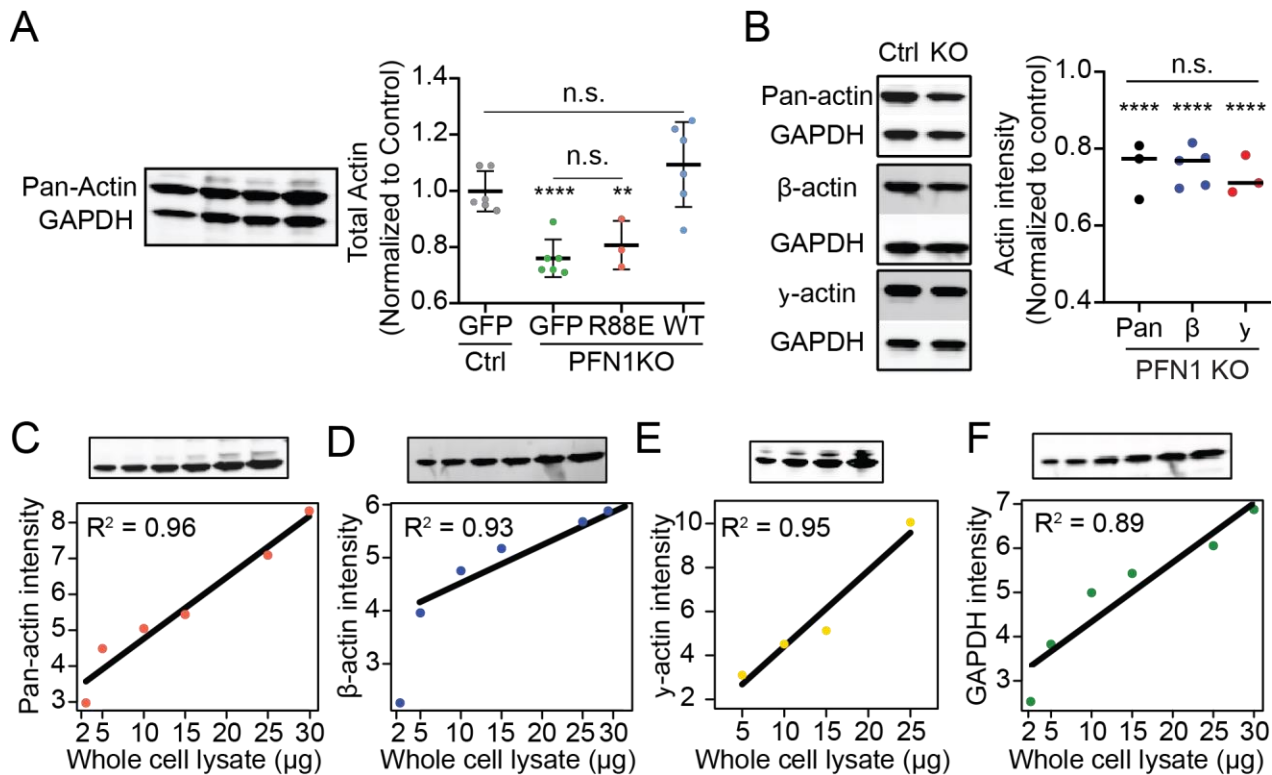
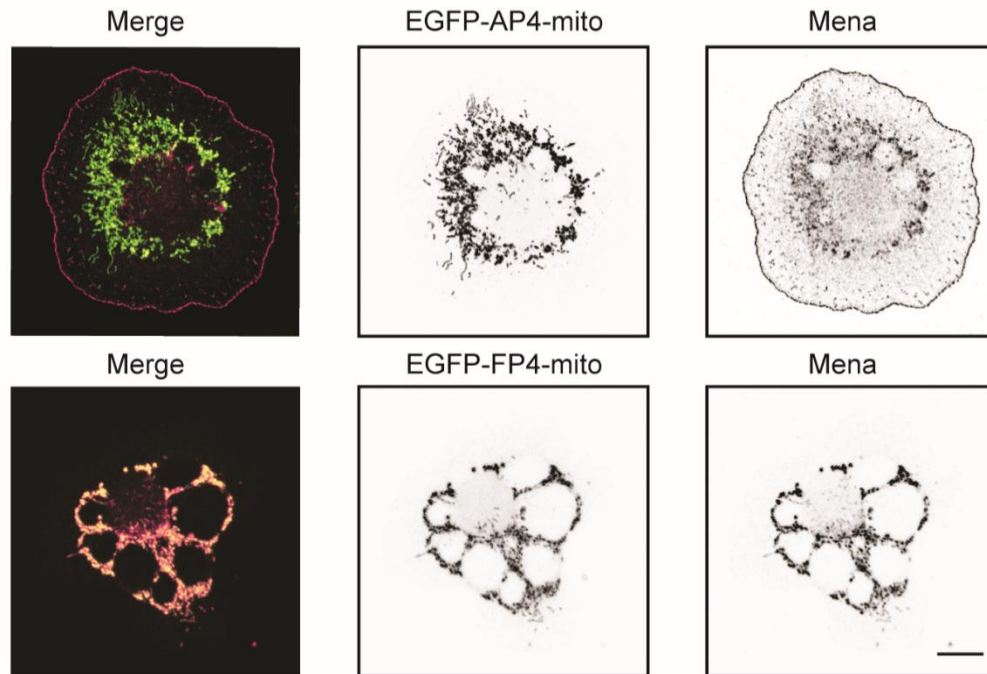


