

Supporting Information

Lesson from the stoichiometry determination of the cohesin complex; a short protease mediated elution increases the recovery from crosslinked antibody-conjugated beads

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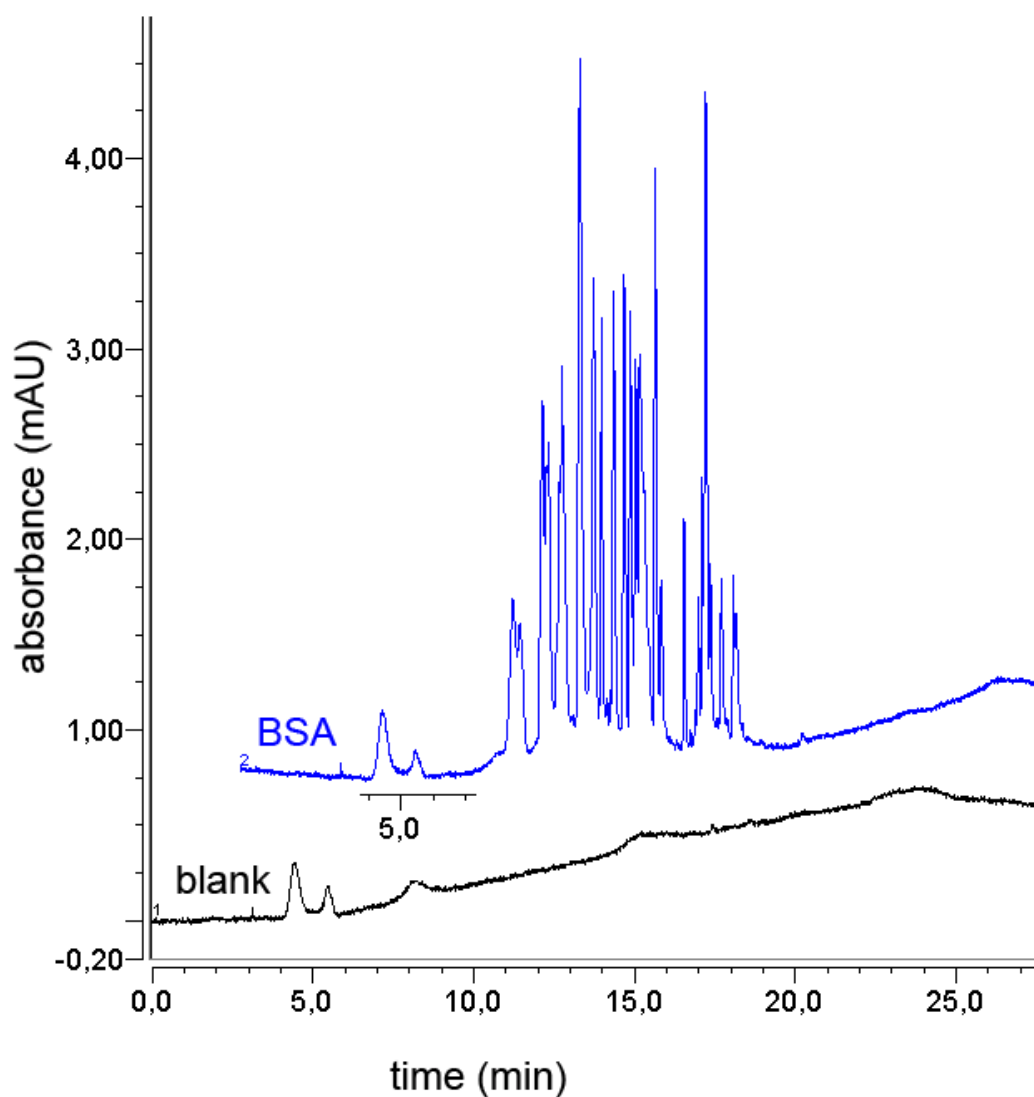
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Table of contents:

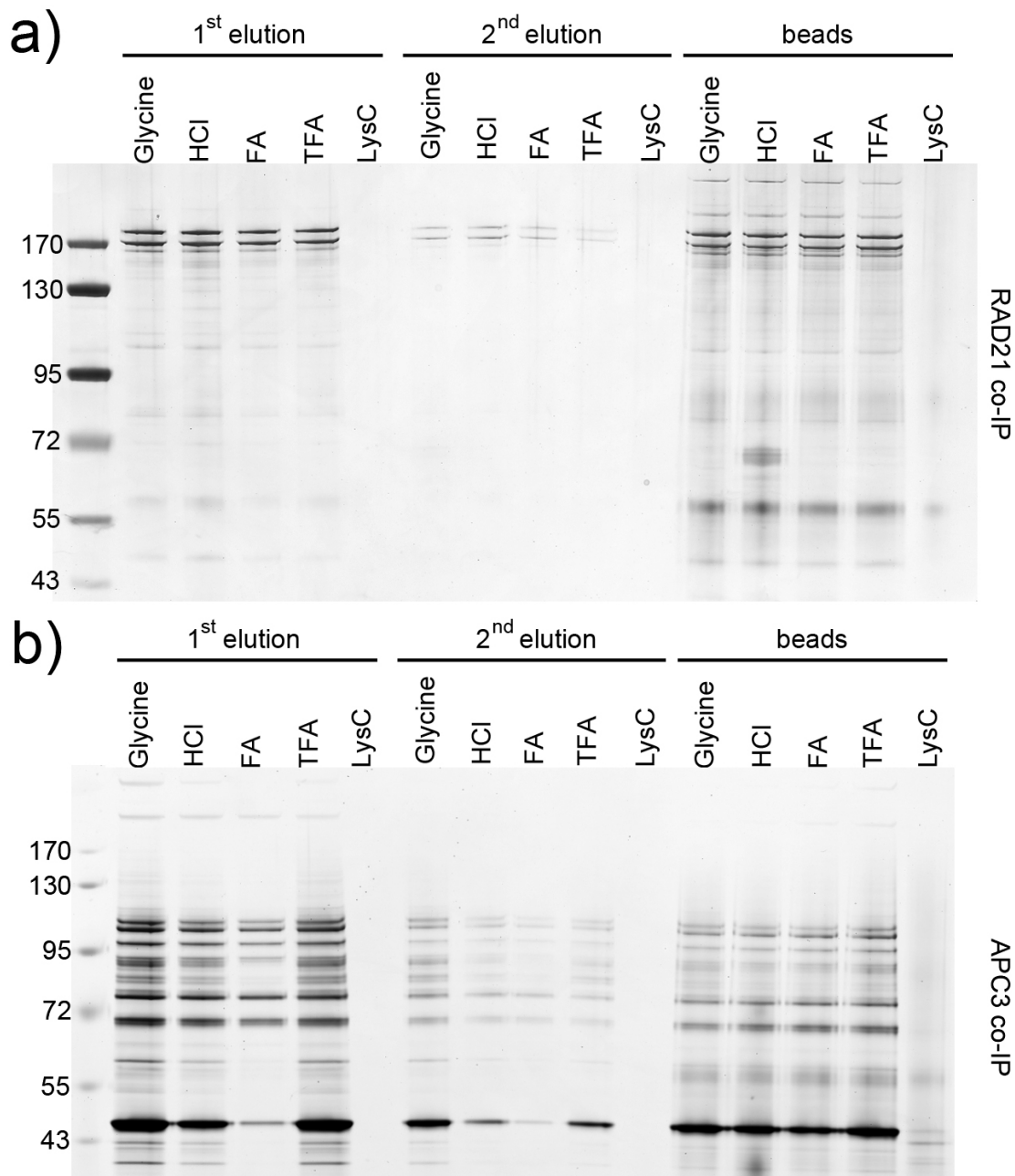
Figure S1	Separation of digested BSA using a monolithic column	3
Figure S2	SDS-PAGE of cohesin and APC/C purifications	4
Figure S3	Relative quantification of cohesin subunits	5
Figure S4	Relative quantification of APC/C subunits	6
Figure S5	XICs of α APC3 rabbit antibody peptides	7
Figure S6	Workflow of the EtEP strategy	8
Figure S7	Calibration curves of STAG1 specific peptides	9
Figure S8	Absolute quantification cohesin – peptide level	10
Figure S9	LC-SRM chromatogram of cohesin quantification	10
Figure S10	LC-SRM chromatogram of STAG1 specific peptide EDLLVLR	11
Table S1	SRM transitions used for absolute quantification of cohesin peptides	12-14
Table S2	Protein identified from 1, 4 and 16 h of pre-digestion (cohesin)	15-16
Table S3	Protein identified from 1, 4 and 16 h of pre-digestion (APC/C)	17-21
Table S4	Proteins identified from 15, 30 and 60 min LysC pre-digestion	22
Figures S11-13	Results obtained using trypsin to perform protease elution	23-24

