

Supplemental figures

Experimental evidence for the correlation between RNA structural fluctuations and the frequency of beneficial mutations

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A

	Mutation number																	Fitness	LoE fitness
	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17		
genotype_01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0
genotype_02	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1.4	0.34
genotype_03	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2.1	0.74
genotype_04	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1.9	0.64
genotype_05	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1.9	0.64
genotype_06	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.8	0.59
genotype_07	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1.5	0.41
genotype_08	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1.8	0.59
genotype_09	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.8	0.59
genotype_10	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.9	0.64
genotype_11	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1.7	0.53
genotype_12	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	4.3	1.46
genotype_13	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	2.3	0.83
genotype_14	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2.2	0.79
genotype_15	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1.9	0.64
genotype_16	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1.9	0.64
genotype_17	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	3.3	1.19
genotype_18	0	0	0	1	1	1	0	0	0	1	1	0	0	0	0	0	0	2.4	0.88
genotype_19	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	4.0	1.39
genotype_20	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.1	1.13
genotype_21	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	4.5	1.5
genotype_22	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	6.6	1.89
genotype_23	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	5.2	1.65
genotype_24	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	1	0	3.8	1.34
genotype_25	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	5.7	1.74
genotype_26	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	5.5	1.7
genotype_27	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	9.5	2.3
genotype_28	1	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	7.0	1.95
genotype_29	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	1	0	6.0	1.79
genotype_30	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	5.1	1.63
genotype_31	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	7.5	2.0
genotype_32	1	1	1	1	0	1	0	0	0	1	1	0	0	0	0	0	0	6.3	1.84
genotype_33	1	1	1	1	1	0	0	1	1	0	0	1	0	0	0	0	0	8.8	2.2
genotype_34	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	7.5	2.0
genotype_35	1	1	1	1	1	1	0	1	1	0	0	0	1	0	0	0	0	13.4	2.6
genotype_36	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	1	13.5	2.6
genotype_37	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	11.2	2.4
genotype_38	1	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	16.5	2.8
genotype_39	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	11.6	2.5
genotype_40	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	12.7	2.5
genotype_41	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	1	0	10.0	2.3
genotype_42	1	1	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	18.0	2.9
genotype_43	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	11.2	2.4
genotype_44	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	11.1	2.4
genotype_45	1	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	1	17.9	2.9
genotype_46	1	1	1	1	1	0	1	1	1	0	0	1	0	0	0	0	0	20.3	3.0
genotype_47	1	1	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0	13.1	2.6
genotype_48	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	17.0	2.8
genotype_49	1	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	1	28.3	3.3
genotype_50	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	16.9	2.8
genotype_51	1	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	28.9	3.4
genotype_52	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	1	0	11.2	2.4
genotype_53	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	30.4	3.4
genotype_54	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	17.1	2.8
genotype_55	1	1	1	1	0	1	0	0	0	1	1	0	0	0	0	0	1	15.2	2.7
genotype_56	1	1	1	1	1	0	1	1	1	0	0	1	0	0	0	1	0	17.8	2.9
genotype_57	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	32	3.5
genotype_58	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1	24	3.2
genotype_59	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	1	29	3.4
genotype_60	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	30	3.4
genotype_61	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	27	3.3
genotype_62	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	31	3.4
genotype_63	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0	0	34	3.5
genotype_64	1	1	1	1	1	1	1	1	0	1	0	1	0	0	0	0	0	25	3.2
genotype_65	1	1	1	1	1	1	0	1	0	0	0	1	0	0	0	0	0	38	3.6
genotype_66	1	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	24	3.2
genotype_67	1	1	1	1	1	1	1	0	1	1	1	0	0	0	0	0	0	26	3.3
genotype_68	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0	25	3.2
genotype_69	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	20	3.0
genotype_70	1	1	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	30	3.4
genotype_71	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	31	3.4
genotype_72	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	40	3.7
genotype_73	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0	46	3.8
genotype_74	1	1	1	1	1	0	1	1	1	1	1	0	0	0	0	0	0	41	3.7
genotype_75	1	1	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	51	3.9
genotype_76	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	37	3.6

B

Mutation number	
M1	A186G
M2	C167U
M3	U203C
M4	1995CAC delete
M5	1998CAC delete
M6	G743A
M7	155 insert
M8	A2033G
M9	G2064A
M10	G1600A
M11	A1523U
M12	C1895U
M13	G1723A
M14	G1829U
M15	U1913C
M16	A1652G
M17	A623G

Figure S1. Mutations in the RNAs used in this study.

A) Mutations introduced into each genotype. B) Mutation list.

