Using cytoplasmic distribution of phosphorylated tyrosine (pY) residues associated with TCR clusters, we next measured signaling activity specific to each TCR cluster within constrained synapse motifs (*17*) (see also fig. S3). At early time points, pY patterns were similar in both native and repatterned synapses (Fig. 3, A and B). However, at 5 min, TCR clusters in the natively pattered IS were observed only in the c-SMAC region and had very low pY levels (Fig. 3C). In contrast, TCR clusters that had been stably restrained to the periphery of the contact area by the substrate grids retained high specific pY levels (Fig. 3D). This effect was restricted to the periphery, because TCR clusters trapped in more central regions of spatially modified synapses lost their pY signal in a time frame similar to those observed in native synapses. The duration of TCR-pY signaling thus correlated with radial position of the TCR rather than with cluster size. Overall, the extent of specific pY associated with TCR clusters above the local background was significantly greater in the IS that had been spatially constrained by the grid (Fig. 3E).

Another key measure of signaling activity is the flux of intracellular Ca^{2+} induced by TCR antigen recognition, which integrates the outputs of all TCR signaling events in the IS (*18*). The integrated Ca^{2+} response was significantly higher in cells with spatially constrained IS as compared with those with native synapses (Fig. 3F). Thus, mechanical trapping of TCR in the IS periphery augments early TCR-associated pY levels, as well as the elevation of cytoplasmic Ca^{2+}.

These experiments provide insight into how signaling is extinguished in individual TCR clusters in the IS, which may be attributed to temporal or spatial processes such as recruitment of inhibitors (*19*) or changes in the actin cytoskeleton that feed back on signaling (*20*). The hybrid live cell–supported membrane platform made it possible to physically impede receptor translocation to prevent c-SMAC formation, allowing us to resolve that radial location represents a critical parameter in the IS. In physiological terms, it is possible that some APCs may use their own cytoskeletons to restrict transport of pMHC or costimulatory molecules in a related manner. Impeding TCR cluster translocation to the c-SMAC might thus represent a means of augmenting T cell activation (*21*, *22*). Potentially, the ability to induce spatial modifications in model cell-cell interfaces could be useful in exploring spatial organization of membrane domains and proteins on the cell surface, receptor signaling activity, and cytoskeletal regulation processes.

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Regulation of Yeast Replicative Life Span by TOR and Sch9 in Response to Nutrients

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Calorie restriction increases life span in many organisms, including the budding yeast *Saccharomyces cerevisiae*. From a large-scale analysis of 564 single-gene–deletion strains of yeast, we identified 10 gene deletions that increase replicative life span. Six of these correspond to genes encoding components of the nutrient-responsive TOR and Sch9 pathways. Calorie restriction of *tor1Δ* or *sch9Δ* cells failed to further increase life span and, like calorie restriction, deletion of either *SCH9* or *TOR1* increased life span independent of the Sir2 histone deacetylase. We propose that the TOR and Sch9 kinases define a primary conduit through which excess nutrient intake limits longevity in yeast.

Calorie restriction (CR) is the only intervention known to increase life span in yeast, worms, flies, and mammals, but the molecular mechanism for this phenomenon has not been clear. In yeast, CR due to reduced glucose concentration of the culture medium increases replicative life span (the number of daughter cells produced by a given mother cell before senescence) by 20 to 40% (*1*–*3*). This increased life span has been attributed to activation of Sir2 (*1*), a histone deacetylase that is dependent on NAD (the oxidized form of nicotinamide adenine dinucleotide) (*4*) and that promotes longevity by inhibiting the formation of extrachromosomal ribosomal DNA (rDNA) circles (ERCs) in the nucleolus (*5*). Recently, however, the link between Sir2 and CR has been called into question with the discovery that Sir2 is not required for life-span extension by CR (*3*).

To identify genes that regulate longevity in the budding yeast, a large-scale analysis of replicative life span was conducted with the *MATα* haploid open reading frame (ORF) deletion collection, a set of ~4800 single-gene–deletion strains (*6*). Because replicative life-span analysis requires labor-intensive micromanipulation of daughter cells from mother cells, fewer than 80 different genes have been previously examined for their effect on replicative life span (*7*). Here we examined the replicative aging properties of 564 single-gene–deletion strains (Fig. 1A; table S1).

