Supplemental Materials

Supplementary Table S1
Supplementary Table S2
Supplementary Table S3
Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3
Supplementary Figure S4
Supplementary Note 1
Supplementary Note 2
| Genotype | Cervical | Thoracic | Lumbar | Sacral | Lumbar + Sacral |
|----------|----------|----------|--------|--------|----------------|
| +/-      | 7        | 13       | 6      | 4      | 10             |
| +/-      | 7        | 13       | 6      | 4      | 10             |
| +/-      | 7        | 13       | 6      | 4      | 10             |
| +/-      | 7        | 13       | 6      | 4      | 10             |
| +/-      | 7        | 13       | 6      | 4      | 10             |
| +/-      | 7        | 13       | 6      | 4      | 10             |
| +/-      | 7        | 13       | 5      | 5      | 10             |
| +/-      | 7        | 13       | 5      | 4      | 9              |
| +/-      | 7        | 13       | 5      | 4      | 9              |
| +/-      | 7        | 13       | 5      | 4      | 9              |
| +/-      | 7        | 13       | 5      | 4      | 9              |
| +/-      | 7        | 13       | 5      | 4      | 9              |

**Table S1. The number of vertebrae in newborn mice.**

The numbers of vertebrae in the newborn mice were counted after the mice were stained.
| Gene Symbol | Gene_Name | Probe ID | Cluster | Ratio1 | Ratio2 | Ratio3 | Avg. |
|-------------|-----------|---------|---------|--------|--------|--------|------|
| Nrarp       | Notch-regulated ankyrin repeat protein | 315756  |         | 0.04   | 0.10   | 0.05   | 0.06 |
| Hes5        | hairy and enhancer of split 5 (Drosophila) | 562022  |         | 4.56   | 4.77   | 3.51   | 4.20 |
| Lfng        | lunatic fringe gene homolog (Drosophila) | 882835  |         | 1.37   | 1.43   | 1.10   | 1.28 |
| Hes7        | hairy and enhancer of split 7 (Drosophila) | 558449  | Notch   | 1.18   | 1.20   | 0.57   | 0.87 |
| Hes1        | hairy and enhancer of split 1 (Drosophila) | 406680  |         | 1.20   | 0.91   | 0.53   | 0.79 |
| Hey1        | hairy/enhancer-of-split related with YRPW motif 1 | 814590 |         | 1.55   | 1.13   | 1.32   | 1.31 |
| Nkd1        | naked cuticle 1 homolog (Drosophila) | 394939  |         | 1.04   | 1.20   | 1.10   | 1.11 |
| Axin2       | axin2 | 357287  |         | 1.19   | 1.19   | 0.62   | 0.91 |
| Lef1        | lymphoid enhancer binding factor 1 | 928212  | Wnt     | 1.18   | 1.20   | 1.05   | 1.14 |
| Msgn1       | mesogenin 1 | 733438  |         | 1.00   | 0.88   | 1.53   | 1.08 |
| T           | brachyury | 661161  |         | 0.89   | 0.77   | 1.03   | 0.88 |
| Dusp1       | dual specificity phosphatase 1 | 303843  |         | 1.05   | 0.89   | 1.07   | 1.00 |
| Dusp4       | dual specificity phosphatase 4 | 672437  |         | 0.99   | 0.84   | 1.11   | 0.97 |
| Dusp6       | dual specificity phosphatase 6 | 932169  | FGF     | 0.97   | 0.70   | 0.75   | 0.79 |
| Spry2       | sprouty homolog 2 (Drosophila) | 872375  |         | 0.90   | 0.68   | 1.12   | 0.87 |
| Spry4       | sprouty homolog 4 (Drosophila) | 529472  |         | 1.00   | 0.95   | 0.74   | 0.88 |
| Rho5        | reproductive homeobox on X chromosome, 5 | 535766  |         | 3.60   | 2.50   | 2.15   | 2.63 |
| Car4        | carbonic anhydrase 4 | 349705  | -*      | 2.42   | 2.11   | 2.30   | 2.27 |
| Gpr133      | G protein-coupled receptor 133 | 716011  |         | 2.22   | 2.67   | 2.06   | 2.29 |

Table S2. Summary of microarray analysis results.

