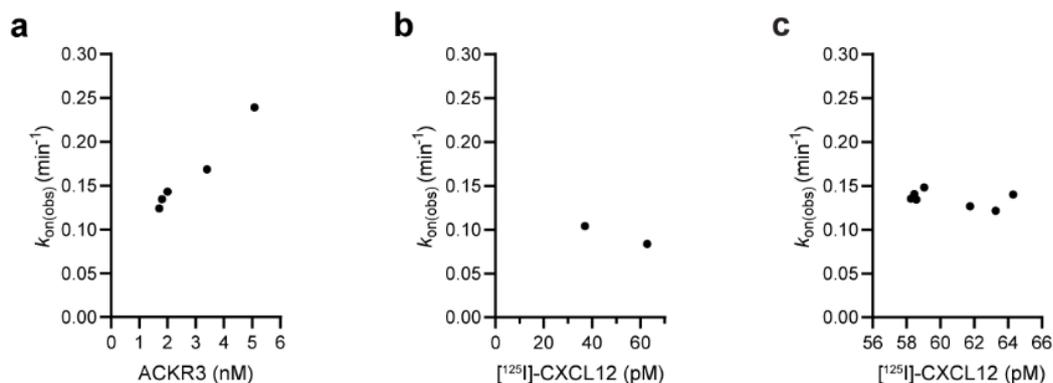


## Supplementary



**Figure S1.** The observed association rate constant  $k_{\text{on}(\text{obs})}$  depends on the ACKR3 concentration but not on the [ $^{125}\text{I}$ ]-CXCL12 concentration. **(a)**  $k_{\text{on}(\text{obs})}$  increases with increasing ACKR3 concentration.  $k_{\text{on}(\text{obs})}$  were determined at various ACKR3 concentrations but constant [ $^{125}\text{I}$ ]-CXCL12 concentration (~61 pM); **(b,c)**  $k_{\text{on}(\text{obs})}$  and the [ $^{125}\text{I}$ ]-CXCL12 concentration are uncorrelated. Different [ $^{125}\text{I}$ ]-CXCL12 concentrations were tested at 1.6 nM **(b)** and 2.0 nM **(c)** ACKR3. All experiments were performed in duplicates. Subtraction of the non-specific binding to empty nanodiscs (NDs) from the total binding to receptor containing NDs yielded the specific binding. One-phase association models were fitted to the specific binding curves using GraphPad Prism version 9.3.0 (GraphPad Software, Inc., San Diego, CA) to obtain  $k_{\text{on}(\text{obs})}$ . Different buffer conditions were used for the tests as follows: **(a)** 200 mM NaCl, 3.98% (*v/v*) glycerol, 60 mM HEPES pH 7.5, 0.04% (*w/v*) BSA; **(b)** 213 mM NaCl, 3.98% (*v/v*) glycerol, 66 mM HEPES pH 7.5; **(c)** as **(a)** but with 5 mM CaCl<sub>2</sub>.