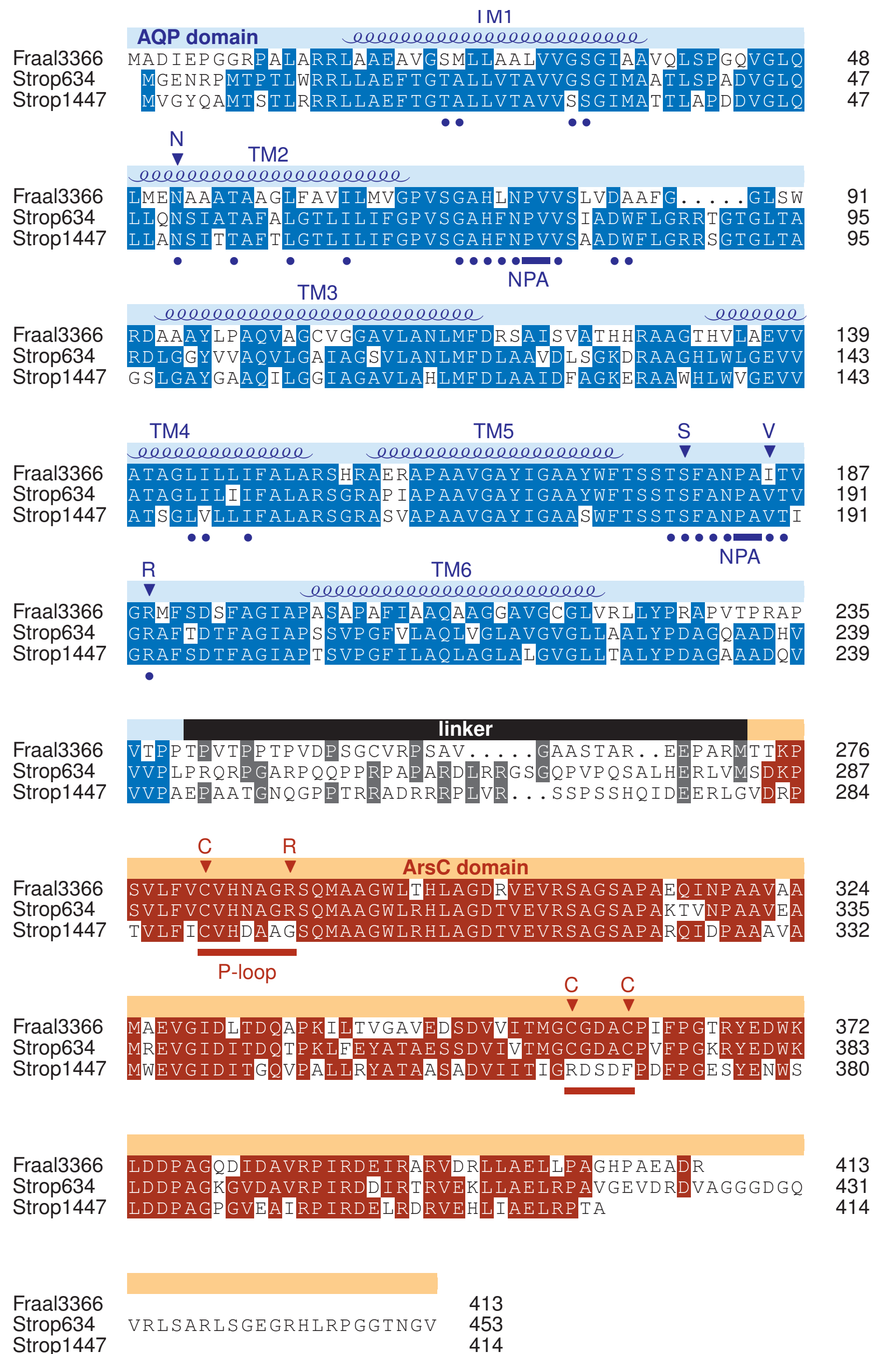

Fig. S1. Alignment of aquaglyceroporin-ArsC fusion proteins from *F. alni* (Fraal3366) and *S. tropica* (Strop634, Strop1447). Labelled are the putative transmembrane domains (TM1-6), the NPA-motifs, the channel-lining residues (blue dots), the residues of the aromatic/arginine selectivity filter (blue triangles), the linker region, and the catalytic domains (red bars) as well as residues in the ArsC domain (red triangles).

