Supplementary Material

Table S1. Tabular Listing of all used Primers and Oligonucleotides for Homologous Recombination. Homology arms are underlined, melting temperature annotated as T_M.

Incorporation	Direction	Homolgy	Sequence [5′ → 3′]	T_M [°C]
codon-opt S_{full}/S_1	Forward	Bacmid	AAATCAAAGAACTGCTCCTCAGTGGATGTTGCCTTTACTTCTAGGCCTGTGCCACCATGTTCGTCTTCCTGGTC	80.5
codon-opt S_{full}	Reverse	Bacmid	GCGGTTGGAATAATAGCGAGAACAGAGAAATAGCGGCAAAAATAATACCCTTAGGTGTAATGCAGCTTCACGCC	78.8
codon-opt S_1	Reverse	Bacmid	GCGGTTGGAATAATAGCGAGAACAGAGAAATAGCGGCAAAAATAATACCCTTACCGGGCTCTTCTGGGAGAGT	79.5
C-StrepII S_1 opt	Forward	Bacmid	GCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTCACGAATTCGCAGTGCGTCAATCTGACAACTCGG	80.6
C-StrepII S_1 opt	Reverse	Bacmid	GAAATAGCGGCAAAAATAATACCCTTACTTCTCGAACTGGGGGTGGCTCCACCGGGCTCTTCTGGGAGAGT	81.5
rpsL-neo for ΔSV40	Forward	Bacmid	GTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGTCAGATCGGCCTGGTGATGATGGCGGGATCG	83.0
rpsL-neo for ΔSV40	Reverse	Bacmid	GCACTGTGAGGAGACCAGAGGCAGCAGGACCAGGAAGACGAACATGGTGGCTCAGAAGAACTCGTCAAGAAGGCG	84.1
ΔSV40	Reverse	Bacmid	GCACTGTGAGGAGACCAGAGGCAGCAGGACCAGGAAGACGAACATGGTGGCACCGGTGGATCCCGGGCCCG	87.3
rpsL-neo for ΔSP & IL-2- S_{full}/S_1	Forward	Bacmid	GAACTGCTCCTCAGTGGATGTTGCCTTTACTTCTAGGCCTGTGCCACCATGGCCTGGTGATGATGGCGGGATCG	83.8
rpsL-neo for ΔSP &IL-2- S_{full}/S_1	Reverse	Bacmid	CTATTAGTATAAGCAGGTGGCAGCTGAGTCCGAGTTGTCAGATTGACGCACTGTCAGAAGAACTCGTCAAGAAGGCG	81.1
IL-2-S_{full}/S_1	Oligo	Bacmid	GAACTGCTCCTCAGTGGATGTTGCCTTTACTTCTAGGCCTGTGCCACCATGTACAGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTCACGAATTCGCAGTGCGTCAATCTGACAACTCGGACTCAGCTGCCACCTGCTTATACTAATAG	85.4
ΔSP-S_{full}/S_1	Oligo	Bacmid	GAACTGCTCCTCAGTGGATGTTGCCTTTACTTCTAGGCCTGTGCCACCATGCAGTGCGTCAATCTGACAACTCGGACTCAGCTGCCACCTGCTTATACTAATAG	83.9
ΔSV40-$S_β$/ $S_γ$	Forward	Bacmid	CGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACCGGTGCCACCATGTTCGTCTTCCTGGTC	86.3
ΔSV40-$S_β$/ $S_γ$	Reverse	Bacmid	CGGTTGGAATAATAGCGAGAACAGAGAAATAGCGGCAAAAATAATACCCTTAGGAGCCACAGCTACAGC	78.3

Table S2. Tabular Listing of Primers used for mRNA Splicing Analysis by PCR. Melting temperature annotated as T_M.

Primer	Orientation	Sequence	T_M [°C]
#1 5′UTR	Forward	GAGACGCCATCCACGCTGT	61
#2 Linker fwd	Forward	GCAGTCGACGGTACCGCG	62.8
#3 WT S_1 1 fwd	Forward	GGGACCAATGGTACTAAGAGG	59.8
#4 WT S_1 2 fwd	Forward	GATCTCTGCTTTACTAATGTCTATGCA	60.4
#5 WT S_2 fwd	Forward	GTTGAGGCTGAAGTGCAAATTG	58.4
#6 opt S_1 1 fwd	Forward	CGGCACCAATGGCACAAAGC	61.4
#7 opt S_1 2 fwd	Forward	CTGTGCTTTACCAACGTCTAC	57.9
#8 opt S_2 fwd	Forward	CAGAATGTGCTGTACGAGAAC	57.9
#9 3′UTR rev	Reverse	GAGGCCGAGTTTGTCAGAAAGC	62.1

Table S3. Nucleotide Sequence for Elements used in HAdV-C5 Expression Cassette for Spike Protein Expression.

