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Supplemental Information

Intrinsic Cell Polarity Coupled to Growth Axis Formation in Tobacco BY-2 Cells

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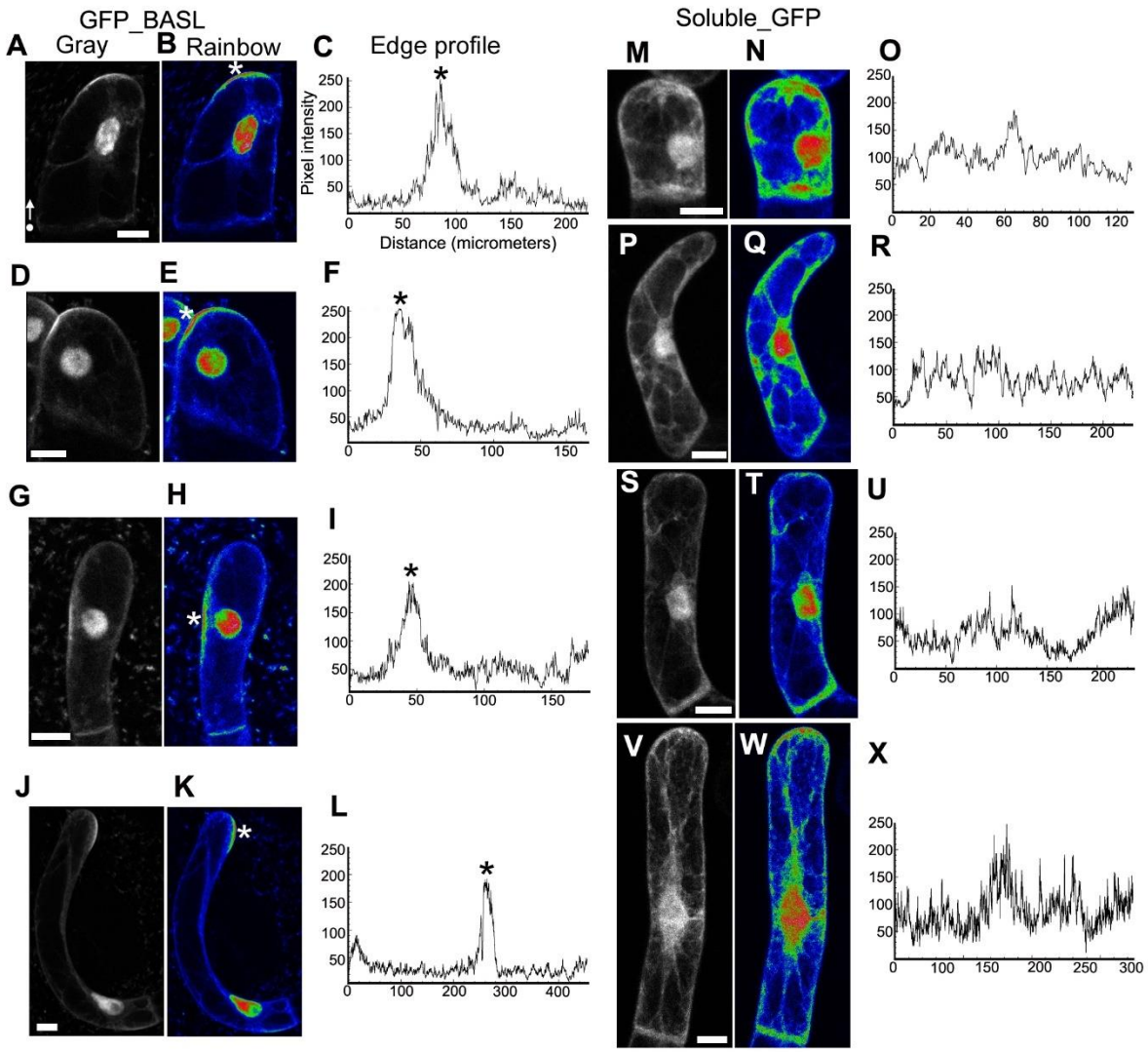

Figure S1: Localisation of 35S:GFP-BASL and 35S:GFP in BY-2 cells. Related to Figure 1.

