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Supplemental information

P2X7 receptor is essential for cross-dressing of bone marrow-derived dendritic cells

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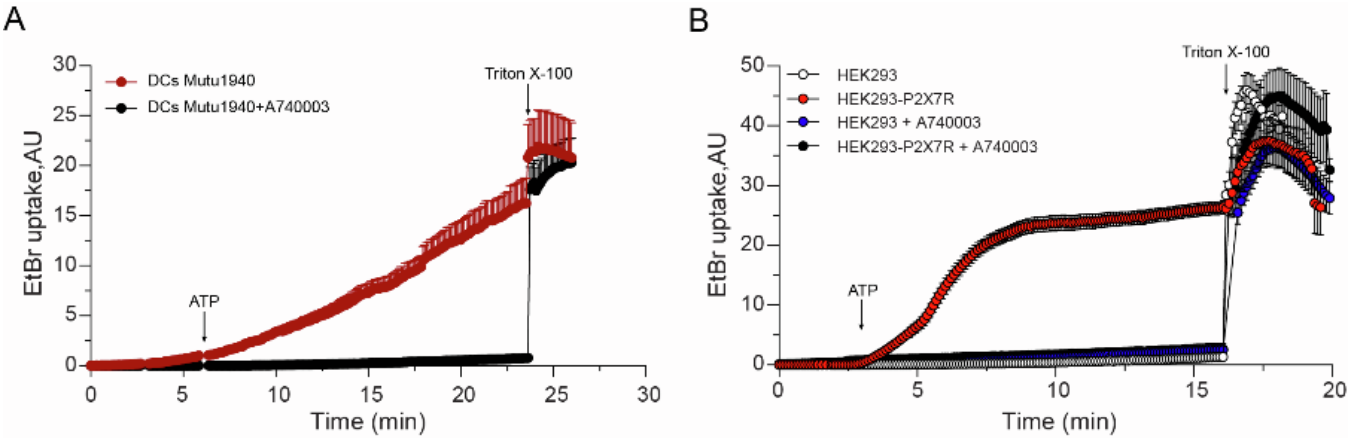
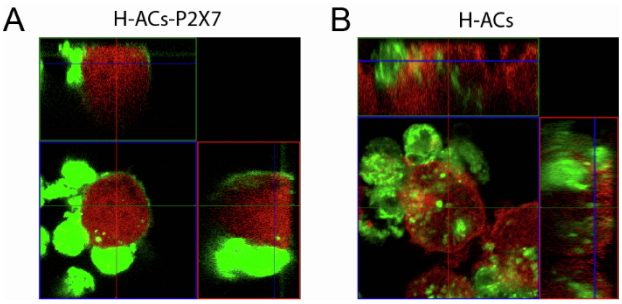


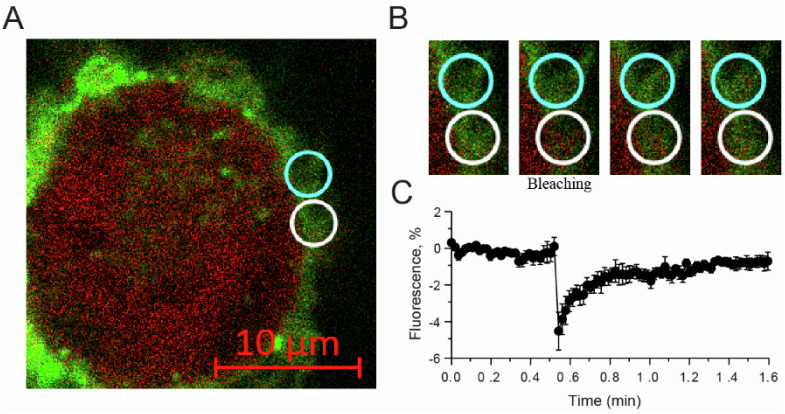
Supplementary figure 1. P2X7 expressed in MutuDCs and HEK293 P2X7 is functional. Related to Figure 1

A) MutuDCs were challenged with ATP (1 mM) to induce receptor activation increasing EtBr uptake, in the presence and absence of previous treatment with the P2X7 receptor antagonist A740003 (100μM) MutuDCs. **B)** The functionality of the P2X7 receptor expressed in HEK293 cells was evaluated using ATP and A740003, as previously described. Triton X-100 (0.3%) was added to the cells as a positive EtBr uptake control.



