Cell-cycle-gated feedback control mediates desensitization to interferon

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Introduction

Hypothesis

- Does pretreatment with IFN-α result in desensitization?
- Can a computational model be created to predict IFN responses?
- What role does the cell cycle play in the IFN response?

Results

IFN-α pretreatments confer opposite effects depending on their durations

Fig 1A. HeLa Reporter Cell Line

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Fig. 1D Schematic of IFN-α pretreatment experiments

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Fig. 1D A diagram of the microfluidic set-up

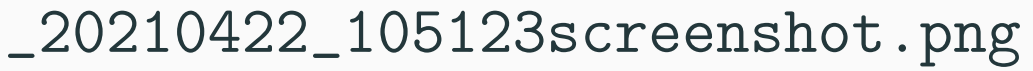

Fig. 1B Time Lapse of Cells treated for IFN-α for 48 hours

Fig. 1B Time traces of nuclear/cytoplasmic STAT1-mCherry and PIRF9-YFP signals of the cell

Fig. 1C Averaged time traces of nuclear/cytoplasmic STAT1-mCherry, PIRF9-YFP

Fig. Supp 1D Time course western blots showing the dynamics of phosphorylation (pY701), IRF9 and expression of STAT1

Fig. 1E PIRF9-driven YFP induction response to the second IFN-α treatment

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USP18 is responsible for desensitization induced by the prolonged IFN-α pretreatment

Fig. 2A Time-lapse images of STAT1 nuclear translocation

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Fig. Supp. 2A Western blots of USP18 expression in WT and USP18-KD cells

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Fig. 2B YFP induction response to the second IFN-α treatment in USP18-KD

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Fig. 1F Amounts of PIRF9-YFP induction by the second IFN-α stimulation

