Supporting Information

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SI Materials and Methods

Animal Maintenance. C57BL/6N and $p27^{Kip1}$ knockout mice ($p27^{-/-}$) were housed in an animal facility maintained under a 12-h light-dark schedule. Conception was ascertained by the presence of a vaginal plug corresponding to embryonic day 0 (E0). $p27^{-/-}$ mice were routinely genotyped by PCR using WT5 (5′- CCT GGA GCG GAT GGA CGC CAG ACA -3′) and WT3 (5′- CAC CAA ATG CCG GTC CTC AGA GTT -3′) primers for detecting wild-type *$p27^{Kip1}$* and KO5 (5′- GGG CTT TAG AAA TAG AGA ATG - CTG -3′) and KO3 (5′- ATG CTC CAG ACT GCC TTG GGA AAA -3′) for detecting knockout gene. $p27^{-/-}$ mice were obtained by cross-breeding heterozygous ($p27^{+/-}$) females with $p27^{+/-}$ males.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Administration. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and the TCDD-treated mice were handled exclusively at an approved facility at the National Institute for Environmental Studies, Tsukuba, Japan, to avoid the confounding effects of environmental contamination on the results.

GABA Immunohistochemistry. Fixed postnatal day (P) 21 brains were embedded in paraffin and sectioned coronally into 4-μm sections with a rotary microtome. GABA immunohistochemistry was performed as described previously using anti-GABA antibody (Chemicon International) (1). The sections were counterstained with cresyl violet. The numbers of GABA-positive and GABA-negative cells were counted in each bin (that is, in sections measuring 250 μm in width and 25 μm in height, lined serially from the pial surface to the white matter in the primary somatosensory area of the neocortex). Glial and non-GABAergic cells were distinguished by their morphological characteristics as described previously (1). Three nonadjacent sections from three brains were analyzed.

Cumulative Labeling Analysis. Pregnant mice were administered bromodeoxyuridine (BrdU) [50 μg body weight $(bw)^{-1}$; Sigma] by i.p. injection every 3 h starting from 9:00 AM on E12. The mice were exposed to BrdU for 2, 4, and 6.5 h before killed. Embryonic forebrains were immersion-fixed in 70% ethanol and embedded in paraffin and sectioned coronally at 4 μm; the sections were processed for immunohistochemistry using anti-BrdU antibody (Becton Dickinson) as described previously (2). The sections were counterstained with basic fuchsin. The BrdU labeling index (LI), the ratio of the number of BrdU-labeled nuclei to the total number of nuclei, was used as the analytical index. Analysis was conducted at the dorsomedial cerebral wall in a sector measuring 100 μm in length in the coronal plane. Nuclei of endothelial cells were not counted. The average LI was calculated for each bin across a series of three to four brains obtained from the embryos of two to three litters. The number of nuclei counted for this report was ~80,000.

Q Fraction Analysis. Cells in the Q fraction were identified using two S-phase tracers, iododeoxyuridine (IdU) and BrdU (Sigma), as previously described (3–5). Briefly, pregnant C57BL/6N mice exposed to TCDD or corn oil were administered IdU [50 μg $(g\ bw)^{-1}$] by i.p. injection at 7:00 AM on E12. At 9:00 AM, the animals were divided into two groups: (*i*) one group in which BrdU [50 μg $(g\ bw)^{-1}$] injection was administered every 3 h and then, the mice were killed 7 h later (Q experiment), and (*ii*) a second group in which a single BrdU injection was administered followed by killing of the animals 7 h afterward (P + Q experiment). Both sets of forebrains were fixed in 4% phosphate-buffered formaldehyde, embedded in paraffin, sectioned serially into 4-μm sections, and double-immunostained with anti-IdU/BrdU (Becton Dickinson) and anti-BrdU antibodies (AbD Serotec). The number of IdU-positive/BrdU-negative blue nuclei was counted in the dorsomedial ventricular zone (VZ) of six nonadjacent sections from three brains obtained from the embryos of two to three litters.

Expression of Cell Cycle Regulatory Genes. E12 cerebral walls of animals exposed to either TCDD or corn oil were carefully resected under a dissecting microscope using microscissors and snap-frozen in liquid nitrogen. Total RNA was isolated using the RNeasy Protect kit (Qiagen), and the mRNA was purified using the MicroPolyA Pure kit (Ambion) for generating biotinylated cDNA probes. The biotinylated probes were hybridized to cDNA expression arrays (GE array Q series Mouse Cell Cycle Gene Array; Superarray) in accordance with the manufacturer's protocol. Signal intensities were quantified by Scanalyze 2 imaging software (Michael B. Eisen, University of California, Berkeley, CA). Signal data for all genes were analyzed and consolidated using the GE Array Analyzer software (Superarray). In each experiment, the signal intensity was subtracted from the background signals and normalized by comparison with the signal intensity of β-actin. The results from experiment conducted in triplicate were analyzed.

Immunoblot Analysis. Snap-frozen E12 cerebral walls were initially washed in ice cold PBS with a proteinase inhibitor mixture (Complete Mini; Roche) and homogenized manually and mechanically by pellet/pestle in ice-cold lysis buffer not containing detergent (10 mM Hepes-KOH, pH 7.8, 10 mM KCl, 0.1 mM EDTA, pH 8.0, 1 mM DTT, Complete Mini as proteinase inhibitor). To optimize the destruction levels of the cellular membrane, samples were stained with H&E and examined under an optical microscope. After centrifugation and removal of the supernatant, lysis buffer with 0.1% Nonidet P-40 (Sigma) was applied to the nuclear fraction. Genomic DNA was fragmented using an ultrasonic sonicator, and SDS/PAGE was performed using 2 μg nuclear protein. Separated proteins were blotted onto supported nitrocellulose membranes using a semidry blotting apparatus (BioRad). Immunoblot analyses were conducted using anti-$p27^{Kip1}$, anti-$p15^{INK4b}$, anti-cyclin E, anti-Skp2, anti–β-actin (Santa Cruz Biotechnology), anti-cyclin D1, anti-CDK2, anti-CDK4 (Sigma), anti-AKT (Cell Signaling Technology), and anti-Jab-1 (GeneTex) antibodies. The signal intensity of $p27^{Kip1}$ was quantified using the Scion Image imaging software (Scion). In each experiment, the signal intensities were standardized by comparison with the intensities of β-actin. The results of experiments conducted in triplicate were analyzed.

Statistical Analysis. All experiments were performed a minimum of three times. Statistical comparison of the datasets was performed by a two-tailed paired Student *t* test using Microsoft Excel 2003 software.

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