

SUPPLEMENTARY INFORMATION

Serine 26 in Early Growth Response-1 is Critical for Endothelial Proliferation, Migration and Network Formation

Fernando S. Santiago, Yue Li and Levon M. Khachigian

Fig S1. Sequences and chromatographs from sequenced WT cells.

Alignment of *Egr-1* nucleotide sequences in WT cells with human *Egr-1* (NM_001964.3). Forward sequences are shown in the left upper panel while reverse complement sequences are shown in the upper right panel. The red box indicates the location of TCG (Ser26). Lower panels show the sequencing data. The translated amino acid sequence (partial) is shown. **A**, WT1; **B**, WT1; **C**, WT3 cells.

Fig S2. Sequences and chromatographs from sequenced MUT cells.

Alignment *Egr-1* nucleotide sequences in MUT cells with human *Egr-1* (NM_001964.3). Forward sequences are shown in the left upper panel while reverse complement sequences are shown in the upper right panel. The red box indicates the location of the TCG>GCG mutation (Ser26>Ala26). TCG (Ser26). Lower panels show the sequencing data. Green box indicates the location of silent PAM site. The translated amino acid sequence (partial), including the Ser26>Ala mutation (in red) is shown. **A**, M26A; **B**, M26B; **C**, M26C cells.

Fig S3. Sequences and chromatographs from sequenced DEL cells.

Alignment *Egr-1* nucleotide sequences in MUT cells with human *Egr-1* (P18146). Forward sequences are shown in the left upper panel while reverse complement sequences are shown in the upper right panel. Red box indicates the location of TCG (Ser26) in the reference sequence. Black box indicates the location of the 4-nucleotide deletion. Lower panels show the sequencing data. The translated amino acid sequence is shown, including nonsense sequences (in red, due to frameshift) and premature termination (*). **A**, DEL1; **B**, DEL2; **C**, DEL3 cells.

Fig S4. Copy number analysis in WT and M26 cells.

Copy number was determined by quantitative real-time PCR across the *Egr-1* region and Ct values were normalized against Ct values for PCR across a control gene (*Sp1*).

Fig S5. PCR of CRISPR/Cas9 clones.

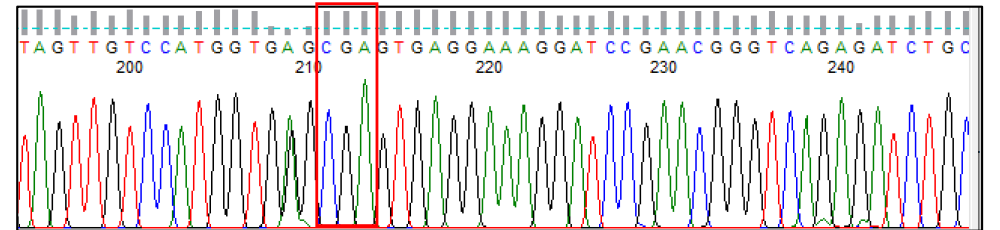
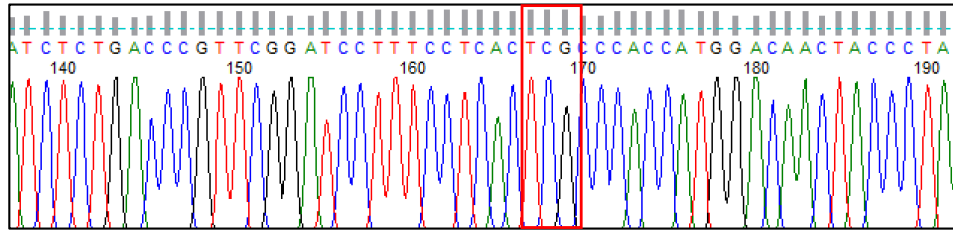
Long PCR of human *Egr-1* to test for insertions or indels downstream of Ser26. DNA size markers are shown.