Several target genes of the Notch, Wnt, and FGF signaling pathways are displayed. The ratios of the expression levels (Nrarp<sup>−/−</sup> / wild type) were calculated using normalized signal intensities (n = 3). *, Genes that had a more than two-fold difference for all three analyses. The functions in the segmentation clock remain unclear.
| Individual no. | Cervical | Thoracic | Lumbar | Sacral | Lumbar+Sacral |
|---------------|----------|----------|--------|--------|---------------|
| 1             | 7        | 13       | 5      | 5      | 10            |
| 2             | 7        | 13       | 6      | 4      | 10            |
| 3             | 7        | 13       | 6      | 5      | 11            |
| 4             | 7        | 13       | 7      | 4      | 11            |
| 5             | 7        | 13       | 6      | 4      | 10            |
| 6             | 7        | 13       | 7      | 4      | 11            |
| 7             | 7        | 13       | 7      | 4      | 11            |
| 8             | 7        | 13       | 6      | 4      | 10            |
| 9             | 7        | 13       | 7      | 4      | 11            |
| 10            | 7        | 13       | 6      | 4      | 10            |
| 11            | 7        | 13       | 6      | 4      | 10            |
| **Mean ± s.e.m.** | **7.0 ± 0.0** | **13.0 ± 0.0** | **6.3 ± 0.18** | **4.2 ± 0.12** | **10.5 ± 0.15** |

**Table S3. Number of vertebrae in gamma-secretase inhibitor-treated mice.**

An inhibitor, 0.1 mg/kg of LY411,575, was administered three times from E 7.5 to E 9.5, and the animals were then sacrificed on postnatal day 1 to prepare skeletal sample (mean ± s.e.m.).
Figure S1. Generation of Nrarp-deficient mice.

(A) Targeting strategy. The whole coding region of Nrarp was replaced by IRES-LacZ and PGK-neo (inverted orientation). (B) Southern blot analysis. The 3’-external probe was used to detect 6.6-kb (wild-type) and 4.5-kb (mutant) fragments in SpeI-digested genomic DNA. wt, wild-type; KO, knockout. (C) Whole-mount in situ hybridization for Nrarp at E 10.5. Expression was observed in the central nervous systems and the PSM (left, arrow) of wild-type embryos. In Nrarp−/− embryos, Nrarp expression was completely absent (right). (D) β-galactosidase activity was detected in the regions where Nrarp was expressed. Scale bar, 1mm.
Figure S2. The shape and size of somites are not affected in Narp<sup>−/−</sup>.

Whole-mount in situ hybridization for Uncx4.1 at the indicated stages. Dorsal views of caudal part of embryos are presented. There were no significant differences in shape, symmetry, and polarity of somites between the wild-type embryos and the Narp<sup>−/−</sup> embryos. The top side is the anterior region. Scale bars, 500 μm.
Figure S3. Notch signaling inhibition disrupts somite genesis. Whole-mount in situ hybridization for Uncx4.1 of embryos that were treated with 0.3 mg/kg LY411,575 (A) or 1.0 mg/kg LY411,575 (B). Defects were observed in somite segmentation and somite patterning (A) and in general development under high dose of inhibitor (B). Scale bars, 1 mm.
Figure S4. Pace of general development is identical between Nrarp mutant embryos and wild-type littermates. (A) whole-mount in situ hybridization of Uncx4.1 in E10.5 embryos. White asterisks indicate non-specific staining. (B,C) in situ hybridization of Fgf8 in E11.5 embryos. (B) Lateral views of limb buds. Fgf8 mRNA expression domain revealed the apical ectoderm ridge (AER). Anterior is towards the left. Black arrows and white arrows indicate anterior end and posterior end of Fgf8 expression domain in the AER, respectively. Narrow bands of AER are detected identically in Nrarp +/- and their wild-type littermates. We detected no differences in morphology of limb buds and their size. (C) Frontal views of the anterior half. Fgf8 mRNA was detected at op and mx.
Differentiation levels of these organs are observed identically between mutant and their wild-type littermates. Anterior is towards the top. FLB, forelimb bud; HLB, hind limb bud; op, olfactory pits; mx, maxillary process; md, mandibular process. Scale bars, 500 μm.
Mathematical analysis of the Notch activity-dependent periodicity

Basic formulation of the model

To examine the dependence of the Hes7 expression period on the Notch activity, we studied the original model developed by Lewis (1) by following the mathematical analysis in ref (1). Consider a model of two variables, protein \( p(t) \) and mRNA \( m(t) \), formulated as,

\[
\frac{dp(t)}{dt} = am(t - T_p) - bp(t) \tag{S1}
\]

\[
\frac{dm(t)}{dt} = \frac{k}{1 + p^2(t - T_m)/p_0^2} - cm(t) \tag{S2}
\]

where \( a \) and \( k \) represent protein production rates per mRNA molecule and maximum rate of mRNA, respectively, \( b \) and \( c \) are degradation rates, \( p_0 \) is a gain parameter, and \( T_p \) and \( T_m \) are time delays for protein and mRNA generation.

