SUPPLEMENTAL MATERIALS

Body Mass Index in Multiple Sclerosis modulates ceramide-induced DNA methylation and disease course.

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Baseline demographics and clinical characteristics of a primary cohort			
Treatment naïve CIS/RRMS patients (n=54)	Normal BMI (n=27)	High BMI (n=27)	p value
BMI (mean±SD) (min-max)	21.8±1.9 (18.1-24.7)	30.2±5.0 (25.2-44.9)	<0.001
Age (mean±SD) (min-max)	37.4±10.0 (18.0-58.4)	39.3±11.8 (20.6-60.2)	0.51
Sex Female (n) Male (n)	 20 7	 14 13	0.09
Race Caucasian (n) African American(n) Latino (n)	 20 6 3	 18 10 2	0.53
EDSS (median±SD) (min-max)	2±1.1(0-3.5)	1±1.0 (0-3.0)	0.68
Disease duration in months (mean±SD)	42.7±52.9	34.5±45.1	0.58

Table S1. Baseline demographics and clinical characteristics of a primary cohort. Therapy-naïve RRMS patients were grouped by normal or high BMI. Range and/or average of BMI, age, and disease duration were calculated for each BMI category. Statistical significance for BMI, age, and disease duration (based on date of diagnosis) was assessed by Student's t-test. Sex and race was also recorded for each BMI category and statistical significance was assessed by Chi-squared test. Range and median of EDSS was calculated and statistical significance between the two BMI groups was assessed by Mann-Whitney U test.

Baseline demographics and clinical characteristics of a validation cohort			
Therapy naïve RRMS patients (n=93)	Normal BMI (n=42)	High BMI (n=51)	p value
BMI (mean±SD) (min-max)	21.4±1.9 (16.8-24.7)	29.6±4.4 (25.0-44.4)	<0.001
Age (mean±SD) (min-max)	35.17±9.49 (18.3-61.6)	42.23±10.90 (18.0-68.6)	0.001
Sex Female (n) Male (n)	 31 11	 23 28	0.005
EDSS (median±SD) (min-max)	1.51±1.47 (0-6.5)	1.5±1.35 (0-6.0)	0.99

Table S2. Baseline demographics and clinical characteristics of a validation cohort. Therapy-naïve RRMS patients were grouped by normal or high BMI. Range and average of BMI and age were calculated for each BMI category. Statistical significance was assessed by Student's t-test. Sex was also recorded for each BMI category and statistical significance was assessed by Chi-squared test. Range and median of EDSS was calculated and statistical significance between the two BMI groups was assessed by Mann-Whitney U test.

Baseline demographics of a healthy control cohort for immunophenotyping and MRI measurements			
Healthy Controls (n=50)	Normal BMI (n=13)	High BMI (n=37)	p value
BMI (mean±SD) (min-max)	21.5±2.3 (17.8-24.8)	30.2±4.5 (25.2-39.1)	<0.001
Age (mean±SD) (min-max)	33.1±12.9 (19.7-55.7)	42.4±13.9 (20.0-72.4)	0.01
Sex Female (n) Male (n)	 5 8	 16 21	0.76

Table S3. Baseline demographics of a healthy control cohort for immunophenotyping and MRI measurements. Healthy controls were grouped by normal or high BMI. Range and averageof BMI and age were calculated for each BMI category and statistical significance for BMI and age was assessed by Student's t-test. Sex was also recorded for each BMI category and statistical significance was assessed by Chi-squared test.

Baseline demographics of a healthy control cohort for lipidomics			
Healthy Controls (n=40)	Normal BMI (n=20)	High BMI (n=20)	p value
BMI (mean±SD) (min-max)	22.6±1.1 (21.0-24.0)	29.4±3.4 (26.0-38.0)	<0.001
Age (mean±SD) (min-max)	39.5±16.2 (19.0-67.0)	36.1±8.6 (24.0-54.0)	0.42
Sex Female (n) Male (n)	 10 10	 10 10	0.99

Table S4. Baseline demographics of a healthy control cohort for lipidomics. Healthy controls were grouped by normal or high BMI. Range and average of BMI and age were calculated for each BMI category and statistical significance assessed by Student's t-test. Sex was also recorded for each BMI category and statistical significance was assessed by Chi-squared test.

