Supplementary Materials

Molecular Biology of the Cell

King and Campellone

King and Campellone, 2023

Supplemental Material

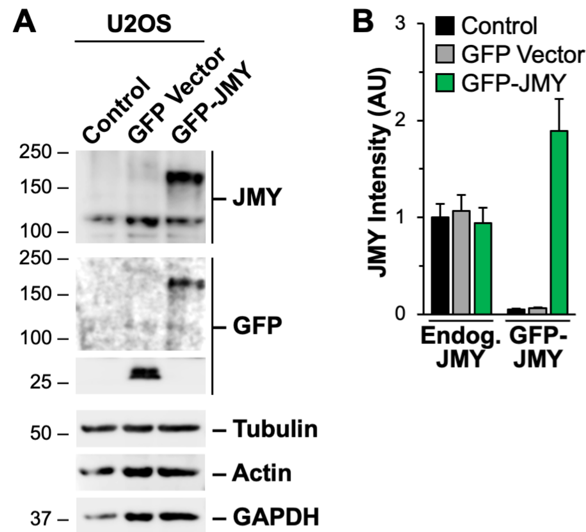

Figure S1. JMY expression in cells stably encoding GFP-JMY. (A) Lysates from control nontransfected U2OS cells or U2OS cells stably encoding GFP or GFP-JMY were immunoblotted with antibodies to JMY, GFP, tubulin, actin, and GAPDH. **(B)** For quantification, the JMY band intensities for endogenous JMY and GFP-JMY were normalized to the loading control intensities. The endogenous control value for JMY was set to 1. Each bar is the mean intensity ±SD from 3 blots. AU = Arbitrary Units. Note that endogenous JMY exhibited a prominently nuclear localization in U2OS cells at steady state, while GFP-JMY maintained a mostly cytosolic presence (Figure 1A). These differences in the nucleo-cytoplasmic distribution of endogenous compared to tagged JMY imply that GFP-JMY is more useful for studying cytosolic rather than nuclear activities of JMY.

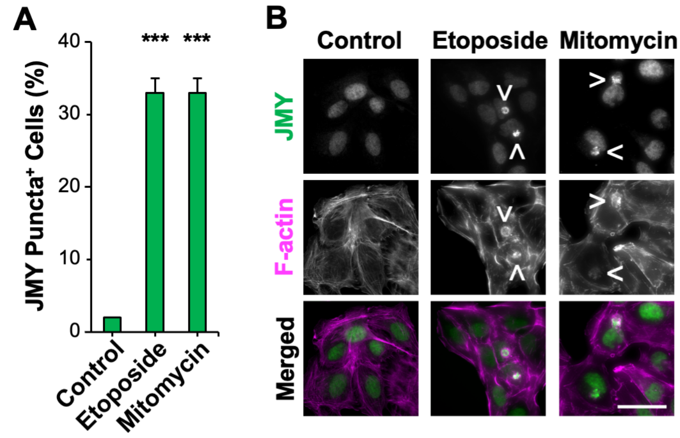

Figure S2. JMY forms cytosolic puncta within juxtanuclear F-actin-rich territories in response to different DNA damaging agents. (A-B) U2OS cells were treated with a media control, 10μM etoposide, or 60μM mitomycin c for 6h before being fixed and stained with a JMY antibody (green) to visualize endogenous JMY and phalloidin to visualize F-actin (magenta). In (A), the % of cells with JMY puncta was calculated. Each bar represents the mean ± SD from 3 experiments (n = 1,314-1,387 cells per bar). ***p<0.001 (ANOVA, Tukey post-hoc tests) are comparisons to the control sample. In (B), arrowheads highlight examples of cytosolic JMY puncta and F-actin-rich territories. Scale bar: 25μm.

