Supporting Information

Alsiö et al. 10.1073/pnas.1215707110

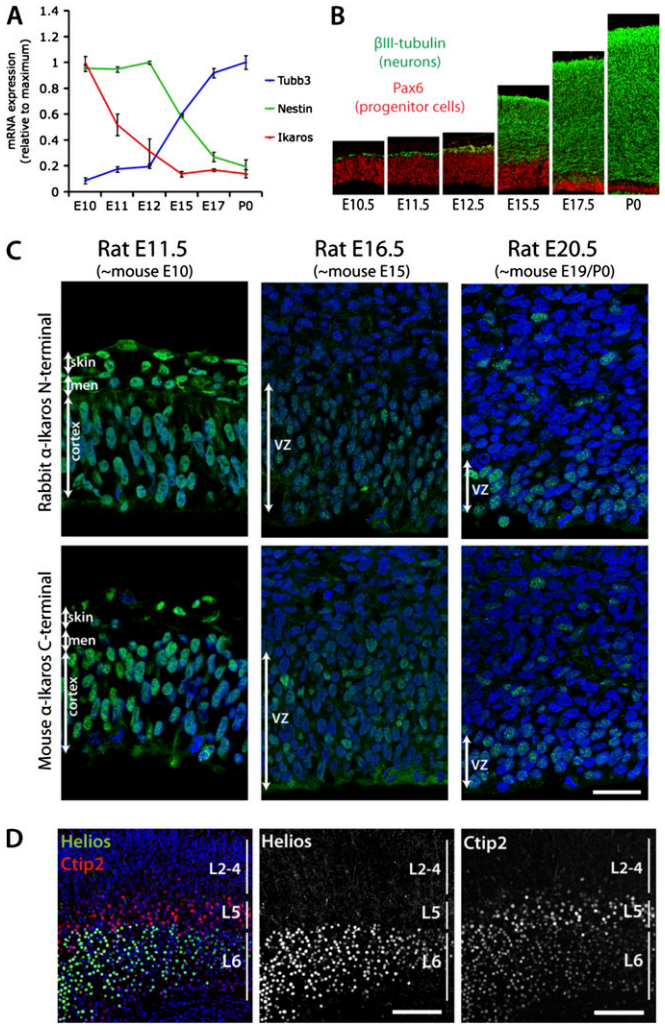

Fig. S1. Expression of Ikaros family members in the developing cerebral cortex. (*A*) Real-time quantitative RT-PCR (qRT-PCR) assay for Ikaros, Nestin (progenitor cells), and Tubb3 (βIII-tubulin, neurons) in cortex from embryonic day (E) 10.5 to postnatal day (P) 0 shows that Ikaros levels in the cortex decrease faster than the progenitor cell marker Nestin. Expression was normalized to the average expression of six housekeeping genes (GAPDH, β-actin, TBP, UBC, YWHAZ, and SDHA). For each gene, values are shown relative to the time point with the highest expression level and represent the mean normalized expression ± SEM ($n = 3$ independent RNA extractions). (*B*) Cortex stained for neurons (βIII-tubulin) and progenitor cells (Pax6) at the same developmental stages as for the qRT-PCR assays reported above. In agreement with the qRT-PCR results, this shows that Ikaros levels in the cortex decrease faster than the proportion of progenitor cells. (*C*) Two different Ikaros antibodies show similar expression in ventricular zone (VZ) progenitor cells in the rat cortex. men, meninges. (Scale bar: 25 μm.) (*D*) Helios is expressed in layer (L) 6 neurons (weakly Ctip2^{+} neurons) in the P0 cortex. (Scale bar: 100 μm.)

