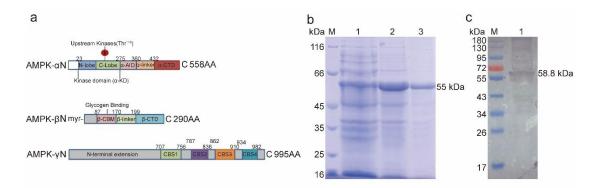
Supplementary Materials

WSSV exploits AMPK to activate mTORC2 signaling for viral proliferation by enhancing aerobic glycolysis

Peng Zhang^{1#}, Hai-Jing Fu^{1#}, Li-Xia Lv¹, Chen-Fei Liu¹, Chang Han¹, Xiao-Fan Zhao¹, Jin-Xing Wang^{1,2,*}

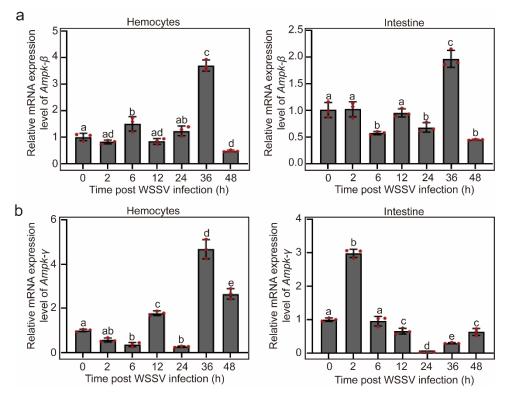
The PDF contains: Supplementary Figure 1-9 Supplementary Table 1 Uncropped and unedited blot images: Supplementary Figure 10-33



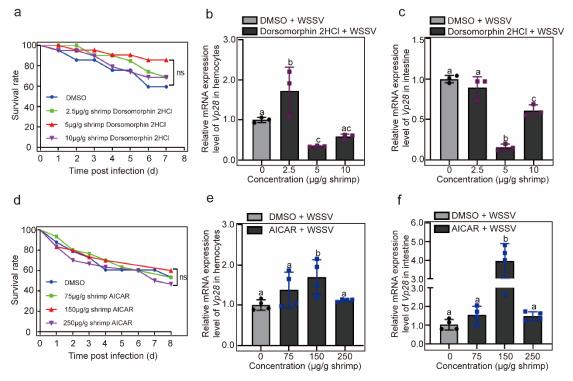
Supplementary Figure 1 Domain architectures of the AMPK complex and antibody preparation of AMPKα. a The domain architectures of three AMPK subunits from *M. japonicus*. **b** Recombinant expression in *E. coli* and purification of AMPKα. Lane 1, total proteins of *E. coli* with AMPKα-pGEX4T-2 without IPTG induction; Lane 2, Total proteins of the *E. coli* with IPTG induction; Lane 3, Purified recombinant AMPKα; lane M, protein molecular mass markers. **c** Western blotting using AMPKα polyclonal antibodies to detect AMPKα in normal hemocytes of shrimp. Lane M, protein molecular mass markers; Lane 1, the native AMPKα in hemocytes detected using AMPKα polyclonal antibodies.

M. japonicus P. Vannamei AKE50479.1 S. parammosain AKA44626. N. lugens XP. 039286472.1 A. cerana XP. 016919334.1 H. sapiens AAH69822.1 M. musculus NP 835279.2 B. mori ABQ62953.1 R. norvegicus NP_076481.1 H. armigera XP 021182528.1 D. piexippus XP_025227627.1 D. reiro XP 700831.4 S. scrofa NP 999431.1 G. gailus NP_001034692.1 O. aries XP 004017088.2 C. livia XP_005508087.1	HS RS-GSIDETIKIEHNIE MS	40 	INF <mark>QKIES</mark> LDVV <mark>G</mark> KIKREIONIKLFRH INF <mark>QK</mark> IFSLDVVGKIRREIONIKLFRH	VISTPIE FVIEV SCELET 103 VISTPIE FVIEV SCELET 104 VISTPIE FVIEV SCELET 104 VISTPIE FVVEV SCELET 101 PICK VISTPIE FVVEV SCELET 101 PICK VISTPIE FVVEV SCELET 109 PICK VISTPIE FVVEV SCELET 100 VISTPIE FVVEV SCELET 103 VISTPIE FVVEV SCELET 103 VISTPIE FVVEV SCELET 103 VISTPIE FVVEV SCELET 103 PICK VISTPIE FVVEVSCELET 103 PICK VISTPIE FVVEST SCELET 103 PICK VISTPIE FFVEST SCELET 103 PICK VISTPIE FVVEST SCELET 103 PICK VISTPIE FV
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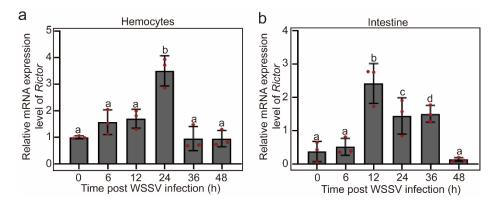
Supplementary Figure 2 The amino acid sequence alignment of AMPK*a* proteins from different species. Blue Boxed residues show the conserved activation ring. Red Boxed residues show the conserved phosphorylation sites.



