

Article

## Fold Change of Nuclear NF- $\kappa$ B Determines TNF-Induced Transcription in Single Cells

Robin E.C. Lee<sup>1, 2</sup>, Sarah R. Walker<sup>3, 4, 5</sup>, Kate Savery<sup>1</sup>, David A. Frank<sup>3, 4, 5</sup>, Suzanne Gaudet<sup>1, 2</sup>, , 

Presentation by Jingyu Zhang  
Nov. 30, 2015

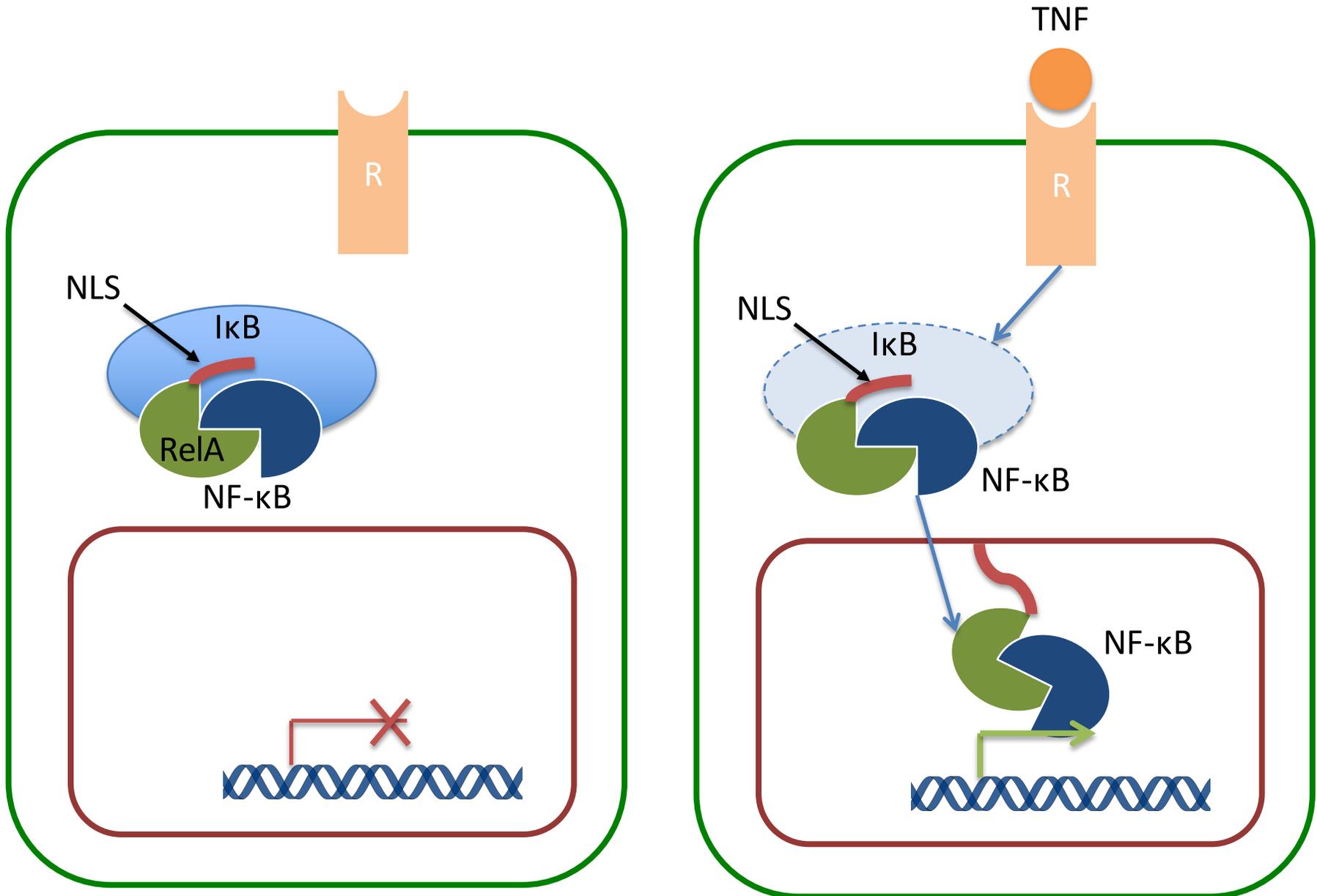
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## Fold Change of Nuclear **NF- $\kappa$ B** Determines **TNF**-Induced Transcription in Single Cells

