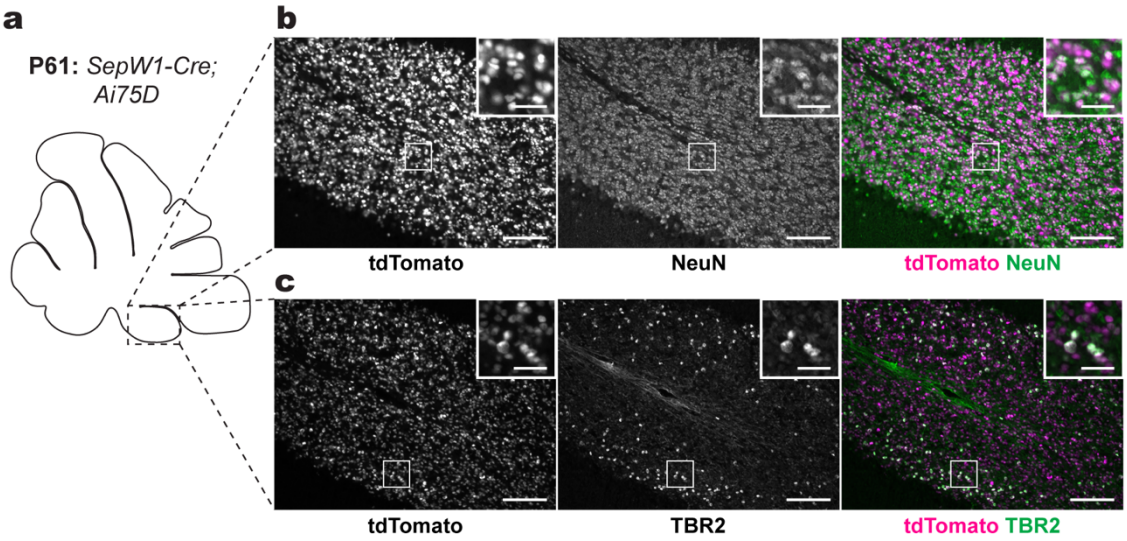
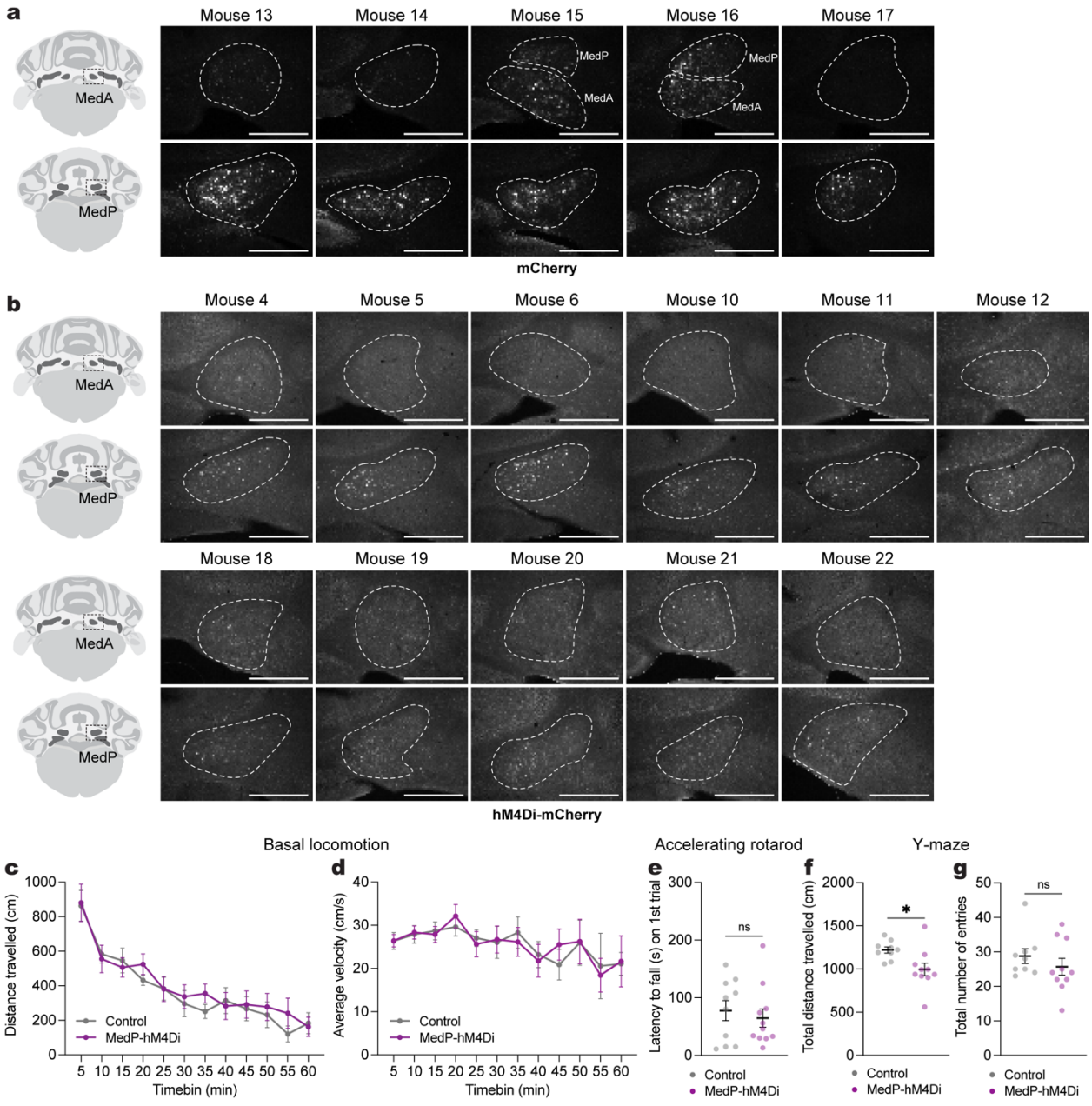


Supplementary Fig. 1 | *SepW1-Cre* recombines in granule cells and unipolar brush cells.

a, Schematic representation of sagittal plane of an adult mouse showing where **b** and **c** images were acquired.

b, Immunofluorescence images of tdTomato (magenta) and NeuN (green) co-expressing granule cells in adult *SepW1-Cre; Ai75D* mice. Scale bars = 100 um; inset scale bars = 25 um.

c, Immunofluorescence images of tdTomato (magenta) and TBR2 (green) co-expressing unipolar brush cells in adult *SepW1-Cre; Ai75D* mice. Scale bars = 100 um.



Supplementary Fig. 2 | Motor coordination during behavior is largely intact after acute chemogenetic inhibition of adult MedP eCN.

a, Schematic (left) and representative images of mCherry expression (right) in MedA (upper row) and MedP (lower row) CN in the five control mice. The other six control mice were Cre-negative (treated with CNO). Scale bars = 500 um.

b, Schematic (left) and representative images of hM4Di-mCherry expression (right) in MedA and MedP CN in the eleven MedP-hM4Di mice. Scale bars = 500 um.

c, Distance travelled during basal locomotion by 5 min time bins (n=11 per group). Repeated measure two-way ANOVA: no main effect of time (P = 0.0609), chemogenetics (P = 0.9730) or interaction (P = 0.9975).

