Table S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source or reference
S. gordonii strains		
CH1	Parental strain Challis (sequenced genome)	(13)
DL1	Parental strain Challis	(8)
UB1968	DL1; 266 bp SGO_2006 replaced by 913 bp *aad9* with no terminator	This study
UB1890	DL1; 1960 bp SGO_2005 replaced by 1158 bp *aad9* with terminator	This study
UB1964	DL1; 1454 bp SGO_2004 replaced by 1158 bp *aad9* with terminator	This study
UB1545	*hsa*::*aphA3* (Kn^r)	(7)
UB1360	Δ(*sspA sspB*)::*aad9* (Sp^r)	(6)
UB1552	*hsa*::*aphA3* Δ(*sspA sspB*)::*aad9* (Kn^r Sp^r)	(7)
UB2031	DL1; *hsa*::*aphA3 padA*::*aad9* (Kn^r Sp^r)	This study
E. coli strains		
DH5α	F^- ϕ80d *lacZ* ΔM15 Δ(*lacZYA-argF*)*U169 deoR recA1 endA1 hsdR17*(r_k -m_k^+) *supE44* λ^1 *thi-1 gyrA96 relA1*	Invitrogen
JM109	F' *traD36 proA*$^-$*B*$^-$ Δ*(lacZ)M15/*Δ*(lac-proAB) glnV thi*	New England BioLabs
BL21	F^- *ompT hsdS*$_B$ (*r*$_B^-$*m*$_B^-$) *gal dcm* (DE3) pLysS (Cm^R)	Novagen
Plasmids		
pGEM-7:sp^R	Spectinomycin-resistance (Sp^r) *aad9* determinant with terminator in pGEM-7Zf, (Ap^r Sp^r)	(8)
pET46	PCR product cloning vector incorporating x6 His NH_2 tag, (Ap^r)	Novagen
pBluescript II SK(+)	Cloning vector, (Ap^r)	Invitrogen
pBS:sp^Rt^-	Spr *aad9* determinant with no terminator (sp^Rt^-) in pBluescriptII SK(+), (Ap^r Sp^r)	This study
pBS:sp^Rt^-Δ2006	Sp^r determinant in pBS:sp^Rt^- flanked by SGO_2006 and SGO_2005 regions, (Ap^r Sp^r)	This study
pGEM-7:sp^RΔ2005	Sp^r determinant in pGEM-7:sp^R flanked by SGO_2005 DNA amplicons, (Ap^r Sp^r)	This study
pGEM-7:sp^RΔ2004	sp^r determinant in pGEM-7:sp^R flanked by SGO_2004 DNA amplicons, (Ap^r Sp^r)	This study

Table S2. Primers used in this study

Primer	Primer location in genome	Sequence[a]	Annealing temperature[b]
*Sst*2006usF	2069663-2069686	(TAGAGC)TCGATATTTGTACAGTGCTTGCTC	66
*Bam*2006usR	2069366-2069387	(TAGGATCC*T)AG*CTAGCCCACTCTACTACCAG	66
*Eco*2006dsF	2069078-2069098	(ATGAA)TTCACGATGAAGCAGCGCTTG	62
*Xho*2006dsR	2068668-2068690	(TACTCGA)GTTCTGGACTAACATATGTTGTC	62
2006tfJ	2069902-2069925	GTCTTCGACTGGCTTGAATATAGG	66
*Xho*2005usF	2068881-2068903	(ATCTCG)AGTTGTTCGAGCTGATGATCCTC	66
*Eco*2005usR	2068511-2068535	(AT)GAATTCGCATCAGTATATACATTCG	66
*Hin*d2005dsF	2066528-2066550	(ATAAGCTT)CTGATCTTCAAGGCTAAGATTCG	64
*Sst*2005dsR	2065799-2065821	(ATGAGCT)CTGATAACCGGTTTGTCTGTACC	64
2005tfJ	2069089-2069112	AGTAATGACAGACTTTCACGATGA	64
*Xho*2004usF	2057867-2057888	(TACTC)GAGCTGACACATGTCCCTACTG	62
*Mun*2004usR	2057542-2057563	(ATCAAT)TGAGTCGTAATCTCTGGTTGAG	62
*Hin*d2004dsF	2056062-2056086	(ATAAGCT)TCAAGTAATTTACCATGGCACTATC	64
*Bam*2004dsR	2055682-2055704	(ATGGATC)CTAGAGTATTGAGGTTGTTGTGG	64
2004tfJ	2058006-2058028	CTATGCTCTTCTGGTTACTATCG	64
HP1F	2068889-2068867	(GACGACGACAAGATGG)ATGATCCTCTGAATATTGAGACG	55
HP2R	2067913-2067938	(GAGGAGAAGCCCGGTTT)ATTGCTCATTAACATAGAAGGAAACAT	55
HP3R	2066920-2066943	(GAGGAGAAGCCCGGTTT)AGTCTTTTGTCTTGGCATGGGCATC	55
HP4R	2065006-2065029	(GAGGAGAAGCCCGGTTT)ATTCTTCCAAGGTATCAGAAAGTAC	55

[a] Primers were designed based on the annotated genome sequence of *S. gordonii* CH1 (GenBank accession number CP000725). Some primers were engineered with additional sequence (in parentheses) for cloning e.g. to generate restriction sites (underlined), or to encode an in-frame translational stop codon (italicized).

[b] *S. gordonii* CH1 chromosomal DNA template was amplified by PCR with an initial denaturation of 95 °C, 30 cycles of denaturation at 95 °C, annealing at the noted temperatures and extension at 72 °C, followed by a final extension at 72 °C for 10 minutes.

Table S3. Characteristics of ORFs interrupted in *S. gordonii* DL1 by allelic replacement

S. gordonii CH1 SGO number [a]	ORF size [b]		Leader peptide (aa) [b, c]	Putative functional sub-domain [d]		Region of ORF replaced by *aad9* [b,e, f]		Predicted MW of encoded protein [g]	
	bp	aa		Type	Location in ORF (aa)[b]	bp	aa	Parent	Mutant
SGO_2006	564	187	27-28	thioredoxin	56-172	253-519	85-173	21924	9531
SGO_2005	10941	3646	33-34	vWF type A	72-229	479-2438	161-813	397305	9618
SGO_2004	2424	807	21-22	G5	140-698	431-1885	145-630	86908	18391

[a] ORFs were located in the annotated genome sequence of *S. gordonii* CH1 (GenBank accession number CP000725).
[b] bp and aa indicate base pairs and amino acid residues, respectively.
[c] Predicted signal peptide cleavage site for Gram-positive bacteria, determined using Signal P (3)
[d] The presence of protein families of thioredoxin (PF00085) and vWF (von Willebrand Factor) type A domain (VWA; PF00092) were identified using BlastP (1).The presence of a thioredoxin domain in SGO_2006 and vWA in SGO_2005 suggests that these ORFs may be involved in cytochrome maturation (10) and platelet-binding to subendothelial connective tissue (reviewed in ref. 12). The protein family of G5 (PF07501) was determined using Pfam (5). Seven 78-amino acid G5 direct repeats were found in SGO_2004; the presence of such repeats is implicated in binding to *N*-acetylglucosamine (2).
[e] The *aad9* gene has its own putative promoter region. In allelic replacement of SGO_2005 and SGO_2004, the *aad9* gene had a terminator region; in SGO_2006, the *aad9* gene did not have this terminator.
[f] The reverse primer used to generate the upstream *S. gordonii* DL1 amplicon in SGO_2006 was engineered with a translational stop codon (Table S2), thereby truncating the encoded protein upstream of the *aad9* gene.
[g] Predicted molecular mass (Daltons) of the encoded protein (including the predicted signal peptide) in the *S. gordonii* CH1 parent and respective mutant, in which part of the putative functional sub-domain was replaced by the *aad9* gene

