Supplementary Material

Vitronectin and its interaction with PAI-1 suggests a functional link to vascular changes in AMD pathobiology

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Supplementary Table S1. Primer sequences, target and application in expression cloning or real-time quantitative PCR.

| Target	Primer/probe name	Primer/probe Sequence (5'-3')	Application	Polymerase/ MasterMix
PAI-1	PAI-1-EcoRI-F	**F:**gaattcATGCAGATGTCTCCAGCCCTCA	Cloning of pEXPR-IBA103-PAI-1	GoTaq® DNA Polymerase (Promega Corporation, Madison, WI, USA)
	PAI-1-XhoIwostop-R	**R:**ctcgagGGGTTCCATCACTTGGCCCAT		
	PAI-1-seq1-F	**F:**acagacgcgatcttggtcca	Sequencing of pEXPR-IBA103-PAI-1	AmpliTaq® DNA Polymerase (BigDye Terminator v1.1 Cycle Sequencing Kit, Thermo Fisher)
	PAI-1-seq2-F	**F:**TATACTGAGTTCACCACGCCCG		
Expression vector (flanking cloning sites)	pEXPR-IBA3-F	**F:**GAGAACCCACTGCTTACTGGC		
	pEXPR-IBA3-R	**R:**TAGAAGGCACAGTCGAGG		
PAI-1	FH1_SERPINE1	**F:**GCTGCAGAAAGTGAAGATCG	Real-Time quantitative PCR	Takyon™ DNA Polymerase (Takyon™ Low ROX Probe 2X MasterMix dTTP blue; Eurogentec, Seraing, Liège, Belgium)
	RH1_SERPINE1	**R:**GTCCATGATGATCTCCTCGG		
	PH1_SERPINE1	**P:**(6FAM)GTGGCCTCCTCATCCAACAGCTGTC(OQA)		
HPRT1	FH1_HPRT1	**F:**CTTTGCTTTCCTTGGTCAGG		
	RH1_HPRT1	**R:**TCAAATCCAACAAAGTCTGGC		
	PH1_HPRT1	**P:**(6FAM)GCTTGCTGGTGAAAAGGACCCCACG(OQA)		

F:Forward; R: Reverse; P: Probe

Supplementary Table S2. Antibodies, their dilution, application, and origin.

Antibodies	Dilution	Application	Manufacturer (Country)
α-VTN (MAB2349)	1:5000 1:250	WB IF	R&D Systems, Inc., Minneapolis, MN, USA
α-PAI-1 (11907S)	1:1000 1:100	WB IF	Cell Signaling Technology, Danvers, MA, USA
α-ACTB (A5441)	1:10000	WB	Sigma-Aldrich, St. Louis, MO, USA
Goat Anti-Mouse IgG, Peroxidase conjugated	1:10000	WB	(Calbiochem) Merck Chemicals GmbH, Darmstadt, Germany
Goat Anti-Rabbit IgG, Peroxidase conjugated	1:10000	WB	(Calbiochem) Merck Chemicals GmbH, Darmstadt, Germany
Goat Anti-Mouse IgG, Alexa Fluor ® 594 conjugated	1:800	IF	ThermoFisher Scientific, Waltham, MA, USA
Goat Anti-Rabbit IgG, Alexa Fluor ® 488 conjugated	1:800	IF	ThermoFisher Scientific, Waltham, MA, USA

