Supplementary Material

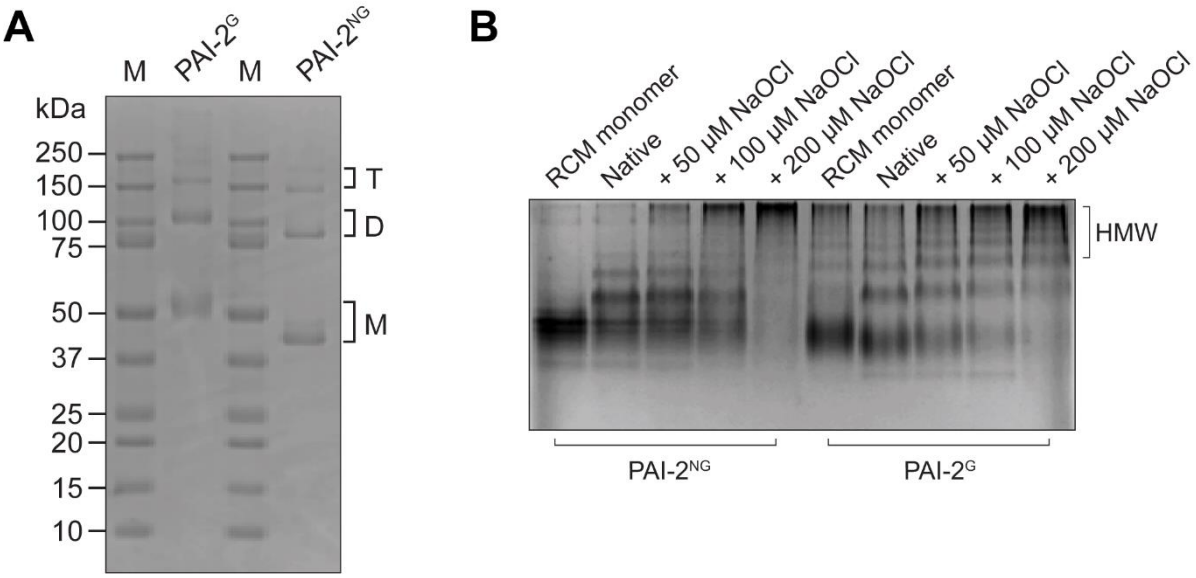


Figure S1. SDS-PAGE and native PAGE analyses of PAI-2^{G} and PAI-2^{NG}. (A) PAI-2^{G} and PAI-2^{NG} were separated by non-reducing SDS-PAGE using a 4–12% Bis-Tris gel. Protein molecular weight standards (lane M) are included for comparison, with sizes as indicated in kDa. Positions of PAI-2^{G} and PAI-2^{NG} monomers (M), dimers (D) and trimers (T) are marked. (B) PAI-2^{G} or PAI-2^{NG} pre-treated with 0–200 µM NaOCl were separated by native PAGE using an 8% Tris-glycine gel. A reduced, S-carboxymethylated sample (RCM monomer) is included as a standard to define the migratory position of the monomer for each PAI-2^{G} and PAI-2^{NG}. High molecular weight oligomers and complexes (HMW) are marked. Note the lower isoelectric point of PAI-2^{G} (pI=4.4), versus PAI-2^{NG} (pI=5), which affects migration under native PAGE conditions (approximate running pH=8).

