

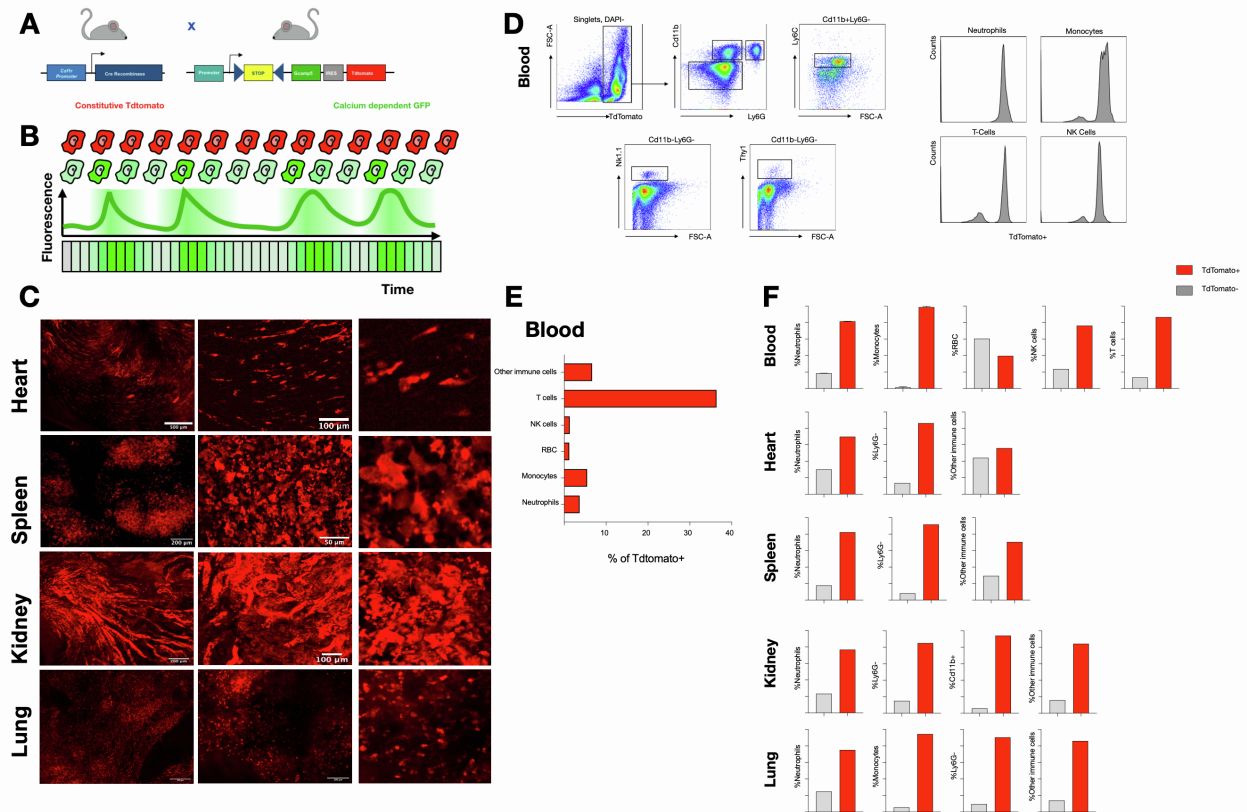
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Supplemental information

