MALAT1 promotes colorectal cancer cell proliferation/migration/invasion via PRKA kinase anchor protein 9

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Supplemental Data

Table S1 Clinic pathological characteristic of patients

Characteristics	CRC tissues with metastases (n)	CRC tissues without metastases (n)
	9	18
Gender		
Male	5	10
Female	4	8
Age(years)		
<50	6	16
⩾50	3	2
Tumour site		
Proximal colon	3	6
Distal colon	4	6
Rectum	2	6
Tumour size (cm in diameter)		
<5	2	10
⩾5	7	8
Tumour differentiation		
Good	2	6
Moderate	4	6
Poor	3	6
T-stage		
1-2	2	10
3	6	8
4	1	0
N-stage		
0	0	18
1-2	9	0
Distant metastasis		
0	8	18
1	1	0

Fig. S1

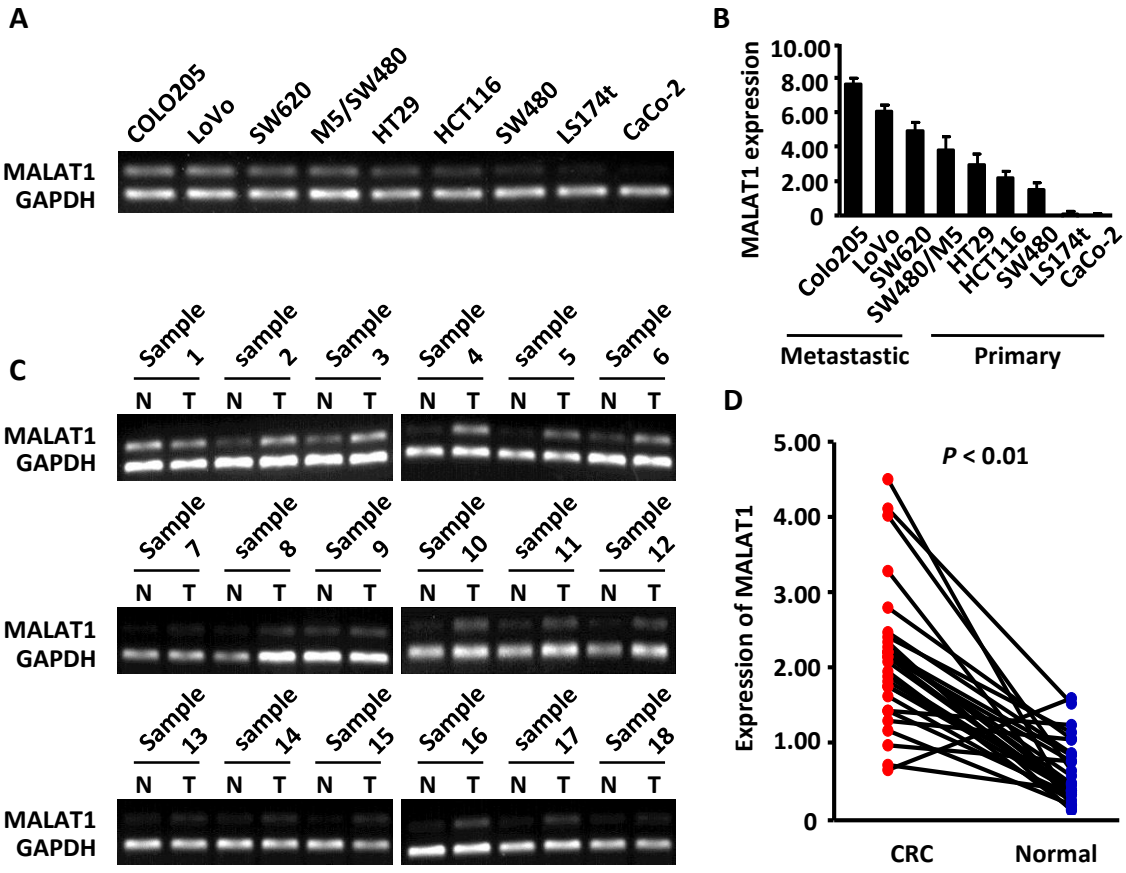

Figure S1. The expression of MALAT1 was up-regulated in metastatic CRC cells and human primary CRC tissues. (A) Semi-quantitative analyses of MALAT1 expression in CRC cell lines. (B) Expression of MALAT1 in CRC cell lines was quantified by real-time PCR and normalized to GAPDH. (C, D) MALAT1 levels of 18 paired CRC and adjacent normal tissues detected by semi-quantitative analysis (C) and real-time-PCR (D). The means level of MALAT1 expression in CRC was significantly higher than that in normal tissues.

