Supplementary Material for:

Ticks from Diverse Genera Encode Chemokine-Inhibitory Evasin Proteins

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Supplementary Table

Supplementary Figures

Table S1: Accession numbers of all sequences in the final evasin database

Sequence ID	Database	Species
KY696668.1	Genbank	*Ixodes holocyclus*
KY696671.1	Genbank	*Ixodes holocyclus*
KY696667.1	Genbank	*Ixodes holocyclus*
KY696674.1	Genbank	*Rhipicephalus microplus*
JAA60714.1	Genbank	*Rhipicephalus pulchellus*
G3MSV3_9ACAR	UniprotKB	*Amblyomma maculatum*
G3ML03_9ACAR	UniprotKB	*Amblyomma maculatum*
G3MSV4_9ACAR	UniprotKB	*Amblyomma maculatum*
JAA57173.1	Genbank	*Rhipicephalus pulchellus*
JAG92275.1	Genbank	*Amblyomma americanum*
JAA60749.1	Genbank	*Rhipicephalus pulchellus*
JAA71836.1	Genbank	*Ixodes ricinus*
JAA70458.1	Genbank	*Ixodes ricinus*
JAA65577.1	Genbank	*Ixodes ricinus*
JAA67769.1	Genbank	*Ixodes ricinus*
JAA67752.1	Genbank	*Ixodes ricinus*
JAA67622.1	Genbank	*Ixodes ricinus*
JAB77289.1	Genbank	*Ixodes ricinus*
JAA67762.1	Genbank	*Ixodes ricinus*
JAA67760.1	Genbank	*Ixodes ricinus*
JAB76903.1	Genbank	*Ixodes ricinus*
JAC18893.1	Genbank	*Amblyomma cajennense*
JAC24826.1	Genbank	*Amblyomma parvum*
JAC27776.1	Genbank	*Amblyomma triste*
JAC27143.1	Genbank	*Amblyomma parvum*
JAC27149.1	Genbank	*Amblyomma parvum*
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JAC18902.1	Genbank	*Amblyomma cajennense*
JAG91726.1	Genbank	*Amblyomma americanum*
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JAC19608.1	Genbank	*Amblyomma cajennense*
JAC19605.1	Genbank	*Amblyomma cajennense*
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JAC19000.1	Genbank	*Amblyomma cajennense*
JAC18987.1	Genbank	*Amblyomma cajennense*

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JAC19684.1	Genbank	*Amblyomma cajennense*
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JAC19681.1	Genbank	*Amblyomma cajennense*
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JAC27265.1	Genbank	*Amblyomma parvum*
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KY696675.1	Genbank	*Rhipicephalus microplus*

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JAA60817.1	Genbank	*Rhipicephalus pulchellus*
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JAC19108.1	Genbank	*Amblyomma cajennense*
JAC27217.1	Genbank	*Amblyomma parvum*
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JAC30561.1	Genbank	*Amblyomma triste*
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JAC30567.1	Genbank	*Amblyomma triste*
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JAA67927.1	Genbank	*Ixodes ricinus*
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GADI01003291.1/1-158	VectorBase	*Ixodes ricinus*
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JAC93372.1	Genbank	*Ixodes ricinus*
JAC93373.1	Genbank	*Ixodes ricinus*
GBBN01015718.1/1-151	VectorBase	*Ixodes scapularis*
GBBN01015718.1/1-151	VectorBase	*Ixodes scapularis*
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JAA65508.1	Genbank	*Ixodes ricinus*
JAC93369.1	Genbank	*Ixodes ricinus*
JAA71946.1	Genbank	*Ixodes ricinus*
JAC93366.1	Genbank	*Ixodes ricinus*
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JAC93396.1	Genbank	*Ixodes ricinus*
JAA65581.1	Genbank	*Ixodes ricinus*
JAC93383.1	Genbank	*Ixodes ricinus*
JAA68201.1	Genbank	*Ixodes ricinus*
JAA67791.1	Genbank	*Ixodes ricinus*
JAA65625.1	Genbank	*Ixodes ricinus*
GBIH01001355.1/1-133	VectorBase	*Ixodes ricinus*

