SUPPLEMENTAL DATA

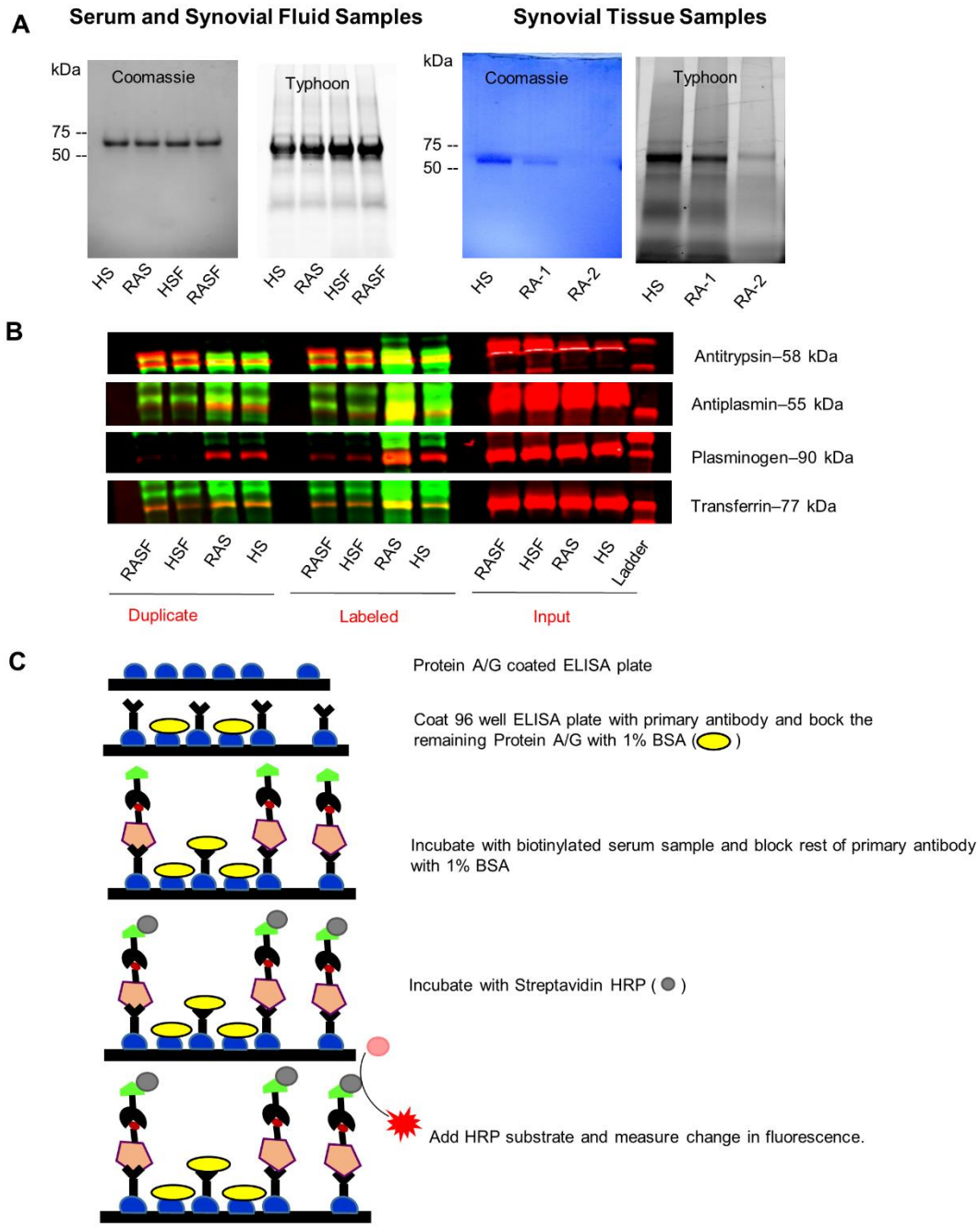

Figure S1 Quantification of citrullinated proteins from RA samples and quantification of specific citrullinated proteins by western blot and ELISA, related to Figure 1 AND 3. A) Coomassie and Typhoon images of Rh-PG labeled serum, synovial fluid and synovial tissue samples. Where, HS = healthy serum, RAS = RA serum, HSF = healthy synovial fluid and RASF = RA synovial fluid. Synovial tissue samples were obtained from two different confirmed RA patients numbed RA-1 and RA-2. B) Western blot analysis of antitrypsin, antiplasmin, plasminogen and transferrin. The healthy and RA serum and synovial fluid samples were labeled with biotin-PG and citrullinated fractions were isolated

