

Article

Fold Change of Nuclear NF- κ B Determines TNF-Induced Transcription in Single Cells

Robin E.C. Lee^{1, 2}, Sarah R. Walker^{3, 4, 5}, Kate Savery¹, David A. Frank^{3, 4, 5}, Suzanne Gaudet^{1, 2}, , 

Presentation by Jingyu Zhang
Nov. 30, 2015

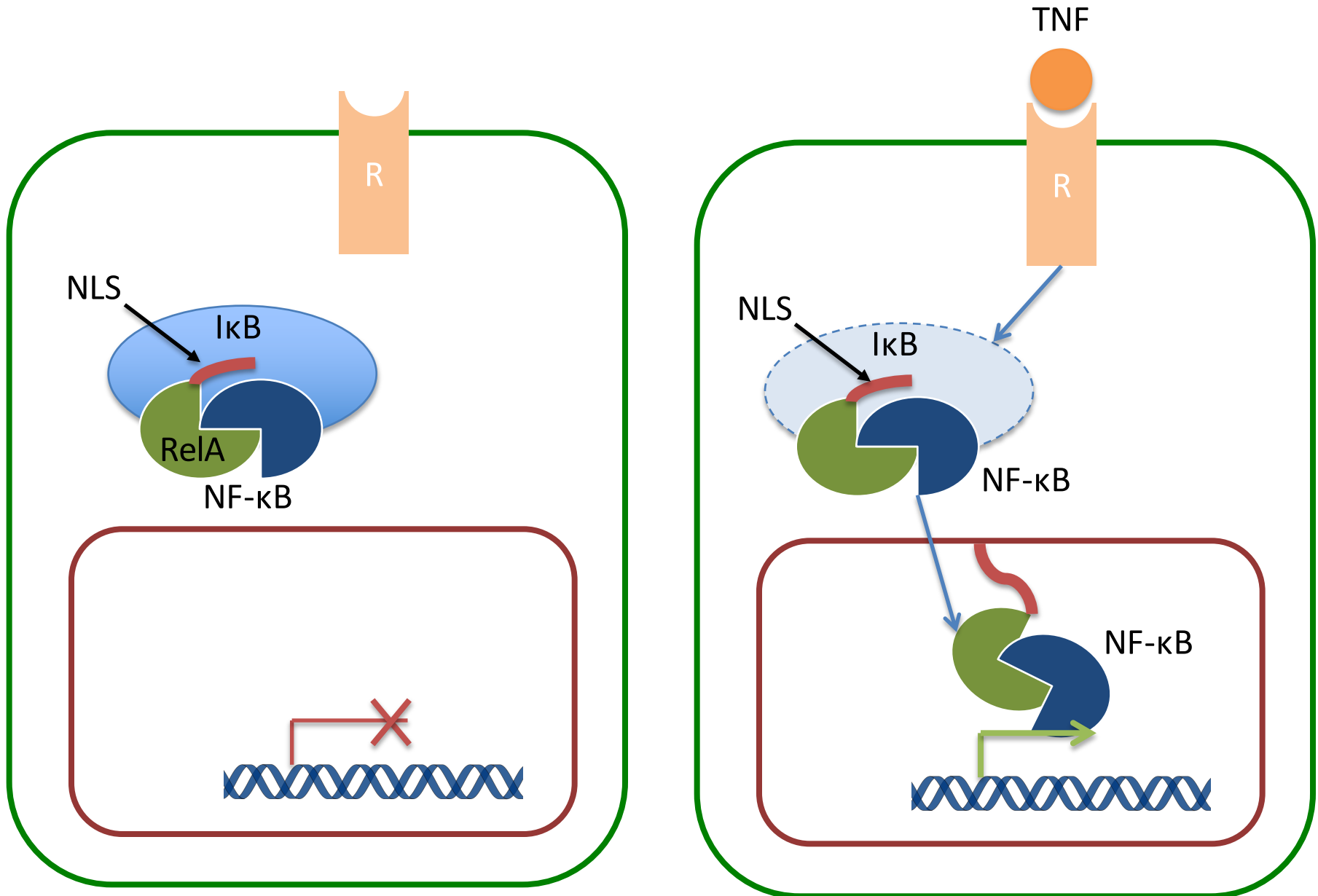
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Fold Change of Nuclear **NF- κ B** Determines **TNF**-Induced Transcription in Single Cells

