

1 Pseudorabies Virus Infection Accelerates Degradation of the Kinesin-3 Motor KIF1A

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3 Running Title: Pseudorabies Virus Accelerates KIF1A Degradation

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10 Word Count: Abstract (231),

11 Main Text (5744)

12 Keywords: alphaherpesvirus, pseudorabies virus, kinesin, KIF1A, proteasomal degradation,  
13 axonal sorting.

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16

17 **Abstract**

18 Alphaherpesviruses, including pseudorabies virus (PRV), are neuroinvasive pathogens that  
19 establish life-long latency in peripheral ganglia following the initial infection at mucosal  
20 surfaces. The establishment of latent infection and the subsequent reactivations during which  
21 newly-assembled virions are sorted into and transported anterogradely inside axons to the initial  
22 mucosal site of infection, rely on axonal bidirectional transport mediated by microtubule-based  
23 motors. Previous studies using cultured peripheral nervous system (PNS) neurons have  
24 demonstrated that KIF1A, a kinesin-3 motor, mediates the efficient axonal sorting and transport  
25 of newly-assembled PRV virions. In this study, we report that KIF1A, unlike other axonal  
26 kinesins, is an intrinsically unstable protein prone to proteasomal degradation. Interestingly, PRV  
27 infection of neuronal cells leads not only to a non-specific depletion of KIF1A mRNA, but also  
28 to an accelerated proteasomal degradation of KIF1A proteins, leading to a near depletion of  
29 KIF1A protein late in infection. Using a series of PRV mutants deficient in axonal sorting and  
30 anterograde spread, we identified the PRV US9/gE/gI protein complex as a viral factor  
31 facilitating the proteasomal degradation of KIF1A proteins. Moreover, by using compartmented  
32 neuronal cultures that fluidically and physically separate axons from cell bodies, we found that  
33 the proteasomal degradation of KIF1A occurs in axons during infection. We propose that PRV  
34 anterograde sorting complex, gE/gI/US9, recruits KIF1A to viral transport vesicles for axonal  
35 sorting and transport, and eventually accelerates the proteasomal degradation of KIF1A in axons.

36 **Importance**

37 Pseudorabies virus (PRV) is an alphaherpesvirus related to human pathogens herpes simplex  
38 virus -1, -2 and varicella zoster virus. Alphaherpesviruses are neuroinvasive pathogens that  
39 establish life-long latent infections in the host peripheral nervous system (PNS). Following  
40 reactivation from latency, infection spreads from the PNS back via axons to the peripheral  
41 mucosal tissues, a process mediated by kinesin motors. Here, we unveil and characterize the  
42 underlying mechanisms for a PRV-induced, accelerated degradation of KIF1A, a kinesin-3  
43 motor promoting the sorting and transport of PRV virions in axons. We show that PRV infection  
44 disrupts the synthesis of KIF1A, and simultaneously promotes the degradation of intrinsically  
45 unstable KIF1A proteins by proteasomes in axons. Our work implies that the timing of motor  
46 reduction after reactivation would be critical because progeny particles would have a limited  
47 time window for sorting into and transport in axons for further host-to-host spread.

48 **Introduction**

49

50 Alphaherpesviruses, including human pathogens herpes simplex virus-1 and -2, varicella-zoster  
51 virus, and the veterinary pathogen pseudorabies virus (PRV), are neuroinvasive pathogens that  
52 establish and maintain life-long latency in the sensory and automatic ganglia of the peripheral  
53 nervous system (PNS) of their mammalian hosts following initial infection at mucosal surfaces (1-  
54 3). Latently infected neurons in peripheral ganglia undergo occasional reactivation, during which  
55 viral genomes are actively replicated and new viral particles assembled. Spread of infection to new  
56 hosts requires that these progeny virions be transported to the initial site of epithelial infection,  
57 where replication and subsequent host-to-host transmission occur. On rare occasions, infection  
58 spreads from the PNS to the central nervous system, often with serious consequences such as  
59 herpes simplex encephalitis (HSE).

60

61 Axonal bidirectional transport mechanisms are crucial for the virions and virus proteins to move  
62 efficiently through the PNS axons, which are often centimeters to meters long (4). In uninfected  
63 neurons, microtubule-based motors, including minus-ended directed cytoplasmic dynein and plus-  
64 ended directed kinesin superfamily motors, mediate fast axonal transport of RNAs, proteins,  
65 organelles, and vesicles between cell bodies and axonal termini for the maintenance and proper  
66 functioning of axons (5, 6). Neuroinvasive viruses such as the alphaherpesviruses and rabies virus  
67 have evolved to adopt existing axonal transport machinery for efficient neuroinvasion and  
68 subsequent spread to new hosts. A notable example is rabies virus (RABV), whose phosphoprotein  
69 (P) binds to LC8 dynein light chain, and was shown to hijack the P75NTR-dependent transport to  
70 facilitate fast retrograde axonal transport in sensory DRG neurons (7).

71

72 Initially after infection of peripheral tissues, alphaherpesvirus virions bind to PNS axon terminals,  
73 and viral capsids are released into axons where they recruit cytoplasmic dynein, via inner tegument  
74 proteins UL36 and UL37 (8, 9). This complex then moves in the MT minus-end direction towards  
75 the cell body and deposits the viral DNA inside the nucleus. After reactivation of a latent PNS  
76 infection, kinesin-superfamily motors are recruited both for viral egress at cell bodies, as well as  
77 for sorting into axons and subsequent transport of viral structural proteins and newly-assembled  
78 virions (10-14). Previous studies in sympathetic PNS neurons revealed that a subpopulation of  
79 fully-enveloped PRV virions in transport vesicles recruit KIF1A, a kinesin-3 motor, through the  
80 interactions with a gE/gI/US9 tripartite membrane protein complex in lipid rafts at late Golgi  
81 network compartments (e.g., late endosomes or the trans-Golgi network (TGN)) (14, 15). After  
82 this interaction, fully enveloped virions in transport vesicles are sorted into axons followed by  
83 unidirectional anterograde transport before egressing along the axonal shaft (16). In PRV infected  
84 neurons, ectopic expression of dominant-negative KIF1A proteins significantly reduced the  
85 number of PRV particles transported in the anterograde direction, demonstrating the critical role  
86 of KIF1A in sorting virus particles into axons and also in subsequent transport (14). In this report,  
87 we focus on a curious observation made by Kramer et al, who noted that the amount of KIF1A in  
88 infected cultured neuronal cells decreased significantly late after PRV infection (14).

89

90 In uninfected neurons, the kinesin motor KIF1A mediates fast axonal transport of membranous  
91 vesicles including synaptic vesicle precursors and dense core vesicles, and is critical in dendrite  
92 morphogenesis and synaptogenesis (17-19). Neurons lacking KIF1A showed impaired transport  
93 in synaptic vesicle precursors, marked neuronal degeneration, and death in both cultured neurons

94 and *in vivo* (20). Despite its functional importance, several lines of evidence suggest that KIF1A  
95 motors are degraded upon the completion of their axonal transportation runs, and are not recycled  
96 for further use. Studies with a ligature applied to the mouse sciatic nerve revealed increased  
97 antibody staining of KIF1A in the proximal (close to the cell body), but not distal (close to the  
98 axonal terminal), region of ligature, suggesting that once KIF1A motors entered axons, they did  
99 not return to the cell bodies (e.g. with dyneins) (21). UNC-104, the KIF1A homolog in *C. elegans*,  
100 was shown to be degraded by ubiquitin-related pathways at synapses of mechanosensory neurons  
101 (22). More importantly, the stability of UNC-104 protein strongly correlates with the motor's  
102 specific binding to cargo, implying a potential mechanistic link between motor degradation and  
103 cargo release (22).

104  
105 In this study, we investigated KIF1A protein dynamics during PRV infection both in neuronal cell  
106 lines and in primary neurons. Here, we report that KIF1A (kinesin-3), unlike kinesin-1 and kinesin-  
107 2 motors, is intrinsically prone to proteasomal degradation in uninfected neuronal cells. We further  
108 showed that PRV infection blocks new synthesis of this motor, and that viral anterograde-sorting  
109 complex US9/gE/gI specifically targets KIF1A for proteasomal degradation, resulting in a near  
110 depletion of the protein in 24 hours following infection. Using compartmented neuronal cultures  
111 that fluidically and physically separate axons from cell bodies, we found that the proteasomal  
112 degradation of KIF1A proteins occurs in axons. Moreover, using a series of PRV mutants deficient  
113 in anterograde spread, and using adenovirus vectors to express the viral proteins in neuronal cells,  
114 we discovered that the PRV US9/gE/gI protein complex targets specifically KIF1A motors for  
115 proteasomal degradation. Altogether, these results suggest that progeny PRV particles recruit  
116 KIF1A motors for efficient axonal sorting and anterograde transport that leads to the degradation

117 of the motor protein in axons. Since new synthesis of KIF1A is also blocked by PRV infection,  
118 these results suggest progeny particles have a limited time window for sorting into and transport  
119 in axons for further host-to-host spread.

