

SUPPLEMENTARY INFORMATION

Engineering Broad-spectrum Inhibitors of Inflammatory Chemokines from Subclass A3 Tick Evasins

Shankar Raj Devkota¹, Pramod Aryal¹, Rina Pokhrel¹, Wanting Jiao², Andrew Perry³,
Santosh Panjekar^{1,4}, Richard J. Payne^{5,6}, Matthew C.J. Wilce¹, Ram Prasad Bhusal^{1,*} and
Martin J. Stone^{1,*}

¹Monash Biomedicine Discovery Institute, and Department of Biochemistry and Molecular
Biology, Monash University, Clayton, VIC 3800, Australia

²Ferrier Research Institute, Victoria University of Wellington, Wellington 6140, New
Zealand; Maurice Wilkins Centre for Molecular Biodiscovery, Auckland 1142, New Zealand

³Monash Bioinformatics Platform, Monash Biomedicine Discovery Institute, Monash
University, Clayton, VIC 3800, Australia

⁴Australian Synchrotron, ANSTO, Clayton, VIC 3168, Australia

⁵School of Chemistry, The University of Sydney, Sydney, NSW 2006, Australia

⁶Australian Research Council Centre of Excellence for Innovations in Peptide and Protein
Science, The University of Sydney, NSW 2006, Australia

*Correspondence: ram.bhusal@monash.edu; Tel.: +61-3-9905-5937;

martin.stone@monash.edu; Tel.: +61-3-9902-9246

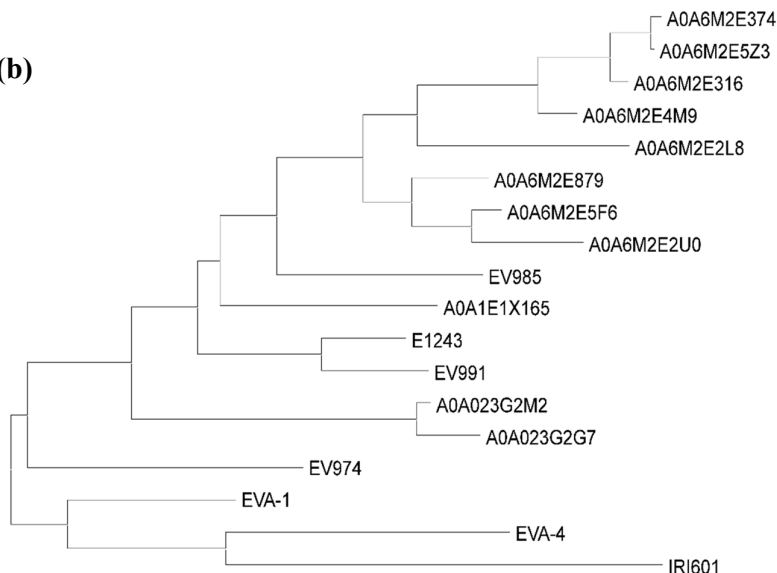
(a)

IRI601	(IRI) -----GPAPSAKENEKAPLCL-----PQ	35
EVA-4	(RSA) -----EVPQMTSSSAPDLEE	15
EV974	(ACA) -----ENTQQ	5
EVA-1	(RSA) -----	0
A0A6M2E2L8	(ATL) -----EE	2
A0A023FT45/EV985	(APA) -----DEESE---ELGAS	10
A0A6M2E4M9	(ATL) -----EPAATAT---PSCLEDPCNSTSSSGEES EDDY -----	29
A0A6M2E316	(ATL) -----AHEEDSHEPAVTGS---ASSLDDTS NRT SASGEEI EDDYDD -----	38
A0A6M2E374	(ATL) -----DSHEPAVNES---ATSL--- NNRT SASGEEI EDDYDD -----	31
A0A6M2E5Z3	(ATL) -----DSHEPAVNES---ATSL--- NNRT SASGEEI EDDYDD -----	31
A0A6M2E879	(ATL) -----EPAATAT---PSCLEDPS NST SSSDASEESES EDDYDD -----	38
A0A6M2E5F6	(ATL) -----LQDASRESDDTGTSTHES	17
A0A6M2E2U0	(ATL) -----HDASLQTDVTDASLQS	16
A0A023G2M2	(ATT) -----KDL---SSTTEETAVGYSSTTDNINASLGTTETTTDQA	34
A0A023G2G7	(ATT) -----KDL---SSTTEETVVGYSSTTDNINASLGTTETTTDGT	34
A0A1E1X165	(AAL) -----TDPG NST ECT TNTT	13
A0A0C9S461/E1243	(AAM) -----GSAR NHT EDN	10
A0A023FFD0/EV991	(ACA) -----ENGE GT Q PP Y YN	13

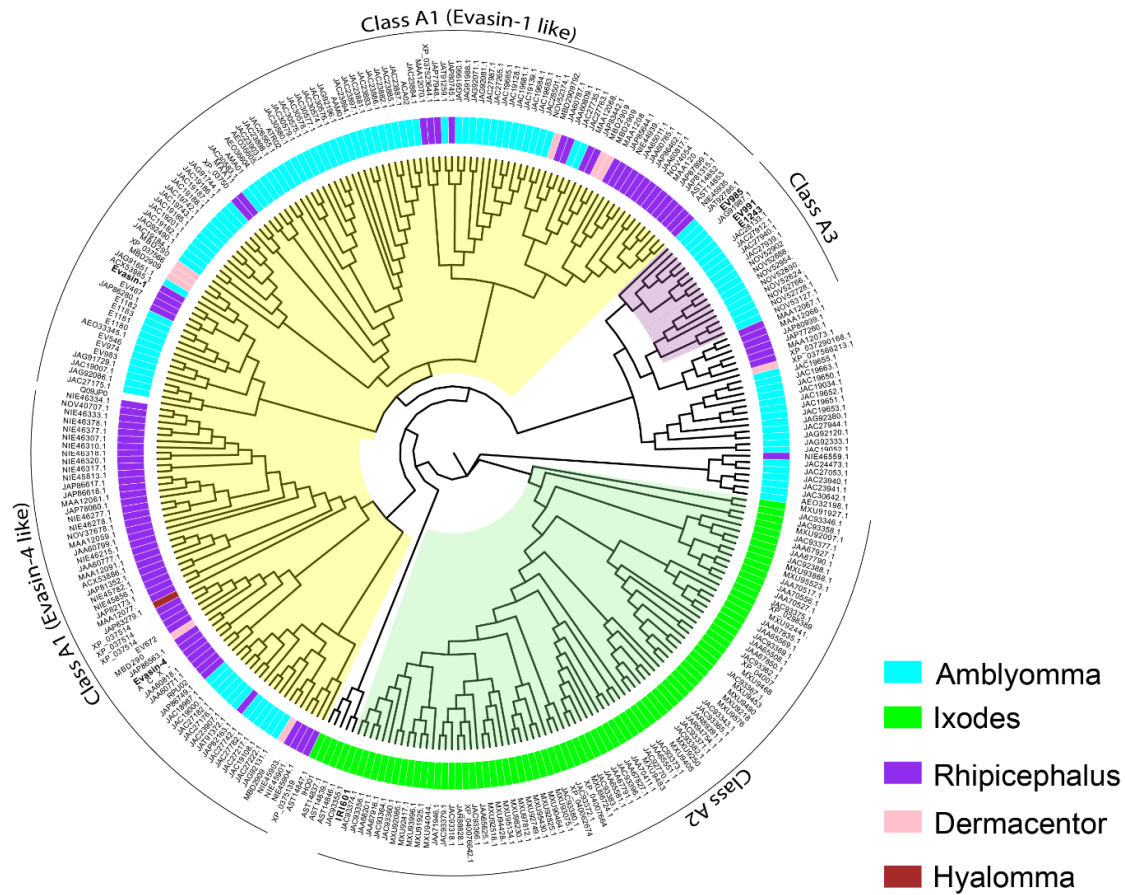
IRI601	ESLINNRDPNGCNYQLLPYFTEDGMGGGLAIDCSKSCPEGT---HETVVDGNSC V AKVD	92
EVA-4	-- EDDY TAYAPLT CYF ---- TNST LGLLAPPNC SVL CNST TTWFNETSPNNAS CL LTVD	68
EV974	EEQDYDYG --TDT CP FPV-- LANKTN -KAKFV GCHQK CNGG---DQKLT DGT CYV VER	56
EVA-1	-- EDDE DY G -DLGG CP FLV-- AENKTG -YPTIV ACKQD CNGT ---TETAP NGTR CF SGD	51
A0A6M2E2L8	ALTDDFIEN TE CA CPVQ Q ---LEN ANGT VLRAP GCTYF CGTV --- SC PIPD DY PC YAVTL	56
A0A023FT45/EV985	TDV DY E EL DAN CT CPA P A---LTSTRNNKH YPLG CIY NCSSY --- NCT IPD GT PC YVTLT	64
A0A6M2E4M9	----DSIVL GE CY CLAP H ---LH TN QGNVSRV VG CIY CEGR --- NCT AE EDY PC YDTTL	79
A0A6M2E316	---N YNS GA LGG CY CPAA H ---LR TR QGNFSRV AG CIY CEER --- NCT VEHD Y PC YDITL	90
A0A6M2E374	-- NY DSAVL GG CY CPAP N ---LL NL QGNFSRL AG CVI CEER --- NCT VKH DY PC YDITL	83
A0A6M2E5Z3	-- NY DS TV L GG CY CPAP N ---LL NL QGNFSRL AG CIY CEER --- NCT VKH AY PC YDITL	83
A0A6M2E879	NQ TD DY Y PQ AE CL CPV F V---LR NT M NST SK PPG CIY CE SK--- NCT IPD GE AC YNISF	92
A0A6M2E5F6	NET DDY Y PT GG CT CPV I V---L NN P NST V KPPG CIY PC GA V --- NCT LT DE EP CY NISL	71
A0A6M2E2U0	NET DDY Y PT D GCT CPIT N ---L NN H SS AT KPPG CVY SC GT V --- NCT LT DE EP CY NISL	70
A0A023G2M2	TTE Y Y DY GLE NCT CS YPT ---LR NS SM N -LTATV GCHQI CDGI --- NCT L PE GR CTY LQS	87
A0A023G2G7	TTE Y Y DY EL DNCT CS HPT ---LR NS SM N -VTGAV GCSLP CNGV --- NCT LPD GTN CY TLGS	87
A0A1E1X165	EDY DY Y EL DNCT CS HPT ---LY NT NG T MP KQV GCEYH CGTE --- NCK VP VGTA CY DLNI	67
A0A0C9S461/E1243	STE Y Y DY EEAR CA CPAR H ---L NT NG T V LK LG CHYF CNGT --- LCT APD GY PCY NLTA	64
A0A023FFD0/EV991	ST DY Y N Y ED FK CT CPAP H ---L NT NG T V MK PI G YYT CNVT --- RC TAPD TY PCY NLTE	67

IRI601	SLSKEE-----ATV MLG AC DKGS CK PEN PPQ HLTV TL MVEGE EKEE ---	133
EVA-4	FLTQDAILQENQ PY NC SVGH CD NGT CAG PP RHAQ C -----W-----	104
EV974	KVWDRMT--P MLW Y E CP L GE CK NG V CE DL R KKED C -----RKG NGE E K ---	97
EVA-1	EGLRRMT--ANL PYD CP L G CS NG DC IP KET YEV C -----YRR NRD KK N ---	94
A0A6M2E2L8	ETFAQMA--PRVE HT CL P G IC QNGT CV PSAN E EAC -----MK G V Y IE---	96
A0A023FT45/EV985	GEVKEHLQIGSTV PNC T CGL CR NGT CV SN GT VEE C -----FAVE E IE ET ---	108
A0A6M2E4M9	EVFYTME--V NLT Q EC VP MC RNGT CV TT GE KEE C -----HK-----	114
A0A6M2E316	ELFSAME--V NAT Q NC FP MC RNGT CV TT GE KEE C -----FK-----	125
A0A6M2E374	EEFFAME--V NAT Q DC FP IC RNGT CV TT GE QEE C -----FK-----	118
A0A6M2E5Z3	EEFSAME--V NAT Q DC FP IC RNGT CV TT GE QEE C -----FK-----	118
A0A6M2E879	VEYKNMK--V NQT Q N CL GS CNGT CV PD GQ EE Q C -----YQ IEA -----	130
A0A6M2E5F6	QAYKLME--I NMT Q S CL GT CQNGT CV PN GQ EE Q C -----Y S S-----	107
A0A6M2E2U0	LEFGSME--V NKT Q S CL LT CN NGT CV PD GK EL C -----Y L GED-----	108
A0A023G2M2	QLASVQLQARSFE HTCQV G CH NGT C IL NGT VEG C -----Y PL VP K -----	128
A0A023G2G7	KLTSVQLQARTIE QSCQ AG VCH NGT C IL NGT VEL C -----F PL VP K KT TL S	133
A0A1E1X165	TVVNTW--KT NT TH PC VP GI CNG DM CR KNGT TEE C -----F K AT F -----	105
A0A0C9S461/E1243	QQVRTL--T TY P N T S CA V G V C M K GT CV KNGT ME Q C -----F K TP-----	101
A0A023FFD0/EV991	HQAKNL--T TS P T TL CA V GN CD H GI CV FNGT KEL C -----F K AP N LEE---	108