Figure S1



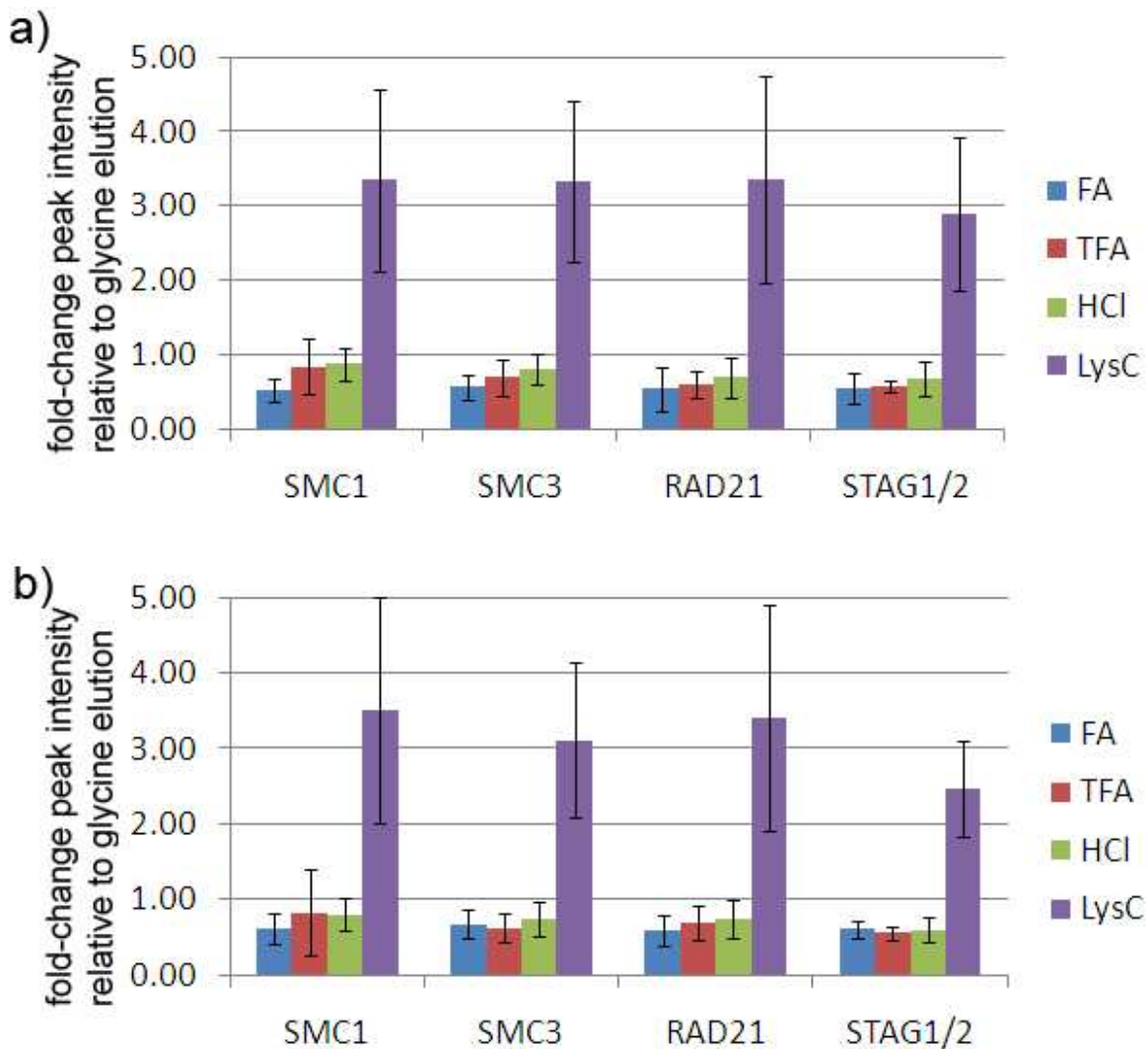
UV chromatogram of 1 pmol digested BSA (blue) and blank (black) separated on a Dionex Ultimate nanoHPLC equipped with a 200 μ m I.D. monolithic column (PepSwift from Dionex, Amsterdam, The Netherlands). For better illustration chromatograms are displayed with a 5% time and a 15% signal offset.

Figure S2



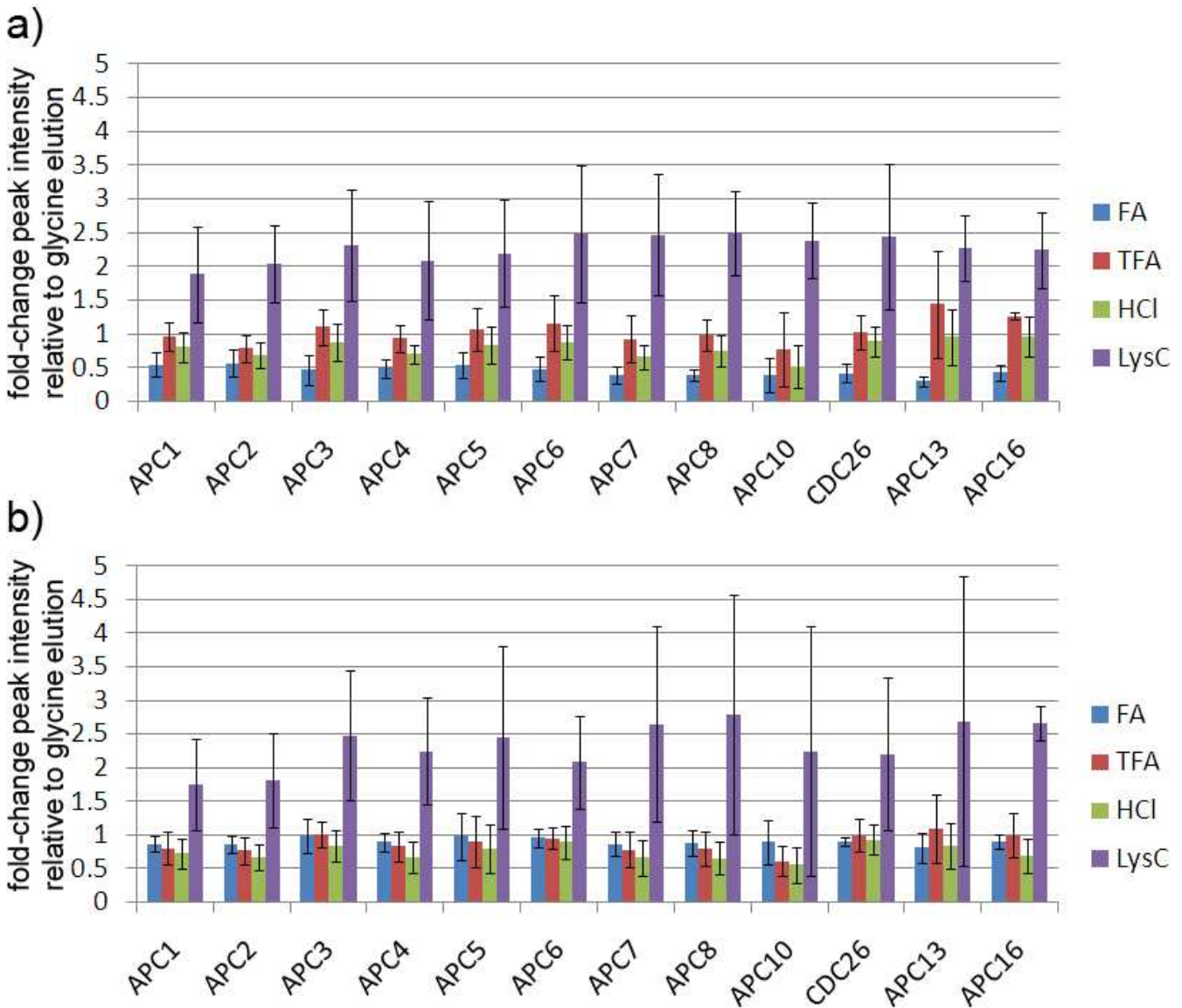
SDS-PAGE of cohesin and APC/C purifications eluted using different reagents. After incubation with S-20 HeLa extract and a total of 7 washing steps, the 30 μ l antibody beads were eluted with 60 μ l of 100 mM glycine pH 2.0, 100 mM HCl, 100 mM FA, 100 mM TFA and by incubation with 500 ng LysC after re-suspension of beads in 60 μ l 0.5 M TEAB, respectively. 50% of the eluate was used for labelfree quantification on Orbitrap XL and 50% was used for SDS-PAGE. In lanes denoted “beads”, the remaining proteins bound to beads after 2-step elution were eluted by boiling in Laemmli-buffer for 5 min at 95°C.

Figure S3



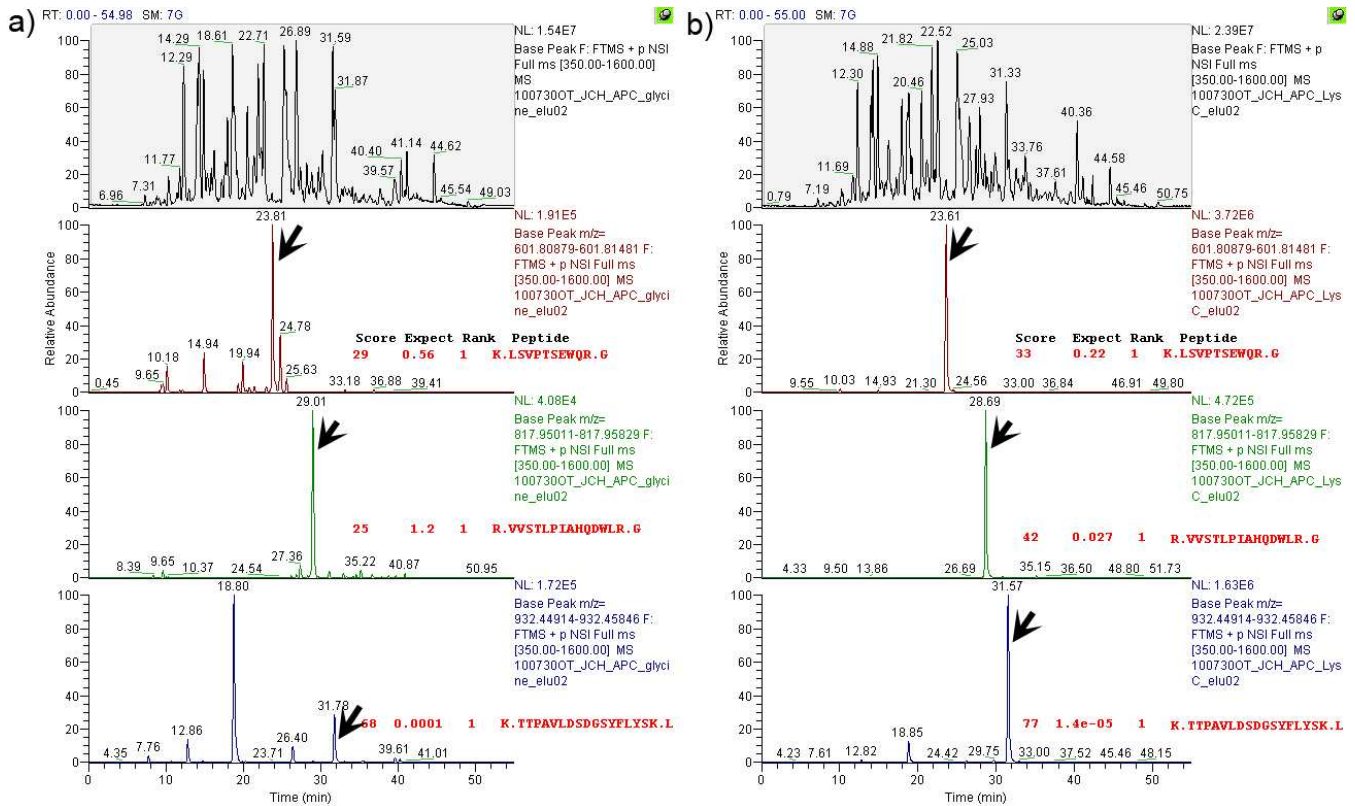
Label-free quantification of cohesin subunits by XIC from preparations using different elution protocols relative to glycine elution. Cohesin was purified by immunoadsorption and eluted using 5 different elution protocols. XICs were extracted using Proteome Discoverer Software (v 1.2) and peptides containing no chemical modification (such as methionine oxidation or S,T,Y-phosphorylation) were quantified relative to the glycine elution. a) Technical repeat 1 and b) technical repeat 2. Shown are the mean \pm S.D. of relative measurements of peptides that were identified in all 5 preparations. The minimum number of peptides per subunit used for quantification was 5.

