which acts downstream of Ras. However, NgBR overexpression or knockdown did not significantly affect the activity of the Akt signaling pathway in NSCLC cells. These results were further confirmed by pharmacologically blocking the MEK/ERK signaling pathway by using the MEK inhibitor U0126 or by performing a rescue experiment with an NgBR cDNA or siRNA.

Thus, the results of the present study indicate the importance of NgBR in NSCLC progression and highlight molecular mechanisms underlying NgBR-induced migration, invasion, and metastasis of NSCLC cells. However, additional clinical investigations should be performed to determine NgBR as a therapeutic target for preventing tumor metastasis in patients with NSCLC.

Conflicts of interest

The authors declare that they do not have any conflicts of interest related to this study.

Acknowledgements

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Appendix A. Supplementary data

The following are the supplementary data related to this article:

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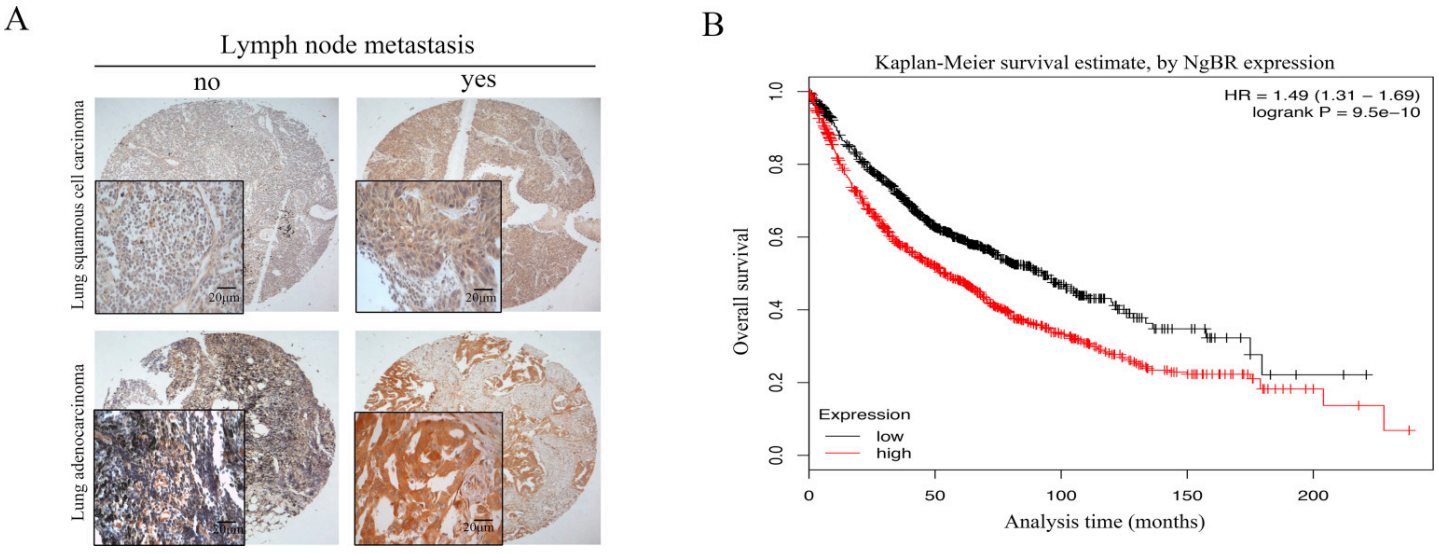

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Fig. S1. A. Immunohistochemical staining of high NgBR expression in NSCLC-metastasized lymph nodes of lung adenocarcinoma and lung squamous cell carcinoma. Scale bar, 20μm and 200μm. B. NgBR expression is negatively

associated with overall survival of NSCLC patients. NgBR (NUS1) mRNA expression values were retrieved from lung cancer profiling dataset deposited in a Kaplan-Meier Plotter (probe ID: 215207_x_at NUS1). Kaplan-Meier analysis of overall survival with high or low NgBR expression of 2437 NSCLC patients (p=9.5e-10, log-rank test).

