2 Materials

2.1 Instruments

Analytical scale	MR 2002	Sartorius
Precission scale	MC1 Analytical AC1205	Sartryos
pH-meter	CG 840	Schott
Photometers	6300	JenWay
	DU® 7400	Beckman
Vortex mixer	7-2020	neoLAB
Thermal block	Thermomixer 5437	Eppendorf
Thermocycler	PTC-200	MJ Research
Lightcycler	iCycler	Bio-Rad
37 °C incubator	MIR-153	SANYO
Culture shaker	HT	INFORS
Tissue culture incubator	Steri-Cult 200	Forma Scientific
Power supplies	Power PAC 300	Bio-Rad
	Power Pack P25	Biometra
PAGE gel chamber	Min 2-D cell	Bio-Rad
DNA gel chamber	HG370 / HG330	Savant
Blotting device	Fast Blot B33	Biometra
Gel documentation	Video Graphic Printer UR890CE	Sony
	UV Table	Biometra
	Transluminator	Roth
	Video Monitor WV-BM900	Panasonic
French Press	Cell FA-031	SIM-AMINCO
Sonicator	SH213G	Bandelin electronics
Tissue homogenizer	Ultra-turrax T25	IKA-Werke
Centrifuges	5417R	Eppendorf
	J2-MC	Beckman
	Biofuge 28RS	Heraeus
Chromatography systems	Äkta explorer	GE Healthcare
	LKB-FRAC-200	GE Healthcare
	Uvicord SII	GE Healthcare
	LCC-501 PLUS	GE Healthcare
	LKB-REC-102	GE Healthcare
	LKB-Pump P-500	GE Healthcare
	Vison Workstation	Applied Bioscience
Chromatography columns	HiTrap chelating column	GE Healthcare
	HiTrap S cation exchange column	GE Healthcare
	Superdex 75 HiLoad 16/60	GE Healthcare
	Superdex 75 HiLoad 26/60	GE Healthcare
	Superdex 200 HiLoad 26/60	GE Healthcare
	PoRos Talon Superflow column	Applied Bioscience
CD Spectrometer	J720	Jasco
Concentrators	Amicon Ultra	Millipore

Crystallization robot	Hydra-Plus-One	Matrix Technologies
	Hydra II	Matrix Technologies
Pipetting robot	Lizzy XXL	Zinsser
Crystallization plates	Crystal quick flat bottom	Greiner Bio-One
Crystal observation system	Light table KL 2500LCD	Leica
	Microscope M420	Leica
	Camera DXC-390P	Sony
Crystal plates storage system	Homebase	The Automation Partnership
Nylon loops	CryoLoops	Hampton Research
Pins	CrystalCap Copper	Hampton Research
Home x-ray source	rotating anode RU H2B	Rigaku
	dtb345 image plate	MAR-research
	mirror system	MaxFlux
	cryo-cooling	OXFORD cryosystems

2.2 Chemicals, enzymes, kits

Standard chemicals were purchased from Roth Chemikalien or Sigma-Aldrich.

