In silico guided reconstruction and analysis of ICAM-1-binding *var* genes from *Plasmodium falciparum*.

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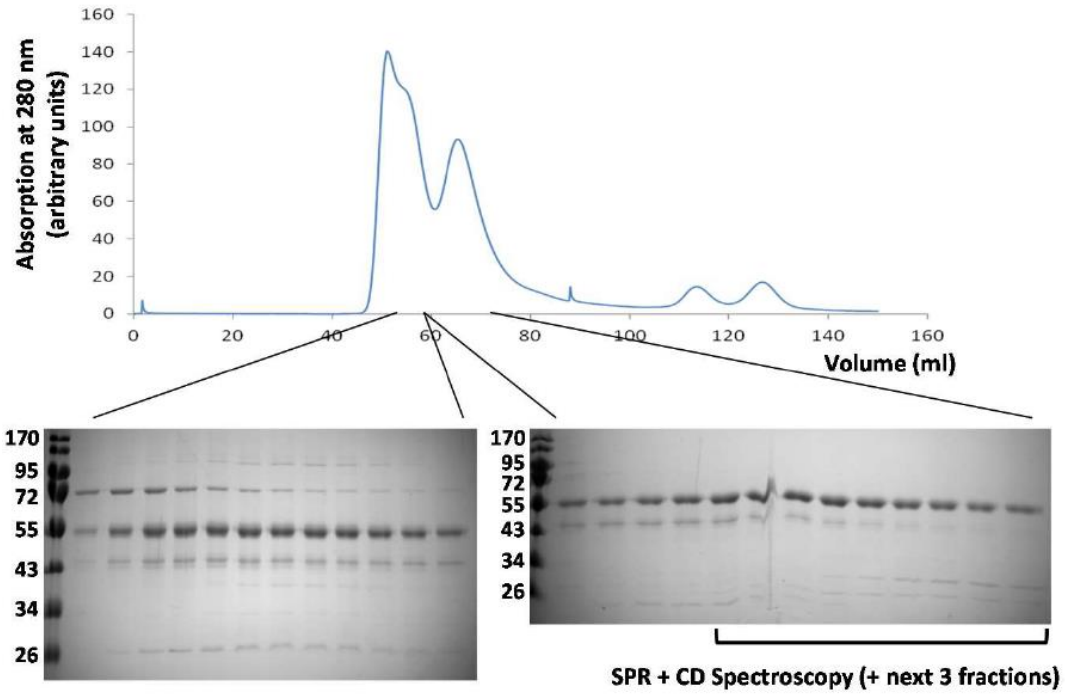
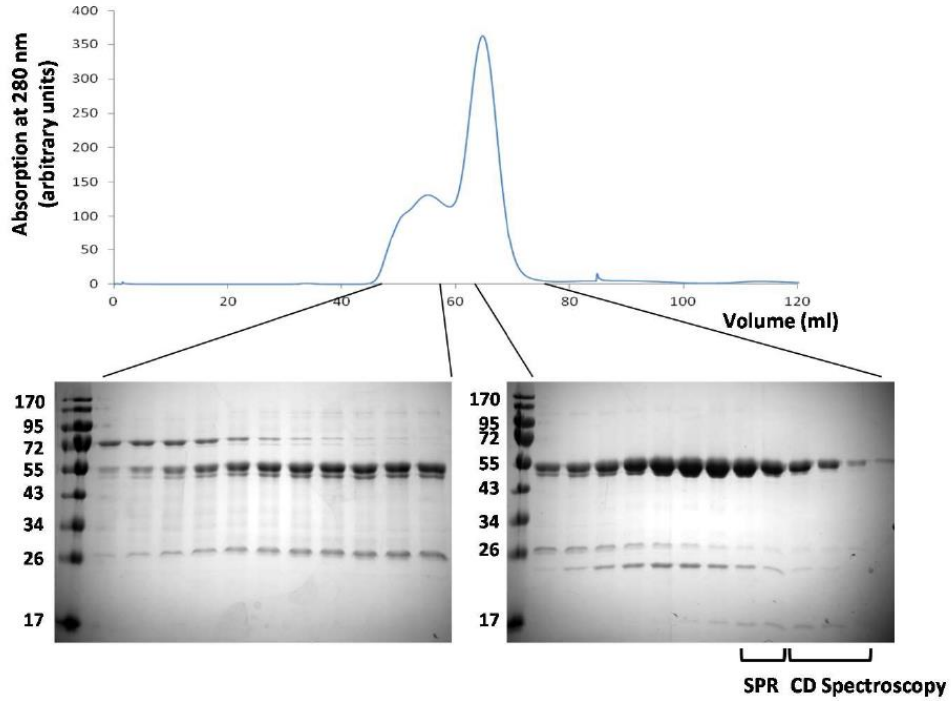
Supplementary Information