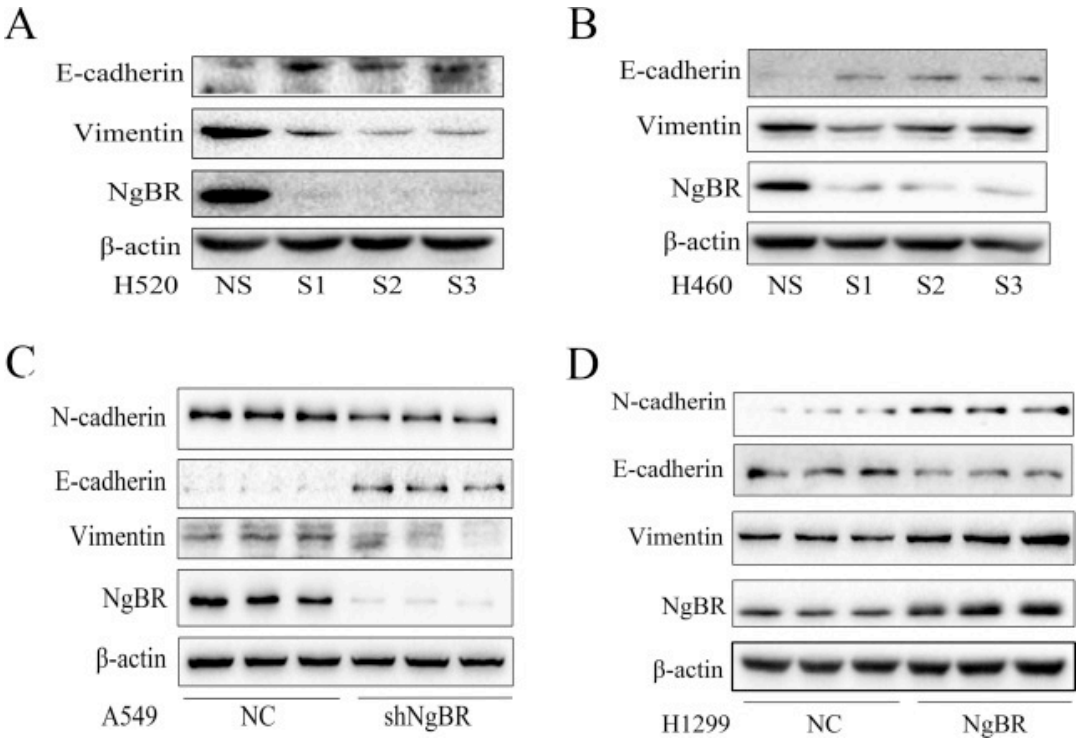

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Fig. S2. NgBR knockdown and overexpression in NSCLC cells. A, H520 and B, H460cells were transfected with NgBR siRNA (S1, S2 and S3) or All-Star non-silencing siRNA (NS) for 72h and subjected to whole-cell lysate extraction and Western blot analysis. C, stably NgBR shRNA or nonspecific control transfected A549cells, and D, stably pIRES-NC (NC) and pIRES-NgBR (NgBR) transfected H1299cells were grown and subjected to Western blot analysis.