using streptavidin agarose. Bound proteins were eluted, separated by SDS-PAGE and electrotransferred to PVDF membrane. Membranes were then treated with the appropriate primary antibody against the protein of interest followed by the corresponding secondary antibody (Licor IRDye®800CW) and streptavidin (Licor IRDye®680CW). The red band represents the input level of the specific protein of interest whilst the green band shows the level of citrullination of that same protein. C) Detailed work flow of ELISA assay used to quantitate the specific citrullinated protein from RA patient samples.

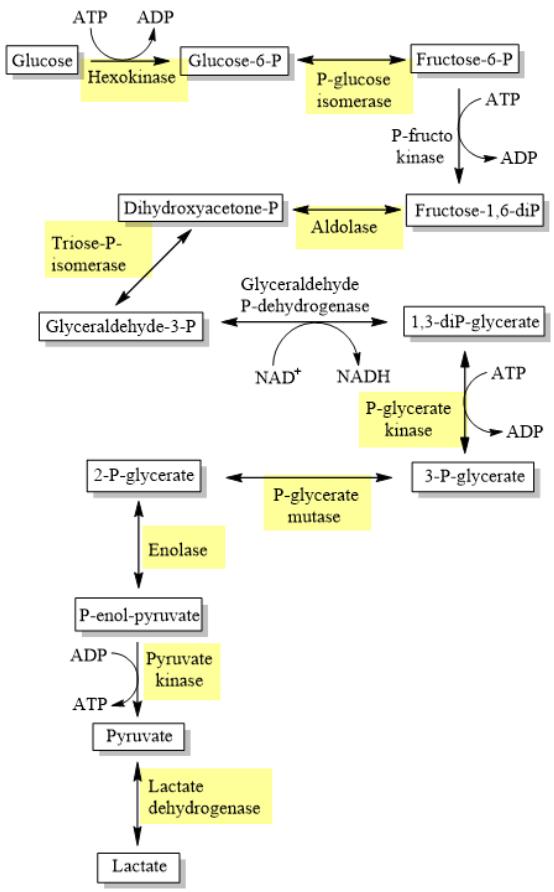

Figure S2. Citrullinated glycolytic enzymes discovered from RA synovial tissues are highlighted in yellow, related to STAR Methods

