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Computational analysis of 4-1BB-induced NF κ B signaling suggests improvements to CAR cell design

Vardges Tserunyan¹ and Stacey D. Finley^{1,2,3*}

Abstract

Background: Chimeric antigen receptor (CAR)-expressing cells are a powerful modality of adoptive cell therapy against cancer. The potency of signaling events initiated upon antigen binding depends on the costimulatory domain within the structure of the CAR. One such costimulatory domain is 4-1BB, which affects cellular response via the NF κ B pathway. However, the quantitative aspects of 4-1BB-induced NF κ B signaling are not fully understood.

Methods: We developed an ordinary differential equation-based mathematical model representing canonical NF κ B signaling activated by CD19scFv-4-1BB. After a global sensitivity analysis on model parameters, we ran Monte Carlo simulations of cell population-wide variability in NF κ B signaling and quantified the mutual information between the extracellular signal and different levels of the NF κ B signal transduction pathway.

Results: In response to a wide range of antigen concentrations, the magnitude of the transient peak in NF κ B nuclear concentration varies significantly, while the timing of this peak is relatively consistent. Global sensitivity analysis showed that the model is robust to variations in parameters, and thus, its quantitative predictions would remain applicable to a broad range of parameter values. The model predicts that overexpressing NEMO and disabling IKK β deactivation can increase the mutual information between antigen levels and NF κ B activation.

Conclusions: Our modeling predictions provide actionable insights to guide CAR development. Particularly, we propose specific manipulations to the NF κ B signal transduction pathway that can fine-tune the response of CD19scFv-4-1BB cells to the antigen concentrations they are likely to encounter.

Keywords: Adoptive cell therapy, CAR cells, 4-1BB signaling, NF κ B pathway, Mathematical modeling, Information theory

Background

In the last two decades, adoptive cell therapy has emerged as a powerful tool for combatting cancer [1]. One successful approach is to confer adoptive immune cells with specificity against antigens expressed on cancer cells by engineering them to express chimeric antigen

receptors (CAR) [2]. Currently, T cells are the predominant immune cell type used for CAR-based immunotherapy, and multiple CAR-T cell therapies have received FDA approval [3]. CARs feature an antigen recognition domain derived from single-chain variable fragments (scFv) of monoclonal antibodies, while their cytoplasmic portion includes different intracellular signaling domains. The first generation of CARs had a single cytoplasmic CD3 ζ signaling domain connected to the recognition domain by a transmembrane linker. Later advances resulted in second-generation CARs, which have one additional signaling domain originating from natively

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occurring costimulatory receptors [4]. 4-1BB (also referred to as CD137) is one such costimulatory domain frequently incorporated in the structure of the CAR [5].

The effects mediated by the 4-1BB domain in immune cells have been tracked to the Nuclear Factor κ B (NF κ B) signaling pathway [6–8], which is comprised of two branches, termed canonical and non-canonical NF κ B signaling [9, 10]. The canonical NF κ B signaling is triggered in a matter of minutes after stimulation, while the non-canonical system is slower and depends on protein translation to exert its effect [11]. In addition, there is a functional difference between the two branches: the sets of genes activated by each are not identical [12]. The genes of many cytokines, such as those of IL-1 α / β [13, 14], IL-6 [15], IL-10 [16] and IFN γ [17], as well as perforin [18], show experimental evidence of canonical NF κ B regulation. Meanwhile, the non-canonical NF κ B pathway was found to be responsible for improved survival rates among 4-1BB-bearing second-generation CAR-T cells [8]. Thus, investigating NF κ B signaling initiated by 4-1BB can help gain a deeper understanding of the observed biological features of CAR cells.

Computational systems biology provides tools to explore signaling systems without the need for expensive and prolonged experimentation. For example, this approach was successful in describing T cell receptor-induced activation of MAPK/ERK signaling [19]. Recently, our research group successfully utilized an ordinary differential equation (ODE)-based model to describe MAPK signaling in a heterogeneous CAR-T cell population [20, 21]. Several mathematical models have been published for NF κ B signaling as well. Some of these mathematical models have mostly focused on downstream events of the pathway, replacing the dynamics of IKK β with an assumed activation profile [22–24], while other models have incorporated a more explicit account of upstream processes [25–27]. Mathematical modeling was also used to analyze the NF κ B pathway as an information transmission channel. This work has shown that the pathway can encode approximately 1 bit of information at most, i.e., whether the extracellular stimulus is present or absent [28, 29]. Insights from the information-theoretic analysis of NF κ B signal transmission were extended to the expression patterns of genes regulated by NF κ B [30]. However, none of these analyses address NF κ B signaling in the context of CAR cells. In addition, while some published models include aspects of NF κ B activation via 4-1BB [31], they are based on a logic-gate approach and do not capture the dynamic chemical interactions in response to stimulation.

Hence, in our current work, we set out to develop a mechanistic ODE-based model of canonical NF κ B signaling initiated by antigen binding to a CD19scFv-4-1BB.

We applied the model to quantitatively investigate the features of 4-1BB-mediated NF κ B signaling. We found that in response to antigen binding, 4-1BB initiates a transient increase in the nuclear concentration of NF κ B. The timing of peak NF κ B concentration is relatively consistent, while its magnitude shows a much greater dependence on the antigen level. Next, we found that very few of the model parameters have a significant impact on the activation time and magnitude of the NF κ B pathway, indicating that the behavior of the model would be robust to large variations in kinetic parameters and protein concentrations. Finally, by analyzing the mutual information between antigen concentration and successive levels of the NF κ B pathway in conditions of simulated variability, we proposed potential improvements to CD19scFv-4-1BB cells. Specifically, our results suggest that the overexpression of NEMO or the disabling of IKK β deactivation would make NF κ B activation level more suited to the encountered antigen concentration. Altogether, we provide a quantitative framework that can be used to guide the development and optimization of 4-1BB-induced CAR signaling.

