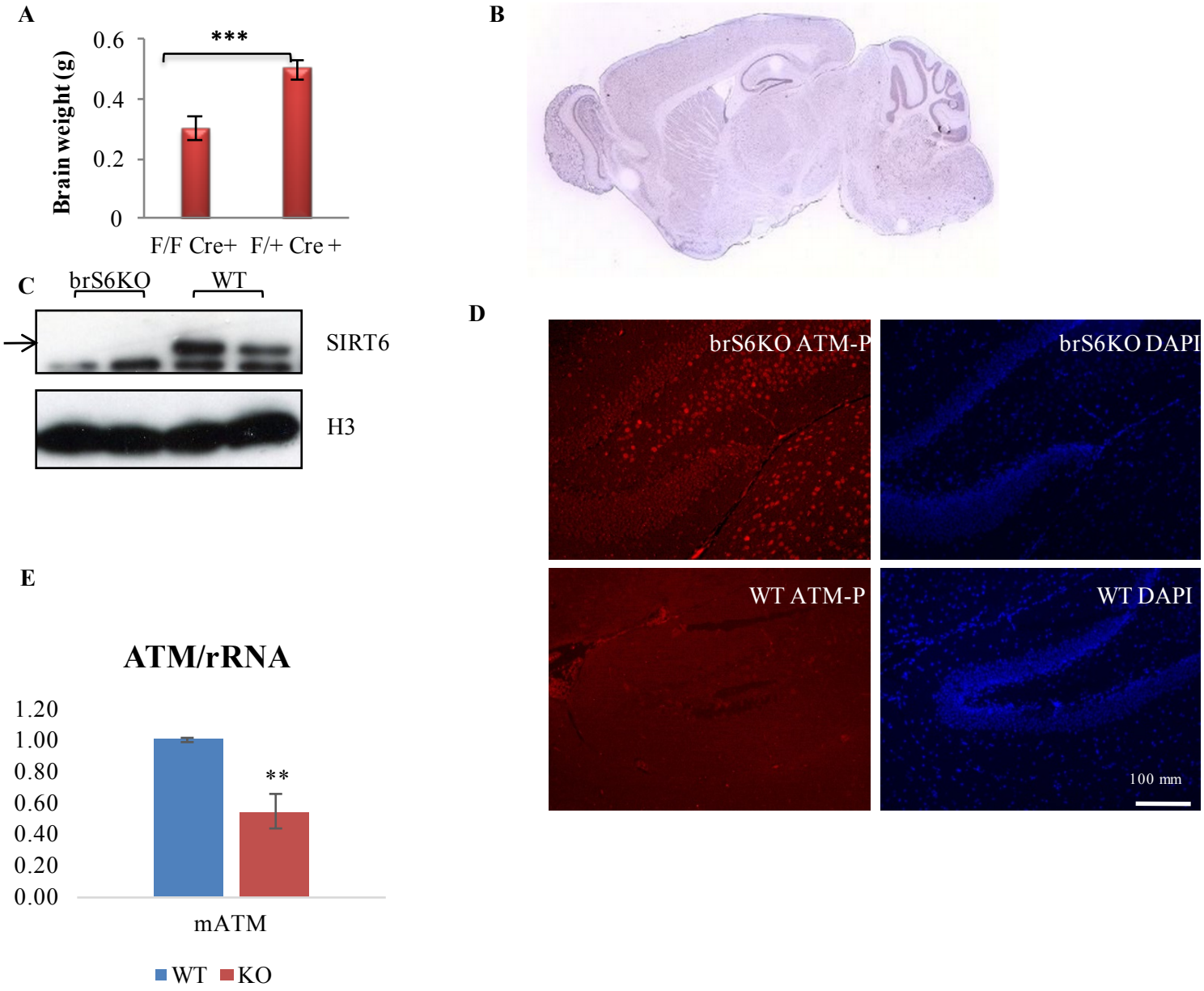
