**Supplementary Table 2**: Mutations of the Hairpin Ribozyme

| Symbol of the Mutation[1] | $k_{rel}$[2] | Length[3] | Type[4] | Location[5] | Ref. [6] |
|---|---|---|---|---|---|
| c+4a; u+5a; g+6c | 0.0000 | 50 | MP | H1 | 1 |
| G6A | 0.0000 | 50 | MP | H1 | 1 |
| G6C | 0.0000 | 50 | MP | H1 | 1 |
| G6U | 0.0000 | 50 | MP | H1 | 1 |
| u+7a, u+8a, u+9a | 1.3182 | 50 | MP | H1 | 1 |
| u+9a | 0.6818 | 50 | MP | H1 | 1 |
| a-3c; U12G | 1.0000 | 50 | BP | H2 | 2 |
| a-3g; U12C | 3.0000 | 50 | BP | H2 | 2 |
| a-3u; U12A | 1.0000 | 50 | BP | H2 | 2 |
| c-2a, a-3c | 0.0000 | 50 | MP | H2 | 1 |
| G11A | 0.0000 | 50 | MP | H2 | 1 |
| G11C | 0.0000 | 50 | MP | H2 | 1 |
| G11U | 0.0000 | 50 | MP | H2 | 1 |
| g-4c, u-5a | 0.0364 | 50 | MP | H2 | 1 |
| U12A | 0.05-0.2 | 50 | MP | H2 | 1 |
| u-5a | 1.1364 | 50 | MP | H2 | 1 |
| Δ C13; ΔA14 | >1.0000 | 48 | L | H2 | 3 |
| Δ U12; ΔC13; ΔA14 | 0.9000 | 47 | L | H2 | 3 |
| Δ A1; Δ A2; Δ A3; Δ C4; Δ C13; Δ A14 | 0.9000 | 44 | L | H2 | 3 |
| Δ A1 | >1.0000 | 49 | L | H2 | 3 |
| Δ A1; Δ A2 | >1.0000 | 48 | L | H2 | 3 |
| Δ A1; Δ A2; Δ A3 | >1.0000 | 47 | L | H2 | 3 |
| Δ A1; Δ A2; Δ A3; Δ C4 | >1.0000 | 46 | L | H2 | 3 |
| Δ A14 | >1.0000 | 49 | L | H2 | 3 |
| A15G | 0.8000 | 50 | MP | H3 | 4 |
| A15U; U49A | 1.1500 | 50 | BP | H3 | 5 |
| A18G; C17A; U46C; G47U | 0.6000 | 50 | BP | H3 | 4 |
| A18U | 0.0500 | 50 | MP | H3 | 4 |
| A50G | 0.7500 | 50 | SS | H3 | 5 |
| C16G; G48C | 0.0000 | 50 | BP | H3 | 5 |
| C17A; G47C | 0.0500 | 50 | MP | H3 | 4 |
| C17G | 0.0000 | 50 | MP | H3 | 5,6 |
| C17G; G47C | 0.2100 | 50 | BP | H3 | 5 |
| C17U; G47C | 0.1000 | 50 | MP | H3 | 4 |
| C18U; G47C | 0.0500 | 50 | MP | H3 | 4 |
| Δ A50 | 0.4900 | 49 | L | H3 | 5 |
| Δ U49; Δ A50 | 0.3800 | 48 | L | H3 | 5 |