Chemicals

[^{3}H]palmitate, 40-60 Ci mmol^{-1}	GE Healthcare
Agar, granulated	BD Bioscience
Agarose, elektrophoresis grade	Invitrogen
Ammonium persulfate (APS)	Serva
Ampicillin sodium sulfate	Roth
Betaine	Sigma
Biotin	Sigma
Cerulein	Sigma
Chloramphenicol	Roth
Complete Mini EDTA-free protease inhibitor tablets	Roche
Complete protease inhibitor tablets	Roche
Desthiobiotin (DTB)	Sigma
D-panthothenic acid	Sigma
External well factor solution	Bio-Rad
Glutathion (GSH)	Boehringer Mannheim
Glutathion sepharose	GE Healthcare
Isopropyl-thiogalactoside (IPTG)	AppliChem
Kanamycin sulfate	Roth
L-amino acids	Fluka
Myo-inositol	Sigma
N,N,N',N'-tetramethylethylenediamine (TEMED)	Serva
Nicotinic acid	Merck
Ni-Nitrilotriacetic acid agarose	Qiagen
Nitrocellulose membrane	Schleicher & Schuell
Nucleic acids	Fluka
Peptone	BD Bioscience
Phenylmethylsulfonyl fluoride (PMSF)	Sigma
Polyvinylidene fluoride membrane	Roth
Protein G sepharose	GE Healthcare
Pyridoxine HCl	Fluka
Rotiphorese acrylamide/bisacrylamide (37.5:1, 30 %) solution	Roth
seleno-methionine	Acros organics
Strep-Tactin MacroPrep resin	IBA
Talon Superflow Metal Affinity resin	BD Biocscience
Thiamin	Sigma
Tryptone	AppliChem
Yeast extract	MP Biomedicals
α-flag antibody (M2)	Sigma
α-HA Affinity Matrix	Roche
α-HA antibody (Y11)	Santa Cruz
α-His Horseradish peroxidase (HRP) conjugate	Qiagen
α-mouse HRP conjugate	Jackson Labs
α-myc antibody (9E10)	Santa Cruz
α-rabbit HRP conjugate	Jackson Labs
Overnight express instant TB medium	Novagen

Enzymes

Restriction enzymes	*Bam*HI, *Not*I, *Sal*I, *Nde*I, *Xho*I, *Dpn*I	NEB
	*Esp*3I	Fermentas
Polymerases	*Taq*	Promega, Roboklon
	Pfu	Stratagene
	Turbo *Pfu*	Stratagene
	Pfu Plus !	Roboklon
	T4 polymerase	Roboklon
Ligase	T4 DNA ligase	Promega, Roboklon
Reaction buffers	as supplied by manufaturer	
Proteases	TEV Protease	Konrad Büssow
	PreScission	Konrad Büssow
Reverse transcriptase	M-MLV (H-)	Promega
Nuclease	DNAseI	Roche
	Benzonase	Roche

Kits

QIAprep Spin Miniprep Kit	Qiagen
QIAGEN Plasmid Maxiprep Kit	Qiagen
QIAquick PCR Purification/Gel Extraction Kit	Qiagen
RNeasy Mini Kit	Qiagen
QuantiTect SYBR Green PCR Kit	Qiagen
Enhanced Chemiluminescence (ECL)	GE Healthcare

2.3 Strains and plasmids

Bacterial strains

E.coli BL21(DE3)		Novagen
E.coli B834(DE3)		Novagen
E.coli SCS1 Rosetta		Stratagene
E.coli DH5α		Invitrogen

Yeast strains

S. serevisiae AH22 *ura*3	MATa; *ura*3Δ; *leu*2-3, 112; *his*4-519;*can*1	Christine Lang
S. serevisiae Y25984	MAT a/α; *his3Δ1/his3Δ1*; *leu2Δ0/leu2Δ0*; *lys2Δ0/LYS2*; *MET15/met15Δ0*; *ura3Δ0/ura3Δ0*; *YKR068c*::*kanMX4/YKR068c*	EUROSCARF

Cell lines

HEK293	Human embryonic kidney fibroblasts transformed with adenovirus genes E1A and E1B	DSZM

Template clones

Bet3	PSF500000092	Christine Lang
Bet3p	PSF500001485	Christine Lang
Mum2	PSF3300102711	Konrad Büssow
Sedl	IRAKp961O1427	RZPD
synbindin	IRATp970C019	RZPD
Tpc5	IRALp962F0644	RZPD
Tpc6A	IRAUp969C0218	RZPD
Tpc6B	DKFZp667C045	RZPD

