

6 Materials

6.1 Bacterial strains

Bacterial strain	Gene product
BL21-CodonPlus (DE3)-RP	F' <i>ompT hsdS</i> (r _B ⁻ m _B ⁻) dcm ⁺ tet ^r gal λ(DE3) endA The [argU proL Cam ^r] (Stratagene, Germany)
C41 (DE3)pLysS	F' <i>ompT gal hsdS_B</i> (r _B ⁻ m _B ⁻) dcm lon λDE3 pLysS
DH5α	F'/endA1 <i>hsdR17</i> (r _K ⁻ m _K ⁺) sup E44 thi-1 recA1 gyrA (Nal ^r) relA1 (lacZYA-argF) U169 (80dlac(lacZ) M15)
TOP10F	F'[{lac ^q Tn10 (Tet ^R)}] mcrA D (mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 deoR recA1 araD139 Δ(ara-leu) 7697 galU galK rpsL endA1 nupG

6.2 Plasmids

Vector	Addgene no.	supplier	comment
Lentiviral vectors			
pLL3.7	11795		
pLVTHM	12247		
pLVPT-tTR-KRAB	11642		
pLV-tTRKRAB-red	12250		
Packaging plasmids			
pMD2.G	12259		VSVG
psPax2	12260		2 nd generation
pRSV-REV	12253		3 rd generation
pMDLg/pRRE	12251		3 rd generation
Mammalian expression vectors			
pcDNA3		Invitrogen	
pcDNA3-myc			Myc-tag
pEGFP-N1		clontech	GFP-tag
pGL3-control		Promega	luciferase

Vector	Addgene no.	supplier	comment
Bacterial expression vectors			
pGex4T3		Invitrogen	GST-tag
pET28c		Novagen	His-tag

6.3 Cell lines

Cell type	comment	mutation	ATTC no.
HeLa	Cervix adenocarcinoma		CCL-2
293T	HEK 293T/17Kidney, SV40 T antigen		CRL-11268
IF6	Melanoma	B-raf	
HT29	Colon, colorectal adenocarcinoma	K-/H-/N-ras, B-raf	HTB-38
Capan1	Pancreas, adenocarcinoma	K-ras	HTB-79
HT1080wt	fibrosarcoma	N-ras	CCL-121
Sk-ov-3	Ovary, adenocarcinoma	Erb-B2/HER2	HTB-77
Jurkat	Lymphoblast, T-cell leukemia		TIB-152
HUVEC	Human Umbilical Vein Endothelial Cells		

6.4 Enzymes

T4-DNA-Polymerase, T4-DNA ligase, Klenow-Polymerase, *XhoI*, *XbaI*, *HpaI*, *EcoRI*, *BamHI*, *DpnI*, *EcoRI*, *HindIII*, *Clal*, *MluI* from NEB; Taq-Polymerase (in house), Lysozyme from Sigma, Expand long template DNA polymerase from Roche, Benzoylase from Merk.

6.5 Oligonucleotides

Primer:

Oligoname	Comment	Sequence
3' Bap37 HindIII (pET28c)	PHB 2 - His-tag	CTTCATTTCTTACCCTTGATGAGGCTGTCAC
5' Bap37 BamHI (pET28c)	PHB2 - His-tag	TCATGGCCCAGAAGCTTGAAGGACTTG
3' PHB AS243 HindIII	C-term del. PHB1	CTATCATTCCAGCTTGCGCAGCTC
PHB neu 5' (EcoRI)	PHB1 GST-tag	AATTC ATG GCT GCC AAA GTG TTT GA
PHB neu 3' (XhoI)	PHB1 GST-tag	GAC CTG GGG CAG CTG GAG GA

Oligoname	Comment	Sequence
5' PHB AS243 BamHI	C-term del. PHB1	TCGCTGCAGAGGACATCGCGTA
CS003	3 point mutations	AGCAGGAAGCCGAGAGAGCCAGGTTTGTGGT
CS003a	2 point mutations	ACCACAAATCTCGCCCCTCTGCTTCCTGCT
3' EcoRI PHB-GFP (EGFP-N1)	PHB1 GFP-tag	GAGAATTCTCTGGGGCAGCTGGAGGAGCAC
5' XhoI PHB-GFP (pEGFP-N1)	PHB1 GFP-tag	GACTCGAGGCCACCATGGCTGCCAAAG
rev PHB 3'UTR XhoI	PHB - 3'UTR	GACGGAAGGTCTGGGTGTCATTATTGAC
for PHB 3'UTR EcoRI	PHB - 3'UTR	AATTCGGGCCACCCCTGCCTG

