

Supplementary Figure 1: Separation of cell populations from LUAD tissues

A. Graphical representation of the isolation of cell populations. **B.** Quality control after cell separation. Sorted CD45⁺ and CD45⁻ cells from non-tumor (N) and tumor (T) areas of LUAD tissues were stained with an anti-CD45 antibody and analyzed by flow cytometry. n=3 independent experiments.

Supplementary Figure 2: Expression of the homo-P2RX7A trimer

hP2RX7 HEK or purified CD45⁻ cells were permeabilized or not with 1% triton and stained with the conformational anti-P2RX7 antibody to characterize intracellular (intra) and membrane expression (Mb) of P2RX7. This result showed that CD45⁻ cells from a tumor area of LUAD patients did not retained P2RX7 within the cytoplasm.

Supplementary Figure 3: Characterization of P2RX7 activity

A. Representative dot plots are shown. hP2RX7 HEK cells were stimulated for 5 min with the indicated doses of BzATP (a stable analogue of ATP) and stained with both TO-PRO-3 (APC) and Fluo-4-AM (FITC) to assay for large pore opening and intracellular Ca²⁺ variations, respectively. **B.** Dose response curves showing large pore opening (TO-PRO-3 uptake) and Ca²⁺ channel activation in response to BzATP in live cells. n=3 independent experiments.

Supplementary Figure 4: P2RX7 activity in LUAD patients

Representative dot plot showing a time course of macropore opening in purified CD45⁺ cells (**A**) or CD45⁻ cells (**B**) isolated from LUAD tissue. The percentage of TO-PRO-3 positive cells in response to BzATP (250 μM) is shown. To demonstrate that P2RX7 is involved in the TO-PRO-3 uptake, cells were pretreated with a specific P2RX7 antagonist (GSK13700319A) 30 min before adding BzATP for an additional 30 min.

Supplementary Figure 5 : P2RX7 splice variant expression in human peripheral blood mononuclear cells

A. Primers used to analyze *P2RX7* splice variant expression in human peripheral blood mononuclear cells (PBMC). **B.** Representative image showing qualitative PCR. We were unable to generate primers specific for *P2RX7D* and *E*.

Supplementary Figure 6 : Primers used in quantitative PCR experiments

A. Primers used to analyze *P2RX7* splice variant expression by qPCR. **B.** Expression vectors coding for human *P2RX7A*, *P2RX7B*, *P2RX7H* and *P2RX7J* were used to produce specific amplicons of each *P2RX7* mRNA. The number of molecules was calculated and serial dilutions, to verify the linear amplification of each mRNA after qPCR, were performed.

Supplementary Figure 7: *P2RX7* expression in LUAD

P2RX7 expression obtained from the TCGA database showing down-regulation of *P2RX7* in LUAD tissues (T) versus non-tumor tissues (N). *P2RX7* expression was compared in LUAD tissues and adjacent non-tumor tissues. All stage: 57 LUAD tissues and paired non-tumor tissues; Stage I-II: 42 LUAD tissues and paired non-tumor tissues; Stage III-IV: 15 LUAD tissues and paired non-tumor tissues (unpaired Student's *t* test).

Supplementary Figure 8: Characterization of tagged *P2RXA* and *P2RXA* AB receptors

A. Hemi-venus expressing cells are not GFP positive. HEK cells were transiently transfected with *P2RX7A* and *P2RX7B* tagged with venus 1 or venus 2 sequences. Representative images of *P2RX7B*-v1, *P2RX7B*-v2, *P2RX7A*-v1 and *P2RX7A*-v2 showing that only cells expressing the trimeric conformation of *P2RX7A* are stained with the anti-*P2RX7*. *P2RX7* (red), v1-v2 complementation (green), nucleus (blue). **B.** Representative illustration of the quantification of v1-v2 (GFP) and *P2RX7A* (red) positive cells. The distance between tagged-*P2RX7* and *P2RX7A* was calculated using Image J software.

