1. Introduction

Apolipoprotein B mRNA-editing catalytic polypeptide (APOBEC) deaminases are a family of proteins, which include AID, APOBEC1, 2, 3A, 3B, 3C, 3DE, 3F, 3G, 3H, and 4 [1e4]. APOBEC3 (A3) proteins are antiviral factors that inhibit viruses and transposable elements, both of which use reverse transcription during their life cycle [1e4]. The antiviral functions of A3 proteins have been extensively studied in human immunodeficiency virus 1 (HIV-1) and hepatitis B virus (HBV) infections. In the case of HIV-1, A3G-induced hypermutation of viral DNA inhibits HIV-1 replication either by base excision repair (BER)-mediated DNA cleavage or accumulation of destructive mu- tations in the viral genome [1e4]. Furthermore, deaminase-independent antiviral activities are reported; A3G is proposed to block elongation of HIV-1 and HBV DNA by reverse transcription through a deaminase-independent mechanism [5,6]. In addition, binding of A3G with viral RNA is proposed to be important for inhibiting reverse transcription of retroviral genomes [7]. Human papillomaviruses (HPVs) are small double-stranded DNA viruses, and a subset of HPVs are recognized as causative agents of anogenital and oropharyngeal cancers [8e10], where HPV16 accounts for at least 50% of cervical cancer cases worldwide [11]. The HPV16 genome is a 7.9-kb closed circular DNA comprising at least eight open reading frames (ORFs) (E1, E2, E4, E5, E6, E7, L1, and L2) and a noncoding long control region (LCR). The LCR con- tains viral replication origin and an early promoter responsible for transcription of E6 and E7 oncogenes required for cellular trans- formation, while L1 and L2 encode the viral capsid proteins [8,9,11]. HPV16 infects the basal cell in cervical epithelia and establishes its genome as extrachromosomal episomes. Viral replication and capsid expression are induced in synchrony with host cell differ- entiation, and virions are assembled in the upper layer of epithelia and released into cell surroundings

once the host cell is exofoliated after terminal differentiation [9]. We have recently demonstrated that the HPV16 E2 gene is hypermutated by endogenous A3A and A3G induced by interferon b (IFN-b) in W12 cells [12], human cervical keratinocytes derived from a CIN1 biopsy that maintains HPV16 episomes [13]. Despite the observed hypermutation, the levels of HPV16 episomal genomes were not affected under the condition of A3s up-regulation. Thus, the pathophysiological relevance of A3 proteins in HPV infection remains unknown. To examine the antiviral role for A3 proteins, particularly in the virion assembly, we utilized an HPV16 pseudovirion (PsV) system [14]. Our results suggest that A3A and A3C exert their antiviral activities during the assembly phase of the HPV16 virion.

2. Material and methods

2.1. Cell culture

293FT cells were purchased from Life Technologies and main- tained in DMEM (10% FBS, 0.1 mM Non-Essential Amino Acids, 6 mM L-glutamine, 1 mM Sodium Pyruvate, and Penicillin/Strep- tomycin), as instructed by the supplier. HeLa cells were maintained in DMEM (10% FBS, Penicillin/Streptomycin).

2.2. Plasmid construction

To create pEF-nLuc, the NanoLuc coding sequence from the pNL1.1 vector (Promega, N1001) was subcloned into the pEF-BOS- EX vector [15]. pHPV16-L1/L2 was previously described [16]. FLAG-tagged green fluorescent protein (GFP), A3A (Acc. No. XM_005261387), A3F (Acc No. NM_145298) and A3G (Acc No. NM_021822) expression vectors were previously described [12,17]. For the FLAG-A3C expression vector, an ORF of human A3C (NM_014508) was amplified by RT-PCR with forward (50 -AAA- GAATTCATGAATCCACAGATCAGAA-30) and reverse (50 -AAAACTC-GAGTCACTGGAGACTCCCGTAG-30) primers using cDNA derived from HepG2 cells. The fragment was then cloned into pCMV3TAG1A (Invitrogen). For the FLAG-A3H expression vector, an ORF of human A3H (FJ376616) was amplified by RT-PCR with for- ward (50 -

AAAGAATTCATGGCTCTGTTAACAGCCGAA-30) and reverse (50 - AATAGTCGACTCAGACCTCAGCATCACACA-30) primers using cDNA derived from CaSki (human keratinocyte cell line) cells. The fragment was subsequently cloned into pCMV3TAG1A. Successful construction of plasmids was confirmed by DNA sequencing.

