

# Epitome mapping of E6 and E7 proteins from high-risk oncogenic HPV types 16, 18 and 45

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## Research

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# Abstract

**Background** It is crucial to reveal entire epitomes of more target homologous proteins and specificity of each mapped B cell epitope (BCE) within them for the development of high-risk (hr-) human papillomavirus (HPV) type-specific diagnostic reagents.

**Methods** Recombinant E6 and -E7 oncoproteins from HPV16/18/45 were immunized mice to prepare Rabbit antisera. Overlapping 16mer/8mer-peptides for two rounds of antigenic peptide and fine BCE motif mapping were expressed as GST188 fusion proteins. Fine BCEs were delineated by Western blot and sequence alignment.

**Results** In this work, we decoded six epitomes of E6 and E7 oncoproteins from three HPV types 16, 18 and 45 that are the most common hr-HPVs in cervical cancer patients worldwide, in which total 35 fine BCEs (8, 6 and 4 for E6; 7, 6 and 4 for E7) were mapped using rabbit antisera to respective recombinant proteins. The specificity of each mapped BCE among 20 defined or possible hr-HPVs was delineated by sequence alignment based on BCE minimal motif. According to similarities of immune responses to E6/E7 existed among rabbit and humans, 7 human-recognizing (HR) BCE motifs in HR-peptides of HPV16/18-E6 and E7 proteins were delimited by comparing with corresponding rabbit-recognizing BCEs. Also, the unique BCE distribution within three delineated E7 epitomes was confirmed, in which almost mapped BCEs were clustered at the first half of the molecules, suggesting that it may be a common characteristic of hr-HPV E7 proteins.

**Conclusions** The results would form the basis for identifying HR-BCEs and developing serodiagnostic reagents used in HPV-based cervical cancer screening and HPV-positive women managing.

## Background

Cervical cancer is one of the most common cancers in women, which is associated with persistent infection of one or multiple oncogenic human papillomavirus (HPV) types [1–3]. Until now, more than 200 HPV types have been established, of which twelve HPVs are defined as high risk (hr) oncogenic HPVs by the World Health Organization (WHO) [4] and HPV16, 18 and 45 are the most common hr-HPV types responsible for 94% of cervical cancer worldwide [5]. In addition, other six or eight HPV types are considered as possibly hr-oncogenic types as well [6, 7]. Ubiquitous HPV is minute non-enveloped DNA virus, which circular double-stranded genome encodes two capsid proteins (L1 and L2) and six nonstructural proteins including E6 and E7. Much research has been proved that early proteins E6 and E7 are consistently expressed in the virus life cycle, although they are not incorporated into virions [8, 9] and are oncoproteins correlated with progression to the malignant lesions during hr-HPV infection [10–12], since they affect the cell cycle by modulating the activity of p53 tumor suppressor protein and retinoblastoma protein (pRB) [13–16].

Based on possible diagnostic and/or epidemiological implications, it has been an active area for serological diagnosis of patients with HPV infection [17–22] and epitope mapping of HPV proteins by

rabbit/murine polyclonal antibodies (pAbs), murine monoclonal antibodies (mAbs) and human sera against HPV viruses [23–30]. Difficulties with serology for E6 and E7 proteins of HPVs are associated with existence of many defined and possible hr-HPV types with remarkably divergent sequences, the weak immune response to HPV after natural infection compared with that of most other viral infections due to little tissue destruction related with HPV infection [31, 32], and cases of co-infection by multiple hr-HPV types. Thus, it is usually not the best choice to use a recombinant (r-) protein and/or longer synthetic antigenic peptide mapped within them for the serological detection of HPV infections due to possible lack of type-specificity and/or sensitivity, although only such antigens could be used previously [18, 33, 34].

It had been a challenge to delineate all fine BCEs on target viral proteins by rabbit and murine pAbs, including followed by determining HPV type-specific and conservative BCEs of them. In previous studies, using the biosynthetic peptide (BSP) method with simple, economic and reliable merits [35, 36], we have decoded three complete IgG-epitomes of oncogenic E6, E7 and major capsid L1 proteins from hr-HPV58 with rabbit pAbs raised against respective r-proteins. The specificity and conservativeness of each mapped BCE among defined and possible hr-HPVs were determined via homologous sequence alignments based on BCE minimal motif. The similarities of immune responses to L1 protein between rabbit and murine were investigated and 18 cross-reactive BCEs were identified in the decoded L1-epitome with murine pAbs against HPV58 L1-VLPs [30].

In the present work, our main aims are: i) decoding six entire non-conformational IgG-epitomes of E6 and E7 proteins from hr-HPV16/18/45 using rabbit pAbs generated against respective bacteria-expressed r-proteins; ii) determining specificity of each mapped BCE among 20 hr-HPV homologous proteins; iii) delimitating human-recognizing (HR) BCE motifs on HPV16/18-E6 and E7 via sequence alignment between rabbit-recognizing (RR) BCE motifs and respective corresponding HR-antigenic peptides, according to similarities of immune responses between rabbit and humans.

## Materials And Methods

### Immunization of rabbits

Sixteen male New Zealand white rabbits with weight of  $2.0 \pm 0.5$  kg were purchased from SIPPR-BK Lab Animal Co., Ltd. (Shanghai, China) and randomly divided into four groups ( $n = 4$ ). The preparation of rabbit antisera to respective r-E6 and -E7 oncoproteins from HPV16/18/45, of which these two viral transcripts of HPV16 and 45 were expressed in the form of E6-E7 fusion proteins (for short: r-16/45-E6E7 protein), was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals, and was approved by the Ethics Committee of Shanghai Institute of Planned Parenthood Research (2012-08). These rabbits were immunized intramuscularly with 1 mg of respective purified *Escherichia coli* (*E. coli*)-expressed target r-proteins emulsified in 0.5 mL of complete Freund's adjuvant and 0.5 ml of PBS at multiple sites on rabbit's back. Three booster immunizations of 0.5 mg r-protein in incomplete Freund's adjuvant were administered at 3-week intervals. Sera were collected from each rabbit seven days after each vaccination and stored at  $-70^{\circ}\text{C}$ .

## Plasmids, cell strains and antibodies

The thermo-inducible pBV221 (VT1884), IPTG-inducible pET-28a (VT1207) and pRSET-A (VT1924) expression plasmids were purchased from YouBio (Hunan, China), which were used in expressing E6 and E7 proteins of HPV16 (GenBank ID: K02718), 18 (X05015) and 45 (X74479), respectively. The plasmid pXXGST-3 to express short peptides for epitope mapping was constructed as described earlier [36]. *E. coli* strain BL21 (DE3) pLysS competent cells were purchased from CWBIO IT Group (Beijing, China). The mAbs against His6-tag (ab77824) and HPV18-E6 (mAb C1P5 [37], 12-6080) were purchased from ARP Inc. (Belmont, MA, USA) and Abcan Inc. (Shanghai, China), which were used to confirm the expressed target proteins of HPV16/18/45, respectively. The goat anti-rabbit/mouse IgG conjugated to horseradish peroxidase (HRP) (Proteintech Group, USA) was purchased from Shanghai Sangon Co., China.

## Other reagents and materials

DNA ligase, restriction enzymes *Bam*HI and *Sal*I were purchased from Takara Co., Ltd (Dalian, China). QIAprep Spin Miniprep Kit QIAquick Gel Extraction Kit (QIAGEN, Duesseldorf, Germany), prestained molecular weight markers (Shanghai Shisheng Cell Biotechnol, China), 0.2 µm nitrocellulose membrane (Whatman GmbH, Germany), enhanced chemiluminescence (ECL) plus Western blot detection kit, and other general chemicals were obtained from Shanghai Sangon Co., Ltd, China. All genes encoding E6 and E7 proteins of three hr-HPVs and DNA fragments encoding short peptides with different length were synthesized by the SBS Genetech Co., Ltd, Shanghai. DNA sequencing of inserts in each r-clone was performed by Shanghai Generay Biotech Co., Ltd, China.