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Introduction



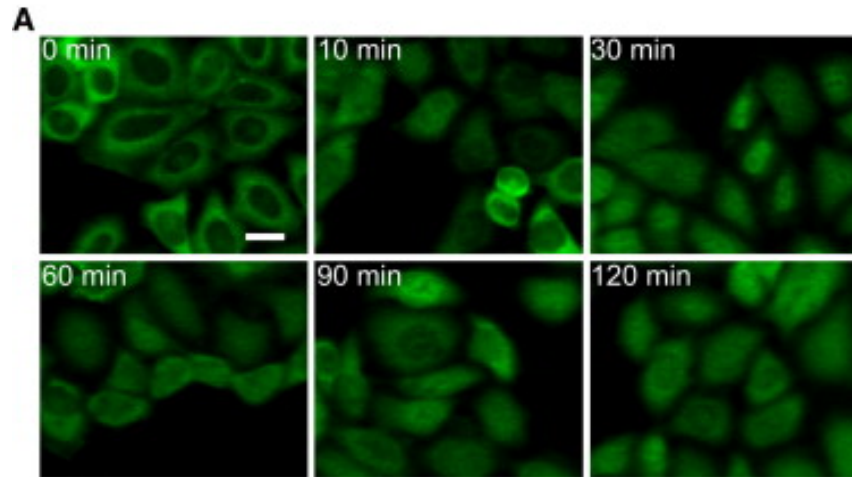
Motivation

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

Methods

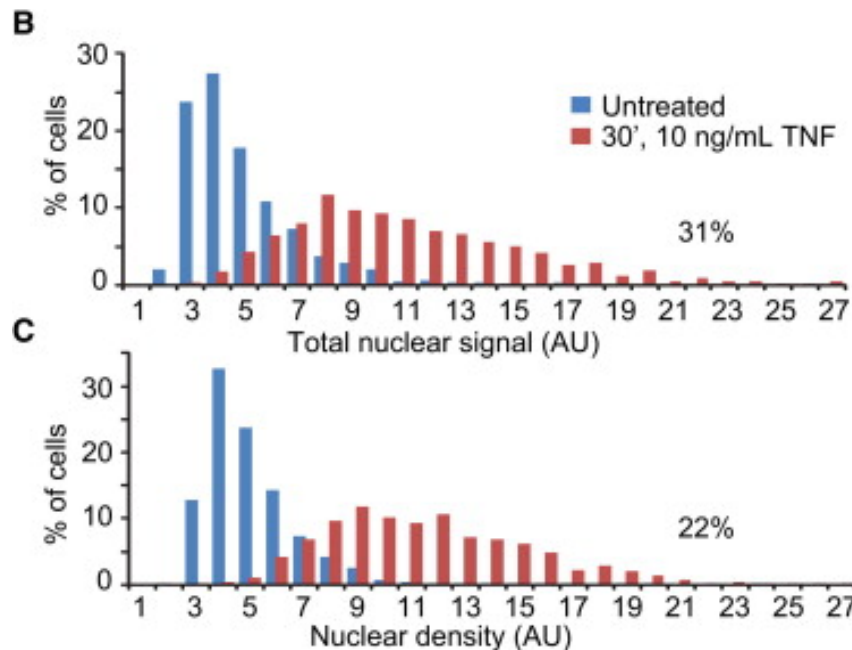
- Experiments
 - Cell line: HeLa
 - Immunofluorescence imaging and analysis
 - Live-cell imaging and analysis
 - smFISH microscopy and image analysis
- Model
 - I1-FFL (D2FC) model
 - direct promotion (D2F) model

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- κ 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

