**Immunohistochemical Localization of Calbindin D28-k, Parvalbumin, and Calretinin in the Superior Olivary Complex of Circling Mouse**

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**Supplementary Manuscript**

**PCR Analysis Method**

Postnatal day 16 (P16) heterozygote (+/cir) and homozygote (cir/cir) cirlcing mice were obtained from the mating of cir/cir males with +/cir females. To prevent genetic mixings, both +/cir and cir/cir mice were completely separated from wild-type (+/+) mice, which were purchased from the laboratory animal company (Samtako Bio Korea). Polymerized chain reaction (PCR) analysis was performed to differentiate +/cir and cir/cir mice using genomic DNA obtained from mouse tails. The genomic DNA was isolated according to the manufacturer’s instructions (Bioneer Corporation, Daejeon, Korea). The cir/cir mouse was identified by absence of the tmie gene (Chung et al., 2007). PCR was performed with primers designed to amplify the exon 1 coding region of the tmie gene (forward, 5’ AGCTGTAGCTCTGAAATCT 3’; reverse, 5’ TCTGGCAGAATGCATGGAGGCT 3’; (Chung et al., 2007). One hundred ng of template DNA was used in a final reaction volume of 20 µl containing 10 mM Tris-HCl (pH 9.0), 40 mM KCl, 1.5 mM MgCl2, 250 mM dNTP, 20 pmol of each primer, and 1U Taq DNA polymerase (Bioneer Corporation, CA, USA). PCR was carried out in a thermal cycler (C1000TM; Bio-Rad, Singapore). The PCR was performed in two steps. The first step consisted of denaturation at 96°C for 5 min, annealing at 59°C for 1 min, and extension at 72°C for 1 min at the end of 4 cycles. The second step included denaturation at 96°C for 1 min, annealing at 59°C for 1 min, extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min at the end of 30 cycles. Electrophoresis was performed at 93 V for 1 hour at 25°C to identify the amplification fragments of the tmie gene fragment (Fig 1).

**Supplementary Figures**

**Supplementary fig. 1. PCR analysis of the *tmie* gene fragment in wild-type (+/+), heterozygous (+/cir), and homozygous (cir/cir) mice.**

Primer pairs designed for the sequences of the *tmie* gene were used and an equivalent amount of each PCR reaction derived from each template were electrophoresed in each lanes of a 1.5% agarose gel. The expected *tmie* gene products (562 bp) representing the exon 1 coding region were amplified from the +/+ and +/cir mice DNA, but not from cir/cir mice DNA. Sizes of molecular standards are indicated on the left (in bp).

**Supplementary fig. 2. Expression of CaBPs in SOC of circling mice**

****Western blot analysis was used to detect the expressions of CaBPs in homogenates of brain stem region containing SOC in P16 +/+, +/cir, and cir/cir mice. The expression levels of CaBPs were quantified with the Image Studio software (Li-Cor, USA). Statistical analysis was carried out by one-way ANOVA with Bonferroni's post-hoc test using GraphPad Prism (GraphPad Software, USA). *p*< 0.05 was considered significant. Values are mean±SD (n=6). \**p*<0.05, \*\**p*<0.01, and \*\*\**p*<0.001 compared with +/+ mice.