



Figures and figure supplements

Genetic mechanisms control the linear scaling between related cortical primary and higher order sensory areas

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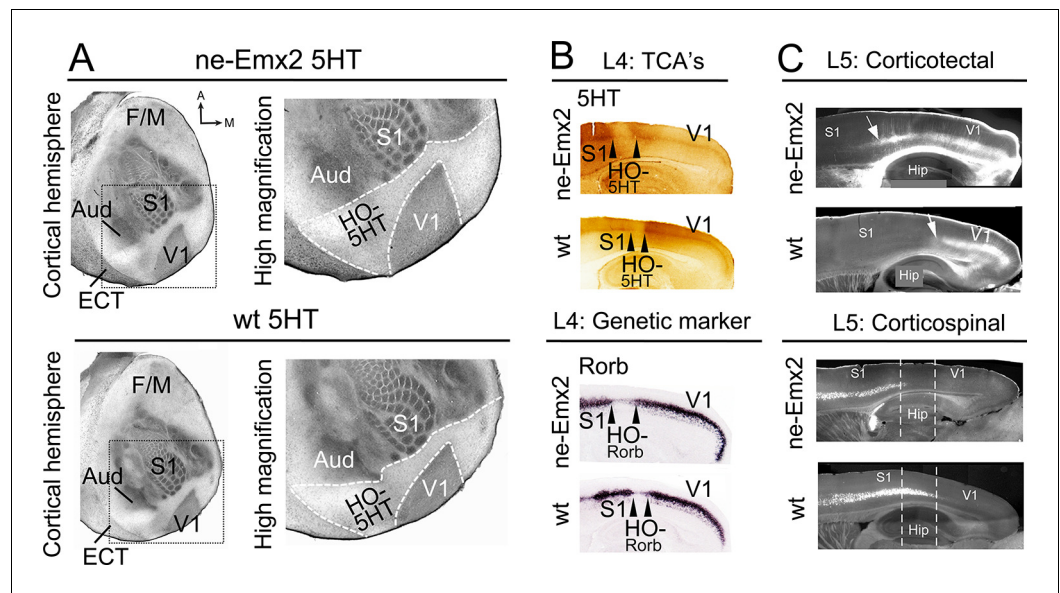


Figure 1. Increased V1 and higher order sensory area sizes in *ne-Emx2* cortices (A) Serotonin (5HT) staining on postnatal day (P) 7 tangential sections of the flattened cortex reveals targeting patterns of TCAs revealing primary sensory area borders and the border of the neocortex to the ECT. 5HT staining is not detectable in the region containing the retrosplenial cortex and the higher order sensory areas surrounding V1 (HO-5HT). In *Emx2*-overexpressing brains (*ne-Emx2*), V1 and HO-5HT appear larger (compare dotted outlines in higher magnification images), compared to wt brains. (B) Targeting of TCAs in cortical layer 4 (L4) was revealed on P7 sagittal cortex sections by 5HT staining, whereas L4 genetic area borders were revealed by in situ hybridization for *Rorb*. In *ne-Emx2* brains, the V1 border shifts anteriorly. Higher order areas surrounding V1 are characterized by low 5HT/*Rorb* staining (between arrowheads, HO-5HT and HO-*Rorb*), which in *ne-Emx2* brains appear overall larger (compare area between arrowheads). (C) In L5, an expansion (see arrows) of corticotectal projection neurons (retrogradely labeled by Dil injections into the superior colliculus) is apparent in *ne-Emx2* brains, to the expense (see dotted lines) of L5 corticospinal projection neurons (retrogradely labeled by Dil injections into the pyramidal decussation). Main axes: A: anterior; M: medial; F/M: frontal/motor cortex; S1: primary somatosensory cortex; Aud: auditory areas; V1: primary visual cortex, ECT: entorhinal cortex. 5HT, serotonin; L5, cortical layer 5; TCAs, thalamocortical axons; wt, wildtype.

DOI: <http://dx.doi.org/10.7554/eLife.11416.003>

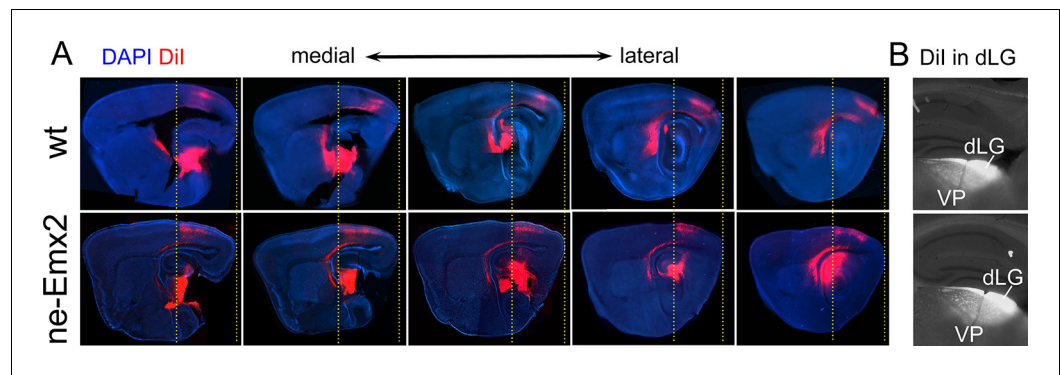


Figure 1—figure supplement 1. Anterior shifted cortical boundary formed by TCAs from the dLG in *ne-Emx2* brains. (A) In P7 brains, Dil (red dye) crystals were inserted into the thalamic dorsal lateral geniculate nucleus (dLG) to label the projections of TCAs into the primary visual cortex (V1). In sagittal sections at five different medial to lateral levels, TCAs in *Emx2*-overexpressing brains (*ne-Emx2*) extend more anteriorly, compared to wt brains (compare dotted lines). Across genotypes, Dil labeling shows a sharp border to Dil-negative cortical tissues. (B) Images show representative Dil staining close to the injection sites in the dLG revealing robust and comparable dye filling in the dLG in wt and *ne-Emx2* brains. dLG, dorsal lateral geniculate nucleus; TCA, thalamocortical axons; wt, wildtype.

