

Supplementary File S1

This file contains:

1.- HH-sgRNAS-HDV Sequences

2.- EMBOSS Needle pairwise alignment of *S. cerevisiae* Crz1 (P53968) with *K. phaffii* C4R2J3 and of *S. cerevisiae* Rim101 (P33400) and *A. nidulans* PacC (Q00202) with *phaffii* C4R535

3.- Supplementary Methods for proteomics analyses

HH-sgRNAs-HDV Sequences

These sequences were cloned into the vector BB3cH_pGAP_23* _pLAT1_Cas9 as described in the main text. The gene-specific sequences are underlined.

>Crz1-sgRNA-1

tgaagacgccatgTTCACGCTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCCGTGAATC
AGATAAAAACGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT
GAAAAAGTGGCACCAGAGTCGGTGCTTTTGGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGC
TGGGCAACATGCTTCGGCATGGCGAATGGGACAGCTTTGGACTgcttgcgctcttct

>Crz1-sgRNA-8 (248 bp)

tgaagacgccatgTTACGCCTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCCGCTAAAC
ATGGCAGACCAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT
GAAAAAGTGGCACCAGAGTCGGTGCTTTTGGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGC
TGGGCAACATGCTTCGGCATGGCGAATGGGACAGCTTTGGACTgcttgcgctcttct

> Rim101-gRNA-4 (248 bp)

tgaagacgccatgGGCCCACTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCTGGGCCAT
TCCAGTGCAAGTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT
GAAAAAGTGGCACCAGAGTCGGTGCTTTTGGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGC
TGGGCAACATGCTTCGGCATGGCGAATGGGACAGCTTTGGACTgcttgcgctcttct

> Rim101-gRNA-26 (248 bp)

tgaagacgccatgACACAGCTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTCCTGTGTTG
TTAGCAATGGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT
GAAAAAGTGGCACCAGAGTCGGTGCTTTTGGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGC
TGGGCAACATGCTTCGGCATGGCGAATGGGACAGCTTTGGACTgcttgcgctcttct

```
#####  
#  
#  
# Aligned_sequences: 2  
# 1: ScRim101  
# 2: C4R535 1  
# Matrix: EBLOSUM62  
# Gap_penalty: 10.0  
# Extend_penalty: 0.5  
#  
# Length: 792  
# Identity: 178/792 (22.5%)  
# Similarity: 263/792 (33.2%)  
# Gaps: 351/792 (44.3%)  
# Score: 434.5  
#  
#  
#####
```

3

C4R535	499	-----NSNSKNSSISVNQKSSVNTLRNLTGLLDKLDINEANGSESS	539
ScRim101	553	SEIED-----DFVEMLGIVNIIKDYLLCCVMEDLDDE	584
C4R535	540	SDVEDSELSTTSSDEETEDININDFLKHRQLVQLIQEY-----	577
ScRim101	585	ESEDKDEENAFLESLEKLSLQNMGTNSVRI-LTRYPKILV	625
C4R535	578	-----IODALKOSKAVEOSATTMKEGSSLYPTIVV	608