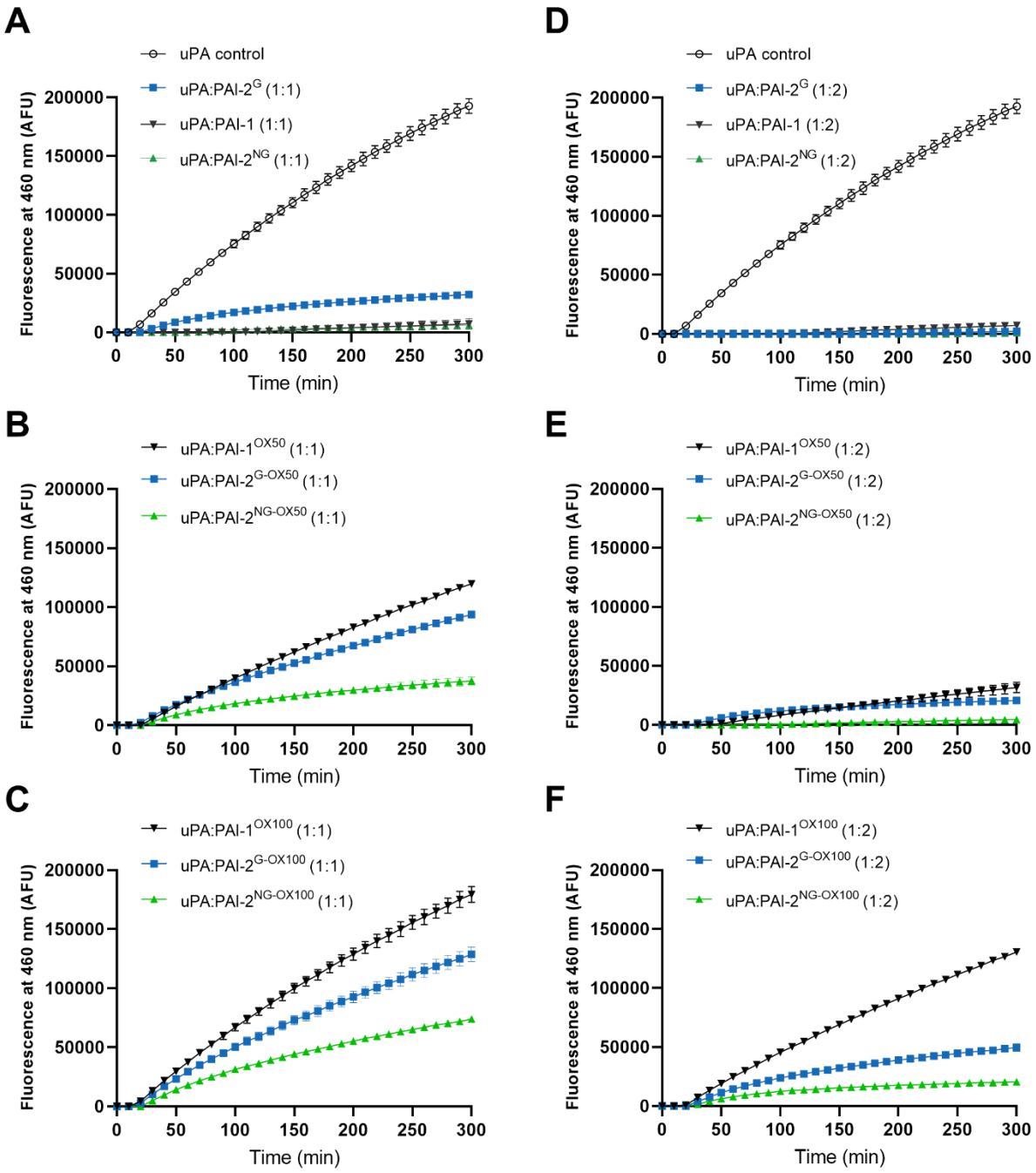


Figure S2. Effect of hypochlorite treatment on uPA inhibitory activity of PAIs at different molar ratios of PAI to uPA. Representative kinetic uPA activity plots showing the inhibitory effect of (A) Native PAIs, (B) 50 μM NaOCl pre-treated (OX50) PAIs and (C) 100 μM NaOCl pre-treated (OX100) PAIs at a 1:1 molar ratio of uPA:PAI; or (D) Native PAIs, (E) OX50 PAIs and (F) 100OX PAIs at a 1:2 molar ratio of uPA:PAI. Data points show the mean fluorescence ± SD for triplicate determinations at each time point. The fluorescence of uPA control (no PAIs) was included as a control. The average rate of change in fluorescence is presented in Figure 2 of the main article.

