

S5 Fig. Evidence for glycosylation of CSP in *P. vivax* VK210 salivary gland sporozoites. (A) Representative extracted ion chromatograms (XIC) of the doubly-charged ions of the CSP peptide ATVGTEWTPCSVTCGVGVR from *P. vivax* VK210 salivary gland sporozoites. The largest chromatographic peak (green) was produced by a species with a mass matching the peptide plus a deoxyhexose, which we presume to be O-fucose. An unidentified peptide of similar mass (purple) created a shoulder peak. Though the MS2 spectrum for this species was not confidently mapped to a peptide sequence, it was distinct from the identifying spectra of the CSP peptide (not shown; scan number 23860). The unmodified peptide (orange) was observed co-eluting with the putatively-fucosylated species, consistent with loss of the gas-phase-labile modification due to collision-induced dissociation (CID) within the mass analyzer source, as well as eluting as a distinct peak at 80.7 min. These data suggest that the majority of CSP in the sample was modified with a single O-fucose, but that some unmodified CSP was also present. Representative collision-induced dissociation (CID) fragmentation spectra of the unmodified (B) and glycosylated (C) peptides confirmed the peptide sequence. CID of the peptide lacking O-fucose (B) provided confirmation of the assignments of the fragment spectra obtained from the O-fucosylated species (C). Fragment ions are annotated as b-ions (blue) and y-ions (red). The unfragmented peptide is designated M (yellow) with addition of protons (H) and neutral loss of deoxyhexose (dHex). Fucose is a deoxyhexose. Fragmentation spectra of the O-fucose glycan intact, so the location of the glycan could not be confirmed, but based on the presence of the O-fucosylation motif, we presume that the Thr of CSVTCG was modified.