

Dim2	S.c.	MVAPTALKKATVTPVSGQDGGSSRIIGINNTESID-EDDDDDVLLDSDNNTAKEEVEGE--EGSRKTHE	67
	C.t.	MPAPTALKQPPPAPE-----QQA-APAITNENEDELLIDIQQA--AATLTDPNAAEPPPEETME	55
	A.n.	MPAPTALRQTAEVAMAE-----TPV-APTM-SEQDEEILIDAQTS--ADQVSAGLA-PEIEPTQD	55
	H.s.	TEARPA-----	7
	A.t.	MAESTQMEVETATE-----	14
Krr1	S.c.	MVSTHNRD-----	12
	C.t.	MPSTHKKD-----	12
	A.n.	MPSTYKRD-----	12
	H.s.	MASPSLERPEK-----GA--GK-SE--FR-NQKPKPEN	27
	A.t.	MAVEEIIAHEE-----QNI--EKREKKKGK-HEKPKPWD	31

Dim2	S.c.	SKTVV-----VDDGKPRFTASKTQG-NKIKFESRKIMVPPHRMTPLRNSWTKIYPPLVEHLKLQVRM	130
	C.t.	-NEMA-----VDEEGRPRFAPGKNID--PIRRIETRKIPIPPHRMSALKANWTKIYPPLVDHCKLQVRM	116
	A.n.	-SDMR-----IDEEGRPLFTPAKDT--RVYRVESRKVPVPPHRMTPLKASWARIYPPLVEHLKLQVRM	116
	H.s.	-----KRPVFPPLCGDGL-LSGKEETRKIPV PANRYTPLKENWMIYFTPIVEHLGLQIRF	61
	A.t.	--GT-----VPLPKPTFKPLKAHEM-SDGKVQFRKIAVPPNRYSPKKAWLDIYTPYIQMVKVDIRM	74
Krr1	S.c.	-TD-D-----IDKWKIEEFKEEDNASGQPF AEEESFMTLFPKYRESYLKTIWINDVTRALDK-HNIACVL	73
	C.t.	-TD-D-----IDKWKIEPFLPEHSS-G-PFLEESFMTLFPKYRERYLKDCWPLVTKALEK-HGIAATL	71
	A.n.	-TD-D-----IDKWKVEPFAEDNVAG-SFAEESFATLFPKYREVYLKEAWPVVTRALEK-FGIACTL	72
	H.s.	-QD-ESELLTVPDGWKEPAFSKEDNP-R-GLLEESFATLFPKYREAYLKECWPLVQKALNE-HYVNATL	92
	A.t.	-DDPN-----IDRWITIEKFDPAWNP-T-GMTETSFFSTLFPQYREKYLQECWPRVESALKE-YGVACKL	91

**KH1**

Dim2	S.c.	NLKTGSVELRTNPKFTDPGALQKGADEFKAFITLGFDDISIALRLDDL YIETFEVKDVK-TLTDHLS	199
	C.t.	NIKERKVELRSS-KYTVSNEALQMGADFVSFAFMGFDIDDAIALLRLDSL YIQSFDIKDVRQTLGPDALS	185
	A.n.	NIKNRAVELRTS-RFTTDTTEALQKGEDFVKAFITLGFDDDAIALLRLDDL YIRSFEIRDIK-ALNGEHL	184
	H.s.	NLKS RNVEIRTC-KETKDVSAITKAADFVKAFILGFQVEDALALIRLDDL FLESFEITDVK-PLKGDHLS	129
	A.t.	NLKARKVELKTR-ADTPTDISNLQKSADFVHAFMLGFDIPDAISLLRMDL YVESFEIKDVK-TLKGHLS	142
Krr1	S.c.	DLVEGSMTVKTTR-KTYDPAIILKARDL I KLLARSVPFPAQAVKILQ-DDMACDVIKIGNFV-T-NKERFV	139
	C.t.	DIVEGSMTVKTTR-KTYDPAIILKARDL I KLLARSVPAPQALKILE-DGMACDVIKIRSMV-R-NKERFV	137
	A.n.	DLVEGSMTVKTTR-KTFDPAAILKARDL I KLLARSVPVQQALKILE-DDVACDVIKIRNQV-R-NKERFV	138
	H.s.	DLIEGSMTVCTTR-KKTFDPIIILKARDL I KLLARSVSEFQAVRILQ-DDVACDVIKIGSLV-R-NKERFV	158
	A.t.	NLVEGSMTVSTTR-KKTRDPIIIVKARDL I KLLARSVPAPQALKILE-DEVQCDVIKIGNLV-R-NKERFV	157

**KH1**

**KH2**

Dim2	S.c.	RAIGRIAGKDGKTKFAIENATRTRIVLADSKIHILGGFTHIGMARESVVSLILGSP-PPGKVYGNLRIVAS	229
	C.t.	RAIGRIAGKDGKTKFAIENATKTRIVLAGSKVHILGAFENIGMARESVVSLV LGA-QPGKVYNNLRIVAS	254
	A.n.	RAIGRCAGKDGRTKFAIENATRTRIVADQKIHLGAVKNVDCAQQAIVSLILGSP-PPGKVYGNLRKVAS	253
	H.s.	RAIGRIAGKGGKTKFTIENVTTRTRIVLADVKHVHILG SFQNKMARTAICNLILGN-PPSKVYGNIRAVAS	198
	A.t.	RAIGRLSGKGGKTKFAIENSTKTRIVIADTRIHLGAFSNIKVARS SLCSLIMGSP-PA GKVYSKLRVSA	211
Krr1	S.c.	KRRQRLVGPNGNTLKALELLTKCYILVQGNTVSAMGPFKGLKEVRRVVEDCMKNI-HP IYHIKEL-MIKR	207
	C.t.	KRRQLLGQNGTLLKALELLTQTYILVHGNTVSMGGYKGLKEVRRVVEDTMNNI-HP IYHIKEL-MIKR	205
	A.n.	KRRQRLGPGGNTLKALELLTSTYILVQGNTVSAMGPFKGLKEVRRRI INDCMANI-HP IYHIKEL-MIKR	206
	H.s.	KRRQRLIGPKGNTLKALELLTNCYIMVQGNTVSAIGPFGSLKEVRRVVDIMKNI-HP IYNIKSL-MIKR	226
	A.t.	KRRQRLVGPNSNTLKALEILLTNCYILVQGNTVVAAMGPFKGLKQLRRLVEDCVQNIHMPVYHIKTL-MMKK	226