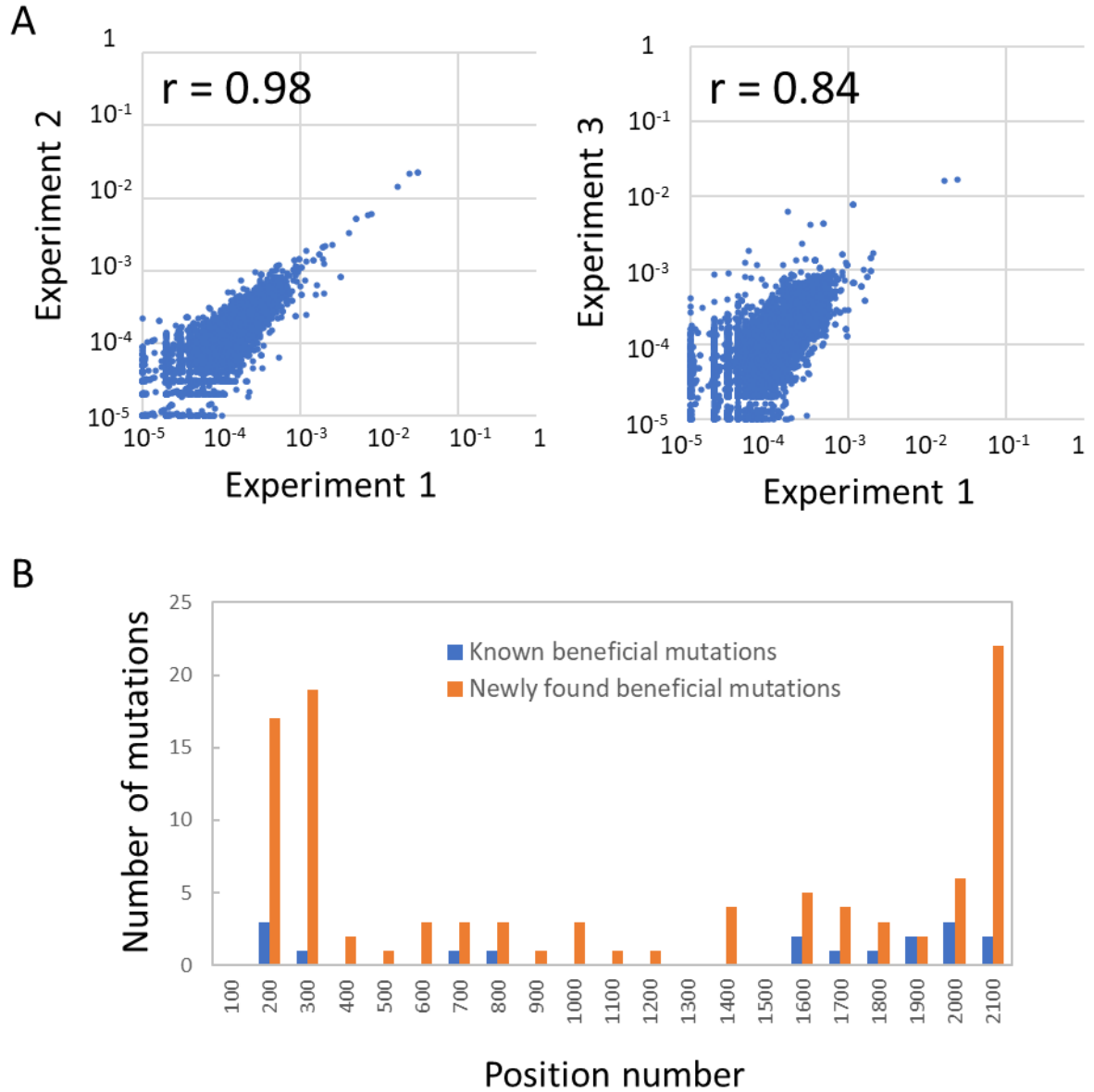


Figure S2. Analysis of the newly found beneficial mutations.

A) Reproducibility of the measurement of beneficial mutations. The frequency of the beneficial mutations of the genotype 01 were measured by the method depicted in Fig. 2A three times independently and compared the measured frequencies between the experiments. Correlation coefficient, r , was shown on each panel. B) Comparison of mutational position between the already-known beneficial mutations and the newly found beneficial mutations. The already-known 17 beneficial mutations are found in the previous study (Ichihashi et al 2015) and shown in Fig. S1B. The newly found beneficial mutations are the top-100 most frequent mutations in the distribution of the genotype 01 (Fig. 2B).

