CoREST complex-selective HDAC inhibitors show pro-synaptic effects and an improved safety profile to enable treatment of synaptopathies

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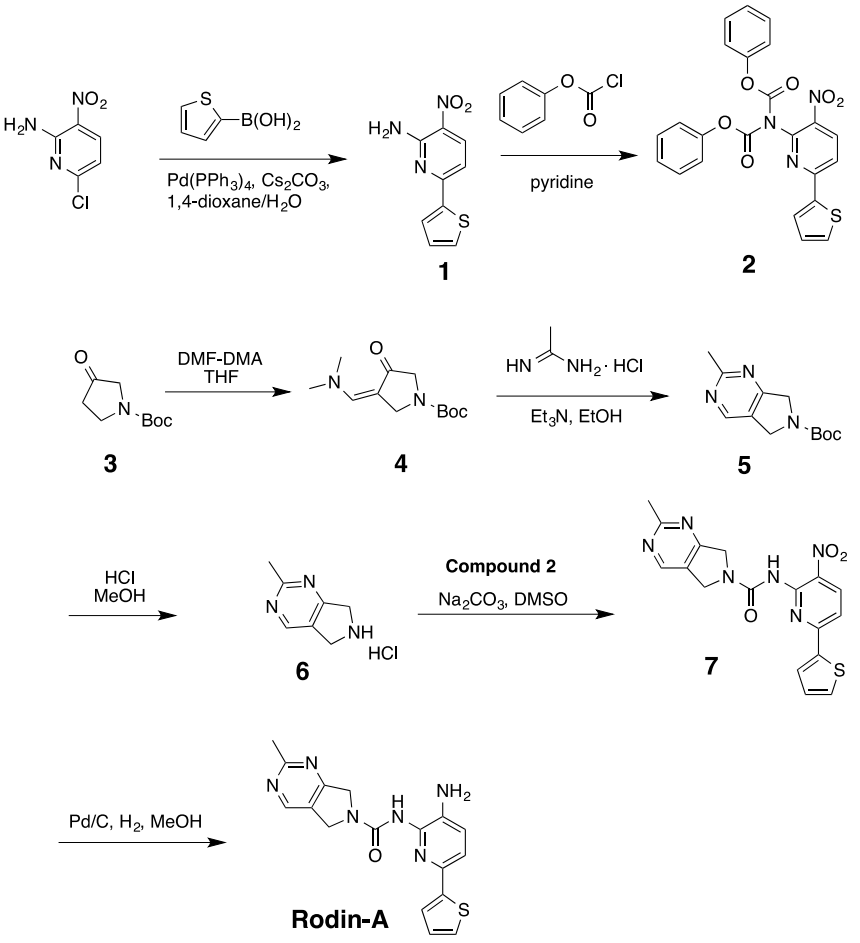
Supporting Information.

Fig. S2. Hippocampal dendritic spine density following 14 days of dosing with Rodin-A.

Experimental procedures for the synthesis of new compounds:

Thin layer chromatography spots were visualized by UV light (254 and 365 nm). Purification by column and flash chromatography was carried out using silica gel (200-300 mesh). Solvent systems are reported as the ratio of solvents. NMR spectra were recorded on a Bruker 400 (400 MHz) spectrometer. ^{1}H chemical shifts are reported in δ values in ppm with tetramethylsilane (TMS, = 0.00 ppm) as the internal standard. LCMS spectra were obtained on an Agilent 1200 series 6110 or 6120 mass spectrometer with ESI (+) ionization mode.

Synthesis of Rodin-A:



Synthesis of Compound 1: A mixture of 6-chloro-3-nitropyridin-2-amine (10.00 g, 57.6 mmol), thiophen-2-ylboronic acid (8.12 g, 63.4 mmol) and Cs_2CO_3 (37.56 g, 115.2 mmol) in dioxane/H_2O (200 mL/20 mL) was treated with $Pd(PPh_3)_4$ (2.44g, 2.88 mmol) under an N_2 atmosphere. The mixture was stirred at 95 °C for 2 h and then concentrated *in vacuo*. The residue was dissolved with EtOAc (200 mL) and the solution was washed with brine (100 mL × 3). The organic layer was dried over anhydrous Na_2SO_4 and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (PE : EtOAc = 5 : 1 ~ 3 : 1) to give **1** (10.0 g, 79%) as a yellow solid

Synthesis of Compound 2: To a stirred solution of **1** (1.30 g, 5.88 mmol) in pyridine (20 mL) was added phenyl carbonochloridate (2.29 g, 14.7 mmol) in dropwise fashion. After the addition was completed, the mixture was heated to 50 °C and stirred for 4 h. The mixture was then concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel (PE : EtOAc = 8: 1 ~ 3 : 1) to give **2** (2.4 g, 89%) as a yellow solid.

Synthesis of Compound 4: A solution of tert-butyl 3-oxopyrrolidine-1-carboxylate (**3**) (150.0 g, 809.8 mmol) and DMF-DMA (289.5 g, 2.4 mol) in THF (1500 mL) was stirred at 70 °C for 16 h. The solution was then concentrated *in vacuo* to give **4** as a crude product, which was used directly in the next step without further purification.

Synthesis of Compound 5: To a solution of **4** (809.8 mmol, crude product from last step) in EtOH (1000 mL) was added Et_3N (409.7 g, 4.0 mol) and acetimidamide hydrochloride (306.2 g, 3.2 mol). The resulting solution was stirred at 80 °C for 24 h. After the mixture was cooled to room temperature, the mixture was diluted with water (500 mL) and extracted with DCM (500 mL × 3). The combined organic layers were washed with brine (500 mL × 3), dried over anhydrous Na_2SO_4