2.3. PsV preparation and infectivity assay

We closely followed the protocol provided by Buck et al. [14]. In brief, 293FT cells were cotransfected using Lipofectamine 2000 (Life technologies) with pEF-nLuc, pHPV16-L1/L2, and pFLAG-A3 proteins, as per the manufacturer's instruction. Two days after transfection, the cells were harvested and lysed with phosphate- buffered saline (PBS) containing 0.5% Triton-X 100, 1 mM ATP, 25 mM ammonium sulfate, 1 mg/mL RNase A (Sigma), 50 mg/ml DNase I grade II (Roche), and 0.1% Plasmid-Safe (Epicentre). The lysates were incubated for at least 16 h at 37 C to induce maturation of PsVs, followed by addition of sodium chloride at a final concentration of 0.85 M. The lysates were incubated on ice for 10 min and centrifuged at 4 C at 5000 g for 5 min. 2 x 10 4 HeLa cells per well of a 24 well plate were incubated with the superna- tant (high salt extract, HSE) with a 2000-fold dilution of the culture media, unless noted. Two days later, the cells were harvested by trypsin digest, and the luciferase activity was measured using the Nano-Glo Luciferase Assay system (Promega), as per the manufac- turer's instruction. Challenge of HSEs from the GFP control to a well without HeLa cells yielded a luciferase activity of ~300e400 rela- tive luciferase units (data not shown), comparable to the value of the empty wells. Thus, the residual luciferase activity in HSEs was considered to be negligible. In addition, Buck et al. demonstrated that challenging HSEs allowed 293H cells to express the reporter gene in a L1 or L2 dependent manner [18]. Hence, we justified challenging HSE as a method to assess infectivity of PsVs. 2.4. Western blotting Western blotting was performed as previously described

[12,17]. The antibodies used in this study were: rabbit anti-GAPDH (G9545, Sigma), horseradish peroxidase (HRP)-conjugated antirabbit IgG (GE Healthcare), mouse anti-FLAG (M2, Sigma), mouse anti-Myc (9E10, sc-40, Santa-Cruz), mouse anti-HPV16 L1 (ab69, Abcam), and antimouse IgGeHRP (GE Healthcare). Signal development was performed using ECL Western Blotting Detection Reagents (Amer- sham) and signal detection was achieved using the LAS1000 imager system (FujiFilm). 2.5. Copy number determination of pEF-nLuc by qPCR described [12]. To prepare nuclease-resistant DNA, 2 ml of HSEs were added to 10 ml of extraction buffer (10 mM Tris pH 8.0, 150 mM NaCl, 25 mM EDTA, 1% NP-40, and 200 mg/ml proteinase K) at 50 C overnight to degrade PsV capsid proteins. The resulting extracts were diluted 10-fold in double-distilled water, incubated at 95 C for 7 min to heat-inactivate proteinase K, and subjected to qPCR. For quantification of the pEF-nLuc copy number, forward (50 -TCCTTGAACAGGGAGGTGTGT-30) and reverse (50 -CGATCTTCAGCC- CATTTTCAC-30) primers were used. Specificity and linearity of the quantification were confirmed using pEF-nLuc as a template (data not shown). Copy numbers of pEF-nLuc in total cell lysates or HSEs were determined by qPCR. Serially diluted pEF-nLuc was used to obtain the standard curve for calculating the copy numbers of each sample. 2.6. Immunoprecipitation Cells were lysed with PBS containing 1% Triton-X 100, 0.5% Tween 20, and a complete protease inhibitor cocktail tablet (Roche). Immune complexes were collected using an Anti-FLAG M2 Affinity Gel or an Anti-c-Myc Agarose Affinity Gel (Sigma, A2220 and A7470, respectively), as per the manufacturer's instructions. Precipitated complexes were purified using Micro Bio-Spin Chro- matography Columns (BioRad, #732e6304) and eluted with buffer containing 6% SDS, 50 mM TriseHCl, and 150 mM NaCl. 2.7. Statistical analysis Statistical analyses were performed using GraphPad Prism (GraphPad Software). The two-tailed unpaired t-test was used for determining significance by qPCR and luciferase assay. P values of <0.05 obtained between experimental groups were considered statistically significant. In all graphs displayed in this study, error

bars indicate the standard error of the mean from duplicate or triplicate samples.

