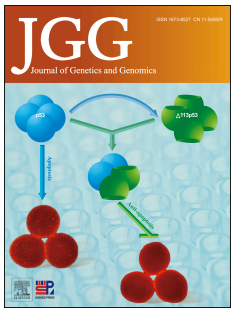
Accepted Manuscript



CDK5-mediated phosphorylation of CP190 may regulate locomotor activity in adult female *Drosophila*

Chin-Tong Ong, Wei-Qi Lin

PII: S1673-8527(17)30195-9

DOI: 10.1016/j.jgg.2017.09.012

Reference: JGG 573

To appear in: *Journal of Genetics and Genomics*

Received Date: 9 March 2017

Revised Date: 26 August 2017

Accepted Date: 15 September 2017

Please cite this article as: Ong, C.-T., Lin, W.-Q., CDK5-mediated phosphorylation of CP190 may regulate locomotor activity in adult female *Drosophila*, *Journal of Genetics and Genomics* (2017), doi: 10.1016/j.jgg.2017.09.012.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

CDK5-mediated phosphorylation of CP190 may regulate locomotor activity in adult female *Drosophila*

Chin-Tong Ong [a,b,*], Wei-Qi Lin [a].

[a] Temasek Life Sciences Laboratory, National University of Singapore, Singapore 117604, Singapore.

[b] Department of Biological Sciences, National University of Singapore, Singapore 117543, Singapore.

* Corresponding author.

Email address: chintong@tll.org.sg (C.-T. Ong)

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

The three-dimensional organization of the genome plays a crucial role in regulating gene expression patterns in metazoans (Ong and Corces, 2014). The nuclear architectural proteins are known to facilitate the formation of topological domains within the genome through mediating inter- and intra-chromosomal interactions. In vertebrate, CCCTC-binding factor (CTCF) is the main architectural protein that mediates long-range chromosomal interactions among its DNA binding sites through a process that is stabilized by cohesin (Parelho et al., 2008; Wendt et al., 2008). In *Drosophila*, there are several sequence-specific DNA-binding architectural proteins that bind distinct genomic sites, including the *Drosophila* homolog of CTCF (dCTCF), Suppressor of Hairy-wing [Su(Hw)], and boundary element-associated factor 32 (BEAF-32). These DNA-binding proteins recruit two common accessory architectural proteins, centrosomal protein 190 (CP190) and Modifier of mdg4 [Mod(mdg4)], which contain BTB (Broad-Complex, Tramtrack and Bric a brac) domains necessary for protein complex assembly and their *in vivo* functions (Pai et al., 2004; Gerasimova et al., 2007). The functional consequences of such long-range DNA interactions depend on the types of regulatory elements that architectural proteins bring together. For instance, architectural proteins may act as insulators that block transcription when chromosomal looping between two of their DNA binding sites abolishes enhancer-promoter communication. On the other hand, interaction between two of their DNA binding sites that brings an enhancer into close proximity with a promoter would facilitate activation of transcription. The different classes of architectural proteins may also contribute to distinct enhancer-promoter interaction networks between housekeeping and developmentally regulated genes (Cubenas-Potts et al., 2016). However, how the functions of different nuclear architectural proteins are regulated during development remains poorly understood.

Studies indicated that the DNA occupancy of the nuclear architectural proteins and the stability of the interactions between these proteins may be modulated by specific post-translational modifications. For instance, vertebrate CTCF undergoes phosphorylation, SUMOylation (Small Ubiquitin-like Modifier conjugation) and poly(ADP-ribosyl)ation (Yu et al., 2004), and the activity of CP190 protein can be regulated by SUMOylation (Capelson and Corces, 2006), ubiquitination (Capelson and Corces, 2005) and poly(ADP-ribosyl)ation (Ong et al., 2013). These post-translational modifications may alter the type and the strength of long-range DNA interactions mediated by architectural proteins in the genome, which in turn leads to differential gene expression.

Interestingly, phosphoproteome analysis of *Drosophila* embryos and cell lines revealed that CP190 protein is extensively phosphorylated, although the types of kinases involved and the functional significances remain unknown (Bodenmiller et al., 2007; Zhai et al., 2008; Sopko et al., 2014).