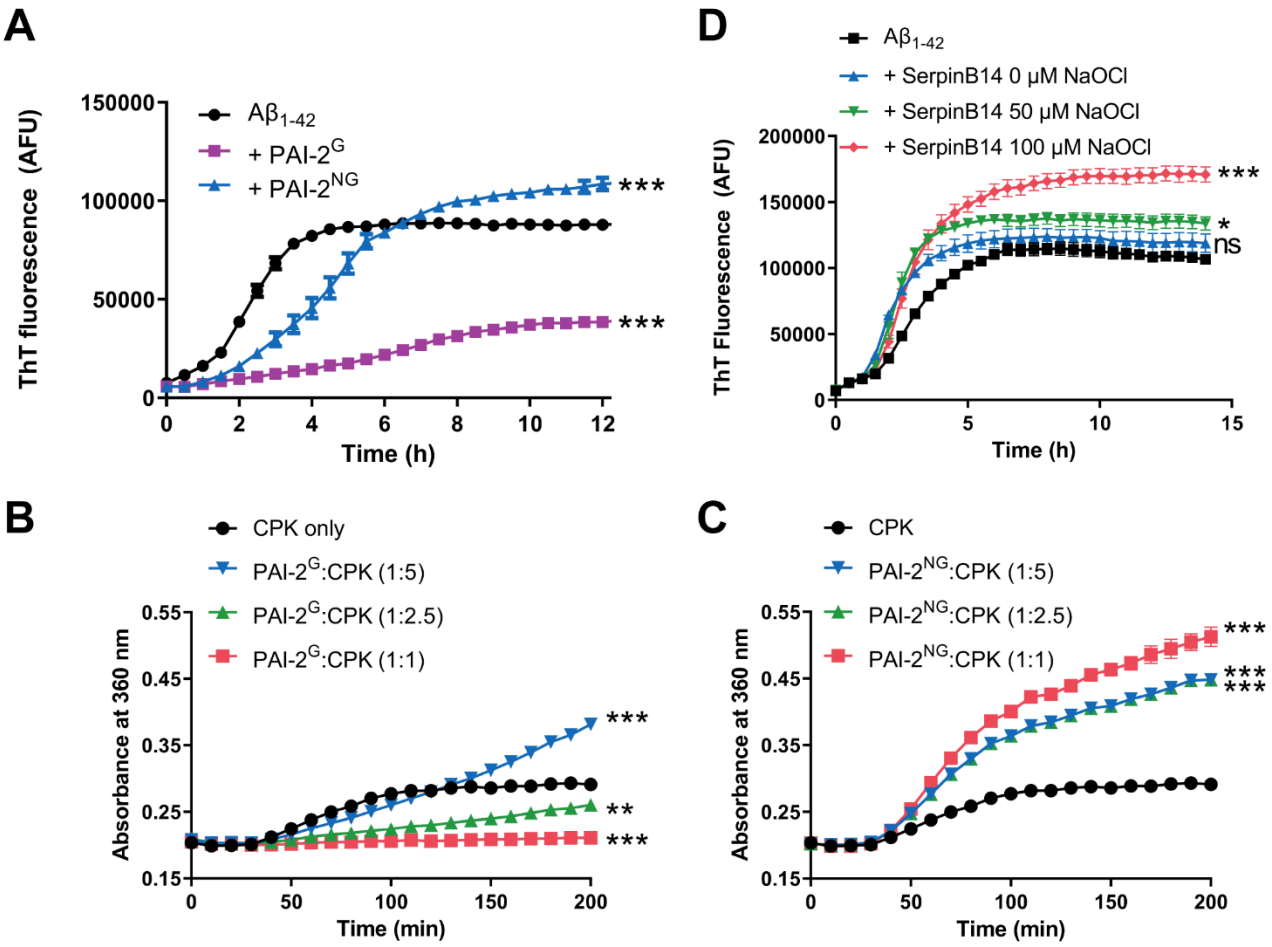
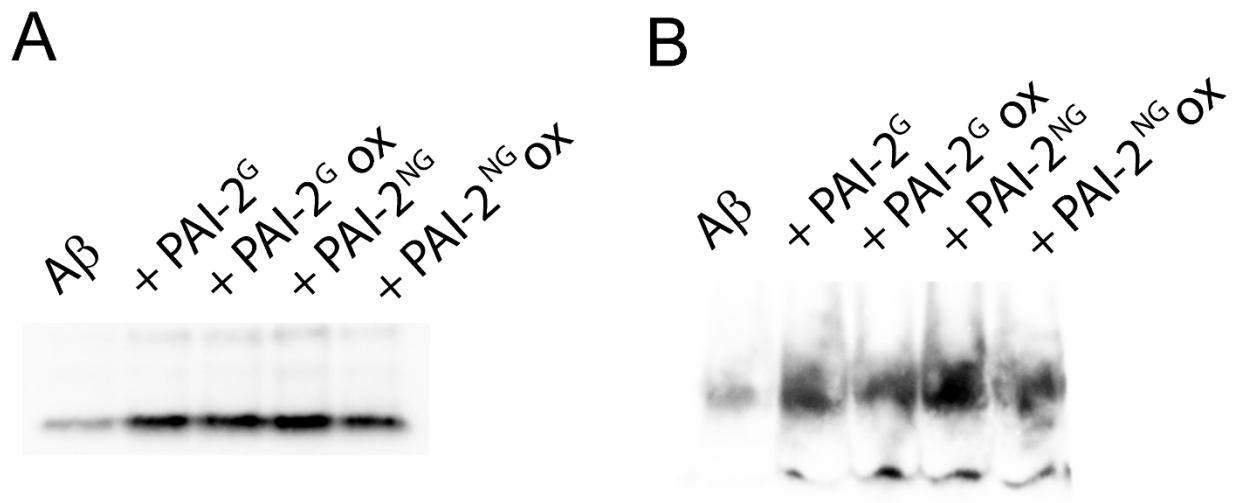


