Supplementary Table and Figures

PfMSA180 is a novel *Plasmodium falciparum* vaccine antigen that interacts with human erythrocyte integrin associated protein (CD47)

Hikaru Nagaoka[1], Chisa Sasaoka[1], Takaaki Yuguchi[1], Bernard N. Kanoi[1], Daisuke Ito[2], Masayuki Morita[1], Rachanee Udomsangpetch[3], Jetsumon Sattabongkot[4], Tomoko Ishino[5], Takafumi Tsuboi[1], Eizo Takashima[1,*]

[1] Division of Malaria Research, Proteo-Science Center, Ehime University, 3 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan;
[2] Division of Medical Zoology, Department of Microbiology and Immunology, Faculty of Medicine, Tottori University, 86 Nishi-cho, Yonago, Tottori 683-8503, Japan;
[3] Center for Research and Innovation, Faculty of Medical Technology, Mahidol University, Salaya, Nakhosn Pathom 73170, Thailand;
[4] Mahidol Vivax Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand;
[5] Division of Molecular Parasitology, Proteo-Science Center, Ehime University, Toon Ehime 791-0295, Japan

* Corresponding author
Address: Division of Malaria Research, Proteo-Science Center, Ehime University, Japan, 3 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan. Tel.: (+81) 89 927 9939. E-mail address: takashima.eizo.mz@ehime-u.ac.jp

Table S1: List of primers used in this study

Protein	Primer sequence	Target amino acid range
PfMAS170Tr1-F	CTCGAGGAAAATGTTAATAATAAAAACTGTAATGAG	
PfMAS170Tr1-R	GCGGCCGCCTATGATACATACACATTATCCTCTTCAG	E22 – S263
PfMAS170Tr2-F	CTCGAGGCTACAAAAGGAAATCAAAAAGAAGAAACTG	
PfMAS170Tr2-R	GCGGCCGCTACATCAATTACGTCTACTTTTGTTTGTA	A264 – D501
PfMAS170Tr3-F	CTCGAGATTGCAAATACTATTTATGTAAATGTTGG	
PfMAS170Tr3-R	GCGGCCGCTATGGGAAATATCTAGAATTATGATAATATGC	I508 – P723
PfMAS170Tr4-F	CTCGAGGCAAAAAATTTTTATAACATATCTAATGAG	
PfMAS170Tr4-R	GCGGCCGCTATGGATATTGATTATTATTTGTTTGTTCA	A805 – P1093
PfMAS170Tr5-F	CTCGAGCTCTTTGATACTGATAAAAAAAGAAATTAT	
PfMAS170Tr5-R	GCGGCCGCTACTATGGATTTCTAAAATCTAGTGCATC	L1193 – P1455
AMIGO2_F	GGGCGGATATCTCGAATGTCGTTACGTGTACACAC	
AMIGO2_R	AAAACTAGTGCGGCCCTAATGGTGATGGTGATGGTGGTTAAATGCCTCATGAGCAT	M1 - N397
BCAM_F	GGGCGGATATCTCGAGAGGTGCGCTTGTCTGTA	
BCAM_R	AAAACTAGTGCGGCCCTAATGGTGATGGTGATGGTGGCCACGGCCATGACGGCC	E32 – A555
CD44_F	GGGCGGATATCTCGACAGATCGATTTGAATATAACC	
CD44_R	AAAACTAGTGCGGCCCTAATGGTGATGGTGATGGTGTTCTGGAATTTGGGGTGTC	Q21 – E606
CD47_F	GGGCGGATATCTCGAAAAACAAAATCTGTAGAATTCAC	
CD47_R	AAAACTAGTGCGGCCCTAATGGTGATGGTGATGGTGTGGAGAAAACCATGAAACAAC	K24 – S139
CD55_F	GGGCGGATATCTCGAGACTGTGGCCTTCCCCCA	
CD55_R	AAAACTAGTGCGGCCCTAATGGTGATGGTGATGGTGAGTCAGCAAGCCCATGGT	D35 – T381
CD58_F	GGGCGGATATCTCGATCCCAACAAATATATGGTGTT	
CD58_R	AAAACTAGTGCGGCCCTAATGGTGATGGTGATGGTGTCTGTGTCTTGAATGACCG	S30 – R215
CD59_F	GGGCGGATATCTCGACTGCAGTGCTACAACTGTC	
CD59_R	AAAACTAGTGCGGCCCTAATGGTGATGGTGATGGTGGGGATGAAGGCTCCAGGC	L26 – P128
CD99_F	GGGCGGATATCTCGAGATTTATCTGATGCCCTTCC	
CD99_R	AAAACTAGTGCGGCCCTAATGGTGATGGTGATGGTGGCCTGGGGCGTCGGCCTC	D27 – G125
ERMAP_F	GGGCGGATATCTCGACACGCAGGGGATGCCGGC	
ERMAP_R	AAAACTAGTGCGGCCCTAATGGTGATGGTGATGGTGTGAGGGGGAGAGACTCCC	H30 – S154
F11-Receptor_F	GGGCGGATATCTCGACACTCTTCTGAACCTGAAG	
F11-Receptor_R	AAAACTAGTGCGGCCCTAATGGTGATGGTGATGGTGGACCCCCACATTCCGCTC	H32 – V238
ICAM4_F	GGGCGGATATCTCGAGCGCTGGGACGCCGGACT	
ICAM4_R	AAAACTAGTGCGGCCCTAATGGTGATGGTGATGGTGGCCACCATGTATGGCCAT	A23 – G272
NPTN_F	GGGCGGATATCTCGACAGAACGAGCCAAGGATTG	
NPTN_R	AAAACTAGTGCGGCCCTAATGGTGATGGTGATGGTGCAGGTGGCTCCGCACCCT	Q29 – L221
SEMA7A_F	GGGCGGATATCTCGACAGGGCCACCTAAGGAGC	
SEMA7A_R	AAAACTAGTGCGGCCCTAATGGTGATGGTGATGGTGGTGGACCAGCAAGCCAAG	Q45 – H666