To determine if the *in vivo* function of CP190 is regulated by phosphorylation, the protein was immunoprecipitated (IP) from S2 cells with either IgG or CP190 antibodies. The pull-down fraction was probed with either pan-phosphoserine (pSer) or 34B2 antibody followed by CP190 antibody. The pSer antibody recognizes all types of phosphorylated serine, whereas the 34B2 antibody is specific for MAPK (mitogen-activated protein kinase) or CDK (cyclin-dependent kinase) phosphor-substrates PXS*P and S*PX(R/K) (* denotes phosphorylation). Consistent with earlier studies (Bodenmiller et al., 2007; Zhai et al., 2008; Sopko et al., 2014), we detected phosphoserine products whose molecular weight correspond to that of the CP190 protein (Fig. 1A, top panel). The recognition of a 190 kDa band by the 34B2 antibody further suggests that the CP190 protein may undergo *in vivo* phosphorylation within either PXS*P or S*PXR/K motifs (Fig. 1A, bottom panel). Mass spectrometry analyses of the excised CP190 band (Fig. S1A) identified 19 phosphorylated sites, 14 of which were previously identified by phosphoproteome analyses of *Drosophila* lysates (Fig. 1B, white ovals) and 5 are novel sites (Fig. 1B, green ovals). The phosphorylated S233 and S392 residues belong to the SPPS and the consensus SPX(R/K) motifs, respectively. These motifs are predicted computationally to be substrates of either MAPKs or CDKs (Figs. 1B and S1B).

In order to determine if the phosphorylation of S233 and S392 is mediated by either MAPKs or CDKs, GST-tagged wild-type or mutant truncated CP190 proteins (GST-CP190Δ, residues 193–407; Fig. 1B) were purified and subjected to *in vitro* kinase reactions with CDK1/cyclin B, CDK5/p25 or MAPK (Fig. 1C). CDK1 (the mammalian orthologue of *Drosophila* Cdc2) is the catalytic subunit of the cyclin B/CDK1 heterodimer required for checkpoint control of the mitotic phase and cell-cycle progression; CDK5 is an atypical CDK important for regulating differentiation processes in post-mitotic neurons. Wild-type GST-CP190Δ undergoes both CDK1- and CDK5-mediated phosphorylation (Fig. 1C). This CDK-mediated phosphorylation was mildly affected by S233A mutation but was severely impaired by S392A mutation (Fig. 1B–D). The phosphorylation level of the S233A/S392A double-mutant (named as AA) protein was slightly lower than that of the S392A single-mutant protein, although this difference is not statistically significant (Fig. 1B–D). On the other hand, the phosphorylation of GST-CP190Δ by MAPK was unaffected by mutations at S233

and S392 (Fig. 1C). These results suggest that the CP190 may be phosphorylated by the CDKs instead of MAPKs.

Previous studies showed that the centrosomal binding domain (CENT; residues 385–508) is necessary for CP190 to bind microtubules and centrosomes during mitosis (Fig. 1B) (Oegema et al., 1995; Butcher et al., 2004). It is thus possible that CDK1-mediated phosphorylation of S392 may be involved in targeting CP190 to microtubules and centrosomes during mitosis. To test this hypothesis, endogenous CP190 protein in Kc167 cells was first knockdown by prolonged treatment with double-stranded RNA (dsRNA) designed to target the untranslated regions of the endogenous mRNA. The endogenous level of CP190 protein was significantly depleted upon 6–7 consecutive rounds of dsRNA treatment (Fig. S2A). In a separate experiment, Kc167 cells were transfected with either myc-tagged wild-type or double-mutant (AA) full-length CP190 plasmids during the fifth dsRNA treatment. The cells were further treated with two additional rounds of dsRNA and harvested 48 h after transient expression of myc-tagged CP190 (Fig. S2B). The localization patterns of myc-tagged CP190 in these cells were then visualized by immunostaining with antibodies against myc epitope and the centrosome marker γ-tubulin (Raff et al., 1993). Consistent with earlier studies, knocking down endogenous CP190 protein has no effect on mitosis (Butcher et al., 2004). Interestingly, both the wild-type and double-mutant myc-tagged CP190 proteins had proper centrosomal localization (co-localized with γ-tubulin) in the majority of the mitotic cells (81%–82%), indicating that the phosphorylation at S233 and S392 is not necessary for its association with the centrosome (Fig. S2C).

