Supplementary Information

β2-microglobulin is a systemic pro-aging factor that impairs cognitive function and neurogenesis

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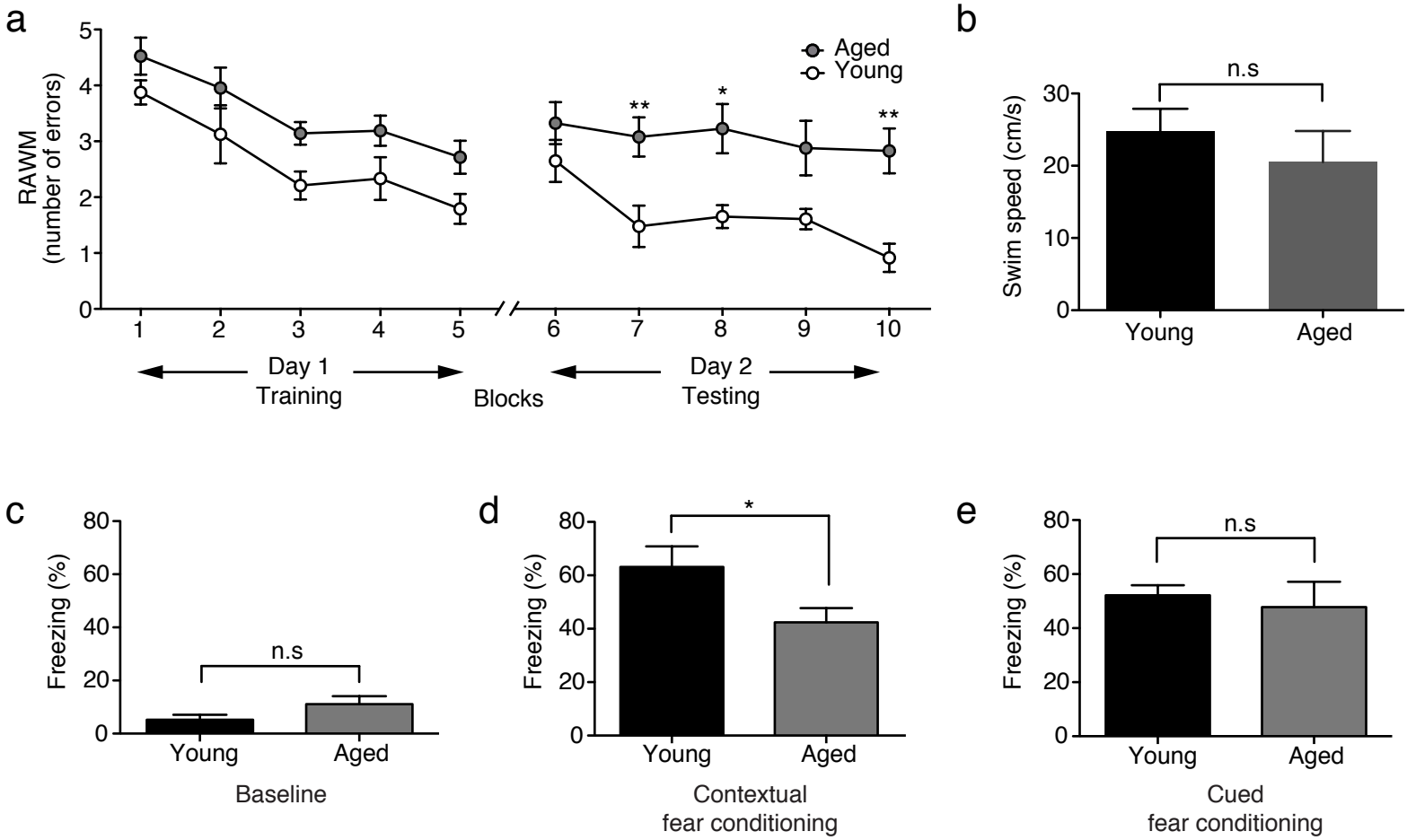
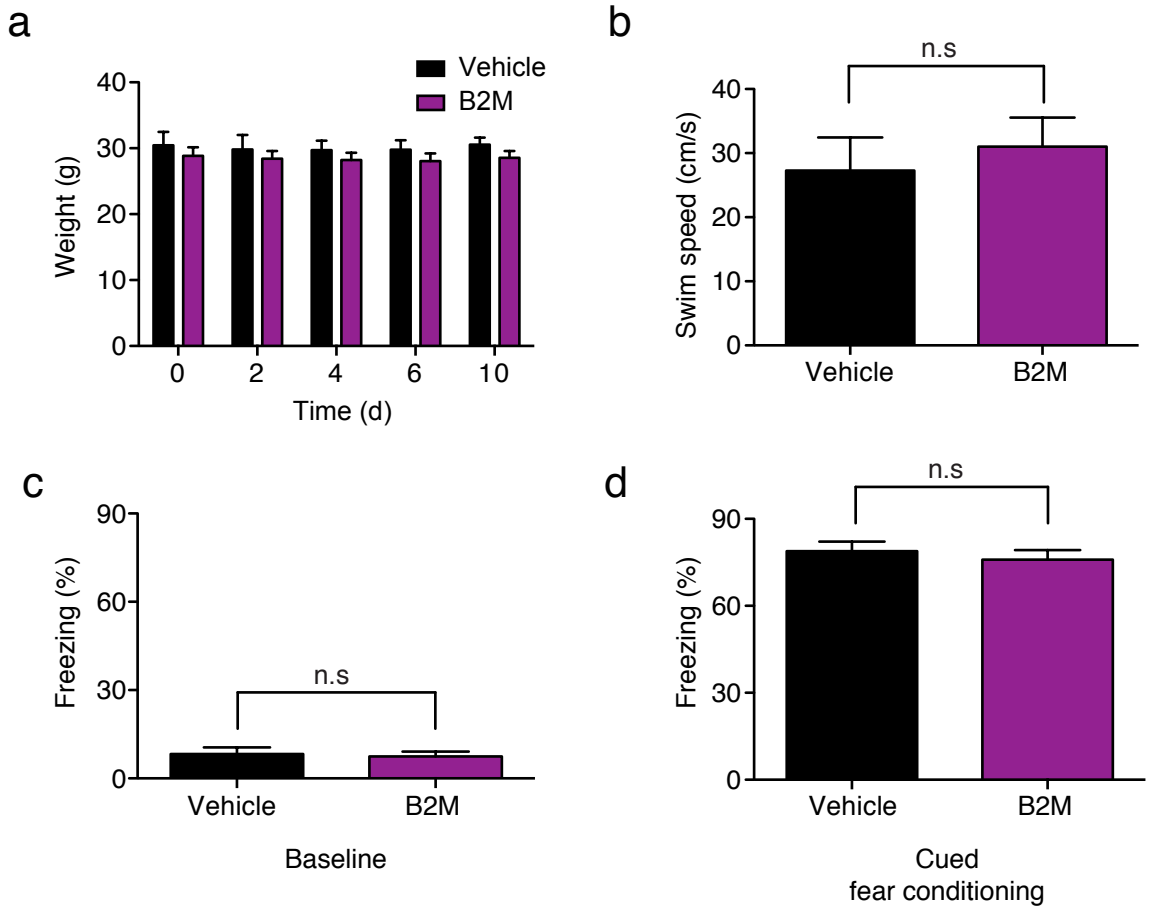
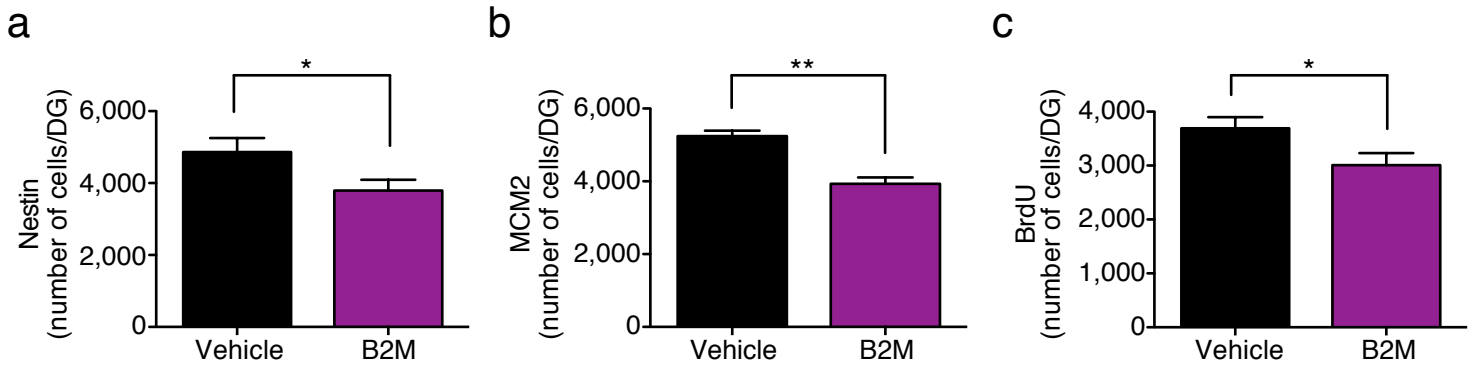