If we introduce a new variable \( p_{adv}(t) = p(t + T_p) \), we have

\[
\frac{dp_{adv}(t)}{dt} = am(t) - bp_{adv}(t) \tag{S3}
\]

\[
\frac{dm(t)}{dt} = \frac{k}{1 + p_{adv}^2(t - T)/p_0^2} - cm(t), \tag{S4}
\]

where \( T = T_m + T_p \). To discuss the qualitative characteristics of the system, we introduce dimensionless variables and parameters as follows:

\[
\tau = t/T, \quad \beta = bT, \quad \gamma = cT, \quad \kappa = ak/bcp_0, \quad P(\tau) = bpc_{adv}(t)/ak, \quad M(\tau) = cm(t)/k,
\]

where the parameters, \( k \) and \( p_0 \), which regulate mRNA production rate, are aggregated into a single parameter \( \kappa \), by which we can incorporate a Notch activity level into the model. Using these notations, the ODEs are transformed as

\[
\frac{1}{\beta} \frac{dP(\tau)}{d\tau} = -\{P(\tau) - M(\tau)\} \tag{S5}
\]

\[
\frac{1}{\gamma} \frac{dM(\tau)}{d\tau} = -\{M(\tau) - f(P(\tau - 1))\} \tag{S6}
\]
\[ f(x) = \frac{1}{1 + (\kappa x)^2}. \] (S7)

Note that \( \beta \) and \( \gamma \) are Notch-independent constants and that the variables, \( P(\tau) \) and \( M(\tau) \), are the protein and mRNA levels normalized by the production rates \( a \) and \( k \), respectively. These normalizations, in which both of the \( P(\tau) \) and \( M(\tau) \) have a range of \((0,1)\), allow us to evaluate the protein and mRNA levels as the production rate scale. Equations (S5) and (S6) indicate that \( P(\tau) \) converges to \( M(\tau) \) exponentially with the \( \beta \) time scale, and that \( M(\tau) \) approaches to the function of \( P(\tau-1), f(x) \), exponentially with the \( \gamma \) time scale.

**Notch activity-dependent oscillation amplitude**

Taking an analysis similar to that in ref (1), if the reactions are very fast due to large \( \beta \) and \( \gamma \) (so the right sides of Eqs. (S5) and (S6) can be approximated as zero), they have two quasi-steady states, \((P_{\text{max}}, M_{\text{max}})\) and \((P_{\text{min}}, M_{\text{min}})\), which satisfy

\[
P_{\text{max}} = M_{\text{max}}, \quad P_{\text{min}} = M_{\text{min}},
\]

\[
M_{\text{max}} = \frac{1}{1 + (\kappa P_{\text{max}})^2}, \quad M_{\text{min}} = \frac{1}{1 + (\kappa P_{\text{min}})^2}.
\]

In the case of oscillation, where the maximum is larger than the minimum, the pair of \((P_{\text{max}}, P_{\text{min}})\) and \((M_{\text{max}}, M_{\text{min}})\) are the solutions to the quadratic equation \( x^2 - x + 1/\kappa^2 = 0 \). Therefore,

\[
P_{\text{max}} = M_{\text{max}} = \frac{1 + \sqrt{1 - 4/\kappa^2}}{2},
\] (S8)

\[
P_{\text{min}} = M_{\text{min}} = \frac{1 - \sqrt{1 - 4/\kappa^2}}{2},
\] (S9)

where \( 2 > \kappa \) for the oscillation case (Fig. S-I).

![Figure S-I: Dependence of oscillation amplitude on \( \kappa \) corresponding to Notch activity. The maximum and minimum values of quasi-steady states described by Eq. (S8) and (S9) are shown.](image-url)
**Notch activity-dependent oscillation period**

As analyzed in ref (1), the oscillation period of the model expressed as Eqs. (S5)-(S7) can be approximated as \(2(T+1/\beta+1/\gamma)\) when \(\kappa\) changes little. If \(\kappa\) has a different value from a control because of experimental manipulation (Nrarpp KO in this work), it is important to take a computational approach to examine the \(\kappa\)-dependent oscillation period.

The numerical simulation using Eqs. (S5)-(S7) with various \(\kappa\) values shows that the oscillation period has a positive correlation with \(\kappa\), as shown in the left column of Fig. S-II. The figures in the right column show changes in the distribution of \(P(\tau)\) values (blue points in the left column figures), suggesting that the term during which \(P(\tau)\) is very small (bottom bars in the histograms) accounts for prolongation of the period by the increase in \(\kappa\). Note that the period increases despite the decrease in most \(P(\tau)\) values except for the bottom in the left column of Figure S-II: Comparison of the time courses of \(P(\tau)\) and the histograms of the difference between the two distributions of \(P(\tau)\) (blue points on the left) obtained by two \(\kappa\) values for an identical calculation time (right). The values of \(\kappa\) and the period are shown in each figure.