## Molecular cloning

The molecular cloning of synthesized DNA fragments was done as described previously [36], which is briefly described as follows: i) designing all plus and minus strands of DNA fragments encoding each designed 8/16mer-peptides of the target proteins, having cohesive ends for *Bam*HI and *Sal*I restriction enzymes at their 5' and 3' ends; ii) making 100 µM stock solution of each synthesized DNA fragment by adding appropriate volume of double distilled H<sub>2</sub>O (ddH<sub>2</sub>O) into tubes, respectively; iii) taking respectively 5 µl of the stock solution from two tubes in pairs into a 1.5 ml of new tube, and add 90 µl of ddH<sub>2</sub>O to make 5 µM final concentration of each DNA fragment; iv) heating tubes up to 94°C in an electric heating block, anneal for 5 min, and then allowed them to cool gradually to room temperature (RT); v) performing the ligation reactions using above each annealed DNA fragment and vector of pXXGST-3 digested with *Bam*HI and *Sal*I enzymes at 16°C overnight; vi) using the above ligation mixture in each tube to transform the *E. coli* BL21 (DE3) competent cells, and growing overnight each clone on solid Luria Broth (LB)-ampicillin (amp) plates at 37°C; vii) growing overnight each clone from LB-amp plates in 3 ml of liquid LB-amp medium at 30°C, and repeating once after dilution 1:50 in fresh LB-amp medium to an OD<sub>600</sub> of 0.6-0.7. The clones were induced at 42°C for 4-5 h; viii) harvesting each cell pellets by centrifugation, and then running sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) to screen r-clones according to the specific band of 8/16mer-peptide fusion protein shown on the

gel; ix) conducting DNA sequencing of all synthesized DNA fragments inserted into plasmid pXXGST-3 from r-clones verified by SDS-PAGE analysis.

### **Expression and purification of HPV16/18/45-E6 and -E7 proteins**

The pET-28a, pBV221 and pRSET-A plasmids were used to express respective E6 and E7 proteins from HPV16/18/45 in prokaryotic system. Briefly, various steps involved in their expression were as follows: i) the synthesized and sequenced DNA fragments with *Bam*HI and TAA-*Sa*I cohesive end on their 5' and 3' ends encoding HPV16/45-E6E7 fusion proteins were inserted into the polyclonal region of pET-28a plasmid, as well as the DNA fragments encoding HPV18-E6 and -E7 proteins were inserted into the *Eco*RI and *Sa*I cloning sites of pBV221 as well as the *Bam*HI and *Eco*RI sites of pRSET-A, respectively; ii) the resultant r-plasmid expressing respective r-proteins was transformed into the BL21(DE3) pLysS *E. coli* strain; iii) several r-clones were picked-up from LB plate and first grown in 3 mL of LB containing 100  $\mu\text{g mL}^{-1}$  ampicillin at 30°C with continuous shaking at 200 rpm overnight. Next day, 60  $\mu\text{L}$  of cell suspension was inoculated in 3 mL of fresh LB and grown until the cell density reached 0.6–0.8 at OD<sub>600</sub>, and then further grown for 4 h at 42°C to induce expression of r-protein; iv) the whole bacterial pellets harvested from induced clones were first used to run SDS-PAGE for screening positive r-clones: the uninduced cell total protein was used as negative control; and v) the expression of respective r-proteins was further confirmed by utilizing the mAb against His6-tag and mAb C1P5.

The respective expressed r-proteins were harvested using the method of PAGE-preparation [38]. Briefly, the steps involved: i) cell pellet (2 g) obtained from induced culture of the respective clone was suspended in 5 mL of sonication buffer followed by sonication; ii) the inclusion bodies were collected by centrifugation; iii) the purified inclusion bodies were solubilized in 25 mL of Tris-HCl buffer supplemented with 8 M urea and 70 mM  $\beta$ -mercaptoethanol; iv) the supernatant containing r-protein was finally collected after centrifugation; v) the supernatant was subjected to reversed electrophoresis by rearranging the gel-carrying plate when the bromophenol blue band reached 1-1.5 cm from the bottom of the gel, and 8 fractions of 3-4 mL each were collected after the bromophenol blue had run out of the gel into a collecting trough between the gel and a dialysis membrane used to isolate the upper tank buffer; vi) from each collected fraction, 0.2 mL was taken and the r-protein was precipitated by sequentially adding trichloroacetic acid and acetone; vii) the sample was dissolved in 20  $\mu\text{L}$  4 × sample loading buffer, analyzed and quantified (together with 2  $\mu\text{g}$  BSA standard) by SDS-PAGE; viii) finally, the identified fractions were pooled and the precipitated r-proteins were used as immunogens.

### **Biosynthesis of 8/16mer-peptides**

The designed overlapping 16mer/8mer-peptides for two rounds of antigenic peptide and fine BCE motif mapping of E6 and E7, which all had an overlap of 8 and 7 aa residues respectively, were expressed as GST188 fusion proteins as described earlier [36]. Briefly, the synthesized annealed DNA fragments encoding each 8/16mer-peptide, incorporating *Bam*HI and TAA-*Sa*I cohesive end on their 5' and 3' ends, were inserted into the polyclonal region downstream of GST188 gene in pXXGST-3 plasmid; BL21 (DE3)

pLysS *E. coli* cells were transformed with r-plasmids; respective two or three clones grown on the LB-amp plates were picked-up to conduct thermo-inducible expression and the harvested cell pellet was first used to run 15% SDS-PAGE to determine respective r-clones according to the specifically expressed short peptide fusion proteins with about 23 and 24 kDa, since there is no any visible specific band in the weak antigenic area of proteins from non-recombinant clone. Finally, the r-clones expressing 8/16mer-peptides as fusion protein were confirmed by DNA sequencing of inserted gene fragments. All r-clones contained each fusion peptide were stored at -20°C.

## **ELISA**

Serum antibody titers were determined by ELISA using respective r-protein as the antigen. ELISA plates (96-well; Sigma-Aldrich Inc., USA) were coated with 400 ng of r-E6, -E7 or -E6E7 protein/well. After overnight incubation at 4 °C, excess liquid was removed and non-specific sites were blocked by the addition of 200 µL of 3% BSA in PBS (pH 7.4) for 1 h at RT. Following blocking, plates were washed three times in PBST (PBS supplemented with 0.05% Tween-20), and then serially diluted rabbit antisera in PBS were added and incubated for 2 h at RT. After washing three times with PBST, goat anti-rabbit IgG conjugated to HRP at an optimized dilution of 1:1000 in PBS was added to each well and incubated for 1 h at RT and washed as described above. Plates were incubated with 0.4 mg/mL orthophenylenediamine in 50 mM citrate phosphate buffer, pH 5.2 with 0.015% (v/v) H<sub>2</sub>O<sub>2</sub> as the substrate to develop color. The reaction was stopped with 50 µL of 4N H<sub>2</sub>SO<sub>4</sub> and the absorbance was read at 490 nm with 630 nm as reference filter using an ELX 800 Universal Micorplate Reader (Bio-TEK Instruments, Inc., USA).

## **SDS-PAGE and Western blotting**

Cell pellets obtained from 3 mL culture of expressed r-proteins and peptide fusion proteins were boiled in 400 µL of 1 × sample loading buffer (50 mM Tris-HCl, 2% SDS, 0.1% bromophenol blue, 10% glycerol and 100 mM β-mercaptoethanol) for 5 min and proteins were separated by 15% SDS-PAGE under reducing conditions. Two gels running same samples were either stained with Coomassie brilliant blue G-250 for analyzing the bands of fusion proteins or performed for Western blotting by electrotransferring the proteins onto 0.2 µm nitrocellulose membrane. The complete transfer of proteins was ensured by staining the nitrocellulose membrane with 0.1% (w/v) Ponceau S. Nitrocellulose membrane was subsequently processed for Western blotting using rabbit pAbs against r-E6, -E7 or -E6E7 fusion protein (1:300 dilution in PBS containing 0.05% Tween 20 and 1% skim milk powder) or preimmune rabbit serum. Specific antigen-antibody reaction on the membrane was visualized by using goat anti-rabbit IgG or goat anti-mouse IgG (only for identification of expressed HPV18-E6) conjugated to HRP at 1:2000-dilution. The blot was developed by using the ECL plus Western blot detection reagents.

## **Sequence alignment of homologous HPVs**

The aligned E6 and E7 sequences of prototypes from hr-HPV16 to hr-HPV82 within Table 1 are available from GenBank, NCBI. Their accession numbers are, K02718 (HPV16), X05015 (HPV18), X74472 (HPV26), J04353 (HPV31), M12732 (HPV33), M74117 (HPV35), M62849 (HPV39), X74479 (HPV45), M62877

(HPV51), X74481 (HPV52), X74482 (HPV53), EF177181 (HPV56), D90400 (HPV58), X77858 (HPV59), U31794 (HPV66), D21208 (HPV67), DQ080079 (HPV68a), X94165 (HPV73) and AB027021 (HPV82), and low risk (lr) HPV accession numbers are: AF092932 (HPV6), M14119 (HPV11), X74478 (HPV40), M73236 (HPV42), AJ620205 (HPV43) and U31788 (HPV44).

