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Supplemental Material to:

Kenji F Shoji, Pablo J Sáez, Paloma Harcha, Hector L Aguila, and Juan C Sáez

Pannexin1 channels act downstream of P2X7 receptors in ATP-induced murine T-cell death

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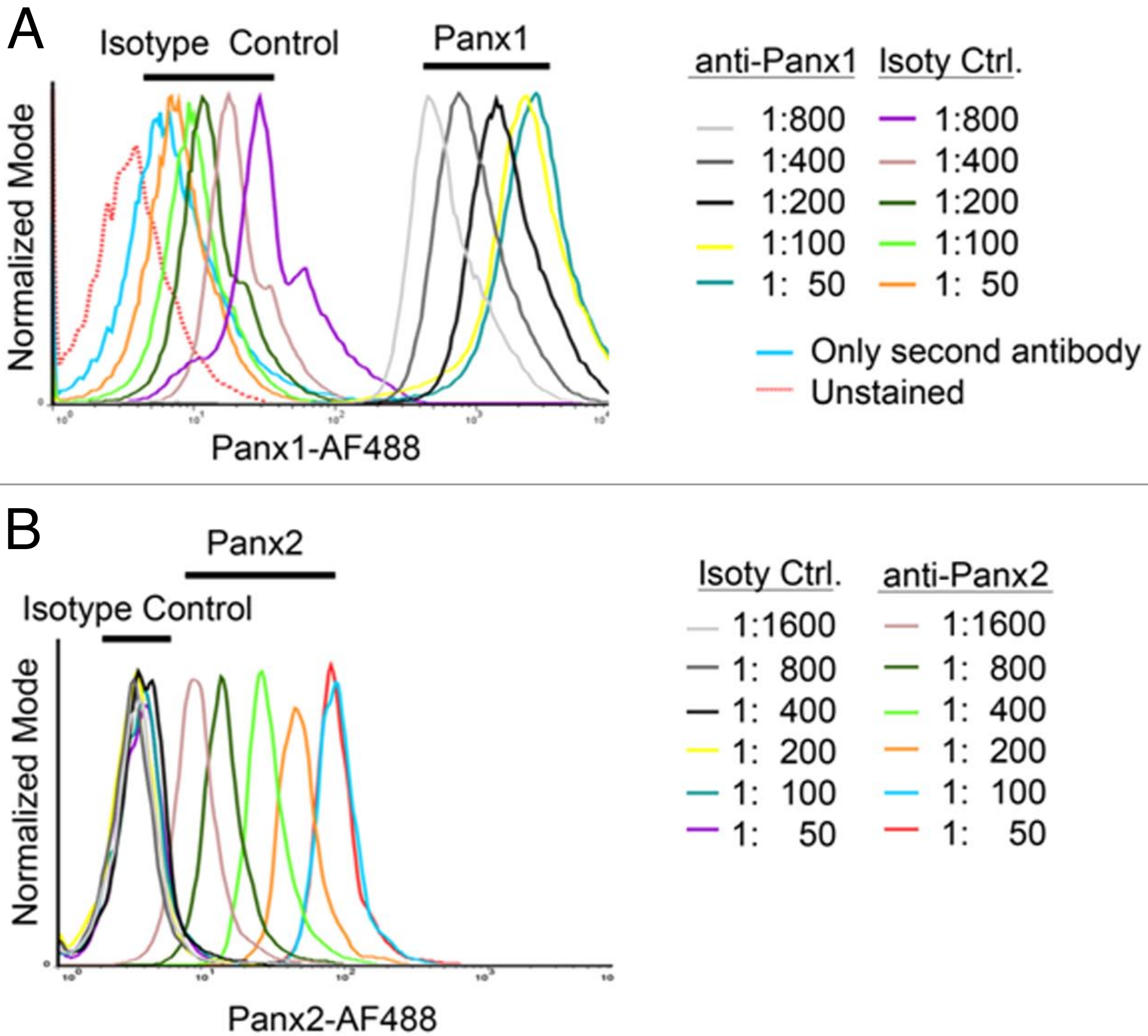
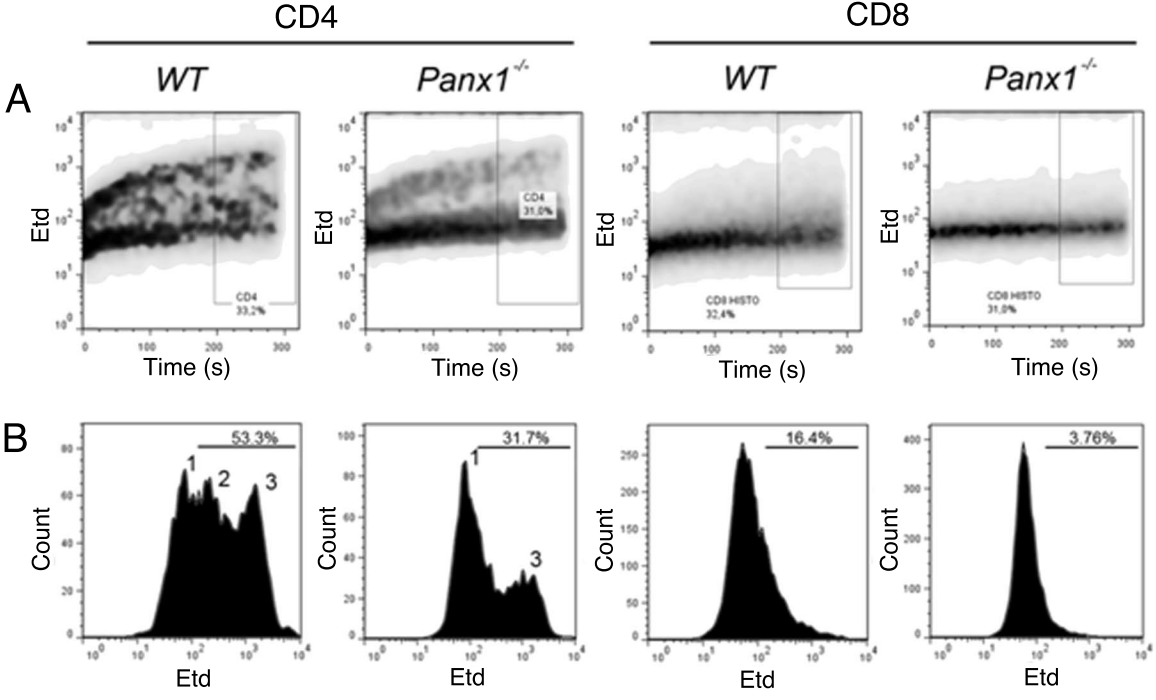