An iterative method was designed to identify ~95% of strains with mean replicative life span at least 30% longer than wild type (*8*). For each single-gene–deletion strain, replicative life span was initially determined for five individual mother cells. If the mean life span was less than 26 generations, the strain was classified as not-long-lived (NLL). This lower cutoff value is predicted to result in misclassification of a long-lived strain less than 5% of the time (fig. S1). If the mean life span was less than 20, the strain was classified as short-lived (SL). If the mean life span was greater than 36, the strain was putatively classified as long-lived (LL),

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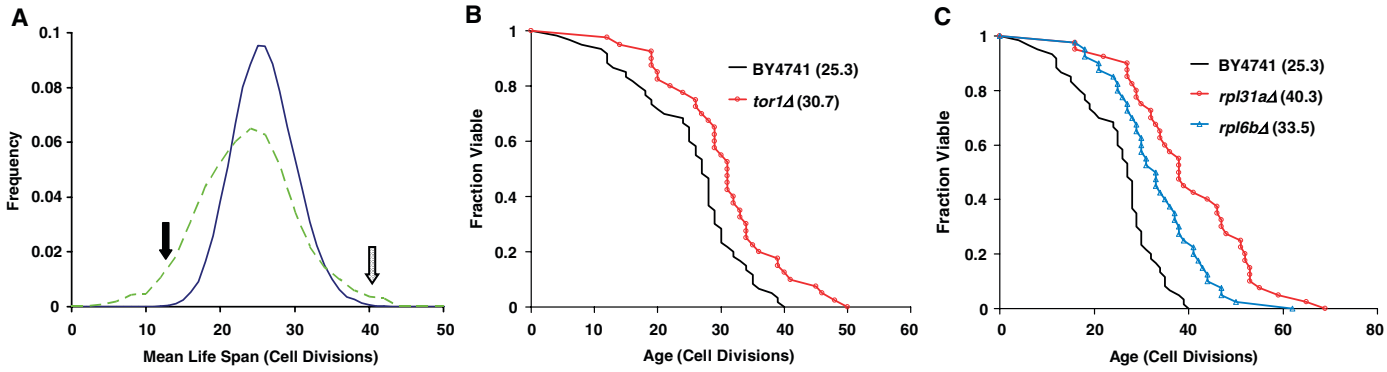

Fig. 1. TOR activity is an important modifier of yeast longevity. (**A**) The distribution of observed strain mean life spans for 564 single-gene–deletion mutants (broken line) shows an overrepresentation of short-lived (dark arrow) and long-lived (light arrow) mutants relative to expected mean life-span distribution (solid lines) for wild-type cells of the same sample size (n = 5). (**B**) Deletion of *TOR1* increases life span. (**C**) Deletion of either *RPL31A* or *RPL6B*, ribosomal proteins transcriptionally regulated by TOR, increases life span. Mean life spans are shown in parentheses.

and an additional 10 cells were examined. This upper cutoff value is predicted to result in misclassification of a strain with wild-type life span less than 2% of the time. For the remaining strains with a five-cell mean life span between 26 and 36 generations, an additional five cells were analyzed (one iteration), and the same classification scheme was applied. This process was repeated until every strain was either classified as SL, NLL, or LL or until replicative life span had been determined for a minimum of 15 cells for each unclassified strain. The replicative life-span data for strains from which at least 15 mother cells had been assayed were compared with cell life-span data from wild-type mothers, matched by experiment, by using a Wilcoxon rank-sum test to generate a P value. Strains with $P \leq 0.1$ were classified as LL, and strains with $P > 0.1$ were classified as having a life span not significantly extended (NSE). Of the 564 strains analyzed, 114 were classified as SL, 254 as NLL, 152 as NSE, and 44 as LL. Although nearly 20% of the gene deletions resulted in a significantly shortened life span, relatively few of these are likely to represent a true premature aging phenotype, because dysregulation of many different cellular processes will decrease fitness and longevity (*9*). For this reason, we focused on genes that, when deleted, resulted in increased replicative life span, reasoning that the proteins encoded by these genes must impede the normal aging process.