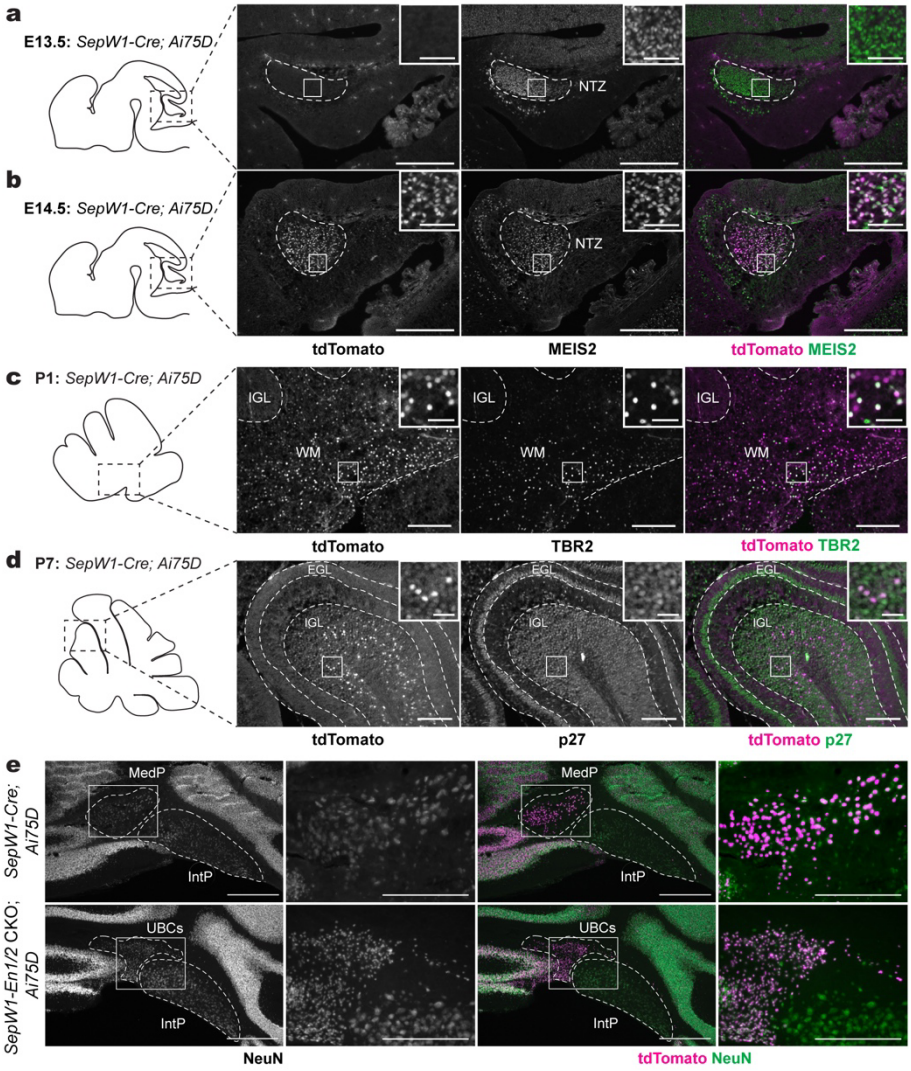
d, Average velocity during basal locomotion by 5 min time bins (n=11 per group). Repeated measure two-way ANOVA: main effect of time ($F_{4.870,97.41}$ = 28.43, P < 0.0001), but not of chemogenetics (P = 0.7000) or interaction (P = 0.8668).

e, Latency to fall on the first trial of the accelerating rotarod test (MedP-hM4Di: n=11, control: n=10; Mann Whitney *U* test: *U* = 48, P = 0.6412).

f, Total distance travelled in the Y-maze (MedP-hM4Di: n=10, control: n=9; two-tailed unpaired t-test: t_{17} = 2.648, P = 0.0169).

g, Total number of arm entries in the Y-maze (MedP-hM4Di: n=10, control: n=9; Mann Whitney *U* test: *U* = 30, P = 0.2326).

ns, not significant: $P \geq 0.05$. Data are presented as mean values ± SEM.



Supplementary Fig. 3 | *SepW1-Cre* recombines in the developing excitatory cerebellar neurons and *SepW1-En1/2* CKOs have preferential loss of MedP CN.

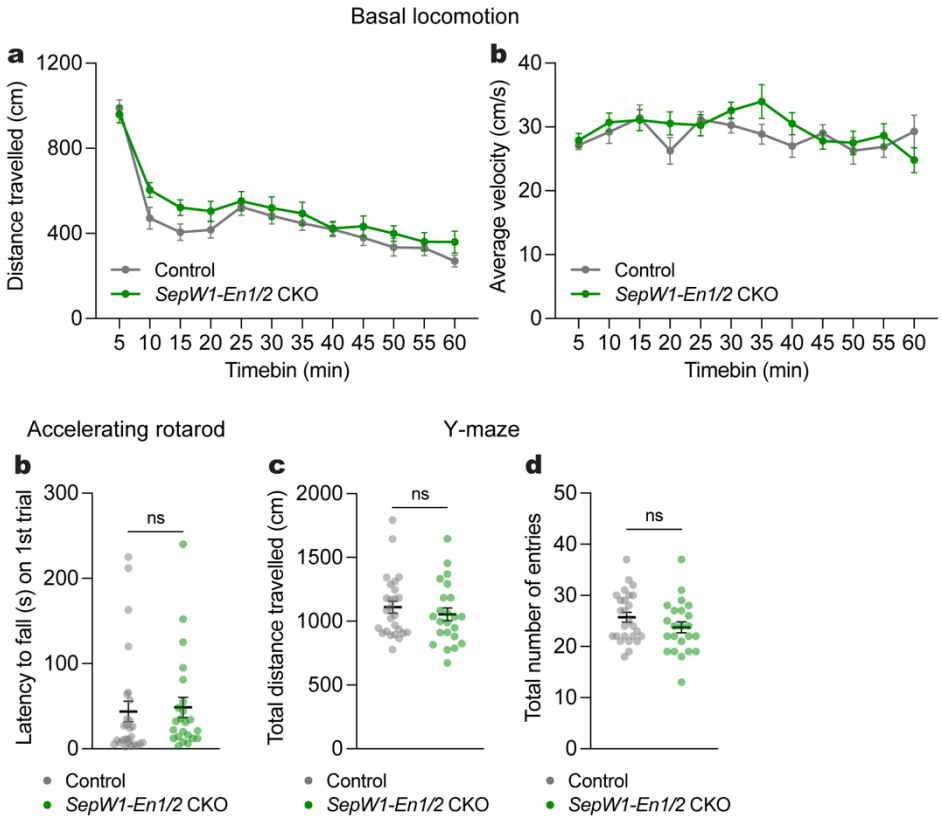
a,b, Schematic (left) and representative images (right) of sagittal sections stained for tdTomato (magenta) in *SepW1-Cre; Ai75D* mice showing recombination at E14.5 (**b**) but not at E13.5 (**a**) in eCN in the NTZ marked by MEIS2 (green). NTZ = nuclear transitory zone.

c, Schematic (left) and representative sagittal image (right) of tdTomato (magenta) expression in *SepW1-Cre; Ai75D* mice showing recombination at postnatal day (P1) in TBR2+ (green) unipolar brush cells.

d, Schematic (left) and representative sagittal images (right) of tdTomato (magenta) expression in *SepW1-Cre; Ai75D* mice showing recombination at P7 in p27+ (green) differentiated granule cells in the internal granule cell layer (IGL), but not in proliferating granule cell precursors in the external granule cell layer (EGL). Scale bars = 100 um; inset scale bars = 20 um.

e, Representative images of coronal sections stained for NeuN (single channel), NeuN (green) and tdTomato (magenta) co-labeling in the posterior CN of *SepW1-Cre; Ai75D* and *SepW1-En1/2* CKO; *Ai75D* mice (*SepW1-Cre/+; En1*$^{flox/flox}$*; En2*$^{flox/flox}$*; R26*$^{LSL\text{-}nls\text{-}tdTomato/+}$). NeuN labeling near the MedP of mutants are ectopic unipolar brush cells that are not TBR2+ or MEIS2+ (confirmed in Krishnamurthy et al., 2024). Abbreviations: MedP=Posterior medial; IntP=Posterior interposed. Scale bars for low magnification = 500 um; scale bars for high magnification = 100 um.