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# Introduction



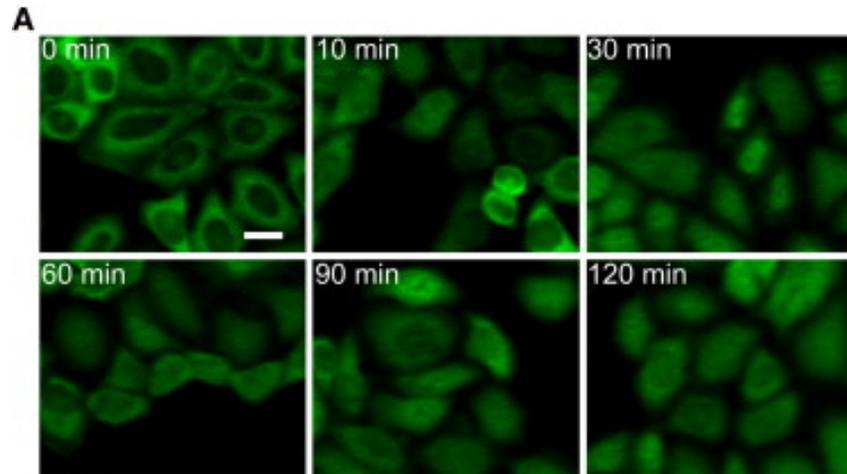
# Motivation

- NF- $\kappa$ B deregulation is associated with disease.
- The nuclear NF- $\kappa$ B levels have considerable variability from cell to cell.
- What is the most important aspect of NF- $\kappa$ B changes? Which determines the TNF-induced transcription via NF- $\kappa$ B?

# Methods

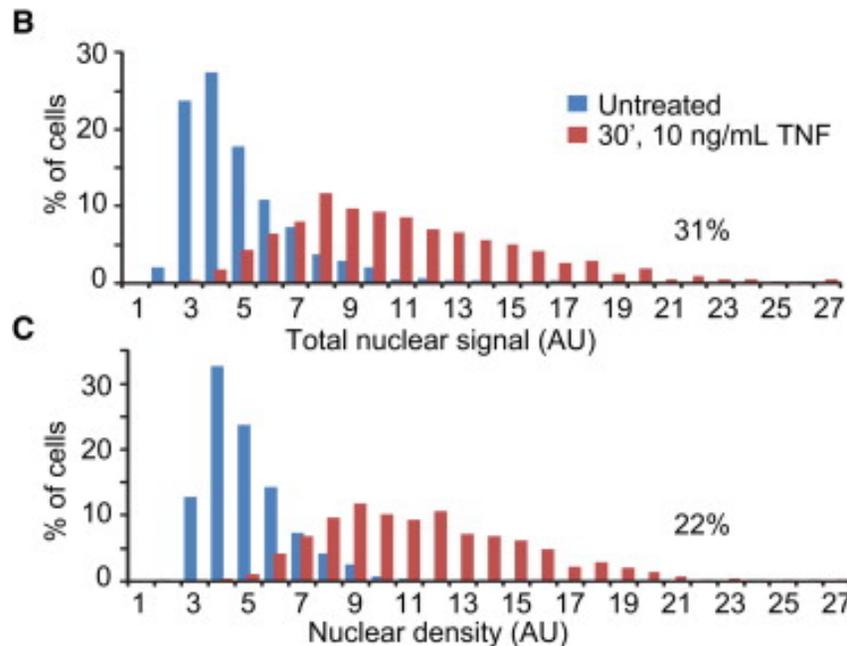
- Experiments
  - Cell line: HeLa
  - Immunofluorescence imaging and analysis
  - Live-cell imaging and analysis
  - smFISH microscopy and image analysis
- Model
  - I1-FFL (D2FC) model
  - direct promotion (D2F) model

# Results



## Method:

Fixed-cell RelA immunofluorescence imaging and analysis



## Conclusion:

The timing and intensity of RelA translocation in response to TNF vary among cells.

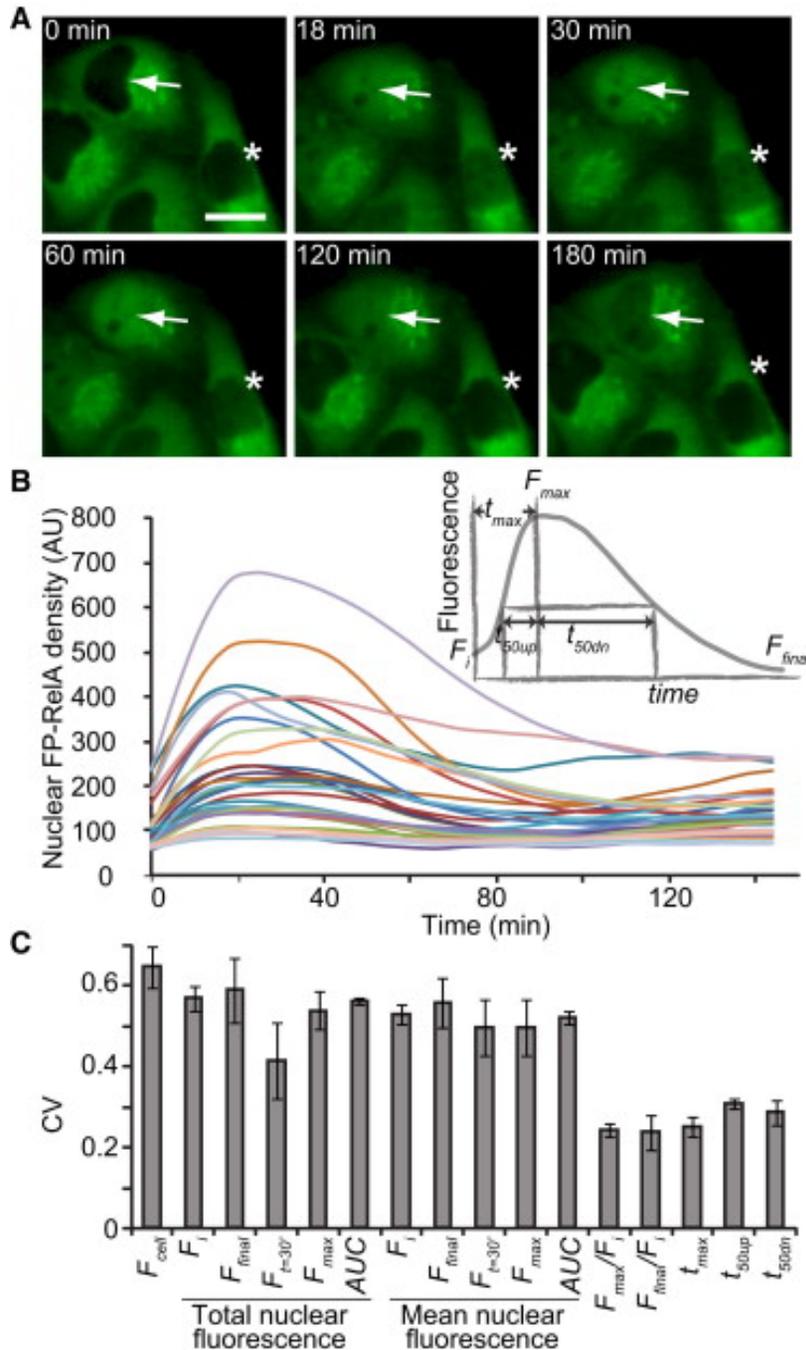
**Figure 1.** TNF-Induced NF- $\kappa$ B Subcellular Localization Is Variable.