(b)



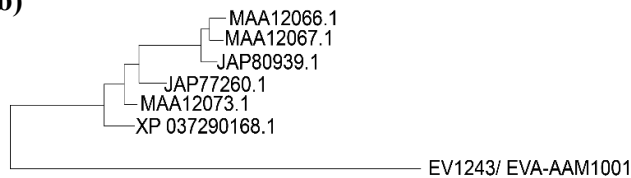
Supplementary Figure 1. Sequences and features of class A3 evasins. (a) Multiple sequence alignment (obtained using Clustal Omega) of the sequences of class A3 evasins, along with the sequences of EVA-P974 (EVP974, class A1), EVA-1 (Evasin-1, class A1), EVA-4 (Evasin-4, class A1) and EVA-IRI601 (IRI601, class A2). Sequence names are the primary accession code found in UniProt. Sequence A0A0C9S461 corresponds to EVA-AAM1001. Conserved cysteines (yellow), additional cysteines (red), conserved glycine (green), putative tyrosine sulfation sites (pink) and putative glycosylation sites (sky blue) are highlighted. Abbreviations used for tick species are *ATL*, *A. tuberculatum*; *AAM*, *A. americanum*; *ACA*, *A. cajennense*; *APA*, *A. parvum*; *ATR*, *A. triste*; *IHO*, *Ixodes ricinus* and *RSA*, *R. sanguineus*. (b) The unrooted tree (ClustalO, IQ-TREE) showing the relationships between the sequences.



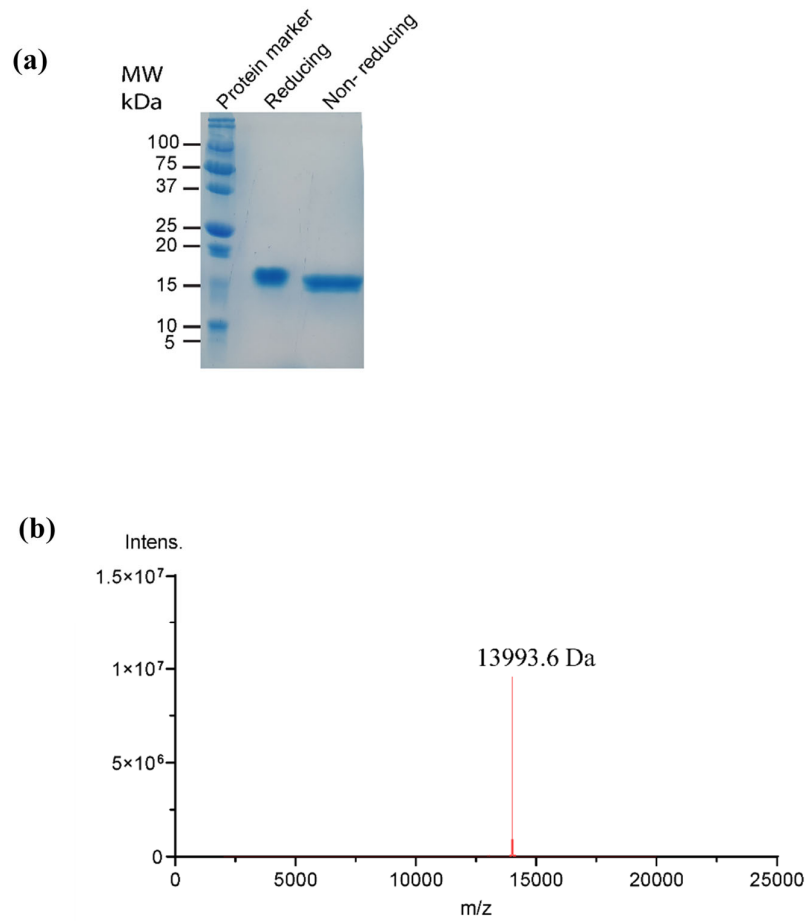
Supplementary Figure 2. Neighbour-joining tree of class A evasin proteins. A midpoint-rooted, neighbour-joining tree represented as a cladogram, based on MUSCLE alignments of the class A evasins. Background colours show subclasses: A1 (light yellow), A2 (green) and A3 (purple). The genus of each node is indicated by coloured segments in the outer ring and shown in the legend. The figure is generated based on the sequences available in *Front. Cell. Infect. Microbiol.*, 22 October 2021 Sec. Parasite and Host Volume 11 – 2021.

EV1243/AAM1001	(AAM)	-----RNHTEDNSTEY--DYEEARACPARHLNNTNGTVLKLGGCHYFCN	44
JAP80939.1	(RAP)	-LSMCNNQAPAESGKNINVKVISIRPDSVPVSGTVCTIPVLR---GNSLRPVPVGGCHFCR	55
MAA12067.1	(RZA)	-LSMCNNQAPAE-----SVSRPDSVPVSGTVCSIPVLR---GNSLRPVPVGGCHFCR	46
MAA12066.1	(RZA)	-LSMCNNQAPAE-----SVSRPDSVPVSGTVCSIPVLR---GNSLRPVPVGGCHFCR	46
MAA12073.1	(RZA)	AVSMCNSPAPAGSGKKISVQVVISIRPESRSASTICRIPFLH---GKLTMRKVPVGCCLFCR	56
JAP7260.1	(RAP)	-LSMCNNPAPAESGKKISVQVVISIRPESRSASTICRIPFLH---GKLTMRKVPVGCCLFCR	55
XP_037290168.1	(RMI)	-VSMCNPPELAASGKNISVKVISIRPESRSRETVCITPFLH---GQHTMRKVPVGCCLLCA	55

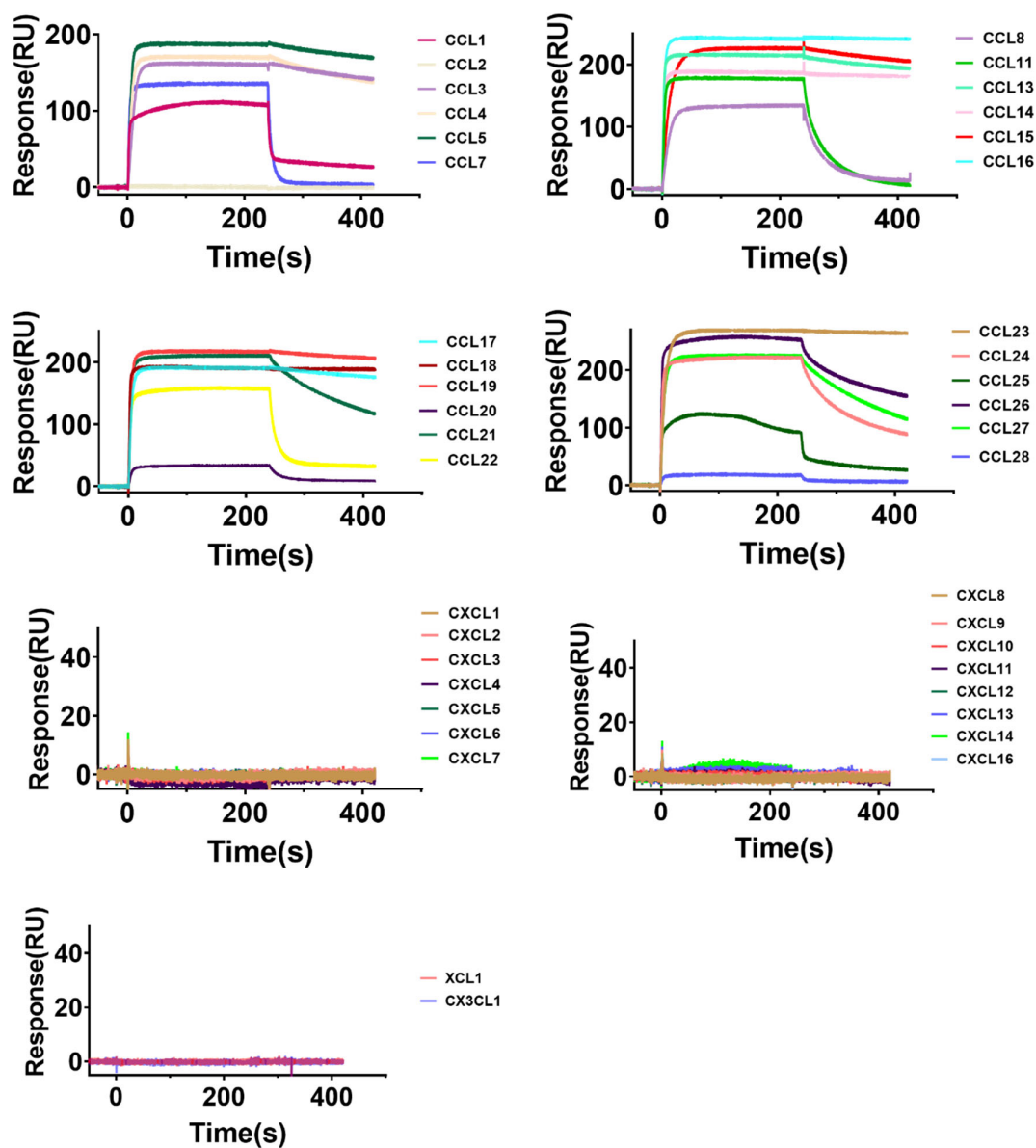
EV1243/AAM1001	GTLC ^T APD ^G GP ^C CYN ^L TAQ ^V VRT ^L LT ^T YP ^N TS ^C AV ^G VC ^M KGT ^C V ^K NGT ^M EQ ^C FK ^T P-----	98
JAP80939.1	DMNET ^L PD ^K TC ^P YA ^I PP ^L EAL ^R MK ^P PHARR ^S CP ^L GL ^C ENG ^V CK ^P TG ^K Y ^E IC ^S ET ^I AT ^I IK ^M	114
MAA12067.1	DMNET ^L PD ^K TC ^P YA ^I PP ^L EAL ^R MK ^P PHARR ^T CP ^L GL ^C ENG ^V CK ^P TG ^K Y ^E IC ^S ET ^I AT ^I IK ^M	105
MAA12066.1	DMNET ^L PD ^K TC ^P YA ^I PP ^L EAL ^R MK ^P PHARR ^T CP ^L GL ^C ENG ^V CK ^P TG ^K Y ^E IC ^S ET ^I AT ^I IK ^M	105
MAA12073.1	EMNRT ^L QD ^K TC ^P YA ^I PP ^L EA ^L Q ^M K ^L RAP ^R SP ^C PL ^G LC ^E NGE ^C T ^P TG ^E Y ^E LC ^S EMAS ^Y DK ^N	115
JAP77260.1	EMNRT ^L QD ^E TP ^C YA ^I PP ^L EA ^L Q ^M K ^L RAP ^R SP ^C PL ^G LC ^E NG ^V CK ^P TG ^K Y ^E IC ^S ET ^I AT ^I IK ^M	114
XP_037290168.1	ETNRT ^L QD ^E TP ^C YV ^I PP ^Q EAL ^R MK ^L GA ^P RR ^L CL ^G LC ^E NGA ^C K ^P TG ^K Y ^E LC ^S ETAS ^Y DQ ^M	114



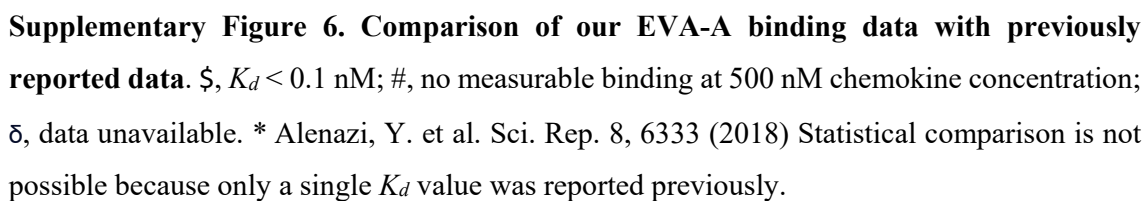
Supplementary Figure 3. Sequences of C₁₀ evasins from *Rhipicephalus* species. (a) Multiple sequence alignments of a fourth subfamily of class A evasin-like proteins, all encoded by tick species from the genus *Rhipicephalus* and all containing the eight conserved cysteine residue from class A1 evasins (highlighted in yellow) as well as two additional cysteine residues (highlighted red) in positions distinct from class A3 evasins. Sequence names are the primary accession codes found in UniProt. Abbreviations used for tick species are *RAP*, *R. appendiculatus*; *RZA*, *R. zambeziensi* and *RMI*, *R. microplus*. (b) The unrooted tree (ClustalO, IQ-TREE) showing a separate clade of evasin-like sequences from *Rhipicephalus* species.



