A PDZ-kinase allosteric relay mediates Par complex regulator exchange

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1 Abstract

2 The Particle in Kinase C (aPKC) to pattern substrates. Two upstream regulators of the Par complex,
Cdc42 and Par-3, bind separately to the complex to influence its activity in different ways. Each
regulator binds a distinc 3 Cdc42 and Par-3, bind separately to the complex to influence its activity in different ways. Each
regulator binds a distinct member of the complex, Cdc42 to Par-6 and Par-3 to aPKC, making it uncle-
how they influence one 4 regulator binds a distinct member of the complex, Cdc42 to Par-6 and Par-3 to aPKC, making it
how they influence one another's binding. Here we report the discovery that Par-3 binding to aF
regulated by aPKC autoinhibitio 5 regulated by aPKC autoinhibition and link this regulation to Cdc_42 and Par-3 binding to aPKC is
regulated by aPKC autoinhibition and link this regulation to Cdc_42 and Par-3 exchange. The Par-6 PDZ
domain activates aPK 6 regulated by aPKC autoinhibition and link this regulation to Cdc42 and Par-3 exchange. The Par-6 PD.
domain activates aPKC binding to Par-3 via a novel interaction with the aPKC kinase domain. Cdc42
and Par-3 have opposite 7 regulated by an anti-transmister and minimizing summary surregulation by Summary Prince (match of 2014)
domain activates aPKC binding to Par-3 via a novel interaction with the aPKC kinase domain. Cdc42
and Par-3 have oppos 8 and Par-3 have opposite effects on the Par-6 PDZ-aPKC kinase interaction: while the Par-6 kinase
domain interaction competes with Cdc42 binding to the complex, Par-3 binding is enhanced by the
interaction. The differential 9 domain interaction competes with Cdc42 binding to the complex, Par-3 binding is enhanced by the
interaction. The differential effect of Par-3 and Cdc42 on the Par-6 PDZ interaction with the aPKC
kinase domain forms an allo 10 interaction. The differential effect of Par-3 and Cdc42 on the Par-6 PDZ interaction with the aPKC
kinase domain forms an allosteric relay that connects their binding sites and is responsible for the
negative cooperativity 11 interaction. The differential effects of Particular effects of Parameters of Rinding Sites and is responsible for the negative cooperativity that underlies Par complex polarization and activity.
Introduction
The Par comple 12

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14 Introduction

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kinase a summinism an allocative relay time complex interacting sites and is rependenced in
negative cooperativity that underlies Par complex polarization and activity.
Introduction
The Par complex plays a central role in neformative, that underlies the complex polarization and activity.
The Par complex plays a central role in establishing and maintaining cortical
animal cells, including epithelial, immune and neural stem cells (1–5). The c animal cells, including epithelial, immune and neural stem cells (1–5). The complex is composed of the
proteins atypical kinase C (aPKC) and Par-6, with the catalytic activity of aPKC providing the primary
output of the c 16 proteins atypical kinase C (aPKC) and Par-6, with the catalytic activity of aPKC providing the primary
output of the complex (6–8). Several upstream regulators bind directly to the Par complex to precisely
control its loc 17

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prother of the complex (6–8). Several upstream regulators bind directly to the Par complex to precisel
control its localization and activity. Regulators include the Rho GTPase Cdc42, which is thought to
activate aPKC acti activate aPKC activity, and the multi-PDZ protein Par-3 (Bazooka or Baz in *Drosophila*), which is
thought to repress activity (9–12). The opposing effects of the Par regulators are facilitated by neg:
cooperativity that e 20 activate aPKC activity, and the multi-PDZ protein Par-3 (Bazooka or Baz in *Drosophila*), which is
21 thought to repress activity (9–12). The opposing effects of the Par regulators are facilitated by no
22 cooperativit

control its localization and activity. Regulators include the Rho GTPase Cdc42, which is thought to
activate aPKC activity, and the multi-PDZ protein Par-3 (Bazooka or Baz in *Drosophila*), which is
thought to repress act 21

cooperativity that ensures only one of the regulators is bound to the complex at a time (13). Given that
each regulator binds to a different member of the Par complex, Cdc42 to Par-6 (14–18) and Par-3 to
aPKC (19–22), it 22

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each regulator binds to a different member of the Par complex, Cdc42 to Par-6 (14–18) and Par-3 to
aPKC (19–22), it has been unclear how they might influence one another's binding. Here we examine
the mechanism by which n each regulator binds to a minimum of the Party Company Diety of the Party Diety as $\frac{1}{2}$, and $\frac{1}{2}$, \frac and the mechanism by which negative cooperativity is implemented in the Par complex to support the distinct activities induced by Cdc42 and Par-3.
The combined regulation by Cdc42 and Par-3 ensure that the Par complex is a distinct activities induced by Cdc42 and Par-3.
The combined regulation by Cdc42 and Par-3 ensure that the Par complex is activated at the prope
time and in the appropriate membrane domain, and the presence of both regula The combined regulation by $Cdc42$ and Par-3 e
time and in the appropriate membrane domain
required for Par-mediated polarity in a wide ar
both $Cdc42$ and Baz are apically enriched durin
disrupts localization of the Par co 27

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the approaches the approximate members of systems (23–28). In the *Drosophila* neuroblas
both Cdc42 and Baz are apically enriched during asymmetric cell division and disruption of either
disrupts localization of the Par c 29

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- The combined required for Par-mediated polarity in a wide array of systems (23–28). In the *Drosophila* neuroblast,
both Cdc42 and Baz are apically enriched during asymmetric cell division and disruption of either
disrupt required for Par-mediated polarity in a wide array of systems (23–28). In the Drosophila neurobiast,
both Cdc42 and Baz are apically enriched during asymmetric cell division and disruption of either
disrupts localization o disrupts localization of the Par complex (10, 29). In cdc42 mutants, Par-6 and aPKC are both found
Pa 31 disrupts localization of the Par complex (10, 29). In cdc42 motalits, Par-6 and aPKC are both found in $Page$

32 and sequentially to properly localize the Par complex (10). In the *C. elegans* zygote, Par-3 maintains the
Par complex in an inactive state while coupling it to actomyosin-generated cortical flows which moves
the complex 33 and sequentially to properly localize the Par complex (10). In the C. elegans zygote, Par-3 maintains the
Par complex in an inactive state while coupling it to actomyosin-generated cortical flows which moves
the complex to 34 reader of the complex toward the anterior cortex (30, 31). At the anterior cortex, GTP-bound Cdc42 stimulates
aPKC activity. The localization of the Par complex in *C. elegans* is consistent with distinct Par-3-bound
and C 35 aPKC activity. The localization of the Par complex in *C. elegans* is consistent with distinct Par-3-bound
and Cdc42-bound pools, suggesting that the Par complex switches between regulator bound states (
This exchange bet 36 an KC activity. The localization of the Par complex in C. elegans is consistent with distinct Par-3-bound
and Cdc42-bound pools, suggesting that the Par complex switches between regulator bound states (9)
This exchange bet 37 This exchange between Cdc42 and Par-3-bound states can be recapitulated *in vitro* with purified
components demonstrating that negative cooperativity underlies complex switching and that no
additional factors are required 38 39 40

