

Supplementary:

Material and methods:

Apoptosis and cell cycle analyses.

HEK293 cells were transfected with either empty vector, pcdna/ING1b WT or pcdna/ING1b E195A. After 48 hours apoptosis was analysed on a Gallios cytometer (Beckman Coulter) using PE & active caspase-3 apoptosis kit (BD Pharmigen) and cell cycle was analysed using DAPI staining (SIGMA) using Kaluza 1.3 and FlowJo vX softwares.

Figure legends:

Figure S1. A. HEK293 cell lysates co-expressing HA/SUMO and pcdna/ING1b were co-immunoprecipitated with α HA and subjected to ING1b IB. Cell lysates were immunoblotted with α ING1b, α HA and α actin to confirm equal loading. **B.** U2OS cell lysates expressing either empty vector or ING1b and HA/SUMO1 was subjected to α ING1b IP and IB with ING1b and β -actin antibodies. SM : SUMO Modified ING1b ; UM : UnModified ING1b ; Ig : immunoglobulin. **C.** U2OS cell lysates transfected with empty vector or pcdna/ING1b were subjected to α ING1b IP and IB with ING1b and actin antibodies. ++ or + denotes high or low exposure.

Figure S2 A. HA/Ubc9 dose dependent ING1b sumoylation. U2OS cell lysates coexpressing Flag/SUMO, HA/Ubc9 and ING1b were subjected to Flag IP followed by α ING1b IB. Cell lysates were probed for HA, Flag and β -actin to confirm protein expression and equal protein loading. **B.** U2OS cell lysates overexpressing Flag/ING1b were subjected to Flag IP followed by α Ubc9 IB. Lysates were probed for Flag and β -actin to confirm protein expression and equal protein loading.

Figure S3 A. HEK293 cell lysates co-expressing pcdna/ING1b, HA/SUMO, HA/Ubc9, Flag/PIAS3 and Flag/PIAS4 were directly subjected to SDS-PAGE electrophoresis and α ING1b to detect modified (SM) and unmodified (UM) ING1b and with α HA, α Flag and α β -actin antibodies. ++ or + denotes high or low exposure.

Figure S4 U2OS cell lysates expressing either empty vector, pcdna/ING1b WT or pcdna/ING1b S199A and Flag/SUMO were subjected to α Flag IP followed by IB with α ING1b, α Flag and α β -actin to detect ING1b sumoylation, protein expression and protein loading controls respectively.

Figure S5 U2OS cell lysates expressing either empty vector, ING1b WT or ING1b E195A were subjected to α Sin3a IP followed by IB with α ING1, α Sin3a and α β -actin to detect ING1b interaction with Sin3a, protein expression and protein loading controls respectively.

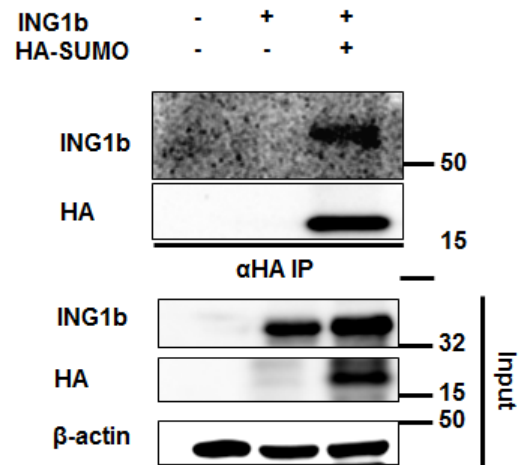
Figure S6 HEK293 cells were transfected with either empty vector, ING1b WT or ING1b E195A and assessed for apoptosis (**A**) and cell cycle (**B**) by flow cytometry 48 hours later. **A.** Apoptosis was evaluated by the use of active caspase-3 staining. Bar graph represents the percentage of positive active caspase 3 cells \pm SD. (*P<0.05; **P<0.01; ***P<0.001; NS, not significant). **B.** Cell cycle was analyzed by visualization of DNA content stained with DAPI.

Figure S7 A. Efficiency of immunoprecipitations in ChIP experiments. *ISG15* (**A**) and *DGCR8* promoters (**B**) were immunoprecipitated with ING1 or IgG antibodies from U2OS cells expressing empty vector, pcdna/ING1b and pcdna/ING1b E195A \pm SD. In (**A**) and (**B**), ING1b immunoprecipitations were significantly greater than IgG immunoprecipitations following an ING1b WT induced expression. (*p<0.05; **p<0.01; ***p<0.001; NS, not significant).