Figure S4



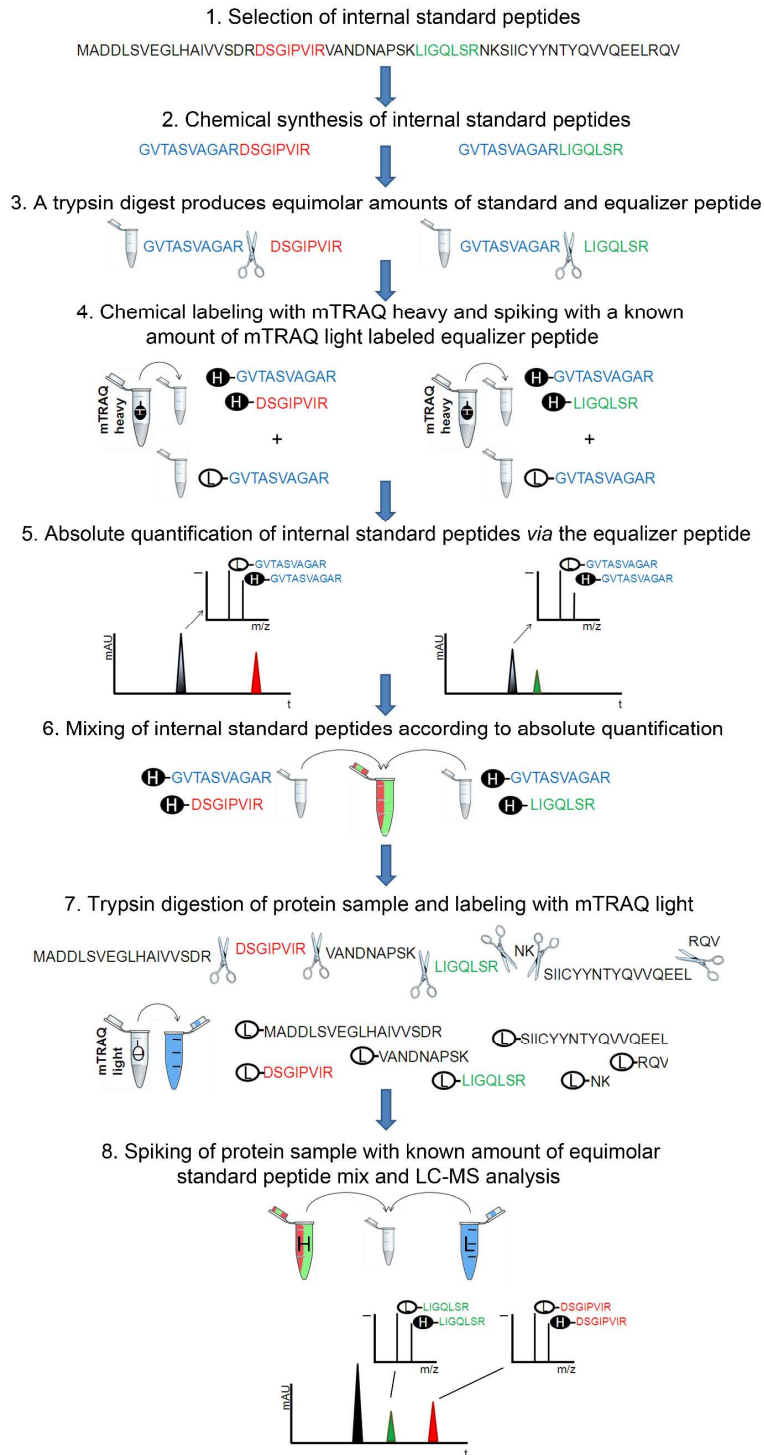
Labelfree quantification of APC/C subunits by XIC from preparations using different elution protocols relative to glycine elution. APC/C was purified by immunoadsorption and eluted using 5 different elution protocols. XICs were extracted using Proteome Discoverer Software (v 1.2) and peptides containing no chemical modification (such as methionine oxidation or S,T,Y-phosphorylation) were quantified relative to the glycine elution. a) Technical repeat 1 and b) technical repeat 2. Shown are the mean \pm S.D. of relative measurements of peptides that were identified in all 5 preparations. The minimum number of peptides per subunit used for quantification was 3.

Figure S5



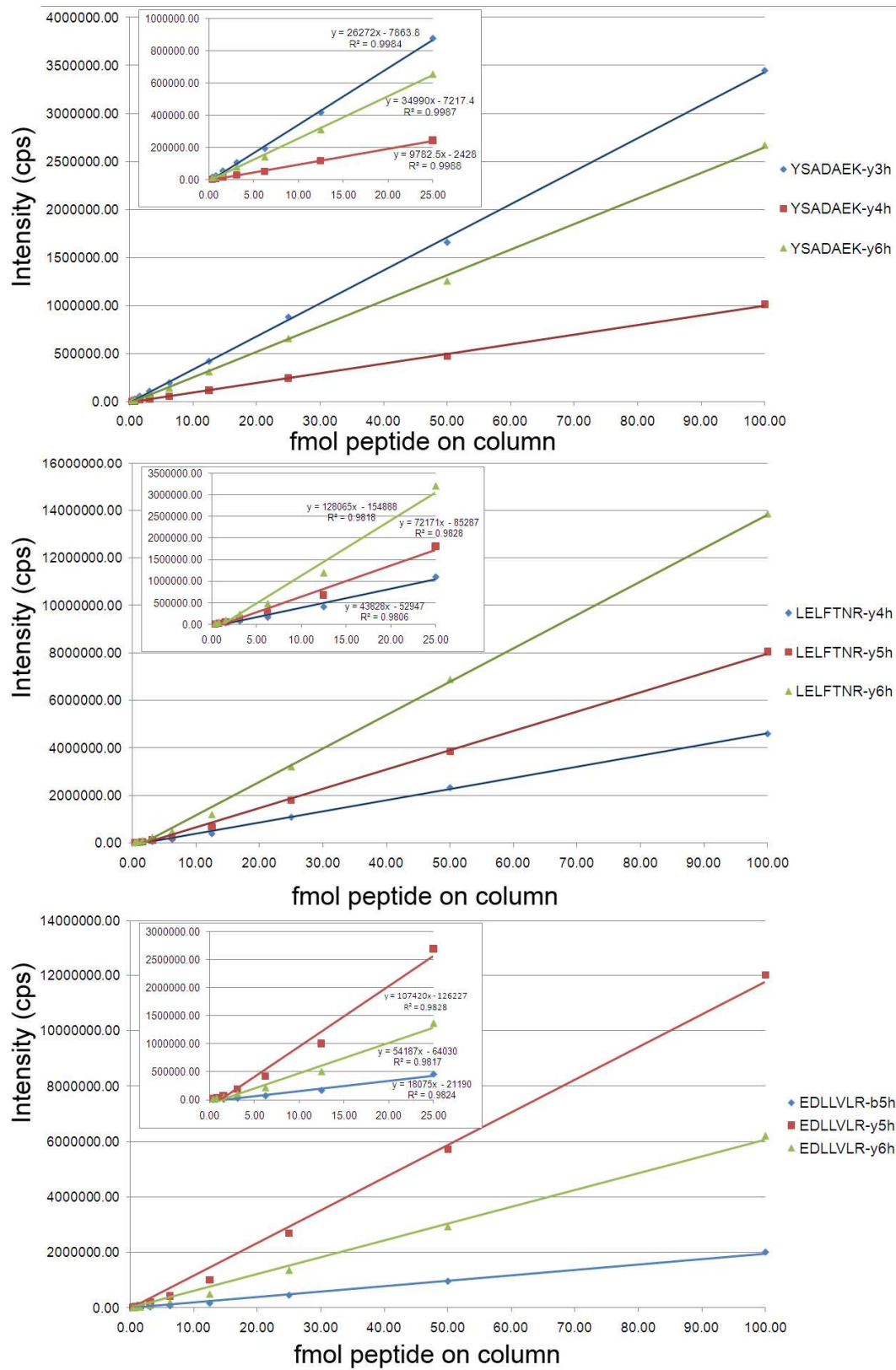
Base peak chromatogram and XICs of α APC3 rabbit antibody peptides identified after glycine elution (a) compared to 1 hour LysC elution (b). A total of 7 antibody peptides stemming from the Fc region were identified in LysC elution and 3 were identified after glycine elution, 3 were in common. XICs of these 3 peptides were extracted using Xcalibur software (version 2.0.7) with a mass tolerance of 5 ppm. XICs of peptides after LysC elution were on average 20-fold more intense than after glycine elution. In red, score, expect, rank and peptide sequence are given from the Mascot result.

