Taxanes are microtubule-targeting drugs that represent one of the most widely used classes of cancer chemotherapeutics, with demonstrated clinical efficacy across a wide-spectrum of tumor types. In patients with gastric cancer taxanes are used in both first- and second-line treatments. However, intrinsic drug resistance limits their efficacy, the molecular basis of which remains poorly elucidated. Here, we report the discovery of a novel truncated variant of the microtubule plus-end binding protein (+TIP) CLIP-170, hereafter CLIP-170S, which we found enriched in taxane resistant gastric cancer cell lines and in tumor biopsies from patients with gastric cancer refractory to cabazitaxel. Importantly, CLIP-170S knock-down entirely reversed taxane resistance *in vitro* and *in vivo*. We identified by mass spectrometry that CLIP-170S is missing the first N-terminal Cap-Gly motif. Surprisingly, this deletion enhances CLIP-170S' binding affinity for microtubules resulting in the formation of longer comets that extend beyond microtubule plus-ends. This aberrant binding of CLIP-170S to microtubules impairs taxane access to its high-affinity but kinetically unfavorable binding site in the MT lumen. Ectopic CLIP-170S expression rendered sensitive cells resistant to taxanes while its re-expression in CLIP-170-KD cells rescues the original drug-resistant phenotype. Taken together, these data identify CLIP-170S as a previously unrecognized short isoform of CLIP-170 with high affinity for microtubules, which in turn results in taxane resistance by impairing taxane access to its luminal binding site of microtubules.

P818

Rapid binding to protofilament edge sites controls tip tracking of EB1 at growing microtubule tips in cells

S. J. Gonzalez, K. T. Vu, R. Shoemaker, T. A. Reid, R. R. Goldblum, M. K. Gardner; University of Minnesota, Minneapolis, MN.

EB1 tracks growing microtubule plus-ends, thus targeting key cellular proteins to growing microtubule plus-ends that normally have little or no affinity for microtubules. However, the mechanism for how EB1 rapidly identifies and binds to growing microtubule plus-ends remains unknown. Live cell imaging in cells revealed that treatment with the cancer drug Eribulin, which binds to the edge sites of newly growing protofilaments, led to a dramatic decrease in EB1 tip tracking with increasing drug concentration. Thus, we created a stochastic model to ask whether protofilament edge binding could control EB1 tip tracking. In our model, we found that without rapid binding to newly formed protofilament edge sites, EB1 was unable to properly tip track growing microtubules. To test this prediction, we performed cell-free assays, and found that Eribulin prevented protofilament edge binding on stabilized, damaged microtubules, and also suppressed EB1 tip tracking on dynamic microtubules, consistent with simulation predictions. Thus, we conclude that rapid EB1 binding to GTP-tubulin at protofilament edge sites plays an important role in proper EB1 accumulation at the growing microtubule plus end.

P819

Characterization of the Tubulin-binding cofactor B in *Toxoplasma gondii*, a zoonotic parasite

I. Delgado[1,2], S. Francisco[1], D. Santos[1], S. Zúquete[1], A. Leitão[1], H. Soares[1,3], S. Nolasco[1,3]; [1]CIISA, FMV-UL, Lisboa, PORTUGAL, [2]FMV-ULHT, Lisboa, PORTUGAL, [3]ESTeSL-IPL, Lisboa, PORTUGAL.

Tubulin cofactors participate in the folding, dimerization, and dissociation pathways of the tubulin dimer, being implicated in the control of tubulin proteostasis and consequently in the control of microtubule (MT) dynamics *in vivo*. We identified a gene coding for a Tubulin cofactor B in the *T. gondii*

genome and characterized its protein (TgTBCB) in the tachyzoite stage. TgTBCB showed a polarized localization at the anterior region of tachyzoites, both intracellular and extracellular, being present under the conoid and in close association with the polar ring and the subpellicular MTs. It did not co-localize with the apical complex secretory vesicles, but the interaction with rhoptries and micronemes cannot be excluded. Tachyzoites overexpressing TgTBCB showed a significant decrease in the ability to form lysis plaques in host cell monolayers, attributable to a proportional reduction in the invasion efficiency. No differences were observed in replication and egress efficiencies. The *TgTbcb* knockout strains showed a complete depletion of the protein and viability no longer than one week in cell culture. These strains showed a strong reduction in their ability to invade the host cell and in their replication rate. In the absence of TBCB, cells presented an altered axis of division resulting in abnormal division. Some parasites showed the loss of the correct division axis and some parasites presented four daughter cells forming instead of two. To better understand TBCB's role in the regulation of the *T. gondii* invasion and replication, we used a promiscuous biotin ligase BirA to identify the TBCB interactome. For this we produced BirA and BirA-TBCB overexpression strains, to perform pull-down of biotinylated proteins coupled with identification through mass spectrometry (nanoLC-TripleTOF). This screening will contribute to the identification of the key regulators of parasite invasion and replication, establishing a platform to identify therapeutic molecular targets to control parasite infection.

P820

Mechanism of kinetochore fiber maturation by Augmin

A. C. Almeida[1], A. J. Pereira[1], J. S. Oliveira[1], D. Drpic[2], L. P. Cheeseman[1], J. Damas[3], H. A. Lewin[4], D. M. Larkin[5], P. Aguiar[6], H. Maiato[1]; [1]Chromosome Instability and Dynamics, i3S, University of Porto, Porto, PORTUGAL, [2]CeMM, Research Center for Molecular Medicine of the Austrian Academy of Science, Vienna, AUSTRIA, [3]The Genome Center, University of California, Davis, CA, [4]Department of Evolution and Ecology, College of Biological Sciences, The Genome Center, University of California, Davis, CA, [5]Department of Comparative Biomedical Sciences, Royal Veterinary College, University of London, London, UNITED KINGDOM, [6]Instituto Nacional de Engenharia Biomédica (INEB), i3S, University of Porto, Porto, PORTUGAL.

Accurate chromosome segregation during mitosis is required to prevent chromosomal instability, a hallmark of human cancers implicated in tumor evolution, metastasis and drug resistance. In mammals, chromosome segregation relies on the formation and maturation of a thick bundle of microtubules that attach at the kinetochore region of each chromosome to form kinetochore fibers (k-fibers). How k-fibers mature from initial kinetochore-microtubule attachments remains a key fundamental question. Here we used the low chromosome number (N=3) and distinctively large kinetochores (up to 2 µm in length) of Indian muntjac cells, to directly investigate the molecular mechanism underlying k-fiber maturation. By combining functional analyses of 65 conserved mitotic proteins, with fixed- and live-cell super-resolution CH-STED nanoscopy, we identified Augmin as the main driver of k-fiber maturation. Augmin is an octameric Y-shaped complex that recruits γ-tubulin to pre-existing microtubules, triggering microtubule nucleation and contributing to rapid microtubule amplification in the spindle. Surprisingly, we found that Augmin promoted kinetochore microtubule turnover by sustaining centrosome-independent microtubule growth from kinetochores and poleward flux. Tracking of microtubule growth events within k-fibers revealed an angular dispersion of ~40°, consistent with Augmin-mediated branched microtubule nucleation. Indeed, Augmin depletion reduced the frequency of microtubule growth events within k-