

Figure S1. AS-IV induces apoptosis in GH3 cells. (A) Western blot analysis of the effects of AS-IV on (B) T and (C) clv PARP expression in GH3 cells. (D) Western blot analysis of the effects of AS-IV on the relative expression of (E) pro- and (F) clv casp-3 in GH3 cells. (G) Transmission electron microscopy of GH3 cells treated with AS-IV. The black arrows indicate cell vesicles, the blue arrows indicate the formation of apoptotic bodies; red arrows indicate mitochondrial changes. *** $P < 0.001$, ** $P < 0.01$. AS-IV, astragaloside IV; T, Total; clv, cleaved; casp, caspase; ns, not significant.

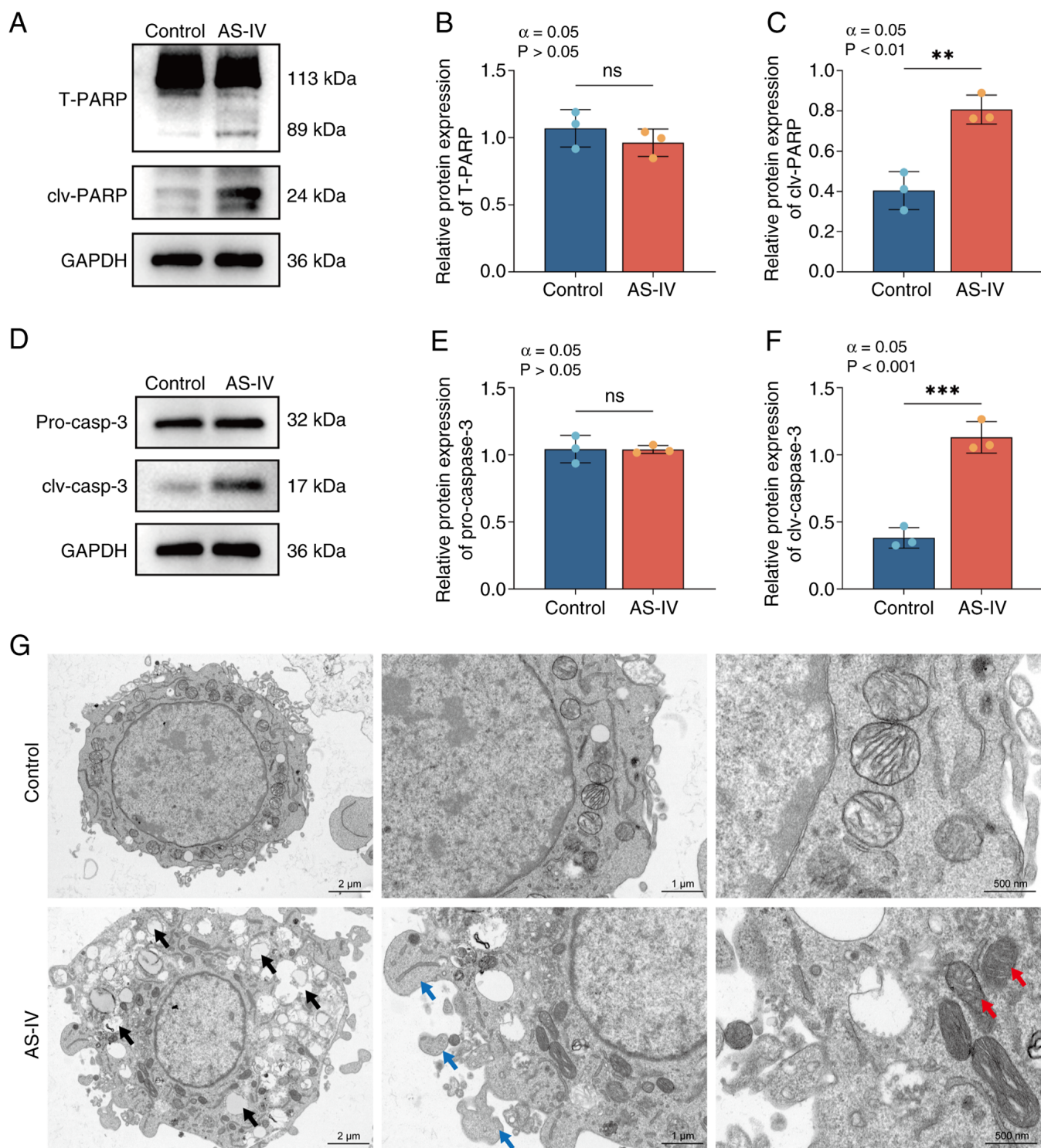


Figure S2. AS-IV specifically binds to TUBB4B, and overexpression of TUBB4B enhances the sensitivity of GH3 cells to AS-IV. (A) Thermal displacement of cells following AS-IV treatment. (B) Changes in TUBB4B protein melting curves in the Vector, (C) OE-TUBB4B and (D) KD2-TUBB4B group. (E) Protein melting curves after TUBB4B regulation. (F) Comparative IC_{50} values for the Vector, OE-TUBB4B, and KD2-TUBB4B groups were 112.8, 60.4 and 292.1 μM , respectively. AS-IV, Astragaloside IV; TUBB4B, tubulin beta 4B class IVb; OE, overexpression; CETSA, Cellular Thermal Shift Assay; KD, knockdown; IC_{50} , half-maximal inhibitory concentration.

