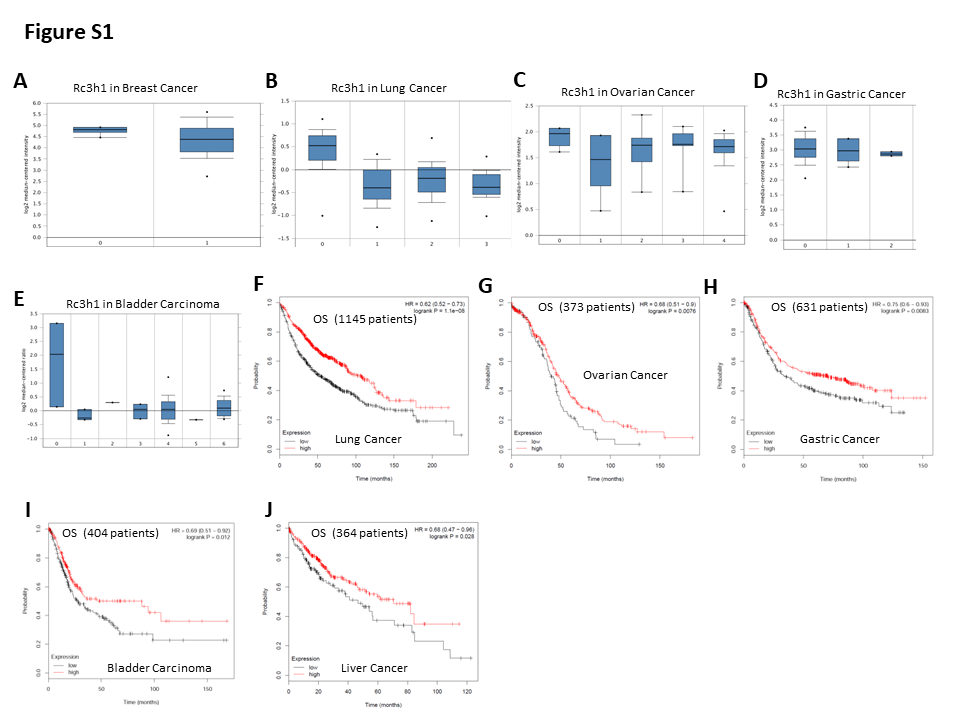
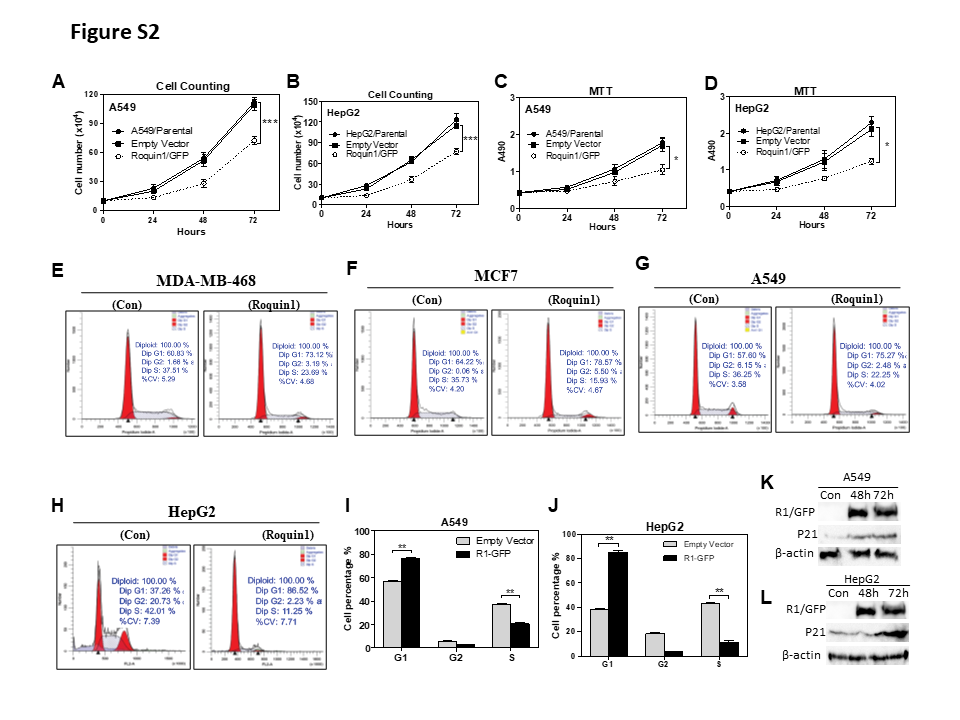
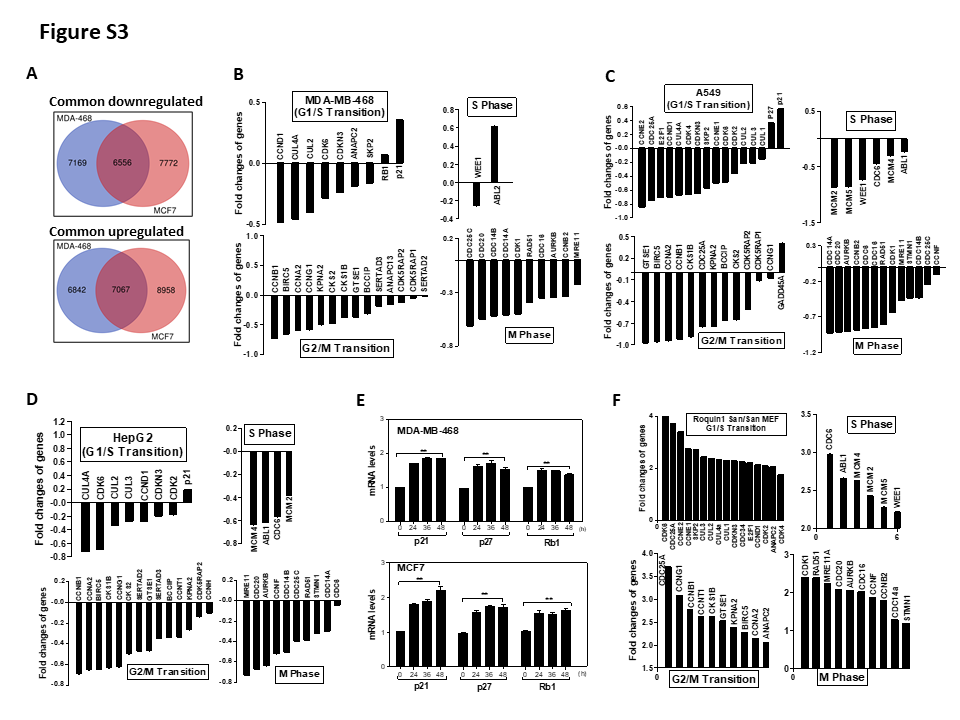
**Additional file 1:**



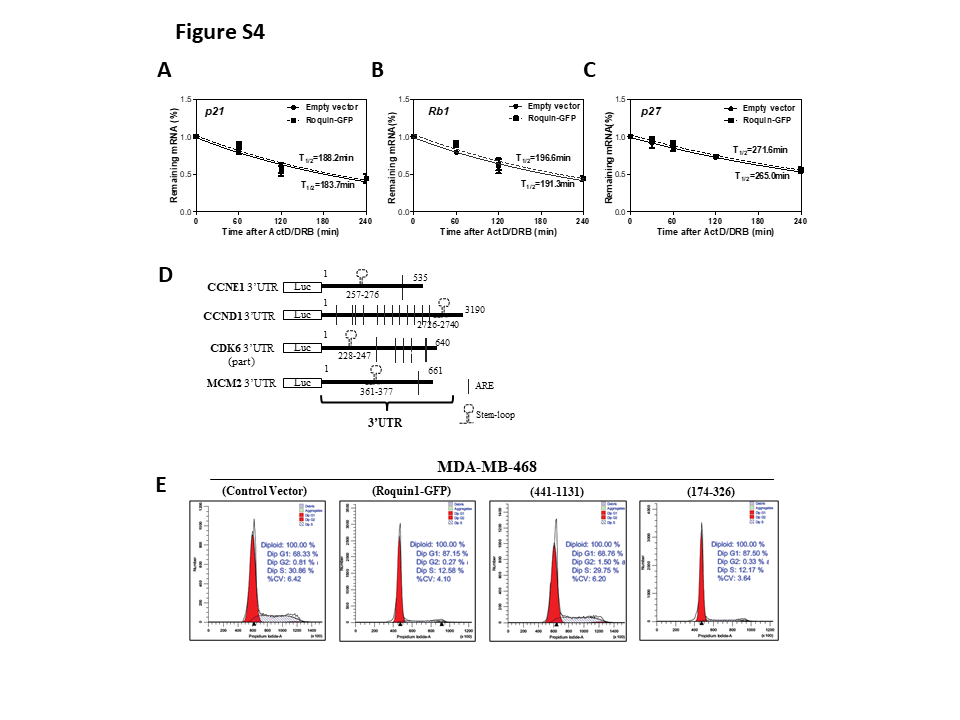
**Figure S1.** Roquin1 expression is reduced in several human cancers, and positively associated with patient survival. **a** Comparison of Roquin1 mRNA expression between normal (0) (*n* = 7) and ductal breast carcinoma (1) (*n* = 40). **b** Comparison of Roquin1 mRNA expression among normal (0) (n = 65), large cell lung carcinoma (1) (*n* = 19), lung