

Supplementary Material

Mapping the native interaction surfaces of PREP1 with PBX1 by cross-linking mass-spectrometry and mutagenesis.

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Supplementary Figures

Figure S1

Panel A: Cross-linking mass-spectrometry workflow.

Panel B: SDS-PAGE of purified samples (PREP1-PBX1 and MEIS1-PBX1 complexes) and their BS³ cross-link. In lane 1, the PREP1-PBX1 purified complex. In lanes 2-4 the PREP1-PBX1 cross-linked samples, used for mass-spectrometry analysis. In lane 5, the MEIS1-PBX1 purified complex. In lanes 6-8 the MEIS1-PBX1 cross-linked samples, used for mass-spectrometry analysis. The experiment was repeated twice, and the SDS PAGE shown is representative of one of the two experiments.

Panel C: cartoon model summarizing the overall folding of the PBX1-PREP1 complex, as comprehended from the XLMS data. The most relevant inter molecular interactions between domains are highlighted with a star, and the corresponding peptides identified are listed. In grey the PBX1 model, in purple the PREP1 model.

Panel D: cartoon model summarizing the overall folding of the PBX1-MEIS1 complex, as understood from the XLMS data. The most relevant inter molecular interactions between domains are highlighted with a star, and the corresponding peptides identified are listed. In grey the PBX1 model, in azure the MEIS1 model.

Figure S2

Panel A and B: HR1 domains are highlighted in the red box, HR2 domains in the green box and the homeodomain (HD) is highlighted in the magenta box. The HR3 domain of PREP1 is highlighted in the dark green box. Stars above and below the sequence indicate the reactive lysines (K) in the cross-linking experiments. Heptad repeats of PREP1 and MEIS1 are highlighted in red.

Panel C: The PBX1,2,3 alignment. The PBC-A domain is highlighted in the grey box, the PBC-B in the orange box and the homeodomain (HD) in the magenta box. The dark-green boxes show the heptad repeats in the PBC-A analysed by mutations, while the red box shows the heptad repeat in the PBC-B domain. Stars above the sequence indicate the reactive lysines (K) in the cross-linking experiments.

Figure S3

Circular Dichroism (CD) spectroscopic analysis of PREP1 and PBX1 proteins used in this study. All PREP1 mutants analyzed are those tested in the binding assays reported in **Figures 2C** and **2D**. All PBX1 deletion mutants analyzed are those tested in the binding assays reported in Figure 3B and **3C**. All PBX1 point mutants analyzed are those tested in the binding assay in **Figure 3D**.

Panel A: Overlay of CD spectra of wild-type PREP1 and point-mutants in the HR1 region.

Panel B: Overlay of CD spectra of wild-type PREP1 and point-mutants in the HR2 region

Panel C: Overlay of CD spectra of wild-type PREP1 and point-mutants in the HR3 region

Panel D: Overlay of CD spectra of wild-type PREP1 and the quadruple mutants HR1m and HR2m.

Panel E: An overlay of CD spectra of the wild-type protein and of the Δ43-53, Δ77-84, Δ105-113 and Δ197-204 analogs is shown.

Panel F: Overlay of CD spectra of wild-type PBX1 and point-mutants in I43, I46, L47, I50 and I53 within the PBC-A region

Panel G: Overlay of CD spectra of wild-type PBX1 and point-mutants in L77, V80, L81 and I84 within the PBC-A region

Panel H: Overlay of CD spectra of wild-type PBX1 and point-mutants in L105, L108 and L112 within the PBC-A region.

Panel I: Overlay of CD spectra of wild-type PBX1 and point-mutants in I197, I204 and I211 within the PBC-B region.

Figure S4

Expression levels of PREP1-GFP constructs in total lysates after retroviral infection of A549 cells. Immunoblot with anti-GFP (**Panel A**), anti-PBX1 (**Panel B**) and anti-vinculin (**Panel C**). In Panel B, above the PBX1 signal, it is also visible at 160 KDa the signal of vinculin, as anti-PBX1 was probed after vinculin.

In **Panel D** are shown the whole membranes of the immunoblots shown in **Figure 4B**.

Figure S5

Flow-cytometry analysis of A549 cells expressing wild-type and mutant PREP1-GFP fusion proteins used for the pull-down and for the immunofluorescence experiments. A549 were retrovirally transduced with wild-type PREP1-GFP (**Panel A**); PREP1-GFP HR1m (L63A/L66A/L67A/L70A) (**Panel B**); PREP1-GFP HR2m (I122A/L125A/L129A/L132A), (**Panel C**); and PREP1-GFP ΔHR1+2 (**Panel D**) for stable expression. In addition, A549 were transduced with GFP (**Panel E**). In **Panel F** are shown control, not infected, cells. The figure shows the percentage of expression for PREP1-GFP wild-type and mutants in A549 cells.

Figure S6

SDS-PAGE of GST-affinity purified heterologous proteins expressed in *E. Coli* and of the constructs used for time-resolved fluorescence immunoassay (TR-FIA) and ELISA. Details of the samples purified are indicated next to each SDS-PAGE.

Supplementary Tables

Supplementary Table S1: List of primers used for PREP1 mutagenesis

Mutation	Forward 5'->3'	Reverse 5'->3'
PREP1 L63A	CAACAAAGCTAATAATGAAATGCTGGATGCCTATAAATGGCCTGC	GCAGGCCATTATAGGCATCCAGCATTCCATTATTAGCTTTGTTG
PREP1 L66A	CACATTTCAAAACAACAAAGCTAATGCTGGAAATAGTGGATCTGGATGCCTATAATGG	CCATTATAGGCATCCACTATTCAGCATTAGCTTTGTTGTTGAAAAATGTG
PREP1 L67A	TTTCAAACAACAAAGCTCTAATGAAATAGTGGATGCCCTATAATGGCCTG	CAGGCCATTATAGGCATCCACTATTCAGCATTAGCAGCTTTGTTGAA
PREP1 L69A	TAGATTGTTCACATTTCAAACAAACCGCAGCTAATAATGGAAATAGTGGATCTGGAAATAGTGGATG	GGCATCCACTATTCAGCATTAGCTGGTTGTTGAAATGTGAACAACTCA
PREP1 L70A	CATCCACTATTCATTAGCTTGCGTTGAAAAATGTGAACAACTCACACA	TGTGTAGATTGTTCACATTTCAAACGCCAAGCTAATGGAAATAGTGGATG
PREP1 L117A	TGGATTGCTTTACCATTGCATTATCAGTTCTGGATCTTCACAAAAGAAAGG	CCTTCTTTGTGAAGATCCAGAAACTGATAATGCAAATGGTAAAGCAGCCA
PREP1 I122A	GAATGCGAAACCTGGCTGCTTTACCTAAATTATCAGTTC	ATCCAGAAACTGATAATTAAATGGTAAAGCAGCCAATGGTAAAGCAGCCA
PREP1 L124A	AATGGTAAAGCAATCCAGGTTGCGCATTCTTCATTG	GGTTTGGCATTTCAGAATGCGCAAAGCTGGATTGCTTTAC
PREP1 L125A	CAAGAAGATGAATGCCAACCTGGATTGCTTTACCTAAATTATTAATTATCAGT	ACTGATAATTAAATGGTAAAGCAATCCAGGTTGCGCATTCTTCAGT
PREP1 L129A	ACCTTTCCAGCTCAAGAGCATGAATGCCAAACCTGGAT	ATCCAGGTTGCGCATTCTGCTCTGAGCTGGAAA
PREP1 L130A	CCTTTCCAGCTCAGCAAGATGAATGCCAAACCTGGAT	GGT
PREP1 L132A	GAGTTCTTAACCTTTCCGCTCAAGAAGATGAATGCC	ATCCAGGTTGCGCATTCTGCTGAGCTGGAAA
PREP1 L245A	GATGCTGAGATCTGGTTGCCTGTAAGCTGGAG	GGT
PREP1 L249A	TGATGCAAGATGCTGGCATCTGGTTAACTGTAAGCTGGAG	CTCCCAGCTTCAGTTACAGGCAAACCAAGATCTCAGCA
PREP1 L251A	GAACCATCATTTGATGCAAGGCCTGAGATCTGGTTAACTG	TC
PREP1 L252A	AGATGAACCATCATCTTGATGCGCGATGCTGAGATCTTGGTTAAC	CCAGCTTCAGTTACAGTTAAACCAAGATGCCAGCATCTGAGATGATGATGTTAC

Supplementary Table S2: List of primers used for PBX1 mutagenesis

Mutation	Forward 5'->3'	Reverse 5'->3'
PBX1 I43A	GCTGTAAAATGTCTCCAGCGCTGCTTCCTCC CGC	GCGGGAGGAAGCAGGACGCTGGAGACATTTCAC AGC
PBX1 I46A	GATGGTCATAATTGCTGTAAAGCGTCTCCAAT GTCCTGCTTCCTC	GAGGAAGCAGGACATTGGAGACGCTTACAGCA AATTATGACCATC
PBX1 L47A	GTCATAATTGCTGTGCAATGTCTCCAATGTCC TGCTTCCTCC	GGAGGAAGCAGGACATTGGAGACATTGCACAG CAAATTATGAC
PBX1 I50A	ACTCTGGTCTGTGATGGTCATAGCTGCTGAA AATGTCTCCAATG	CATTGGAGACATTACAGCAAGCTATGACCAT CACAGACCAGAGT
PBX1 I53A	CTCATCCAAACTCTGGTCTGTGGCGGTATAAT TTGCTGTAAAATG	CATTTACAGCAAATTATGACGCCACAGACCA GAGTTGGATGAG
PBX1 L77A	TTTCACACAACACATTAACACGCGGAGCTCA TTCTGTGGC	GCCACAGAACGCTGCGCGTTAACATGTGT TGTGTGAAA
PBX1 V80A	TTTTTCTTGTGATTCACACAACGCATTAAACA AGGCAGGCTTCATT	AATGAAGCCTGCCTGTTAACATGCCTGTGTA AATCAAAGAAAAAA
PBX1 L81A	AACTGTTTTCTTGATTCACACGCCACATT AAACAAGGCAGGCTTCAT	ATGAAGCCTGCCTGTTAACATGTGGCGTGTGAA ATCAAAGAAAAAAACAGTT
PBX1 I84A	GGATACTCAAAACTGTTTTCTTGCTTCAC ACAACACATTAACAAAGGCAG	CTGCCTGTTAACATGTGTTGTGAAAGCCAAG AAAAAAACAGTTTGAGTATCC
PBX1 L105A	GTCCAGCCGCATGCCCTGGGGTCTGTG	CACAGACCCCCAGGCCATGCCCTGGAC
PBX1 L108A	AACAGCATGTTGTCGCCCGCATCAGCTGGGG	CCCCAGCTGATGCCGGCGACAACATGCTGTT
PBX1 L112A	ACGCCTTCCGCTAACGCCATGTTGCCAGCCG	CGGCTGGACAACATGGCGTTAGCGGAAGGCCT
PBX1 I197A	GCTGACCATCCGCTAGCCTCCTTGGGAGAT G	CATCTCCCCAAAGGAGGCTGAGCGGATGGTCAG C
PBX1 I204A	GCTGAACCTGCGGTGGCGATGCTGACCATCCG C	GGGATGGTCAGCATGCCACCGCAAGTCAG C
PBX1 I211A	CTTGAGCTGCATCTGGCGGAGCTGAACCTGCG G	CCGCAAGTTCAGCTCCGCCAGATGCAGCTAA G
PBX1 Δ 43-55	CGGGAGGAAGCAGGACAGAGTTGGATGAGG	CCTCATCCAAACTCTGGCCTGCTCCCTCCCG
PBX1 Δ 77-84	GCCACAGAACATGAAGCCTGCCAAAGAAAAAAACA GTTTGAGT	ACTCAAAACTGTTTTCTTGGCAGGCTTCAT TCTGTGGC
PBX1 Δ 105-113	CCACGCCTTCCGCTGGGGTCTGTG	CACAGACCCCCAGGCCAGGCGTGG

PBX1 Δ 197-204	AGCTGAAC TTGCGGTGCTCCTTGGGAGATG	CATCTCCCAAAGGAGCACCGCAAGTCAGCT
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Supplementary Table S3: List of primers used for cloning PREP1-GFP into retroviral vector pBABE

Primer name	Sequence 5'-3'
PREP1FX	ATCTCGAGGCCACCATGATGGCTACACAGACA
PREP1RK	CGGGTACCATCTGCAGGGAGTCACTGT
PREP1SnF	CGTACGTAGCCACCATGATGGCTACA
GFPSSaR	GCGTCGACTTACTTGTACAGCTCGTCCA
GFPSSnF	CGTACGTAATGGTGAGCAAGGGCGA

