A Conserved Bcd1 Interaction that Is Essential for Box C/D snoRNP Biogenesis

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Supporting Information

Included Supporting Information: Supplementary Figure S1-3 and Tables S1-S3.

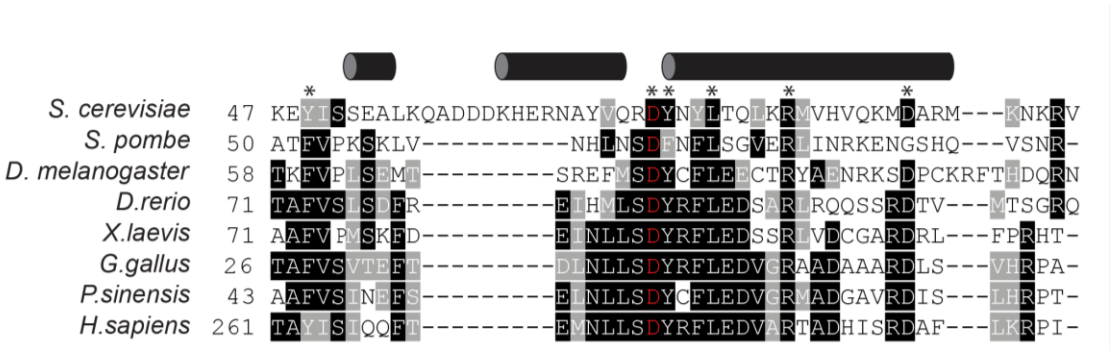

Figure S1. Sequence alignment of the fragments of Bcd1 corresponding to *S.cerevisiae* residues 47-97 from different organisms. Very conserved and less conserved residues are outlined in black and grey, respectively. The residues which were mutated in Figure 1D are marked with asterisks. The conserved Asp72 is colored in red. The secondary structure prediction of *S. cerevisiae* Bcd1 is shown on the top, with cylinders depicting alpha helices. This figure is related to Figure 1.

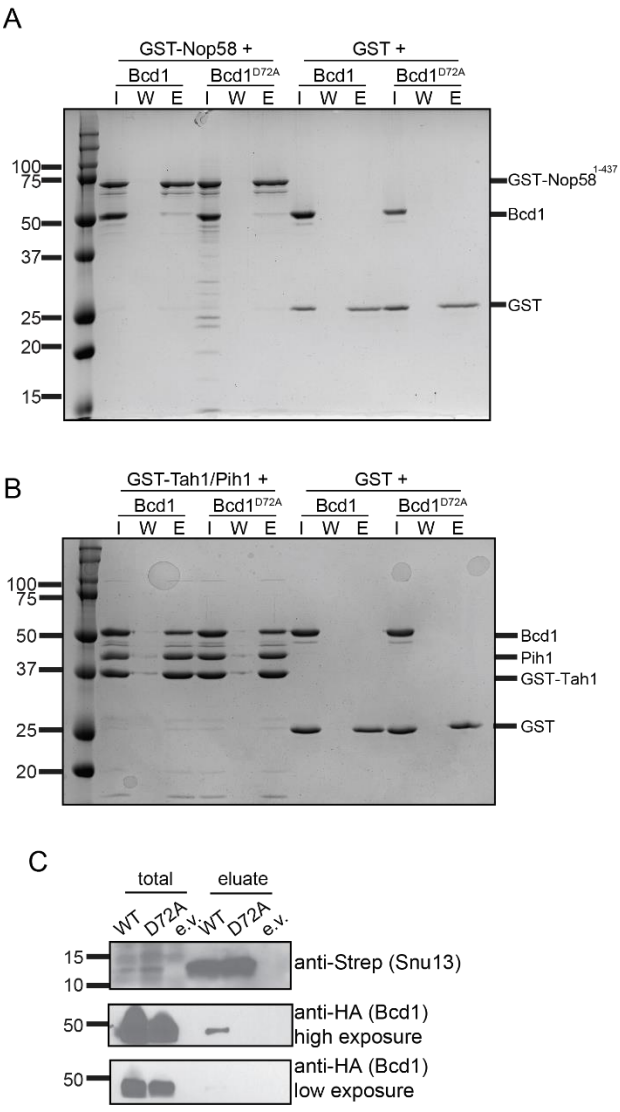

Figure S2. (A) Bcd1 and Bcd1-D72A variant bind to GST-Nop58$^{1-437}$ but not to GST alone. (B) Bcd1 and Bcd1-D72A bind to GST-Tah1/Pih1 but not to GST alone. (C) Strep-tagged Snu13 can pull down Bcd1 but not Bcd1-D72A variant from total yeast lysate. This figure is related to Figures 2 and 3.