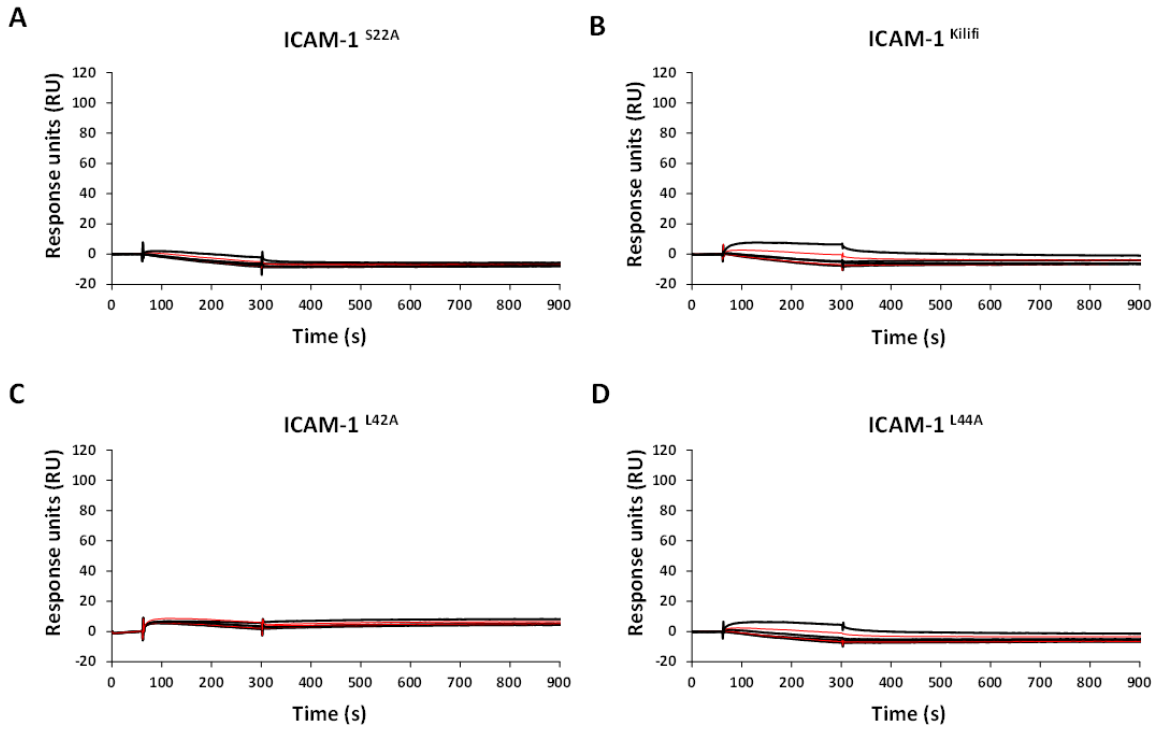
S1 Fig. Purification of BC12aDBLβ by gel filtration.



S2 Fig. Purification of J1aDBLβ by gel filtration.

