Supplemental materials and methods

Methods used in mouse behavior tests

Open Field

The Open Field test was carried out according to the standardized phenotyping screens developed by the Eumorphia protocol (Holter et al., 2015). The test apparatus (ActiMot, TSE) was a square-shaped frame with two pairs of light-beam strips, each pair consisting of one transmitter strip and one receiver strip. These basic light barrier strips were arranged at right angles to each other in the same plane to determine the X and Y coordinates of the animal, and thus its location (XY frame). With two further pairs of uni-dimensional light-barrier strips (Z1 and Z2), rearing could be detected in addition to location.

All animals were transported to the test room and left undisturbed for at least 30 minutes before the testing started. Then each animal was placed individually into the middle of one side of the arena facing the wall and allowed to explore it freely for 20 min. After each trial, the test arena was cleaned carefully with a disinfectant. For data analysis, the arena was divided by the computer in two areas, the periphery defined as a corridor of 8 cm width along the walls and the remaining area representing the center of the arena (42% of the total arena in our TSE-system).

The following parameters were recorded: distance traveled, resting and permanence time as well as speed of movement for the whole arena, the periphery and the center. Additionally, rearing frequency, percentage distance traveled and percentage time spent in the center as well as the latency to first entry in center and center entry frequency were calculated.

Transfer arousal
Mice were removed from their home cage and transferred to the center of a viewing arena. The immediate reaction of the mouse to the new environment was recorded as extended freeze (freeze for a period longer than 5 seconds), brief freezes, or immediate movement.

**Rotarod**

The rotarod (Bioseb, Chaville, France) was used to measure fore limb and hind limb motor coordination, balance and motor learning ability (Jones and Roberts, 1968). The unit consists of a rotating spindle and five individual lanes for each mouse. All mice were placed on the Rotarod at an accelerating speed from 4 to 40 rpm for 300 seconds with 15 min between each of the three trials performed. The mean latency to fall off the Rotarod during the trials was recorded and used in subsequent analysis. In addition, the reason for the trial end (falling, jumping or rotating passively) was recorded.

**Acoustic startle**

Acoustic startle and its prepulse inhibition were assessed using a startle apparatus setup (Med Associates Inc., VT, USA) including four identical sound-attenuating cubicles. The protocol is based on the Eumorphia protocol (www.empress.har.mrc.ac.uk), adapted to the specifications of our startle equipment. Background noise was 65 dB, and startle pulses were bursts of white noise (40 msec). A session was initiated with a 5-min-acclimation period followed by five presentations of leader startle pulses (110 dB) that were excluded from statistical analysis. Trial types included prepulse alone trials at four different sound pressure levels (67, 69, 73, 81 dB), and trials in which each prepulse preceded the startle pulse (110 dB) by a 50 msec inter-stimulus interval. Each trial type was presented 10 times in random order,
organized in 10 blocks, each trial type occurring once per block. Inter-trial intervals varied from 20-30 sec.

**Hot plate**

The mice were placed on a metal surface maintained at 52 ± 0.2 C (TSE GMBH, Germany) (Eddy and Leimbach, 1953). Locomotion of the mouse on the hot plate was constrained by 20 cm high Plexiglas wall to a circular area with a diameter of 28 cm. Mice remained on the plate until they performed one of three behaviors regarded as indicative of nociception: hind paw lick (h.p. licking), hind paw shake/flutter (h.p. shaking) or jumping. Each mouse was tested only once since repeated testing leads to profound changes in response latencies. The latency was recorded to the nearest 0.1 s. To avoid tissue injury 30 s cut-off time was used.
Deng et al Supplemental Figure S1: The mRNA expression patterns of (A) pluripotency markers, (B) ectoderm markers, (C) mesoderm markers, and (D) endoderm markers during WT and DKO ESCs differentiation. Data were obtained from 3 independent ESC clones and are presented as Mean ± SEM (* P < 0.05, ** P < 0.01).
Deng et al Supplemental Figure S2: The comparison of transcriptome (mRNA-seq) between each individual replicate and the average of 3 independent WT or DKO ESC clones at (A) day 0, (B) day 2, (C) day 4, (D) day 6 and (E) day 8 of embryoid body differentiation.