Forward WT1

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Query 70 CTCTCCAGCCTGCTCGTCCAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCC 129
Sbjct 262 CTCTCCAGCCTGCTCGTCCAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCC 321
Query 130 GCTGCAGATCTCTGACCCGTTTCGGATCCTTTCTCACTCGCCACCATGGACAACACTACCC 189
Sbjct 322 GCTGCAGATCTCTGACCCGTTTCGGATCCTTTCTCACTCGCCACCATGGACAACACTACCC 381
Query 190 TAAGCTGGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGG 249
Sbjct 382 TAAGCTGGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGG 441
```

Reverse Complement WT1

```
Query 124 CTGCTCGTCCAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGAT 183
Sbjct 271 CTGCTCGTCCAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGAT 330
Query 184 CTCTGACCCGTTTCGGATCCTTTCTCACTCGCTCACCATGGACAACACTACCCCTAAGCTGGA 243
Sbjct 331 CTCTGACCCGTTTCGGATCCTTTCTCACTCGCTCACCATGGACAACACTACCCCTAAGCTGGA 390
Query 244 GGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGGGGCCCCAGA 303
Sbjct 391 GGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGGGGCCCCAGA 450
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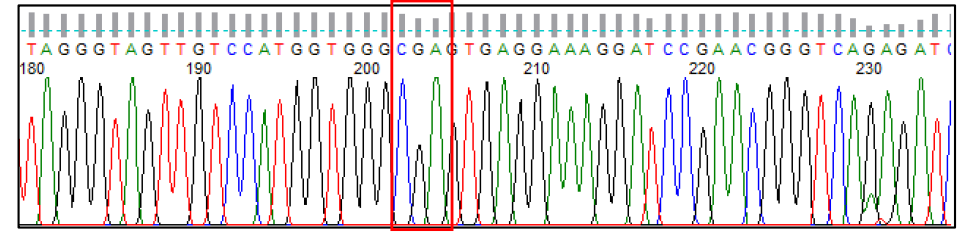
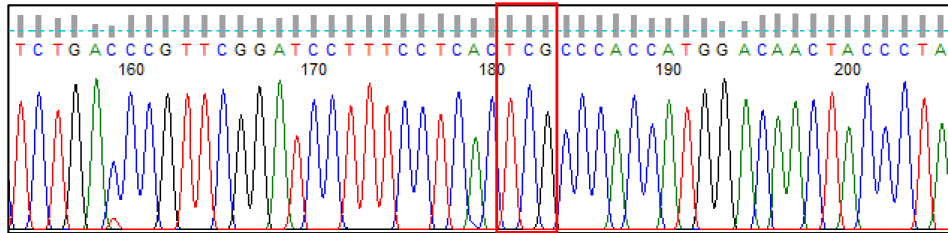
FIG. S1A

Forward WT2

Query	90	AGCCTGCTCGTCCAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCA	149
Sbjct	268	AGCCTGCTCGTCCAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCA	327
Query	150	GATCTCTGACCCGTTTCGGATCCTTTCTCACTCGGCCACCATGGACAACACCCTAAGCT	209
Sbjct	328	GATCTCTGACCCGTTTCGGATCCTTTCTCACTCGGCCACCATGGACAACACCCTAAGCT	387
Query	210	GGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGGGGCCCC	269
Sbjct	388	GGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGGGGCCCC	447

Reverse Complement WT2

Query	136	CAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATCTTGACCC	195
Sbjct	280	CAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATCTTGACCC	339
Query	196	GTTTCGGATCCTTTCTCACTCGGCCACCATGGACAACACCCTAAGCTGGAGGAGATGAT	255
Sbjct	340	GTTTCGGATCCTTTCTCACTCGGCCACCATGGACAACACCCTAAGCTGGAGGAGATGAT	399
Query	256	GCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGGGGCCCCAGAGGgcagcgg	315
Sbjct	400	GCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGGGGCCCCAGAGGGCAGCGG	459