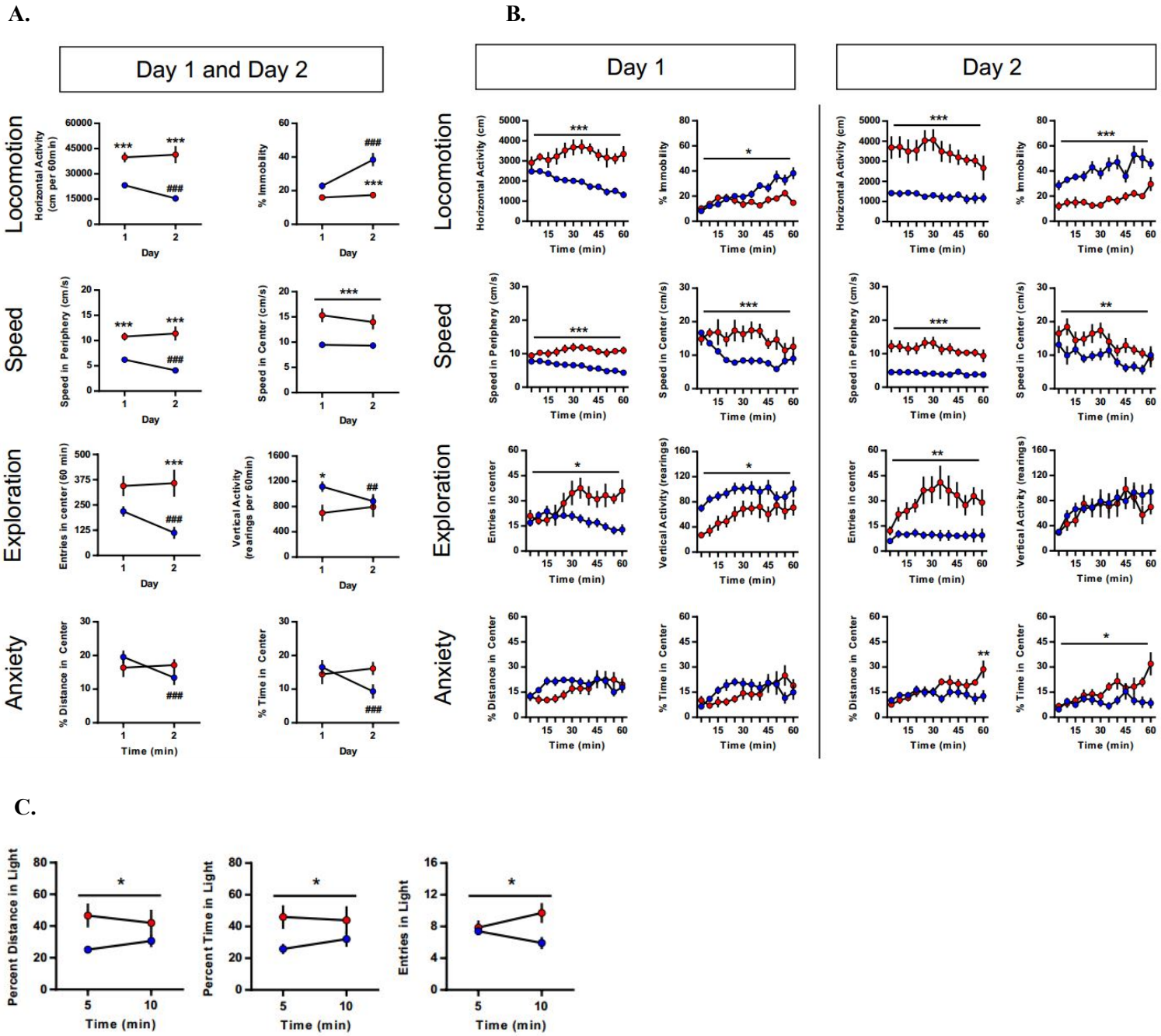
Supplemental Figures and Tables:

Supplementary Figure 1.



Supplementary Figure 1. Related to Figure 1
A. Brain weight in grams of brS6KO mice Het (F/+) or KO (F/F) mice. Mice were 3-5 months old (Het n=12, KO n=7) **B.** SIRT6 mRNA expression by in situ hybridization from ALLEN brain atlas. **C.** Protein blot showing SIRT6 deletion in the brS6KO mice. **D.** Immunofluorescence of brain sections of 4-month-old brS6KO or WT mice (WT n=4 ,KO n=4) with ATM-p antibody, **E.** qPCR of ATM in WT (n=4) and brS6KO (n=4) brains.

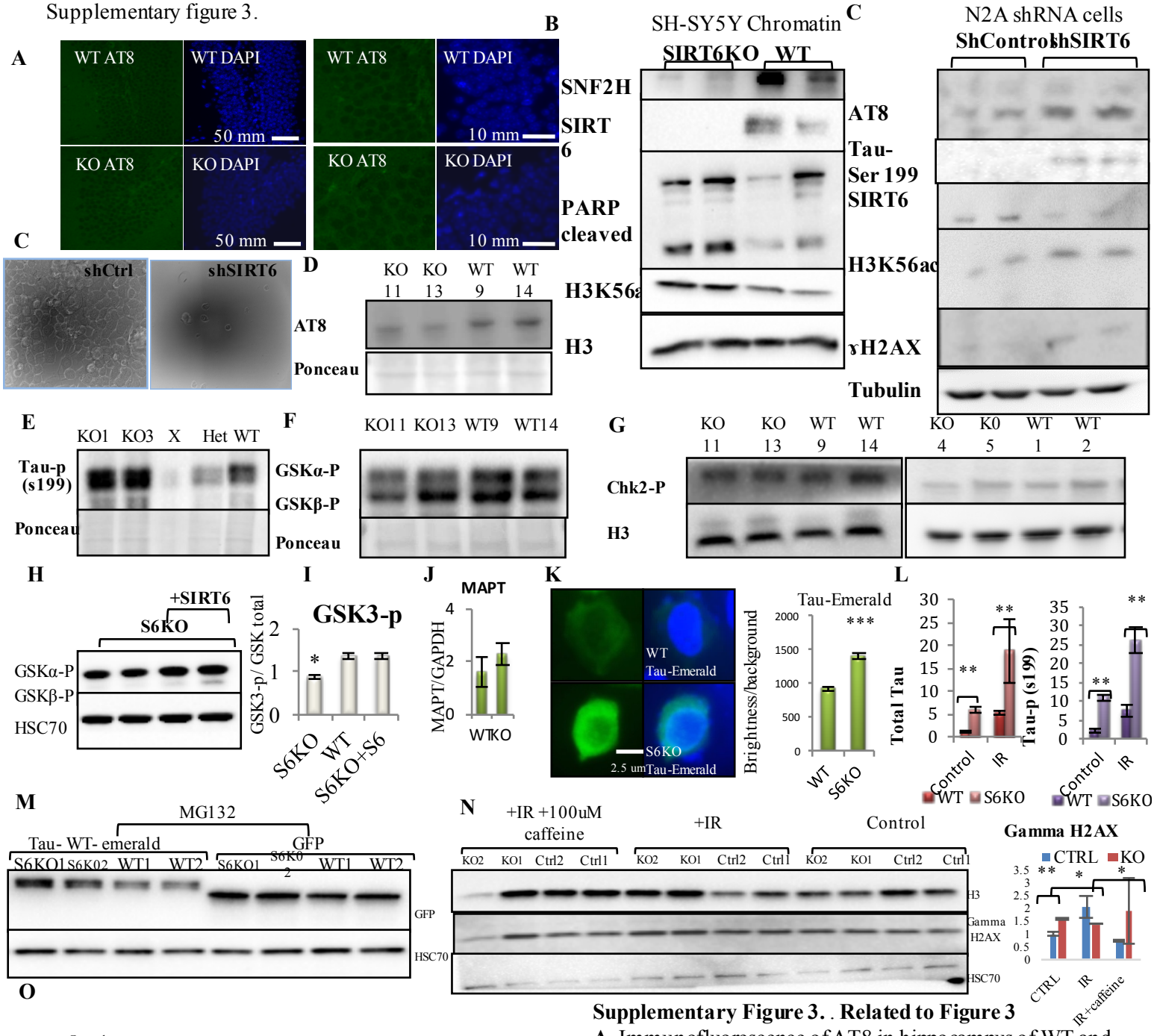
Supplementary Figure 2.



Supplementary Figure 2. Related to Figure 2

A-B. SIRT6 deletion (SIRT6KO) enhances spontaneous locomotor activity over two consecutive days in the open field test (60 min each session). Overall, the speed of brSIRT6KO mice is enhanced in the periphery and in the center of the arena (25% of total surface). This enhancement in horizontal activity results in an increase in the number of visits in the center of the arena at the expense of the number of rearing events. Overall, these data suggest an increase in ambulation rather than exploratory behavior. Finally, brain-specific SIRT6 deletion has a minimal impact on innate anxiety as measured in the open field since the animals spent similar percent of time and distance in the center of the arena on day 1. Note the robust habituation effect on day 2 in control mice that is not observed in brSIRT6KO mice. **C.** SIRT6 deletion (SIRT6KO) increases the percent time, percent distance and number of entries in the light-dark test (10 min session). *$p<0.05$, **$p<0.005$, ***$p<0.0005$.

