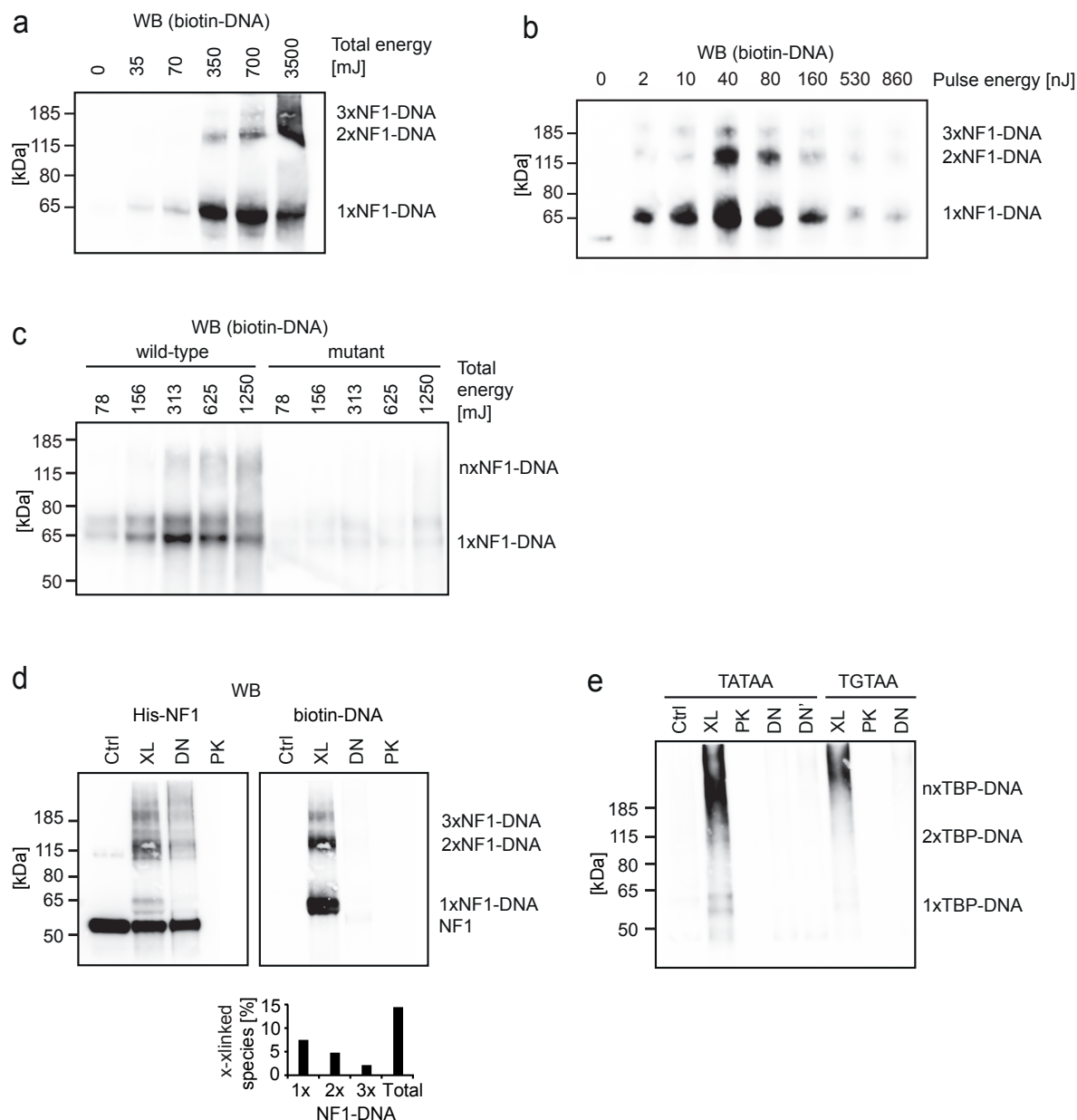


Supplementary Information

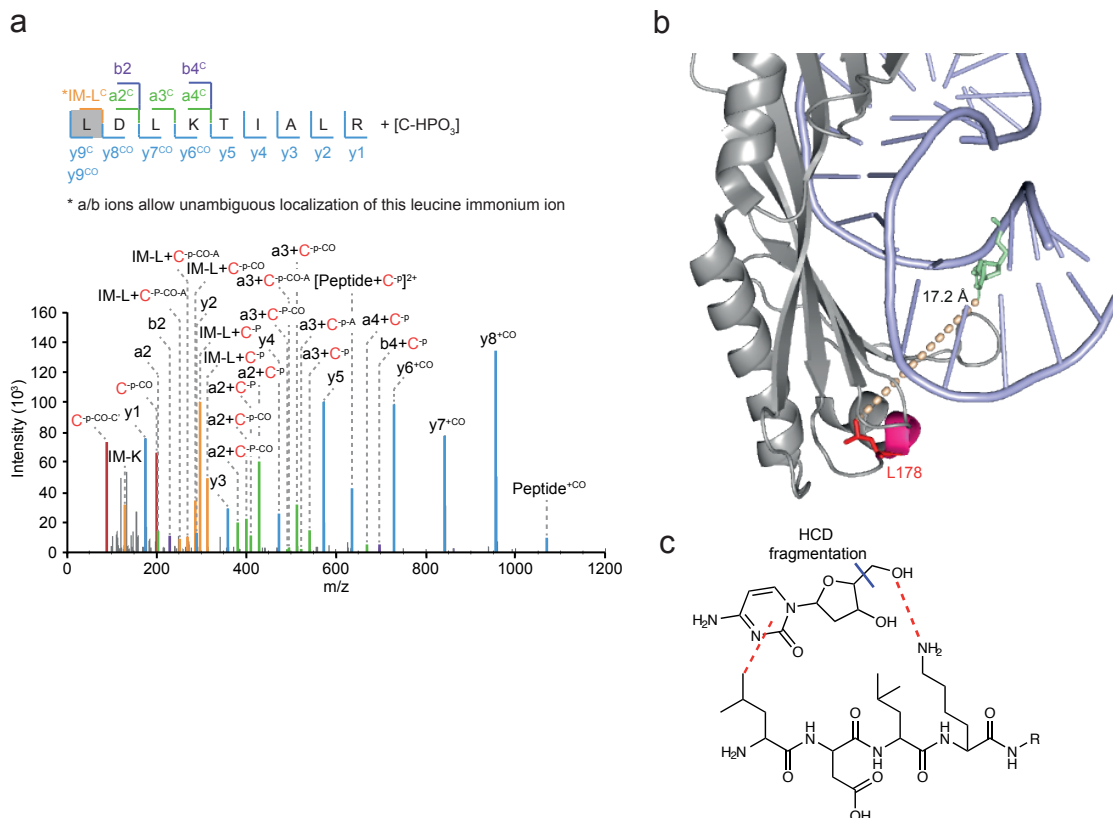
Reim et al.: 'Atomic-resolution mapping of transcription factor-DNA interactions by femtosecond laser crosslinking and mass spectrometry'



Supplementary Figure 1: Assessment of crosslinking efficiencies. **a**, Titration of total energy with constant pulse energy of 7 nJ. Full scale version of Western-blot depicted in Fig. 2b. **b**, Titration of pulse energy with constant total energy of 1J. Full scale version of Western-blot depicted in Fig. 2c. **c**, NF1-DNA crosslinking is specific for a wild type consensus motif. Full scale version of Western-blot depicted in Fig. 2d. **d**, Western blot probed by anti-His antibody (left) or Streptavidin-HRP conjugate (right) following membrane stripping. Full scale version of Western-blot depicted in Fig. 2e. **e**, Percentage of crosslinked species were extrapolated from the mono-NF1 band reflecting 7.5% of all NF1 (see Fig. 2e) according to their relative intensities in the biotin-DNA blot. **f**, Anti biotin-DNA Western blots of UV-crosslinked TBP-DNA complex with wild type (TATAA) or point-mutated (TGTA) consensus motif. Full scale version of Western-blot depicted in Fig. 2f.