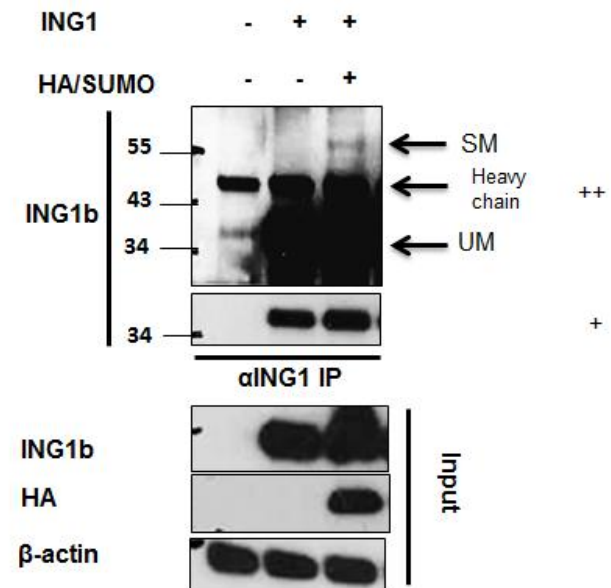
Supplemental figures with legends:

FigureS1

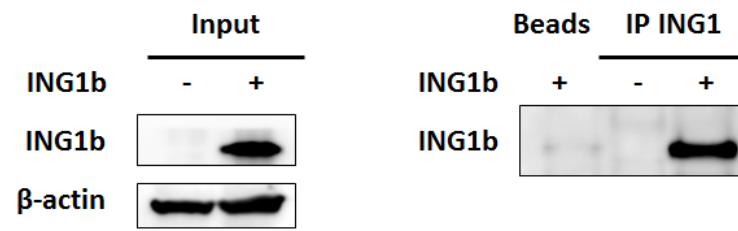
A.



B.

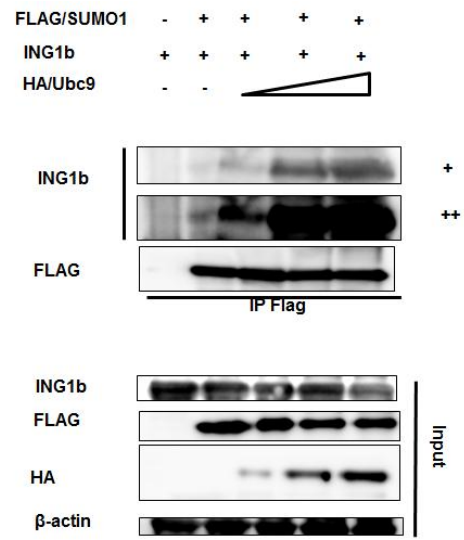


C.

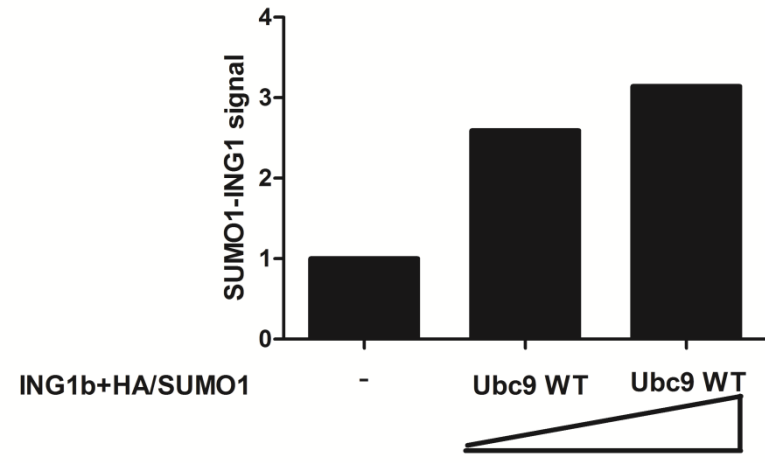


FigureS2

A.



B.



C.

