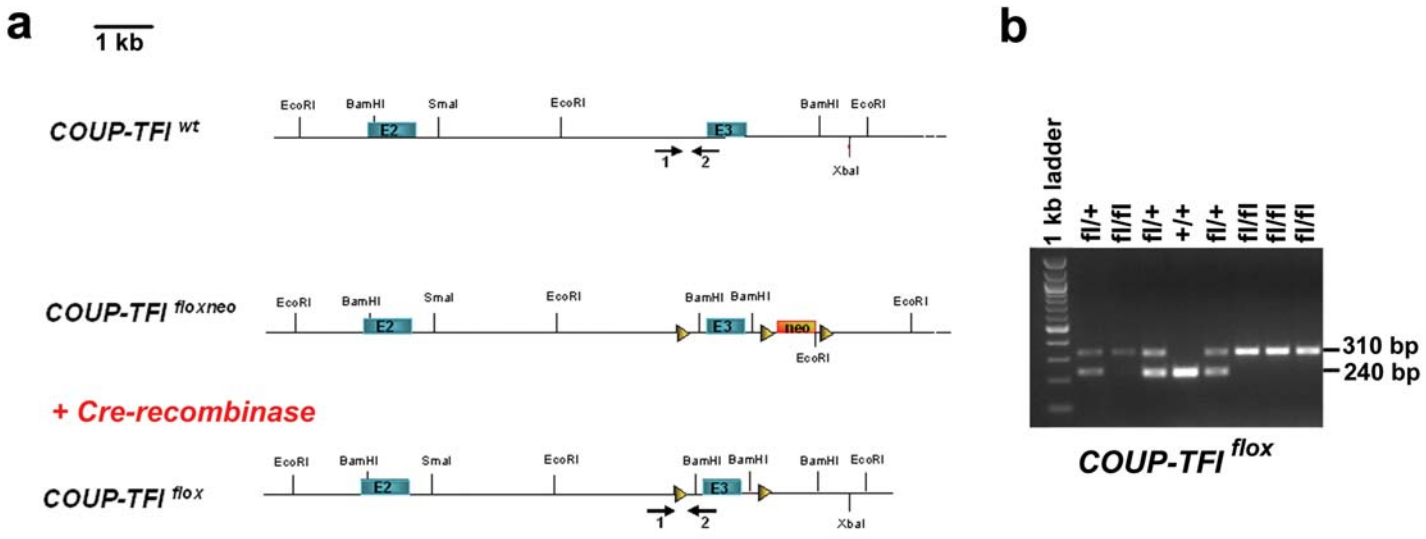
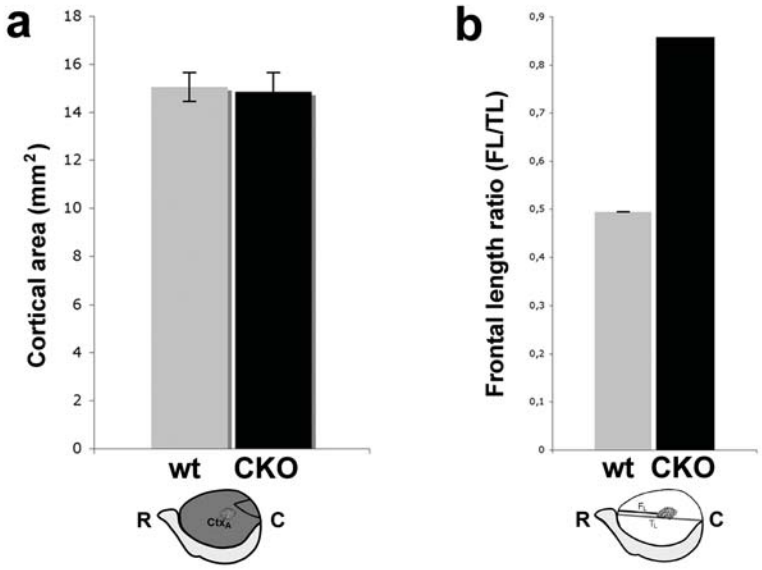


