Supporting Information

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SI Materials and Methods

Cloning, expression, refolding and purification of the monomeric recombinant collagen-binding domain of human GPVI. The human GP6 cDNA clone MGC:138168 (IMAGE:8327431) was used as a template to amplify the sequence encoding the GPVI collagen-binding domain by PCR. The amino acid sequence of the gpVICBD construct used for binding experiments is as follows:

6HisTrx=
MGHHHHHHGSGMSDKIIHLTDDSFDTDVLKADGAIL-
VDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNI-
DQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSK-
GQLKEFLDANLAGSGSGLVPR

gpVICBD=
GSGPLPKPSLQALPSSLVPLEKPVLRCQGPPGVDLYRL-
EKLSSSRYQDQAVLFIPAMKRSLAGRYRCSYQNGSL-
WSLPSDQLELVATGVFAKPSLSAQPGPAVSSGGDVTL-
QCQTRYGFDQFALYKEGDPAPYKNPERWYRASFPIITV-
TAAHSGTYRCYSFSSRDPYLWSAPSDPLELVVTGAS

The thrombin cleavage results in a release of gpVICBD.

The recombinant construct was expressed at 25 °C in the *Escherichia coli* BL21 (DE3) (Novagen) after isopropyl-β-Dthiogalactopyranoside induction (final concentration of 1 mM) for 16 h. Insoluble protein was dissolved in denaturing buffer (8 M Guanidinium hydrochloride, 10 mM DTT), quickly diluted with a 10-fold excess of a refolding buffer [1 M arginine, 50 mM Tris·HCl, 50 mM sodium phosphate, 5 mM EDTA, 10 mM reduced glutathione, 1 mM oxidized glutathione (pH 8.8)] at room temperature, chilled to 4 °C, and incubated for 16 h. The refolded protein was extensively dialyzed against loading buffer [20 mM Tris·HCl, 150 mM NaCl (pH 8)]. Initial purification and thioredoxin removal were done as generally described by Boudko et al. (1). The purity of gpVICBD higher than 95% was determined by SDS/PAGE. A correct mass of gpVICBD was confirmed by MS.

1. Boudko SP, et al. (2010) The NC2 domain of collagen IX provides chain selection and heterotrimerization. *J Biol Chem* 285(31):23721–23731.