5 Materials

5.1 Instruments and equipment

Agarose gel chambers	BioRad
Cell culture plates and flasks	TPP
Centrifuges	Eppendorf 5417R
	Eppendorf 5402
	Eppendorf 5416
	Beckmann Avanti J-25
	Beckmann J6-HC
	IEC MicroMax
CO_2 incubators	Nuaire US Autoflow
	Binder
Cryo tubes	Nalgene
Electroporation cuvettes	Biorad
Flowcytometer	LRSII, BD
FPLC	BioRad Biological Workstation
	Model 2128 fraction collector
Freezers	Forma Scientific
Gel documentation	Herolab
Gel dryer	Savant Stacked Gel Dryer SGD 300
	Hoefer Slab Gel Dryer GD2000
Heat blocks	Techne DB3
Hyperfilms	Amersham Biosciences
Incubators	Memmert
	Infors HT
Magnetic Particle Concentrator 6	Dynal
Magnetic stirrer	Heidolph
Microplate reader Mithras LB 940	Berthold technologies
Microscope Axioplan 2	Zeiss
Microscope cell culture	Zeiss TELAVAL 31
Microtiter plates	TPP
Microwave	Privileg 9029GD
N_2-tank	CRONOS
Overhead rotator (Intelli-mixer)	Neolab
Petri dishes	Peske Medizintechnik
pH-meter	Knick
Photometers	Pharmacia Biotech GeneQuant II
	Pharmacia Biotech Novaspec II
Plastic cuvettes	Sarstedt
Power supplies	Biorad Model 200/2.0 Power Supply
	ST305 Pharmacia
	Electrophoresis Power Supply EPS3500
Precision cuvettes	Hellma
Prescision scales	Sartorius BP 310S
	Sartorius AC 210P
	Scaltec SP061
PVDF-membrane	Millipore
SDS PAGE chambers	Biorad Mini Protean II Cell

Semi-dry blotter	PHASE
Sequencer	Applied Biosystems 310
Streptavidin-Microtiter plates	Biotez
Superose 6 column	Pharmacia
Thermocycler	Biometra T3
Thermomixer	Eppendorf 5436
Tissue culture hoods	BDK
Ultrasonic processor UP 200s	Dr. Hielscher GmBH
UV-table	Biometra TI 2
Vortexer	Heidolph Reax 2000
Water bath	Haake F3, Julabo MP

5.2 Chemicals

Acrylamide/Bisacrylamide	Roth
Agarose	Invitrogen
Ammonium persulfate	Sigma
Ampicillin sodium sulfate	Roche
anhydrotetracycline (AHT)	IBA
Bacto agar	Roth
Bacto tryptone	Roth
Bacto yeast extract	Roth
β-Glycerophosphate	Sigma
β-Mercaptoethanol	Merck
Bovine serum albumin (BSA)	Roth
Bradford reagent	Biorad
Brefeldin A	Sigma
Bromphenolblue	Biorad
mCD4 (L3T4)-beads	Dynal
Complete protease inhibitor tablets	Roche
DAPI (4,6-Diamidin-2-phenylindol-dihydrochlorid)	Roche
Detachabeads mCD4	Dynal
Developing solution for Xray films	Sigma
DMEM	PAA
DMSO	Sigma
DNA bp ladder	Invitrogen
dNTPs	Amersham Biosciences
DTT	Sigma
Energy Regeneration Solution (ERS)	Boston Biochemicals
Ethidium bromide	Roche
Fetal calf serum (FCS)	GibcoBRL, PAA
Fixation solution for X ray films	Sigma
Glutathione (GSH)	Boehringer Mannheim
Glutathione-Sepharose 4B	Amersham Biosciences
mIL-2	Roche
Imperial protein stain	Pierce
Isopropyl-b-D-thiogalactoside (IPTG)	Biomol
Kanamycin	PAA
L-Glutamine	PAA
Lipofectamine 2000 reagent	Invitrogen