Fig. S2

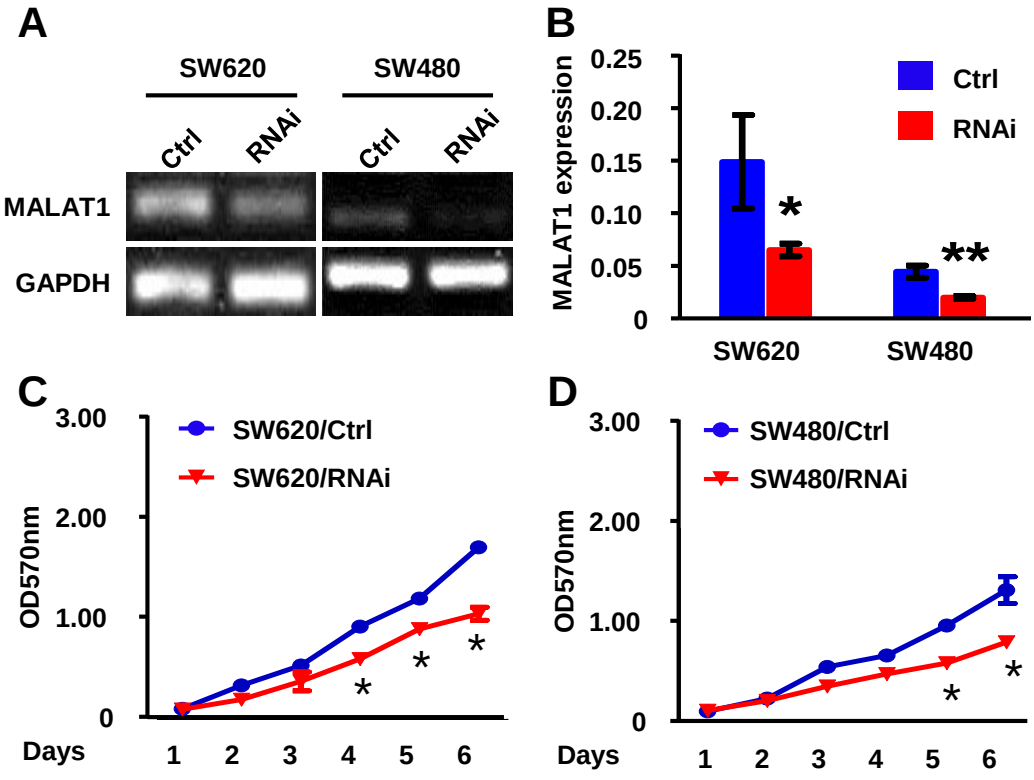

Figure S2. Knockdown of MALAT1 decreased CRC cell growth *in vitro.* (A) MALAT1 expression in SW620 and SW480 cells was effectively blocked by MALAT1 siRNA. (B) quantification of MALAT1 expression shown in A. *P < 0.05, **P <0.01.compared to scramble siRNA-transfected cells (Ctrl). (C, D) Knockdown of MALAT1 inhibited the proliferation of SW620 (C) and SW480 cells (D) as detected by CCK8 assays. The results were reproducible in three independent experiments. *P < 0.05 compared to Ctrl group for the corresponding cells.

