2 Material

2.1 Chemicals

Chemicals which were used in the present work are listed below.

Table 1 List of chemicals.

Designation	Provider
Acrylamide	Applichem GmbH
Antibiotic/Antimycotic (A/A)	gibco® by life technologies™
Blasticidin	Invitrogen
Bovine serum albumin (BSA)	Carl Roth GmbH & Co. KG
Bromphenol blue	Applichem GmbH
Carbenicillin	Applichem GmbH
CellMask™ Deep Red Plasma membrane stain	Thermo Fisher Scientific
dNTP's	New England BioLabs GmbH
Dulbecco's Modified Eagle's Medium (DMEM)	gibco® by life technologies™
EDTA	Applichem GmbH
Ethanol	VWR
Ethidium bromide 1% *Biochemica*	Applichem
Fetal calf serum (FCS)	Life technologies
GeneRuler™ 1 kb Plus DNA Ladder	Thermo Fisher Scientific
GeneRuler™ 100 bp DNA Ladder	Thermo Fisher Scientific
Goat serum (normal)	Dako
Hoechst 33342 dye	Applichem GmbH
Hygromycin B	Invitrogen by Thermo Fisher Scientific
Isopropanol	Applichem GmbH
KCl	Applichem GmbH
KH_2PO_4	Applichem GmbH
LB-Agar-Powder according to Miller	Applichem GmbH
LB-Medium-Powder according to Miller	Applichem GmbH
Lipofectamine 2000	Invitrogen AG
$MgSO_2$	Applichem GmbH
Na_2HPO_4	Carl Roth GmbH & Co. KG

D. Dannheisig, *Impact of Survivin Acetylation on its Biological Function*, BestMasters, DOI 10.1007/978-3-658-18623-4_2

NaCl	Carl Roth GmbH & Co. KG
Opti-MEM®	gibco® by life technologies™
Penicillin/Streptomycin (P/S)	gibco® by life technologies™
Phosphate buffered saline (PBS)	gibco® by life technologies™
Roti®-Histofix	Carl Roth GmbH & Co. KG
Sodium azide	Applichem GmbH
Staurosporin	Biomol GmbH
Tet System fetal bovine serum (FBS)	Clone Tech (Takara)
Tetracycline	Applichem GmbH
TRIS-HCl	Applichem GmbH
Triton X-100	Applichem GmbH
TrypLE™ Express	gibco® by life technologies™
Tryptone	Applichem GmbH
$MgCl_2$	Applichem GmbH
Yeast extract	Applichem GmbH
Zeocin®	Invitrogen™

2.2 Buffers, solutions and media

Buffers, solutions and media which were used in the present work are listed below. If not denoted, buffers, solutions and media were prepared with Millipore H_2O.

Table 2 List of buffers, solutions and media.

Designation	Application	Composition
Cultivation medium (A)	Growth of **untranfected** parental Flp-In HeLa cell line	DMEM 120 U/ml Penicillin 120 µg/ml Streptomycin 10 % (v/v) FCS 200 µg/ml Zeocin 15 µg/ml Blasticidin
Cultivation medium (B)	Growth of stable Flp-In HeLa cell line	DMEM 120 U/ml Penicillin 120 µg/ml Streptomycin 10 % (v/v) Tet system FBS 400 µg/ml Hygromycin 15 µg/ml Blasticidin

Cultivation medium (C)	Growth of HeLa Kyoto cell line	DMEM 10 % (v/v) FCS 100 U/ml Penicillin G 100 µg/ml Streptomycin sulphate 250 µ/ml Amphotericin B
LB agar	Prokaryotic cell growth	25 g/L LB-Medium-Powder according to Miller in ddH_2O
LB medium	Prokaryotic cell growth	40 g/L LB-Agar-Powder according to Miller in ddH_2O
NEB Ab dilution buffer	PLA and IF	1 % (w/v) BSA 0,3 % (v/v) Triton X-100 in 1x PBS
NEB blocking solution	PLA and IF	5 % (v/v) normal goat serum 0,3 % (v/v) Triton X-100 in 1x PBS
Phosphate buffered saline (PBS) pH= 7,4	Washing steps	137 mM NaCl 2,7 mM KCl 10 mM Na_2HPO_4 2 mM KH_2PO_4
SOB medium	Transformation	0,5 g/L yeast extract 20 g/L tryptone 0,6 g/L NaCl 0,2 g/L KCl 10 mM $MgCl_2$ 10 mM $MgSO_4$
NaAc/PBS	Storage of samples and prevention of microbial growth	0,1 % sodium azide in PBS

2.3 Oligonucleotides

Oligonucleotides which were used in the present work are listed below. The respective oligonucleotides served as PCR or sequencing primers and were either synthesized or provided by Eurofins Genomics (Ebersberg, Germany).