## Results

### Expression, purification and immunization of E6 and E7 proteins

To obtain rabbit sera against respective immunogens for decoding IgG-epitomes of E6 and E7 proteins from HPV16/18/45, expression of all target proteins was carried out employing *E. coli* expression system. Since E6 and E7 proteins of HPV16 and 45 failed to be solely expressed in *E. coli* with many various prokaryotic expression plasmids, they were finally expressed in the form of fusion protein in *E. coli* (Fig. 1A and 1C), respectively, using the synthesized fusion genes encoding E6E7 proteins of HPV16 and 45, which were inserted into pET28a plasmid. The expression of HPV18-E6 and -E7 proteins was achieved using pBV221 and pRSET-A plasmids, respectively (Fig. 1B). All r-E6E7 fusion proteins of HPV16/45, r-E6 and -E7 proteins of HPV18 were purified with the PAGE-preparation method, and identified with mAb to His6-tag or mAb C1P5, respectively (Fig. 1A-C). After obtaining four r-proteins with the purity higher than 95%, they were used as immunogens to immunize rabbits, which were emulsified in complete Freund's and incomplete Freund's adjuvants. The collected anti-sera were used to determine the Ab titers by ELISA and the results showed that all r-proteins elicited high levels of antigen-specific Ab titers (Supplementary Fig. 1).

### E6 and E7 epitomes of HPV16

HPV16 is the first to be found that it is associated with invasive cervical cancer [39, 40] and the most prevalent HPV type in the world. HPV E6 and E7 oncoproteins can regulate the cell cycle through reacting with p53 and pRB to contribute the progression of invasive cervical cancer [9-12]. However, only limited information were available about the humoral immune responses to E6 and E7 proteins, in particular linear BCEs, although for them there were several studies on BCE mapping with sera from cervical cancer patients with HPV16-positive, murine pAbs and mAbs [24-27]. Thus, it is still necessary to delineate all BCEs and type-specific BCEs in HPV16-E6 and E7.

To reveal non-conformational IgG-epitome of HPV16-E6 with 158 aa, in the first round of antigenic peptide mapping, 19 of 16mer-peptides (a1 to a19, a19 is 14mer) with an overlap of 8 aa covering the full-length sequence of E6 were expressed as r-protein fused with GST188 carrier in *E. coli* using the pXXGST-3 plasmid. For the second round of fine BCE identification, 75 octapeptides (a20 to a94) with an overlap of 7 aa corresponding all reactive 16/14mer-peptides shown in Western blotting were expressed in *E. coli*. In epitome mapping of HPV16-E7 protein, 12 of overlapping 16mer-peptides (b1 to b12, b12 is 10mer) and 56 overlapping 8mer-peptides (b13-b68) corresponding all reactive 16mer-peptides were employed.

As shown in Fig. 2A, Western blotting with the mixed rabbit sera to r-E6E7 revealed 10 antigenic 16/14mer-peptides of E6. Further, using 8 sets of octapeptides corresponding 10 antigenic 16/14mer-peptides, 8 fine BCE motifs (<sup>12</sup>PQER<sup>15</sup>, <sup>35</sup>LECVYCK<sup>41</sup>, <sup>47</sup>REVDYDF<sup>52</sup>, <sup>61</sup>YRDGNPY<sup>67</sup>, <sup>90</sup>LYGTTLEQ<sup>97</sup>, <sup>109</sup>RCINC<sup>113</sup>, <sup>131</sup>RFHNIRG<sup>137</sup> and <sup>155</sup>ETQL<sup>158</sup>) were identified according to respective common sequences within continue reactive octapeptides (Fig. 2B and Supplementary Table 1), which were named as 16/E6-1 to 16/E6-8. The 16/E6-8 motif in reactive a89 located at the end of a19 were identified using 5 extra octapeptides (a90–a94), wherein the respective aa residue from N-terminal of a89 was sequentially substituted with alanine (A). Similarly, 7 antigenic 16/10mer-peptides of E7 were identified (Fig. 2C), 7 BCE motifs (<sup>7</sup>TLHEYML<sup>13</sup>, <sup>12</sup>MLDLQ<sup>16</sup>, <sup>21</sup>DLYC<sup>24</sup>, <sup>30</sup>DSSEE<sup>34</sup>, <sup>36</sup>DEIDGP<sup>41</sup>, <sup>42</sup>AGQAEP<sup>47</sup> and <sup>95</sup>SQKP<sup>98</sup>) were precisely mapped employing 5 sets of octapeptides according to common sequences within reactive octapeptides (Fig. 2D and Supplementary Table 2), which were named as 16/E7-1 to 16/E7-7. The 16/E7-7 motif in reactive b63 located at the end of b12 were determined using 5 extra octapeptides (b64–b68), wherein the respective aa residue from N-terminal of b63 was sequentially substituted with A.

The position of 15 delineated BCEs on two predicted three-dimensional (3D) structures of HPV16-E6 and E7 proteins has been shown in Fig. 3A and 3D, wherein 3D structures of E6 and E7 are predicted according to the previously described method [41]. Of all mapped BCEs on these two proteins, 2 BCEs (16/E6-5 and 16/E6-6) on E6 and 1 BCE (16/E7-7) on E7 were located in predicted  $\alpha$ -helical domains, respectively. The linear schematic diagrams of decoded E6 and E7-epitomes in the predicated 3D structures of E6 and E7 showed two obvious features: i) the distribution pattern of eight BCEs on E6 presenting disperse state; and ii) except for a BCE 16/E7-7 at the C-terminal of E7, the rest of 6 BCEs being situated in the first half of the protein, like those (7/8) of the HPV58-E7 epitome [30].

## E6 and E7 epitomes of HPV18

HPV 18 is associated predominantly with adenocarcinomas and adenosquamous carcinomas compared with squamous cell carcinomas [42, 43] and is the second most common type in invasive cervical cancer worldwide and in China [5, 42]. It is essential to reveal all BCEs on HPV18-E6 and E7, in particular the type-specific BCEs, for developing specifically diagnostic reagent of HPV18. Although there have been studies on epitope mapping of HPV18-E6 and E7 using different methods including phage display library and/or peptide pin screening and sera from cervical cancer patients with HPV18-positive, rabbit pAbs and murine mAbs [27-29], the so-called BCEs revealed are longer antigenic peptides, and information about their fine and type-specific BCEs is still lacking.

For epitome mapping of HPV18-E6, 19 of overlapping 16mer-peptides (c1 to c19, c19 is 14mer) covering the full-length sequence of HPV18-E6 with 158 aa were used in the first round of BCE mapping. In the second round of fine BCE identification, 7 set of 57 octapeptides (c20 to c76) corresponding 8 mapped antigenic 16/14mer-peptides were used. As shown in Fig. 4A, 8 reactive peptides within c1-c19, and 6 fine BCEs (<sup>6</sup>DPTRR<sup>10</sup>, <sup>76</sup>RELRYH<sup>81</sup>, <sup>86</sup>YGDYTL<sup>91</sup>, <sup>113</sup>NPAEKLRLH<sup>121</sup>, <sup>133</sup>HYRGQ<sup>137</sup> and <sup>154</sup>RETQV<sup>158</sup>) were identified according to their common sequences within consecutively reactive octapeptides (Fig. 4B-4D and Supplementary Table 3), which were named as 18/E6-1 to 18/E6-6. Because of failing to find any

reactive band within a set of overlapping octapeptides (c45-c53) corresponding reactive peptide c15, the nonapeptide BCE of 18/E6-4 was identified by using another group of 10mer-peptides (c54-c61). The 18/E6-6 motif in reactive c71 located at the end of Pc19 was identified using five extra octapeptides (c72-c76), wherein the respective aa residue from N-terminal of c71 was sequentially substituted with A.

In epitome mapping of HPV18-E7, 12 of overlapping 16mer-peptides (d1 to d12, d12 is 17mer) spanning the full-length sequence of HPV18-E7 with 105 aa were used in the first round of BCE mapping, and 6 sets of 63 octapeptides (d13 to d75) were used in the second round of fine BCE identification. Finally, 6 fine BCEs (<sup>10</sup>DIVL<sup>13</sup>, <sup>20</sup>EIPVDLL<sup>26</sup>, <sup>30</sup>QLSDSE<sup>35</sup>, <sup>38</sup>NDEID<sup>42</sup>, <sup>46</sup>HQHL<sup>49</sup>, and <sup>101</sup>CASQQ<sup>105</sup>) were identified by Western blotting with rabbit pAbs to HPV18-E7 according to consecutively reactive bands (Fig. 4F) and their common sequence within respective reactive octapeptides (Supplementary Table 4), which were named as 18/E7-1 to 18/E7-6. Of them, the 18/E7-6 motif in reactive d71 located at the end of d12 was identified with four extra octapeptides (d72-d75), wherein the respective aa residue from N-terminal of d71 was sequentially substituted with A.