This exchange between Cdc42 and Par-3-bound states can be recapitulated *in vitro* with purified
components demonstrating that negative cooperativity underlies complex switching and that no
additional factors are required additional factors are required for switching (13).
Cdc42 and Par-3 bind distinct sites on the Par complex making it likely that an allosteric mechanis
underlies their competitive binding. Par-6 contains a CRIB motif that additional factors are required for surfaining (13).
Cdc42 and Par-3 bind distinct sites on the Par cor
underlies their competitive binding. Par-6 contair
Cdc42 (Fig. 1A) (14, 15, 18). Upon binding, Cdc42
PDZ domain which 41 ounderlies their competitive binding. Par-6 contains a CRIB motif that selectively binds GTP-bound
Cdc42 (Fig. 1A) (14, 15, 18). Upon binding, Cdc42 induces an allosteric transition in the adjacent Par-4
PDZ domain which i 42 Cdc42 (Fig. 1A) (14, 15, 18). Upon binding, Cdc42 induces an allosteric transition in the adjacent Pa
PDZ domain which influences the PDZ's affinity for the transmembrane receptor Crumbs (32). The
kinase domain and an adj 43 PDZ domain which influences the PDZ's affinity for the transmembrane receptor Crumbs (32). The
kinase domain and an adjacent PDZ Binding Motif (PBM) of aPKC primarily interact with the second of
three Par-3 PDZ domains (19 44 For a comain and an adjacent PDZ Binding Motif (PBM) of aPKC primarily interact with the second
three Par-3 PDZ domains (19), though other interactions have been reported (14, 15, 33–35). The or
reported biochemical intera 45 kinase areas and an adjacent PDD and the interactions have been reported (14, 15, 33–35). The only
reported biochemical interaction between aPKC and Par-6 is through their PB1 domains (36, 37),
though the Par-6 PDZ domain 46 reported biochemical interaction between aPKC and Par-6 is through their PB1 domains (36, 37),
though the Par-6 PDZ domain has been proposed to inhibit the aPKC kinase domain activity via an
unknown mechanism (38). Using 47 reported biochemical interaction bases of the inhibit the aPKC kinase domain activity via a
unknown mechanism (38). Using purified components in an affinity pulldown assay, we were able
identify the elements of the Par com 48 unknown mechanism (38). Using purified components in an affinity pulldown assay, we were able to
identify the elements of the Par complex that are required for Cdc42 and Par-3 complex switching.
we uncover a direct intera 49 identify the elements of the Par complex that are required for Cdc42 and Par-3 complex switching. H
we uncover a direct interaction between the PDZ domain of Par-6 and the kinase domain of aPKC th
allows Cdc42 to toggle t 50 identify the elements of the Party in the PDZ domain of Par-6 and the kinase domain of aPKC that
allows Cdc42 to toggle the affinity of the Par complex for Par-3, providing a mechanism for how
complex switching may occur i 51 we uncover a direct interaction between the Parton between the Parton between the kinases of defate interactio
allows Cdc42 to toggle the affinity of the Partomplex for Par-3, providing a mechanism for how
complex switchin 52 complex switching may occur *in vivo*.
Results
Par-3, binding to aPKC is autoinhibited and activated by Par-6
The region of Par-3 containing a short basic region followed by its PDZ2 domain (BR-PDZ2; here
PDZ2) binds to th 53

54 Results

complex switching may occur *in vivo.*
Results
Par-3 binding to aPKC is autoinhibit
The region of Par-3 containing a short
PDZ₂) binds to the aPKC kinase doma
interaction is essential for aPKC mem 55 Par-3 binding to aPKC is autoinhibited and activated by Par-6

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interaction is essential for aPKC membrane recruitment and polarization (19, 20). The interact
high affinity, with an overall ΔG° of 7.9 kcal/mol ($K_d = 1.3 \mu$ M) (19). We examined Par-3 PDZ 2 b
full-length aPKC to de 58

PDZ2) binds to the aPKC kinase domain and its PDZ Binding Motif (KD-PBM; Fig. 1A) and this
interaction is essential for aPKC membrane recruitment and polarization (19, 20). The interaction is
high affinity, with an overal high affinity, with an overall ΔG° of 7.9 kcal/mol ($K_d = 1.3 \mu$ M) (19). We examined Par-3 PDZ2 binding
full-length aPKC to determine if the known intramolecular interactions within aPKC influence Par-3
binding. The 59

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high affinity, with an overall ∆G° of 7.9 Kcal/mol (Kd = 1.3 μM) (19). We examined Par-31 DZ2 binding to
full-length aPKC to determine if the known intramolecular interactions within aPKC influence Par-3
binding. The inte inding. The interaction between Par-3 PDZ2 and full length aPKC was significantly reduced compa
to the KD-PBM alone (Fig. 1B), suggesting that Par-3's interaction with the KD-PBM is repressed wh
Page 61

binding. The interaction between Par-3 interaction with the KD-PBM is repressed when
to the KD-PBM alone (Fig. 1B), suggesting that Par-3's interaction with the KD-PBM is repressed when
Page 2 62 to the KD-PBM alone (Fig. 1B), suggesting that Party is interaction with the KD-PBM is representation with the KD-PBM is represented when P

- 63
- the regulatory module is added *in trans* (Fig. 1C-D), confirming that it interferes with Par-3
the aPKC regulatory module is added *in trans* (Fig. 1C-D), confirming that it interferes with Par-3
binding to aPKC. Thus, in 64
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- where perfect to Par-3 binding). We are constructed in the SPC of Par-3 binding to aPKC regulatory module is added *in trans* (Fig. 1C-D), confirming that it interferes with Par-3 binding to aPKC. Thus, in addition to its the ar KC regolatory module is added *in trans* (Fig. 1C-D), commining that it interferes with rar-3
binding to aPKC. Thus, in addition to its established role in regulating catalytic activity (39),
autoinhibition of aPKC autoinhibition of aPKC regulates binding to Par-3.
Autoinhibition of Par-3 binding to aPKC raises the question of how binding to Par-3 become
Previously, we found that the Par complex, which consists of aPKC bound to Par-Autoinhibition of Par-3 binding to aPKC raises the
Previously, we found that the Par complex, which of
with a similar affinity (ΔG° of 7.5 kcal/mol; Kd = 2.5
length Par-6 activates aPKC's ability to bind Par-3 if
to 69
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- Previously, we found that the Par complex, which consists of aPKC bound to Par-6, binds to Par-3 PDZ2
with a similar affinity (ΔG° of 7.5 kcal/mol; Kd = 2.5 µM) to aPKC KD-PBM alone suggesting that full-
length Par-6 with a similar affinity (ΔG° of 7.5 kcal/mol; Kd = 2.5 µM) to aPKC KD-PBM alone suggesting that full-
length Par-6 activates aPKC's ability to bind Par-3 (19). When we compared the binding of Par complex
to Par-3 wi Length Para-distribution Particle application 2013). The binding of full length aPKC to Par-3, we found that the Par complex bound
substantially better (~8-fold) than full length aPKC alone to Par-3 PDZ2 (Fig. 2A-B) confir substantially better (~8-fold) than full length aPKC alone to Par-3 PDZ2 (Fig. 2A-B) confirmine
6 overcomes aPKC autoinhibition of Par-3 binding. These results indicate that the Par complex bound that the Par
2 binding sit 73
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- regulatory module), and other elements within Par-6 that disrupt this repression. 76
- 77 The Par-6 CRIB-PDZ promotes aPKC binding to Par-3
- We sought to identify the Par-6 elements that activate Par-3 binding to aPKC. Par-6 interacts with regulatory module), and other elements within Far-0 that disrupt this repression.
The Par-6 CRIB-PDZ promotes aPKC binding to Par-3
We sought to identify the Par-6 elements that activate Par-3 binding to aPKC. Par
aPKC via 78
- a Par-3 binding site (the aPKC KD-PBM), an element that represses the Par-3 binding site (the aPKC
regulatory module), and other elements within Par-6 that disrupt this repression.
The Par-6 CRIB-PDZ promotes aPKC binding and a paramagement of a Par-3 binding site (the aPM), and then that repression increments within Par-6 that disrupt this repression.
The Par-6 CRIB-PDZ promotes aPKC binding to Par-3
We sought to identify the Par-6 element aPKC via a PB1-PB1 interaction [Fig. 1A; (36, 37)] suggesting that the Par-6 PB1 could be responsible
activating aPKC's Par-3 binding. To determine if the Par-6 PB1 domain is sufficient to activate Par-
binding we examined 79
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- activating aPKC's Par-3 binding. To determine if the Par-6 PB1 domain is sufficient to activate Par-3
binding we examined the binding to full length aPKC in the presence of Par-6 PB1 ("Par complex
 $\triangle CRIB-PDZ''$) and found tha 82
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- binding we examined the binding to full length aPKC in the presence of Par-6 PB1 ("Par complex

