Supporting Information

Figure S1

Schematic calcium life imaging experiment.

Lymphocytes adhere on a poly-L-lysine-coated glass cover slip in a sandwiched chamber, which permits solution exchanges within less than 1 sec. Excess nonadherent cells can be removed by a single flush with buffer solution. For measurement the chamber can be placed on an inverted microscope equipped with a 30X S Fluor object lens. Cells are alternately illuminated at 340 and 387 nm and emission signals at 468 - 550 nm recorded with a CCD camera.

Figure S2

Ca^{2+} imaging in classified Tcon and Treg.

Full-screen image of Fura-2 emission at 387 nm before (a) and after (b) adding PBMC to Treg. (c) Color-coded image detail of the same experiment at a single measurement point. Warmer colors (yellow/*red*) indicate high $[Ca^{2+}]_i$ while colder colors (*blue*/*pink*) indicate low $[Ca^{2+}]_i$. (d) Corresponding image section with fixed and stained cells after the live-cell imaging experiment: red anti-FOXP3, blue anti-CD4, green anti-CD25.

Figure S3

Subcellular localization of NFAT.

(a) Resting PBMC were fixed and stained with anti-NFAT (green) and DAPI for the nuclei (blue). No or only minor overlay of NFAT and DAPI signals indicate the cytoplasmatic localization of NFAT in resting cells. (b) PBMC were stimulated with TG to induce sustained elevation of $[Ca^{2+}]_i$ and fixed after 20 minutes. Nuclear translocation of NFAT results in an overlay of the anti-NFAT and DAPI fluorescence signals.

Video S1

Inhibition of Ca^{2+} signals in Tcon by prestimulated Treg.

Color-coded live-cell imaging experiment with prestimulated Treg seeded on the cover slip of the live-cell imaging chamber followed by insertion of PBMC and T-cell stimulation with anti-CD3 mAb. Color scale covers 25 - 500 nM of $[Ca^{2+}]_i$ concentration with colder colors (blue/pink) indicating low $[Ca^{2+}]_i$ and warmer colors (yellow/red) indicating high $[Ca^{2+}]_i$. The anti-CD3 stimulus induces no or only marginal elevation of $[Ca^{2+}]_i$ in the majority of Tcon (while prestimulated Treg predominantly generate oscillatory Ca^{2+} signals). Respective annotations highlight the different stages of the ongoing experiment. A chronometer shows the elapsed time. Tcon and Treg are marked with orange (Tcon) and green (Treg) arrows. The classification of Tcon and Treg is illustrated by cross-fading of the postexperimental immunofluorescence staining at the end of the video (red anti-FOXP3, blue anti-CD4).

Video S2

Effective anti-CD3 mAb induced Ca^{2+} signaling in Tcon in the absence of Treg.

PBMC are seeded on the cover slip of the live-cell imaging chamber without Treg and stimulated with anti-CD3 mAb. Contrary to the experimental settings visualized in Video S1, anti-CD3 mAb induces substantial Ca^{2+} signals in the majority of Tcon. Data presentation corresponds to Video S1.