The position of 12 mapped BCEs on two predicted 3D structures of HPV18-E6 and E7 proteins has been shown in Fig. 3B and 3E. Of mapped BCEs, except for 2 BCEs (18/E6-1 and 18/E6-6), 4 BCEs on E6 and 1 BCE (18/E7-6) on E7 were shown to be located in respective  $\alpha$ -helical domains. The linear schematic diagram of mapped BCEs on HPV18-E6 showed that most (5/6) of mapped BCEs are clustered at the C-terminal except for 18/E6-1 at the N-terminal (Fig. 3B), which is different with HPV16-E6 (Fig. 3A) but similar with HPV58-E6 [30]. Interestingly, like the epitomes of HPV16/58-E7 proteins, the majority (5/6) of mapped BCEs on HPV18-E7 are clustered at the N-terminal except for 18/E7-6 at the C-terminal (Fig. 3E).

## **E6 and E7 epitomes of HPV45**

The HPV45 has been determined as the third most prevalent hr-HPV type involved in invasive cervical carcinoma, and found in 4-9% of cervical cancers worldwide [3, 5], which is closely related to HPV18 and belongs to HPV species 7 in the genus alpha-papillomavirus [44]. Unlike HPV16 and 18 that have been studied intensively, little is known about the biological properties of HPV45, but it has been reported that HPV45-E7 protein can be expressed in cervical cancer biopsies [45, 46], and is a transforming protein that binds to pRB and induce pRB-degradation and anchorage-independent cell cycle progression [47]. Furthermore, to our knowledge, the study on BCE mapping of HPV45-E6 and E7 proteins has not yet been carried out up to now.

For epitome mapping of HPV45-E6 and E7 proteins, 19 and 12 of overlapping 16mer-peptides (e1 to e19 for E6 and e19 is 14mer; f1 to f12 for E7, f11 and f12 are 17mer) representing full-length sequences of E6 with 158 aa and E7 with 106 aa were used to map antigenic peptides by rabbit pAbs against HPV45-E6E7 r-protein. After identification of 5 and 6 reactive peptides on E6 and E7 respectively (Fig. 5A and 5C), 6 sets of 29 octapeptides (e20-e48) for E6 and 47 octapeptides (f13 to f59) for E7 were used to reveal each fine BCE motif, respectively.

As shown in Fig. 5B, the consecutively reactive bands among e21-e25, e34-e37 and e43-e47 and a single reactive band of e28 in 3 sets of octapeptides were observed, and thus 4 fine BCEs (<sup>6</sup>DPKQ<sup>9</sup>, <sup>9</sup>QRPYKLPD<sup>16</sup>, <sup>80</sup>YYSNS<sup>84</sup> and <sup>113</sup>NPAE<sup>116</sup>) were identified according to each common sequence within reactive octapeptides (Supplementary Table 5), which were named as 45/E6-1 to 45/E6-4. The number of mapped BCEs in HPV45-E6 epitome is less than those (8 and 6) in HPV16 and 18-E6 epitomes, but it is identical with that in HPV58-E6 epitome [30]. For HPV45-E7 epitome mapping, 4 fine BCEs (<sup>18</sup>QNELD<sup>22</sup>, <sup>39</sup>EEEN<sup>42</sup>, <sup>47</sup>HAQL<sup>50</sup> and <sup>50</sup>LPARRAEP<sup>57</sup>) were identified according to respective common sequences within reactive octapeptides in f13-f27, f28-f43 and f44-f59 (Fig. 5D, Supplementary Table 6), which were designed as 45/E7-1 to 45/E7-4.

The position of delineated reactive 8 BCEs on two predicted 3D structures of HPV45-E6 and E7 proteins are shown in Fig. 3C and 3F. Of mapped 8 BCEs on E6 and E7, only one (45/E6-3) was located in a  $\beta$ -sheet domain (Fig. 3C). Their linear schematic diagrams of mapped BCEs in E6 and E7-epitomes has been shown in the predicated 3D structures of E6 and E7. There is no distinct BCEs distribution property for E6 (Fig. 3C), and the epitome of HPV45-E7 showed the same feature of BCE distribution pattern with HPV16/18/58-E7, in which all four mapped BCEs were clustered at the N-terminal (Fig. 3F).

### **Specificity of mapped BCEs among hr-HPV homologous proteins**

Since it has known that early E6 and E7 oncoproteins are consistently expressed in the HPV life cycle [8, 9] and they are associated with cervical cancer during hr-HPV infection [10-12], the goal of most E6/E7 epitope mapping studies mainly was to identify the antibody-reactive peptides using sera from cervical cancer patients with HPV16/18-positive and rabbit/murine pAbs [23, 27, 29]. Obviously, it is imperative to map all type-specific BCEs on E6 and E7 of each hr-HPV among homologous proteins for the development of highly specific and sensitive detection reagents.

Based on sequence alignment method, eight BCEs marked by an asterisk were defined as completely type-specific since the same and similar sequences with an aa mutation were not found in known and possible hr-HPVs, also including known Ir-HPV types such as HPV6, HPV11, HPV40, HPV42, HPV43 and HPV44, which are 16/E7-1, 16/E7-2, 16/E7-7, 18/E6-5, 18/E7-2, 18/E7-6, 45/E7-1 and 45/E7-3 (Table 1). In addition, the other 9 BCEs marked by two asterisks could also be considered to be completely or highly type-specific, which are 16/E6-3, 16/E6-5, 16/E6-8, 18/E6-1, 18/E6-4, 18/E7-3, 18/E7-4, 45/E6-1, and 45/E6-4, as their similar peptides present only in another HPV type, although the cross-reactivity of each similar peptide with respective rabbit pAbs remains to be determined. In short, 8 completely and 9 possible completely type-specific BCEs would lay a foundation for the development of BCE-based diagnostic reagents and/or chips targeting HPV16, 18 and 45 in the future.

### **Delimitation of HR-BCE motifs within known HR-antigenic peptides**

Based on the similarities of Ab responses to HPV18-E6 and E7 between rabbit and human [29], we investigate the cross-reactivity of mapped RR-BCEs with human sera to HPV16/18 by comparing sequences of mapped RR-BCEs with HR-peptides. Specifically, the RR-BCEs recognized by human serum

can be determined according to whether they are present within corresponding HR-peptides identified by sera from patients with HPV16 and 18-positive. The results showed that 9 in 27 mapped RR-BCEs on E6 and E7 of HPV16/18 were determined to be recognized by human sera to HPV16/18 based on their presence in 8 known HR-peptides. Of them, 5 RR-BCEs including 16E6-5, 16E7-1, -2, -3, and -4 are present in 3 HR-peptides (E6:6, E7:1 and E7-DLYCYEQLNDSSEE of HPV16) [23, 27] as well as 4 RR-BCEs including 18E6-1, -2, -3 and 18E7-1 are present in 5 HR-peptides (pepE6/1, pepE6/2, pepE6/4, pepE7/1 and pepE701) [29]. Additionally, there was none RR-BCE of HPV16-E7 in the HR-E7:4 peptide [23], and two HR-BCEs (DEIDGVNH and LPARRA) mapped in the pepE7/1 and pepE701 peptides [29] did not exist in the decoded HPV18-E7 epitome, suggesting the differences in Ab responses to HPV16/18-E7 between both species.

Due to the similarities between 9 mapped RR-BCEs and eight known HR-peptides, we further delimited HR-BCE motifs within HR-peptides based on each RR-BCE motif. As a result, three HR-BCE motifs named h-16/E6-1, h-16/E7-1 and h-16/E7-2 were delimited as LYGTTLE, DLYC and DSSEE, of which the first HR-BCE motif in HR-peptide E6:6 YSKISEYRHYCYSLYGTTLE [23] is less a residue than RR-BCE 16/E6-5 motif, and the latter 2 HR-BCE motifs located at both ends of HR-peptide DLYCYEQLNDSSEE [27] are identical to RR-BCEs 16/E7-3 and 16/E7-4. Additionally, 2 HR-BCEs (TLHEYML and MLDLQ) in the middle of E7:1-HGDTPTTLHEYMLDLQPETTD [23] are identical to BCEs 16E7-1 and 16/E7-2, but it could not be determined whether one or 2 HR-BCEs exist in HR-peptide E7:1 because the potential differences of antibody responses among rabbit and humans, and an overlap of 2 residues between these 2 RR-BCEs.

Taking the same method and based on four HR-peptides of HPV18-E6 and E7 proteins [29], 3 fine HR-BCEs named h-18/E6-1 to -3 were delimited as DPTRR, RELRHY and RETQV motifs based on their common sequences in HR-MARFFDPTRRPYKLPDL (pepE6/1) and RR-BCE 18/E6-1, FYSRIRELRHYSDSVYGD (pepE6/2) and RR-BCE 18/E6-2, as well as NRARQEPLQRRRRETQV (pepE6/4) and RR-BCE 18/E6-6, respectively. Another HR-BCE HQHL was shared in HR-IDGVNHHQHLPARRAEPQR (pepE7/1) and RR-BCE 18/E7-4, although 1 HR-BCE (LPARRA) in this HR-peptide was mapped by human sera against HPV18 virus [29].