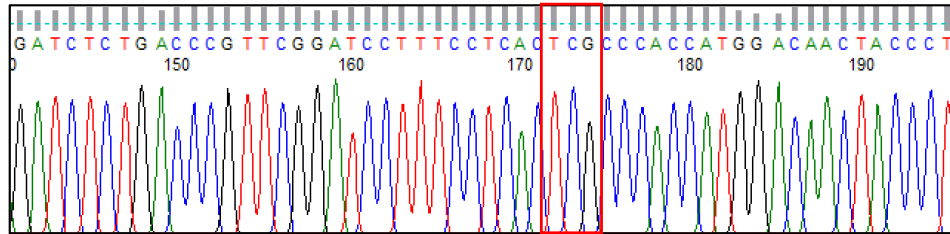


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FIG. S1B

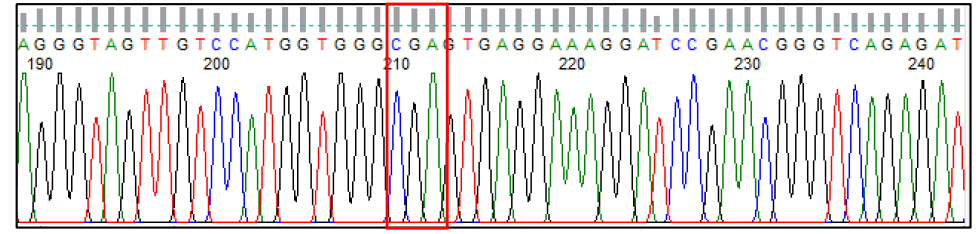
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Query 131 CCCCCTGCAGATCTCTGACCCGTTTCGGATCCTTTCTCACTCGCCACCATGGACAAC 190
Sbjct 318 CCCCCTGCAGATCTCTGACCCGTTTCGGATCCTTTCTCACTCGCCACCATGGACAAC 377
Query 191 ACCCTAAGCTGGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCTCGGCGCCG 250
Sbjct 378 ACCCTAAGCTGGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCTCGGCGCCG 437
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Reverse Complement WT3

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Query 138 CAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATCTCTGACCC 197
Sbjct 280 CAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATCTCTGACCC 339
Query 198 GTTCGGATCCTTTCTCACTCGCCACCATGGACAACACCCTAAGCTGGAGGAGATGAT 257
Sbjct 340 GTTCGGATCCTTTCTCACTCGCCACCATGGACAACACCCTAAGCTGGAGGAGATGAT 399
Query 258 GCTGCTGAGCAACGGGGCTCCCCAGTTCTCGGCGCCGCGGGGCCCCAGAgggcagcgg 317
Sbjct 400 GCTGCTGAGCAACGGGGCTCCCCAGTTCTCGGCGCCGCGGGGCCCCAGAGGGCAGCGG 459
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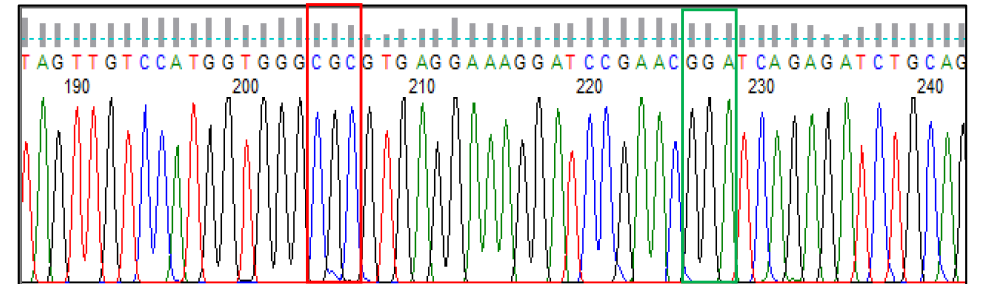
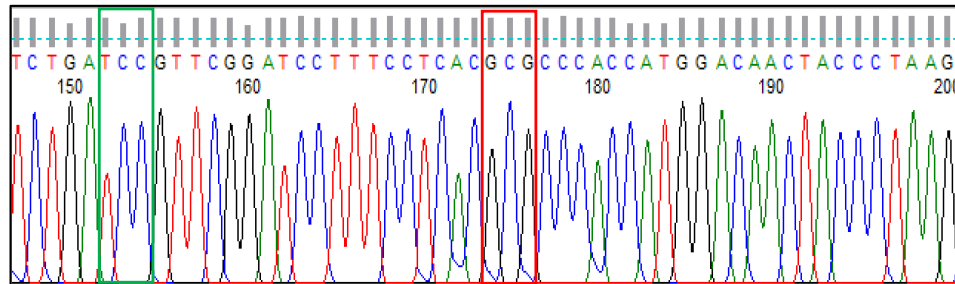
FIG. S1C

