Supplemental material

Table S1 (The list of primers for PCR)

Fig. S1 (The nucleotide sequence alignment of Oka IE62 and IE62 mutants.)

Fig. S2 (The nucleotide sequence alignment of C-terminal region of IE62 and IE62ΔS2.)

Fig. S3 (The interaction of the IE62 SRT with EAP results in the formation of globular structures.)

References, supplemental figures

Table S1. The list of primers for PCR

Primers	Sequence
IE62SRT-F	5’-ccggtaccgctagcgccaccatggatacgccgccgatg-3’
IE62SRT-R2	5’-gggtttaaacgctgagtttgtctgtctcgatggaccaccga-3’
IE62SRT-R1	5’-gggtttaaacgacgactctttggctttttctccactgggct-3’
IE62N-F1	5’-cctctagagctagcgccaccatggatacgccgccgatg-3’
IE62N-R1	5’-gggaattcacgactctttggctttttctccactgggct-3’
IE62N-F2	5’-gggaattccaaccgggatatcgatccatcagcggtccc-3’
IE62N-R2	5’-taacggcggtacccgcccgtccggtggca-3’
YSRT-F3	5’-cctgaattcccggcaccccgggtccggagcatctcc-3’
SRT-F5	5’-aacgaattcgtttcggcattatccgga-3’
SRT-R5	5’-gatcgatatcccggttggatatt-3’
SRT-F6	5’-aacgaattcgtttcgtcttcatcctct-3’
TAD-P4-F	5’-cccgaattcggtaccgccgtttaaacatggcgtcggagaacaagcagcgccccggctccccg-3’
TAD-P4-R	5’-cagagccagctgtcgcggcgagacggcgtc-3’
NYFP-F	5’-tttgaattcgccaccatggtgagcaagggcga-3’
NYFP-R	5’-tttgcggccgcttaggtaccgtcctcgatgttgtg-3’
CYFP-F	5’-tttgaattcgccaggatgggcagcgtgcagctc-3’
CYFP-R	5’-tttgcggccgcttaggtacccttgtacagctcgt-3’
YIE62-F	5’-tttgaattcatggatacgccgccgatgcagcgctct-3’
YIE62-R2	5’-tttcttaagaaaaaaatgctttatttatgttagctcttcgccaacatc-3’
IETAD1-F	5’-tttggtaccatggccagccagcgcagcgacttcgcc-3’
IETAD89-R2	5’-tttgcggccgcttagccggcggccgagggcgaccgc-3’
YVP16-F	5’-tttggtacctcgacggcccccccgaccgatgtcagc-3’
VP16-R2	5’-tttgcggccgcttactacccaccgtactcgtcaatt-3’
YEAP-F	5’-cctggtaccatggcccccgtgaagaagctggtggtgaaggg-3’
YEAP-R	5’-gaagcggccgcttaatcctcgtcttcctcctcttcttcgtcctg-3’
YETIF-F	5’-ggatctagaatgtttgctgccggggaagagaacgat-3’
YETIF-R	5’-ggaaagctttcaaatgtcaaacatctggttgagctcatcgcc-3’
Y62SRT-F	5’-cctggtaccatggatacgccgccgatgcagcgctctaca-3’
Y62SRT-R3	5’-aacttaaggatattttgtttcgtcgacccaccatcatt-3’
YSRT-F	5’-cctggtaccgcaccccgggtccggagcatctccatctca-3’
YSRT-R	5’-gaagcggccgcttgccttcgtcgctgtcgctgtcgtcggagga-3’
YSRT-R3	5’-cctgatatcccggttgctcctcctcgccgccttcgtcgctgtcgct-3’