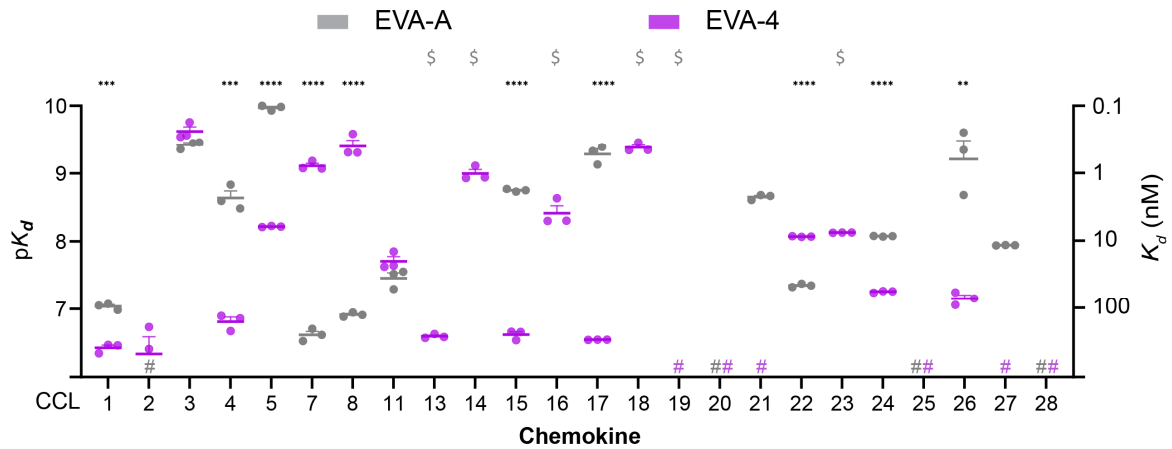
Supplementary Figure 4. Quality Control Analysis of purified EVA-A. (a) SDS-PAGE of wild type EVA-A under reducing and non-reducing conditions and stained with colloidal Coomassie blue. (b) Purified EVA-A (with a C-terminal linker-AVI tag; sequence ((GGGS)₃ GLNDIFEAQKIEWHE) was analysed by liquid chromatography-electrospray ionisation mass spectrometry (LC MS-ESI). The expected mass of EVA-A with five disulfide bonds is 13994.42 Da.



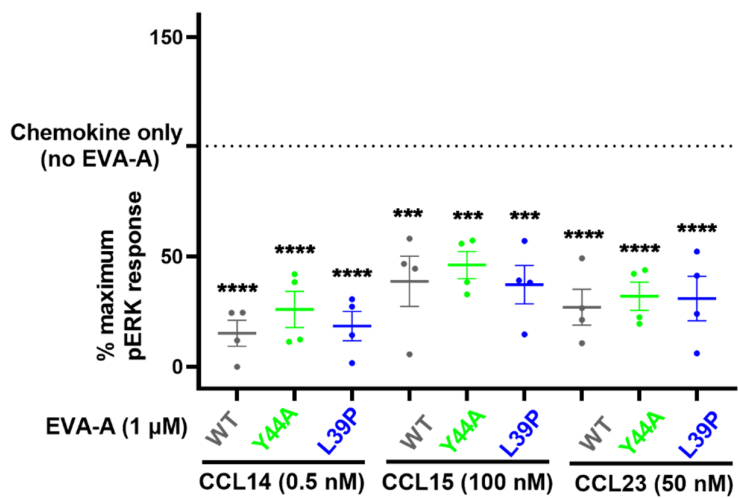
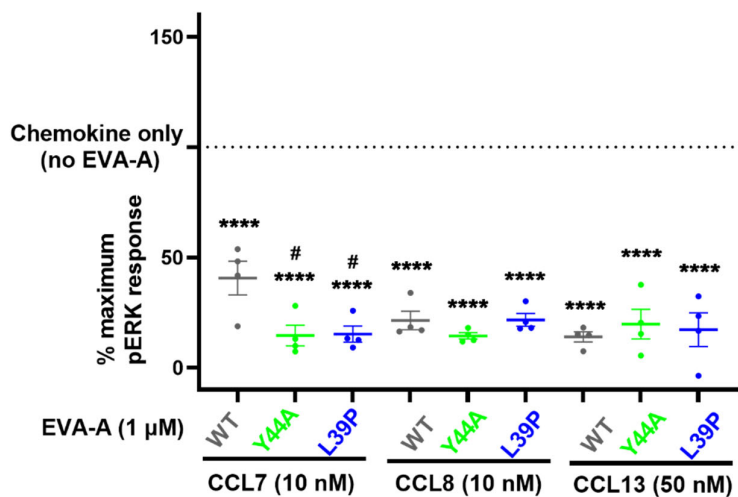
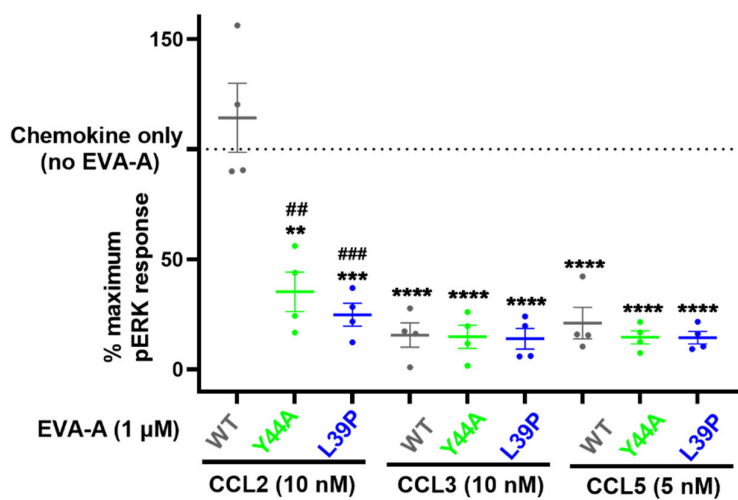
Supplementary Figure 5. EVA-A selectively binds to multiple human CC chemokines. Screening of EVA-A with multiple human chemokines (500 nM) by SPR using single-cycle kinetics at a single concentration. EVA-A selectively binds to CC chemokines but not to other classes of chemokines.



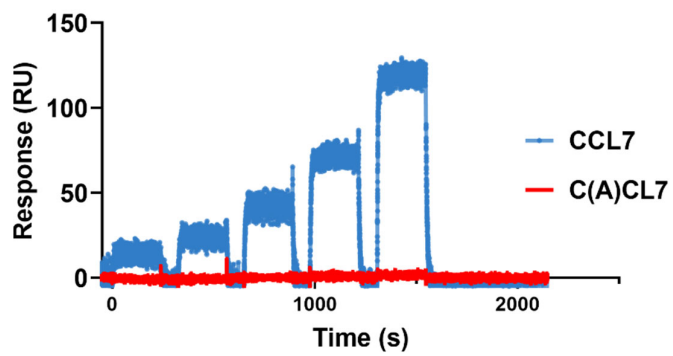
Supplementary Figure 6. Comparison of our EVA-A binding data with previously reported data. \$, $K_d < 0.1$ nM; #, no measurable binding at 500 nM chemokine concentration; ⚪, data unavailable. * Alenazi, Y. et al. Sci. Rep. 8, 6333 (2018) Statistical comparison is not possible because only a single K_d value was reported previously.



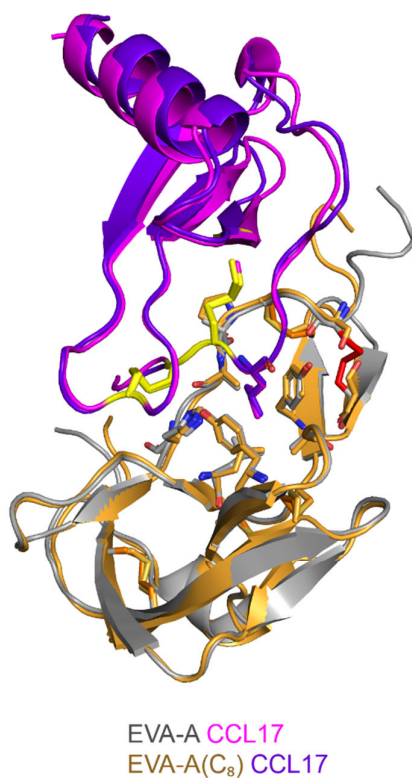
Supplementary Figure 7. Comparison of binding affinities of EVA-A and EVA-4. pK_d values for EVA-4 (expressed in human embryonic kidney cells) are from Aryal, P. et al. *J. Biol. Chem.* 298 (2022) DOI: <https://doi.org/10.1016/j.jbc.2022.102382>. Data are presented as mean ± SEM from three independent experiments. \$, K_d < 0.1 nM; #, no measurable binding at 500 nM chemokine concentration; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (two-tailed t test with Holm-Šídák correction for multiple comparisons).



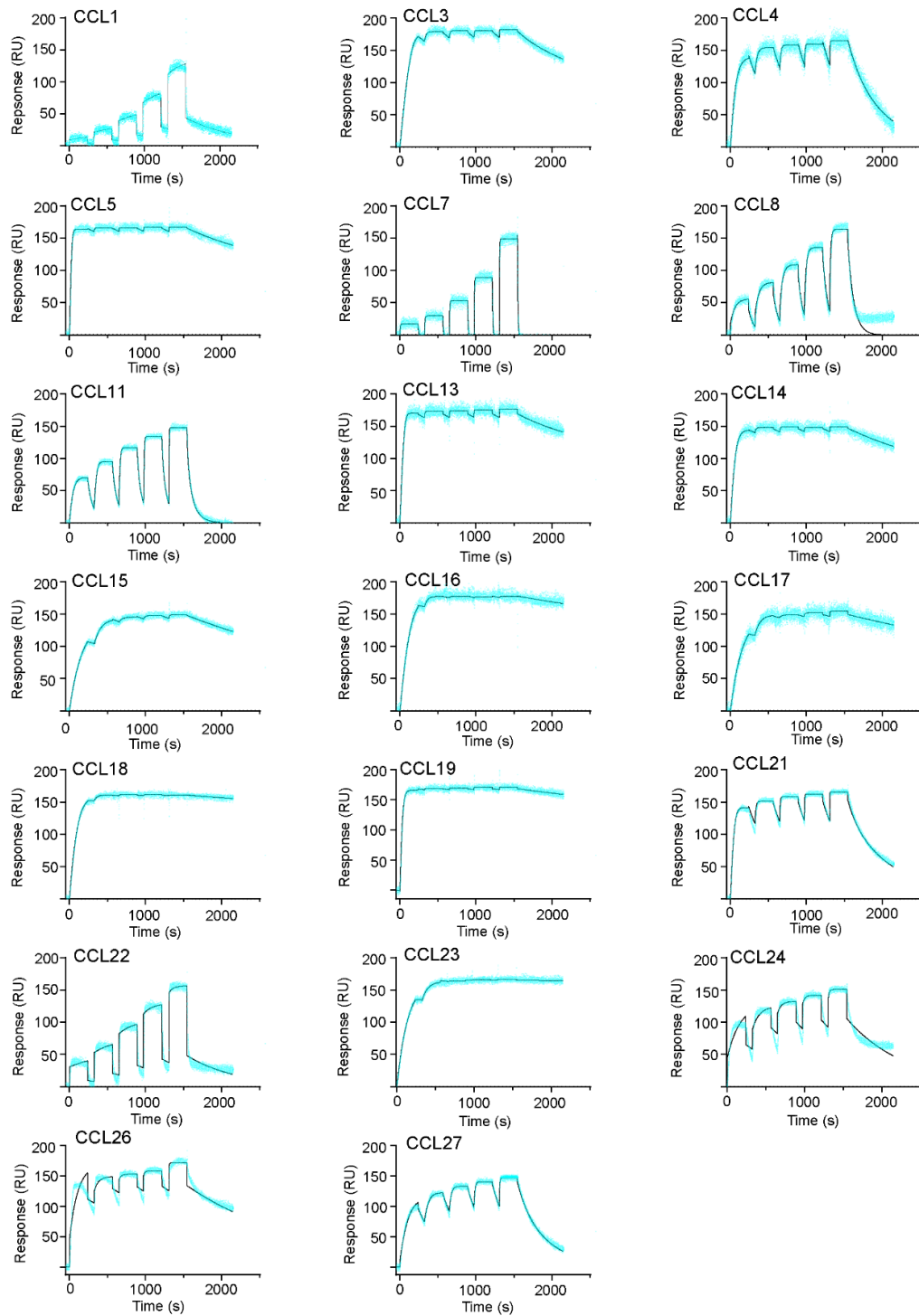
Supplementary Figure 8. EVA-A, EVA-A(L39P) and EVA-A(Y44A) inhibit the ability of chemokines to stimulate receptor mediated phosphorylation of extracellular signal-regulated kinase 1/2 (ERK). THP-1 monocytes that endogenously express the chemokine receptors CCR1 and CCR2 were treated with the chemokines (ligands of CCR1 and/or CCR2, at the indicated concentrations) or treated with mixtures of the chemokines (at the indicated concentrations) and evasins (at 1 μ M concentration). The observed level of ERK phosphorylation (pERK response) was normalised to the response for each chemokine alone. Data points represent the average $\pm$ SEM of four independent experiments, each conducted in duplicate. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ relative to chemokine signals (one-way ANOVA with Dunnett's multiple comparisons test). # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ relative to chemokine signals inhibited by wild type EVA-A (one-way ANOVA with Dunnett's multiple comparisons test).