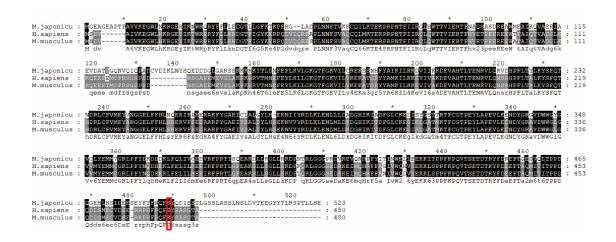
Supplementary Figure 3 Expression profiles of AMPKs in shrimp challenged by WSSV. a The expression patterns of $Ampk\beta$ in hemocytes and intestine of the shrimp analyzed using qPCR. b The expression patterns of $Ampk\beta$ in hemocytes and intestine of the shrimp. All results shown are means \pm SD for experiments performed at least three times. Multiple different comparisons were analyzed with log-rank test using the GraphPad Prism 8.0 software and a significant difference was accepted at P < 0.05.



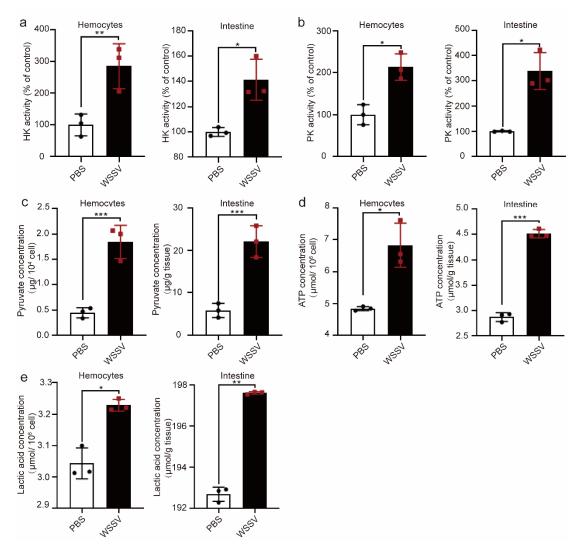
Supplementary Figure 4 Survival analysis of shrimp injected with different concentrations of Dorsomorphin 2HCL and AICAR, and WSSV replication analysis in the shrimp following WSSV infection. a Survival rate of shrimp injected with different concentrations of Dorsomorphin 2HCL. b, c Vp28 expression in hemocytes (b) and intestine (c) of shrimp injected with different concentrations of Dorsomorphin 2HCL following WSSV infection. Shrimp injected with the same amount of DMSO were used as controls. d Survival rate of shrimp injected with different concentrations of AICAR. e, f Vp28 expression in hemocytes (e) and intestine (f) of shrimp injected with different concentrations of AICAR. Following WSSV infection. Shrimp injected with the same amount of DMSO in each group were used as controls. All results shown are means \pm SD for experiments performed at least three times. Multiple different comparisons were analyzed with log-rank test using the GraphPad Prism 8.0 software and a significant difference was accepted at P < 0.05.