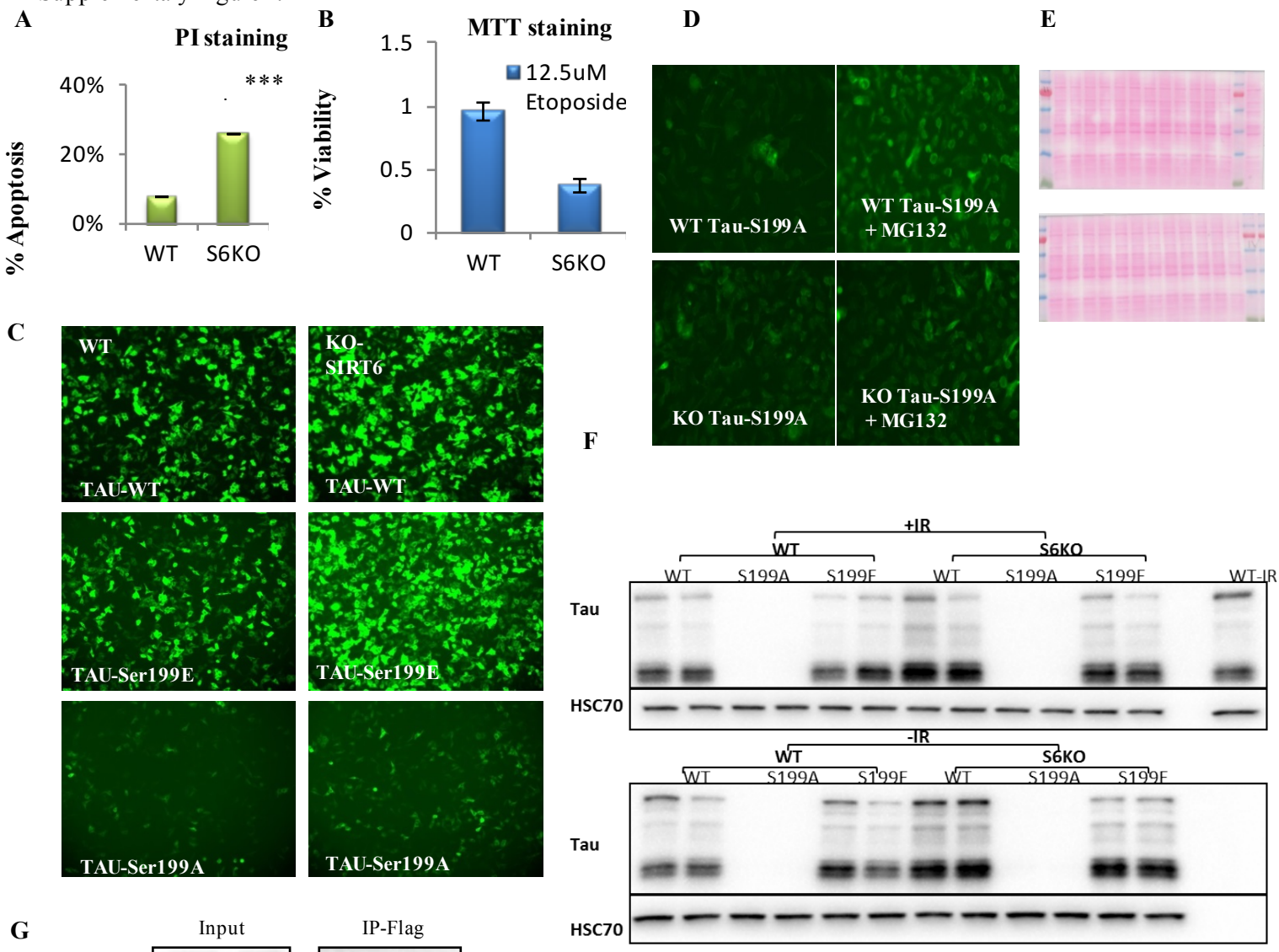
Supplementary figure 3.



Supplementary Figure 3. . Related to Figure 3

A. Immunofluorescence of AT8 in hippocampus of WT and brS6KO mice. **B.** Western blot of protein extracts from SH-SY5Y KO cells or N2a shRNA cells for SIRT6 and control (empty gRNA or shRNA scramble). Microscope image show N2a cells, which were not divided and had impaired morphology **D, E.** Another set of animals showing Tau-p in mouse brains. With two antibodies AT8 and Ser199 **F, G.** GSK3α/β and Chk2 activation in mouse brains. **H-I.** Rescue of GSK3 inhibition by transfectin SIRT6 WT protein. **J.** qPCR for Tau (MAPT gene) in mouse brains (n=3 WT and 3 KO). **K.** Microscope image of SHSY-5Y WT and KO cells transfected with Tau-emerald (n=75 each). **L.** quantification of western presented in figure 3D **M.** SHSY-5Y cells were treated with MG132 for 12hours and protein levels were measured in cells transfected with Tau-Emerald or GFP by GFP antibody. **N.** Protein blot for H2AX phosphorylated to measure ATM inhibition with caffeine. **O.** Protein blot from figure 3, showing most of the samples in same gel, to denote the changes with and without IR.

