Detailed methods for determination of Neuroscores: Part 1

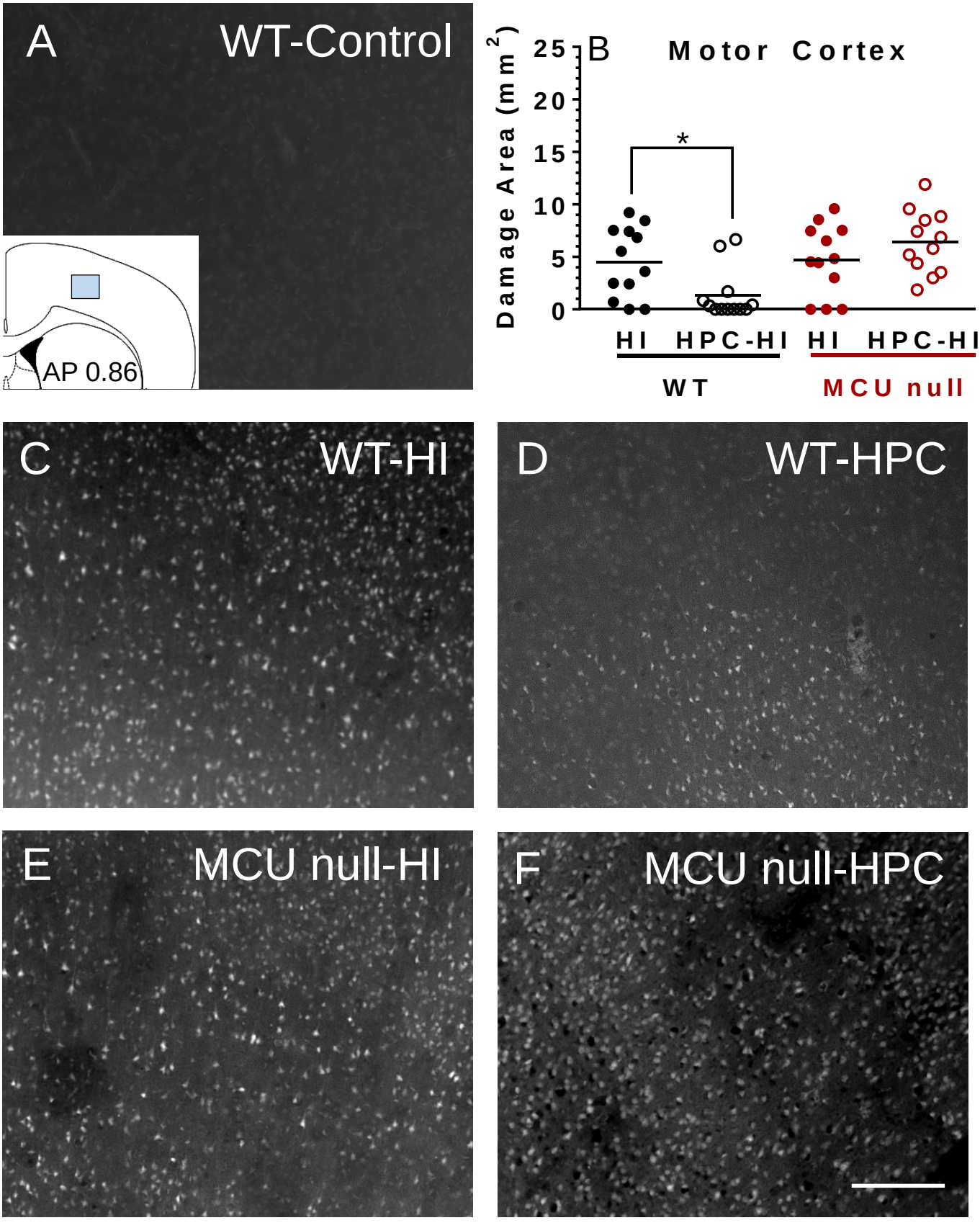
Assessment of general behavioural deficits