3. Results

3.1. A3A and A3C reduce infectivity of HPV16 PsV

We previously reported that A3s are abundantly expressed in W12 cells, compared to AID or A1 [12]. Furthermore, our RT-qPCR analysis revealed that both HPV16 (-) and (+) cervical tissues ex- press A3s (data not shown). These findings motivated us to explore the possibility that A3 proteins influence the assembly of the HPV16 virion by utilizing the production system of PsV [14]. Each expression vector producing A3 proteins or GFP was co-transfected with an HPV16 capsid expression vector (pHPV16-L1/L2) and a luciferase reporter plasmid (pEF-nLuc). Two days later, the cell lysates containing PsV were prepared under high salt conditions. These lysates (called HSEs) were further treated with a nuclease to digest DNA not encapsidated into the PsV, while leaving encapsi- dated reporter plasmids intact. To determine infectivity, HeLa cells seeded in a well of 24-well plate were challenged with 0.25 ml of HSEs prepared from 293FT cells overexpressing each A3, and the luciferase activity was determined 48 h after infection (Fig 1A). As shown in Fig. 1A, HSEs prepared from A3A, A3C, A3F, and A3G were less infectious than that prepared from GFP. The reduced infectivity of PsV in the presence of A3G, A3F and A3H could be partly attributed to decreased capsid protein (L1) levels in HSEs of A3 transfected cells compared with that of GFP transfected cells (Fig. 1B). It is currently unknown why L1 protein levels decreased in A3 transfected cells compared with GFP transfected cells. To compare infectivity of PsVs produced from A3 transfected cells that contained varying amounts of the L1 capsid protein, we

titrated GFP HSE by 2-fold serial dilution, and determined relation between luciferase activity and L1 protein levels, by plotting the L1 level against the luciferase activity for both GFP and each A3 protein (Fig. 1C). As expected, the amount of L1 protein and luciferase ac- tivity correlated well across the serially diluted GFP HSE. Plots of A3A, A3C, and A3H were positioned below the curve of GFP (Fig. 1C). This result indicates that the infectivity of A3A, A3C, and A3H HSEs was lower than the GFP HSE, even after normalizing for the amount of L1. A3F and A3G plotted to the same line as GFP (Fig. 1C), indicating that A3F and A3G reduced L1 protein levels but did not reduce the infectivity of the PsV. A3H expression slightly reduces infectivity even after normalization (Fig. 1C right side); however, decrease of L1 protein level was significant (Fig. 1B right side). Therefore, it is not easy to determine whether A3H decreases PsV infectivity. Taken together, we concluded that A3A and A3C attenuate HPV16 PsV infectivity.

3.2. Expression of A3A, but not A3C, affects encapsidation of PsV DNA We then investigated how A3A and A3C act to decrease PsV infectivity. First, total DNA was purified from the producer cells and reporter plasmid levels were determined. As shown in Fig. 2A, the copy numbers of the reporter plasmid in total DNA were compa- rable between each sample. Because A3A is thought to hyper- mutate foreign DNA [19], DNA sequencing of the reporter plasmid was performed. A reporter gene fragment (560 bp) was amplified from the total DNA of A3A-expressing producer cells and the fragment was cloned into the pGEM-T Easy Vector (Promega). This vector was transformed into Escherichia coli and 24 clones were randomly selected. DNA sequencing of these clones (24 x 560= 13440 bp) revealed an absence of C-to-T and G-to-A mutations (data not shown). These results suggest that neither differences in transfection efficiency or foreign DNA hypermutation can explain the reduction of infectivity by A3A (Figs. 1C and 2A). Furthermore, we determined reporter plasmid levels in the HSEs. Because HSEs were obtained following nuclease digestion, reporter plasmid levels in HSEs should reflect the level of encap-

encap- sidated PsV DNA. As expected from the decreased L1 protein levels in the HSEs compared to the GFP HSE, all A3 samples exhibited a lower copy number of the reporter plasmid than the GFP control (Fig. 2B). To evaluate the copy number of the reporter plasmid per single PsV, copy numbers of serially diluted HSE from GFP trans- fected cells were determined and plotted along with A3 proteins against L1 levels (Fig. 2C). Only A3A exhibited a reduced reporter plasmid copy number as measured per L1 level. Other groups compared the infectivity of PsVs by adjusting the amount of reporter plasmid [20, 21]. To evaluate infectivity of A3 HSEs per pseudogenome, the same data used in Figs. 1 and 2 were plotted according to the copy number of the reporter plasmid. (Supplementary Fig. 1). Infectivity of A3A and A3C per pseudoge- nome was lower than that of GFP, however, L1 levels per psuedo- genome were higher for A3A HSE and equivalent for A3C to that of GFP, while in A3F, A3G, and A3H HSEs, both infectivity and L1 protein levels per pseudogenome were lower than those of GFP (Supplementary Fig. 1, lower). Even after conducting different an- alyses, we obtained the same conclusion; the decreased infectivity of HSEs from A3F, A3G, and partly A3H, could be attributed to the decreased L1 expression, while A3A reduce infectivity by reducing pseudogenome per PsV. Taken together, these results suggests that A3A interferes with encapsidation of the reporter plasmid, whereas A3C decreases PsV infectivity by a mechanism other than encapsidation of the pseudogenome.

