Supplementary Information

Interactions between sirtuins and fluorogenic small-molecule substrates offer insights into inhibitor design

Hua-Li Wang,[a,‡] Sha Liu,[a,‡] Zhu-Jun Yu,[a] Chengyong Wu,[a] Linna Cheng,[a] Yuxi Wang,[a] Kai Chen,[a] Shu Zhou,[a] Qiang Chen,[a] Yamei Yu,[a,*] and Guo-Bo Li[a,*]

[a]*Key Laboratory of Drug Targeting and Drug Delivery System of Ministry of Education, West China School of Pharmacy, and Laboratory of Biotherapy and Cancer Center, Sichuan University, Chengdu 610041, P. R. China.*

[‡] H.-L. Wang and S. Liu contributed equally to this work.

[*] Correspondence: liguobo@scu.edu.cn (G.-B. Li); yamei_yu@scu.edu.cn (Y. Yu).

Table of Contents

Supplementary Figures

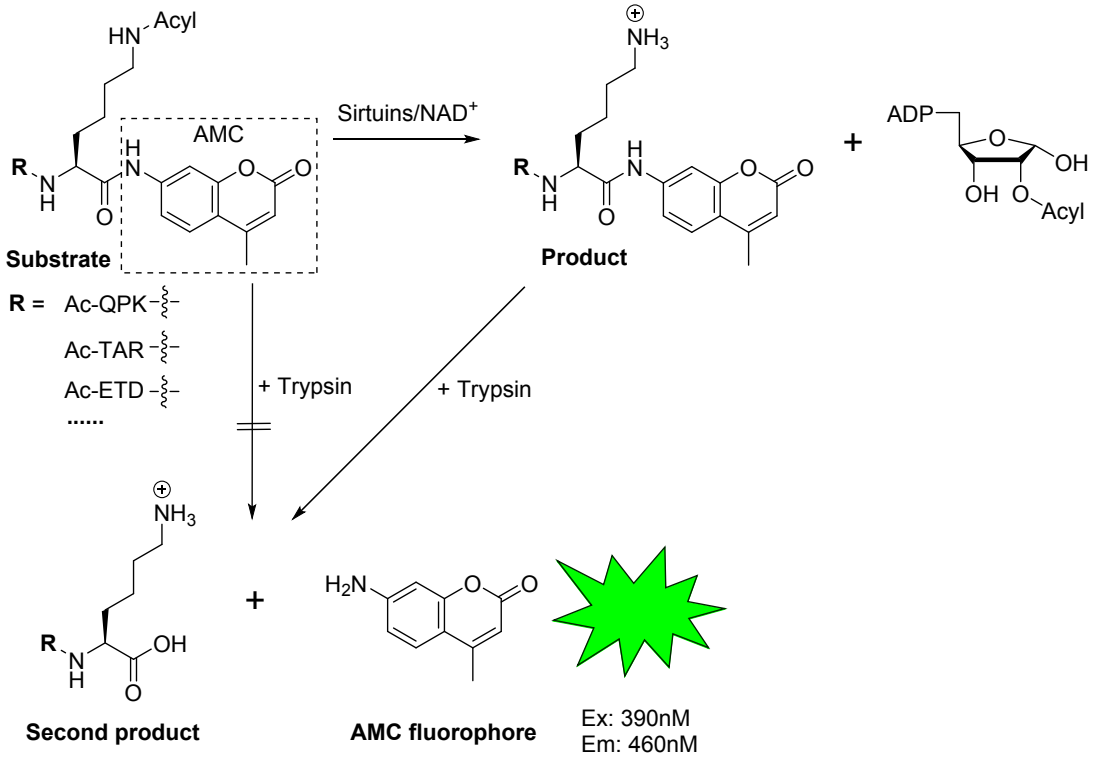

Fig. S1 A trypsin-coupled sirtuin activity assay using fluorogenic substrates.

