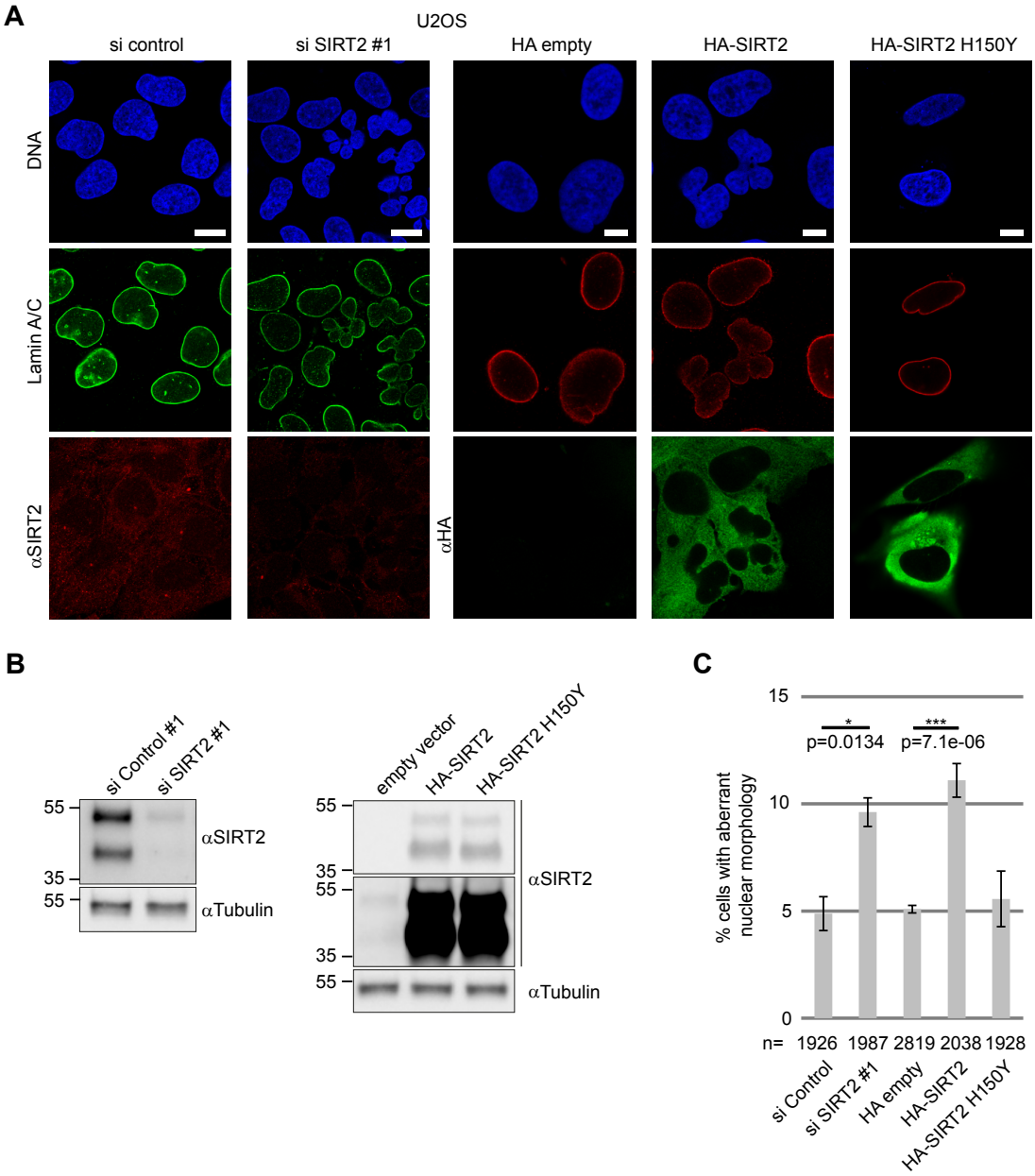

Fig. S1. SIRT2 depletion and overexpression in U2OS cells cause nuclear shape defects. (A) Immunofluorescence images of U2OS cells transfected with siSIRT2 and siControl for 48 h or with HA-SIRT2 and HA-SIRT1 H150Y and stained with anti-laminA/C as a marker of nuclear lamina. Scale bars: 10 µm. **(B)** Western blots showing SIRT2 silencing efficiency and overexpression levels. Tubulin was used as a loading control. **(C)** Quantification of lobulated nuclei caused by silencing or overexpression of SIRT2.

