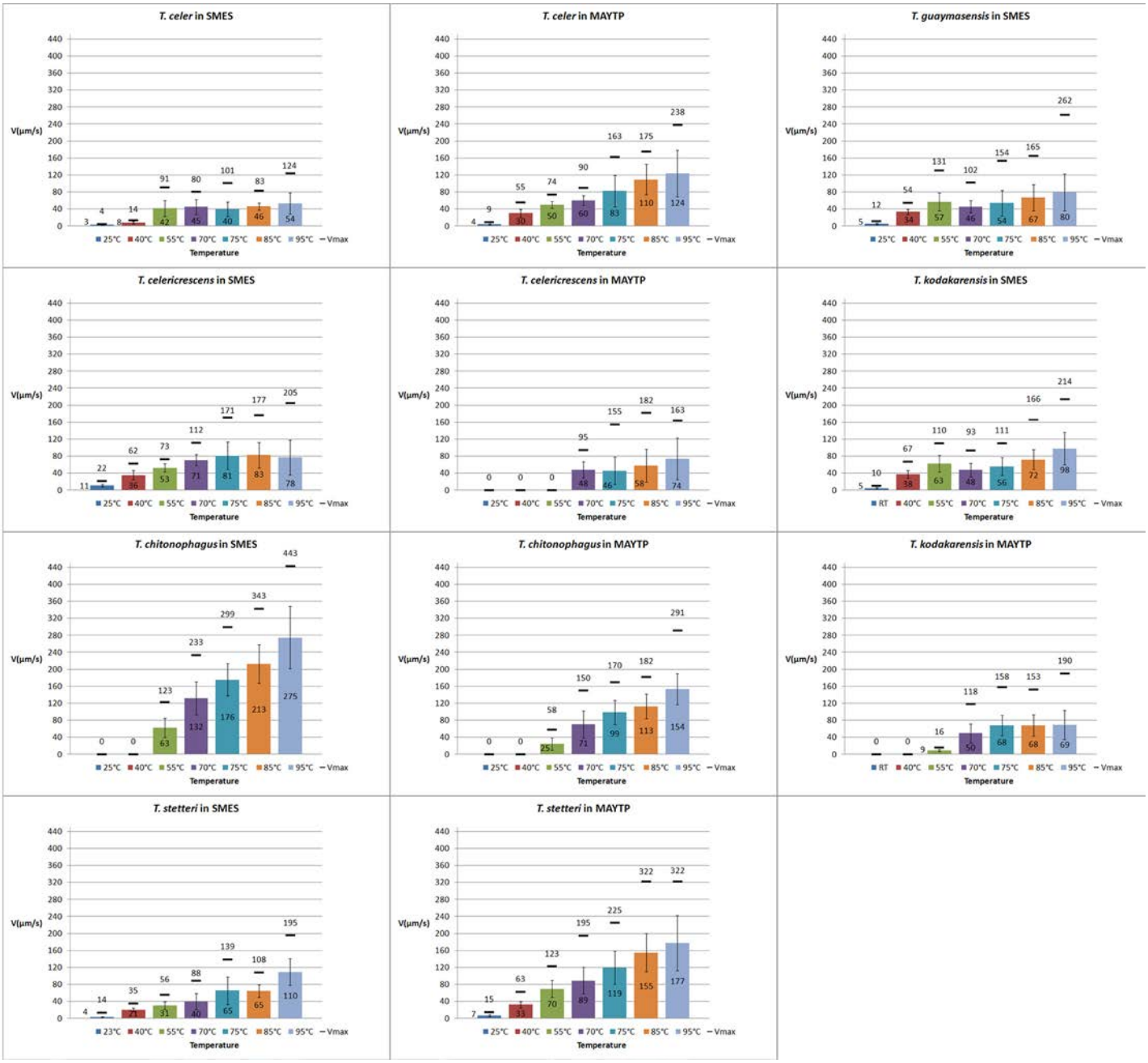
Figure S1. Comparison of swimming speeds for 15 different *Thermococcus* species.

Motility was measured in poor SMES medium and rich MAYTP medium. Swimming speeds (given in µm/s) are indicated by differently coloured columns. Values are the mean from 3 different experiments each. The black bars above the columns give the maximum speeds observed. (A) summarizes the data for fast swimming species; (B) summarizes the data for relatively slow swimming species – note the difference in the scale of ordinates between (A) and (B).

