

Melgar-Locatelli et al. – Supplementary Information

## Supplementary Table 1

**Supplementary Table 1.** Summary of non-statistically significant differences in cognitive, neurogenic and neuroplastic analysis across experimental diets.

Test	Variable	Statistical Test	F Value	P Value
Body weight gain	Diet	Repeated measures ANOVA	$F(2, 45) = 0.198$	$p = 0.821$
	Day	Repeated measures ANOVA	$F(7, 315) = 14.530$	$p < 0.001$
	Diet x Day	Repeated measures ANOVA	$F(14, 315) = 2.439$	$p = 0.003$
Elevated plus maze	Total locomotion	One-Way ANOVA	$F(2, 45) = 0.700$	$p = 0.502$
	Time in open arms	One-Way ANOVA	$F(2,45) = 0.857$	$p = 0.431$
	Latency to open arms	One-Way ANOVA	$F(2, 45) = 1.003$	$p = 0.375$
Open field test	Locomotor activity	One-way ANOVA	$F(2,45) = 5.790$	$p = 0.006$
	Time in center	One-way ANOVA	$F(2,45) = 0.048$	$p = 0.953$
Place memory recognition test	Object memory	One-way ANOVA	$F(2,45) = 1.291$	$p = 0.285$
Recognition memory locomotion	Diet	Repeated measures ANOVA	$F(2,45) = 0.969$	$p = 0.387$
	Session	Repeated measures ANOVA	$F(2,90) = 18.108$	$p < 0.001$
	Diet x Session	Repeated measures ANOVA	$F(4,90) = 0.927$	$p = 0.452$
Habituation in the Water maze	Thigmotaxis	One-way ANOVA	$F(2,45) = 0.717$	$p = 0.494$
Locomotion during the visible platform in the water maze	Diet	Repeated measures ANOVA	$F(2, 45) = 1.086$	$p = 0.346$
	Trial	Repeated measures ANOVA	$F(7, 315) = 12.663$	$p < 0.001$
	Diet x Trial	Repeated measures ANOVA	$F(14, 315) = 0.656$	$p = 0.817$
Path length during the reference memory training	Diet	Repeated measures ANOVA	$F(2, 45) = 0.456$	$p = 0.637$
	Trial	Repeated measures ANOVA	$F(29, 1305) = 7.683$	$p < 0.001$
	Diet x Trial	Repeated measures ANOVA	$F(58, 1305) = 0.960$	$p = 0.158$
Latency during the reference memory training	Diet	Repeated measures ANOVA	$F(2, 45) = 1.926$	$p = 0.158$
	Trial	Repeated measures ANOVA	$F(29, 1305) = 8.333$	$p < 0.001$
	Diet x Trial	Repeated measures ANOVA	$F(58, 1305) = 0.850$	$p = 0.781$
Swimming velocity during the reference memory training	Diet	Repeated measures ANOVA	$F(2, 45) = 1.943$	$p = 0.155$
	Trial	Repeated measures ANOVA	$F(29, 1305) = 6.380$	$p < 0.001$
	Diet x Trial	Repeated measures ANOVA	$F(58, 1305) = 0.701$	$p = 0.957$
Long-term memory retention	Diet	Repeated measures ANOVA	$F(2, 45) = 0.454$	$p = 0.638$
	Quadrant	Repeated measures ANOVA	$F(1,45) = 14.469$	$p < 0.001$
	Diet x Quadrant	Repeated measures ANOVA	$F(2,45) = 0.375$	$p = 0.689$
	Platform crossings	One-way ANOVA	$F(2, 45) = 1.063$	$p = 0.354$
Short-term acquisition	Diet	Repeated measures ANOVA	$F(2,45) = 0.904$	$p = 0.412$

