Supplemental Materials

Molecular Biology of the Cell

Tang *et al*.

Supplemental Figure 1. Validation of WAVE expression in WAVE knockout cell lines.

(A) Sequencing of CRISPR-Cas9 targeted genomic loci (*Wasf1*, *Wasf2*) in WAVE knockout cell lines. Genomic sequences (upper) and the corresponding amino acids (lower) in the alleles of each cell lines are shown. The inserted nucleotides are in green, and the deleted (also struck through) nucleotides are in red. Mutated amino acids are in red. Positions of the early stop codons are indicated. **(B)** Specificity of antibodies against WAVE1 or WAVE2. Western blots showing WAVE1 or WAVE2 signals in 20 μg of lysates from B16 cells with or without ectopic expression of EGFP-WAVE1 or EGFP-WAVE2 from plasmids (0.8 or 1.6 μg). GAPDH signal was shown for equal loading of lysates. The asterisks indicate breakdown products of WAVE proteins due to overexpression of WAVE from plasmids. **(C)** Same western blots as in Figure 1A, showing a wider molecular weight range (50-150 kDa). Lysates from B16-F1 cells probed with anti-WAVE1 and anti-WAVE2 antibodies. **(D)** Western blot showing that WAVE3 is undetectable in B16-F1 cells (WT and WAVE1/2 KO) but is present in MDA-MB-231 cells. GAPDH signal shows equal loading of lysates. **(E)** Representative cell images showing distribution of Abi1 subunit of WAVE regulatory complex at the leading edge, determined by immunofluorescence. Scale Bar, 10 μm. **(F)** Abi1 density (mean ± SD) at the leading edge quantified from cell images as in (E). Data pooled from two independent experiments (left to right: n = 124, 128, 97 cells). **(G)** Western blots showing levels of Sra-1 and Nap-1 subunits of WAVE regulatory complex in WT and WAVE KO cells. Tubulin signal shown for equal loading of lysates.

Supplemental Figure 2. Actin ultrastructure at the leading edge and effects of WAVE1 and WAVE2 knockouts on cell migration.

(A) Additional examples of platinum replica electron micrographs of actin organization at the leading edge of WT, W1KO (#6), W2KO (#16), and W1/2KO (#20) B16-F1 cells, as in Figure 1D. Scale bars, 200 nm. **(B)** Trajectories of individual WT, W1KO (#6), W2KO (#16), and W1/2 KO (#20) cells migrating randomly on laminin-coated surfaces over 10 h. (n = 86, 107, 107, 149 trajectories for WT, W1KO, W2KO, and W1/2KO). **(C)** Average migration speeds (mean ± SEM) calculated from cell migration path length divided by time. Data pooled from three independent experiments for W2KO(#6), W2KO(#11), W1KO(#1) and W1/2KO(#7), and four independent experiments for W1KO(#6) and W1/2KO(#20). Data pooled from four independent experiments for each WT shown. Number of cells analyzed for each condition: WT n=86, W1KO(#6) n=107,

W2KO(#16) n=107, W1/2KO(#20) n=149; WT n=110, W1KO(#1) n=51, W2KO(#11) n=80, W1/2KO(#20) n=188. **(D)** Migration distances (mean ± SEM) over 10 h, defined as the shortest distance between start and end points. Analyzed for the same cells as in (C). **(E)** Frequency of pausing (mean ± SEM) during the 10 h cell migration window, analyzed for individual cells and then averaged, for the same cells as in (C). **(F)** Mean square displacement (MSD) analysis of cell migration (mean ± SEM) for the same cells as in (C). One-way ANOVA and Tukey's multiple comparison tests were performed in (C) and (D). Kruskal-Wallis test and Dunn's multiple comparison tests were performed in (E). **** $p < 0.0001$, ns ($p > 0.05$), not significant.

Supplemental Figure 3. Ectopic expression of different EGFP-WAVE proteins in WAVE KO cell lines.