**GXXG**

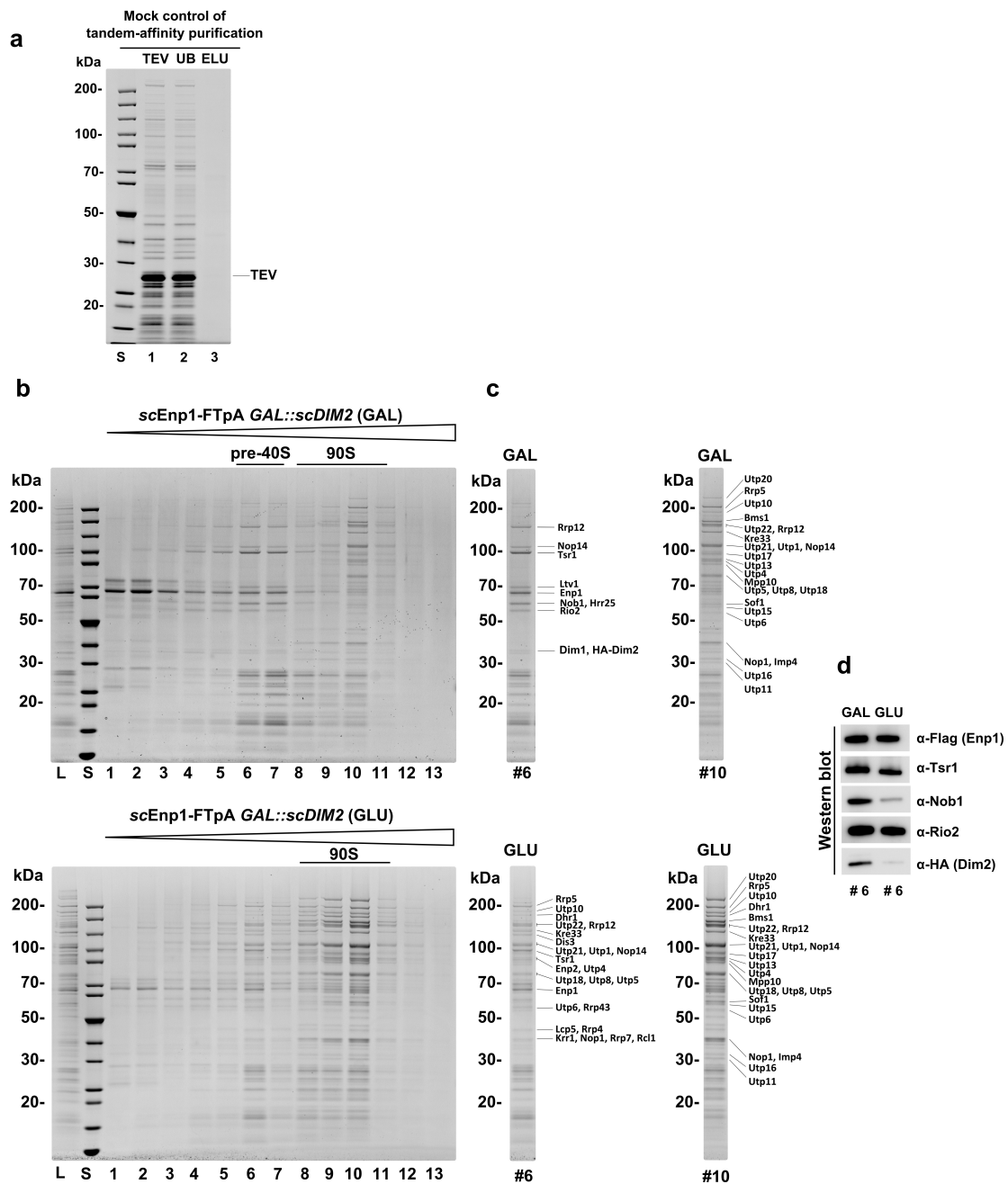
**KH2**

Dim2	S.c.	RLKERY-----	274
	C.t.	RMKERW-----	260
	A.n.	RMKERF-----	259
	H.s.	RSADRF-----	204
	A.t.	--RLNE-----	215
Krr1	S.c.	ELAKRPELANEDWSRFLPMFKKRNVA-RKKPKKIRNVEKKVYTPFPQAQLPRKVDLEIESGGEYFLSKREK	276
	C.t.	ELAKDPALAHEDWSRYLPQFKKRTL SKRRPKFKINDKSKPYTPFPQAPEKSKIDLQIESGGEYFLSKEAK	275
	A.n.	ELAKDPTLANESWDRFLPMFKKRTL SKRRVFPKVTDKTKKVVYTPFPQAPEKSKVDLQIESGGEYFLSKEAK	276
	H.s.	ELAKDS ELRSQSWERFLPQFKHKNVNRKEPKKK--TVKKEYTPFPQPQGSQIDKELASGGEYFLKANQK	294
	A.t.	ELKDPALANESWDRFLPTFRKKNVK-QKKPKS---KEKKPYTPFPQPQPSKIDMLQIESGGEYFMSDKKK	292

Dim2	S.c.	-----	274
	C.t.	-----	260
	A.n.	-----	259
	H.s.	-----	204
	A.t.	-----	215
Krr1	S.c.	QMKKLNEQKEKQMERIEIQEERAKDFIAP EEEAY-----	311
	C.t.	QRAAEAERAERAKRQKKEEKREKREKFPPEEDGGKK-----	312
	A.n.	DRQKKEEVMERQRLKREEKMKEREKAFVPEELDAEEKKE-K---KEKKEK-----KEKKKR	330
	H.s.	KRQKMEAIKAKQAEAISKQEERNKAFIPPEKPKIVKPKKEA-STEAKIDVASI-----KEKVKK	352
	A.t.	SEKKWQEKQEKQSEKSTENKRKRDRASFLPEEPMNNNSNANKSSEDGKNDITELTNSLKSKTRELKQKKTTH	363

Dim2	S.c.	-----	274
	C.t.	-----	260
	A.n.	-----	259
	H.s.	-----	204
	A.t.	-----	215
Krr1	S.c.	-----KPNQN	316
	C.t.	-----KKR-----KVKHGEE	322
	A.n.	KRDSEAADVDDGAEK-KEKKKKKSKSKEDASDGEA	367
	H.s.	AKNKKLGALTAEEIALKMEADE-----KSKKKK	318
	A.t.	ERVNAEEYIAGPSSSAD-KSSK-----KSKKIRD	391

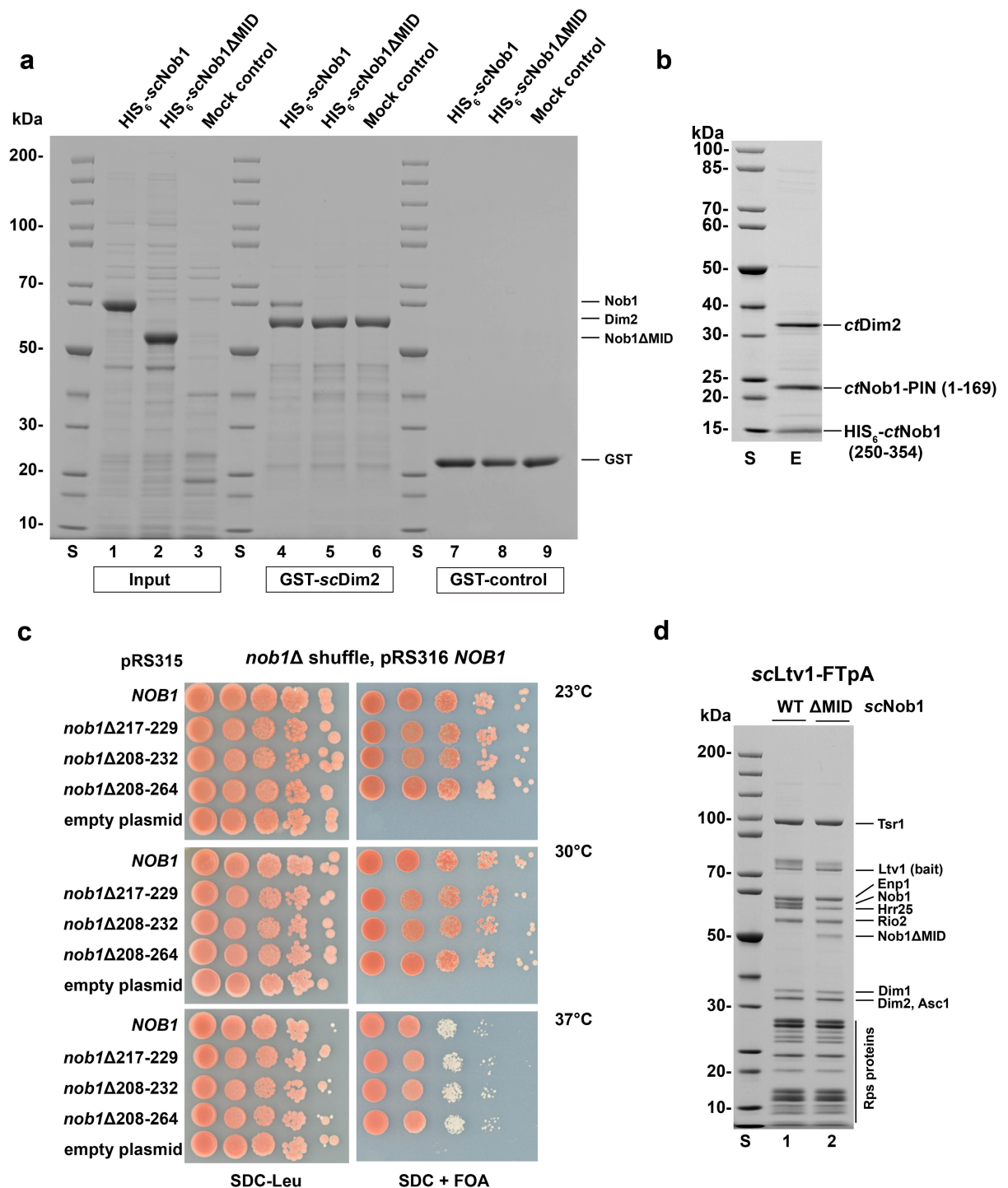
**Supplementary Figure 1. Multiple-sequence alignment of KH domain proteins Dim2 and Krr1 from different species.** For the alignment, orthologs from the following species were used: *Saccharomyces cerevisiae* (S.c.), *Chaetomium thermophilum* (C.t.), *Aspergillus niger* (A.n.), *Homo sapiens* (H.s.) and *Arabidopsis thaliana* (A.t.). The default color scheme of ClustalX/Jalview<sup>1</sup> was used with e.g. hydrophobic residues in blue, acidic residues in violet and basic residues in red. Both proteins contain two tandem KH domains (KH1, KH2) in row. In both Dim2 and Krr1, KH1 (KH-like) lacks the conserved GXXG motif, predicted to be required for RNA/DNA binding, which however is present in KH2 of Dim2 and Krr1. Krr1 contains a conserved C-terminal extension absent in Dim2.



