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Supplemental information

m^{6}A-induced repression of SIAH1 facilitates alternative splicing of androgen receptor variant 7 by regulating CPSF1

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Supplementary Table S1. Sequence of primers, siRNA, and shRNA used in the study

shSIAH1 (1)	sense (5'-3')	GATCCGCAATTTAGGCATCAATGTAAGCTTACATTGATGC CTAAATTGCTTTTTT
	anti-sense (5'-3')	CTAGAAAAAAGCAATTTAGGCATCAATGTAAGCTTACAT TGATGCCTAAATTGCG
shSIAH1#2	sense (5'-3')	GATCCTAATGGACTTATGCTGATGCAGCTGCATCAGCATA AGTCCATTATTTTTT
	anti-sense (5'-3')	CTAGAAAAAATAATGGACTTATGCTGATGCTGCATCAGC ATAAGTCCATTAG
shAR-v7 (2)	Sense (5'-3')	CCGGAAGGCTAATGAGGTTTATTTTCTCGAGAAAATAAA CCTCATTAGCCTTTTTTTG
	anti-sense (5'-3')	AATTCAAAAAAAGGCTAATGAGGTTTATTTTCTCGAGAA AATAAACCTCATTAGCCTT
CPSF1 siRNA-1	CCAGATGATCAGCGTCAAGAA	
CPSF1 siRNA-2	AGGGCGGATCTTGATCATGGA	
Alkbh5 siRNA-1	ACAAGTACTTCTTCGGCGA	
Alkbh5 siRNA-2	GCGCCGTCATCAACGACTA	
siRNA control (siCont)	TTCTCCGAACGTGTCACGA	
qPCR for SIAH1	sense	TACTCCACCTTCTCTGTACTCCTG
	anti-sense	CTCATTTCTTTTCTCTTCCTTGTC
qPCR for CPSF1	sense	TACCTGTTCCTGGGTTCTCG
	anti-sense	CGCATCCACTCGCTTCTTCT
qPCR for AR-FL	sense	CAGTGGATGGGCTGAAAAAT
	anti-sense	GGAGCTTGGTGAGCTGGTAG
qPCR for AR-v7	sense	GCAATTGCAAGCATCTCAAA
	anti-sense	CAACCCCAACGTCAAAGTCT
qPCR for Alkbh5	sense	TGTCCGTGTCCTTCTTTAGCG
	anti-sense	GCCGTATGCAGTGAGTGATTTC
qPCR for Mettl3	sense	GCAGGCTCAACATACCCGTACT
	anti-sense	AGACATTCTCTCCCCAACTCCAT
qPCR for Mettl14	sense	TTGCAGCACCTCGATCATTTAT
	anti-sense	CTTAGTCTTCCCAGGATTGTTTTTA
qPCR for WTAP	sense	GGAAAACATCCTTGTAATGCGAC
	anti-sense	GCTGGACTTGCTTGAGGTACTG
qPCR for FTO	sense	GCTGTGCCATTGTGTATGTCTG
	anti-sense	ATGTCCACTTCATCTTGTCCGT
qPCR for nup210	sense	AAGGAGAAGTCTTTTGGGTGGC
	anti-sense	GCTGGAGAAGGTCAGGGTAGTG
qPCR for slc3a2	sense	GGGGACTAACTCCTCCGACCT
	anti-sense	GGAGCCTTGCCTGAGACAAACT
qPCR for β-actin	sense	CTGGAACGGTGAAGGTGACA

	anti-sense	AAGGGACTTCCTGTAACAATGCA

1. Ko HR, Jin EJ, Lee SB, et al.: SIAH1 ubiquitin ligase mediates ubiquitination and degradation of Akt3 in neural development. The Journal of biological chemistry 294: 15435-15445, 2019.