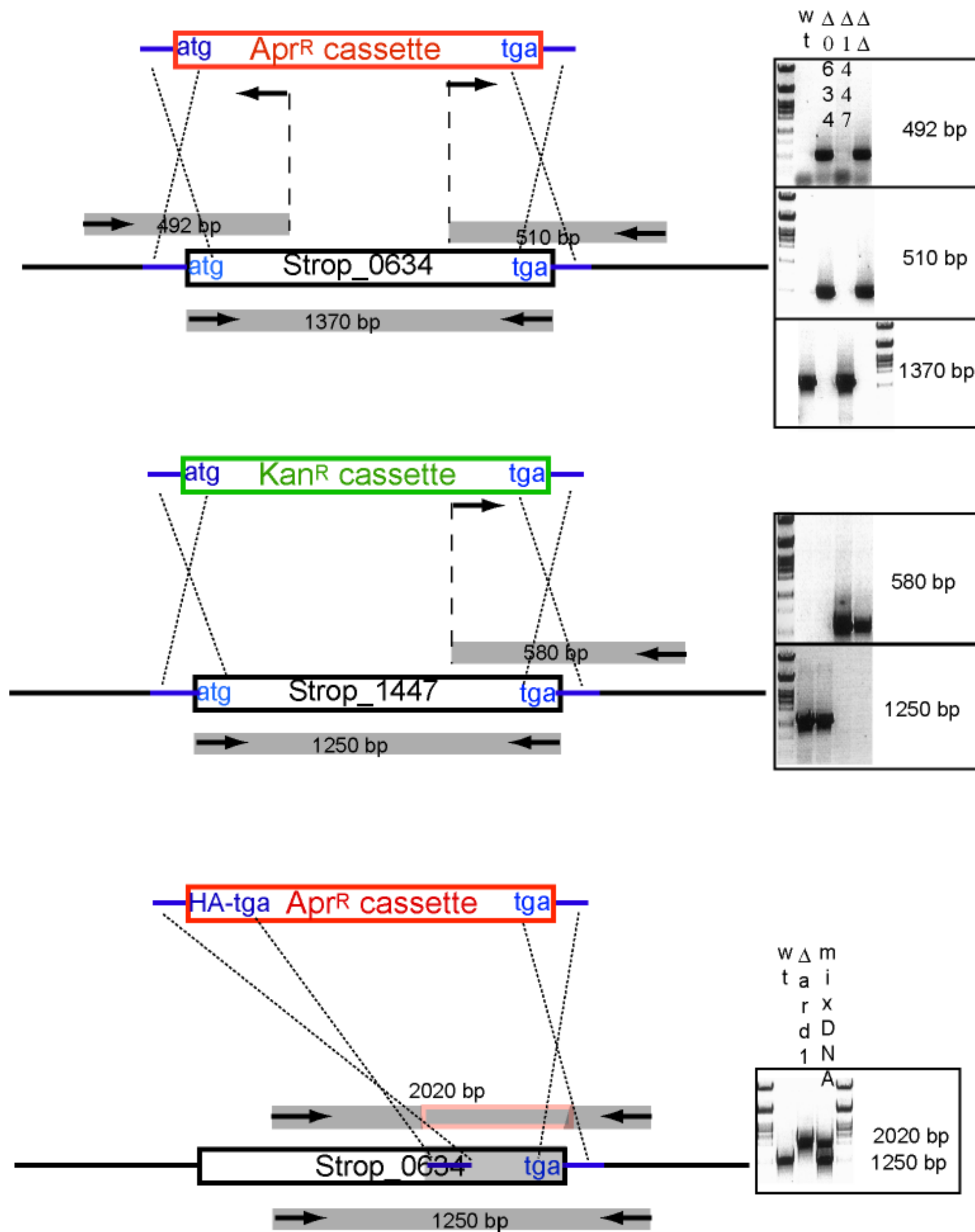

Fig. S2. Confirmation by PCR of correct gene targeting in *S. tropica* deletion strains, see Materials and Methods for details. Δ0634 denotes the *strop634* deletion strain, Δ1447 the *strop1447* deletion, ΔΔ the double deletion, and Δard1 the partial deletion of strop634 leaving the channel domain intact and removing the ArsC domain alone.