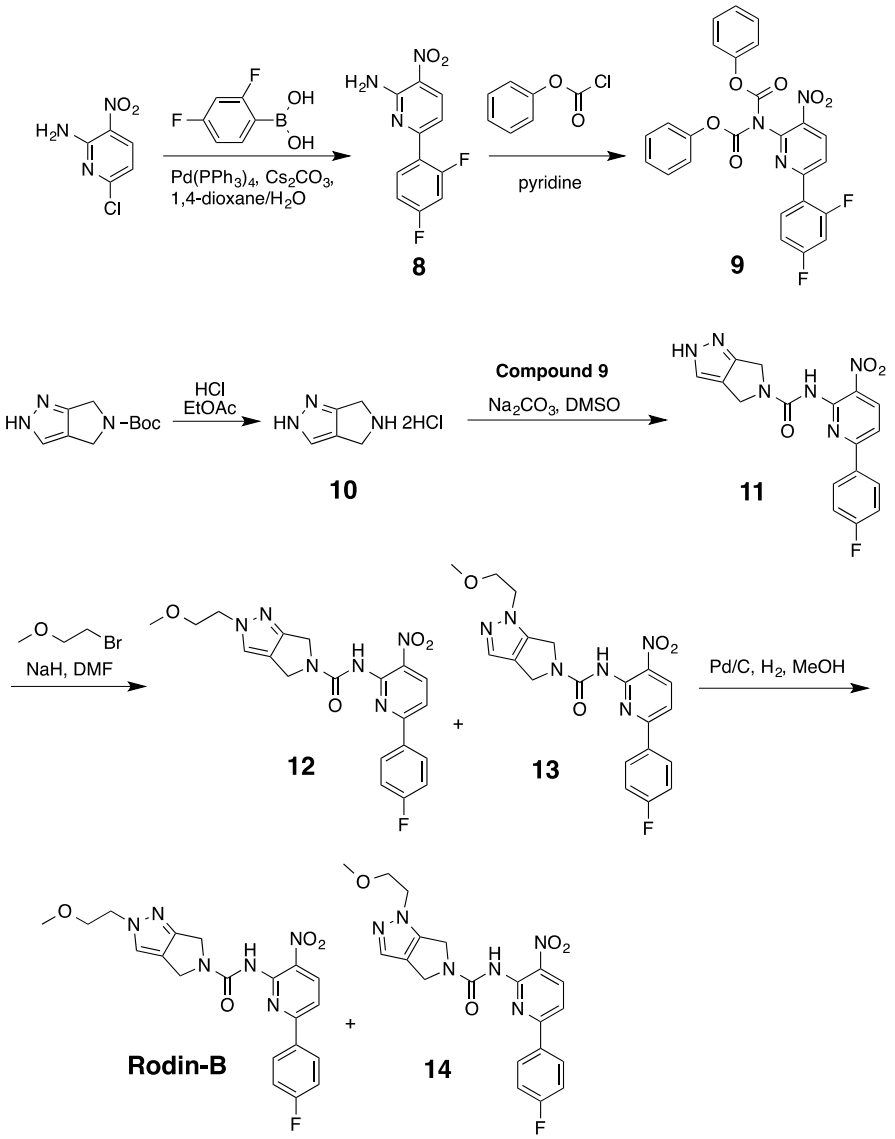
and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (PE : DCM = 10 : 1 ~ 1 : 2) to give **5** (105.0 g, 55%) as a brown solid.

Synthesis of Compound 6: To a solution of **5** (105.0 g, 446.3 mmol) in DCM (1000 mL) was added TFA (333 mL) dropwise. The reaction mixture was stirred at room temperature for 1 h, whereupon the solution was concentrated *in vacuo* to give **6** as a crude product which was used directly in the next step.

Synthesis of Compound 7: A mixture of **6** (325.1 mmol, crude product from last step) and **2** (75.0 g, 162.5 mmol) in DMSO (750 mL) was stirred at room temperature for 10 min, then Na_2CO_3 (137.8 g, 1.3 mol) was added, and the reaction mixture was stirred at room temperature for 2 h. The mixture was then diluted with water (1000 mL) and extracted with EtOAc (500 mL × 3). The combined organic layers were washed with brine (500 mL × 3), dried over anhydrous Na_2SO_4 and then concentrated *in vacuo.* The residue was purified by column chromatography on silica gel (PE : EA = 10 : 1 ~ 1 : 2) to give **7** (44.0 g, 71%) as a yellow solid..

Synthesis of Rodin-A : A mixture of **7** (44.0 g, 115.1 mmol) and Pd/C (22.0 g) in MeOH (250 mL) and DCM (250 mL) was stirred at room temperature for 1 h under a H_2 atmosphere. Pd/C was removed by filtration through Celite. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography on silica gel (DCM : MeOH = 50 : 1 ~ 15 : 1) to give **Rodin-A** (26.0 g, 64%) as a light yellow solid. LC/MS (ESI^+), m/z (M+H) = 353. **^{1}H NMR Data (400 MHz, DMSO-d_6)** δ 8.70 (s, 1H), 8.60 (s, 1H), 7.53 – 7.47 (m, 2H), 7.42 – 7.40 (q, J = 4.0 Hz, 1H), 7.13 – 7.07 (t, J = 8.0 Hz, 1H), 7.06 – 7.05 (t, J = 1.2 Hz, 1H), 5.18 (s, 2H), 4.78 – 4.75 (d, J = 10.4 Hz, 4H), 2.64 (s, 3H).

Synthesis of Rodin-B:



Synthesis of Compound 8.

A mixture of 6-chloro-3-nitropyridin-2-amine (4.58 g, 26.4 mmol), 2,4-difluorophenylboronic acid (5.00 g, 31.7 mmol) and Cs_2CO_3 (25.73 g, 79.2 mmol) in dioxane/H_2O (100 mL/10 mL) was treated with $Pd(PPh_3)_4$ (1.10 g, 0.95 mmol) under a N_2 atmosphere. The mixture was stirred at 100 ^oC for 2 h and then concentrated *in vacuo*. The residue was dissolved with EtOAc (200 mL) and the solution was washed with brine (100 mL × 3). The organic layer was dried over anhydrous

Na_2SO_4 and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (PE : EtOAc = 7 : 1 ~ 5 : 1) to give **8** (4.0 g, 61%) as a yellow solid. MS 252.1 $[M + H]^+$.

Synthesis of Compound 9.

A stirred solution of **8** (4.0 g, 15.94 mmol) in pyridine (60 mL) was treated with phenyl carbonochloridate (7.50 g, 47.81 mmol) dropwise at 0 ^oC. After the addition was completed, the mixture was stirred at 50 ^oC for 4 h, then was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (PE : DCM = 3: 2 ~ 1 : 1) to give **9** (7.1 g, 91%) as a yellow solid. MS 492.1 $[M + H]^+$.

Synthesis of Compound 10.

A solution of tert-butyl 4,6-dihydropyrrolo[3,4-c] pyrazole-5(2H)-carboxylate (205 mg, 0.98 mmol) in HCl/EA (4N, 8 mL) was stirred at room temperature for 1 h. The solvent was then removed *in vacuo* to give **10** as a crude product. MS 110.1 $[M + H]^+$.

Synthesis of Compound 11.

A mixture of **10** (crude product from last step) and **9** (400 mg, 0.82 mmol) in DMSO (8 mL) was treated with Na_2CO_3 (691 mg, 6.52 mmol) and then stirred at room temperature for 2 h. The mixture was dlitued with water (20 mL), extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine (20 mL × 3), dried over anhydrous Na_2SO_4 and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (DCM : MeOH = 100 : 1 ~ 50 : 1) to give **11** (270 mg, 86%) as a yellow solid. MS 387.1 $[M + H]^+$.

