SUPPORTING INFORMATION

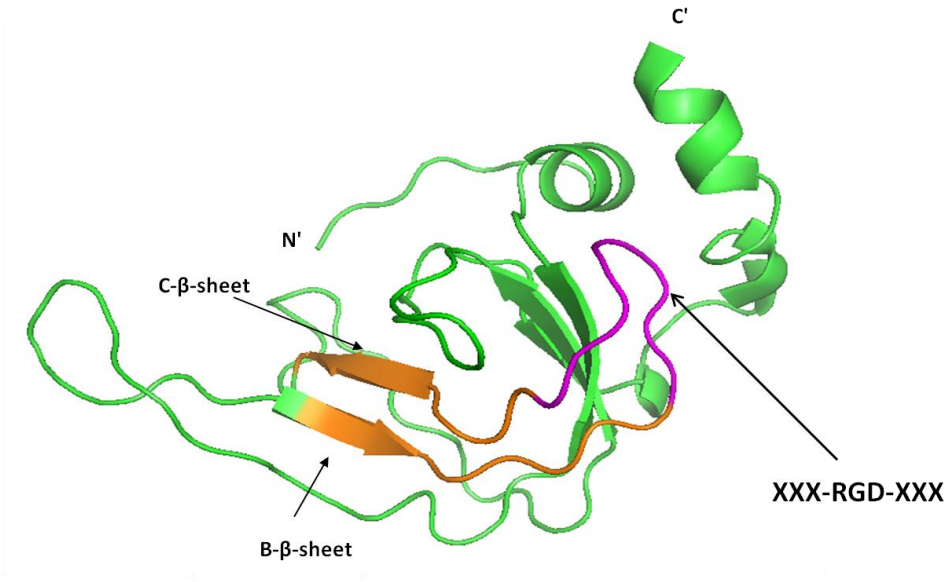

Fig. S1. N-TIMP2 structure and library position**.** The B-C loop is located between the B-β sheet and C-β sheet at positions 43-66 (orange and pink). The N-TIMP2$_{RGD}$ library, located at the apex of the B-C loop, corresponding to the location of the RGD motif in fibrinogen, is located at positions 51-59 (pink). Based on 1BQQ.pdb

Supplementary Table 1. Clones identified in Sort 5.

Clone	Loop sequence*	Repetitions
Clone 1	SDQ-RGD-NAP	1
Clone 2	PEP-RGD-NAR	1
Clone 3	HTV-RGD-MPS	1
Clone 4	QEP-RGD-MPV	2
Clone 5	KTA-RGD-MPA	1
Clone 14	HGL-RGD-MPS	1
Clone 15	SMN-RGD-TIP	2
Clone 17	SQA-RGD-MPN	1
Clone 18	SEV-RGD-VPN	1

*Amino acids at positions 51-59.