R indicates Pearson correlation coefficient.
Deng et al Supplemental S3. Epigenetic markers around gene Olig1&2. (A) Genome browser snapshot visualizes mRNA expression level of gene Olig1&2 and various histone modification markers around gene Olig1&2 in WT and DKO ESCs. Numbers in parenthesis indicate the scales of the y-axis, in RPM. (B) ChIP-qPCR analysis of IgG control (related to Figure 3 B-E) in indicated groups and regions around gene Olig1&2. Data were obtained from two independent ESC clones (biological replicates) and presented as Mean ± SEM.
**Deng et al Supplemental Figure S4. In Vitro HMT Assay.** (A) Substrates on a Coomassie-stained polyacrylamide gel: lane 1: core histones from H1-depleted poly-nucleosome preparation, note there is no H1 band; lane 2: free histone H3; lane 3: histone H1; lane 4: wild-type HMGN1; lane 5: wild-type HMGN2; lane 6: mutant HMGN1 S20,24E. (B) HMT assays show the H3K27me3 levels when free histone H3 was used as substrate and pre-mixed with different combination of H1 and HMGNs as indicated and then subjected to the HMT reactions with EZH2 complex. (C) Quantification of panel B. Relative level of H3K27me3 has been normalized to that of H3. Data were obtained from 2 independent experiments and are presented as Mean ± SEM.
Deng et al. Supplemental Figure S5. Loss of HMGNs leads to neurological phenotypes. (A) Open field test shows decreased rearing activity in DKO mice. (B) Altered transfer arousal in female DKO mice suggests reduced neurotransmission activity. (C-D) Altered behavior in rotarod tests suggests of impaired balance and motor coordination. (E) Decreased acoustic startle reactivity in DKO mice suggests reduced sensorimotor performance and impaired rate of neurotransmission (BN: background noise, ns: not significant). (F) Increased hot plate response time in DKO mice suggesting decreased rate of signal transmission along nerve fibers. All the data were obtained from 15 mice per genotype per gender and are presented as Mean ± SEM (* P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001).
Supplemental Table S1: ChIP-qPCR primers used in Figure 3 and Figure 4.

| Site | Primer sequences                                      | Product Size |
|------|-------------------------------------------------------|--------------|
| 1    | F: TGGAGTGCTTTACCGCACAC R: TGTCCCTTGGCGCGAGATTCCTC | 146 bp       |
| 2    | F: ATGTTGCTCTACTCCAGGAC R: AATACAGTCAAAGCGCAACG     | 120 bp       |
| 3    | F: TTAGACGAGACGCAGAGCAC R: CACGCCAGCGTCTACTACTAATC | 158 bp       |
| 4    | F: CCGCTGTTGGCAGAGATACCT R: CTTGGGGAGGCGAAGTACG     | 151 bp       |
| 5    | F: CTCCTTGGGCTGGGCTACTC R: TTGGCTGACACCTGCAAATC     | 108 bp       |
| 6    | F: CGGCTATTCTCGTATCCG T: CATGCCAGGAAAACAAAGCTG      | 121 bp       |
| 7    | F: GTGGGCTATGTAACCTGGG R: TAGTTTCAAGCTGCCCTCC       | 124 bp       |
| 8    | F: CTTTTGTGTCGGCATTG ACA R: CGTAACAGCGAACAACCTG GC  | 119 bp       |
| 9    | F: ATGCACCGGAGGTCATAAG R: CCCCTGGGGGATAGGGAGTTA     | 130 bp       |
| 10   | F: GCACCTGGTGATCTGGCTCTC R: TTCTGACCTGCTGTGGCAC     | 120 bp       |