WB = Western Blot, IF = Immunofluorescence
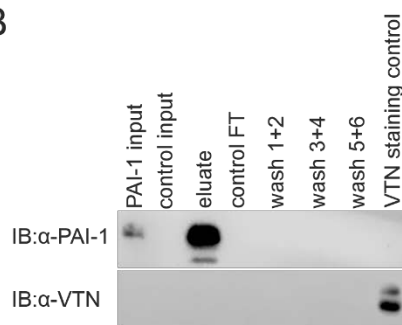
Supplementary Figure S1
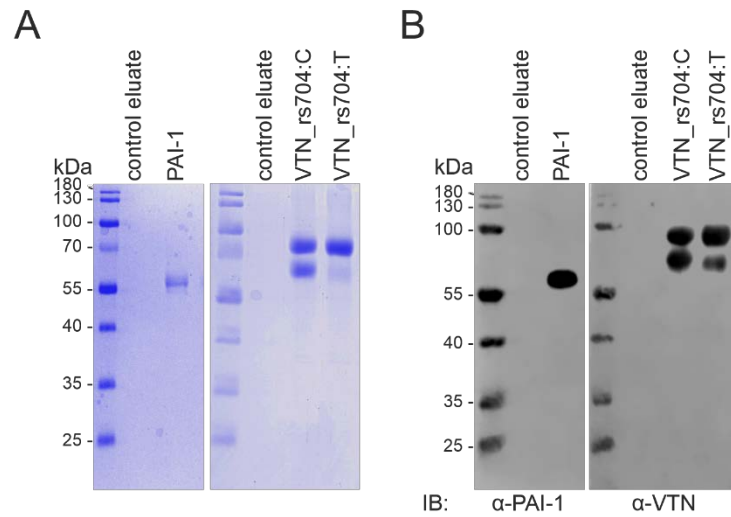
SUPPLEMENTARY FIGURE S1. Purification of Strep-tagged PAI-1, VTN_rs704:C and VTN_rs704:T. Purified recombinant proteins were subjected to SDS-PAGE and analysed *via* (A) Coomassie blue staining and (B) immunoblot (IB) analysis using antibodies against PAI-1 and vitronectin. Eluate obtained from cultivation medium of HEK293-EBNA transfected with empty pEXPR-IBA103 vector and subjected to the same purification procedure served as a control (control eluate).
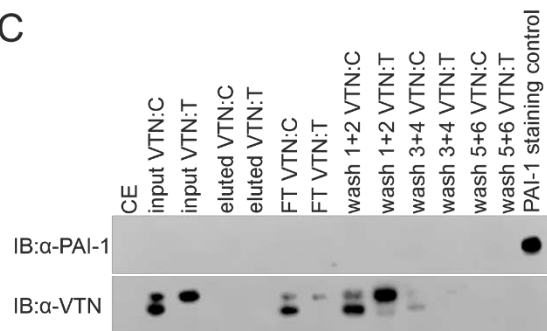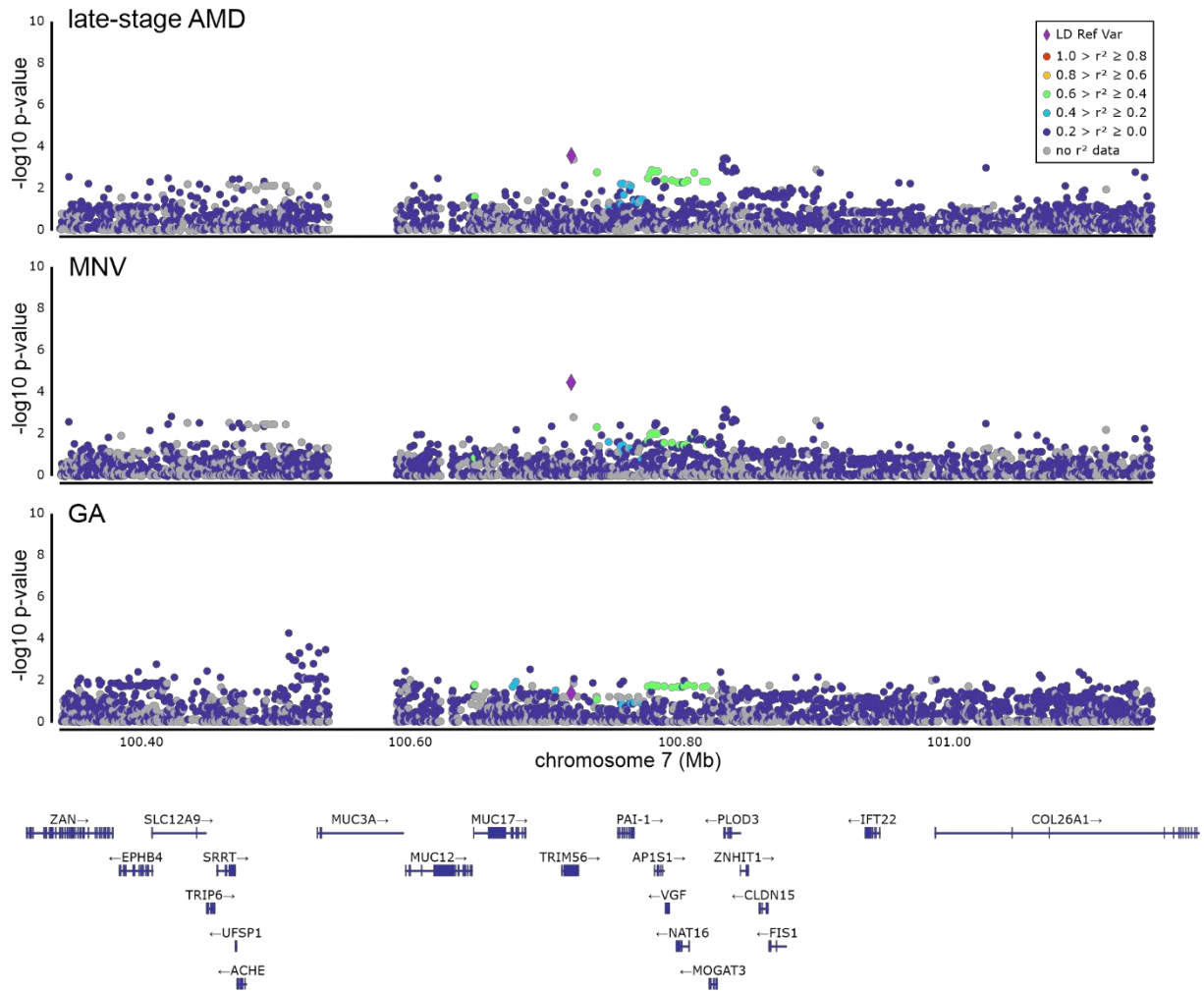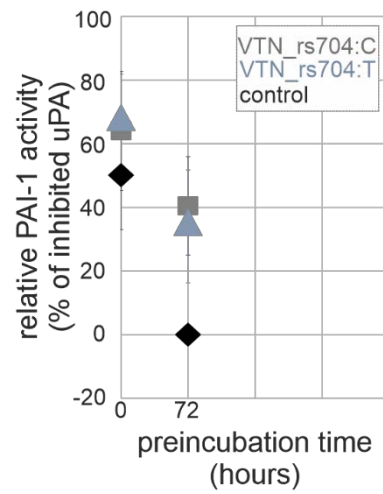
Supplementary Figure S2
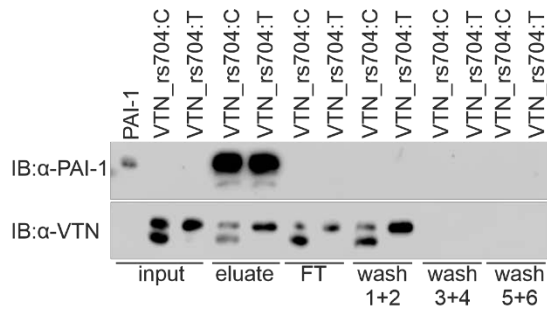
SUPPLEMENTARY FIGURE S2. Controls for the co-precipitation experiments. (A) Full-size western blots of Figure 2A, also revealing flow through (FT) and wash fractions (wash) prior to PAI-1 elution. (B) Vitronectin-free control input was loaded onto columns coupled with PAI-1 and subjected to the identical experimental procedure described for the vitronectin-containing input. After immunoblot (IB) analyses with antibodies against vitronectin, no staining was detected in the experimental fractions analysed. (C) Potential non-specific binding of vitronectin to the column material was tested by loading the vitronectin-containing input onto columns free of PAI-1 (coupled with empty control eluate; CE) and subjecting it to the same experimental procedure as described above. After IB analysis with antibodies against vitronectin and PAI-1, vitronectin was not detected in the elution fractions, but only in flow through and wash fractions, excluding non-specific binding of vitronectin to the column material.
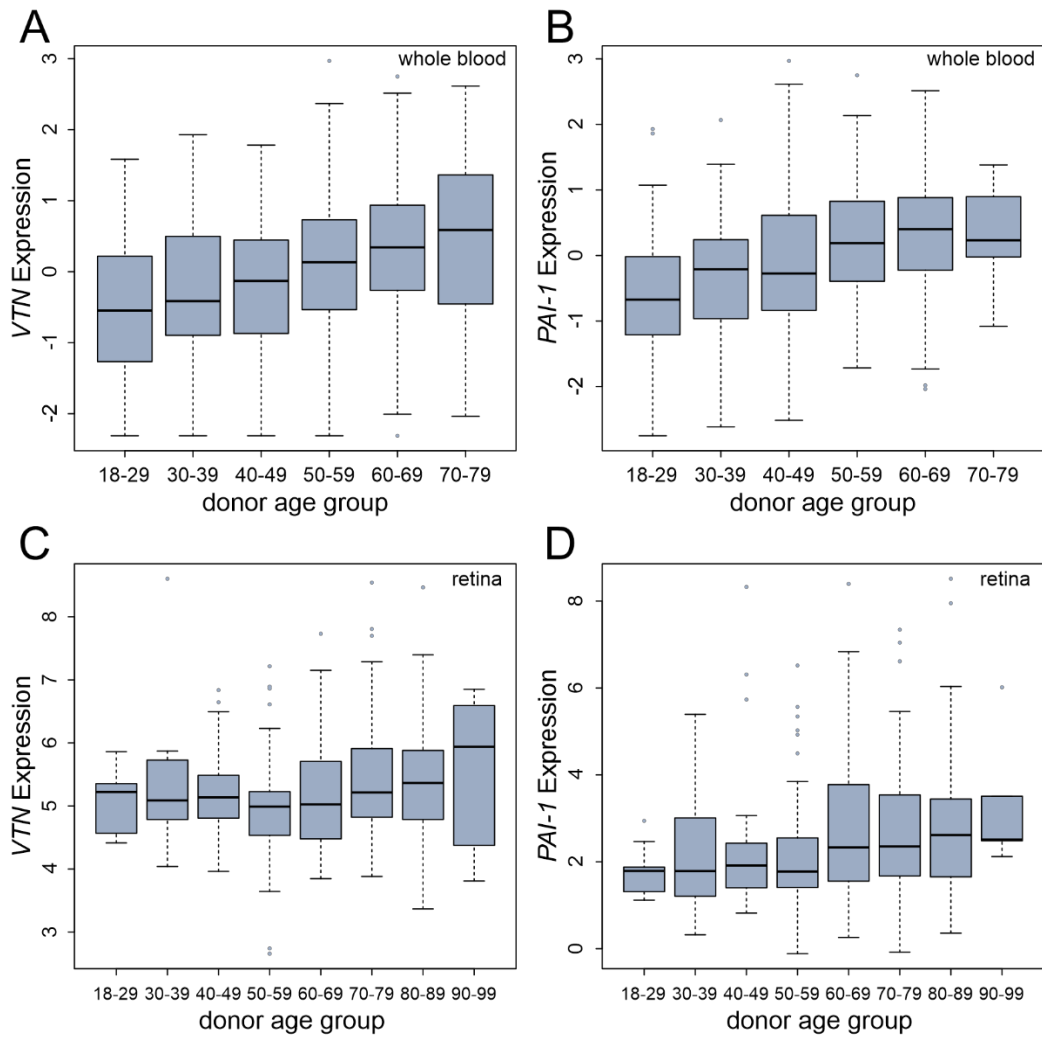
Supplementary Figure S3