The lack of mitotic defects in Kc167 cells coupled with the targeting of double-mutant CP190 to the centrosomes suggests that CDK1-mediated phosphorylation of CP190, established through *in vitro* experiments, is unlikely to have a significant physiological function. As such, we turned our attention towards addressing the possible link between CDK5-mediated phosphorylation and the *in vivo* activity of CP190. To this end, we used epistatic analysis to test the effect of mutant *p35* gene, which encodes the co-activator subunit of CDK5 in *Drosophila* (Trunova and Giniger, 2012), on the insulator activity using the reporter strain *cut-6* (ct^6). The ct^6 reporter strain was generated previously by inserting the *gypsy* retrotransposon, which contains multiple Su(Hw) binding sites, between the enhancer and promoter sequences of the *cut* gene (Ong et al., 2013). Binding of Su(Hw), Mod(mdg4)2.2 and CP190 proteins to the *gypsy* transposon blocked the communication

between these regulatory elements and led to the abolishment of the *cut* gene expression. As a result, adult ct^6 flies had cut wing margins (Fig. 1E, left panel). The absence of Mod(mdg4)2.2 protein in $mod(mdg4)^{T6}$ mutants impaired insulator activity, leading to partial expression of the *cut* gene and most adults flies having one or few notches in the otherwise round wing margin (Fig. 1E, I to IV). Surprisingly, the combination of *p35* and $mod(mdg4)^{T6}$ double mutations led to more severely cut wing margins in the majority of adult flies, indicating that down-regulation of CDK5 activity via *p35* mutation could increase the insulator activity of *gypsy* transposon (Fig. 1F). This result suggests that attenuating CDK5-mediated phosphorylation of CP190 may enhance its *in vivo* insulator activity at the *gypsy* transposon.

To further address the functional significance of the phosphorylation at S233 and S392 and avoid possible pleiotropic effects caused by *Cdk5*/*p35* mutation, we generated transgenic flies with either wild-type or AA double-mutant CP190 transgene in the null CP190 background ($CP190^{P11/H31-2}$) (Pai et al., 2004) (see Supplementary materials and methods for details). Three independent wild-type (Wt258, Wt521, and Wt502) or double-mutant (AA4, AA10, and AA22) lines with insertion sites located in the autosomes were used for further analyses (Fig. S3). Both wild-type and double-mutant transgenes were able to rescue null *CP190* lethality. Comparable level of CP190 protein was also detected between the wild-type and double-mutant transgenic lines, suggesting that these animals have similar dosage of *CP190* transgenes (Figs. 1G, S5A and S5B). Consistent with the above results that mutation in the *p35* gene leads to enhanced insulator activity in the ct^6 reporter strain, the AA double-mutant transgenic lines, which cannot be phosphorylated by CDK5, exhibited cut wing margins similar to the wild-type transgenic lines (Fig. S4A). However, when introduced into the $mod(mdg4)^{T6}$ background, the AA double-mutant *CP190* transgene did not appear enhanced cut wing phenotype (Fig. S4B). As endogenous *CP190* gene remained intact in the $mod(mdg4)^{T6}$ flies, this result suggests that AA double-mutant CP190 protein did not have a dominant negative effect. As insulator activity is regulated by protein-protein interaction between different nuclear architectural proteins, we asked if AA double-mutant CP190 exhibits differential binding affinity to its DNA-binding partner, dCTCF. Interestingly, AA double-mutant CP190 pulled down more dCTCF compared to wild-type CP190 in adult flies (Fig. S4C, $n = 5$, $P < 0.01$). The higher binding affinity between AA double-mutant CP190 and dCTCF is consistent with the

increased insulator activity observed in *p35* mutant flies. This result also suggests that the genomic distribution patterns of CP190 may be regulated by CDK5-mediated phosphorylation.