ACRIB-PDZ") and found that Par-3 PDZ2 bound with an affinity similar to that of full length aPKC ald

(Fig. 2A-B). Thus, bindi binding we hadden in the binding of the bar-3 PDZ2 bound with an affinity similar to that of full length aPKC
(Fig. 2A-B). Thus, binding of the Par-6 PB1 domain is insufficient to relieve aPKC autoinhibition a
facilitate h 2002 2003). Thus, binding of the Par-6 PB1 domain is insufficient to relieve aPKC autoinhibition and
(Fig. 2A-B). Thus, binding of the Par-6 PB1 domain is insufficient to relieve aPKC autoinhibition and
facilitate high a (Fig. 2011)
Facilitate high affinity Par-3 binding.
Aside from a PB1 domain, Par-6 contains a CRIB domain, which is known to bind Cdc42 (14, 15, 18),
a PDZ domain that interacts with other polarity proteins such as Stardus Familie in go anning tan glancing.
Aside from a PB1 domain, Par-6 cont
a PDZ domain that interacts with oth
Par-6 CRIB-PDZ was added to Par co
B). Binding was not fully restored, lik
CRIB-PDZ domains are covalently at 85 a PDZ domain that interacts with other polarity proteins such as Stardust and Crumbs (32, 40). When
Par-6 CRIB-PDZ was added to Par complex $\triangle CRIB$ -PDZ we observed enhanced binding to Par-3 (Fig 2A-
B). Binding was not full 86 Par-6 CRIB-PDZ was added to Par complex $\triangle CRIB$ -PDZ we observed enhanced binding to Par-3 (Fig 2
B). Binding was not fully restored, likely because of effective concentration effects when the PB1 and
CRIB-PDZ domains are co 87 88 B). Binding was noticely placed to the concentration effects when the PB1 and CRIB-PDZ domains are covalently attached. Importantly, however, activation of Par-3 binding by Par-
CRIB-PDZ *in trans* indicates that the coval 89 CRIB-PDZ *in trans* indicates that the covalent linkage between PB1 and CRIB-PDZ is not required to
relieve aPKC autoinhibition.
Page 3 90 CRIB-PDZ *in trans* indicates that the covalent linkage between PB1 and CRIB-PDZ is not required to relieve aPKC autoinhibition.
91 relieve aPKC autoinhibition.
Page 91

92 The Par-6 PDZ domain binds to the aPKC kinase domain

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- How does napker and Par-6 outside of the PB1-PB1 interaction, but our data suggest that Par-6 CRIB-I
may interact directly with aPKC. We tested if CRIB-PDZ could bind to either the N-terminus of aPK
which contains the regu which contains the regulatory module (PB1-C1), or the C-terminus of aPKC, which contains the kinase
and PBM domains (KD-PBM). We found that Par-6 CRIB-PDZ bound directly to aPKC KD-PBM, but no
PB1-C1 (Fig. 3A). When we exa 96
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- may interact directly with aPKC. We tested if CRIB-PDZ could bind to either the N-terminus of aPKC,
which contains the regulatory module (PB1-C1), or the C-terminus of aPKC, which contains the kinase
and PBM domains (KD-PB 99
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- and PBM domains (KD-PBM). We found that Par-6 CRIB-PDZ bound directly to aPKC KD-PBM, but not
PB1-C1 (Fig. 3A). When we examined the interaction at higher resolution, we found that Par-6 PDZ
(lacking the CRIB motif) bound PB1-C1 (Fig. 3A). When we examined the interaction at higher resolution, we found that Par-6 PDZ
(lacking the CRIB motif) bound aPKC KD-PBM with a similar affinity to that of Par-6 CRIB-PDZ (Fig. 3B)
Interestingly, despite (lacking the CRIB motif) bound aPKC KD-PBM with a similar affinity to that of Par-6 CRIB-PDZ (Fig. Interestingly, despite the interaction involving a PDZ domain, the PBM (PDZ-binding motif) of aPK
was not required, though (laternary and the Internation involving a PDZ domain, the PBM (PDZ-binding motif) of aPKC
Interestingly, despite the interaction involving a PDZ domain, the PBM (PDZ-binding motif) of aPKC
was not required, though binding Interaction between aPKC and Par-6 outside of the PB1-PB1 heterodimerization, between the
aPKC KD and Par-6 PDZ.
Par-3 PDZ2 and Par-6 CRIB-PDZ bind cooperatively to aPKC
Given that both Par-3 PDZ2 and Par-6 PDZ appear to b 101
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104 Par-3 PDZ2 and Par-6 CRIB-PDZ bind cooperatively to aPKC