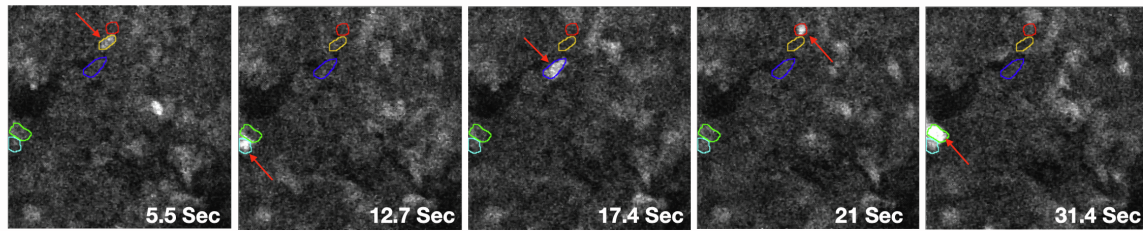
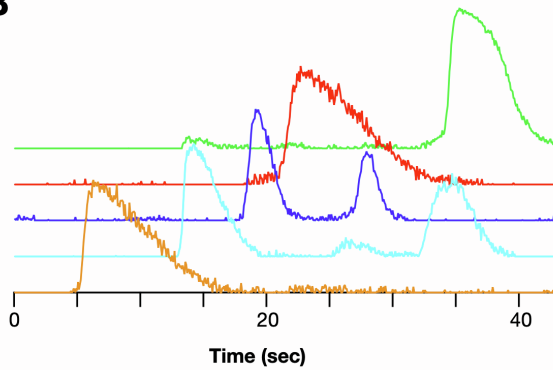
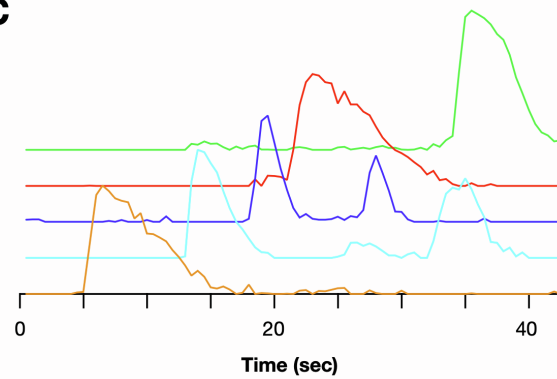
**Macrophage calcium reporter mice reveal
immune cell communication *in vitro* and *in vivo***

Nika Taghdiri, David M. Calcagno, Zhenxing Fu, Kenneth Huang, Rainer H. Kohler, Ralph Weissleder, Todd P. Coleman, and Kevin R. King

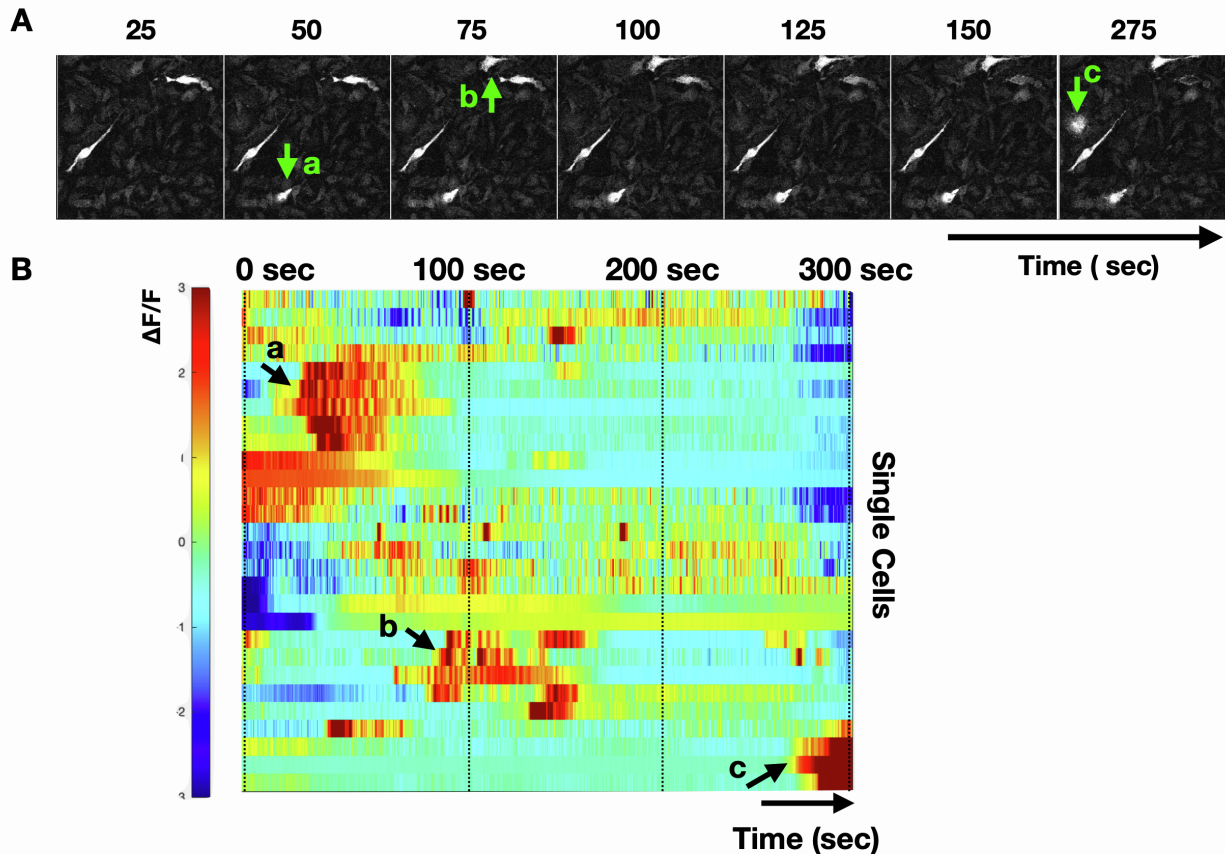
SUPPLEMENTARY INFORMATION (SI)



