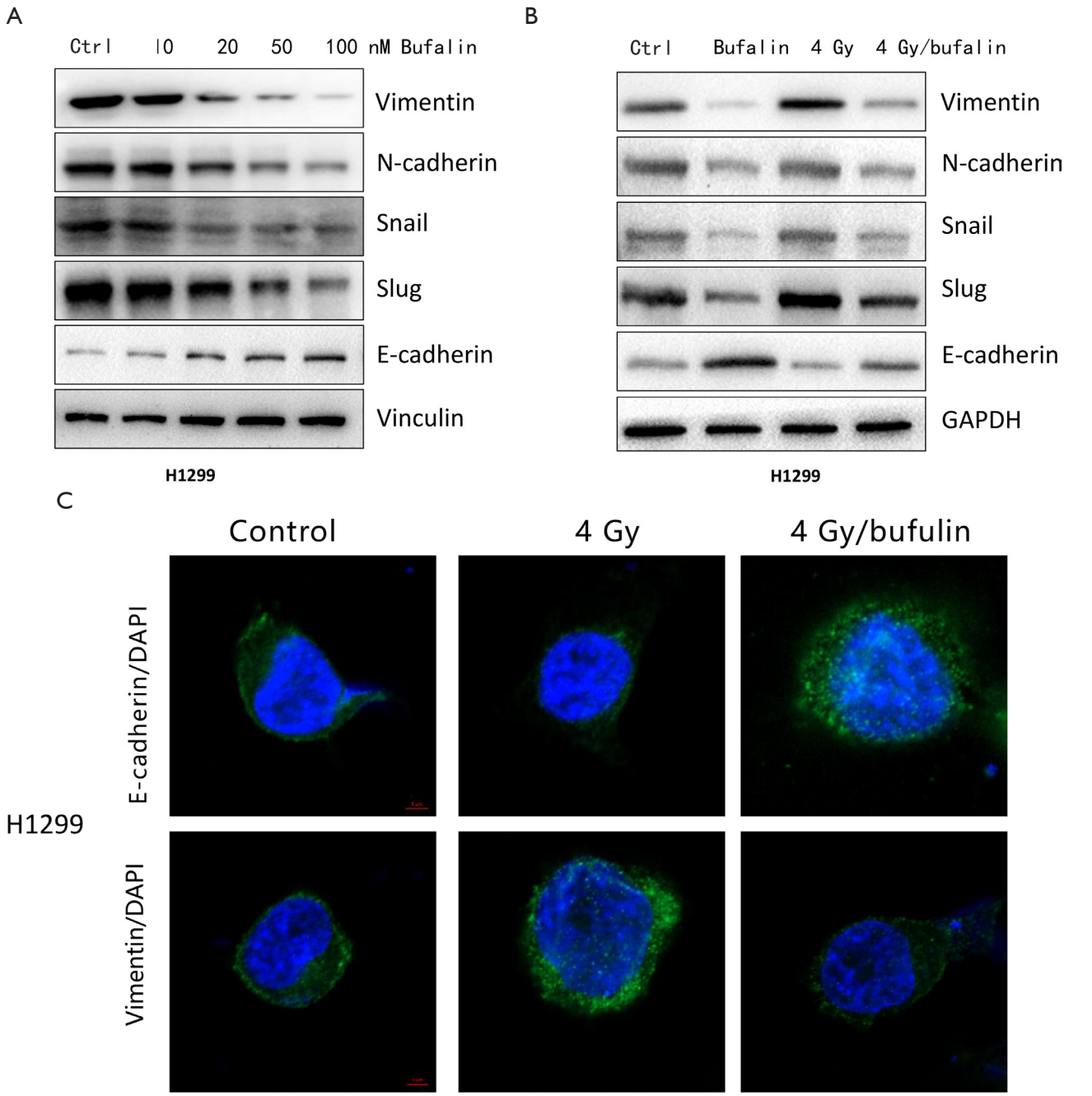

Figure S1 Bufalin inhibits EMT and abrogates radiation-induced EMT in H1299 cells. (A) Western blot analysis for protein levels of vimentin, N-cadherin, Snail, Slug, and E-cadherin after treatment of H1299 cells with increasing concentrations of bufalin. (B) Western blot to assess protein levels of vimentin, N-cadherin, Snail, Slug, and E-cadherin after treatment of H1299 cells with DMSO, bufalin, radiation, and combined treatment (50 nM bufalin + radiation 4 Gy). (C) Cells were treated with radiation (4 Gy) or radiation plus bufalin, the changes of vimentin and E-cadherin in H1299 cells were visualized by immunofluorescence microscopy (magnification ×200). Ctrl, control; DMSO, dimethyl sulfoxide; EMT, epithelial-mesenchymal transition.