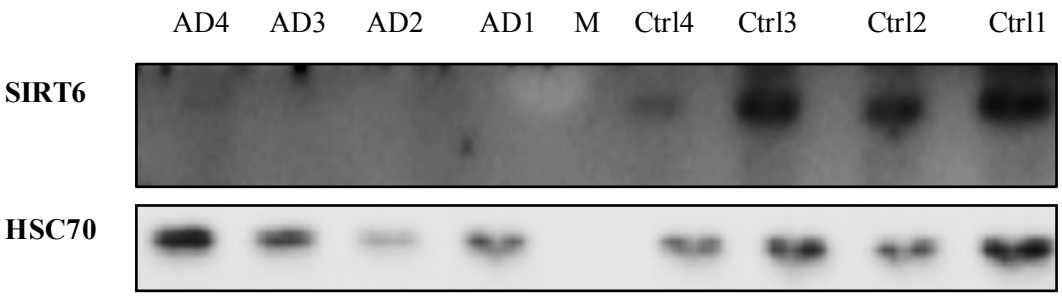
Supplementary Figure 4.



Supplementary Figure. Related to Figure 4

A. Apoptosis measured by Propidium Iodide staining (PI), measured by FACS **B.** MTT assay to measure cell viability. **C.** Microscope image of SHSY-5Y WT and KO cells transfected with Tau-emerald WT or Ser-199 Alanine or Glutamic acid mutants. **D** Microscope image of SHSY-5Y WT and KO cells transfected with Tau-emerald Ser 199 Alanine and treated with MG132 for 4hrs. **E-F** Protein blots for SHSY-5Y WT and KO cells transfected with Tau-emerald Ser 199 Alanine or Glutamic acid with and without IR. Ponceau is shown to confirm protein were loaded equally, in addition to HSC70. For comparison between gels first sample of the second gel appears in the first gel. **G**. Immunoprecipitation of Flag-SIRT6 showing GSK3 interaction. **H**. SHSY-5Y WT and KO cells +/- IR were probed for Tau280Ac, and quantified by normalizing to total Tau.

Supplementary figure 5.



Supplementary Figure 5. Related to Figure 5
Protein blot of SIRT6 in AD and non-demented controls.

