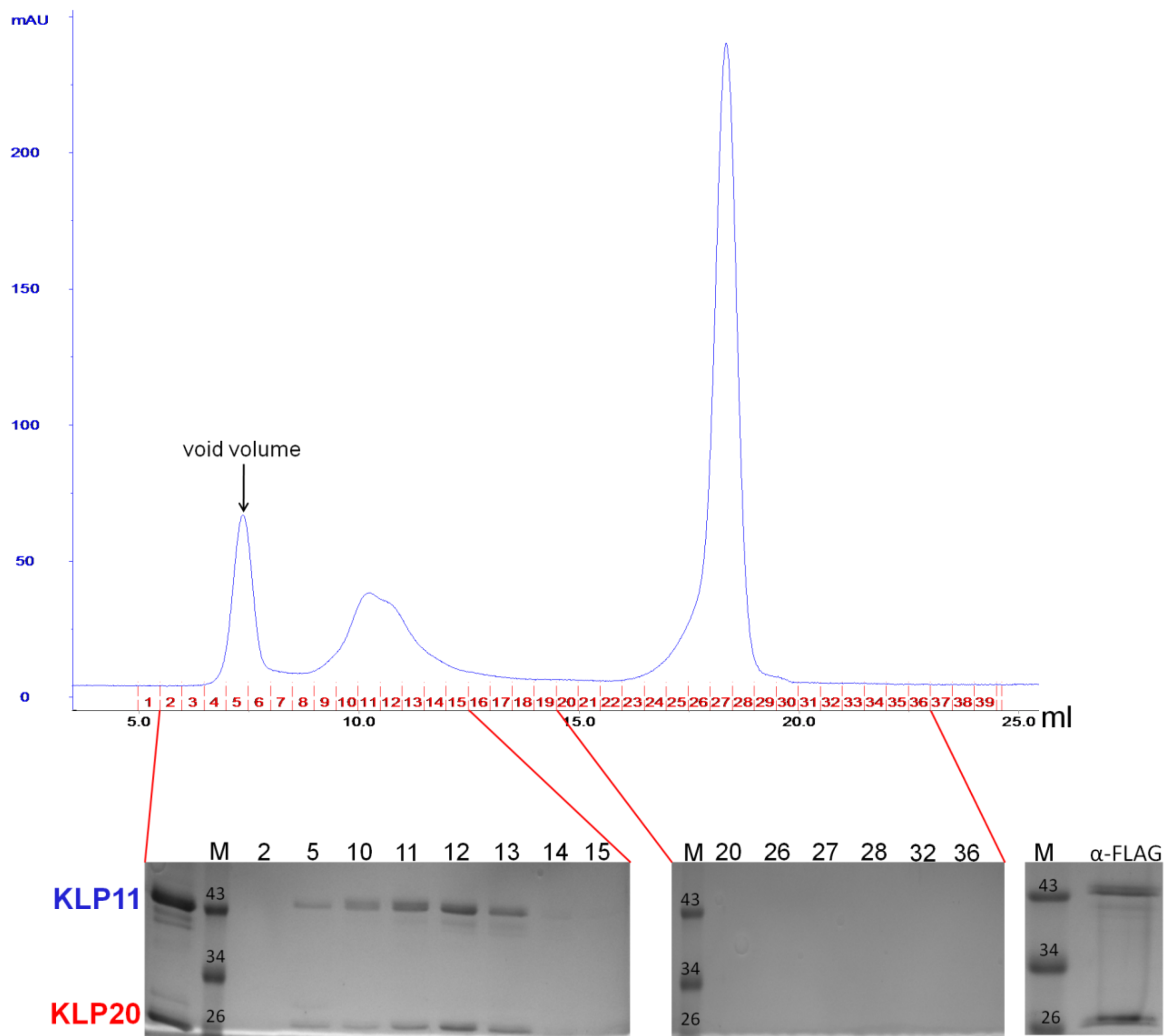
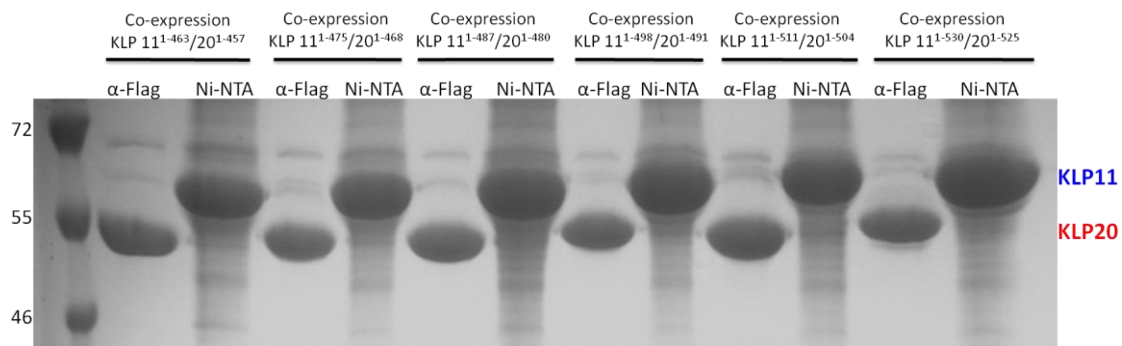
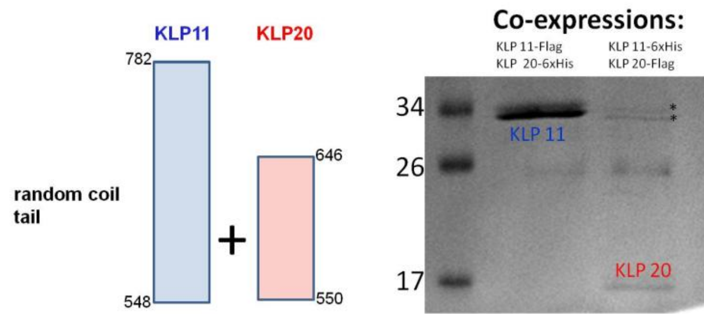


