Supplementary Information

Supplementary Methods

Fasting blood glucose levels and intraperitoneal glucose tolerance test

Fasting blood glucose levels and glucose tolerance were measured in 6-, 8-, or 11-month-old mice. In short, mice were fasted overnight in clean cages with free access to drinking water. The following morning, two drops of blood were collected from the tail vein, and fasting glucose levels were measured using a handheld glucometer (Contour Next EZ Glucose Meter, Bayer). Glucose (2 g/kg body weight in 0.1 ml sterile water) was then injected intraperitoneally, and blood glucose levels were measured at 5, 15, 30, 60, 90, and 120 min post injection. At the end of the experiment, mice were returned to their cages with their experimental diet provided ad libitum.

Blood ketone levels

Fasted blood ketone levels were measured in 6-, 8-, or 11-month-old mice after fasted blood glucose levels were measured. Mice were fasted overnight with access to drinking water. The following morning, two drops of blood were collected from the tail vein, and blood ketone levels were measured using a handheld ketone meter (Precision Xtra Blood Glucose and Ketone Monitoring System, Abbott). We repeated blood ketone level measurements after mice were re-fed, at least 24 hours after the fasting period ended.

Blood collection and plasma preparation

Blood was collected by cardiac puncture using a sterile 25G x 5/8 needle and EDTA-coated 1 mL syringe. Blood was transferred into EDTA-coated tubes (SAI Infusion Technologies) and centrifuged at 1500 x g for 15 min at room temperature (RT). The supernatant was transferred into polypropylene tubes (Fisher Scientific) and centrifuged again as described above. Plasma was then aliquoted into clean polypropylene tubes and stored at -80°C until use.

Cholesterol analysis

Plasma samples were submitted to the Laboratory of Comparative Biology at Memorial Sloan Kettering Cancer Center (MSKCC) where total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured spectrophotometrically using a Beckman Coulter AU680 analyzer.

Behavioral analysis

Novel object recognition (NOR)

NOR was performed during the dark phase of the light-dark cycle to allow these nocturnal animals to carry out cognitive tasks when they are most active. NOR was carried out for 4 days with a 5-min trial on each day. The first two days consisted of habituation trials where mice were allowed to freely explore the testing arena - a large opaque rectangular box (54.9cm x 39.3cm x 32cm) with a sawdust-covered bottom. On the training day, mice were allowed to explore two identical objects (Intact Tissue-Tek O.C.T. Compound Bottles, Cat No. 25608-930) inside the testing arena. On the testing day, one of the objects was replaced with a novel one (stacked DUPLO® blocks), and the mice were allowed to interact with both objects. In order to prevent animals from using any olfactory cues, the objects and the box were cleaned with 20% ethanol after each trial. Object exploration was defined as the length of time the animal spent sniffing or touching the surface of the object with its nose and/or forepaws within a distance of 1 cm from the object. The time spent exploring both objects during training and testing was used to calculate the novel-to-familiar object exploration ratio. A ratio >1 indicates a preference for the novel object. A clear preference for one of the objects indicates that the animal could remember which of the two objects had previously been explored. We also examined locomotor ability of all animals by determining total distance moved and velocity to control for the potential confounding effect of obesity on object exploration. The behavioral performance was recorded and tracked for automated analysis of locomotion using EthoVision XT software (Noldus). Object exploration behavior was scored manually by two investigators who were unaware of mouse genotypes using EthoVision XT software.

Contextual fear conditioning

At 6 months-of-age, cognitive ability was examined using the contextual fear conditioning paradigm (NIR Video Fear Conditioning, MED-VFC2-SCT-M, Med Associates Inc.). On the training day, mice were allowed to explore the test chamber for 2 min. Baseline freezing was examined to ensure that there were no preexisting differences between groups. After 2 and 5 min, mice received a mild foot shock (0.7 mA, 2 s) and were returned to their cages 30 s after the last shock. On the following day, mice were placed in the same test chamber for 5 min, and contextual

memory was scored as percent immobility after 5 min using NIR Video Freeze software (Med Associates).