Scale bars in **a**, **b**, **c** = 250 um; inset scale bars = 50 um.



Supplementary Fig. 4 | Motor coordination during behaviors is not altered in *SepW1-En1/2* CKOs.

a, Distance travelled during basal locomotion by 5 min time bins (*SepW1-En1/2* CKOs: n=24, littermate controls: n=27). Repeated measure two-way ANOVA: main effect of time ($F_{5.410,265.1}$ = 71.20, P < 0.0001), but not of genotype (P = 0.2081) or interaction (P = 0.2042).

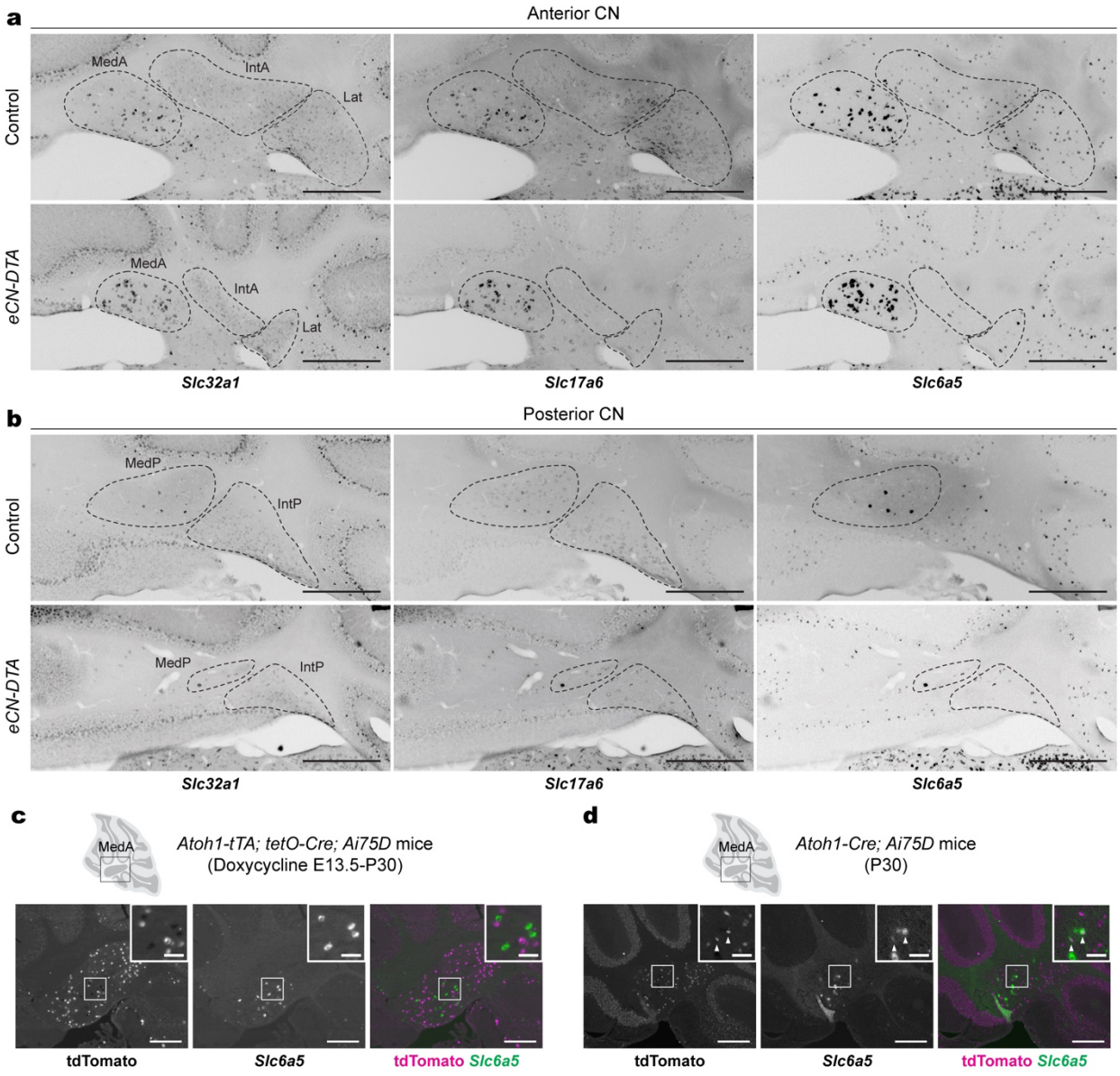
b, Average velocity during basal locomotion by 5 min time bins (*SepW1-En1/2* CKOs: n=24, littermate controls: n=27). Repeated measure two-way ANOVA: main effect of time ($F_{7.996,390.3}$ = 2.386, P = 0.0162), but not of genotype (P = 0.3429) or interaction (P = 0.1806).

c, Latency to fall on the first trial of the accelerating rotarod test (*SepW1-En1/2* CKOs: n=23, littermate controls: n=27; Mann-Whitney *U* test: *U* = 240.5, P = 0.1759).

d, Total distance travelled in the Y-maze (*SepW1-En1/2* CKOs: n=23, littermate controls: n=26; Mann-Whitney *U* test: *U* = 266, P = 0.5184).

e, Total number of arm entries in the Y-maze (*SepW1-En1/2* CKOs: n=23, littermate controls: n=26; two-tailed unpaired t-test: $t_{47} = 1.397$, $P = 0.1689$).

ns, not significant: $P \geq 0.05$. Data are presented as mean values ± SEM.



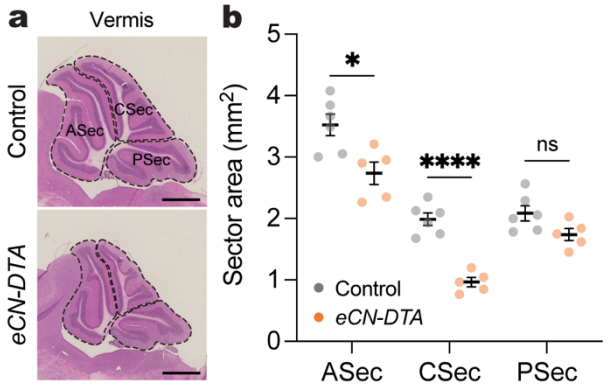
Supplementary Fig. 5 | Remaining CN neurons in *eCN-DTA* mice are inhibitory neurons.

a,**b**, Representative images of coronal sections of triple RNA *in situ* analysis of *Slc32a1*, *Slc17a6* and *Slc6a5* with single channel expression in anterior (**a**) and posterior (**b**) CN of *eCN-DTA* mice and littermate controls. Dotted outlines indicate the CN subregions. Abbreviations: MedA=Anterior medial; MedP=Posterior medial; IntA=Anterior interposed; IntP=Posterior interposed; Lat=Lateral. Scale bars = 500 um.

c, Representative images from the MedA region of double RNA *in situ* hybridization and immunofluorescence for *Slc6a5* and tdTomato in P30 *Atoh1-tTA; tetO-Cre; Ai75D* (*Atoh1-tTA/+;*

tetO-Cre; R26$^{LSL\text{-}nls\text{-}tdTomato/+}$) mice treated with doxycycline from E13.5 until P30. *Slc6a5*+ CN neurons are not labeled by the *Atoh1-tTA* transgene (tdTomato as a readout). Scale bars = 250 um; inset scale bars = 50 um.

d, Representative images from the MedA region of double RNA *in situ* hybridization and immunofluorescence for *Slc6a5* and tdTomato in P30 *Atoh1-Cre; Ai75D* mice. Subset of *Slc6a5*+ CN neurons are labeled by the *Atoh1-Cre* transgene (tdTomato as a readout). Scale bars = 250 um; inset scale bars = 50 um.