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JAA70749.1	Genbank	*Ixodes ricinus*
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JAC93374.1	Genbank	*Ixodes ricinus*
JAA67827.1	Genbank	*Ixodes ricinus*
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JAA55737.1	Genbank	*Rhipicephalus pulchellus*
JAA68608.1	Genbank	*Ixodes ricinus*
JAB75472.1	Genbank	*Ixodes ricinus*
JAG92019.1	Genbank	*Amblyomma americanum*
A0A0C9R596	UniprotKB	*Amblyomma americanum*
KY696661.1	Genbank	*Ixodes holocyclus*
JAG91989.1	Genbank	*Amblyomma americanum*
JAC19654.1	Genbank	*Amblyomma cajennense*
JAC24842.1	Genbank	*Amblyomma parvum*
JAC27912.1	Genbank	*Amblyomma triste*
JAC27939.1	Genbank	*Amblyomma triste*
JAC27940.1	Genbank	*Amblyomma triste*
JAB71388.1	Genbank	*Ixodes ricinus*
JAA68126.1	Genbank	*Ixodes ricinus*
JAB70275.1	Genbank	*Ixodes ricinus*
JAC92340.1	Genbank	*Ixodes ricinus*
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P0C8E7	UniprotKB	*Rhipicephalus sanguineus*
C9W1N8	UniprotKB	*Rhipicephalus sanguineus*
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JAC19652.1	Genbank	*Amblyomma cajennense*

Figure S1. Simulated fluorescence anisotropy binding curves. Shown are the fluorescence anisotropy competitive displacement curves simulated for binding of evasins to chemokines with 10 different K_d values (in the range 1-1000 nM, different curves on the same panel) and for four different binding affinities between the chemokine and the fluorescent peptide (Fl-Pep) in the four panels (20 nM, top panel; 50 nM, second panel; 100 nM, third panel; and 200 nM, bottom panel). In all simulations the concentrations of chemokine (100 nM) and Fl-Pep (10 nM) are held constant.

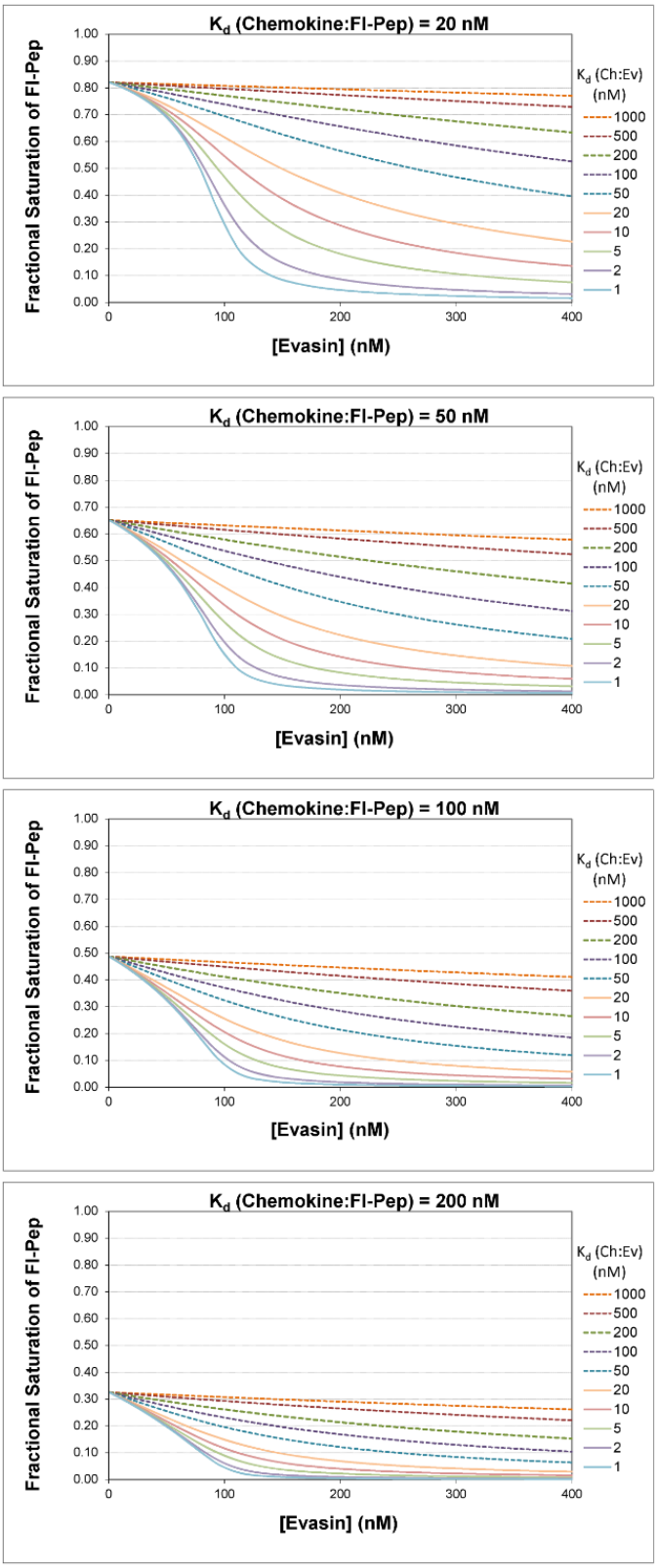

Figure S2. Chemokine binding curves of candidate evasins. Shown are the fluorescence anisotropy competitive displacement curves obtained for binding of nine evasin candidates against a panel of 6 human CC chemokines. Data points represent the mean anisotropy ± SEM from duplicate assays performed three times independently. Solid lines are the best fits to a competitive displacement model (see *Experimental Procedures* for details).

