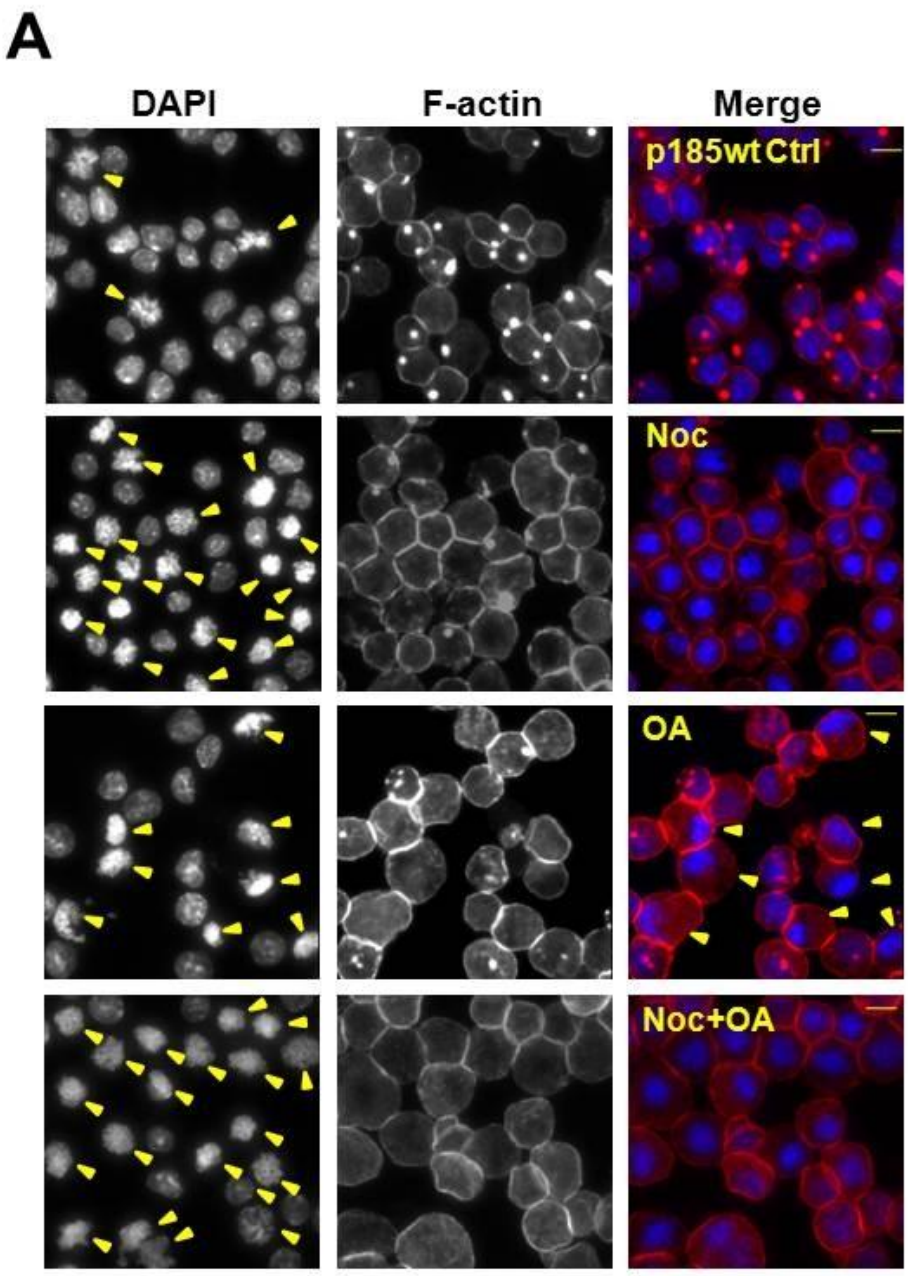
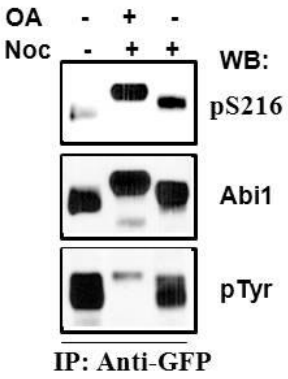
Zhuang et al. Figure S1A