## Discussion

Using antigenic peptides to replace intact r-proteins in serological test of HPV infection is a great progress, but an ideal peptide reagent used in viral antibody detection should be a shorter peptide pool based on BCE motif and/or combines as many type-specific BCEs as possible in a r-peptide in order to improve specificity and/or sensitivity of serological diagnosis. Clearly, the key to achieve this goal is that entire IgG-epitomes of several target viral proteins and all type-specific BCEs in them can be revealed. Now, it is achievable since all linear BCEs present in a protein and type-specific BCEs in them has been able to be delineated using rabbit/murine pAbs using the BSP method specific for BCE mapping [35, 36], which has been used in many studies of mapping BCEs on different viral protein using rabbit pAbs and BSPs [30, 48–52]. Our previous study has revealed three IgG-epitomes of E6, E7 and L1 proteins from

HPV58 virus [30]. In this study, we decoded two sets of six IgG-epitomes of HPV16/18/45-E6 and E7 proteins using this BSP method and all type-specific BCEs within them.

The decoded homologous epitomes of E6 or E7 showed obvious differences for the number of mapped RR-BCEs (Fig. 3) and defined type-specific BCEs (Table 1) in respective corresponding proteins, even in two proteins with high aa homology, for example, E6 and E7 proteins from closely related alpha viruses HPV18 and 45 share 81% and 78% aa homology, respectively [53]. These differences, including the irregular or regular distribution characteristics of BCEs in respective homologous epitomes, indicated the necessity to carry out epitomics study on two sets of homologous proteins from HPV16/18/45. HPV18-E6 epitome mapping with rabbit pAbs shows that 6 fine BCEs all are in four longer antigenic peptides mapped in the previous study [29], suggesting the credibility of mapped HPV18-E6 epitome. Moreover, the 18/E6-3 BCE ( $^{86}\text{YGD}\text{TLE}^{91}$ ) of HPV18-E6 was almost identical to the E6-2 BCE ( $^{84}\text{YGD}\text{TLE}^{88}$ ) of HPV58-E6 protein [30], indicating that the rabbit immune system could recognize the same antigenic site in various proteins. Interestingly, rabbits could recognize not only the similar antigenic region ( $^{91}\text{YGT}\text{TLE}^{96}$ ) with one residue mutation in HPV16-E6, but also the mapped 16/E6-5 motif ( $^{90}\text{LYGT}\text{TLEQ}^{97}$ ) with two more residues on its N and C-terminals compared with 18/E6-3 motif.

To our knowledge, the BCE minimal motifs mapped using mAbs and chemically synthesized peptides (CSPs) together with ELISA were 4mer-6mer peptides [24, 28, 29, 54], whereas the BCE motifs mapped using pAbs and BSPs together with Western blotting can be the shortest 3mer-peptides [30, 55] and the longest 9mer-peptide (18/E6-4) mapped in this study, even longer 10mer-peptide [52]. In addition to contributing to reveal complete BCEs on a protein using animal sera and their specificity among homologous proteins, the BCE motif identification has two other implications: i) helping to the design of multi-BCE peptide diagnostic reagents and/or vaccines, which would improve the accuracy and sensitivity of serological diagnosis by combining many specific BCEs as possible, and prevent potential harmful antibody cross-reactivity to other key proteins in human body, that is, it should avoid using the mapped 3mer and 4mer-peptide BCEs if they are used as candidates of preventive vaccine; and ii) conducting to understand the possible regular differences of BCE motifs located the same site that are mapped by various species pAbs and/or mAbs, for instance, the PTRR and ELRHY motifs [29, 36] mapped by mAbs E6-18-1 and C1P5 are less one residue at the N-terminal of them than the DPTRR and RELRHY motifs of 18/E6-1 and 18/E6-2 mapped by rabbit pAbs. Similarly, the delimited HR-BCE h-16E6-1 motif is less one residue at its C-terminal compared with the RR-BCE 18/E6-5.

The rabbit pAbs is the most frequently used probe in epitope mapping because it is relatively easy to prepare and is available in sufficient quantities for use. However, it is difficult to determine whether the BCEs delineated in BCE mapping with rabbit pAbs could be recognized by other species, humans in particular, because only limited information is available by using traditional CSP method together with ELISA test before [29]. Now, with the establishment of BSP methods specific for BCE mapping by Western blotting, researchers have been able to provide relatively systematic information based on BCE level. Three BCEs combined in a r-peptide vaccine of the  $\beta$ -hCG subunit all were recognized by rabbit and murine antisera as well as induced respective antibodies in both species [56], and 13 of 18 BCEs mapped

by rabbit pAbs to HPV58-L1 were reacted with murine pAbs to HPV58 L1-VLPs [30]. All 19 and 9/13 RR-BCEs on nucleoprotein (NP) and hemagglutinin (H) proteins of peste des petits ruminants virus (PPRV) were recognized by sera from goat with PPRV-positive, respectively [48, 49]. All 5 and 6/9 RR-BCEs on NP of Crimean-Congo hemorrhagic fever virus (CCHFV) and Gn protein of Guertu virus (GTV) were reacted with sera from sheep CCHFV/GTV-infected, respectively [50–52]. These information reflected good similarities of antibody responses to the same proteins among different animal species.

Similarly, there was evidence that 3 of 4 RR-peptides on HPV18-E6 can be recognized by human sera to HPV18 in ELISA test [29]. In this study, we provide more information on this through delimitation of 7 HR-BCE motifs in HR-peptides of HPV16/18-E6 and E7 according to RR-BCE motifs. The delimited results are non-experimental but credible and acceptable, because two species can recognize the same antigenic site and the BCE motif recognized by another species pAbs can be delimited. Based on mapped RR-E6/1 17mer-peptide and murine-recognizing (MR) BCE PTRR, the RR-BCE motif in RR-peptides could be delimited as PTRR according to the MR-BCE motif [29], which has been identified as DPTRR in this study and only shows one residue difference between both. Similarly, RR-BCE (RELRHY) mapped in this work has also one residue difference compared with MR-BCE (ELRHY) [36] identified by HPV18-E6 mAb C1P5. The similar result was also found between RR and HR-BCEs such as 16/E6-5 and h-16E6-1. In short, it should be a feasible and reliable method to determine HR-BCE motifs in known HR-peptides according to the mapped RR/MR-BCE motifs.

Mapping of BCE minimal motif is very important, since it is the basis of decoding complete IgG-epitome of a target protein and then revealing specificity or conservativeness of each mapped BCE among homologous proteins efficiently by sequence alignment [30]. The antibody-recognizing BCE motif is one of major features to distinguish different antibodies as well, which obviously conduces to determine whether two or more mAbs with various IgG isotypes are identical in the function of binding target protein. For example, two mAbs E6-18-1 (IgG3) and E6-18-2 (IgG1) derived from HPV18-E6 as well as mAbs 8C6 and 1B12 produced by M2 protein of influenza A virus all can be classified as the same antibodies, since they recognize the same tatrapeptide PTRR or pentapeptide EPTIR, respectively [29, 57]. More importantly, it helps in overcoming reproducibility crisis or disorder present in antibody application that was mentioned a few years ago [58] for knowing BCE motif of each antibody, as currently users can conveniently employ BSP method to verify the obtained antibody quality of different batches or whether it is a target antibody needed if researchers or suppliers can provide each antibody-recognizing BCE motif, but which has not been included in the disorder solution as a required parameter [58, 59]. In short, the meaning of fine BCE mapping should be emphasized in the fields related to BCE mapping and antibody research henceforth.

In conclusion, six complete IgG-epitomes of HPV16/18/45-E6 and E7 proteins were decoded, and 17 of mapped 35 BCEs on E6 and E7 proteins were determined as possible type-specific BCEs among 20 compared HPV types. A nonapeptide BCE on E6 of HPV18 was identified, and the unique BCE distribution characteristics on E7 proteins of HPV16/18/45 were confirmed. Seven HR-BCE motifs on E6 and E7 of HPV16/18 in known HR-peptides were delimited according to the similarity of immune responses

among rabbit and humans. The results will facilitate the delimitation of HR-BCE motifs and the development of multi-BCE peptide diagnostic reagents and/or BCE peptide chips for the HPV type-specific detection, and lead to a better understanding of similarity and difference of immune responses to the same protein among different species and epitope biology.

