Supplemental Data

The C-terminal region of A-kinase anchor protein 350 (AKAP350A) enables formation of microtubule-nucleation centers and interacts with pericentriolar proteins

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List of Material included:

Truncation of AKAP350A	Vector	Restriction sites	Primers: S/AS
F2F3Δ1 (1882-3907)	pEGFP-C1	HindIII/BamHI	gagc AAGCTTcgAGCTTCCGGCAGAAGCAGGAAGCCAC gagcggatccTCATCTCCGCATGCCGGCGTGGA
F2F3Δ2 (2182-3907)	pEGFP-C1	HindIII/BamHI	gagcAAGCTTcgAAGCCCGAACTGAGCCTGGAAGTGCAG gagcggatccTCATCTCCGCATGCCGGCGTGGA
F2F3Δ3 (2482-3907)	pEGFP-C1	HindIII/BamHI	gagcAAGCTTcgTACTTCAAGTCCTTCGAAGAGAACGGCAAGGGCA gagcggatccTCATCTCCGCATGCCGGCGTGGA
F3Δ1 (2762-3907)	pEGFP-C3	XhoI/BamHI	gagcCTCGAGAAAAAGGCCTGCATGTTCGAGCCCCTGC gagcggatccTCATCTCCGCATGCCGGCGTGGA
F3Δ2 (3285-3907)	pEGFP-C3	XhoI/BamHI	gagcCTCGAGGACGCACAGCTGTCCGAGGAACAGGGAC gagcggatccTCATCTCCGCATGCCGGCGTGGA
F3Δ3 (3458-3907)	pEGFP-C3	XhoI/BamHI	gagcCTCGAGCGCATCCTGTACCAGAATCTGAACGAGCCT gagcggatccTCATCTCCGCATGCCGGCGTGGA
Inhibitory Region (1882-2182)	mCherry-C1	SalI/BamHI	gagcGTCGACAGCTTCCGGCAGAAGCAGGAAGCCAC gagcggatccTCAGGCCTCCACGGCGCCGAAGTGCTTTCT
Inhibitory Region (1882-2182)	pBD-Bam	BamHI/SalI	gagcggatccAGCTTCCGGCAGAAGCAGGAAGCCA gagcgtcgacGGCCTCCACGGCGCCGAAGTGCTTTCT
Promoting Region (2762-3458)	mCherry-C3	XhoI/BamHI	gagcCTCGAGAAAAAGGCCTGCATGTTCGAGCCCCTGC gagcggatccTCACCGGCTCTCCCGCTTTTCCTCCCGTTC
Promoting Region (2762-3458)	pBD-Bam	BamHI/SalI	gagcggatccAAAAAGGCCTGCATGTTCGAGCCCCTG gagcgtcgacCCGGCTCTCCCGCTTTTCCTCCCGTTC