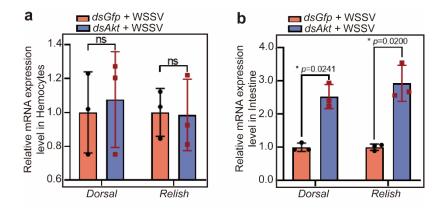
Supplementary Figure 5 Expression profiles of Rictor in hemocytes (a) and intestines (b) of shrimp challenged by WSSV analyzed using qPCR. All results shown are means \pm SD for experiments performed at least three times. Multiple different comparisons were analyzed with log-rank test using the GraphPad Prism 8.0 software and a significant difference was accepted at P < 0.05.



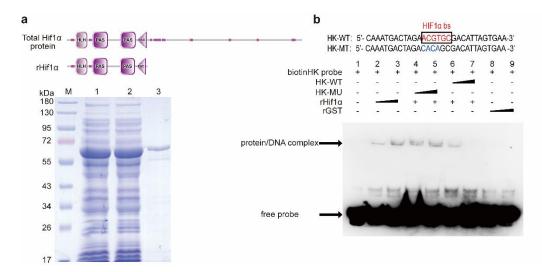
Supplementary Figure 6 Alignment of shrimp AKT amino acid sequence with AKTs of other species. The conserved phosphorylation sites were boxed in red.



Supplementary Figure 7 Changes in the enzyme activities and glycolytic metabolites during WSSV infection in shrimp. a HK activity analysis in hemocytes and intestine of shrimp challenged by WSSV, PBS injection was used as a control. b PK activity analysis in hemocytes and intestine of shrimp challenged by WSSV, PBS injection was used as a control. c Pyruvate level analysis in hemocytes and intestine of shrimp challenged by WSSV, PBS injection was used as a control. d ATP level analysis in hemocytes and intestine of shrimp challenged by WSSV, PBS injection was used as a control. e LA level analysis in hemocytes and intestine of shrimp challenged by WSSV, PBS injection was used as a control. All results are shown by means \pm SD for experiments were performed at least three times; the data were analyzed statistically using Student's t test. *, P < 0.05; **, P < 0.01; ***, P < 0.001.



Supplementary Figure 8 The expression of *Dorsal* and *Relish* in *Akt*-knockdown shrimp challenged by WSSV analyzed by qPCR. a. Hemocytes, b. Intestine. All results are shown by means \pm SD for experiments were performed at least three times; the data were analyzed statistically using Student's t test. *, P < 0.05; **, P < 0.01; ***, P < 0.001.



Supplementary Figue 9 The binding activity of rHIF1 α to binding site in HK promoter region was detected by EMSA *in vitro*. a Domain architectures of HIF1 α and recombinant expression in *E. coli* and purification of HIF1 α . Lane 1, total proteins of *E. coli* with pGEX4T-2/HIF α with 0.1 mM IPTG induction; Lane 2, Total proteins of the *E. coli* with 0.5 mM IPTG induction; Lane 3, Purified recombinant HIF α ; lane M, protein molecular mass markers. **b** Upper panel: synthesized probe sequences containing binding site from promoter sequence of HK. Lower panel: EMSA was performed to validate the interaction of rHIF1 α with its binding site in HK promoter. Lane 1: negative group (without rHIF1 α); Lane 2-3: positive groups (1, 3 µg rHIF1 α); Lane 4-5: mutant HK group (3 µg rHIF1 α incubated with two different concentrations of mutant HK probes; HK-MU, mutant HK probes without biotin); Lane 6-7: wild HK group (3 µg rHIF1 α incubated with two different concentrations of wild HK probes; HK-WT, wide HK probes without biotin); Lane 8-9: GST control protein (1, 3 µg rGST).