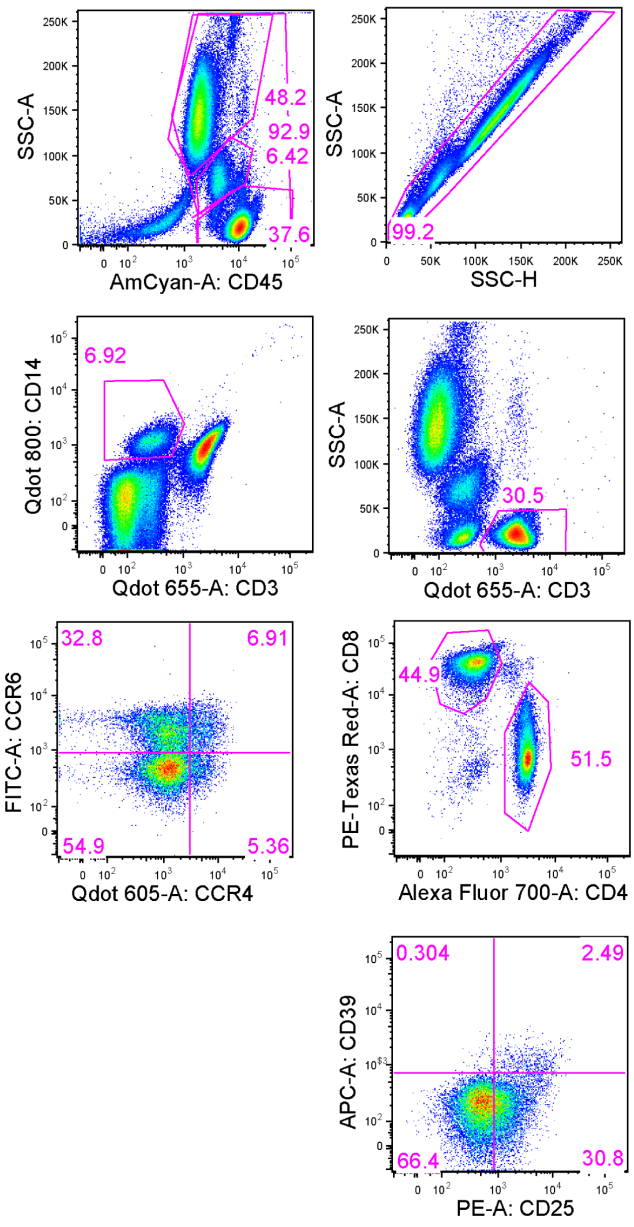

Fig. S1. Gating strategy for immunophenotyping. Immune cell populations from fresh whole blood were quantified using flow cytometry. Gating strategy shown by representative FACs plots. Monocytes were identified as CD45+CD3-CD14+ cells. CD8 T cells were identified as CD45+CD3+CD8+ cells. CD4 T cells were identified as CD45+CD3+CD4+ cells. CD4 subsets were further defined as CCR4-CCR6- (naïve/Th1), CCR4+CCR6+ (Th17), and CD127-CD25+ (Treg).