120

121 **Results**

122

123 **In uninfected, differentiated PC12 cells, the steady state concentration of KIF1A protein is  
124 achieved through rapid protein synthesis and proteasomal degradation.**

125

126 We tested the hypothesis that in differentiated PC12 cells axonal kinesins undergo rapid  
127 degradation while protein synthesis continues. To determine the role of the proteasome in this  
128 process, we treated uninfected differentiated PC12 cells with the proteasomal inhibitor MG132 for  
129 2, 4, 6, and 8 hours, and monitored the protein concentration of three different kinesin motors:  
130 KIF5 (kinesin-1, and the conventional kinesin), KIF3A (kinesin-2), and KIF1A (kinesin-3).  
131 MG132 treatment led to more than a 2-fold increase in KIF1A concentration within 6 hours post  
132 treatment, indicating that KIF1A protein rapidly accumulated upon proteasome inhibition (Figure  
133 1A and B). However, this surge in KIF1A concentration was ablated when PC12 cells were  
134 simultaneously treated with MG132 and cycloheximide, a protein synthesis inhibitor, and KIF1A  
135 concentration remained at the similar levels as in untreated cells. These experiments demonstrated  
136 that KIF1A proteins are degraded by the proteasomes with new proteins efficiently made to  
137 maintain protein steady state levels. Consistent with this idea, in cells treated with CHX alone,  
138 KIF1A concentration dropped by over 50% within 4 hours of treatment (Figure 1A and B).

139

140 In contrast to KIF1A, neither KIF5 nor KIF3A protein concentration increased significantly during  
141 MG132 treatment (Figure 1A, C and D). With both MG132 and CHX treatments, KIF5 and KIF3A  
142 concentrations appeared unchanged in the 8 h treatment period (Figure 1A, C and D), suggesting  
143 that while both proteins are degraded by proteasomes, their rate of degradation is much slower  
144 than that of KIF1A. As a result, the half-lives of both KIF5 and KIF3A proteins were much longer  
145 (> 8 hours) than the half-life of KIF1A in CHX-treated PC12 cells.

146

147 **PRV infection induces specific reduction of KIF1A protein late in infection**

148

149 The inherent instability of KIF1A in uninfected cells prompted us to explore an observation  
150 previously made in our laboratory: the concentration of KIF1A protein, the major kinesin  
151 facilitating axonal sorting and transport of PRV virions, decreased late in PRV infection of  
152 sympathetic neuronal cells. This observation might explain the significant numbers of immobile  
153 PRV particles in axons late in infection (23). Accordingly, we infected differentiated PC12 cells  
154 with PRV Becker at an MOI of 20 for 3, 8, 12, 16, 20, and 24 hours, and monitored KIF1A protein  
155 levels by quantitative western blot methods. Similar to the previous report, we found that the  
156 KIF1A protein concentration dropped by ~90% at 24 hpi (Figure 2A and B, Figure S1). To  
157 determine if PRV infection affects the steady state concentrations of other kinesin motors, we  
158 monitored the protein levels of KIF5 and KIF3A, two kinesins well-characterized in mediating  
159 axonal transport. Neither KIF5 nor KIF3A protein levels were affected by PRV infection (Figure  
160 2).

161

162 **PRV infection reduces both KIF1A and KIF5B transcripts.**

163

164 Alphaherpesviruses promote the instability of many host cell transcripts early in infection, leading  
165 to reduced protein synthesis (24-27). We hypothesized that the loss of KIF1A proteins in PRV-  
166 infected PC12 cells resulted from such mRNA degradation. We therefore measured the amounts  
167 of KIF1A transcripts in PRV infected cells by qRT-PCR. Differentiated PC12 cells were infected  
168 with PRV Becker and the attenuated vaccine strain PRV Bartha at an MOI of 20 for 3, 8, 12, 16,  
169 20, 24 hours. Infected cells were collected for RNA extraction and qRT-PCR analysis of KIF1A,  
170 KIF5B, and GAPDH mRNA level (Figure 3). In PRV Becker-infected cells, we detected a  
171 significant and uniform reduction of KIF1A, KIF5B, and GAPDH transcripts as early as 8 hpi, and  
172 their respective concentrations dropped by more than 80% at 16 hpi (Figure 3A).

173

174 Compared to PRV Becker infection, PRV Bartha infection decreased KIF1A transcripts at a slower  
175 rate, starting at 12 hpi, with more than 80% loss at 20 hpi (Figure 3B). Reduction of GAPDH and  
176 KIF5B transcripts occurred with comparable rates and magnitude to the KIF1A transcripts. These  
177 experiments showed that KIF1A transcripts were reduced in PRV-infected PC12 cells, but with  
178 different kinetics after infection by different PRV strains. Such transcriptional regulation may be  
179 one reason why KIF1A protein levels drop significantly during PRV infection. However, this  
180 effect was not specific for KIF1A, because KIF5B transcripts were also reduced after infection.  
181 Therefore, the specific loss of KIF1A and not KIF5B proteins in infected neurons is not due to  
182 reduction of transcript levels.

183

184 **KIF1A is degraded by the proteasome during PRV infection in differentiated PC12 cells and**  
185 **in primary SCG neurons.**

186 We found that KIF1A (but not other kinesins) is rapidly degraded by the proteasome in uninfected  
187 PC12 cells. We also knew from previous studies that complexes of PRV US9 and KIF1A contain  
188 the E3-ubiquitin ligases NEDD4 and EP0, suggesting that KIF1A may be degraded via the  
189 ubiquitin-proteasome pathway after PRV infection (14). To examine this possibility, we infected  
190 differentiated PC12 cells with PRV Becker at an MOI of 20, followed by treatment with the  
191 proteasomal inhibitor MG132 at 12 hpi. In mock-infected cells, KIF1A proteins accumulated  
192 rapidly, doubling the concentration in 8 hours, after MG132 treatment (Figure 4A and C). However,  
193 in PRV Becker infected cells, MG132 treatment led to an effective stabilization of KIF1A protein  
194 levels where the protein concentration remained mostly unchanged during the treatment period  
195 (Figure 4B and C). This finding suggests that, in the late phase of infection, new KIF1A synthesis  
196 is blocked but proteasomal degradation continues, leading to the accelerated loss of KIF1A during  
197 PRV infection.

198

199 These studies were done in differentiated PC12 cells that have similar attributes to sympathetic  
200 neurons. We performed similar experiments in primary rat superior cervical ganglionic neurons  
201 (SCG) cultured in compartmented chambers where we could examine kinesin motor concentration  
202 in isolated axons. We cultured primary sympathetic rat SCG neurons in trichambers, in which  
203 axons (in the neurite compartment, N) were fluidically and physically separated from cell bodies  
204 (in the soma compartment, S) (Figure 5A). SCG cell bodies in the S compartment were infected  
205 with PRV Becker for 24 hours and extracts from the S and N compartments were collected for  
206 western blot analysis. The concentration of KIF1A protein decreased dramatically both in cell  
207 bodies and axons after PRV infection (Figure 5B).

208

209 We further determined if the loss of KIF1A protein is mediated by proteasomes in isolated SCG  
210 axons during infection. SCG neurons cultured in trichambers were infected with PRV Becker in  
211 the S compartment, followed by MG132 treatment in the N compartment at 8 hpi. KIF1A protein  
212 levels were reduced significantly in both S and N compartments after PRV Becker infection  
213 (Figure 5C). Moreover, axonal KIF1A levels were partially restored by MG132 treatment in the  
214 N-compartment (Figure 5C). We concluded that PRV infection accelerates the degradation of  
215 KIF1A proteins by proteasomes in axons.

216

217 **Expression of both PRV early and late genes are involved in accelerated KIF1A degradation**  
218 **during infection.**

219

220 We next searched for viral proteins that might accelerate KIF1A protein degradation. First, we  
221 determined if early events such as virion entry and tegument protein delivery would be enough to  
222 promote KIF1A degradation. We infected PC12 cells either with UV treated PRV Becker virions  
223 (UVPRV) or with a PRV mutant that lacks IE180 (PRV HKO146), the single master immediate  
224 early transcriptional activator of viral genes required for RNA transcription and DNA replication  
225 (28, 29). Both UVPRV and PRV HKO146 (grown in IE180-complementing cells) enter the cells,  
226 release outer tegument proteins (including vhs, the virion host shutoff protein), and deposit viral  
227 DNA in the nucleus, but neither can initiate *de novo* viral protein synthesis. We found that after  
228 either infection, KIF1A was not degraded (Figure 6A). We concluded that PRV entry into PC12  
229 cells is not sufficient to accelerate KIF1A protein degradation.

230

231 We next determined if PRV immediate-early and early, or late genes were involved in KIF1A  
232 protein degradation. We pretreated differentiated PC12 cells with the herpesvirus DNA replication  
233 inhibitor AraT for 15 minutes, and infected them with PRV Becker for 8, 12, 16, 20, 24 hours.  
234 AraT inhibition should have minimal effects on early viral gene expression, but should severely  
235 diminish late gene expression. As expected, we detected comparable amounts of the early protein,  
236 EP0, in both AraT treated and untreated cells during infection, whereas the accumulation of the  
237 late protein VP5 (also major capsid protein, MCP) was severely affected by the AraT treatment  
238 (Figure 6B). AraT treatment led to a minor, though statistically significant, stabilization of KIF1A  
239 protein in the later stage of infection (Figure 6B-C). This experiment demonstrated that while PRV  
240 IE and/or E gene products could promote a reduction in KIF1A protein levels (e.g. potentially  
241 through the reduction of KIF1A transcripts), viral proteins accumulating in the late phase of  
242 infection are also required for efficient degradation of KIF1A.