Of the 44 single-gene–deletion strains initially classified as LL, 13 result in a significant increase in replicative life span (Table 1). Verification was accomplished by determining the replicative life span for the corresponding gene deletion strain from the haploid *MAT***a** deletion collection and, in select cases, by generating a new deletion allele in the parental BY4742 strain. Of the 13 genes, *FOB1* served as a proof of principle that our method can identify a true-positive aging gene, because deletion of *FOB1* is known to increase life span by reducing the formation of ERCs (*10*). In two cases, gene deletions conferring increased life span occurred in overlapping ORFs encoded on opposite strands (*REI1* contains *YBR266C*; *IDH2* overlaps *YOR135C*), and longevity was comparable for overlapping deletion pairs (table S2). The identification of two different overlapping gene pairs from this screen suggests that a high fraction of true-positive genes were successfully identified.

The most striking feature of the 10 (excluding the overlapping dubious ORFs and *FOB1*) newly identified aging genes is that 6 are implicated in the TOR signaling pathway. TOR proteins are highly conserved from yeast to humans and regulate multiple cellular processes in response to nutrients, including cell size, autophagy, ribosome biogenesis and translation, carbohydrate and amino acid metabolism, stress response, and actin organization (*11*). Yeast has two TOR proteins, Tor1 and Tor2. Tor2 is essential and, therefore, not represented in the deletion collection. Deletion of *TOR1* was identified from this screen and found to increase both mean and maximum life span by ~20% (Fig. 1B). Two downstream targets of Tor1 and Tor2 were also identified: Ure2, which regulates activity of the nitrogen-responsive transcription factor Gln3, and Rom2, a proposed activator of protein kinase C (*12*, *13*). Deletion of three genes that are transcriptionally up-regulated by TOR increased life span: *YBR238C*, a gene of unknown function (*14*), and *RPL31A* and *RPL6B*, encoding two components of the large ribosomal subunit (Fig. 1C). Not all TOR-regulated ribosomal protein gene deletions examined conferred increased life span. Unlike the case in most multicellular eukaryotes, many of the ribosomal protein genes are duplicated in yeast (e.g., *RPL31A* and *RPL31B*), which allows for viable deletion of either paralog (but not both simultaneously). The relative importance of each paralog for ribosomal function, perhaps reflecting differential expression levels, may determine the longevity phenotype on deletion, with the gene coding for the more abundant member of the pair more likely to influence life span. Consistent with this idea, *rpl31aΔ* mother cells are long-lived and slow growing, whereas *rpl31bΔ* mother cells are not (fig. S2).

Table 1. Long-lived deletion strains. From a screen of 564 single-gene–deletion strains, 13 genes were found to increase replicative life span when deleted. GDP, guanosine diphosphate; GTP, guanosine 5′-triphosphate; PI3-like kinase, a kinase like phosphatidylinositol 3-kinase.

Deletion strain	Protein function
bre5Δ	Ubiquitin protease
fob1Δ	rDNA replication fork barrier protein
idh2Δ	Isocitrate dehydrogenase
rei1Δ	Protein of unknown function with similarity to human ZPR9
rom2Δ	GDP-GTP exchange factor for Rho1p
rpl31aΔ	Ribosomal protein L31
rpl6bΔ	Ribosomal protein L6
tor1Δ	PI3-like kinase involved in regulation of cell growth
ure2Δ	Regulator of nitrogen catabolite repression
ybr238cΔ	Protein of unknown function
ybr255wΔ	Protein of unknown function
ybr266cΔ	Hypothetical ORF overlapping *REI1*
yor135cΔ	Hypothetical ORF overlapping *IDH2*

Protein kinase A (PKA) and Sch9 are nutrient-responsive protein kinases that modulate replicative aging in yeast (*1*, *15*). Mutations that decrease PKA activity increase replicative life span and have been suggested as genetic models of CR (*1*, *3*). TOR is thought to act both upstream and parallel to PKA, whereas Sch9 is thought to act in a pathway parallel to PKA and TOR (*16*, *17*). TOR, PKA, and Sch9 regulate the expression of common downstream targets, including ribosomal proteins, such as Rpl31a and Rpl6b (*18*, *19*). CR of *tor1Δ* or *sch9Δ* cells failed to significantly increase the life span of these long-lived mutants (Fig. 2, A and B), which indicates that, similar to PKA, Sch9 and TOR are targets of CR in yeast. CR by growth