3.3. Binding of A3 proteins with L1 capsid protein in vitro Because the encapsidation process failed to explain the decreased infectivity of PsVs in A3C HSE, we next explored whether A3C affects PsV infectivity by interactions with the capsid proteins. To this end, we expressed Myc-tagged L1 and FLAG-tagged A3 proteins in 293FT cells. Co-immunoprecipitation (IP) experiments revealed that FLAG-A3C, -A3F, -A3G, and -A3H, co-precipitated Myc-L1, while almost no GAPDH, FLAG-GFP and negligible FLAG- A3A proteins were precipitated with Myc-L1 (Fig. 3 middle). Consistent with this result, IP complexes of Myc-L1 exhibit detectable levels of FLAG-A3C, 3F, 3G, and 3H, but not GFP and A3A (Fig. 3 bottom). Of note, FLAG-A3C most efficiently pulled down

Myc-L1 among these four (Fig. 3 middle). The distinguished binding property of A3C for L1 was also demonstrated by another co-IP experiment, using total lysates from 293FT cells transfected with pHPV16-L1/L2, pEF-nLuc, and FLAG-A3s, from which we obtained HSEs (Supplementary Fig. 2). Collectively, these data indicate that A3C efficiently (while A3F and A3G modestly) binds to the L1 capsid protein in vitro. 4. Discussion In this study, we investigated the role of APOBEC3 proteins during the assembly phase of the HPV16 virion. Using the PsV production system, which recapitulates the assembly phase of the HPV16 virion, we demonstrated that A3A and A3C reduce the PsV infectivity when co-expressed in 293FT cells (Fig. 1). The levels of the encapsidated pseudogenome were decreased in PsVs prepared from A3A transfected cells, but not other A3s transfected cells (Fig. 2). To clarify the mechanism underling the reduced infectivity, we examined whether A3 proteins bind to the HPV16 L1 capsid. A3C efficiently bound to L1, but A3A did not (Fig. 3, Supplementary Fig. 2), implying that the A3C binding to L1 may impair PsV infec- tivity. Notably, A3A and A3C localize both in the nucleus and cytoplasm, whereas A3F and A3G are distributed to the cytoplasm [22].

Unexpectedly, co-transfection of pHPV16-L1/L2 with pFLAG-A3s resulted in decreased L1 expression (Fig. 1B, Supplementary Fig. 2, top). Meanwhile, Myc-L1 was not obviously decreased by

co-expression with FLAG-A3s (Fig. 3, top). We do not deny the possibility that A3s are involved in the degradation of L1 protein or mRNA, which does not target Myc-tagged L1. We have demonstrated that A3C binds to HPV16 L1 in vitro, which implies the mechanism how A3C reduces the infectivity. Binding capacity of L1 with heparan sulfate proteoglycans (HSPGs) is proposed to be important for the primary attachment on the cell surface, internalization, and uncoating of the capsid, to allow the pseudogenome to enter the nucleus [23]. Therefore, it is intriguing to speculate that A3C blocks either of these steps, by binding with L1, to deprive HSPGs of its access to the PsVs. During preparing this manuscript, Warren et al. reported that A3A could decrease the infectivity of HPV16 PsV, which is consis- tent with this study [21]. However, they concluded that A3C does not affect HPV16 PsV infectivity. In their study, recombinant HPV16 genomic DNA with a GFP reporter gene, driven by SV40 promoter, was used as a pseudogenome and infectivity of PsVs prepared from A3C-expressing cells was determined by GFP expression in infected cells. In this study, a vector encoding a luciferase reporter gene driven by EF1a promoter was used, therefore, the discrepancy be- tween that study and the present work can be attributed to the differences in reporter gene and cell lines used. In summary, this study provides for the first time the evidence for the involvement of A3 proteins in interference with HPV16 virion assembly. Further studies are required to clarify the specific mechanism of how A3 proteins mediate this infectivity defect.

Conflict of interest
The authors declare no conflicts of interest.

Acknowledgments
We thank Ms. Shimadzu for technical support. This study was supported by the Founding Program for Next Generation World- Leading Researchers and a Grant-in-Aid for Young Scientists (B) from the Japan Society for the Promotion of Science, Tekeda Science Foundation, the Hokkoku Foundation for Cancer Research, and The Yasuda Medical Foundation.

Appendix A. Supplementary data Supplementary data related to this article can be found at http:// dx.doi.org/10.1016/j.bbrc.2014.12.103.