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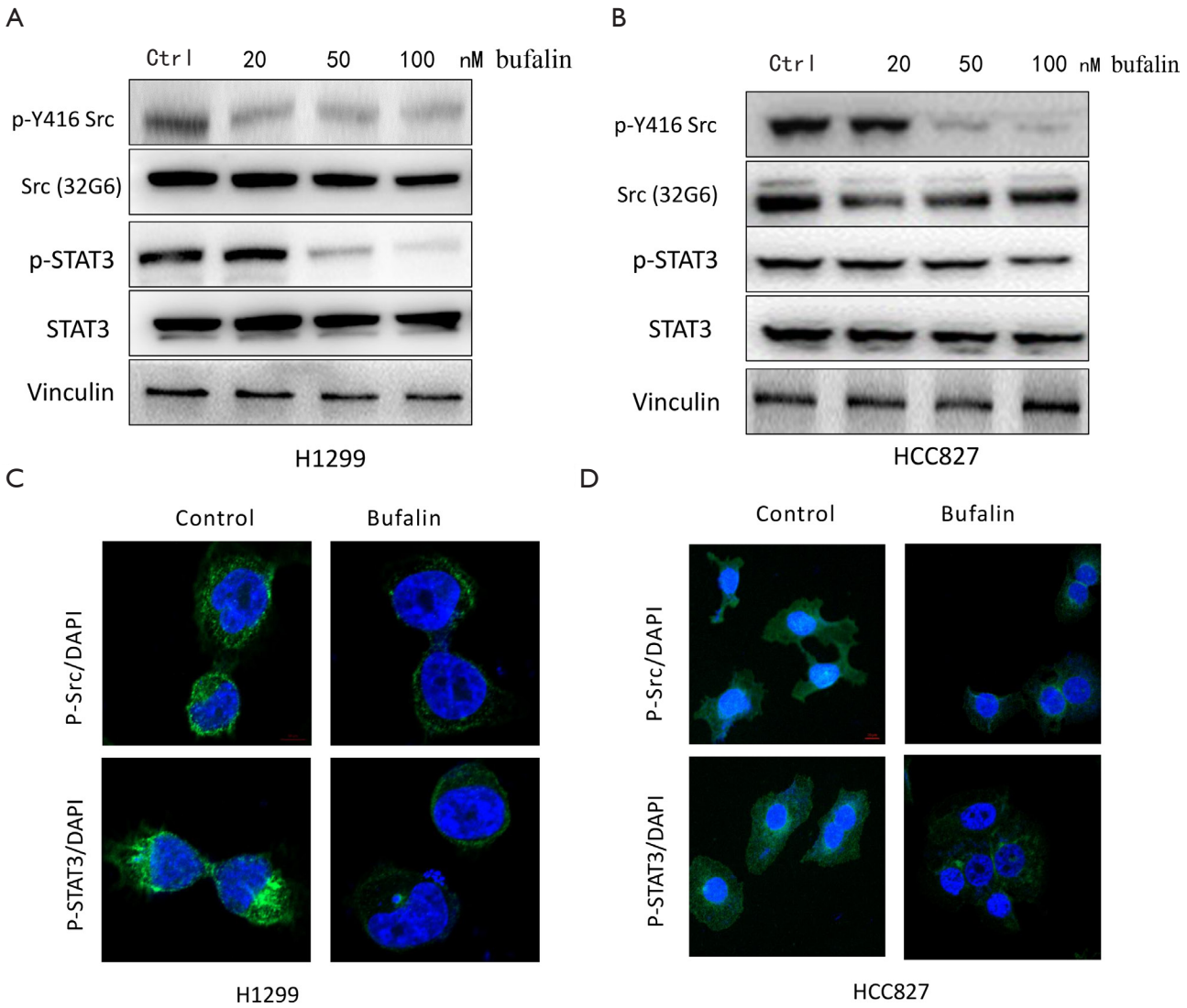

Figure S2 Bufalin reduced the phosphorylation of Src-Y416 and p-STAT3 in H1299 and HCC827 cells. (A,B) Western blot analysis for protein levels of p-Y416 Src, Src (32G6), p-STAT3, and STAT3 after treatment of H1299 and HCC827 cells with increasing concentrations of bufalin. (C,D) Representative immunofluorescence micrographs of H1299 and HCC827 cells stained with antibodies against p-Y416 SRC and p-STAT3 (magnification ×200). Ctrl, control; DMSO, dimethyl sulfoxide.