Supple	ementary Table 1. Sequences of prime	rs used in the	present	t study	
		Gene			
Drimore	$S_{accurrence}(5, 2)$	target and	Tm^1	A^2	Efficier
Primers	Sequence (5'-3')	GenBank	(°C)	(bp)	y ³ (%)
			. /	× 17	
RT-PCR and qP	CR				
IE1-RT-F	GACTCTACAAATCTCTTTGCCA	Iel,	51.9	500	100.55
IE1-RT-R	CTACCTTTGCACCAATTGCTAG	AF332093	50.8	502	123.57
VP28-RT-F	AGCTCCAACACCTCCTCCTTCA	Vp28,	60.6	1.60	101.00
VP28-RT-F	TTACTCGGTCTCAGTGCCAGA	AY422228	57.8	162	101.20
β -actin-RT-F	AGTAGCCGCCCTGGTTGTAGAC	Actb,	55.3	2.40	105 50
β -actin-RT-R	TTCTCCATGTCGTCCCAGT	GU645235	54.6	240	107.78
$Ef-1\alpha$ -RT-F	GGATTGCCACACCGCTCACA	Efla,	61.4		
$E_{f-1\alpha}$ -RT-R	CACAGCCACCGTTTGCTTCAT	AB458256	58.9	223	98.83
AMPKα-RT-F	ACAAGCGTTTTGCTGATGC	Ampka,	57.0		
AMPKα-RT-R	CTCGCAAAGGTGCTATTCTC	OL364937	55.3	156	101.56
AMPKβ-RT-F	GATGGCAAAGATAGTGAAGAGC	Ampkb,	56.2		
			56.3	196	116.83
AMPKβ-RT-R	GTAGGTCCACAATGGCAACA	OL364938			
AMPKγ-RT-F	TCAAACGCCTCACGGAAAC	Ampkr	59.1	204	10 < 0.1
AMPKγ-RT-R	CGATGTAGCGGCAAGAATGT	XP042865	58.7	284	126.91
		543			
CAMKK-RT-F	TGATGGGGACGTGGAAGAA	Camkk	58.9		
CAMKK-RT-R	CGACACCGGATACTTCAGGA	XP042859	58.1	216	108.27
		770	50.1		
HK-RT-F	GCTTAGATTTCATCCGCACAG	Hk,	57.8	283	89.40
HK-RT-R	CAGTCAGCGTCAGTAGCATTG	OL364939	56.8	203	69.40
PFK-RT-F	GGAGAAGTTGCCGTATTTGA	Pfk,	55.4	170	02 72
PFK-RT-R	CACGATAGTCTGGGCTGTTT	OL364940	55.1	178	83.73
PK-RT-F	CTCGTGGTGATTTGGGAATT	Pk,	56.3		
PK-RT-R	TGAGTCAGTGGGCAGATGGA	OL364941	56.9	255	92.88
Glut1-RT-F	AACTGCGGACTGAACACCTC	Glut1,	54.7		
Glut1-RT-R	TCTTCGTAGGGCTCTTCGTG	OL364942	56.9	162	93.60
HIF1α-RT-F	CCCAGAAGAACAGCGAGAA	Hifla	54.7		
HIF1α-RT-R	TCAGGTAGGCAATGGTAAGC	XP042868		171	102.30
1111 ¹ 10-K1-K	ICAOOTAOOCAATOOTAAOC	035	54.5	1/1	102.30
Rictor-RT-F	TCAGGGCAGTGTTATGTTGG	Rictor,	55		
Rictor-RT-R	AAGGCGAGGTTAAGGAAAAG	OK143319	52.2	162	81.72
AKT-RT-F	GTTGACTGGTGGGGGTTATGGA	Akt,	57.1		
AKT-RT-R		KP419299	54.0	243	106.06
	GGTGATGTAGAAGGGGTGATT	KP419299	34.0		
Recombinant exp AMPKα-EX-F	TACTCAGTCGACATC	GAGGTCCC		GA	
AMPKα-EX-R	TACTCAGCGGCCGCTTAACGAGCTAGTTCTGT TACTCAGTCGACGGTCATTACCAGATCGGA				
AMPKα-EX-F2					
AMPKα-EX-R2	TACTCAGCGGCCGCC	ITCTGGAAC	CATTC	AIG	
SiRNA					
Primers		ce (5'-3')			
GFP- Oligo1	GATCACTAATACGACTCACTATA				
GFP- Oligo2	AACAAGAATTGGGACAACTCCCC				
GFP- Oligo3	AAGGAGTTGTCCCAATTCTTGCC	CTATAGTGA	GTCGT	CATTA(GTGATC
GFP- Oligo4	GATCACTAATACGACTCACTATA	GGGCAAGAA	ATTGG	GACAA	CTCCTT
AMPKa-Oligo1	GATCACTAATACGACTCACTATAGGGGGCTGCACCAGAAGTTATATTT				
AMPKa-Oligo2	AAATATAACTTCTGGTGCAGCCCCTATAGTGAGTCGTATTAGTGATC				
AMPKa-Oligo3					
AMPKα-Oligo4	AAGCTGCACCAGAAGTTATATCCCTATAGTGAGTCGTATTAGTGATC GATCACTAATACGACTCACTATAGGGATGGCCGTCTGGTTCTTGCTT				
AMPKβ-Oligo1					
AMPKβ-Oligo2	GATCACTAATACGACTCACTATAGGGCCTACTCTCCAGGGAGAATTT				
AMPKβ-Oligo3	AAATTCTCCCTGGAGAGTAGGCCCTATAGTGAGTCGTATTAGTGATC AACCTACTCTCCAGGGAGAATCCCTATAGTGAGTCGTATTAGTGATC				
AMPRp-Oligos AACCTACTCCCAGGGAGAATCCCTATAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTATTAGTGAGTCGTAGTGAGTCGTATTAGTGAGTCGTAGTGAGTCGTATTAGTGAGTCGTAGTGAGTCGTATTAGTGAGTCGTAGTGAGTCGTATTAGTGAGTCGTAGTGAGTCGTAGTGAGTCGTAGTGAGTCGTATTAGTGAGTCGTGGGGGGTTCTCCCCTGGAGAGGTAGGT					
	UAICACIAAIACUACICACIAIAC	JUGATICICC		AUAUI	AUUII
RNAi					