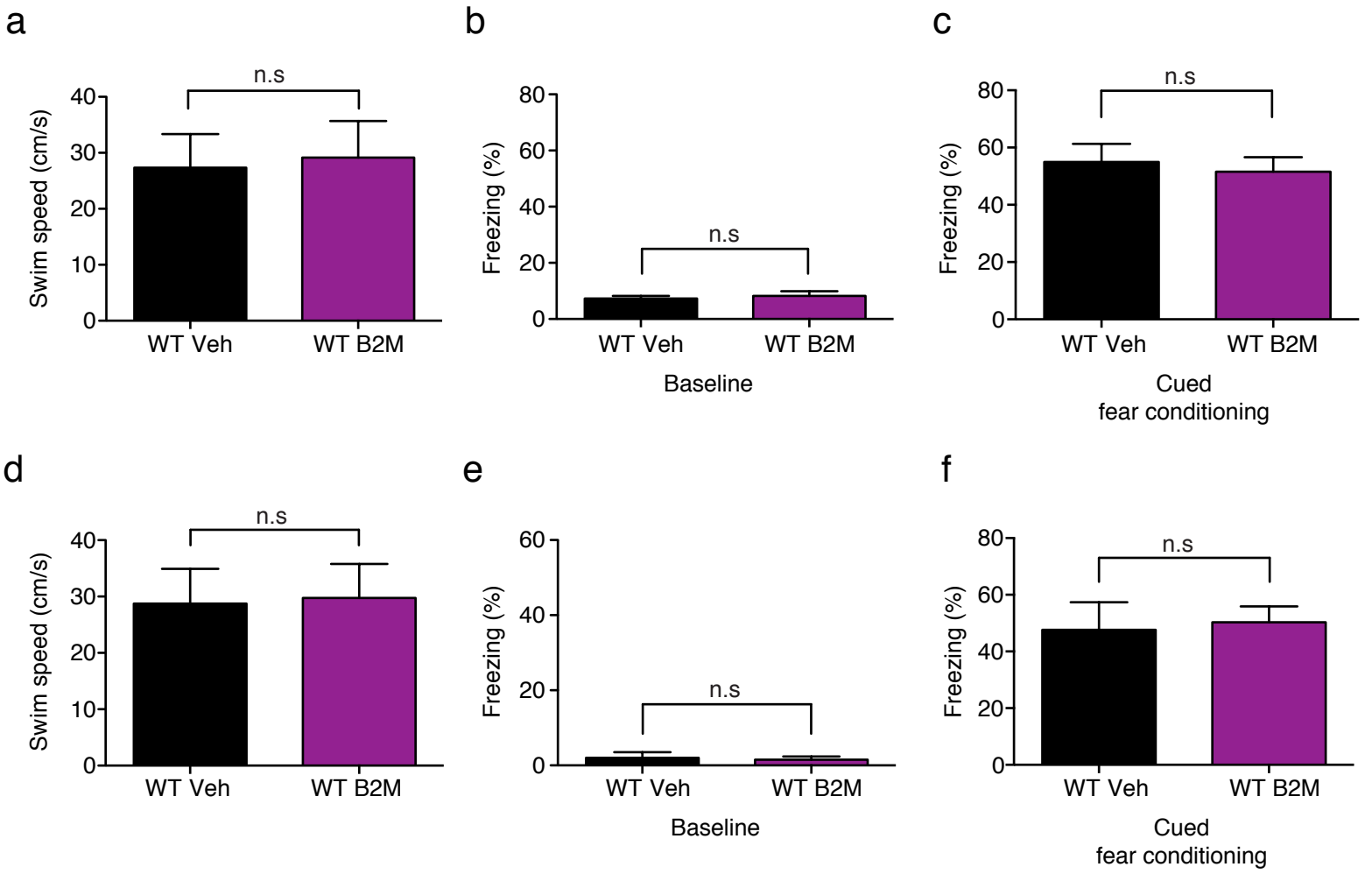
Supplementary Figure 1. Aging characterization of hippocampal dependent learning and memory. **a-e**, Learning and memory was examined during normal aging in young (3 months) versus aged (18 months) animals using RAWM (**a,b**) and contextual fear conditioning (**c-e**) paradigms. **a**, Aged mice demonstrate impaired learning and memory for platform location during the testing phase of the RAWM task. Cognitive deficits were quantified as the number of entry arm errors made prior to finding the target platform. **b**, No differences in swim speeds of were detected between young and aged animals. **c**, Young and aged animals exhibited similar baseline freezing time during fear conditioning training. **d**, During contextual fear conditioning aged mice demonstrate decreased freezing time during contextual memory testing. **e**, No differences in cued memory were detected 24 hours after training. Data from 10 mice per group. Data represented as mean ± SEM; *P<0.05; **P<0.01; n.s., not significant; t-test (**a-c**,**e**), repeated measures ANOVA, Bonferroni post-hoc test (**d**).