# Results

## Method:

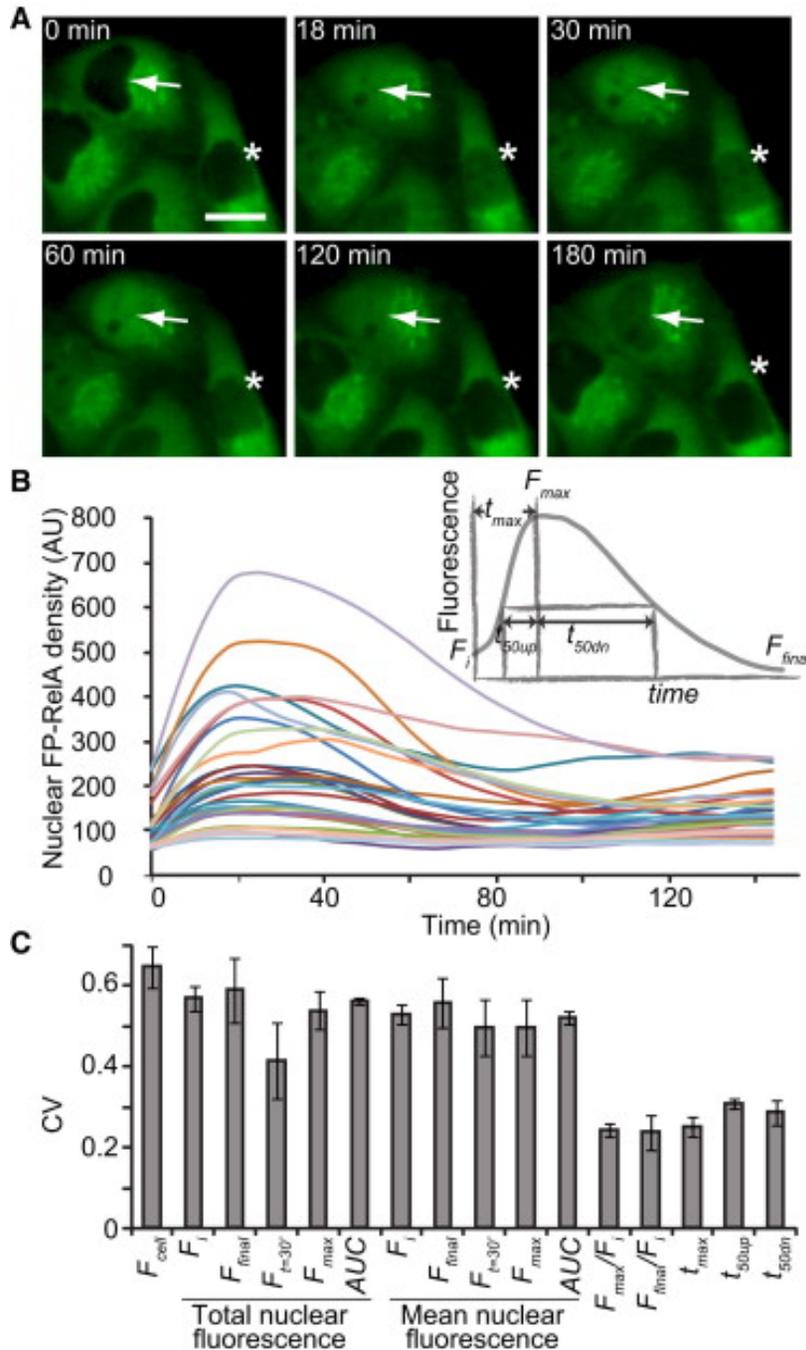
Stably-expressing EGFP-RelA cell line  
Living cell imaging and analysis.