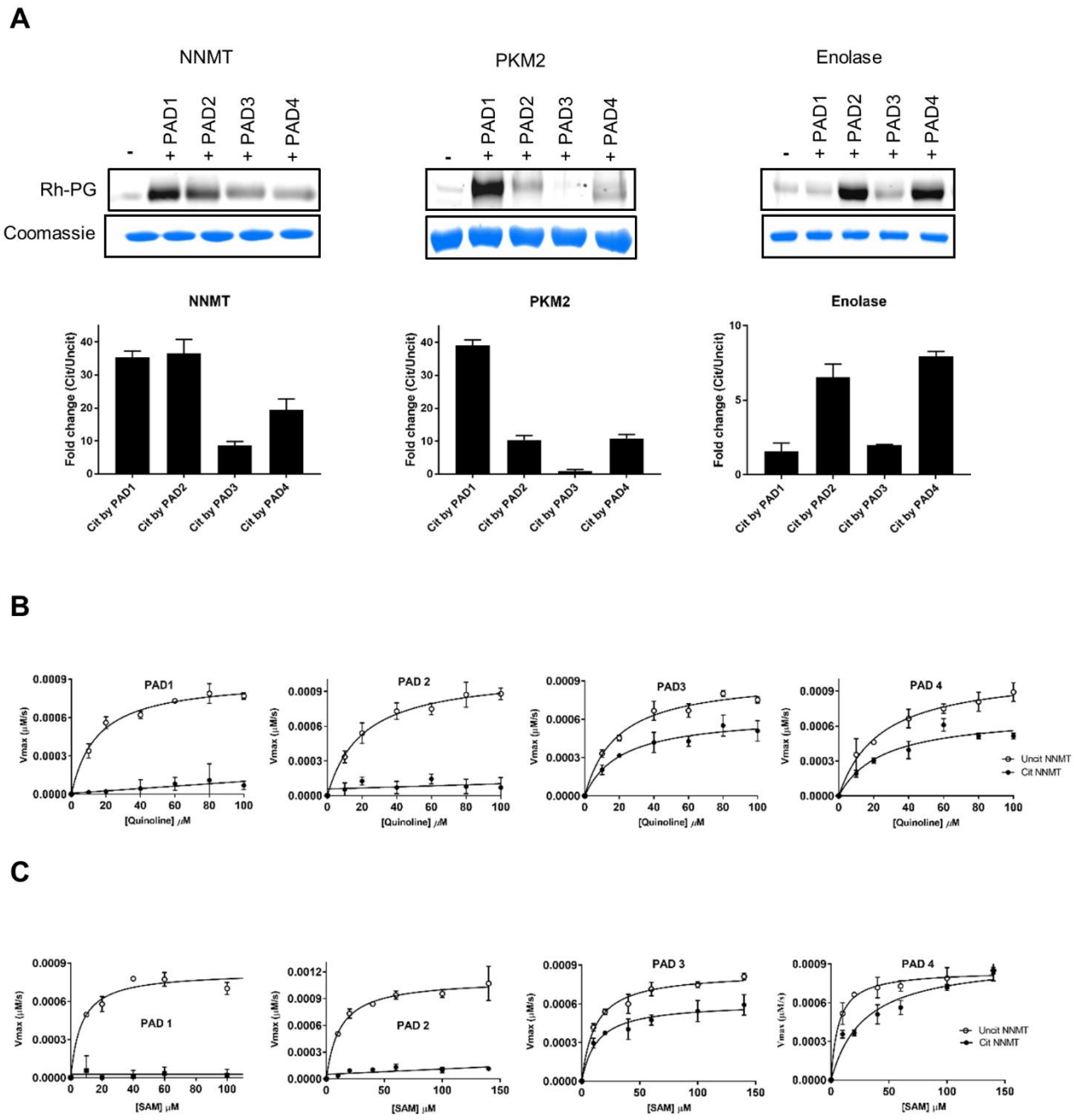

Figure S3. Citrullination of NNMT, PKM2, and enolase and activity of citrullinated NNMT, related to STAR Methods. A) Citrullination of NNMT, PKM2 and enolase (10 µM each). The proteins were citrullinated by PAD1, PAD2, PAD3 and PAD4 and then labelled with Rh-PG probe. Proteins were separated by SDS-PAGE and rhodamine fluorescence imaged (top). Coomassie images (bottom) confirm equal loading. The band fluorescence intensities were further quantified, normalized against uncitrullinated proteins and plotted against PAD1, PAD2, PAD3 and PAD4. B) Michaelis-Menten plots showing activity of citrullinated and uncitrullinated NNMT against quinoline. Activity of NNMT (0.3

μM) was measured against various concentrations of quinoline (0, 10, 20, 40, 60, and 80, and 100 μM) in the presence of a fixed concentration of SAM (100 μM) by monitoring the formation of 1-methyl quinoline (λ ex = 330 nm; λ em = 405 nm) fluorometrically. C) Michaelis-Menten plots showing activity of citrullinated and uncitrullinated NNMT against SAM. Activity of NNMT (0.3 μM) was measured against various concentrations of SAM (0, 10, 20, 40, 60, and 80, and 100 μM) in the presence of a fixed quinoline concentration (100 μM) by monitoring the formation of 1-methyl quinoline (λ ex = 330 nm; λ em = 405 nm) fluorometrically.