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- new interaction between aPKC and Par-6 outside of the PB1-PB1 heterodimerization, between the
aPKC KD and Par-6 PDZ.
Par-3 PDZ2 and Par-6 CRIB-PDZ bind cooperatively to aPKC
Given that both Par-3 PDZ2 and Par-6 PDZ appear influenced one another's binding. The presence of Par-6 CRIB-PDZ significantly enhanced Par-3 binding
to full length aPKC (Figure 2) indicating positive cooperativity of the interaction. To confirm binding
cooperativity, w 106
- new interaction between a resolution between and the Particle Cambridge of the Par-3 PDZ2 and Par-6 CRIB-PDZ bind cooperatively to aPKC
Given that both Par-3 PDZ2 and Par-6 PDZ appear to bind the aPKC KD, we sought to dete Par-3 PDZ2 and Par-6 CF
Given that both Par-3 PD.
influenced one another's
to full length aPKC (Figure
cooperativity, we examine 107
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- to full length aPKC (Figure 2) indicating positive cooperativity of the interaction. To confirm binding
cooperativity, we examined if Par-3 could also enhance binding of Par-6 CRIB-PDZ to aPKC. In the
absence of Par-3, Par to full also enhance binding of Par-6 CRIB-PDZ to aPKC. In the
absence of Par-3, Par complex ΔCRIB-PDZ only binds weakly to Par-6 CRIB-PDZ (Fig. 4A). In the
presence of Par-3 PDZ2, the binding of aPKC to Par-6 CRIB-PDZ wa absence of Par-3, Par complex $\triangle CRIB$ -PDZ only binds weakly to Par-6 CRIB-PDZ (Fig. 4A). In the
presence of Par-3 PDZ2, the binding of aPKC to Par-6 CRIB-PDZ was increased greater than 20-fol
(Fig. 4B) demonstrating that Pa absence of Par-3, PDZ2, the binding of aPKC to Par-6 CRIB-PDZ was increased greater than 20-f
(Fig. 4B) demonstrating that Par-3 and Par-6 CRIB-PDZ significantly enhance one another's bind
aPKC KD-PBM.
Cdc42 displaces Parpresence of Aar₋₃ Para-₄ Instanting of an extended to the Para-Alex Hamburg greater than 2001.
(Fig. 4B) demonstrating that Par-3 and Par-6 CRIB-PDZ significantly enhance one another's binding
aPKC KD-PBM.
Cdc42 displa 111
- 112
- 113 Cdc42 displaces Par-6 CRIB-PDZ from aPKC KD-PBM to regulate Par-3 binding to aPKC
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- (Fig. 4B) demonstrating that Party State in Fig. 4B) and Party Statement Party Statement Paramage 14
aPKC KD-PBM.
Cdc42 displaces Par-6 CRIB-PDZ from aPKC KD-PBM to regulate Par-3 binding to aPKC
The Par-6 PDZ interaction an market wand
Cdc42 displaces
The Par-6 PDZ i
(Figure 4) sugge
affinity of Par-6
lost, returning a (Figure 4) suggest a possible mechanism for Cdc42 and Par-3 complex switching. If Cdc42 reduces the affinity of Par-6 PDZ for aPKC KD, the positive cooperativity of Par-6 PDZ and Par-3 binding would lost, returning aPKC t 115
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- affinity of Par-6 PDZ for aPKC KD, the positive cooperativity of Par-6 PDZ and Par-3 binding would be
lost, returning aPKC to the low Par-3 affinity state. Cdc42 is known to induce an allosteric change in th
Par-6 PDZ dom affinity of Para-Figure Cooking State Cooking State Positive Cooking and State Cooking and Par-6 PDZ domain when it binds the CRIB motif (41), providing a possible mechanism for altering the Par-6 PDZ affinity for the aPKC 117
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- Par-6 PDZ domain when it binds the CRIB motif (41), providing a possible mechanism for altering the
Par-6 PDZ affinity for the aPKC KD. We directly tested the effect of constitutively active Cdc42
(Cdc42^{061L}; hereafter Par-6 PDZ affinity for the aPKC KD. We directly tested the effect of constitutively active Cdc42
(Cdc42^{061L}; hereafter Cdc42) on the interaction between Par-6 CRIB-PDZ and aPKC KD-PBM and four
that Cdc42 reduced the aff \footnotesize (Cdc42^{061L}; hereafter Cdc42) on the interaction between Par-6 CRIB-PDZ and aPKC KD-PBM an
that Cdc42 reduced the affinity of the aPKC KD-PBM interaction with Par-6 CRIB-PDZ to an ext
it was virtually undetectable 120 (Cdc42⁰⁶¹¹; hereafter Cdc42) on the interaction between Par-6 CRIB-PDZ and aPKC KD-PBM and found
121 that Cdc42 reduced the affinity of the aPKC KD-PBM interaction with Par-6 CRIB-PDZ to an extent that
122 it was vir 121
- that Consistent with this observation, we previously it was virtually undetectable in our assay (Fig. 5A-B). Consistent with this observation, we previously
Page 4 122 it was virtually undertained in our assay (Fig. 5A-B). Consistent with this observation, we previously $Page$

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found that Cdc42 has a higher affinity for Par-0 CKID-PDZ alone (∆GP of 7.6 Kcal/finite, $R_d = 2.2 \mu m$)
than for the Par complex, (ΔG° of 7.1 kcal/mole; $K_d = 5.4 \mu M$) (13). Thus, while the Par-6 CRIB-PDZ
interaction than for the Par complex, (ΔG° of 7.1 kcal/mole, Kd° 5.4 μM) (13). Thus, while the Par-6 CRIB-PDZ
interaction with aPKC KD-PBM enhances the affinity for Par-3 (i.e. positive cooperativity), it lower:
affinity for Cdc42 (i affinity for Cdc42 (i.e. negative cooperativity). We confirmed this behavior using reconstituted Par
complex with the *trans* CRIB-PDZ (Fig. 6A) by determining if Cdc42 displaced Par-3 binding in this
context. We observed 127