Supplementary Table 1. Sequences of primers used in the present study

GFP-Ri-F	TAATACGACTCACTATAGGGGGGGTGTACAGCTCGTCCATGC
GFP-Ri-R	TAATACGACTCACTATAGGGCTTGTACAGCTCGTCCATGC
CAMKK-Ri-F	GCGTAATACGACTCACTATAGGGCAATGGGCGTGACCTTAT
CAMKK-Ri-R	GCGTAATACGACTCACTATAGGTCAAGCGACCTCTTCCTGT
Rictor-Ri-F	GCGTAATACGACTCACTATAGGGAAGCAGATGGGAAAAATA
Rictor-Ri-R	GCGTAATACGACTCACTATAGGCAAGCGGAATGAATAGGAA
Raptor-Ri-F	GCGTAATACGACTCACTATAGGGAGACATAATGGAACAGAAG
Raptor-Ri-R	GCGTAATACGACTCACTATAGGTTGGATGAAAGAGTAACACA
AKT-Ri-F	GCGTAATACGACTCACTATAGGGGGGCGAGAAGCTCAAAGAA
AKT-Ri-R	GCGTAATACGACTCACTATAGGAACCCCACCAGTCAACACCT

1. Annealing temperature (°C)

2. Amplicon size (bp)

3. qPCR assay efficiencies of related genes (%)

Uncropped and unedited blot/gel images

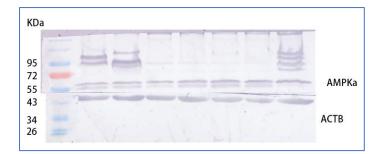


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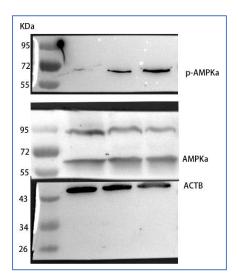
Supplementary Figure 10. Western blots of Figure 1b. The blot image is from two blot membranes.

KDa 95 72 55		AMPKa	
43	~		АСТВ
34 26		1	

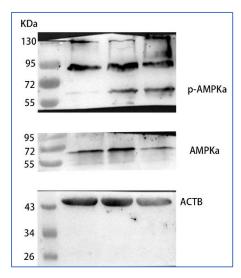
Supplementary Figure 11. Western blots of Figure 1e. The blot image is from two blot membranes.



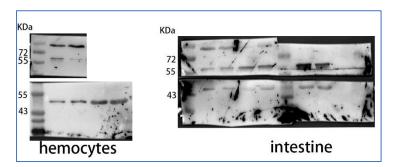
Supplementary Figure 12. Western blots of Figure 1f. The blot image is from the same blot membrane.