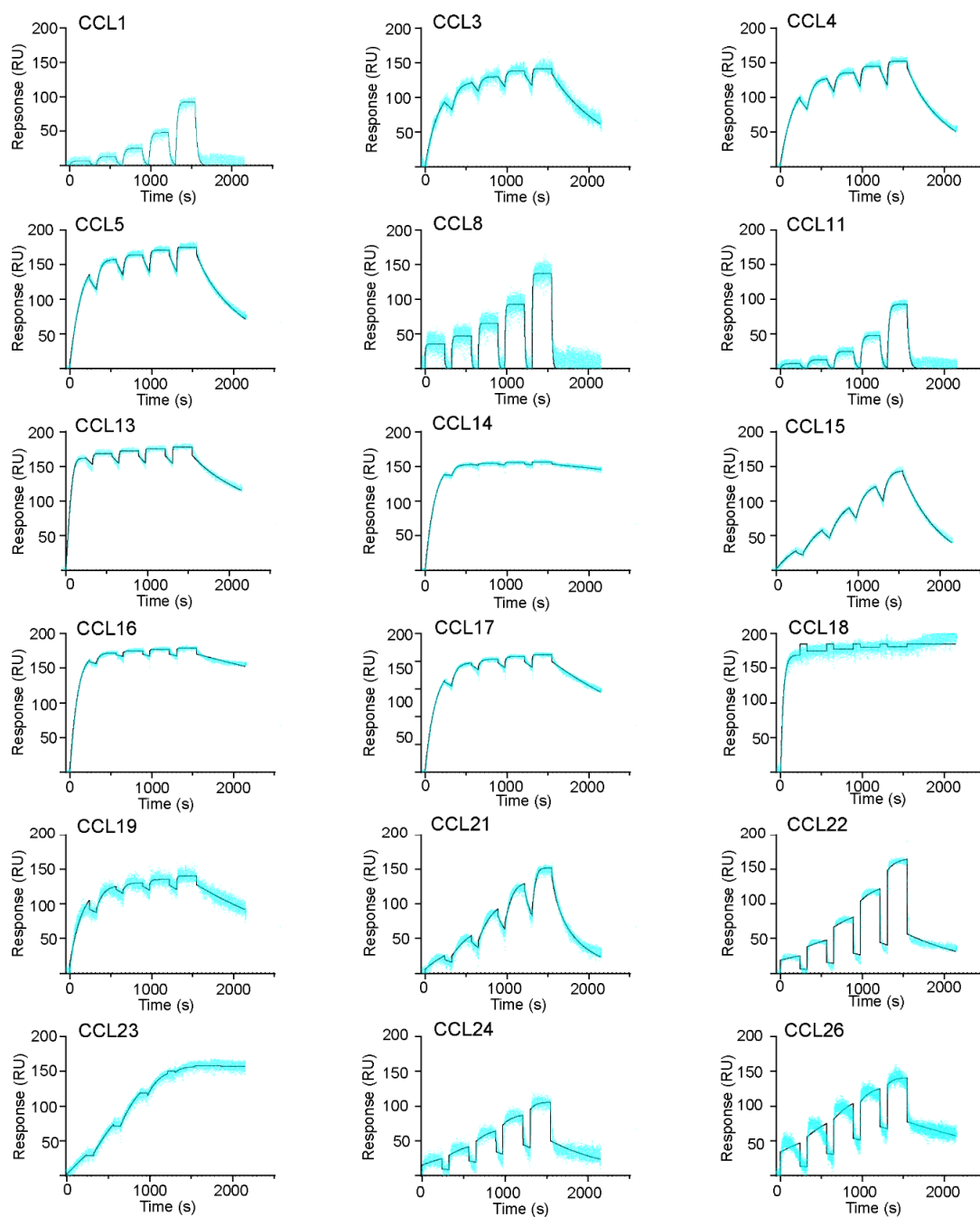
Supplementary Figure 9. SPR sensorgrams for CCL7 and C(A)CL7. Single-cycle kinetics SPR sensorgrams for CCL7 (sky blue) and C(A)CL7 (red) binding to EVA-A.



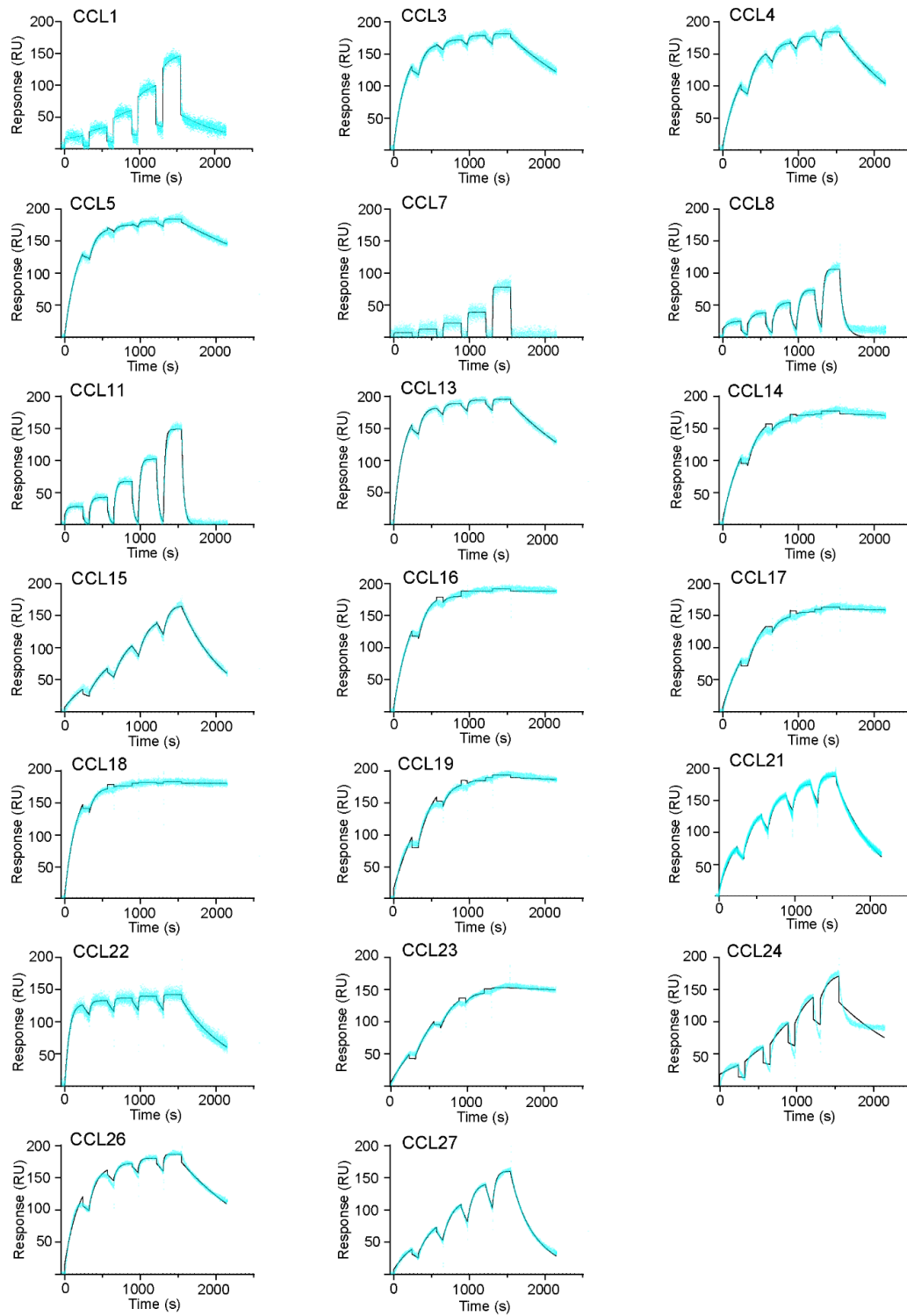
Supplementary Figure 10. Superimposition of EVA-A CCL17 and EVA-A(C₈) CCL17.
Overlay of EVA-A and EVA-A(C₈) CCL17 shows the same fold despite the removal of an additional disulfide bond.



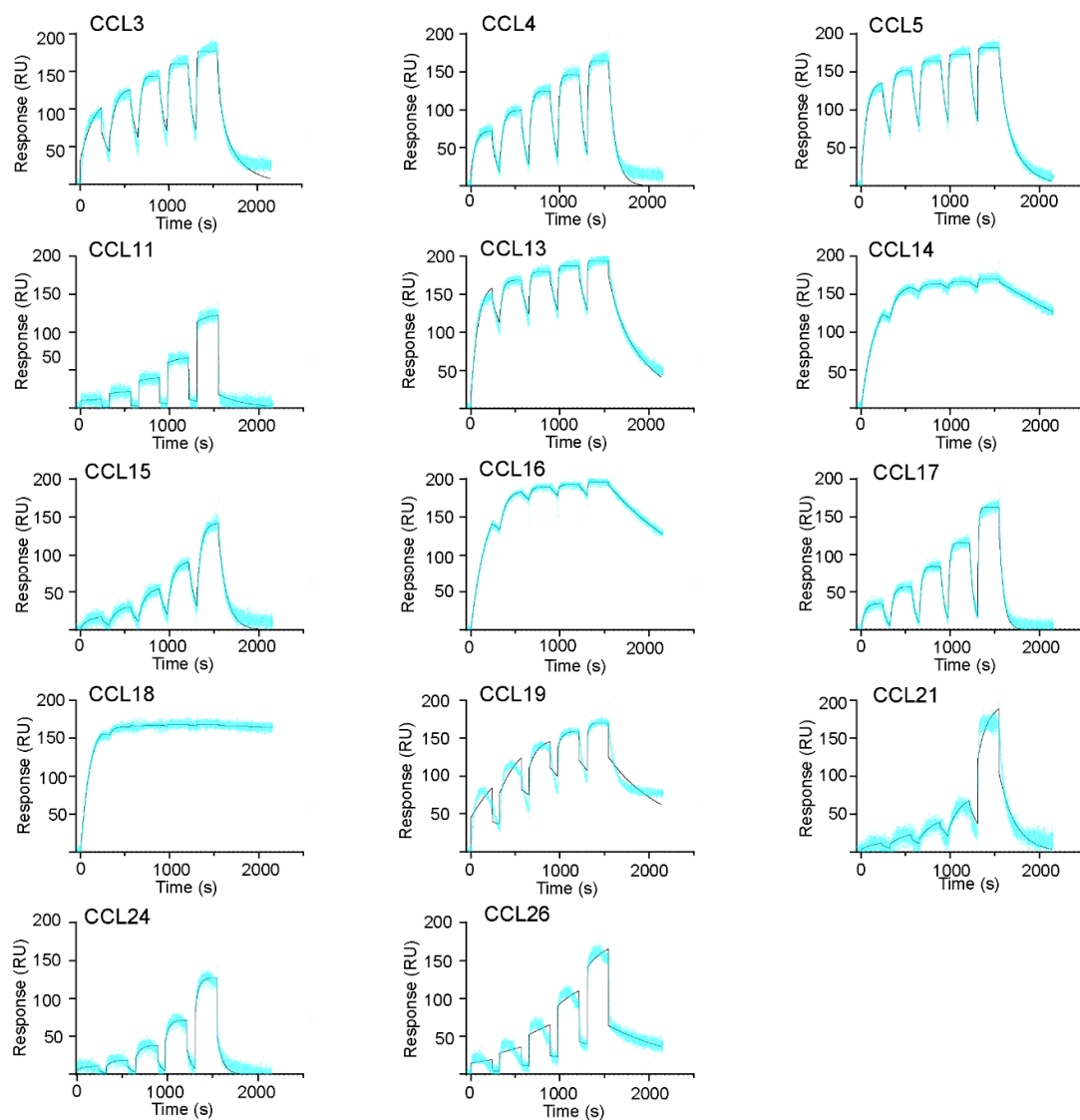
Supplementary Figure 11. Representative surface plasmon resonance (SPR) sensorgrams showing the binding of wild type EVA-A to CC chemokines, with experimental data in cyan and fitted curves in black.