adenocarcinoma (2) (*n* = 45), and squamous cell lung carcinoma (3) (*n* = 27). **c** Comparison of Roquin1 mRNA expression among normal (0) (*n* = 5), ovarian clear cell large cell adenocarcinoma (1) (*n* = 7), ovarian endometrioid adenocarcinoma (2) (*n* = 9), ovarian mucinous adenocarcinoma (3) (*n* = 9), and ovarian serous adenocarcinoma (4) (*n* = 20). **d** Comparison of Roquin1 mRNA expression in normal (0) (*n* = 31), diffuse gastric adenocarcinoma (1) (*n* = 6), and gastric adenocarcinoma (2) (*n* = 2). **e** Comparison of Roquin1 mRNA expression in normal (0) (*n* = 3), bladder cancer (1) (*n* = 3), bladder squamous cell carcinoma (2) (*n* = 1), bladder urothelial carcinoma (3) (*n* = 3), infiltrating bladder urothelial carcinoma (4) (*n* = 34), bladder papillary urothelial carcinoma (5) (*n* = 1), and superficial bladder cancer (6) (*n* = 17). **f-j** Kaplan-Meier overall survival curve of patients with lung cancer (**f**), ovarian cancer (**g**), gastric cancer (**h**), bladder carcinoma (**i**), and liver cancer (**j**) having low and high tumor Roquin1 transcripts.



