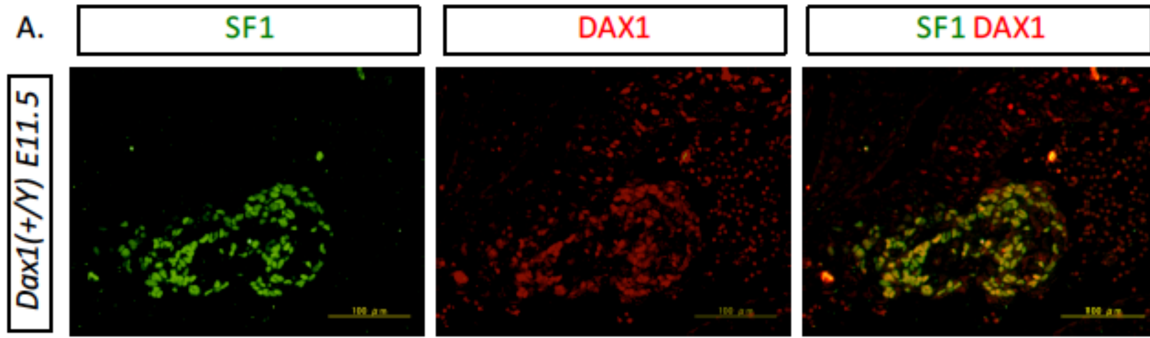
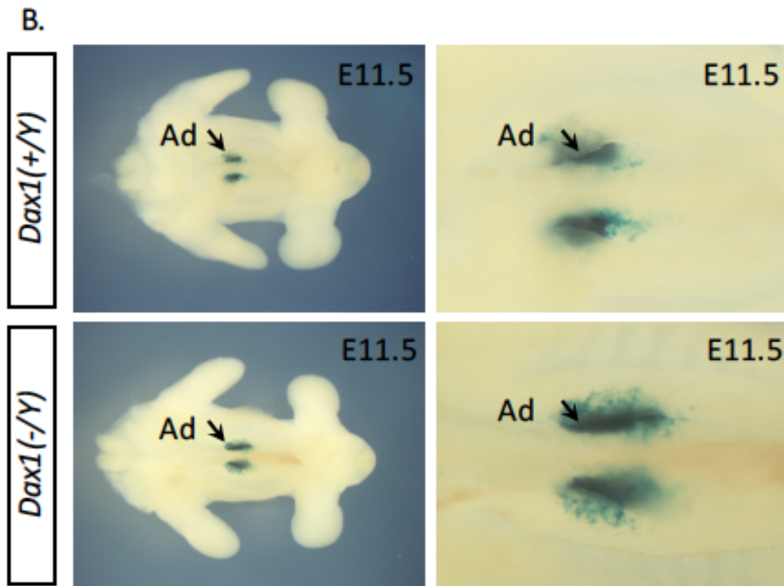

