Supplementary figure legends

Fig. S1. Final laminar location is not altered by T-cad-overexpression.

T-cad and *eyfp* expression vectors were coelectroporated into cells destined to become upper layer cells (E14.5 mouse brain). (A) In the T-cad-transfected brain (right), numerous cells were found to be detained in the deep layers at P1, whereas most labeled cells had migrated to the upper layers in the control (left). Flanking images show DAPI-staining. Scale bar: 100 μm. (B) The cortical depth was divided into 10 equal segments from segment 1 (SP) to segment 10 (pial surface). Percentage of fluorescent labeled cells in each segment is shown cumulatively in Y-axes. After the birth (P0), 37% of labeled cells were located in the deep segments (segment #1-6) in T-cad-overexpressing brain, whereas only 23% of labeled cells were found in the control (T-cad, 300 cells from 3 brains vs control, 300 cells from 3 brains; Mann-Whitney *U* test, $p < 0.0001$). However, the difference was much smaller at P6, indicating that final laminar positions are not affected by the overexpression (T-cad, 200 cells from 2 brains vs control, 200 cells from 2 brains; $p > 0.1$). (C) T-cad shRNA construct (piGENEmU6/Tcad1631) and *eyfp* expression vector were cotransfected into deep layer cells of the rat neocortex. The migration patterns of shRNA transfected cells were not significantly different from those in the control at P0–1 (shRNA, 734 cells from 2 brains vs control, 616 cells from 3 brains; Mann-Whitney *U* test, $p > 0.1$) and P6–7 (shRNA, 1132 cells from 3 brains vs control, 458 cells from 2 brains; $p > 0.05$).

Fig. S2. Cellular identity persists in T-cad-overexpressing upper layer cells.

(A) *In situ* hybridization for *mef-2c*, an upper-layer-specific transcription factor, was performed on sections of normal and T-cad-overexpressing mouse brains at P1. *mef-2c* was expressed in the upper layers of the control cortex (left). *mef-2c* was also expressed in T-cad-overexpressing cells, which accumulated in the IZ (bordered by dashed lines, right), suggesting that T-cad-expressing cells display normal cellular identity. (B-E) Immunostaining for Ctip2, a marker for subcortical projection

neurons in layer V, was performed on a section of T-cad-overexpressing mouse brain at P3. No Ctip2 signal (magenta) was detected in T-cad-overexpressing cells (green), which were settled down in the upper layer (C), detained in the layer V (D), and accumulated in the IZ or the SVZ (E). Scale bars: 100 μm (A), 250 μm (B), 50 μm (C-E).

Fig. S3. The origins of T-cad-overexpressing upper layer cell axons which project to the abnormal direction.

T-cad and *eyfp* were coelectroporated into mouse cortex at E14.5, and the brain was sectioned at P3. (A) T-cad-overexpressing upper layer cells occasionally accumulated in the IZ or SVZ (asterisk). (B) Higher magnification view of the boxed area in (A). The abnormal projections originated from cells which accumulated in the SVZ and IZ (A,B) and migrated into the CP (C,D). From the accumulated cells, abnormally oriented axons directly entered the IC (B), although callosal projections were observed as well (A, arrowheads). (C,D) The aberrant projections also occurred from the cells which migrated into the CP (arrowheads), although the majority of labeled axons were oriented normally to the medial direction (arrows). These axons turned abnormally to the lateral direction and misrouted in the upper part of the IZ, which is the primary pathway of T-cad-positive subcortical projection neurons (arrowheads in C,D). Scale bars: 250 μm (A), 100 μm (B-D).

Fig. S4. Homophilic interactions by T-cad contribute to axonal extension.

