Supplementary Figure, Table, and Movie Legend

Figure S1. (related to Figure 1) IHC staining of RBP2 protein in normal and cancerous human lung tissues. Shown are 1 example of normal adjacent tissue and 1example of tumor section.

Figure S2. (related to Figure 2B) Knockdown of RBP2 inhibits growth of lung cancer cell A549. RBP2 depletion decreases cell proliferation. Data are shown as mean ± SD from 3 independent assays. *, $p < 0.05$ vs. scramble control. The statistics was determined by student's t-test.

Figure S3. (related to Figure 3D) RBP2 knockdown impairs formation of metastatic nodules in lungs of nude mice. A. Photographs show mouse lungs taken at week 6 post tail vein injection of CL1-5 luc2 cells with scramble shRNA, shRBP2 KD1 or KD2 into female nude mice. Each group contains five mice. B, bioluminescent images of the mouse lungs in A at endpoint.

Figure S4. (related to Figure 4) Construction of shRNA-resistant HA-tagged RBP2 plasmids. The targeting sequence of RBP2 shRNA KD1 and the corresponding peptide sequence of RBP2 are shown in the upper panel. The KD1-resistant RBP2 construct was generated by changing eight nucleotides (mark in red) of wild type RBP2 cDNA without altering the corresponding amino acid sequences. 2μg of plasmids was introduced into CL1-5 cells with scramble shRNA or shRBP2 KD1 for 48 hr using Lipofectamine 2000. The HA-RBP2-resistant,

but not HA-RBP2 plasmid, can be expressed in cells depleted of RBP2 by KD1 (compare lane 6 to 5). α-tubulin is served as a loading control.

Figure S5. (related to Figure 5) The protein level of p21 is increased in gastric AGS cell line but decreased in lung cancer cells when RBP2 is depleted. Total protein extracts (50μg) were separated on SDS-PAGE and transferred onto nitrocellulose membrane for western using antibodies against RBP2 (Abnova), p21 (BD pharmingen) and α-tubulin (Sigma).

Figure S6. (related to Figures 5C and 5D) RBP2 knockdown effect on G1 cyclin gene expression and the efficiency of p27 depletion. A. RBP2 depletion slightly decreases mRNA levels of cyclins D1 and E1 in gastric cancer cell AGS, but suppresses their expression greatly in lung cancer cells A549 and CL1-5. Relative mRNA levels of indicated genes were analyzed by RT-qPCR and normalized to the level of actin. B. RT-PCR shows that p27 siRNA used in Fig 5D reduces p27 mRNA level to around 25%.

Figure S7. (related to Figure 6D) Successful expression of exogenous ITGB1 in RBP2-depeletd H1299 cells does not restore cell growth defect. A, 2μg of pcDNA3.1/V5-His (vector) or ITGB1-v5 plasmid was transfected into RBP2-knocked down H1299 cells for 48 h using Lipofectamine 2000. Antibodies against RBP2, V5 and p84 detected the corresponding protein by western. p84 is served as a loading control. B, RBP2-depleted H1299 cells were transfected with vector alone or plasmid expressing ITGB1-v5, followed by analysis of cell proliferation by the Real-Time Cell Analyzer (RTCA) Dual Plate (DP) system (xCELLigence, Roche Diagnostics GmbH). Data are shown as mean ± SD from 3 independent assays. The

statistics were determined by repeated-measure ANOVA. ***, $p < 0.001$. NS, not significant.

SUPPLEMENTARY TABLE LEGEND:

Table S1. RBP2-regulated genes involved in tumorigenesis and metastasis. Shown are genes with at least 1.5-fold (log 2) change. p-value < 0.05, determined by statistical t-test.

SUPPLEMENTARY MOVIE LEGEND:

Videos of cells depicted in Figure 3A are provided as online supplementary data. Cell movement from cells lentivirally infected with scramble shRNA, RBP2 KD1, or RBP2 KD2 are shown in movie 1, 2 and 3, respectively.