Fragment 3 (2691-3907)	pBD-Bam	BamHI/SalI	gagcggatcc CTCGAGGCCCTGAGAGCCGAGA gagcgtcgac TCATCTCCGCATGCCGGCGTG
F2F3Δ1 (1882-3907)	pBD-Bam	BamHI/SalI	gagcggatcc AGCTTCCGGCAGAAGCAGGAAGCCAC gagcgtcgac TCATCTCCGCATGCCGGCGTG
F3Δ1 (2762-3907)	pBD	EcoRI/SalI	gagc GAATTC AAAAAGGCCTGCATGTTCGAGCCCCTGC gagc GTCGAC TCATCTCCGCATGCCGGCGTGGA
F3Δ2 (3285-3907)	pBD	EcoRI/SalI	gagc GAATTC GACGCACAGCTGTCCGAGGAACAGGGAC gagc GTCGAC TCATCTCCGCATGCCGGCGTGGA
F3Δ3 (3458-3907)	pBD	EcoRI/SalI	gagc GAATTC CGCATCCTGTACCAGAATCTGAACGAGCCT gagc GTCGAC TCATCTCCGCATGCCGGCGTGGA
Gene/Ref	**Vector**	**Restriction sites**	**Primers: S/AS**
Cep170 TRANSOMIC BC143762	mCherry-C1	SalI/SacII	GAGCGTCGACATGAGCTTAACATCCTGGTTTTTGGTGAGC GAGCCCGCGGTCATTCTTGTACTGTAACATCTTCCTCTTCCC
Cep170	pEGFP-C1	SalI/SacII	re-cloned from mCherry-C1
Cep170	pAD	NdeI/SalI	ctcgagcatatg ATGAGCTTAACATCCTGGTTTTTGGTGAGC tatatagtcgacTCATTCTTGTACTGTAACATCTTCCTCTTCCC
Cep170(1-852)	pAD	NdeI/SalI	ctcgagcatatg ATGAGCTTAACATCCTGGTTTTTGGTGAGC tatatagtcgac TCAAAGTTCTATGGGTATGTTTGGGCTGGG
Cep170(1-1112)	pAD	NdeI/SalI	ctcgagcatatg ATGAGCTTAACATCCTGGTTTTTGGTGAGC tatatagtcgac TCATGAAGCTTCACCAAGTCGTGCTCTG
Cdk5RAP2(Cep215) TRANSOMIC BC140794	mCherry-C2	EcoRI/SalI	GAGCGAATTCATGATGGACTTGGTGTTGGAAGAGGACGT GAGCGTCGAC TCAGGAGCCTGGTCTGCTGGGA
Cdk5RAP2(Cep215)	pAD	EcoRI/SalI	re-cloned from mCherry-C2
Cdk5RAP2(Cep215)	pBD-GAL4	EcoRI/SalI	re-cloned from mCherry-C2
Cep68 NM 015147.2	mCherry-C2	EcoRI/SalI	GAGCGAATTC ATGGCCCTGGGTGAAGAAAAGGC GAGCGTCGAC TTAAACCCCTTCACATGGGTGCTCC
Cep68	pAD	EcoRI/SalI	re-cloned from mCherry-C2
Cep68	pMyc-C2	EcoRI/SalI	re-cloned from mCherry-C2