Alignment *A. nidulans* (En) PacC vs *K. Phaffi* C4R535

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#####
#
# Aligned_sequences: 2
# 1: En_pacC
# 2: C4R535
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 835
# Identity:      153/835 (18.3%)
# Similarity:   236/835 (28.3%)
# Gaps:         384/835 (46.0%)
# Score: 450.0
#
#
#####
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En_pacC	1	-----	0
C4R535	1	MFGNTGLPSISSNIARADNEQRTSNTTFNVHPTTYLNP SIANN TALIAN K	50
En_pacC	1	-----MLGAMAEAEAVAPVAVPTTQE QPTSQ PAAA-----	29
C4R535	51	LNGIQHDFN ASATSSSSNNESASPN SVASYTDS SVQSPSA APEKPEFP SL	100
En_pacC	30	---QVTTVTSPSVTATAAAATAAVAS PQAN-----GN-----	58
C4R535	101	KFHPRQT TTTG PLLSSDLLNVQA HNQNQQF NHPHQ NLYPLD GNDLNA PW	150
En_pacC	59	---AASP VAPASSTS-----RPAEE-----LTCM	79
C4R535	151	NSYDSQRPRASSMNTT PFTVQTSSLMATGVGKR KPRKQLD DSNGPFOCK	200
En_pacC	80	WQCSEKLPTPE SLYEHCERH VGRKSTNNLN LTCOWGSCRTT TVKR DHI	129
C4R535	201	WEECPLVFDDAKVLYQHLCDFHVG RKNKNL SLNCKWDGCDVSTV KR DHI	250
En_pacC	130	TSHIRVH VPLKPKC DFCGKA FKRPQDLKKHVKTH ADDSVLVRSP EPGSR	179
C4R535	251	TSHLRVHVPLKPF GCNLCNKKFKRPQDLKKHVKTHAD DNE LQEIT----	295
En_pacC	180	NPDMFMFGNGKGYAAAHYFEPALNPVPSQGYAHGPPQYYQA HHAPQP-SN	228
C4R535	296	-----PPT EYSVHHQKVPM SF	311
En_pacC	229	PSYGNVYYALNTGPEPHQASYE SKKR GY-DALNEFFG---DLKRRQ FDP	273
C4R535	312	P-----DSRKR AFNDVLSQNF GTFYEEIKRSKI QP	341
En_pacC	274	NSYAAVGQRLLSLQNL SLPVLTAAPLPEYQAMPAPVAVASG--PYGGGPH	321
C4R535	342	MYNSDIATKLNSLGNM---IPTNQPFQQQQH LQPLPYHGHSAYNHSNH	388
En_pacC	322	-----PAPAYHLPP---MSNVRTKNDLINIDQFLQQMQDTIYEND	358
C4R535	389	GSLQDQHMSLPVP-QSFPPTNKVSNIPTQH ELFEANSFFNQLSNTM----	433
En_pacC	359	DNVAAAGVAQPGAHYIHNGISYRTHSPPTQLPSAHATTQT TAGPIISNT	408
C4R535	434	DHTA-----H HOAHVHPOPLENAHVDAEN---YIEVH	462

En_pacC	409	SAHSPSSSTPALTPPSSAQSYTSGRSPISLPSAHRVSPPHESGSSMYPRL	458
C4R535	463	DDHHHAAHNQALYP-----TINVPGGNYSFFYPQI	492
En_pacC	459	PSATDGMTSGYTAASSAAPST-----LG GIFNDERRRYTGGLQRAR	502
C4R535	493	SNRYETNSNSKNSSISVNQKSSVNTLRNLTGLLDKLDINEANG-----SE	537
En_pacC	503	PASRAASESMDLSSDDKESGE-----RTPKQISASLIDPALHSGSP	543
C4R535	538	SSTDVEDSELSTTSDEETEDININDFLKHRQLVLQLIQEYIQDALKQSKA	587
En_pacC	544	GEDDVTRTAKAAATEVAERSDVQSEWVEKVRLIEYL RNYIANRLERGEFS D	593
C4R535	588	VEQSATTMTKEGSSL YPTIVV-----	608
En_pacC	594	DSEQEQDQE QE QDQE EQDQE QGQDRVSRSPVSKADVDMEGVERDSLPRS	643
C4R535	609	-----	608
En_pacC	644	PRTVP IKT DGESAEDSVMYPTIRGLDEDGDSKMPS	678
C4R535	609	-----	608