DOI: <http://dx.doi.org/10.7554/eLife.11416.004>

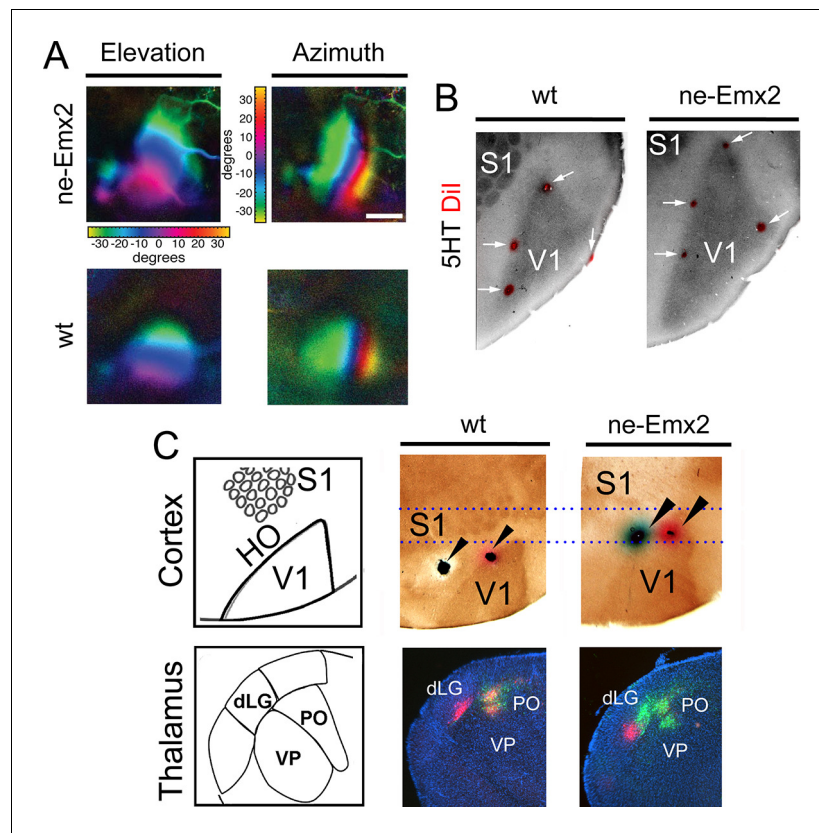


Figure 2. Enlarged functional V1 topographic maps in *ne-Emx2* mice. **(A)** Fourier intrinsic signal optical imaging reveals topographic visual response maps to light bars that were moved across the visual field of the retina (up and down: elevation maps; left to right: azimuth maps). Visual responses in V1 produced intrinsic signal maps that were indistinguishable in strength and the color-coded axes of azimuth and elevation were organized in the same way in all tested brains (*wt*: $n = 6$, *ne-Emx2*: $n = 6$). The elevation/azimuth representations were expanded in *ne-Emx2* animals, revealing that overall their V1 retinotopic maps were larger. **(B)** 5HT staining performed on flattened tangential cortex section reveals cortical area borders including V1. On representative images ($n = 6$ per genotype), the red dots lining the perimeter of the 5HT-stained V1 indicates Dil injection sites that were made after the recordings adjacent to the border of the derived V1 intrinsic response maps, determined by Fourier intrinsic optical imaging. **(C)** Schematics depict caudal cortical sensory areas and main sensory thalamus divisions. In *wt* brains ($n = 15$), cortical dual tracer injections (red tracer (Dil) injected around V1/HO border; green tracer (DiD) into HO; injection site location (arrowheads) was identified by 5HT staining) showed retrogradely labeled red cells in the dLG and the PO, whereas green labeled cells were only present in the PO. Dotted lines show that dual tracer injections in *ne-Emx2* brains ($n = 17$) were administered at more anterior coordinates (red tracer into V1; green tracer around the V1/HO border) compared with *wt* brains. In *ne-Emx2* brains, retrogradely labeled red cells were apparent in the dLG, whereas green cells were present in the dLG and the PO, revealing normal thalamocortical connectivity patterns, but an anterior shifted V1/HO border in *ne-Emx2* brains. 5HT, serotonin; dLG, dorsal lateral geniculate nucleus; PO, posterior thalamic nucleus; S1, primary somatosensory cortex; VP, ventroposterior nucleus; V1, primary visual cortex; *wt*, wildtype.