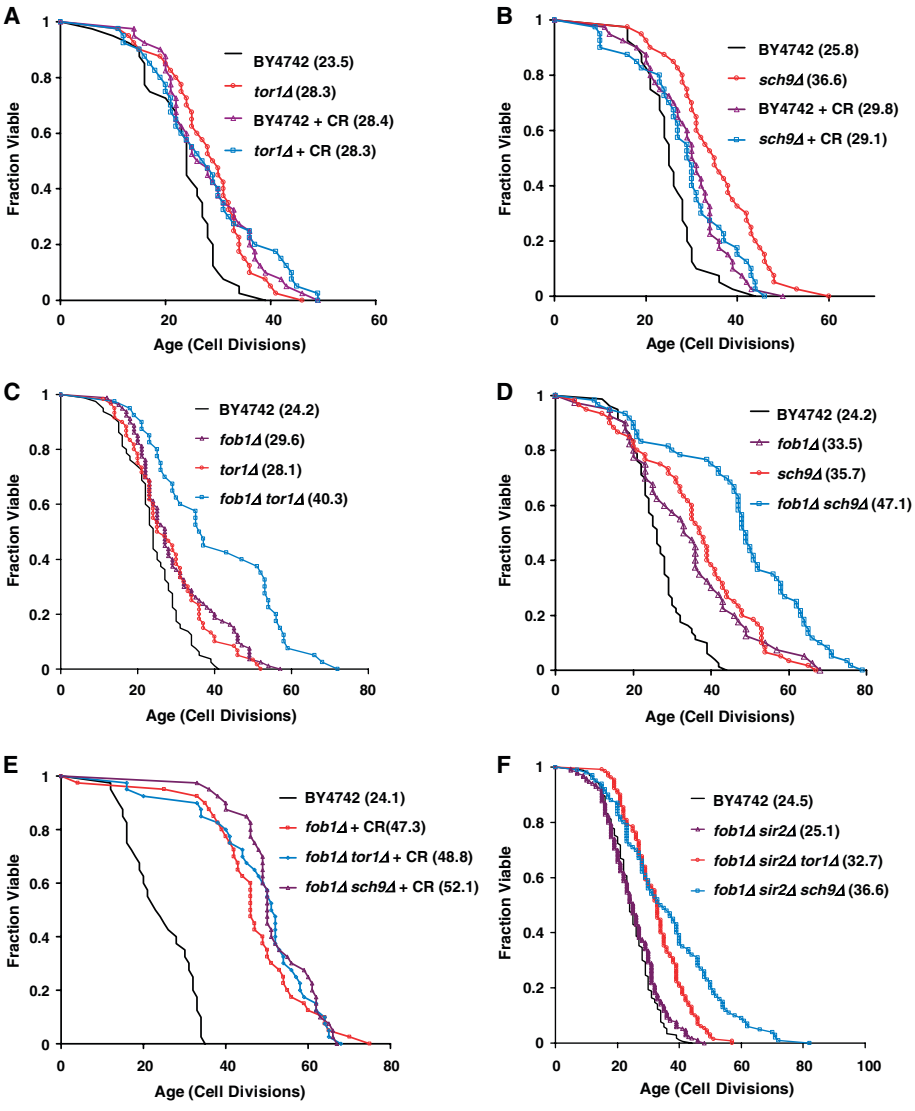

Fig. 2. *TOR1* or *SCH9* deletion mutants are genetic mimics of CR. (**A**) CR fails to further increase the life span of cells lacking *TOR1*. (**B**) CR fails to further increase the life span of cells lacking *SCH9*. (**C**) Deletion of *TOR1* increases life span additively with deletion of *FOB1*. (**D**) Deletion of *SCH9* increases life span additively with deletion of *FOB1*. (**E**) Deletion of either *TOR1* or *SCH9* fails to increase the life span of calorie-restricted *fob1Δ* cells. (**F**) Deletion of either *TOR1* or *SCH9* increases the life span of *sir2Δ fob1Δ* double-mutant cells. Mean life spans are shown in parentheses.