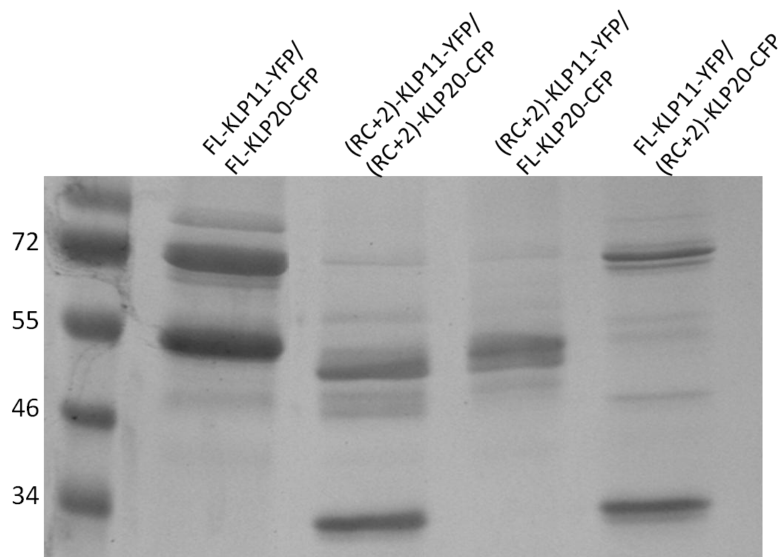
Supplementary Figure 1. Size exclusion chromatography of KLP11/20-C reveals its heterodimeric state. The left lane shows the protein loaded onto the column after the purification using the Flag-tagged KPL20 polypeptide chain. The eluted fractions under the protein peak (10-13) show both interacting partners in a 1:1 molar ratio. The lack of protein bands (20-36) demonstrates that no monomeric subunits were present after the Flag co-precipitation. The right panel shows the re-co-precipitation of the pooled fractions 11 and 12 via the FLAG-tagged KLP20 subunit.



