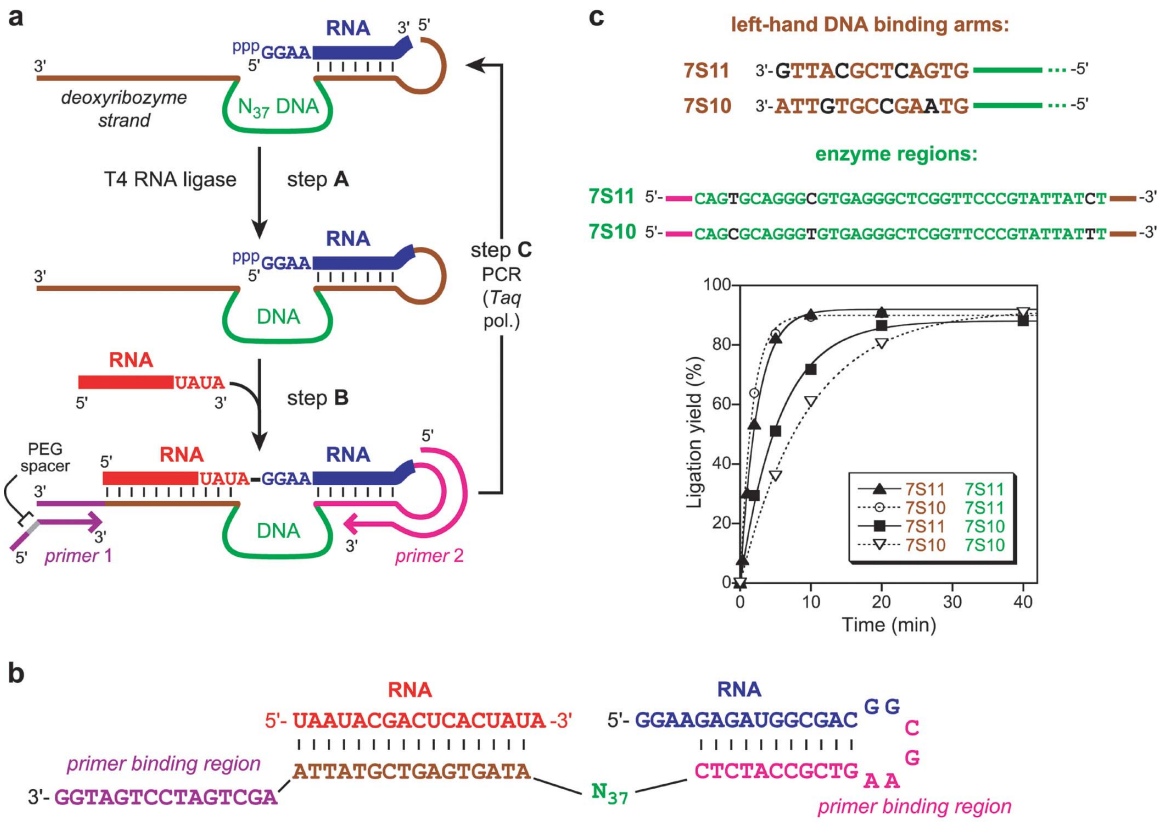
## Supplementary Methods

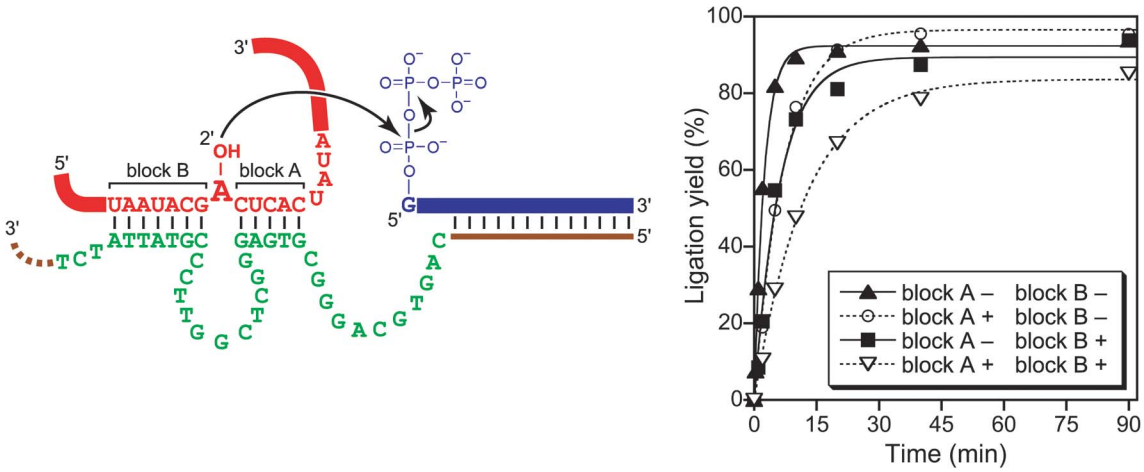
**Selection strategy and new deoxyribozymes.** The selection strategy is summarized in Supplementary Figure 1a. For full experimental details, see reference 15. Briefly, in step A of selection, T4 RNA ligase catalyzes the joining of the right-hand RNA substrate to the deoxyribozyme strand, which contains the randomized DNA $N_{37}$ region. In step B, DNA enzyme sequences are selected for competence to ligate the left-hand RNA substrate to the right-hand substrate (50 mM HEPES, pH 7.5, 150 mM NaCl, 2 mM KCl, 40 mM $MgCl_2$, 37 °C, 13 h). Finally, in step C, the successful DNA enzymes are amplified by PCR, and the desired single-stranded products are retained for input into step A of the subsequent selection round. Steps A–C are each completed by denaturing PAGE purification of the desired single-stranded products. Seven selection rounds were performed, after which time the activity of the DNA pool reached a plateau at 32% ligation activity. Individual DNA enzymes were cloned and surveyed for ligation activity using methods described in reference 15; 7S11 and 7S10 were identified by automated sequencing from the individual clones with significant ligation activity.