Supplementary Table S4: PREP1-PBX1 cross-linked peptides Experiment #1

Protein	Title	Spectrum Number	Precursor Mass (Da)	Peptide	Peptide Mass (Da)	Modifications	Evalue	Score	Precursor Mass Error (ppm)	
			Charge				pLINK			
sp P40424 PBX1_HUMAN (87)-sp P40424 PBX1_HUMAN (195)			26							
	CB_4a_GluC.9754.9754.5.0.dta	5	2279,343935	KTVLSR(1)-TRPISPKIER(7)	2279,344428	null	8,80E-10	1,29E-13	-0,21629	
	CB_4a_GluC_bis.10057.10057.5.0.dta	5	2279,345545	KTVLSR(1)-TRPISPKIER(7)	2279,344428	null	3,11E-10	2,40E-10	0,490053	
	CB_4a_GluC_bis.10103.10103.4.0.dta	4	2279,345922	KTVLSR(1)-TRPISPKIER(7)	2279,344428	null	9,04E-12	5,64E-10	0,655452	
	CB_4a_GluC_bis.10136.10136.5.0.dta	5	2279,345446	KTVLSR(1)-TRPISPKIER(7)	2279,344428	null	2,72E-09	1,49E-09	0,44662	
	CB_4a_GluC.9742.9742.4.0.dta	4	2279,345322	KTVLSR(1)-TRPISPKIER(7)	2279,344428	null	2,04E-12	9,28E-08	0,392218	
	CB_4a_GluC_bis.10028.10028.4.0.dta	4	2279,345963	KTVLSR(1)-TRPISPKIER(7)	2279,344428	null	9,66E-14	1,27E-07	0,673439	
	CB_4a_GluC.9830.9830.4.0.dta	4	2279,345500	KTVLSR(1)-TRPISPKIER(7)	2279,344428	null	1,75E-09	1,99E-06	0,471188	
	CB_4a_GluC_bis.10197.10197.4.0.dta	4	2279,346156	KTVLSR(1)-TRPISPKIER(7)	2279,344428	null	1,82E-07	9,40E-06	0,758113	
	CB_4a_GluC_bis.10059.10059.3.0.dta	3	2279,346376	KTVLSR(1)-TRPISPKIER(7)	2279,344428	null	2,11E-10	3,52E-04	0,854632	
	CB_4a_GluC_bis.9696.9696.3.0.dta	3	1881,122483	KTVLSR(1)-TRPISPKET(7)	1881,116681	null	2,24E-25	2,56E-13	3,084338	
	CB_4a_GluC.9403.9403.3.0.dta	3	1881,123337	KTVLSR(1)-TRPISPKET(7)	1881,116681	null	6,36E-18	7,08E-12	3,555867	
	CB_4a_GluC.9477.9477.4.0.dta	4	1881,123865	KTVLSR(1)-TRPISPKET(7)	1881,116681	null	1,04E-07	3,62E-10	3,819008	
	CB_4a_GluC_bis.9767.9767.4.0.dta	4	1881,122834	KTVLSR(1)-TRPISPKET(7)	1881,116681	null	2,72E-09	5,97E-10	3,270929	
	CB_4a_GluC.9402.9402.4.0.dta	4	1881,123738	KTVLSR(1)-TRPISPKET(7)	1881,116681	null	1,09E-12	8,91E-10	3,773822	
	CB_4a_GluC_bis.9693.9693.3.0.dta	4	1881,122834	KTVLSR(1)-TRPISPKET(7)	1881,116681	null	2,81E-12	2,19E-09	3,260297	
	CB_4a_GluC_bis.7689.7689.3.0.dta	3	1881,122554	KTVLSR(1)-TRPISPKET(7)	1881,116681	null	8,23E-15	9,02E-09	3,122082	
	CB_4a_GluC_bis.9761.9761.4.0.dta	4	1881,122729	KTVLSR(1)-TRPISPKET(7)	1881,116681	null	6,47E-02	4,63E-02	3,209796	
	CB_4a_GluC_bis.9679.9679.4.1.dta	4	1881,123027	KTVLSR(1)-TRPISPKET(7)	1881,116681	null	8,25E-02	2,06E-01	3,373528	
	CB_4a_GluC.10439.10439.3.0.dta	3	1623,972658	KTVLSR(1)-HPSKE(5)	1623,967905	null	2,58E-12	1,21E-12	2,926782	
	CB_4a_GluC_bis.10770.10770.3.0.dta	3	1623,972863	KTVLSR(1)-HPSKE(5)	1623,967905	null	4,00E-14	2,31E-10	3,053016	
	CB_4a_GluC_bis.10893.10893.4.0.dta	4	2022,201093	KTVLSR(1)-TRPISPKIER(5)	2022,195653	null	2,49E-08	1,54E-09	2,690145	
	CB_4a_GluC_bis.10538.10538.4.0.dta	4	2022,201371	KTVLSR(1)-HPSKE(5)	2022,195653	null	9,33E-09	1,57E-08	2,82762	
	CB_4a_GluC.10816.10816.3.0.dta	3	2123,244962	KTVLSR(1)-TRPISPKEE(7)	2123,243326	null	5,14E-10	1,27E-08	0,770519	
	CB_4a_GluC_bis.11167.11167.3.0.dta	3	2123,246189	KTVLSR(1)-TRPISPKEE(7)	2123,243326	null	1,80E-11	2,38E-06	1,348409	
	CB_4a_GluC_bis.11151.11151.4.0.dta	4	2123,243463	KTVLSR(1)-TRPISPKEE(7)	2123,243326	null	4,32E-07	5,24E-06	0,064524	
	CB_4a_GluC_bis.11121.11121.4.4.dta	4	2123,243416	KTVLSR(1)-TRPISPKEE(7)	2123,243326	null	9,03E-07	4,01E-02	0,042388	
sp P40424 PBX1_HUMAN (87)-sp P55347-2 PKNX1_HUMAN (55)			3							
	CB_4a_GluC_bis.11198.11198.4.1.dta	4	2621,122684	KTVLSR(1)-SQTPMDVDXQAYR(9)	2621,196588	Oxidation[M](5)	1,90E-21	7,04E-13	-1,287482	
	CB_4a_GluC.10847.10847.2.0.dta	4	2621,394558	KTVLSR(1)-SQTPMDVDXQAYR(9)	2621,196588	Oxidation[M](5)	1,84E-20	1,73E-10	-0,774396	
sp P40424 PBX1_HUMAN (153)-sp P40424 PBX1_HUMAN (242)			4	1732,051638	KTVLSR(1)-KOAYIK(1)	1732,047881	null	9,61E-14	2,75E-11	2,169109
	CB_4a_GluC_bis.9730.9730.4.0.dta	5								
	CB_4a_GluC_bis.9353.9353.3.0.dta	3	1904,025457	AKLSQIR(2)-FNFKNQATE(4)	1904,023512	null	2,03E-08	1,42E-07	1,021521	
	CB_4a_GluC_bis.9111.9111.3.0.dta	3	1904,026698	AKLSQIR(2)-FNFKNQATE(4)	1904,023512	null	4,27E-08	7,03E-06	1,673299	
	CB_4a_GluC_bis.8168.8168.3.0.dta	3	2060,131075	AKLSQIR(2)-RNFKNQATE(5)	2060,124614	null	1,42E-11	7,17E-05	3,136218	
	CB_4a_GluC.7938.7938.3.0.dta	3	2060,131447	AKLSQIR(2)-RNFKNQATE(5)	2060,124614	null	2,97E-11	7,94E-05	3,31679	
	CB_4a_GluC_bis.8161.8161.4.0.dta	4	2060,127337	AKLSQIR(2)-RNFKNQATE(5)	2060,124614	null	3,60E-07	1,05E-04	1,321765	
sp P40424 PBX1_HUMAN (195)-sp P40424 PBX1_HUMAN (308)			3							
	CB_4a_GluC.9054.9054.3.0.dta	3	1815,003442	TRPISKE(7)-ANIYAAK(7)	-1815,00099	null	1,42E-13	3,24E-06	1,350963	
	CB_4a_GluC_bis.9713.9713.3.0.dta	3	2213,235603	TRPISKE(7)-ANIYAAK(7)	2213,228738	null	2,06E-13	4,87E-04	3,101803	
	CB_4a_GluC.9418.9418.3.0.dta	3	2213,237968	TRPISKE(7)-ANIYAAK(7)	2213,228738	null	2,95E-13	1,07E-02	4,179414	
sp P40424 PBX1_HUMAN (153)-sp P55347-2 PKNX1_HUMAN (268)			6							
	CB_4a_GluC.9116.9116.5.0.dta	5	2275,308994	AKLSQIR(2)-GVLPKHATNVMR(5)	2275,306604	null	2,59E-14	8,99E-12	1,050408	
	CB_4a_GluC.9098.9098.4.0.dta	4	2275,30977	AKLSQIR(2)-GVLPKHATNVMR(5)	2275,306604	null	1,80E-09	5,47E-07	1,391461	
	CB_4a_GluC_bis.9247.9247.4.0.dta	4	2275,311392	AKLSQIR(2)-GVLPKHATNVMR(5)	2275,306604	null	2,21E-12	1,07E-06	2,104332	
	CB_4a_GluC.8127.8127.4.0.dta	4	2291,304888	AKLSQIR(2)-GVLPKHATNVMR(5)	2291,301518	Oxidation[M](11)	4,63E-09	9,68E-06	1,470778	
	CB_4a_GluC_bis.9249.9249.5.0.dta	5	2275,310163	AKLSQIR(2)-GVLPKHATNVMR(5)	2275,306604	null	2,98E-07	1,77E-05	1,76196	
	CB_4a_GluC_bis.8304.8304.4.0.dta	4	2291,300449	AKLSQIR(2)-GVLPKHATNVMR(5)	2291,301518	Oxidation[M](11)	1,22E-05	7,53E-05	-0,466547	
sp P40424 PBX1_HUMAN (153)-sp P40424 PBX1_HUMAN (308)			1							
	CB_4a_GluC_bis.10187.10187.3.0.dta	3	1702,389814	AKLSQIR(2)-ANIYAAK(7)	1702,384949	null	3,38E-18	5,98E-11	2,856749	
sp P40424 PBX1_HUMAN (153)-sp P55347-2 PKNX1_HUMAN (153)			5							
	CB_4a_GluC.7888.7888.2.0.dta	2	1661,894684	AKLSQIR(2)-TKMNSE(2)	1661,889002	null	2,27E-05	6,71E-11	3,419001	
	CB_4a_GluC.7867.7867.3.0.dta	3	1661,891897	AKLSQIR(2)-TKMNSE(2)	1661,889002	null	5,46E-11	1,01E-09	1,741994	
	CB_4a_GluC_bis.8111.8111.3.0.dta	3	1661,890569	AKLSQIR(2)-TKMNSE(2)	1661,889002	null	7,08E-12	4,24E-08	0,942903	
	CB_4a_GluC.6717.6717.3.0.dta	3	1677,887834	AKLSQIR(2)-TKMNSE(2)	1677,883916	Oxidation[M](13)	3,70E-08	9,69E-08	2,335084	
	CB_4a_GluC_bis.6937.6937.3.0.dta	3	1677,888735	AKLSQIR(2)-TKMNSE(2)	1677,883916	Oxidation[M](13)	1,09E-08	1,80E-05	2,87207	
sp P40424 PBX1_HUMAN (308)-sp P55347-2 PKNX1_HUMAN (268)			4							
	CB_4a_GluC.9835.9835.4.1.dta	4	2210,21193	ANIAYAK(7)-GVLPKHATNVMR(5)	2210,211314	null	7,09E-14	1,19E-10	0,278706	
	CB_4a_GluC_bis.10205.10205.3.0.dta	3	2210,215623	ANIAYAK(7)-GVLPKHATNVMR(5)	2210,211314	null	1,68E-20	2,52E-16	1,949587	
	CB_4a_GluC_bis.10196.10196.4.0.dta	4	2210,217331	ANIAYAK(7)-GVLPKHATNVMR(5)	2210,211314	null	2,66E-14	2,12E-08	2,722364	
	CB_4a_GluC_bis.9187.9187.4.1.dta	4	2226,213222	ANIAYAK(7)-GVLPKHATNVMR(5)	2226,206228	Oxidation[M](11)	4,08E-13	3,72E-05	3,141668	
sp P40424 PBX1_HUMAN (242)-sp P40424 PBX1_HUMAN (308)			2							
	CB_4a_GluC_bis.9103.9103.3.0.dta	3	1995,031981	RWNFKQATE(5)-ANIYAAK(7)	1995,029323	null	1,93E-15	1,95E-06	1,332311	
	CB_4a_GluC.8843.8843.3.0.dta	3	1995,034195	RWNFKQATE(5)-ANIYAAK(7)	1995,029323	null	2,37E-13	2,00E-06	2,442069	
sp P55347-2 PKNX1_HUMAN (134)-sp P55347-2 PKNX1_HUMAN (268)			1							
	CB_4a_GluC_bis.9395.9395.4.0.dta	4	2191,192764	LEVKVNE(3)-GVLPKHATNVMR(5)	2191,190244	null	1,81E-05	4,72E-04	1,15006	

Table legend

Protein
Title
Spectrum Number
Charge
Precursor Mass
Peptide
Peptide Mass
Modifications
Evalue
Score
Precursor Mass Error

Accession number of the proteins involved in cross linking; the site position of the xLys on the protein is reported in brackets
File name .dat (and corresponding .tif) of the ms2 annotated spectrum (as reported in the repository)
Frequency of the ms2 spectra
Charge of the fragmented ion
Observed mass from the precursor ion (Da)
Sequence of cross-linked peptide pair, the site position of the Lys involved in cross-linking is reported in brackets
Theoretical mass of cross-linked peptide (Da)
Cys Carbamidomethyl or Met Oxidation

Criteria applied for manual evaluation of spectra confidently identified = E-value < 0,001 (corresponding to FDR < 0,05); the majority of the ions of the spectrum are assigned and 4 consecutive b or y ions for each xLys peptide pair observed

Supplementary Table S5: PREP1-PBX1 looped peptides Experiment #1

Protein	Title	Spectrum Number	Precursor Mass (Da)	Peptide	Peptide Mass (Da)	Modifications	Evalue	Score	Precursor Mass Error pLINK (ppm)
		Charge							
sp P40424 PBX1_HUMAN (292)(293)		7							
	CB_4a_GluC.10473.10473.2.0.dta	2	1521,797828	YKKNIGKFQEE(2)(3)	1521,794702	null	3,66E-50	2,10E-02	2,054154
	CB_4a_GluC.7545.7545.3.0.dta	3	1257,7674	IRYKKNIGK(4)(5)	1257,767706	null	3,55E-15	3,40E-02	-0,243288
	CB_4a_GluC.7564.7564.2.0.dta	2	1257,768681	IRYKKNIGK(4)(5)	1257,767706	null	9,76E-28	3,98E-02	0,775183
	CB_4a_GluC.7255.7255.2.0.dta	2	988,584147	YKKNIGK(2)(3)	988,582546	null	9,15E-14	3,48E-02	1,61949
	CB_4a_GluC.7178.7178.2.0.dta	2	988,584185	YKKNIGK(2)(3)	988,582546	null	2,68E-19	4,00E-02	1,657929
	CB_4a_GluC_bis.7423.7423.2.0.dta	2	988,583137	YKKNIGK(2)(3)	988,582546	null	4,14E-26	7,56E-02	0,597826
	CB_4a_GluC.7173.7173.2.0.dta	2	988,584425	YKKNIGK(2)(3)	988,582546	null	2,07E-18	8,70E-02	1,900701
sp P55347-2 PKNX1_HUMAN (334)(335)		4							
	CB_4a_GluC.3713.3713.2.0.dta	2	1463,844217	KKTAQNRPVQR(1)(2)	1463,844048	null	1,90E-16	5,05E-02	0,115449
	CB_4a_GluC.3698.3698.3.0.dta	3	1463,844439	KKTAQNRPVQR(1)(2)	1463,844048	null	2,49E-26	6,40E-02	0,267105
	CB_4a_GluC.3372.3372.2.0.dta	2	983,565388	KKTAQNR(1)(2)	983,563206	null	1,21E-30	5,90E-02	2,218464
	CB_4a_GluC_bis.3462.3462.2.0.dta	2	983,565925	KKTAQNR(1)(2)	983,563206	null	2,59E-23	1,16E-01	2,764439
sp P55347-2 PKNX1_HUMAN (331)(333)		2							
	CB_4a_GluC.4661.4661.2.0.dta	2	1390,689554	SSCSETPKTKK(8)(10)	1390,688191	Carbamidomethyl[C](3)	2,04E-26	3,10E-02	0,98009
	CB_4a_GluC_bis.4856.4856.2.0.dta	2	1390,688445	SSCSETPKTKK(8)(10)	1390,688191	Carbamidomethyl[C](3)	2,27E-44	7,11E-02	0,182643
sp P55347-2 PKNX1_HUMAN (260)(262)		5							
	CB_4a_GluC.7682.7682.4.0.dta	4	1835,962896	LSILHQDDGSSKNKR(12)(14)	1835,960913	null	3,46E-28	4,87E-02	1,080088
	CB_4a_GluC.8535.8535.3.0.dta	3	1835,968132	LSILHQDDGSSKNKR(12)(14)	1835,960913	null	4,19E-32	6,15E-02	3,932001
	CB_4a_GluC_bis.8825.8825.3.0.dta	3	1835,967448	LSILHQDDGSSKNKR(12)(14)	1835,960913	null	1,28E-65	6,90E-02	3,559444
	CB_4a_GluC_bis.8904.8904.3.0.dta	3	1835,967345	LSILHQDDGSSKNKR(12)(14)	1835,960913	null	3,93E-33	9,89E-02	3,503343
	CB_4a_GluC_bis.9017.9017.3.0.dta	3	1835,960322	LSILHQDDGSSKNKR(12)(14)	1835,960913	null	4,63E-24	1,25E-01	-0,321902

