Annexin A2 Stabilizes Oncogenic JAG1 Intracellular Domain by Inhibiting Proteasomal Degradation in Glioblastoma Cells

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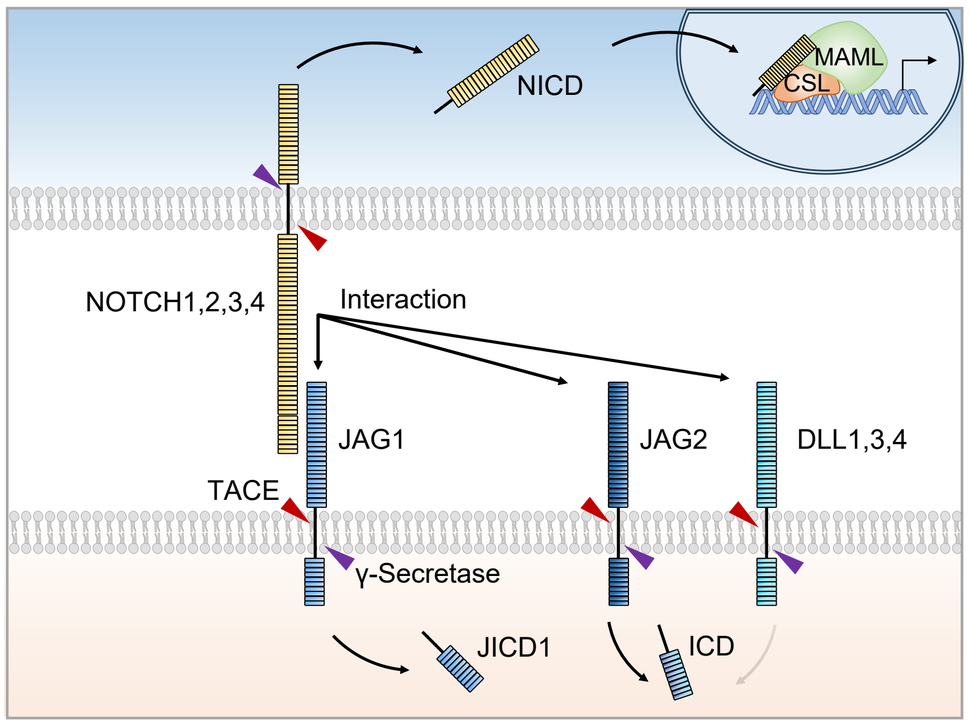

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LN18 JICD1-HA-FLAG

Immunoprecipitation (HA)

Mass spectrometry

Identification of JICD1-binding proteins

A list of JICD1 binding proteins

FLNA	PRDX1	FUBP1
ANXA2	HSP90AA1	HSPA1A
PDIA4	HSP90AB1	LMNB1
HNRNPU	NARF	TRIM25
BCAR1	CSK	HNRNPH3
ACTA1	HSPA2	G6PD
BCAR3	TUBA1C	HNRNPH1
GRP	HNRNPH2	HNRNPM
DBN1	GSR	TUBB6
EEF1G	ALB	XRCC6
TRIM21	H6PD	HSPA5
HSPA8	HDLBP	PDIA6
EEF1A1	FUS	DDX17
IGHG3	SRP72	
SH3GL1	TCP1	

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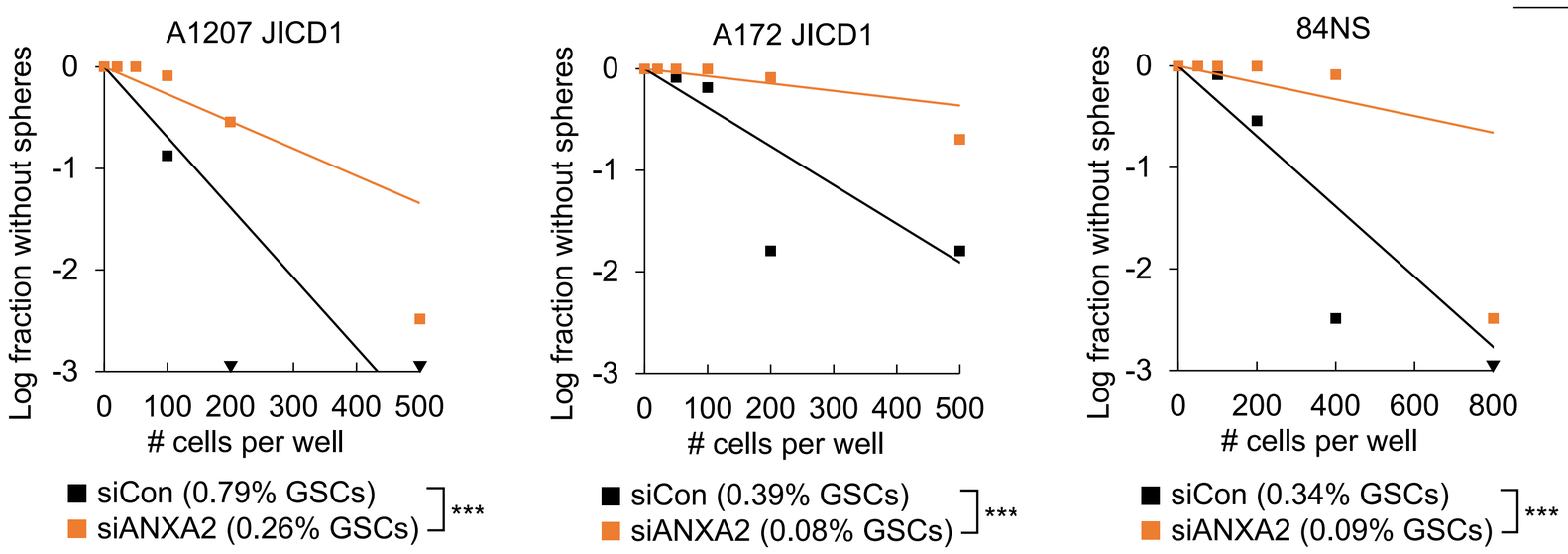

