Supplemental Information

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Supplemental Materials and Methods

Measurement of the delay for expression of Dll1 WT and mutant genes
To measure the delay for expression of Dll1 WT and mutant genes, we used the optogenetic control tool of gene expression (Imayoshi et al., 2013). The light-inducible Dll1 WT gene and Dll1 mutant gene reporters were constructed, as follows. Dll1 WT reporter (Dll1-luciferase fusion reporter) sequence and Dll1 type1/type2 mutant reporter sequences were obtained by BAC recombineering. To obtain the light-inducible Dll1 WT reporter, the region from the ATG sequence of Dll1 gene to the 3’UTR sequence was retrieved by T2A-pUAS vector, including Tol2 transposon target sites for integration of DNA fragments into genomic DNA and the repeated UAS sequences with TATA box, GAVPO target sites. To obtain the light-inducible Dll1 type1 mutant reporter, the region from the ATG sequence of Dll1 cDNA to the 3’UTR was retrieved by T2A-pUAS vector. In the case of Dll1 type2 mutant reporter, the region from the ATG of Dll1 cDNA, inserted to the first exon of Dll1 gene, to the 3’UTR of Dll1 gene was retrieved. The retrieving vector also included the sequence of hGAVPO under the control of PGK promoter, puromycin-resistant gene for clone selection, and mCherry coding sequence. These light-inducible Dll1 WT and mutant reporter constructs were introduced into NIH3T3 cell lines and integrated into genomic DNA by Tol2 transposon. Puromycin-resistant clones were collected by FACS, according to mCherry expression. Established stable cell lines carrying either Dll1 WT reporter or Dll1 type1/type2 mutant reporter were seeded to 24-well dishes in 10%FBS/DMEM, including 1mM luciferin. After 1 min blue light stimulation, the expression of each reporter was measured by photonmultiplier tube (CL24B-LIC/B, Churitsu Electric Corp.) for 5-sec exposures with 3-min intervals. Signals were obtained by counts per second (cps).

RT-PCR analysis
Dll1 RNA expression in wild-type and Dll1 type 2 mutant mice were examined by RT-PCR. The following primers were used.
F1: GGTCAGGGATACACACAGCAAACG;
F3: AAAGGCACCTCGGGGTCTATGTGAC;
R1: CATTCACTCGGGCTATATCCTTGG;
R2: AGGCCGGCTGAGTGCATTCTGGG.

2
Supplemental Figures

A

targeting vector

Neo +

Neo -

Neo -

Neo -

1kb

B

C

D

E

F

G

H

I

Measurements of half-life

[Graph showing measurements of half-life with expression levels over time]

Expression level (AU)

0 20 40 60 80 100 120

Time (min)
Figure S1. Generation and analysis of Dll1-luciferase knock-in (KI) mice, Related to Figures 1 and 2. (A) Strategy for generation of Dll1-luciferase KI mice. (B) In situ hybridization of *Unx4.1* in heterozygous (wt/DL) and homozygous (DL/DL) Dll1-Fluc KI mice at E10.5. (C) In situ hybridization of *Unx4.1* in wild-type (wt/wt), heterozygous (wt/DE), and homozygous (DE/DE) Dll1-Eluc KI mice at E10.5. (D) In situ hybridization of *Unx4.1* in heterozygous (wt/DR) and homozygous (DR/DR) Dll1-Rluc KI mice at E10.5. (E) Bone staining of wild-type (wt/wt), heterozygous (wt/DL), and homozygous (DL/DL) Dll1-Fluc KI mice at postnatal day 1 (P1). (F) Bone staining of wild-type (wt/wt), heterozygous (wt/DR), and homozygous (DR/DR) Dll1-Rluc KI mice at P0. (G) Bioluminescence imaging (Luc, Fluc) and immunocytochemistry (ICC) for Dll1 in neural progenitors of Dll1-Fluc KI embryos. BF, bright field. (H) Immunostaining for Dll1 (left) and bioluminescence imaging (right) of the PSM (dorsal view) of a Dll1-Rluc KI embryo at E10.5. (I) Half-lives of wild-type Dll1 and Dll1-Fluc proteins were measured by treatment with cycloheximide (20 μM). Relative protein levels were measured after addition of cycloheximide at time = 0.
Figure S2.Dll1 expression in the presomitic mesoderm, Related to Figure 1. 