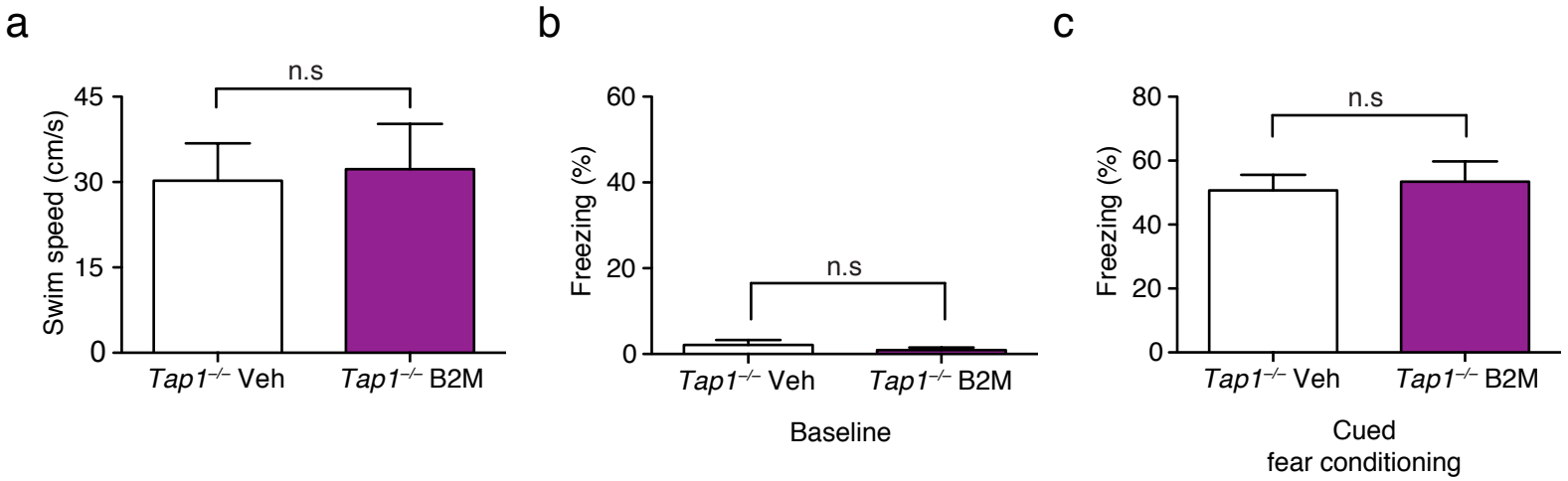
Supplementary Figure 2. Weight, swim speeds and cued memory are not altered by systemic B2M administration. **a-d**, Young adult (3 months) mice were injected intraorbitally with B2M or PBS (vehicle) control five times over 12 days prior to behavioral testing. **a**, Average mouse weight of B2M and vehicle treated groups. **b**, Swim speeds of mice injected with B2M or vehicle during the testing phase of the RAWM. **c,d**, Conditioned fear was displayed as freezing behavior. **c**, Animals from all treatment groups exhibited similar baseline freezing time during training. **d**, No differences in cued memory were detected between groups when re-exposed to the conditioned stimulus (tone and light) in a novel context 24 hours after training. Data from 10 mice per group. All data represented as Mean+SEM; n.s. not significant; t-test.



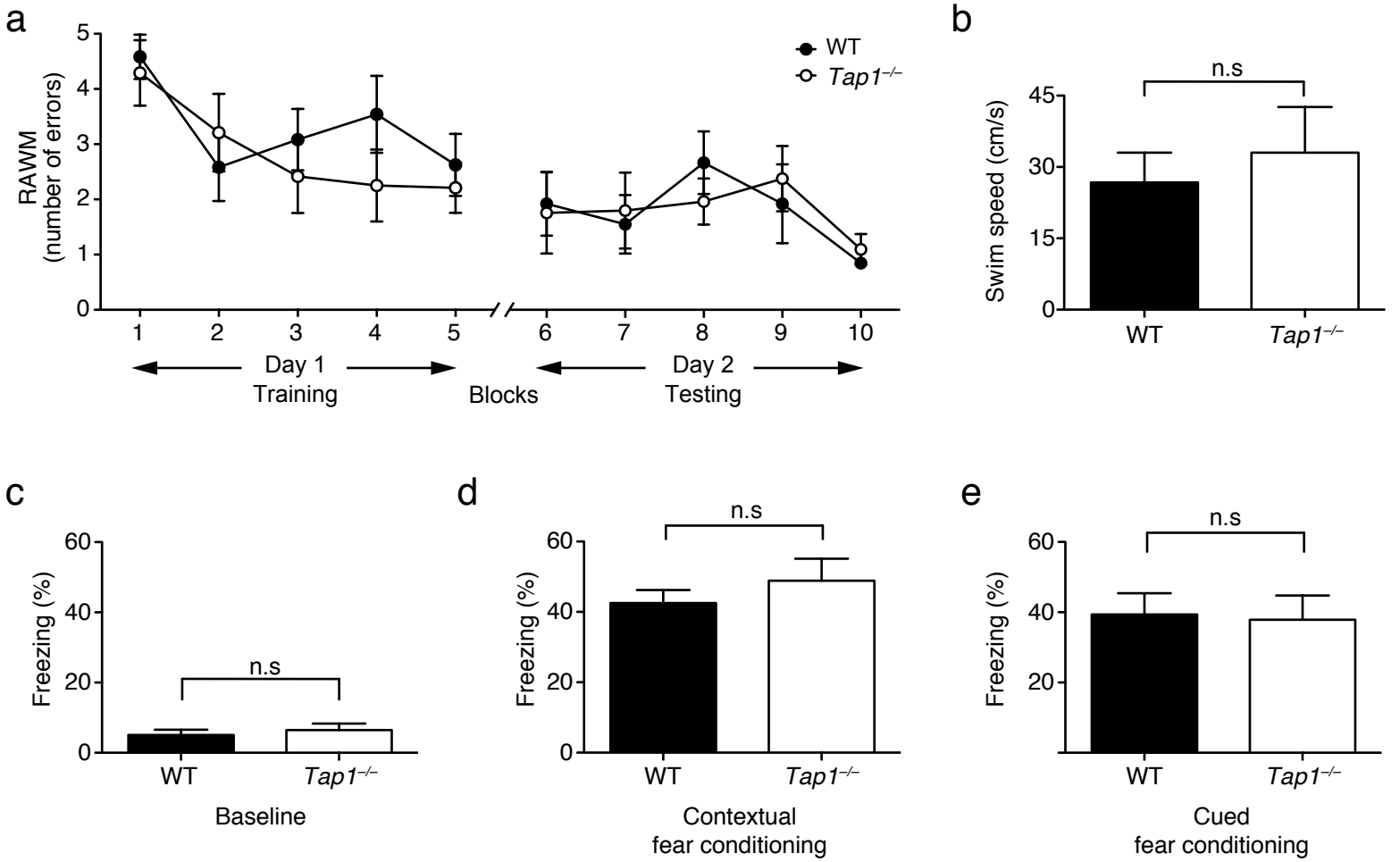
Supplementary Figure 3. Systemic administration of B2M decreases neurogenesis in the DG of young animals. **a,b**, Young adult mice (3 months) were injected with B2M or PBS (vehicle) control through intraorbital injections five times over 12 days. Prior to euthanasia Bromodeoxyuridne (BrdU) was administered by intraperitoneal injections for three days. Quantification of Nestin-positive (**a**), MCM2-positive (**b**), and BrdU-positive (**c**) cells in the dentate gyrus (DG) after treatment. Data from 7 B2M treated and 8 vehicle treated mice. All data represented as Mean+SEM; *P< 0.05; **P<0.01; t-test.