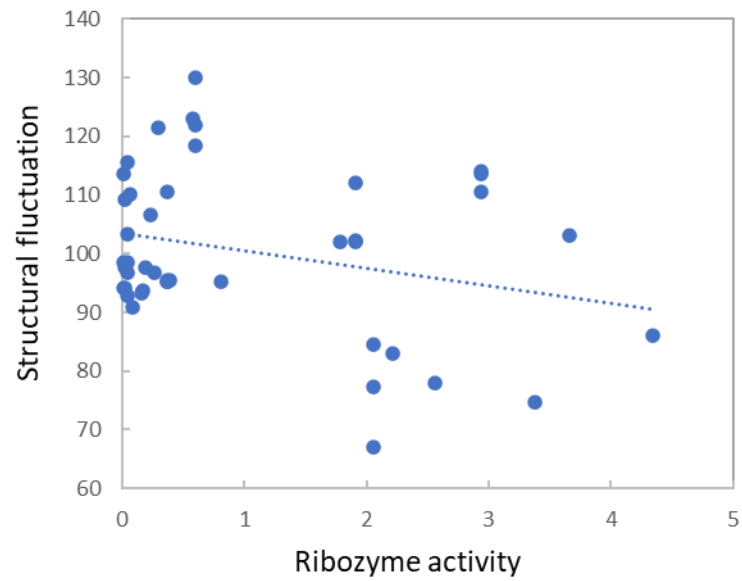


Figure S3. Relationship between the structural fluctuations and the ribozyme activities.

The sequence and ribozyme activity data were obtained from previous paper (Lehman N, Joyce GF. 1993. Evolution in vitro: analysis of a lineage of ribozymes. *Curr Biol* 3: 723–734). The structural fluctuations were predicted by the method described in the Method section.

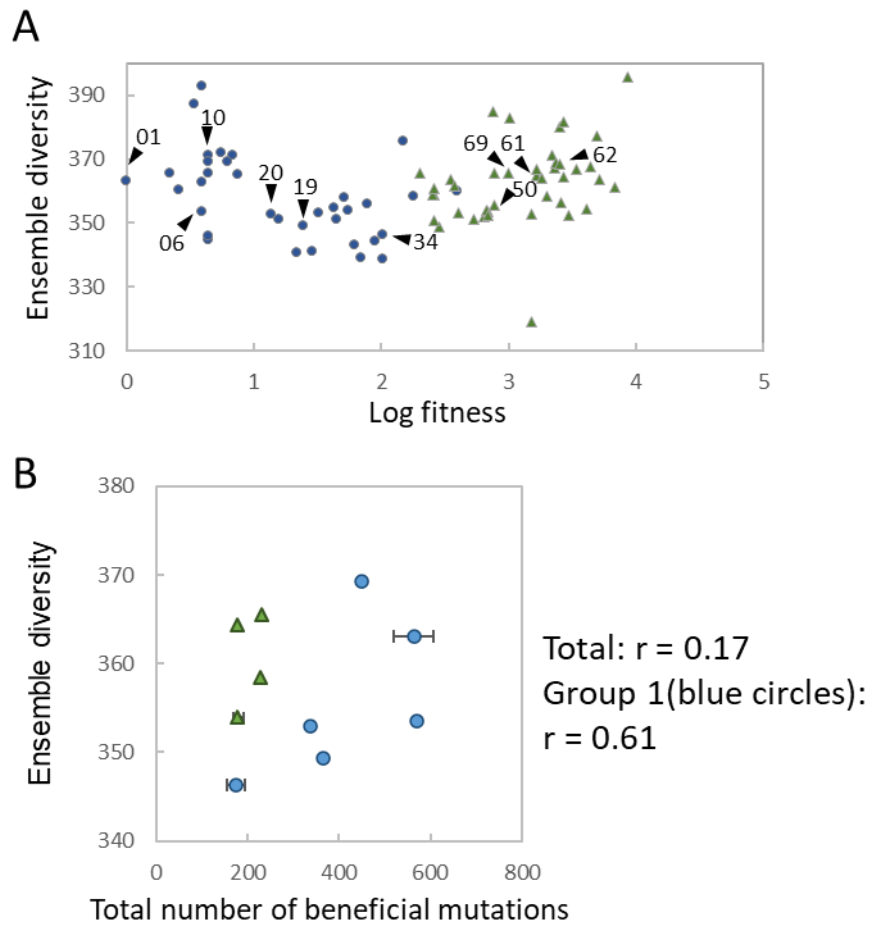


Figure S4. Result with the ensemble diversity instead of the structural fluctuation

A) Relationship between the fitness and ensemble diversity of the 76 genotypes. B) Relationship between the total number of beneficial mutations and ensemble diversity. The RNAs in the groups 1 and 2 are shown with blue circles and green triangles, respectively. Ensemble diversities were calculated by Vienna RNA web service with the same energy models (Mathews model 2004) as that used for the calculation of the structural fluctuations.