Primer for qRT-PCR

gene	Acc.no.	Sequence	Position (bp)
Prohibitin	NM_002634	CTTTGACTGCCGTTCTCGAC	271
		TGGGTGATTAGTTCTCCAGC	498
Prohibitin2	NM_007273	ATGGCCAGAACTTGAAGGAC	1
		CACTCCACCGATCCGATTGAA	174
GAPDH	NM_002046	GGTATCGTGGAAGGACTCATGAC	531
		ATGCCAGTGAGCTTCCCGTTCAG	718
EGFR	X00588	GACTCCGTCCAGTATTGATC	133
		GAACATCCTCTGGAGGCTG	351

Sequences shRNAs

Name	Acc.no.	Sequence	Position (bp)
shPHB	NM_002634	GCGGAGAGAGCCAGATTTG (ss)	650-668
shPHBx	NM_002634	TTATATAATGTGGATGCTGGG (as)	152-173
shPHBy	NM_002634	GTGTTTGAGTCCATTGGCA (ss)	86-104
shPHBz	NM_002634	GACACATCTGACCTTCGGGAA (ss)	583-603
shPHB2-0	NM_007273	GACAGAGAGGGCCAAGGAC (ss)	689-707
shPHB2-1	NM_007273	(G)AATTGTGCAGGCCGAGGGTGA (ss)	857-877
shLuci	M15077	AACTTACGCTGAGTACTTCGA (ss)	513-533

shRNA oligos

Name	strand/oligosequence
shPHB	ss 5' TGCGGAGAGAGCCAGATTTGTTCAAGAGACAAATCTGGCTCTC TCCGCTTTTTTC 3'
	as 5' TCGAGAAAAAAGCGGAGAGAGCCAGATTTGTCTCTTGAACAAT CTGGCTCTCTCCGCA 3'
shPHBx	ss 5' TGCCCAGCATCCACATTATATAATTCAAGAGATTATATAATGTG GATGCTGGGTTTTTTC 3'
	as 5' GAGAAAAAACCCAGCATCCACATTATATAATCTCTTGAATTATA TAATGTGGATGCTGGGCA 3'
shPHBy	ss 5' TGTGTTTGTAGTCCATTGGCATTCAAGAGATGCCAATGGACTCA AACACTTTTTTC 3'
	as 5' GAGAAAAAGTGTTTGAGTCCATTGGCATCTCTTGAATGCCAAT GGACTCAAACACA 3'
shPHBz	ss 5' TGACACATCTGACCTTCGGGAATTCAAGAGATTCCCGAAGGTC AGATGTGTCTTTTTTC 3'
	as 5' GAGAAAAAGACACATCTGACCTTCGGGAATCTCTTGAATTC CCGAAGGTCAGATGTGTCA 3'
shPHB2-0	ss 5' TGACAGAGAGGGCCAAGGACTTCAAGAGAGTCCTTGGCCCT CTCTGTCTTTTTTC 3'
	as 5' GAGAAAAAGACAGAGAGGGCCAAGGACTCTCTTGAAGTCCTT GGCCCTCTGTCA 3'
shPHB2-1	ss 5' TGAATTGTGCAGGCCGAGGGTGATTCAAGAGATCACCTCGG CCTGCACAATTTTTTC 3'
	as 5' GAGAAAAATTGTGCAGGCCGAGGGTGATCTCTTGAATCACCC TCGGCCTGCACAATTCA 3'
shLuci	ss 5' ACCACGCGTCCCCAACTTACGCTGAGTACTTCGATTCAAGAGA TCGAAGTACTCAGCGTAAGTTTTTTGGAAAT 3'
	as 5' TATCGATTTCCAAAAAACTTACGCTGAGTACTTCGATCTCTTG AATCGAAGTACTCAGCGTAAGTTGGGGA 3'

