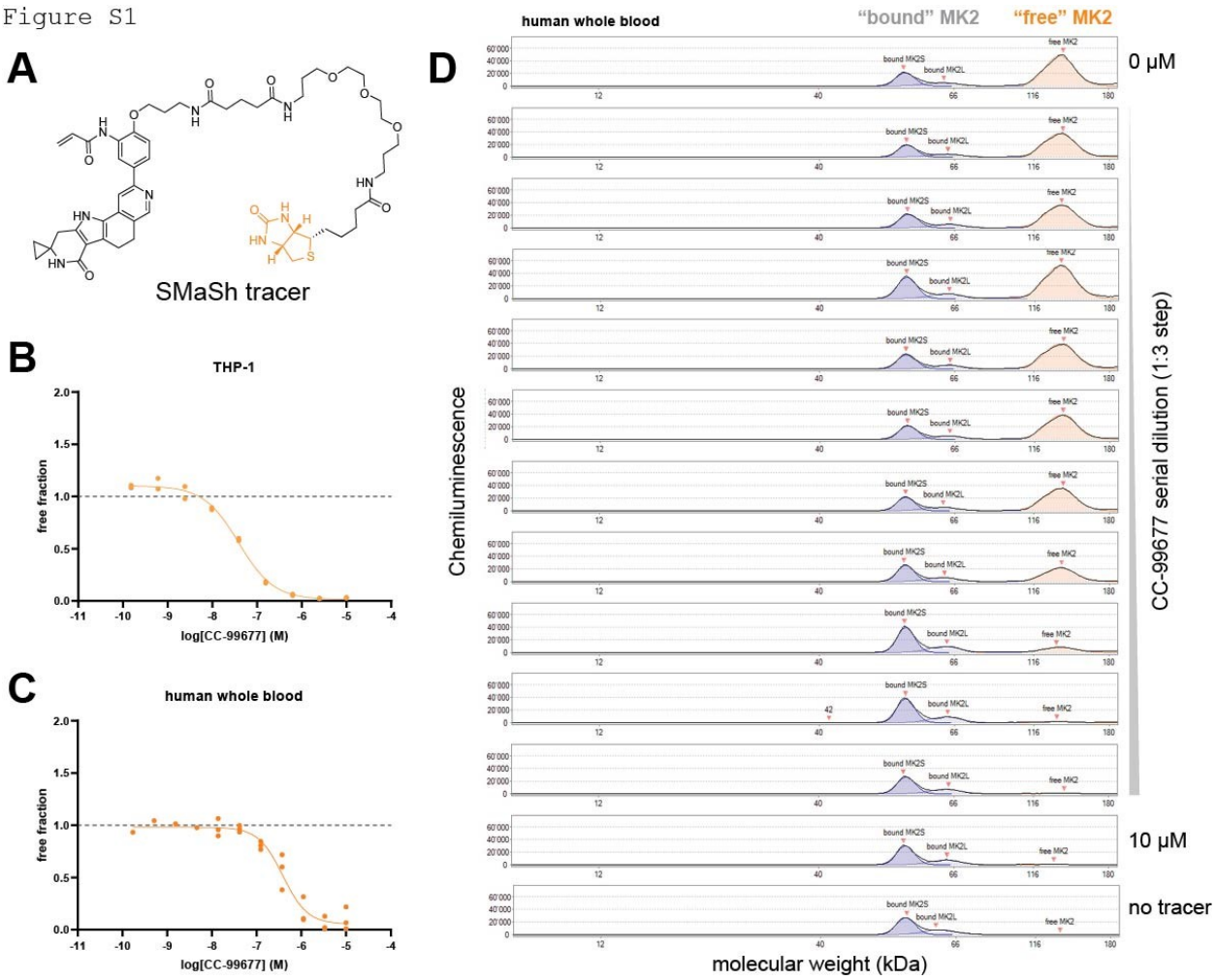
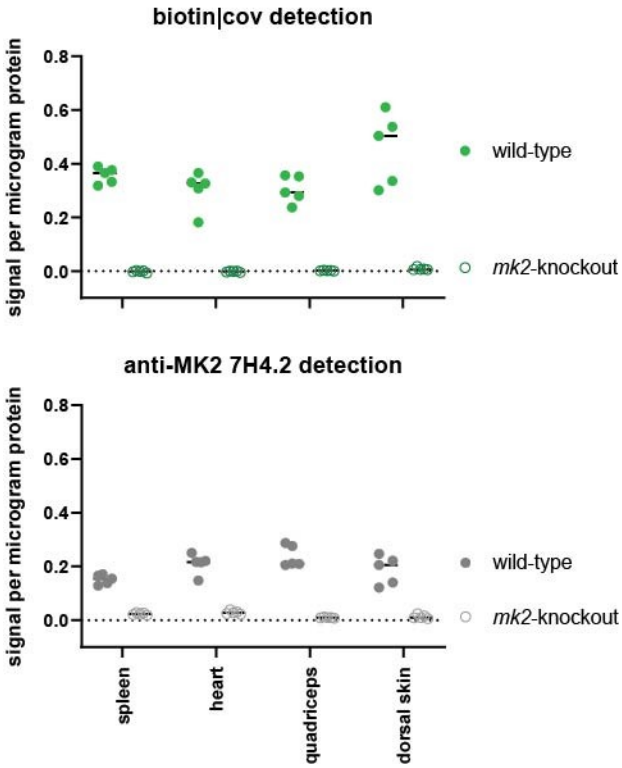
Figure S1



