

HMGB1 Promotes Prostate Cancer Development and Metastasis by Interacting with Brahma-Related Gene 1 and Activating the Akt Signaling Pathway

Running title: *HMGB1* promotes cell growth and metastasis in PCa

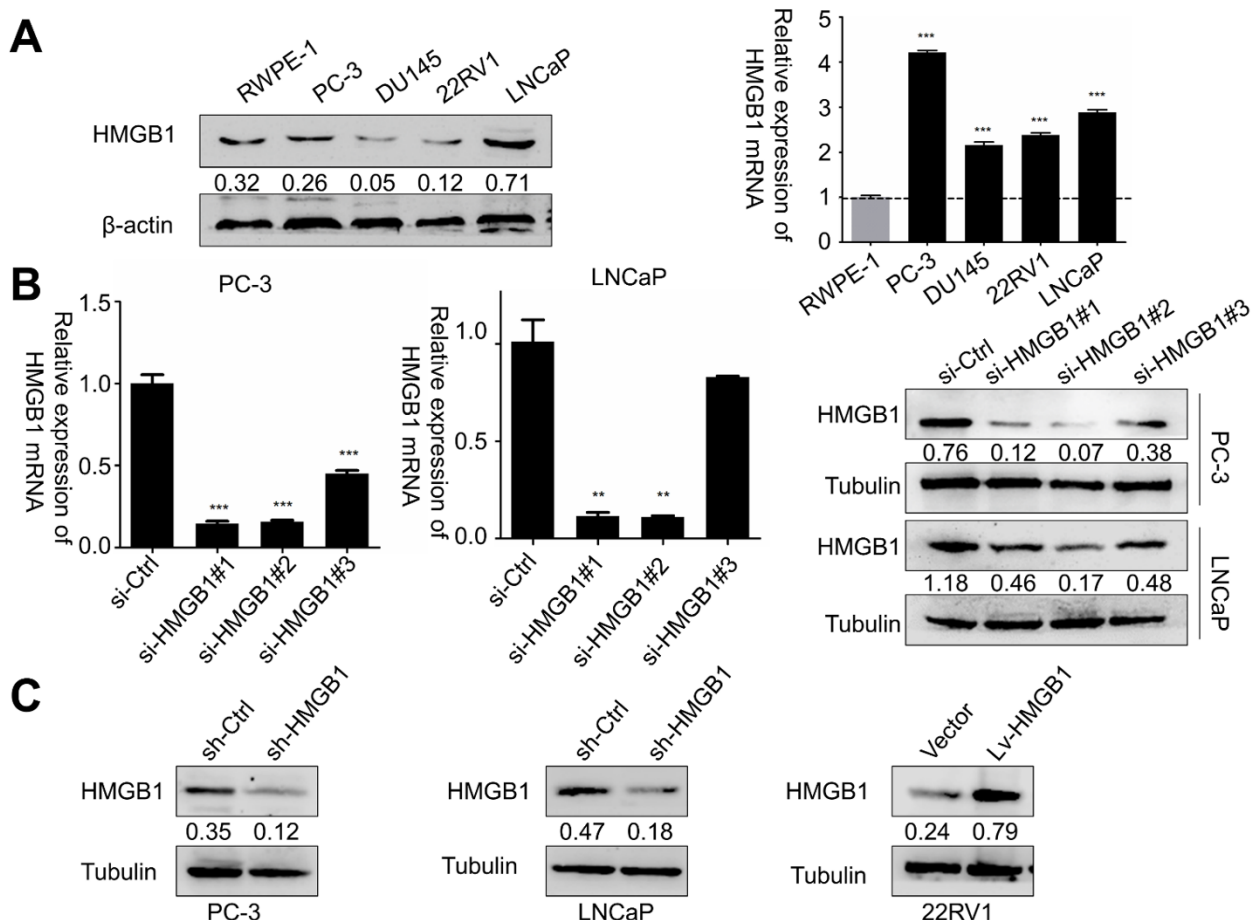
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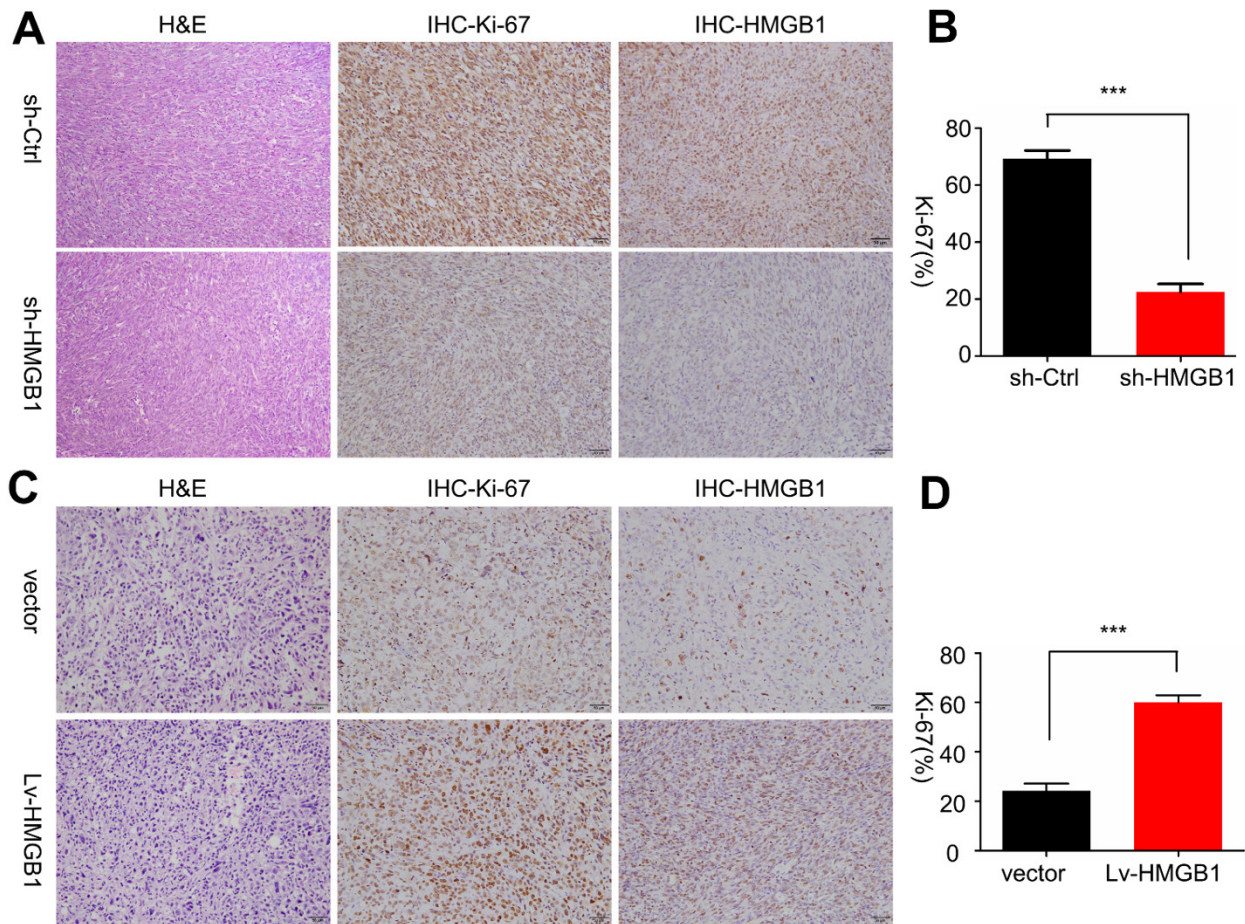
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Supplemental Data