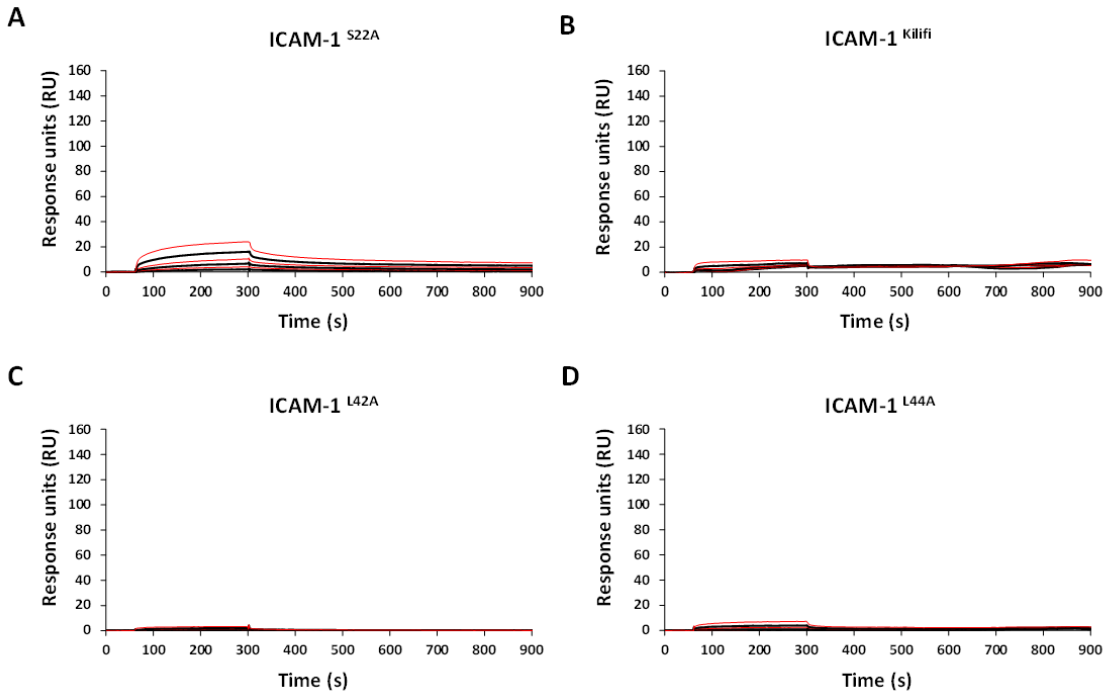
For S1 Fig and S2 Fig, DBLβ domains were passed through a HiLoad 16/600 Superdex 75 prep grade column (GE healthcare) and 1 ml fractions collected between 38 and 100 ml. For the BC12DBLβ gel filtration, SDS-PAGE of samples is from fractions 11-22 (left) and 23-35 (right). Fractions pooled for

use in SPR and CD spectroscopy are indicated (black bar). For J1aDBLβ gel filtration, SDS-PAGE of samples is from fractions 11-21 (left) and 22-34 (right). Fractions pooled for use in SPR and CD spectroscopy are indicated (black bars).



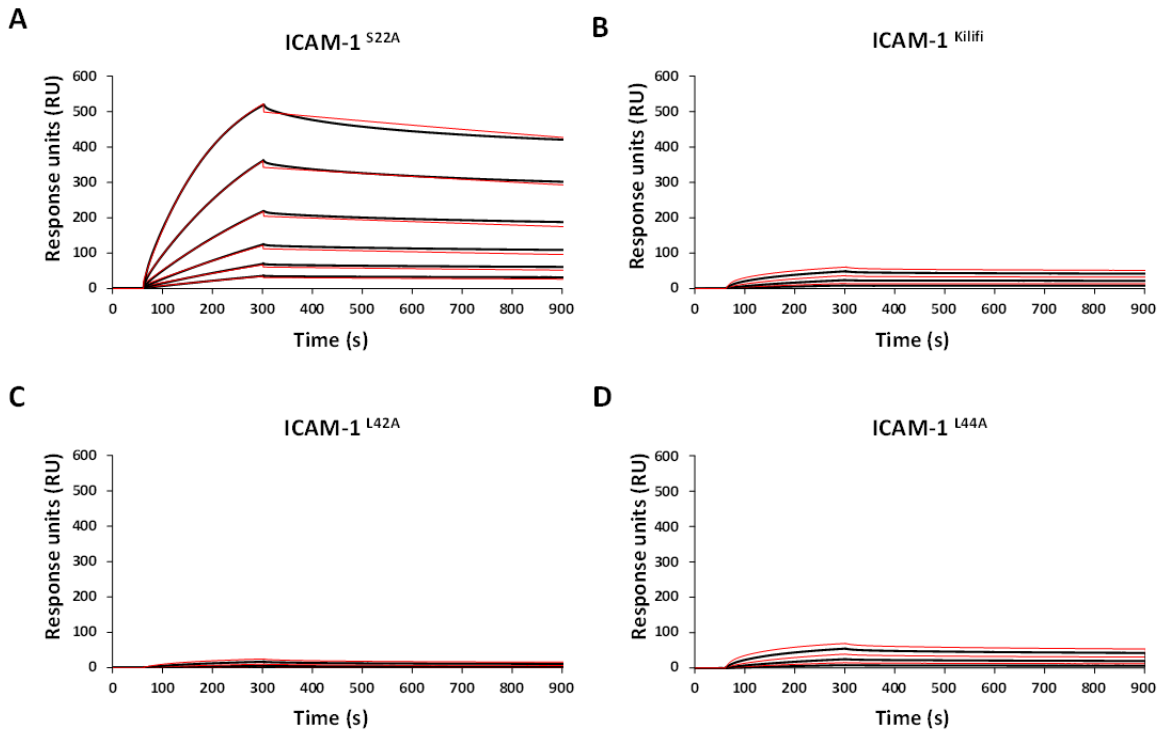
S3 Fig. BC12a$^{DBL\beta}$ response to ICAM-1 mutant proteins.