Methods

Model structure

By performing an extensive literature search, we compiled a list of key protein–protein interactions and chemical reactions, which form the core of the activation of the canonical NF κ B pathway. In our model, in response to antigen binding, the 4-1BB domain of the CAR induces the formation of a signalosome, in which TRAF2 trimers play a key role [7, 32, 33]. Acting as a ubiquitin-ligase, TRAF2 promotes the attachment of long K63 ubiquitin chains to RIP1 [34, 35]. Once RIP1 is ubiquitinated, its K63 ubiquitin chains enable the docking of TAB1/2/3, the adaptor subunits of the TAK enzymatic complex [36]. Similarly, the IKK multimeric complex is docked with NEMO (also known as IKK γ) as an adaptor subunit to the K63 ubiquitin chains of RIP1 [37]. The protein TAK1, the enzymatic subunit of the TAK complex, phosphorylates the β subunit of the IKK complex in a ubiquitin-dependent manner [38]. IKK β activation is transitory since phosphorylation of a cluster of 10 serine residues at a regulatory domain deactivates it [39, 40]. Catalytically active IKK β phosphorylates the protein I κ B α , targeting it for proteasomal degradation [10, 41, 42]. In resting cells, I κ B α tightly associates with cytoplasmic NF κ B, preventing it from shuttling to the nucleus. However, the degradation of I κ B α under the influence of catalytically active IKK β releases cytoplasmic NF κ B from this sequestration and makes it free to translocate to the nucleus, where it can function as a transcription factor and alter the expression profile of the cell [9]. In this work, we do

not explicitly consider the transmembrane domain, as we have not uncovered experimental evidence indicating that the transmembrane domain has a direct effect on NF κ B signaling. Rather, 4-1BB domain's recruitment of TRAF proteins seems to be the defining event that triggers downstream signaling.

These reactions were represented as interaction rules in RuleBender (v2.3.1) [43]. Then, RuleBender was used to generate a corresponding set of ordinary differential equations (ODEs) describing the temporal dynamics of the system. MATLAB (v2019a/b) was used to integrate the system of ODEs to obtain time courses of the species involved. Kinetic parameters and protein concentrations were compiled from prior models of different components of the system or direct experimental measurements, with a detailed list provided in Additional file 1.

Activation profiles and dose response curves

We simulate binding of CD19, a common target for CAR therapies, to the antigen recognition domain of the CAR. Prior experimental measurements of the concentration of CD19 estimated its surface concentration to have a mean of 3.4 molecules per μm^2 on NALM-6, a human acute lymphoblastic leukemia-derived cell line, while the CD19 concentration on primary myeloma cells was found to be in the range of 0.16–5.2 μm^2 [44]. We took these values as a basis for our simulations of activation profiles and dose response curves but used the wider range of $10^{-0.5}$ – $10^{2.5}$ molecules per μm^2 in order to gain a complete picture of dynamic behaviors captured by the model in a broader range of antigen concentrations.

Sensitivity analysis

We performed sensitivity analysis to determine how strongly model inputs (kinetic parameters and initial protein concentrations) affect the quantitative predictions of the model. We use the extended Fourier Amplitude Sensitivity Test (eFAST) method of quantifying global sensitivity of a mathematical model to underlying parameter values [45]. The sensitivity coefficients are obtained by simultaneously varying parameters of interest at different pre-assigned signature frequencies and evaluating a system output for each subsequent value of the parameter vector. Then, the Fourier transform of sequential values of system output is taken to reveal its spectral composition. The underlying assumption is that if a parameter influences a system output to a significant extent, then its periodic variation according to a certain signature frequency would cause large variation in the model output at that frequency, as revealed by a higher amplitude in the Fourier transform. The significance of different parameters is evaluated in comparison with the sensitivity coefficient attained by a “dummy” parameter, a parameter with

no involvement in the system at all. Following the spectral decomposition of the output, if the amplitude at any system parameter's characteristic frequency is lower than that for the “dummy” parameter, the system parameter is taken to have no significant impact on system output.

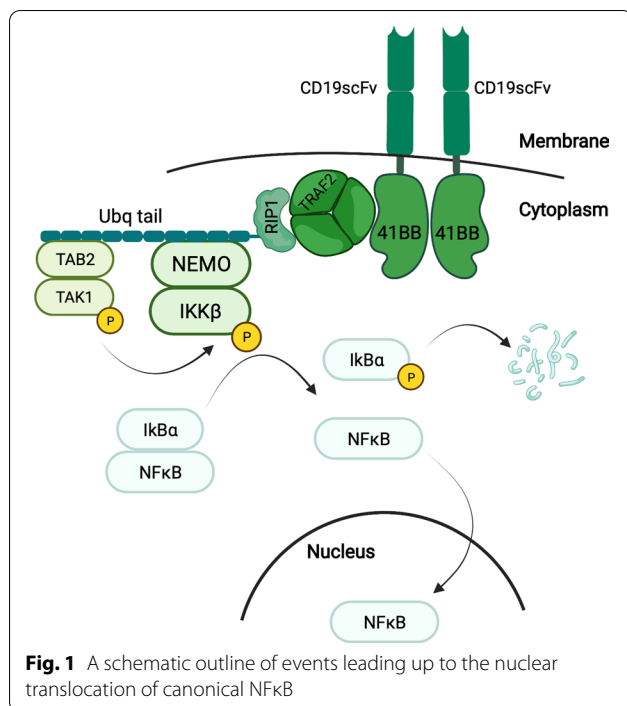
Monte Carlo simulations

We considered a population of CAR-expressing cells by accounting for cell-to-cell variability in protein concentrations. Motivated by prior experimental findings [46], we modeled the intrinsic noise of the population response by assuming that proteins involved in signal transduction in each cell have varying initial concentrations subject to a lognormal distribution. We chose the location parameter of each distribution so that the median concentration across the CAR cell population would equal the accepted value of the protein concentration. The scale parameter was taken to be the same for all proteins, and different hypothesized values of this scale parameter were investigated to gain a more complete picture (termed “noise parameter” throughout). We included variability in target antigen concentration by assuming a lognormal distribution and choosing the location and scale parameters such that the distribution would mostly lie within the measured range of 0.16–5.2 μm^2 for myeloma cells and have a mean of approximately 3.4 μm^2 as measured for lymphoma cells [44]. This random sampling with subsequent simulation was repeated 2000 times to yield representative distributions.

Mutual information

Mutual information is a quantity measuring the mutual dependence of two random variables [47]. As such, its value is zero if and only if two random variables are independently distributed, and a positive number if they depend on each other. We used the Kraskov algorithm for computing mutual information based on distribution entropies, with the entropies estimated through a K -nearest neighbors procedure [48].