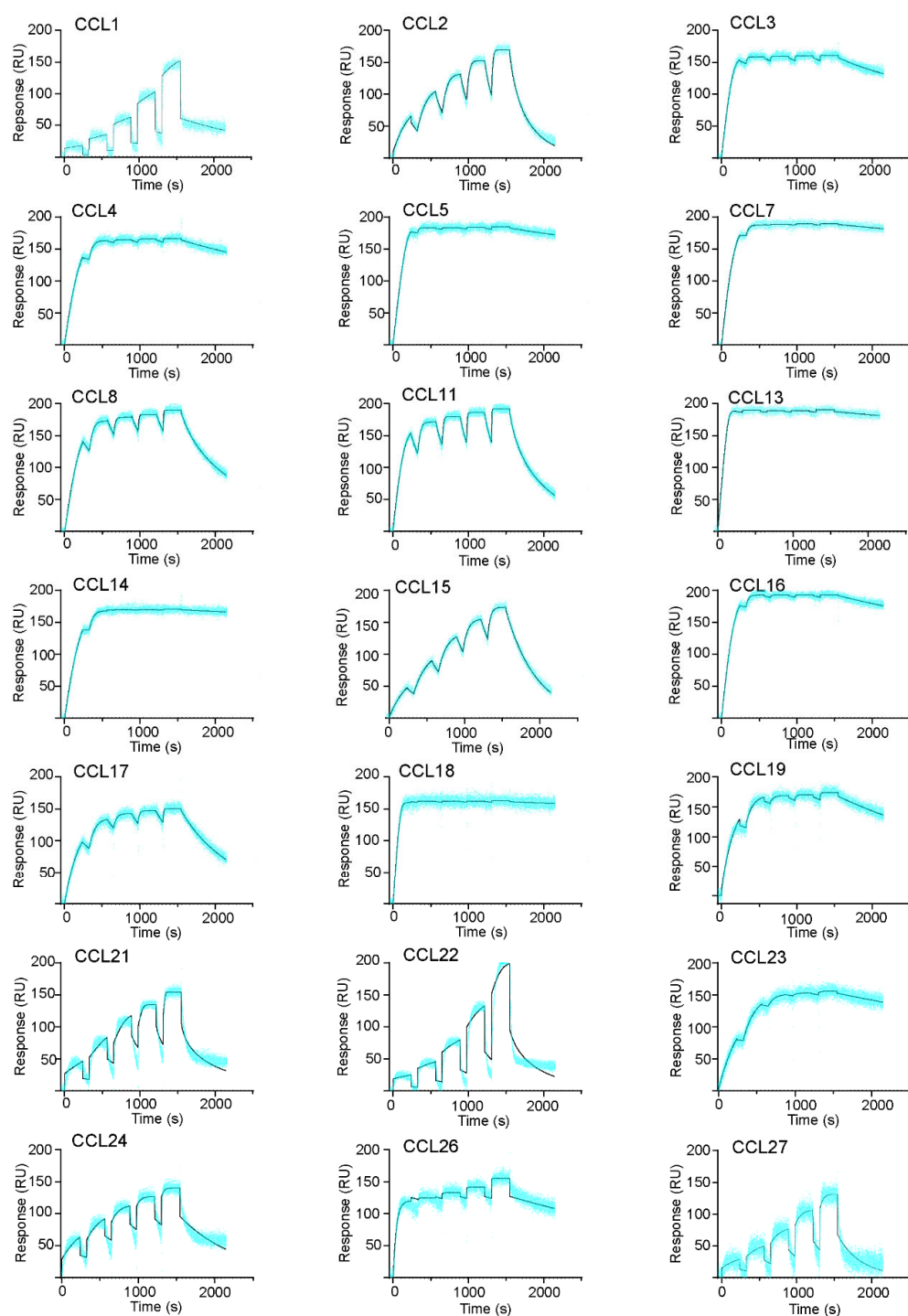
Supplementary Figure 12. Representative surface plasmon resonance (SPR) sensorgrams showing the binding of EVA-A(21-102) to CC chemokines, with experimental data in cyan and fitted curves in black.



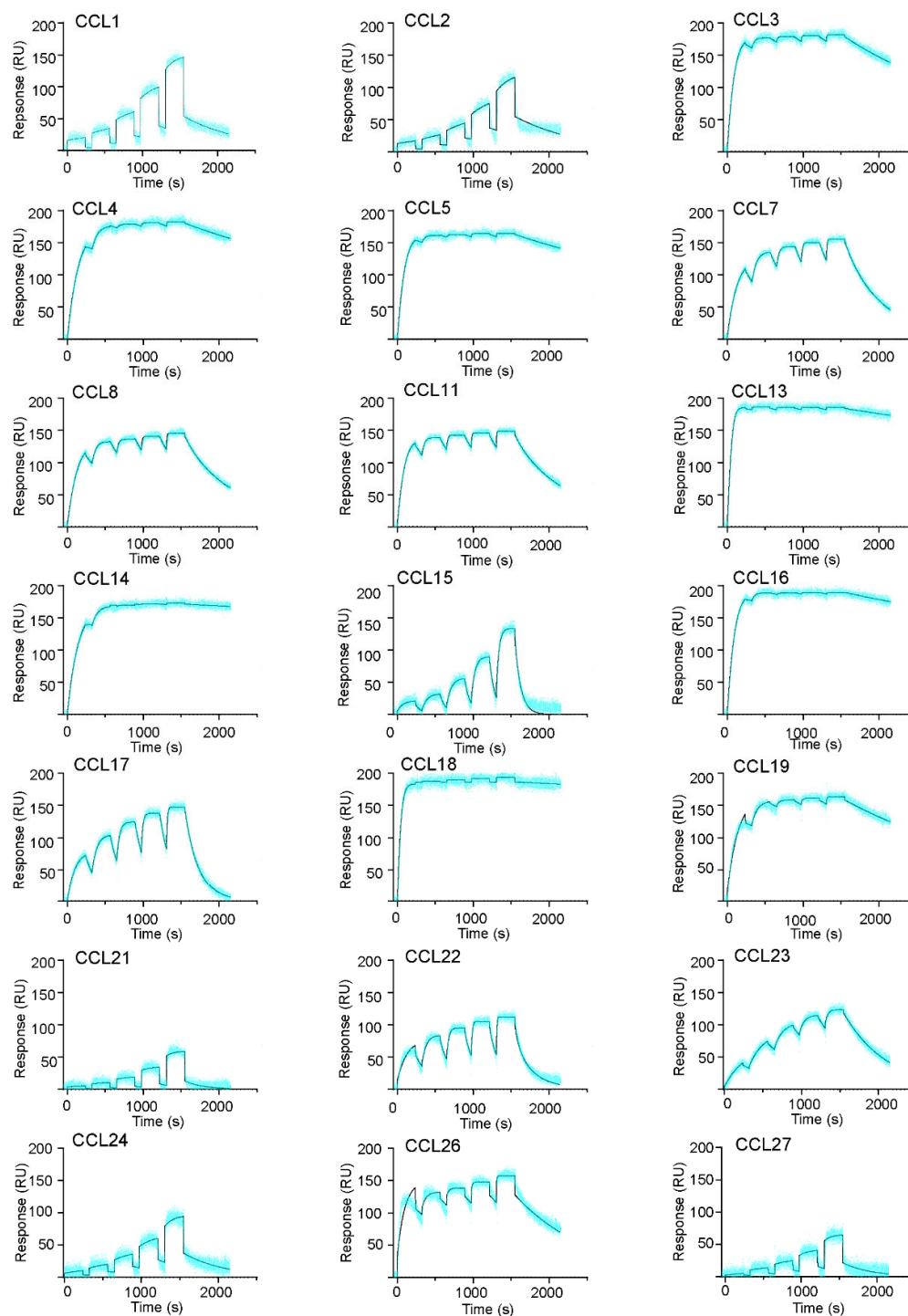
Supplementary Figure 13. Representative surface plasmon resonance (SPR) sensorgrams showing the binding EVA-A(1-98) to CC chemokines, with experimental data in cyan and fitted curves in black.



Supplementary Figure 14. Representative surface plasmon resonance (SPR) sensorgrams showing the binding of EVA-A(C₈) to CC chemokines, with experimental data in cyan and fitted curves in black.

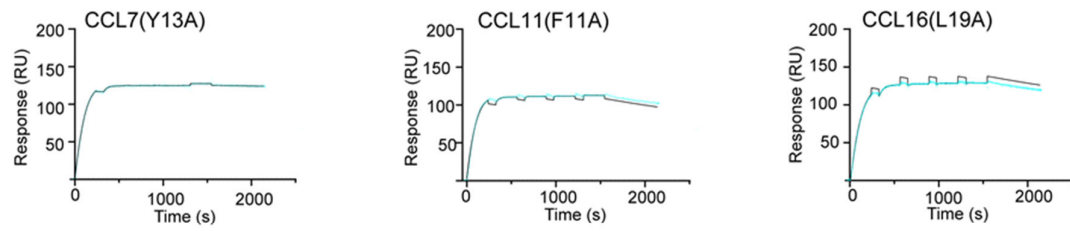


Supplementary Figure 15. Representative surface plasmon resonance (SPR) sensorgrams showing the binding of EVA-A(L39P) to CC chemokines, with experimental data in cyan and fitted curves in black.

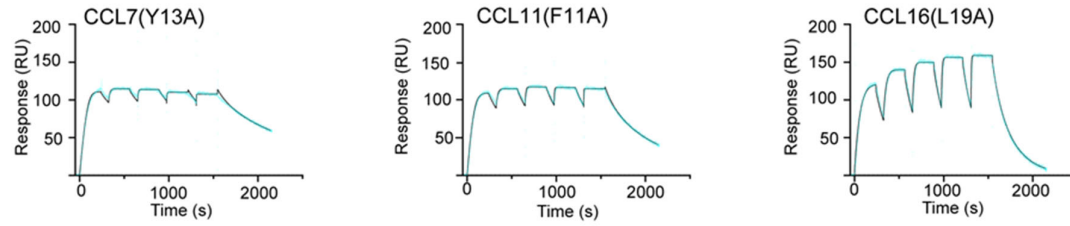


Supplementary Figure 16. Representative surface plasmon resonance (SPR) sensorgrams showing the binding of EVA-A(Y44A) to CC chemokines, with experimental data in cyan and fitted curves in black.

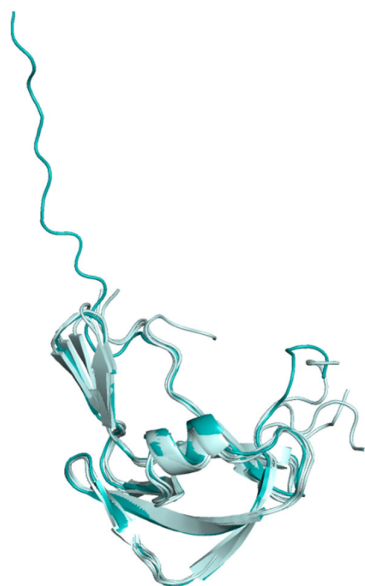
EVA-A(L39P)



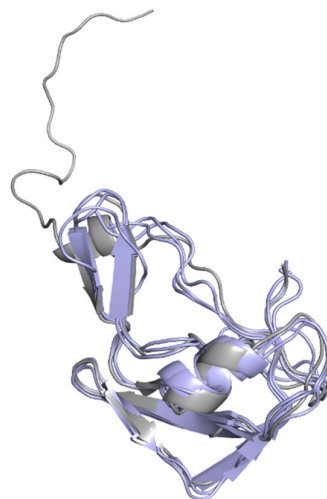
EVA-A(Y44A)



Supplementary Figure 17. Representative surface plasmon resonance (SPR) sensorgrams showing the binding of EVA-A mutants to the chemokine (CCL7, CCL11 and CCL16) variants prepared by substitution of CC+1 residue by alanine, with experimental data in cyan and fitted curves in black.



