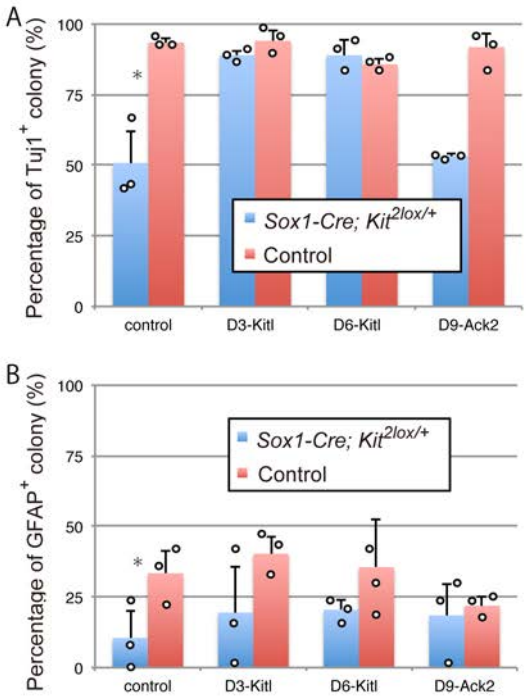
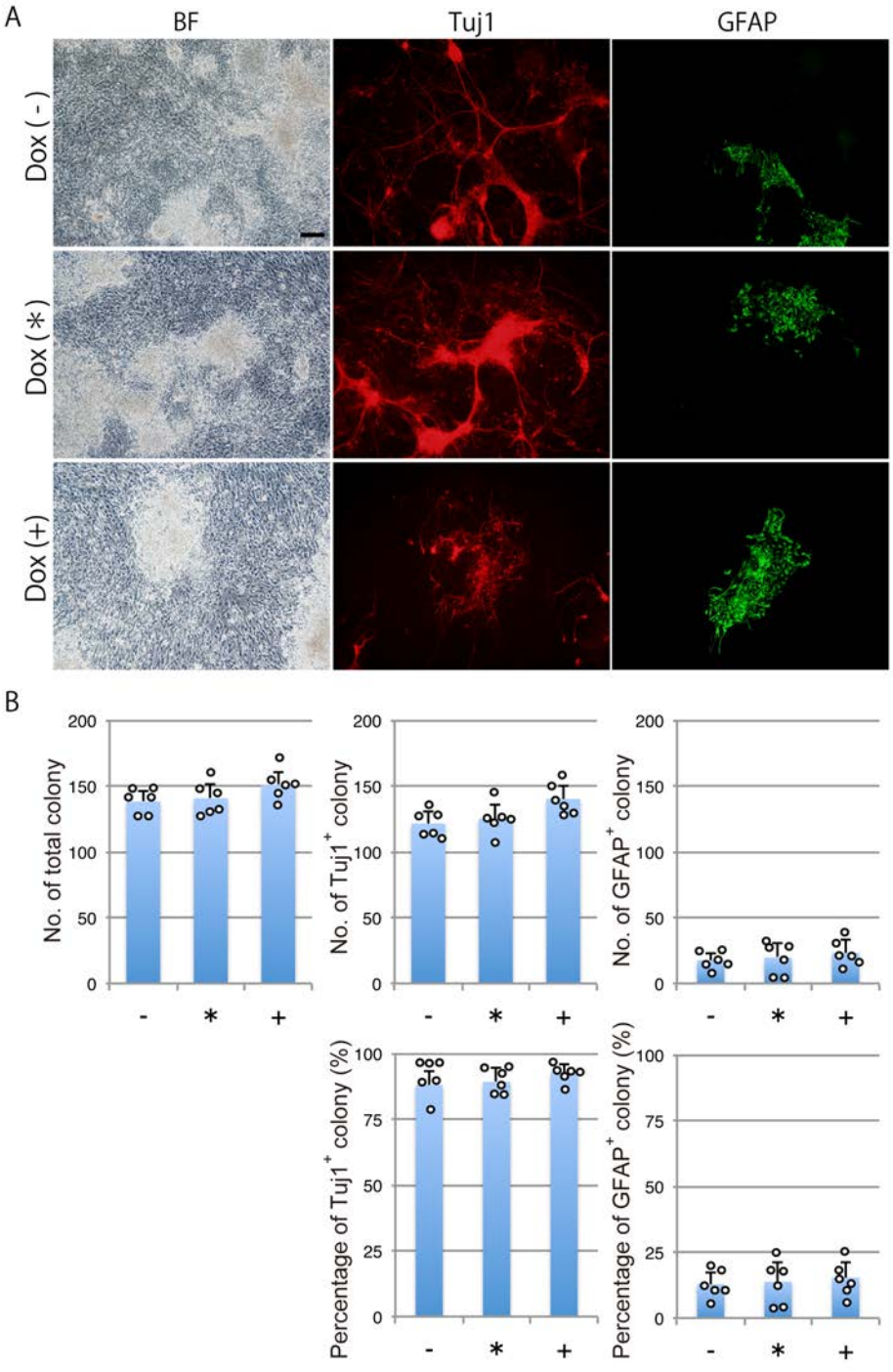
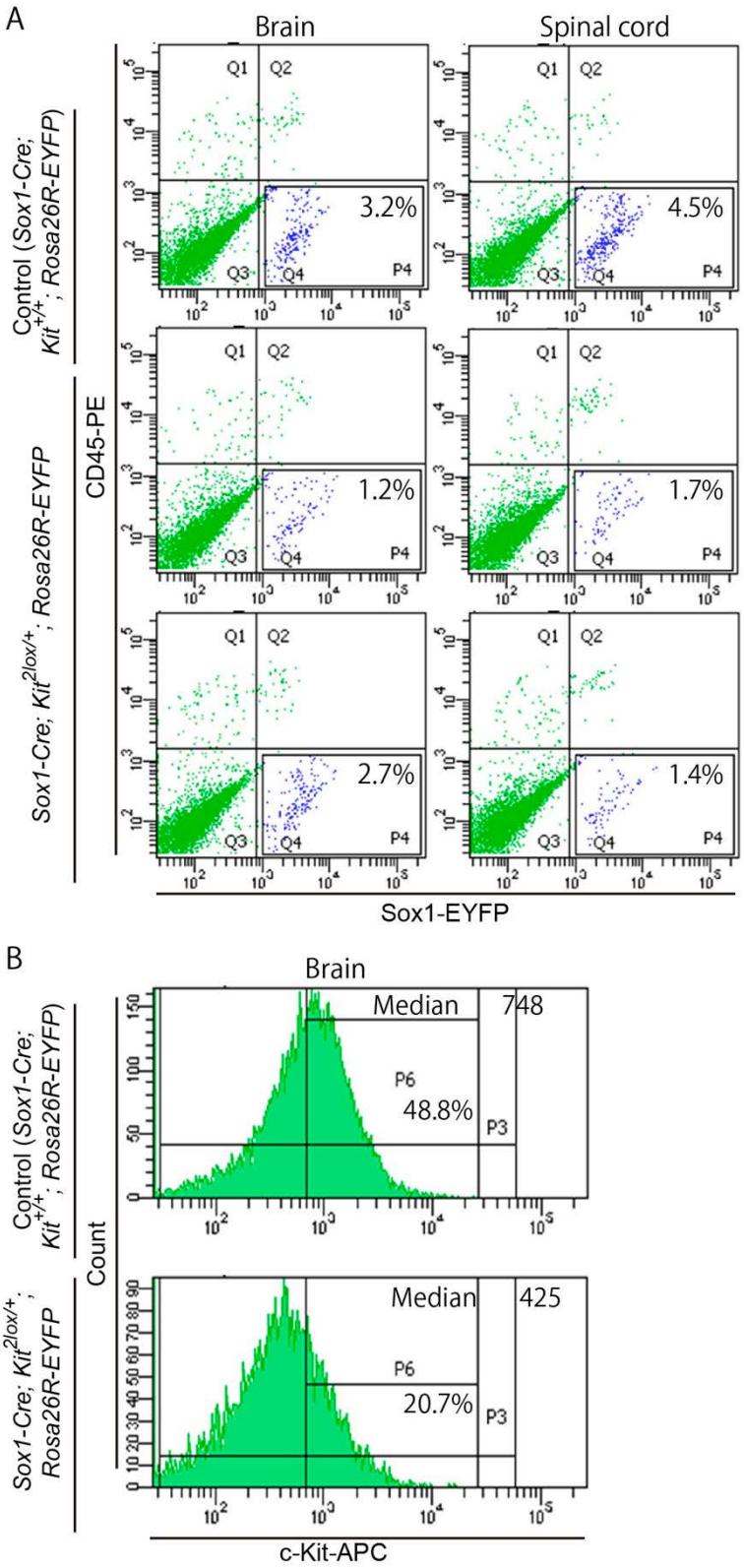
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



