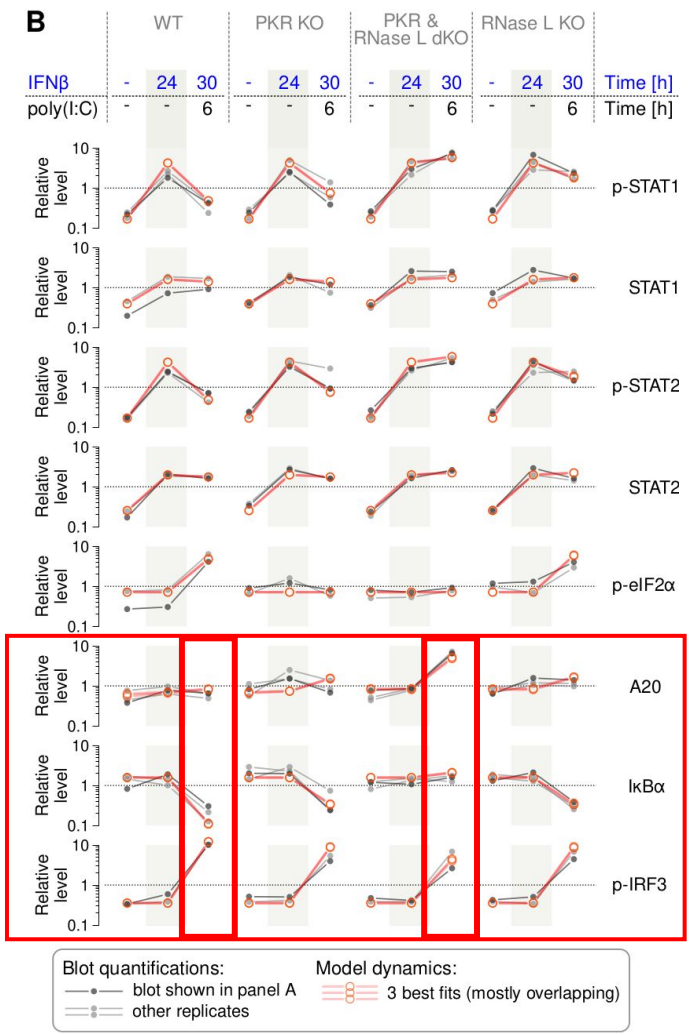




Second poly(I:C) arm is also RNase L- and PKR-dependent

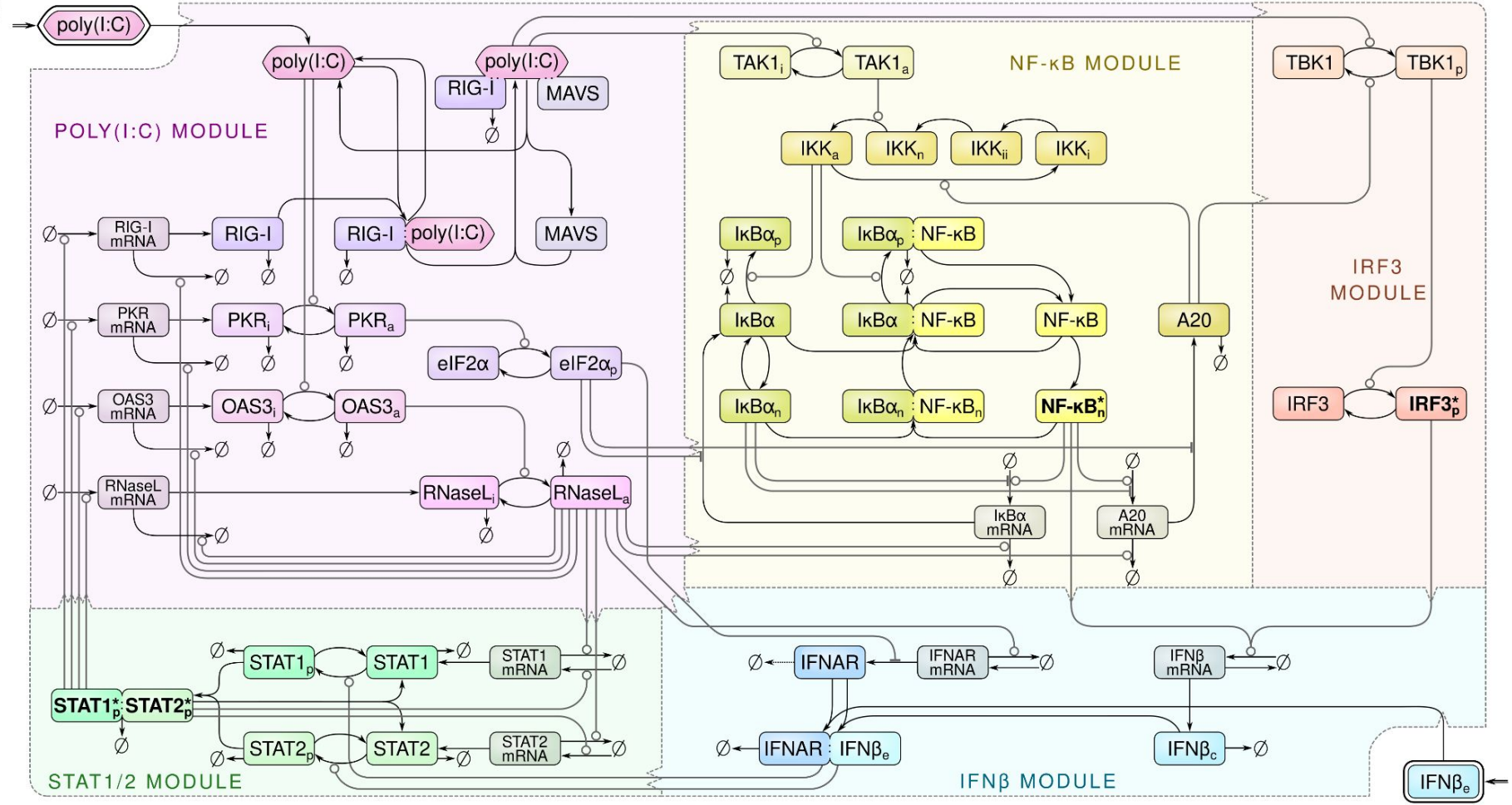






Knock-out of RNase L and PKR leads to accumulation of A20 and IκBα and thus attenuation of IRF3 phosphorylation (IRF3 activation is indispensable for IFNβ synthesis).

Computational model: ODEs, big but “finite”, constrained by data (identifiable parameters)