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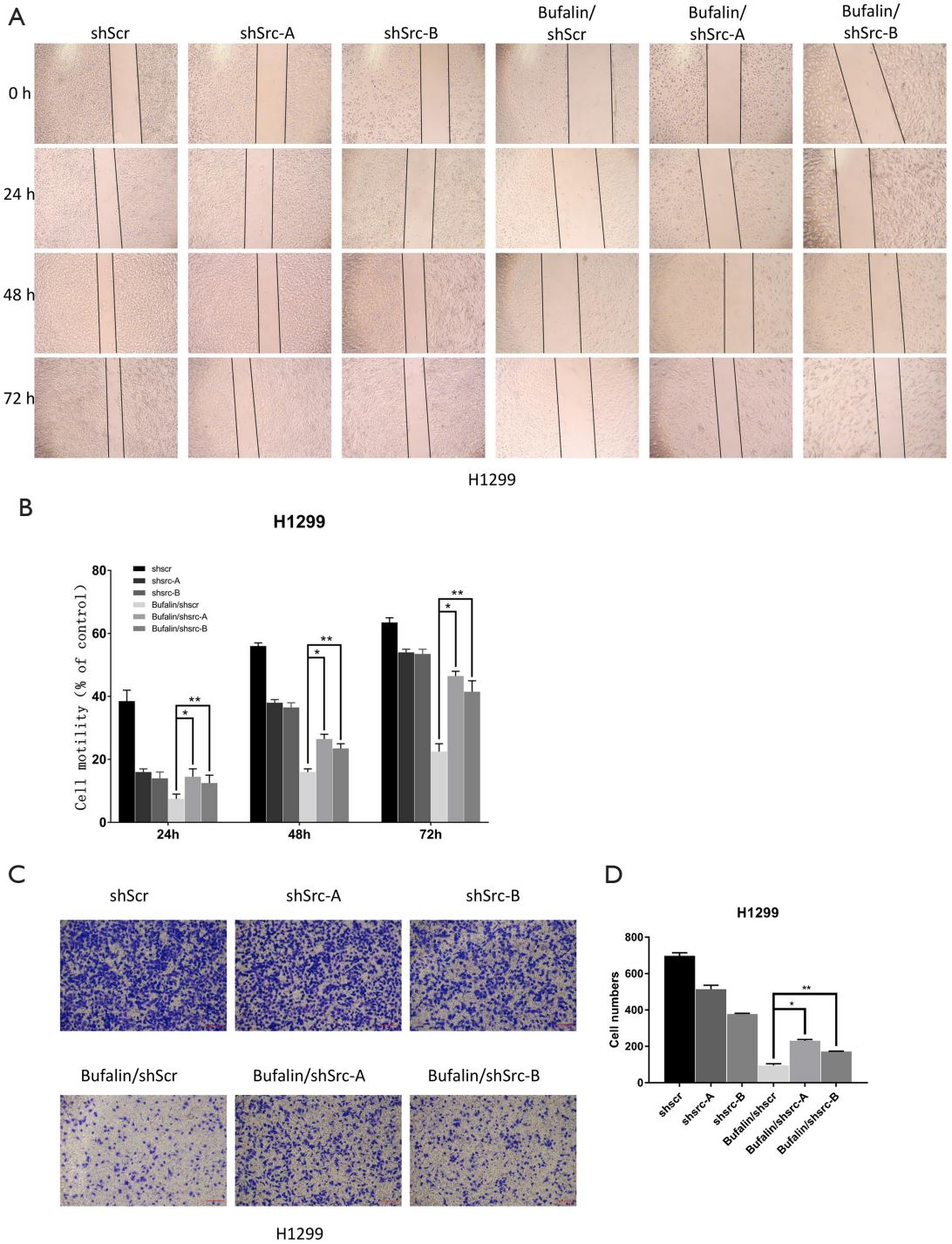

Figure S3 Depletion of Src abrogated bufalin-inhibited cell migration and invasion in H1299 cells. Wound healing assay and transwell migration assay were performed in H1299 cells. (A,B) The cells infected with PLKO.1-shScr and pLKO.1-Src-shRNAs (shSrc-A and shSrc-B) were treated with 50 nM Bufalin. The wound closure was monitored under a microscope for 72 h and photographs were taken at 0, 24, 48 and 72 h after wound generation (50× magnification), and the cell motility (% of control) was calculated. (C,D) The relative migration was calculated by counting the number of stained cells. Crystal violet staining; scale bar, 100 μm. The data are expressed as the mean ± SD of three independent experiments. *, P<0.05 *vs.* the cells infected with pLKO.1-Src-A-shRNAs virus; **, P<0.05 *vs.* the cells infected with pLKO.1-Src-B-shRNAs virus. shScr, scramble shRNA; SD, standard deviation.