Plasmids

pET-28 KARL	*E. coli* expression vector with kanamycin resistance, T7*lac* Promotor , N-terminaler His-Tag, Thrombin cleavage site	Klaas Max
pET-28 KoT	*E. coli* expression vector with kanamycin resistance,T7*lac* Promotor , no tag	Kerstin Böhm
pETDuet-1	*E. coli* dual expression vector with ampicillin resistance, T7*lac* Promotor , His-tag for cassette 1, no tag for cassette 2	Novagen
pQTEV	*E. coli* expression vector with ampicillin resistance, T7*lac* Promotor , N-terminaler His-Tag, TEV protease cleavage site	Konrad Büssow
pQLinkH	*E. coli* expression vector with ampicillin resistance,T7*lac* Promotor , N-terminaler His-Tag, TEV protease cleavage site, LINK sequences	Konrad Büssow
pGEX-4T1	Expression vector with ampicillin resistance, *tac* Promotor, N-terminaler GST-Tag, Thrombin cleavage site	GE Healthcare

pGEX-6P1	*E. coli* expression vector with ampicillin resistance, *tac* Promotor, N-terminaler GST-Tag, PreScission cleavage site	GE Healthcare
pYEXTHS-BN	*S. serevisiae* expression vector with ampicillin resistance, *CUP*1 promotor, N-terminaler His-Tag, C-terminal StrepII-tag, *URA3*, *LEU2d*,	Christine Lang
pTL-HA 1	Vector for cell culture expression, with ampicillin resistance, T7 and SV 40 promotor, N-terminal HA-tag	Ulrich Stelzl
pRK5-myc	Vector for cell culture expression, with ampicillin resistance, *CMV* promotor, N-terminal myc-tag	Daniel Krappmann
pcDNA3-flag	Vector for cell culture expression, with ampicillin resistance, *CMV* promotor, N-terminal flag-tag	Daniel Krappmann
pGEM	Vector for cloning of PCR products with T-overhangs, with ampicillin resistance, *T7* and *SP6* promotor, disrupted *lacZ* gene	Promega

2.4 Media and buffers

Culture media

LB medium	Tryptone	10 g l^{-1}
	Yeast extract	5 g l^{-1}
	NaCl	10 g l^{-1}
SB medium	Yeast extract	24 g l^{-1}
	Tryptone	12 g l^{-1}
	Glycerol	3.75 g l^{-1}
	KH_2PO_4	2.3 g l^{-1}
	K_2HPO_4	12.5 g l^{-1}
LB agar	LB + agar	15 g l^{-1}
YPD	Yeast extract	10 g l^{-1}
	Peptone	20 g l^{-1}
	Glucose (40%)	50 ml l^{-1}
Sporulation medium	Potassium actetae	10 g l^{-1}
	Yeast extract	1 g l^{-1}
	Dextrose	0.5 g l^{-1}
YPD agar	YPD + agar	20 g l^{-1}
SD-ura plates	Yeast nitrogen base w/o amino acids	6,7 g l^{-1}
	Glucose	20 g l^{-1}
	Agar	20 g l^{-1}
	Histidine	20 mg l^{-1}
	Leucine	20 mg l^{-1}
WM VIII	Sucrose	50 g l^{-1}
	$NH_4H_2PO_4$	0.25 g l^{-1}
	NH_4Cl	2.8 g l^{-1}
	$MgCl_2$ x $6H_2O$	0.25 g l^{-1}
	$CaCl_2$ x $2H_2O$	12.5 g l^{-1}
	KH_2PO_4	2.0 g l^{-1}
	$MgSO_4$ x $7H_2O$	0.55 g l^{-1}
	Myo-inositol	0.075 gl^{-1}
	Sodium glutamate	10 g l^{-1}
	Trace elements (1000x)	1 ml
	Vitamins (250x)	4 ml
	Histidine (20 mg ml^{-1})	1 ml
Trace elements (1000x)	$ZnSO_4$ x $7H_2O$	1.75 g l^{-1}
	$FeSO_4$ x $7H_2O$	0.5 g l^{-1}
	$CuSO_4$ x $5H_2O$	0.1 g l^{-1}
	$MnCl_2$ x $4H_2O$	0.1 g l^{-1}
	Na_2MoO_4 x $2H_2O$	0.1 g l^{-1}
	EDTA	10 mM
Vitamins (250x)	Nicotinate	2.5 g l^{-1}
	Pyridoxine	6.25 g l^{-1}
	Thiamine	2.5 g l^{-1}
	Biotin	0.625 g l^{-1}
	Phanthotenate	12.5 g l^{-1}