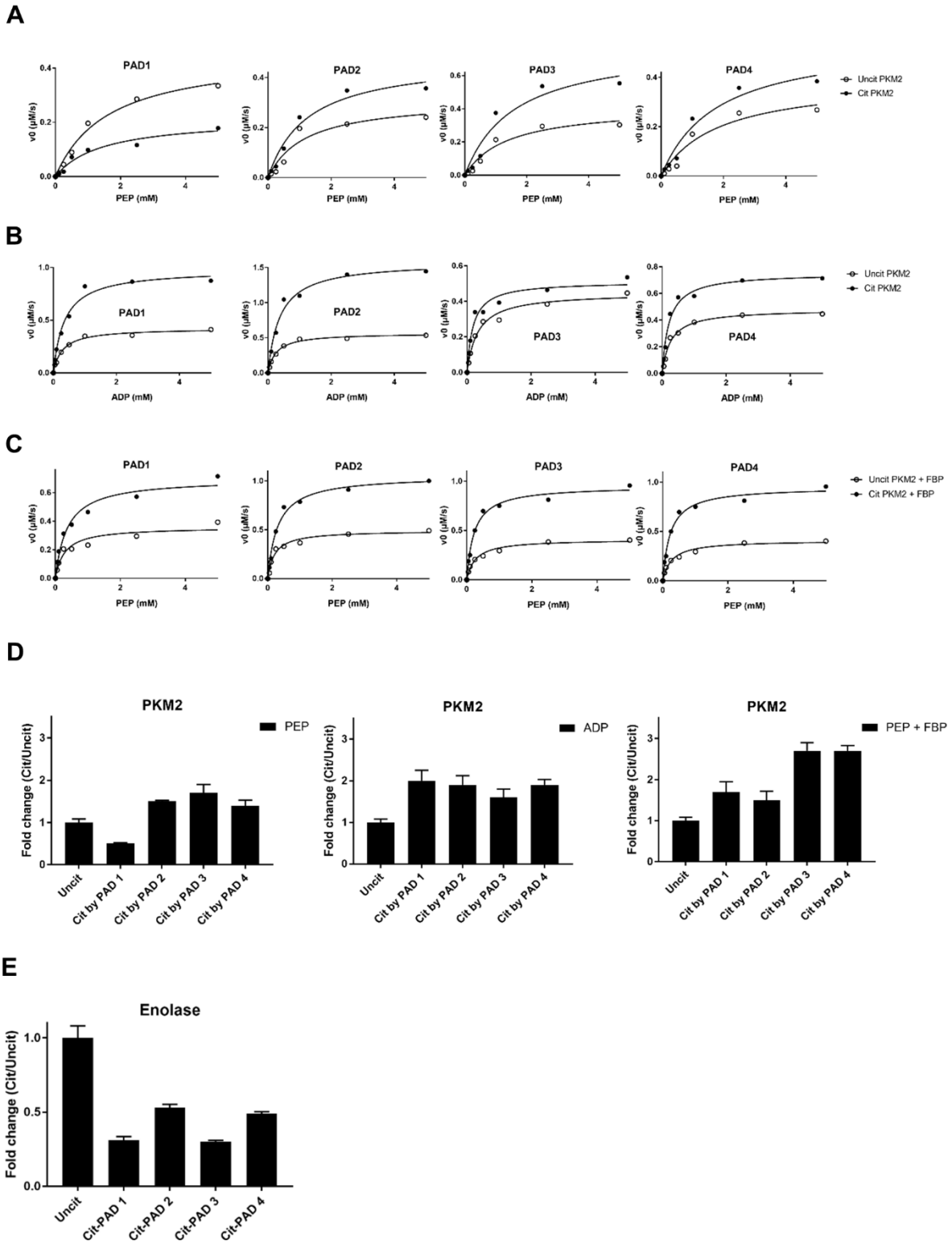

Figure S4. Activity of citrullinated PKM2 and enolase, related to STAR Methods. A) Michaelis-Menten plots showing activity of citrullinated and uncitrullinated PKM2 against phosphoenol pyruvate

(PEP). Activity of PKM2 (0.6 µM) was measured against various PEP concentrations (0.05-5 mM) in the presence of saturating concentration of ADP (10 mM) using the LDH coupled assay. The oxidation of NADH was measured at 340 nm. B) Michaelis-Menten plots showing activity of citrullinated and uncitrullinated PKM2 against ADP. Activity of PKM2 (0.6 µM) was measured against various ADP concentrations (0.05-5 mM) in the presence of a saturating concentration of PEP (10 mM) using the LDH coupled assay. The oxidation of NADH was measured at 340 nm. C) Michaelis-Menten plots showing the activity of citrullinated and uncitrullinated PKM2 against phosphoenol pyruvate (PEP) in the presence of fructose 1,6-biphosphate (FBP). PKM2 (10 µM) was incubated in the presence and absence of FBP (1 mM) for 30 min at 37 °C before citrullinating it with PADs. Activity of PKM2 (0.6 µM) was measured against various PEP concentrations (0.05-5 mM) in the presence of a saturating concentration of ADP (10 mM) using the LDH coupled assay. The oxidation of NADH was measured at 340 nm. D) Fold change in activity of citrullinated/uncitrullinated PKM2 measured from Supplementary Figures 4A, 4B and 4C. E) Fold change in activity of citrullinated/uncitrullinated enolase. The enolase activity of citrullinated and uncitrullinated enolase (0.5 µM) was measured against various concentrations of 2-phosphoglycerate (0.05-5 mM) using the LDH/PKM2 coupled assay. The oxidation of NADH was measured at 340 nm and the data was fit to the Michaelis-Menten equation.