243

244 **PRV anterograde-sorting complex US9/gE/gI promotes the accelerated degradation of**  
245 **KIF1A proteins.**

246

247 PRV particles in transport vesicles use KIF1A for sorting into axons and subsequent transport. The  
248 recruitment of KIF1A to transport vesicles containing enveloped PRV particles at the TGN  
249 requires viral membrane protein US9. This interaction is stabilized by the heterodimer of gE/gI.  
250 We had noted previously that two ubiquitin ligases, NEDD4 and viral EP0, co-purified with US9  
251 and KIF1A (14). Since KIF1A instability results from proteasomal degradation, we hypothesized  
252 that the tripartite complex US9/gE/gI might be involved in accelerating KIF1A degradation. We  
253 infected differentiated PC12 cells with PRV Becker and two PRV mutants that were deleted for

254 US9, gE, and gI genes, and are deficient in axonal sorting and anterograde spread: PRV Bartha  
255 and PRV BaBe (PRV Becker with the Us region of Bartha). Infection with either mutant lacking  
256 US9, gE, and gI partially restored KIF1A proteins during PRV infection (Figure 7A and B). As  
257 both of these mutant viruses degrade host transcripts presumably through early proteins, KIF1A  
258 levels show a comparable decrease between 8-12 hpi to Becker infection. However, we did not  
259 detect the rapid decrease in KIF1A protein levels after 12 h in Bartha and BaBe infections,  
260 suggesting that the tripartite complex help accelerate the degradation of KIF1A by proteasomes  
261 (Figure 1A and B). We further infected PC12 cells with two PRV US9 mutants deficient in  
262 anterograde spread, PRV 161 (US9-null), and PRV 172 (Y49-50A US9, functionally null). In  
263 PRV172 infections, all US9, gE, and gI proteins were present, but the dityrosine mutation within  
264 the acidic cluster of US9 gene disrupted the formation of the tripartite complex, and made the virus  
265 particles incapable of recruiting KIF1A and anterograde spread (14, 30, 31). Both PRV 161 and  
266 PRV 172 infections restored KIF1A proteins in magnitudes comparable to infections with PRV  
267 Bartha and Babe (Figure 7A and B). These results suggested that the formation of the tripartite  
268 complex is necessary for the accelerated loss of KIF1A proteins.

269  
270 We further determined if the US9/gE/gI complex could target KIF1A for degradation independent  
271 of PRV infection. We transduced differentiated PC12 cells with adenovirus vectors expressing the  
272 US9, gE, and gI protein, either individually or in combination, for 1, 2, 3, 4, 5 days. Neither the  
273 control Ad-GFP nor Ad-GFP-US9 transduction resulted in KIF1A protein instability, but when  
274 PC12 cells were transduced with equal units of the three adenovirus vectors expressing US9, gE,  
275 and gI, the KIF1A protein was completely degraded by 5 days post transduction (Figure 7C and

276 Figure S2). We concluded that a functional anterograde sorting complex could augment the  
277 proteasomal degradation of KIF1A protein independent of infection by PRV.  
278 Altogether these results suggest that PRV infection leads to the depletion of KIF1A proteins in  
279 infected neurons both by blocking new protein synthesis and also specifically targeting the motor  
280 for anterograde spread of progeny late in infection, which eventually accelerates proteasomal  
281 degradation of KIF1A.

282

## 283 **Discussion**

284

285 A major distinction of alphaherpesviruses from most of other neuroinvasive viruses is their ability  
286 to travel in both anterograde and retrograde directions in axons and in neuronal circuits (4). The  
287 anterograde transport of newly assembled herpesvirus virions is crucial for the viral life cycle  
288 because it ensures that virus particles are sorted into axons to facilitate inter-host transmission.  
289 KIF1A mediates the sorting and transport of PRV egressing virions in axons, with the dynamics  
290 of transport involving long segment runs with high velocity ( $V_{max} \sim 2.4\mu\text{m/s}$ ) (16). The  
291 anterograde transport of PRV virions was greatly hindered in the neurons expressing the dominant-  
292 negative form of KIF1A protein (14). In this study, we demonstrated that KIF1A is intrinsically  
293 prone to degradation by the proteasomes, and that PRV infection accelerates such degradation and  
294 results in near depletion late in infection. The steady state concentration of KIF1A protein in  
295 uninfected neuronal cells is achieved through rapid protein synthesis and proteasomal degradation.  
296 Upon PRV infection, KIF1A mRNA was degraded in a nonspecific manner, reducing KIF1A  
297 protein synthesis while the existing proteins were gradually degraded by the proteasome. In  
298 addition, adenovirus vectors expressing proteins constituting the PRV anterograde sorting

299 complex US9/gE/gI induced a decline in KIF1A protein level independent of PRV infection,  
300 suggesting the US9/gE/gI tripartite complex accelerates the proteasomal degradation of KIF1A  
301 and contributes, alongside PRV host shutoff, to the decline in KIF1A protein during PRV infection.  
302 Importantly, using compartmented primary neuron cultures we showed that proteasomal  
303 degradation of KIF1A is accelerated in axons after PRV infection.

304

305 **The intrinsic instability of the KIF1A protein.**

306

307 The kinesin motor KIF1A mediates fast axonal transport of membranous vesicles including  
308 synaptic vesicle precursors and dense core vesicles, and is critical in synaptogenesis (17, 18). In  
309 differentiated PC12 cells, KIF1A protein levels decreased rapidly upon treatment with the protein  
310 synthesis inhibitor cycloheximide (CHX), and showed an apparent half-life of approximately 4  
311 hours. The half-lives of kinesin-1 and -2 motors were longer under the same condition (>8 hours).  
312 Our results are consistent with a previous proteomics study using rat cortical neurons, where  
313 KIF1A had the 8<sup>th</sup> shortest half-life in among ~2800 proteins measured ( $t_{1/2} = 0.42$  day) (32),  
314 suggesting that this is a consistent characteristic of KIF1A in both CNS and PNS neurons. The  
315 decline in KIF1A concentration is blocked when PC12 cells were treated simultaneously with  
316 CHX and MG132, demonstrating that the loss of KIF1A protein was predominately mediated by  
317 proteasomes. Our results confirmed another previous proteomics study where the half-life of the  
318 KIF1A protein increased and the degradation rate dropped upon inhibition of proteasomes (33).

319

320 UNC-104, the KIF1A homolog in *C. elegans*, was shown to be degraded at synapse regions *in vivo*  
321 in an ubiquitin-dependent manner. Moreover, the stability of the motor was linked to its capacity

322 for cargo binding (22). It was also proposed that the degradation of Liprin $\alpha$ 1, an cargo adaptor for  
323 KIF1A, by either proteasome or CaMKII phosphorylation was required for the “unloading” of  
324 LAR (leukocyte common antigen- related) family of receptor protein tyrosine phosphatases at  
325 synapses (34, 35). These studies imply a potential mechanistic link between motor degradation  
326 and cargo release. Since we also observed proteasomal degradation of KIF1A in fluidically and  
327 physically separated axons during PRV infection, KIF1A may be actively degraded in axons by  
328 proteasomes upon release of viral cargo.

329  
330 The relative stabilities of KIF5 and KIF3A proteins were unexpected, since several lines of  
331 evidence suggested that both motors are actively degraded in axons (36-39). However, unlike  
332 KIF1A, both KIF5 and KIF3A also mediate short distance transport of cellular cargoes within cell  
333 bodies, and may be recycled for future use. The particular short half-life of KIF1A is consistent  
334 with the idea that KIF1A is a specialized motor for axonal transport.

335  
336 **The reduction of KIF1A mRNA upon PRV infection.**  
337  
338 The dynamic steady state level of KIF1A protein in uninfected cells suggests that, in PRV-infected  
339 cells, KIF1A protein concentration may be sensitive to viral host shutoff or mRNA instability  
340 induced by infection. Alphaherpesviruses reduce/block the synthesis of some cellular proteins after  
341 infection to facilitate viral gene expression and to evade host antiviral responses. The virion host  
342 shutoff protein (vhs) is an endoribonuclease encoded by the viral UL41 gene (40-42). HSV-1 vhs  
343 enters the infected cells as a part of incoming virion tegument, and subsequently interacts with  
344 translation initiation factors eIF4H, eIF4B, and eIF4A to degrade host mRNAs and halts host

345 protein translation (43, 44). Infection of PC12 cells with inactivated virions (UV'd, IE180-null  
346 PRV) did not lead to the decline in KIF1A protein. We conclude that tegument proteins like vhs  
347 delivered by entering particles are not sufficient for the reduction in KIF1A protein during  
348 infection.

349

350 We further analyzed the accumulation of cellular mRNAs during infection and compared the levels  
351 of kinesin motor transcripts. qRT-PCR analysis of PRV-infected PC12 cells revealed that the  
352 levels of KIF1A, KIF5B, and GAPDH transcripts all decreased significantly at similar rates,  
353 starting as early as 3 h.p.i., and reaching almost full depletion at 12 h.p.i. The reduction of KIF1A  
354 mRNA reduced the rate of new protein synthesis. This reduction of new KIF1A proteins, coupled  
355 with the active degradation of existing KIF1A motors by proteasomes, leads to the significant  
356 decrease in KIF1A protein concentration in infected neuronal cells.