References
[1] R. Goila-Gaur, K. Strebel, HIV-1 Vif, APOBEC, and intrinsic immunity, Retro- virology 5 (2008) 51. lomavirus 16 minor capsid protein L2, Virology 434 (2012) 110e117.
[2] R.S. Harris, M.T. Liddament, Retroviral restriction by APOBEC proteins, Nat. Rev. Immunol. 4 (2004) 868e877.
[3] M.H. Malim, APOBEC proteins and intrinsic resistance to HIV-1 infection, Philos. Trans. R Soc. Lond. B Biol. Sci. 364 (2009) 675e687.
[4] V.C. Vieira, M.A. Soares, The role of cytidine deaminases on innate immune responses against human viral infections, BioMed. Res. Int. 2013 (2013) 683095.
[5] Y. Iwatani, D.S. Chan, F. Wang, K.S. Maynard, W. Sugiura, A.M. Gronenborn, I. Rouzina, M.C. Williams, K. Musier-Forsyth, J.G. Levin, Deaminase-indepen- dent inhibition of HIV-1 reverse transcription by APOBEC3G, Nucleic Acids Res. 35 (2007) 7096e7108.
[6] D.H. Nguyen, S. Gummuluru, J. Hu, Deamination-independent inhibition of hepatitis B virus

reverse transcription by APOBEC3G, J. Virol. 81 (2007) 4465e4472.
[7] K. Belanger, M. Savoie, M.C. Rosales Gerpe, J.F. Couture, M.A. Langlois, Binding of RNA by APOBEC3G controls deamination-independent restriction of ret- roviruses, Nucleic Acids Res. 41 (2013) 7438e7452.
[8] J. Bodily, L.A. Laimins, Persistence of human papillomavirus infection: keys to malignant progression, Trends Microbiol.
19 (2011) 33e39. [9] H. Zur Hausen, Papillomaviruses and cancer: from basic studies to clinical application, Nat. Rev. Cancer 2 (2002) 342e350.
[10] A.R. Kreimer, A.K. Chaturvedi, HPV-associated oropharyngeal cancerseare they preventable? Cancer Prev. Res (Phila) 4 (2011) 1346e1349.
[11] M.A. Stanley, M.R. Pett, N. Coleman, HPV: from infection to cancer, Biochem. Soc. Trans. 35 (2007) 1456e1460.
[12] Z. Wang, K. Wakae, K. Kitamura, S. Aoyama, G. Liu, M. Koura, A.M. Monjurul, I. Kukimoto, M. Muramatsu, APOBEC3 deaminases induce hypermutation in human papillomavirus 16 DNA upon Beta interferon Stimulation, J. Virol. 88 (2014) 1308e1317.
[13] M.A. Stanley, H.M. Browne, M. Appleby, A.C. Minson, Properties of a non- tumorigenic human cervical keratinocyte cell line, International journal of cancer, J. Int. du Cancer 43 (1989) 672e676.
[14] D.V.P. Christopher, B. Buck, Douglas R. Lowy, John T. Schiller, Generation of HPV pseudovirions using transfection and their use in neutralization assays, in: J.D. Clare Davy (Ed.), Human Papillomavirus Methods and Protocols, Humana Press, London, 2006, pp. 445e462.
[15] S. Mizushima, S. Nagata, pEF-BOS, a powerful mammalian expression vector, Nucleic Acids Res. 18 (1990) 5322.
[16] S. Nakao, S. Mori, K. Kondo, K. Matsumoto, H. Yoshikawa, T. Kanda, Mono- clonal antibodies recognizing cross-neutralization epitopes in human papil- lomavirus 16 minor capsid protein L2,

Virology 434 (2012) 110e117.
[17] K. Kitamura, Z. Wang, S. Chowdhury, M. Simadu, M. Koura, M. Muramatsu, Uracil DNA glycosylase counteracts APOBEC3G-induced hypermutation of hepatitis B viral genomes: excision repair of covalently closed circular DNA, PLoS Pathog. 9 (2013) e1003361.
[18] C.B. Buck, D.V. Pastrana, D.R. Lowy, J.T. Schiller, Efficient intracellular assembly of papillomaviral vectors, J. Virol. 78 (2004) 751e757.
[19] M.D. Stenglein, M.B. Burns, M. Li, J. Lengyel, R.S. Harris, APOBEC3 proteins mediate the clearance of foreign DNA from human cells, Nat. Struct. Mol. Biol. 17 (2010) 222e229.
[20] Z. Surviladze, R.T. Sterk, S.A. DeHaro, M.A. Ozbun, Cellular entry of human papillomavirus type 16 involves activation of the phosphatidylinositol 3-ki- nase/Akt/mTOR pathway and inhibition of autophagy, J. Virol. 87 (2013) 2508e2517.
[21] C.J. Warren, T. Xu, K. Guo, L.M. Griffin, J.A. Westrich, D. Lee, P.F. Lambert, M.L. Santiago, D. Pyeon, APOBEC3A functions as a restriction factor of human papillomavirus, J. Virol 89 (2015) 688e702.
[22] L. Lackey, E.K. Law, W.L. Brown, R.S. Harris, Subcellular localization of the APOBEC3 proteins during mitosis and implications for genomic DNA deami- nation, Cell Cycle 12 (2013) 762e772.
[23] K.F. Richards, M. Bienkowska-Haba, J. Dasgupta, X.S. Chen, M. Sapp, Multiple heparan sulfate binding site engagements are required for the infectious entry of human papillomavirus type 16, J. Virol. 87 (2013) 11426e11437.
) 868e877.