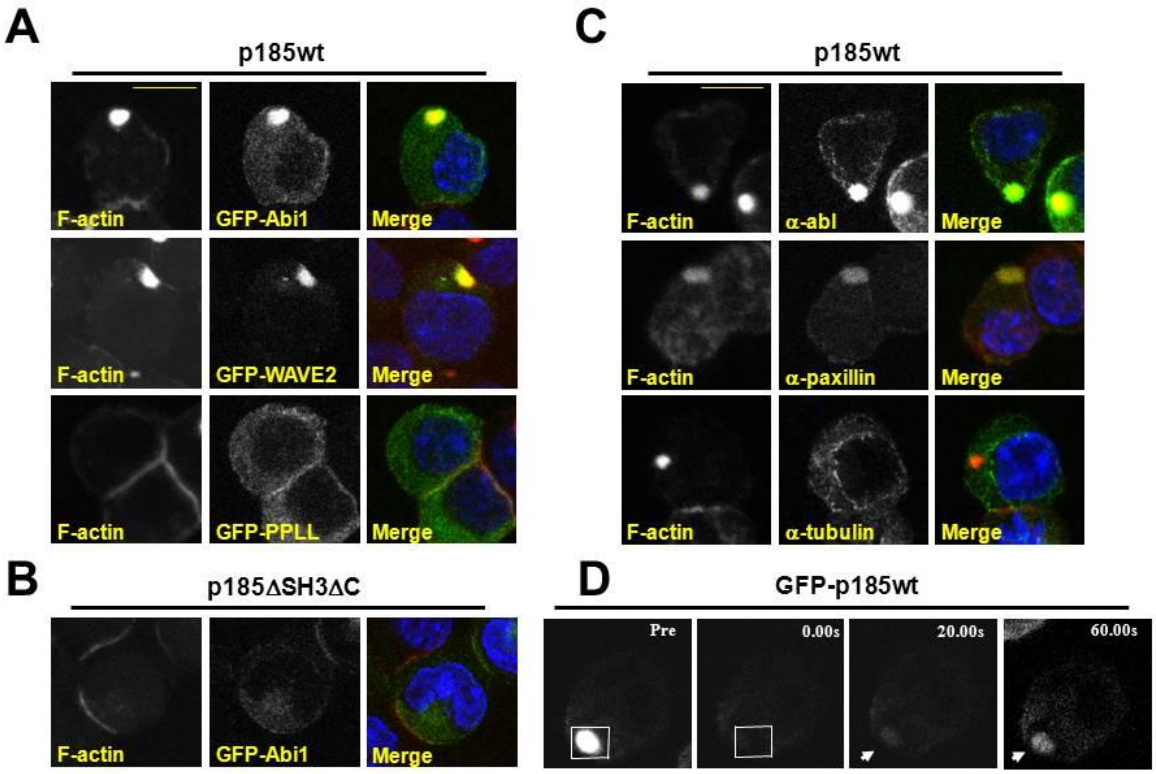
B

Cells	Cell number counted (n)	Cells with spots (%)
Mitotic 185wt cells	33	0
Interphase p185wt cells	188	166 (88%)
Mitotic p185wt cells treated by nocodazole	156	0