Molecular weight marker (protein)	Biorad, MBI Fermentas
Mowiol 4-88	Calbiochem
NP-40	Fluka
OptiMEM I	Gibco
Penicillin/Streptomycin	PAA
PMA	Calbiochem
poly dIdC	Roche
Protein-G-Sepharose	Amersham Biosciences
RPMI 1640	PAA
Sodium pyruvate	PAA
Strep-Tactin Superflow resin	IBA
TEMED	Biorad
Triton-X-100	Serva
Trypan blue	Sigma
Trypsin/EDTA	PAA
Tween 20	Sigma

5.3 Enzymes and Kits

BigDye Terminator v 1.1Cycle Sequencing	Applied Biosystems
DeepVent-DNA-Polymerase	New England Biolabs
Dual Luciferase Reporter Assay System Kit	Promega
Qiagen Plasmid Maxi Kit	Qiagen
Qiaquick Gel Extraction and PCR Purification Kit	Qiagen
QiaPrep Spin Miniprep Kit	Qiagen
Restriction endonucleases and buffers	Amersham, New England Biolabs
5x Sequencing buffer	Applied Biosystems
T4-DNA Ligase	usb

5.4 Bacteria

E. coli DH5α		Gibco
E. coli BL21-(DE3)pLysS	BL21(DE3) are lysogens of the bacteriophage λDE3 and therefore carry a chromosomal copy of the T7 RNA polymerase gene under control of the *lacUV5* promoter. BL21(DE3)pLysS possess an additional plasmid that encodes for the T7 lysozyme (pLysS), a natural inhibitor of T7 RNA polymerase. Thus, basal expression of T7 RNA polymerase prior to induction is suppressed, which provides tighter control of protein expression and stabilizes recombinants encoding target proteins that affect cell growth and viability.	Novagen
E. coli BL21-CodonPlus(DE3)-RIL	BL21-CodonPlus(DE3)-RIL contain extra genes for tRNAs that recognize the arginine codons AGA and AGG, the isoleucine codon AUA, and the leucine codon CUA. Those tRNAs most frequently restrict translation of heterologous proteins from organisms that have AT-rich genomes.	Stratagene
E. coli SCS10	SCS 10 are deficient for adenine and cytosine methylases.	Stratagene

5.5 Eukaryotic cell lines

HEK293 cells	Human embryonic kidney cells transformed with parts of adenovirus 5 DNA
COS-7 cells	African green monkey kidney cells transformed by a mutant SV-40 virus
Jurkat T cells	Human T cell line (acute T cell leukemia)
Phoenix cells	Amphotrophic packaging cell line, HEK293 derived, transformed with adenovirus E1a
Jurkat NEMO -/- cells	gift from S. C. Sun (Pennsylvania State University, Pennsylvania, USA)
Jurkat NEMO -/- reconstituted cells (with HA NEMO WT and L329P)	gift from J. D. Ashwell (National Institutes of Health, Bethesda, USA)

5.6 Recombinant proteins

Ubiquitin wt, K63R and K48R (human)	Boston Biochemicals
UbcH13/Uev1a heterodimer complex (human)	Boston Biochemicals
Ubiquitin activating enzyme E1 (rabbit)	Boston Biochemicals
GST Malt1 482-813 WT, 6R and 11R	affinity-purified from *E. coli* BL21(DE3)Codon Plus-RIL (see 6.6.1)
GST TRAF6	affinity-purified from *E. coli* BL21(DE3)Codon Plus-RIL (see 6.6.1)
StrepIKKγ WT, L329P and Y308S	affinity-purified from *E. coli* BL21(DE3)pLysS (see 6.5.1)