DOI: <http://dx.doi.org/10.7554/eLife.11416.005>

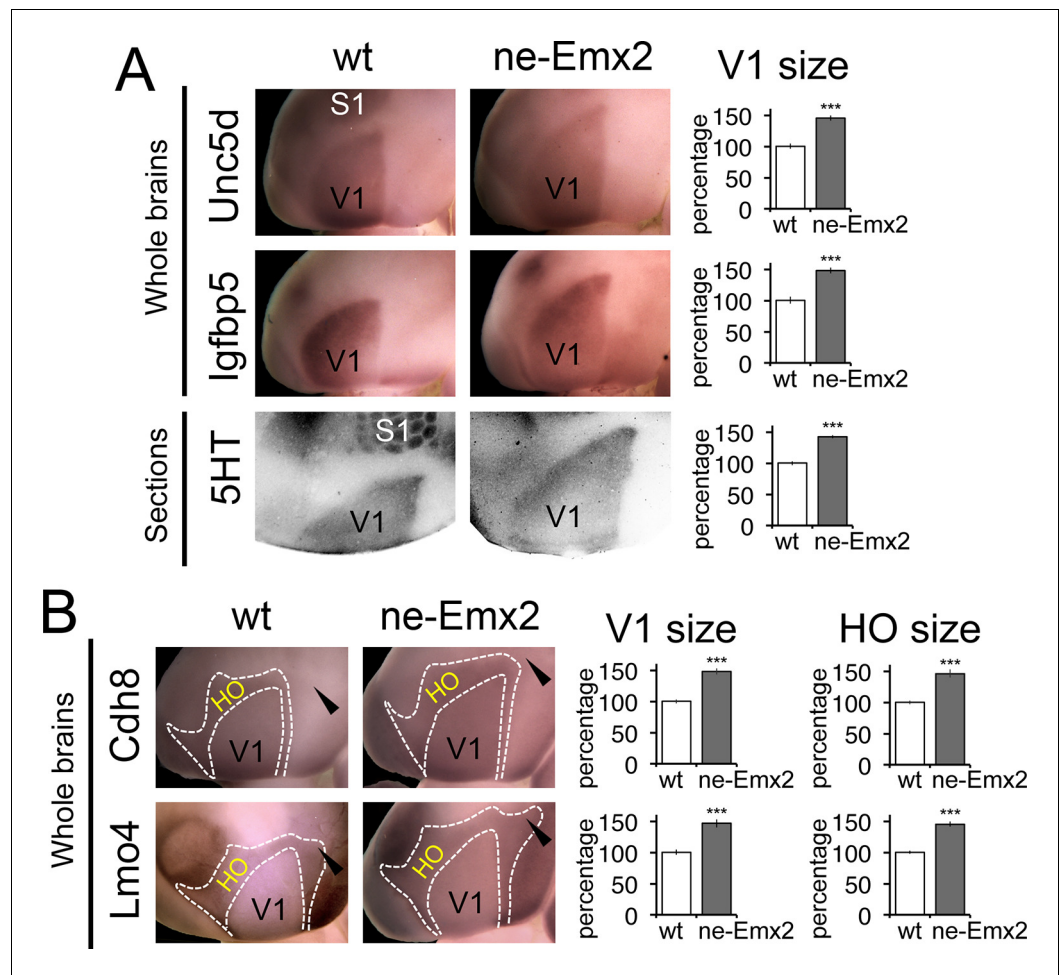


Figure 3. Proportionally increased V1 and HO sizes in *ne-Emx2* cortices. **(A)** Schematic shows sensory area outlines in the caudal neocortex (12). WMISH with the molecular V1 marker genes *Unc5d* (wt: *n* = 5, *ne-Emx2*: *n* = 6) and *Igfbp5* (wt: *n* = 11, *ne-Emx2*: *n* = 6) at P7 highlights increased V1 size in *ne-Emx2* brains using whole un-sectioned brains. Quantification of V1 size using 5HT-stained P7 flattened cortical sections similarly reveals larger V1 sizes in *ne-Emx2* brains (*n* = 11), compared to wt brains (*n* = 13). **(B)** WMISH for molecular markers that label both V1 and HO (dotted outlines: *Cdh8*, *Lmo4*: high expression in HO, lower in V1; for each probe and genotype *n* = 6) reveal that V1 as well as HO sizes in *ne-Emx2* are larger compared with wt brains. *Cdh8* is not expressed around the anteromedial edge of V1 (arrowheads). Quantifications in **Figures 3** and **4** show mean values as percent of wt, error bars indicate standard error of the mean; asterisks highlight statistical significance according to unpaired t-test. 5HT, serotonin; S1, primary somatosensory cortex; WMISH, whole mount in situ hybridization; V1, primary visual cortex; wt, wildtype.