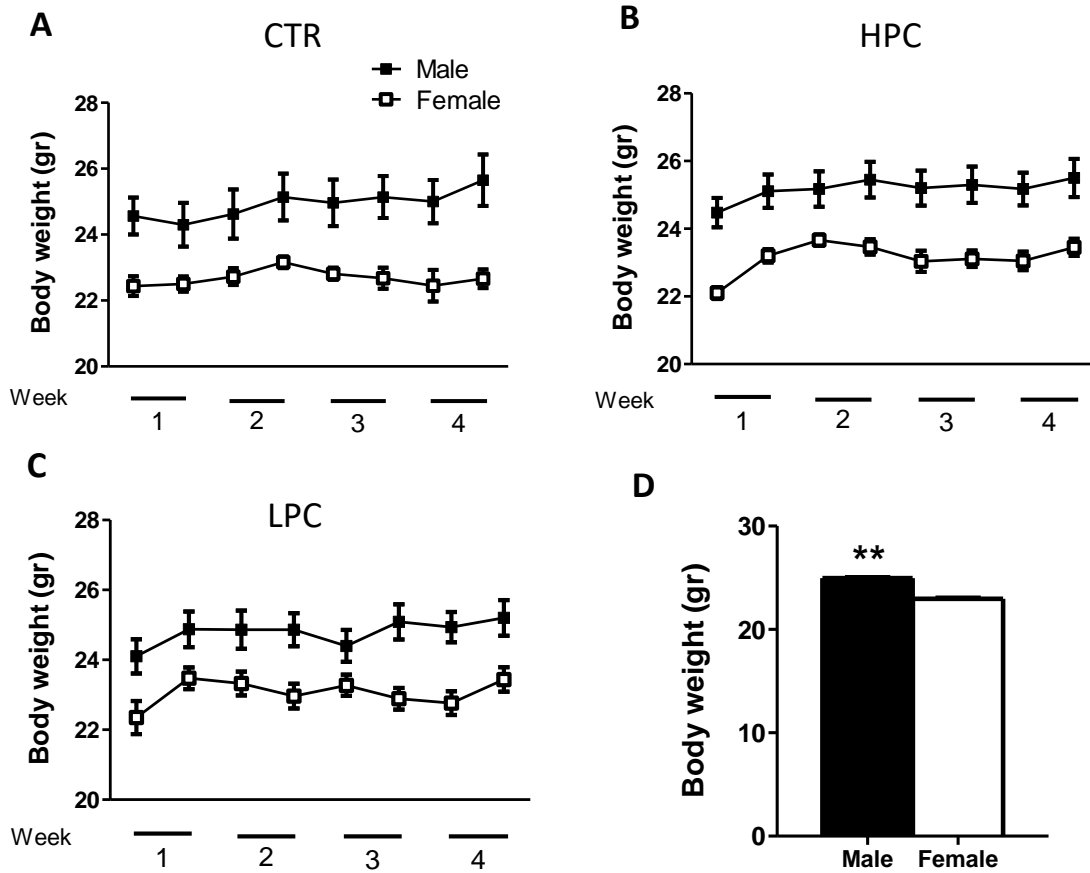
	Quadrant	Repeated measures ANOVA	$F(1,45) = 0.000$	$p = 0.991$
	Diet x Quadrant	Repeated measures ANOVA	$F(2,45) = 0.539$	$p = 0.587$
	Platform crossings	One-way ANOVA	$F(2,45) = 0.819$	$p = 0.447$
Adult hippocampal neurogenesis markers	Type 1 DCX+ neurons	One-way ANOVA	$F(2,21) = 0.956$	$p = 0.400$
	BrdU/NeuN	One-way ANOVA	$F(2,9) = 0.885$	$p = 0.446$
Basal synaptic transmission	Diet	Repeated measures ANOVA	$F(2,20) = 0.239$	$p = 0.790$
	Voltage	Repeated measures ANOVA	$F(6, 120) = 751.40$	$p < 0.001$
	Diet x Voltage	Repeated measures ANOVA	$F(12, 120) = 0.239$	$p = 0.996$
Long-term potentiation (LTP)	Diet	Repeated measures ANOVA	$F(2,20) = 0.867$	$p = 0.436$
	Time	Repeated measures ANOVA Repeated measures ANOVA	$F(124, 2480) = 77.791$	$p < 0.001$
	Diet x Time	Repeated measures ANOVA	$F(248, 2480) = 0.885$	$p = 0.894$
Paired-pulse facilitation (PPF)	Diet	Repeated measures ANOVA	$F(2,20) = 0.177$	$p = 0.839$
	Time	Repeated measures ANOVA	$F(11, 220) = 8.363$	$p < 0.001$
	Diet x Time	Repeated measures ANOVA	$F(22, 220) = 0.785$	$p = 0.742$

## Supplementary Table 2

**Supplementary Table 2.** Summary of the nutritional composition based in labelling of the selected cocoas, per 100 g of product.

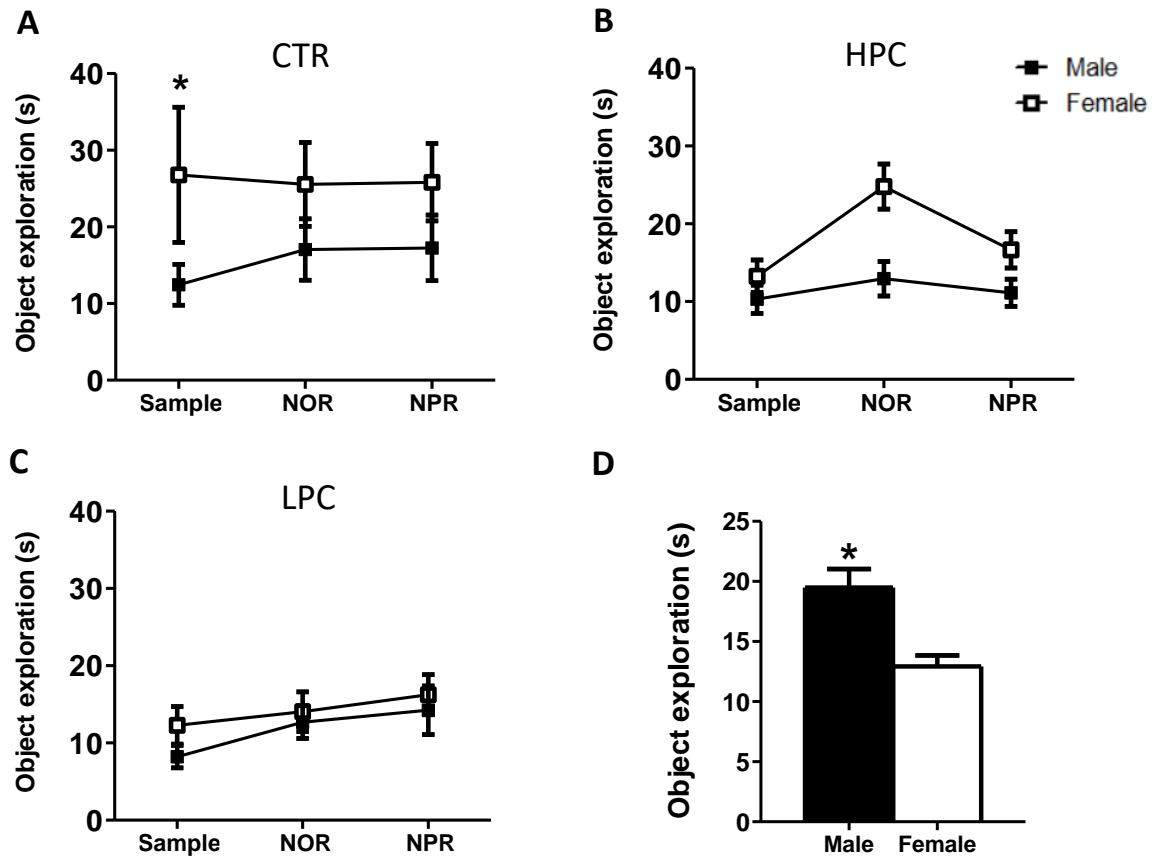
	<b>HPC</b>	<b>LPC</b>
<b>Energy (KJ)</b>	1338.88	1251.02
<b>Fat (g)</b>	12	12
<b>Saturated fat (g)</b>	7	7.4
<b>Carbohydrates (g)</b>	28	14.1
<b>Sugars (g)</b>	1.4	1.9
<b>Protein (g)</b>	19	22

