Expanded View Figures

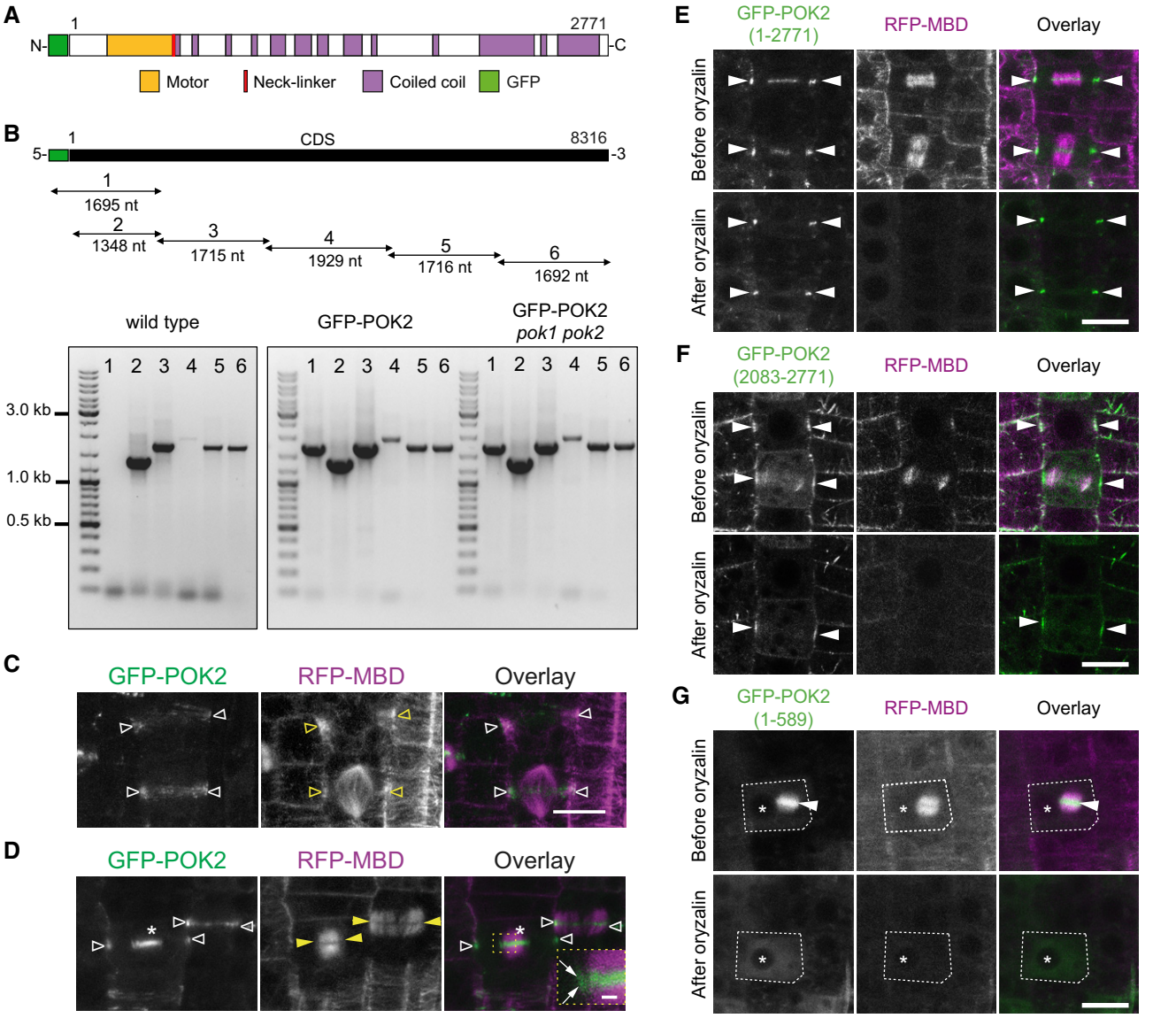

Figure EV1. POK2 expression and microtubule dependencies of POK2 protein domains.

A GFP-POK2 protein organization.

B Graphical representation of transgene coding sequence (CDS) and relative position of PCR fragments (consecutive numbers and fragment sizes are indicated) amplified from cDNA derived from wild type, transgenic line (GFP-POK2), and rescue line (GFP-POK2 *pok1 pok2*). Primer combinations are given in Appendix Table S2.