Synthesis of Compounds 12 and 13.

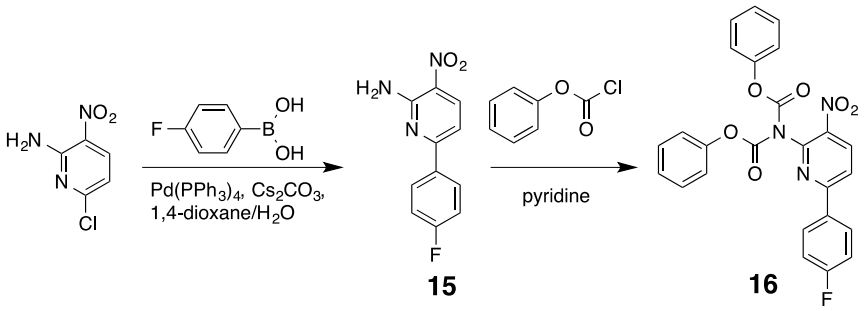
To a solution of **11** (270 mg, 0.70 mmol) in DMF (5 mL) was added NaH (60% in mineral oil) (84 mg, 2.10 mmol) while being cooled by an ice bath. The resulting mixture was allowed to warm to room temperature and was stirred at room temperature for 30 min. Then 1-bromo-2-

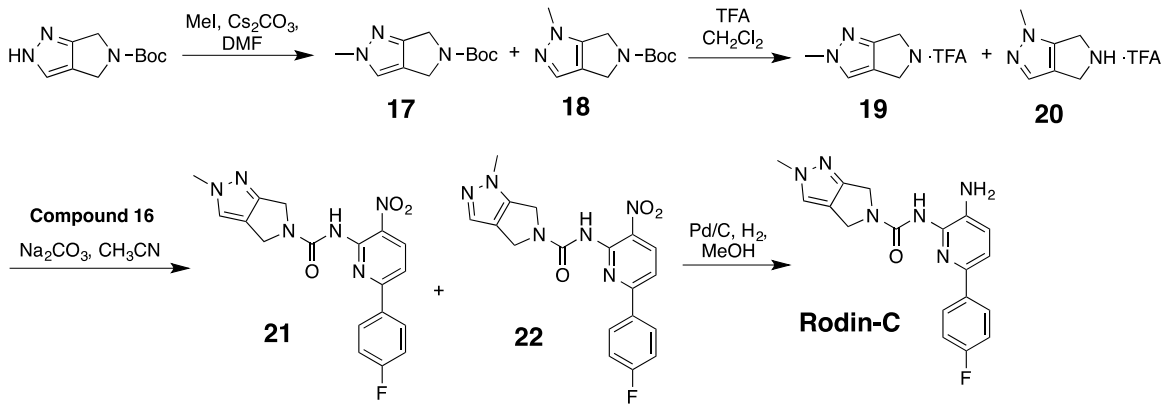
methoxyethane (194 mg, 1.40 mmol) was added to the above mixture and stirred at room temperature for 2 h. The mixture was quenched with water (15 mL) and then extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine (10 mL × 3), dried over anhydrous Na_2SO_4 and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (DCM : MeOH = 100 : 1 ~ 30 : 1) to give a mixture of **12** and **13** (300 mg, 97%) as a yellow solid. MS 445.0 $[M + H]^+$.

Synthesis of Rodin-B and Compound 14.

A mixture of **12** and **13** (300 mg, 0.68 mmol) and Pd/C (300 mg) in MeOH (15 mL) was stirred at room temperature for 1 h under H_2 atmosphere. Pd/C was then removed by filtration through Celite. The filtrate was concentrated and the residue was purified by column chromatography on silica gel (DCM : MeOH = 100 : 1 ~ 30 : 1) to give **Rodin-B** and **14**. The mixture was purified via reverse phase HPLC, using chiral separation (Column: Chiralcel OJ-3; Solvent: MeOH/MeCN=1/1; Flow rate: 2 mL/min; RT_{1667} = 1.74 min, RT_{1667A} = 0.93 min) to give **Rodin-B** (80 mg, 28%) as a white solid (MS 415.1 $[M + H]^+$) and **14** (40 mg, 14%) as a white solid. MS 415.1 $[M + H]^+$. **^{1}H NMR Data (400 MHz, DMSO-d_6)** δ 8.48 (s, 1H), 7.98 – 7.91 (m, 1H), 7.57 (s, 1H), 7.41 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.32 – 7.26 (m, 1H), 7.18 – 7.13 (m, 2H), 5.25 (s, 2H), 4.52 (s, 4H), 4.27 – 4.24 (t, *J* = 5.4 Hz, 2H), 3.69 – 3.66 (t, *J* = 5.2 Hz, 2H), 3.24 (s, 3H).

Synthesis of Rodin-C:





Synthesis of Compound 15. A mixture of 6-chloro-3-nitropyridin-2-amine (10.0 g, 57.6 mmol), 4-fluorophenylboronic acid (8.87 g, 63.4 mmol) and Cs_2CO_3 (37.56 g, 115.2 mmol) in dioxane/H_2O (200 mL/20 mL) was treated with $Pd(PPh_3)_4$ (2.44 g, 2.9 mmol) under N_2 atmosphere. The mixture was stirred at 95 °C for 2 h and then concentrated *in vacuo*. The residue was dissolved with EtOAc (200 mL) and the solution was washed with brine (100 mL × 3). The organic layer was dried over anhydrous Na_2SO_4 and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (PE : EtOAc = 5 : 1 ~ 3 : 1) to give **15** (11.2 g, 83%) as a yellow solid.

Synthesis of Compound 16. A stirred solution of **15** (3.0 g, 13.0 mmol) in pyridine (60 mL) was treated with phenyl carbonochloridate (4.45 g, 28.5 mmol) dropwise. After the addition was completed, the mixture was heated to 50 °C and stirred at that temperature for 4 h. The mixture was then concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel (PE : EtOAc = 8: 1 ~ 3 : 1) to give **16** (5.2 g, 84%) as a yellow solid.

