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Experimental procedures

Disorder prediction

The following servers were used to predict the disordered parts of STIL: DisEMBL, DripPred, FoldUnfold, GlobPlot, IUPred, Prelink, RONN, SEG, DisPro and Spritz[1]. The full length sequence of *human* STIL (1-1288) was subjected to disorder prediction using the default parameters for all servers.

Protein expression and purification

DNAs encoding fragments of STIL were amplified by PCR using primers with Sal1 restriction sites at the 5' and Not1 restriction sites at the 3'. PCR products were purified by clean-up kits (Promega), cleaved by Sal1 and Not1 and purified again. The insert fragments were ligated into the pHIS parallel 2HLT cut vector. The HLT vector encodes for a tag containing six His residues, a lipoamyl domain that increases the solubility of the fusion protein and a Tev protease cleavage site for cleaving the HLT domain, and is under the induction of IPTG[2]. The DNA was transformed into competent JM109 bacteria, and then colonies were screened for the STIL fragments insertion plasmids and verified by sequencing. HLT STIL4$_{200\text{-}450}$, HLT STIL5$_{450\text{-}700}$ and HLT STIL6$_{500\text{-}650}$ vectors were transformed into E.coli BL21(DE3) pLysS cells (Novagen). Transformed bacteria cells were grown at 37°C in 2xYT medium. At OD_{600nm} of 0.7, 0.8 mM IPTG was added to start the induction. Different temperatures of induction and different times of induction duration were screened to optimize the conditions for the expression of soluble proteins. The optimal conditions chosen were 26°C and 4 hours of IPTG induction. Bacteria were harvested, sedimented as pellet and were kept at -80°C. Bacteria were lysed using microfluidyzer and the soluble fraction was purified using nickel Sepharose 4ml column. Elution was performed using an imidazole gradient. The proteins eluted in 100% elution buffer (containing 20 mM TrisHCl pH = 8, 0.5 M NaCl, 5 mM βMe, 10% glycerol and 250 mM imidazole) and were further purified using size exclusion chromatography Sephacryl S200 500 ml column. Elution was performed with buffer of 20mM TrisHCl pH = 8, 0.3 M NaCl, 5 mM βMe and 2% glycerol. This buffer was also used as the storage buffer. The protein purity was confirmed by coomassie staining of SDS-PAGE gel.

Circular Dichroism (CD)

9 μM HLT STIL4$_{200\text{-}450}$ were dissolved in 20 mM TrisHCl buffer pH = 7.5, 150 mM NaCl, 2.5 mM βMe and 2% glycerol. 5 μM HLT STIL5$_{450\text{-}700}$ were dissolved in 20 mM TrisHCl buffer pH = 7.5, 50 mM Na_2SO_4, 0.5 mM βMe, 2% glycerol. 9 μM HLT STIL6$_{500\text{-}650}$ were dissolved in 20 mM TrisHCl buffer

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pH = 7.5, 100 mM NaCl, 0.5 mM βMe, 5% glycerol. The proteins concentrations were measured using UV spectrophotometer (UV-1650PC, Shimadzu, Japan). CD spectra of the above STIL fragments were recorded using a J-810 spectropolarimeter (Jasco) in a 0.1 cm quartz cuvette for far-UV CD spectroscopy, in a spectral range of 195 nm to 260 nm.

Urea denaturation

12 μM HLT STIL4$_{200-450}$ were dissolved in 25 mM Hepes buffer pH=7, 150 mM NaCl, 1 mM βMe and 2% glycerol; 2.5 μM HLT STIL5$_{450-700}$ and 8 μM HLT STIL6$_{500-650}$ were dissolved in 20 mM TrisHCl buffer pH=7.5, 50 mM Na_2SO_4, 0.5 mM βMe and 2% glycerol. The proteins were incubated with 0.01M, 0.1M, 0.5M, 2M, 4M and 6M urea for 2 hours in ice. CD spectra of all conditions for the three proteins were recorded using a J-810 spectropolarimeter (Jasco) in a 0.1 cm quartz cuvette for far-UV CD spectroscopy, in a spectral range of 195 nm to 260 nm.