Figure S3. Effect of PAI-2^{G} and PAI-2^{NG} on the aggregation of Aβ$_{1–42}$ and creatine phosphokinase, and the effect of SerpinB14 on the aggregation of Aβ$_{1–42}$ following hypochlorite treatment. (A) Aβ$_{1–42}$ (3 µM) was incubated with 25 µM ThT in PBS at 28°C ± 20 µg/mL of PAI-2^{G} or PAI-2^{NG}. (B and C) Creatine phosphokinase (CPK; 6.5 µM) was incubated at 43°C with shaking ± (B) PAI-2^{G} or (C) PAI-2^{NG} at molar ratios of 1:1. 1:2.5 or 1:5 (PAI-2:CPK). Turbidity indicative of CPK aggregation was monitored by measuring absorbance at 360 nm of the solution. (D) Aβ$_{1–42}$ was incubated as before except ± SerpinB14 that had been pre-treated with 0–100 µM hypochlorite at a 1:10 molar ratio (SerpinB14:Aβ$_{1–42}$). All data are means ± SEM (n=3) and are representative of three independent experiments. One-way ANOVA with post-hoc Tukey's HSD was conducted on the assay endpoints to identify non-significant (ns) and significant differences (*** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$). Statistical results are shown based on comparisons with Aβ$_{1–42}$ or CPK only.



	Soluble Aβ	P value
+ PAI-2^{G}	3.07 ± 0.72	< 0.05
+ PAI-2^{G} ox	3.16 ± 1.21	< 0.05
+ PAI-2^{NG}	3.54 ± 1.23	< 0.01
+ PAI-2^{NG} ox	3.01 ± 0.43	< 0.05