Fig S1

a

MNRIFYFCLFTILFWLSLVSGENVNNKNCNEKNRKAILLALLKNSLVDNKDYNNSEELKYALEHIQNSELYPKDSKKFDKFIDEFFSYYNIHVNFTDEEKRILHISGVFKEFY
VDVDNLNKDEMKEYFKKNYEKGLSLINLIVHSNLIIQQFDHDIIDKKKVHEQNTNTNKTLEYISDNLNDLINFKNIHLNNNSTGDFIIKLYTNYVNYINPYQTNPLPNTPHYYE
HKNFHTKEHYIYDEEIVNPNMDNINTHTEEDNVYVSATKGNQKEETEKKENHENNNAINPKYMNYETYYKKIFNAIFEQIDKLNKTLFEIKNKNNSETNENISESNSGNPEL
NNENSYSVKLSSSSPNSTNKESLIFPYTYYNPYYMFRLTNNFKENDEGLKNENNINNNEDNQNDNMNIVLGKIHNILKDFNINENIMTNKMSAPLIMTIILNFFKKYMAENK
FNLPMSSEVENKINKSNNKALLQQSNKDTPIHKKKEIRNKKIQTKVDVIDEKTKKKIANTIYVNVGQSGINGFFNFFDFREKSIDSNIFDLLHVMEDMKIFDIFQTIIFIQKFTE
NVCASYCMNITDVLELSHYDMIFYDKMVFHFSKDGMMIKTDKKYLYNLKEFENILNLLNINANTIALNCTCKFYVDVNYTYSEQYKMHLKGYLHKMNEFDYINNFSASYLL
NQLIIFQDKFNYIKMNGKLPIDDPKNIYNMNNVHDTAYYHNSRYFPTKDMPSLEDNFYEHLKYPDINTIHIYYNASPVKLNEVNDLKTIIIDEIKSKIFYINSYRVGDQFFPTYS
NLGKDDHDLEHSAKNFYNISNENGDNTFNNNNNNNMDNKKRMYNYNKHKDNDSRYTDNSNKNRDNSNKNRDNYNRNKDKNNTNRDNYNRYKDNNYYYNNSDNNN
YNERKRYIRKKTYNKLSYFNLPSLKSIYNNKIKGNSEEFSFDNELPEQTESFPLNKPQDHEAFYNLKKHHTNVYEPNDEEKQNEQKLKDQIKITSDILYKDIEENKNTEDVL
LIETITINNGTTSNTIENNKDSNKEAENSNTEQNDNNNNDNNNNINNNNNNNDNKEEDMNENNNNSKVTGDSVENINEQTNNNQYPNTEYNTIQRSINAKYLIFFFKNLH
VWKTDLFCQNINYMNNYLNSIQYNKTLTFDINYDTNAVTIYFTDNITYTVKVNLEYLVFLLEKISLITFVEDLCSLFDTDKKRNYKNLTEFLENTERINTFVRNHMMLSNEQFI
NKNKYAKELAEISTSNLFYPKKDIILRSTPYNNIILDEKDIYQTIFIYMDDMLTEKMVNDTWITPYAFVVYSKSKKDMQGNNIKIEQNKNITKYSRSAIDKYVHYEYKRISENLN
RFFMESNSNAPQFNENYKEYTIIIYNDNPSMPNIVLTTTINVFNVSFLQSIMEMLLNIKANQQFFSYKGKFIPINAFITLENKINYIFFNYIPLENYVNNGDALDFRNP