NMM (new minimal medium)	10x amino acids /ATGC	100 ml
	NaCl (5 M)	1.7 ml
	$(NH_4)_2SO_4$ (2.5 M)	3.0 ml
	Glucose (2 M)	10 ml
	$MgSO_4$ (1M)	1 ml
	Mn, Zn, Cu, MoO_4 (10 mg l^{-1} each)	0.1 ml
	Ca^{2+} (1 g l^{-1})	1 ml
	$FeSO_4$ (1 g l^{-1})	3 ml
	Thiamine (20 g l^{-1})	0.5 ml
	Biotin (2 g l^{-1})	5 ml
	KH_2PO_4 (1 M)	55 ml
	K_2HPO_4 (1 M)	100 ml
	H_2O	to 1 l
		pH 7.0
10x amino acids/ATGC	L-amino acids (each)	0.5 g l^{-1}
	Nucleic acids (each)	0.5 g l^{-1}
	NaCl	137 mM
	KCl	2.5 mM
	Na_2HPO_4	10 mM
	KH_2PO_4	1.76 mM
		pH 7.0

Molecular Biology

TAE-buffer	Tris HCl	40 mM
	Sodium acetate	5 mM
	EDTA	1 mM
	Acetic acid	to pH 8
Ethidium bromide solution	TAE Puffer with ethidium bromide	10 mg ml^{-1}
DNA sample buffe 5x	EDTA	100 mM
	Xylencyanol	0,05% (v/v)
	Glycerol	30% (v/v)
	Bromphenol blue in TAE-Puffer	0.05% (w/v)
DNA molecular weight Marker	1 kb ladder (NEB)	0.5 µg $µl^{-1}$
TFB 1	$RbCl_2$	100 mM
	$MnCl^2$	50 mM
	Potassium acetate	30 mM
	$CaCl_2$	10 mM
	Glycerol	15%
	Acetic acid	to pH 5.8
TFB 2	$RbCl_2$	10 mM
	$CaCl_2$	75 mM
	Glycerol	15%
	MOPS	10 mM
	NaOH	to pH 6.8
PEG mix	PEG 3350	40% (w/v)
	Lithium acetate	0.1 M

Protein purification

His-lysis buffer	NaH_2PO_4	50 mM
	NaCl	300 mM
	Imidazole	10 mM
		pH 8
His-wash buffer	NaH_2PO_4	50 mM
	NaCl	300 mM
	Imidazole	20mM
		pH 8
His-elution buffer	NaH_2PO_4	50 mM
	NaCl	300 mM
	Imidazole	250 mM
		pH 8
Yeast lysis	NaH_2PO_4	100 mM
	NaCl	300 mM
	Triton X-100	1%
		pH 8
Talon wash buffer	NaH_2PO_4	100 mM
	NaCl	300 mM
	Imidazole	5 mM
		pH 8
Talon elution buffer	NaH_2PO_4	100 mM
	NaCl	300 mM
	Imidazole	150 mM
		pH 7
Denaturing buffer	NaH_2PO_4	100 mM
	Tris HCl	10 mM
	Urea	8 M
		pH 8.0
Buffer W	Tris	100 mM
	NaCl	150 mM
	EDTA	1 mM
		pH 8.0
GST-lysis buffer, phosphate buffered saline (PBS)	NaCl	137 mM
	KCl	2,7 mM
	Na_2HPO_4	8 mM
	KH_2PO_4	1,47 mM
GST-elution buffer	Glutathione, reduced	5 mM
	Tris HCl	50 mM
		pH 8.0
Gel-filtration buffer Tpc6B	Tris	20 mM
	NaCl	100 mM
	DTT	1 mM
		pH 7.4
Gel-filtration buffer Bet3:Tpc6B	Tris	25 mM
	NaCl	250 mM
	β-mercaptoethanol	5 mM
		pH 7.4