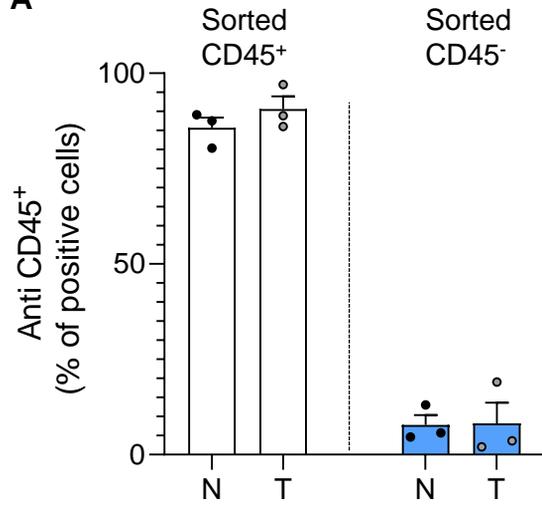
Supplementary Figure 9: Characterization of *P2RX7B* isoform in HEK transfected cells

A. HEK cells were transfected with empty vector, *P2RX7A* or *P2RX7B*. 50 ug of total protein were analyzed by western blotting using the anti-extracellular loop anti *P2RX7* antibody. In cells transfected with *P2RX7A* we observed a band of 70 kDa. In cells transfected with *P2RX7B*, we observed two bands, one of 55 kDa which corresponds to *P2RX7B* and one at lower molecular which likely corresponds to

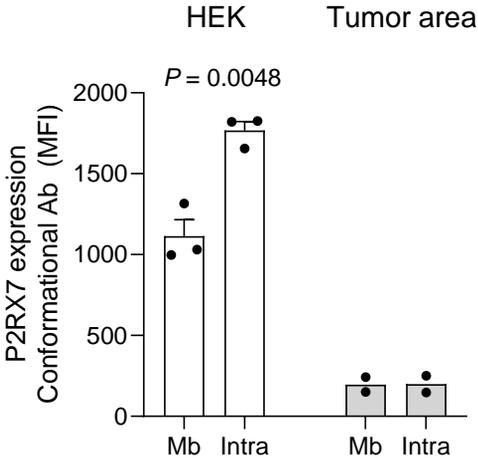
non-glycosylated protein. Expression of two bands in cells transfected with P2RX7B were previously observed in Adinolfi et al, 2010. **B.** Immunofluorescence analysis of HEK cells expressing either P2RX7A or P2RX7B. Cells were stained with the conformational anti-P2RX7 antibody (left panel). These results showed that P2RX7B is not recognized by the conformational antibody. The right panel illustrates HEK cells transfected with v1+v2-P2RX7B showing GFP fluorescence at the membrane (asterisk). **C.** P2RX7B expressed in HEK cells is activated by BzATP and induced increased Ca^{2+} concentration but not large pore opening. n=4 independent experiments.