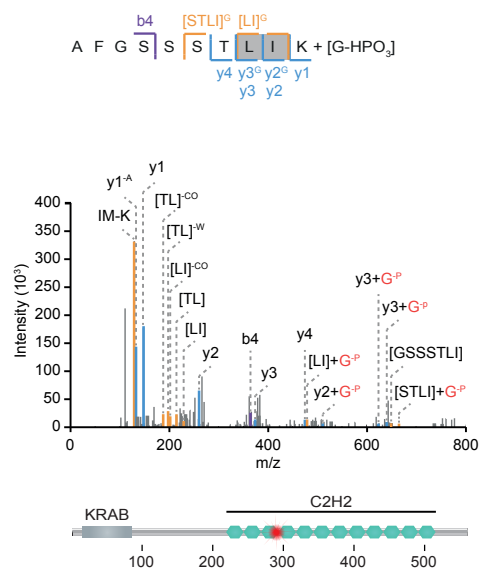
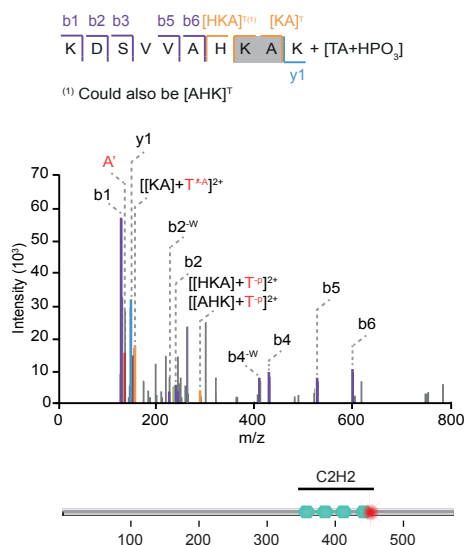
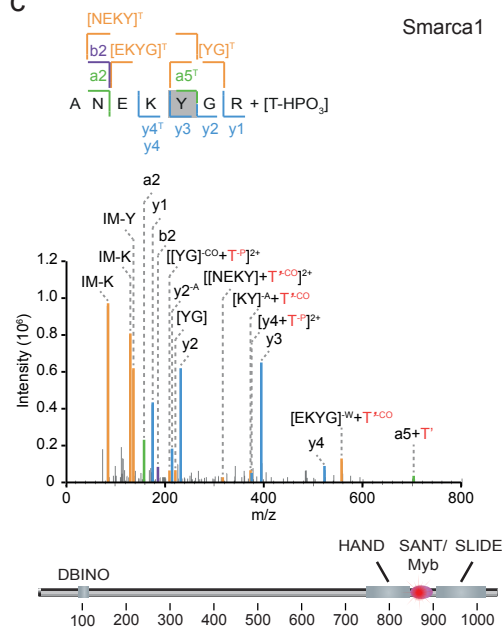
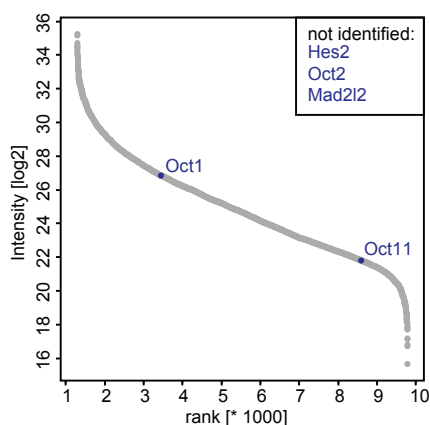
NFIC_PIG	1	MYSSPLCLTQDEFHFFIEALLPHVRAFAYTWFNLQARKRKYFKKHEKRMSKDEERAVKDE
NFIC_HUMAN	1	MYSSPLCLTQDEFHFFIEALLPHVRAFAYTWFNLQARKRKYFKKHEKRMSKDEERAVKDE
NFIC_RAT	1	MYSSPLCLTQDEFHFFIEALLPHVRAFAYTWFNLQARKRKYFKKHEKRMSKDEERAVKDE
NFIC_PIG	61	LLGEKAEVKQKWASRLAKL RK DIRPE C EDFVLA IT GKKAPGCVLSNP DQ KGKMRRIDC
NFIC_HUMAN	61	LLGEKAEVKQKWASRLAKL RK DIRPE C EDFVLS IT GKKAPGCVLSNP DQ KGKMRRIDC
NFIC_RAT	61	LLGEKAEVKQKWASRLAKL RK DIRPE C EDFVLA VT GKKAPGCVLSNP DQ KGKMRRIDC
NFIC_PIG	121	LR QADKVWRDLVMVILFKGIPL E TDGERLVKAAQCGH V LQVQPHHIGVAVKELDLYL
NFIC_HUMAN	121	LR QADKVWRDLVMVILFKGIPL E TDGERLVKAAQCGHPVLCVQPHHIGVAVKELDLYL
NFIC_RAT	121	LR QADKVWRDLVMVILFKGIPL E TDGERLVKAA A CAHPVLCVQPHHIGVAVKELDLYL
NFIC_PIG	181	AYFVRERDAEQSGSPRAGMGSDQEDSKPITLDTTDFQESFVTSGVFSVTELIQVSRTPVV
NFIC_HUMAN	181	AYFVRERDAEQSGSPRTGMGSDQEDSKPITLDTTDFQESFVTSGVFSVTELIQVSRTPVV