Figure S1

A

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961
pOka IE62     AGAGCGTTTTTGGCCCTGCCGGGAAGATCCCACGCACCAGACCCGATTGAGGATGACAGC
IE62          AGAGCGTTTTTGGCCCTGCCGGGAAGATCCCACGCACCAGACCCGATTGAGGATGACAGC
IE62ΔS        AGAGCGTTTTTGGCCCTGCCGGGAAGATCCCACGCACCAGACCCGATTGAGGATGACAGC
IE62ΔS-S      AGAGCGTTTTTGGCCCTGCCGGGAAGATCCCACGCACCAGACCCGATTGAGGATGACAGC
              ************************************************************

pOka IE62     CCAGTGGAGAAAAAGCCAAAGAGTCGTGAGTTTGTTTCGTCTTCATCCTCTTCCTCGTCG
IE62          CCAGTGGAGAAAAAGCCAAAGAGTCGTGAGTTTGTTTCGTCTTCATCCTCTTCCTCGTCG
IE62ΔS        CCAGTGGAGAAAAAGCCAAAGAGTCGTGAATTC---------------------------
IE62ΔS-S      CCAGTGGAGAAAAAGCCAAAGAGTCGTGAATTCCCGGCACCCCGGGTCCGGAGCATCTCC
              ***************************

pOka IE62     TGGGGATCGTCATCGGAGGATGAAGACGATGAACCCCGGCGCGTTTCGGTGGGAAGTGAA
IE62          TGGGGATCGTCATCGGAGGATGAAGACGATGAACCCCGGCGCGTTTCGGTGGGAAGTGAA
IE62ΔS        ------------------------------------------------------------
IE62ΔS-S      ATCTCATCGTCGTCCTCGTCCTCGTCCTCGATGGACGAGGACGACCAGGCGGATGGTGCC


pOka IE62     ACTACAGGCAGCAGGTCCGGACGCGAACACGCCCCTTCCCCGTCAAATTCGGATGATTCG
IE62          ACTACAGGCAGCAGGTCCGGACGCGAACACGCCCCTTCCCCGTCAAATTCGGATGATTCG
IE62ΔS        ------------------------------------------------------------
IE62ΔS-S      GGGGCGAGTAGCTCCTCTTCGTCGTCCTCCGACGACAGCGACAGCGACGAAGGCGGCGAG


pOka IE62     GACTCAAATGATGGTGGGTCGACGAAACAAAATATCCAACCGGGATATCGATCCATCAGC
IE62          GACTCAAATGATGGTGGGTCGACGAAACAAAATATCCAACCGGGATATCGATCCATCAGC
IE62ΔS        ------------------------------------CAACCGGGATATCGATCCATCAGC
IE62ΔS-S      GAGGAG------------------------------CAACCGGGATATCGATCCATCAGC
                                                  ************************

pOka IE62     GGTCCCGATCCGAGGATTCGTAAGACCAAACGTCTTGCGGGGGAACCGGGGCGCCAGAGA
IE62          GGTCCCGATCCGAGGATTCGTAAGACCAAACGTCTTGCGGGGGAACCGGGGCGCCAGAGA
IE62ΔS        GGTCCCGATCCGAGGATTCGTAAGACCAAACGTCTTGCGGGGGAACCGGGGCGCCAGAGA
IE62ΔS-S      GGTCCCGATCCGAGGATTCGTAAGACCAAACGTCTTGCGGGGGAACCGGGGCGCCAGAGA
              ************************************************************

