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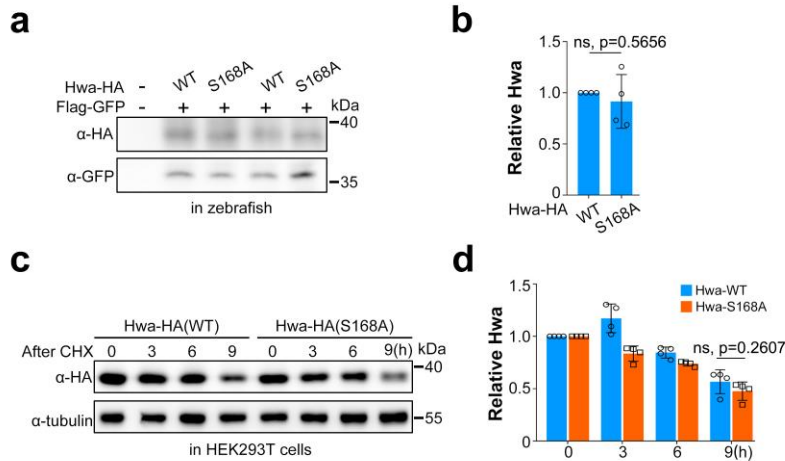
Supplementary Information for
A Huluwa phosphorylation switch regulates embryonic axis
induction

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Zhao Wang, Yaohui Li, Yunjing Wan, Weixi Chen, Shiqian Qi, Xianming Mo, Anming
Meng, Bo Xiang, Jing Chen

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This file includes:

- 1. Supplementary Fig. 1- 11**
- 2. Source data of Supplementary information :**

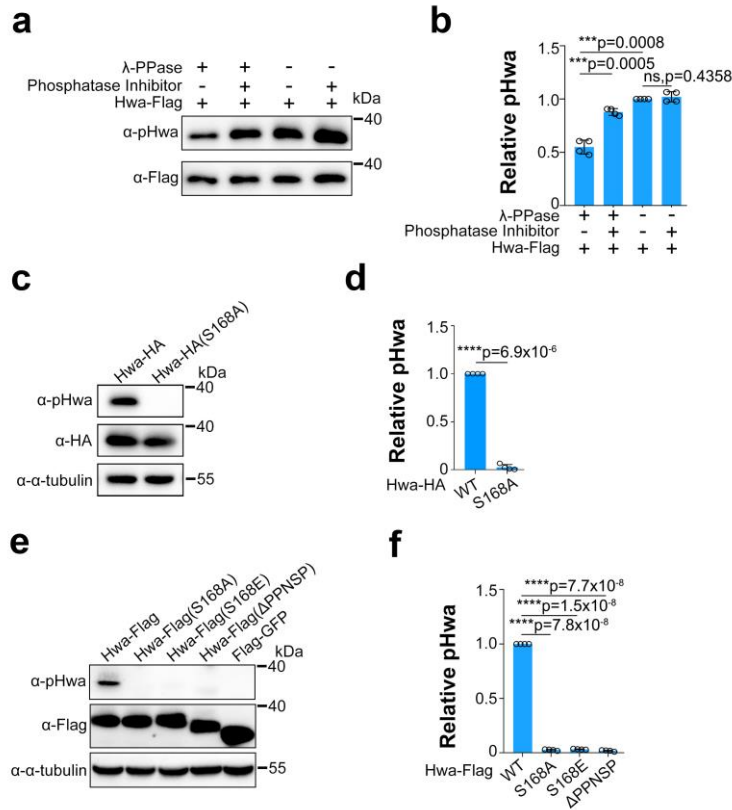


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17 **Supplementary Fig. 1. S168A point mutation does not affect the stability of Hwa.**

18 (a) Immunoblotting of Hwa-HA and Flag-GFP in zebrafish embryos injected with 200
 19 pg WT or S168A mutant *hwa*-HA mRNA with 200 pg Flag-*gfp* mRNA at the 1-cell
 20 stage and harvested at 4 hpf. (b) Quantifications of relative Hwa protein levels in
 21 embryos treated as in (a), N=4. (c) Immunoblotting of Hwa in cells transfected with
 22 wild-type or S168A Hwa-HA plasmids, followed by treatment with cycloheximide
 23 (CHX; 100 μ g/ml) and harvesting at the indicated time points. (d) Quantifications of
 24 relative Hwa protein levels treated as in (c), N=4. GFP (a) and α -tubulin (c) were used
 25 as references for quantification. (b & d) A two-tailed unpaired t test was performed and
 26 data were presented as mean \pm SD. N, number of biological replicates; Significant
 27 differences are indicated by ns \geq 0.05, * p < 0.05, ** p < 0.01, *** p < 0.001, and **** p
 28 < 0.0001. Source data are provided at the end of the Supplementary Information file.

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31 **Supplementary Fig. 2. Verification of the specificity of the pHwa antibody.**

32 (a) Immunoblotting of pHwa from samples treated with λ -PPase or λ -PPase &
 33 phosphatase inhibitor. (b) Quantifications of relative pHwa treated as in (a), N=4. (c)
 34 Immunoblotting of pHwa in HEK293T cells transfected with wild-type or S168A Hwa-
 35 HA mutant. (d) Quantifications of relative pHwa levels treated as in (c), N=4. (e)
 36 Immunoblotting of pHwa in HEK293T cells transfected with wild-type or mutants of
 37 Hwa-Flag. (f) Quantifications of relative pHwa treated as in (e), N=4. (b, d & f) A two-
 38 tailed unpaired t test was performed and data were presented as mean \pm SD. N, number
 39 of biological replicates; Significant differences are indicated by ns \geq 0.05, *p < 0.05,
 40 **p < 0.01, ***p < 0.001, and ****p < 0.0001. Source data are provided at the end of
 41 the Supplementary Information file.

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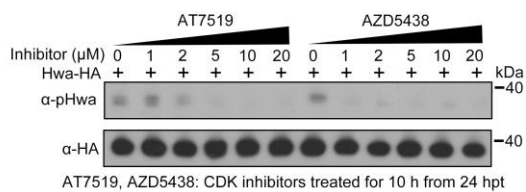
a

Locations (AA)	Phosphorylated Sites	HMM Bit Score	E-value	Catalytic Kinases	HMMs	Logo
168	VPPNSPVLR	1.8	0.032	CDK	HMM	

<http://kinasephos.mbc.nctu.edu.tw/predict.php>

CDK substrate	SPxxK/R
Hwa	VPPNSPVLR

b



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Supplementary Fig. 3. AT7519 and AZD5438 decreased pHwa in HEK293T cells.