NFIC_RAT	181	AYFVRERDAEQSS S SPRTGVGSDQEDSKPITLDTTDFQESFVTSGVFSVTELIQVSRTPVV
NFIC_PIG	241	TGTGPNFSLGELQGHMAYDLNPASTGMRRLTPSTSSSGSKRHKSGSMEEDVDTSPGGDYY
NFIC_HUMAN	241	TGTGPNFSLGELQGHLAYDLNPASTGLRRTLTPSTSSSGSKRHKSGSMEEDVDTSPGGDYY
NFIC_RAT	241	TGTGPNFSLGELQGHLAYDLNPAS A GMRRLTPSTSSSGSKRHKSGSMEEDVDTSPGGDYY
NFIC_PIG	301	TSPSSPTSS N NRNWTEDMEGGISSPVKKTEMDKSPFNSPSPQDSPRLSSFTQHHRPVIADV
NFIC_HUMAN	301	TSPSSPTSSSRNWTEDMEGGISSPVKKTEMDKSPFNSPSPQDSPRLSSFTQHHRPVIADV
NFIC_RAT	301	TSP N SPTSSSRNWTEDMEGGISSPVKKTEMDKSPFNSPSPQDSPRLSSFTQHHRPVIADV
NFIC_PIG	361	SGIARSPHPSSALHFPTTSILPQTASTYFPHTAIRYPHNLNPQDPLKDLVSLACDPASQQ
NFIC_HUMAN	361	SGIARSPHPSSALHFPTTSILPQTASTYFPHTAIRYPHNLNPQDPLKDLVSLACDPASQQ
NFIC_RAT	361	SGIARSPHPSSALHFPA T ILPQTASTYFPHTAIRYPHNLNPQDPLKDLVSLACDPAT Q Q
NFIC_PIG	421	PGPLNGSGQLKMS S SHCLSAQMLAPPPGLPRLALPPATK P T--SEGGSSSPTSPSYST P FG
NFIC_HUMAN	421	PGPLNGSGQLKMPS S SHCLSAQMLAPPPGLPRLALPPATK P ATTSEGGATSPSPSYSPD
NFIC_RAT	421	PGEP A ----- L -RPARELQTV E L-----
NFIC_PIG	479	TSPANRSFVGLGPRDPTGIYQAQSWYLG
NFIC_HUMAN	481	TSPANRSFVGLGPRDPAGIYQAQSWYLG
NFIC_RAT	438	-----WD--