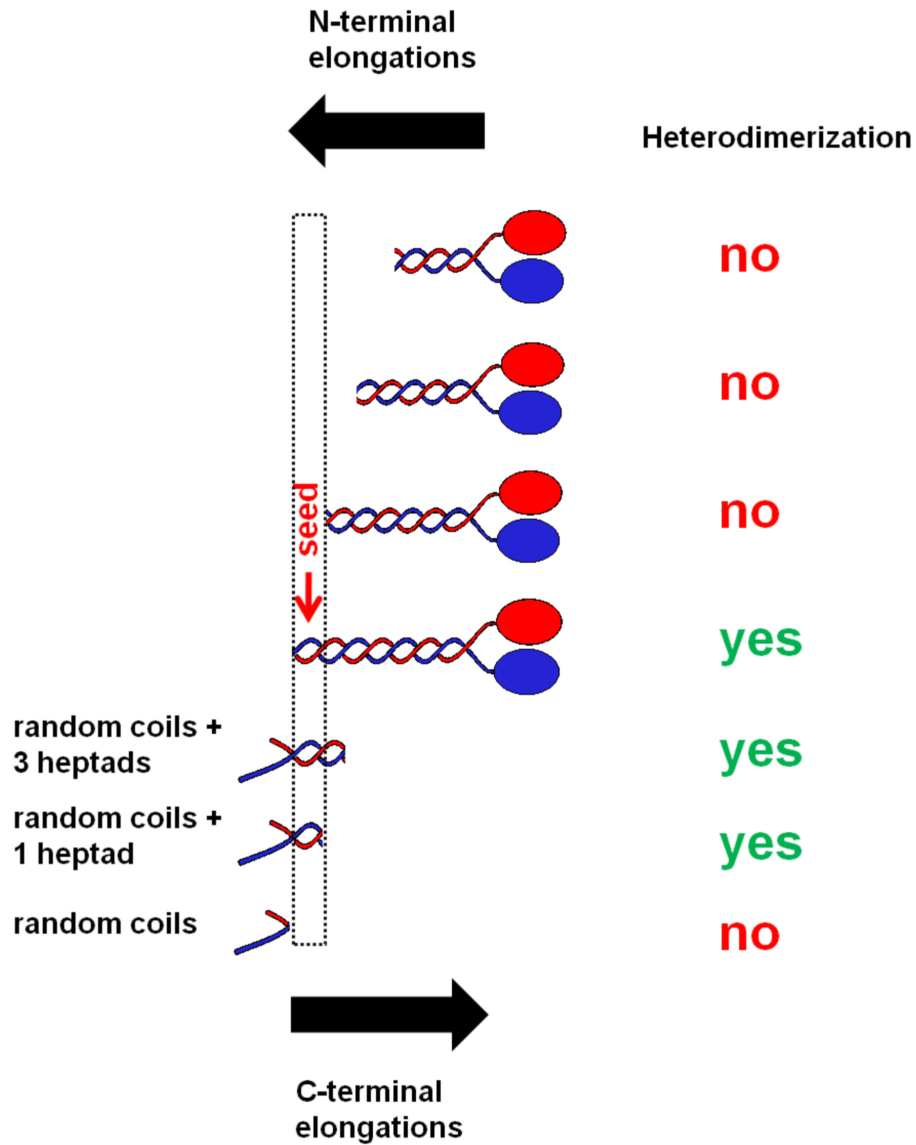
Supplementary Figure 2. C-terminal truncations lead to dimer formation only if the entire stalk domain is included in both motor domains (KLP11$^{1\text{-}548}$/20$^{1\text{-}550}$, Figure 3B). None of the six stepwise elongations of the N-terminal halves (Figure 2) of the motor domains is sufficient for heterodimerization. To exclude the possibility that lack of protein expression is responsible for the obtained results, for each case the cell lysate was divided in two equal parts for Ni-NTA and α-Flag purification, respectively, to demonstrate that both polypeptides were expressed properly. Marker protein sizes are shown in kDa.