## Abbreviations

BCE, mapped B cell epitope; BSP, biosynthetic peptide; CSPs, chemically synthesized peptides; HPV, human papillomavirus; hr, high-risk; HR, human-recognizing; mAbs, monoclonal antibodies; MR, murine-recognizing; pAbs, polyclonal antibodies; pRB, retinoblastoma protein; r, recombinant; RR, rabbit-recognizing; WHO, World Health Organization;

## Declarations

### Author contributions

WX Xu, C Xu and J Li conceived and designed the experiments. HP Tang, WB Lian, JM Zhan, YP He, F Xue and L Wang performed the experiments. XW Xu, J Wang, F Zhang and J Li wrote the paper.

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### Availability of data and materials

All the data and materials used in this study are included in the manuscript.

### Ethics approval and consent to participate

Animal experiment was granted by the ethical committee of the Shanghai Institute of Planned Parenthood Research (2012-08). Consent to participate is not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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## Table

**Table 1. Specific analysis of HPV16/18/45 BCEs among known and probable hr-HPVs.**

HPV BCE No.	HPV types and sequence alignment analysis																			
	16	18	31	33	35	39	45	51	52	56	58	59	26	53	66	67	68	70	73	82
16E6-1	-	-	■	-	■	■	-	-	-	●	-	-	-	-	-	-	■	-	-	-
16E6-2	-	-	■	-	■	-	-	-	-	■	-	-	-	-	-	-	-	-	-	-
16E6-3**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16E6-4	-	-	-	-	-	-	-	■	■	-	-	-	-	-	-	■	-	-	-	-
16E6-5**	-	-	-	-	-	-	-	-	-	-	▲	-	-	-	-	-	-	-	-	-
16E6-6	-	-	■	■	■	-	-	-	■	-	■	-	-	-	-	-	-	-	-	-
16E6-7	-	■	■	■	■	-	-	-	■	-	■	■	-	-	-	-	-	-	-	-
16E6-8**	-	-	-	▲	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16E7-1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16E7-2*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16E7-3	-	■	-	●	●	■	■	-	■	■	■	■	-	-	■	●	■	●	■	■
16E7-4	-	■	■	■	●	-	-	-	■	-	■	-	-	-	-	■	-	-	-	■
16E7-5	-	-	-	-	■	■	-	-	-	-	-	-	-	-	-	-	■	-	-	-
16E7-6	-	-	●	-	■	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16E7-7*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18E6-1**	-	-	-	-	-	-	-	-	-	-	-	▲	-	-	-	-	-	-	-	-
18E6-2	-	-	-	-	-	■	■	-	-	-	-	■	-	-	-	-	■	●	-	-
18E6-3	■	-	■	■	■	-	■	■	■	■	●	■	-	-	-	■	-	-	-	■
18E6-4**	-	-	-	-	-	-	-	-	▲	-	-	-	-	-	-	-	-	-	-	-
18E6-5*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18E6-6	-	-	-	-	■	●	●	-	-	-	●	-	-	-	-	-	-	●	-	-
18E7-1	-	-	■	-	■	-	-	-	-	■	-	●	-	-	-	-	-	-	-	●
18E7-2*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18E7-3**	-	-	-	-	-	-	-	-	▲	-	-	-	-	-	-	-	-	-	-	-
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18E7-6*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45E6-1**	-	-	-	-	-	-	-	-	-	-	-	▲	-	-	-	-	-	-	-	-
45E6-2	-	-	-	-	-	-	-	-	-	-	-	●	-	-	-	-	-	-	-	-
45E6-3	-	-	-	-	-	■	-	-	-	-	-	-	-	-	-	-	■	-	-	-
45E6-4**	-	▲	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45E7-1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45E7-2	-	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45E7-3*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
45E7-4	-	●	-	-	-	-	-	-	-	-	-	■	-	-	-	-	-	-	-	-

An asterisk indicates those completely specific BCEs among known and probable hr-HPVs, which have no the same and similar sequences with an aa mutation among homologous proteins. Two asterisks indicate those BCEs that occur only in one HPV and have possible cross-reactivity due to existence of an aa mutation among them, which are shown by ▲ symbol. The ● and ■ symbols indicate those 100%

conserved BCEs and BCEs that have an aa mutation but exist in at least two HPVs, or together with a conserved BCE. “-” means no the same or similar sequences.

## Figures

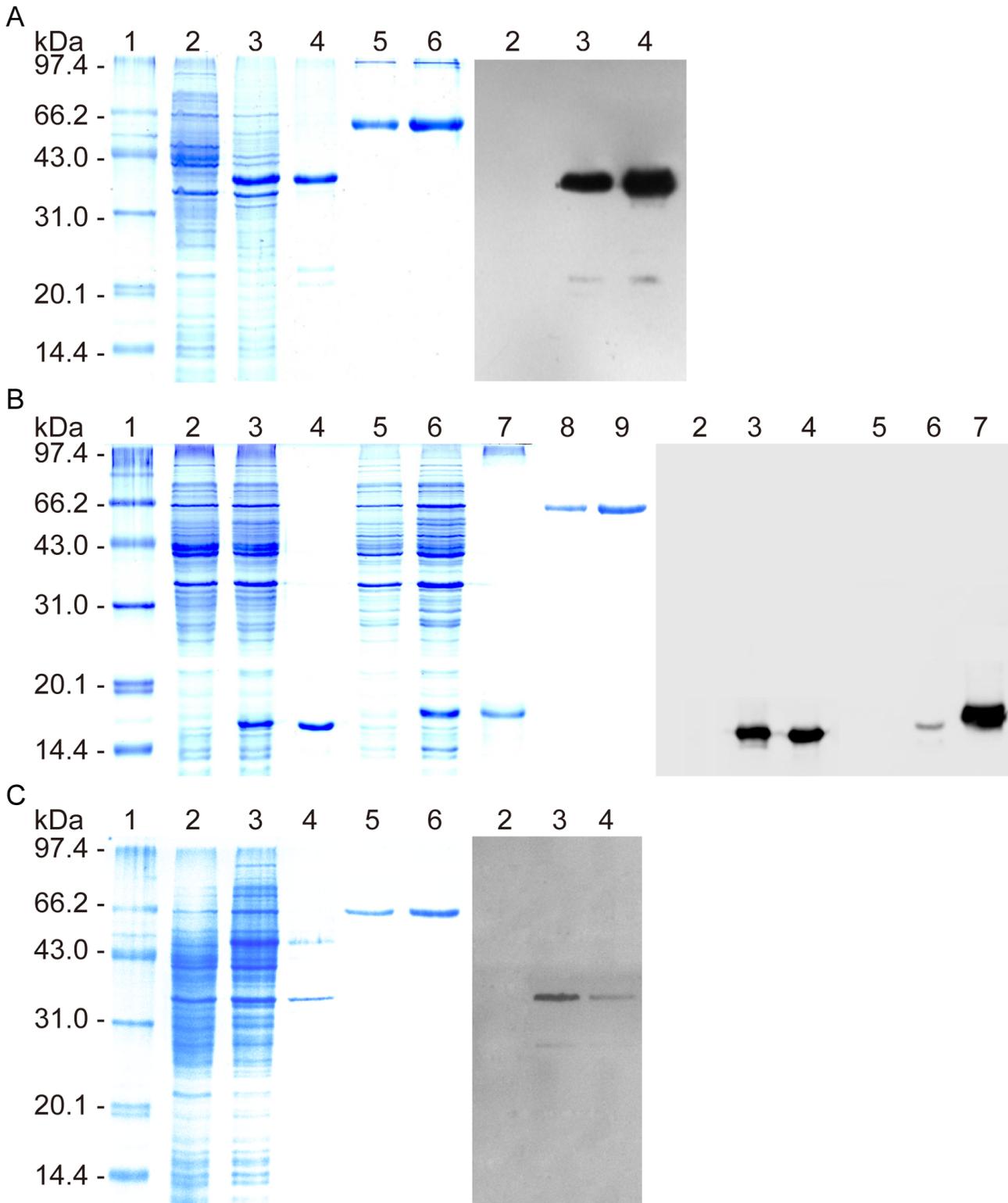
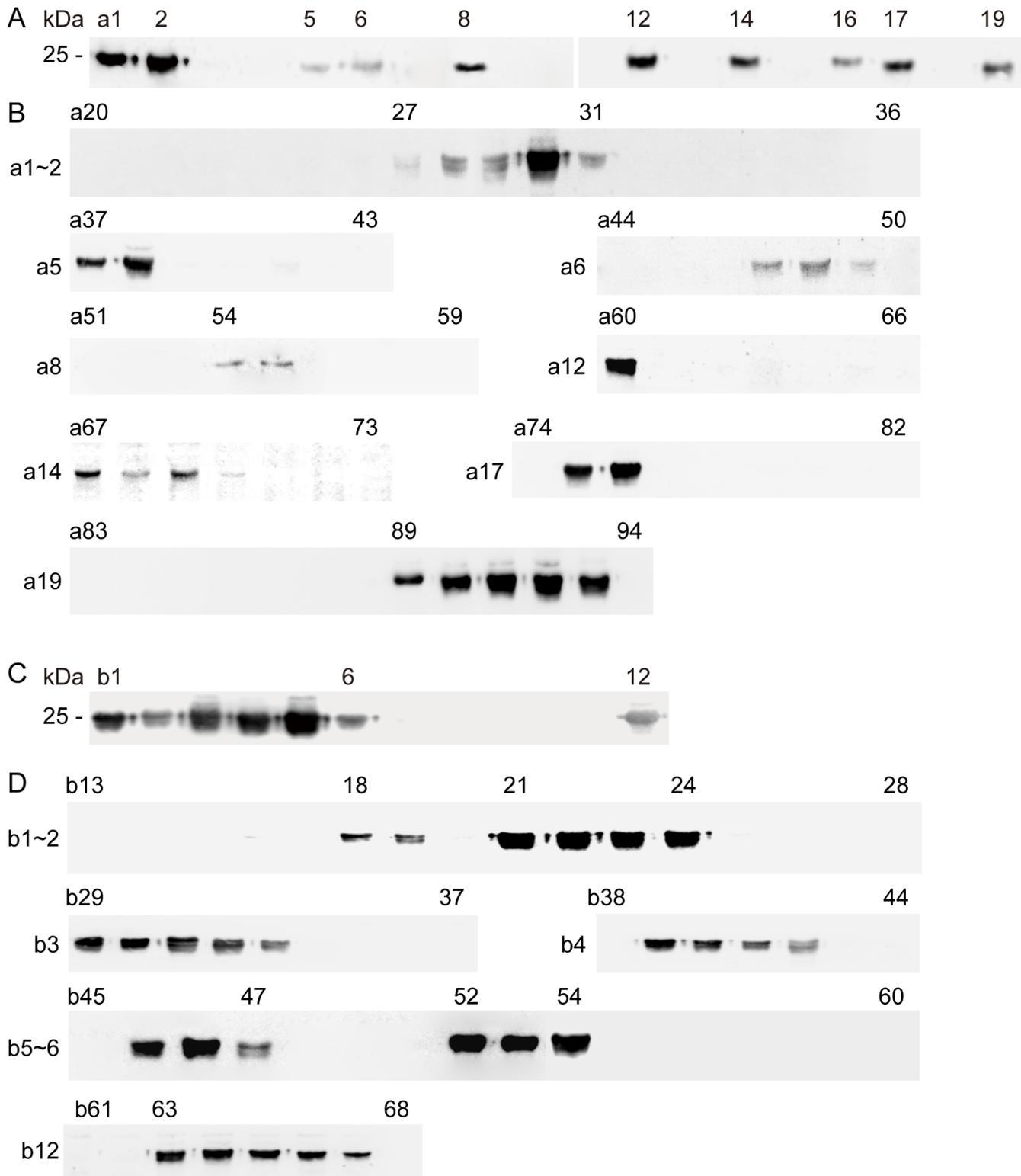