Supplementary Figure 1

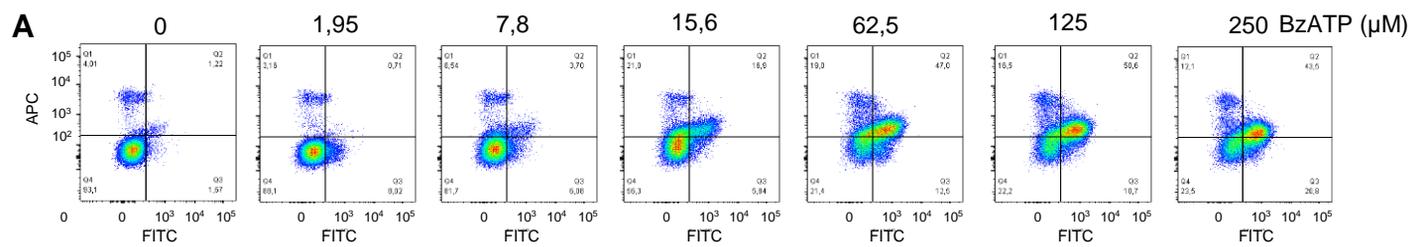
A



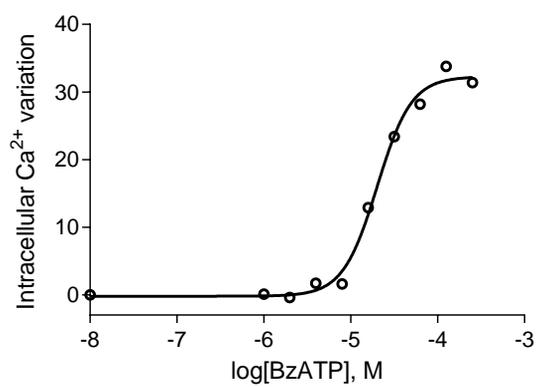
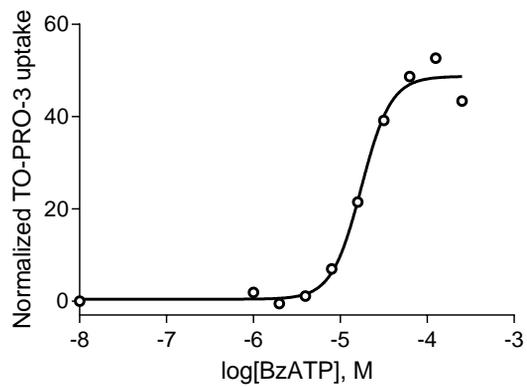
Supplementary Figure 2



