**SUPPLEMENTAL DATA**

Figure S1

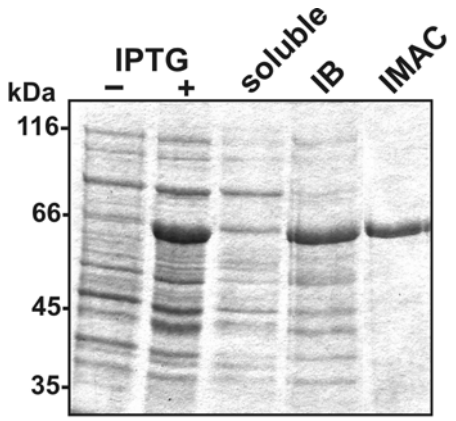


**Figure S1. Expression and isolation of active recombinant tpSTK1.** Coomassie-stained SDS-PAG. Identical amounts of cells from an *E. coli* clone harboring plasmid pPROEX-Htb encoding recombinant tpSTK1 were loaded before (-) and after 3 hours of induction with IPTG (+). After cell lysis equal aliquots of the 23,000 xg supernatant (soluble) and pellet (inclusion bodies = IB) were loaded. After solubilization of the inclusion bodies with urea, adsorption of the solubilize material to Ni-NTA agarose, and refolding on the column, the majority of recombinant tpSTK1 eluted with 200 mM imidazole (immobilized metal affinity chromatography = IMAC).

Figure S2

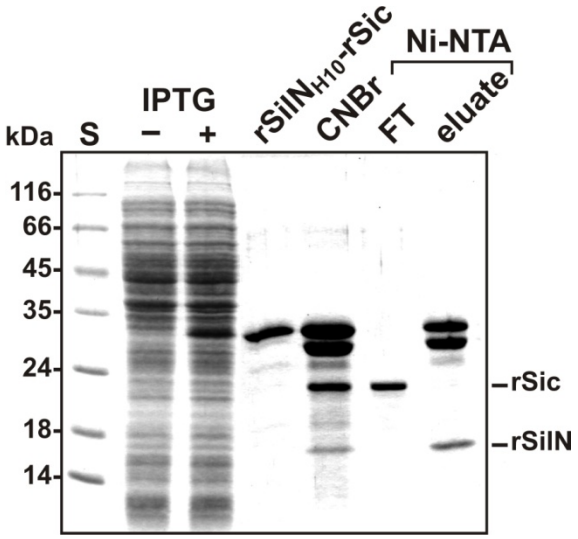


**Figure S2. Expression of the rSilN$_{H10}$-rSic fusion protein and isolation of rSic.** Coomassie-stained SDS-PAG. **S**: molecular mass standard. **IPTG:** Identical amounts of cells from an *E. coli* clone harboring plasmid pET28a/rSilN$_{H10}$-rSic were loaded before (-) and after 3 hours (+) of induction with IPTG. **rSilN$_{H10}$-rSic**: Silaffin-silacidin fusion protein after purification by IMAC. **CNBr:** Silaffin-silacidin fusion protein after CNBr treatment. **Ni-NTA:** CNBr fragments of rSilN$_{H10}$-rSic that did not bind (**FT**) and did bind (**eluate**) to Ni-NTA resin.

Figure S3

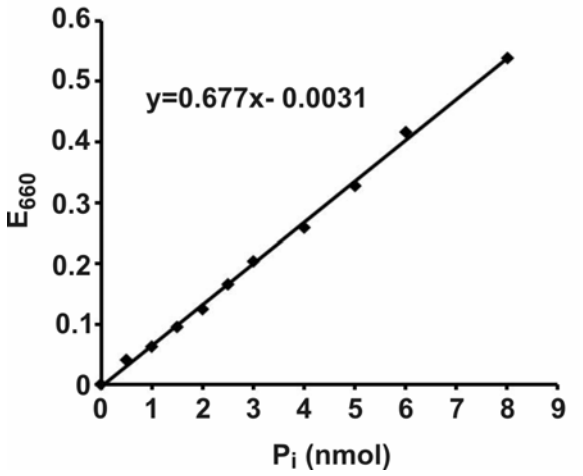


**Figure S3. Quantification of inorganic phosphate using the phosphomolybdate method (40).**

**Table S1. Analysis of phosphate content in silaffins and reference compounds.** The chemically bound phosphate was determined according to a published method (40). Phosphorylated silaffins rSil3 (rSil3-P) and rSilC (rSilC-P) were purified by RP-HPLC and quantified as previously described for the unphosphorylated silaffins (36), ATP (contains 3 phosphate residues pre molecule) and glycerophosphate (GlcP; contains 1 phosphate residue per molecule) were used as purchased. Inorganic phosphate obtained after hydrolysis was calculated according to the inorganic phosphate reference shown in Figure S2.