## Supplementary Figure 1



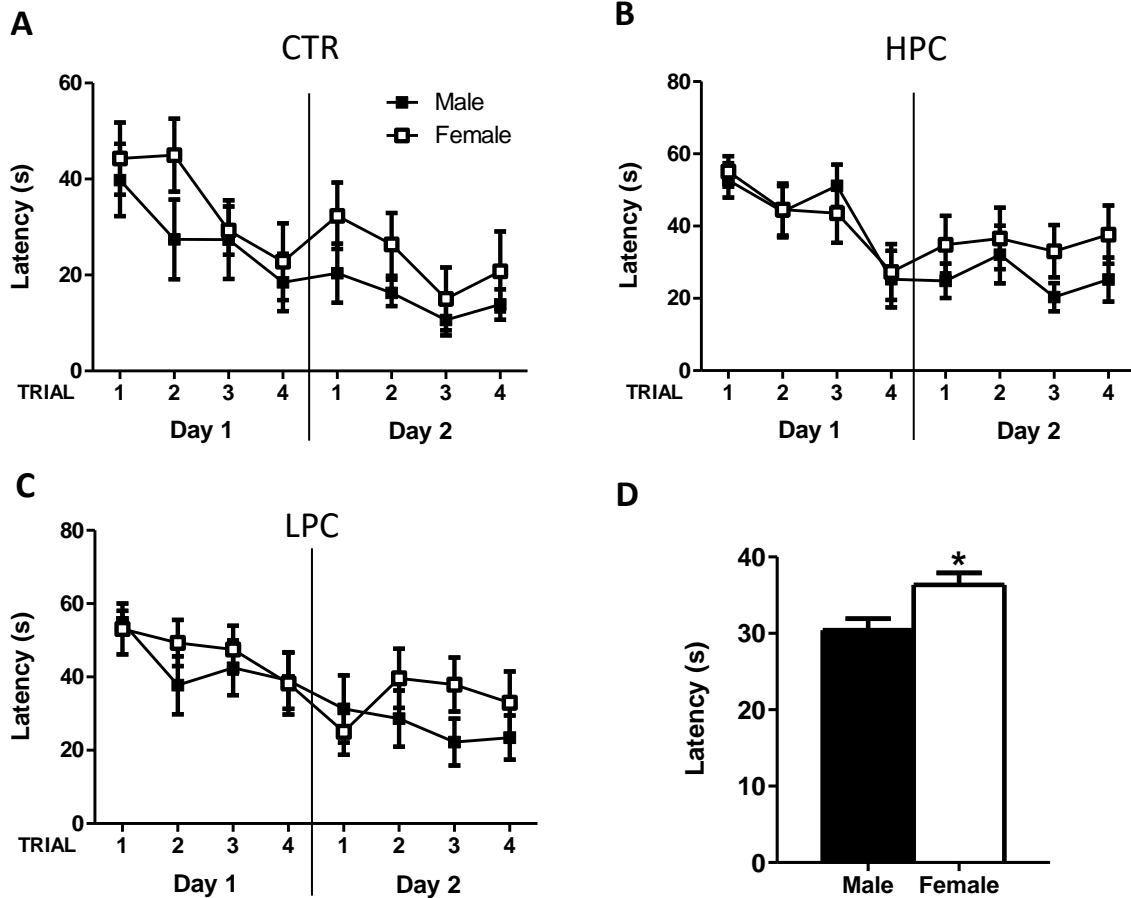
**Supplementary Figure 1.** Body weight differences with regard to sex. Irrespective of their dietary treatment, male mice exhibited a higher body weight compared to females [repeated measures ANOVA 'diet x day x sex' on body weight data: effect for 'diet':  $F(2, 42) = 0.315$ ,  $p = 0.731$ ; 'day':  $F(7, 294) = 15.169$ ,  $p < 0.001$ ; 'sex':  $F(1, 42) = 29.304$ ,  $p < 0.001$ ; 'diet x day':  $F(14, 294) = 2.546$ ,  $p = 0.002$ ; 'diet x sex':  $F(2, 42) = 0.143$ ,  $p = 0.867$ ; 'sex x day':  $F(7, 294) = 3.003$ ,  $p = 0.005$ , 'diet x sex x day':  $F(14, 294) = 0.987$ ,  $p = 0.466$ ]. For clarity, graphs A-C show data per individual diet treatment and day, while (D) shows data collapsed by sex. Results are expressed as mean  $\pm$  SEM; differences between sexes across all groups:  $**p \leq 0.001$  (D).