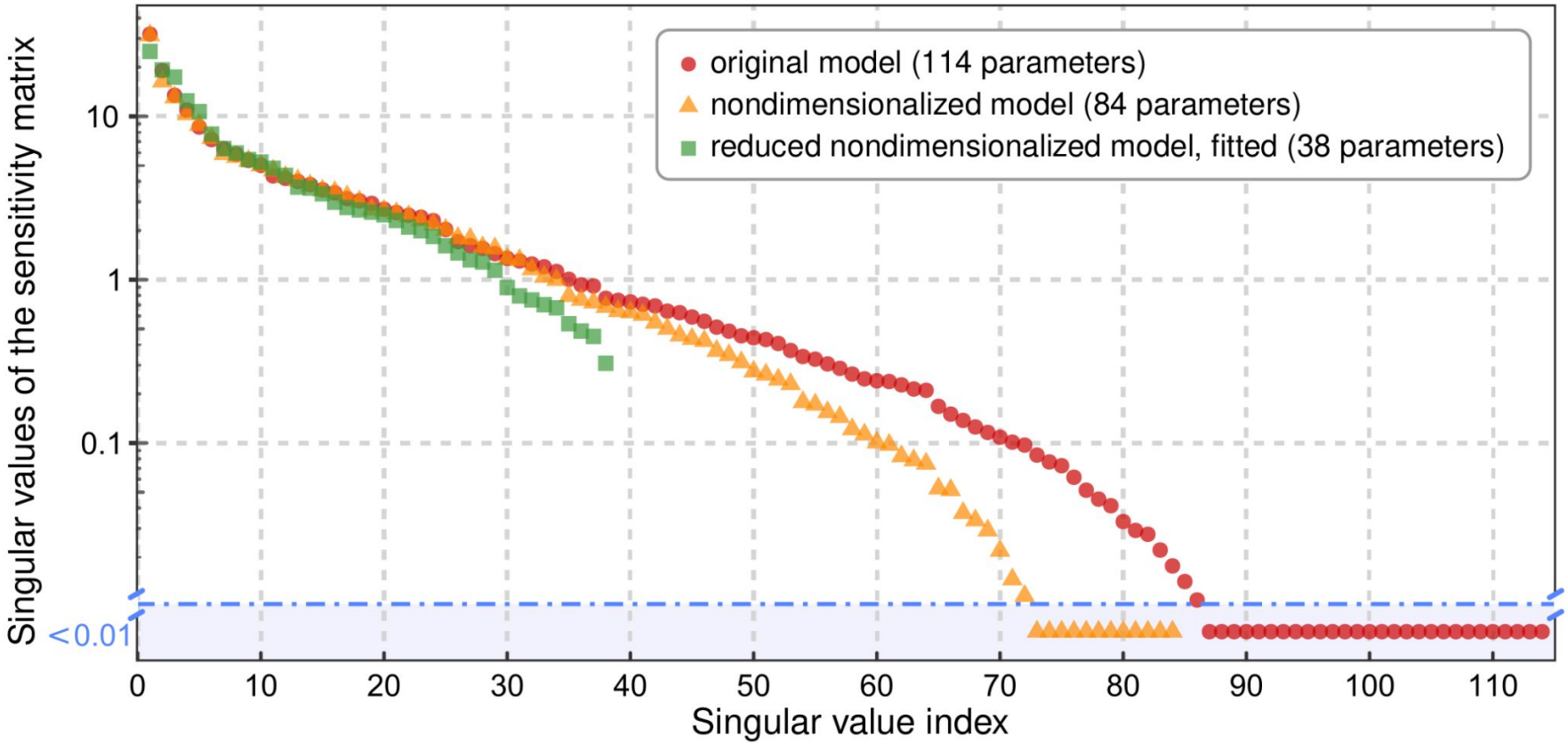
Model complexity, data used for parameter fitting, and the fitted computational model

Model complexity	
Number of variables in the model	**53**
Number of independent model parameters	**38**