A



B

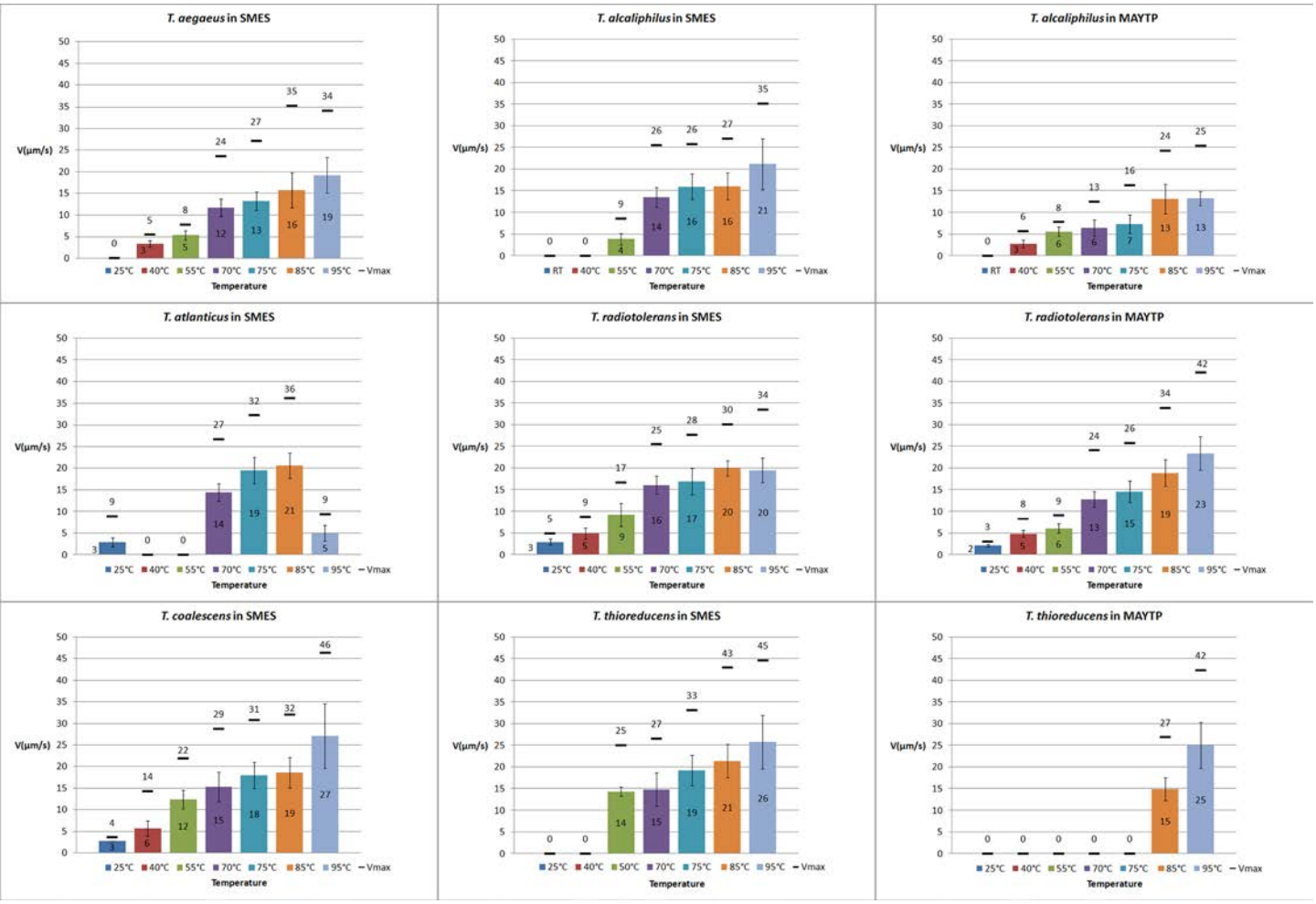
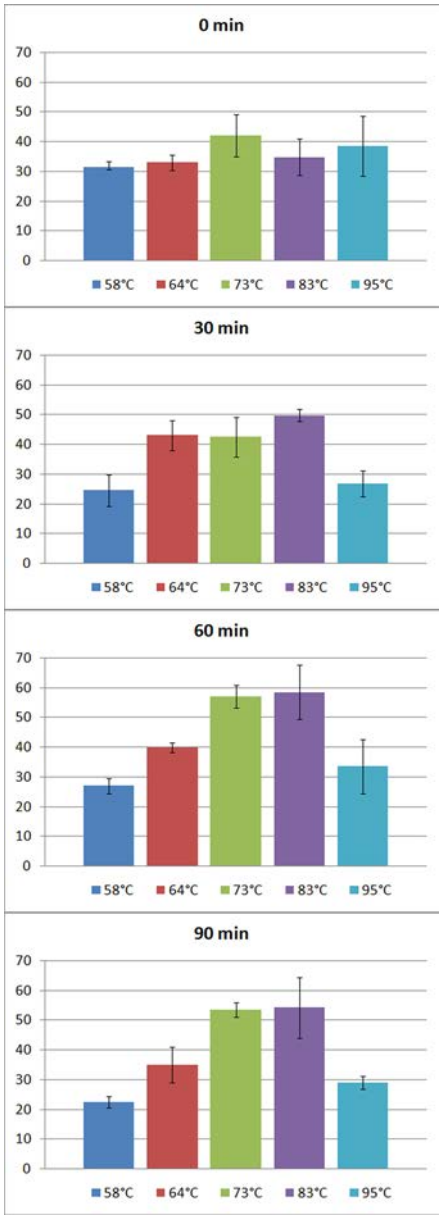