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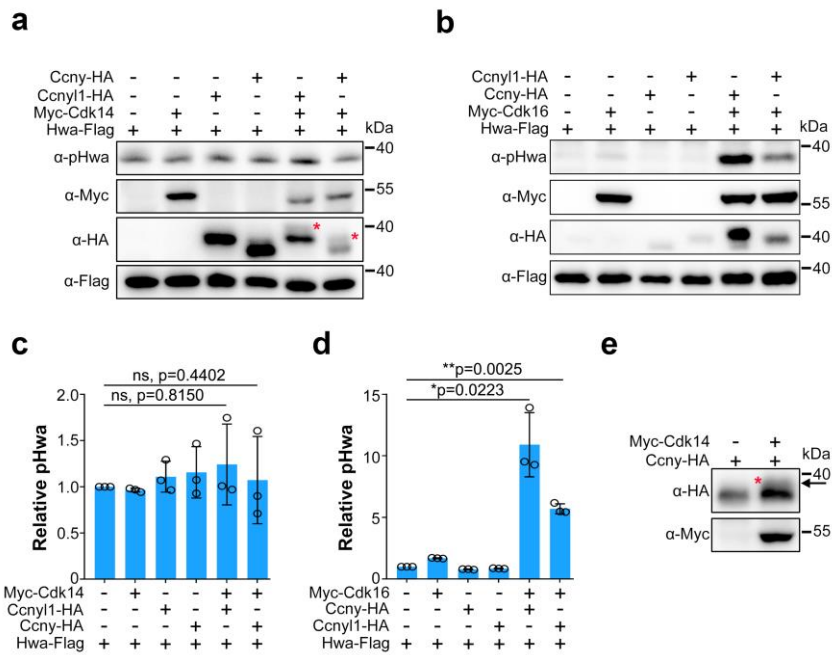
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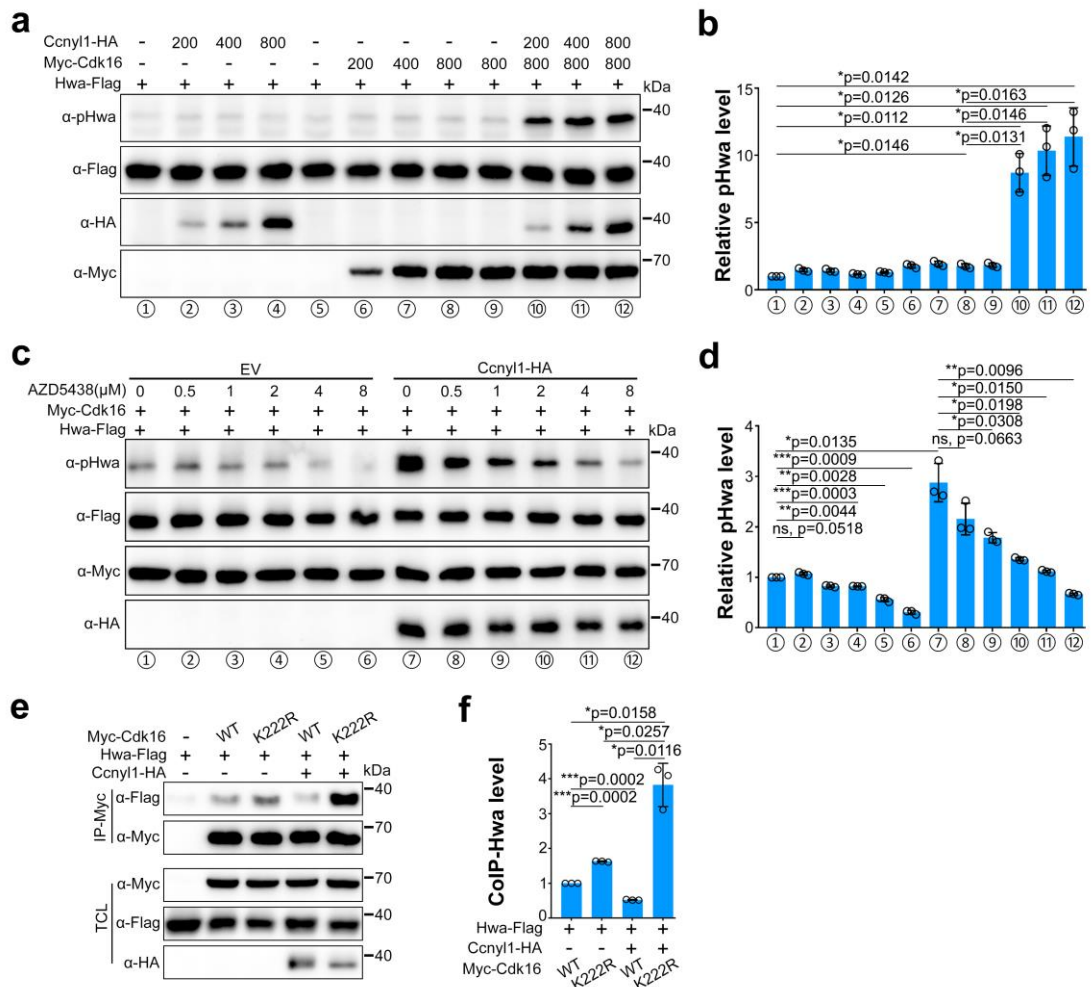
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55 **Supplementary Fig. 4. Cdk16, but not Cdk14, elevated p_{Hwa} with Ccny/Ccny11.**
56 (a) Immunoblotting of p_{Hwa} in HEK293T cells transfected with Hwa-Flag and Myc-
57 Cdk14, in the absence or presence of Cyclin Y proteins (Ccny/Ccny11-HA). (b)
58 Immunoblotting of p_{Hwa} in HEK293T cells transfected with Hwa-Flag and Myc-
59 Cdk16, in the absence or presence of Cyclin Y (Ccny/Ccny11-HA). Total Hwa-Flag
60 was applied as the reference. (c) and (d) Statistics of relative p_{Hwa} in (a) and (b),
61 respectively, N=3. (e) Immunoblotting of phosphorylated Ccny (a migration-shift band
62 indicated by the black arrow) in HEK293T cells transfected with Ccny-HA alone or
63 cotransfected with Myc-Cdk14. The red star indicates the migration-shift band of
64 phosphorylated Ccny/Ccny11 in (a & e). Total Hwa proteins (a & b) were used as
65 references for quantification. (c & d) A two-tailed unpaired t test was performed and
66 data were presented as mean ± SD. N, number of biological replicates; Significant
67 differences are indicated by ns ≥ 0.05, *p < 0.05, **p < 0.01, ***p < 0.001, and ****p
68 < 0.0001, with individual p-values shown. Source data are provided at the end of the
69 Supplementary Information file.

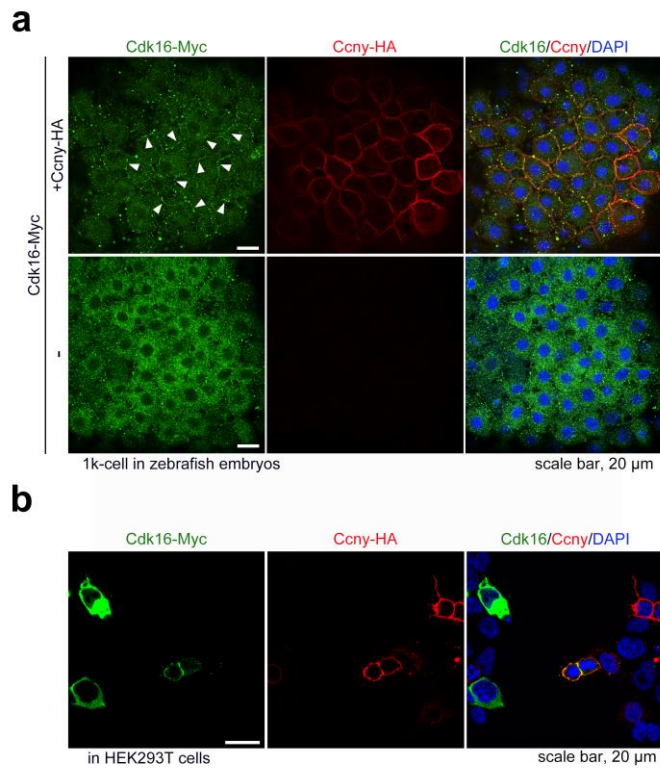
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72 **Supplementary Fig. 5. Cdk16 interacted with and phosphorylated Hwa.**

73 (a) Immunoblotting of phosphorylated Hwa protein at Ser168 (pHwa) from HEK293T
74 cells transfected with Hwa-Flag and Myc-Cdk16, in the absence or presence of Ccnyl1-
75 HA. (b) Quantifications of relative pHwa levels in HEK293T cells treated as in (a),
76 N=3. (c) Immunoblotting of pHwa from HEK293T cells cotransfected with Myc-
77 Cdk16 and Ccnyl1-HA, followed by treatment with different doses of the CDK
78 inhibitor AZD5438 (0-8 μM). (d) Quantifications of relative pHwa levels in HEK293T
79 cells treated as in (c), N=3. (e) Coimmunoprecipitation of Hwa-Flag with wild-type and
80 K222R mutant Myc-Cdk16 in the absence or presence of Ccnyl1-HA. The kinase-dead
81 form Cdk16 showed a stronger interaction with Hwa in the presence of Ccnyl1-HA. (f)
82 Quantifications of relative Co-IP Hwa in HEK293T cells treated as in (e), N=3. Total
83 Hwa proteins (a, c & e) were used as references for quantification in immunoblotting.
84 (b, d & f) A two-tailed unpaired t test was performed and data were presented as
85 mean ± SD. N, number of biological replicates; Significant differences are indicated by
86 ns ≥ 0.05, *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001, with individual p-
87 value illustrated. Source data are provided at the end of the Supplementary Information
88 file.