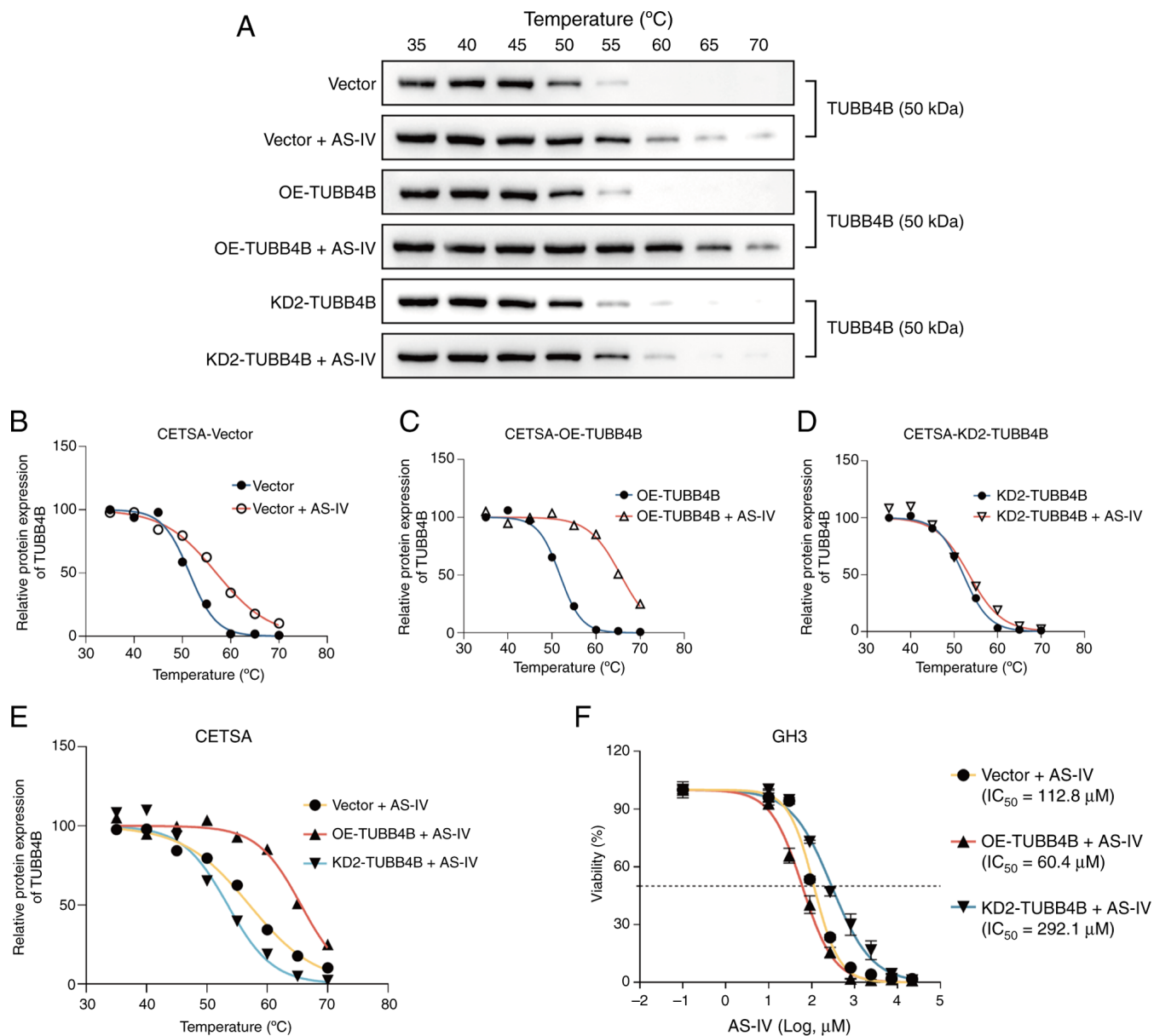


Figure S3. The TUBB4B and STMN1 proteins interact with each other. (A) Immunofluorescence colocalization of TUBB4B (green) and STMN1 (red) protein. (B) Western blot analysis of the effects of TUBB4B on the expression of (C) STMN1, (D) p-STMN1, (E) ERK and (F) p-ERK. ***P<0.001, **P<0.01, *P<0.05. TUBB4B, tubulin β 4B class IVb; STMN1, stathmin 1; p-, phospho-; OE, overexpression; KD, knockdown.