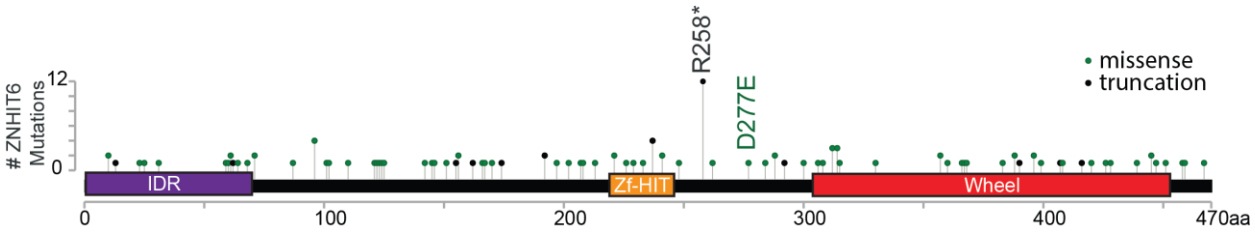

Figure S3. Analyses of ZNHIT6 cancer mutations. A stop mutation in ZNHIT6 which may result in ZNHIT6 protein lacking the conserved D277 and the wheel domain is associated with cancer.

Table S1: Yeast strains used in this work

Strain name	Description	Background	Reference
yHG000	WT	BY4741	GE Dharmacon
yHG057	*tetO7:BCD1*	R1158 (derived from BY4741)	GE Dharmacon
yHG458	*bcd1-D72A*	BY4741	This work

Table S2: Oligonucleotides used in this work

Use	Type	Gene	Sequence (5' to 3')
Northern probes		U3	CATAGGATGGGTCAAGATCATCGCGCC
		U14	CGATGGGTTCGTACTCCTACCGTGG
		U24	GGTATGTCTCATTCGGAACTCAAAGTTCCATC TGAAGTAGC
		snR8	CACTCGCGCAGCTACCGATCTGGGCCAATGG GAGAC
		snR35	GAACAAAATGATGATCTCTCCGATGGACTTGA CGC
		snR38	GAGAGGTTACCTATTATTACCCATTCAGACAG GGATAACTG
		snR46	CTTCCCTTTGGAAATCGGAAATTCAATGATAT GCCCTATGCCC
		snR48	GGAGAGTACTTAAACTTCACATCCTAACATTA GAGATGCCAG
		snR51	TGTAGTCATCAATTAGCCCC
		scR1	ATCCCGGCCGCCTCCATCAC
		18S	CATGGCTTAATCTTTGAGAC
		25S	GCCCGTTCCCTTGGCTGTG
		003 (A2-A3)	TGTTACCTCTGGGCCC
		B (D-A2)	GCTCTCATGCTCTTGCC
		E (E-C2)	GGCCAGCAATTTCAAGTTA
Site-directed mutagenesis	Y49A	*BCD1*	CAAACACATGATCCTAAGGAGGCTATATCGAG TGAGGCG
	D72A	*BCD1*	GCTTATGTCCAGAGGGCCTACAACTATCTGAC GC
	Y73A	*BCD1*	GCTTATGTCCAGAGGGACGCCAACTATCTGA CGCAG
	D72A,Y73A	*BCD1*	CGAAATGCTTATGTCCAGAGGGCCGCCAACT ATCTGACGCAGTTGAAGC
	L76A	*BCD1*	CCAGAGGGACTACAACTATGCGACGCAGTTG AAGCGAATG
	R81A	*BCD1*	CTATCTGACGCAGTTGAAGGCAATGGTGCAT GTACAAAAG
	D89A	*BCD1*	GGTGCATGTACAAAAGATGGCTGCTAGAATGA AGAACAAG
Cloning	For	*BCD1*	CGATCCCCGGGATGGCGGTGTTGTGTGGTGT ATGC
	Rev	*BCD1*	CGATCCTCGAGTCATGCAGTGAGGAAATCCAT GGATAAGC
	For	*TAH1*	CGTCAGGATCCATGAGCCAATTTGAAAAGCAG AAGG
	Rev	*TAH1*	CGTCACTCGAGTCAGGACCGGTCGTATCCCT CCGG
	For	*PIH1*	CGATCGGCGCCATGGCCGATTTCTTATTGAGA CC
	Rev	*PIH1*	CGTCACTCGAGTTATATATATATATATAGTGTG CGTTCTTTGC
	For	*RSA1*	CGATAGGCGCCATGAATTATAATAACTTTGAA AATTCGAAGGG
	Rev	*RSA1*	CGATACTCGAGTTACTTATTTCTAGTAAAGTTG GCTCTACTC

