Correlating short-term Ca^{2+} responses with long-term protein expression after activation of single T cells

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Supplementary material

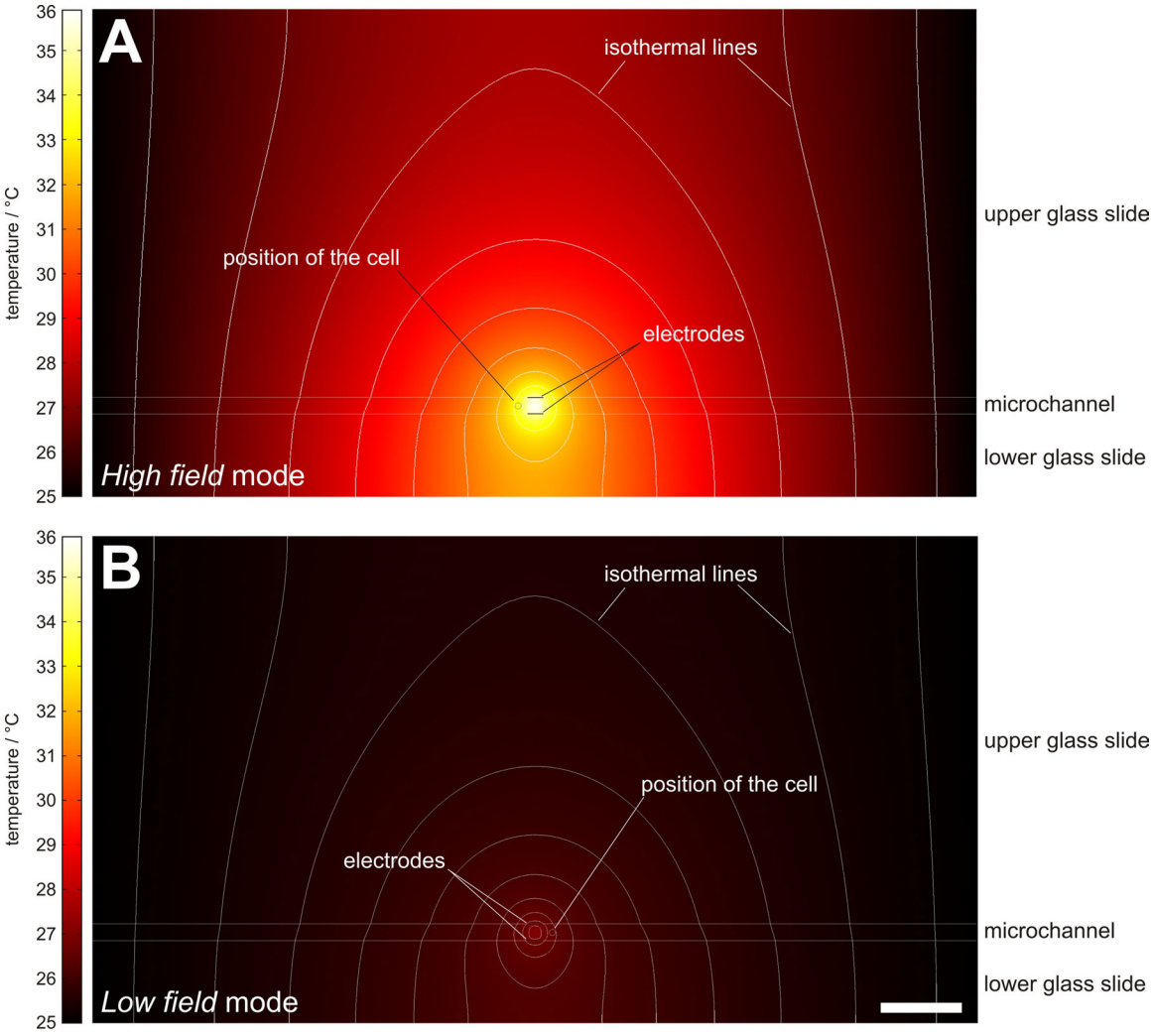

Fig. S1: Quantification of the temperature distribution in the DEP chip while applying an electric field to the electrodes. Cross-section of the DEP chip. The microchannel is formed by two glass slides that are spaced apart from each other through a 30 µm thick SU-8 polymer spacer. The upper and lower glass slides are 700 and 150 µm thick, respectively, and equipped with congruent electrode structures at the inner faces. Application of a voltage between these electrodes leads to ohmic warming of the microchannel, since it is filled with electrically conductive cell culture medium. For both *high-field* (A) and *low-field* (B) configuration (see methods section for details) the resulting shift of the microchannel temperature was modeled in 2D using the finite element software COMSOL Multiphysics (COMSOL, Burlington, MA, USA) and superimposed to a room temperature of 25 °C. For numerical modeling, we employed the *Conductive Media DC* and the *General Heat Transfer* module. Internal boundaries were set to *continuity*. The heat transfer coefficients of the external boundaries were set to *infinite* (side walls) and *insulating* (top and bottom walls), respectively. The heat capacities C, electrical conductivities σ, densities ρ and heat conductivities k of the channel medium and the glass slides were set to $C_m = 4181$ J kg^{-1} K^{-1}, $C_g = 820$ J kg^{-1} K^{-1}, $\sigma_m = 1.4$ S m^{-1}, $\sigma_g = 25$ µS m^{-1}, $\rho_m = 1$ g cm^{-3}, $\rho_g = 2.51$ g cm^{-3}, $k_m = 0.604$ W m^{-1} K^{-1} and $k_g = 1.1$ W m^{-1} K^{-1}, respectively. Electrode voltages were set to $U = 3$ V and 1.2 V for *high field* and *low field* configuration, respectively. Scale bar, 150 µm.