As the *Cdk5* gene is known to regulate neurogenesis, and the documented age-dependent loss of locomotor activity is due to defective axon patterning in *p35* mutant (Connell-Crowley et al., 2007; Trunova and Giniger, 2012), we asked if AA double-mutant *CP190* transgenic lines exhibit differential behavior patterns when compared to their wild-type counterparts. Negative geotaxis is a well-studied age-dependent behavior where flies climb up the wall of a cylinder after being tapped to its bottom (Grotewiel et al., 2005). Wild-type *W1118* strain was first used to establish the experimental parameters and test the reproducibility of the assay (Fig. S5C). 10-day-old female and 20-day-old male transgenic lines were subsequently collected for the climbing assays. Interestingly, we observed an unexpected distribution shift in their locomotor activity when comparing female flies from three independent wild-type and double-mutant transgenic lines. 49%–50% of the female double-mutant lines (AA4, $n = 208$; AA10, $n = 161$; AA22, $n = 147$) climbed above the height of 14 cm within 10 sec time-frame, whereas only 27%–33% of the wild-type transgenic lines (Wt258, $n = 211$; Wt521, $n = 171$; Wt502, $n = 279$) managed to cover similar height ($P < 0.01$, Figs. 1G). The observation that double-mutant female transgenic lines exhibited significantly faster rate of climbing appeared to be sex-linked since the overall distribution was not statistically different between the wild-type and AA double-mutant male transgenic lines ($P > 0.05$, Fig. S5D). Consistent with the above notion that AA double-mutant CP190 protein may not have a dominant negative effect, all the wild-type and AA transgenic lines show similar locomotor activity in heterozygous $CP190^{H31\text{-}2}/+$ genetic background (Fig. S5E and F). We also did not observe significantly difference in stress response between wild-type and AA double-mutant female transgenic lines. After 24 h of dry starvation, 71%–93% of mutant female flies survived compared to 80%–92% of wild-type flies (data not shown). Taken together, our data indicate that mutations (S233A and S392A) that prevent CDK5-mediated phosphorylation of CP190 may enhance locomotor activity of female flies.

As all the *CP190* transgenes tested were not inserted in the sex chromosomes, the above result suggests that the effect of *CP190* gene may be biased towards female flies. To test this possibility and rule out the potential side effects caused by random insertion of P-elements, further analysis were conducted with previously characterized hypomorphic *CP190* mutant. Generated by ethyl

methanesulfonate (EMS) mutagenesis, the *CP190*$^{H4\text{-}1}$ allele produces a truncated CP190 protein (Fig. 1B; residues 1–765) that has been reported to impair insulator activity (*CP190*$^{P11/H4\text{-}1}$, Fig. 1H) (Pai et al., 2004). Interestingly, the ratio of female to male eclosed hypomorphic *CP190* mutants was 1:2.2, inconsistent with the expected 1:1 ratio (Fig. 1I). More than 75% of males survived till Day 10, whereas only 47% of females managed to stay alive (Fig. 1I). Finally, the locomotor activity of hypomorphic female flies was more severely impacted when compared to male flies (Fig. 1J). Taken together, these results suggest that female flies succumbed to loss of functional CP190 protein more than male flies.

