Architecture of the Human Mitochondrial Iron-Sulfur Cluster Assembly Machinery

Oleksandr Gakh[1#], Wasantha Ranatunga[1#], Douglas Y. Smith IV[1], Eva-Christina Ahlgren[2], Salam Al-Karadaghi[2], James R. Thompson[3], and Grazia Isaya[1]

[1]Departments of Pediatric and Adolescent Medicine and Biochemistry and Molecular Biology and Mayo Clinic Children's Research Center, Mayo Clinic, Rochester, MN 55905, USA; [2]Center for Molecular Protein Science, Institute for Chemistry and Chemical Engineering, Lund University, P. O. Box 124, SE-221 00 Lund, Sweden; [3]Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, Minnesota 55905, USA

[#]These authors contributed equally to this work.

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To whom correspondence should be addressed: Grazia Isaya, Mayo Clinic, 200 First St. SW, Stabile 7-52, Rochester, MN 55905. Tel.: 507-266-0110; Fax: 507-266-9315; E-mail: isaya@mayo.edu.

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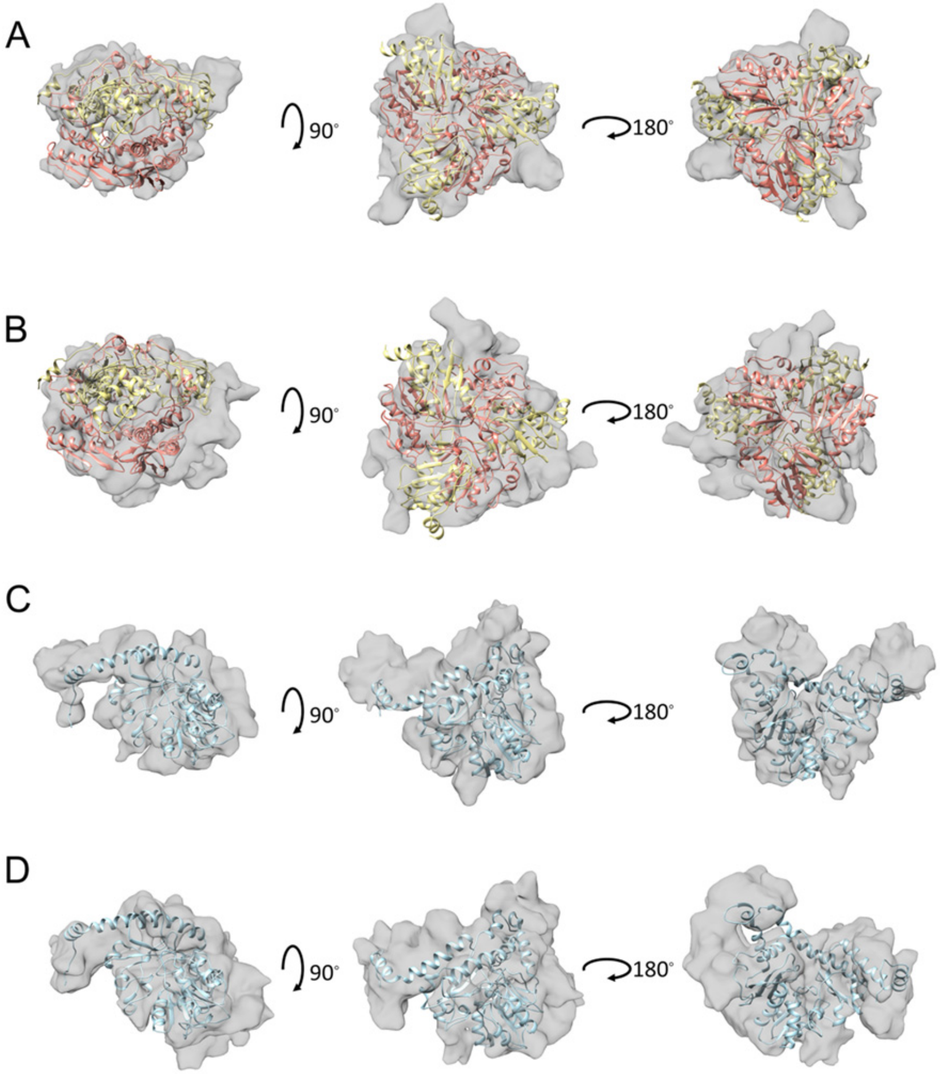

Supplemental Figure S1. (*A-B*), the EM density maps of the refined 3D models without symmetry applied and with 432 symmetry applied were segmented using Chimera. Segmentation revealed volumes with similar shapes around the 3-fold symmetry axes. Shown is the simulated structure of the $[\mathrm{FXN}^{42\text{-}210}]_3 \bullet [\mathrm{ISCU}]_3$ sub-complex docked into one of these volumes from the 3D model without symmetry applied (*A*) compared to the model with 432 symmetry applied (*B*). Cross-correlation functions were 0.54 and 0.62 for the 3D model without and with 432 symmetry applied, respectively. (*C-D*), segmentation also revealed volumes with similar shapes around the 4-fold symmetry axes of the two 3D models. Shown is the simulated structure of NFS1 monomer docked into one of these volumes from the 3D model without symmetry applied (*C*) compared to the model with 432 symmetry applied (*D*). Cross-correlation functions were 0.56 and 0.57 for the 3D model without and with 432 symmetry applied, respectively.

Supplemental Table S1. Mass spectrometry analysis of protein bands

Protein	**Band*****		**Band****		**Band***		**ISCU**	
	TSC	**% TSC**	**TSC**	**% TSC**	**TSC**	**% TSC**	**TSC**	**% TSC**
FXN	164	61%	41	24%	6	3%	11	5%
NFS1	97	36%	108	62%	22	13%	27	13%
ISCU	6	2%	23	13%	140	80%	157	77%
ISD11	0	0%	2	1%	8	5%	8	4%
TSC, Total Spectrum Count, total number of spectra associated with each protein including replicates.								

The ISCU band and the three bands denoted by asterisks in Fig. 1G were analyzed by tandem mass spectrometry as described in Experimental Procedures. The most abundant protein in band* is ISCU (80% of the total spectra identified), and the overall protein composition of this band is very similar to that of the actual ISCU band. These data suggest that band* is a faster migrating form of ISCU, which may result from *intra*-molecular disulfide bonds that make ISCU more compact and increase its mobility. The most abundant protein in band** is NFS1 (62% of the total spectra identified), suggesting a faster migrating form of NFS1 due to *intra*-molecular disulfide bonds. The presence of both FXN and ISCU peptides in this band and its apparent molecular mass of ~44 kDa may also be consistent with the presence of FXN$^{42\text{-}210}$-ISCU adducts. However, given that band** is barely detectable (Fig. 1G), only negligible amounts of these species may be present. The most abundant protein in band*** is FXN$^{42\text{-}210}$ (61% of the total spectra identified), although significant amounts of NFS1 are also present (36% of the total spectra identified). It is unlikely band*** represents a FXN$^{42\text{-}210}$-NFS1 adduct given its apparent molecular mass of ~46 kDa. On the other hand, the presence of NFS1 in band*** could have resulted from diffusion from the NFS1 band immediately underneath. In addition, we detected the same ~46 kDa band by non-reducing SDS-PAGE analysis of individually expressed and purified oligomeric FXN$^{42\text{-}210}$ (not shown). Therefore, band*** most likely represents a slower migrating form of FXN$^{42\text{-}210}$, probably dimer given the apparent molecular mass of ~46 kDa. This species may result from an *inter*-molecular disulfide bond between FXN$^{42\text{-}210}$ subunits. It is unlikely that *inter*-molecular disulfide bonds are required to stabilize oligomeric FXN$^{42\text{-}210}$ as the protein contains only one Cys residue, Cys-50, which is outside of the N-terminal region necessary and sufficient for oligomerization (residues 56-78) (1). In addition, in the structural model of the complex, the position of the N-terminal region, which was modeled on the basis of the cross-linking data, is not compatible with the formation of disulfide bonds between adjacent FXN$^{42\text{-}210}$ subunits. Thus, this region of the protein, which is predicted to be highly flexible, may sample alternative configurations some of which may favor disulfide bond formation between adjacent FXN$^{42\text{-}210}$ subunits. Accordingly, the ~46 kDa band was also detected by non-reducing SDS-PAGE analysis of purified monomeric FXN$^{42\text{-}210}$ (not shown).

1. O'Neill, H. A., Gakh, O., and Isaya, G. (2005) Supramolecular assemblies of human frataxin are formed via subunit-subunit interactions mediated by a non-conserved amino-terminal region. *J. Mol. Biol.* **345**, 433-439

Supplemental Table S2. Analysis of cross-linked peptides and distances between pairs of cross-linked amino acids in complex structure

Supplemental Table S2a, p.1

Cross-linked peptides			Distances (Å) measured in structure				False Discovery Rate (FDR) of cross-linked peptides					
FXN$^{42-210}$	FXN$^{42-210}$	Figure	FXN$^{42-210}$ intra-monomer	FXN$^{42-210}$-FXN$^{42-210}$ intra-trimer	FXN$^{42-210}$-FXN$^{42-210}$ inter-trimers	# of peptides						
Single digestion GluC							≤5 %	≤10%	≤15%	≤25 %	≤35 %	≤60%
$T_{93}T_{94}Y_{95}E$	$FFEDLADK_{116}PYTFE$					1				1		
T_{93}	K_{116}		35.8 ± 1.5	31.2 ± 1.1	28.1 ± 0.8							
T_{94}	K_{116}		33.3 ± 0.9	32.9 ± 1.1	26.3 ± 1.1							
Y_{95}	K_{116}	6B,C,D	31.9 ± 1.4	34.2 ± 1.6	26.3 ± 1.2							
							≤5 %	≤10%	≤15%	≤25 %	≤35 %	≤60%
$ET_{102}LDS_{105}LAE$	$FFEDLADK_{116}PY_{118}T_{119}FE$					1				1		2
T_{102}	K_{116}	6B,D	21.9 ± 0.5	45.2 ± 1.6	23.4 ± 1.6							
S_{105}	K_{116}	6B,D	17.6 ± 0.5	48.6 ± 1.7	18.8 ± 1.6							
Double digestion GluC&AspN							≤5%	≤10%	≤15%	≤25%	≤35 %	≤60%
$LTK_{192}ALK_{195}TK_{197}L$	$DLS_{201}S_{202}LAY_{205}S_{206}GK_{208}$					6	4		2			
K_{192}	S_{201}		14.4±07	39.9±0.9	31.5±1.3							
K_{192}	S_{202}		17.5±0.7	37.7±1.1	29.8±2.0							
K_{192}	Y_{205}	6B	23.0±1.5	31.0±1.1	27.1±2.3							
K_{192}	S_{206}		20.3±1.6	34.0±1.3	30.2±2.0							
K_{192}	K_{208}		19.0±1.0	37.8±1.4	31.7±1.2							
K_{195}	S_{201}	6B	16.9±0.7	44.8±1.1	31.9±2.3							
K_{195}	S_{202}		19.4±0.9	42.6±1.3	28.4±2.5							
K_{195}	Y_{205}	6D	26.0±1.3	36.0±1.3	26.5±2.5							
K_{195}	S_{206}		23.3±1.5	38.9±1.4	29.4±2.2							
K_{195}	K_{208}		21.0±1.1	42.5±1.4	29.8±1.3							
K_{197}	S_{201}		12.1±0.9	44.8±1.2	35.8±2.3							
K_{197}	S_{202}		14.2±1.2	43.0±1.5	32.1±2.0							
K_{197}	Y_{205}		21.1±1.3	35.9±1.4	31.2±2.0							
K_{197}	S_{206}		18.4±1.8	38.5±1.5	34.0±1.7							
K_{197}	K_{208}	6B	15.1±1.5	42.2±1.8	34.4±1.0							
T_{191}	K_{208}		16.2±1.1	39.1±1.5	32.2±0.9							
T_{196}	K_{208}		18.8±1.4	43.8±1.5	31.6±1.2							
							≤5 %	≤10%	≤15%	≤25 %	≤35 %	≤60%
$DLS_{201}S_{202}LAY_{205}S_{206}GK_{208}$	$DWT_{169}GK_{171}NWVY_{175}S_{176}H$					3	3					
K_{208}	T_{169}		19.4±2.3	25.0±2.1	48.0±1.4							
K_{208}	K_{171}	6B	15.2±2.0	25.2±2.1	44.8±1.3							
K_{208}	Y_{175}		17.9±1.4	24.6±1.7	43.5±0.7							
K_{208}	S_{176}	6C	21.0±1.5	21.5±1.8	44.0±1.2							
S_{201}	K_{171}		15.5±1.8	24.0±1.8	38.1±1.4							
S_{202}	K_{171}		15.3±1.8	26.3±2.1	39.8±1.2							
Y_{205}	K_{171}	6C	8.1±1.6	22.5±1.6	45.0±1.5							
S_{206}	K_{171}	6C	9.4±1.9	21.2±2.0	43.7±1.0							

Supplemental Table S2a, p.2

Cross-linked peptides			Distances (Å) measured in structure				False Discovery Rate (FDR) of cross-linked peptides					
FXN$^{42-210}$	**FXN$^{42-210}$**	**Figure**	**FXN$^{42-210}$ intra-monomer**	**FXN$^{42-210}$-FXN$^{42-210}$ intra-trimer**	**FXN$^{42-210}$-FXN$^{42-210}$ inter-trimers**	**# of peptides**						
							≤5 %	**≤10%**	**≤15%**	**≤25 %**	**≤35 %**	**≤40%**
DVSFGSGVLTVK$_{135}$LGG	DLGTYVINK$_{147}$QTPNK$_{152}$QIWLSSPSSGPK$_{164}$RY					4				1		3
K$_{135}$	K$_{147}$		17.6±0.7	33.0±1.8	17.3±3.9							
K$_{135}$	K$_{152}$	6D	24.3±0.7	28.4±1.5	17.5±2.7							
K$_{135}$	K$_{164}$	6B,D	18.4±0.8	34.7±1.1	14.3±2.2							
LOW SCORE PEPTIDE							**≤5 %**	**≤10%**	**≤15%**	**≤25 %**	**≤35 %**	**≤40%**
DK$_{116}$PY$_{118}$TFEDY$_{123}$	DVSFGSGVLTVK$_{135}$LGGDLGTYVINK$_{147}$QTPNK$_{152}$QIWLSSPSSGPK$_{164}$RY					3						3
K$_{116}$	K$_{135}$	6B	18.0±2.9	52.2±2.2	26.0±1.8							
K$_{116}$	K$_{147}$		27.7±1.1	46.4±1.3	37.7±2.1							
K$_{116}$	K$_{152}$		33.0±0.7	40.5±1.1	36.8±3.0							
K$_{116}$	K$_{164}$		29.4±1.2	37.7±0.7	33.6±1.9							
Y$_{118}$	K$_{135}$	6B	17.8±2.8	55.9±1.8	30.3±3.2							
Y$_{118}$	K$_{147}$		29.0±1.5	46.5±1.1	32.7±2.4							
Y$_{118}$	K$_{152}$		34.5±0.7	40.7±1.1	33.9±2.0							
Y$_{118}$	K$_{164}$		28.6±1.4	40.6±0.5	30.5±1.7							
Y$_{123}$	K$_{135}$	6B,D	7.7±2.5	51.1±3.1	22.6±1.9							
Y$_{123}$	K$_{147}$	6B,D	21.6±0.8	39.2±0.6	22.5±3.1							
Y$_{123}$	K$_{152}$		28.2±1.2	33.9±0.6	24.2±2.2							
Y$_{123}$	K$_{164}$	6B,D	22.9±1.2	36.7±1.4	21.7±1.6							

Supplemental Table S2b, p.1

Cross-linked peptides			Distances (Å) measured in structure				False Discovery Rate (FDR) of cross-linked peptides					
ISCU	**ISCU**	**Figure**	**ISCU intra-monomer**	**ISCU-ISCU intra-trimer**	**ISCU-ISCU inter-trimers**	**# of peptides**						
Single digestion GluC							**≤5 %**	**≤10%**	**≤15%**	**≤25 %**	**≤35 %**	**≤60%**
AEK$_{166}$K$_{167}$	DAIK$_{147}$AALADY$_{153}$K$_{154}$LK$_{156}$QEPK$_{160}$K$_{161}$GE					21	12	3	6			
K_{166}	K_{147}	6F	9.5 ± 1.0	24.4 ± 0.9	46.5 ± 0.7							
K_{166}	Y_{153}		14.0 ± 1.0	23.4 ± 1.1	56.1 ± 0.8							
K_{166}	K_{154}	6F,G	11.7 ± 1.0	20.2 ± 2.2	56.4 ± 0.7							
K_{166}	K_{156}	6G	16.6 ± 1.0	22.2 ± 2.0	60.8 ± 0.8							
K_{166}	K_{160}		10.8 ± 0.4	13.0 ± 1.8	58.8 ± 0.5							
K_{166}	K_{161}	6F,G	9.1 ± 0.6	11.2 ± 1.7	57.2 ± 0.4							
K_{167}	K_{147}		6.9 ± 0.8	25.6 ± 0.8	45.0 ± 1.1							
K_{167}	Y_{153}		12.7 ± 1.3	24.3 ± 1.1	54.6 ± 1.2							
K_{167}	K_{154}	6F	11.0 ± 1.1	21.9 ± 1.1	55.1 ± 1.0							
K_{167}	K_{156}	6F	15.7 ± 1.4	23.4 ± 1.4	59.4 ± 1.2							
K_{167}	K_{160}	6F,G	11.5 ± 0.4	15.0 ± 1.0	57.9 ± 0.4							
K_{167}	K_{161}		10.9 ± 0.6	13.8 ± 0.9	56.5 ± 0.4							
							≤5 %	**≤10%**	**≤15%**	**≤25 %**	**≤35 %**	**≤60%**
WVK$_{110}$GK$_{112}$T$_{113}$VEE	ALT$_{119}$IK$_{121}$NT$_{123}$DIAK$_{127}$E					23	17			6		2
K_{110}	T_{119}		13.9 ± 0.5	30.4 ± 0.7	45.8 ± 1.1							
K_{110}	K_{121}		15.1 ± 0.3	33.6 ± 0.6	39.4 ± 1.2							
K_{110}	T_{123}		17.3 ± 0.2	38.6 ± 0.6	35.8 ± 1.2							
K_{110}	K_{127}	6F	16.1 ± 0.5	43.5 ± 0.6	36.0 ± 1.2							
K_{112}	T_{119}		11.2 ± 0.2	25.3 ± 0.7	46.8 ± 1.2							
K_{112}	K_{121}	6F,G	14.3 ± 0.5	28.4 ± 0.6	40.4 ± 1.2							
K_{112}	T_{123}		16.8 ± 0.6	33.3 ± 0.7	36.8 ± 1.3							
K_{112}	K_{127}	6F	16.4 ± 0.9	38.4 ± 0.6	37.5 ± 1.3							
T_{113}	K_{121}		15.0 ± 0.4	25.7 ± 0.6	42.5 ± 0.9							
T_{113}	K_{127}		18.9 ± 0.9	35.6 ± 0.6	39.1 ± 1.0							
							≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
AEK$_{166}$K$_{167}$	K$_{160}$K$_{161}$GEAE					15	10	3	2			5
K_{166}	K_{160}		10.8 ± 0.4	13.0 ± 1.8	58.8 ± 0.5							
K_{166}	K_{161}	6F,G	9.1 ± 0.6	11.2 ± 1.7	57.2 ± 0.4							
K_{167}	K_{160}	6F,G	11.5 ± 0.4	15.0 ± 1.0	57.9 ± 0.4							
K_{167}	K_{161}		10.9 ± 0.6	13.8 ± 0.9	56.5 ± 0.4							