Table legend

Protein Accession number of the proteins involved in cross linking; the site position of the xl-Lys on the protein is reported in brackets
 Title File name .dat (and corresponding .tif) of the ms2 annotated spectrum (as reported in the repository)
 Spectrum Number Frequency of the ms2 spectra
 Charge Charge of the fragmented ion
 Precursor Mass Observed mass from the precursor ion (Da)
 Peptide Sequence of cross-linked peptide pair, the site position of the Lys involved in cross-linking is reported in brackets
 Peptide Mass Theoretical mass of cross-linked peptide (Da)
 Modifications Cys Carbamidomethyl or Met Oxidation
 Evalue
 Score pLink score
 Precursor Mass Error precursor mass error in ppm

Criteria applied for manual evaluation of spectra confidently identified = E-value < 0,001 (corresponding to FDR < 0,05); the majority of the ions of the spectrum are assigned and 4 consecutive b or y ions for each xl peptide pair observed

Supplementary Table S6: PREP1-PBX1 cross-linked peptides Experiment #2

Protein	Title	Spectrum Number	Precursor Mass (Da)	Peptide	Peptide Mass (Da)	Modifications	Value	Score	Precursor Mass Error (ppm)
			Charge				pLINK		
sp P40424 PBX1_HUMAN (87)-sp P40424 PBX1_HUMAN (195)			19						
	CB FB_180416_Prep1_Tryp_GluC_3.11563.11563.5.0.dta	5	2279,359429	KTVLSIR(1)-TRPISPKEIER(7)	2279,344428	null	8,55E-16	1,85E-13	6,581278
	CB FB_180416_Prep1_Tryp_GluC_3.11664.11664.5.0.dta	5	2279,358459	KTVLSIR(1)-TRPISPKEIER(7)	2279,344428	null	9,13E-10	1,20E-10	6,155717
	CB FB_180416_Prep1_Tryp_GluC_3.11478.11478.5.0.dta	5	2279,359209	KTVLSIR(1)-TRPISPKEIER(7)	2279,344428	null	1,21E-12	8,71E-10	6,484759
	CB FB_180416_Prep1_Tryp_GluC_3.11562.11562.4.0.dta	4	2279,360169	KTVLSIR(1)-TRPISPKEIER(7)	2279,344428	null	3,95E-13	9,19E-09	6,905933
	CB FB_180416_Prep1_Tryp_GluC_3.11659.11659.4.0.dta	4	2279,357757	KTVLSIR(1)-TRPISPKEIER(7)	2279,344428	null	6,42E-10	1,10E-08	5,847734
	CB FB_180416_Prep1_Tryp_GluC_3.11758.11758.4.0.dta	4	2279,356112	KTVLSIR(1)-TRPISPKEIER(7)	2279,344428	null	1,48E-09	1,51E-07	5,126035
	CB FB_180416_Prep1_Tryp_GluC_3.11469.11469.4.0.dta	4	2279,360279	KTVLSIR(1)-TRPISPKEIER(7)	2279,344428	null	2,97E-11	1,73E-07	6,954193
	CB FB_180416_Prep1_Tryp_GluC_3.12296.12296.4.0.dta	4	2279,357191	KTVLSIR(1)-TRPISPKEIER(7)	2279,344428	null	8,73E-13	3,78E-07	5,599417
	CB FB_180416_Prep1_Tryp_GluC_3.11572.11572.4.0.dta	4	2279,360169	KTVLSIR(1)-TRPISPKEIER(7)	2279,344428	null	1,14E-06	2,64E-04	6,905933
	CB FB_180416_Prep1_Tryp_GluC_3.11476.11476.3.0.dta	3	2279,358399	KTVLSIR(1)-TRPISPKEIER(7)	2279,344428	null	1,94E-17	2,04E-01	6,129394
	CB FB_180416_Prep1_Tryp_GluC_3.12723.12723.4.0.dta	4	2022,200532	KTVLSIR(1)-LISPKEIE(5)	2022,195653	null	1,79E-09	2,71E-13	2,412724
	CB FB_180416_Prep1_Tryp_GluC_3.12418.12418.3.0.dta	3	1623,97707	KTVLSIR(1)-PISKE(5)	1623,967905	null	8,36E-09	4,14E-13	5,643584
	CB FB_180416_Prep1_Tryp_GluC_3.11070.11070.3.0.dta	3	1881,125879	KTVLSIR(1)-TRPISPKE(7)	1881,116681	null	3,88E-13	1,41E-11	4,889649
	CB FB_180416_Prep1_Tryp_GluC_3.11132.11132.4.0.dta	4	1881,126678	KTVLSIR(1)-TRPISPKE(7)	1881,116681	null	6,42E-10	8,72E-11	5,314397
	CB FB_180416_Prep1_Tryp_GluC_3.12293.12293.3.0.dta	3	2123,256433	KTVLSIR(1)-TRPISPKE(7)	2123,243326	null	1,05E-13	5,35E-07	6,173103
	CB FB_180416_Prep1_Tryp_GluC_3.12987.12987.3.0.dta	3	2123,257251	KTVLSIR(1)-TRPISPKE(7)	2123,243326	null	1,70E-10	6,78E-06	6,558363
	CB FB_180416_Prep1_Tryp_GluC_3.12892.12892.4.0.dta	4	2123,256962	KTVLSIR(1)-TRPISPKE(7)	2123,243326	null	7,76E-09	1,42E-05	6,42225
	CB FB_180416_Prep1_Tryp_GluC_3.12986.12986.4.0.dta	4	2123,257839	KTVLSIR(1)-TRPISPKE(7)	2123,243326	null	5,07E-06	2,36E-04	6,835298
	CB FB_180416_Prep1_Tryp_GluC_3.11976.11976.4.1.dta	4	2408,401589	EKTVLSIR(2)-TRPISPKE(7)	2408,387016	null	1,28E-11	2,34E-04	6,050938
sp P40424 PBX1_HUMAN (87)-sp P55347-2 PKNX1_HUMAN (55)			16						
	CB FB_180416_Prep1_Tryp_GluC_3.11125.11125.3.0.dta	3	1732,056322	KTVLSIR(1)-KQAIYR(1)	1732,047881	null	2,74E-15	7,28E-14	4,873422
	CB FB_180416_Prep1_Tryp_GluC_3.11118.11118.4.0.dta	4	1732,05599	KTVLSIR(1)-KQAIYR(1)	1732,047881	null	1,64E-12	2,32E-13	4,681741
	CB FB_180416_Prep1_Tryp_GluC_3.13027.13027.4.0.dta	4	2621,414281	KTVLSIR(1)-SQTPMDVKQAIYR(9)	2621,396588	Oxidation[M](5)	6,85E-27	5,08E-13	6,749456
	CB FB_180416_Prep1_Tryp_GluC_3.13130.13130.4.0.dta	4	2621,413312	KTVLSIR(1)-SQTPMDVKQAIYR(9)	2621,396588	Oxidation[M](5)	1,26E-21	2,37E-10	6,379805
	CB FB_180416_Prep1_Tryp_GluC_3.1052.13052.3.0.dta	3	2621,413207	KTVLSIR(1)-SQTPMDVKQAIYR(9)	2621,396588	Oxidation[M](5)	1,73E-16	9,91E-10	6,33975
	CB FB_180416_Prep1_Tryp_GluC_3.14385.14385.4.0.dta	4	2605,41678	KTVLSIR(1)-SQTPMDVKQAIYR(9)	2605,401674	null	3,34E-19	5,45E-08	5,797954
	CB FB_180416_Prep1_Tryp_GluC_3.13152.13152.3.0.dta	3	2621,411985	KTVLSIR(1)-SQTPMDVKQAIYR(9)	2621,396588	Oxidation[M](5)	2,63E-12	2,21E-01	5,873587
	CB FB_180416_Prep1_Tryp_GluC_3.14202.14202.4.0.dta	4	3383,816763	KTVLSIR(1)-GVSPPPVESQTPMVDVKQAIYR(17)	3383,787757	Oxidation[M](13)	2,73E-18	6,33E-08	8,572051
	CB FB_180416_Prep1_Tryp_GluC_3.14099.14099.4.0.dta	4	3383,811298	KTVLSIR(1)-GVSPPPVESQTPMVDVKQAIYR(17)	3383,787757	Oxidation[M](13)	1,16E-23	2,83E-07	6,956997
	CB FB_180416_Prep1_Tryp_GluC_3.14298.14298.4.0.dta	4	3383,818207	KTVLSIR(1)-GVSPPPVESQTPMVDVKQAIYR(17)	3383,787757	Oxidation[M](13)	3,01E-16	9,27E-07	8,998791
	CB FB_180416_Prep1_Tryp_GluC_3.14395.14395.4.0.dta	4	3383,809634	KTVLSIR(1)-GVSPPPVESQTPMVDVKQAIYR(17)	3383,787757	Oxidation[M](13)	6,11E-11	1,12E-06	6,46524
	CB FB_180416_Prep1_Tryp_GluC_3.14207.14207.5.0.dta	5	3383,812066	KTVLSIR(1)-GVSPPPVESQTPMVDVKQAIYR(17)	3383,787757	Oxidation[M](13)	4,39E-15	7,49E-06	7,183961
	CB FB_180416_Prep1_Tryp_GluC_3.15396.15396.5.0.dta	5	3367,819835	KTVLSIR(1)-GVSPPPVESQTPMVDVKQAIYR(17)	3367,792843	null	7,06E-06	1,11E-05	8,014745
	CB FB_180416_Prep1_Tryp_GluC_3.15133.15133.4.0.dta	4	3367,820597	KTVLSIR(1)-GVSPPPVESQTPMVDVKQAIYR(17)	3367,792843	null	3,12E-21	4,33E-04	8,241006
	CB FB_180416_Prep1_Tryp_GluC_3.14497.14497.4.4.dta	4	3383,823445	KTVLSIR(1)-GVSPPPVESQTPMVDVKQAIYR(17)	3383,787757	Oxidation[M](13)	1,79E-22	8,11E-04	10,546761
	CB FB_180416_Prep1_Tryp_GluC_3.15386.15386.4.0.dta	4	3367,821689	KTVLSIR(1)-GVSPPPVESQTPMVDVKQAIYR(17)	3367,792843	null	2,78E-16	2,69E-03	8,565254
sp P40424 PBX1_HUMAN (242)-sp P40424 PBX1_HUMAN (308)			3						
	CB FB_180416_Prep1_Tryp_GluC_3.12344.12344.2.0.dta	2	1967,983303	NFNKQATE(4)-ANIYAAK(8)	1967,970809	null	3,35E-08	1,27E-07	6,348671
	CB FB_180416_Prep1_Tryp_GluC_3.10255.10255.2.0.dta	2	1995,03927	RNFNKQATE(4)-ANIYAAK(7)	1995,029323	null	4,74E-10	1,58E-06	4,985892
	CB FB_180416_Prep1_Tryp_GluC_3.11996.11996.2.0.dta	2	1838,940423	NFNKQATE(4)-ANIYAAK(7)	1838,928221	null	2,06E-05	9,65E-05	6,635387
sp P40424 PBX1_HUMAN (65)-sp P40424 PBX1_HUMAN (195)			6						
	CB FB_180416_Prep1_Tryp_GluC_3.6884.6884.5.0.dta	5	2498,35256	KHALNCHR(1)-TRPISPKEIER(7)	2498,34075	Carbamidomethyl[C](20)	7,54E-15	6,75E-15	4,727137
	CB FB_180416_Prep1_Tryp_GluC_3.6794.6794.5.0.dta	5	2498,351333	KHALNCHR(1)-TRPISPKEIER(7)	2498,34075	Carbamidomethyl[C](20)	1,89E-12	2,93E-13	4,236011
	CB FB_180416_Prep1_Tryp_GluC_3.5318.5318.4.0.dta	4	2100,125903	KHALNCHR(1)-TRPISPKE(7)	2100,113002	Carbamidomethyl[C](6)	1,22E-12	1,64E-10	6,143003
	CB FB_180416_Prep1_Tryp_GluC_3.5234.5234.5.0.dta	5	2100,124388	KHALNCHR(1)-TRPISPKE(7)	2100,113002	Carbamidomethyl[C](6)	2,19E-08	4,82E-09	5,421613
	CB FB_180416_Prep1_Tryp_GluC_3.5334.5334.3.0.dta	3	2100,125128	KHALNCHR(1)-TRPISPKE(7)	2100,113002	Carbamidomethyl[C](6)	1,80E-11	2,06E-04	5,773975
	CB FB_180416_Prep1_Tryp_GluC_3.7590.7590.5.0.dta	5	2342,252694	KHALNCHR(1)-TRPISPKE(7)	2342,239647	Carbamidomethyl[C](19)	9,97E-12	1,58E-06	5,57031
sp P40424 PBX1_HUMAN (195)-sp P40424 PBX1_HUMAN (308)			4						
	CB FB_180416_Prep1_Tryp_GluC_3.11089.11089.4.0.dta	4	2213,236968	TRPISPKEIER(7)-ANIYAAK(7)	2213,228738	null	3,44E-13	3,21E-09	3,718549
	CB FB_180416_Prep1_Tryp_GluC_3.10503.10503.2.0.dta	2	1815,015195	TRPISPKE(7)-ANIYAAK(7)	1815,00099	null	4,42E-11	8,87E-07	7,826442
	CB FB_180416_Prep1_Tryp_GluC_3.10488.10488.3.0.dta	3	1815,014094	TRPISPKE(7)-ANIYAAK(7)	1815,00099	null	4,98E-13	3,89E-03	7,219831
	CB FB_180416_Prep1_Tryp_GluC_3.12600.12600.3.0.dta	3	2057,135549	TRPISPKE(7)-ANIYAAK(7)	2057,127636	null	2,92E-12	3,53E-05	3,846626
sp P40424 PBX1_HUMAN (153)-sp P40424 PBX1_HUMAN (308)			5						
	CB FB_180416_Prep1_Tryp_GluC_3.11614.11614.2.0.dta	2	1702,9955	AKLSQIR(2)-ANIYAAK(7)	1702,984949	null	1,36E-11	4,07E-19	6,195592
	CB FB_180416_Prep1_Tryp_GluC_3.11612.11612.3.0.dta	3	1702,996352	AKLSQIR(2)-ANIYAAK(7)	1702,984949	null	1,76E-20	9,18E-11	6,69589
	CB FB_180416_Prep1_Tryp_GluC_3.11707.11707.3.0.dta	3	1702,995935	AKLSQIR(2)-ANIYAAK(7)	1702,984949	null	2,46E-15	1,43E-09	6,451026
	CB FB_180416_Prep1_Tryp_GluC_3.11922.11922.3.1.dta	3	1702,995032	AKLSQIR(2)-ANIYAAK(7)	1702,984949	null	1,91E-11	5,76E-09	5,92078
	CB FB_180416_Prep1_Tryp_GluC_3.11638.11638.4.0.dta	4	1702,99401	AKLSQIR(2)-ANIYAAK(7)	1702,984949	null	1,32E-06	1,41E-05	5,320658
sp P40424 PBX1_HUMAN (153)-sp P40424 PBX1_HUMAN (242)			2						
	CB FB_180416_Prep1_Tryp_GluC_3.10639.10639.2.0.dta	2	1904,031906	AKLSQIR(2)-NFNKQATE(4)	1904,023512	null	7,04E-13	1,60E-17	4,408559
	CB FB_180416_Prep1_Tryp_GluC_3.8994.8994.4.0.dta	4	2060,133109	AKLSQIR(2)-RNFKQATE(5)	2060,124614	null	2,60E-06	4,93E-04	4,123537
sp P40424 PBX1_HUMAN (87)-sp P40424 PBX1_HUMAN (308)			2						
	CB FB_180416_Prep1_Tryp_GluC_3.13460.13460.2.0.dta	2	1704,012436	KTVLSIR(1)-ANIYAAK(7)	1704,00535	null	5,08E-13	8,15E-20	4,158438
	CB FB_180416_Prep1_Tryp_GluC_3.13455.13455.3.0.dta	3	1704,011255	KTVLSIR(1)-ANIYAAK(7)	1704,00535	null	3,11E-12	1,32E-10	3,465365
sp P55347-2 PKNX1_HUMAN (134)-sp P55347-2 PKNX1_HUMAN (140)			3						
	CB FB_180416_Prep1_Tryp_GluC_3.11343.11343.2.0.dta	2	1953,946564	LEKVNNE(3)-LCKDFCSR(3)	1953,94077	Carbamidomethyl[C](2) Carbamidomethyl[C](6)	1,32E-10	1,51E-13	2,965289
	CB FB_180416_Prep1_Tryp_GluC_3.11333.11333.3.0.dta	3	1953,948688	LEKVNNE(3)-LCKDFCSR(3)	1953,94077	Carbamidomethyl[C](2) Carbamidomethyl[C](6)	3,46E-10	7,45E-09	4,052323
	CB FB_180416_Prep1_Tryp_GluC_3.11446.11446.3.0.dta	3	1953,948843	LEKVNNE(3)-LCKDFCSR(3)	1953,94077	Carbamidomethyl[C](2) Carbamidomethyl[C](6)	5,08E-05	3,80E-03	4,13165
sp P40424 PBX1_HUMAN (87)-sp P40424 PBX1_HUMAN (153)			2						
	CB FB_180416_Prep1_Tryp_GluC_3.11934.11934.4.0.dta	4	1769,113021	KTVLSIR(1)-AKLSQIR(2)	1769,10064	null	1,27E-09	1,52E-11	6,998471