The 6 general deficit categories were scored as follows: *1. Hair.* Mouse observed on an open bench top (OBT) without interference: 0 - Hair neat and clean, 1 - Localized piloerection and dirty hair in 2 body parts (typically nose and eyes); 2 - Piloerection and dirty hair in more than 2 body parts. *2. Ears*. Mouse on observed on an OBT without interference. Mouse then stimulated by snapping fingers. 0 – Normal reaction, ears are stretched laterally and behind; 1 – Partial reaction, ears stretched laterally but not behind (one or both); 2 – No reaction to noise. *3. Eyes.* Mouse on OBT. Observed with no interference. 0 - Open, clear, quickly follow the surrounding environment/movement. 1 - Open and characterized by aqueous mucus. Slowly follow the surrounding environment; 2 - Open and characterized by dark mucus; 3 - Ellipsoidal shaped and characterized by dark mucus; 4 - Closed. *4. Posture*. Mouse placed on the palm and swung gently. 0 - The mouse stands up in the upright position with the back parallel to the palm. During the swing, it stands rapidly; 1 - The mouse stands humpbacked. During the swing, it flattens the body to gain stability; 2 - The head or part of the trunk lies on the palm; 3 - The mouse lies on one side, barely able to recover into the upright position; 4 - The mouse lies in a prone position, not able to recover/stand. *5. Spontaneous Activity*. Mouse on OBT. No interference. 0 - The mouse is alert and explores actively; 1 - The mouse seems alert but it is calm and sluggish; 2- The mouse explores intermittently and sluggishly, 3 - The mouse is somnolent and numb, few movements on the spot; 4 - No spontaneous movements. *6. Epileptic behaviour*. Mouse on OBT. The worst behaviour is recorded during the whole observation period. 0 – None; 3 - The mouse is reluctant to handling, shows hyperactivity; 6 - The mouse is aggressive, stressed and stares at environment; 9 - The mouse shows hyper-excitability, chaotic movements and presence of convulsion during handling or after handling; 12 - Generalized seizures associated with wheezing and unconsciousness.

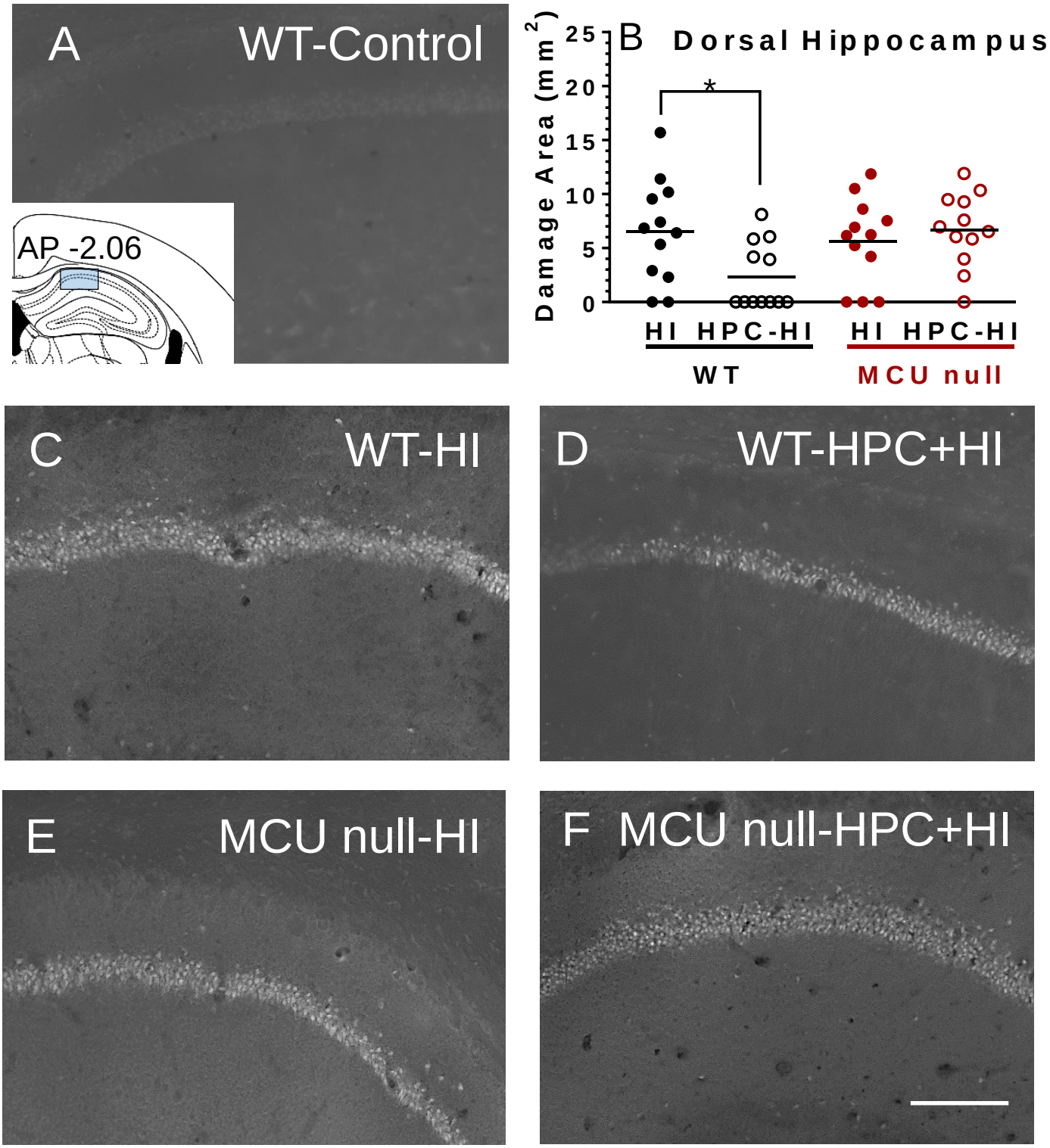
Detailed methods for determination of Neuroscores: Part 2

