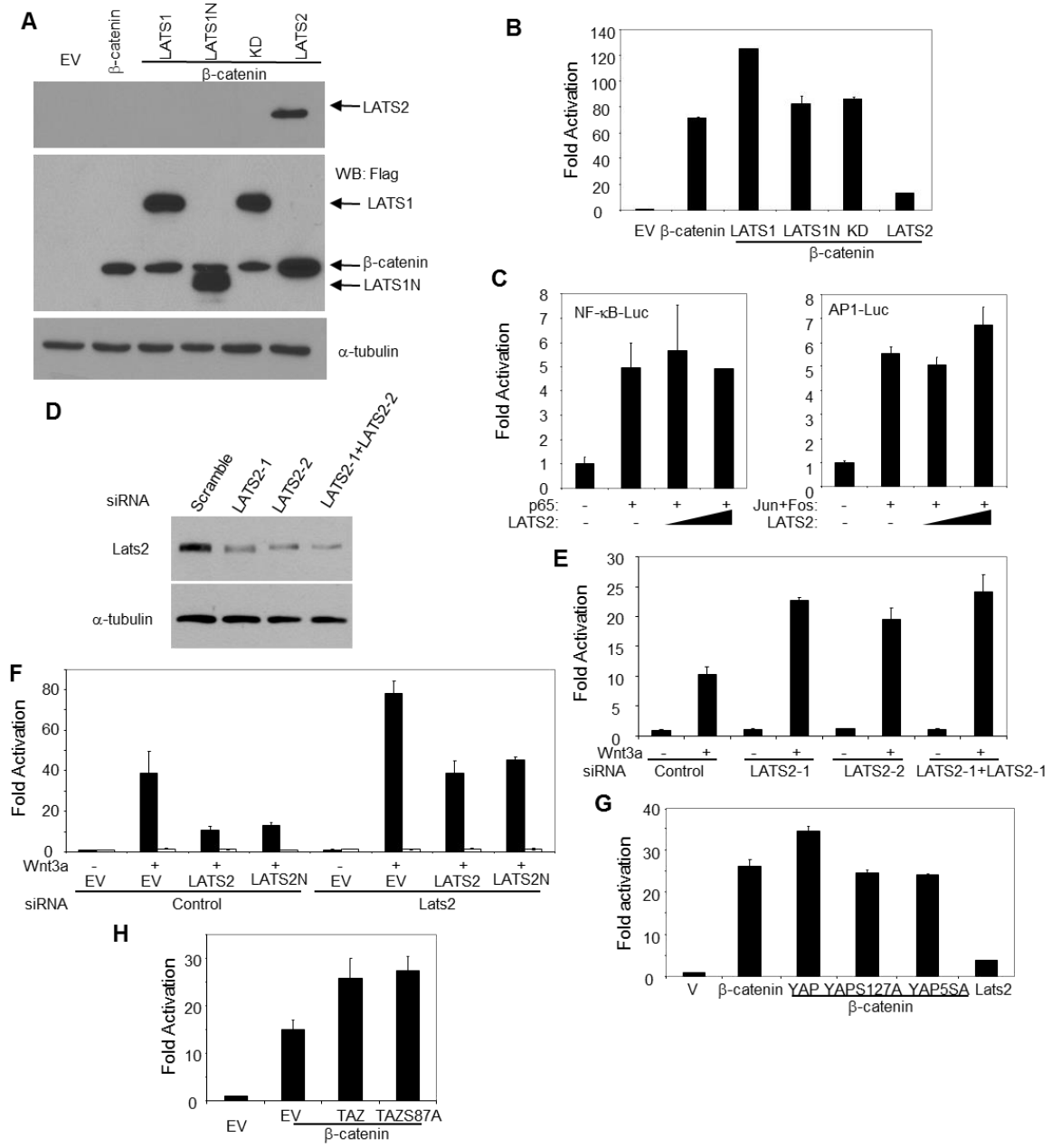

Fig. S1. LATS2 specifically inhibits β-catenin-mediated transcripton, Related to Fig. 1.

(A) The expression of LATS1, LATS2 and β-catenin was detected by Western blot analysis. KD, LATS1 kinase dead mutation.

(B) Over-expression of LATS1 did not inhibit β-catenin-mediated transcription. KD, LATS1 kinase dead mutation.