C, D Co-localization of GFP-POK2 and microtubule marker RFP-MBD. (C) Prophase cells displaying preprophase bands and pro-spindle (yellow triangles), respectively, and filamentous GFP-POK2 (white triangles). (D) Telophase cells displaying phragmoplasts (yellow arrowheads) and GFP-POK2 at the division site (white triangles) and at the phragmoplast midzone (asterisk). The inset shows the leading edge of the phragmoplast (arrows) at a higher magnification. Images are Z-projections. Scale bar 10 μm. Relates to Fig 1 and Movies EV1 and EV2.

E–G Root meristem cells expressing (E) GFP-POK2 (1–2,771), (F) GFP-POK2 (2,083–2,771), or (G) GFP-POK2 (1–589) along with the microtubule marker RFP-MBD are shown before (upper row) and after (lower row) treatment with 10 μM oryzalin. Images of the same cells are shown in each case. Arrow heads point to GFP signal at the division site (E and F) and to the phragmoplast midzone (G, dashed line indicates cell outline). The asterisks mark a conspicuous vacuole present before and after treatment. Note the persistence of GFP signal at the division site in (E) and (F), despite the depolymerization of phragmoplast microtubules and cytosolic GFP in (G). Images are maximum Z-projections. Scale bars indicate 10 μm. Relates to Figs 1–3.

