# Identification of fibrinogen as a natural inhibitor of MMP-2

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**Running title:** Fibrinogen inhibits MMP-2

**Key words:** Matrix metalloproteinase, MMP-2, Fibrinogen, MMP-2 deficiency

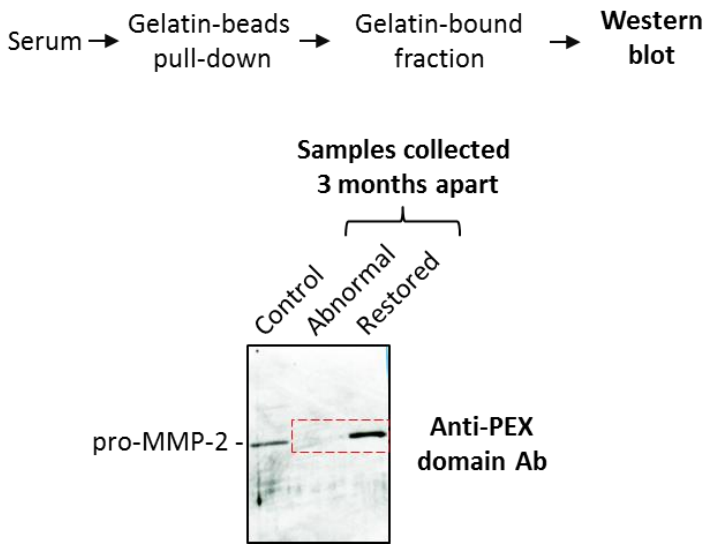



**Figure S1: Restoration of binding of serum MMP-2 to gelatin when serum fibrinogen levels normalized.** Western blot (using the anti-PEX domain antibody) showing MMP-2 in the gelatin-bound fraction indicating the restoration of binding of MMP-2 to gelatin in a second serum sample from the donor of the abnormal serum collected three months after the first collection. PEX, hemopexin-like domain; Ab, antibody.

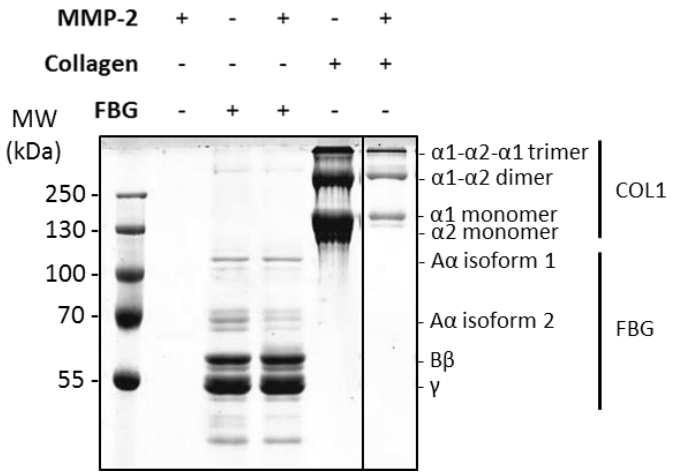



**Figure S2: Cleavage of purified collagen type I and FBG by MMP-2.** SDS-PAGE of fibrinogen (50 μg) and collagen type I (50 μg) degradation by recombinant MMP-2 (12 nM) after incubation for 24 hours at 37°C.

