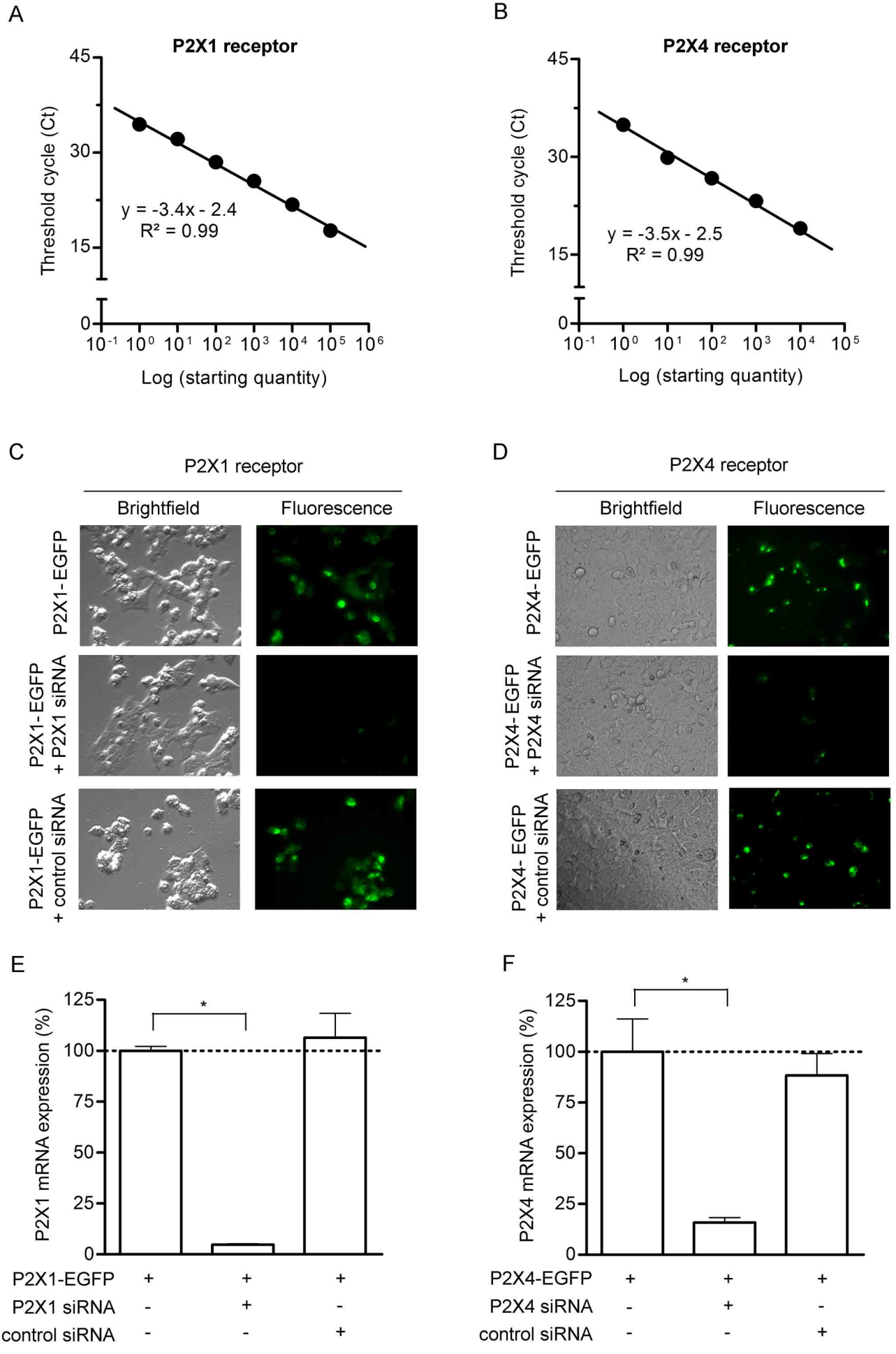
Figure S1. Assessment of P2X1 and P2X4 receptor mRNA expression and silencing

Real-time RT-PCR assays were performed with the primer pairs shown in **Material and Methods**. Real-time RT-PCR amplified mRNA products with an efficiency of >95% and amplification was linear over a 5 or 6 log range of concentrations. Quantification of gene expression was made within the range of Ct values shown on the standard curves **(A, B)**. HEK 293 cells were transfected with P2X1-EGFP or P2X4-EGFP alone, with nonsense control siRNA, or with siRNA targeting P2X1 or P2X4 receptors. Cells were visualized by fluorescence microscopy and P2X1 and P2X4 receptor mRNA expression were determined by real-time RT-PCR 48 h later. Silencing with P2X1 or P2X4 receptor siRNA inhibited P2X1-EGFP or P2X4-EGFP protein **(C and D)** and mRNA (**E and F**) expression, respectively. Data represent means ± SEM, *$p \leq 0.05$, $n = 3$, two-tailed unpaired Student's t-test.

Figure S2. T-cell activation enhances Ca^{2+} entry at the immune synapse

Jurkat cells in Ca^{2+}-free medium were loaded with Fluo-4, stimulated with anti-CD3/CD28 antibody-coated beads, and after 30 min, Ca^{2+} was restored to a final concentration of 2 mM. Fluorescence intensity was monitored in 200 ms intervals. Confocal laser scanning images show rapid influx of Ca^{2+}, originating at the cell-bead interaction site. Graphs depict the increase in fluorescence intensity.


A
P2X1 receptor
Threshold cycle (Ct)
y = -3.4x - 2.4
R² = 0.99
Log (starting quantity)
B
P2X4 receptor
Threshold cycle (Ct)
y = -3.5x - 2.5
R² = 0.99
Log (starting quantity)
C
P2X1 receptor
Brightfield
Fluorescence
P2X1-EGFP
P2X1-EGFP + P2X1 siRNA
P2X1-EGFP + control siRNA
D
P2X4 receptor
Brightfield
Fluorescence
P2X4-EGFP
P2X4-EGFP + P2X4 siRNA
P2X4-EGFP + control siRNA
E
P2X1 mRNA expression (%)
P2X1-EGFP
P2X1 siRNA
control siRNA
F
P2X4 mRNA expression (%)
P2X4-EGFP
P2X4 siRNA
control siRNA

Supplemental Fig. S2