

European Journal of Immunology

Supporting Information

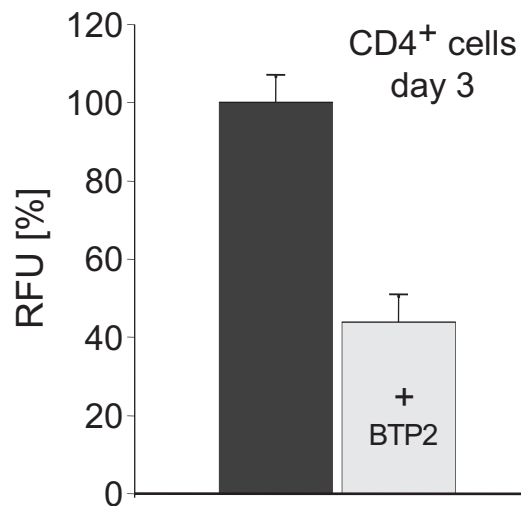
for

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Calcium dependence of T cell proliferation following focal stimulation

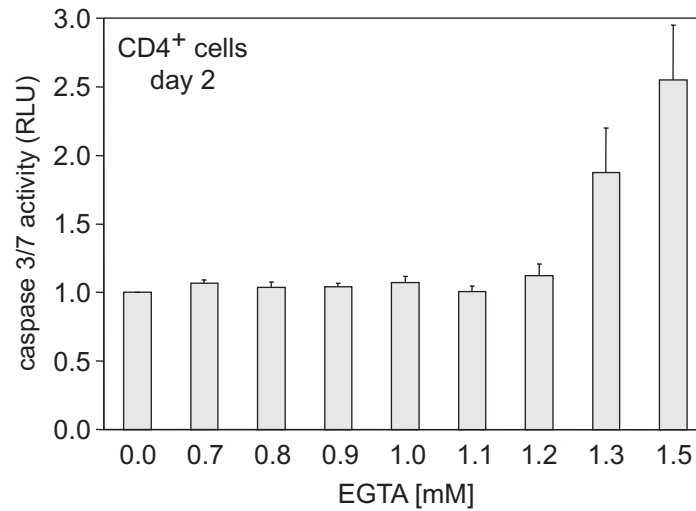
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Supplementary Figure 1



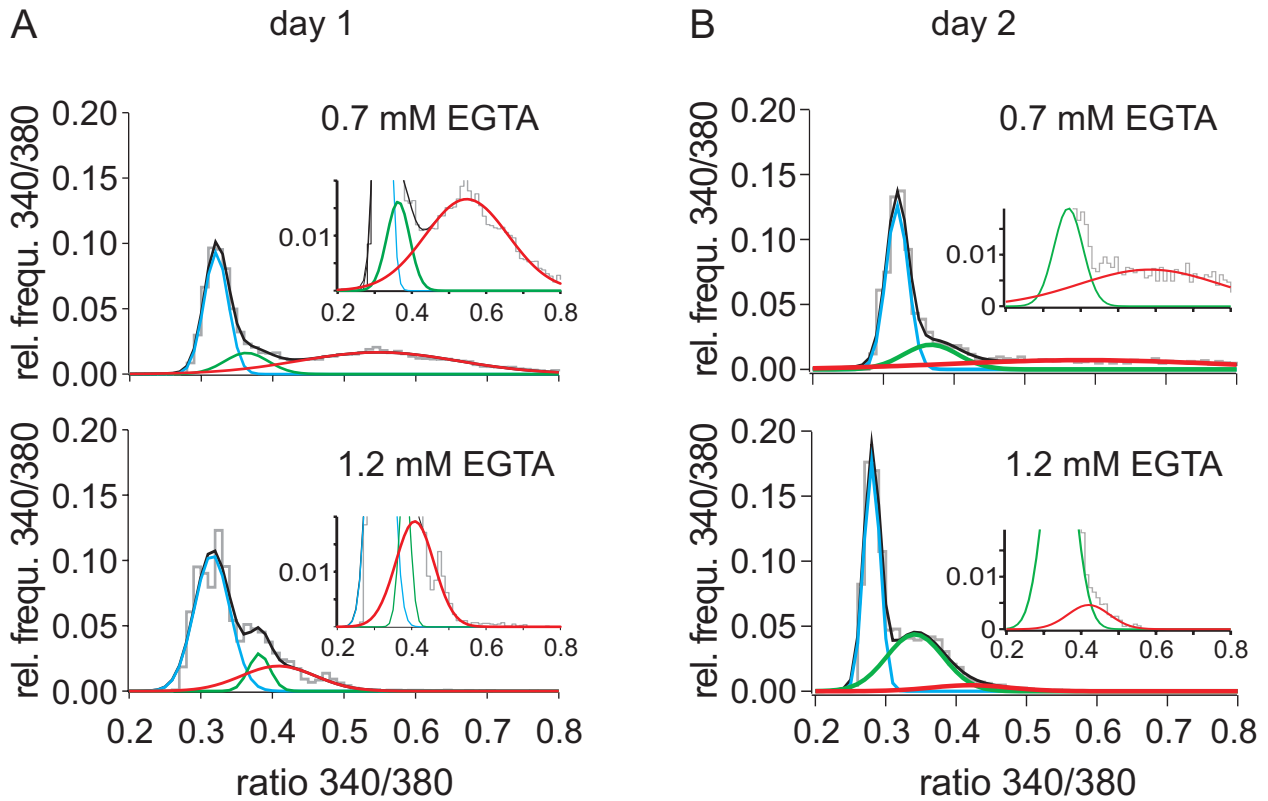
Supplementary Figure 1. Proliferation of anti-CD3/CD28 coated bead stimulated CD4⁺ T-cells is reduced by the addition of 200 nM BTP2. Proliferation was measured either with (grey bar) or without (black bar) the addition of 200 nM BTP2. CD4⁺ T-cells were stimulated with anti-CD3/CD28 coated beads over 3 days. Proliferation is expressed as relative fluorescence units (RFU). Proliferation under control conditions (without BTP2) was set to 100% (raw data were 10763 +/- 715 RFU). Data are means +/- SD of 2 different donors each measured as triplicates.

Supplementary Figure 2



Supplementary Figure 2. Apoptosis rates of non-stimulated CD4⁺ T-cells are almost constant under Ca²⁺ limiting conditions up to 1.2 mM EGTA. CD4⁺ T-cells were incubated with different concentrations of EGTA present without any stimulation added for two days. Apoptosis was measured as caspase 3/7 activity in relative luminescence units (RLU, raw data under control conditions were 8563 +/- 1495). Activity under control conditions (no EGTA added) was set to 1. Data are shown as mean of 3 donors +/- SD.

Supplementary Figure 3



taken from Figure 5B

Supplementary Figure 3. Histogram analysis of $[Ca^{2+}]_i$ (expressed as ratio 340/380) of $CD4^+$ T-cells. Only cells which were in contact with an anti-CD3/CD28 coated bead during the experiment were analyzed at day 1 (A) and at day 2 (B, taken from Figure 5B). Extracellular $[Ca^{2+}]$ are 207 μM (0.7 mM EGTA) and 24 μM (1.2 mM EGTA). The raw data (grey trace) were fitted with 3 different Gaussian fits which were presented as a blue trace (1st Gaussian distribution), a green trace (2nd Gaussian distribution) and a red trace (3rd Gaussian distribution). The black trace represents the sum of the 3 different Gaussian fits. The insets highlight the shape of the red trace (3rd Gaussian distribution).