JEM

Supplemental material

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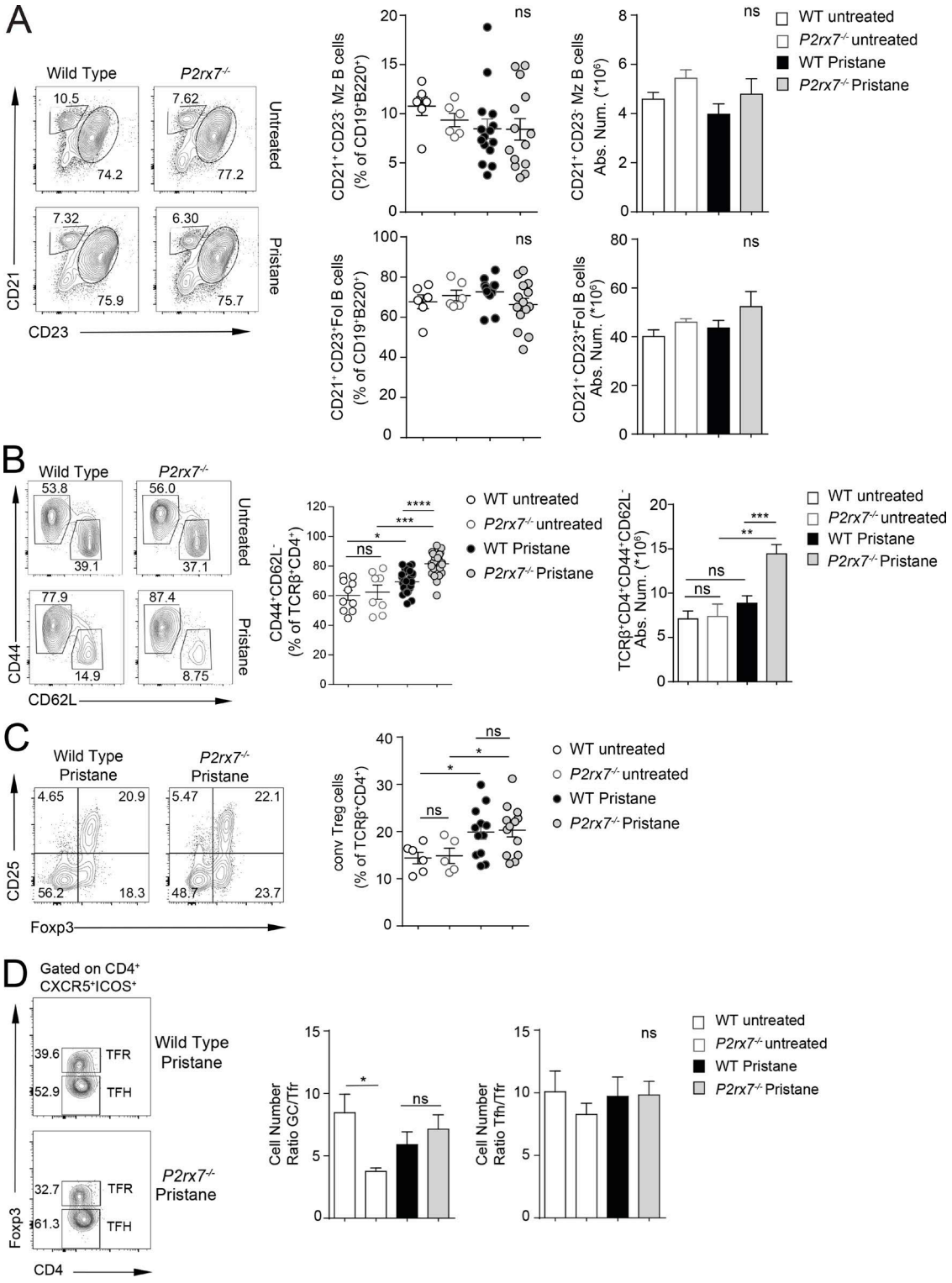