                                                                     1,380
pOka IE62     CAGAAATCATTTTCCCTGCCGCGATCCAGAACCCCGATAATTCCCCCGGTGTCGGGGCCG
IE62          CAGAAATCATTTTCCCTGCCGCGATCCAGAACCCCGATAATTCCCCCGGTGTCGGGGCCG
IE62ΔS        CAGAAATCATTTTCCCTGCCGCGATCCAGAACCCCGATAATTCCCCCGGTGTCGGGGCCG
IE62ΔS-S      CAGAAATCATTTTCCCTGCCGCGATCCAGAACCCCGATAATTCCCCCGGTGTCGGGGCCG
              ************************************************************
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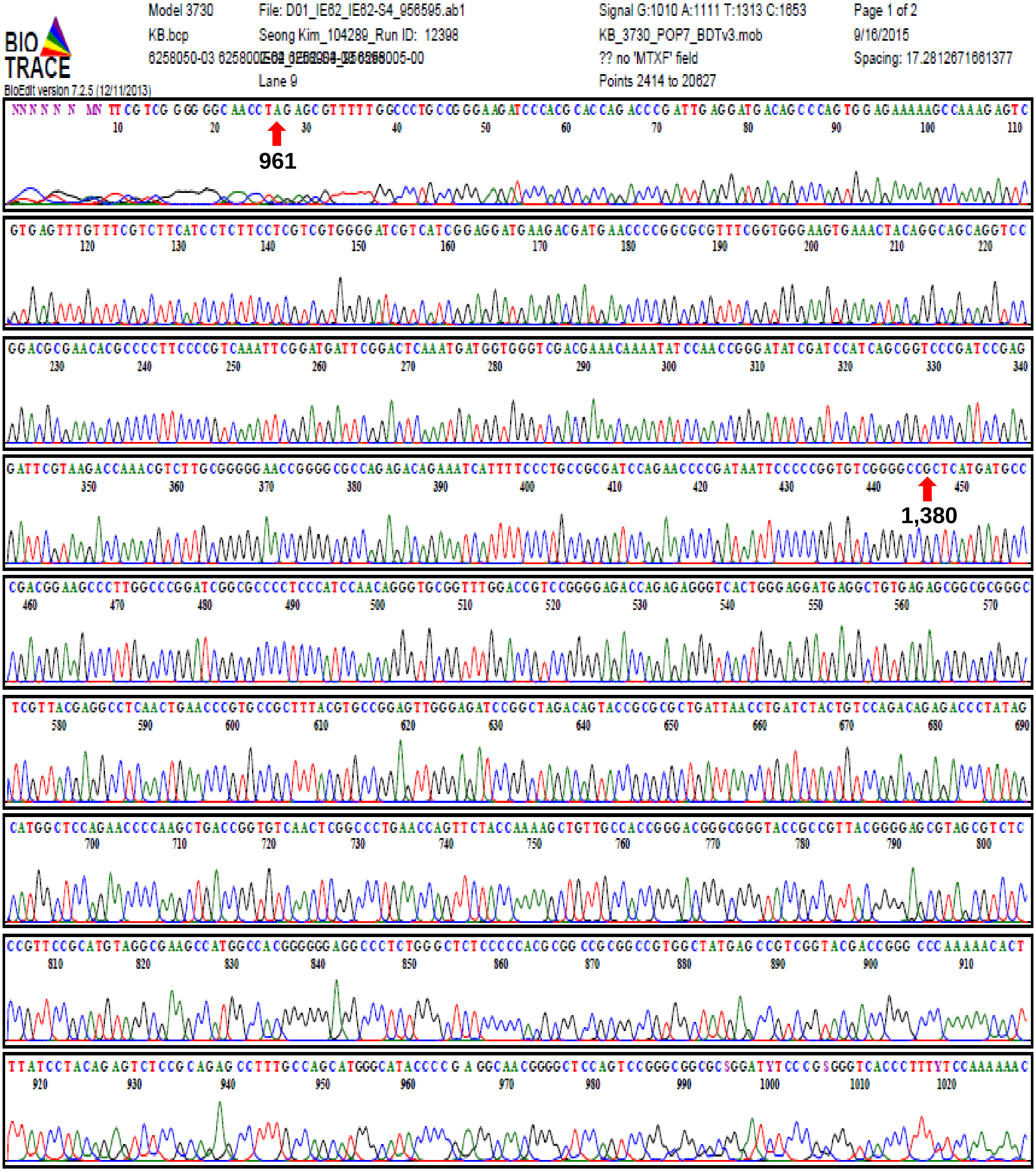
Figure S1. The nucleotide sequence alignment of Oka IE62 and IE62 mutants. (A) The nucleotide sequences of pOka IE62 (Accession: AB097933) (1) are completely the same with the IE62 gene (2) used in this studies. All numbers refer to the number of nucleotide sequence from the first ATG of IE62 gene. Dashes represent gaps introduced for maximal alignment. The serine-rich tracts (SRTs) of VZV IE62 and EHV-1 IEP are in blue and red, respectively. Asterisk (*), conserved nucleotide sequences. DNA sequencing results of intact IE62 (B), IE62ΔS (C), and IE62ΔS-IEP SRT (IE62ΔS-S) genes (D). The start and end nucleotides of the sequence alignment are indicated by arrows.