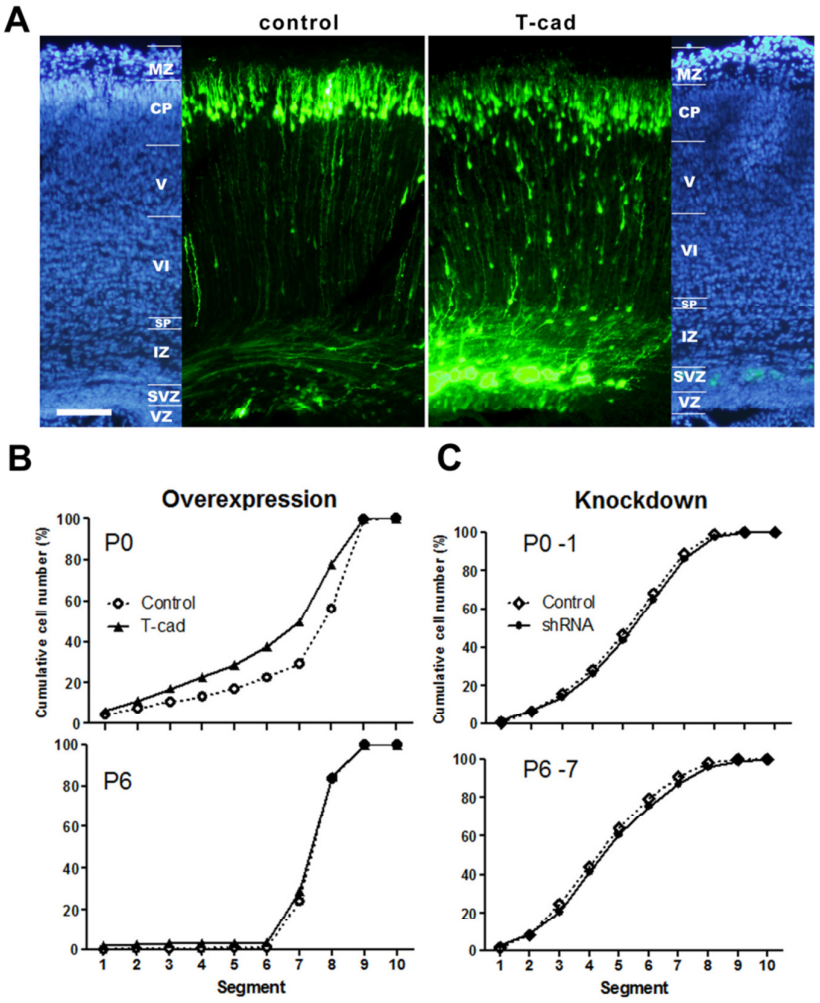
(A, B) L-cells were plated and transfected with *dsred* and *T-cad* expression plasmids. After 12-24 hours, dissociated cortical cells from E15 rat were transfected with *eyfp* and *T-cad* expression plasmids and were plated onto the transfected L-cell cultures. After two days in culture, neurites from cortical cells (green) tended to grow on adjacent L-cells (magenta). (C, D) Neurites from cortical cells transfected with *eyfp* alone (green) scarcely grew on L-cells (magenta) transfected with *dsred* alone. Scale bars: 50 μm. (E) Preferential growth of cortical neurons on L-cells was quantified and compared between the three groups: (1) co-culture of T-cad-negative cortical neurons

and T-cad-negative L-cells, (2) T-cad-negative neurons and T-cad-transfected L-cells, (3) T-cad-transfected neurons and T-cad-transfected L-cells. Axon growth of EYFP-labeled cortical cells on the adjacent L-cells was classified into three categories in Grow along: axon grows along L-cell, Pass through: axon partly grows on L-cell, and Grow away: axon hardly grows on L-cell. The distribution of neurites is significantly different between the groups (1) and (3), and between the groups (2) and (3). Pairwise distribution comparison *p* values were calculated using Pearson's chi-spuare test.