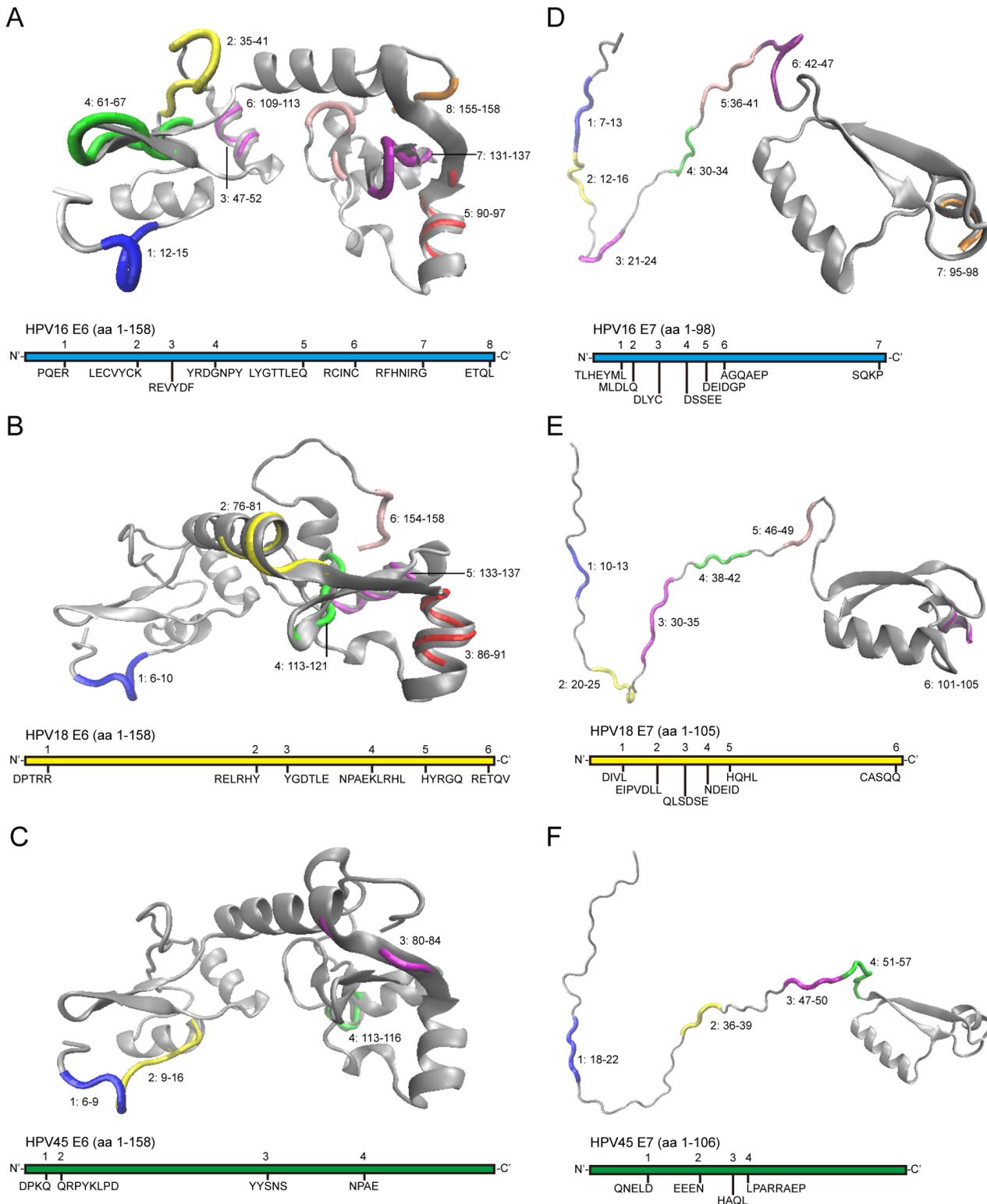
Figure 2

Recombinant E6E7, E6 and E7 proteins. (A-C) SDS-PAGE analysis of expressed and purified r-E6E7, r-E6 and r-E7 proteins from HPV16, 18 and 45 and Western blot identification with the mAbs. Lane 1, pre-stained protein markers; (A,C) lanes 2-3, uninduced and induced cell proteins harboring r-E6E7 of HPV16 and 45; lane 4, purified respective r-proteins; lanes 5-6, 2 and 4  $\mu\text{g}$  of bovine serum albumin. (B) lanes 2-3 and 5-6, uninduced and induced cell proteins harboring r-E6 and E7 of HPV18; lane 4 and 7, purified respective r-proteins; lanes 8-9, 2 and 4  $\mu\text{g}$  of bovine serum albumin. The mAb against His6-tag was used to identify all the expressed and purified target r-proteins except E6 of HPV18 (lanes 3-4 in Fig. 1B) that were identified using mAb C1P5.