Table S4. Quantitative RT-PCR expression of SGO_2005 and SGO_2004

$\Delta\Delta C_t$ Analysis of SGO_2005

Experiment	Strain	SGO_2005 C_t	*gyrA* C_t	ΔC_t	$\Delta\Delta C_t$	Fold Change $(2^{-(\Delta\Delta Ct)})^a$
1	DL1	25.06	24.43	0.64	0	1
	UB1890	26.74	25.59	1.15	1.52	0.70
	UB1964	25.83	25.27	0.55	-0.08	1.06
2	DL1	25.48	25.72	-0.24	0	1
	UB1968	23.14	23.32	-0.18	0.06	0.96

$\Delta\Delta C_t$ Analysis of SGO_2004

Experiment	Strain	SGO_2004 C_t	*gyrA* C_t	ΔC_t	$\Delta\Delta C_t$	Fold Change $(2^{-(\Delta\Delta Ct)})^a$
1	DL1	24.51	24.43	0.08	0	1
	UB1890	26.55	25.59	0.96	0.88	0.54
	UB1964	26.03	25.27	0.75	0.67	0.63
2	DL1	24.93	25.72	-0.79	0	1
	UB1968	22.62	23.32	-0.70	0.09	0.94

[a] Relative expression normalized to *gyrA* of the mutant strains with disrupted SGO_2004 (strain UB1964), SGO_2005 (strain UB1890) or SGO_2006 (strain UB UB1968) as compared to strain DL1 in same experiment.

Primer	Sequence 5'- 3'	T_m (ºC)
2005 Upstream	AATCTTCGCAATGGCTTCTG	60.36
2005 Downstream	GGCAACCCTATCAAAGATGC	59.54
2004 Upstream	AAGATCAAGCGAAACCAGGA	59.81
2004 Downstream	CAAAGCCATTTGCATGTTCA	60.64
gyrA Upstream	GAACGTCGAACGGAGCTTAT	57.86
gyrA Downstream	ACCTCCACGTTTTTGAGCTG	58.77

SUPPLEMENTAL MATERIALS AND METHODS

RNA was prepared from mid-log *S. gordonii* cells grown in Todd Hewitt broth, as previously described (13). Briefly, cultures were extracted with hot acid-phenol and precipitated RNA was suspended in DEPC water and purified via an RNeasy Midi spin column (QIAGEN). After DNase treatment to remove any remaining DNA, the RNA was purified with a second RNeasy midi spin column, eluted in RNase-free H_2O with 0.4 Units RNaseOUT™ (Invitrogen) /µl to prevent degradation, and stored in portions at -80ºC.

Primer3 software (http://frodo.wi.mit.edu/primer3/input.htm (11) was used to design primers for real-time PCR. Input parameters for primer pairs were a 150-bp target amplicon size, 20-bp primer sequence length, a G-C content of 40-60% for the primer sequences, and an annealing temperature range of 59 - 61ºC. All primers were checked against the *S. gordonii* genome sequence to confirm specificity.

RNA from strains DL1, UB1890,UB1964 and UB1968 were templates in real-time PCR to determine the expression of SGO_2005, SGO_2004 and *gyrA*. The iScript One-Step RT-PCR Kit with SYBR Green (BioRad) was used according to the manufacturer's directions for reverse transcription and labeling of cDNA. Real-time data were collected using the MyiQ™ Single-Color Real-Time PCR Detection System (Bio-Rad). Data were analyzed using the Livak ($2^{-\Delta\Delta Ct}$) Method (9) and expression of SGO_2005 or SGO_2004 were reported as a fold-changes normalized to *gyrA* expression in each strain.

Legends to Supplemental Figures

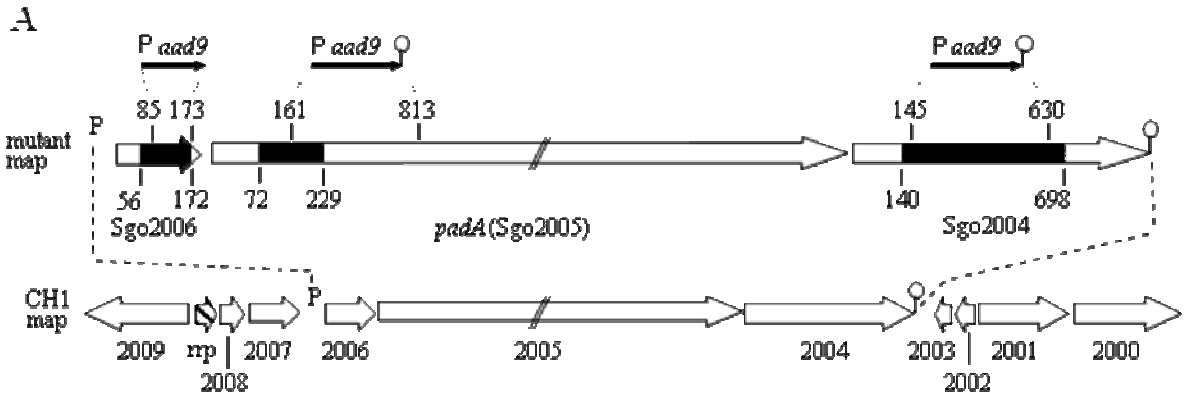
Fig. S1. Panel A: diagrammatic representation of a 19,060-bp *S. gordonii* Challis CH1 (Genbank CP000725) chromosomal region. The arrows in the CH1 map indicate the direction of transcription and relative size and position of each ORF. A 4,500-bp region within SGO_2005 (at 1000-2500 amino acids) is indicated by hatch marks. ORFs designated in the CH1 annotated sequence are labeled with SGO numbers. A predicted promoter (P) sequence was found upstream of SGO_2006 using BPROM software available from Softberry (Mount Kisco, NY; www.softberry.com) and a putative terminator (stemloop) sequence was found downstream of SGO_2004 using mfold (14). The hatched arrow labeled "rrp" is not designated in the current version of the *S. gordonii* CH1 annotated genome. However, this ORF is present and a BlastP search (1), determined that it encodes a 50S ribosomal protein L33 with 84 % amino acid identity to spr1822 (NP_359414) in *S. pneumoniae* R6 (Genbank AE007317). The expanded mutant map above shows details of the location of the putative functional subdomains (in black) of SGO_2006 (thioredoxin domain; PF00085), SGO_2005 (von Willebrand Factor type A domain; PF00092) and SGO_2004 (seven G5 domain direct repeats of 78 amino acids each; PF07501). The amino acid position of these domains is indicated by the numbers below each arrow. The amino acid numbers above each arrow designate the position of the cloned fragment containing the *aad9* gene (encoding spectinomycin resistance, Sp^R), amplified from pGEM7:*aad9* with its native promoter and with (SGO_2005 and SGO_2004), or without (SGO_2006), a transcriptional terminator. Linear fragments from plasmids containing the appropriate homologous streptococcal DNA flanking the directionally-cloned *aad9*-containing amplicon were used to construct three individual gene mutants by allelic replacements in SGO_2006, SGO_2005 and SGO2004. Details of construction of the mutant strains are provided in the **Materials and Methods** section. Panel B: predicted product of each ORF shown in the *S. gordonii* CH1 chromosomal map in panel A.