Synthesis of Compound 17 and Compound 18. A mixture of tert-butyl 4,6-dihydropyrrolo[3,4-c]pyrazole-5(2H)-carboxylate (314 mg, 1.50 mmol), Cs_2CO_3 (978 mg, 3.00 mmol) and iodomethane (320 mg, 2.25 mmol) in DMF (6 mL) was stirred at room temperature for 16 h. The mixture was diluted with water (18 mL), extracted with EtOAc (10 mL × 3), and then the combined

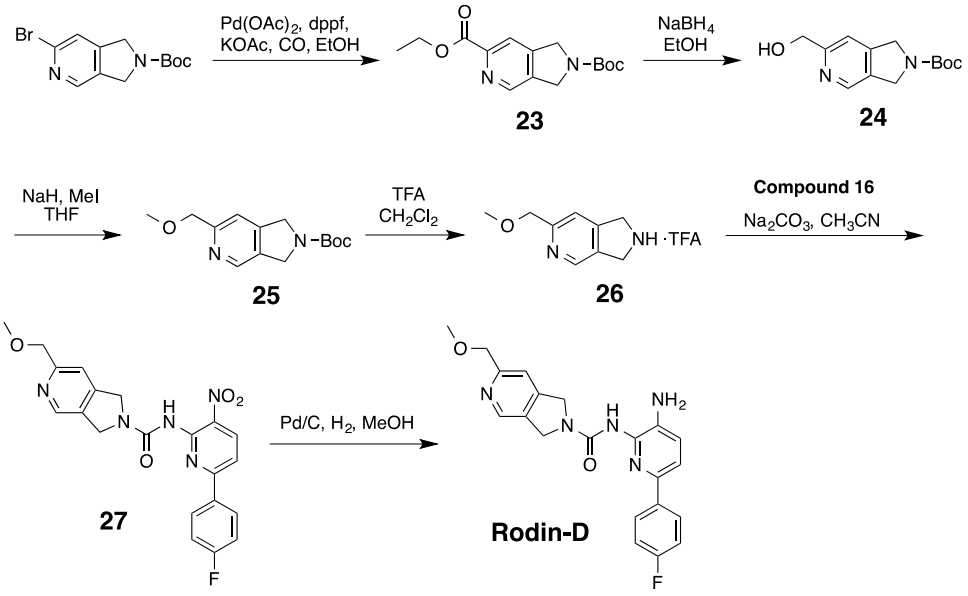
organic layers were washed with brine (10 mL × 3), dried over anhydrous Na_2SO_4 and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (DCM : MeOH = 100 : 1 ~ 50 : 1) to give a mixture of **17** and **18** (270 mg, 81%) as a yellow solid.

Synthesis of Compound 19 and 20. To a solution of **17** and **18** (270 mg, 1.21 mmol) in DCM (6 mL) was added TFA (2 mL) dropwise while cooling with an ice bath. The reaction mixture was allowed to warm to room temperature, and was stirred at room temperature for 1 h. The solvent was then removed *in vacuo* to give a mixture of **19** and **20** as a crude product.

Synthesis of Compounds 21 and 22. A mixture of **19** and **20** (crude product from last step) and **16** (286 mg, 0.61 mmol) in acetonitrile (10 mL) was stirred at 50 ^oC for 30 min, then Na_2CO_3 (581 mg, 6.05 mmol) was added into the above mixture, and the reaction was stirred at 50 ^oC for 1 h. The mixture was then cooled to room temperature, and Na_2CO_3 was removed by filtration. The filtrate was concentrated *in vacuo*, and the resulting residue was purified by column chromatography on silica gel (DCM : MeOH = 200 : 1) to give **21** (100 mg, 44%) as a yellow solid and **22** (50 mg, 22%) as a yellow solid.

Synthesis of Rodin-C. A mixture of **21** (100 mg, 0.26 mmol) and Pd/C (100 mg) in MeOH (5 mL) was stirred at room temperature for 1 h under a H_2 atmosphere. Pd/C was then removed by filtration through Celite, the filtrate was concentrated, and the residue was purified by Prep-TLC (DCM : MeOH = 15 : 1) to give **Rodin-C** (50 mg, 55%) as a yellow solid. LC/MS (ESI^+), m/z (M+H) = 353. **^{1}H NMR Data (400 MHz, DMSO-d_6)** δ 8.45 (s, 1H), 7.98 (dd, *J* = 8.8, 5.6 Hz, 2H), 7.57 (d, *J* = 8.2 Hz, 2H), 7.22 (t, *J* = 8.9 Hz, 2H), 7.17 (d, *J* = 8.2 Hz, 1H), 5.16 (s, 2H), 4.51 (s, 4H), 3.85 (s, 3H).

Synthesis of Rodin-D:



Synthesis of Compound 23. A mixture of tert-butyl 3-bromo-5H-pyrrolo[3,4-b]pyridine-6(7H)-carboxylate (820 mg, 2.75 mmol), potassium acetate (540 mg, 5.5 mmol), dppf (111 mg, 0.08 mmol) and palladium acetate (8.5 mg, 0.03 mmol) in ethanol (20 mL) was stirred at 100 ^{o}C for 12 h under a CO atmosphere at 1.5 MPa. The reaction mixture was cooled to room temperature and filtered through Celite. The filtrate was concentrated *in vacuo* and the residue was dissolved with DCM (50 mL), washed with brine (10 mL × 3), dried over anhydrous Na_2SO_4 and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (PE : EtOAc = 8 : 1 ~ 3 : 1) to give **23** (670 mg, 84%) as a white solid.

Synthesis of Compound 24. A mixture of **23** (500 mg, 1.7 mmol) and $NaBH_4$ (390 mg, 10.2 mmol) in ethanol (50 mL) was stirred at room temperature for 12 h. The reaction mixture was concentrated *in vacuo* and and the residue was dissolved with DCM (50 mL), washed with brine (10 mL × 3), dried over anhydrous Na_2SO_4 and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (DCM : MeOH = 100 : 1 ~ 20 : 1) to give **24** (350 mg, 81%) as a white solid.

Synthesis of Compound 25. To a mixture of **24** (150 mg, 0.6 mmol) in THF (5 mL) was added NaH (60% in mineral oil) (96 mg, 2.4 mmol) at room temperature. The resulting mixture was stirred at room temperature for 30 min, whereupon MeI (170 mg, 1.2 mmol) was added dropwise. The resulting mixture was stirred at room temperature for 30 min. The solution was then diluted with water (10 mL), extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine (10 mL × 3), dried over anhydrous Na_2SO_4 and then concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (PE : EtOAc = 8 : 1 ~ 3 : 1) to give **25** (60 mg, 38%) as a yellow solid

Synthesis of Compound 26. To a solution of **25** (60 mg, 0.23 mmol) in DCM (4 mL) was added TFA (2 mL) dropwise, with the reaction being cooled with an ice bath. The reaction mixture was allowed to warm to room temperature, and was stirred at room temperature 1 h. The solvent was then removed *in vacuo* to give **26** as a crude product which was used without further purification.