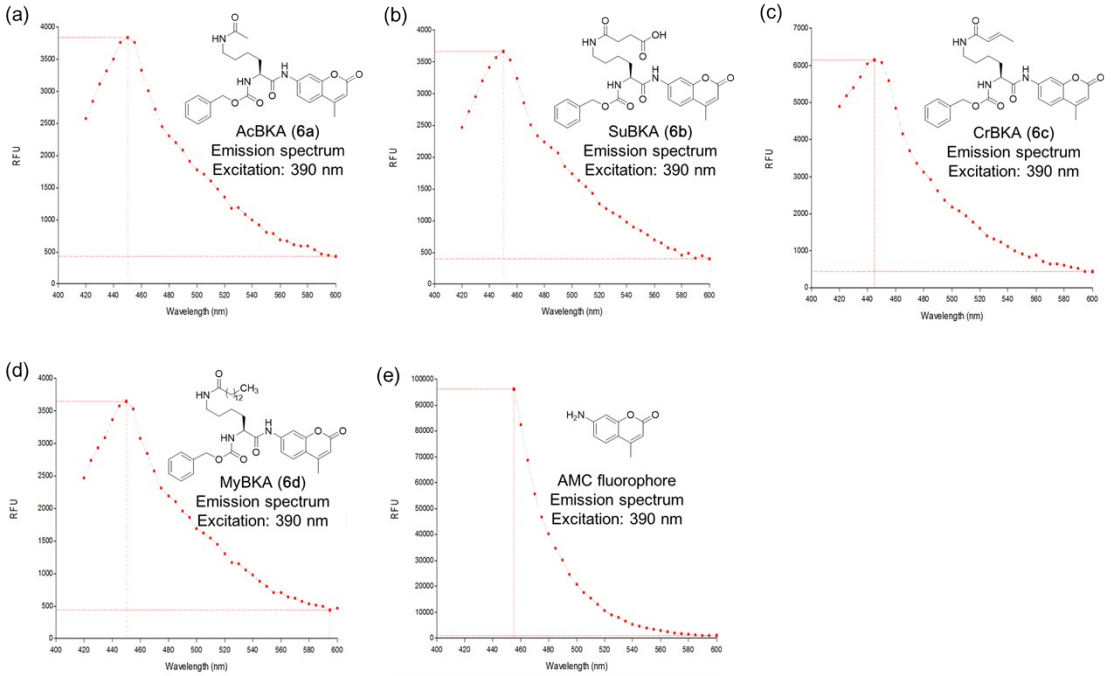

Fig. S2 Emission spectra of AcBKA (**6a**), SuBKA (**6b**), CrBKA (**6c**), MyBKA (**6d**), and AMC fluorophore obtained with excitation of 390 nm. Note, the RFU of AMC fluorophore at excitation of < 455 nm is beyond the scope of the microplate reader (BioTek Cytation 3). The fluorescence intensity of AMC (460 nm, emission) is significantly stronger than that of all of the four small-molecule substrates (**6a-6d**), indicating that these substrates are suitable for simultaneous detection of sirtuin mediated deacylation at 390 nm (excitation) and 460 nm (emission).

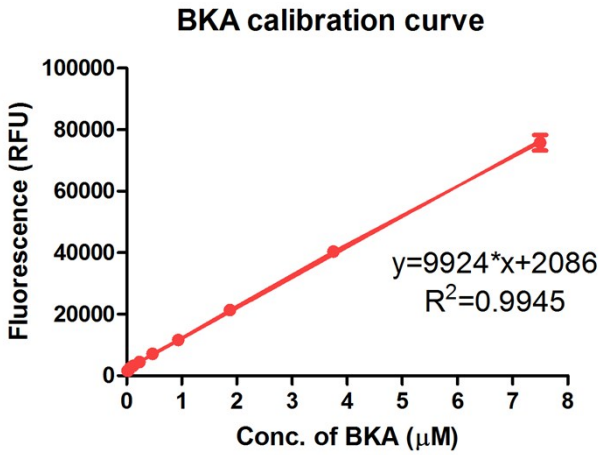

Fig. S3 Calibration curve for the hydrolysis product of BKA (0.015 μM to 7.5 μM) by trypsin using Corning 96-well plate. The BKA-fluorescence relationship obtained by treating saturating trypsin to different BKA concentrations for 24h.