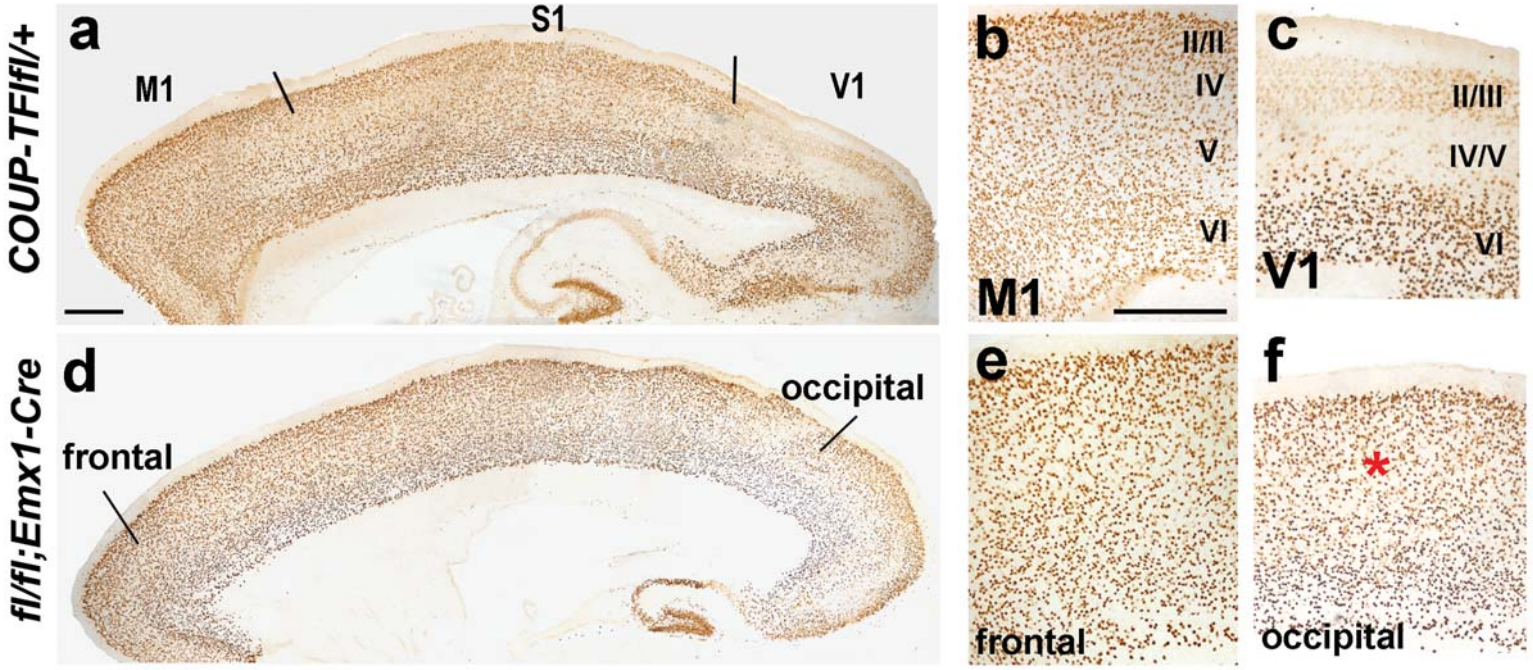
Supplementary Figure 1. Generation of *COUP-TFI* flox mice.

(a) To generate the *COUP-TFI*^{flox} allele in embryonic stem cells (ES), the *Cre-recombinase* was electroporated in ES clones heterozygous for the *COUP-TFI*^{floxneo} allele and excised the neomycin (neo) cassette. Triangles indicate the presence of *loxP* sites. E2, exon2; E3, exon3. (b) PCR genotyping on wild type (+/+), heterozygous (fl/+) and homozygous (fl/fl) mice using the primers indicated in (a) as black arrows.



