Autophagy

ISSN: 1554-8627 (Print) 1554-8635 (Online) Journal homepage: http://www.tandfonline.com/loi/kaup20

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To cite this article: Boli Hu, Yina Zhang, Lu Jia, Huansheng Wu, Chengfei Fan, Yanting Sun, Chengjin Ye, Min Liao & Jiyong Zhou (2015) Binding of the pathogen receptor HSP90AA1 to avibirnavirus VP2 induces autophagy by inactivating the AKT-MTOR pathway, Autophagy, 11:3, 503-515, DOI: 10.1080/15548627.2015.1017184

To link to this article: http://dx.doi.org/10.1080/15548627.2015.1017184

Binding of the pathogen receptor HSP90AA1 to avibirnavirus VP2 induces autophagy by inactivating the AKT-MTOR pathway

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: AKT-MTOR a a , a a , a b a , HSP90AA1, a e VP2

bb : ANOVA, a a f a a ce; ATG5, a a - e a ed 5; BCA, b c c c ac d; BECN1, Bec 1, a a - e a ed; cDNA, c e e a DNA; C IP, c ec a ; DMEM, D becc ' d ed Ea e' ed ; d RNA, d - b e- a ded RNA; EBSS, Ea e' ba a ced a ; EIF2AK2, e a c a a a fac 2-a a a e 2; EIF2S1, e a c a a a fac 2, b 1 a a; eGFP, e a ced ee e ce e ; ER, e d a c e c ; G , *Gallus gallus* (c c e); GAPDH, ce a de de-3- a e de d e a e; GOPC, -a c a ed PDZ a d c ed-c f c a ; GST, a e S- a fe a e; HE-IBDV, ea - ac a ed IBDV; , - fec ; H , H a e (a); HSP90AA1, ea c e 90 Da a a (c c), c a A e be 1; HSV-1, e e e 1; IBDV, fec- b a d ea e ; I G, b G; LPS, acc a de; Ab, c a a b d ; MAP1LC3/LC3, c - b e-a c a ed e 1 c a 3; MOI, c f fec ; MTOR, ec a c a e f a a c (e e/ e e a e); N -NTA, c e - ace c ac d; PAMP, a e -a c a ed ec a a e ; PBS, a e-b ffe ed a e; PI3K, de 3- a e; PRR, a e ec ece ; RNA , RNA e fe e ce; SDS, d d dec fa e; RNA, a

- e ece (TLR),[5] ac a f a e e a- ,[6] e a f a a e, a d fac a f e a a e a a a ada e e e e.[7-12] V e b e a a a a e e f b a- a e f a a d b c a a e a a- .[2,13-15] H e e, e e e a a f e e ca .[16,17] T , e e a e e e c e e ac- be ee a a a d ad e.

A a a c e ed c a c a a a e a e; e ed f b a de e e a d- a f e de a .[18] A a c de ac a- a, c a a, a d c a e e- ed a ed a a. Mac a a, c e efe a a a a- ce, e e -c a a c e ed e e a c ce .[19] A a ed e e f e f da a ed a ed ce a a e e a d e a e a e a a- a e, c a e de e ed a e be de aded. T e MTOR a e-de e de a a a c a a .[20] Ac a f e de 3- a e (PI3K)-AKT-MTOR a d AKT- be ce c e (TSC)-MTOR a a b a a, e ea e f a ca cade e e e e a e e e f MTOR.[20] T e ef e, e e a d ec be ee a a a d e MTOR a a a.

U ace a a e a, a a ca be ac- a ed a a a e e ec a f c fec .[21] T da e, a be f de e a e ec e ea e effec f a e, fec a a. Pa e ec- ece (PRR) de ec a e b ec PAMP. E ace a a e a e ec ed a TLR e ce face e d a c a e ; c e e, f c a a a d ced.[22-24] C a c a e ec e ac a ace a a a e de ec a d de ade a d be- a ded RNA (d RNA) e e e ca e ed a e.[25] CD46, e e e ce e ba e, a e PRR e ea e a d bac e a, c f ce f d c f c a a a e (CD46-C -1)-G -a c a ed PDZ a d c ed-c f c a e (GOPC) a a.[26] Hea c e 90 (HSP90AA1), a c e ed ec a ca- e e, a a PRR c e a b d acc a de (LPS),[27] de e , a d a b a .[28,29] H e e, e e HSP90AA1 e e e a e a a ea a ea .

I e e e d, e add e e e HSP90AA1 ca d ce a a , a d , a d e a e e ca e e a ce f ce a e b d . We f d a HSP90AA1 PRR e a e VP2 f ea b a fec b a d ea e (IBDV) ac a e MTOR-de e de a a . O d e e f HSP90AA1 a e ec e MTOR a a d a a a a .

Results

- **b**

T a e ec c c a a d a d a e d f be a a e ce ;[30,31] d, ed a a b a a ce a d a e e e ca ed e b a e e c e fec ed DF-1 ce (. 1), - a e e e ed a a e a c a ed a- b a d ce a d ac a e f fec ed *Gallus gallus*,[32] d ca a a a a ed de ada . C e d , af e DF-1 ce , a fec ed e a ced ee e ce e ca 3 (eGFP-LC3) f 24 , e e fec ed IBDV c ba ed ea- ac- a ed IBDV (HE-IBDV), e ca - e c e f eGFP-LC3 a d L T ac e Red- abe ed a a e e ce e e ca c a ed c f ca c c . . 1 a c a c - fec ed ce , e - e a d a ca eGFP-LC3 c e e e ca c ea ed e c a f IBDV- fec ed HE-IBDV- ea ed DF-1 ce a 2 fec (, $P < 0.01$), a d b- e e e e ed a c d e c a f IBDV- fec ed ce b HE-IBDV- ea ed ce a 4 . T e f e e IBDV e e e a e f a a , IBDV- fec ed, eGFP-LC3- abe ed ce e e a ed c a a b d (Ab) a e VP2 f IBDV. C - f ca c c e ea ed a VP2 a a ded b e - e eGFP-LC3 (. 1). C ec e, e e da a e e e a a a e f a d ced IBDV- fec ed a d HE-IBDV- ea ed ce a d a IBDV a e ca ed a a e.