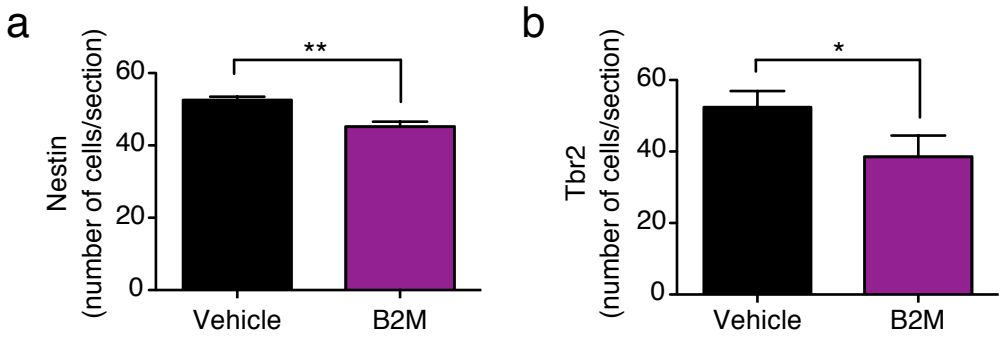
Supplementary Figure 4. Swim speeds and cued memory are not altered by local B2M administration in WT mice. **a-c**, Young adult wild type (WT) mice were given bilateral stereotaxic injections of B2M or PBS (vehicle) control six days (**a-c**) or 30 days (**d-f**) prior to behavioral testing. **a,d**, Swim speeds of mice injected with B2M or vehicle during the testing phase of the RAWM. **b,e**, Animals from all treatment groups exhibited similar baseline freezing time during fear conditioning training. **c,f**, No differences in cued memory were detected between groups when re-exposed to the conditioned stimulus (tone and light) in a novel context 24 hours after training. Data from 10 mice per group. All data represented as Mean+SEM; n.s. not significant; t-test.



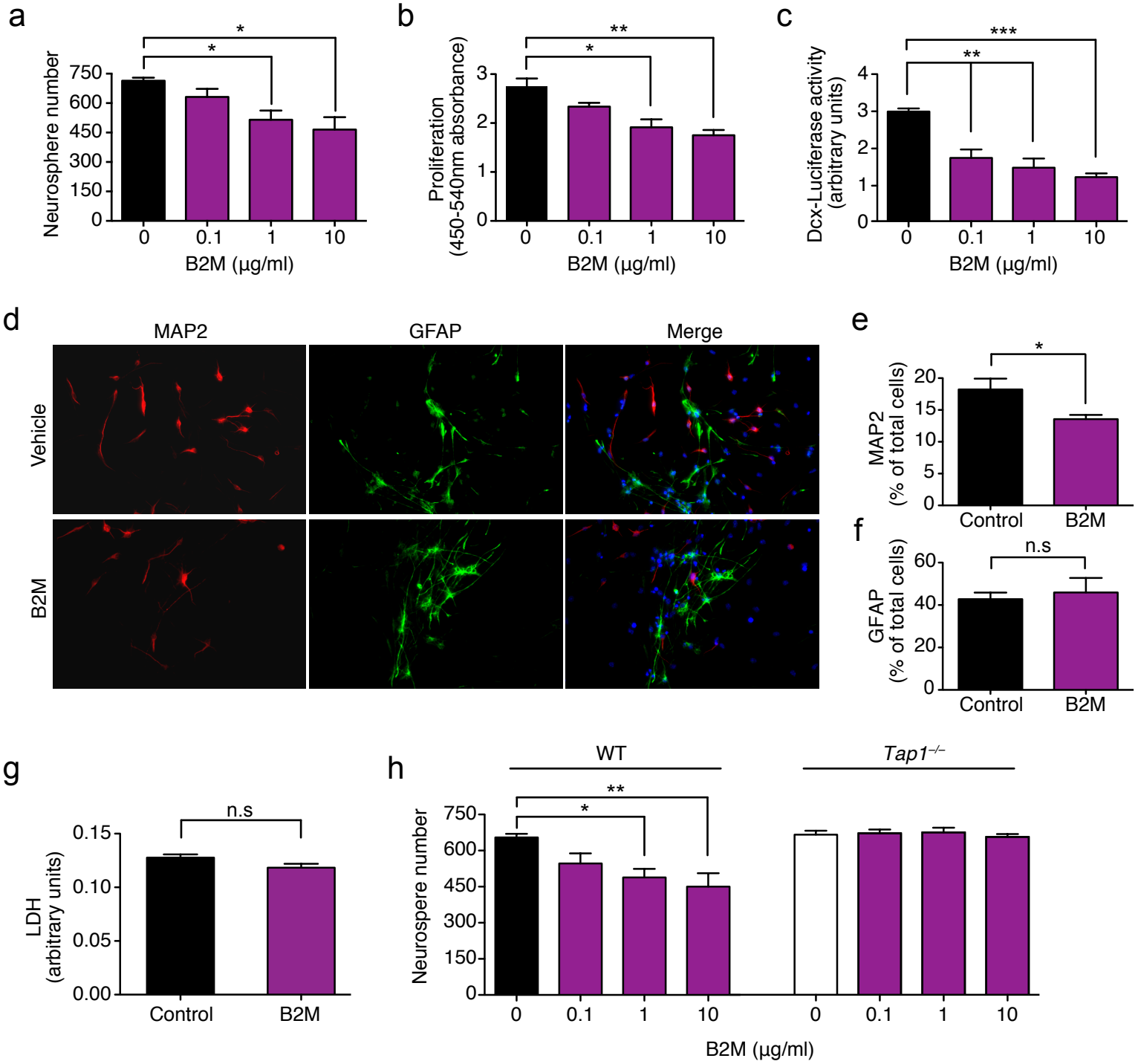
Supplementary Figure 5. Swim speeds and cued memory are not altered by local B2M administration in *Tap1*$^{-/-}$ mice. **a-c**, Young adult *Tap1*$^{-/-}$ mice were given bilateral stereotaxic injections of B2M or PBS (vehicle) control six days (**a-c**) prior to behavioral testing. **a**, Swim speeds of mice injected with B2M or vehicle during the testing phase of the RAWM. **b**, Animals from all treatment groups exhibited similar baseline freezing time during fear conditioning training. **c**, No differences in cued memory were detected between groups when re-exposed to the conditioned stimulus (tone and light) in a novel context 24 hours after training. Data from 10 mice per group. All data represented as Mean+SEM; n.s. not significant; t-test.



