Supplement

c-Myc is targeted to the proteasome for degradation in a SUMOylation-dependent manner, regulated by PIAS1, SENP7 and RNF4

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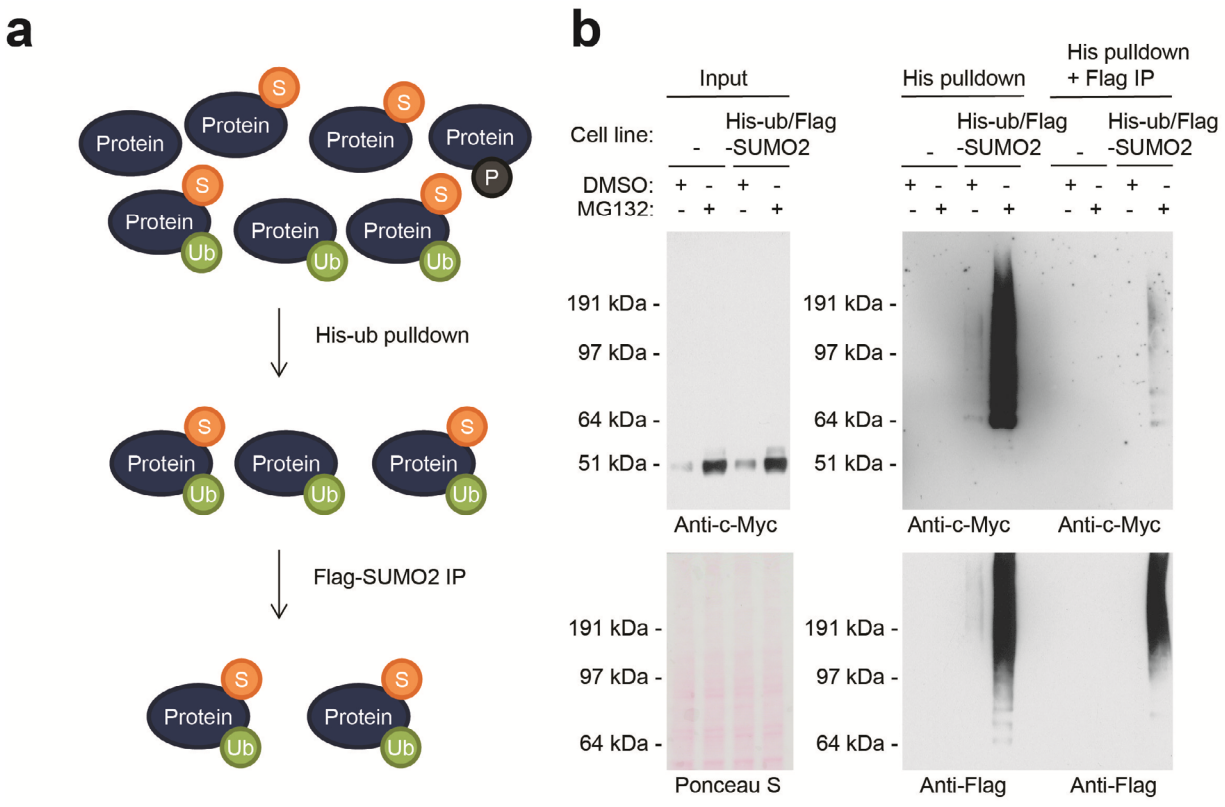

Figure S1. Ubiquitin-SUMO co-modification of c-myc. A) Cartoon depicting our strategy to identify co-modification of c-Myc by ubiquitin and SUMO. Lysates of cells expressing His-tagged ubiquitin in combination with Flag-tagged SUMO2 contained all modified and unmodified proteins. First, a His-ubiquitin pulldown was performed to enrich for ubiquitylated proteins. Subsequently, the ubiquitin and SUMO2 co-modified proteins were selected from this sample via Flag-SUMO2 immunopurification (IP). B) U2OS cells stable expressing His-ubiquitin and Flag-SUMO2 were treated for six hours with DMSO or the proteasome inhibitor MG132. Input samples were taken and a double purification was performed as described above. Input samples, single and double purified samples were separated by SDS-PAGE, transferred onto a membrane and incubated with antibodies to detect c-Myc or the Flag-tag. Equal amounts of starting material were used for the single purification and for the double purified sample, enabling a comparison of these samples.

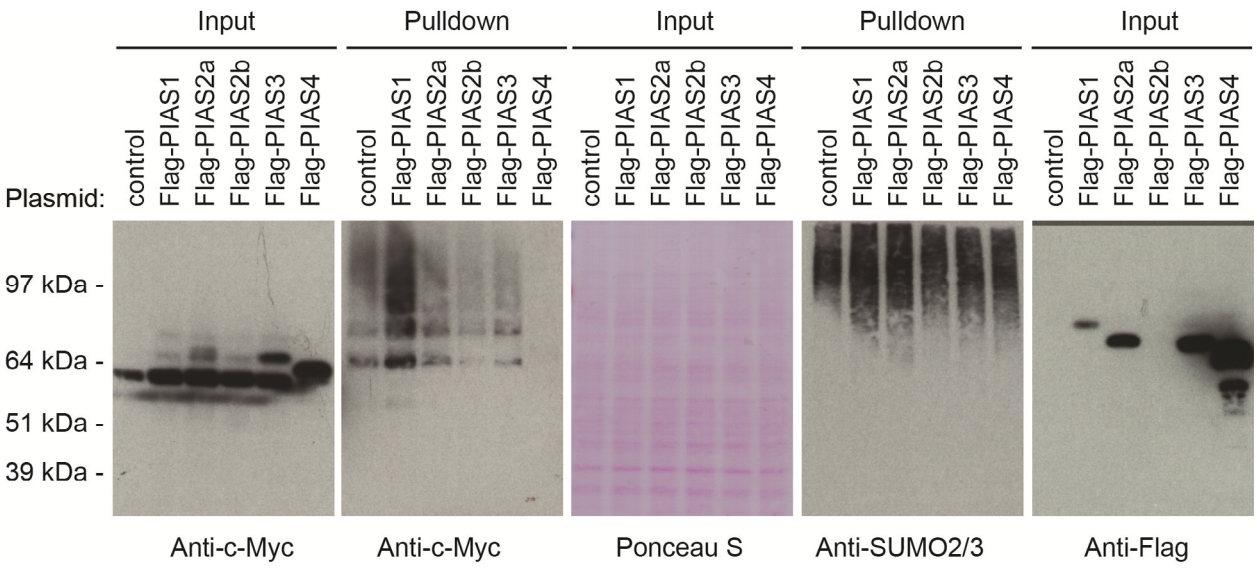

Figure S2. PIAS1 overexpression enhances endogenous c-Myc SUMOylation. U2OS cells stably expressing His-SUMO2 were transfected with a control plasmid or Flag-tagged PIAS constructs. Two days after transfection these cells were treated for six hours with the proteasome inhibitor MG132, lysed and a His-SUMO2 pulldown was performed. Input and His pulldown samples were separated by SDS-PAGE, transferred onto a membrane and incubated with antibodies to detect c-Myc, SUMO2/3 and the Flag-tag.