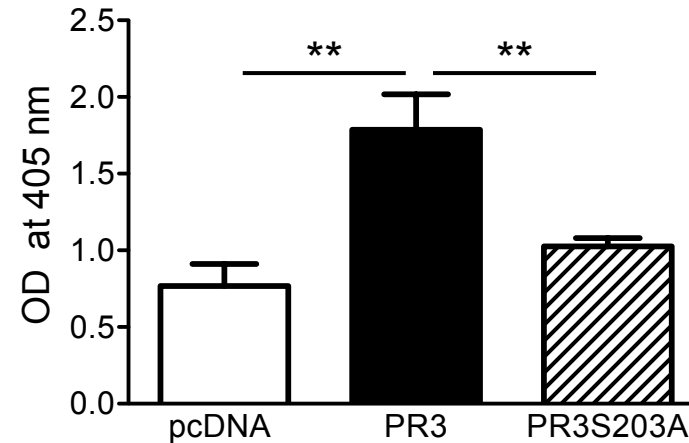


Revision 2- Supplemental Figure 1
Millet et al

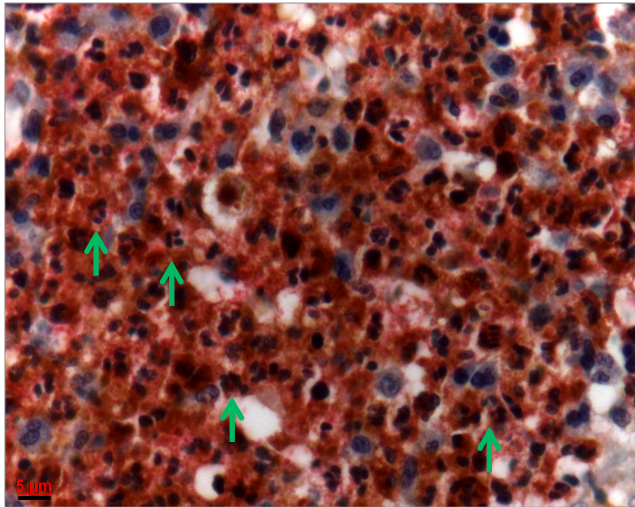


Supplemental Figure 1: PR3S203A is inactive enzymatically.

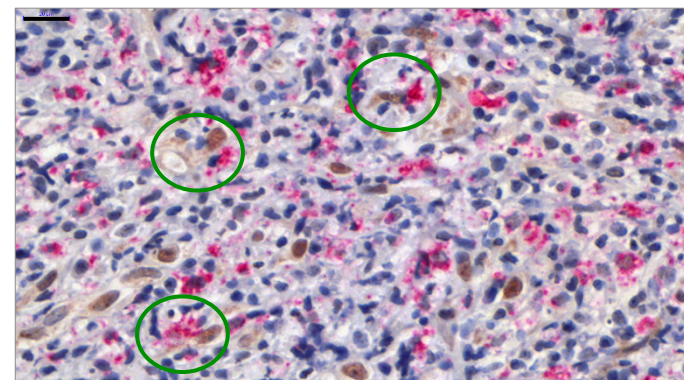
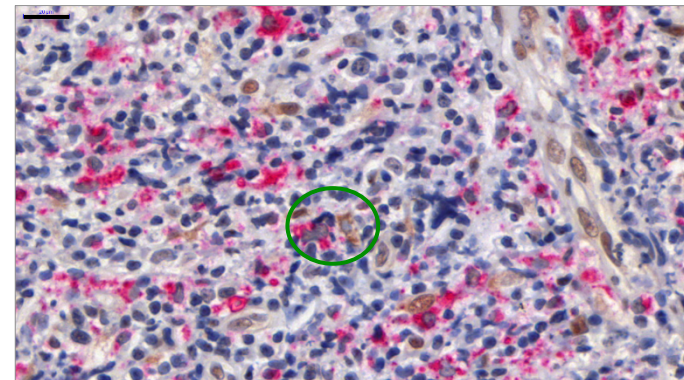
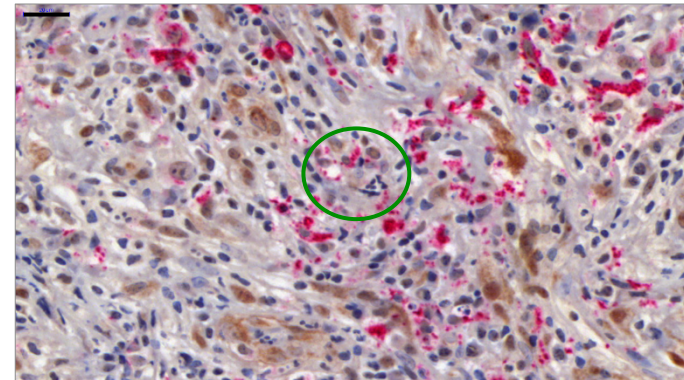
Control RBL transfected with the empty pcDNA plasmide, RBL/PR3 and RB/PR3S203A have been solubilized in PBS supplemented with 1% Triton-X100 and protein concentration has been adjusted at 0.5 mg/ml. Serine protease activity was measured using the chromogenic substrate Boc-Ala-Pro-Nval-SBzl and was quantified by the OD at 405 nm as previously described in Witko-Sarsat et al. 1999 Am J Resp Cell Mol Biol 20: 729-36.