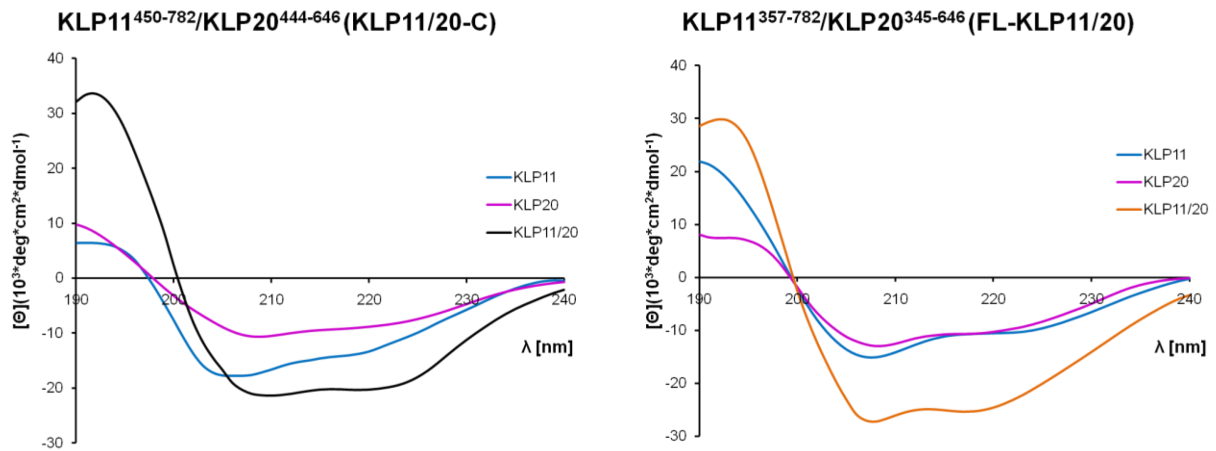
Supplementary Figure 3. Random coil tails do not dimerize. None of the attempts to co-purify the co-expressed random coil tails from the cell lysate were successful. The identity of all protein bands was confirmed by LC-MS/MS (*=keratin). Marker protein sizes are shown in kDa.



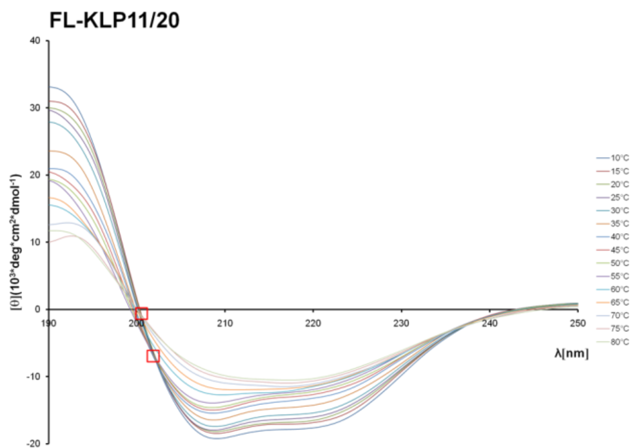
Supplementary Figure 4. Co-expression of the FRET constructs. Differentially tagging the FRET KLP11 and KLP20 constructs (Flag-tag and 6xHis-tag, respectively) enabled dimer purification in a 1:1 ratio. FL-KLP11-YFP/FL-KLP20-CFP comprises the entire stalk; (RC+2)-KLP11-YFP/(RC+2)-KLP20-CFP serves as a positive control; (RC+2)-KLP11-YFP/FL-KLP20-CFP and FL-KLP11-YFP/(RC+2)-KLP20-CFP serve as negative controls. Marker protein sizes are shown in kDa.

