Supporting Information

Immunoblot

Proteins were separated on 10% SDS gels and transferred to nitrocellulose membranes. After 30 min incubation in blocking solution (5% milk-PBS-TWEEN 0.1%), membranes were incubated overnight with anti-HA-HRP conjugated (clone HA-7, Sigma) dissolved in fresh blocking solution. Membranes were washed 3× in PBS-TWEEN 0.1% and developed using ECL Western Blotting detection reagent (GE-Healthcare, Little Chalfont, UK).

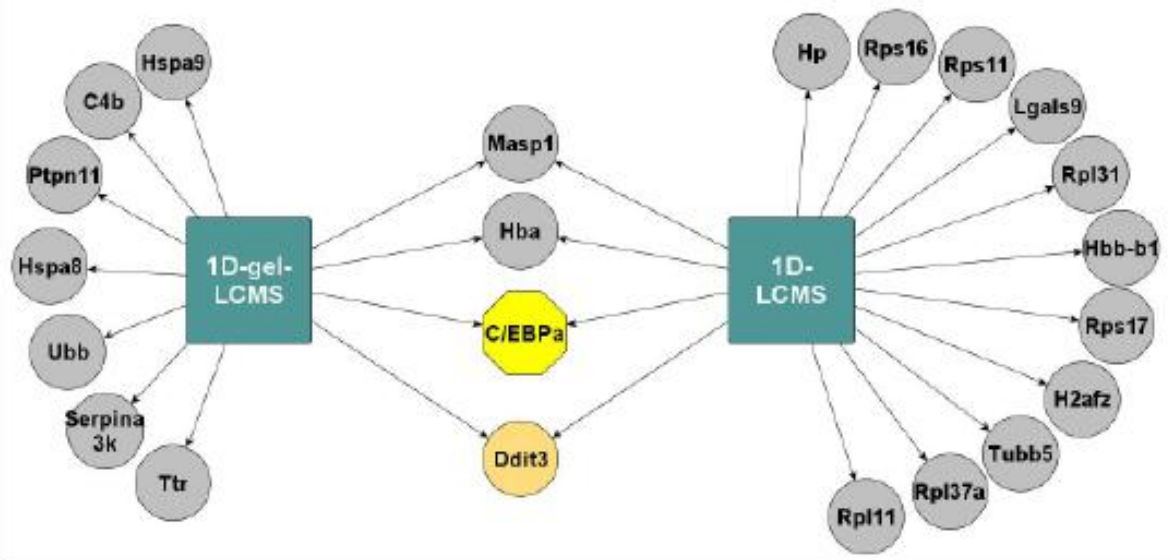
In vitro protein cross-linking

Tagged C/EBPalpha was purified using StrepTactin sepharose beads (IBA) and eluted with biotin as described in the material and methods section for the one-step affinity purification. Three percent of the eluted material was cross-linked with reagents of different solubility and spacer arm distance to reveal the formation of higher molecular mass complexes of C/EBPalpha. Increasing amounts of BS^3 (bis[sulfosuccinimidyl] suberate, water soluble, 11.4Å, Thermo Fisher Scientific Inc., Waltham, MA, USA); FA (formaldehyde, water soluble, 0Å, Sigma-Aldrich); DSG (disuccinimidyl glutarate, water insoluble, 7.7Å, Thermo Fisher Scientific Inc.) and DFDNB (1,5-difluoro-2,4-dinitrobenzene, water insoluble, 3Å, Thermo Fisher Scientifc Inc.) were assessed. The samples were then separated on a NuPAGE 3-8% Tris-acetate gel (Invitrogen) and C/EBPalpha was detected by anti HA-immunoblotting.

Strategy	Elution	Background proteins	Sensitivity	Resolution	Throughput
STREP-1D-gel-LCMS	biotin	ribosomal proteins	high	high	Low
STREP-1D-LCMS	biotin	carboxylase proteins	moderate	low	High
STREP-2D-LCMS	biotin	carboxylase proteins	high	high	moderate
HA-1D-gel-LCMS	SDS buffer	keratin proteins	high	moderate	Low
HA-1D-LCMS	formic acid	keratin proteins	low	low	High
HA-2D-LCMS	formic acid	Golgi-associated proteins	high	high	moderate

Table S1. Comparison of the six different affinity purification strategies described in the study. Advantages and disadvantages of each strategy are as indicated.