357

358 **The PRV anterograde sorting complex US9/gE/gI directs more KIF1A motors to be  
359 degraded by proteasomes.**

360

361 US9 forms a complex with two other membrane proteins, gE and gI to sort progeny PRV virions  
362 in transport vesicles into axons (14, 15)(J. Scherer, I. B. Hogue, Z. A. Yaffe, N. S. Tannet, B. Y.  
363 Winer, M. Vershinin, L. W. Enquist, submitted for publication). We have previously identified  
364 KIF1A as an interaction partner of PRV US9 protein (14). In addition, we had noted that two  
365 ubiquitin ligases, NEDD4 and viral EP0, co-purified with US9 and KIF1A (14). Since we also  
366 showed accelerated proteasomal degradation of KIF1A during infection, we hypothesized that the  
367 PRV anterograde sorting complex, gE/gI/US9, could independently recruit KIF1A to viral

368 transport vesicles for axonal sorting and transport, and eventually promote the proteasomal  
369 degradation of KIF1A in axons. We investigated this hypothesis using adenovirus vectors to  
370 express the components of the anterograde sorting complex (one by one and in combination) in  
371 neuronal cells without PRV infection. In cells transduced with adenovirus vectors expressing US9,  
372 gE, and gI, US9 interacts with the gE/gI heterodimer in lipid rafts, and the US9, gE, and gI complex  
373 was observed to undergo axonal anterograde transport together with long-range motility  
374 characteristic of anterogradely-moving PRV virions in axons (J. Scherer, I. B. Hogue, Z. A. Yaffe,  
375 N. S. Tanneti, B. Y. Winer, M. Vershinin, L. W. Enquist, submitted for publication). PRV envelope  
376 proteins, US9/gE/gI, when expressed together in neuronal cells, induced a significant decline in  
377 KIF1A protein concentration. Therefore we concluded that the viral anterograde sorting complex  
378 US9/gE/gI promotes the accelerated loss of KIF1A proteins.

379  
380 This result is consistent with experiments where PC12 cells were infected with PRV strains that  
381 fail to express a functional US9/gE/gI complex to recruit KIF1A to undergo anterograde spread.  
382 These PRV mutants still promote host shutoff, leading to the decline of KIF1A proteins late in  
383 infection. However, the rate of decline is slower than that observed after wildtype PRV infection,  
384 suggesting that the US9/gE/gI complex accelerates the degradation of KIF1A by the proteasomes.  
385 The inhibition of late gene expression (e.g. US9) by AraT treatment also led to a minor yet  
386 statistically significant stabilization of KIF1A protein concentration. We concluded that the PRV  
387 anterograde sorting complex US9/gE/gI directs more KIF1A motors to be degraded by  
388 proteasomes. This might shed light on the mechanisms underlying the intrinsic instability of  
389 KIF1A protein, which potentially stem from the interaction of the motor with its cellular cargoes.

390

391 **PRV infection induces the accelerated loss of KIF1A protein through two separate  
392 mechanisms.**

393

394 Our model to account for our results is shown in Figure 8. KIF1A motors, mediating the  
395 anterograde transport of the vesicles in axons critical for synapse formation and function, are  
396 inherently prone to proteasomal degradation. Such intrinsic instability results in the dynamic  
397 steady state level of KIF1A protein maintained through rapid protein synthesis and proteasomal  
398 degradation in neuronal cells (Fig. 8A). The balance between protein synthesis and degradation is  
399 disrupted by PRV infection through two separate mechanisms (Fig. 8B). First, PRV infection  
400 promotes the nonspecific loss of KIF1A mRNAs, and reduces the synthesis of KIF1A protein. The  
401 concentration of existing KIF1A protein continues to decline through default proteasomal  
402 degradation. Second, PRV infection accelerates the degradation of KIF1A protein through the  
403 PRV anterograde sorting complex US9/gE/gI. We propose that US9 recruits KIF1A to transport  
404 vesicles containing enveloped virions or light particles, as well as vesicles with viral glycoproteins  
405 for axonal sorting and transport, and eventually this complex promotes the proteasomal  
406 degradation of KIF1A in axons.

407

408 Our work implies that after reactivation from latently infected PNS neurons, the targeted loss of  
409 KIF1A motor proteins leads to a limited time window for sorting into and transport of viral  
410 particles in axons for further host-to-host spread. Indeed, the loss of available KIF1A motors over  
411 the course of PRV infection results in reduced recruitment of the motor by US9 (14). While our  
412 work has focused on PRV, it would be important to know if infection by other alphaherpesviruses  
413 promotes differential degradation of axonal motor proteins. The anterograde transport of HSV-1

414 has been shown to be mediated by kinesin-1 (KIF5A, KIF5B, and KIF5C) and kinesin-3 (KIF1A)  
415 motors (11, 45)(E. Engel and L.W. Enquist, unpublished data). It is therefore of clinical  
416 importance to determine if HSV-1 infection induces similar loss in human neurons *in vitro*. Such  
417 a loss of kinesin-1 and kinesin-3 motors after reactivation from PNS neuron could have significant  
418 consequence for long term neuronal function. Further work is required to understand whether PNS  
419 neurons can tolerate the loss of specific kinesin motors during reactivation episodes, and whether  
420 the motor concentrations are restored after reactivation if those neurons survive.

421

422 **Materials and Methods:**

423

424 **Cell lines and viruses.** Porcine kidney epithelial cells (PK15, ATCC) were maintained in  
425 Dulbecco modified Eagle medium (DMEM, Hyclone) supplemented with 10% fetal bovine serum  
426 (FBS, Hyclone) and 1% penicillin-streptomycin (Hyclone). PK15 cells were used to propagate and  
427 determine titers of all PRV strains used in this study. 293A cells (Invitrogen) were used to  
428 propagate and determine the titer of adenovirus vectors used in this study. The neuronal PC12 cell  
429 line has been used extensively to model primary neurons infection of PRV and to reproduce the  
430 US9-associated transport phenotype (14, 46). PC12 cells were cultured on dishes coated with type  
431 1 rat tail collagen (BD Bioscience, Bedford, MA) in 85% RPMI 1640 (Thermo Fisher)  
432 supplemented with 10% horse serum(Life Technologies) and 5% FBS (47). PC12 cells were  
433 differentiated in RPMI 1640 supplemented with 1% horse serum and nerve growth factor (NGF,  
434 2,5S, Invitrogen) at 100 ng/ml (47). Differentiation medium was replaced every third day for eight  
435 days before infection.

436

437 The PRV viral strains used in this study include both wild-type laboratory stain and recombinants.  
438 PRV Becker is a wildtype strain (25, 26). PRV Bartha is an attenuated vaccine strain (48, 49). PRV  
439 BaBe is a viral recombinant in the PRV Becker background with deletions from the unique short  
440 (US) region of PRV Bartha (50). HKO146 lacks the only PRV immediate early gene IE180 and  
441 expresses hSyn Cre-t2a-venus in US9 loci in the PRV Becker background (H. Oyibo, P.  
442 Znamenskiy, H. V. Oviedo, L. W. Enquist, A. Zador, unpublished data). PRV161 harbors a  
443 complete deletion of the US9 gene in the PRV Becker background (51). PRV172 harbors two  
444 tyrosine to alanine substitutions (Y49 Y50 to AA) in the PRV Becker background (30). Individual  
445 replication-deficient adenovirus vectors expressing GFP, GFP-US9, gE-mCherry, gI-mTurquoise  
446 2 were previously reported (14, 52).

447  
448 **Primary neuronal culture.** Embryonic superior cervical ganglia (SCG) neurons were isolated and  
449 cultured as previously described (53). Briefly, superior cervical ganglia (SCG) were isolated from  
450 day 17 Sprague-Dawley rat (Hilltop Labs) embryos, plated and maintained on poly-DL-ornithine  
451 (Sigma-Aldrich) and laminin (Invitrogen) coated dishes (Falcon or MatTek) in neuronal medium  
452 made with neurobasal medium (Gibco) supplemented with 1% penicillin-streptomycin with 2 mM  
453 glutamine (Invitrogen), 2% B-27 (Gibco), and 100 ng/ml neuronal growth factor (NGF, 2.5S  
454 (Invitrogen)). On two days post plating, 1 mM cytosine-D-arabinofuranoside (AraC; Sigma-  
455 Aldrich) was added to selectively eliminate dividing non-neuronal cells in culture for two days.  
456 SCG neurons were allowed to differentiate for at least 14 days before infection.

457  
458 Compartmented neuronal cultures were prepared on poly-ornithine and laminin coated plastic  
459 dishes. Parallel grooves were etched into the plastic dish, and 1% methylcellulose in neuronal

460 medium was added to the region that would underlie the middle compartment of the isolator rings.  
461 CAMP320 isolator rings (Tyler Research) were coated with autoclaved silicone vacuum grease on  
462 one side and gently applied to the dish so that the etched grooves extended across three  
463 compartments. Dissociated SCG neurons were plated in the soma compartment (S), and were  
464 allowed to extend axons along the grooves through the M compartment and into the neurite (N)  
465 compartment for 14-28 days.

466

467 **Viral infection in neuronal culture.** Triplicates of differentiated PC12 cells were infected with  
468 the indicated PRV strain at a multiplicity of infection (MOI) of 20 at the indicated hours post  
469 infection. Infected cells from each triplicate were harvested and combined into a single sample for  
470 further biochemical analysis. Compartmented SCG neuronal cultures were infected in the soma (S)  
471 compartment for 24 hours. Neuronal cell bodies from the S compartments and axons from the N  
472 compartments were harvested for biochemical analysis. The proteasome inhibitor MG132  
473 (Millipore Sigma) was prepared in DMSO and added at a concentration of 2.5  $\mu$ M. The herpesvirus  
474 DNA replication inhibitor thymine 1- $\beta$ -D-arabinofuranoside (AraT) (Millipore Sigma) was added  
475 at a concentration of 100 $\mu$ g/ml 15 minutes prior to infection.