b

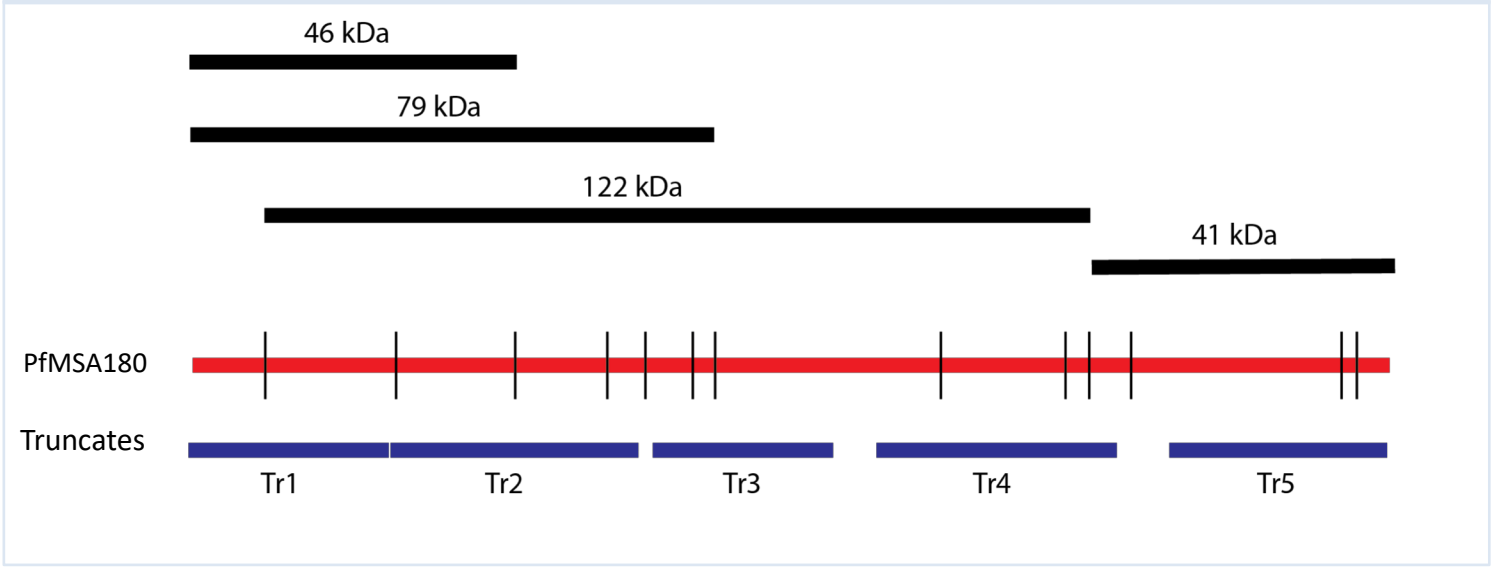

Figure S1: SUB1 recognition sites (Ile/Leu/Val/Thr-Xaa-Gly/Ala-Paa (not Leu) ; Xaa) identified by previous study [1] were used for prediction of PfSUB1 sites in PfMSA180 amino acid sequence. a) The amino acid sequence of PfMSA180. The predicted PfSUB1 sites are highlighted. b) Schematic presentation of the predicted PfSUB1 cleavage sites in PfMSA180 (vertical lines). Blue lines present the position of the truncates indicated. Black horizontal lines indicate predicted products due to the partial digestion of PfMSA180.