Results are given as the mean \pm SEM from three independent experiments. (**p<0.01, ANOVA)

A



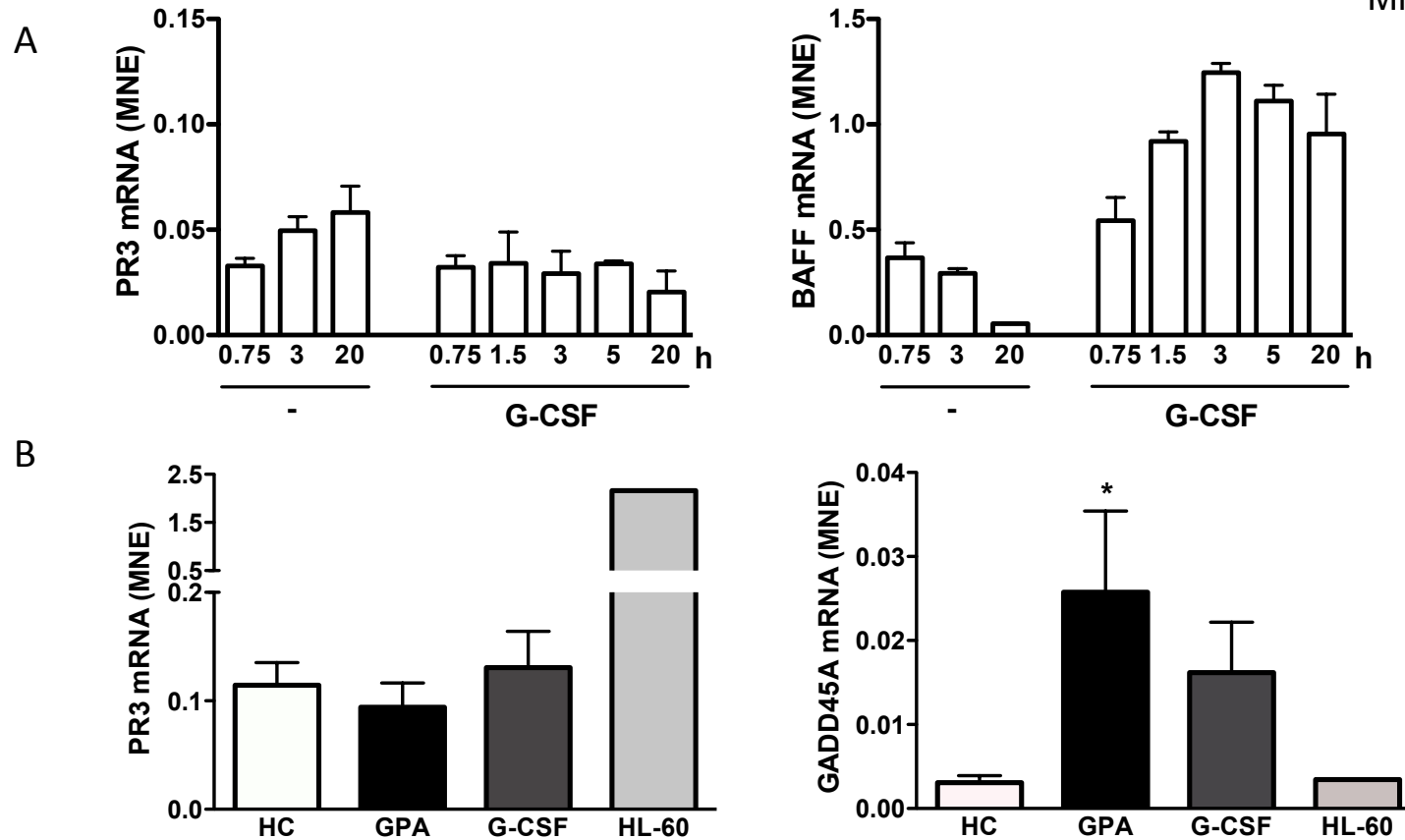
B



Supplemental Figure 2. Detection of pDC, macrophages and neutrophils, appearing in close vicinity in granulomatous inflammation, using IHC.