DOI: <http://dx.doi.org/10.7554/eLife.11416.006>

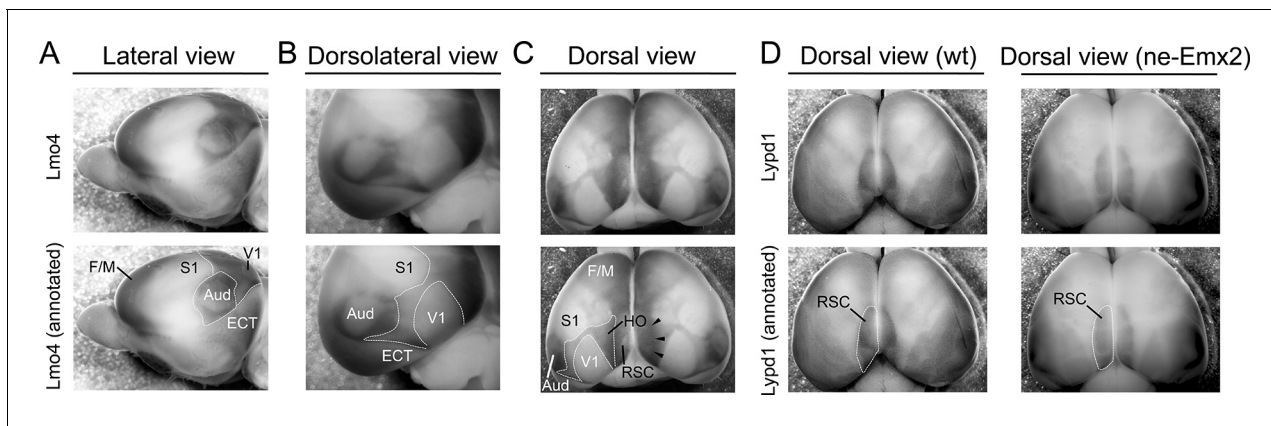


Figure 3—figure supplement 1. *Lmo4* expression delineates primary and higher order cortical area boundaries in whole mount brains. (A) WMISH for *Lmo4* using P7 brains reveals gene expression borders that delineate cortical regions. Their approximated outlines are annotated in the lower panels (A-D: dotted lines). Using images showing a lateral view of the brain, auditory areas (Aud) can be delineated by a ring of strong *Lmo4* expression. Similarly, a line of strong *Lmo4* expression delineates the border between the neocortex and the ECT. The perimeter of the primary visual cortex (V1) cannot be clearly seen in the lateral view. (B) In the dorsolateral view, *Lmo4* expression readily reveals the ECT and auditory area outlines. In addition, the V1 dimensions and the caudal border of the primary somatosensory cortex (S1) are clearly identifiable. (C) In the dorsal view, primary cortical area borders are identifiable using *Lmo4* staining. In addition, lower *Lmo4* expression domains in the caudal cortex appear to outline a cortical region that includes higher order visual areas around V1. In addition, this region (HO) contains adjacent cortical areas like the ventral posterior temporal cortex up to the border of the auditory areas and ECT but seem to exclude the retrosplenial cortex (RSC) medially, which appears to be delineated by a narrow higher expression stripe of *Lmo4* (arrowheads). (D) *Lypd1* expression is low in F/M cortex, S1 and V1, but very strong around the medial cortical pole, where its expression appears to overall overlap with the RSC. Comparing *Lypd1* and *Lmo4* expression in the medial cortex suggests that the outlined HO in (C) and in **Figures 3–4** do not contain much of the RSC. The *Lypd1* gene expression domain around the RSC in *ne-Emx2* brains appears enlarged compared with wt brains. Aud, auditory areas; ECT, entorhinal cortex; F/M, frontal/motor; RSC, retrosplenial cortex; S1, primary somatosensory cortex; V1, primary visual cortex; wt, wildtype.

