Glaucoma is associated with plasmin proteolytic activation mediated through oxidative inactivation of neuroserpin

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Legends to supplementary figures

S1: Microbeads were injected weekly into the rat eyes to induce increased IOP (2 months). Microbead injected eyes demonstrated a sustained increase in IOP compared to the control eyes (*$p<0.05$) as measured by I-care tonometer.

S2: Multiple alignment of human, mouse and rat neuroserpin amino acid sequence indicating that the protein primary structure is highly conserved (red) amongst the three species. Reactive site Arg-Met bond of the serpin is highlighted in the box (cyan).

S3: Western blot showing tissue plasminogen activator (tPA) expression in the **A.** human and **B.** rat retina. The relative intensities of bands in WB were quantified and plotted. Actin was used as internal loading control. (n=4 each; *$p<0.05$). **C.** Time dependent tPA enzymatic assay was carried out (0-8 hrs) from human and rat retinal lysates from control and glaucoma samples (n=5 each) and data plotted. Dotted lines represent allosteric sigmoidal curve regression analysis.

S4: Western blot showing tissue urokinase-type plasminogen activator (uPA) expression in the **A.** human and **B.** rat retina. The relative intensities of bands in WB were quantified and plotted. Actin was used as internal loading control (n=3 each). **C.** Time dependent uPA enzymatic assay was carried out (0-8 hrs) from human and rat retinal lysates from control and glaucoma samples (n=5 each, *$p<0.05$) and data plotted. Dotted lines represent allosteric sigmoidal curve regression analysis.

S5: Plasminogen peptides identified following neuroserpin immunoprecipitation and subjecting the trypsin digest of eluted proteins to mass spectrometric analysis.

S6: The relative intensities of bands in WB from WT and SOD mice (figure 9) were quantified and plotted. **A.** quantification of relative lane intensities (figure 9A) (*$p<0.05$). **B.** quantification of relative band intensities (figure 9E) (*$p<0.05$). **C.** quantification of relative band intensities (figure 9F) (*$p<0.05$).

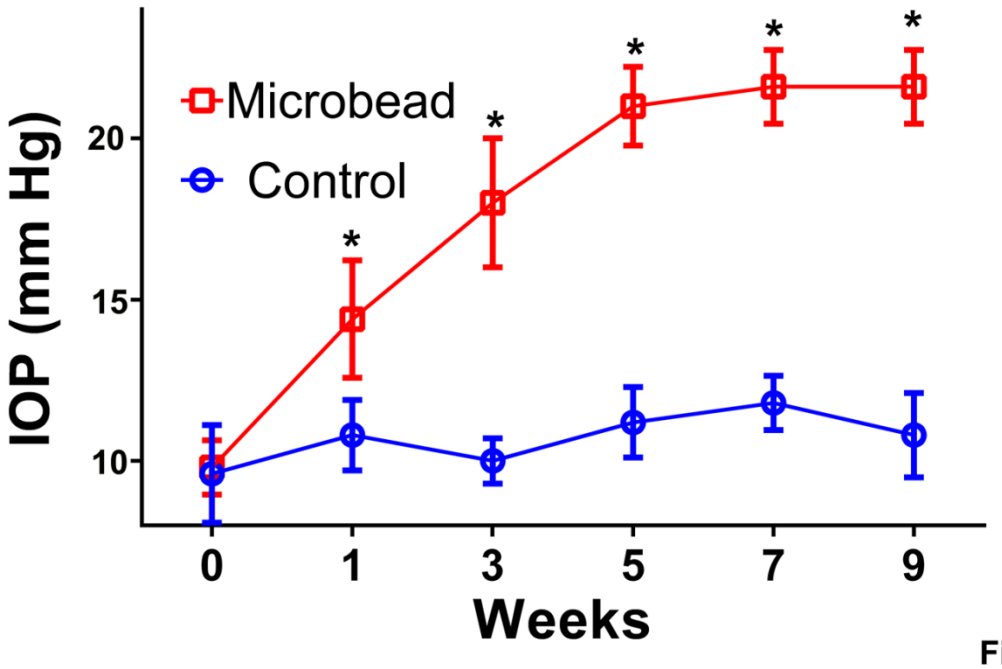
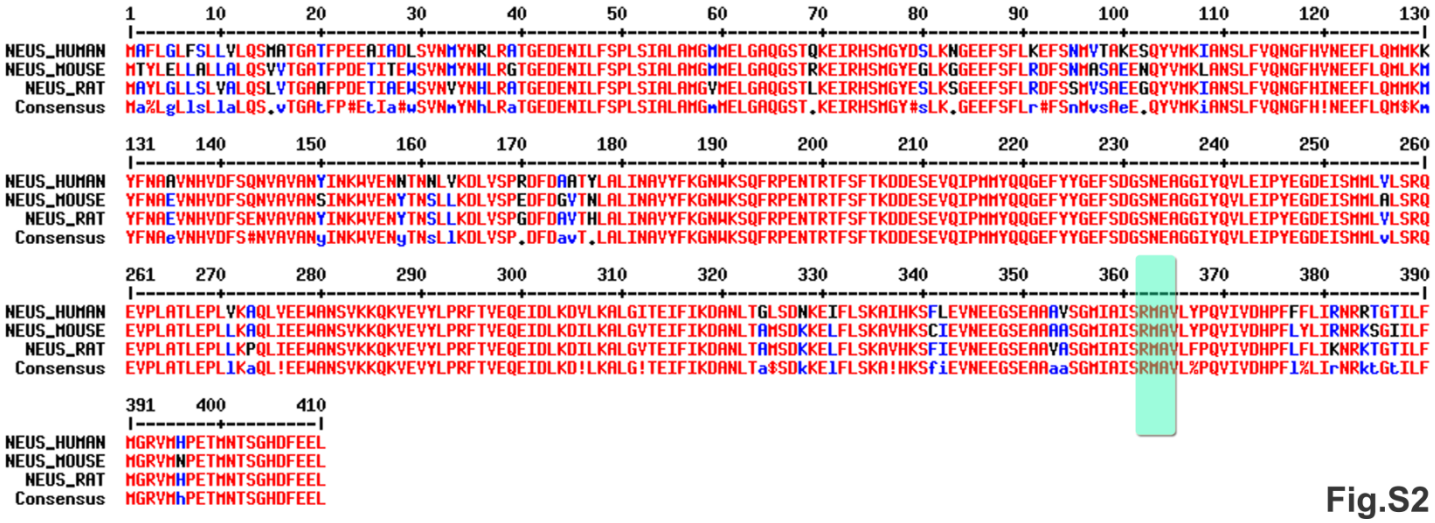