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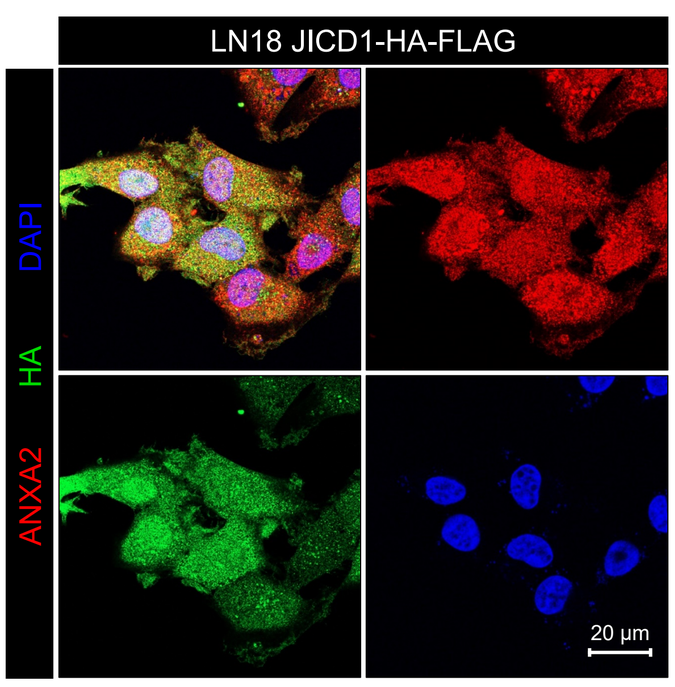

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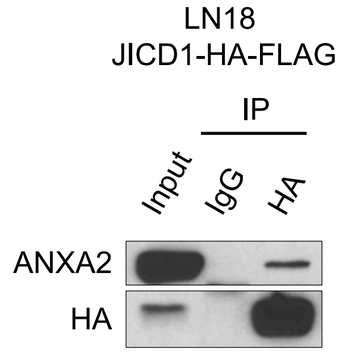

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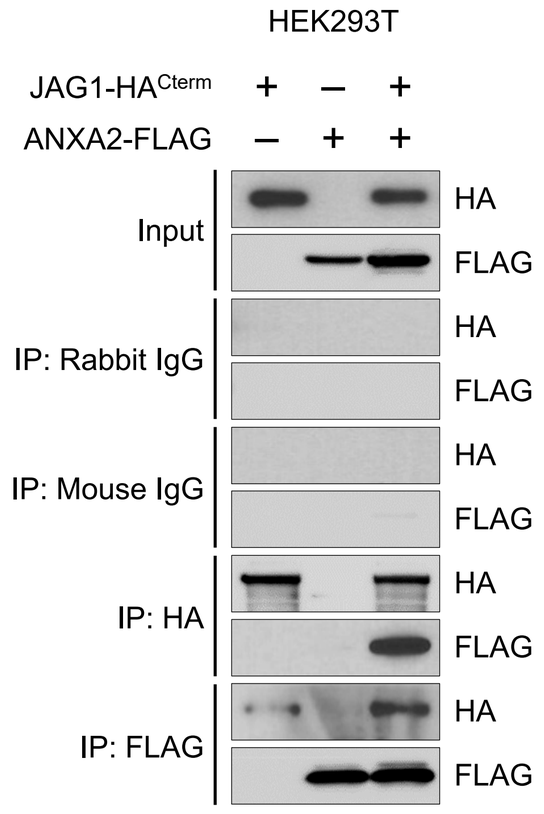

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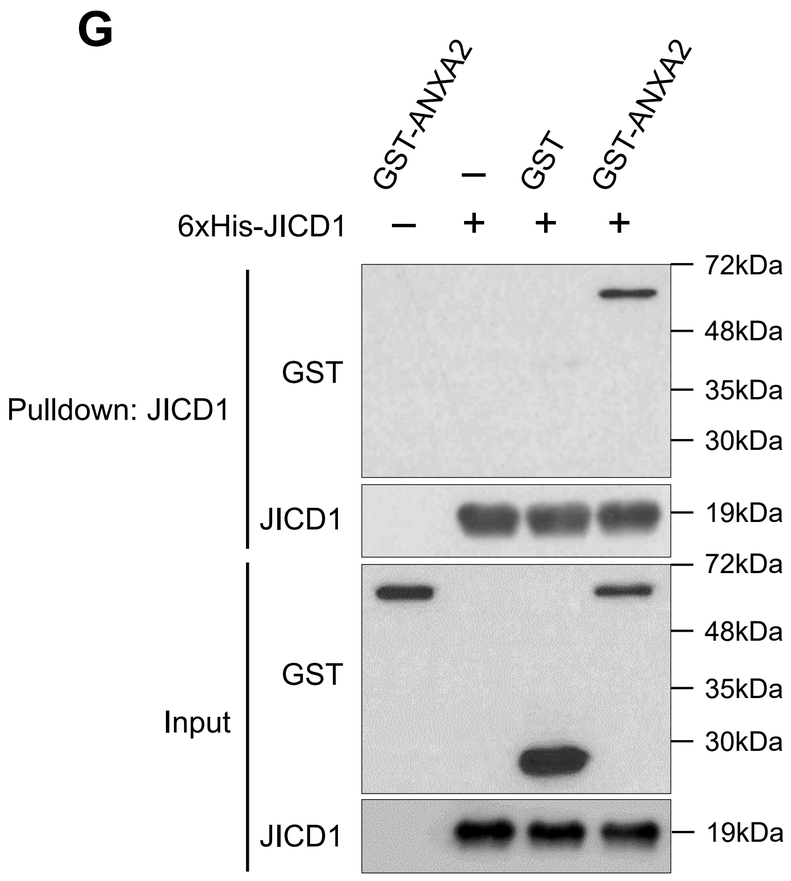