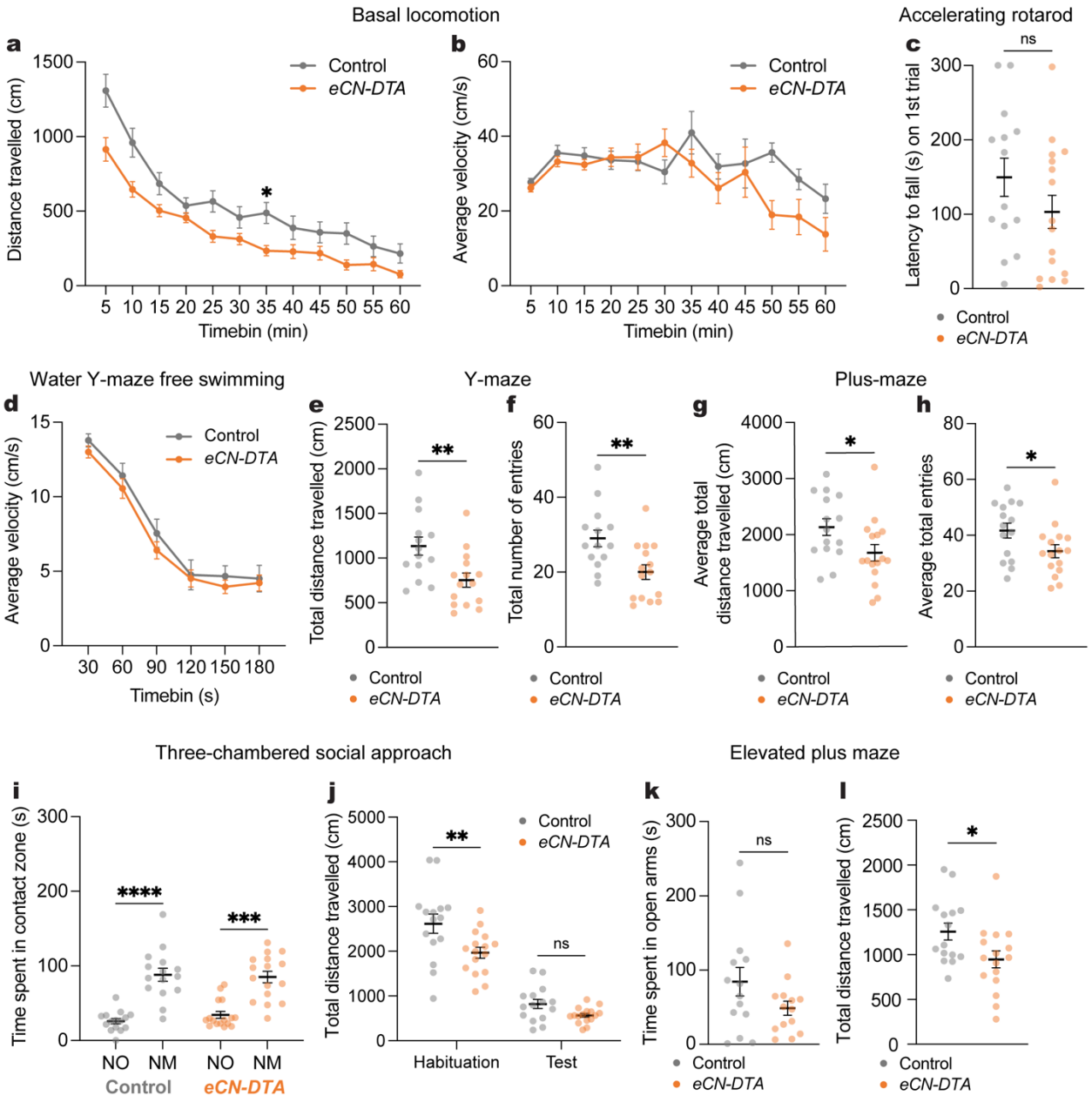


Supplemental Fig. 6 | *eCN-DTA* mice show reduced growth in the anterior and central vermis.

a, Representative images of H&E labeled vermis in *eCN-DTA* and littermate control mice. Anterior, central and posterior sectors (ASec, CSec, and PSec, respectively) are outlined in dotted lines. Scale bars = 1 mm.

b, Quantification of sector area in eCN-DTA mice (n=5) compared to littermate controls (n=6). Ordinary two-way ANOVA: main effect of genotype ($F_{1,9}$ = 17.96, P = 0.0022), main effect of sector ($F_{1.693,15.23}$ = 264.6, P < 0.0001), and interaction ($F_{2,18}$ = 10.65, P = 0.0009); with post hoc two-tailed t-tests with uncorrected Fisher's LSD for effect of genotype for ASec ($t_{8.779}$ = 3.100, P = 0.0131), CSec ($t_{8.706}$ = 8.001, P < 0.0001), and PSec (P = 0.0540).

ns, not significant: $P \geq 0.05$. Data are presented as mean ± SEM.



Supplementary Fig. 7 | Motor coordination during behavior is task-dependent in *eCN-DTA* mice.

a, Distance travelled during basal locomotion by 5 min time bins (*eCN-DTA* mice: n=16, littermate controls: n=15). Repeated measure two-way ANOVA: main effect of time ($F_{5.410,156.9}$ = 100.4, P < 0.0001), main effect of genotype ($F_{1,29}$ = 8.210, P = 0.0077), and interaction ($F_{11,319}$ = 2.629, P =

0.0032); with post hoc two-tailed t-tests with Šídák correction for effect of genotype for 30-35 min ($t_{20.48}$ = 3.245, P = 0.0466) but no other comparisons (P ≥ 0.05).

b, Average velocity during basal locomotion by 5 min time bins (*eCN-DTA* mice: n=16, littermate controls: n=15). Repeated measure two-way ANOVA: main effect of time ($F_{5.283,153.2}$ = 5.057, P = 0.0002), but not of genotype (P = 0.0883) or interaction (P = 0.0589).

c, Latency to fall on the first trial of the accelerating rotarod test (*eCN-DTA* mice: n=16, littermate controls: n=14; Mann-Whitney *U* test: *U* = 72.50, P = 0.1034).

d, Average swimming velocity during a three-minute swim (*eCN-DTA* mice: n=16, littermate controls: n=15). Repeated measure two-way ANOVA: main effect of time ($F_{5,145}$ = 137.8, P < 0.0001), but not of genotype (P = 0.3829) or interaction (P = 0.9235).

e, Total distance travelled during the Y-maze test (*eCN-DTA* mice: n=15, littermate controls: n=14; two-tailed unpaired t-test: t_{27} = 3.027, P = 0.0054).

f, Total number of arm entries in the Y-maze (*eCN-DTA* mice: n=15, littermate controls: n=14; two-tailed unpaired t-test: t_{27} = 2.969, P = 0.0062).

g, Average total distance travelled during two days of testing in the plus-maze (*eCN-DTA* mice: n=16, littermate controls: n=15: two-tailed unpaired t-test: t_{29} = 2.211, P = 0.0350).

h, Average total number of entries during two days of testing in the plus-maze (*eCN-DTA* mice: n=16, littermate controls: n=15: two-tailed unpaired t-test: t_{29} = 2.114, P = 0.0432).

i, Total time in which the animal's nose was within the contact zone of a novel mouse (NM) and novel object (NO) during the three-chambered social approach test (*eCN-DTA* mice: n=16, littermate controls: n=15). Repeated measure two-way ANOVA: main effect of location ($F_{1,29}$ = 53.64, P < 0.0001), but not of genotype (P = 0.5828) or interaction (P=0.4639); with post hoc two-tailed t-tests with Šídák correction for effect of location for littermate controls (t_{29} = 5.614, P < 0.0001) and *eCN-DTA* mice (t_{29} = 4.731, P = 0.0001) mice.