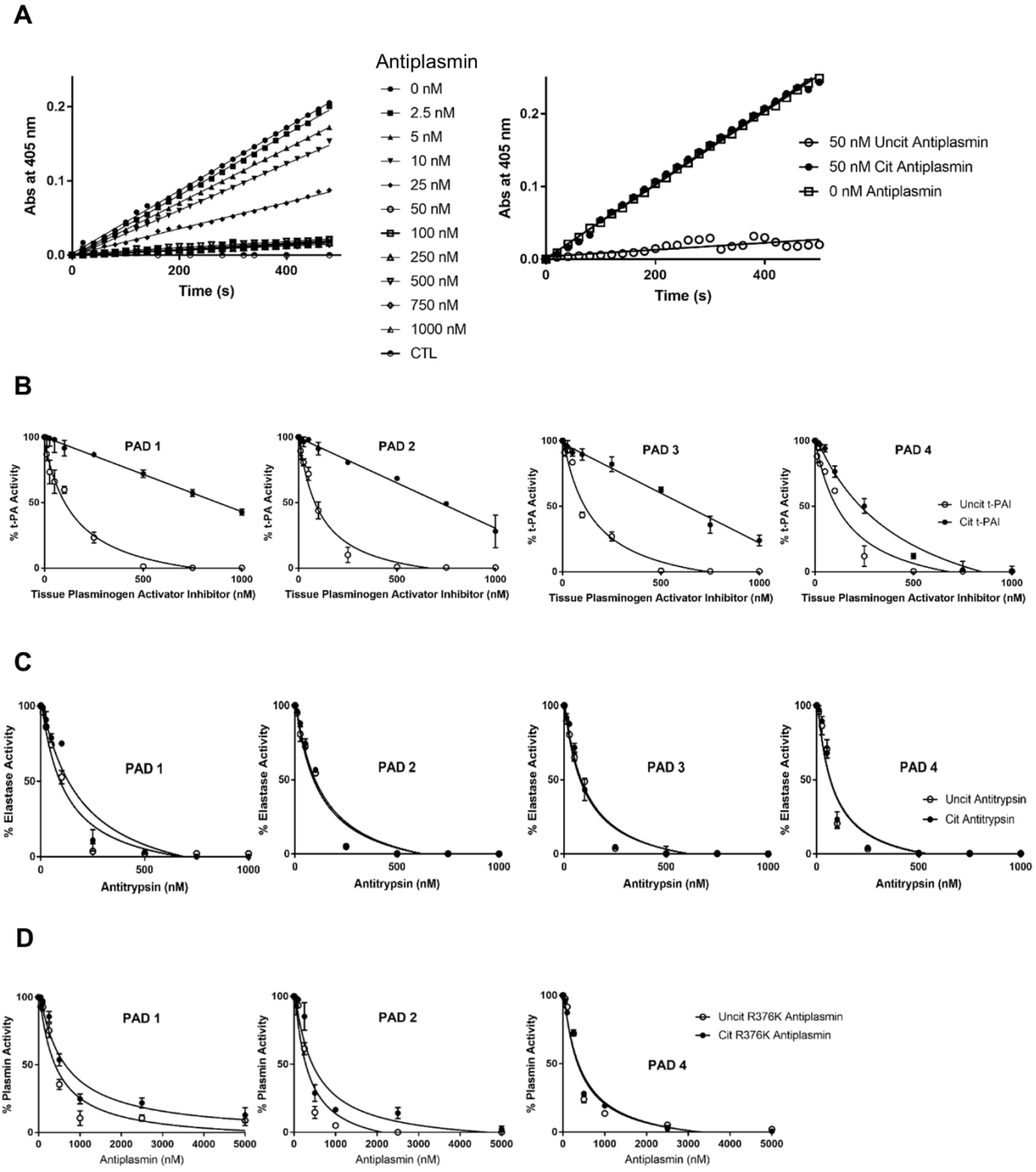

Figure S5. Functional effect of citrullination on Serpins, related to Figures 4 and 5. A) Inhibition of plasmin (50 nM) by various concentrations of antiplasmin (0, 2.5, 5, 10, 25, 50, 100, 250, 500, 750 and 1000 nM) and inhibition of plasmin (50 nM) by citrullinated and uncitrullinated antiplasmin (50 nM). A fixed concentration of plasmin was mixed with various concentrations of antiplasmin and incubated for

