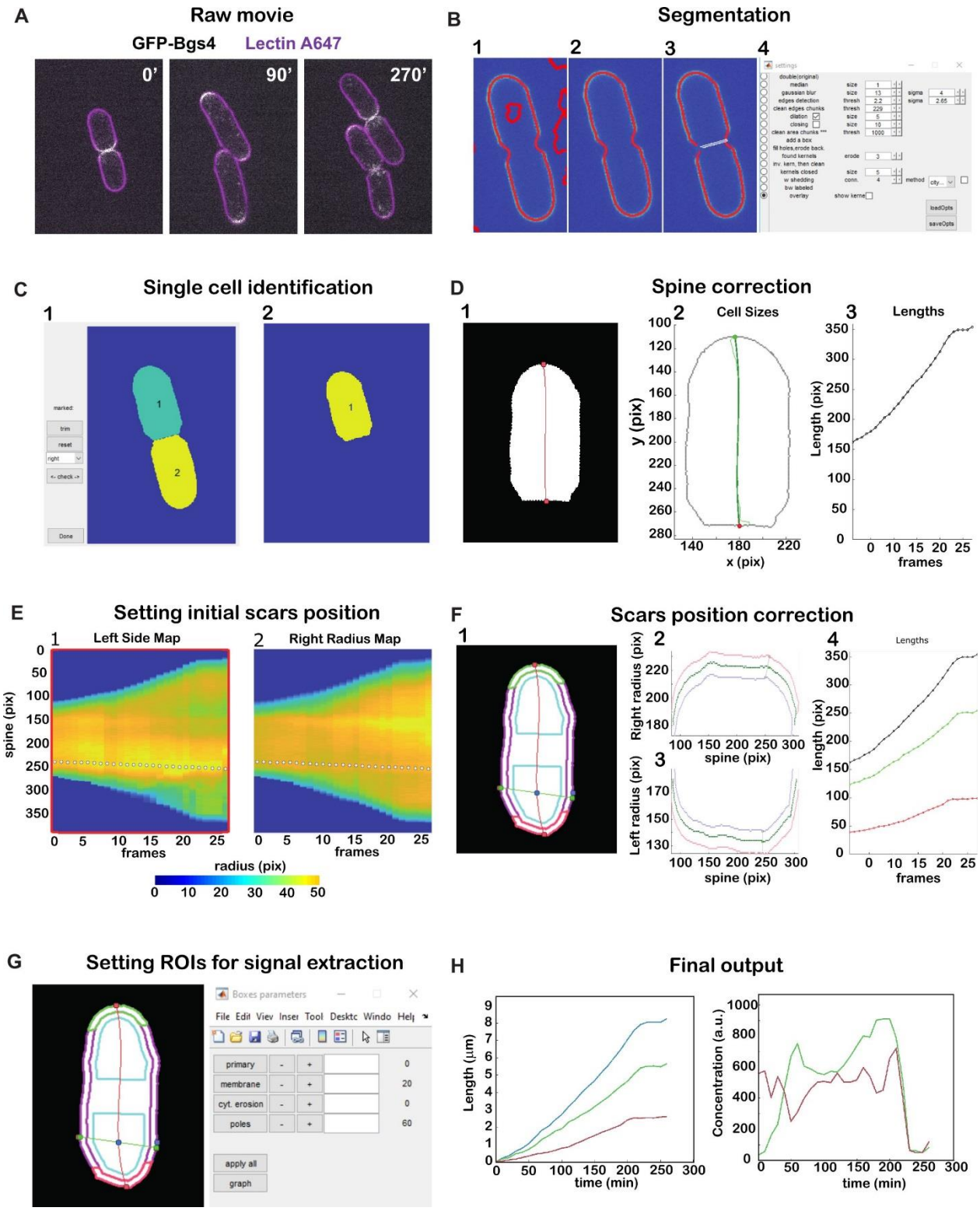

Figure S1. Image analysis workflow. (A) Raw images have two signals: one from labeled cell walls (Lectin-A647), which we use to segment the cells, and the other from labeled proteins of interest (i.e. GFP-Bgs4). The image analysis package provides a first tool for organization of the input data into separate stage positions, adapted to Metamorph outputs. **(B)** Segmentation. The program aims to delineate cells present in the field of view using the signal of the stained cell wall, or other signals (Bright field, phase, DIC..). Initial segmentation is typically not good (1), and the user needs to adjust a number of parameters (3) to get a good segmentation (2). In case when it is not possible to get proper deliniation (weak contour signal), the user can draw edges manually; this happens often for the separation between young daughter cells, since there is not enough Lectin signal at the division site (4). **(C)** Identification of individual cells. The user chooses a cell of interest (i.e. "1") and removes the unwanted segment ("2" and others if present). The tool provides the means to quickly remove unwanted ROIs from the whole time lapse). Importantly, in this step the direction of the new end is set; this information is used in the following analysis to distinguish the poles. **(D)** Spine correction. The total length of the current cell is calculated form the central line of the ROI ("spine"). The position of the endpoints of this line can be revised manually if required. **(E)** Setting initial scars position. In this step the user sees a 2D surface; the x-dimension is time, the y-dimension is cell length, and the colored intensity (blue = low, yellow = high) is the radius of a cell to the left of the spine (1) and to the right (2). The wider radii corresponding to the scar location should appear brighter in these surfaces, and the user manually draws a line (shown with small white circles) thus setting the scar position for the whole time lapse. **(F)** Scar position correction. The software provides a tool to correct manually the positions of the endpoints of each scar. Radii profiles (1,2) are used as the reference for positioning the scars; the curves showing the evolution of lengths in time (green curve for the old end and red curve for the new end) can also help guiding scar positioning. **(G)** Setting ROIs for signal extraction. The user can set the sizes of various ROIs: the membrane width (purple), old/new end ROI along the spine (green/red), and cytoplasm (cyan). **(H)** Typical data output for cell length (OE in green, NE in red and total cell length in blue) and polarity concentration evolution at both ends in a single cell.