A.
SF1
DAX1
SF1 DAX1
Dax1(+/Y) E11.5


B.
Dax1(+/Y)
E11.5
Ad
E11.5
Ad
Dax1(-/Y)
E11.5
Ad
E11.5
Ad

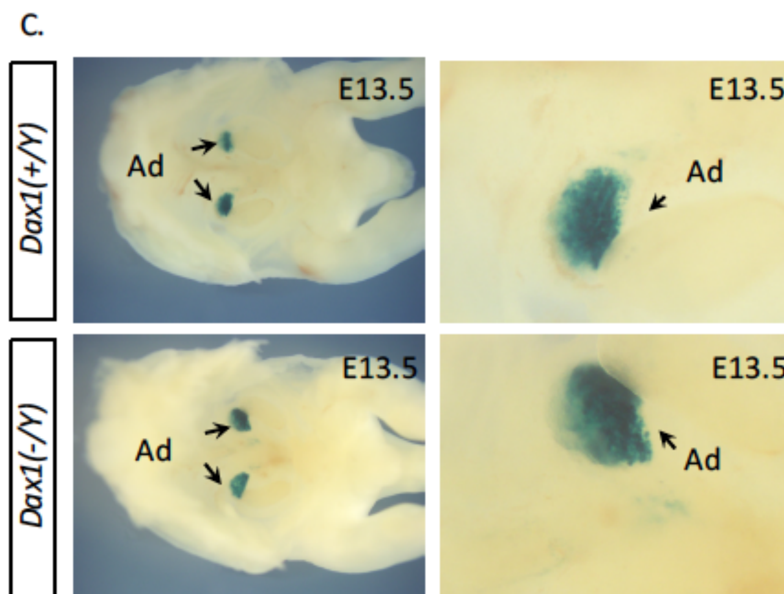

C.
Dax1(+/Y)
E13.5
Ad
E13.5
Ad
Dax1(-/Y)
E13.5
Ad
E13.5
Ad

Fig. S1. Dax1 is required for fine-tuning of Ad4BP/Sf1 expression in adrenal progenitor. (A) Expression of Ad4BP/Sf1 and Dax1 in adrenogonadal region of E11.5 embryo (immunostaining). While both genes are expressed in the gonad and adrenal, Dax1 is expressed in a broader region posterior to the adrenal. Scale bar represents 100 μm. (B) Views of whole-mount X-gal stained Ad4BP-lacZ-FAdE Tg wild type or Dax1 KO E11.5 embryos (N=4. Left panel: lower power view. Right panel: higher power view). Arrow indicates adrenal gland location in embryo. (C) Views of whole-mount X-gal stained Ad4BP-lacZ-FAdE Tg wild type or Dax1 KO E13.5 embryos (N=3. Left panel: lower power view. Right panel: higher power view). Arrow indicates adrenal gland location in embryos.

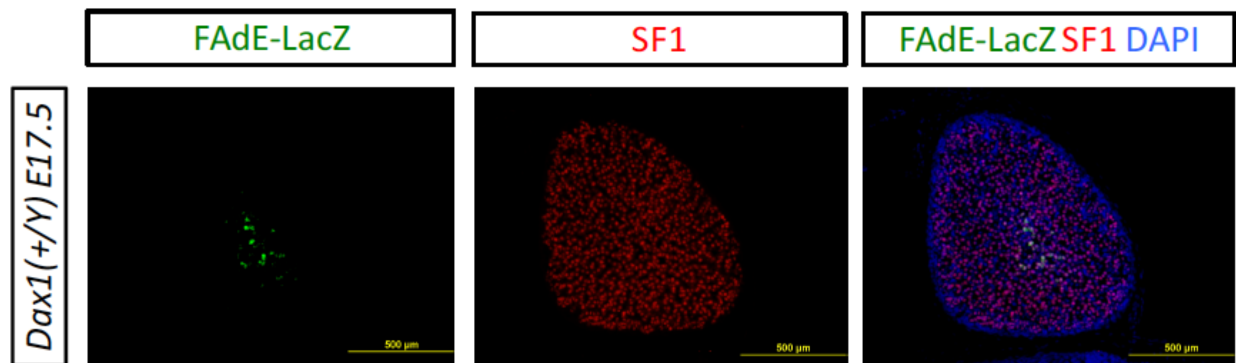


Fig S2. FAdE-lacZ expression is largely extinguished in the adrenal gland of E17.5 WT male mice. FAdE activity in WT mice during early development stages as shown by immunostaining with LacZ (E17.5). LacZ staining is shown in green channel with Sf1 stating in red channel. DAPI is used for nuclear counterstaining. Scale bar represents 500 μm.