Figure S1. **Phenotypic analysis of B cells, T effector cells, and T reg cells in WT and *P2rx7*$^{-/-}$ mice. (A)** Representative contour plots of B2 B cells in the spleen of WT and *P2rx7*$^{-/-}$ mice. Frequencies and absolute numbers from untreated WT (n = 6) and *P2rx7*$^{-/-}$ (n = 6) mice and treated WT (n = 15) and *P2rx7*$^{-/-}$ (n = 14) mice of marginal zone (Mz) and follicular (Fol) B cells gated as CD21^{+}CD23^{-} and CD21^{lo}CD23^{+} cells (as depicted in contour plots), respectively, among CD19^{+}B220^{+} total B cells. **(B)** CD44 and CD62L staining of TCRβ^{+}CD4^{+} cells. Numbers indicate percentages of CD44^{+}CD62L^{-} (effector) and CD44^{-}CD62L^{+} (naive) cells within displayed quadrants; untreated WT (n = 10), *P2rx7*$^{-/-}$ (n = 8), treated WT (n = 29), and *P2rx7*$^{-/-}$ (n = 29) mice. **(C)** Representative contour plots for CD25 and Foxp3 on gated CD4^{+} T cells and frequency of conventional T reg cells in the spleen of the indicated mice. Untreated WT (n = 6) and *P2rx7*$^{-/-}$ (n = 5) mice; treated WT (n = 12) and *P2rx7*$^{-/-}$ (n = 13) mice. **(D)** Representative contour plots used to define Tfh and Tfr cells on gated CXCR5^{+}ICOS^{+} CD4^{+} T cells. Bar graphs (mean ± SEM) represent ratios of GC B/Tfr (left) and Tfh/Tfr (right) cells in the spleen of the same mice as above. Each dot in the graphs represents an individual mouse (mean ± SEM is shown). Two-tailed Mann–Whitney *U* test. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. ns, not significant.

