Modeling predicts differences in CAR T cell signaling due to biological variability

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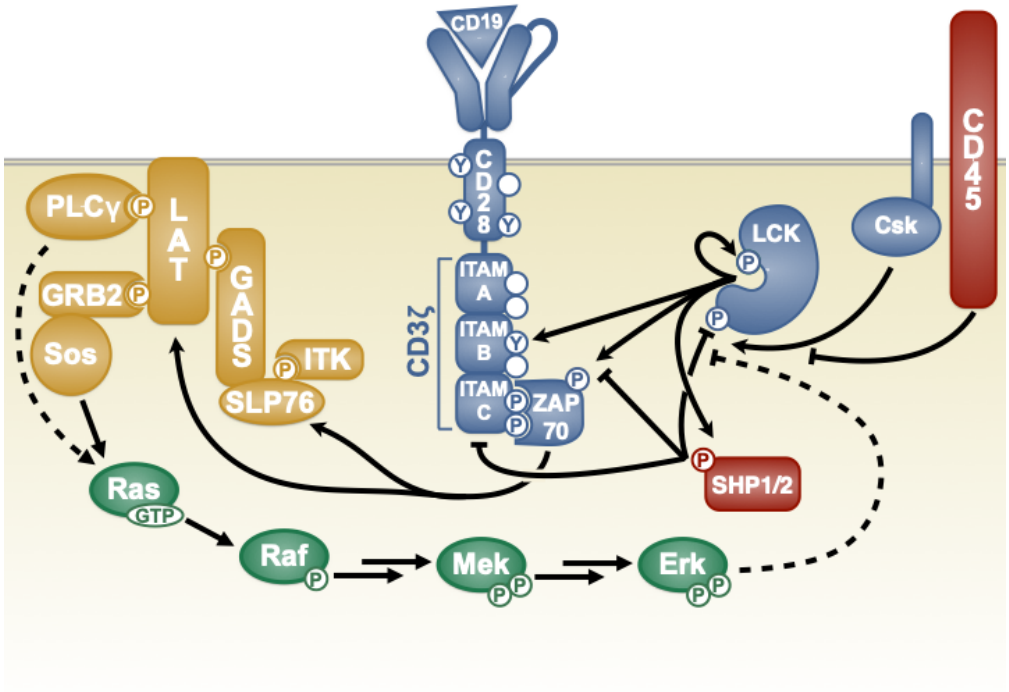
Supplementary Information