| Symbol of the Mutation[1] | $k_{rel}$[2] | Length[3] | Type[4] | Location[5] | Ref. [6] |
|---|---|---|---|---|---|
| G19C | 0.0100 | 50 | MP | H3 | [5] |
| G19C; C45G | 0.2500 | 50 | BP | H3 | [5] |
| G19U | 0.0500 | 50 | MP | H3 | [4] |
| G19U; C45A; C16U; G48A; A15U; U49A | 0.9000 | 50 | BP | H3 | [4] |
| G19U; C45G; C17U; G47A; A15G; U49C | 0.7000 | 50 | BP | H3 | [4] |
| G47C | 0.0000 | 50 | MP | H3 | [5,6] |
| G48C | 0.0000 | 50 | MP | H3 | [5] |
| U46A | 0.1000 | 50 | MP | H3 | [4] |
| U49A | 0.8000 | 50 | MP | H3 | [5] |
| U49C | 0.6000 | 50 | MP | H3 | [5] |
| A28U; U34A | 1.1300 | 50 | BP | H4 | [5] |
| A30C | 0.0462 | 50 | SS | H4 | [6] |
| C27A; G35U | 0.8500 | 50 | BP | H4 | [4] |
| C27G | 0.0000 | 50 | MP | H4 | [5,6] |
| C27G; G35C | 0.1000 | 50 | BP | H4 | [6] |
| C27G; G35C | 0.1000 | 50 | BP | H4 | [5,6] |
| C27G; G35U | 0.5000 | 50 | MP | H4 | [4] |
| C27U; G35C | 0.4500 | 50 | MP | H4 | [4] |
| C29A | 0.5000 | 50 | MP | H4 | [4] |
| C29G; G33C | 0.1200 | 50 | BP | H4 | [5] |
| C29G; G35C | 0.0000 | 50 | MP | H4 | [5] |
| G33C | 0.1000 | 50 | MP | H4 | [5] |
| G33U | 0.7000 | 50 | MP | H4 | [4] |
| G35A | 0.1000 | 50 | MP | H4 | [4] |
| G35C | 0.0200 | 50 | MP | H4 | [4] |
| G35C | 0.0000 | 50 | MP | H4 | [5] |
| G35U | 0.0200 | 50 | MP | H4 | [4] |
| U34A | 0.1100 | 50 | MP | H4 | [5] |
| A10C | 0.0000 | 50 | SS | LA | [7] |
| A10G | 0.0493 | 50 | SS | LA | [7] |
| A10U | 0.0000 | 50 | SS | LA | [7] |
| A7C | 0.1644 | 50 | SS | LA | [7] |
| A7C; A20C | 1.0400 | 50 | SS | LA | [5] |
| A7G | 0.5205 | 50 | SS | LA | [7] |
| A7U | 0.2603 | 50 | SS | LA | [7] |
| A9C | 0.0056 | 50 | SS | LA | [7] |
| A9G | 0.0019 | 50 | SS | LA | [7] |
| A9U | 0.0014 | 50 | SS | LA | [5,7] |
| G8A | 0.0000 | 50 | SS | LA | [7] |

| Symbol of the Mutation[1] | $k_{rel}$[2] | Length[3] | Type[4] | Location[5] | Ref. [6] |
|---|---|---|---|---|---|
| G8C | 0.0000 | 50 | SS | LA | 5,7 |
| G8U | 0.0000 | 50 | SS | LA | 7 |
| u+2a | 0.0742 | 50 | SS | LA | 8 |
| u+2c | 0.0142 | 50 | SS | LA | 8 |
| u+2g | 0.0250 | 50 | SS | LA | 8 |
| A20C | 0.8100 | 50 | SS | LB | 2,5 |
| A20G | 0.4041 | 50 | SS | LB | 2,6 |
| A20U | 1.3699 | 50 | SS | LB | 2,6 |
| A22C | 0.0000 | 50 | SS | LB | 6 |
| A22G | 0.0000 | 50 | SS | LB | 6 |
| A22U | 0.0000 | 50 | SS | LB | 6 |
| A23C | 0.0000 | 50 | SS | LB | 6 |
| A23G | 0.0000 | 50 | SS | LB | 6 |
| A23U | 0.0000 | 50 | SS | LB | 6 |
| A24C | 0.0000 | 50 | SS | LB | 5,6 |
| A24G | 0.0055 | 50 | SS | LB | 6 |
| A24G; U37C | 0.0000 | 50 | SS | LB | 5 |
| A24U | 0.0068 | 50 | SS | LB | 6 |
| A26C | 0.1781 | 50 | SS | LB | 6 |
| A26G | 0.0055 | 50 | SS | LB | 6 |
| A26U | 0.0082 | 50 | SS | LB | 6 |
| A38C | 0.0000 | 50 | SS | LB | 6 |
| A38G | 0.0000 | 50 | SS | LB | 6 |
| A38U | 0.0000 | 50 | SS | LB | 5,6 |
| A40G | 0.0031 | 50 | SS | LB | 6 |
| A40U | 0.0454 | 50 | SS | LB | 6 |
| A40U | 0.0300 | 50 | SS | LB | 5 |
| A43C | 0.0000 | 50 | SS | LB | 2,6 |
| A43C; G21U | 0.0000 | 50 | SS | LB | 2 |
| A43G | 0.0846 | 50 | SS | LB | 6 |
| A43G; G21U | 0.0000 | 50 | SS | LB | 2 |
| A43U | 0.0000 | 50 | SS | LB | 2,5 |
| A43U; G21U | 0.0000 | 50 | SS | LB | 2 |
| C25A | 0.0000 | 50 | SS | LB | 6 |
| C25G | 0.0000 | 50 | SS | LB | 4,6 |
| C25G; G36C | 0.0000 | 50 | BP | LB | 5 |
| C25U | 0.0000 | 50 | SS | LB | 6 |
| C44A | 0.6538 | 50 | SS | LB | 6 |
| C44G | 0.1000 | 50 | SS | LB | 6 |