Figure S6



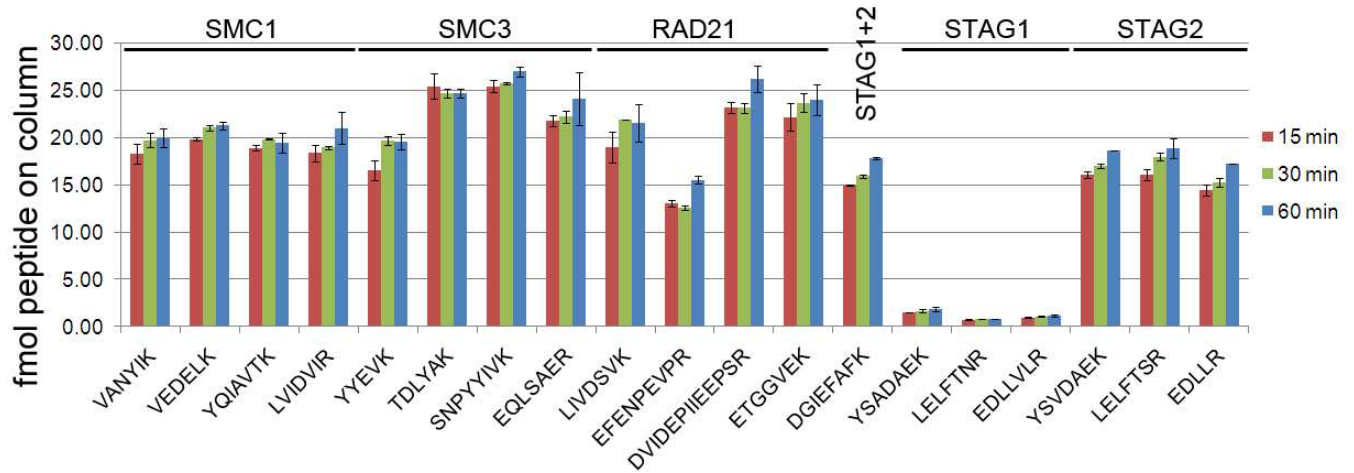
Workflow of the Equimolarity through Equalizer Peptide strategy (EtEP) for the generation of an equimolar mixture of internal reference peptides.

Figure S7



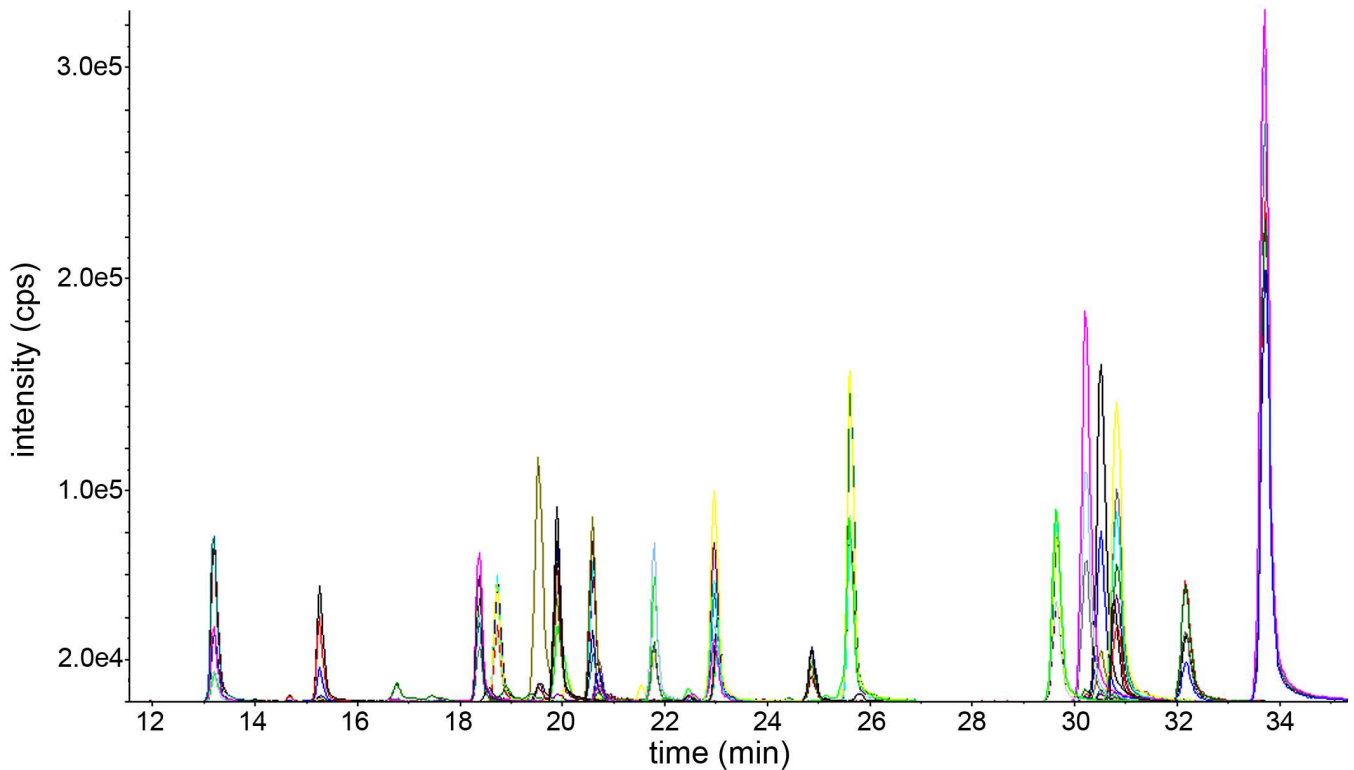
Calibration curves of STAG1 specific peptides. Peptide amounts from 390 amol to 100 fmol were measured in triplicate by scheduled SRM on a 5500 QTRAP instrument.

Figure S8



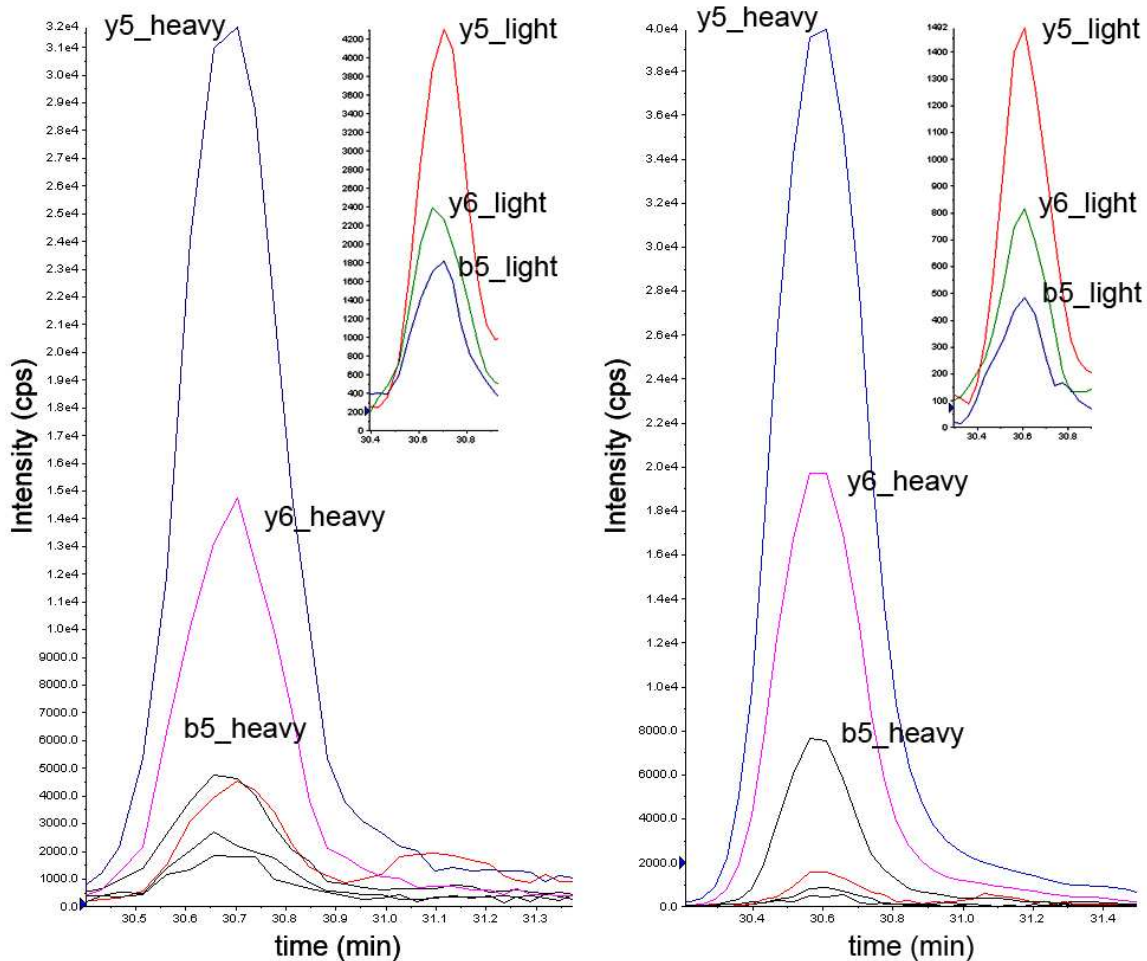
Absolute quantification of SMC1, SMC3, RAD21 and STAG1, STAG2 peptides using scheduled SRM on a 5500 QTRAP after 15, 30 and 60 min LysC elution. Measurements are mean \pm S.D. of duplicate measurements.

Figure S9



LC-SRM chromatogram of cohesin peptides spiked with 10 fmol internal reference peptides. A target scan time of 2 sec in the scheduled SRM method led to at least 12 data points across the eluting peaks.

Figure S10



LC-SRM chromatogram of STAG1 specific peptide EDLLVLR after (a) 30 min LysC elution and (b) HCl elution. Transitions 499.3→710.4 (b5 fragment) after HCl elution was below our intensity threshold of 500 cps. 10 fmol internal reference peptides spiked.