The specific nucleotide sequences of the RNA substrates and deoxyribozyme strand are shown in Supplementary Figure 1b, which is colored appropriately for illustrating the PCR amplification step C of selection. Note that the brown portion of the deoxyribozyme strand originates by *Taq* polymerase extension during each round of selection, and thus these nucleotides are particularly susceptible to mutation during the selection process. Indeed, sequences of the deoxyribozymes revealed that numerous mutations accumulated within this region of each DNA enzyme (see below). In contrast, the right-hand DNA binding arm (shown in pink) originates solely in a DNA primer prepared by solid-phase synthesis and not by *Taq* polymerase extension. Therefore these nucleotides are not susceptible to mutation during selection, and no mutations are observed.

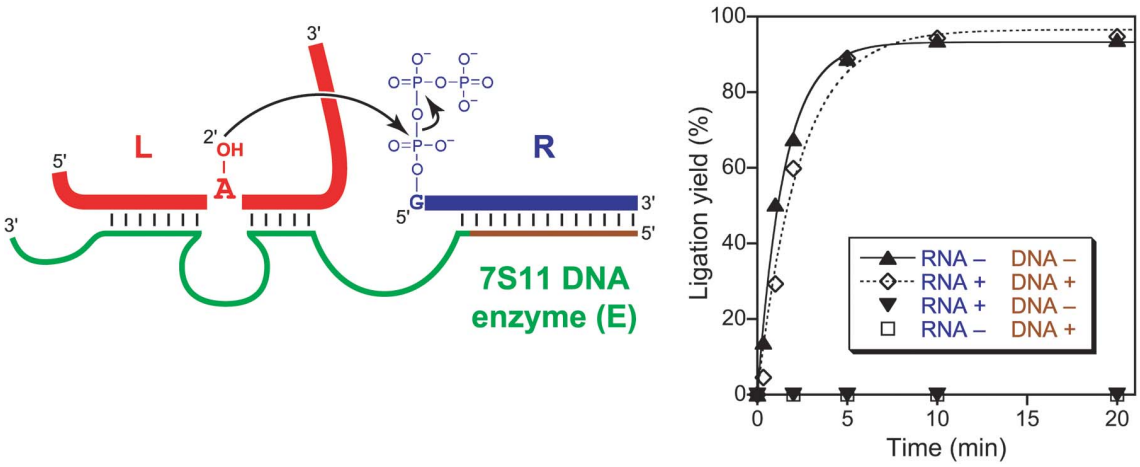
Shown in Supplementary Figure 1c are ligation assays with chimeras of the 7S11 and 7S10 deoxyribozymes. These differ at several nucleotides (black) in both the left-hand DNA binding arm (brown) and the enzyme region itself (green). The assays demonstrate that the specific binding arm sequence is inconsequential, which is consistent with the finding that this binding arm may be truncated without affecting activity (**Fig. 3**). Furthermore, the three nucleotide differences between the enzyme regions of 7S11 and 7S10 are not particularly important. We have not yet investigated further the modest functional effects attributable to the three nucleotide differences between the 7S11 and 7S10 enzyme region sequences.