Synthesis of Compound 27. A mixture of **26** (0.23 mmol, crude product from last step), **16** (80 mg, 0.17 mmol) and Na_2CO_3 (122 mg, 1.15 mmol) in acetonitrile was stirred at 50 ^oC for 3 h. After the reaction was completed according to LCMS. Na_2CO_3 was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (DCM : MeOH = 100 : 1 ~ 50 : 1) to give **27** (70 mg, 73%) as a yellow solid.

Synthesis of Rodin-D. A mixture of **27** (70 mg, 0.16 mmol) and Pd/C (70 mg) in MeOH (5 mL) was stirred at room temperature for 1 h under a H_2 atmosphere. Pd/C was then removed by filtration through Celite, the filtrate was concentrated, and the residue was purified by prep-TLC (DCM : MeOH = 15 : 1) to give **Rodin-D** (48 mg, 78%) as a yellow solid. LC/MS (ESI^+), m/z (M+H) = 394. **^{1}H NMR Data (400 MHz, DMSO-d_6)** δ 8.55 (d, *J* = 8.1 Hz, 2H), 7.98 (dd, *J* = 8.9, 5.6 Hz,

2H), 7.57 (d, *J* = 8.2 Hz, 1H), 7.44 (s, 1H), 7.22 (t, *J* = 8.9 Hz, 2H), 7.17 (d, *J* = 8.2 Hz, 1H), 5.19 (s, 2H), 4.83 (s, 4H), 4.53 (s, 2H), 3.38 (s, 3H).

Recombinant HDAC biochemical assay (Nanosyn):

Table S1. Summary of HDAC protein composition and respective substrate peptides used in recombinant HDAC biochemical assays

Assay name	Expression Construct	Regulatory subunit	Substrate peptide
HDAC1	Full length Human HDAC1 with C-terminal His-tag and C-terminal FLAG-tag, expressed in baculovirus expression system.	None	FAM-TSRHK(Ac)KL-NH2
HDAC2	Full length Human HDAC2 with C-terminal FLAG-tag, expressed in baculovirus expression system.	None	FAM-TSRHK(Ac)KL-NH2
HDAC3	Full length Human HDAC3 with C-terminal His-tag, co-expressed with Human NCOR2 , a.a. 395- 489, N-terminal GST-tag, in baculovirus expression system.	NCOR2	FAM-RHKK(Ac)-NH2
HDAC4	Full length Human HDAC4 with N-terminal GST-tag, expressed in baculovirus expression system.	None	FAM-TSRHK(TFAc)KL-NH2
HDAC5	Full length Human HDAC5 with N-terminal GST-tag, expressed in baculovirus expression system.	None	FAM-TSRHK(TFAc)KL-NH2
HDAC6	Full length Human HDAC6 with N-terminal HIS-tag, expressed in baculovirus expression system.	None	FAM-RHKK(Ac)-NH2
HDAC7	Full length Human HDAC7 with N-terminal GST-tag, expressed in baculovirus expression system.	None	FAM-TSRHK(TFAc)KL-NH2
HDAC8	Full length Human HDAC8 with N-terminal HIS-tag, expressed in baculovirus expression system.	None	FAM-TSRHK(TFAc)KL-NH2
HDAC9	Human HDAC9 catalytic domain a.a. 604-1066 with C-terminal His-tag, expressed in baculovirus expression system.	None	FAM-TSRHK(TFAc)KL-NH2

HDAC10	Full length Human HDAC10 with N-terminal HIS-tag, expressed in baculovirus expression system.	None	FAM-TSRHK(Ac)KL-NH2
HDAC11	Full length Human HDAC11 with N-terminal HIS-tag, expressed in baculovirus expression system.	None	FAM-TSRHK(TFAc)KL-NH2

HDAC reactions are assembled in 384 well plates (Greiner) in a total volume of 20 μL as follows: 1) HDAC proteins are pre-diluted in the assay buffer comprising: 100mM HEPES, pH 7.5, 0.1% BSA, 0.01% Triton X-100, 25mM KCl and dispensed into 384 well plate (10uL per well); 2)Test compounds are serially pre-diluted in DMSO and added to the protein samples by acoustic dispensing (Labcyte Echo). Concentration of DMSO is equalized to 1% in all samples; 3) Control samples (0%-inhibition in the absence of inhibitor, DMSO only) and 100%-inhibition (in the absence of enzyme) are assembled in replicates of four and used to calculate the %-inhibition in the presence of compounds; 4) The reactions are initiated by addition of 10μL of the FAM-labeled substrate peptide pre-diluted in the same assay buffer. Final concentration of substrate peptide is 1μM (HDAC1-10) and 2μM (HDAC11); 5) The reactions are allowed to proceed at room temperature; 6) Following incubation, the reactions are quenched by addition of 50 μL of termination buffer (100 mM HEPES, pH7.5, 0.01% Triton X-100, 0.1% SDS); 7) Terminated plates are analyzed on a microfluidic electrophoresis instrument (Caliper LabChip® 3000, Caliper Life Sciences/Perkin Elmer) which enables electrophoretic separation of de-acetylated product from acetylated substrate. A change in the relative intensity of the peptide substrate and product is the parameter measured. Activity in each test sample is determined as the product to sum ratio (PSR): P/(S+P), where P is the peak height of the product, and S is the peak height of the substrate. Percent inhibition (P_{inh}) is determined using the following equation: $P_{inh} = (PSR_{0\%inh} - PSR_{compound})/(PSR_{0\%inh} - PSR_{100\%inh})*100$, in which: $PSR_{compound}$ is the product/sum ratio in the presence of compound, $PSR_{0\%inh}$ is the product/sum ratio in the absence of compound and the

$PSR_{100\%inh}$ is the product/sum ratio in the absence of the enzyme. To determine IC_{50} of compounds (50%-inhibition) the %-inh data (P_{inh} versus compound concentration) are fitted by a 4-parameter sigmoid dose-response model using XLfit software (IDBS).

Data collection and processing statistics for co-crystal structure of Rodin-A bound to HDAC2

HDAC2 produced in insect cells was concentrated to 8 mg/ml in 25 mM TRIS/HCl 7.6, 250 mM NaCl, 0.125 mM TCEP. The protein was crystallized from 49% PEG600, 0.1 M CHES/NaOH pH 8.5 by mixing 0.1 µl of the protein solution with 0.1 µl of the reservoir solution and incubating the drop over reservoir solution at 20 °C (sitting drop).

