SUPPLEMENTARY MATERIAL

Heparin binding. We characterized the glycosaminoglycan (GAG) binding properties of CC-CXCL8 by measuring its affinity to immobilized heparin. GAGs are highly sulfated and negatively charged, and CXCL8 is known to predominantly bind to cell surface heparan sulfate on the endothelial cells. Heparin, like heparan sulfate, is highly sulfated and negatively charged, and previous studies have shown binding properties of CXCL8 to both GAGs are similar. Therefore, measuring binding affinity allows a simple but effective method for assessing GAG binding properties of CC-CXCL8.

WT or CC-CXCL8 (~0.3 mg in 5 mM sodium phosphate buffer, pH 7) was loaded onto a HiTrap Heparin affinity column (GE LifeSciences). At these concentrations, both WT and CC-CXCL8 are predominantly dimers. The proteins were eluted from the heparin column using a step gradient (50 mM) of 0 to 1 M sodium chloride in the same buffer, and their concentration was determined using UV absorption spectroscopy at 215 and 280 nm. Both CC-CXCL8 and WT CXCL8 eluted from the heparin column at ~0.7 M NaCl, indicating that the CXC motif plays no role in GAG binding (Fig. S1).

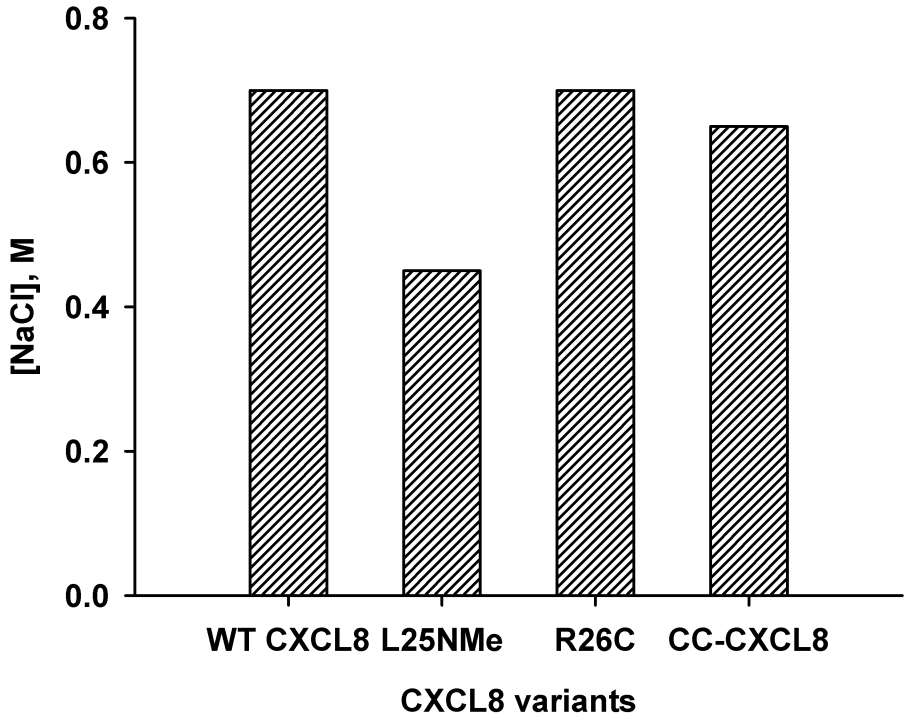

Fig. S1. Heparin binding affinity of CC-CXCL8. The salt concentration at which CC-CXCL8 is eluted is shown. The data for the WT CXCL8 and also that of R26C trapped dimer (1) and L25NMe trapped monomer (2) are also shown for comparison.

1. Rajarathnam, K., Prado, G. N., Fernando, H., Clark-Lewis, I., and Navarro, J. (2006) *Biochemistry* **45**, 7882-7888
2. Rajarathnam, K., Clark-Lewis, I., and Sykes, B. D. (1995) *Biochemistry* **34**, 12983-1299