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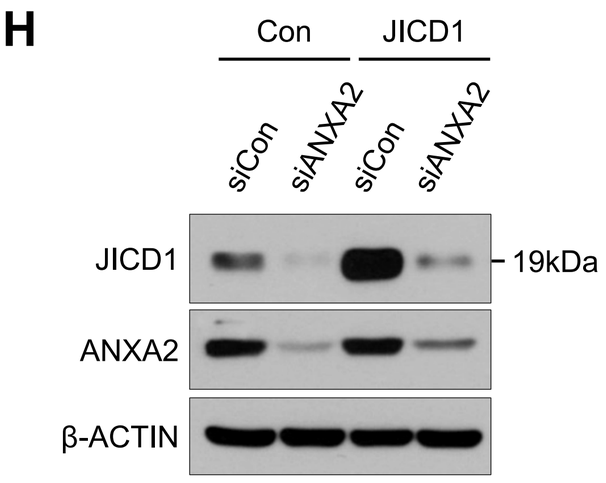

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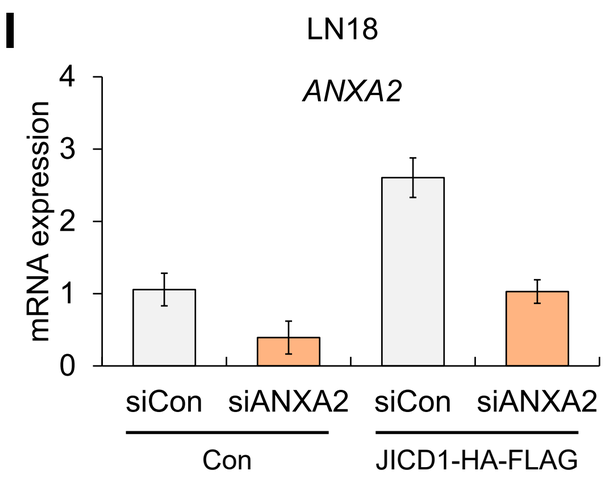

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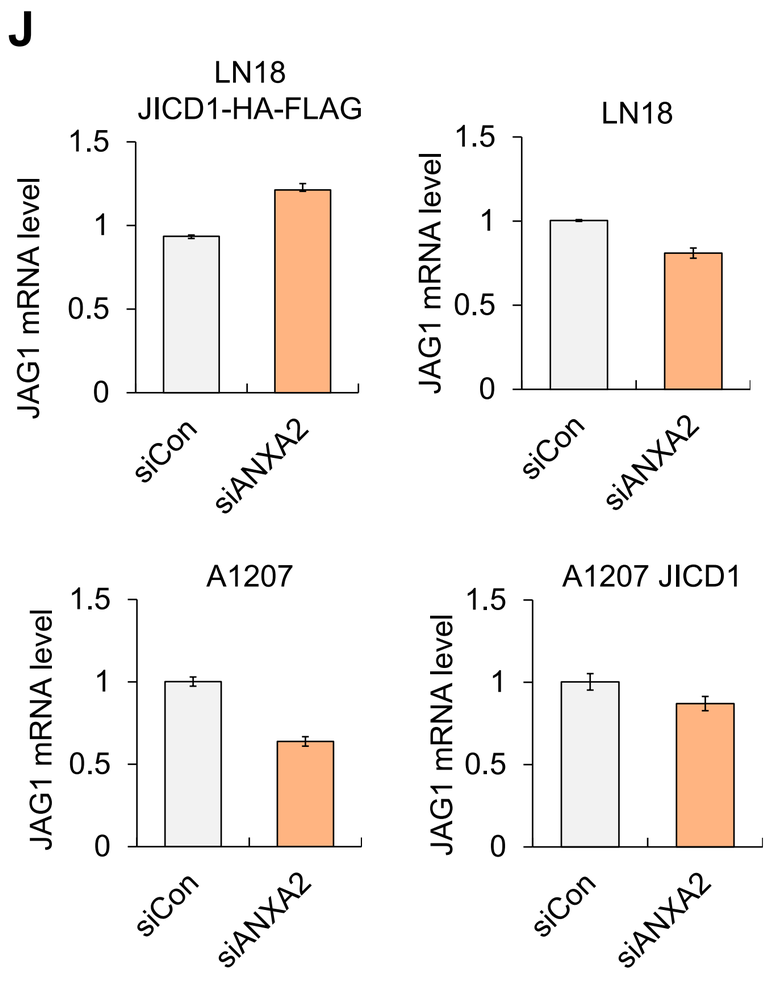


Supplementary Figure S1. Annexin A2 (ANXA2) is a JAG1 intracellular domain (JICD1)-binding protein.

(A) A graphic description of the NOTCH signaling pathway. NICD; NOTCH intracellular domain, CSL; centromere-binding protein 1/suppressor of hairless/lag-1, MSML; mastermind-like protein.

(B) A schematic presentation of JICD1-binding protein identification and a list of identified proteins.

(C) Limiting dilution sphere-forming assay performed following ANXA2 knockdown in other JICD1-expressing cell lines. ***$p < 0.001$.

(D) Microscopic images of immunofluorescence staining of HA and ANXA2 in LN18 cells expressing JICD1-HA-FLAG. Scale bar = 20 μm.

(E) Immunoprecipitation against HA in LN18 JICD1-HA-FLAG cells.