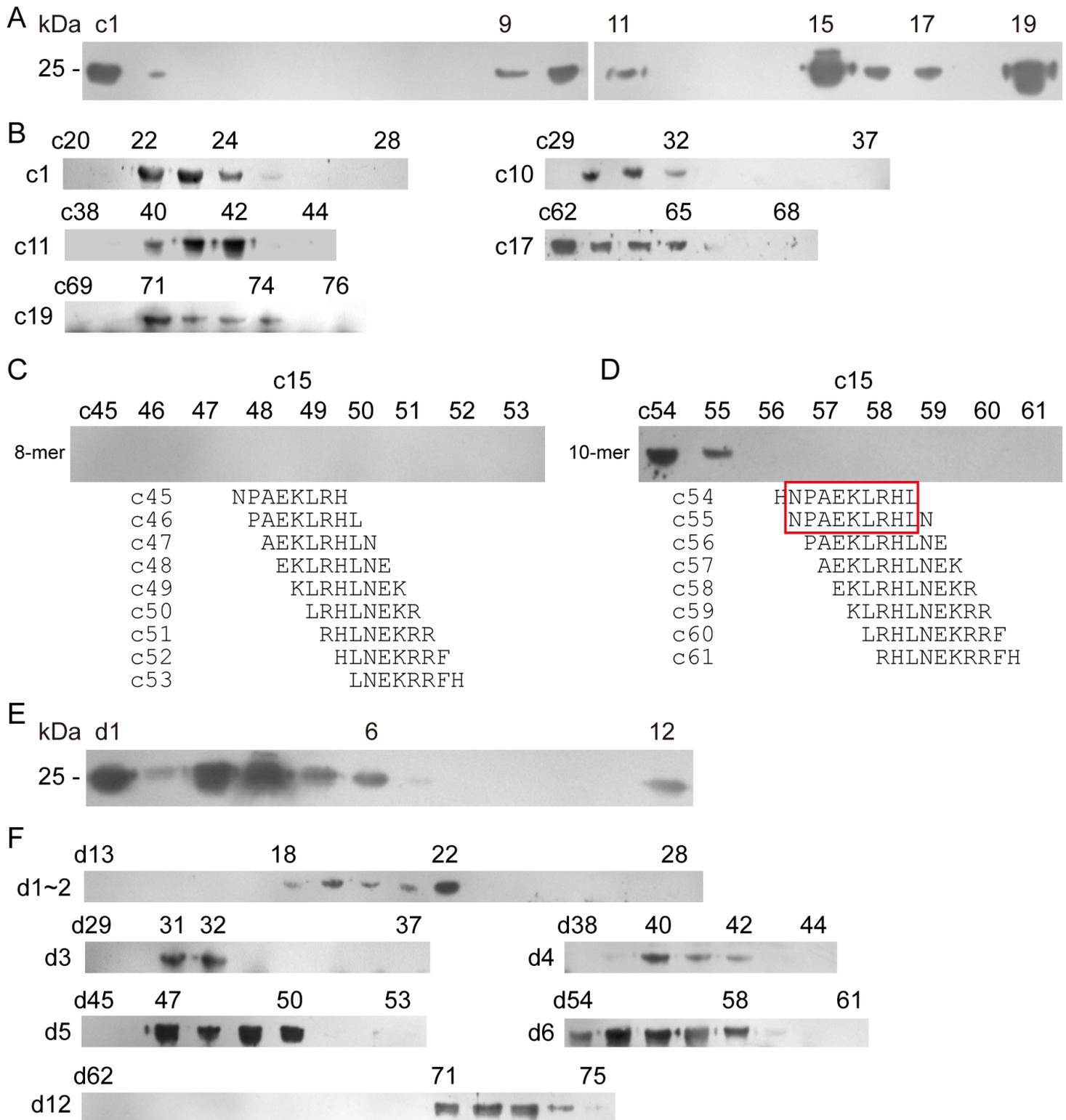
**Figure 4**

Epitope mapping of HPV16-E6 and E7 proteins. (A, C) Western blotting of 16mer-peptides using rabbit pAbs to HPV16-E6E7 fusion protein. (B, D) Western blotting of 8mer-peptides corresponding 10 and 7 reactive 16mer-peptides of E6 and E7. The number above each membrane represents overlapping 16mer a1 to a19 for E6 and b1-b12 for E7 as well as 8mer a20-a94 for E6 and b13-b68 for E7.



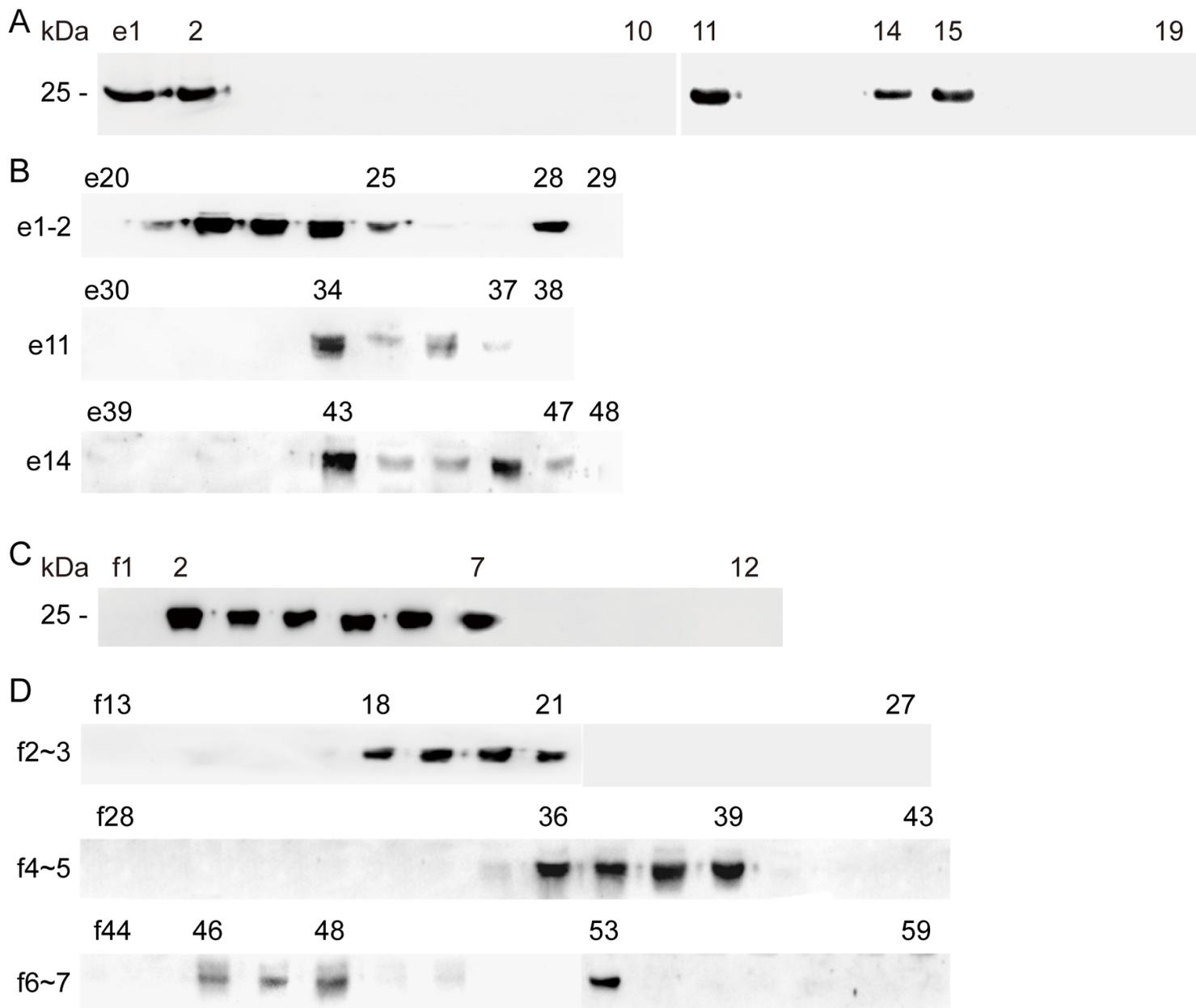
**Figure 5**

Positioning of mapped BCEs on the predicted 3D structures of E6 and E7 proteins. (A-C) Position of BCEs on the predicted 3D structures of HPV16/18/45-E6. (D-F) Position of BCEs on the predicted 3D structures of HPV16/18/45-E7. The positioned BCEs in A-F are highlighted with various colors. The line schematics of decoded six epitomes are under respective predicted 3D structures. The 3D structure prediction of HPV16/18/45-E6 and E7 all are according to the previous method<sup>41</sup>.



**Figure 8**

Epitope mapping of HPV18-E6 and E7 proteins. (A, E) Western blotting of 16mer-peptides (c1-c19 and d1-d12) with rabbit pAbs to E6 or E7 of HPV18. (B, C) Western blotting of 8mer-peptides (c20-c53 and c62-c76) corresponding 9 reactive 16mer-peptides of E6. (D) Western blotting of eight 10mer-peptides (c54-c61) corresponding the reactive 16mer c15. (F) Western blotting of 8mer-peptides (d13-d75) corresponding 7 reactive 16mer-peptides of E7.



**Figure 10**

Epitome mapping of HPV45-E6 and E7 proteins. (A, C) Western blotting of 16mer-peptides (e1 to e19 for E6 and f1-f12 for E7) with rabbit pAbs to HPV45-E6E7 fusion protein. (B) Western blotting of 8mer peptides (e20-e48) corresponding 5 reactive 16mer-peptides of E6. (D) Western blotting of 8mer peptides (f13-f59) corresponding 6 reactive 16mer-peptides of E7.

## Supplementary Files

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