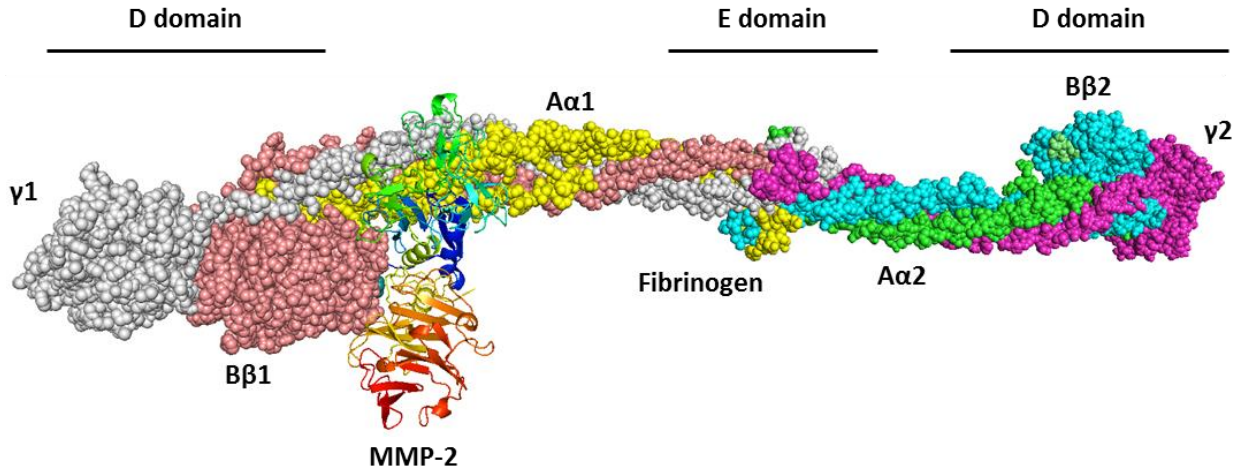



**Fig. S3: Molecular docking of FBG and MMP-2.** Whole annotated image of molecular interactions between MMP-2 and FBG presented in Fig. 7a.

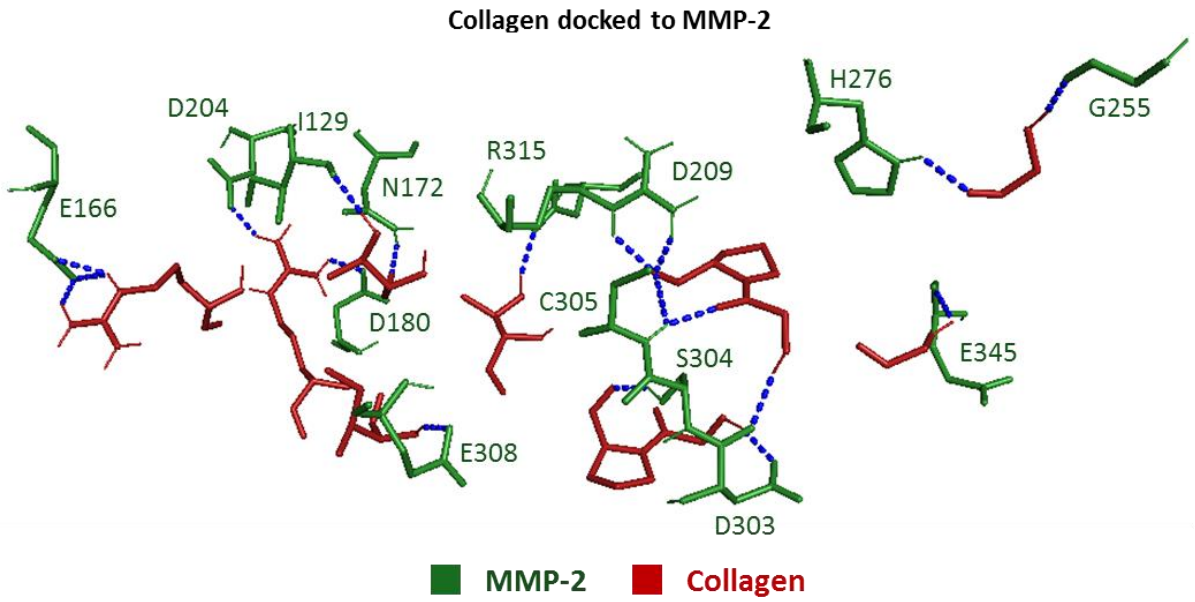



**Figure S4: Molecular docking of collagen to MMP-2.** Labelled residues of MMP-2 (green) that form hydrogen bonds (blue dotted lines) with collagen residues (red) are presented. Interacting MMP-2 residues span over the catalytic domain and the fibronectin type II repeats 1,2 and 3.