Immunofluorescence

Mice were deeply anesthetized by intraperitoneal (IP) injection of 2.5% tribromoethanol and perfused with 0.9% ice cold saline containing heparin. For fixed brain tissue, brains were removed and the left hemisphere was fixed in 2% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB) for 24 hours at 4°C with gentle agitation. Brains were then dehydrated in 30% sucrose in PBS for 48 h at 4°C, rapidly frozen on dry ice, and sectioned at a thickness of 30 µm using a microtome. Sections were collected into PBS, transferred into cryoprotectant (30% glycerol, 30% ethylene glycol, 40% 0.1 M PB), and stored at -80°C. For analysis, sections were quickly rinsed in PBS, mounted onto slides using a gelatin-based mounting medium (0.35% gelatin, 20% ethanol, and 0.04% sodium azide in PBS), and dried overnight. The next day, sections were washed in PBS. For frozen brain tissue, brains were removed, and the left hemisphere was rapidly frozen on dry ice and stored at -80 °C. Brains were sliced into 30 µm sections using a cryostat, thaw-mounted onto slides, and stored at -80 °C until use. All sections were blocked in 5% normal donkey serum with 0.3% Triton X-100 for 1 hour at RT. Prior to staining, sections were allowed to adjust to RT and fixed in 50% ethanol/50% methanol solution for 10 min at -20°C. Primary antibodies were diluted in 3% normal donkey serum with 0.3% Triton X-100 and 0.02% sodium azide, and sections were incubated with primary antibody overnight at 4°C. The following antibodies and dilutions were used: mouse anti-6E10 Alexa Fluor 488 (1:1000, BioLegend), rat anti-CD11b (1:10, DSHB), rabbit anti-fibrinogen (1:500, Dako), and goat anti-collagen IV (1:500, Millipore). The following day, sections were incubated with an appropriate fluorescent secondary antibody (Alexa Fluor, donkey anti-host species, Life Technologies) for 1 hour at RT. Coverslips were added using Vectashield Mounting Medium (Vector). Sections were imaged at 20x magnification using Eclipse Ti2-E inverted epifluorescence microscope (Nikon) or at 63x magnification using LSM 880 Airyscan NLO inverted laser scanning confocal microscope (Zeiss). Images had consistent exposure time and gain for each stain between all sections from all animals, and only identical brain regions were compared between groups. To compare Aβ plaque load across several cohorts of mice, brain slices obtained from 6-, 8-, and 11-month-old mice were stained and imaged simultaneously. Exposure time, gain, and threshold settings were kept consistent for all cohorts.

Images were thresholded manually using NIS-Elements software (Nikon Instruments, Inc.) to subtract background from apparent positive signal; threshold settings were kept consistent for all experimental images. The intensity of the signal was quantified as percent area for each section. Positive areas from each section of each animal were then averaged and compared between groups using GraphPad Prism software.

Immunohistochemistry

Mouse epididymal white adipose tissue (WAT) was used for fibrinogen immunohistochemistry. After mouse perfusion, WAT was removed and placed in 10% formalin for 24 hours at RT and then transferred to 70% ethanol for 24 hours at RT. Fixed WAT was embedded in paraffin, sectioned at a thickness of 5 μm using a microtome, and mounted onto glass slides. Glass slides were placed in a 58°C oven for 1.5 hours to melt the paraffin. Tissue sections were then re-hydrated, incubated in Proteinase K solution at 37°C for 20 min to unmask epitopes, washed in TBS, and blocked in 10% normal goat serum 5% BSA solution. Tissues were incubated in rabbit anti-fibrinogen antibody (1:200, Dako) at 4°C overnight. The next day, tissue sections were washed and incubated in biotinylated anti-rabbit antibody (1:200, Vector Laboratories) for 1 hour at RT. The staining was developed using the Vectastain ABC kit (Vector Laboratories) and the DAB Peroxidase Substrate Kit (Vector Laboratories) according to manufacturer's instructions. To visualize cell morphology, tissue sections were stained using hematoxylin. Coverslips were added with Vectamount AQ Aqueous Mounting Medium (Vector Laboratories). Images were collected on Revolve upright epifluorescence microscope (Echo), and fibrinogen staining was quantified using Fiji (NIH).

Western Blotting

Mice were deeply anesthetized by IP injection of 2.5% tribromoethanol and perfused with 0.9% ice cold saline containing heparin. Mouse cortical tissue was dissected and stored at -80 ^{0}C before protein extraction. Protein extraction was performed as reported previously [1] with modifications. In brief, cortical samples were homogenized in QIAzol lysis reagent (Qiagen) using a hand-held tissue homogenizer (Polytron PT 1200E; VWR) and incubated at room temperature for 3 min with added chloroform. Samples were centrifuged at 12000 x g for 15 min at 4 °C. The aqueous phase was removed for RNA isolation, and DNA was precipitated with ethanol. Isopropanol was added to the remaining supernatant, and the samples were centrifuged at 12000 x g for 15 min at 4 °C.

Protein pellets were washed twice with 95% ethanol, homogenized in RIPA lysis buffer (Millipore, Cat No.20-188) containing a protease inhibitor cocktail (Sigma-Aldrich), and sonicated. Total protein concentrations were measured using Pierce BCA Protein Assay Kit (Thermo Scientific). Protein samples (10 μg) were boiled at 95 °C with 4x Laemmli sample buffer for 5 min. Samples were separated on 4-20% Criterion TGX Stain-Free Protein Gel (Bio-Rad) and transferred to 0.2 μm PVDF (polyvinylidene difluoride) membrane using Trans-Blot® Turbo™ Transfer System (Bio-Rad). After blocking with 5% BSA in Tris-buffered saline with 0.1% Tween 20 for 1 hr at RT, membranes were incubated overnight at 4 °C with purified anti-β-amyloid antibody (6E10, BioLegend, 1:1000) and HRP-conjugated anti-GAPDH monoclonal antibody (Proteintech, 1:10,000). Blots were further incubated with secondary antibody (HRP-conjugated sheep anti-mouse IgG; GE Healthcare, 1:10,000) for 2 h. Bands were detected via Clarity Western ECL substrate (Bio-Rad) and scanned using ChemiDoc Imaging System (Bio-Rad). After target bands were detected, band intensity was analyzed using NIH Image J software. Proteins were normalized to GAPDH, used as a loading control.

