Attenuated strain of CVB3 with a mutation in the CAR-interacting region protects against both myocarditis and pancreatitis

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Running title: Mutations in the CVB3 genome affecting disease phenotype

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Table S1: Amino acid and nucleotide codons of VP1 771-790

Amino acid position	Amino acids	Codons
771	Arginine (R)	AGG
772	Asparagine (N)	AAC
773	Glycine (G)	GGA
774	Valine (V)	GTT
775	Tyrosine (Y)	TAC
776	Glycine (G)	GGC
777	Isoleucine (I)	ATC
778	Asparagine (N)	AAC
779	Threonine (T)	ACG
780	Leucine (L)	CTA
781	Asparagine (N)	AAC
782	Asparagine (N)	AAC
783	**Methionine (M)**	**ATG**
784	**Glycine (G)**	**GGC**
785	**Threonine (T)**	**ACG**
786	Leucine (L)	CTA
787	Tyrosine (Y)	TAT
788	Alanine (A)	GCA
789	Arginine (R)	AGA
790	Histidine (H)	CAT

Note: Bold residues (M, G and T) are partially buried, but they interact with CAR. Underlined residues (Y, G, I, L, A, L, Y, R, and H) are buried, but do not interact with CAR.

Table S2: List of primers used for the creation of CVB3 mutant viruses

Sl No.	**Primer name**	**Sequence**
1	SPEI-P2 Reverse Primer	5' ggagtcttgacccactagtgattctttc 3'
2	XHOI-P3 Forward Primer	5' accagggtactcgagtgtttttag 3'
3	Mt 1 Forward Primer	5' ccaggaacggagttgccggcatcaa 3'
4	Mt 2 Forward Primer	5' ccaggaacggagtttacgccatcaacac 3'
5	Mt 3 Forward Primer	5' ttacggcgccaacacgctaaac 3'
6	Mt 4 Forward Primer	5' atcaacacggcaaacaacatg 3'
7	Mt 5 Forward Primer	5' ccaggaacggagttgccgccgccaacacg 3'
8	Mt 6 Forward Primer	5' aaacaacatgggcacggcatatgcaag 3'
9	Mt 7 Forward Primer	5' gggcacgctagctgcaagacatg 3'
10	Mt 8 Forward Primer	5' gggcacgctatatggaagacatgt 3'
11	Mt 9 Forward Primer	5' gggcacgctatatgcagcacatgtcaac 3'
12	Mt 10 Forward Primer	5' atatgcaagagctgtcaacgctgg 3'
13	Mt 11 Forward Primer	5' gggcacggcagctggagcagctgtcaacgc 3'
14	SPE-CVB Forward Primer	5' ataccaatcccatgtgcttttag 3'
15	SPE-CVB Reverse Primer	5' aaccgataagggctagtgtggcag 3'

Table S3: The list of mutations introduced within VP1 771-790 in the CVB3 genome

Amino acid position	**Residue/s to be mutated to Alanine/Glycine**	**Original codon**	**Mutated codon**
VP1 775	Y to A (Tyrosine to Alanine)	**TAC**	**GCC**
VP1 776	G to A (Glycine to Alanine)	**GGC**	**GCC**
VP1 777	I to A (Isoleucine to Alanine)	**ATC**	**GCC**
VP1 780	L to A (Leucine to Alanine)	**CTA**	**GCA**
VP1 775-777	YGI to AAA (Tyrosine, Glycine and Isoleucine to Alanine, Alanine and Alanine)	**TACGGCATC**	**GCCGCCGCC**
VP1 786	L to A (Leucine to Alanine)	**CTA**	**GCA**
VP1 787	Y to A (Tyrosine to Alanine)	**TAT**	**GCT**
VP1 788	A to G (Alanine to Glycine)	**GCA**	**GGA**
VP1 789	R to A (Arginine to Alanine)	**AGA**	**GCA**
VP1 790	H to A (Histidine to Alanine)	**CAT**	**GCT**
VP1 786-790	LYARH to AAGAA (Leucine, Tyrosine, Alanine, Arginine and Histidine to Alanine, Alanine, Glycine, Alanine and Alanine)	**CTATATGCAAGACAT**	**GCAGCTGGAGCAGCT**

Table S4: List of original and mutated nucleotide sequences of VP1 771-790 for the derivation of infectious mutant viruses

Mutations	Sequences (original, top; and mutated, bottom)
1. Y to A (775)	AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT AGGAACGGAGTT**GC**CGGCATCAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT
2. G to A (776)	AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT AGGAACGGAGTTTACG**C**CATCAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT
3. I to A (777)	AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT AGGAACGGAGTTTACGGC**GC**CAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT
4. L to A (780)	AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT AGGAACGGAGTTTACGGCATCAACACG**GC**AAACAACATGGGCACGCTATATGCAAGACAT
5. YGI to AAA (775-777)	AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT AGGAACGGAGTT**GC**CG**C**C**GC**CAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT
6. L to A (786)	AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACG**GC**ATATGCAAGACAT
7. Y to A (787)	AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTA**GC**TGCAAGACAT
8. A to G (788)	AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATG**G**AAGACAT
9. R to A (789)	AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATGCA**GC**ACAT
10. H to A (790)	AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATGCAAGA**GC**T
11. LYARH to AAGAA (786-790)	AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACGCTATATGCAAGACAT AGGAACGGAGTTTACGGCATCAACACGCTAAACAACATGGGCACG**GC**A**GC**TG**G**A**GC**A**GC**T