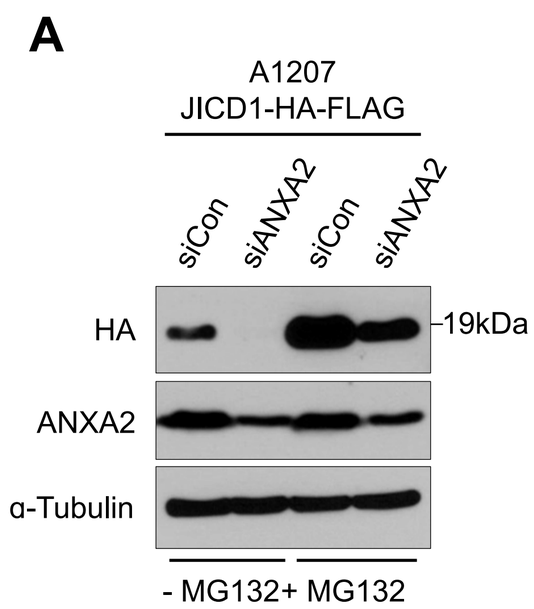
(F) Immunoprecipitation against HA and FLAG in HEK293T cells transduced with JAG1-HACterm and ANXA2-FLAG.

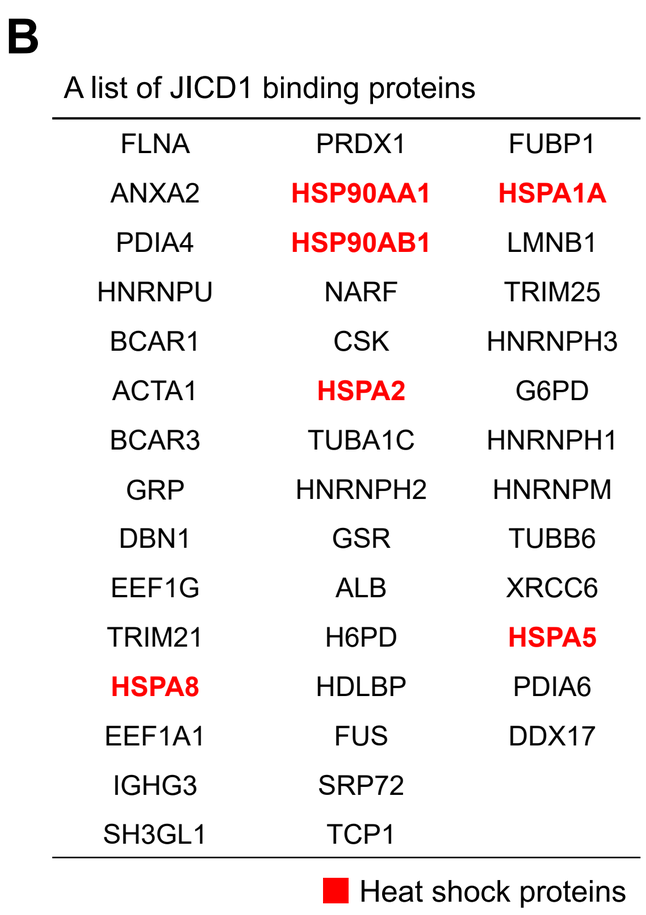
(G) In vitro binding assay using the purified recombinant JICD1 and GST-ANXA2. The pull-down against JICD1 in the absence of JICD1 was used as a control.

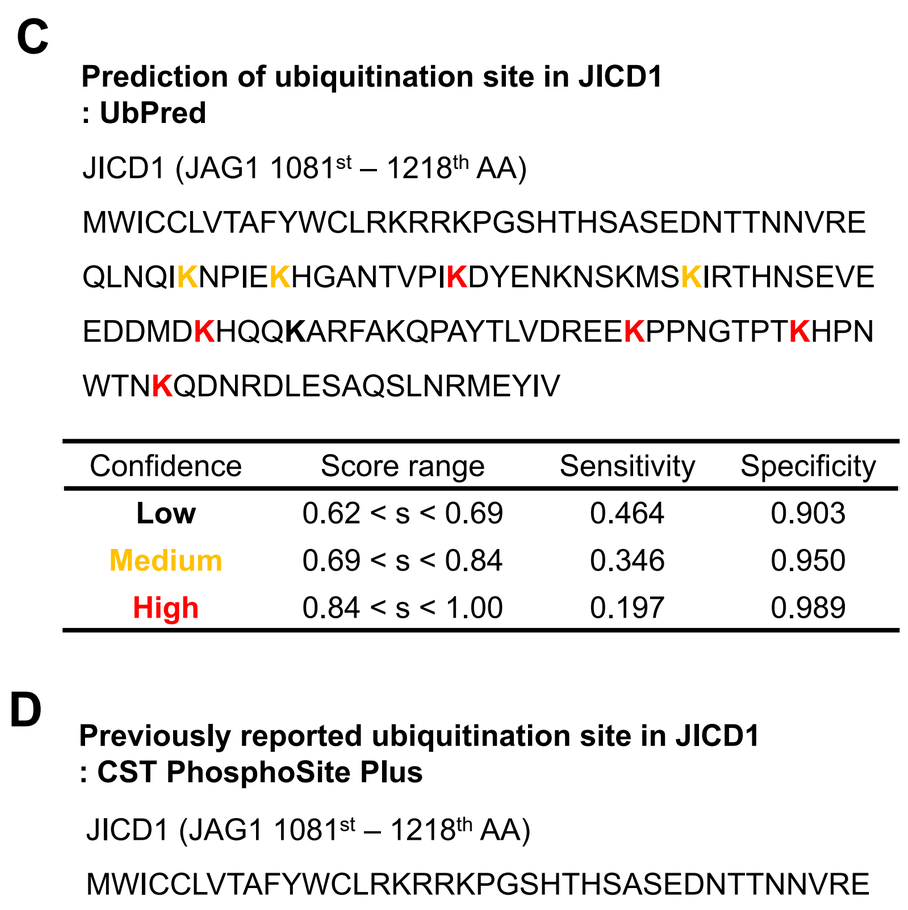
(H) Western blotting results showing that ANXA2 knockdown decreases JICD1 protein level in A1207 cells.

