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Supplementary information

Anti-PfGARP activates programmed cell death of parasites and reduces severe malaria

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Supplementary Text

Protein processing of PfGARP

P. falciparum exports proteins to the RBC cytoplasm and membrane using the *Plasmodium* export element (PEXEL) motif followed by its cleavage in the endoplasmic reticulum by plasmepsin V, as well as by other, non-PEXEL mediated mechanisms [1]. PfGARP encodes an amino terminal signal sequence/transmembrane region (aa 1-22) and an appropriately located PEXEL element (aa 48-52). To determine whether parasites process and cleave the PEXEL element, we probed parasite extracts using peptide-specific antisera generated against peptides that flank the PEXEL element (aa 31-48 and aa 504-522). As expected, both peptide-specific antibodies recognized full length (aa 1-673) recombinant PfGARP while only the antibodies raised against aa 504-522 recognized rPfGARP-A (aa 410-673). Only the antibodies raised against aa 504-522 recognized native PfGARP in trophozoite-infected RBCs confirming that the PEXEL element (and therefore the signal sequence/transmembrane domain) in PfGARP is processed and cleaved from the mature protein (**Extended Data Fig. 10**). Because mature PfGARP lacks a transmembrane domain and consensus sequences for glycophosphatidylinositol or palmitic acid anchors, the mechanism of attachment of PfGARP to the exofacial surface of the RBC membrane remains unknown but may include interactions with RBC or trophozoite-infected RBC surface proteins. Supporting this notion, recent evidence indicates that a highly charged 28 aa peptide from PfGARP (aa 417-444) containing 5 lysine-rich repeats is able to bind to Band 3 [2].

1 Boddey, J. A. *et al.* Export of malaria proteins requires co-translational processing of the PEXEL motif independent of phosphatidylinositol-3-phosphate binding. *Nature communications* **7**, 10470, doi:10.1038/ncomms10470 (2016).

2 Almukadi, H. *et al.* Human erythrocyte band 3 is a host receptor for Plasmodium falciparum glutamic acid-rich protein. *Blood* **133**, 470-480, doi:10.1182/blood-2018-07-865451 (2019).