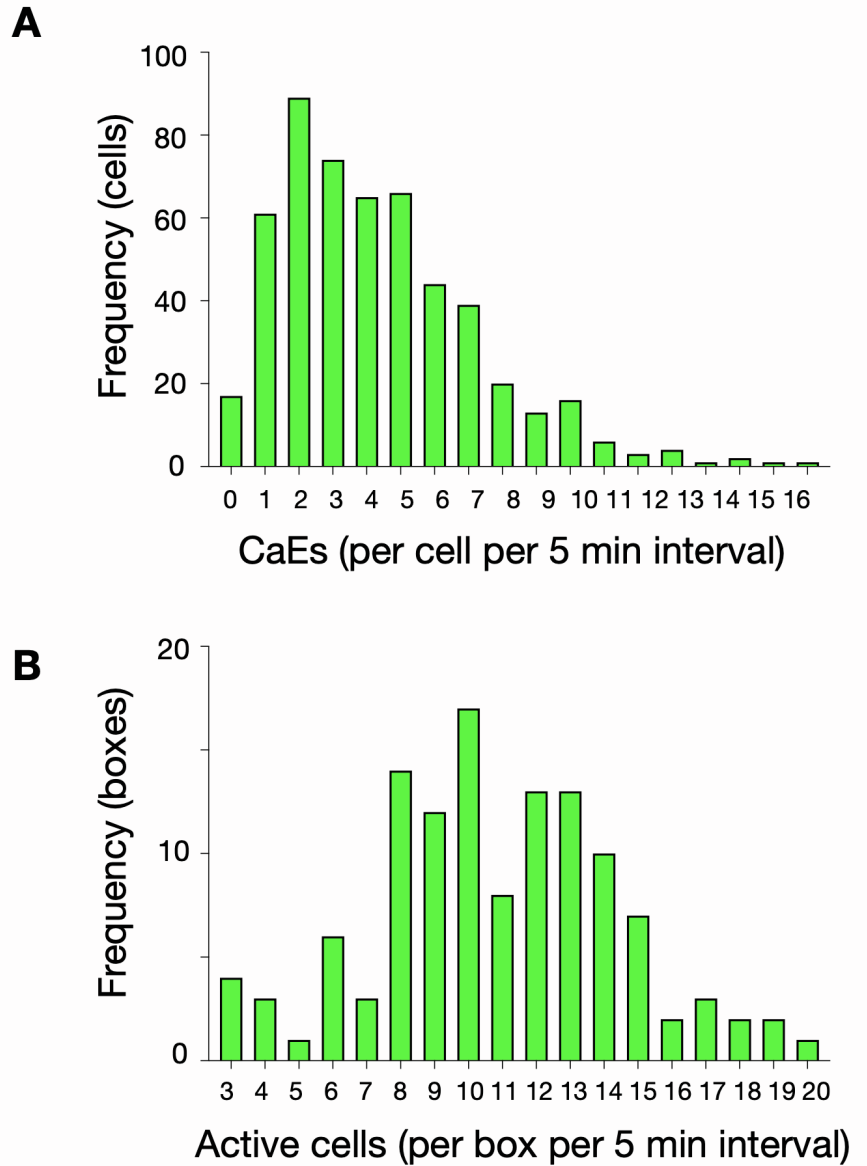
Supplemental Figure 1 Design, construction, and characterization of a *Csfr1^{Cre}GCaMP5^{fl}* calcium reporter for non-destructive quantification of innate immune cell dynamics, Related to Figure 1. A) Illustration of mouse breeding strategy. *Csfr1-cre* mice were crossed with floxed-STOP *GCaMP5* inducible reporter mice to create an innate immune cell specific reporter. B) Cartoon illustrating that *tdTomato* is constitutively expressed as a reference and dynamic calcium-dependent *GCaMP5* signals are quantified ratio-metrically C) Spatial distribution of *tdTomato*⁺ cells in solid organs (heart, spleen, kidney, lung) at low (left), medium (middle), and high (right) magnification (scale bars from left to right, 500 μ m, 100 μ m and 10 μ m). D) Gating strategy for flow sorting of immune subsets. E) Percentage of total *tdTomato*^{high} cells in the peripheral blood from the *Csfr1^{Cre}GCaMP5^{fl}* mouse. F) Percentage of each subset in each tissue compartment that is *tdTomato*⁺.