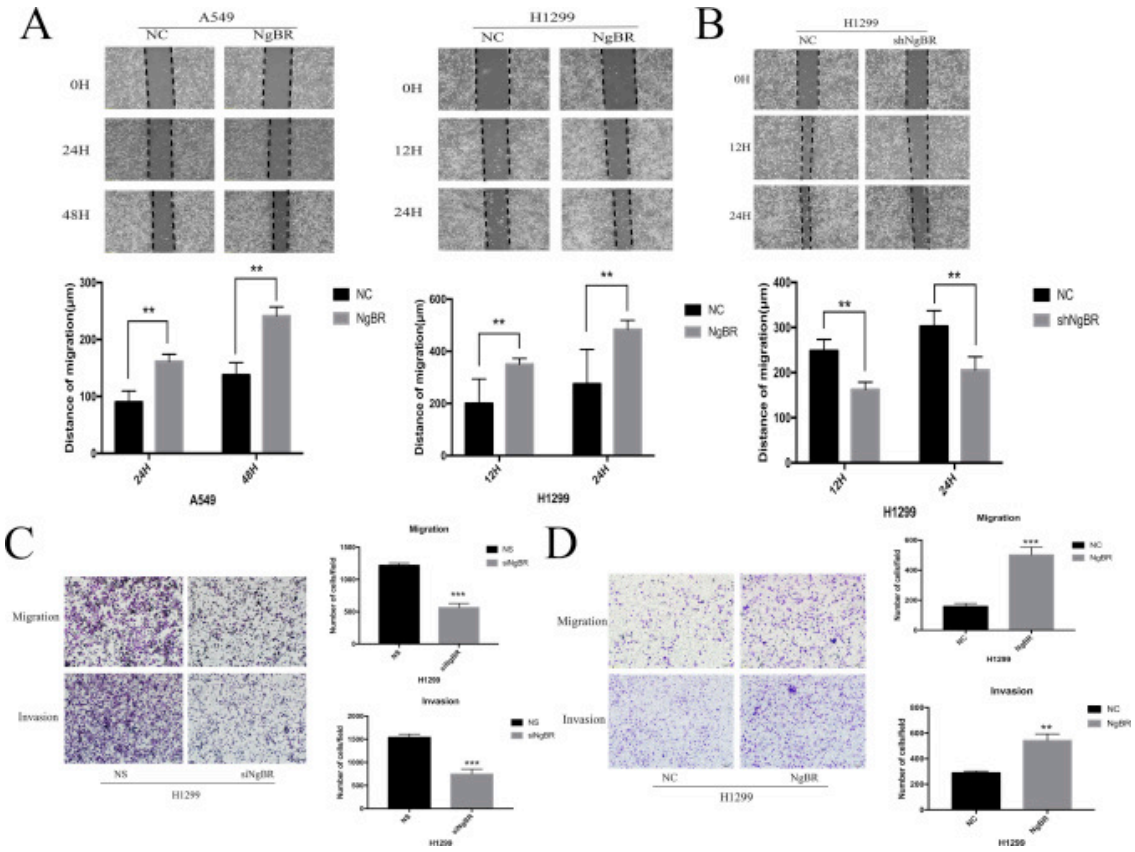

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Fig. S3. Effect of NgBR knockdown and overexpression on the migration and invasion capacity of NSCLC cells. A, wound healing assays were performed in stably transfected A549 and H1299 cells with NgBR and NC. B, wound healing assay in stably transfected H1299 cells with shNgBR and NC. Images were taken at 0 h, 24 h, and 48 h in A549 cells and at 0 h, 12 h, and 24 h in H1299 cells. Scale bar, 200 μm. Error bar, SD of three independent experiments. **p < 0.01. C, transwell migration and invasion assay in H1299 cells transfected with NgBR siRNA (S1, S2 and S3) or All-Star non-silencing siRNA (NS) for 72 h. D, transwell migration and invasion assays were performed in stably NgBR or NC transfected H1299 cells. Images were taken at 8 h (migration) and 12 h (invasion). Scale bar, 100 μm. Error bar, SD of three independent experiments. **p < 0.01 and ***p < 0.001.