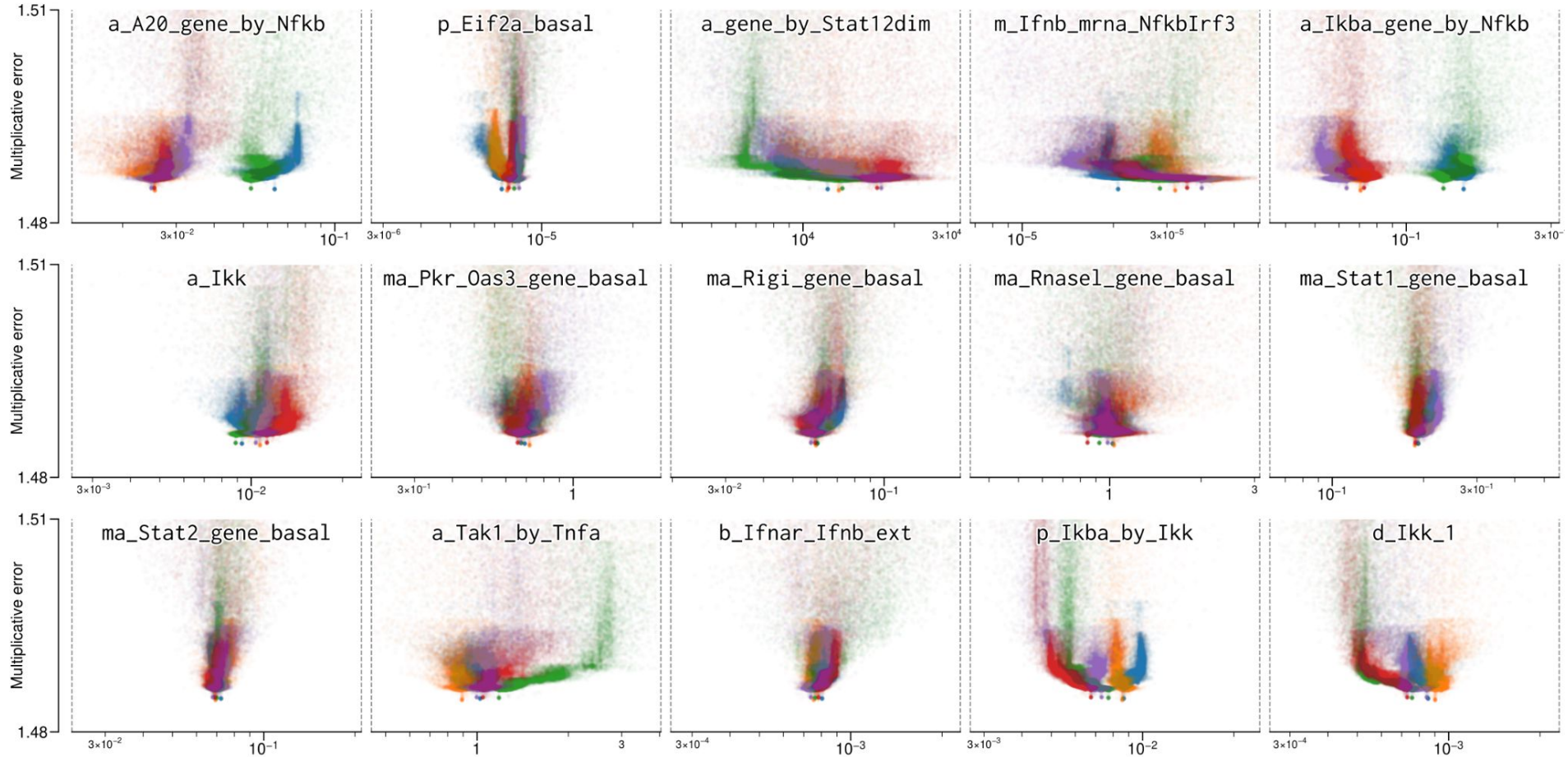
Statistics of data used for fitting (WT and four A549 KO cell lines)	
Number of experimentally measured variables	**25**
Number of independent experimental data points used for parameter fitting (dimensionality of the measurement vector space)	**2915**
Average multiplicative error between experimental replicates (for Western blots)	**1.24**

Parameter fitting	
Average multiplicative error between data and the worst of 10 best fits	**1.49**
Max pairwise error between 10 best fits	**1.04**
Maximum s.d. of log-fitted parameters (among 10 best fits)	**0.19**

Identifiability reached by nondimensionalization and systematic model reduction



Practical identifiability: “convergence” of independent stochastic fits



Conclusions

In IFNβ-primed cells, poly(I:C) via activation of RNase L and PKR:

- terminates STAT1/2 activity and leads to degradation of STAT1/2-regulated mRNAs,
- triggers activation of NF-κB and IRF3 leading to synthesis of IFNβ and IL-6, IL-8.