476

477 **Adenovirus vectors transduction.** The adenovirus vectors expressing GFP, GFP-US9, gE-  
478 mCherry, and gI-mTurquoise-2 were reported previously (14, 52). Adenovirus vectors were  
479 propagated in 293A cells, and cell-associated virus was harvested in serum-free DMEM media.  
480 Adenoviral transduction of differentiated PC12 cells was performed for up to five days to ensure  
481 the expression of fluorescent transgene(s) in the majority of cells (>90%).

482

483 **Quantitative Western blotting analysis.** Neuronal cell lysates were prepared in  
484 radioimmunoprecipitation assay buffer (RIPA) (50mM Tris-HCl, 150mM NaCl, 5mM EDTA, 1%  
485 NP-40, 0.1% SDS, 0.1% Triton X-100, and 1% sodium deoxycholate, pH 8.0) supplemented with  
486 1 mM dithiothreitol (DTT, Sigma-Aldrich) and protease inhibitor cocktail (Sigma-Aldrich). Cell  
487 lysates were kept on ice for one hour and centrifuged at 13,200 rpm at 4°C for 10 minutes. Cleared  
488 cell lysates were transferred to a new tube and mixed with 4X LDS sample loading buffer  
489 (Invitrogen) supplemented with 240 mM DTT. The samples were heated at 95°C for 10 minutes  
490 and centrifuged at 4°C for 10 minutes to remove potential protein aggregates before proteins were  
491 separated in gradient polyacrylamide gels (4-12%) (Invitrogen). Proteins were transferred onto  
492 nitrocellulose membranes (Whatman) by semidry transfer (Bio-Rad). Membranes were blocked in  
493 5% nonfat dry milk in Tris buffer saline with 0.1% Tween 20 (TBS-T) for 1 hour at room  
494 temperature. Primary and secondary antibodies for immunoblot analysis were prepared in 1% milk  
495 in TBS-T. Protein bands were imaged by LI-COR Odyssey CLx imaging system. The signal  
496 intensity was quantified using LI-COR Image Studio Lite software.

497  
498 Antibodies used in this study included a mouse monoclonal KIF1A antibody (Clone 16, BD, at  
499 1:2000), a mouse monoclonal kinesin heavy chain antibody (MAB-1614, Millipore Sigma, at  
500 1:1000), a mouse monoclonal KIF3A antibody (Clone K2.4, Covance Inc., at 1:1000), a mouse  
501 monoclonal β-Actin antibody (Sigma, at 1:10000), a monoclonal US9 antibody (IA8, DSHB, at  
502 1:100), a rabbit polyclonal GST-EP0 antibody at 1:2000 (54), a mouse monoclonal PRV VP5  
503 antibody at 1:1000 (55), a mouse monoclonal PRV US3 antibody at 1:10000 (56), a rabbit  
504 polyclonal gE cytoplasmic tail antibody at 1:1000 (57), a rabbit polyclonal gI antibody, a generous  
505 gift from K. Bienkowska-Szewczyk, at 1:1000 (58). IRDye secondary antibodies included 800CW

506 donkey anti-mouse IgG, 800CW donkey anti-rabbit IgG, and 680RD donkey anti-rabbit at 1:15000  
507 (LI-COR).

508

509 **qRT-PCR analysis.** Differentiated PC12 cells infected with PRV were harvested and subjected to  
510 total RNA extraction using an RNeasy Plus Mini Kit (Qiagen). First strand cDNA synthesis of  
511 isolated RNAs was performed with SuperScript III First Strand synthesis kit with Oligo (dT)  
512 primer (Invitrogen). Quantitative reverse transcription PCR was performed using Eppendorf  
513 Realplex<sup>2</sup> Mastercycler with reaction mixtures prepared with KAPA SYBR Fast Universal qPCR  
514 Kit (KAPA Biosystems). The PCR primers for KIF1A, KIF5B, GAPDH, and 18s rRNA were  
515 reported elsewhere (59). The qRT-PCR reaction samples were prepared in triplicates. The relative  
516 abundance of RNA in each sample was calculated using the  $-\Delta\Delta C_T$  method normalized to 18s  
517 rRNA and subsequently to uninfected samples.

518

519 **Statistical analysis.** One-way analysis of variance (ANOVA) with Tukey's posttest, and two-way  
520 ANOVA with Sidak's posttest were performed using GraphPad Prism 6. Values in text, graphs,  
521 and figure legends throughout the manuscript are means  $\pm$  standard errors of the means (SEM).

522

523 **Image production.**

524 Cartoon illustrations were made with web application BioRender under Princeton University  
525 license for education use.

526

527 **Ethics Statement.** All animal work was performed in accordance with the Princeton Institutional  
528 Animal Care and Use Committee (protocols 1947-16). Princeton personnel are required to adhere

529 to applicable federal, state, local and institutional laws and policies governing animal research,  
530 including the Animal Welfare Act and Regulations (AWA); the Public Health Service Policy on  
531 Humane Care and Use of Laboratory Animals; the Principles for the Utilization and Care of  
532 Vertebrate Animals Used in Testing, Research and Training; and the Health Research Extension  
533 Act of 1985.

534

### 535 **Acknowledgement**

536 We thank Halina Staniszewska-Goracznik for excellent technical and logistical support, and the  
537 rest members of the Enquist Lab for their critical comments on the projects. This project was  
538 funded by NIH, National Institute of Neurological Disorders and Stroke (NINDS) RO1 NS033506  
539 and RO1 NS060699 (L.W.E.).

540

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704 **Figure legends**

705 **Fig. 1. KIF1A protein undergoes rapid accumulation and degradation in neuronal cells upon**  
706 **MG132 and CHX treatments.**

707 (A) Differentiated PC12 cells were treated with the translation inhibitor cycloheximide,  
708 proteasome inhibitor MG132, or both cycloheximide and MG132 for 2, 4, 6, 8, hours. Cells were  
709 harvested at indicated time points and lysates were analyzed by Western blot to monitor protein  
710 levels of KIF1A, KIF5, KIF3A, and actin. (B-D) KIF1A, KIF5, and KIF3A protein levels were  
711 measured by band intensities and normalized to actin protein levels for each time interval.  
712 Normalized values were again normalized to that of Mock 0 hour samples. Values are means plus  
713 SEMs (error bar) from three independent experiments. n.s., not statistically significant, \*\*, P<0.01,  
714 \*\*\*, P<0.001, \*\*\*\*, P<0.0001 for the indicated comparison at 8 hours post treatment.

715

716 **Fig. 2. KIF1A protein concentration is reduced during PRV infection in neuronal cells.**

717 (A and B) Differentiated PC12 cells were mock-infected or infected with PRV Becker for 3, 8, 12,  
718 16, 20, 24 hours with MOI of 20. Cells were harvested at the time intervals shown, and lysates  
719 were analyzed by Western blot to monitor protein levels of KIF1A, KIF5, KIF3A, and actin.  
720 Kinesin levels were measured by band intensities and normalized with respect to actin levels at  
721 each time interval. Normalized values were again normalized to that of Mock 0 hour samples.  
722 Values are means plus SEMs (error bars) from four independent experiments. n.s., not statistically  
723 significant, \*\*\*, P<0.001, \*\*\*\*, P<0.0001.

724

725 **Fig. 3. The abundance of KIF1A, KIF5B, and GAPDH transcripts is significantly reduced in**  
726 **PRV-infected neuronal cells.**

727 Differentiated PC12 cells were infected with (A) PRV Becker or (B) PRV Bartha for 3, 8, 12, 16,  
728 20, 24 hours with MOI of 20. Cells were harvested at the time intervals shown. Total RNA were  
729 extracted and qRT-PCR quantification was performed to measure KIF1A, KIF5B, and GAPDH

730 mRNA levels using 18S rRNA as internal control at each time interval. The normalized values are  
731 then normalized to that of Mock 0 hour samples. Values are means plus SEMs (error bars) from  
732 three independent experiments.

733

734 **Fig. 4. KIF1A is degraded by the proteasome in the late phase of PRV infection.**

735 Differentiated PC12 cells were (A) mock-infected or (B) infected with PRV Becker for 8, 12, 16,  
736 20, 24 hours with MOI of 20. In a parallel experiment, the proteasomal inhibitor, MG132, was  
737 added at 12 hours post infection at the concentration of 2.5  $\mu$ M. KIF1A levels were analyzed by  
738 Western blot and normalized to actin levels at each time interval. Normalized values were again  
739 normalized to that of Mock 0 hour samples. Values are means plus SEMs (error bars) from three  
740 independent experiments. \*\*, P<0.01 for comparison between PRV-infected and PRV-  
741 infected+MG132 samples.

742

743 **Fig. 5. PRV infection of compartmented primary neuronal cultures leads to reduction of**  
744 **KIF1A protein and proteasomal degradation in axons.**

745 (A) Illustration of *in vitro* culture of primary superior cervical ganglion neurons using modified  
746 Campenot chambers that include the S (soma), M (methocel), and N (neurite, axon termini)  
747 compartments.  $10^6$  PFU of PRV Becker was added in the S (soma) compartment. (B and C) SCG  
748 neurons were infected at the Soma (S) compartment with PRV Becker for 24 hours. (B) Cell bodies  
749 and axons from S and N compartments were collected at the time intervals show, and lysate were  
750 analyzed by Western blot to monitor the levels of KIF1A and Actin. (C) DMSO or MG132 (2.5  
751  $\mu$ M) were added to the N (Neurite) compartment at 8 hpi. Cell bodies and axons from S and N

752 compartments were collected at the time intervals show, and lysate were analyzed by Western blot  
753 to monitor the levels of KIF1A and Actin.