**Supplementary Figure 2. Dim2 depletion leads to arrest of 90S biogenesis visualized by sucrose gradient analysis of Enp1-FTpA derived particles.** (a) Mock control revealing the specificity of split-tag tandem-affinity purification. Wild-type yeast W303 lysate, devoid of tagged bait proteins, was passed first over IgG-sepharose, followed by TEV elution and a second affinity-purification step via  $\alpha$ -FLAG beads. The final FLAG eluate was TCA precipitated and analyzed by SDS-PAGE and Coomassie staining. Loading: 10% of TEV eluate (lane 1), 10% of the unbound fraction (lane 2), 85% of TCA-precipitated Flag eluate (lane 3). S, molecular weight

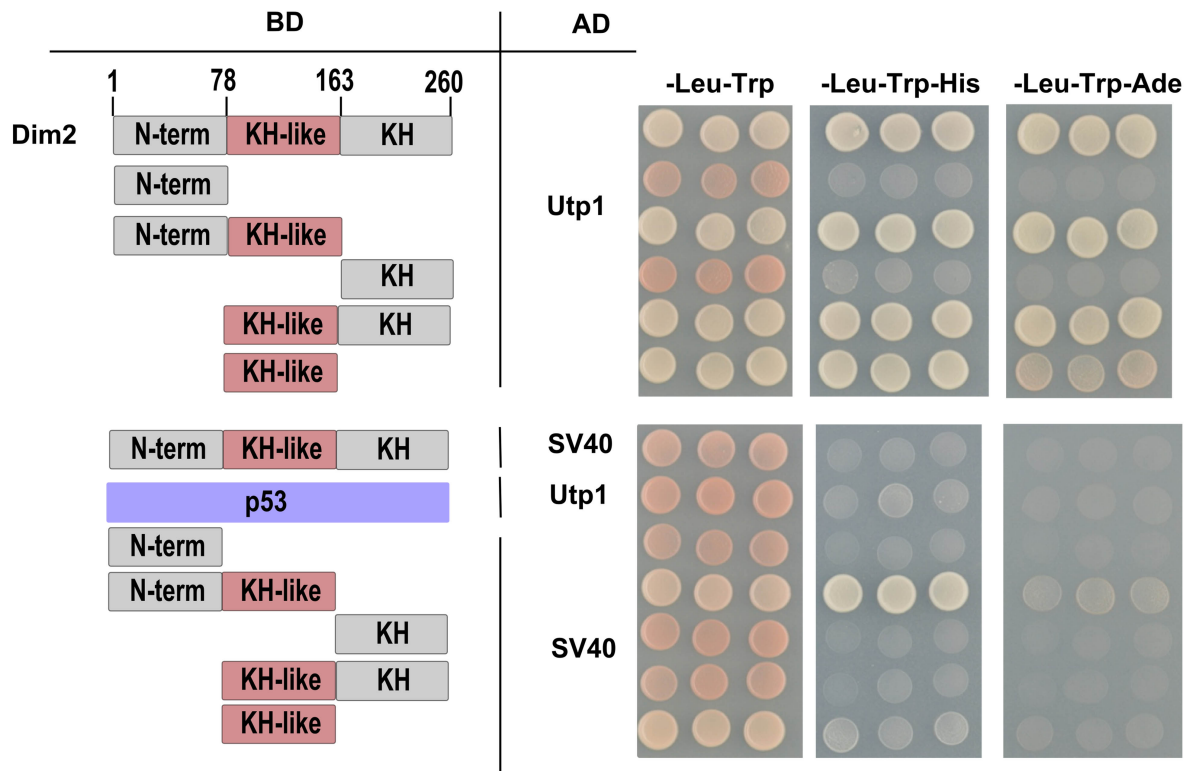
protein standard. (b) Eluates derived from scEnp1-FTpA tandem affinity-purification under conditions of Dim2 expression (GAL) and depletion (GLU, 8h), respectively, were analyzed by sucrose gradient (15-40%) centrifugation. Gradient fractions (1-13) were TCA-precipitated and analyzed by SDS-PAGE (4-12%) and Coomassie staining. (c) Shown are selected fractions 6 and 10 from the sucrose gradient analysis, for which the labeled bands were identified by mass spectrometry. (d) Western blots analysis of sucrose gradient fractions 6 under Dim2 expression (GAL) and Dim2 repression (GLU) conditions with the indicated antibodies.





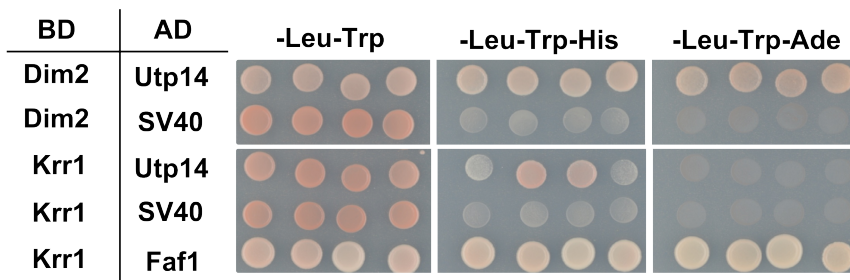
**Supplementary Figure 3. Biochemical and *in vivo* analyses of the conserved Dim2-Nob1 interaction.** (a) *In vitro* binding assay using yeast *Saccharomyces cerevisiae* GST-scDim2 and indicated HIS<sub>6</sub>-scNob1 constructs (lanes 1-2). BL21 extract was used as mock control (lane 3). After the binding reaction, final SDS eluates (lanes 4-9) were analyzed by SDS-PAGE and Coomassie staining. Note that full-length Nob1, but not Nob1ΔMID

( $\Delta$  208-264) binds to Dim2. S, molecular weight protein standard. (b) Complex formation between distinct Nob1 domains and Dim2. Recombinant *ctDim2*, *ctNob1* PIN (1-169) and HIS<sub>6</sub>-*ctNob1* Mid-ZnF (250-354) were co-expressed in *E. coli* B121 and affinity-purified utilizing SP-Sepharose and Ni-NTA. The final eluate was analyzed by SDS-PAGE (4-12%) and Coomassie staining. S, molecular weight protein standard; E, eluate. (c) Yeast Nob1 shuffle strain was transformed with single copy plasmids, either empty (pRS315), or containing the indicated *NOB1* constructs, all under the authentic *NOB1* promoter and terminator. Growth was analyzed after three days at the indicated temperatures on SDC-Leu and SDC + FOA plates. (d) Tandem affinity purification of integrated *scLtv1*-FTpA from the shuffled yeast Nob1 shuffle strain, carrying either plasmid based *scNob1* WT or *scNob1*  $\Delta$ MID ( $\Delta$ 208-264). The two consecutive purification steps involve the ProtA- in the first and the Flag-tag in the second step. Final Flag eluates were TCA-precipitated and analyzed by SDS-PAGE (4-12%) and Coomassie staining. The indicated protein bands were identified by mass spectrometry. S, molecular weight protein standard.

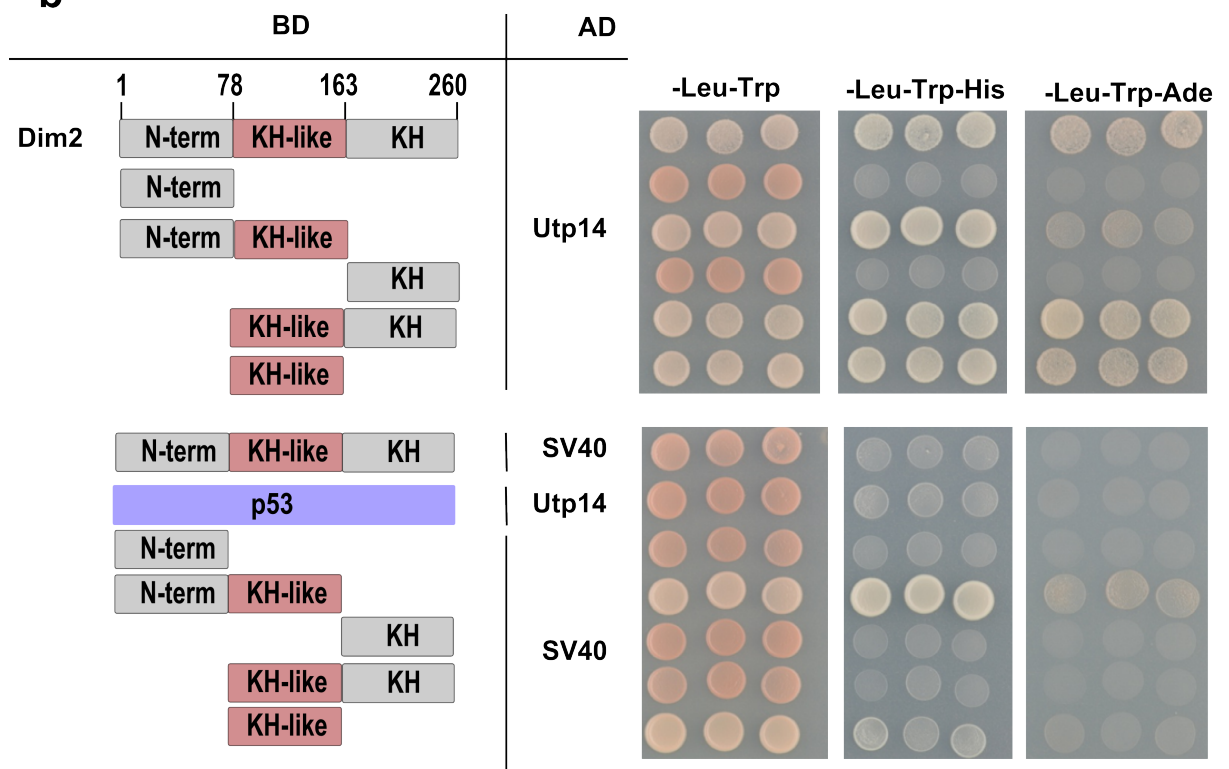


**Supplementary Figure 4** Dim2 utilizes its KH-like domain for interaction with Utp1. Yeast 2-hybrid tests using Dim2 and Utp1 constructs. Three individual transformants, derived from the tester strain PJ69-4a and harboring the indicated bait (BD) and prey (AD) plasmids, were analyzed by growth on SDC-Leu-Trp (plating efficiency), SDC-Leu-Trp-His (weak interaction) and SDC-Leu-Trp-Ade plates (strong interaction). SV40 and p53 served as negative controls. The growth was analyzed after two days at 30°C.