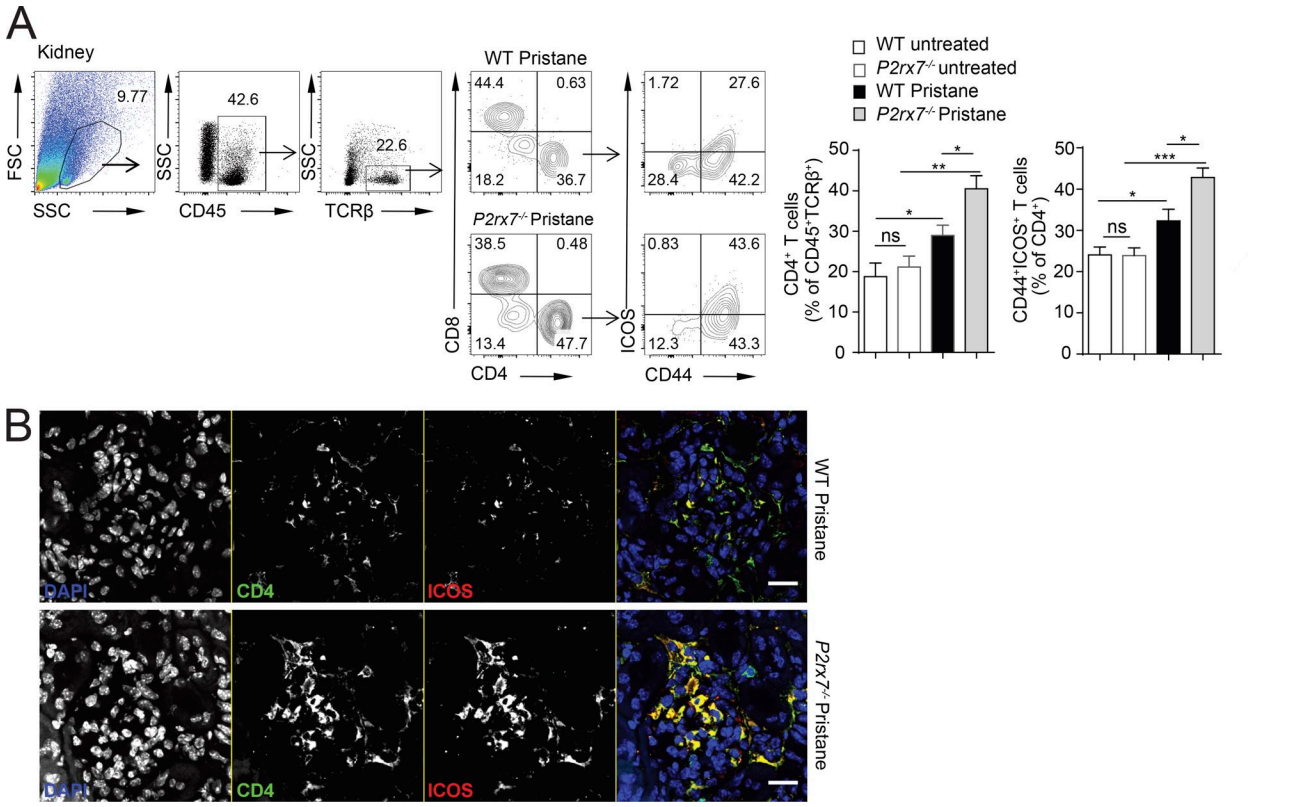

Figure S2. **Increase of ICOS⁺ CD4 T cells in kidneys in pristane-treated *P2rx7*$^{-/-}$ mice. (A)** Gating strategy for quantifying kidney-infiltrating CD44⁺ICOS⁺ effector CD4 T cells, and frequency of CD4⁺ and CD44⁺ICOS⁺ T cells from at least five animals. FSC, forward scatter; SSC, side scatter. Mean ± SEM, two-tailed Mann–Whitney *U* test. **(B)** Representative confocal immunofluorescence images of fixed kidney cryosection stained with anti-CD4 (green), anti-ICOS (red) antibodies and DAPI (blue) showing ICOS⁺ T cells within glomeruli of WT and *P2rx7*$^{-/-}$ mice. Bars, 20 µm. *, P < 0.05; **, P < 0.01; ***, P < 0.001. ns, not significant.

