Supplementary materials

Supplementary Figure 6. Interaction of FLAP1 with ER and p300. (**A**) GST fusion proteins analyzed by Coomassie blue staining: GST fusion proteins were analyzed by SDS-PAGE and Coomassie blue staining. Full size GST fusion proteins are indicated by dots on the left side of the stained band. (**B & C**) GST-pulldown assays: in vitro translated Flag-FLAP1 was incubated with the indicated GST fusion proteins (approximately 1 μg) bound to beads. Bound fractions were analyzed by immunoblot with anti-Flag antibodies. (**D**) Mammalian one hybrid assays: GK1-Luc (a Gal4-responsive reporter plasmid), pM-ERAF1 and pM-ERAF2 (0.2 μg of each as indicated, encoding the N-terminal activation domain of ER or the C-terminal ligand binding domain of ER fused to Gal4 DBD) were transfected along with 0.2 μg of pCMV-FLAP1 and pCMV-p300 vectors. For pM-ERAF2 (assays 5-8), 100 μM estradiol was included after transfection. (**E**) FLAP1 but not Fli-I LRR fragment binds to p300 KIX domain: GST pull down assays were performed as described above, using in vitro translated Flag-FLAP1 or Flag-LRR and GST-KIX. (**F**) Competition assay: in vitro translated FLAP1 protein was incubated with purified GST-KIX protein in the presence of in vitro translated LRR and Gelsolin fragments of Fli-I, as indicated. The relative in vitro translation efficiency of FLAP1 and LRR is shown in E; in vitro translation efficiency of the Gelsolin-like fragment was similar to that of LRR (data not shown). Five times excess amounts of LRR and Gelsolin-like fragments were included as competitors for FLAP1. The GST fusion protein bound fractions were analyzed by immunoblot with anti-FLAP1 antibody.

Fig.6 (Supplementary figure)