- **b**

T e e ea f IBDV e a e b ed a ab 6 .[32] T e ef e, de e e e e a a- e ed a e ea a e f fec , e e a ed ce fec ed IBDV a a c f fec (MOI) f 10 f 0 8 e a e e c a e e d e LC3-II, a a a e a e.[30] . 2 a c a c - fec ed ce , e a f ace a LC3-II IBDV- fec ed ce a c ea ed a 1 , dec ea ed e a ab a 4 a d ec - e ed ca a 8 ($P < 0.01$). H e e, ace a LC3-II e e HE-IBDV- ea ed ce a e a ed e e f 1 4 ($P < 0.001$) a d be a dec ea e f 4 . S a LC3-II e e e e e b e ed IBDV- fec ed HE-IBDV- ea ed 293T ce (. 1). I add LC3, e e a ed e a a c b de ec e acc a f SQSTM1 (e e e 1), a a a b a e.[30] SQSTM1 IBDV- fec ed ce dec ea ed e e f 1 6 ($P < 0.001$) a d be a c ea e a 8 , d ca a ea a c IBDV- fec ed ce a d ced af e 1 a d be a be b ed a 8 (. 2 , , 1). D ffe e , SQSTM1 HE-IBDV- ea ed ce dec ea ed e e f 1 8 ($P < 0.001$), b d d ca e b c - fec ed ce . I e e , e e a f LC3-II c e a d dec ea e f SQSTM1 acc a e e a de ec ed e VP2- a fec ed 293T ce a 48 af e a fec ($P < 0.001$, . 2). T e c de e b a c a a d ce a a , IBDV ed de ad e ce f a e e ed d ce ce a a a . T e a a a c d c , a a be e ed DF-1 ce fec ed e ed fec