Peptide array screening

An array of 32 partly overlapping peptides derived from the STIL-binding protein CHFR[3] was synthesized by INTAVIS Bioanalytical Instruments AG, Koeln, Germany. The peptides were acetylated at their N-termini and attached to a cellulose membrane via their C-termini through an amide bond. The array was washed with 50 mM TrisHCl pH = 7.5, ionic strength of 150 mM (adjusted by NaCl), 0.05% Tween20 and 2.5% milk. 4.5 μM HLT STIL4$_{200-450}$, 5 μM HLT STIL5$_{450-700}$ or 4 μM HLT STIL6$_{500-650}$ were dissolved in TrisHCl pH = 7.5, ionic strength of 150 mM (adjusted by NaCl), 5 mM βMe, 2% glycerol, 0.3 mM $ZnCl_2$, 0.05% Tween20 and 2.5% milk, and incubated with the array at 4°C with shaking overnight. After four washings with 50 mM TrisHCl pH = 7.5 buffer (adjusted by NaCl to an ionic strength of 150 mM) the array was incubated with anti His HRP conjugated antibody at room temperature for 1 hour and then washed again four times with the Tris buffer. Immunodetection was performed using chemiluminiscence with ECL reagents. 4 μM HLT were also screened for binding the array as a control.

Fluorescence anisotropy

Measurements were performed generally as described[4]. 100 nM fluorescein-labeled peptides were dissolved in 25 mM Hepes pH = 7, 150 mM NaCl, 2% glycerol and 2 mM βMe. Different concentration

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of 250 µl protein in the same buffer (HLT STIL4$_{200-450}$ or HLT STIL6$_{500-650}$) were titrated into the peptides solutions in additions of 5 µl. Dissociation constants were calculated using the origin software by fitting of the anisotropy to the Hill equation $R = R_0 + (R_\infty - R_0)\frac{X^n}{k^n + X^n}$, where R is the measured anisotropy, R_0 is the anisotropy at time zero (free peptide), R_∞ is the anisotropy at the end of the titration (peptide in complex), K is the association constant, n is Hill coefficient and X is the added protein concentration.

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Table S1: Amino acid composition of STIL 400-700compared to other IDRs *

Amino acid	STIL 400-700	p53 1-61[5]	APC 800-2843[6]	AXIN1 295-500[7]	ASPP2 693-918[8]
Ala	3.7%	1.6%	5.5%	6.8%	4.9%
Gly	4.0%	1.6%	5.1%	6.8%	4.9%
Ile	4.7%	1.6%	4.1%	2.4%	3.5%
Leu	8.3%	13.1%	5.1%	6.3%	8.4%
Met	1.3%	4.9%	1.3%	1.5%	1.3%
Phe	2.3%	3.3%	1.9%	1.0%	0.4%
Pro	**15.0%**	**16.4%**	**7.9%**	**11.7%**	**17.7%**
Trp	0.3%	3.3%	0.3%	1.0%	0.0%
Val	4.3%	3.3%	4.0%	7.3%	5.8%
Asn	7.0%	3.3%	5.8%	2.4%	6.2%
Cys	3.7%	0.0%	1.1%	1.5%	0.0%
Gln	7.0%	6.6%	5.3%	4.4%	2.7%
Ser	**13.0%**	**11.5%**	**17.2%**	**7.8%**	**11.5%**
Thr	2.3%	3.3%	6.4%	2.9%	4.4%
Tyr	2.0%	0.0%	1.7%	2.4%	4.0%
Arg	3.7%	0.0%	5.4%	10.2%	4.4%
His	5.6%	0.0%	2.0%	4.9%	1.3%
Lys	3.7%	1.6%	7.5%	2.9%	4.4%
Asp	3.0%	13.1%	5.3%	5.3%	3.5%
Glu	5.3%	11.5%	7.1%	10.7%	10.6%
Overall Hydrophobicity:	44%	49%	35%	45%	47%

*The sequence of the STIL IDR was compared with IDRs of different representative proteins. Residues colored grey are hydrophobic, residues colored green are hydrophilic, residues colored blue are positively charged and residues colored red are negatively charged.

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Table S2: Binding of the CHFR peptides to the STIL fragments*

Peptide	*Kd* (μM) for interaction with HLT STIL6$_{500-650}$	*Kd* (μM) for interaction with HLT STIL4$_{200-450}$
CHFR $_{475-489}$	0.84 ± 0.02	3.9 ± 0.4
CHFR $_{482-496}$	2.02 ± 0.09	TWTM
CHFR $_{517-531}$	0.37 ± 0.02	NM
CHFR $_{531-545}$	0.74 ± 0.04	4.8 ± 0.7
CHFR $_{524-538}$	2.2 ± 0.6	TWTM
CHFR $_{594-608}$	0.69 ± 0.06	NM
CHFR $_{643-657}$	NM	4 ± 1

*Dissociation constants of the interactions between the STIL fragments and the CHFR derived peptides as calculated by fitting of the anisotropy curves to the Hill equation, as described in materials and methods. NM-not measured, TWTM- too weak to measure.