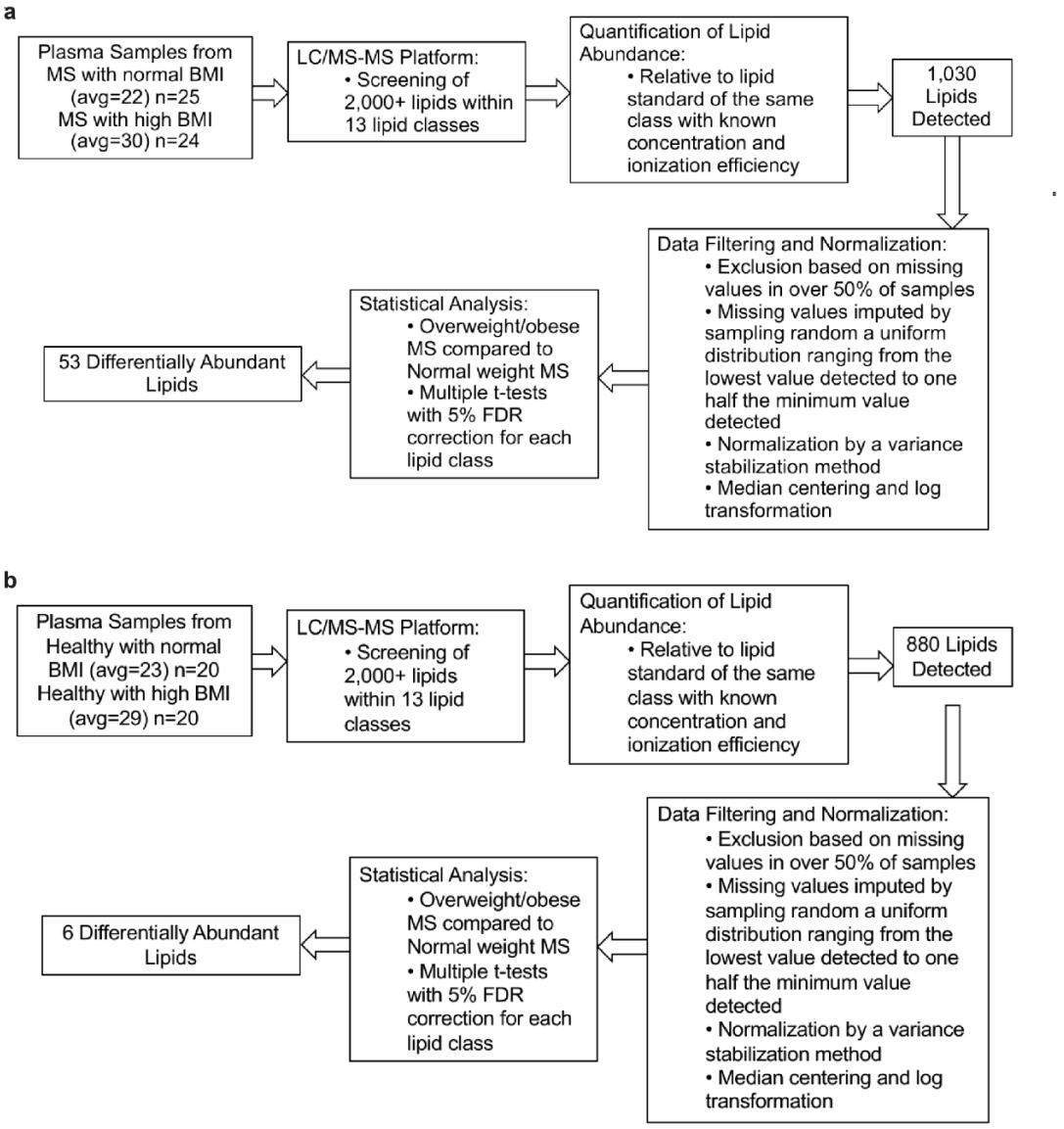

Fig. S2. Pipeline for lipidomic analyses. (a-b) Unbiased lipidomic analysis was performed on plasma samples from MS patients (n=48) and healthy individuals (n=40) using an MS/MS^{ALL} platform. Lipids that differed in abundance due to BMI were assessed within each class using multiple t-tests with 5% FDR correction, $q<0.05$ were considered significant.