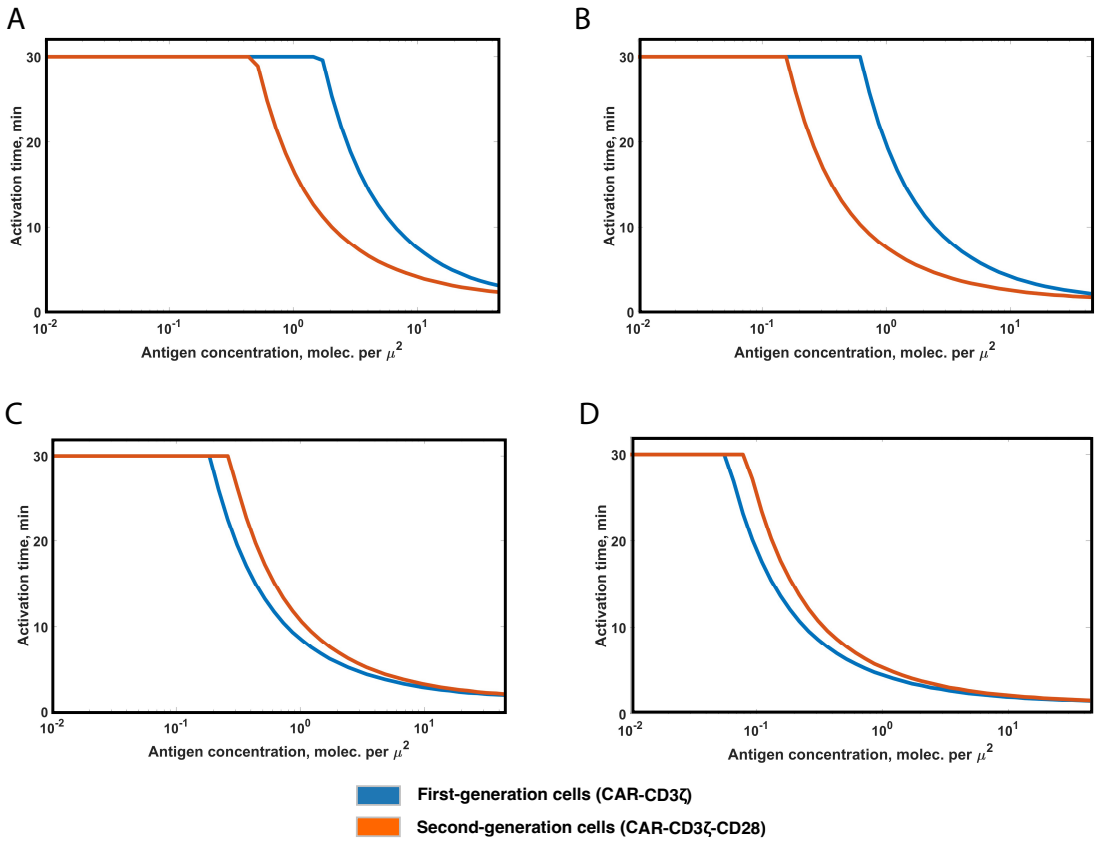
Supplementary Table 1. Performance of the gradient boosted tree ensemble on various datasets.

Construct used: / Antigen conc.:	First-generation	Second-generation
"Low"	R^2 = 0.905±0.0008 EV = 0.905±0.0008	R^2 = 0.900±0.0004 EV = 0.900±0.0004
"High"	R^2 = 0.816±0.0024 EV = 0.816±0.0024	R^2 = 0.807±0.0035 EV = 0.807±0.0035

