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# **Evasin-4, a tick-derived chemokine-binding protein with broad selectivity can be modified for use in preclinical disease models**

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## C-terminus 6His tagged constructs



MAFKYWVFAAVLYARQWLSTKG  
Evasin-4 signal peptide

MTFKACIAIITALCAMQVIC  
Evasin-1 signal peptide

MRALLARLLL CVLVVSDSKG  
uPA signal peptide

EVPQMTSSSA PDLEEEDDYT AYAPLTCYFT NSTLGLLAPP NCSVLCNSTT TWFNETSPNN  
ASCLLTVDLFL TQDAILQENQ PYNCSVGHCD NGTCAGPPRH AQCWHHHHHH

## N-terminus 6His tagged construct



MRALLARLLL CVLVVSDSKG GSPNSHHHHH HGSPNSLETD EVPQMTSSSA PDLEEEDDYT AYAPLTCYFT  
NSTLGLLAPP NCSVLCNSTT TWFNETSPNN ASCLLTVDLFL TQDAILQENQ PYNCSVGHCD NGTCAGPPRH AQCW

## Untagged construct



MRALLARLLL CVLVVSDSKG EVPQMTSSSA PDLEEEDDYT AYAPLTCYFT NSTLGLLAPP NCSVLCNSTT  
TWFNETSPNN ASCLLTVDLFL TQDAILQENQ PYNCSVGHCD NGTCAGPPRH AQCW

## Fc constructs



MRALLARLLL CVLVVSDSKG *EPKSSDKTHT CPPCPAPPVA GPSVFLFPPK PKDTLMISRT PEVTCVVVDV*  
*SHEDPEVKFN WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYCKVSNK ALPSSIEKTI*  
*SKAKGQPREP QVYTLPPSRE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTPP VLDSGSGFFL*  
*YSKLTVDKSR WQQGNVFSCS VMHEALHNHY TQKSLSLSPG KSGSGSGGEV PQMTSSSAPD LEEEDDYTAY*  
*APLTCYFTNS TLGLLAPPNC SVLCNSTTTW FNETSPNNAS CLLTVDFLTQ DAILQENQPY NCSVGHCDNG*  
*TCAGPPRHAQ CW*



MRALLARLLL CVLVVSDSKG EVPQMTSSSA PDLEEEDDYT AYAPLTCYFT NSTLGLLAPP NCSVLCNSTT  
TWFNETSPNN ASCLLTVDLFL TQDAILQENQ PYNCSVGHCD NGTCAGPPRH AQCWGSGSGG *GEPKSSDKTH*  
*TCPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NQYVDGVEVH NAKTKPREEQ*  
*YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPSSIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL*  
*TCLVKGFYPS DIAVEWESNG QPENNYKTPP PVLDSGSGFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNN*  
*YTQKSLSLSP GK*

**Fig. S1 Sequences of Evasin-4 constructs.** Signal peptide sequences are in blue, caspase 8 clivage site in red, 6His tag underlined, Fc moiety in italic and linker sequence underlined with dots.

A

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ccl1 : -----*-----20-----40-----60-----80-----100-----120-----140-----*-----: 73
ccl3 : -----SLAADTPTRACCFQTSQQLQNF-----ADFF-----SSQSKKFG-----LRR-----SQ-----CQDSEHWKYSLIELSA-----: 69
ccl5 : -----SPYSSDPTCCCFAMIAEPLRAHAKKFF-----YDGGKSNPAAVVVTRRANQCVCANEKKVVREYINSLEMS-----: 68
ccl7 : -----QFVGINSTGCFYINNKIKKFF-----EQRRU-----SNGPREA-----LRR-----LQ-----CQDQTKRWDSDSHDKKQTPKFL-----: 76
ccl8 : -----QFDSVSIPIICCFENVINRKHQRFESYTRINIQCPKEAVIERTTRGRGVEYCADEKERVWDSMKKHLDQIFQNLKP-----: 76
ccl11 : -----GPASVPTTCCFENLANRRHQLQRFESYTRINIQCPKEAVIERTTRGRGVEYCADEKERVWDSMKKHLDQIFQNLKP-----: 74
ccl14 : -----TKTSSSRGPFYHPSGCFYITTYRIRKQF-----MNYE-----NSQSKKFG-----LRR-----GHS-----CRKSRKWDYDYLKEN-----: 74
ccl15 : QFINDAETELMMSKPLENFVVLNSFHF-AAACCTSSISQSLCSLY-----KQFF-----SSQSKKFG-----LRR-----GHS-----CRKSGFVDCRKKPFYSI-----: 92
ccl16 : -----QPKVPEWVNTPTSCCLKRYEKVLRRIIVGGR-K-ALNCHLPLAIVVTRRNEVCTNENDDVQEYIKDPNLPPLPRLNLTSTVKIITA-KNGQPQLLNSQ-----: 97
ccl17 : -----ARGTNVGRCCLEFKGATLRRKIKWY-----QTSEDCSRDAIVVTVCGAIGSDENNRKRVKNAVYIQSLERS-----: 71
ccl18 : -----AQVGNKEICGLVYINNKIKKFF-----VLYFE-----SPQPKFG-----LRR-----LQ-----CQDNRKWDKYSLKINA-----: 69
ccl19 : -----GTNDAREGLVQCFYINNKIKKFF-----RSHVLLKKGQVEVYVTRRANQCVCANEKKVVREYINSLEMS-----: 77
ccl21 : -----SDGGAQCFLELVQCFYINNKIKKFF-----RQKQFESLGSLEF-----LRR-----RQAF-----CQDKELWQCLQF-----DRTFSPQKPAQCRKDRGASKTGKRGKSGK--GCKRT--ERSQTPKGF-----: 111
ccl22 : -----GPYGANMEDSVCCRDVYRYRLHLRVVKEHY-WGSDSCPRPGVLLITFRDREICADERVEVTKMIINKISQ-----: 69
ccl23 : RVTKDAETEFMMSKPLENFVLLDRFHATSADCCISYTPRSSIPLSFY-EANSSQSKKFGVIEITTRGRGVEYCADEKERVWDSMKKHLDQIFQNLKP-----: 99
ccl24 : -----VVIPSECCMFVSKRHEHNRVYVQLSSRSTGLRAGVIEITTRGRGVEYCADEKERVWDSMKKHLDQIFQNLKP-----: 93
ccl25 : -----QGVFECGLAH-YFEGAVVRRARVIGEVSSGNLEAEYLER-----HKK-----DAKSRVGRALDARNVFAKLHNN---TQTFGAGPHAVKLLSSGNLSSSKFNFNIPSSSKRNVLLISANSGL-----: 127
ccl26 : -----TRGSDISKCCCFQSHKHLHWTV-----RSEF-----SNGSQRALVIEITTRGRGVEYCADEKERVWDSMKKHLDQIFQNLKP-----: 71
          CC          6p          6          5          C          6f          t          C          p          wv          SL          KTFKQL