Table S1. A summary of cloning of AKAP350A-truncation mutants used in mapping of functional regions of AKAP350A and AKAP350A-binding proteins.

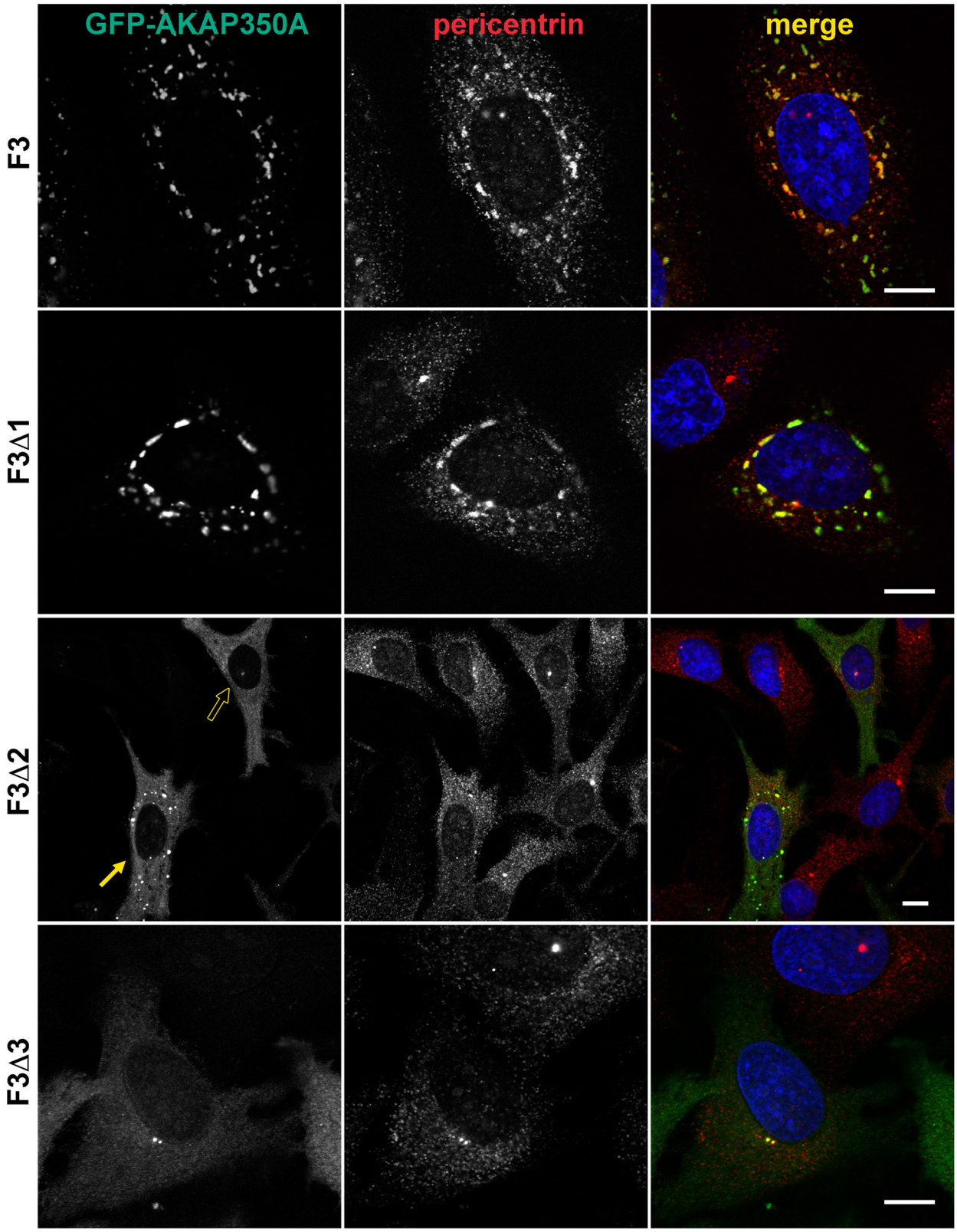


Figure S1. Mapping of AKAP350A regions responsible for formation of supernumerary MTNCs (see schematic on Figure 8). HeLa cells were transected with truncations of synthetic EGFP-AKAP350A, fixed with cold methanol and stained for pericentrin (red). Note: lower magnification was used for F3Δ2 to include two transected cells with different phenotypes (hollow arrow indicates single centrosome, and solid arrow indicates cell with numerous MTNCs). Bar = 10 μm.