(C) Over-expression of LATS2 did not inhibit the NF-κB and AP-1 reporter activities. (D) Knockdown of LATS2 by two different siRNAs.

(E) Knockdown of LATS2 by two different siRNAs enhanced STopflash reporter activities induced by Wnt3a.

(F) The restoration of LATS2 and LATS2N abolished the enhancement of STopflash reporter activities by LATS2 knockdown.

(G) Over-expression of YAP did not inhibit β-catenin mediated transcription.

(H) Over-expression of TAZ did not inhibit β-catenin-mediated transcription.

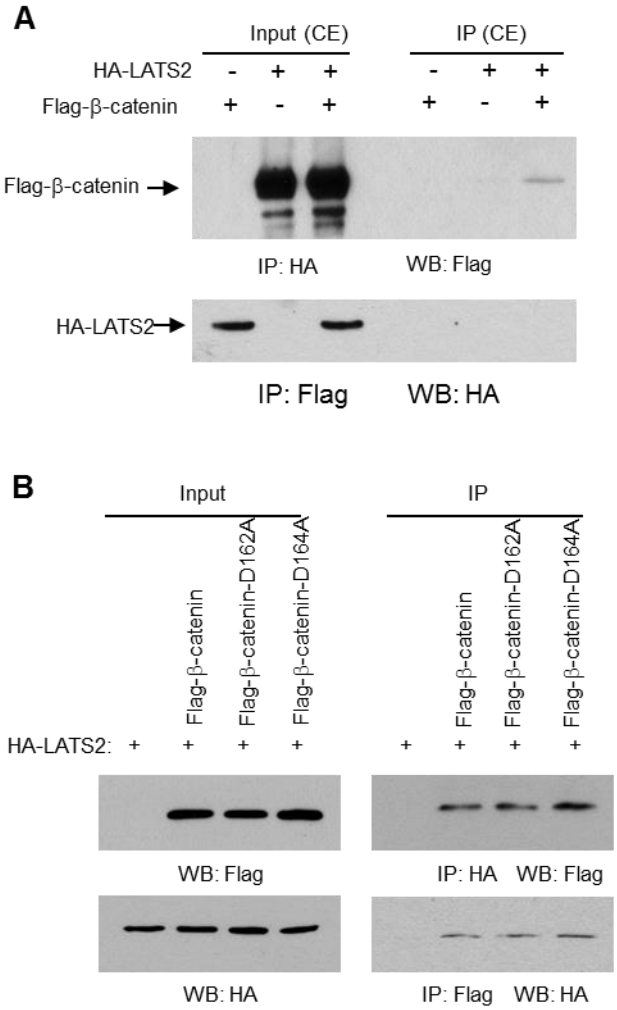

Fig. S2. LATS2 weakly interacts with β-catenin in the cytoplasm, Related to Fig. 2.
(A) LATS2 weakly interacts with β-catenin in the cytoplasm.
(B) The mutation of D162 or D164 residue of β-catenin did not affect the interaction between LATS2 and β-catenin.