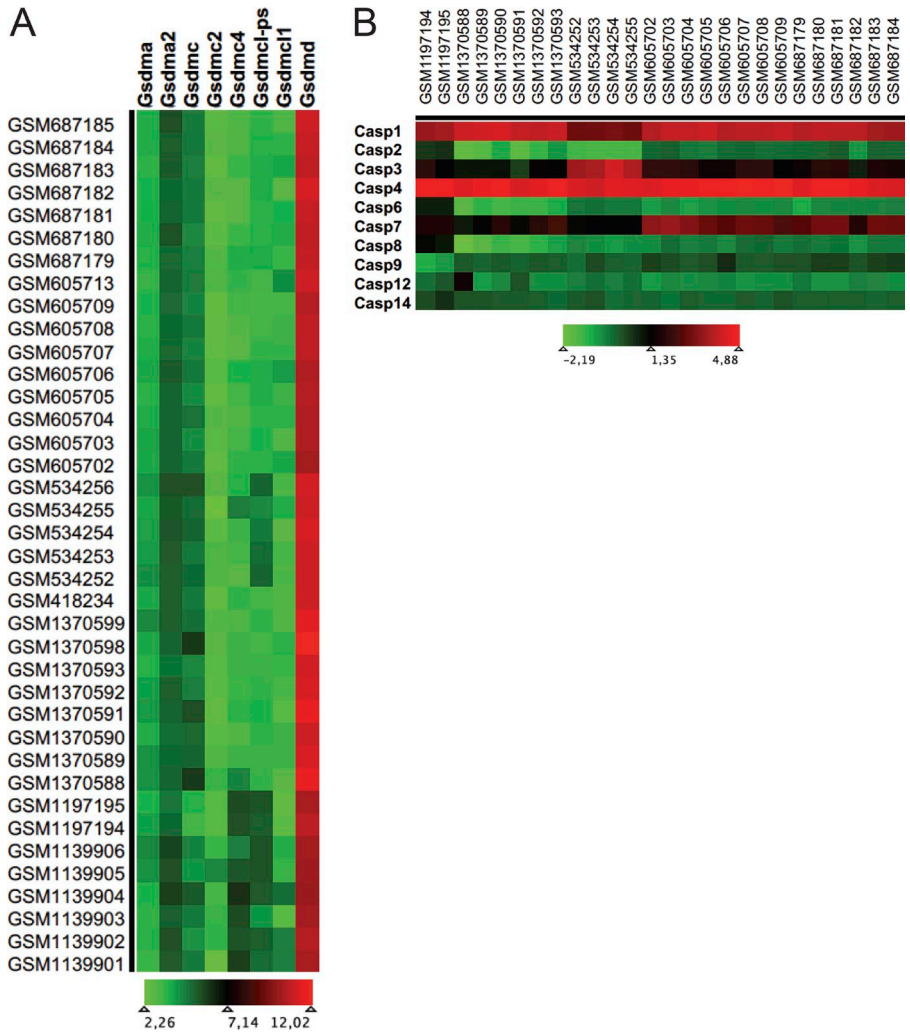

Figure S3. **Gasdermins and caspases expression in naive CD4 and Tfh cells. (A)** Heat map representing the log2 expression levels of genes encoding mouse gasdermins in CD4 naive and Tfh cells based on data derived from publicly available gene expression datasets (numbers on the left are accession codes). **(B)** Heat map showing fold changes of caspases in Tfh compared with naive CD4 cells (accession codes on top).

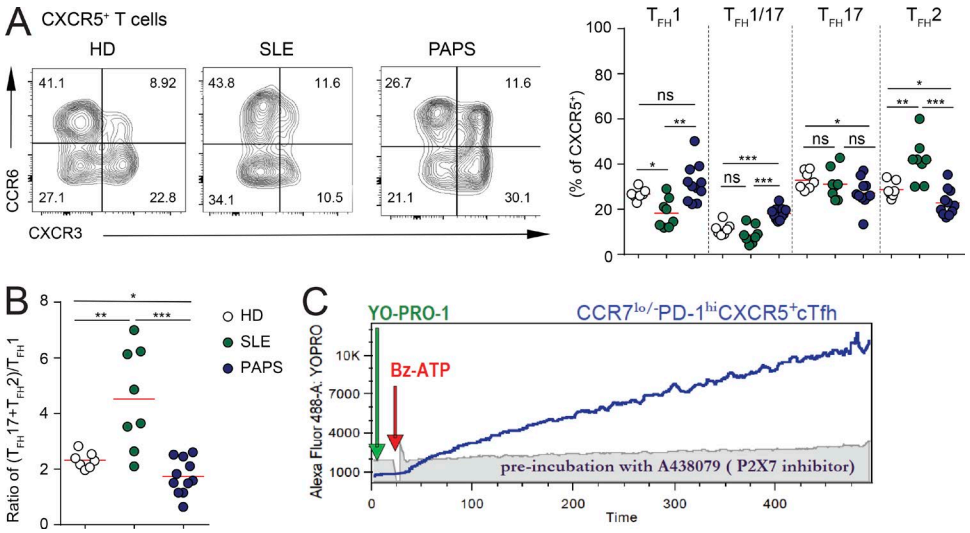

Figure S4. **Distribution of functional cTfh cell subsets in CXCR5⁺ cells from HDs and SLE and PAPS patients. P2X7-dependent YOPRO-1 uptake in cTfh cells. (A)** Representative contour plots of CXCR5⁺ cTfh cells from healthy (HD), SLE, and PAPS subjects for CCR6 and CXCR3, and distribution of Tfh1, Tfh1/17, Tfh17, and Tfh2 cells in individual subjects from different groups (HD, *n* = 7; SLE, *n* = 8; and PAPS, *n* = 11; see Results for details). **(B)** Ratio between (Tfh17 + Tfh2) and Tfh1 cells in the same subjects. **(C)** Representative uptake of YO-PRO-1 in electronically gated $CCR7^{lo}PD\text{-}1^{hi}$ $CXCR5^{+}$ T cells stimulated with BzATP (0.1 mM) or preincubated before stimulation with A438079 to allosterically block pore formation. Two-tailed Mann–Whitney *U* test. *, P < 0.05; **, P < 0.01; ***, P < 0.001. ns, not significant.