We computed the mutual information between antigen levels and five different stages of signal transduction: concentration of antigen-bound CAR, amount of TRAF2 bound to the 4-1BB domain, amount of RIP1 bound to the TRAF2 signalosome, concentration of enzymatically active TAB2/TAK1, peak concentration of enzymatically active NEMO/IKK β , and peak nuclear concentration of NF κ B. In order to quantify the amount of information reaching a certain level along signal transduction, we randomized the concentrations of all proteins upstream of that level, leaving the rest of the protein concentrations unchanged. Then, we stimulated the system by a randomly sampled antigen concentration. Since only the steps leading up to the

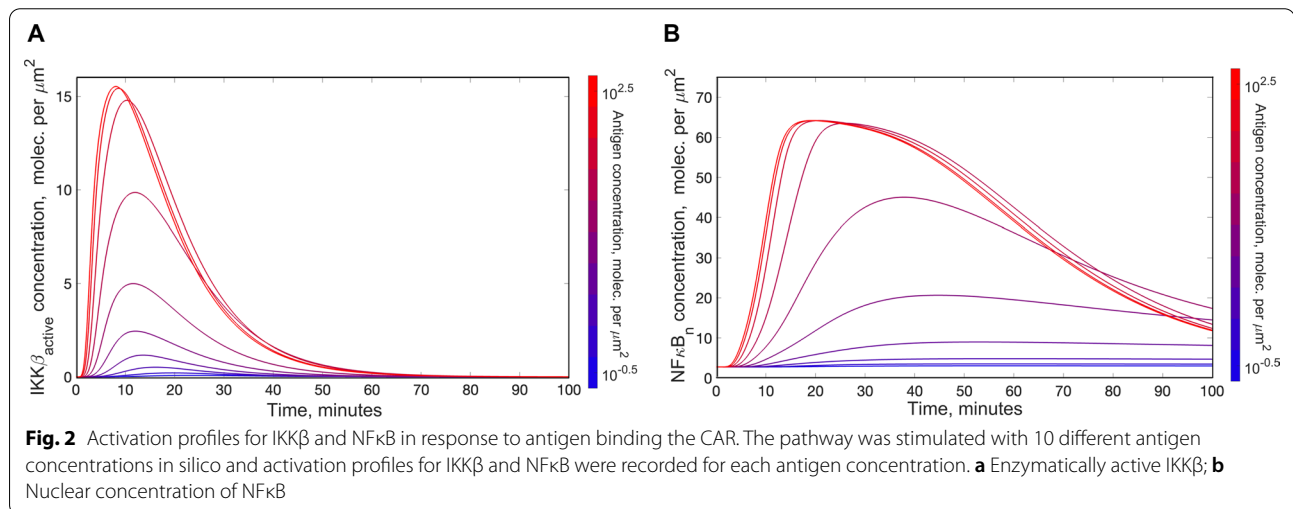


Results

Model structure and dose response

In order to obtain a quantitative description of signaling events triggered by antigen binding CD19scFv-4-1BB cells, we developed an ODE-based mechanistic model (Fig. 1, Supplemental File). The biochemical reactions included in the model are based on prior experimental and modeling studies available in literature. Given the paucity of quantitative experimental measurements for 4-1BB in immune cells, the model is largely based on data from studies performed using fibroblasts. We utilize this model to investigate various aspects of 4-1BB signaling and generate novel hypotheses about how to enhance the signaling promoted by this co-stimulatory domain.

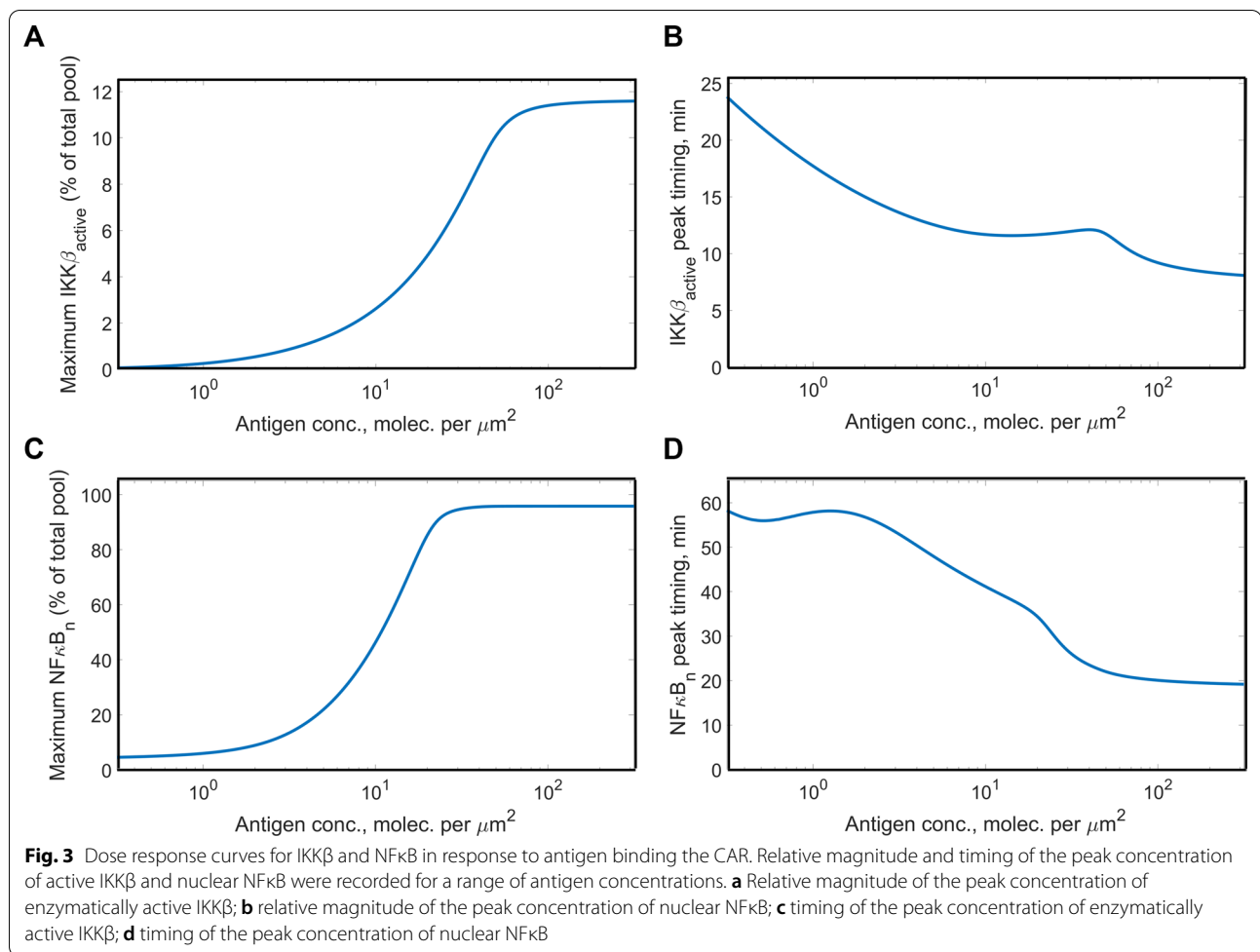
To understand the signaling dynamics predicted by our model, we generated time courses for enzymatically active IKKβ, which is a key convergence point for NFκB-activating signals [49–51], and nuclear NFκB for a range of physiologically relevant antigen concentration values (Fig. 2). The model predicts that in response to persistent antigen binding, there is only transient activation of IKKβ. The IKKβ concentration is predicted to exhibit a rapid-onset, short-term peak typically occur-