Post-translational modifications of nuclear architectural proteins may be strategy to regulate three-dimensional genome architecture and elicit specific transcriptional programs during development. Here, we showed that mutations of S233 and S392 in CP190 could improve locomotor activity of adult female flies. Consistent with this sex-biased effect, loss of functional CP190 protein impacts female more than male flies (Fig. 1J). Results from *in vitro* kinase assay and the effect of *p35* mutation on *gypsy* transposon suggest that CDK5-mediated phosphorylation may potentially regulate the function of CP190 at a genome-wide level. However, our data cannot rule out the possible indirect effects caused by *p35* mutation on *gypsy* function. Also, it remains to be determined how reduced phosphorylation by CDK5 in the absence of *p35* co-activator may enhance insulator activity at the *ct*^{6} locus. Contrary to the progressive loss of locomotive ability in young *p35* mutant flies (Connell-Crowley et al., 2007), we observe higher mobility in young adult female flies at 10 days old when S233 and S392 were both mutated. This discrepancy would suggest that phosphorylation of CP190 may be regulating genes in other cell types that are distinct from those targeted by *Cdk5/p35* in the neurons. The sex-linked phenotypes caused by phosphor-mutant (AA) *CP190* transgene and hypomorphic *CP190* mutation (*CP190*$^{P11/H4\text{-}1}$) is intriguing. In mammals, CTCF mediates selective chromatin loop formation between enhancer and distinct promoter elements within the protocadherin clusters to establish diverse neuronal identity and self-avoidance in individual neurons (Golan-Mashiach et al., 2012; Monahan et al., 2012). It is possible that CP190 may perform an analogous function in *Drosophila* and regulate a subset of sex-linked genes that might be crucial for normal development and animal survival. Further genome-wide profiling of specific cell populations isolated from wild-type and phosphoserine-mutant *CP190* flies would

address this hypothesis and provide further mechanistic insight on how phosphorylation of CP190 may regulate animal locomotive behavior during development.

Acknowledgments

The mass spectrometry analysis was carried out in part by the Emory Integrated Proteomics Core (EIPC), which is subsidized by the Emory University School of Medicine and is one of the Emory Integrated Core Facilities. Additional support for EIPC was provided by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR000454. The content is solely the responsibility of the authors and does not necessarily reflect the official views of the National Institutes of Health. We are also grateful to Victor Corces for critical reading and comments on this manuscript. This work was supported by core funding from Temasek Life Sciences Laboratory, Singapore.

References

Bodenmiller, B., Malmstrom, J., Gerrits, B., Campbell, D., Lam, H., Schmidt, A., Rinner, O., Mueller, L.N., Shannon, P.T., Pedrioli, P.G., Panse, C., Lee, H.K., Schlapbach, R., Aebersold, R., 2007. PhosphoPep-a phosphoproteome resource for systems biology research in *Drosophila* Kc167 cells. Mol. Syst. Biol. 3, 139.

Butcher, R.D., Chodagam, S., Basto, R., Wakefield, J.G., Henderson, D.S., Raff, J.W., Whitfield, W.G., 2004. The *Drosophila* centrosome-associated protein CP190 is essential for viability but not for cell division. J Cell Sci 117, 1191-1199.

Capelson, M., Corces, V.G., 2005. The ubiquitin ligase dTopors directs the nuclear organization of a chromatin insulator. Mol Cell 20, 105-116.

Capelson, M., Corces, V.G., 2006. SUMO conjugation attenuates the activity of the gypsy chromatin insulator. EMBO J 25, 1906-1914.

Connell-Crowley, L., Vo, D., Luke, L., Giniger, E., 2007. *Drosophila* lacking the Cdk5 activator, p35, display defective axon guidance, age-dependent behavioral deficits and reduced lifespan. Mech Dev 124, 341-349.