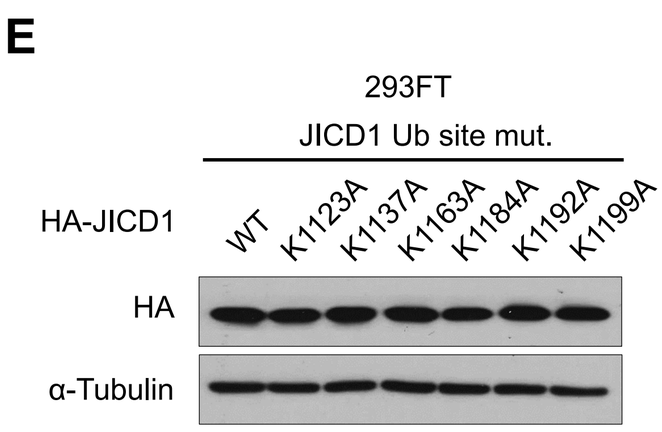
(I) qRT-PCR showing that ANXA2 expression is increased by JICD1 and is downregulated by ANXA2 knockdown.

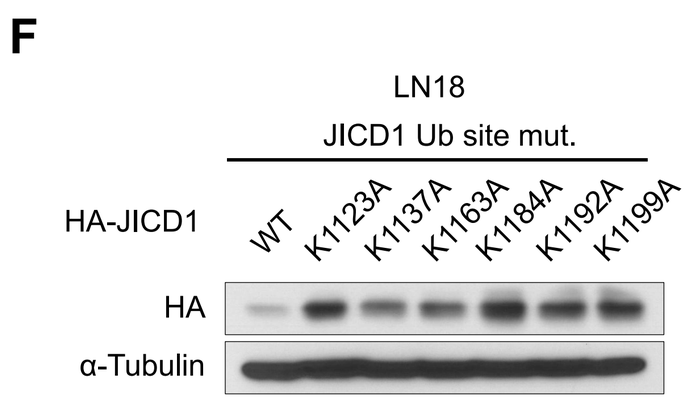
(J) qRT-PCR demonstrating the mRNA level following ANXA2 knockdown in LN18 JICD1-HA-FLAG, LN18, A1207, A1207 JICD1 cells.

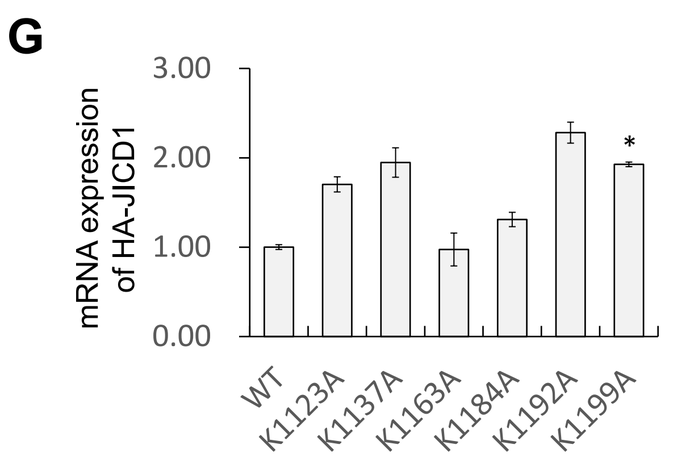












Supplementary Figure S2. The transcriptional expression of JAG1 was not changed after the ANXA2 knockdown.

(A) The protein level of JICD1-HA-FLAG following ANXA2 knockdown combined with CQ treatment in A1207-HA-FLAG cells.

(B) A list of HSP70 and HSP90 family proteins detected as JICD1-binding proteins.

(C, D) Possible ubiquitination sites in JICD1 predicted by using the (C) UbPred and (D) CST PhosphoSite Plus.

(E, F) The protein level of JICD1 with mutants in possible ubiquitination sites in (E) HEK 293T and (F) LN18 cells.

(G) Level of JICD1 mRNA with mutants in possible ubiquitination sites in LN18 cells. *$p < 0.05$.