Table 1
Related to Fig. 2 and Sup Fig. 2

Related to supplementary Methods		Two-way ANOVA Repeated Measure over time (Post-hoc: Bonferroni)					
Statistical analysis.		Interaction		Time		Genotype	
		F	p	F	p	F	p
Fig. 2A	Horizontal activity	F (1, 17) = 10.63	P = 0.0046	F (1, 17) = 4.654	P = 0.0456	F (1, 17) = 37.53	P < 0.0001
Fig. 2B	Vertical activity	F (1, 17) = 10.58	P = 0.0047	F (1, 17) = 1.757	P = 0.2025	F (1, 17) = 2.397	P = 0.1400
Fig. 2C	% Immobility	F (1, 17) = 9.449	P = 0.0069	F (1, 17) = 13.47	P = 0.0019	F (1, 17) = 14.28	P = 0.0015
Fig. 2D	% Time center	F (1, 17) = 14.37	P = 0.0015	F (1, 17) = 5.455	P = 0.0320	F (1, 17) = 0.5466	P = 0.4698
Fig. 2G	% Freezing	F (2, 34) = 15.88	P < 0.0001	F (2, 34) = 29.43	P < 0.0001	F (1, 17) = 17.21	P = 0.0007
Fig. S2A	Horizontal activity	F (1, 17) = 10.63	P = 0.0046	F (1, 17) = 4.654	P = 0.0456	F (1, 17) = 37.53	P < 0.0001
Fig. S2A	% Immobility	F (1, 17) = 9.449	P = 0.0069	F (1, 17) = 13.47	P = 0.0019	F (1, 17) = 14.28	P = 0.0015
Fig. S2A	Speed in periphery	F (1, 17) = 12.40	P = 0.0026	F (1, 17) = 3.742	P = 0.0699	F (1, 17) = 37.77	P < 0.0001
Fig. S2A	Speed in center	F (1, 17) = 0.6121	P = 0.4447	F (1, 17) = 1.006	P = 0.3298	F (1, 17) = 22.52	P = 0.0002
Fig. S2A	Entries in Center	F (1, 17) = 10.14	P = 0.0054	F (1, 17) = 6.030	P = 0.0251	F (1, 17) = 11.74	P = 0.0032
Fig. S2A	Vertical activity	F (1, 17) = 10.58	P = 0.0047	F (1, 17) = 1.757	P = 0.2025	F (1, 17) = 2.397	P = 0.1400
Fig. S2A	% Distance center	F (1, 17) = 10.74	P = 0.0044	F (1, 17) = 6.616	P = 0.0198	F (1, 17) = 0.008594	P = 0.9272
Fig. S2A	% Time center	F (1, 17) = 14.37	P = 0.0015	F (1, 17) = 5.455	P = 0.0320	F (1, 17) = 0.5466	P = 0.4698
Fig. S2B Day1	Horizontal activity	F (11, 187) = 4.832	P < 0.0001	F (11, 187) = 3.135	P = 0.0007	F (1, 17) = 36.60	P < 0.0001
Fig. S2B Day1	% Immobility	F (11, 187) = 6.119	P < 0.0001	F (11, 187) = 9.943	P < 0.0001	F (1, 17) = 5.576	P = 0.0304
Fig. S2B Day1	Speed in periphery	F (11, 187) = 4.564	P < 0.0001	F (11, 187) = 2.700	P = 0.0030	F (1, 17) = 36.94	P < 0.0001
Fig. S2B Day1	Speed in center	F (11, 187) = 2.710	P = 0.0029	F (11, 187) = 3.401	P = 0.0003	F (1, 17) = 20.04	P = 0.0003
Fig. S2B Day1	Entries in Center	F (11, 187) = 6.861	P < 0.0001	F (11, 187) = 2.723	P = 0.0028	F (1, 17) = 6.269	P = 0.0228
Fig. S2B Day1	Vertical activity	F (11, 187) = 1.293	P = 0.2313	F (11, 187) = 5.792	P < 0.0001	F (1, 17) = 8.110	P = 0.0111
Fig. S2B Day1	% Distance center	F (11, 187) = 3.367	P = 0.0003	F (11, 187) = 4.232	P < 0.0001	F (1, 17) = 0.9125	P = 0.3528
Fig. S2B Day1	% Time center	F (11, 187) = 3.118	P = 0.0007	F (11, 187) = 4.018	P < 0.0001	F (1, 17) = 0.3438	P = 0.5654
Fig. S2B Day2	Horizontal activity	F (11, 187) = 2.079	P = 0.0237	F (11, 187) = 3.528	P = 0.0002	F (1, 17) = 32.53	P < 0.0001
Fig. S2B Day2	% Immobility	F (11, 187) = 1.020	P = 0.4302	F (11, 187) = 3.636	P = 0.0001	F (1, 17) = 15.02	P = 0.0012
Fig. S2B Day2	Speed in periphery	F (11, 187) = 1.972	P = 0.0333	F (11, 187) = 2.828	P = 0.0019	F (1, 17) = 33.27	P < 0.0001
Fig. S2B Day2	Speed in center	F (11, 187) = 0.8199	P = 0.6202	F (11, 187) = 2.653	P = 0.0035	F (1, 17) = 11.76	P = 0.0032
Fig. S2B Day2	Entries in Center	F (11, 187) = 4.100	P < 0.0001	F (11, 187) = 5.435	P < 0.0001	F (1, 17) = 14.87	P = 0.0013
Fig. S2B Day2	Vertical activity	F (11, 187) = 1.339	P = 0.2058	F (11, 187) = 8.538	P < 0.0001	F (1, 17) = 0.2142	P = 0.6494
Fig. S2B Day2	% Distance center	F (11, 187) = 3.924	P < 0.0001	F (11, 187) = 4.182	P < 0.0001	F (1, 17) = 1.393	P = 0.2542
Fig. S2B Day2	% Time center	F (11, 187) = 3.671	P < 0.0001	F (11, 187) = 4.820	P < 0.0001	F (1, 17) = 4.546	P = 0.0479
Fig. S2F-G	% Distance	F (1, 17) = 2.518	P = 0.1310	F (1, 17) = 0.01411	P = 0.9068	F (1, 17) = 6.346	P = 0.0221
Fig. S2F-G	% Time	F (1, 17) = 1.244	P = 0.2801	F (1, 17) = 0.3191	P = 0.5796	F (1, 17) = 4.794	P = 0.0428
Fig. S2F-G	Entries	F (1, 17) = 3.235	P = 0.0899	F (1, 17) = 0.03661	P = 0.8505	F (1, 17) = 8.114	P = 0.0111