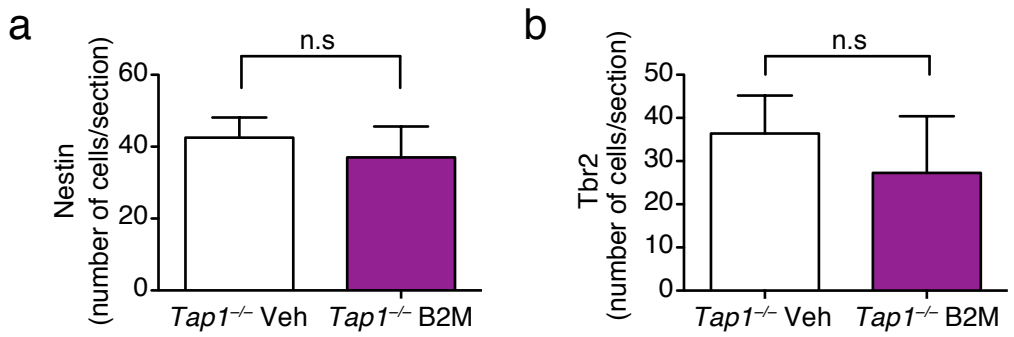
Supplementary Figure 6. No difference in hippocampal dependent learning and memory are observed between WT and *Tap1*$^{-/-}$ mice. **a-e**, Learning and memory was examined in adult wild type (WT) and *Tap1*$^{-/-}$ animals using RAWM (**a,b**) and contextual fear conditioning (**c-e**) paradigms. **a**, No differences in learning and memory for platform location were observed during the testing phase of the RAWM task. Cognitive deficits were quantified as the number of entry arm errors made prior to finding the target platform. **b**, No differences in swim speeds were detected between genotypes. **c**, All animals exhibited similar baseline freezing time during fear conditioning training. **d**, During contextual fear conditioning animals demonstrated no differences in freezing time during contextual memory testing. **e**, No differences in cued memory were detected 24 hours after training. Data from 10 mice per group. All data represented as Mean±SEM; n.s., not significant; t-test (**b-e**), repeated measures ANOVA, Bonferroni post-hoc test (**a**).



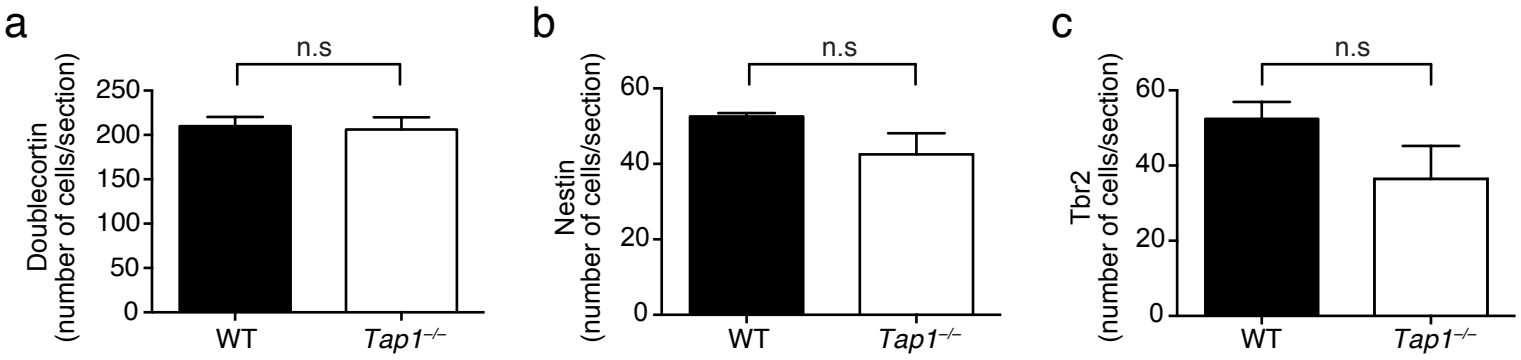
Supplementary Figure 7. Local administration of B2M decreases neurogenesis in the DG of young WT animals. **a,b**, Young adult (3 months) wild type (WT) mice were given unilateral stereotaxic injections of B2M or vehicle control into contralateral dentate gyrus (DG). Quantification of Nestin-positive (**a**), and Tbr2-positive (**c**) progenitors in the DG after treatment. Data from five mice per group. All data represented as Mean+SEM; *P< 0.05; **P<0.01; t-test.