Figure S1

B

IE62



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BioEdit version 7.2.5 (12/11/2013)

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9/16/2015
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Figure S1

C

IE62ΔS

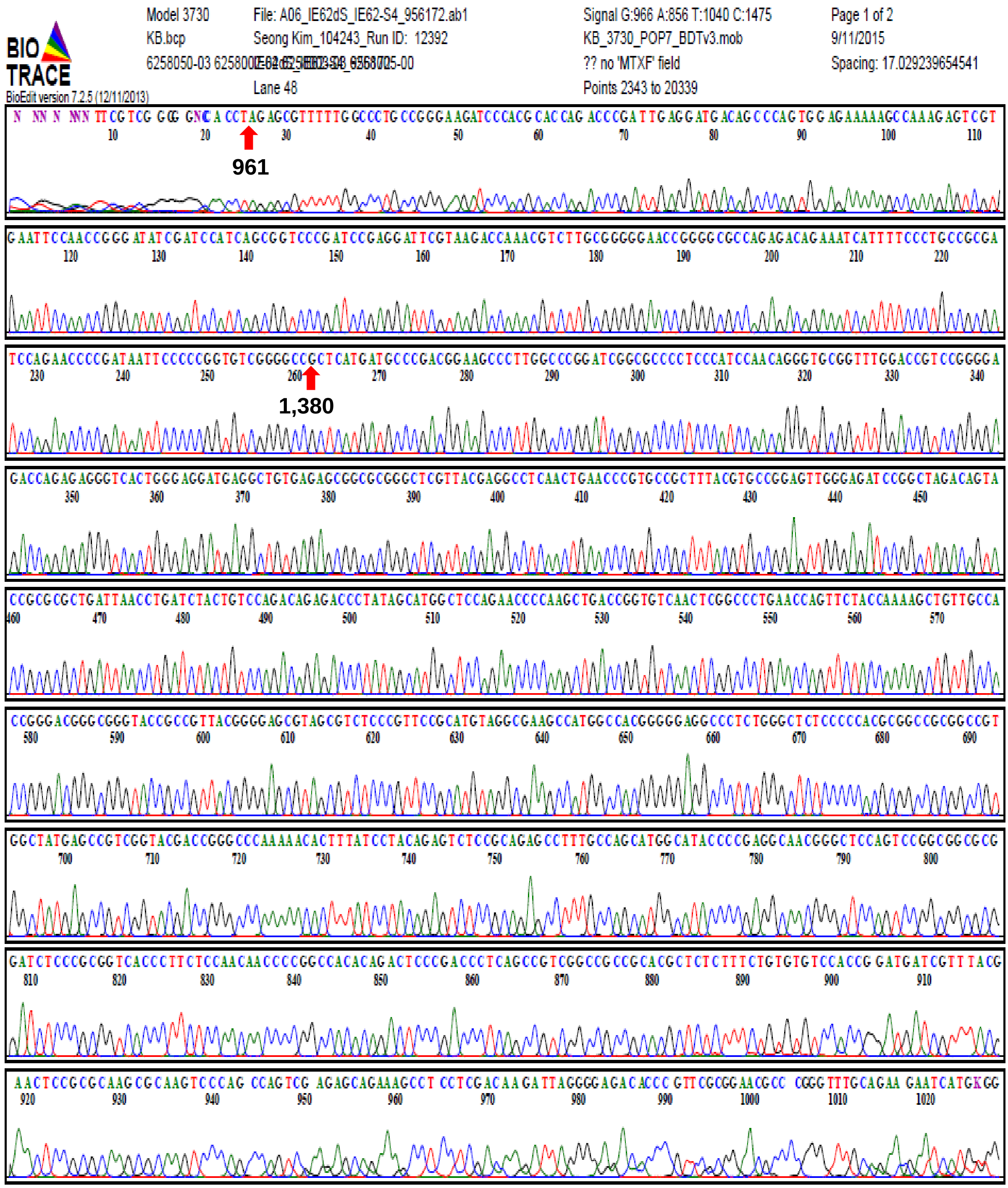
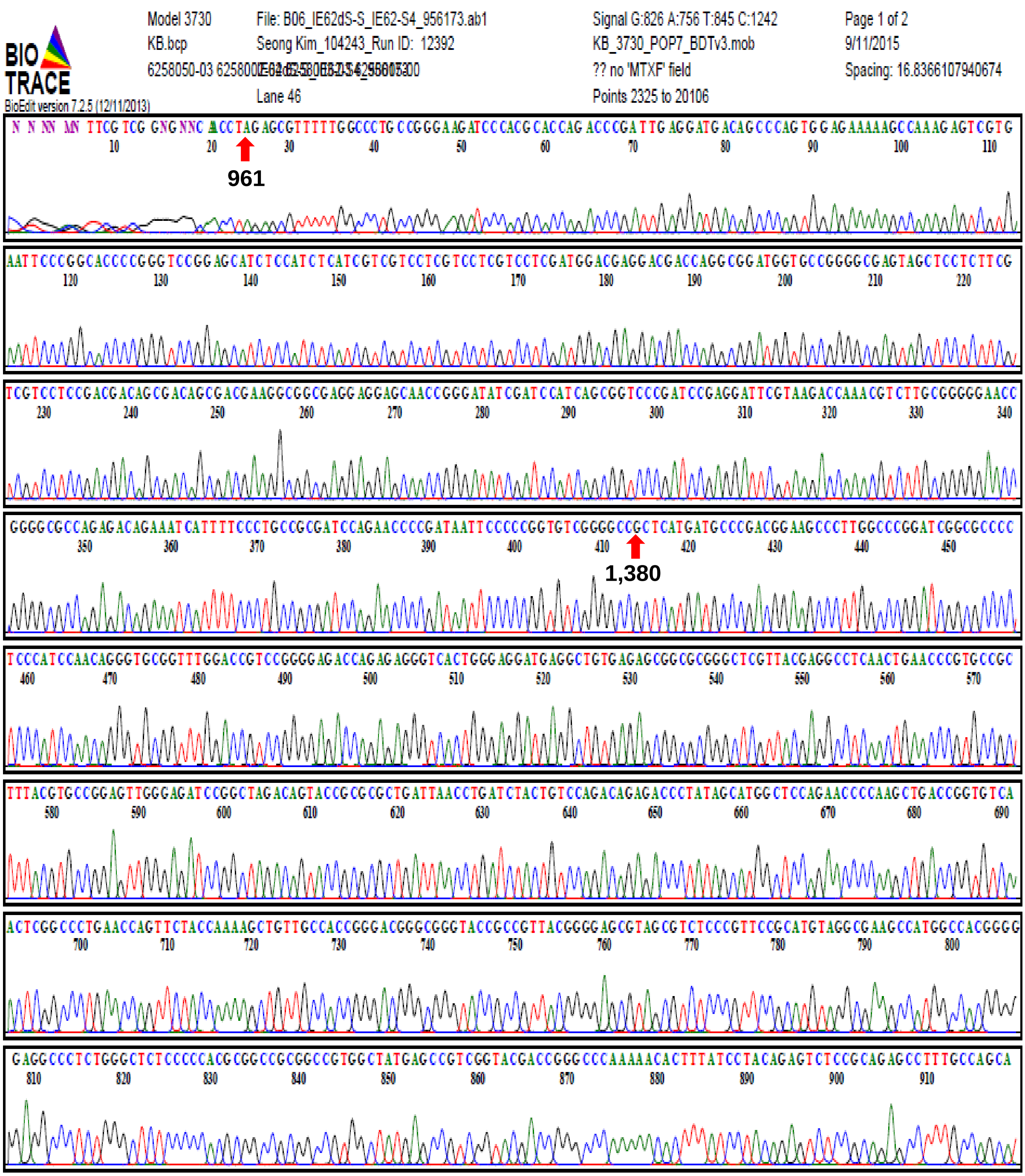