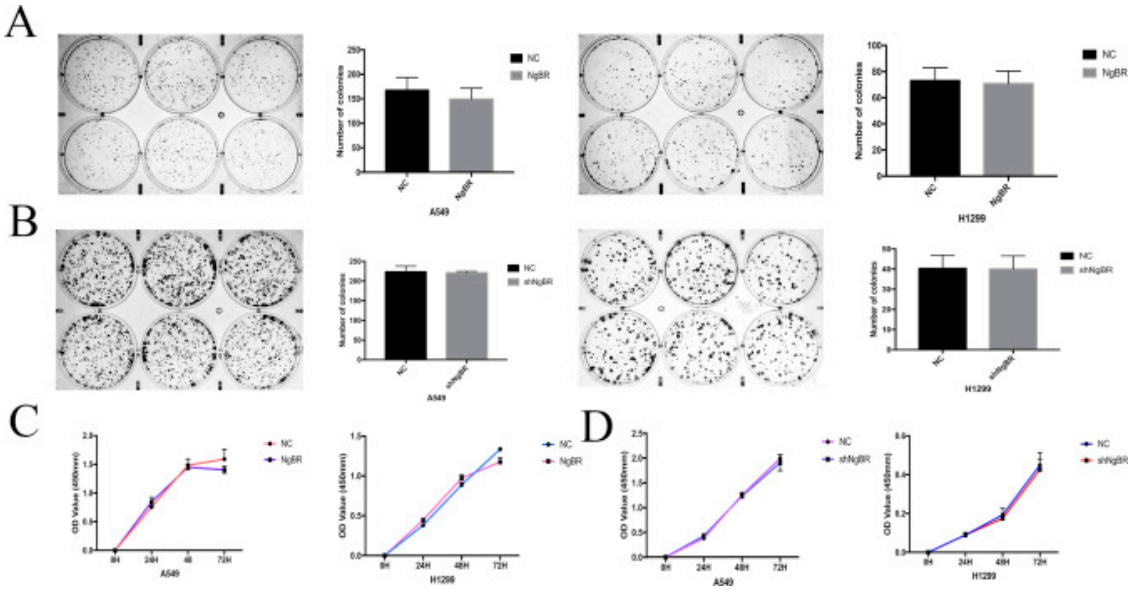

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Fig. S4. Effect of NgBR knockdown and overexpression on NSCLC cell proliferation. A and B, the colony formation experiments and C and D, CCK-8 assays were performed in either A549 transfected with NgBR plasmid DNA or H1299cells stably transfected with shNgBRi, respectively.

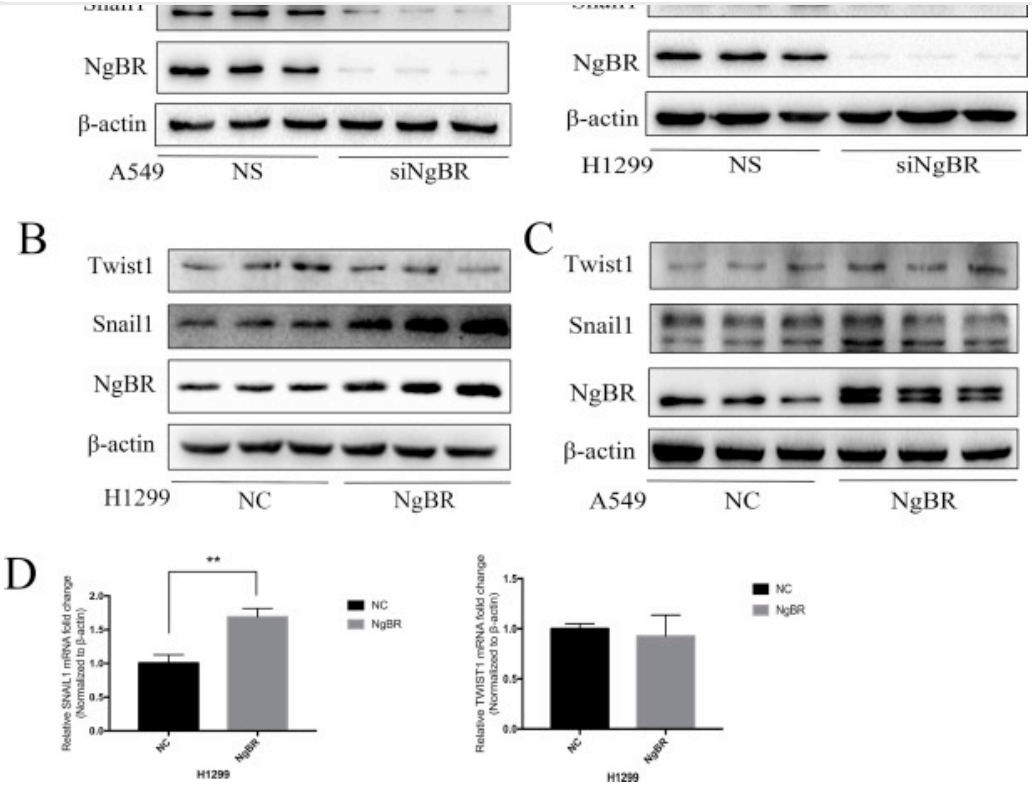

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Fig. S5. Effect of NgBR knockdown and overexpression on the expression of Snail1 and Twist1 in NSCLC cells. A, A549 and H1299 cells were transfected with siRNA-targeting NgBR or All-Star non-silencing siRNA (NS) for 72h and then subjected to whole-cell lysates extraction and Western blot. B, stably NgBR or NC transfected H1299 cells were grown and then subjected to whole-cell lysate extraction and Western blot analysis. C, A549 cells were transfected with NgBR and NC for 72h and then subjected to whole-cell lysate extraction and Western blot analysis. D, stably NgBR transfected H1299 cells were grown and then subjected to qRT-PCR analysis of Snail1 and Twist1 mRNA levels. β-actin was used as a normalized control. Error bar, SD of three independent experiments. **$p < 0.01$.