30 min at room temperature. The reaction mixtures were then placed in a 96-well plate followed by the rapid addition of the plasmin substrate D-Val-Leu-Lys-pNA (1 mM). Hydrolysis of the latter was monitored spectrophotometrically at 405 nm. **B)** IC_{50} plots showing inhibition of tissue plasminogen activator (t-PA) by various concentrations of citrullinated and uncitrullinated tissue plasminogen activator inhibitor (t-PAI). A fixed concentration of t-PA (100 nM) was mixed with various concentrations of citrullinated or uncitrullinated t-PAI (0, 10, 25, 50, 100, 250, 500, 750 and 1000 nM) and incubated for 30 min at room temperature. The reaction mixtures were placed in a 96-well plate followed by the rapid addition of the t-PA substrate methyl sulfonyl D-hexahydrotyrosine-Gly-Arg-pNA (1 mM). Hydrolysis of the latter was monitored spectrophotometrically at 405 nm. **C)** IC_{50} plots showing inhibition of neutrophil elastase by various concentrations of citrullinated and uncitrullinated antitrypsin. A fixed concentration of neutrophil elastase (100 nM) was mixed with various concentrations of citrullinated or uncitrullinated antitrypsin (0, 10, 25, 50, 100, 250, 500, 750 and 1000 nM) and incubated for 30 min at room temperature. The reaction mixtures were then placed in a 96-well plate followed by the rapid addition of the neutrophil elastase substrate methoxy-succ-Ala-Ala-Pro-Val-pNA (1 mM). Hydrolysis of the latter was monitored spectrophotometrically at 405 nm. **D)** IC_{50} plots showing inhibition of plasmin by various concentrations of citrullinated and uncitrullinated R376K antiplasmin. A fixed concentration of plasmin (50 nM) was mixed with various concentrations of citrullinated or uncitrullinated R376K antiplasmin (0, 50, 100, 250, 500, 1000, 2500 and 5000 nM) and incubated for 30 min at room temperature. The reaction mixtures were placed in a 96-well plate followed by the rapid addition of the plasmin substrate D-Val-Leu-Lys-pNA (1 mM). Hydrolysis of the latter was monitored spectrophotometrically at 405 nm.

Table S1 Activity of uncitrullinated and citrullinated NNMT, related to Figure 4 and S3.

Table S1: Activity of uncitrullinated and citrullinated NNMT.				
Quinoline varied				
Uncitrullinated k_{cat}/K_m ($M^{-1}s^{-1}$)	200 ± 30	300 ± 30	170 ± 20	140 ± 10
Citrullinated by: k_{cat}/K_m ($M^{-1}s^{-1}$)	PAD1 3 ± 1	PAD2 2 ± 1	PAD3 100 ± 10	PAD4 100 ± 10
Fold change (Cit/Uncit)	0.015	0.006	0.580	0.700
SAM varied				
Uncitrullinated k_{cat}/K_m ($M^{-1}s^{-1}$)	300 ± 30	300 ± 30	240 ± 50	300 ± 30
Citrullinated by: k_{cat}/K_m ($M^{-1}s^{-1}$)	PAD1 1 ± 1	PAD2 2 ± 1	PAD3 150 ± 10	PAD4 100 ± 20
Fold change (Cit/Uncit)	0.003	0.006	0.620	0.330

Table S2 Activity of uncitrullinated and citrullinated PKM2, related to Figure 4 and S4.

Table S2: Activity of uncitrullinated and citrullinated PKM2.