Supplementary Figure 2: Sequence alignment of Nuclear Factor 1/C in pig, human and rat. Crosslinked peptides of the pig Nuclear Factor 1/C are highlighted in pale blue. The crosslinked amino acid is indicated by a red star above the amino acid or above the bracket around a stretch of amino acids. Amino acids are depicted in red if their mutation was found to significantly reduce DNA binding in rat NF1¹.



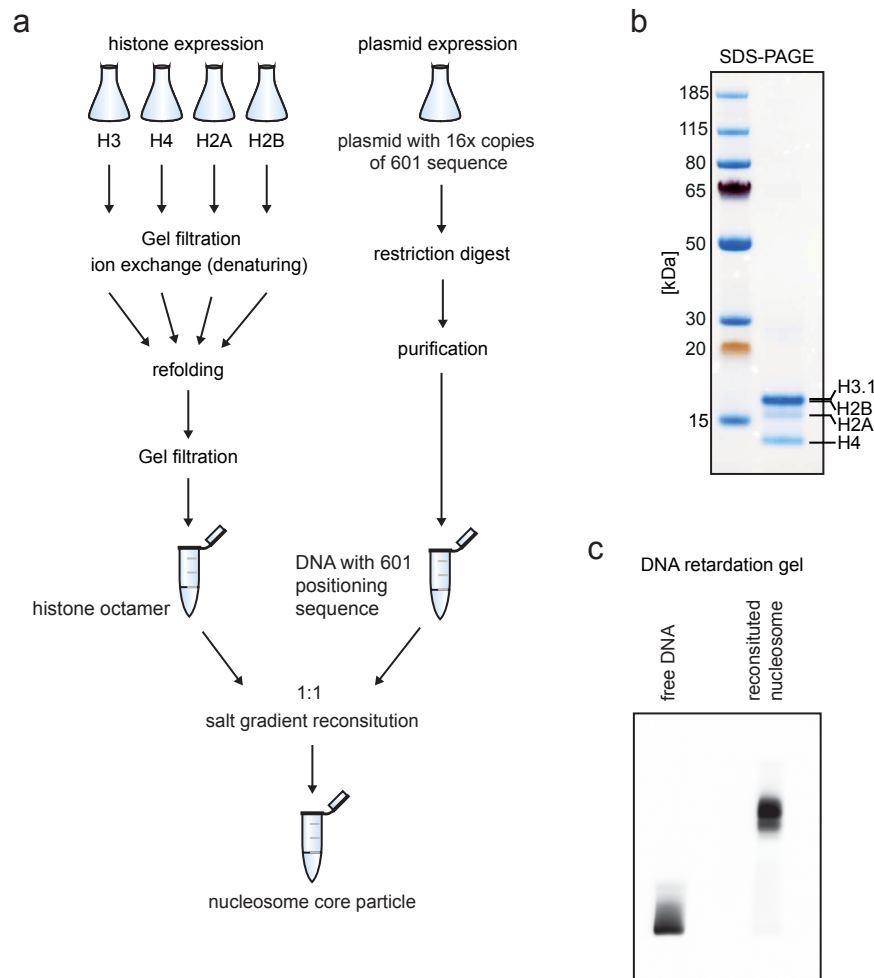
Supplementary Figure 3: Spectrum and structural information of the crosslinked LDLKTIALR peptide. **a**, MS2 ion series and spectra of the peptide LDLKTIALR crosslinked to a cytosine. Abbreviations in the MS2 spectra and MS2 ion series: +CO: adduct of carbon monoxide. Other abbreviations as in Fig. 4 and 5. **b**, Location of the crosslink of the amino acid L178 to deoxycytidine (light green) in the crystal structure (PDB ID: 1C9B²). The crosslinked amino acid is depicted in red and the peptide 178LDLKTIALR186 in pink. **c**, Proposed scheme of the double crosslink of L178 and K181 to the deoxycytidine and creation of the CO adduct on the y6-y9 ion series after HCD fragmentation as observed in Supplementary Figure 3a. The blue line indicates bond breakage after HCD fragmentation. Dashed, red lines represent the possible covalent bonds of the two amino acids to the nucleotide. R: amino acids TIALR.