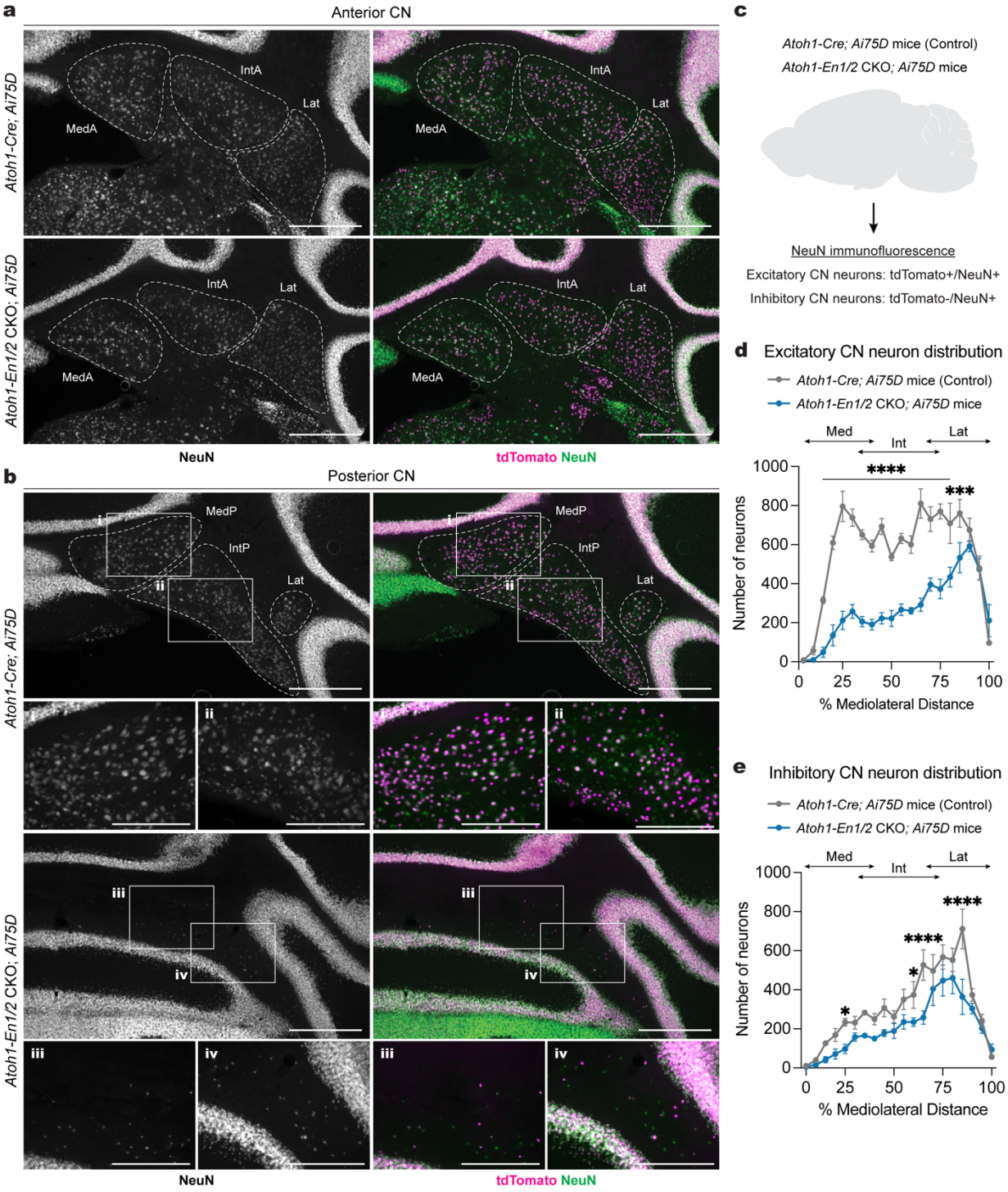
j, Total distance travelled during habituation and test phases of the three-chambered social approach test (*eCN-DTA* mice: n=16, littermate controls: n=15). Repeated measure two-way

ANOVA: main effect of phase ($F_{1,29}$ = 358.9, P < 0.0001), genotype ($F_{1,29}$ = 7.130, P = 0.0123), and interaction ($F_{1,29}$ = 5.461, P = 0.0266); with post hoc two-tailed t-tests with Šídák correction for effect of genotype for the habituation phase (t_{58} = 3.433, P = 0.0022) but not test phase (t_{58} = 1.344, P = 0.3343).

k, Total time spent in the open arms of an elevated plus maze (*eCN-DTA* mice: n=14, littermate controls: n=14; two-tailed unpaired t-test: t_{26} = 1.662, P = 0.1086).

l, Total distance travelled in an elevated plus maze (*eCN-DTA* mice: n=15, littermate controls: n=16; two-tailed unpaired t-test: t_{29} = 2.320, P = 0.0276).

ns, not significant: $P \geq 0.05$. Data are presented as mean values ± SEM.



Supplementary Fig. 8 | Inhibitory CN neurons are significantly reduced across the mediolateral axis in *Atoh1-En1/2* CKOs.

a,**b**, Representative images of immunofluorescent staining NeuN (singe channel) and NeuN (green), tdTomato (magenta) co-labeling in of coronal sections of the anterior (**a**) and posterior

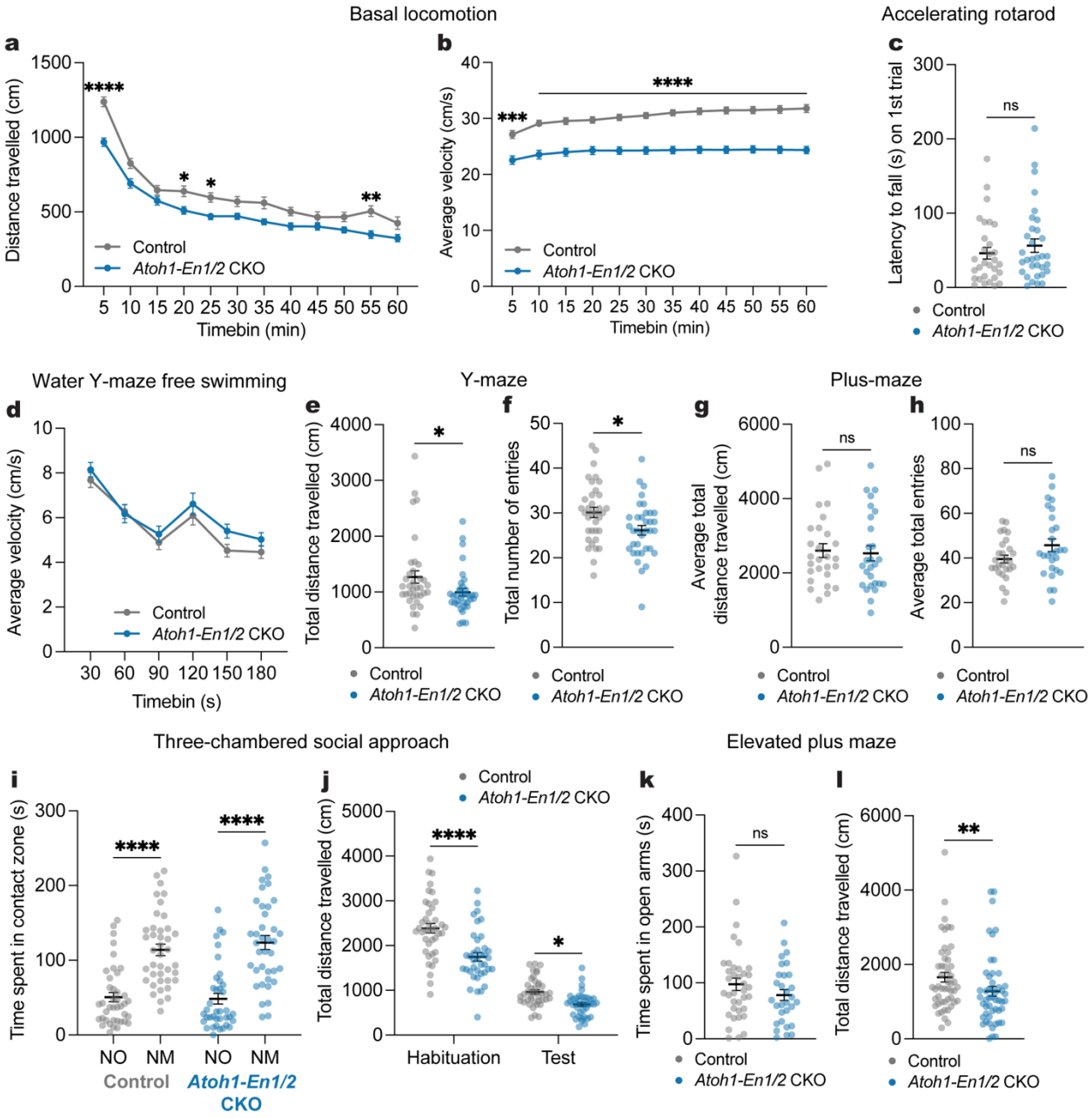
(**b**) CN of *Atoh1-Cre; Ai75D* and *Atoh1-En1/2* CKO; *Ai75D* mice. Abbreviations: MedA=Anterior medial; MedP=Posterior medial; IntA=Anterior interposed; IntP=Posterior interposed; Lat=Lateral. Scale bars = 500 um, scale bars for **i-iv** = 100 um.