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90 **Supplementary Fig. 6. Cdk16 was recruited to plasma membrane by Ccny.**

91 (a) Immunofluorescence of Cdk16-Myc and Ccny-HA in zebrafish embryos at the 1k-

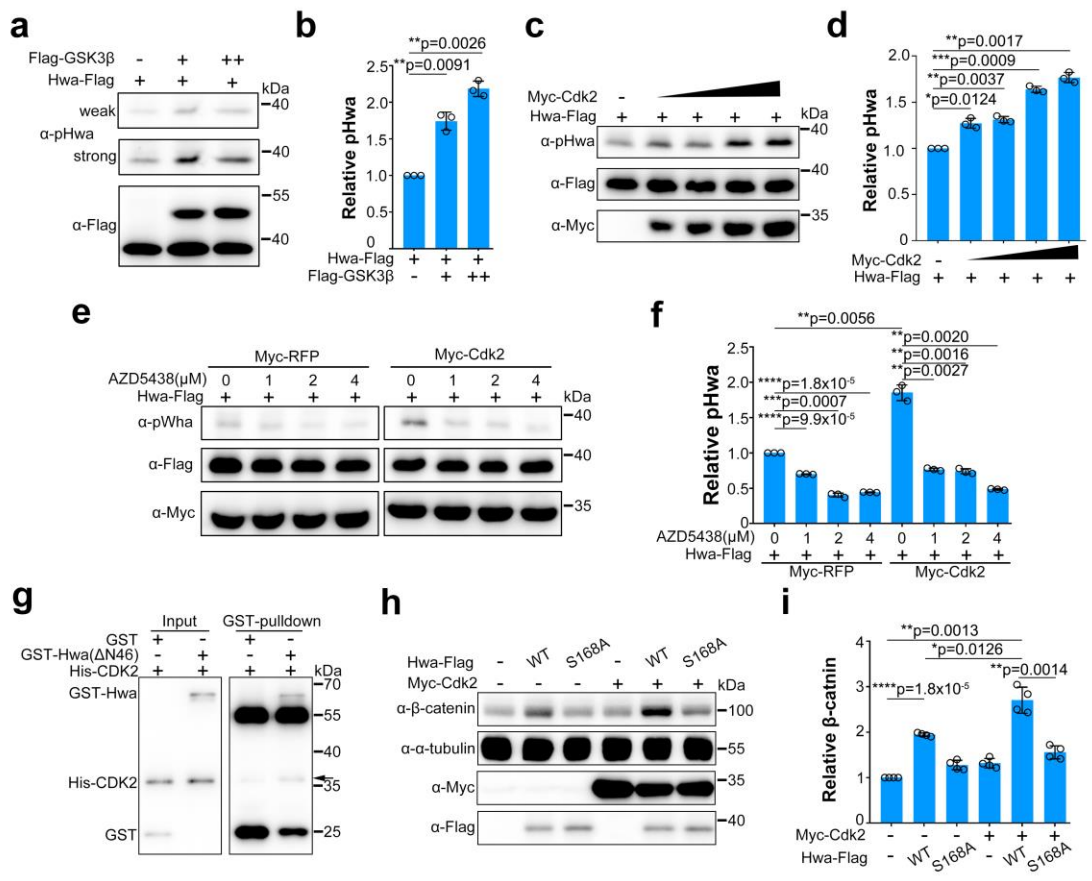
92 cell stage, with arrowheads indicating the plasma membrane located Cdk16-Myc, N=3.

93 (b) Immunofluorescence of Cdk16-Myc and Ccny-HA in HEK293T cells, N=3. Cells

94 were stained with DAPI in blue; Scale bar, 20 μm. N, number of biological replicates.

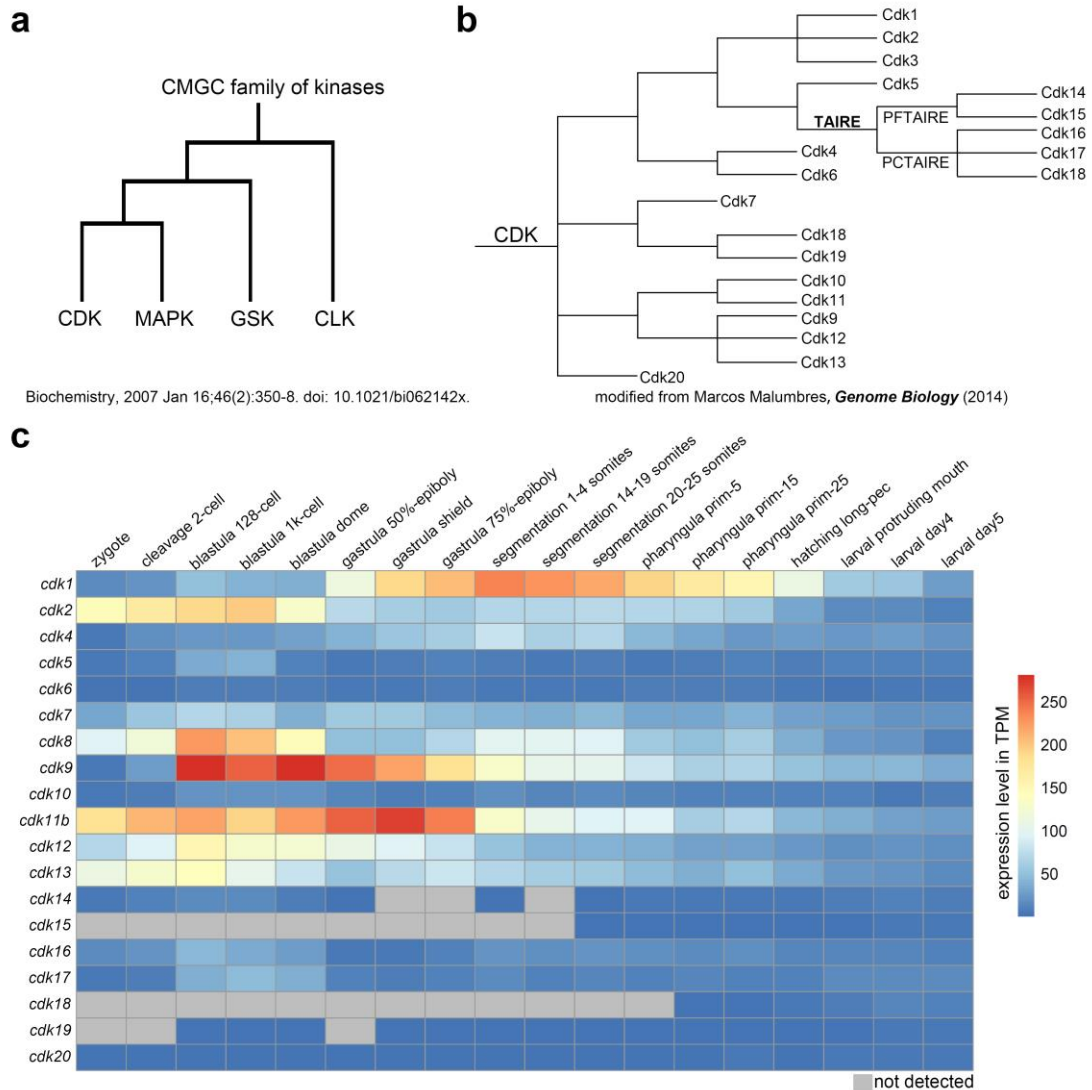
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Supplementary Fig. 7. GSK3β and Cdk2 phosphorylates Hwa at Ser168.

(a) Immunoblotting of pHwa from HEK293T cells transfected with Hwa-Flag with or without Flag-tagged human GSK3β. (b) Quantifications of relative pHwa levels in HEK293T cells treated as in (a), N=3. (c) Immunoblotting of pHwa from HEK293T cells transfected with Hwa-Flag with different doses of Myc-Cdk2. (d) Quantifications of relative pHwa levels in HEK293T cells treated as in (c), N=3. (e) Immunoblotting of pHwa from HEK293T cells cotransfected Hwa-Flag with Myc-RFP or Myc-Cdk2, followed by treatment with different doses of the inhibitor AZD5438 (0-4 μM). (f) Quantifications of relative pHwa levels in HEK293T cells treated as in (e), N=3. (g) *in vitro* GST-pulldown assay of His-CDK2 with GST-Hwa(ΔN46) or GST, N=3. The arrow indicated the His-CDK2 protein. (h) Immunoblotting of cytosolic/active β-catenin in HEK293T cells transfected with Hwa-Flag (WT, S168A) alone or together with Myc-Cdk2. (i) Quantifications of relative pHwa levels in HEK293T cells treated as in (h), N=4. Total Hwa (a, c & e) and α-tubulin (h) proteins were used as references in immunoblotting. (b, d, f & i) A two-tailed unpaired t test was performed and data were presented as mean ± SD. N, number of biological replicates; Significant differences are indicated by ns ≥ 0.05, *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001, with individual p-value labeled. Source data are provided at the end of the Supplementary Information file.