(A,J) Structures of Dll1-Fluc (A) and Dll1-Eluc (J) KI alleles. (B,K) Time-lapse imaging of Dll1 protein expression in the PSM of Dll1-Fluc (B) and Dll1-Eluc (K) KI mice at E10.5. The signal intensity is also shown by pseudocolors (lower panels). (C,L) Bioluminescence images of the PSM in Dll1-Fluc (C) and Dll1-Eluc (L) KI mice. (D,M) Spatiotemporal profiles of Dll1 protein expression obtained from (B,K). (E,N) Quantification of Dll1 protein expression in the PSM. The intensity of Dll1 protein expression was quantified along the red lines in (D and M). (F,O) Temporal differences in the rate of change in Dll1 protein expression in the PSM of Dll1-Fluc (F) and Dll1-Eluc (O) KI mice at E10.5. The signal intensity is also shown by pseudocolors (lower panels). (G,P) Bioluminescence images of temporal differences in the rate of change in Dll1 protein expression in the PSM of Dll1-Fluc (G) and Dll1-Eluc (P) KI mice. (H,Q) Spatiotemporal profiles of temporal differences in the rate of change in Dll1 protein expression obtained from (F,O). (I,R) Quantification of temporal differences in the rate of change in Dll1 protein expression along the red lines in (H,Q).
Figure S3. Time-lapse imaging analysis of Dll1 expression in neural progenitors, Related to Figure 2. (A,B) Quantification of Dll1 protein expression in neural progenitors obtained from Dll1-Fluc KI mice at E10.5. (C,D) Quantification of Dll1 mRNA expression in neural progenitors obtained from pDll1-Ub-Fluc transgenic mice at E10.5. (E,F) Distribution of periods of Dll1 protein (E) and Dll1 mRNA (F) oscillations.
Figure S4. Dll1 expression in neural progenitors, Related to Figure 2.
(A) Structure of 6-kb Dll1 promoter-driven Dll1-Fluc reporter. (B,C) Bioluminescence imaging of neural progenitors transfected with 6-kb Dll1 promoter-driven Dll1-Fluc reporter and pEF-mCherry. (D) Quantification of Dll1 protein expression in neural progenitors in (B,C).
Figure S5. Quantification of the delay in Dll1 expression and strategies for generation of Dll1 type 1 and type 2 mutant mice, Related to Figures 3-7.

(A) Structures of the hGAVPO expression vector and Dll1 type 1 and type 2 mutant and wild-type (WT) reporters. (B) The time course of luciferase activities after blue light illumination. n=4. (C) The initial 90-min time course is magnified. (D,E) Strategies for generation of Dll1 type 1 (D) and type 2 (E) mutant mice.
Figure S6. Analysis of Dll1 RNA expression from Dll1 type 2 KI mutant mice, Related to Figures 3-7. (A) Genomic structures of Dll1 locus of wild-type (WT) and Dll1 type 2 KI mutant mice (KI). (B) mRNA structures expressed from Dll1 locus of wild-type (WT) and Dll1 type 2 KI mutant mice (KI). The positions of the primers are indicated. (C,D) RT-PCR was performed with primers F1 and R1 (C) and F3 and R2 (D). While the 3’UTR sequence was inserted in the first exon of Dll1 locus of Dll1 type 2 KI mutant mice, the downstream region (C, wt; D, mut) was also continuously transcribed at a comparable level to the upstream region (C, mut), suggesting that the inserted 3’UTR did not stop transcription. Furthermore, Dll1 mRNA expression levels were comparable between the wild type and Type 2 mutant embryos (see Figure 5A), suggesting that the nonsense-mediated decay did not occur.
Figure S7. Time-lapse imaging analysis of Dll1 expression in the presomitic mesoderm (PSM) of *Dll1* type 2 mutant mice, Related to Figure 3.

(A) Structure of *Dll1* type 2 mutant mouse gene. (B) Time-lapse imaging of Dll1 protein expression in the PSM (lateral view) of a *Dll1* type 2 mutant mouse at E10.5. The signal intensity is also shown by pseudocolors (lower panels). (C) Bioluminescence image of the PSM of a *Dll1* type 2 mutant mouse. (D) Spatiotemporal profile of Dll1 protein expression obtained from (B). (E) Quantification of Dll1 protein expression in the PSM. The intensity of Dll1 protein expression was quantified along the red line in (D). (F) Temporal differences in the rate of change in Dll1 protein expression in the PSM of a *Dll1* type 2 mutant mouse at E10.5. The signal intensity is also shown by pseudocolors (lower panels). (G) Bioluminescence image of temporal differences in the rate of change in Dll1 protein expression in the PSM of a *Dll1* type 2 mutant mouse at E10.5. (H) Spatiotemporal profile of temporal differences in the rate of change in Dll1 protein expression obtained from (F). (I) Quantification of temporal differences in the rate of change in Dll1 protein expression along the red line in (H).
Figure S8. Analysis of neural marker expression in Dll1 type 1 and type 2 mutant mice, Related to Figure 7. (A-C) Hes1 protein and Dll1 mRNA expression in the telencephalon of wild-type (A) and homozygous Dll1 type 1 and type 2 mutant mice (B,C) at E12.5. (D,E) Quantification of Hes1 protein and Dll1 mRNA expression in the ventricular zone shown in (A-C). (F-H) Higher magnification of the telencephalon of wild-type and homozygous Dll1 type 1 and type 2 mutant mice for NICD, Hes1, and Neurog2 protein expression at E12.5. (I-K) Box plots of NICD, Hes1, and Neurog2 protein expression levels at E12.5. *p<0.05, Student t test. Scale bar: (A-C) 50µm.
Figure S9. Size defects of neural development of Dll1 type 1 and type 2 mutant mice, Related to Figure 7. (A-C) In situ hybridization of Hes5 in the telencephalon of wild-type (A) and homozygous Dll1 type 2 mutant mice (B) at E12.5. A boxed region in (B) is enlarged in (C). (D,E) The proportions of the ventricular zone in the cortex of Dll1 type 1 and type 2 mutant mice at E10.5-E14.5. (F) The proportions of Neurod+ regions in the cortex at E12.5. (G) The dorsal views of wild-type, heterozygous and homozygous Dll1 type 1 mutant brains. (H) The relative brain sizes of wild-type, heterozygous and homozygous Dll1 type 1 mutant mice at E17. *p<0.05, **p<0.01, ***p<0.001, Student t test. *p<0.05, Student t test. Scale bars: (A,B) 500µm; (G) 2mm.
Figure S10. Light-induced Dll1 expression by the hGAVPO system, Related to Figure 7. (A) The structures of pPGK-hGAVPO and pUAS-Dll1 type1 mutant reporter. (B,C) pPGK-hGAVPO and pUAS-Dll1 type1 mutant reporter were co-transfected into NS cells, and Dll1 expression was induced by blue light exposure at 3-hr (B) and 30-min (C) intervals. Dll1 expression was monitored with the luciferase activities.
A