c, Experimental design for quantifying excitatory and inhibitory CN neurons in *Atoh1-Cre; Ai75D* (*Atoh1-Cre/+; R26*$^{LSL\text{-}nls\text{-}tdTomato/+}$) and *Atoh1-En1/2* CKO; *Ai75D* (*Atoh1-Cre/+; En1*$^{flox/flox}$*; En2*$^{flox/flox}$*; R26*$^{LSL\text{-}nls\text{-}tdTomato/+}$) mice.

d, Quantification and distribution of excitatory CN neurons in half of the cerebellum (every second section). Two-way ANOVA: main effect of % mediolateral distance ($F_{19,120}$ = 31.38, P < 0.0001), genotype ($F_{1,120}$ = 400.1, P < 0.0001), and interaction ($F_{19,120}$ = 8.830, P < 0.0001); with post hoc two-tailed t-tests with uncorrected Fisher's LSD for effect of genotype for bin 10-80% (list of t value for each bin: t_{120} = 4.029, 7.169, 8.828, 7.267, 6.726, 6.087, 7.103, 4.759, 5.516, 5.122, 7.85, 5.06, 6, 4.169; all P values: P < 0.0001), for bins 80-85% (t_{120} = 3.435, P = 0.0008), and no other comparisons ($P \geq 0.05$). Abbreviations: Med=medial; Int=interposed; Lat=lateral.

e, Quantification and distribution of inhibitory CN neurons in half of the cerebellum (every second section). Two-way ANOVA: main effect of % mediolateral distance ($F_{19,120}$ = 23.97, P < 0.0001), and genotype ($F_{1,120}$ = 50.81, P < 0.0001), but not interaction ($F_{19,120}$ = 1.659, P = 0.0531); with post hoc two-tailed t-tests with uncorrected Fisher's LSD for effect of genotype for bin 20-25% (t_{120} = 2.094, P = 0.0384), bin 55-60% (t_{120} = 2.141, P = 0.0343), bin 60-65% (t_{120} = 4.114, P < 0.0001), bin 80-85% (t_{120} = 5.316, P <0.0001), and no other comparisons ($P \geq 0.05$). Abbreviations: Med=medial; Int=interposed; Lat=lateral.

Data are presented as mean values ± SEM.



Supplementary Fig. 9 | Motor coordination during behavior is task-dependent in *Atoh1-En1/2* CKOs.

a, Distance travelled during basal locomotion by 5 min time bins (*Atoh1-En1/2* CKOs: n=33, littermate controls: n=35). Repeated measure two-way ANOVA: main effect of time ($F_{7.310,482.5}$ = 171.0, P < 0.0001), genotype ($F_{1,66}$ = 15.45, P = 0.0002), and interaction ($F_{11,726}$ = 3.120, P = 0.0004); with post hoc two-tailed t-tests with Šídák correction for effect of genotype on 0-5 min

($t_{65.21}$ = 6.250, P < 0.0001), 15-20 min ($t_{61.20}$ = 3.012, P = 0.0443), 20-25 min ($t_{59.87}$ = 3.358, P = 0.0163), 50-55 min ($t_{60.88}$ = 3.556, P = 0.0088), and no other comparisons ($P \geq 0.05$).

b, Average velocity during basal locomotion by 5 min time bins (*Atoh1-En1/2* CKOs: n=33, littermate controls: n=35). Repeated measure two-way ANOVA: main effect of time ($F_{2.435,160.7}$ = 171.0, P < 0.0001), genotype ($F_{1,66}$ = 57.43, P = 0.0002), and interaction ($F_{11,726}$ = 4.381, P < 0.0001); with post hoc two-tailed t-tests with Šídák correction for effect of genotype on 0-5 min ($t_{65.45}$ = 4.355, P = 0.0006) and 5-60 min (t value for each bin: $t_{65.45}$ = 4.355, t_{61} = 5.693, $t_{63.58}$ = 5.927, $t_{62.8}$ = 5.916, $t_{63.54}$ = 6.755, $t_{61.95}$ = 7.332, $t_{62.74}$ = 7.867, $t_{65.1}$ = 7.819, $t_{65.56}$ = 7.863, $t_{65.83}$ = 7.695, t_{66} = 7.698, $t_{65.53}$ = 7.589, all P values: P < 0.0001).

c, Latency to fall on the first trial of the accelerating rotarod test (*Atoh1-En1/2* CKOs: n=32, littermate controls: n=30; Mann-Whitney *U* test: *U* = 417, P = 0.3792).

d, Average swimming velocity during a three-minute swim (*Atoh1-En1/2* CKOs: n=35, littermate controls: n=31). Repeated measure two-way ANOVA: main effect of time ($F_{5,320}$ = 0.5563, P < 0.0001), but not of genotype (P = 0.1350) and interaction (P = 0.7335).

e, Total distance travelled during the Y-maze (n=35 per genotype; Mann-Whitney *U* test: *U* = 405, P = 0.0144).

f, Total number of arm entries in the Y-maze (n=35 per genotype; two-tailed unpaired t-test: t_{68} = 2.548, P = 0.0131).

g, Average total distance travelled during two days of testing in the plus-maze (n=27 per genotype; Mann-Whitney *U* test: *U* = 332.5, P = 0.5857).

h, Average total number of arm entries during two days of testing in the plus-maze (n=27 per genotype; two-tailed unpaired t-test: t_{52} = 1.839, P = 0.0717).

i, Total time in which the animal's nose was within the contact zone of a novel mouse (NM) and novel object (NO) during the three-chamber social approach test (*Atoh1-En1/2* CKOs: n=38, littermate controls: n=40). Repeated measure two-way ANOVA: main effect of location ($F_{1,152}$ = 81.64, P < 0.0001), but not of genotype (P = 0.6191) or interaction (P = 0.4360); with post hoc