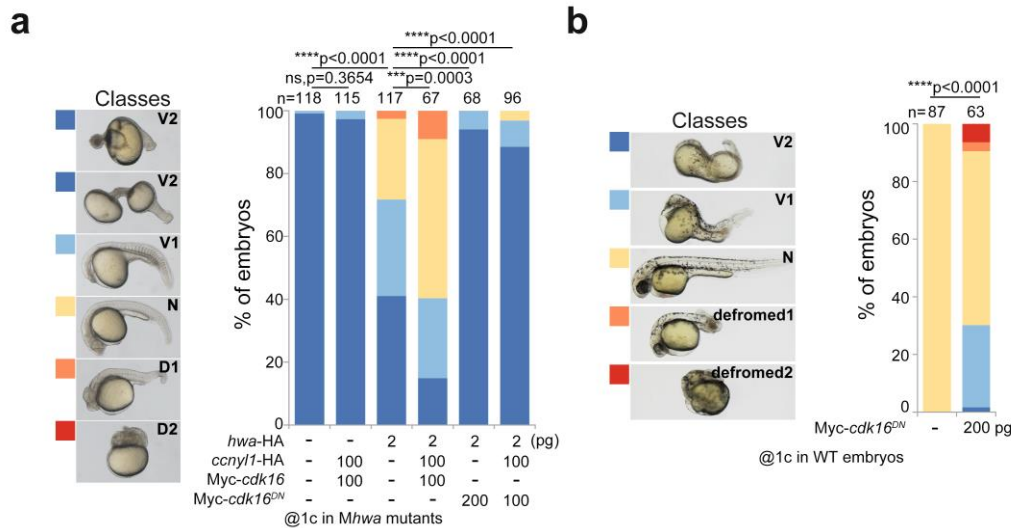
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119 **Supplementary Fig. 8. CMGC kinase family and expression profile of *cdk* genes**
 120 **in zebrafish.**

121 (a) The classification of the CMGC kinase family. (b) Members of the CDK subfamily,
 122 derived from Marcos Malumbres, *Genome Biology* (2014). (c) Expression levels of
 123 different *cdk* genes during zebrafish early developmental stages were plotted with data
 124 from Expression Atlas. Expression levels are displayed in TPM (transcripts per million)
 125 with color coding, while gray color indicates “not detected”.

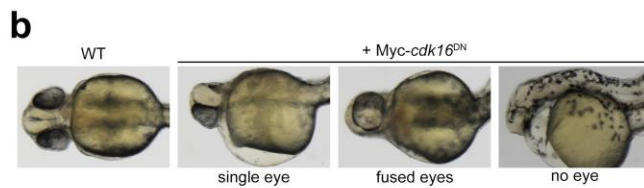
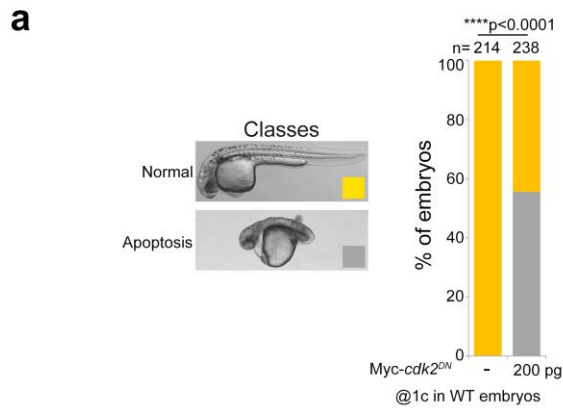
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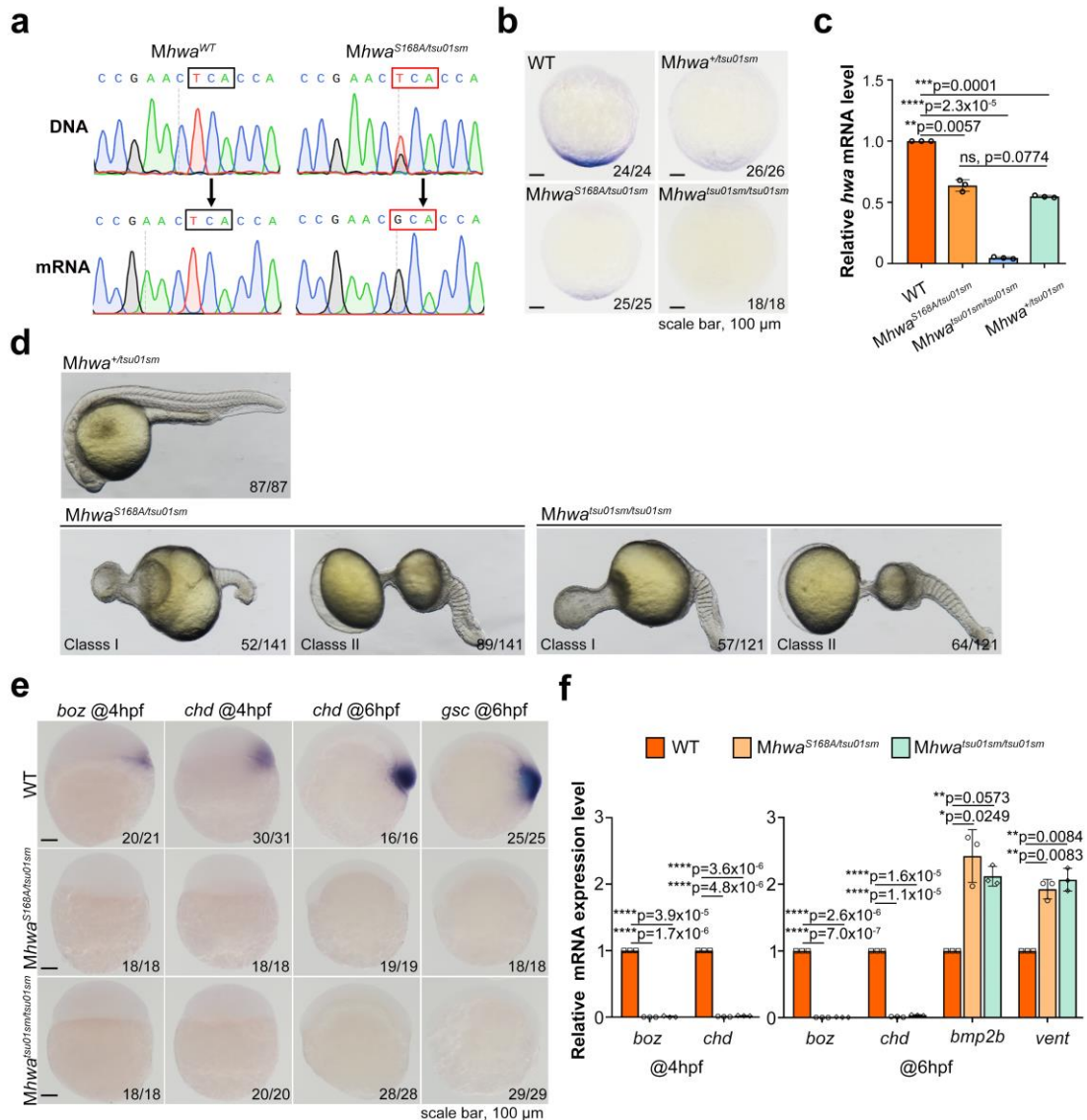
129 **Supplementary Fig. 9. Cdk16^{DN} hindered the axis-inducing function of Hwa.**
 130 (a) Rescue efficiency of *Mhwa*^{tsu01sm} mutant embryos with a low dosage of *hwa* mRNA
 131 (2 pg) together with wild-type and the dominant negative form of *Myc-cdk16*^{DN} mRNA,
 132 N=2. *Myc-cdk16* mRNA coexpressed with *ccny11*-HA mRNA failed to rescue the
 133 *Mhwa*^{tsu01sm/tsu01sm} mutant phenotype without *hwa* mRNA. (b) Effects of overexpression
 134 of dominant negative *cdk16* (*cdk16*^{DN}) mRNAs in WT embryos: some embryos shown
 135 deformed phenotypes, N=2. V2<V1<N<D1<D2; V, ventralized; N, normal; D,
 136 dorsalized. (a & b) A two-tailed Fisher's exact test was performed to evaluate
 137 differences between treatments (all phenotypes were divided into two groups:
 138 Unchanged [Class I] and Changed [Class II-V or Class II-IV]). N, number of biological
 139 replicates; n, total number of embryos in each treatment; Significant differences are
 140 indicated by ns ≥ 0.05, *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. Source
 141 data are provided at the end of the Supplementary Information file.
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144 **Supplementary Fig. 10. Phenotypes of *Myc-cdk2*^{DN} and *Myc-cdk16*^{DN}**
 145 **overexpression in WT embryos**

146 (a) Effects of overexpression of 200 pg dominant negative *cdk2* (*cdk2*^{DN}) mRNA in
 147 wild-type embryos, N=4. (b) Injection of 200 pg *Myc-cdk16*^{DN} mRNA in WT embryos
 148 at the 1-cell stage resulted in some embryos with single-eye/fused-eyes/no-eye
 149 phenotypes, which was similar to that of blocking nodal signaling. (a) A two-tailed
 150 Fisher's exact test was performed to evaluate differences between treatments. N,
 151 number of biological replicates; n, total number of embryos in each treatment;
 152 Significant differences are indicated by ns \geq 0.05, * p < 0.05, ** p < 0.01, *** p < 0.001,
 153 and **** p < 0.0001. Source data are provided at the end of the Supplementary
 154 Information file.