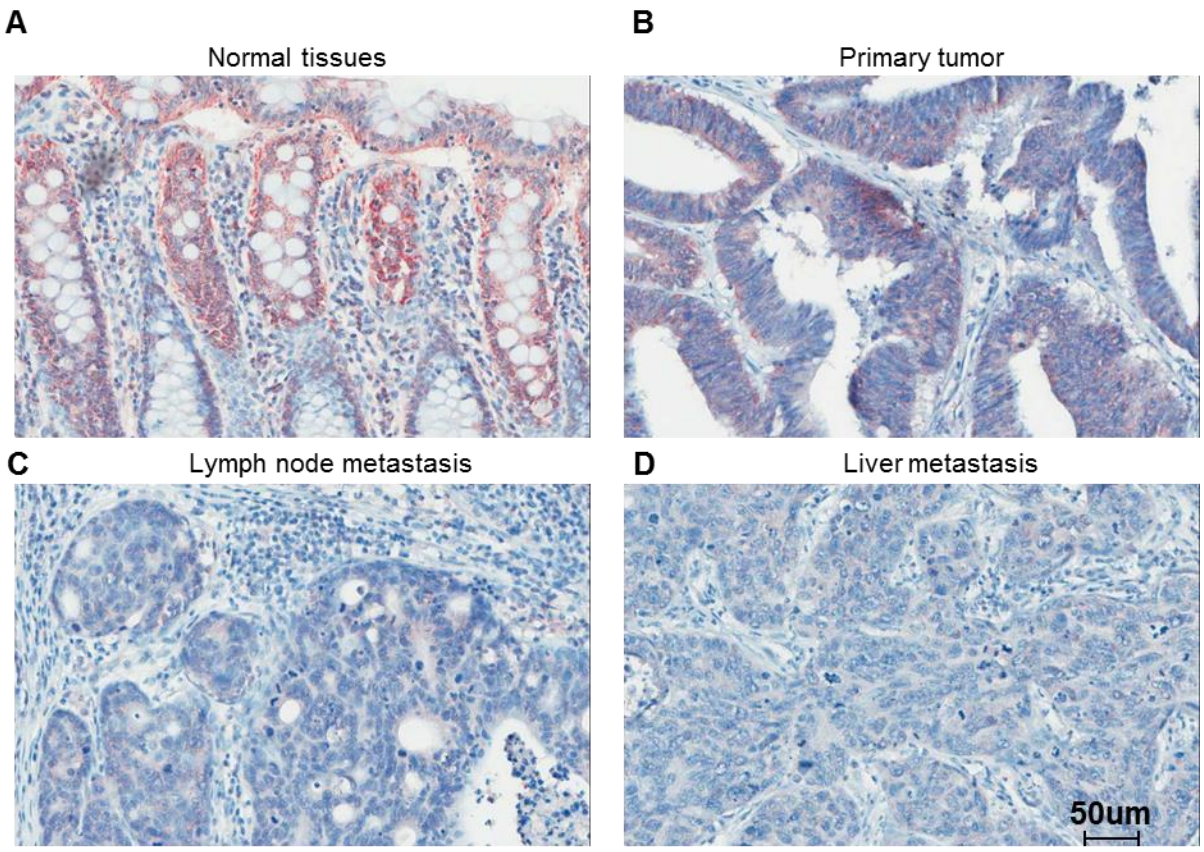

Fig. S3. LATS2 expression is reduced in metastatic human colorectal cancers, Related to Fig. 3.
(A) LATS2 expression in normal colorectal tissues.
(B) LATS2 expression in primary human colorectal cancer tissues.
(C) LATS2 expression in lymph node metastasis.
(D) LATS2 expression in liver metastasis.