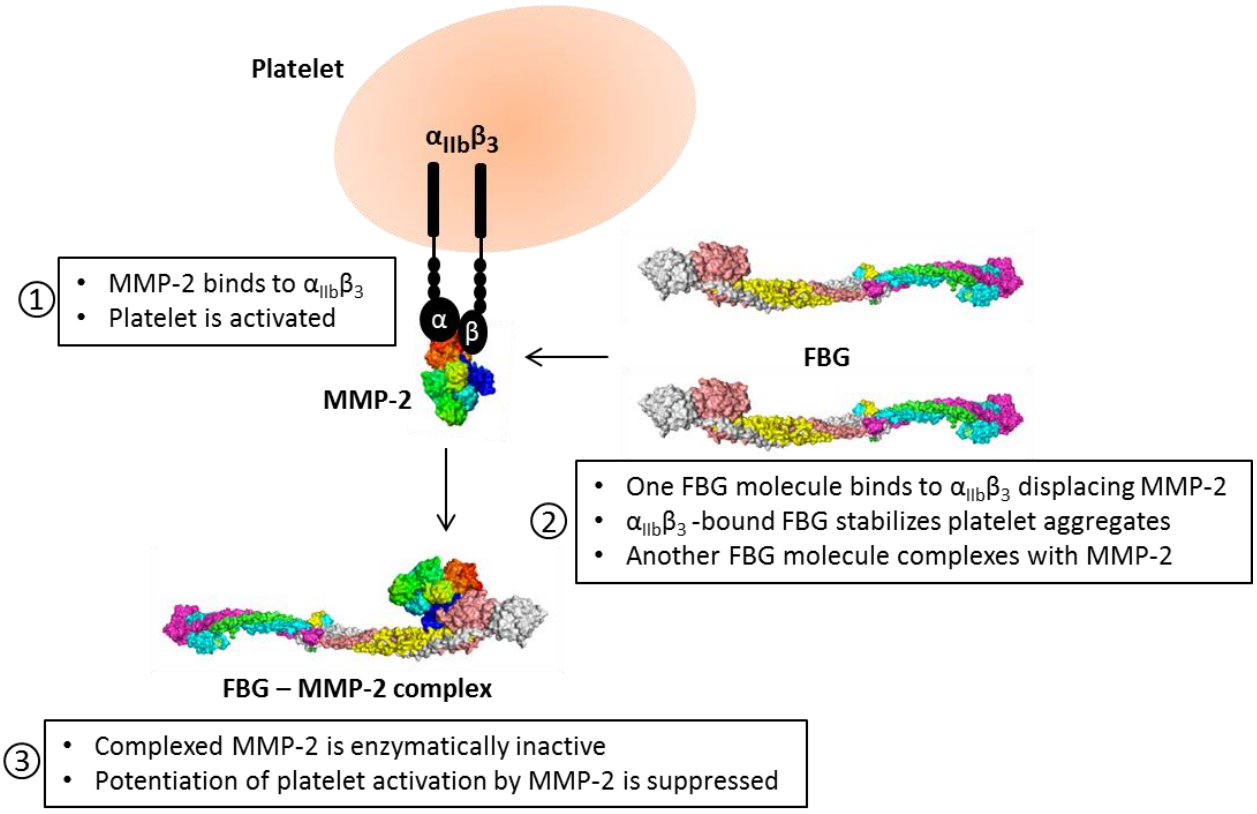



**Figure S5: Illustration of a hypothesized anti-aggregatory function