## Supplementary Figure 2



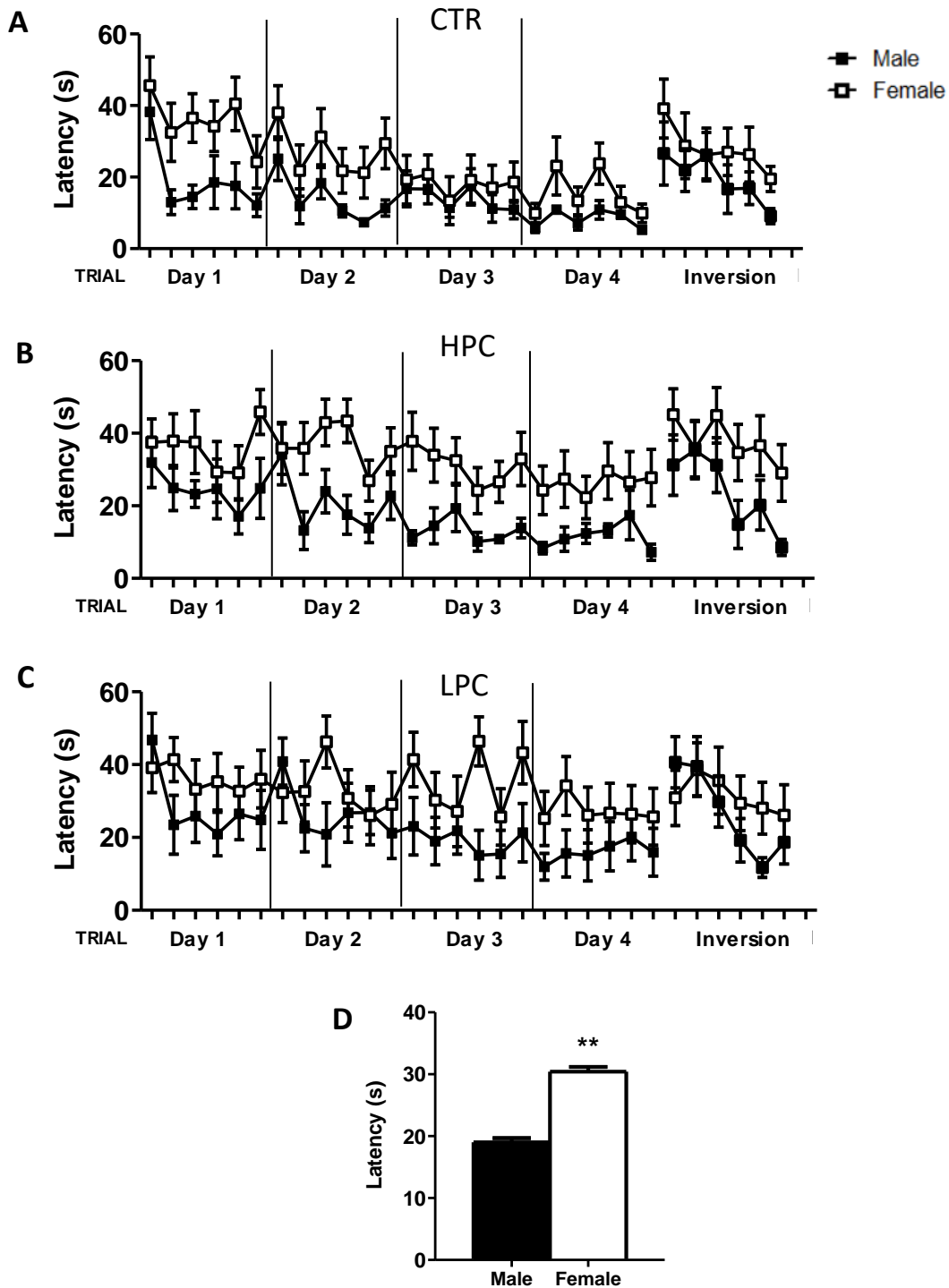
**Supplementary Figure 2.** Time spent exploring objects, differentiated by sex. When examining the cumulative data across all groups and sessions, males consistently spent more total time exploring objects than females [repeated measures ANOVA 'diet x trial x sex': effect for 'diet':  $F(2, 42) = 3.591$ ,  $p = 0.036$ ; 'session':  $F(2, 84) = 4.379$ ,  $p = 0.016$ ; 'sex':  $F(1, 42) = 6.899$ ,  $p = 0.012$ ; 'diet x session':  $F(4, 84) = 1.340$ ,  $p = 0.262$ ; 'diet x sex':  $F(2, 42) = 0.852$ ,  $p = 0.434$ ; 'sex x session':  $F(2, 84) = 0.280$ ,  $p = 0.756$ ; 'diet x sex x trial':  $F(4, 84) = 1.318$ ,  $p = 0.270$ ]. For clarity, graphs A-C are shown per individual diet treatment. Results are expressed as mean  $\pm$  SEM; differences between sexes across all groups: \* $p \leq 0.05$ .