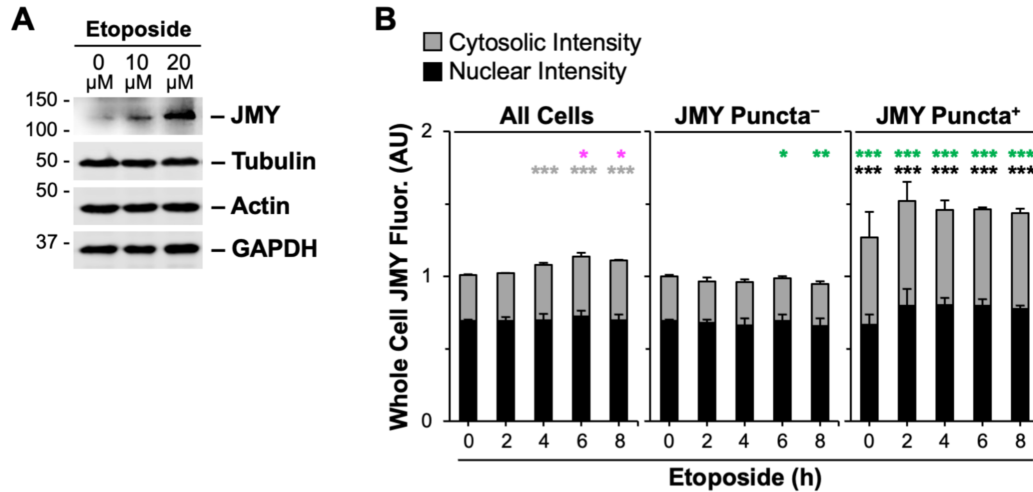

Figure S3. JMY expression increases in the cytosol during DNA damage-induced apoptosis. (A) Lysates from U2OS cells treated with 0, 10, or 20μM etoposide were immunoblotted with antibodies to JMY, tubulin, actin, and GAPDH. **(B)** Cells were treated as in Figure 1A-C. Data from Figure 1C were replotted to show the mean whole cell JMY fluorescence in the full bar. The gray portion of the bar is the mean cytosolic JMY intensity, and the black portion is the mean nuclear JMY intensity. Each bar is the mean ± SD from 3 experiments (n = 310-348 cells per timepoint). Magenta significance stars refer to comparisons of the total population whole cell fluorescence values at each timepoint to the 0min timepoint, and gray significance stars are to the cytosolic intensity value. Green significance stars refer to comparisons of whole cell fluorescence values for puncta-negative or puncta-positive populations to the total population at each timepoint, and black significance stars are for the cytosolic intensity values. *$p<0.05$; **$p<0.01$; ***$p<0.001$ (ANOVA, Tukey post-hoc tests).

