Supporting Information

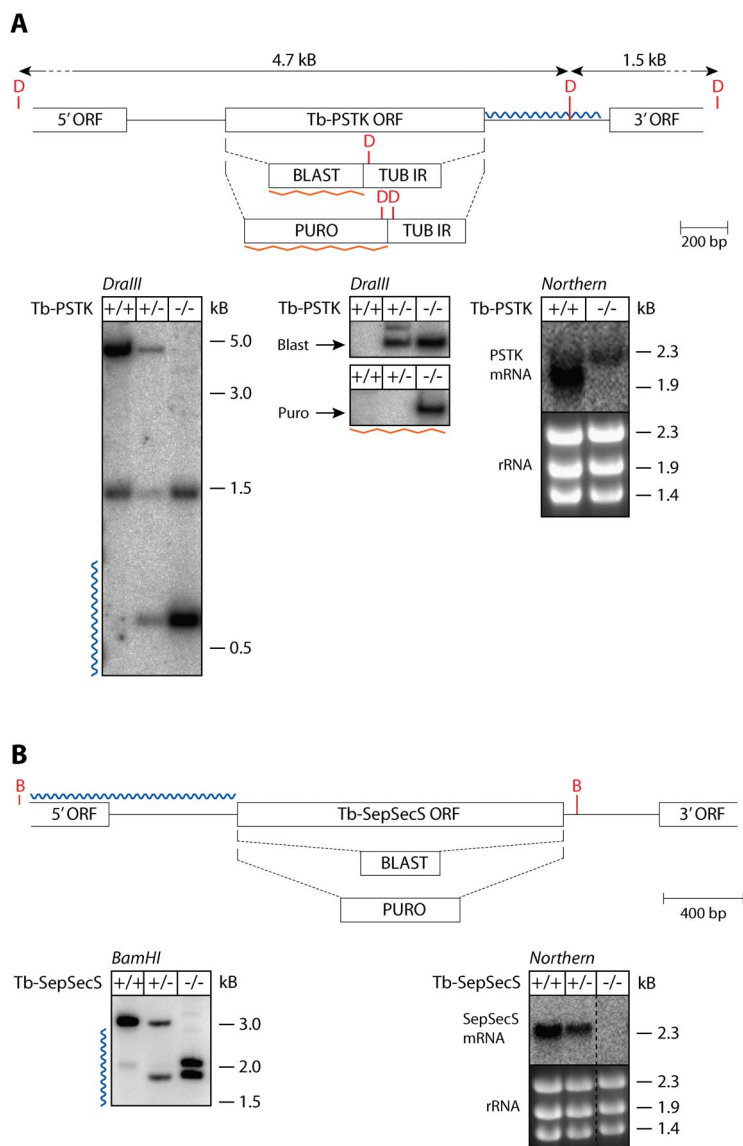## Aeby et al. 10.1073/pnas.0901575106

Fig. S1. Generation of Tb-PSTK and Tb-SepSecS KO cell lines. (*A*) (*Upper*) Schematic to scale drawing of the WT Tb-PSTK-encoding locus and the situation after homologous recombination leading to replacement of the two loci by blasticidine (BLAST) and puromycin (PURO) resistance genes, respectively. DraIII (D) restriction sites are indicated. The lengths of the 2 DraIII fragments of WT cells that hybridize with the 3′ flank of the PSTK locus (jagged line) are indicated. Tb-PSTK is expressed only at very low level. Thus, to express sufficient amounts of the BLAST and the PURO genes the 3′-flanking region (TUB IR) of the highly expressed α-tubulin gene was inserted between the stop codons of the resistance marker genes and their endogenous 3′-flanking regions. (*Lower*) (*Left* and *Center*) DraIII-digested genomic DNA of the parent cell line (+/+) and the resulting cell lines after replacement of the first (+/−) and the second (−/−) PSTK allele were hybridized with a probe corresponding to either the 3′-flank of the PSTK locus (jagged line) or with probes recognizing the BLAST and PURO genes (wiggly lines), respectively. (*Right*) Total RNA from the parent and the Tb-PSTK KO cell lines was analyzed by Northern blots using the Tb-PSTK ORF as a probe. The position of the Tb-PSTK mRNA is indicated. An unspecific cross-reacting band comigrating with cytosolic rRNA is present in both cell lines. The ethidium bromide stain shows the rRNA region that serves as a loading control and as size markers. (*B*) (*Upper*) Schematic to scale drawing of the WT Tb-SepSecS-encoding locus and the situation after homologous recombination leading to replacement of the two loci by blasticidine (BLAST) and puromycin (PURO) resistance genes, respectively. BamHI (B) restriction sites are indicated. (*Lower*) (*Left*) Southern blot of BamHI-digested genomic DNA of the indicated cell lines using a probe corresponding to the 5′-flank of the Tb-SepSecS ORF gene. (*Right*) Northern blot of total RNA from the indicated cell lines probed for the Tb-SepSecS ORF.