Fig. S1. The importance of Ser117 for sumoylation at Lys123 in SREBP-1a. COS-1 cells were transfected with expression plasmids for Flag-SUMO-1 and the wild type (WT) or a mutant version of GST-SREBP1a, as indicated. Thirty-six h later, the cells were harvested, lysed, and subjected to GST pull-down with glutathione-Sepharose resins as described under "EXPERIMENAL PROCEDURES". Aliquots of GST pull-down were subjected to SDS/PAGE and immunoblot (IB) with anti-Flag or anti-GST antibodies.

Fig. S2. IGF-1-induced phosphorylation of Lys123 enhances SREBP-1a transcriptional activity. COS-1 cells were transfected with expression plasmids for either 300 ng of the wild type (WT) or a mutant version of Gal4-SREBP-1a, 100 ng of pG5Luc containing five copies of the Gal4 binding sites, and 10 ng pRL-CMV. The cells were cultured with medium A containing 5% LPDS supplemented with 1 μg/ml 25-hydroxychoresterol plus 10 μg/ml cholesterol for 48 h in order to decrease the amount of endogenous SREBPs in the nucleus. The cells were incubated with or without 30nM IGF-1 for the last 6 h, and then luciferase assays were performed. The promoter activity driven by wild type Gal4-SREBP-1a in the absence of IGF-1 is represented as 1. All data are presented as the mean ± S.D. values of three independent experiments performed in triplicate. *, $p<0.05$; **, $p<0.01$.

Fig. S3. SREBP-2 is modified with SUMO-1, -2 and -3 at Lys464. COS-1 cells were transfected with expression plasmids for His-SUMO-1/-2/-3 and the wild type (WT) or a mutant version of Flag-SREBP2 (K464R), as indicated. Thirty-six h later, the cells were harvested, and the whole cell extracts (WCE) subjected to immunoprecipitation (IP) with anti-Flag antibodies. Aliquots of WCE and pellets of IP were subjected to SDS/PAGE and immunoblot (IB) with anti-His or anti-Flag antibodies.

Fig. S4. Sumoylation is not sufficient for the recruitment of the HDAC3 complex. (*A* and *B*) HEK293 cells were transfected with expression plasmids for HA-HDAC3 and either wild type (WT) or a mutant version of GST-SREBP-2, as indicated. The whole cell extracts (WCE) were subjected to GST pull-down with glutathione-Sepharose resins, as described under "EXPERIMENAL PROCEDURES". Aliquots of GST pull-down were subjected to SDS/PAGE and immunoblot (IB) with anti-HA or anti-GST antibodies. Aliquots of WCE were subjected to SDS/PAGE and IB with anti-HA antibodies.

Fig.S1

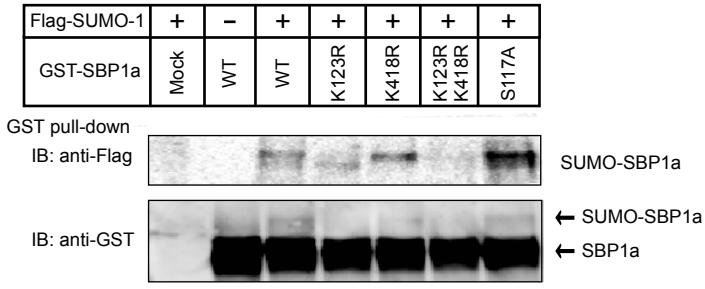

Flag-SUMO-1
+
−
+
+
+
+
+
GST-SBP1a
Mock
WT
WT
K123R
K418R
K123R K418R
S117A
GST pull-down
IB: anti-Flag
SUMO-SBP1a
IB: anti-GST
← SUMO-SBP1a
← SBP1a