### Supplementary Figure 3



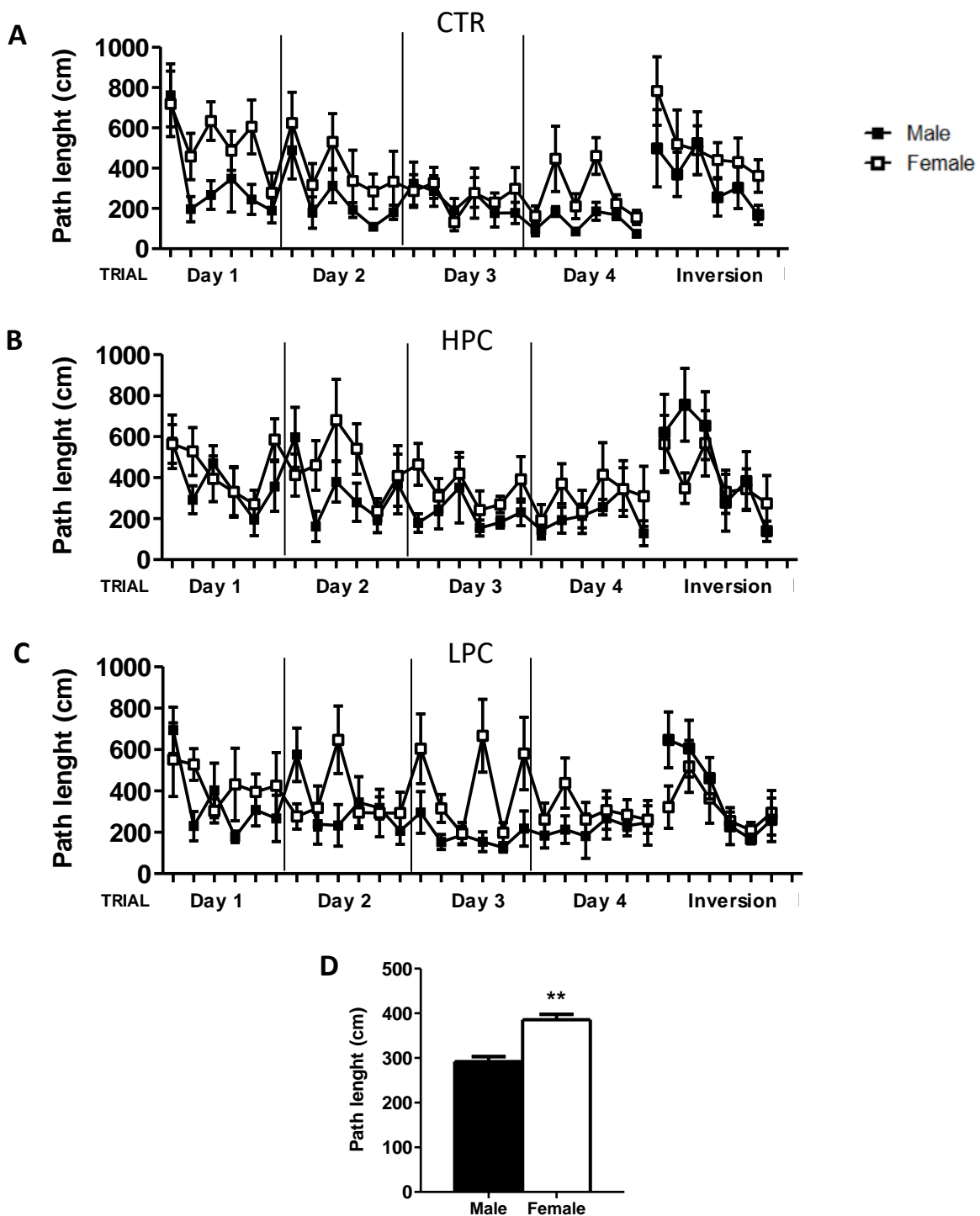
**Supplementary Figure 3.** Latency results in the water maze during the visible platform training presented by sex. When considering all groups collectively, female mice demonstrated an increased time required to reach the platform compared to males [repeated measures ANOVA 'diet x trial x sex' on latency data: effect for 'diet':  $F(2, 42) = 8.605$ ,  $p < 0.001$ ; 'trial':  $F(7, 294) = 12.26$ ,  $p < 0.001$ ; 'sex':  $F(1, 42) = 5.015$ ,  $p = 0.030$ ; 'diet x trial':  $F(14, 294) = 0.635$ ,  $p = 0.835$ ; 'diet x sex':  $F(2, 42) = 0.119$ ,  $p = 0.888$ ; 'sex x trial':  $F(7, 294) = 0.672$ ,  $p = 0.696$ ; 'diet x sex x trial':  $F(14, 294) = 0.448$ ,  $p = 0.958$ . For clarity, graphs A-C are shown per individual diet treatment. Results are expressed as mean  $\pm$  SEM; differences between sexes across all groups: \* $p \leq 0.05$  (D).