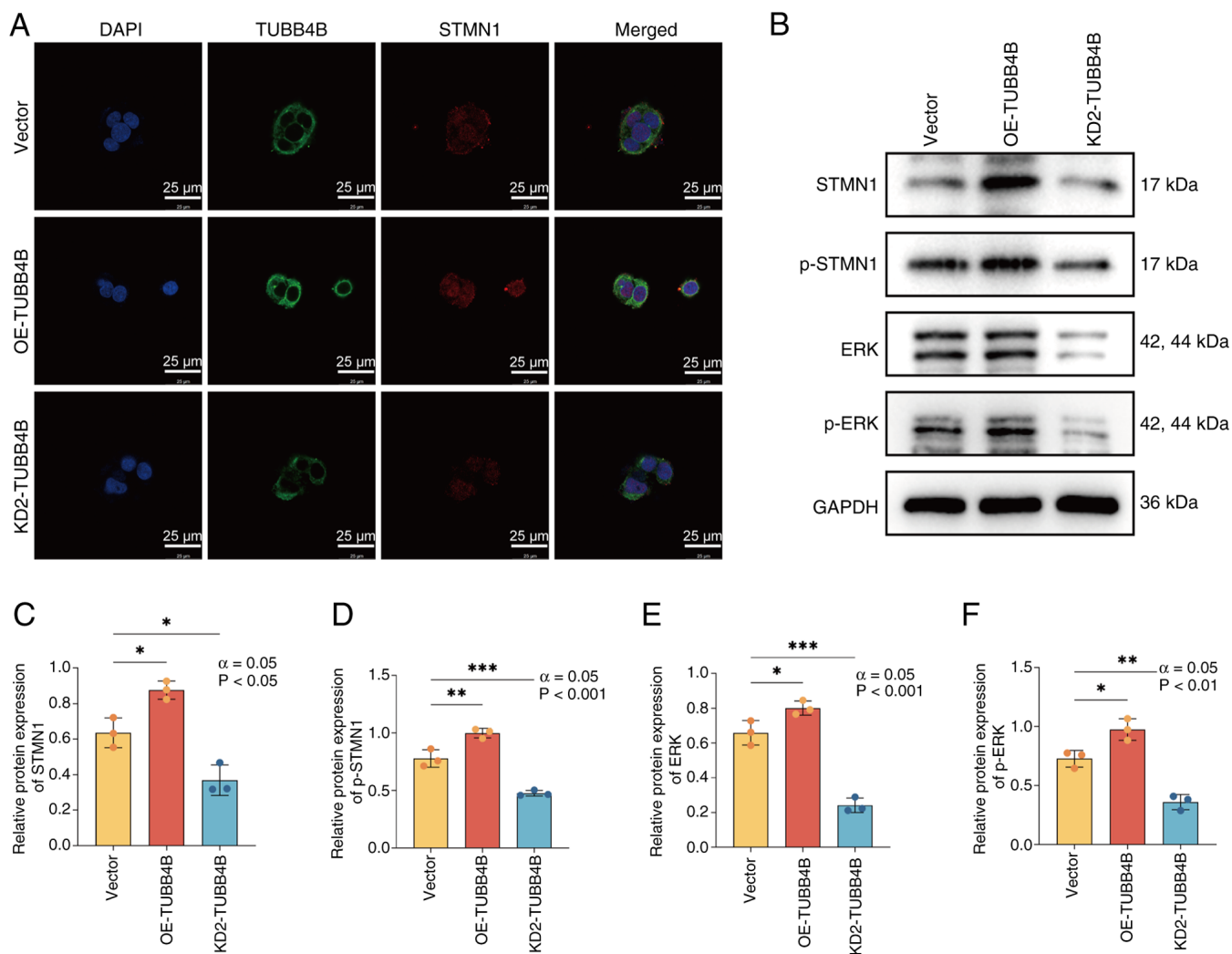


Figure S4. AS-IV induces apoptosis in xenograft tumors by targeting TUBB4B. (A) Western blotting was used to assess protein expression of (B) pro- and (C) clv-casp3 in tumor tissues from different groups. *** $P < 0.001$, ** $P < 0.01$. clv, cleaved; casp, caspase; OE, overexpression; TUBB4B, tubulin beta 4B class IVb; KD, knockdown; ns, not significant; AS-IV, astragaloside IV.

