cDNA sequences

a) HPV16 E1C

5'-
ATGGCTGATCCTGCAGATTCTAGGTGGCCTTATTTACATAATAGATTGGTGGTGTTTACATTTCCTAATGAGTTTCCAT
TTGACGAAAACGGAAATCCAGTGTATGAGCTTAATGATAAGAACTGGAAATCCTTTTTCTCAAGGACGTGGTCCAGATTA
AGTTTGCACGAGGACGAGGACAAGGAAAACGATGGAGACTCTTTGCCAACGTTTAAATGTGTGTCAGGACAAAATACTA
ACACATTATGA
-3'

b) HPV16 E1C-RNAmut

5'-
TGGTATAACAGGATTAAGAAACCAATACAAAGGCTACATCCTCACTTAGATGAAAGCAAACGCAGAATAATGATTA
CTTTTTCGATACGTGAAACATATCCCATGGTAGTCCAAAGACTTGAAAGTCTATCACCTCTAGGGCCCTTTTCTGGATATAA
ACGCCAAGTTGAATCCGTATTTGGAGGTACGATGGATCAGTCTGGATGAGACGTGCTTCATTTATATCGTAAGTAGGGTCG
ACCAAGAACC
-3'

c) HPV16 E1C-Promut

5'-
TGATAGTAACCTGCAGATTCTAGGTGGCCTTATTTACATAATAGATTGGTGGTGTTTACATTTCCTAATGAGTTT
CCATTTGACGAAAACGGAAATCCAGTGTATGAGCTTAATGATAAGAACTGGAAATCCTTTTTCTCAAGGACGTGGTCCAGAT
TAAGTTTGCACGAGGACGAGGACAAGGAAAACGATGGAGACTCTTTGCCAACGTTTAAATGTGTGTCAGGACAAAATACTA
ACACATTATGA
-3'

Color code: In red, green and blue are the three stop codons

Alignments (using Clustal Omega software of EMBL-EBI)

a) cDNA sequence alignment of HPV16 E1C and HPV16 E1C-RNAmut showing not more than 6 base pair homologies

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HPV16_E1C        --------ATGGCTGATCCTGCAGATTCTAGGTG----GCCTTATTTACATAATAGA---
HPV16_E1C-RNAmut TGGTATAACAGGATTAAGAAACCAATACAAAGGCTACATCCTCACTTAGATGAAAGCAAA
                         **  *      *  ** * *     *** * *** ** * **

HPV16_E1C        ---TTGGTGGTGTTTACATTT------CCTAATGAGTTTCCATTTG-ACGAAAACGGAAA
HPV16_E1C-RNAmut CGCAGAATAATGATTACTTTTTCGATACGTGAAACATATCCCATGGTAGTCCAAAGACTT
                     *  ** **** ***       * * *     * ***  * * *      ** *

HPV16_E1C        TCCAGTGTATGAGCTTAATGATAAGAACTGGAAATCCTTTTTCTCAAGGACGTGGTCCAG
HPV16_E1C-RNAmut GAAAGTCT-------------ATCACCTCTAGGGCCCTTTTCTGGATATAAACGCCAA-
                   *** *              *  * **  *  ** ****** *      * * *

HPV16_E1C        ATTAAGTTTGCACGAGGACGAGGACAAGGAAAACGATGGAGACTCTTTGCCAACGTTTAA
HPV16_E1C-RNAmut GTTGAATCCGTATTTGGAGGT-ACGATGGATCAGTCTGGATGAGACGTGCTTCATTTATA
                  ** * *  * *   *** *      * ***  *   ****     ***    **  *

HPV16_E1C        ATGTGTGTCAGGACAAAATACTAACACATTATGA 249
HPV16_E1C-RNAmut TCGTAAGTAGGGTCGACCAAGAACC--------- 249
                  **  **  ** ** *    *  * *
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b) cDNA sequence alignment of HPV16 E1C and HPV16 E1C-Promut

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HPV16_E1C        ATGGCTGATCCTGCAGATTCTAGGTGGCCTTATTTACATAATAGATTGGTGGTGTTTACA
HPV16_E1C-Promut TGATAGTAACCTGCAGATTCTAGGTGGCCTTATTTACATAATAGATTGGTGGTGTTTACA
                 * *******************************************************