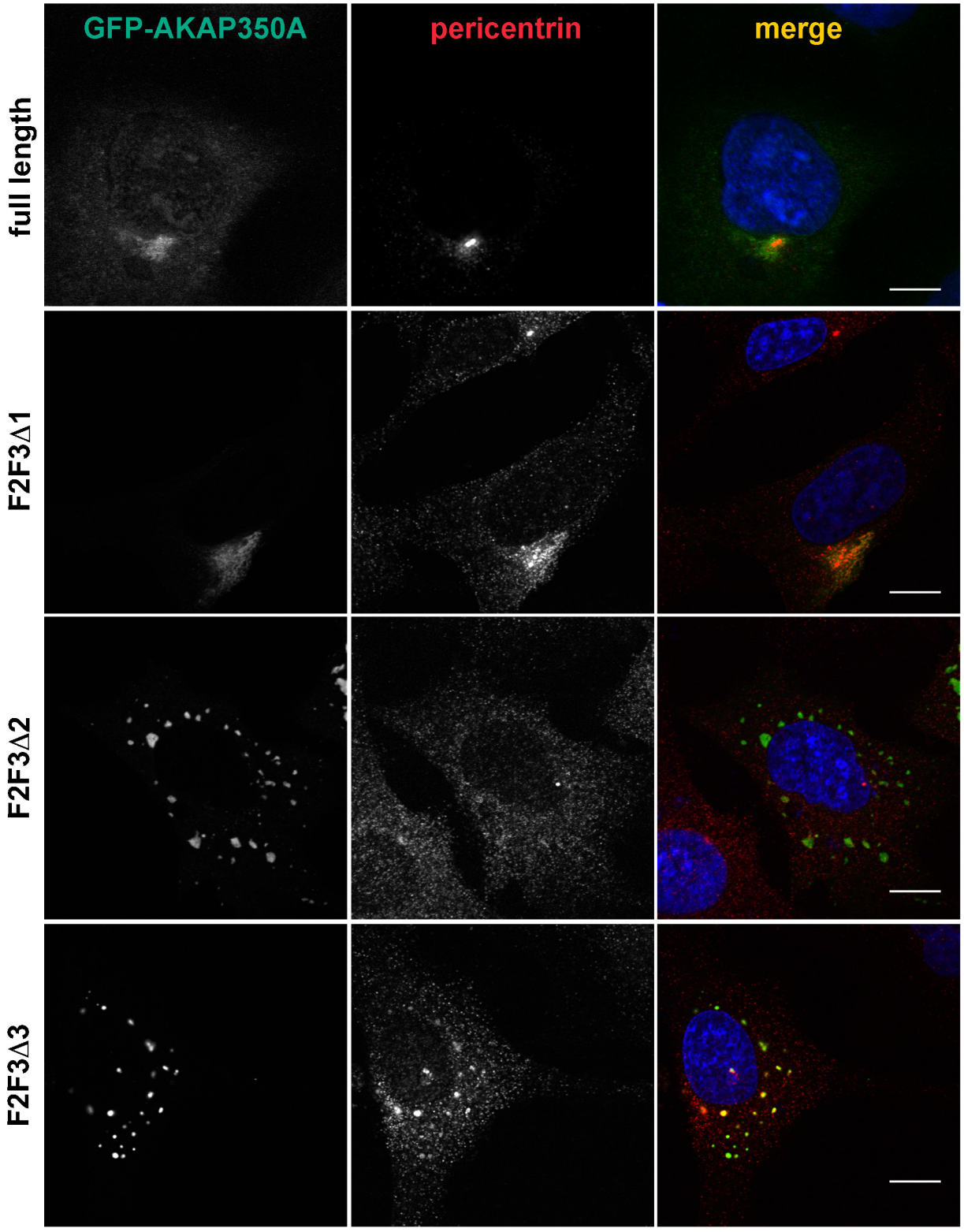


Figure S2. Mapping of AKAP350A regions responsible for formation of supernumerary MTNCs (see schematic on Figure 8). HeLa cells were transfected with full length synthetic AKAP350A or truncations of synthetic EGFP-AKAP350A as indicated at the left, fixed with cold methanol and stained for pericentrin (red). Bar = 10 µm.

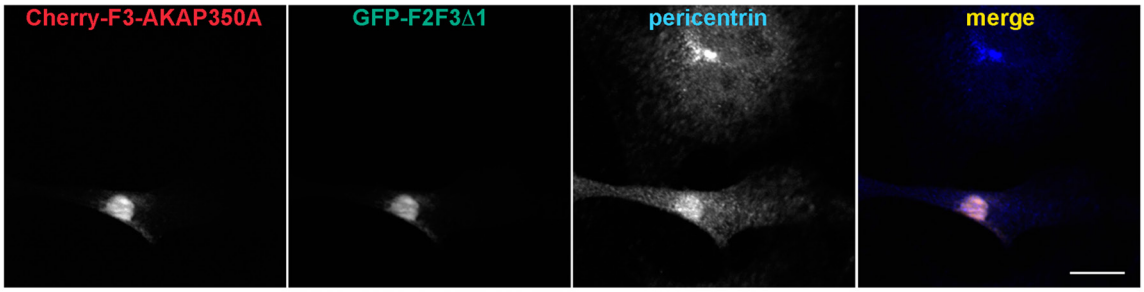


Figure S3. Dual over-expression of EGFP-F2F3Δ1-AKAP350A with Cherry-F3-AKAP350A restores single centrosome phenotype and led to enlargement of PCM. HeLa cells were fixed with methanol and stained for pericentrin (blue). Bar 10 = μm.