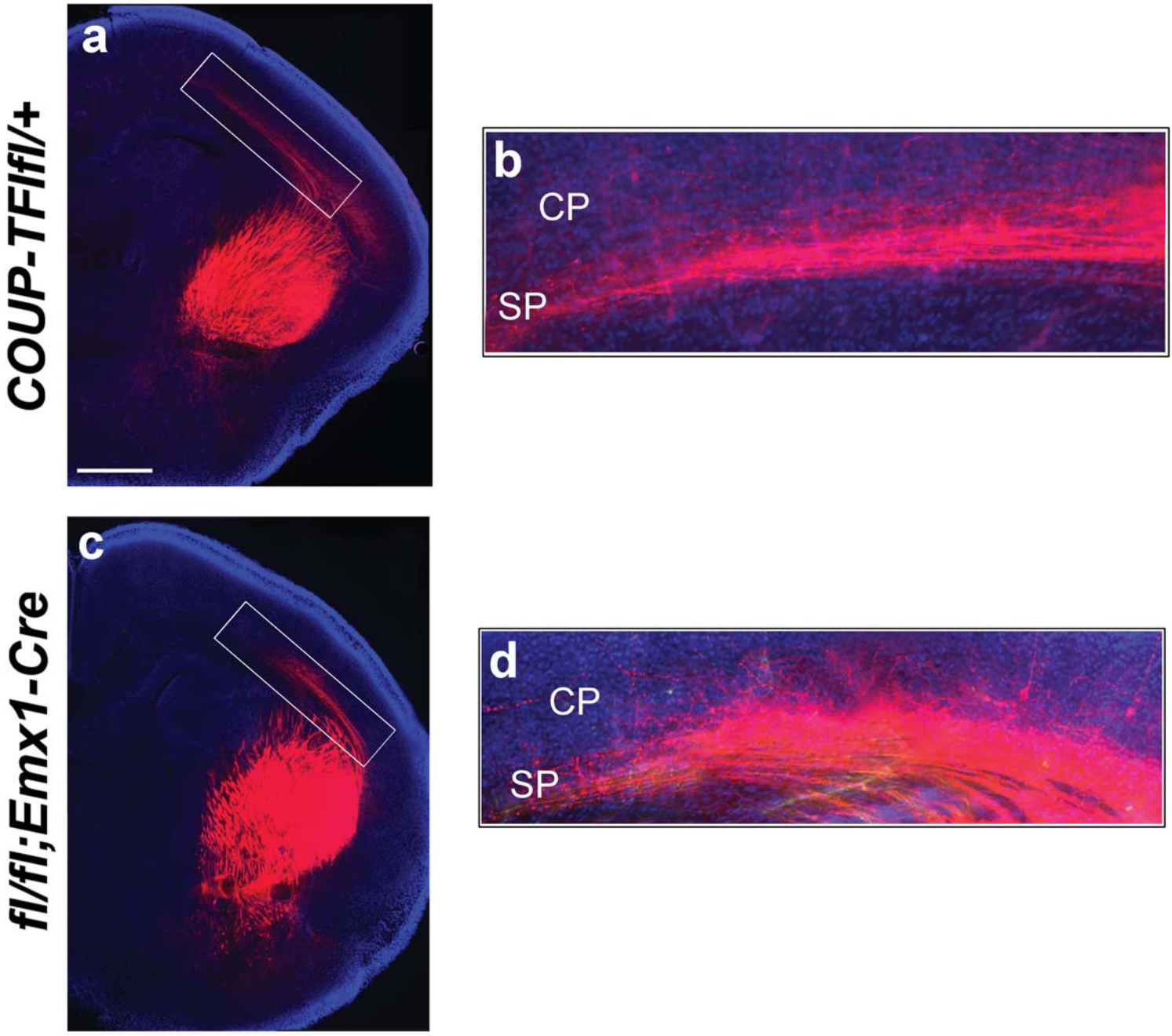
Supplementary Figure 2. Caudal shift of primary sensory areas in *COUP-TFIfl/fl*^{Emx1Cre} brains.