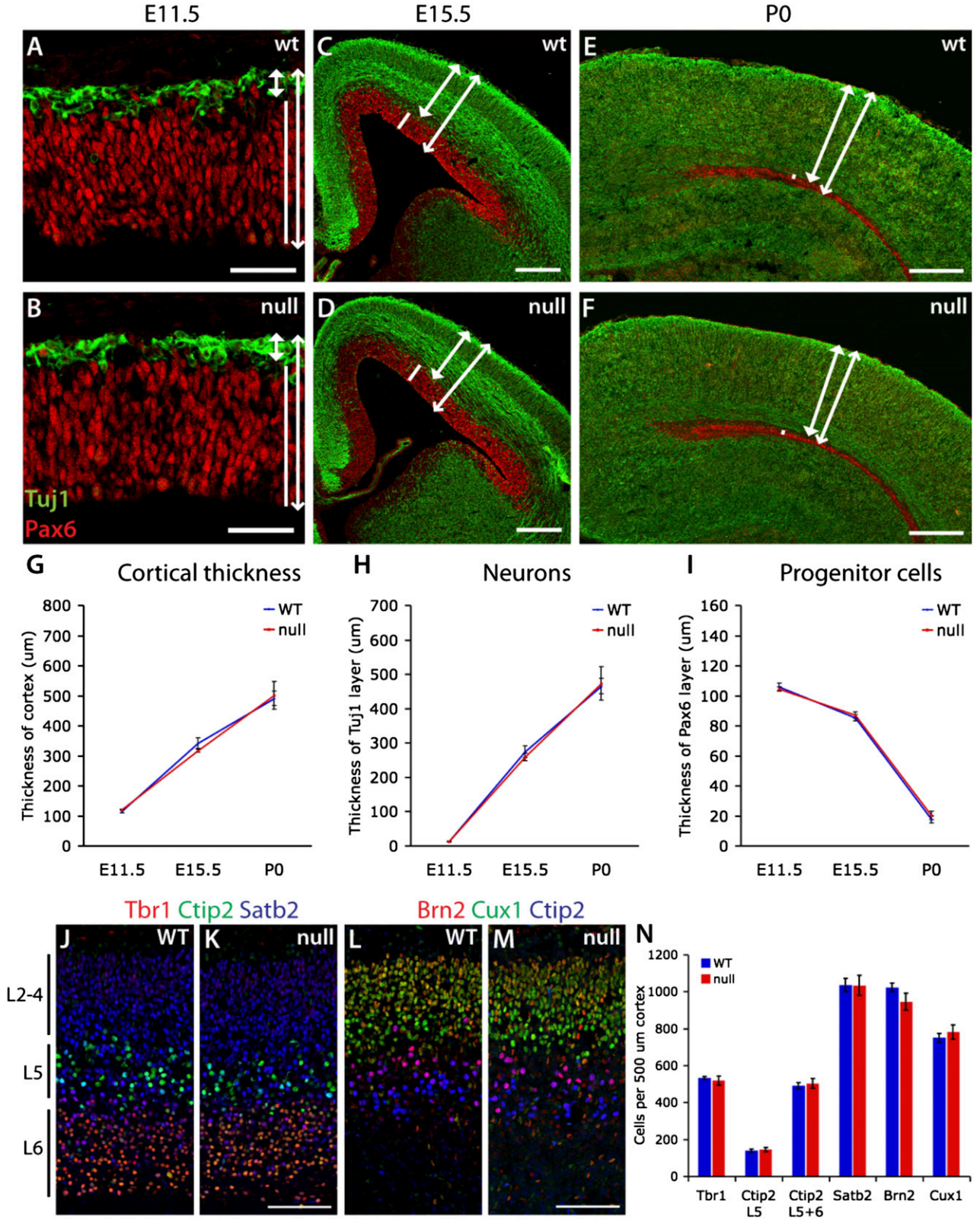

Fig. S2. Ikaros mutant mice have no detectable cortical phenotype. (*A–F*) Immunohistochemistry for Tuj1 (neurons, green) and Pax6 (progenitor cells, red) on cortical sections at embryonic day (E) 11.5, E15.5, and postnatal day (P) 0 shows no anatomical differences between WT and Ikaros mutant cortex. Cortical thickness (*G*; lines with open arrowheads in *A–F*), thickness of the Tuj1^{+} neuronal layer (*H*; lines with filled arrowheads in *A–F*), and thickness of the Pax6^{+} progenitor cell layer (*I*; lines with no arrowheads in *A–F*) are indistinguishable in Ikaros mutant and WT littermates. (*J–M*) P0 cortical sections stained for deep layer-specific transcription factors Tbr1 (layer 6) and Ctip2 (strongly labeled cells in layer 5 and weakly labeled cells in layer 6) and for upper layer-specific transcription factors Satb2 (most cells in layers 2–4 but also some in layers 5–6), Brn2 (layers 2–5), and Cux1 (layers 2–4). (*N*) Quantifications of neurons expressing the different transcription factors showed no significant differences between Ikaros mutant and WT littermates: E11.5 ($n = 4$), E15.5 ($n = 4$ mutant/3 wt), P0 ($n = 3$ mutant/4 wt). Values shown are mean ± SEM. L2-4, layers 2–4; L5, layer 5; L6, layer 6. (Scale bars: *A* and *B*, 50 μm; *C–F*, 200 μm; *J–M*, 100 μm.)