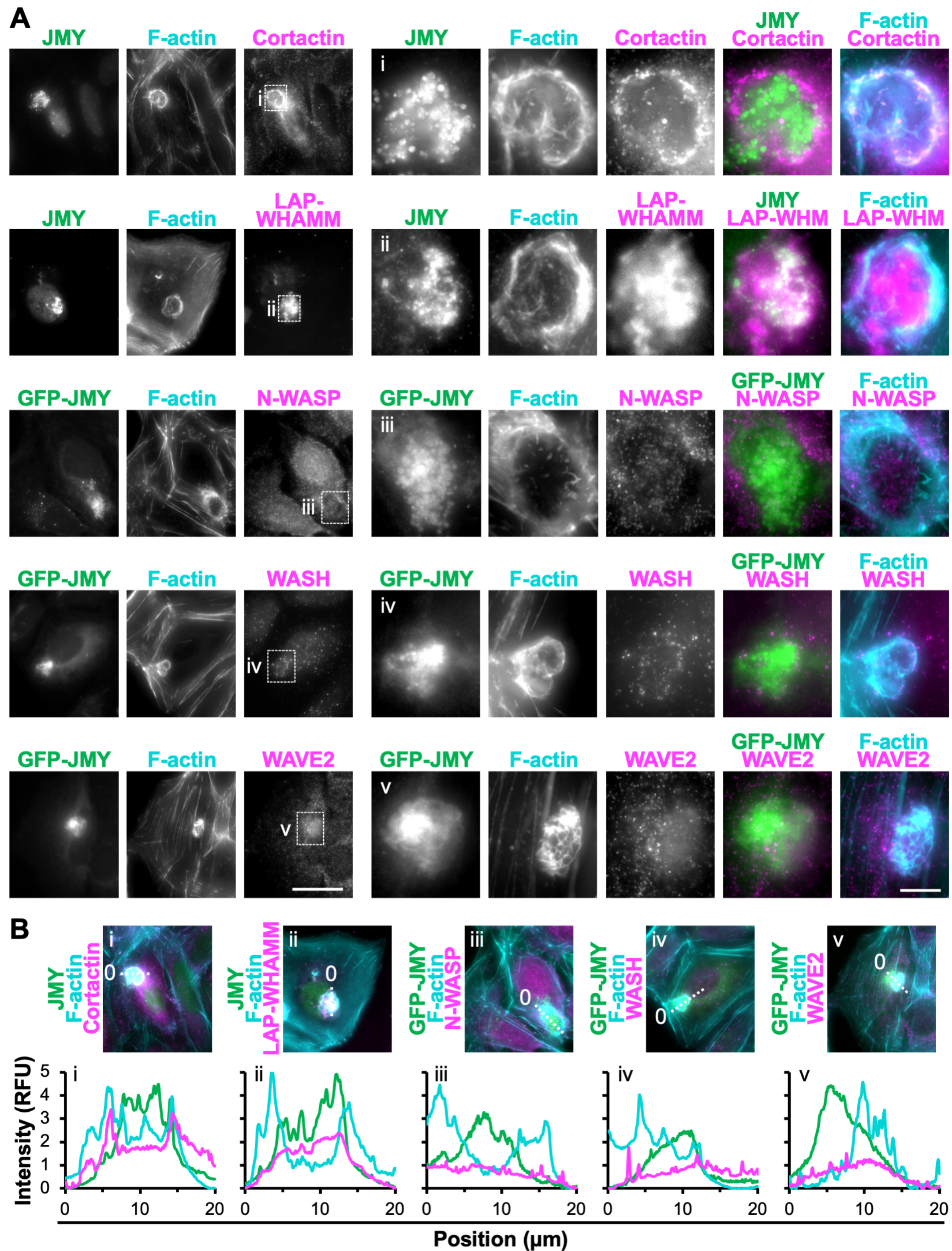

Figure S4. Cortactin and WHAMM, but not representatives of other subgroups within the WASP family, are enriched within apoptotic F-actin-rich territories. (A) Nontransfected U2OS cells or transfected U2OS cells expressing GFP-JMY (green) or LAP-WHAMM (magenta) were treated with 10μM etoposide for 6h, fixed, and stained with phalloidin (F-actin; cyan) and antibodies to JMY (green), Cortactin, N-WASP, WASH, or WAVE2 (each in magenta). Magnifications (i-ii) depict F-actin territories with JMY, Cortactin, and LAP-WHAMM, while (iii-v) show territories without enrichment of N-WASP, WASH, or WAVE2. Scale bars: 25μm, 5μm. **(B)** 20μm lines were used to measure the pixel intensity profiles. RFU = relative fluorescence units.