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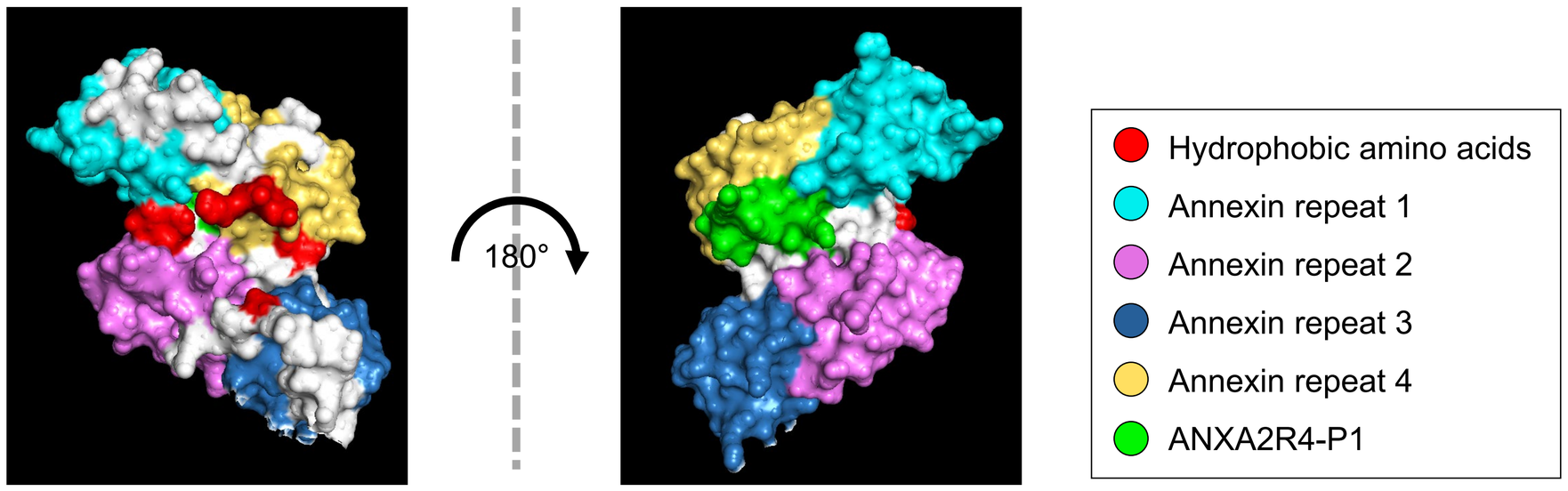

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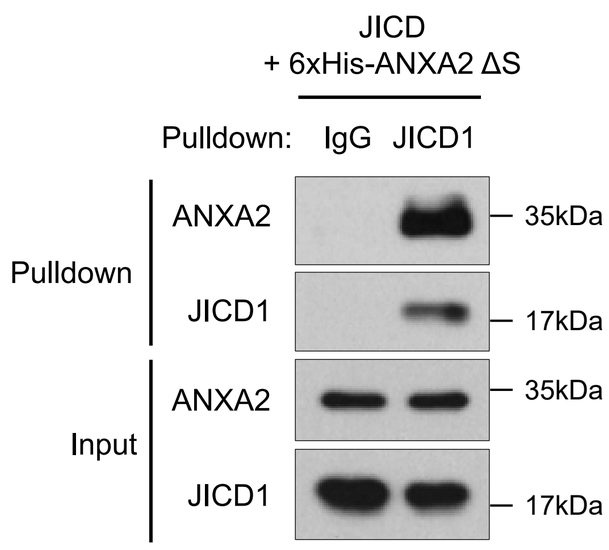

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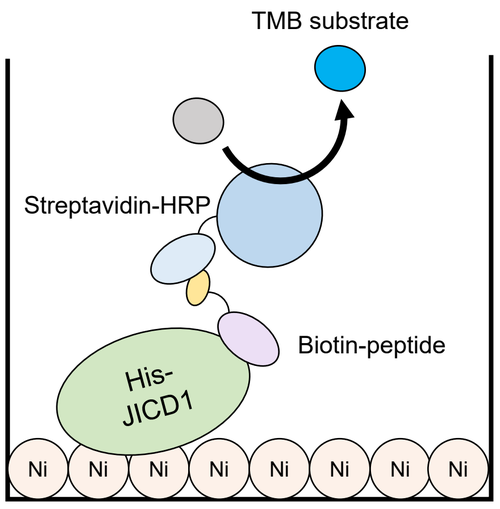
Peptide	Sequence	Length
R4P-1	LYDSMKGKGTRDKVLIRIMVS	21
R4P-2	RSEVDMLKIRSEFKRK	16
R4P-3	YGKSLYYYIQQ	11
R4P-4	DTKGDYQKALLYL	13

D



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	Locus	Amino acid sequence	Similarity
ANXA2 R4P-1		LYD------S------MKGKGTRDK-V--LIRIMVS	
ANXA2 isoform 2	(274 – 294)	LYD------S------MKGKGTRDK-V--LIRIMVS	100(21/21)
	(109 – 123)	YDASELKAS------MKGLGT	38(8/21)
	(198 – 217)	LYD------AG-----VKRKGT-D--VPKWISIM	57(12/21)
ANXA6 isoform 1	(260 – 280)	LFK------A------MKGLGTRDN-T--LIRIMVS	76(16/21)
	(608 – 628)	LYK------S------MKGAGTDEK-T--LTRIMVS	76(16/21)
	(103 – 121)	D------A------ISGIGTDEK-C--LIEILAS	52(11/21)
	(439 – 459)	YD------AKQLKKAMEGAGTDEK-A--LI	47(10/21)
	(29 – 35)	LYT------A------MKG	24(5/21)
	(376 – 381)	MKGLGT	24(5/21)
ANXA1	(282 – 302)	LHQ------A------MKGVGTRHK-A--LIRIMVS	71(15/21)
	(127 – 143)	MKGLGTDED-T--LIEILAS	52(11/21)
ANXA4 isoform a	(256 – 276)	LYK------S------MKGLGTDDN-T--LIRVMVS	76(16/21)
	(91 – 106)	LYDVQELRRA------MKGAGT	38(8/21)
	(29 – 34)	MKGLGT	24(5/21)
ANXA5	(255 – 275)	LYY------A------MKGAGTDDH-T--LIRVMVS	71(15/21)
	(90 – 110)	LYD------AYELKHALKGAGTNEK-V--L	57(12/21)
	(28 – 33)	MKGLGT	24(5/21)
ANXA11 isoform 1	(444 – 460)	MRGAGTKDR-T--LIRIMVS	62(13/21)
	(213 – 215)	MKG	14(3/21)
ANXA8 isoform 1	(300 – 320)	LYY------A------MKGAGTRDG-T--LIRNIVS	67(14/21)
	(140 – 160)	LHD------A------MKGLGTKEG-V--IIEILAS	62(13/21)
	(30 – 36)	LYK------S------MKG	28(6/21)
ANXA13 isoform a	(254 – 273)	LYK------S------MKGAGTDEE-T--LIRIVV	67(14/21)
	(99 – 110)	MKGLGTDES-V--LI	43(9/21)
	(148 – 154)	LKKILVS	19(4/21)
	(28 – 32)	KGMGT	19(4/21)
ANXA7 isoform 1	(403 – 422)	LYY------A------MKGAGTDDS-T--LVRIVV	62(13/21)
	(239 – 259)	YD------AWSLRKAMQGAGTQER-V--LI	47(10/21)
	(176 – 178)	MKG	14(3/21)
ANXA9	(292 – 299)	V--LIRILIS	28(6/21)
	(184 – 188)	KGGRD	10(2/21)
ANXA3	(102 – 114)	S------MKGAGTNED-A--LI	38(8/21)
	(35 – 45)	GTDEK-M--LISIL	28(6/21)
	(262 – 278)	LKGIGT-DEFT--LNRIMVS	47(10/21)
	(226 – 230)	D------S------IKG	19(4/21)
ANXA10	(92 – 118)	LYD------AHELWHAMKGVGTDEN-C--LIEILAS	57(12/21)
	(267 – 275)	NK-T--VIRILI	19(4/21)