(A,D,G,J) Examples of BY-2 cells transformed with GFP-BASL in transient assays. (B, E, H, K) Corresponding Rainbow LUT images. (C, F, I, L) Corresponding edge intensity/profiles. Note: GFP-BASL often labels the domed tips of terminal cells in filaments, though it may also label side walls (e.g. G, H, I). (M,P,S,V) Examples of BY-2 cells transformed with soluble GFP. (N,Q,T,W) Corresponding Rainbow LUT images. (O,R,U,X) Corresponding edge intensity profiles. Note in edge profiles, pixel intensities tend to be relatively high along cross-walls between cells (peaks on right-hand side of plots). The dot and arrow in (A) mark the origin and direction of the edge intensity profile, respectively. The asterisks mark polarised BASL. Bar = 15 µm.

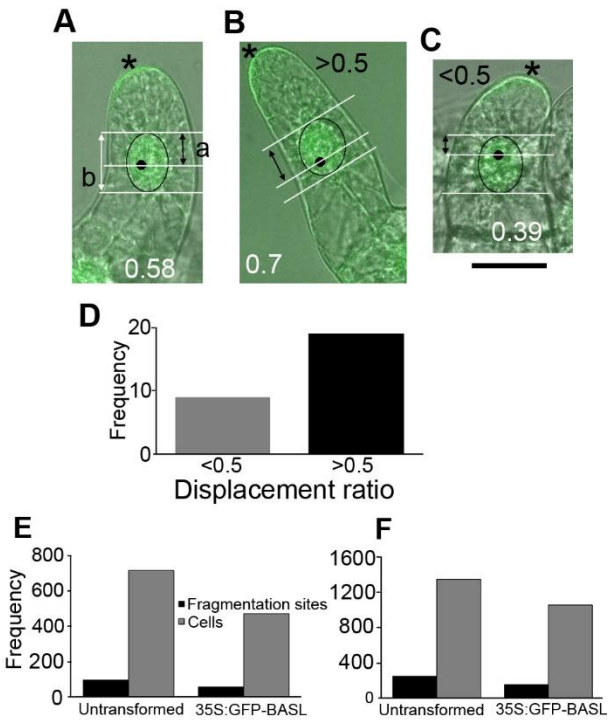

Figure S2: Properties of cells expressing 35S::GFP-BASL. Related to Figure 1.

(A-C) Images of premitotic cells expressing 35S::GFP-BASL taken 4 hours before mitosis showing their nuclei colocalise with cell's centroid (back spot). The nucleus overlapped the cell's centroid In 30/31 cells (obtained from 2 replicates) – although not centrally aligned with it in 28 cells. A displacement ratio was estimated for each of the 28 cells where the nucleus was not centrally aligned with the centroid. Cells were divided into two halves by drawing a line through the centroid (A). The proportion of the nucleus inside the cell half containing polarised BASL (asterisk) was estimated by dividing length a (distance between centroid line and edge line facing cell end with GFP-BASL; black arrows) by length b (total length of the nucleus; white arrows) (A). A value of 0.5 indicates the nucleus is centrally aligned, >0.5 indicates a greater proportion of the nucleus is inside the cell half with polarised BASL (B), and <0.5 indicating a greater proportion of the nucleus is inside the cell half without polarised BASL (C). Asterisks indicate cell end with polarised BASL. White numbers indicate the displacement ratio. Bar = 30 µm. (D) Histogram showing the displacement of the nuclei in the 28 cells, where the nucleus was not centrally aligned with the centroid. 19 cells had nuclei displaced towards, and 9 cells nuclei away from, the cell's end with polarised BASL. This distribution was not significantly different from a uniform distribution where there was an equal probability of being skewed towards either end of the cell (chi square = 3.571, p=0.06, 1 degree of freedom). (E-F) Plots showing the number of fragmentation sites in cells from untransformed or cultures expressing 35S:GFP-BASL (2 independent experiments). Cultures were quantified 4 days after subculture into fresh media. (Experiment 1 (E) = untransformed culture gave 95 fragmentation sites out of 718 cells, compared to 35S::GFP-BASL which gave 56 fragmentation sites out of 474 cells; Experiment 2 (F) untransformed culture gave 244 fragmentation sites out of 1347 cells, compared to 35S::GFP-BASL which gave 154 fragmentation sites out of 1052 cells). The frequencies of fragmentation sites in untransformed compared to transgenic cultures were not significantly different. [Experiment 1 (E): chi square = 0.40, p-value =0.53, 1 degree of freedom. Experiment 2 (F): chi square = 3.7, p-value =0.054, 1 degree of freedom].