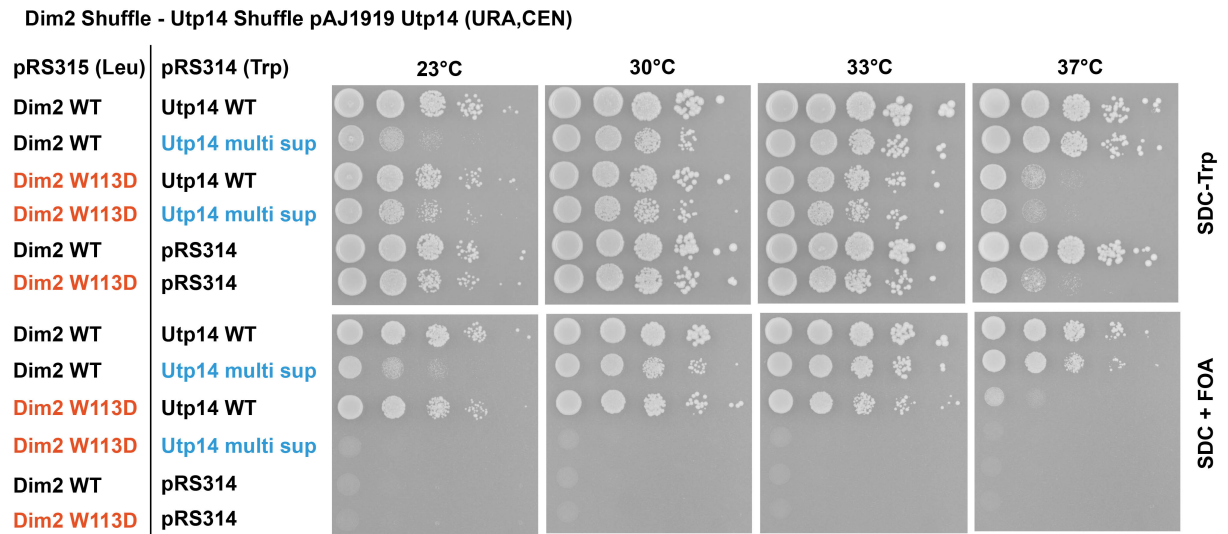
**a**



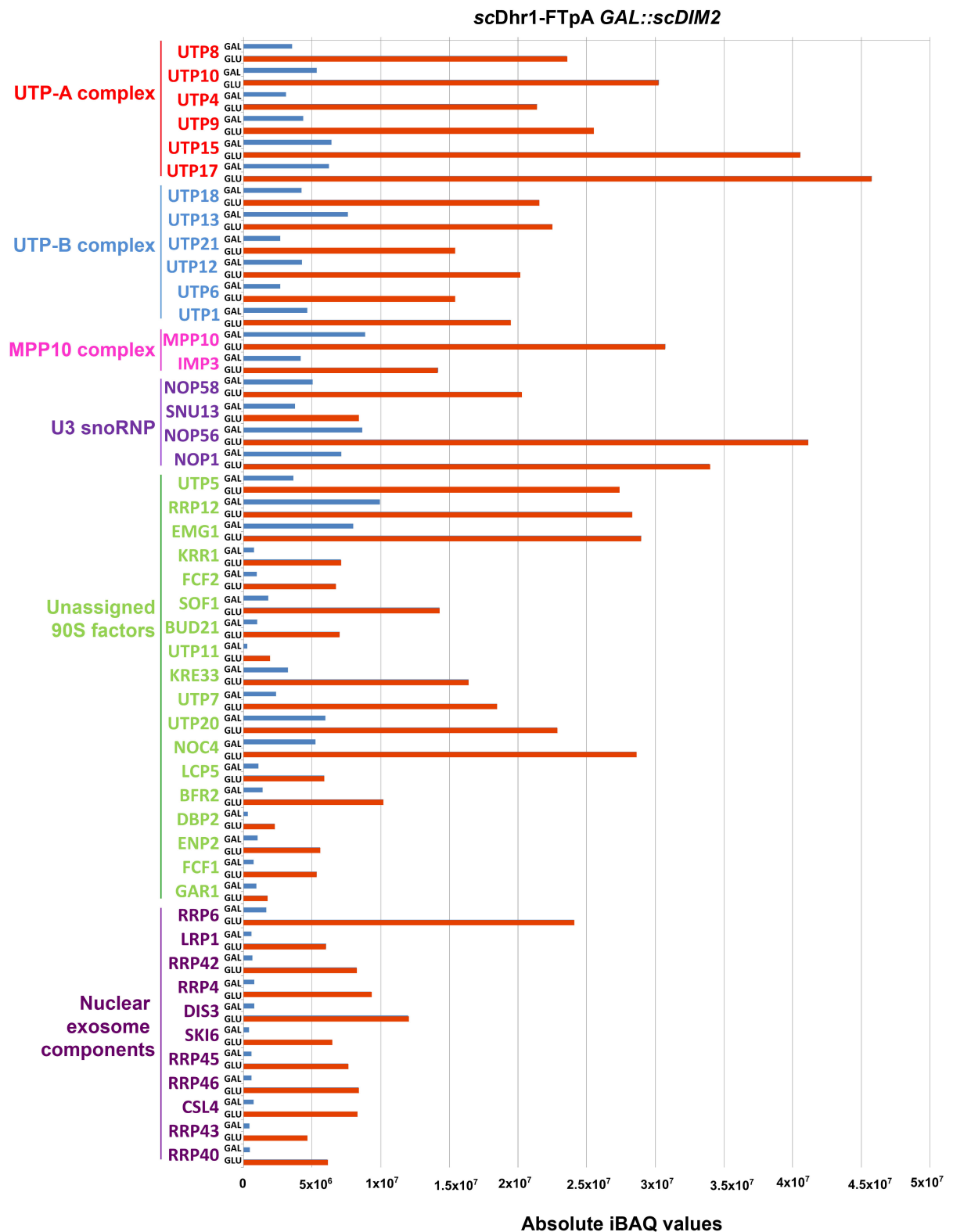
**b**



**Supplementary Figure 5. Dim2 interacts via its KH-like domain with Utp14.** (a, b) Yeast 2-hybrid tests using the indicated Dim2 and Utp14 constructs. Three individual transformants, derived from the tester strain PJ69-4a and harboring the indicated bait (BD) and prey (AD) plasmids, were analyzed by growth on SDC-Leu-Trp (plating efficiency), SDC-Leu-Trp-His (weak interaction) and SDC-Leu-Trp-Ade plates (strong interaction). SV40 and p53 served as negative controls. The growth was analyzed after two days at 30°C.



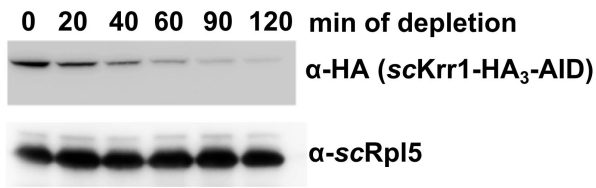
**Supplementary Figure 6. Synthetic lethal interaction between mutant alleles, Dim2 W113D and Utp14 multi sup.** The genetic analysis was carried out with a *dim2Δ utp14Δ* double shuffle strain, transformed with the indicated plasmids carrying *DIM2* wild-type or *dim2* W113D mutant alleles, and *UTP14* wild-type *utp14* multi sup mutant. Cells were grown on SDC-Trp or SDC + FOA plates. Growth was monitored at the indicated temperatures after 3 days. No growth on 5-FOA plates indicates synthetic lethality.