given level were randomized, any variation at this level would reflect upstream stochasticity and not an artifact of the simulation. On the other hand, by randomizing all upstream proteins, we ensured that the contribution of every upstream component to overall stochasticity is accounted for. By performing this procedure for five different levels of the signal transduction pathway, we were able to track how much mutual information is shared between the antigen concentration and a given level of the signaling pathway.

ring 10–15 min after stimulation (Fig. 2a). We observed a similar transient peak for the nuclear concentration of NFκB. Here, the peak was broader, with the maximum concentration occurring after approximately 20–40 min of stimulation with physiologically relevant antigen concentrations (Fig. 2b).

Next, we obtained dose response curves for the activation of IKKβ and NFκB (Fig. 3). Notably, only a small fraction of the total IKKβ pool (<15%) was activated even at its maximal level and with saturating antigen levels (Fig. 3a). Peak concentration achieved by active IKKβ



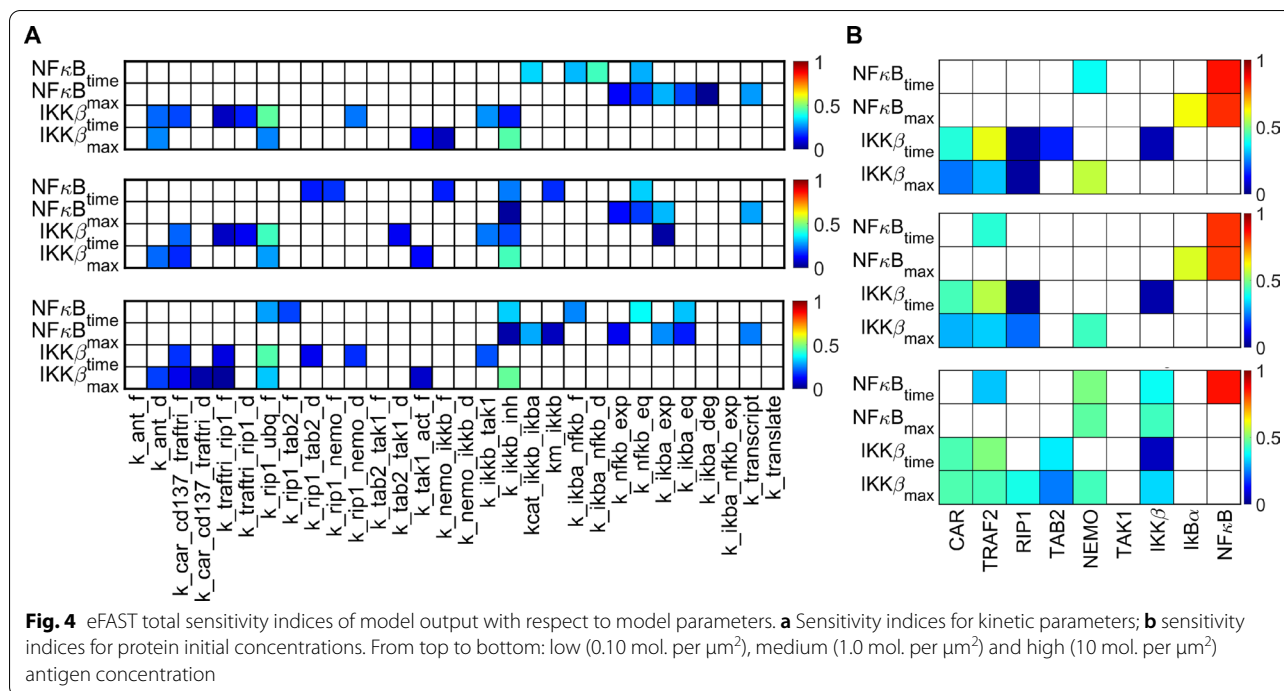
had a sigmoidal dependence on antigen concentration and was sensitive to antigen levels. A 1000-fold change in antigen concentration results in a 193-fold change in active IKK β peak concentration. In contrast, the timing of the IKK β peak was relatively robust to the antigen concentration. Specifically, a 1000-fold range of antigen concentrations produces only a threefold change in the time at which IKK β peaked (Fig. 3b). The nuclear concentration achieved by NF κ B increased with increasing antigen concentration (21-fold change in the response range) and showed a sigmoidal dependence on antigen concentration (Fig. 3c). In addition, the timing of peak nuclear NF κ B concentration was relatively consistent for different antigen concentrations (2.9-fold change in the response range, Fig. 3d).

Sensitivity of the output to kinetic parameters and protein concentrations

Once we established basic features of pathway activation predicted by our model, we proceeded to evaluate how the model output is impacted by the model parameters.

This would allow us to see how sensitive the quantitative predictions from our model are with respect to choices of specific values for kinetic constants and protein initial concentrations. To this end, we used the Extended Fourier Amplitude Sensitivity Test (eFAST), a method of global sensitivity analysis, on the kinetic constants for regimes of low, medium and high antigen stimulation (Fig. 4a). We evaluated the impact of the kinetic constants on four measures of the system output: (1) the timing of peak active IKK β concentration, (2) the magnitude of peak active IKK β concentration, (3) the timing of peak nuclear NF κ B concentration and (4) the magnitude of peak nuclear NF κ B concentration.