CV (coefficient of variation)  
= standard deviation / mean



**Figure 2.** TNF-Induced NF- $\kappa$ B Translocation Varies in Live Cells.

# Results



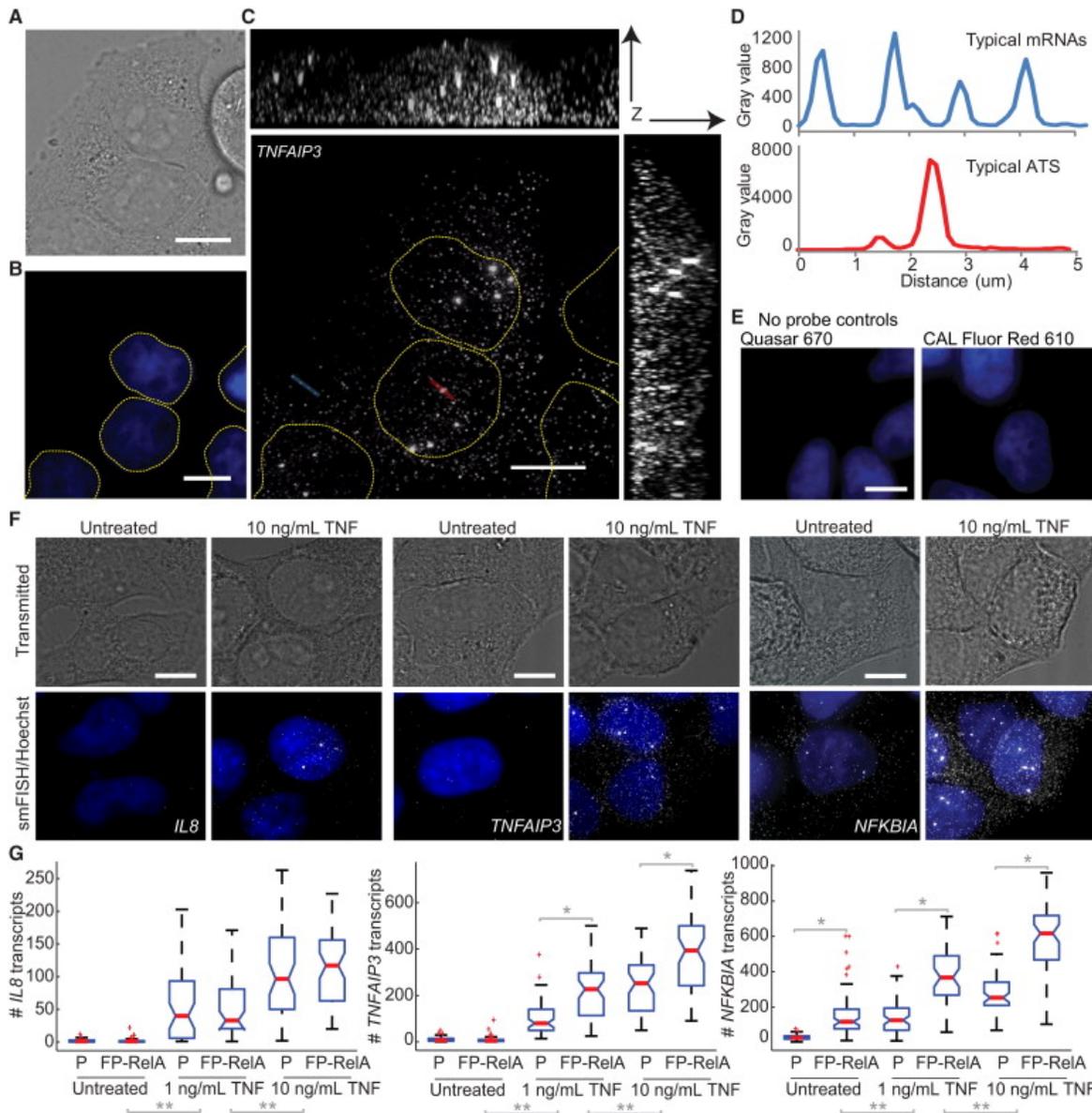
## Conclusion:

The 'Descriptor' is important to present the cell-to-cell variability in response to TNF.

The fold change of nuclear RelA is less variable than absolute RelA abundance.

**Figure 2.** TNF-Induced NF- $\kappa$ B Translocation Varies in Live Cells.

# Results



## Method:

Single-molecule fluorescent *in situ* hybridization (smFISH)