6.6 Antibodies

Primary antibodies

Antibodies	Origin	Dilution	supplier	Orderno.
Actin, mAb	Mouse	1:5000 (IB)	Sigma	A5441
Calnexin	Rabbit	1:3000 (IB)	Stressgene	610823
c-Raf (C-12), pAb	Rabbit	1:1000 (IB)	Santa Cruz	sc-133
c-Raf phospho (Ser338) (56A6)	Rabbit	1:1000 (IB)	Cell Signaling Technology	9427S
Erk; phospho-p44/42 MAPK, mAb	Mouse	1:1000 (IB)	Cell Signaling Technology	9106L
Erk2 (C-14), pAb	Rabbit	1:800 (IB)	Santa Cruz	sc-154
GM130	Mouse	1:1000 (IB)	BD Transduction	610822

Antibodies	Origin	Dilution	supplier	Orderno.
Hsp60,mAb	Mouse	1:1000 (IB)	Stressgen	SPA-806
Prohibitin, mAb	Mouse	1:1000 (IB)	NeoMarkers	MS-261-P0
Prohibitin, pAb	Rabbit	1:1000 (IB) 1:100 (IF)	NeoMarkers	RB-292-PO
PHB2 (Bap37, REA),pAb	Rabbit	1:1000 (IB)	Biologend	611801
Tom20, mAb	Mouse	1:100 (IF)	BD Transduction	612278
Tom22 (1C9-2) mAb	Mouse	1:1000 (IB)	GeneTex, Inc.	GTX 10436
Tubulin clone DM1A, mAb	Mouse	1:2000 (IB)	Sigma	T-9026
VDAC1, pAb	Rabbit	1:1000 (IB)	Abcam	ab 3434-100
EGFR (528), mAb	Mouse	1:100 (IF)	Santa Cruz	sc-120
EGFR, pAb	Rabbit	1:1000 (IB)	Santa Cruz	sc-03

Secondary antibodies

Antibodies for IB	Dilution	Supplier
ECL™ sheep anti-mouse peroxidase	1:3000	Amersham Bioscience
ECL™ donkey anti-rabbit peroxidase	1:3000	Amersham Bioscience

Antibodies for IF	Dilution	Supplier
Goat anti-rabbit, Cy™3-conjugatd	1:100	Jackson Immuno Research
Donkey anti-mouse, Cy™3-conjugatd	1:100	Jackson Immuno Research
Goat anti-rabbit, Cy™2-conjugatd	1:100	Jackson Immuno Research
Donkey anti-mouse, Cy™2-conjugatd	1:100	Jackson Immuno Research

6.7 Chemical reagents

Chemical	supplier
Accutase	Sigma-Aldrich
Agarose, low melting point	Sigma-Aldrich
CCCP	Sigma-Aldrich
dNTPs	Invitrogen
Doxycycline hyclate	Sigma-Aldrich

Chemical	supplier
ECL plus	NEN
Gluthatione Sepharose™ 4B	Amersham
Hoechst33258	Sigma-Aldrich
Mitotracker Orange	Molecular Probes
Propidium Iodide	Sigma-Aldrich
Polybrene	Sigma-Aldrich
Protein A Sepharose	Amersham
Protein G Sepharose	Amersham
Saponin	Sigma-Aldrich
TMRE	Molecular Probes

Other reagents, which are not cited here, are from Biomol, Biorad, Fluka, Merck, Roth, Serva and Sigma.

6.8 Kits

Name	supplier
ATP Bioluminescence Assay Kit HSII	Roche
Cell Proliferation ELISA, BrdU (chemiluminescence)	Roche
TNT® T7 Quick coupled Transcription/Translation System	Promega
Endofree Maxi-Plasmidpreparation kit	Qiagen
RNeasy-Kit	Qiagen
Gel Extraction kit	Qiagen
PCR-purification kit	Qiagen
Quantitect™SYBR®Green RT-PCR Kit	Qiagen
Caspase-Glo	Promega
Thermoscript™ RT-PCR kit	Invitrogen

6.9 Buffers

Common buffers

Buffer	composition
10x PBS	150 mM NaCl, 8.4 mM Na ₂ HPO ₄ , 1.8 mM NaH ₂ PO ₄ , pH 7.4

Cloning of lentiviral vectors

Buffer	composition
Annealing Buffer	100 mM potassium acetate, 30 mM HEPES-KOH pH 7.4 (adjust pH with KOH), 2 mM Mg acetate
10x ligase buffer (blund end)	500 mM Tris-HCl (pH 7.5), 100 mM MgCl ₂ , 100 mM DTT, 5 mM ATP, 250 µg/ml BSA