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156 **Supplementary Fig. 11. The endogenous function of the S168A mutation in**
 157 **zebrafish embryos.**

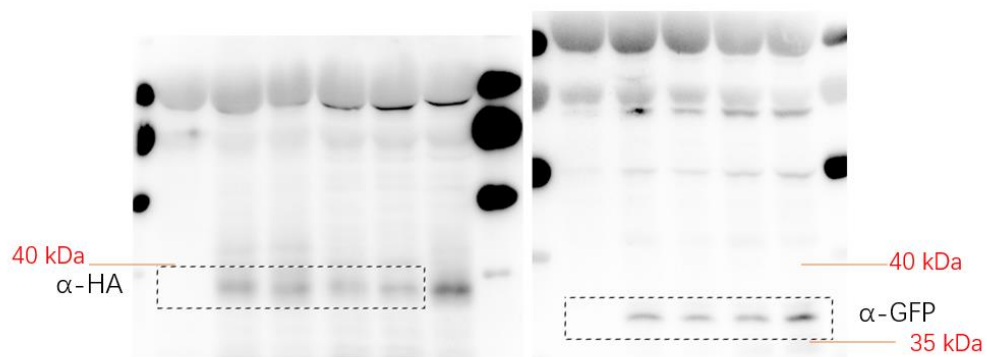
158 (a) Sanger sequencing of WT and *Mhwa*^{S168A/tsu01sm} females (DNA in the upper panel)
 159 and derived embryos (mRNA in the lower panel) confirmed the successful construction
 160 of the *hwa*^{S168A} mutant line. WISH (b) and RT-qPCR (c) showed that the *hwa* mRNA
 161 levels were similar in *Mhwa*^{S168A/tsu01sm} and *Mhwa*^{+/tsu01sm} embryos, while the expression
 162 was almost absent in *Mhwa*^{tsu01sm/tsu01sm} embryos, N=3. (d) Phenotypes of
 163 *Mhwa*^{S168A/tsu01sm} embryos were similar to those from *Mhwa*^{tsu01sm/tsu01sm} mutants at 24
 164 hpf and could be divided into two classes: both lacking an embryonic axis; while
 165 *Mhwa*^{+/tsu01sm} embryos were all normal. (e) Loss of expression of dorsal/organizer
 166 markers (*boz* and *chd* at 4 hpf; *chd* and *gsc* at 6 hpf) was detected by WISH in
 167 *Mhwa*^{S168A/tsu01sm} and *Mhwa*^{tsu01sm/tsu01sm} embryos. (f) Marker genes were quantified by
 168 RT-qPCR at 4 hpf and 6 hpf for WT, *Mhwa*^{S168A/tsu01sm} and *Mhwa*^{tsu01sm/tsu01sm} embryos,
 169 N=3. (c & f) A two-tailed unpaired t test was performed and data were presented as
 170 mean ± SD. N, number of biological replicates; Significant differences are indicated by
 171 ns ≥ 0.05, *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001, with individual p-

172 value labeled in each graph. Source data are provided at the end of the Supplementary
173 Information file.

174 **Source data of supplementary information :**

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176 **Source data of Supplementary Fig. 1:**



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178 Supplementary Fig.1a, uncropped scans of all blots, detailed information has been labeled in the figure.

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					Average	STDEV	p-value
WT-HA	1	1	1	1	1	0	
S168A-HA	1.254411	0.990007	0.687156	0.730356	0.915483	0.262572	0.565557

182 Supplementary Fig.1b, Specific values of quantifications of relative Hwa protein levels in embryos.

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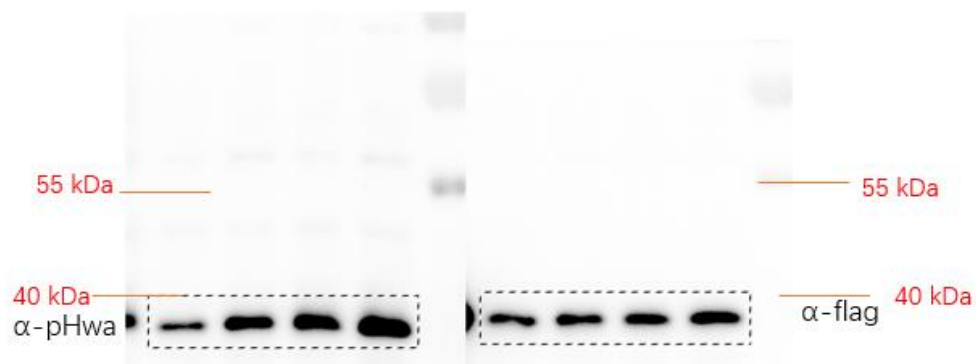
Supplementary Fig.1c, uncropped scans of all blots, detailed information has been labeled in the figure.

					Average	STDEV
WT-0	1	1	1	1	1	0
WT-3	1.304104	1.094027	1.019931	1.271177	1.17231	0.137225
WT-6	0.835441	0.881294	0.774472	0.889713	0.84523	0.052858
WT-9	0.396793	0.63434	0.600264	0.633806	0.566301	0.114124
S168A-0	1	1	1	1	1	0
S168A-3	0.729183	0.879277	0.847093	0.885779	0.835333	0.07276
S168A-6	0.740513	0.705717	0.754782	0.756497	0.739377	0.023556
S168A-9	0.347149	0.523938	0.49762	0.536679	0.476347	0.087653

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Supplementary Fig.1d, Specific values of quantifications of relative Hwa protein levels.

193 **Source data of Supplementary Fig. 2:**



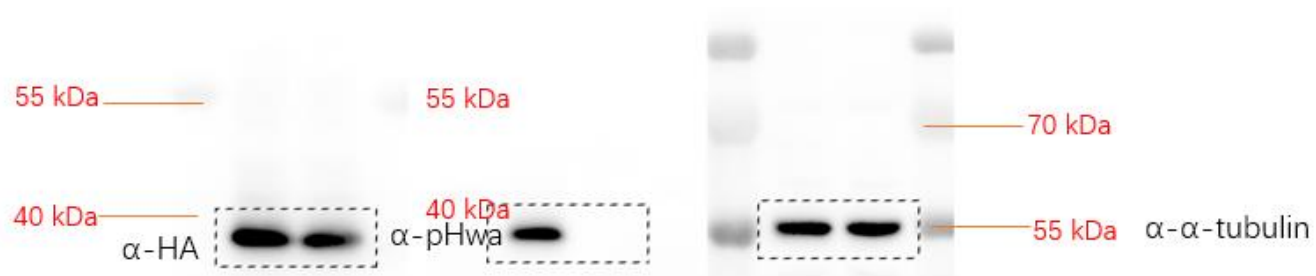
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195 Supplementary Fig.2a, uncropped scans of all blots, detailed information has been labeled in the figure.