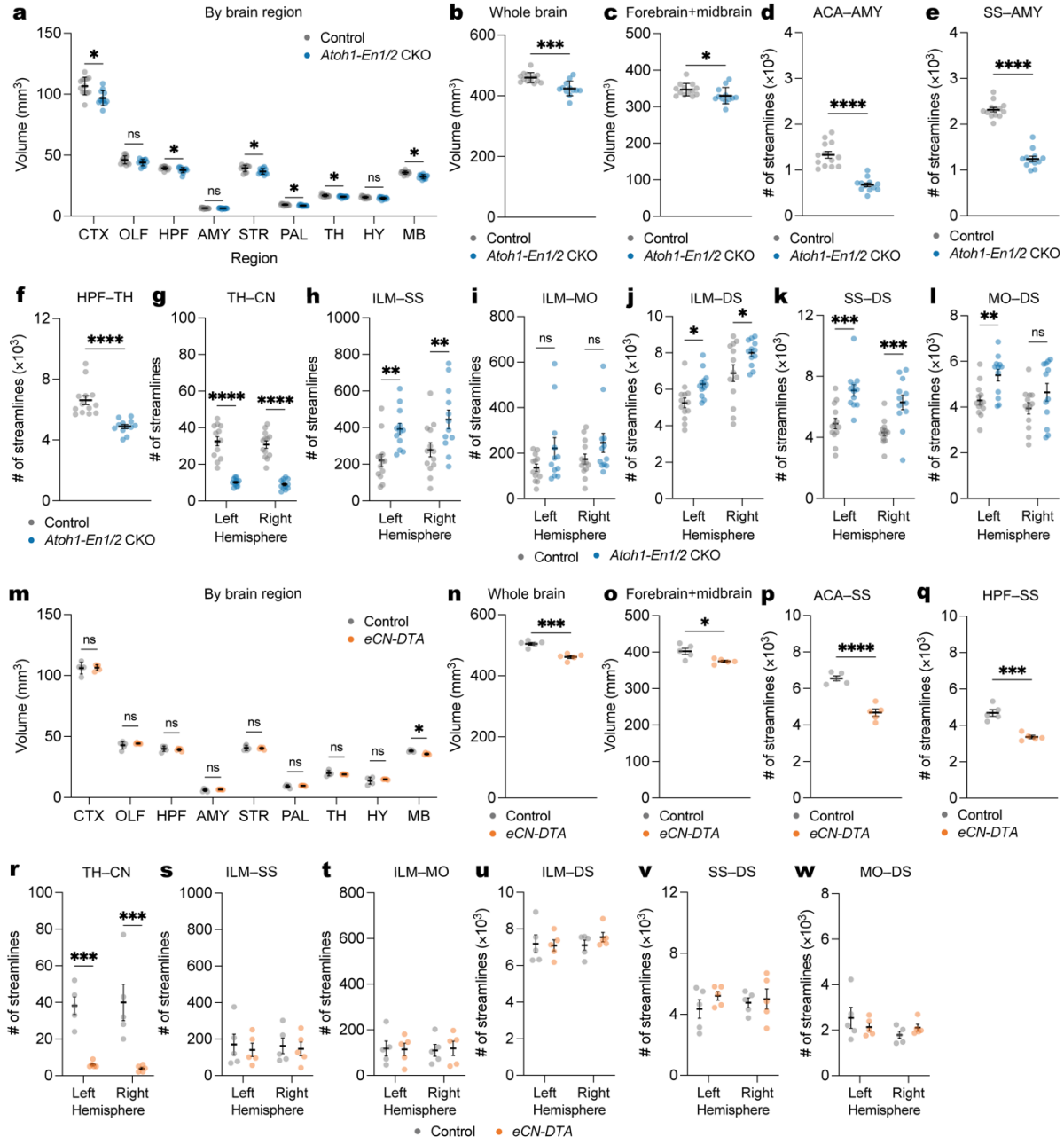
two-tailed t-tests with Šídák correction for effect of location for *Atoh1-En1/2* CKOs (t_{152} = 6.854, $P < 0.0001$) and littermate controls (t_{152} = 5.913, $P < 0.0001$).

j, Total distance travelled during habituation and test phases in the three-chamber social approach test (*Atoh1-En1/2* CKOs: n=38, littermate controls: n=40). Repeated measure two-way ANOVA: main effect of phase ($F_{1,76}$ = 487.6, $P < 0.0001$), genotype ($F_{1,76}$ = 22.58, $P < 0.0001$), and interaction ($F_{1,76}$ = 10.31, P = 0.0019); with post hoc two-tailed t-tests with Šídák correction for effect of genotype for the habituation phase (t_{152} = 5.772, $P < 0.0001$) and test phase (t_{152} = 2.491, P = 0.0275).

k, Total time spent in the open arms of the elevated plus maze (n=50 per genotype; Mann-Whitney *U* test: *U* = 878, P = 0.01).

l, Total distance travelled in the elevated plus maze (n=50 per genotype; Mann-Whitney *U* test: *U* = 467, P = 0.2705).

ns, not significant: $P \geq 0.05$. Data are presented as mean values ± SEM.



Supplementary Fig. 10 | Brain volume and connectivity changes in *Atoh1-En1/2* CKO and eCN-DTA mice.

a, Quantification of regional brain volumes in *Atoh1-En1/2* CKOs (n=12) compared to littermate controls (n=13). Two-tailed unpaired t-tests to test for effect of genotype on CTX (t_{23} = 3.633, P = 0.0014), HPF (t_{23} = 2.329, P = 0.03), STR (t_{23} = 2.612, P = 0.016), PAL (t_{23} = 3.143, P = 0.005),

TH (t_{23} = 3.052, P = 0.006), MB (t_{23} = 5.665, P < 0.0001), HB (t_{23} = 11.16, P < 0.0001), CB (t_{23} = 11.15, P < 0.0001), other comparisons $P \geq 0.05$.

b, Quantification of whole brain volume in *Atoh1-En1/2* CKOs compared to littermate controls (*Atoh1-En1/2* CKOs: n=12, littermate controls: n=13; two-tailed unpaired t-test: t_{23} = 4.298, P = 0.0003).

c, Quantification of forebrain and midbrain combined volume in *Atoh1-En1/2* CKOs compared to littermate controls (*Atoh1-En1/2* CKOs: n=12, littermate controls: n=13; two-tailed unpaired t-test: t_{23} = 2.087, P = 0.0481).

d, Quantification of average (left and right hemispheres) ACA-AMY tractography in *Atoh1-En1/2* CKOs compared to littermate controls (*Atoh1-En1/2* CKOs: n=12, littermate controls: n=13; two-tailed unpaired t-test: t_{23} = 14.35, P < 0.0001).

e, Quantification of average (left and right hemispheres) SS-AMY tractography in *Atoh1-En1/2* CKOs compared to littermate controls (*Atoh1-En1/2* CKOs: n=12, littermate controls: n=13; Mann-Whitney *U* test: *U* = 0, P < 0.0001).

f, Quantification of average (left and right hemispheres) HPF-TH tractography in *Atoh1-En1/2* CKOs compared to littermate controls (*Atoh1-En1/2* CKOs: n=12, littermate controls: n=13; two-tailed unpaired t-test: t_{23} = 4.298, P = 0.0003).

g, Quantification of TH-CN tractography in *Atoh1-En1/2* CKOs (n=12) compared to littermate controls (n=13). Ordinary two-way ANOVA: main effect of genotype ($F_{1,46}$ = 166.5, P < 0.0001), but not of hemisphere (P = 0.3838) or interaction (P = 0.8437); with post hoc two-tailed t-tests with uncorrected Fisher's LSD for effect of genotype for left hemisphere (t_{46} = 9.265, P < 0.0001) and right hemisphere (t_{46} = 8.985, P < 0.0001).