1. Withers-Martinez, Chrislaine, et al. "Plasmodium subtilisin-like protease 1 (SUB1): insights into the active-site structure, specificity and function of a pan-malaria drug target." *International journal for parasitology* 42.6 (2012): 597-612.

Fig S2 A

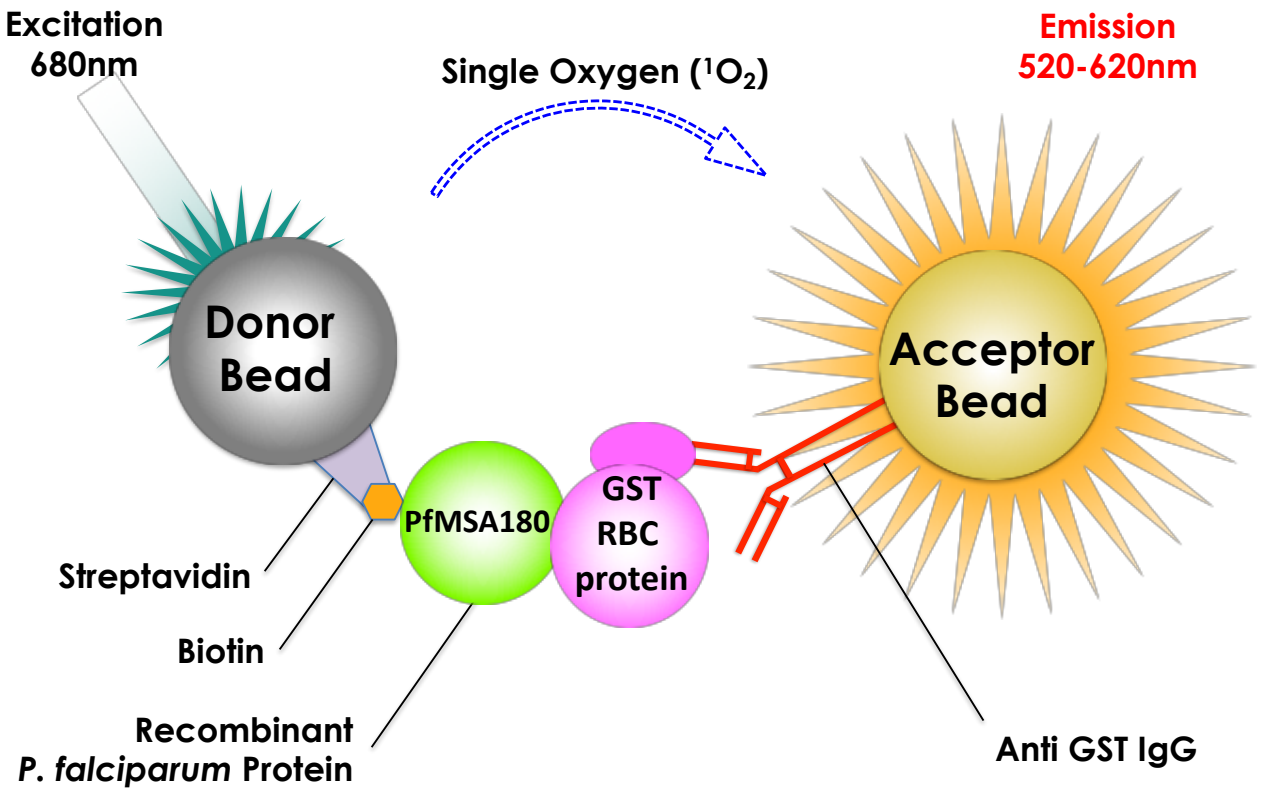

Figure S2A: Schematic representation of AlphaScreen protein-protein interaction assay.
GST tagged erythrocyte proteins were incubated with mono-biotinylated recombinant PfMSA180 After incubation, the streptavidin-conjugated donor beads and protein G conjugated acceptor beads – with a pre-attached anti-GST antibody were added to the mixture. If ligand-receptor complex is formed, it brings the beads into close proximity. Thus, excitation of the donor beads causes release of singlet oxygen molecules that trigger energy transfer and subsequent luminescent light emission at 520-620 nm from the acceptor beads. This reaction occurs only when the distance between donor and acceptor beads is within 200 nm.

