SUPPLEMENTAL DATA

Supplementary Table 1. VL CDR3 sequences of dual specific scFvs E7 variants.

scFv	VL CDR3[a]
E7	^{112}LNGN^{115}
J9	^{112}SEPR^{115}
P8	^{112}QDPR^{115}
C1	^{112}YERY^{115}
F13	^{112}IGNY^{115}
J5	^{112}ESPA^{115}

[a]numbering of the amino acid residues according to IMGT(29,30).

Supplementary Table 2. PCR primers used to generate the human CXCL10 and human CXCL9 mutants by QuikChange site-directed mutagenesis.

Template	Mutant	Primer sequence[a]
Human CXCL10	hum10N$_{13}$	F 5′ CGCTGTACCTGCATC<u>AAC</u>ATTAGTAATCAACCT 3′ R 5′ AGGTTGATTACTAAT<u>GTT</u>GATGCAGGTACAGCG 3′
	hum10D$_{13}$	F 5′ CGCTGTACCTGCATC<u>GAT</u>ATTAGTAATCAACCT 3′ R 5′ AGGTTGATTACTAAT<u>ATC</u>GATGCAGGTACAGCG 3′
	hum10H$_{13}$	F 5′ CGCTGTACCTGCATC<u>CAT</u>ATTAGTAATCAACCT 3′ R 5′ AGGTTGATTACTAAT<u>ATG</u>GATGCAGGTACAGCG 3′
Human CXCL9	hum9N$_{13}$	F 5′ CGCTGTTCCTGCATC<u>AAC</u>ACCAACCAAGGGACT 3′ R 5′ AGTCCCTTGGTTGGT<u>GTT</u>GATGCAGGAACAGCG 3′

[a]The mutant codons are underlined. F, forward; R, reverse; E, elongation.

SUPPLEMENTAL EXPERIMENTAL PROCEDURE

QuikChange site-directed mutagenesis- Three human CXCL10 mutants**,** hum10N$_{13}$, hum10D$_{13}$, and hum10H$_{13}$ and one human CXCL9 mutant, hum9N$_{13}$, were generated by QuikChange site-directed mutagenesis. Residues were numbered according to the target sequence. The recombinant pET43.1a plasmids containing mature human CXCL10 (hum10) or human CXCL9 (hum9) were used for PCR mutagenesis using specific primer pairs (Supplementary Table 2). PfuUltra II HotstartPCR Master Mix (Agilent technologies) was used for PCR and PCR products were then digested with DpnI (New England Biolabs) for 2 hours. The recombinant plasmids were controlled by sequencing before transformation into Tuner™ (DE3) competent bacteria (Novagen) for recombinant protein production.

SUPPLEMENTAL FIGURE LEGENDS

<u>Supplementary Figure 1.</u> Dual specific scFv binding to human CXCL10 and CXCL9 mutated at position 13. Specific binding of E7, J9, P8, F13, C1 and J5 dual specific scFvs to a panel of CXCL10 (A) and CXCL9 (B) mutants was assessed in an ELISA. The indicated NusA-fusion chemokines were immobilized and incubated with scFvs. Coating was controlled using specific anti NusA mAb and NusA protein was also added to the assay as negative control (data not shown). Results are expressed as mean ± S.D of duplicates. hum10, human CXCL10; hum9, human CXCL9.

Supplementary Figure 1.