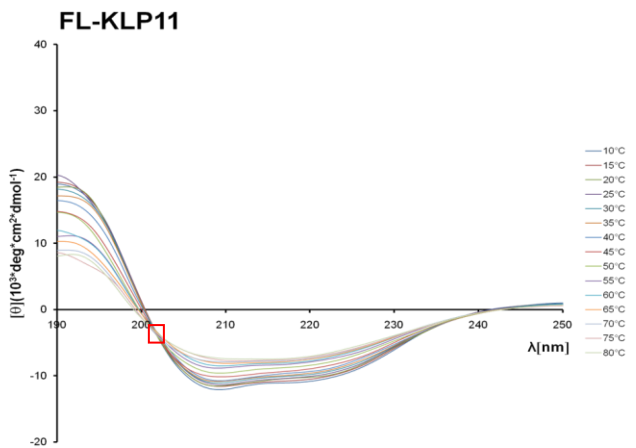


Supplementary Figure 5. Identification of the dimerization seed by systematic N-and C-terminal elongations of truncated KLP11 and KLP20 polypeptide chains. No dimerization occurs unless the C-terminal end of the stalk is present. C-terminally truncated constructs must thus be elongated to include the entire stalk, whereas elongation of random coil tails by only 10 amino acids already leads to dimerization.



Supplementary Figure 6. KLP11 and KLP20 prefer hetero- to homodimer formation under native conditions. For both KLP11/20-C and FL-KLP11/20, the spectra show significantly more secondary structure for the heterodimers (higher absorption at 208 nm and 222 nm and higher maximum at 195 nm). In support of spectra, the $[\theta]_{222}/[\theta]_{208}$ ratio, index for coiled-coil content is higher for the heterodimers (0.97 for KLP11/20-C; 0.89 FL-KLP11/20, see Table 1), indicating that motor domains indeed do not form homodimers but remain in monomeric state.


