

Fig. S1. Increased suppressive function by P2X7 deficient T_{regs} . Suppression assay performed with WT (black line) or $p2x7^{-/-}$ (red line) T_{regs} and WT responder T_{conv} cells. Cell proliferation was determined by ^3H -thymidine incorporation.

Fig. S2. Correlation between ERK phosphorylation and variations in intracellular ATP concentration. WT and $p2x7^{-/-}$ T_{regs} were stimulated for 90 min with CD3 mab or CD3 mab and IL-6. ERK phosphorylation determined in flow cytometry and expressed as mean fluorescence intensity (MFI) was correlated with intracellular ATP concentration expressed as fold increase with respect to baseline level. The degree of correlation was measured by the Pearson coefficient.

Fig. S3. Impaired phosphorylation of ERK in $p2x7^{-/-}$ T_{regs} . FACS profiles showing staining with anti-phospho ERK mab in sorted T_{regs} from C57BL/6 and $p2x7^{-/-}$ mice either unstimulated or stimulated with CD3 mab for 90 min in the presence of irradiated splenocytes from $cd3\epsilon^{-/-}$ mice.

Fig. S4. Increase in intracellular ATP and ROR γ t transcript levels in T_{regs} by TCR signal strength. Top panel. Intracellular ATP variations by stimulation of sorted WT T_{regs} with 0.5 $\mu\text{g}/\text{ml}$ (black lines) or 5 $\mu\text{g}/\text{ml}$ (red lines) CD3 mab. The P value was calculated by analysis of variance (ANOVA). Lower panel. RT-PCR of ROR γ t mRNA from WT T_{regs} stimulated for 6 days in the presence of the indicated concentrations of CD3 mab. Means \pm SE are displayed (n=3).

Fig. S5. P2X7 mediated expression of IL-17 in T_{regs} . RT-PCR of IL-17 mRNA from WT (black bars) and $p2x7^{-/-}$ (red bars) T_{regs} stimulated for 96 h in the presence of ARL, IL-6 and IL-6 + FICZ. One representative experiment out of two. AU: arbitrary unit

Fig. S6. T_{regs} conversion by P2X antagonism. Percentages of CD25^{high}Foxp3⁺ cells by FACS analysis at day 7 after stimulation of naïve T cells from C57BL/6 and *p2x7*^{-/-} mice. Cells were stimulated for the first 48 h with CD3 mab and the indicated P2X antagonist/s in the presence of irradiated splenocytes from *cd3ε*^{-/-} mice and then maintained in 50 U/ml IL-2. For selective antagonism of P2 receptors, 30 μM NF449 for P2X1, 100 μM 5-BDBD for P2X4, 10 μM A-740003 or 10 μM A-438079 for P2X7 (all from Tocris Bioscience) were used. Statistical analysis was performed by using a Student's *t*-test. Mean±SD are shown (n=4; **: *P* <0.01, ***: *P* <0.001).

Fig. S7. T_{regs} generation by oATP and additive effect of TGF-β. **A.** Percentages of CD4⁺CD25^{high}Foxp3⁺ cells (T_{regs}) recovered at day 7 from naïve T cells stimulated for 48 h with CD3 mab, irradiated splenocytes and IL-2 or anti-CD3/28 coated microbeads. Black bars, control; red bars, oATP 100 μM. **B.** RT-PCR of Foxp3 and RORγt transcripts from naïve T cells stimulated in the presence of the indicated agents. **C.** Flow cytometry for CD25 and Foxp3 expression in naïve cells stimulated by CD3 mab, splenocytes and IL-2 as described above, in the presence of the indicated agents for the initial 48 h culture.

Fig. S8. Effect of TGF-β and oATP treatment on expression of genes interacting within the TGF-β pathway (<http://www.netpath.org>). The hierarchical cluster plot shows the median centralized relative expression data of differentially expressed genes resulting from the comparison of TGF-β, TGF-β+oATP and oATP treated with untreated T_{conv} cells. Genes showing a similar expression profile are clustered together (left tree) as well as samples showing a similar expression behavior among the TGF-β pathway (top tree).