ICAM-1 mutant proteins were coupled to a sensor chip surface (1000 RU) and BC12a$^{DBL\beta}$ was injected at 30 µl/min with an association time of 240 seconds and a dissociation time of 600 seconds. Shown are sensorgrams for the binding of BC12a$^{DBL\beta}$ to ICAM-1^{S22A} (A), ICAM-1^{Kilifi} (B), ICAM-1^{L42A} (C) and ICAM-1^{L44A} (D). Data (black lines) are modelled to a 1:1 global interaction model (red lines). Data shown in S5 Data.



S4 Fig. J1a$^{DBL\beta}$ response to ICAM-1 mutant proteins.

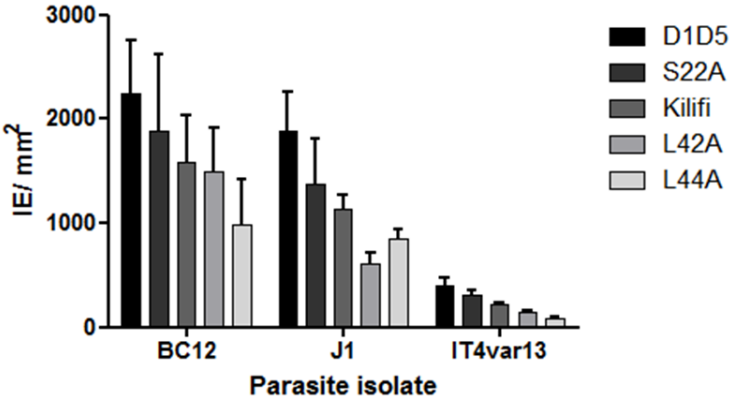
ICAM-1 mutant proteins were coupled to a sensor chip surface (1000 RU) and J1a$^{DBL\beta}$ was injected at 30 µl/min with an association time of 240 seconds and a dissociation time of 600 seconds. Shown are sensorgrams for the binding of J1a$^{DBL\beta}$ to ICAM-1^{S22A} (A), ICAM-1^{Kilifi} (B), ICAM-1^{L42A} (C) and ICAM-1^{L44A} (D). Data (black lines) are modelled to a 1:1 global interaction model (red lines). Data shown in S6 Data.



S5 Fig. IT4var13$^{DBL\beta}$ response to ICAM-1 mutant proteins.

ICAM-1 mutant proteins were coupled to a sensor chip surface (1000 RU) and IT4var13$^{DBL\beta}$ was injected at 30 µl/min with an association time of 240 seconds and a dissociation time of 600 seconds. Shown are sensorgrams for the binding of IT4var13$^{DBL\beta}$ to ICAM-1^{S22A} (A), ICAM-1^{Kilifi} (B), ICAM-1^{L42A} (C) and ICAM-1^{L44A} (D). Data (black lines) are modelled to a 1:1 global interaction model (red lines). Data shown in S7 Data.

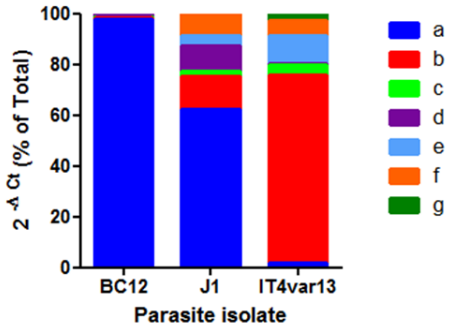
A



B

	ICAM-1^{S22A}	ICAM-1^{Kilifi}	ICAM-1^{L42A}	ICAM-1^{L44A}
BC12 (n=3)	0.708	0.387	0.318	0.134
J1 (n=3)	0.428	0.139	0.033	0.059
IT4var13 (n=4)	0.325	0.061	0.016	0.007

C



S6 Fig. Adhesion of BC12, J1 and IT4var13 parasite isolates to ICAM-1 proteins under flow conditions.