(a) Measurement of neocortical surface area on P7 pups. The overall surface area is not significantly different between genotypes (15.05 ± 0.6 mm^2 for controls, n = 6; 14.85 ± 0.8 mm^2 for conditional mutants, n = 6; *P*= 0.85 by unpaired Studentís t test). (b) Histogram of frontal length ratio: ratio of FL (length from the rostral pole of neocortex to the rostral edge of PMBSF) to TL (total length of neocortex from rostral pole to the occipital pole). Compared with controls (0.4938±0.0171; n=6), the frontal ratio is significantly increased in homozygous mutants (0.8560±0.050; n=5; *P*<0.0001; by unpaired Studentís t test). Bars are the means, the error bars are SEM. WT, wild type; CKO, *COUP-TFIfl/fl*^{Emx1Cre} pups.

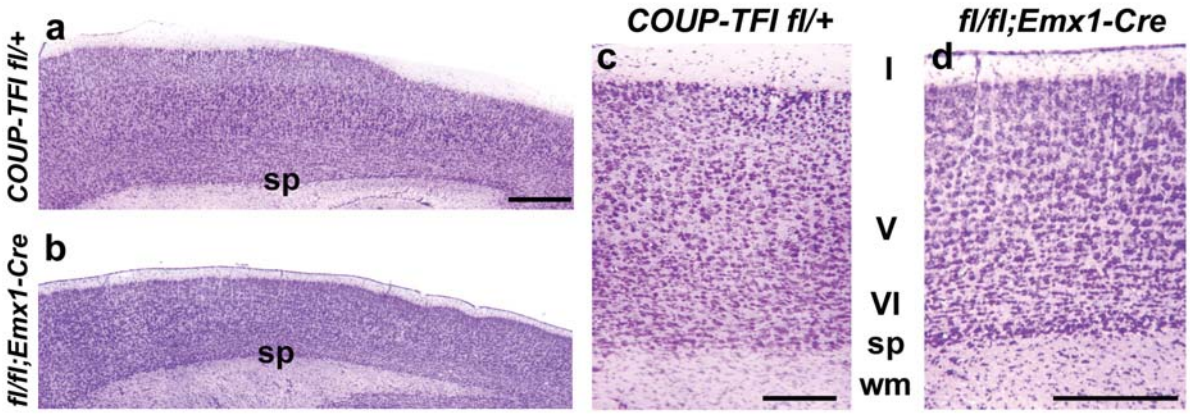


Supplementary Figure 3. Differences in Tbr1 staining along the rostro-caudal axis.