(A) Nuclei of PMN detected as PR3 (brown staining) and LL37 (red staining) forming a microabscess appear fragmented (green arrows), which may be indicative of ongoing karyorrhexis. Nuclei are counterstained in blue.

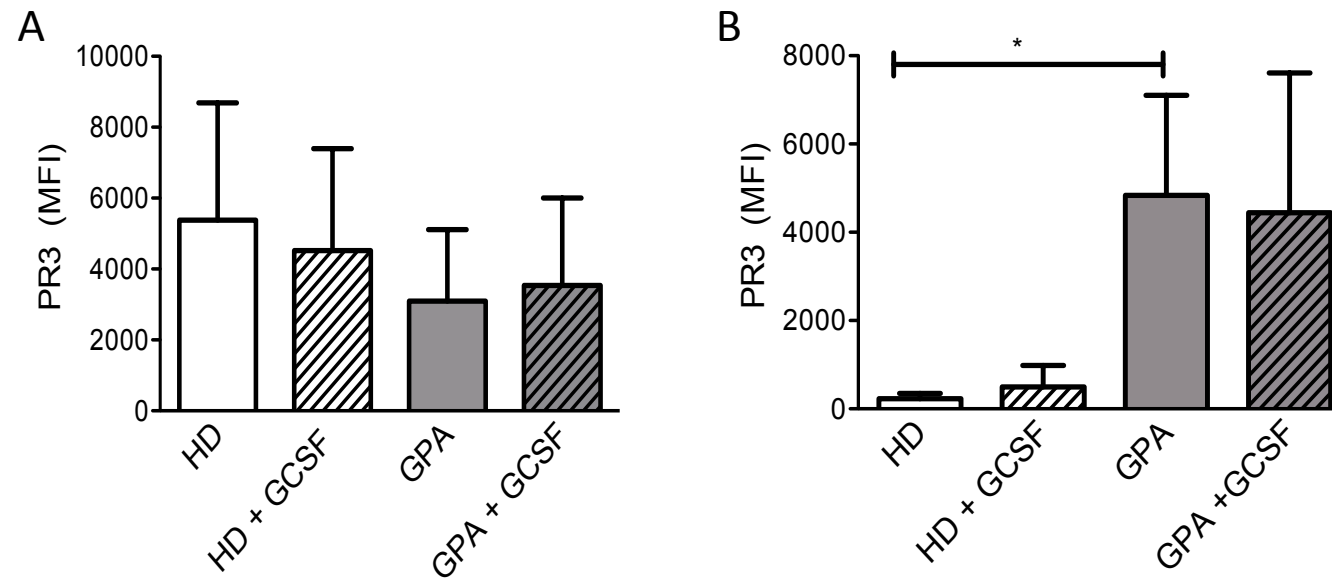
(B) Some CD123⁺ cells (brown staining) are located near to CD68⁺ cells (red staining, macrophages) and also to neutrophils in granulomatous inflammation of the upper respiratory tract in GPA (green circles). Nuclei are counterstained in blue. Data displays typical images representative of 6 GPA patients. Scale bars: 5 μm (A) and 20 μm (B)



Supplemental Figure 3: G-CSF does not modulate PR3 mRNA expression.

(A) Incubation of neutrophils in vitro with G-CSF does not increase the levels of PR3 mRNA expression. Total RNA was extracted from neutrophils cultured with and without 1000 U/ml G-CSF for the times indicated, before measuring PR3, BAFF/BLyS, and RPL32/GADD45A mRNA expression by RT-qPCR. Data are depicted as mean normalized expression (MNE) units after RPL32 mRNA normalization. A representative experiment out of 3 is shown. Error bars represent standard errors calculated from triplicate qPCR reactions. (B) Neutrophils isolated from GPA patients, G-CSF-treated donors and healthy individuals express equivalent levels of PR3 mRNA. PR3 and GADD45A mRNA expression in neutrophils isolated from healthy donors (HD, n=13), GPA patients (GPA, n=13), G-CSF-treated donors (G-CSF, n=11) and HL-60 promyelocytic cells (HL-60), as revealed by RT-qPCR. Gene expression data (mean \pm SEM) are depicted as mean normalized expression (MNE) units after RPL32 mRNA normalization. * $p < 0.05$, by one-way ANOVA followed by Tukey's post test.