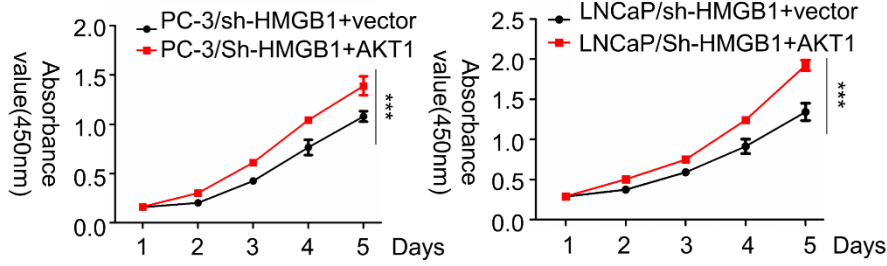
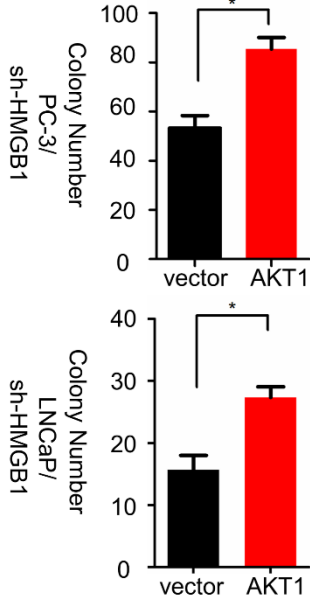
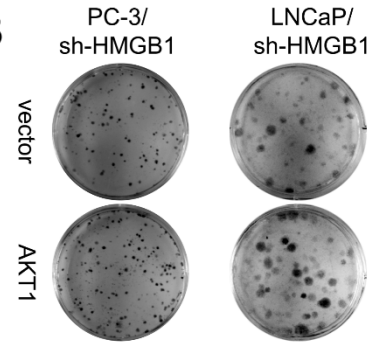
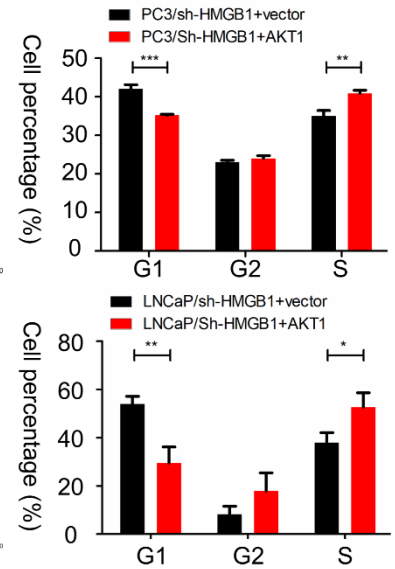
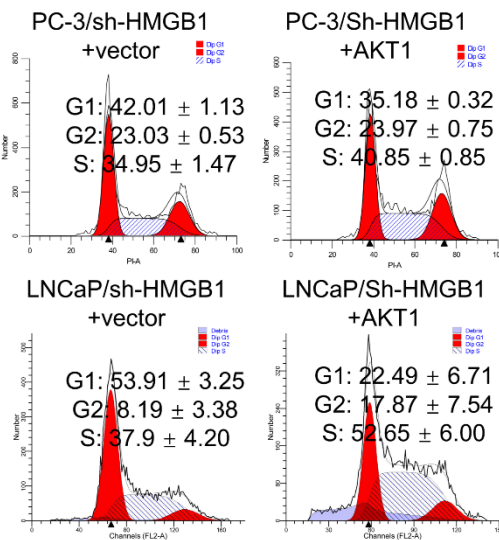
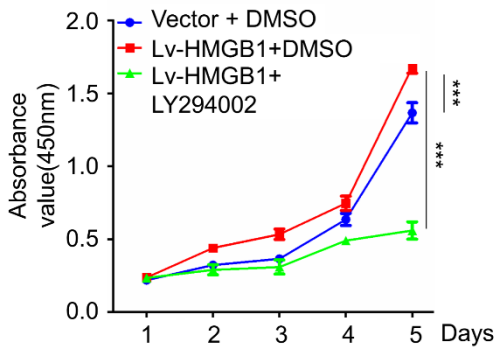
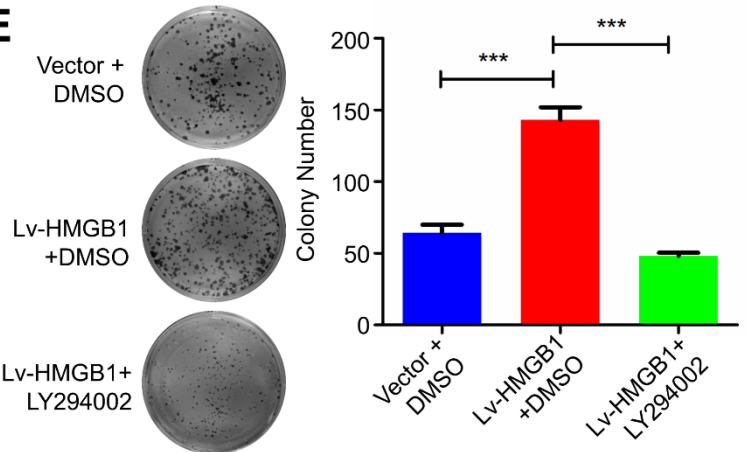
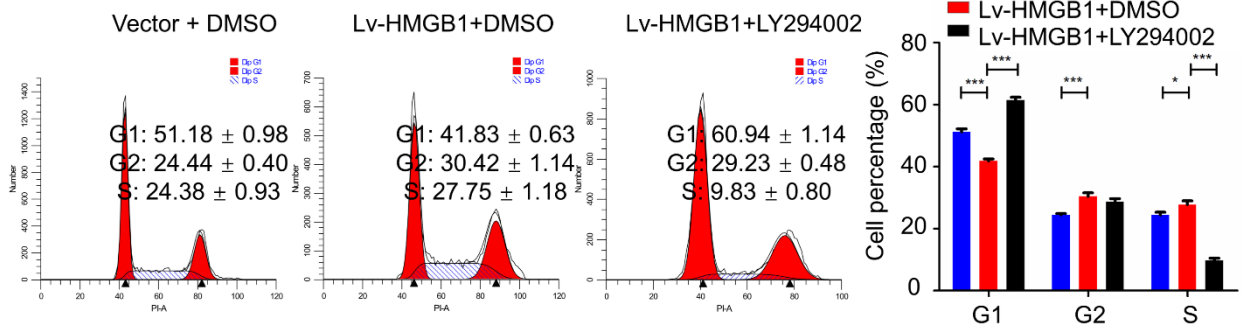