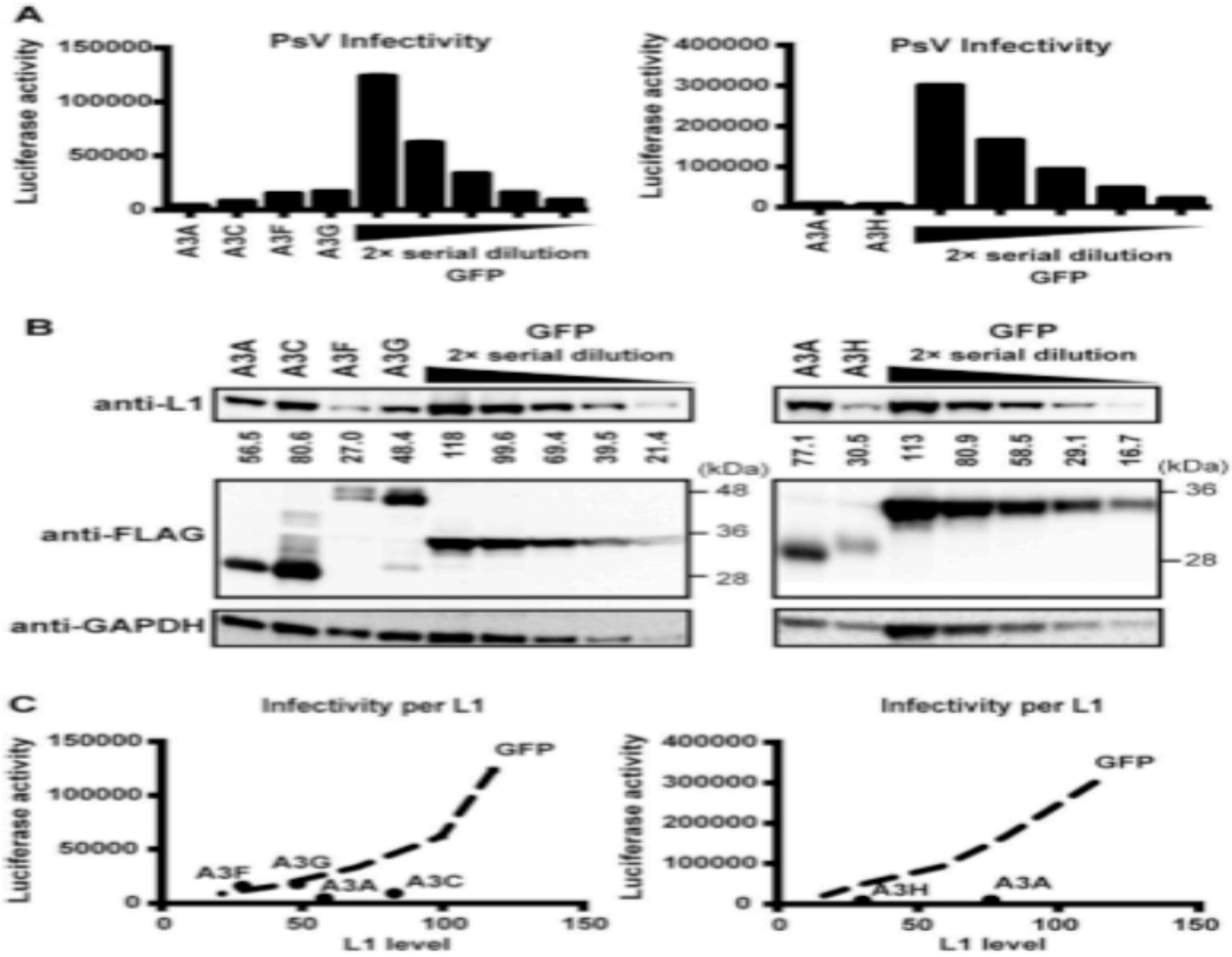

Fig. 1. A3 protein expression decreased infectivity of HPV16 PsV. (A) 293FT cells were cotransfected with an L1/L2 expression vector (pHPV16-L1/L2), reporter plasmid (pEF- nLuc), and an expression vector producing each A3 protein or GFP. Transfected 293FT cells were harvested at 48 h after transfection and HSE-containing PsVs were prepared. 100 ml of HSE was prepared from a well of a 6-well plate of transfected 293FT cells. 0.25 ml of each HSE (0.25% of the HSE fraction) was used to challenge HeLa cells seeded in a well of a 24-well plate. Cells were harvested and luciferase activity was determined 48 h post infection. For control cells transfected with pFLAG-GFP, 0.25, 0.125, 0.0625, and 0.03125 ml of HSEs were used to challenge to HeLa cells to obtain a standard curve of L1 protein level and infectivity. (B) Protein levels of HPV16-L1, FLAG- A3 proteins, and GAPDH in 2 ml (2% of a 6-well plate) of each HSE were determined by immunoblotting followed by densitometry. For control cells transfected with pFLAG- GFP, L1 protein levels in 2.0, 1.0, 0.5, and 0.25 ml of HSEs were determined. (C) Each HSE was plotted according to its L1 protein level and luciferase activity. Serially diluted HSEs from GFP transfected cells were plotted to compare infectivity of HSEs containing different amounts of L1. Errors bars in (A) and (C) represent the standard deviations of three independent experiments.