References

[1] Kopec AM, Rivera PD, Lacagnina MJ, Hanamsagar R, Bilbo SD. Optimized solubilization of TRIzol-precipitated protein permits Western blotting analysis to maximize data available from brain tissue. J Neurosci Methods. 2017;280:64-76.

Supplementary Figure Legends

Figure S1. HFD induced hyperglycemia and hyperlipidemia, affected glucose metabolism, and caused ketogenesis in 6-month-old mice. **A** HFD impaired glucose clearance in WT and AD mice in a glucose tolerance test (GTT). **B** HFD-fed WT mice showed higher blood glucose levels after GTT, compared to CON-fed mice, as indicated by area under the curve (AUC). **C** HFD increased blood glucose levels in fasted WT and AD mice. **D** HFD increased blood ketone levels in fasted WT, but not AD mice. **E** HFD increased blood ketones in non-fasted WT and AD mice. **F** HFD increased the level of plasma cholesterol in WT and AD mice. **G, H** HFD induced a small, non-significant increase in plasma HDL levels but not plasma LDL levels in WT and AD mice. For **A**-**C**, **F**-**H**: n=8-11 mice/group. Results are from one representative experiment. For **D**, **E**: n=12-14 mice/group. Results are from two independent experiments. Statistical analysis performed by two-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure S2. HFD consumption starting at 1 month reduced AD-related pathology in 6-month-old AD mice. **A** Representative images of Aβ staining of whole brain slices of 6-month-old AD mice at Bregma 1.18mm, -0.94mm, and -1.70mm. **B** Representative images of CD11b staining used to visualize microglial cells in the RSC of 6-month-old mice after 20 weeks of experimental diet. Staining was quantified in the RSC, the boxed area on the left of each image and enlarged on the right. **C** HFD reduced the presence of microglia in the RSC of 6-month-old AD mice. n=11-13 mice per group. Results are combined from two independent experiments. Statistical analysis performed by Student's t-test. ***p<0.001

Figure S3. HFD did not affect locomotion during training or testing in 6-month-old mice during novel object recognition (NOR) test. HFD did not affect the total distance moved or velocity during NOR training (**A, B**) or testing (**C, D**) days in WT or AD mice. n=8-12 mice per group. Results are from one representative experiment. Statistical analysis performed by two-way ANOVA.

Figure S4. HFD consumption starting at 1 month did not affect Aβ/fibrinogen co-deposition in the retrosplenial cortex (RSC) of 6-month-old AD mice. **A** Representative triple-stained confocal microscopy images show Aβ deposits (green, 6E10) and fibrinogen extravasation (red) from collagen IV-positive blood vessels (purple) in the brain parenchyma of AD CON, but not AD HFD mice. Scale bar=50 μm. **B** HFD consumption caused a small, non-significant decrease in the level of Aβ/fibrinogen co-deposition in the RSC of AD mice. n=8-9 mice per group. Results are from one representative experiment. Statistical analysis performed by Student's t-test.

