Supplementary Figure 9. *in vitro* splenocyte stimulation with nalfurafine to determine optimal dose range for *in vitro* restimulation experiments. (a, b) Splenocytes were stimulated with ConA and a range of doses of nalfurafine (100 pM-10 μM) or vehicle. Following 72 hours of stimulation, supernatant was removed and analyzed for IL-10 and IFNγ by ELISA. A dose range of 1-1000 nM was deemed appropriate for further analysis. (c, d) Splenocytes were either unstimulated or stimulated with ConA and a range of doses of nalfurafine (1 nM-1000 nM) or vehicle (v). After 72 hrs, cells were stimulated with PMA (50 ng mL⁻¹), Ionomycin (500 ng mL⁻¹) and GolgiStop/Monensin (1 μg/10⁶ cells) for 4 hrs. Cells were then prepared for flow cytometry analysis of IL-10 and IFNγ cytokines. Results are combined from 3 independent experiments. A 50 nM dose of nalfurafine was determined an appropriate range driving cytokine changes in response to ConA compared to unstimulated.