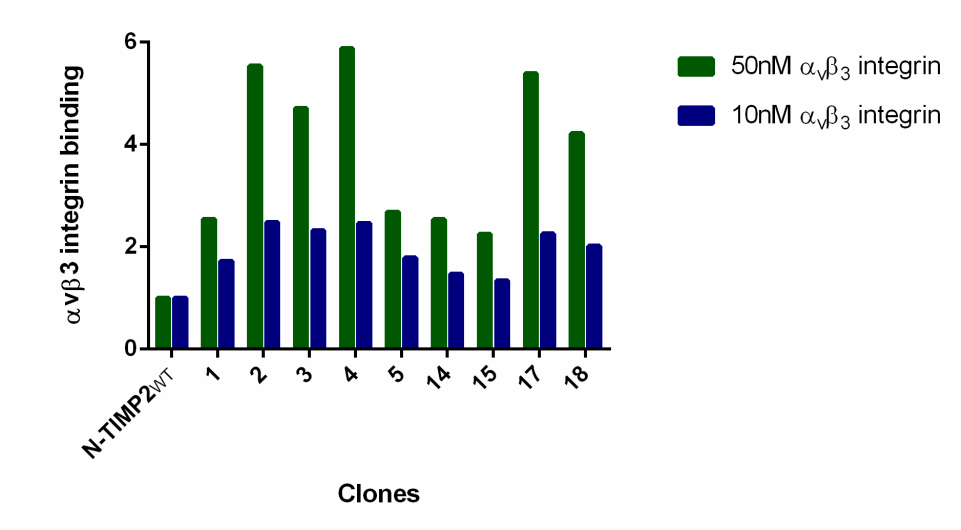

Fig. S2. Binding of the YSD N-TIMP2$_{RGD}$ sort 5 clones to soluble integrin $\alpha_v\beta_3$. Clones binding to integrin $\alpha_v\beta_3$ were normalized to N-TIMP2$_{WT}$.

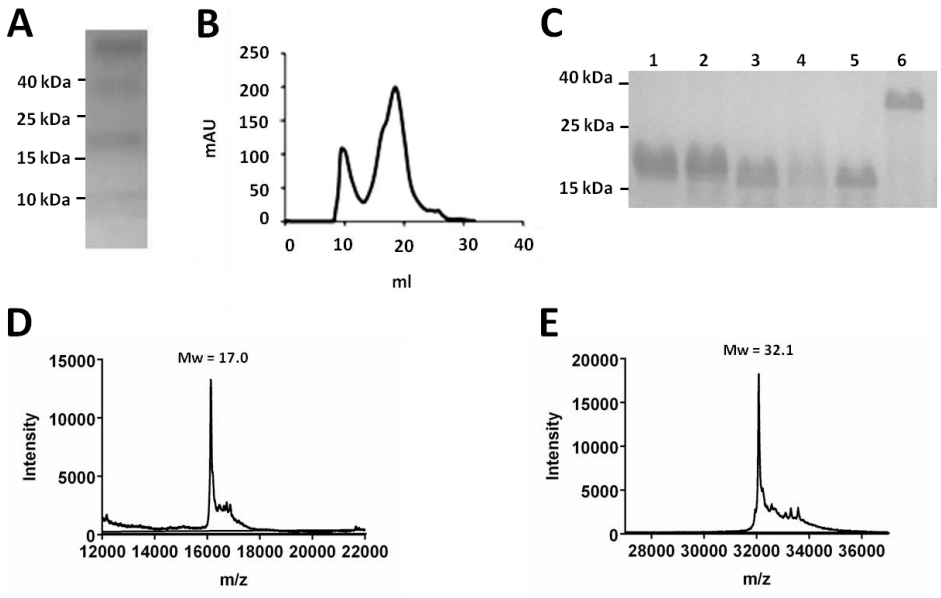

Fig. S3. N-TIMP2$_{WT,RGD}$ purification. (*A*) SDS-PAGE results of N-TIMP2$_{WT,RGD}$ (after nickel column chromatography). Four proteins of different sizes were detected, including a protein of the desired size (~17 kDa). (*B*) Size-exclusion purification of N-TIMP2$_{WT,RGD}$. The right peak represents the desired protein. (*C*) SDS-PAGE of all the N-TIMP2 variants (after size-exclusion chromatography). Lanes 1-6 represent N-TIMP2$_{WT}$, N-TIMP2$_{5M}$, N-TIMP2$_{WT,RGD}$, N-TIMP2$_{5M,RGD}$, Ala-N-TIMP$_{WT,RGD}$ and N-TIMP2$_{HD}$, respectively. Bands of the correct sizes were detected (32 kDa for the N-TIMP2$_{HD}$ and 17 kDa for all the other variants). (*D*) Mass spectrometry analysis of N-TIMP2$_{WT,RGD}$. (*E*) Mass spectrometry analysis of N-TIMP2$_{HD}$.