T. aegaeus in SMES
T. alcaliphilus in SMES
T. alcaliphilus in MAYTP
T. atlanticus in SMES
T. radiotolerans in SMES
T. radiotolerans in MAYTP
T. coalescens in SMES
T. thioreducens in SMES
T. thioreducens in MAYTP
V(µm/s)
Temperature
RT
25°C
40°C
50°C
55°C
70°C
75°C
85°C
95°C
Vmax

Figure S2 Thermotaxis behaviour of *Thermococcus stetteri* in the TGFD. After filling the glass capillary with a freshly grown culture of *T. stetteri* a temperature gradient from 58 to 95°C was established in the TGFD. The numbers of swimming cells (focus plane in the middle of the capillary; y-axis) were counted from 4 different movies and four different fields of observation for each temperature point at 0, 30, 60 and 90 min each.



Comment. Thermotaxis is defined as the active movement of organisms to temperatures supporting their life. The TGFD is a device by which we could ask if such a thermotaxis can be observed. Thermotaxis will not be seen for individual cells which stay in the focus plane for one

second or less, but rather for a cell community. Cells filled into the glass capillary are evenly distributed over its total length and therefore, +/- equal numbers of cells are seen through the five observation holes at the beginning of an experiment. By applying a temperature gradient, cells showing thermotaxis should accumulate over time in their preferred temperature range. Such behaviour indeed could be observed for *T. stetteri* in a temperature gradient of 58 to 95°C. Ca. 50% of actively swimming cells moved from the lower and upper temperature range to a region in the capillary between 73 to 83 °C (see Figure S2). Since the number of adherent cells (top and bottom focus plane of the capillary) did not change during the 90 minutes of experimentation, but clearly the number of actively swimming cells, we state to have demonstrated thermotaxis by this experiment.

Thermotaxis generally is believed to be of importance for organisms; from human sight it seems logic for any organism to move to (and stay in) a place having a temperature supporting its life. Ample experimental evidence for thermotaxis of eukaryotes is available, especially for plants, amoeba, *Caenorhabditis elegans* (1) and human sperm (2). In the case of prokaryotic microorganisms thermotaxis generally is believed to occur; hard data, however, for this – to the best of our knowledge – are available only by two reports for *Escherichia coli* (3, 4); in this species methylation of chemoreceptors can alter the preference for heat or cold (5). In case of the bacterium *Thermotoga maritima* responses to changes in temperature have been reported (6); no data, however, on a potential accumulation of cells at a certain temperature, were given. For the archaeon *Sulfolobus solfataricus* data on swimming speed and run time in dependence of temperature and pH have been reported (7), but again no measurements of a potential accumulation of cells at a certain temperature were shown.