EVA-P overlaid with simulated structures



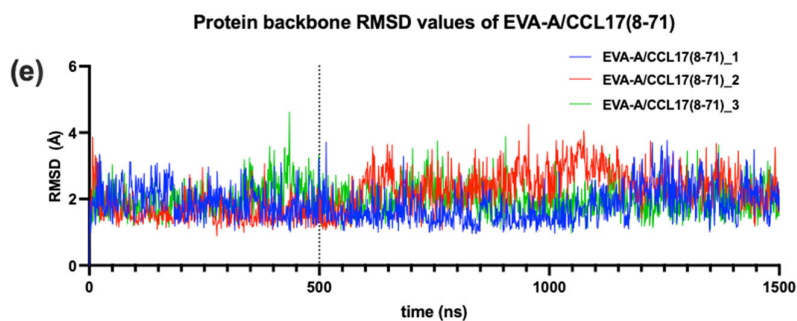
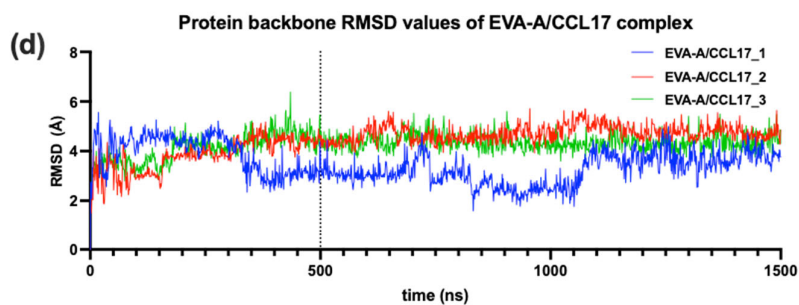
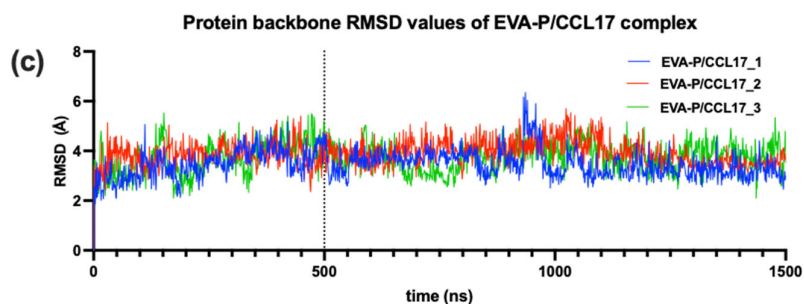
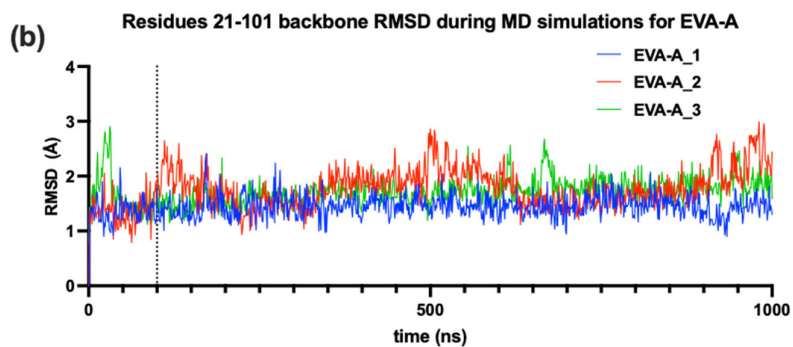
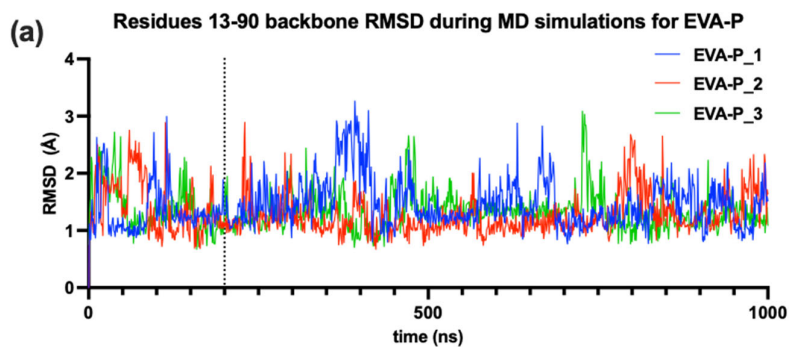
EVA-A overlaid with simulated structures

Supplementary Figure 18. Superimposition of the representative conformations of EVA-A and EVA-P from each of the three replicates MD simulations. (a) Figure showing the overlay of WT EVA-P (green teal) structure with three MD representative structures of EVA-P (pale cyan). (b) Figure showing the overlay of WT EVA-A (grey) structure with three MD representative structures of EVA-A (light blue). Both EVA-P and EVA-A retain the same fold over the MD simulations.

<i>Residue no:</i>	<i>22</i>	<i>25</i>	<i>39</i>	<i>44</i>	<i>51</i>	<i>57</i>
A0A0C9S461 (AAM)	C	P	L	Y	C	Y
A0A6M2E2L8 (ATL)	C	P	A	Y	C	Y
A0A6M2E4M9 (ATL)	C	L	V	Y	C	Y
A0A6M2E3I6 (ATL)	C	P	V	Y	C	Y
A0A6M2E5Z3 (ATL)	C	P	L	Y	C	Y
A0A6M2E374 (ATL)	C	P	L	Y	C	Y
A0A6M2E879 (ATL)	C	P	P	Y	C	E
A0A6M2E5F6 (ATL)	C	P	P	Y	C	E
A0A6M2E2U0 (ATL)	C	P	P	Y	C	E
A0A023G2M2 (ATT)	C	S	T	Q	C	T
A0A023G2G7 (ATT)	C	S	A	L	C	T
A0A023FT45 (APA)	C	P	P	Y	C	T
A0A1E1X165 (AAL)	C	P	Q	Y	C	T
A0A023FFD0 (ACA)	C	P	P	Y	C	Y

Supplementary Figure 19. Residues forming the CC+1 binding pocket in class A3 evasins.

Residues numbers are for EVA-A. Residues conserved with EVA-A are highlighted in pink.



Supplementary Figure 20. Protein backbone RMSD values during MD simulations. (a) Protein backbone RMSD values of residues 13-90 during MD simulations for N-terminal truncated EVA-P. Each simulation was conducted for 1 μ s. All three replicates appeared to be equilibrated after 200 ns, thus simulation periods 200 ns – 1 μ s were analysed. Total time for analysis was 800 ns x 3 = 2.4 μ s. (b) Protein backbone RMSD values of residues 21-101 during MD simulations for N-terminal truncated EVA-A. Each simulation was conducted for 1 μ s. All three replicates appeared to be equilibrated after 100 ns, thus simulation periods 100 ns – 1 μ s were analysed. Total time for analysis was 900 ns x 3 = 2.7 μ s. (c) Protein backbone RMSD values of EVA-P/CCL17 complex during MD simulations. The trajectories were first aligned by backbone atoms of EVA-P and then the overall protein (including both EVA-P and CCL17) RMSD values were calculated. For EVA-P/CCL17 complex, the simulations appeared to have equilibrated after 500 ns of each simulation, therefore, time periods 500 ns – 1.5 μ s were analysed. Total time for analysis was 3 μ s. (d) Protein backbone RMSD values of EVA-A/CCL17 complex during MD simulations. The trajectories were first aligned by backbone atoms of EVA-A and then the overall protein (including both EVA-P and CCL17) RMSD values were calculated. The overall protein backbone RMSD values appeared to have equilibrated after 350 ns in both simulations 2 and 3, but simulation 1 showed large fluctuations. Examination of the trajectories in simulation 1 showed the fluctuation may be due to movements of the flexible N-terminal region of CCL17 (residues 1-8). To confirm this, protein RMSD values including all backbone of EVA-A and backbone of residues 8 to 71 of CCL17 are plotted in (e). (e) Protein backbone RMSD values of EVA-A/CCL17(residue 8-71) complex during MD simulations. When the flexible N-terminal region has been removed from the RMSD calculation, it is clear simulation 1 has also equilibrated. Therefore, trajectories between 500 ns – 1.5 μ s from each simulation were used for analysis. The total time for analysis was 3 μ s.

Supplementary Table 1. Kinetic and equilibrium parameters for EVA-A binding to chemokines: fitted values of the association rate constant (k_a) and dissociation rate constant (k_d), the equilibrium dissociation constants (K_d) and negative logarithm of the equilibrium dissociation constant K_d in M (pK_d), presented as mean $\pm$ SEM from three independent SPR experiments. The representative K_d value listed is calculated from the mean value of pK_d .

Chemokine	$k_a \times 10^5$ [M⁻¹s⁻¹]	$k_d \times 10^{-3}$ [s⁻¹]	pK_d	K_d [nM]
CCL1	0.16 $\pm$ 0.03	1.55 $\pm$ 0.07	6.99 $\pm$ 0.08	107
CCL2	#	#	#	#
CCL3	14.8 $\pm$ 1.1	0.63 $\pm$ 0.09	9.38 $\pm$ 0.04	0.42
CCL4	4.40 $\pm$ 0.15	1.13 $\pm$ 0.15	8.60 $\pm$ 0.07	2.59
CCL5	38.7 $\pm$ 14.4	0.046 $\pm$ 0.171	9.943 $\pm$ 0.003	0.11
CCL7	2.03 $\pm$ 1.67	57.3 $\pm$ 50.8	6.61 $\pm$ 0.10	256
CCL8	3.17 $\pm$ 0.17	64.9 $\pm$ 16.6	6.72 $\pm$ 0.11	200
CCL11	11.6 $\pm$ 3.4	57.3 $\pm$ 25.9	7.36 $\pm$ 0.10	45.6
CCL13	51.4 $\pm$ 5.7	\$	\$	\$
CCL14	12.8 $\pm$ 1.5	\$	\$	\$
CCL15	1.76 $\pm$ 0.03	0.31 $\pm$ 0.00	8.75 $\pm$ 0.01	1.77
CCL16	10.6 $\pm$ 2.0	\$	\$	\$
CCL17	4.52 $\pm$ 0.81	0.23 $\pm$ 0.03	9.28 $\pm$ 0.07	0.54
CCL18	19.6 $\pm$ 10.9	\$	\$	\$
CCL19	15.8 $\pm$ 0.4	\$	\$	\$
CCL20	#	#	#	#
CCL21	12.4 $\pm$ 1.3	2.73 $\pm$ 0.14	8.65 $\pm$ 0.02	2.23
CCL22	0.33 $\pm$ 0.003	0.04	7.35 $\pm$ 0.01	44.6
CCL23	2.29 $\pm$ 0.01	\$	\$	\$
CCL24	1.82 $\pm$ 0.19	1.48 $\pm$ 0.12	8.09 $\pm$ 0.01	8.19
CCL25	#	#	#	#
CCL26	19.4 $\pm$ 12.4	0.81 $\pm$ 0.19	9.16 $\pm$ 0.28	1.04
CCL27	3.18 $\pm$ 0.03	3.58 $\pm$ 0.03	7.95 $\pm$ 0.00	11.3
CCL28	#	#	#	#

\$, ($K_d < 0.1$ nM, $pK_d > 10$, $k_d < 10^{-4}$ s⁻¹)

#, no measurable binding at 500 nM chemokine concentration

Supplementary Table 2. Kinetic and equilibrium parameters for EVA-A (21-102) binding to chemokines: fitted values of the association rate constant (k_a) and dissociation rate constant (k_d), the equilibrium dissociation constants (K_d) and negative logarithm of the equilibrium dissociation constant K_d in M (pK_d), presented as mean $\pm$ SEM from three independent SPR experiments. The representative K_d value listed is calculated from the mean value of pK_d .