## Conclusion:

The three targeted genes have distinct patterns of sensitivity to RelA abundance

RelA may not be an adequate descriptor of this transcription-inducing signal

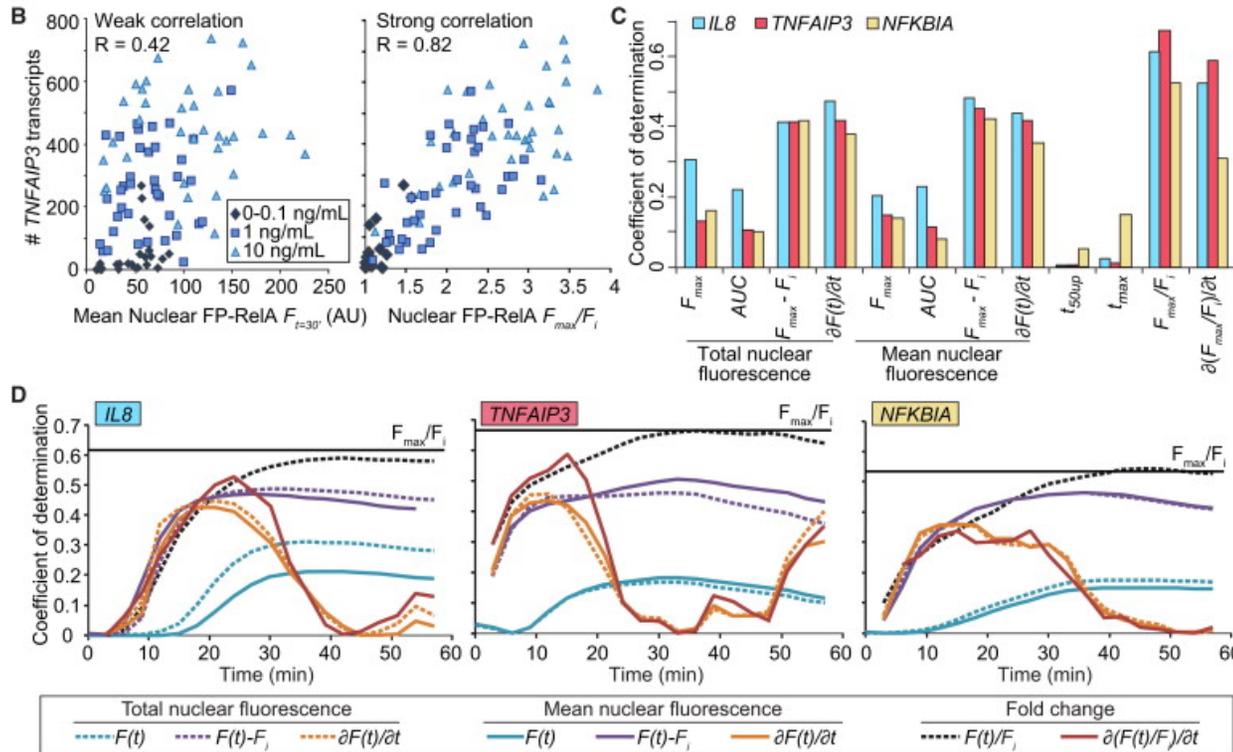
**Figure 3.** Variability of TNF-Induced NF-κB-Dependent Transcription Is Transcript Specific.

# Results

$y_i$ : observed values;  
 $f_i$ : predictable values;  
 $R^2$ : Coefficient of determination.

$$SS_{\text{tot}} = \sum (y_i - \bar{y})^2, \quad SS_{\text{res}} = \sum_i (y_i - f_i)^2$$

$$R^2 \equiv 1 - \frac{SS_{\text{res}}}{SS_{\text{tot}}}$$

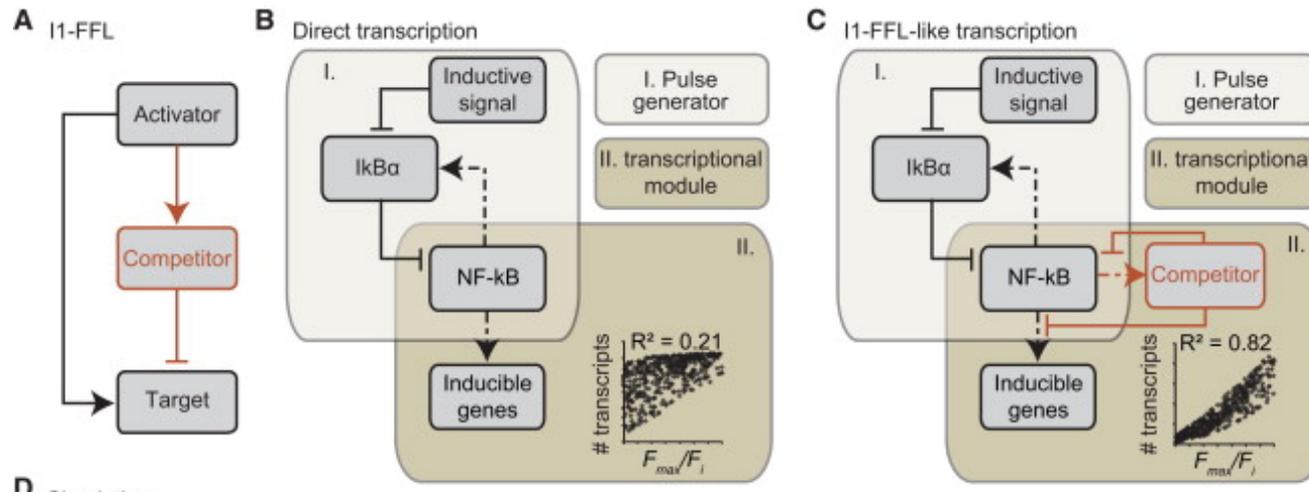


## Conclusion:

NF- $\kappa$ B transcription regulation system is capable of fold-change detection.