Supplemental Table S2b, p.2

Cross-linked peptides			Distances (Å) measured in structure				False Discovery Rate (FDR) of cross-linked peptides					
ISCU	ISCU	Figure	ISCU intra-monomer	ISCU-ISCU intra-trimer	ISCU-ISCU inter-trimers	# of peptides						
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
PK$_{160}$K$_{161}$GE	DAIK$_{147}$AALADY$_{153}$K$_{154}$LK$_{156}$QE					7	4	1	2			
K_{160}	K_{147}	6F,G	14.7 ± 1.1	15.9 ± 0.8	56.4 ± 1.1							
K_{160}	Y_{153}		11.3 ± 0.8	12.9 ± 1.2	65.9 ± 1.2							
K_{160}	K_{154}	6F	7.6 ± 0.9	10.7 ± 1.2	66.5 ± 1.0							
K_{160}	K_{156}	6F,G	10.0 ± 0.4	14.0 ± 1.2	70.8 ± 1.1							
K_{161}	K_{147}		15.1 ± 0.8	16.3 ± 0.9	55.4 ± 0.9							
K_{161}	Y_{153}		13.5 ± 0.6	14.7 ± 1.2	65.1 ± 0.9							
K_{161}	K_{154}	6G	9.7 ± 0.6	11.8 ± 1.3	65.4 ± 0.8							
K_{161}	K_{156}		13.1 ± 0.3	15.6 ± 1.3	69.8 ± 0.9							
							≤5 %	≤10%	≤15%	≤25 %	≤35 %	≤60%
AEK$_{166}$K$_{167}$	ALT$_{119}$IK$_{121}$NT$_{123}$DIAK$_{127}$E					7	3	1	1	2		1
K_{166}	T_{119}		7.0 ± 1.5	18.3 ± 0.8	44.5 ± 0.6							
K_{166}	K_{121}	6F,G	11.5 ± 0.8	24.5 ± 0.9	38.6 ± 0.5							
K_{166}	T_{123}		16.4 ± 0.8	27.7 ± 1.1	33.8 ± 0.5							
K_{166}	K_{127}		20.9 ± 1.0	29.7 ± 1.3	33.1 ± 0.6							
K_{167}	T_{119}		7.9 ± 1.5	19.6 ± 1.0	43.3 ± 0.7							
K_{167}	K_{121}		10.7 ± 1.3	25.6 ± 1.0	37.3 ± 0.6							
K_{167}	T_{123}		15.8 ± 1.3	28.7 ± 1.2	32.4 ± 0.6							
K_{167}	K_{127}	6F,H	20.5 ± 1.2	29.9 ± 1.3	31.2 ± 0.6							
							≤5 %	≤10%	≤15%	≤25 %	≤35 %	≤60%
PK$_{160}$K$_{161}$GE	WVK$_{110}$GK$_{112}$T$_{113}$VEE					2		1		1		
K_{160}	K_{110}	6G	23.2 ± 1.2	16.5 ± 0.8	57.4 ± 1.2							
K_{160}	K_{112}	6F	20.9 ± 1.2	12.5 ± 0.9	58.3 ± 1.5							
K_{160}	T_{113}		17.7 ± 1.2	8.8 ± 0.9	60.8 ± 1.3							
K_{161}	K_{110}		23.1 ± 1.0	18.9 ± 0.8	57.1 ± 1.0							
K_{161}	K_{112}		20.0 ± 1.1	14.6 ± 0.9	57.8 ± 1.4							
K_{161}	T_{113}		16.8 ± 1.1	10.9 ± 0.9	60.1 ± 1.2							
							≤5 %	≤10%	≤15%	≤25 %	≤35 %	≤60%
PK$_{160}$K$_{161}$GE	PK$_{160}$K$_{161}$GE					4			2	2		
K_{160}	K_{160}	6G	NA	11.8 ± 0.7	69.1 ± 0.2							
K_{160}	K_{161}		3.8 ± 0.04	10.5 ± 0.5	67.6 ± 0.3							
K_{161}	K_{160}		3.8 ± 0.04	10.5 ± 0.5	67.6 ± 0.3							
K_{161}	K_{161}		NA	8.4 ± 0.6	66.1 ± 0.1							

Supplemental Table S2b, p.3

Cross-linked peptides			Distances (Å) measured in structure				False Discovery Rate (FDR) of cross-linked peptides					
ISCU	ISCU	Figure	ISCU intra-monomer	ISCU-ISCU intra-trimer	ISCU-ISCU inter-trimers	# of peptides						
							≤5 %	≤10%	≤15%	≤25 %	≤35 %	≤60%
ALT$_{119}$IK$_{121}$NT$_{123}$DIAK$_{127}$E	LCLPPVK$_{135}$LHCS$_{139}$MLAE					2	2					
K$_{121}$	K$_{135}$		13.5 ± 0.8	44.8 ± 1.3	17.2 ± 1.6							
K$_{121}$	S$_{139}$		10.5 ± 1.1	41.8 ± 1.4	20.5 ± 1.1							
K$_{127}$	K$_{135}$	6F,H	12.0±0.7	46.4±1.5	12.9±1.5							
K$_{127}$	S$_{139}$	6H	11.2±1.1	43.7±1.4	15.5±1.2							
Double digestion GluC&AspN							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
AEK$_{166}$K$_{167}$	DY$_{153}$K$_{154}$LK$_{156}$QEPK$_{160}$K$_{161}$GE					18	6	3	7	2		
K$_{166}$	Y$_{153}$		14.0 ± 1.0	23.4 ± 1.1	56.1 ± 0.8							
K$_{166}$	K$_{154}$		11.7 ± 1.0	20.2 ± 2.2	56.4 ± 0.7							
K$_{166}$	K$_{156}$		16.6 ± 1.0	22.2 ± 2.0	60.8 ± 0.8							
K$_{166}$	K$_{160}$		10.8 ± 0.4	13.0 ± 1.8	58.8 ± 0.5							
K$_{166}$	K$_{161}$		9.1 ± 0.6	11.2 ± 1.7	57.2 ± 0.4							
K$_{167}$	Y$_{153}$		12.7 ± 1.3	24.3 ± 1.1	54.6 ± 1.2							
K$_{167}$	K$_{154}$		11.0 ± 1.1	21.9 ± 1.1	55.1 ± 1.0							
K$_{167}$	K$_{156}$		15.7 ± 1.4	23.4 ± 1.4	59.4 ± 1.2							
K$_{167}$	K$_{160}$		11.5 ± 0.4	15.0 ± 1.0	57.9 ± 0.4							
K$_{167}$	K$_{161}$		10.9 ± 0.6	13.8 ± 0.9	56.5 ± 0.4							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
AEK$_{166}$K$_{167}$	DK$_{54}$T$_{55}$S$_{56}$K$_{57}$NVGT$_{61}$GLVGAPACG					5	2	1	2			
K$_{166}$	K$_{54}$		28.0±1.1	30.1±1.6	66.3±1.1							
K$_{166}$	T$_{55}$		25.3±1.3	26.5±1.7	64.1±0.9							
K$_{166}$	S$_{56}$		21.9±1.2	24.1±1.6	62.8±0.8							
K$_{166}$	K$_{57}$	6F	20.6±1.1	24.9±1.9	62.7±0.9							
K$_{166}$	T$_{61}$		17.8±1.2	30.1±1.1	53.4±1.4							
K$_{167}$	K$_{54}$		27.3±1.0	29.3±2.0	64.3±1.4							
K$_{167}$	T$_{55}$		24.9±1.1	25.7±2.1	62.1±1.1							
K$_{167}$	S$_{56}$		21.6±1.0	23.6±1.9	61.0±0.9							
K$_{167}$	K$_{57}$		19.9±1.1	24.9±2.1	61.0±0.9							
K$_{167}$	T$_{61}$		15.2±1.6	30.8±1.1	51.6±1.6							

Supplemental Table S2b, p.4

Cross-linked peptides			Distances (Å) measured in structure				False Discovery Rate (FDR) of cross-linked peptides					
ISCU	**ISCU**	**Figure**	**ISCU intra-monomer**	**ISCU-ISCU intra-trimer**	**ISCU-ISCU inter-trimers**	**# of peptides**						
							≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
K$_{82}$GK$_{84}$IV	WVK$_{110}$GK$_{112}$TVEE					5	1	1	1	2		
K$_{82}$	K$_{110}$		13.8±0.5	25.2±0.4	58.2±0.6							
K$_{82}$	K$_{112}$	6F	11.1±0.9	23.8±0.8	59.7±1.7							
K$_{84}$	K$_{110}$		7.3±0.3	30.9±0.6	54.7±0.4							
K$_{84}$	K$_{112}$		5.7±1.0	29.9±0.8	56.7±0.8							
							≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DY$_{153}$K$_{154}$LK$_{156}$QEPK$_{160}$K$_{161}$GE	DK$_{54}$T$_{55}$S$_{56}$K$_{57}$NVGT$_{61}$GLVGAPACG					4	3		1			
K$_{154}$	K$_{54}$		17.5±0.4	19.9±0.9	71.4±0.8							
K$_{154}$	T$_{55}$		15.3±0.3	16.5±0.9	69.6±0.5							
K$_{154}$	S$_{56}$		11.8±0.3	14.4±0.6	68.7±0.6							
K$_{154}$	K$_{57}$		9.5±0.5	15.6±1.0	68.6±0.6							
K$_{154}$	T$_{61}$		10.8±0.2	24.6±0.4	58.5±0.7							
K$_{156}$	K$_{54}$	6F	14.7±0.6	14.9±0.9	74.6±0.8							
K$_{156}$	T$_{55}$		13.4±0.4	11.8±1.0	72.8±0.7							
K$_{156}$	S$_{56}$		10.1±0.4	10.1±0.6	72.1±0.4							
K$_{156}$	K$_{57}$		6.7±0.3	12.0±0.8	72.2±0.4							
K$_{156}$	T$_{61}$		11.0±0.4	22.4±0.05	62.1±0.5							
K$_{160}$	K$_{54}$	6G	23.7±0.8	20.6±1.1	75.5±1.5							
K$_{160}$	T$_{55}$		21.4±0.6	17.1±1.1	73.4±1.2							
K$_{160}$	S$_{56}$		17.7±0.6	14.1±1.2	72.3±1.1							
K$_{160}$	K$_{57}$		15.4±0.8	14.6±1.7	72.4±1.1							
K$_{160}$	T$_{61}$		17.4±0.9	19.7±1.2	62.9±1.6							
K$_{161}$	K$_{54}$		25.8±0.7	24.0±1.0	75.1±1.3							
K$_{161}$	T$_{55}$		23.2±0.5	20.6±1.0	72.9±1.0							
K$_{161}$	S$_{56}$		19.6±0.6	17.5±1.1	71.7±0.9							
K$_{161}$	K$_{57}$		17.8±0.9	17.4±1.6	71.7±0.9							
K$_{161}$	T$_{61}$		19.7±0.7	21.5±1.1	62.2±1.4							
Y$_{153}$	K$_{54}$		15.1±0.6	20.0±1.0	69.8±0.7							
Y$_{153}$	K$_{57}$		7.8±0.4	17.1±0.9	67.3±0.5							

Supplemental Table S2b, p.5

Cross-linked peptides			Distances (Å) measured in structure				False Discovery Rate (FDR) of cross-linked peptides					
ISCU	ISCU	Figure	ISCU intra-monomer	ISCU-ISCU intra-trimer	ISCU-ISCU inter-trimers	# of peptides						
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
PK$_{160}$K$_{161}$GE	DY$_{153}$K$_{154}$LK$_{156}$QE					4	1		1	2		
K$_{160}$	Y$_{153}$		11.3 ± 0.8	12.9 ± 1.2	65.9 ± 1.2							
K$_{160}$	K$_{154}$		7.6 ± 0.9	10.7 ± 1.2	66.5 ± 1.0							
K$_{160}$	K$_{156}$		10.0 ± 0.4	14.0 ± 1.2	70.8 ± 1.1							
K$_{161}$	Y$_{153}$		13.5 ± 0.6	14.7 ± 1.2	65.1 ± 0.9							
K$_{161}$	K$_{154}$		9.7 ± 0.6	11.8 ± 1.3	65.4 ± 0.8							
K$_{161}$	K$_{156}$		13.1 ± 0.3	15.6 ± 1.3	69.8 ± 0.9							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
DIAK$_{127}$E	WVK$_{110}$GK$_{112}$TVEE					3		1	1	1		
K$_{127}$	K$_{110}$		16.1 ± 0.5	43.5 ± 0.6	36.0 ± 1.2							
K$_{127}$	K$_{112}$		16.4 ± 0.9	38.4 ± 0.6	37.5 ± 1.3							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
DVMK$_{74}$LQIQV	DK$_{54}$T$_{55}$S$_{56}$K$_{57}$NVGT$_{61}$GLVGAPACG					2	2					
K$_{74}$	K$_{54}$		22.8±1.0	30.1±1.1	57.1±1.0							
K$_{74}$	T$_{55}$		22.3±0.8	27.2±0.9	55.7±1.1							
K$_{74}$	S$_{56}$		20.9±0.3	27.2±0.7	55.4±0.6							
K$_{74}$	K$_{57}$	6F	19.5±0.3	29.9±0.6	55.6±0.7							
K$_{74}$	T$_{61}$		7.6±0.2	38.5±0.7	45.6±0.4							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
n-MHK$_{37}$K$_{38}$VVDHYENPRNVGS$_{51}$L	DK$_{54}$T$_{55}$S$_{56}$K$_{57}$NVGT$_{61}$GLVGAPACG					1	1					
N-term												
K$_{37}$	K$_{54}$		NM	NM	NM							
K$_{37}$	T$_{55}$		NM	NM	NM							
K$_{37}$	S$_{56}$		NM	NM	NM							
K$_{37}$	K$_{57}$		NM	NM	NM							
K$_{37}$	T$_{61}$		NM	NM	NM							
K$_{38}$	K$_{54}$		NM	NM	NM							
K$_{38}$	T$_{55}$		NM	NM	NM							
K$_{38}$	S$_{56}$		NM	NM	NM							
K$_{38}$	K$_{57}$		NM	NM	NM							
K$_{38}$	T$_{61}$		NM	NM	NM							
S$_{51}$	K$_{54}$		9.0±0.3	26.0±1.0	69.3±1.4							
S$_{51}$	K$_{57}$		12.3±0.6	23.8±1.3	67.6±1.5							

Supplemental Table S2b, p.6

Cross-linked peptides			Distances (Å) measured in structure				False Discovery Rate (FDR) of cross-linked peptides					
ISCU	ISCU	Figure	ISCU intra-monomer	ISCU-ISCU intra-trimer	ISCU-ISCU inter-trimers	# of peptides						
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
WVK$_{110}$GK$_{112}$TVEE	DK$_{54}$T$_{55}$S$_{56}$K$_{57}$NVGT$_{61}$GLVGAPACG DVMK$_{74}$LQIQV					1	1					
K$_{110}$	K$_{54}$		19.7±0.6	33.7±1.2	62.8±1.0							
K$_{110}$	T$_{55}$		17.1±0.8	31.4±0.9	61.5±0.8							
K$_{110}$	S$_{56}$		16.4±0.6	29.8±0.5	60.3±0.4							
K$_{110}$	K$_{57}$		17.8±0.8	26.3±0.9	59.5±0.4							
K$_{110}$	T$_{61}$		16.0±0.4	26.3±1.2	48.0±0.5							
K$_{110}$	K$_{74}$		16.8±0.8	31.2±1.6	41.0±0.9							
K$_{112}$	K$_{54}$		21.3±1.1	32.0±1.6	65.6±1.1							
K$_{112}$	T$_{55}$		18.0±1.2	30.9±1.4	64.1±1.0							
K$_{112}$	S$_{56}$		16.7±1.4	27.4±1.2	62.7±0.9							
K$_{112}$	K$_{57}$		18.2±1.6	24.2±1.3	61.8±0.9							
K$_{112}$	T$_{61}$		18.4±0.8	23.0±1.4	50.5±1.0							
K$_{112}$	K$_{74}$		20.3±0.9	27.5±1.7	43.9±1.3							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
ALT$_{119}$IK$_{121}$NT$_{123}$	DAIK$_{147}$AALA					1	1					
T$_{119}$	K$_{147}$		8.8±0.5	25.2±1.0	42.2±1.0							
K$_{121}$	K$_{147}$		9.6±0.4	31.3±1.1	36.0±0.9							
T$_{123}$	K$_{147}$		14.4±0.5	34.4±1.1	31.3±0.9							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
ALT$_{119}$IK$_{121}$NT$_{123}$	DY$_{153}$K$_{154}$LK$_{156}$QEPK$_{160}$K$_{161}$GE					1	1					
T$_{119}$	K$_{154}$		14.2±0.6	20.6±1.3	52.3±1.0							
K$_{121}$	K$_{154}$		18.5±0.5	26.8±1.2	46.1±0.8							
T$_{123}$	K$_{154}$		23.6±0.5	30.9±1.2	41.4±0.8							
T$_{119}$	K$_{156}$		19.3±0.6	22.3±1.2	56.5±1.1							
K$_{121}$	K$_{156}$		23.2±0.5	28.0±1.0	50.3±0.9							
T$_{123}$	K$_{156}$		28.4±0.5	32.2±1.1	45.5±0.9							
T$_{119}$	K$_{160}$		16.0±0.8	13.2±0.6	54.6±0.7							
K$_{121}$	K$_{160}$		21.0±0.7	19.5±0.6	48.6±0.6							
T$_{123}$	K$_{160}$		26.2±0.7	23.8±0.6	43.8±0.6							
T$_{119}$	K$_{161}$		14.3±0.8	12.5±0.7	53.3±0.5							
K$_{121}$	K$_{161}$		19.8±0.6	19.0±0.6	47.5±0.5							
T$_{123}$	K$_{161}$		24.8±0.5	23.3±0.6	42.7±0.5							
K$_{121}$	Y$_{153}$		18.7±0.5	30.3±1.2	45.6±1.0							