213 Cubenas-Potts, C., Rowley, M.J., Lyu, X., Li, G., Lei, E.P., Corces, V.G., 2016. Different enhancer
214 classes in Drosophila bind distinct architectural proteins and mediate unique chromatin interactions
215 and 3D architecture. Nucleic Acids Res.
216 Gerasimova, T.I., Lei, E.P., Bushey, A.M., Corces, V.G., 2007. Coordinated control of dCTCF and
217 gypsy chromatin insulators in Drosophila. Mol Cell 28, 761-772.
218 Golan-Mashiach, M., Grunspan, M., Emmanuel, R., Gibbs-Bar, L., Dikstein, R., Shapiro, E., 2012.
219 Identification of CTCF as a master regulator of the clustered protocadherin genes. Nucleic Acids
220 Res 40, 3378-3391.
221 Grotewiel, M.S., Martin, I., Bhandari, P., Cook-Wiens, E., 2005. Functional senescence in
222 Drosophila melanogaster. Ageing Res Rev 4, 372-397.
223 Monahan, K., Rudnick, N.D., Kehayova, P.D., Pauli, F., Newberry, K.M., Myers, R.M., Maniatis, T.,
224 2012. Role of CCCTC binding factor (CTCF) and cohesin in the generation of single-cell diversity
225 of protocadherin-alpha gene expression. Proc Natl Acad Sci U S A 109, 9125-9130.
226 Oegema, K., Whitfield, W.G., Alberts, B., 1995. The cell cycle-dependent localization of the CP190
227 centrosomal protein is determined by the coordinate action of two separable domains. J Cell Biol
228 131, 1261-1273.
229 Ong, C.T., Corces, V.G., 2014. CTCF: an architectural protein bridging genome topology and
230 function. Nat Rev Genet 15, 234-246.
231 Ong, C.T., Van Bortle, K., Ramos, E., Corces, V.G., 2013. Poly(ADP-ribosyl)ation regulates
232 insulator function and intrachromosomal interactions in Drosophila. Cell 155, 148-159.
233 Pai, C.Y., Lei, E.P., Ghosh, D., Corces, V.G., 2004. The centrosomal protein CP190 is a component
234 of the gypsy chromatin insulator. Mol Cell 16, 737-748.
235 Parelho, V., Hadjur, S., Spivakov, M., Leleu, M., Sauer, S., Gregson, H.C., Jarmuz, A., Canzonetta,
236 C., Webster, Z., Nesterova, T., Cobb, B.S., Yokomori, K., Dillon, N., Aragon, L., Fisher, A.G.,
237 Merkenschlager, M., 2008. Cohesins functionally associate with CTCF on mammalian chromosome
238 arms. Cell 132, 422-433.
239 Raff, J.W., Kellogg, D.R., Alberts, B.M., 1993. Drosophila gamma-tubulin is part of a complex
240 containing two previously identified centrosomal MAPs. J Cell Biol 121, 823-835.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

241 Sopko, R., Foos, M., Vinayagam, A., Zhai, B., Binari, R., Hu, Y., Randklev, S., Perkins, L.A., Gygi,
242 S.P., Perrimon, N., 2014. Combining genetic perturbations and proteomics to examine
243 kinase-phosphatase networks in Drosophila embryos. Dev Cell 31, 114-127.
244 Trunova, S., Giniger, E., 2012. Absence of the Cdk5 activator p35 causes adult-onset
245 neurodegeneration in the central brain of Drosophila. Dis Model Mech 5, 210-219.
246 Wendt, K.S., Yoshida, K., Itoh, T., Bando, M., Koch, B., Schirghuber, E., Tsutsumi, S., Nagae, G.,
247 Ishihara, K., Mishiro, T., Yahata, K., Imamoto, F., Aburatani, H., Nakao, M., Imamoto, N.,
248 Maeshima, K., Shirahige, K., Peters, J.M., 2008. Cohesin mediates transcriptional insulation by
249 CCCTC-binding factor. Nature 451, 796-801.
250 Yu W, Ginjala V, Pant V, Chernukhin I, Whitehead J, Docquier F, Farrar D, Tavoosidana G,
251 Mukhopadhyay R, Kanduri C, Oshimura M, Feinberg AP, Lobanenkov V, Klenova E, Ohlsson R.,
252 2004. Poly(ADP-ribosyl)ation regulates CTCF-dependent chromatin insulation. Nat. Genet. 36,
253 1105-1110.
254 Zhai, B., Villen, J., Beausoleil, S.A., Mintseris, J., Gygi, S.P., 2008. Phosphoproteome analysis of
255 Drosophila melanogaster embryos. J Proteome Res 7, 1675-1682.

