**Method for MST experiments**

Human Sirt3 protein was labeled with DY-647P1-NHS-Ester in 1x PBS pH 7.4, 0.05 % Pluronic F-127. A 2:1 molar excess of reactive dye was used over protein, in order to preferentially label one lysine within the protein. Free dye was removed using a gravity-flow size exclusion column and the labeled protein was buffer exchanged into 47 mM Tris-HCl pH 8.0, 129 mM NaCl, 2.5 mM KCl, 0.94 mM MgCl2, 5% DMSO, 0.05% Tween-20 (reaction buffer). The concentration of the labeled Sirt3 was determined in a Monolith NT.115 Pico instrument (NanoTemper Technologies GmbH) with a calibration series of free dye. Labeled Sirt3 was mixed with varying concentrations of ligands, and thermophoresis was measured at 25 °C (excitation wavelength 653 nm, emission wavelength 672 nm, LED-power 15%, laser-power 80%) using a Monolith NT .115 Pico instrument. All experiments were performed in reaction buffer. Dissociation constants were determined with MO.Affinity Analysis software (NanoTemper Technologies GmbH) by nonlinear fitting (one-site and two-site fitting equations). Each experiment was repeated at least twice.