PROX1_MOUSE	577	QEGLSPNHLKKAKLMFFFYTRYPSNNMLKTYFSVKNRCITSQLIKWFSNFREFYYIQME
PROX2_MOUSE	433	QEGLSPGHLKKAKLMFFFYTRYPSSSLKAYFPDQVFNRCITSQMIKWFSNFREFYYIQME
PROS_DROME	1546	S-TLTPMHLRKAALMFFWVRYPSSAVLKMYPFDIKFNKNNTAQLVKWFSNFREFYYIQME
PROX1_MOUSE	637	KYARQAINDGVSTSEELSTTRDCELYRALNMHYNKANDFEVPERFLEVAQITLREFFNAI
PROX2_MOUSE	493	KYARQALSDGITNAQALAVLRDSELFRLVNLTHYNGKDNFEVDPDFLETAALTKEFFRAV
PROS_DROME	1605	KYARQAVTEGIKTPDDLILAGDSELYRVLNLHYNRNNHIEVEPQNFREFYVESLREFFRAI
PROX1_MOUSE	697	IAGKDVDPSSWKKAIFYKVICKLDSEVPEIFKSPNCIQEILHE
PROX2_MOUSE	553	LAGKDSDPSSWKKPIYKVISKRLSDVPEMLKSPNFLGLFPS
PROS_DROME	1665	QGGKDEQSWKKSIYKIISRMDPEYKFSKSPFLQGL--E