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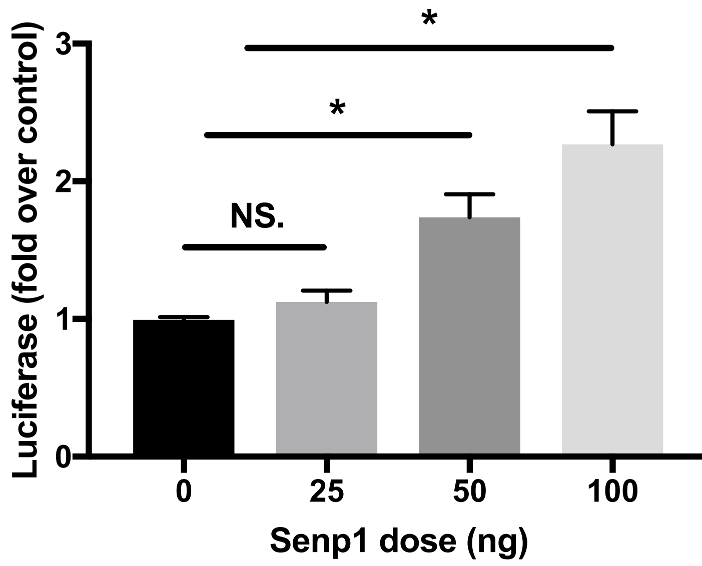
