Wu et al., http://www.jcb.org/cgi/content/full/jcb.201306032/DC1

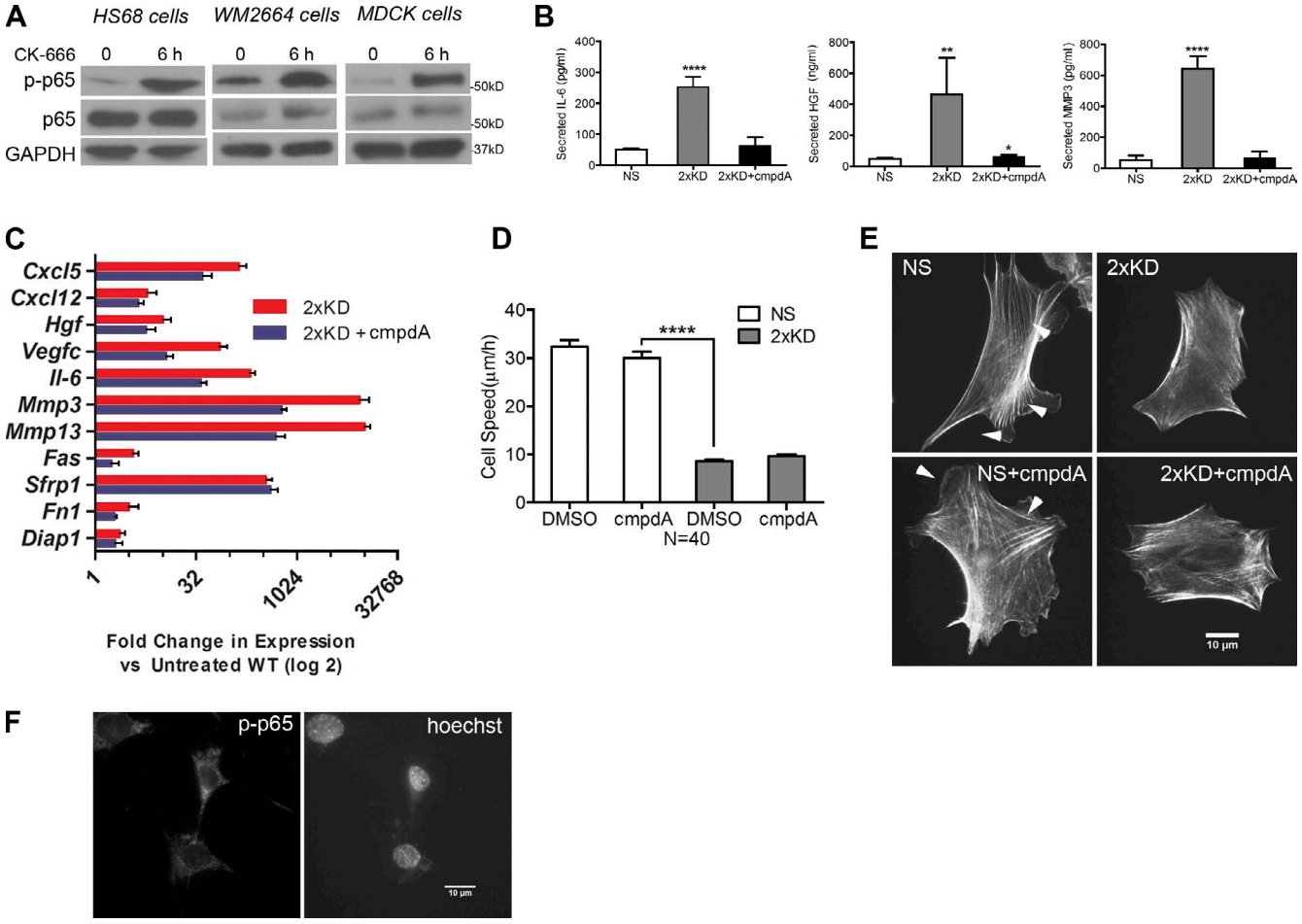


Figure S1. **NF-κB is activated in Arp2/3-inhibited cells and regulates the secretory phenotype in Arp2/3-deficient or -inhibited cells.** (A) Inhibition of Arp2/3 complex caused increased NF-κB activation in multiple cell lines. HS68 (human foreskin fibroblasts), WM2664 (human melanoma cells), and MDCK (epithelial cells) were treated with 100 μM CK666 for 0 or 6 h before being lysed. Western blot showed p-p65, p65, and GAPDH levels in these three cell lines under the indicated treatment. (B) ELISAs showing secreted IL-6, HGF, and MMP3 levels in 2×KD cells with and without cmpdA treatment compared with NS cells. ****, P<0.001; **, P<0.01 by Student's *t* test. (C) qRT-PCR showing the expression fold changes of the indicated genes compared with wild-type IA32 cells. 2×KD cells were treated with or without 5 μM cmpdA for 12 h before being harvested for qRT-PCR. Fold changes were analyzed using Pfaffl method. (D) Graph showing cell speed of NS and 2×KD cells with 0.5% DMSO or 5 μM cmpdA treatment. *n* = 40 for each group. ****, P < 0.001. (E) Phalloidin staining showed F-actin in NS and 2×KD cells. Lamellipodia in NS cells were pointed out by white arrowheads. (F) Immunofluorescent images showing $p65^{-/-}$ MEFs stained with p-p65 and Hoechst. Error bars show 95% CI.

