



Extended Data Figure 6 | Saturation binding analysis of C5aR1 mutants with ^{3}H NDT9513727. **a**, Saturation binding of ^{3}H NDT9513727 to solubilized cell lysate containing wild-type C5aR1. **b**, Single experiment showing fluorescence size-exclusion analysis of solubilized cell lysates containing indicated mutant variants of C5aR1 with a C-terminal green fluorescent protein (GFP) tag, in 1% digitonin. **c–h**, Saturation binding of ^{3}H NDT9513727 to solubilized cell lysates containing indicated

mutant variants of C5aR1. Data are representative from three biologically independent experiments performed in duplicate \pm s.d. K_D values are inset with s.d. in parentheses. The mean $\text{p}K_D$ values (s.e.m. in brackets) are 6.59 (0.08), 6.42 (0.2), 6.28 (0.21) and 6.65 (0.15) for the wild-type, L125F, T129L and T217L mutants, respectively. The datasets for A128F, T129F and W213L could not be analysed unambiguously owing to marked loss of specific binding.