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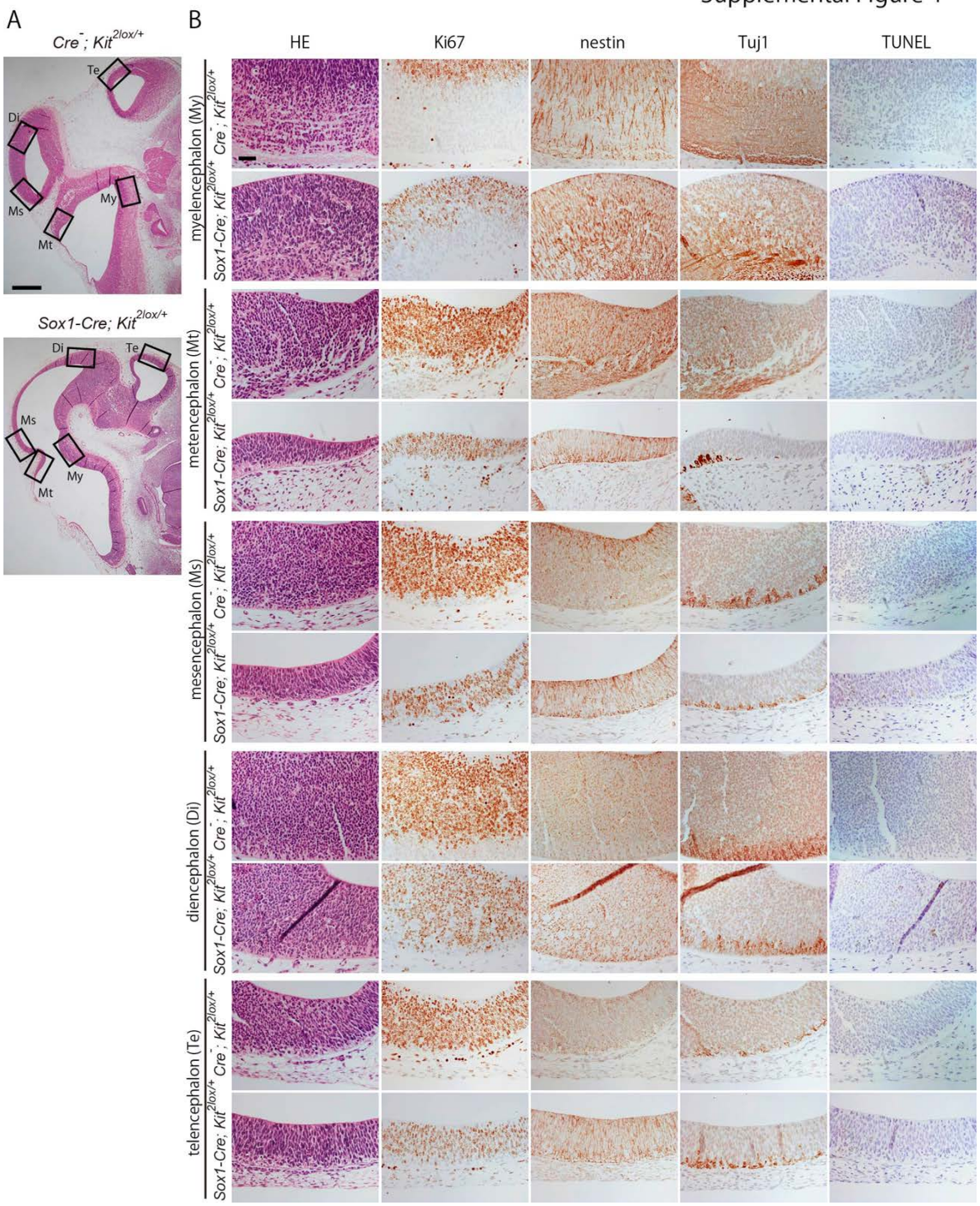