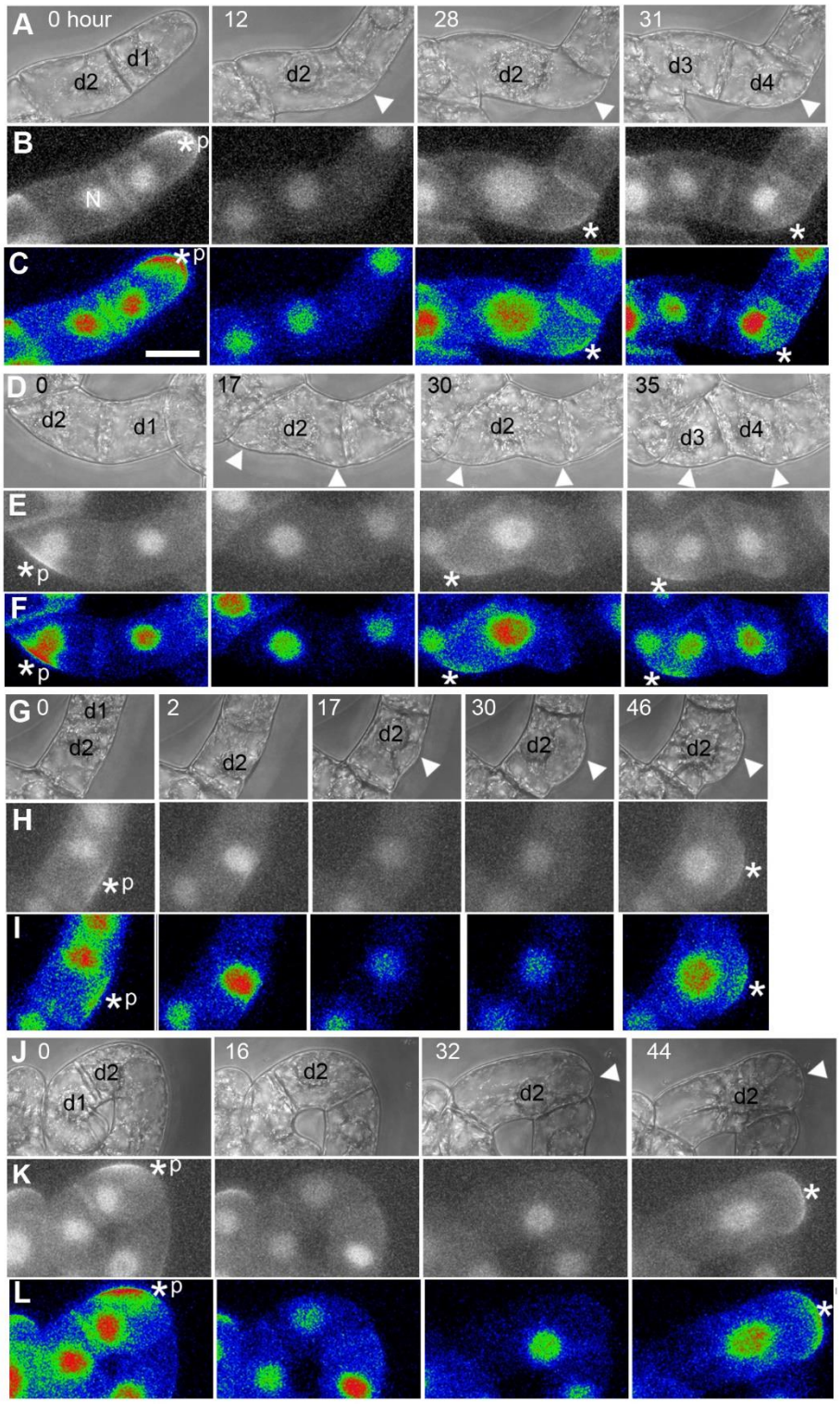

Figure S3: Examples of fragmentaion sites developing in filaments expressing 35S::GFP-BASL. Related to Figure 1.