Crystals of HDAC2 in complex with the ligand Rodin-A were prepared by soaking crystals of HDAC2 with the ligand at 2 mM diluted from a 100 mM DMSO stock solution into reservoirs solution for 2 days. For data collection the crystal was frozen directly in liquid nitrogen. Diffraction data of the HDAC2 ligand complex containing the ligand Rodin-A were collected at the Diamond Light Source (DLS, Oxford, England) (Table S2).

The structure was solved and refined to a final resolution of 2.25 Å (Table S3). The crystals contain three monomers of HDAC2 in the asymmetric unit with basically the same overall conformation. The model comprises residues Gly12 to Pro379. The structure of human Histone deacetylase 2 (HDAC2) in complex with ligand Rodin-A shows a single α/β domain that includes an eight stranded β sheet sandwiched between 13 α helices (Fig. 1B-C).

Table S2. Data collection and processing statistics for Rodin-A

Ligand	**Rodin-A**
X-ray source	I02 (DLS[1])
Wavelength [Å]	0.9282
Detector	PILATUS 6M
Temperature [K]	100
Space group	P 2_1 2_1 2_1
Cell: a; b; c; [Å]	93.32; 98.92; 141.22
α; β; γ; [◦]	90.0; 90.0; 90.0
Resolution [Å]	2.25 (2.50-2.25)
Unique reflections	61258 (16103)
Multiplicity	2.8 (2.8)
Completeness [%]	97.2 (95.5)
R_{sym} [%][3]	7.7 (42.2)
R_{meas} [%][4]	9.5 (51.9)
Mean(I)/sd[5]	9.65 (2.49)

[1] DIAMOND LIGHT SOURCE (DLS, Oxford, England)

[2] values in parenthesis refer to the highest resolution bin.

[3] $Rsym = \frac{\sum_h \sum_i^{n_h} \left| \hat{I}_h - I_{h,i} \right|}{\sum_h \sum_i^{n_h} I_{h,i}}$ with $\hat{I}_h = \frac{1}{n_h} \sum_i^{n_h} I_{h,i}$

where $I_{h,i}$ is the intensity value of the ith measurement of h

[4] $Rmeas = \frac{\sum_h \sqrt{\frac{n_h}{n_h - 1}} \sum_i^{n_h} \left| \hat{I}_h - I_{h,i} \right|}{\sum_h \sum_i^{n_h} I_{h,i}}$ with $\hat{I}_h = \frac{1}{n_h} \sum_i^{n_h} I_{h,i}$

where $I_{h,i}$ is the intensity value of the ith measurement of h

[5] calculated from independent reflections

Table S3. Refinement statistics for Rodin-A[1]

Ligand	**Rodin-A**
Resolution [Å]	81.02-2.25
Number of reflections (working /test)	59839 / 1384
R_{cryst} [%]	20.6
R_{free}[%][2]	23.1
Total number of atoms:	
Protein	8866
Water	235
Ligand	75
Deviation from ideal geometry: [3]	
Bond lengths [Å]	0.012
Bond angles [°]	1.34
Bonded B's [Å^2] [4]	3.2
Ramachandran plot: [5]	
Most favoured regions [%]	91.1
Additional allowed regions [%]	8.9
Generously allowed regions [%]	0.0
Disallowed regions [%]	0.0

[1] Values as defined in REFMAC5, without sigma cut-off
[2] Test-set contains 2.3% of measured reflections
[3] Root mean square deviations from geometric target values
[4] Calculated with MOLEMAN
[5] Calculated with PROCHECK

LSD1 and LSD1-CoREST recombinant enzymatic assay description (Nanosyn):

Demethylase activity of LSD1 or LSD1-CoREST complex was measured by microfluidic electrophoresis mobility shift assay. The reactions were assembled in a total volume of 25 uL in a reaction buffer comprising: 50 mM TRIS-HCl (pH 8.0), 50 mM NaCl, 0.01% Tween 20, 1mM DTT, 1% DMSO (from compound) and 3nM LSD1 enzyme (BPS Biosciences, cat#50100). When indicated, CoREST protein (BPS Biosciences, cat#50274) was added to a final concentration of 10 nM. Serially diluted compounds were pre-incubated with enzyme and the reactions were initiated by addition of 0.5uM of FAM-labeled H3K4me peptide. After 30 min incubation at RT (25^{o}C) the reactions were quenched by addition of 45 uL of termination buffer (100 mM Tris:HCl,

pH8.5, Lys C peptidase 0.03 units per mL). After 30min of incubationwith Lys C (at RT), the reactions were analyzed on LabChip® 3000 microfluidic electrophoresis instrument (Caliper Life Sciences).

The enzymatic de-methylation of the peptide results in additional cleavage site for the Lys C peptidase enabling electrophoretic separation of the cleaved product from substrate peptide. As substrate and product peptides are separated, two peaks of fluorescence are observed. Change in the relative fluorescence intensity of the substrate and product peaks is the parameter measured, reflecting enzyme activity. In the presence of an inhibitor, the ratio between product and substrate is altered: the signal of the product decreases, while the signal of the substrate increases. Activity in each sample is determined as the product to sum ratio (PSR): P/(S+P), where P is the peak height of the product peptide and S is the peak height of the substrate peptide. Negative control samples (0%- inhibition in the absence of inhibitor) and positive control samples (100%-inhibition, in the absence of the enzyme) were assembled in replicates of four and are used to calculate %-inhibition values for each inhibitor at each concentration. Percent inhibition (Pinh) is determined using following equation:

Pinh = (PSR0% - PSRinh)/(PSR0% - PSR100%)*100 , where PSRinh is the product sum ratio in the presence of inhibitor, PSR0% is the average product sum ratio in the absence of inhibitor and PSR100% is the average product sum ratio in 100%-inhibition control samples;

The IC50 values of inhibitors were determined by a 4 parameter sigmoidal dose-response model (XLfit 4 software).

Table S4. Inhibition of LSD1 and LSD1-CoREST activity as measured in recombinant enzymatic assays. Compounds were screened against LSD1 and the LSD1-CoREST complex to measure ability to inhibit LSD1 activity. Literature control compounds GSK-LSD1 and SP2509

showed potent inhibition of LSD1 activity in both the isolated enzyme and the LSD1-CoREST complex assay. None of the HDACi tested showed any inhibition of LSD1 activity.

Compound	**Rodin-A**	**Rodin-B**	**Rodin-C**	**Rodin-D**	**CI-994**	**GSK-LSD1***	**SP2509***
LSD1 IC_{50} (μM)	>150	>150	>150	>150	>150	0.015	0.95
LSD1-CoREST IC_{50} (μM)	>150	>150	>150	>150	>150	0.003	0.136

All reported compound IC_{50} values are N=1. *average of two runs.