HPV16_E1C        TTTCCTAATGAGTTTCCATTTGACGAAAACGGAAATCCAGTGTATGAGCTTAATGATAAG
HPV16_E1C-Promut TTTCCTAATGAGTTTCCATTTGACGAAAACGGAAATCCAGTGTATGAGCTTAATGATAAG
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              ************************************************************
HPV16_E1C          AACTGGAAATCCTTTTTCTCAAGGACGTGGTCCAGATTAAGTTTGCACGAGGACGAGGAC
HPV16_E1C-Promut   AACTGGAAATCCTTTTTCTCAAGGACGTGGTCCAGATTAAGTTTGCACGAGGACGAGGAC
              ************************************************************

HPV16_E1C          AAGGAAAACGATGGAGACTCTTTGCCAACGTTTAAATGTGTGTCAGGACAAAATACTAAC
HPV16_E1C-Promut   AAGGAAAACGATGGAGACTCTTTGCCAACGTTTAAATGTGTGTCAGGACAAAATACTAAC
              ************************************************************

HPV16_E1C          ACATTATGA
HPV16_E1C-Promut   ACATTATGA
              *********
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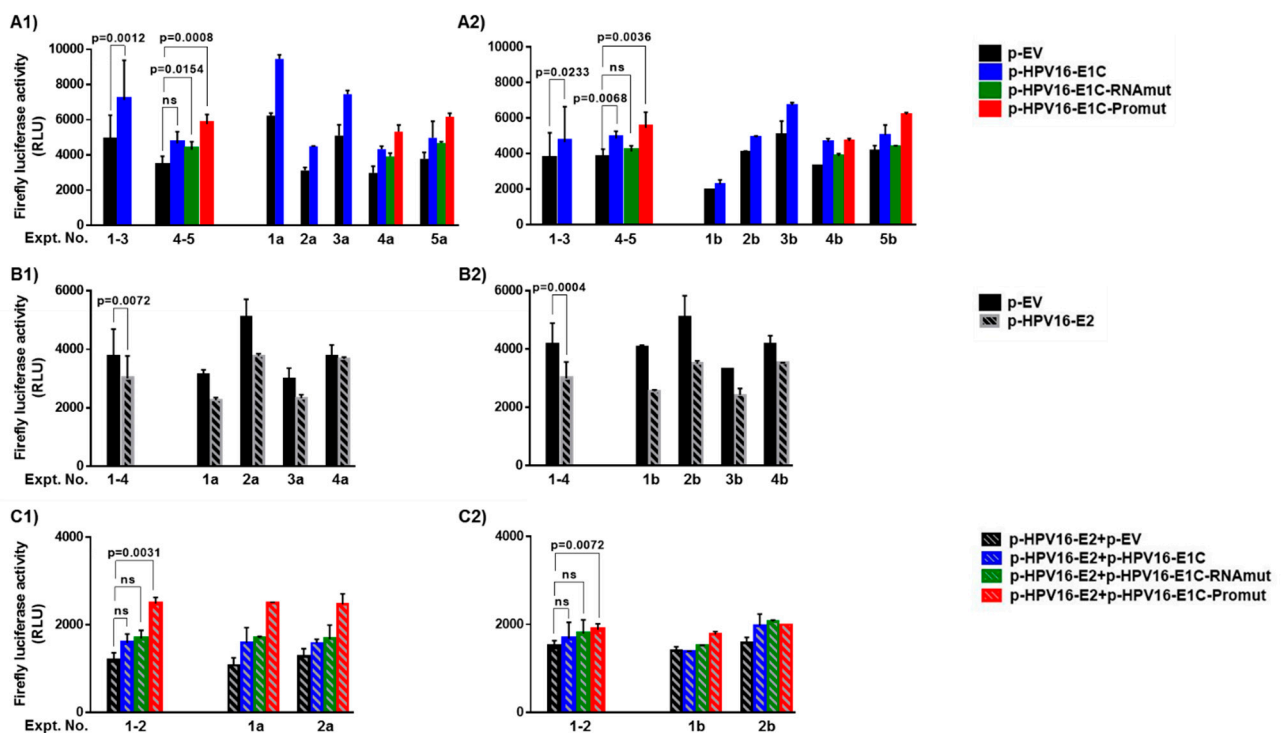**Figure S1:** cDNA sequence alignments of HPV16 E1C, HPV16 E1C-RNAmut and HPV16 E1C-Promut