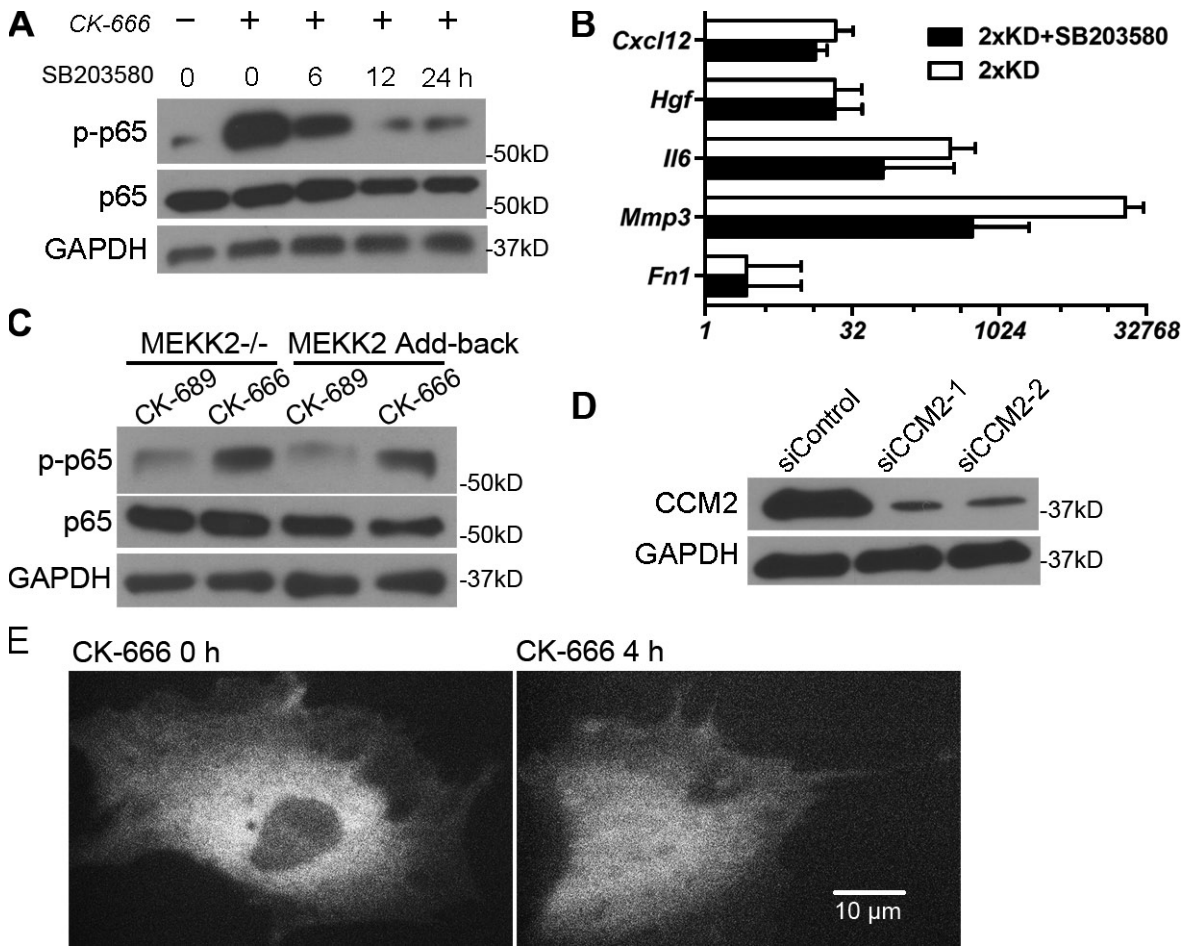
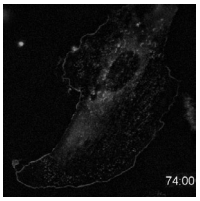


Figure S2. **p38 MAPK and the MEKK3–OSM complex are involved in the secretory phenotype in Arp2/3-deficient or -inhibited cells.** Video 1 shows a representative cell expressing Arp2-GFP (with Arp2 depletion) treated with sorbitol to induce hyperosmotic stress. (A) Preinhibition of p38 MAPK for >12 h abrogated the effect of Arp2/3 inhibition on NF-κB activation. IA32 cells were pretreated with 10 µM p38 MAPK inhibitor SB203580 for the indicated times before adding 100 µM CK666. p-p65, p65, and GAPDH were blotted. (B) qRT-PCR showing the expression fold changes of the indicated genes compared with wild-type IA32 cells. 2×KD cells were treated with or without 10 µM SB203580 for 12 h before being harvested for qRT-PCR. Fold changes were analyzed using Pfaffl method. Error bars show 95% CI. (C) MEKK2 does not regulate loss of Arp2/3-induced NF-κB activation. MEKK2$^{-/-}$ or MEKK2$^{-/-}$/MEKK2 add-back MEFs were treated with 100 µM CK666 or its inactive control compound CK689 for 6 h before harvesting cells. p-p65, p65, and GAPDH were blotted. (D) CCM2 is effectively silenced using siRNA. IA32 cells treated with control siRNA (siControl) and two CCM2 siRNAs (siCCM2-1 and siCCM2-2) were harvested and blotted for CCM2 and GAPDH. (E) Fluorescent images showing a representative YPET-CCM2–expressing cell before (left) and after (right) 4 h of CK666 treatment.



Video 1. **Representative cell expressing Arp2-GFP (with Arp2 depletion) treated with sorbitol to induce hyperosmotic stress.** Localization of Arp2-EGFP upon hyperosmotic shock IA32 cells stably expressing Arp2-EGFP were imaged every minute. Sorbitol-containing medium was added at 8 min as indicated to stress cells with hyperosmotic condition (0.25 M sorbitol). Images were captured using an inverted microscope (IX-81; Olympus) with a 60×, 1.42 NA objective, a charge-coupled device camera (C4742-80-12AG; Hamamatsu Photonics), and an automated X-Y stage with MetaMorph imaging software.

Table S1 is provided online as an Excel file and shows the RNA-Seq results of this study.