Expanded View Figures

A

5'-WSWSWSWSWSWSWSWSWSWSWSWSWSWSWS -3'

B

Figure EV1. Schematic representing the cellular barcoding strategy.

A   Briefly, a double-stranded oligonucleotide is synthesized bearing a semi-random sequence formed by the alternation of weak (W = A or T) and strong bases (S = G or C) flanked by two fixed sequences that serve as primer binding sites for PCR amplification. This pool of DNA molecules is cloned in a vector that has homology sequences which promote the integration of the vector into the 18s rRNA locus in *Leishmania* genome.

B   After transfection, barcoded parasites are selected with hygromycin, as the barcoding vector also has a hygromycin-resistance gene. The eGFP gene allow to monitor by flow cytometry the potential presence of remaining non-barcoded cells after hygromycin selection.

Figure EV2. Additional genomic changes associated with the Sb^{III}-Flash selection performed on the barcoded BPK282 population.

A   Heatmap depicting the allele frequencies of SNPs and indels identified in protein coding regions compared to the reference genome. An additional annotation bar display to which group (control or Sb^III^-exposed) a sample belongs. Samples are named as Px_cPOPy for controls, and Px_SePOPy for the Sb^III^-exposed groups, with x being the number of passages and y being the replicate number. The initial population is named as 'Before Sb^III^'.

B   Copy number variation in the MRPA locus. The Y axis represents the median read count of 5 kb bins normalized by the median count of chromosome 23 and reflects the average copy number per haploid genome of the MRPA locus.
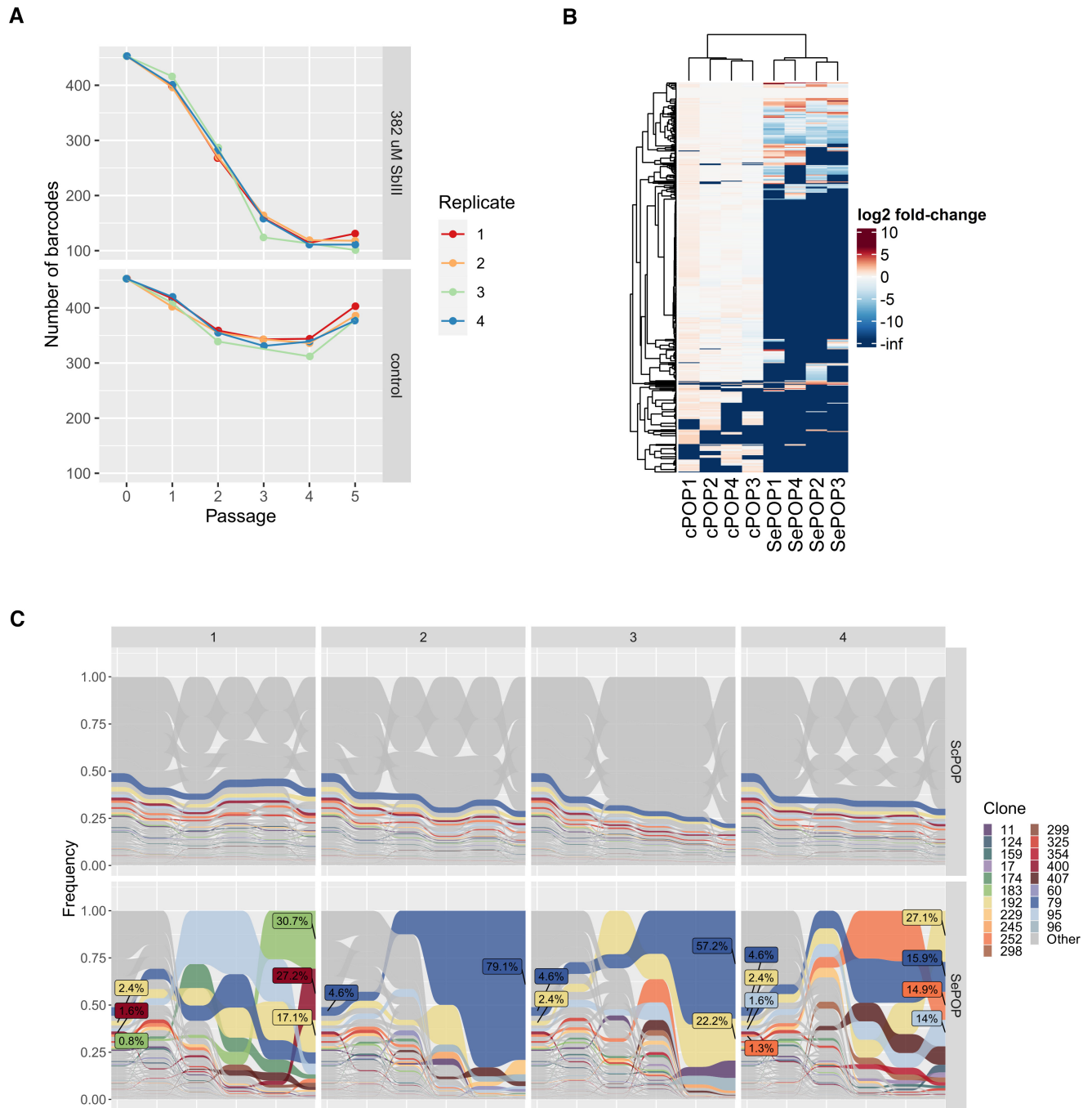
A

B

C

Figure EV3. Supporting figures for main Fig 2.

