**Supplement Inventory:**

Supplementary experimental procedures: Describes the experimental procedure used in Supplement Figures S1.

**Supplement Figures:**

Supplement Figure 1: Shows the expression levels of SIRT2 isoforms in cortical tissues of R6/2 and knock-in HD mice. The data validates the presence of sirtuin 2 as a molecular target for the inhibitor AK-7 in the HD mouse models used in the study (Figures 1-3).

Supplement Figure 2: Describes the organic synthesis scheme and analysis of the synthesized brain-permeable SIRT2 inhibitor AK-7 (Figures 1-3).

## Extended experimental procedure

### Supplementary experimental procedure for Figure S1:

### Protein extraction from tissues

Frozen cortical tissues from mice were homogenized in PBS containing Complete EDTA-free Protease Inhibitor Cocktail (Roche Applied Science, USA) and 1 mM PMSF (phenylmethanesulfonylfluoride), using a Kontes Pellet Pestle (Kimble/Kontes, USA). They were then sonicated with a Branson Sonifier (Branson Ultrasonic Corp., USA) and lysed overnight at 4 ºC in 3X volume of 63 mM Tris buffer pH 6.8, 2% SDS, 10% glycerol, 1 mM DTT, Complete EDTA-free Protease Inhibitor Cocktail and 1 mM PMSF.

Protein concentration in the lysates was determined with the BCA protein assay kit (Pierce, Thermo Scientific, USA) and the appropriate volume of each sample was diluted in PBS and 3X SDS sample buffer (New England BioLabs, USA) for SDS-PAGE analysis.

### Proteins SDS-PAGE and immunoblotting

Protein lysates, containing SDS sample buffer, were boiled at 100 ºC for 2 min and then subjected to SDS-PAGE. Proteins were transferred onto a 0.2 μm Immobilon-P membrane (Millipore, USA) and then blocked with a 5% milk solution in PBS-Tween for 1 h.

Blots were probed overnight at 4 ºC with primary antibodies against the proteins of choice in 5% milk solution in PBST. Antibodies against actin (A2066), α-tubulin (T6074), acetylated α-tubulin (T6793) and SIRT2 (S8447) were purchased from Sigma (Sigma-Aldrich, USA). The rabbit polyclonal SIRT2 antibody (S8447) has been previously characterized (Maxwell et al, HMG, 2011) and validated in SIRT2 KO mouse strains (Beirowski et al, PNAS, 2011; Bobrowska et, PloS One, 2012). SIRT2.1 and SIRT2.2 isoforms are typically visualized as a double band on western blot, where slow migrated species likely represent a phosphorylated and less active protein (Pandithage et al, J. Cell Biology, 2008). SIRT2.3 isoform is detected in adult brain and

accumulated in aging CNS (Maxwell et al, 2011), but could also represent a cleavage fragment of SIRT2.2.

The antibody against GADPH (glyceraldehyde 3-phosphate dehydrogenase) (MAB374) was purchased from Millipore (Millipore, USA). The antibody against HDAC6 (NB100-91805) was purchased from Novus Biologicals (Novus Biologicals, USA). Antibodies were used at concentrations recommended by their manufacturers.

Secondary detection was performed incubating the blots with HRP-conjugated secondary antibodies in 3% milk solution in PBST for 2 h at room temperature. Proteins were visualized using an ECL detection substrate (Pierce, Thermo Scientific, USA).

**Supplemental References**

Pandithage, R., Lilischkis, R., Harting, K., Wolf, A., Jedamzik, B., Lüscher-Firzlaff, J., Vervoorts, J., Lasonder, E., Kremmer. E., Knöll. B., Lüscher, B. (2008) The regulation of SIRT2 function by cyclin-dependent kinases affects cell motility. J Cell Biol. *180*, 915-29.