Table S1. **Demographic, clinical, and laboratory characteristics of SLE and PAPS patients and HDs**

Characteristic	SLE (*n* = 42)	PAPS (*n* = 14)	HD (*n* = 31)
Female/male, *n*	41/1	8/6	29/2
Median age, yr (range)	43 (26–76)	51 (39–68)	41 (25–62)
Median disease duration, yr (range)	17 (0.5–48)	13.65 (2–24)	NA
Onset <18 yr	12 (28)	0 (0)	NA
Laboratory tests			
ANA positivity	40 (95)	6 (42.8)	NA
Anti-ENA positivity	19 (45)	0 (0)	NA
Anti-SSA/Ro	13 (30.9)	NA	NA
Anti-Sm	6 (14.3)	NA	NA
Anti-RNP	6 (14.3)	NA	NA
Anti-dsDNA positivity[a]	22 (52.4)	1 (7.14)	NA
Low titer	7 (16.7)	1 (7.14)	NA
Medium/high titer	15 (36)	0 (0)	NA
LA	12 (28.5)	13 (93)	NA
Anti-β2GPI IgG[b]	5 (11.9)	12 (85.7)	NA
Anti-β2GPI IgM[b]	2 (4.7)	5 (35.7)	NA
Anti-CL IgG[b]	7 (16.6)	13 (92.8)	NA
Anti-CL IgM[b]	2 (4.7)	2 (14.3)	NA
Low C3[c]	19 (45.2)	4 (28.6)	NA
Low C4[c]	27 (64.3)	7 (50)	NA
Hypergammaglobulinemia[d]	14 (33)	3 (21)	NA
Clinical manifestations (ever)			
SLEDAI-2K, median (IQR)	4 (2–6)	NA	NA
Skin rash	9 (21.4)	NA	NA
Alopecia	2 (4.76)	NA	NA
Hematologic involvement[e]	12 (28.6)	NA	NA
Arthritis	11 (26)	NA	NA
Myositis	1 (2.4)	NA	NA
Glomerulonephritis	2 (4.76)	NA	NA
Thrombotic APS	6 (14)	11 (78.6)	NA
Obstetric APS	0 (0)	14 (28.6)	NA
Therapy			
Equivalent prednisone dose			
Median cumulative dose, mg (IQR)	36.5 (15–58.4)	NA	NA
Median daily dose, mg/die (IQR)	5 (2.5–10)	NA	NA
Ongoing DMARDs			
Hydroxychloroquine	28 (66.7)	9 (64)	NA
Mycophenolate mofetil	8 (19)	1 (7)	NA
Azathioprine	5 (11.9)	0 (0)	NA
Methotrexate	3 (7)	0 (0)	NA
Cyclosporine	5 (11.9)	0 (0)	NA

Data are *n* (%) unless noted otherwise. Anti-CL, anti-cardiolipin; anti-ENA, anti–extractable antinuclear antigens; IQR, interquartile range; DMARD, disease-modifying antirheumatic drug; GPL/MPL, IgG/IgM antiphospholipid units; LA, lupus anticoagulant; NA, not applicable.
[a]Anti-dsDNA has been detected as previously reported (Ingegnoli et al., 2014); anti-dsDNA titers were defined as low if <40 UI/ml, medium/high if >40 UI/ml.

[b]Anti-β2GPI and anti-CL positivity was defined as medium/high titer according to 2006 revised classification criteria for APS (>99th percentile for anti-β2GPI and >40 GPL/MPL for anti-CL; Miyakis et al., 2006).
[c]Low C3 was defined as <80 mg/dL; low C4 as <15 mg/dL.
[d]Hypergammaglobulinemia: >20% of total serum proteins.
[e]Hematologic involvement: leukopenia, thrombocytopenia based on SLEDAI-2K definitions (Gladman et al., 2002) and Coombs-positive hemolytic anemia.

References

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Ingegnoli, F., R. Gualtierotti, T. Schioppo, A. Orenti, P. Boracchi, C. Lubatti, C. Mastaglio, V. Galbiati, A. Murgo, S. Zeni, et al. 2014. Fibrosis biomarkers in isolated Raynaud's phenomenon: too little, too soon? *Ann. Rheum. Dis.* 73:940–941. https://doi.org/10.1136/annrheumdis-2013-204009
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