| | **molec. (nmol $P_i$)** | **$E_{660}$** | **$P_i$ calc (nmol)** | **$P_i$:molec.** |
|---|---|---|---|---|
| **rSil3-P** | 0.1 | 0.016 | 0.28 | **2.8** |
| | 0.75 | 0.152 | 2.30 | **3.1** |
| | 1.1 | 0.216 | 3.24 | **2.9** |
| **rSilC-P** | 0.17 | 0.012 | 0.14 | **0.8** |
| | 1.26 | 0.112 | 1.40 | **1.1** |
| | 1.85 | 0.178 | 1.79 | **1.0** |
| **GlcP** | 0.50 | 0.013 | 0.39 | **0.8** |
| | 2.00 | 0.113 | 2.01 | **1.0** |
| | 5.00 | 0.272 | 4.36 | **0.9** |
| **ATP** | 0.25 | 0.046 | 0.73 | **2.9** |
| | 0.50 | 0.102 | 1.56 | **3.1** |
| | 2.50 | 0.521 | 7.73 | **3.1** |

Figure S4

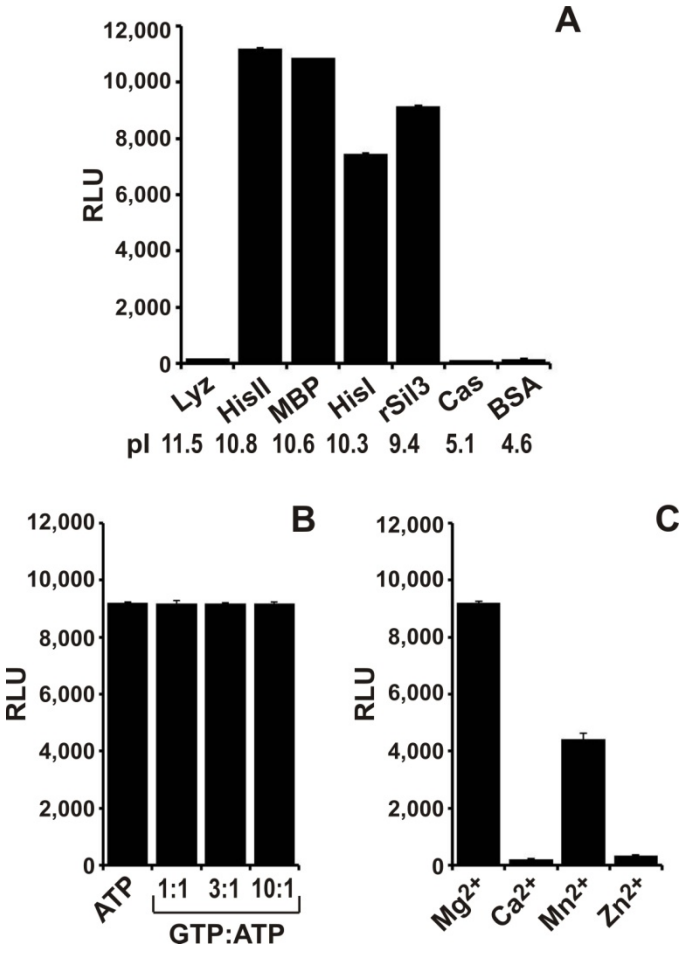


**Figure S4. Substrate specificity and co-factor dependence of recombinant tpSTK1.** Each measurement was repeated at least three times. The brackets in the bars indicate standard deviations. RLU = relative luminescence units. ***A***, Kinase activity with commercial substrate proteins (Lysozyme = Lyz, Histone II = HisII, Myelin basic protein = MBP, Histone I = HisI, dephosphorylated Casein = Cas, bovine serum albumin = BSA). For comparison the activity with recombinant rSil3 as substrate is included. The proteins are ordered according to their isoelectric points (pI). ***B***, Kinase activity with rSil3 in the presence of ATP and increasing concentrations of GTP (molar ratios of the nucleotides are indicated). The consumption of ATP was not affected by the presence of GTP indicating that GTP cannot replace ATP as co-substrate for recombinant tpSTK1 (note: GTP cannot substitute for ATP in the luciferase-dependent reporter reaction). ***C***, Metal-ion dependence of kinase activity. Metal-ion free recombinant tpSTK1 was incubated under kinase reaction conditions in the presence of equal concentrations of the indicated cations (no other polyvalent metal ions were present).

Figure S5

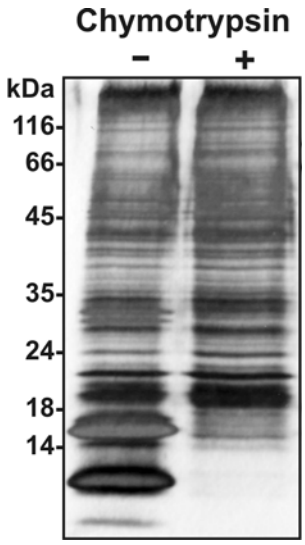


**Figure S5. Chymotrypsin treatment of intact membranes.** Silver-stained SDS-PAG. F4 membranes (0.25 mg/ml protein) were incubated for 1 hour at 4 °C in the absence (-) or presence (+) of 0.3 mg/ml chymotrypsin.