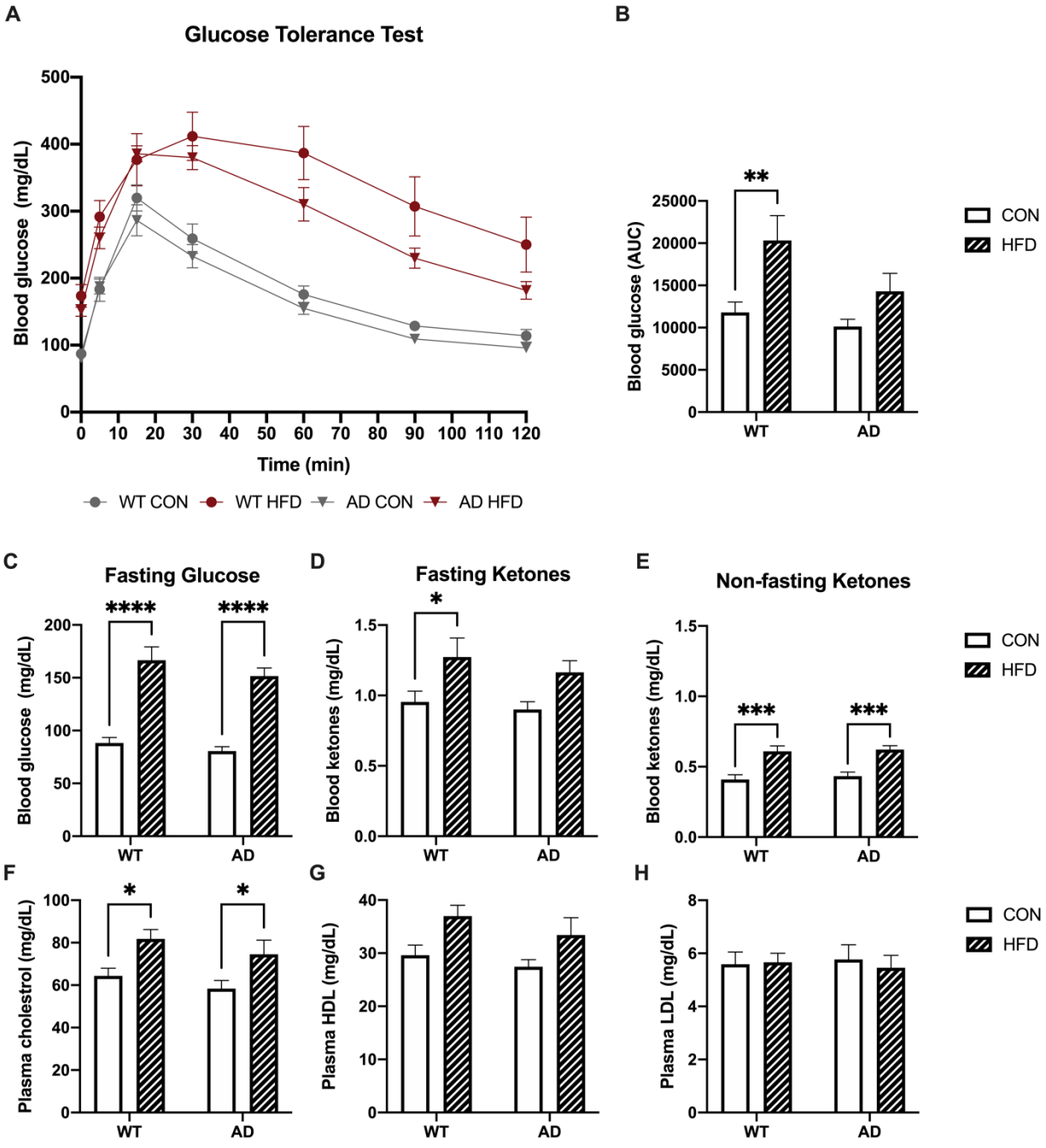
Figure S5. HFD consumption starting at 3 months, but not at 6 months, improved Aβ pathology and cognition in AD mice. **A** HFD consumed starting at 3 months (Cohort I) reduced Aβ deposition throughout the brains of 8-month-old AD mice. **B** Quantification of Aβ staining throughout the brains of Cohort I 8-month-old AD mice showed a decrease in overall Aβ pathology. Each symbol represents a single Bregma point. **C** HFD increased the novel-to-familiar object exploration ratio in 8-month-old AD mice during NOR testing. **D, E** HFD significantly reduced the total distance moved and velocity during NOR testing, restoring locomotion to WT levels in 8-month-old AD mice. **F** HFD consumption starting at 6 months (Cohort II) increased Aβ deposition throughout the brains of 11-month-old AD mice. **G**

Quantification of Aβ staining throughout the brains of Cohort II 11-month-old AD mice showed an increase in overall Aβ pathology. Each symbol represents a single Bregma point. **H-J** HFD did not affect object exploration (**H**), distance moved (**I**), or velocity (**J**) during NOR testing day in 11-month-old AD mice. For **A** and **B**, n=4-8 mice per group. For **C-E**, n=5-9 mice per group. For **F** and **G**, n=4-9 mice per group. For **H-J**, n=8 mice per group. Results are from one representative experiment. Statistical analysis performed by Student's t-test (**B**, **G**) or two-way ANOVA (**C-E**; **H-J**). *p<0.05, **p<0.01.

Figure S6. HFD consumption starting at 3 months-of-age, but not 6-months-of-age, increased white adipose tissue (WAT) fibrinogen deposition in WT and AD mice but did not affect Aβ/fibrinogen interaction in the brain parenchyma. **A, C** Representative images of WAT fibrinogen staining in 8-month-old mice (**A**) and 11-month-old mice (**C**) after 20 weeks of experimental diet. **B, D** HFD increased percentage of fibrinogen-positive cells in WAT of 8-month-old, but not 11-month-old WT and AD mice. **E,G** Representative triple-stained images of Aβ deposits (green, 6E10) and fibrinogen extravasation (red) from collagen IV-positive blood vessels (purple) in the brain parenchyma of 8- (**E**) and 11- (**G**) month-old AD CON, but not AD HFD mice. Scale bar=50 μm. **F, H** HFD had a small but insignificant effect on the interaction between Aβ and fibrinogen in the RSC of 8- (**F**) and 11- (**H**) month-old AD mice. For **A** and **B**, n=5-6 mice per group, 3 slices per animal. For **C** and **D**, n=4-5 mice per group, 3 slices per animal. For **E** and **F**, n=4-5 mice per group, 3 slices per animal. For **G** and **H**, n=5-6 mice per group, 3 slices per animal. Results are from one representative experiment. Statistical analysis performed by two-way ANOVA. **p<0.01. Scale bar=130μm in **A, C**; 50 μm in **E, G**.

