



**Figure S3 *Plasmodium berghei* CSP in mosquitoes. A: CSP associates with lipophorin particles in mosquito hemolymph.**

Twenty infected females were anaesthetized and bled in 350  $\mu$ l buffer. The extract was centrifuged and subjected to lipophorin immunoprecipitation (see Methods). Western blot with anti-lipophorin antibody (top panel) or CSP antibody (bottom panel), note that the secondary antibody reveals the mouse primary antibody used in the IP (IgG heavy chain) in addition to the CSP signal. Some released CSP associates with Lipophorin particles, while a large fraction remains in lipophorin-depleted supernatant. **B: CSP is released both without and with a GPI anchor and GPI-linked released CSP associates with lipophorin.**

Triton X-114 protein fractionation (see Methods) of raw mosquito hemolymph extracts, immunoprecipitated lipophorin captured on sepharose beads and post-immunoprecipitation supernatant. CSP present in hemolymph extracts partitions in both aqueous (no GPI anchor) and detergent (GPI anchor) phases. CSP present on immunoprecipitated lipophorin exclusively partitions in the detergent phase (GPI anchored). Most lipophorin protein bound to antibodies and sepharose beads remained attached to the beads throughout the detergent fractionation process. No GPI-linked CSP remains in the lipophorin-depleted post-IP supernatant. **C: Codon-optimized CSP is expressed in transgenic mosquitoes.** Western blot was performed on mosquito hemolymph and whole body (carcass) extracts prepared at the indicated times after blood feeding with antibodies recognizing CSP, Vitellogenin (to monitor *Vg* promoter activity) and PPO2 (used as a loading control). Note that although both CSP $\Delta$ GPI (left panel) and full-length CSP (right panel) expressions are highly induced by the blood meal (in addition to constitutive expression under the control of the *Lp* promoter, right panel) in tissues (carcasses), only CSP $\Delta$ GPI is released to high levels in hemolymph. Also note that CSP appears to be cleaved inefficiently in transgenic mosquitoes compared to the *P. berghei* control, suggesting that mosquitoes lack the protease that usually cleaves CSP. Antibodies to Lipophorin, Vitellogenin and PPO2 are described in Rono Rono *et al.* (2010) and Fraiture *et al.* (2009).