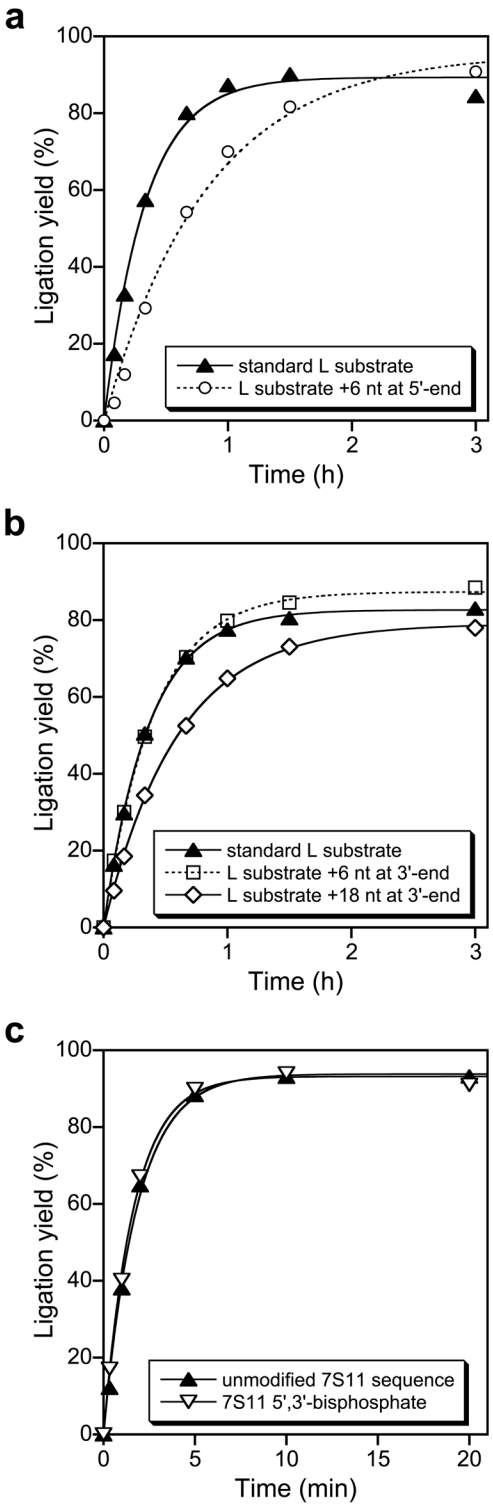

**Supplementary Figure 1**. Selection strategy and new deoxyribozymes. See text for details. (a) Summary of the selection strategy. (b) Nucleotide sequences. (c) Ligation assays for chimeras of the 7S11 and 7S10 deoxyribozymes (see also **Figure 3**). $k_{obs}$ values (top to bottom as in legend, $min^{-1}$): 0.42, 0.60, 0.18, 0.10.



