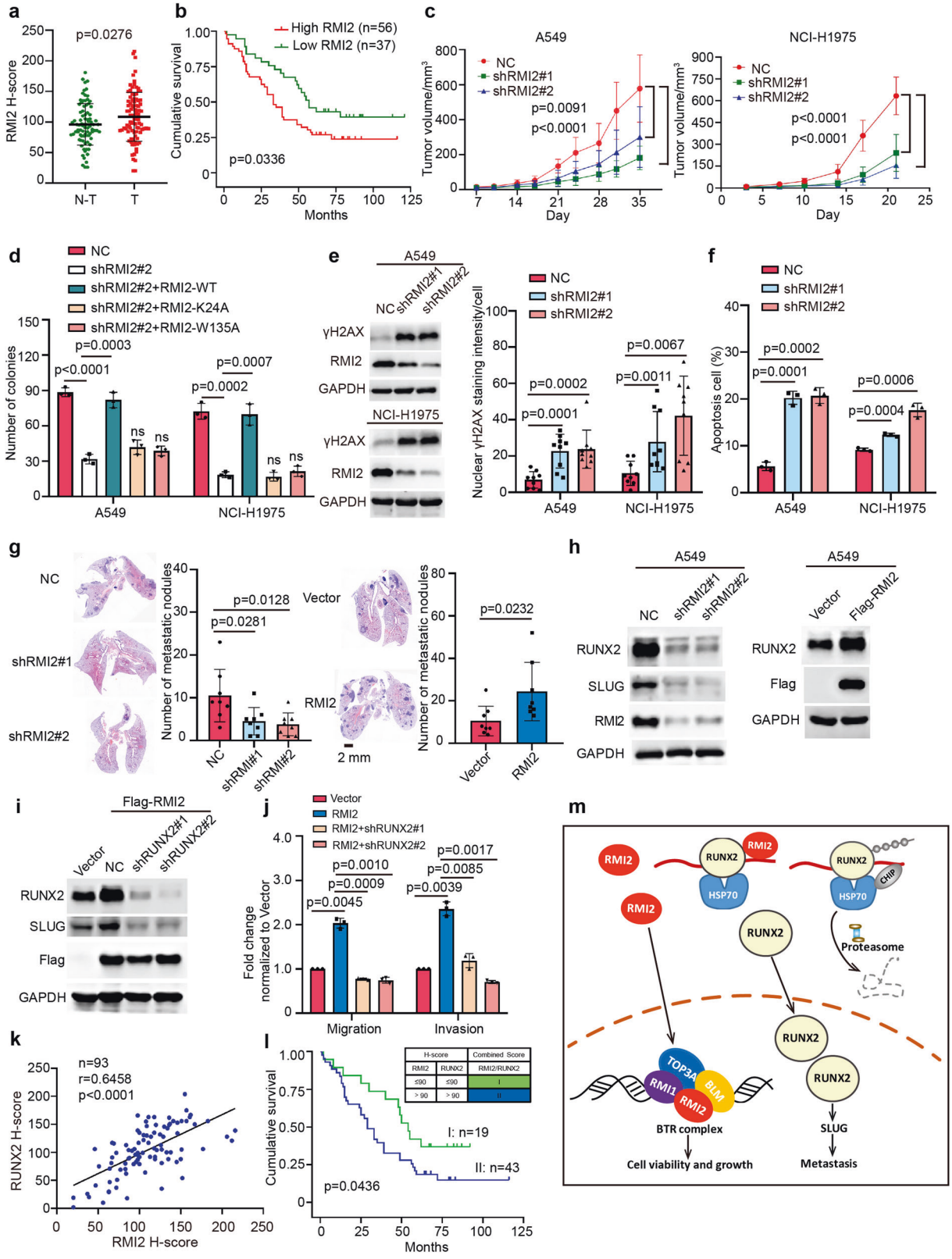
LETTER OPEN

RMI2 plays crucial roles in growth and metastasis of lung cancer

Signal Transduction and Targeted Therapy



CHIP could not be detected in the Flag-RMI2/RUNX2 complex in NCI-H1975 cells (Supplementary Fig. S6d), suggesting that the scarcely misfolded RUNX2 may exist when RMI2 is overexpressed in cells, and ubiquitination of RUNX2 mediated by CHIP was decreased by RMI2 (Supplementary Fig. S6e). Furthermore, the decrease of RUNX2 by knocking down RMI2 was significantly rescued with proteasome inhibitor bortezomib and lysosomal inhibitor Bafilomycin A1 (Supplementary