Table S1

Q1 [m/z]	Q3 [m/z]	t [min]	Fragment	Protein	CE	CXP
607.84	656.39	35	DGIEFAFK-y4h	STAG1+2	36	22
603.83	652.39	35	DGIEFAFK-y4L	STAG1+2	36	22
607.84	785.43	35	DGIEFAFK-y5h	STAG1+2	35	27
603.83	781.43	35	DGIEFAFK-y5L	STAG1+2	35	27
607.84	955.54	35	DGIEFAFK-y7h	STAG1+2	35	33
603.83	951.54	35	DGIEFAFK-y7L	STAG1+2	35	33
828.43	587.2	32.2	DVIDEPIIEEPSR-b4h	RAD21	46	20
826.43	583.2	32.2	DVIDEPIIEEPSR-b4L	RAD21	46	20
828.43	716.2	32.2	DVIDEPIIEEPSR-b5h	RAD21	46	25
826.43	712.2	32.2	DVIDEPIIEEPSR-b5L	RAD21	46	25
828.43	940.51	32.2	DVIDEPIIEEPSR-y8h	RAD21	48	32
826.43	940.51	32.2	DVIDEPIIEEPSR-y8L	RAD21	48	32
789.49	502.3	22.3	EDLLR-b3h	STAG2	58	17
785.49	498.3	22.3	EDLLR-b3L	STAG2	58	17
789.49	615.2	22.3	EDLLR-b4h	STAG2	57	21
785.49	611.2	22.3	EDLLR-b4L	STAG2	57	21
789.49	516.31	22.3	EDLLR-y4h	STAG2	58	18
785.49	516.31	22.3	EDLLR-y4L	STAG2	58	18
501.31	714.42	33.1	EDLLVLR-b5h	STAG1	35	25
499.31	710.42	33.1	EDLLVLR-b5L	STAG1	35	25
501.31	613.3	33.1	EDLLVLR-y5h	STAG1	35	21
499.31	613.3	33.1	EDLLVLR-y5L	STAG1	35	21
501.31	728.3	33.1	EDLLVLR-y6h	STAG1	34	25
499.31	728.3	33.1	EDLLVLR-y6L	STAG1	34	25
630.82	664.2	24.7	EFENPEVPR-b4h	RAD21	39	23
628.82	660.2	24.7	EFENPEVPR-b4L	RAD21	39	23
630.82	711.4	24.7	EFENPEVPR-y6h	RAD21	40	25
628.82	711.4	24.7	EFENPEVPR-y6L	RAD21	40	25
630.82	987.2	24.7	EFENPEVPR-y8h	RAD21	36	34
628.82	987.2	24.7	EFENPEVPR-y8L	RAD21	36	34
976.52	802.3	16.3	EQLSAER-b6h	SMC3	66	28
972.52	798.3	16.3	EQLSAER-b6L	SMC3	66	28
976.52	575.31	16.3	EQLSAER-y5h	SMC3	69	20
972.52	575.31	16.3	EQLSAER-y5L	SMC3	69	20
976.52	703.2	16.3	EQLSAER-y6h	SMC3	69	24
972.52	703.2	16.3	EQLSAER-y6L	SMC3	69	24
504.28	576.35	14.6	ETGGVEK-y4h	RAD21	32	20
500.28	572.35	14.6	ETGGVEK-y4L	RAD21	32	20
504.28	633.2	14.6	ETGGVEK-y5h	RAD21	32	22
500.28	629.3	14.6	ETGGVEK-y5L	RAD21	32	22
504.28	734.3	14.6	ETGGVEK-y6h	RAD21	32	25
500.28	730.3	14.6	ETGGVEK-y6L	RAD21	32	25
518.8	537.28	32.8	LELFTNR-y4h	STAG1	31	19
516.8	537.28	32.8	LELFTNR-y4L	STAG1	31	19
518.8	650.36	32.8	LELFTNR-y5h	STAG1	31	22
516.8	650.36	32.8	LELFTNR-y5L	STAG1	31	22

518.8	779.4	32.8	LELFTNR-y6h	STAG1	31	27
516.8	779.4	32.8	LELFTNR-y6L	STAG1	31	27
505.29	510.27	33.5	LELFTSR-y4h	STAG2	31	18
503.29	510.27	33.5	LELFTSR-y4L	STAG2	31	18
505.29	623.35	33.5	LELFTSR-y5h	STAG2	31	21
503.29	623.35	33.5	LELFTSR-y5L	STAG2	31	21
505.29	752.39	33.5	LELFTSR-y6h	STAG2	32	26
503.29	752.39	33.5	LELFTSR-y6L	STAG2	32	26
531.34	477.32	27.5	LIVDSVK-y3h	SMC3	33	16
527.34	473.32	27.5	LIVDSVK-y3L	SMC3	33	16
531.34	592.34	27.5	LIVDSVK-y4h	SMC3	34	20
527.34	588.34	27.5	LIVDSVK-y4L	SMC3	34	20
531.34	804.49	27.5	LIVDSVK-y6h	SMC3	33	28
527.34	800.49	27.5	LIVDSVK-y6L	SMC3	33	28
486.32	585.2	36.6	LVIDVIR-b4h	SMC1	29	20
484.62	581.2	36.6	LVIDVIR-b4L	SMC1	29	20
486.32	387.27	36.6	LVIDVIR-y3h	SMC1	29	13
484.62	387.27	36.6	LVIDVIR-y3L	SMC1	29	13
486.32	714.45	36.6	LVIDVIR-y6h	SMC1	27	25
484.62	714.45	36.6	LVIDVIR-y6L	SMC1	27	25
636.37	769.4	26.8	SNPYYIVK-b5h	SMC3	39	27
632.36	765.4	26.8	SNPYYIVK-b5L	SMC3	39	27
636.37	666.4	26.8	SNPYYIVK-y4h	SMC3	39	23
632.36	662.4	26.8	SNPYYIVK-y4L	SMC3	39	23
636.37	926.55	26.8	SNPYYIVK-y6h	SMC3	39	32
632.36	922.55	26.8	SNPYYIVK-y6L	SMC3	39	32
499.79	525.32	20.3	TDLYAK-y3h	SMC3	30	18
495.79	521.32	20.3	TDLYAK-y3L	SMC3	30	18
499.79	638.4	20.3	TDLYAK-y4h	SMC3	30	22
495.79	634.4	20.3	TDLYAK-y4L	SMC3	30	22
499.79	753.4	20.3	TDLYAK-y5h	SMC3	30	26
495.79	749.4	20.3	TDLYAK-y5L	SMC3	30	26
498.31	592.2	23.4	VANYIK-b4h	SMC1	30	20
494.3	588.2	23.4	VANYIK-b4L	SMC1	30	20
498.31	681.41	23.4	VANYIK-y4h	SMC1	30	23
494.3	677.41	23.4	VANYIK-y4L	SMC1	30	23
498.31	752.44	23.4	VANYIK-y5h	SMC1	27	26
494.3	748.44	23.4	VANYIK-y5L	SMC1	27	26
510.79	533.34	21.5	VEDELK-y3h	SMC1	29	18
506.79	529.34	21.5	VEDELK-y3L	SMC1	29	18
510.79	648.37	21.5	VEDELK-y4h	SMC1	29	22
506.79	644.37	21.5	VEDELK-y4L	SMC1	29	22
510.79	777.2	21.5	VEDELK-y5h	SMC1	29	27
506.79	773.2	21.5	VEDELK-y5L	SMC1	29	27
555.84	620.3	24.7	YQIAVTK-b4h	SMC1	34	21
551.84	616.3	24.7	YQIAVTK-b4L	SMC1	34	21
555.84	675.45	24.7	YQIAVTK-y5h	SMC1	32	23
551.84	671.45	24.7	YQIAVTK-y5L	SMC1	32	23
555.84	803.51	24.7	YQIAVTK-y6h	SMC1	32	28

551.84	799.51	24.7	YQIAVTK-y6L	SMC1	32	28
536.28	491.29	16.8	YSADA EK-y3h	STAG1	35	17
532.28	487.29	16.8	YSADA EK-y3L	STAG1	35	17
536.28	606.32	16.8	YSADA EK-y4h	STAG1	34	21
532.28	602.32	16.8	YSADA EK-y4L	STAG1	34	21
536.28	764.39	16.8	YSADA EK-y6h	STAG1	35	26
532.28	760.39	16.8	YSADA EK-y6L	STAG1	35	26
550.3	491.29	20	YSVDA EK-y3h	STAG2	33	17
546.29	487.29	20	YSVDA EK-y3L	STAG2	33	17
550.3	606.2	20	YSVDA EK-y4h	STAG2	35	21
546.29	602.2	20	YSVDA EK-y4L	STAG2	35	21
550.3	792.42	20	YSVDA EK-y6h	STAG2	31	27
546.29	788.42	20	YSVDA EK-y6L	STAG2	31	27
495.28	600.2	22.3	YYEVK-b3h	SMC3	32	21
491.27	596.2	22.3	YYEVK-b3L	SMC3	32	21
495.28	390.28	22.3	YYEVK-y2h	SMC3	30	13
491.27	386.28	22.3	YYEVK-y2L	SMC3	30	13
495.28	682.39	22.3	YYEVK-y4h	SMC3	30	24
491.27	678.39	22.3	YYEVK-y4L	SMC3	30	24

SRM transitions used for absolute quantification of cohesin peptides.