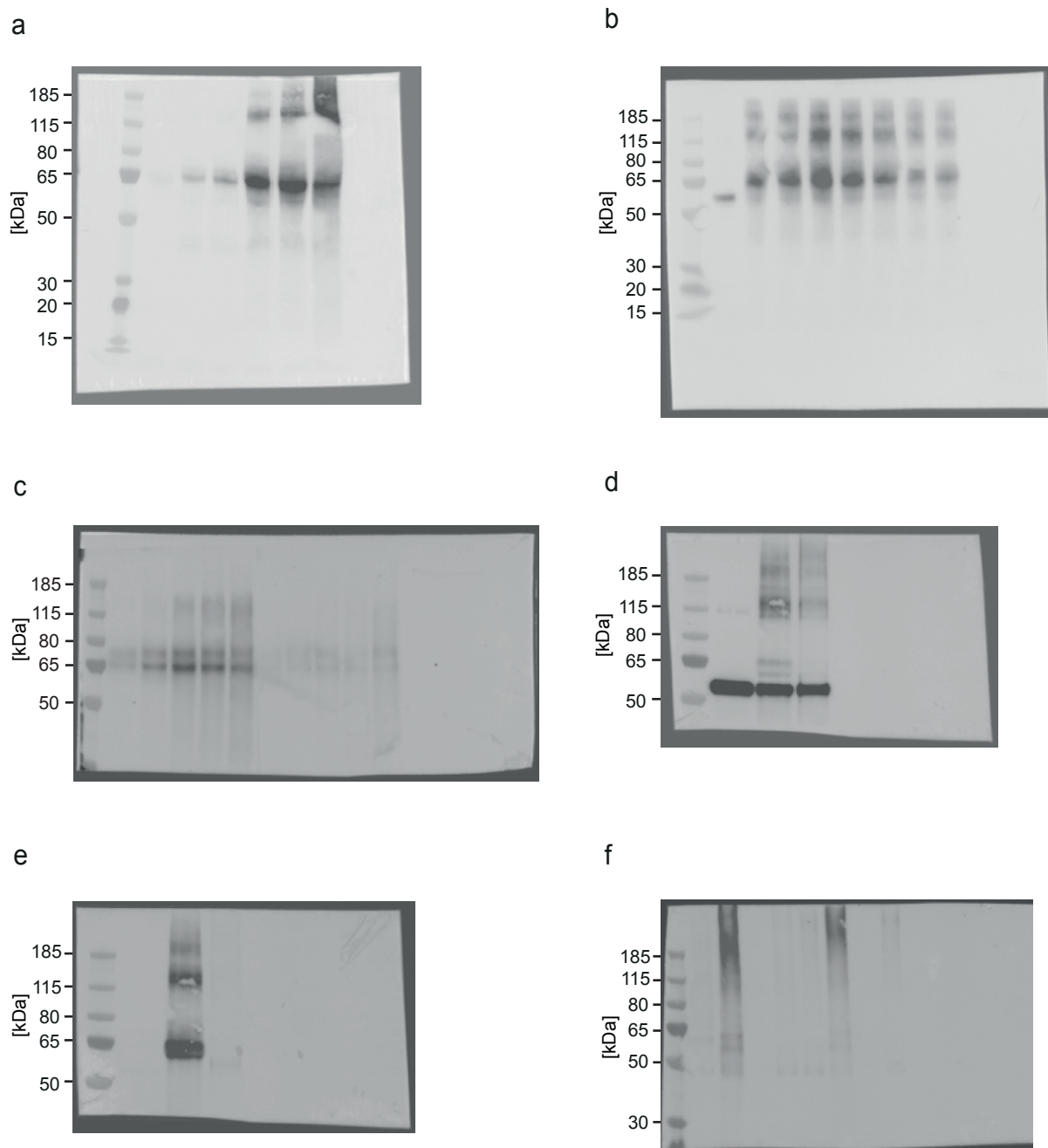


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peptide and the crosslinked amino acids are highlighted in green or with a red star, respectively. **b**, Ranked log₂ protein intensity of a deep proteomic analysis of mouse ES cells (E14TG2a) identifies expression of Oct1 and Oct11. **c-e**, MS/MS spectra of high-confidence crosslinks obtained from the in-vivo crosslinking experiment including the global transcription factor Smarca1 and the two zinc finger proteins Zfp91 and Znf354c. Abbreviations in the MS/MS spectra as in Fig. 4 and schematic representation of the crosslink location on the protein as in Fig. 6d-e.



Supplementary Figure 5: Reconstitution of recombinant human nucleosome core particles. **a**, Schematic workflow of experimental workflow to generate recombinant core particles adapted from ³⁻⁵. Individual histone proteins are expressed in *E. coli* and sequentially purified by Gel filtration and Strong Cation Exchange chromatography. Histones are combined and refolded followed by purification of histone octamers by Gel filtration. The DNA is generated as plasmid containing 16 copies of the strong nucleosome positioning 601 sequence. After restriction digest, individual fragments were purified, mixed together with the histone octamer in a 1:1 molar ratio, and nucleosome core particles reconstituted by dialysis applying a salt gradient. **b**, Reconstituted nucleosomes were separated on a SDS-PAGE and proteins visualized by Colloidal Blue Staining. **c**, Comparison of reconstituted nucleosome or the same amount of 601 DNA on a DNA retardation gel revealed 100% reconstitution efficiency.



Supplementary Figure 6: Original Western blots. **a**, Full scale, original version of Western-blot shown in Figure 2b and Supplementary Figure 1a. **b**, Full scale, original version of Western-blot depicted in Figure 2c and Supplementary Figure 1b. **c**, Full scale, original version of Western-blot shown in Figure 2d and Supplementary Figure 1c. **d**, Full scale, original version of anti His-NF1 Western-blot shown in Figure 2e (left blot) and Supplementary Figure 1d (left blot). **e**, Full scale, original version of anti biotin-DNA Western-blot shown in Figure 2e (right blot) and Supplementary Figure 1d (right blot). **f**, Full scale, original version of western blot probed by Streptavidin-HRP conjugate depicted in Figure 2f and Supplementary Figure 1e.

Supplementary References

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