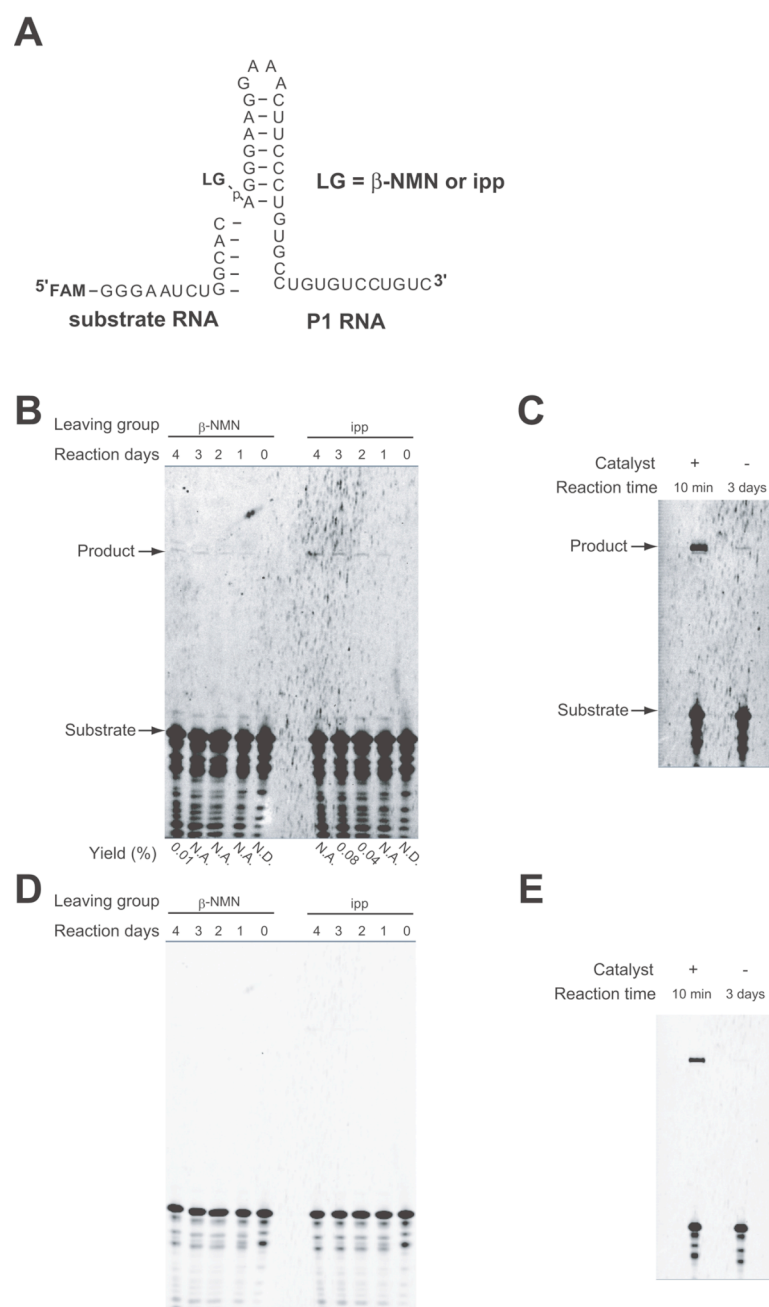
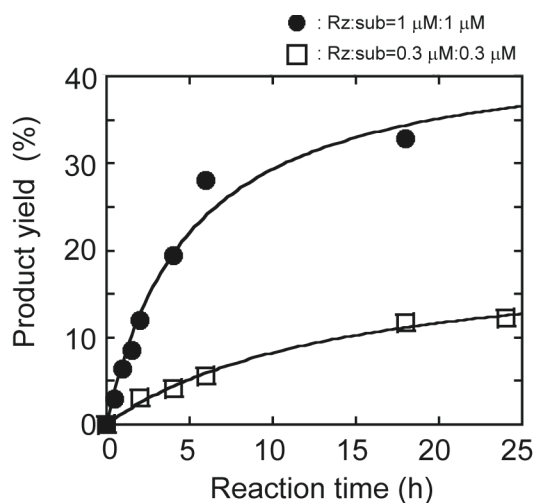
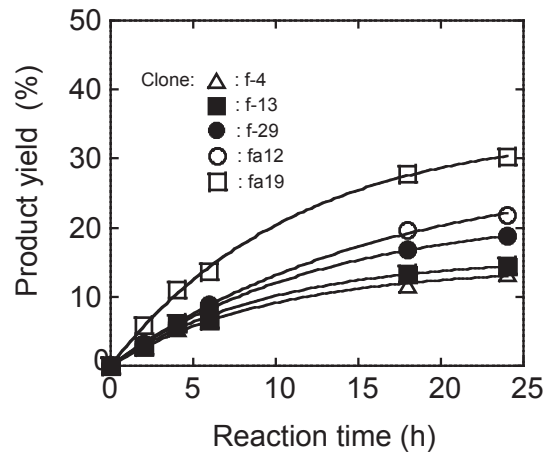
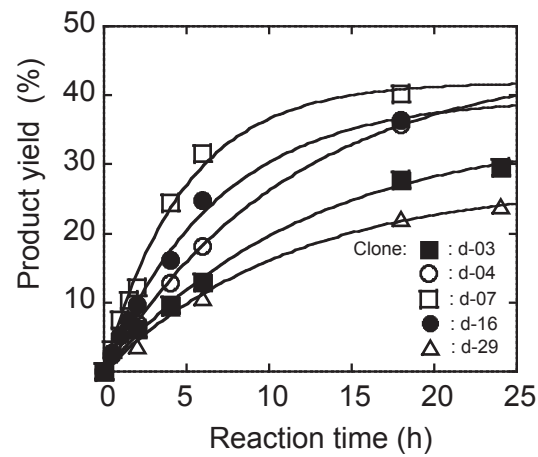