Bright field (A, D, G, J) and corresponding GFP-BASL images (B, E, H, K) and Rainbow LUT images (C, F, I, L) showing temporal relationship between fragmentation and polarised BASL . Cells were tracked immediately after cell divison in daugher cells either without (A-C) or containing the parential BASL patch (D-L). (A -C) Polarised BASL patch is absent in internal daughter cell d2 (0 hours). A patch becomes prominent at the bulge in d2 (28 hours). Cell d2 then divides (31 hours) giving rise to cells

d3 and d4. (D-F) The parental BASL patch occupies a lateral site in daughter cell d2. The parental patch disappears (17 hours) and a new patch forms at the same site after a bulge forms (30 hours). The cell then divides (35 hours). Note: cell d2 forms another bulge on opposite side of the cell, which was not marked by parental BASL. (G-I) The parental patch of GFP-BASL occupies a lateral site in cell d2 (0 hours). It then disappears (2 hours) and a new patch forms after a bulge forms (46 hours). (J-L) The parental patch of GFP-BASL is located on a lateral face of cell d2 (0 hours). It disappears (16 hours) and a new patch appears at the new tip breaking from the filament axis (44 hours). Arrow head = fragmentation site, asterisk indicates polarised BASL, d1, d2 = daughter cells, d3, d4 = daughters of d2. Bar = 30 µm. t=0 refers to 56 (A-C), 59 (D-F), 93 (G-I), and 72 (J-L) hours after start of imaging.

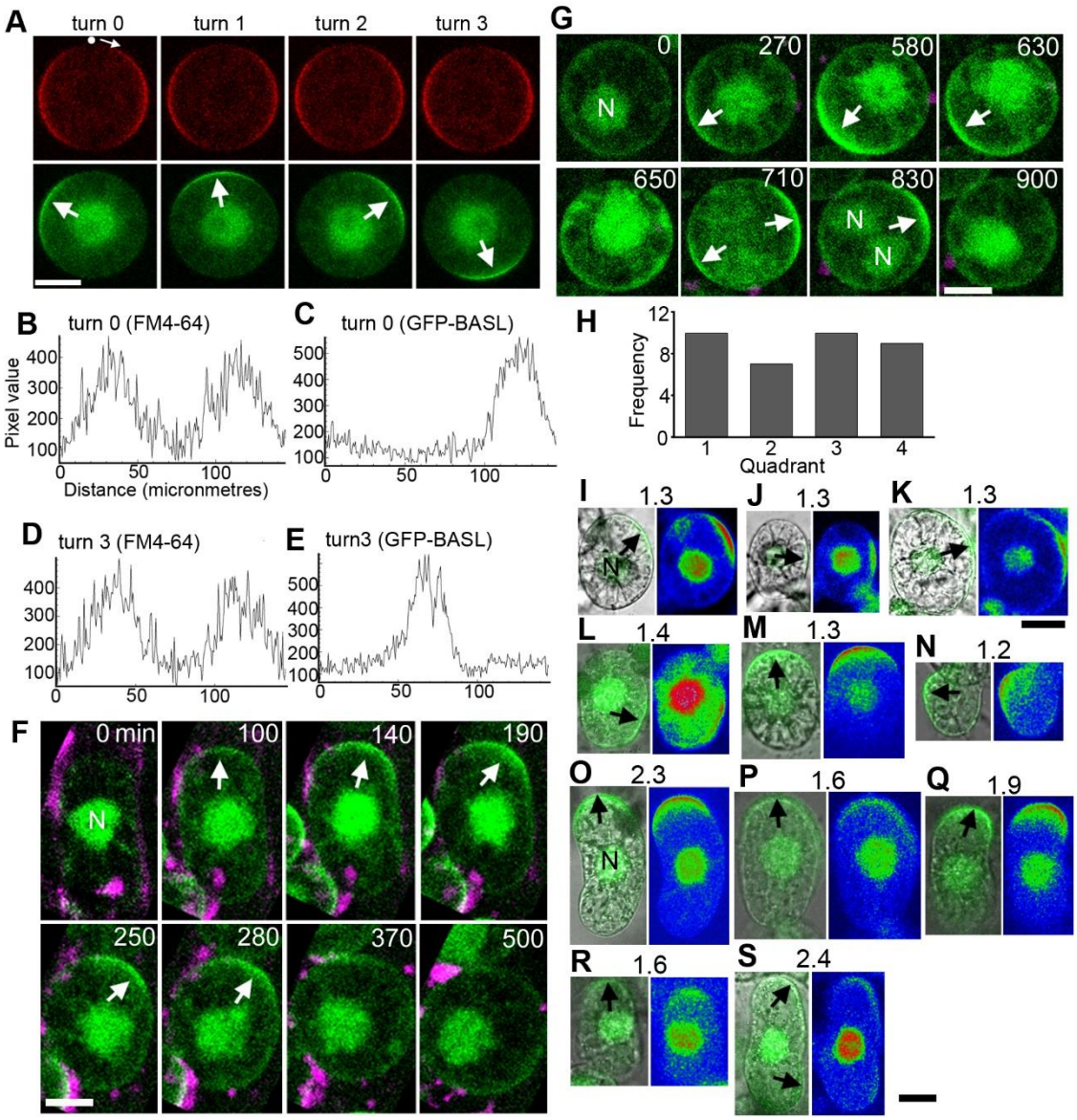

Figure S4. Behaviour of polarised BASL in protoplasts and regenerating protoplasts. Related to Figures 2 and 4.