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					average	STDEV
Ctr	1	1	1	1	1	0
Inhibitor	1.061319	0.980177	1.065272	0.979649	1.021604	0.048168
PPase	0.604031	0.476713	0.605391	0.511423	0.54939	0.065435
PPase+Inhibitor	0.906551	0.842963	0.905817	0.860212	0.878886	0.0323

199 Supplementary Fig.2b, Specific values of quantifications of relative pHwa.

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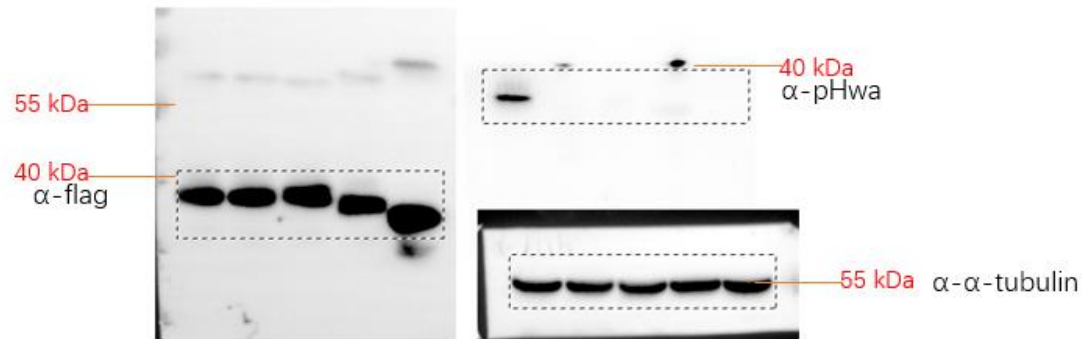


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202 Supplementary Fig.2c, uncropped scans of all blots, detailed information has been labeled in the figure.
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pHwa					Average	STDEV	p-Value
WT	1	1	1	1	1	0	
S168A	0.00816	0.022938	0.06629	0.003767	0.025289	0.028538	6.91E-06

206 Supplementary Fig.2d, Specific values of quantifications of relative pHwa levels.
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209 Supplementary Fig.2e, uncropped scans of all blots, detailed information has been labeled in the figure.

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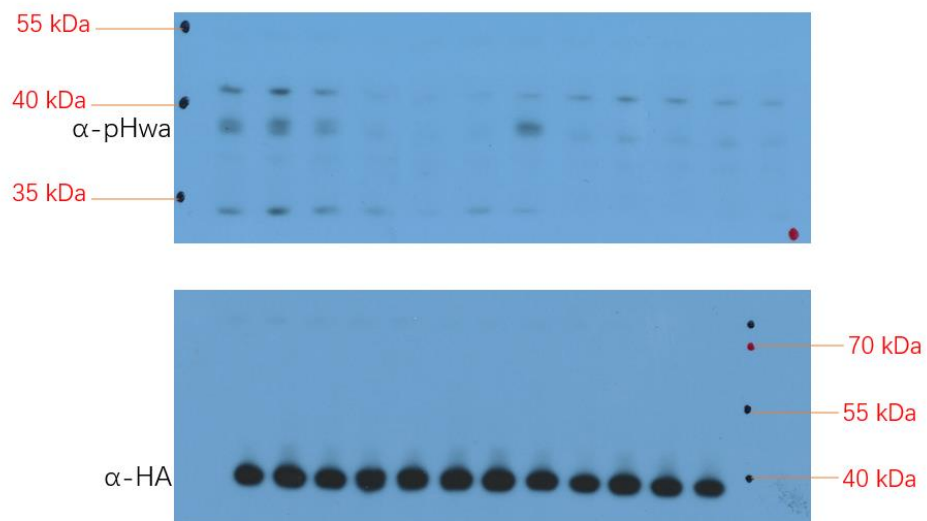
					Average	STDEV	P-Value
Flag-Hwa(WT)	1	1	1	1	1	0	
Flag-Hwa(S168A)	0.018082	0.030109	0.032583	0.025168	0.026485	0.006394	7.81E-08
Flag-Hwa(S168E)	0.029132	0.033351	0.037518	0.03077	0.032693	0.003656	1.49E-08
Flag-Hwa(dPPNSp)	0.019458	0.021089	0.009231	0.02395	0.018432	0.006409	7.67E-08

213 Supplementary Fig.2f, Specific values of quantifications of relative pHwa .

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218 **Source data of Supplementary Fig. 3:**

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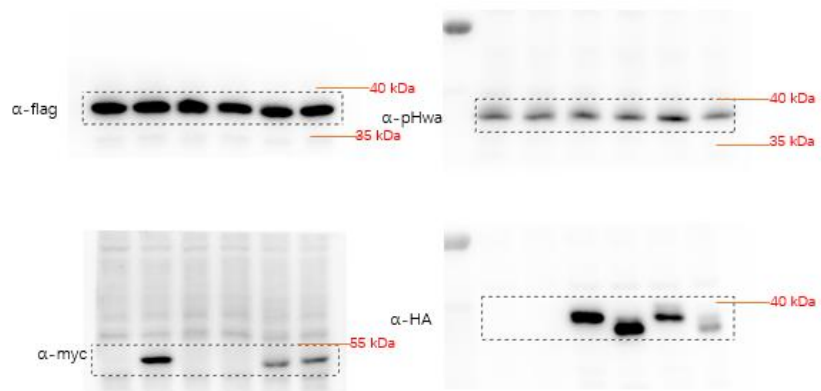


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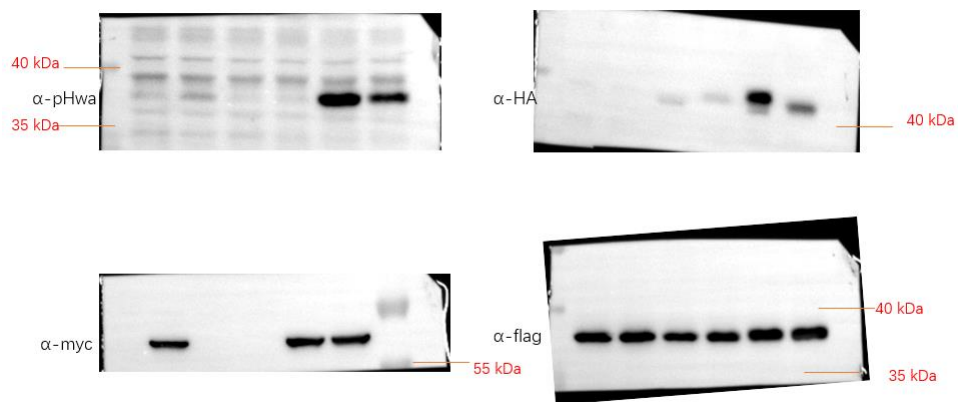
221 Supplementary Fig.3b, uncropped scans of all gels, detailed information has been labeled in the figure.

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223 **Source data of Supplementary Fig. 4:**



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225 Supplementary Fig.4a, uncropped scans of all blots, detailed information has been labeled in the figure.
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228 Supplementary Fig.4b, uncropped scans of all blots, detailed information has been labeled in the figure.

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				Average	STDEV
EV	1	1	1	1	0
Cdk14	0.967145	0.941568	0.982109	0.963607	0.020501
Ccny	1.29237	0.964266	1.069514	1.108717	0.167528
Ccny11	1.467017	0.928605	1.074769	1.156797	0.278421
Cdk14+Ccny	1.746816	0.970754	1.007165	1.241578	0.437927
Cdk14+Ccny11	1.607466	0.900232	0.710281	1.07266	0.472794

230 Supplementary Fig.4c, Statistics of relative pHwa protein.

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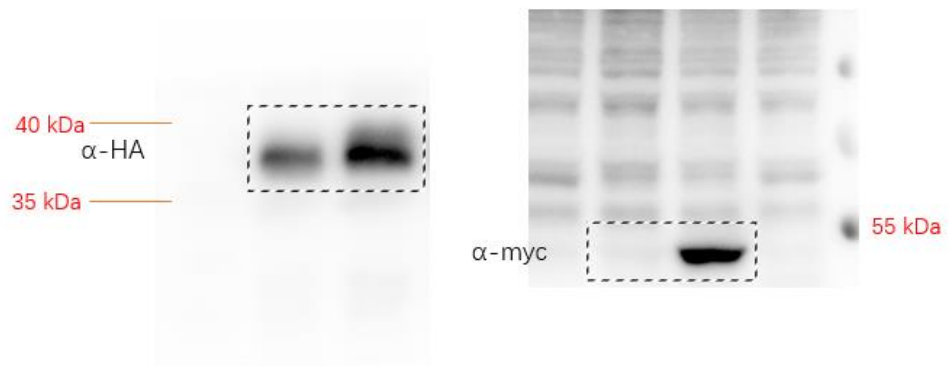
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				Average	STDEV
EV	1	1	1	1	0
Cdk16	1.693286	1.6602	1.713051	1.688845	0.026704
Ccny	0.798929	0.753302	0.802192	0.784808	0.027333
Ccny11	0.836635	0.844341	0.885442	0.855473	0.026239
Cdk16+Ccny	13.90698	9.264641	9.534279	10.90197	2.60591
Cdk16+Ccny11	6.171502	5.482484	5.445777	5.699921	0.408814