Fig. S1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NEUS_MOUSE MTYLELLALLALQSVVTGATFPDETITEWSVNMYNHLRGTGEDENILFSPLSIALAMGMMELGAQGSTRKEIRHSMGYEGLKGGEEFSFLRDFSNMASAEENQYVMKLANSLFVQNGFHVNEEFLQMLKM
NEUS_RAT MAYLGLLSLVALQSLVTGAAFPDETIAEWSVNVYNHLRATGEDENILFSPLSIALAMGVMELGAQGSTLKEIRHSMGYESLKSGEEFSFLRDFSSMVSAEEGQYVMKIANSLFVQNGFHINEEFLQMMKM
Consensus Ma%LgLlsLlaLQS.vTGAtFP#EtIa#wSVNmYNhLRaTGEDENILFSPLSIALAMGmMELGAQGST.KEIRHSMGY#sLK.GEEFSFLr#FSnMvsAeE.QYVMKiANSLFVQNGFH!NEEFLQM$Km
131 140 150 160 170 180 190 200 210 220 230 240 250 260
NEUS_HUMAN YFNAAVNHVDFSQNVAVANYINKWVENNTNNLVKDLVSPRDFDAATYLALINAVYFKGNWKSQFRPENTRTFSFTKDDESEVQIPMMYQQGEFYYGEFSDGSNEAGGIYQVLEIPYEGDEISMMLVLSRQ
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Consensus YFNAeVNHVDFS#NVAVANgINKWVENgTNsLlKDLVSP.DFDavT.LALINAVYFKGNWKSQFRPENTRTFSFTKDDESEVQIPMMYQQGEFYYGEFSDGSNEAGGIYQVLEIPYEGDEISMMLvLSRQ
261 270 280 290 300 310 320 330 340 350 360 370 380 390
NEUS_HUMAN EVPLATLEPLVKAQLVEEWANSVKKQKVEVYLPRFTVEQEIDLKDVLKALGITEIFIKDANLTGLSDNKEIFLSKAIHKSFLEVNEEGSEAAAVSGMIAISRMAVLYPQVIVDHPFFFLIRNRRTGTILF
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Consensus EVPLATLEPLlKaQL!EEWANSVKKQKVEVYLPRFTVEQEIDLKD!LKALG!TEIFIKDANLTa$SDkKElFLSKA!HKSfiEVNEEGSEAAaaSGMIAISRMAVL%PQVIVDHPFl%LIrNRktGtILF
391 400 410
NEUS_HUMAN MGRVMHPETMNTSGHDFEEL
NEUS_MOUSE MGRVMNPETMNTSGHDFEEL
NEUS_RAT MGRVMHPETMNTSGHDFEEL
Consensus MGRVMhPETMNTSGHDFEEL