Supplemental Table S2b, p.7

Cross-linked peptides			Distances (Å) measured in structure				False Discovery Rate (FDR) of cross-linked peptides					
ISCU	ISCU	Figure	ISCU intra-monomer	ISCU-ISCU intra-trimer	ISCU-ISCU inter-trimers	# of peptides						
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
PK$_{160}$K$_{161}$GE	ALT$_{119}$IK$_{121}$NT$_{123}$					1		1				
K$_{160}$	T$_{119}$		16.0±0.8	13.2±0.6	54.6±0.7							
K$_{160}$	K$_{121}$	6G	21.0±0.7	19.5±0.6	48.6±0.6							
K$_{160}$	T$_{123}$		26.2±0.7	23.8±0.6	43.8±0.6							
K$_{161}$	T$_{119}$		14.3±0.8	12.5±0.7	53.3±0.5							
K$_{161}$	K$_{121}$		19.8±0.6	19.0±0.6	47.5±0.5							
K$_{161}$	T$_{123}$		24.8±0.5	23.3±0.6	42.7±0.5							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
AEK$_{166}$K$_{167}$	K$_{82}$GK$_{84}$IV					1			1			
K$_{166}$	K$_{82}$	6F	16.2±1.7	15.4±1.5	58.2±0.7							
K$_{166}$	K$_{82}$		18.1±1.7	19.0±2.0	54.3±0.4							
K$_{167}$	K$_{84}$	6G	17.2±1.1	15.0±1.5	56.7±0.4							
K$_{167}$	K$_{84}$		18.6±1.0	18.0±1.8	52.5±0.7							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
WVK$_{110}$GK$_{112}$TVEE	ALT$_{119}$IK$_{121}$NT$_{123}$					1			1			
K$_{110}$	T$_{119}$		13.9 ± 0.5	30.4 ± 0.7	45.8 ± 1.1							
K$_{110}$	K$_{121}$		15.1 ± 0.3	33.6 ± 0.6	39.4 ± 1.2							
K$_{110}$	T$_{123}$		17.3 ± 0.2	38.6 ± 0.6	35.8 ± 1.2							
K$_{112}$	T$_{119}$		11.2 ± 0.2	25.3 ± 0.7	46.8 ± 1.2							
K$_{112}$	K$_{121}$		14.3 ± 0.5	28.4 ± 0.6	40.4 ± 1.2							
K$_{112}$	T$_{123}$		16.8 ± 0.6	33.3 ± 0.7	36.8 ± 1.3							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
n-MHK$_{37}$K$_{38}$VVDHYENPRNVGS$_{51}$L	DVMK$_{74}$LQIQV					1				1		
N-term	K$_{74}$		NM	NM	NM							
K$_{37}$	K$_{74}$		NM	NM	NM							
K$_{38}$	K$_{74}$		NM	NM	NM							
S$_{51}$	K$_{74}$	6F	16.7±1.4	34.8±1.5	49.0±1.4							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
DIAK$_{127}$E	DAIK$_{147}$AALA					1				1		
K$_{127}$	K$_{147}$		17.9±0.6	35.3±1.3	30.0±0.8							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
DIAK$_{127}$E	LCLPPVK$_{135}$LHCS$_{139}$MLAE					1			1			
K$_{127}$	K$_{135}$	6H	12.0±0.7	46.4±1.5	12.9±1.5							
K$_{127}$	S$_{139}$	6H	11.2±1.1	43.7±1.4	15.5±1.2							

Supplemental Table S2b, p.8

Cross-linked peptides			Distances (Å) measured in structure				False Discovery Rate (FDR) of cross-linked peptides					
ISCU	ISCU	Figure	ISCU intra-monomer	ISCU-ISCU intra-trimer	ISCU-ISCU inter-trimers	# of peptides						
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
DAIK$_{147}$AALA	DY$_{153}$K$_{154}$LK$_{156}$QE					1				1		
K$_{147}$	Y$_{153}$		9.9±0.1	26.3±1.0	52.1±0.6							
K$_{147}$	K$_{154}$		10.4±0.2	22.9±1.0	53.0±0.5							
K$_{147}$	K$_{156}$		14.6±0.1	22.9±1.0	57.0±0.5							

Supplemental Table S2c, p.1

Cross-linked peptides			Distances (Å) measured in structure				# of peptides	False Discovery Rate (FDR) of cross-linked peptides					
NFS1	**NFS1**	**Figure**	**Monomer**	**Dimer**	**Trimer**	**Tetramer**							
Single digestion GluC								**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
IGVK$_{212}$QPIAE	Y$_{317}$DHK$_{320}$RIS$_{323}$K$_{324}$LSE						15	7	2	2			
K$_{212}$	Y$_{317}$		20.5 ± 1.0	74.3 ± 1.9	59.6 ± 2.3	40.0 ± 2.3							
K$_{212}$	K$_{320}$	7B	16.4 ± 0.9	71.2 ± 2.2	64.5 ± 2.3	43.9 ± 1.9							
K$_{212}$	S$_{323}$		12.8 ± 0.4	66.2 ± 2.2	67.5 ± 1.9	48.2 ± 1.5							
K$_{212}$	K$_{324}$	7B	16.0 ± 0.3	66.4 ± 2.3	66.4 ± 2.2	50.2 ± 1.8							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
MS$_{437}$PLWE	HK$_{157}$CVLDS$_{162}$CRS$_{165}$LE						2	1			1		
S$_{437}$	K$_{157}$	7C	40.2 ± 1.1	19.0 ± 0.9	55.5 ± 3.7	67.6 ± 1.2							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
IGVK$_{212}$QPIAE	K$_{425}$CIQHVK$_{431}$RLREMS$_{437}$PLWE						2		1			1	
K$_{212}$	K$_{425}$	7B	26.1 ± 0.8	49.5 ± 2.3	53.1 ± 2.3	66.1 ± 1.0							
K$_{212}$	K$_{431}$		33.1 ± 1.0	42.0 ± 1.6	48.9 ± 3.7	73.8 ± 3.5							
K$_{212}$	S$_{437}$		41.3 ± 0.8	31.7 ± 1.5	42.9 ± 3.4	76.3 ± 3.7							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
MS$_{437}$PLWE	Y$_{317}$DHK$_{320}$RIS$_{323}$K$_{324}$LSE						2	2					
S$_{437}$	K$_{320}$	7D	44.8 ± 0.8	47.2 ± 1.4	27.7 ± 3.9	65.0 ± 2.9							
S$_{437}$	K$_{324}$		39.2 ± 0.9	44.0 ± 1.5	32.2 ± 3.9	69.3 ± 2.7							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
*S$_{99}$EAAME	IGVK$_{212}$QPIAE						2	1	1				
S$_{99}$	K$_{212}$		37.2 ± 1.2	80.0 ± 2.1	57.8 ± 3.0	30.5 ± 1.5							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
GFQVTYLPVQK$_{180}$SGIIDLK$_{187}$E	S$_{365}$LLMALK$_{371}$DVALS$_{376}$S$_{377}$GS$_{379}$ACT$_{382}$ S$_{383}$AS$_{385}$LE						1					1	
K$_{180}$	S$_{365}$	7C	22.2 ± 1.3	27.3 ± 1.6	64.1 ± 2.9	76.6 ± 2.7							
K$_{180}$	K$_{371}$	7B	21.5 ± 1.6	37.6 ± 2.0	64.3 ± 1.6	66.7 ± 2.8							
K$_{180}$	S$_{376}$		19.7 ± 1.4	42.3 ± 3.0	74.5 ± 2.0	64.2 ± 3.9							
K$_{180}$	S$_{377}$	14F	18.4 ± 1.9	39.7 ± 2.7	76.2 ± 2.6	61.0 ± 2.8							
K$_{180}$	S$_{379}$	14F	19.0 ± 1.4	38.4 ± 2.2	78.9 ± 2.9	56.9 ± 3.6							
K$_{180}$	T$_{382}$		24.7 ± 1.6	38.7 ± 2.9	77.7 ± 2.9	55.4 ± 2.2							
K$_{180}$	S$_{383}$		26.0 ± 1.2	40.9 ± 2.1	80.6 ± 2.6	51.6 ± 3.4							
K$_{180}$	S$_{385}$		26.9 ± 1.7	39.7 ± 1.4	85.6 ± 2.4	48.5 ± 3.9							
K$_{187}$	S$_{365}$		36.4 ± 1.5	37.4 ± 3.2	70.5 ± 3.7	72.3 ± 2.1							
K$_{187}$	K$_{371}$		35.5 ± 1.9	46.3 ± 2.2	72.2 ± 2.3	63.5 ± 2.4							
K$_{187}$	S$_{376}$		29.8 ± 1.0	53.6 ± 2.9	82.1 ± 2.5	60.4 ± 3.6							
K$_{187}$	S$_{377}$		28.4 ± 1.7	52.3 ± 3.6	83.3 ± 3.3	62.2 ± 3.0							
K$_{187}$	S$_{379}$		27.5 ± 0.8	51.1 ± 2.2	85.5 ± 3.2	59.7 ± 3.6							

Supplemental Table S2c, p.2

Cross-linked peptides			Distances (Å) measured in structure					False Discovery Rate (FDR) of cross-linked peptides					
NFS1	**NFS1**	**Figure**	**Monomer**	**Dimer**	**Trimer**	**Tetramer**	**# of peptides**						
K$_{187}$	T$_{382}$		32.9 ± 1.4	51.6 ± 2.6	83.8 ± 2.7	58.9 ± 3.1							
K$_{187}$	S$_{383}$		32.6 ± 1.1	53.8 ± 1.9	86.7 ± 2.5	55.8 ± 2.8							
K$_{187}$	S$_{385}$		31.5 ± 1.6	53.5 ± 1.0	90.7 ± 4.0	53.4 ± 3.7							
Double digestion GluC&AspN								**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DHK$_{320}$RIS$_{323}$K$_{324}$LSE	RLIQNIMK$_{335}$S$_{336}$LP						4	1		1	2		
K$_{320}$	K$_{335}$	7B	23.3±0.3	66.1±2.0	48.3±2.4	57.7±2.1							
S$_{323}$	K$_{335}$		18.8±0.5	60.7±2.1	52.4±2.3	62.9±2.0							
K$_{324}$	K$_{335}$	7B	17.2±0.2	61.5±2.1	50.7±2.5	64.0±2.2							
K$_{320}$	S$_{336}$		25.0±0.2	64.5±2.0	45.6±2.5	59.7±2.1							
K$_{324}$	S$_{336}$		18.8±0.2	60.2±2.1	47.0±2.4	65.5±2.1							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
S$_{99}$EAAME	MGSSHHHHHHSSGLVPRGSHMLEVLR PLYMDVQATT$_{67}$PL						3			3			
S$_{99}$	T$_{67}$		29.0±0.7	64.2±2.9	39.4±2.6	24.4±2.9							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
MEY$_{317}$	DHK$_{320}$RIS$_{323}$K$_{324}$LSERLIQNIMK$_{335}$SLPD VVMNG						3		1	1	1		
Y$_{317}$	K$_{320}$		5.5±0.2	85.6±1.0	58.3±2.1	56.1±1.9							
Y$_{317}$	K$_{324}$	7B	10.9±0.4	81.7±1.5	53.1±2.0	54.7±1.8							
Y$_{317}$	K$_{335}$		27.9±0.4	70.1±1.8	44.6±2.8	53.7±1.6							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DMK$_{248}$I	IGRICS$_{223}$S$_{224}$RK$_{226}$VY$_{228}$FHT$_{231}$DAAQAV GK$_{239}$IPL						2	1	1				
K$_{248}$	S$_{223}$		6.6±0.4	90.4±2.0	101.2±2.9	27.1±2.7							
K$_{248}$	S$_{224}$		10.0±0.4	92.1±2.0	103.6±3.0	27.2±2.8							
K$_{248}$	K$_{226}$	7B,E	10.6±0.4	91.2±1.9	103.2±3.0	22.0±2.7							
K$_{248}$	Y$_{228}$		8.9±0.3	86.4±1.9	97.8±2.9	22.9±2.3							
K$_{248}$	T$_{231}$		9.9±0.2	79.5±2.2	88.9±2.7	30.8±2.0							
K$_{248}$	K$_{239}$		19.6±1.0	78.9±2.5	81.2±2.2	39.1±2.2							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
S$_{99}$EAAME	HK$_{157}$CVL						1	1					
S$_{99}$	K$_{157}$	7E	39.5±1.1	64.3±1.4	48.4±2.9	18.7±4.9							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DMK$_{248}$I	DVVMNGDPK$_{347}$HHY$_{350}$PGCINLS$_{357}$FA Y$_{360}$VE						1	1					
K$_{248}$	K$_{347}$	7B	22.9±0.4	72.4±1.6	81.1±2.4	47.0±2.0							
K$_{248}$	Y$_{350}$		19.9±0.8	72.1±1.5	79.2±2.4	44.2±1.6							
K$_{248}$	S$_{357}$		32.5±0.9	58.0±2.3	68.3±3.1	51.3±4.4							
K$_{248}$	Y$_{360}$		40.6±0.8	51.5±1.8	65.9±3.6	52.4±1.8							

Supplemental Table S2c, p.3

Cross-linked peptides			Distances (Å) measured in structure					False Discovery Rate (FDR) of cross-linked peptides					
NFS1	**NFS1**	**Figure**	**Monomer**	**Dimer**	**Trimer**	**Tetramer**	**# of peptides**						
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
S_{365}LLMALK$_{371}$	DAAQAVGK$_{239}$IPL						1	1					
S_{365}	K_{239}		32.5±1.6	42.2±1.8	43.5±2.4	51.6±2.5							
K_{371}	K_{239}	7B	23.8±1.6	49.5±1.9	44.1±1.7	52.6±2.1							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
S_{365}LLMALK$_{371}$	DLK$_{450}$S$_{451}$IK$_{453}$WT$_{455}$QH-c						1	1					
S_{365}	K_{450}		27.3±2.2	32.4±2.2	49.7±2.9	86.0±2.4							
K_{371}	K_{450}		29.5±2.1	30.3±3.8	40.6±3.6	75.7±1.8							
S_{365}	K_{453}		26.8±2.6	31.3±1.4	52.5±2.6	82.0±2.0							
K_{371}	K_{453}	7B	27.7±2.3	27.8±2.6	43.5±2.7	71.4±2.8							
K_{371}	S_{451}		29.3±1.5	29.3±3.0	42.4±2.9	75.0±2.7							
K_{371}	T_{455}	7C	27.8±3.9	25.3±3.2	48.0±2.7	70.0±3.7							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
GFQVT$_{174}$Y$_{175}$LPVQK$_{180}$S$_{181}$GIIDLK$_{187}$ELE	DHK$_{320}$RIS$_{323}$K$_{324}$LS$_{326}$ERLIQNIMK$_{335}$S$_{336}$LP						1	1					
K_{180}	K_{320}		25.3±1.5	62.3±1.5	60.1±2.5	42.9±1.7							
K_{180}	S_{323}		20.6±1.4	58.0±3.0	64.9±2.2	47.8±1.6							
K_{180}	K_{324}	7B	22.0±1.0	57.1±1.6	63.1±2.5	48.9±1.8							
K_{180}	S_{326}		18.3±1.0	51.7±1.4	66.7±1.9	52.4±1.5							
K_{180}	K_{335}	7B	16.3±1.0	43.1±1.8	72.1±2.9	65.9±1.8							
K_{180}	S_{336}		19.1±0.8	41.1±1.8	68.8±3.1	67.4±1.6							
K_{187}	K_{320}		31.6±1.2	70.1±2.0	75.0±2.6	37.4±1.3							
K_{187}	S_{323}		28.3±1.0	65.8±2.3	79.4±2.4	42.1±1.1							
K_{187}	K_{324}		30.6±0.9	65.0±2.4	78.6±1.9	43.3±1.5							
K_{187}	S_{326}		28.6±0.5	60.6±2.3	81.1±2.0	47.2±1.1							
K_{187}	K_{335}		29.7±0.8	51.3±3.3	81.3±2.5	59.4±1.7							
K_{187}	S_{336}		33.0±0.7	47.8±2.7	77.0±2.9	61.5±1.6							
T_{174}	K_{320}	7E	37.7±1.2	62.7±2.8	75.3±3.0	24.7±1.5							
Y_{175}	K_{320}		35.3±1.3	61.7±3.5	71.9±2.4	27.0±1.7							
S_{181}	K_{320}		22.4±1.5	65.7±1.4	62.0±1.6	44.2±1.9							
T_{174}	K_{324}		36.9±0.6	57.3±2.7	79.4±2.2	31.0±1.7							
Y_{175}	K_{324}		34.2±0.7	55.4±2.4	75.1±2.9	33.0±1.7							
S_{181}	K_{324}		19.3±1.0	60.6±1.5	64.7±2.5	50.3±2.0							
T_{174}	K_{335}		35.8±1.0	44.6±2.7	84.7±2.9	47.6±1.7							
Y_{175}	K_{335}		32.2±1.0	42.7±2.5	83.0±2.4	50.1±1.8							
S_{181}	K_{335}		16.6±1.0	46.8±1.8	70.1±3.2	67.8±1.2							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
S_{365}LLMALK$_{371}$	GFQVT$_{174}$Y$_{175}$LPVQK$_{180}$S$_{181}$GII						1		1				
K_{371}	T_{174}		34.5±2.7	36.7±2.2	74.3±1.4	50.3±2.2							
K_{371}	Y_{175}		30.9±2.6	35.2±2.5	71.8±1.4	51.8±2.4							