Supplementary Figures

Figure S1:



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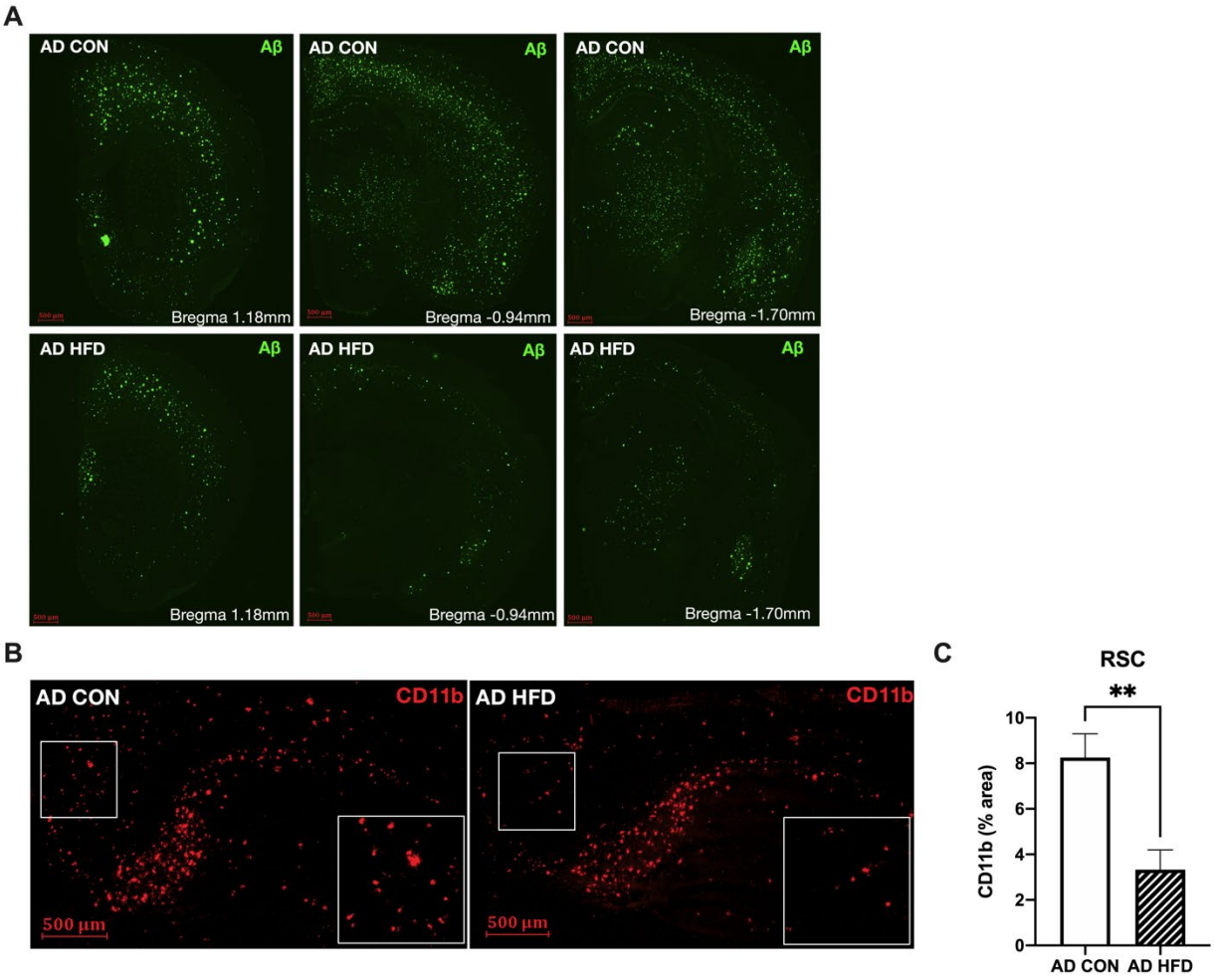

A
AD CON
Aβ
Bregma 1.18mm
Bregma -0.94mm
Bregma -1.70mm
AD HFD
B
CD11b
500 µm
C
RSC
**
CD11b (% area)
10
8
6
4
2
0
AD CON
AD HFD

Figure S3:

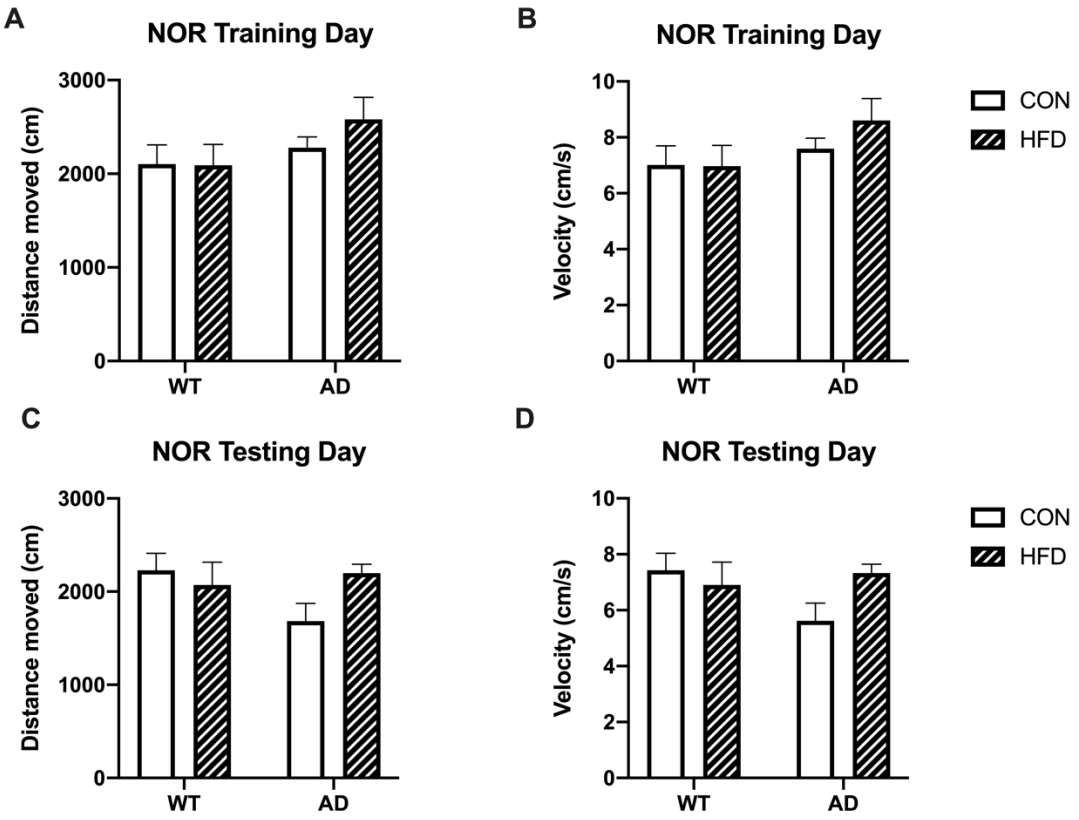

A
NOR Training Day
Distance moved (cm)
3000
2000
1000
0
WT
AD
B
NOR Training Day
Velocity (cm/s)
10
8
6
4
2
0
WT
AD
CON
HFD
C
NOR Testing Day
Distance moved (cm)
3000
2000
1000
0
WT
AD
D
NOR Testing Day
Velocity (cm/s)
10
8
6
4
2
0
WT
AD
CON
HFD

Figure S4:

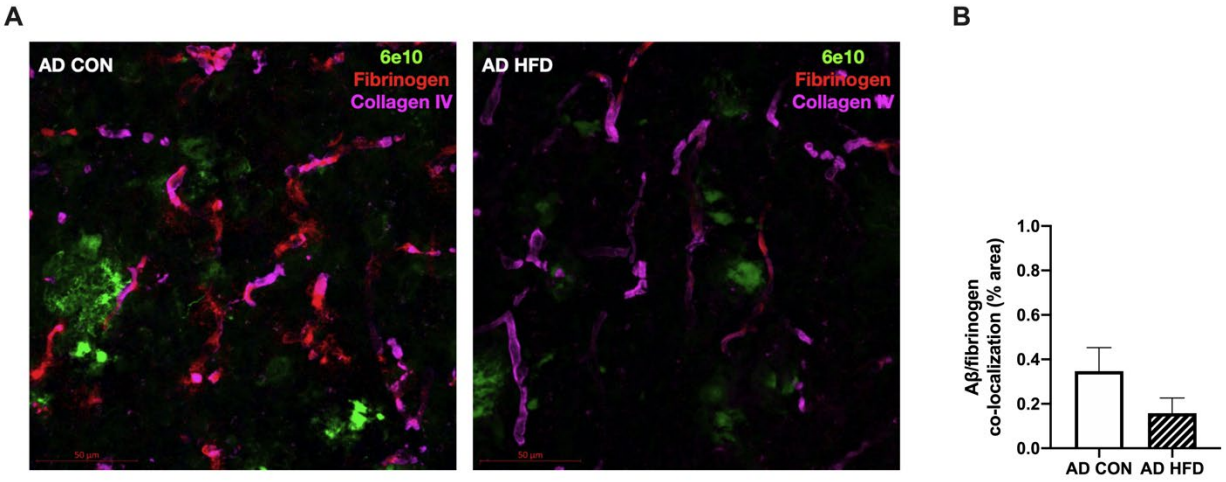

A
AD CON
6e10
Fibrinogen
Collagen IV
AD HFD
6e10
Fibrinogen
Collagen IV
B
Aβ/fibrinogen co-localization (% area)
1.0
0.8
0.6
0.4
0.2
0.0
AD CON
AD HFD

Figure S5:

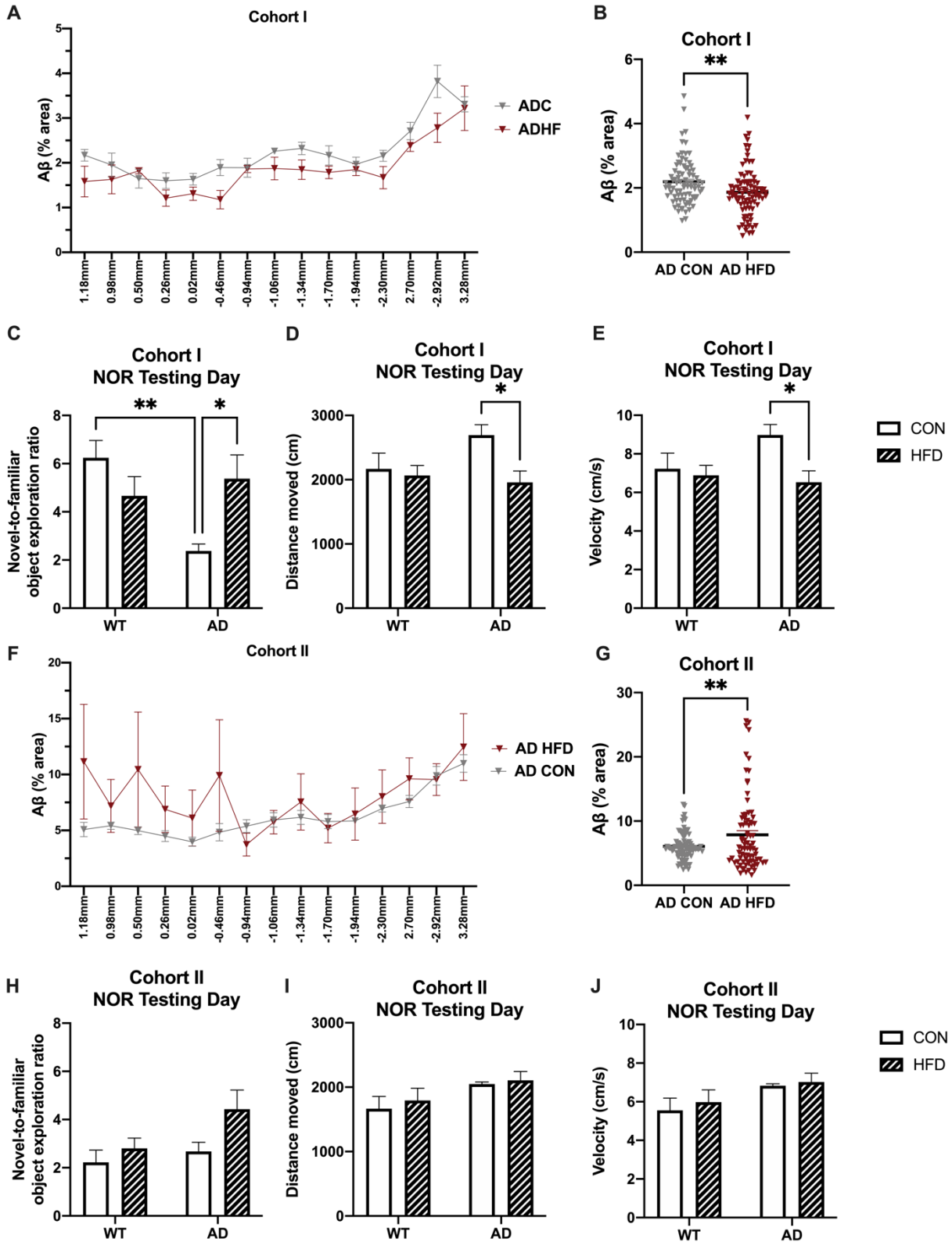
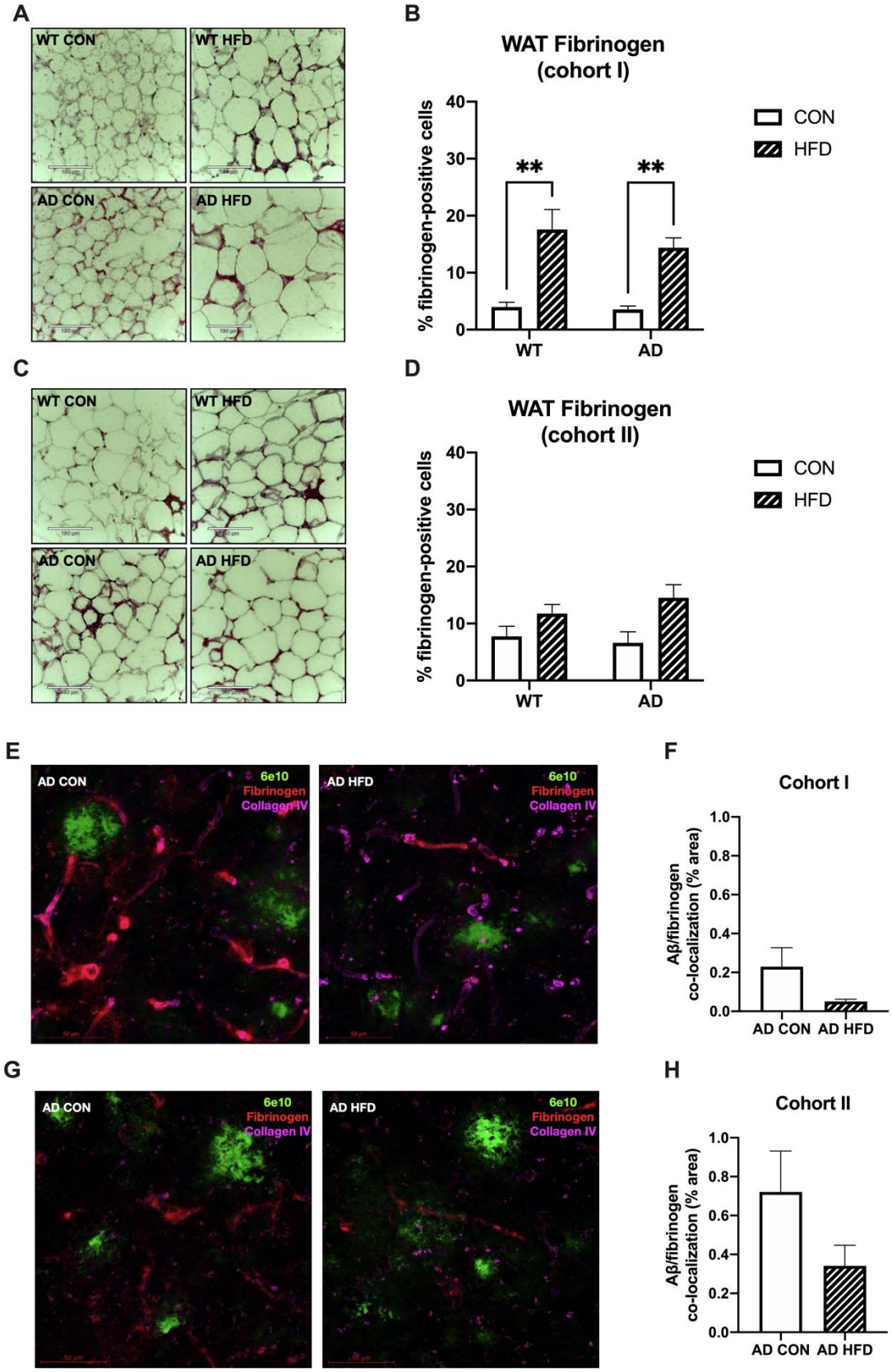