**Table S2. Protease accessibility of CCRase activity in F4 membranes.** The activity of this ER-located enzyme was measured after treatment of intact and detergent-solubilized (1 % w/w Igepal) F4 membranes (0.21 mg/ml total protein) with 1.0 mg/ml chymotrypsin. The listed numbers represent the averages from three independent experiments.

| | **CCRase (nmol cyt $c_{red}$/min)** |
|---|---|
| intact membranes | 1.81 |
| intact membranes +chymotrypsin | 1.75 |
| solubilized membranes +chymotrypsin | 0.01 |

Figure S6

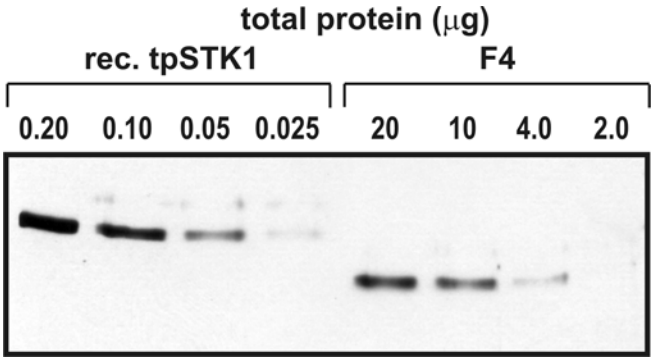


**Figure S6. Western blot for estimation of native tpSTK1 content of F4 membranes.** Recombinant tpSTK1 was isolated by IMAC and gel elution after SDS-PAGE. Protein concentrations were determined using the BCA assay (46). The Western blot was probed with 0.2 μg/ml anti-tpSTK1 IgG. The intensity of the signal for native tpSTK1 in F4 membranes containing 10 μg of total protein is about as strong as the intensity of the signal for 50 ng recombinant tpSTK1.

Figure S7

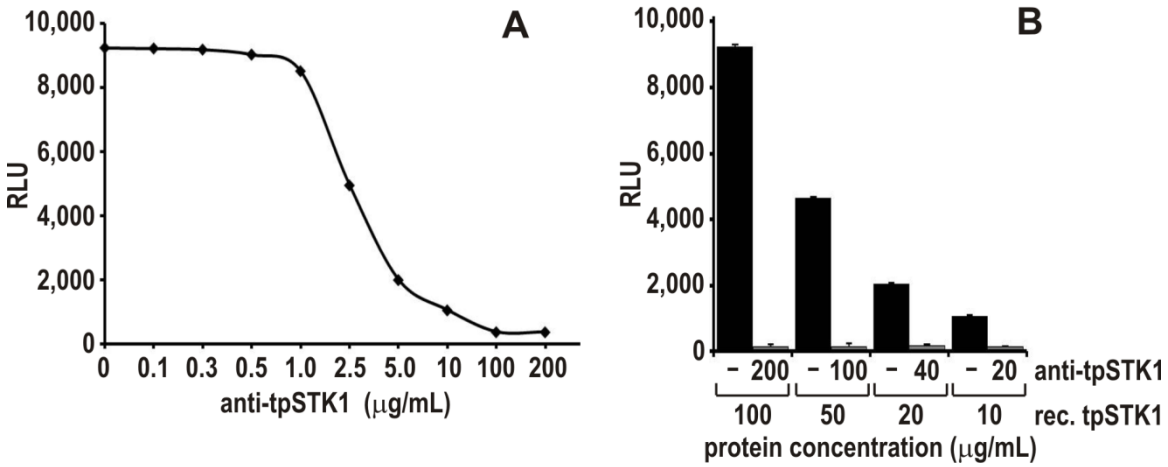


**Figure S7. Inhibition of recombinant tpSTK1 by anti-tpSTK1 IgG in vitro.** Recombinant silaffin rSil3 was used as substrate protein. ***A***, Kinase activity of recombinant tpSTK1 (100 μg/ml) in the presence of increasing concentrations of anti-tpSTK1 IgG. Virtually complete inhibition of kinase activity (<5 % of maximum kinase activity) can be achieved at an antibody concentration of ≥100 μg/ml. ***B***, Kinase activity of recombinant tpSTK1 in the presence of anti-tpSTK1 IgG at a constant tpSTK1:IgG ratio (1:2 by mass), but decreasing overall protein concentration. The kinase remained virtually inactive at all protein concentrations indicating that the tpSTK1:IgG complexes are stable even at high dilution.