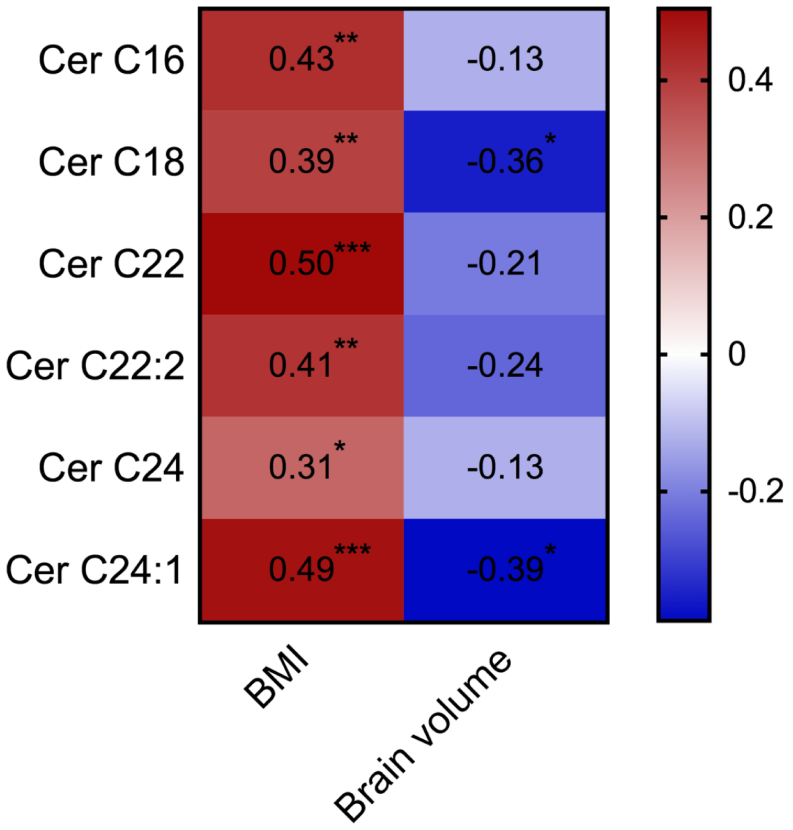

Fig. S3. Correlations between ceramide abundance and brain volume reveal specificity for ceramideC18 and C24:1. Pearson's correlations were performed between ceramide abundance and BMI and brain volume of MS patients (n=36) and a heat map was generated based on the correlation coefficients (r). P values<0.05 were considered significant (*p<0.05, **p<0.01, ***p<0.001).

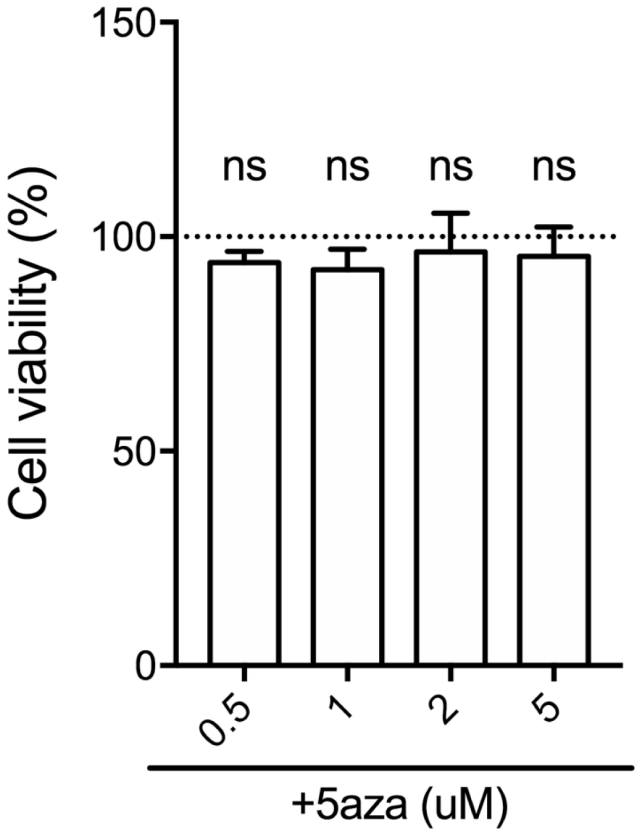

Fig. S4. 5aza is not toxic to cultured monocytes. Cultured monocytes (i.e. THP1 cells) were exposed to increasing doses of 5-aza-2'-deoxycytidine (5aza) (0, 0.5, 1, 2, and 5 uM) for 24 hours. MTT assay was performed to assess cell viability after treatment. Cell viability was normalized to untreated cells (dotted line) and given as a percentage. Dunnett's multiple comparisons test was performed to assess differences with 5aza treatment compared to untreated cells (n=3 independent experiments, n=5 biological replicates/group). *p* values<0.05 were considered significant.