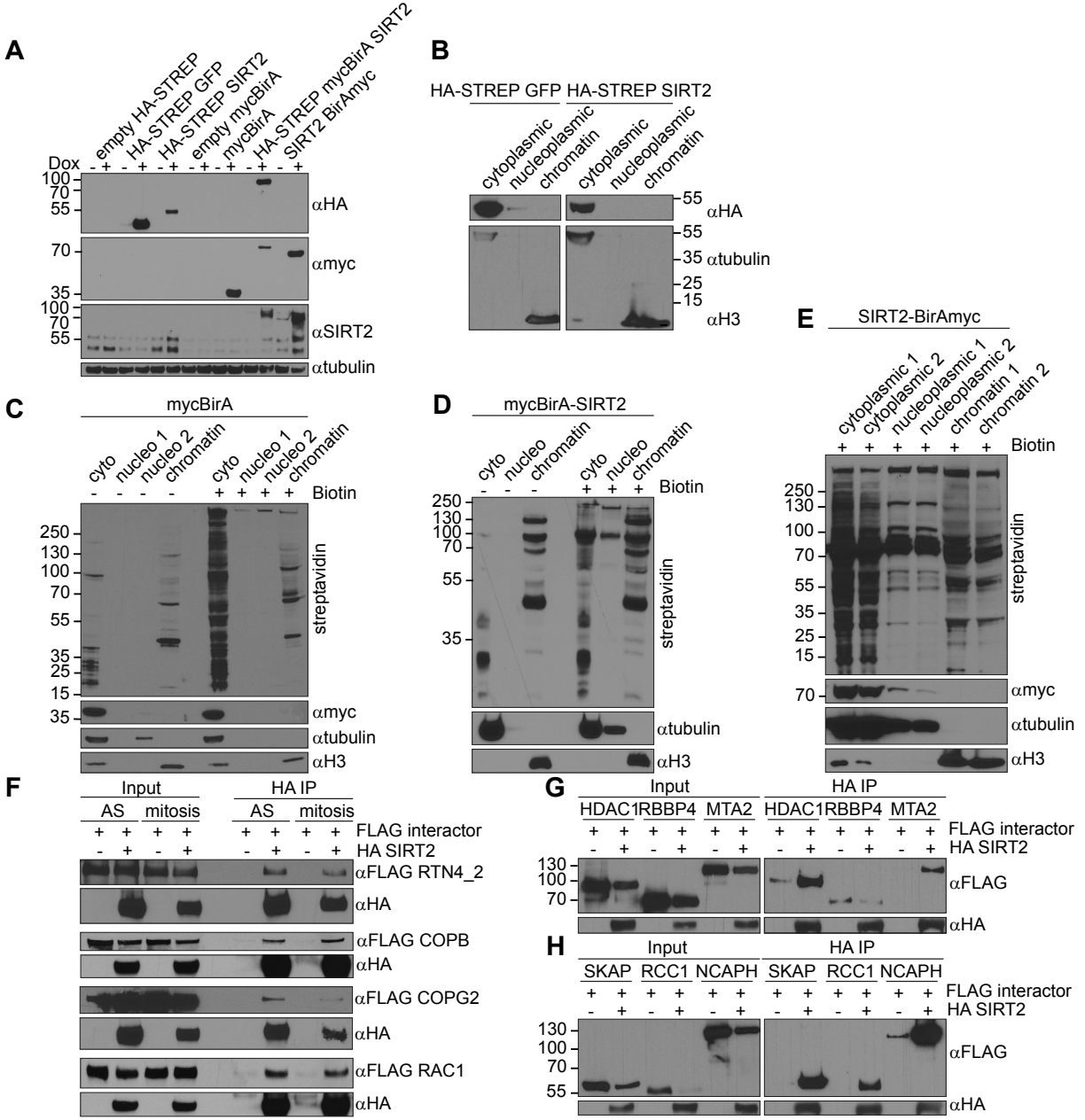

Fig. S2. Biochemical fractionation of TAP and BioID cell lines shows cytoplasmic localization of SIRT2. (A) SIRT2 expression levels in stable cell lines. The SIRT2 antibody recognizes two endogenous SIRT2 isoforms. **(B)** Cytoplasmic localization of GFP and SIRT2 in stable cell lines. **(C)** Cytoplasmic localization of mycBirA and predominant cytoplasmic biotinylation upon biotin addition. **(D,E)** mycBirA-SIRT2 and SIRT2-BirAmyc cell lines show cytoplasmic localization of SIRT2 and predominant cytoplasmic biotinylation as well as weaker chromatin biotinylation. **(F-H)** Validation of SIRT2 interactions by anti-HA co-immunoprecipitation from HEK293T cells overexpressing HA-SIRT2 and FLAG-tagged interactors.