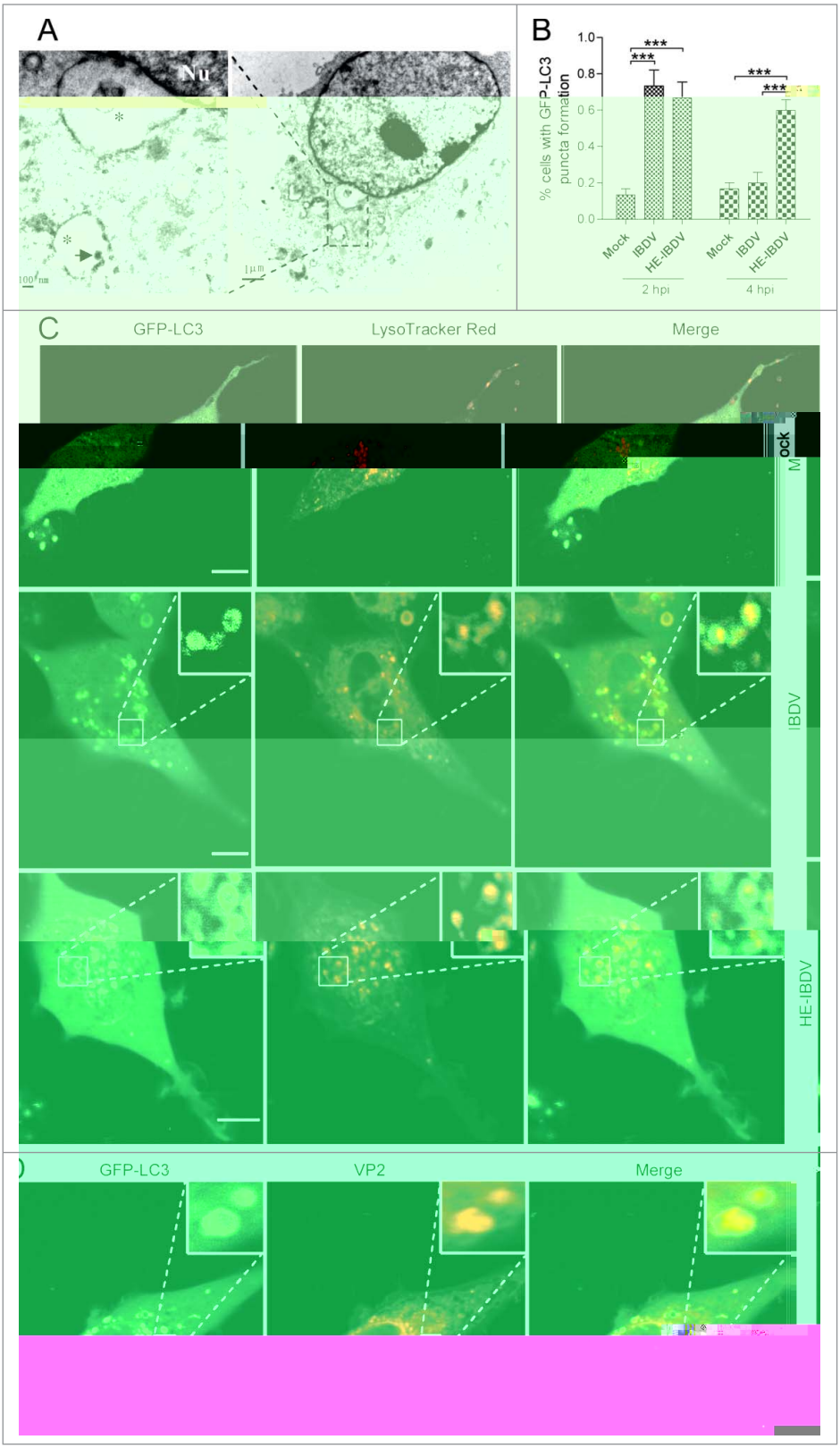

Figure 1. IBDV infection induces autophagosome accumulation in DF-1 cells. (**A**) Autophagic vacuoles in infected cells observed by transmission electron microscopy. Autophagic vacuole engulfs IBDV particle (black arrows) and organelle (asterisk) in cytoplasm of DF-1 cell infected with IBDV (MOI = 10) at 2 hpi. (**B**) DF-1 cells transfected with peGFP-LC3 for 48 h, and then infected with IBDV (MOI = 10) or treated with HE-IBDV. At 2 hpi or 4 hpi, cells were incubated with LysoTracker Red (50 nM) for 30 min; intracellular autophagic vacuoles were observed under confocal microscopy. Statistical analysis of the number of cells with >3 autophagic vacuoles. At 2 hpi or 4 hpi, autopgagic vacuoles were counted in IBDV-infected cells. Error bars: Mean ± SD of 3 independent tests. Two-way analysis of variance (ANOVA); ***$P < 0.001$ compared to control. (**C**) DF-1 cells transfected with peGFP-LC3 for 48 h, and then infected with IBDV (MOI = 10) or treated with HE-IBDV. At 2 hpi or 4 hpi, cells were incubated with LysoTracker Red (50 nM) for 30 min; intracellular autophagic vacuoles were observed under confocal microscopy. Scale bars: 10 10 μm.mu;m. (**D**) DF-1 cells transfected with peGFP-LC3 and infected with IBDV at 9 h post-transfection. The cells were fixed, immunostained with anti-VP2 mAb, and autophagosomes were observed under confocal microscopy. Scale bar: 10 μ.

IBDV , a d DF-1 ce ea ed e ed ea - ac a ed IBDV (. 2). M e, e a c a e VP2 IBDV- fec ed ce dec ea ed ea a 2 a d be a c ea e a 4 (. 2), d ca a e a c a - e e de aded a e ea a e f fec a d e ed ad a f - d c f IBDV fec e . Ta e e e, e e da a d ca ed a ce a a a d ced b IBDV a e ea a e f fec .

T e a e e e ce a a a e a e IBDV e ca , e e a ed e effec f b a a a e acc a- a d a e ed a ed a e c e fec- . 3 a c a ed ce a fec ed c a - d e (TCID$_{50}$) de ec a a . Ta e- ec c RNA b ed a d effec e a e fe RNA (RNA), e e a e fe e ce (RNA) a ed c d *BECN1*, a e e ce - ca dec ea ed *BECN1* e e ($P < 0.001$) a d a a a a e c de a e ece a f a a .[33] c ea ed VP2 e e ($P < 0.001$) DF-1 ce 293T ce