of FBG-mediated inhibition of MMP-2 activity.**

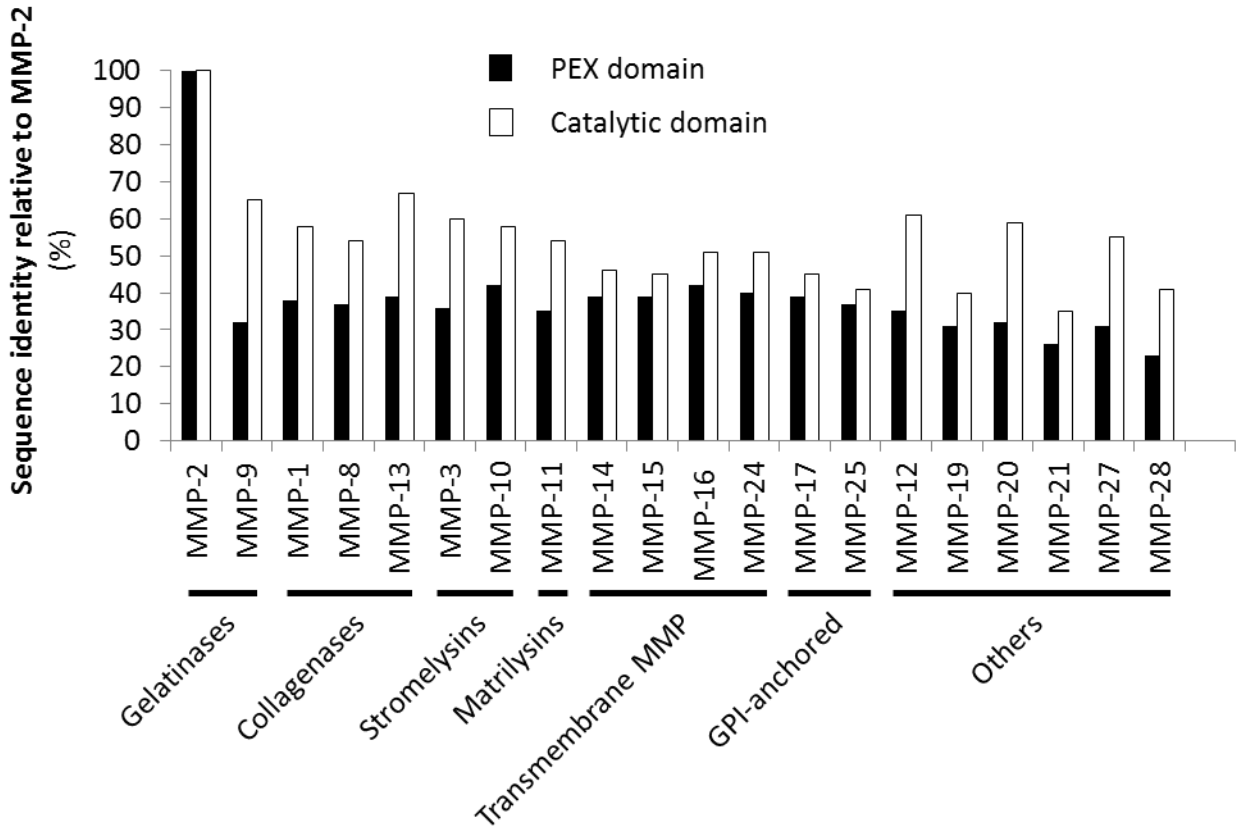