Supplemental Figure 1: *HMGB1* expression in human PCa cell lines and establishment of stable expression *HMGB1* cells. (A) Western blot and Real-time PCR analysis of *HMGB1* expression in PCa cell lines. (B) RNAi-silencing of *HMGB1* in shRNA-transduced stable PC-3 and LNCaP cells. (C) Western

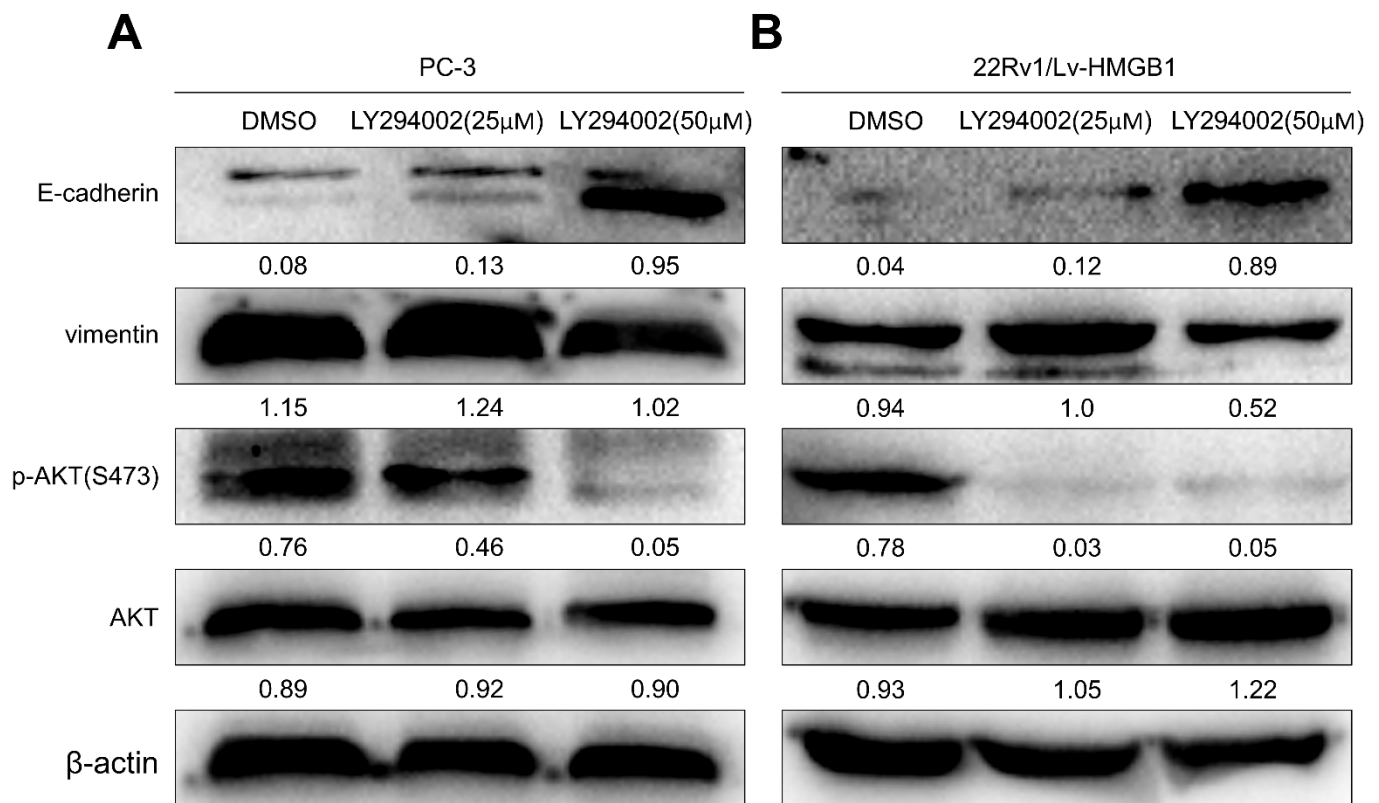
blotting analyses of *HMGB1* in specific shRNA-transduced stable PC-3, LNCaP cells and 22Rv1 cell with ectopic expression of *HMGB1*. *α-Tubulin* was used as a loading control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.



