



Supplementary figure 2: In vitro sumoylation reaction. The SDS gel shows the in vitro sumoylation reaction with GST-Gilz fusion protein with or without ATP (lanes 1 and 2), with the positive control RanGAP₁ (lanes 3 and 4), and with the negative control GST (lanes 5 and 6). Each reaction was performed with (+) or without (-) the ATP necessary to the enzymatic reaction. No differences in sumoylated proteins were seen in the GST-Gilz lanes with (lane 1) or without (lane 2) the ATP or compared to the GST lane 5.

Method: In vitro sumoylation was performed using the “SUMOylation Kit” purchased from Enzo Life Sciences. (cat. # UW8955) by following the company instructions. Briefly, assay components (dH₂O, 10x SUMOylation buffer, 20x Mg-ATP, 20x SUMO E₁, 20x SUMO E₂, 20x RG₁, 20x SUMO_{1/2/3}). The target protein was GST-Gilz and the target protein negative control was GST) were added to 0,5 mL eppendorf tubes. Tubes were mixed gently, incubated at 30°C for 60 minutes. Assays were quenched by addition of 20µL 2x SDS-PAGE Gel Loading Buffer and analysed by western blotting.