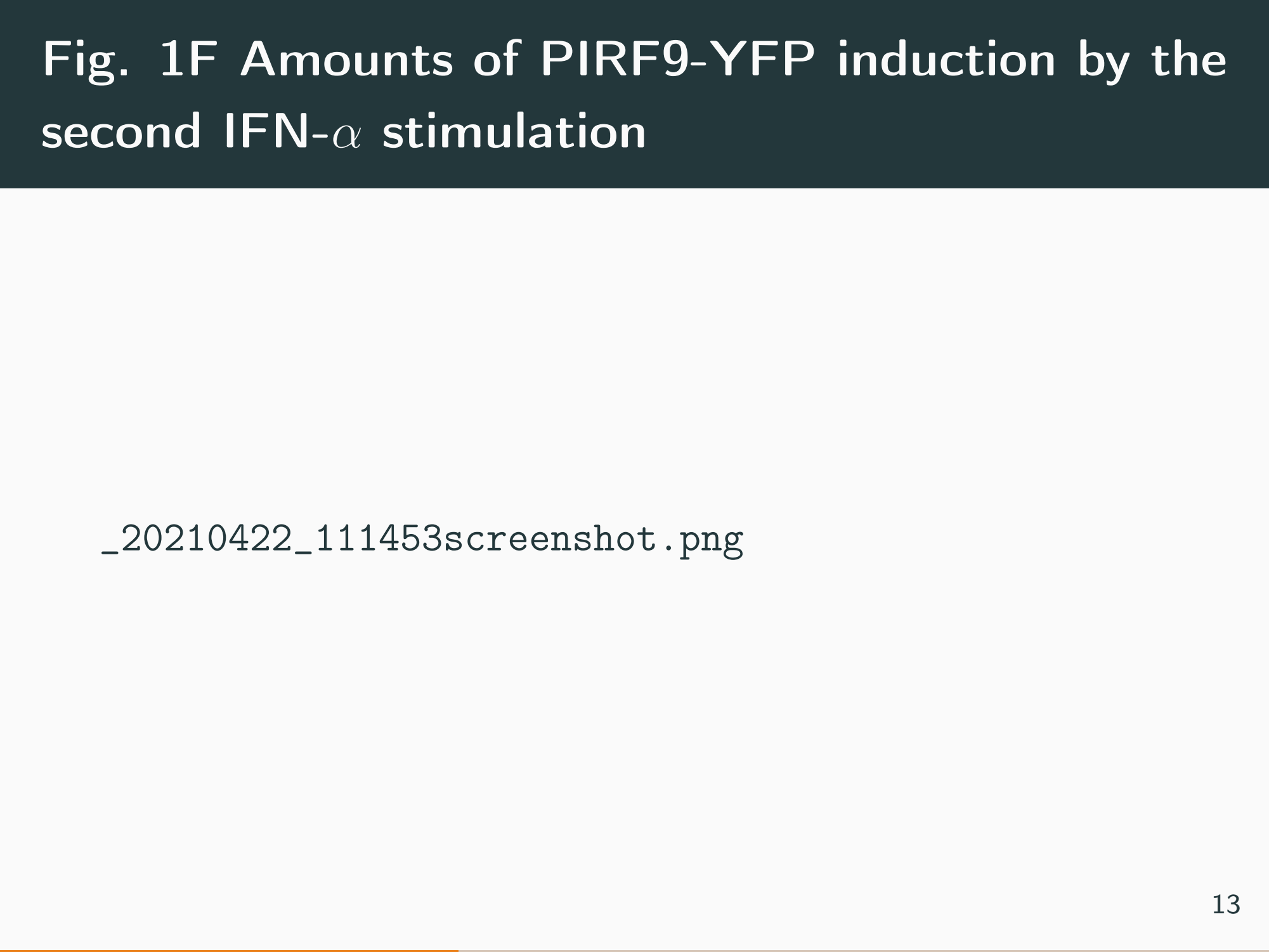

Fig. 2C Amounts of PIRF9-YFP induction in USP18-KD cells by the second IFN-α stimulation

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Computational modeling suggests a delayed negative feedback loop through USP18