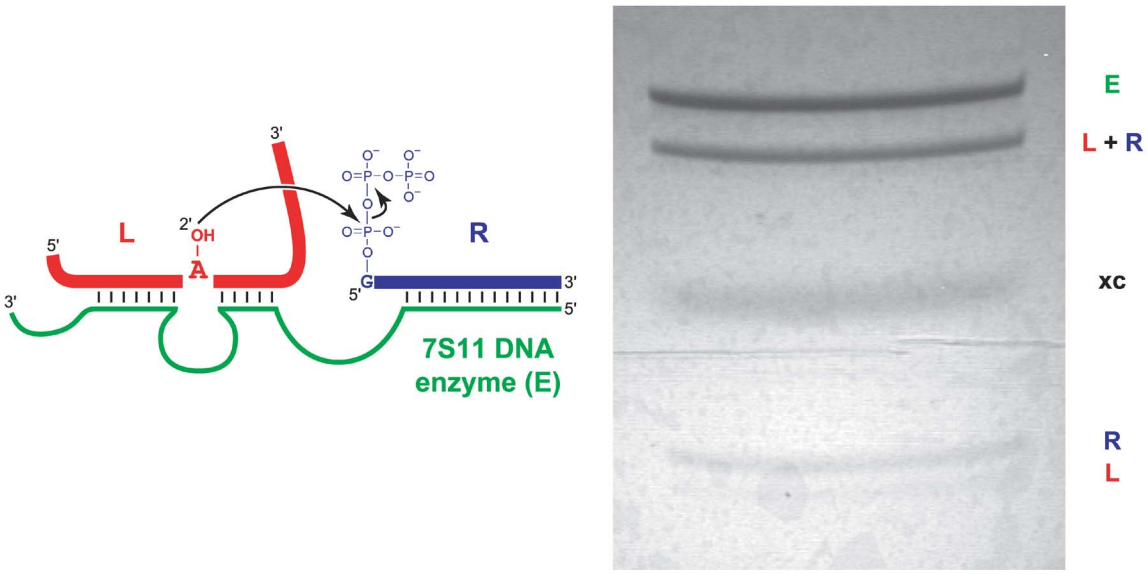

**Supplementary Figure 2**. Ligation assays for the successful combinations of RNA substrates and DNA enzymes of **Figure 4b**. The symbol – denotes retaining both the original RNA and DNA sequences of the indicated block A or B, whereas + denotes transversions of both the RNA and DNA of the indicated block (A↔C or G↔U/T). $k_{obs}$ values (top to bottom as in legend, $min^{-1}$): 0.42, 0.14, 0.16, 0.081.




**Supplementary Figure 3**. Demonstrating Watson-Crick base pairing between the R substrate (blue) and the corresponding DNA binding arm (brown). The symbol – denotes retention of the original nucleotide sequence (RNA or DNA), whereas + denotes transversion (A↔C or G↔U/T). $k_{obs}$ values: original sequences, 0.67 $min^{-1}$; transversion sequences, 0.43 $min^{-1}$. Only those combinations of RNA and DNA that maintained Watson-Crick base pairing supported ligation activity. The first five nucleotides of R (5′-GGAAG) were not altered in any of the combinations.




**Supplementary Figure 4**. Assaying modified L substrates and 7S11 deoxyribozyme. (a) Extending the 5′-end of $k_{obs}$ values: standard substrate, 3.0 $h^{-1}$; extended substrate, 1.2 $h^{-1}$. (b) Extending the 3′-end of L. $k_{obs}$ values: standa substrate, 2.8 $h^{-1}$; L +6 nt, 2.5 $h^{-1}$; L +18 nt, 1.7 $h^{-1}$. For panels a and b, the L substrate was extended at its 5′-end b 6 nt (5′-GGUCGU...) or at its 3′-end by either 6 nt (...CAGCAG-3′) or 18 nt (...CAGCAGAGCUGAUCCUGA-3′ (c) Phosphorylating both ends of the 7S11 DNA enzyme. $k_{obs}$ values: unmodified 7S11, 0.55 $min^{-1}$; bisphosphate, 0.61 $min^{-1}$. Data in panels a and b were obtained in 50 mM HEPES, pH 7.5; data in panel c were obtained in 50 m CHES, pH 9.0 (all data were obtained at 40 mM $Mg^{2+}$ and 37 °C).




**Supplementary Figure 5**. Gel image (20% denaturing PAGE) from the preparative ligation experiment described in the Methods section. xc = xylene cyanol dye.