DOI: <http://dx.doi.org/10.7554/eLife.11416.007>

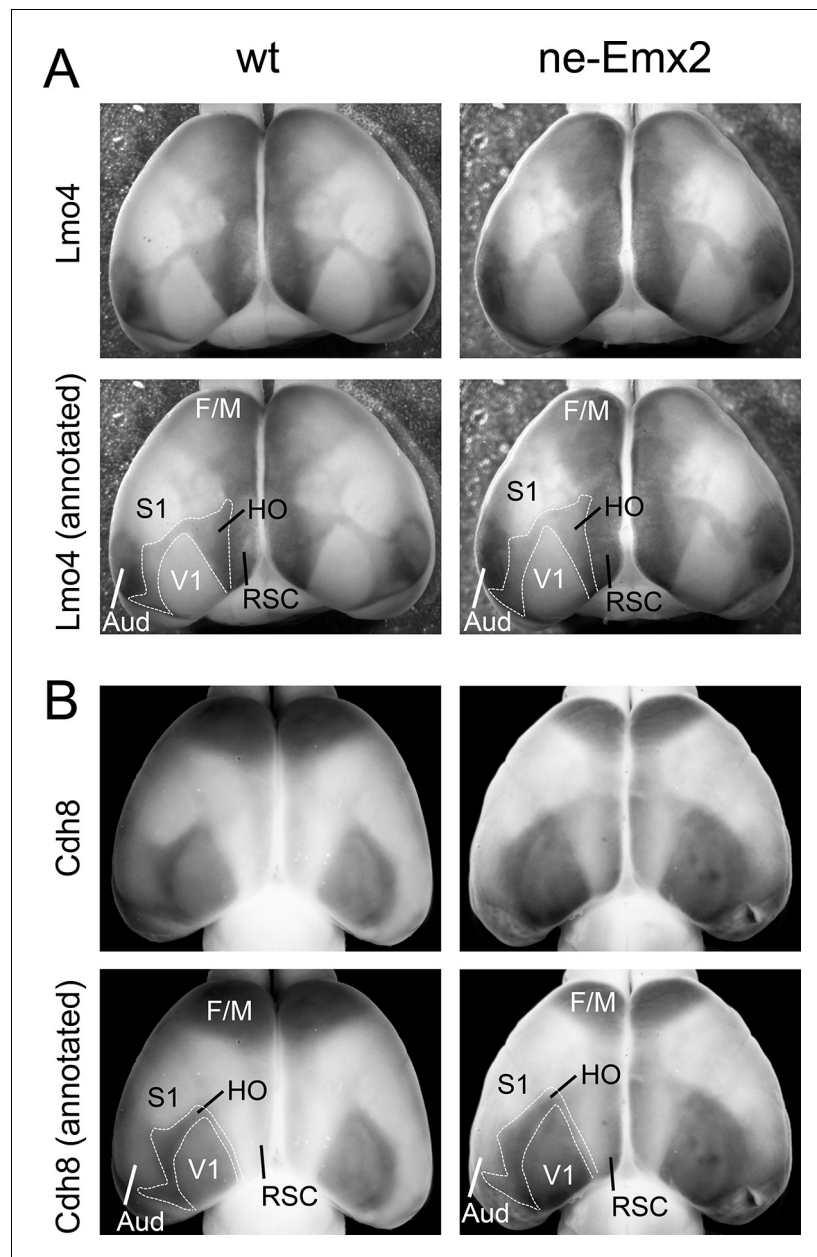


Figure 3—figure supplement 2. *Lmo4* and *Cdh8* expression marks primary and caudal extrastriate cortical regions. (A) Primary cortical area borders are apparent by *Lmo4* staining on WMISH brains at P7. (A-B: raw images in upper panels, annotated images in lower panels). The low *Lmo4* expression domain in the caudal cortex appears to outline a joint HO that contains higher order visual areas around V1 and the ventral posterior temporal cortex up to the borders to the auditory areas and ECT laterally, but seem to exclude the RSC medially. In *ne-Emx2* brains the *Lmo4*-positive HO complex overall appears larger than compared to wt brains. (B) Low *Cdh8* expression labels V1, which is surrounded by a domain of higher *Cdh8* expression labeling higher order visual areas between V1 and S1 in the medial cortex up to the borders to the auditory areas laterally and the ECT cortex caudally, respectively. In *ne-Emx2* brains the *Cdh8*-positive HO complex overall appears larger compared to wt brains. Aud, auditory areas; ECT, entorhinal cortex; F/M, frontal/motor; RSC, retrosplenial cortex; S1, primary somatosensory cortex; V1, primary visual cortex; WMISH, whole mount in situ hybridization; wt, wildtype.