Forward M26A

Query	68	CGACACCAGCTCTCCAGCCTGCTCGTCCAGGATGGCCGCGGCCAAGGCCGAGATGCAGCT	127
Sbjct	253	CGACACCAGCTCTCCAGCCTGCTCGTCCAGGATGGCCGCGGCCAAGGCCGAGATGCAGCT	312
Query	128	GATGTCCCCGCTGCAGATCTCTGATCCGTTTCGGATCCTTTCCTCACGCGCCACCATGGA	187
Sbjct	313	GATGTCCCCGCTGCAGATCTCTGATCCCGTTTCGGATCCTTTCCTCACGCGCCACCATGGA	372
Query	188	CAACTACCCTAAGCTGGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGG	247
Sbjct	373	CAACTACCCTAAGCTGGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGG	432

Reverse Complement M26A

Query	132	CAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATCTCTGATCC	191
Sbjct	280	CAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATCTCTGATCC	339
Query	192	GTTTCGGATCCTTTCCTCACGCGCCACCATGGACAACACCCTAAGCTGGAGGAGATGAT	251
Sbjct	340	GTTTCGGATCCTTTCCTCACGCGCCACCATGGACAACACCCTAAGCTGGAGGAGATGAT	399
Query	252	GCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCGGGGCCCCAGAGggcagcgg	311
Sbjct	400	GCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCGGGGCCCCAGAGGGCAGCGG	459



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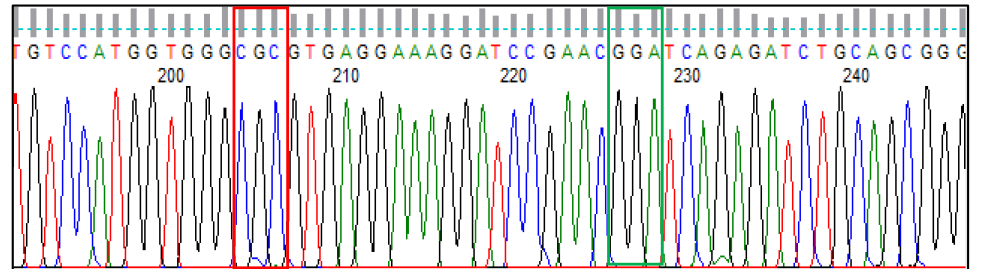
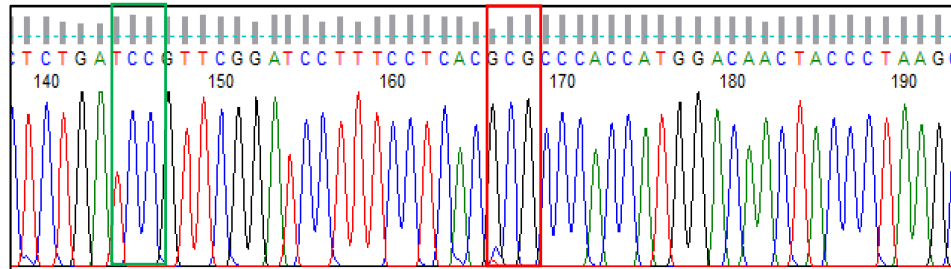
FIG. S2A

Forward M26B

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Sbjct	261	GCTCTCCAGCCTGCTCGTCCAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCC	320
Query	128	CGCTGCAGATCTCTGATCCGTTTCGGATCCTTTCTCACGCGCCACCATGGACAACACTACC	187
Sbjct	321	CGCTGCAGATCTCTGATCCGTTTCGGATCCTTTCTCACGCGCCACCATGGACAACACTACC	380
Query	188	CTAAGCTGGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCG	247
Sbjct	381	CTAAGCTGGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCG	440

