Supplementary Fig. S1. Thermogram of the LC8-IC_{L7Δ} interaction. ITC plot was recorded at 5 °C, in 50 mM Na_{2}PO_{4}, 50 mM NaCl, pH 6.3. The solid line corresponds to the non-linear least squares fit for an A + B to AB binding model, and gives a binding constant (K_{d}) of 10.1 µM.

Supplementary Fig. S2. Secondary structure and association states of IC mutants. (A) Overlay of far UV CD spectra of IC_{TL7} (o), IC_{TL7154C} (Δ), IC_{TL7219C} (□), and IC_{L7Δ} (x). (B) Analytical gel filtration elution profiles of IC_{TL7} (solid line) and the IC_{TL7219C-dimer} (dashed line). Data were collected at a flow rate of 0.5 ml/min in buffer composed of 50 mM sodium phosphate, 0.4 M NaCl, 1 mM sodium azide, pH 7.5.

Supplementary Fig. S3. Effect of increasing GndCl concentration on IC_{TL7} mutants. (A) Fluorescence emission spectra of unbound IC_{TL7219C-TMR} without (Δ) or with (▲) 1.5 M GndCl. (B) Fluorescence emission spectra of Tctex1/LC8-bound IC_{TL7219C-TMR} without (●) or with (▲) 1.5 M GndCl. The spectrum of IC_{TL7219C-TMR} without GndCl (Δ) is shown for comparison.

Supplementary Fig. S4. Effect of Tctex1/LC8 binding on fluorescence emission spectra of “lightly labeled” IC_{TL7} mutants. Fluorescence emission spectra of unbound (o) or bound (●) (A) IC_{TL7154C-TMR}, and (B) IC_{TL7219C-TMR}. “Lightly labeled” indicates a labeling efficiency of 3% (see materials and methods). Fluorescence spectra were recorded with the excitation and emission slit widths set to 5 nm each.

Supplementary Fig. S5. GndCl titration of IC_{L7Δ}. Overlay of ¹H-¹⁵N HSQC spectra of IC_{L7Δ} collected in 50 mM sodium phosphate, 50 mM NaCl, 1 mM NaN₃, pH 6.3, with 0 M (black), 0.1 M (green), 0.3 M (blue) and 0.5 M (magenta) GndCl. With increasing denaturant concentration, several residues shift towards the center of the spectrum. (B) GndCl unfolding profile of IC_{L7Δ} monitored by far UV CD spectroscopy. Data were recorded in 10 mM sodium phosphate pH 6.3, with 0 - 1.1 M GndCl. At lower protein concentrations, data were too noisy to be reliable and at higher protein concentrations, the protein precipitated.
S.5

A

Normalized Ellipticity at 222 nm

GndCl concentration, M

B

Normalized Ellipticity at 222 nm

GndCl concentration, M