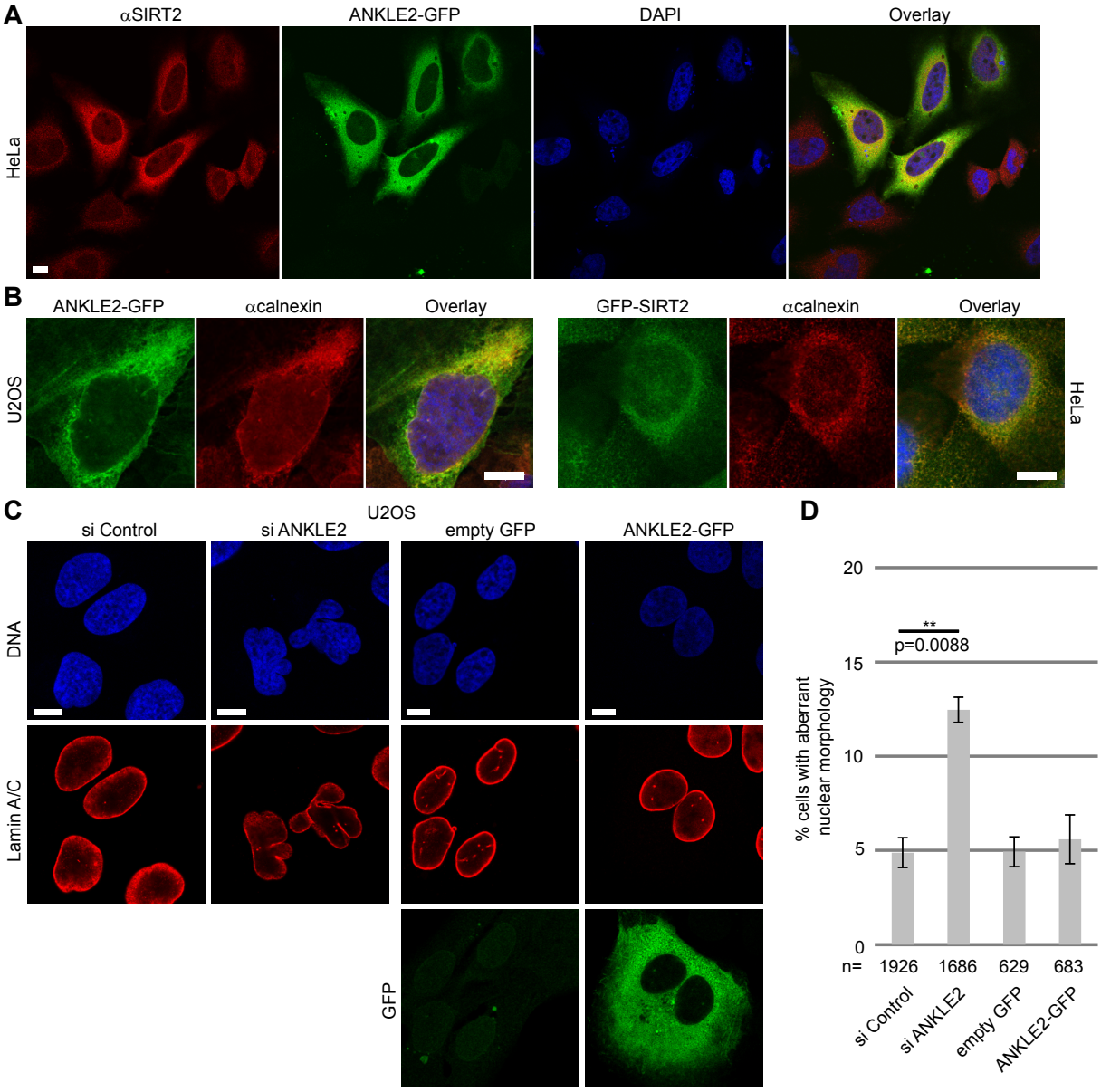

Fig. S3. ANKLE2 depletion in U2OS cells causes nuclear shape defects. (A) Immunofluorescence images showing cytoplasmic localization of endogenous SIRT2 and ANKLE2-GFP in HeLa cells. Scale bars: 10 µm. **(B)** Immunofluorescence images showing ER localization of GFP-SIRT2 in HeLa and ANKLE2-GFP in U2OS cells. Calnexin was used as an ER marker. Scale bars: 10 µm. **(C)** Immunofluorescence images of U2OS cells transfected with siANKLE2 and siControl, or empty GFP/ANKLE2-GFP for 48 h and stained with anti-laminA/C as a marker of nuclear lamina. Scale bars: 10 µm. **(D)** Quantification of lobulated nuclei caused by silencing or overexpressing ANKLE2 based on two independent experiments.