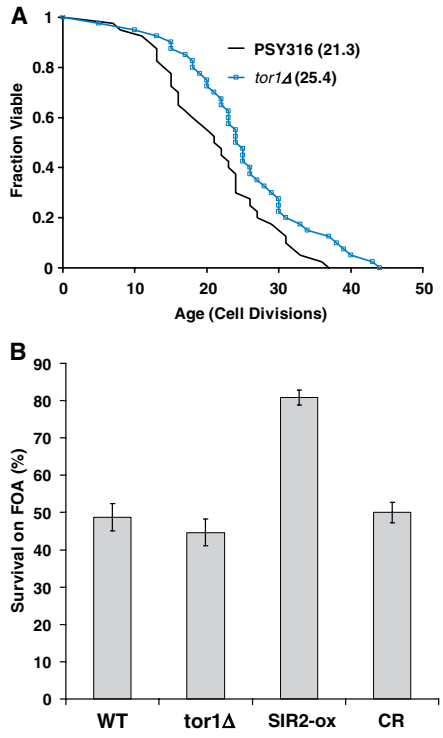

Fig. 3. Decreased TOR activity, like CR, is a strain-independent modifier of replicative life span. (**A**) Deletion of *TOR1* increases life span in the PSY316 background. Mean life spans are shown in parentheses. (**B**) Deletion of *TOR1* and CR have no effect on Sir2-dependent silencing of a telomeric *URA3* marker gene, as measured by survival in the presence of 5-FOA, in the PSY316 background. An extra copy of Sir2 (SIR2-ox) increases silencing of telomeric *URA3*. WT is wild-type.

on low glucose, or mutations resulting in decreased PKA activity, increase life span additively with deletion of *FOB1* (*3*). Deletion of *TOR1* or deletion of *SCH9* also resulted in an additive increase in life span when combined with deletion of *FOB1* (Fig. 2, C and D). The already long life span of the *sch9Δ fob1Δ* or *tor1Δ fob1Δ* mother cells was not further increased by CR (Fig. 2E). Life-span extension by CR also occurs independently of Sir2, as long as ERC formation is kept low through deletion of *FOB1* (*3*). Deletion of either *TOR1* or *SCH9* also increased the life span of *sir2Δ fob1Δ* cells (Fig. 2F). These epistasis experiments suggest that decreased activity of the nutrient-responsive kinases Sch9 and TOR in response to CR results in increased replicative life span in yeast.

Life-span extension by CR in yeast was initially characterized in the short-lived strain background PSY316 (*1*). PSY316 is unique among yeast strains used for longevity studies in that, although CR increases life span by 30 to 40%, deletion of *FOB1* or overexpression of *SIR2* fails to result in increased life span (*20*). To determine whether TOR activity is a general or strain-specific determinant of replicative life span, we examined the effect of *TOR1* deletion on life span and Sir2 activity in the PSY316 background. Deletion of *TOR1* significantly increased life span in PSY316, but had no effect on Sir2-dependent silencing at telomeres, similar to the effect of CR by growth on low glucose (Fig. 3, A and B). Thus, like CR, decreased TOR activity is a strain-independent mechanism to achieve enhanced longevity in yeast.

TOR activity is a primary determinant of replicative aging in yeast, and genetic analysis indicates that Sir2-independent life-span extension by CR is mediated by reduced signaling through TOR, Sch9, and PKA, resulting in down-regulation of ribosome biogenesis. Recently, an alternative model has suggested that Sir2-independent CR is caused by decreased ERC formation, resulting from nuclear relocalization and activation of the Sir2 homolog Hst2 (*21*). However, as long as ERC formation is maintained at a low level, CR increases life span to a greater extent in cells lacking Sir2 than in cells where Sir2 is present, seemingly inconsistent with Hst's simply playing a role redundant to Sir2's. CR increases life span additively with deletion of *FOB1*, which suggests a mechanism for CR that is independent of ERCs. ERCs also affect aging only in yeast, whereas the longevity-promoting role of CR has been evolutionarily conserved.

Decreased activity of TOR and Sch9 orthologs increases life span in *Caenorhabditis elegans* (*22*, *23*) and *Drosophila melanogaster* (*24*), as does mutation of the TOR-regulated S6 kinase (*24*), which promotes ribosomal protein maturation in multicellular eukaryotes. Therefore, the data presented here are consistent

with a model whereby CR increases life span through a highly conserved, Sir2-independent signaling network from nutrients to ribosomes.

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Golgi Duplication in *Trypanosoma brucei* Requires Centrin2

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Centrins are highly conserved components of the centrosome, which in the parasitic protozoan *T. brucei* comprises the basal body and nucleates the flagellum used for locomotion. Here, we found TbCentrin2 in an additional bi-lobed structure near to the Golgi apparatus. One lobe was associated with the old Golgi, and the other became associated with the newly forming Golgi as the cell grew. Depletion of TbCentrin1 inhibited duplication of the basal body, whereas depletion of TbCentrin2 also inhibited duplication of the Golgi. Thus, a Centrin2-containing structure distinct from the basal body appears to mark the site for new Golgi assembly.