Fig. S3

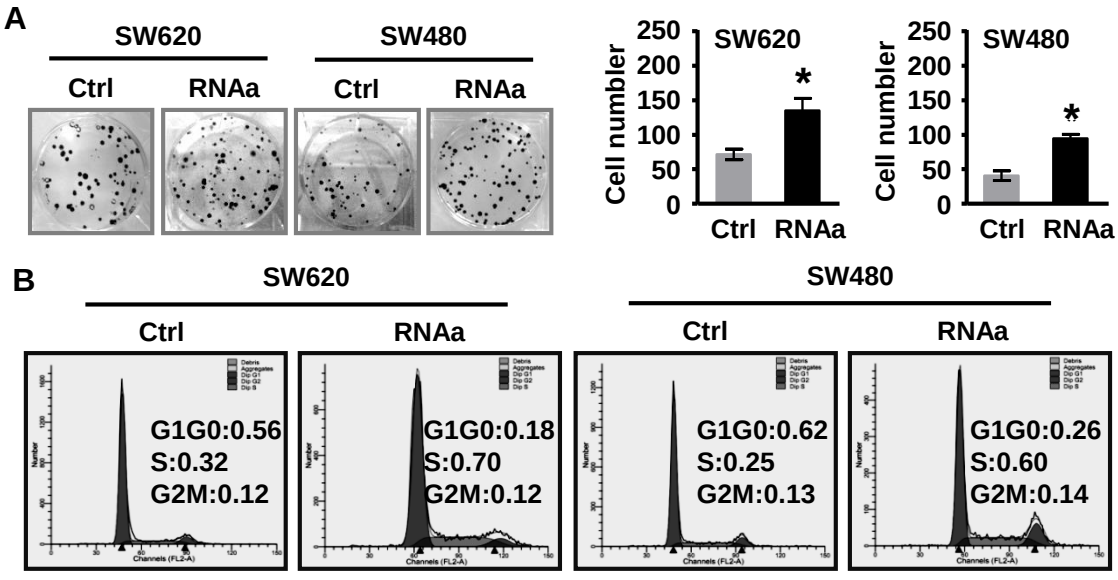

Figure S3. Overexpression of MALAT1 enhanced CRC cell proliferation *in vitro.* (A) SW620 or SW480 cells with MALAT1 overexpression by RNA activation (RNAa) were plated, and colony formation were examined. MALAT1 overexpression by RNAa promoted CRC colony formation. *P<0.01 compared to scramble RNA group (Ctrl). (B) RNAa activation-induced MALAT1 overexpression promoted CRC cell cycle progression to S phase as detected by Flow Cytometry, consistent with the cell proliferation.