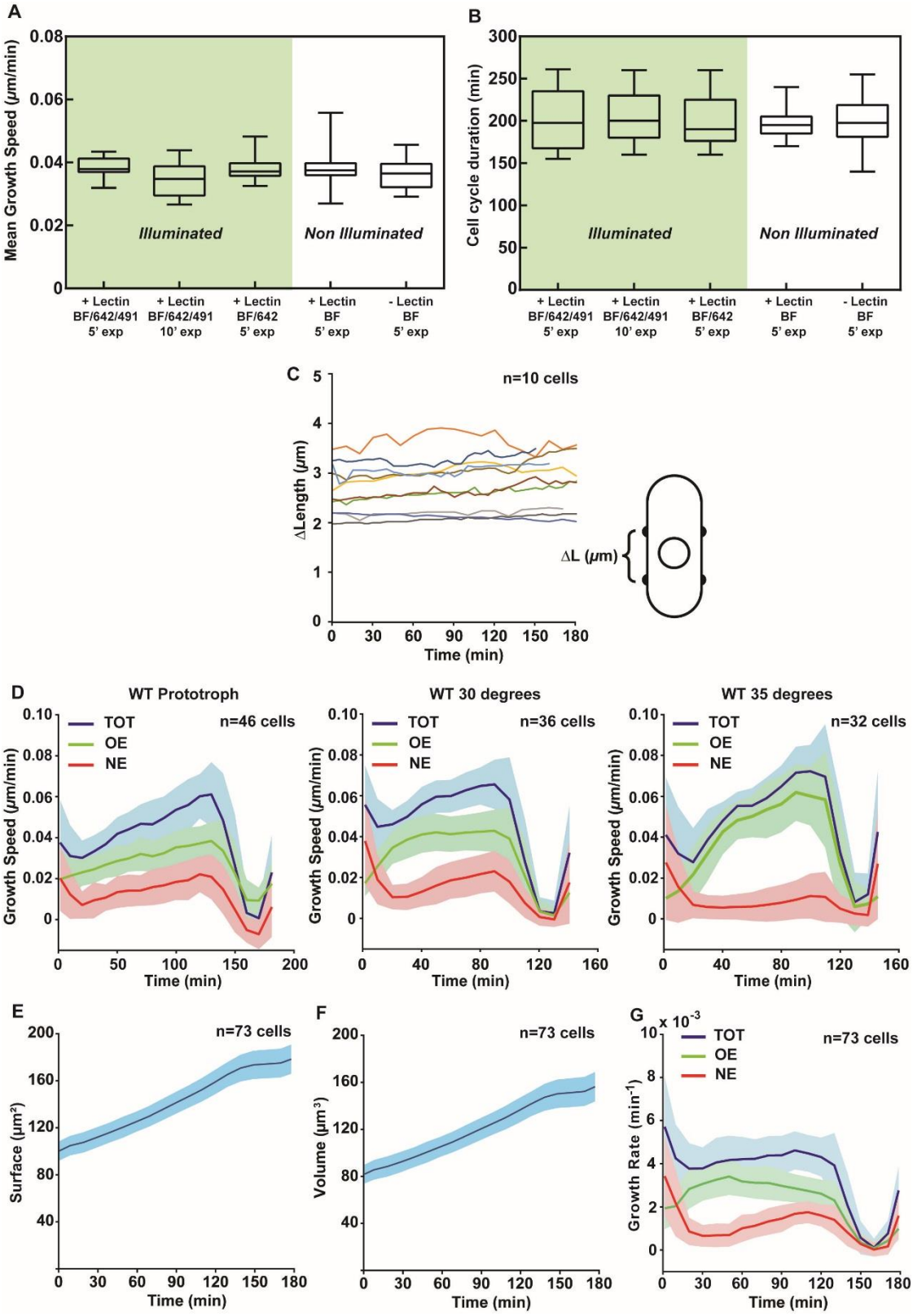

Figure S2. Controls for the imaging and image analysis methodology, and impact of temperature and auxotrophies on growth speeds. (A) Mean growth speeds of cells imaged under the indicated conditions (n > 20 cells for each conditions). **(B)** Mean cell cycle time (computed from the end of septation to the end of the next septation), for cells imaged under the indicated conditions (n > 20 cells for each conditions). Error bars represent standard deviations. **(C)** Evolution of the distance between two birth scars plotted as a function of time over the cell cycle for 10 individual cells (represented by a different color). **(D)** Growth speed for the OE, NE and total cell of a WT prototroph strain (n=46 cells, left), a WT laboratory strain grown at 30°C (n=36, middle) and a WT laboratory strain grown at 35°C (n=32, middle). The dark line represent the average and colored shades delimit +/- the standard deviation. **(E-F)** Evolution of the surface and volume along the cell cycle, computed as the sum of the perimeter or area, respectively, of cross sections along the cell spline axis (n=73 cells). (G) Growth rate computed as the growth speed divided by cell length for the OE, NE and total length of WT cells (n=73 cells).