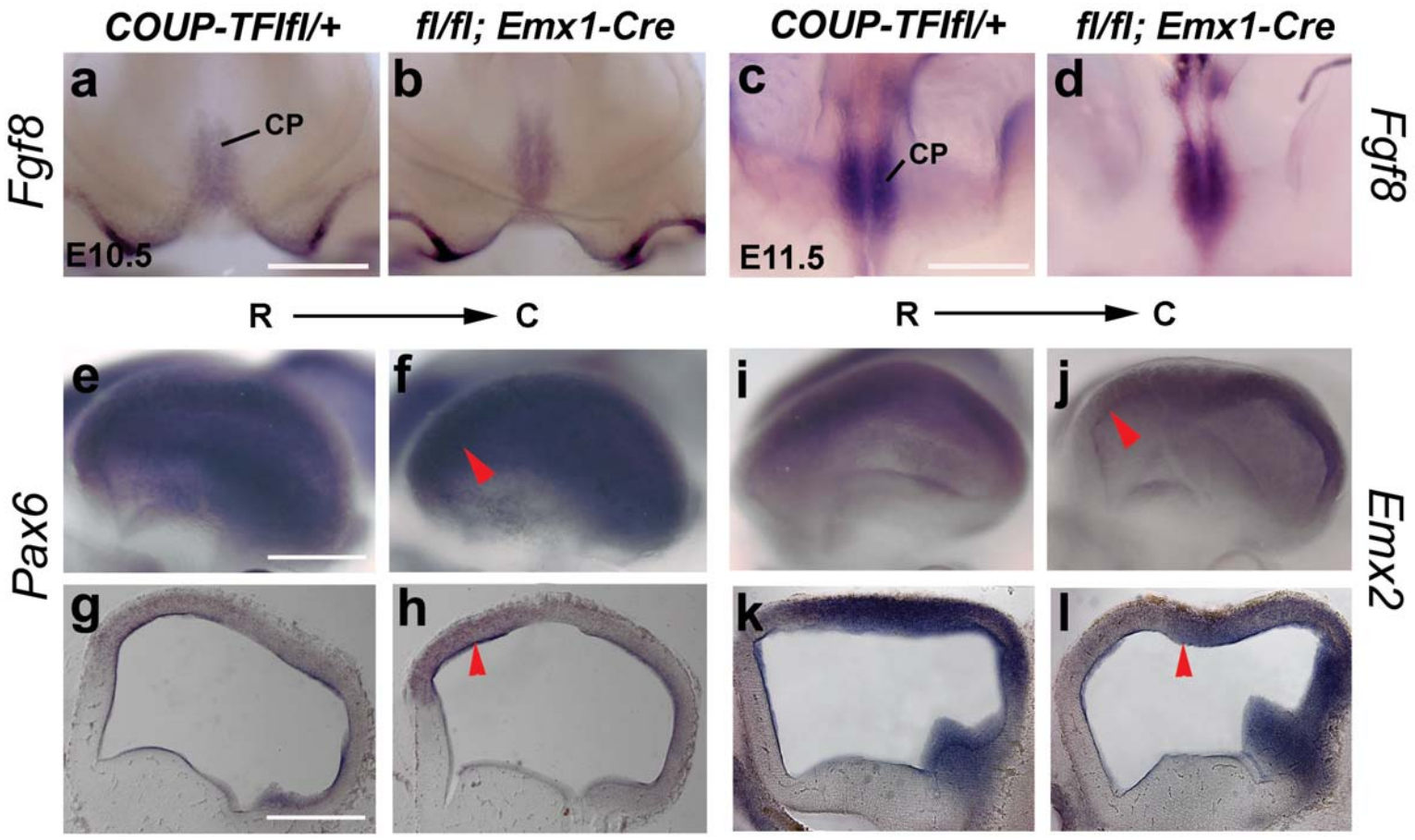
(a, d) Parasagittal sections of P8 control (*COUP-TFIfl/+*) and *COUP-TFIfl/fl*^{Emx1Cre} mutant (*fl/fl; Emx1-Cre*) brains immunostained with Tbr1 (b, c: higher magnifications in M1 and V1 of a; e, f: higher magnifications in frontal and occipital regions of d). (a-c) Along the rostro-caudal axis Tbr1 is expressed strongest in layer VI, however levels of Tbr1 expression varies in an area-specific manner, as previously described[25]. In presumptive motor area (M1), levels of Tbr1 are high in all layers (see detail of M1 in (b), whereas in presumptive V1 Tbr1 is almost absent in layers IV and V, and low in layers II/III (detail of V1 in (c). (d-f) In *COUP-TFIfl/fl*^{Emx1Cre} mutants layer-specific differences of Tbr1 expression levels are almost lost, and the Tbr1 expression pattern in occipital cortex (d, f) is similar to the one observed in frontal cortex (d, e), suggesting that the occipital cortex has acquired Tbr1 properties of the frontal cortex. The asterisk in (f) shows high ectopic expression of Tbr1 in layers II-V, a pattern that resembles the one observed in frontal cortex (e). Scale bar: 200μm.



Supplementary Figure 4. Thalamocortical axons project to the subplate and innervate the cortical plate in *COUP-TFIfl/fl*^{Emx1Cre} mutant brains. (a-d) Coronal sections taken at rostral levels of E18.5 control (*COUP-TFIfl/+*) and *COUP-TFIfl/fl*^{Emx1Cre} mutant (*fl/fl; Emx1-Cre*) fetuses counterstained with Hoechst in which a crystal of DiI was inserted in the dorsal thalamus (dTh) (see also diagram in Fig. 7). b, d are higher magnification of the boxed areas of a, c. Note how in the *COUP-TFIfl/fl*^{Emx1Cre} mutant (c, d) thalamic axons do reach the subplate (SP) and start to innervate the cortical plate (CP). The amount of axons reaching the SP and CP can vary between fetuses and is not significantly different between wild type and *COUP-TFIfl/fl*^{Emx1Cre} embryos.