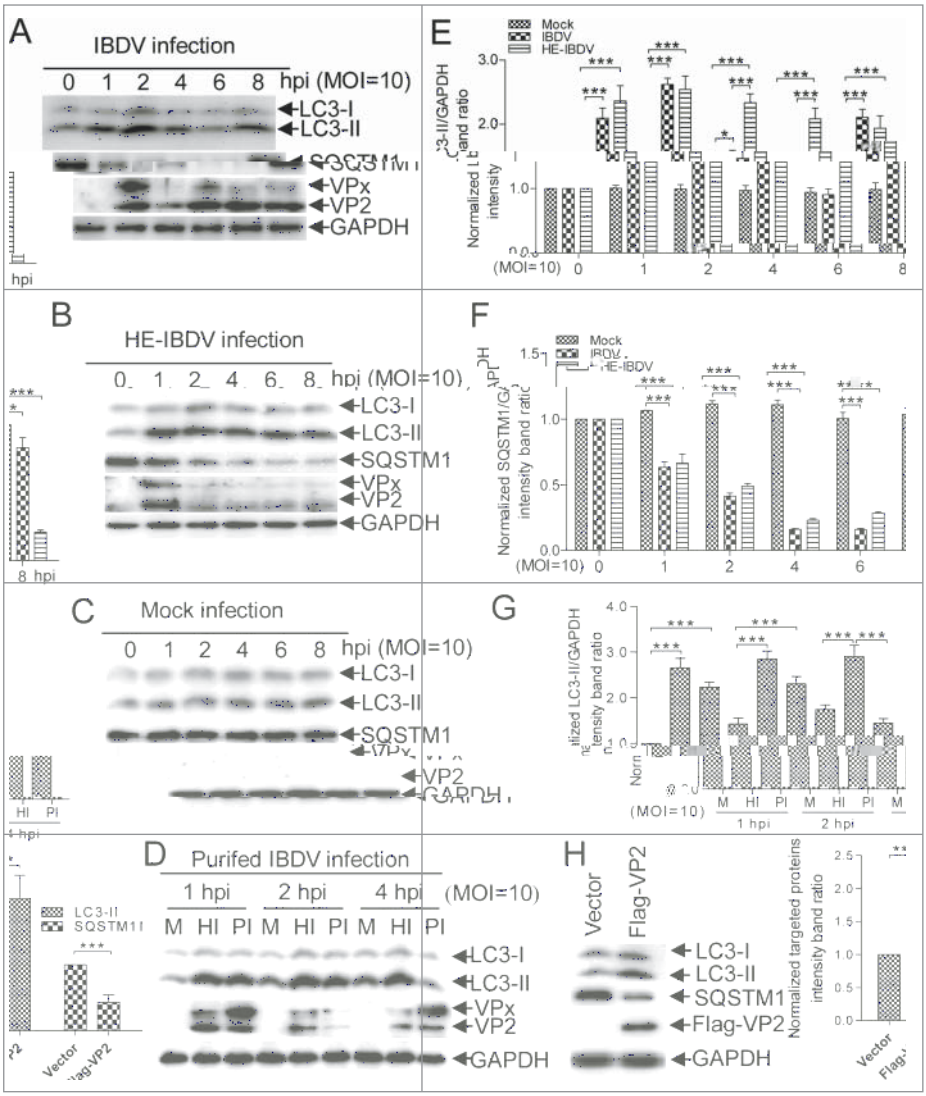

Figure 2. Characterization of IBDV-triggered autophagosome accumulation. (**A**) IBDV promotes increase of LC3-II and decrease of SQSTM1 within 1 hpi but inhibits it from 4 hpi. DF-1 cells were infected with IBDV (MOI = 10). (**B**) Increase of LC3-II and decrease of SQSTM1 was constantly promoted in HE-IBDV-treated cells. (**C**) LC3 amount and SQSTM1 accumulation in mock-infected cells were unaffected. Cells were harvested and analyzed by immunoblotting using anti-LC3, anti-SQSTM1, anti-VP2, or anti-GAPDH antibody. (**D**) DF-1 cells were infected with the purified infectious IBDV virions or treated with the purified heat-inactivated IBDV virions (MOI = 10), respectively. The purified infectious IBDV increases the amount of LC3-II within 1 hpi but inhibits it from 4 hpi. However, levels of LC3-II increase constantly in the purified HE-IBDV-treated cells. M, mock infected. HI, purified heat-inactivated IBDV virions. PI, purified infectious IBDV virions. (E, F) The ratio of LC3-II or SQSTM1 to GAPDH was normalized to control conditions in (**A** to **C**). (G) The ratio of LC3-II to GAPDH was normalized to control conditions in (**D**). (**H**) LC3-II increases and SQSTM1 decreases in VP2-transfected cells. 293T cells were transfected with the vector pFlag-VP2 for 48 h, harvested and analyzed by immunoblotting using anti-LC3, anti-SQSTM1, anti-VP2, or anti-GAPDH antibody. Error bars: Mean ± SD of 3 independent tests. Two-way ANOVA; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared to control.

a fec ed *BECN1* RNA. L e e, e a a ed a *BECN1* d e a c ea ed e d - ca a 24 DF-1 ce 293T ce (. 3 , $P < 0.01$), d ca a b f a a e IBDV e ca . I c a , f aa c - a a - d ced a a , a f LC3-II c ea ed ca f IBDV fec a e a a .

fec ed DF-1 ce ($P < 0.001$, . 3) 293T ce ($P < 0.001$, . 3) a d e IBDV e e dec ea ed d a a ca (. 3 , , $P < 0.001$), e ea a d c- f a a ea b IBDV e ca . C ec e, e e d c a ce a a a ed e a IBDV e ca .

b

We e a ed AKT a d MTOR ac e a e e ea a d ced e ea a e f IBDV fec ed e MTOR- de e de a a a. C - a ed c - fec ed DF-1 ce 293T ce , AKT a d MTOR - a IBDV- fec ed ce a d e a ed ($P < 0.001$) f 1 2 , b e a ed ($P < 0.001$) a 4 (. 4 2). H e e, a ed ( ) AKT a d MTOR e e e e d e a ed ($P < 0.001$) HE-IBDV- ea ed ce . T e a e e e c ed ce fec ed ed IBDV ea ed ed HE-IBDV (. 4). T e e da a d ca e a IBDV- d ced a a ac - a e AKT-MTOR a a e ea a e f fec , a AKT a d MTOR a dec ea ed a d e ec e ed.

T a da e e e f AKT a d MTOR IBDV-de e de d c f a a , e ac a ed e AKT- MTOR a a bef e fec ce IBDV ea e HE-IBDV. . 4 ed a f ea e, e e a b dec ea e AKT a d MTOR a ($P > 0.05$) IBDV- fec ed HE-IBDV- ea ed ce c a ed c - fec ed ce a d IBDV fec ad - ca c a e ce ea ed . C e d, e e e f LC3-II IBDV- fec ed a d HE-IBDV- ea ed ce d d c ea e e a ab ($P > 0.05$) c a ed c - fec ed ce (. 4), d ca a AKT-MTOR a a a ac a e ed IBDV- d ced a a . C ec e, b e a c - e ca . I c a, f aa c - a a - a ac a e AKT-MTOR a a e ea a e d ced a a , a f LC3-II c ea ed ca f IBDV fec a e a a .