Fig.S2

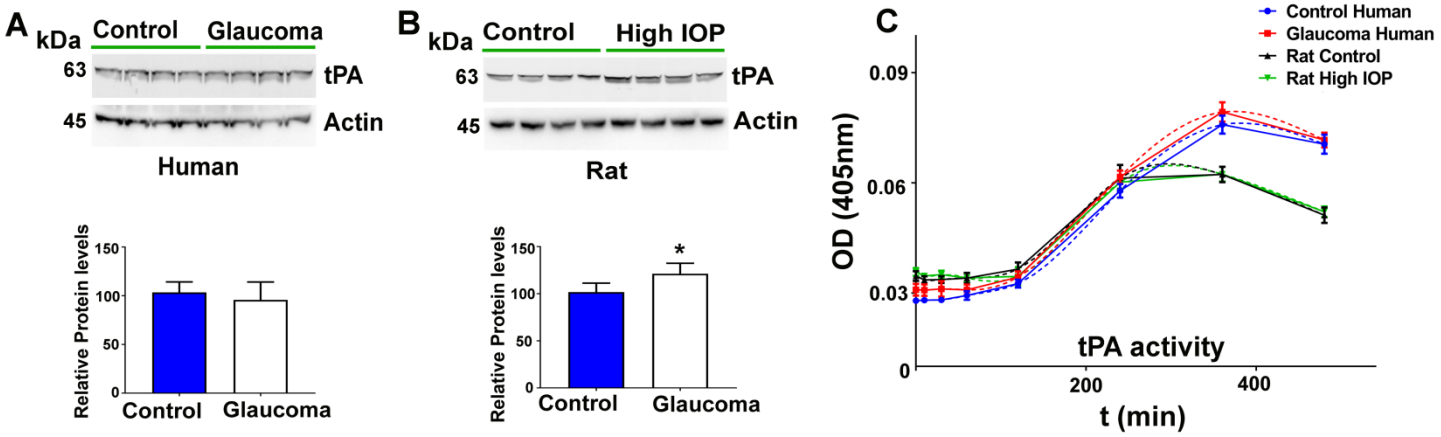
A
kDa
Control
Glaucoma
63
tPA
45
Actin
Human
Relative Protein levels
150
100
50
0
Control
Glaucoma
B
kDa
Control
High IOP
63
tPA
45
Actin
Rat
Relative Protein levels
150
100
50
0
*
Control
Glaucoma
C
Control Human
Glaucoma Human
Rat Control
Rat High IOP
OD (405nm)
0.09
0.06
0.03
0
tPA activity
200
400
t (min)

Fig S3

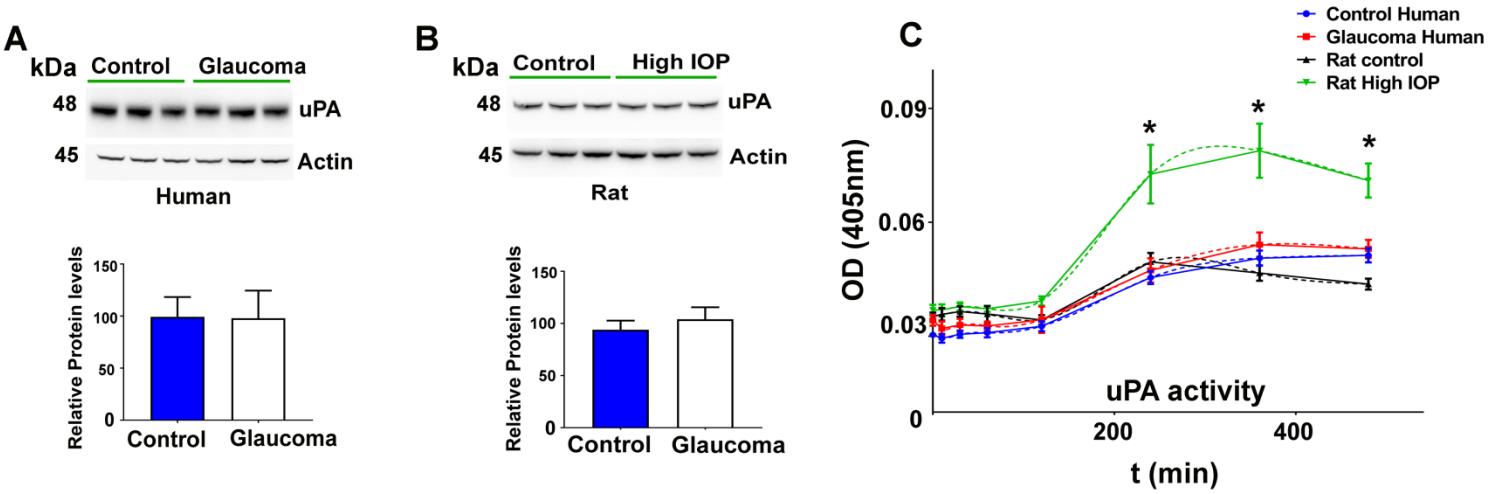
A
kDa
Control
Glaucoma
48
uPA
45
Actin
Human
Relative Protein levels
150
100
50
0
Control
Glaucoma
B
kDa
Control
High IOP
48
uPA
45
Actin
Rat
Relative Protein levels
150
100
50
0
Control
Glaucoma
C
Control Human
Glaucoma Human
Rat control
Rat High IOP
OD (405nm)
0.09
0.06
0.03
0
*
*
*
uPA activity
200
400
t (min)