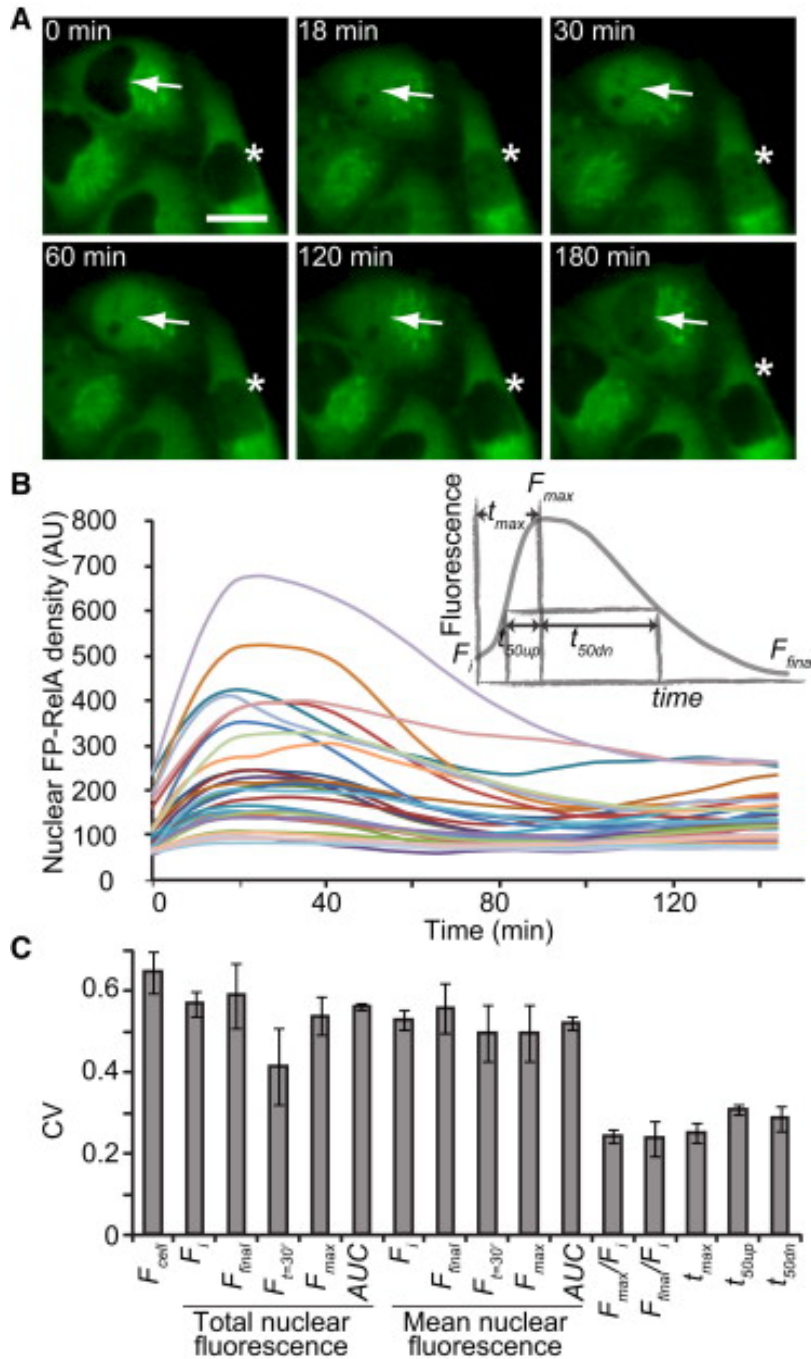
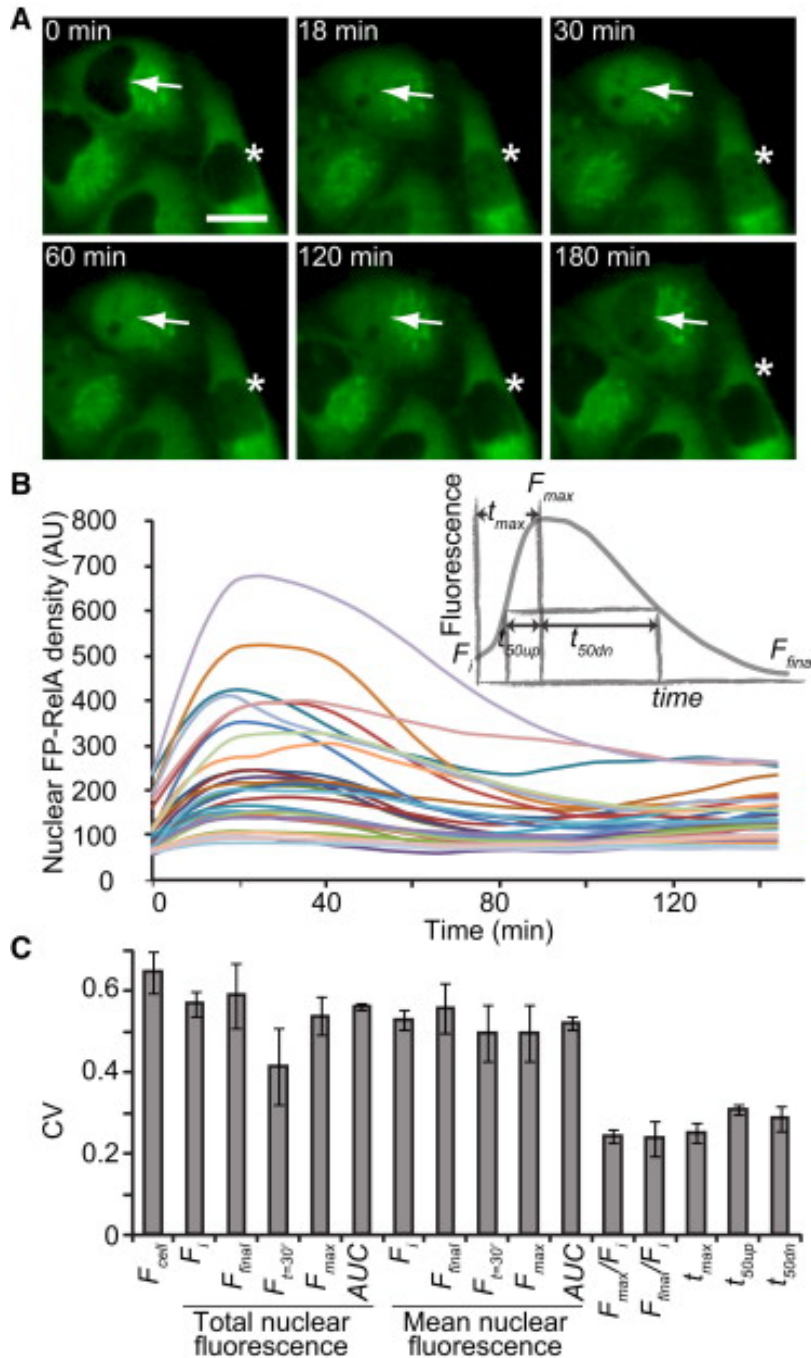


Figure 2. TNF-Induced NF- κ B Translocation Varies in Live Cells.