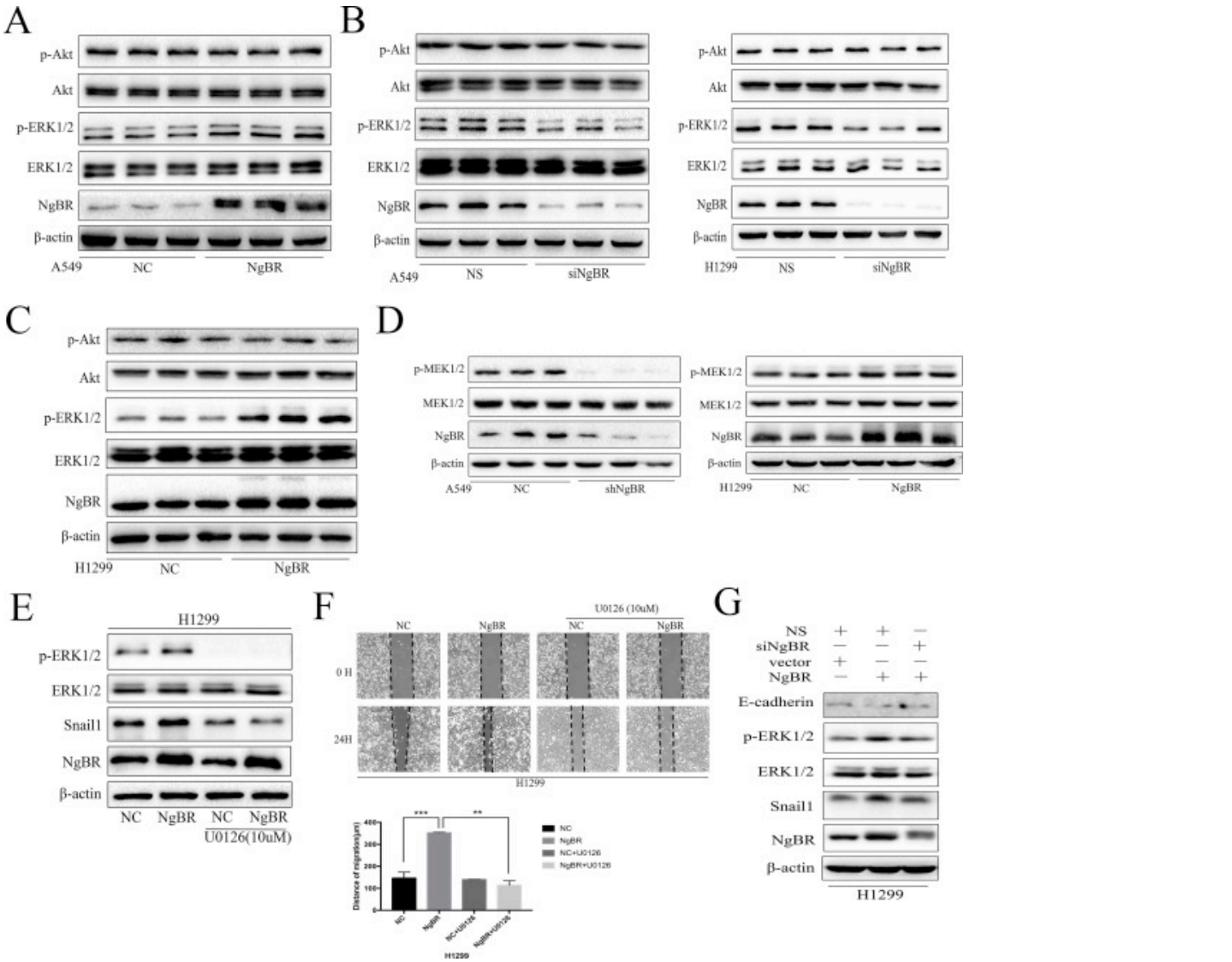

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Fig. S6. Effect of NgBR knockdown and overexpression on the regulation of MEK1/2/ERK1/2 pathway in NSCLC cells. A, A549cells were transfected with NgBR and NC for 72h and then subjected to whole-cell lysate extraction and Western blotting. B, A549 and H1299cells were transfected with siRNA-targeting NgBR or All-Star non-silencing siRNA (NS) for 72h and then subjected to whole-cell lysate extraction and Western blotting. C, stably NgBR or NC transfected H1299cells were grown and then subjected to whole-cell lysate extraction and Western blotting. D, stable A549 and H1299cells were grown and then subjected to whole-cell lysate extraction and Western blot analysis for *p*-MEK1/2. Total Mek1/2 and β-actin levels were used as loading controls. E, stably pIRES-NC (NC) or pIRES-NgBR (NgBR) transfected H1299cells were grown and treated with or without U0126 (10μM) for 24h and then subjected to Western blot analysis for *p*-ERK1/2, ERK1/2 and Snail1. F, stably pIRES-NC (NC) or pIRES-NgBR (NgBR) H1299 cells were grown and treated with or without U0126 (10μ M) for 24 h and then subjected to wound-healing assay. Images were taken at 0 h and 24 h. Scale bar, 200 μm (upper panel). Quantitative data on the low panel. Error bar, SD of three independent experiments. **<0.01 and ***p < 0.001. G, stably pIRES-NC (NC) or pIRES-NgBR (NgBR) transfected H1299 cells were grown and then transfected with siNgBR or NS for 48 h and then subjected to Western blot analysis of E-cadherin, *p*-ERK1/2, and Snail1 levels. Total ERK1/2 and β-actin levels were used as loading controls.