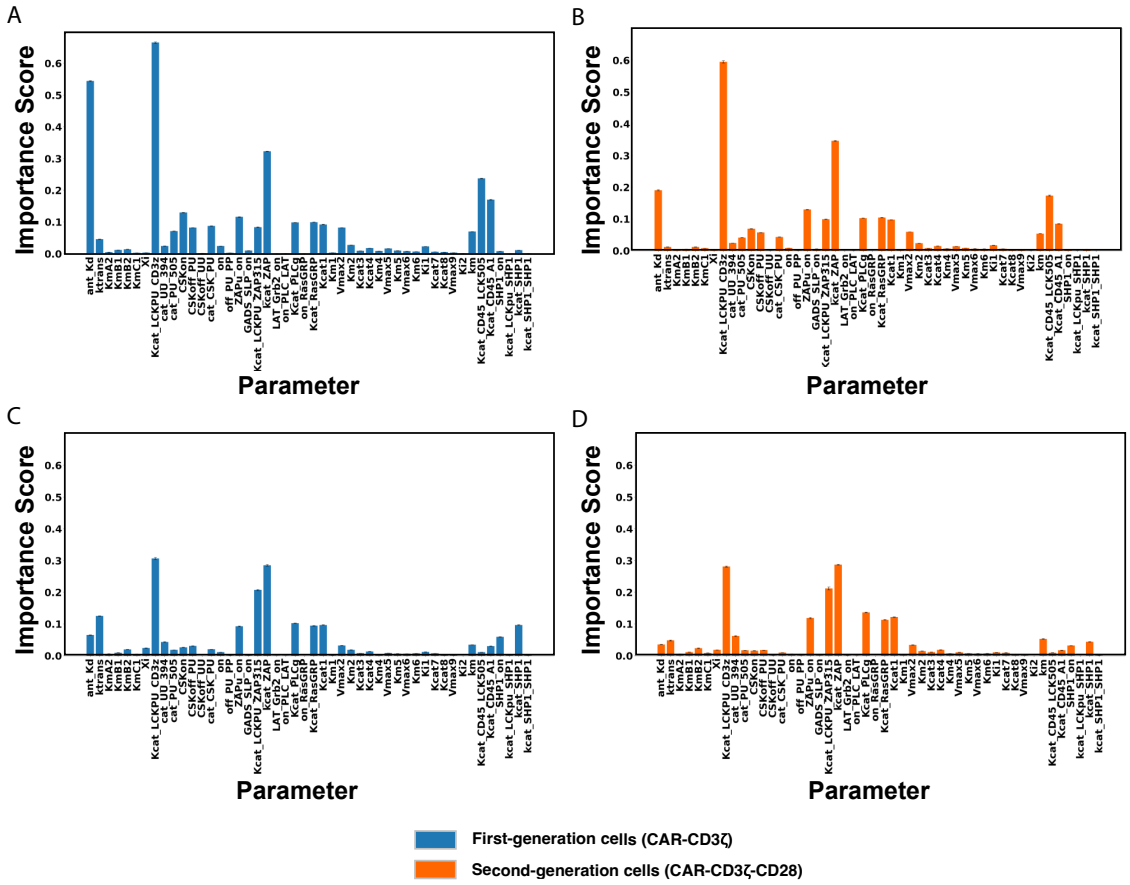
Supplementary Figures



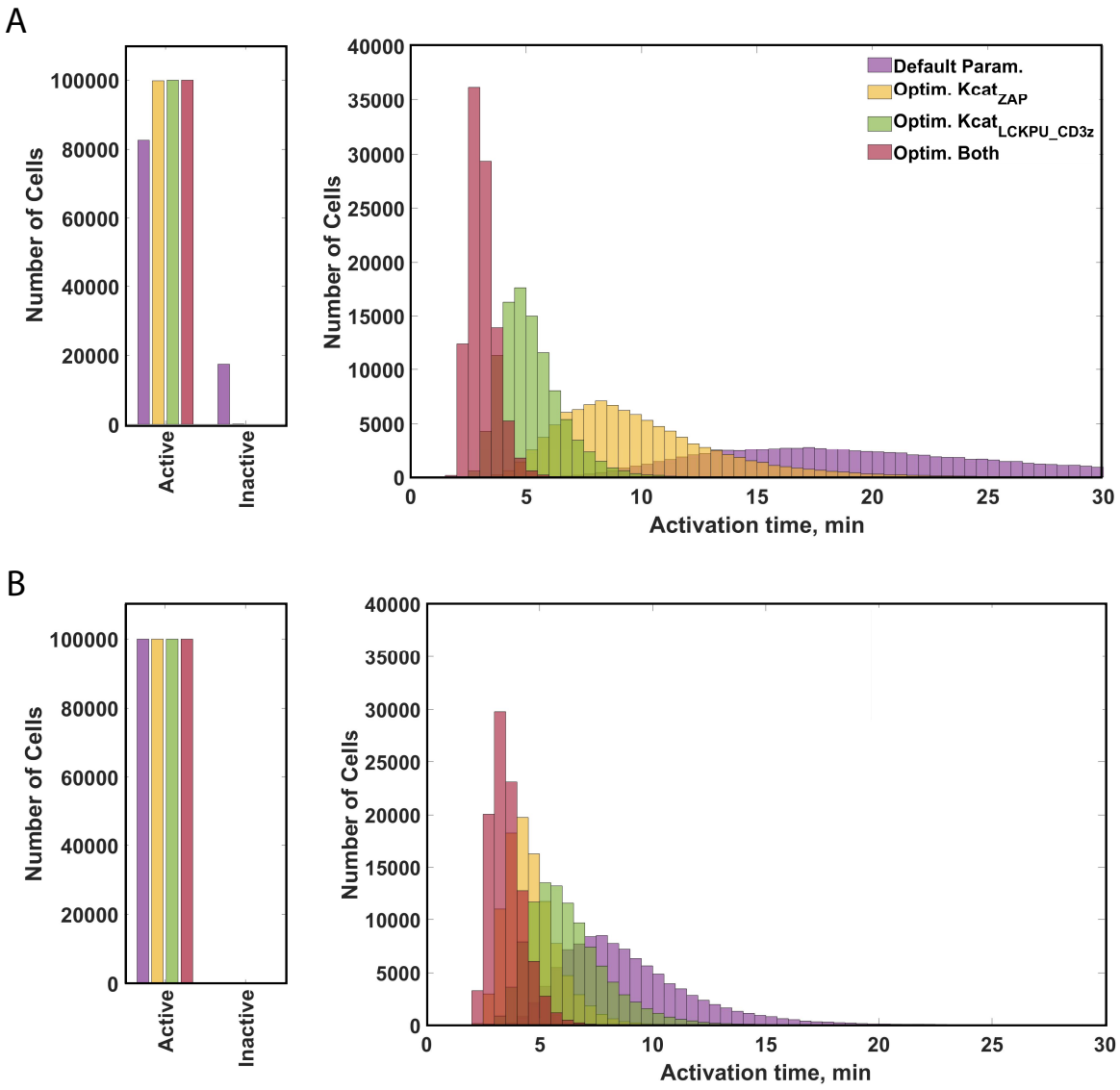
Supplementary Figure 1. Schematic of CAR T cell signaling model. We consider four modules. *Module I* (blue): CAR activation by LCK (whose catalytic activity is regulated by autophosphorylation and the inhibitory kinase, CSK). *Module II* (red): inhibition of CAR activation by phosphatases CD45 and SHP1/2. *Module III* (yellow): formation of the LAT signalosome, a multi-protein complex. *Module IV* (green): downstream signaling in the MAPK pathway, leading to ERK activation. Lines with bar indicate inhibition; lines with arrowheads indicate activation steps.



