Monaco et al., http://dx.doi.org/10.1083/jcb.201602001

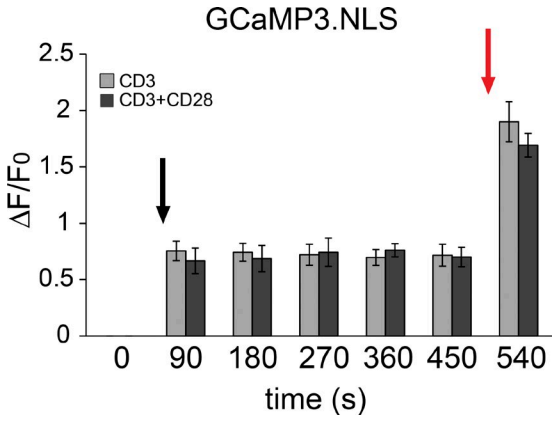

Figure S1. **Stimulation with CD3 or CD3 + CD28 induces similar nuclear calcium transients in T cells.** Nuclear calcium transients were measured by flow cytometry in T cells transfected with GCaMP3.NLS. The cells were prestimulated with CD3 antibodies or with a combination of CD3 and CD28 antibodies. The baseline was measured for 90 s. Anti–mouse IgGs (black arrow) were added for antibody cross-linking, and calcium signals were measured for 9 min. Ionomycin (red arrow) was added to obtain the maximum signal. The histograms show mean GFP fluorescence ($\Delta F/F_0$) from three independent experiments. Error bars represent SEM.

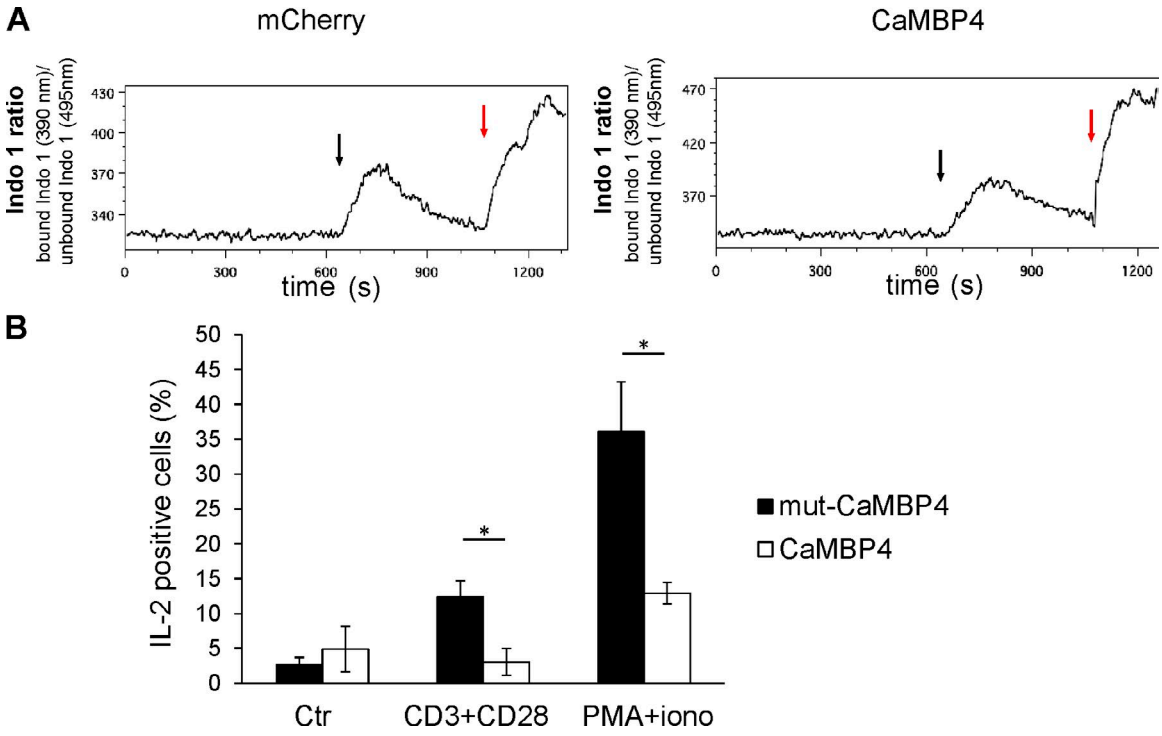

Figure S2. **CaMBP4 does not alter TCR-induced calcium transients, and its effect on T cell activation is caused by inhibition of the calcium/calmodulin complex.** (A) Representative experiments showing calcium transients in T cells transfected with mCherry or CaMBP4.mCherry (CaMBP4). Cells were loaded with the ratiometric calcium indicator indo-1 and prestimulated with CD3 antibodies. The baseline was measured for 10 min. Anti–mouse IgGs (black arrows) were added for antibody cross-linking, and calcium signals were measured for 9 min. Ionomycin (red arrows) was added to obtain the maximum signal. The indo-1 ratio was calculated from the violet (390 nm) and green (495 nm) emission. (B) T cells were transfected with CaMBP4.mcherry (CaMBP4) or a mutant inactive form of CaMBP4.mCherry unable to bind to CaM (mut-CaMBP4). The cells were stimulated with a combination of CD3 and CD28 antibodies or PMA and ionomycin or left unstimulated (ctr). IL-2 expression was analyzed by flow cytometry with IL-2 FITC-conjugated antibodies. The histograms show the mean percentage of FITC-positive cells from three independent experiments. Statistically significant differences are indicated with asterisks (*, $P < 0.05$). Error bars represent SEM.