5.7 Vectors and oligonucleotides

General Vectors	
pEF4 3xFlag	three Flag sequences in pEF4HIS-C (Stratagene) between *Hind*III/*Kpn*I restriction sites, N-terminal tag (provided by D. Krappmann)
pcDNA3 1xFlag	Flag sequence between *Hind*III/*Bam*HI sites in pcDNA3 (Invitrogen), N-terminal tag (provided by D. Krappmann)
6xNF-κB reporter plasmid	six NF-κB sites in pGL2-Basic (M. Bergmann), expression of Firefly luciferase
Tkluc plasmid	*Renilla* luciferase gene under control of *herpes simplex* virus thymidine kinase promotor
pMCSV	Retroviral vector, IRES sequence enables simultaneous expression of Thy1.1 (CD90.1) and cloned constructs (V. Heissmeyer).
Malt1 constructs	
MycMalt1	N-terminal tag, *Sal*I/*Not*I in pRK5 vector, RZPD-clone BM016367 (E. Wegener)
3xFlagMalt1	cloned *Bam*HI/*Not*I in pEF4 3xFlag (E. Wegener)
3xFlagMalt1 N-terminal deletion mutants	Constructs (aa 314-318, 482-813, 548-813, 612-813 and 684-813) were PCR-amplified and cloned *Bam*HI/*Not*I in pEF4 3xFlag (E. Wegener).
GSTMalt1 fl and 482-813	transferred from 3xFlagMalt1/3xFlagMalt1 482-813 vector into pGEX 6p1 (Amersham) using *Bam*HI/*Not*I restriction sites

3xFlagMalt1 612-813 lysine mutants:	Sequential introduction of point mutations in 3xf Malt1 612-813 *via* PCR-based mutagenesis and cloning using the *Bam*HI/*Not*I sites in pEF4 3xFlag. All constructs were amplified using the following 5'and 3'end primers and specific internal primers (changed bases are underlined): 5' primer (Malt1 1870 *Bam*HI s): 5' TAT GGA TCC GAG ATA ATA ATG TGT GAT GCC TAC G 3' primer (Malt1 *Not*I as): 5' AT AGC GGC CGC TCA TTT TTC AGA AAT TCT GAG CCT GTC
KK633,37RR	internal mutagenesis primer rev (Malt1-K633,37-as): 5' TTC TTC AGG TGT GCC T**C**T ATT TGC ATC T**C**T TGG ATC AAT ATC
KK650,54RR	internal mutagenesis primer rev (Malt1-K650,54R-as): 5' GGT ATA GAG GCA ATG C**C**T GGG AAG ATC C**C**T TGA TAC CAA G
KK666,68RR	internal mutagenesis primer rev (Malt1-K666,68R-as): 5' C TGT GAA GAC TAG ATG TTC C**C**T TAA T**C**T TTG CAG TGA ACT GAG
6R	lysines 633, 637, 650, 654, 666 and 668 mutated to arginines through sequential introduction of point mutations
11R	lysines 633, 637, 650, 654, 666, 668, 691, 698, 703, 713 and 813 mutated to arginines through sequential introduction of point mutations using the additional primers: 3' primer (Malt1 K813R *Not*I as): 5' ATA GCG GCC GCT CAT CTT TCA GAA ATT CTG internal mutagenesis primer (Malt1-K691R-as): 5' TCA CTT CCT GCC TGT C**C**T CTA CAG internal mutagenesis primer (Malt1-K698,703R-as): 5' CAT GTC TAA T**C**T AGC AAT GAG AGG T**C**T CCC AAC ATT C internal mutagenesis primer (Malt1-K713R-as): 5' G AAA GCA AGT C**C**T CCT TCC CAA ACC
3xFlagMalt1 f.l. lysine mutants: 6R 11R 5R.B	Mutations were first introduced into pGEX GST Malt1 from the respective 3xFlagMalt1 612-813 constructs *via Xba*I/*Not*I cloning. The GST Malt1 vector was purified from *E.coli* SCS10 (methylase deficiency) and was partially digested with *Xba*I (1µl for 10 min). Full length Malt1 was then transferred *via Bam*HI/*Not*I digestion into the pEF4 3xFlag vector.
3xFlagMalt1 2EA	Point mutations were first introduced into GST Malt1 through a three step PCR-based mutagenesis. With the first PCR a C-terminal Megaprimer was amplified containing the E795A mutation, which was used in a second PCR to produce a second C-terminal Megaprimer containing both (E642A and E795A) mutations. This second Megaprimer was used together with the 5' *Bam*HI primer to generate a full length Malt1 construct, which was then cloned *Bam*HI/*Not*I into the pGEX-6p1 PKA vector and from there finally transferred *via Bam*HI/*Not*I digestion into the pEF4 3xFlag vector. 5' primer (Malt1 *Bam*HI s): 5' ATA GGA TCC GTG TCG CTG TTG GGG GAC C 3' primer (Malt1 *Not*I as): see 3xFlagMalt1 612-813 lysine mutants internal mutagenesis primer (Malt1-E806A): 5' GTG CCA GTA G**C**G ACA ACT GAT GAA ATA CC internal mutagenesis primer (Malt1 E653A s): 5' ACA CCT GAA G**C**A ACT GGC AGC TAC