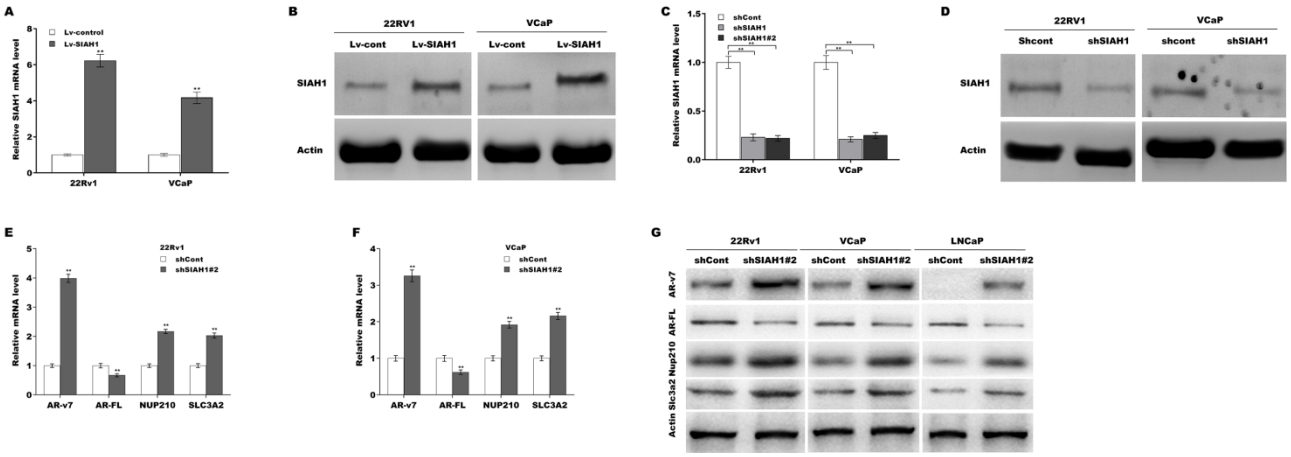
2. Fan L, Zhang F, Xu S, et al.: Histone demethylase JMJD1A promotes alternative splicing of AR variant 7 (AR-V7) in prostate cancer cells. Proceedings of the National Academy of Sciences of the United States of America 115: E4584-E4593, 2018.

Table S2. Genes encoding RBP and splicing factors selected by gene ontology terms in AmiGO

Table S3. Splicing factors concurrently dysregulated in PCa in these datasets

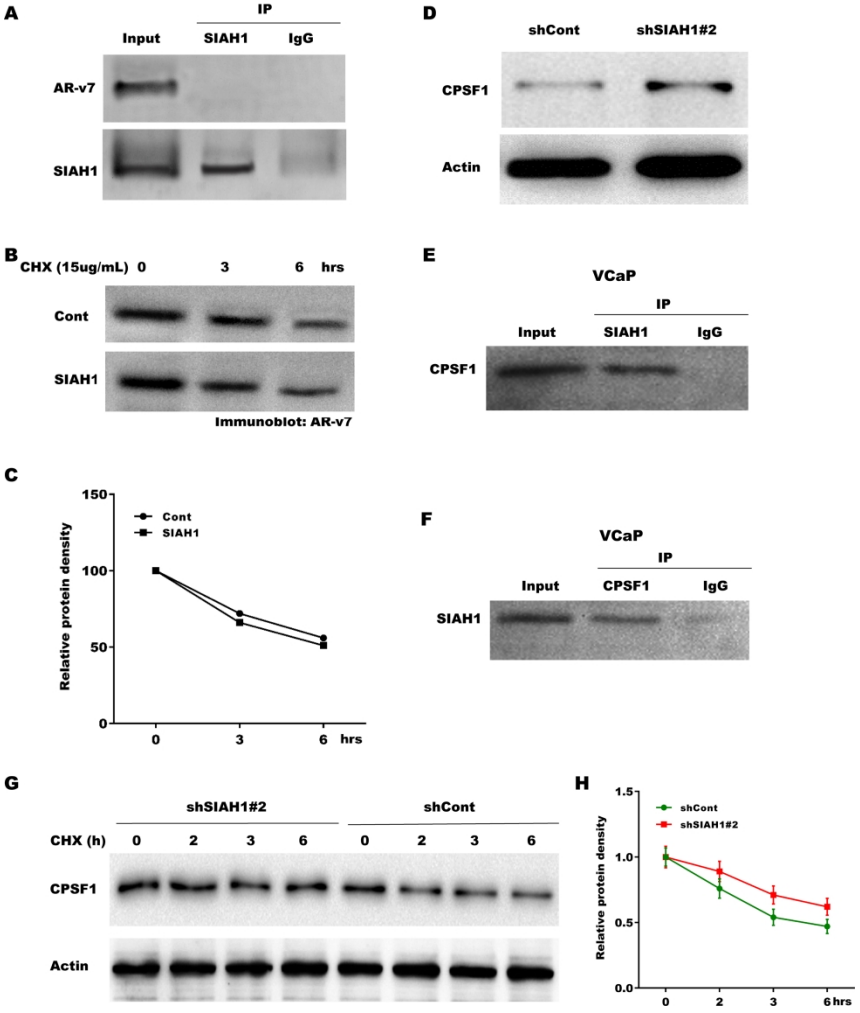
GSE54460	GSE6919	RBP	GSE3325	diff	GeneSymbol
TRUE	TRUE	TRUE	TRUE	up	CPSF1,PABPC1
FALSE	TRUE	TRUE	TRUE	down	MBNL2;LGALS3