A.



B.

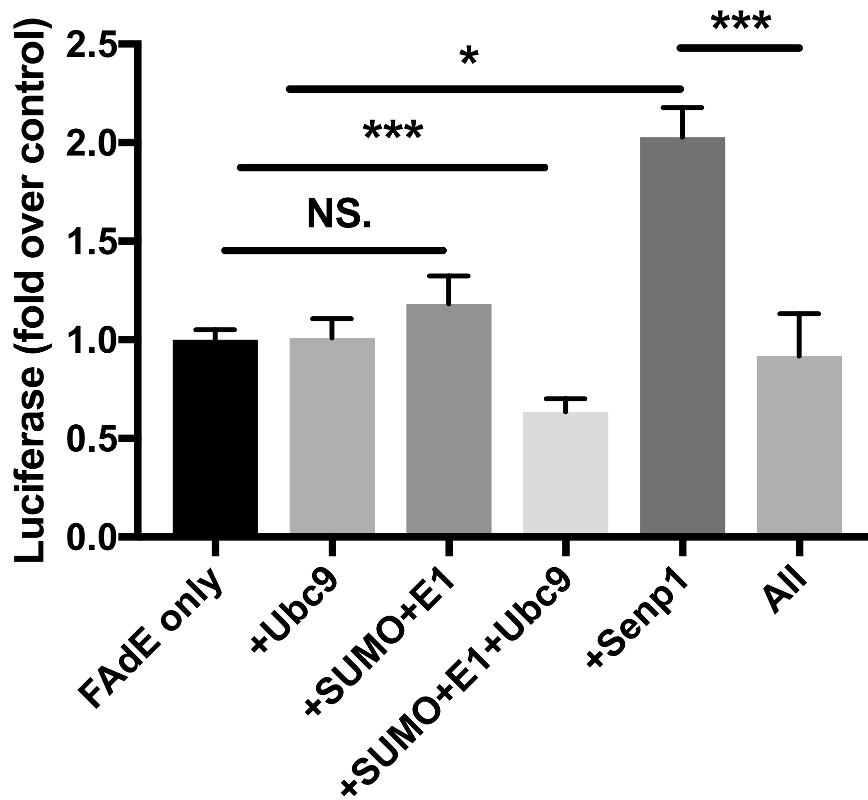
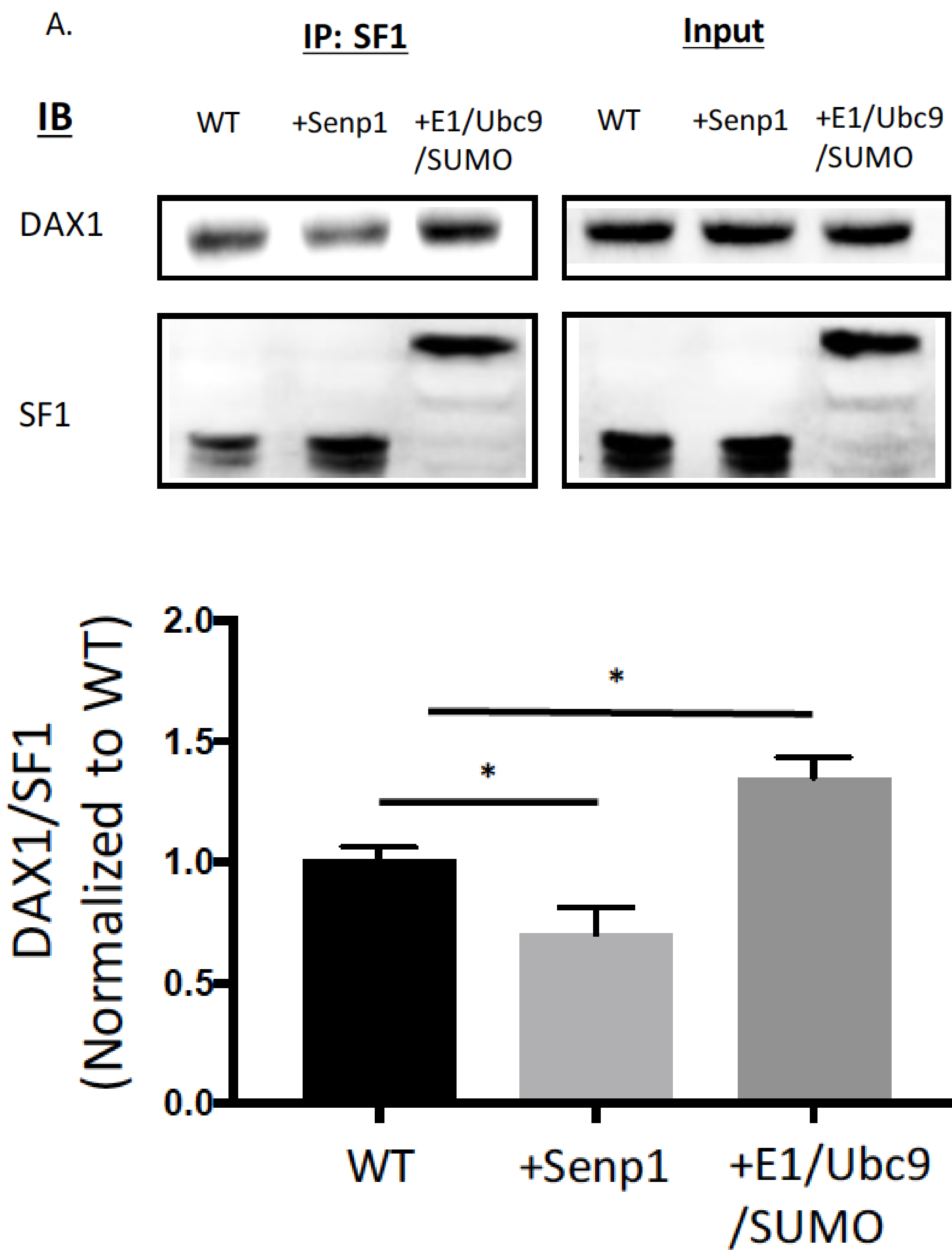