Table S2

Protein	Accession Number	Trypsin			LysC		
		1 h	4 h	16 h	1 h	4 h	16 h
Structural maintenance of chromosomes protein 3	IPI00219420	55	42	29	55	57	46
Structural maintenance of chromosomes protein 1A	IPI00291939	56	43	30	57	53	51
cohesin subunit SA-2 isoform a	IPI00470883	26	26	13	31	29	19
Double-strand-break repair protein rad21 homolog	IPI00006715	21	17	9	26	22	20
Isoform 1 of Myosin-9	IPI00019502	5	38	24	5	10	10
Isoform 1 of Protein-L-isoaspartate(D-aspartate) O-methyltransferase	IPI00411680	7	8	8	7	8	6
Isoform 1 of Heat shock cognate 71 kDa protein	IPI00003865	10	16	11	11	12	10
Isoform 1 of Gephyrin	IPI00016006 (+1)	8	17	8	12	12	10
Isoform 1 of Protein-L-isoaspartate O-methyltransferase domain-containing protein 2	IPI00884185	5	12	11	7	9	9
Actin, cytoplasmic 1	IPI00021439 (+4)	6	9	10	8	8	8
2-oxoglutarate dehydrogenase, mitochondrial	IPI00098902	7	8	4	10	11	5
Isoform 1 of Centrosomal protein of 72 kDa	IPI00465040	2	14	8	12	13	11
Molybdenum cofactor sulfurase	IPI00304895	4	13	9	5	4	5
Serine/threonine-protein kinase 12	IPI00176642	2	8	6	5	4	5
Transcription elongation factor B polypeptide 1	IPI00300341	3	5	4	4	4	3
Isoform 1 of Protein-L-isoaspartate O-methyltransferase domain-containing protein 1	IPI00162744	3	5	4	3	2	3
Stress-70 protein, mitochondrial	IPI00007765	4	5	3	5	7	4
Metalloproteinase inhibitor 3	IPI00218247	1	4	2	3	3	4
Isoform Short of Ubiquitin carboxyl-terminal hydrolase 25	IPI00219886	3	10	4	4	3	1
Isoform 1 of Phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 2	IPI00016932	9	0	0	7	10	2
Isoform 1 of Wings apart-like protein homolog	IPI00375330 (+2)	9	1	0	11	8	3
Isoform 1 of Clathrin heavy chain 1	IPI00024067 (+1)	0	3	3	2	0	1
Isoform 2 of Protein LSM14 homolog A	IPI00410590 (+2)	0	4	4	1	1	2
Tubulin, beta	IPI00645452 (+1)	1	5	4	2	2	3
Isoform Non-muscle of Myosin light polypeptide 6	IPI00335168 (+3)	0	3	2	3	2	2
Lipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase complex, mitochondrial	IPI00003944	9	0	1	8	6	2
THO complex subunit 4	IPI00328840	1	5	3	3	3	1
Tubulin alpha-1A chain	IPI00180675 (+3)	3	5	3	3	1	3
60S acidic ribosomal protein P0	IPI00008530	2	4	2	2	3	2
Transcription elongation factor B polypeptide 2	IPI00026670 (+2)	1	1	3	1	2	2
Isoform 1 of Cell division protein kinase 9	IPI00301923 (+1)	1	3	3	4	2	2
60S ribosomal protein L18	IPI00215719	2	2	2	3	2	3

Heat shock 70 kDa protein 1A/1B	IPI00304925 (+1)	1	5	2	1	2	2
Isoform 1 of Polymerase I and transcript release factor	IPI00176903 (+1)	2	3	3	2	1	1
Thyroid hormone receptor-associated protein 3	IPI00104050	0	2	0	7	4	7
60S ribosomal protein L13	IPI00465361	3	1	3	3	2	2
Cohesin subunit SA-1	IPI00025158 (+1)	5	2	0	5	4	1
Isoform 1 of 60S ribosomal protein L11	IPI00376798 (+1)	2	2	2	1	2	2
Myosin regulatory light chain 12B	IPI00033494 (+3)	1	3	1	2	1	1
ATP-dependent RNA helicase DDX3X	IPI00215637	0	5	1	3	1	1
Isoform 1 of RNA-binding protein 39	IPI00163505 (+1)	0	5	3	1	0	1
60S ribosomal protein L7a	IPI00299573 (+2)	2	2	2	2	2	1
Histone H4	IPI00453473	3	2	2	3	2	2
Nuclease-sensitive element-binding protein 1	IPI00031812	1	5	3	1	2	1
Transcription factor ETV6	IPI00013159	4	0	0	4	4	1
Isoform 1 of N(2),N(2)-dimethylguanosine tRNA methyltransferase	IPI00020508 (+1)	1	5	1	2	1	1
60S ribosomal protein L24	IPI00306332 (+3)	0	3	2	1	1	1
60S acidic ribosomal protein P2	IPI00008529	3	1	1	3	1	2
60S ribosomal protein L4	IPI00003918	1	3	3	2	0	0
Activated RNA polymerase II transcriptional coactivator p15	IPI00221222	1	3	1	0	0	1
Isoform C1 of Heterogeneous nuclear ribonucleoproteins C1/C2	IPI00216592 (+3)	0	0	0	5	5	4
Ribosomal protein L14 variant	IPI00555744 (+2)	1	2	1	2	1	1
Isoform 1 of 60S ribosomal protein L12	IPI00024933	1	1	2	1	2	1
Isoform 1 of Bcl-2-associated transcription factor 1	IPI00006079 (+4)	1	1	0	1	2	2
Thyroid transcription factor 1-associated protein 26	IPI00329594	2	2	0	2	0	1
Enhancer of rudimentary homolog	IPI00029631	0	1	1	2	0	2
PDS5, regulator of cohesin maintenance, homolog A isoform 2	IPI00303063 (+1)	3	0	0	3	4	1
HSPA5 protein	IPI00003362	0	5	2	1	1	0
60S ribosomal protein L7	IPI00030179 (+3)	1	2	2	1	1	1
Vimentin	IPI00418471	1	0	0	2	3	1
40S ribosomal protein S10	IPI00008438 (+1)	0	3	0	1	1	1
Isoform 2 of Centrosomal protein of 97 kDa	IPI00238469 (+2)	0	3	2	0	0	0
40S ribosomal protein S25	IPI00012750	1	1	2	1	1	1

Proteins identified from 1 h, 4 h and 16 h pre-digestion. 1 h and 4 h pre-digested samples were further digested over night. Spectra acquired on LTQ Orbitrap were searched against the human IPI database and compared using Scaffold Q+ software (v 3.0, Proteome Software). Listed are the identified proteins along with the number of unique peptides with a Mascot score >25; > 2 peptides in at least one elution. Cohesin subunits and known interactors are in bold. Sample amounts loaded onto column: 1 h Trypsin

pre-digestion 1/2 of eluate from immunoabsorption; 4 h Trypsin pre-digestion 1/2 of eluate; 16 h Trypsin pre-digestion 1/10th of eluate; 1 h LysC pre-digestion 1/2 of eluate; 4 h LysC pre-digestion 1/2 of eluate; 16 h of pre-digestion 1/4 of eluate

Table S3

Protein	Accession Number	Trypsin			LysC		
		1 h	4 h	16 h	1 h	4 h	16 h
Isoform 1 of Plectin-1	IPI00014898 (+7)	141	28	24	75	58	118
Isoform 3 of Spectrin alpha chain, brain	IPI00843765	91	19	29	67	50	73
Isoform 2 of Filamin-A	IPI00302592 (+2)	64	18	19	64	48	57
Isoform Long of Spectrin beta chain, brain 1	IPI00005614	78	18	21	64	46	72
Actin, cytoplasmic 1	IPI00021439 (+1)	15	7	8	12	12	11
Isoform 1 of Myosin-9	IPI00019502	61	18	14	34	33	47
Anaphase-promoting complex subunit 1	IPI00033907	38	13	5	39	36	31
Isoform 1 of Anaphase-promoting complex subunit 7	IPI00008248 (+3)	20	8	9	20	19	21
Isoform 1 of Anaphase-promoting complex subunit 5	IPI00008247	23	9	8	23	21	18
Isoform 1 of Cell division cycle protein 23 homolog	IPI00005822	24	9	10	24	21	20
Cell division cycle protein 27 homolog	IPI00294575 (+1)	17	5	4	18	19	16
Isoform 1 of Anaphase-promoting complex subunit 4	IPI00002551 (+1)	18	11	2	18	15	14
Isoform 1 of Anaphase-promoting complex subunit 2	IPI00002549 (+1)	21	10	4	22	18	20
Isoform 5 of Caldesmon	IPI00333771 (+1)	18	3	13	18	17	19
Vimentin	IPI00418471	21	5	9	18	14	18
filamin-B isoform 1	IPI00900293	32	9	6	25	15	23
Isoform 1 of Cell division cycle protein 16 homolog	IPI00022091 (+2)	11	8	4	15	15	13
Keratin, type II cytoskeletal 1	IPI00220327	21	7	10	8	10	21
LIM domain and actin-binding protein 1 isoform a	IPI00883896 (+1)	11	2	5	13	10	9
Keratin, type I cytoskeletal 9	IPI00019359	18	4	7	8	4	15
Alpha-actinin-4	IPI00013808	25	5	4	9	7	19
Isoform 1 of Heat shock cognate 71 kDa protein	IPI00003865	15	7	4	18	17	12
Isoform 1 of Drebrin	IPI00003406 (+1)	14	2	5	14	10	12
Tax_Id=9606 Gene_Symbol=TPM1 tropomyosin alpha-1 chain isoform 5	IPI00970818	13	2	5	11	10	9
Transferrin receptor protein 1	IPI00022462	15	8	3	4	2	9
Anaphase-promoting complex subunit CDC26	IPI00169387	3	1	2	5	5	6
Keratin, type II cytoskeletal 2 epidermal	IPI00021304	16	4	5	1	1	18
Tubulin alpha-1C chain	IPI00218343	7	5	2	7	7	7
Ras GTPase-activating-like protein	IPI00009342	22	5	0	5	1	11