(A) Representative images of F-actin (stained by Alexa^{594}-phalloidin) and EGFP signals in WAVE1/2 KO (#20) cells ectopically expressing EGFP-WAVE1, EGFP-WAVE2, or EGFP-WAVE3. Scale bar, 10 μm. **(B)** Representative images (for independent cell line clones not displayed in main figures) showing F-actin stained with Alexa^{568}-phalloidin. Scale bar, 10 μm. Highlighted regions (dotted boxes) shown at higher magnification. Scale bar, 3 μm. **(C)** Representative images (for independent cell line clones not displayed in main figures) showing distribution of Arp2/3 complex (p16/ARPC5 subunit) at the leading edge determined by immunofluorescence. Scale bar, 10 μm. Highlighted regions (dotted boxes) shown at higher magnification; scale bar, 3 μm. (**D**) Cell migration speeds (mean ± SD) for WT, WAVE1/2 KO (#20), and WAVE1/2 KO (#20) cells ectopically expressing EGFP-WAVE1 or EGFP-WAVE2. Data pooled from three independent experiments (left to right: n = 137, 141, 120, 144 cells). **(E)** Representative images of F-actin stained by Alexa^{594}-phalloidin in WT and WAVE2 KO (#11) cells with or without the expression of EGFP-WAVE1 (EGFP signal also shown). Scale bar, 10 μm. **(F)** Density of F-actin at the lamellipodia of cells (mean ± SD). Data pooled from two independent experiments (left to right: n = 100, 113, 88 cells). Kruskal-Wallis test and Dunn's multiple comparison test was performed in (D). One-way ANOVA and Tukey's multiple comparison tests were performed in (F). **** $p < 0.0001$, * $p < 0.05$, ns ($p > 0.05$), not significant.

Supplemental Figure 1

A

W1 KO (#1):

WAVE1:
~~ATG CCG T~~TG GTG AAA AGA AAC ATC GAC CCT CGG CAC TTG TGC CAC ACA GCA CTG CCC
No product translated

~~ATG CCG~~ TTG GTG AAA AGA AAC ATC GAC CCT CGG CAC TTG TGC CAC ACA GCA CTG CCC
No product translated

W1 KO (#6):

WAVE1:
~~ATG CCG TTG GTG AAA AGA AAC ATC GAC CCT CGG CAC TTG TGC CAC ACA GCA CTG CCC~~
No product translated

W2 KO (#11):

WAVE2:
ATG CCG TTA GTA ACC AGG AAA ACA TCG AGC CAA GGC ACC TGT GCC GTC AGA ... STOP
M P L V T R K T S S Q G T C A V R ... @ aa 44

ATG CCG TTA GTA ACC AGG ATA CAT CGA GCC AAG GCA CCT GTG CCG TCA GAC ... STOP
M P L V T R I H R A K A P V P S D ... @ aa 20

ATG CCG TTA GTA ACC AGG AA~~CA~~T CGA GCC AAG GCA CCT GTG CCG TCA GAC GTT ... STOP
M P L V T R N R A K A P V P S D V ... @ aa 19

W2 KO (#16):

WAVE2:
ATG CCG TTA GTA ACC AGG ~~A~~ACA TCG AGC CAA GGC ACC TGT GCC GTC AGA CGT ... STOP
M P L V T R T S S Q G T C A V R R ... @ aa 43

ATG CCG TTA GTA ACC AGG AAA CAT CGA GCC AAG GCA CCT GTG CCG TCA GAC ... STOP
M P L V T R I H R A K A P V P S D ... @ aa 20

ATG CCG TTA GTA ACC AGG A~~AC~~AT CGA GCC AAG GCA CCT GTG CCG TCA GAC GTT ... STOP
M P L V T R N R A K A P V P S D V ... @ aa 19

W1/2 KO (#7):
Derived from W2 KO (#11)