(A): Individual biochip channels were coated with 4 µl of 50 µg/ml of either ICAM-1^{D1D5}, ICAM-1^{S22A}, ICAM-1^{Kilifi}, ICAM-1^{L42A} or ICAM-1^{L44A}. Recently ICAM-1-selected IEs were passed through the channel at 3% parasitaemia, 2% haematocrit for 8 minutes. Bound parasites were counted in 7-10 fields and adjusted to IE/mm^2. Bars represent the mean of 3 (BC12, J1) or 4 (IT4var13) independent experiments with the standard error of the mean (SEM) shown. Binding data shown in S1 Data. (B):

P-values as a result of unpaired t-tests comparing each ICAM-1 mutant protein to the wild type ICAM-1^{D1D5}. (C): RT-qPCR of cDNA isolated from each parasite culture. RT-qPCR was carried out using BC12 and J1 DBLα tag primers (a-e and a-f, respectively) and primers to IT4 ICAM-1 binding *var* genes (a: 01, b:13, c:14, d:16, e:27, f:31, g:41). Primers are listed in Table S1. Ct values were normalised against the ASL internal control gene to give $2^{-\Delta Ct}$ values and are shown as percentage of total for each isolate.

Table S1. Primer sequences

DBLα tag, UPS and Exon 2 previously published primers

Target	T_A (°C)	Primer name	Sequence 5'-3'
DBLα tag †	47	DBLαAF'	GCACG(A/C)AGTTT(C*/T)GC
DBLα tag †	47	DBLαBR	GCCCATTC(G/C)TCGAACCA
UPS A ‡	52	upsA-5'	ATTAYATTTGTTGTAGGTGA
UPS B ‡	52	17DBLa-5'	ATGTAATTGTTGTTTTTTTTTTTGTTAGAATATTTAAA
UPS C ‡	52	5B1-5'	CACATATARTACGACTAAGAAACA
Exon 2 ᵹ	48	Ex2-reg	TCTTCATAYTCRCTTTC

† (Bull *et al.*, 2005), ‡ (Mugasa *et al.*, 2012), ᵹ (Lavstsen *et al.*, 2012)

ICAM-1 binding isolate RT-qPCR primers

Target	T_A (°C)	Primer name	Sequence 5'-3'
DBLα tag BC12a	60	E-195F	AAGCGGAAAAACACTACGAAGAT
DBLα tag BC12a	60	E-196R	TAGCCATAGATGGACTTTCACTA
DBLα tag BC12b	60	E-197F	AAGCGCAACACAAAAGCATTATGCA
DBLα tag BC12b	60	E-198R	TTTGTTATATGTTATATTTCCTCCATTACA
DBLα tag BC12d	60	E-201F	GACGAGAGGGAGGACGAA
DBLα tag BC12d	60	E-202R	ATTATCACCACCACATGTTTTTCGA
DBLα tag BC12e	60	E-203F	AGATCTCGCTACAAAAAAGACGGT
DBLα tag BC12e	60	E-204R	TAGCAGTCGTACCGTATGTGAT
DBLα tag BC12f	60	E-205F	ACGGATCCCAAAGCGAAAGA
DBLα tag BC12f	60	E-206R	TTGGGCTCCACTTTGTCCAT
DBLα tag J1a	60	E-183F	TCGTACTACAAAAATGATAATGACCG
DBLα tag J1a	60	E-184R	ATGCCCACCCTTAATGGAAGA
DBLα tag J1b	60	E-185F	AGACGAATGGGAAGGTACCAGA

DBLα tag J1b	60	E-186R	AGCCGAACTTCCTCCTTCAGA
DBLα tag J1c	60	E-211F	AGGCAGCRAAAGACCACTAC
DBLα tag J1c	60	E-212R	TTCTCCTGAACCACATGTATTTCTA
DBLα tag J1d	60	E-213F	AAGAAGAACAATAAGAATCCGGCAA
DBLα tag J1d	60	E-214R	TCTAAAATATTCAGCATTATCTGTTACA
DBLα tag J1e	60	E-215F	AGACGAATGTGAAGACGAATGT
DBLα tag J1e	60	E-216R	TGCACACGCTCTTTGTCGAA
DBLα tag J1f	60	E-217F	AGAGGGAAGAAAGGCGCAA
DBLα tag J1f	60	E-218R	AGAGTCATACCATCATCTGCGAT
DBLα tag PCM7a	60	αE-3F	TGGATTGATGAACGGCGCACA
DBLα tag PCM7a	60	αE-4R	TACCACTCTTAACGTCACACG
DBLα tag PCM7b	60	αE-1F	AGATCGCTACCAAGATACTGA
DBLα tag PCM7b	60	αE-2R	CATTCCAAAGAGTCATACCAT
DBLα tag PCM7c	60	E-269F	AAGATGAACAAATTAGGGAATACTGGT
DBLα tag PCM7c	60	E-270R	TGGCATTTATCCAGACTCCAAGT
DBLα tag PCM7d	60	E-271F	ACAATGATGATACTGACAAAAACTATTACA
DBLα tag PCM7d	60	E-272R	AGGCTCTCCATTGACACATCTA
DBLα tag PCM7e	60	E-273F	TACAAACTCGCTACGGAAGTGAT
DBLα tag PCM7e	60	E-274R	AGTTCCATCGATACACTGGCA