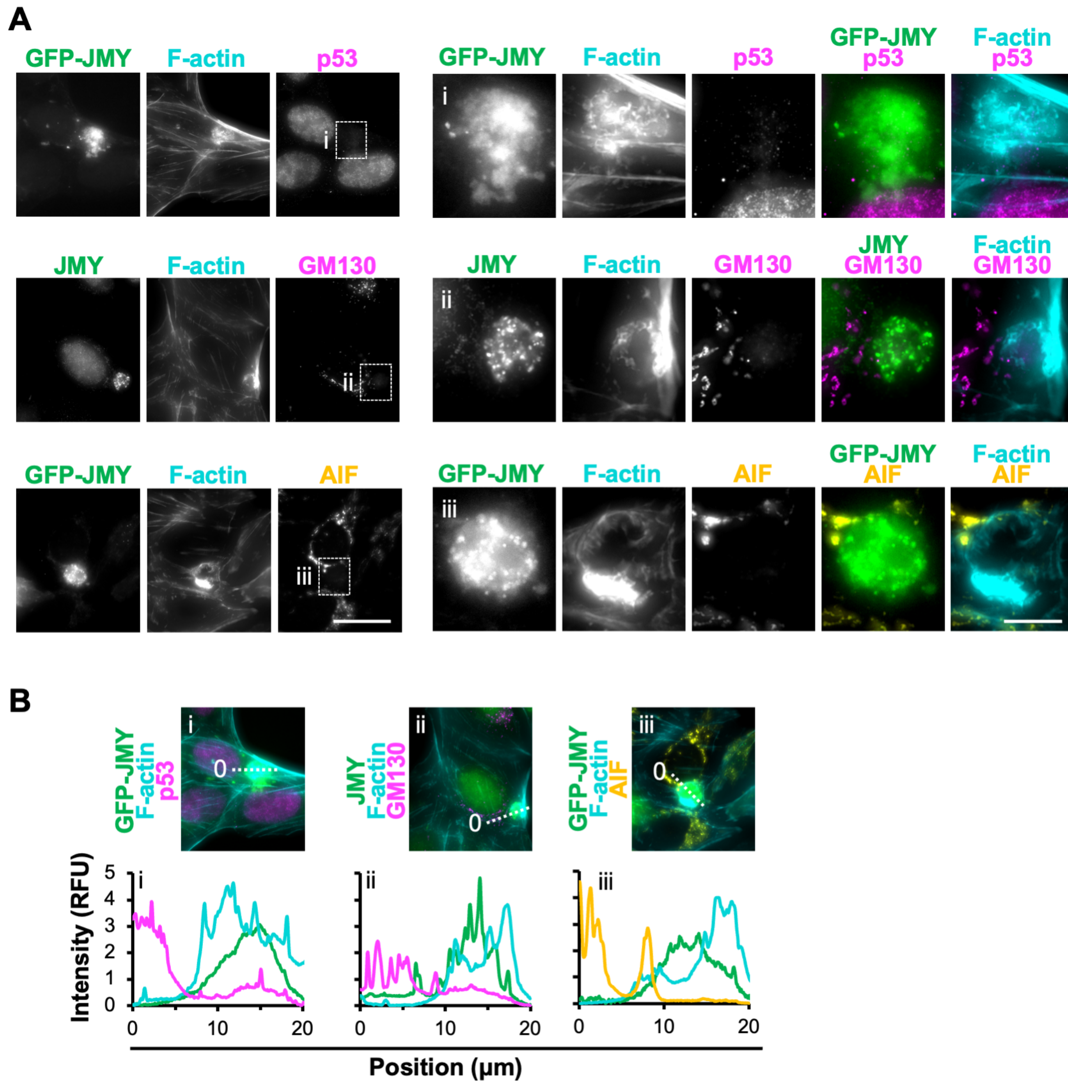

Figure S5. p53 and the Golgi are not recruited to apoptotic F-actin-rich territories. (A) U2OS cells or U2OS cells encoding GFP-JMY (green) were treated with etoposide for 6h, fixed, and stained with phalloidin (F-actin; cyan) and antibodies to JMY (green), p53 (magenta), GM130 (magenta), or AIF (yellow). Magnifications (i-iii) depict JMY and F-actin territories lacking p53, Golgi (GM130), or AIF. Scale bars: 25µm, 5µm. **(B)** 20µm lines were used to measure the pixel intensity profiles.