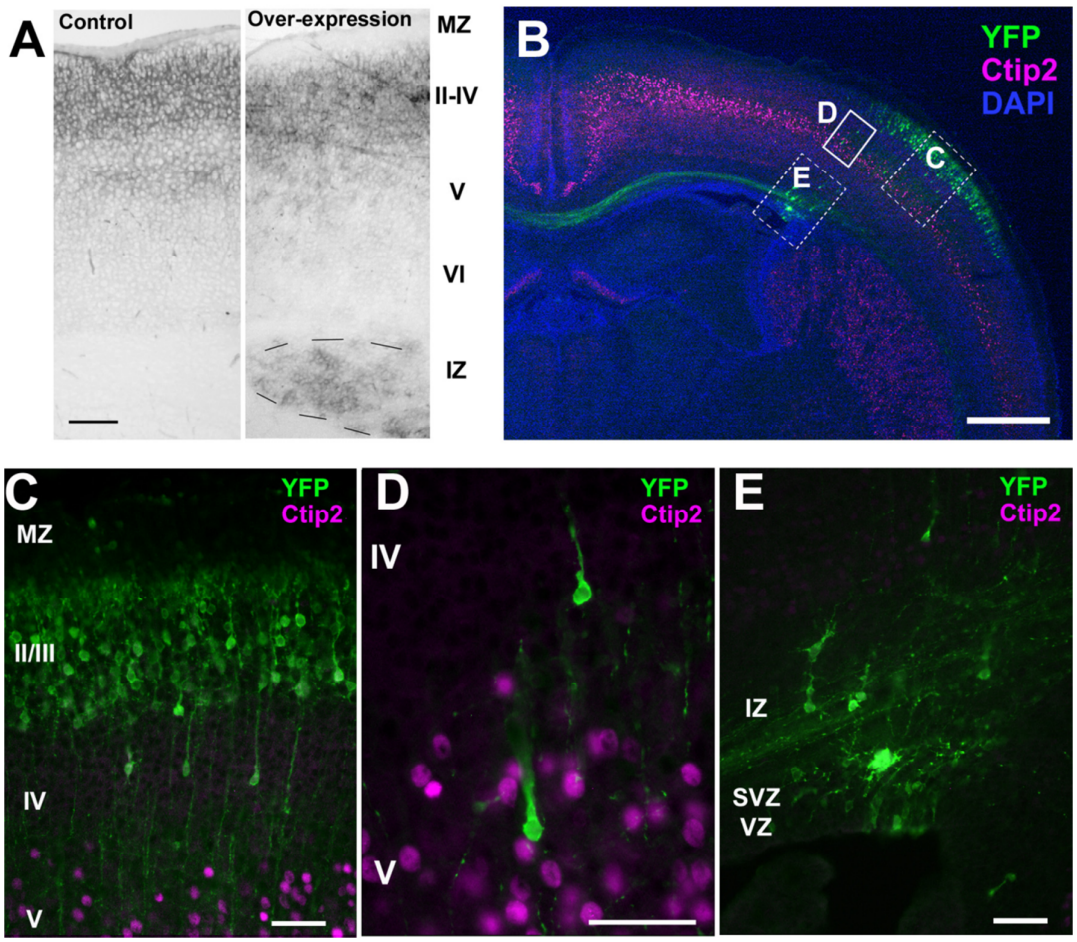
Fig. S5. *In vivo* knockdown of T-cad by shRNA.

One of the shRNA constructs (piGENE-mU6/T-cad689) and fluorescent reporter plasmid (*dsred*) were coelectroporated into cells which were destined to become deep layer cells (E15.0 rat). Ten micrometer-thick cryosections were prepared at E18.5, when endogenous T-cad is still detectable on cell bodies of cortical cells. (A) In the control, T-cad expression was observed in the CP and SP. Higher magnification views show DsRed and T-cad double positive cells (arrows). (B) Endogenous T-cad was hardly detected in the shRNA transfected cells migrating in the CP (arrows). (C) The proportion of T-cad-expressing cells to the DsRed-labeled cells was calculated in the CP. Endogenous T-cad was detected only in 20% of the shRNA transfected cells, whereas it was found in 63% of DsRed-labeled cells in the control, indicating that T-cad expression was effectively down-regurated by the shRNA in approximately 70% of the originally T-cad-expressing population. The data were collected from 2 embryos each. (D) Immunostaining for Tbr1 (magenta), a marker for layer VI corticothalamic projection neurons, shows that shRNA transfected cells (green) express Tbr1, suggesting that the cellular property had not been changed. Scale bars: 50 μm (upper panel in A,B), 10 μm (lower panel in A,B), 10 μm,(D).