Fig S4

Plasminogen peptides	
1	KVYLSECK
2	WSSTSPHRPR
3	FSPATHPSEGLEENYCR
4	ELRPWCFTTDPNK
5	RWELCDIPRCTTPPPSSGPTYQCLK
6	NLDENYCRNPDGK
7	TPENFPCK
8	RAPWCHTTNSQVR
9	APWCHTTNSQVR
10	KCQSWSSMTPHR
11	CQSWSSMTPHR
12	TPENYPNAGLTMNYCR
13	HSIFTPETNPR
14	NPDGDVGGPWCYTTNPR
15	LSSPAVITDKVIPACLPSPNYVVADR
16	VIPACLPSPNYVVADR
17	VCNRYEFLNGR
18	FVTWIEGVMR

Fig. S5

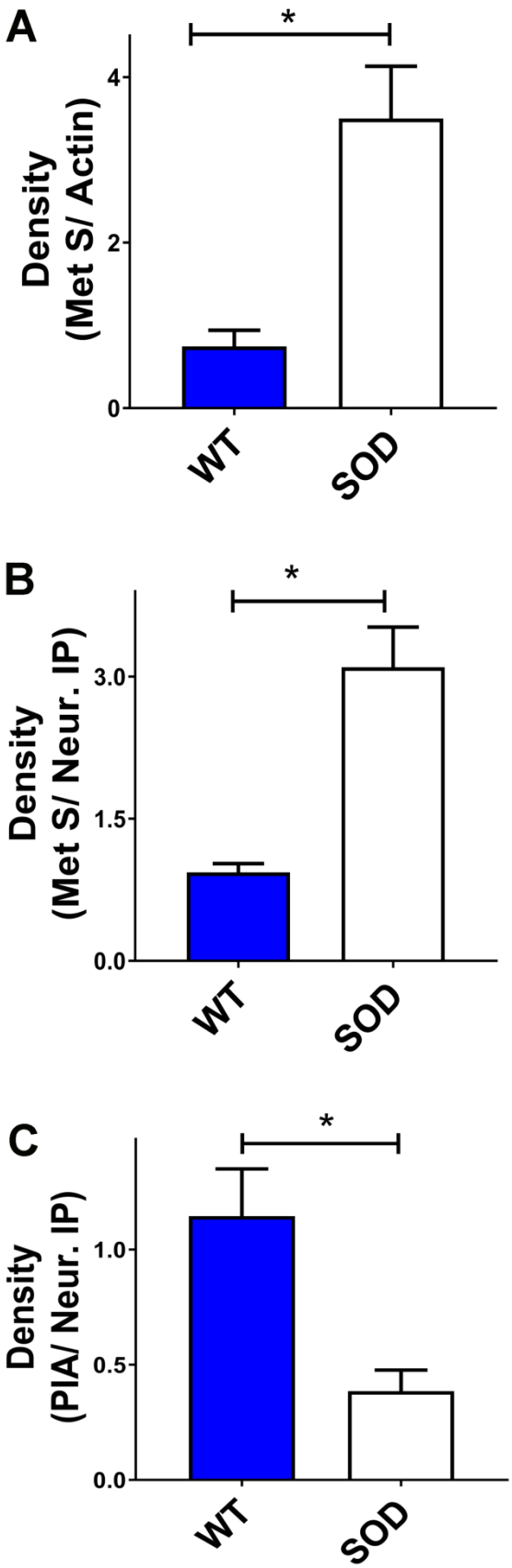
A
*
Density
(Met S/ Actin)
4
2
0
WT
SOD
B
*
Density
(Met S/ Neur. IP)
3.0
1.5
0.0
WT
SOD
C
*
Density
(PIA/ Neur. IP)
1.0
0.5
0.0
WT
SOD

Fig S6