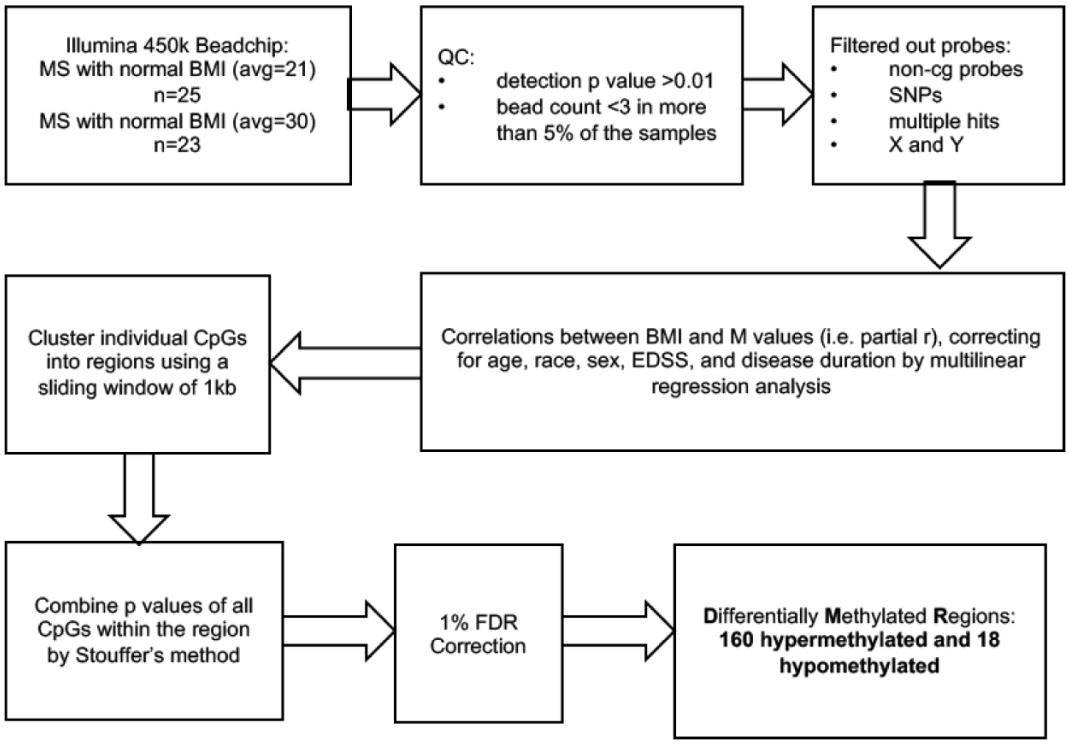

Fig. S5. Pipeline for DNA methylation analysis in monocytes of MS patients. DNA methylation analysis was conducted on DNA from monocytes of MS patients using the Infinium HumanMethylation450 BeadChip. Samples from MS patients with high BMI (n=23) were compared to those with normal BMI (n=25). Differentially methylated regions (DMRs) were identified by a 1kb sliding window and were determined by evaluating the combined p values of CpG sites within the region using Stouffer's method with a 1% FDR correction.