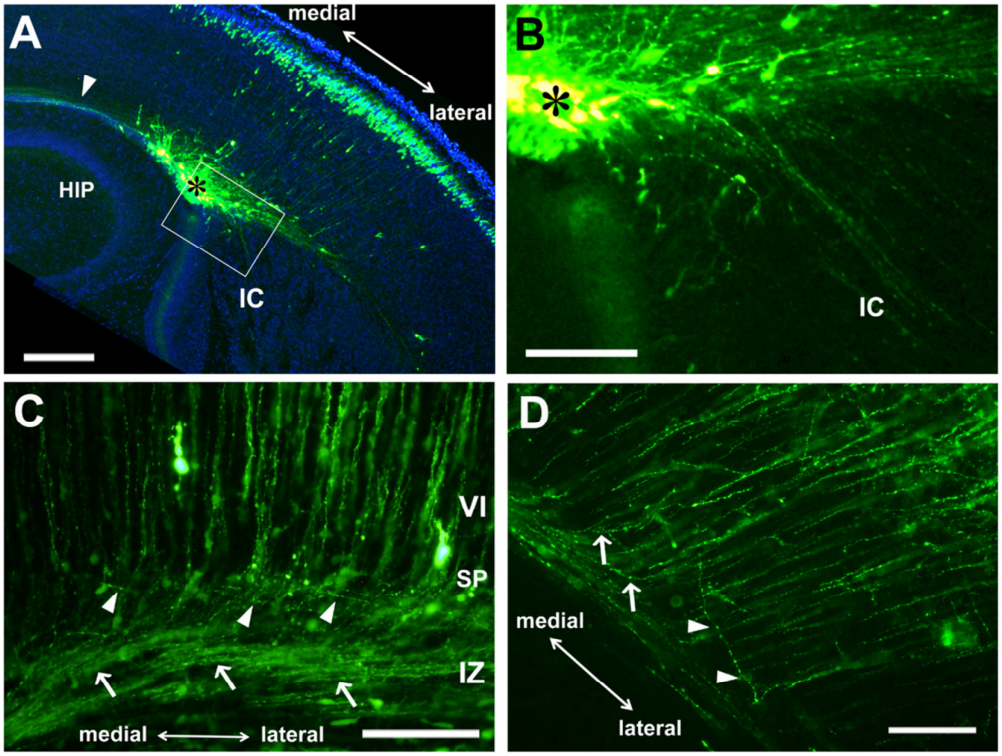
Supplementary figure S1



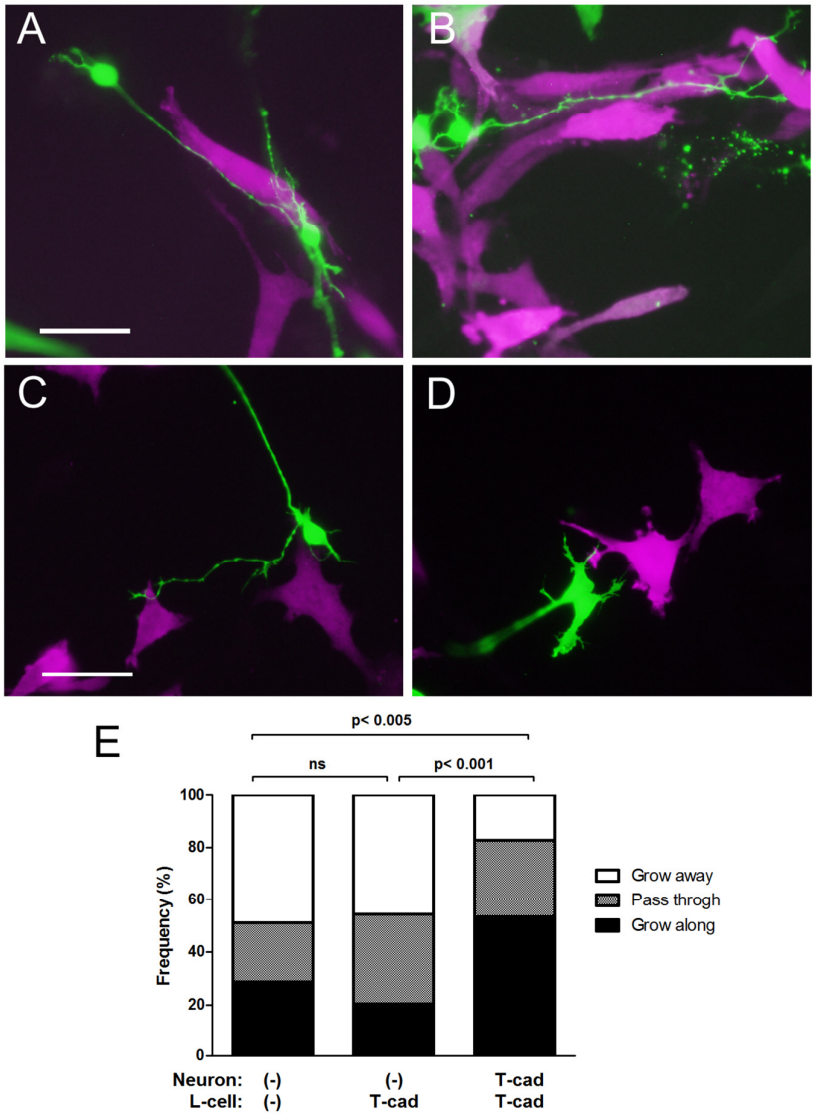
Supplementary figure S2



Supplementary figure S3



Supplementary figure S4



Supplementary figure S5