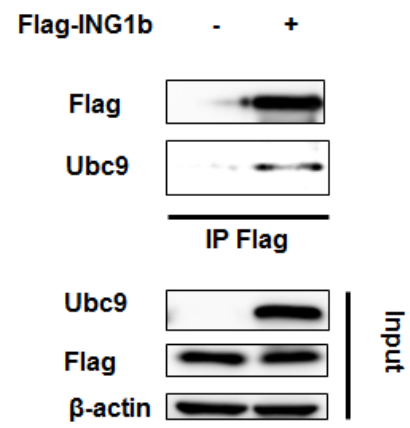


Figure S3

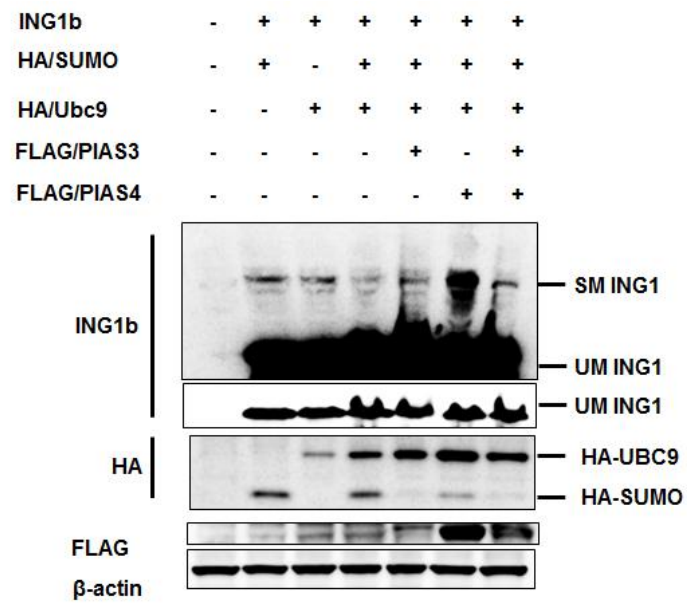


Figure S4

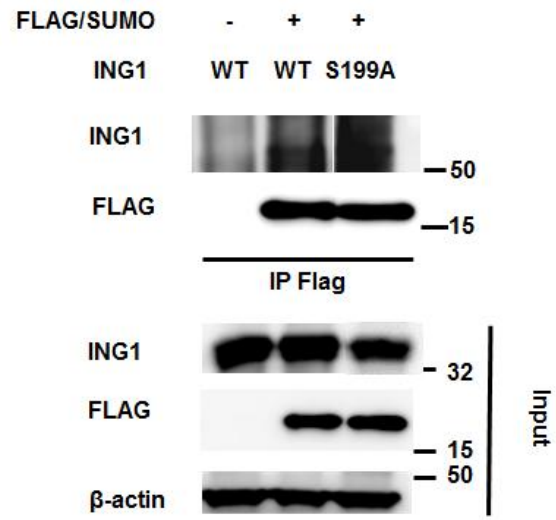


Figure S5

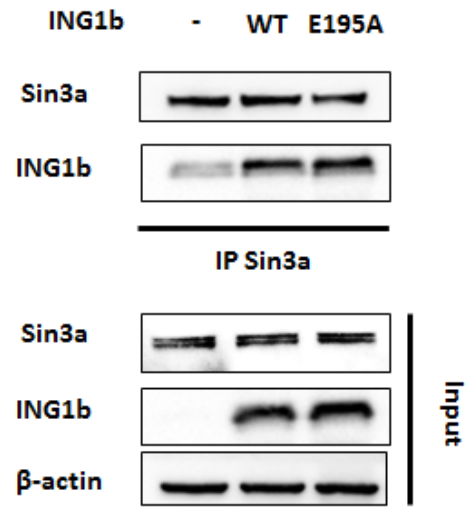
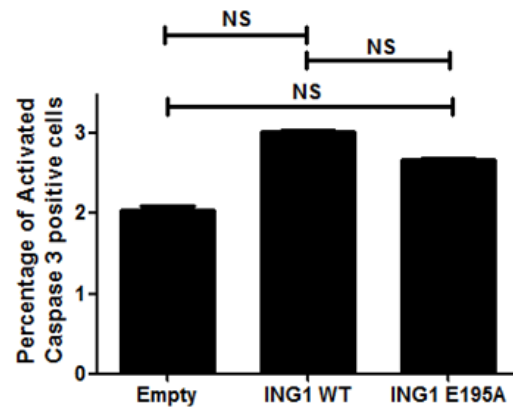


Figure S6

A.



B.

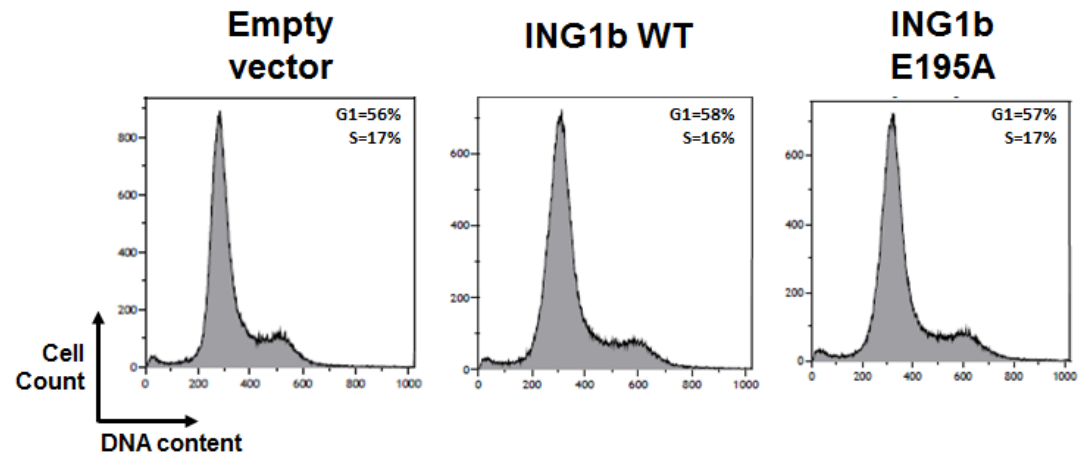


Figure S7