**Supplementary Figure titles and legends:**

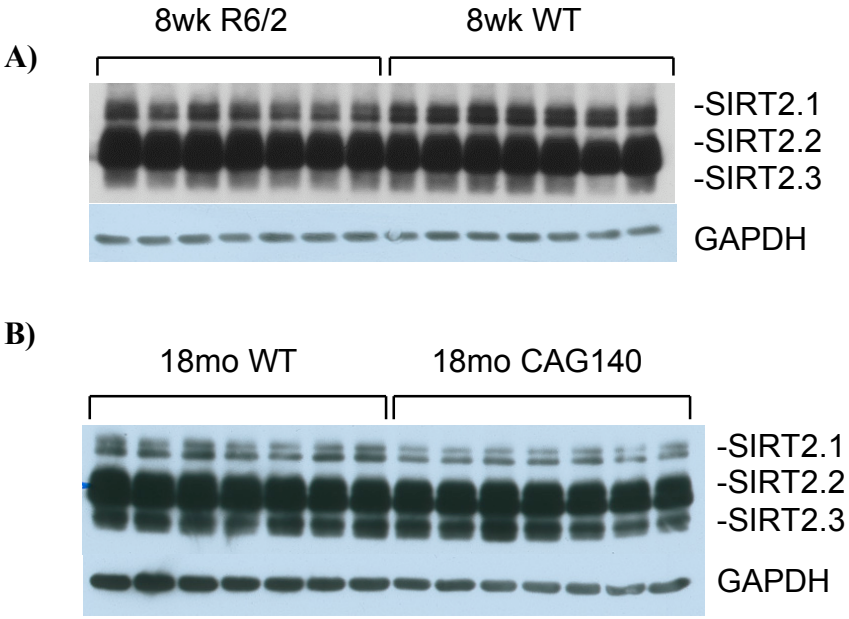
**Figure S1**: **Levels of SIRT2 in brain tissues of R6/2 and knock-in HD mice targeted by SIRT2 inhibitor AK-7 (associated with Figures 1-3)**. **A)** Levels of SIRT2 and GAPDH proteins in cortices of age-matched wild type and R6/2 transgenic HD mice at 8 weeks of age as detected by western blots. **B)** Levels of SIRT2 and GAPDH proteins in cortices of age-matched wild-type and CAG-140 knock-in HD mice at 18 months of age as detected by Western blots. Quantification of SIRT2 isoforms (not shown) shows no dramatic difference in SIRT2 expression between HD and wild-type animals with exception of SIRT2.1, which levels were slightly reduced in R6/2 mice. There were no progressive disease changes detected in either SIRT2 isoform levels in R6/2 or 140CAG mice as they age. SIRT2.1 and SIRT2.2 isoforms, visualized

as double bands by immunostaining, and SIRT2.3 isoform are marked -SIRT2.1, -SIRT2.2, and -SIRT2.3 respectively. The expression pattern of the SIRT2 isoforms in striata of wild-type, R6/2, and 140CAG knock-in mice was identical to that in cortices and therefore not shown.

**Figure S2: Synthesis, purification and analysis of compound AK-7 (*3-(azepan-1-ylsulfonyl)-N-(3-bromophenyl) benzamide*) (associated with Figures 1-3). A)** To a solution of 3-(chlorosulfonyl)benzoic acid (**1**, 1.0 equiv, ca. 0.1 *M)* in dichloromethane, was slowly added hexamethyleneimine (3.1 equiv) at 0 °C with stirring. The mixture was stirred at room temp for 12 h. The solvent was removed by a stream of nitrogen gas, and 1 *M* aqueous NaOH (> 10 equiv) was added to the mixture, which was extracted with ether three times. The aqueous fraction was then acidified with 3 *M* aqueous HCl to ~pH 1. The mixture turned cloudy during the addition. The resulting precipitate was collected by suction filtration on a Buchner funnel, washed with distilled water, and dried overnight at reduced pressure to give 3-(azepan-1-ylsulfonyl)benzoic acid (**2**, 50-60%), which was used without further purification. To a solution of **2** (1.0 equiv, ca. 0.1 *M*), 3-bromoaniline (1.1 equiv), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 1.3 equiv), and 4-dimethylaminopyridine (DMAP, 0.1 equiv) were added. The mixture was stirred at room temp for 12 h, and then diluted with excess EtOAc. The organic mixture was washed with 1 *M* aqueous HCl (2 times), 1 *M* aqueous $NaHCO_3$ (2 times), and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to yield a crude product, which was purified by silica gel column chromatography to give a white solid (AK-7, 60-70 %); mp 157 °C; **B)** $^{1}$H NMR (500 MHz, $CDCl_3$) δ 8.22 (t, *J* = 1.6 Hz, 1H), 8.12-8.08 (m, 2H), 7.97 (t, *J* = 1.8 Hz, 1H), 7.95 (dt, *J* = 7.7, 1.3 Hz, 1H), 7.66 (t, *J* = 7.8 Hz, 1H), 7.58 (d, *J* = 8.1 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.26 (t, *J* = 8.0 Hz, 1H), 3.30 (t, *J* = 5.9 Hz, 4H), 1.72 (br s, 4H), 1.62-1.56 (m, 4H). **C)** $^{13}$C NMR (125.8 MHz, $CDCl_3$) δ 164.26, 140.30, 138.79, 135.66, 131.41, 130.41, 129.98, 129.91, 127.96, 124.95, 123.33, 122.76, 118.81, 48.34, 29.11, 26.83. **D)** HRMS (ESI) *m/z*