Gel-filtration buffer Mum2 complexes	Tris	20 mM
	NaCl	200 mM
	DTT	2 mM
		pH 7.5
Cation exchange buffer (low salt)	Tris	50 mM
	DTT	1 mM
		pH 7.5
Cation exchange buffer (high salt)	Tris	50 mM
	NaCl	1 M
	DTT	1 mM
		pH 7.5
SDS-PAGE		
Electrophoresis buffer	Tris	25 mM
	Glycine	190 mM
	SDS	0.1% (w/v)
Stacking gel buffer (4x)	Tris	0.5 M
	SDS	0.4% (w/v)
		pH 6.8
Stacking gel	30 % acrylamide /0,8 % (w/v) Bisacrylamide stock	0.4 ml
	Stacking gel buffer	0.75 ml
	dH_2O	1.85 ml
	APS 10% (w/v)	30 µl
	TEMED	5 µl
Separation gel buffer (4x)	Tris	1.5 M
	SDS	0.4% (w/v)
		pH 8.8
Separation gel 15% (w/v)	30 % acrylamide /0,8 % (w/v) Bisacrylamide stock	5 ml
	Separation gel buffer	2.5 ml
	dH_2O	2.5 ml
	APS 10% (w/v)	75 µl
	TEMED	10 µl
SDS-sample buffer 2x	SDS	3% (w/v)
	Glycerol	20% (w/v)
	β-mercaptoethanol	3% (v/v)
	Bromphenol blue	0.05% (w/v)
	EDTA	10 mM
Protein standard	97, 67, 45, 29, 21, 12.5, 6.5 kDa	
Coomassie stain	Coomassie R-250	0.25% (w/v)
	Methanol	45% (v/v)
	dH_2O	45% (v/v)
	Acetic acid, glacial	10% (v/v)
Destain solution	Methanol	40% (v/v)
	dH_2O	50% (v/v)
	Acetic acid, glacial	10% (v/v)

Silver staining		
Fixation solution	Acetone (50 % v/v)	60 ml
	Tri chlor acetic acid (50 % v/v)	1.5 ml
	Formaldehyde (37%)	25 µl
Reducing solution	$Na_2S_2O_3$ (10 % w/v)	100µl
	dH_2O	60 ml
Silver stain solution	$AgNO_3$ (20 % w/v)	800 µl
	Formaldehyde (37%)	600 µl
	$Na_2S_2O_3$ (10 % w/v)	25 µl
	dH_2O	60 ml
Development solution	Na_2CO_3	1.2 g
	Formaldehyde (37%)	25 µl
	$Na_2S_2O_3$ (10 % w/v)	25 µl
	dH_2O	60 ml
Western blot		
Semi-dry transfer buffer	Tris HCl	25 mM
	Glycine	150 mM
	Methanol	10%
		pH 8.3
PBS-T	PBS	1 l
	Tween-20	1 ml
Cell culture		
HEK293 culture medium	DMEM/F-12	
	Fetal Calf Serum	10%
	Sodium pyruvate	1 mM
	Penicillin	100 U ml^{-1}
	Streptomycin	0.1 mg ml^{-1}
Trypsin/EDTA	Trypsin	0.5 mg ml^{-1}
	EDTA	0.22 mg ml^{-1}
2x HBS	NaCl	280 mM
	HEPES	50 mM
	Na_2HPO_4	0.2 mM
		pH 7.12
PBS (sterile)	NaCl	137 mM
	KCl	2,7 mM
	Na_2HPO_4	8 mM
	KH_2PO_4	1,47 mM
Immuno-precipitation		
CoIP Buffer	NaCl	150 mM
	Hepes	50 mM
	NP-40	0.2%
	Glycerol	1 mM
	DTT	1 mM
		pH 7.5
HEK293 lysis	CoIP buffer	
	DTT	1 mM
	NaF	10 mM
	Na_3VO_4	0.2 mM
	β-glycerophosphate	8 mM
	Complete Protease Inhibitor	1 tablet in 50 ml

2.5 Synthetic oligonucleotides

Synthetic oligonucleotides were ordered from MWG Biotech AG or Eurogentech.