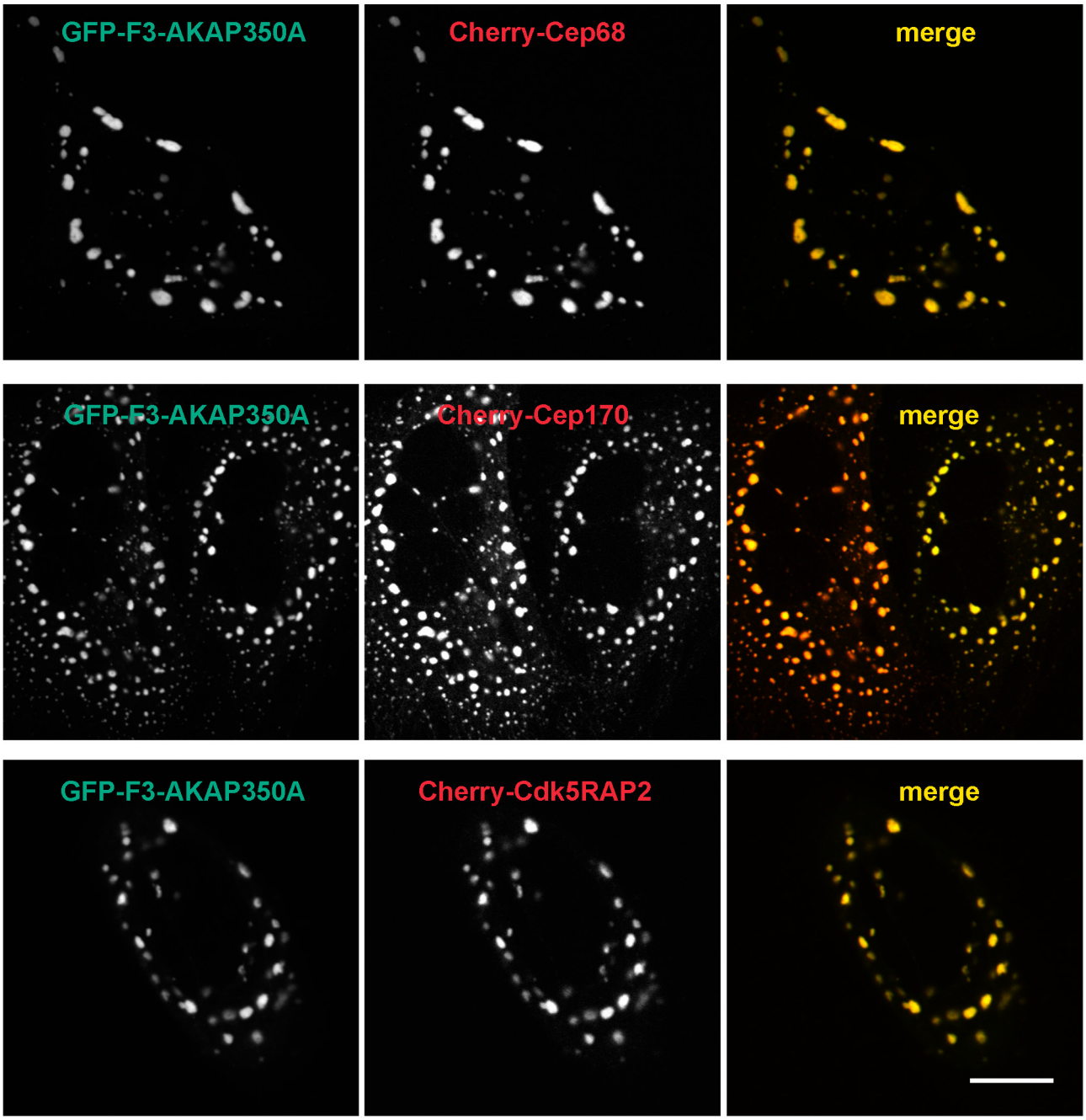
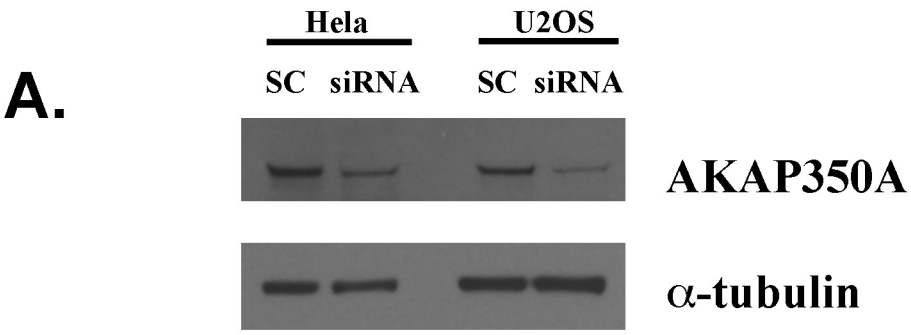


Figure S4. mCherry-fused chimeras of Cdk5RAP2, Cep170 and Cep68 colocalized with over-expressed of EGFP-F3-AKAP350A on supernumerary MTNCs. HeLa cells were fixed with methanol or with 4% PFA for Cdk5RAP2 staining, and immunostained for endogenous Cdk5RAP2, Cep170 or Cep68 (red). The degree of co-localization between EGFP-F3-AKAP350A (green) and mCherry-fused Cdk5RAP2/Cep170/Cep68 (red) were quantified using Pearson's Correlation Coefficient (PCC). PCCs were determined using JACOP plug-in of ImageJ software. PCC: Cep68:AKAP350 0.98±0.02; Cep170:AKAP350 0.95±0.04; Cdk5RAP:AKAP350 0.97±0.03. Bar 15 = µm (applies to all images).