**Figure 4.** Transcriptional Responses to TNF Are Determined by the Fold Change of Nuclear NF- $\kappa$ B

# Results



**Figure 5.** An I1-FFL Model of NF- $\kappa$ B-Mediated Transcription Recapitulates Experimental Transcriptional Patterns

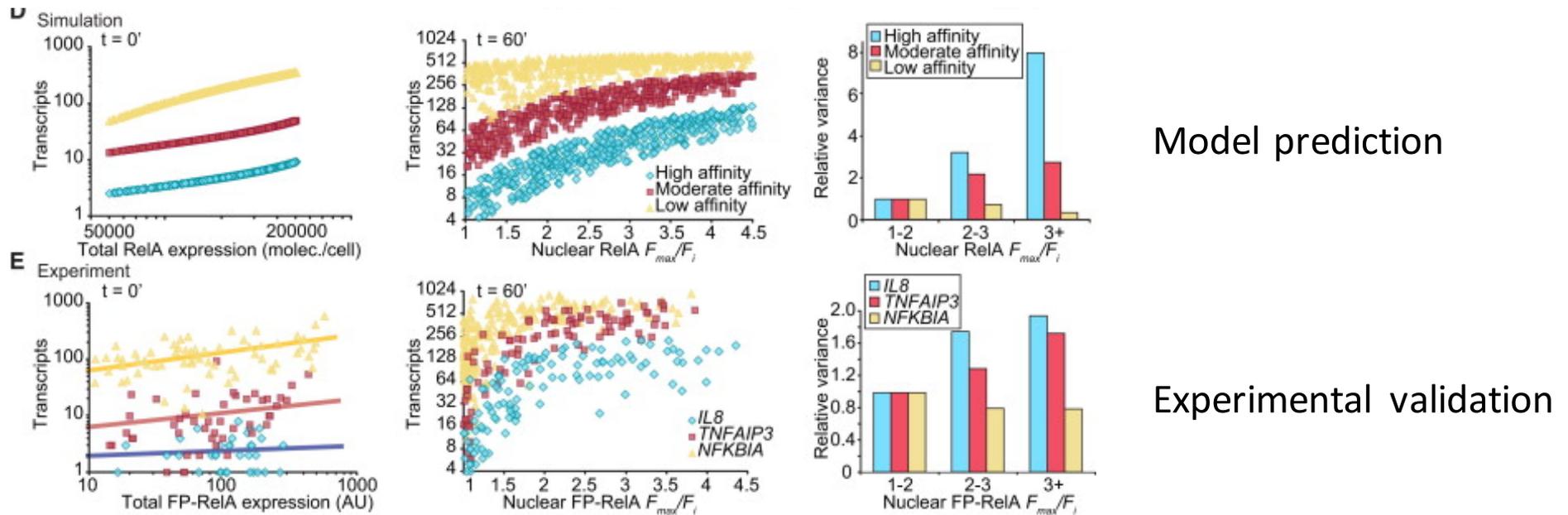
**Direct transcription**

$$mRNA_i(t) = c1a_i \times \frac{\left(\frac{nNFkB(t)}{k_{NFkB_i}}\right)^{h_i}}{\left(\frac{nNFkB(t)}{k_{NFkB_i}}\right)^{h_i} + 1}$$

**I1-FFL-like transcription**

$$mRNA_i(t) = c1a_i \times \frac{\left(\frac{nNFkB(t)}{k_{NFkB_i}}\right)^{h_i}}{\left(\frac{nNFkB(t)}{k_{NFkB_i}}\right)^{h_i} + \left(\frac{Competitor(t)}{k_{Comp_i}}\right)^{h_i} + 1}$$