Figure S1. Overexpression and knockdown of SIAH1, respectively.



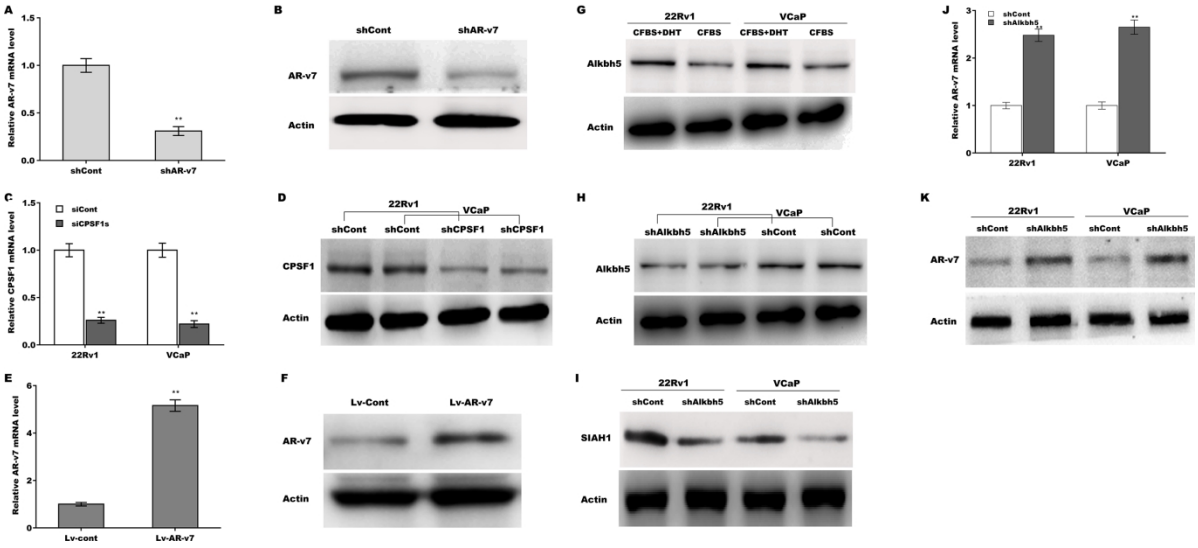
(A) qPCR assays of SIAH1 expression in 22Rv1 and VCaP cells after SIAH1 overexpression. (B) Western Blot analysis for SIAH1 expression in 22Rv1 and VCaP cells after SIAH1 overexpression. qPCR (C) and western blot (D) assays of SIAH1 expression in 22Rv1 and VCaP cells after treatment with shSIAH1 or shSIAH1#2. qPCR assays of AR-v7, AR-FL, Nup210, and Slc3a2 expression in 22Rv1 (E) and VCaP (F) cells after treatment with shSIAH1#2. (G) Western blot assays of AR-v7, AR-FL, Nup210, and Slc3a2 expression in 22Rv1,VCaP, and LNCaP cells after treatment with shSIAH1#2. $^{**}p<0.01$.

Figure S2. SIAH1 regulates CPSF1 portein stability.