GSTMalt1 482-813 6R und 11R	PCR amplified from the respective 3xFlagMalt1 constructs and cloned between *Bam*HI/*Not*I sites into pGEX-6p1. 5' primer (Malt1 1480-1504 *Bam*HI s): 5' TAT GGA TCC CAA GGA GCA GAA GCT TTT GAA ATC C 3' primer: Malt1 *Not*I as (for 6R) and Malt1 K813R *Not*I as (for 11R)
3xFlagMalt1 WT, 6R, 11R, 2EA and aa 314-813 in pMCSV	Cloning was performed using the Gateway technology (Invitrogen). 3xFlag tagged constructs were cloned from the respective pEF vectors into pENTR11+*Hind*III vector (V. Heissmeyer) *via Hind*III/*Not*I restriction and were then transferred into the pMCSV vector through an LR recombination reaction (E. Wegener).
TRAF contructs	
FlagTRAF6	*Sal*I/*Not*I in pRK5 vector, N-terminal tag (P. Baeuerle, Tularik)
FlagTRAF6	aa 289-522, *Sal*I/*Not*I in pRK5 vector, N-terminal tag (P. Baeuerle)
MycTRAF6	PCR amplified from FlagTRAF6, cloned in pRK5 between *Sal*I/*Not*I sites using the following primers: 5' primer (TRAF6-*Sal*I-s): 5' ATA GTC GAC AGT GAG TCT GCT AAA CTG TGA AAA C 3' primer (TRAF6-*Not*I-as): 5' ATA GCG GCC GCT CAT ACC CCT GCA TCA GTA CTT CG
HisXTRAF6	PCR amplified from FlagTRAF6, cloned in pcDNA4 between *Eco*RI/*Not*I sites using the following primers: 5'primer (TRAF6-*Eco*RI-s): 5'ATA GAA TTC GTG AGT CTG CTA AAC TGT GAA AAC 3' primer (TRAF6-*Not*I-as): 5' ATA GCG GCC GCT CAT ACC CCT GCA TCA GTA CTT CG
FlagTRAF2	*Sal*I/*Not*I in pRK5 vector, N-terminal tag (P. Baeuerle, Tularik)
IKKγ constructs	
FlagIKKγ	cloned *via Eco*RI/*Xho*I sites in pcDNA3 1xFlag vector (S. Col Arslan)
FlagIKKγ L329P Y308S	Point mutations were introduced through PCR-based mutagenesis and constructs were cloned *Eco*RI/*Xho*I in pcDNA3 1xFlag vector using the following primers: 5' primer (hNEMO_Nter_*Eco*RI): 5' GAC TGA ATT CAA TAG GCA CCT CTG GAA GAG C 3' primer (hNEMO_Cter _*Xho*I): 5' GAC TCT CGA GCT ACT CAA TGC ACT CCA TGA C internal mutagenesis primer (NEMO L329P as): 5' CAG CTG CTC CTG C**G**G GAG CTC CTT CTT CTC GG internal mutagenesis primer (NEMO Y308S as): 5' GAA GTC CGC CTT G**G**A GAT ATC CGC CTG GGC
StrepIKKγ	cloned into pASK-IBA5plus vector using *Sac*II/*Nco*I sites (S. Col Arslan)
StrepIKKγ L329P Y308S	IKKγ point mutants were amplified from the respective FlagIKKγ constructs and cloned *Sac*II/*Nco*I in the pASK-IBA5plus vector. 5' primer (hNEMO_Nter_*Sac*II): 5' GACTCCGCGGCGAATAGGCACCTCTGGAAG 3' primer (hNEMO_Cter_*Nco*I_stop): 5' GACTCCATGGCTACTCAATGCACTCCATGAC
Bcl10 constructs	
3xFlagBcl10	cloned between *Bam*HI/*Xba*I sites in pEF4 3xFlag (D. Krappmann)
3xFlagBcl10 K110R and K17/18R	mutation introduced in 3xFlagBcl10 by PCR based mutagenesis (L. Lavitas and S. Jungmann)