Element	Sequence
CMV Promotor	TCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCA TTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACG TCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATA TGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCC CAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCT ATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTC ACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAA ATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGG
SV40 Intron	TAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGA GTAAGTTTAGTCTTTTTGTCTTTTATTTCAGGTCCCGGATCCGGTGGTGGTGCAAATCAA AGAACTGCTCCTCAGTGGATGTTGCCTTTACTTCTAG
IL-2 Signal Peptide	TACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTCACGAATTCG
StrepII Tag	TGGAGCCACCCCCAGTTCGAGAAG

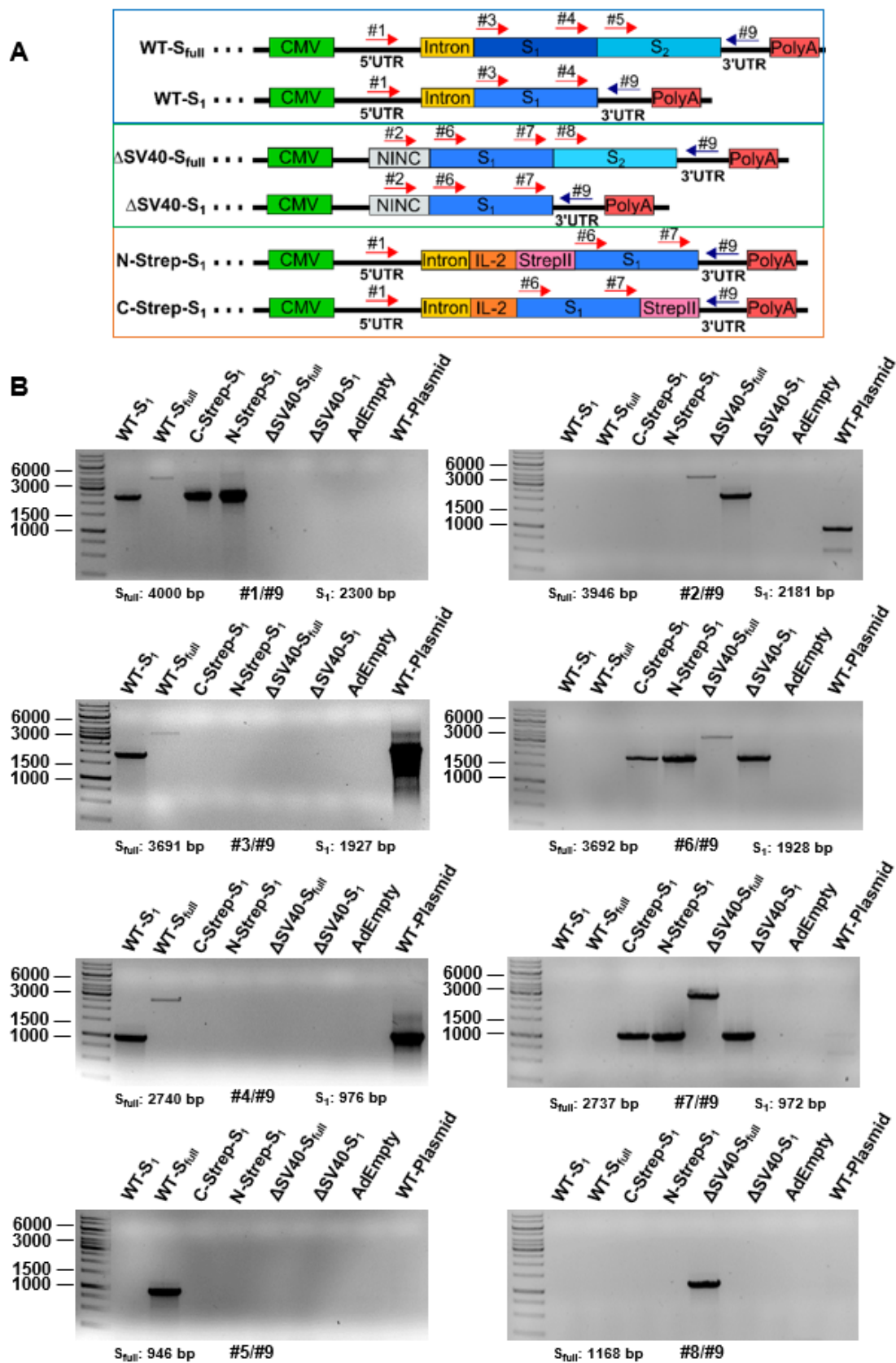**Figure S1. Analysis of mRNA Splicing by PCR. (A)** Schematic representation of primer placement for amplification of in cDNA transcribed mRNA. **(B)** A549 cells were infected with a panel of viral vectors for 48 h and total RNA was isolated. RNA was reversely transcribed into cDNA, then PCR was performed with appropriate primers. PCR products were subsequently separated by agarose gel.
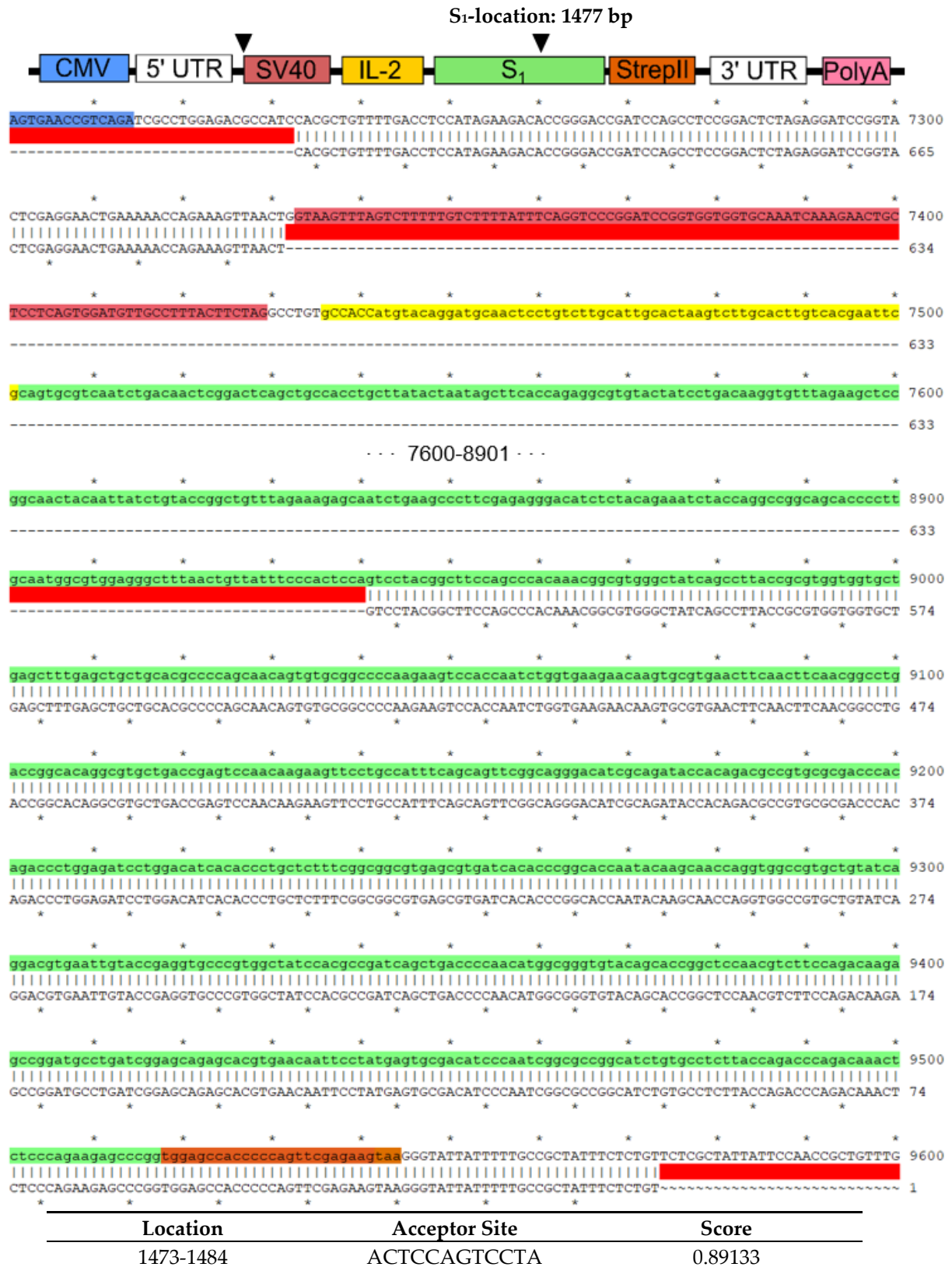
Figure S2. **Results of Splicing Analysis.** Additional band at around 1000 bp from C-Strep-S$_1$ (Figure 5) was excised from agarose gel, DNA was extracted using phenol-chloroform extraction and subsequently analyzed via Sanger sequencing. Sequence alignment was performed using the plasmid editor ApE (https://jorgensen.biology.utah.edu/wayned/ape/). Splice Acceptor site prediction was performed using the SpliceRover Splice Site Predictor tool (http://bioit2.irc.ugent.be/rover/splicerover).