Reverse Complement M26B

Query	122	TGCTCGTCCAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATC	181
Sbjct	272	TGCTCGTCCAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATC	331
Query	182	TCTGATCCGTTTCGGATCCTTTCTCACGCGCCACCATGGACAACACTACCCTAAGCTGGAG	241
Sbjct	332	TCTGATCCGTTTCGGATCCTTTCTCACGCGCCACCATGGACAACACTACCCTAAGCTGGAG	391
Query	242	GAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGGGGCCCCAGAG	301
Sbjct	392	GAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGGGGCCCCAGAG	451



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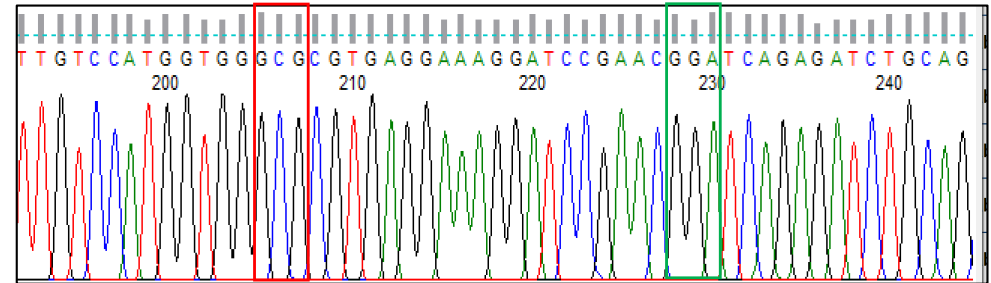
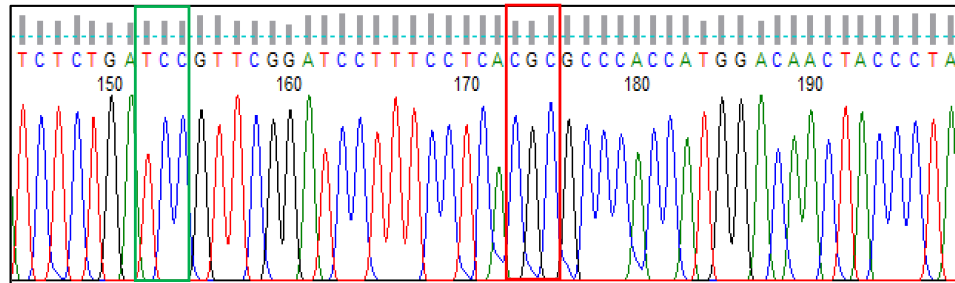
FIG. S2B

Forward M26C

Query	85	CCTGCTCGTCCAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGA	144
Sbjct	270	CCTGCTCGTCCAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGA	329
Query	145	TCTCTGATCCGTTTCGGATCCTTTCTCACTCGCCACCATGGACAACCTACCCTAAGCTGG	204
Sbjct	330	TCTCTGATCCGTTTCGGATCCTTTCTCACTCGCCACCATGGACAACCTACCCTAAGCTGG	389
Query	205	AGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCTCGGCGCCGCCGGGGCCCCAG	264
Sbjct	390	AGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCTCGGCGCCGCCGGGGCCCCAG	449