C

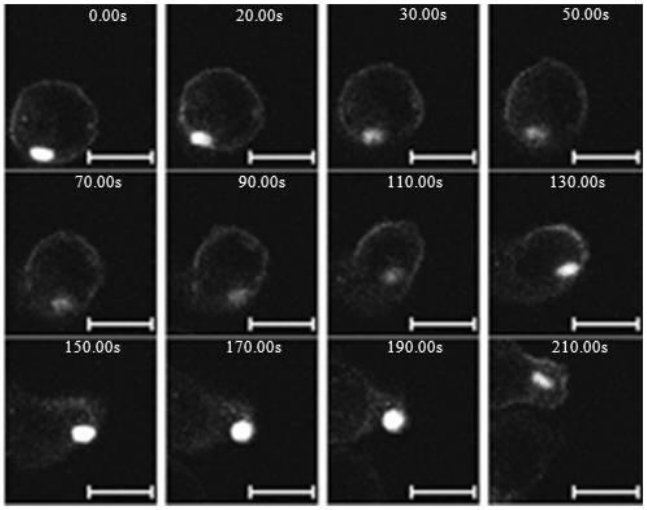


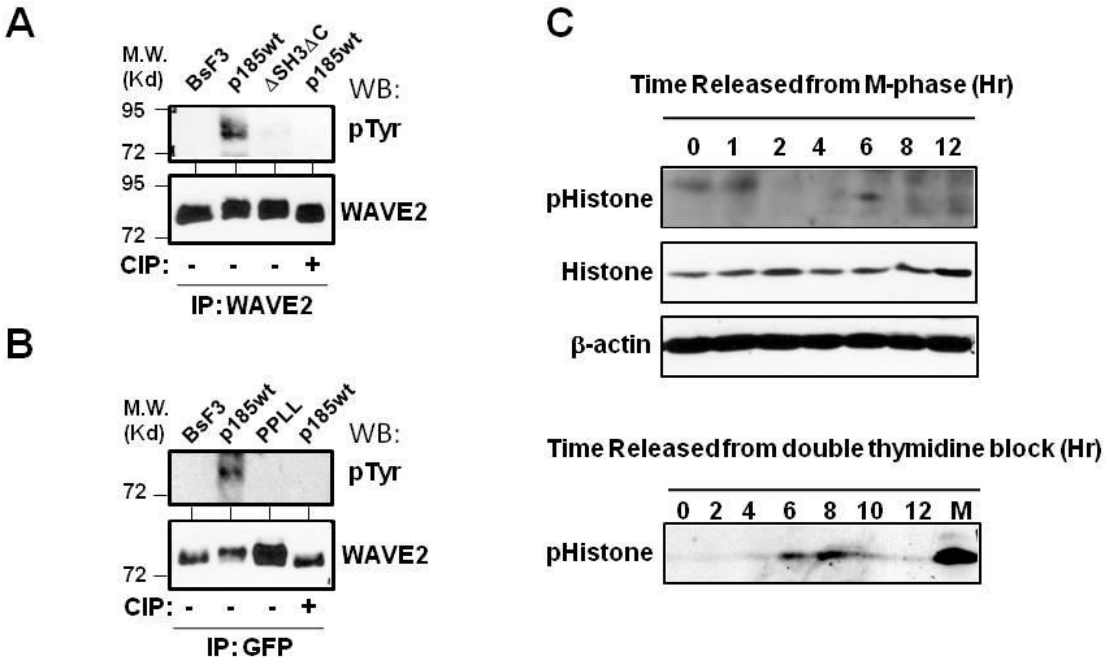
Zhuang et al. Figure S2A-D



Zhuang et al. Figure S2E

E




A
M.W. (Kd)
BsF3
p185wt
ΔSH3ΔC
p185wt
WB:
95
72
pTyr
95
72
WAVE2
CIP: - - - +
IP: WAVE2
B
M.W. (Kd)
BsF3
p185wt
PPLL
p185wt
WB:
72
pTyr
72
WAVE2
CIP: - - - +
IP: GFP
C
Time Released from M-phase (Hr)
0 1 2 4 6 8 12
pHistone
Histone
β-actin
Time Released from double thymidine block (Hr)
0 2 4 6 8 10 12 M
pHistone