Supplemental Table S2c, p.4

Cross-linked peptides			Distances (Å) measured in structure				# of peptides	False Discovery Rate (FDR) of cross-linked peptides					
NFS1	**NFS1**	**Figure**	**Monomer**	**Dimer**	**Trimer**	**Tetramer**							
K$_{371}$	K$_{180}$		21.5 ± 1.6	37.6 ± 2.0	64.3 ± 1.6	66.7 ± 2.8							
K$_{371}$	S$_{181}$		22.6±1.4	40.4±1.4	62.0±1.8	67.4±1.7							
S$_{365}$	K$_{180}$		22.2 ± 1.3	27.3 ± 1.6	64.1 ± 2.9	76.6 ± 2.7							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
HK$_{157}$CVL	S$_{365}$LLMALK$_{371}$						1			1			
K$_{157}$	S$_{365}$	7C	27.1±2.0	23.7±2.6	66.0±2.6	59.5±2.9							
K$_{157}$	K$_{371}$	7B	23.8±2.8	29.5±2.3	64.9±1.3	48.0±2.2							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
VLRPLY$_{60}$MDVQATT$_{67}$PL	RLIQNIMK$_{335}$S$_{336}$LPDVVMNG						1			1			
Y$_{60}$	K$_{335}$		NM	NM	NM	NM							
T$_{67}$	K$_{335}$		26.9±1.0	52.5±2.6	58.3±1.3	70.3±1.4							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
S$_{365}$LLMALK$_{371}$	DVQAT$_{66}$T$_{67}$PL						1			1			
K$_{371}$	T$_{66}$		NM	NM	NM	NM							
K$_{371}$	T$_{67}$		14.3±1.3	38.4±2.3	46.3±2.2	59.0±2.5							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DLAHS$_{404}$S$_{405}$IRFGIGRFT$_{414}$T$_{415}$EE	YDHK$_{320}$RISK$_{324}$LSERLIQNIMK$_{335}$SLP						1			1			
S$_{404}$	K$_{320}$		32.6±0.8	50.2±1.7	48.5±2.6	42.8±2.1							
S$_{405}$	K$_{320}$		29.5±0.8	53.5±1.9	50.9±1.7	41.9±1.4							
T$_{414}$	K$_{320}$	7B	12.7±0.5	73.1±1.3	50.8±2.2	58.8±2.3							
T$_{415}$	K$_{320}$		12.4±0.5	75.7±1.3	50.3±2.0	62.4±2.2							
S$_{404}$	K$_{324}$		28.6±0.8	45.6±1.8	52.2±2.5	48.2±2.1							
S$_{405}$	K$_{324}$		25.8±0.8	48.5±1.4	54.4±2.2	47.5±1.4							
T$_{414}$	K$_{324}$		10.4±0.4	68.9±1.0	51.7±2.2	64.9±2.1							
T$_{415}$	K$_{324}$	7B	10.0±0.3	71.6±0.9	51.0±1.7	66.6±1.3							
S$_{404}$	K$_{335}$		21.4±1.4	34.6±2.3	66.5±1.7	64.6±1.9							
S$_{405}$	K$_{335}$	7B	19.7±1.4	37.5±2.3	68.2±1.4	64.2±1.2							
T$_{414}$	K$_{335}$		19.7±0.7	58.9±1.6	48.9±2.3	65.0±1.9							
T$_{415}$	K$_{335}$		20.3±0.5	62.2±1.8	46.3±2.4	63.8±1.7							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
*S$_{99}$EAAME	K$_{425}$CIQHVK$_{431}$RLRE						1			1			
S$_{99}$	K$_{425}$		46.6±1.4	69.3±1.5	41.9±2.2	41.2±2.7							
S$_{99}$	K$_{431}$		56.0±1.4	66.6±1.6	39.3±2.8	44.1±0.8							
								≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
IGVK$_{212}$QPIAE	DPK$_{347}$HHY$_{350}$PGCINLS$_{357}$FAY$_{360}$VE						1				1		
K$_{212}$	K$_{347}$		9.6±0.3	63.5±2.3	75.0±2.2	53.1±2.1							
K$_{212}$	Y$_{350}$		5.3±0.4	61.0±1.9	73.0±2.2	50.0±1.7							
K$_{212}$	S$_{357}$		18.0±0.7	45.8±1.9	65.3±2.8	57.3±2.4							
K$_{212}$	Y$_{360}$		26.7±0.6	39.4±2.4	64.2±3.1	63.5±1.4							

Supplemental Table S2d, p.1

Cross-linked peptides			Distances (Å) measured in structure		False Discovery Rate (FDR) of cross-linked peptides					
ISD11	**ISD11**	**Figure**	**ISD11-ISD11**	**# of peptides**						
Single digestion GluC					**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
NRDMPRT$_{91}$-c	NK$_{44}$NVK$_{47}$DPVE	7G	24.0 ± 0.8	1					1	
Double digestion GluC&AspN										
ISD11	**ISD11**				**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DK$_{80}$LIIE	IQT$_{54}$LVNK$_{58}$AK$_{60}$R			3	2		1			
K$_{80}$	T$_{54}$		33.3±0.0							
K$_{80}$	K$_{58}$	7G	27.6±0.0							
K$_{80}$	K$_{60}$	7G	27.2±0.0							
					≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
NK$_{44}$NVK$_{47}$	n-MAQVLS$_{11}$LY$_{13}$RAMLRE			2	1			1		
K$_{44}$	N-term		24.4±0.0							
K$_{44}$	S$_{11}$		24.5±0.0							
K$_{44}$	Y$_{13}$	7G	21.3±0.0							
K$_{47}$	N-term		29.2±0.0							
K$_{47}$	S$_{11}$		29.5±0.0							
K$_{47}$	Y$_{13}$	7G	26.5±0.0							
					≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
NK$_{44}$NVK$_{47}$	IQT$_{54}$LVNK$_{58}$AK$_{60}$R			1				1		
K$_{44}$	T$_{54}$		11.4±0.0							
K$_{44}$	K$_{58}$	7G	13.7±0.0							
K$_{44}$	K$_{60}$		17.2±0.0							
K$_{47}$	T$_{54}$		11.2±0.0							
K$_{47}$	K$_{58}$		15.5±0.0							
K$_{47}$	K$_{60}$	7G	19.2±0.0							

Supplemental Table S2e, p.1

Cross-linked peptides			Distances (Å) measured in structure									
FXN$^{42-210}$	ISCU	Figure	FXN$^{42-210}$-ISCU intra-dimer	FXN$^{42-210}$-ISCU intra-trimer	FXN$^{42-210}$-ISCU inter-trimers	# of peptides	False Discovery Rate (FDR) of cross-linked peptides					
Single digestion GluC							≤5 %	≤10%	≤15%	≤25 %	≤41 %	≤60%
$T_{93}T_{94}Y_{95}$ERLAE	P$K_{160}K_{161}$GE					6	2		1		3	
T_{93}	K_{160}		32.1 ± 1.2	23.9 ± 1.5	44.8 ± 1.5							
T_{94}	K_{160}		32.6 ± 1.4	24.7 ± 1.4	45.2 ± 1.8							
Y_{95}	K_{160}		30.8 ± 1.6	23.5 ± 1.4	47.8 ± 1.7							
T_{93}	K_{161}	9A	28.8 ± 1.1	22.5 ± 1.4	43.2 ± 1.4							
T_{94}	K_{161}		29.2 ± 1.4	23.0 ± 1.4	43.3 ± 1.8							
Y_{95}	K_{161}	9A,B	27.3 ± 1.5	21.5 ± 1.3	45.7 ± 1.7							
							≤5 %	≤10%	≤15%	≤25 %	≤35 %	≤60%
$T_{93}T_{94}Y_{95}$ERLAE	AE$K_{166}K_{167}$					10	2		1	2	2	3
T_{93}	K_{166}		30.1 ± 2.2	14.2 ± 1.5	34.8 ± 1.6							
T_{94}	K_{166}		29.5 ± 2.3	15.0 ± 1.6	34.7 ± 1.8							
Y_{95}	K_{166}	9A,B	26.9 ± 2.3	14.3 ± 1.4	37.2 ± 1.8							
T_{93}	K_{167}		33.4 ± 1.9	13.4 ± 2.0	33.8 ± 1.2							
T_{94}	K_{167}		32.8 ± 2.1	14.6 ± 1.8	34.8 ± 0.8							
Y_{95}	K_{167}	9B	30.3 ± 2.1	14.4 ± 2.0	37.5 ± 2.0							
							≤5 %	≤10%	≤15%	≤25 %	≤35 %	≤60%
ET_{102}LDS_{105}LAE	AE$K_{166}K_{167}$					1	1					
T_{102}	K_{166}	9A	24.2 ± 1.6	19.9 ± 1.6	44.5 ± 0.9							
S_{105}	K_{166}		27.8 ± 1.8	22.9 ± 1.7	44.4 ± 0.8							
T_{102}	K_{167}	9B	27.1 ± 1.8	20.1 ± 2.3	44.3 ± 0.8							
S_{105}	K_{167}		30.3 ± 1.8	22.6 ± 2.3	43.2 ± 0.9							
Double digestion GluC&AspN							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
KGALEVLFQGPGHMART_{44}	DHYENPRNVGSLD$K_{54}T_{55}S_{56}K_{57}$NVGT_{61}GLVGAPACG					1	1					
T_{44}	K_{54}		31±1.0	23.7±1.7	68.5±1.3							
T_{44}	K_{57}		25.0±1.0	25.1±1.5	67.5±1.4							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
DWT_{169}GK_{171}NWV$Y_{175}S_{176}$H	LCLPPVK_{135}LHCS_{139}MLAEDAIK_{147}AALA					1			1			
T_{169}	K_{135}		32.1±1.9	29.3±1.9	30.0±1.6							
K_{171}	K_{135}		32.6±1.2	25.8±1.4	26.9±0.7							
Y_{175}	K_{135}	9A,C	22.5±1.5	37.6±1.4	23.2±2.5							
S_{176}	K_{135}		24.1±1.3	37.7±1.2	25.0±2.9							
T_{169}	K_{147}		24.0±2.0	21.3±1.1	47.9±1.5							
K_{171}	K_{147}	9A,B	23.8±1.2	17.1±1.0	45.5±0.5							

Supplemental Table S2e, p.2

Cross-linked peptides			Distances (Å) measured in structure				False Discovery Rate (FDR) of cross-linked peptides					
FXN$^{42-210}$	ISCU	Figure	FXN$^{42-210}$-ISCU intra-dimer	FXN$^{42-210}$-ISCU intra-trimer	FXN$^{42-210}$-ISCU inter-trimers	# of peptides						
Y$_{175}$	K$_{147}$	9A	18.2±0.5	27.6±1.2	41.9±2.0							
S$_{176}$	K$_{147}$		18.5±1.6	28.4±1.0	43.4±2.0							
K$_{171}$	S$_{139}$		29.5±1.4	23.2±1.0	34.6±0.2							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
DGVS$_{181}$LHE	DAIK$_{147}$AALA					1				1		
S$_{181}$	K$_{147}$	9A	18.8±0.5	25.3±1.1	40.8±1.8							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
DWT$_{169}$GK$_{171}$NWVY$_{175}$S$_{176}$H	DIAK$_{127}$E					1			1			
T$_{169}$	K$_{127}$		26.2±1.3	27.0±1.0	35.2±1.7							
K$_{171}$	K$_{127}$		24.9±1.3	25.0±0.9	32.0±1.1							
Y$_{175}$	K$_{127}$	9A	18.4±0.8	34.5±0.5	31.2±1.8							
S$_{176}$	K$_{127}$		21.3±0.7	30.8±0.6	32.2±2.0							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
T$_{93}$T$_{94}$Y$_{95}$E	AEK$_{166}$K$_{167}$					1			1			
T$_{93}$	K$_{166}$		30.1 ± 2.2	14.2 ± 1.5	34.8 ± 1.6							
T$_{94}$	K$_{166}$		29.5 ± 2.3	15.0 ± 1.6	34.7 ± 1.8							
Y$_{95}$	K$_{166}$	9A,B	26.9 ± 2.3	14.3 ± 1.4	37.2 ± 1.8							
T$_{93}$	K$_{167}$		33.4 ± 1.9	13.4 ± 2.0	33.8 ± 1.2							
T$_{94}$	K$_{167}$		32.8 ± 2.1	14.6 ± 1.8	34.8 ± 0.8							
Y$_{95}$	K$_{167}$	9B	30.3 ± 2.1	14.4 ± 2.0	37.5 ± 2.0							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
LTK$_{192}$ALK$_{195}$TK$_{197}$L	ALT$_{119}$IK$_{121}$NT$_{123}$					1			1			
K$_{192}$	T$_{119}$		18.9±0.8	39.6±0.8	27.0±0.7							
K$_{192}$	K$_{121}$	9A	13.9±0.8	41.9±1.0	21.0±0.8							
K$_{192}$	T$_{123}$	9C	8.8±0.7	45.6±1.0	18.7±1.1							
K$_{195}$	T$_{119}$		23.0±0.7	43.1±0.8	27.3±0.8							
K$_{195}$	K$_{121}$	9A,C	18.2±0.6	45.0±1.0	21.6±0.9							
K$_{195}$	T$_{123}$		13.4±0.5	48.7±1.0	20.3±1.3							
K$_{197}$	T$_{119}$		20.2±1.0	38.1±1.1	33.7±0.8							
K$_{197}$	K$_{121}$	9A	17.1±0.7	39.6±1.0	27.9±0.9							
K$_{197}$	T$_{123}$		13.4±0.6	43.4±1.0	26.1±1.2							
T$_{191}$	K$_{121}$		14.6±0.6	39.3±1.0	24.2±1.0							
T$_{197}$	K$_{121}$		18.9±0.7	43.2±0.9	25.3±1.0							
LOW SCORE PEPTIDES							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
DYDVSFGSGVLTVK$_{135}$LGG	DK$_{54}$TSK$_{57}$NVGTGLVGAPACGDVM K$_{74}$LQIQV					1					1	
K$_{135}$	K$_{54}$		54.9±1.9	63.0±1.4	50.8±3.2							
K$_{135}$	K$_{57}$		50.2±1.8	55.1±1.7	42.5±3.6							
K$_{135}$	K$_{74}$	9C	42.1±1.8	53.0±1.8	30.1±1.5							

Supplemental Table S2e, p.3

Cross-linked peptides			Distances (Å) measured in structure				False Discovery Rate (FDR) of cross-linked peptides					
FXN$^{42-210}$	ISCU	Figure	FXN$^{42-210}$-ISCU intra-dimer	FXN$^{42-210}$-ISCU intra-trimer	FXN$^{42-210}$-ISCU inter-trimers	# of peptides						
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
DVSFGSGVLTVK$_{135}$LGG	AEK$_{166}$K$_{167}$-c					3						3
K$_{135}$	K$_{166}$		32.9±1.3	38.3±2.0	20.5±0.8							
K$_{135}$	K$_{167}$	9C	33.0±1.3	39.0±1.6	22.8±2.7							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
DVSFGSGVLTVK$_{135}$LGG	PK$_{160}$K$_{161}$GE					1						1
K$_{135}$	K$_{160}$		43.7±1.6	40.1±1.5	32.0±2.3							
K$_{135}$	K$_{161}$	9C	41.1±1.5	38.5±1.4	30.7±2.7							
							≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤40%
DATCTPRRASSNQRGLNQIWNVK$_{69}$K$_{70}$Q SVYLMNLRK$_{80}$SGTLGHPGSL	AEK$_{166}$K$_{167}$-c					2						2
T$_{49}$	K$_{166}$		21.8±2.9	29.7±1.6	51.7±2.1							
T$_{51}$	K$_{166}$		21.7±3.5	33.0±2.3	48.8±2.8							
S$_{56}$	K$_{166}$		22.7±2.4	34.6±2.2	53.2±3.3							
S$_{57}$	K$_{166}$		19.5±2.2	31.7±2.2	53.0±3.5							
K$_{69}$	K$_{166}$	9A	23.8±0.9	28.7±1.5	65.6±0.9							
K$_{70}$	K$_{166}$		25.9±1.0	28.6±1.8	68.9±0.9							
S$_{72}$	K$_{166}$		25.3±1.0	25.5±1.8	67.6±0.4							
K$_{80}$	K$_{166}$	9B	26.1±1.9	19.1±2.7	56.5±1.0							
S$_{81}$	K$_{166}$		25.4±2.2	16.6±2.7	53.8±0.4							
T$_{83}$	K$_{166}$		21.0±2.4	11.4±2.6	52.3±2.1							
T$_{49}$	K$_{167}$	9A	19.1±2.1	31.4±1.1	51.3±2.0							
T$_{51}$	K$_{167}$		18.5±3.0	35.0±1.7	47.9±2.6							
S$_{56}$	K$_{167}$		19.4±2.4	36.4±1.2	51.8±3.2							
S$_{57}$	K$_{167}$	9A	16.3±2.4	33.5±1.3	51.6±3.5							
K$_{69}$	K$_{167}$		22.8±1.3	28.6±1.6	63.8±1.2							
K$_{70}$	K$_{167}$	9A	25.2±1.4	28.9±1.4	67.4±1.2							
S$_{72}$	K$_{167}$		25.2±1.0	25.0±2.2	65.8±0.9							
K$_{80}$	K$_{167}$		27.4±1.2	16.6±2.7	55.5±1.3							
S$_{81}$	K$_{167}$		27.1±1.7	14.0±2.7	52.7±1.0							
T$_{83}$	K$_{167}$		23.1±2.3	9.2±2.2	51.1±1.5							