## Supplementary Figure 4



**Supplementary Figure 4.** During reference memory training, irrespective of their dietary treatment, female mice consistently exhibited an extended time to reach the platform compared to males [repeated measures ANOVA 'diet x trial x sex' on latency measures: effect for 'diet':  $F(2, 42) = 2.370$ ,  $p = 0.106$ ; 'trial':  $F(29, 1218) = 8.303$ ,  $p < 0.001$ ; 'sex':  $F(1, 42) = 12.769$ ,  $p < 0.001$ ; 'diet x trial':  $F(58, 1218) = 0.847$ ,  $p = 0.786$ ; 'diet x sex':  $F(2, 42) = 0.302$ ,  $p = 0.741$ ; 'sex x trial':  $F(29, 1218) = 1.106$ ,  $p = 0.319$ , 'diet x sex x trial':  $F(58, 1218) = 0.868$ ,  $p = 0.749$ ]. For clarity, graphs A-C are shown per individual diet treatment. Results are expressed as mean  $\pm$  SEM; differences between sexes across all groups: \*\* $p \leq 0.001$  (D).

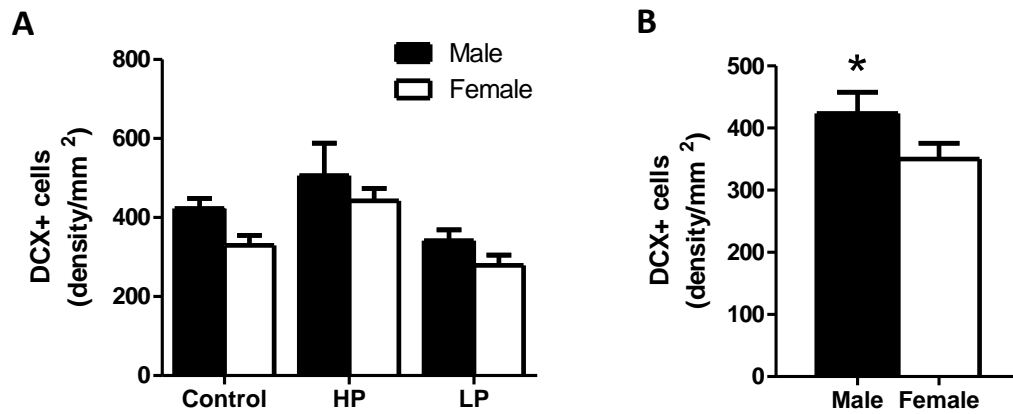
## Supplementary Figure 5



**Supplementary Figure 5.** (A-C) Path length during the reference memory training showed no significant variation between sexes within the same group. (D) Nonetheless, when considering the overall data across all groups, female mice swam greater distance compared to male [repeated measures ANOVA 'diet x trial x sex' on path length data: effect for 'diet':  $F(2, 42) = 1.931$ ,  $p = 0.158$ ; 'trial':  $F(29, 1218) = 7.028$ ,  $p < 0.001$ ; 'sex':  $F(1, 42) = 27.118$ ,  $p < 0.001$ ; 'diet x trial':  $F(58, 1218) = 1.156$ ,  $p = 0.204$ ; 'diet x sex':  $F(2, 42) = 6.056$ ,  $p = 0.005$ ; 'sex x trial':  $F(29, 1218) = 1.893$ ,  $p = 0.003$ , 'diet x sex x trial':  $F(46, 966) = 0.869$ ,  $p = 0.746$ . For clarity, graphs A-C are shown per individual diet treatment. Results are expressed as mean  $\pm$  SEM; differences between sexes across all groups: \*\* $p \leq 0.001$  (D).