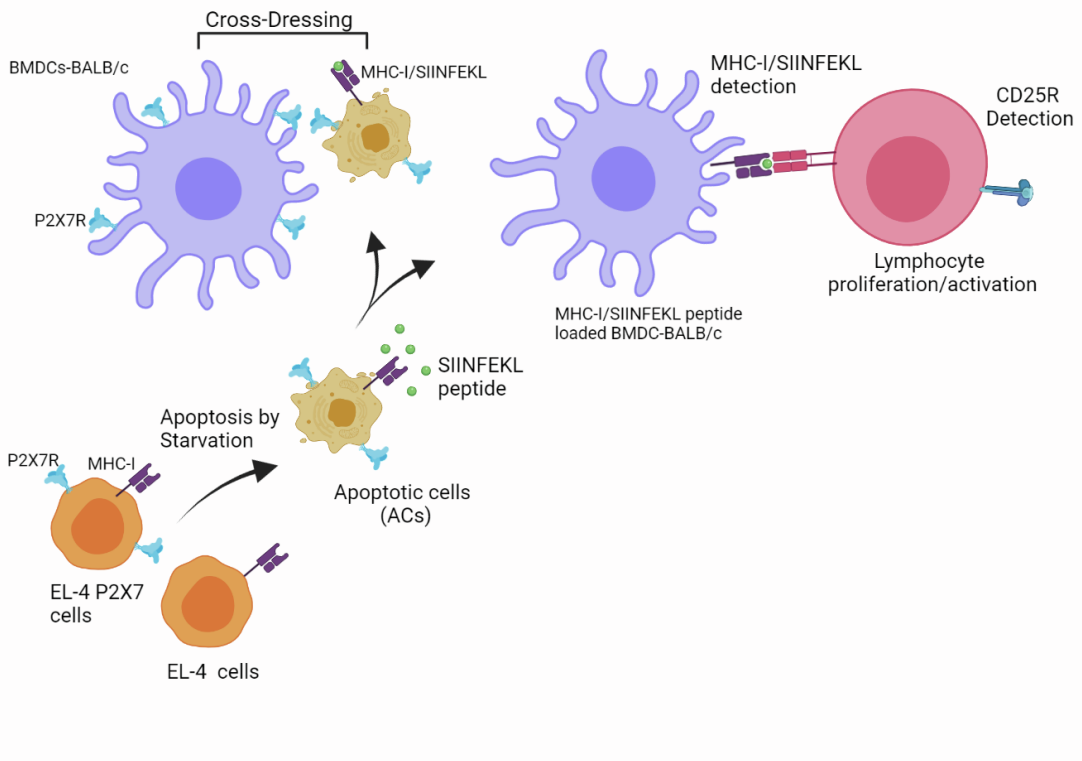
Supplementary figure 2. H-ACs P2X7 expression determines CM membrane distribution on DCs. Related to Figure 1

MutuDCs were challenged with **A)** CM H-ACs-P2X7 and **B)** CM H-ACs. The photo gallery was obtained 60-90 minutes after the cell was challenged. Picture diameter was fixed at 50 x 50μm on the XY plane. Mutu cells express constitutively GFP which is shown as red pseudocolor while CM is depicted as green pseudocolor.



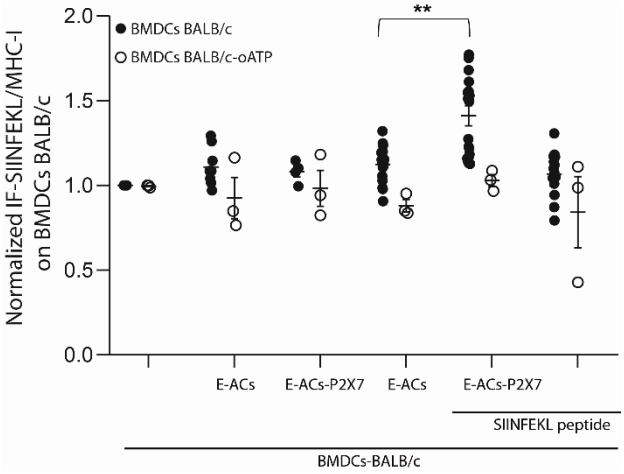
Supplementary figure 3. P2X7 H-ACs labeled with CellMarker (CM) favor the membrane transference to MutuDC. Related to Figure 1