Reverse Complement M26C

Query	75	GCTCCAGCCCCGGGCTGCACccccccGCCCCGACACCAGCTCTCCAGCCTGCTCGTCCAG	134
Sbjct	223	GCTCCAGCCCCGGGCTGCACCCCCCGCCCCGACACCAGCTCTCCAGCCTGCTCGTCCAG	282
Query	135	GATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATCTCTGATCCCGTT	194
Sbjct	283	GATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATCTCTGATCCCGTT	342
Query	195	CGGATCCTTTCTCACTCGCCACCATGGACAACCTACCCTAAGCTGGAGGAGATGATGCT	254
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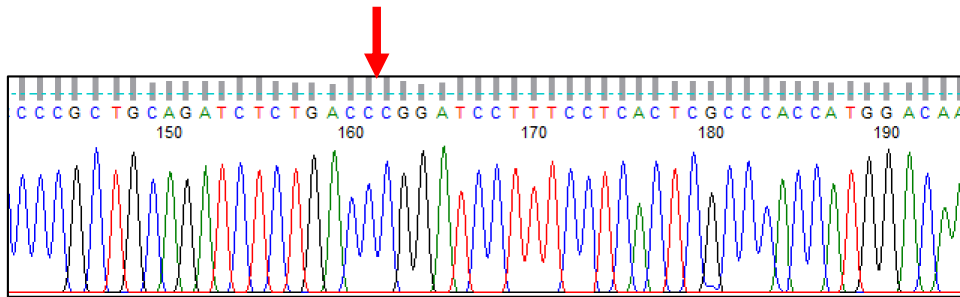


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FIG. S2C

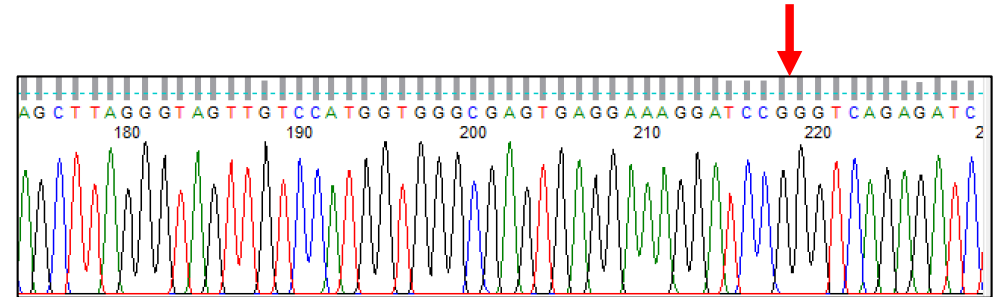
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Sbjct	329	ATCTCTGACC CCGTT CGGATCCTTTCCTCACTCGCCACCATGGACAACACCCTAAGCTG	388
Query	208	GAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGGGGCCCA	267
Sbjct	389	GAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGGGGCCCA	448



Reverse Complement DEL1

Query	134	CAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATCTCTGACC-	192
Sbjct	280	CAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATCTCTGACC CC	339
Query	193	---CGGATCCTTTCCTCACTCGCCACCATGGACAACACCCTAAGCTGGAGGAGATGAT	249
Sbjct	340	GTT CGGATCCTTTCCTCACTCGCCACCATGGACAACACCCTAAGCTGGAGGAGATGAT	399
Query	250	GCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGGGGCCCAAGAGGgcagcgg	309
Sbjct	400	GCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCCGGGGCCCAAGAGGgcagcgg	459