Supplement 1. **SMaSh target occupancy of CC-99677.**

(A) Structure of the biotinylated, covalent tracer used in the SMaSh assay for detecting "free" MK2. (B) Concentration-response profiles of CC-99677 after a 3-hour exposure in human THP-1 cells. Plots show data from two independent experiments. (C) Concentration-response profiles of CC-99677 after an 18-hour exposure in human whole blood, followed by stimulation with $1\ \mu g/mL$ lipopolysaccharide for an additional 3 hours. Plots show data from 3 individual healthy whole blood donors. (D) Representative Simple Western electropherograms of individual CC-99677 concentrations from one whole blood donor, as shown in Figure S1C. Total MK2 was calculated as the sum of the peak areas for bound plus free MK2. Note that bound MK2 is detected as two separate peaks, which correspond to CC-99677 bound to long (L) and short (S) isoforms of MK2 [20].

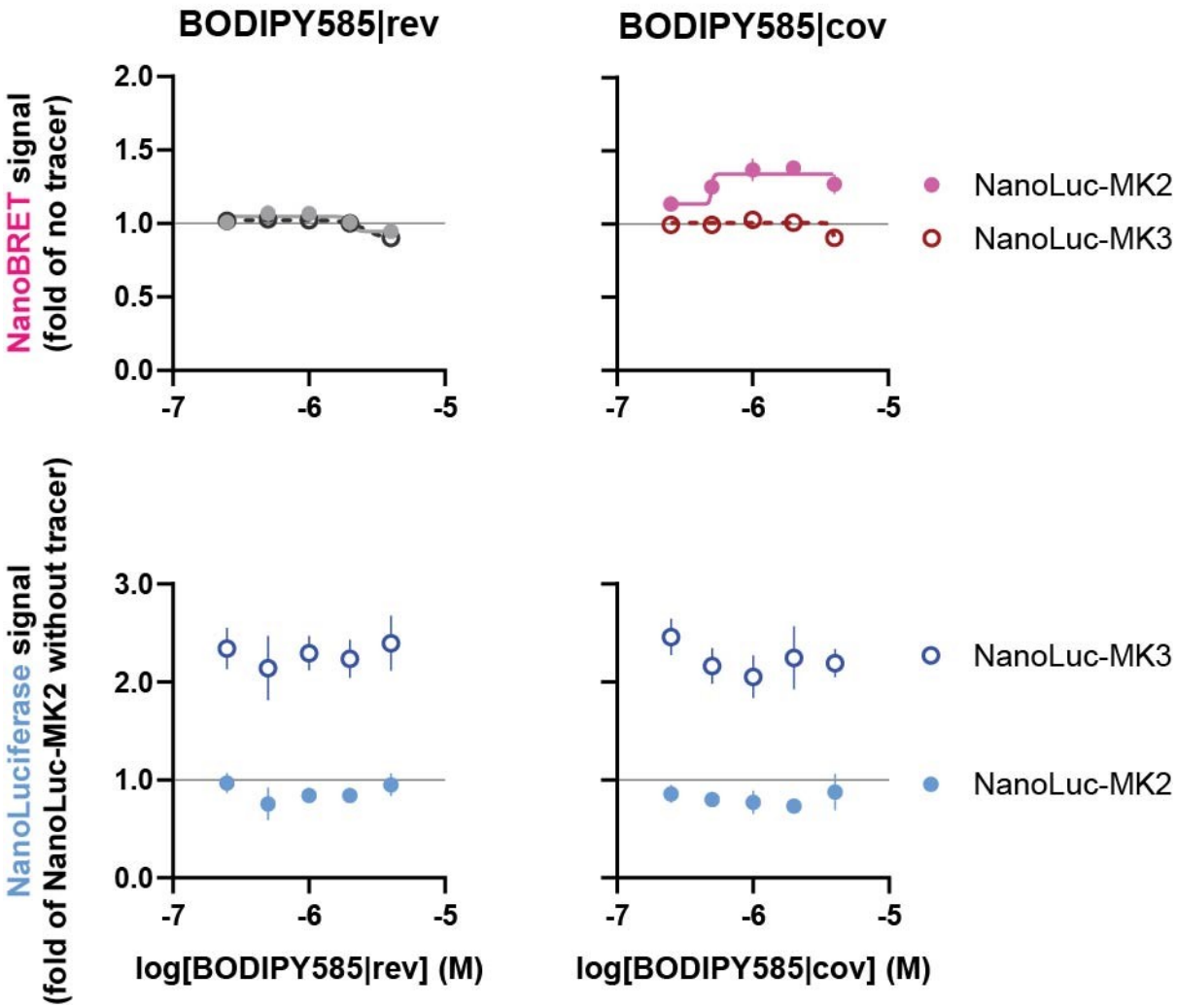
Figure S2



Supplement 2. **Measurement of endogenous MK2 in mouse tissue by dual HTRF**.

MK2 abundance in tissue from naïve wild-type and *mk2*-knockout mice. Snap-frozen tissue was homogenized in IP Lysis Buffer containing 1X HALT protease inhibitor (5 µL per milligram of spleen or heart; 2 µL per milligram of quadriceps or dorsal skin), resulting in average total protein concentrations of 28 mg/mL spleen; 32 mg/mL heart; 31 mg/mL quadriceps; and 6 mg/mL dorsal skin. The neat lysates were loaded into white 1536-well plates, in quintuplet, for measuring MK2 abundance with the dual HTRF method. Signal for biotin|cov-bound and anti-MK2 7H4.2-detected protein represent the same total population of MK2, so differences in magnitude reflect quantum efficiency of energy transfer from terbium cryptate plus quantum yield of the two acceptor fluorophores. Each datum represents an average of technical replicate signal normalized to total protein concentration per animal. Data from 5 individual mice per genotype are shown.