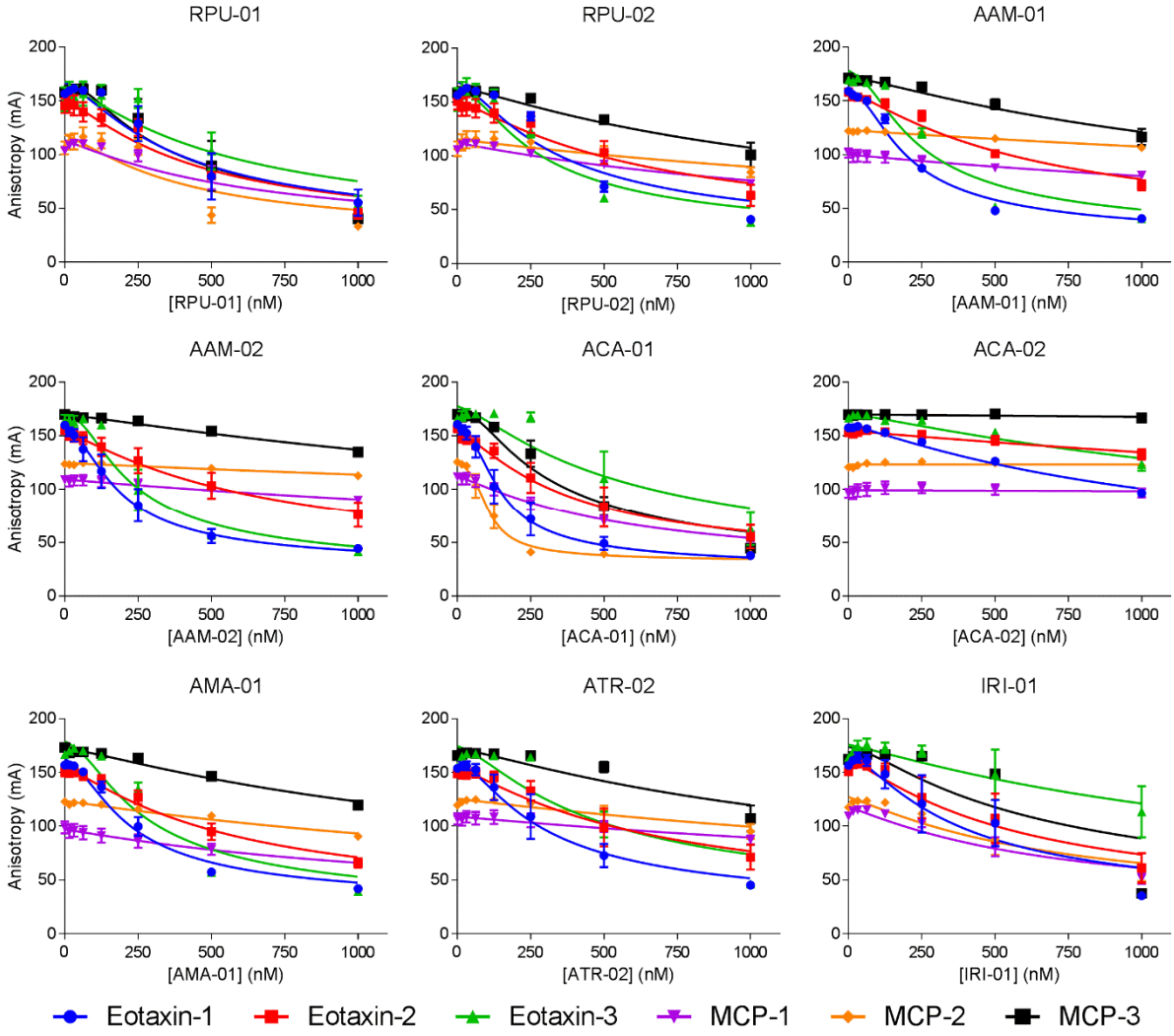

Figure S3. Chemokine binding affinity profiles of purified evasins. *(A-E)*Affinities (pK_d values), derived from the competitive fluorescence anisotropy binding curves (Figs. 3 and 4) for binding of purified evasins to each of five CC chemokines. Each panel shows the binding profile for one evasin: *(A)* RSA evasin-4, *(B)* RPU-01, *(C)* ACA-01, *(D)* IRI-01, and *(E)* IHO-01. Data represent the average ± SEM of values from three independent experiments, each recorded in duplicate. Significance (multiple t-tests) is shown as * p< 0.05, ** p<0.01 and *** p < 0.001.