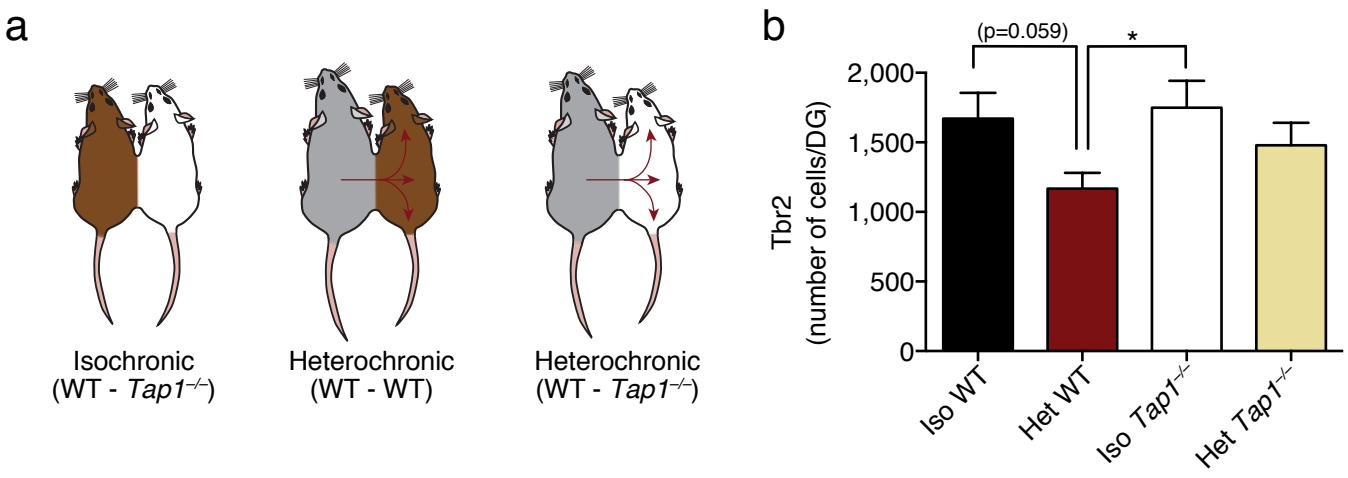
Supplemenatry Figure 8. B2M inhibits NPC function and neuronal differentiation in vitro. **a,b**, Primary postnatal hippocampal neural progenitor cells (NPCs) were derived from wild type (WT) mice and exposed to soluble B2M under self-renewal conditions. Number of neurospheres was quantified (**a**) and BrdU-positive NPCs were detected by measuring immunostaining absorbance at 450-550nm (**b**). **c**, Primary postnatal hippocampal NPCs derived from Dcx-reporter mice, in which luciferase expression is controlled under the Dcx promoter, were exposed to B2M under differentiation conditions. Dcx reporter gene activity was measured as luciferase activity. **d-f**, Primary postnatal hippocampal NPCs were derived from WT mice and exposed to B2M under differentiation conditions. Representative images of differentiated neurons (MAP2, red) and astrocytes (GFAP, green) are shown (**d**). Neurons (**e**) and astrocytes (**f**) are quantified as a percent of total cells. **g**, Primary postnatal hippocampal NPCs were exposed to B2M under self-renewal conditions, and cytotoxicity was measured by lactate dehydrogenase (LDH) detection. **h**, Primary postnatal hippocampal NPCs were derived from WT and *Tap1*$^{-/-}$ mice. Number of neurospheres was quantified after NPC exposure to B2M under self-renewal conditions. In vitro data are representative of three independent experiments done in triplicate. All data represented as bar graphs with Mean+SEM; *P< 0.05; **P< 0.01; ***P< 0.001 ANOVA, Tukey's post-hoc test (**a-c,h**), and t-test (**e-g**).



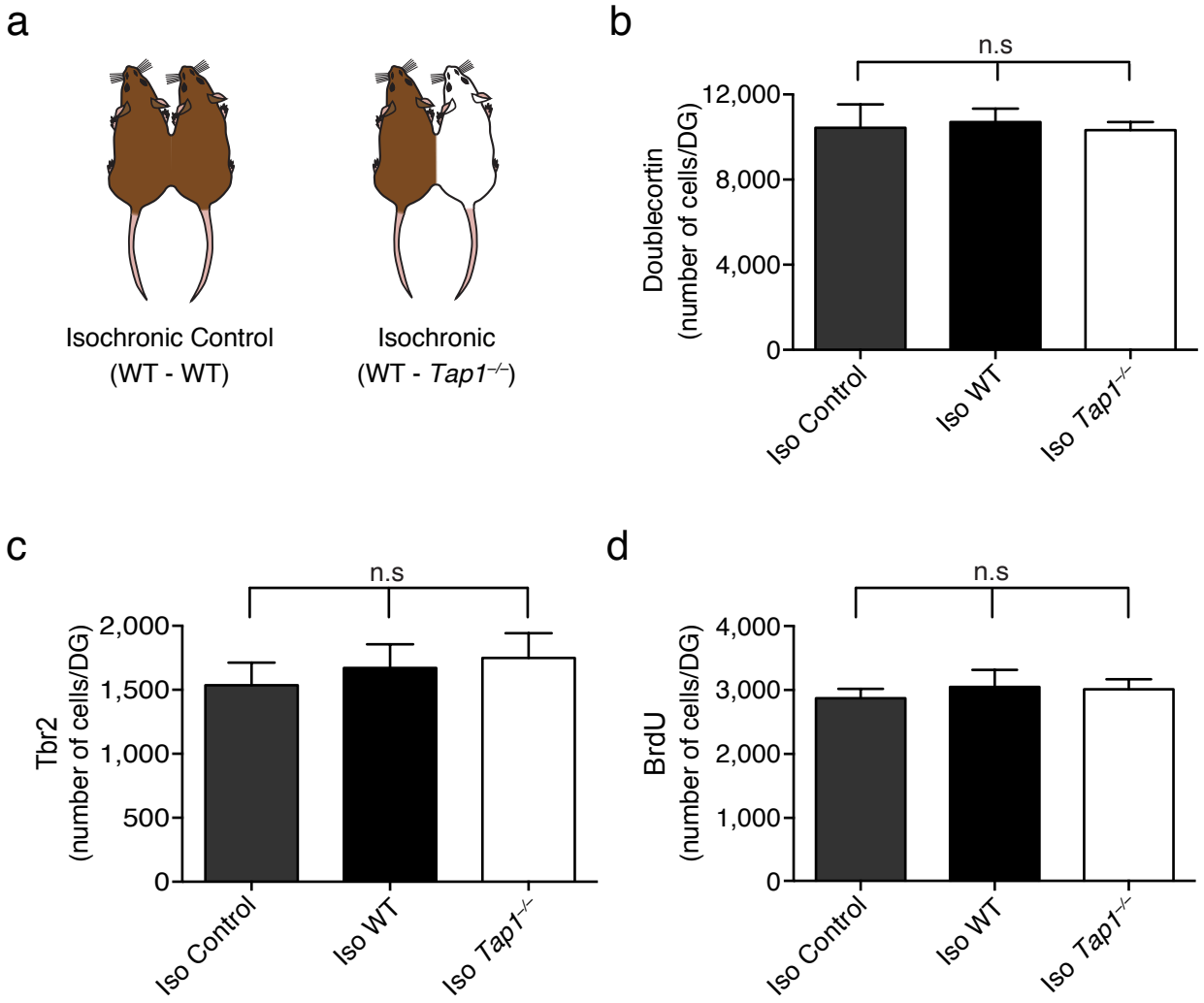
Supplementary Figure 9. Local administration of B2M does not elicit changes in neurogenesis in the DG of young *Tap1*$^{-/-}$ mice. **a,b**, Young adult (3 months) *Tap1*$^{-/-}$ mice were given unilateral stereotaxic injections of B2M or vehicle control into contralateral dentate gyrus (DG). Quantification of Nestin-positive (**a**), and Tbr2-positive (**b**) progenitors in the DG after treatment. Data from five mice per group. All data represented as Mean+SEM; *P< 0.05; **P<0.01; t-test.