Pharmacokinetic studies.

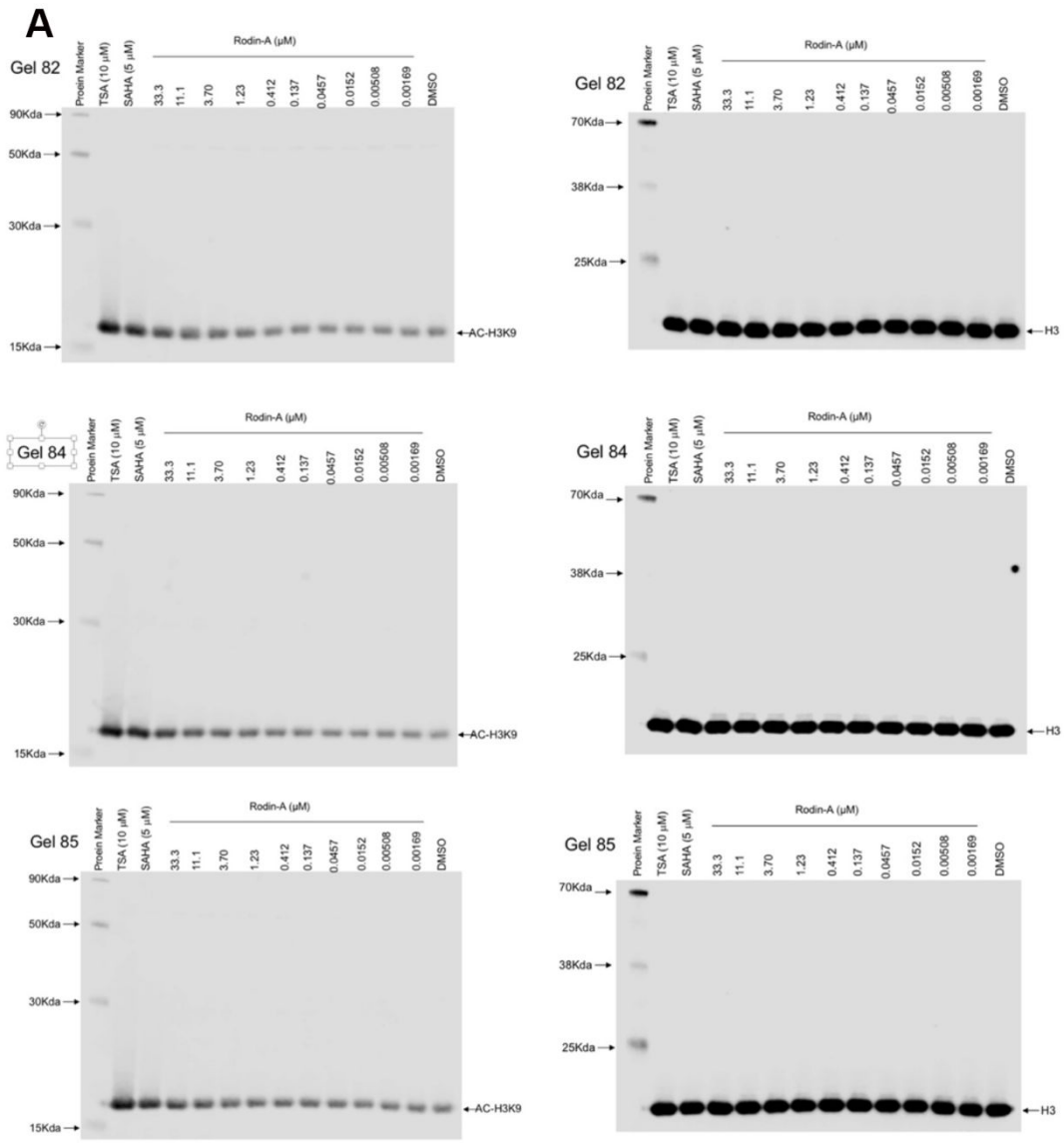
Mice were given free access to food and water and were dosed via oral gavage at 10 mg/kg (0.2 mg/mL in 20% HPβCD in water. For blood sample collection, the animal was restrained manually and approximately 150 μL blood/time point was collected into K_2EDTA tube via retro-orbital puncture. Blood sample was put on ice and centrifuged to obtain plasma sample (2000 g, 5 min under 4 ℃) within 15 minutes. For brain sample collection, at the designated time point post dosing, a mid-line incision was made in the animals scalp and the skin was retracted. Using small bone cutters and rongeurs, removed the skull overlying the brain. Removed the brain using a spatula and rinse with cold saline. Placed the brain in screw-top tubes, and then stored the tubes under -70 ℃ until analysis. Samples were collected from blood and brain at 0.25, 0.5, 1, 4, 12, and 24 hours, with N=3 at each time point per sample.

Table S5. Pharmacokinetic parameters for CI-994 and Rodin-A in mice.

Parameter	CI-994	Rodin-A
Dose	10 mg/kg	10 mg/kg
Species	CD1 mouse	C57 mouse
Formulation	20% HPβCD in water	20% HPβCD in water
Plasma Cmax (ng/mL)	5713	3023
Plasma AUC (hr*ng/mL)	10126	2668
Brain Cmax (ng/mL)	988	289
Brain/Plasma Ratio	0.17	0.09
Tmax (hour)	0.5	0.25
In vitro mouse plasma protein binding, fraction unbound (fu), %	56.5	25.1
In vitro mouse brain protein binding, fraction unbound (fu), %	36.1	29.7
Free plasma Cmax (ng/mL)	3228	759
Free Brain Cmax (ng/mL)	357	86
Kpuu (predicted unbound brain/unbound plasma ratio)	0.11	0.11

Table S6. Pharmacokinetic parameters from microdialysis experiment with Rodin-A.