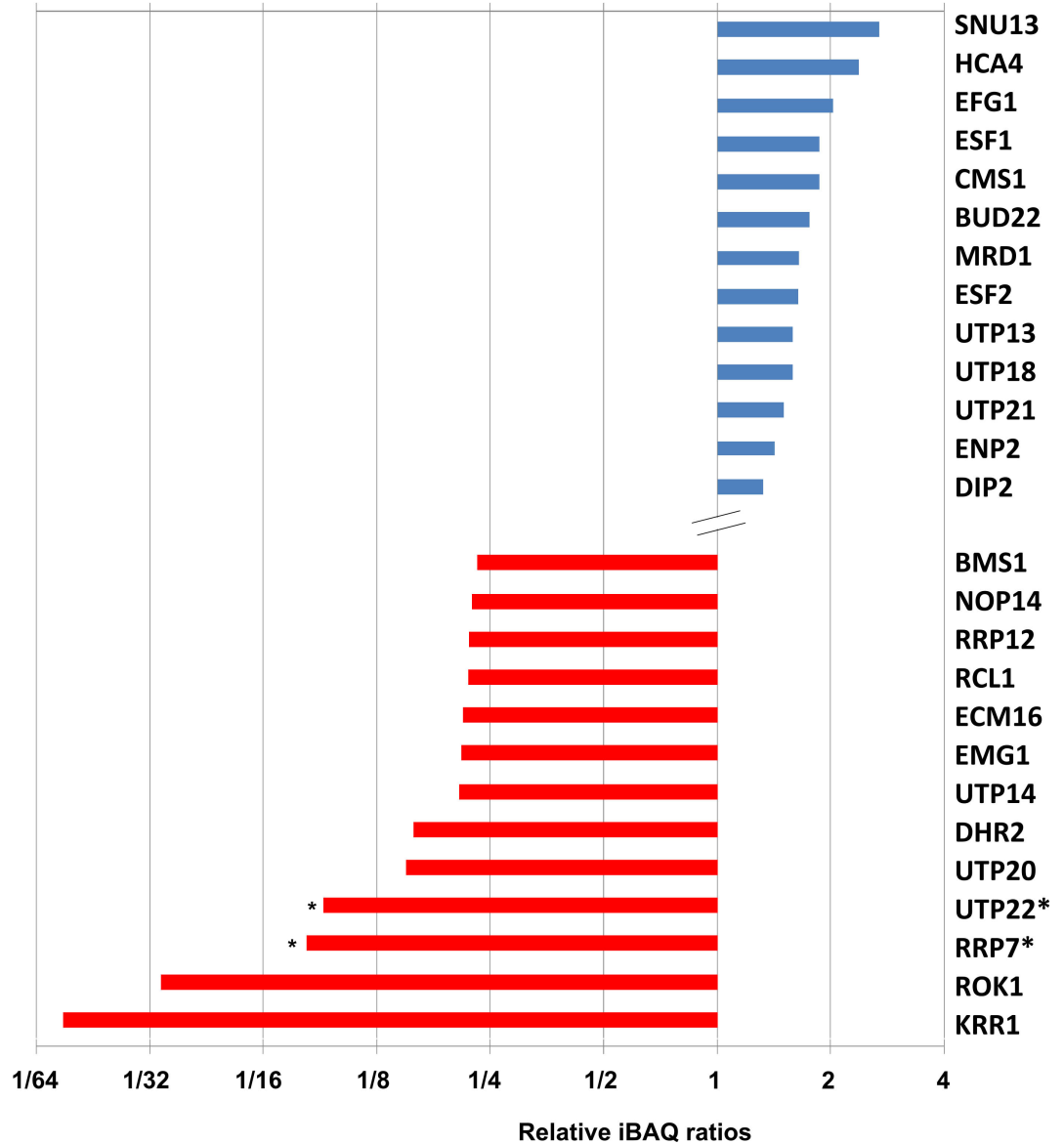
**Supplementary Figure 7. Dhr1 co-purifies increased amounts of 90S factors upon Dim2 depletion.** Semi-quantitative mass spectrometry of the Dhr1-FTpA eluates derived from experiment Fig. 3c. The iBAQ (Intensity Based Absolute Quantification) numbers derived from semi-quantitative mass

spectrometry analysis of the Dim2 expressing (GAL) and Dim2 depletion (GLU) conditions were normalized to the iBAQ value of the Dhr1 bait protein. The absolute iBAQ values of co-purified 90S factors derived from depleted versus expressing Dim2 cells are shown. The corresponding numerical data are shown in Supplementary Data 1.

**a**



**b**

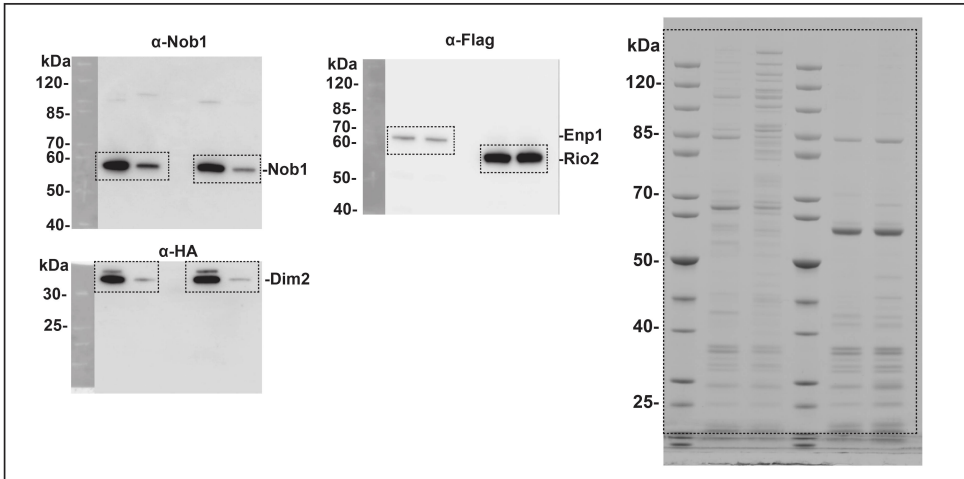


**Supplementary Figure 8. Krr1 depletion leads to a reduction of UTP-C complex members Utp22 and Rrp7.** (a) Western blot analysis revealing Krr1 depletion in yeast cells. scKrr1-HA<sub>3</sub>-AID degenon strain was grown in YPD medium, before degradation of Krr1 was induced by addition of indole-3-acetic acid (Auxin) to the medium. Cells were collected at indicated time

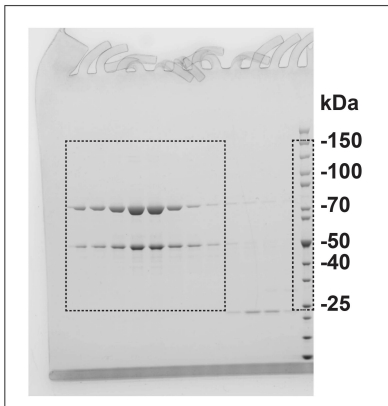


points (min of depletion). Whole cell lysates were analyzed by SDS-PAGE and Western blotting utilizing the indicated antibodies. (b) Semi-quantitative mass spectrometry of the Utp10-FTpA eluates derived from scKrr1-HA<sub>3</sub>-AID expressing (- Auxin) and scKrr1-HA<sub>3</sub>-AID depleted (+ Auxin) cells (see Fig. 4c, lane 1 and 2). The iBAQ (Intensity Based Absolute Quantification) numbers derived from semi-quantitative mass spectrometry analysis of the two different Utp10-FTpA eluates were normalized to the iBAQ value of the Utp10 bait protein. The relative iBAQ ratio of co-purified 90S factors derived from depleted versus expressing Krr1 cells are shown. The corresponding numerical data are shown in Supplementary Data 1.