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130 Discussion

complex with the *trans* CRIB-PDZ (Fig. 6A) by determining if Cdc42 displaced Par-3 binding in this
context. We observed a significant reduction in Par-3 binding upon addition of Cdc42 along with
displacement of the CRIB-P complex with the trans CRIB-PDZ (Fig. 0A) by determining if Cdc42 displaced Par-3 binding in this
context. We observed a significant reduction in Par-3 binding upon addition of Cdc42 along with
displacement of the CRIB-PDZ displacement of the CRIB-PDZ (Fig. 6A-B).
Discussion
A critical step in current models for Par-mediated polarity is the exchange between Par-3- and Co
bound Par complex. These regulators promote distinct activities with th discussion
A critical step in current models for Par-me
bound Par complex. These regulators prom
a polarized, active Par complex. Par-3 main
actomyosin-driven cortical movements tha 131 A critical step in consideration of the modelities polarity is the antiting a combined action resulting
a polarized, active Par complex. Par-3 maintains the complex in an inactive state while coupling it to
actomyosin-driv 132 a polarized, active Par complex. Par-3 maintains the complex in an inactive state while coupling it to
actomyosin-driven cortical movements that polarize the complex (30, 31). Conversely, Cdc42 activates
aPKC's catalytic 133 actomyosin-driven cortical movements that polarize the complex (30, 31). Conversely, Cdc42 activat
aPKC's catalytic activity (12, 16), which is essential for polarizing substrates. Due to their differing
effects on aPKC c 134 aPKC's catalytic activity (12, 16), which is essential for polarizing substrates. Due to their differing
effects on aPKC catalytic activity, Par-3 and Cdc42 form mutually exclusive interactions with the
complex (9). This 135 aPKC's catalytic activity (12, 16), which is essential for polarizing substrates. Due to their differing
effects on aPKC catalytic activity, Par-3 and Cdc42 form mutually exclusive interactions with the
complex (9). This r 136 effects on all the complex (9). This regulator exchange is driven by negative cooperativity in their coupled interact
with the complex (13). However, the physical features of the complex that couple Par-3 and Cdc4
binding 137 provide the complex (13). However, the physical features of the complex that couple Par-3 and Cdc42
binding were previously unknown. Our research has uncovered a crucial internal Par complex
interaction between the Par-6 P 138 binding were previously unknown. Our research has uncovered a crucial internal Par complex
interaction between the Par-6 PDZ domain and the aPKC catalytic domain (KD) that plays a key ro
the exchange process. Both Cdc42 b 139 interaction between the Par-6 PDZ domain and the aPKC catalytic domain (KD) that plays a k
the exchange process. Both Cdc42 binding to Par-6 CRIB-PDZ and Par-3 binding to aPKC KD-
coupled to the interaction with the kinase 140 the exchange process. Both Cdc42 binding to Par-6 CRIB-PDZ and Par-3 binding to aPKC KD-PBM are
coupled to the interaction with the kinase domain. Notably, Par-3 and Cdc42 exert opposite effects on
this interaction: Par-3 141 the exchange processes to the kinase domain. Notably, Par-3 and Cdc42 exert opposite effects on
this interaction: Par-3 binding enhances it (positive cooperativity), whereas Cdc42 reduces it (negative
cooperativity). These 142 coupled to the interaction. Par-3 binding enhances it (positive cooperativity), whereas Cdc_4 2 reduces it (negative cooperativity). These opposing actions of Par-3 and Cdc_4 2 support a mechanism for Par-3 and Cdc_4 2 com 143 this interactivity). These opposing actions of Par-3 and Cdc42 support a mechanism for Par-3 and Cdc42
complex exchange. The CRIB-PDZ binding to KD-PBM promotes Par-3 binding to aPKC but is
detrimental to Cdc42 binding, th 144 complex exchange. The CRIB-PDZ binding to KD-PBM promotes Par-3 binding to aPKC but is
detrimental to Cdc42 binding, thus facilitating the exchange between Par-3- and Cdc42-bound states
the complex.
How might Cdc42 influen 145 146 147

complex exchange. The change of the CRIB-PLA is the exchange between Par-3- and Cdc42-bound
detrimental to Cdc42 binding, thus facilitating the exchange between Par-3- and Cdc42-bound
the complex.
How might Cdc42 influence determines to Cac₄₂ and any measurements in a manning contraction in your analyse of the exchange in the complex.
How might Cdc₄₂ influence the Par-6 CRIB-PDZ interaction with the aPKC KD-PBM? The CRIB and PDZ
domains the complete
How might Co
domains of Pa
through the P
upon Cdc42 b
motifs (41). O 148 domains of Par-6 are structurally linked, with the CRIB forming an extension of a beta-sheet that runs
through the PDZ domain (18). The CRIB-PDZ linkage mediates an allosteric change in the PDZ domain
upon Cdc42 binding to 149 through the PDZ domain (18). The CRIB-PDZ linkage mediates an allosteric change in the PDZ domain
upon Cdc42 binding to the CRIB, changing the PDZ's structure and its affinity for standard PDZ bindin
motifs (41). Our resul 150 upon Cdc₄₂ binding to the CRIB, changing the PDZ's structure and its affinity for standard PDZ binding
motifs (41). Our results suggest that it also alters the PDZ's affinity for the aPKC kinase domain. Cdc42
binding to 151 upon Care CRIB, our results suggest that it also alters the PDZ's affinity for the aPKC kinase domain. Cdc42
binding to the CRIB increases the PDZ's affinity for standard COOH-terminal PBM ligands (41), or does
Page 5 152 binding to the CRIB increases the PDZ's affinity for standard COOH-terminal PBM ligands (41), or does
Page 153 binding to the CRIB increases the CRIB increases the PDZ's affinity for standard COOH-terminal PBM ligands (41), or does not

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- no masses the affinity of the PDZ for the kinase domain. The opposing effects of Cdc42 on Par-6
ligands suggest that the kinase domain binds the Par-6 PDZ elsewhere from its PBM binding sit
The biochemical properties of Pa 159
- digands suggest that the kinase domain binds the Par-6 PDZ elsewhere from its PBM binding site.
The biochemical properties of Par-3 PDZ2 and Par-6 PDZ suggest a mechanism for how they
simultaneously interact with aPKC KD-P ligands suggest and the kinase domain and the Far Part of Baronner from the Paramas Site.
The biochemical properties of Par-3 PDZ2 and Par-6 PDZ suggest a mechanism for how they
simultaneously interact with aPKC KD-PBM. Bo Figure 1 Simultaneously interact with aPKC KD-PBM. Both the aPKC KD and PBM are required for hightar-3 PDZ2 binding (19), suggesting that this PDZ forms a canonical PDZ-PBM interaction alcontacts with the kinase domain. Pa Par-3 PDZ2 binding (19), suggesting that this PDZ forms a canonical PDZ-PBM interaction alongside
contacts with the kinase domain. Par-6 PDZ binding depends primarily on the aPKC KD rather than the
PBM, suggesting that it Partial strategy (19), 1933 and anti-12 District and the anti-12 and this Properties of the SPKC KD rather than the
PBM, suggesting that it forms a non-canonical interaction, potentially on a surface outside of its PBM
bin 160
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- PBM, suggesting that it forms a non-canonical interaction, potentially on a surface outside of its PBM
binding site. The Par-6 PDZ and Par-3 PDZ2 bind cooperatively to aPKC KD-PBM, suggesting that they
contact one another Pamy angles in a non-calonical interaction, perfection, presenting consistent a non-calonization binding site. The Par-6 PDZ and Par-3 PDZ2 bind cooperatively to aPKC KD-PBM, suggesting that the contact one another once th 162
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- binding site with the Para-Gard Complex. Our results suggest how these interactions
mediate complex exchange between Cdc42 and Par-3. Interestingly, in the structure of the Par complex
with the substrate Lethal giant larva mediate complex exchange between Cdc42 and Par-3. Interestingly, in the structure of the Par com
with the substrate Lethal giant larvae (Lgl), Lgl binds the Par-6 PDZ in a manner that precludes its
interaction with the aP 164
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- with the substrate Lethal giant larvae (Lgl), Lgl binds the Par-6 PDZ in a manner that precludes its
interaction with the aPKC kinase domain, suggesting that it may disrupt this interaction (42). Future
efforts will be dir interaction with the aPKC kinase domain, suggesting that it may disrupt this interaction (42). Futu
efforts will be directed at understanding how the interactions might alter other Par complex functi
such as aPKC catalytic 166
- interaction with the aPK catalytic activity and membrane recruitment.
Experimental Procedures
Such as aPKC catalytic activity and membrane recruitment.
Experimental Procedures
Data availability 167
- efforts will be directed at understanding how the interactions might alter other Particle Chinactic,
such as aPKC catalytic activity and membrane recruitment.
Experimental Procedures
Data availability
All data are containe 168
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- such as a we catalytic activity and membrane recruitment.
Experimental Procedures
All data are contained within the manuscript.
Cloning F
Data availability
All data are contained wit
Cloning
GST-, MBP- and his-tagge
cloning (New Fngland Bio 171
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All data are cont
Cloning
GST-, MBP- and
cloning (New Eng
addition to an N-
terminal his-tag. Cloning
GST-, MBP- and his-tagged constructs were c
cloning (New England BioLabs), Q5 mutagene
addition to an N-terminal MBP tag, the aPKC
terminal his-tag. Par complex components (al
described (20, 39). Please see the Key GST-, M
GST-, M
cloning
addition
terminal
describe
Expressi cloning (New England BioLabs), O5 mutagenesis (New England BioLabs) or traditional methods.
addition to an N-terminal MBP tag, the aPKC PB1-C1 (residues 1-225) construct also contained a
terminal his-tag. Par complex compo 174
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- addition to an N-terminal MBP 1ag, the aPKC PB1-C1 (residues 1-225) construct also contained a C-
terminal his-tag. Par complex components (aPKC and his-Par-6) were cloned into pCMV as previous
described (20, 39). Please s 176
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- (Thermofisher) or ExpiFectamine (Thermofisher) according to the manufacturer's protocol. After 48 described (20, 39). Please see the Key Resources table for additional information on specific construct:
Expression
Par complex, full length aPKC and full length aPKC with Par-6 PB1 were expressed in HEK 293F cells
(Thermo described (20, 39). Please see the Key Resources table for additional information on specific complex, full length aPKC and full length aPKC with Par-6 PB1 were expressed in HEK 293F cells
(Thermofisher), as previously des 182
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- Par comple
(Thermofisl
media (The
(Thermofisl
hours, cells
lysis buffer
stored at -8 (Thermofisher), as previously described (20, 39). Briefly, cells were grown in FreeStyle 293 expression
media (Thermofisher) in shaker flasks at 37°C with 8% CO2. Cells were transfected with 293fectin
(Thermofisher) or Ex Figure 20, and a (Thermofisher) in shaker flasks at 37° C with 8% CO2. Cells were transfected with 293fectin
(Thermofisher) or ExpiFectamine (Thermofisher) according to the manufacturer's protocol. After 48
hours, cel (Thermofisher) or ExpiFectamine (Thermofisher) according to the manufacturer's protocol. After hours, cells were collected by centrifugation (500g for 5 min). Cell pellets were resuspended in niclearly is buffer [50mM NaH hours, cells were collected by centrifugation (500g for 5 min). Cell pellets were resuspended in nickel
hours, cells were collected by centrifugation (500g for 5 min). Cell pellets were resuspended in nickel
lysis buffer [lysis buffer [50mM NaH2PO4, 300 mM NaCl, 10 mM Imidazole, pH 8.0] and then frozen in liquid N2 a
stored at -80°C.
Page 184 stored at -80° C.