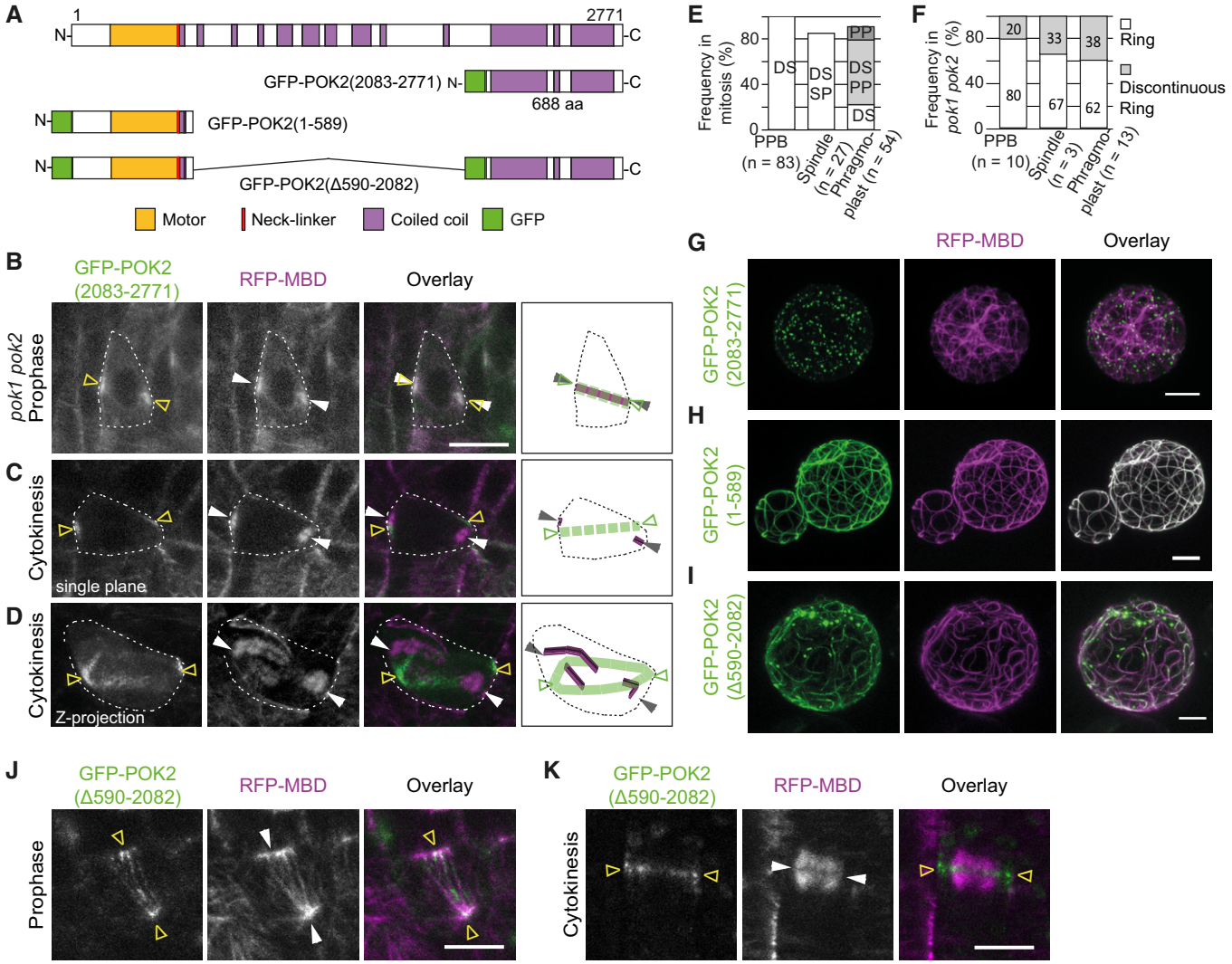

Figure EV2. Localization patterns of different POK2 fusion proteins in transgenic lines and transiently expressed in protoplasts.

A Graphical representation of protein domain organization of POK2, GFP-POK2 (2,083–2,771), GFP-POK2 (1–589) and GFP-POK2 (Δ590–2,082).

B–D (B) GFP-POK2 (2,083–2,771) co-localizes with the preprophase band (arrow heads) (C and D) Mis-alignment of GFP-POK2 (2,083–2,771) (triangles) and phragmoplasts (arrow heads). (B and C) are single plane images. (D) shows a maximum Z-projection. Cartoons illustrate microtubule (magenta) and GFP-POK2 (2,083–2,771) subcellular distribution in cells, outlined by dashed lines.

E Frequency (%) of mitotic cells displaying GFP-POK2 (2,083–2,771) in wild type. Numbers indicate the percentage of cells that showed GFP-POK2 (2,083–2,771) association with preprophase band (PPB), spindle (SP), phragmoplast (PP), and division site (DS).

F Frequency (%) of complete and discontinuous rings at the division site of *pok1 pok2* mutants during cell division.

G–I Transient co-expression of (G) GFP-POK2 (2,083–2,771), (H) GFP-POK2 (1–589), and (I) GFP-POK2 (Δ590–2,082) with the microtubule reporter RFP-MBD in *Arabidopsis* protoplasts. Note the complete co-localization of GFP-POK2 (1–589) and microtubules.

J, K Localization of GFP-POK2 (Δ590–2,082) (triangles) in (J) prophase cell with a preprophase band (arrow heads) and (K) telophase cell with a phragmoplast (arrow heads).

Data information: Scale bars indicate 10 μm.