WAVE1:
ATG ~~C~~CGT TGG TGA AAA GAA ACA TCG ACC CTC GGC ACT TGT GCC ACA CAG CAC TGC
M R W *

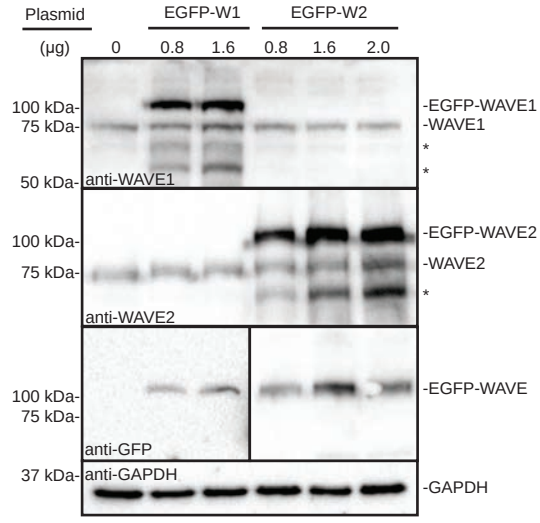
WAVE2: see W2 KO (#11)

W1/2 KO (#20):
Derived from W2 KO (#11)

WAVE1:
ATG CA~~CGTTGGTG~~A AAA GAA ACA TCG ACC CTC GGC ACT TGT GCC ACA CAG CAC TGC ... STOP
M Q K E T S T L G T C A T Q H C ... @ aa 27

WAVE2: see W2 KO (#11)

B



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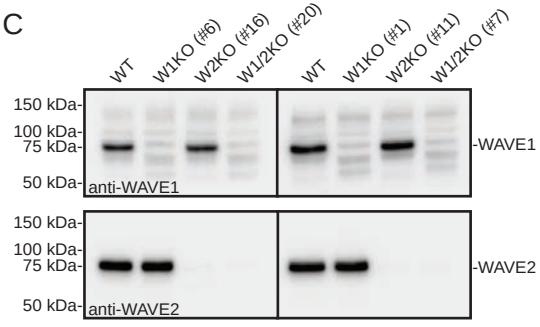

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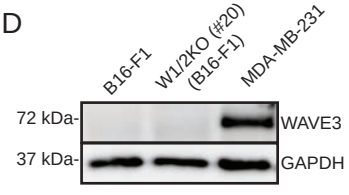

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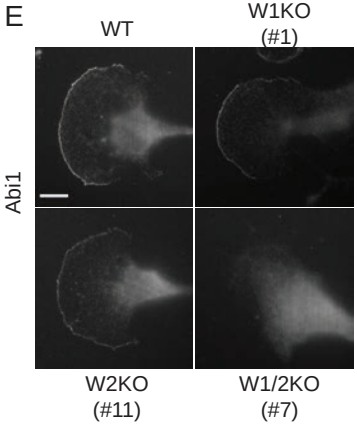

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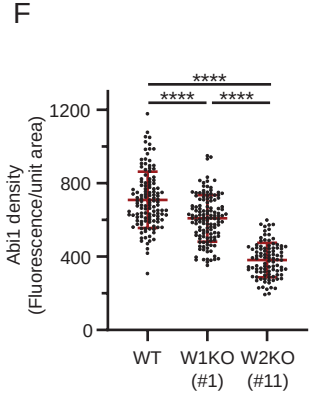