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- cells, grown overnight at 37°C on LB + ampicillin (Amp; 100 mg/mL). Resulting colonies were selected and used to inoculate 100mL of LB + Amp starter cultures. Cultures were grown at 37°C to an OD600 $(0.6-1.0 \text{ and then diluted into } 2L \text$ 187
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- and used to inoculate 100mL of LB + Amp starter cultures. Cultures were grown at 37°C to an OD600 co.6-1.0 and then diluted into 2L LB + Amp starter cultures. Cultures were grown at 37°C to an OD600 co.6-1.0 and then dilut o.6-1.0 and then diluted into 2L LB + Amp cultures. At an OD600 of 0.8-1.0 expression was induced with 0.5 mM IPTG for 2-3 hours. Cultures were centrifuged at 4400g for 15 minutes to pellet cells. Media was removed and pel o.5 mM IPTG for 2-3 hours. Cultures were centrifuged at 4400g for 15 minutes to pellet cells. Media was
removed and pellets were resuspended in nickel lysis buffer [50mM NaH2PO4, 300 mM NaCl, 10 mM
Imidazole, pH 8.0], GST removed and pellets were resuspended in nickel lysis buffer [50mM NaH2PO4, 300 mM NaCl, 10 mM
Imidazole, pH 8.0], GST lysis buffer [1XPBS, 1 mM DTT, pH 7.5] or Maltose lysis buffer [20 mM Tris, 200
mM NaCl, 1 mM EDTA, 1 mM 191
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- remidazole, pH 8.0], GST lysis buffer [1XPBS, 1 mM DTT, pH 7.5] or Maltose lysis buffer [20 mM Tris, 20
mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.5], as appropriate. Resuspended pellets were frozen in liquid
N2 and stored at -80° IMM NaCl, 1 MM EDTA, 1 MM DTT, pH 7.5], as appropriate. Resuspended pellets were frozen in liquid
N2 and stored at -80°C.
Purification
Resuspended E.coli pellets were thawed and cells were lysed by probe sonication using a N2 and stored at -80°C.

Purification

Resuspended E.coli pellets were thawed and cells were lysed by probe sonication using a Sonicator

Dismembrator (Model 500, Fisher Scientific; 70% amplitude, 0.3/0.7s on/off pulse, 3 Purification
Resuspended E.coli pelle
Dismembrator (Model 5
pellets were lysed simila
Lysates were centrifuge
were aliquoted, frozen in Resuspende
Dismembrat
pellets were
Lysates wer
were aliquot
His-tagged _|
(Thermofish Dismembrator (Model 500, Fisher Scientific; 70% amplitude, 0.3/0.7s on/off pulse, 3x1 min). 293F ce
pellets were lysed similarly using a microtip probe (70% amplitude, 0.3/0.7s on/off pulse, 3x1 min). 293F ce
pellets were 197
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- pellets were lysed similarly using a microtip probe (70% amplitude, 0.3/0.7s on/off pulse, 4x1 min).
Lysates were centrifuged at 27,000g for 20 min to pellet cellular debris. GST-tagged protein lysates
were aliquoted, froz pellet cellular debris. GST-tagged protein lysate
were aliquoted, frozen in liquid N2 and stored at -80°C.
His-tagged protein lysates, except for aPKC KD-PBM and KDAPBM, were incubated with HisPur C
(Thermofisher) resin fo Lysate aliquoted, frozen in liquid N2 and stored at -80°C.