Yeast strains

Strain	Genotype	Source
BY4742	MATα his3-1, leu2Δ0, lys2Δ0, ura3Δ0	EUROSCARF
Δfps1	BY4742, yll043w::KanMX	EUROSCARF
Δacr2	BY4742, ypr200c::kanMX4	EUROSCARF
Δacr2,3	BY4742, acr2,3::LEU2	This study
Δfps1Δacr2,3	BY4742, yll043w::KanMX, acr2,3::LEU2	This study
Δfps1Δacr2,3 Δycf1	BY4742, yll043w::KanMX, acr2,3::LEU2, ycf1::HIS3	This study

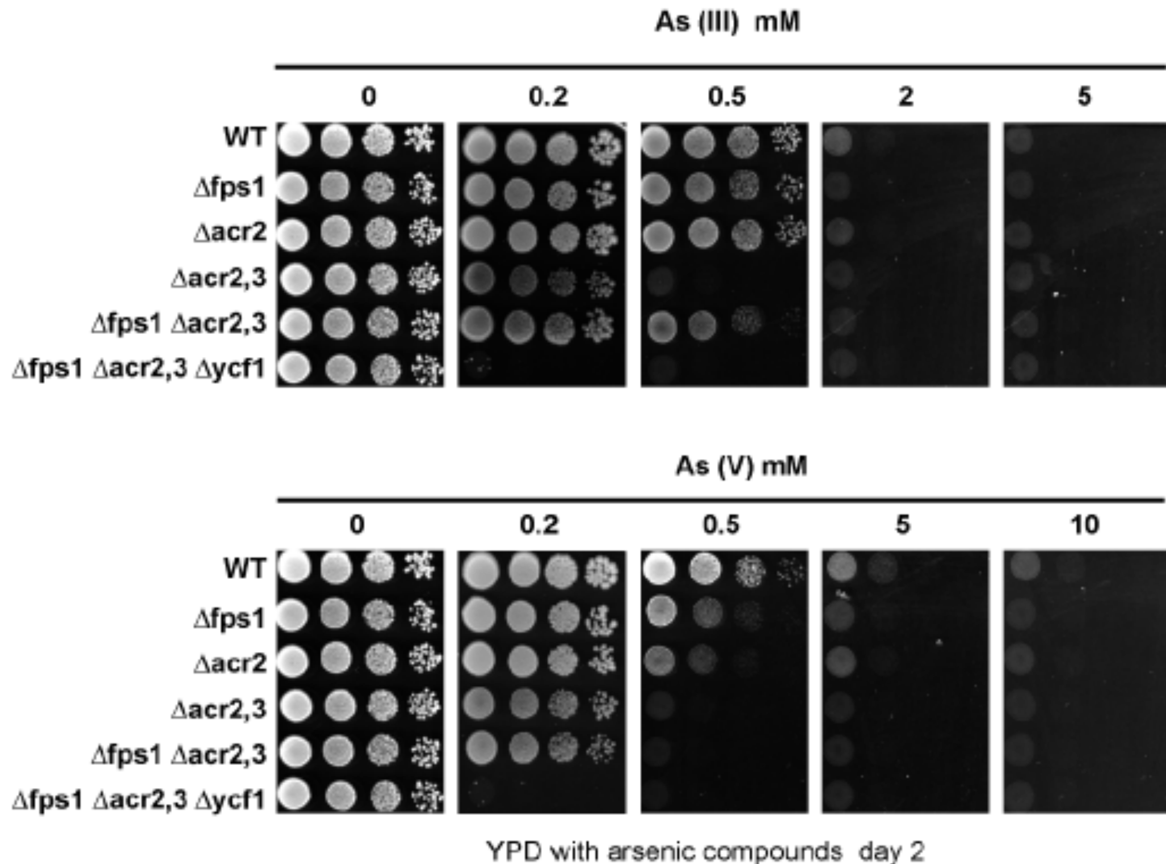

Fig. S3. Yeast strains used in this study and their sensitivity for arsenite and arsenate monitored after 3 days, see Materials and Methods for details. Note that apparent differences in the sensitivity levels compared to Fig. 2 in the main text are solely due to a shorter observation period of only 3 days vs. 4 days.