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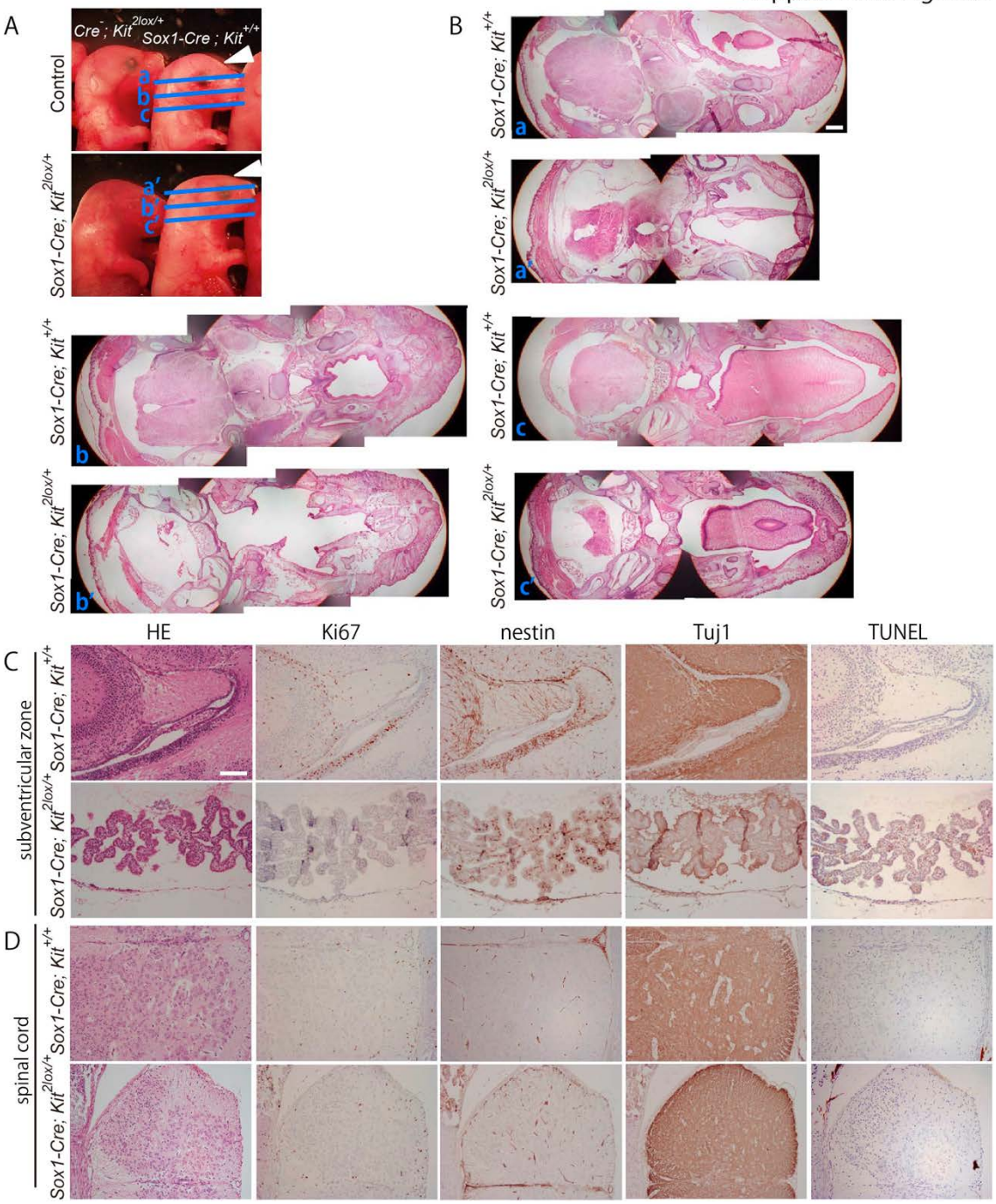