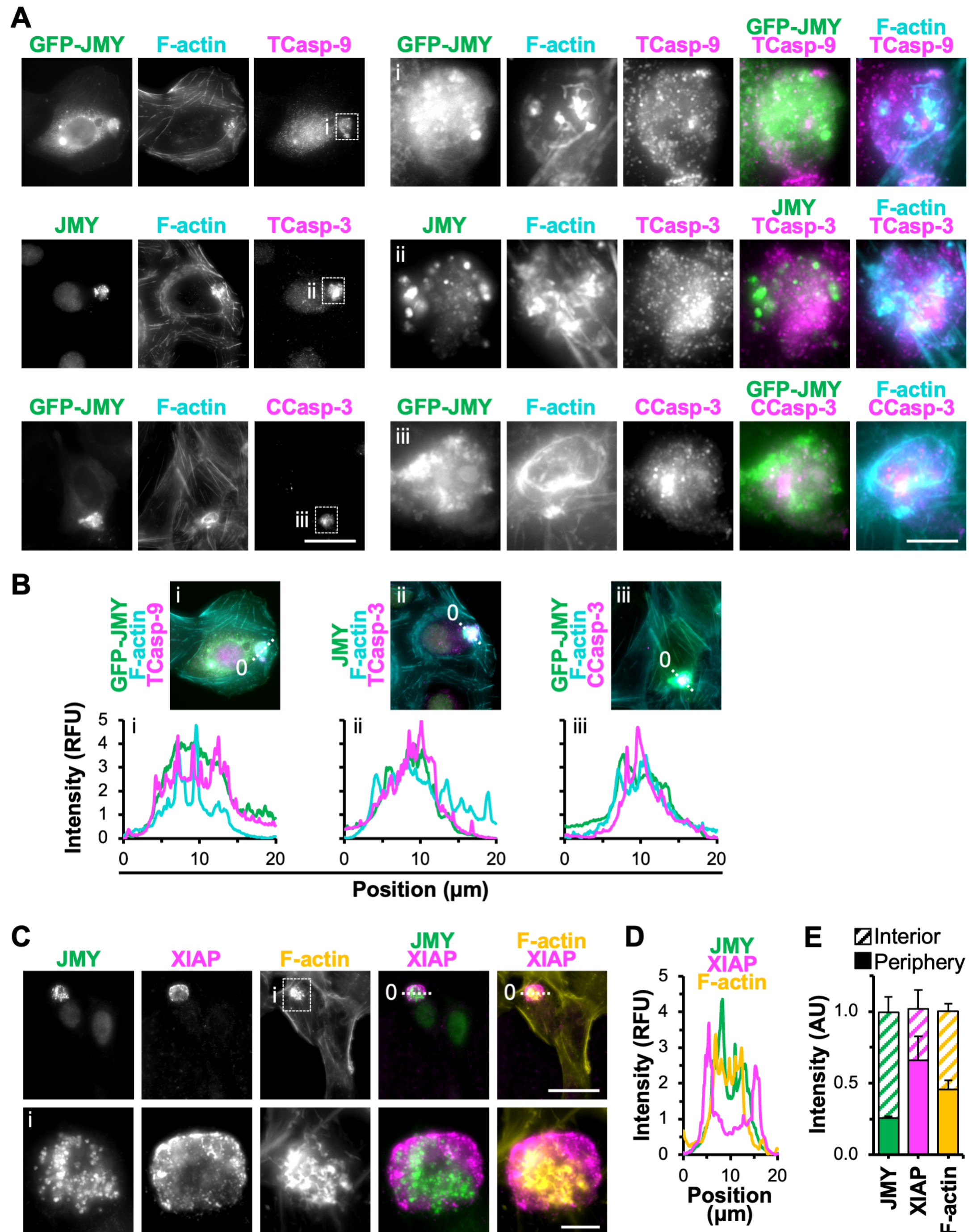

Figure S6. Initiator caspase-9 and executioner caspase-3 are found in apoptotic F-actin-rich territories. (A) U2OS cells or U2OS cells encoding GFP-JMY (green) were treated with etoposide for 6h, fixed, and stained with phalloidin (F-actin; cyan), antibodies to JMY (green), and antibodies that recognize total caspase-9 (TCasp-9; magenta), total caspase-3 (TCasp-3; magenta), or active caspase-3 cleaved at Asp175 (CCasp-3; magenta). Magnifications (i-iii) depict territories containing total or cleaved caspases. Scale bars: 25µm, 5µm. **(B)** 20µm lines were used to measure the pixel intensity profiles. **(C)** U2OS cells were stained with antibodies to JMY (green) and XIAP (magenta), and with phalloidin (F-actin; yellow). Magnifications (i) depict an F-actin-rich territory surrounded by XIAP. **(D)** 20µm lines were used to measure the pixel intensity profiles. **(E)** The JMY, XIAP, and F-actin fluorescence intensities were measured for the whole territory as well as the interior and peripheral portions (n = 17 territories).