Fig. S2. Features of PadA (3646 aa residues) primary sequence include: leader peptide (1-33 aa); N-terminal region (blue print, equivalent to Fragment II in Fig. 1) comprising vWF-like domain (72-229 aa, underlined) and two cysteine residues (C55 and C508, purple shaded); fourteen aa residue repeat blocks (1329-3485 aa, green, yellow and blue shading); cell wall anchorage region (3541-3618 aa); sortase motif LPKTG (3614-3618 aa, purple shaded).. For repeat blocks numbers 1 (green shaded), 13 (green shaded) and 14 (shaded light blue) the sequences tend to diverge. Repeat blocks 1-12 each contain 148-152 aa residues, while repeat blocks 13 and 14 contain 176 aa residues and 179 aa residues respectively. More specifically there is duplication of two super repeat blocks each of 755 aa residues consisting of 5 aa residue repeat blocks: repeat blocks 2-6, 1479-2230 aa residues (designated RP1, 755 aa) (red typeface underlined in red) and repeat blocks 7-11, 2231-2982 aa residues (designated RP2, 755 aa) (black typeface underlined in black). The von Willebrand factor type A (vWA) domain is the prototype for a domain of widespread occurrence involved in a variety of cellular functions (4). It possesses no significant sequence similarity to any known protein structure. Secondary structure predictions suggest a largely alternating pattern of six alpha-helices and six beta-strands. A protein fold for this domain is proposed to correspond to a doubly-wound open twisted beta-sheet structure flanked by alpha-helices (α/β para-rossman type of fold). In vWf the domain forms multimers.

Fig. S3. Analysis of recombinant PadA polypeptides by SDS-PAGE stained with Coomassie Blue. Fragment I (346 aa residues) with predicted M_r 36.2 kDa showed retarded migration in SDS-PAGE with apparent M_r approximately 53 kDa. Fragment II (657 aa residues) with predicted M_r 73 kDa migrated at apparent Mr 80 kDa. Fragment III (1295 as residues) showed predicted and apparent M_r 142 kDa. M, molecular mass markers in kilodaltons.

Fig. S4 Glycoprotein (GP) IIb/IIIa transfected CHO cells or mock infected cells (vector only), were incubated with 10 μg/ml phycoerythrin (PE) labelled primary antibody (mouse anti-CD41, clone SZ22) or isotype control antibody for 20 min at room temperature. The samples were then diluted in 1 ml PBS and analyzed on a FACS-CAlibur flow cytometer (San Jose, CA, USA) using CellQuest Pro software. Panel A, separation of mock or GBIIbIIIa transfected cells; panel B, CD41 gated binding levels for mock or GPIIbIIa transfected CHO cells; panel C, Western immunoblot of lysates from mock or transfected cells reacted with anti-CD41 antibody and peroxidase-conjugated anti-mouse secondary antibody, showing GPIIbIIIa expression.

Fig. S5. Alignment of amino acid sequences of vWf (GenBank accession number P04275) and PadA. There is no significant amino acid sequence identity. Shaded yellow is the A1 domain of vWf showing only weak homology to PadA. The motif in vWf interacting with platelet GPIb is highlighted in green. Shaded red is the C1 domain of vWf again showing only weak homology with PadA. The vWf motif interacting with platelet GPIIbIIIa is highlighted in green.



B

SGO_2009: zinc proteinase
rrp: 50S ribosomal protein L33 homolog
SGO_2008: preprotein translocase SecE component-related protein
SGO_2007: transcription termination/antitermination factor NusG
SGO_2006: thioredoxin signature protein
SGO_2005: LPXTG cell wall surface protein (PadA)
SGO_2004: LPXTG cell wall surface protein
SGO_2003: structural tRNA (Cys tRNA)
SGO_2002: hypothetical protein
SGO_2001: *rpsB* ribosomal protein S2
SGO_2000: translation elongation factor Ts

Supplemental material

Figure S1. Structure of the *padA* locus and sites of insertion of *aad9* to generate mutants.