Figure S1

D

IE62ΔS-IEP SRT



BIO TRACE
BioEdit version 7.2.5 (12/11/2013)

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Lane 46

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Points 2325 to 20106

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NNNNMNTTCGTCGGNGNNCACCTAGAGCGTTTTTGGCCCTGCCGGGAAGATCCCACGCACCAGACCCGATTGAGGATGACAGCCCAGTGGAGAAAAAGCCAAAGAGTCGTG

AATTCCCGGCACCCCGGGTCCGGAGCATCTCCATCTCATCGTCGTCCTCGTCCTCGTCCTCGATGGACGAGGACGACCAGGCGGATGGTGCCGGGGCGAGTAGCTCCTCTTCG

TCGTCCTCCGACGACAGCGACAGCGACGAAGGCGGCGAGGAGGAGCAACCGGGATATCGATCCATCAGCGGTCCCGATCCGAGGATTCGTAAGACCAAACGTCTTGCGGGGGAACC

GGGGCGCCAGAGACAGAAATCATTTTCCCTGCCGCGATCCAGAACCCCGATAATTCCCCCGGTGTCGGGGCCGCTCATGATGCCCGACGGAAGCCCTTGGCCCGGATCGGCGCCCC

TCCCATCCAACAGGGTGCGGTTTGGACCGTCCGGGGAGACCAGAGAGGGTCACTGGGAGGATGAGGCTGTGAGAGCGGCGCGGGCTCGTTACGAGGCCTCAACTGAACCCGTGCCGC

TTTACGTGCCGGAGTTGGGAGATCCGGCTAGACAGTACCGCGCGCTGATTAACCTGATCTACTGTCCAGACAGAGACCCTATAGCATGGCTCCAGAACCCCAAGCTGACCGGTGTCA

ACTCGGCCCTGAACCAGTTCTACCAAAAGCTGTTGCCACCGGGACGGGCGGGTACCGCCGTTACGGGGAGCGTAGCGTCTCCCGTTCCGCATGTAGGCGAAGCCATGGCCACGGGG

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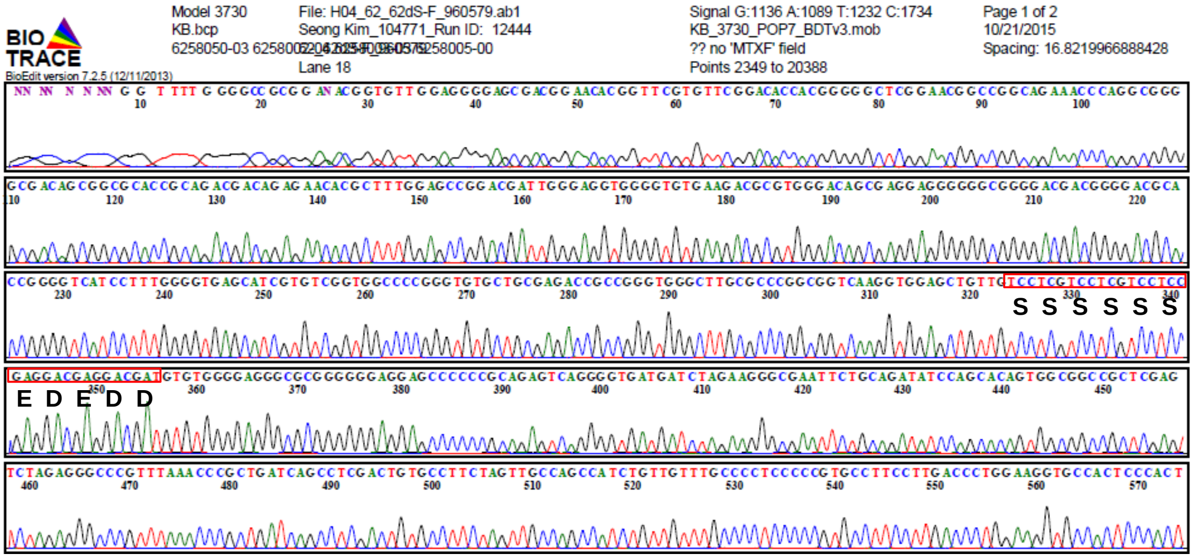
Figure S2