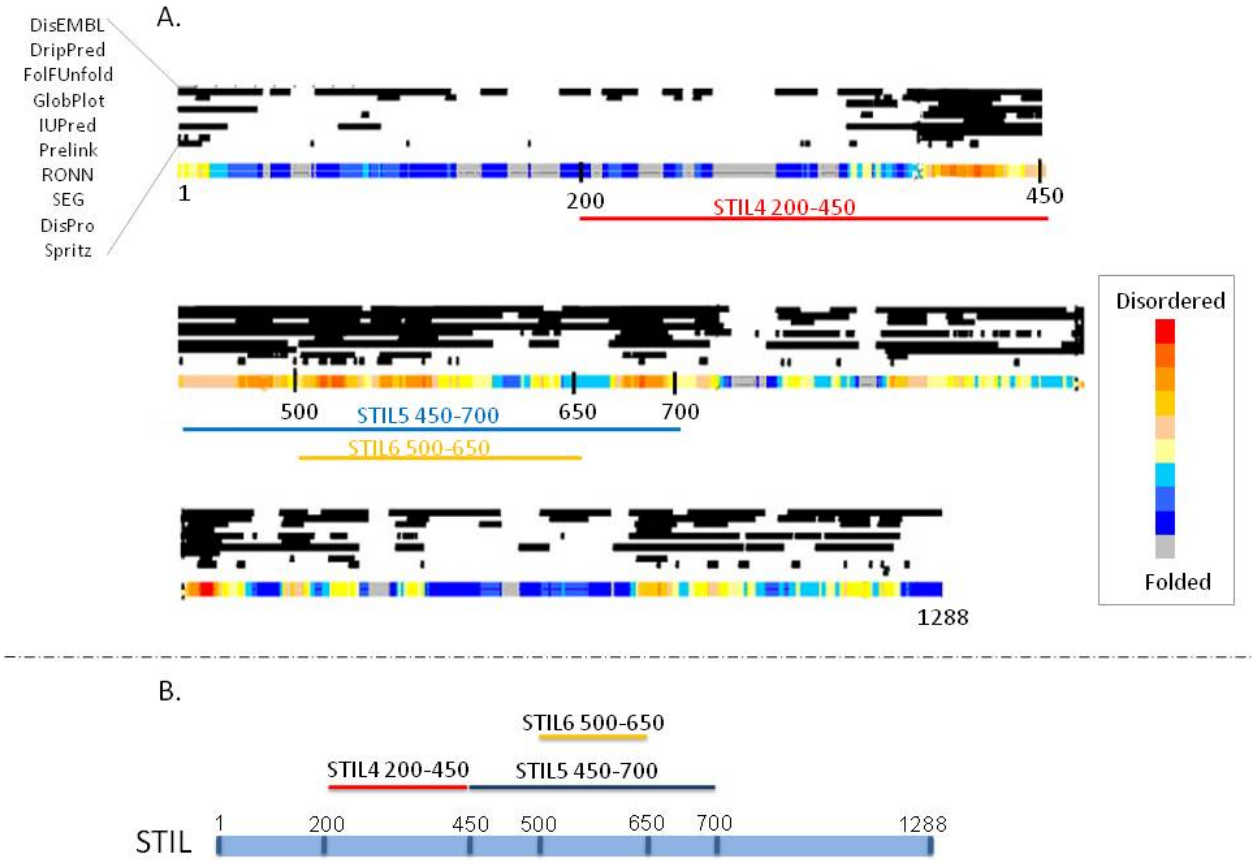

Figure S1: Disorder prediction of STIL and its division into fragment. (A) Ten servers were used to predict the disordered fragments of STIL: DisEMBL, DripPred, FoldUnfold, GlobPlot, IUPred, Prelink, RONN, SEG, DisPro and Spritz[1]. Residues that are predicted to be disordered are colored black. Each line represents a different server. Residues predicted to be disordered by all the servers are colored red and residues predicted to be folded by all the servers are colored gray. (B) The central part of the protein was divided into three fragments: $STIL4_{200-450}$, $STIL5_{450-700}$ and $STIL6_{500-650}$.