Fig. 3A Simple kinetic model of the IFN-driven gene regulatory network

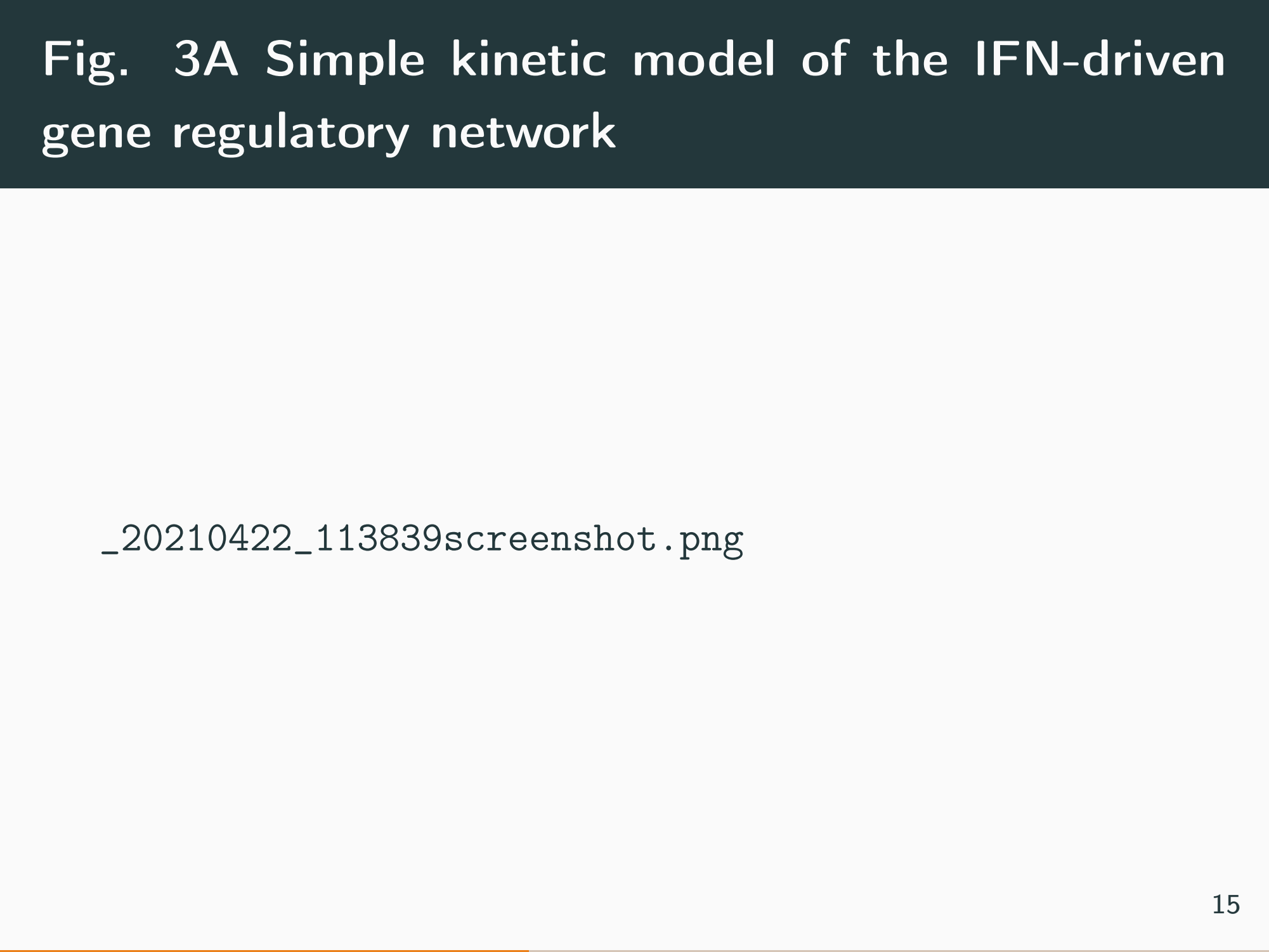

Fig. Supp 3A kinetic model of the IFN-driven gene regulatory network with parameters