a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C4b
Ptpn11
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Ubb
Serpina 3k
Ttr
1D-gel-LCMS
Masp1
Hba
C/EBPa
Ddit3
1D-LCMS
Hp
Rps16
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Rpl31
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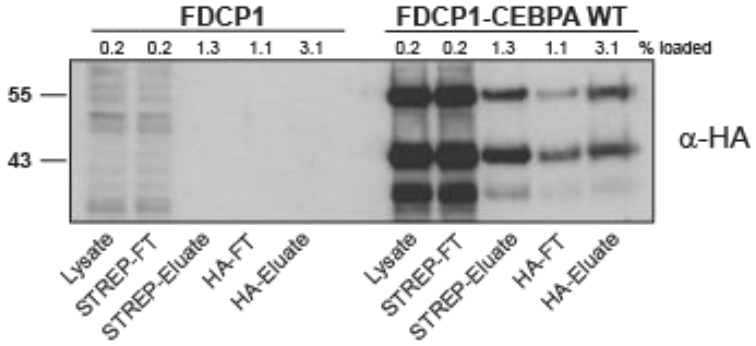
Rpl37a
Rpl11

b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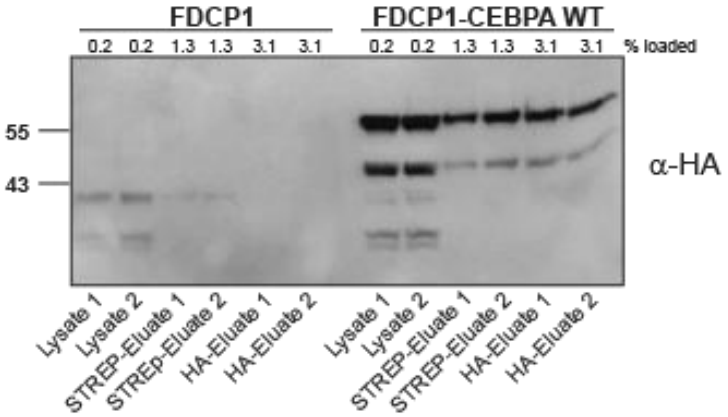
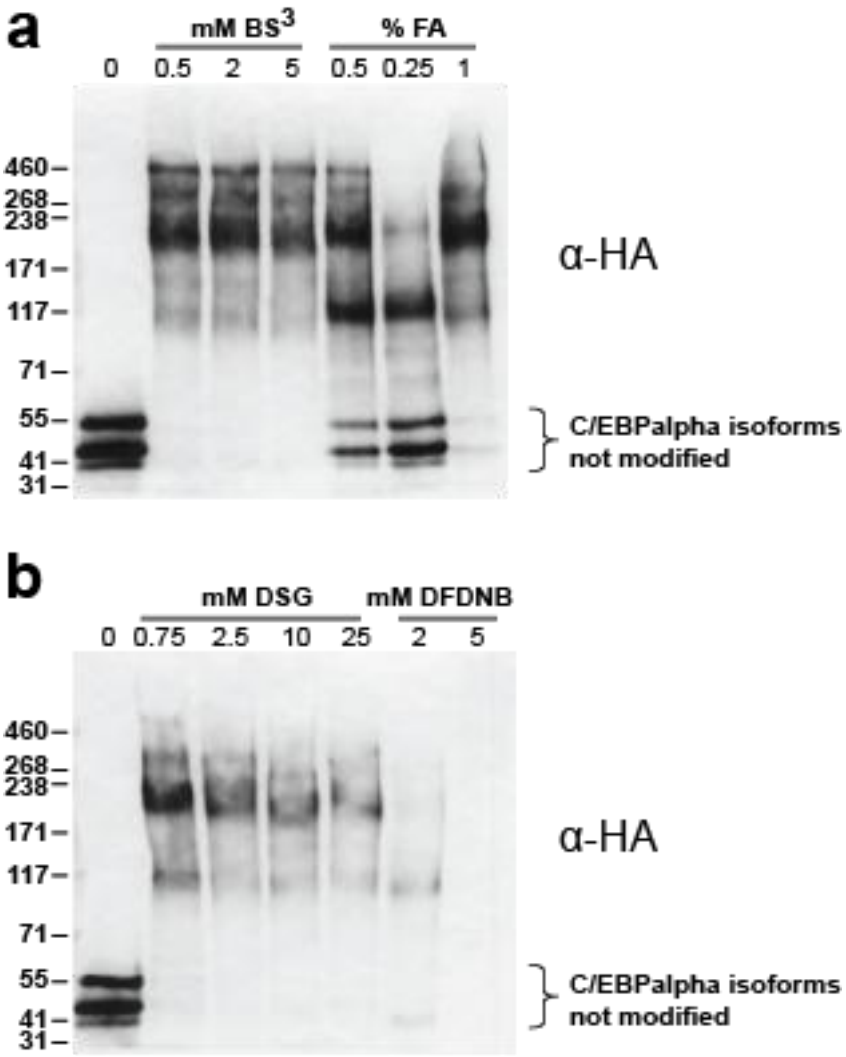
0.2 0.2 1.3 1.1 3.1
0.2 0.2 1.3 1.1 3.1 % loaded
55
43
α-HA
Lysate
STREP-FT
STREP-Eluate
HA-FT
HA-Eluate