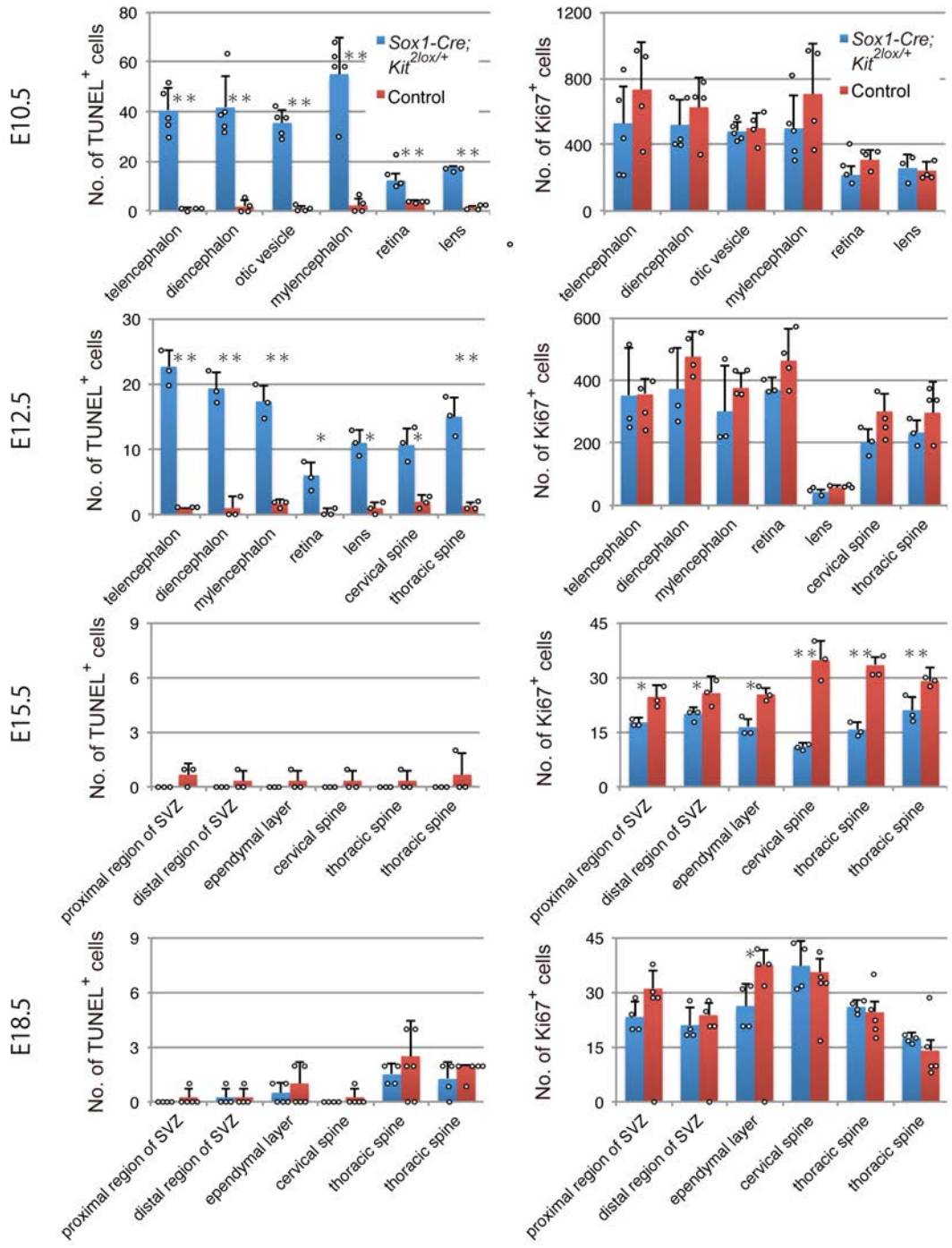
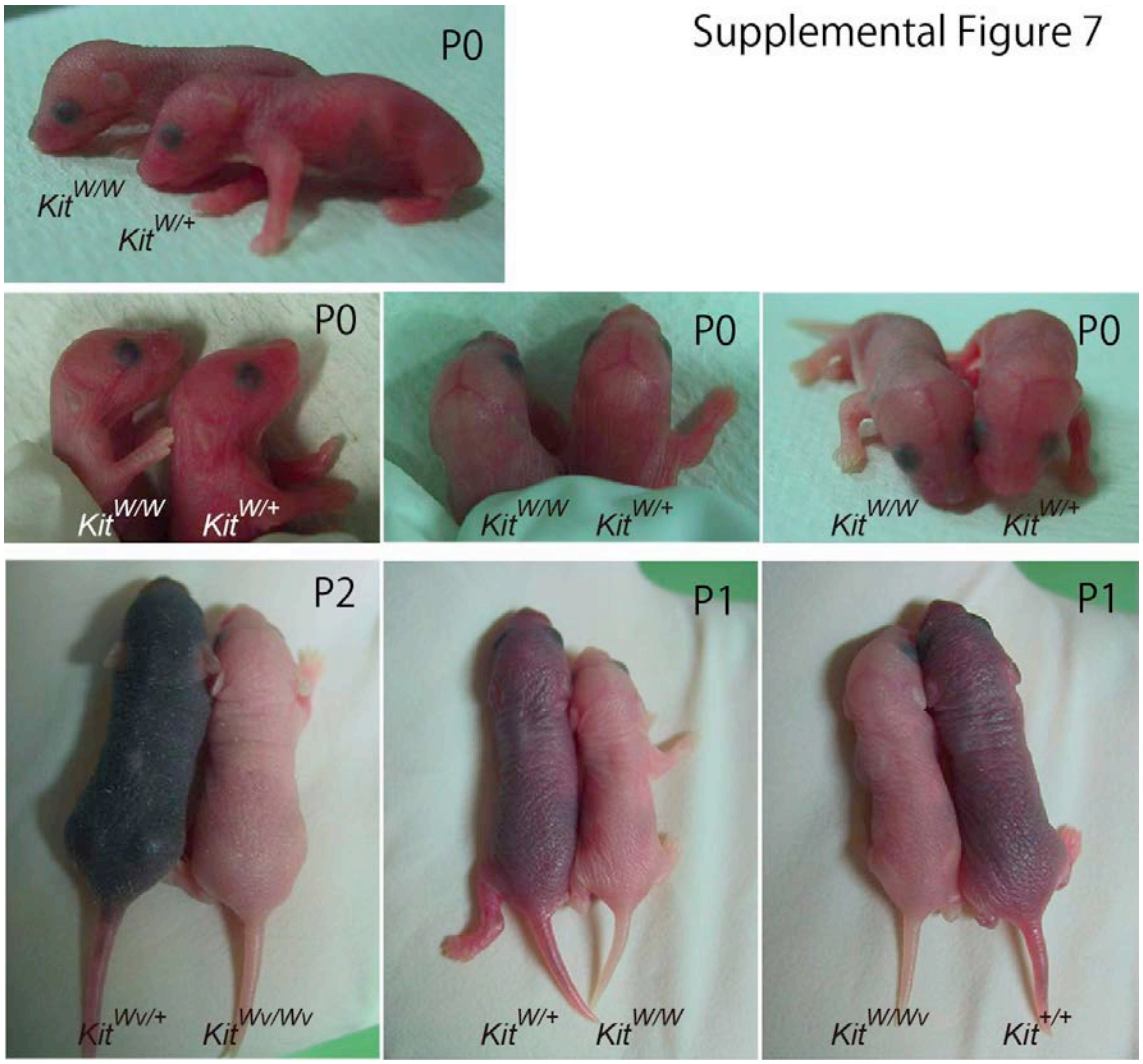