Supplemental Table S2f, p.1

Cross-linked peptides			Distances (Å) measured in structure						# of peptides	False Discovery Rate (FDR) of cross-linked peptides					
FXN$^{42-210}$	**NFS1**	**Figure**	**2-fold #1**	**2-fold #2**	**2-fold #3**	**3-fold**	**4-fold**	**C-term**							
Single digestion GluC										**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
*ET$_{102}$LDS$_{105}$LAE	IGVK$_{212}$QPIAE								3	1				2	
T$_{102}$	K$_{212}$		42.2 ± 1.5	67.2 ± 1.6	67.5 ± 0.7	52.1 ± 2.8	60.3 ± 2.1	57.9 ± 1.5							
S$_{105}$	K$_{212}$	9D	39.3 ± 1.1	70.9 ± 1.7	68.7 ± 0.8	49.1 ± 3.3	56.2 ± 2.1	60.4 ± 1.4							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
T$_{93}$T$_{94}$Y$_{95}$ERLAE	K$_{425}$CIQHVK$_{431}$RLREMS$_{437}$PLWE								2				2		
T$_{93}$	K$_{425}$	9D	47.1 ± 1.8	54.2 ± 2.0	76.2 ± 1.2	44.6 ± 2.5	87.0 ± 1.9	30.1 ± 0.9							
T$_{94}$	K$_{425}$		47.4 ± 1.7	55.2 ± 1.9	74.4 ± 1.2	46.1 ± 2.5	84.3 ± 1.9	34.0 ± 0.9							
Y$_{95}$	K$_{425}$		45.8 ± 1.7	54.4 ± 22	71.8 ± 1.4	49.1 ± 2.2	82.0 ± 1.7	35.9 ± 0.8							
T$_{93}$	K$_{431}$		55.7 ± 1.5	56.7 ± 2.4	75.4 ± 1.2	46.8 ± 3.0	95.6 ± 1.8	32.6 ± 0.9							
T$_{94}$	K$_{431}$		55.8 ± 1.5	59.9 ± 2.8	74.0 ± 1.1	49.9 ± 2.2	92.8 ± 1.9	36.5 ± 1.0							
Y$_{95}$	K$_{431}$		54.3 ± 1.8	58.2 ± 2.6	71.6 ± 1.5	52.9 ± 2.0	90.5 ± 1.6	38.6 ± 1.0							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DLADK$_{116}$PY$_{118}$T$_{119}$FE	SNNIAIK$_{136}$GVARFYRSRK$_{146}$K$_{147}$HLIT$_{151}$T$_{152}$Q T$_{154}$EHK$_{157}$CVLDS$_{162}$CRS$_{165}$LE								1				1		
K$_{116}$	K$_{136}$	9D,F	21.3 ± 1.2	85.1 ± 1.0	65.2 ± 1.2	33.6 ± 4.6	36.9 ± 2.5	78.8 ± 0.9							
K$_{116}$	K$_{146}$		35.4 ± 1.2	98.7 ± 1.3	76.0 ± 1.0	41.9 ± 5.5	48.3 ± 2.0	91.0 ± 1.1							
K$_{116}$	K$_{147}$		33.2 ± 1.7	96.9 ± 1.2	72.4 ± 1.1	40.9 ± 5.5	47.7 ± 2.2	87.4 ± 1.1							
K$_{116}$	T$_{151}$		27.5 ± 1.3	88.2 ± 1.2	64.6 ± 1.0	42.7 ± 5.5	43.4 ± 2.1	78.0 ± 1.0							
K$_{116}$	T$_{152}$		27.9 ± 1.2	86.8 ± 1.3	62.9 ± 1.0	44.9 ± 5.5	43.8 ± 2.1	75.8 ± 1.0							
K$_{116}$	T$_{154}$		25.9 ± 1.2	82.9 ± 1.4	58.1 ± 1.1	46.0 ± 4.9	42.8 ± 2.4	70.4 ± 1.0							
K$_{116}$	K$_{157}$	9D	21.0 ± 1.4	81.1 ± 1.4	52.7 ± 0.9	39.4 ± 5.0	41.4 ± 3.3	66.1 ± 0.9							
K$_{116}$	S$_{162}$		21.1 ± 1.8	85.2 ± 1.4	58.0 ± 1.3	33.1 ± 4.5	41.8 ± 2.3	72.1 ± 1.1							
K$_{116}$	S$_{165}$		25.4 ± 1.9	90.1 ± 1.5	59.6 ± 1.4	35.1 ± 3.7	46.2 ± 2.1	75.0 ± 1.1							
Y$_{118}$	K$_{136}$		19.5 ± 1.0	82.5 ± 1.2	61.3 ± 1.1	35.6 ± 4.8	38.2 ± 2.8	83.1 ± 0.7							
Y$_{118}$	K$_{146}$		33.6 ± 0.9	96.0 ± 1.5	72.2 ± 0.9	43.5 ± 5.4	49.9 ± 2.5	95.4 ± 0.7							
Y$_{118}$	K$_{147}$		30.7 ± 1.1	94.4 ± 1.3	68.7 ± 1.0	42.7 ± 5.3	49.2 ± 2.5	91.8 ± 0.7							
Y$_{118}$	K$_{157}$		16.8 ± 1.7	79.4 ± 1.5	48.5 ± 0.9	42.3 ± 5.0	41.2 ± 3.4	70.4 ± 0.6							
T$_{119}$	K$_{136}$		21.9 ± 1.1	80.6 ± 1.3	59.8 ± 1.2	37.8 ± 4.5	39.3 ± 2.5	83.3 ± 1.4							
T$_{119}$	K$_{146}$		36.1 ± 1.3	94.1 ± 1.6	71.2 ± 1.0	46.4 ± 5.1	51.5 ± 2.1	95.8 ± 1.6							
T$_{119}$	K$_{147}$		33.4 ± 1.5	92.5 ± 1.4	67.6 ± 0.9	45.4 ± 4.9	50.5 ± 2.0	92.3 ± 1.5							
T$_{119}$	K$_{157}$		18.4 ± 1.3	77.6 ± 1.5	47.2 ± 0.9	43.5 ± 4.7	41.2 ± 3.2	71.0 ± 1.4							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
LTK$_{192}$ALK$_{195}$TK$_{197}$LDLSSLAYSGK$_{208}$DA	S$_{99}$EAAME								1			1			
K$_{192}$	S$_{99}$		33.7 ± 1.6	31.9 ± 2.6	52.4 ± 2.0	55.4 ± 1.4	15.7 ± 3.5	65.6 ± 1.4							
K$_{195}$	S$_{99}$		34.2 ± 2.0	36.0 ± 2.6	55.2 ± 2.2	54.7 ± 1.8	13.9 ± 4.1	70.3 ± 1.4							
K$_{197}$	S$_{99}$		28.7 ± 2.3	38.9 ± 2.7	60.3 ± 1.9	51.4 ± 2.8	15.8 ± 3.0	67.0 ± 1.9							
K$_{208}$	S$_{99}$	9D	20.9 ± 1.4	41.7 ± 1.8	67.2 ± 1.8	49.7 ± 3.0	25.5 ± 1.9	54.9 ± 1.3							

Supplemental Table S2f, p.2

Cross-linked peptides			Distances (Å) measured in structure							False Discovery Rate (FDR) of cross-linked peptides					
FXN$^{42-210}$	**NFS1**	**Figure**	**2-fold #1**	**2-fold #2**	**2-fold #3**	**3-fold**	**4-fold**	**C-term**	**# of peptides**						
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
LTK$_{192}$ALK$_{195}$TK$_{197}$LDLSSLAYSGK$_{208}$DA	GES$_{365}$LLMALK$_{371}$DVALS$_{376}$S$_{377}$GS$_{379}$ACT$_{382}$ S$_{383}$AS$_{385}$LE								1			1			
K_{192}	S_{365}		27.9 ± 2.3	69.5 ± 2.0	29.9 ± 2.9	70.2 ± 4.9	64.8 ± 1.4	59.3 ± 0.9							
K_{195}	S_{365}		27.3 ± 2.5	73.9 ± 1.9	27.6 ± 2.3	71.4 ± 5.0	62.0 ± 1.8	60.7 ± 0.8							
K_{197}	S_{365}		27.6 ± 2.7	71.8 ± 2.2	32.1 ± 2.0	65.1 ± 4.7	66.4 ± 2.1	54.1 ± 0.9							
K_{208}	S_{365}		34.2 ± 2.2	62.4 ± 1.7	44.3 ± 2.8	52.2 ± 4.3	78.6 ± 1.9	40.6 ± 0.7							
K_{192}	K_{371}	9D	22.6 ± 2.1	59.5 ± 2.3	30.4 ± 1.1	67.1 ± 3.2	54.4 ± 1.1	55.8 ± 1.8							
K_{195}	K_{371}	9D,14F	22.3 ± 2.5	63.8 ± 2.2	29.2 ± 1.1	68.1 ± 3.2	51.5 ± 1.4	57.9 ± 1.9							
K_{197}	K_{371}	9D	20.8 ± 2.6	62.1 ± 2.7	34.8 ± 1.4	62.0 ± 2.9	56.2 ± 1.7	51.7 ± 1.9							
K_{208}	K_{371}		24.1 ± 1.5	53.6 ± 2.3	47.1 ± 1.9	50.3 ± 3.0	68.9 ± 1.5	37.2 ± 2.1							
K_{192}	S_{376}		14.8 ± 2.2	58.7 ± 1.9	28.6 ± 1.7	54.9 ± 3.2	50.7 ± 2.5	61.0 ± 1.4							
K_{195}	S_{376}		12.4 ± 2.5	63.4 ± 1.9	29.6 ± 2.3	55.6 ± 3.2	47.9 ± 2.8	63.7 ± 1.6							
K_{197}	S_{376}		11.1 ± 2.8	62.8 ± 2.0	34.4 ± 2.5	49.4 ± 2.8	51.3 ± 3.1	57.9 ± 1.5							
K_{208}	S_{376}		20.6 ± 1.8	56.6 ± 1.6	43.7 ± 1.9	38.6 ± 2.4	62.5 ± 2.5	43.4 ± 1.7							
K_{192}	S_{377}		16.3 ± 2.3	61.9 ± 2.0	28.4 ± 2.5	54.4 ± 3.8	53.6 ± 2.5	63.3 ± 2.2							
K_{195}	S_{377}		13.7 ± 2.2	66.6 ± 2.0	28.4 ± 1.6	55.0 ± 3.9	50.8 ± 2.7	65.9 ± 2.4							
K_{197}	S_{377}		13.8 ± 2.2	66.1 ± 2.1	33.7 ± 3.3	48.7 ± 3.5	53.9 ± 3.0	60.0 ± 2.2							
K_{208}	S_{377}		23.7 ± 2.0	59.8 ± 1.7	42.6 ± 1.6	37.6 ± 2.7	64.8 ± 1.6	45.6 ± 2.8							
K_{192}	S_{379}		17.3 ± 2.0	66.2 ± 2.0	28.5 ± 1.6	52.9 ± 3.1	57.4 ± 1.9	65.6 ± 1.6							
K_{195}	S_{379}	14F	14.4 ± 1.8	70.9 ± 2.0	29.2 ± 2.1	53.5 ± 3.4	54.5 ± 2.3	68.0 ± 1.7							
K_{197}	S_{379}		16.5 ± 1.6	70.6 ± 2.1	33.2 ± 2.6	47.1 ± 3.1	57.4 ± 2.4	62.1 ± 1.6							
K_{208}	S_{379}		28.1 ± 2.0	64.2 ± 2.0	40.9 ± 1.5	35.4 ± 2.2	67.7 ± 1.8	48.0 ± 1.8							
K_{192}	T_{382}		13.3 ± 1.9	65.2 ± 2.5	24.1 ± 1.5	48.0 ± 3.1	57.8 ± 2.3	62.3 ± 0.6							
K_{195}	T_{382}		10.2 ± 2.7	70.1 ± 2.4	25.8 ± 1.4	49.0 ± 3.2	55.4 ± 2.4	64.7 ± 0.5							
K_{197}	T_{382}	9D	14.5 ± 2.1	69.7 ± 2.4	28.9 ± 1.1	42.6 ± 2.8	58.2 ± 3.1	59.0 ± 0.7							
K_{208}	T_{382}		28.2 ± 3.4	63.1 ± 2.9	35.8 ± 1.7	30.2 ± 2.6	67.9 ± 2.8	45.1 ± 1.3							
K_{192}	S_{383}		14.4 ± 2.3	65.8 ± 2.1	25.6 ± 1.2	42.3 ± 3.2	57.1 ± 2.0	64.4 ± 0.3							
K_{195}	S_{383}	14F	10.2 ± 2.2	70.7 ± 2.0	28.3 ± 1.7	46.2 ± 3.3	54.8 ± 2.2	67.0 ± 0.5							
K_{197}	S_{383}		14.7 ± 2.2	70.6 ± 2.2	30.9 ± 2.0	39.7 ± 3.0	57.3 ± 2.7	61.3 ± 0.8							
K_{208}	S_{383}		29.2 ± 3.1	64.5 ± 2.6	36.2 ± 1.3	27.5 ± 2.6	66.4 ± 2.4	47.7 ± 1.6							
K_{192}	S_{385}	9D	19.9 ± 2.9	71.3 ± 2.5	27.9 ± 2.2	45.0 ± 3.0	61.7 ± 1.9	69.4 ± 1.7							
K_{195}	S_{385}	9D	15.0 ± 1.8	76.2 ± 2.5	30.7 ± 2.6	45.8 ± 3.3	59.3 ± 1.9	71.8 ± 1.8							
K_{197}	S_{385}		19.8 ± 1.9	76.3 ± 2.4	32.3 ± 2.6	39.2 ± 3.2	61.2 ± 2.4	66.2 ± 1.6							
K_{208}	S_{385}	9F	34.0 ± 1.8	70.4 ± 2.9	36.1 ± 2.5	26.4 ± 2.1	69.8 ± 2.1	52.8 ± 1.9							
Double digestion GluC&AspN															
FXN	**NFS1**									**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DWT$_{169}$GK$_{171}$NWVY$_{175}$S$_{176}$H	S$_{365}$LLMALK$_{371}$								2	2					
K_{171}	S_{365}		44.7±2.0	54.1±2.3	55.0±3.3	49.8±3.5	85.2±1.9	40.0±1.3							
K_{171}	K_{371}		35.1±1.4	46.2±2.9	55.6±2.3	48.5±2.1	75.9±1.9	32.9±0.9							
T_{169}	K_{371}		37.7±2.4	43.9±2.1	55.3±2.8	52.6±2.4	76.0±1.4	37.5±2.3							
Y_{175}	K_{371}		31.1±1.0	44.8±2.0	46.0±2.4	59.9±2.6	69.8±1.2	44.7±2.3							
S_{176}	K_{371}		33.2±1.2	41.7±2.1	47.0±2.4	61.5±2.5	72.6±1.4	45.1±1.7							

Supplemental Table S2f, p.3

Cross-linked peptides			Distances (Å) measured in structure							False Discovery Rate (FDR) of cross-linked peptides					
FXN$^{42\text{-}210}$	**NFS1**	**Figure**	**2-fold #1**	**2-fold #2**	**2-fold #3**	**3-fold**	**4-fold**	**C-term**	**# of peptides**						
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
VLFQGPGHMART$_{44}$DI	MEY$_{317}$DHK$_{320}$RIS$_{323}$K$_{324}$LSE								2			2			
T_{44}	K_{320}	9D	53.4±1.0	14.0±1.6	57.9±1.8	101.3±1.3	79.0±1.9	56.9±2.3							
T_{44}	K_{324}		49.3±1.1	16.4±1.8	57.4±2.0	100.5±1.3	83.4±1.9	57.2±2.2							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DLGT$_{142}$Y$_{143}$VINK$_{147}$QT$_{149}$PNK$_{152}$QIWL S$_{157}$S$_{158}$PS$_{160}$S$_{161}$GPK$_{164}$RY$_{166}$	S$_{365}$LLMALK$_{371}$								1	1					
K_{147}	S_{365}		50.7±1.8	64.7±1.9	54.5±3.5	58.3±2.3	72.6±1.6	54.3±1.2							
K_{147}	K_{371}		43.5±1.6	56.6±1.5	51.8±2.0	55.6±1.9	63.5±1.6	48.8±1.3							
K_{152}	S_{365}		52.3±1.9	58.5±2.0	56.5±2.7	55.5±1.7	79.8±1.8	51.7±1.8							
K_{152}	K_{371}		44.7±1.3	51.5±1.6	55.8±1.8	54.4±1.8	71.2±1.8	45.2±1.7							
K_{164}	S_{365}		41.8±1.7	58.4±1.9	44.4±3.1	66.9±2.9	74.2±1.3	58.0±1.6							
K_{164}	K_{371}		34.0±0.9	49.4±1.7	43.1±1.9	63.5±2.5	64.7±1.1	50.7±1.3							
T_{142}	K_{371}		37.1±1.8	62.2±2.0	37.0±2.5	67.3±2.3	51.3±1.6	61.2±2.6							
Y_{143}	K_{371}		34.4±1.2	59.9±1.9	38.3±1.4	63.5±1.9	54.2±1.2	57.7±2.6							
T_{149}	K_{371}		47.6±1.3	54.5±1.3	56.8±2.0	56.2±2.1	69.2±1.9	49.1±1.8							
S_{157}	K_{371}		36.2±1.9	55.2±1.8	40.8±2.0	64.9±2.5	58.9±1.2	55.7±2.2							
S_{158}	K_{371}		32.9±1.9	54.8±1.7	37.5±1.9	65.1±1.7	57.8±1.3	54.9±1.2							
S_{160}	K_{371}		30.1±1.7	53.9±2.1	32.0±1.2	71.0±2.0	57.3±1.6	58.7±2.3							
S_{161}	K_{371}		30.7±1.4	51.1±2.0	35.4±1.8	68.7±2.3	60.6±1.4	56.8±2.4							
Y_{166}	K_{371}		36.0±1.5	47.8±1.6	48.0±1.6	58.3±2.3	68.3±1.0	45.9±1.8							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
T$_{93}$T$_{94}$Y$_{95}$E	DYTVEK$_{425}$CIQHVK$_{431}$RLRE								1		1				
T_{93}	K_{425}	9D	47.1 ± 1.8	54.2 ± 2.0	76.2 ± 1.2	44.6 ± 2.5	87.0 ± 1.9	30.1 ± 0.9							
T_{94}	K_{425}		47.4 ± 1.7	55.2 ± 1.9	74.4 ± 1.2	46.1 ± 2.5	84.3 ± 1.9	34.0 ± 0.9							
Y_{95}	K_{425}		45.8 ± 1.7	54.4 ± 22	71.8 ± 1.4	49.1 ± 2.2	82.0 ± 1.7	35.9 ± 0.8							
T_{93}	K_{431}		55.7 ± 1.5	56.7 ± 2.4	75.4 ± 1.2	46.8 ± 3.0	95.6 ± 1.8	32.6 ± 0.9							
T_{94}	K_{431}		55.8 ± 1.5	59.9 ± 2.8	74.0 ± 1.1	49.9 ± 2.2	92.8 ± 1.9	36.5 ± 1.0							
Y_{95}	K_{431}		54.3 ± 1.8	58.2 ± 2.6	71.6 ± 1.5	52.9 ± 2.0	90.5 ± 1.6	38.6 ± 1.0							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
T$_{93}$T$_{94}$Y$_{95}$ERLAE	S$_{365}$LLMALK$_{371}$								1			1			
T_{93}	K_{371}	9D	45.3±1.9	53.9±2.1	67.8±1.6	34.8±2.1	28.4±0.8	24.6±1.5							
T_{94}	K_{371}		45.1±1.7	54.6±1.9	66.1±1.5	36.5±2.3	31.0±0.3	27.5±0.6							
Y_{95}	K_{371}		43.9±1.7	53.5±1.9	63.4±1.8	40.8±3.1	33.8±1.1	30.5±0.9							
LOW SCORE PEPTIDES										**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DATCTPRRASSNQRGLNQIWNVK$_{69}$K$_{70}$Q SVYLMNLRK$_{80}$SGTLGHPGSL	MS$_{437}$PLWE								1						1
K_{69}	S_{437}	9D	29.5±0.8	43.1±1.1	57.8±2.0	71.5±1.6	43.9±2.1	41.6±0.7							
K_{70}	S_{437}		32.7±0.9	40.2±1.1	61.5±2.0	70.1±1.4	41.9±2.2	41.3±1.2							
K_{80}	S_{437}	9D	42.7±1.4	57.9±2.9	53.4±1.5	56.1±2.7	28.7±1.9	23.4±1.3							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤45 %**	**≤60%**
DYDVSFGSGVLTVK$_{135}$LGG	HK$_{157}$CVLDSCRSLE								3					1	2
K_{135}	K_{157}	9D	30.3±1.7	70.6±1.6	45.6±2.6	52.2±2.1	45.8±1.6	71.1±1.1							