Calcium phosphate transfection

Buffer	composition
2x HBS	50 mM Hepes pH 7.05 (±0.05), 10 mM KCl, 12 mM Dextrose, 280 mM NaCl, 1.5 mM Na ₂ HPO ₄ , sterile filter
CaCl ₂	1.25M CaCl ₂

Propidium iodide staining

Buffer	composition
0.5% Saponin	0.5% (w/v) Saponin in PBS
PI staining solution	10 µg/ml propidium iodide, 100 µg/ml RNase A in PBS

Indirect Immunofluorescence

Buffer	composition
4% PFA, pH 7.4	Dissolve 4g of PFA in 50ml H ₂ O. Heat to 60°C while stirring and add 1ml 1M NaOH. Add 10ml 10x PBS, cool down to RT and adjust pH to 7.4 (ca. 1ml 1M HCl) Make up the final volume to 100ml and freeze in aliquots at -20°C
10x TBS	200 mM Tris pH 7.5, 1.54M NaCl, 20 mM EGTA, 20 mM MgCl ₂
Solution A	0.5% Triton-X100 in 1xTBS
Solution B	0.05% Triton-X100 in 1xTBS, 0.2% BSA

Buffer	composition
Solution C	0.05% Triton-X100 in 1xTBS, 1% goat serum
Moviol	13.3% (w/v) Moviol 4-88, 33% (w/v) Glycerin in 0.15 M Tris-HCl, pH 8.5

Soft agar assay

Buffer	composition
Agarose	2% agarose in 1x PBS, autoclaved
	1% low melting agarose in 1x PBS, autoclaved

Mitochondria isolation

Buffer	composition
Solution B	20 mM Hepes-KOH pH7.6, 220 mM Mannitol, 70 mM sucrose, 1 mM EDTA, 0.5 mM PMSF
Solution A	Solution B containing 2mg/ml BSA
Sucrose Buffer	10 mM Hepes-KOH pH7.6, 0.5 mM sucrose

Preparation of competent *E.coli*

Buffer	composition
Tfb1	30 mM K-acetate, 100 mM RbCl, 10 mM CaCl ₂ , 10 mM MnCl ₂ , 15% (v/v) Glycerol, pH 5.8 (acetic acid)
Tfb2	10 mM MOPS, 75 mM CaCl ₂ , 10 mM RbCl, 15% (v/v) Glycerol pH 6.5 (KOH)

SDS-PAGE and Western blot

Buffer	composition
2x sample buffer	20% Glycerin, 100 mM Tris-HCl pH 6.8, 4% SDS, 200 mM DTT, 0.2% bromophenol blue
Running buffer	0.25 M Tris, 1.918 M Glycin, 1% SDS
Transfer buffer	50 mM Tris-HCl, 40 mM Glycin, 20% Methanol
TBS-T	10 mM Tris-HCl pH 7.5, 100 mM NaCl, 0,1% Tween-20
Blocking solution	10% milk powder in 3% (w/v) BSA in TBS-T

Buffer	composition
Stripping buffer	62.5 mM Tris-HCl, pH 6.7, 10 mM β -Mercaptoethanol, 2% (w/v) SDS, 0.05% Tween-20

Protein staining / fixation

Buffer	composition
Ponceau S	2% PonceauS (w/v), 30% TCA (w/v), 30% sulfosalicylic acid (w/v)
Coomassie solution	0.25% (w/v) Coomassie Brilliant Blue R250, 50% Methanol, 10% glacial acetic acid
Destainer	10% ethanol, 10% glacial acetic acid
Fix-solution	25% methanol, 10% glacial acetic acid

GST tagged protein purification from E.Coli

Buffer	composition
Column buffer (4°C)	20 mM Tris pH7.4, 200 mM NaCl, 10 mM DTT, protease inhibitor cocktail
Buffer 1 (4°C)	100 mM Tris pH 8.0, 150 mM NaCl, 1 mM EDTA, 1 mM DTT
Buffer 2 (4°C)	1M urea, 100 mM Na ₂ HPO ₄ , 10 mM Tris pH 8.0, 1 mM DTT
Buffer 3 (RT)	8M urea, 100 mM Na ₂ HPO ₄ , 10 mM Tris pH 8.0, 0.1 mM DTT
Refolding buffer	100 mM Tris pH8.0, 2 mM EDTA, 400 mM L-Arginine, 0.5 mM Glutathione ox., 5 mM Glutathione red., 1 mM ATP