Figure S2. Effect of wild type and mutant E1C expression plasmids on HPV16 upstream regulatory region (URR) without and with E2 plasmid co-transfection. HEK-293T cells were co-transfected with 400 ng of p-HPV16-URR-FLuc reporter plasmid and A1 and A2) 100 ng of either p-EV, p-HPV16-E1C, p-HPV16-E1C-RNAmut or p-HPV16-E1C-Promut, B1 and B2) 100 ng of p-HPV16-E2, C1 and C2) same plasmids as in (A1 and A2) always together with 100 ng of p-HPV16-E2. Mean and standard deviation of firefly luciferase signals (RLU, Relative Light Units) of all experiments combined and of duplicates from the individual experiments (numbered below x-axis) are shown on the left and right respectively. p values are from paired t test. Letters a and b in experimental numbers indicate experiments without and with additional co-transfection of 2.5 ng of p-TATAbox-RLuc respectively.
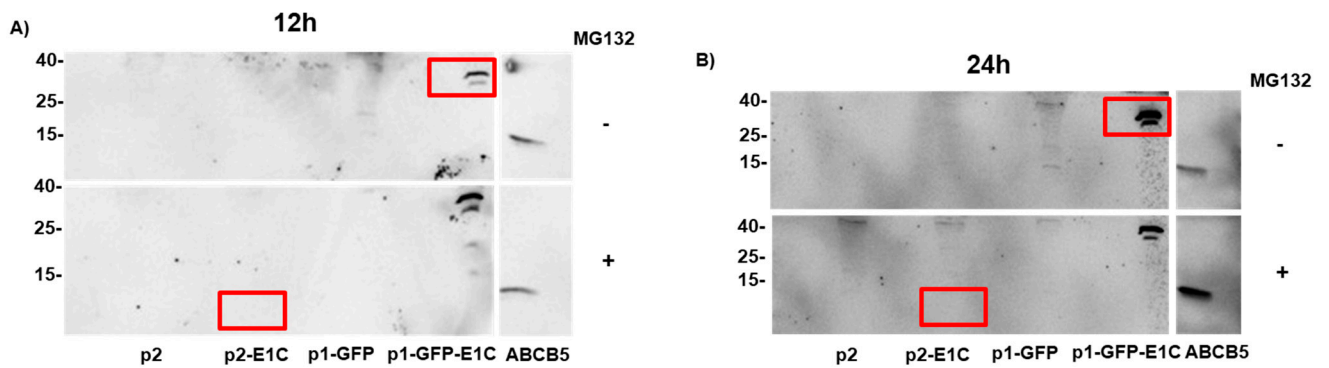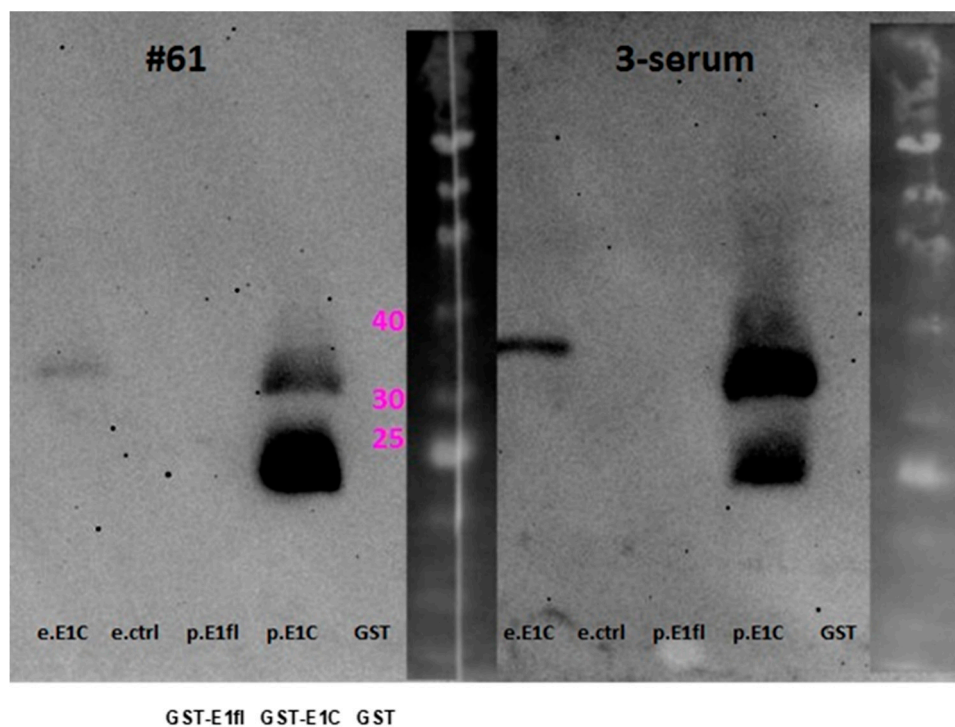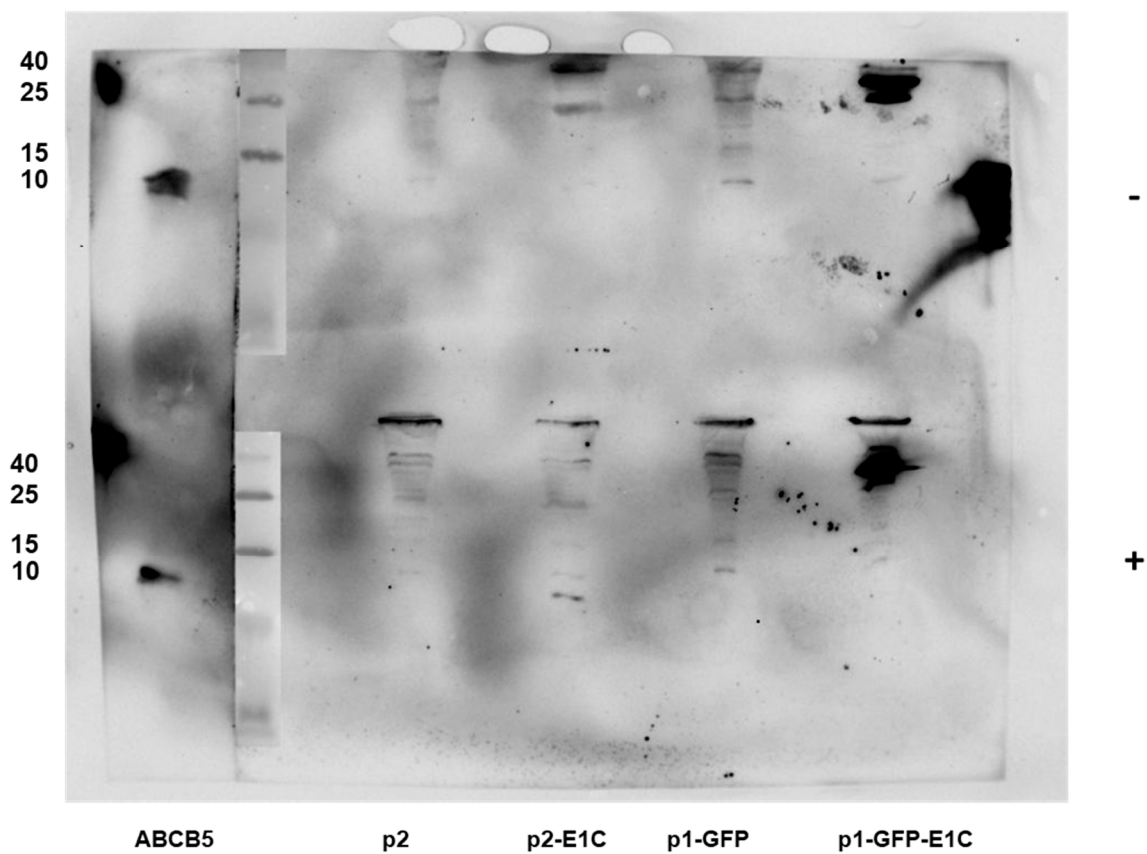
Figure S3. Detection of E1C protein. **A and B**) E1C mAb-stained western blot of lysates (100 µg total protein, prepared 12 h post transfection (**A**) and 24 h post transfection (**B**) from HEK-293T cells transfected with expression plasmids p2, p2-E1C, p1-GFP or p1-GFP-E1C with (bottom) or without (top) proteasome inhibitor MG132 treatment. Size marker (in kDa) is shown on the left and 50 ng of truncated ATP-Binding Cassette sub-family B member 5 (ABCB5)-6XHis protein as positive control for detection of very small proteins on the right. Expected positions of E1C and GFP-E1C proteins are marked by red boxes.