IE62



IE62ΔSS^c

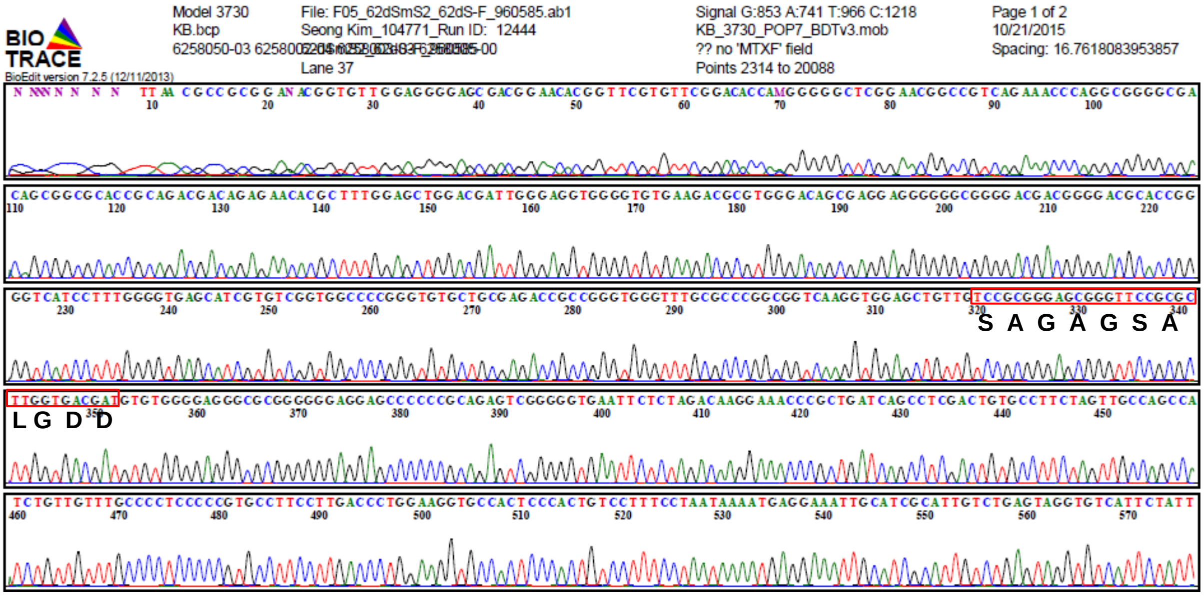


Figure S2. Comparison of C-terminal nucleotide sequences of IE62 and IE62ΔSS^c genes. Eleven amino acid sequences (boxed region: SSSSSSEDEDD) of the SRT^c of IE62 were mutated to SAGAGSALGDD (SRT^c of IE62Δ**SS**^{c}).

Figure S3

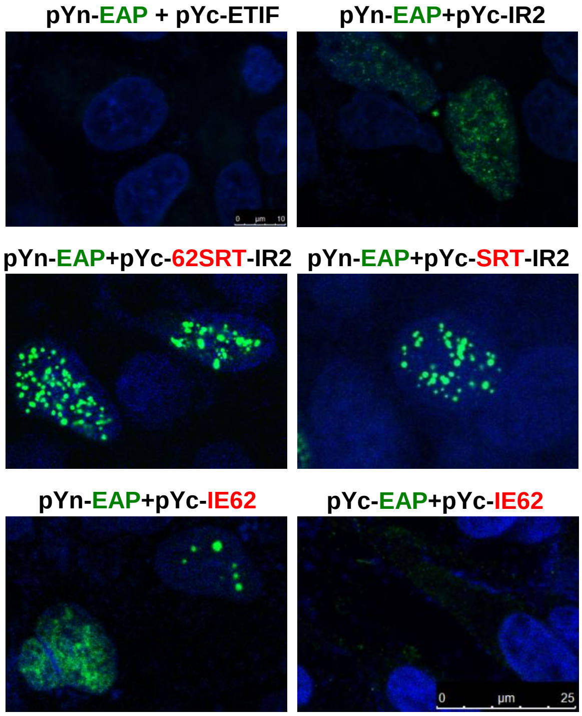


Figure S3. **The interaction of the IE62 SRT with EAP results in the formation of globular structures.** Bimolecular complementation (BiMC) assay to detect the interaction of cellular EAP and SRT-IR2P. Rabbit kidney RK13 cells were cotransfected with the constructs shown above each panel and examined by confocal microscopy at 48h posttransfection.

References, Supplemental Figures

1. **Gomi Y, Sunamachi H, Mori Y, Nagike K, Takahashi M, Yamanishi K.** 2002. Comparison of the complete DNA sequences of the Oka varicella vaccine and its parental virus. J Virol **76**:11447-11459.
2. **Sen N, Sommer M, Che X, White K, Ruyechan WT, Arvin AM.** 2010. Varicella-zoster virus immediate-early protein 62 blocks interferon regulatory factor 3 (IRF3) phosphorylation at key serine residues: a novel mechanism of IRF3 inhibition among herpesviruses. Journal of virology **84:**9240-9253.