h, Quantification of ILM-SS tractography in *Atoh1-En1/2* CKOs (n=12) compared to littermate controls (n=13). Ordinary two-way ANOVA: main effect of genotype ($F_{1,46}$ = 18.16, P < 0.0001), but not of hemisphere (P = 0.1675) or interaction (P = 0.9536); with post hoc two-tailed t-tests

with uncorrected Fisher's LSD for effect of genotype for left hemisphere (t_{46} = 3.055, P = 0.0037) and right hemisphere (t_{46} = 2.972, P = 0.0047).

i, Quantification of ILM-MO tractography in *Atoh1-En1/2* CKOs (n=12) compared to littermate controls (n=13). Ordinary two-way ANOVA: main effect of genotype ($F_{1,46}$ = 5.585, P = 0.024), but not of hemisphere (P = 0.3553) or interaction (P = 0.8322); with post hoc two-tailed t-tests with uncorrected Fisher's LSD for effect of genotype for left hemisphere (t_{46} = 1.822, P = 0.0750) and right hemisphere (t_{46} = 1.520, P = 0.1353).

j, Quantification of ILM-DS tractography in *Atoh1-En1/2* CKOs (n=12) compared to littermate controls (n=13). Ordinary two-way ANOVA: main effect of genotype ($F_{1,46}$ = 11.94, P < 0.0001) and hemisphere ($F_{1,46}$ = 29.03, P < 0.0001), but not of interaction (P = 0.9007); with post hoc two-tailed t-tests with uncorrected Fisher's LSD for effect of genotype for left hemisphere (t_{46} = 3.055, P = 0.0037) and right hemisphere (t_{46} = 2.972, P = 0.0047).

k, Quantification of SS-DS tractography in *Atoh1-En1/2* CKOs (n=12) compared to littermate controls (n=13). Ordinary two-way ANOVA: main effect of genotype ($F_{1,46}$ = 32.43, P < 0.0001), but not of hemisphere (P = 0.0662) or interaction (P = 0.7773); with post hoc two-tailed t-tests with uncorrected Fisher's LSD for effect of genotype for left hemisphere (t_{46} = 4.228, P = 0.0001) and right hemisphere (t_{46} = 3.825, P = 0.0004).

l, Quantification of MO-SS tractography in *Atoh1-En1/2* CKOs (n=12) compared to littermate controls (n=13). Ordinary two-way ANOVA: main effect of genotype ($F_{1,46}$ = 10.60, P = 0.0021), but not of hemisphere (P = 0.0535) or interaction (P = 0.4680); with post hoc two-tailed t-tests with uncorrected Fisher's LSD for effect of genotype for left hemisphere (t_{46} = 2.820, P = 0.0071) and right hemisphere (t_{46} = 1.785, P = 0.0809).

m, Quantification of regional brain volumes in *eCN-DTA* mice (n=5) compared to littermate controls (n=5). Two-tailed unpaired t-tests to test for effect of genotype on MB (t_8 = 4.935, P = 0.001) and CB (t_8 = 3.130, P = 0.014), other comparisons $P \geq 0.05$.

n, Quantification of whole brain volume in *eCN-DTA* mice compared to littermate controls (n=5 per genotype; two-tailed unpaired t-test: $t_8 = 6.346$, P = 0.0002).

o, Quantification of forebrain and midbrain combined volumes in *eCN-DTA* mice compared to littermate controls (n=5 per genotype; two-tailed unpaired t-test: $t_8 = 3.055$, P = 0.0157).

p, Quantification of average (left plus right hemispheres) ACA-SS tractography in *eCN-DTA* mice compared to littermate controls (n=5 per genotype; two-tailed unpaired t-test: $t_8 = 7.743$, P < 0.0001).

q, Quantification of average (left plus right hemispheres) HPF-STR tractography in *eCN-DTA* mice compared to littermate controls (n=5 per genotype; two-tailed unpaired t-test: $t_8 = 6.324$, P = 0.0002).

r, Quantification of TH-CN tractography in *eCN-DTA* mice (n=5) compared to littermate controls (n=5). Ordinary two-way ANOVA: main effect of genotype ($F_{1,16} = 37.89$, P < 0.0001), but not of hemisphere (P = 0.9717) or interaction (P = 0.7233); with post hoc two-tailed t-tests with uncorrected Fisher's LSD for effect of genotype for left hemisphere ($t_{16} = 4.103$, P = 0.0008) and right hemisphere ($t_{16} = 4.613$, P = 0.0003).

s, Quantification of ILM-SS tractography in *eCN-DTA* mice (n=5) compared to littermate controls (n=5). Ordinary two-way ANOVA: no main effect of genotype (P = 0.6080), hemisphere (P = 0.9875) or interaction (P = 0.8883).

t, Quantification of ILM-MO tractography in *eCN-DTA* mice (n=5) compared to littermate controls (n=5). Ordinary two-way ANOVA: no main effect of genotype (P = 0.9440), hemisphere (P = 0.9600) or interaction (P = 0.8281).

u, Quantification of ILM-DS tractography in *eCN-DTA* mice (n=5) compared to littermate controls (n=5). Ordinary two-way ANOVA: no main effect of genotype (P = 0.6155), hemisphere (P = 0.5876) or interaction (P = 0.4505).

v, Quantification of SS-DS tractography in *eCN-DTA* mice (n=5) compared to littermate controls (n=5). Ordinary two-way ANOVA: no main effect of genotype (P = 0.2886), hemisphere (P = 0.8391) or interaction (P = 0.5482).

w, Quantification of MO-SS tractography in *eCN-DTA* mice (n=5) compared to littermate controls (n=5). Ordinary two-way ANOVA: no main effect of genotype (P = 0.8636), hemisphere (P = 0.1664) or interaction (P = 0.1924).

Abbreviations: CTX=cerebral cortex; OLF=olfactory bulb; HPF=hippocampal formation; AMY=amygdala; STR=striatum; PAL=pallidum; TH=thalamus; HY=hypothalamus; MB=midbrain; HB=hindbrain; CB=cerebellum; ILM=intralaminar nuclei; SS=primary somatosensory cortex; MO=primary motor cortex; ACA = anterior cingulate cortex. ns, not significant: $P \geq 0.05$. Data are presented as mean values ± SD for **a**,**b**,**g**,**h** and mean value ± SEM for **c**-**f**,**i**-**k**.

eCN phenotype & behavior	**MedP-hM4Di**	***SepW1-En1/2* CKOs**	***Atoh1-En1/2* CKOs**	***eCN-DTA***
eCN phenotype	MedP eCN inhibited	MedP eCN gone MedA eCN reduced 50%	MedP & IntP eCN gone MedA & IntA eCN reduced 50%	All eCN gone
Negative geotaxis P7 & P11	NA	P	X	X
Righting reflex P7	NA	P	P	X
Footprint	P	P	X	X
Basal locomotion	P	P	X	X
Accelerating rotarod	P	P	X	P
Water Y maze	reversal	P	Initial & reversal	P
Y-maze	P	P	X	P
Plus-maze	NA	NA	X	P
Social Preference	NA	NA	P	P
Elevated Plus Maze	NA	NA	P	P
Grooming	NA	NA	P	P
Diffusion MRI	NA	NA	Increased connectivity thalamo-cortical-striatal	No change
Compensation	NA	From remaining eCN and/or extracerebellar circuits	Extracerebellar circuits interfere with non-motor behaviors	From extracerebellar circuits

Supplementary Fig. 11 | Summary of results.

Legend: ✓ = no difference; NA = not applicable; X = impairment.