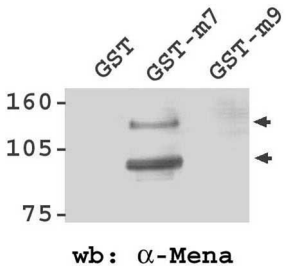
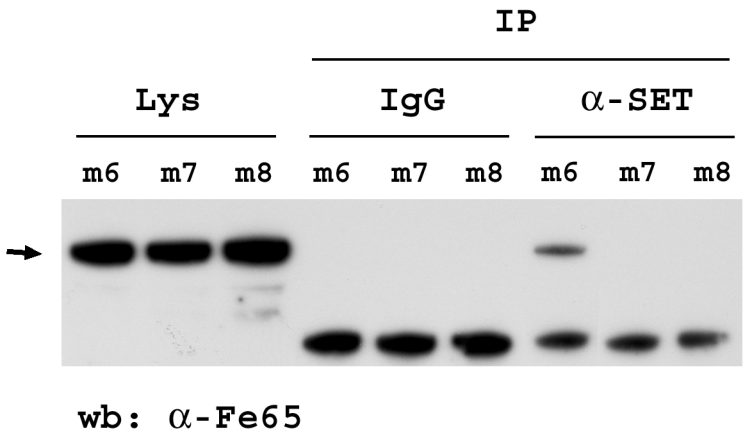
Supplemental material 1

Gal4-Fe65 fl construct (Fig 1A) has been co-transfected in HeLa cells with the G5BCAT vector and/or plasmids driving the expression of fragments of Fe65 tagged with the HA epitope. CAT expression is reported as fold increase compared to the transcription observed when the Gal4 DNA binding domain alone was transfected. The bars indicate the mean values of triplicate experiments + SD.
The co-transfection of the region including the WW domain significantly inhibits the effect of Gal4-Fe65 fl.

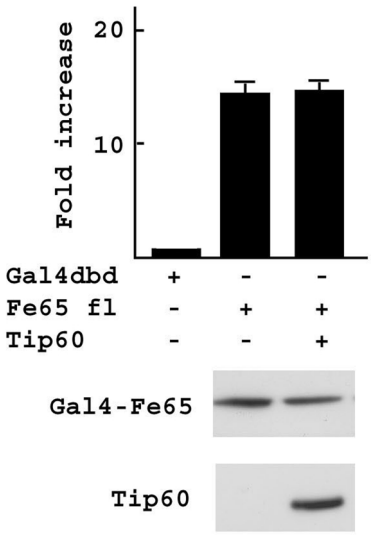


Supplemental material 2

Extracts from HEK293 cellls were challenged with GST or GST-Fe65 fusion proteins containing the regions of Fe65 present in the m7 or m9 proteins of Fig 1A. Pull-down experiments were analyzed by western blot with anti-Mena antibody.
Only the GST-m7 protein, containing the entire WW domain, interacts with Mena, while the GST-m9 protein fails to interact.



Supplemental material 3
HEK293 cells were co-transfected with Gal4-Fe65 constructs m6, m7 or m8 and with SET expression vector. Cell lysates were immunoprecipitated with either mouse IgG or α-SET antibody. Immunoprecipitated proteins were analyzed by western blot with α-Fe65 antibody. Cell lysates (30 μg) were run on the same gel as a control. The result indicates that only m6 protein co-immunoprecipitates with SET, while m7 and m8 proteins, lacking transactivating properties, do not interact with SET.



Supplemental material 4

HeLa cells were co-transfected with G5BCAT and the vectors expressing the Gal4 DNA binding domain or the Gal4-Fe65 fl fusion protein (see Fig 1A) and/or Tip60-HA. The bars indicate the fold increase of CAT expression compared to that obtained by transfecting the Gal4 dbd alone. Mean values of triplicate experiments plus SD are reported.
The overexpression of Tip60 did not further increase the effect of the Gal4-Fe65 fl protein.