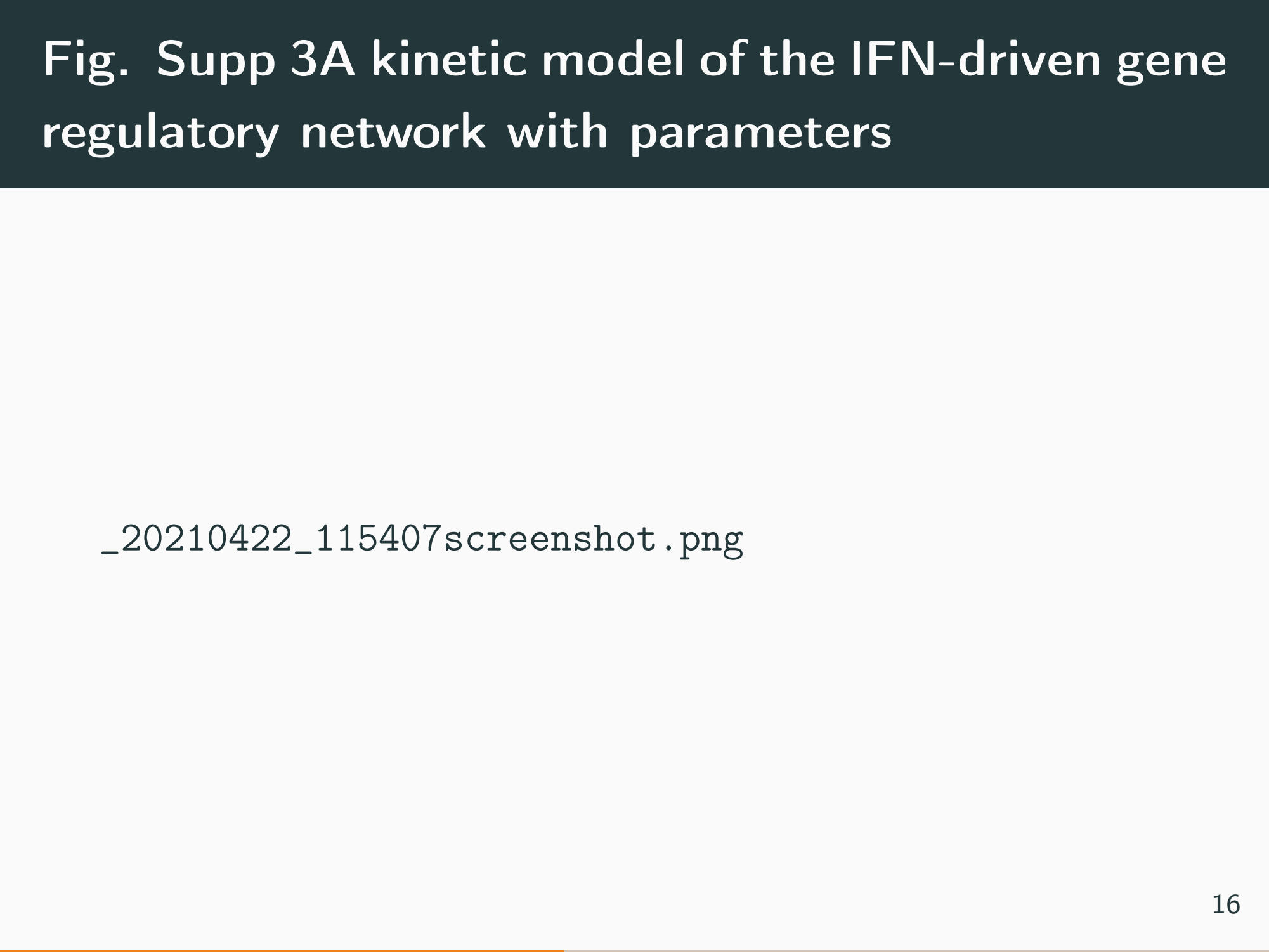

Fig. 3C Amounts of PIRF9-YFP induction by the second IFN- stimulation Predicted by model simulations

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Fig. Supplement 3B Model fitting results

Fig 3D. Experimental design with repetitive IFN pulses

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Fig. 3E Model prediction of the responses to pulse versus sustained IFN inputs

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Fig. 3F Experimental data of the responses to pulse versus sustained IFN inputs

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The kinetics of USP18 upregulation by IFN is heterogeneous in single cells

Fig. 4A Dual reporter cell line schematic

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Fig. 4B Time traces of PIRF9-YFP and PUSP18-CFP of a single cell in response to IFN-