His-tagged protein lysates, except for aPKC KD-PBM and KDΔPBM, were incubated with HisPur Col

(Thermofisher) resin for 30 min at 4°C and then washed 3x with nic Mate and Peter, measuring the amore the RKC KD-PBM and thermofisher) resin for 30 min at 4°C and then washed
100µM ATP and 5mM MgCl2 were added to the first and
1.5mL fractions with nickel elution buffer [50 mM NaH2
For al 201
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- His-tagged protein lysates, except for aPKC KD-PBM and KDΔPBM, were incubated with HisPur Cobalt
(Thermofisher) resin for 30 min at 4°C and then washed 3x with nickel lysis buffer. For 293F lysates,
100μM ATP and 5mM MgCl (Thermorial small and small and second washes. Proteins were eluted in o.gr) and second washes. Proteins were eluted in o.gr) and fractions with nickel elution buffer [50 mM NaH2PO4, 300 mM NaCl, 300 mM Imidazole, pH 8 Fo 204
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- 1. 5mL fractions with nickel elution buffer [50 mM NaH2PO4, 300 mM NaCl, 300 mM Imidazole, pH 8.
For all proteins expressed in E.coli, fractions containing protein were pooled, buffered exchanged int
final buffer [20mM HE For all proteins expressed in E.coli, fractions containing protein were pooled, buffered exchanged into
final buffer [20mM HEPES pH 7.5, 100 mM NaCl and 1 mM DTT] using a PD10 desalting column
(Cytiva), concentrated using (Cytiva), concentrated using a Vivaspin20 protein concentrator spin column (Cytiva), aliquoted,
in liquid N2 and stored at -80°C. For 293F-expressed constructs, proteins were further purified u
anion exchange chromatograp anion exchange chromatography on an AKTA FPLC protein purification system (Amersham
Biosciences). Following his-purification fractions were pooled and buffered exchanged into 20mM
HEPES pH 7.5, 100 mM NaCl, 1 mM DTT, 100 µ Biosciences). Following his-purification fractions were pooled and buffered exchanged into a
HEPES pH 7.5, 100 mM NaCl, 1 mM DTT, 100 μM ATP and 5 mM MgCl2 using a PD10 desaltii
(Cytiva). Buffer-shifted protein was inject HEPES pH 7.5, 100 mM NaCl, 1 mM DTT, 100 µM ATP and 5 mM MgCl2 using a PD10 desalting coll
(Cytiva). Buffer-shifted protein was injected onto a Source Q (Cytiva) column and eluted over a salt
gradient of 100-550mM NaCl. Fr 212
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- Final buffer [20mM HEPES pH 7.5, 100 mM NaCl and 1 mM DTT] using a PD10 desalting column
(Cytiva), concentrated using a Vivaspin20 protein concentrator spin column (Cytiva), aliquoted, frozen
in liquid N2 and stored at -80 (Cytiva), aliquoted, frozen in liquid N2 and stored at -80°C. For 293F-expressed constructs, proteins were further purified using
anion exchange chromatography on an AKTA FPLC protein purification system (Amersham
Bioscie or and the discussion of the desired protein(s) were pooled, buffered
exchanged into 20 mM HEPES pH 7.5, 100 mM NaCl, 1 mM DTT, 100 μM ATP, and 5 mM MgCl2 usir
PD10 desalting column (Cytiva), concentrated using a Vivaspin 214
- exchanged into 20 mM HEPES pH 7.5, 100 mM NaCl, 1 mM DTT, 100 μM ATP, and 5 mM MgCl2 P
PD10 desalting column (Cytiva), concentrated using a Vivaspin20 protein concentrator spin colu
(Cytiva), aliquoted, frozen in liquid N 215
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- (Cytiva). Buffer-shifted protein was injected onto a Source Q (Cytiva) column and eluted over a salt
gradient of 100-550mM NaCl. Fractions containing the desired protein(s) were pooled, buffered
exchanged into 20 mM HEPES PD10 desalting column (Cytiva), concentrated using a Vivaspin20 protein concentrator spin column
(Cytiva), aliquoted, frozen in liquid N2 and stored at -80°C.
Due to solubility issues, aPKC KD-PBM and KDΔPBM were expresse (Cytiva), aliquoted, frozen in liquid N2 and stored at -80°C.
Due to solubility issues, aPKC KD-PBM and KDΔPBM were expressed in E. coli and his-purified parti
under denaturing conditions. Following sonication and centrif (Cyton), and consider the and stored and KDAPBM were
Due to solubility issues, aPKC KD-PBM and KDAPBM were
under denaturing conditions. Following sonication and cent
fraction was discarded and the insoluble pellet was resu 217
- Due to solubility issues, aPKC KD-PBM and KDΔPBM were expressed in E. coli and his-purified partially under denaturing conditions. Following sonication and centrifugation (described above), the soluble fraction was discar 218
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- fraction was discarded and the insoluble pellet was resuspended in 50mM NaH2PO4, 300 mM NaCl, and MM Imidazole, 8M Urea pH 8.0. Centrifugation was repeated (27,000g for 20 min) and the resulting soluble phase was incubated 220
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mM Imidazole, 8M Urea pH 8.o. Centrifugation was repeated (27,000g for 20 min) and the resulting
soluble phase was incubated with HisPur Ni-NTA resin (ThermoFisher) for 30 min at 4°C. Resin was
washed and eluted as descri soluble phase was incubated with HisPur Ni-NTA resin (ThermoFisher) for 30 min at 4°C. Resin was washed and eluted as described above. Purified protein was aliquoted, frozen in liquid N2 and stored above and store of the washed and eluted as described above. Purified protein was aliquoted, frozen in liquid N2 and store -80°C.
-80°C.
Qualitative binding assays
For qualitative binding ("GST pulldown") assays, GST lysates were incubated with -80°C.
Qualitative binding assays
For qualitative binding ("GST pulldown") assays, GST lysates were incubated with glutathione agarose
resin (GoldBio) for at least 30 min at 4°C and then washed 3x 5 min washes at room temp Qualita
For qua
resin ((or qualitative binding ("G.
The qualitative binding ("G.
The sin (GoldBio) for at least
Single School (SoldBio) for at least 225 For qualitative binding ("GST") pulled with pulled by $\frac{1}{2}$ and then washed $\frac{1}{3}$ s min washes at room temp with binding Pesin (GoldBio) for at least 30 min at 4°C and then washed $\frac{1}{3}$ s min washes at room te 226

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- with rotational mixing. Soluble proteins were added to GST-bound proteins, as indicated, and
incubated at room temperature with rotational mixing for 6o min. Resin was then washed 3x with
binding buffer and proteins were e 228
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- incubated at room temperature with rotational mixing for 60 min. Resin was then washed 3x w
binding buffer and proteins were eluted with $4 \times$ LDS sample buffer (ThermoFisher). Samples v
on a Bis-Tris gel and stained with binding buffer and proteins were eluted with 4X LDS sample buffer (ThermoFisher). Samples were
on a Bis-Tris gel and stained with Coomassie Brilliant Blue R-250 (GolBio). Band intensities of repli
were quantified using Ima on a Bis-Tris gel and stained with Coomassie Brilliant Blue R-250 (GolBio). Band intensities of replicates
were quantified using ImageJ (v1.53a). The normalized band intensity was determined by averaging the
intensity of e were quantified using ImageJ (v1.53a). The normalized band intensity was determined by averaging the intensity of either full length aPKC or KD-PBM signal within the experiment, as appropriate, and then dividing the band i 232
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- intensity of either full length aPKC or KD-PBM signal within the experiment, as appropriate, and then
dividing the band intensity of each individual value by that mean value. The data was visualized and
analyzed using the dividing the band intensity of each individual value by that mean value. The data was visualized and
analyzed using the DABEST (43) software packages. Confidence intervals were estimated using the
bootstrap method as imple 234
- analyzed using the DABEST (43) software packages. Confidence intervals were estimated using the
bootstrap method as implemented in DABEST.
Acknowledgments
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- 237 Acknowledgments
- bootstrap method as implemented in DABEST.
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Author Contributions 238 This work was supported by NIH grants R35GM127092 and T32HD007348.