Supplementary Figure 3

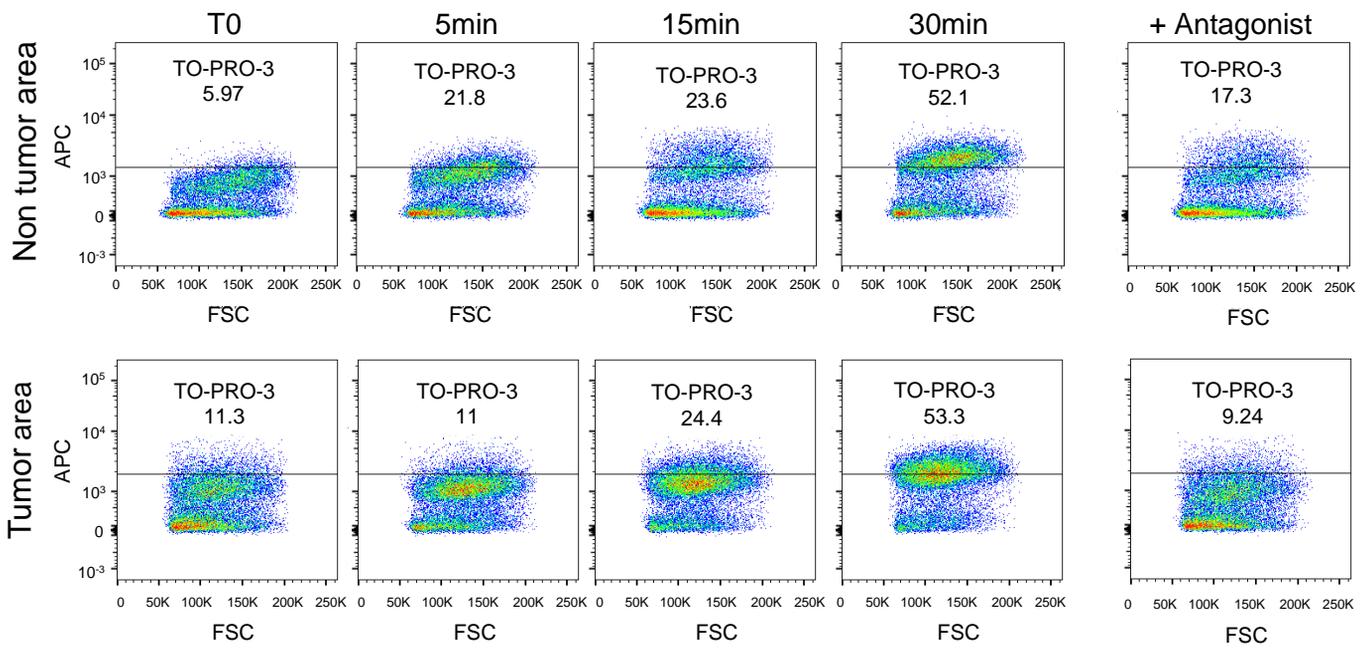


B

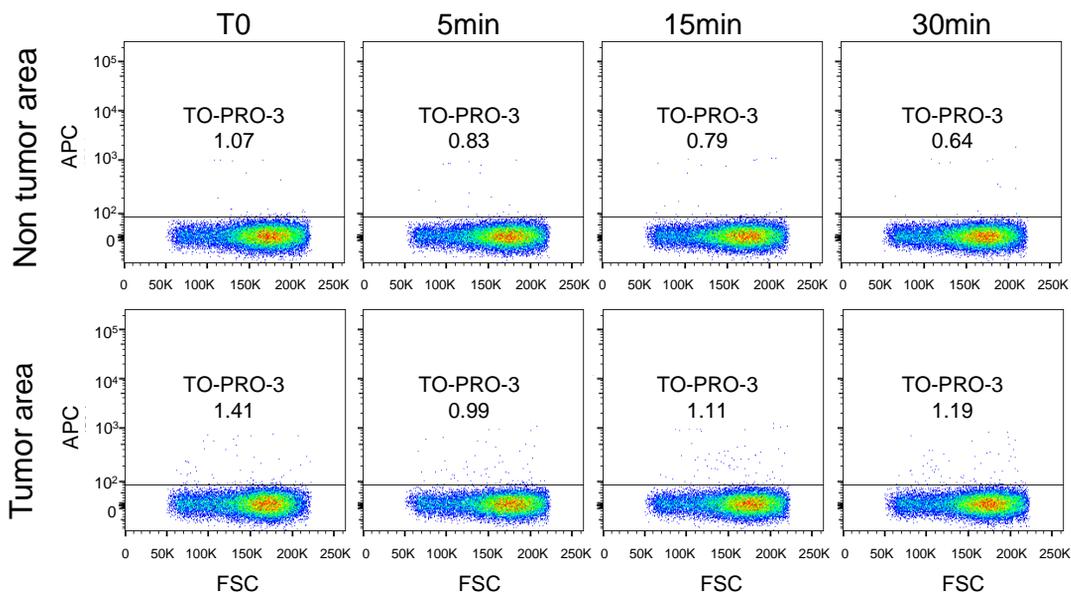


Supplementary Figure 4

A



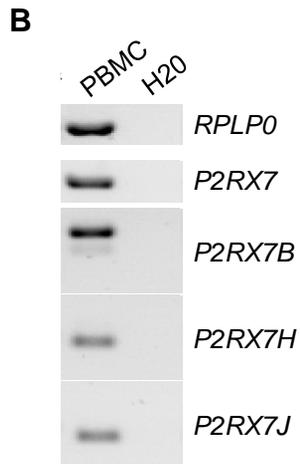
B



Supplementary Figure 5

A

Specificity	Forward primer	Reverse primer	MW
<i>P2RX7(A, B, H)</i>	GAACCAGCAGCTACTAGGGAGAAG	GAACCAGCAGCTACTAGGGAGAAG	476
<i>P2RX7B</i>	CGGCCACAACACTACACCACGAG	CGGCCACAACACTACACCACGAG	527
<i>P2RX7H</i>	CAAGGTCAGCCGAGATTCAG	CAAGGTCAGCCGAGATTCAG	850
<i>P2RX7J</i>	TTTCAGATGTGGCAATTCAGATA	TTTCAGATGTGGCAATTCAGATA	150