FL-KLP11/20
[θ](10³*deg*cm²*dmol⁻¹)
40
30
20
10
0
-10
-20
190
200
210
220
230
240
250
λ[nm]
10°C
15°C
20°C
25°C
30°C
35°C
40°C
45°C
50°C
55°C
60°C
65°C
70°C
75°C
80°C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30
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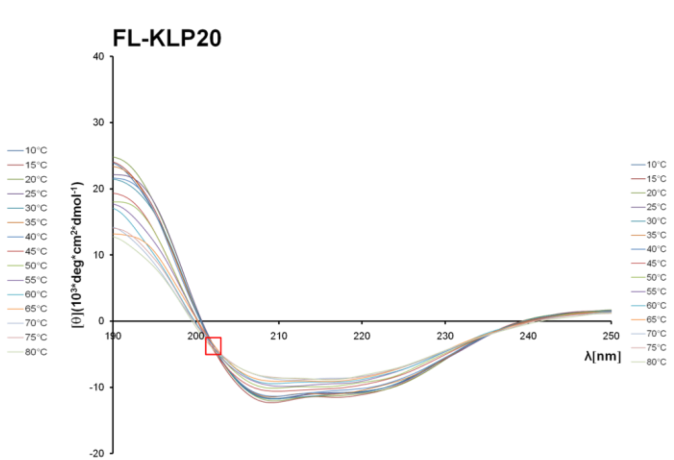
10
0
-10
-20
190
200
210
220
230
240
250
λ[nm]
10°C
15°C
20°C
25°C
30°C
35°C
40°C
45°C
50°C
55°C
60°C
65°C
70°C
75°C
80°C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30
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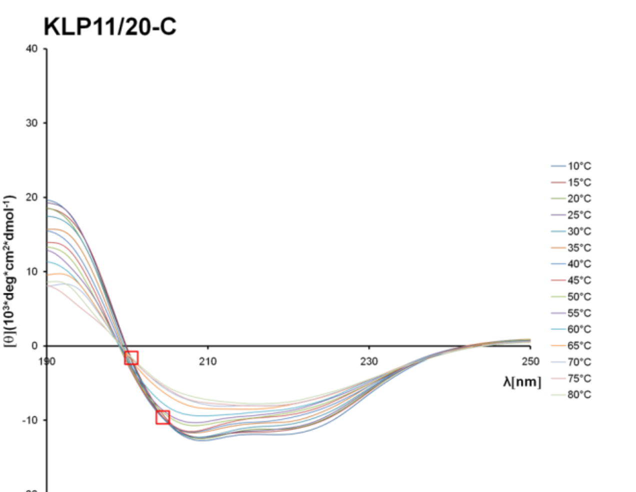
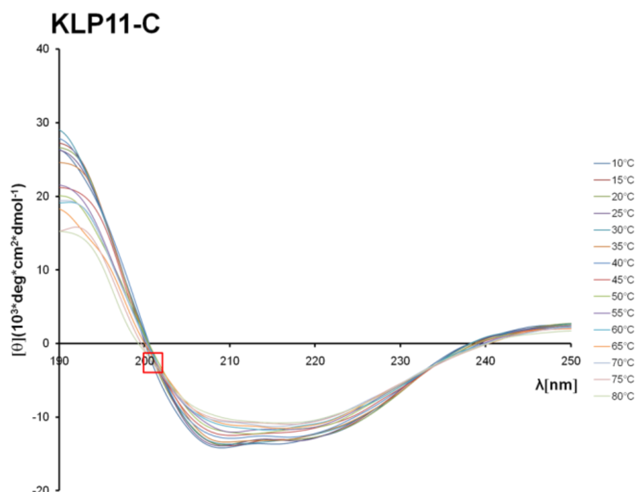
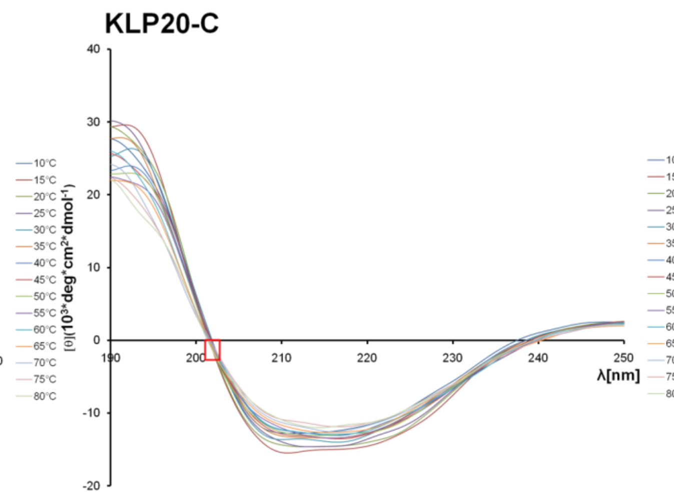
10
0
-10
-20
190
200
210
220
230
240
250
λ[nm]
10°C
15°C
20°C
25°C
30°C
35°C
40°C
45°C
50°C
55°C
60°C
65°C
70°C
75°C
80°C


KLP11/20-C
[θ](10³*deg*cm²*dmol⁻¹)
40
30
20
10
0
-10
-20
190
210
230
250
λ[nm]
10°C
15°C
20°C
25°C
30°C
35°C
40°C
45°C
50°C
55°C
60°C
65°C
70°C
75°C
80°C


KLP11-C
[θ](10³*deg*cm²*dmol⁻¹)
40
30
20
10
0
-10
-20
190
200
210
220
230
240
250
λ[nm]
10°C
15°C
20°C
25°C
30°C
35°C
40°C
45°C
50°C
55°C
60°C
65°C
70°C
75°C
80°C


KLP20-C
[θ](10³*deg*cm²*dmol⁻¹)
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30
20
10
0
-10
-20
190
200
210
220
230
240
250
λ[nm]
10°C
15°C
20°C
25°C
30°C
35°C
40°C
45°C
50°C
55°C
60°C
65°C
70°C
75°C
80°C

Supplementary Figure 7. Temperature dependant CD measurements of monomeric and dimeric KLP11/20-C and FL-KLP11/20. While denaturation of monomeric constructs is a 2-state process (one isodichroic point (red square)), heterodimers denaturate in a 3-state process (two isodichroic points). The two states are α-helix and unfolded state; the three states are coiled-coil, α-helix and unfolded state.