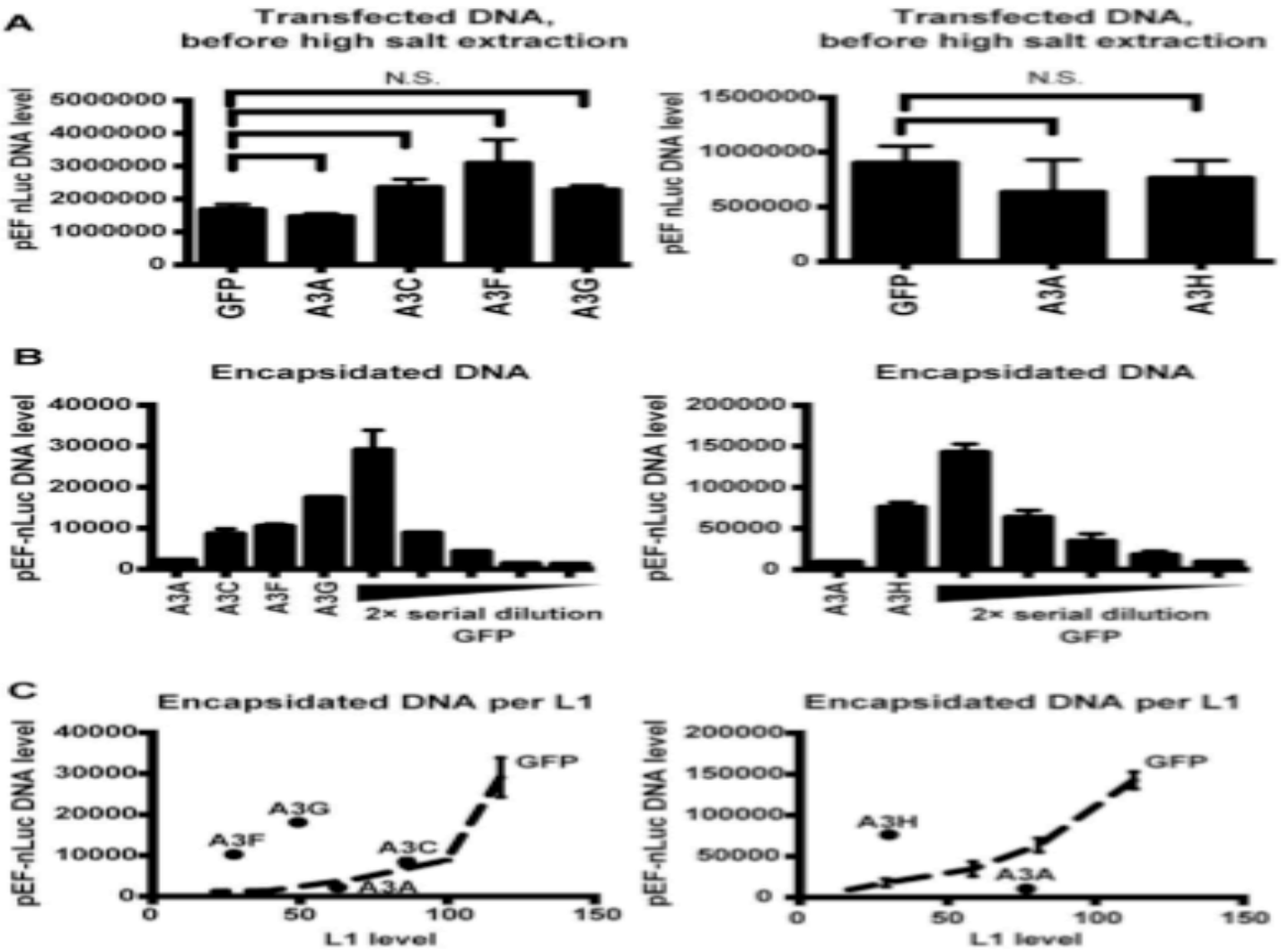

Fig. 2, top). Meanwhile, Myc-L1 was not obviously decreased by Fig. 2. A3A expression, but not that of other A3s, decreased the amount of encapsi- dated DNA. (A, B) Total DNA from 293FT cells (A) or corresponding HSEs (B) were subjected to qPCR to measure copy numbers of the reporter plasmid (pEF-nLuc). Re- porter plasmid levels in HSEs were equivalent to encapsidated reporter plasmid levels, as HSEs were prepared after DNaseI treatment. Reporter plasmid levels from 0.167 ml of each HSE were determined. For the GFP control, reporter plasmid levels from 0.167 ml of the HSE and its serial dilution were determined. N.S. ¼ not statistically signi ficant. (C) HSEs from each transfected