| Symbol of the Mutation[1] | $k_{rel}$[2] | Length[3] | Type[4] | Location[5] | Ref. [6] |
|---|---|---|---|---|---|
| C44U | 0.1000 | 50 | SS | LB | 6 |
| Δ A24 | 0.0000 | 49 | L | LB | 4 |
| Δ A26 | 0.0000 | 49 | L | LB | 5 |
| Δ U42 | 0.0000 | 49 | L | LB | 5 |
| G21A | 0.0685 | 50 | SS | LB | 6 |
| G21C | 0.1301 | 50 | SS | LB | 2,6 |
| G21U | 0.0753 | 50 | SS | LB | 6 |
| G21U; A20C | 0.1500 | 50 | SS | LB | 2 |
| G21U; A20G | 0.0030 | 50 | SS | LB | 2 |
| G21U; A20U | 0.0100 | 50 | SS | LB | 2 |
| G36A | 0.0269 | 50 | SS | LB | 6 |
| G36C | 0.0215 | 50 | SS | LB | 6 |
| G36U | 0.1231 | 50 | SS | LB | 6 |
| U37A | 0.7692 | 50 | SS | LB | 6 |
| U37A; A43U | 0.0000 | 50 | SS | LB | 5 |
| U37C | 1.3077 | 50 | SS | LB | 6 |
| U37G | 0.3077 | 50 | SS | LB | 6 |
| U39A | 0.9231 | 50 | SS | LB | 6 |
| U39C | 0.6154 | 50 | SS | LB | 6 |
| U39G | 0.2462 | 50 | SS | LB | 6 |
| U41A | 0.0177 | 50 | SS | LB | 6 |
| U41C | 0.0923 | 50 | SS | LB | 6 |
| U41G | 0.0000 | 50 | SS | LB | 6 |
| U42A | 0.0015 | 50 | SS | LB | 6 |
| U42C | 0.0300 | 50 | SS | LB | 5 |
| U42G | 0.0000 | 50 | SS | LB | 6 |
| U43U | 0.0023 | 50 | SS | LB | 6 |

**(1) Symbol of the mutation:** We used the conventional descriptions of the mutations. For a point mutation the original nucleotide is listed first, the location (position) of the mutation second, and then the nucleotide to with the original was replaced. So for example the mutation U42C means that uracil at position 42 is replaced by a cytosine. Deletions begin with a Δ symbol, followed by the position and the original nucleotide, thus the mutation ΔA50 means that the adenine at position 50 is deleted.

If there is more than one mutation in a mutant, then the mutations are comma separated. Some deletions and insertions have textual descriptions see the references for the exact sequence.

**(2) Relative enzymatic activity ($k_{rel}$):** The relative activity is the ratio of the enzymatic activity of the mutant and the wild-type ribozyme.

**(3) Length:** The length of the ribozyme.

**(4) Type of mutation:** Based on structural changes and activity loss, we can discern the following types of mutations:

*Base pair change (BP)*: The base pair is either reversed or changed to another base pair. The structure does not change. The base paired positions in the substrate binding regions are also considered base paired, and if the base pair is changed to another base-pair then it is listed as a BP mutation.

*Mutation affecting a single stranded region (SS):* The point mutation affects one of the single stranded regions of the ribozyme, mainly the two loops. The structure does not change.
*Mispair (MP):* The mutation disrupts one of the base pairs, so the pair could not form and the structure of the molecule changes locally (an internal bulge is formed). The base paired positions in the substrate binding regions are also considered base paired, and if the base pair is perturbed it is listed as a MP mutation.
*Change in length (L):* The mutation is either a deletion or an insertion.

**(5) Location of the mutation:** The helical regions (H1, H2, H3, H4) or loop (LA or LB) where the mutation occurs.

**(6) Reference:** The source of information for the given ribozyme.

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8. Walter, N.G., Chan, P.A., Hampel, A., Millar, D.P. & Burke, J.M. A base change in the catalytic core of the hairpin ribozyme perturbs function but not domain docking. *Biochemistry* **40**, 2580-2587 (2001).