A
Isotype Control
Panx1
Normalized Mode
Panx1-AF488
anti-Panx1
1:800
1:400
1:200
1:100
1: 50
Isoty Ctrl.
1:800
1:400
1:200
1:100
1: 50
Only second antibody
Unstained
B
Panx2
Isotype Control
Normalized Mode
Panx2-AF488
Isoty Ctrl.
1:1600
1: 800
1: 400
1: 200
1: 100
1: 50
anti-Panx2
1:1600
1: 800
1: 400
1: 200
1: 100
1: 50


CD4
CD8
WT
Panx1-/-
WT
Panx1-/-
A
Etd
Time (s)
CD4
33,2%
CD4
31,0%
CD8 HISTO
32,4%
CD8 HISTO
31,0%
B
Count
Etd
53.3%
31.7%
16.4%
3.76%
1
2
3

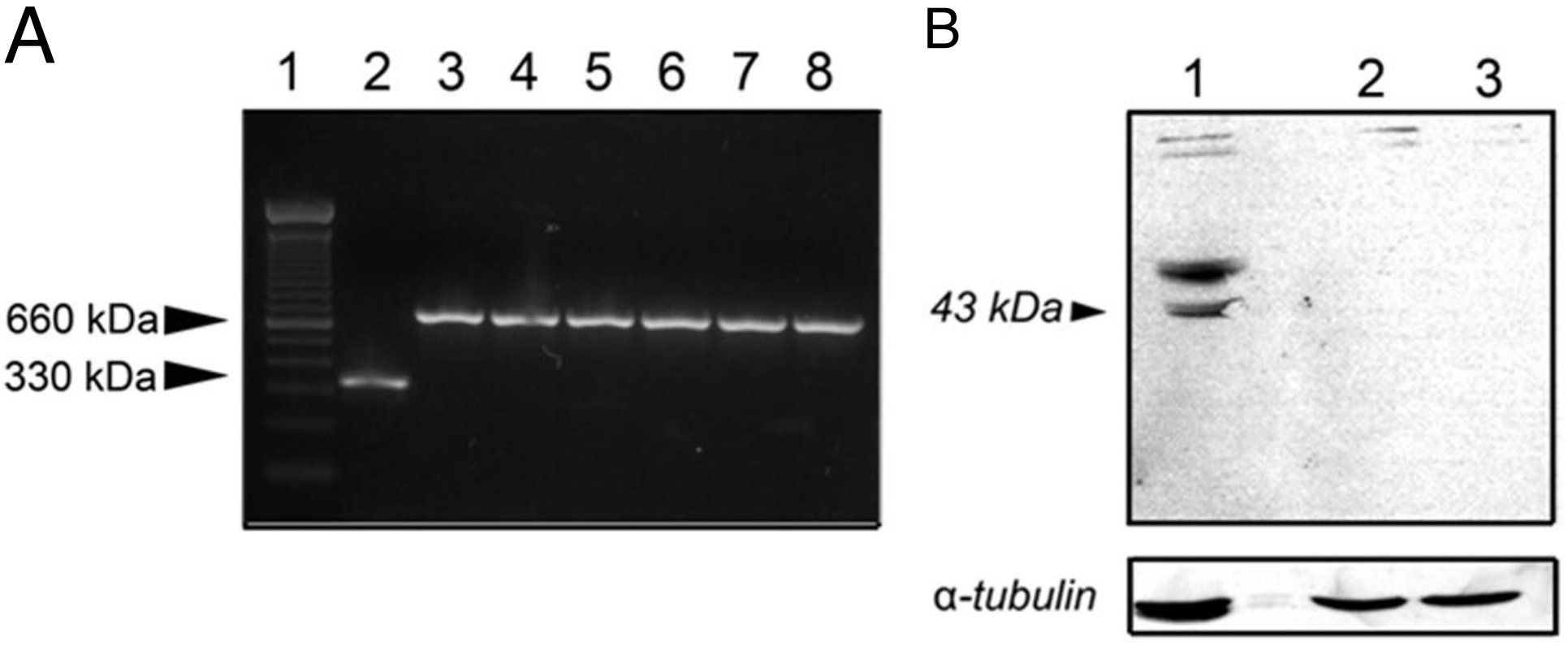

A
1 2 3 4 5 6 7 8
660 kDa
330 kDa
B
1 2 3
43 kDa
α-tubulin

Figure S1. Titration of Panx1 and Panx2 antibodies. Representative histogram plots showing the titration curves done for rabbit anti-Panx1 **(A)** and anti-Panx2 **(B)** antibodies. Isotype IgGs were used as non-specific controls. On the right side, the different treatments applied are listed and denoted by the corresponding line color.

Figure S2. Different pathways of dye uptake in CD4 T cells. T cells derived from WT or Panx1$^{-/-}$ mice were labeled with anti-CD4 conjugated to AF488 and anti-CD8 conjugated to APC and incubated with 1 mM ATP. Cells were monitored in real time with flow cytometry and displayed as densitometric analysis **(A)** or as histogram **(B)**. Real time flow cytometric experiments were done, and representative densitometric curves showing the Etd uptake in $CD4^+$ or $CD8^+$ gated cells were taken. **(A)** Representative Etd kinetics between WT or Panx1$^{-/-}$ cells are shown. **(B)** Etd uptake histogram showing the frequency of dye uptake by responding cells from WT or Panx1$^{-/-}$ mice, in either $CD4^+$ or $CD8^+$ T cells.

Figure S3. Targeted disruption in mouse Panx1$^{-/-}$ gene. Evidence for complete Panx1 deletion was obtained by RT-PCR analysis from tail DNA from 8-week-old mice and western blot analyses using total PLNs. **(A)** PCR experiments yielded a 330-bp for wild type mouse (line **1**) and 660-bp for C57B6 / Panx1$^{-/-}$(lines **2-8**). **(B)** Representative immunoblot showing Panx1 in mouse PLNs from wild type (line **1**) and Panx1$^{-/-}$ mice (lines **2,3**)(100 μg of total protein/lane). In wild type, immunoreactive bands are likely to corres

pond to the non-glycosylated (43 kDa) and glycosylated forms (60 kDa) of Panx1. At the bottom, immune blot showing α-tubulin used as protein loading controls.