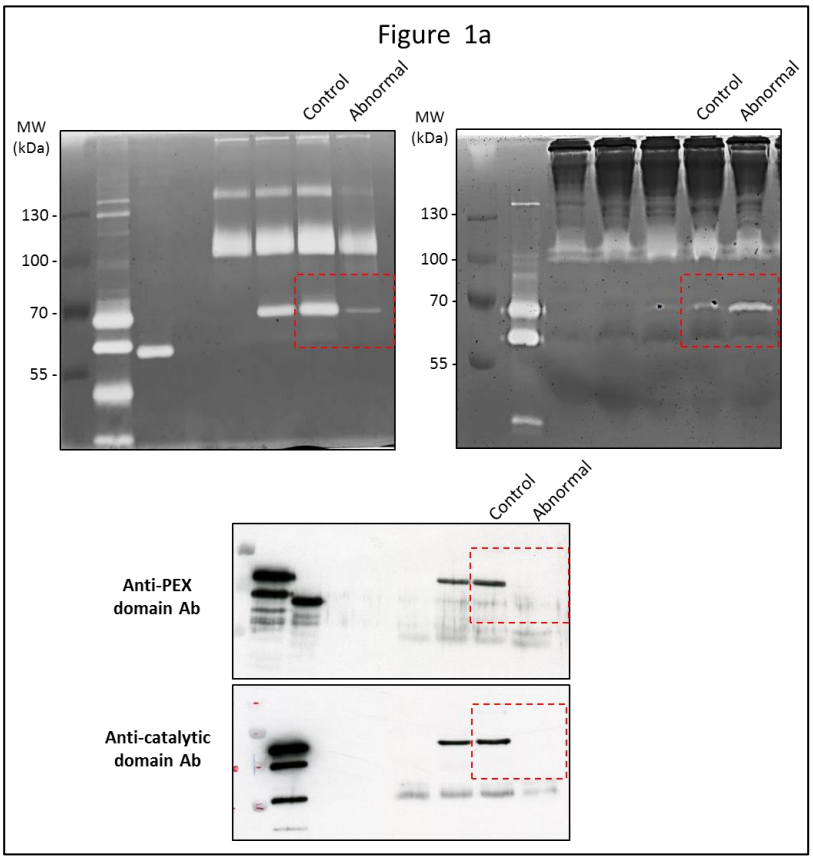
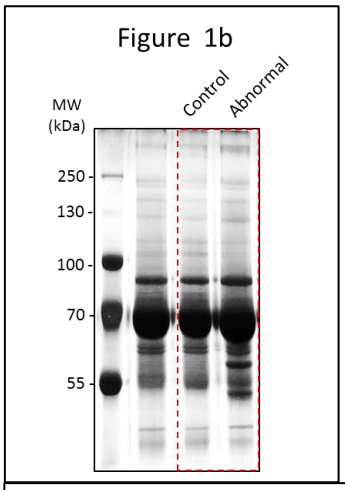
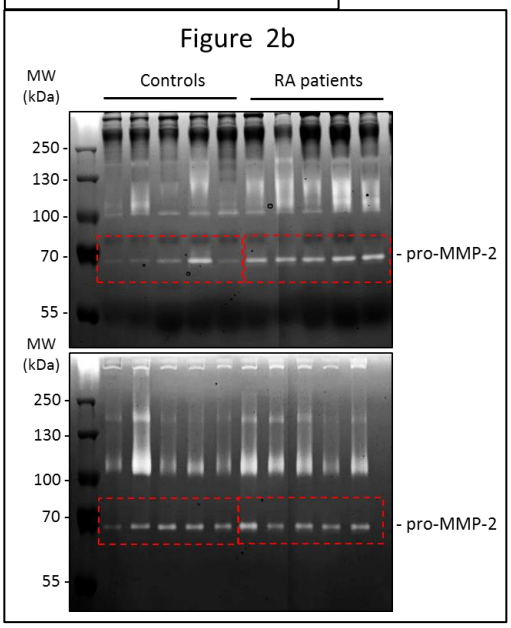