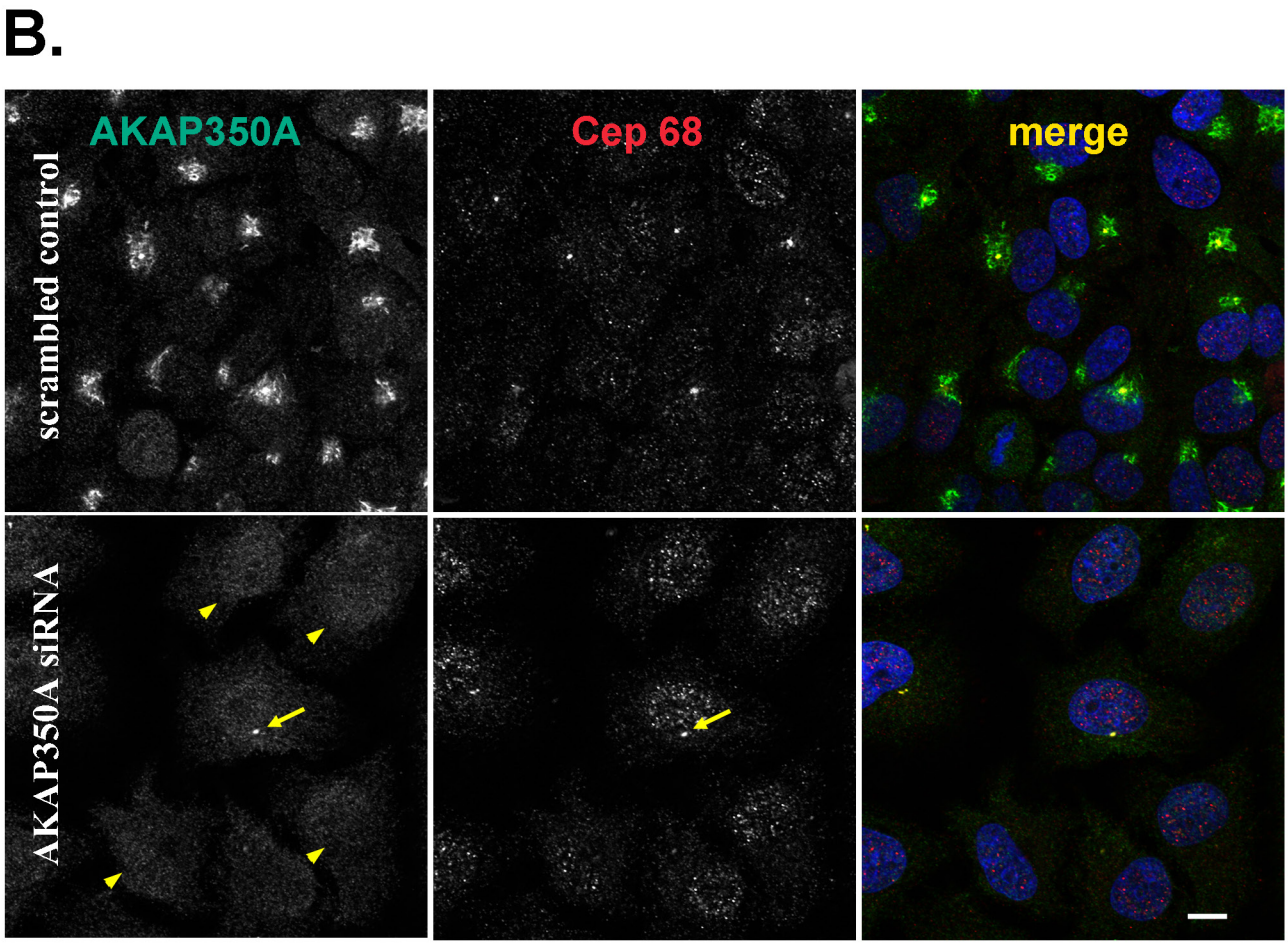


Figure S5. Depletion of AKAP350A by siRNA interference. A. Western blotting was performed for AKAP350A using 14G2 anti-AKAP350A monoclonal antibody and α-tubulin as a loading control. Dual detection was performed on the same membrane for both AKAP350A and α-tubulin using Odyssey Li-Cor system.
B. HeLa cells were transfected with either non-specific scrambled RNA duplexes or siRNA duplexes specific for AKAP350A, fixed and dual stained for AKAP350A (green in merged images) and Cep68 (red in merged images). Arrows indicate cells with AKAP350 at centrosome, co-localized with Cep68. Arrowheads indicate cells with displacement of both AKAP350A and Cep68. Bar = 10 μm.