IQGAP1

Isoform 2 of Tropomyosin alpha-3 chain	IPI00218319 (+1)	8	2	3	10	6	7
Isoform 2 of 4F2 cell-surface antigen heavy chain	IPI00027493 (+5)	11	4	0	7	6	12
Isoform 1 of Myoferlin	IPI00021048 (+1)	19	4	2	2	1	12
Isoform 1 of Nexilin	IPI00180404 (+1)	10	4	5	11	6	9
Tubulin beta-2C chain	IPI00007752	7	3	4	10	8	6
Isoform 2 of Annexin A2	IPI00418169 (+1)	10	1	1	8	6	8
Isoform 2 of Fizzy-related protein homolog	IPI00099464	8	0	2	15	9	5
Isoform 1 of Protein-L-isoaspartate O-methyltransferase domain-containing protein 2	IPI00884185	7	1	0	9	9	6
Isoform 1 of Brain acid soluble protein 1	IPI00299024	8	1	4	10	8	8
Anaphase-promoting complex subunit 10	IPI00007088	5	2	2	5	4	4
Isoform 1 of Spectrin beta chain, brain 2	IPI00012645	18	2	3	3	3	5
Isoform 1 of Protein-L-isoaspartate(D-aspartate) O-methyltransferase	IPI00411680	7	2	3	6	4	6
Isoform 1 of Myosin-Ib	IPI00376344 (+2)	11	2	1	4	0	8
Isoform 4 of A-kinase anchor protein 2	IPI00816415	13	0	0	3	4	9
Isoform 1 of Tropomyosin beta chain	IPI00013991	5	2	1	5	4	4
HSPA5 protein	IPI00003362	10	2	0	9	6	6
Heat shock protein HSP 90-beta Neuroblast differentiation-associated protein AHNAK	IPI00414676	6	0	1	7	4	5
cDNA FLJ45706 fis, clone FEBRA2028457, highly similar to Nucleolin	IPI00021812	6	2	1	5	2	7
Heat shock 70 kDa protein 1A/1B	IPI00444262 (+1)	5	3	2	8	3	5
DEP domain-containing protein 1B cDNA FLJ55574, highly similar to Calnexin	IPI00304925 (+2)	6	3	0	6	6	5
Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	IPI00293897	5	4	0	10	6	3
Anaphase-promoting complex subunit 16	IPI00020984 (+1)	8	1	1	4	1	6
Isoform 1 of Clathrin heavy chain 1	IPI00550451	6	2	2	4	3	5
Transcription elongation factor B polypeptide 1	IPI00060893	4	1	1	3	3	3
Actin filament-associated protein 1 similar to actin alpha 1 skeletal muscle protein	IPI00024067 (+1)	10	1	1	2	2	4
F-box only protein 5	IPI00300341	5	0	1	4	4	2
Isoform Non-muscle of Myosin light polypeptide 6	IPI00398154 (+1)	6	1	1	1	1	5
Isoform 1 of Phostensin	IPI00886911 (+1)	1	0	1	1	1	1
Isoform 1 of Serine/threonine-protein kinase Nek2	IPI00016633	3	0	0	7	5	1
Isoform 1 of Protein-L-isoaspartate O-methyltransferase domain-containing protein 1	IPI00335168 (+3)	5	0	1	4	3	4
Ras-related protein Rab-11B	IPI00152853 (+1)	5	0	1	3	1	4
Keratin, type II cytoskeletal 5	IPI00021331	5	0	0	7	3	4
haptoglobin isoform 2 preproprotein	IPI00162744	4	1	0	3	3	2
Isoform 1 of Protein phosphatase 1	IPI00020436 (+4)	5	2	2	2	1	5
	IPI00009867	8	1	0	0	0	4
	IPI00478493 (+2)	0	0	0	0	0	1
	IPI00183002	9	2	1	1	1	3

regulatory subunit 12A

Histone H4	IPI00453473	4	1	1	4	4	4
Cytospin-A	IPI00178072	6	2	0	1	0	4
Calmodulin	IPI00075248 (+4)	3	1	2	2	2	3
Elongation factor 1-alpha	IPI00025447 (+2)	5	0	0	3	1	1
60S ribosomal protein L7a	IPI00299573 (+2)	3	3	0	3	2	3
Ras-related protein Rab-7a	IPI00016342	4	2	0	1	1	3
HLA class I histocompatibility antigen, A-68 alpha chain	IPI00472882	3	2	1	0	1	4
Tubulin, beta	IPI00645452	2	2	1	3	1	2
Nuclease-sensitive element-binding protein 1	IPI00031812 (+1)	2	0	2	4	4	1
Isoform 1 of 60S ribosomal protein L12	IPI00024933	3	2	1	2	2	2
Myosin regulatory light chain 12B	IPI00033494 (+4)	3	1	0	4	2	4
40S ribosomal protein S6	IPI00021840	3	0	0	3	2	2
Putative uncharacterized protein IFI35	IPI00008613 (+2)	4	0	0	3	1	1
60S ribosomal protein L23	IPI00010153 (+1)	2	1	0	2	1	1
Thyroid hormone receptor-associated protein 3	IPI00104050	0	0	0	7	8	1
Histone H1.2	IPI00217465 (+2)	3	2	1	3	3	3
Cell division cycle protein 20 homolog	IPI00329526	2	2	0	3	1	2
60S acidic ribosomal protein P0	IPI00008530	4	0	0	2	1	2
40S ribosomal protein S4, X isoform	IPI00217030	4	0	0	2	3	2
Keratin, type II cytoskeletal 7	IPI00306959	4	2	0	1	0	5
60S ribosomal protein L13	IPI00465361	2	2	0	2	2	2
Protein FAM101B	IPI00166945	3	1	0	2	2	3
Isoform 1 of Tropomyosin alpha-4 chain	IPI00010779	2	0	1	2	2	2
N-myc-interactor	IPI00012450	2	2	0	4	2	1
Annexin A5	IPI00329801 (+1)	4	2	0	2	0	1
Isoform 1 of Ras-related protein Rab-1A	IPI00005719 (+1)	4	0	0	0	1	1
Isoform Long of Sodium/potassium-transporting ATPase subunit alpha-1	IPI00006482 (+1)	6	1	0	0	0	2
Isoform 1 of 60S ribosomal protein L11	IPI00376798 (+1)	3	1	1	3	3	1
Ras-related protein Rap-1b	IPI00015148	3	1	2	2	1	2
60S ribosomal protein L4	IPI00003918	2	1	1	3	2	2
Coronin-1C_i3 protein	IPI00867509 (+1)	3	0	1	3	1	3
Isoform DPI of Desmoplakin	IPI00013933	5	1	2	1	0	0
60S ribosomal protein L18	IPI00215719	2	1	1	3	2	2
60S ribosomal protein L27a	IPI00456758	2	1	0	2	2	1
A kinase (PRKA) anchor protein 2	IPI00073769	3	0	0	3	2	4
60S ribosomal protein L6	IPI00329389 (+2)	2	0	1	3	0	0
60S ribosomal protein L24	IPI00306332 (+3)	1	0	0	2	1	1
Annexin A6	IPI00221226 (+1)	5	1	1	0	0	3
Peroxiredoxin-1	IPI00000874 (+1)	3	1	0	0	0	0
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	IPI00003348	3	0	1	1	1	2
40S ribosomal protein S16	IPI00221092	2	0	0	3	2	1
Annexin A1	IPI00218918	3	2	0	0	0	1
Isoform 1 of Leucine-rich repeat flightless-interacting protein 2	IPI00007277 (+3)	4	0	0	1	0	2

epiplakin	IPI00010951	4	0	0	0	0	2
60S ribosomal protein L7	IPI00030179 (+2)	2	0	1	1	1	2
Isoform 1 of Myosin-XVIIIa	IPI00760846 (+2)	4	1	0	0	0	3
Stress-70 protein, mitochondrial	IPI00007765	5	1	0	2	1	1
Microtubule-actin cross-linking factor 1, isoform 4	IPI00432363	3	1	0	0	0	1
60S ribosomal protein L3	IPI00550021 (+2)	1	0	0	3	3	1
Tropomyosin 1 alpha variant 6	IPI00384369	2	0	1	2	2	2
Isoform 2 of Nucleophosmin	IPI00220740 (+2)	2	0	0	1	1	1
Isoform 1 of L-lactate dehydrogenase A chain	IPI00217966 (+3)	2	0	0	3	2	1
Isoform 2 of Reticulon-4	IPI00298289	3	1	0	1	0	1
Coronin-2A	IPI00023736	3	0	0	1	1	3
60S ribosomal protein L8	IPI00012772 (+2)	2	1	1	2	2	2
Isoform 2 of Heat shock protein HSP 90-alpha	IPI00382470 (+1)	3	2	0	2	2	1
40S ribosomal protein S14	IPI00026271	2	0	0	2	1	0
Isoform 2 of Tropomyosin beta chain	IPI00220709	1	0	1	1	1	2
Isoform 1 of S-phase kinase-associated protein 1	IPI00301364	2	0	0	2	2	1
Isoform 1 of Vesicle-associated membrane protein 7	IPI00020887 (+1)	4	1	0	0	0	2
neurabin-2	IPI00045550	5	0	1	0	0	2
Coiled-coil domain-containing protein 96	IPI00166414	1	0	1	1	1	1
17 kDa protein	IPI00173589 (+2)	2	0	0	2	2	1
Anaphase-promoting complex subunit 13	IPI00029244	2	1	0	2	2	2
Tropomodulin-3	IPI00005087	4	2	1	0	1	1
60S ribosomal protein L23a	IPI00021266 (+4)	2	0	1	2	0	2
Isoform 1 of Heterogeneous nuclear ribonucleoprotein M	IPI00171903 (+1)	2	0	0	0	0	2
40S ribosomal protein S7	IPI00013415 (+3)	1	0	0	0	2	0
Isoform 1 of Mitotic checkpoint serine/threonine-protein kinase BUB1 beta	IPI00141933 (+3)	0	0	0	5	3	0
Isoform 1 of Heterogeneous nuclear ribonucleoprotein U-like protein 1	IPI00013070 (+2)	0	0	0	5	2	1
40S ribosomal protein S19	IPI00215780	1	1	0	0	0	2
Pyrroline-5-carboxylate reductase 2	IPI00470610	1	0	0	3	3	1
Histone H2A type 1-B/E	IPI00026272 (+10)	0	0	0	0	1	0
Isoform 2 of Ankycorbin	IPI00292953 (+5)	3	0	1	0	0	2
Galectin-1	IPI00219219	1	0	0	0	0	2
Keratin, type I cytoskeletal 14	IPI00384444	4	0	0	0	0	2
Protein kinase, cAMP-dependent, regulatory, type II, alpha, isoform CRA_b	IPI00063234 (+1)	4	0	0	0	0	1
Isoform 2 of Myosin-Ic	IPI00010418 (+2)	3	0	0	0	0	0
Isoform 2 of Tropomyosin alpha-4 chain	IPI00216975	2	0	1	1	1	1
Dermcidin	IPI00027547 (+1)	2	1	0	0	0	2