MutuDCs were challenged with CM H-ACs-P2X7 for ninety minutes. **A)** Membrane CM localization was confirmed by fluorescence recovery after Photo-bleaching of a region of interest as summarized, a general plane is shown. **B)** A close-up of different time points shows the bleached zone (white circles) and the control zone (turquoise circles). **C)** Data were quantified and plotted, and then normalized with respect to the fluorescence obtained on the control zone. Data are represented as mean ± SEM. MutuDC expresses eGFP (red pseudocolor) and CM H-ACs-P2X7 (green pseudo-color). Scale bar 10µm.



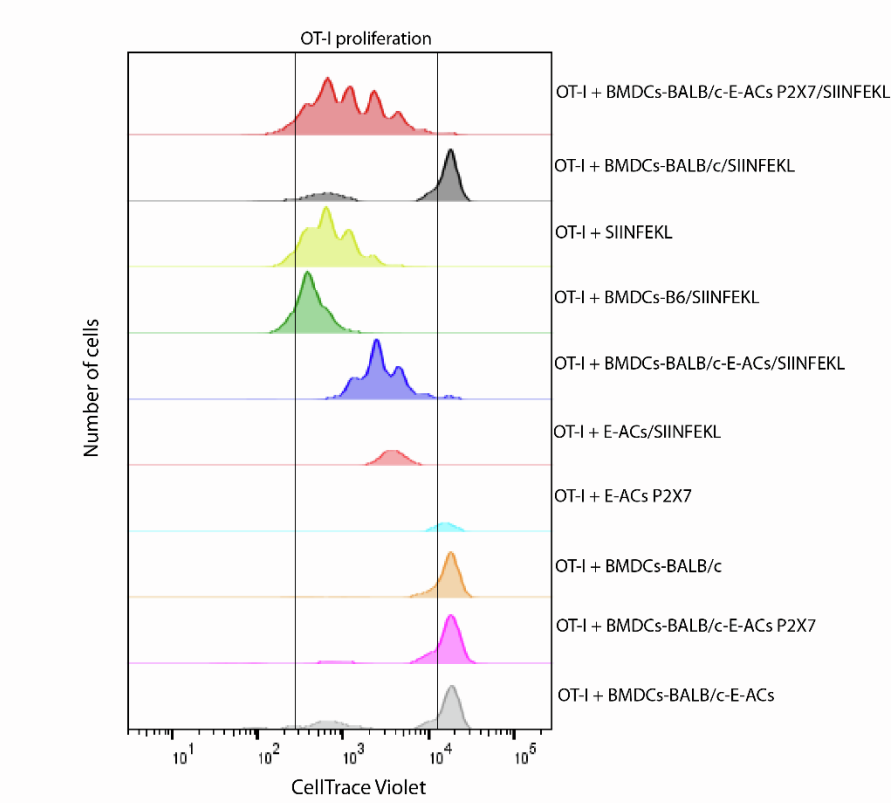
Supplementary figure 4. Schematic representation for the detection of the SIINFEKL / MHC-I complex in BMDC-BALB / c, CD25R and proliferation of OT-I lymphocytes. Related to Figure 3

BMDCs-BALB / c were incubated with the antigenic sources: E-ACs and E-ACs-P2X7, coming from EL4 cells without and with P2X7R, respectively, using a previously described starvation protocol. These ACs were incubated with SIINFEKL peptide for 24 hours. The transfer of the SIINFEKL / MHC-I complex from these ACs to the surface of the BM-DCs BALB / c was measured by CD11c + PI- gating. Subsequently, these same BALB / c BM-DCs loaded with SIINFEKL / MHC-I (complex from ACs or ACs-P2X7) were incubated with OT-1 lymphocytes (with specific transgenic-TCRs for the SIINFEKL / MHC-I complex, same EL -4 haplotype) to measure proliferation and lymphocytes T activation (CD25 + CD8 +)