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1. Mitotic inhibition of F-actin assembly in p185^{wt} cells. A and B. The p185^{wt} cells were treated with 200 ng/ml nocodazole for 12 hours (Noc), 1 μM okadaic acid for 4 hours (OA), or 200 ng/ml nocodazole for 12 hours with addition of 1 μM okadaic acid in the last 2 hours (Noc+OA). The treated cells, together with untreated cells (p185wt) as control, were fixed, permeabilized, and stained with Alexa-conjugated phalloidin and DAPI to visualize F-actin (red) and nuclei (blue), respectively. The stained cells were analyzed and images captured by fluorescence microscopy (A). Mitotic cells are indicated by arrowheads. *Bar:* 10 μm. Actin-enriched structures in mitotic and interphase p185^{wt} cells were counted in 10 randomly selected areas from three independent experiments (B). C. The p185^{wt} cells expressing GFP-Abi1 were treated with nocodazole alone or nocodazole plus OA, as described in A. Cell lysates were immunoprecipitated by anti-GFP antibody and immunoprecipitates were subjected to western blot analysis using indicated antibodies.

Fig. S2. Abi1 is required for Bcr-Abl to induce the assembly of an invadopodium-like structure in Ba/F3 cells. A and B. Abi1/WAVE2 co-localizes with the F-actin rich structure and is required for its assembly in p185^{wt} cells. The Ba/F3 cells expressing wild type p185$^{Bcr-Abl}$ (A, p185wt) or a mutant Bcr-Abl defective in binding to Abi1 (B, p185ΔSH3ΔC) were transfected by retroviral vectors expressing GFP-tagged wild type Abi1 (GFP-Abi1), a mutant Abi1 defective in binding to Bcr-Abl (GFP-PPLL), or WAVE2 (GFP-WAVE2), as indicated. Cells were fixed, permeabilized, and stained with Alexa-conjugated phalloidin and DAPI to visualize F-actin (red) and nuclei (blue), respectively. The stained cells were analyzed and images were captured by 2-photon confocal microscopy. C. Colocalization of Bcr-Abl and paxillin with the F-actin rich structure. The p185^{wt} cells were fixed, permeabilized, and immunostained with indicated primary antibodies and FITC-conjugated secondary antibodies. The cells were then counterstained with Alexa-conjugated phalloidin and DAPI to visualize F-actin (red) and nuclei (blue), respectively. The stained cells were analyzed and images captured by 2-photon confocal microscopy (A). A part of the data presented in A-C has been published previously (17). D. Rapid turnover of the invadopodium-like structure in Bcr-Abl-transformed Ba/F3 cells. Ba/F3 cells transformed by GFP-tagged p185^{wt} (GFP-p185^{wt}) were analyzed by fluorescence recovery after photobleach (FRAP) using 2-photon confocal microscopy. The GFP-p185^{wt}, which co-localizes with F-actin rich structure as demonstrated in C, was photobleached at the time 0 and the images were taken afterward at the indicated time intervals to follow the recovery of GFP flurescence. Pre: pre-photobleaching. E. Time-lapse microscopy analysis of the Ba/F3 cells transformed by GFP-p185^{wt} to show dynamic changes in shape and location of the GFP-p185^{wt}, which co-localizes with the invadopodium-like structures. The Ba/F3 cells transformed by GFP-p185^{wt} were analyzed by 2-photon confocal microscopy and GFP fluorescence images were taken every 20 seconds.

Fig. S3. Biochemical analyses of the WAVE2 phosphorylation and cell synchronization. A and B: Treatment of the WAVE2 immunoprecipitates with CIP. The WAVE2 immunoprecipitates prepared as in Fig 2A were treated with 10 units CIP for 30 minutes and then subjected to western blot (WB) analysis using indicated antibodies. C. Analysis of cell synchronization of p185^{wt} and Hela cells. The p185^{wt} GFP-Abi1 cells synchronized by nocodazole treatment (upper panel) and Hela cells synchronized by double thymidine block (lower panel) were released from arrest and collected at various time points, as indicated. The Hela cells synchronized at M-phase by nocodazole treatment is also included (M in lower panel). The cell lysates containing 80 μg total proteins were analyzed by western blot for serine 10 phosphorylation of histone H3 using anti-pSer10 histone H3 antibody (pHistone) and, for total histone H3 and β-actin using respective antibodies, as indicated.