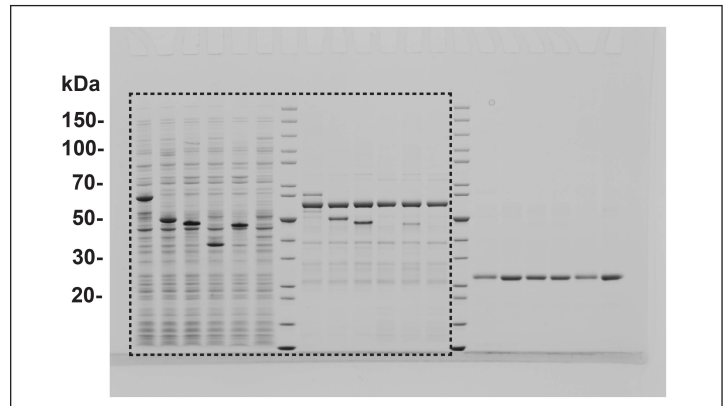
**Figure 1a**



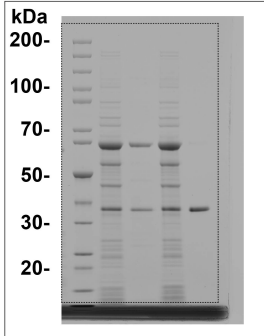
**Figure 1b**



**Figure 1d**



**Figure 1e**



**Figure 2a**

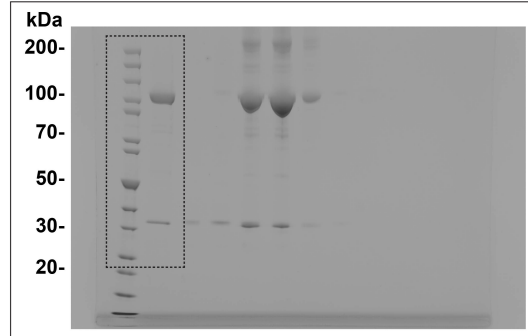


Figure 2c

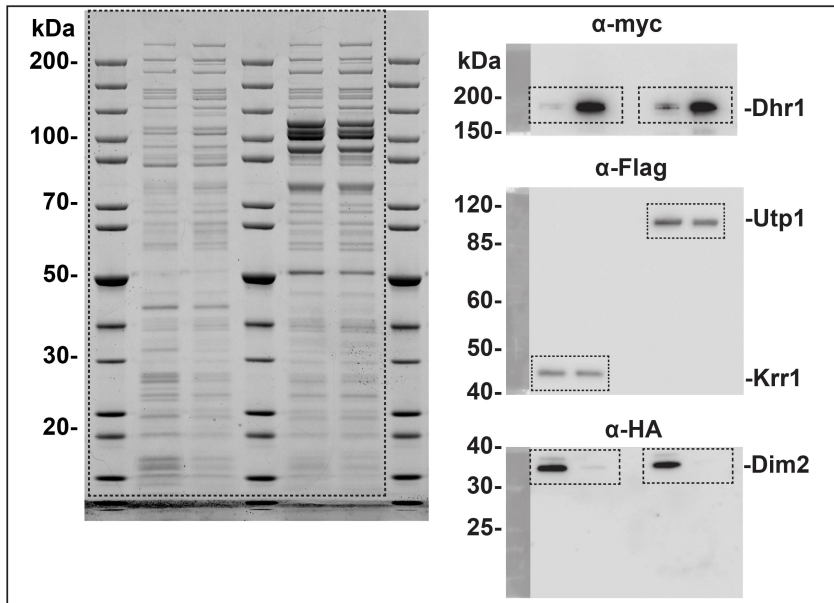


Figure 3b

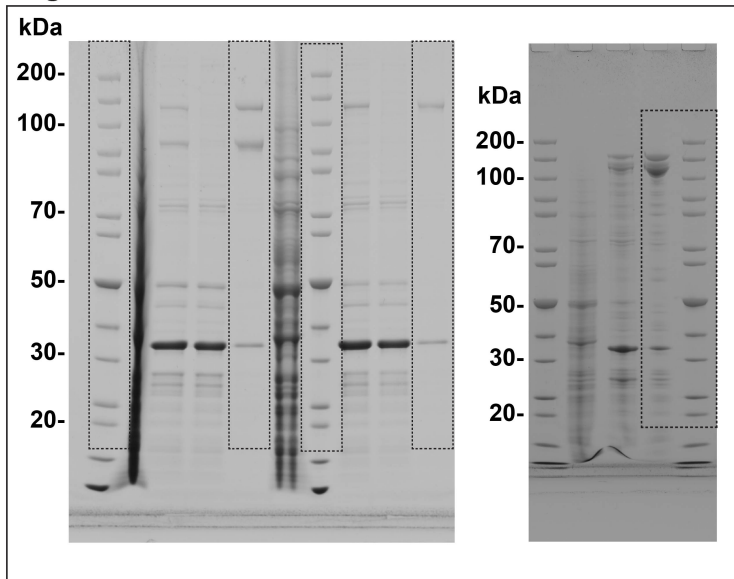


Figure 3c

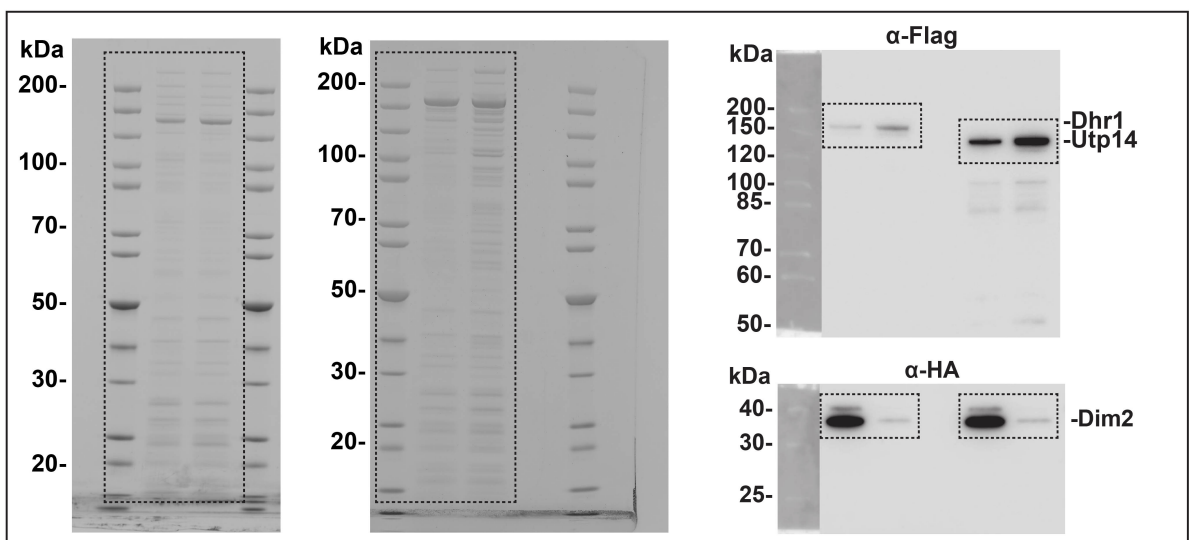


Figure 3d

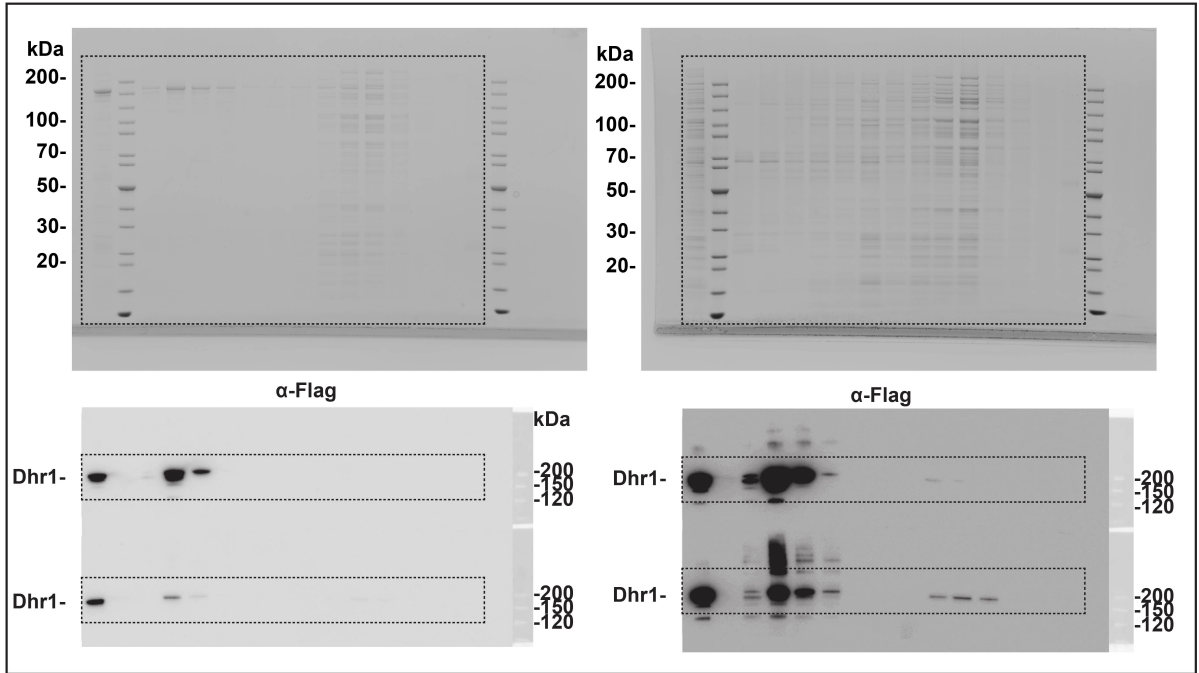


Figure 4a

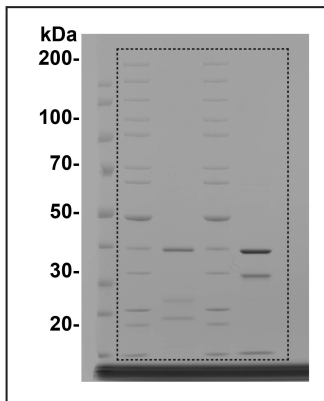


Figure 4b

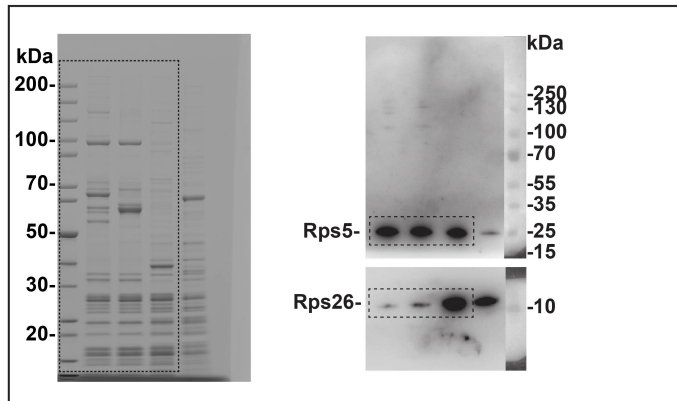


Figure 5a

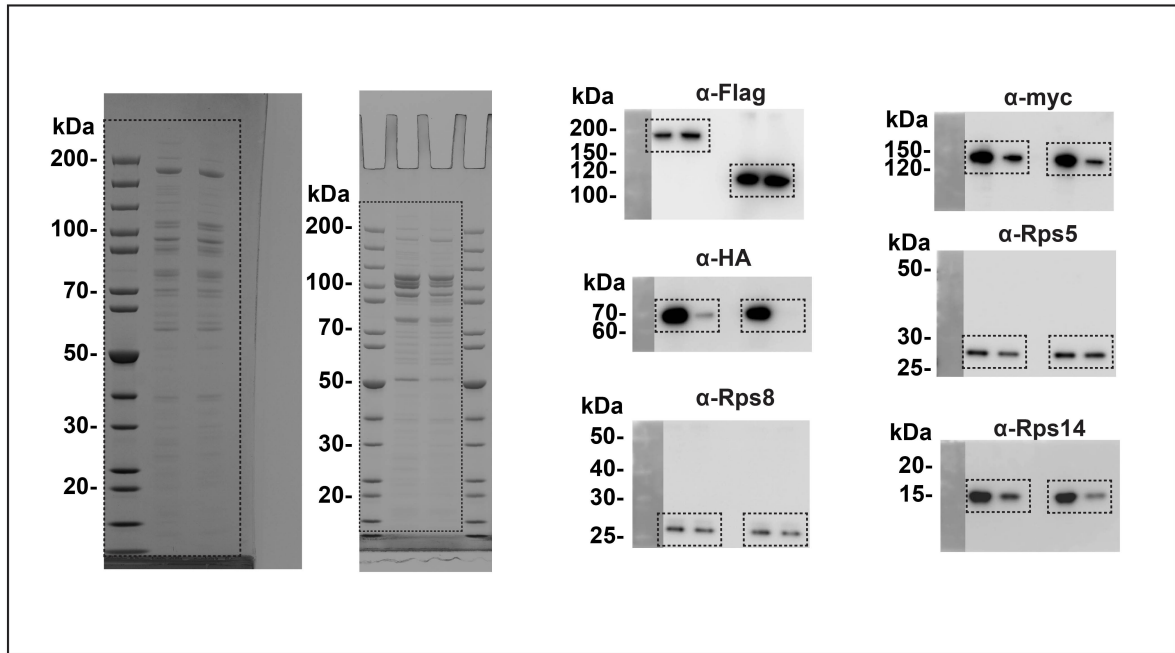
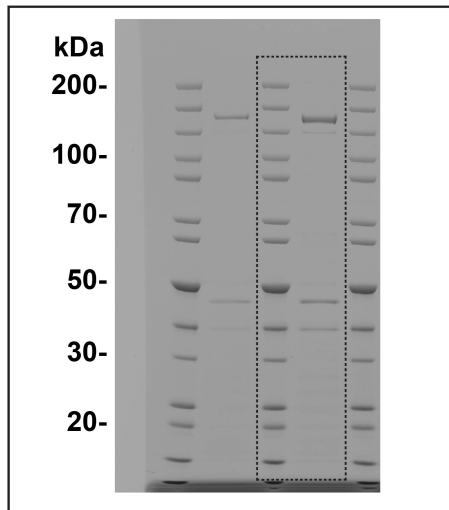


Figure 5b



Supplementary Figure 9. Uncropped scans of Western blots and Coomassie-stained SDS-polyacrylamide gels included in main figures.

**Supplementary Table 1: Plasmids used in this study**

<b>Name</b>	<b>Genotype</b>	<b>Reference</b>
YCPlac111 HA-scDim2	<i>CEN; LEU2, AmpR, P<sub>GAL</sub> HA-DIM2</i>	This study
petMBP-ctDim2	<i>KanR, P<sub>T7</sub> MBP-ctDIM2</i>	This study
petDuet HIS <sub>6</sub> -ctNob1 1-354	<i>AmpR, P<sub>T7</sub> HIS<sub>6</sub>-ctNOB1 1-354</i>	This study
pet24d ctDim2	<i>KanR, P<sub>T7</sub> ctDIM2</i>	This study
petDuet HIS <sub>6</sub> -ctNob1 250-354 , ctNob1 1-169	<i>AmpR, P<sub>T7</sub> HIS<sub>6</sub>-ctNOB1 250-354, P<sub>T7</sub> ctNOB1 1-169</i>	This study
pMT-LEU2 ProtA-TEV-ctDim2	<i>2μ, LEU2, AmpR, P<sub>GAL1-10</sub> PROTA-TEV-ctDIM2</i>	This study