(A) Rotation of protoplast stained with FM4-64 (red) and expressing GFP-BASL (green). The protoplast was turned 3 times (turn1-3), by rotating the sample. Note: the axial labelling pattern of FM4-64 is insensitive to turning, while GFP-BASL changes location. (B-E) Edge proile plots of the protoplast shown in A at turn 0 (B: FM4-64, C: GFP-BASL) and turn 3 (D: FM4-64, E: GFP-BASL). The axial labelling pattern of FM4-64 gives rise to 2 peaks (B,D). Polarised GFP-BASL displays a single peak (E,F). The location of the GFP-BASL peak changes location when the protoplast was turned; the FM4-64 peaks do not. (F) Appearance of GFP-BASL (green) during protoplast formation. GFP-BASL is initially present in the nucleus (0 min, N). A cap of polarised BASL then appears (100 min) and intensifies (140–280 min) before disappearing when the protoplast forms (500 min). The cell wall has been stained with Direct Fast Red B (magenta). (G) Polarised GFP-BASL shifting location in a dividing protoplast. GFP-BASL is initally present in the nucleus (0 h, N). A cap of polarised GFP-BASL

appears (270 min) at 8 o'clock. Polarised BASL disappears just before divison (650 min) and then reappears in a new postion (2 o'clock) after the nucleus has divided (830 min; note: 2 nuclei are present). The appearance of BASL at 2'oclock is preceded by a transitional state in which polarised GFP-BASL forms two caps at 8- and 2'oclock (710 min). Polarised GFP-BASL disappears after the daughter nuclei fuse into one structure (900 min). Note: cells in protoplasting solution do not form new cell walls following nuclear division. (H) Histogram showing the orientation of polarised BASL is not coordinated in protoplasts. 41 protoplasts were divided into quadrants within the imaging plane, 10 cap centres were in the first quadrant (12-3 o'clock; e.g. Figure 2D, protoplast a, 600 min), 7 in the second (3-6 o'clock, e.g. Figure 2D, protoplast c, 600 min), 10 in the third (6-9 o'clock; e.g. Figure 2D, protoplast d, 150 min) and 9 in the fourth (9-12 o'clock; e.g. Figure 2D, protoplast a, 150 min). This distribution is not significantly different from random (χ^2 for deviation from random orientation = 0.667, p=0.881, 3 degrees of freedom). Eight cells had a cap on the top surface (e.g. Figure 2D, protoplast c), which is consistent with a random distribution given that the cap occupies 18% of the protoplast surface area (7 out of 40 cells are expected to have a cap on the top surface if polarity is randomly oriented). Three of the 41 cells had two caps; two cells with both caps on their side surfaces and one with a cap on the top surface and the other on a side surface. (I-N) Polarised GFP-BASL displays diverse orientations in ovoid-shaped cells with relatively low aspect ratios. Left-hand panels show merge of bright field and GFP-BASL channels. The right-hand panels show corresponding Rainbow LUT images (of the GFP channel). Numbers indicate aspect ratio. Out of 40 cells with relatively low aspect ratios (1.1 to 1.5), 20 and 18 cells had polarised BASL on long or short sides, respectively. The numbers for short versus long side was not significantly different (χ^2 for deviation from random orientation = 0.4, p=0.527, 1 degree freedom). (O-R) Polarised GFP-BASL tends to be on one of the short sides of sausage-shaped cells with relatively high aspect ratios. 19/20 cells with higher aspect ratios (1.6 to 3.2) had polarised BASL only on a short side. The bias for short versus long side was significant (χ^2 for deviation from random orientation =16.2, p=0.00006, 1 degree freedom). Measurements were obtained from 3 replicates. (S) Example of polarised BASL located on both a long and short side of sausage-shaped cell. Arrows mark polarised GFP-BASL. Bar: A, I-N = 20 µm; F-G = 15 µm; O-S = 30 µm. t=0 in panels F,G is a few minutes after resuspension in protoplasting solution. See also Video S2.