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Supplementary Information for

Human pregnancy zone protein stabilizes misfolded proteins including preeclampsia- and Alzheimer's-associated amyloid beta peptide

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Supplementary Information Text

PZP co-localises with Aβ in extravillous trophoblasts (EVT).
This cluster of cells captured in preeclamptic placenta (Fig. S4A) indicates that PZP and Aβ co-localize in the EVT cytoplasm (Fig. S4B). An unexpected finding clearly visible using confocal microscopy was the nuclear localization of PZP into trophoblasts with perturbed morphology. In some EVT nuclei the blue DAPI fluorescence was entirely replaced by that of PZP whereas in other cells the two proteins coexisted as illustrated by the merged purple staining (Fig. S4 C&D).

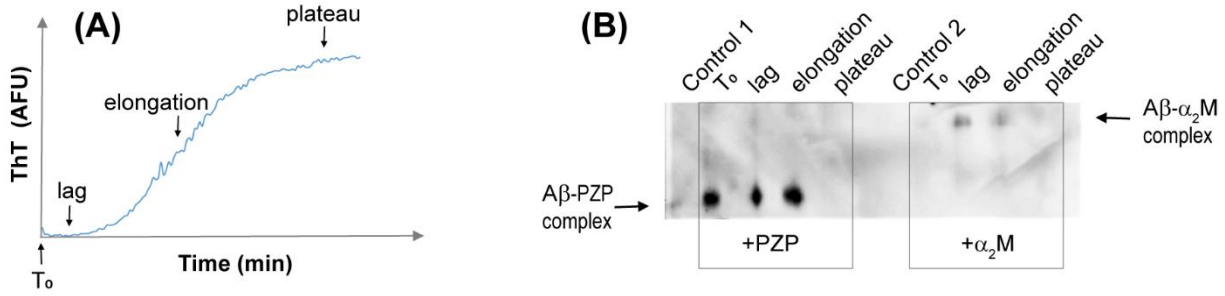


Fig. S1. Image of a native Western blot showing the co-migration of monomeric and aggregated $A\beta_{1\text{-}42}$ with native PZP or α_2M. (A) $A\beta_{1\text{-}42}$ (5 µM) was induced to aggregate using the standard protocol for ThT assay involving incubation at 32°C with constant shaking and aliquots were removed as indicated at T_0 and during the lag, elongation and plateau phase. **(B)** $A\beta_{1\text{-}42}$ as described in (A) was subsequently incubated with native PZP or α_2M at a 1:10 molar ratio (αM-to-$A\beta_{1\text{–}42}$) for 30 min at ambient room temperature. The migration of $A\beta_{1\text{-}42}$ was probed for by native Western blot analysis. $A\beta_{1\text{-}42}$ incubated in the absence of αM (control 1- taken at T_0 and control 2 taken at elongation, as in (A)) migrate to the end of the gel and are not visible. $A\beta_{1\text{-}42}$ retained at the approximate positions of the native PZP dimer and the native α_2M tetramer are indicated.

Fig. S2. **Native gel image of recombinant ECAM.** Image of an Instant Blue stained 3-8% Tris acetate native gel showing the migration of recombinant purified ECAM and reduced α_2M.

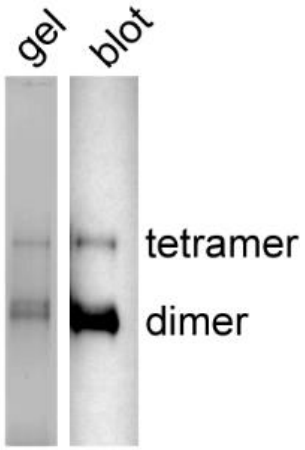


Fig. S3. **Preferential binding of PZP antibody to dimeric PZP.** PZP-protease complexes were generated by pre-incubation of PZP with chymotrypsin at a 2:1 molar ratio of PZP-to-chymotrypsin for 30 min at 37°C. Equivalent amounts of the preparation were then analysed by native gel electrophoresis or native Western blot, with probing of the blot accomplished using PZP antibody.