## Supplementary Figure 6



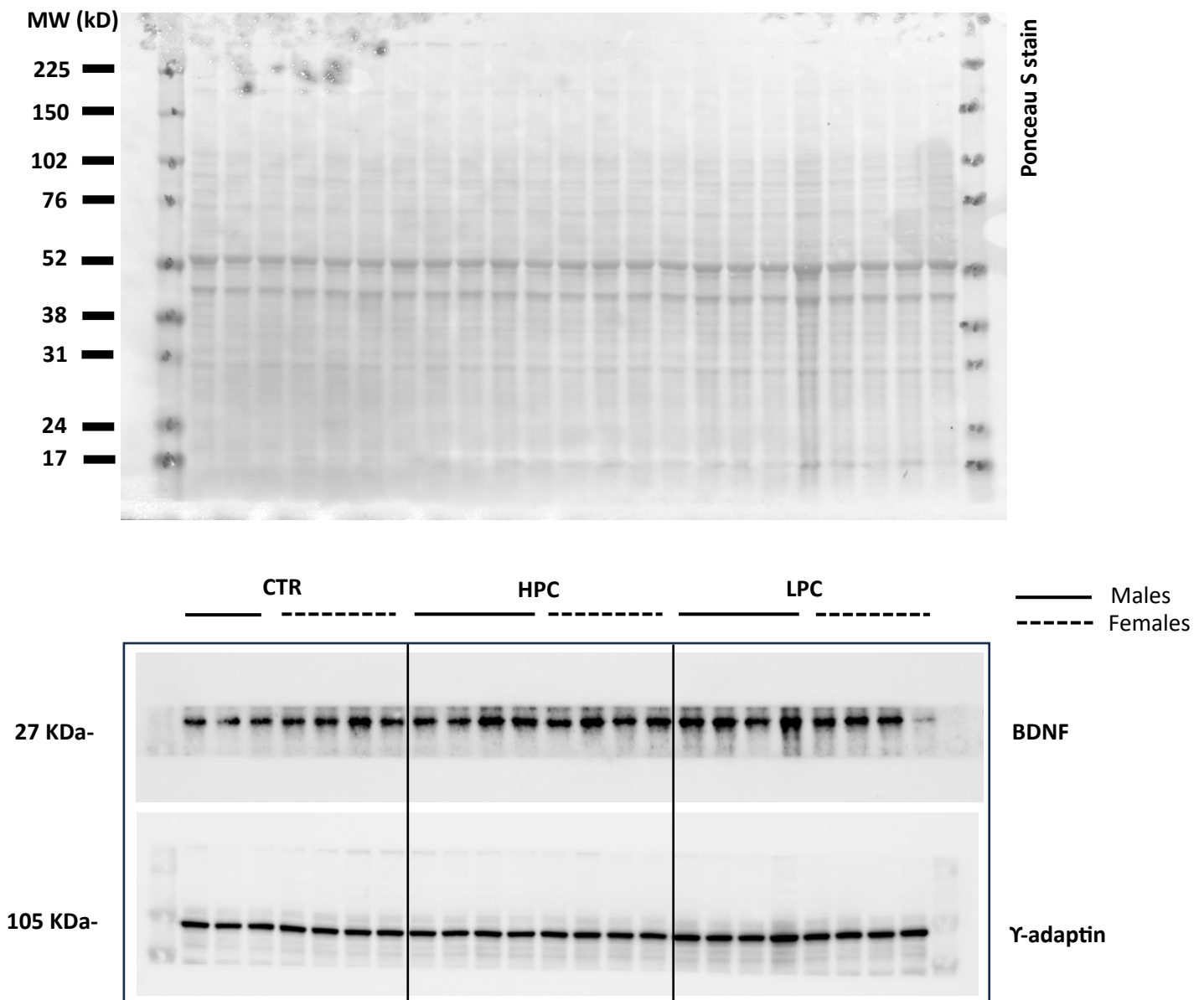
**Supplementary Figure 6.** While there were no sex differences in the total number of DCX+ cells within individual groups (A), the overall data revealed a higher total count of these cells in males compared to females (B). Two-Way ANOVA: 'Sex':  $F(1, 20) = 5.133$ ,  $p = 0.035$ ; 'diet':  $F(2,20) = 8.701$ ,  $p = 0.002$ . Results are expressed as mean  $\pm$  SEM; differences between sexes across all groups:  $*p \leq 0.05$ .

## Extra information

Considering the significant sample size ( $n = 42$ ), BDNF and  $\gamma$ -adaplin immunoblottings were performed on distinct membranes. Specifically, two membranes were designated for hippocampal analysis (Gels 1-2), while two were used for the prefrontal cortex (Gels 3-4). Red Ponceau Staining and individual membranes are shown below. BDNF and  $\gamma$ -adaplin immunoblottings conducted on Gels 1 and 4 are represented in Fig.4.

### HIPPOCAMPUS (unedited blots)

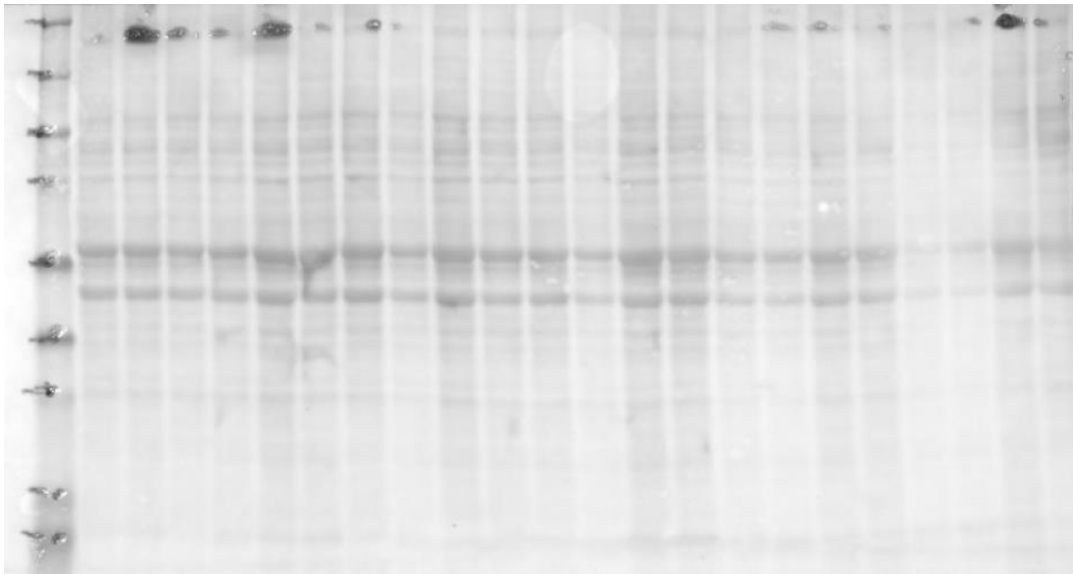
#### GEL 1



### GEL 2

MW (kD)