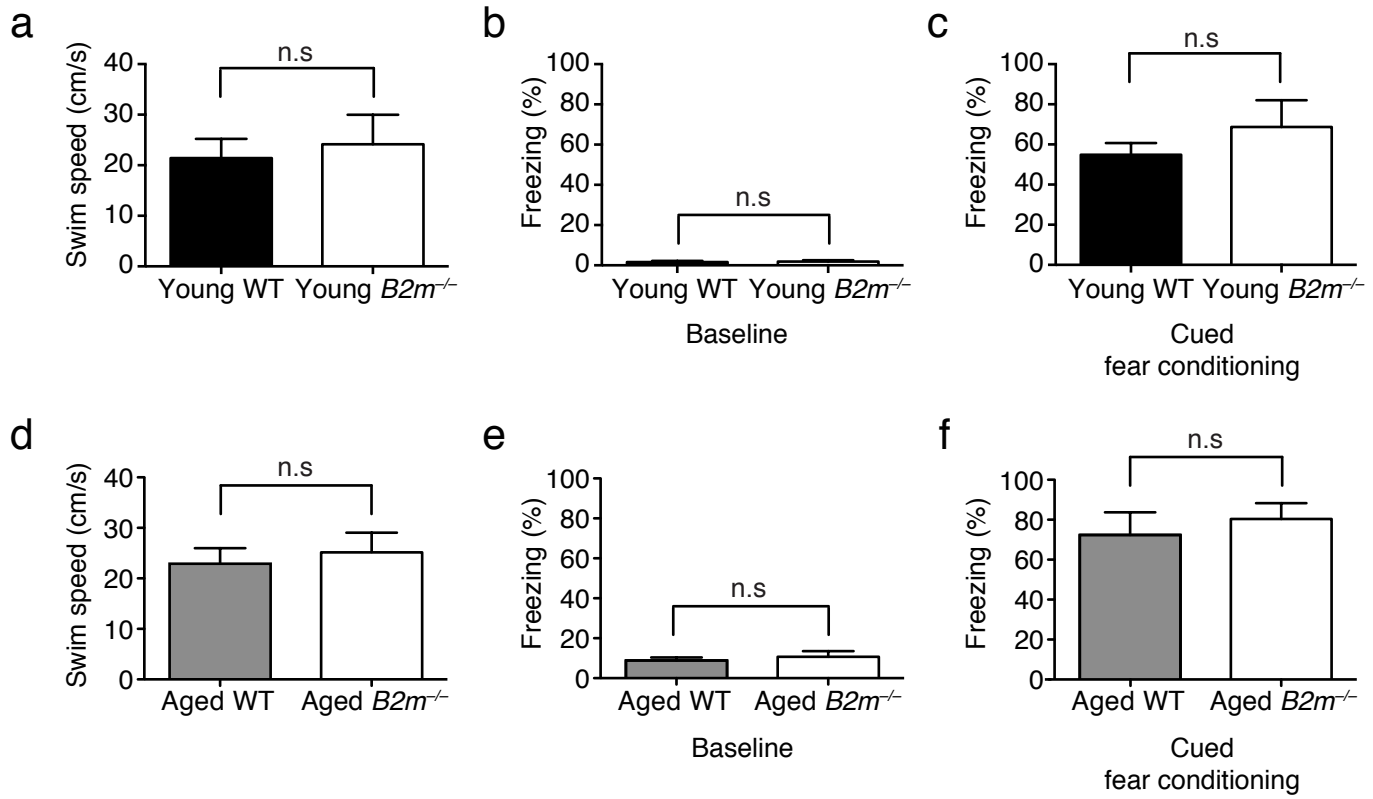
Supplementary Figure 10. No differences in neurogenesis are observed in the DG of young WT and *Tap1*$^{-/-}$ animals. **a-c**, Quantification of Doublecortin (Dcx)-positive (**a**), Nestin-positive (**b**),and Tbr2-positive (**c**) cells in the dentate gyrus (DG) of young adult (3 months) wild type (WT) and *Tap1*$^{-/-}$ mice. Data from 5 mice per group. All data represented as Mean+SEM; n.s. not significant; t-test (**a-c**).



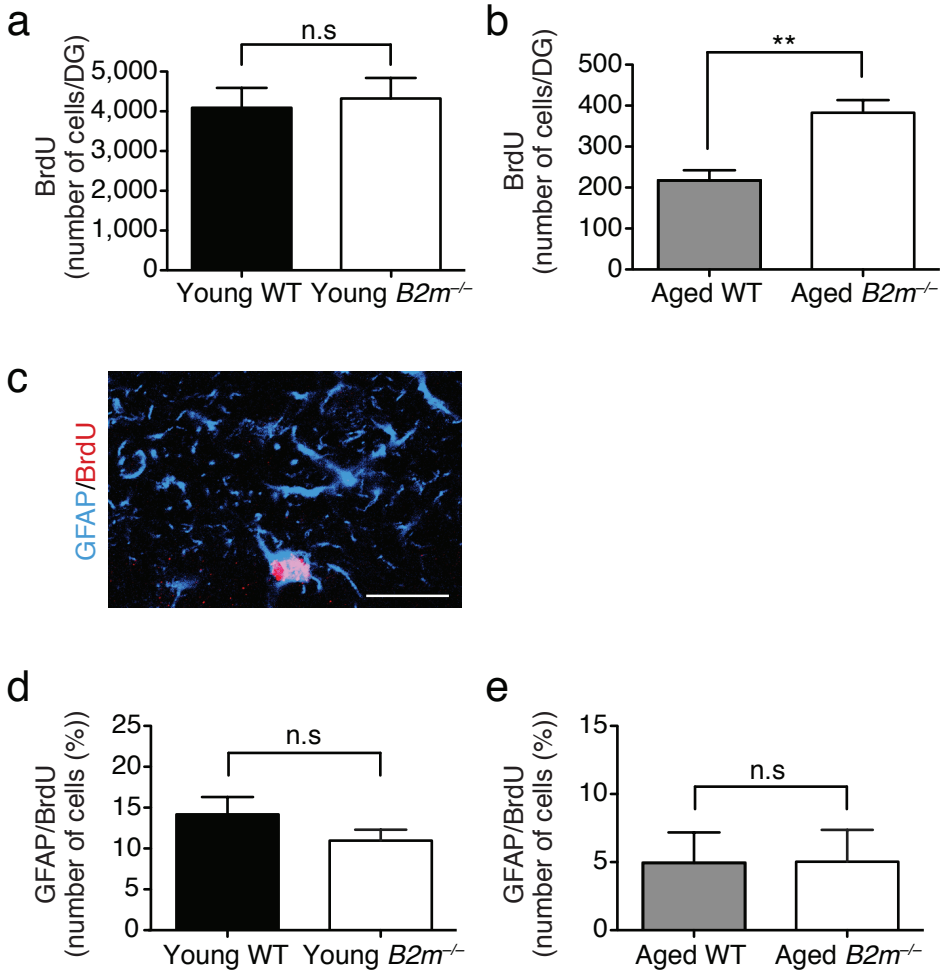
Supplementary Figure 11. Reducing endogenous MHC I surface expression mitigates in part the decrease in neuronal progenitor cell number in young mice after heterochronic parabiosis. **a**, Schematic of young wild type (WT) and *Tap1*$^{-/-}$ isochronic parabionts and young WT and *Tap1*$^{-/-}$ heterochronic parabionts. **b**, Quantification of the T-box transcription factor Tbr2 immunostaining of young isochronic and heterochronic parabionts five weeks after parabiosis Data from 8 young isochronic WT, 6 young isochronic *Tap1*$^{-/-}$, 8 young heterochronic WT, and 8 young heterochronic *Tap1*$^{-/-}$ parabionts. All data represented as Mean+SEM; *P< 0.05; ANOVA, Tukey's post-hoc test.