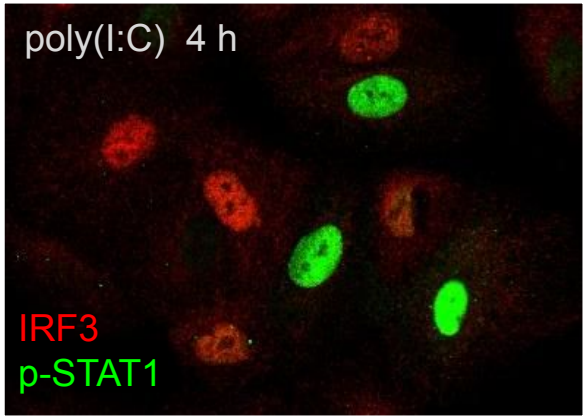
Thus poly(I:C), a viral RNA analog:

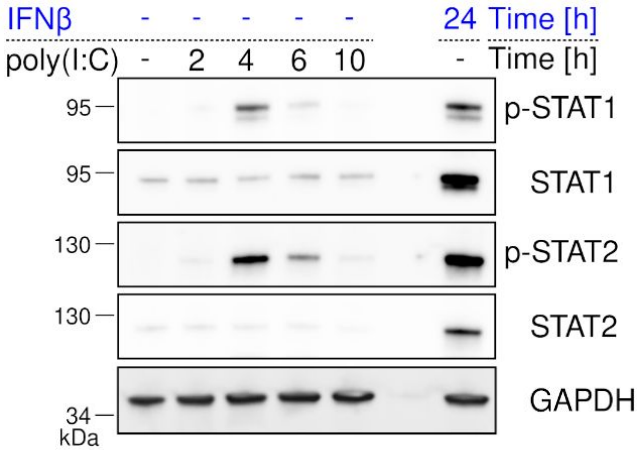
turns IFNβ-responding cells (antiviral state)
into IFNβ-producing cells (signaling state)

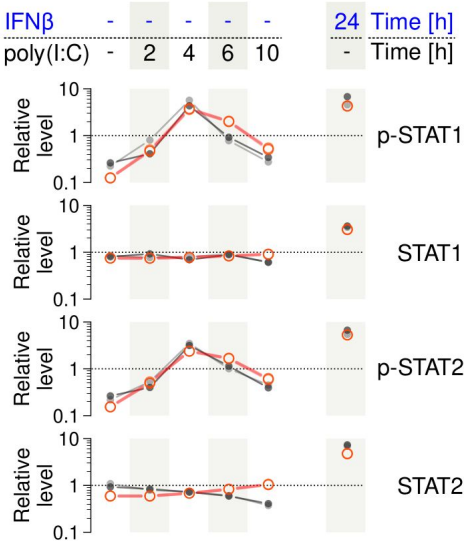
The process involves five regulatory modules and is recapitulated by an identifiable model of innate immune response to non-self RNA.

Questions?

What happens when naive cells are stimulated by poly(I:C)?







Simply:

- poly(I:C) will activate NF-κB and IRF3 leading to production of IFNβ,
- IFNβ will trigger STAT1/2 activation,
- poly(I:C) will activate also RNase L and PKR
- RNase L and PKR will lead to depletion of IFNAR and termination of STAT1/2 activity.

As a consequence we see short pulse of STAT1/2 activity.

Thank you!