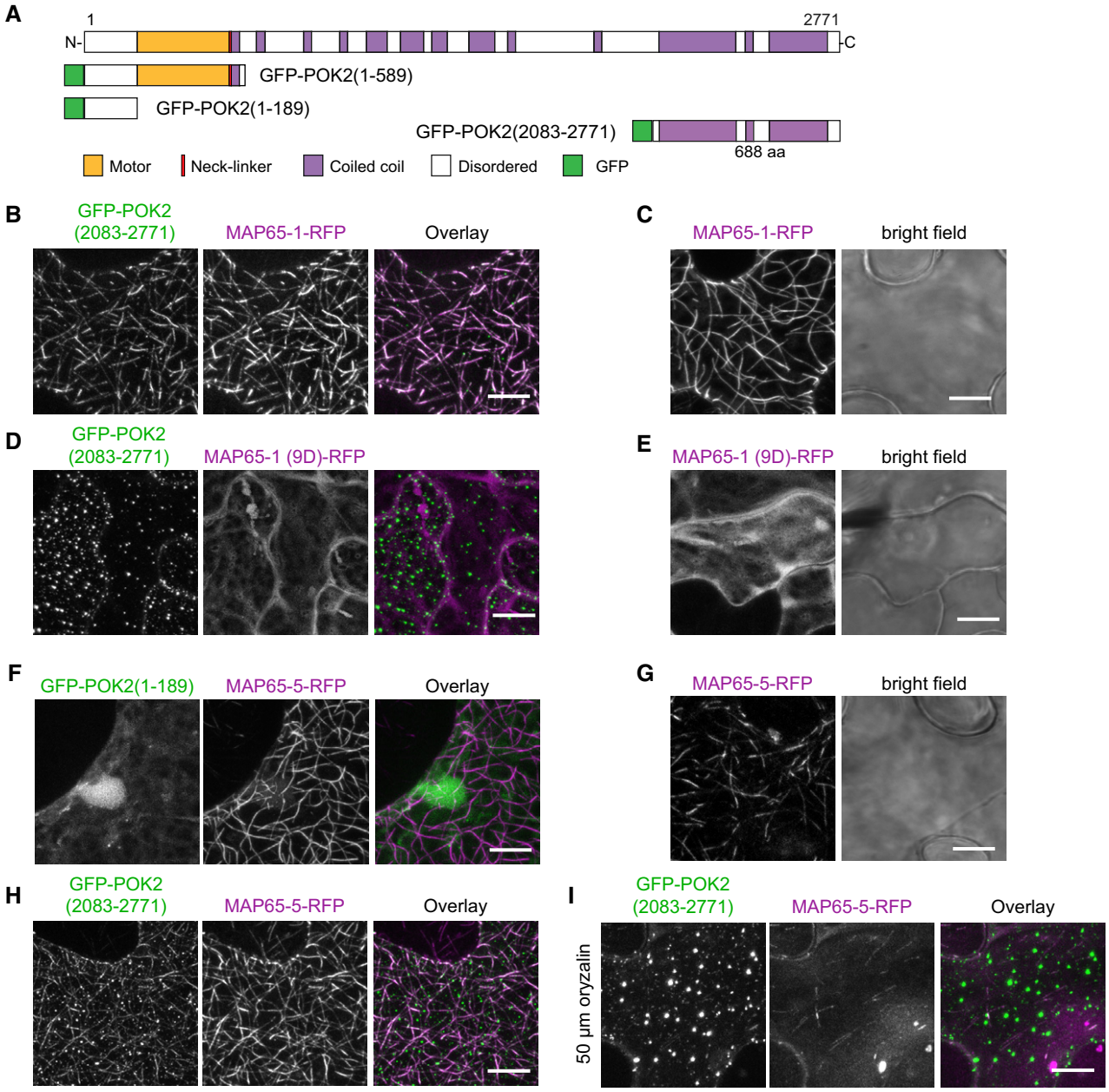

Figure EV3. Transient co-expression of fusion proteins in tobacco abaxial leaf epidermal cells.

A Domain organization and overview of fusion proteins.

B–I Transient co-expression of GFP-POK2 (2,083–2,771) and (B) MAP65-1-RFP, (D) MAP65-1(9D)-RFP, (H and I) MAP65-5-RFP in tobacco abaxial leaf epidermal cells. Note that microtubules are labeled by both fusion proteins in cells co-expressing GFP-POK2 (2,083–2,771) and MAP65-1-RFP, and MAP65-5-RFP. In cells expressing MAP65-1(9D), which does not bind microtubules, and MAP65-5-RFP treated with oryzalin to depolymerize microtubules, GFP-POK2 (2,083–2,771) displays punctate clusters, similar to the pattern observed when expressed alone (Fig 6G). Single expression of (C) MAP65-1-RFP, (E) MAP65-1(9D)-RFP, and (G) MAP65-5-RFP. Note that MAP65-1(9D)-RFP is cytosolic. (F) Co-expression of GFP-POK2 (1–189) with MAP65-5 RFP. Scale bar indicates 10 µm.