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Fig. S-II. Because the parameters, $T$, $\beta$, and $\gamma$, are constant in the numerical calculation, only $\kappa$ can contribute to prolonging of the term with small $P(\tau)$.

To discuss an approximate dependency of the period on $\kappa$, we focus on the characteristics of the function $f(x)$ defined by Eq. (S7). Figure S-III, illustrating $f(x)$, suggests that $f(x)$ becomes small if $x$ takes on a large value, and vice versa (see Eqs. (S6) and (S7)), and it indicates that $f(x)$ is a monotonically decreasing function of $x$ and its slope is steeper with larger $\kappa$. If we here define the threshold, $S$, at which the variable $f(x)$ shifts to the increasing phase when $x$ decreases (see Fig. S-III), the very small $P(\tau)$ in the Fig. S-II is equivalent to $f(x)$ under $S$. Then the threshold of $x$ in terms of $S$ is given by

$$x_{th} = \frac{1}{\kappa} \sqrt{\frac{1}{S} - 1}.$$  \hspace{1cm} (S10)

When $P(\tau)$ increases by following $M(\tau)$, $P(\tau-1)$ decreases from nearly one to $x_{th}$ according to Eqs. (S6) and (S7). Since these equations have a form of the ODE, $dx/dt = -x$, thus, the amount of the $P(\tau-1)$ decrease can be approximated by an exponential function,

$$e^{-D/\alpha} \approx \left( \frac{1}{S} - 1 \right)^{1/2},$$  \hspace{1cm} (S11)

where $D$ is a time interval necessary for $P(\tau-1)$ to decrease from nearly one to $x_{th}$ with a time constant $\alpha$. By transforming this equation, we get

$$D \approx \alpha \ln \kappa - \frac{\alpha}{2} \ln \left( \frac{1}{S} - 1 \right),$$  \hspace{1cm} (S12)

suggesting that the $\kappa$-dependent prolongation of the period has a logarithmic form of $\kappa$. This approximation shows good agreement with the numerical simulation with large $\kappa$, as shown in Fig. S-IV, where the amplitude of the oscillation is given by

$$A(\kappa) = P_{\text{max}} - P_{\text{min}} = \sqrt{1 - 4/\kappa^2},$$  \hspace{1cm} (S13)
suggesting that the amplitude increases as $\kappa (>2)$ increases and has an upper limit as shown in Fig. S-IV.

References
1. J. Lewis, *Curr Biol* **13**, 1398 (2003).

| Parameter | Value (unit)       | Meaning                                      |
|-----------|-------------------|----------------------------------------------|
| $a$       | 0.5 (molecules/min) | Production rate for mRNA                     |
| $b, c$    | 0.11 (1/min)      | Degradation rate for NICD, mRNA, and protein |
| $p_0$     | 13 (min)          | Gain value of Hes7 for inhibition of protein production |
| $T_p$     | 15 (min)          | Time delay for mRNA nuclear export and protein production |
| $T_m$     | 20 (min)          | Time delay for Hes7 nuclear imports           |
Supplementary Note 2

Statistical analysis of somite number

1 Outline
To statistically evaluate the difference in the average somite number between two genotypes \( Nrarp^{-/-} \) and \( Nrarp^{+/+} \), a paired t-test was first performed; the difference was not significant for E 8.5, while it was statistically significant for E 10.5 (\( p<0.02 \)) and E 11.5 (\( p<0.01 \)). Next, to evaluate the statistical difference in terms of inference, such as the confidence interval (CI), we developed a generative model that simulates the statistical generation process of somite number of each embryo. This model includes a parameter \( m_t \) that represents the mean genetic effect of a somite number decrease in genotype \( t=Nrarp^{-/-} \) relative to the number of somites in \( t=Nrarp^{+/+} \). A 95% CI of the extension in the segmentation clock period was estimated after obtaining a 95% CI of the parameter \( m_t \) by applying statistical inference to the generative model.

Note that overall statistical significance was evaluated with the simple paired t-test, and the parameters were then estimated to further examine the physical meaning of the significance, if any. In the following, we define the generative model to evaluate the parameters and describe the inference technique we used.