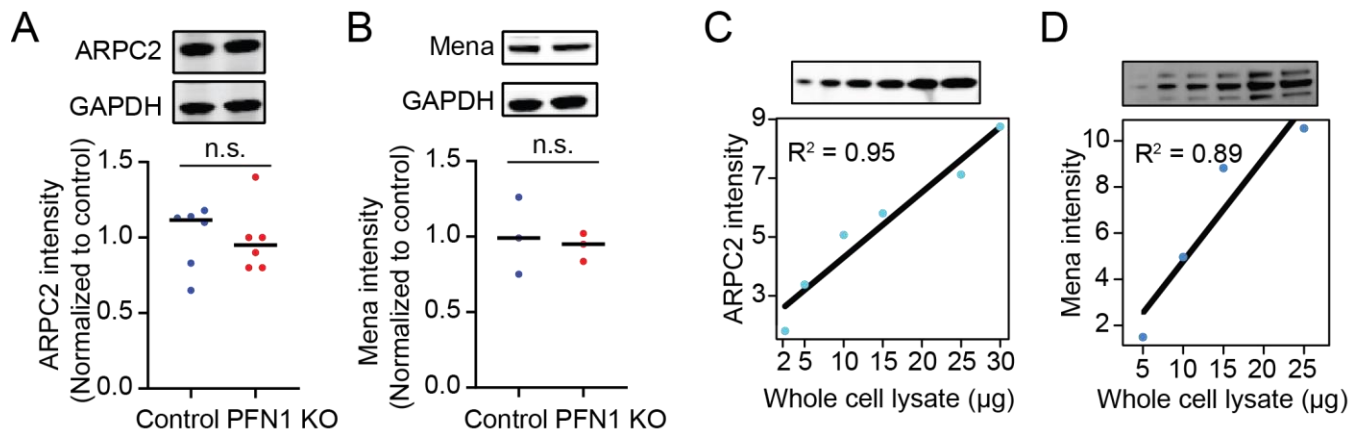
**Figure S1. Differentially expressed genes between control and PFN1 KO cells, related to Figure 1.** Volcano plot of differentially expressed genes upon deletion of PFN1. Criteria for significance is an adjusted p value  $\leq 0.01$  where significance is indicated in orange. Points are labelled based on adjusted p value, the top 15 up-regulated genes and 14 down-regulated genes (after removal of PFN1).