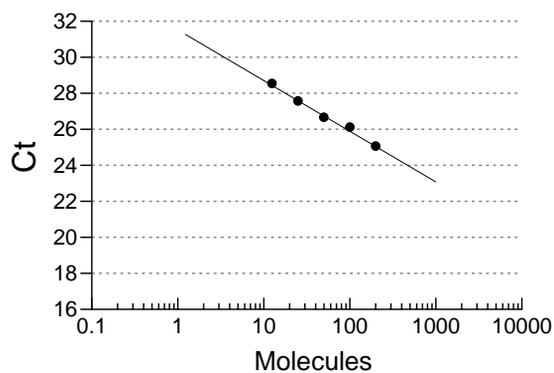
Supplementary Figure 6

A

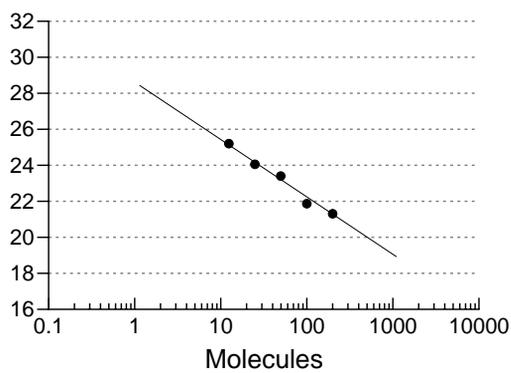
Specificity	Forward primer	Reverse primer	MW
<i>P2RX7 (A, B, H)</i>	ATACAGTTTCGTCGCCTTG	AACGGATCCCGAAGACTTTT	133
<i>P2RX7B</i>	GACATTATCCAGCTGGTTG	GACAAGCGCTGCGTTAGTCAC	125
<i>P2RX7H</i>	CAAGGTCAGCCGAGATTCAG	CAAGGTCAGCCGAGATTCAG	111
<i>P2RX7J</i>	TTCAGATGTGGCAATTCAGATA	TTCAGATGTGGCAATTCAGATA	150

B

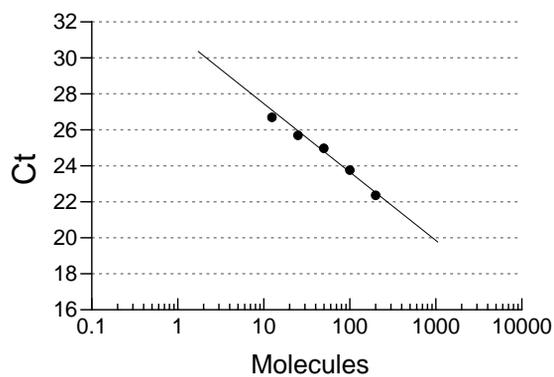
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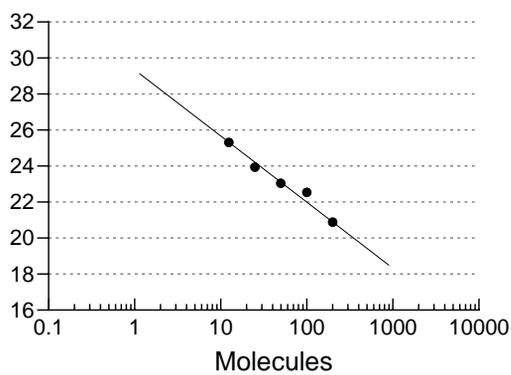
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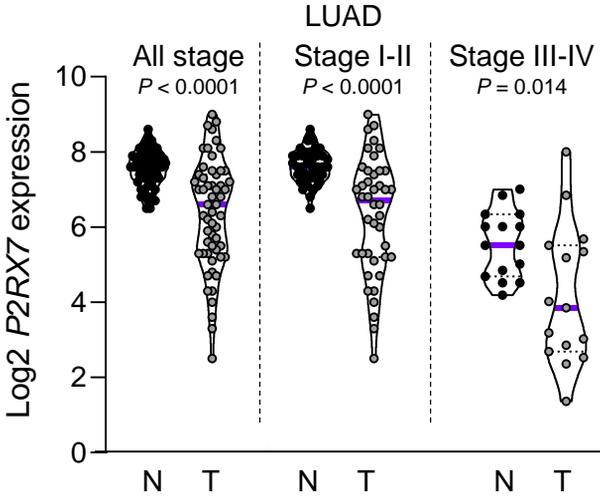
P2RX7H