Organelle duplication helps to ensure propagation through successive generations. For the Golgi apparatus, a number of models have been put forward, which differ as to the role played by the old Golgi in the construction of the new (*1*). What has not been addressed is the mechanism that determines the location for assembly of the new Golgi. In the budding yeast, *Pichia pastoris*, this location appears to be random, based on the probability of components reaching a critical mass for assembly (*2*). In several parasitic protozoa and in other protists, however, this location seems to be defined (*1*, *3*). In *T. brucei*, for example, there is a single Golgi, and a new copy is assembled at a fixed distance away (*4*). This new assembly site is somehow related to the new basal body (*5*); inhibition of basal-body segregation inhibits that of the Golgi (*4*) and inhibits division of the replicated kinetoplast, which contains all of the mitochondrial DNA (*6*). Basal bodies in particular and centrosomes in general have been implicated in the biogenesis of a number of membrane-bound organelles, in a variety of organisms (*6*, *7*), prompting us to study further their role in Golgi duplication.

Centrins are Ca^{2+}-binding proteins that are highly conserved and essential components of all centrosomes (*8*). The monoclonal antibody, 20H5, raised against *Chlamydomonas reinhardtii* Centrin (*9*), labels centrosomes in a wide range of organisms. It can also stain the basal bodies in *T. brucei* at different stages of the cell cycle (Fig. 1). The basal bodies are closely associated with the kinetoplast and mediate the division of the replicated kinetoplast (*6*).

The 20H5 antibody stained an additional, bi-lobed structure (Fig. 1). Early in the cell cycle, the old Golgi was adjacent to one lobe (Fig. 1A), whereas the new Golgi was later seen to be adjacent to the other, more posterior lobe (Fig. 1B), suggesting that it might be marking the site for new assembly. As the new Golgi grew and increasingly separated from the old (Fig. 1C), the bi-lobed structure itself duplicated, so that one remained with the old Golgi and one with the new (Fig. 1, D and E). This occurred at about the same time as the division of the replicated kinetoplast (Fig. 1E).

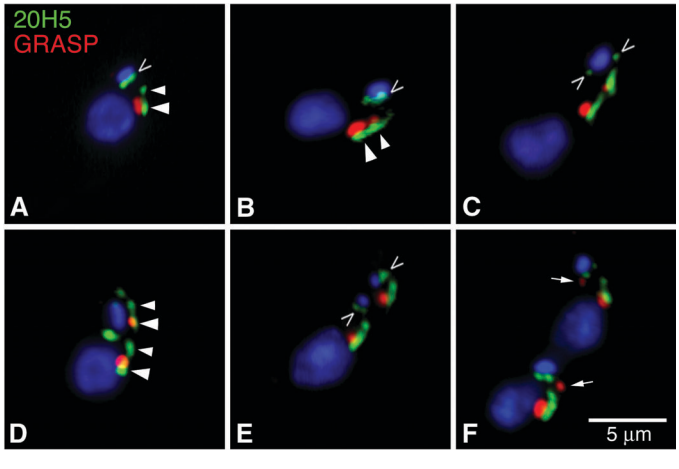

Fig. 1. A Centrin-containing structure associated with the Golgi. Gallery of images through the cell cycle of cells triple labeled for Golgi [anti–Golgi Reassembly Stacking Protein (GRASP), red], Centrin (20H5, green), and DNA [4′,6′-diamidino-2-phenylindole (DAPI), blue]. Basal bodies [open arrowheads (**A** and **B**)] underwent duplication (**C** and **D**) and mediated the division of the replicated kinetoplast (**E**). Centrin associated with the Golgi was present in a bi-lobed structure (solid arrowheads), with one lobe near to the old Golgi (A) and the other more toward the posterior of the parasite, marking the site where the new Golgi was undergoing assembly [compare (A) and (B)]. Increasing separation of the old and new Golgi (C) was accompanied by duplication of this bi-lobed structure [(C) to (E)] at about the same time as the replicated kinetoplast divided (E). (**F**) Just before cytokinesis, additional Golgi (arrows) appeared that were not associated with Centrin. Scale bar, 5 μm.

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