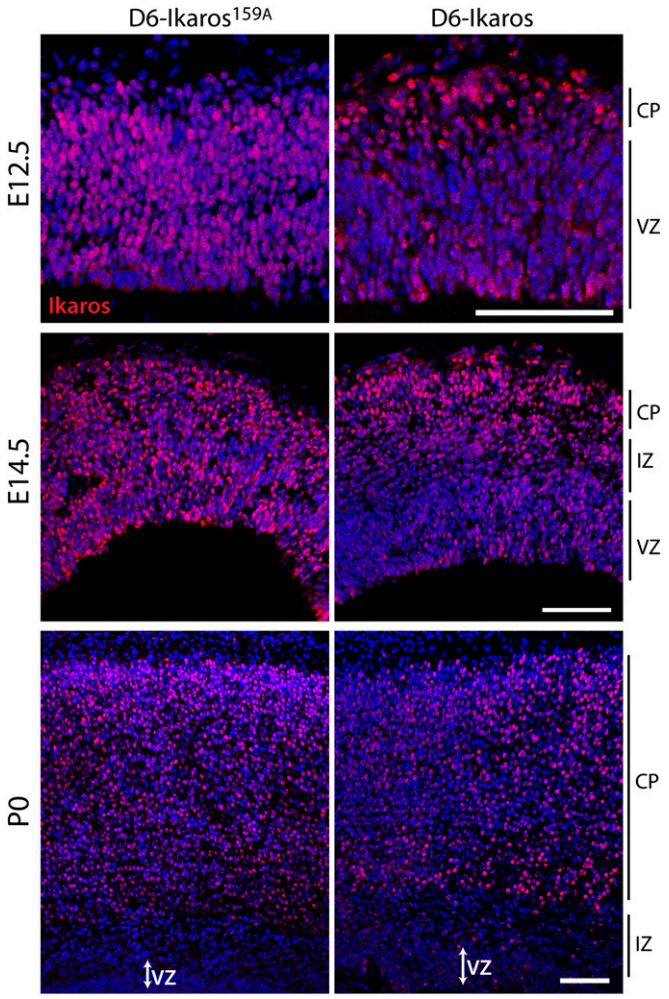

Fig. S3. Transgenic Ikaros expression from the D6 promoter in D6-Ikaros and D6-Ikaros^{159A}. Immunostaining for Ikaros in WT, D6-Ikaros^{159A}, and D6-Ikaros cortex at embryonic day (E) 12.5, E14.5, and postnatal day (P) 0 shows that even though Ikaros levels are gradually reduced in the ventricular zone (VZ) of D6-Ikaros from E12.5, transgenic Ikaros expression is still detected at levels higher than in WT at E14.5. CP, cortical plate; IZ, intermediate zone. (Scale bars: 100 μm.)