235 Supplementary Fig.4d, Statistics of relative pHwa protein.

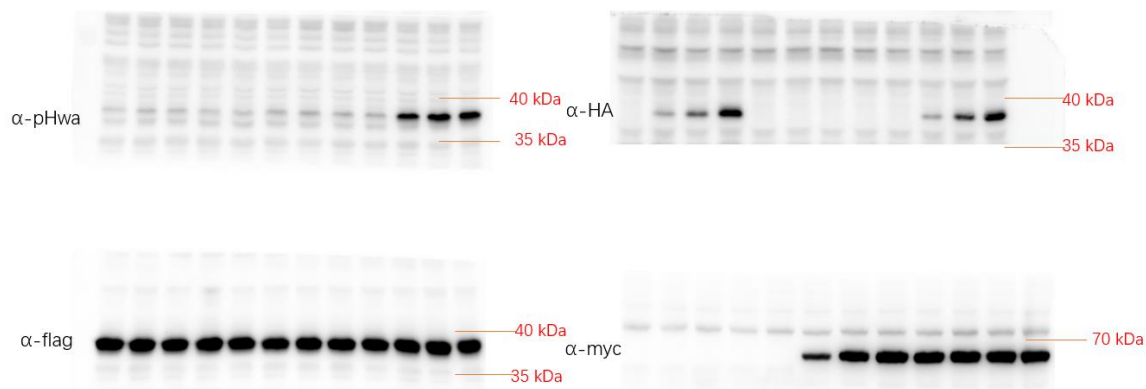
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239 Supplementary Fig.4e, uncropped scans of all blots, detailed information has been labeled in the figure.
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241 **Source data of Supplementary Fig. 5:**



242
243 Supplementary Fig.5a, uncropped scans of all blots, detailed information has been labeled in the figure.

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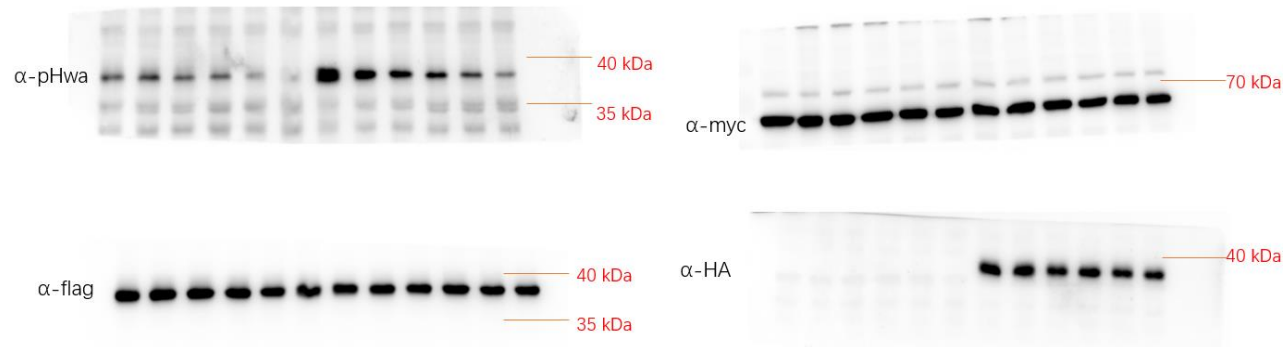
				Average	STDEV
Cdk16-1	1	1	1	1	0
Cdk16-2	1.427467	1.605504	1.363785	1.465585	0.125287
Cdk16-3	1.379244	1.551027	1.338199	1.422823	0.112908
Cdk16-4	1.142319	1.22073	1.132826	1.165292	0.048245
Cdk16-5	1.298487	1.354297	1.231817	1.294867	0.06132
Cdk16-6	1.819608	1.894624	1.615389	1.77654	0.144513
Cdk16-7	1.915496	2.143667	1.783137	1.947433	0.182375
Cdk16-8	1.730444	1.936633	1.618729	1.761935	0.161275
Cdk16-9	1.80057	2.008938	1.690429	1.833313	0.161759
Cdk16-10	8.79421	10.07848	7.237377	8.703356	1.422729

Cdk16-11	10.63168	12.01579	8.383637	10.3437	1.833123
Cdk16-12	11.87496	13.23049	8.995402	11.36695	2.162766

246 Supplementary Fig.5b, Specific values of quantifications of relative pHwa levels in HEK293T cells.

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250 Supplementary Fig.5c, uncropped scans of all blots, detailed information has been labeled in the figure.

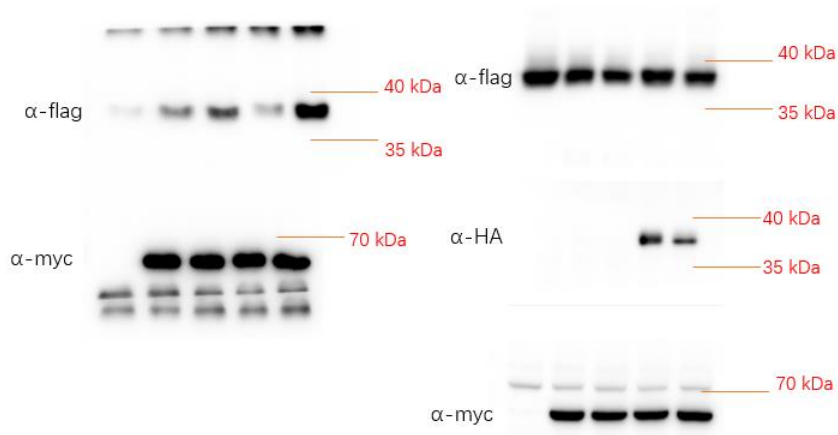
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				Average	STDEV
EV_AZD 0	1	1	1	1	0
EV_AZD 0.5	1.043391	1.10078	1.066871	1.070347	0.028852
EV_AZD 1	0.801592	0.829972	0.840798	0.82412	0.020247
EV_AZD 2	0.817593	0.817212	0.827173	0.820659	0.005644
EV_AZD 4	0.577243	0.51176	0.585513	0.558172	0.040406
EV_AZD 8	0.325747	0.261532	0.325182	0.304153	0.036913
Cdk16+Ccn11_AZD 0	2.694781	3.315196	2.621445	2.877141	0.381135
Cdk16+Ccn11_AZD 0.5	1.981456	2.515808	1.961509	2.152924	0.314425

Cdk16+Ccnyl1_AZD 1	1.74355	1.900792	1.702903	1.782415	0.104512
Cdk16+Ccnyl1_AZD 2	1.342739	1.392122	1.336692	1.357185	0.030408
Cdk16+Ccnyl1_AZD 4	1.141979	1.096022	1.112227	1.116743	0.023309
Cdk16+Ccnyl1_AZD 8	0.685284	0.646207	0.670499	0.66733	0.01973

252 Supplementary Fig.5d, Specific values of quantifications of relative pHwa levels in HEK293T cells.

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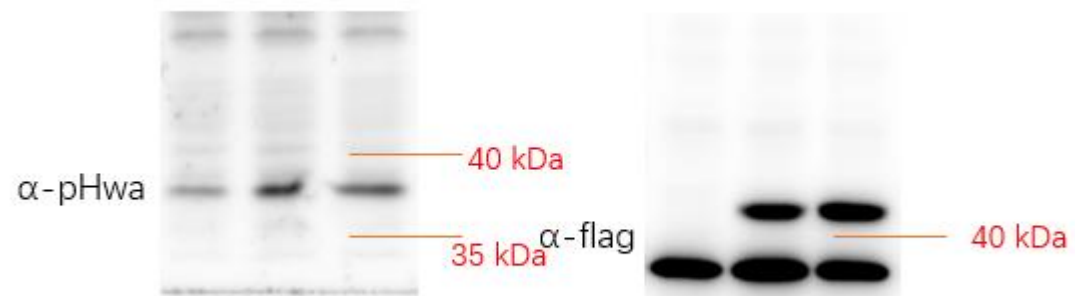
255 Supplementary Fig.5e, uncropped scans of all blots, detailed information has been labeled in the figure.

256

				Average	STDEV
CDK16_WT	1	1	1	1	0
CDK16_K222R	1.639776	1.608304	1.632062	1.626714	0.016404
CDK16_WT+Ccnyl1	0.517029	0.516846	0.535308	0.523061	0.010607
CDK16_K222R+Ccnyl1	3.112329	4.265601	4.095706	3.824545	0.62262

257 Supplementary Fig.5f, Specific values of quantifications of relative Co-IP Hwa in HEK293T cells.

258 **Source data of Supplementary Fig. 7:**



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260 Supplementary Fig.7a, uncropped scans of all blots, detailed information has been labeled in the figure.