Results



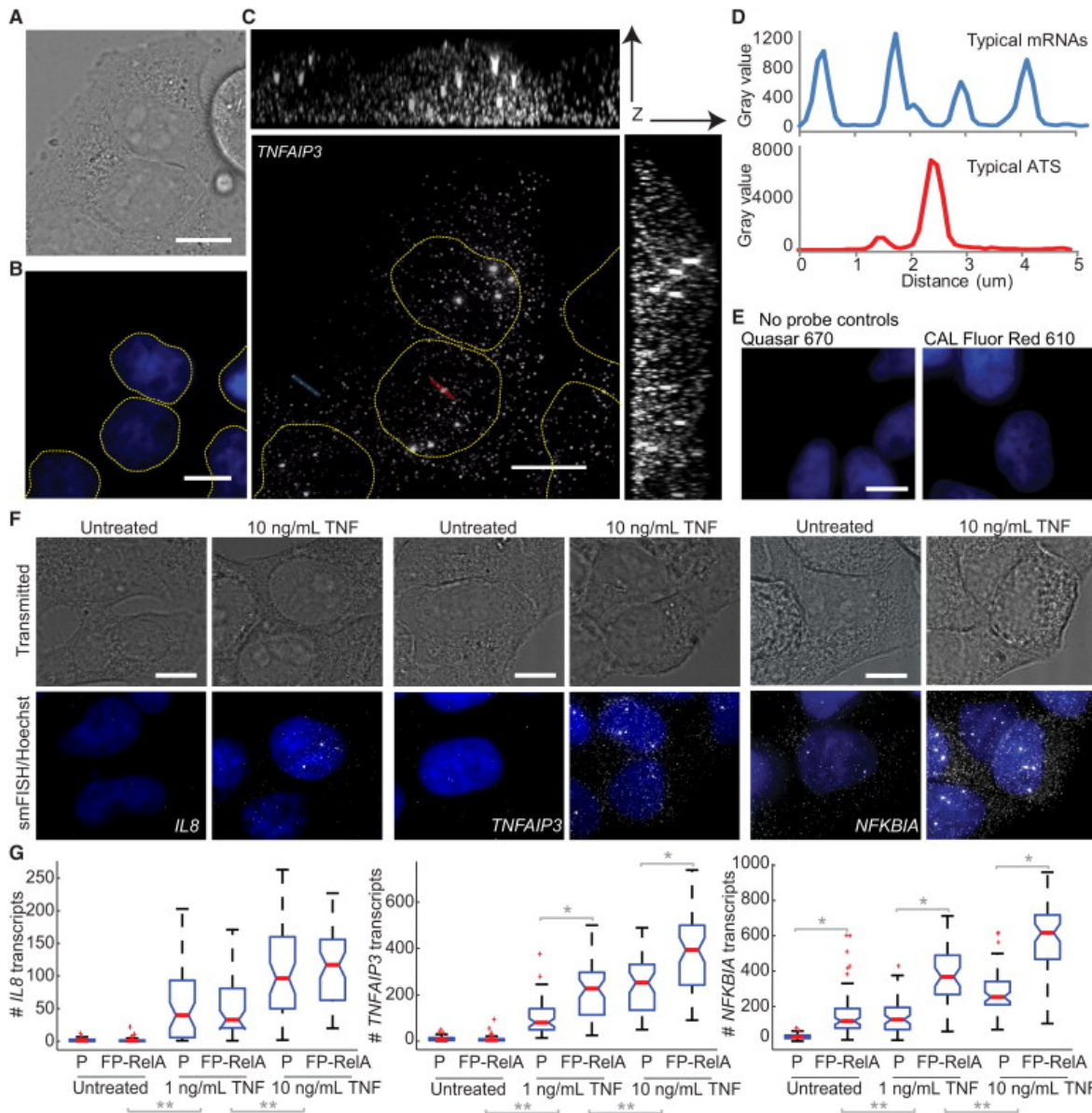
Conclusion:

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- κ 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