		CB_FB_180416_Prep1_Tryp_GluC_3.11952.11952.3.0.dta	3	1769,115016	KTVLSIR(1)-AKLSQIR(2)	1769,10064	null	5,98E-09	2,06E-10	8,126163
sp P40424 PBX1_HUMAN (308)-sp P55347-2 PKNX1_HUMAN (134)			1							
		CB_FB_180416_Prep1_Tryp_GluC_3.11861.11861.2.0.dta	2	1618,87949	ANIYAAK(7)-LEKVNE(3)	1618,868589	null	8,79E-10	1,43E-09	6,733715
sp P40424 PBX1_HUMAN (65)-sp P40424 PBX1_HUMAN (308)			1							
		CB_FB_180416_Prep1_Tryp_GluC_3.7161.71614.0.dta	4	1923,013309	KHALNCHR(1)-ANIYAAK(7)	1923,001671	Carbamidomethyl[C](6)	2,84E-08	3,57E-09	6,051997
sp P40424 PBX1_HUMAN (65)-sp P40424 PBX1_HUMAN (74)			3							
		CB_FB_180416_Prep1_Tryp_GluC_3.14528.14528.3.0.dta	3	2510,260131	KHALNCHR(1)-MKPALFNVLC(2)	2510,246384	Carbamidomethyl[C](10);Oxidation[M](1);Carbamidomethyl[C](20)	1,40E-13	1,05E-08	5,476355
		CB_FB_180416_Prep1_Tryp_GluC_3.14544.14544.5.0.dta	5	2510,25566	KHALNCHR(1)-MKPALFNVLC(2)	2510,246384	Carbamidomethyl[C](10);Oxidation[M](1);Carbamidomethyl[C](20)	1,33E-07	3,01E-05	3,695255
		CB_FB_180416_Prep1_Tryp_GluC_3.15057.15057.3.0.dta	3	2494,267085	KHALNCHR(1)-MKPALFNVLC(2)	2494,25147	Carbamidomethyl[C](10);Carbamidomethyl[C](20)	1,24E-10	1,76E-04	6,260395
sp P40424 PBX1_HUMAN (65)-sp P40424 PBX1_HUMAN (87)			1							
		CB_FB_180416_Prep1_Tryp_GluC_3.7771.77714.2.dta	4	1989,127062	KHALNCHR(1)-KTVLSIR(1)	1989,117362	Carbamidomethyl[C](6)	1,89E-09	1,77E-06	4,876535
sp P40424 PBX1_HUMAN (308)-sp P55347-2 PKNX1_HUMAN (268)			4							
		CB_FB_180416_Prep1_Tryp_GluC_3.11541.11541.3.0.dta	3	2210,227111	ANIYAAK(7)-GVLPKHATNVMR(5)	2210,211314	null	5,37E-18	1,82E-06	7,14728
		CB_FB_180416_Prep1_Tryp_GluC_3.11549.11549.4.0.dta	4	2210,226008	ANIYAAK(7)-GVLPKHATNVMR(5)	2210,211314	null	3,25E-11	7,44E-04	6,648233
		CB_FB_180416_Prep1_Tryp_GluC_3.10416.10416.4.0.dta	4	2226,211772	ANIYAAK(7)-GVLPKHATNVMR(5)	2226,206228	Oxidation[M](11)	2,67E-12	9,16E-04	6,982282
		CB_FB_180416_Prep1_Tryp_GluC_3.11626.11626.3.0.dta	3	2210,227032	ANIYAAK(7)-GVLPKHATNVMR(5)	2210,211314	null	1,75E-12	3,67E-02	7,111537
sp P40424 PBX1_HUMAN (87)-sp P55347-2 PKNX1_HUMAN (268)			2							
		CB_FB_180416_Prep1_Tryp_GluC_3.11800.11800.5.0.dta	5	2276,339091	KTVLSIR(1)-GVLPKHATNVMR(5)	2276,327005	null	9,81E-10	2,21E-06	5,30943
sp P40424 PBX1_HUMAN (87)-sp P55347-2 PKNX1_HUMAN (73)			5	2292,330628	KTVLSIR(1)-GVLPKHATNVMR(5)	2292,321919	Oxidation[M](11)	1,93E-04	4,63E-03	3,799205
		CB_FB_180416_Prep1_Tryp_GluC_3.10849.10849.5.0.dta	2							
		CB_FB_180416_Prep1_Tryp_GluC_3.9431.9431.3.0.dta	3	2107,082479	KTVLSIR(1)-KCEQSTQGSE(1)	2107,069859	Carbamidomethyl[C](2)	8,13E-15	2,26E-06	5,98936
		CB_FB_180416_Prep1_Tryp_GluC_3.9468.9468.2.0.dta	2	2107,078565	KTVLSIR(1)-KCEQSTQGSE(1)	2107,069859	Carbamidomethyl[C](2)	8,89E-11	1,37E-02	4,131804
sp P40424 PBX1_HUMAN (308)-sp P55347-2 PKNX1_HUMAN (140)			1							
		CB_FB_180416_Prep1_Tryp_GluC_3.12398.12398.3.0.dta	3	1972,974595	ANIYAAK(7)-LCKDFCSR(3)	1972,96184	Carbamidomethyl[C](2);Carbamidomethyl[C](6)	4,02E-14	3,87E-06	6,464899
sp P40424 PBX1_HUMAN (153)-sp P55347-2 PKNX1_HUMAN (268)			5							
		CB_FB_180416_Prep1_Tryp_GluC_3.10373.10373.5.0.dta	5	2275,318296	AKLSQIR(2)-GVLPKHATNVMR(5)	2275,306604	null	4,37E-08	5,39E-06	5,138648
		CB_FB_180416_Prep1_Tryp_GluC_3.10482.10482.5.0.dta	5	2275,320514	AKLSQIR(2)-GVLPKHATNVMR(5)	2275,306604	null	1,05E-08	1,26E-05	6,113462
		CB_FB_180416_Prep1_Tryp_GluC_3.9111.9111.4.0.dta	4	2291,312125	AKLSQIR(2)-GVLPKHATNVMR(5)	2291,301518	Oxidation[M](11)	6,34E-06	4,94E-02	4,629247
		CB_FB_180416_Prep1_Tryp_GluC_3.10378.10378.4.0.dta	4	2275,318867	AKLSQIR(2)-GVLPKHATNVMR(5)	2275,306604	null	1,54E-09	1,33E-01	5,389603
		CB_FB_180416_Prep1_Tryp_GluC_3.9117.9117.3.0.dta	3	2291,308453	AKLSQIR(2)-GVLPKHATNVMR(5)	2291,301518	Oxidation[M](11)	3,74E-11	1,97E-01	3,026664
sp P55347-2 PKNX1_HUMAN (140)-sp P55347-2 PKNX1_HUMAN (151)			2							
		CB_FB_180416_Prep1_Tryp_GluC_3.11965.11965.3.0.dta	3	2219,118045	LCKDFCSR(3)-YIACLTK(6)	2219,102025	Carbamidomethyl[C](2);Carbamidomethyl[C](6);Carbamidomethyl[C](15)	4,45E-10	2,28E-04	7,219136
		CB_FB_180416_Prep1_Tryp_GluC_3.11951.11951.4.0.dta	4	2219,117154	LCKDFCSR(3)-YIACLTK(6)	2219,102025	Carbamidomethyl[C](2);Carbamidomethyl[C](6);Carbamidomethyl[C](15)	4,25E-07	1,79E-02	6,817623
sp P40424 PBX1_HUMAN (65)-sp P55347-2 PKNX1_HUMAN (134)			7							
		CB_FB_180416_Prep1_Tryp_GluC_3.6041.6041.4.0.dta	4	1903,987187	KHALNCHR(1)-LEKVNE(3)	1903,980601	Carbamidomethyl[C](6)	8,86E-05	2,83E-04	3,459069
		CB_FB_180416_Prep1_Tryp_GluC_3.5852.5852.4.0.dta	4	1903,988696	KHALNCHR(1)-LEKVNE(3)	1903,980601	Carbamidomethyl[C](6)	1,42E-05	4,64E-04	4,251619
		CB_FB_180416_Prep1_Tryp_GluC_3.6247.6247.4.0.dta	4	1903,992024	KHALNCHR(1)-LEKVNE(3)	1903,980601	Carbamidomethyl[C](6)	2,53E-05	8,60E-04	5,999536
		CB_FB_180416_Prep1_Tryp_GluC_3.6148.6148.4.0.dta	4	1903,989296	KHALNCHR(1)-LEKVNE(3)	1903,980601	Carbamidomethyl[C](6)	7,15E-05	2,07E-03	4,566748
		CB_FB_180416_Prep1_Tryp_GluC_3.6454.6454.4.0.dta	4	1903,987391	KHALNCHR(1)-LEKVNE(3)	1903,980601	Carbamidomethyl[C](6)	8,19E-05	3,38E-03	3,566213
		CB_FB_180416_Prep1_Tryp_GluC_3.5854.5854.3.0.dta	3	1903,987774	KHALNCHR(1)-LEKVNE(3)	1903,980601	Carbamidomethyl[C](6)	5,41E-06	4,19E-03	3,76737
		CB_FB_180416_Prep1_Tryp_GluC_3.6859.6859.4.2.dta	4	1903,989531	KHALNCHR(1)-LEKVNE(3)	1903,980601	Carbamidomethyl[C](6)	1,98E-05	1,12E-02	4,690174
sp P40424 PBX1_HUMAN (87)-sp P55347-2 PKNX1_HUMAN (25)			1							
		CB_FB_180416_Prep1_Tryp_GluC_3.11184.11184.3.0.dta	3	1686,972671	KTVLSIR(1)-LKTEQD(2)	1686,963545	null	2,04E-05	7,15E-04	5,40972
sp P40424 PBX1_HUMAN (74)-sp P40424 PBX1_HUMAN (195)			1							
		CB_FB_180416_Prep1_Tryp_GluC_3.17071.17071.3.0.dta	3	2402,264905	MKPALFNVLC(2)-TRPISPKE(7)	2402,245703	Carbamidomethyl[C](10);Oxidation[M](1)	5,00E-13	3,83E-03	7,993354
sp P40424 PBX1_HUMAN (195)-sp P55347-2 PKNX1_HUMAN (268)			1							
		CB_FB_180416_Prep1_Tryp_GluC_3.9166.9166.5.0.dta	5	2801,557108	TRPISPKE(7)-GVLPKHATNVMR(5)	2801,545307	Oxidation[M](11)	2,37E-08	1,81E-02	4,212318
sp P40424 PBX1_HUMAN (153)-sp P55347-2 PKNX1_HUMAN (151)			1							
		CB_FB_180416_Prep1_Tryp_GluC_3.11216.11216.4.0.dta	4	1949,137576	AKLSQIR(2)-YIACLTK(6)	1949,125135	Carbamidomethyl[C](4)	6,73E-06	5,36E-02	6,382864
sp P40424 PBX1_HUMAN (65)-sp P55347-2 PKNX1_HUMAN (25)			1							
		CB_FB_180416_Prep1_Tryp_GluC_3.6023.6023.3.0.dta	3	2905,338727	KHALNCHR(1)-LKTEQDPNCSEPAE(2)	2905,320197	Carbamidomethyl[C](9);Carbamidomethyl[C](24)	4,19E-17	2,90E-01	6,377954
sp P40424 PBX1_HUMAN (153)-sp P55347-2 PKNX1_HUMAN (153)			1							
		CB_FB_180416_Prep1_Tryp_GluC_3.7547.7547.3.0.dta	3	1677,891223	AKLSQIR(2)-TKMNSE(2)	1677,883916	Oxidation[M](13)	3,92E-04	3,55E-01	4,35489