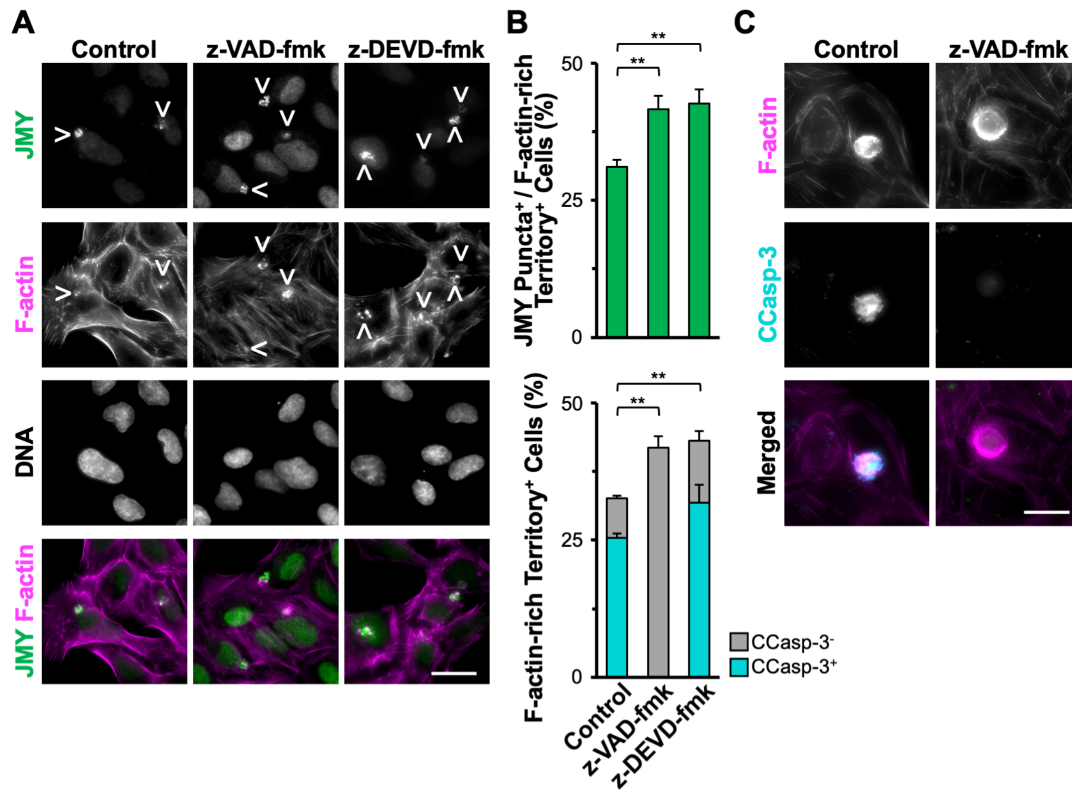

Figure S7. The formation of JMY puncta and F-actin-rich territories is not dependent on caspase activity. (A) U2OS cells were treated with 10μM etoposide (control), 10μM etoposide plus 50μM z-VAD-fmk, or 10μM etoposide plus 50μM z-DEVD-fmk for 6h before being fixed and stained with a JMY antibody (green), phalloidin (F-actin; magenta), and DAPI (DNA). Arrowheads highlight examples of JMY puncta and F-actin-rich territories. **(B)** The % of cells with JMY puncta within F-actin-rich territories was calculated. Each bar represents the mean ± SD from 3 experiments (n = 2,414-2,502 cells per bar). Cells were also stained with an antibody to cleaved caspase-3 (CCasp-3; cyan). The fraction of F-actin-rich territory-positive cells that were (CCasp-3$^+$) or were not (CCasp-3$^-$) enriched for cleaved caspase-3 was quantified. Each bar represents the mean ± SD from 3 experiments (n = 2,434-2,566 cells per bar). **(C)** Etoposide-treated control cells possess F-actin-rich territories containing cleaved caspase-3 while etoposide-treated cells co-treated with the pan-caspase inhibitor z-VAD-fmk have F-actin-rich territories lacking cleaved caspase-3. Scale bars: 25μm. **p<0.01 (ANOVA, Tukey post-hoc tests).

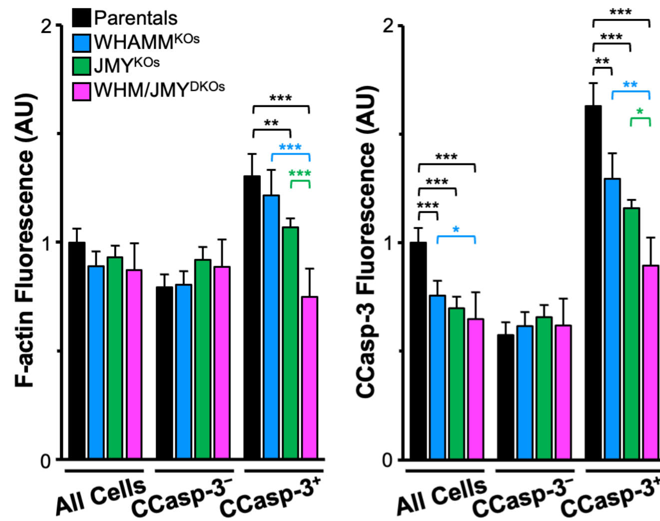

Figure S8. The amount of F-actin and caspases within apoptotic cells is enhanced by WHAMM. Cells were treated as in Figure 8A. Whole cell fluorescence values for F-actin and cleaved caspase-3 were measured in individual cells and normalized to the parental samples. Values for the CCasp-3-postitve cell population alone appear in Figure 8G. Each bar is the mean ± SD from 3 experiments (n = 607-866 cells per sample). *p<0.05; **p<0.01; ***p<0.001 (ANOVA, Tukey post-hoc tests).

Table S1. Cell Lines.

<table>
<tr><td colspan="5">Parental Cells</td></tr>
<tr><td colspan="2">Cell Line</td><td colspan="3">Source</td></tr>
<tr><td colspan="2">eHAP</td><td colspan="3">Horizon Genomics (C669)</td></tr>
<tr><td colspan="2">HAP1</td><td colspan="3">Horizon Genomics (C631)</td></tr>
<tr><td colspan="2">U2OS</td><td colspan="3">UC Berkeley Cell Culture Facility</td></tr>
<tr><td colspan="5">HAP1 Derivatives</td></tr>
<tr><td colspan="2">KO Cell Line</td><td>Mutation</td><td>Predicted AAs</td><td>Source</td></tr>
<tr><td colspan="2">JMY^{KO-1A}</td><td>17bp deletion
in exon 1 of 11</td><td>145/988,
4 post-shift</td><td>Horizon Genomics
(HAP1_JMY_28380-03)
King et al., 2021</td></tr>
<tr><td colspan="2">JMY^{KO-2}</td><td>2bp deletion
in exon 2 of 11</td><td>362/988,
3 post-shift</td><td>Horizon Genomics
(HZGHC002631c007)
King et al., 2021</td></tr>
<tr><td colspan="5">eHAP Derivatives</td></tr>
<tr><td colspan="2">KO Cell Line</td><td>Mutation</td><td>Predicted AAs</td><td>Source</td></tr>
<tr><td colspan="2">WHAMM^{KO-2}</td><td>10bp deletion
in exon 2 of 10</td><td>204/809,
7 post-shift</td><td>Horizon Genomics
Mathiowetz et al., 2017
King et al., 2021</td></tr>
<tr><td colspan="2">WHAMM^{KO-4}</td><td>7bp deletion
in exon 4 of 10</td><td>321/809,
17 post-shift</td><td>Horizon Genomics
(HZGHC001060c001)
King et al., 2021</td></tr>
<tr><td rowspan="2">WHAMM/JMY^{DKO-1}</td><td>WHAMM:</td><td>10bp deletion
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7 post-shift</td><td rowspan="2">Horizon Genomics
(HZGHC004884C012)
King et al., 2021</td></tr>
<tr><td>JMY:</td><td>16bp deletion
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64 post-shift</td></tr>
<tr><td rowspan="2">WHAMM/JMY^{DKO-2}</td><td>WHAMM:</td><td>10bp deletion
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7 post-shift</td><td rowspan="2">Horizon Genomics
(HZGHC004884C007)
King et al., 2021</td></tr>
<tr><td>JMY:</td><td>35bp deletion
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33 post-shift</td></tr>
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Table S2. Plasmids.