ACCEPTED MANUSCRIPT

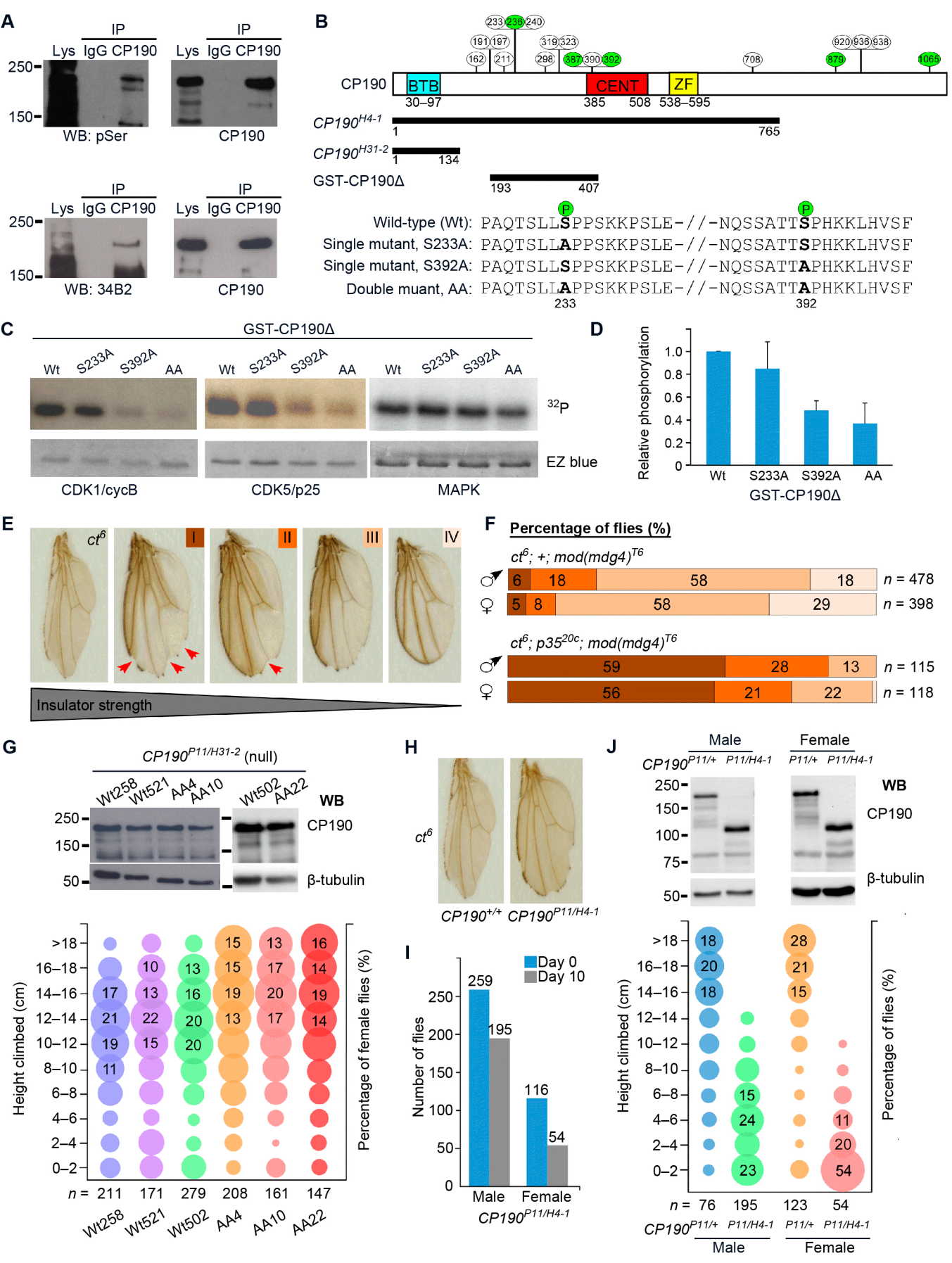
Figure legend

Fig.1. Phosphorylation of CP190 regulates locomotor activity in female flies

A: Immunoprecipitation (IP) of S2 cell lysates (Lys) with either IgG or CP190 antibody was followed by Western blot (WB) analyses using phosphoserine (pSer and 34B2) and CP190 antibodies. **B**: Diagram depicting the different functional domains and the 19 phosphorylation sites in CP190. BTB, Broad-Complex, Tramtrack and Bric a brac; CENT, centrosomal binding domain; ZF, zinc finger domain. Green and white ovals denote novel and previously identified phosphorylation sites. The truncated proteins produced by mutant *CP190* alleles are depicted by the black bars. Amino acid sequences of different *CP190* mutants used in this study were shown below. **C**: *In vitro* kinase assay of wild-type and mutant truncated GST-CP190 (GST-CP190Δ) proteins with the indicated recombinant kinases. **D**: Quantification of CDK-mediated phosphorylation of GST-CP190Δ using wild-type CP190 protein as the reference. The relative phosphorylation levels of both S392A mutant and AA double mutant were significantly impaired ($P < 0.05$, $n = 4$). **E**: Severity of the cut wing margin phenotype (highlighted by red arrow heads) correlates with the level of insulator strength at the ct^6 locus. **F**: Distribution of wing margin phenotypes in ct^6*; +; mod(mdg4)*^{T6} and ct^6*; p35*^{20c}*; mod(mdg4)*^{T6} lines. Chi-square test was performed. No significant difference was observed between the male and female files of the same phenotype ($P > 0.05$), while significant difference was observed between the same sex from two genotypes ($P > 0.001$). **G**: Double-phosphor mutant (AA) female flies exhibit faster locomotor activity. Top: Western blot analysis of the protein expression of wild-type and AA double-mutant *CP190* transgenes in adult female files in the null *CP190* background (*CP190*$^{P11/H31-2}$). For