A**B****C**

Supplemental Figure 2 $Csf1r^{Cre}GCaMP5^{fl}$ sampling frequency determination, Related to Figure 2. A) Calcium elevations were recorded from a population of cells at 15Hz. B) Quantification of calcium fluorescence versus time for multiple cells across time at 15Hz. C) Down-sampling was performed, and every 7th sample was plotted to show the similarity of calcium elevation tracings. This led to selection of 2Hz as the sampling frequency used throughout the manuscript.



Supplemental Figure 3 Example of $Csf1r^{Cre}GCaMP5^{fl}$ macrophage calcium reporter dynamics following immunogenic double-stranded DNA stimulation in vitro, Related to Figure 2. A) Montage of time-lapse imaging. Newly calcium-overloaded macrophages indicated in a, b, and c precipitate non-fatal calcium fluctuations in neighboring macrophages. B) Heatmap illustration of hierarchically clustered macrophage dynamics.



Supplemental Figure 4 Histograms of in vivo $Csf1^{Cre}GCaMP5^{fl}$ calcium reporter dynamics, Related to Figure 5. A) Histogram illustrating distribution of number of calcium elevations per cell per 5-minute interval. B) Histogram illustrating distribution of number of active cells per box per 5-minute interval.

SUPPLEMENTAL MOVIES

M1a. Csf1r^{Cre}GCaMP5^{fl} Macrophages – vehicle control stimulation, Related to Figure 1.

M1b. Csf1r^{Cre}GCaMP5^{fl} Macrophages - dsDNA stimulation, Related to Figure 1.

(0.008 Hz sampling)

in vitro

M2. Csf1r^{Cre}GCaMP5^{fl} macrophages - complexed dsDNA stimulation, Related to Figure 2.

(15 Hz sampling)

in vitro

M3a. Csf1r^{Cre}GCaMP5^{fl} macrophages - vehicle control stimulation, Related to Figure 2.

M3b. Csf1r^{Cre}GCaMP5^{fl} macrophages - complexed dsDNA stimulation, Related to Figure 2.

(2 Hz sampling)

in vitro

M4. Csf1r^{Cre}GCaMP5^{fl} reporter - MC38-H2B-mCherry tumor cells, Related to Figure 3.

(2 Hz sampling)

in vivo

M5. Csf1r^{Cre}GCaMP5^{fl} reporter - MC38-H2B-mCherry tumor cells, Related to Figure 5.

(2 Hz sampling)

in vivo

M6a. Csf1r^{Cre}GCaMP5^{fl} reporter - MC38-H2B-mCherry tumor cells, Related to Figure 3.

M6b. Csf1r^{Cre}GCaMP5^{fl} reporter - MC38-H2B-mCherry tumor cells, Related to Figure 3.

(2 Hz sampling)

in vivo