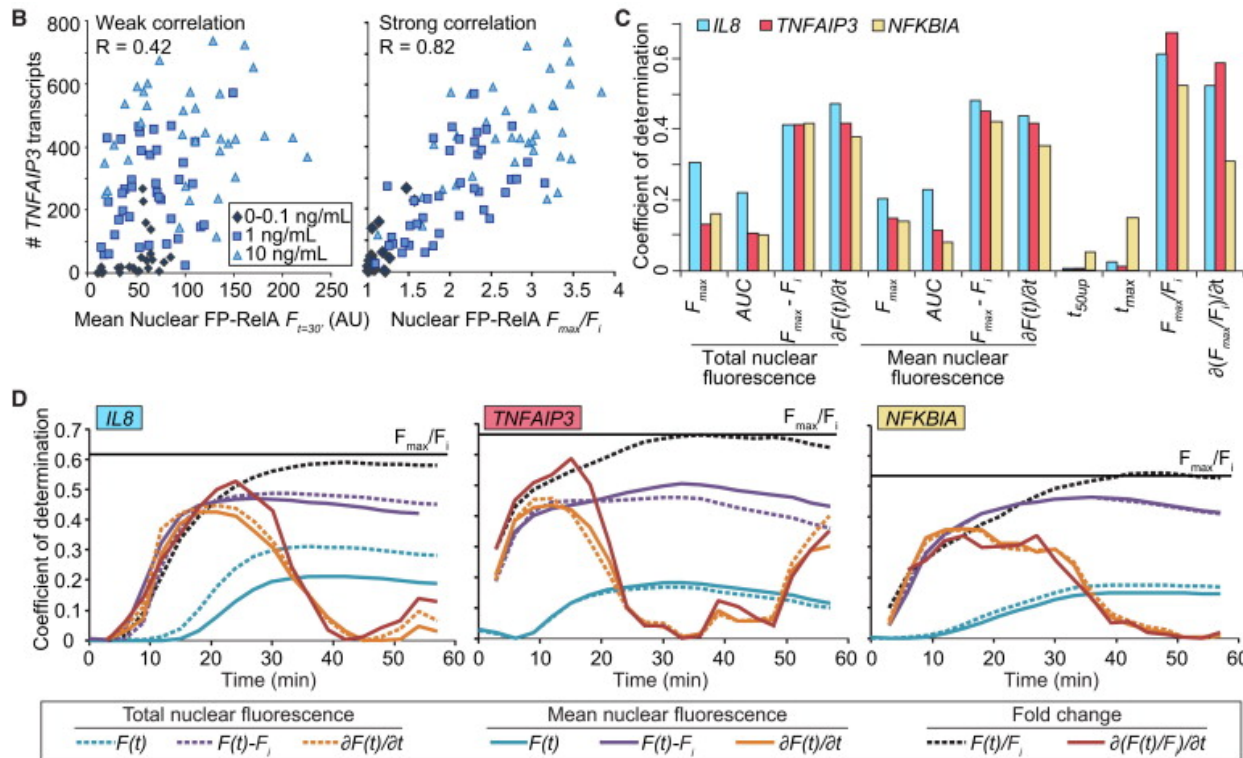
Figure 3. Variability of TNF-Induced NF-κB-Dependent Transcription Is Transcript Specific.

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- κ 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- κ B

Results

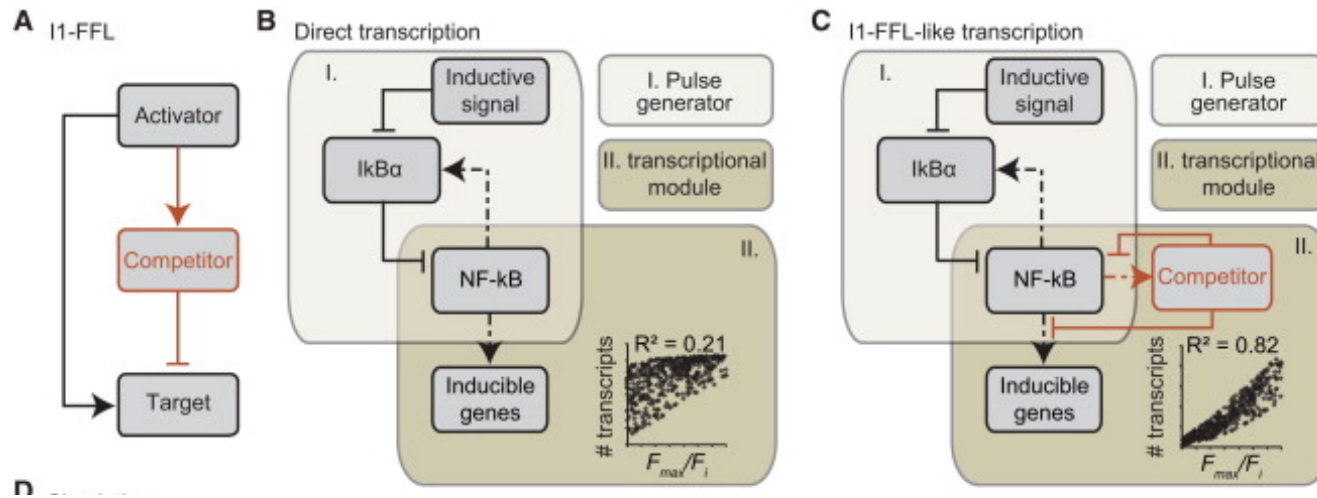


Figure 5. An I1-FFL Model of NF- κ B-Mediated Transcription Recapitulates Experimental Transcriptional Patterns