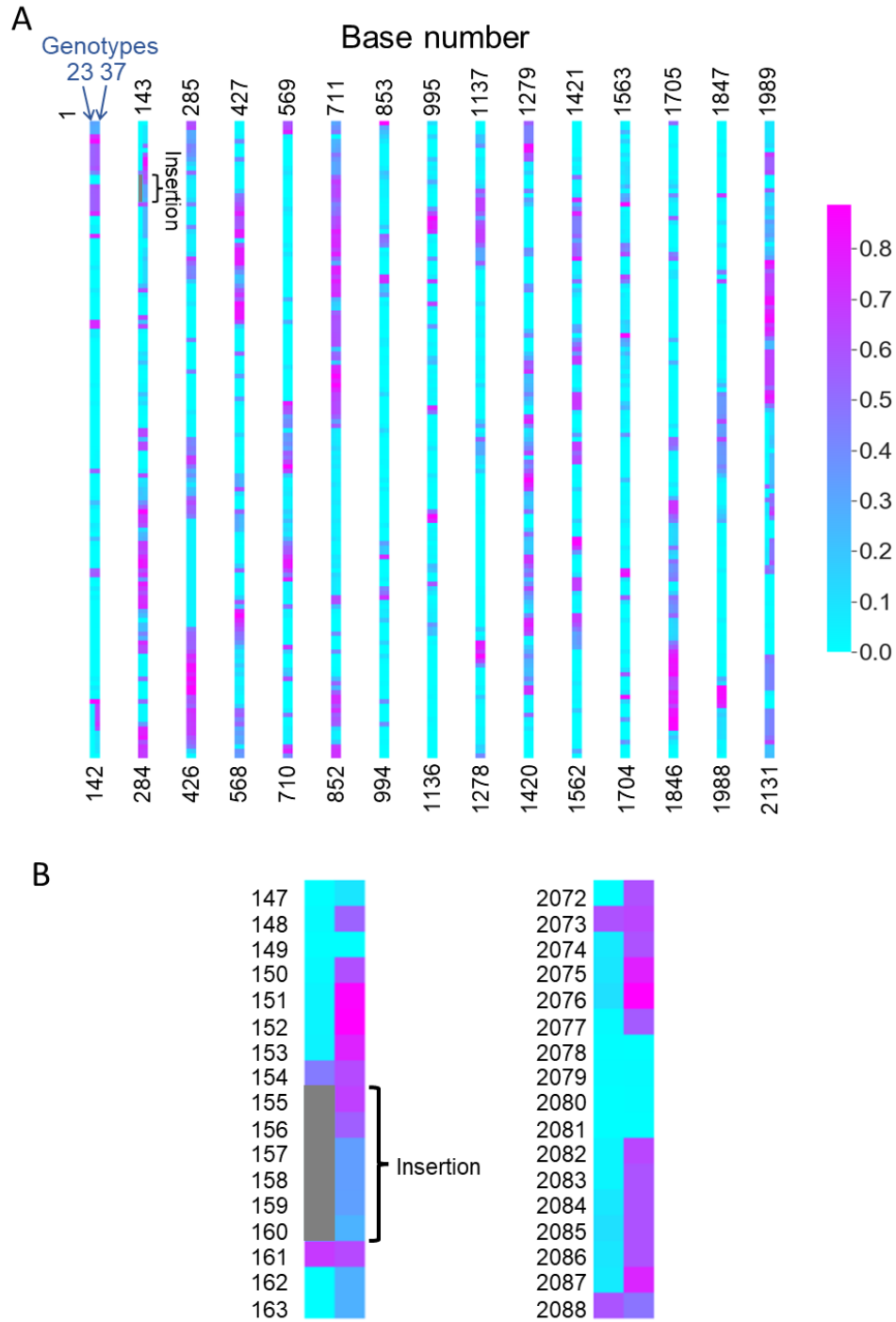


Figure S5. The effect of the 155 insertion on the local structure fluctuations.

A) The structural fluctuations (average Hamming distance of the two secondary structures) were calculated at each position between all combinations of the 1000 structures for genotypes 23 and 37. The genotype 37 has an additional 155 insertion (M7) to the genotype 23. The values of each base represented as a heat map. B) Two regions (147-163 and 2072-2088), where the local structural fluctuations were changed by the insertion, were picked up.