225  
150  
102  
76  
52  
38  
31  
24  
17



CTR

HPC

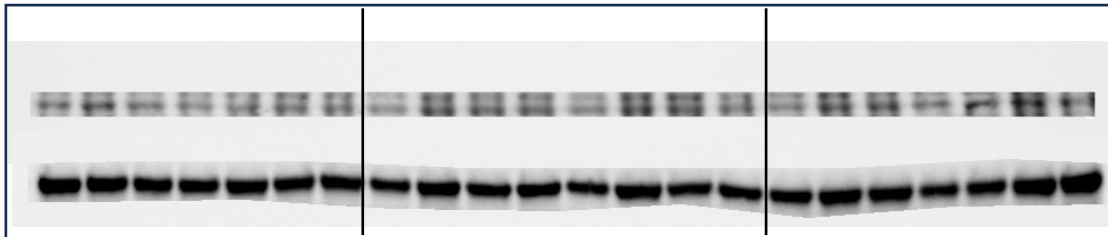
LPC

— Males

- - - Females

27 KDa-

105 KDa-

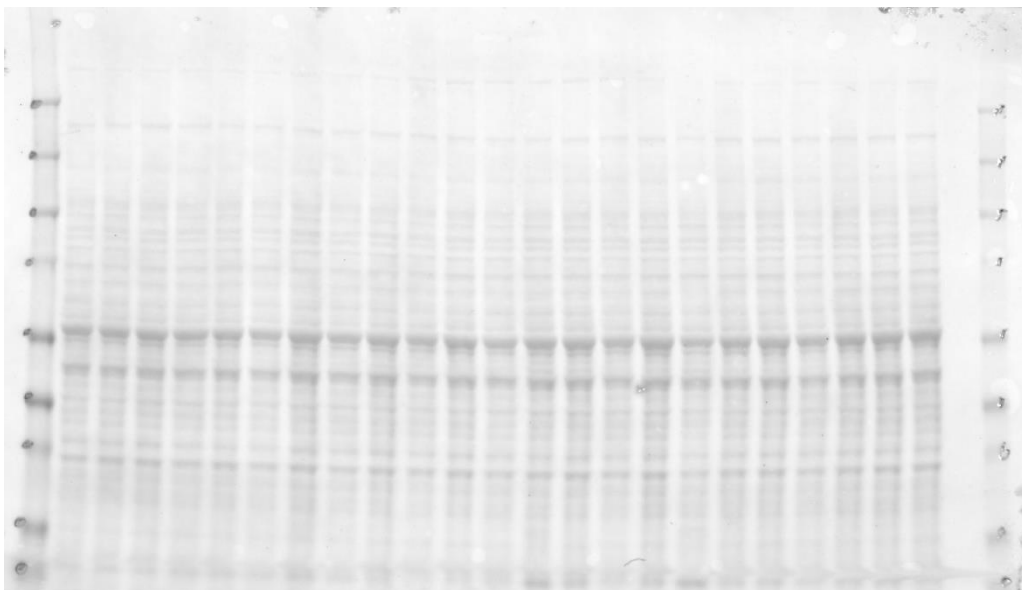


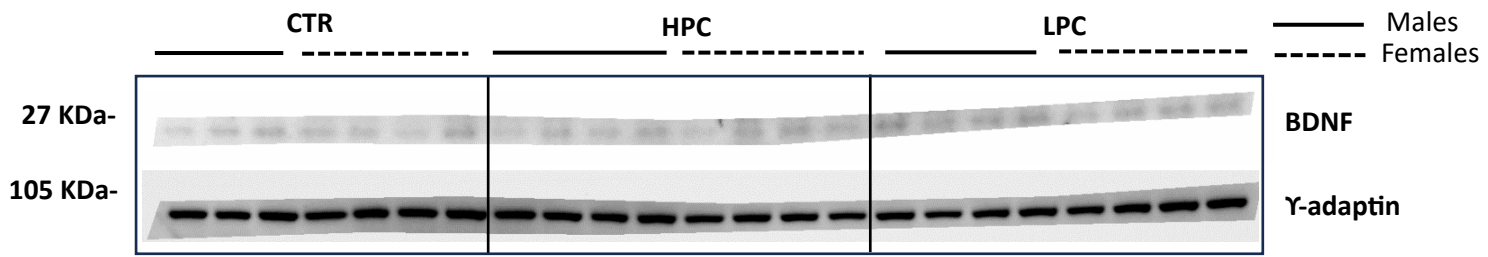
*PREFRONTAL CORTEX (unedited blots)*

### GEL 3

MW (kD)

225  
150  
102  
76  
52  
38  
31  
24  
17





**GEL 4**