Supplemental Figure 2: *HMGB1* and *Ki-67* immunohistochemical staining of xenograft tumor tissues. (A, C) Representative photographs of tumor tissue structures (H&E) and *HMGB1* and *Ki-67* immunohistochemical staining of xenograft tumor tissues derived from control (sh-Ctrl) or silencing *HMGB1* (sh-*HMGB1*) shRNA-contained PC-3 (A) or overexpression of *HMGB1* (Lv-*HMGB1*) 22Rv1 cells (B) as indicated. Magnification: 200x. (B, D) Quantification of *HMGB1* and *Ki-67* expression. *** $P < 0.001$ compared to the sh-Ctrl or empty vector group, n = 6.

A**B****C****D****E****F**

Supplemental Figure 3: *HMGB1* activates *Akt* signaling pathways. (A-B) Transfection of constitutively activated *Akt* (myr-*Akt*) into PC3-sh*HMGB1* and LNCaP-sh*HMGB1* cells restored proliferation, as determined by CCK-8 assays and colony formation assays. (C) Upon myr-*Akt* transfection into PC3-*HMGB1*-shRNA and LNCaP-*HMGB1*-shRNA cells, the percentage of cells in G₁ phase decreased and the percentage in S phase increased. (D, E and F) Inhibition of the *Akt* signaling blocks the promoting effect of *HMGB1*-overexpression on cell proliferation and cell cycle progression of PCa cells as determined by CCK-8 assay (D), colony formation assay (E) and flow cytometry (F) after treatment with LY294002 (50 μM). Error bars represent mean ± SD from 3 independent experiments; **P*<0.05, ***P*<0.01 and ****P*<0.001.



Supplemental Figure 4: LY294002 blocked *Akt* phosphorylation in PC-3 and 22Rv1/Lv-*HMGB1* cells. Western blot analysis was performed to evaluate p - *Akt*, *Akt* and EMT markers expression under LY294002 treatment for 48 h in PC-3(A) and 22Rv1/Lv-*HMGB1*(B). p - Akt: phosphor - *Akt*.

Supplemental Table1: The information of proteins analyzed from LC-MS-MS.

Supplemental Table2: The characteristics of patients and the stain score of *HMGB1* and *BRG1*.