SUPPLEMENTARY FIGURE S3. Association of the genetic locus harboring the *PAI-1* gene with late-stage AMD phenotypes (all) and subgroups neovascular AMD (NV-AMD) and geographic atrophy (GA-AMD). The analysis within the IAMDGC dataset revealed only a minor association signal at this locus and low level of linkage disequilibrium (LD) among genetic variants in the selected intervals hampering association mapping. The variant with the smallest p-value, namely rs28709821, is presented as purple diamond and serves as linkage disequilibrium (LD) reference variant. Plots were generated with Locuszoom [26, 27]. A gene map with direction of transcription is shown at the bottom.

Supplementary Figure S4

SUPPLEMENTARY FIGURE S4. PAI-1 activity assay after 0 and 72 hours preincubation of PAI-1 and vitronectin. VTN_rs704:C (grey squares), VTN_rs704:T (ice-blue triangles), or control eluate (control, black diamonds) were mixed with PAI-1 and incubated at 37°C. Aliquots of vitronectin/PAI-1 or control eluate/PAI-1 mixtures were subjected to the PAI-1 activity assay after 0 and 72 hours, measuring PAI-1-dependent uPA activity. The data were calibrated against the values obtained for control measurements at 72 hours (uPA at its maximum activity in our experimental setting, 100%). To better visualize the extent of PAI-1 inhibition and thus the inhibitory capacity of PAI-1, the calibrated uPA activity was subtracted from 100 % (maximal uPA activity). Data represent the mean ± SD of 3 replicates.

Supplementary Figure S5

SUPPLEMENTARY FIGURE S5. Gene expression of *VTN* and *PAI-1* in human blood and retina separated by age groups. Donor individuals from whole blood samples (n = 556) [20] and healthy retinal tissues (n = 311) [22] were grouped according to their age at tissue donation. Shown are gene expression of (A) *VTN* and (B) *PAI-1* in whole blood and (C-D) in retinal tissue samples.