GST tagged protein purification from SF9 cells

Buffer	composition
Lysis buffer	25 mM Tris, pH 7.6, 150 mM NaCl, 10 mM Na ₄ P ₂ O ₇ , 10% glycerin, 0.75% NP-40, 25 mM β -glycerophosphate, 25 mM NaF, protease inhibitors
Wash buffer	25 mM Tris, pH 7.6, 300 mM NaCl, 10 mM Na ₄ P ₂ O ₇ , 10% glycerin, 0.2% NP-40, 25 mM β -glycerophosphate, 25 mM NaF, protease inhibitors

Binding assay

Buffer	composition
Binding buffer	20 mM Hepes pH 7.8, 300 mM NaCl, 1% Tween-20, protease inhibitors
Wash buffer A	20 mM Hepes pH 7.8, 400 mM NaCl, 1% Tween-20, 10 mM DTT
Wash buffer B	20 mM Hepes pH 7.8, 50 mM NaCl, 1% Tween-20, 10 mM DTT
Wash buffer C	20 mM Hepes pH 7.8, 400 mM NaCl, 1 mM DTT
Elution buffer	50 mM Tris pH 7.8, 20 mM Gluthatione, protease inhibitors

Immunoprecipitation

Buffer	composition
Chaps buffer	150mM NaCl, 10mM HEPES pH7.4, 1% Chaps (w/v), protease inhibitors

PCR buffer

Buffer	composition
10x Taq buffer	100 mM KCl, 100 mM (NH ₄) ₂ SO ₄ , 100 mM Tris-HCl, pH 8.5, 20 mM MgSO ₄ , 1% Triton-X 100

Miniprep with boiling

Buffer	composition
STET buffer	8% sucrose, 5% TritonX-100, 50mM EDTA, 50mM Tris-HCl, pH 8.0, store at 4°C

6.10 Media and supplements

Buffer	composition
LB-culture medium	LB-Broth from Life Technologies prepared according to the manufacturer's instructions
Cell culture medium	Gibco

6.11 Appliances and consumable materials

HERA cell 150 incubator (Heraeus), Mini-PROTEAN III electrophoresis cell (BIORAD), Dounce homogenizer (Kimble Kontes), shaking incubator (New Brunswick), Mini Trans-Blot[®] Electrophoretic Transfer Cell (BIORAD), Monolight 3096 microplate luminometer (BD Bioscience), Image analyzer LAS-3000 (Fujifilm Life science), PhosphorImager FLA-3000 (Fujifilm Life science), Imaging Plate Bas-MS 2325 (Fujifilm), Robocycler (Stratagene), PCR machine (PerkinElmers), Centrifuge 5417 C (Eppendorf), Sorvall RC-5B (Kendro Laboratory Products), fluorescence microscope (Zeiss), confocal microscope (Leica), inverted microscope Leica DMIL, Chromaphor camera (Scion Corp.), Abi Prism 7900 (AME Biosystems), FACS Calibur (BD), FACS Aria (BD), ELISA Reader (Molecular Devices), ODO - BioRobot 8000 (Bigneat Containment Technology), TL-100 ultracentrifuge (Beckmann)

Hyperfilm[™]-ECL High performance chemiluminescence film (Amersham Biosciences), PVDF-membrane (PerkinElmer Life science), Whatman 3mm chromatography paper (Schleicher and Schüll), 96 well flat bottom plate, white rimmed (Costar), Falcon tubes with cell strainer (BD), materials for cell culture from Greiner and TPP, Amicon Ultra -15 (30k) (Millipore), Durapore[™] 0.45 µm (Millipore), Multiscreen HTS[™],HV 0.45 µm (Millipore), 96 well PCR-plates (Abgene), optical adhesive covers (Abgene)

6.12 Software

Windows 2000, XP (Microsoft), MS Office (Microsoft), OSX (apple), Mozilla Firefox, Adobe Acrobat 7.0 (Adobe), Photoshop 6.0 (Adobe), Corel Draw 11 (Corel), ACT-1.2 (Nikon), Visicapture (Scion Corp.), Reference Manager 11 (Thompson ISI research soft), CellQuest Pro (BD), Image Reader LAS-3000 (Fujifilm Life Science), BasReader 3.14 (Fujifilm), Aida Image analyzer 4.03 (Fujifilm), Vector NTI 10 (Invitrogen), Chromas (Technelysium Pty), QIAsoft 4.1.4.7 (Qiagen), SDS 2.2.2 (Applied Biosystems), ImageJ