# Results



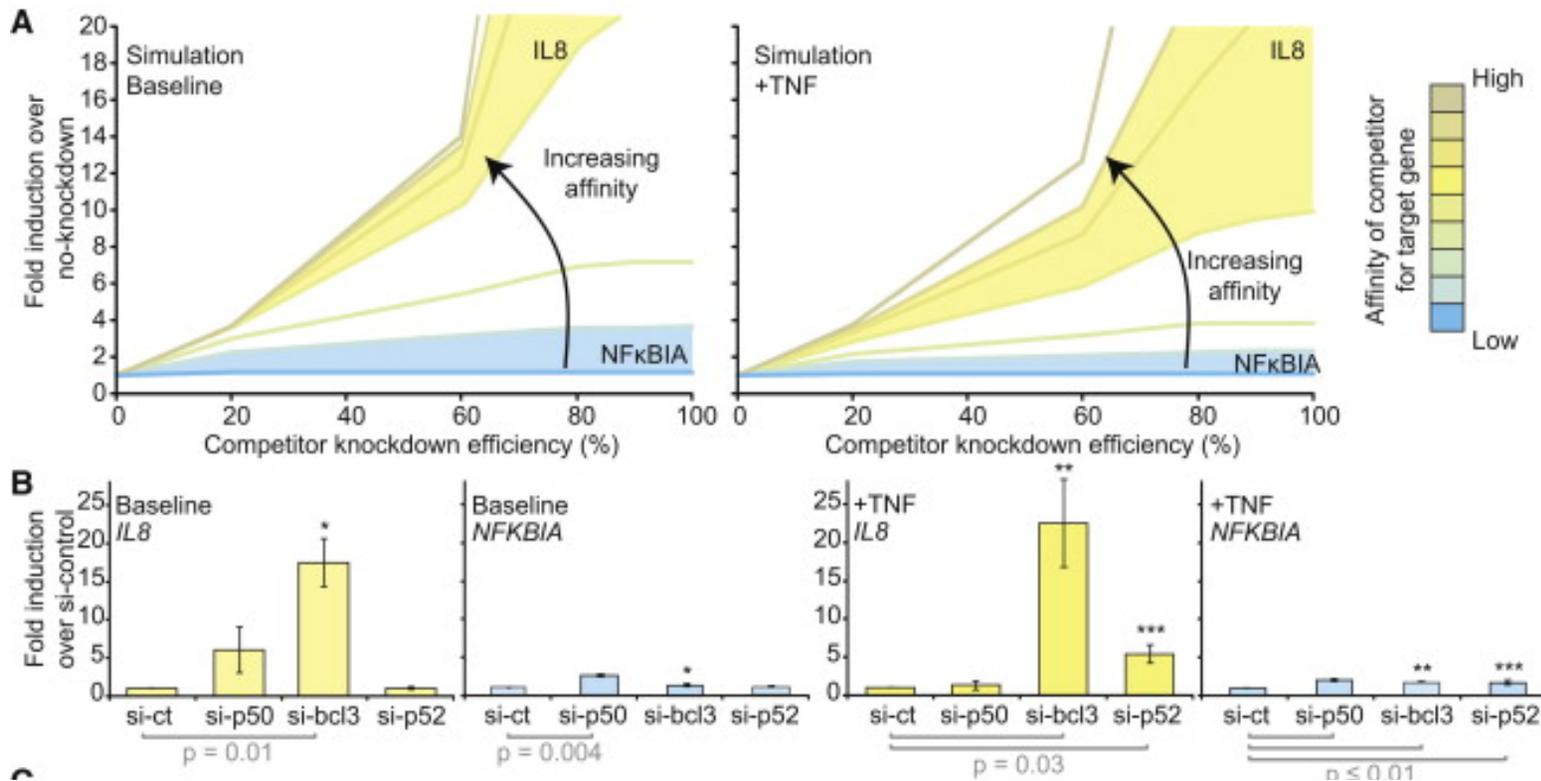
**Figure 5.** An I1-FFL Model of NF- $\kappa$ B-Mediated Transcription Recapitulates Experimental Transcriptional Patterns

## Conclusion:

High affinity of competitor for a promoter–inducible, depending on fold changes;

Low affinity of competitor for a promoter–constitutive (like D2F)

# Results



**Figure 6.** Individual Genes Show Different Sensitivity to Knockdown of Candidate Competitors.

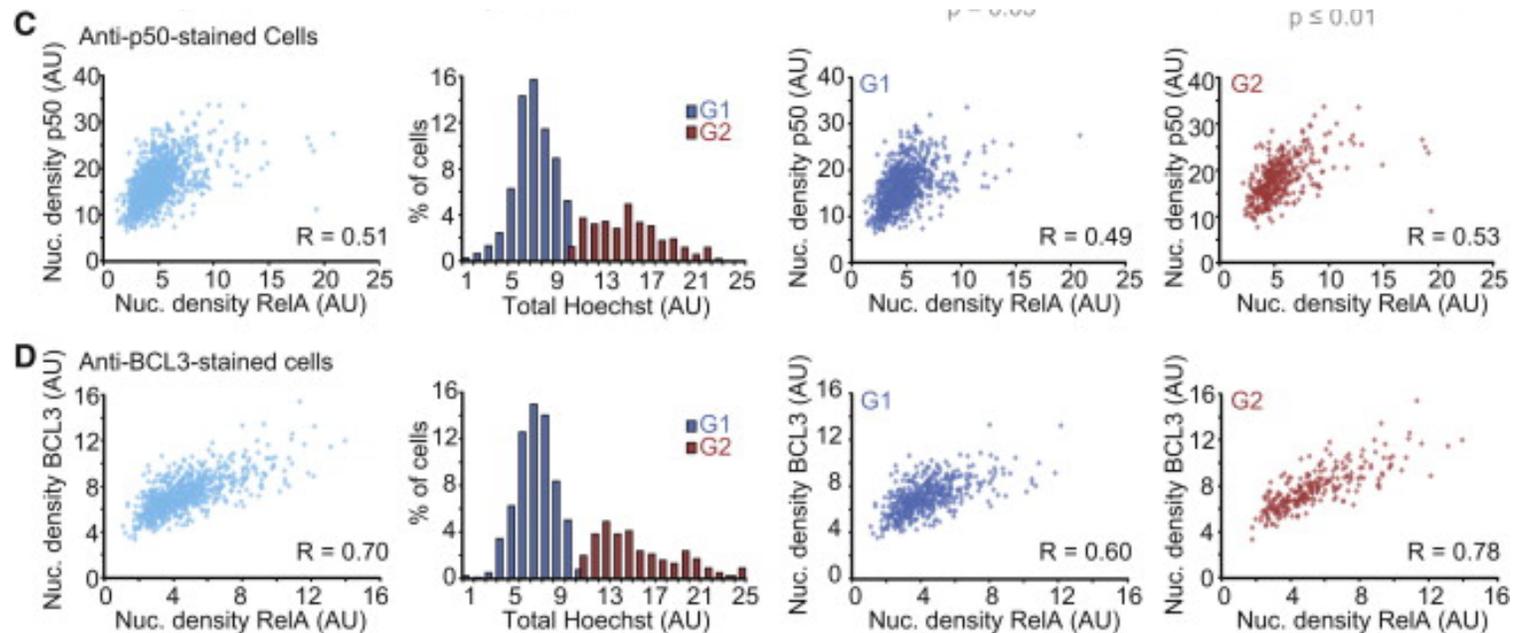
## Method:

siRNA knockdown  
qRT-PCR

## Conclusion:

Knockdown of the competitor increased transcription of genes with high-affinity for competitor but less impact on the low affinity gene.