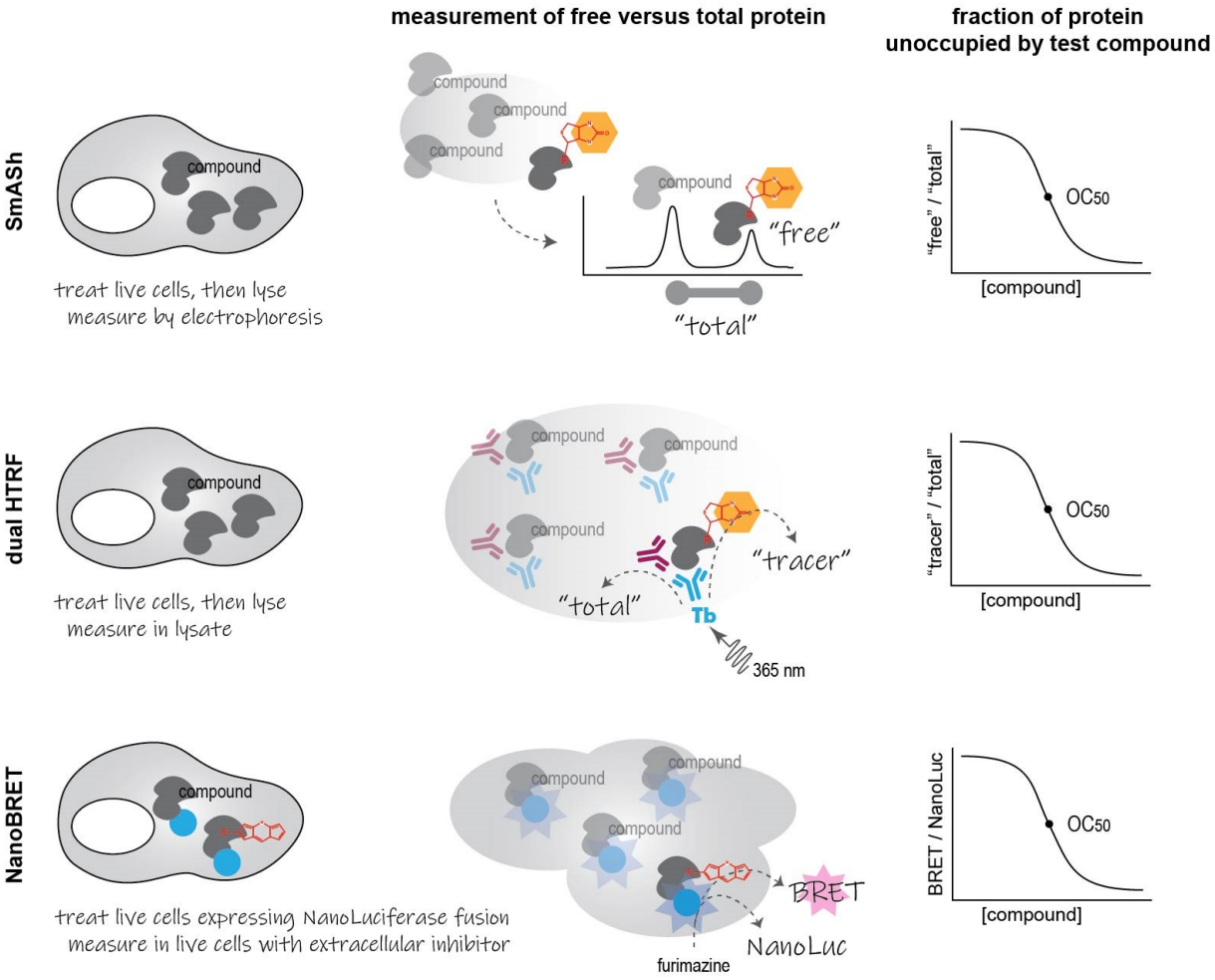
Figure S3



Supplement 3. **Paralog specificity of BODIPY585|cov tracer in the NanoBRET assay**.

Binding data for BODIPY|rev and BODIPY585|cov tracers in HeLa cells overexpressing amino-terminal MK2 or MK3 fusions. (top row) Ratiometric NanoBRET signal, after subtraction of no-tracer control ratios. (bottom row) NanoLuciferase signal from the two constructs, normalized to NanoLuc-MK2 no-tracer control wells. Signal was measured 1 hour after addition of tracer to cells using a CLARIOstar Plus (average of 5 replicates with standard deviation is shown).

Figure S4



Supplement 4. **Schematic comparison of the three target engagement assays**.