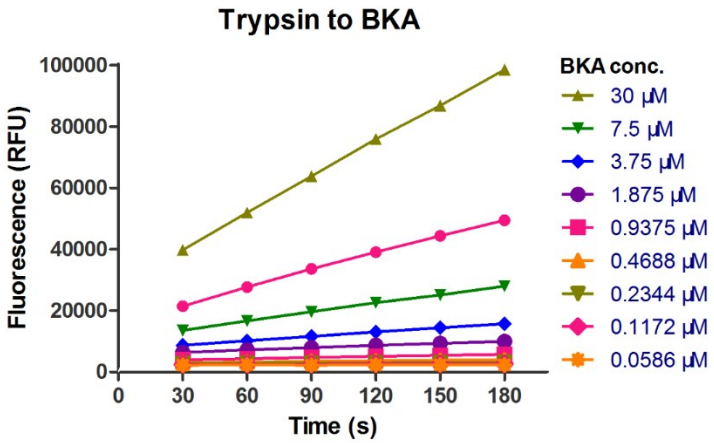

Fig. S4 The hydrolysis activity of trypsin to the template molecule BKA. The initial rate velocities of trypsin (2 $U \cdot \mu L^{-1}$) hydrolysing BKA (0.05 μM to 30 μM) obtained by recording the fluorescence values every 30 seconds.

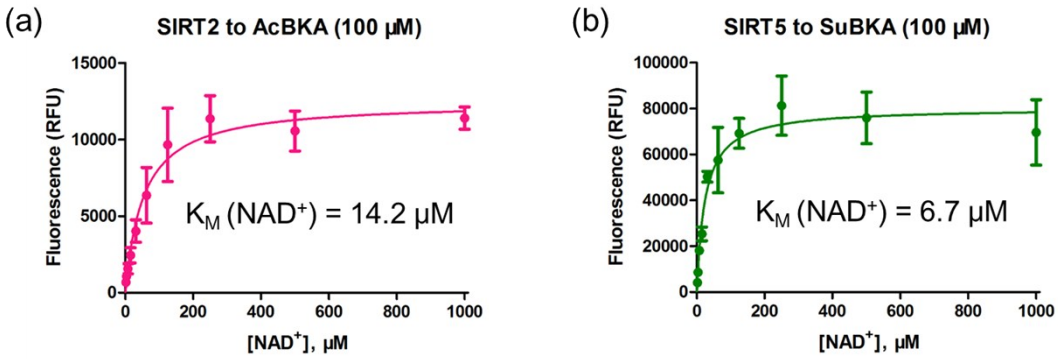

Fig. S5 The catalytic activities of SIRT2 to AcBKA and SIRT5 to SuBKA obtained with NAD^+ (1000 μM ~ 2μM) and AcBKA/MyBKA (100 μM) in the assay buffer (a total of 60 μL solutions) for 4 h at 37 ℃ and 140 rpm. The results revealed evidence for the NAD^+-dependent catalysis nature of sirtuins.

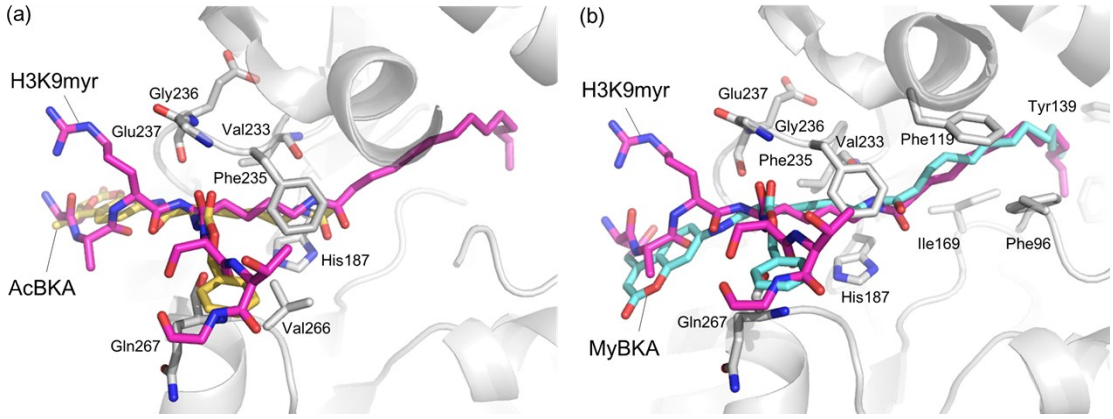

Fig. S6 Comparison of the SIRT2:H3K9myr (PDB ID: 4Y6L)[1] crystal structure with the predicted binding modes of AcBKA and MyBKA with SIRT2, revealing similarity between the SIRT2 catalyzed mechanisms for AcBKA/MyBKA and the peptide substrate H3K9myr.