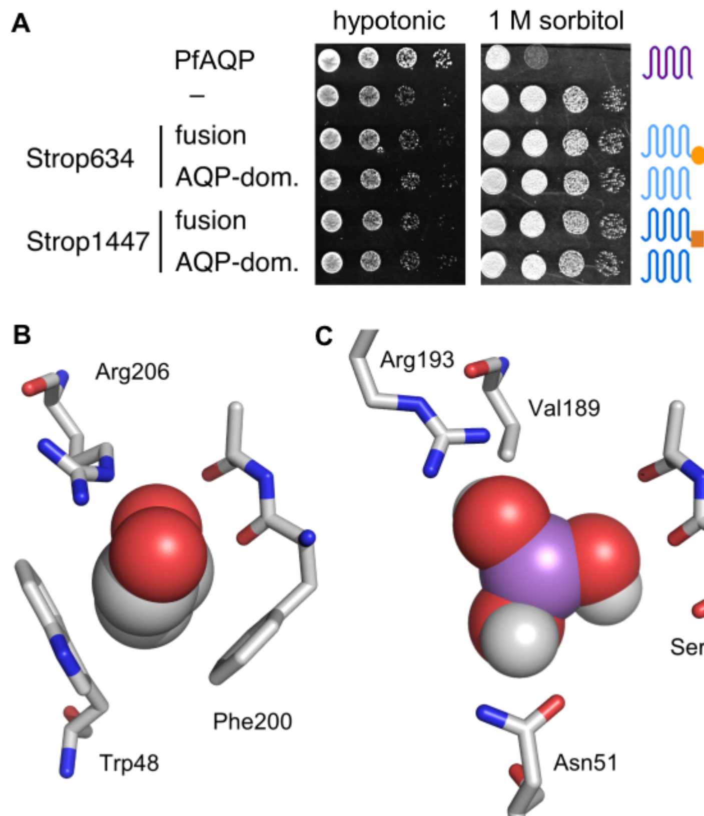

Fig. S4. Insufficient glycerol permeability of Strop634 and Strop1447 and structure model of the aromatic/arginine selectivity filter. **A.** Yeast cells lacking the endogenous aquaglyceroporin (Δ*fps1*) and expressing PfAQP, Strop634 or Strop1447 full-length proteins or the channel domains alone were grown overnight in selective liquid medium (SD –Ura) containing 1 M glycerol in order to increase the internal osmolarity. The cells were then spotted in serial dilutions on hypotonic agar medium (left panel) or on isotonic agar medium supplemented with 1 M non-permeating sorbitol (right panel) (for details see: Uzcategui NL, et al. (2004) Cloning, heterologous expression and characterization of three aquaglyceroporins from Trypanosoma brucei. *J Biol Chem* **279**: 42669-42676). Under hypotonic conditions (left panel), functional glycerol channels permit rapid adjustment of the internal osmolarity by release of glycerol sustaining cell growth (see control aquaglyceroporin PfAQP). Yet, Strop634 and Strop1447 did not increase growth over the non-expressing control cells. Under isotonic 1 M sorbitol conditions (right panel), functional aquaglyceroporins will release glycerol due to the outward chemical glycerol gradient, yet, sorbitol cannot permeate the cell membrane. The resulting hyperosmotic stress will drain water out of the cells and inhibit growth (see PfAQP control). Again, Strop634 and Strop1447 were non-functional. Both assays indicate absent or too low glycerol permeability of Strop634 and Strop1447 to complement the Fps1-deletion phenotype. **B.** Shown is the aromatic/arginine selectivity filter of the *E. coli* aquaglyceroporin (PDB # 1FX8) which is virtually identical to that of PfAQP (PDB # 3C02) and **C.** a model of the respective Strop634 region.

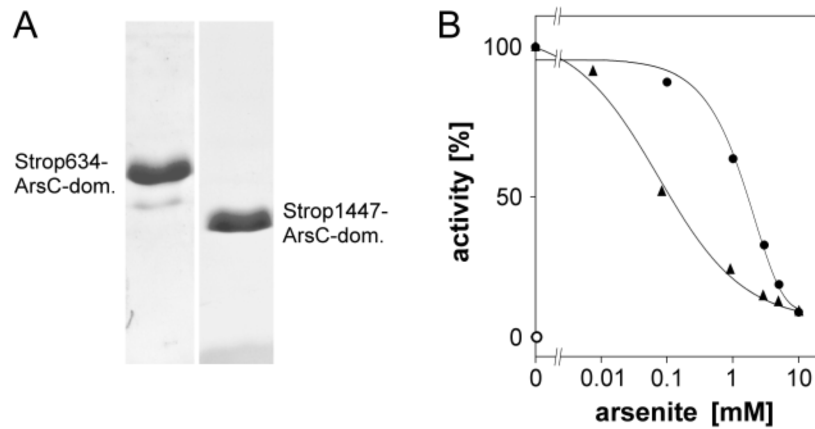

Fig. S5. Expression of the Strop634 and Strop1447 ArsC domains and sensitivity of thioredoxin reductase for arsenite. **A.** Coomassie stain of the affinity purified ArsC domains (19 and 15 kD, resp.). **B.** Inhibition of the Strop634/thioredoxin/thioredoxin reductase cascade by arsenite (filled circles). Thioredoxin reductase activity with 5,5'-Dithio-Bis (2-Nitrobenzoic Acid) as a substrate (filled triangles) was highly sensitive to arsenite identifying this enzyme rather than the Strop634 ArsC domain as the main target for arsenite inhibition.