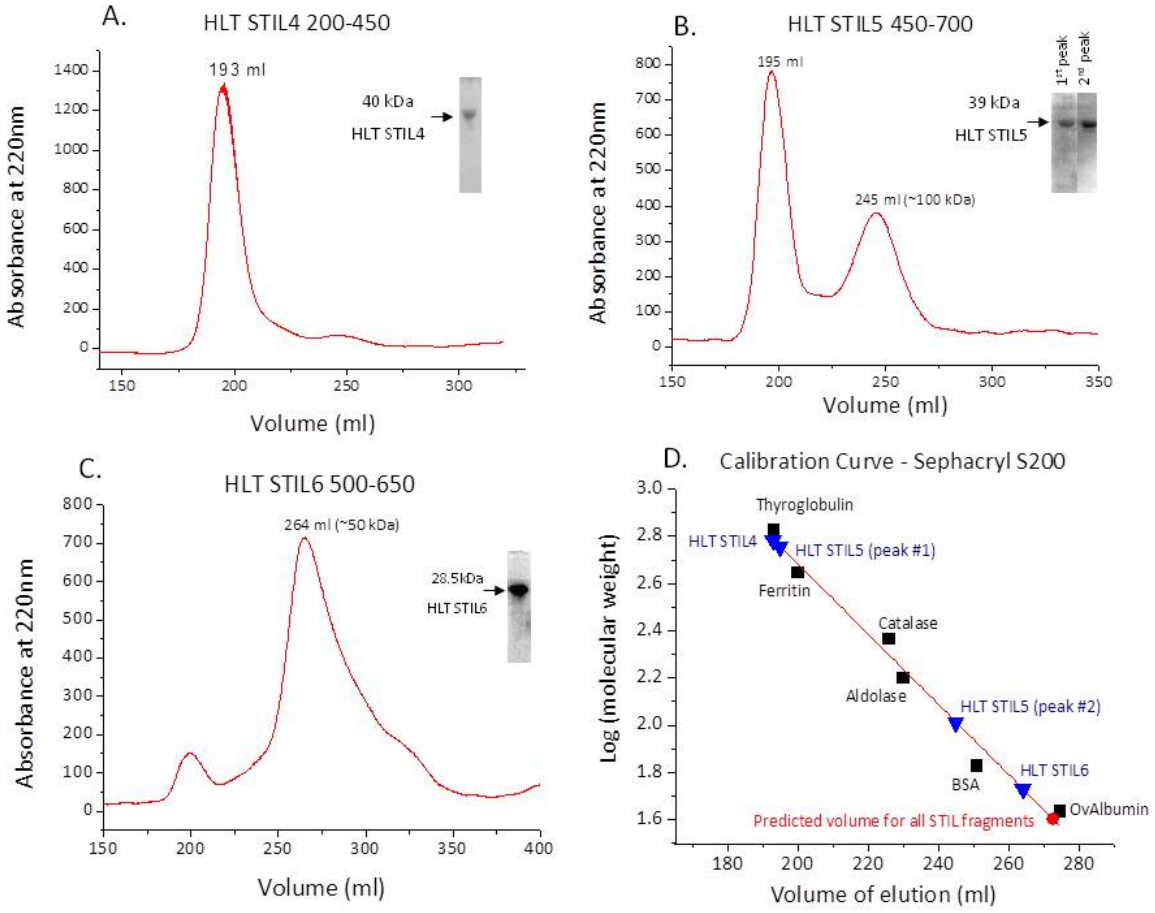

Figure S2: Size exclusion chromatography of the three recombinant STIL fragments. The proteins were purified by Nickel affinity followed by size exclusion chromatography. (A) The elution profile of HLT STIL4$_{200\text{-}450}$ from the sephacryl S200 preparative column, as monitored by UV absorbance at 220 nm. (B) The elution profile of HLT STIL5$_{450\text{-}700}$ from the sephacryl S200 preparative column as monitored by UV absorbance at 220 nm. (C) The elution profile of HLT STIL6$_{500\text{-}650}$ from the sephacryl S200 preparative column as monitored by UV absorbance at 220 nm. Coomassie staining of the SDS-PAGE gel of the purified protein is shown at the right side of each panel. (D) A calibration curve of the Sephacryl S200 preparative column (in black) with the positions of HLT STIL4$_{200\text{-}450}$, HLT STIL5$_{450\text{-}700}$ and HLT STIL6$_{500\text{-}650}$: predicted elution volume (in red) and experimental elution volume (in blue).

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