Supplemental Experimental Procedures:

Human samples:

Case	Gender	Age	C-Score	B-score	A-score	Braak	Montine
C-01	**male**	**70**	**0**	**0**	**0**	**--**	**not**
C-02	**male**	**72**	**0**	**0**	**0**	**--**	**not**
C-03	**female**	**75**	**0**	**0**	**0**	**--**	**not**
C-04	**female**	**55**	**0**	**0**	**0**	**--**	**not**
AD-01	**male**	**86**	**3**	**3**	**3**	**VI**	**high**
AD-02	**female**	**77**	**3**	**3**	**2**	**VI**	**intermediate**
AD-03	**male**	**76**	**3**	**3**	**3**	**VI**	**high**
AD-04	**female**	**83**	**2**	**2**	**2**	**III**	**intermediate**

C, controls; AD, Alzheimer´s disease; for ABC-Scores see Montine et al., 2012
A-score: Thal phase for Abeta plaques
B-score: Braak and Braak NFT stage
C-score: CERAD neuritic plaque score
Braak: Braak staging

Behavioral Tests: (See statistics in Supplementary table-1)

Mice were housed four per cage in a 12-hour (7:00 A.M. to 7:00P.M.) light/dark colony room at 22-24°C, with ad libitum access to food and water. All animals were handled and experiments were conducted in accordance with procedures approved by the Institutional Animal Care and Use Committee at the Massachusetts General Hospital in accordance with NIH guidelines. Behavioral tasks were performed as previously described by McAvoy et al. (2015) (McAvoy et al., 2015) in the following order: open field test (days 1-2), light-dark choice test (day 2), novel-object recognition test (days 3-4) and contextual fear conditioning test (days 5-7). Prior to each experiment, mice were allowed to habituate in a quiet, darkened room for at least 1 hour.

Open Field: Motor activity was quantified in four Plexiglas open-field boxes (41 x 41cm, Kinder Scientific with infrared photobeams to record x-y-z ambulatory movements) over 60 minutes. After each trial, the whole apparatus was cleaned thoroughly with water followed by ethanol (70%). A software (MotorMonitor, Kinder Scientific) defined grid lines that divided the open field into center (25% of total area) and periphery areas. Overall locomotor activity was quantified as the total distance traveled in centimeters (horizontal activity) and the immobility time percent. The speed (cm/s) was calculated for each area (center and periphery). Exploratory behavior was measured by both the number of entries to the center, and number of rearing events (vertical activity). Finally, anxiety behavior was analyzed by the percent of time spent and percent of distance traveled in the center compartment. This procedure was performed on two consecutive days in order to evaluate behavioral habituation, which manifests as a decrease of exploration of a previously encountered environment.

Light-Dark: The light-dark test was conducted in the open-field chamber at the end of day 2. A dark plastic box that is opaque to visible light but transparent to infra-red covered one-half of the chamber area, thus creating dark and light compartments of an equal size. An opening at floor level in the center of one wall of the dark compartment allowed passage between the light and dark compartments. The light compartment was brightly illuminated (900 lx). The mouse was placed in the dark compartment and allowed to freely explore both compartments for 10 minutes. The percent of distance travelled, percent of time spent, number of entries and the latency to first enter the light compartment were recorded.

Novel Object Recognition: The novel object recognition test was conducted in the same open-field chamber on day 3, to which mice had been extensively exposed during the first two experimental days (i.e. two 60-minute sessions). Mice were given four blocks of two 5-minute trials of exploration of two objects with an inter-trial interval of 5 minutes within each block, and 90 minutes between the blocks. Two different objects were placed in the open field during the sample phase (A-B). The objects were made of interlocked plastic pieces of various shape and color. They were cleansed thoroughly with ethanol (70%) between trials to ensure the absence of olfactory cues. The memory of the original two objects (A-B) was tested 24 hours later by placing the mice back in the open field for a 10-minute session and randomly exchanging one of the familiar objects for a novel one in a counterbalanced manner (A-C). Typically, a strong bias of exploration towards the new object is suggestive of a good retention of the familiar pair of objects. Measurement of direct approaches of each object was based strictly on active exploration, where mice had forelimbs within a circle of 10 cm around an object, head oriented towards it or touching it with their nose. Exploration of the novel object was expressed as a percentage of the total object exploration during the initial sample session (day 3, A-B) and final test session (day 4, A-C). Each session was videotaped and the general locomotor activity was analyzed using View-Point Life Sciences software.