Figure S4. Effect of PAI-2 on the solubility of Aβ1-42 as assessed by (A) native Western Blot analysis and (B) streptavadin-biotin pull down assay. PAI-2^{G} and PAI-2^{NG} were pre-treated with 200 μM NaOCl in PBS and then extensively dialysed to generate PAI-2^{G}ox and PAI-2^{NG}ox, respectively (see Methods 2.3). All samples of PAI-2 were then biotinylated following the manufacturer's instructions (Sigma-Aldrich). $A\beta_{1-42}$ (5 μM) was incubated with 25 μM ThT in PBS at 30°C ± 1 μM PAI-2^{G}, PAI-2^{NG}, PAI-2^{G}ox or PAI-2^{NG}ox overnight. Following centrifugation, the soluble protein fraction was recovered and subjected to native Western blot analysis for $A\beta_{1-42}$ detected using a monoclonal anti-Aβ1-42 antibody (W02). Panel (A) shows a representative Western blot image of $A\beta_{1-42}$. Densitometry analysis was performed and the mean soluble Aβ1-42 relative to the control sample containing Aβ1-42 alone is reported (n = 3 ± SD). All forms of PAI-2 increased the solubility of Aβ1-42, compared to Aβ1-42 alone, as determined using one-way ANOVA and post-hoc Tukey's HSD analysis. However, there was no difference between the different PAI-2 forms under the conditions used. Panel (B) shows a Western Blot of $A\beta_{1-42}$ pulled down with biotinylated PAI-2 recovered using Dynabeads™ MyOne™ Streptavidin C1 according to the manufacturer's instructions (Invitrogen). Washing was performed using PBS, pH 7.4 containing 0.01% [w/v] BSA to minimise the non-specific binding of Aβ1-42 to the beads. The recovered protein was separated by reducing SDS-PAGE using a 4–12% Bis-Tris gel prior to Western blot analysis.

