**Table of Contents:**

1. Supplemental Figures

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2. Computational Modeling

a. Description of the overall modeling

• Supplemental Figure1: The whole model framework

b. TNF production model

• Supplemental Figure 2: Predicting mRNA dynamics based on nascent suggesting a decrease of processing in myd88-/-

• Supplemental Figure 3: Predicting nascent mRNA

• Supplemental Figure 4: Predicting pro-TNF and ELISA data

• Supplemental Figure 5: Connecting all modules of TNF production model

c. Whole model: TLR + TNF production + TNFR

• Supplemental Table1: Molecular Species in the whole model

• Supplemental Table2: Model reactions and parameters.

3. References

***Computational Modeling***

**A. Description of the overall modeling construction.**

The whole model framework is shown in Supplemental Figure 1. It is constitutive by TNF production, TNFR module, TLR module, and IKK-NFkB module. In this work, we construct the mathematical models following the steps as below:

**Step1 Constructing the model for the TNF production module.** The goal of this step is to 1) identify the genotype-specific parameters 2) find a parameter set to predict all nascent TNF mRNA, mature TNF mRNA, pro-TNF and secreted TNF by input the measured NFkB activities into the model 3) test the potential regulating mechanism by TRIF and/or MyD88.

**Step2 Connect TNF production model with TLR and IKK-NFkB modules (with/without TNFR module) to predict dynamics after LPS stimulation for wild-type, myd88-/- and trif-/- macrophage.** The TLR and IKK-NFkB modules are constructed before and documented elsewhere. The goal of this step is to 1) identify to test whether how strong the auto-crine TNF feedback is in TLR4 signaling. 2) How does the feedback relate to the MyD88 and TRIF.

**Step3 Connect TNF production model with TLR model (with/without TNFR module) to predict responses after CpG and Poly I:C stimulation.** At this stage, we compared results with and without including TNFR model, i.e. *tnfr-/-* condition to test and predict whether autocrine functions in a stimulus-specific manner.

In the supplement text here, we will emphasize on the construction of the TNF production module. Details of TLR module, TNFR module and IKK-NFkB module are documented elsewhere. Details about the reactions and parameters of the whole model can be found in supplemental Table 2.

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**Supplemental Figure 1: The whole model framework. It is constituted by the TNF module, TLR module, TNFR module and IKK-NFkB module.**

**B. TNF production model**

The TNF production is modeled by the ODEs as below:







In this model, we use a Hill equation with a fixed Hill coefficient nH=2 to describe the NFkB induced transcription of tnf. The RNA processing to make nascent mRNA become mature mRNA is simulated by mass action. Similar mass action apply for the mature mRNA degradation, translation, protein degradation and secretion. The secreted TNF is detected by ELISA, and we assumed a slow decay rate of the secreted TNF so that the time integrated secreted TNF flux will be the detected TNF.

In order to measure the discrepancy between our simulation and the experimental data, we used root-mean-square deviation (RMSD) defined as:



, in which n is the number of measured data points, is the data predicted by model simulation , is the experimental data points.

At this step, we measures the output and input for each equation of the model, and parameterizing it to identify genotype-specific parameters, which can be considered as prediction and will be either tested or confirmed by experiments or by previous publications. The output of this step is that we will have a working model to predict dynamics of nascent TNF mRNA, mature TNF mRNA, pro-TNF on the plasma membrane and secreted TNF based on the input of NFkB for wild-type, myd88-/- and trif-/- macrophage in response to LPS stimulation.

**Predicting mature mRNA dynamics.** We started the parameter exploration with Eqn (1.2), because we have measured the mRNA half-lives, leaving only one free parameter kpr to determine the value.

The mRNA degradation rate is measured at 30 mins post stimulation. As suggested by the data, the mRNA half-life seems controlled by the p38 activity. The p38 activity seems to be reach at peak at 30 mins and return to basal at the 60 mins. So we assumed a linear dependence of the p38 with the degradation rate for the wild-type and myd88-/- and constant at the trif-/-. So  and



In figure 2F, this is corresponding to the mechanism that TRIF regulates mRNA half-life. To simulate the mechanism that MyD88 regulating the mRNA half-life, we use  and  equal to the right side of eqn. (1.5). To simulate MyD88 and TRIF both regulate mRNA half-life, only is equal to eqn. (1.5) right side, and . Setting all degradation rates are equal to 0.07min-1 is corresponding to the no half-life regulation regime.

Then we first start to predict processing rate kpr by assuming it is the same in different genotypes. We use nascent mRNA as input and interpreted by the same time steps as numerical solving the equations.

**a) Same processing rate cannot capture the lowest mRNA profile in MyD88-/-.** The fitting is not dependent of processing rate (Supplemental Figure 2A), because we scaled all the date to the maximum in wild-type which eliminated the effect of changing kpr. While mature mRNA dynamics in wild-type and trif-/- fit relatively well, the predicted mature mRNA in MyD88-/- is much higher than wild-type and trif-/- after 60mins (Supplemental Figure 2B), which is actually reflecting the dynamical relationships in the nascent mRNA profiles. This suggests that the processing should be less efficient in myd88-/-. Therefore, we introduced a parameter fold\_pr\_mko to capture the fold of reduction in kpr in myd88-/-. Indeed, previous work has shown that PKR activity is important for processing nascent TNF RNA to mRNA (Osman 1999), and that PKR activation is at least partially mediated by MyD88 (Horng 2001).

**b) fold\_pr\_mko>2 is suggested by the data (Supplemental Figure 2 C and D).** Because the fit is independent on kpr, we set it as a constant 0.4 min-1. Then we scan the value of fold\_pr\_mko. It turns out we cannot determine one exact value for fold\_pr\_mko, but only a range larger than 2 (Supplemental figure 2C). And the mature mRNA dynamics can recapture by the model (Supplemental Figure 2D).