Table legend

Protein
 Title
 Spectrum Number
 Charge
 Precursor Mass
 Peptide
 Peptide Mass
 Modifications
 Evaluate
 Score
 Precursor Mass Error

Accession number of the proteins involved in cross linking; the site position of the xl-Lys on the protein is reported in brackets
 File name .dat (and corresponding .tif) of the ms2 annotated spectrum (as reported in the repository)
 Frequency of the ms2 spectra
 Charge of the fragmented ion
 Observed mass from the precursor ion (Da)
 Sequence of cross-linked peptide pair, the site position of the Lys involved in cross-linking is reported in brackets
 Theoretical mass of cross-linked peptide (Da)
 Cys Carbamidomethyl or Met Oxidation
 pLink score
 precursor mass error in ppm

Criteria applied for manual evaluation of spectra confidently identified = E-value < 0,001 (corresponding to FDR < 0,05); the majority of the ions of the spectrum are assigned and 4 consecutive b or y ions for each xl peptide pair observed

Supplementary Table S7: PREP1-PBX1 looped peptides Experiment #2

Protein	Title	Spectrum Number	Precursor Mass (Da)	Peptide	Peptide Mass (Da)	Modifications	Evalue	Score	Precursor Mass Error (ppm)
		Charge						pLINK	
sp P55347-2 PKNX1_HUMAN (260)(262)		9							
	CB_FB_180416_Prep1_Tryp_GluC_3.10137.10137.2.0.dta	2	1835,968731	LSILHQDDGSSKNKR(12)(14)	1835,960913	null	4,06E-79	9,32E-17	4,258261
	CB_FB_180416_Prep1_Tryp_GluC_3.9933.9933.2.0.dta	2	1835,968955	LSILHQDDGSSKNKR(12)(14)	1835,960913	null	3,28E-43	8,73E-16	4,380268
	CB_FB_180416_Prep1_Tryp_GluC_3.9926.9926.2.0.dta	2	1835,968956	LSILHQDDGSSKNKR(12)(14)	1835,960913	null	2,62E-45	2,04E-14	4,380812
	CB_FB_180416_Prep1_Tryp_GluC_3.10070.10070.3.0.dta	3	1835,969641	LSILHQDDGSSKNKR(12)(14)	1835,960913	null	2,13E-32	4,98E-13	4,753914
	CB_FB_180416_Prep1_Tryp_GluC_3.10308.10308.2.1.dta	2	1835,966195	LSILHQDDGSSKNKR(12)(14)	1835,960913	null	1,15E-39	1,37E-12	2,876968
	CB_FB_180416_Prep1_Tryp_GluC_3.9827.9827.2.0.dta	2	1835,968925	LSILHQDDGSSKNKR(12)(14)	1835,960913	null	3,81E-22	1,35E-10	4,363927
	CB_FB_180416_Prep1_Tryp_GluC_3.10421.10421.2.1.dta	2	1835,972486	LSILHQDDGSSKNKR(12)(14)	1835,960913	null	2,17E-20	2,18E-06	6,303511
	CB_FB_180416_Prep1_Tryp_GluC_3.3614.3614.2.0.dta	2	1029,536995	DGSSKNKR(5)(7)	1029,532299	null	3,30E-11	2,04E-05	4,561294
	CB_FB_180416_Prep1_Tryp_GluC_3.2982.2982.2.0.dta	2	914,508000	GSSKNKR(4)(6)	914,505361	null	6,42E-08	4,85E-04	2,890087
sp P55347-2 PKNX1_HUMAN (102)(105)		7							
	CB_FB_180416_Prep1_Tryp_GluC_3.16715.16715.2.0.dta	2	1620,733702	KEGPFFCEDPE(1)(4)	1620,724967	Carbamidomethyl[C](8)	7,42E-13	9,39E-13	5,389563
	CB_FB_180416_Prep1_Tryp_GluC_3.17175.17175.2.0.dta	2	1620,735116	KEGPFFCEDPE(1)(4)	1620,724967	Carbamidomethyl[C](8)	2,28E-12	3,77E-08	6,262012
	CB_FB_180416_Prep1_Tryp_GluC_3.17040.17040.2.0.dta	2	1620,7374	KEGPFFCEDPE(1)(4)	1620,724967	Carbamidomethyl[C](8)	1,78E-16	5,57E-08	7,671258
	CB_FB_180416_Prep1_Tryp_GluC_3.16874.16874.2.0.dta	2	1620,737029	KEGPFFCEDPE(1)(4)	1620,724967	Carbamidomethyl[C](8)	2,62E-14	1,37E-06	7,442348
	CB_FB_180416_Prep1_Tryp_GluC_3.16794.16794.2.0.dta	2	1620,733904	KEGPFFCEDPE(1)(4)	1620,724967	Carbamidomethyl[C](8)	4,84E-16	3,36E-06	5,514199
	CB_FB_180416_Prep1_Tryp_GluC_3.17331.17331.2.1.dta	2	1620,735117	KEGPFFCEDPE(1)(4)	1620,724967	Carbamidomethyl[C](8)	1,64E-13	3,83E-04	6,295331
	CB_FB_180416_Prep1_Tryp_GluC_3.17230.17230.2.2.dta	2	1620,734928	KEGPFFCEDPE(1)(4)	1620,724967	Carbamidomethyl[C](8)	1,12E-15	3,98E-03	6,146015
sp P40424 PBX1_HUMAN (292)(293)		1							
	CB_FB_180416_Prep1_Tryp_GluC_3.8063.8063.2.0.dta	2	988,587947	YKKNIGK(2)(3)	988,582546	null	4,04E-17	1,41E-11	5,463378
sp P40424 PBX1_HUMAN (85)(87)		1							
	CB_FB_180416_Prep1_Tryp_GluC_3.14434.14434.2.0.dta	2	1324,826938	IKEKTVLSIR(2)(4)	1324,819797	null	1,10E-30	8,95E-10	5,390167

Table legend

Protein Accession number of the proteins involved in cross linking; the site position of the xl-Lys on the protein is reported in brackets
 Title File name .dat (and corresponding .tif) of the ms2 annotated spectrum (as reported in the repository)
 Spectrum Number Frequency of the ms2 spectra
 Charge Charge of the fragmented ion
 Precursor Mass Observed mass from the precursor ion (Da)
 Peptide Sequence of cross-linked peptide pair, the site position of the Lys involved in cross-linking is reported in brackets
 Peptide Mass Theoretical mass of cross-linked peptide (Da)
 Modifications Cys Carbamidomethyl or Met Oxidation
 Evalue
 Score pLink score
 Precursor Mass Error precursor mass error in ppm

Criteria applied for manual evaluation of spectra confidently identified = E-value < 0,001 (corresponding to FDR < 0,05); the majority of the ions of the spectrum are assigned and 4 consecutive b or y ions for each xl peptide pair observed

Supplementary Table S8: MEIS1-PBX1 cross-linked peptides Experiment #1

Protein	Title	Spectrum Number	Precursor Mass	Peptide	Peptide Mass	Modifications	Evalue	Score	Precursor Mass Error
			(Da)		(Da)			pLINK	(ppm)
		Charge							
sp O00470 MEIS1_HUMAN (195)-sp P40424 PBX1_HUMAN (153)		3							
	CB_FB_180212_Meis1_Tryp_GluC_1.10186.10186.3.0.dta	3	2204,156338	GGGSKSDSEDIR(4)-AKLSQIR(2)	2204,151609	null	5,30E-10	2,95E-10	2,145497
	CB_FB_180212_Meis1_Tryp_GluC_1.10350.10350.3.0.dta	3	2333,20422	EGGSKSDSEDIR(5)-AKLSQIR(2)	2333,194197	null	7,48E-08	1,20E-09	4,295828
sp P40424 PBX1_HUMAN (153)-sp P40424 PBX1_HUMAN (242)	CB_FB_180212_Meis1_Tryp_GluC_1.12142.12142.2.0.dta	2	1904,037031	AKLSQIR(2)-NFNKQATE(4)	1904,023512	null	4,64E-16	1,04E-08	7,100227
	CB_FB_180212_Meis1_Tryp_GluC_1.10613.10613.4.0.dta	4	2060,126643	AKLSQIR(2)-RNFKQATE(5)	2060,124614	null	2,02E-06	2,45E-02	0,984892
		3							
sp O00470 MEIS1_HUMAN (195)-sp P40424 PBX1_HUMAN (242)	CB_FB_180212_Meis1_Tryp_GluC_1.10412.10412.3.0.dta	3	2469,137814	EGGSKSDSEDIR(5)-NFNKQATE(4)	2469,137469	null	3,56E-09	2,79E-05	0,139725
	CB_FB_180212_Meis1_Tryp_GluC_1.10433.10433.2.0.dta	2	2469,138872	EGGSKSDSEDIR(5)-NFNKQATE(4)	2469,137469	null	5,00E-04	1,24E-01	-1,456784
	CB_FB_180212_Meis1_Tryp_GluC_1.10274.10274.3.0.dta	3	2340,102758	GGGSKSDSEDIR(4)-NFNKQATE(4)	2340,094881	null	2,42E-10	6,81E-05	3,366103
sp O00470 MEIS1_HUMAN (178)-sp P40424 PBX1_HUMAN (153)		1							
	CB_FB_180212_Meis1_Tryp_GluC_1.12464.12464.3.0.dta	3	1921,09885	YISCLKGK(6)-AKLSQIR(2)	1921,093837	Carbamidomethyl[C](4)	1,35E-19	4,97E-12	2,609451
		1							
sp P40424 PBX1_HUMAN (153)-sp P40424 PBX1_HUMAN (308)	CB_FB_180212_Meis1_Tryp_GluC_1.13196.13196.3.0.dta	3	1702,990327	AKLSQIR(2)-ANIYAAK(7)	1702,984949	null	1,94E-20	1,53E-11	3,157984
		2							
	CB_FB_180212_Meis1_Tryp_GluC_1.6521.6521.5.0.dta	5	1927,002383	LEKVHE(3)-KHALNCHR(1)	1926,996585	Carbamidomethyl[C](6)	1,19E-06	7,50E-09	3,008827
sp O00470 MEIS1_HUMAN (195)-sp P40424 PBX1_HUMAN (308)	CB_FB_180212_Meis1_Tryp_GluC_1.6538.6538.3.0.dta	3	1927,005073	LEKVHE(3)-KHALNCHR(1)	1926,996585	Carbamidomethyl[C](6)	7,21E-09	1,88E-08	4,404782
	CB_FB_180212_Meis1_Tryp_GluC_1.11496.11496.3.0.dta	1							
		3	2268,109023	EGGSKSDSEDIR(5)-ANIYAAK(7)	2268,098907	null	2,57E-16	1,27E-08	4,460123
sp P40424 PBX1_HUMAN (297)-sp P40424 PBX1_HUMAN (308)	CB_FB_180212_Meis1_Tryp_GluC_1.15874.15874.2.0.dta	1							
	CB_FB_180212_Meis1_Tryp_GluC_1.15874.15874.2.0.dta	2	1851,960175	NIGKFQEE(4)-ANIYAAK(7)	1851,948622	null	2,73E-06	1,15E-05	6,238294
		1							
sp O00470 MEIS1_HUMAN (178)-sp O00470 MEIS1_HUMAN (195)	CB_FB_180212_Meis1_Tryp_GluC_1.10734.10734.4.2.dta	4	2357,175313	YISCLKGK(6)-GGGSKSDSEDIR(4)	2357,165206	Carbamidomethyl[C](19)	5,96E-07	8,33E-05	4,287778
		1							
	CB_FB_180212_Meis1_Tryp_GluC_1.12715.12715.3.0.dta	3	2482,166163	EGGSKSDSEDIR(5)-NIGKFQEE(4)	2482,15787	null	3,02E-07	3,53E-03	3,341045
sp O00470 MEIS1_HUMAN (126)-sp O00470 MEIS1_HUMAN (132)		1							
	CB_FB_180212_Meis1_Tryp_GluC_1.18959.18959.3.1.dta	3	2516,350802	DIAVFAKQIR(7)-AEKPLFSSNPE(3)	2516,3394	null	2,36E-08	2,21E-02	4,531185