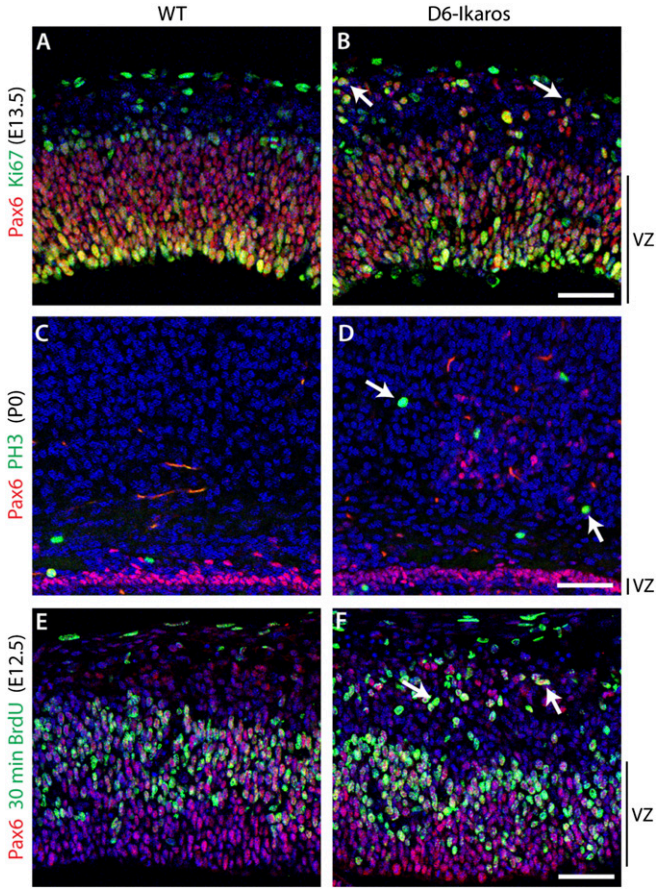

Fig. S4. Ectopic Pax6^{+} cells in the D6-Ikaros cortex are proliferating progenitor cells. (*A* and *B*) Ectopic Pax6 cells (red) are positive for Ki67 (green, marker of proliferative cells) in the D6-Ikaros embryonic day (E) 13.5 cortex. (C and *D*) Some ectopic Pax6 cells (red, indicated by arrows) are positive for phosphohistone H3 (PH3; green, marker of mitotic cells) in the D6-Ikaros postnatal day (P) 0 cortex. (*E* and *F*) Ectopic Pax6 cells (red, indicated by arrows) incorporated BrdU (i.e., were in S-phase) in D6-Ikaros E12.5 cortex. VZ, ventricular zone. (Scale bars: 50 μm.)

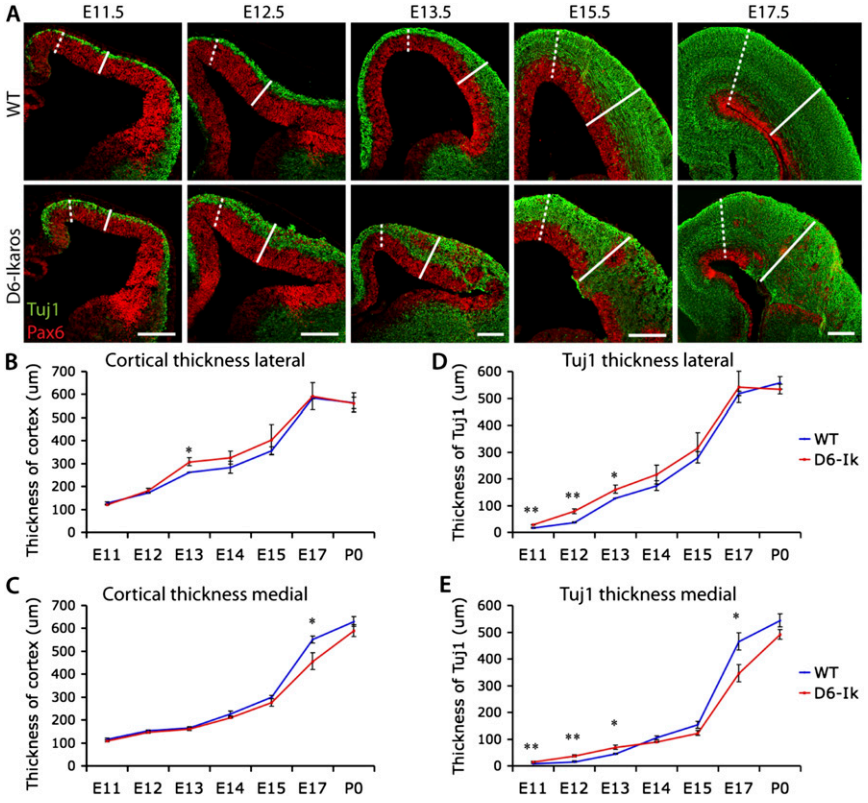

Fig. S5. Cortical neurogenesis is altered in the Ikaros-overexpressing cortex. (*A*) Immunostaining for progenitor cells (Pax6, red) and neurons (Tuj1, green) at various stages of cortical development, as indicated, in WT (*Upper*) and D6-Ikaros (*Lower*) cortical sections. White lines indicate positions of measurements in *B*–*C*. (*B*) Total thickness of cortex was similar in D6-Ikaros and WT littermates at most developmental stages, both laterally (*C*) and medially (*D*). (*D*–*E*) The Tuj1$^+$ neuronal layer (cortical plate and intermediate zone) was significantly thicker in D6-Ikaros than in WT littermates at the early stages from embryonic day (*E*) 11.5 to E13.5 but similar to WT at later stages. In *B*–*E*, values represent mean ± SEM [$n = 3$ brains (E11.5–E17.5) or $n = 6$ brains (postnatal day [P] 0)]. *$P < 0.05$; **$P < 0.01$. (Scale bars: 200 μm.)