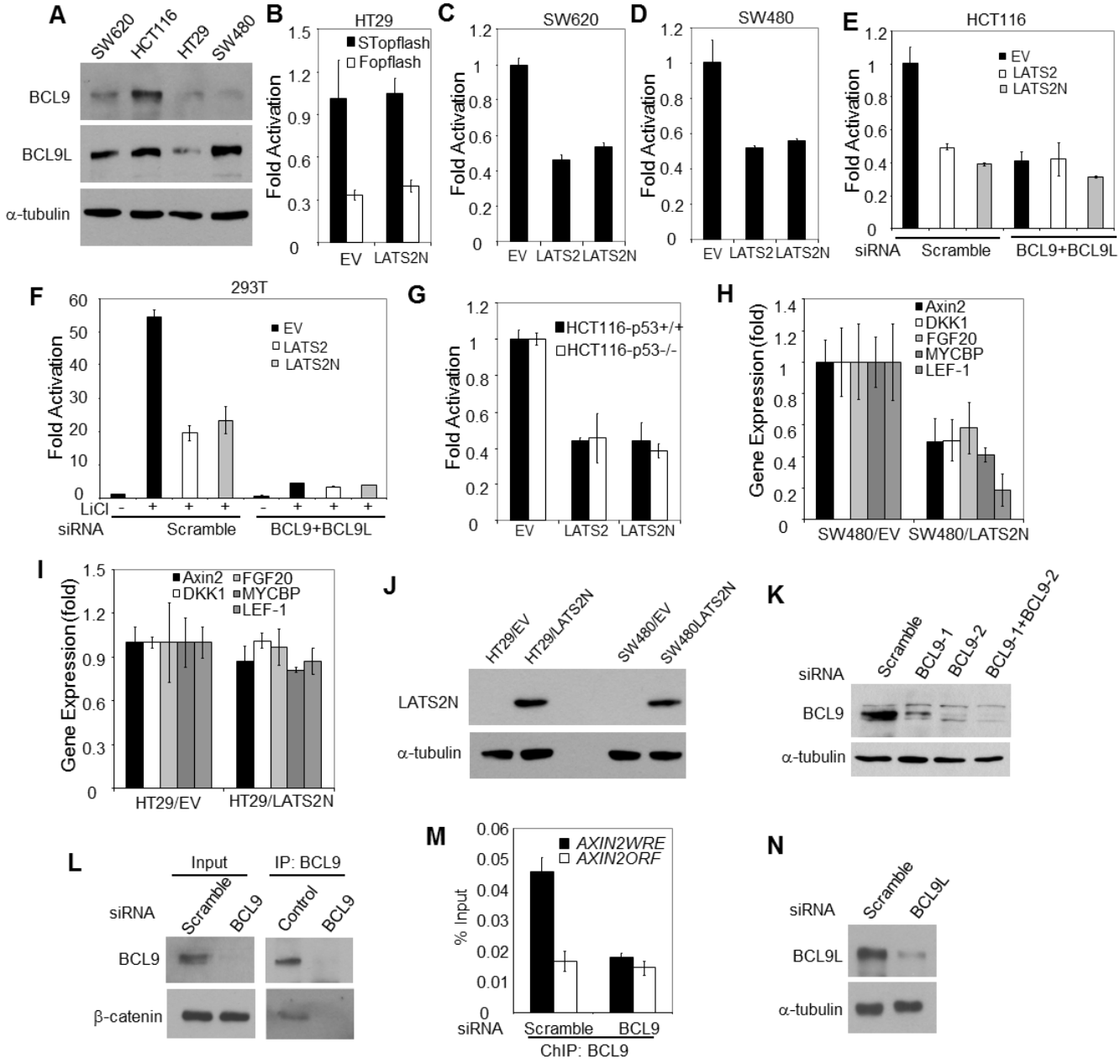

Fig. S4. LATS2 inhibits β-catenin/BCL9/BCL9L-mediated transcription, Related to Fig. 5.

(A) The expression of BCL9 and BCL9L in human colorectal cancer cell lines.

(B) LATS2N could not inhibit STopflash reporter activities in HT29 cells.

(C and D) Over-expression of LATS2 or LATS2N inhibited STopflash reporter activities in SW620 and SW480 cells.

(E) LATS2 and LATS2N were unable to inhibit STopflash reporter activities in BCL9- and BCL9L-depleted HCT116 cells.

(F) LATS2 and LATS2N were unable to inhibit STopflash reporter activities in BCL9- and BCL9L-depleted 293T cells.

(G) LATS2 and LAST2N inhibited STopflash reporter activities independent of p53.

(H) LAST2N inhibited the endogenous expression of Wnt target genes in SW480 cells. SW480/EV, SW480 cells expressing empty vector; SW480/LATS2N, SW480 cells expressing LAST2N.

(I) LAST2N could not inhibit the endogenous expression of Wnt target genes in HT29 cells. HT29/EV, HT29 cells expressing empty vector; HT29/LATS2N, HT29 cells expressing LAST2N.

(J) Confirmation of LATS2N expression in SW480 cells and HT29 cells.

(K) Confirmation of the specificity of anti-BCL9 antibodies by siRNAs.

(L) BCL9 knockdown abolished the β-catenin/BCL9 complex.

(M) Confirmation of the specificity of anti-BCL9 antibodies by ChIP assays.