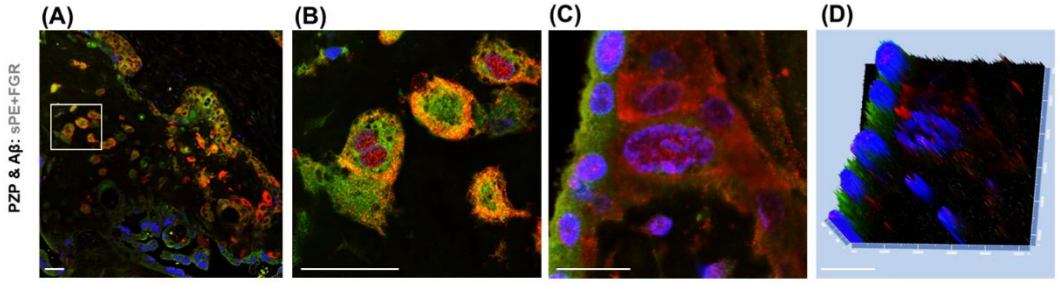
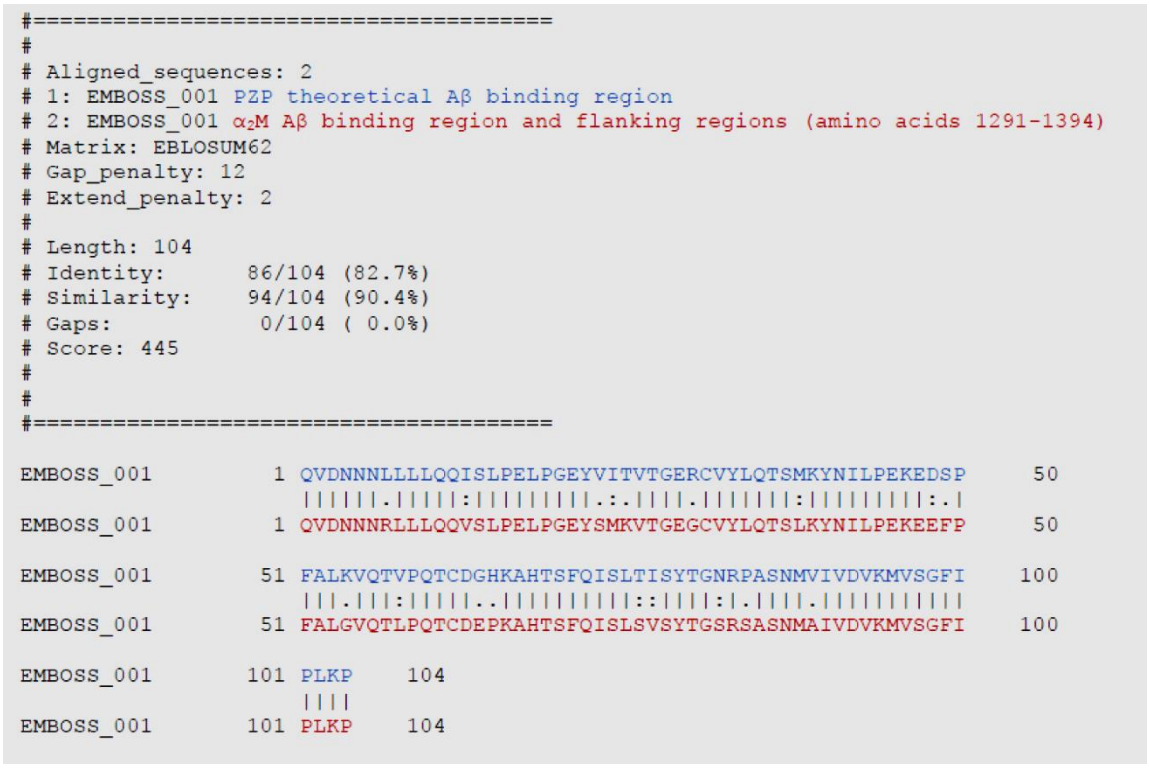


Fig. S4 Intracellular location of PZP and Aβ in SPE placenta (A) Extravillous trophoblasts co-staining for PZP (red) and Aβ (green) in various patterns. **(B)** The boxed region in A shown at higher magnification. **(C)** Higher magnification image of the region demarcated by the box in panel A showing co-localization of PZP with DAPI (blue) in some trophoblast nuclei visualized as purple fluorescence persistent after 3D z-stack reconstruction **(D)**. Scale bar represents 50 µm.