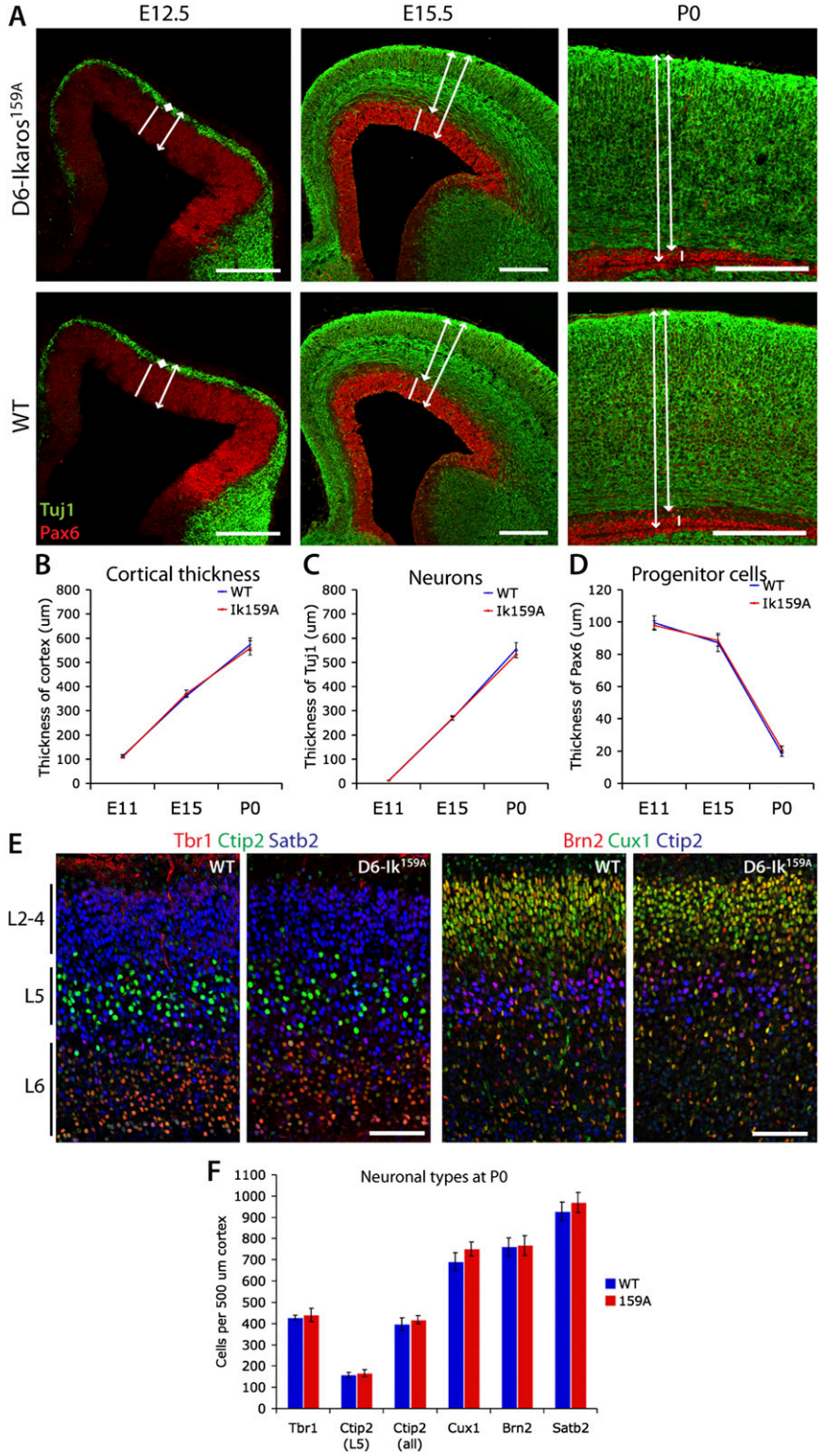

Fig. S6. Cortical expression of the DNA-binding deficient form of Ikaros, Ikaros^{159A}, has no detectable effect on cortical development. (*A*) Immunohistochemistry for Tuj1 (neurons, green) and Pax6 (progenitor cells, red) on cortical sections at embryonic day (E) 11.5, E15.5, and postnatal day (P) 0 shows no anatomical differences between WT (*Upper*) and D6-Ikaros^{159A} (*Lower*) cortex. Thickness of the cortex (*B*; lines with open arrowheads in *A*), the Tuj1^{+} neuronal layer (*C*; lines with filled arrowheads in *A*), and the Pax6^{+} progenitor cell layer (*D*; lines with no arrowheads in *A*) are indistinguishable in D6-Ikaros^{159A} and WT littermate cortex. (*E*) P0 cortical sections stained for deep layer-specific transcription factors Tbr1 (layer 6) and Ctip2 (strongly labeled cells in layer 5 and weakly labeled cells in layer 6) and for upper layer-specific transcription factors Satb2 (most cells in layers 2–4 but also some in layers 5–6), Brn2 (layers 2–5), and Cux1 (layers 2–4). (*F*) Quantifications of neurons expressing the different transcription factors showed no significant differences between D6-Ikaros^{159A} and WT littermates: E11.5 ($n = 5$ D6-Ikaros^{159A}/4 wt), E15.5 ($n = 3$), P0 ($n = 3$). Values shown are mean ± SEM. L2-4, layers 2–4; L5, layer 5; L6, layer 6. (Scale bars: *A*, 200 μm; *E*, 100 μm.)