Based on our results, the responses of both IKK β and NF κ B appear to be relatively robust with respect to variations in the kinetic parameters. In fact, only very few parameters are shown to significantly impact the timing and magnitude of their peak concentrations. For example, at most eight of the 30 kinetic constants influence the magnitude or timing of nuclear NF κ B across the range of antigen concentrations we considered



(considering a sensitivity index > 0.3). Particularly, at low antigen concentrations, nuclear import/export rates of NF κ B and I κ B α strongly influence peak nuclear NF κ B concentration. At higher antigen concentrations, parameters related to the enzymatic activity of IKK β exert more influence.

Next, we performed eFAST on the initial concentrations of the proteins involved in signal transduction (Fig. 4b). We observed a very strong dependence of the timing and magnitude of peak nuclear NF κ B concentration on the total cellular amount of NF κ B. In addition, with non-saturating antigen concentrations, the NF κ B response is predicted to depend on the amount of I κ B α , the protein that sequesters NF κ B in the cytoplasm. Interestingly, while the activation of the upstream protein IKK β depends on the amounts of many of the upstream proteins, those upstream species do not significantly affect NF κ B.

The robustness of model output to kinetic parameters and initial concentrations of pathway proteins demonstrates that the dynamics of the NF κ B pathway predicted by the model would remain essentially similar across a wide range of parameter values. This indicates that the quantitative insights derived from the model would be applicable to a broad range of systems, including engineered CD19scFv-4-1BB cells, even though the model is based on studies performed on fibroblasts. This provides confidence that we can gain biological insights from the model predictions.

Mutual information carried by the NF κ B pathway about antigen concentration

Signal transduction pathways are subject to biochemical noise, which can prevent downstream messengers from serving as an exact reflection of the extracellular signal [52]. In the case of CD19scFv-4-1BB signaling, this means that the activation of the canonical NF κ B pathway and subsequent changes in gene expression patterns mediated by the nuclear localization of NF κ B may not necessarily match the amount of antigen to which the cell is exposed. In order to make assessments about the accuracy of signal transduction of canonical NF κ B signaling in CD19scFv-4-1BB cells, we simulated a population of CD19scFv-4-1BB cells in which the accurate population-level response to target antigen was precluded by stochastic variations in the intracellular concentrations of proteins involved in signaling. We were particularly interested in how accurately each successive level of the transduction pathway reflects the amount of antigen stimulation. To this end, we performed simulations by stochastically varying all protein concentrations upstream from a given level of the pathway. We also varied the antigen concentration level. Then, we computed the mutual information between the distribution of responses at this level and the distribution of antigen concentrations (Fig. 5). This allowed us to track how the amount of information carried by the pathway changes at each level of signal transduction. Since experimental measurements on the intrinsic variability of CD19scFv-4-1BB cells were not available, we performed

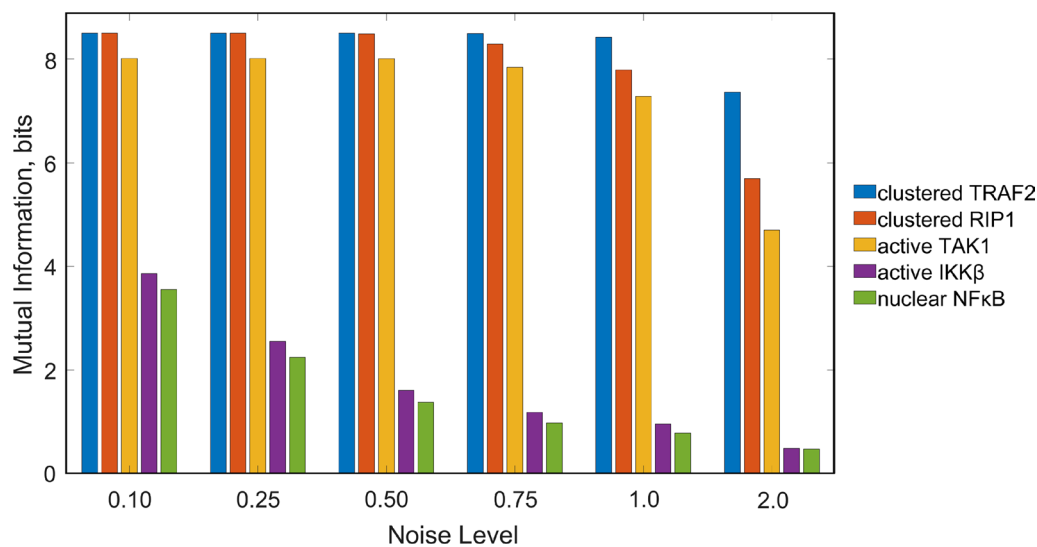


Fig. 5 Information transduction along the canonical NF κ B pathway. Calculated mutual information in bits between antigen concentration and concentration of clustered TRAF2, clustered RIP1, enzymatically active TAK1, enzymatically active IKK β and nuclear NF κ B

our simulations for multiple values of the noise parameter. In this way, we were able to identify common observable trends that characterize the system, regardless of a certain variability level considered.

We can visually observe differences between the distribution of peak NF κ B nuclear concentrations and the antigen distribution (Additional file 2: Fig. S1). Interestingly, we observed that for the same underlying distribution of antigen concentrations, higher levels of intrinsic noise diminish the strength of NF κ B signaling (Additional file 2: Fig. S1).

