SUPPLEMENTARY FIGURES

Suppl Fig. 1 – Subcellular localisation of endogenous Daxx and C/EBPβ. HepG2 cells were seeded on coverslips, fixed and stained with antibodies against Daxx and C/EBPβ. The cells were examined by confocal laser scanning microscopy.

Suppl Fig. 2 – GST pull-down experiment. GST-Daxx1-751 and GST were immobilized on glutathione-sepharose and incubated with in vitro translated, ^{35}S-methionine labelled C/EBPβ. Bound proteins as well as a sample of the input material (10 %) were analyzed by 10 % SDS-PAGE and analyzed by autoradiagraphy. The left panel Coomassie blue staining of the GST-proteins used in the pull down experiments.

Suppl Fig. 3 – GST pull-down experiment. QT6 cells were transfected with expression vectors for Flag-C/EBPβ (5 μg) and Flag-SENP1 (5 μg), as indicated above the lanes. Cells were lysed after 24 hours and protein extracts were incubated with GST-Daxx1-571 and GST immobilized on glutathione-sepharose. Bound proteins as well as samples of the input material (10 %) were analyzed by 10 % SDS-PAGE and analyzed by western blotting with Flag antibodies. The left panel Coomassie blue staining of the GST-proteins used in the pull down experiments.

Suppl Fig. 4 - Electrophoretic mobility shift assay (upper panel). QT6 cells were transfected with expression vectors for C/EBPβ (3 μg) and HA-Daxx (5 and 10 μg), as indicated at the top. Nuclear extracts prepared from the cells were then subjected to an electrophoretic mobility shift experiment with a radiolabeled oligonucleotide containing a consensus C/EBP binding site. Lane 1 shows the free oligonucleotide without added cell extract and lanes 2-5 show binding reaction with the respective extracts. Preparation of nuclear extract and binding reactions were carried out as described before (15). The amounts of C/EBPβ and Daxx present in the nuclear extract were analyzed by SDS-PAGE and western blotting (bottom panels).

Suppl Fig. 5 - Hela cells were transfected with 1,3 μg of expression vectors for GFP-Daxx (a-c) or GFP-Daxx and Flag-C/EBPβ (d-f). Endogenous PML was detected with a primary anti-PML antibody and a TRITC-coupled secondary antibody. The white arrows indicate co-localisation of GFP-Daxx with endogenous PML in PODs.