**Figure S6: Comparison of amino acid sequence similarity of MMPs relative to MMP-2**. The amino acid sequences of catalytic and the hemopexin-like domains of MMPs were aligned with MMP-2. The data is presented as percent identity relative to MMP-2.



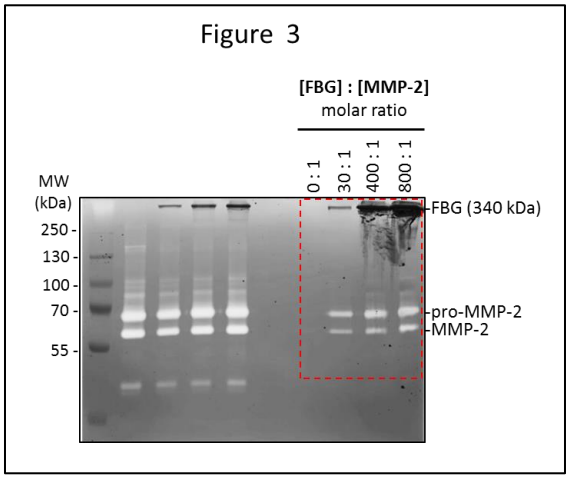




**Figure S7: Full length gels and blots for figures 1a, 1b, 2b and 3.**

| Denotation | Condition | Age | Sex | Denotation | Condition | Age | Sex |
|---|---|---|---|---|---|---|---|
| C1 (abnormal) | Asymptomatic | 30 | M | R4 | RA | 70 | F |
| C2 | Control | 69 | F | R5 | RA | 76 | F |
| C3 | Control | 41 | M | R6 | RA | 36 | F |
| C4 | Control | 69 | F | R7 | RA | 64 | M |
| C5 | Control | 60 | M | R8 | RA | 68 | F |
| C6 | Control | 77 | F | R9 | RA | 35 | F |
| C7 | Control | 44 | M | R10 | RA | 42 | F |
| C8 | Control | 35 | F | R11 | RA | 40 | M |
| C9 | Control | 43 | F | R12 | RA | 69 | F |
| C10 | Control | 59 | F | R13 | RA | 45 | F |
| C11 | Control | 42 | F | R14 | RA | 34 | F |
| C12 | Control | 53 | F | R15 | RA | 56 | F |
| C13 | Control | 34 | F | R16 | RA | 66 | M |
| C14 | Control | 37 | F | R17 | RA | 57 | F |
| C15 | Control | 65 | F | R18 | RA | 40 | F |
| C16 | Control | 71 | M | R19 | RA | 36 | F |
| C17 | Control | 36 | F | R20 | RA | 66 | F |
| C18 | Control | 69 | F | R21 | RA | 69 | F |
| C19 | Control | 45 | F | R22 | RA | 74 | F |
| C20 | Control | 67 | F | R23 | RA | 44 | M |
| R1 | RA | 53 | F | R24 | RA | 58 | F |
| R2 | RA | 58 | M | R25 | RA | 47 | F |
| R3 | RA | 38 | F | | | | |

**Table S1**: **Demographic information of the total cohorts of sera donors assessed.**

| | **Fibrinogen docked to MMP-2** | **Marimastat docked to MMP-2** |
|---|---|---|
| **Hydrogen bonds** | Y110, N111, E166, A167, E177, G181, Y182, P183, L190, A192, G200, D204, H407, E412, H413, S414, Q415, P423 | H413, P423 |
| **Other interacting residues** | F112, F113, P114, R115, R127, D164, G165, D168, I169, M170, H178, G179, D180, F184, D185, G189, L191, H193, V201, T311, I424, Y425 | F184, G189, L190, A192, H193, A194, L399, V400, H403, H407, L420, A422, I424, Y425, T426 |
| **Target domain(s) of MMP-2** | Catalytic<br>Fibronectin type II repeat 2 | Catalytic |

**Table S2: A comparison of MMP-2 residues interacting (within 5Å) with FBG and Marimastat.**

| | **Collagen docked to MMP-2** |
|---|---|
| **Hydrogen bonds** | I129, E166, N172, D180, D204, D209, G255, H276, D303, S304, C305, E308, R315, E345 |
| **Other interacting residues** | R127, G130, Y131, T132, P133, G165, M170, F173, H178, G200, V201, A278, C291, F293, S301, Y302, T306, T307, G309, R310, D312, G313, T335, S344, G346, A347, K386 |
| **Target domain(s) of MMP-2** | Catalytic<br>Fibronectin type II repeat 1<br>Fibronectin type II repeat 2<br>Fibronectin type II repeat 3 |

**Table S3: A comparison of MMP-2 residues interacting (within 5Å) with collagen in the presence or absence of FBG.**