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

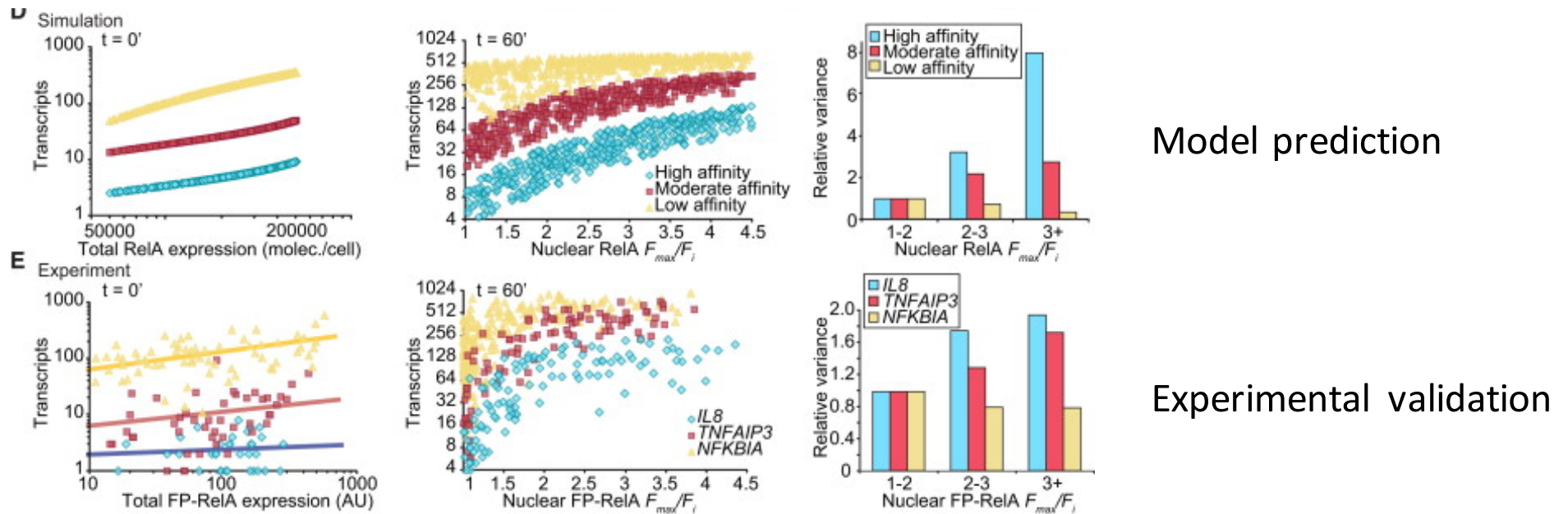


Figure 5. An I1-FFL Model of NF- κ B-Mediated Transcription Recapitulates Experimental Transcriptional Patterns

Conclusion:

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

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

Results

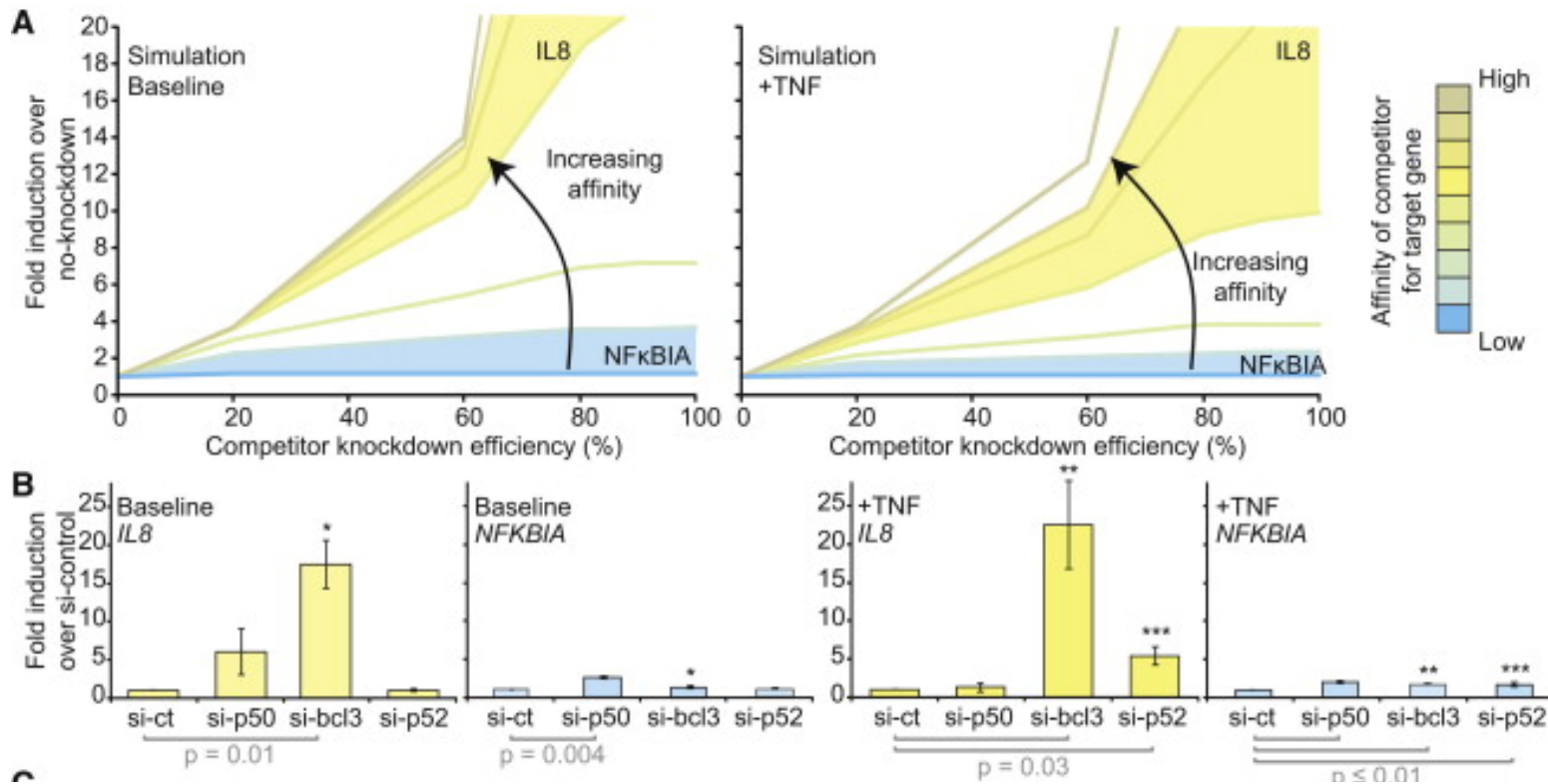


Figure 6. Individual Genes Show Different Sensitivity to Knockdown of Candidate Competitors.