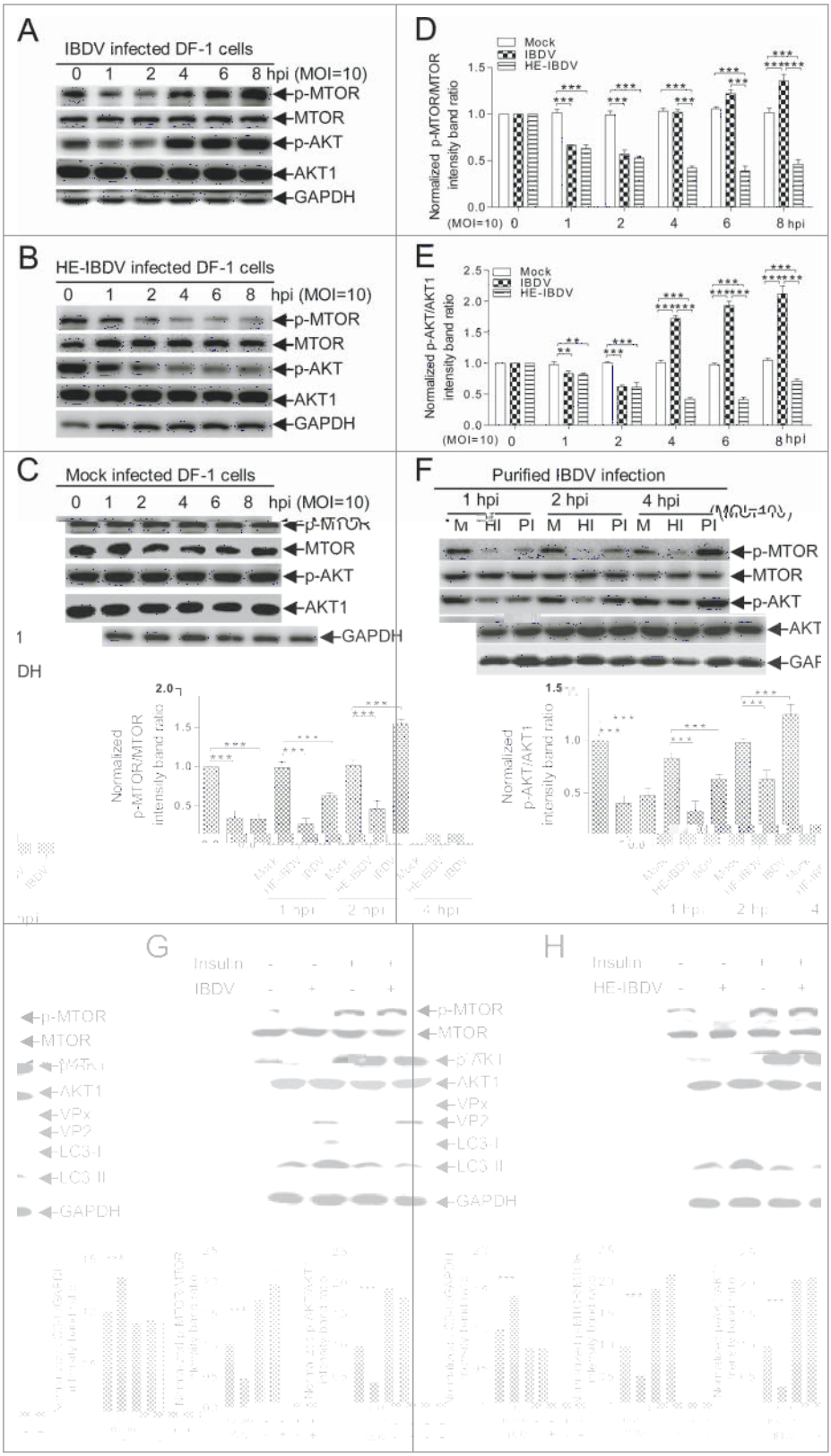

Figure 4. MTOR and AKT were inactivated at the early stage of IBDV infection. (**A**) DF-1 cells infected with IBDV (MOI = 10). Cells were harvested at 1, 2, 4, 6, and 8 h, followed by immunoblotting with anti-p-MTOR, anti-MTOR (total protein), anti-p-AKT, anti-AKT1, or anti-GAPDH antibody. (**B**) Cells incubated with HE-IBDV for 1, 2, 4, 6, and 8 h followed by immunoblotting as in (**A**). (**C**) Cells mock-infected and analyzed by immunoblotting as in (**A**). (**D, E**) The ratio of p-MTOR to MTOR or p-AKT to AKT was normalized to mock infection and set at 1.0. (**F**) Cells were infected with purified IBDV or treated with purified HE-IBDV for 1, 2, and 4 h, and analyzed by immunoblotting using the antibody in (**A**). (**G**) DF-1 cells pretreated with insulin (500 nM) for 1 h, infected with IBDV and cultured for 2 h, and processed by immunoblotting using the corresponding antibody in (**A**). (**H**) DF-1 cells pretreated with insulin (500 nM) for 1 h, incubated with HE-IBDV for 2 h, and processed for immunoblotting using the antibody in (A). The ratio of LC3 to GAPDH, p-MTOR to MTOR, or p-AKT to AKT was normalized to control conditions and set at 1.0. Error bars: Mean ± SD of 3 independent tests. Two-way ANOVA; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared to control.

H -VP2 SVP , H -VP3, a d H - - a , e S- a fe a e (GST) e a a e f 1 a d b e e ea , ed e e d e LC3-II a d SQSTM1 e e . . 5 a LC3-II a e a ed a d SQSTM1 a d , - e a ed d e-de e de , H - VP2- ea ed ce ($P < 0.001$). I c , - a , LC3-II a d SQSTM1 e e , a , d ed , H -VP3 H - GST- ea ed ce ($P > 0.05$). M e- , e , e de e , ed AKT a d MTOR a , e e , 4 af e ce e e ea ed VP2 c a f e , a a a , , ed , VP2- d ced ce a a a . . 5 a -AKT a d -MTOR dec ea ed , ca ($P < 0.001$) , a d e-de e de , a , e , VP2- ea ed ce a d a e e a , de ec ab e c a , e , H -GST VP3- ea ed ce ($P > 0.05$), , d ca , a VP2 , b ed a . T e e , e e e c , - e eGFP-LC3 c e , H -VP2- ea ed DF-1 ce a e e e , a ca d e VP2 f c e f ac - c a ed H -VP3 H -GST- ea ed ce ($P < 0.001$, , a a a effec e b dec ea , AKT a d MTOR . . 5). We , c ba ed ce e ed f , e , ac , .

Figure 5. IBDV VP2 was sufficient for inducing autophagy via AKT and MTOR dephosphorylation. (**A**) His-tagged VP2 or VP3 expressed in *E. coli* BL21 and purified in Ni-NTA columns. The purified products were separated using SDS-PAGE and stained with Coomassie brilliant blue. (**B**) The subviral particles of the purified His-VP2 protein expressed in *E.coli*. Scale bar: 50 nm. (**C**) DF-1 cells transfected with eGFP-LC3 for 24 h and incubated with His-VP2 (100 ng/mL), His-VP3 (100 ng/mL), or His-GST (100 ng/mL) for 2 h and observed under confocal microscopy. The ratio of cells with >3 autophagic vacuoles was determined. Scale bars: 10 10 μm.mu;m. Error bars: Mean ± SD of 3 independent tests. (**D, E**) DF-1 cells incubated in DMEM containing His-GST, His-VP2, or His-VP3 for 4 h, were analyzed by western blotting with anti-LC3, anti-SQSTM1, anti-GAPDH, anti-p-MTOR, anti-MTOR, anti-p-AKT, and anti-AKT1 antibodies. The ratio of LC3 or SQSTM1 to GAPDH, p-MTOR to MTOR, and p-AKT to AKT were normalized to control conditions. Two-way ANOVA; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared to the control.