cell were plotted according to L1 protein level and copy number of reporter plasmid. Serially diluted HSEs from GFP transfected cells were plotted to compare the copy numbers between HSEs containing different amount of L1. Errors bars represent standard deviations of two independent experiments.

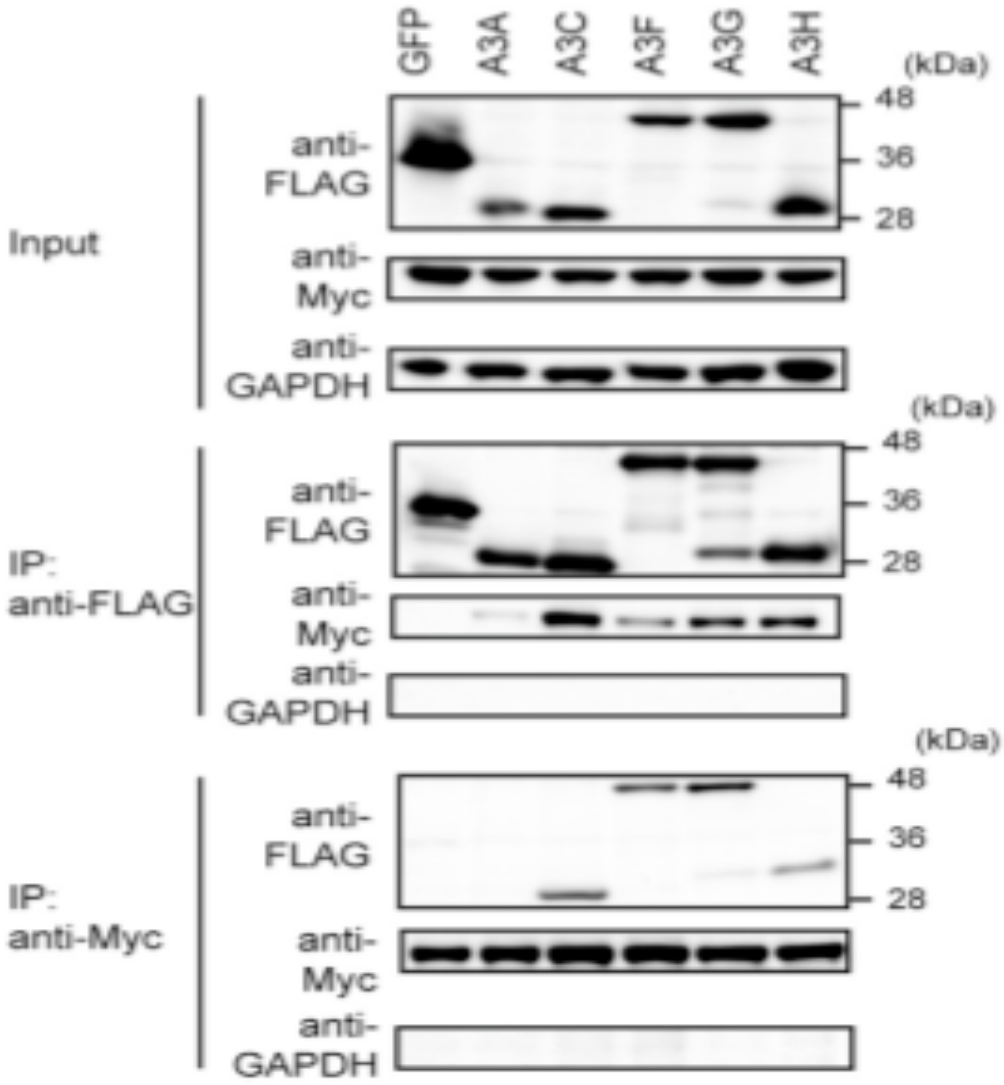

Fig. 3. Physical interaction of A3 proteins with HPV16 L1. 293FT cells were transfected with FLAG-tagged A3 proteins and Myc-tagged L1 and immunoprecipitated by anti- FLAG or anti-Myc antibody. Immunoprecipitated samples as well as the input were immunoblotted by anti-FLAG and anti-Myc antibodies. GAPDH blot was used as a control. Displayed is representative of two independent experiments.