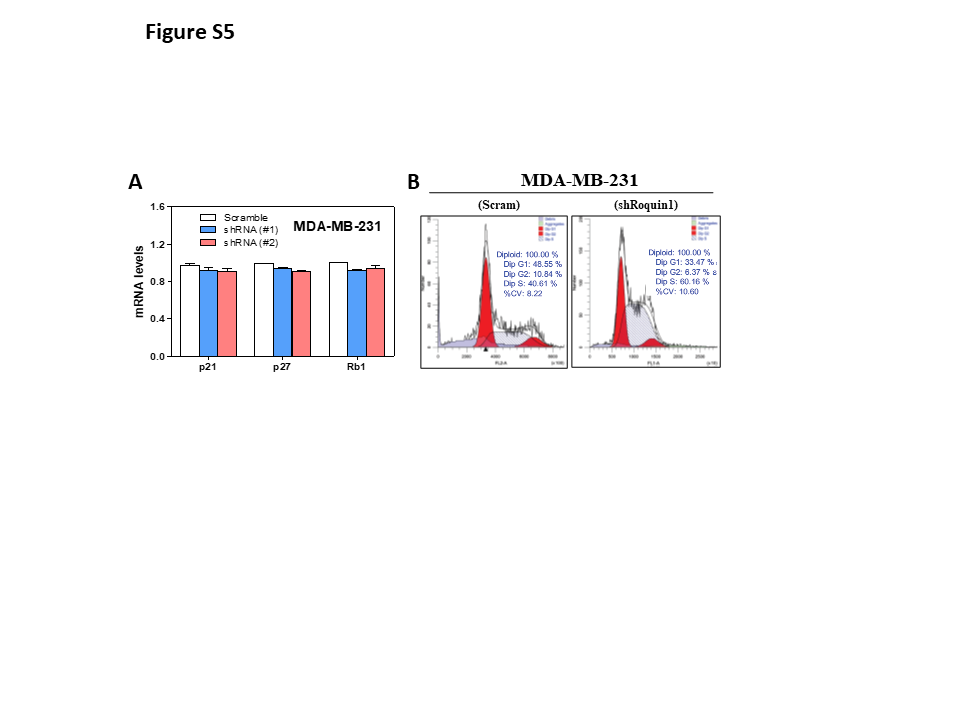
**Figure S2.** Roquin1 inhibits cell proliferation and induces G1/S phase cell cycle arrest in tumor cells. **a-b** Cell counting was carried out every 24 h in A549 (**a**) and HepG2 (**b**) cells with Roquin1/GFP overexpression. \*\*\**P* < 0.001. **c-d** MTT assay was performed in A549 and HepG2 cells to measure cell proliferation after Roquin1 overexpression. \**P* < 0.05. **e-h** Representative cell cycle histograms showing cell cycle analyses of MDA-MB-468 (**e**), MCF7 (**f**), A549 (**g**), and HepG2 (**h**) cells after Roquin1 overexpression. **i-j** Cell cycle analysis was carried out in A549 (**i**) and HepG2 (**j**) cells after Roquin1 overexpression, and the percentages of different cell phases were quantified. \*\**P* < 0.01. **k-l** The protein level of cell cycle inhibitor p21 was measured by immunoblotting with an anti-p21 antibody at different time points after Roquin1 overexpression in A549 (**k**) and HepG2 (**l**) cells. β-actin was used as a loading control.



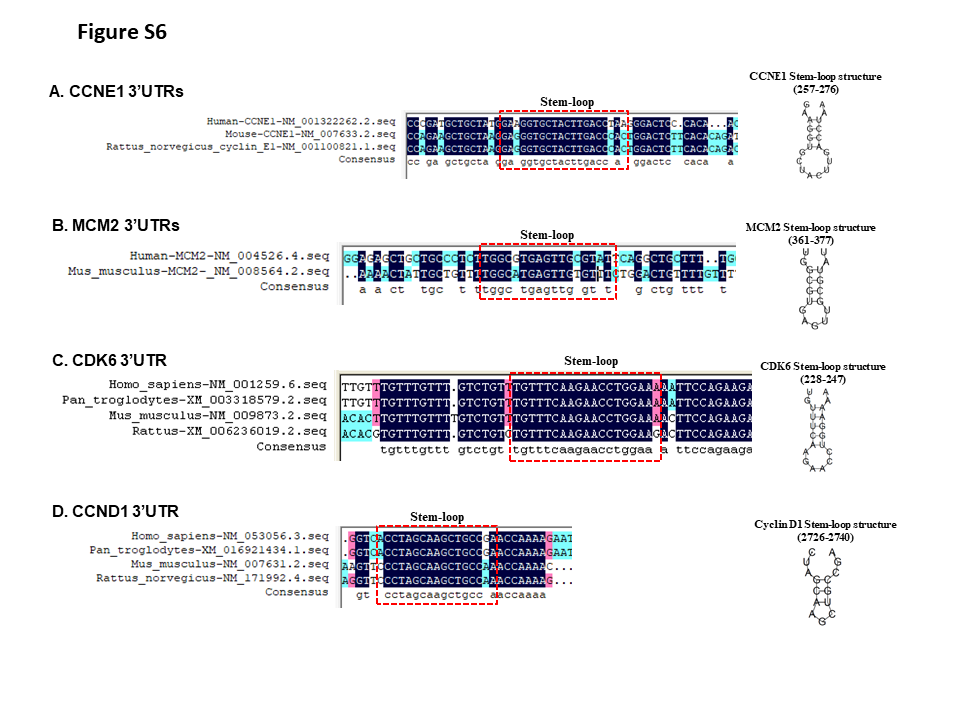
**Figure S3.** Roquin1 selectively inhibits the mRNA expression of cell cycle-promoting genes. **a** Venn diagrams showing the common down-regulated (upper) and up-regulated genes (bottom) by Roquin1 in breast tumor cells. **b-d** The expression levels of cell cycle–related genes affected by Roquin1 were