Contextual Fear Conditioning: Contextual fear conditioning was conducted in four fear-conditioning chambers (Coulbourn Habitest) with clear front and back Plexiglass walls, aluminum side walls, and stainless-steel bars as a floor. The chamber was lit from above with a dim light, ventilated with a fan, and encased by a sound-dampening cubicle. Mouse behavior was recorded by digital video cameras mounted above the conditioning chamber. Freezeframe and Freezeview software (Actimetrics) were used for recording and analyzing freezing behavior, respectively. Between each trial, the whole apparatus was cleaned thoroughly with water followed by ethanol (70%). The contextual fear conditioning protocol consisted of a single 2-second footshock of 0.7 mA, 180 seconds after placement of the mouse in the training context. The mouse was taken out 20 seconds after termination of the footshock and returned to its home cage. Freezing levels were quantified over the initial 180 seconds prior to the shock. This protocol was repeated on three consecutive days.

TUNEL Staining and Analysis: TUNEL Label Mix (Cat. No. 11767291910) and TUNEL Enzyme (Cat. No. 11767305001) from Roche were used in brain slices staining. Briefly: Slides were deparafinized 10 minutes with Xylene and rehydrated gradually with EtOH 100% 5'x2, 70% 5', 50% 5', 30% 5'. The brain slides were then washed with DDW 5'x3. Slides were permeabilized with Citrate buffer and heat. After permeabilization, the protocol from Roche was followed.

Over 10 pictures from each brain cortex were taken, to account for possible variability within the tissue. Each slide was analyzed for TUNEL (green staining) to co-localize with DAPI staining, preventing false positive results. The average for the 10-15 pictures from each mouse's brain cortex was calculated, and this was the value of the sample in the following Student's t-test. P-value for TUNEL: $p=0.0024$.

Antibody List:

Antibody	Company	Catalog number	Dilution
SIRT6	abcam	Ab88494	1:1000
ɣH2AX	abcam	Ab2893	1:1000
H3	Santa Cruz Biotechnology	Sc-10809	1:3000
H3k56 acetylated	abcam	Ab76307	1:2000
Tau	abcam	Ab32057	1:1000
Tau phosphorylated (s199)	abcam	Ab4749	1:1000
PHF-Tau (AT8)	Thermo	MN1020	1:1000
β Tubulin	Hybridoma bank	E7-s	3:1000
SNF2H	Novus Biologicals	NB100-55310	1:1000
Chek2	Millipore	05-649	1:1000
PARP cleaved	Cell Signaling	9542s	1:1000
GSK- α/β- phosphorylated	Cell Signaling	9396	1:1000
GSK- α/β	Cell Signaling	9396	1:1000
vinculin	abcam	Ab129002	1:1000
ATM- phosphorylated	Millipore	05-740	1:1000
Goat anti rabbit IgG H&L (HRP)	abcam	Ab6721	1:10,000

Rabbit anti-mouse IgG H&L (HRP)	abcam	Ab97046	1:10,000
Alexa Fluor 647-conjugated donkey anti-mouse	Jackson ImmunoResearch	715-605-150	1:200
Alexa Fluor 488-conjugated donkey anti-rabbit	Jackson ImmunoResearch	711-545-152	1:200
Alexa Fluor 594-conjugated donkey anti-rabbit	Jackson ImmunoResearch	711-585-152	1:200
Alexa Fluor 647-conjugated donkey anti-rabbit	Jackson ImmunoResearch	711-605-152	1:200
Alexa Fluor 488-conjugated donkey anti-mouse	Jackson ImmunoResearch	715-545-150	1:200
HSC 70	Almog diagnostic	Sc-7289	1:1000

Supplemental References:

McAvoy, K., Russo, C., Kim, S., Rankin, G., and Sahay, A. (2015). Fluoxetine induces input-specific hippocampal dendritic spine remodeling along the septotemporal axis in adulthood and middle age. Hippocampus *25*, 1429–46.

Montine, T.J., Phelps, C.H., Beach, T.G., Bigio, E.H., Cairns, N.J., Dickson, D.W., Duyckaerts, C., Frosch, M.P., Masliah, E., Mirra, S.S., et al. (2012). National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol. *123*, 1–11.