Supplemental Figure 6



Supplemental Figure 7



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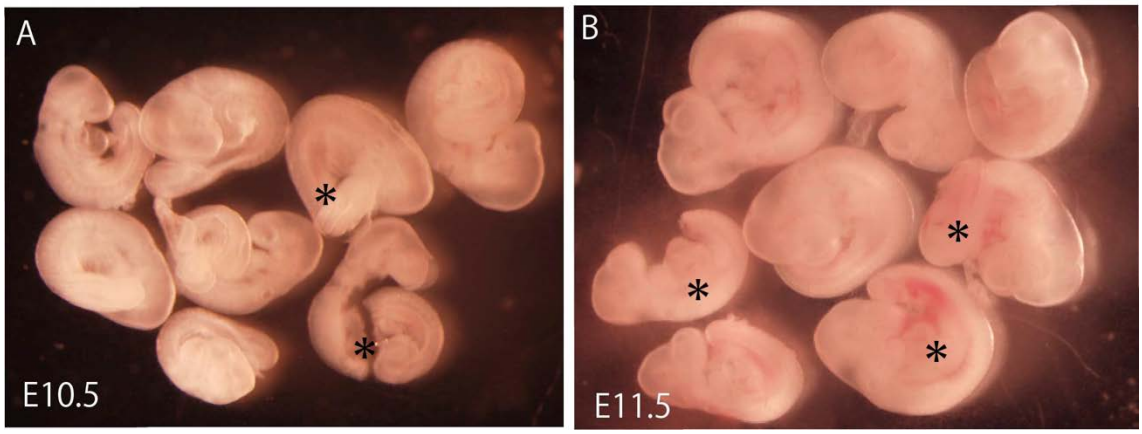
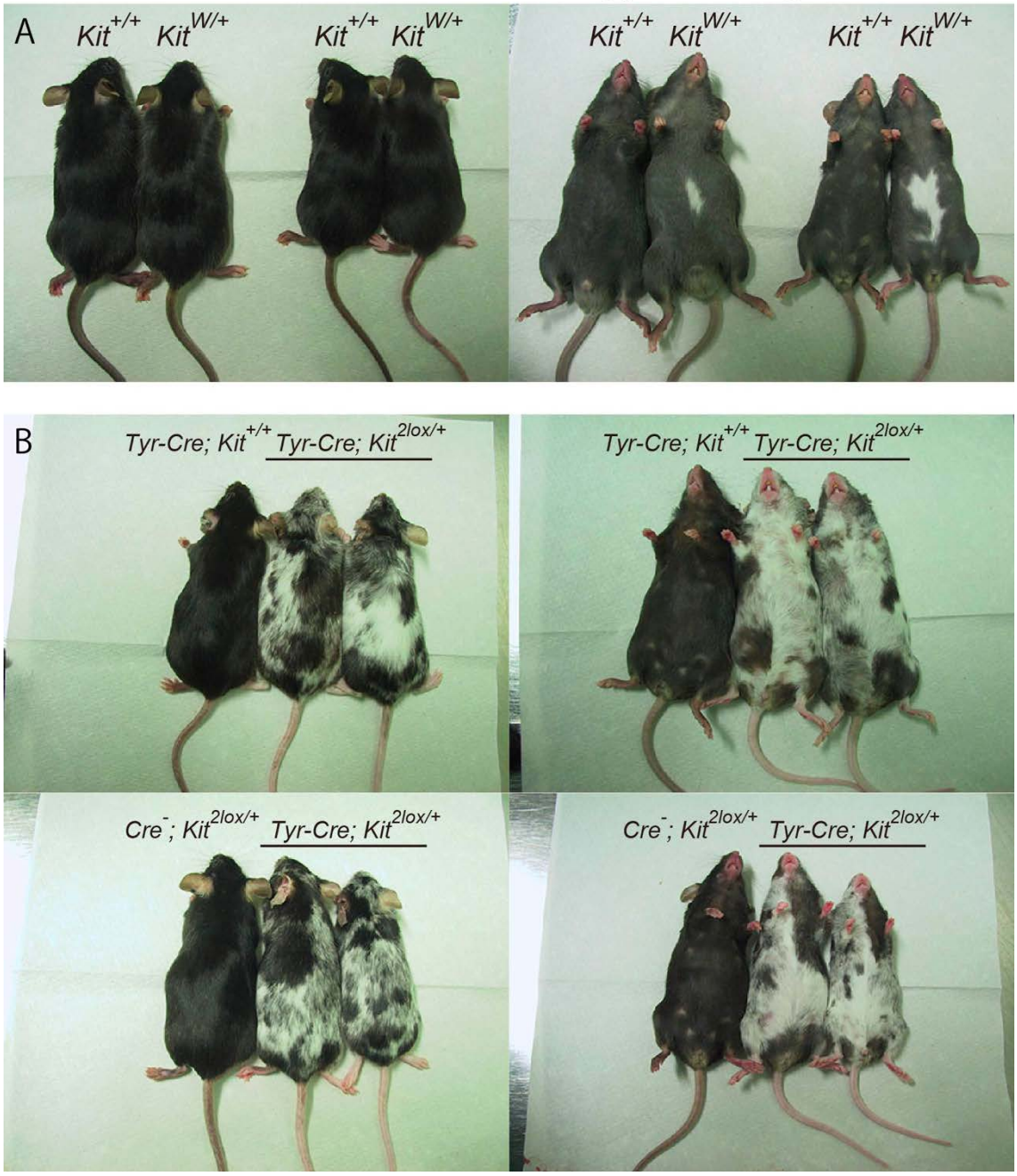
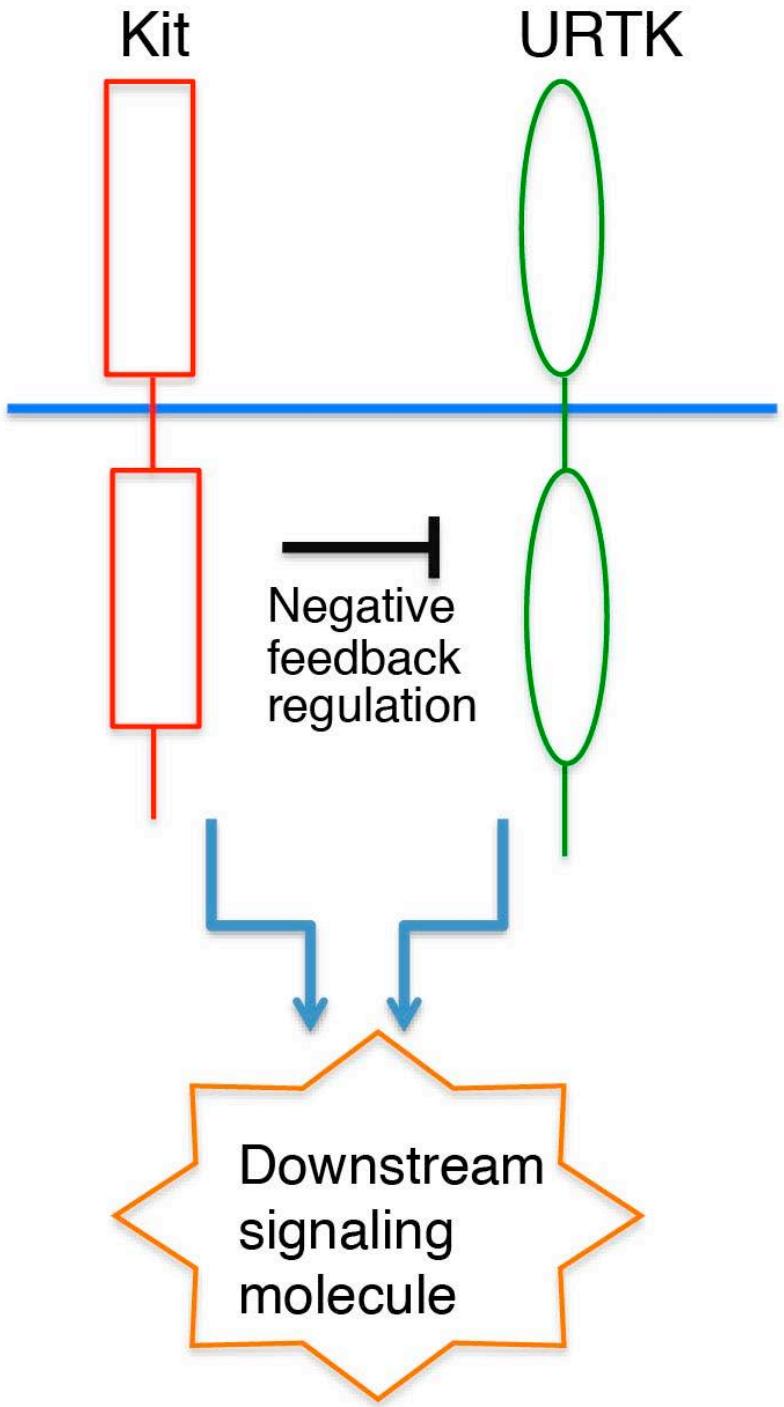