Table 3 List of oligonucleotides.

Designation	Application	Sequence (5'→3')
myc-tag-fw [423]	forward primer [sequencing] PCR forward primer [clone screening]	ATG-GAG-CAG-AAG-CTT-ATC-TCT-G
Surv-rv	PCR reverse primer [clone screening]	TTA-ATC-CAT-GGC-AGC-CAG-CTG
Crm1-fw [2148]	forward primer [sequencing]	GTC-TCT-CTG-AAG-TGC-CTC-ACT-GAG
Crm1-rv [2149]	reverse primer [sequencing]	CAG-CGA-CCA-TCT-GTG-GAT-CAT-TGG-ATC-G
CMV-F	forward primer [sequencing]	GCA-AAT-GGG-CGG-TAG-GCG-T
pCDNA3.1-R	reverse primer [sequencing]	TAG-AAG-GCA-CAG-TCG-AGG-CT
EGFP-C1-F	forward primer [sequencing]	GAA-GCG-CGA-TCA-CAT-GGT-C
BamHI-Surv-fw [464]	PCR forward primer [cloning]	AAA-GGA-TCC-ACG-GTG-CCC-CGA-CGT-TG
EcoRI-Surv-rv [1518]	PCR reverse primer [cloning]	TTT-GAA-TTC-TTA-ATC-CAT-CGC-AGC-CAG
BamHI-Crm1-fw	PCR forward primer [cloning]	AAA-GGA-TCC-ACC-CAG-CAA-TTA-TGA-CAA-TG
NotI-Crm1-rv	PCR reverse primer [cloning]	TTT-GCG-GCC-GCT-TAA-TCA-CAC-ATT-TCT-TC

2.4 Plasmids

Plasmids which were used in the present work are listed below. The respective application is denoted. Newly generated constructs were sequenced by LGC Genomics GmbH (Berlin, Germany) before experiments were performed.

Table 4 List of plasmids.

Designation	Encoding for	Application	Reference
pC3-Cerulean	Cerulean	FRET [negative control]	Cecilia Vallet
pC3-Cerulean-Citrine	Cerulean-Citrine fusion protein	FRET [positive control]	Cecilia Vallet
pC3-Cerulean-Surv_WT	Cerulean-Survivin (WT) fusion protein	FRET	Cecilia Vallet
pC3-Cerulean-Surv-F101A-L102A	Cerulean-Survivin (F101A + L102A) fusion protein	FRET	Cecilia Vallet
pC3-Cerulean-Surv-K129A	Cerulean-Survivin (K129A) fusion protein	FRET	this work
pC3-Cerulean-Surv-K129E	Cerulean-Survivin (K129E) fusion protein	FRET	this work
pC3-Cerulean-Surv-K129Q	Cerulean-Survivin (K129Q) fusion protein	FRET	this work
pC3-Cerulean-Surv-K129R	Cerulean-Survivin (K129R) fusion protein	FRET	this work
pC3-Citrine	Citrine	FRET [negative control]	Cecilia Vallet
pC3-Citrine-Crm1	Citrine-Crm1 fusion protein	FRET	this work
pC3-Citrine-Surv_WT	Citrine-Survivin (WT) fusion protein	FRET	Cecilia Vallet
pC3-Citrine-Surv-F101A-L102A	Citrine-Survivin (F101A + L102A) fusion protein	FRET	Cecilia Vallet
pC3-Citrine-Surv-K129A	Citrine-Survivin (K129A) fusion protein	FRET	this work
pC3-Citrine-Surv-K129E	Citrine-Survivin (K129E) fusion protein	FRET	this work
pC3-Citrine-Surv-K129Q	Citrine-Survivin (K129Q) fusion protein	FRET	this work

pC3-Citrine-Surv-K129R	Citrine-Survivin (K129R) fusion protein	FRET	this work
pC3-myc-Surv-K129A	myc-tagged survivin (K129A)	Cloning [PCR template]	Britta Unruhe
pC3-myc-Surv-K129E	myc-tagged survivin (K129E)	Cloning [PCR template]	Cecilia Vallet
pC3-myc-Surv-K129Q	myc-tagged survivin (K129Q)	Cloning [PCR template]	Cecilia Vallet
pC3-myc-Surv-K129R	myc-tagged survivin (K129R)	Cloning [PCR template]	Cecilia Vallet
pET41b-GST-Crm1 [1266]	GST-PreSc-Crm1 fusion protein	Cloning [PCR template]	Sandra Bäcker

2.5 Enzymes

Enzymes which were used in the present work are listed below.