754

755 **Fig. 6. Accelerated proteasomal degradation of KIF1A during infection requires the**  
756 **expression of both PRV early and late proteins.**

757 (A) *De novo* PRV viral protein synthesis is required for KIF1A degradation. Differentiated PC12  
758 cells were mock-infected or infected with PRV Becker, UV'd PRV Becker, or IE180-null PRV  
759 (HKO146) for 24 hours with MOI of 20. Cells were harvested and lysates were analyzed by  
760 Western blot to monitor the levels of KIF1A, actin, and VP5 at indicated time intervals. (B)  
761 Differentiated PC12 cells were mock-treated or treated with AraT (100 $\mu$ g/ml) starting 15 minutes  
762 before being infected with PRV Becker for 0, 8, 12, 16, 20, 24 hours. Samples were collected at  
763 the indicated time intervals. Lysates were subjected to Western Blot analysis to measure protein  
764 levels of KIF1A, actin, VP5, and EP0. (C) For every infection, KIF1A protein abundance were  
765 measured by band intensities and normalized with respect to actin levels at each time interval.  
766 Normalized values were then normalized to that for Mock 0 hour samples. Values are means plus  
767 SEMs (error bars) from three independent experiments. \*, P<0.05 for PRV Becker vs. PRV Becker  
768 with AraT comparison at specified time interval.

769

770 **Fig. 7. PRV anterograde-spread complex US9/gE/gI promotes the accelerated degradation of**  
771 **KIF1A proteins.**

772 (A) Differentiated PC12 cells were infected with PRV Becker, or different PRV mutants defective  
773 in anterograde spread (results from PRV BaBe and PRV161 infections are shown) for 0, 8, 12, 16,  
774 20 ,24 hours. Cells were harvested and lysates were analyzed by Western blot to monitor KIF1A

775 and actin protein Level. (B) For every infection, KIF1A levels were measured by band intensities  
776 and normalized with respect to actin levels at each time interval. Normalized values were then  
777 normalized to that for Mock 0 hour samples. Values are means plus SEMs (error bars) from seven  
778 independent experiments. \*, P<0.05 for all mutants vs. PRV Becker at specified time interval. (C)  
779 Differentiated PC12 cells were transduced with Adenoviral vectors expressing GFP, GFP-US9, or  
780 GFP-US9/gE-mCherry/gI-mTurquoise 2. Cells were collected at indicated days post transduction  
781 (d.p.t.) and subjected to Western Blot Analysis to monitor KIF1A and Actin level.

782

783 **Fig. 8. PRV infection induces the accelerated loss of KIF1A protein through two separate**  
784 **mechanisms.**

785 (A) In uninfected cells, KIF1A protein is inherently prone to be degraded by the proteasome. The  
786 steady state concentration of KIF1A protein is achieved through rapid protein synthesis and  
787 proteasomal degradation. (B) PRV infection induces KIF1A mRNA degradation and reduced  
788 protein synthesis, while the existing KIF1A proteins undergo default proteasomal degradation.  
789 Furthermore, the proteasomal degradation of the existing KIF1A proteins is accelerated by the  
790 PRV gE/gI/US9 complex in the late phase of infection. Images were created with BioRender.

791

# Figure 1

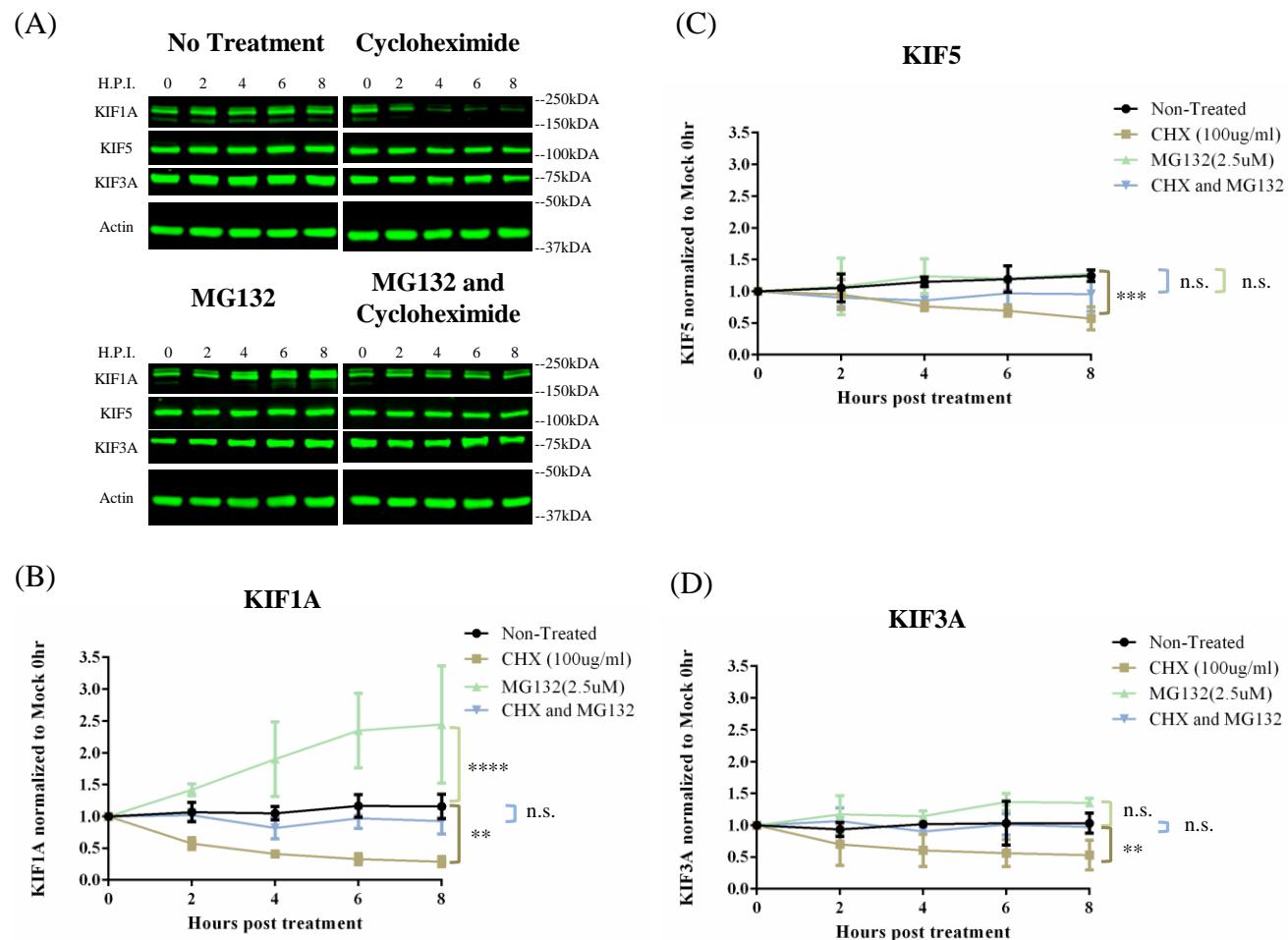


Fig. 1. KIF1A protein undergoes rapid accumulation and degradation in neuronal cells upon MG132 and CHX treatments.

# Figure 2

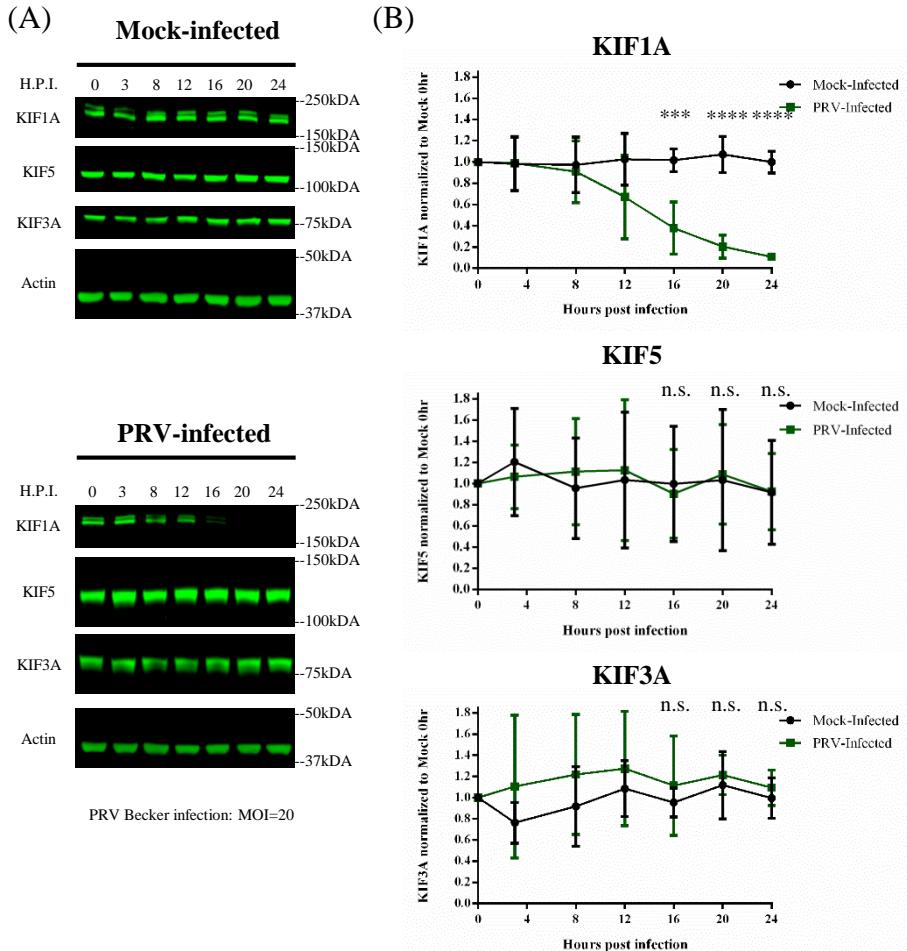
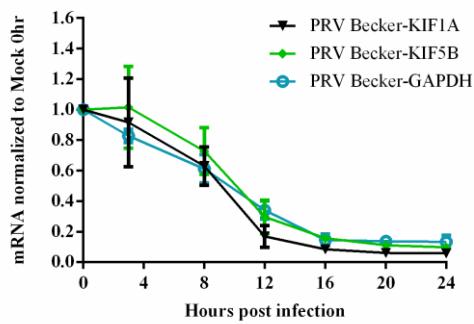


