

## Supplementary Methods

### Score test

*Mecp2* WT and KO mice were scored every other day to evaluate the effect of CBDV treatment on Hindlimb claspings, gait, breathing, tremor, mobility and general condition. Each of the six symptoms was scored from 0 to 2 (0 corresponds to the symptom being absent or the same as in the WT animal; 1 when the symptom was present; 2 when the symptom was severe). Specifically: For tremor: the mouse was observed while standing on the flat palm of the hand. 0 = no tremor; 1 = intermittent mild tremor; 2 = continuous tremor or intermittent violent tremor. For hind limb claspings: 0 = WT; hind limbs splay outward when suspended by the tail; 1 = one hind limb is pulled into the body or forelimbs are stiff and splayed outward without motion; and 2 = one hind limb is pulled into the body and forelimbs are stiff and splayed outward without motion and might form a widened bowl shape or both hind limbs are pulled into the body with or without abnormal forelimb posture. For breathing: movement of flanks were observed while the animal was standing still. 0 = normal breathing; 1 = periods of regular breathing interspersed with short periods of more rapid breathing or with pauses in breathing; 2 = very irregular breathing-gasping or panting. For mobility: the mouse was observed when placed on bench, then when handled gently and scored as follows: 0 = as WT; 1 = reduced movement when compared with WT: extended freezing period when first placed on bench and longer periods spent immobile; 2 = no spontaneous movement when placed on the bench; mouse can move in response to a gentle prod or a food pellet placed nearby. For general condition: the mouse was observed for indicators of general well-being such as coat condition, eyes and body stance. 0 = clean shiny coat, clear eyes, and normal stance; 1 = eyes dull, coat dull/ungroomed, and somewhat hunched stance; 2 = eyes crusted or narrowed, piloerection, and hunched posture. For gait: 0 = as WT; 1 = hind limbs spread wider than WT when ambulating and/or a lowered pelvis when ambulating; and 2 = lack of full strides by hind limbs resulting in a dragging of hindquarters.

**Table S1**

	DILUTION	SUPPLIER
Rabbit polyclonal anti-BDNF	1:1000	Millipore, Italy
Goat polyclonal anti-IGF-1	1:1000	Millipore, Italy
Rabbit polyclonal anti-pAKT Ser473	1:1000	Cell Signaling, Danvers, MA
Rabbit polyclonal anti-phospho-rpS6	1:1000	Cell Signaling, Danvers, MA
Rabbit polyclonal anti-pERK1/2	1:1000	Cell Signaling, Danvers, MA
Rabbit polyclonal anti-cannabinoid CB1 receptor (CB1)	1:1000	Cayman Chemical, Ann Arbor, MI
Rabbit polyclonal anti-cannabinoid CB2 receptor (CB2)	1:1000	Cayman Chemical, Ann Arbor, MI
Rabbit polyclonal anti-N-acyl-phosphatidylethanolamine-hydrolysing phospholipase D (NAPE-PLD)	1:3000	Cayman Chemical, Ann Arbor, MI
Rabbit polyclonal anti-fatty acid amide hydrolase (FAAH)	1:2000	Cayman Chemical, Ann Arbor, MI
Goat polyclonal anti-diacylglycerol Lipase $\alpha$ (DAGL $\alpha$ )	1:1000	Abcam, Cambridge, UK
Rabbit polyclonal anti-monoacylglycerol lipase (MAGL)	1:1000	Cayman Chemical, Ann Arbor, MI