analyzed by RNA-seq in MDA-MB-468 (**b**), A549 (**c**), and HepG2 (**d**) cells. The expression of genes promoting G1/S, S phase, G2/M, and M phase transition was downregulated; and the expression of cell cycle–inhibiting genes were upregulated. **e** The mRNA expression levels of indicated cell cycle–inhibiting genes were measured by qPCR in MDA-MB-468 and MCF7 cells at different time points, including *p21*, *p27*, *p16*, and *Rb1*. **f** Cell cycle–related genes were regulated by Roquin1 in Roquin1san/san MEF cells. The expression of genes promoting G1/S, S phase, G2/M, and M phase transition were upregulated.



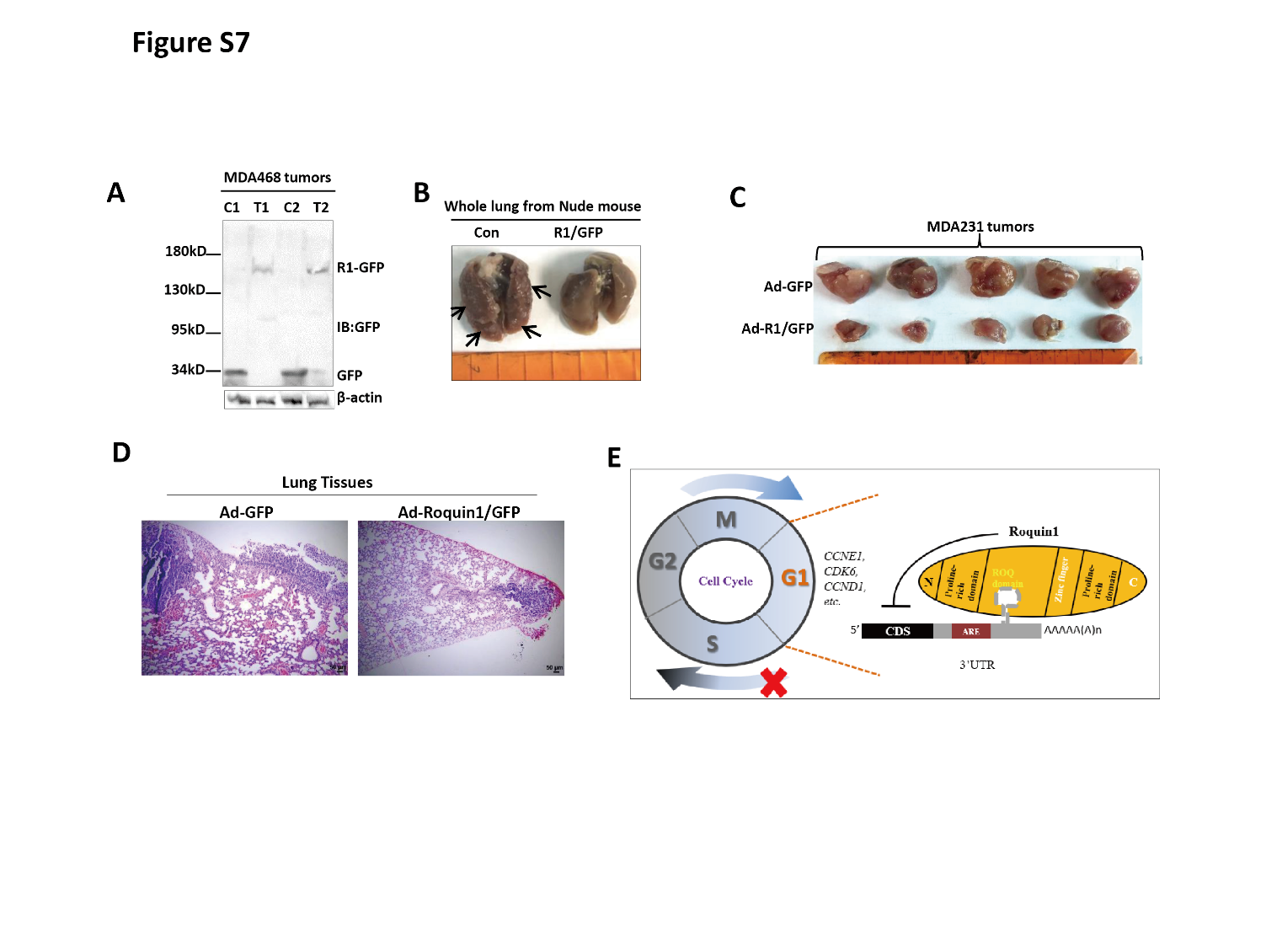
**Figure S4.** Roquin1 destabilizes the mRNAs of cell cycle-promoting genes via the ROQ domain. **a**–c The half-lives of cell cycle–inhibiting genes, including *p21* (**a**)*, p16* (**b**)*,* and *p27* (**c**) were measured by qPCR in Roquin1-expressing MDA-MB-468 cells. **d** Schematic representation of the luciferase reporter constructs containing 3’UTRs sequences of *CCNE1*, *CCND1*, *CDK6* (part), and *MCM2*. **e** Representative cell cycle histograms showing cell cycle analyses of MDA-MB-468 cells after overexpression of Roquin1/GFP, aa 441-1131, and aa 174-326 truncated mutations.



**Figure S5.