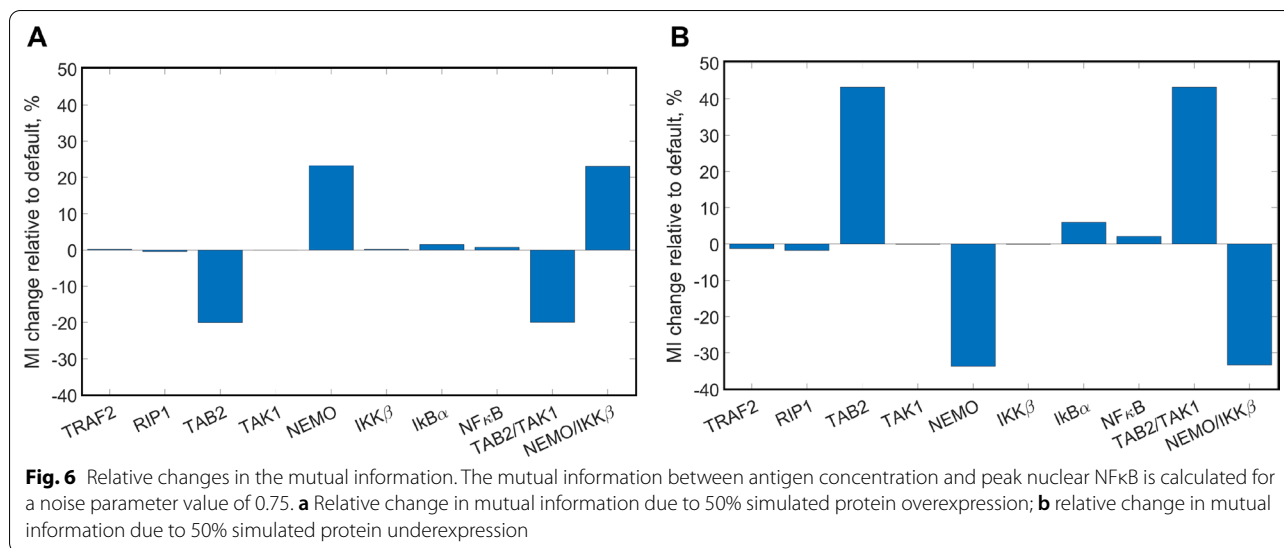
Our analysis of the mutual information provides a quantitative measure of differences between the antigen distribution and the distribution of concentrations for successive steps in the signaling pathway (Fig. 5). We predict that the proteins recruited early in signal transmission exhibit a relatively small loss of information following antigen stimulation. By definition, the mutual information between two distributions cannot exceed the marginal entropies of each of those distributions [47]. The marginal entropy for the distribution of antigen concentrations we used was 8.79, while the mutual information at the level of TRAF2 clustering was close to this upper limit, between 7.36 and 8.50 bits, depending on the intrinsic noise level. Interestingly, most information loss consistently occurred between the enzymatic activation of the TAB2/TAK1 complex and enzymatic activation of the NEMO/IKK β complex. In some conditions, there was up to a tenfold reduction in the mutual information at this stage. Meanwhile, signal transduction between enzymatically active IKK β and the nuclear translocation

of NF κ B seemed relatively accurate with only a small reduction in mutual information. In summary, we find that for all noise levels, mutual information is predicted to decrease as the signal is transduced through the pathway, with the steps directly leading up to the activation of IKK β as the least accurate in signal transduction.

Suggested improvements to CAR design based on mutual information

A primary goal of CAR therapy is to enhance the on-target cytolytic activity of CAR cells while avoiding side effects. Some of these side effects include cytokine release syndrome, on-target-off-tumor effects, and graft-versus-host disease [53]. We hypothesized that if secondary messengers more accurately reflect the amount of antigen encountered by the engineered CAR cell, CAR cells would show a more specific response to antigen binding. Particularly, we suggest that increasing the mutual information between the antigen levels and the nuclear concentration of NF κ B would affect the therapeutic performance of cells in a manner such that the cells do not become unnecessarily active when encountering only trace amounts of the target, while showing enhanced activity levels when encountering higher amounts of antigen.

Thus, we applied the model to investigate how changing the initial concentration of each protein involved in signal transduction affects the mutual information between antigen concentration and nuclear NF κ B concentration (Fig. 6). We repeated our Monte Carlo simulations, this time increasing or decreasing by 50% the



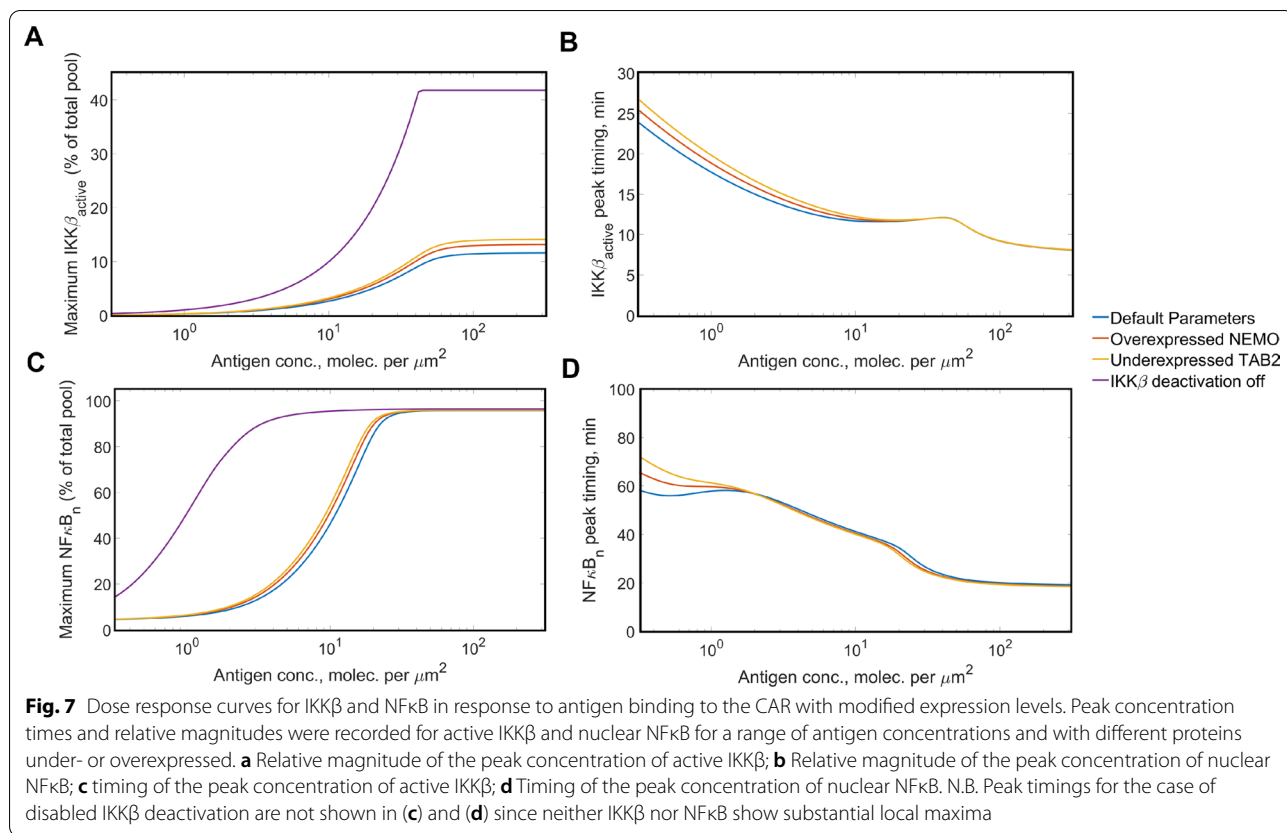
initial concentrations for individual proteins. We also considered modulating pairs of proteins that together form multimeric enzymes, such as TAB2/TAK1 and NEMO/IKK β , to check for possible synergistic effects. The analysis showed that it is possible to increase mutual information by 23% by overexpressing NEMO (Fig. 6a). The overexpression of NEMO yielded the same increase as its joint overexpression with IKK β , indicating a lack of synergistic effects. Mutual information decreased by 20% when TAB2 was overexpressed separately or in conjunction with TAK1. We believe that these results indicate that overexpressing NEMO experimentally would make the nuclear NFκB concentration in response to activation more reflective of the extracellular antigen concentration. Thus, the potency of the cellular response triggered by NFκB would have a closer correspondence to the targets encountered. When repeating the same procedure for decreased protein concentrations (Fig. 6b), the estimated mutual information between antigen levels and nuclear NFκB is predicted to increase by 43% with TAB2 underexpressed but is reduced by 33% when underexpressing NEMO.