**Table S1:** List of primary antibodies used for Western blot analysis

Supplementary figures

Figure S1

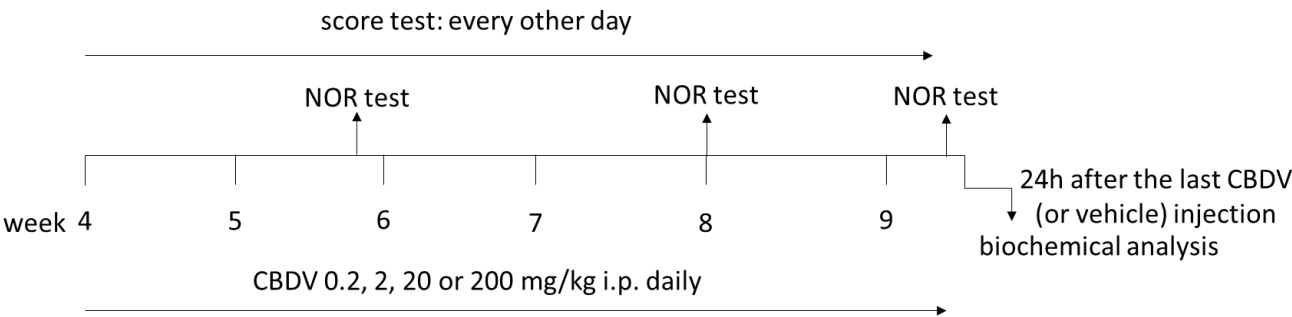
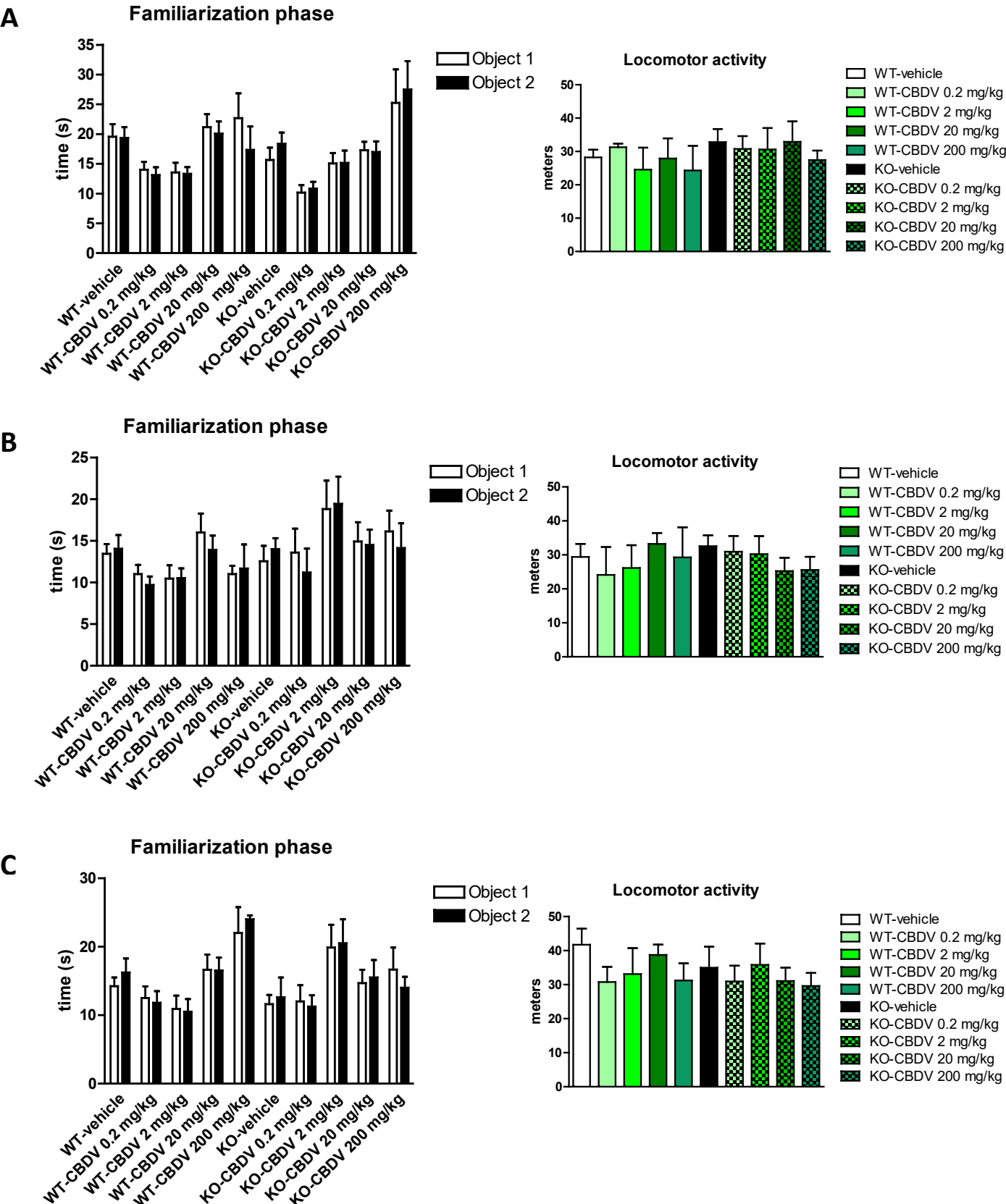