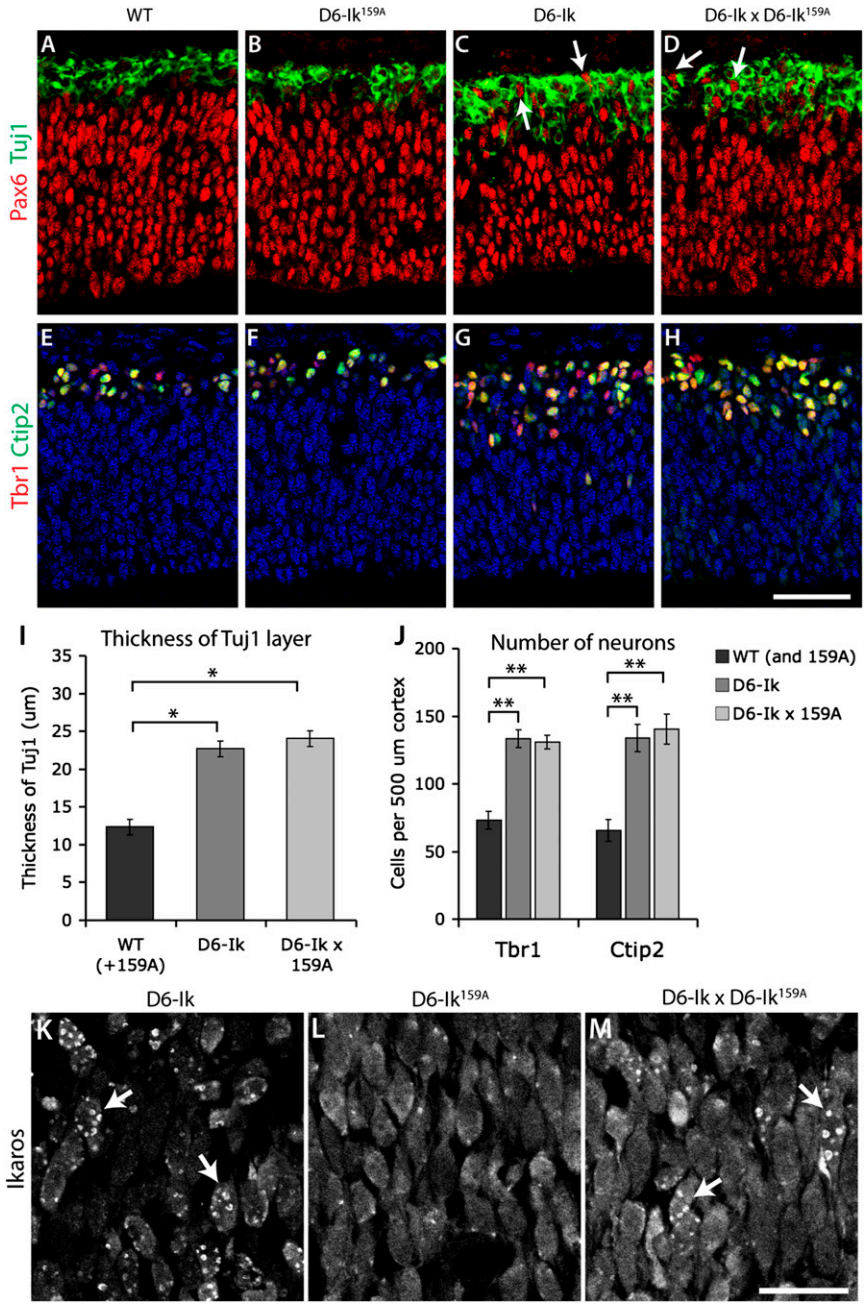

Fig. S7. Ikaros^{159A} does not act as a dominant negative in the cerebral cortex. (*A–D*) Double transgenic D6-Ikaros × D6-Ikaros^{159A} does not rescue the D6-Ikaros phenotype. Staining for Pax6 (progenitor cells) and Tuj1 (neurons) at embryonic day (E) 12.5 shows that both D6-Ikaros and double transgenic D6-Ikaros × D6-Ikaros^{159A} embryos have ectopic Pax6^{+} progenitor cells in the Tuj1^{+} neuronal layer (arrows in *C* and *D*). Both D6-Ikaros and double transgenic embryos also have a wider Tuj1^{+} neuronal layer than WT and D6-Ikaros^{159A}. Staining for the neuronal transcription factors Tbr1 and Ctip2 shows that both D6-Ikaros (*G*) and the double transgenic embryos (*H*) have more neurons than WT (*E*) or D6-Ikaros^{159A} (*F*). (*I*) Quantifications of the thickness of Tuj1 from *A–D*. (*J*) Quantifications of the number of Tbr1^{+} and Ctip2^{+} neurons from *E–H*. For the quantifications, all embryos are from the same litter to avoid small interlitter differences in age that greatly affect the numbers of neurons and D6-Ikaros^{159A} were counted together with the WT because they were previously shown to have identical numbers of neurons at E12.5 ($n = 3$ WT/D6-Ikaros^{159A}, $n = 3$ D6-Ikaros, $n = 2$ double