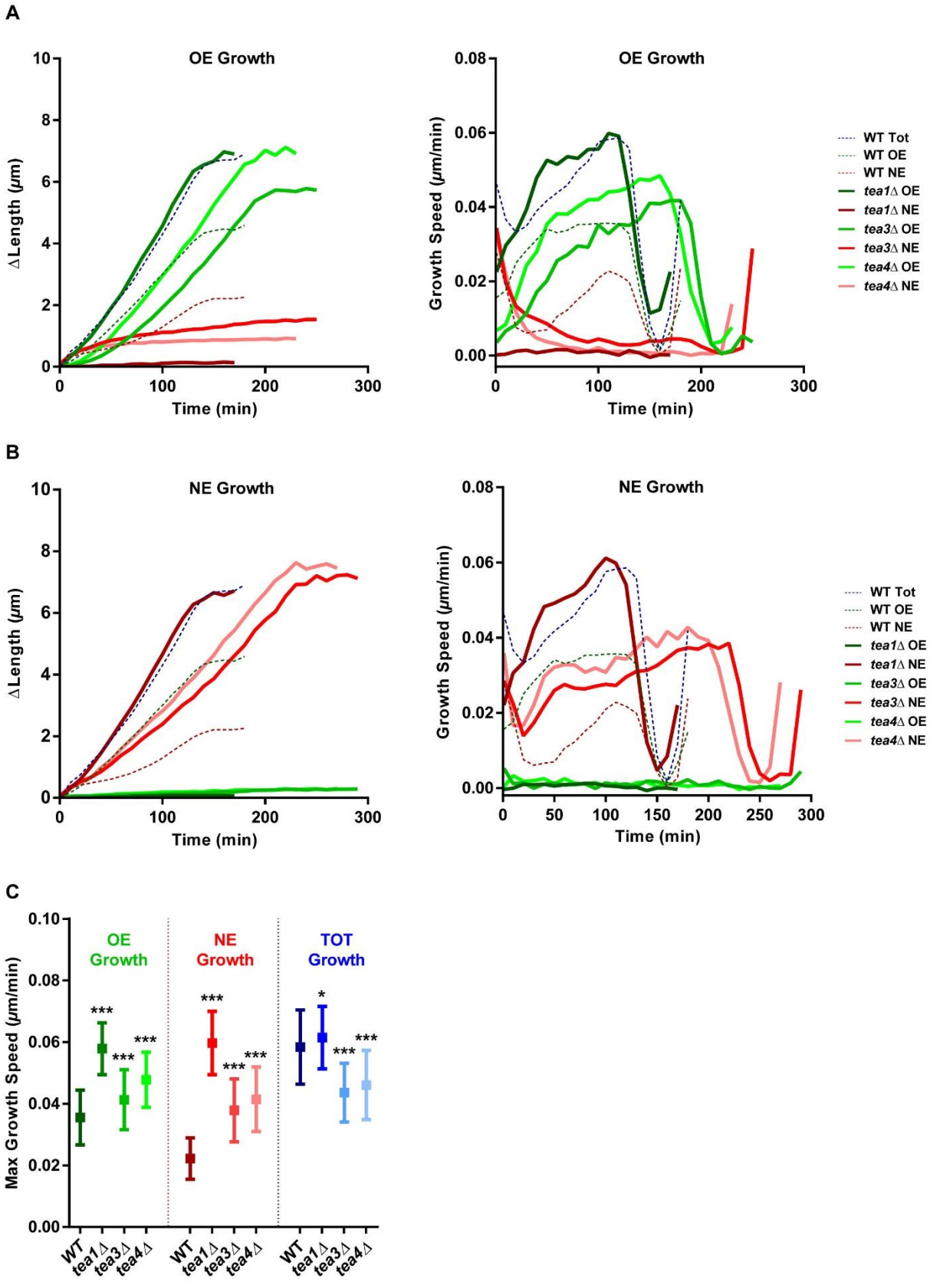

Figure S3. Tip growth speeds of monopolar tea mutants. (A) Comparison of Total, OE and NE lengths expansions and Growth Speed (GS) of WT (dotted lines, n=73 cells) and *tea1Δ* (n=16), *tea3Δ* (n=12) and *tea4Δ* (n=25) old end growing cells during the cell cycle. **(B)** Comparison of Total, OE and NE lengths expansions and Growth Speed (GS) of WT (dotted lines, n=73 cells) and *tea1Δ* (n=35), *tea3Δ* (n=10) and *tea4Δ* (n=14) new end growing cells during the cell cycle. (C) Mean maximum growth speed, computed as an average on 20min around the maximum of the speed profile, for the indicated mutant and tips. Results between genotypes for OE, NE and total growth were compared by using a two-tailed Mann–Whitney test. *, $P < 0.1$; **, $P < 0.01$; ***, $P < 0.001$. Error bars are standard deviations.

Table S1. Strains used in this study

IDENTIFIER	Mat Type	Genotype	Auxtotrophy	Source
NM291	h-	WT	ALU-	Lab stocks
NM439	h-	GFP-atb2:KanMX	LU-	Lab stocks
NM123	h-	CRIB-GFP:Ura	ALU	Lab stocks
NM256	h+	bgs4::ura4 GFP-bgs4-Leu	LUH-	Lab stocks
NM244	h+	rlc1-GFP:KanMX	ALU-	Lab stocks