** Knocking down Roquin1 enhances breast tumor cell cycle progression. **a** The mRNA expression levels of indicated cell cycle–inhibiting genes were measured after Roquin1 knockdown by infecting lentivirus expressing shRNA/Scramble and shRNA/Roquin1 by qPCR in MDA-MB-231 cells*.* **b** Representative cell cycle histograms showing cell cycle analyses of MDA-MB-231 cells after knocking down Roquin1.



**Figure S6.** Putative stem-loop structure in the 3’UTRs of cell cycle-promoting genes. **a**–**d** The 3’UTR sequences from different species for each cell cycle–promoting gene, including *CCNE1* (**a**), *MCM2* (**b**), *CDK6* (**c**), and *CCND1* (**d**), was aligned using DNAMAN software. The stem-loop sequences were predicted by RNAfold web server to fold a secondary stem–loop structure (right) and indicated by red box.



**Figure S7.** Roquin1 suppresses breast tumor growth and metastasis. **a** Total protein was extracted from tumor tissues and used to detect Roquin1/GFP expression by immunoblotting with an anti-GFP antibody. **b** Whole lungs from nude mouse bearing MDA-MB-468/GFP or MDA-MB-468/Roquin1/GFP tumors was collected and compared. **c** MDA-MB-231 tumors treated with control adenovirus (Ad-GFP) or Roquin1-expressing adenovirus (Ad-R1/GFP). **d** H&E staining of lung sections of tumor-bearing mice treated with control adenovirus or Roquin1-expressing adenovirus. Scale bar, 50µm. **e** A proposed work model of cell cycle-promoting genes regulation by Roquin1.