Table legend

Protein	Accession number of the proteins involved in cross linking; the site position of the xl-Lys on the protein is reported in brackets
Title	File name .dat (and corresponding .tif) of the ms2 annotated spectrum (as reported in the repository)
Spectrum Number	Frequency of the ms2 spectra
Charge	Charge of the fragmented ion
Precursor Mass	Observed mass from the precursor ion (Da)
Peptide	Sequence of cross-linked peptide pair, the site position of the Lys involved in cross-linking is reported in brackets
Peptide Mass	Theoretical mass of cross-linked peptide (Da)
Modifications	Cys Carbamidomethyl or Met Oxidation
Evalue	
Score	pLink score
Precursor Mass Error	precursor mass error in ppm

Criteria applied for manual evaluation of spectra confidently identified = E-value < 0,001 (corresponding to FDR < 0,05); the majority of the ions of the spectrum are assigned and 4 consecutive b or y ions for each xl peptide pair observed

Supplementary Table S9: MEIS1-PBX1 looped peptides Experiment #1

Protein	Title	Spectrum Number	Precursor Mass	Peptide	Peptide Mass	Modifications	Evalue	Score	Precursor Mass Error
			(Da)		(Da)			pLINK	(ppm)
		Charge							
sp P40424 PBX1_HUMAN(292)(293)		10							
	CB_FB_180212_Meis1_Tryp_GluC_1.14086.14086.2.0.dta	2	1521,799292	YKKNIKGKFQEE(2)(3)	1521,794702	null	1,55E-41	7,83E-16	3,016176
	CB_FB_180212_Meis1_Tryp_GluC_1.9800.9800.2.0.dta	2	988,584694	YKKNIKG(2)(3)	988,582546	null	3,44E-15	2,18E-13	2,172808
	CB_FB_180212_Meis1_Tryp_GluC_1.9708.9708.2.0.dta	2	988,584559	YKKNIKG(2)(3)	988,582546	null	1,66E-19	5,37E-13	2,036249
	CB_FB_180212_Meis1_Tryp_GluC_1.9896.9896.2.0.dta	2	988,585122	YKKNIKG(2)(3)	988,582546	null	1,39E-15	2,00E-12	2,605751
	CB_FB_180212_Meis1_Tryp_GluC_1.9931.9931.2.1.dta	2	988,586181	YKKNIKG(2)(3)	988,582546	null	4,90E-17	7,44E-11	3,676982
	CB_FB_180212_Meis1_Tryp_GluC_1.14223.14223.2.0.dta	2	1392,762496	YKKNIKGKFQEE(2)(3)	1392,752114	null	3,74E-35	1,91E-11	7,454306
	CB_FB_180212_Meis1_Tryp_GluC_1.10317.10317.2.0.dta	2	1257,771428	IRYKKNIGK(4)(5)	1257,767706	null	1,18E-19	5,78E-11	2,959211
	CB_FB_180212_Meis1_Tryp_GluC_1.10345.10345.3.0.dta	3	1257,770542	IRYKKNIGK(4)(5)	1257,767706	null	1,50E-16	1,52E-09	2,254788
	CB_FB_180212_Meis1_Tryp_GluC_1.13716.13716.2.0.dta	2	1790,984771	IRYKKNIGKFQEE(4)(5)	1790,979862	null	8,64E-42	1,24E-09	2,740958
	CB_FB_180212_Meis1_Tryp_GluC_1.13704.13704.3.0.dta	3	1790,984642	IRYKKNIGKFQEE(4)(5)	1790,979862	null	1,55E-46	1,54E-08	2,66893
sp P40424 PBX1_HUMAN(293)(297)		2							
	CB_FB_180212_Meis1_Tryp_GluC_1.14784.14784.2.0.dta	2	1521,799196	YKKNIKGKFQEE(3)(7)	1521,794702	null	1,37E-11	2,43E-06	2,953092
	CB_FB_180212_Meis1_Tryp_GluC_1.15713.15713.2.0.dta	2	1230,642114	KNIGKFQEE(1)(5)	1230,636423	null	3,46E-09	9,67E-05	4,624437
sp O00470 MEIS1_HUMAN(270)(271)		5							
	CB_FB_180212_Meis1_Tryp_GluC_1.3713.3713.2.0.dta	2	1024,580156	PDKDKKR(5)(6)	1024,578521	null	1,29E-22	2,73E-14	1,595778
	CB_FB_180212_Meis1_Tryp_GluC_1.3695.3695.3.0.dta	3	1024,579677	PDKDKKR(5)(6)	1024,578521	null	1,88E-15	4,06E-14	1,128269
	CB_FB_180212_Meis1_Tryp_GluC_1.3874.3874.3.0.dta	3	1024,578817	PDKDKKR(5)(6)	1024,578521	null	2,04E-16	2,60E-12	0,288899
	CB_FB_180212_Meis1_Tryp_GluC_1.4435.4435.3.1.dta	3	1024,579647	PDKDKKR(5)(6)	1024,578521	null	5,08E-13	1,74E-09	1,098988
	CB_FB_180212_Meis1_Tryp_GluC_1.4439.4439.2.0.dta	2	1024,580785	PDKDKKR(5)(6)	1024,578521	null	2,11E-15	7,60E-09	2,209689

Table legend

Protein	Accession number of the proteins involved in cross linking; the site position of the xl-Lys on the protein is reported in brackets
Title	File name .dat (and corresponding .tif) of the ms2 annotated spectrum (as reported in the repository)
Spectrum Number	Frequency of the ms2 spectra
Charge	Charge of the fragmented ion
Precursor Mass	Observed mass from the precursor ion (Da)
Peptide	Sequence of cross-linked peptide pair, the site position of the Lys involved in cross-linking is reported in brackets
Peptide Mass	Theoretical mass of cross-linked peptide (Da)
Modifications	Cys Carbamidomethyl or Met Oxidation
Evalue	
Score	pLink score
Precursor Mass Error	precursor mass error in ppm

Criteria applied for manual evaluation of spectra confidently identified = E-value < 0,001 (corresponding to FDR < 0,05); the majority of the ions of the spectrum are assigned and 4 consecutive b or y ions for each xl peptide pair observed

Supplementary Table S10: MEIS1-PBX1 cross-linked peptides Experiment #2

Protein	Title	Spectrum Number	Precursor Mass	Peptide	Peptide Mass	Modifications	Evalue	Score	Precursor Mass Error
		(Da)		(Da)				pLINK	(ppm)
Charge									
8									
sp Q00470 MEIS1_HUMAN(195)-sp P40424 PBX1_HUMAN(153)	CB_FB_180416_Meis1_Tryp_GluC_7.8044.8044.3.0.dta	3	2333.204641	EGGSKSDSEDITR(5)-AKLSQIR(2)	2333.194197	null	9.12E-14	2.54E-17	4.476267
	CB_FB_180416_Meis1_Tryp_GluC_7.8131.8131.4.0.dta	4	2333.204031	EGGSKSDSEDITR(5)-AKLSQIR(2)	2333.194197	null	4.01E-12	8.35E-15	4.214823
	CB_FB_180416_Meis1_Tryp_GluC_7.8034.8034.4.0.dta	4	2333.203856	EGGSKSDSEDITR(5)-AKLSQIR(2)	2333.194197	null	1.24E-14	7.58E-14	4.139818
	CB_FB_180416_Meis1_Tryp_GluC_7.8024.8024.4.0.dta	4	2333.203901	EGGSKSDSEDITR(5)-AKLSQIR(2)	2333.194197	null	5.45E-12	1.98E-06	4.159105
	CB_FB_180416_Meis1_Tryp_GluC_7.7868.7868.4.0.dta	4	2204.163668	GGSKSDSEDITR(4)-AKLSQIR(2)	2204.151609	null	6.80E-09	6.21E-15	5.471039
	CB_FB_180416_Meis1_Tryp_GluC_7.7933.7933.4.1.dta	4	2204.163795	GGSKSDSEDITR(4)-AKLSQIR(2)	2204.151609	null	1.90E-03	3.54E-01	5.528658
	CB_FB_180416_Meis1_Tryp_GluC_7.7342.7342.3.0.dta	3	1847.941575	EGGSKSDE(5)-AKLSQIR(2)	1847.934425	null	5.00E-03	3.44E-05	3.869185
	CB_FB_180416_Meis1_Tryp_GluC_7.7158.7158.3.0.dta	3	1718.898544	GGGSKSDE(4)-AKLSQIR(2)	1718.891837	null	9.49E-04	3.64E-05	3.901933
sp Q00470 MEIS1_HUMAN(195)-sp P40424 PBX1_HUMAN(242)		4							
	CB_FB_180416_Meis1_Tryp_GluC_7.8109.8109.3.0.dta	3	2469.14994	EGGSKSDSEDITR(5)-NFNKQATE(4)	2469.137469	null	3.05E-07	8.12E-07	5.050752
	CB_FB_180416_Meis1_Tryp_GluC_7.7999.7999.3.0.dta	3	2340.107503	GGGSKSDSEDITR(4)-NFNKQATE(4)	2340.094881	null	1.70E-04	4.90E-05	5.393798
	CB_FB_180416_Meis1_Tryp_GluC_7.6996.6996.3.0.dta	3	2625.258863	GGGSKSDSEDITR(5)-RNFKQATE(5)	2625.238571	null	2.36E-06	3.20E-04	7.729583
	CB_FB_180416_Meis1_Tryp_GluC_7.6823.6823.4.0.dta	4	2496.208006	GGGSKSDSEDITR(4)-RNFKQATE(5)	2496.195983	null	1.01E-05	1.73E-03	4.816529
sp P40424 PBX1_HUMAN(87)-sp P40424 PBX1_HUMAN(195)		9							
	CB_FB_180416_Meis1_Tryp_GluC_7.10344.10344.5.0.dta	5	2279.355629	KTVLISR(1)-TRPISPKIER(7)	2279.344428	null	4.68E-14	6.69E-20	4.914132
	CB_FB_180416_Meis1_Tryp_GluC_7.10313.10313.4.0.dta	4	2279.35591	KTVLISR(1)-TRPISPKIER(7)	2279.344428	null	1.44E-10	3.01E-14	5.037413
	CB_FB_180416_Meis1_Tryp_GluC_7.10396.10396.4.0.dta	4	2279.356188	KTVLISR(1)-TRPISPKIER(7)	2279.344428	null	1.93E-08	1.14E-12	5.159378
	CB_FB_180416_Meis1_Tryp_GluC_7.10321.10321.3.0.dta	3	2279.356111	KTVLISR(1)-TRPISPKIER(7)	2279.344428	null	1.07E-13	4.81E-07	5.125597
	CB_FB_180416_Meis1_Tryp_GluC_7.9873.9873.3.0.dta	3	1881.127616	KTVLISR(1)-TRPISPKIER(7)	1881.116681	null	2.27E-17	3.10E-19	5.813037
	CB_FB_180416_Meis1_Tryp_GluC_7.9960.9960.3.0.dta	3	1881.12797	KTVLISR(1)-TRPISPKIER(7)	1881.116681	null	8.58E-10	7.88E-18	6.001223
	CB_FB_180416_Meis1_Tryp_GluC_7.9896.9896.4.0.dta	4	1881.127158	KTVLISR(1)-TRPISPKIER(7)	1881.116681	null	2.48E-09	3.55E-14	5.569564
	CB_FB_180416_Meis1_Tryp_GluC_7.9916.9916.4.0.dta	4	1881.127146	KTVLISR(1)-TRPISPKIER(7)	1881.116681	null	1.09E-04	6.54E-06	5.563185
	CB_FB_180416_Meis1_Tryp_GluC_7.11441.11441.3.0.dta	3	2123.256909	KTVLISR(1)-TRPISPKIER(7)	2123.243326	null	3.64E-14	2.81E-14	6.397288
sp P40424 PBX1_HUMAN(242)-sp P40424 PBX1_HUMAN(308)		3							
1 CB_FB_180416_Meis1_Tryp_GluC_7.10682.10682.2.0.dta	2	1838.942311	NFNKQATE(4)-ANIYAAK(7)	1838.928221	null	4.63E-09	4.20E-13	7.662072	
5 CB_FB_180416_Meis1_Tryp_GluC_7.10994.10994.2.0.dta	2	1967.983086	NFNKQATE(4)-EANIYAAK(8)	1967.970809	null	7.38E-09	1.31E-12	6.238406	
6 CB_FB_180416_Meis1_Tryp_GluC_7.9248.9248.2.0.dta	2	1995.036839	RNFNKKQATE(5)-ANIYAAK(7)	1995.029323	null	3.88E-10	7.07E-10	3.767363	
sp Q00470 MEIS1_HUMAN(195)-sp P40424 PBX1_HUMAN(308)		2							
1 CB_FB_180416_Meis1_Tryp_GluC_7.8812.8812.3.0.dta	3	2139.067974	GGGSKSDSEDITR(4)-ANIYAAK(7)	2139.056319	null	5.26E-12	3.28E-19	5.401915	
2 CB_FB_180416_Meis1_Tryp_GluC_7.9005.9005.3.0.dta	3	2268.109765	GGGSKSDSEDITR(5)-ANIYAAK(7)	2268.098907	null	3.00E-10	2.53E-12	4.787269	
sp P40424 PBX1_HUMAN(153)-sp P40424 PBX1_HUMAN(308)		3							
1 CB_FB_180416_Meis1_Tryp_GluC_7.10384.10384.3.0.dta	3	1702.996098	AKLSQIR(2)-ANIYAAK(7)	1702.984949	null	1.77E-18	1.07E-18	6.54674	
2 CB_FB_180416_Meis1_Tryp_GluC_7.10395.10395.2.0.dta	2	1702.996694	AKLSQIR(2)-ANIYAAK(7)	1702.984949	null	5.50E-10	1.16E-16	6.896714	
3 CB_FB_180416_Meis1_Tryp_GluC_7.10643.10643.3.2.dta	3	1832.028783	AKLSQIR(2)-EANIYAAK(8)	1832.027537	null	2.21E-16	2.76E-07	6.138554	
sp P40424 PBX1_HUMAN(153)-sp P40424 PBX1_HUMAN(242)		5							
1 CB_FB_180416_Meis1_Tryp_GluC_7.9535.9535.2.0.dta	2	1904.036346	AKLSQIR(2)-RNFKQATE(4)	1904.023512	null	2.41E-09	4.92E-14	6.740463	
2 CB_FB_180416_Meis1_Tryp_GluC_7.9520.9520.3.0.dta	3	1904.035873	AKLSQIR(2)-RNFKQATE(4)	1904.023512	null	6.43E-09	1.47E-10	6.492042	
4 CB_FB_180416_Meis1_Tryp_GluC_7.8182.8182.3.0.dta	3	2060.136096	AKLSQIR(2)-RNFKQATE(5)	2060.124614	null	3.43E-08	2.50E-10	5.573449	
5 CB_FB_180416_Meis1_Tryp_GluC_7.8167.8167.3.0.dta	3	2060.136096	AKLSQIR(2)-RNFKQATE(5)	2060.124614	null	3.23E-04	4.26E-09	5.573449	
6 CB_FB_180416_Meis1_Tryp_GluC_7.8190.8190.4.0.dta	4	2060.136032	AKLSQIR(2)-RNFKQATE(5)	2060.124614	null	5.39E-05	1.00E-08	5.542383	
sp Q00470 MEIS1_HUMAN(195)-sp P40424 PBX1_HUMAN(87)		2							
1 CB_FB_180416_Meis1_Tryp_GluC_7.9528.9528.4.0.dta	4	2334.228502	EGGSKSDSEDITR(5)-KTVLISR(1)	2334.214597	null	3.20E-12	2.44E-12	5.957036	
2 CB_FB_180416_Meis1_Tryp_GluC_7.8044.8044.3.1.dta	3	2334.207154	GGGSKSDSEDITR(4)-EKTVLISR(2)	2334.214597	null	6.23E-02	6.74E-02	-3.188653	
sp P40424 PBX1_HUMAN(195)-sp P40424 PBX1_HUMAN(308)		4							
1 CB_FB_180416_Meis1_Tryp_GluC_7.9408.9408.3.0.dta	3	1815.0174	TRPISPKIE(7)-ANIYAAK(7)	1815.00099	null	2.20E-12	2.29E-09	9.041317	
2 CB_FB_180416_Meis1_Tryp_GluC_7.9432.9432.2.0.dta	2	1815.015919	TRPISPKIE(7)-ANIYAAK(7)	1815.00099	null	3.23E-08	1.43E-08	8.224789	
3 CB_FB_180416_Meis1_Tryp_GluC_7.9874.9874.4.1.dta	4	2213.238917	TRPISPKIE(7)-ANIYAAK(7)	2213.228738	null	1.26E-06	4.44E-07	4.599163	
4 CB_FB_180416_Meis1_Tryp_GluC_7.9890.9890.3.0.dta	3	2213.241247	TRPISPKIE(7)-ANIYAAK(7)	2213.228738	null	9.52E-11	1.96E-04	5.651924	
sp Q00470 MEIS1_HUMAN(126)-sp Q00470 MEIS1_HUMAN(132)		1							
2 CB_FB_180416_Meis1_Tryp_GluC_7.15195.15195.3.0.dta	3	2516.346402	DIAVFAKQIR(7)-AEKPLFSSNP(3)	2516.3394	null	1.23E-05	1.63E-03	2.782614	
sp P40424 PBX1_HUMAN(87)-sp P40424 PBX1_HUMAN(153)		2							
1 CB_FB_180416_Meis1_Tryp_GluC_7.10616.10616.4.0.dta	4	1769.111238	KTVLISR(1)-AKLSQIR(2)	1769.10064	null	9.51E-09	2.66E-18	5.990615	
2 CB_FB_180416_Meis1_Tryp_GluC_7.10622.10622.3.0.dta	3	1769.112603	KTVLISR(1)-AKLSQIR(2)	1769.10064	null	5.04E-09	4.61E-18	6.762193	
sp P40424 PBX1_HUMAN(87)-sp P40424 PBX1_HUMAN(242)		2							
1 CB_FB_180416_Meis1_Tryp_GluC_7.11056.11056.3.0.dta	3	1905.056021	KTVLISR(1)-NFNKQATE(4)	1905.043912	null	1.78E-08	2.06E-14	6.356284	
2 CB_FB_180416_Meis1_Tryp_GluC_7.11069.11069.2.0.dta	2	1905.05776	KTVLISR(1)-NFNKQATE(4)	1905.043912	null	2.00E-07	2.54E-14	7.269124	
sp Q00470 MEIS1_HUMAN(161)-sp P40424 PBX1_HUMAN(308)		1							
1 CB_FB_180416_Meis1_Tryp_GluC_7.9617.9617.3.0.dta	3	1641.89188	LEKVH(3)-ANIYAAK(7)	1641.884573	null	1.74E-14	7.81E-12	4.450374	
sp Q00470 MEIS1_HUMAN(161)-sp Q00470 MEIS1_HUMAN(195)		1							
1 CB_FB_180416_Meis1_Tryp_GluC_7.7254.7254.4.1.dta	4	2272.10574	LEKVH(3)-EGGSKSDSEDITR(5)	2272.093821	null	5.50E-05	3.74E-09	5.245822	

