Supporting Information for

Biochemical and structural analyses suggest that plasminogen activators coevolved with their cognate protein substrates and inhibitors

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A) Full-length plasminogen

B) Sequence of hμPlg

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MASMTGGQQMGRGSPGRVAAPSFDCGKPQVEPKKCPGRVVGGCVAHPHSWPWQVSLRTRFGM
HFCGGTLISPEWVLTAAHCLEKSPRPSSYKVILGAHQEVNLEPHVQEIEVSRLFLEPTRKDI
ALLKLSSPAVITDKVIPACLPSPNYVVADRTECFVTGWGETQGTFGAGLLKEAQLPVIENKV
CNRYEFLNGRVQSTELCAGHLAGGTDSCQGDSGGPLVCFEKDKYILQGVTSWGLGCARPNKP
GVYVRVSRFVTWIEGVMRNN
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C) Sequence of zfμplg

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MASMTGGQQMGRGSPGRVESLKCGQPATKPKRCFGRIVGGCVSKPHSWPWQISLRTRGKIHF
CGGTLIDPQWVVTAAHCLERSDSPSAYKIMLGIHTERATESSKQERDVTKIIKGPAGTDIAL
LKLDRPALINDKVSPVCLPEKDYIVPSNTECYVTGWGETQDTGGEGYLKETGFPVIENKVCN
RPSFLNGRVKDHEMCAGNIEGGNDSCQGDSGGPLVCYAQNTFVLQGVTSWGLGCANAMKPGV
YTRVSKFVDWIERSIKEN
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Figure S1. Sequence of expressed wt huPlg and wt zfuPlg. A) shows the schematic of the plasminogen domain architecture. The domain nomenclature is: Pan-apple domain, Pap; kringles 1 to 5, K1 to K5 and serine protease domain, SPD. B) protein sequence of the produced human μPlg. C) protein sequence of the produced zebrafish μPlg. In both B) and C) the peptide facilitating expression in inclusion bodies is highlighted in gray, the linker connecting the SPD and K5 is underlined. The activation loop is located between the two Cys residues marked with boxes with the scissile bond shown with an arrow.