Plasmids					
Description	Vector	Species	AA	R.E. Sites	Source
pGFP	pCDNA3::GFP (pKC425)	N/A	N/A	N/A	Campellone et al., 2008
pGFP-JMY	pGFP	Mouse	1-983	BamHI-NotI	King et al., 2021
pGFP-JMY(ΔCA)	pGFP	Mouse	1-941	BamHI-NotI	King et al., 2021
pGFP-JMY(ΔWWW)	pGFP	Mouse	1-856 / 934-983	BamHI-NotI	King et al., 2021
pKC-LAP-C1	pIC113	N/A	N/A	N/A	Campellone et al., 2008
pLAP-WHAMM	pKC-LAP-C1	Human	1-809	KpnI-BamHI	Campellone et al., 2008
pACT-Lifeact-MCH	pACT::MCH	N/A	N/A	SpeI-BglII	Ohkawa & Welch, 2018

Table S3. Immunofluorescence and Immunoblotting Reagents.

Primary Antibodies (Immunofluorescence)

Target	*Probe*		*Conc.*	*Identifier*
AIF (Fig 4, S5)	anti-AIF	Rabbit	1:1,000	Cell Signaling Technology (5318)
Apaf-1 (Fig 5, 6)	anti-Apaf-1	Mouse	1:250	R&D Systems (MAB868)
Arp3 (Fig 3)	anti-ARP3	Mouse	1:1,000	Sigma (A5979)
ArpC2 (Fig 3, 5)	anti-p34-Arc	Rabbit	1:1,000	EMD Millipore (07-227-I)
BAX (Fig 4)	anti-BAX	Mouse	1:500	Proteintech (60267-1-Ig)
Caspase-9 (Fig S6)	anti-Total Caspase-9	Rabbit	1:500	Cell Signaling Technology (9502)
Caspase-3 (Fig S6)	anti-Total Caspase-3	Mouse	1:500	Cell Signaling Technology (9668)
Cleaved Caspase-3 (Fig 6, 7, 8, S6, S7)	anti-Cleaved Caspase-3 (Asp175)	Rabbit	1:1,000	Cell Signaling Technology (9664)
Cortactin (Fig S4)	anti-Cortactin	Mouse	1:2,000	EMD Millipore (05-180)
Cyto *c* (Fig 5, 6)	anti-Cytochrome *c*	Mouse	1:500	Cell Signaling Technology (12963)
GM130 (Fig S5)	anti-GM130	Mouse	1:1,000	BD Transduction Labs (610822)
JMY (Fig 1-5, S2-S7)	anti-JMY	Rabbit	1:1,000	Proteintech (25098-1-AP)
mtDNA (Fig 4)	anti-DNA	Mo IgM	1:100	EMD Millipore (CBL-186)
N-WASP (Fig S4)	anti-N-WASP	G Pig	1:1,000	Duleh et al., 2010
p53 (Fig S5)	anti-p53	Rabbit	1:1,000	Proteintech (10442-1-AP)
STRAP (Fig 3)	anti-STRAP	Mouse	1:1,000	Proteintech (66712-1-Ig)
Tubulin (Fig 3)	anti-Beta-Tubulin	Mouse	1:2,000	DSHB (E7)
WASH (Fig S4)	anti-WASH	Rabbit	1:1,000	Duleh et al., 2010
WAVE2 (Fig S4)	anti-WAVE2	Rabbit	1:1,000	Cell Signaling Technology (3659)
WHAMM (Fig 3, 5)	anti-WHAMM	Rabbit	1:250	Shen et al., 2012
XIAP (Fig 6, S6)	anti-XIAP	Mouse	1:1,000	Proteintech (66800-1-Ig)

Primary Antibodies (Immunoblotting)

Target	*Probe*		*Conc.*	*Identifier*
Actin (Fig S1, S3)	anti-Beta-Actin	Mouse	1:10,000	Proteintech (66009-1-Ig)
GAPDH (Fig S1, S3)	anti-GAPDH	Mouse	1:10,000	Proteintech (60004-1-Ig)
GFP (Fig S1)	anti-GFP	Mouse	1:1,000	Santa Cruz (sc9996)
JMY (Fig S1, S3)	anti-JMY	Rabbit	1:1,000	Proteintech (25098-1-AP)
Tubulin (Fig S1, S3)	anti-Beta-Tubulin	Mouse	1:10,000	DSHB (E7)