Fig 4C. Distributions of PIRF9 and PUSP18 activation times in single cells

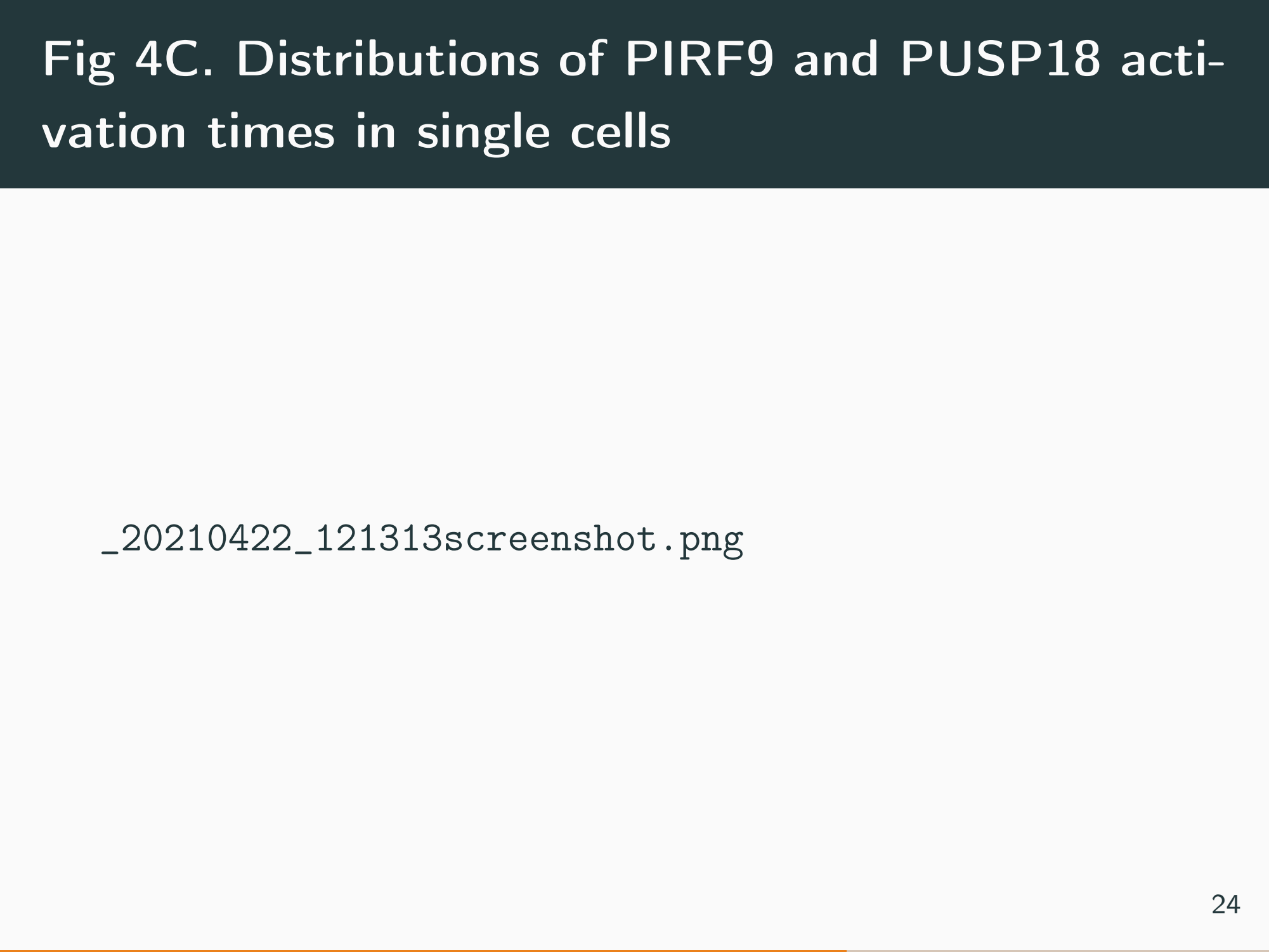

Fig 4D. Distributions of delay times in single cells

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Fig 4E. Representative time traces of PIRF9 and PUSP18 in a single cell from each group

Cell cycle phases differentially regulate USP18 expression

Fig 4E. Delay times as a function of the percentages of cell cycle progression upon IFN treatment onset

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Fig 5A. Delay times in cells treated with different cell cycle perturbation

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Fig 5B. CDK2 activity reporter Schematic

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Fig 5C. Nuclear DHB and PUSP18-driven gene expression

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Fig 5D. Effect of decitabine on DNA methylation and nucleosome occupancy

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Fig 5D. Distribution of delay times upon decitabine treatment

Cell-cycle-gated feedback control shapes single-cell responses to repetitive IFN inputs