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      Leader peptide
1     MKDFLKKVLILFTVLLMSMPSSVLNLGTSVVRADDPLNIETRRIDEHTTITQNGCYRKIE
61    KTDATDWTVPRKPIDLVILQDASGSFRTTIPSVKNALKRLTTYVSPEQYDENDPHLVKTD  vWF-like domain
121   DPRTTDRVFVASYQGLDQVRYFENNDFSGNPANVYTDANSTGKNYTYGNSGLTSDQNKVH
181   NFIDNIAVDGGTPTVPAIDDTIAQYNRVKGNMENGRKTVFLLVTDGVANGYRLPGTNTVV
241   MDKSWTRTDAIQKAWRVDSYPEAAQDIIGRANELKAAGNQLKAAVGSEGSVVVGFWERVD
301   NFTEKYYQYGPAYLNGFGNTINIGDNRSVQAIFHDALQSMASPDKVVNGKNVSFYVNEQN
361   NIDVFSQKILESVAAALVKDDITGEFDITEGYKVDAIRINGKKIVPKVTDPSKEIRGTIT
421   QTGNKVKISVPDSVFNPGKNSFDYDLSKEARAPETDEDSEVDPPENYVPEKEEITVPELT
481   GKFKAGDFETRQIGGRNQTVEVQKLEYCYPSATKTVKDADASNDIGVIPDPLELTKKPSY
541   SAQLSKKDEEFTYTVDYNFNNVPYEFEKNVMLTDPIDYRLEVVSHSAQGPDGQSWPTRVV
601   TQQDAGGNSQSVVVADVPPQGKDYNYLIMKKAKLKMTVRLKEEYRKNQASKAYLAILQNN
661   NGYGLVNQGNIMWNGEDDSPNQDAHAKTKDKASTIRRSNPIYVKPPLDTEVDKKVNEKEH
721   EGLQADGEEFEYKVTAPWPGIADKFTLTDTVVDELEIVPNSAKVTVAGKSYNALTKAISI
781   NGQTIDITLDKAQLTSLNRLISRRGGSEVQEIELIFKAKIRPGADLSKYKKNGAVNIPNT
841   ADVILNDKKKTSKEVTVTPPKPKEPTVSKKINNTLDSLVTFDGQPYTYNITTAVPSDVAG
901   YKKFVISDKLDADLEFDGQASISGPLADVFEIQTNGQTVTATVKEGKFKELAKYSFVELT
961   IPAKVKAGVTGKTIENKAKISFTNENNVAKEVESNPVTVTPPPVTKKINENLDHLDIATG
1021  QPYKYNVKTTLPSDITSYKEFVITDTLEDELSVINEGTDKPVISGPAAEFFDVTVSGQKV
1081  TATMKNFAGASALAGQEIELVIPAKINDGVTRSNIPNKATFSFKDKNDHKGEKETIPVTV
1141  TPPTEPNVSKKINGDQDNATIAAETDFTYNIKTTLPNDIDTYKSFAITDTLDENLGVVNP
1201  EPSISEEAKKFFDITVSGNTVTATMKDFAKASALANKEIELVIHAKVKKESVLPEIPNTA
1261  KITYTNKNNESKEKETEPVKVTPPPITKKVNGKDQEDLASLTSTFKYTVDSKVPIVADKF
1321  VLSDTLEEVLTFDGDATVTIDGQTVTDVTVAKKDQKLTVTFDKDQVKKYAGKAVQVAFDA
1381  KIKSGYTVDQLVAKYPNGDKAAIPNKASFVVNDNPETEKFSNPVTVTPPPPNTPEIEKKV
1441  NGADSYNLQTRLEEFTYSLNTAMPTNATEFTVTDELKSVLEFAGKKGDVQVKIDGKAAND  RP1 755 aa
1501  QATISTDKNTLTVAFAEKAVKANAGKSIEVTFKAKIREGANLLDYLVPGQGIRIPNKASY
1561  DIDHNPKFHKDSNEVPVTPPSPEQPPIEKDVNDKAEATLEARDEEFTYHVKTKIPYEATA
1621  FNITDTLKEVLDFSGEKGQAEATVDGKKLSDDHIAINGQTITVTLNQEELKANADKEIKL
1681  TFKAKIRPNANLAAYVVGDKVVINNQASYNVDLPDNPGVHKDSNIVPVTPPSPEKPEIEK
1741  TVNDAKEATLANRDEIFTYKVKTKVPFDATAFSIDDTIKDVLEFADAGSATLNGEALEAD
1801  RISIADQKITLTLTEDQVKNNGGKEVVLTFKAKIRQGANLSGYIEKGKTVINNQASYNAA
1861  FPNDPNFHKDSNIVPVTPPNPENPPIEKKVNEAESANLGARDEEFTYTIDTTVPLDVTGF
1921  AVYDTIEKVLEFSGENGQASATVDGQPLDASHITIKGQKITVKLTEDEAKALGGKAVHVS
1981  FKAKIKAGANLSDYIEKDGTTRIYNTAKYNFNNDPGTEQSSKPVPVIPPTPTEPELKKEV
2041  NGKEAETLANRDDVFTYTVKTTVPQDATAFSISDSLVPVLEFAGEDAEASLTLNGEKLDA
2101  KQIKLKDQTISAELTEAQVKANGGKEVVLNFKAKIREGANLADYIEADGVTRVPNKASYV
2161  ANFPHRPKVEKDSNIVPVTPPSPENPPVEKKVNNKPSATLDSRDEEFTYTIDTKVPVDAT
2221  GFKITDELKDVLEFSGKKGQAEVTVDGDKDVIEDSQITVDKQVLTVTLTKDQVKKYGNKA  RP2 755 aa
2281  VHVSFKAKIRKNVSLAGYIEADGVTRIPNIAKYIINDDPKTEKSTEPVPVIPPSPEEPGI
2341  KKEVNGQPEATLKERYEEFTYKVTTSVPQDATAFSVSDTLVPVLEFSGEKGQATATLDGQ
2401  EIDANRINVADQTISMALTEDEVKANGGKEVTLTFKAKIREGANLSAYIEKGKTSIPNTA
2461  SYTAGFPNRPEIHKDSNRVPVTPPTPEEPEIKKDVNGKEEETLANRNDEFTYHINTKVPF
2521  DATAFSINDELKDVLEFADGTGRATASLNGQALDADRISINGQTITVNLTEEQVKNNGGH
2581  DVNLTFTAKIRQGVNLSGYIKDGKTSIPNKASYRVDFPNNPGVTKDSNEVPVTPPSPENP
2641  PIEKKVNEAESANLGARDEEFTYTIDTTVPLDVTGFAVYDTIEKVLEFSGENGQASATVD
2701  GQPLDASHITIKGQKITVKLTEDEAKALGGKAVHVSFKAKIKAGANLSDYIEKDGTTRIY
2761  NTAKYNFNNDPGTEQSSKPVPVIPPTPTEPELKKEVNGKEAETLANRDDVFTYTVKTTVP
2821  QDATAFSISDKLEDVLEFAGESSATLAGEDLKADQITTDGQIIKLTLTEDQVKANGGKEV
2881  VLNFKAKIREGANLSAYMKADKAEVPNKASYTVGFPNKPAVTKDSNEVPVTPPSPEQPPI
2941  EKDVNSKPSETIADRTEEFTYNIHTTMPQDATGFTVTDELKDVLEFAGDVQVTLGGKKAD
3001  AAVAKNGQTLEVTFPEETVKANGGKKVQVTFKAKIKADADLTPYETANSYSVPNTASYLI
3061  NNNPTSKKETKPVTVEVPKQPGPEVTKKINRTLDHLDVDRDVPYMYNVNTQIPKDIRLYK
3121  EFTVTDTLEPVLEITGTPVAYVDGYATDAVETKVEGNTVTVTVKDFARISGYKEIQLYIP
3181  AKLKADSDLSAYENQTVPNKATIAFKDSNGKNGTKESNPVTVRPRDPEKPEEPKPNEPAK
3241  TVGPADGSNPSTAYRLKELKEGFRFDVTAKVPTDPVDESGNPIKDAQGRDVKTELNSFTV
3301  TDELEKVLKVDRVAVKVEENKVAEAIAKITAKIEKAESDLKELEGKETNGTFAKKLAEAE
3361  KKVEELTAQLAAAKEKAAAAPATPAPASDSDAGNATATPAPADNNAEVAALEESLKAAQA
3421  ELEQLKADGAKAGNLATPEEQKVEQDKLNKNLEQLKESKEKLEKALEAFTTVNDKGEITD
3481  EALAKIAKVTVEGQKVTVEVTDKAVLEALKGSTFRVIIYSSIKDGADLSSYLNKENNETK
3541  IPNKATVTFNDKPKVTNTVNVYPPEPTTPPQTPPHTPPTTPGTPPPTTPDTPPAPKGDLP
3601  PAPTPEPEKPKNILPKTGTSATMVNEVIIGMILVLMGLLLRRKPKH                CWA
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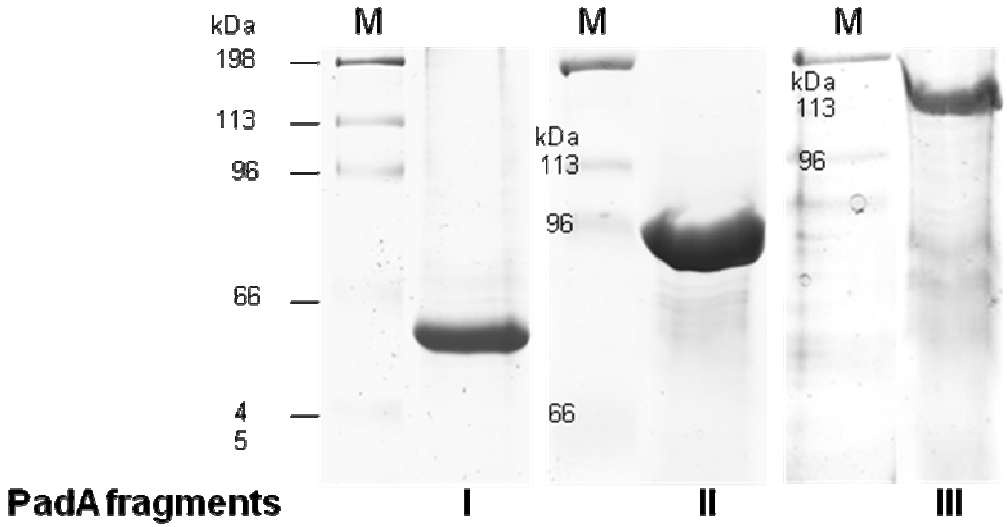
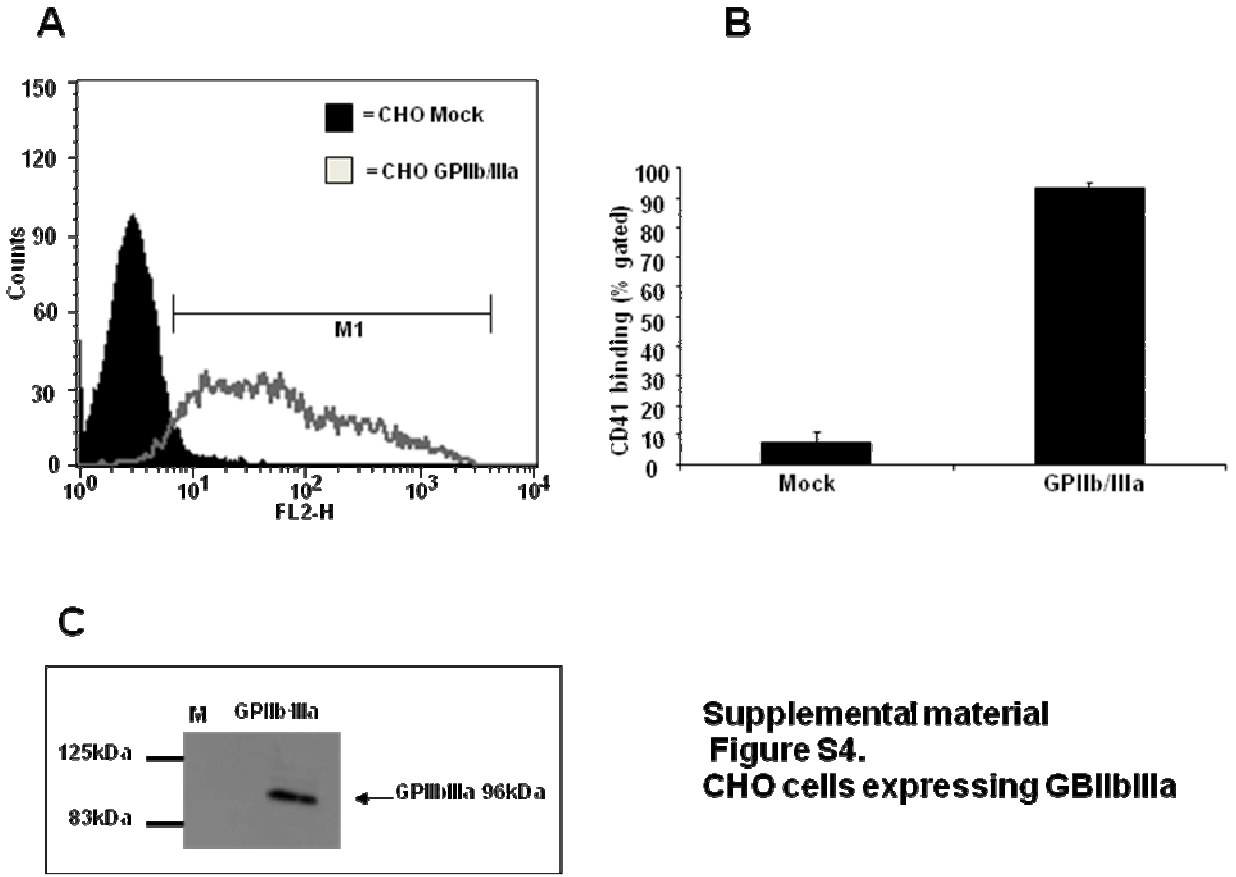
Supplemental material
Figure S2. Primary sequence of PadA highlighting regions of interest and potential functional significance



