During transmission from the vertebrate host to mosquito vector malaria parasites must adapt to a rapid change in environment when encountering the hostile mosquito gut. To establish a successful mosquito infection, the parasite develops into a specialised motile ookinete form capable of invading the mosquito midgut. Ookinete formation is a strictly regulated developmental process orchestrated by dynamic signalling networks. In eukaryotes, signalling networks are often regulated by post-translational modifications (PTMs) that can significantly diversify protein function. Ubiquitylation is a reversible PTM utilised by eukaryotes to regulate protein activity, localisation and stability. While ubiquitylation has been explored in Plasmodium erythrocytic stages, the role of this crucial PTM in malaria transmission is poorly understood. Leveraging Plasmodium berghei to investigate early host-vector transition stages of the parasite, we identified ~1400 ubiquitin associated proteins, and >600 ubiquitylation sites in 240 unique proteins. We discovered several E3 ligases (writers of ubiquitin) in the ubiquitylome interaction networks indicating a vital role during parasite transmission. A systematic genetic and molecular analysis revealed five E3 ligases are essential for Plasmodium transmission. In particular two E3 ligases (RING-E3, U-Box-E3) regulated ookinete development where removal of the E3 ligases revealed crucial roles in maintaining cell morphology and motility. The E3-deficient parasites exhibit a severely reduced or complete inability to establish a mosquito infection. Currently we are investigating the spatial and temporal expression of the E3 enzymes and how they regulate the ookinete's cytoskeleton. Our results demonstrate prevalent ubiquitylation in the parasite proteome during transmission, uncovering essential roles for E3 ligases that could inform new transmission blocking strategies.