A
Cohort I
Aβ (% area)
ADC
ADHF
1.18mm
0.98mm
0.50mm
0.26mm
0.02mm
-0.46mm
-0.94mm
-1.06mm
-1.34mm
-1.70mm
-1.94mm
-2.30mm
2.70mm
-2.92mm
3.28mm
B
Cohort I
**
Aβ (% area)
AD CON
AD HFD
C
Cohort I
NOR Testing Day
**
*
Novel-to-familiar object exploration ratio
WT
AD
D
Cohort I
NOR Testing Day
*
Distance moved (cm)
WT
AD
E
Cohort I
NOR Testing Day
*
Velocity (cm/s)
WT
AD
CON
HFD
F
Cohort II
Aβ (% area)
AD HFD
AD CON
1.18mm
0.98mm
0.50mm
0.26mm
0.02mm
-0.46mm
-0.94mm
-1.06mm
-1.34mm
-1.70mm
-1.94mm
-2.30mm
2.70mm
-2.92mm
3.28mm
G
Cohort II
**
Aβ (% area)
AD CON
AD HFD
H
Cohort II
NOR Testing Day
Novel-to-familiar object exploration ratio
WT
AD
I
Cohort II
NOR Testing Day
Distance moved (cm)
WT
AD
J
Cohort II
NOR Testing Day
Velocity (cm/s)
WT
AD
CON
HFD

Figure S6:


A
WT CON
WT HFD
AD CON
AD HFD
B
WAT Fibrinogen
(cohort I)
% fibrinogen-positive cells
40
30
20
10
0
**
**
WT
AD
CON
HFD
C
WT CON
WT HFD
AD CON
AD HFD
D
WAT Fibrinogen
(cohort II)
% fibrinogen-positive cells
40
30
20
10
0
WT
AD
CON
HFD
E
AD CON
6e10
Fibrinogen
Collagen IV
AD HFD
6e10
Fibrinogen
Collagen IV
F
Cohort I
Aβ/fibrinogen
co-localization (% area)
1.0
0.8
0.6
0.4
0.2
0.0
AD CON
AD HFD
G
AD CON
6e10
Fibrinogen
Collagen IV
AD HFD
6e10
Fibrinogen
Collagen IV
H
Cohort II
Aβ/fibrinogen
co-localization (% area)
1.0
0.8
0.6
0.4
0.2
0.0
AD CON
AD HFD