Assessment of focal behavioural deficits

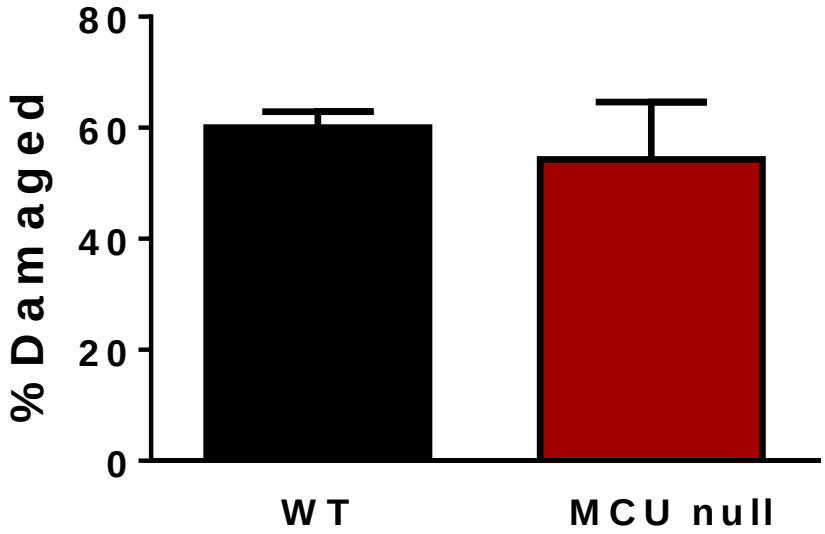
The 7 focal deficits categories were scored as follows: *1. Body Symmetry.* Mouse on OBT, observation of resting behaviour, the nose-tail line observed. 0 - Normal (normal posture, tail is straight); 1 - Slight asymmetry (body leans on one side with fore- and hind-limbs beneath the body, tail is slightly bent); 2 - Moderate asymmetry (body leans on one side with fore and hind-limbs stretched out, tail is slightly bent); 3 - Prominent asymmetry (body bent, one side is on the OBT, tail is bent); 4 - Extreme asymmetry (highly bent, on one side constantly lies on the OBT). *2. Gait.* Mouse on OBT, undisturbed movements. 0 - Normal. Flexible, symmetric and quick; 1 - Stiff, inflexible. Walks humpbacked, slower than normal; 2 - Limping with asymmetric movements; 3 - Trembling, drifting, falling; 4 - Does not walk spontaneously (gently pushed with pen to stimulate walking. If mouse takes no more than 3 steps, then receives a score of 4). 3.*Climbing.* Place mouse on a gripping surface 45 degrees to OBT. Placed in the centre of the surface and observed. 0 - Normal, climbs quickly to the top; 1 - Climbs with strain, limb weakness apparent; 2 - Holds onto slope, does not slip or climb; 3 - Slides down slope, unsuccessful effort to prevent fall; 4 - Slides immediately, no effort to prevent fall. *4. Circling behaviour*. Mouse observed on OBT. 0 - Absent. Turns equally to both sides; 1 - Predominately one sided turns; 2 - Circles to one sides, not constantly; 3 - Circles constantly to one side; 4 - Pivoting, swaying or no movement. *5. Forelimb symmetry*. Mouse suspended by the tail. Movements and position of forelimbs are observed. 0 - Normal. Both forelimbs are extended towards the bench and move actively; 1 - Light asymmetry. Contralateral limb does not extend entirely; 2 - Marked asymmetry. Contralateral forelimb bends towards the trunk. The body slightly bends on ipsilateral side; 3 - Prominent asymmetry. Contralateral forelimb adheres to the trunk; 4 - Slight asymmetry. Body/limb movement. *6. Compulsory circling.* Forelimbs on bench. Hind-limbs are suspended by the tail. This position reveals the presence of contralateral limb palsy. 0 - Absent. Normal extension of both forelimbs; 1 - Tendency to turn to one side. The mouse extended both forelimbs, but starts to turn preferably to one side; 2 - Circles to one side. The mouse turns towards one side with a slower movement compared to healthy mice; 3 - Pivots to one side sluggishly. The mouse turns towards one side failing to perform a complete circle; 4 - Does not advance. The front part of the trunk lies on the bench. Slow and brief movements. *7. Whisker Response*. Mouse on the bench. Using a pen, whiskers touch gently at the tip of the ears from behind, first one on the side with a lesion, then on the contralateral side. 0 - Normal symmetrical response. The mouse turns the head towards the stimulated area and withdraws from the stimulus; 1 - Light asymmetry, the mouse withdraws slowly when stimulated on the ischemic side. Normal response on the contralateral side; 2 - Prominent asymmetry. No response when stimulated on the side contralateral to ischemic injury. Normal response on the contralateral side; 3 - Absent response on the contralateral side, slow response when stimulated on the ipsilateral side; 4 - Absent response, no response when stimulated on the ipsilateral or contralateral sides.