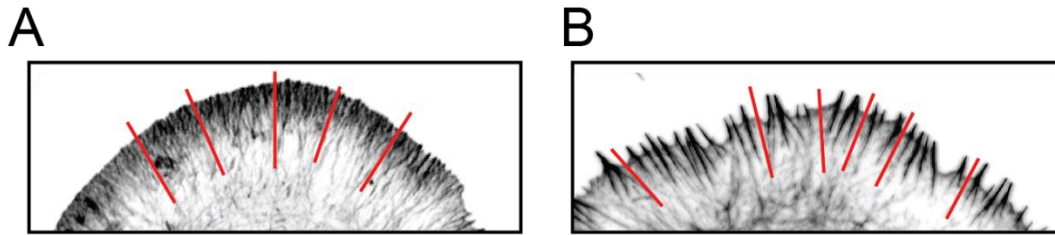
**Figure S2. Actin expression in PFN1 KO cells, related to Figure 1. (A)** Western blot analysis and quantification of pan-actin expression in control and PFN1 KO cells normalized to GAPDH as a loading control.  $n = 6$  for control + GFP, PFN1 KO + GFP, PFN<sup>WT</sup> and  $n = 3$  for PFN1 KO + PFN<sup>R88E</sup>. **(B)** Western blot analysis and quantification of pan/ $\beta$ / $\gamma$ -actin expression in PFN1 KO cells normalized to GAPDH as a loading control.  $n = 3$  for expression of pan and  $\gamma$ -actin,  $n = 5$  for  $\beta$ -actin expression. **(C)** Western blot and quantification of linearity of pan-actin signal. From left to right 2.5, 5, 10, 15, 25, 30 $\mu$ g of whole cell lysate **(D)** Western blot and quantification of linearity of  $\beta$ -actin signal. From left to right 2.5, 5, 10, 15, 25, 30 $\mu$ g of whole cell lysate. **(E)** Western blot and quantification of linearity of  $\gamma$ -actin signal. From left to right 5, 10, 15, 25 $\mu$ g of whole cell lysate **(F)** Western blot and quantification of linearity of GAPDH signal. From left to right 2.5, 5, 10, 15, 25 $\mu$ g of whole cell lysate.



**Figure S3. Expression of EGFP-FP4-Mito construct sequesters Mena/VASP, related to Figures 3,5,7.** Representative images of PFN1 KO cells expressing EGFP-FP4-mito or EGFP-AP4-mito and immuno-stained for Mena. Scale bar is 10  $\mu$ m.



**Figure S4. Mena and ARPC2 expression in PFN1 KO cells, related to Figure 4. (A)** Western blot and quantification of ARPC2 in control and PFN1 KO cells normalized to GAPDH as a loading control. N=6 for both control and PFN1 KO. **(B)** Western blot of Mena in control and PFN1 KO cells normalized to GAPDH as a loading control. N = 3 for both control and PFN1 KO. **(C)** Western blot and quantification of linearity of ARPC2 signal. From left to right 2.5, 5, 10, 15, 25 $\mu\text{g}$  of whole cell lysate **(D)** Western blot and quantification of linearity of Mena signal. From left to right 2.5, 5, 10, 15, 25 $\mu\text{g}$  of whole cell lysate. The signal from 2.5 $\mu\text{g}$  was weak and thus not used.



**Figure S5. Diagram illustrating how measurements were made for linescans in the absence (A) and presence (B) of filopodia or linear arrays, related to Figures 2G, 5L, 6H, 7B,E. (A)** Lines were drawn perpendicular to the leading edge of the cell to measure actin intensity in the lamellipodia in the absence of filopodia-like protrusions. **(B)** Lines were drawn perpendicular to the leading edge of the cell and in between protrusions when present.