Supplementary Tables

Table S1 Sirtuin functions and substrates.[2-6]

Subclass	Enzyme	Activity	Localization	Representative non-histone substrates
Class I	SIRT1	Deacetylase Depropionylase Debutyrylase	Nucleus	HDAC1, PARP1, p53, FOXO1, FOXO3A, PGAM1, ACECS1, PTP1B, S6K1
	SIRT2	Deacetylase Depropionylase Debutyrylase Defattyacylase	Cytoplasm Nucleus	Tubulin, keratin 8, PAR3, PR, FOXO1, FOXO3A, p300, NF κB, HIF1α
	SIRT3	Deacetylase Depropionylase Debutyrylase Defattyacylase	Mitochondria	LCAD, VLCAD, HMGCS2, SDHA, ACECS2, GDH, IDH2, MRPL10, PDP1, SOD2, PDH, FOXO3, GOT2
Class II	SIRT4	Deacetylase ADP-ribosylase Lipoamidase Delipoylase Debiotinylase	Mitochondria	GDH, IDE, SLC25A5, PDH, MCD
Class III	SIRT5	Deacetylase Desuccinylase Demalonylase Deglutarylase	Mitochondria	CPS1, HMGCS2, PDH, SDH, SOD1, GAPDH
Class IV	SIRT6	Deacetylase ADP-ribosylase Defattyacylase	Nucleus	CtIP, GCN5, SNF2H, G3BP, FOXO3, PARP1, MYC, HIF1α, NF-κB, TNF, USP10
	SIRT7	Deacetylase	Nucleus	MYC, H3K18, PAF53, HIF1α, HIF2α, ELK4, MYBBP1A, TFIIIC2, p53, mTOR, CUL4B, GABPβ1

Table S2 Data collection and refinement statistics.

Structure	SIRT5:SuBKA
PDB ID	5XHS
Radiation Source	BL19U1
Processing	
Space Group	$P\ 2_1\ 2\ 2_1$
Unit Cell Dimensions a, b, c (Å)	42.13, 55.27, 124.30
Unit Cell Dimensions α, β, γ (˚)	90.00, 90.00, 90.00
*Mol/ASU	1
Resolution Range (outer shell) (Å)	39.9-2.19 (2.27-2.19)
Number of Unique Reflections	14762 (979)
Completeness (%)	99.7% (97.9)
I/σ(I) (outer shell)	10.3 (2.0)
R_{merge} (outer shell)	0.234 (0.665)
Wilson B Factor (Å^2)	31.09
Refinement	
Overall B Factor (Å^2)	34.46
Protein B Factor (Å^2)	34.02
Ligand B Factor (Å^2)	55.48
Water B Factor (Å^2)	35.00
‡RMSD from Ideal Bond Length (Å)	0.012
RMSD from Ideal Angles (˚)	1.204
R_{work} (%)	0.174
R_{free} (%)	0.225

*Mol/ASU = molecules per asymmetric unit; ‡RMSD = root mean square deviation.

Reference

1 J. L. Feldman, K. E. Dittenhafer-Reed, N. Kudo, J. N. Thelen, A. Ito, M. Yoshida and J. M. Denu, *Biochemistry*, 2015, **54**, 3037-3050.

2 R. H. Houtkooper, E. Pirinen and J. Auwerx, *Nat. Rev. Mol. Cell Biol.*, 2012, **13**, 225-238.

3 M. S. Bonkowski and D. A. Sinclair, *Nat. Rev. Mol. Cell Biol.*, 2016, **17**, 679-690.

4 A. Chalkiadaki and L. Guarente, *Nat. Rev. Cancer*, 2015, **15**, 608-624.

5 B. Chen, W. Zang, J. Wang, Y. Huang, Y. He, L. Yan, J. Liu and W. Zheng, *Chem. Soc. Rev.*, 2015, **44**, 5246-5264.

6 L. Yang, X. Ma, Y. He, C. Yuan, Q. Chen, G. Li and X. Chen, *Sci. China Life Sci.*, 2017, **60**, 249-256.