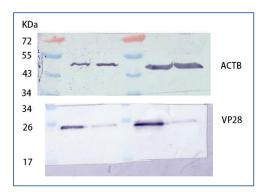
Supplementary Figure 13. Western blots of Figure 1g. The blot image is from three blot membranes.



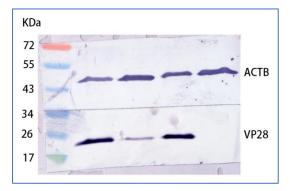
Supplementary Figure 14. Western blots of Figure 1h. The blot image is from three blot membranes.



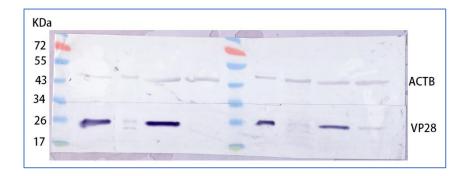
Supplementary Figure 15. Western blots of Figure 2b. The blot image is from three blot membranes.



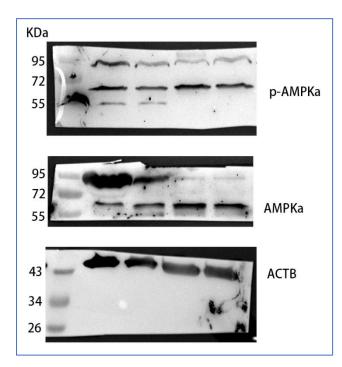
Supplementary Figure 16. Western blots of Figure 2d. The blot image is from two blot membranes.



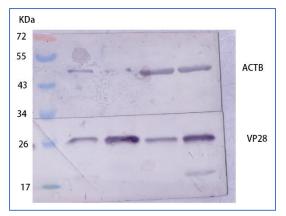
Supplementary Figure 17. Western blots of Figure 2i. The blot image is from the same blot membrane.



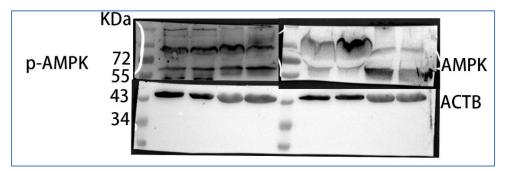
Supplementary Figure 18. Western blots of Figure 3b. The blot image is from the same blot membrane.



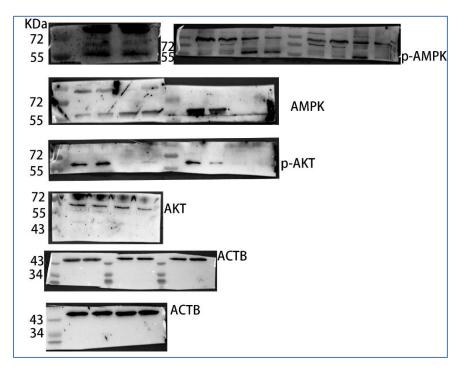
Supplementary Figure 19. Western blots of Figure 3c. The blot image is from three blot membranes.



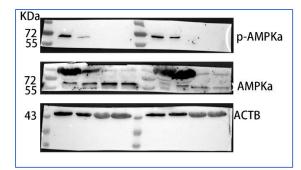
Supplementary Figure 20. Western blots of Figure 3f. The blot image is from the same blot membrane.



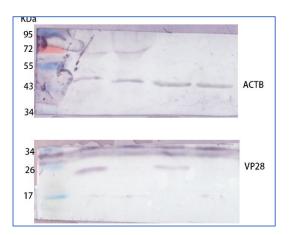
Supplementary Figure 21. Western blots of Figure 3g. The blot image is from the same blot membrane.



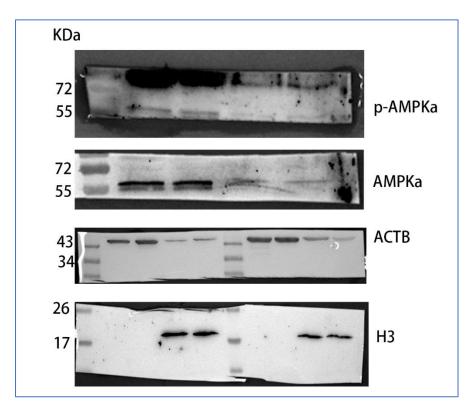
Supplementary Figure 22. Western blots of Figure 4c. The blot image is from seven blot membranes.