3xFlagBcl10 L41Q	cloned *Bam*HI/*Xba*I in pEF 3xFlag, point mutation L41Q (D. Krappmann)
3xFlag-NLS-Bcl10	Oligonucleotide containing the NLS sequence of the SV40 large T antigen (PKKKRKV) introduced in *Bam*HI restriction site. Oligo s 5' GATCTGCGGCCGCTCCCAAGAAGAAGAGGAAGGTCG Oligo as 5' GATCCGACCTTCCTCTTCTTCTTGGGAGCGGCCGCA
3xFlag-NLS-Bcl10 K110R and K17,18R	NLS Oligonucleotide (see 3xFlag-NLS-Bcl10) introduced in *Bam*HI site of 3xFlagBcl10 K110R or 3xFlagBcl10 K17,18R
GFPBcl10	cloned *Bam*HI/*Xba*I in pEGFPC1 (Clonetech), N-terminal tag (S. Jungmann)
GFPBcl10 L41Q	cloned *Bam*HI/*Xba*I in pEGFP C1, point mutation L41Q (S. Jungmann)
GFPBcl10 L41Q K105,110R	point mutations introduced in GFPBcl10 L41Q (S. Jungmann)
GFP-NLS-Bcl10	NLS Oligo (see 3xFlag-NLS-Bcl10) introduced in *Bam*HI site of GFPBcl10
GFPBcl10-SUMO	Bcl10 was cloned between the *Hind*III and *Bam*HI restriction sites in GFP SUMO (pEGFP C1) using the following primers: 5' primer (Bcl10_*Hind*III_s_EGFP): 5' CGA AGC TTC TGA GCC CAC CGC ACC GTC C 3' primer (Bcl10_*Bam*HI_as_EGFP): 5' TAT GGA TCC TTG TCG TGA AAC AGT ACG TGA TC
Ubiquitin(-like) modification constructs	
MycUbc9	cloned *Sal*I/*Not*I in pRK5, N-terminal tag (E. Wegener)
MycSUMO1	aa 1-97 (processed form with C-terminal glycine), cloned *Sal*I/*Not*I in pRK5, N-terminal tag (E. Wegener)
GFPSUMO1	aa 1-97 (processed form with C-terminal glycine), transferred from 3xFlagSUMO (E. Wegener) by *Bam*HI/*Xba*I restriction digest in pEGFPC1
HAUbiquitin	HA tag (*Hind*III/*Bam*HI) and ubiquitin (*Bam*HII/*Xba*I) were cloned into pEF4C (V. Welteke)
IKK and IκBα constructs	
MycIKKβ K44A	catalytically inactive IKKβ mutant (K44A) in pRK5, N-terminal tag (gift from M. Karin)
FlagIκBα ΔN	IκBα aa 71-317 in pcDNA3 Flag vector (E.Kärgel)

All constructs were cloned from human cDNA.