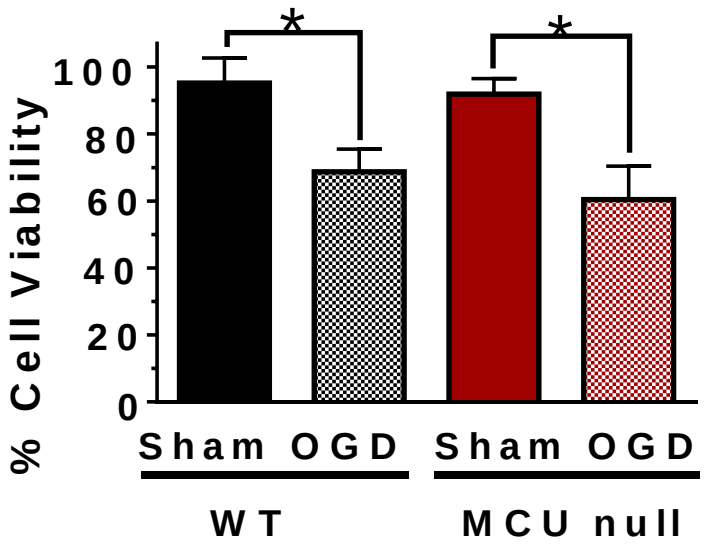
Supplementary Figure 1. Fluorojade (FJ)-positive neurons damaged by HI brain injury in the motor cortex of WT (A, C, D) and MCU nulls (E, F) subjected to sham conditions or HPC. (B) HI damage quantified by determining the area occupied by FJ-positive cells within the indicated sector of CA1 (insert, top left panel). *p<0.05, Two-way ANOVA followed by Bonferroni's post-hoc test. Scale bar = 150 µm.



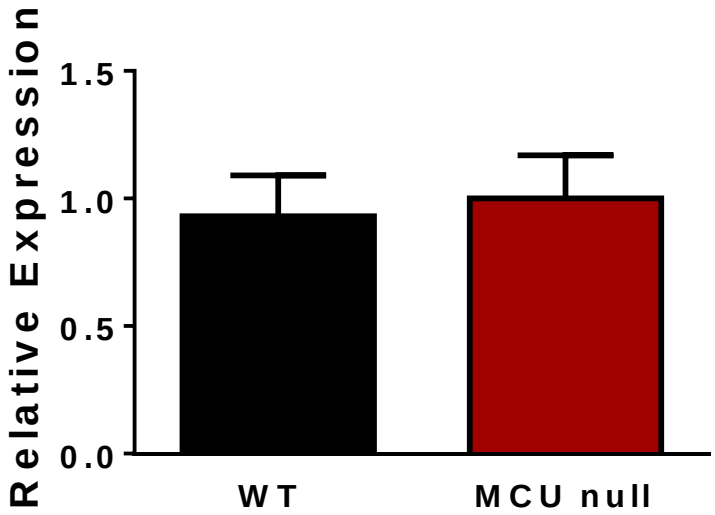
Supplementary Figure 2. Fluorojade (FJ)-positive neurons damaged by HI brain injury in the CA1 region of the dorsal hippocampus of WT (A, C, D) and MCU nulls (E, F) subjected to sham conditions or HPC. (B) HI damage quantified by determining the area occupied by FJ-positive cells within the indicated sector of CA1 (insert, top left panel). *p<0.05, Two-way ANOVA followed by Bonferroni's post-hoc test. Scale bar = 150 µm.



Supplementary Figure 3. Relative percentage of damaged mitochondria detected 2 hr after HI in 1024 electron microscopic images of the ipsilateral CA1 region in WT or MCU null mice. Unpaired t-test, $p>0.05$. Each bar represents the mean ± SEM (n=4/group).



Supplementary Figure 4. Primary neurons obtained from either WT or MCU null were subject to either sham or 2h OGD. Cell viability was measured 24 h following OGD using the MTT assay. Global MCU ablation in neurons did not confer protection to OGD. *p<0.05, Two-way ANOVA followed by Bonferroni's post-hoc test (n=4/group).



Supplementary Figure 5. Relative mRNA levels for the Complex I member (MT-ND2) detected by qRT-PCR in MCU null and WT cortical neurons. Unpaired t-test, $p>0.05$. Each bar represents the mean ± SEM (n=7/group).