Fig. S4

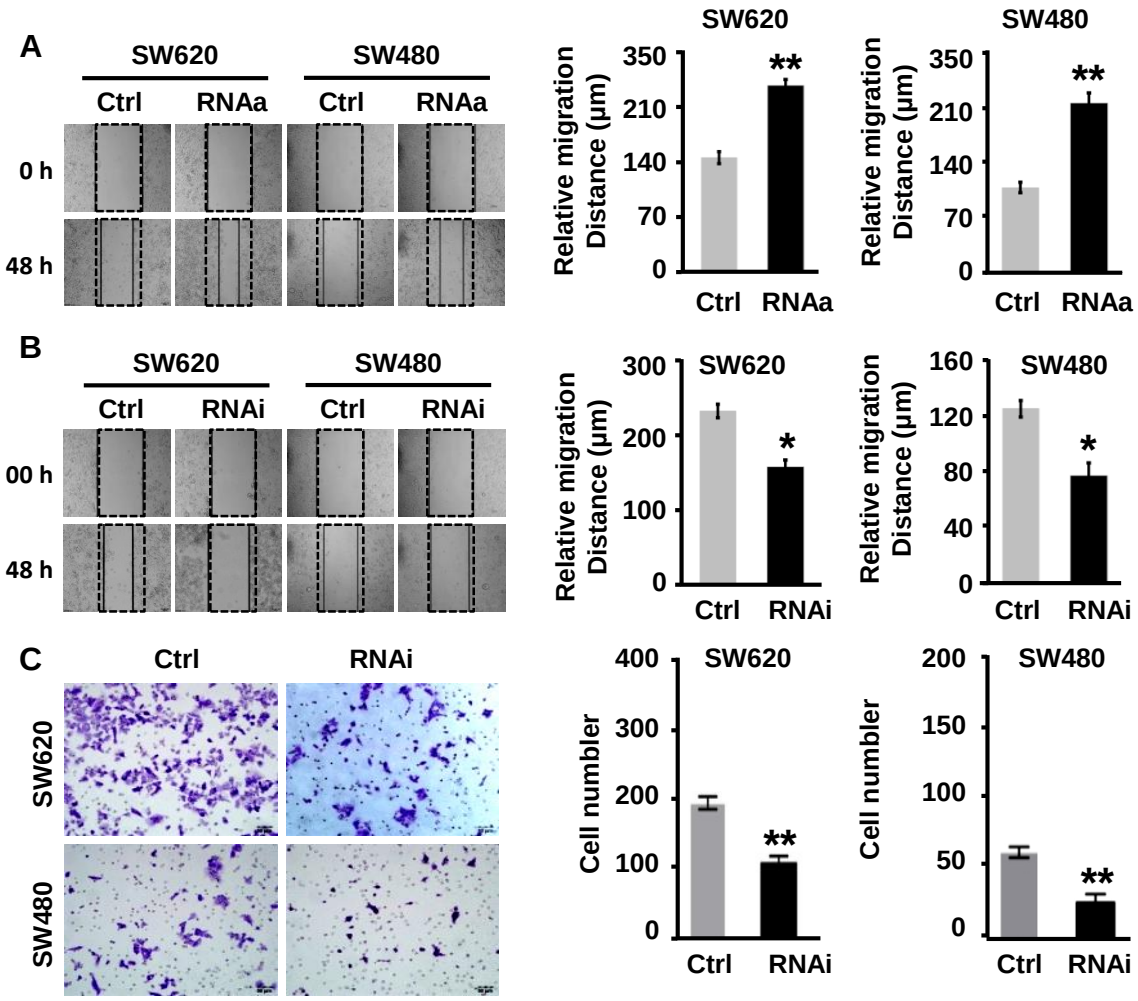

Figure S4. Effect of MALAT1 on CRC cell migration and invasion *in vitro.* (A, B) Wound-healing assays showed that overexpression of MALAT1 (RNAa) promoted while MALAT1 knockdown by siRNA (RNAi) blocked the migration of SW620 or SW480 cells as compared to scramble RNA treatment (Ctrl). (C) MALAT1 knockdown blocked CRC cell invasive potency as shown in matrigel invasion assays. Data were presented as mean ± SD. The results were reproducible in three independent experiments. * $P < 0.05$, ** $P < 0.01$ compared control treatments (Ctrl).