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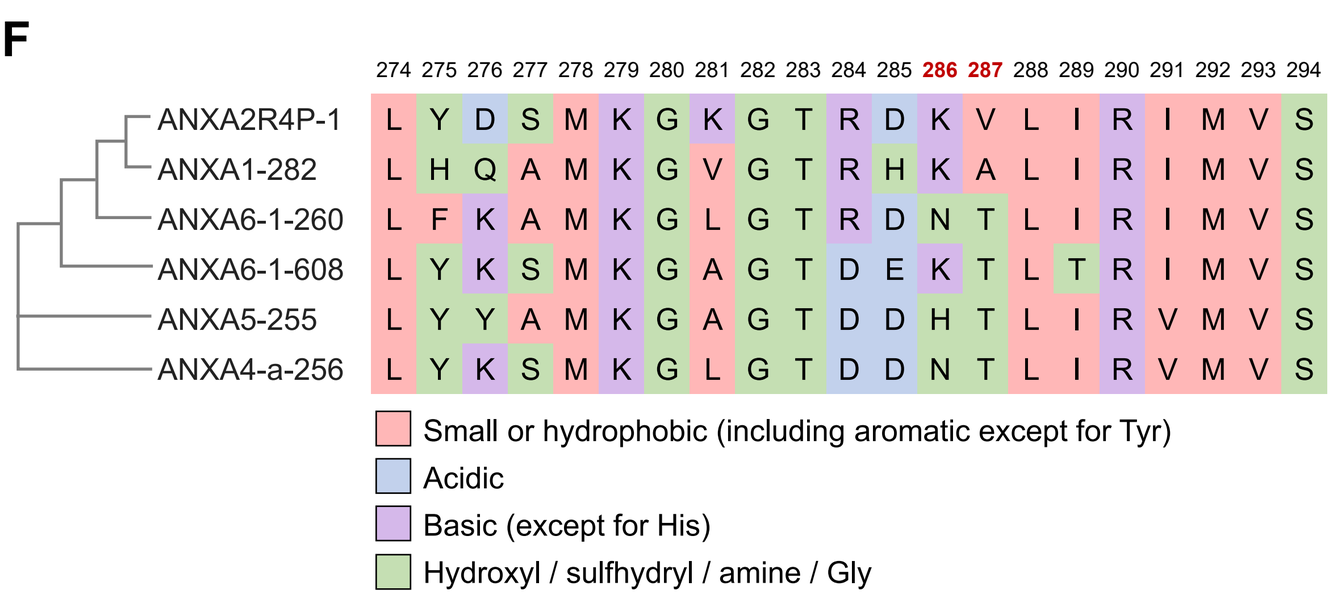


Supplementary Figure S3. R4P-1 region of ANXA2 directly binds to JICD1.

(A) Three-dimensional structural presentation of ANXA2 (PDB: 1XJL) with an indication of Annexin repeats, R4P-1.