IT4 RT-qPCR primers (Viebig *et al.*, 2007)

Target	T_A (°C)	Primer name	Sequence 5'-3'
IT4var01	60	IT4var01F	GATCCGCCAGCAAAAGAAG
IT4var01	60	IT4var01R	CCCCCTTTATATTTTTGTCTGC
IT4var13	60	IT4var13F	GTAAACATCAGGCGTGTAAGG
IT4var13	60	IT4var13R	TGTTCCTCTCCGCTGAAGA
IT4var14	60	IT4var14F	CAAGATGGAAGCGGTAAAG
IT4var14	60	IT4var14R	CATGCATTATCCCAAAGAT
IT4var16	60	IT4var16F	ATGGTAGACAAGCTGTTCGTTT
IT4var16	60	IT4var16R	AGCACAGGCTCCTACTGAATT
IT4var27	60	IT4var27F	CAATAACGACAACCCTGGCA
IT4var27	60	IT4var27R	TGGTGTCTTCGTCGGTTTTT
IT4var31	60	IT4var31F	ACTGGTCGTAAAGGTGCACA
IT4var31	60	IT4var31R	CTCCCTTCAAATCACTTCCC
IT4var41	60	IT4var41F	AACATATGTTTGATAGAATTG
IT4var41	60	IT4var41R	TGGCATCTGTAGGCACGAA

Primers designed against *var* database hits to ICAM-1 binding isolates

Target	T_A (°C)	Primer name	Sequence 5'-3'
XX0156-C.g40 (BC12a)	52	E-227F	TAGTGAAAGTCCATCTATGGCTA
XX0156-C.g40 (BC12a)	52	E-228R	TCCATTCATCATACAGTTTCTGACT
XX0156-C.g40 (BC12a)	52	E-229F	TGGTGATGGACAAACAGAAATTGAA
XX0156-C.g40 (BC12a)	52	E-230R	ACGCCTTTCTACCACCAGCA
XX0156-C.g40 (BC12a)	52	E-231F	ACCTATTATGAGATCCAATCCATGT
XX0156-C.g40 (BC12a)	52	E-232R	TTTCTCATATTTATCGCAGGCGTTT
XX0156-C.g40 (BC12a)	52	E-233F	TGCAATCACAGGAGTATGAGACA
XX0156-C.g40 (BC12a)	52	E-234R	TTCTTTAGGTTTTGGGAATATTGTATCT
XX0156-C.g40 (BC12a)	52	E-235F	AGGTCCCTCCAGAATTTTTGC
XX0156-C.g40 (BC12a)	52	E-236R	TGGGTGGCACACAAATACTAC
XX0156-C.g40 (BC12a)	52	E-237F	AGTCTCAATGCCGCCTGCT
XX0156-C.g40 (BC12a)	52	E-238R	TTGACCCCATTCTTCAAGGTAAC
XX0156-C.g40 (BC12a)	52	E-239F	TGTCACACTTAAAGATGAAAGTAGT
XX0156-C.g40 (BC12a)	52	E-240R	ATGGGGATTCTTCACGATTTTTCATA
XX0156-C.g40 (BC12a)	52	E-241F	ATGAGGAAAAAGTCAGTGGGAAA
XX0156-C.g40 (BC12a)	52	E-242R	AGTGAACGCAGCAAAACCGAT
XX0156-C.g40 (BC12a)	52	E-242F	ATCGGTTTTGCTGCGTTCACT
XX0004-C.g20 (J1a)	52	E-257F	TCTTCCATTAAGGGTGGGCAT
XX0004-C.g20 (J1a)	52	E-258R	ACATTTACTAATTTCTTTCCTCCATTCT
XX0004-C.g20 (J1a)	52	E-259F	TGATGAAGATAATGACTGTGAAACGA
XX0004-C.g20 (J1a)	52	E-260R	ACATATGTTCTCTTCTTTGTGGCA
XX0004-C.g20 (J1a)	52	E-261F	TGAAAATCATTCCAATCGTAATCCTAA
XX0004-C.g20 (J1a)	52	E-262R	ACTATTTGGAGGGAGTAATTCTTGTA
XX0004-C.g20 (J1a)	52	E-263F	ATGGGACAAAATGCAACTGAAATACT
XX0004-C.g20 (J1a)	52	E-264R	ATTATCGCCACTAAATGTAACGGTTT
XX0137-C.g35 (J1a)	52	E-309F	AAAGGTGACCAAACAGATGAAAAGAA
XX0137-C.g35 (J1a)	52	E-310R	AGTGATAAATATCTATCTCCATCTGTTGT
XX0137-C.g42 (J1b)	52	E-243F	ATGCCAACTTAATCACTCTCTTCAT
XX0137-C.g42 (J1b)	52	E-243R	ATGAAGAGAGTGATTAAGTTGGCAT
XX0137-C.g42 (J1b)	52	E-244R	TGTTTCAGCACAGAACGCGTT
XX0137-C.g42 (J1b)	52	E-245F	AGTGGTACTAATGATAAAGAAAAAGGAA
XX0137-C.g42 (J1b)	52	E-246R	TGTGGGTCTGGGCACTTGT
XX0137-C.g42 (J1b)	52	E-247F	AAGAAACAAAACGCATTAAGGACAT
XX0137-C.g42 (J1b)	52	E-248R	TGTGGGAGGAGGTTTGGAA
XX0137-C.g42 (J1b)	52	E-249F	ATATAGTAGTACGTGGTGTTGCT
XX0137-C.g42 (J1b)	52	E-250R	AGTTTTGCAGTCCTTTTCTTTACCAT
XX0137-C.g42 (J1b)	52	E-251F	AGGTAGTGAGATAACTTTTGATGATA
XX0137-C.g42 (J1b)	52	E-252R	TTCATCACTATTATCTGCTGGTTCA
XX0137-C.g42 (J1b)	52	E-253F	TGAACCAGCAGATAATAGTGATGAA

