Supporting Material:

The small heat shock protein Hsp27 affects assembly dynamics and structure of keratin intermediate filament networks

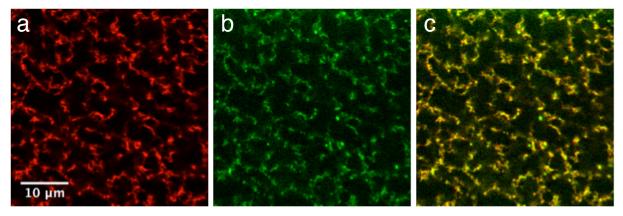


FIGURE S1 Keratin and Hsp27 colocalize in vitro. (a) Keratin is labeled with Atto647N . (b) Hsp27 is labeled with FAM. (c) Overlay of both channels. Channels where recorded separately and no cross excitation/emission of keratin was detected. Keratin concentration was $c_{\rm K}$ = 10 μ M. The ratio of Hsp27 to keratin was R = 0.3 .

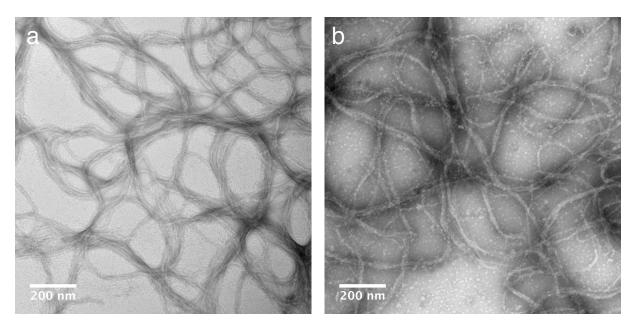


FIGURE S2 Filament elongation and bundling can be temporally separated by first assembling filaments by a drop in pH, followed by the initiation of bundling via the addition of salt (a). If Hsp27 is added simultaneous with the salt, after filament elongation, bundling seems to be reduced slightly (b). However, the effect is much less pronounced compared to Hsp27 added before initiation of filament assembly. This suggests that Hsp27 can only influence kinetic trapping in a significant manner if elongation and bundling proceed simultaneously. The protein concentration of the sample applied to the grid was $c_{27} = 2 \mu M$. All steps where carried out at room temperature.

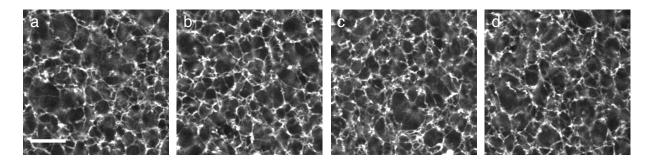


FIGURE S3 Representative confocal images taken at randomly chosen positions throughout the sample, R=6 (a-b). The standard deviation of the quantified fineness of the images can then serve as a measure for the mesoscopic homogeneity of the sample. The scale bar denotes 10 μ m.

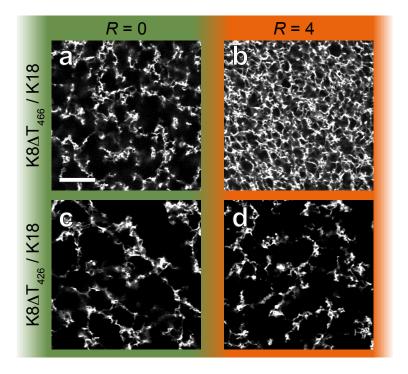


FIGURE S4 Comparison of the Hsp27 effect for the partial tail truncation mutants K8 Δ T₄₆₆ (a and b) and K8 Δ T₄₂₆ (c and d) . The keratin concentration was c_K =6 μ M for all samples. All images are confocal slices. The scale is the same for all images. The scale bar denotes 10 μ m.

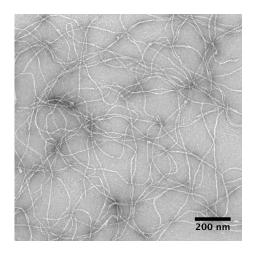
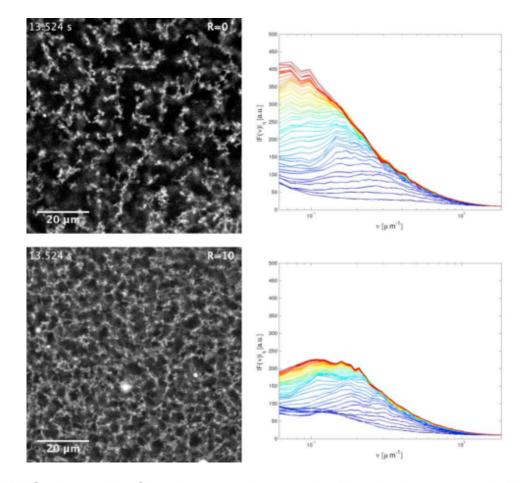


FIGURE S5 The depicted sample has a composition similar to that shown in Fig. 3a, only without salt. The image shows the expected 10 nm wide single filaments in contrast to the filament bundles in Fig. 3a.



MOVIE S1 Assembly of keratin networks (see also Fig. 2 in the main text). The movie (a still of the movie is depicted above) shows the assembly process of a keratin network without (R=0, top) and with Hsp27 (R=10, bottom). In addition to the confocal image (left) the structure factor $|F(v)|_{\varphi}$ is displayed (right). The corresponding time is shown in the top left corner.