Parameter	Rodin-A
Dose	20 mg/kg
Species	C57 mouse
Formulation	20% HPβCD in water
Plasma @ Brain Cmax (μM)	2.552
Mouse plasma fu (%)	25.1
Free plasma @ brain Cmax (μM)	0.64
Free Cmax brain, dialysate (μM)	1.507
Kpuu (free brain/plasma ratio)	2.4


A
Gel 82
Protein Marker
TSA (10 μM)
SAHA (5 μM)
Rodin-A (μM)
33.3
11.1
3.70
1.23
0.412
0.137
0.0457
0.0152
0.00508
0.00169
DMSO
90Kda
50Kda
30Kda
15Kda
AC-H3K9
Gel 82
70Kda
38Kda
25Kda
H3
Gel 84
AC-H3K9
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AC-H3K9
Gel 85
H3

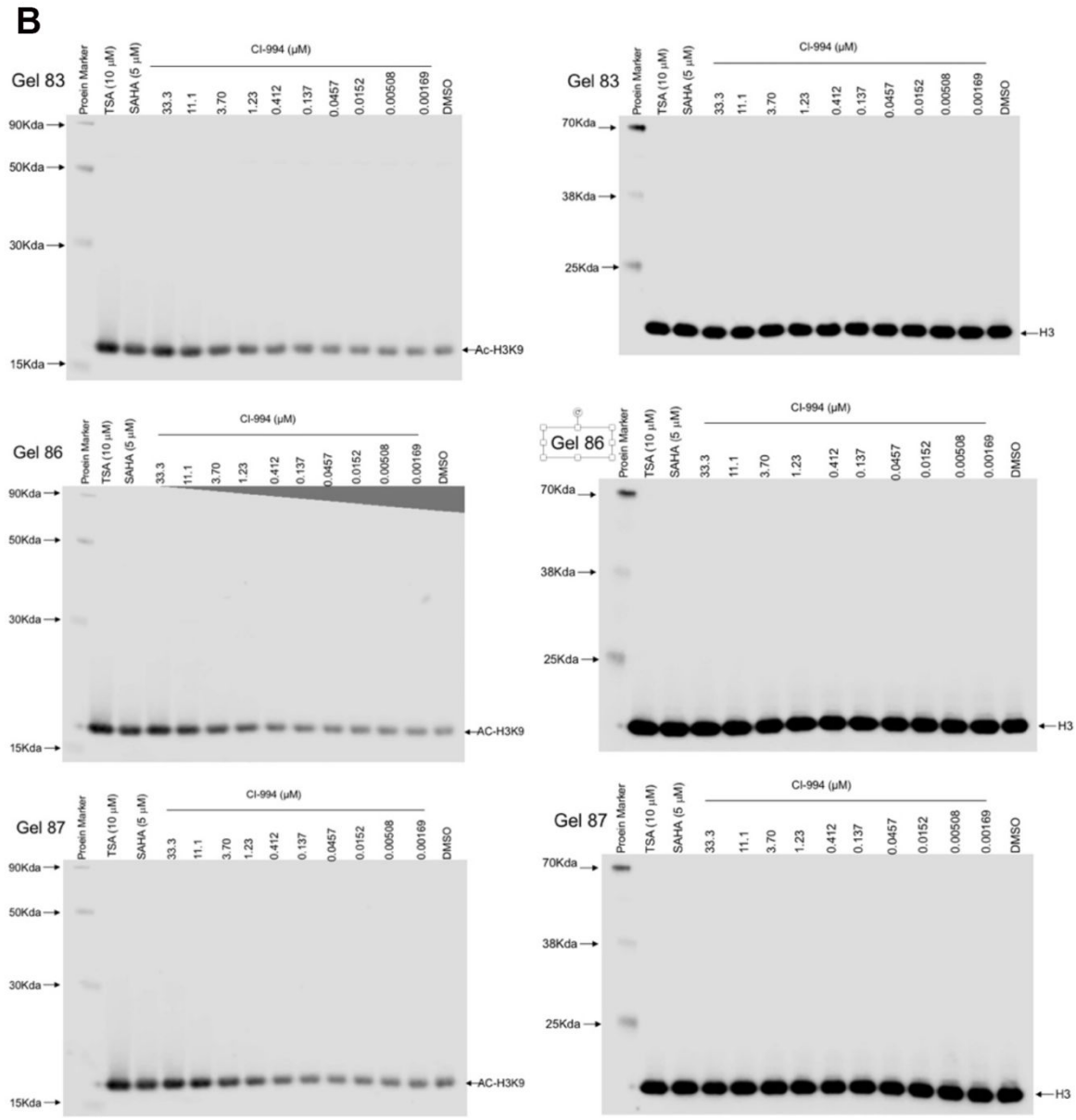

Fig. S1. Full Western Blots of Acetylation at Histone 3, position Lysine 9 for Rodin-A and CI-994. Dose-response effects of (**A**) Rodin-A and (**B**) CI-994 on acetylation at position H3K9. Bands are scanned and normalized as described in methods.

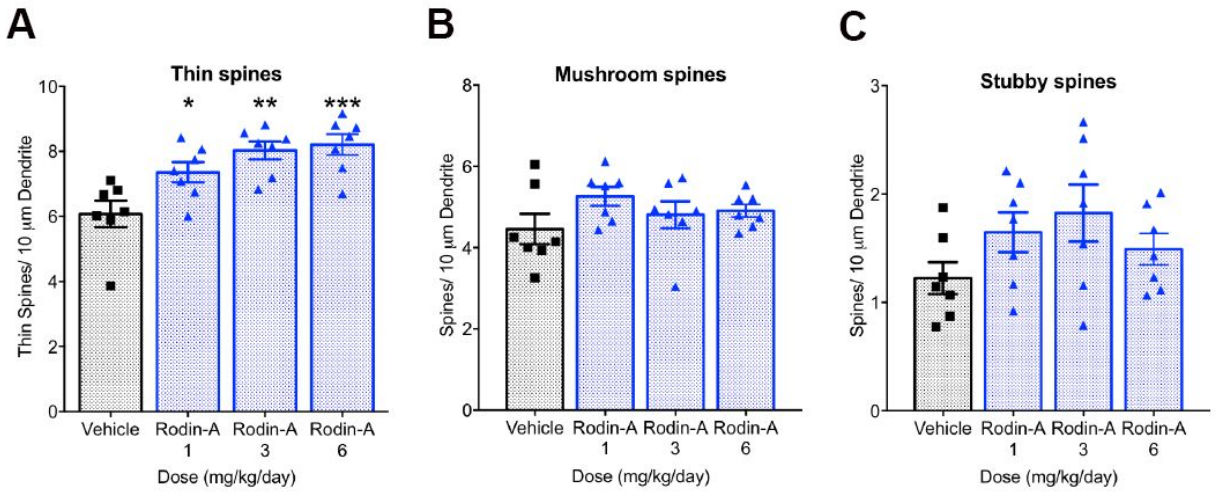

Fig. S2. Hippocampal dendritic spine density following 14 days of dosing with Rodin-A. Quantification of density of thin **(A)**, mushroom **(B),** and stubby (**C**) spines following 14 days treatment with vehicle or Rodin-A (n=7 animals each group). Significant increase in thin spines with all tested doses of Rodin-A (mean ± SEM, One-way ANOVA and Dunnett's post-hoc analysis. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$).