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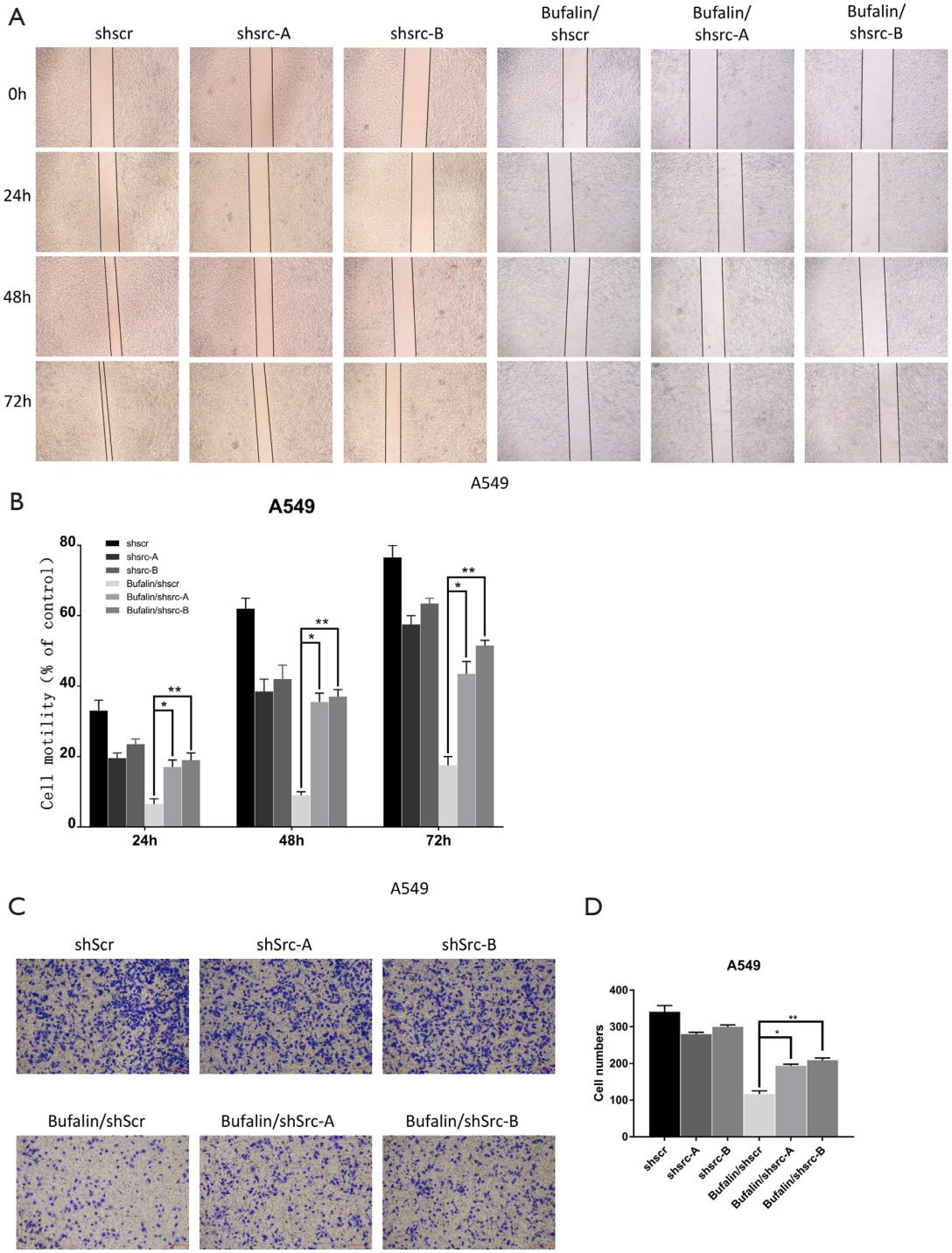

Figure S4 Depletion of Src abrogated bufalin-inhibited cell migration and invasion in A549 cells. (A,B) Wound healing assay and transwell migration assay were performed in A549 cells. The cells infected with PLKO.1-shScr and pLKO.1-Src-shRNAs (shSrc-A and shSrc-B) were treated with 50 nM bufalin. The wound closure was monitored under a microscope for 72 h, and photographs were taken at 0, 24, 48, and 72 h after wound generation (50× magnification), and the cell motility (% of control) was calculated. (C,D) The relative migration was calculated by counting the number of stained cells. Crystal violet staining; scale bar, 100 μm. The data are expressed as the mean ± SD of three independent experiments. *, P<0.05 *vs.* the cells infected with pLKO.1-Src-A-shRNAs virus; **, P<0.05 *vs.* the cells infected with pLKO.1-Src-B-shRNAs virus. shScr, scramble shRNA; SD, standard deviation.

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