Fig. S5

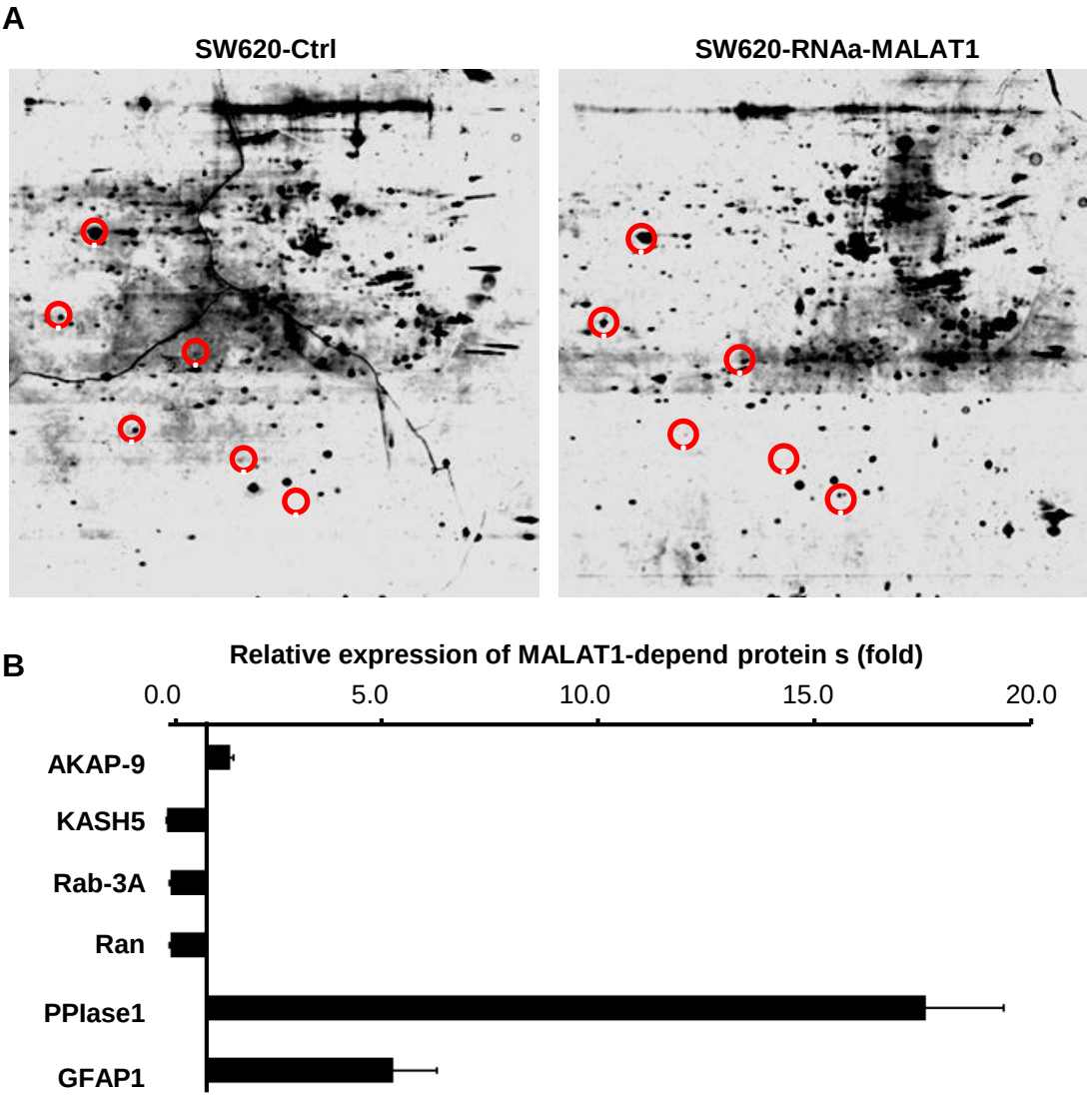

Figure S5. Proteomics analyses identified potential MALAT1 gene targets. (A) Distribution of differentially expressed proteins in SW620 cells with MATAL1 overexpression by RNAa (SW620-RNAa-MALAT1) vs control SW620 (SW620-Ctrl) in 2D gel electrophoresis. The red circles represent the proteins up- or down- regulated by MALAT1. (B) Three proteins were significantly up-regulated in MALAT1-activated cells including AKAP-9 (AKAP-9), Peptidyl-prolyl cis-tRNAs isomerase A (PPIase1), and Glial fibrillary acidic protein (GFAP1). Meanwhile, Coiled-coil domain-containing protein 155 (KASH5), Ras-related protein Rab-3A (Rab-3A), and GTP-binding nuclear protein RNA (Ran) were down-regulated.

Fig S6

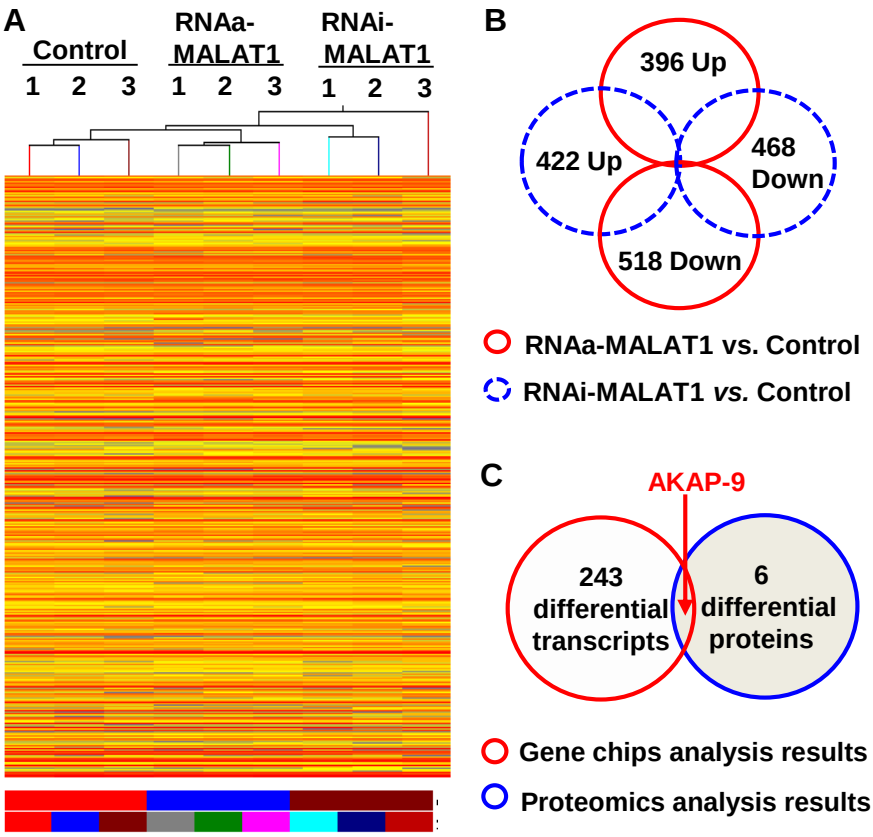

Figure S6. Identification of MALAT1-regulated genes by microarray and proteomics analysis. (A) Hierarchical cluster diagram of 243 differentially expressed genes among MALAT1 overexpressed (RNAa-MALAT1), MALAT1 blocked (RNAi-MALAT1), and control SW480 cells. (B) Venn diagrams of the differentially-regulated genes between RNAa-MALAT1 and control SW480 cells or between RNAi-MALAT1 and control SW480 cells. (C) Venn diagrams of genes regulated by MALAT1 as identified by cross-comparing the results from microarray with that of proteomics analysis. Only AKAP-9 was altered in both assays.