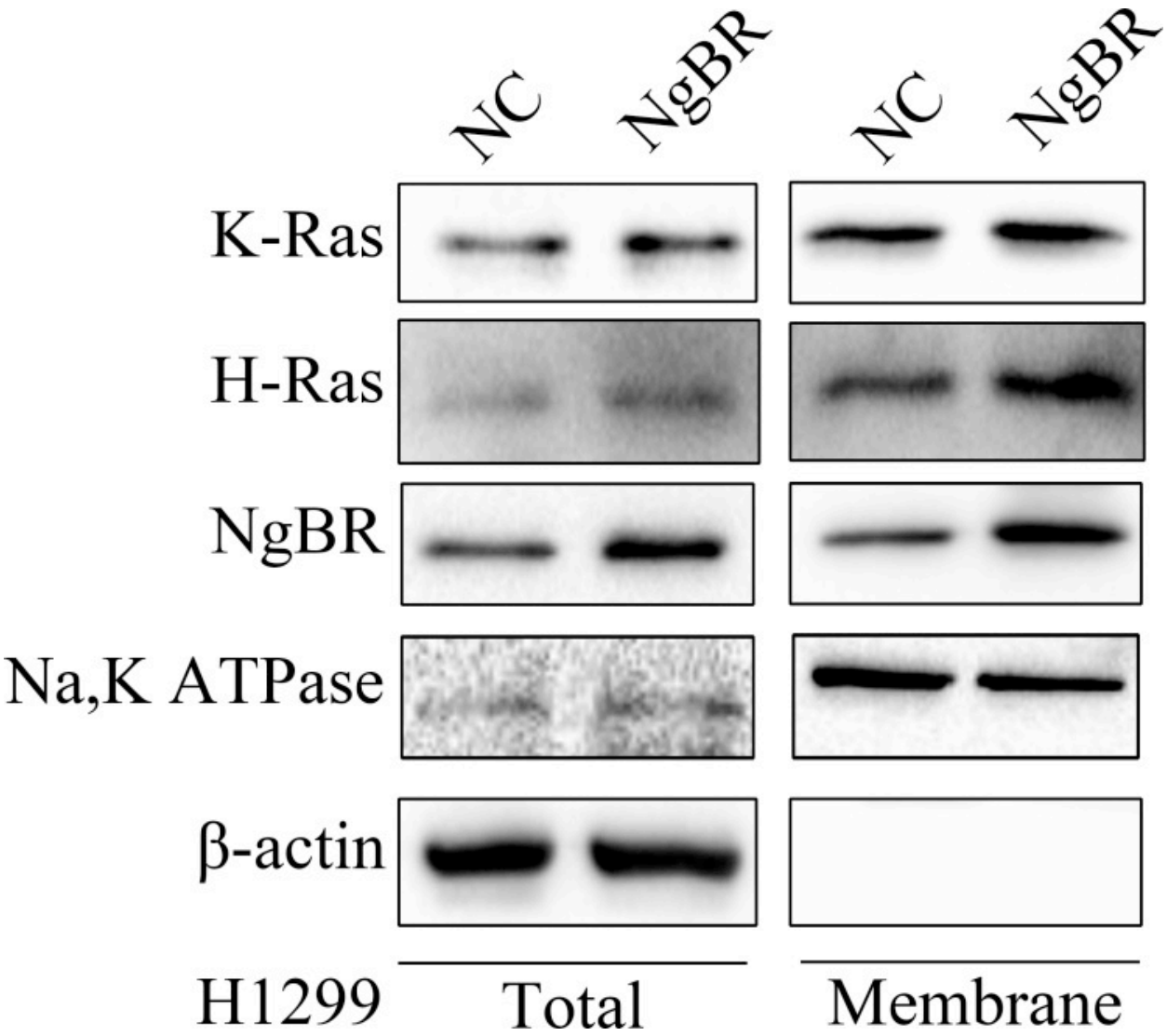

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Fig. S7. Effect of NgBR overexpression on the regulation of gene expression in H1299cells. Stably NgBR or NC transfected H1299cells were grown and subjected to membrane and cytoplasmic protein extraction and Western blot analysis of K-Ras and