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				Average	STDEV	P-Value
GSK3b-	1	1	1	1	0	
GSK3b+	1.825649	1.797905	1.599728	1.741094	0.12321	0.009088
GSK3b++	2.29869	2.091101	2.161732	2.183841	0.105546	0.002639

264 Supplementary Fig.7b, Specific values of quantifications of relative pHwa levels in HEK293T cells.

265



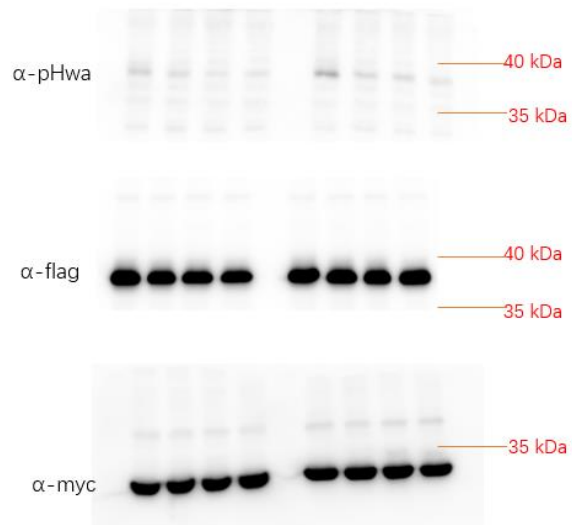
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Supplementary Fig.7c, uncropped scans of all blots, detailed information has been labeled in the figure.

				Average	STDEV
Cdk2-0	1	1	1	1	0
Cdk2-1	1.260741	1.225759	1.329658	1.272053	0.052865
Cdk2-2	1.289344	1.296922	1.350254	1.312173	0.033196
Cdk2-3	1.614944	1.676971	1.624961	1.638959	0.033299
Cdk2-4	1.756869	1.826192	1.716371	1.766477	0.055537

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Supplementary Fig.7d, Specific values of quantifications of relative pHwa levels in HEK293T cells.

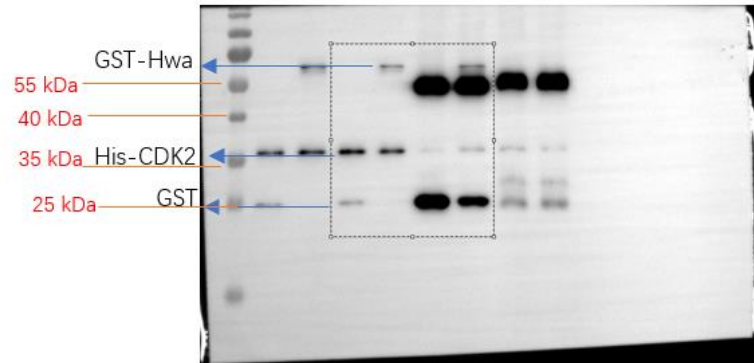


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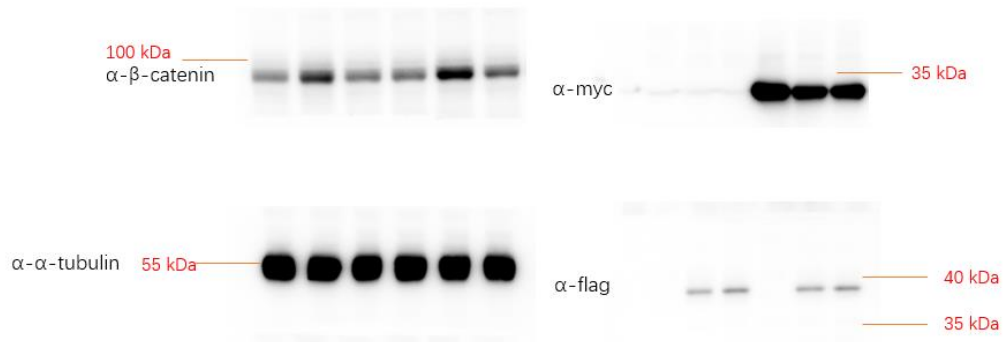
Supplementary Fig.7e, uncropped scans of all blots, detailed information has been labeled in the figure.

				Average	STDEV
EV_AZD-0	1	1	1	1	0
EV_AZD-1	0.703841	0.694373	0.70273	0.700315	0.005176
EV_AZD-2	0.369688	0.419241	0.414047	0.400992	0.027234
EV_AZD-4	0.435893	0.443512	0.442551	0.440652	0.004149
Cdk2_AZD-0	1.864454	1.734913	1.956351	1.851906	0.111251
Cdk2_AZD-1	0.763578	0.746115	0.785151	0.764948	0.019554
Cdk2_AZD-2	0.727468	0.710568	0.778845	0.73896	0.03556
Cdk2_AZD-4	0.472208	0.485426	0.492921	0.483518	0.010487

276 Supplementary Fig.7f, Specific values of quantifications of relative pHwa leves in HEK293T cells.



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 278 Supplementary Fig.7g, uncropped scans of blot, detailed information has been labeled in the figure.
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 283 Supplementary Fig.7h, uncropped scans of all blots, detailed information has been labeled in the figure.

284

					Average	STDEV
EV	1	1	1	1	1	0
Hwa-WT	1.930155	1.96052	1.98722	1.899468	1.944341	0.037926
Hwa-S168A	1.398092	1.323582	1.195585	1.179132	1.274098	0.10489
EV+Cdk2	1.459175	1.307877	1.271426	1.216135	1.313653	0.104088
Hwa-WT+Cdk2	2.430845	3.043058	2.836223	2.499181	2.702327	0.288095
Hwa-S168A+Cdk2	1.721601	1.631977	1.409564	1.460514	1.555914	0.145782

285 Supplementary Fig. 7i, Specific values of quantifications of relative pHwa levels in HEK293T cells.

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289 **Source data of Supplementary Fig. 9:**

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	V2	V1	N	D1	D2	
Sum	ClassI	ClassII	ClassIII	ClassIV	ClassV	Total
Mhwa	117	1	0	0	0	118
GFP 2pg+cdk16+ccnyl1 100+100pg	112	3	0	0	0	115
hwa-HA 2pg+GFP 200pg	48	36	30	3	0	117
hwa-HA 2pg+cdk16+ccnyl1 100+100pg	10	17	34	6	0	67
hwa-HA 2pg+cdk16-DN 200pg	64	4	0	0	0	68
hwa-HA 2pg+cdk16-DN-ccnyl2 100+100pg	85	8	3	0	0	96

291 Supplementary Fig.9a, Specific statistics in Fig.9a, numbers represent the number of zebrafish embryos.

292

293

WT	V2	V1	N	deformed1	deformed2	
	ClassI	ClassII	ClassIII	ClassIV	ClassV	Total
AB	0	0	87	0	0	87
AB+cdk16-DN200	1	18	38	2	4	63

294 Supplementary Fig.9b, specific statistics in Fig.9b, numbers represent the number of zebrafish embryos.

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298 **Source data of Supplementary Fig. 10:**

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WT embryos			
Summary	Normal	Apoptosis	Total
uninjection	214	0	214
cdk2 DN	121	117	238

300 Supplementary Fig.10a, specific statistics in Fig.10a, numbers represent the number of zebrafish embryos.

301

302 **Source data of Supplementary Fig. 11:**

303

				Average	STDEV
WT	1	1	1	1	0
S168A/tsu01sm	0.64551	0.586957	0.681206	0.637891	0.047584
tsu01sm/tsu01sm	0.054354	0.045769	0.03851	0.046211	0.007931
wt/tsu01sm	0.540034	0.546307	0.558175	0.548172	0.009213

304 Supplementary Fig.11c, specific statistics of relative hwa mRNA levels

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4 hpf	WT			S168A/tsu01sm			tsu01sm/tsu01sm		
<i>boz</i>	1	1	1	0.003911	5.35E-05	4.94E-05	0.019432237	0.000209	0.001636
<i>chd</i>	1	1	1	0.0066	0.005645	0.01257	0.018297691	0.02469	0.020903
6 hpf	WT			S168A/tsu01sm			tsu01sm/tsu01sm		
<i>chd</i>	1	1	1	0	0.002796	0.000716	0.003762596	0.002397	0.007758
<i>gsc</i>	1	1	1	0.004144	0.015092	0.012061	0.040408956	0.035526	0.027256
<i>bmp</i>	1	1	1	1.969831	2.701364	2.599216	2.015618358	2.287804	2.053604
<i>vent</i>	1	1	1	1.756268	2.009843	2.012396	1.902025732	2.056002	2.241871

308 Supplementary Fig.11f, relative mRNA expression levels of the organizer-specific genes (*boz*, *chd*, *gsc*, *bmp2b*, and *vent*) at different stages.

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