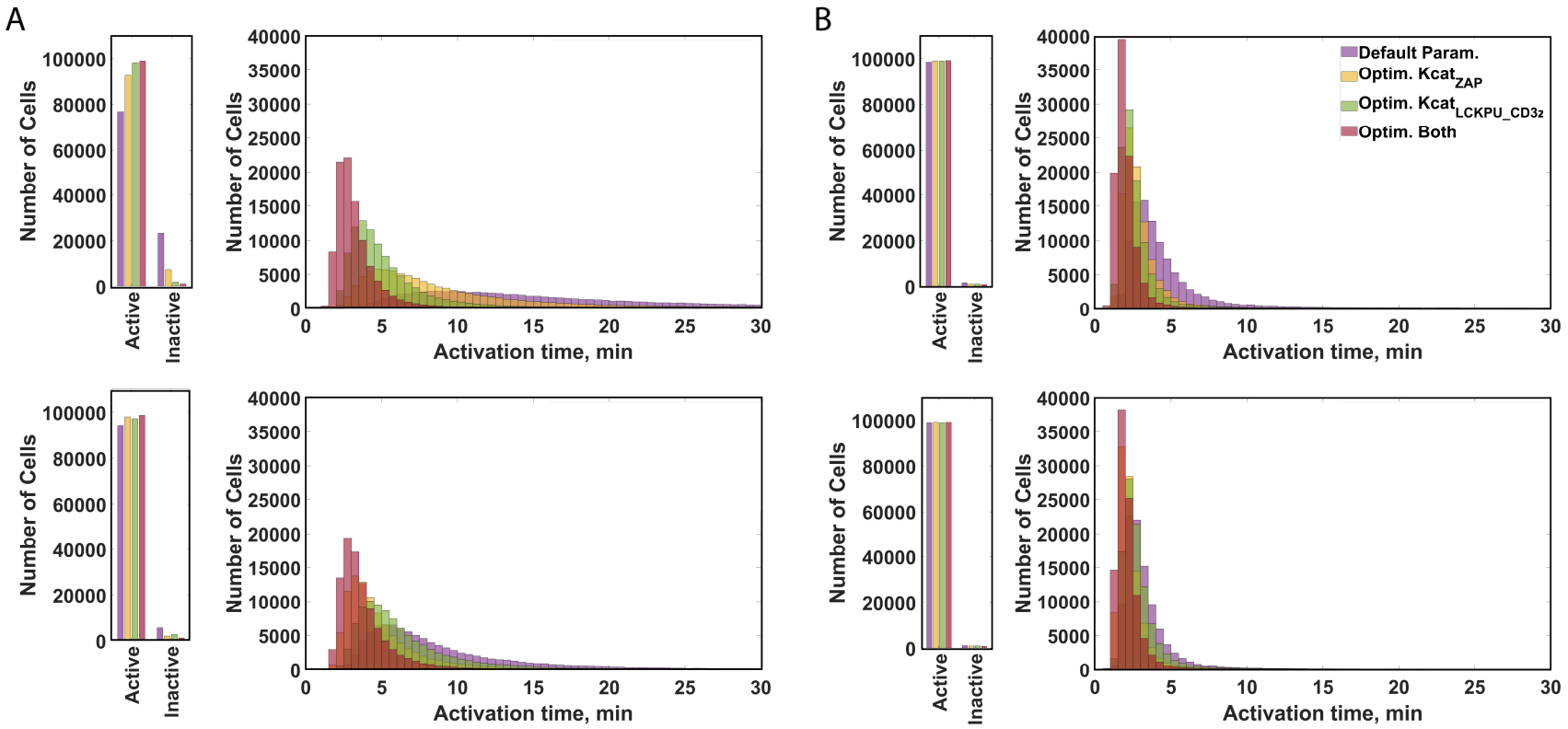
Supplementary Figure 2. Dose response curves of cell activation times. We varied the level of antigen exposure and simulated the activation time for different conditions. (A) Default parameters; (B) Optimized value for *Kcat_ZAP*, (C) Optimized value for *Kcat_LCKPU_CD3z*, (D) Optimized values for both *Kcat_ZAP* and *Kcat_LCKPU_CD3z*.