Sequencing primer

Sp6-primer	5' ATTTAGGTGACACTATAG
T7-primer	5' TAATACGACTCACTATAGGG
BGH reverse	5' TAGAAGGCACAGTCGAGGCTG
pGEX s (for pGEX-6p1)	5' GGCGACCATCCTCCAAAATCG G
pGEX as (for pGEX-6p1)	5' CCGAAACGCGCGAGGCAGATCG
pASK_IBA_Nter_sequencing	5' GAGTTATTTTACCACTCCCT
pASK_IBA_Cter_sequencing	5' CGCAGTAGCGGTAAACG

siRNAs

siKO:	s	GCAACGGUGAACGGUUAAUUCAAUC
	as	UAUUGAAUUAACCGUUCACCGUUGC
siTRAF6.1:	s	GCACAGCAGUGCAAUGGAAUUUAUA
	as	UAUAAAUUCCAUUGCACUGCUGUGC
siTRAF6.2:	s	CCAGCUCCUGUAGCGCUGUAACAAA
	as	UUUGUUACAGCGCUACAGGAGCUGG
siTRAF6.3:	s	CCACGAAGAGAUAAUGGAUdTdT
	as	AUCCAUUAUCUCUUCGUGGdTdT

5.8 Antibodies

Primary antibodies

Bcl10 (331.3, C17)	Santa Cruz Biotech
Carma1	Abcam
hCD3 (HIT3a)	BD Pharmingen
mCD3 (145-2C11)	BD Pharmingen
hCD28 (CD28.2)	BD Pharmingen
mCD28 (37.51)	BD Pharmingen
mCD16/CD32 (Fc Block)	BD Pharmingen
c-myc (9E10)	Santa Cruz Biotech
Flag M2	Sigma
Flag M2-FITC	Sigma
Flag M5	Sigma
HA (Y-11)	Santa Cruz Biotech
ICN hamster	MP Biomedicals
IκBα (C-21)	Santa Cruz Biotech
IκBα (L35A5)	Cell Signaling
IKKγ (FL-419)	Santa Cruz Biotech
IKKγ mAb	BD Transduction Laboratories
mIL-2-FITC	eBioscience
Malt1	Genentech
Malt1 (B12, C16 and H300)	Santa Cruz Biotech
Thy1.1-APC	eBioscience
TRAF6 (D10 and H274)	Santa Cruz
Ubiquitin (FK2)	Biomol

Secondary antibodies

HRP- conjugated anti-rabbit	JacksonImmunoResearch
HRP- conjugated anti-mouse	JacksonImmunoResearch
HRP- conjugated anti-goat	JacksonImmunoResearch
anti-mouse-IgG1a-FITC	BD Biosciences
anti-mouse IgG1 and anti-mouse IgG2a	BD Pharmingen
Alexa Fluor 546 goat anti-mouse (Cy3)	Molecular Probes, Invitrogen
Alexa Fluor 488 donkey anti-mouse (FITC)	Molecular Probes, Invitrogen

5.9 Buffers and solutions

Molecular Biology		
TBE buffer	Tris Boric acid EDTA	50 mM 50 mM 1 mM
DNA sample buffer	Bromphenolblue Xylencyanol Glycerin	1% (w/v) 1% (w/v) 40% (v/v)
Bacteria		
LB medium	Bacto tryptone Bacto yeast extract NaCl	10 g/l 5 g/l 10 g/l
LB Agar	Bacto tryptone Bacto yeast extract NaCl Agar	10 g/l 5 g/l 10 g/l 15 g/l
SOB medium	Bacto tryptone Bacto yeast extract NaCl KCl $MgCl_2$ $MgSO_4$ pH 6.7-7	20 g/l 10 g/l 10 mM 2.5 mM 10 mM 10 mM
TB buffer	PIPES pH 6.7 $MnCl_2$ $CaCl_2$ KCl	10 mM 55 mM 15 mM 250 mM
Ampicillin (1000x)		100 mg/ml
Kanamycin (1000x)		20 mg/ml
SDS PAGE		
Stacking gel buffer	Tris pH 6.8	1M
Stacking gel (1ml)	H_2O Acrylamid mix Stacking gel buffer 10 % SDS 10 % APS TEMED	680 µl 170 µl 130 µl 10 µl 10 µl 1 µl
Separation gel 8% (5 ml)	H_2O Acrylamid mix Separation gel buffer 10 % SDS 10 % APS TEMED	2.3 ml 1.3 ml 1.3 ml 50 µl 50 µl 3 µl
Separation gel 10% (5 ml)	H_2O Acrylamid mix Separation gel buffer 10 % SDS 10 % APS TEMED	1.9 ml 1.7 ml 1.3 ml 50 µl 50 µl 2 µl
Separation gel buffer	Tris pH 8.8	1.5 M

