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Supplementary Materials for

β-Arrestin–Dependent Activation of the Cofilin Pathway Is Required for the Scavenging Activity of the Atypical Chemokine Receptor D6

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Fig. S1. D6 stimulates cofilin phosphorylation through a G protein–independent Rac1-PAK1 signaling pathway to sustain chemokine degradation in BeWo cells.

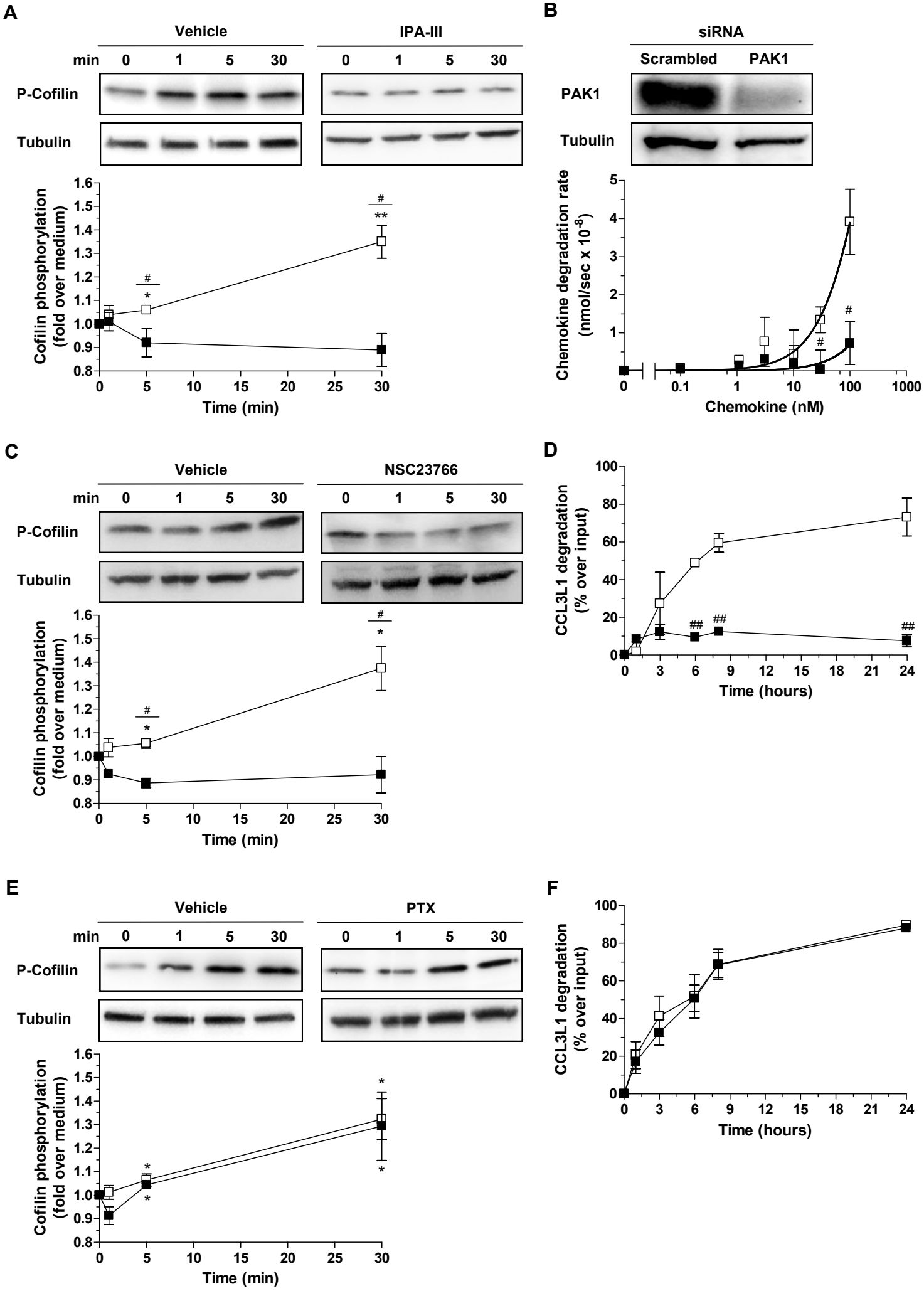
A
Vehicle
IPA-III
min 0 1 5 30
0 1 5 30
P-Cofilin
Tubulin
Cofilin phosphorylation (fold over medium)
Time (min)
B
siRNA
Scrambled
PAK1
PAK1
Tubulin
Chemokine degradation rate (nmol/sec x 10-8)
Chemokine (nM)
C
Vehicle
NSC23766
min 0 1 5 30
0 1 5 30
P-Cofilin
Tubulin
Cofilin phosphorylation (fold over medium)
Time (min)
D
CCL3L1 degradation (% over input)
Time (hours)
E
Vehicle
PTX
min 0 1 5 30
0 1 5 30
P-Cofilin
Tubulin
Cofilin phosphorylation (fold over medium)
Time (min)
F
CCL3L1 degradation (% over input)
Time (hours)

Fig. S1. D6 stimulates cofilin phosphorylation through a G protein–independent Rac1-PAK1 signaling pathway to sustain chemokine degradation in BeWo cells. (**A**) Representative Western blotting analysis of cells pre-treated for 1 hour at 37°C with vehicle (empty squares) or 5 µM IPA-III (filled squares) before being stimulated with 100 nM CCL3L1. (**B**) Measurement of chemokine degradation in cells after treatment with 50 nM scrambled siRNA (empty squares) or PAK1-specific siRNA (filled squares) for 72 hours followed by incubation with 0.1 nM ^{125}I-labeled CCL4 and the indicated concentrations of unlabelled CCL4. Cells were then analyzed by Western blotting to determine their amounts of PAK1 and tubulin. (**C**) Representative Western blotting analysis of cells pretreated for 1 hour at 37°C with vehicle (empty squares) or 200 µM NSC23766 (filled squares) before being stimulated with 100 nM CCL3L1. (**D**) CCL3L1 degradation in cells after treatment with vehicle (empty squares) or 200 µM NSC23766 (filled squares) followed by stimulation with 1 nM CCL3L1 for the indicated times. (**E**) Representative Western blotting analysis of cells pretreated for 16 hours at 37°C with vehicle (empty squares) or PTX (100 ng/ml, filled squares) before being stimulated with 100 nM CCL3L1. (D) CCL3L1 degradation in cells after treatment with vehicle (empty squares) or PTX (100 ng/ml) (filled squares) followed by stimulation with 1 nM CCL3L1 for the indicated times. For each Western blotting analysis, the relative amounts of phosphorylated cofilin (P-Cofilin) in CCL3L1-treated cells were determined by densitometric analysis and then normalized to the amounts of tubulin, and are expressed as the fold-increase relative to those of untreated cells. Data were analyzed by unpaired Student's *t* test in (B), (D), and (F). $P \leq 0.05$ for stimulated versus untreated cells; $^{\#}P \leq 0.05$ and $^{\#\#}P \leq 0.01$ for vehicle-treated cells versus inhibitor-treated or knockdown cells. Results are representative of three experiments.