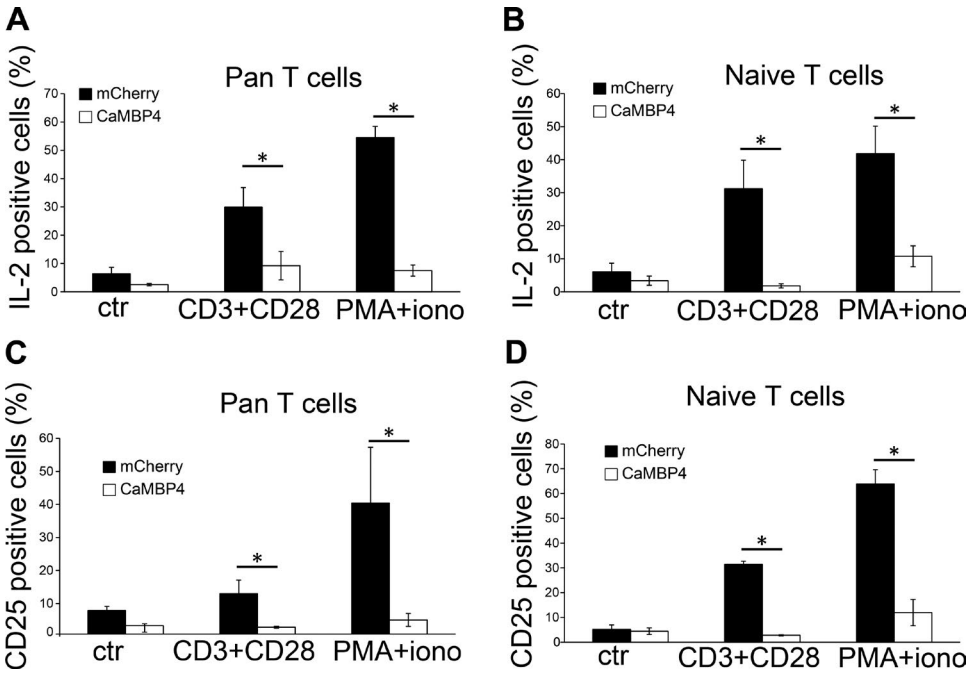

Figure S3. **Nuclear calcium controls the expression of activation markers in both pan-T cells and naive T cells.** Comparison of the effects of CaMBP4 on the activation of naive T cells and pan-T cells (total circulating T cells including naive, memory, and regulatory T cells). Pan-T cells (A and C) and naive T cells (B and D) were extracted from blood of the same donor. The cells were transfected with expression vectors for CaMBP4.mCherry (CaMBP4) or mCherry and stimulated with a combination of CD3 and CD28 antibodies or PMA and ionomycin or were left unstimulated (ctr). The expression of IL-2 (A and B) and CD25 (C and D) was analyzed by flow cytometry with FITC-conjugated antibodies. The histograms show the mean percentage of FITC-positive cells from three independent experiments. Statistically significant differences are indicated with asterisks (*, $P < 0.05$). Error bars represent SEM.