Fig. 2. KIF1A protein concentration is reduced during PRV infection in neuronal cells.

# Figure 3

(A)



(B)

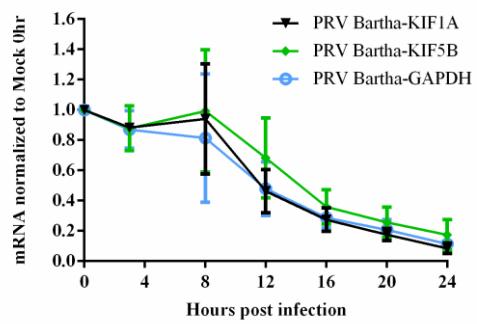


Fig. 3. The abundance of KIF1A, KIF5B, and GAPDH transcripts is significantly reduced in PRV-infected neuronal cells.

# Figure 4

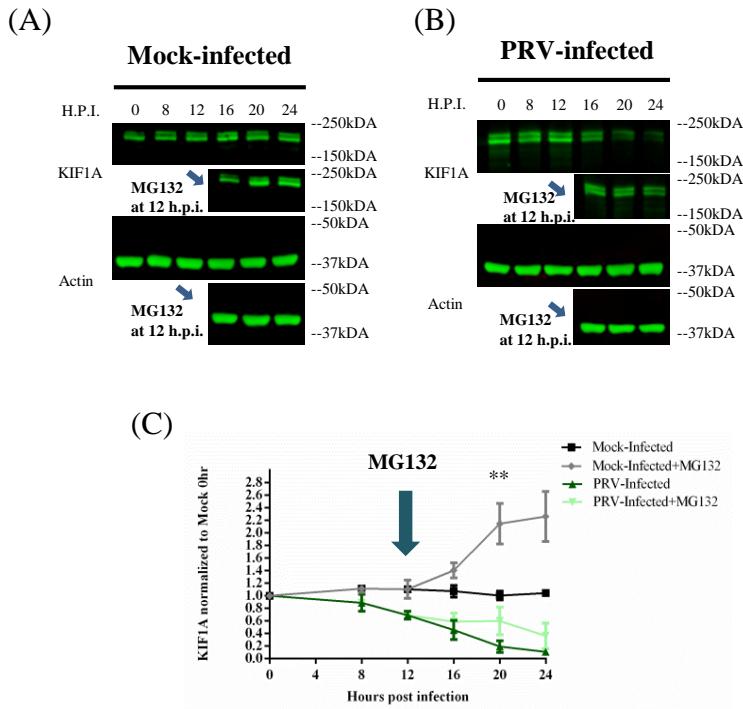


Fig. 4. KIF1A is degraded by the proteasome in the late phase of PRV infection.

# Figure 5

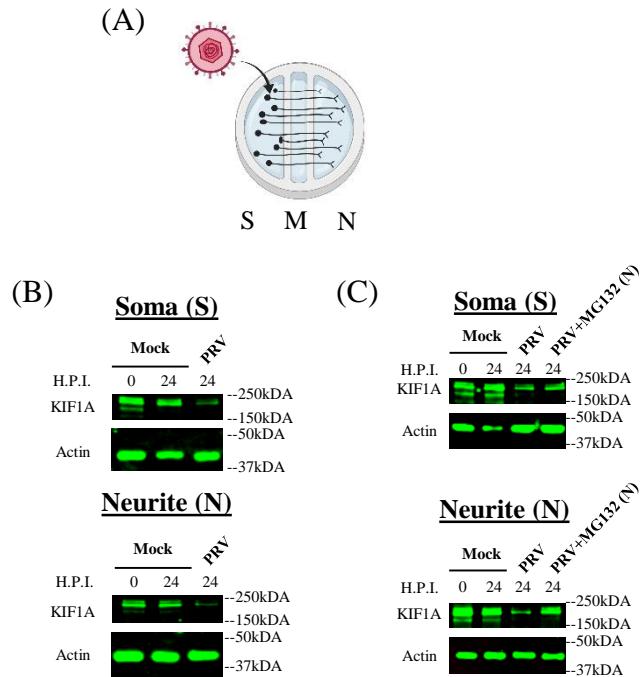


Fig. 5. PRV infection of compartmented primary neuronal cultures leads to reduction of KIF1A protein and proteasomal degradation in axons.

# Figure 6

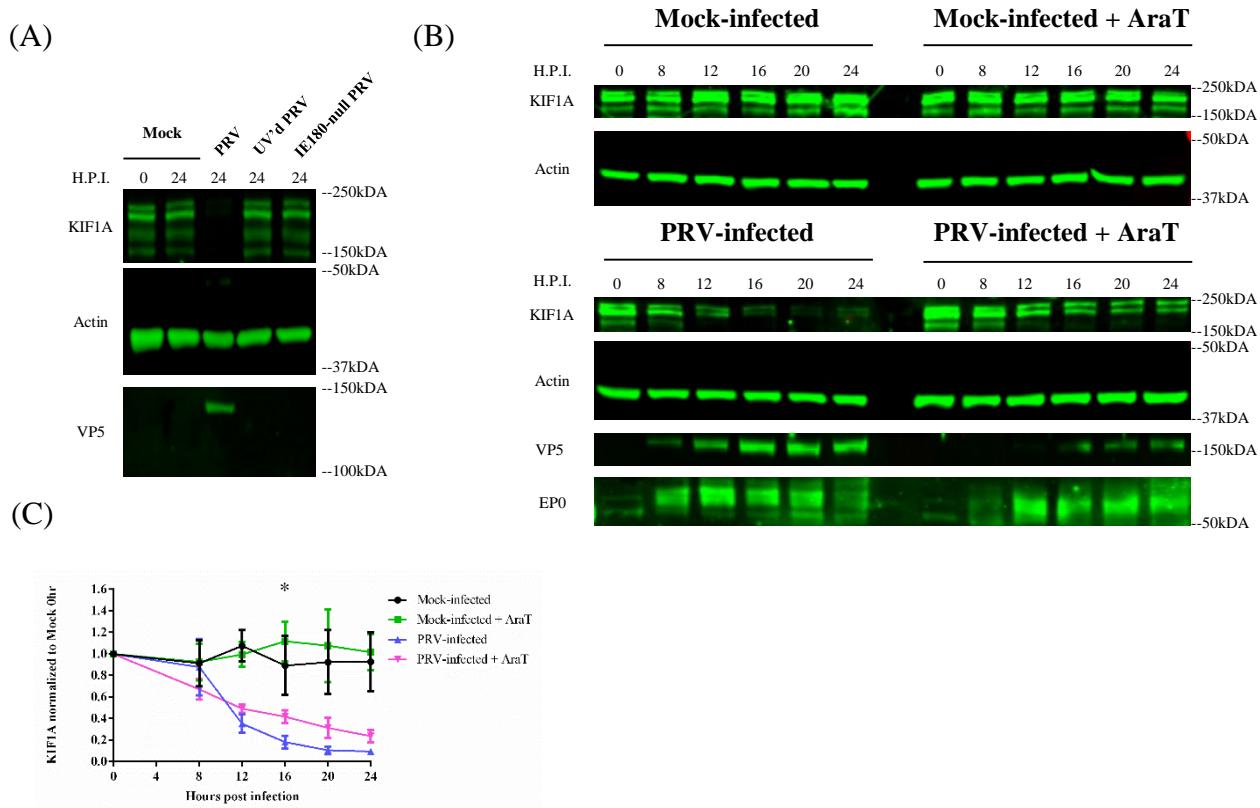
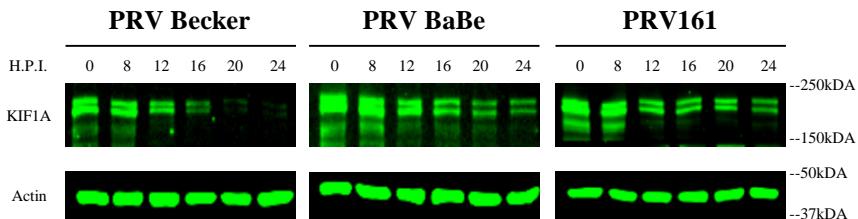


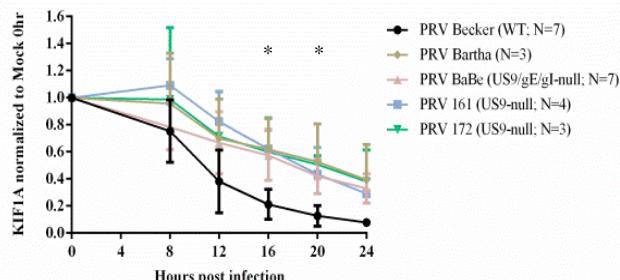
Fig. 6. Accelerated proteasomal degradation of KIF1A during infection requires the expression of both PRV early and late proteins.