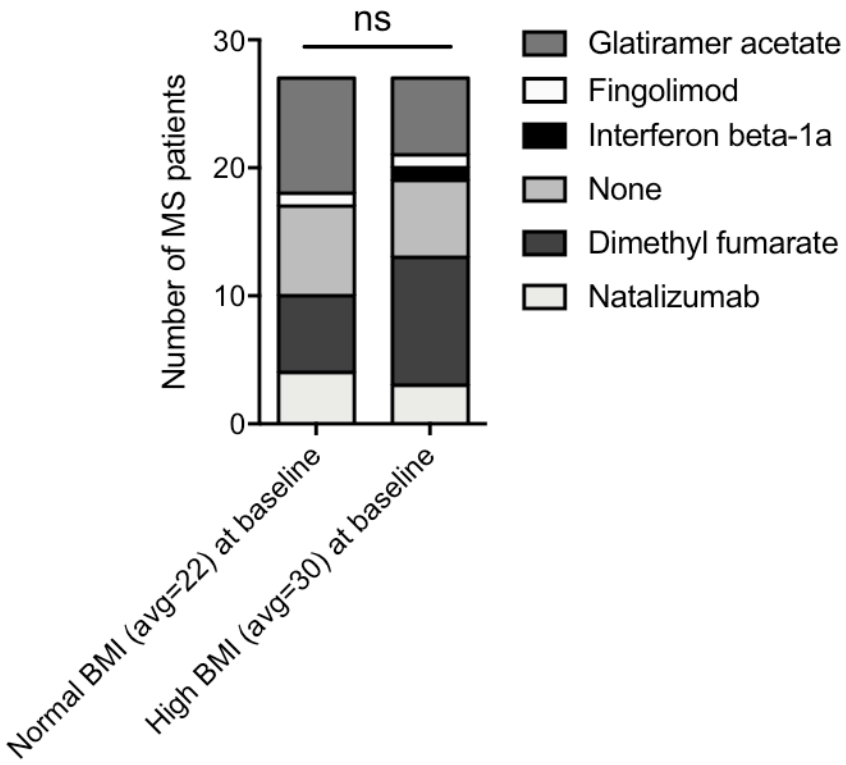

Fig. S6. Therapies of MS patients in the primary cohort. MS patients were on one of the following therapies at the two-year follow-up: 1. Glatiramer acetate, 2. Fingolimod, 3. Interferon beta-1a, 4. None, 5. Dimethyl fumarate, and 6. Natalizumab. Chi-squared test was performed to assess whether the distribution of therapies differed in MS patients with normal (n=27) or high BMI (n=27). *p* values<0.05 were considered significant.

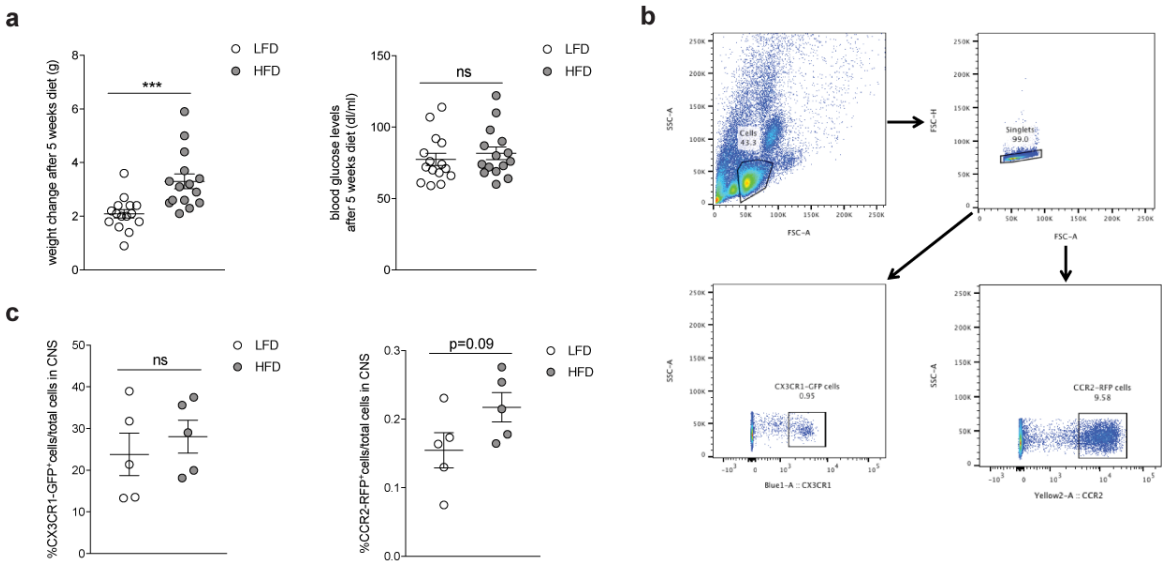

Fig. S7. High fat diet induces weight gain and monocytic infiltration into the CNS. (a) Female C57/bl6 mice were fed a low fat (LFD) or high fat diet (HFD) for 5 weeks beginning at 4 weeks of age. Weight change and blood glucose levels were evaluated after 5 weeks of diet. Statistical differences between LFD (white) and HFD (gray) mice were assessed by Student's t-test (***p<0.001) (n=15 mice/group). **(b-c)** Microglia and infiltrating monocytes were identified as CX3CR1-GFP^{+} or CCR2-RFP^{+}, respectively, by flow cytometry in female CX3CR1$^{GFP/+}$;CCR2$^{RFP/+}$ mice that had been given a low fat (LFD) or high fat diet (HFD) for 5 weeks beginning at 4 weeks of age. Percentage of microglia (CX3CR1-GFP^{+}) and monocytes (CCR2-RFP^{+}) of total cells in the CNS were calculated in the two groups. Statistical differences between LFD (white) and HFD (gray) mice were assessed by Student's t-test, $p<0.05$ considered significant (n=5 mice/group).