Table legend

Protein
Title
Spectrum Number
Charge
Precursor Mass
Peptide
Peptide Mass
Modifications
Evaluate
Score
Precursor Mass Error

Accession number of the proteins involved in cross linking; the site position of the xi-Lys on the protein is reported in brackets
File name .dat (and corresponding .tif) of the ms2 annotated spectrum (as reported in the repository)
Frequency of the ms2 spectra
Charge of the fragmented ion
Observed mass from the precursor ion [Da]
Sequence of cross-linked peptide pair, the site position of the Lys involved in cross-linking is reported in brackets
Theoretical mass of cross-linked peptide [Da]
Cys Carbamidomethyl or Met Oxidation
pLink score
precursor mass error in ppm

Criteria applied for manual evaluation of spectra confidently identified = E-value < 0.001 (corresponding to FDR < 0.05); the majority of the ions of the spectrum are assigned and 4 consecutive b or y ions for each xi peptide pair observed

Supplementary Table S11: MEIS1-PBX1 looped peptides Experiment #2

Protein	Title	Spectrum Number	Precursor Mass	Peptide	Peptide Mass	Modifications	Evalue	Score	Precursor Mass Error
		Charge	(Da)		(Da)		pLINK		(ppm)
sp P40424 PBX1_HUMAN (85)(87)		1							
	CB_FB_180416_Meis1_Tryp_GluC_7.12585.12585.2.0.dta	2	1324,829242	IKEKTVLISR(2)(4)	1324,819797	null	3,42E-11	1,27E-03	7,129271
sp P40424 PBX1_HUMAN (292)(293)		1							
	CB_FB_180416_Meis1_Tryp_GluC_7.7278.7278.2.0.dta	2	988,587026	YKKNIGK(2)(3)	988,582546	null	1,24E-16	5,26E-03	4,531741
sp Q00470 MEIS1_HUMAN (305)(306)		1							
	CB_FB_180416_Meis1_Tryp_GluC_7.8586.8586.2.1.dta	2	1096,607915	QKKQLAQD(2)(3)	1096,599648	null	5,46E-07	4,05E-02	7,538759

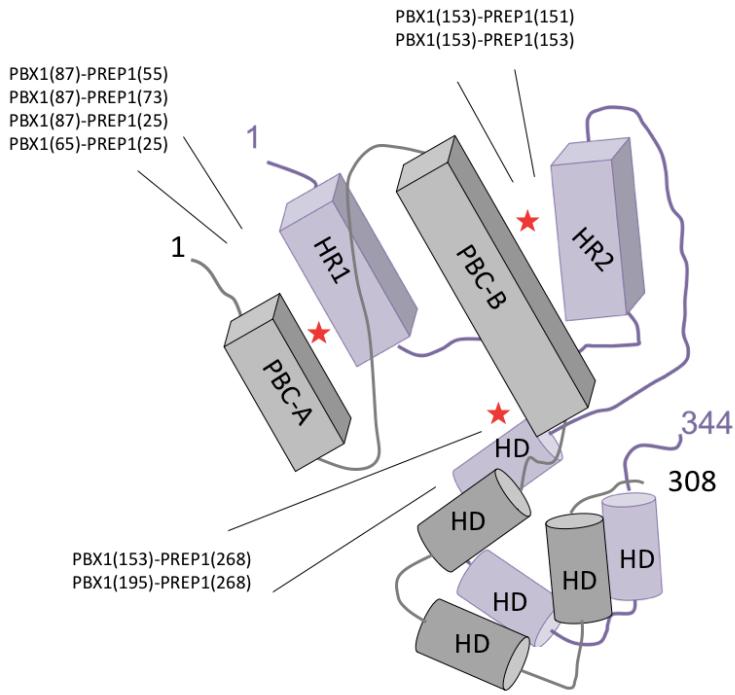
Table legend

Protein	Accession number of the proteins involved in cross linking; the site position of the xl-Lys on the protein is reported in brackets
Title	File name .dat (and corresponding .tif) of the ms2 annotated spectrum (as reported in the repository)
Spectrum Number	Frequency of the ms2 spectra
Charge	Charge of the fragmented ion
Precursor Mass	Observed mass from the precursor ion (Da)
Peptide	Sequence of cross-linked peptide pair, the site position of the Lys involved in cross-linking is reported in brackets
Peptide Mass	Theoretical mass of cross-linked peptide (Da)
Modifications	Cys Carbamidomethyl or Met Oxidation
Evalue	
Score	pLink score
Precursor Mass Error	precursor mass error in ppm

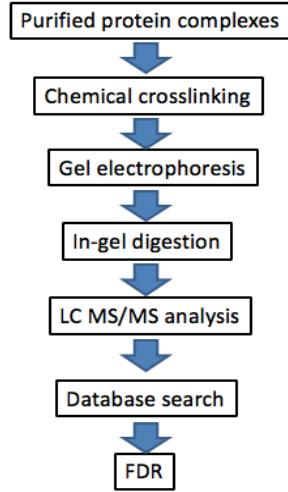
Criteria applied for manual evaluation of spectra confidently identified = E-value < 0,001 (corresponding to FDR < 0,05); the majority of the ions of the spectrum are assigned and 4 consecutive b or y ions for each xl peptide pair observed

Supplementary S1

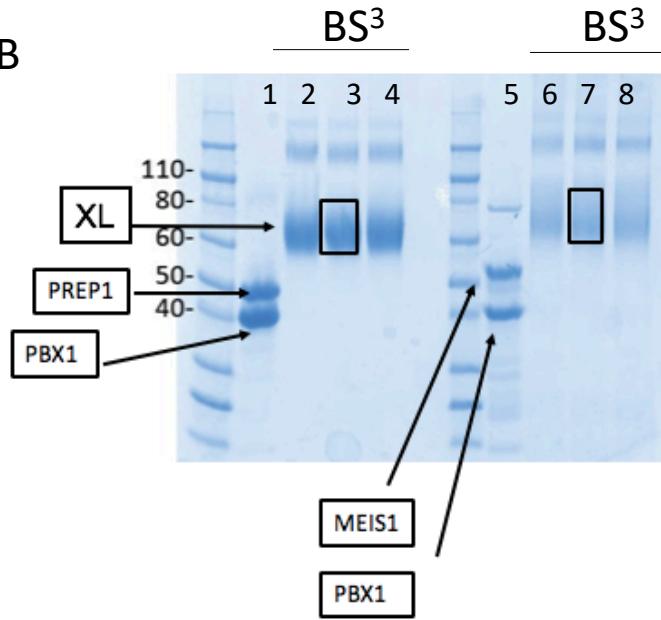
C



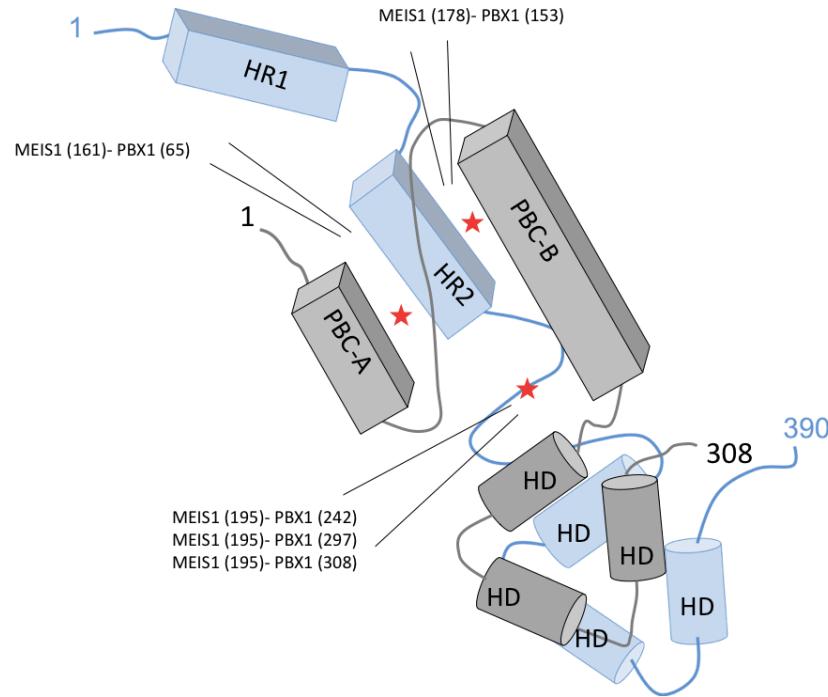
A



B



D



A

PREP1

B MEIS1

Sequence alignment diagram showing the alignment of MAQRYYDDLPHGGMDGVGIPSTM... and ATCTPREPGVAGGDVCSSSFNEDIAVFAKQIRAEKPLF... with their respective PDB structures. The alignment highlights several conserved regions with blue boxes and specific residues with blue asterisks. Secondary structure elements are shown as blue lines above the sequences.