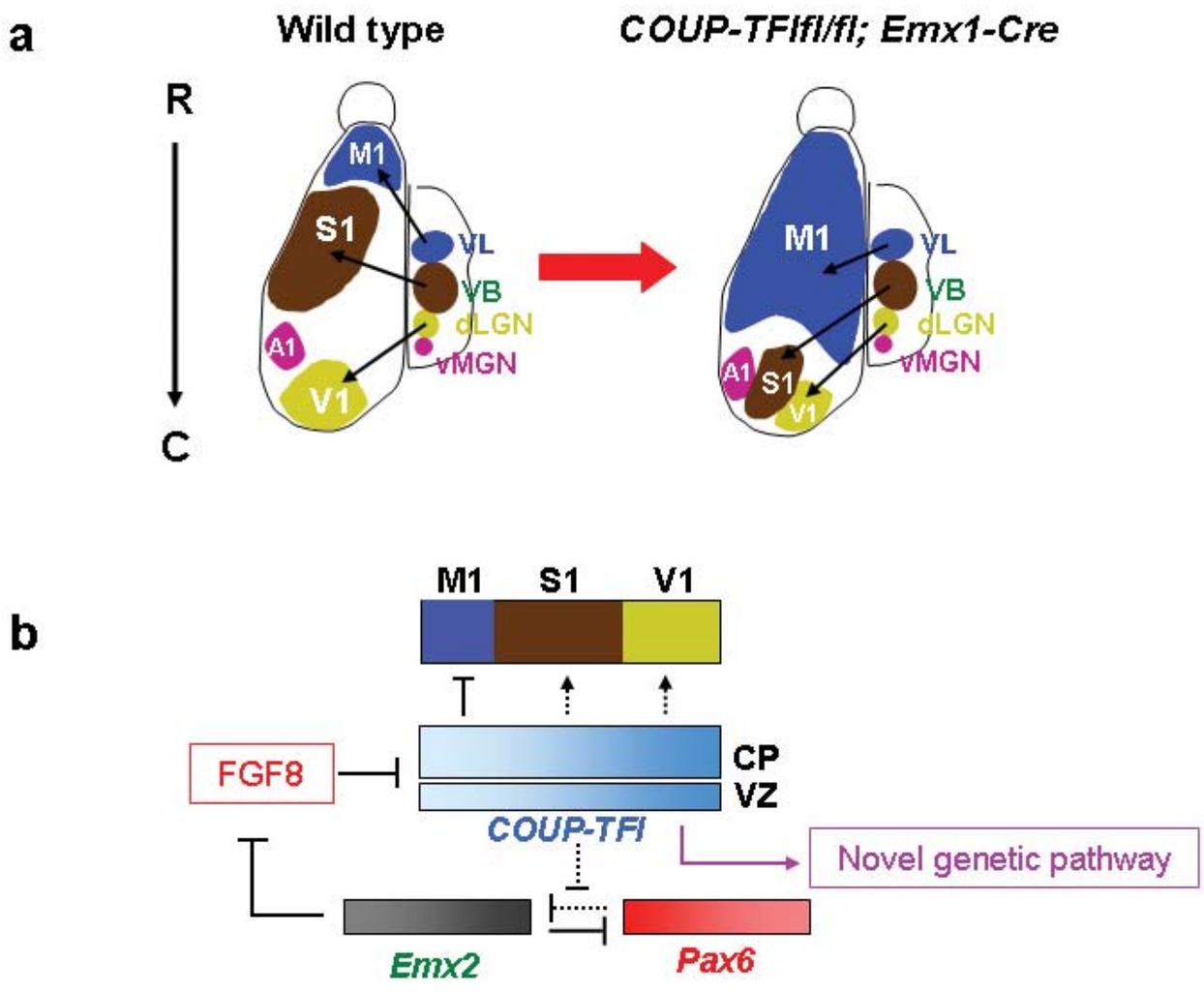


Supplementary Figure 5. A well defined subplate layer is present in *COUP-TFIfl/fl*^{Emx1Cre} mutant cortex. (a-d) Nissl stained parasagittal sections of P7 control (*COUP-TFIfl/+*) and *COUP-TFIfl/fl*^{Emx1Cre} mutant (*fl/fl; Emx1-Cre*) brains. Panels c, d are higher magnification of a radial traverse through the level of parietal cortex in a and b. A well defined subplate (sp) layer is present in the *fl/fl; Emx1-Cre* mutant cortex and the density of cells is qualitatively indistinguishable from wild type. wm, white matter. Roman numbers indicate cortical layers. Scale bar, 0.5 mm (a, b) and 0.2mm (c, d).



Supplementary Figure 6. Rostral expression of *Pax6* and *Emx2* are altered but *Fgf8* expression is not affected in *COUP-TFIfl/fl^{Emx1Cre}* embryos.

(a-d) Frontal views of whole-mount E10.5 and E11.5 control (*COUP-TFIfl/+*) and *COUP-TFIfl/fl^{Emx1Cre}* mutant (*fl/fl; Emx1-Cre*) embryos hybridized with *Fgf8*. Note that expression of *Fgf8* in the commissural plate (CP) is unchanged in *COUP-TFIfl/fl^{Emx1Cre}* mutant embryos (b, d). (e, f, i, j) Lateral views of E11.5 whole-mount telencephalons hybridized with Pax6 (e, f) and Emx2 (i, j). Note how in COUP-TFIfl/fl^{Emx1Cre} embryos *Pax6* expression is slightly up-regulated, whereas *Emx2* expression is down-regulated (arrowheads in f, j). (g, h, k, l) Sagittal sections of whole-mount E11.5 telencephalons hybridized with *Pax6* (g, h) and Emx2 (k, l). In *COUP-TFIfl/fl^{Emx1Cre}* mutant embryos the rostral expression of *Pax6* is up-regulated, while *Emx2* expression is down-regulated (arrowheads in h, l). R, rostral; C, caudal. Scale bars: 500 μm.