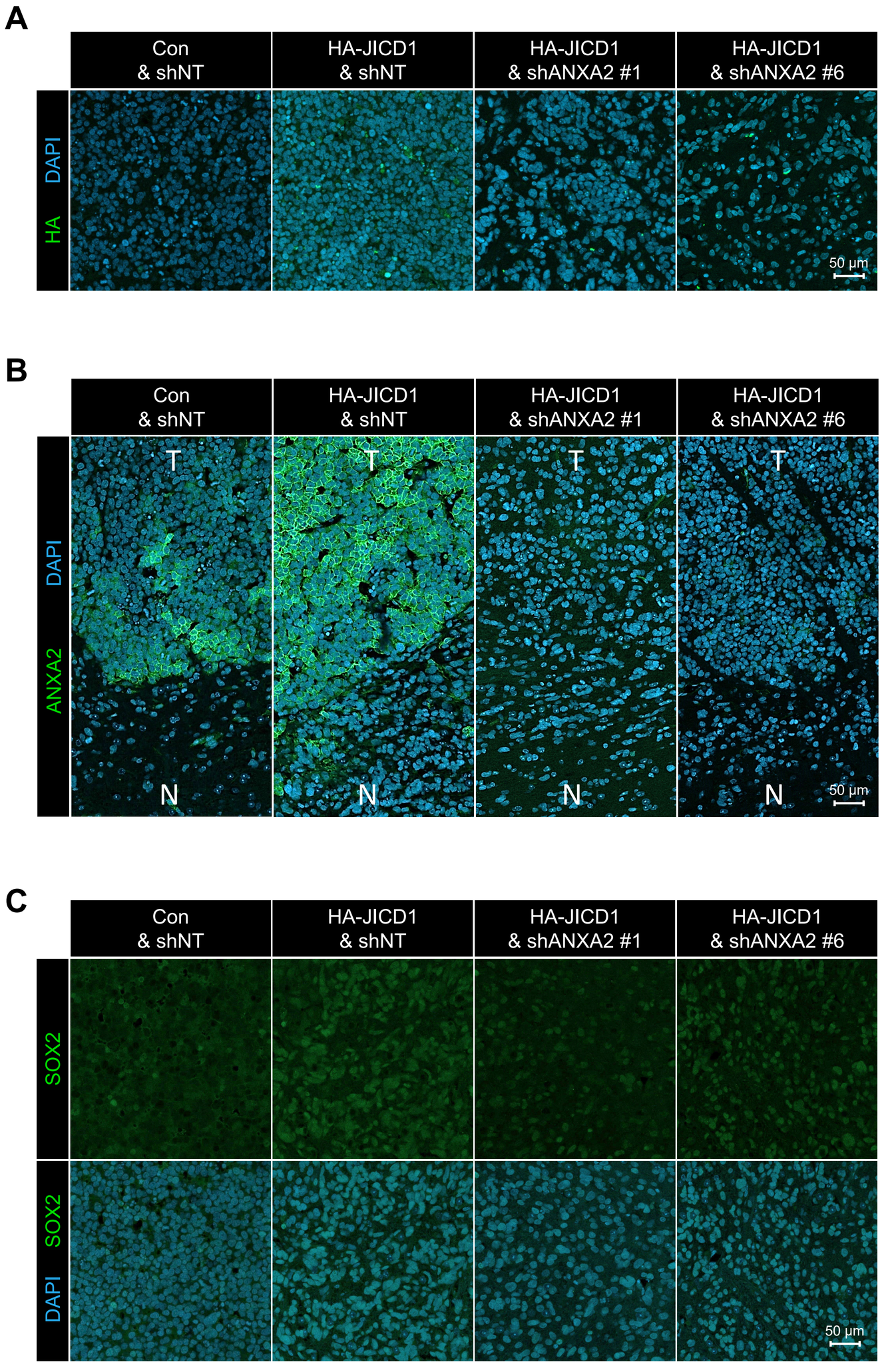
(B) In vitro binding assay using the recombinant JICD1 and 6xHis-tagged ANXA2 lacking S domain (ANXA2ΔS).

(C) Sequences of ANXA2 R4 peptides, R4P-1, 2, 3, and 4.

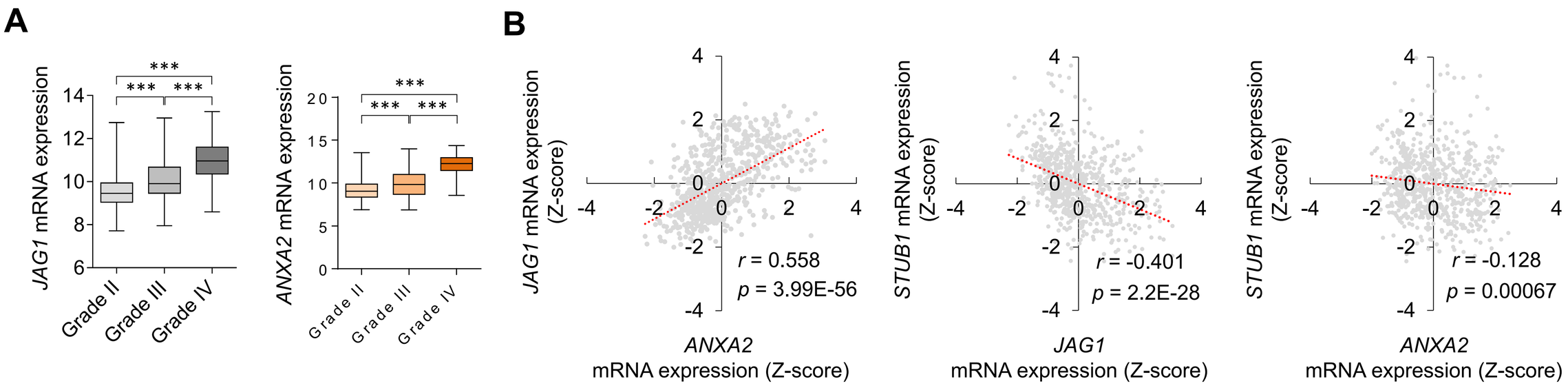
(D) A graphic description of the JICD1-peptide binding assay.

(E) The result from the Basic Local Alignment Search Tool search for the proteins having a similar amino acid sequence to R4P-1.

(F) Clustering of the amino acid sequences of the peptides used in Figure S2E.



Supplementary Figure S4. Immunofluorescence images for (A) HA-JICD1, (B) ANXA2, and (C) SOX2. T: Tumor, N: Normal. Scale bar = 50 μm.



Supplementary Figure S5. JICD1–ANXA2 interaction correlates with cancer aggressiveness in patients with glioma.

(A) The mRNA expression levels of *JAG1* (left) and *ANXA2* (right) in GBM patients depending on the WHO grade. Unpaired two-tailed t-tests: ***$p < 0.001$.

(B) Correlation between JAG1, ANXA2, and STUB1 in TCGA database.

(C) Survival time of patients with glioma according to expressions of ANXA2 and STUB1. The patients were divided into two groups (JAG1 high and STUB1 low versus JAG1 low and STUB1 high) based on their mRNA expression (mean ± SEM). *p*-value was calculated with a log-rank (Mantel-Cox) test. ***$p < 0.001$.