Figure S1: Timeline of the experiments

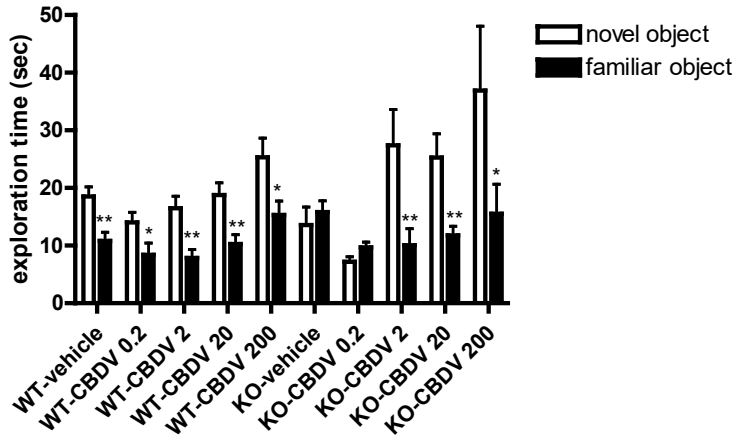
Figure S2



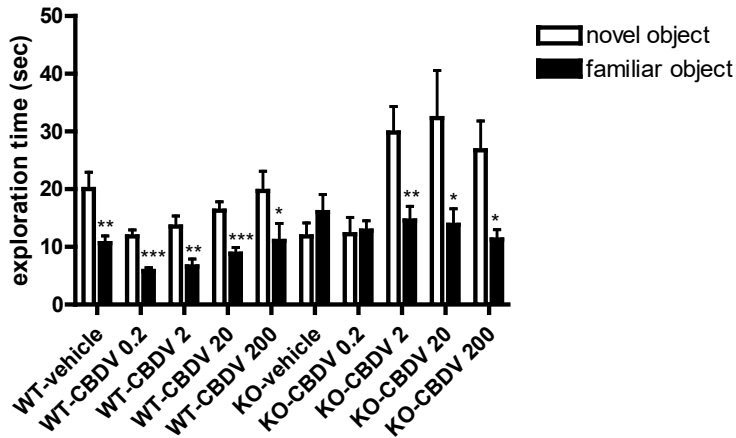
**Figure S2:** Exploration times and locomotor activity in vehicle- and CBDV-treated *Mecp2* WT and KO during the familiarization phase of the NOR test at PND 41 (A), 56 (B) and 66 (C). Left panels show the time spent exploring the two identical objects during the 10-minute session; right panels report total distance moved. Data are expressed as mean  $\pm$  S.E.M. and analyzed using Student t test or two-way ANOVA.