pMT-TRP1 Flag <sub>3</sub> -ctUtp1	<i>2μ, TRP1, AmpR, P<sub>GAL1-10</sub> FLAG<sub>3</sub> -ctUTP1</i>	This study
pGBKT7 ctDim2	<i>2μ, TRP1, KanR, GAL4BD-cMyc-ctDIM2</i>	Ref. <sup>2</sup>
pGADT7 ctUtp14	<i>2μ, LEU2, AmpR, GAL4AD-HA<sub>3</sub>-ctDIM2</i>	Ref. <sup>2</sup>
pVA3-1	<i>2μ, TRP1, AmpR, GAL4 DNA-BD-MURINE p53 (72-390)</i>	Clontech Laboratories, Inc
pTD1-1	<i>2μ, LEU2, KanR, GAL4 AD-SV40 (84-708)</i>	Clontech Laboratories, Inc
pMT-TRP1 Flag <sub>3</sub> -ctUtp14	<i>2μ, TRP1, AmpR, P<sub>GAL1-10</sub> FLAG<sub>3</sub> ctUTP14</i>	This study
pMT-URA3 ctUtp14	<i>2μ, URA3, AmpR, P<sub>GAL1-10</sub> ctUTP14</i>	This study
pMT-URA3 HA <sub>3</sub> -ctDhr1	<i>2μ, URA3, AmpR, P<sub>GAL1-10</sub> HA<sub>3</sub>-ctDHR1</i>	This study
pMT-LEU2 ProtA-TEV-ctKrr1	<i>2μ, LEU2, AmpR, P<sub>GAL1-10</sub> PROTA-TEV-ctKRR1</i>	This study
pMT-TRP1 Flag <sub>3</sub> -ctRps14	<i>2μ, TRP1, AmpR, P<sub>GAL1-10</sub> FLAG<sub>3</sub> ctRPS14</i>	This study
pMT-URA3 ctRps1	<i>2μ, URA3, AmpR, P<sub>GAL1-10</sub> ctUtp22</i>	This study
pMT-TRP1 Flag <sub>3x</sub> -ctFap7	<i>2μ, TRP1, AmpR, P<sub>GAL1-10</sub> FLAG<sub>3</sub> ctFAP7</i>	This study
pMT-TRP1 Flag <sub>3</sub> -ctRrp7	<i>2μ, TRP1, AmpR, P<sub>GAL1-10</sub> FLAG<sub>3</sub> ctRRP7</i>	This study
pMT-URA3 HA <sub>3</sub> -ctUtp22	<i>2μ, URA3, AmpR, P<sub>GAL1-10</sub> HA<sub>3</sub>-ctUTP22</i>	This study
pGBKT7 ctDim2 N-term	<i>2μ, TRP1, KanR, GAL4BD-cMyc-ctDIM2 1-78 aa</i>	This study
pGBKT7 ctDim2 KHlike	<i>2μ, TRP1, KanR, GAL4BD-cMyc-ctDIM2 79-163</i>	This study