SDS electrophoresis buffer	Tris pH 7.3 SDS Glycine	25 mM 0.1% (w/v) 192 mM
6x SDS sample buffer	DTT SDS Tris pH 6.8 EDTA Glycerol Bromphenol blue	10% (w/v) 12% (w/v) 300 mM 12 mM 40% (v/v) spatula tip
Western Blotting		
Blotting buffer	Tris pH 8.3 Glycine Methanol SDS	48 mM 39 mM 20% (v/v) 0.037 % (w/v)
PBS (Phosphate buffered saline)	NaCl KCl Na_2HPO_4 KH_2PO_4	137 mM 2.7 mM 10 mM 1.7 mM
PBS-T	PBS	+ 0.1% Tween-20 (f.c.)
Stripping buffer A	Glycine pH 2.2 SDS Tween 20	0.1 M 0.1% (w/v) 1% (v/v)
Stripping buffer B	Tris pH 6.7 SDS ß-Mercaptoethanol	80 mM 10% (w/v) 71 µl / 10 ml (freshly added)
Solution A (4 °C)	Luminol 0.1 M Tris pH 8.6	50 mg 200 ml
Solution B (RT, dark)	Para-hydroxy-coumaric acid DMSO	11 mg 10 ml
Cell culture		
2x HBS (HEPES buffered saline) buffer	HEPES pH 7.12 NaCl Na_2HPO_4	25 mM 280 mM 1.2 mM
2 x HBS-P buffer	HEPES pH 7.0 NaCl Na_2HPO_4	50 mM 280 mM 1.5 mM
Trypsin/EDTA	Trypsin EDTA	0.5 mg/ml 0.22 mg/ml
TAC (Tris-Ammonium-Chloride) lysis buffer	Tris pH 7.2 NH_4Cl	20 mM 0.83% (v/v)
Immunoprecipitation		
Co-immunoprecipitation buffer (CoIP buffer)	HEPES pH 7.5 NaCl Glycerol NP-40	25 mM 150 mM 1 mM 0.2% (v/v)
Protease and phosphatase inhibitor additives (added for all cell lysates)	Complete protease inhibitor tablets (Roche) ß-glycerophosphate DTT sodium vanadate NaF	1 / 50 ml 8 mM 1 mM 300 µM 10 mM

Ubiquitination lysis buffer	CoIP buffer	+ 1% SDS w/v (f.c.)
Protein purification		
StrepNEMO lysis buffer	Tris pH 8 NaCl DTT Complete protease inhibitors	100 mM 150 mM 1 mM 1 x
E.coli lysis buffer (for GST TRAF6 and GST Malt1 constructs)	HEPES pH 7.5 NaCl $MgCl_2$ Glycerol Triton-X-100 DTT Complete protease inhibitors	50 mM 150 mM 2 mM 10% (v/v) 0.1% (v/v) 1mM 1 x
GST elution buffer	HEPES pH 7.2 NaCl $MgCl_2$ Glutathione	20 mM 150 mM 10 mM 10 mM
Pull-down experiments		
1% Triton buffer	HEPES pH 7.5 NaCl Glycerol Triton-X-100	25 mM 150 mM 1 mM 1%
0.1% Triton buffer	HEPES pH 7.5 NaCl Glycerol Triton-X-100	25 mM 150 mM 2 mM 0.1%
Triton dilution buffer	HEPES pH 7.5 NaCl Glycerol	25 mM 150 mM 3 mM
***In vitro* ubiquitination assay**		
buffer P1	HEPES pH 7.2 $MgCl_2$	20 mM 10 mM
buffer P4	HEPES pH 7.2 NaCl $MgCl_2$	20 mM 150 mM 10 mM
Immunofluorescence		
Mowiol solution	Mowiol 4-88 Glycerin in 12 ml Tris pH 8.5	2.4 g 6 g 0.2 M