A   Total number of different barcodes identified in each population at each timepoint in the Sb[III]-exposed populations (top) and the controls (bottom).

B   Heatmap displaying the Sb[III]-associated fold-change of each lineage (rows) in each population (columns) at passage 5.

C   Frequency of each barcoded lineage along the 5 passages in the ScPOP1-4 (top) and SePOP1-4 (bottom) populations. This is similar to main Fig 2F but includes the ScPOP1-4 for comparison. Only lineages that reached a frequency higher than 1% at passage 5 in at least one of the SePOP populations are colored.
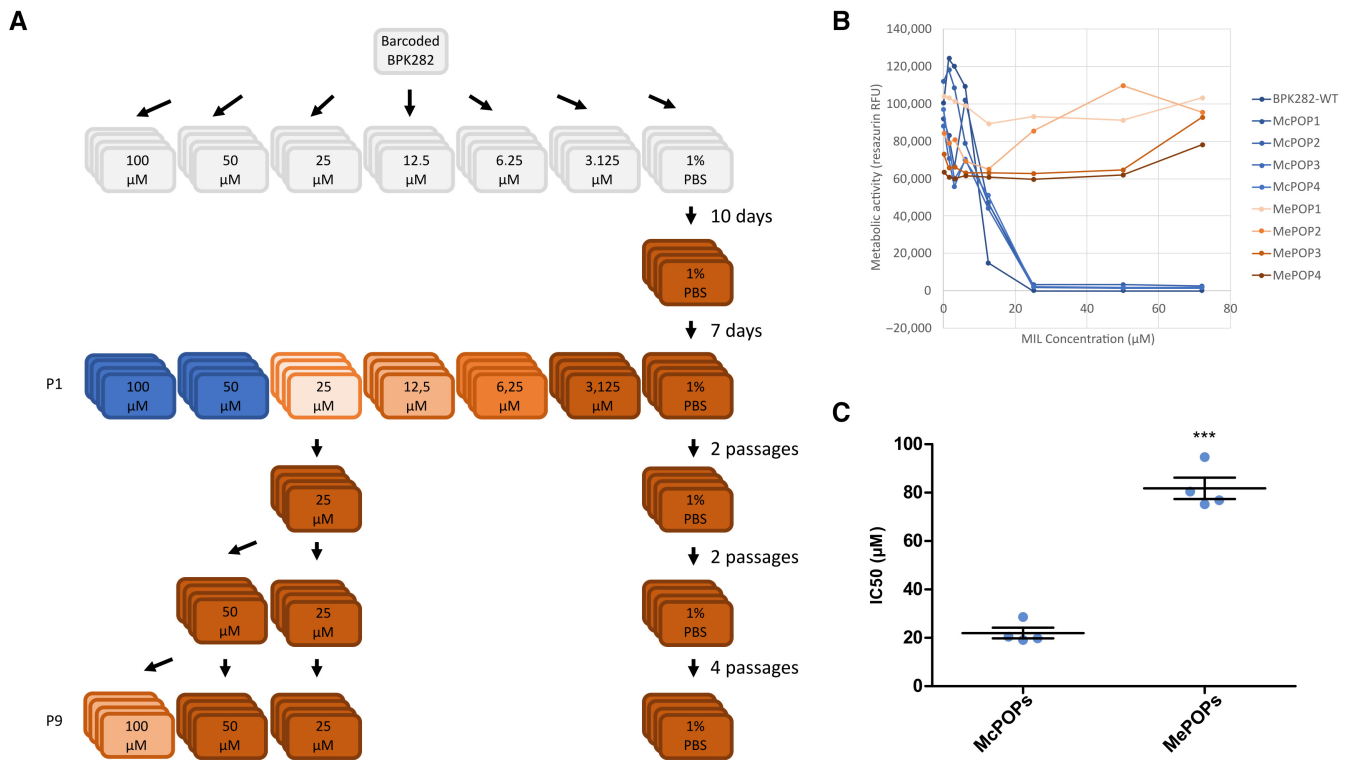
Figure EV4. Supporting figures for flash selection with miltefosine.

A Schematic representing the experimental design. Colors indicate the lack of viable parasites (blue) or the relative observed number of parasites at day 7 compared to the controls (darker brown = more cells).

B Dose–response to miltefosine of the metabolic activity of the populations at the first passage after exposure to the drug, estimated with the resazurin assay.

C Difference in IC50 of the same populations one passage later. ***$P < 0.001$ (*t*-test).