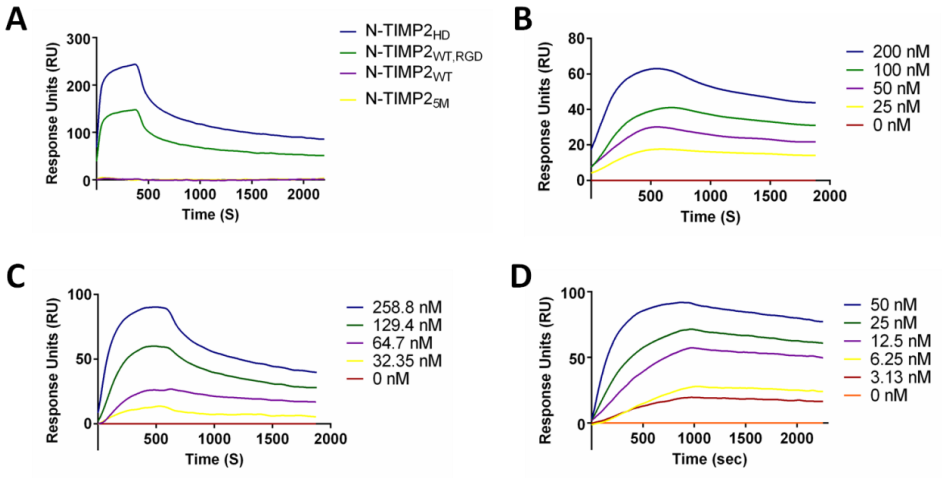

Fig. S4. Surface plasmon resonance sensorgrams. (*A*) N-TIMP2$_{WT,RGD}$ and N-TIMP2$_{HD}$ but not N-TIMP2$_{WT}$ and N-TIMP2$_{5M}$ bind to integrin $\alpha_v\beta_3$. Each variant (1 μM) was allowed to flow over integrin $\alpha_v\beta_3$ immobilized on a chip, and binding was detected as an increase in response units. (*B*) Ala-N-TIMP2$_{WT,RGD}$ binds to integrin $\alpha_v\beta_3$. N-TIMP2$_{5M,RGD}$ binds both integrin $\alpha_v\beta_3$ (*C*) and MMP-14$_{CAT}$ (*D*).

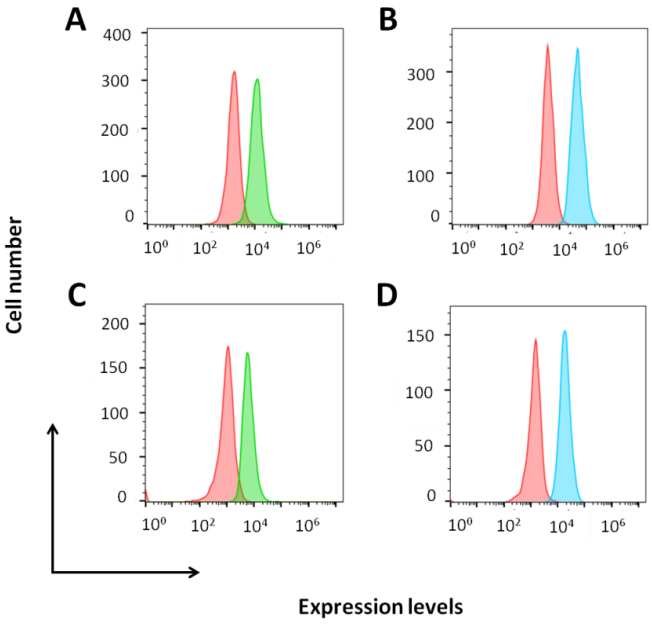

Fig. S5. Expression of MMP-14 (green) and integrin $\alpha_v\beta_3$ (blue) on TIME and U87MG cell lines compared to the control (red). Expression of MMP-14 (*A*) and integrin $\alpha_v\beta_3$ (*B*) on the U87MG cell line. Expression of MMP-14 (*C*) and integrin $\alpha_v\beta_3$ (*D*) on the TIME cell line.