Norway grants

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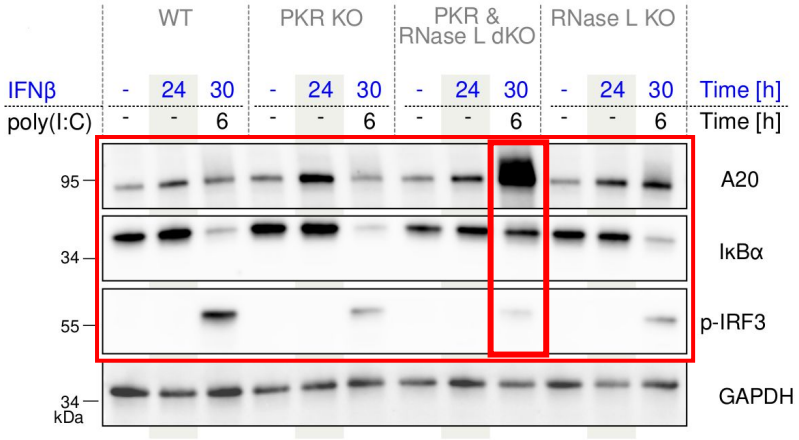
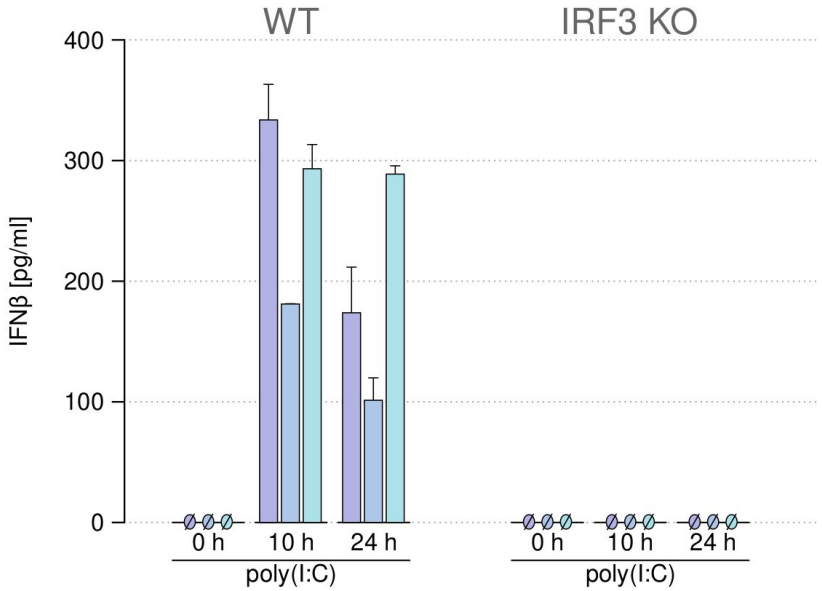
Conundrum



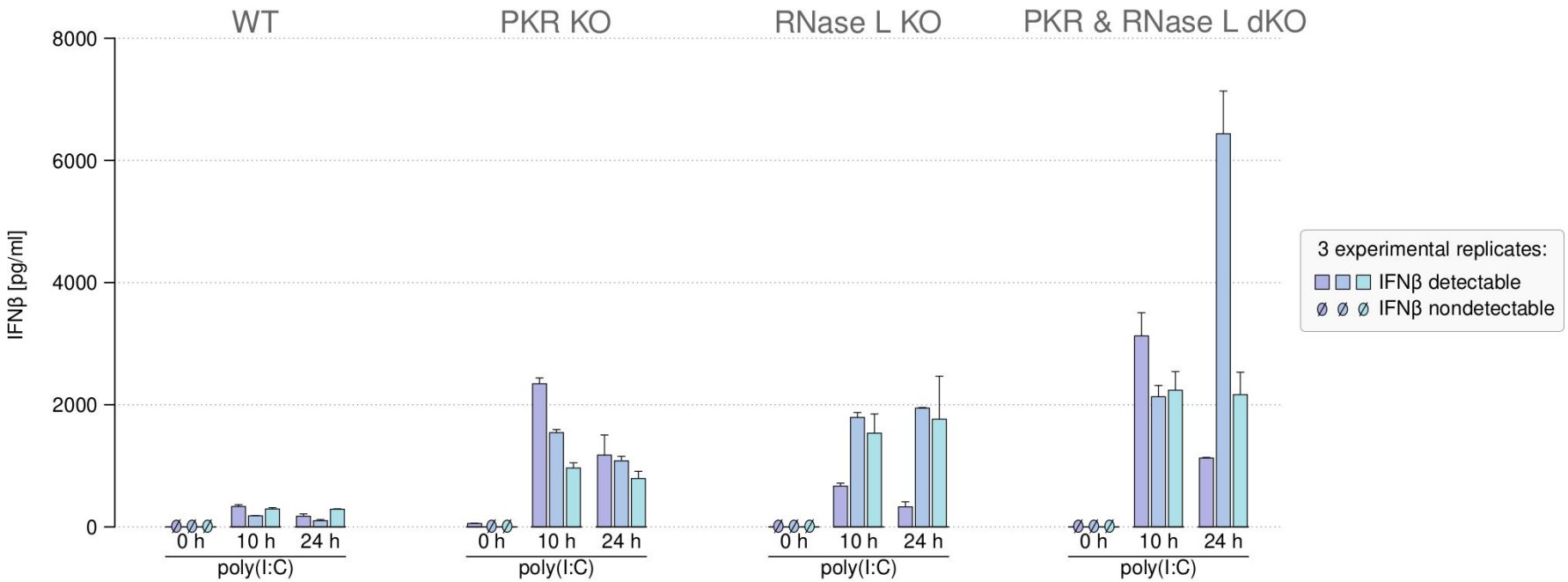
PKR and Rnase L dKO attenuate IRF3 activation