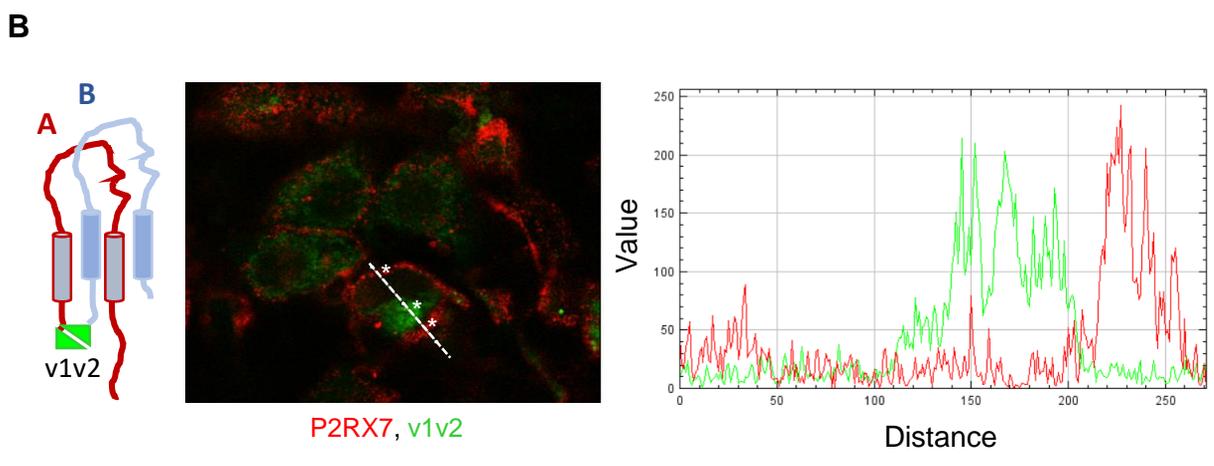
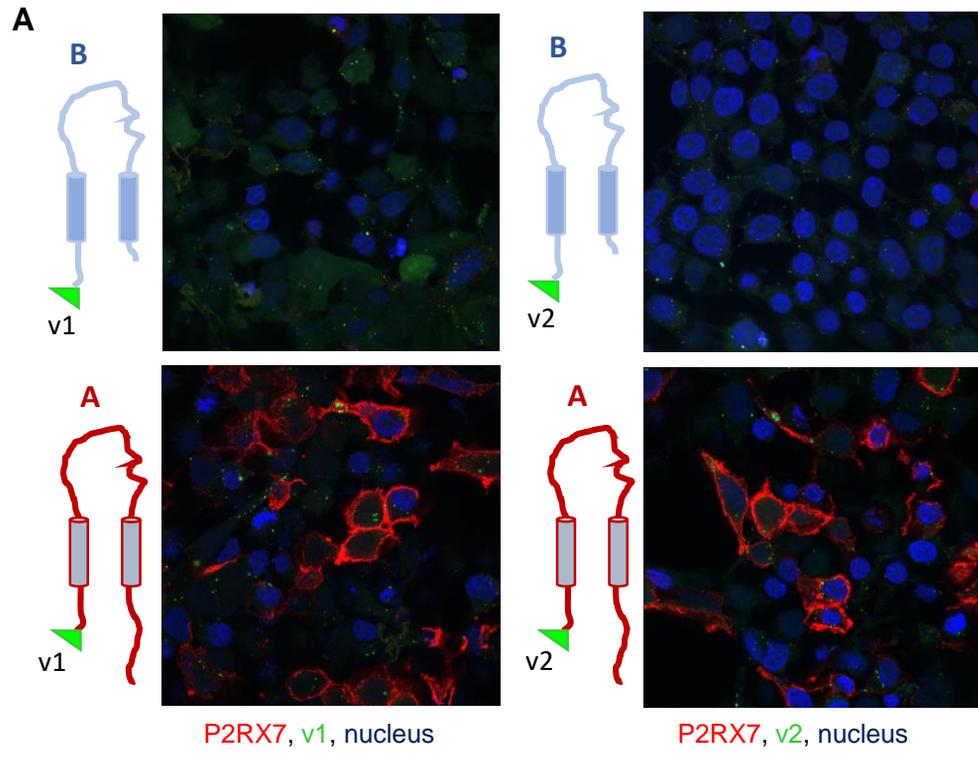
P2RX7J



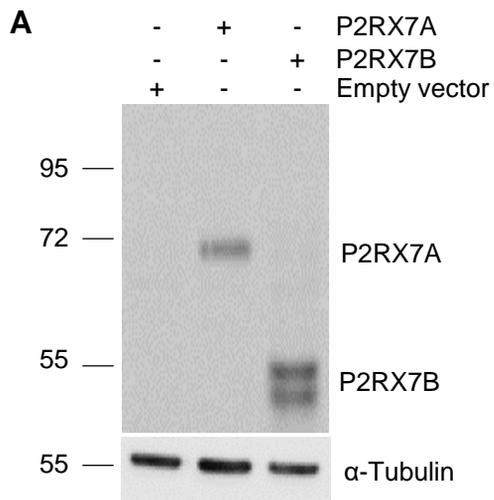
Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure 9



B Conformational antibody