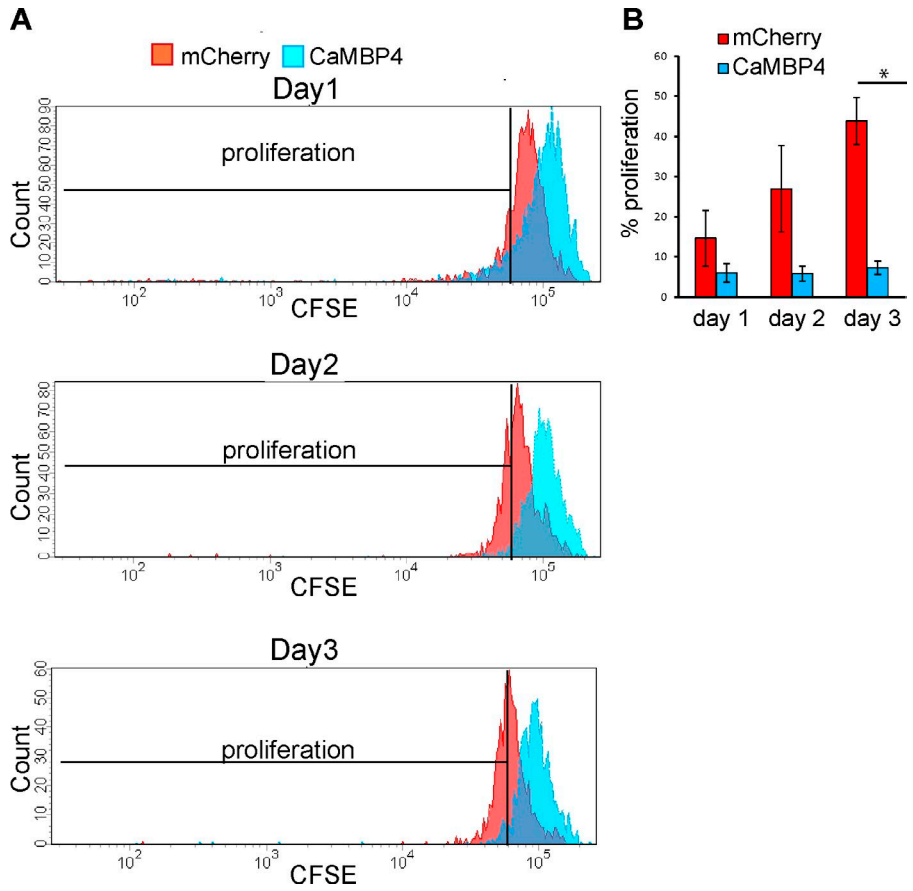

Figure S4. **Blockade of nuclear calcium abolished the proliferative response of T cells in an MLR.** Responder cells (T cells) and stimulator cells (PBMCs) were isolated from different donors. To measure the proliferation of the responder T cells only, PBMCs were treated with ionizing radiation to block proliferation. Responder T cells transfected with mCherry or CaMBP4.mCherry (CaMBP4) were labeled with CFSE and cultured with PBMCs for 3 d. Proliferation was calculated as the percentage of cells in which CFSE fluorescence decreased over time. (A) Representative FACS experiment showing T cell proliferation. The proliferation gate was set for both populations to the basal CFSE fluorescence immediately (i.e., 1 h) after CFSE loading. The analysis was restricted to gated mCherry-positive cells. (B) Histogram showing the mean percentage of proliferating T cells in three independent experiments. Statistically significant differences are indicated with asterisks (*, $P < 0.05$). Error bars represent SEM.

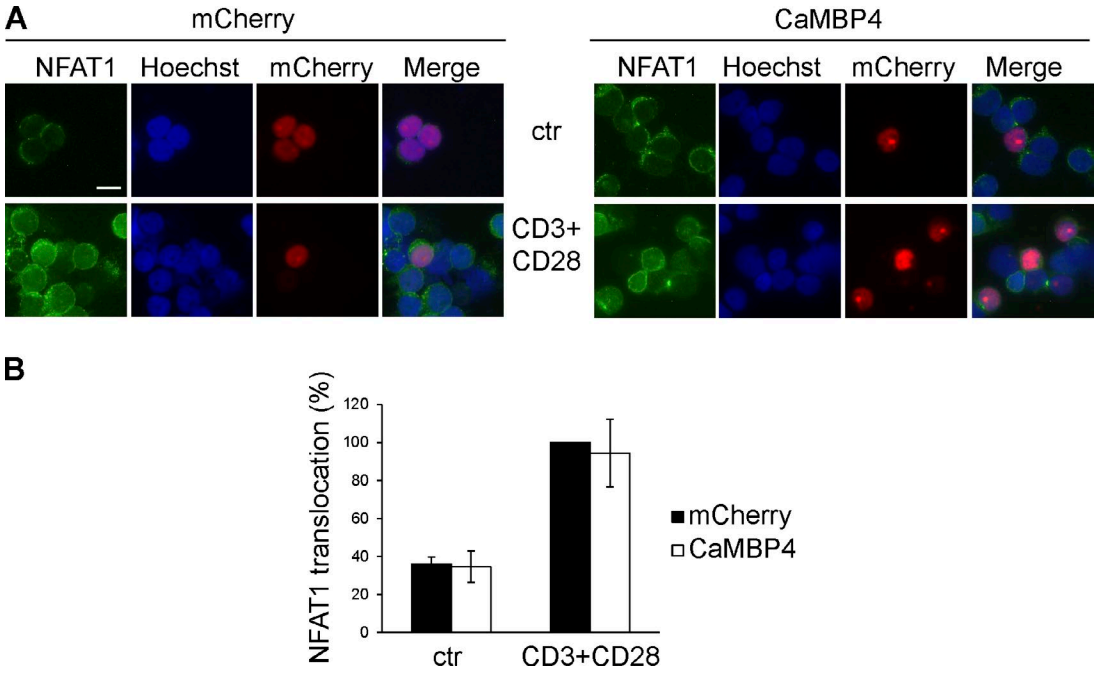

Figure S5. **Nuclear calcium signaling does not regulate nuclear translocation of NFAT1.** (A) T cells transfected with expression vectors for CaMBP4. mCherry (CaMBP4) or mCherry were either left unstimulated (ctr) or were stimulated with a combination of CD3 and CD28 antibodies. Cells were stained with antibodies against NFAT1. Nuclei were stained with Hoechst. Bar, 5 µm. (B) Quantitative analysis of NFAT1 nuclear localization in three independent experiments. Error bars represent SEM.