Supplemental material
Figure S3.
SDS-PAGE gels of purified PadA protein fragments I. II and III.



Supplemental material
Figure S4.
CHO cells expressing GBIIbIIIa

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vWf             --MIPARFAGVLLALALILPGTLCAEGTRGRSSTARCSLFG------------------- 39
PadA            MKDFLKKVLILFTVLLMSMPSSVLNLGTSVVRADDPLNIETRRIDEHTTITQNGCYRKIE 60
                   :  :.  :: .* : :*.::   **    :    .:

vWf             ----------------------SDFVNTFDGSMYSFAGYCSYLLAGGCQKRSFSIIG-- 74
PadA            KTDATDWTVPRKPIDLVILQDASGSFRTTIPSVKNALKRLTTYVSPEQYDENDPHLVKTD 120
                                    ..* .*: .   ::    :*: .   ::..  ::

vWf             DFQNGKRVSLSVYLGEFFDIHLFVNGTVTQGDQRVSMPYASKGLYLETEAGYYKLSGEAY 134
PadA            DPRTTDRVFVASYQGLDQVRYFENNDFSGNPANVYTDANSTGKNYTYGNSGLTSDQNKVH 180
                * :. .** :: * *     ::  *.   :  :  : . ::   *   ::*  . ..:.:

vWf             GFVARIDGSG--------------------NFQVLLSDRYFNKTCGLCGNFNIFAEDDFM 174
PadA            NFIDNIAVDGGTPTVPAIDDTIAQYNRVKGNMENGRKTVFLLVTDGVANGYRLPGTNTVV 240
                .*: .*  .*                   *::   .  ::  * *:...:.: . : .:

vWf             TQEGTLTSDPYDFAN--------SWALSSGEQWCERASPPSSSCNISSGEMQKGLWEQCQ 226
PadA            MDKSWTRTDAIQKAWRVDSYPEAAQDIIGRANELKAAGNQLKAAVGSEGSVVVGFWERVD 300
                 ::.   :*. : *         :  : .  :  : *.   .:.  *.*.:  *:**: :

vWf             LLKS---------TSVFARCHPLVDPEPFVALCEKTLCECAGGLECACPALLEYARTCAQ 277
PadA            NFTEKYYQYGPAYLNGFGNTINIGDNRSVQAIFHDALQSMASPDKVVNGKNVSFYVNEQN 360
                 :..          . *..   : * ... *: ..:* . *.  : .    :.:  .  :

vWf             EGMVLYGWTDHSACSPVCPAGMEYRQCVSPCARTCQSLHINEMCQERCVDGCSCPEGQLL 337
PadA            NIDVFSQKILESVAAALVKDDITGEFDITEGYKVDAIRINGKKIVPKVTDPSKEIRGTIT 420
                :  *:     .*..:.:   .:  .  ::   :.      .:    : .* ..  .* :

vWf             DEGLCVESTECPCVHSGKRYPPGTSLSRDCNTCICRNSQWICSNEECPG------ECLVT 391
PadA            QTGNKVKISVPDSVFNPGKNSFDYDLSKEARAPETDEDSEVDPPENYVPEKEEITVPELT 480
                : *  *: :   .*..  : . . .**::..:    :.. : . *:           :*

vWf             GQSHFKSFDNRYFTFSGICQYLLARDCQDHSFSIVIETVQCADDRDAVCTRSVTVRLPGL 451
PadA            GKFKAGDFETRQIGGRNQTVEVQKLEYCYPSATKTVKDADASNDIGVIPDPLELTKKPSY 540
                *: :  .*:.* :   .    :   :    * : .:: .:.::* ..:      .: *.