DOI: <http://dx.doi.org/10.7554/eLife.11416.008>

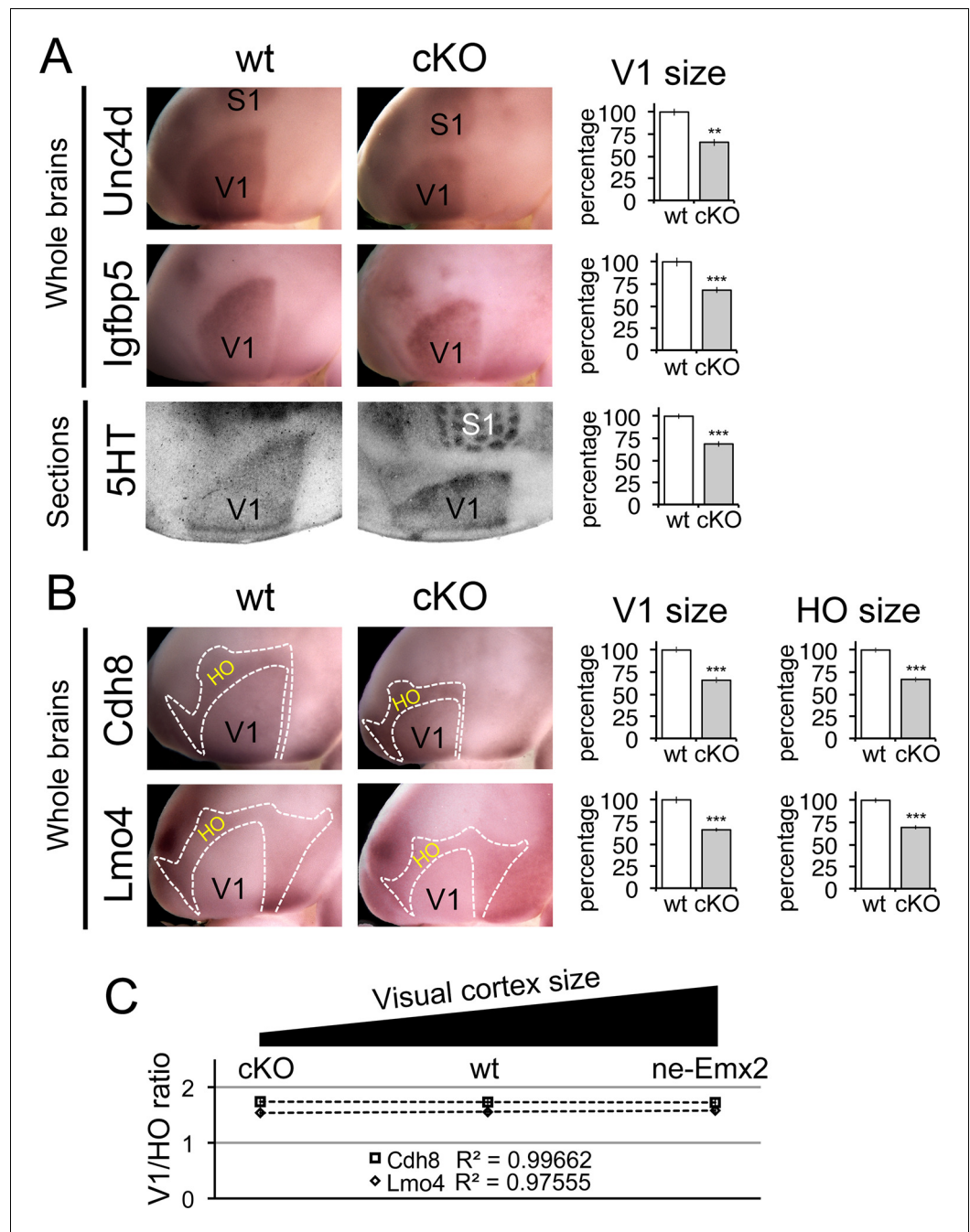


Figure 4. Proportionally decreased V1 and HO sizes in *cKO* cortices. WMISH for V1 (**A**: *Unc5d*; wt: $n = 5$, *ne-Emx2*: $n = 6$, *Igfbp5*; wt: $n = 11$, *ne-Emx2*: $n = 8$) or V1 and HO marker genes (**B**: *Cdh8*; wt: $n = 6$, *ne-Emx2*: $n = 6$; *Lmo4*; wt: $n = 5$, *ne-Emx2*: $n = 5$) at P7 conversely reveals decreased sizes (~70% of wt size) of V1 and HO in brains that were derived from *Emx1-IRES-Cre*-mediated cortex-specific conditional *Emx2* mutant brains (*cKO*), compared with wt brains. Quantification of V1 size using 5HT staining on P7 flattened cortical sections (**A**: wt: $n = 15$, *cKO*: $n = 10$) reveals similar reductions of V1 size in *cKO* brains. (**C**) The ratio between quantified V1 and HO sizes derived from WMISH-stained brains with decreased (*cKO*), normal (wt), and increased (*ne-Emx2*) V1 sizes demonstrates linear scaling of HO size in response to bi-directional changes of V1 size. S1, primary somatosensory cortex; V1, primary visual cortex; WMISH, whole mount in situ hybridization; wt, wildtype.

DOI: <http://dx.doi.org/10.7554/eLife.11416.009>

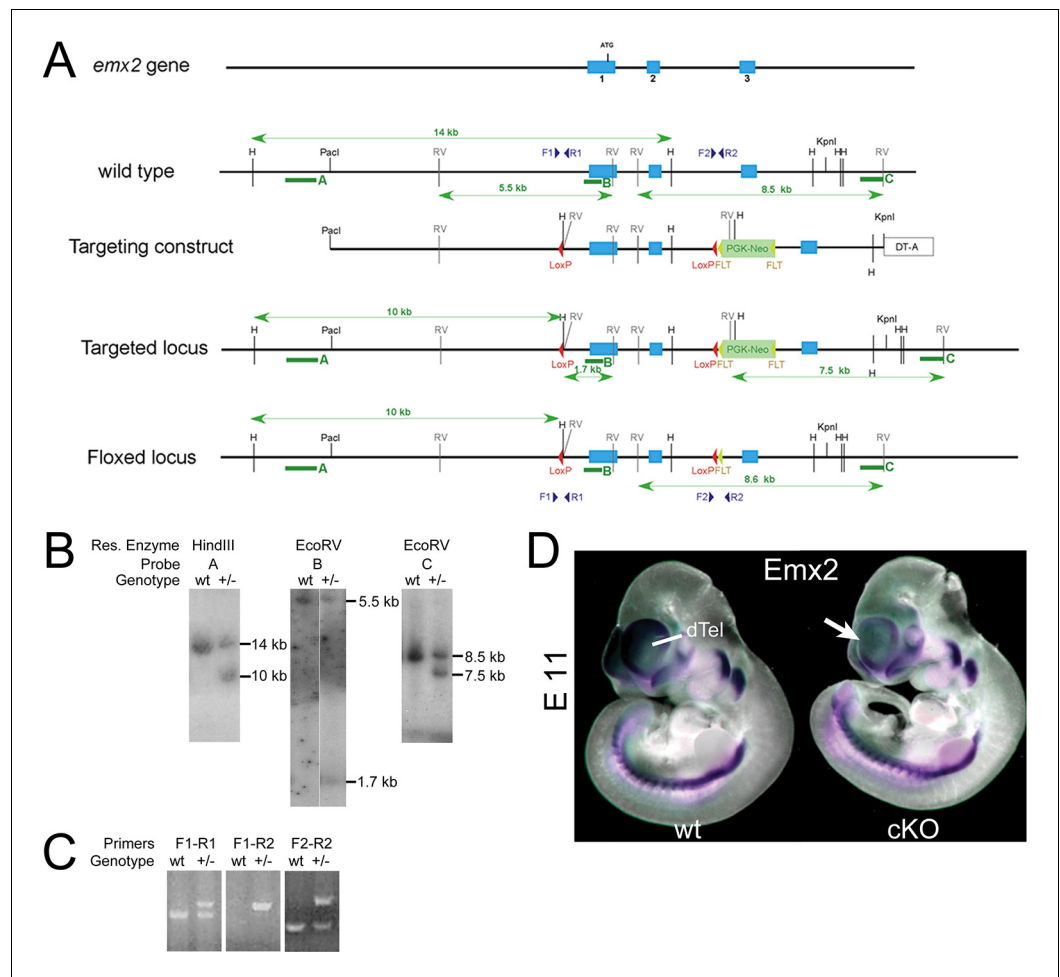


Figure 4—figure supplement 1. Generation of *Emx2*-floxed mice and confirmation of cortex specific *Emx2* deletion. (A) Targeting strategy of the *Emx2* locus: Blue shapes indicate exons of the *Emx2* gene. Red and yellow triangles indicate *LoxP* and *FRT* sites. RV, EcoRV; H, HindIII indicate restriction enzyme cutting sites. The targeting construct included *LoxP* sites flanking *Emx2* exons 1 and 2 followed by a *FRT*-site-flanked *PGK-Neo* cassette. (B) Southern blot hybridization of wt and *Emx2* floxed heterozygous (+/-) embryonic stem cell clones with probes A, B, and C (denoted by green arrows in A). Blots of HindIII digested genomic DNA that were hybridized with probe A revealed a 14kb *Emx2* wt band and a 10 kb *Emx2* floxed band. EcoRV digestion and hybridization with probe B revealed a 5.5kb *Emx2* wt band and a 1.7 kb *Emx2* floxed band. EcoRV digestion and hybridization with probe C revealed a 8.5kb *Emx2* wt band and a 7.5kb *Emx2* floxed band. (C) Genomic DNA from wt and *Emx2* heterozygous (+/-) embryonic stem cell clones was amplified by PCR using combinations of the primers (F1, R1, F2, R2, genomic location indicated in A) revealing a *Emx2* wt PCR product (~300 bp) and a *Emx2* floxed PCR product (~400 bp). (D) WMISH at E11 using a *Emx2* probe showed that *Emx2* expression was intact in the entire embryo in homozygous *Emx2* floxed embryos (*Emx2*^{fl/fl}; considered as wt control). Conversely, WMISH using *Emx1*-IRES-Cre-mediated conditional *Emx2* knock out animals (*Emx2*^{fl/fl}-*Emx1*-IRES-Cre⁺; cKO) reveals specific deletion of *Emx2* activity from cortical progenitors in the dTel, while leaving *Emx2* expression in the rest of the embryo intact. dTel, dorsal telencephalon; PCR, polymerase chain reaction; WMISH, whole mount in situ hybridization; wt, wildtype.