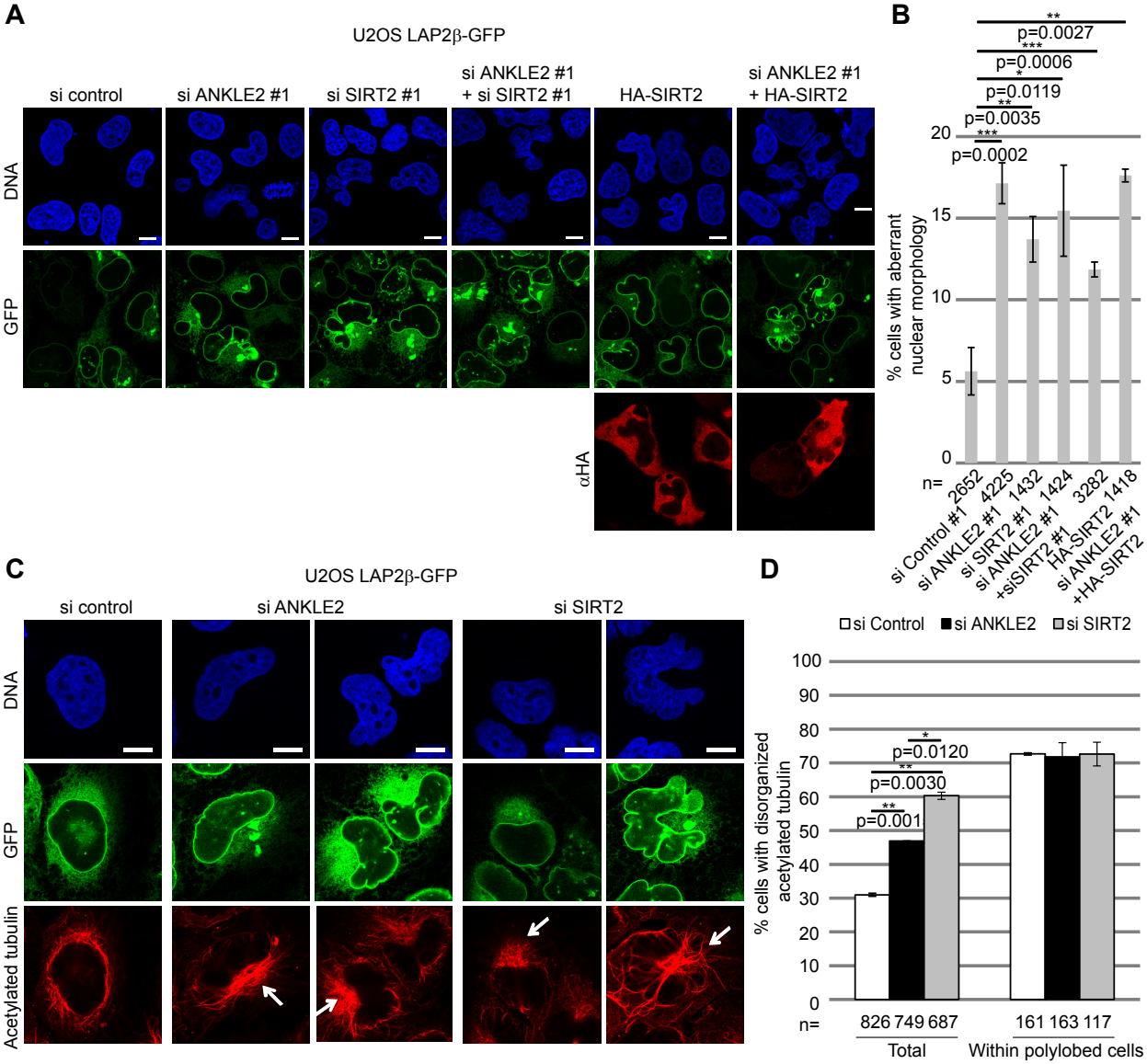

Fig. S4. SIRT2 downregulation or overexpression cannot complement ANKLE2 downregulation phenotype and nuclear shape defects caused by SIRT2 depletion are not an indirect result of tubulin acetylation. (A) Immunofluorescence images of U2OS LAP2β-GFP cells transfected with siControl, siANKLE2, siSIRT2, siANKLE2+siSIRT2, HA-SIRT2 and siANKLE2+HA-SIRT2 for 48 h. Scale bars: 10 µm. **(B)** Quantification of lobulated nuclei for conditions under (A). **(C)** Immunofluorescence images of acetylated tubulin in U2OS LAP2β-GFP cells transfected with siANKLE2, siSIRT2 and siControl for 48 h. Scale bars: 10 µm. **(D)** Quantification of aggregates of acetylated tubulin due to siSIRT2 or siANKLE2 in all cells compared to only those that exhibit the polylobed phenotype for two independent experiments.

Table S1

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Table S2

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Table S3

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Table S4

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