239 **Author Contributions**

Bootstrap method as implements in DAB
This work was supported by NIH grants R35
Author Contributions
E.V., R.R.P. and K.E.P. designed the experi 240 E.V., R.R.P. and K.E.P. designed the experiments, analyzed the data, prepared figures and 241 wrote the manuscript. E.V. and R.R.P. performed the experiments.

242 **Declaration of Interests**

- 243 The authors have no competing interests to declare.
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Figure 1: Par-3 binding to aPKC is autoinhibited by the regulatory domain of aPKC.

A. Schematic of the domain architecture of and interactions between the Par complex proteins (aPKC and Par-6) and known regulators Par-3 (Bazooka in Drosophila) and Cdc42.

B. Par-3 binding to aPKC kinase domain with its associated PDZ-binding motif (KD-PBM) or full length aPKC (aPKC FL). Solid phase (glutathione resin)-bound glutathione-S-transferase (GST) or GST-fused Par-3 PDZ2 with its associated basic region (BR-PDZ2) incubated with aPKC KD-PBM or full length (aPKC FL). Shaded regions indicate the fraction applied to the gel (soluble phase or solid phase components after incubation with indicated soluble components and washing).

C. Par-3 binding to aPKC KD-PBM and/or regulatory domain (aPKC PB1-C1). Labeling as described in (B).

D. Gardner-Altman estimation plot of normalized band intensity of aPKC KD-PBM binding to Par-3 BR-PDZ2 in the presence or absence of its regulatory domain (aPKC PB1-C1). The results of each replicate (filled circles) are shown along with mean and standard deviation (gap and bars adjacent to filled circles). The mean difference is plotted on the right as a bootstrap sampling distribution (shaded region) with a 95% confidence interval (black error bar). **E.** Summary of Par-3 interactions with aPKC.

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Figure 2: Par-3 binding to aPKC is activated by Par-6.

A. Par-3 binding to aPKC in the presence or absence of various Par-6 domains. Solid phase (glutathione resin)-bound glutathione-S-transferase (GST)-fused Par-3 PDZ2 with its associated basic region (BR-PDZ2) incubated with full length aPKC (aPKC FL), Par complex (full length aPKC and Par-6), Par complex ∆CRIB-PDZ (aPKC with the Par-6 PB1 domain) or Par complex ∆CRIB-PDZ plus Par-6 CRIB-PDZ. Shaded regions indicate the fraction applied to the gel (soluble phase or solid phase components after incubation with indicated soluble components and washing). Inset shows enlargement of the last 4 lanes as indicated.

B. Cumming estimation plot of the normalized band intensity of aPKC binding to Par-3 BR-PDZ2 under the indicated conditions shown in (A). The result of each replicate (filled circles) along with the mean and SD (gap and bars next to circles) are plotted in the top panel and the mean differences are plotted in the bottom panel as a bootstrap sampling distribution (shaded region) with a 95% confidence interval (black error bar).

C. Summary of Par-3 interactions with Par complex.

Figure 3: Par-6 PDZ binds directly to the kinase domain of aPKC.

A. Par-6 CRIB-PDZ binding to the various domains of aPKC. Solid phase (glutathione resin)-bound glutathione-S-transferase (GST)-fused Par-6 CRIB-PDZ incubated with the regulatory module of aPKC (PB1-C1) or aPKC kinase domain (KD) with its PDZ-binding motif (PBM). Shaded regions indicate the fraction applied to the gel (soluble phase or solid phase components after incubation with indicated soluble components and washing). **B.** Binding of individual domains of Par-6 to aPKC KD or KD-PBM. Solid phase (glutathione resin)-bound glutathione-S-transferase (GST)-fused Par-6 CRIB-PDZ, GST-fused Par-6 CRIB or GST-fused Par-6 PDZ incubated with either aPKC KD-PBM or aPKC KD only. Shaded regions indicate the fraction applied to the gel (soluble phase or solid phase components after incubation with indicated soluble components and washing).

Figure 4: Par-6 CRIB-PDZ and Par-3 PDZ2 bind cooperatively to aPKC.

A. Binding of Par-6 CRIB-PDZ to aPKC in the presence or absence of Par-3. Solid phase (glutathione resin)-bound glutathione-S-transferase (GST)-fused Par-6 CRIB-PDZ incubated with Par complex ∆CRIB-PDZ in the presence or absence of Par-3 PDZ2 and its associated basic region (BR-PDZ2). Shaded regions indicate the fraction applied to the gel (soluble phase or solid phase components after incubation with indicated soluble components and washing). **B.** Gardner-Altman estimation plot of normalized band intensity of aPKC binding to Par-6 CRIB-PDZ in the absence or presence of Par-3 BR-PDZ2.

C. Summary of Par-6 CRIB-PDZ and Par-3 BR-PDZ2 cooperative binding to aPKC

Figure 5: Cdc42 displaces Par-6 CRIB-PDZ from the aPKC kinase domain (KD).

A. Binding of Par-6 CRIB-PDZ to Cdc42 and aPKC KD with its PDZ binding motif (KD-PBM). Solid phase (glutathione resin)-bound glutathione-S-transferase (GST)-fused Par-6 CRIB-PDZ incubated with Cdc42 Q61L, aPKC KD-PBM or both Cdc42 and aPKC KD-PBM.

B. Gardner-Altman estimation plot of normalized band intensity of aPKC KD-PBM binding to Par-6 CRIB-PDZ in the absence or presence of Cdc42 Q61L.

C. Summary of Cdc42's effect on the Par-6 CRIB-PDZ interaction with the aPKC KD.

Figure 6: Cdc42 toggles the interaction between Par-6 CRIB-PDZ and aPKC kinase domain (KD) to influence Par-3 binding.

A. Binding of Par-3 to aPKC in the presence of Par-6 CRIB-PDZ with or without Cdc42. Solid phase (glutathione resin)-bound glutathione-S-transferase (GST)-fused Par-3 PDZ2 with its associated basic region (BR-PDZ2) incubated with Par complex (full length aPKC and Par-6) or Par complex ∆CRIB-PDZ (full length aPKC plus the Par-6 PB1 domain) with Par-6 CRIB-PDZ and Cdc42Q61L as indicated. Shaded regions indicate the fraction applied to the gel (soluble phase or solid phase components after incubation with indicated soluble components and washing). Inset shows enlargement of the last 4 lanes.

B. Cumming estimation plot of normalized band intensity of aPKC binding to Par-3 BR-PDZ2 under the indicated conditions from (A)**.** The result of each replicate (filled circles) along with the mean and SD (gap and bars next to circles) are plotted in the top panel and the mean differences are plotted in the bottom panel as a bootstrap sampling distribution (shaded region) with a 95% confidence interval (black error bar).

C. Summary of PDZ-kinase mediated regulator exchange. Par-3 BR-PDZ2 binds with low affinity to aPKC because of autoinhibition. Par-6 relieves autoinhibition through its PDZ domain interacting with the aPKC kinase domain leading to high affinity Par-3 binding. Cdc42 inhibits the PDZ-kinase interaction to restore aPKC to its low Par-3 affinity state.