

Figure S1. Positions of amino acid mutations in the structure

The amino acid position and the population name in which the mutation was observed is in parentheses. (a) The mutational positions in A2 were mapped on protein data bank 5MNT. (b) The mutational positions in A2 were mapped in the same manner as (a), but the positions shown in gray filled circles represent the amino acid that interacts with the RNA genome [29]. (c) The mutational positions in coat protein were mapped on protein data bank 4L8H. (d) The mutational positions in β -subunit of Q β replicase with the structure of EF-Tu and EF-Ts (protein data bank 3AVT).

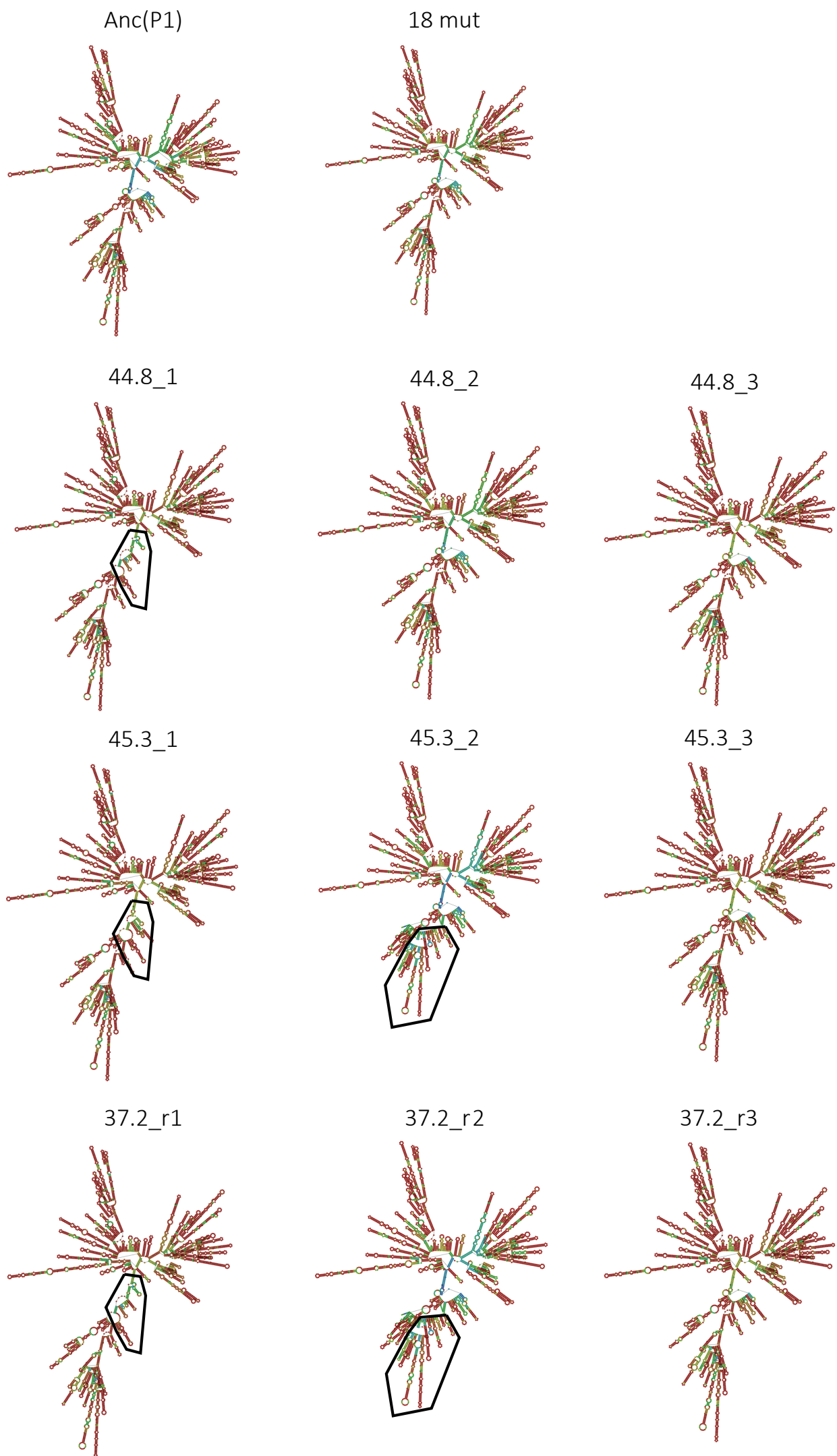


Figure S2. Secondary structure of starting Q β and thermal adapted Q β genomes
 Anc(P1) is a starting phage cultured from 37.2°C in the previous study [7]; 18 mut is a starting phage in this study. Minor differences were observed in the closed region.