	Gene	Symbol	logFC	PValue
	Arp2/3 Complex subunit 1b	Arpc1b	0.290	1.6E-07
	Arp2/3 Complex subunit 2	Arpc2	0.056	2.7E-01
	Arp2/3 Complex subunit 3	Arpc3	0.103	9.5E-02
	Arp2/3 Complex subunit 4	Arpc4	0.102	4.3E-02
	Arp2/3 Complex subunit 5	Arpc5	0.167	1.0E-03
	Capping Actin Protein	Capza1	-0.109	2.3E-01
	Capping Actin Protein	Capza2	-0.129	3.5E-02
	Capping Actin Protein	Capzb	-0.036	4.5E-01
	Cell Division Cycle 42	Cdc42	0.038	4.5E-01
	Cofilin-1	Cfl1	-0.212	1.2E-05
	Calponin-1	Cnn1	0.272	8.6E-01
	Coronin 1A	Coro1a	0.126	3.8E-01
	Coronin 1B	Coro1b	0.115	2.6E-02
	Cortactin	Ctnn	0.147	4.4E-03
	Dishevelled Associated Activator of Morphogenesis 1	Daam1	0.196	7.7E-03
	Diaphanous Related Formin 1	Diap1	0.379	5.1E-08
	Diaphanous Related Formin 2	Diap2	0.255	7.7E-03
	Diaphanous Related Formin 3	Diap3	0.028	6.1E-01
	Destrin, actin depolymerizing factor	Dstn	0.187	1.7E-04
	Enah	Enah	-0.051	3.4E-01
	Enah/Vasp-Like	Evl	-0.165	3.8E-03
	Ezrin	Ezr	-0.062	3.6E-01
	Formin Homology 2 Domain Containing 1	Fhod1	0.431	1.9E-06
	Formin Like 1	Fmnl1	-0.033	5.9E-01
	Formin Like 2	Fmnl2	0.061	2.7E-01
	Formin Like 3	Fmnl3	-0.077	2.6E-01
	Gelsolin	Gsn	0.777	3.7E-17
	Inverted formin-2	Inf2	0.456	8.7E-12
	Junction Mediating And Regulatory Protein	Jmy	0.063	3.4E-01
	Myocardin Related (MAL)	Mkl1	0.088	0.15679
	Profilin-2	Pfn2	0.228	2.9E-05
	Tropomyosin1	Tpm1	-0.120	8.2E-02
	Twinfilin-1	Twf1	-0.137	3.4E-02
	Twinfilin-2	Twf2	0.154	5.8E-02
	Vasodilator Stimulated Phosphoprotein	Vasp	-0.004	9.4E-01
	Vinculin	Vcl	-0.261	7.2E-07
	Vimentin	Vim	0.238	1.4E-06
	Wiskott-Aldrich syndrome protein family member 1	Wasf1	-0.240	6.9E-05
	WD Repeat Domain 1	Wdr11	0.026	0.66484
	Zyxin	Zyx	0.184	9.8E-03

**Table S1. Genes of actin binding proteins with no expression change between control and PFN1 KO CAD cells, related to Figure 1. Criteria for differential expression of genes: logFC >±0.5 and a p value of <0.0001**

Gene	Symbol	logFC	PValue
Cordon-bleu WH2 repeat	Cobll1	+0.89	1.45E-47
Gelsolin	Gsn	+0.78	3.67E-17
Was/Wasl 1	Wipf1	+0.71	1.07E-12
Arp2/3 Complex subunit 5-like	Arpc5l	+0.56	1.05E-18
Villin-1	Vil1	+0.55	4.84E-11
Formin Homology 2 Domain Containing 3	Fhod3	+0.55	1.11E-04
$\beta$ -actin	Actb	-0.49	3.29E-26
Dishevelled-associated activator of Morphogenesis	Daam2	-0.53	3.38E-23
Thymosin $\beta$ -4	Tmsb4x	-0.94	7.63E-49
Gamma-actin	Actg1	-1.21	1.50E-39

**Table S2. Differentially expressed genes of actin binding proteins between control and PFN1 KO cells, related to Figure 1.** Criteria for differential expression of genes: logFC  $>\pm 0.5$  and a P value of  $<0.0001$