In the case of *T. stetteri* we obtained evidence for thermotaxis, an ability which should be of great advantage for microorganisms living in habitats characterized by very steep temperature gradients. These data, therefore, for the first time indicate the occurrence of thermotaxis in *Archaea*. In principle, one might expect every organism analysed in the temperature-organ to show thermotaxis; we could observe it only for *T. stetteri* and *T. celericrescens* (to a lesser extent – data not shown). One reason that thermotaxis is not easy to measure lies in the fact that cells have to swim quite a distance (at least 1 cm = 10,000 cell lengths) to reach optimal conditions. Motility, however, does consume substantial amounts of energy and therefore it might be possible that during the experiment, cells already were energetically exhausted. In the case of both, *T. stetteri* and *T. celericrescens* this obviously was not the case, because cells of both species at the end of the experiment clearly were motile.

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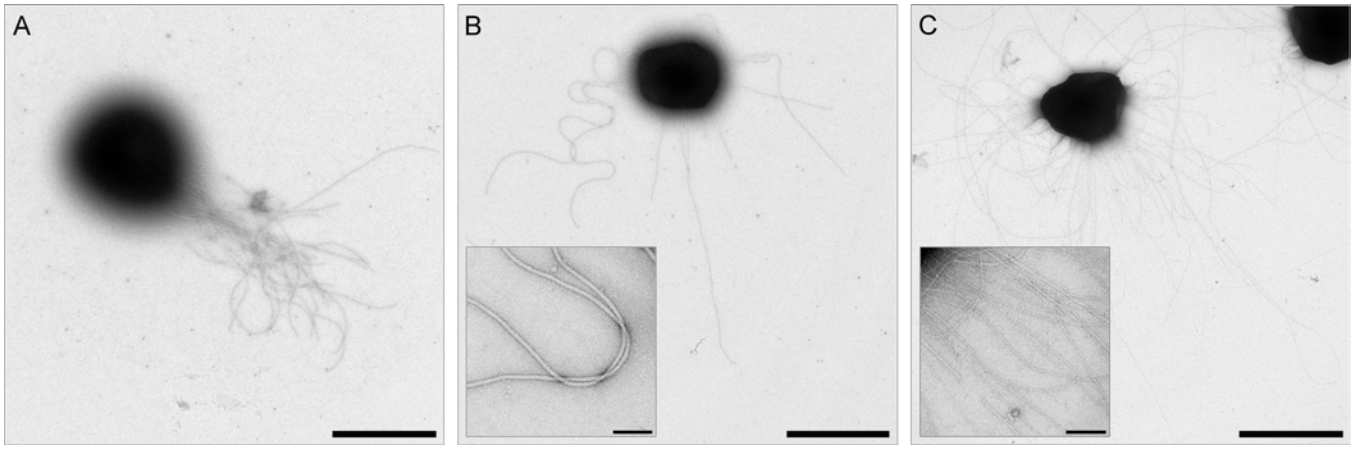
Figure S3 Comparison of different cell appendages. Transmission electron micrographs of negatively stained cells. (A) *T. stetteri* in MAYTP medium possessing a tuft of true flagella; (B) *T. alcaliphilus* in SMES medium with some single flagella; (C) *T. gorgonarius* in MAYTP medium showing many polytrichous appendages. Enlargements demonstrate the differences in diameter between true flagella (10-12 nm) in (B) and thinner filaments (5-8 nm) in (C). Bars = 1 µm, in enlargements = 100 nm.



SUPPLEMENTAL MATERIAL - MOVIES

Movie 1 is real time; movies 2 and 3 are ca. 3-fold time compressed, to reduce their size. The movies show cells in temporal, but not in spatial temperature gradients. Some drift of liquid is frequently observed during heating due to minute air bubbles in the capillaries. The movies originally were recorded as TIFF files for each single frame; to reduce their data size, they have been converted to JPEG format using ImageJ.

Movie 1 Reaction of *Methanocaldococcus villosus* to a temperature shift from room temperature to 90°C.

Movie 2 Reaction of *Methanocaldococcus villosus* to a temperature shift from room temperature to 90°C, after 6 months of “cold-ageing” at 6°C.

Movie 3 Reaction of *Methanocaldococcus villosus* cells analysed in movie 2 to a temperature gradient from 90 to 140°C.