vWf             HNSLVKLKHGAGVAMDG------------------QDIQLPLLKGDLRIQHTVTASVRLS 493
PadA            SAQLSKKDEEFTYTVDYNFNNVPYEFEKNVMLTDPIDYRLEVVSHSAQGPDGQSWPTRVV 600
                  .* * ..    ::*                   * :* ::. . :  .  : ..*:

vWf             YGEDLQMDWD----------GRGRLLVKLSPVYAGKTCGLCGNYNGNQG----------- 532
PadA            TQQDAGGNSQSVVVADVPPQGKDYNYLIMKKAKLKMTVRLKEEYRKNQASKAYLAILQNN 660
                  :*   : :          *:.   : :. .    *  *  :*. **.

vWf             --------DDFLTPSGLAEPRVEDFGNAWKLHGDCQDLQKQHSDPCALNPRMTRFSEEAC 584
PadA            NGYGLVNQGNIMWNGEDDSPNQDAHAKTKDKASTIRRSNPIYVKPPLDTEVDKKVNEKEH 720
                        .:::  .   .*. : ..:: .  .  :  :  : .*   .   .:..*:

vWf             AVLTSPTFEACHRAVSPLPYLRNCRYDVCSCSDGRECLCG-----------ALASYAAAC 633
PadA            EGLQADGEEFEYKVTAPWPGIADKFTLTDTVVDELEIVPNSAKVTVAGKSYNALTKAISI 780
                  * :   *  ::..:* * : :    . :  *  * : .             : * :

vWf             AGRGVRVAWREPGRCELNCPKGQVYLQCGTPCNLTCRSLSYPDEECNEACLEGCFCPP-- 691
PadA            NGQTIDITLDKAQLTSLNRLISRRGGSEVQEIELIFKAKIRPGADLSKYKKNGAVNIPNT 840
                 *: : ::  :.   .**   .:   .     :*  ::   *. : .:   :*..  *

vWf             -GLYMDERGDCVPKAQCPCYYDGEIFQPEDIFSDHHTMCYCEDG-FMHCTMSGVPGSLLP 749
PadA            ADVILNDKKKTSKEVTVTPPKPKEPTVSKKINNTLDSLVTFDGQPYTYNITTAVPSDVAG 900
                 .: :::: .   :.  .     *   .:.* .  .::   :.  : :   :.**..:

vWf             DAVLSSPLSHRSKRSLSCRPPMVKLVCPADNLRAEGLECTKTCQNYDLECMSMGCVSGCL 809
PadA            YKKFVISDKLDADLEFDGQASISGPLADVFEIQTNGQTVTATVKEGKFKELAKYSFVELT 960
                   :  . .  :. .:. :..:   :. . :::::*   * * :: .:: ::  ..

vWf             CPPGMVR------------------------HENRCVALERCPCFHQGKE------YAPG 839
PadA            IPAKVKAGVTGKTIENKAKISFTNENNVAKEVESNPVTVTPPPVTKKINENLDHLDIATG 1020
                 *. :                           *.. *::   *  :: :*       *.*

vWf             ETVKIGCNTCVCRDRK----WNCTDHVCDATCSTIGMAHYLTFDG-----LKYLFPGECQ 890
PadA            QPYKYNVKTTLPSDITSYKEFVITDTLEDELSVINEGTDKPVISGPAAEFFDVTVSGQKV 1080
                :. * . :* :  * .    :  ** : *  .     :.  .:.*     :.  ..*:
```

```
vWf             YVLVQDYCGS----------------NPGTFRILVGNKGCSHPSVKCKKR---------V 925
PadA            TATMKNFAGASALAGQEIELVIPAKINDGVTRSNIPNKATFSFKDKNDHKGEKETIPVTV 1140
                 . ::::.*:               * *. *  : **.    . * .::         *

vWf             TILVEGGEIELFDG---------EVNVKRPMKDETHFEVVESGRYIILLLGKALSVVWDR 976
PadA            TPPTEPNVSKKINGDQDNATIAAETDFTYNIKTTLPNDIDTYKSFAITDTLDENLGVVNP 1200
                *  .* .  : ::*         *.:..  :*     ::     : *    .    * :

vWf             HLSISVVLKQTYQEKVCG----------LCGNFDGIQNNDLTSSNLQVEEDPVDFGNSW 1025
PadA            EPSISEEAKKFFDITVSGNTVTATMKDFAKASALANKEIELVIHAKVKKESVLPEIPNTA 1260
                . ***   *: :: .*.*            .. : . : : :  :::: *.   :: *:

vWf             KVSSQCADTRKVPLDSSPATCHNNIMKQTMVDSSCR----------ILTSDVFQDCNKL 1074
PadA            KITYTNKNNESKEKETEPVKVTPPPITKKVNGKDQEDLASLTSTFKYTVDSKVPIVADKF 1320
                *::    :...   ::.*..     :.:.: ... .            : *.*   .:*:

vWf             VDPEPYLDVCIYDTCSCESIG---------------------------------------- 1095
PadA            VLSDTLEEVLTFDGDATVTIDGQTVTDVTVAKKDQKLTVTFDKDQVKKYAGKAVQVAFDA 1380
                * .:.  :*  :*  :  :*.

vWf             ------------------DCACFCDTIAAYAHVCAQHGKVVTWRTATLCP---------- 1127
PadA            KIKSGYTVDQLVAKYPNGDKAAIPNKASFVVNDNPETEKFSNPVTVTPPPPNTPEIEKKV 1440
                                 * *.: :. :  .:  .:  *. .  *.*  *

vWf             QSCEERNLRENGYECEWRYNSCAPACQVTCQHPEPLACPVQCVEG---------CHAHCP 1178
PadA            NGADSYNLQTRLEEFTYSLNTAMPTNATEFTVTDELKSVLEFAGKKGDVQVKIDGKAAND 1500
                :..:. **: .  *  :  *:. *:  .    .: * . :: .            :*

vWf             PGKILDELLQTCVDPEDCPVCEVAGRRFASGKKVTLNPSDPEHCQICHCDVVNLTCEACQ 1238
PadA            QATISTDKNTLTVAFAEKAVKANAGKSIEVTFKAKIREGANLLDYLVPGQGIRIPNKASY 1560
                 ..*  :     *   : .*   **: :    *..:. .      :   : :.:. :*.

vWf             EPGG----------LVVPPTDAPVSPTTLYVEDISEPPLHD------------------- 1269
PadA            DIDHNPKFHKDSNEVPVTPPSPEQPPIEKDVNDKAEATLEARDEEFTYHVKTKIPYEATA 1620
                : .           : *.*...  .*    *:* :*..*.