Supplementary Figure 12. No differences in neurogenesis are observed in the DG of young isochronic WT and *Tap1*$^{-/-}$ parabionts. **a**, Schematic of young WT and *Tap1*$^{-/-}$ isochronic parabionts. **b-d**, Quantification of Dcx-positive (**b**), Tbr2-positive (**c**), and BrdU-positive (**d**) cells in the dentate gyrus (DG) of young WT and *Tap1*$^{-/-}$ isochronic parabionts five weeks after parabiosis. Data from 8 young isochronic WT control, 6 young isochronic WT, and 6 young isochronic *Tap1*$^{-/-}$. All data represented as Mean+SEM; n.s. not significant; t-test (**a**); ANOVA, Tukey's post-hoc test (**c-d**).



Supplementary Figure 13. Swim speeds and cued memory are not altered in young and aged *B2m*$^{-/-}$ animals. **a-d**, Learning and memory was assessed in young (3 months) and aged (17 months) wild type (WT) and B2m knock out (*B2m*$^{-/-}$) mice by RAWM (**a,d**) and fear conditioning (**b,c,e,f**). **c,d**, Young (**a**) and aged (**d**) animals exhibited similar baseline freezing time during fear conditioning training regardless of genotype. **b,e**, No differences in baseline freezing were detected in young (**b**) or aged (**e**) animals. **c,f**, No difference in cued memory were detected between genotypes in young (**c**) or aged (**f**) animals when mice were re-exposed to the conditioned stimulus (tone and light) in a novel context 24 hours after training. Data from 10 young WT, 10 young *B2m*$^{-/-}$, 8 aged WT, and 12 aged *B2m*$^{-/-}$ mice. All data represented as Mean+SEM; n.s. not significant; t-test.



Supplementary Figure 14. Absence of endogenous B2M increases proliferation but not astrocyte differentiation in an age-dependent manner in vivo. **a-c**, To assess proliferation young (3 months) and aged (16 months) wild type (WT) and B2m knock out (*B2m*$^{-/-}$) mice were administered BrdU by intraperitoneal injections for three days prior to euthanasia. **b,c**, Immunostaing of BrdU-positive cells was quantified in the DG of young (b) and aged (c) animals. Data from 8 young and 10 aged mice per genotype. **c-e**, To examine astrocyte differentiation WT and *B2m*$^{-/-}$ mice were administered BrdU by intraperitoneal injections for six days and euthanized 28 days later. **c**, Representative confocal microscopy from the DG of brain sections immunostained for BrdU (red) in combination with GFAP (blue; scale bar: 25μm). **d,e**, Quantification of the relative number of BrdU and GFAP-double positive cells out of the total BrdU-positive cells in the young (**d**) and aged (**e**) DG of WT and *B2M*$^{-/-}$ animals. Data from 8 mice per group (3 sections per mouse). All data represented as Mean+SEM; **P< 0.01; n.s. not significant; t-test.

Supplementary Table 1.

Normal aging subject inclusion criteria	Normal aging subject exclusion criteria
• Age: Subject meets age cutoffs for entry to the specific diagnostic group. • Informant: Presence of an informant for all subjects. • General health: good enough to complete study visits. • Body Mass Index (BMI): 18 - 34 • Stable medications for 4 weeks before the visit to draw blood or CSF. • Permitted medications include: AChE-inhibitors, Memantine, HRT (estrogen +/- progesterone, Lupron), Thyroid hormone, Antidepressants, statins. • Normal basic laboratory tests: BUN, creatinine (will allow creatinine up to 1.5), B12, TSH. • MMSE > 27/30 (exemptions if low education and control status established by detailed evaluation) • Memory performance on logical Memory within normal limits. • CDR = 0 • Neurological exam is normal, i.e. no evidence of stroke, Parkinsonism or major abnormalities.	• Vision and/or hearing too impaired (even with correction) to allow compliance with psychometric testing • Medical problems: unstable, poorly controlled, or severe medical problems or diseases. • Cancer in the past 12 months (excludes squamous CA of the skin or stage 1 prostate CA). • Contraindications to lumbar puncture: Bleeding disorder, use of Coumadin, heparin or similar anticoagulant, platelets < 100,000; deformity or surgery affecting lumbosacral spine which is severe enough to make lumbar puncture difficult, cutaneous sepsis at lumbosacral region. • Neurological disorders: neurodegenerative diseases such as Alzheimer's Disease, Parkinson's Disease, CJD, FTD, PSP; stroke in past 12 months or severe enough residual effects of earlier stroke to impair neurological or cognitive function; Multiple sclerosis; epilepsy • Psychiatric disorders: schizophrenia, bipolar affective disorder • Active/ uncontrolled depression: by history or GDS score • Drug or alcohol abuse in past 2 years • Exclusionary medications (in 4 weeks before visit to draw blood or CSF) • Neuroleptics/ atypical antipsychotics • Anti-Parkinson's Disease medications (L-dopa, dopamine agonists) • CNS stimulants: modafinil, Ritalin • Antiepileptic drugs (exceptions for Neurontin or similar newer AEDs given for pain control) • Insulin treatment • Cortisone (oral prohibited – topical or inhaler use allowed), anti-immune drugs (e.g. methotrexate, cytoxan, IVIg, tacrolimus, cyclosporine) or antineoplastic drugs • Anti-HIV medications

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