PREP1

HR1
52-79

HR2
110-154

HR3
242-252

HD
257-325

MEIS1

HR1
71-95

HR2
137-180

HD
275-338

PBX1

PBC-A
45-122

PBC-B
147-234

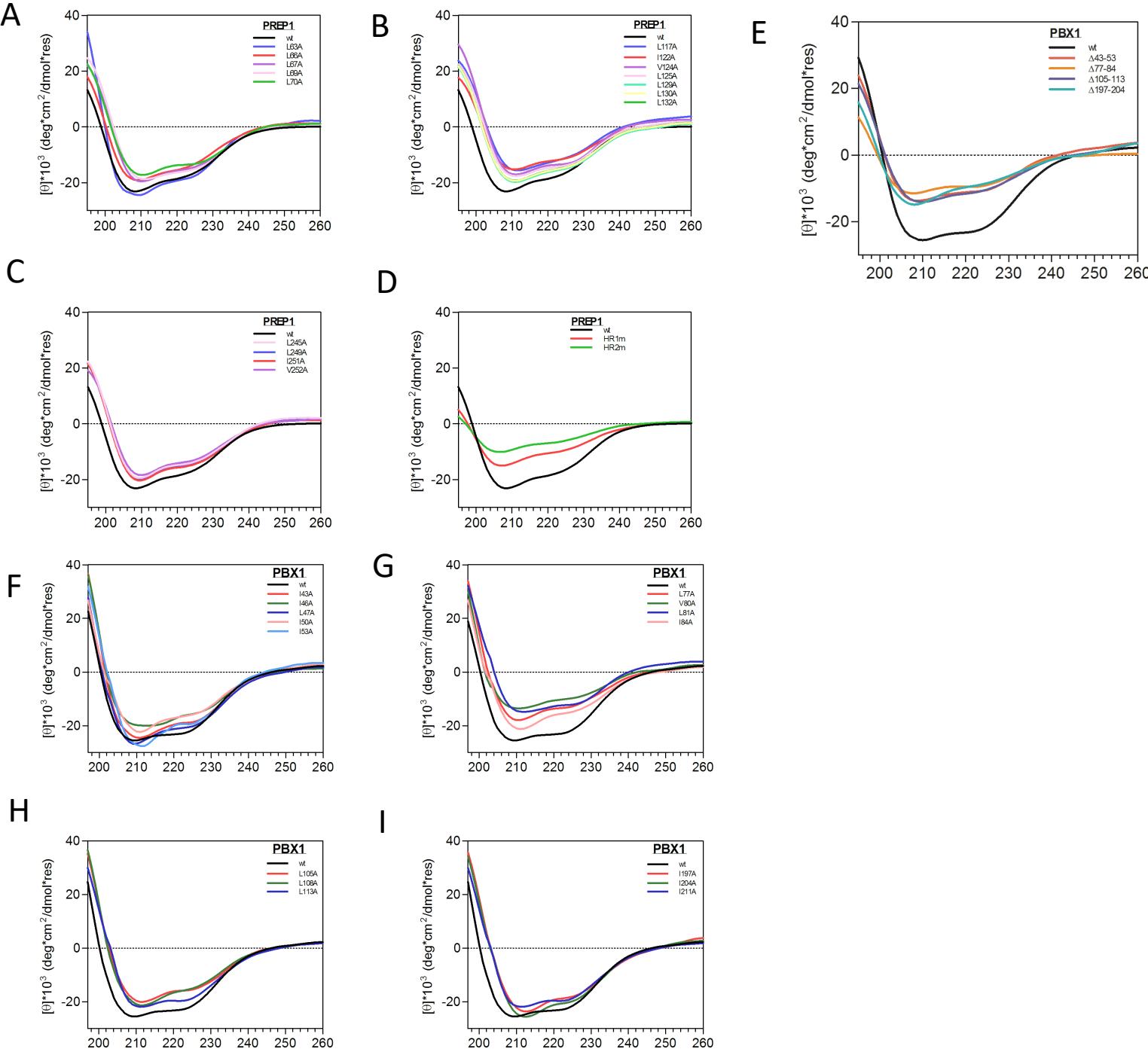
HD
245-305

C

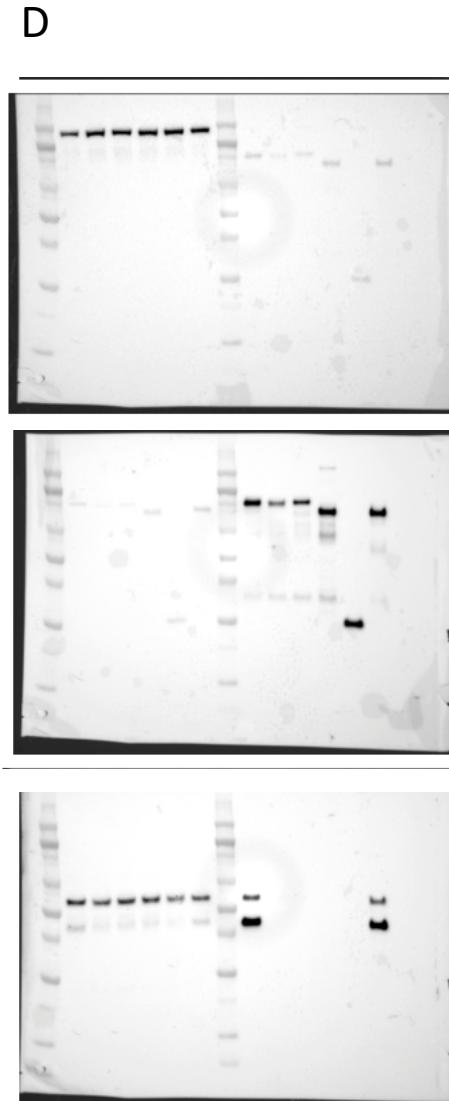
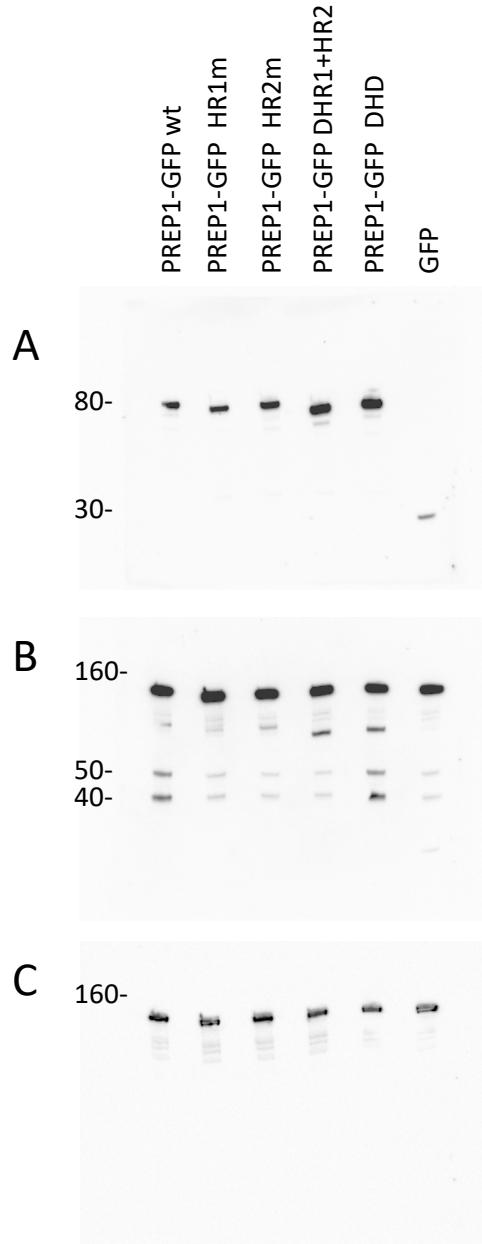
*K involved in intra and inter cross-links

*K involved in looped cross-links

Supplementary S3



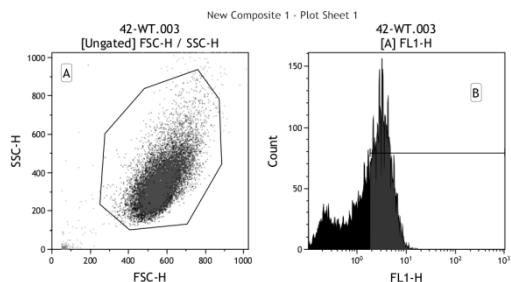
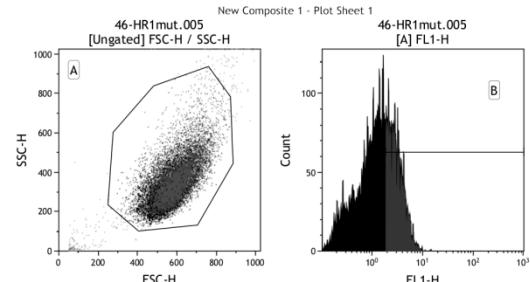
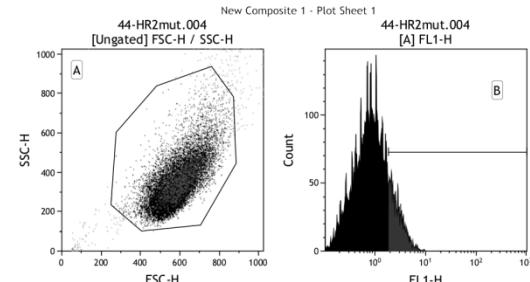
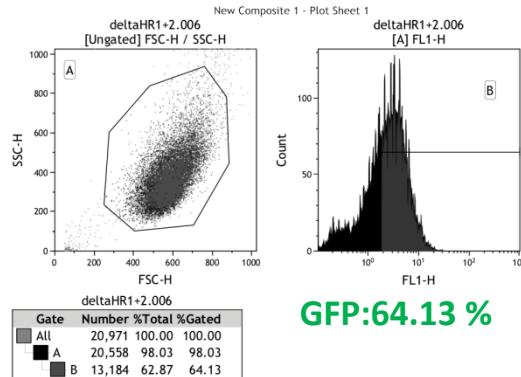
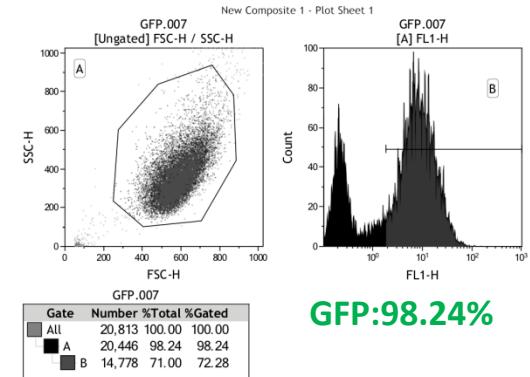
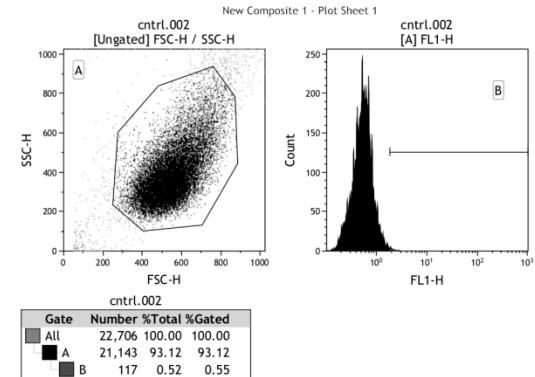
Supplementary S4



Anti vinculin

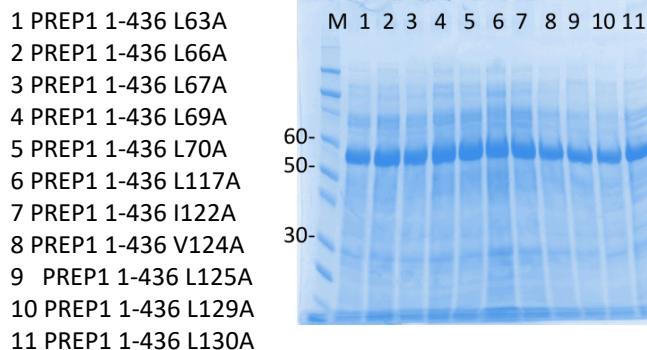
Anti-GFP

Anti-PBX1

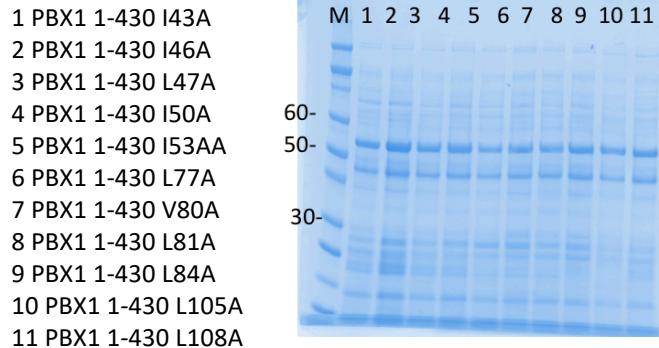
A**A549 GFP-PREP1 wt****B****A549 GFP-PREP1 HR1m****C****A549 GFP-PREP1 HR2m****D****A549 GFP-PREP1 Δ HR1+HR2****E****A549 GFP****F****A549 control**

Supplementary S6

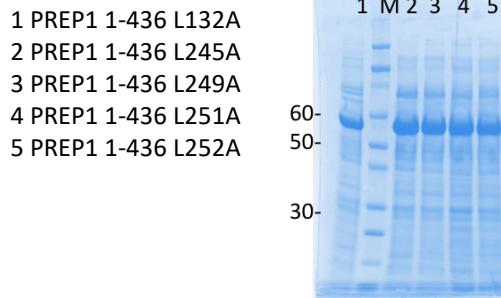
A



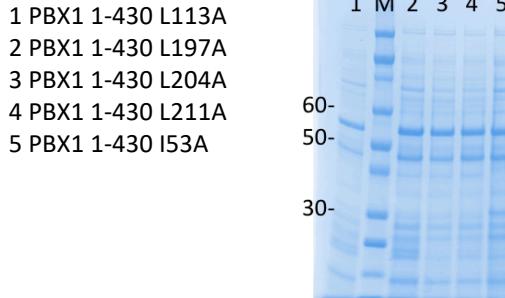
B



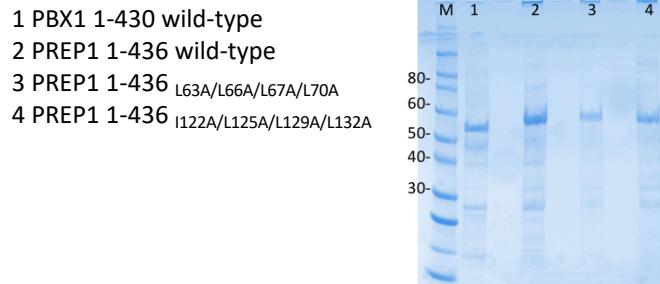
C



D



E



F

1 MEIS1 1-390