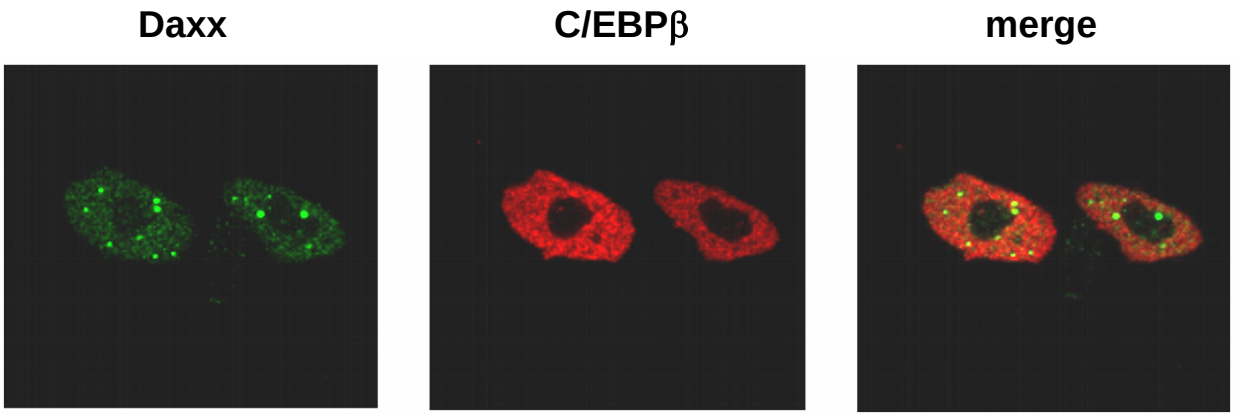
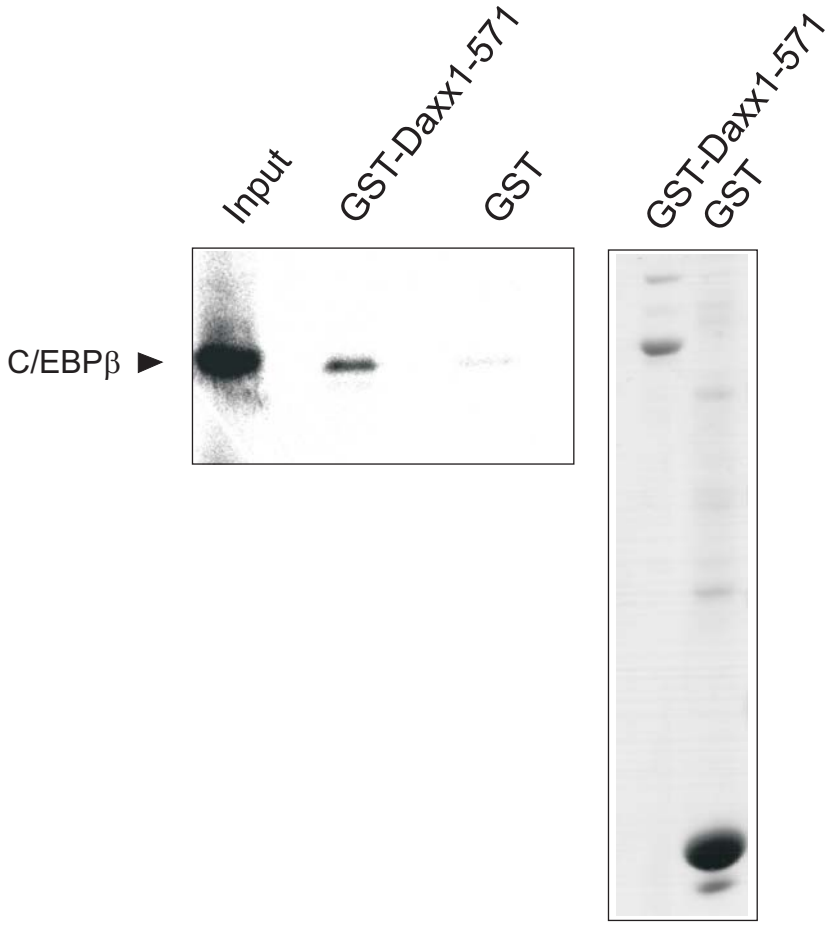
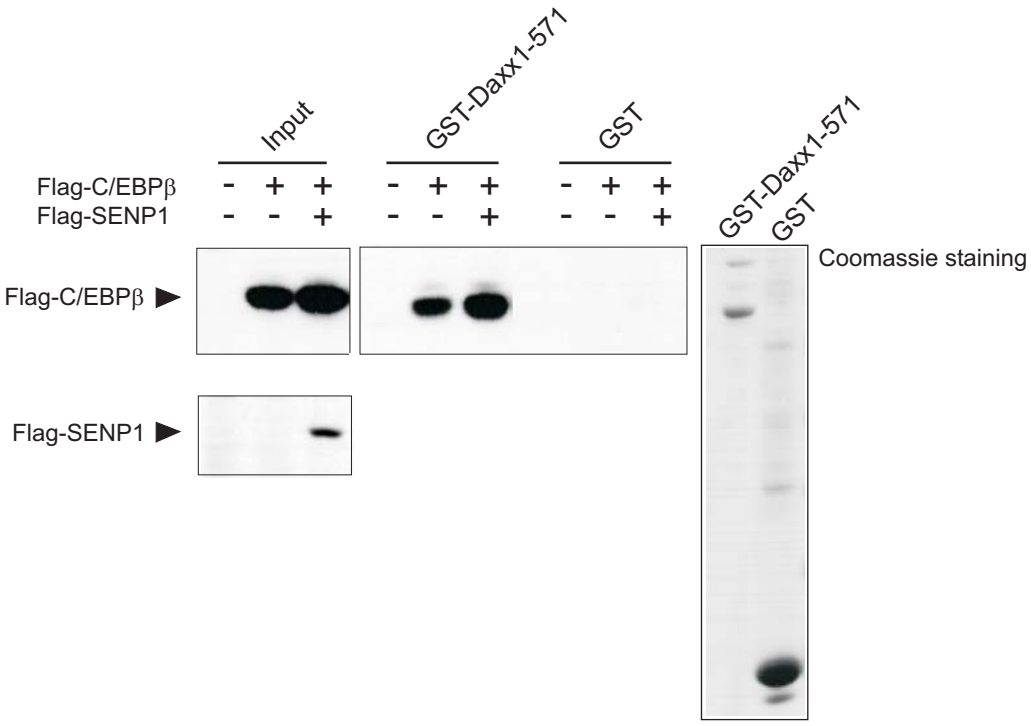