# Figure 7

(A)



(B)



(C)

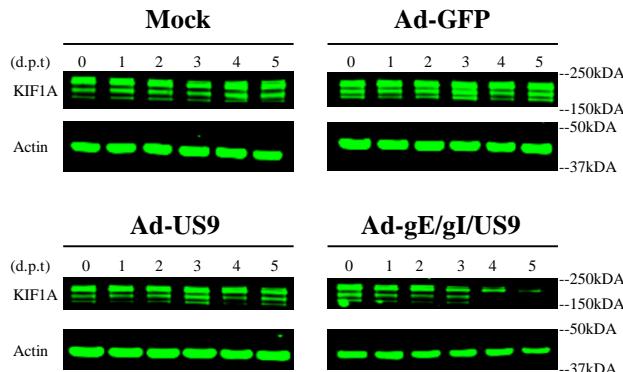
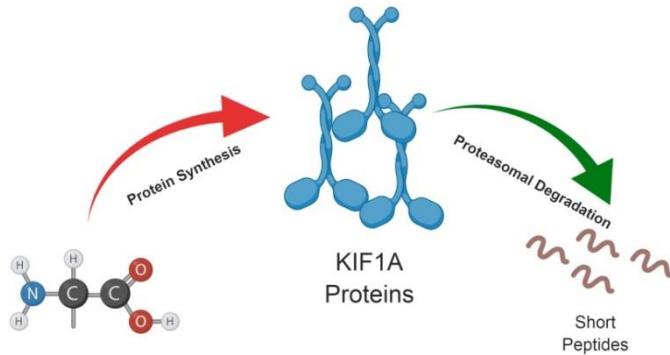


Fig. 7. PRV anterograde-spread complex US9/gE/gI promotes the accelerated degradation of KIF1A proteins.

# Figure 8 (MODEL)

(A)



(B)

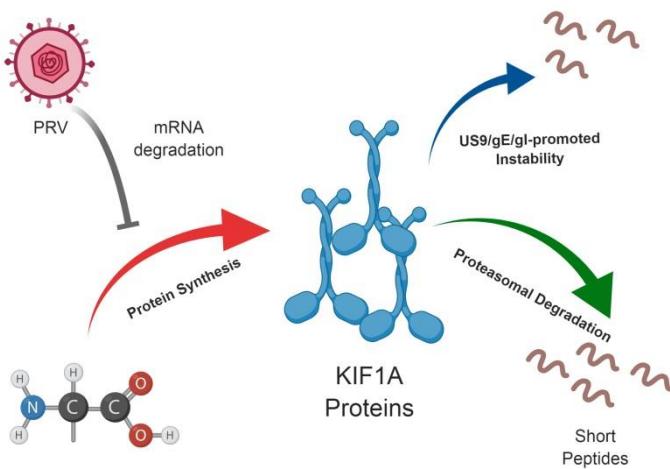


Fig. 8. PRV infection induces the accelerated loss of KIF1A protein through two separate mechanisms.

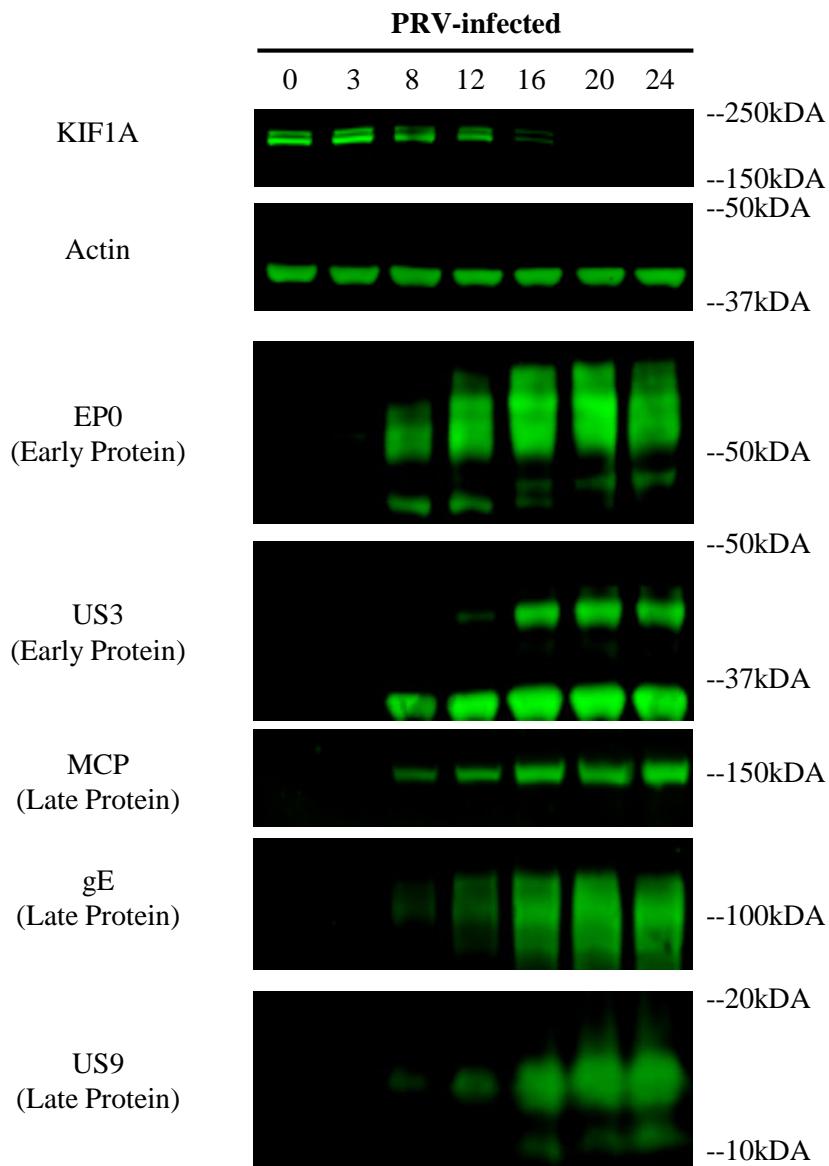
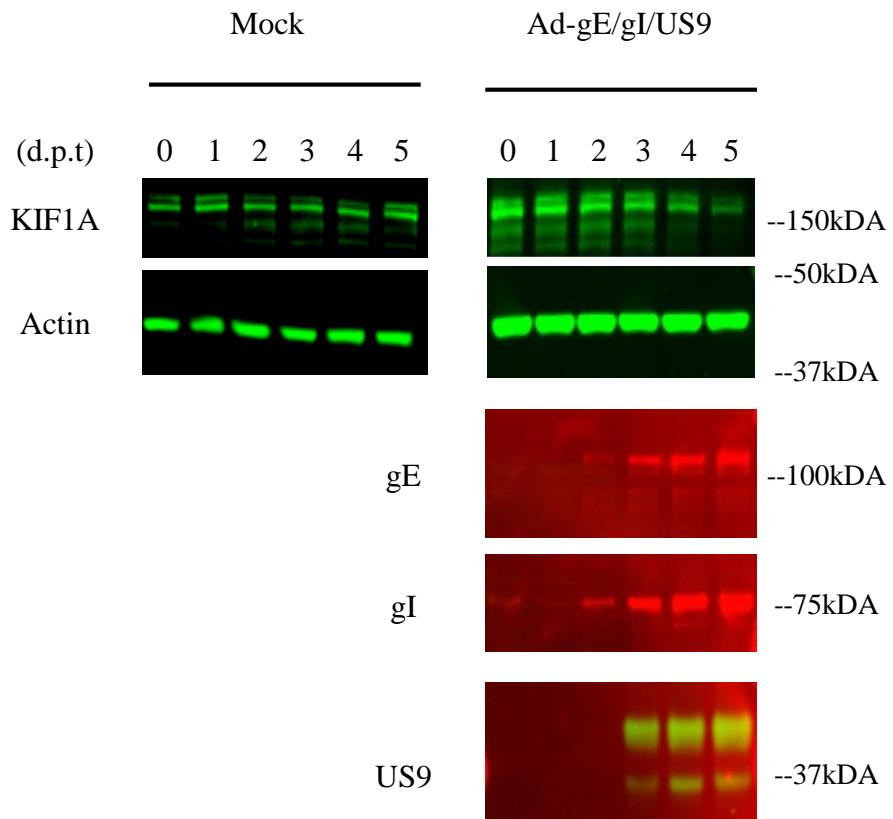


Figure S1. PRV early and late gene expressions in differentiated PC12 cells.



**Figure S2. Expression of transduced genes US9, gE, and gI in differentiated PC12 cells.**