Table 5 List of enzymes including enzyme class, kit and provider.

Enzyme class	Designation	Kit	Provider
Ligase	T4 DNA ligase	TaKaRa DNA Ligation Kit Ver. 2.1	TaKaRa Bio Company
	Ligase	Duolink® Detection Reagents Orange in Situ	OLINK BioScience (Sigma)
Polymerase	Expand High Fidelity Enzyme Mix [Taq DNA pol.+ Tgo DNA pol.]	Expand High Fidelity PCR system	Roche Diagnostics GmbH
	Polymerase	Duolink® Detection Reagents Orange in Situ	OLINK BioScience (Sigma)
	BamHI-HF®	-	New England BioLabs®

Restriction endonucleases	EcoRI-HF	-	New England BioLabs®
	NotI-HF	-	New England BioLabs®
Peptidases	TrypLE™ Express	-	gibco® by life technologies
Proteases	Proteinase K	NucleoSpin® tissue	Macherey-Nagel

2.6 Antibodies

Antibodies which were used in the present work are listed below.

Table 6 List of primary (Ab_1) and secondary antibodies (Ab_2).

Designation	Origin	Dilution factor	Application	Provider
α-myc	mouse	1:1,000	PLA and IF [primary antibody]	Cell Signalling Technology®
α-Crm1	rabbit	1:1,000	PLA and IF [primary antibody]	Novus Biologicals
α-mouse IgG Alexa Fluor® 488	goat	1:10,000	IF [secondary antibody]	Invitrogen/Thermo Fisher Scientific
α-rabbit IgG Alexa Fluor® 568	goat	1:10,000	IF [secondary antibody]	Invitrogen/Thermo Fisher Scientific

2.7 Organisms

Bacterial strains

For cloning approaches, *Escherichia coli* XL2-Blue™ (Stratagene, USA) were used. In general, growth of bacteria occurred at 37 °C in LB media or on LB-agar plates containing 100 µg/ml carbenicillin. The respective genotype is listed below.

Table 7 List of bacterial strains.

E. coli strain	Genotype
XL2-Blue™	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F*' proAB lacI*^{q}*ZΔM15 Tn10* (Tet^{r}) Amy Cam^{r}]

Eukaryotic cell lines

In the context of FRET interaction studies, HeLa Kyoto cells were used. Proximity Ligation and Apoptosis Assays were performed in Flp-In™ T-Rex™ HeLa cells. An overview of the respective cell lines is shown below.

Table 8 List of eukaryotic cell lines.

Cell line	ID	Origin
HeLa Kyoto	(RRID) CVCL_1922	Epithelial cervix-adenocarcinoma (*Homo sapiens*)
Flp-In™ T-REx™ HeLa (derived from HeLa.P3)	(JRCB) 0649.1	

Flp-In® T-Rex HeLa cell line

The Flp-In® T-Rex HeLa cell line was generated via usage of a site-specific Flp-Recombinase/FRT system provided by the company invitrogen.

The Flp-Recombinase specifically targets the 34 bp Flp-Recombination target (FRT) sequence within the parental host cell genome and catalyzes a recombinational event (**Figure 16**).

Figure 16 Molecular insight into 34 bp Flp-Recombinase target (FRT) site. Recognition of target region by Flp-Recombinase is achieved by two 14 bp repeats, whereas double-strand breakage and re-ligation occurs in the enclosed 8 bp linker area (indicated by triangles).

The Flp-In™ T-Rex™ system is commonly used to generate stable and inducible mammalian cell lines. For a successful genomic integration of the gene of interest three different components were required: (1) Flp-In host cell line, (2) pcDNA5 vector and (3) pOG44. In general, co-transfection of both plasmids followed by diverse selection steps are sufficient to generate a desired cell line within four weeks (**Figure 17**).

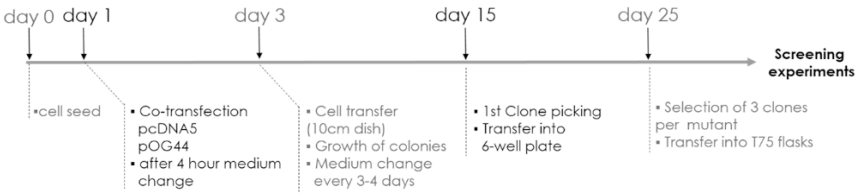

Figure 17 General timeline concerning genomic integration of mutational survivin sequences into HeLa host cell line. After cell were seeded (day 0), co-transfection with pcDNA5-myc-Surv (WT, F101A+L102A, K129A, K129E, K129Q, K129R) and pOG44 (encoding Flp-Recombinase) was performed (day 1). On day 3, cells were transferred into 10 cm culture dishes and fed with selective medium every 3-4 days until visible colonies were grown. On day 15, six clones of each mutant were picked and transferred into a 6-well plate. Selection was achieved by use of DMEM containing 15 µg/ml blasticidin and 400 µg/ml hygromycin. On day 25, three clones of each mutant were transferred into T75 culturing flasks and grown for screening experiments. Clones were tested for Zeocin sensitivity, lack of β-Galactosidase activity and expression of myc-tagged survivin mutants.