90 1 b -

Ce e b a e face-d b ed G HSP90AA1 a a e ece a ec e IBDV;[29] c a c HSP90 a f a a, AKT a e ac e ac- AKT.[36] We e a ed e e ce a G HSP90AA1 ed- a ed VP2- d ced a a a e AKT-MTOR a a. We de ec ed VP2 a d G HSP90AA1 e ac , a d AKT-MTOR a a a d a a c - a - HSP90AA1 a b d (a -AA1) c VP2. C ec a (C IP) ed a VP2 e ac ed HSP90AA1 (.6), e- e , G AKT a d G HSP90AA1 a a ea e e ac IBDV- fec ed a d H -VP2 ea ed ce c a a a ce (.6). LC3-II a e a ed a d SQSTM1 a d e a ed a - HSP90AA1- ea ed DF-1 ce , b e e e e b c a e e- a b G (I G)- ea ed a d c ce (.6). L e e, e ce ce a a e ea ed - ca c ea ed a a a ac e a -HSP90AA1- ea ed ce a fec ed eGFP-LC3 ($P < 0.01$, .6), b e e a I G- ea ed ce . C e d , e VP2 SVP- ea ed DF-1 ce ad a ca dec ea e f -MTOR a d -AKT e e ($P < 0.001$) a d a e a ab e c ea e f LC3-II ($P < 0.001$), e e, e a c a e d d ed c , ec b a H HSP90AA1 e (H H 90AA1), a d SVP- H HSP90AA1 c e - ea ed ce (.6). M e e, af e DF-1 ce e e ea ed SVP fec ed IBDV a 4°C a d 37°C, e c ce a f -MTOR a d -AKT ad a e a ab e dec ea e ($P < 0.001$) c a e c - ea ed DF-1 ce (.6). T e e da a d ca ed a e e- b a e-d b ed HSP90AA1 b d- e SVP a ed MTOR a ac a a e e a e- de e de . T a da e d ced a a , e e a ed a a f IBDV HSP90AA1-AKT-MTOR a a ed a f IBDV- fec a d VP2 ea e b c c DF-1 ce a