C)

MG132

40
25

15
10

−

40
25

15
10

+

ABCB5   p2   p2-E1C   p1-GFP   p1-GFP-E1C

F)

40

25

15
10

MG132   −   +   −   +
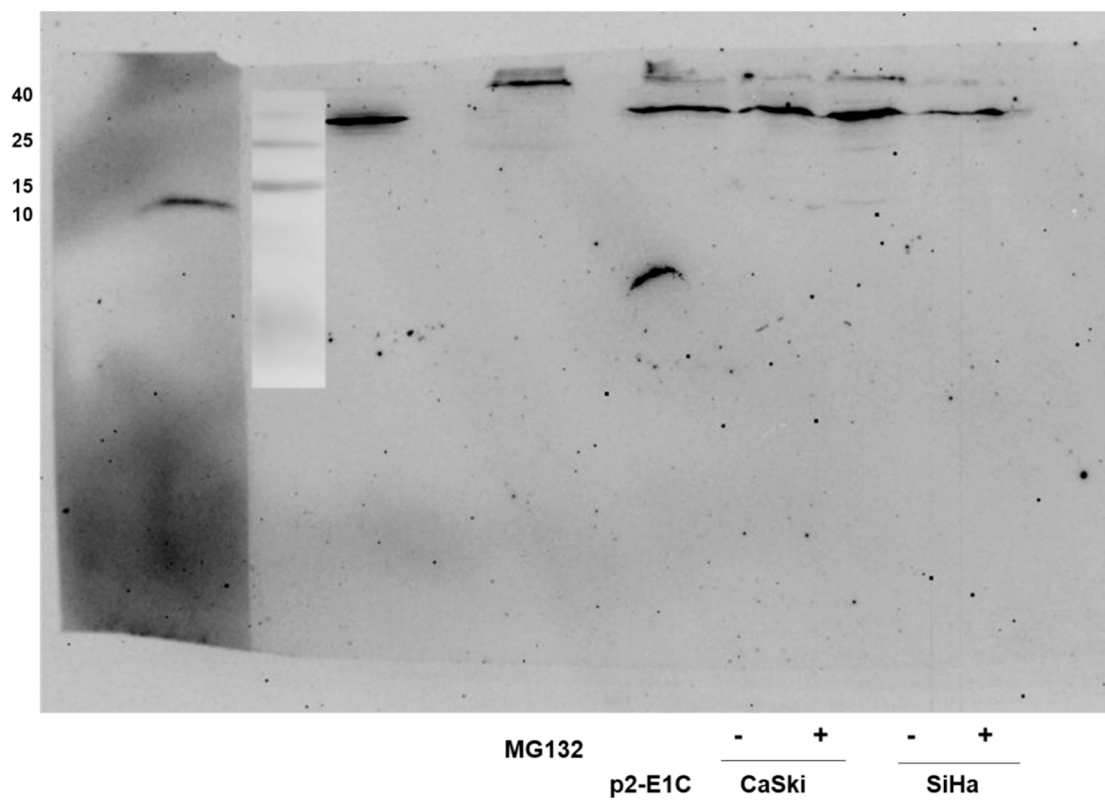
p2-E1C   CaSki   SiHa