each sample, $n = 5$. Bottom: The size of each filled circle is proportional to the percentage of flies that climbed the indicated height (cm). The top four groups of each genotype were indicated with the number of flies in parenthesis. Chi-square test was performed and significant difference was observed between wild-type and AA female transgenic flies ($P < 0.002$). **H**: Hypomorphic *CP190*$^{P11/H4-1}$ mutant exhibits impaired insulator activity at ct^6 locus. **I**: The number of hypomorphic mutant flies eclosed (Day 0) and alive on Day 10. **J**: Locomotor activity

is more impaired in female *CP190*$^{P11/H4\text{-}1}$ mutants than in the male *CP190*$^{P11/H4\text{-}1}$ flies. Top: Western blot analysis of the protein expression of heterozygous and hypomorphic *CP190* mutants. Bottom: The hypomorphic *CP190*$^{P11/H4\text{-}1}$ mutants have significant sex-biased impairment in their locomotor activity compared to their heterozygous *CP190*$^{P11/+}$ counterparts ($P < 0.0001$).

ACCEPTED MANUSCRIPT


A
IP
Lys IgG CP190
250
150
WB: pSer
CP190
WB: 34B2
B
CP190
BTB
CENT
ZF
30–97
385
508
538–595
765
134
193
407
Wild-type (Wt): PAQTSLLSPPSKKPSLE-//-NQSSATTSPHKKLHVSF
Single mutant, S233A: PAQTSLLAPPSKKPSLE-//-NQSSATTSPHKKLHVSF
Single mutant, S392A: PAQTSLLSPPSKKPSLE-//-NQSSATTAPHKKLHVSF
Double muant, AA: PAQTSLLAPPSKKPSLE-//-NQSSATTAPHKKLHVSF
233
392
C
GST-CP190Δ
Wt
S233A
S392A
AA
32P
EZ blue
CDK1/cycB
CDK5/p25
MAPK
D
Relative phosphorylation
GST-CP190Δ
E
ct6
I
II
III
IV
Insulator strength
F
Percentage of flies (%)
ct6; +; mod(mdg4)T6
n = 478
n = 398
ct6; p35 20c; mod(mdg4)T6
n = 115
n = 118
G
CP190P11/H31-2 (null)
Wt258
Wt521
AA4
AA10
Wt502
AA22
WB
CP190
β-tubulin
Height climbed (cm)
Percentage of female flies (%)
n = 211 171 279 208 161 147
H
ct6
CP190+/+
CP190P11/H4-1
I
Day 0
Day 10
Number of flies
Male
Female
J
Male
Female
CP190P11/+
P11/H4-1
WB
CP190
β-tubulin
Height climbed (cm)
Percentage of flies (%)
n = 76 195 123 54