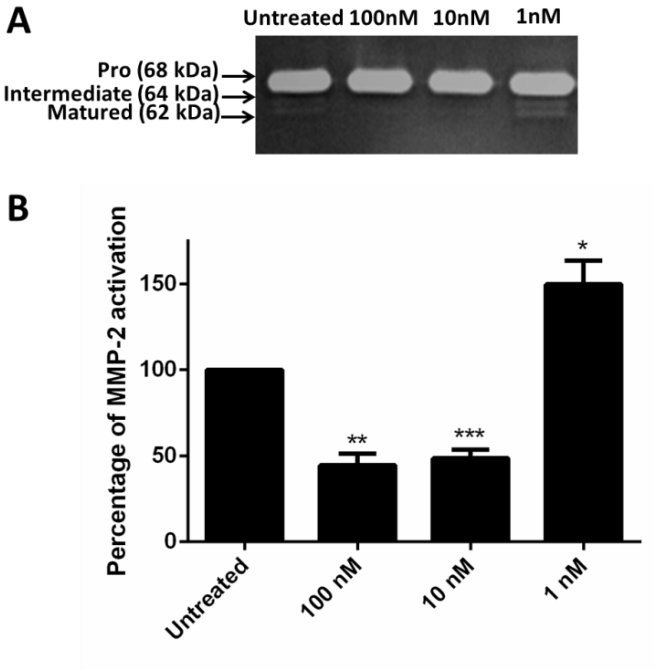

Fig. S6. Effect of the FL-TIMP2 concentration on pro-MMP-2 activation. The U87MG cell line was treated with different concentrations of FL-TIMP2 (100 nM, 10 nM and 1 nM). (*A*) Gelatin zymography gel results. (*B*) Percentage of catalytically active MMP-2. The catalytically active MMP-2, i.e. intermediate + matured forms, of each treatment was normalized to untreated cells. At high concentrations of FL-TIMP2, inhibition of pro-MMP-2 activation was observed, while at low concentrations an increase in pro-MMP-2 was detected. Error bars represent SD. Statistical analysis was performed by Student's t-test compared to the untreated control; **P*< 0.05, ***P*< 0.005, ****P*< 0.001, n = 3.

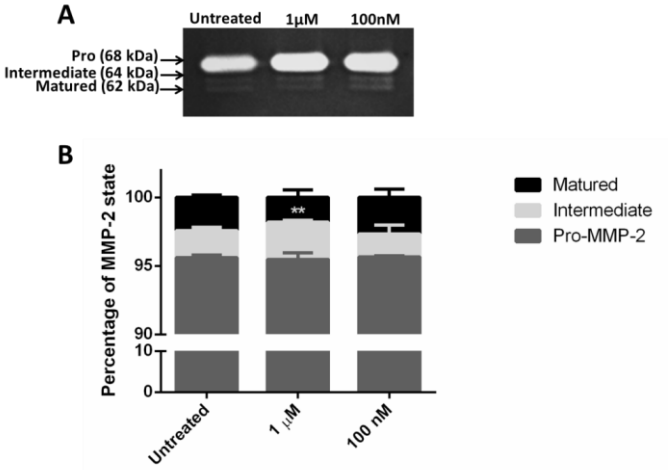

Fig. S7. Effect of cRGD treatment on the percentage of intermediate MMP-2. The U87MG cell line was treated with different concentrations of cRGD (1 µM and 100 nM). *(A)* Gelatin zymography gel results. (*B*) Quantification of the percentage of each MMP-2 form, i.e., pro/intermediate/matured [the band intensity of each MMP-2 state, i.e., pro-MMP-2 (68 kDa), intermediate MMP-2 (64 kDa) and matured MMP-2 (62 kDa), was divided by the total band intensity of all the MMP-2 forms, i.e., pro+ intermediate+ matured MMP-2]. Processing of the intermediate form was significantly inhibited by 1 µM of cRGD. Error bars represent SEM. Statistical analysis was performed by Student's t-test compared to the untreated control; ***P*< 0.005, n = 3.