vWf             FYCSRLLDLVFLLDGSSRLSEAEFEVLKAFVVDMMERLRISQKWVRVAVVEYHDGSHAYI 1329
PadA            FNITDTLKEVLDFSGEKGQAEATVDGKKLSDDHIAINGQTITVTLNQEELKANADKEIKL 1680
                *  :  *. *: :.*..  :** .:  *    .:  . :     :.   :: : ...  :

vWf             GLKDRKRPSE--LRRIASQVKYAGSQVASTSEVLKYTLFQIFSKID-----RPEASRIAL 1382
PadA            TFKAKIRPNANLAAYVVGDKVVINNQASYNVDLPDNPGVHKDSNIVPVTPPSPEKPEIEK 1740
                 :* : **.      :..:    ..*.: . :: . . .:  *:*       ** ..*

vWf             LLMASQEPQRMSRNFVRYVQGLKKKKVIVIPVGIGPHANLKQIRLIEKQAPENKAFVLSS 1442
PadA            TVNDAKEATLANRDEIFTYKVKTKVPFDATAFSIDDTIKDVLEFADAGSATLNGEALEAD 1800
                 :  ::*.   .*: :   :  .*  . . ...*.   :         .*. *   : :.

vWf             VDELEQQRDEIVSYLCDLAPEAPPPTLPPHMAQVTVGPGLLGVSTLG-------PKRNSM 1495
PadA            RISIADQKITLTLTEDQVKNNGGKEVVLTFKAKIRQGANLSGYIEKGKTVINNQASYNAA 1860
                  .: :*:  :.    ::  :.   .: .. *::  *..* *    *       .. *:

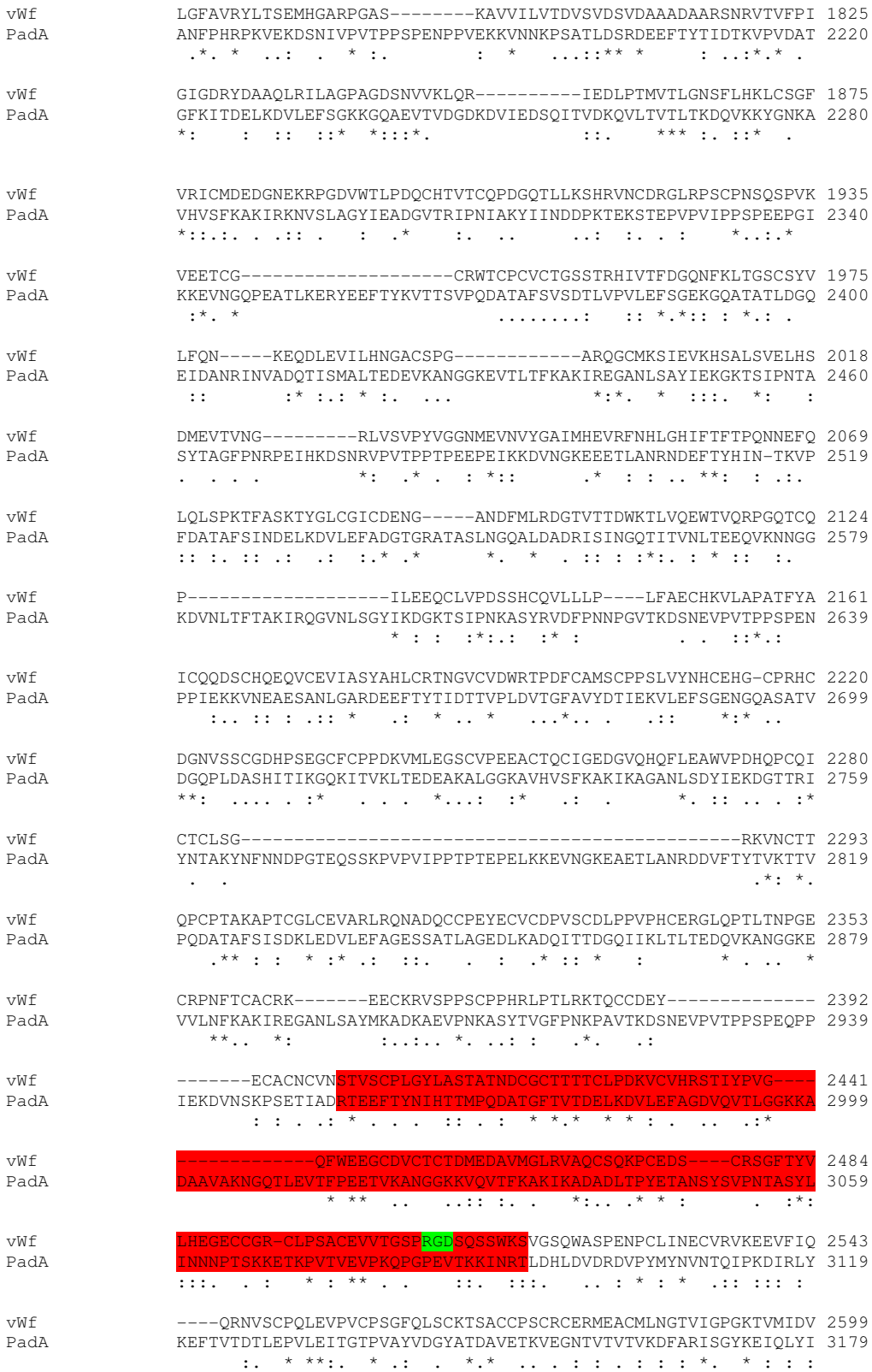
vWf             VLDVAFVLEGSDKIGEADFNRSKEFMEEVIQRMDVG-------------QDSIHVTVLQY 1542
PadA            FPNDPNFHKDSNIVPVTPPNPENPPIEKKVNEAESANLGARDEEFTYTIDTTVPLDVTGF 1920
                . : . . :.*: :  :  * .:  :*: ::. : .             : :: : *  :

vWf             SYMVTVEYPFSEAQSKGDILQRVREIRYQGGNRTNTGLALRYLSDHSFLVSQGDREQAPN 1602
PadA            AVYDTIEKVLEFSGENGQASATVDGQPLDASHITIKGQKITVKLTEDEAKALGGKAVHVS 1980
                :   *:*  :. : .:*:    *     :..: * .*  :     ..   : *.:    .

vWf             LVYMVTGNPASDEIKRLPGDIQVVPIGVGPNANVQELERIG--------WPNAPILIQDF 1654
PadA            FKAKIKAGANLSDYIEKDGTTRIYNTAKYNFNNDPGTEQSSKPVPVIPPTPTEPELKKEV 2040
                :   :....  .:  .  *  ::   .     *    *: .         *. * * ::.