Supplementary Figure 7. Role for COUP-TFI in patterning the neocortex into primary areas.

(a) Diagram summarizing how axons from individual thalamic nuclei project to a unique set of cortical areas in wild type and *COUP-TFIfl/fl*^{Emx1Cre} (*COUP-TFIflf/fl; Emx1-Cre*) mice. Although the frontal cortex, which includes the motor area M1, is caudally expanded and the primary sensory areas are reduced and caudally shifted, the topographic projections between the different thalamic nuclei and the cortical areas are maintained in *COUP-TFIfl/fl*^{Emx1Cre} mutants.

(b) COUP-TFI is expressed in a low rostro-medial to high caudo-lateral expression gradient in progenitors across the ventricular zone (VZ) and in post-mitotic neurons across the cortical plate (CP). Our study hints to a model whereby COUP-TFI directly participates in the specification of the positional identity of all primary areas by positively regulating primary sensory areas, such as somatosensory (S1) and visual (V1) areas, and negatively regulating the frontal cortex, which includes M1. Previous studies have shown that rostral expression of *COUP-TFI* is repressed by the signaling molecule FGF8, and that EMX2 represses *Fgf8* expression. *Emx2* and *Pax6* have complementary expression gradients, which are established by mutual negative cross-regulation and under the partial regulation of COUP-TFI. Our data suggest that the slight changes in *Pax6* and *Emx2* expression that we observe in *COUP-TFIfl/fl*^{Emx1Cre} mutants cannot account for the massive anterior expansion and hypothesize the presence of a novel genetic pathway through the action of other not yet identified downstream players controlled by COUP-TFI.