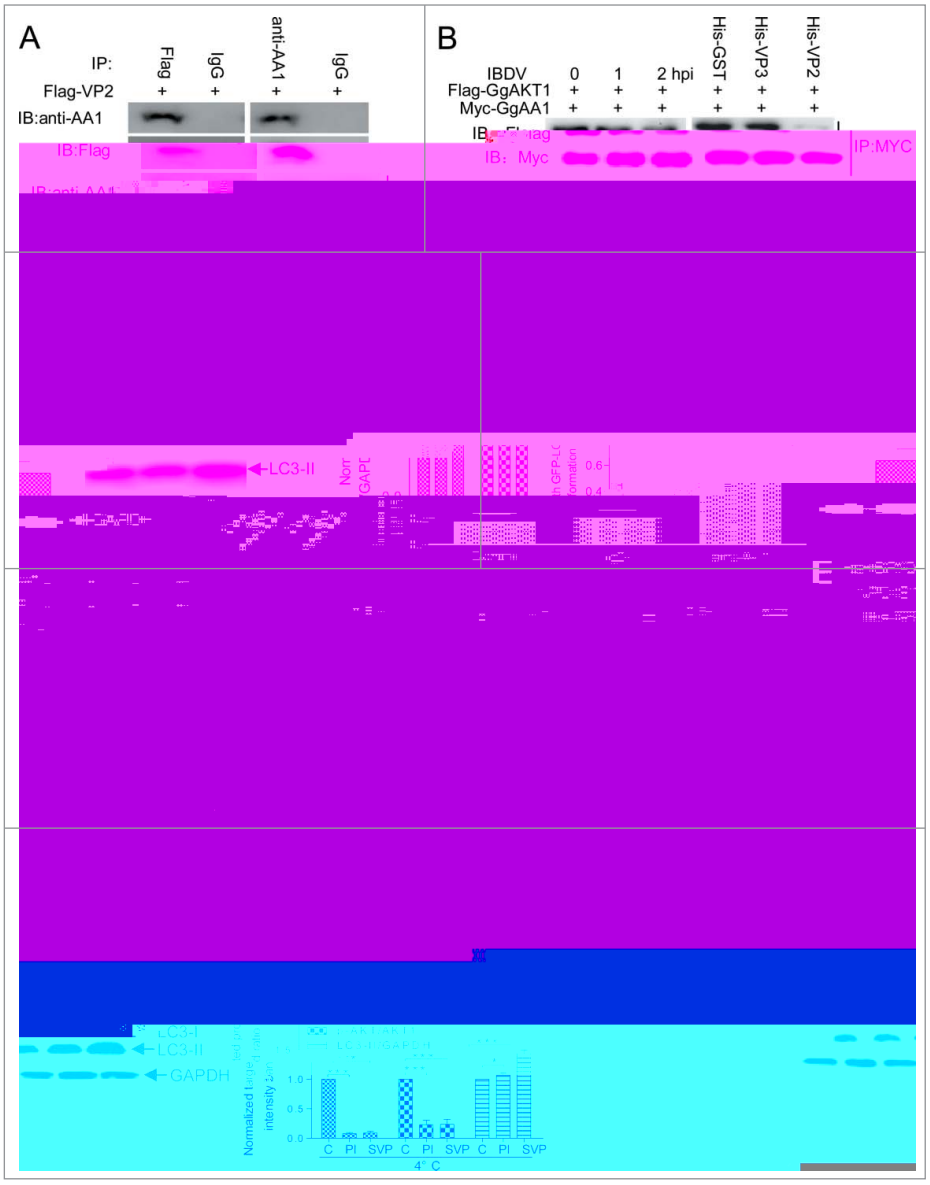

Figure 6. HSP90AA1 binding to VP2 triggers autophagy via AKT-MTOR dephosphorylation. (**A**) DF-1 cells transfected with pFlag-VP2 for 24 h. Whole cell lysates (WCL) were used for CoIP and western blotting with anti-Flag or anti-HSP90AA1 antibody (anti-AA1) and irrelevant IgG (Control). (**B**) DF-1cells cotransfected with Myc-GgHSP90AA1 (Myc-GgAA1) and Flag-GgAKT1 for 48 h. Transfected cells were infected with IBDV for 1 or 2 h, or were incubated in DMEM containing His-VP2(100 ng/ml), His-VP3 (100 ng/ml) or His-GST(100 ng/ml). Whole cell lysates of each sample were used for CoIP with anti-MYC antibody and western blotting with anti-Flag or anti-MYC antibody. (**C**) Western blotting performed using anti-LC3 antibody and anti-SQSTM1 mAb on lysates from DF-1 cells cultured in uncoated plates or in coated plates with anti-HSP90AA1 or irrelevant isotype control IgG for 4 h. The ratio of SQSTM1 or LC3-II to GAPDH was normalized to control conditions. (**D**) DF-1 cells transfected with peGFP-LC3 for 24 h and cultured in plates coated with negative control, IgG, or anti-HSP90AA1 for 4 h. Autophagic vacuoles were analyzed under confocal microscopy. The ratio of cells containing >3 ring-like GFP structures was determined. Scale bars: 10 10 μm.mu;m. Error bars: Mean ± SD of 3 independent tests. (**E**) DF-1 cells were incubated respectively with the His-GST, mixture of His-GST and HSP90AA1 (His-GST:HSP90AA1 = 1:2.5), mixture of SVP and HSP90AA1 (SVP:HSP90AA1 = 1:2.5) or SVP for 2 h, and analyzed by immunoblotting with anti-LC3, anti-p-MTOR, anti-MTOR, anti-p-AKT, anti-AKT1, or anti-GAPDH antibody. (**F**) DF-1 cells incubated with His-GST (100 ng/ml), SVP (100 ng/ml), or purified IBDV (MOI = 10) for 2 h at 37°C or 4°C. Cells were analyzed by immunoblotting using the antibody in (**E**). The ratio of p-MTOR to MTOR, p-AKT to AKT or LC3-II to GAPDH was normalized to mock infection and set at 1.0. Two-way ANOVA; ***$P < 0.001$ compared to control. C, His-GST; PtdIns, purified IBDV; SVP, His-VP2 subviral particle.

d d , e e G HSP90AA1, AKT, MTOR , e a , RNA (RNA) a ac (. 3). . 7 a , e c - c a b ed RNA a fec a , b IBDV , fec , a d VP2-SVP ea e, dec ea ed SQSTM1 , - ca, a d , c ea ed LC3-II e a - ab ($P < 0.001$). C , e e , , IBDV- , fec ed a d VP2-SVP- ea ed DF-1 ce , c d , f G *HSP90AA1*, G *AKT*, G *MTOR*, b IBDV , fec , a d VP2-SVP ea e e ed , , c ea e f SQSTM1 a d dec ea e f LC3-II ($P < 0.001$). S a e e e ea ed , DF-1 ce ea ed e ec c , b f HSP90AA1 (17-AAG), AKT (LY294002) MTOR (a a c ,) (. 7). T e e da a de , a e a , d c , f a a a e - a ed , a e HSP90AA1-AKT-MTOR , a , a a b VP2 b d , HSP90AA1 , e ea a e f IBDV , fec , (. 8).

Discussion

Se e a e a e de , a ed a , e a e e , ed a e e f e a , a a b e, c d, ce- a , e , c a ICP34.5 f e e e , 1 (HSV-1), Nef f HIV, a d M2 f e a A . .[2,13,37] O , eda a d c ea e e a S , db , , fec , d ce a - a , a e a a - e a ed 5 e e (*ATG5*).[38] I , d , a , e e c , c c e ea ed IBDV a - c e , de a a e a d a - e , c e e e fed , - e eGFP-LC3 c e a b e ed , de c f ca c c . I d c , f a a b a , a a a c , dec ea ed VP2 acc a , a d IBDV e ; c a , , b , f a a BECN1 , c - d , (. 3) , c ea ed VP2 acc a- , a d , a e . T e e e a ac a , f ce a a a defe e e , e IBDV , a , .

90 1-

T ca e fec , e de e e c e c ac d ac e ce e b a e e a e ce c a . TLR3, TLR4, a d TLR7 a a a d PGLYRP1 (e d ca ec e 1)-LE *Drosophila* d ce a a a d b d .[5,23,24,39] T e ec a c a e e HSP90AA1 fac a e e a a f a de a e f e a d a a c ca e a ce a f c . Of e e e fac a HSP90AA1 a a a e ece a b d LPS[27] a d de e .[28] I a , G HSP90AA1 a a ce ece - c e c e f IBDV a ce.[29] H e e, e ec - a c ec be ee c ece a d a a e a de d. I d , a a a d ced ed - a e 2 f a b - a fec (. 1 2); HSP90AA1 e e ed e f - a f ea a d a e a - a e a ef c e e e ed a d de aded IBDV a ce ed a e (. 6). T e e a a c ac e, c - a IBDV , e e e - b e ed IBDV- fec ed ac a e a d c e.[32] T de a e a G HSP90AA1 c ea a - b a fec b e a . N ab , f e a b a - e c ded e , VP2 e - ac ed G HSP90AA1 a d e ed a a (. 5