vWf             ETLPREAPDLVLQRCCSGEGLQIPTLSPAPDCSQPLDVILLLDGSSSFPASYFDEMKSFA 1714
PadA            NGKEAETLANRDDVFTYTVKTTVPQDATAFSISDSLVPVLEFAGEDAEASLTLNGEKLDA 2100
                :    *:     :         :*  :.* . *:.*  :* : *..: .:  ::  *  *

vWf             KAFISKAN-IGPRLTQVSVLQYGSITTIDVPWNVVPEKAHLLSLVDVMQREGGPSQIGDA 1773
PadA            KQIKLKDQTISAELTEAQVKANGGKEVVLNFKAKIREGANLADYIEADGVTRVPNKASYV 2160
                * :  * : *...**:..*   *.  .:      : * *:* . ::.      *.: . .
```


vWf LGFAVRYLTSEMHGARPGAS--------KAVVILVTDVSVDSVDAAADAARSNRVTVFPI 1825
PadA ANFPHRPKVEKDSNIVPVTPPSPENPPVEKKVNNKPSATLDSRDEEFTYTIDTKVPVDAT 2220
vWf GIGDRYDAAQLRILAGPAGDSNVVKLQR----------IEDLPTMVTLGNSFLHKLCSGF 1875
PadA GFKITDELKDVLEFSGKKGQAEVTVDGDKDVIEDSQITVDKQVLTVTLTKDQVKKYGNKA 2280
vWf VRICMDEDGNEKRPGDVWTLPDQCHTVTCQPDGQTLLKSHRVNCDRGLRPSCPNSQSPVK 1935
PadA VHVSFKAKIRKNVSLAGYIEADGVTRIPNIAKYIINDDPKTEKSTEPVPVIPPSPEEPGI 2340
vWf VEETCG----------CRWTCPCVCTGSSTRHIVTFDGQNFKLTGSCSYV 1975
PadA KKEVNGQPEATLKERYEEFTYKVTTSVPQDATAFSVSDTLVPVLEFSGEKGQATATLDGQ 2400
vWf LFQN-----KEQDLEVILHNGACSPG------------ARQGCMKSIEVKHSALSVELHS 2018
PadA EIDANRINVADQTISMALTEDEVKANGGKEVTLTFKAKIREGANLSAYIEKGKTSIPNTA 2460
vWf DMEVTVNG---------RLVSVPYVGGNMEVNVYGAIMHEVRFNHLGHIFTFTPQNNEFQ 2069
PadA SYTAGFPNRPEIHKDSNRVPVTPPTPEEPEIKKDVNGKEEETLANRNDEFTYHIN-TKVP 2519
vWf LQLSPKTFASKTYGLCGICDENG-----ANDFMLRDGTVTTDWKTLVQEWTVQRPGQTCQ 2124
PadA FDATAFSINDELKDVLEFADGTGRATASLNGQALDADRISINGQTITVNLTEEQVKNNGG 2579
vWf P----------ILEEQCLVPDSSHCQVLLLP----LFAECHKVLAPATFYA 2161
PadA KDVNLTFTAKIRQGVNLSGYIKDGKTSIPNKASYRVDFPNNPGVTKDSNEVPVTPPSPEN 2639
vWf ICQQDSCHQEQVCEVIASYAHLCRTNGVCVDWRTPDFCAMSCPPSLVYNHCEHG-CPRHC 2220
PadA PPIEKKVNEAESANLGARDEEFTYTIDTTVPLDVTGFAVYDTIEKVLEFSGENGQASATV 2699
vWf DGNVSSCGDHPSEGCFCPPDKVMLEGSCVPEEACTQCIGEDGVQHQFLEAWVPDHQPCQI 2280
PadA DGQPLDASHITIKGQKITVKLTEDEAKALGGKAVHVSFKAKIKAGANLSDYIEKDGTTRI 2759
vWf CTCLSG----------RKVNCTT 2293
PadA YNTAKYNFNNDPGTEQSSKPVPVIPPTPTEPELKKEVNGKEAETLANRDDVFTYTVKTTV 2819
vWf QPCPTAKAPTCGLCEVARLRQNADQCCPEYECVCDPVSCDLPPVPHCERGLQPTLTNPGE 2353
PadA PQDATAFSISDKLEDVLEFAGESSATLAGEDLKADQITTDGQIIKLTLTEDQVKANGGKE 2879
vWf CRPNFTCACRK-------EECKRVSPPSCPPHRLPTLRKTQCCDEY---------- 2392
PadA VVLNFKAKIREGANLSAYMKADKAEVPNKASYTVGFPNKPAVTKDSNEVPVTPPSPEQPP 2939
vWf -------ECACNCVNSTVSCPLGYLASTATNDCGCTTTTCLPDKVCVHRSTIYPVG---- 2441
PadA IEKDVNSKPSETIADRTEEFTYNIHTTMPQDATGFTVTDELKDVLEFAGDVQVTLGGKKA 2999
vWf ----------QFWEEGCDVCTCTDMEDAVMGLRVAQCSQKPCEDS----CRSGFTYV 2484
PadA DAAVAKNGQTLEVTFPEETVKANGGKKVQVTFKAKIKADADLTPYETANSYSVPNTASYL 3059
vWf LHEGECCGR-CLPSACEVVTGSPRGDSQSSWKSVGSQWASPENPCLINECVRVKEEVFIQ 2543
PadA INNNPTSKKETKPVTVEVPKQPGPEVTKKINRTLDHLDVDRDVPYMYNVNTQIPKDIRLY 3119
vWf ----QRNVSCPQLEVPVCPSGFQLSCKTSACCPSCRCERMEACMLNGTVIGPGKTVMIDV 2599
PadA KEFTVTDTLEPVLEIITGTPVAYVDGYATDAVETKVEGNTVTVTVKDFARISGYKEIQLYI 3179

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vWf             CTTCRCMVQVGVIS------------------GFKLECRKTTCNPCPLGYKEENNTGECC 2641
PadA            PAKLKADSDLSAYENQTVPNKATIAFKDSNGKNGTKESNPVTVRPRDPEKPEEPKPNEPA 3239
                 :. :.  ::.. .                 . . *.. .* .*      ** :..* .

vWf             GRCLPTACTIQLRGGQIMTLKRDETLQDGCDTHFCKVNERGEYFWEKR------------ 2689
PadA            KTVGPADGSNPSTAYRLKELKEGFRFDVTAKVPTDPVDESGNPIKDAQGRDVKTELNSFT 3299
                    *:  :    . ::  **..  ::  ...    *:* *: : : :

vWf             ------------------------------------------------------------
PadA            VTDELEKVLKVDRVAVKVEENKVAEAIAKITAKIEKAESDLKELEGKETNGTFAKKLAEA 3359

vWf             ----------------------------------------------------VTGCPP 2695
PadA            EKKVEELTAQLAAAKEKAAAAPATPAPASDSDAGNATATPAPADNNAEVAALEESLKAAQ 3419
                                                                       ..

vWf             FDEHKCLAEGGKIMKIPGTCCDTCEEPECNDITARLQYVKVGSCKSEVEVDIHYCQGKCA 2755
PadA            AELEQLKADGAKAGNLATPEEQKVEQDKLNKNLEQLKESKEKLEKALEAFTTVNDKGEIT 3479
                 : .:  *:*.*  ::. .  :. *: : *.   :*:  *    *:   .     :*: :

vWf             SKAMYSIDINDVQDQCSCCSPTRTEPMQVALHCTNGSVVYHEVLNAMECKCSPRKCSK-- 2813
PadA            DEALAKIAKVTVEGQKVTVEVTDKAVLEALKGSTFRVIIYSSIKDGADLSSYLNKENNET 3539
                .:*: .*    *:.*    . * .  ::.   .*   ::* .: :. : ..  .* .:

vWf             ------------------------------------------------------------
PadA            KIPNKATVTFNDKPKVTNTVNVYPPEPTTPPQTPPHTPPTTPGTPPPTTPDTPPAPKGDL 3599

vWf             ------------------------------------------------
PadA            PPAPTPEPEKPKNILPKTGTSATMVNEVIIGMILVLMGLLLRRKPKH 3646
```

Supplemental material

Figure S5. Alignment of vWf and PadA amino acid sequences

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