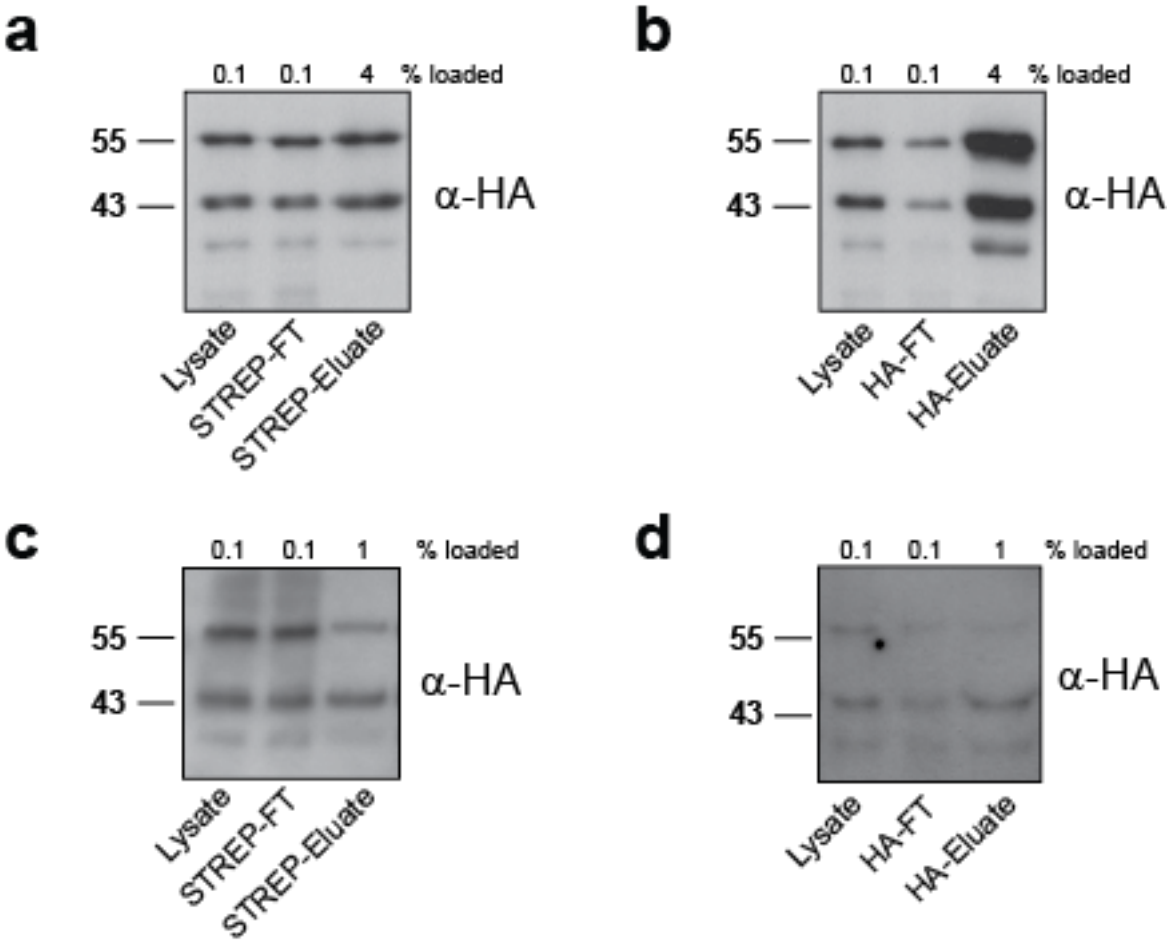
c


FDCP1
FDCP1-CEBPA WT
0.2 0.2 1.3 1.3 3.1 3.1
0.2 0.2 1.3 1.3 3.1 3.1 % loaded
55
43
α-HA
Lysate 1
Lysate 2
STREP-Eluate 1
STREp-Eluate 2
HA-Eluate 1
HA-Eluate 2

Supplementary Figure 1. 2-step STREP-HA purifications of C/EBPalpha analysed by 1D-gel- and 1D-gel-free LCMS analyses. a, Network of the proteins identified by both AP-MS strategies, after filtering the negative controls. C/EBPalpha is coloured in yellow. Ddit3, the single interactor previously described in the literature is indicated in orange. b, Anti-HA-immunoblot of STREP-HA purifications eluted with SDS buffer from control FDCP-1 and C/EBPalpha-STREP-HA-expressing FDCP-1 cells. c, Anti-HA-immunoblot of STREP-HA purifications eluted with formic acid from control FDCP-1 and C/EBPalpha-STREP-HA-expressing FDCP-1 cells. The samples of both biological replicates were analysed to compare technical reproducibility (number 1 and 2).



Supplementary Figure 2. STREP-purified C/EBPalpha cross-linked with different reagents. a, BS^3 and FA; b, DSG and DFDNB. Anti HA-immunoblot analyses were performed to detect C/EBPalpha.



Supplementary Figure 3. Anti-HA immunoblots of 1-step STREP- and HA- purifications of C/EBPalpha from C/EBPalpha-STREP-HA-expressing FDCP-1 cells. a,b, for analysis by 1D-gel-LCMS. c,d, for analysis by 1D-LCMS.