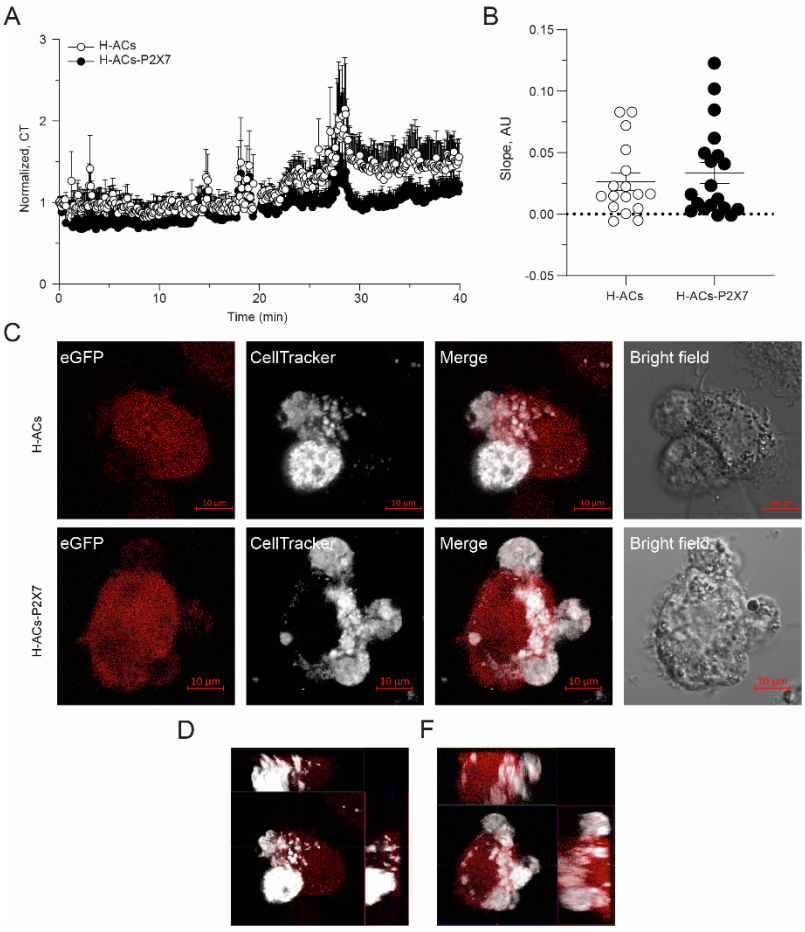
Supplementary figure 5. Detection of the SIINFEKL / MHC-I (H-2^b) complex on the surface of BMDC-BALB/c. Related to Figure 3

Detection of the fluorescence intensity of the SIINFEKL /MHC-I (H-2^b) complex normalized on the surface of BMDCs-BALB / c under the different conditions used. Fluorescence intensity (MFI) was normalized dividing the MFI of the SIINFEKL/MHC-I (H-2^b) complex of the different experimental groups with the MFI of the SIINFEKL/MHC-I complex of the BMDC-BALB/c. alone. The solid lines represent the presence of the components in each experimental group. Black circles correspond to untreated BMDC-BALB/c, and open circles indicate that the BMDC-BALB/c were pre-treated with 300 μM oATP. n = 15. Data are represented as mean ± SEM. The asterisks ** represent statistically significant differences. $p<0.01$.



Supplementary figure 6. Qualitative analysis of T-I lymphocyte proliferation. Related to Figure 4

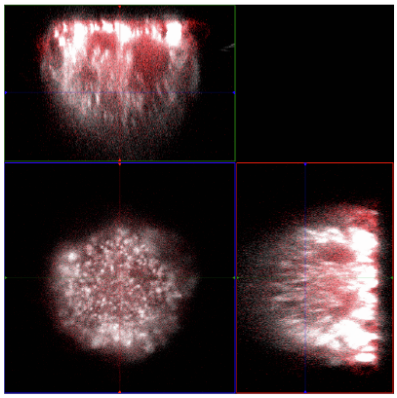
All controls performed for the OT-I proliferation analysis are shown. The table shows on the right side the description of the treatment belonging to the proliferation histogram on the left. The box in the image indicates the area of cell proliferation. The first peak from right to left indicates OT-I without proliferating (outside the highlighted box). The Y axis shows number of cells and the X axis shows fluorescence intensity for the CellTrace Violet probe.