In order to elucidate the mechanism by which the overexpression of NEMO and underexpression of TAB2 cause an increase in mutual information between antigen exposure and NFκB activation, we obtained dose response curves for peak concentrations of active IKK β (Fig. 7a) and nuclear NFκB (Fig. 7c), and their timings (Fig. 7b, d) in each of those cases. We can see that in cases of intermediate and high antigen concentrations but not low concentrations, the overexpression of NEMO causes an increase in peak concentration attained by active IKK β , compared to the default concentration levels (Fig. 7a). The enhancing effect of NEMO overexpression

on nuclear NFκB peak concentration (Fig. 7c) is limited to intermediate antigen concentrations. Thus, NEMO overexpression alters the response of the canonical NFκB pathway in a way, such that the pathway is slightly more active in case of moderate antigen concentrations without showing such an enhancement at lower antigen concentrations.

By plotting similar dose response curves for the case of TAB2 underexpression (Fig. 7), we saw an effect by which the decreased amount of TAB2 caused more IKK β activation. This was highly surprising, since the TAB2/TAK1 complex is known to phosphorylate and activate the NEMO/IKK β complex, where TAB2 and NEMO are the docking subunits of each multimeric enzyme [36, 37]. We hypothesized that since both TAB2 and NEMO dock at K63 ubiquitin tails attached to RIP1, the underexpression of TAB2 would make more docking sites available to NEMO and, thus, increase the amount of IKK β available for activation. Indeed, when we ran simulations to compare concentrations of ubiquitin-bound TAB2 and NEMO, we saw that with underexpressed TAB2, there is a greater abundance of ubiquitin-bound NEMO accompanying the decrease in ubiquitin-bound TAB2 (Additional file 2: Fig. S2). This can also explain the greatly reduced mutual information when TAB2 is overexpressed (Fig. 6a), since in this case, TAB2 would out-compete NEMO for access to docking sites. Thus, both over- and underexpression studies underline the centrality of NEMO abundance for enhancing the response of the canonical NFκB pathway.

Finally, given the importance of IKK β activity for nuclear translocation of canonical NFκB, we investigated how altering the IKK β activation profile may affect signal transduction. Specifically, we tested whether more



persistent IKK β activity resulting from disabled deactivation would enhance canonical NF κ B signaling for low and intermediate antigen concentrations. We were motivated by the fact that such manipulations were shown to be feasible experimentally [39]. As expected, the model predicted that lasting IKK β activation (Additional file 2: Fig. S3A) results in more persistent nuclear concentration of NF κ B upon antigen stimulation (Additional file 2: Fig. S3B).

In order to test the consequences of this hypothetical manipulation on accuracy of signal transduction, we repeated our simulations to compute the mutual information carried by the pathway when IKK β deactivation is disabled. Notably, the mutual information between antigen concentration and nuclear NF κ B concentration showed a dramatic increase for all values of the noise parameter (Additional file 2: Fig. S4). In order to find out the reasons for this increase, we plotted dose response curves for the system under disabled IKK β deactivation (Fig. 7). Deactivation of IKK β increases the peak level of IKK β for intermediate to high antigen concentrations (Fig. 7a). On the other hand, there was a substantial amplification of the NF κ B response in low and intermediate antigen concentration conditions, with the amplification observed for the intermediate range being much

more dramatic (Fig. 7c). This suggests that if IKK β deactivation is absent, the canonical NF κ B pathway would be highly sensitive to antigen concentrations of the order of 1 molecule per μm^2 , without demonstrating as high activation levels for much lower antigen concentrations. Interestingly, the timing of the peak IKK β and NF κ B were not significantly affected by IKK β deactivation (Fig. 7b, d).

Discussion

We present a novel mathematical model of NF κ B signaling mediated by 4-1BB. Importantly, the model predicts signaling features that agree with what is already known about NF κ B signaling. Particularly, prior measurements have shown that IKK β activity peaks 10–15 min after stimulation, and nuclear NF κ B peaks approximately 30 min after stimulation in the dynamic range of the pathway [54]. Thus, the model generates dynamics that have been observed experimentally, and it can be used to investigate the effects of 4-1BB stimulation.

We applied this model to investigate various characteristics of 4-1BB-mediated signaling. By examining the dose response of active IKK β and NF κ B, we found that the magnitudes of their transient peaks are sensitive to antigen concentration, while the timing of their peaks

changes very little within the range of physiologically relevant stimulus concentrations. Next, we used a global sensitivity analysis to identify parameters that have a significant influence on the magnitude of pathway activation. Particularly, our results highlight the influence of NF κ B nuclear import/export parameters in determining the peak nuclear concentration of NF κ B for most levels of antigen concentrations. Finally, our analysis of mutual information between antigen concentration and successive levels of the NF κ B pathway enabled us to suggest specific strategies for enhancing the accuracy of NF κ B signaling initiated by 4-1BB. Particularly, overexpression of NEMO and disabling of IKK β deactivation can greatly increase the information transmission capabilities of the NF κ B pathway. Thus, we provide quantitative insights to motivate bioengineering efforts for CAR cells, which are feasible with modern experimental techniques [39, 55, 56]. Prior experimental studies have shown that such targeted manipulations can greatly increase the therapeutic potential of CAR cells. Specifically, partially impeding the phosphorylation of the CD3 ζ ITAM sequences was found to increase CAR cell persistence and confer superior anti-tumor ability [57], while directing the CAR gene insert to specific loci can result in steadier expression levels and diminished tonic signaling [58]. Thus, our modeling analysis provides biologically relevant insight that can be similarly applied to engineered immune cells. CD19scFv-4-1BB Natural Killer (NK) cells are a particularly promising platform for future work. It has been shown that NK-based adoptive cell therapies tend not to suffer from graft-versus-host disease [55–57] and cytokine release syndrome [58–60], disadvantages that frequently accompany CAR-T cell-based therapy [53]. Thus, modeling-driven design improvements to CD19scFv-4-1BB NK cells motivated by a greater understanding of cell signaling would contribute to further progress in CAR development.