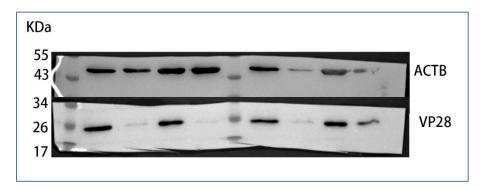
Supplementary Figure 23. Western blots of Figure 4f. The blot image is from three blot membranes.



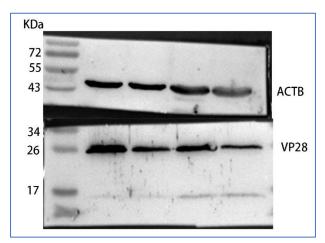
Supplementary Figure 24. Western blots of Figure 4h. The blot image is from two blot membranes.



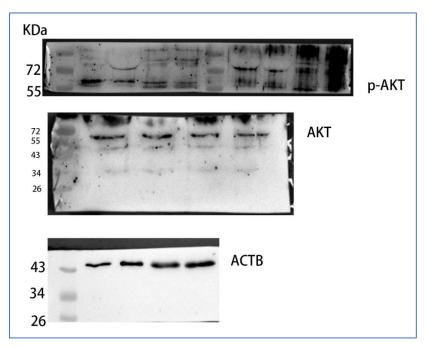
Supplementary Figure 25. Western blots of Figure5b. The blot image is from four blot membranes.



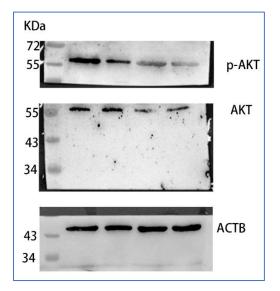
Supplementary Figure 26. Western blots of Figure 6c. The blot image is from the same blot membrane.



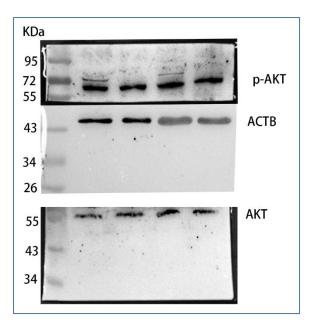
Supplementary Figure 27. Western blots of Figure 6h. The blot image is from the same blot membrane.



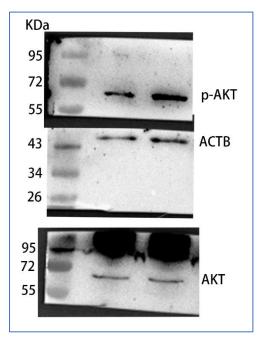
Supplementary Figure 28. Western blots of Figure 7a. The blot image is from three blot membranes.



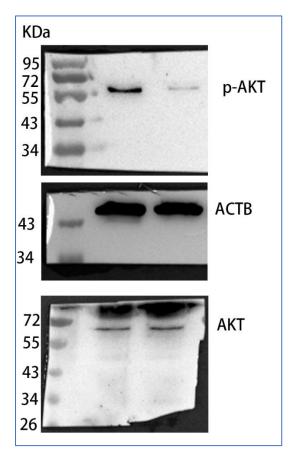
Supplementary Figure 29. Western blots of Figure 7b. The blot image is from three blot membranes.



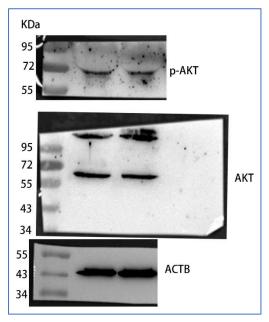
Supplementary Figure 30. Western blots of Figure 7c. The blot image is from three blot membranes.



Supplementary Figure 31. Western blots of Figure 7d. The blot image is from three blot membranes.



Supplementary Figure 32. Western blots of Figure 7e. The blot image is from three blot membranes.



Supplementary Figure 33. Western blots of Figure 7f. The blot image is from three blot membranes.