Supplemental Table S2g, p.1

Cross-linked peptides			Distances (Å) measured in structure							False Discovery Rate (FDR) of cross-linked peptides					
ISCU	**NFS1**	**Figure**	**2-fold #1**	**2-fold #2**	**2-fold #3**	**3-fold**	**4-fold**	**C-term**	**# of peptides**						
Single digestion GluC										**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
*K$_{166}$K$_{167}$	IIFT$_{124}$S$_{125}$GAT$_{128}$E								3	3					
K$_{166}$	T$_{124}$		39.1 ± 2.1	38.1 ± 2.2	37.9 ± 2.7	51.1 ± 2.0	60.2 ± 1.7	53.5 ± 1.9							
K$_{166}$	S$_{125}$		35.5 ± 2.1	35.6 ± 2.2	37.4 ± 2.7	51.1 ± 1.8	60.6 ± 1.7	50.1 ± 1.9							
K$_{166}$	T$_{128}$		38.9 ± 1.2	42.2 ± 2.0	35.0 ± 2.5	50.3 ± 2.2	68.5 ± 1.9	55.8 ± 2.2							
K$_{167}$	T$_{124}$		40.4 ± 2.4	37.3 ± 1.5	36.6 ± 2.9	53.8 ± 1.6	60.9 ± 1.5	56.6 ± 1.6							
K$_{167}$	S$_{125}$		35.9 ± 1.6	34.4 ± 1.3	34.8 ± 1.8	53.6 ± 1.0	60.9 ± 1.6	53.3 ± 1.6							
K$_{167}$	T$_{128}$	9E	39.3 ± 1.5	40.6 ± 1.8	32.2 ± 2.0	52.6 ± 1.4	69.0 ± 1.6	58.5 ± 1.8							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
PK$_{160}$K$_{161}$GE	VDY$_{421}$T$_{422}$VEK$_{425}$CIQHVK$_{431}$RLRE MS$_{437}$PLWE								1			1			
K$_{160}$	Y$_{421}$	9E	28.0 ± 1.0	23.1 ± 1.6	66.8 ± 1.2	76.2 ± 1.0	85.9 ± 1.5	32.4 ± 1.2							
K$_{160}$	T$_{422}$		30.7 ± 1.3	25.2 ± 1.2	64.1 ± 1.1	43.7 ± 0.9	87.0 ± 1.1	35.3 ± 1.2							
K$_{160}$	K$_{425}$	9E	29.3 ± 1.2	25.9 ± 1.2	63.3 ± 1.3	72.5 ± 1.0	90.2 ± 1.0	35.3 ± 1.0							
K$_{160}$	K$_{431}$		33.7 ± 2.3	33.7 ± 1.5	64.2 ± 1.9	72.7 ± 1.6	99.6 ± 0.7	40.9 ± 0.7							
K$_{160}$	S$_{437}$		36.4 ± 2.6	39.2 ± 1.8	61.1 ± 2.1	68.5 ± 1.8	105.6 ± 1.6	45.5 ± 0.9							
K$_{161}$	Y$_{421}$		29.7 ± 1.3	26.5 ± 1.4	66.9 ± 1.1	73.7 ± 1.0	84.5 ± 1.5	32.5 ± 1.6							
K$_{161}$	T$_{422}$		32.0 ± 1.2	28.7 ± 1.3	64.3 ± 0.9	71.4 ± 1.0	85.4 ± 1.1	35.1 ± 1.6							
K$_{161}$	K$_{425}$		30.9 ± 1.1	29.3 ± 1.4	63.4 ± 1.1	70.3 ± 1.0	88.6 ± 0.9	35.0 ± 1.4							
K$_{161}$	K$_{431}$		35.9 ± 2.1	36.2 ± 1.6	64.4 ± 1.6	70.8 ± 1.6	97.9 ± 0.8	40.6 ± 1.0							
K$_{161}$	S$_{437}$		38.6 ± 2.5	40.8 ± 1.7	61.2 ± 1.8	66.8 ± 1.7	103.7 ± 1.5	44.5 ± 1.2							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
WVK$_{110}$GK$_{112}$TVEE	PS$_{389}$Y$_{390}$VLRAIGT$_{397}$DE								2				1		1
K$_{110}$	S$_{389}$		29.7 ± 1.5	66.5 ± 1.9	31.7 ± 2.6	41.6 ± 1.6	76.4 ± 2.5	61.6 ± 1.1							
K$_{110}$	Y$_{390}$		30.2 ± 1.4	67.1 ± 2.1	35.5 ± 3.5	43.5 ± 1.0	75.9 ± 2.6	63.7 ± 1.0							
K$_{110}$	T$_{397}$		28.4 ± 0.9	66.4 ± 1.4	35.5 ± 2.7	50.4 ± 2.2	81.7 ± 2.4	58.5 ± 0.9							
K$_{112}$	S$_{389}$		32.8 ± 1.2	65.3 ± 2.1	36.4 ± 2.9	38.8 ± 1.6	75.9 ± 2.6	56.9 ± 1.1							
K$_{112}$	Y$_{390}$		33.7 ± 1.6	66.2 ± 2.1	40.3 ± 3.7	41.0 ± 1.0	75.6 ± 2.3	59.1 ± 1.0							
K$_{112}$	T$_{397}$		33.2 ± 1.2	65.3 ± 1.6	40.1 ± 2.4	47.4 ± 2.0	81.8 ± 2.3	54.3 ± 0.8							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
ALT$_{119}$IK$_{121}$NT$_{123}$DIAK$_{127}$ELCLPPVK$_{135}$LHC SMLAE	PS$_{389}$Y$_{390}$VLRAIGT$_{397}$DE								1				1		
K$_{121}$	S$_{389}$		31.8 ± 1.0	58.9 ± 2.0	29.1 ± 1.5	50.7 ± 2.1	73.3 ± 2.4	65.4 ± 1.0							
K$_{121}$	Y$_{390}$		34.5 ± 1.6	59.8 ± 2.4	32.9 ± 1.6	53.1 ± 1.7	73.4 ± 2.4	67.8 ± 1.1							
K$_{121}$	T$_{397}$		32.8 ± 1.3	60.9 ± 1.5	34.2 ± 1.2	59.4 ± 2.4	78.3 ± 2.0	64.6 ± 0.7							
K$_{127}$	S$_{389}$		23.3 ± 1.1	68.6 ± 1.9	30.5 ± 1.5	49.9 ± 3.0	64.5 ± 2.4	68.4 ± 1.1							
K$_{127}$	Y$_{390}$	9E	25.6 ± 1.0	69.4 ± 2.4	34.4 ± 1.7	51.9 ± 2.8	64.4 ± 2.4	71.1 ± 1.0							
K$_{127}$	T$_{397}$		26.7 ± 1.0	70.8 ± 1.6	33.3 ± 1.6	59.3 ± 3.4	69.4 ± 2.0	67.2 ± 1.2							
K$_{135}$	S$_{389}$	14F	25.4 ± 2.6	64.0 ± 1.4	23.4 ± 1.7	61.7 ± 2.0	67.6 ± 2.3	78.5 ± 1.4							
K$_{135}$	Y$_{390}$	14F	28.2 ± 2.3	64.1 ± 1.7	26.1 ± 1.4	63.1 ± 2.4	68.9 ± 1.5	81.1 ± 1.3							
K$_{135}$	T$_{397}$		27.2 ± 3.1	67.4 ± 1.3	25.8 ± 2.0	71.0 ± 2.3	72.1 ± 1.5	77.7 ± 1.3							

Supplemental Table S2g, p.2

Cross-linked peptides			Distances (Å) measured in structure							False Discovery Rate (FDR) of cross-linked peptides					
ISCU	**NFS1**	**Figure**	**2-fold #1**	**2-fold #2**	**2-fold #3**	**3-fold**	**4-fold**	**C-term**	**# of peptides**						
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DAIK$_{147}$AALADY$_{153}$K$_{154}$LK$_{156}$QE	MS$_{437}$PLWE								1			1			
K$_{147}$	S$_{437}$		24.5 ± 2.0	52.9 ± 0.9	46.1 ± 1.8	75.3 ± 2.4	93.2 ± 0.6	47.7 ± 0.8							
K$_{154}$	S$_{437}$		30.5 ± 1.7	43.9 ± 1.0	54.8 ± 1.7	70.2 ± 1.9	102.9 ± 0.6	43.1 ± 0.6							
K$_{156}$	S$_{437}$		29.8 ± 1.3	41.3 ± 0.8	57.3 ± 1.9	71.2 ± 1.8	107.5 ± 0.5	43.7 ± 0.7							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
PK$_{160}$K$_{161}$GE AEK$_{166}$K$_{167}$	S$_{99}$EAAME								4			1		3	
K$_{160}$	S$_{99}$		43.4 ± 1.1	33.1 ± 1.0	63.4 ± 2.4	72.3 ± 2.2	47.1 ± 2.5	40.1 ± 0.7							
K$_{161}$	S$_{99}$		40.5 ± 1.3	32.9 ± 0.7	62.9 ± 2.4	69.1 ± 2.0	44.9 ± 2.6	38.4 ± 1.0							
K$_{166}$	S$_{99}$		38.2 ± 2.2	26.9 ± 1.8	55.9 ± 3.9	66.5 ± 3.2	37.5 ± 2.7	45.1 ± 1.7							
K$_{167}$	S$_{99}$	9E	40.7 ± 1.8	25.1 ± 0.8	54.7 ± 3.2	69.1 ± 2.9	37.8 ± 2.4	47.8 ± 1.9							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
PK$_{160}$K$_{161}$GE	Y$_{317}$DHK$_{320}$RIS$_{323}$K$_{324}$LSE								1	1					
K$_{160}$	Y$_{317}$		41.6 ± 1.0	31.1 ± 1.1	73.1 ± 1.1	83.6 ± 1.0	72.8 ± 2.3	39.8 ± 1.2							
K$_{160}$	K$_{320}$	9E	42.7 ± 1.1	32.8 ± 1.2	71.3 ± 0.8	81.9 ± 0.7	77.4 ± 2.0	42.3 ± 1.4							
K$_{160}$	S$_{323}$		41.6 ± 1.4	32.2 ± 1.3	67.6 ± 0.7	78.2 ± 0.8	80.6 ± 1.6	42.5 ± 1.6							
K$_{160}$	K$_{324}$	9E	40.8 ± 1.2	31.9 ± 1.2	70.2 ± 0.6	80.7 ± 0.7	82.4 ± 1.8	41.9 ± 1.4							
K$_{160}$	S$_{326}$		38.5 ± 1.4	30.1 ± 1.3	64.9 ± 0.6	75.4 ± 0.7	83.1 ± 1.5	40.8 ± 1.5							
K$_{161}$	Y$_{317}$		41.3 ± 1.2	34.2 ± 1.3	73.6 ± 1.0	81.1 ± 0.8	71.7 ± 2.4	40.3 ± 1.5							
K$_{161}$	K$_{320}$		42.7 ± 1.2	35.9 ± 1.3	71.9 ± 0.7	79.6 ± 0.5	76.2 ± 2.1	42.5 ± 1.8							
K$_{161}$	S$_{323}$		41.8 ± 1.5	35.5 ± 1.4	68.3 ± 0.8	75.9 ± 0.6	79.2 ± 1.7	42.4 ± 2.1							
K$_{161}$	K$_{324}$		41.3 ± 1.3	35.4 ± 1.3	70.8 ± 0.7	78.4 ± 0.6	81.1 ± 1.8	42.1 ± 1.8							
K$_{161}$	S$_{326}$		38.9 ± 1.5	33.5 ± 1.6	65.6 ± 0.7	73.1 ± 0.6	81.6 ± 1.5	40.5 ± 2.0							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
WVK$_{110}$GK$_{112}$TVEE	VDY$_{421}$T$_{422}$VE								1	1					3
K$_{110}$	Y$_{421}$		13.4 ± 1.3	41.3 ± 1.0	54.8 ± 1.0	72.2 ± 1.6	69.4 ± 1.1	39.6 ± 1.2							
K$_{110}$	T$_{422}$	9E	13.2 ± 1.1	44.2 ± 1.1	52.4 ± 0.9	69.3 ± 1.7	70.2 ± 1.0	42.0 ± 1.1							
K$_{112}$	Y$_{421}$	9E	17.5 ± 2.1	41.1 ± 1.2	58.1 ± 1.0	68.2 ± 1.8	71.0 ± 1.3	36.7 ± 1.5							
K$_{112}$	T$_{422}$		18.0 ± 1.6	44.0 ± 1.2	55.8 ± 0.7	65.4 ± 1.9	71.6 ± 0.9	38.9 ± 1.3							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
*DAIK$_{147}$AALADY$_{153}$K$_{154}$LK$_{156}$QE	IIFT$_{124}$S$_{125}$GAT$_{128}$E								1	1					
K$_{147}$	T$_{124}$		38.1 ± 1.6	40.8 ± 1.1	37.7 ± 2.5	52.6 ± 0.9	59.5 ± 2.6	61.0 ± 0.7							
K$_{147}$	S$_{125}$		34.0 ± 1.2	38.4 ± 1.1	35.6 ± 1.9	52.7 ± 1.3	58.9 ± 2.2	57.6 ± 0.6							
K$_{147}$	T$_{128}$	9E	35.7 ± 0.5	44.3 ± 1.2	31.4 ± 1.9	51.3 ± 0.9	67.5 ± 2.6	62.1 ± 0.6							
K$_{154}$	T$_{124}$		44.0 ± 1.2	42.6 ± 1.2	44.3 ± 1.9	53.9 ± 0.9	66.6 ± 2.5	54.9 ± 0.7							
K$_{154}$	S$_{125}$		40.5 ± 1.3	39.7 ± 1.2	43.8 ± 1.9	53.5 ± 0.8	67.0 ± 2.6	51.5 ± 0.7							
K$_{154}$	T$_{128}$		42.8 ± 0.6	44.0 ± 0.9	39.8 ± 2.0	50.8 ± 1.0	75.0 ± 2.5	55.8 ± 0.7							
K$_{156}$	T$_{124}$		48.1 ± 1.2	43.7 ± 1.1	47.0 ± 2.6	57.4 ± 1.3	70.7 ± 2.3	56.1 ± 0.7							
K$_{156}$	S$_{125}$		44.7 ± 1.3	40.9 ± 1.3	45.8 ± 1.8	57.0 ± 0.9	70.6 ± 1.8	52.9 ± 0.7							
K$_{156}$	T$_{128}$		46.5 ± 0.5	44.3 ± 0.8	41.2 ± 2.2	53.7 ± 1.5	78.6 ± 1.8	57.0 ± 0.5							

Supplemental Table S2g, p.3

Cross-linked peptides			Distances (Å) measured in structure							False Discovery Rate (FDR) of cross-linked peptides					
ISCU	**NFS1**	**Figure**	**2-fold #1**	**2-fold #2**	**2-fold #3**	**3-fold**	**4-fold**	**C-term**	**# of peptides**						
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
ALTIK$_{121}$NTDIAK$_{127}$ELCLPPVK$_{135}$LHCSML	VDY$_{421}$T$_{422}$VE								1		1				
K$_{121}$	Y$_{421}$	9E	26.6 ± 0.7	41.5 ± 1.4	48.9 ± 1.2	76.8 ± 1.9	64.7 ± 1.3	47.0 ± 1.2							
K$_{121}$	T$_{422}$		26.4 ± 0.8	43.4 ± 1.4	46.8 ± 1.0	75.3 ± 2.1	65.8 ± 0.9	49.3 ± 1.1							
K$_{127}$	Y$_{421}$	9E	27.8 ± 1.0	50.6 ± 1.7	44.5 ± 0.9	76.6 ± 2.0	56.4 ± 1.0	52.1 ± 1.2							
K$_{127}$	T$_{422}$		26.6 ± 1.1	52.8 ± 1.7	42.8 ± 0.6	72.6 ± 1.6	57.2 ± 0.7	54.1 ± 1.0							
K$_{135}$	Y$_{421}$		33.5 ± 1.5	50.1 ± 1.6	35.2 ± 1.4	87.5 ± 1.8	54.0 ± 1.2	60.3 ± 1.3							
K$_{135}$	T$_{422}$		31.9 ± 1.8	51.7 ± 1.7	33.2 ± 1.7	85.2 ± 2.2	55.6 ± 1.2	62.7 ± 1.1							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
K$_{166}$K$_{167}$	PS$_{389}$Y$_{390}$VLRAIGT$_{397}$DE								1	1					
K$_{166}$	S$_{389}$		42.2 ± 1.3	49.9 ± 2.6	33.5 ± 2.8	50.5 ± 2.7	82.4 ± 1.6	60.0 ± 2.7							
K$_{166}$	Y$_{390}$		44.3 ± 1.2	50.7 ± 2.8	36.7 ± 2.9	52.8 ± 2.6	83.5 ± 2.4	61.6 ± 2.7							
K$_{166}$	T$_{397}$		42.3 ± 1.3	51.8 ± 2.6	40.8 ± 1.6	58.1 ± 1.7	88.7 ± 2.1	59.6 ± 2.4							
K$_{167}$	S$_{389}$		41.6 ± 2.6	49.7 ± 2.7	30.1 ± 2.6	52.6 ± 1.6	83.6 ± 2.5	62.9 ± 2.0							
K$_{167}$	Y$_{390}$	9E	43.5 ± 2.0	50.7 ± 3.0	32.6 ± 2.5	55.3 ± 1.6	83.4 ± 1.9	65.0 ± 2.2							
K$_{167}$	T$_{397}$		40.4 ± 1.3	50.7 ± 2.1	37.5 ± 1.5	60.3 ± 0.7	88.3 ± 1.4	62.2 ± 1.5							
Double digestion GluC&AspN										**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DAIK$_{147}$AALA	DVALS$_{376}$S$_{377}$GS$_{379}$ACT$_{382}$S$_{383}$AS$_{385}$ LEPS$_{389}$Y$_{390}$VLRAIGT								3	2		1			
K$_{147}$	S$_{376}$		24.3±1.1	40.2±1.9	30.7±0.6	56.2±1.8	72.8±2.4	52.9±1.0							
K$_{147}$	S$_{377}$		26.4±1.5	44.1±1.6	29.2±1.5	55.2±1.7	77.1±1.5	55.7±1.4							
K$_{147}$	S$_{379}$		30.3±1.4	48.5±2.1	25.9±1.0	52.3±1.3	79.1±2.4	59.4±0.9							
K$_{147}$	T$_{382}$		28.8±1.4	48.0±1.5	21.1±1.6	46.4±2.0	79.9±2.6	57.3±0.6							
K$_{147}$	S$_{383}$	9E	31.6±1.6	49.4±1.6	19.7±1.6	45.1±1.6	78.9±2.3	59.7±0.8							
K$_{147}$	S$_{385}$	14F	37.2±1.3	55.1±1.6	17.6±1.7	42.9±1.9	82.4±1.5	65.5±1.0							
K$_{147}$	S$_{389}$		36.2±0.9	53.7±1.8	26.5±1.7	51.2±1.0	81.4±2.0	65.3±1.2							
K$_{147}$	Y$_{390}$		38.4±1.1	54.7±1.8	28.5±0.9	53.6±0.8	82.8±1.3	67.3±1.3							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
ALT$_{119}$IK$_{121}$NT$_{123}$	DLK$_{450}$S$_{451}$IK$_{453}$WT$_{455}$QH-c								2	1		1			
T$_{119}$	K$_{450}$		27.6±1.8	49.5±2.2	37.2±1.6	61.5±2.6	88.4±2.4	32.9±2.3							
K$_{121}$	K$_{450}$	9E	24.9±2.4	55.7±2.2	32.0±1.5	66.4±2.2	80.5±2.8	37.7±2.4							
T$_{123}$	K$_{450}$		26.8±2.2	59.9±2.4	27.6±1.7	68.8±2.1	77.2±2.6	37.8±2.1							
T$_{119}$	K$_{453}$		24.8±2.7	48.0±2.8	31.5±2.1	55.3±2.4	81.3±2.8	33.0±1.9							
K$_{121}$	K$_{453}$	9E	20.5±2.9	53.7±2.8	26.7±2.1	61.0±2.4	76.7±2.4	36.6±2.2							
T$_{123}$	K$_{453}$		21.5±2.8	57.2±2.7	21.9±2.1	64.3±2.9	73.4±2.8	36.8±2.3							
K$_{121}$	S$_{451}$		23.5±2.0	54.5±2.1	30.1±1.2	64.0±1.4	79.2±2.7	35.9±2.5							
K$_{121}$	T$_{455}$		19.2±3.2	51.9±2.3	22.3±1.8	61.6±2.5	74.7±2.4	41.5±2.8							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
*PK$_{160}$K$_{161}$GE	RLIQNIMK$_{335}$S$_{336}$LP								2	2					
K$_{160}$	K$_{335}$		42.8±2.1	38.4±1.9	66.9±1.1	77.0±1.2	97.1±1.5	47.4±1.5							
K$_{160}$	S$_{336}$		40.0±2.3	36.3±2.1	66.8±1.0	76.6±1.1	97.2±1.7	44.9±1.5							
K$_{161}$	K$_{335}$		44.3±2.0	41.6±1.9	67.9±1.1	75.2±1.3	95.6±1.5	47.3±1.9							
K$_{161}$	S$_{336}$		41.6±2.3	39.5±2.1	67.6±1.0	74.7±1.3	95.6±1.7	44.8±1.9							