NM04	h+	cdc25-22	ALU-	Lab stocks
NM299	h+	tea1::ura4	AL-	Lab stocks
DE040	h-	tea3::KanMX	ALU-	Lab stocks
DB162	h+	tea4::KanMX	ALU-	Lab stocks
NM341	h+	tea1-3GFP-KanMX	LU-	Lab stocks
DE039	h+	tea4-GFP:KanMX	ALU-	Lab stocks
DE003	h-	pom1-GFP-kanMX	AL-	Martin lab (University of Lausanne, Switzerland)
NM311	h-	for3-3GFP-ura4	ALU-	Lab stocks
NM335	h-	bud6-3GFP-KanMX	ALU-	Lab stocks
DB332	h-	Pact1-LAGFP:leu1+	UL	Balasubramaian lab (U. Warwick, UK)
AH282	h-	leu1::KanMX6-P3nmt1-pkc1(HR1-C2)-mECitrine	ALU-	Wu lab (Ohio State University, USA)
ST43	h-	rgf1-GFP:kanR	ALU-	Gould lab (Vanderbilt Univeristy, USA)
DE005	h-	sec8-GFP-ura4+	L-	Martin lab (University of Lausanne, Switzerland)
NM253	h-	bgs1::ura4 GFP-bgs1-Leu	LUH-	Lab stocks
ST15	h-	myo52-3GFP::KanMX	ALU-	Lab stocks
NM348	h-	tea1::NatMX bgs4::ura4 GFP-bgs4-Leu	AL-	Lab stocks
AH151	h-	tea1::NatMX CRIB-3GFP:ura		Lab stocks
ST31	h+	tea1::ura4 Pact1-LAGFP:leu1+	A-	Lab stocks
ST45	h+	tea1::ura4 rgf1-GFP:kanR	AL-	This study
MAD5933	*A. nidulans*	RabEp::GFP-RabE::pyrGAf, pyroA4, pyrG89, nkuAΔ::bar?		Peñalva lab (University of Madrid, Spain)