Table S1. List of templates, primers, and polymerase used for Q β genome sequencing.

PCR products	PCR product preparation				Sequencing	
	Template	Primer name	Primer sequence (5'→3')	DNA polymerase	Primer name	Primer sequence (5'→3')
Qt & Qi & seq_3368 product	2nd strand cDNA	Qt	CCAGTGAGCAGAGTGACGAGGACTC GAGCTCAAGCTTTTTTTTTTTTTTTTT	Pfu Ultra II Fusion HS DNA polymerase (Agilent Technologies, Santa Clara, CA, USA)	A2_1	ATGCCTAAATTACCGCGTGGTCTGC
		Qi	ACGAGGACTCGAGCTCAAGC		A2_489	TTTACTGCGCAGACTGCGTG
		seq_3368	TATCATGACGGTCTGCCAG		seq_3368	TATCATGACGGTCTGCCAG
					seq_3752	AATCCCAGTCAACACGAGCG
					A153C_f	TTTATCGACTCTTCCGACACGCATC
Qi & beta 775 product	1st strand cDNA with poly(T) and poly(A)	Qi	ACGAGGACTCGAGCTCAAGC	Pfu Ultra II Fusion HS DNA polymerase	beta_end	TTACGCCTCGTGTAGAGACGCAACC
		beta_775	CGGTTACACATTCGAGCTCG		seq_512	GGACTCACTATACGGTGACG
					beta_775	CGGTTACACATTCGAGCTCG
					beta_273	GTGATTACGGACCATAACAAG
					beta_561	CGGCAAGCACTACTATTCTG
A2_489 & seq_2281 product	1st strand cDNA with poly(T) and poly(A)	A2_489	TTTACTGCGCAGACTGCGTG	Phusion™ High-Fidelity DNA polymerase (New England Biolabs, Ipswich, MA, USA)	seq_2281	ATACTTACCTGTCCCTGGCG
		seq_2281	ATACTTACCTGTCCCTGGCG		seq_2870	AGATCTAGTGTAGCGAACGG
					seq_3368	TATCATGACGGTCTGCCAG
					A2_489	TTTACTGCGCAGACTGCGTG
					A2_2997	ACAGACGTGACATCCGGCTC
coat_2498 & seq_1722 product	1st strand cDNA with poly(T) and poly(A)	coat_2498	CAGGCATATGCTGACGTGAC	Phusion™ High-Fidelity DNA polymerase	seq_1722	GCAATTGTGCGCTGAGAGAG
		seq_1722	GCAATTGTGCGCTGAGAGAG		seq_2281	ATACTTACCTGTCCCTGGCG
					coat_2498	CAGGCATATGCTGACGTGAC
					RT_1932	TGCTTATTGCTCTCTTAGCG
seq_512 & RT_1932 product	1st strand cDNA with poly(T) and poly(A)	seq_512	GGACTCACTATACGGTGACG	Phusion™ High-Fidelity DNA polymerase	seq_512	GGACTCACTATACGGTGACG
		RT_1932	TGCTTATTGCTCTCTTAGCG		seq_1112	TCTGTCTTACTGTTCTTAGG
					seq_1722	GCAATTGTGCGCTGAGAGAG
					RT_1932	TGCTTATTGCTCTCTTAGCG
					beta_815	CGGTAGTGTGTACCTACGAG
seq_2234 & A153C_f product	1st strand cDNA with poly(T) and poly(A)	seq_2234	GGAGGCTCGTAAACCTCCTC	Phusion™ High-Fidelity DNA polymerase	seq_2234	GGAGGCTCGTAAACCTCCTC
		A153C_f	TTTATCGACTCTTCCGACACGCATC		seq_2870	AGATCTAGTGTAGCGAACGG
					seq_3368	TATCATGACGGTCTGCCAG
					A153C_f	TTTATCGACTCTTCCGACACGCATC
					A2_2997	ACAGACGTGACATCCGGCTC
					coat_2498	CAGGCATATGCTGACGTGAC
					A2_489	TTTACTGCGCAGACTGCGTG
		A2_1158	ACGAGCAATCTTCCGTTCCG			