2.5.1 Gene-specific primers

primer	sequences (5'→3')	restriction site
Tpc6B_f	ga gga tcc atg gcg gat gag gcg t	*Bam*HI
Tpc6B_r	cac tgc ggc cgc tca cgc ttc tgt atc atc acc tga aat t	*Not*I
Tpc5_f	cgt ctc gga tcc atg gag gcg cgc ttc a	*Esp*3I
Tpc5_r	cgt ctc cgg cgc tca gcg gcc ctc cag g	*Esp*3I
synbindin_f	ga gga tcc atg gcg att ttt agt gtg tat gtg g	*Bam*HI
synbindin_r	gac tgc ggc cgc tca tga ccc agg tcc aaa agt tcc	*Not*I
mycBet3_f	tag tcg aca tcg agg cag gcgaaccgtggcaccgag	*Sal*I
mycBet3_r	ta tgc ggc cgc tta ttc ctc tcc agc tgg aag att gtc	*Not*I
Bet3-duet1_f	gcg gat ccg tcg agg cag gcg aac cgt	*Bam*HI
Bet3-duet1_r	cgc agg ctt aag tta ttt ttc gaa ctg cgg gtg gct	
Tpc6B-duet2-f	g agg tcc cat atg gcg gat gag gcg t	*Nde*I
Tpc6B-duet2-r	gc ctc gag tca cag ctt ctg tat cat cac ctg aaa tt	*Xho*I
Tpc6A-duet2-f	gc cat atg gcg gat act gtg ttg ttt gag	*Nde*I
Tpc6A-duet2-r	gc ctc gag tta gga ttt cgg aat cac cac	*Xho*I

2.5.2 Vector-specific primers

primer	sequences (5'→3')	vectors
pGEX fwd	cca aaa tcg gat ctg gtt c	pGEX
pGEX rev	cag agg ttt tca ccg tca tca	
T7 promotor	taa tac gac tca cta tag gg	pET
T7 terminator	gct agt tat tgc tca gcg g	
pQTEV3U	tat aaa aat agg cgt atc acg agg	pQLink
pQTEV3L	cca gtg att ttt ttc tcc att tt	
pQE for	gta tca cga ggc cct ttc gtc t	pQTEV
pQE rev	cat tac tgg atc tat caa cag gag	
DuetUP2	ttg tac acg gcc gca taa tc	pETDuet
DuetDOWN1	gat tat gcg gcc gtg tac aa	

2.5.3 Oligos for QuikChange Mutagenesis

Only forward primers are listed. Reverse primer have reverse complement sequence.

primer	sequences (5'→3')
Bet3C68S_f	gct aga acg gca ttg cca cgc agt gag aat tta gtg
Bet3pC80S_f	gct cgg tca aat gtt ggg agg agc cat gac ttt cgg
Bet3C68A_f	tcc ggt caa atg ttg gga ggg ccc atg act ttc ggg aaa ct
Bet3A82L_f	gat gtc att gcc aag ctg gtg ttc aag atg tac ttg ggc
Bet3A82V_f	gat gtc att gcc aag gtg gtg ttc aag atg tac ttg ggc
Bet3R67E_f	tcg gtc aaa tgt tgg gga gtg cca tga ctt tcg gga aac t
Tpc6Bchim1_f	gag atg gtg tct gga gtg tac tgt aag gac tat gaa gtg gaa aac gga cga tgt att
Tpc6Bchim2_f	gtg tac tgt aag gac tat gaa aat gat gaa gat gtg att act aag ctg gaa aac atg
Tpc6Bscram_f	agg ggg agg tgg aaa acg gag aac tta tta cta agc tgg aaa aca t
Bet3scram_f	tgg tca ccc agc tat gta aga aag ttc gca atg atg aag atg tga ata a

2.5.4 Primer for quantitative RT-PCR

primer	sequences (5'→3')
tpc6a_f	att cga gtt tct gca cac cga
tpc6a_r	ggg cag gga tgc cac gga g
tpc6b_f	cgt tgt ttt tgc ttc tc
tpc6b_r	aga ctt cag ctg tta caa ta
bet3_f	cta tga aaa cga cga aga cgt g
bet3_r	ctc ttc tcc agc tgg gag gtt g
dT_{15}	ttt ttt ttt ttt ttt (Promega)