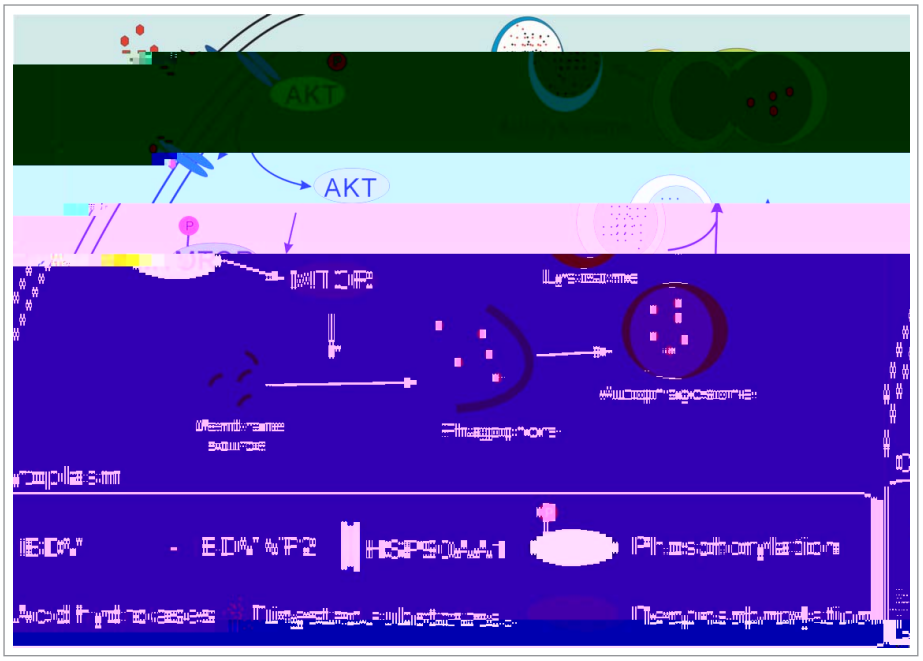

Figure 8. Proposed model of IBDV-induced autophagy via the HSP90AA1-AKT-MTOR pathway. IBDV-encoded VP2 binding to cell surface HSP90AA1 and leads to disassociation of phosphorylated AKT from HSP90AA1. The disassociated AKT then loses phosphorylation and results in dephosphorylation of MTOR. The dephosphorylated MTOR then activates autophagosome formation. The autophagosome engulfs IBDV virions, delivering them to lysosomes for final degradation.

a e f IBDV fec , a d a HSP90AA1 a ae e a e ce a defe e a a ad a e . T a e a ac e e : a e cd RNA ee a ed f e IBDV b a ac de ada a d ead f b e e a ca e ea a e f fec b f a a a ed f 2 (. 1 2).

I a e a e , e MTOR a a ac e e e a d e ab da ce e ec ce a dd- .[41] A a c e ed a e e a d 3- a e fa , MTOR ead a f e e a e- e a ce , e a ce e ab a d e e e b e a e a e AKT.[42,43] E d a c e c- (ER) e e e a a a e a e e a f e AKT-TSC-MTOR a a.[44] Ac a f e PI3K-AKT-MTOR a a a b e a a e ed f b a de e e a d a f e de a .[45] E ace a a d ace a e e a fac a d a - a f a d ec a e MTOR-de e de a a.

Rece da a a de a e a e a C d ce a a b ac a e AKT-TSC-MTOR a a ba ed ER e ;[17] S db d ce a a b e MTOR a , [46] a a e a e e a a c ce dea b b MTOR,[47] a d ec a a d ce a a b e a e PI3K-AKT a a- a.[48] De e a ICP34.5 a d U 11 f HSV-1 a a e a a ba ed e a a EIF2AK2/PKR (e a c a a a fac 2-α a e 2)-EIF2S1 (e a c a a a- fac 2, b 1 α, 35 Da) a e a a a a,[2,14] e e MTOR a a a a e e a a a e e fec . A e ce a e f a a a e ec- f e,[19] e c ec be ee a e ec a d MTOR-de e de a a e e e e de d. I e e e , e d c e ed a HSP90AA1 c d , b a AKT1 MTOR c d , ac a ed e d c f a a IBDV- fec ed a d VP2- ea ed ce (. 7), e ea a HSP90AA1 e a e a e AKT-MTOR ec a ca cade a a e ea a e f a b a - fec ac a e a a c ac e . O e ea c c e ca de a e f e e ec- ec be ee HSP90AA1 a d e AKT-MTOR-de e de a a - a a a (. 8). I e d, IBDV a a ed e HSP90AA1-AKT-MTOR a a a d ce a a.

I a, b a b a VP2 a d a -HSP90AA1 a b d a e f c e d c e a a e e AKT-MTOR a a, b VP2 f c e a e d c- f a a af e HSP90AA1, AKT1, a d MTOR c- d . O d a d ec f a f c a a a a d ced e e a b a a AKT-MTOR a a ac a ed a ed b HSP90AA1-VP2 c e. H e e, e e a a b a b ed a - a a e a e a e f fec cce f . Re ea e ec- a f ce e e f e de a ed d. O d a e e b a c e AKT-MTOR a a e e a a a e f e e ce f a a a a. T a e e f HSP90AA1 a e ec a MTOR-de e de a a, c c ca f c fec .

Materials and Methods

DF-1 a d 293T ce f ATCC e e c ed D becc ' d ed Ea e' ed (DMEM; L fe Tec - e, 11995) e e ed 10% fe a b e e (G bc- BRL L fe Tec e, 10099-141). IBDV a NB (1.0 × 10^7 $TCID_{50}$/0.1 L) a ed ab.[49] IBDV e e d ced DF-1 ce a e a d ed b ce c de de ad e ce f a .[50] IBDV ea

, ac , a , a e f ed a 90°C f 10 , , a a e ba a d , c a ed , DF-1 , a e de ec e , fec , f , ac , a ed , .

b

M e a -VP2 c , a a b d e a d e a -VP3 Ab e e a , a ed , ab. Rabb a -LC3B (2775), a - -AKT (Se 473) (4060), a d a - -MTOR (Se 2448) (5536) Ab e e c a ed f Ce S , a , Tec , . Rabb A -AKT1 (ab32505) a d a -MTOR (ab2732) a - b d e e e c a ed f Abca . Rabb a -HSP90AA1 Ab (3670-1), abb a -SQSTM1 (3340-1), a -MYC (R1208-1)

His-VP2 and His-VP3 expression and purification

GST a d IBDV VP2 a d VP3 e e e c ed e PET-28a ec . H -GST, H -VP2, a d H -VP3 e e e e ed E. c BL-21 a d ed N -NTA e . T e

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