\[
\text{Cell 1} \quad \text{Delta-Notch} \quad \text{Cell 2}
\]

\[
\frac{d}{dt} X_1(t) = -v \frac{K_1^m}{K_1^m + X_1(t - \tau_1)^m} \frac{K_2^n}{K_2^n + X_2(t - \tau_2)^n} - rX_1(t)
\]

\[
\frac{d}{dt} X_2(t) = -v \frac{K_1^m}{K_1^m + X_2(t - \tau_1)^m} \frac{K_2^n}{K_2^n + X_1(t - \tau_2)^n} - rX_2(t)
\]

B

C

D

Shimojo_Fig S11
Figure S11. Mathematical modeling for amplitude/oscillation death of coupled oscillators, Related to Figures 3-7.

(A) The schematic of the mathematical model. This model consists of two variables $x_1(t)$ and $x_2(t)$ (where the unit of time is hour), representing Hes1 levels in two neighboring cells. Here, $\tau_1$ is the time required for Hes1 to affect its own formation in the same cell through the negative feedback loop. The interaction between cells is simplified in the following manner. DII is inhibited by Hes1 in the same cell and activates Hes1 in the other cell. This interaction can be regarded as the mutual inhibition between two cells with delay $\tau_2$.

(B) The dynamical equations of this model. The interpretations of other parameters is as follows: $v$ is the maximum synthesis rate, $r$ is the degradation rate, $K_1$ and $K_2$ correspond to the typical amounts of Hes1 that account for the repression, $m$ and $n$ are the Hill coefficients. In numerical simulations, we set $v = 10$, $r = 2$, $K_1 = 1$, $K_2 = 2$, $m = 2$, $n = 2$, $\tau = 3/4$ and observe the dependence of dynamical behavior on $\tau_2$.

(C) Time series of $x_1$ and $x_2$ for different $\tau_2$ values. For $\tau_2 = 0.7$ and 1.75, in-phase (a) and anti-phase (c) oscillations were observed, respectively. In both cases, when $\tau_2$ was decreased by 0.2 h, $x_1$ and $x_2$ showed quenching of oscillation (b,d). Time series of $x_1$ and $x_2$. (a) $\tau_2 = 0.7$. (b) $\tau_2 = 0.5$. (c) $\tau_2 = 1.75$. (d) $\tau_2 = 1.55$.

(D) Bifurcation diagram. To see $\tau_2$ dependence, the maximum and minimum values of $x_i$ ($i = 1, 2$) were measured after a transient time. The maximum and minimum values are identical when $x_i$ undergoes oscillation quenching, while they are different when $x_i$ undergoes self-sustained oscillation.
Figure S12. Summary of defects observed in Dll1 type 1 and type 2 mutant mice. When \( \tau_z \), the time required for coupling between cells, is decreased or increased (corresponding to Dll1 type 1 or type 2 mutant, respectively), in-phase and out-of-phase Dll1-Hes oscillations would undergo “amplitude/oscillation death.” Steady Dll1 expression seems to be non-functional for tissue development, leading to defects similar to those of Dll1 loss-of-function mutations.
SUPPLEMENTAL MOVIE LEGENDS

Movie S1. Dll1 mRNA expression in the PSM at E10.5.

Movie S2. Dll1 protein expression in the PSM at E10.5.

Movie S3. Dll1 mRNA expression in the developing nervous system at E10.5.

Movie S4. Dll1 mRNA expression in cultured neural progenitors at E14.5.

Movie S5. Dll1 protein expression in cultured neural progenitors at E12.5.

Movie S6. Dll1 protein expression (upper) and its temporal rate changes (lower) in the PSM of heterozygous and homozygous Dll1 type 1 mutant mice at E10.5.