Fig. 1 RMI2 plays crucial roles in growth and metastasis of lung cancer. **a** IHC staining of the primary human LUAD tissue microarray and adjacent noncancerous tissues. Scatter plot graph showing a statistical analysis of RMI2 expression in LUAD and adjacent noncancerous tissues. Data are means ± s.e.m. $p = 0.0276$ by student's *t*-test. **b** Overall survival curves were generated based on the protein levels of RMI2 in LUAD tissue microarray. $p = 0.0336$ using Kaplan–Meier plots and compared with the log-rank test. **c** The indicated stable cells were xenografted subcutaneously on the flank of nude mice ($n = 8$/group). Visible tumors were measured twice a week. Data are means ± s.e.m. of tumor volume. *p* values were calculated by two-way ANOVA. **d** The indicated stable cells were subjected to colony formation assay. The bars indicate the s.e.m. The results are expressed as the mean ± s.e.m. ($n = 3$). *p* values were calculated by student's *t*-test. n.s no significance. **e** The indicated stable cells were analyzed by western blotting and Nuclear γH2AX staining intensities per cell were quantified. Data are means ± s.e.m. *p* value was calculated by student's *t*-test versus the shNC control. **f** Annexin-V and propidium iodide staining for apoptosis are quantified in the indicated stable cells. Data are means ± s.e.m. *p* value was calculated by student's *t*-test versus the shNC control. **g** The indicated stable NCI-H1975 cells were injected into randomized athymic nude mice by tail-vein injection. Representative images of H&E-stained sections in the dissected lungs after inoculation for 6 weeks are shown, and the metastatic nodules were quantified based on the H&E-stained lung sections. Data are means ± s.e.m., $n = 8$, and the *p* values were calculated by student's *t*-test. **h**, **i** The indicated stable cells were subjected to Western blotting. **j** The A549 stable cells were subjected to migration and invasion assays. The columns were mean of three independent experiments. Data are means ± s.e.m. *p* values were calculated by student's *t*-test versus the shNC control. **k** The co-high and co-low subgroups of RMI2 and RUNX2 were determined based on the combination of both RMI2 and RUNX2 staining values. $n = 93$. Coefficient of correlation (r) and *p* value were calculated by the nonparametric Spearman's test. **l** Kaplan–Meier survival analyses of the co-high and co-low subgroups of RMI2 and RUNX2. $p = 0.0436$ was calculated by the log-rank test. **m** A proposed model for the roles of RMI2 in lung cancer growth and metastasis

Fig. 6f), as the aggregated proteins are generally degraded by proteosome and/or lysosome. These results determine that RMI2 may act as a chaperon to stabilize RUNX2 by facilitating its fold, which is independent of BTR complex.

There was a strong positive correlation between RMI2 and RUNX2 at their protein levels using 39 fresh-frozen human LUAD tissues (Supplementary Fig. S6a, b), which was further validated by IHC using a tumor tissue microarray (Fig. 1k and Supplementary Fig. S6c). The Kaplan–Meier survival analysis showed that lung cancer patients with low and high levels of both RMI2 and RUNX2 predicted better and poorer survivals, respectively (Fig. 1l). These results illustrate that elevated RMI2 is correlated with high RUNX2 in lung cancer.

In summary, we provide evidences for the first time that RMI2 is critical for growth and metastasis of lung cancer, which is dependent- and -independent of BTR complex, respectively, indicating that RMI2 may be a promising therapeutic target for lung cancer. As illustrated in Fig. 1m, synthesized in cytoplasm, RMI2 can be shuttled to nucleus, where it acts as BTR complex to maintain genomic integrity, which is required for cell viability and tumor growth. On the other hand, RMI2 may function as a chaperon molecule, which is independent of the BTR complex, to facilitate the fold of RUNX2, which in turn to avoid the degradation of RUNX2 by CHIP, consequently, SLUG is transcriptionally up-regulated by more RUNX2 in the nucleus to promote cancer metastasis.

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ADDITIONAL INFORMATION

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