Supplementary figure 7. H-ACs-P2X7 labeled with CellTracker (CT) are incorporated into DCs in a vesiculated pattern in early times. Related to Figure 5

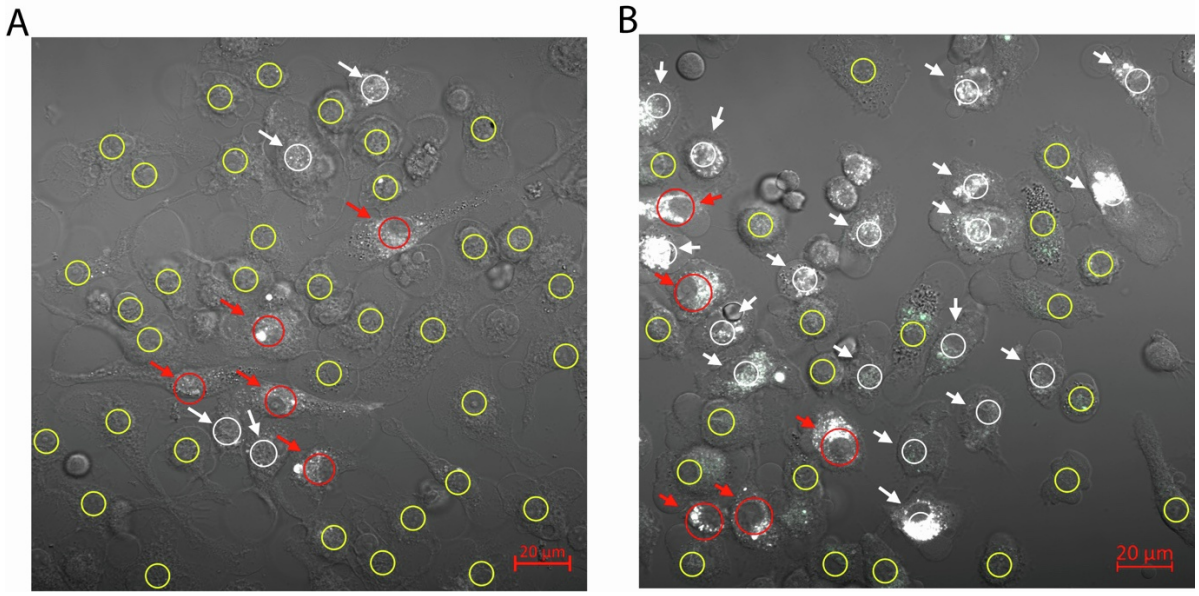
HEK293 cells were loaded with CellTracker™ (CT) before generating ACs by deprivation and stored in darkness until their use. MutuDCs were challenged with CT H-ACs (open circles) or CT H-ACs-P2X7 (closed circles Mutu to measure the fluorescein transfer during the time). **A)** Normalized CT fluorescence against unchallenged cells, measured on 15-20 challenged cells from 3-5 independent experiments **B)** Slope analysis from the time-lapse experiment. **C)** Representative's images of MutuDC1940 challenged with CT H-ACs or CT H-ACs-P2X7 after 90 minutes. From left to right: MutuDC1940 expressing eGFP (red pseudocolor), CT (white

pseudocolor), merged, and bright field. Orthogonal reconstruction from MutuDCs challenged with **D)** CT H-ACs and **E)** CT H-ACs-P2X7. All picture diameters correspond to 50x 50μm on the XY plane. Scale bars 10μm.



Supplementary figure 8. H-ACs-P2X7 labeled with CellTracker (CT) are incorporated into DCs in a diffuse pattern at late times. Related to Figure 5

Micrography diameter corresponds to 50 x 50μm on the XY plane. MutuDCs express constitutively GFP showed as red pseudocolor and CT as white pseudocolor.



Supplementary figure 9. P2X7-KO BMDCs show a decrease in diffusely distributed CT. Related to Figure 5

BMDCs **A)** WT and **B)** P2X7-KO were challenged with H-ACs-P2X7. After 24 hrs cells were fixed and general bright fields were photographed for quantification. BMDCs without CT (yellow circles), diffuse marked (white arrows), and vesiculated CT (red arrows and circles). Each experiment was repeated at least 3 times analyzing 10-16 fields. Scale bars 20µm.