DOI: <http://dx.doi.org/10.7554/eLife.11416.010>

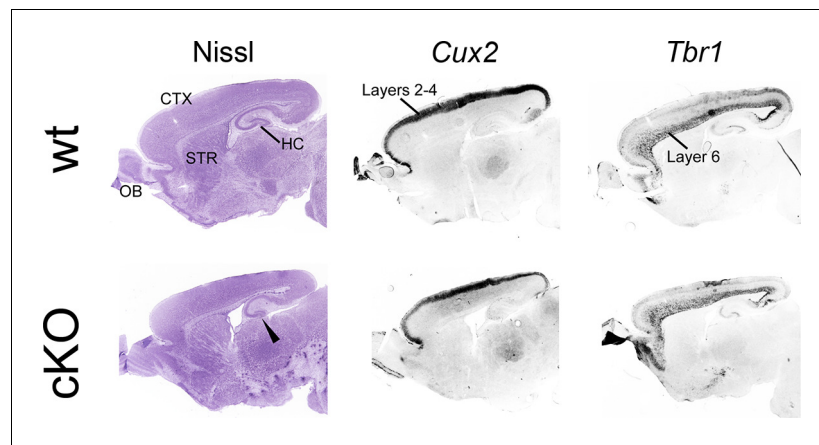


Figure 4—figure supplement 2. Normal cortical neuroanatomy in cKO brains. On P7 sagittal sections, Nissl staining was performed to reveal neuroanatomy. Major forebrain structures are annotated in the wt section. In addition, ISH for the layer-specific marker genes *Cux2* (layers 2-4) and *Tbr1* (layer 6) was carried out. All stains reveal comparable cortical neuroanatomy and comparable cortical layering across genotypes. The only apparent neuroanatomical difference in cKO brains is that the hippocampus appears smaller in cKO brains (arrowhead) compared with wt brain. CTX, cortex; HC, hippocampus; ISH, in situ hybridization; OB, olfactory bulbs; STR, striatum; wt, wildtype.