XX0382-C.g38 (J1d)	52	E-254R	TGTAACCTCATAATAAGAACATTGCCA
XX0382-C.g38 (J1d)	52	E-255F	AGTAATAAAGAGCGTGAGAATAATCCT
XX0382-C.g38 (J1d)	52	E-256R	ACCTCACTTGACGGCCATCT
XX0352-C.g23 (J1d)	52	E-275F	ACAGAAAAGGGGGTAAAGCAGAT
XX0352-C.g23 (J1d)	52	E-276R	TCAATCGCATGCTTGTCTTTATCTTT
XX0352-C.g23 (J1d)	52	E-277F	AAAATCAATGGAAACAAATGGACGAA
XX0352-C.g23 (J1d)	52	E-278R	TGTGATAGACCACATAACATTCCT
XX0352-C.g23 (J1d)	52	E-279F	AGGTAGTGATGTGAGTGATGTAGA
XX0352-C.g23 (J1d)	52	E-280R	ACCTGTTTTCAAACGATTCATACCT
XX0352-C.g23 (J1d)	52	E-281F	AAGCATACATGAAGGATCAGAAGATT
XX0352-C.g23 (J1d)	52	E-282R	CTGTCGCGGCCTTGCATTTA
XX0352-C.g23 (J1d)	52	E-283F	TAAAGAATACAGCATACTTGTTAGTAA
XX0352-C.g23 (J1d)	52	E-284R	TCTGTAAAGTGTTTCTCACCCATA
VAR0141-C.g21 (PCM7a)	55	E-15F	TGTGGTGGAGGAAATCTAAC
VAR0141-C.g21 (PCM7a)	55	E-16R	CTTCTCCGCAGGTTTCGTGACC
VAR0141-C.g21 (PCM7a)	55	E-17F	CATATTGCGAAGCATGTCCGTG
VAR0141-C.g21 (PCM7a)	55	E-18R	GCATCCACATGTGTATCTTC
VAR0141-C.g21 (PCM7a)	55	E-19F	GGCAACACATGTATAACAGG
VAR0141-C.g21 (PCM7a)	55	E-20R	GGTGGCATGTAGATATCTTCGGC
VAR0141-C.g21 (PCM7a)	55	E-21F	GCAAGATTGACACAACGTATTCC
VAR0141-C.g21 (PCM7a)	55	E-22R	CTTACACGTACCACTAACACCCG
VAR0141-C.g21 (PCM7a)	55	E-23F	GTCCTGGTATGCCAGTTGACG
VAR0141-C.g21 (PCM7a)	55	E-24R	GCCATATTTGAGGTTGCATGC
VAR0141-C.g21 (PCM7a)	55	E-25F	GAGGAGGAAGTCACTGACGAC
VAR0141-C.g21 (PCM7a)	55	E-26R	CACCAGTCAACACGCTTGTCAC
VAR0141-C.g21 (PCM7a)	55	E-27F	GATTAAGGCGTTAGAAGCTAGTGG
VAR0141-C.g21 (PCM7a)	55	E-28R	GCAGCGTCAGTGCACGTATCTAG
VAR0141-C.g21 (PCM7a)	55	E-29F	GTGCCATTTCTTGTAGATC
VAR0141-C.g21 (PCM7a)	55	E-30R	CAGTTCTCTGTTCGAATTGCTC
VAR0141-C.g21 (PCM7a)	55	E-31F	GGCCATGTATAGAAAATGG
VAR0141-C.g21 (PCM7a)	55	E-32R	GGAAGCGGACATAACATCGC
XX0488-C.g40 (PCM7d)	52	E-289F	AACCCCTTTCAGAACTTTGTCAT
XX0488-C.g40 (PCM7d)	52	E-289R	ATGACAAAGTTCTGAAAGGGGTT
XX0488-C.g40 (PCM7d)	52	E-290R	TACACGCTTCATTTCCGTCATCT
XX0488-C.g40 (PCM7d)	52	E-291F	TCAAGTGGTGAAGAACATGAAGATA
XX0488-C.g40 (PCM7d)	52	E-292R	TTGACGTCGGGGAGGTAAATA
XX0488-C.g40 (PCM7d)	52	E-293F	ATGAGCGTAATACAGGTGAACCA
XX0488-C.g40 (PCM7d)	52	E-294R	TGCGGTACTATAAACGGTGTTACT
XX0488-C.g40 (PCM7d)	52	E-295F	ACCTGTGGTTAACTTCTTGTTCGA
XX0488-C.g40 (PCM7d)	52	E-296R	ACGCAAACTCCTCGAGATATTCT