Chemokines	$k_a \times 10^5 [\text{M}^{-1}\text{s}^{-1}]$	$k_d \times 10^{-3} [\text{s}^{-1}]$	pK_d	$K_d [\text{nM}]$
CCL1	2.68 $\pm$ 2.54	180.35 $\pm$ 175.82	6.36 $\pm$ 0.10	457
CCL2	#	#	#	#
CCL3	4.49 $\pm$ 0.29	1.44 $\pm$ 0.01	8.49 $\pm$ 0.03	3.23
CCL4	4.61 $\pm$ 0.67	2.18 $\pm$ 0.06	8.31 $\pm$ 0.06	4.95
CCL5	7.77 $\pm$ 0.50	1.95 $\pm$ 0.07	8.60 $\pm$ 0.02	2.52
CCL7	#	#	#	#
CCL8	1.12 $\pm$ 0.97	34.39 $\pm$ 33.16	6.93 $\pm$ 0.29	169
CCL11	4.54 $\pm$ 0.53	218.17 $\pm$ 23.34	6.32 $\pm$ 0.10	507
CCL13	29.40 $\pm$ 0.99	0.87 $\pm$ 0.03	9.53 $\pm$ 0.01	0.30
CCL14	7.03 $\pm$ 0.06	0.08 $\pm$ 0.00	9.95 $\pm$ 0.01	0.11
CCL15	0.63 $\pm$ 0.02	2.14 $\pm$ 0.03	7.47 $\pm$ 0.01	34.0
CCL16	8.31 $\pm$ 0.14	0.19 $\pm$ 0.00	9.65 $\pm$ 0.00	0.22
CCL17	5.20 $\pm$ 0.10	0.54 $\pm$ 0.01	8.98 $\pm$ 0.01	1.04
CCL18	15.87 $\pm$ 3.35	\$	\$	\$
CCL19	3.42 $\pm$ 0.21	0.53 $\pm$ 0.03	8.81 $\pm$ 0.05	1.58
CCL20	#	#	#	#
CCL21	2.24 $\pm$ 0.23	10.57 $\pm$ 1.01	7.33 $\pm$ 0.04	47.53
CCL22	0.26 $\pm$ 0.02	1.61 $\pm$ 0.19	7.22 $\pm$ 0.06	61.60
CCL23	0.87 $\pm$ 0.32	0.04 $\pm$ 0.02	9.40 $\pm$ 0.22	0.51
CCL24	0.46 $\pm$ 0.09	0.91 $\pm$ 0.20	7.70 $\pm$ 0.01	19.93
CCL25	#	#	#	#
CCL26	0.53 $\pm$ 0.06	0.49 $\pm$ 0.05	8.03 $\pm$ 0.02	9.24
CCL27	#	#	#	#
CCL28	#	#	#	#

\$, ($K_d < 0.1$ nM, $pK_d > 10$, $k_d < 10^{-4}$ s $^{-1}$)

#, no measurable binding at 500 nM chemokine concentration

Supplementary Table 3. Kinetic and equilibrium parameters for EVA-A(1-98) binding to chemokines: fitted values of the association rate constant (k_a) and dissociation rate constant (k_d), the equilibrium dissociation constants (K_d) and negative logarithm of the equilibrium dissociation constant K_d in M (pK_d), presented as mean $\pm$ SEM from three independent SPR experiments. The representative K_d value listed is calculated from the mean value of pK_d .

Chemokines	$k_a \times 10^5 [\text{M}^{-1}\text{s}^{-1}]$	$k_d \times 10^{-3} [\text{s}^{-1}]$	pK_d	$K_d [\text{nM}]$
CCL1	0.06 ± 0.02	1.68 ± 2.55	6.43 ± 0.13	406
CCL2	#	#	#	#
CCL3	3.45 ± 0.11	0.58 ± 0.64	8.75 ± 0.02	1.77
CCL4	1.65 ± 0.56	0.87 ± 0.94	8.18 ± 0.21	8.56
CCL5	3.54 ± 0.10	0.33 ± 0.41	8.99 ± 0.02	1.03
CCL7	0.31 ± 0.26	1.79 ± 0.51	6.27 ± 0.34	1014
CCL8	1.73 ± 1.35	34.43 ± 25.29	6.82 ± 0.12	163
CCL11	1.01 ± 0.14	53.65 ± 34.54	6.31 ± 0.01	487
CCL13	4.58 ± 0.05	0.63 ± 0.67	8.85 ± 0.01	1.42
CCL14	2.39 ± 0.29	\$	\$	\$
CCL15	0.53 ± 0.00	1.62 ± 1.73	7.50 ± 0.01	31.67
CCL16	2.58 ± 0.04	\$	\$	\$
CCL17	1.59 ± 0.03	\$	\$	\$
CCL18	4.43 ± 0.27	\$	\$	\$
CCL19	1.44 ± 0.05	0.03 ± 0.01	9.82 ± 0.12	0.16
CCL20	#	#	#	#
CCL21	1.26 ± 0.24	2.33 ± 1.74	7.79 ± 0.14	17.8
CCL22	0.29 ± 0.08	0.84 ± 0.37	6.90 ± 0.00	125
CCL23	1.44 ± 0.33	0.02 ± 0.04	9.48 ± 0.26	0.45
CCL24	0.36 ± 0.06	1.11 ± 0.89	7.55 ± 0.04	28.10
CCL25	#	#	#	#
CCL26	3.57 ± 0.39	0.48 ± 0.67	8.75 ± 0.10	1.89
CCL27	0.95 ± 0.13	4.09 ± 3.67	7.39 ± 0.06	41.73
CCL28	#	#	#	#

\$, ($K_d < 0.1 \text{ nM}$, $pK_d > 10$, $k_d < 10^{-4} \text{ s}^{-1}$)

#, no measurable binding at 500 nM chemokine concentration

Supplementary Table 4. Kinetic and equilibrium parameters for EVA-A(C₈) binding to chemokines: fitted values of the association rate constant (k_a) and dissociation rate constant (k_d), the equilibrium dissociation constants (K_d) and negative logarithm of the equilibrium dissociation constant K_d in M (pK_d), presented as mean $\pm$ SEM from three independent SPR experiments. The representative K_d value listed is calculated from the mean value of pK_d .

Chemokines	$k_a \times 10^5$ [M⁻¹s⁻¹]	$k_d \times 10^{-3}$ [s⁻¹]	pK_d	K_d [nM]
CCL1	#	#	#	#
CCL2	#	#	#	#
CCL3	10.84 $\pm$ 8.78	46.38 $\pm$ 38.37	7.40 $\pm$ 0.02	40.3
CCL4	2.93 $\pm$ 0.98	21.54 $\pm$ 8.32	7.15 $\pm$ 0.02	71.3
CCL5	7.16 $\pm$ 0.12	11.49 $\pm$ 0.28	7.79 $\pm$ 0.00	16.0
CCL7	1.77 $\pm$ 0.53	166.19 $\pm$ 76.01	6.22 $\pm$ 0.24	765
CCL8	#	#	#	#-
CCL11	2.36 $\pm$ 1.22	195.99 $\pm$ 104.04	6.22 $\pm$ 0.14	663
CCL13	14.73 $\pm$ 1.87	4.23 $\pm$ 0.32	8.54 $\pm$ 0.03	2.91
CCL14	3.86 $\pm$ 0.56	0.48 $\pm$ 0.02	8.89 $\pm$ 0.06	1.30
CCL15	0.22 $\pm$ 0.01	12.75 $\pm$ 1.29	6.25 $\pm$ 0.05	577
CCL16	3.96 $\pm$ 0.55	0.75 $\pm$ 0.08	8.72 $\pm$ 0.01	1.90
CCL17	2.54 $\pm$ 0.63	21.69 $\pm$ 2.10	7.04 $\pm$ 0.10	97.1
CCL18	8.37 $\pm$ 0.55	\$	\$	\$
CCL19	2.45 $\pm$ 1.60	3.45 $\pm$ 1.99	7.80 $\pm$ 0.05	16.1
CCL20	#	#	#	#
CCL21	#	#	#	#
CCL22	#	#	#	#
CCL23	0.74 $\pm$ 0.14	1.31 $\pm$ 0.13	7.74 $\pm$ 0.14	20.3
CCL24	0.18 $\pm$ 0.03	8.69 $\pm$ 4.01	6.39 $\pm$ 0.13	447
CCL25	#	#	#	#
CCL26	0.14 $\pm$ 0.02	0.99 $\pm$ 0.03	7.16 $\pm$ 0.05	70.9
CCL27	#	#	#	#
CCL28	#	#	#	#

\$, ($K_d < 0.1$ nM, $pK_d > 10$, $k_d < 10^{-4}$ s⁻¹)

#, no measurable binding at 500 nM chemokine concentration

Supplementary Table 5. Kinetic and equilibrium parameters for EVA-A(L39P) binding to chemokines: fitted values of the association rate constant (k_a) and dissociation rate constant (k_d), the equilibrium dissociation constants (K_d) and negative logarithm of the equilibrium dissociation constant K_d in M (pK_d), presented as mean $\pm$ SEM from three independent SPR experiments. The representative K_d value listed is calculated from the mean value of pK_d .

Chemokines	$k_a \times 10^5$ [M⁻¹s⁻¹]	$k_d \times 10^{-3}$ [s⁻¹]	pK_d	K_d [nM]
CCL1	0.17 $\pm$ 0.02	1.67 $\pm$ 0.64	7.26 $\pm$ 0.08	57.5
CCL2	3.29 $\pm$ 0.16	35.98 $\pm$ 28.10	7.61 $\pm$ 0.04	24.9
CCL3	12.20 $\pm$ 2.87	0.40 $\pm$ 0.02	9.51 $\pm$ 0.09	0.32
CCL4	8.05 $\pm$ 0.48	0.23 $\pm$ 0.04	9.52 $\pm$ 0.02	0.30
CCL5	15.27 $\pm$ 2.49	\$	\$	\$
CCL7	10.50 $\pm$ 1.27	\$	\$	\$
CCL8	9.71 $\pm$ 0.58	2.18 $\pm$ 0.51	8.59 $\pm$ 0.04	2.59
CCL11	14.00 $\pm$ 0.96	3.72 $\pm$ 1.19	8.48 $\pm$ 0.03	3.32
CCL13	32.57 $\pm$ 4.61	\$	\$	\$
CCL14	24.08 $\pm$ 17.06	0.13 $\pm$ 0.08	10.35 $\pm$ 0.03	0.04
CCL15	1.02 $\pm$ 0.03	4.67 $\pm$ 1.93	7.58 $\pm$ 0.03	26.6
CCL16	11.83 $\pm$ 0.17	0.16 $\pm$ 0.04	9.77 $\pm$ 0.04	0.17
CCL17	4.39 $\pm$ 0.31	4.05 $\pm$ 1.40	8.26 $\pm$ 0.23	7.49
CCL18	37.43 $\pm$ 3.21	\$	\$	\$
CCL19	2.78 $\pm$ 0.36	0.36 $\pm$ 0.03	8.88 $\pm$ 0.06	1.34
CCL20	#	#	#	#
CCL21	1.97 $\pm$ 0.31	4.84 $\pm$ 1.01	7.62 $\pm$ 0.05	24.5
CCL22	10.31 $\pm$ 1.50	2.92 $\pm$ 1.34	8.84 $\pm$ 0.05	1.46
CCL23	1.40 $\pm$ 0.30	0.67 $\pm$ 0.50	8.96 $\pm$ 0.08	1.14
CCL24	1.11 $\pm$ 0.17	1.21 $\pm$ 0.10	8.02 $\pm$ 0.02	9.58
CCL25	#	#	#	#
CCL26	26.33 $\pm$ 6.06	0.78 $\pm$ 0.31	9.73 $\pm$ 0.01	0.19
CCL27	0.45 $\pm$ 0.06	3.09 $\pm$ 0.26	7.20 $\pm$ 0.06	63.9
CCL28	#	#	#	#

\$, ($K_d < 0.1$ nM, $pK_d > 10$, $k_d < 10^{-4}$ s⁻¹)

#, no measurable binding at 500 nM chemokine concentration

Supplementary Table 6. Kinetic and equilibrium parameters for EVA-A(Y44A) binding to chemokines: fitted values of the association rate constant (k_a) and dissociation rate constant (k_d), the equilibrium dissociation constants (K_d) and negative logarithm of the equilibrium dissociation constant K_d in M (pK_d), presented as mean $\pm$ SEM from three independent SPR experiments. The representative K_d value listed is calculated from the mean value of pK_d .

Chemokines	$k_a \times 10^5 [\text{M}^{-1}\text{s}^{-1}]$	$k_d \times 10^{-3} [\text{s}^{-1}]$	pK_d	$K_d [\text{nM}]$
CCL1	0.57 ± 0.20	2.56 ± 0.63	7.33 ± 0.10	49.9
CCL2	2.91 ± 2.76	31.99 ± 30.09	6.95 ± 0.13	123
CCL3	5.66 ± 2.03	0.44 ± 0.01	9.06 ± 0.14	0.95
CCL4	3.19 ± 0.72	0.20 ± 0.02	9.19 ± 0.05	0.66
CCL5	9.81 ± 1.88	0.21 ± 0.02	9.66 ± 0.09	0.23
CCL7	4.19 ± 0.55	1.99 ± 0.15	8.32 ± 0.03	4.81
CCL8	4.74 ± 1.33	1.56 ± 0.13	8.45 ± 0.08	3.62
CCL11	8.77 ± 0.96	1.50 ± 0.10	8.76 ± 0.02	1.73
CCL13	26.83 ± 4.64	\$	\$	\$
CCL14	4.81 ± 0.19	0.05 ± 0.01	9.99 ± 0.08	0.11
CCL15	0.79 ± 0.13	11.74 ± 1.63	6.83 ± 0.13	161.30
CCL16	6.64 ± 1.21	0.13 ± 0.01	9.71 ± 0.08	0.20
CCL17	1.66 ± 1.10	5.10 ± 0.50	7.32 ± 0.25	61.9
CCL18	16.93 ± 2.24	\$	\$	\$
CCL19	4.90 ± 0.40	0.36 ± 0.02	9.13 ± 0.05	0.75
CCL20	#	#	#	#
CCL21	0.36 ± 0.09	3.56 ± 0.68	7.00 ± 0.20	118.9
CCL22	7.66 ± 1.35	8.18 ± 1.87	7.98 ± 0.12	11.2
CCL23	1.00 ± 0.15	1.76 ± 0.05	7.74 ± 0.08	18.8
CCL24	0.26 ± 0.01	1.79 ± 0.26	7.18 ± 0.08	68.6
CCL25	#	#	#	#
CCL26	3.81 ± 1.12	1.01 ± 0.20	8.56 ± 0.11	2.94
CCL27	0.52 ± 0.10	6.39 ± 3.53	7.02 ± 0.15	109.7
CCL28	#	#	#	#

\$, ($K_d < 0.1 \text{ nM}$, $pK_d > 10$, $k_d < 10^{-4} \text{ s}^{-1}$)

#, no measurable binding at 500 nM chemokine concentration

Supplementary Table 7. Kinetic and equilibrium parameters for EVA-A binding to chemokines: fitted values of the association rate constant (k_a) and dissociation rate constant (k_d), the equilibrium dissociation constants (K_d) and negative logarithm of the equilibrium dissociation constant K_d in M (pK_d), presented as mean $\pm$ SEM from three independent SPR experiments. The representative K_d value listed is calculated from the mean value of pK_d .