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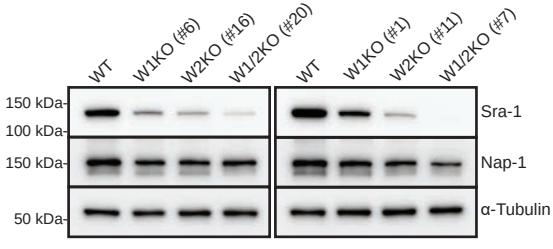
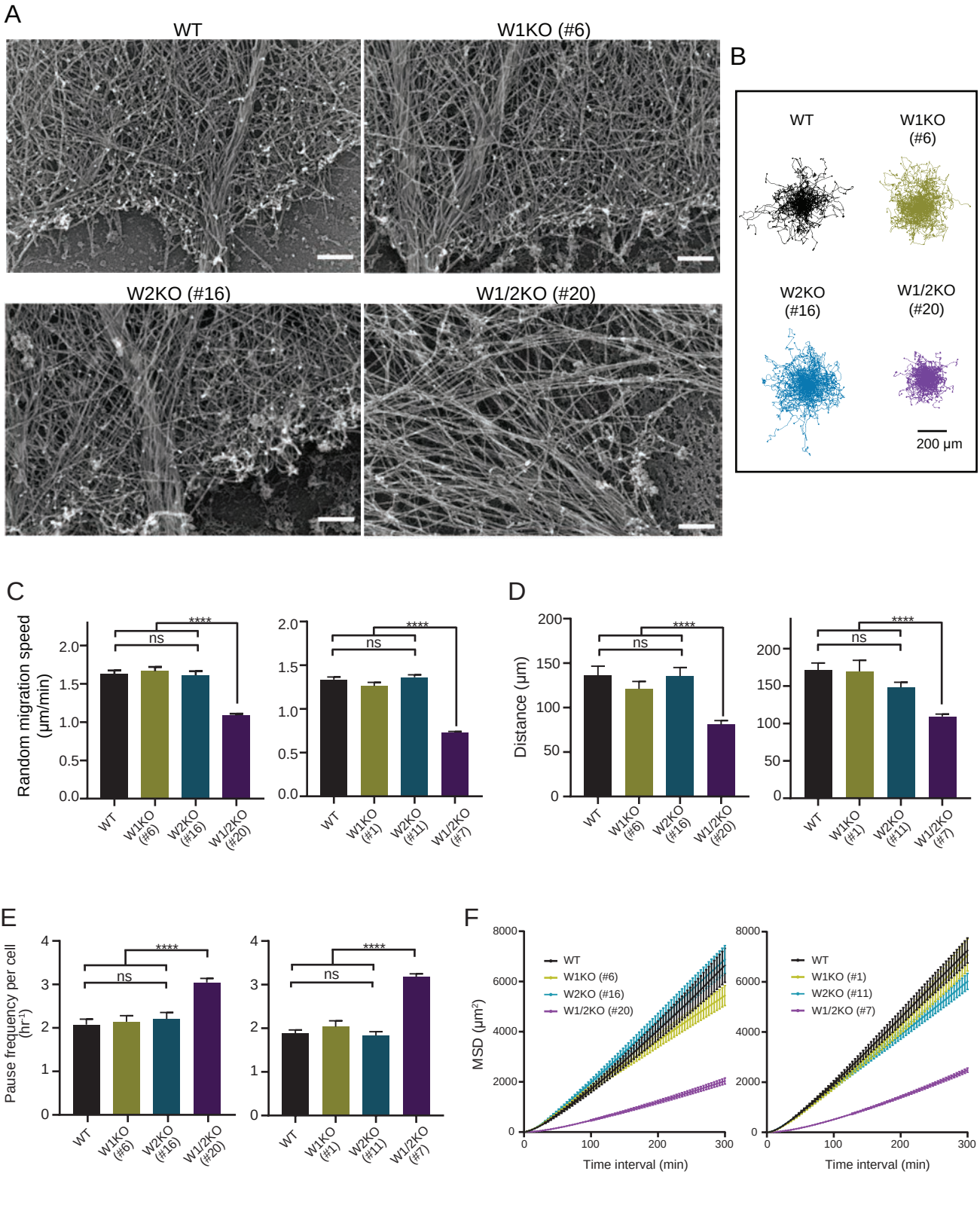


Supplemental figure 2



Supplemental Figure 3