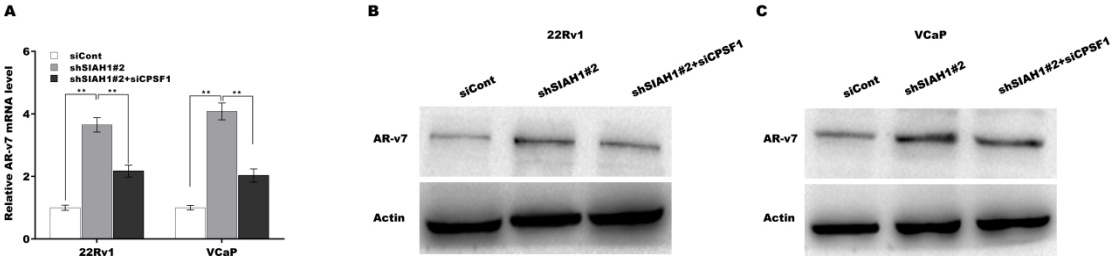
(A) The interaction between SIAH1 and AR-v7 in 22Rv1 cells was determined by Co-IP assay. (B and C) The effect of SIAH1 on the stability of AR-v7 in SIAH1 overexpressed 22Rv1 cells was determined by cycloheximide chase assay. (D) Western blot assays of CPSF1 expression in LNCaP cells after treatment with shSIAH1#2. (E and F) The interaction between SIAH1 and CPSF1 in VCaP cells was determined by reciprocal Co-IP assay. (G and H) The protein level of CPSF1 was assessed by CHX pulse-chase assay in the presence or absence of shSIAH1#2. CHX (50 μg/ml) was used for inhibiting the *de novo* protein synthesis and then relative protein density of CPSF1 was assessed at different time points (0, 2, 3, and 6 h).

Figure S3. Alkbh5 regulates SIAH1 expression.



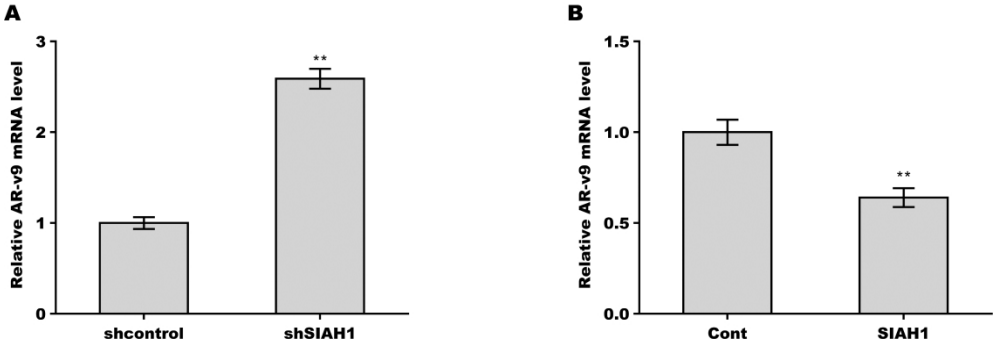
qPCR (A) and western blot (B) analysis of AR-v7 expression in 22Rv1 cells after shAR-v7 treatment. qPCR (C) and Western blot (D) analysis of CPSF1 expression in 22Rv1 and VCaP cells after siCPSF1s treatment. qPCR (E) and Western blot (F) analysis of AR-v7 expression in 22Rv1 cells after Lv-AR-v7 treatment. (G) Western blot analysis of Alkbh5 expression in 22Rv1 and VCaP cells under androgen deprivation. (H) Western blot analysis of Alkbh5 expression in 22Rv1 and VCaP cells after siAlkbh5 treatment. (I) Western blot analysis of SIAH1 expression in 22Rv1 and VCaP cells after Alkbh5 knockdown. qPCR (J) and western blot (K) analysis of AR-v7 expression in 22Rv1 and VCaP cells after Alkbh5 knockdown. $^{**}p<0.01$.

Figure S4. SIAH1 knockdown increased AR-v7 expression in a CPSF1-dependent manner.



qPCR (A) and western blot (B and C) analysis of AR-v7 expression in 22Rv1 and VCaP cells after treatment with shSIAH1#2 in the presence or absence of siCPSF1. $^{**}p<0.01$.

Figure S5. SIAH1 regulates AR-v9 expression.



qPCR analysis of AR-v9 expression in 22Rv1 cells after SIAH1 knockdown (A) or overexpression (B). $^{**}p<0.01$.