PEP varied				
Uncitrullinated				
k_{cat}/K_m ($M^{-1}s^{-1}$)	5.6×10^2	5.1×10^2	5.8×10^2	4.4×10^2
Citrullinated by:	PAD1	PAD2	PAD3	PAD4
k_{cat}/K_m ($M^{-1}s^{-1}$)	2.8×10^2	7.6×10^2	1.0×10^3	6.1×10^2
Fold change (Cit/Uncit)	0.5	1.5	1.7	1.4
ADP varied				
Uncitrullinated				
k_{cat}/K_m ($M^{-1}s^{-1}$)	2.8×10^3	4.5×10^3	2.7×10^3	3.7×10^3
Citrullinated by:	PAD1	PAD2	PAD3	PAD4
k_{cat}/K_m ($M^{-1}s^{-1}$)	5.7×10^3	8.5×10^3	4.3×10^3	7.1×10^3
Fold change (Cit/Uncit)	2.0	1.9	1.6	1.9
PEP varied in the presence of 1 mM FBP				
Uncitrullinated				
k_{cat}/K_m ($M^{-1}s^{-1}$)	1.2×10^4	2.3×10^4	1.5×10^4	1.5×10^4
Citrullinated by:	PAD1	PAD2	PAD3	PAD4
k_{cat}/K_m ($M^{-1}s^{-1}$)	2.0×10^4	3.5×10^4	4.1×10^4	4.1×10^4
Fold change (Cit/Uncit)	1.7	1.5	2.7	2.7

Table S3 Activity of uncitrullinated and citrullinated antiplasmin, C1 inhibitor, tissue plasminogen activator inhibitor (t-PAI) and antitrypsin, related to Figure 4 and S5.

Table S3: IC_{50} values for uncitrullinated and citrullinated serpins.

Antiplasmin				
Uncitrullinated				
IC_{50} (nM)	16.1 ± 1.4	22.9 ± 1.6	19.1 ± 1.4	16.1 ± 1.4
Citrullinated by:	PAD1	PAD2	PAD3	PAD4
IC_{50} (nM)	>1000	>1000	39.5 ± 1.2	>1000
Fold change (Cit/Uncit)	>62.5	>43.7	2.00	>36.6
C1 Inhibitor				
Uncitrullinated				
IC_{50} (nM)	76.3 ± 3.1	53.7 ± 2.9	65.5 ± 3.7	87.1 ± 5.9
Citrullinated by:	PAD1	PAD2	PAD3	PAD4
IC_{50} (nM)	>2000	>2000	337 ± 25	>2000
Fold change (Cit/Uncit)	>26.5	>37.7	5.20	>23.0
Tissue Plasminogen Activator Inhibitor (t-PAI)				
Uncitrullinated				
IC_{50} (nM)	42.2 ± 7.2	37.5 ± 4.4	49.2 ± 3.9	49.7 ± 3.0
Citrullinated by:	PAD1	PAD2	PAD3	PAD4
IC_{50} (nM)	609 ± 21	421 ± 29	327 ± 20	95.6 ± 2.5
Fold change (Cit/Uncit)	14.5	11.4	6.70	1.90

Antitrypsin				
Uncitrullinated				
IC_{50} (nM)	90.7 ± 7.2	88.5 ± 9.2	75.2 ± 7.7	74.7 ± 3.0
Cit by PAD isoforms	PAD1	PAD2	PAD3	PAD4
IC_{50} (nM)	130 ± 10	96.1 ± 8.1	80.4 ± 4.5	77.4 ± 6.5
Fold change				
(Cit/Uncit)	1.4	1.1	1.0	1.1
R376K antiplasmin				
Uncitrullinated	400 ± 10	310 ± 10	375 ± 10	
IC_{50} (nM)				
Citrullinated by:	PAD1	PAD2	PAD4	
IC_{50} (nM)	580 ± 10	470 ± 15	420 ± 10	
Fold change	1.4	1.5	1.1	
(Cit/Uncit)				

Table S4 Activity of uncitrullinated and citrullinated antithrombin in absence and presence of heparin, related to Figure 5.

Table S4: IC_{50} values for uncitrullinated and citrullinated antithrombin

Antithrombin				
Uncitrullinated				
IC_{50} (nM)	100 ± 4	94.9 ± 1.6	120 ± 14	98.5 ± 1.9
Cit by PAD isoforms	PAD1	PAD2	PAD3	PAD4
IC_{50} (nM)	435 ± 15	93.1 ± 5.0	125 ± 10	130 ± 10
Fold change (Cit/Uncit)	4.3	1.0	2.4	2.3
Antithrombin in the presence of 1 mM heparin				
Uncitrullinated				
IC_{50} (nM)	42.4 ± 4.3	28.5 ± 3.4	34.9 ± 2.4	34.7 ± 2.0
Cit by PAD isoforms	PAD1	PAD2	PAD3	PAD4
IC_{50} (nM)	>2000	>2000	260 ± 30	455 ± 10
Fold change (Cit/Uncit)	>47.2	>70.3	7.30	13.1