Chemokines	$k_a \times 10^5 [\text{M}^{-1}\text{s}^{-1}]$	$k_d \times 10^{-3} [\text{s}^{-1}]$	pK_d	$K_d [\text{nM}]$
CCL7(Y13A)	9.66 ± 2.97	\$	\$	\$
CCL11(F11A)	10.99 ± 1.42	0.25 ± 0.003	9.63 ± 0.05	0.24
CCL16(L19A)	7.31 ± 0.95	0.20 ± 0.01	9.57 ± 0.06	0.27

\$, ($K_d < 0.1 \text{ nM}$, $pK_d > 10$, $k_d < 10^{-4} \text{ s}^{-1}$)

Supplementary Table 8. Kinetic and equilibrium parameters for EVA-A(L39P) binding to chemokines: fitted values of the association rate constant (k_a) and dissociation rate constant (k_d), the equilibrium dissociation constants (K_d) and negative logarithm of the equilibrium dissociation constant K_d in M (pK_d), presented as mean $\pm$ SEM from three independent SPR experiments. The representative K_d value listed is calculated from the mean value of pK_d .

Chemokines	$k_a \times 10^5 [\text{M}^{-1}\text{s}^{-1}]$	$k_d \times 10^{-3} [\text{s}^{-1}]$	pK_d	$K_d [\text{nM}]$
CCL7(Y13A)	6.85 ± 0.12	\$	\$	\$
CCL11(F11A)	7.07 ± 0.99	0.35 ± 0.02	9.30 ± 0.04	0.51 \
CCL16(L19A)	5.60 ± 0.34	0.85 ± 0.05	8.82 ± 0.00	1.53 \

\$, ($K_d < 0.1 \text{ nM}$, $pK_d > 10$, $k_d < 10^{-4} \text{ s}^{-1}$)

Supplementary Table 9. Kinetic and equilibrium parameters for EVA-A(Y44A) binding to chemokines: fitted values of the association rate constant (k_a) and dissociation rate constant (k_d), the equilibrium dissociation constants (K_d) and negative logarithm of the equilibrium dissociation constant K_d in M (pK_d), presented as mean $\pm$ SEM from three independent SPR experiments. The representative K_d value listed is calculated from the mean value of pK_d .

Chemokines	$k_a \times 10^5 [\text{M}^{-1}\text{s}^{-1}]$	$k_d \times 10^{-3} [\text{s}^{-1}]$	pK_d	$K_d [\text{nM}]$
CCL7(Y13A)	4.41 ± 0.25	8.80 ± 0.24	7.70 ± 0.02	20.0
CCL11(F11A)	8.02 ± 0.47	2.26 ± 0.07	8.55 ± 0.02	2.83
CCL16(L19A)	1.07 ± 0.22	1.51 ± 0.11	7.83 ± 0.12	15.9

Supplementary Table 10. H-bond established during MD simulations of EVA-P:CCL17 and EVA-A:CCL17. High occupancy hydrogen bonds are in red.

EVA-P:CCL17			EVA-A:CCL17		
Donor	Acceptor	Average	Donor	Acceptor	Average
Cys50 ^{CCL17} Main	Thr16 ^{EVA-P} Main	39.96%	Cys50 ^{CCL17} Main	Ala23 ^{EVA-A} Main	61.77%
Phe19 ^{EVA-P} Main	Cys10 ^{CCL17} Main	62.94%	Ala26 ^{EVA-A} Main	Cys10 ^{CCL17} Main	36.03%
Cys10 ^{CCL17} Main	Phe19 ^{EVA-P} Main	35.03%	Cys10 ^{CCL17} Main	Ala26 ^{EVA-A} Main	17.42%
Val21 ^{EVA-P} Main	Arg8 ^{CCL17} Main	17.12%	His28 ^{EVA-A} Main	Arg8 ^{CCL17} Main	12.45%
Arg8 ^{CCL17} Side	Gly92 ^{EVA-P} Main	12.09%	Arg8 ^{CCL17} Side	Asn30 ^{EVA-A} Side	14.99%
Ala29 ^{EVA-P} Main	Asp33 ^{CCL17} Main	15.68%	Leu37 ^{EVA-A} Main	Asp33 ^{CCL17} Main	51.41%
Asn27 ^{EVA-P} Side	Asp33 ^{CCL17} Side	10.62%	Tyr44 ^{EVA-A} Side	Glu13 ^{CCL17} Side	15.72%

Supplementary Table 11. X-ray diffraction data collection and refinement statistics. Values in parentheses are for the outermost resolution shell.

	EVA-A:CCL7	EVA-A:CCL11	EVA-A:CCL16	EVA-A:CCL17
PDB ID	7SCU	7SCS	7SCT	7SCV
Data collection				
Wavelength (Å)	0.953	0.953	0.953	0.953
Resolution range	48.59 - 1.86 (1.90 - 1.86)	41.58 - 1.51 (1.54 - 1.51)	48.13 - 1.84 (1.88 - 1.84)	44.69 - 2.01 (2.07 - 2.01)
Space group	P 4 ₁ 2 ₁ 2	P 4 ₁ 2 ₁ 2	I 4 2 2	P 3 ₁ 2 1
Cell dimensions				
<i>a</i> , <i>b</i> , <i>c</i> (Å)	71.2 71.2 66.4	75.0 75.0 66.9	96.2 96.2 84.7	88.0 88.0 55.1
<i>a</i> , <i>b</i> , <i>g</i> (°)	90 90 90	90 90 90	90 90 90	90 90 120
<i>R</i> _{merge}	0.102 (1.438)	0.075 (1.130)	0.076 (1.518)	0.114 (1.460)
<i>R</i> _{meas}	0.106 (1.497)	0.077 (1.339)	0.077 (1.549)	0.117 (1.502)
<i>R</i> _{pim}	0.021 (0.299)	0.015 (0.270)	0.015 (0.304)	0.026 (0.346)
<i>I</i> / <i>s</i> (<i>I</i>)	20.0 (2.7)	23.9 (2.9)	23.0 (2.7)	17.4 (2.4)
CC _{1/2}	0.996 (0.670)	1.000 (0.774)	0.999 (0.829)	0.999 (0.699)
Completeness (%)	99.8 (96.5)	99.8 (96.9)	99.9 (98.6)	99.7 (96.5)
Total reflections	377698 (21922)	776078 (33204)	459548 (26148)	330673 (21345)
Unique reflections	14877 (912)	30361 (1433)	17690 (1051)	16612 (1165)
Multiplicity	25.4 (24.0)	25.6 (23.2)	26.0 (24.9)	19.9 (18.3)
Refinement				
<i>R</i> _{work} / <i>R</i> _{free}	0.193 / 0.210	0.184 / 0.206	0.203 / 0.210	0.236 / 0.251
No. of atoms	1214	1331	1248	1177
Macromolecules	1124	1174	1169	1089
Solvent	90	157	79	88
Average B factors	38.3	29.6	50.3	50.4
Macromolecules	38.3	28.4	50.6	50.4
Solvent	38.3	38.6	46.0	50.8
r. m. s. deviations				
Bond lengths (Å)	0.003	0.009	0.007	0.002
Bond angles (°)	0.67	1.21	1.08	0.62
Ramachandran statistics (%)				
Favoured	99.31	97.97	97.28	96.53
Allowed	0.69	2.03	2.72	3.47
Outliers	0	0	0	0
Clashscore	1.38	6.44	2.18	4.78
MolProbity score	0.87	1.36	1.13	1.47

Supplementary Table 11. (continued)

	EVA-A (Y44A): CCL2	EVA-A (Y44A): CCL7	EVA-A (L39P): CCL7	EVA-A(C8): CCL17	EVA-A: CCL7(Y13A)
PDB ID	8FJ0	8FK6	8FK8	8FJ2	8FJ3
Data collection					
Wavelength (Å)	0.953	0.953	0.953	0.953	0.953
Resolution range	47.11 - 2.89 (3.07 - 2.89)	49.25 - 1.74 (1.77 - 1.74)	48.78 - 1.96 (2.01 - 1.96)	46.34 - 2.07 (2.13 - 2.07)	48.55 - 2.07 (2.13 - 2.07)
Space group	P 1 2 ₁ 1	P 4 ₁ 2 ₁ 2	P 4 ₁ 2 ₁ 2	I 2 2 2	P 4 ₁ 2 ₁ 2
Cell dimensions					
<i>a</i> , <i>b</i> , <i>c</i> (Å)	82.4 49.6 88.3	72.3 72.3 67.2	72.5 72.5 65.9	60.6 62.5 92.6	72.2 72.2 65.5
<i>a</i> , <i>b</i> , <i>g</i> (°)	90 112.9 90	90 90 90	90 90 90	90 90 90	90 90 90
<i>R</i> _{merge}	0.427 (2.795)	0.052 (0.351)	0.130 (1.442)	0.210 (1.954)	0.197 (1.411)
<i>R</i> _{meas}	0.465 (3.040)	0.054 (0.358)	0.133 (1.470)	0.219 (2.048)	0.201 (1.440)
<i>R</i> _{pim}	0.180 (1.179)	0.011 (0.069)	0.026 (0.282)	0.062 (0.598)	0.040 (0.285)
<i>I</i> / <i>s(I)</i>	5.4 (1.5)	39.4 (10.4)	18.2 (2.8)	8.7 (2.0)	14.1 (3.4)
CC _{1/2}	0.971 (0.475)	0.999 (0.985)	0.999 (0.810)	0.992 (0.551)	0.998 (0.803)
Completeness (%)	96.2 (90.5)	100 (99.4)	100 (99.4)	99.3 (92.7)	99.9 (98.9)
Total reflections	97403 (14332)	489279 (26691)	335070 (23669)	139936 (8894)	272655 (20935)
Unique reflections	14772 (2250)	18937 (1025)	13129 (903)	10995 (785)	11114 (846)
Multiplicity	6.6 (6.4)	25.8 (26.0)	25.5 (26.2)	12.7 (11.3)	24.5 (24.7)
Refinement					
<i>R</i> _{work} / <i>R</i> _{free}	0.253 / 0.284	0.206 / 0.223	0.241 / 0.262	0.213 / 0.230	0.213 / 0.229
No. of atoms	2886	1226	1078	1096	1160
Macromolecules	1875	1123	1026	1053	1082
Solvent	11	103	52	43	78
Average B factors	62.5	38.9	42.5	57.2	37.1
Macromolecules	62.5	38.8	42.6	57.4	37.2
Solvent	50.3	39.5	40.0	52.5	36.1
r. m. s. deviations					
Bond lengths (Å)	0.002	0.003	0.018	0.008	0.019
Bond angles (°)	0.70	0.63	1.80	1.02	1.67
Ramachandran statistics (%)					
Favoured	89.40	96.62	96.32	97.10	97.18
Allowed	8.99	3.38	3.68	2.90	2.82
Outliers	1.61	0	0	0	0
Clashscore	4.53	1.37	6.20	5.37	7.22
MolProbity score	2.38	1.08	1.59	1.45	1.55

Supplementary Table 12. MD systems setup

		EVA-A free #1	EVA-A free #2	EVA-P free #1	EVA-P free #2	EVA-A complex	EVA-P complex
Box dimension (Å)	a	48.761	56.878	54.251	58.608	79.051	75.884
	b	46.820	52.558	49.125	49.179	59.505	57.778
	c	62.696	49.685	58.233	57.146	53.992	57.552
Total number of atoms		13752	13830	14623	15397	24061	23702
Total number of water molecules		4185	4211	4438	4696	7240	7083
Other molecules	Cl ⁻	5	5	2	2	12	9