Revision 2- Supplemental Figure 4
Millet et al



Supplemental Figure 4. Effect of G-CSF on membrane PR3 expression in basal and apoptotic isolated neutrophils from healthy donors and GPA patients.

(A) MFI of PR3 expression on neutrophils from healthy donors and GPA patients treated for 15 min with medium or G-CSF (B) Same as panel A using apoptotic neutrophils (Annexin V⁺7AAD⁻) from healthy controls and GPA patients. Data are presented as mean \pm SEM, n=7 in GPA and n=4 for healthy controls (HD). (* $p < 0.05$, ANOVA).

Supplemental table 1 Millet et al

Supplemental table 1: Clinical characteristics of patients included in the Neurovasc study (Cochin Hospital, Paris).

AZA: azathioprine; BVAS: Binmingham vasculitis activity score; ENT: ear, nose, throat; F: female; GPA: granulomatosis with polyangiitis; M: male; MPA: microscopic polyangiitis; MPO: microscopic polyangiitis; NA: not available; PR3: proteinase 3; pred: prednisone.

Neurovasc patients	Diagnosis	Age/sex	Clinical manifestations	BVAS	CRP	ANCA	Treatment at the time of disease flare
1	GPA	58/M	Fever, weight loss, ENT, lung	12	195	Anti-PR3	no
2	GPA	85/F	ENT, proteinuria, hematuria	16	14	Anti-PR3	no
3	GPA	67/F	ENT, lung, proteinuria, hematuria	22	34	Anti-PR3	no
4	GPA	42/F	ENT, lung, bowel	9	17	Anti-PR3	no
5	GPA	55/M	Fever, myalgia, scleritis, lung	11	257	Anti-PR3	no
6	GPA	58/M	Fever, myalgia, ENT, lung, proteinuria, hematuria	19	23	Anti-PR3	no
7	GPA	22/F	Fever, weight loss, ENT, lung	15	224	Anti-PR3	no
8	GPA	61/M	Peripheral neuropathy	9	NA	Anti-PR3	AZA 100 mg/j + pred 20 mg/j
9	GPA	57/M	Joint, ENT	7	1,3	Anti-PR3	AZA 100 mg/j
10	GPA	61/M	ENT, lung	11	14	Anti-PR3	no
11	GPA	50/F	ENT, skin, hematuria	14	9,3	Anti-PR3	no
12	GPA	27/M	Fever, joint, ENT, lung, kidney	25	64	Anti-PR3	no
13	GPA	24/M	Fever, ENT, lung, kidney, skin	26	72	Anti-PR3	no
14	GPA	48/M	ENT, lung, hematuria	18	183	Anti-PR3	no
15	GPA	61/M	Weight loss, ENT, lung, peripheral neuropathy	20	57	Anti-PR3	no

Supplemental table 2

Millet et al

Supplemental table 2: Clinical characteristics of patients included in the histopathology Study (Lübeck Hospital, Germany).

AZA: azathioprine; BVAS: Binmingham vasculitis activity score; ENT: ear, nose, throat; F: female; GPA: granulomatosis with polyangiitis; M: male; MPA: microscopic polyangiitis; MPO: microscopic polyangiitis; MTX: Methotrexate; NA: not available; PR3: proteinase 3; pred: prednisone

Histo-pathology patients	Diagnosis	Age/sex	Clinical manifestations	BVAS	CRP (mg l ⁻¹)	ANCA	Treatment at the time of biopsy
1	GPA	71/F	ENT, myalgia	8	4.9	Anti-PR3	no
2	GPA	41/F	ENT, lung	12	18	Anti-PR3	MTX
3	GPA	54/M	fever, ENT, lung, arthralgia, kidney	27	103	Anti-PR3	no
4	GPA	74/F	ENT	6	2	negative	no
5	GPA	69/F	fever, ENT	6	135	negative	MTX + pred
6	GPA	49/M	ENT, night sweat	8	61	Anti-PR3	no