transgenics). Values shown are mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$. (*K–M*) Ikaros^{159A} fails to disrupt the pericentromeric focal localization of WT overexpressed Ikaros in the cortex of double transgenic mice. Overexpressed WT Ikaros is found in typical pericentromeric nuclear foci (*K*, arrows). (*L*) Overexpressed Ikaros^{159A} is found diffusely throughout the nucleus. (*M*) In double transgenic D6-Ikaros × D6-Ikaros^{159A} cortex, both types of staining are visible (arrows point to cells with pericentromeric foci). (Scale bars: *A–H*, 50 μm; *K–M*, 20 μm.)

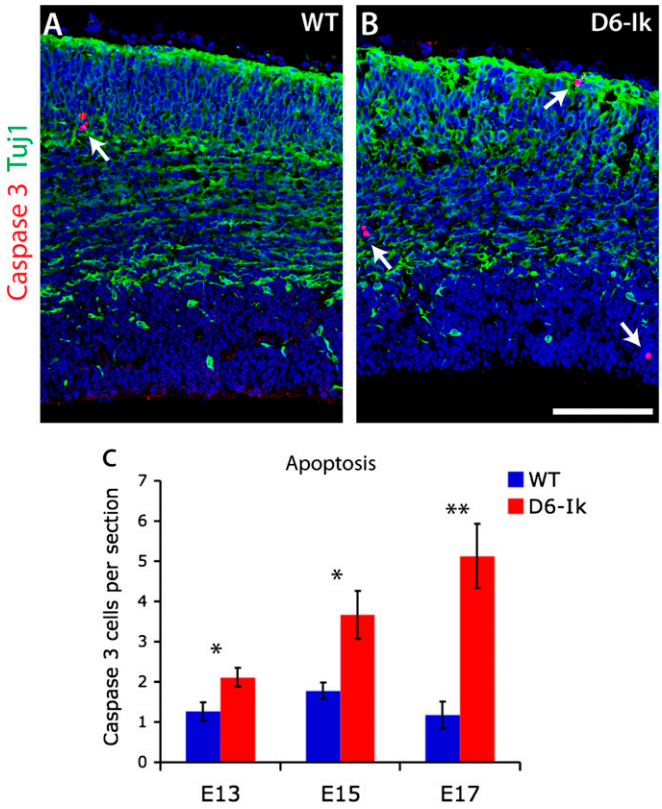

Fig. S8. Increased apoptosis in the D6-Ikaros cortex. (*A* and *B*) Apoptotic cells were identified by cleaved caspase 3 staining (red, arrows) in WT and D6-Ikaros cortex. Images show embryonic day (E) 15.5 cortices. (*C*) Quantification of the number of apoptotic cells in E13.5, E15.5, and E17.5 cortex showed that at all stages examined, D6-Ikaros had increased numbers of apoptotic cells compared with WT littermates, and that this was more severe at later stages: E13.5 and E15.5 ($n = 3$) or E17.5 ($n = 4$). Eight or more sections per brain were quantified, and values shown are mean ± SEM. $*P < 0.05$; $**P < 0.01$. (Scale bar: 100 μm.)