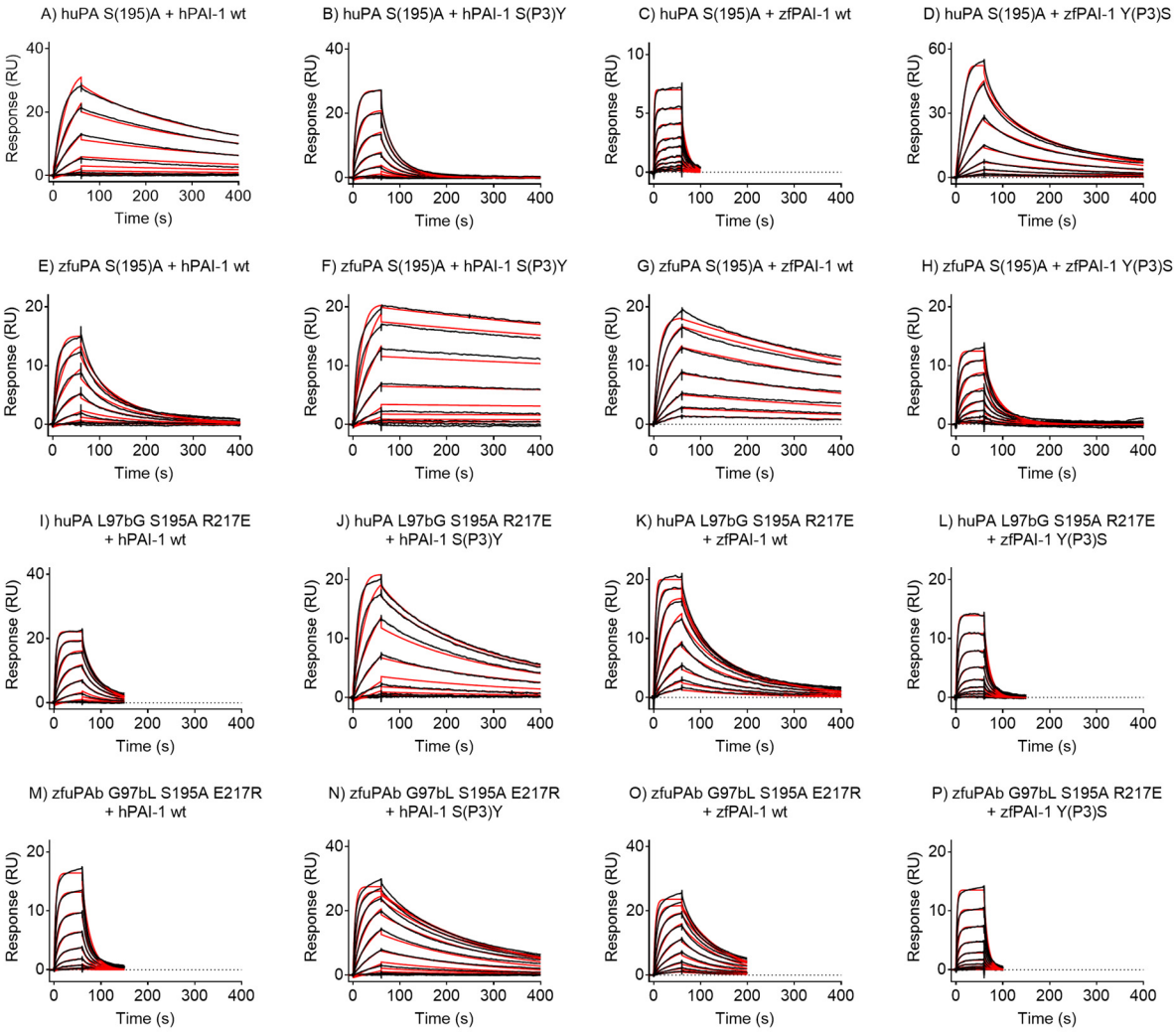

Figure S2. Sensograms representing binding of hPAI-1 and zfPAI-1 variants to huPA S195A (A-D), zfPAI-1 S195A (E-H), huPA L97bG S195A R217E (I-L) and zfuPA G97bL S195A E217R (M-P). Representative raw data of the concentration series is shown in black with the corresponding global fit to the 1:1 binding model given in red. X-axes is kept constant to enable the quick comparison of relative rate constant.

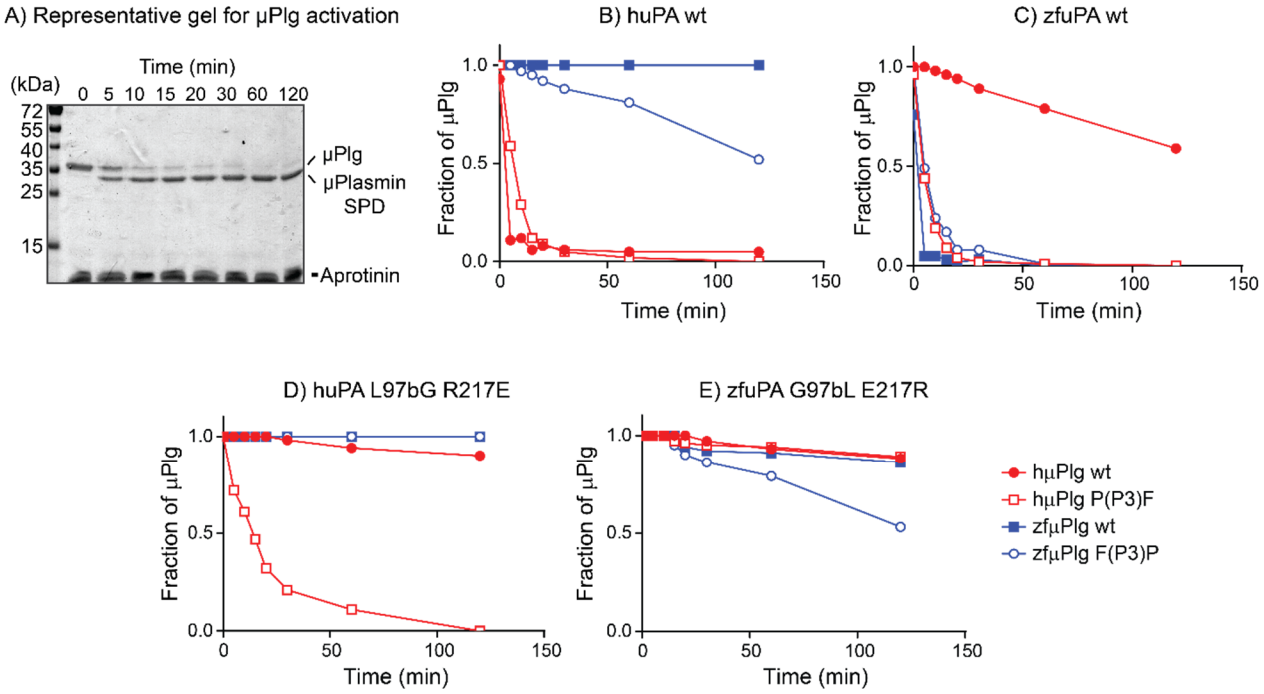

Figure S3. Plasminogen activation followed by SDS-PAGE. A) The representative gel of plasminogen activation. For each tested uPA variant: huPA wt (B); zfuPA wt (C); huPA L97bG R217E (D) and zfuPA G97bL E217R (E) the intensity of band referring to µPlg was measured and plotted against the time.