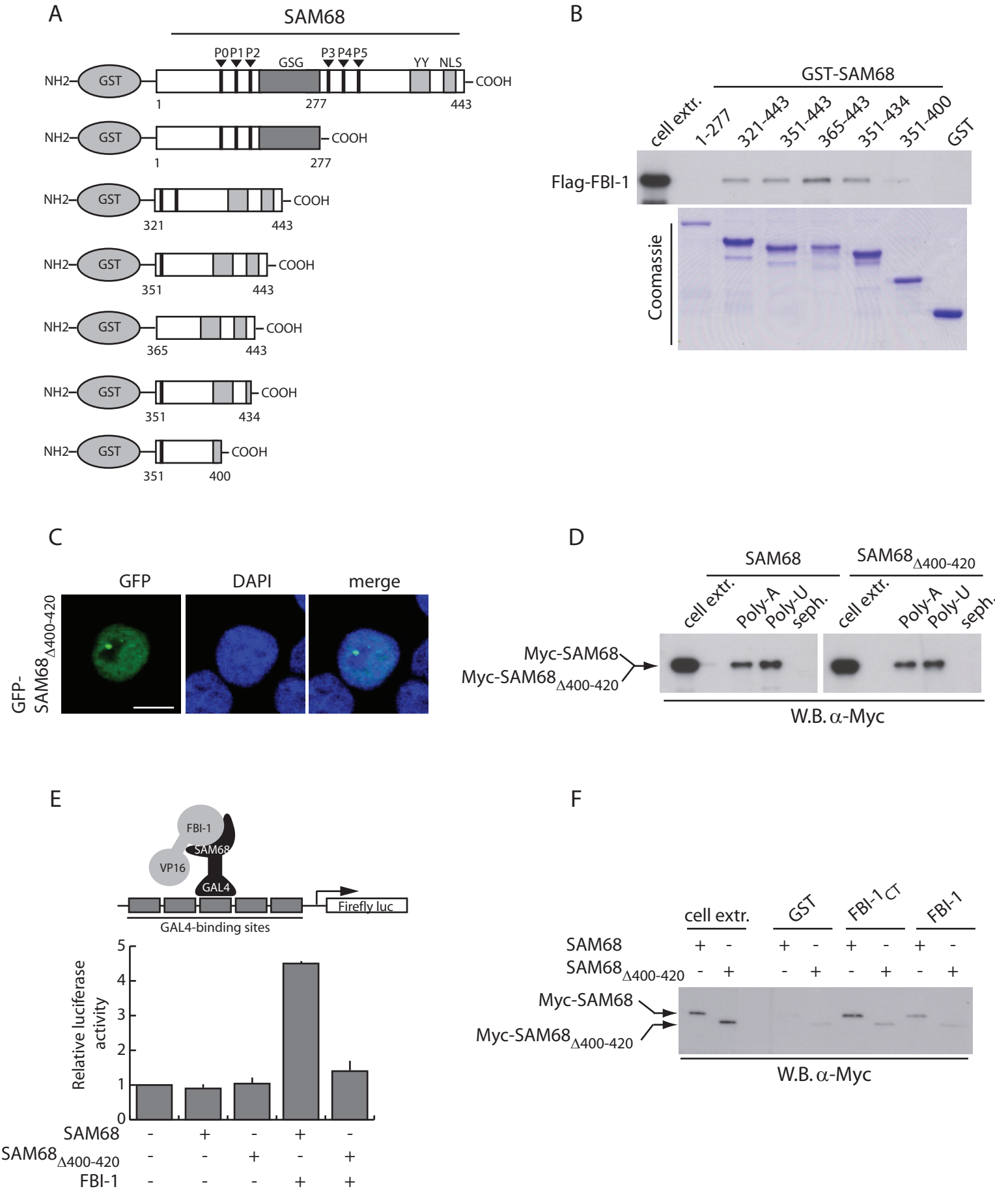
Bielli_Supplemental Figure 2



Supplemental Figure 2. The deletion of the residues 400 to 420 in SAM68 affects its interaction with FBI-1. A) Schematic representation of the structural motifs of SAM68 and of the GST-SAM68 fusion proteins used in the experiments. B) Western blot analysis and Coomassie staining of the pull-down assay between the indicated GST-SAM68 fusion proteins and Flag-FBI-1. GST was used as negative control. C) Analysis of the localization of GFP-SAM68$_{\Delta 400\text{-}420}$ in HEK293T cells by fluorescence microscopy. D) Poly-A/U RNA sepharose pulldown assays using total cell extracts of HEK293T expressing Myc-SAM68 or Myc-SAM68$_{\Delta 400\text{-}420}$. Sepharose (Seph.) was used as control. Western-blot analysis performed using the Myc antibody is shown. E) Scheme of the Two-hybrid mammalian assay (*upper panel*). HEK293T cells were transfected with pACT-VP16-FBI-1 and pBIND-Gal4-SAM68 or pBIND-Gal4-SAM68$_{\Delta 400\text{-}420}$, in the presence of the pG5luc vector and analysed for luciferase activity 24 hrs later. The bar graph (*lower panel*) represents the ratio of firefly/ renilla luciferase activity (mean ± SD, n=3). F) Pull-down assay between cross-linked GST-FBI-1 (FBI-1) or GST-FBI-1$_{274\text{-}584}$ (FBI-1$_{CT}$) and wild type or mutated Myc-SAM68. Extracts from HEK293T expressing Myc-SAM68 or Myc-SAM68$_{\Delta 400\text{-}420}$ were incubated with the cross-linked GST-FBI-1 fusion proteins indicated. Bound proteins were analyzed by Western blot using anti-Myc antibody.