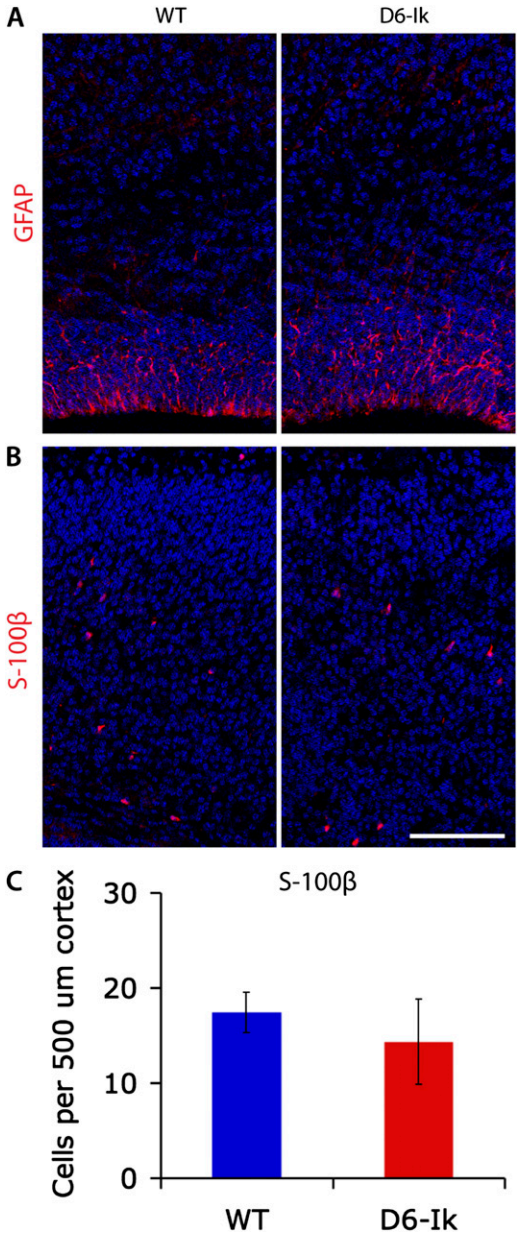

Fig. S9. No observed effect on astrocytes in the D6-Ikaros cortex at postnatal day (P) 0. (*A*) P0 cortical sections stained for the astrocyte protein GFAP in WT and D6-Ikaros cortex. At this stage, GFAP$^+$ radial glia are present in the ventricular zone of both WT and D6-Ikaros cortex. Scattered astrocytes expressing S-100β are present in the cortex at this stage (*B*), with no detectable differences in numbers between the WT and D6-Ikaros cortex (*C*) ($n = 3$). Values shown are mean ± SEM. (Scale bar: 100 μm.)

Table S1. Primers used for semiquantitative RT-PCR

Gene	Forward primer	Reverse primer
Ikaros	ggcctcctttacccagaaag	cagctggtacatggagctga
Helios	taaccagtgcggagcttctt	tgatggcttggtccatcata
Eos	ccttcacacagaagggcaat	gcagatgctctgtacccaca
Pegasus	ggaatacctgacccagcaga	gggacaggttacttcggtca
Aiolos	aagatgaactgcgacgtgtg	tgtagttggcatcgaagcag
β-Actin	tcacccacactgtgcccatctacga	catcggaaccgctcgttgccaatag

Table S2. Primers used for quantitative PCR

Gene	Forward primer	Reverse primer
Ikaros	tggatgtcgatgagggtcaa	gctcatccccttcatctgga
Helios	gaaagcgcaaaagctccact	tctgggtagctgaatcgcataa
Eos	ctgtggccggagctacaaac	tgagtcaggcaccatctcca
Pegasus	tgtccacgaagggttggtct	ctgctctggagaggcaagga
Aiolos	gaaaaagctcgatgcctcagaa	tctcgtacatgtagccgggatt
β-Actin	gggctgtattcccctccatc	tctgacccattcccaccatc
Gapdh	catggccttccgtgttcct	gcggcacgtcagatcca
Sdha	cgcagtttcgaggcttcttc	ccgcaggtctgtttttggag
Tbp	actcctgccacaccagcttc	cgaagtgcaatggtctttaggtc
Ubc	gagccctccttgtgcttgtt	aagacacctcccccatcaca
Ywhaz	atttccatgttgggcacagg	aaagatcatgcggccctttt

Dataset S1. mRNAs with significantly increased levels in the embryonic day 11 D6-Ikaros cortex compared with controls ($P < 0.05$)

Dataset S1

Dataset S2. mRNAs with significantly decreased levels in the embryonic day 11 D6-Ikaros cortex compared with controls ($P < 0.05$)

Dataset S2