Supplementary Figure 3. Permutation importance scores for 48 kinetic parameters used for creating a machine learning model to predict cell activation times. A gradient boosted tree was used to predict the cell activation times based on model kinetic parameters. We show the permutation importance scores for all kinetic parameters under different conditions: (A) CAR-CD3ζ with low antigen concentration, (B) CAR-CD3ζ-CD28 with low antigen concentration, (C) CAR-CD3ζ with high antigen concentration, (D) CAR-CD3ζ-CD28 with high antigen concentration.



Supplemental Figure 4. Comparison of activation of CAR T cells with optimized parameter values. We show the number of cells activated and their activation times, showing shifts in the response of CAR-T cells with optimized kinetic parameters under antigen variation. Note, this is the same simulation data as shown in Fig. 1 and Fig. 6. (A) Comparison of the number of activated cells and their activation times for default and optimized parameter values among cells with first-generation CAR constructs (CAR-CD3ζ); (B) Comparison of the number of activated cells and their activation times for default and optimized parameter values among cells with second-generation CAR constructs (CAR-CD3ζ-CD28).



Supplemental Figure 5. Comparison of activation of CAR T cells with optimized parameter values. We show the number of cells activated and their activation times, showing shifts in the response of CAR-T cells with optimized kinetic parameters under kinetic parameter variation. Note, this is the same simulation data as shown in **Fig. 2 and Fig. 7**. (A) Comparison of the number of activated cells and their activation times for default and optimized parameter values among cells with first-generation CAR constructs (CAR-CD3ζ), top row: low antigen stimulation, bottom row: high antigen stimulation; (B) Comparison of the number of activated cells and their activation times for default and optimized parameter values among cells with second-generation CAR constructs (CAR-CD3ζ-CD28). *Top*: low antigen stimulation, *bottom*: high antigen stimulation.