2 Generative model
It is assumed that the somite number of each litter obeys the following generative model:

\[
x_i = \mu_{l(i)} + m_{t(i)} + \epsilon_i
\]

where \( x_i \), \( \mu_{l(i)} \), \( m_{t(i)} \), and \( \epsilon_i \) denote somite number of the \( i \)-th litter, mean somite number of wild-type littermates in the \( l(i) \)-th pregnant female, mean increase in the somite number of the \( t(i) \)-th genotype, and noise effect (variation) of an individual litter \( i \), respectively. \( l=i(i) \) signifies the pregnant female that conceived the \( i \)-th litter, and \( t=t(i) \) signifies the genotype which the \( i \)-th litter belongs to; \( t=0 \) and \( t=1 \) denote wild type (+/+) and homo type (-/-), respectively. Since the mean increase \( m_t \) is a relative one from the wild type’s somite number, \( m_0=0 \) holds.

It is natural to assume there are two factors leading to the statistical variation in the somite number: a pregnant female-dependent one and an individual litter-dependent one. To dissociate these factors, \( \mu_l \) and \( \epsilon_i \) are assumed to be generated by the following hierarchical model:
\[ \mu_l \sim N(\mu_{E(l)}, \sigma_{\mu}^2) , \quad (2) \]
\[ \varepsilon_i \sim N(0, \sigma^2) , \quad (3) \]

where \( \mu_{E(l)} \), \( \sigma_{\mu}^2 \), and \( \sigma^2 \) are unknown parameters to be estimated. \( a \sim N(b, c^2) \) denotes that \( a \) obeys Gaussian distribution of mean \( b \) and variance \( c^2 \). \( E=E(l) \) denotes the epoch of the \( l \)-th processed pregnant female, either \( E=8.5, \quad E=10.5, \) or \( E=11.5 \).

The parameters of the model should be estimated somehow on the basis of observation; somite number \( x_i \) is observed for each litter. The parameters are divided into three categories: (a) parameters \( \mu_{E(l)} \), \( \sigma_{\mu}^2 \) and \( \sigma^2 \) are point estimated by using the maximum likelihood method; (b) parameter \( \mu_l \) is not estimated, because it is integrated out; and (c) parameter \( m_1 \) is estimated as a 95% confidence interval (CI).

Since equations (1), (2), and (3) define the likelihood function of the parameters, after integrating (marginalized) the likelihood function with respect to \( \mu_l \) ((b)-type), the (a)-type parameters are estimated so as to maximize the marginalized likelihood, and CI of \( m_1 \) ((c)-type) is also obtained.

3 Parameter estimation results

The observed data are summarized in Table S5, and the parameters estimated from them are shown in Table S6.

Table S5. Statistics of somite number data.

| Processing epoch | num. of pregnants | num. of \( Nrarp^{+/+} \) litters | num. of \( Nrarp^{-/-} \) litters |
|------------------|-------------------|---------------------------------|-------------------------------|
| E8.5             | 6                 | 19                              | 19                            |
| E10.5            | 18                | 49                              | 69                            |
| E11.5            | 8                 | 25                              | 25                            |

Table S6. Model estimation results.

| Processing epoch | 95\% CI of \( m_1 \) | \( \mu_E \) | \( \sigma_{\mu} \) | \( \sigma \) |
|------------------|-----------------------|-------------|-------------------|-------------|
| E8.5             | (-1.02, 0.51)         | 12.37       | 2.21              | 1.17        |
| E10.5            | (-0.92, -0.12)        | 39.67       | 3.38              | 1.09        |
| E11.5            | (-2.65, -1.10)        | 52.77       | 2.02              | 1.33        |
The 95% CI of \( m_1 \) in table S6 is consistent with the result of the paired t-test, since the CI for E 8.5 includes zero while those for E 10.5 and E 11.5 do not. The estimate of \( \mu_E \), the average of mean somite number in the pregnant females, was slightly different from the average somite numbers of wild type litters, though the difference was negligible. The smaller estimated standard deviation \( \sigma_\mu \) than \( \sigma \) implies that a high genetic relevance can be found even though there is a large variation in somite number between different pregnant females.

We transformed the 95% CI (-2.6, -1.1) of \( m_1 \) into that of the mean segmentation clock period. The average increase in somite number in 72 hours between E 8.5 and E 11.5 is \( \mu_{E_{11.5}} - \mu_{E_{8.5}} = 40.4 \) in the wild type litter. The 95% CI of the increase in somites in the homo-Nrarp-loss type within the same 72 hours is (37.7, 39.3). Assuming a uniform somite generation process during the 72 hours between E 8.5 and E 11.5, the 95% CI of the segmentation clock period extension during the 72 hours is estimated to be (3.0, 7.5) minutes.