Supplemental Table S2g, p.4

Cross-linked peptides			Distances (Å) measured in structure							False Discovery Rate (FDR) of cross-linked peptides					
ISCU	**NFS1**	**Figure**	**2-fold #1**	**2-fold #2**	**2-fold #3**	**3-fold**	**4-fold**	**C-term**	**# of peptides**						
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DAIK$_{147}$AALA	DLK$_{450}$S$_{451}$IK$_{453}$WT$_{455}$QH-c								2	1		1			
K$_{147}$	K$_{450}$	9E	21.8±2.4	50.6±2.4	40.0±2.1	67.1±1.7	89.9±3.3	40.1±2.9							
K$_{147}$	K$_{453}$	9E	20.9±2.6	50.5±2.9	34.1±2.5	62.4±3.0	85.7±2.5	40.2±1.9							
K$_{147}$	S$_{451}$		21.9±1.9	50.8±2.2	38.0±1.7	65.3±2.2	88.0±2.8	39.2±2.9							
K$_{147}$	T$_{455}$		21.4±2.5	48.6±1.9	28.3±1.3	59.2±3.3	83.8±2.2	44.5±2.1							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DHY$_{43}$ENPRNVGS$_{51}$L	K$_{425}$CIQHVK$_{431}$RLRE								1	1					
Y$_{43}$	K$_{425}$		NM	NM	NM	NM	NM	NM							
S$_{51}$	K$_{425}$	9E	7.3±1.2	40.2±1.0	54.7±1.8	76.4±1.3	84.8±1.6	38.9±1.5							
Y$_{43}$	K$_{431}$		NM	NM	NM	NM	NM	NM							
S$_{51}$	K$_{431}$		14.7±1.6	47.8±1.2	51.8±1.6	77.2±1.8	94.9±1.7	38.6±1.4							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
*DK$_{54}$T$_{55}$S$_{56}$K$_{57}$NVGT$_{61}$GLVGAPACGDVM K$_{74}$LQIQV	DVNDMK$_{248}$I								1			1			
K$_{54}$	K$_{248}$		49.6±0.9	61.1±1.3	65.5±2.7	64.0±2.4	74.7±2.1	68.6±1.4							
T$_{55}$	K$_{248}$		47.8±0.7	61.7±1.3	65.9±2.4	61.8±2.4	72.5±1.5	66.9±1.3							
S$_{56}$	K$_{248}$		49.4±1.1	59.3±1.4	64.3±2.6	62.9±2.4	73.4±1.8	65.3±1.4							
K$_{57}$	K$_{248}$		52.4±1.3	55.7±1.4	61.7±2.6	66.4±2.4	75.8±1.8	65.7±1.4							
T$_{61}$	K$_{248}$		52.3±1.1	48.2±1.6	50.9±2.7	71.6±1.8	72.4±1.8	75.6±1.7							
K$_{74}$	K$_{248}$		50.7±1.2	46.7±1.8	45.2±2.3	72.7±1.5	68.7±1.7	82.5±1.7							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
*DK$_{54}$T$_{55}$S$_{56}$K$_{57}$NVGT$_{61}$GLVGAPACG	IGVK$_{212}$QPIAE								1			1			
K$_{54}$	K$_{212}$	9E	35.9±0.8	50.5±1.2	60.4±2.4	63.6±2.1	81.4±2.1	55.2±1.1							
T$_{55}$	K$_{212}$		34.5±0.6	51.2±1.2	60.5±2.1	61.0±1.9	79.2±1.6	53.5±1.0							
S$_{56}$	K$_{212}$		36.2±0.9	48.6±1.4	60.1±2.6	62.0±1.9	79.6±2.0	52.3±1.1							
K$_{57}$	K$_{212}$		38.9±0.8	44.9±1.4	58.1±2.4	65.2±1.9	81.4±2.1	53.2±1.1							
T$_{61}$	K$_{212}$		37.6±0.3	40.3±1.8	46.0±2.4	71.8±0.8	75.9±2.1	63.9±1.3							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
LCLPPVK$_{135}$LHCSMLAEDAIK$_{147}$AALA	DLAHS$_{404}$S$_{405}$IRFGIGRFT$_{414}$T$_{415}$EE								1			1			
K$_{135}$	S$_{404}$	9E,F	19.4±2.5	60.5±1.0	19.0±2.8	68.8±2.4	65.1±1.6	68.4±1.4							
K$_{135}$	S$_{405}$		22.3±1.4	59.1±0.9	21.3±2.3	69.2±2.2	63.6±1.7	70.4±1.2							
K$_{135}$	T$_{414}$		33.9±1.3	43.1±1.7	35.2±0.8	81.5±1.8	47.1±1.1	63.8±1.2							
K$_{135}$	T$_{415}$		37.0±1.5	42.8±2.0	38.4±0.8	85.1±1.9	46.6±1.3	63.1±1.2							
K$_{147}$	S$_{404}$	9E	25.5±1.6	46.8±1.3	31.2±1.1	58.9±1.9	81.4±1.9	54.3±1.5							
K$_{147}$	S$_{405}$		26.6±0.9	45.8±1.2	33.1±0.7	60.3±1.5	79.7±1.8	55.7±1.5							
K$_{147}$	T$_{414}$	9E	24.5±0.9	27.7±1.2	51.6±1.0	76.3±1.1	64.2±1.2	45.8±1.4							
K$_{147}$	T$_{415}$		25.8±0.8	27.1±1.4	55.1±1.0	80.0±1.1	63.9±1.2	44.7±1.5							
										≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
ALT$_{119}$IK$_{121}$NT$_{123}$DIAK$_{127}$E	S$_{99}$EAAME								1			1			
K$_{121}$	S$_{99}$		36.0±1.1	25.3±1.9	52.6±2.4	62.1±1.3	27.2±3.3	55.0±1.5							
K$_{127}$	S$_{99}$	9E	32.9±1.7	31.3±2.2	54.6±2.2	56.9±1.6	20.1±2.3	63.0±1.8							

Supplemental Table S2g, p.5

Cross-linked peptides			Distances (Å) measured in structure							False Discovery Rate (FDR) of cross-linked peptides					
ISCU	NFS1	Figure	2-fold #1	2-fold #2	2-fold #3	3-fold	4-fold	C-term	# of peptides						
										≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
K$_{82}$GK$_{84}$IV	DVVMNGDPK$_{347}$HHY$_{350}$PGCINL S$_{357}$FAY$_{360}$VE								1		1				
K$_{82}$	K$_{347}$		34.5±1.8	49.3±2.2	67.2±1.4	64.7±0.8	79.5±1.8	47.6±1.4							
K$_{82}$	Y$_{350}$		30.2±1.0	45.9±1.4	61.5±1.2	58.4±0.7	73.7±1.3	44.3±0.9							
K$_{82}$	S$_{357}$		34.5±1.5	50.4±1.6	50.9±0.9	51.2±1.4	87.8±1.4	42.3±1.4							
K$_{82}$	Y$_{360}$		41.6±1.1	56.9±1.1	49.7±1.4	52.0±2.5	97.6±1.6	46.8±1.0							
K$_{84}$	K$_{347}$		28.1±1.6	53.1±2.3	64.1±1.9	63.5±0.6	73.2±1.9	52.9±1.4							
K$_{84}$	Y$_{350}$	9E	23.5±0.9	49.7±1.5	58.8±1.7	57.0±0.7	67.7±1.4	49.9±0.9							
K$_{84}$	S$_{357}$		27.7±1.4	56.0±1.5	47.0±1.1	51.5±2.0	82.3±1.7	45.9±1.4							
K$_{84}$	Y$_{360}$		35.8±1.3	62.9±1.0	45.0±1.2	54.0±2.9	92.2±1.7	49.1±1.1							
										≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
PK$_{160}$K$_{161}$GE	DHK$_{320}$RIS$_{323}$K$_{324}$LSERLIQNIMK$_{335}$ SLP								1			1			
K$_{160}$	K$_{320}$		42.7 ± 1.1	32.8 ± 1.2	71.3 ± 0.8	81.9 ± 0.7	77.4 ± 2.0	42.3 ± 1.4							
K$_{160}$	S$_{323}$		41.6 ± 1.4	32.2 ± 1.3	67.6 ± 0.7	78.2 ± 0.8	80.6 ± 1.6	42.5 ± 1.6							
K$_{160}$	K$_{324}$		40.8 ± 1.2	31.9 ± 1.2	70.2 ± 0.6	80.7 ± 0.7	82.4 ± 1.8	41.9 ± 1.4							
K$_{160}$	K$_{335}$		42.8±2.1	38.4±1.9	66.9±1.1	77.0±1.2	97.1±1.5	47.4±1.5							
K$_{161}$	K$_{320}$		42.7 ± 1.2	35.9 ± 1.3	71.9 ± 0.7	79.6 ± 0.5	76.2 ± 2.1	42.5 ± 1.8							
K$_{161}$	S$_{323}$		41.8 ± 1.5	35.5 ± 1.4	68.3 ± 0.8	75.9 ± 0.6	79.2 ± 1.7	42.4 ± 2.1							
K$_{161}$	K$_{324}$		41.3 ± 1.3	35.4 ± 1.3	70.8 ± 0.7	78.4 ± 0.6	81.1 ± 1.8	42.1 ± 1.8							
K$_{161}$	K$_{335}$		44.1±1.9	41.6±1.9	67.9±1.1	75.2±1.3	95.6±1.5	47.3±1.9							

Supplemental Table S2h, p.1

Cross-linked peptides			Distances (Å) measured in structure		False Discovery Rate (FDR) of cross-linked peptides					
FXN$^{42-210}$	**ISD11**	**Figure**	**FXN$^{42-210}$-ISD11**	**# of peptides**						
Single digestion GluC					**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
*$T_{93}T_{94}Y_{95}$ERLAE	NK_{44}NVK_{47}DPVE			1				1		
T_{93}	K_{44}		32.3 ± 0.1							
T_{94}	K_{44}		36.2 ± 0.7							
Y_{95}	K_{44}		38.7 ± 2.1							
T_{93}	K_{47}		33.9 ± 0.2							
T_{94}	K_{47}		37.5 ± 0.7							
Y_{95}	K_{47}		38.9 ± 0.6							
					≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
*ET_{102}LDS_{105}LAE	n-MAQVLS_{11}LY_{13}RAMLRE			5	1			1	3	
T_{102}	N-term		28.3 ± 0.1							
S_{105}	N-term	9G	28.6 ± 1.3							
Double digestion GluC&AspN					**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
*LTK_{192}ALK_{195}TK_{197}L	NK_{44}NVK_{47}			2	1		1			
K_{192}	K_{44}		36.7 ± 1.3							
K_{192}	K_{47}		39.8 ± 1.3							
K_{195}	K_{44}		37.2 ± 1.3							
K_{195}	K_{47}		40.6 ± 1.1							
K_{197}	K_{44}	9G	33.7 ± 0.1							
K_{197}	K_{47}		37.7 ± 0.2							
T_{191}	K_{44}		36.8 ± 1.1							
T_{196}	K_{44}		36.0 ± 0.1							
T_{191}	K_{47}		40.3 ± 1.2							
T_{196}	K_{47}		39.7 ± 0.0							

Supplemental Table S2h, p.2

Cross-linked peptides			Distances (Å) measured in structure		False Discovery Rate (FDR) of cross-linked peptides					
FXN$^{42\text{-}210}$	**ISD11**	**Figure**	**FXN$^{42\text{-}210}$-ISD11**	**# of peptides**						
LOW SCORE PEPTIDE					**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤45%**
DATCTPRRASSNQRGLNQIWNVK$_{69}$K$_{70}$QSVYLMNLRK$_{80}$SGTLGHPGSL	NK$_{44}$NVK$_{47}$DPVE			2						2
T$_{49}$	K$_{44}$		NM							
K$_{69}$	K$_{44}$	9G	12.9 ± 1.4							
K$_{70}$	K$_{44}$		16.2 ± 1.6							
K$_{80}$	K$_{44}$		26.6 ± 1.1							
K$_{69}$	K$_{47}$		13.7 ± 1.8							
K$_{70}$	K$_{47}$	9G	16.6 ± 2.0							
K$_{80}$	K$_{47}$		31.1 ± 1.0							

Supplemental Table S2h, p.3

Cross-linked peptides			Distances (Å) measured in structure		False Discovery Rate (FDR) of cross-linked peptides					
ISCU	ISD11	Figure	ISCU-ISD11	# of peptides						
Single digestion GluC					≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
*PK$_{160}$K$_{161}$GE	NRDMPRT$_{91}$-c			1					1	
K$_{160}$	T$_{91}$		36.8 ± 0.7							
K$_{161}$	T$_{91}$	9G	35.4 ± 1.1							
					≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
LCLPPVK$_{135}$LHCS$_{139}$MLAEDAIK$_{147}$AALADY$_{153}$K$_{154}$LK$_{156}$QE	NK$_{44}$NVK$_{47}$DPVE			1					1	
K$_{135}$	K$_{44}$		33.0 ± 1.0							
S$_{139}$	K$_{44}$		27.5 ± 2.9							
K$_{147}$	K$_{44}$	9G	21.9 ± 3.0							
Y$_{153}$	K$_{44}$		16.4 ± 2.9							
K$_{154}$	K$_{44}$		20.1 ± 3.0							
K$_{156}$	K$_{44}$		18.2 ± 3.3							
K$_{135}$	K$_{47}$		36.4 ± 4.1							
S$_{139}$	K$_{47}$		29.2 ± 3.0							
K$_{147}$	K$_{47}$		23.6 ± 3.4							
Y$_{153}$	K$_{47}$		17.9 ± 3.4							
K$_{154}$	K$_{47}$		21.4 ± 3.7							
K$_{156}$	K$_{47}$	9G	18.9 ± 4.0		≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
ALT$_{119}$IK$_{121}$NT$_{123}$DIAK$_{127}$ELCLPPVK$_{135}$LHCS$_{139}$MLAE	n-MAQVLS$_{11}$LY$_{13}$RAMLRE			1				1		
K$_{121}$	N-term	9G	24.0 ± 0.7							
K$_{121}$	S$_{11}$		29.3 ± 0.8							
K$_{121}$	Y$_{13}$		30.1 ± 1.1							
K$_{127}$	N-term	9G	24.7 ± 1.8							
K$_{127}$	S$_{11}$		29.3 ± 1.6							
K$_{127}$	Y$_{13}$		29.4 ± 1.6							
K$_{135}$	N-term		35.5 ± 1.1							
K$_{135}$	S$_{11}$		40.0 ± 1.4							
K$_{135}$	Y$_{13}$		39.4 ± 1.8							

Supplemental Table S2h, p.4

Cross-linked peptides			Distances (Å) measured in structure		False Discovery Rate (FDR) of cross-linked peptides					
ISCU	ISD11	Figure	ISCU-ISD11	# of peptides						
					≤5 %	≤10%	≤20%	≤25 %	≤35 %	≤60%
P$K_{160}K_{161}$GE	n-MAQVLS_{11}LY_{13}RAMLRE			3						3
K_{160}	N-term	9G	15.5 ± 3.0							
K_{160}	S_{11}		20.7 ± 2.6							
K_{160}	Y_{13}		23.6 ± 2.2							
K_{161}	N-term		18.7 ± 3.0							
K_{161}	S_{11}		24.1 ± 2.6							
K_{161}	Y_{13}		27.1 ± 2.3							

Supplemental Table S2h, p.5

Cross-linked peptides			Distances (Å) measured in structure		False Discovery Rate (FDR) of cross-linked peptides					
ISD11	**NFS1**	**Figure**	**ISD11-NFS1**	**# of peptides**						
Single digestion GluC					**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
NRDMPRT$_{91}$-c	S$_{365}$LLMALK$_{371}$DVALS$_{376}$S$_{377}$GS$_{379}$ACT$_{382}$S$_{383}$AS$_{385}$LEPS$_{389}$Y$_{390}$VLRAIGT$_{397}$DE			2			2			
T$_{91}$ C-term	K$_{371}$	9G	14.7 ± 1.3							
					≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
*SK$_{21}$RFS$_{24}$AY$_{26}$NY$_{28}$RT$_{30}$Y$_{31}$AVRRIRDAF	RARQQVAS$_{112}$LIGADPRE			1				1		
K$_{21}$	S$_{112}$	9G	30.9 ± 0.6							
Double digestion GluC&AspN										
ISD11	**NFS1**				**≤5 %**	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
NK$_{44}$NVK$_{47}$	Y$_{317}$DHK$_{320}$RIS$_{323}$K$_{324}$LSERLIQNIMK$_{335}$SLP			4	1		3			
K$_{44}$	Y$_{317}$		32.6±0.1							
K$_{44}$	K$_{320}$		30.5±0.4							
K$_{44}$	S$_{323}$		27.0±1.0							
K$_{44}$	K$_{324}$		25.4±0.8							
K$_{44}$	K$_{335}$	9G	19.8±0.6							
K$_{47}$	Y$_{317}$		37.7±0.0							
K$_{47}$	K$_{320}$		35.6±0.4							
K$_{47}$	S$_{323}$		32.7±1.0							
K$_{47}$	K$_{324}$		30.5±0.8							
K$_{47}$	K$_{335}$	9G	23.2±0.6							
					≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DK$_{80}$LIIE	DVALS$_{376}$S$_{377}$GS$_{379}$ACT$_{382}$S$_{383}$AS$_{385}$LEP S$_{389}$Y$_{390}$VLRAIGT			1	1					
K$_{80}$	S$_{376}$		32.4±1.8							
K$_{80}$	S$_{377}$		34.4±0.1							
K$_{80}$	S$_{379}$		34.9±0.6							
K$_{80}$	T$_{382}$		39.5±2.3							
K$_{80}$	S$_{383}$		39.8±0.1							
K$_{80}$	S$_{385}$		36.6±0.8							
K$_{80}$	S$_{389}$		32.3±0.4							
K$_{80}$	Y$_{390}$	9G	30.4±2.6							