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**Supplemental Figure2. Predicting mRNA dynamics based on nascent mRNA experimental data suggesting a decrease of processing in myd88-/-.**

**A-B. Same processing rate cannot capture the lowest mRNA profile in MyD88-/-.** (A) RMSD of fit vs. kpr. (B) The best-fit result of predicted mRNA dynamics for wild-type, myd88-/- and trif-/-.

**C-D. Adding a fold reduction of kpr significantly improves the fit.** (A) RMSD vs. fold reduction of kpr in *myd88-/-*. (B) The best-fit result of predicted mRNA dynamics for wild-type, myd88-/- and trif-/-.

**Predicting nascent mRNA dynamics.** Then we move back to the eqn.(1.1) with adding the additional parameter fold\_pr\_mko (>2). The input is the EMSA data of the NFkB activities in different genotypes. The output is the nascent RNA concentration. The maximum transcription rate Vtr is set as 1 for simplicity. The EC50 Kdtr has the same unit of the NFkB, which is relative fold of the basal condition of the wild-type. Vtr =1, kdtr = 0.5, k\_pr = 0.4.

We first examine whether we can predict myd88-/- by using the predicted **fold\_pr\_mko.**

**a) fold\_pr\_mko >2 predict much higher nascent mRNA in myd88-/- (Supplemental Figure 3A).** This suggested a loss of transcription efficiency in myd88-/- (Supplemental Figure 3A).We modeled this effect by introduce a fold\_increase in the promoter and NFkB binding dissociation affinity Kdtr (fold\_kdtr\_mko). After introducing this factor, we can fit both nascent and mRNA for trif-/- and wild-type data (Supplemental Figure 3B).

**b) The requirement of trif in processing (Supplemental Figure 3C and D).**

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**Supplemental Figure 3. Predicting nascent mRNA dynamics based on NFkB experimental data suggesting a decrease of mRNA processing in trif-/- and a decrease in NFkB regulated transcription in myd88-/-.**

1. Fold of reduction of processing in myd88-/- (ktrfoldmko) predicts higher nascent mRNA in myd88-/-. Left: RMSD vs. ktrfoldmko. Right: nascent mRNA profiles predicted by the model for 4 different ktrfoldmko values.
2. Adding a fold of increase in the NFkB binding constant for myd88-/- (kmtrfoldmko) significantly improve the fit of nascent and mature mRNA data for both myd88-/-. Left: heat-map of the RMSD for both nascent and mature mRNA data against kmtrfoldmko and ktrfoldmko. Middle: best-fit nascent mRNA dynamics for both wild-type and myd88-/-. Right: best-fit mature mRNA dynamics for both wild-type and trif-/-.
3. Same processing rate cannot capture the peak of nascent mRNA production in trif-/-. Left: heat-map of RMSD for nascent mRNA of wild-type and trif-/- data against processing rate (ktr) and NFkB binding constant (Km). Right: best-fit result of nascent mRNA for both wild-type and trif-/-.
4. After introducing a fold reduction in processing rate for the trif-/- (ktrfoldtko), both nascent and mRNA data for trif-/- can be fitted by the model. Left: heat-map of RMSD for nascent mRNA of wild-type and trif-/- data against maximum transcription rate (Vtr) and ktrfoldtko. Middle: best-fit nascent mRNA dynamics for both wild-type and trif-/-. Right: best-fit mature mRNA dynamics for both wild-type and trif-/-.

**Predicting proTNF and secreted TNF.**

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**Supplemental Figure 4. Predicting pro-TNF and secreted TNF dynamics suggesting a reduction of translation and secretion in trif-/-**

1. Model with same translation rates for different genotypes predicts higher pro-TNF in trif-/-. Left: RMSD for pro-TNF data against translation rate (ktl). Right: best-fit result.
2. Adding a fold of decrese in the NFkB binding constant for myd88-/- (kmtrfoldmko) significantly improve the fit of nascent and mature mRNA data for both myd88-/-. Left: heat-map of the RMSD for both nascent and mature mRNA data against kmtrfoldmko and ktrfoldmko. Middle: best-fit nascent mRNA dynamics for both wild-type and myd88-/-. Right: best-fit mature mRNA dynamics for both wild-type and trif-/-..
3. Same processing rate cannot capture the peak of nascent mRNA production in trif-/-. Left: heat-map of RMSD for nascent mRNA of wild-type and trif-/- data against processing rate (ktr) and NFkB binding constant (Km). Right: best-fit result of nascent mRNA for both wild-type and trif-/-.
4. After introducing a fold reduction in processing rate for the trif-/- (ktrfoldtko), both nascent and mRNA data for trif-/- can be fitted by the model. Left: heat-map of RMSD for nascent mRNA of wild-type and trif-/- data against maximum transcription rate (Vtr) and ktrfoldtko. Middle: best-fit nascent mRNA dynamics for both wild-type and trif-/-. Right: best-fit mature mRNA dynamics for both wild-type and trif-/-.

**Connecting the modules**.

So the total parameters are in the table. We simulated eqn 1.1 to 1.3 using only NFkB as input. The result is shown in Supplemental Figure 5, with overall RMSD = 0.2.

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**Supplemental Figure 4. Predicting nascent mRNA (A), mature mRNA (B), pro-TNF (C) and secreted TNF (D) dynamics by TNF production model when use measured NFkB dynamics for wild-type, myd88-/- and trif-/-.** The lines are model simulations. The points are experimental data.

**C. The Whole model**

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**Supplemental Tabel 1.**

There are 55 species in the whole model.