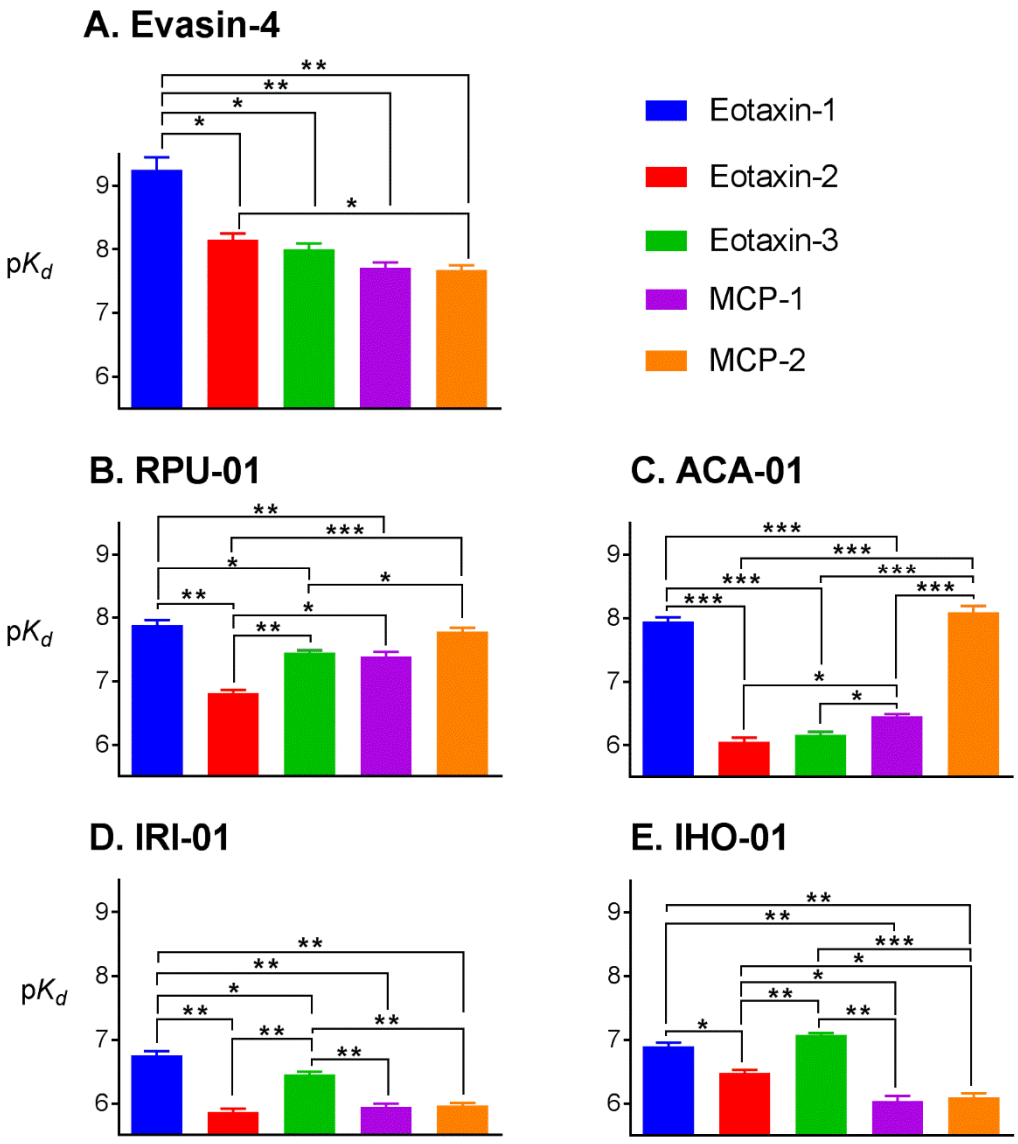

Figure S4. Chemokine inhibition of forskolin-induced cAMP production. Shown are concentration-response curves for inhibition of forskolin (10 μM)-induced production of cAMP in: *(A)* cMyc-FLAG-CCR2 TREx HEK293 cells by the chemokines MCP-1 and MCP-2; or *(B)* cMyc-FLAG-CCR3 FlpIn TREx HEK293 cells by the chemokines eotaxin-1 and eotaxin-2. cAMP was detected via a BRET biosensor, as described in the *Experimental Procedures*. Data points represent the average ± SEM of values from three independent experiments, each recorded in duplicate.