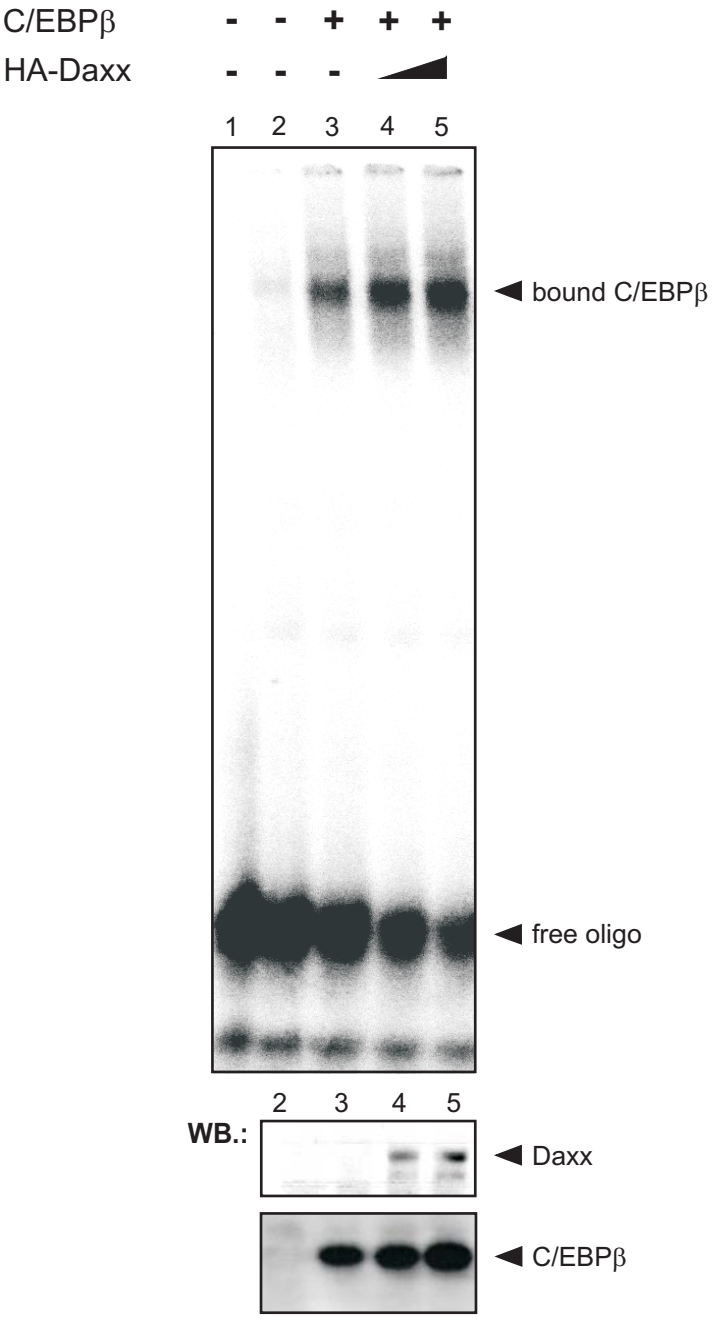
Fig. S1



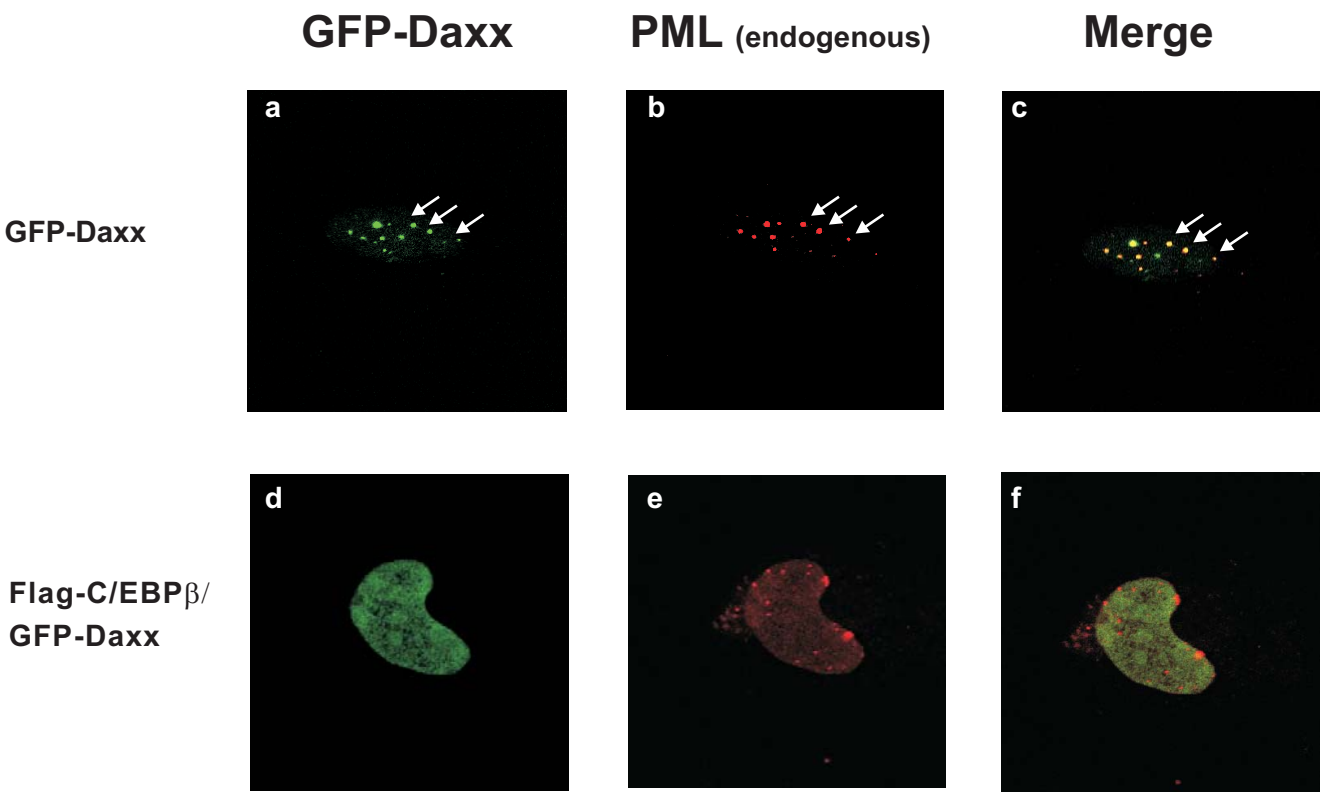
Suppl. Fig 2


Input
GST-Daxx1-571
GST
Flag-C/EBPβ
Flag-SENP1
- + +
- - +
- + +
- - +
- + +
- - +
GST-Daxx1-571
GST
Coomassie staining
Flag-C/EBPβ
Flag-SENP1

Suppl. Fig 3


C/EBPβ - - + + +
HA-Daxx - - - ▲
1 2 3 4 5
bound C/EBPβ
free oligo
2 3 4 5
WB.:
Daxx
C/EBPβ

Suppl. Fig 4


GFP-Daxx
PML (endogenous)
Merge
GFP-Daxx
Flag-C/EBPβ/
GFP-Daxx
a
b
c
d
e
f

Suppl. Fig 5