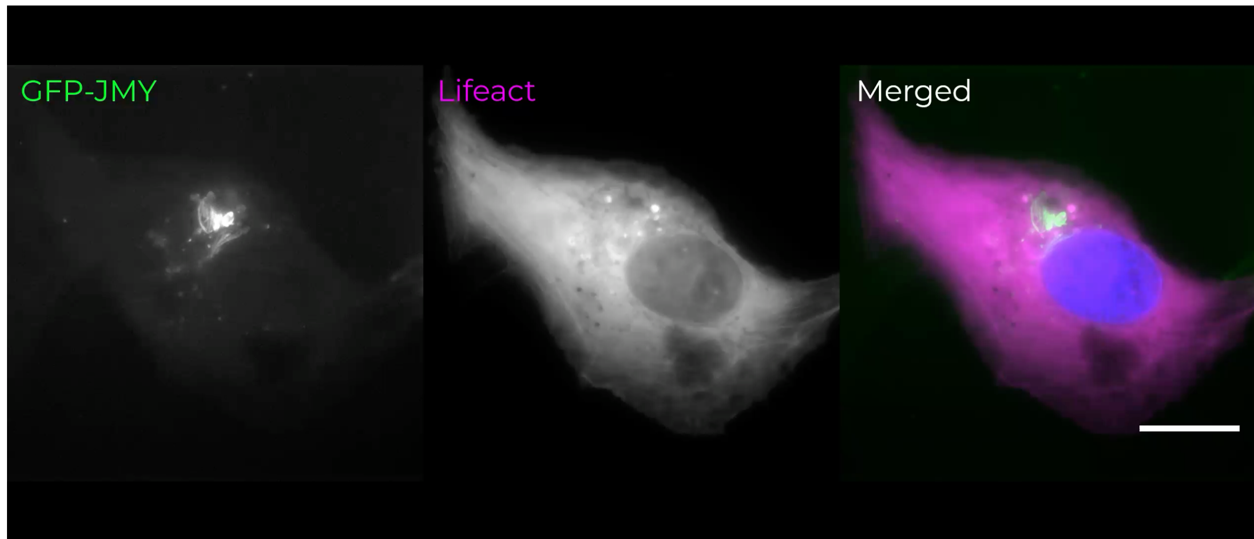
Secondary Antibodies (Immunofluorescence)

Target	*Probe*		*Conc.*	*Identifier*
Mouse IgG	Alexa 488 anti-mouse	Goat	4 μg/ml	Invitrogen (A11029)
Mouse IgG	Alexa 555 anti-mouse	Goat	4 μg/ml	Invitrogen (A21424)
Rabbit IgG	Alexa 488, 555, 647 anti-rabbit	Goat	4 μg/ml	Invitrogen (e.g. A11034)
Guinea Pig IgG	Alexa 555 anti-guinea pig	Goat	4 μg/ml	Invitrogen (A11075)

Secondary Antibodies (Immunoblotting)

Target	*Probe*		*Conc.*	*Identifier*
Mouse IgG	HRP anti-Mouse	Sheep	1:10,000	GE Healthcare (NXA931)
Rabbit IgG	HRP anti-Rabbit	Donkey	1:10,000	GE Healthcare (NA934V)
Mouse IgG	IRDye 680, 800 anti-Mouse	Donkey	0.05 μg/ml	LI-COR (e.g., 926-32212)
Rabbit IgG	IRDye 680, 800 anti-Rabbit	Donkey	0.05 μg/ml	LI-COR (e.g. 926-32213)

Molecular Probes (Fluorescence)			
Target	*Probe*	*Conc.*	*Identifier*
DNA (Fig 1, 2, 7, 8, S7)	DAPI	1 μg/ml	Invitrogen (D1306)
DNA (Fig 2)	Hoescht 33342 Solution	2 μg/ml	Thermo Scientific (62249)
F-actin (Fig 3-7, S3-S6)	Alexa647-Phalloidin	0.4 U/ml	Invitrogen (A22287)
F-actin (Fig 1, 2, 8, S2, S7)	Alexa555-Phalloidin	0.4 U/ml	Invitrogen (A34055)
F-actin (Fig 6)	Alexa350-Phalloidin	0.6 U/ml	Invitrogen (A22281)
Mitochondria (Fig 4)	MitoTracker Red CMH2XRos	500 nM	Invitrogen (M7513)



Video S1. Time-lapse movie showing the formation of punctate GFP-JMY structures and an F-actin-rich territory. Live U2OS cells expressing GFP-JMY (green) and Lifeact-mCherry (magenta) and treated with hoechst (blue) were imaged at 2min intervals 180-260min after exposure to 10µM etoposide. Playback rate = 10 frames per second. Scale bar: 20µm. Still frames are shown in Figure 2.