Tab. S1: Ambient temperature experienced by a cell during its DEP manipulation. Since a dielectrophoretically retained cell is trapped at the position where hydrodynamic and DEP forces balance each other, its distance *d* to the electrodes depends on both the flow rate of the microchannel medium and the applied electrode voltage *U* (see Fig. 6a). To estimate the ambient temperature a cell experienced during its DEP manipulation at *high-field* and at *low-field* configuration (T_{cell}), the temperature calculated in Fig. S1 was integrated over the area corresponding to the respective position of the cell (see Fig. S1). Beyond that, the maximum temperature between the electrodes (T_{max}) was also evaluated.

	U	Flow rate	*d*	T_{cell}	T_{max}
High-field mode	3.0 V	14.4 µl h^{-1}	13.3 µm	33.8 °C	35.9 °C
Low-field mode	1.2 V	0.7 µl h^{-1}	18.9 µm	26.6 °C	27.1 °C

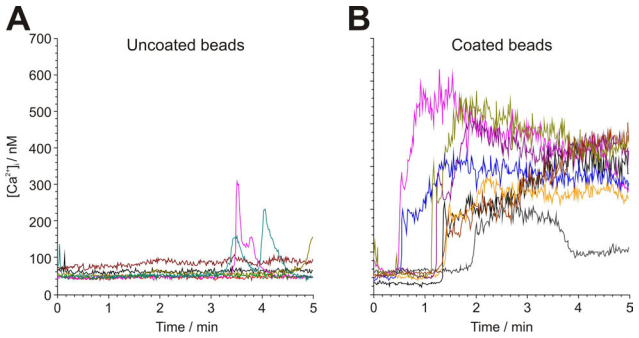

Fig. S2: Ca^{2+} signals after contact formation with antibody-coated and uncoated microbeads. According to the pair formation procedure described in the methods section, single Fura-loaded T cells were contacted with antibody-coated or uncoated microbeads in the DEP chip. Immediately after the bead presentation, the cytosolic Ca^{2+} level was analyzed for a period of 5 min. (A) Ca^{2+} traces of cells that were contacted with uncoated beads (n = 8). (B) Eight representative Ca^{2+} traces elicited by contact formation with antibody-coated beads.

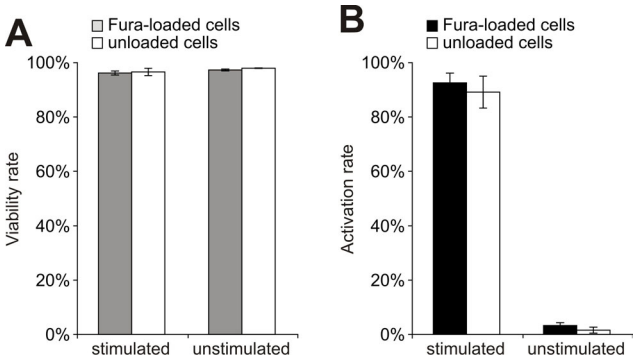

Fig. S3: Influence of the Fura-loading on (A) the viability- and (B) the activation rate of Jurkat T cells. T cell activation in Fura-loaded or unloaded T cells was triggered by their incubation in an anti-CD3/anti-CD28-coated microwell. Alternatively, the cells were left unstimulated by cultivation in an uncoated well. After 16 - 24 h their viability- and activation rates were analyzed as described in the methods section. Error bars, s.e.m.; n = 2.

Tab. S2: Statistical analysis of the data describing the impact of the DEP manipulation on the viability-, activation- and proliferation rate of T cells (see Fig. 4). We used logistic regression analysis (implemented in the glm() function of the R statistical analysis package v2.5.1; http://www.r-project.org) to estimate a relationship between the dichotomous predictor "exposure condition" (*field* exposure or *no field* exposure) or the categorical predictor "exposure time" and either of the three dependent variables "viability rate", "activation rate" and "proliferation rate". The *p*-values for accepting the null hypothesis (no relationship between predictor and dependent variable) are shown for each possible combination. Significant evidence for a relationship between predictor and dependent variable is assumed when $p < 0.05$.

	Viability rate	Activation rate	Proliferation rate
Exposure condition	$p = 0.073$	$p = 0.041$	$p = 0.111$
Exposure time	$p = 0.056$	$p = 0.013$	$p = 0.014$