Alignment *S. cerevisiae* Crz1 vs *K. Phaffi* C4R2J3

ScCrz1	1	MSFSNGNMASYMTSSNGEEQSINNKNKNDIDDNSAYRRNFRNSSNSGSHTF	50
C4R2J3	1	-----	0
ScCrz1	51	QLSD--LDLDVDMR-----MDSANSSEKISKNLSSGIPDSFDSNVNSL	91
C4R2J3	1	-MADQRLDEDFDISRYLSISPIESASIEESINGLMSSWIPPA-KGEIRDS	48
ScCrz1	92	LSPSSGSYSADLNQYSLYKPDLPQQQLQQQQLQQQQQQQQQQQQQKQT	141
C4R2J3	49	LPPNASFEATDSFSTSSYQEIIIPAQVKIKLEFDNDQQPVFYQESQ----	93
ScCrz1	142	PTLKVEQSDTFQWDDILTPADNQHRPSLT-NQFLSPRSNYDGTTRSSGID	190
C4R2J3	94	PV-----YDKHLTVNDQETRSAQDFNQYL----NADAVSRTNSI-	128
ScCrz1	191	SNYSDESNEYH-TPYLYPQDLVS-SPAMSHLTANNDFFDDL----LSVAS	234
C4R2J3	129	SNLSELSTHSHITPPTLLHDQASLSPAL--LSMNSDERNELNLETQLDQ	176
ScCrz1	235	MNSNYLLPVNSH-GYKHISNLDELDDLSTLYSDNNLLSASNNSDFNNSN	283
C4R2J3	177	TSQPYVNQIKTEAAAYEELSELHHRRLERL----TETNLI-----HQD	213
ScCrz1	284	NGIINTADTQNSTIAINKSKVGTNQKMLLTIPSTSTPSPSTHAAPV---T	330

Color codes: CN-binding domain; SRR domain; NLS/NES domains; Zn finger domain

Supplementary Methods for proteomics analyses

Sample preparation

Protein quantification from lysates was performed by tryptophan fluorescence analysis (M-plex). A volume equivalent to 25 µg of protein was brought to 25 µl with water. Then, 25 µl of 2X SDS-containing buffer (10 % SDS, 200 mM triethylammonium bicarbonate (TEAB) pH 8.5, 20 mM tris(2-carboxyethyl)phosphine (TCEP)) was added to reach a final volume of 50 µl. To alkylate cysteine residues, 1 µl of a 1 M iodoacetamide solution was added to reach a final concentration of 20 mM and samples were incubated in the dark for 30 min at 25 °C on a thermomixer at 500 rpm. Samples were acidified with 5 µl of 12 % phosphoric acid (final concentration of 1.2 %) and 330 µl S-Trap buffer (90% methanol, 100 mM TEAB pH 7.1) was added. Proteins were trapped in S-Trap micro columns (ProtiFi, Fairport NY, USA) and washed three times with 150 µl S-Trap buffer. After washing, protein digestion was performed by adding to the column 20 µl of 20 mM TEAB pH 8.0 and 4 µl (2 µg) of 0.5 µg/µl sequencing-grade modified trypsin (Promega). Samples were incubated for 1 h at 47 °C. Then, columns were washed once with 40 µl of 20 mM TEAB pH 8.0 and once with 40 µl of 0.2 % formic acid. To elute the peptides, 35 µl of a solution containing 50 % acetonitrile and 0.2% formic acid was added and columns were centrifuged at 4000 xg for 1 min. Eluted peptides were dried under vacuum and stored at -20 °C. Dried peptides were resuspended in 20 µl of 0.1% aqueous formic acid. Peptide concentration was determined by UV-nanodrop and the peptide concentration was set to 0.25 µg/µl in 0.1 % aqueous formic acid.

DIA LC-MS/MS analysis

An aliquot of 1.2 µl (0.3 µg) of peptides were loaded on EvoTips (EvoSep, Odense, Denmark) according to the vendors instructions using centrifugation. The tips were then subjected to LC-MS/MS analysis using an EvoSep One (EvoSep, Odense, Denmark) connected to an Orbitrap Exploris 480 Mass Spectrometer (Thermo Fisher Scientific) and a custom-made column heater set to 40 °C. Peptides were separated using the 30 SPD default setup. The mass spectrometer was operated in DIA acquisition mode with a total cycle time not exceeding approximately 3 s. For MS1, the following parameters were set: Resolution: 120,000 FWHM (at 200 m/z), Scan Range: 350-1500 m/z, Maximum Injection Time: Auto mode, Normalized AGC Target: Standard. MS2 (SWATH) scans were acquired using the following parameters: Isolation Window: 12 m/z, HCD Collision Energy (normalized): 28 %, Normalized AGC target: 3000 %, Resolution: 15,000 FWHM (at 200 m/z), Precursor Mass Range: 400-900 m/z, Maximal Injection Time: Auto Mode, Data Type: Centroid. In total 42 DIA (MS2) mass windows per MS cycle followed by the one MS1 scan.