Supplemental Figure 9



Supplemental Figure 10



Supplementary Table 1. The penetrance of the brain phenotype in *Sox1-Cre; Kit*$^{2lox/+}$ mice.

Genotype	*Sox1-Cre; Kit*$^{2lox/+}$			Control		
Phenotype / Developmental stage	Brain Hypoplasia	Normal	Penetrance (%)	Brain Hypoplasia	Normal	Penetrance (%)
No. of embryos at E10.5	3	7*	30	0	28	0
No. of embryos at E11.5	10	5*	66.7	0	39	0
No. of embryos at E12.5	12	1	92.3	0	37	0
No. of embryos at E13.5	12	0	100	0	32	0
No. of embryos at E15.5	14	0	100	0	43	0
No. of embryos at E18.5	15	0	100	0	39	0
No. of pups at P0	13	0	100	0	45	0
No. of pups at P1	3	0	100	0	42	0
No. of pups at P2	1	0	100	0	44	0
No. of pups at P7	0	0	-	0	37	0
No. of pups at 3W	0	0	-	0	35	0
No. of pups at 8W	0	0	-	0	54	0

* In these macroscopically normal embryos, four embryos at E10.5 and three embryos at E11.5 showed an increased number of apoptotic cells in each brain.

Supplemental Figure Legends

Supplementary Figure 1. Restoration of the defective neural cell differentiation in *Sox1-Cre; Kit$^{2lox/+}$* ES cells by the addition of recombinant Kitl protein. (A) The percentage of colonies containing Tuj1$^+$ neurons. The culture was conducted by the same method as Figure 1 by using the three independent *Sox1-Cre; Kit$^{2lox/+}$* ES cell lines, and the percentage of the colonies containing Tuj1$^+$ neurons to the total colonies generated in the culture was calculated after 11 days of culture. Recombinant Kit ligand (Kitl) was added to the medium on day 3 or day 6, and Kit antagonistic antibody ACK2 was added to the medium on day 9. **(B)** The percentage of colonies containing GFAP$^+$ glial cells. Cultures conducted as **A** were analyzed for GFAP$^+$ glial cells in the same manner. Error bars show ± SD (n=3 for each group). *P<0.01 by 2-tailed Student's *t*-test.

Supplementary Figure 2. *In vitro* differentiation of neural cells from haploinsufficient *Kit$^{1lox/+}$* ES cells generated from *Rosa26::rtTA; Col1a1::tetO-Cre; Kit$^{2lox/+}$* ES cells by the addition of doxycycline. (A) *In vitro* differentiated Tuj1$^+$ neurons and GFAP$^+$ glial cells with or without Doxycycline (Dox). The culture was conducted by the same method as Figure 1 by using three independent *Rosa26::rtTA; Col1a1::tetO-Cre; Kit^{2lox}/+* ES cell lines. After the addition of Dox from the start of the culture (+) or from day 5 of the culture (*), Tuj1$^+$ neurons and GFAP$^+$ glial cells were analyzed and compared with the culture without Dox (-). **(B)** After 11 days of the culture, the number of total colonies, Tuj1$^+$ colonies, and GFAP$^+$ colonies in each culture were counted and the percentage of these marker positive colonies to total colonies generated were calculated. Error bars show ± SD (n=3 for each group).

Supplementary Figure 3. Reduction of Sox1$^+$ neural lineage cells and decreased Kit expression on their cell surface observed in *Sox1-Cre; Kit$^{2lox/+}$; Rosa26R-EYFP* embryos. (A) Percentage of Sox1$^+$ neural lineage cells. Cells harvested from brain and spinal cord at E12.5 *Sox1-Cre; Kit$^{2lox/+}$; Rosa26R-EYFP* embryos and *Sox1-Cre; Kit$^{+/+}$; Rosa26R-EYFP* embryos were analyzed by flow cytometry. The hematopoietic cell marker, CD45, and a *Sox1-Cre*-induced neural cell lineage tracer, detected as EYFP, were used to discriminate neural cells. CD45-negative and EYFP-positive cells were gated and the percentage was indicated in each panel. **(B)** Kit expression in CD45-negative and EYFP-positive cells. Cells gated in CD45-negative and EYFP-positive areas were analyzed for the expression of cell surface Kit by flow cytometry. The percentage of Kit-positive cells and the median of the Kit expression of the gated cells are indicated in each panel.