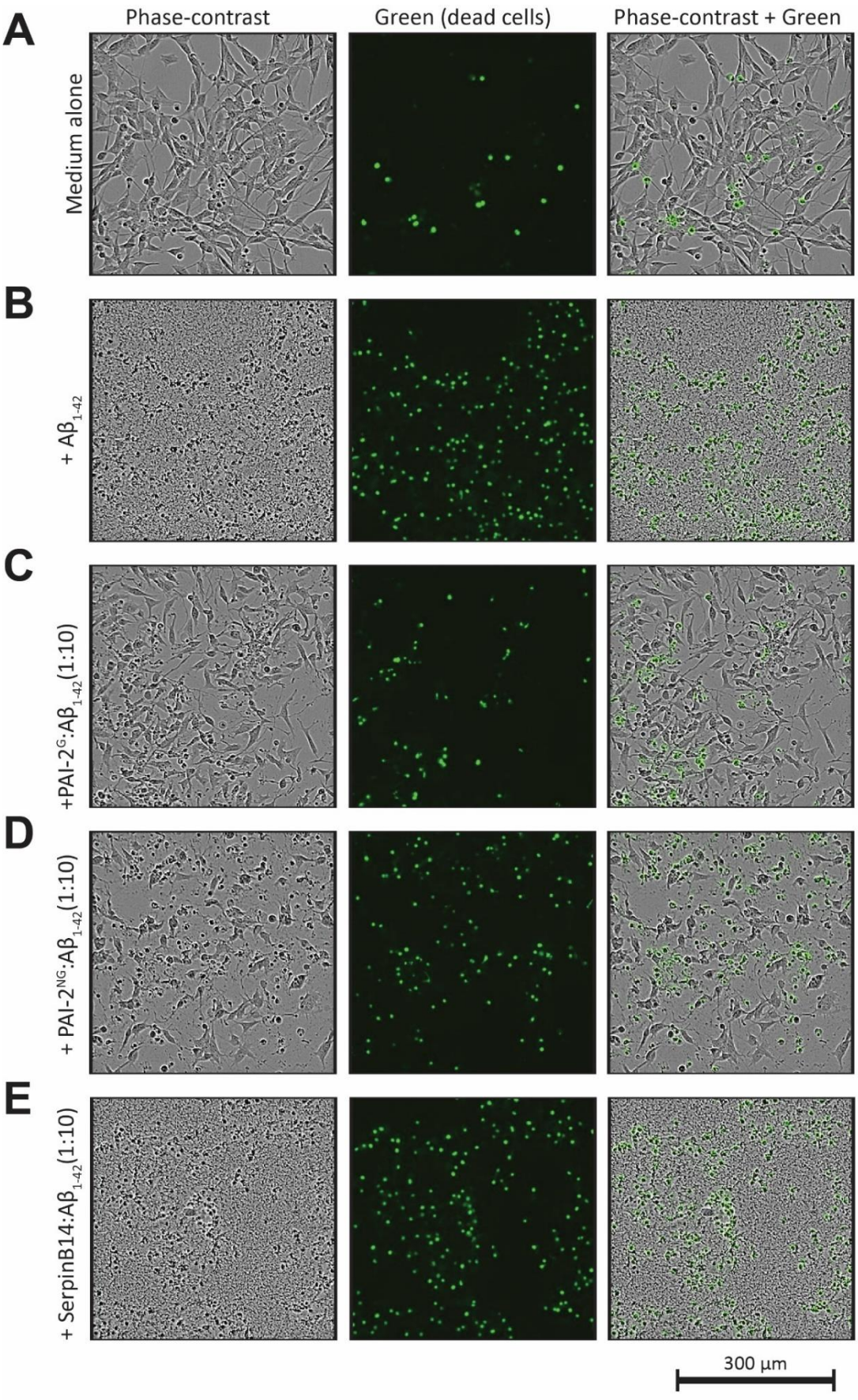


Figure S5. Representative photomicrographs showing the effect of PAI-2 on Aβ1–42-induced cytotoxicity and morphology of SH-SY5Y cells. Images taken at 48 h timepoint from a cytotoxicity experiment as described in Figure 3F for (*A*) medium alone control, (*B*) Aβ1-42,(*C*) PAI-2^{G}:Aβ1-42 (1:10 molar ratio), (*D*) PAI-2^{NG}:Aβ1-42 (1:10 molar ratio) and (*E*) SERPINB14:Aβ1-42 (1:10 molar ratio).

Table S1. Summary of demographic and clinical characteristics of the subjects whose placentas were analysed as part of this study, along with immunohistochemical scores.

Variable	Severe Preeclampsia n=15	Idiopathic Preterm Birth n=15	p value
Maternal age, years *	25 ± 9	29 ± 8	0.146
Race/ethnicity †			
Non-Hispanic White	7 (47)	8 (53)	
Non-Hispanic Black	6 (40)	6 (40)	0.381
Hispanic	2 (13)	0 (0)	
Other	0 (0)	1 (7)	
Nulliparity ‡	11 (73)	7 (47)	0.264
Systolic blood pressure *	169 ± 20	118 ± 13	<0.001
Diastolic blood pressure *	99 ± 10	67 ± 6	<0.001
HELLP manifestations ‡	4 (27)	0 (0)	0.100
Fetal growth restriction ‡	6 (40)	0 (0)	0.017
Neurological symptoms including headache ‡	7 (47)	0 (0)	0.006
Seizures ‡	1 (7)	0 (0)	1.000
Gestational age at delivery, weeks *	30 ± 2	30 ± 2	0.823
Medically indicated delivery for preeclampsia ‡	15 (100)	0 (0)	<0.001
PPROM ‡	6 (40)	0 (0)	0.017
Cesarean delivery ‡	13 (87)	8 (53)	
Birthweight, grams *	1,269 ± 458	1,597 ± 408	0.048
Newborn sex ‡			
Female	8 (53)	10 (67)	0.710
Male	7 (47)	5 (33)	
1 min Apgar §	6 [5 – 7]	7 [5 – 8]	0.344
5 min Apgar §	8 [8 – 9]	9 [8 – 9]	0.162
PAI-2 staining score §	1 [0-4]	2 [1-5]	0.246
PAI-2-staining score distribution †			
Absent or weak (0-1)	8 (53)	4 (27)	0.189
Intermediate (2-3)	2 (13)	6 (40)	
Strong (4-5)	5 (33)	5 (33)	

* Data presented as mean ± standard deviation and compared using Student t-test

† Data presented as n (%) and analysed by Chi-square test

‡ Data presented as n (%) and analysed by Fisher's exact test

§ Data presented as median [interquartile range] and analysed by Mann-Whitney test

Table S2: Summary of ThT fluorescence curves generated by incubating $A\beta_{1-42}$ ± PAI-2 as shown in Fig. 3A and B.