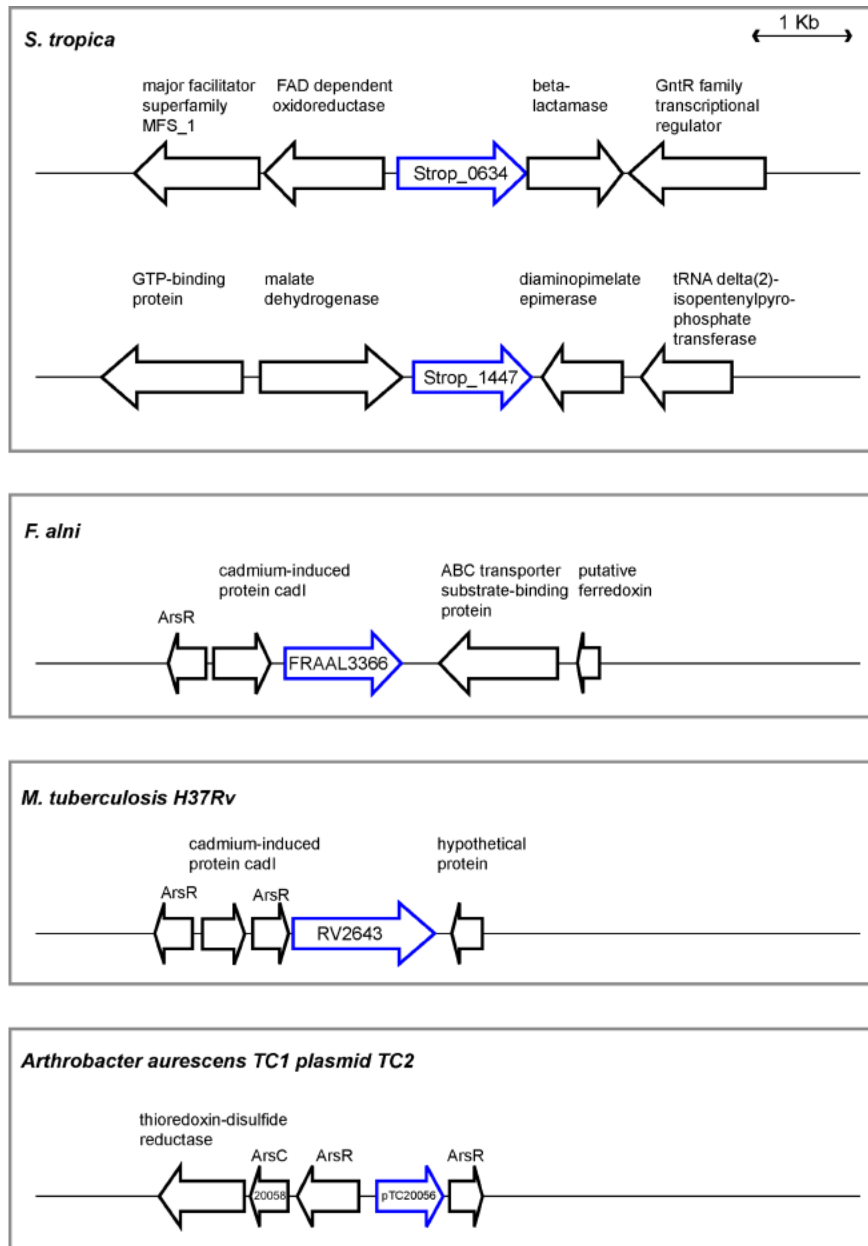

Fig. S6. Genetic environment of arsenite channel-ArsC fusion genes in *S. tropica*, *F. alni*, and *M. tuberculosis*. Pre-fusion homologues of the Strop634 channel and enzyme domain are clustered on an *A. aurescens* plasmid (pTC20058 and pTC20060). ArsR denotes a transcriptional regulator responding to arsenate exposure.