	For	*SNU13*	CGTCAGGATCCATGTCTGCCCCAAACCCAAAGGCTTTC
	Rev	*SNU13*	CGTCACTCGAGTTAAATTAATAAAGTTTCAATCTTGTCC
	For	*HIT1*	CGATAGGATCCATGGTATCTAGTGCAGTTAAATGTGGC
	Rev	*HIT1*	CGATACTCGAGTTATTTCTTCACCGCATTTAATTTATC
	For	*NOP58*	GATCGGATCCATGGCTTACGTTTTAACTGAAACTTCAGCT
	Rev	*NOP58_A437*	TCACTCGAGTTAAGCAGCCTTTGCGGTATCAGCAT
CRISPR primers	Bcd1-D72A repair		CGAACGAAATGCTTATGTCCAGAGGGCCTACAACTATCTAACGCAGTTGAAGCGAATGGT
	Bcd1 sgRNA mutagenesis		CGGGTGGCGAATGGGACTTTTCTGACGCAGTTGAAGCGAAGTTTTAGAGCTAGAAATAGC
	Bcd1-D72A repair amplification-Rev		CCGCTTGTTCTTCATTCTAGCATCCATCTTTTGTACATGCACCATTCGCT
	Bcd1-D72A repair amplification-For		TATCGAGTGAGGCGTTGAAACAGGCGGACGACGACAAGCACGAACGAAAT
Truncations	Start 47	*BCD1*	GACTACCCGGGATGAAGGAGTATATATCGAGTGAGGC
	Start 97	*BCD1*	CGATCCCGGGATGGTTCTGGGGCCTGTGGGCGGC

Table S3: Plasmids used in this work

Plasmid	Description	Backbone	Reference
pHG19	MBP-Bcd1	pSV272	This work
pHG103	MBP-Rsa1	pSV272	This work
pHG119	His-Bcd1	pET23a-TEV	This work
pHG151	His-Bcd1-D72A	pET23a-TEV	This work
pHG104	Tah1	pET24	This work
pHG107	Pih1	pET23a-TEV	This work
pHG108	Snu13	pGEX-6P-2	This work
pHG120	Tah1	pGEX-6P-2	This work
pHG122	Pih1	pSV272	This work
pHG138	$Nop58^{1-437}$	pGEX-6P-2	This work
pHG115	Hit1	pGEX-6P-2	This work
	Rvb1/Rvb2	pET28	Zhou et al., 2017
pHG309	*TEF::BCD1*	pRS413	This work
pHG385	*TEF::bcd1-D72A*	pRS413	This work
pHG3004	*TEF::bcd1-Y73A*	pRS413	This work
pHG373	*TEF::bcd1-D72A,Y73A*	pRS413	This work
pHG379	*TEF::HA-BCD1*	pRS413	This work
pHG3002	*TEF::HA-bcd1-D72A*	pRS413	This work
pHG3031	*TEF::ZNHIT6*	pRS413	This work
pHG3015	*TEF::znhit6-D277A*	pRS413	This work
pHG3170	*TEF::bcd1-Y49A*	pRS413	This work
pHG3171	*TEF::bcd1-L76A*	pRS413	This work
pHG3172	*TEF::bcd1-R81A*	pRS413	This work
pHG3173	*TEF::bcd1-D89A*	pRS413	This work
pHG3040	*TEF::Twin-Strep-BCD1*	pRS413	This work
pHG3073	*TEF::Twin-Strep-D72A*	pRS413	This work
pHG3193	*GPD::Twin-Strep-Snu13*	pRS425	This work
Addgene #60847	pCas	2-micron/pUC	Ryan et al., 2014
pHG3168	pCas-Bcd1	2-micron/pUC	This work