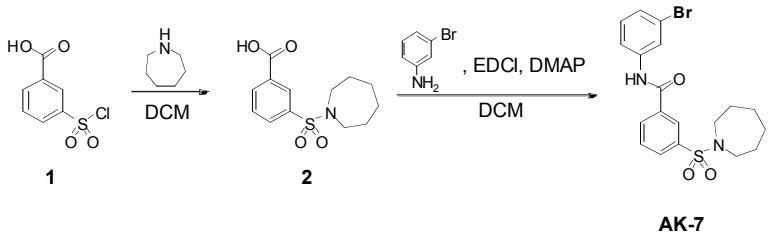
$[M+H]^+$ calcd for $C_{19}H_{22}BrN_2O_3S$ 437.0529, 439.0510, found 437.0540, 439.0525. Anal. ($C_{19}H_{22}BrN_2O_3S$) calcd C 52.18, H 4.84, N 6.41, found C 52.28, H 4.92, N 6.33.

**Supplemental Figure S1**



**Supplemental Figure S2**

**A)**

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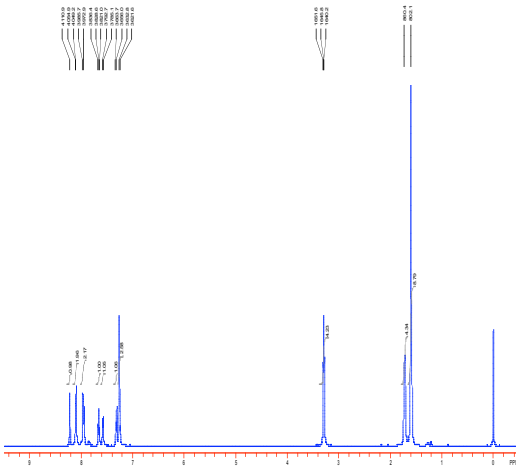

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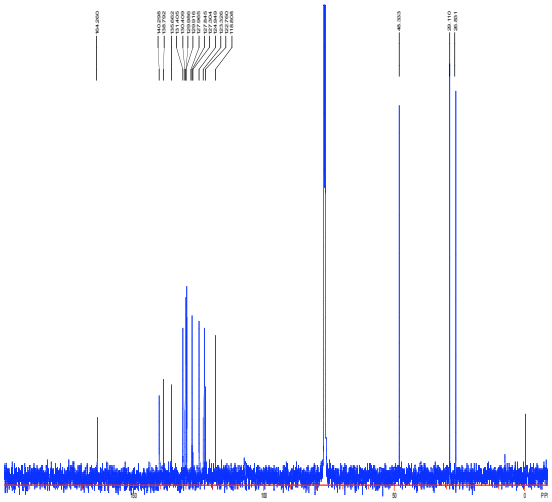

**D)**