(N) Confirmation of BCL9L knockdown by siRNAs

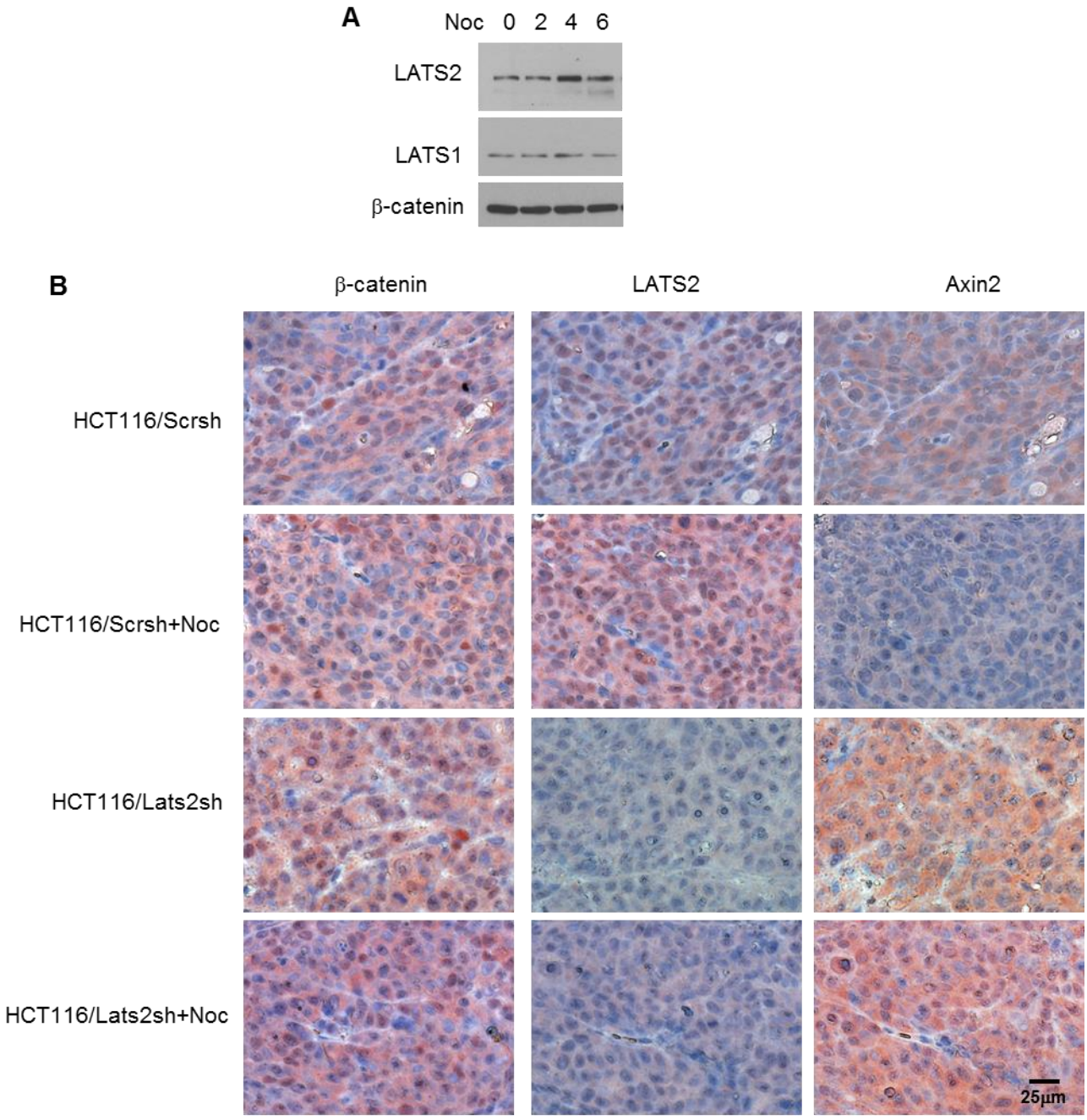

Fig. S5. Nocodazole induces LATS2 in human colorectal cancers, Related to Figure 7.
(A) Nocodazole induced LAST2 expression in SW480 cells.
(B) Nocodazole induced LATS2 to suppress β-catenin-mediated transcription in vivo.

Table S1. Pearson correlation (R) between LATS2 and tumor grade in human colorectal cancers, Related to Fig. 3.

	Tumor Grade	
	Correlation	P Value
Lats2	R=-0.177	<0.05

Table S2. Partial Correlation (R_p) between LATS2 and tumor grade in human colorectal cancers, Related to Fig. 3.

	Tumor Grade	
	Partial Correlation	P Value
Lats2	R_p=-0.178	<0.05

Partial correlation analysis was performed using β-catenin as a control variable.

Table S3. Pearson Correlation between β-catenin and AXIN2 or MMP7 in human colorectal cancers, Related to Fig. 3.

	β-catenin	
	Correlation	P Value
Axin2	R=3.83	<0.001
MMP7	R=3.33	<0.001

Table S4. Partial Correlations (R_p) between LATS2 and AXIN2 or MMP7 in human colorectal cancers, Related to Fig. 3.

	LAT2	
	Partial Correlation	P Value
AXIN2	R_p=-3.11	<0.01
MMP7	R_p=-2.59	<0.01

Partial correlation analysis was performed using β-catenin as a control variable.