Fig. S3. Sf1 SUMOylation modify Sf1 activity on the FAdE enhancer *in vitro*. (A) Inhibition of Sf1 SUMOylation increases its activity on the FAdE enhancer. Y1 cells were plated at $3X10^5$ cells/well in 24-well plates 24 h before transfection and were transfected in triplicate with FAdE Luc (100 ng/well) and Senp1 vector as indicated. Luciferase assays were carried out as described in methods and the data were normalized to the β-galactosidase activity and shown as fold over vehicle. N=4. NS., not significant. *, p<0.05. (B) Enhance SUMOylation of Sf1 inhibit its stimulating effects on FAdE enhancer. Y1 cells were plated at $3X10^5$ cells/well in 24-well plates 24 h before transfection and were transfected in triplicate with FAdE Luc (100 ng/well) and 50ng of Senp1 or Ubc9 or pSA2 or combination of those vectors as indicated (empty vectors were added accordingly for equal amount of vector per transfection. All: combination of Senp1, Ubc9 and pSA2 vectors. Luciferase assays were carried out as described in methods and the data were normalized to the β-galactosidase activity and shown as fold over vehicle. N=4. NS., not significant. *, p<0.05.


A.
IP: SF1
Input
IB
WT
+Senp1
+E1/Ubc9 /SUMO
WT
+Senp1
+E1/Ubc9 /SUMO
DAX1
SF1
DAX1/SF1 (Normalized to WT)
2.0
1.5
1.0
0.5
0.0
*
*
WT
+Senp1
+E1/Ubc9 /SUMO

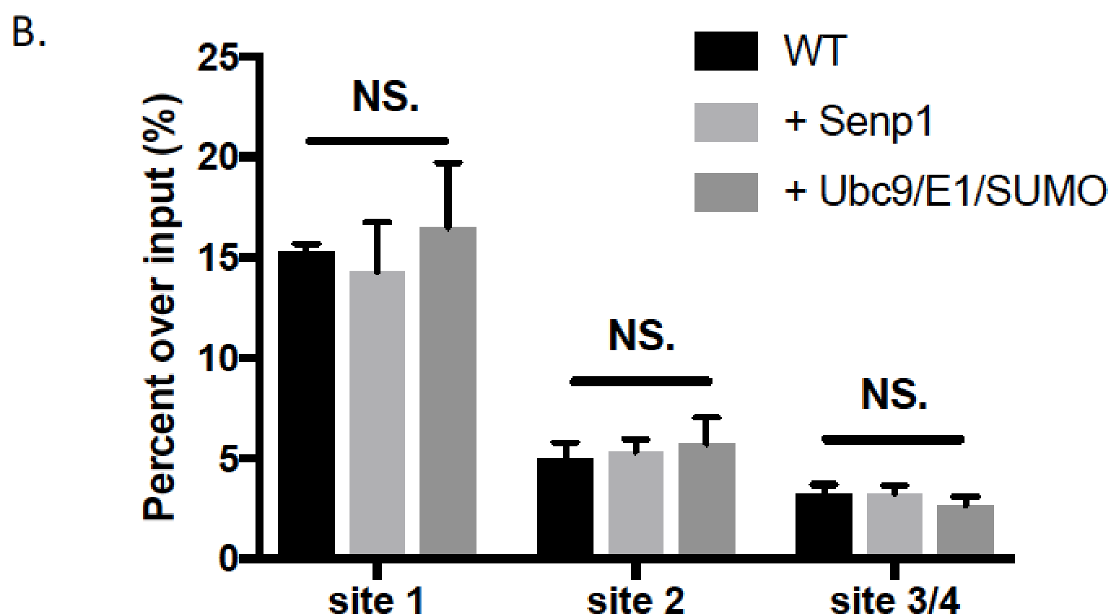


Figure S4. SUMOylation enhances Sf1 binding to Dax1, but does not change its binding affinity to different DNA binding sites in FAdE enhancer. (A) SUMOylation increases Sf1's binding to Dax1. HEK293T cell were plated at 10^6 cells/dish in 10 mm cell culture dish 24 h before transfection and were transfected with 1 µg pcDNA-Sf1, 1 µg pcDNA-Dax1 and 1 µg Senp1 or 1 µg Ubc9+pSA2 vectors. After 48 h, cells were harvested for Co-IP assay. For Co-IP assay, lysates were precipitated using anti-Sf1 antibody and immunoblotted with anti-HA (for Dax1) or Sf1 antibodies. The data were normalized to input and shown as fold over WT Sf1. *, $p<0.05$. N=4. (B). SUMOylation or Dax1 do not change DNA binding capacity of Sf1. Y1cells were plated at $3X10^6$ cells/dish in 10 mm cell culture dish 24 h before transfection and were transfected with 2 µg pcDNA-Dax1 and 2 µg Senp1 or 2 µg Ubc9+pSA2 vectors. After 48h, cells were fixed and processed for ChIP assay using anti-Sf1 antibody. Realtime PCR were performed using three sets of primers aiming at different Sf1 binding sites on FAdE promotor regions. The data were normalized to values obtained for 1% input controls, and the results are presented as percentage of input. N=3.

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