DOI: <http://dx.doi.org/10.7554/eLife.11416.011>

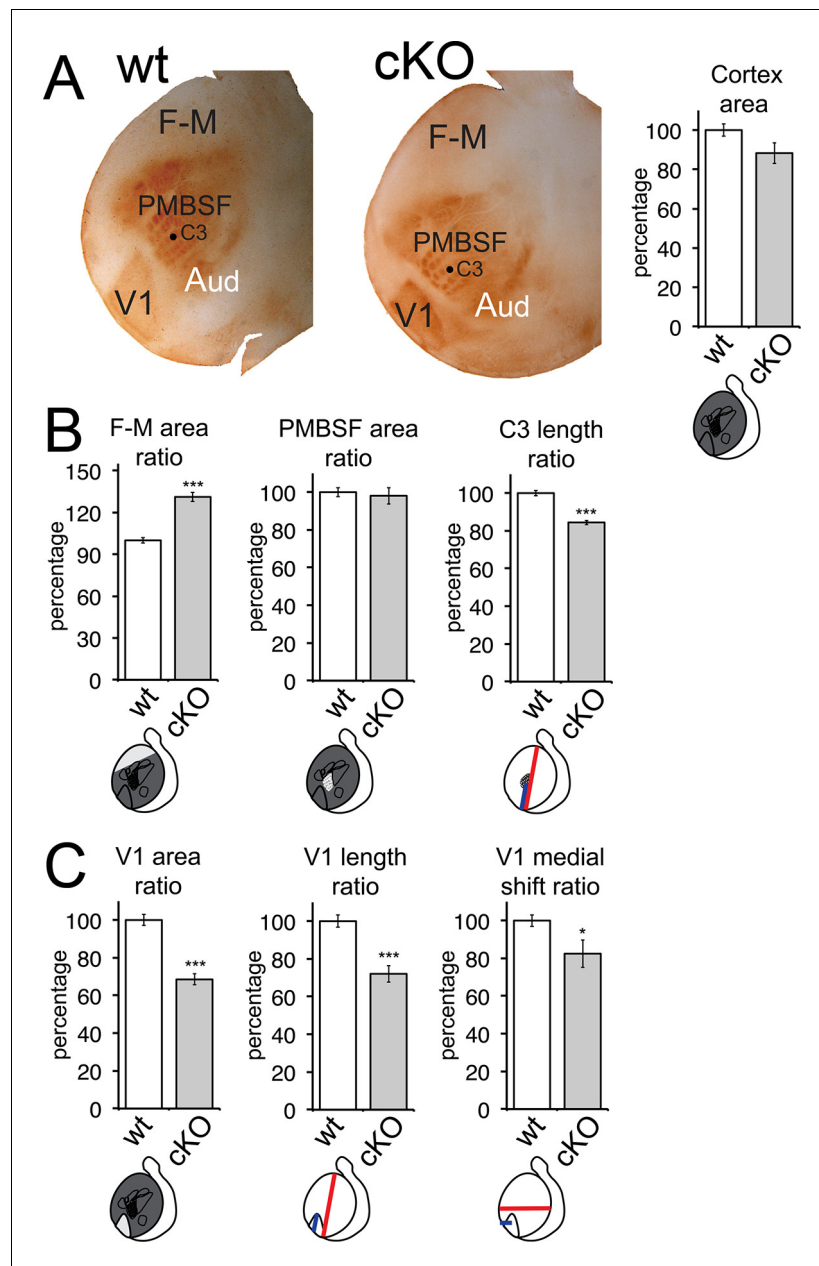


Figure 4—figure supplement 3. Area patterning changes following cortex-specific deletion of *Emx2*. (A) Tangential flattened sections of 5HT-immunostained cortices (wt: n = 15, cKO: n = 10) reveal sensory areas and their borders. Total cortical surface area was reduced by $11.70 \pm 5.25\%$ in cKO brains, without being statistically significant ($p > 0.05$). Further area measurements were normalized to cortical surface area, generating size ratios to facilitate comparisons of area measurements derived from different experiments and mouse lines. Mean ratios of wt brains were defined as 100% and variance calculated using standard error of the mean. Quantified area parameters are depicted in the schematics, below the individual graphs. (B) F/M cortex is larger in cKO brains (F/M area ratio: $131.14 \pm 3.22\%$). S1 size is not affected (PMBSF area ratio: $98.03 \pm 4.37\%$), but overall, indicated by the relative location of PMBSF barrel 'C3' (C3 length ratio: $84.44 \pm 1.16\%$), S1 was located more posteriorly in cKO specimens compared with wt ones. (C) The size of V1 (V1 area ratio: $68.50 \pm 2.95\%$; V1 length ratio: $72.07 \pm 4.39\%$) was reduced and its relative position shifted medially (V1 medial shift ratio: $82.44 \pm 7.29\%$) in cKO brains compared with wt brains. 5HT, serotonin; F/M, frontal/motor; PMBSF, posterior medial barrel sub field; wt, wildtype

DOI: <http://dx.doi.org/10.7554/eLife.11416.012>

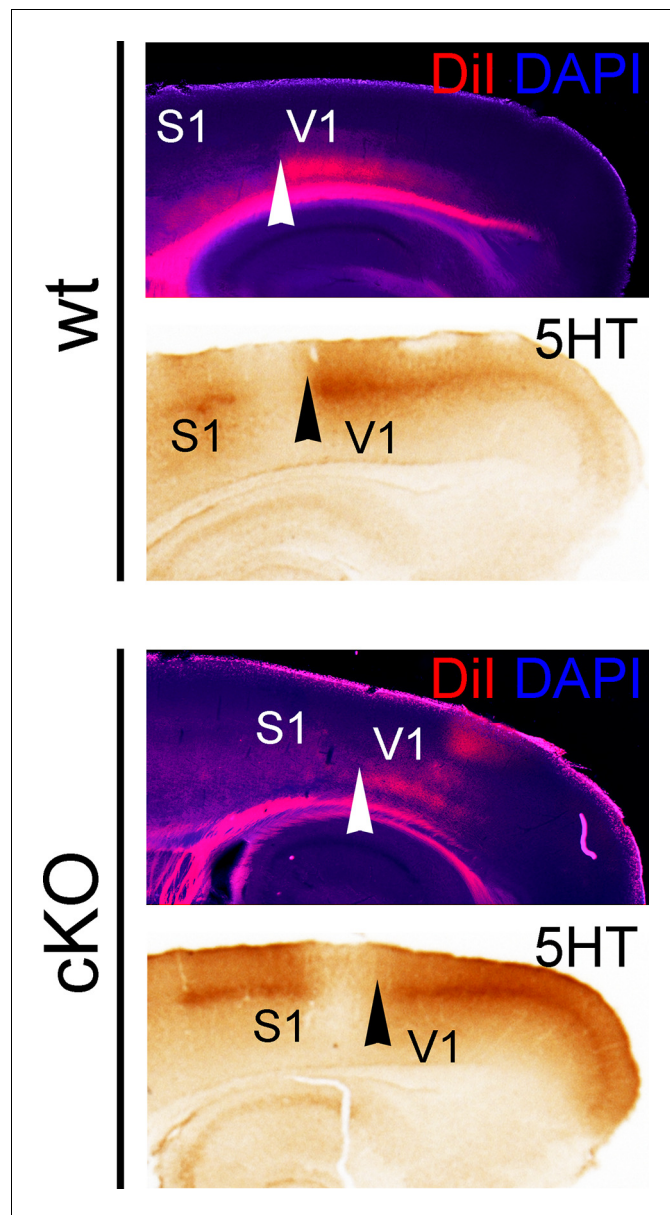


Figure 4—figure supplement 4. Posteriorly shifted V1 border in *cKO* cortices. Adjacent P7 sagittal sections show thalamocortical projections from the dLG to V1, which were either labeled by inserting Dil crystals into the dLG (counterstained with DAPI), or by 5HT immunostaining. Arrowheads denote the anterior border of the V1 area that is innervated by TCAs from the dLG. Both TCA labeling methods reveal that this sharp border is shifted posteriorly in *cKO* brains compared with *wt* brains. 5HT, serotonin; DAPI, 4',6-diamidino-2-phenylindole; S1, primary somatosensory cortex; TCAs, thalamocortical axons; V1, primary visual cortex.

DOI: <http://dx.doi.org/10.7554/eLife.11416.013>