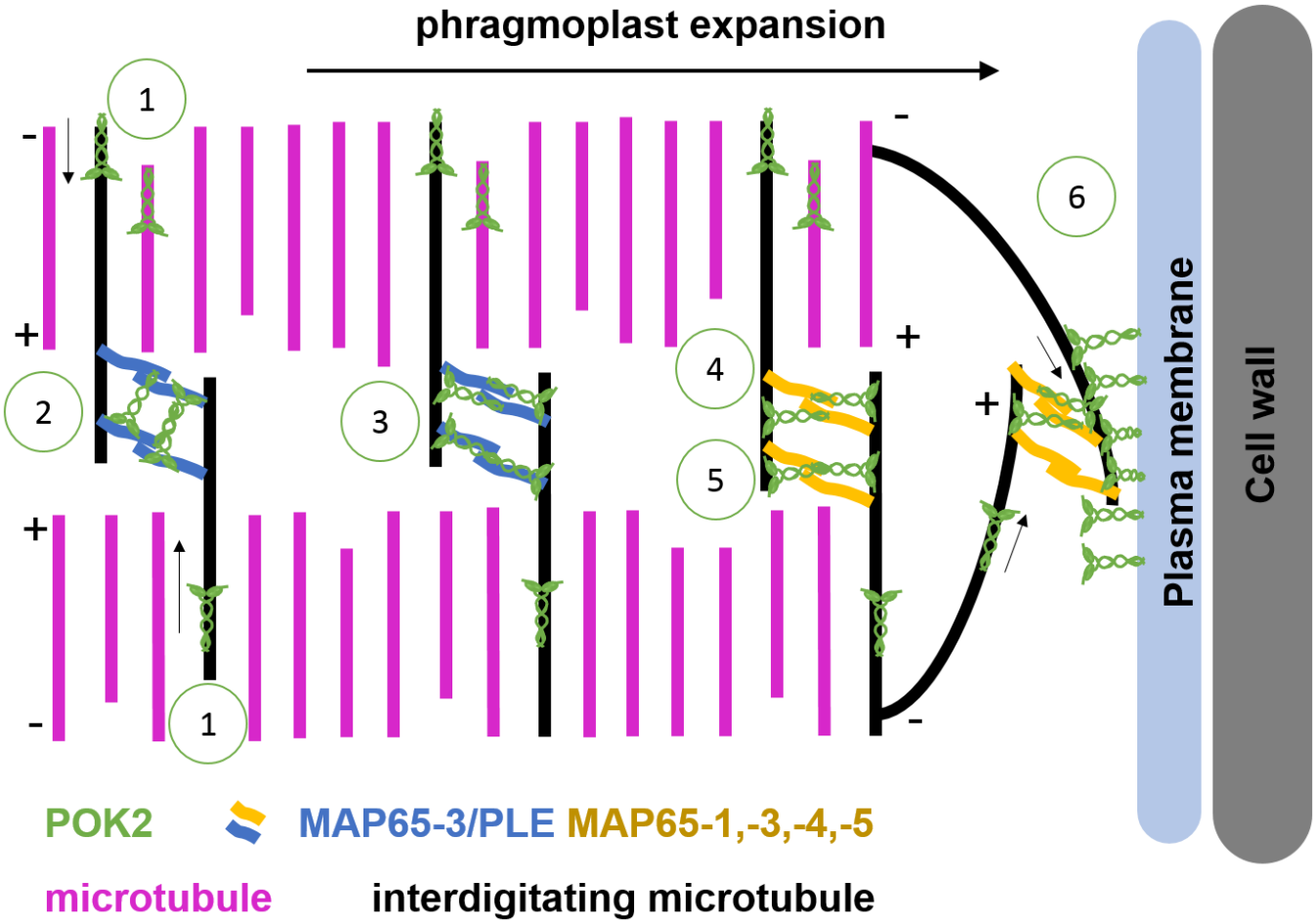

Figure EV4. Putative model for POK2 activities in cytokinesis.

Schematic representation of phragmoplast expansion during cytokinesis. Based on our data and available literature, we propose the following scenarios for POK2 activities during centrifugal phragmoplast expansion: (1) POK2 moves toward the phragmoplast midzone where it associates with MAP65/PLE and other MAP65 dimers. In accordance with HsKif15-microtubule encounters, POK2 forms dimers and/or tetramers and potentially prevents microtubule plus end catastrophe. POK2 interacts with MAP65 isoforms via distinct protein domains. Interaction with MAP65-3/PLE (2, 3) is mediated by both the disordered region (1–189) and the C-terminal domain (2,083–2,771). POK2 might bind between two MAP65-3/PLE dimers (2) or parallel to the axis of one MAP65-3 dimer (3). For interaction with other MAP65 isoforms, dimeric (4) or tetrameric (5) POK2 solely utilizes the C-terminal region. POK2 at the division site (6) is likely dimeric, due to the tethering via its C-terminal region. With their motor domains, POKs might interact with peripheral microtubules directly. Microtubule plus ends (+), microtubule minus ends (–). Arrows indicate direction of POK2 motility.