XX0488-C.g40 (PCM7d)	52	E-297F	TATGGCAAGGAATGTTATGTGCTTT
XX0488-C.g40 (PCM7d)	52	E-298R	ACTTTTGGGTATTTGCAGTATTTGGAA
XX0488-C.g40 (PCM7d)	52	E-299F	TTATGTTCGATAGCTTTTCTGTTCATGA
XX0488-C.g40 (PCM7d)	52	E-300R	TTGTCCAGATCCTCCAAGATAGT
XX0488-C.g40 (PCM7d)	52	E-300F	ACTATCTTGGAGGATCTGGACAA

Gene-specific forward primers used in combination with Ex2-reg for Exon 2 PCR

Target	T_A (°C)	Primer name	Sequence 5'-3'
BC12a	48	241F	ATGAGGAAAAAGTCAGTGGGAAA
J1a	48	310F	ACAACAGATGGAGATAGATATTTATCACT
J1b	48	253F	TGAACCAGCAGATAATAGTGATGAA
J1d	48	285F	TGGTTAGTTGAATGGGGTAAAGAA
PCM7a	48	73F	ATGAAAGAAATATCAGATAAAATAG
PCM7d	48	297F	TATGGCAAGGAATGTTATGTGCTTT

DBLβ expression construct primers (contain BamHI (F) and XhoI (R) restriction sites)

Target	T_A (°C)	Primer name	Sequence 5'-3'
BC12a_DBLβ	52	E-301F	ggtggaggatccCCATGTGCTAAACCCAGTGGT
BC12a_DBLβ	52	E-302R	tccaccctcgagttaACAATTACATGGTGTATCGTGATCAT
J1a_DBLβ	52	E-303F	ggtggaggatccGCTTGTAGTGGAGACCCCA
J1a_DBLβ	52	E-304R	tccaccctcgagttaACACTTACATACATCACCATACCCA

Supplementary data files

S1 Data. Data for flow adhesion assays (S6 Fig).

S2 Data. Data for SPR experiments for Figs 6D, 6E and 6F.

S3 Data. Data for SPR experiments for Figs 6G and 6H.

S4 Data. Kinetic values for SPR experiments

S5 Data. Data for SPR experiments for S3 Fig.

S6 Data. Data for SPR experiments for S4 Fig.

S7 Data. Data for SPR experiments for S5 Fig.