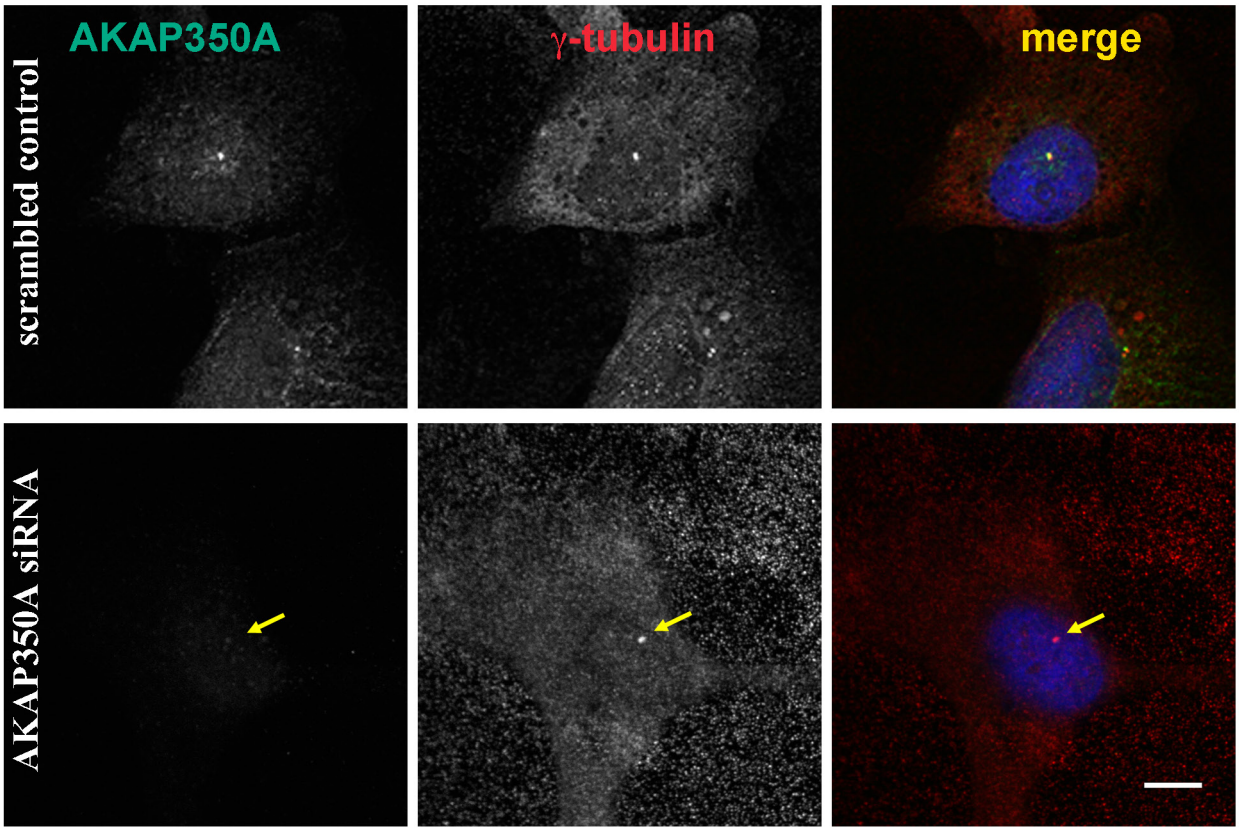


Figure S6. Depletion of AKAP350A by siRNA interference. U2OS cells were transfected with either non-specific scrambled RNA duplexes or siRNA duplexes specific for AKAP350A, fixed and dual stained for AKAP350A (green in merged images) and γ-tubulin (red in merged images). Arrows indicate cell depleted for AKAP350A, but still positive for γ-tubulin. Bar = 10 μm.

Fig.5video1. D*e novo* formation of supernumerary MTNCs induced by over expression of EGFP-F3-AKAP350A. Video was started 6 h following transfection and recorded for 80 minutes. Snapshots of video were taken every 10 min. Nikon Confocal Microscopy.

Fig.5video2. Live imaging of GFP-F3-AKAP350A showing presence of multiple rings of supernumerary MTNCs. Video was started 20h after transfection and recorded for 2h every 2 min. DeltaVision deconvolution microscopy.