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

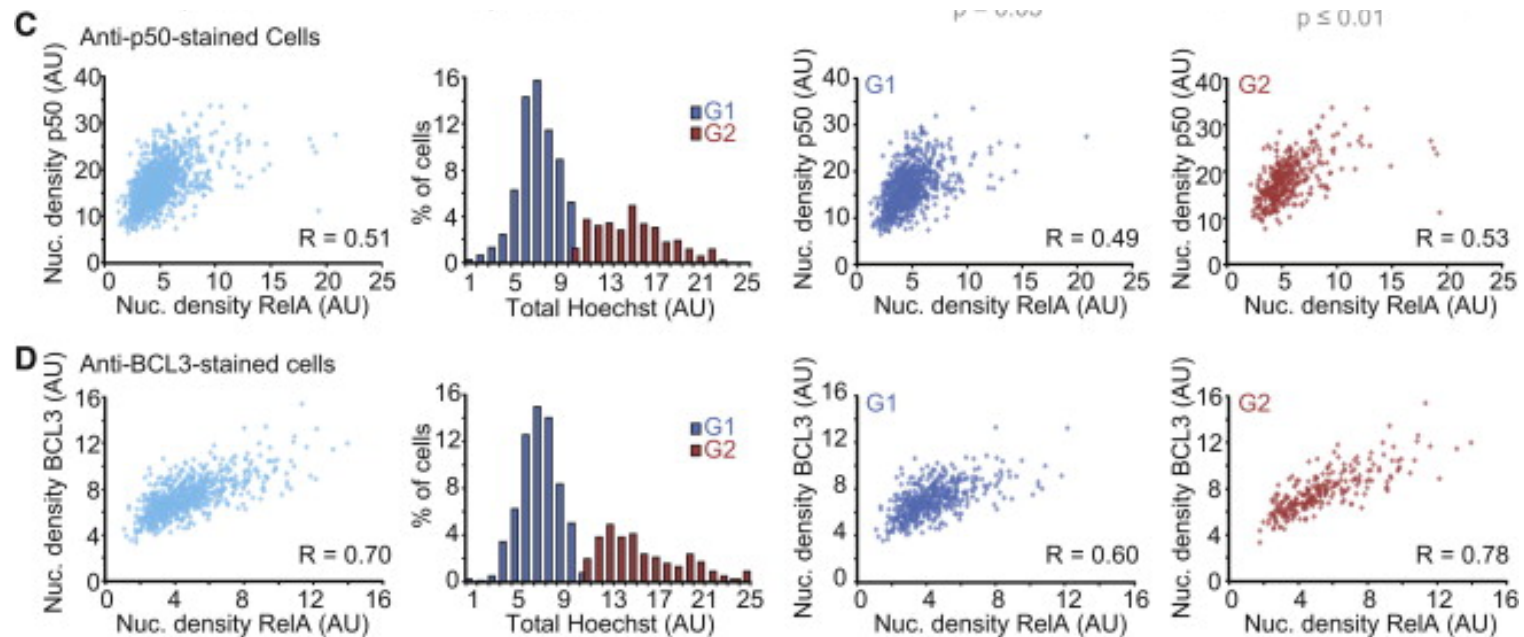


Figure 6. Individual Genes Show Different Sensitivity to Knockdown of Candidate Competitors.

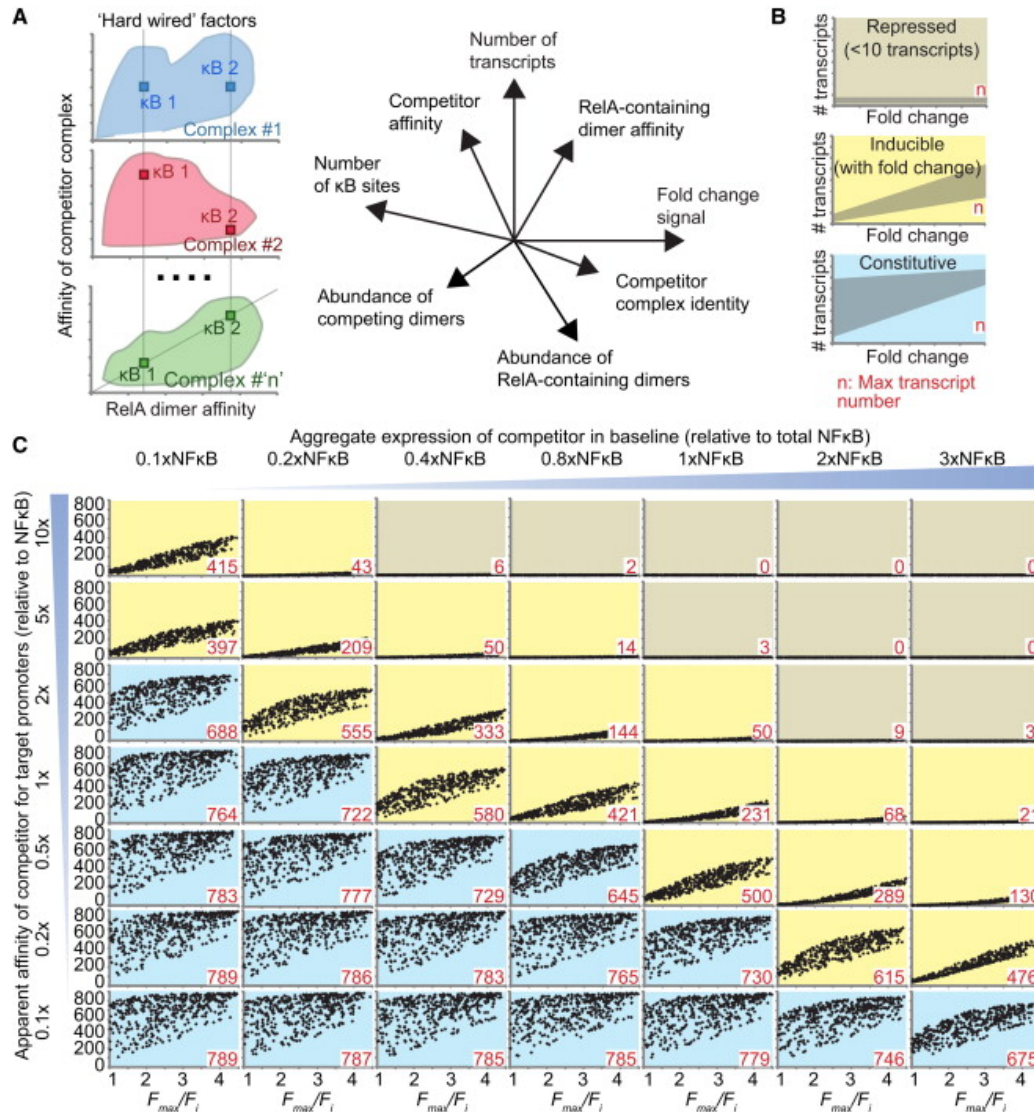
Method:

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

Thank you