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B

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CCL2 : -----*-----20-----40-----60-----80-----100-----120-----*-----: 76
CCL3 : -----SLAADTPACCFEYTSRQLHQNFIALYF-----ETSQCSKPGVIEITTRGRGVEYCADEKERVWDSMKKHLDQIFQNLKP-----: 69
CCL5 : -----SPYSSDPTCCCFAMIAEPLRAHAKKFF-----YDGGKSNPAAVVVTRRANQCVCANEKKVVREYINSLEMS-----: 68
CCL7 : -----QFVGINSTGCFYINNKIKKFF-----EQRRU-----SNGPREA-----LRR-----LQ-----CQDQTKRWDSDSHDKKQTPKFL-----: 76
CCL8 : -----QFDSVSIPIICCFENVINRKHQRFESYTRINIQCPKEAVIERTTRGRGVEYCADEKERVWDSMKKHLDQIFQNLKP-----: 76
CCL11 : -----GPASVPTTCCFENLANRRHQLQRFESYTRINIQCPKEAVIERTTRGRGVEYCADEKERVWDSMKKHLDQIFQNLKP-----: 74
CCL13 : -----FNPQGLAQPDALNVPTSCCFEYTSRQLHQNFIALYF-----ETSQCSKPGVIEITTRGRGVEYCADEKERVWDSMKKHLDQIFQNLKP-----: 82
CCL14 : -----TKTSSSRGPFYHPSGCFYITTYRIRKQF-----MNYE-----NSQSKKFG-----LRR-----GHS-----CRKSRKWDYDYLKEN-----: 74
CCL15 : QFINDAETELMMSKPLENFVVLNSFHF-AAACCTSSISQSLCSLY-----KQFF-----SSQSKKFG-----LRR-----GHS-----CRKSGFVDCRKKPFYSI-----: 92
CCL16 : -----QPKVPEWVNTPTSCCLKRYEKVLRRIIVGGR-K-ALNCHLPLAIVVTRRNEVCTNENDDVQEYIKDPNLPPLPRLNLTSTVKIITA-KNGQPQLLNSQ-----: 97
CCL17 : -----ARGTNVGRCCLEFKGATLRRKIKWY-----QTSEDCSRDAIVVTVCGAIGSDENNRKRVKNAVYIQSLERS-----: 71
CCL22 : -----GPYGANMEDSVCCRDVYRYRLHLRVVKEHY-WGSDSCPRPGVLLITFRDREICADERVEVTKMIINKISQ-----: 69
CCL23 : RVTKDAETEFMMSKPLENFVLLDRFHATSADCCISYTPRSSIPLSFY-EANSSQSKKFGVIEITTRGRGVEYCADEKERVWDSMKKHLDQIFQNLKP-----: 99
CCL24 : -----VVIPSECCMFVSKRHEHNRVYVQLSSRSTGLRAGVIEITTRGRGVEYCADEKERVWDSMKKHLDQIFQNLKP-----: 93
CCL26 : -----TRGSDISKCCCFQSHKHLHWTV-----RSEF-----SNGSQRALVIEITTRGRGVEYCADEKERVWDSMKKHLDQIFQNLKP-----: 71
          CC          6p          6          5          C          66f          T          C          P          wv          6

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C

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ccl4 : APMGSDPPTACCFEYTSRQLHQNFIALYF-----ETSQCSKPGVIEITTRGRGVEYCADEKERVWDSMKKHLDQIFQNLKP-----: 69
ccl20 : -----ASNEDCCLGVYTRHLRFRFVIGFTRQLANEGCDINALIIFTRKKKLSVCANERQTVWVYVRIISKKVKNM-----: 70
          CC          YT          R          L          F6V          5          C          A66F          TK4          VCA1P          23WV          V          L

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**Fig. S2 Human CC chemokines recognized by Evasin-4.** A) Alignment of chemokines bound by Evasin-4 by SPR. B) Alignment of chemokines inhibited by Evasin-4 (chemotaxis assay) C) Alignment of CC chemokines not recognized by Evasin-4. Sequences were taken from Uniprot database and aligned using the Clustal Omega software.