No IFNβ secretion after poly(I:C) in IRF3 KO cells

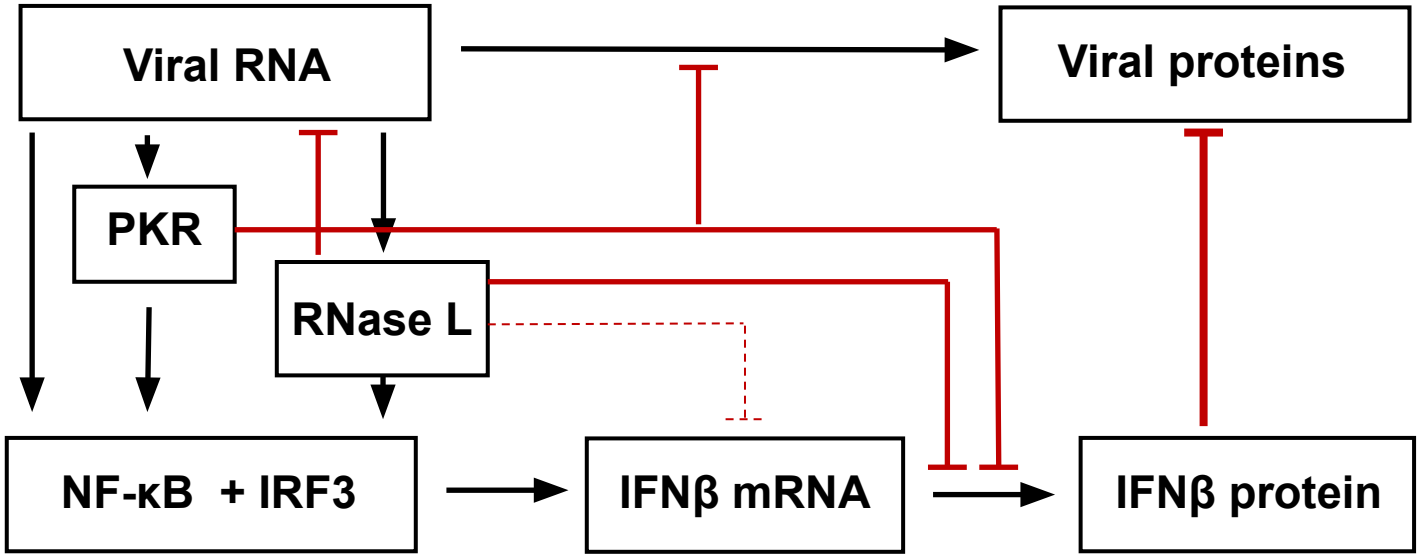
Ergo: PKR and Rnase L dKO will attenuate IFNβ secretion after poly(I:C).

NO, the opposite



Secretion of IFNβ by A549 WT, PKR KO, RNase L KO, and PKR & RNase L double KO cells after stimulation with poly(I:C). ELISA measurements of IFNβ secretion after stimulation with poly(I:C) (0.1 μg/ml) in indicated time points. Error bars represent s.e.m. for two technical replicates in each experiment.

ANY SENSE?


Viral RNA
Viral proteins
PKR
RNase L
NF-κB + IRF3
IFNβ mRNA
IFNβ protein

Yes, when the virus fights back!