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#=======================================
#
# Aligned_sequences: 2
# 1: EMBOSS_001 PZP theoretical Aβ binding region
# 2: EMBOSS_001 α2M Aβ binding region and flanking regions (amino acids 1291-1394)
# Matrix: EBLOSUM62
# Gap_penalty: 12
# Extend_penalty: 2
#
# Length: 104
# Identity:      86/104 (82.7%)
# Similarity:    94/104 (90.4%)
# Gaps:           0/104 ( 0.0%)
# Score: 445
#
#
#=======================================

EMBOSS_001         1 QVDNNNLLLLQQISLPELPGEYVITVTGERCVYLQTSMKYNILPEKEDSP     50
                     ||||||.|||||:|||||||||.:.||||.|||||||:|||||||||:.|
EMBOSS_001         1 QVDNNNRLLLQQVSLPELPGEYSMKVTGEGCVYLQTSLKYNILPEKEEFP     50

EMBOSS_001        51 FALKVQTVPQTCDGHKAHTSFQISLTISYTGNRPASNMVIVDVKMVSGFI    100
                     |||.|||:|||||..||||||||||::||||:|.||||.||||||||||
EMBOSS_001        51 FALGVQTLPQTCDEPKAHTSFQISLSVSYTGSRSASNMAIVDVKMVSGFI    100

EMBOSS_001       101 PLKP    104
                     ||||
EMBOSS_001       101 PLKP    104
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Fig. S5. **Sequence alignment of the cryptic binding site on α_2M for monomeric Aβ and corresponding region of PZP.** Pairwise sequence alignment of the binding site for monomeric Aβ on α_2M as described by (1) and the corresponding region of PZP as performed using EMBOSS stretcher (2).

Table S1: Clinical characteristics of mothers and newborns that contributed placenta samples

Variables	Idiopathic preterm birth (iPTB, n=8)	Severe preeclampsia (sPE, n=11)	P value
Maternal age, *years* *	25 ± 7	25 ± 9	0.953
Nulliparity †	4 (50)	8 (73)	0.377
Non-Caucasian race †	4 (50)	4 (36)	0.658
Systolic blood pressure, *mmHg* *	120 ± 10	164 ± 17	<0.001
Diastolic blood pressure, *mmHg* *	65 ± 5	102 ± 12	<0.001
Dipstick proteinuria ‡	0 [0-0]	3 [2-3]	<0.001
24 h proteinuria, grams *	NA	2.2 ± 1.8	NA
Fetal growth restriction †	0 (0)	7 (64)	0.013
Prelabor rupture of membranes †	3 (38)	0 (0)	0.058
Antenatal steroid administration †	8 (100)	9 (82)	0.485
Magnesium sulfate administration †	5 (63)	11 (100)	0.058
Gestational age at birth, *weeks* *	30 ± 2	30 ± 3	0.453
Cesarean delivery †	4 (50)	11 (100)	0.018
Newborn male sex †	2 (25)	3 (27)	1.000
Birth weight *	1633 ± 351	1076 ± 394	0.005
Apgar 1 min ‡	8 [7-9]	6 [4-7]	0.092
Apgar 5 min ‡	9 [8-9]	8 [8-9]	0.195

* Data presented as mean ± standard deviation and analyzed by Student's t-test
† Data presented as n (%) and analyzed by Fisher's exact test
‡ Data presented as median [interquartile range] and analyzed by Mann-Whitney test

As expected, mothers in the sPE group have higher blood pressures and proteinuria levels and a higher frequency of fetal growth restriction. Their newborns had a lower birthweight and were more often delivered through Cesarean. There were no statistically significant differences in maternal age, nulliparity, antenatal steroid and magnesium sulfate exposure, gestational age at birth, newborn sex distribution and Apgar scores.

References

1. Mettenburg JM, Webb DJ, & Gonias SL (2002) Distinct binding sites in the structure of alpha 2-macroglobulin mediate the interaction with beta-amyloid peptide and growth factors. *The Journal of biological chemistry* 277(15):13338-13345.
2. Myers EW & Miller W (1988) Optimal alignments in linear space. *Comput Appl Biosci* 4(1):11-17.