**Figure S1.** Uncatalyzed reactions between 1  $\mu$ M 5'-FAM-labeled substrate and 1  $\mu$ M isolated P1 RNA. Reactions were performed with 30 mM Tris-HCl (pH 7.5), 50 mM  $\text{MgCl}_2$ , 200 mM KCl at 37°C. (A) Structures of the P1 RNA and the substrate RNA. (B) Time courses of the reaction between 5'-FAM-labeled substrate and P1 region RNA possessing  $\beta$ -NMN or ipp leaving group. N.D., no ligated product was detectable. N.A., the ligated product was detectable but a reliable intensity value was not available due to background noise. In the reaction with the ipp leaving group, the rate can be estimated to be  $1.0 \pm 0.2 \times 10^{-5} \text{ h}^{-1}$ , which was close to the rate of template-directed ligation (approximately  $1.3 \times 10^{-5} \text{ h}^{-1}$ ) measured under similar conditions (50 mM Tris-HCl, pH 7.8, 50 mM  $\text{MgCl}_2$ , 200 mM KCl, 37°C) (Rohatgi *et al.* 1996; *J. Am. Chem. Soc.*, **118**, 3332–3339). (C) The reaction between 5'-FAM-labeled substrate and P1 region RNA possessing ipp leaving group in the presence or absence of a catalyst (derivative of the *trans*-DSL1 ribozyme) that promotes the ligation reaction. To avoid contamination, the reaction with the catalyst was performed after electrophoresis and detection for Figure S1B were completed. (D and E). The gels used in Figures S1B and S1C were visualized under the standard detection conditions.



**Figure S2.** Time courses of the activities of the YFL-1 with different concentrations of the ribozyme and substrate: 1  $\mu\text{M}$  ribozyme and 1  $\mu\text{M}$  substrate (filled circles) and 0.3  $\mu\text{M}$  ribozyme and 0.3  $\mu\text{M}$  substrate (open squares). The half-life of the reaction ( $t_{1/2}$ ) was 5.0 h (1  $\mu\text{M}$ ) or 14.1 h (0.3  $\mu\text{M}$ ). The calculated final product yield (%) was 44% (1  $\mu\text{M}$ ) or 20% (0.3  $\mu\text{M}$ ).

**A****B**

**Figure S3.** The activities of YFL-1 variants

(A) Time courses of the ligation reaction by YFL-1 variants isolated from the first library after 7 rounds of selection.

(B) Time courses of the ligation reaction by YFL-1 variants isolated from the second library after 4 rounds of selection.

Table S1. Comparison of activity of the YFL-1 ribozyme under different conditions

| temp.(°C) | pH  | Mg <sup>2+</sup><br>(mM) | $k_{app}$<br>(10 <sup>-2</sup> ·h·μM) <sup>-1</sup> | Extent of the reaction<br>after 18 h (%) |
|-----------|-----|--------------------------|-----------------------------------------------------|------------------------------------------|
| 37        | 7.5 | 50                       | 20.2                                                | 32.8                                     |
| 37        | 7.5 | 150                      | 45.4                                                | 37.9                                     |
| 37        | 8.0 | 50                       | 61.8                                                | 29.3                                     |
| 37        | 8.0 | 150                      | 34.2                                                | 54.2                                     |
| 42        | 7.5 | 50                       | 10.0                                                | 26.8                                     |
| 42        | 7.5 | 150                      | 12.1                                                | 52.6                                     |
| 42        | 8.0 | 50                       | 71.7                                                | 16.8                                     |
| 42        | 8.0 | 150                      | 106.8                                               | 36.9                                     |
| 32        | 7.5 | 50                       | 3.4                                                 | 36.8                                     |
| 32        | 7.5 | 150                      | 13.1                                                | 55.1                                     |
| 32        | 8.0 | 50                       | 12.4                                                | 37.8                                     |
| 32        | 8.0 | 150                      | 26.4                                                | 60.0                                     |

Table S2. Comparison of activity of the quadruple mutant under different conditions

| temp.(°C) | pH  | Mg <sup>2+</sup><br>(mM) | $k_{app}$<br>(10 <sup>-2</sup> ·h·μM) <sup>-1</sup> | Extent of the reaction<br>after 18 h (%) |
|-----------|-----|--------------------------|-----------------------------------------------------|------------------------------------------|
| 37        | 7.5 | 50                       | 4.4                                                 | 10.4                                     |
| 37        | 7.5 | 150                      | 8.5                                                 | 27.5                                     |
| 37        | 8.0 | 50                       | 7.2                                                 | 12.4                                     |
| 37        | 8.0 | 150                      | 26.4                                                | 18.1                                     |
| 42        | 7.5 | 50                       | 7.2                                                 | 4.4                                      |
| 42        | 7.5 | 150                      | 21.5                                                | 14.0                                     |
| 42        | 8.0 | 50                       | 22.4                                                | 3.1                                      |
| 42        | 8.0 | 150                      | 106.0                                               | 8.1                                      |
| 32        | 7.5 | 50                       | 4.0                                                 | 11.6                                     |
| 32        | 7.5 | 150                      | 5.2                                                 | 23.5                                     |
| 32        | 8.0 | 50                       | 6.0                                                 | 17.0                                     |
| 32        | 8.0 | 150                      | 22.8                                                | 37.0                                     |