Proteins identified from 1 h, 4 h and 16 h pre-digestion. 1 h and 4 h pre-digested samples were further digested over night. Spectra acquired on LTQ Orbitrap were searched against the human IPI database

and compared using Scaffold Q+ software (v 3.0, Proteome Software). Listed are the identified proteins along with the number of unique peptides with a Mascot score >25; > 2 peptides in at least one elution. Cohesin subunits and known interactors are in bold. Sample amounts loaded onto column: 1 h Trypsin pre-digestion 1/2 of eluate from immunoabsorption; 4 h Trypsin pre-digestion 1/4 of eluate; 16 h Trypsin pre-digestion 1/10th of eluate; 1 h LysC pre-digestion 1/2 of eluate; 4 h LysC pre-digestion 1/2 of eluate; 16 h of pre-digestion 1/2 of eluate

Table S4

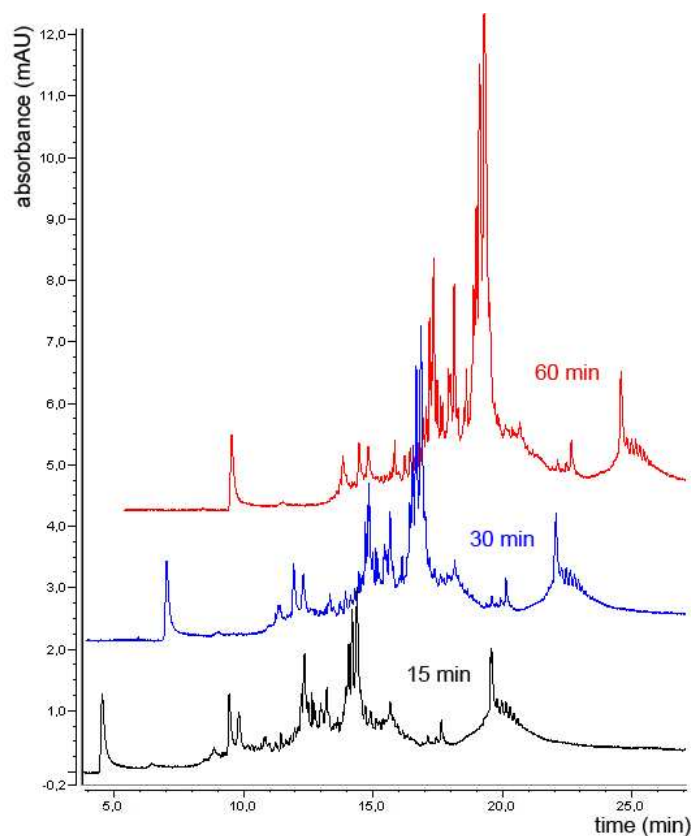
Protein	Accession Number	15 min	30 min	60 min
Structural maintenance of chromosomes protein 1A	IPI00291939	74	71	64
Structural maintenance of chromosomes protein 3	IPI00219420	63	60	64
cohesin subunit SA-2 isoform a	IPI00470883	34	32	32
Double-strand-break repair protein rad21 homolog	IPI00006715	22	23	23
Actin, cytoplasmic 1	IPI00021439 (+4)	10	9	9
Isoform 1 of Protein-L-isoaspartate(D-aspartate) O-methyltransferase	IPI00411680 (+2)	5	6	6
Isoform 1 of Protein-L-isoaspartate O-methyltransferase domain-containing protein 2	IPI00884185	8	6	5
Transcription elongation factor B polypeptide 2	IPI00026670 (+2)	7	6	5
Isoform 1 of Wings apart-like protein homolog	IPI00375330 (+2)	6	5	5
PDS5, regulator of cohesion maintenance, homolog A isoform 2	IPI00303063 (+1)	5	4	4
Transcription elongation factor B polypeptide 1	IPI00300341 (+1)	2	2	3
Isoform 2 of Nucleophosmin	IPI00220740 (+2)	3	3	1
2-oxoglutarate dehydrogenase, mitochondrial	IPI00098902 (+4)	2	3	2
165 kDa protein	IPI00240812 (+2)	4	2	1
Cohesin subunit SA-1	IPI00025158 (+1)	4	3	4
Isoform 1 of Protein-L-isoaspartate O-methyltransferase domain-containing protein 1	IPI00162744	1	2	2
Isoform 1 of Dynein heavy chain 10, axonemal	IPI00784869	2	0	2
Stress-70 protein, mitochondrial	IPI00007765	1	2	1
ubiquitin and ribosomal protein L40 precursor	IPI00456429	2	1	0
Translational activator GCN1	IPI00001159	1	2	2
Isoform 1 of Gephyrin	IPI00016006 (+2)	2	1	0
Zinc finger and BTB domain-containing protein 3	IPI00165014	2	0	1
Molybdenum cofactor sulfurase	IPI00304895	2	1	0
Arginase-2, mitochondrial	IPI00020332	1	2	0
Isoform 1 of Heat shock cognate 71 kDa protein	IPI00003865	3	0	0

Proteins identified from 15, 30 and 60 min LysC elution. Spectra acquired on LTQ Orbitrap were searched against the human IPI database and compared using Scaffold Q+ software (v 3.0, Proteome Software). Listed are the identified proteins along with the number of unique peptides with a Mascot score >25; > 2 peptides in at least one elution. Cohesin subunits and known interactors are in bold.

Results obtained using trypsin to perform protease elution.

Due to the fact that only the LysC – trypsin double digest led to complete sample digestion as judged by the standard deviation of measured peptides within one subunit and by the low number of identified missed cleavage sites in LC-MS/MS experiments, only the LysC elution is discussed in the main article. Results obtained using trypsin to perform protease elution are discussed here.

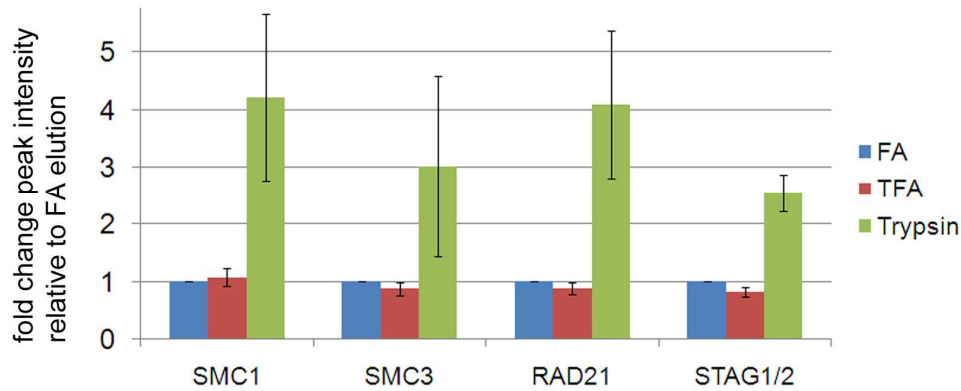
Figure S11



Trypsin elution performed analog to LysC elution experiment shown in figure 3b. Cohesin was purified using α RAD21 antibody beads and protease elution was performed by addition of 500 ng trypsin to 30 μ l antibody beads re-suspended in 60 μ l 0.5 M TEAB. After 15, 30 and 60 min of incubation at 37°C an aliquot was separated using a monolithic column. In contrast to LysC elution signal intensity increases from the 15 min to 60 min time point. As absolute quantification of cohesin peptides did not show an increase in cohesin from the 15 to 60 min time point (Figure S9) the increase in signal intensity can most

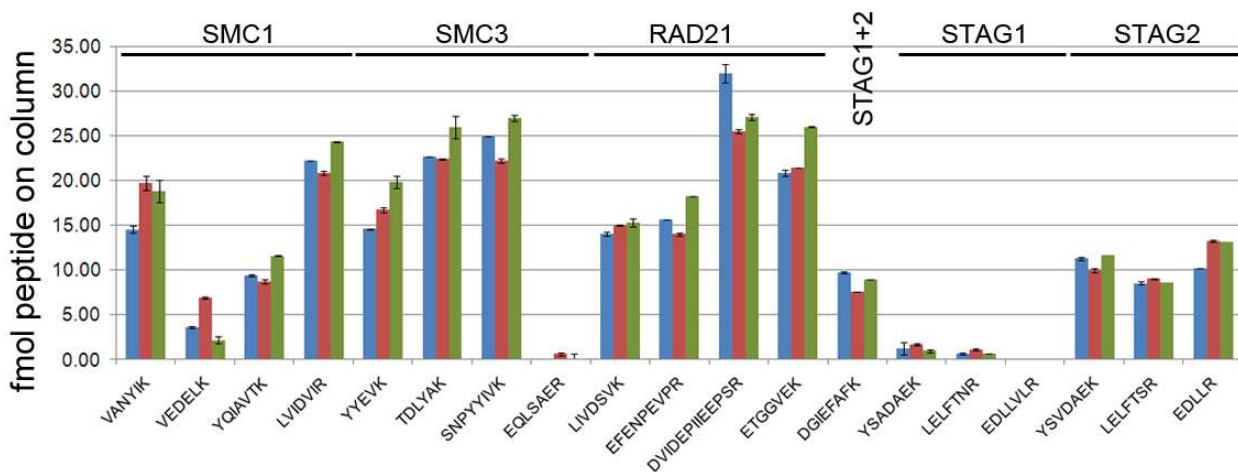
likely explained by cleaved antibody peptides (note: in this experiment approximately 50x the amount of material was loaded compared to experiment shown in figure 1a & 1c).

Figure S12



Labelfree quantification of cohesin subunits by XIC from preparations using different elution protocols relative to FA elution. Data are the mean \pm S.D. of relative measurements of peptides that were identified in all 5 preparations (depending on the size of the subunit but >5). Single measurement.

Figure S13



Absolute quantification of SMC1, SMC3, RAD21 and STAG1, STAG2 peptides using scheduled SRM on a 5500 QTRAP after 15, 30 and 60 min trypsin elution. Measurements are mean \pm S.D. of duplicate measurements. The strong scattering of measured peptides within one subunit indicates incomplete digestion.