Figure 1. Detection of E1C protein. **B**) Reactivity of E1C mAb (hybridoma sub-clone 61/1/2) in western blot with E. coli lysates overexpressing GST, GST-E1C and GST-E1 full-length (fl) fusion proteins. **C**) E1C mAb-stained western blot of lysates (100 µg total protein, prepared 48 h post transfection) from HEK-293T cells transfected with expression plasmids p2, p2-E1C, p1-GFP or p1-GFP-E1C with (bottom) or without (top) proteasome inhibitor MG132 treatment. Size marker (in kDa) is shown on the left and 50 ng of truncated ATP-Binding Cassette sub-family B member 5 (ABCB5)-6XHis protein as positive control for detection of very small proteins on the right. **F**) E1C mAb-stained western blot of lysates (100 µg total protein) from HPV16-positive human cervical cancer cell lines CaSki and SiHa with or without proteasome inhibitor MG132 treatment and, as positive control, from MG132-treated HEK-293T cells transfected with p2-E1C.
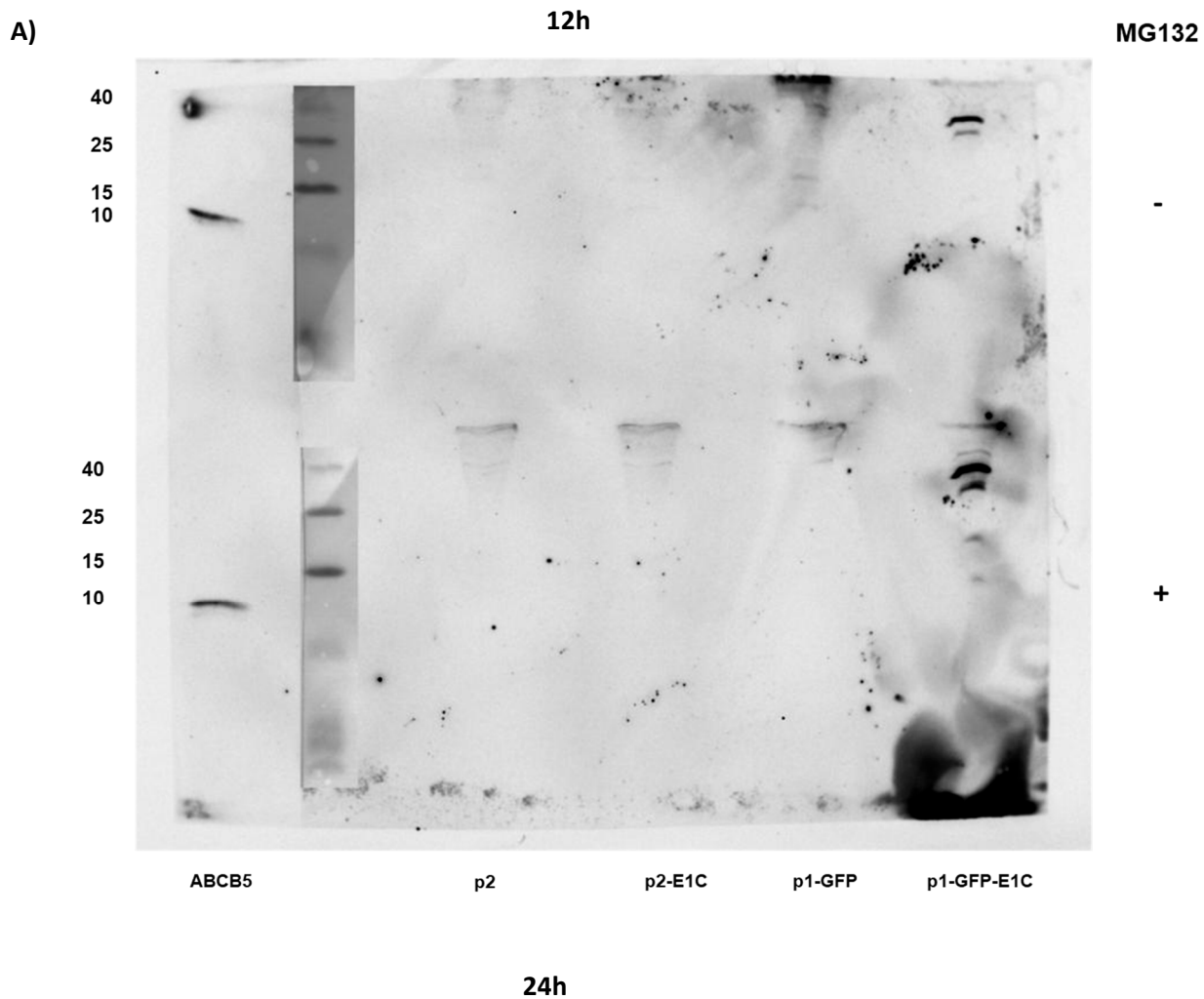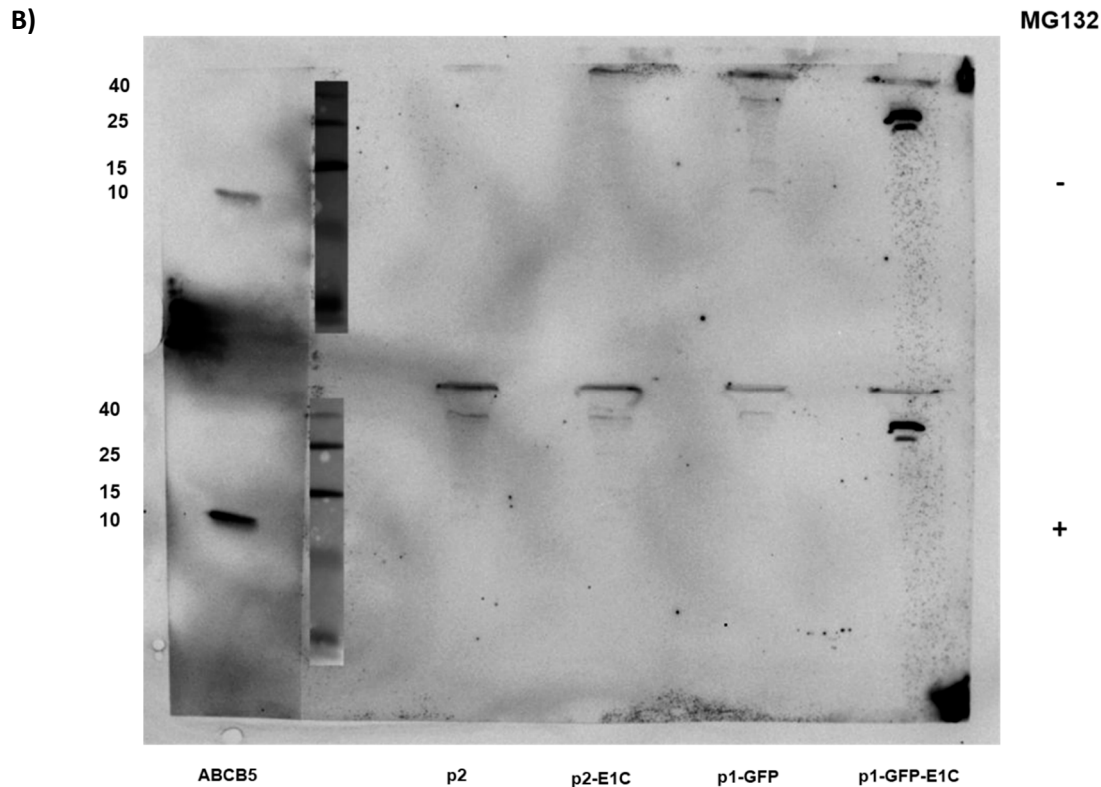
B)

Figure S3. Detection of E1C protein. **A and B**) E1C mAb-stained western blot of lysates (100 µg total protein, prepared 12 h post transfection (**A**) and 24 h post transfection (**B**) from HEK-293T cells transfected with expression plasmids p2, p2-E1C, p1-GFP or p1-GFP-E1C with (bottom) or without (top) proteasome inhibitor MG132 treatment. Size marker (in kDa) is shown on the left and 50 ng of truncated ATP-Binding Cassette sub-family B member 5 (ABCB5)-6XHis protein as positive control for detection of very small proteins on the right.

Figure S4. All the western blots in the manuscript with the molecular marker. Western blots depicted in B), C) and F) of Figure 1. and A) and B) of Figure S3. represent all the western blots in the manuscript together with the molecular marker.