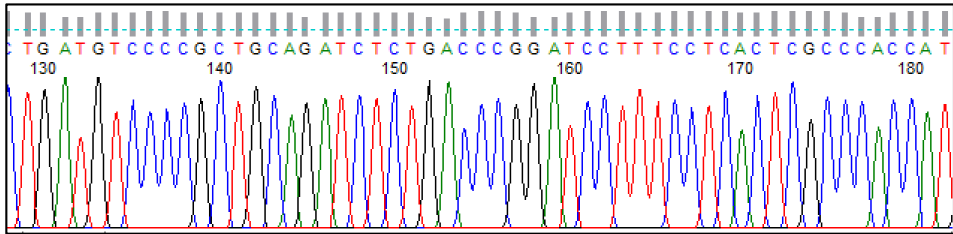


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FIG. S3A

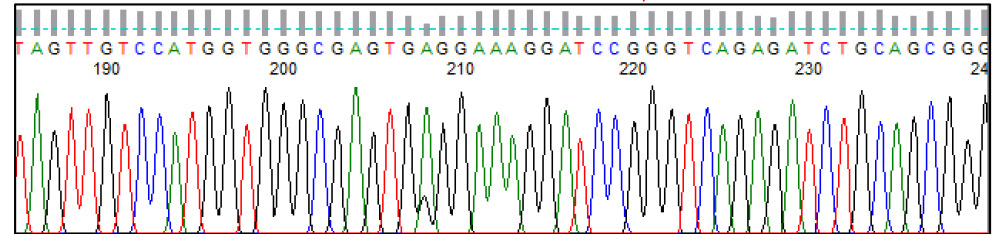
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Query 138 CGCTGCAGATCTCTGACC----GGATCCTTTCTCACTCGCCACCATGGACAAC TACC 193
Sbjct 321 CGCTGCAGATCTCTGACCCTGGTGGATCCTTTCTCACTCGCCACCATGGACAAC TACC 380
Query 194 CTAAGCTGGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCGCCG 253
Sbjct 381 CTAAGCTGGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCGCCG 440
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Reverse Complement DEL2

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Query 132 CAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATCTCTGACC- 190
Sbjct 280 CAGGATGGCCGCGGCCAAGGCCGAGATGCAGCTGATGTCCCCGCTGCAGATCTCTGACC 339
Query 191 ---GGATCCTTTCTCACTCGCCACCATGGACAAC TACCC TAAGCTGGAGGAGATGAT 247
Sbjct 340 GTTGGATCCTTTCTCACTCGCCACCATGGACAAC TACCC TAAGCTGGAGGAGATGAT 399
Query 248 GCTGCTGAGCAACGGGGCTCCCCAGTTCCTCGGCGCCGCGGGGCCCCAGAGGGcagcgg 307
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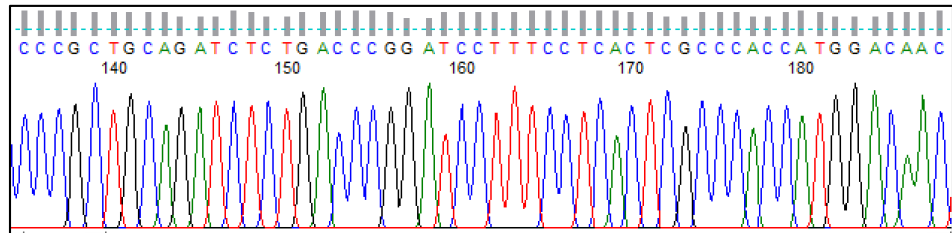
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FIG. S3B

Forward DEL3

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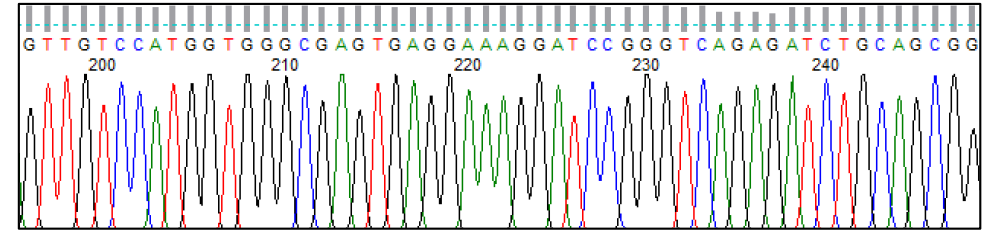
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Query 137  CGCTGCAGATCTCTGACC---CGGATCCTTTTCTCACTCGCCACCATGGACAACCTACC 192
Sbjct 321  CGCTGCAGATCTCTGACCCGGTTCGGATCCTTTTCTCACTCGCCACCATGGACAACCTACC 380
Query 193  CTAAGCTGGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCTCGGCGCCGCCG 252
Sbjct 381  CTAAGCTGGAGGAGATGATGCTGCTGAGCAACGGGGCTCCCCAGTTCTCGGCGCCGCCG 440
  