pGBKT7 ctDim2 Cterm	<i>2μ, TRP1, KanR, GAL4BD-cMyc-ctDIM2 164-260</i>	This study
pGBKT7 ctDim2 N + KHlike	<i>2μ, TRP1, KanR, GAL4BD-cMyc-ctDIM2 1-163</i>	This study
pGBKT7 ctDim2 KHlike + Cterm	<i>2μ, TRP1, KanR, GAL4BD-cMyc-ctDIM2 79-260</i>	This study
pGBKT7 ctKrr1	<i>2μ, TRP1, KanR, GAL4BD-cMyc-ctKRR1</i>	Ref. <sup>2</sup>
pGADT7 ctFaf1	<i>2μ, LEU2, AmpR, GAL4AD-HA<sub>3</sub>-ctFAF1</i>	Ref. <sup>2</sup>
pRS315	<i>CEN, LEU2, AmpR</i>	Ref. <sup>3</sup>
pRS316	<i>CEN,URA3, AmpR</i>	Ref. <sup>4</sup>
pRS315 scDim2	<i>CEN, LEU2, AmpR P<sub>DIM2</sub>-DIM2</i>	This study
pRS316 scDim2	<i>CEN,URA3, AmpR P<sub>DIM2</sub>-DIM2</i>	This study
pAJ1919 scUTP14	<i>CEN, UR3, AmpR, P<sub>UTP14</sub>-UTP14</i>	Ref. <sup>5</sup>
pRS314, scUtp14	<i>CEN,TRP1, AmpR P<sub>UTP14</sub>-UTP14</i>	This study, derived from Ref. <sup>5</sup>
pRS314, scUtp14 multi sup	<i>CEN,TRP1, AmpR P<sub>UTP14</sub>-UTP14 multi sup</i>	This study, derived from Ref. <sup>5</sup>
pRS315 scDim2 W113D	<i>CEN, LEU2, AmpR P<sub>DIM2</sub>-DIM2</i>	This study
pRS315 scNob1	<i>CEN, LEU2, AmpR P<sub>NOB1</sub>-NOB1</i>	Ref. <sup>6</sup>
pRS315 scNob1 Δ 217-229	<i>CEN, LEU2, AmpR P<sub>NOB1</sub>-NOB1 Δ 217-229</i>	This study
pRS315 scNob1 Δ 208-232	<i>CEN, LEU2, AmpR P<sub>NOB1</sub>-NOB1 Δ 208-232</i>	This study
pRS315 scNob1 Δ 208-264	<i>CEN, LEU2, AmpR P<sub>NOB1</sub>-NOB1 Δ 208-264</i>	This study
pet24a HIS <sub>6</sub> -ctNob1 1-476	<i>KanR, P<sub>T7</sub> ctNOB1 1-476</i>	This study
pet24a HIS <sub>6</sub> -ctNob1 169-476	<i>KanR, P<sub>T7</sub> ctNOB1 169-476</i>	This study
pet24a HIS <sub>6</sub> -ctNob1 1-355	<i>KanR, P<sub>T7</sub> ctNOB1 1-355</i>	This study
pet24a HIS <sub>6</sub> -ctNob1 1-294	<i>KanR, P<sub>T7</sub> ctNOB1 1-294</i>	This study

pet24a HIS <sub>6</sub> -ctNob1 1-250	<i>KanR, P<sub>T7</sub>ctNOB1 1-250</i>	This study
pet24a HIS <sub>6</sub> -scNob1	<i>KanR, P<sub>T7</sub>scNOB1</i>	This study
pet24a HIS <sub>6</sub> -scNob1 Δ 208-264 (Δ MID)	<i>KanR, P<sub>T7</sub>scNOB1 Δ 208-264</i>	This study
petDuet HIS <sub>6</sub> -ctDim2, ctNob1	<i>AmpR, P<sub>T7</sub>HIS<sub>6</sub>-ctDIM2, P<sub>T7</sub>ctNOB1</i>	This study
petDuet HIS <sub>6</sub> -ctDim2, ctNob1 W267G	<i>AmpR, P<sub>T7</sub>HIS<sub>6</sub>-ctDIM2, P<sub>T7</sub>ctNOB1 W267G</i>	This study
pGADT7 ctUtp1	<i>2μ, LEU2, AmpR, GAL4AD-HA<sub>3</sub>-ctDIM2</i>	Ref. <sup>2</sup>
pGADT7 ctDhr1	<i>2μ, LEU2, AmpR, GAL4AD-HA<sub>3</sub>-ctDIM2</i>	Ref. <sup>2</sup>
pet24d GST-TEV-ctDim2	<i>KanR, P<sub>T7</sub>GST-TEV.ctDIM2</i>	This study
pet24d GST-TEV-scDim2	<i>KanR, P<sub>T7</sub>GST-TEV.scDIM2</i>	This study

**Supplementary Table 2: Yeast strains used in this study**

<b>Name</b>	<b>Genotype</b>	<b>Reference</b>
W303 α	<i>Mata, ura3-1, trp1-1, his3-11,15, leu2-3,112, ade2-1, can1-100, GAL+</i>	Ref. <sup>4</sup>
PJ69-4a	<i>trp1-901, leu2-3,112, ura3-52, his3-200, gal4D, gal80D, LYS2::GAL1-HIS3, GAL2-ADE2, met2::GAL7-lacZ</i>	Ref. <sup>7</sup>
<i>Enp1</i> -FTpA, <i>dim2</i> Δ shuffle	<i>W303, Mata, dim2::natNT2, enp1-FTpA::HIS3, YCPlac111 HA-scDim2</i>	This study
<i>Rio2</i> -FTpA, <i>dim2</i> Δ shuffle	<i>W303, Mata, dim2::natNT2, rio2-FTpA::HIS3, YCPlac111 HA-scDim2</i>	This study
<i>Krr1</i> -FTpA, <i>dim2</i> Δ shuffle	<i>W303, Mata, dim2::natNT2, krr1-FTpA::HIS3, YCPlac111 HA-scDim2</i>	This study
<i>Utp1</i> -FTpA, <i>dim2</i> Δ shuffle	<i>W303, Mata, dim2::natNT2, utp1-FTpA::HIS3, YCPlac111 HA-scDim2</i>	This study
<i>Dhr1</i> -FTpA, <i>dim2</i> Δ shuffle	<i>W303, Mata, dim2::natNT2, dhr1-FTpA::HIS3, YCPlac111 HA-scDim2</i>	This study
<i>Utp14</i> -FTpA, <i>dim2</i> Δ shuffle	<i>W303, Mata, dim2::natNT2, utp14-FTpA::HIS3, YCPlac111 HA-scDim2</i>	This study
<i>Asc1</i> -FTpA	<i>W303, Mata, Asc1-FTpA::HIS3,</i>	This study



<i>Utp10</i> -FTpA, <i>Krr1</i> -HA <sub>3</sub> -AID, <i>Utp22</i> -myc <sub>13</sub>	W303, <i>Mata</i> , <i>Utp10</i> -FTpA:: <i>natNT2</i> , , <i>P<sub>ADH1</sub></i> - <i>OSTIR1-9xmyc</i> :: <i>TRP1</i> , <i>Krr1</i> -HA <sub>3</sub> - <i>AID</i> :: <i>HIS3MX6</i> , <i>Utp22</i> -13MYC- <i>kanMX6</i>	This study
<i>Krr1</i> -FTpA, <i>dim2</i> Δ shuffle, <i>Dhr1</i> -myc <sub>13</sub>	W303, <i>Mata</i> , <i>dim2</i> :: <i>natNT2</i> , <i>krr1</i> -FTpA:: <i>HIS3</i> , <i>Dhr1</i> -13MYC- <i>kanMX6</i> , <i>YCPlac111</i> HA- <i>scDim2</i>	This study
<i>Utp1</i> -FTpA, <i>dim2</i> Δ shuffle , <i>Dhr1</i> -myc <sub>13</sub>	W303, <i>Mata</i> , <i>dim2</i> :: <i>natNT2</i> , <i>utp1</i> -FTpA:: <i>HIS3</i> , <i>Dhr1</i> -13MYC- <i>kanMX6</i> , <i>YCPlac111</i> HA- <i>scDim2</i>	This study
<i>dim2</i> Δ <i>utp14</i> Δ double shuffle, pRS315 <i>scDim2</i>	W303, <i>Mata</i> , <i>dim2</i> :: <i>natNT2</i> , <i>utp14</i> :: <i>HIS3</i> , pRS315 <i>scDim2</i> , pAJ1919 <i>scUTP14</i>	This study
<i>dim2</i> Δ <i>utp14</i> Δ double shuffle, pRS315 <i>scDim2</i> W113D	W303, <i>Mata</i> , <i>dim2</i> :: <i>natNT2</i> , <i>utp14</i> :: <i>HIS3</i> , pRS315 <i>scDim2</i> W113D, pAJ1919 <i>scUTP14</i>	This study
<i>nob1</i> Δ shuffle	W303, <i>Mata</i> , <i>nob1</i> :: <i>kanMX4</i> , pRS316 <i>scNob1</i>	Ref. <sup>6</sup>
<i>Ltv1</i> -FTpA, <i>nob1</i> Δ shuffle	W303, <i>Mata</i> , <i>nob1</i> :: <i>kanMX4</i> , pRS316 <i>scNob1</i> , FTpA:: <i>HIS3</i>	This study
<i>Utp1</i> -FTpA, <i>Krr1</i> -HA <sub>3</sub> -AID, <i>Utp22</i> -myc <sub>13</sub>	W303, <i>Mata</i> , <i>Utp10</i> -FTpA:: <i>natNT2</i> , , <i>P<sub>ADH1</sub></i> - <i>OSTIR1-9xmyc</i> :: <i>TRP1</i> , <i>Krr1</i> -HA <sub>3</sub> - <i>AID</i> :: <i>HIS3MX6</i> , <i>Utp22</i> -13MYC- <i>kanMX6</i>	This study
<i>Enp1</i> -FTpA, <i>dim2</i> Δ shuffle	W303, <i>Mata</i> , <i>dim2</i> :: <i>natNT2</i> , <i>enp1</i> -FTpA:: <i>HIS3</i> , pRS316 <i>scDim2</i>	This study
<i>Rio2</i> -FTpA, <i>dim2</i> Δ shuffle	W303, <i>Mata</i> , <i>dim2</i> :: <i>natNT2</i> , <i>rio2</i> -FTpA:: <i>HIS3</i> , pRS316 <i>scDim2</i>	This study

## Supplementary - References

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