Fig.S2

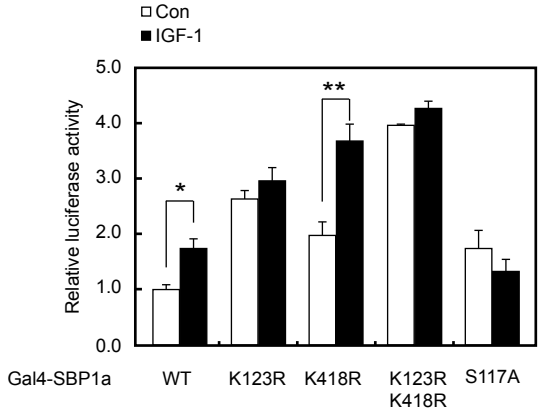

Con
IGF-1
Relative luciferase activity
5.0
4.0
3.0
2.0
1.0
0.0
*
**
Gal4-SBP1a
WT
K123R
K418R
K123R K418R
S117A

Fig.S3

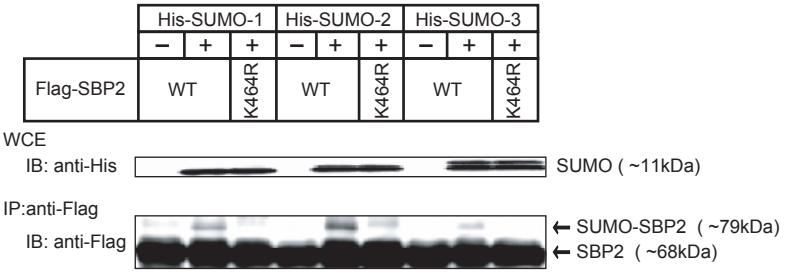
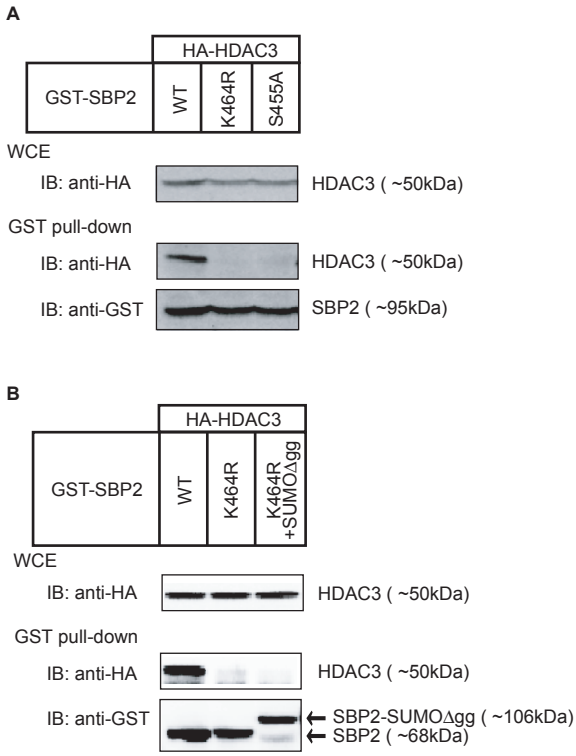

His-SUMO-1
His-SUMO-2
His-SUMO-3
− + + − + + − + +
Flag-SBP2
WT
K464R
WT
K464R
WT
K464R
WCE
IB: anti-His
SUMO (~11kDa)
IP:anti-Flag
IB: anti-Flag
← SUMO-SBP2 (~79kDa)
← SBP2 (~68kDa)

Fig.S4


A
HA-HDAC3
GST-SBP2
WT
K464R
S455A
WCE
IB: anti-HA
HDAC3 (~50kDa)
GST pull-down
IB: anti-HA
HDAC3 (~50kDa)
IB: anti-GST
SBP2 (~95kDa)
B
HA-HDAC3
GST-SBP2
WT
K464R
K464R +SUMOΔgg
WCE
IB: anti-HA
HDAC3 (~50kDa)
GST pull-down
IB: anti-HA
HDAC3 (~50kDa)
IB: anti-GST
SBP2-SUMOΔgg (~106kDa)
SBP2 (~68kDa)