Fig S2B

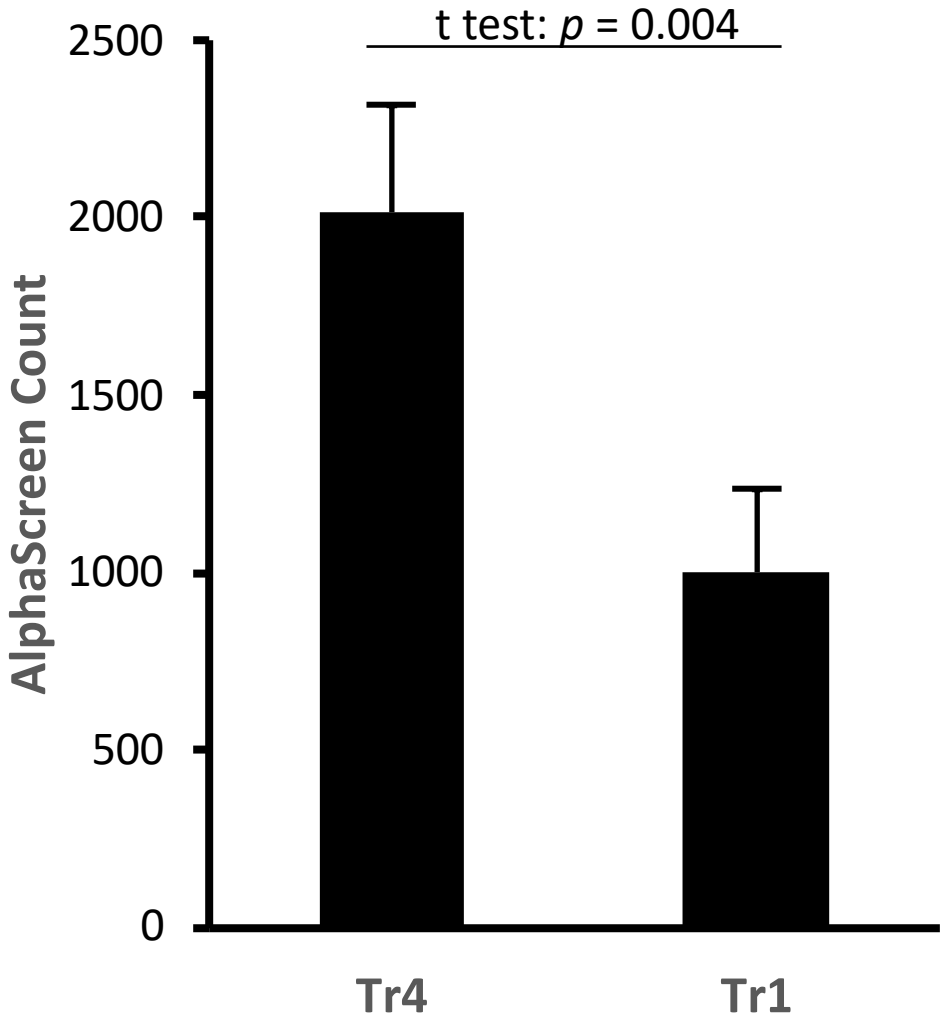

Figure S2B: AlphaScreen reactivity of recombinant PfMSA180-Tr4 and Tr1 to GST-fuse CD47. The AlphaScreen count was significantly higher for Tr4 compared to Tr1 (t test, $p = 0.004$). Data represents mean of a single assay performed in triplicate. Error bars represent standard error of the mean.