Supplemental Table S2h, p.6

Cross-linked peptides			Distances (Å) measured in structure		False Discovery Rate (FDR) of cross-linked peptides					
ISD11	**NFS1**	**Figure**	**ISD11-NFS1**	**# of peptides**						
					≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
SK$_{21}$RFSAYNYRTYAVRRIR	MEY$_{317}$			1	1					
K$_{21}$	Y$_{317}$	9G	10.5±1.1							
					≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DK$_{80}$LIIE	S$_{365}$LLMALK$_{371}$			1			1			
K$_{80}$	S$_{365}$		31.8±1.6							
K$_{80}$	K$_{371}$		31.5±1.3							
					≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DMPRT$_{91}$-c	DVNDMK$_{248}$I			1			1			
T$_{91}$	K$_{248}$	9G	26.7±1.1							
					≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DMPRT$_{91}$-c	DPK$_{347}$HHY$_{350}$PGCINLS$_{357}$FAY$_{360}$VE			1			1			
T$_{91}$	K$_{347}$	9G	9.8±1.1							
					≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
n-MAQVLS$_{11}$LY$_{13}$RAMLRE	RGMRS$_{293}$GT$_{295}$VPT$_{298}$PLVVGLGAACE			1			1			
N-term	S$_{293}$		26.5±0.4							
N-term	T$_{295}$		24.2±0.8							
N-term	T$_{298}$		21.2±1.6							
					≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
NRDMPRT$_{91}$-c	DHK$_{320}$RIS$_{323}$K$_{324}$LSERLIQNIMK$_{335}$SLP			1				1		
T$_{91}$	K$_{320}$	9G	17.4±1.6							
T$_{91}$	K$_{324}$		13.5±0.5							
T$_{91}$	K$_{335}$	9G	11.8±0.2							
					≤5 %	**≤10%**	**≤20%**	**≤25 %**	**≤35 %**	**≤60%**
DMPRT$_{91}$-c	DAAQAVGK$_{239}$IPLDVN			1				1		
T$_{91}$	K$_{239}$	9G	15.9±1.0							

Shown are all of the FXN^{42-210}-FXN^{42-210} (Supplemental Table S2a), ISCU-ISCU (Supplemental Table S2b), NFS1-NFS1 (Supplemental Table S2c), ISD11-ISD11 (Supplemental Table S2d), FXN^{42-210}-ISCU (Supplemental Table S2e), FXN^{42-210}-NFS1 (Supplemental Table S2f), ISCU-NFS1 (Supplemental Table S2g) and FXN^{42-210}-ISD11, ISCU-ISD11 and NFS1-ISD11 (Supplemental Table S2h) cross-linked peptides with FDR (False Discovery Rate) ≤5-≤60% identified as described in Experimental Procedures. Some of the cross-links are shown in Fig. 6-9, as indicated in the table (column labeled Figure). The number of times any given peptide was identified by MS/MS is also shown (column labeled # of peptides). The distance constraints and maximum allowable distance constraints between K-K, K–N-term, N-term–N-term, K-Y, K-S, K-T, N-term–S, N-term–T, and N-term–Y were calculated as described in Experimental Procedures and are shown in the table below. Distances were measured between all possible pairs of cross-linked residues within each of the cross-linked peptides in the simulated half structure of the complex, and are expressed as mean ± S.D. For each distance, we show the mean ± S.D. of 4-12 measurements depending on the geometric arrangement of the four proteins in the complex. The small standard deviations demonstrate that in the structure there are small differences in the orientation of the side chain of any given cross-linked residue among different protein subunits. Distances measured in the $[FXN^{42-210}]_{12}$•$[ISCU]_{12}$•$[NFS1]_{12}$ structure that are equal to or lower than the distance constraints are highlighted in light gray; distances that are equal to or lower than the maximum allowable distance constraints are highlighted in dark gray, and distances greater than the maximum allowable distance constraints are highlighted in yellow. In Supplemental Table S2e, FXN^{42-210}-ISCU *intra-dimer* distances are measured between FXN^{42-210} and ISCU subunits of the same $[FXN^{42-210}]$•[ISCU] heterodimer; FXN^{42-210}-ISCU *intra-trimer* distances are measured between FXN^{42-210} and ISCU subunits of the same $[FXN^{42-210}]_3$•$[ISCU]_3$ sub-complex, and FXN^{42-210}-ISCU *inter-trimers* distances are measured between FXN^{42-210} and ISCU subunits of two adjacent $[FXN^{42-210}]_3$•$[ISCU]_3$ sub-complexes. In Supplemental Table S2a, S2b, S2c or S2d, FXN^{42-210}, ISCU, NFS1 and ISD11 *intra-monomer* distances are measured within individual ISCU, NFS1 and ISD11 subunits, FXN^{42-210}-FXN^{42-210} or ISCU-ISCU *intra-trimer* distances are measured between FXN^{42-210} or ISCU subunits of the same $[FXN^{42-210}]_3$•$[ISCU]_3$ sub-complex, and FXN^{42-210}-FXN^{42-210} or ISCU-ISCU *inter-trimers* distances are measured between FXN^{42-210} or ISCU subunits of two adjacent $[FXN^{42-210}]_3$•$[ISCU]_3$ sub-complexes. In Supplemental Table S2f and S2g, FXN^{42-210}-NFS1 or ISCU-NFS1 *2-fold* #1, *2-fold* #2 and *2-fold* #3 distances are measured at the two-fold axis of the complex with three different modes of interaction of NFS1 with FXN^{42-210} and/or ISCU subunits, *3-fold* distances are measured at the three-fold axis of the complex, *4-fold* distances at the four-fold axis of the complex, and *C-term* distances between FXN^{42-210} or ISCU and the C-terminal region of NFS1. In Supplemental Table S2c, NFS1-NFS1 *monomer*, *dimer*, *trimer* and *tetramer* distances are measured, respectively, within the same NFS1 subunit or between two NFS1 subunits at the two-, three- and four-fold axis of the complex. Peptides beginning with the letter n are N-terminal peptides. NA, not applicable; NM, not measured.

	Distance Constraints (Å)	**Maximum allowable distance constraints (Å)**									
		FXN$^{42-210}$-FXN$^{42-210}$	**FXN$^{42-210}$-ISCU**	**FXN$^{42-210}$-ISD11**	**FXN$^{42-210}$-NFS1**	**ISCU-ISCU**	**ISCU-NFS1**	**ISCU-ISD11**	**ISD11-ISD11**	**ISD11-NFS1**	**NFS1-NFS1**
K-K	24.0	34.2	33.0	32.6	33.0	31.8	31.8	31.4	31.0	31.4	31.8
N-term–K	19.2	29.4	28.2	27.8	28.2	27.0	27.0	26.6	26.2	26.6	27.0
N-term–N-term	14.4	24.6	23.4	23.0	23.4	22.2	22.2	21.8	21.4	21.8	22.2
Y-K	24.1	34.3	33.1	32.7	33.1	31.9	31.9	31.5	31.1	31.5	31.9
S-K	20.1	30.3	29.1	28.7	29.1	27.9	27.9	27.5	27.1	27.5	27.9
T-K	20.1	30.3	29.1	28.7	29.1	27.9	27.9	27.5	27.1	27.5	27.9
N-term–S	15.3	25.5	24.3	23.9	24.3	23.1	23.1	22.7	22.3	22.7	23.1
N-term–T	15.3	25.5	24.3	23.9	24.3	23.1	23.1	22.7	22.3	22.7	23.1
N-term–Y	19.3	29.5	28.3	27.9	28.3	27.1	27.1	26.7	26.3	26.7	27.1

Supplemental Table S3. Contact Surfaces of [FXN$^{42\text{-}210}$]$_6$•[NFS1]$_2$•[ISCU]$_6$ Sub-Complex as Determined by PISA Analysis

Interface	**Buried surface area (Å^2)**	**Residues involdved in hydrogen bonding**	**Residues involdved in salt bridges**	**Residues involdved in hydrophobic interactions**
FXN$^{42\text{-}210}$-ISCU interfaces				
	1646			
		FXN$^{42\text{-}210}$: *N-term* : Gln-64, Lys-69, **Gln-71**, Ser-72, Val-73, Tyr-74, Leu-78, Arg-79, *C-term* : Lys-197, Asp-199, Ser-201, Lys-208. ISCU: *N-term* , *β-sheet* : **Lys-54**, Thr-55, Lys-57, **Val-59**, **Thr-61**, **Leu-63**, **Val-64**, **Gly-65**, **Ala-66**, **Pro-67**, **Gly-70**, **Asp-71**, **Gln-76**, Asp-80, Glu-81, Lys-84; *C-term* , *α2* and *α3* : **Gly-111**, Lys-112, **Thr-113**, **Glu-116**, Thr-119, **Ile-120**, **Lys-121**, **Lys-127**, **Glu-128**, Cys-130, Lys-166.	FXN$^{42\text{-}210}$-ISCU: Asp-178 or Glu-184*–**Lys-121**; Asp-209*–Lys-112; Lys-208–**Glu-116**; Lys-197–**Glu-128**; Asp-199–**Lys-127**; Arg-60*–**Asp-152.**	NA
	682			
		FXN$^{42\text{-}210}$: *L3* : Gly-82, Thr-83, His-86*, **Leu-90**, Asp-91, Glu-92, Thr-93; *C-term* : Lys171, Ser-206. ISCU: *α4* and *α5* : **Asp-144**, **Lys-147**, **Asp-152**, **Lys-154**, Glu-163, Glu-165, Lys-166.	FXN$^{42\text{-}210}$-ISCU: Glu-92–**Lys-147**, His-86*–Glu-163, Asp-91– Lys-167*.	NA

Interface	Buried surface area (Å^{2})	Residues involdved in hydrogen bonding	Residues involdved in salt bridges	Residues involdved in hydrophobic interactions
	331			
		FXN$^{42-210}$: *L6* and *L10* : Asp-139, **Ala-193**, Lys-195. ISCU: Cys-69, Asn-122, His-137.	NA	FXN$^{42-210}$: **Leu-140**, **Pro-159**, Ser-160, **Ser-161**. ISCU: **Pro-133**, **Val-134**, **Leu-136**, **His137**, **Met-140**.
ISCU-ISCU interfaces				
	412			
		ISCU^{a}: *L3* : **Glu-81**, Lys-82, **Gly-83;** *C-term* : Glu-165. ISCU^{b}: *C-term* : Glu-158, Lys-160, Lys-161*.	ISCU^{a}-ISCU^{b}: *C-term* : Glu-158–Lys-57, Lys-161*–Glu-165, Lys-82–Glu-163.	NA
	298			
		ISCU^{1}: **Cys-69**. ISCU^{2}**:** Cys-130	NA	ISCU^{1}: **Leu-131**, **Pro-132**, **Pro-133**, **Val-134**. ISCU^{2}: **Leu-131**, **Pro-132**, **Pro-133**, **Val-134**.
FXN$^{42-210}$-FXN$^{42-210}$ interface				
	1150			
		FXN$^{42-210a}$: *N-term* : Asn-58, Leu-62, Gln-64, Lys-70; *L7* : **Asn-151**; *L8* : **Ser-161**; *L9* : Lys-164, **Arg-165**, Tyr-166, **Asp-167**, Gly-170, Tyr-176, His-177. FXN$^{42-210b}$: *N-term* : Gln-71, **Ser-72**, Gly-82, Thr-83, **Leu-84**; *L3* : Glu-92, Tyr-95; *α3* : Glu-96, Glu-100; *L7* : **Pro-150**,	FXN$^{42-210a}$ (*N-term*)- FXN$^{42-210b}$ (*L3* , *α3* and *L9*): Asp-45*–Arg-79, Asp-91* or Glu-92–His-177, Glu-96 or Glu-100–**Arg-165**, Asp-47*–Lys-208.	NA

Interface	Buried surface area (Å^{2})	Residues involdved in hydrogen bonding	Residues involdved in salt bridges	Residues involdved in hydrophobic interactions
	304			
		FXN$^{42\text{-}210\ \mathit{1}}$ **:** *L6* : Lys-135, Asp-139, **Leu-140**, **Gly-141**. FXN$^{42\text{-}210\ \mathit{2}}$: L3: Thr-93, Thr-94, α3: Arg-97, Glu-101, Tyr-205.	FXN$^{42\text{-}210\ \mathit{1}}$ -FXN$^{42\text{-}210\ \mathit{2}}$: Lys-135–**Glu-101**.	NA
NFS1-NFS1 interface				
	1600			
		NFS1^{a}: *C-term α14* : Gln-444, *L18* : **Ser-377**. NFS1^{b}: *L19* : **Glu-387**; *C-term* : **His-457**.		NFS1^{a}: **Trp-440**, **Val-443**, **Ile-447**, **Leu-449**, **Ile-452**, **Trp-454**, **Gly-446,** Ser-451, Thr-455, **His-457** NFS1^{b}: **Leu-166**, **Leu-386**, **Val-391**, **Lys-157, Cys-158, Arg-164**, Ser-165, **Ser-376**, **Ser-377**, **Ser-383**, **Ala-384**, **Ser-385**, **Pro-388**, **Tyr-390**
NFS1-ISCU interfaces				
	1285			
		NFS1: *N-term* : **Thr-67**, **Asp-70**, **Arg-72**; *α9* : **Lys-371**, **Asp-372**, **Leu-375**, *β10* : **Arg-407**; *α13* : **Glu-418**, Thr-422, Lys-425. ISCU: *β3* : Lys-84, Asp-87, Arg-89, *α1* : **Ser-103**, **Glu-107**, Lys-110, **Gly-111**, Lys-112.	NFS1-ISCU: **Asp-70**–Lys-84, **Asp-372**–Lys-110, **Arg-407**–**Glu-107**, **Glu-418**–Lys-110, Lys-425–Asp-87.	NFS1 (*α8* , *α13* , *L15* , *L23*): **Leu-367**, **Leu-370**, **Val-373**, **Leu-375**, Tyr-421, **Leu-433**, **Leu-439**, **Trp-440**, **Val-443**, **Leu-449**, Ser-451. ISCU: *β1-β3* : **Gly-50, Ser-51, Leu-63, Ala-66, Pro-67, Val-72,** Val-86, Ala-88, **Phe-90, Thr-92, Phe-93, Ile-99**.

Interface	Buried surface area (Å^{2})	Residues involdved in hydrogen bonding	Residues involdved in salt bridges	Residues involdved in hydrophobic interactions
	315			
		NFS1: *L18* , *L19* : **Cys-381**, **Thr-382**, **Ser-383**, **Leu-386**, **Glu-387**. ISCU**:** *L2* **: Ala-68**, **Cys-69**; *L4* : **Lys-91**, **Thr-92**; *L7* : **His-137**.	NFS1-ISCU: **Glu-387**–**Lys-91**.	NA
NFS1-FXN$^{42-210}$ interfaces				
	1895			
		NFS1: *N-term* : **Thr-67**, Leu-69, **Asp-70**, **Arg-72**, **Asp-75**, Tyr-80; *L13* : **Lys-263**, **Gly-264**; *L14* : Gln-282, **Arg-292**, **Thr-295**, **Pro-299**, **Leu-300**, **Val-301**; *L18* : **Cys-381**; *L23* : **Asp-448.** FXN$^{42-210}$**:** *L3* **:** Arg-79, Ser-89, **Leu-90**; *α3* : Glu-96, **Glu-108**, **Glu-111**, **Asp-112**, **Asp-115**, *L4* **:** Lys-116, *α4* **:** Asp-199.	NFS1 and FXN$^{42-210}$: **Asp-70**–Arg-79, **Lys-263**–Asp-199, **Arg-292–Glu-111**.	NA
	926			
		NFS1: (*N-term, L12, L13, α8, α13*): **Arg-72**, **Met-77**, **Lys-239**, **Tyr-260**, Glu-309, **Asp-318**, **Glu-416**, **Glu-417**. FXN$^{42-210}$**:** *N-term* : Arg-43*, Thr-44*, Ile-46*, Asp-47*, Thr-49*, Cys-50*, Arg-54*, Asn-63*.	NFS1-FXN$^{42-210}$: **Asp-75**–Arg-54*, **Glu-416** or **Glu-417**–Arg-43*.	

A coordinate file of the active center of the four-protein complex, $[FXN^{42-210}]_6$•$[NFS1]_2$•$[ISCU]_6$, was extracted from the simulated half-structure of the complex, and was uploaded into the PISA program (www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver). The PISA Interfaces algorithm was used to identify protein-protein interfaces, their buried surface areas (BSA), and amino acid residues involved in hydrogen bonding, salt bridges or hydrophobic interactions between the two contact surfaces. Residues denoted by an asterisk are not conserved, those in bold are conserved in eukaryotes, and all others are conserved in mammals.

Supplemental Table S4. Validation of the protein models generated for the study

Validation Tool	**Protein Models**				
	FXN$^{42\text{-}210}$	**ISCU**	**ISD11**	**NFS1**	**[FXN$^{42\text{-}210}$]$_3$•[ISCU]$_3$**
Ramachandran plot					
Favored region	82.0 %	84.7 %	79.8 %	90.3 %	87.0 %
Allowed region	9.0 %	9.9 %	13.5 %	8.0 %	9.0 %
Disallowed region	9.0 %	5.3 %	6.7 %	1.7 %	4.0 %
ProQ					
Predicted LG score	1.8	4.2	0.39	6.16	2.5
Predicted MaxSub	0.26	0.15	0.085	0.24	0.2
Verify 3D	80.0 %	80.0 %	65 %	80.0 %	63.2 %
I-TASSER					
C-score	-1.3	0.9	-2.7	1.48	
TM-score	0.56±0.15	0.84±0.08	0.4±0.14	0.92±0.06	---

Ramachandran plot identifies whether any given residue is in the favored, allowed or disallowed region, defined using ProCheck parameters (2). Verify 3D determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (α helix, β strand, loop, polar, nonpolar, etc.) and comparing the results to validated structures (3). C-score is a confidence score for estimating the quality of models generated using the I-TASSER web resource. A C-score is typically in the range of [-5 to 2], where a C-score of higher value signifies a model with a higher confidence and vice-versa (4). TM-score is a scale for measuring the structural similarity between two structures. A TM-score >0.5 indicates a model of correct topology and a TM-score<0.17 means a random similarity (4).

2. Morris, A.L., MacArthur, M.W., Hutchinson, E.G. and Thornton, J.M. (1992). Stereochemical quality of protein structure coordinates. *Proteins*, **12**, 345-364.
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4. Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J., and Zhang, Y. (2015). The I-TASSER Suite: protein structure and function prediction *Nat. Methods* **12**, 7-8.