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Reverse Complement DEL3

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Query 191  ---CGGATCCTTTTCTCACTCGCCACCATGGACAACCTAACCTAAGCTGGAGGAGATGAT 247
Sbjct 340  GTTCGGATCCTTTTCTCACTCGCCACCATGGACAACCTAACCTAAGCTGGAGGAGATGAT 399
Query 248  GCTGCTGAGCAACGGGGCTCCCCAGTTCTCGGCGCCGCCGGGGCCCCAGAGggcagcgg 307
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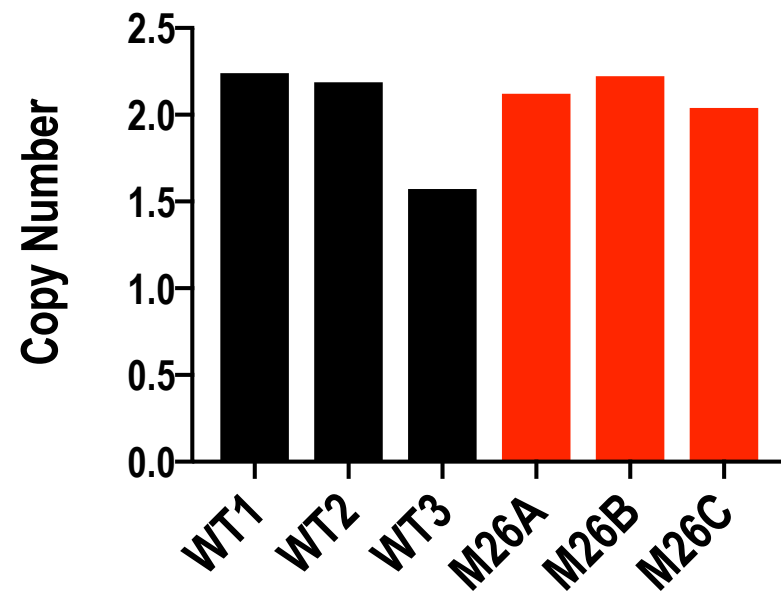


FIG. S4

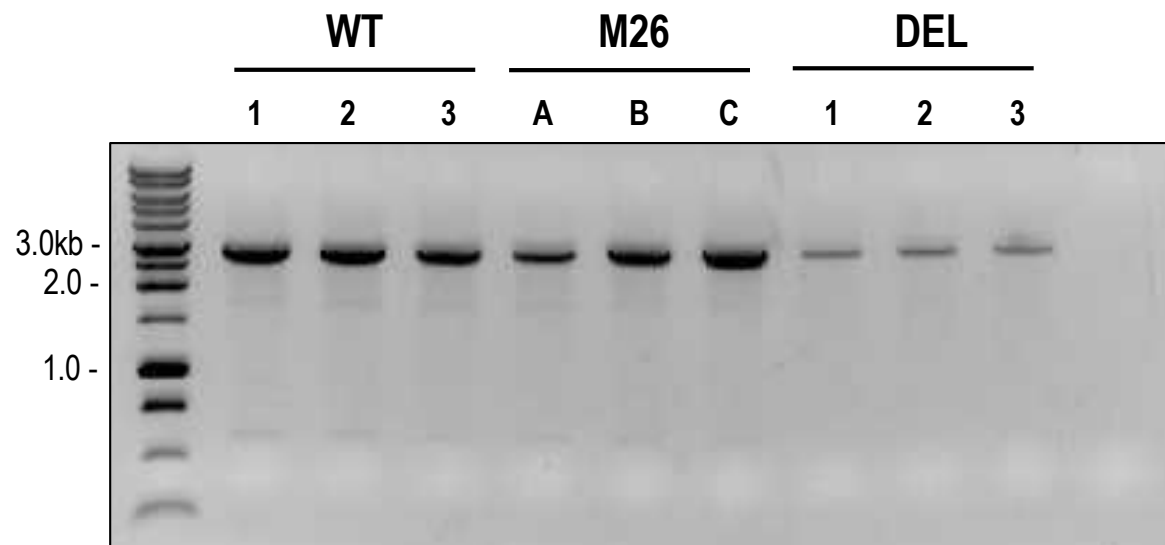


FIG. S5