# Results



**Figure 6.** Individual Genes Show Different Sensitivity to Knockdown of Candidate Competitors.

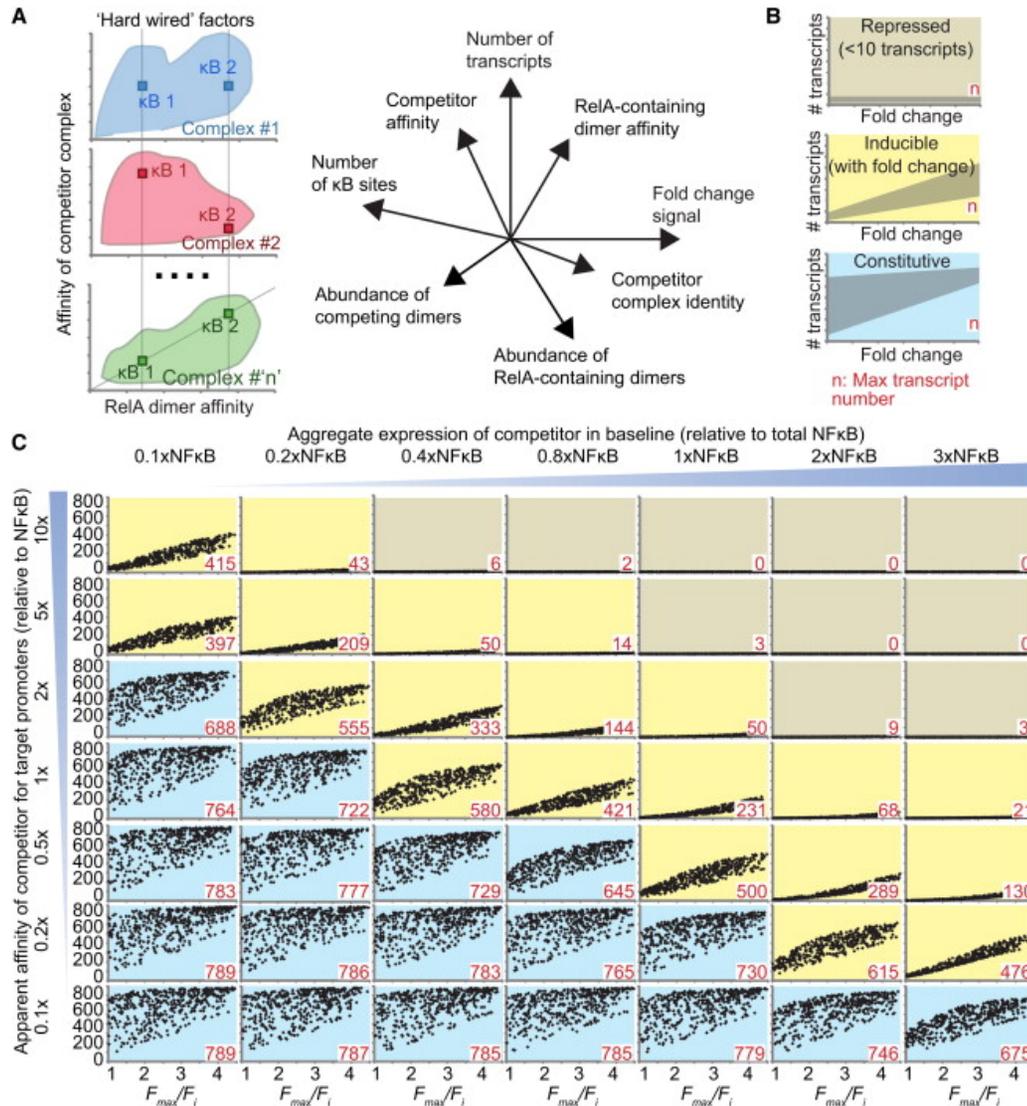
## Method:

siRNA knockdown  
qRT-PCR

## Conclusion:

The nuclear density of competitors, P50 and BCL3 changed correlatively with that of RelA in single cells;

# Results



The establishment and prediction of I1-FFL-like model have to be hard-wired biochemical parameters, which are different case by case.

Noise of protein and epigenetic changes of the promoter of the competitor could alter the competitor:RelA ratio.

**Figure 7.** The Model Explains How Transcription Patterns Are Tuned by Changes to Competitor Affinity and Abundance

# Summary

- The subcellular localization of NF- $\kappa$ B is important for its function as the transcriptional activator at the downstream of TNF pathway;
- Nuclear abundance of NF- $\kappa$ B is vary from cell to cell;
- However, the relationship among NF- $\kappa$ B, TNF, the transcription of the targeted genes can compose a I1-FFL-like motif, -with the competitors;
- The fold-change of NF- $\kappa$ B determines the TNF-induced transcription in single cell.

**Thank you**