**Figure S3**

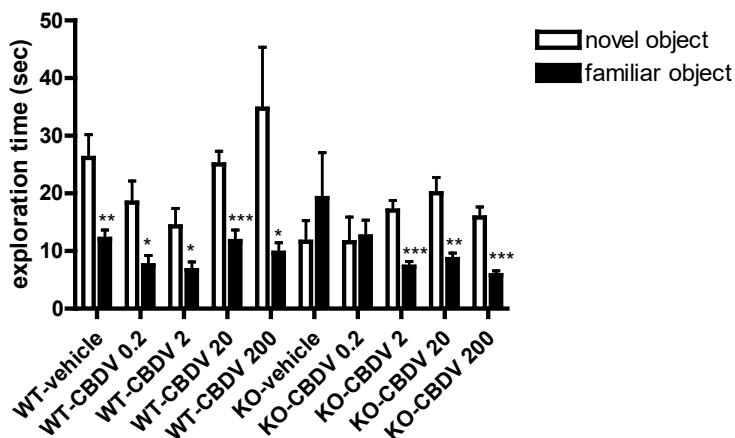
**A Short-term memory - PND 41**



**B Short-term memory - PND 56**

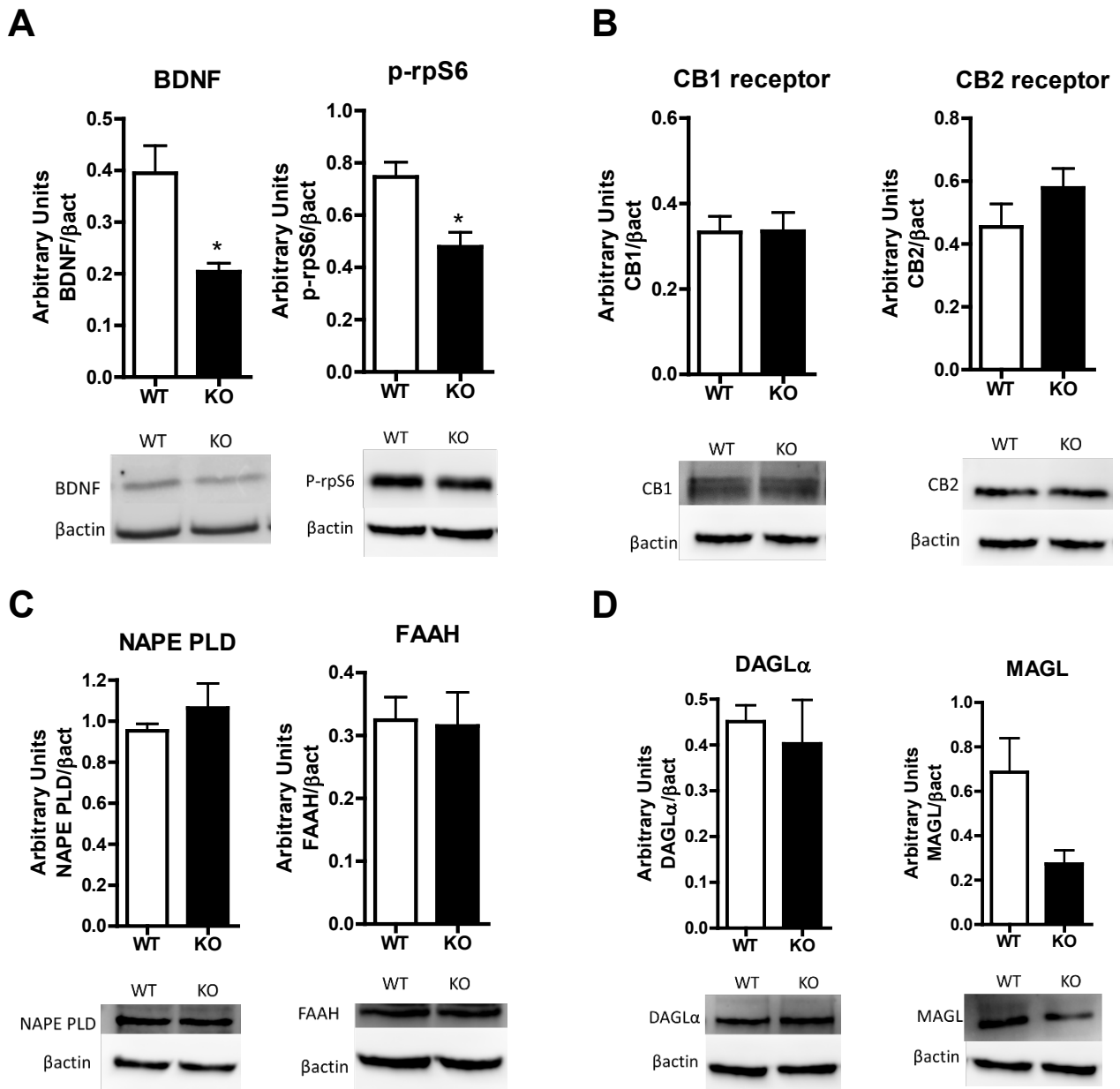


**C Short-term memory - PND 66**



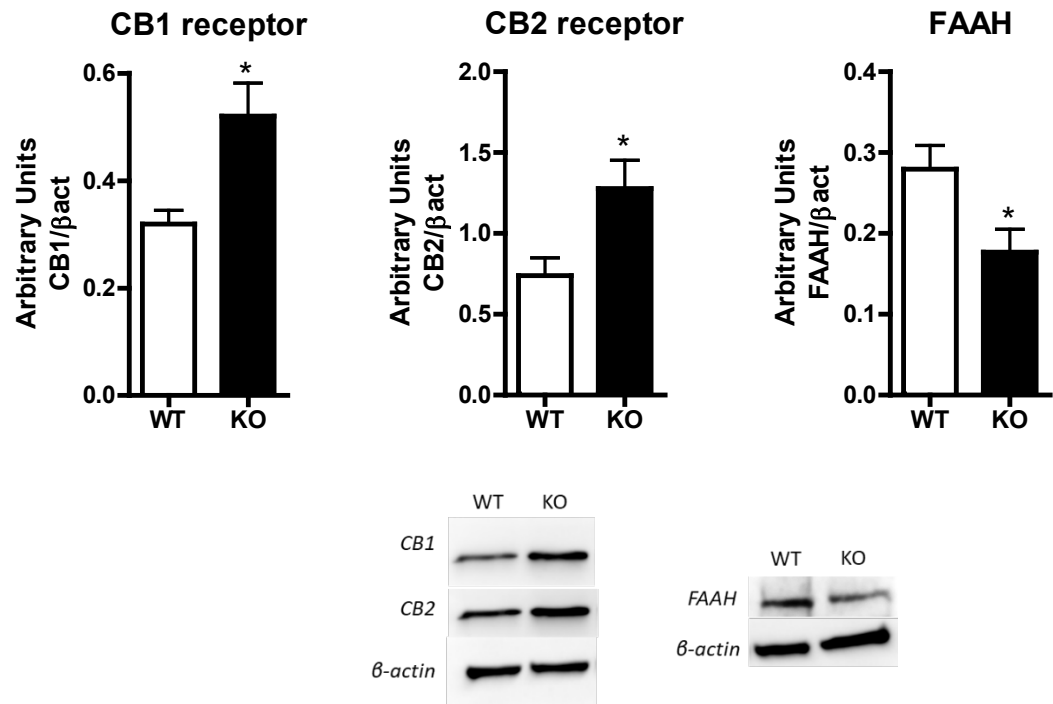
**Figure S3:** Exploration times of the novel object and the familiar object in vehicle- and CBDV-treated *Mecp2* WT and KO during the test phase of the NOR test at PND 41 (A), 56 (B) and 66 (C). Data are expressed as mean  $\pm$  S.E.M. and analyzed using Student t test. \*\*\*p<0.001, \*\*p<0.01, \*p<0.05 vs familiar object.

**Figure S4**



**Figure S4:** Protein levels of (A) BDNF and p-rpS6, (B) CB1 and CB2 receptors, (C) NAPE-PLD and FAAH, (D) DAGL $\alpha$  and MAGL in hemisected brains of pre-symptomatic 4-week-old Mecp2 WT and KO mice as measured by means of Western blot analysis. Data are expressed as mean  $\pm$  S.E.M. of 4 WT and 3 KO mice per group and were analyzed using unpaired Student t test. \* $p < 0.05$  vs WT.

**Figure S5**



**Figure S5:** Protein levels of cannabinoid CB1 and CB2 receptors and FAAH in hemisected brains of naïve 9-weeks-old Mecp2 WT and KO mice (no handling) as measured by means of Western blot analysis. Data are expressed as mean  $\pm$  S.E.M. of 4 mice per group and analyzed using unpaired Student t test. \* $p < 0.05$  vs WT.