Fig. 6A simple model of the IFN-driven gene regulatory network

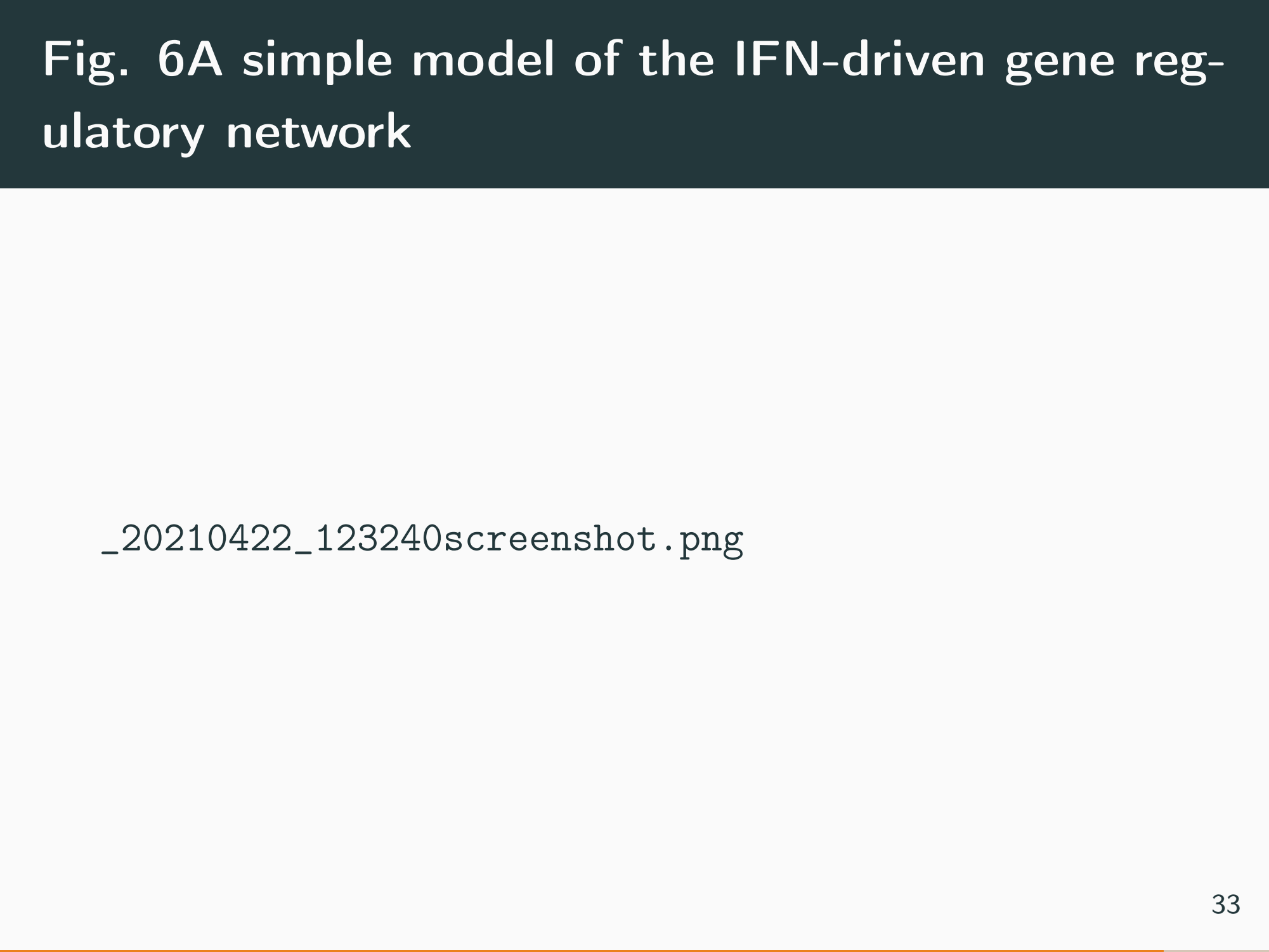

Fig. 6C cell-cycle gated feedback control simulated responses under different pretreatment conditions

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Fig. 6D cell-cycle gated feedback control experimental responses under different pretreatment conditions

Higher levels of USP18 expression by the prolonged pretreatment lead to reduced IRF9 induction upon the second stimulation at the single-cell level, qualitatively in agreement with our experimental data

Conclusion

Conclusion

- The effects of IFN pretreatments depend on their input durations
- The G1 and early S phases enable an open window for immediate USP18 upregulation upon the IFN treatment
    - If they miss the window the USP18 induction has to wait for G1 of the next cell cycle
- SARS-CoV-2 is especially sensitive to type I IFNs
    - IFN pretreatment a potential strategy to prevent SARS-CoV-2 infection