Fig S3

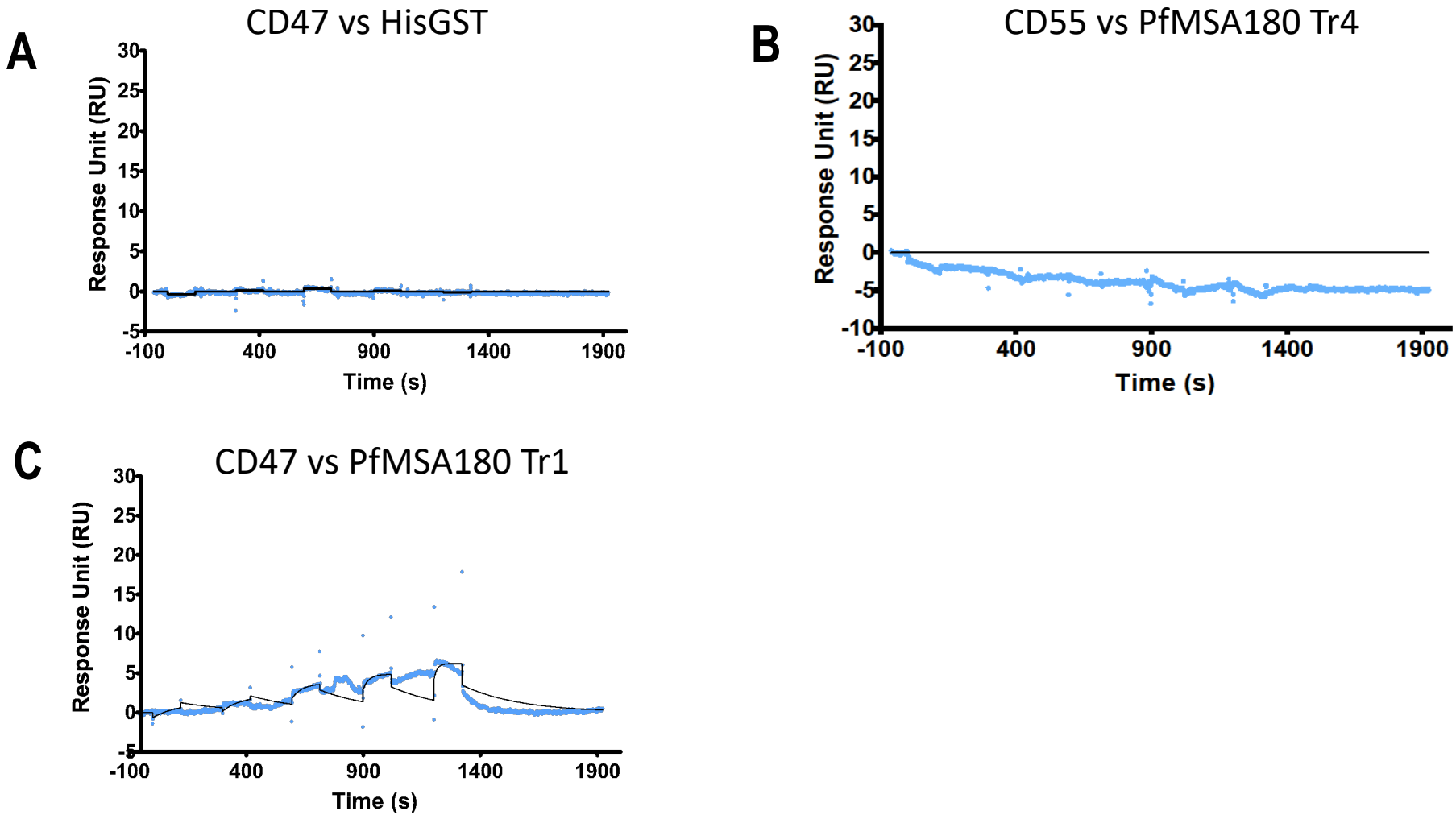

Figure S3: Sensorgram of SPR single-cycle kinetic analysis. Recombinant PfMSA180-Tr4 was immobilized on CM5 chip and used as the ligand while recombinant CD47 was used as analyte. Blue curve represents the actual data-generated sensorgram while black curve indicates line of fit used to calculate kinetics parameters. All assays were performed at an increasing protein concentration of 0.125, 0.25, 0.5, 1 and 2 µM at 120 s contact time and 180 s dissociation time. The dissociation time in the last cycle was extended to 580 s. A. CD47 (ligand) vs HisGST (analyte) B. CD55 (ligand) vs PfMSA180 Tr4 C. CD47 (ligand) vs PfMSA180 Tr1.