Fig. S7

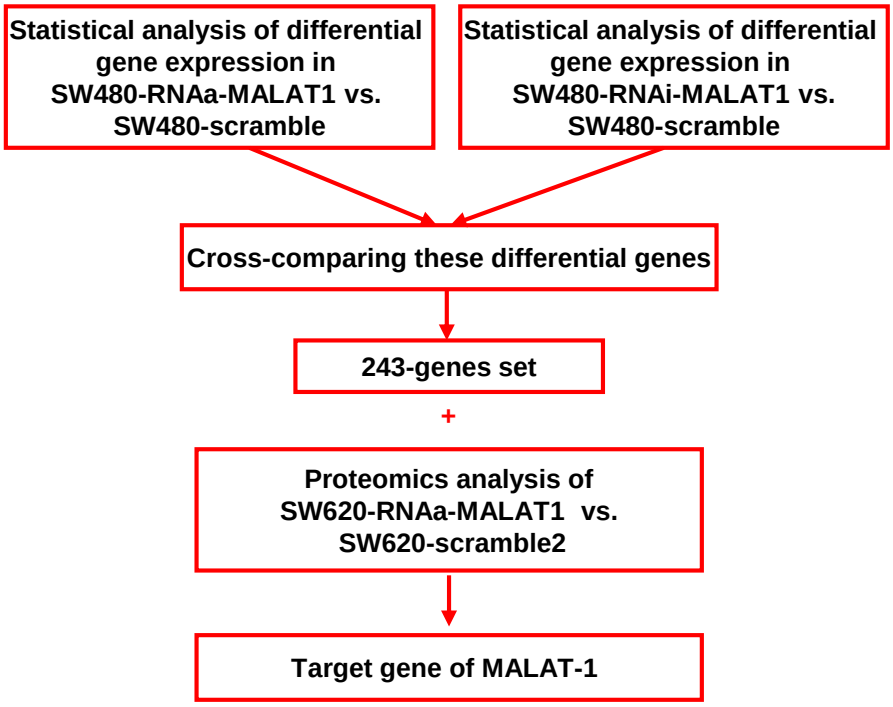

Figure S7. The workflow used for identifying MALAT1-regulated genes.

Fig. S8

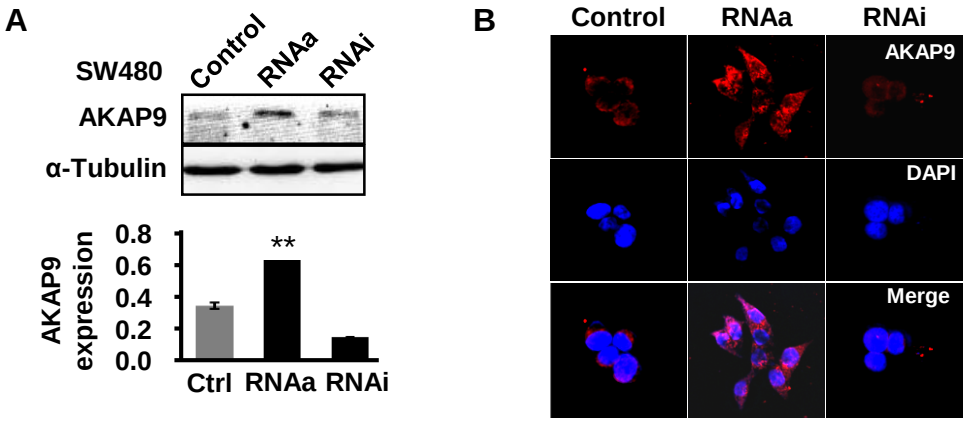

Figure S8. Validation of MALAT1-mediated AKAP-9 expression. (A) AKAP9 protein induction by MALAT1 in SW480 cells. RNAa activation of MALAT1 stimulated AKAP-9 expression as shown by western blot. **P<0.01 compared to other two groups. (B) MALAT1-dependent expression of AKAP-9 in SW480 cells was detected by immunostaining. DAPI stains nuclei.