



Supplementary information, Fig. S1. Identification of a new NAMPT activator (NAT) from high-throughput screening.

a Schematic for the procedure of the high-throughput screening for NAMPT agonists. NMN produced by NAMPT was converted to NADH in a triply-coupled enzyme assay and could be detected by fluorescence measurement (Ex_{340}/Em_{445}). **b** Triply-coupled NAMPT enzyme reactions were performed at room temperature for 20 min with DMSO or 10 μM NAT and started with or without NAM. Progress curves of the enzyme reactions were plotted and data were analyzed with GraphPad Prism. **c** NMNAT1 enzyme assays were carried out in the presence of the indicated concentrations of NAT or NAT-5r. **d** Dose-dependent activation of NAMPT by NAT. NAT was added at the indicated concentrations. The direct NAMPT enzyme assay was performed at room temperature, and reactions were stopped at 2, 4, 6, and 8 min. Progress curves of the enzyme reactions were plotted and data were analyzed with GraphPad Prism. In **(b-d)**, Data are represented as mean \pm SEM from three replicates in a representative experiment. $n=3$ independent experiments. **e** Effect of SBI-797812 on the NAMPT enzyme kinetics in the direct NAMPT assay. Enzyme kinetics curves for NAMPT reactions were plotted in the presence and absence of 3 μM SBI-797812. The velocity of NMN production is shown on the Y-axis as a function of substrate NAM concentration (X-axis). All error bars represent SEM from three replicates. **f** Biacore assay of the interaction between NAMPT and NAT, using a Biacore 8K+ system. NAMPT protein was immobilized on a CM5 chip. The analytes were consisting of serial dilutions of NAT as indicated. Data were analyzed with Biacore Insight Evaluation software.