	Initial lag phase duration (h)	**Maximum rate of aggregation (ΔThT AFU/h) n= 3 ± SD**	**Maximum THT fluorescence (AFU) n= 3 ± SD**
$A\beta_{1-42}$	Negligible	36,037.33 ± 4,353.51	63,225.80 ± 2,005.26
PAI-2^{G}: $A\beta_{1-42}$ (1:10)	2	**7,483.70 ± 940.81 ***	63,225.84 ± 5,034.73 *
PAI-2^{G}: $A\beta_{1-42}$ (1:7.5)	2.4	**3,982.13 ± 1,432.33**	44,299.17 ± 16,177.31 **
PAI-2^{G}: $A\beta_{1-42}$ (1:5)	5	**2,819.45 ± 436.27 ****	**27,566.84 ± 4,240.52 ***
PAI-2^{G}: $A\beta_{1-42}$ (1:2.5)	5.5	**124.11 ± 67.66 ****	**9,188.50 ± 2,158.28 ***
PAI-2^{NG}: $A\beta_{1-42}$ (1:10)	0.5	23,868.11 ± 7,139.39	131,560.20 ± 27,517.30
PAI-2^{NG}: $A\beta_{1-42}$ (1:7.5)	1.5	**17,336.33 ± 8,006.07**	117,279.52 ± 43,669.01
PAI-2^{NG}: $A\beta_{1-42}$ (1:5)	2.5	**5,074.67 ± 701.37**	45,759.50 ± 5,459.84
PAI-2^{NG}: $A\beta_{1-42}$ (1:2.5)	4	**2,674.57 ± 509.52**	**20,800.50 ± 1,919.77**

Bold font denotes reduced compared to $A\beta_{1-42}$ alone; Tukey HSD $p < 0.05$. * or ** Denotes reduced compared to the corresponding sample containing PAI-2^{NG} at the same molar ratio $p < 0.05$ and $p < 0.01$, respectively; Student's t-test

Table S3: Summary of ThT fluorescence curves generated by incubating $A\beta_{1-42}$ ± PAI-2 pre-treated with 0-200 μM NaOCl as shown in Fig. 3C and D.

	Initial lag phase duration (h)	**Maximum rate of aggregation (ΔThT AFU/h) n= 3 ± SD**	**Maximum THT fluorescence (AFU) n= 3 ± SD**
$A\beta_{1-42}$	Negligible	20,207.33 ± 993.57	87,102.66 ± 1,245.23
+ PAI-2^{G} 0 μM NaOCl	2	**4,879.87 ± 1,307.28**	**39,113.67 ± 1,079.35**
+ PAI-2^{G} 50 μM NaOCl	2	**2,961.39 ± 616.85**	**30,615.11 ± 5,361.45**
+ PAI-2^{G} 100 μM NaOCl	2	**2,763.52 ± 769.47**	**33,275.34 ± 2,473.78**
+ PAI-2^{G} 200 μM NaOCl	2	**4,079.08 ± 220.41**	**40,555.00 ± 2,351.17**
+ PAI-2^{NG} 0 μM NaOCl	1.5	**16,107.78 ± 377.20**	109,826.37 ± 6,464.30
+ PAI-2^{NG} 50 μM NaOCl	2	**4,347.00 ± 966.94 ***	**34,817.33 ± 6,261.53 ***
+ PAI-2^{NG} 100 μM NaOCl	2	**4,888.67 ± 279.21 ***	**41,483.67 ± 1,749.58 ***
+ PAI-2^{NG} 200 μM NaOCl	2	**3,457.89 ± 329.73 ***	**31,049.67 ± 3,549.68 ***

Bold font denotes reduced compared to $A\beta_{1-42}$ alone; Tukey HSD $p < 0.05$. * Denotes reduced compared to the corresponding sample containing PAI-2^{NG} 0 μM NaOCl; $p < 0.01$, Student's t-test