Supplementary Figure 4. Reduction of postmitotic neurons in various areas of the E12.5 *Sox1-Cre; Kit$^{2lox/+}$* brain. (A) HE staining of the head region in

E12.5 *Sox1-Cre; Kit$^{2lox/+}$* brains (bottom) and *Cre-; Kit$^{2lox/+}$* brains (top). *Sox1-Cre; Kit$^{2lox/+}$* brains were clearly downsized compared with *Cre-; Kit$^{2lox/+}$* brains. The squared regions were enlarged in **B**. **(B)** Histological analysis in various areas of the brain. Although Ki67^{+} proliferative cells and nestin^{+} cells were reduced in *Sox1-Cre; Kit$^{2lox/+}$* brains, they were almost equally distributed both in *Sox1-Cre; Kit$^{2lox/+}$* brains and *Cre-; Kit$^{2lox/+}$* brains. However, Tuj1^{+} post-mitotic neurons were significantly reduced both in number and range of areas to be distributed. TUNEL^{+} apoptotic cells were frequently detected only in Ki67^{+} and nestin^{+} areas of *Sox1-Cre; Kit$^{2lox/+}$* brains. Te: telencephalon, Di: diencephalon, Ms: mesencephalon, Mt: metencephalon, My: myelencephalon. Scale bars: 500 μm in **A**; 100 μm in **B**.

Supplementary Figure 5. Histological characterization of E18.5 *Sox1-Cre; Kit$^{2lox/+}$* embryos. (A) The head region of the E18.5 *Sox1-Cre; Kit$^{2lox/+}$* and control embryos. The parietal region (indicated by arrowheads) was downsized in *Sox1-Cre; Kit$^{2lox/+}$* embryos. **(B)** HE staining of the head region. Embryos were sectioned around the lines indicated in **A**. In *Sox1-Cre; Kit$^{2lox/+}$* embryos, most areas of the brain were degenerated in comparison with normal counterparts. It should be noted that non-neural areas, including the skull, tongue, and face are indistinguishably developed in both embryos. **(C, D)** Histological analysis of E18.5 *Sox1-Cre; Kit$^{2lox/+}$*brains and spinal cords. Overall number and distribution pattern of each marker-positive cell was similar with that in E15.5. Scale bars: 500 μm in **B**; 100 μm in **C**.

Supplementary Figure 6. Quantitative analysis of the distribution of TUNEL^{+} and Ki67^{+} cells in various areas of *Sox1-Cre; Kit$^{2lox/+}$* embryos at varying developmental stages. TUNEL or Ki67-positive cells in 0.1 mm^{2} of the indicated tissues were counted. Error bars show ± SD (n=3 for each genotype group). *P<0.05 and **P<0.01 by 2-tailed Student's *t*-test.

Supplementary Figure 7. Morphology of *Kit* newborn mutants. Heterozygous and homozygous *Kit^{W}* and *Kit^{Wv}* newborn mutant mice were investigated for their head morphology. As expected from previous reports, although homozygous *Kit$^{W/W}$* and *Kit$^{Wv/Wv}$* newborn mice were small in size, the proportions of the head of *Kit$^{W/W}$*, *Kit$^{W/Wv}$* and *Kit$^{Wv/Wv}$* newborn mice were not different from that of heterozygous or wild type littermate mice.

Supplementary Figure 8. Representative images of E10.5 and E11.5 *Sox1-Cre; Kit$^{2lox/+}$* embryos. (A) E10.5 littermate embryos. The head proportions of E10.5 embryos were indistinguishable in *Sox1-Cre; Kit$^{2lox/+}$* (showed by asterisks) and control embryos. **(B)** E11.5 littermate embryos. In E11.5 embryos, a recognizable reduction of the upper-frontal region was recognized in *Sox1-Cre; Kit$^{2lox/+}$* embryos (showed by asterisks).

Supplementary Figure 9. *Tyr-Cre*-induced *Kit* haploinsufficiency induced a more severe white spot phenotype than that of $Kit^{W/+}$ mice. **(A)** White spots in $Kit^{W/+}$ mice were smaller and restricted to belly skin. **(B)** In *Tyr-Cre;* $Kit^{2lox/+}$ mice, wider white spots were observed on the belly and extended to the back skin.

Supplementary Figure 10. The possible role of an unidentified receptor tyrosine kinase (URTK) compensatory recovery of reduced Kit signaling by a feedback mechanism. Redundancy of Kit and URTK by a responsive circuit. While Kit and URTK perform the same molecular function, they are differently regulated. In the illustrated situation, duplicate URTK is repressed directly or indirectly by Kit so that the URTK protein level responds reciprocally to changes in the Kit protein level. In $Kit^{W/+}$ embryos, reduction of Kit will result in up-regulation of its partner URTK. Given that both RTKs perform similarly, the downstream signaling molecule is constantly activated by the sum of the two RTKs in $Kit^{W/+}$ embryos. This ensures normal development of the brain in germ line $Kit^{W/+}$ or even $Kit^{W/W}$ mutants. In the case of *Sox1-Cre;* $Kit^{2lox/+}$ mice, a sudden decrease of Kit in the developing brain, in which Kit is actually working, might not be balanced fast enough by the responsively up-regulated partner URTK, and thus fails to produce the reduced downstream signaling necessary for prevention of the observed lethal phenotype. Note that RTK is activated by its ligand, and that this simplified model must be more complicated, for example, a feedback system may be targeted to the ligand for URTK. Moreover, the partner of Kit may not even be an RTK, but possibly another cytoplasmic tyrosine kinase.