H-Ras. Na, K ATPase and β-actin were used as markers for the membrane and cytoplasm, respectively.

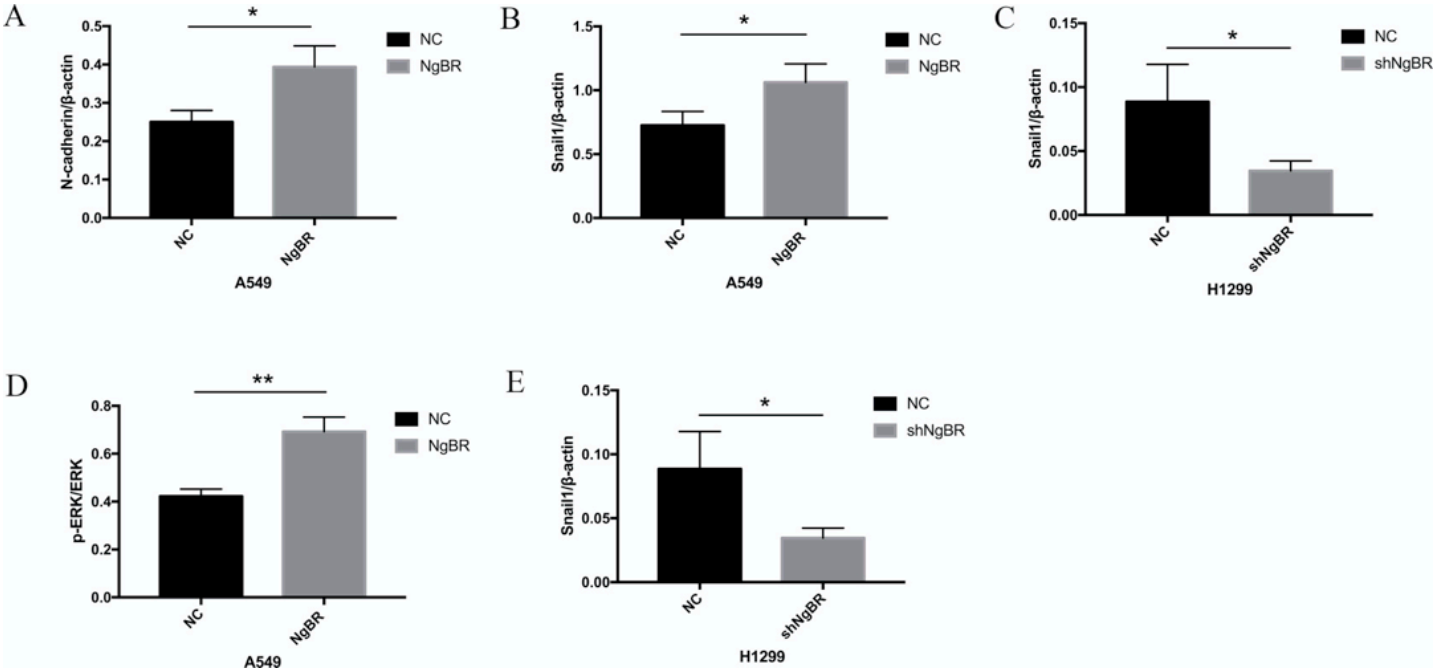

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Fig. S8. Densitometric analysis of related proteins coupled with statistical comparison.

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