Prior experimental measurements of signal transduction by the canonical NF κ B pathway have shown that the channel capacity (i.e., the maximum possible mutual information) of this pathway is approximately equal to 0.92 bits, which implies that it can resolve only $2^{0.92} \approx 2$ levels of extracellular signal [28]. This was interpreted to mean that the pathway is able to only distinguish the presence of the stimulus from its absence. An information theoretic analysis of signaling pathways has shown that even small variations in the number of bits carried by pathways (0.6 bits vs. 0.85 bits) can result in a two-fold difference in the number of cells that exhibit an erroneous response to the extracellular signal [61]. Based on this, we suggest that increasing the mutual information carried by the canonical NF κ B pathway in response to antigen binding the CAR can result in a closer alignment

between CD19scFv-4-1BB cell activity and the antigen concentration it encounters. Thus, our findings can guide further attempts to improve the performance of CAR cells. For example, the predicted improvements can allow the cell to not only to distinguish more accurately whether there is a target or not, but also to discriminate between various concentrations of the target.

Along with the significant findings produced by our work, we recognize some aspects that can be addressed in the future. One limitation is that since direct measurements or estimates were not available for some parameter values, we had to make order-of-magnitude estimates by knowing the timescale at which underlying biological phenomena are observed. Values for other kinetic parameters and protein concentrations were taken from multiple sources, all measured or indirectly estimated in fibroblasts with receptors from the TNFR superfamily triggering the activation of NF κ B. Our motivation for directly applying these data on CD19scFv-4-1BB is the fact that 4-1BB itself is a member of the TNFR superfamily, and like many other members of the same superfamily, transmits signals by means of TRAF proteins [33, 57, 62]. However, more exact numerical results can be obtained if the model is specifically calibrated for the context of CD19scFv-4-1BB-engineered immune cells. We note that quantitative assays via mass-spectrometry [67] have been used to quantify the levels of proteins in NF κ B signaling (reviewed in [68]), along with multiple other signaling networks. More recent iterations of this experimental technique can directly provide absolute concentrations, in contrast to relative concentrations that are then calibrated to a protein of known concentration [69]. Secondly, even though our model accounts for competition between TAB2 and NEMO for docking sites along K-63 ubiquitin tails, this rests on the assumption that each ubiquitin tail has only two docking sites and that the sites do not discriminate between different docking proteins. While the lack of such competition would likely mitigate the negative effect of TAB2 abundance on NEMO/IKK β activity, the strong enhancement of NF κ B signaling in case of persistent NEMO/IKK β activation emphasizes NEMO/IKK β complex's centrality for enhancing the response of the pathway. Finally, another limitation is the assumed distribution of antigen concentrations that will be encountered by the CD19scFv-4-1BB cells. Since it is known that at least 95% of B-cell acute lymphoblastic leukemia (ALL) cells are CD19-positive [70, 71] and that protein concentrations typically follow a lognormal distribution [46], we assumed a lognormal distribution and approximated it to match CD19 antigen levels measured on CD19-positive ALL cells [44]. However, specific choices of the antigen distribution could impact our calculation of mutual information. Thus,

exploring a broader range of possible distributions would help to form a more complete picture. For instance, the same experimental study had shown that some myeloma cells (termed “CD19-negative”) show vanishingly small CD19 expression levels (around 0.001 molecules per μm^2) compared to the rest of the population (around 1 molecule per μm^2). Thus, in the case of myeloma cells, the distribution of CD19 concentrations among the population could be approximated by a bimodal distribution. The percentage of these CD19-negative myeloma cells varied significantly from patient to patient in a range of 10–80% (44). A future study could investigate information transmission capabilities of the NF κ B pathway in the context of a bimodal antigen distribution with different percent contributions from each unimodal component.

Conclusions

Our work focused on developing a mathematical model of CD19scFv-4-1BB-induced NF κ B signaling and analyzing its implications. Specifically, we predicted the dose response curves and different activation metrics of the pathway to elucidate the primary features of the signaling dynamics. Using a sensitivity analysis, we identified model parameters that significantly affect the quantitative predictions of our model. Finally, based on an information-theoretic analysis of NF κ B signaling, we were able to make suggestions for the further improvement of CAR cell design. Particularly, our results demonstrated that overexpressing NEMO and disabling IKK β deactivation are predicted to increase the sensitivity of CD19scFv-4-1BB cells against potential therapeutic targets. Our work provides a predictive, quantitative framework that can guide the development and optimization of 4-1BB-induced CAR signaling.

Abbreviations

ALL: Acute lymphoblastic leukemia; CAR: Chimeric antigen receptor; eFAST: Extended Fourier amplitude test; I κ B α : Inhibitor of NF-kappa-B, alpha; IKK: Inhibitor of NF κ B kinase; NEMO: NF κ B essential modulator; NF κ B: Nuclear factor κ B; NK: Natural killer; RIP1: Ribosome-inactivating protein; TAB2: Transforming growth factor beta activated kinase 1 binding protein 2; TAK: Tat-associated kinase; TRAF2: Tumor necrosis factor receptor associated factor 2; scFv: Single chain variable fragment.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12964-022-00937-w>.

Additional file 1: List of model parameters and their values.

Additional file 2: Supplementary figures.

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Author contributions

SDF proposed and directed the project. VT constructed the mathematical model, performed simulations and developed the figures. VT wrote the draft manuscript. VT and SDF jointly edited the manuscript. SDF provided the funding. All authors read and approved the final manuscript.

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Availability of data and materials

The MATLAB files containing the model used in this work, along with the MATLAB scripts used to perform the analyses presented here are available via GitHub: <https://github.com/FinleyLabUSC/CAR-41BB-NFkB-signaling>.

Declarations

Ethics approval and consent to participate

This article does not represent research with ethics considerations.

Consent for publication

This article does not use any private or personal data.

Competing interests

The authors report no competing interests.

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