In short, the parental host cell line (provided by AG Meyer) offers one FRT-site while a second FRT-site is co-located on a transfer-plasmid with the gene of interest (here: pcDNA5^{TM}/FRT-Survivin constructs). In addition, parental host cell line exhibits a blastidicin (*bsd*) resistance gene which maintains throughout the entire process. Upon co-transfection with the GOI-containing transfer plasmid and the Flp-Recombinase coding pOG44 vector, genomic integration of survivin was achieved. Due to a start codon (ATG) shift, a hygromycin resistance cassette gets functional and was used to select positive clones. Therefore, the stable Flp-In® HeLa cell line was grown in selective media containing 15 µg/ml blasticidin and 400 µg/ml hygromycin. Stable expression of the respective survivin mutant was under control of a modified cytomegalovirus (CMV) promotor into which two tandem copies of the tet operator sequence ($TetO_2$) were inserted. Accordingly, expression was induced by addition of tetracycline (Tet-On system).[132,133]

2.8 Consumables

Consumables which were used in the present work are listed below.

Table 9 List of consumables.

Designation	Provider
35 mm petri dish, 10 mm Microwell No. 1.5 coverglass (0.16- 0.19 mm)	MatTek Corporation
1µ-Slide 8 Well IbiTreat	ibidi GmbH
6 well cultivation plate	Sarstedt AG & Co.
Pipettes (2 ml, 5 ml, 10 ml, 25 ml)	Sarstedt
T75 cell culture flask	Sarstedt AG & Co. KG
96 Well Assay Plate (Black Plate, Clear Bottom with Lid, Tissue Culture Treated Polystyrene)	Corning Inc. costar®
ML10542 Solution Basin	Moonlab plastics®
PCR tubes	BioRad Laboratories GmbH
Micro reaction tubes (1.5 ml, 2 ml)	Sarstedt Ag & Co.

Erlenmeyer flask 50 ml	Technische Glaswerke Ilmenau GmbH
Erlenmeyer flask 500 ml	DURAN Group GmbH

2.9 Kits

In the present work diverse kits were used which are listed below.

Table 10 List of kits.

Designation	Provider
NucleoBond® Xtra Midi/Maxi	Macherey-Nagel GmbH & Co. KG
Nucleo Spin® Plasmid	Macherey-Nagel GmbH & Co. KG
Nucleo Spin® Gel and PCR clean up	Macherey-Nagel GmbH & Co. KG
NucleoSpin® Tissue kit	Macherey-Nagel GmbH & Co. KG
ApoLive-Glo™ Multiplex Assay	Promega
TaKaRa DNA Ligation Kit Ver. 2.1	TaKaRa Bio Company
Expand High Fidelity PCR system	Roche Diagnostics GmbH
Duolink® In Situ PLA probe anti-Rabbit PLUS	OLINK Bioscience (Sigma)
Duolink® In Situ PLA probe anti-Mouse MINUS	OLINK Bioscience (Sigma)
Duolink® Detection reagents Orange In Situ	OLINK Bioscience (Sigma)

2.10 Instruments

Instruments which were used in the present work are listed below.

Table 11 List of instruments.

Designation	Provider
Allegra™ X-22R Centrifuge	Beckmann Coulter™
Confocal microscope SP8	Leica Microsystems GmbH
Centrifuge ROTINA 380/380 R	Hettich GmbH & Co. KG
Centrifuge 5417R	eppendorf
Heraeus Fresco21 Centrifuge	Thermo Scientific
CO_2 Incubator	Binder GmbH
Epifluorescence microscope Olympus-CKX41	Olympus Europe SE & Co. KG

Heating plate	MEDAX GmbH & Co. KG
Thermomixer comfort	Eppendorf GmbH
Vacuum safety aspirator AZ 02	HLC BioTech
Voltage supply source peqPower 300	Peqlab Biotechnologie GmbH
Vortexer Vortex Genie 2	Scientific Industries
Microscope Primo Vert	Zeiss
Agarose chamber	Peqlab Biotechnologie GmbH
Standard analog shaker	VWR®
ST5 CAT	Neo Lab Heidelberg
RS-TR05	Phoenix Instruments
GLOMAX Multi + Detection System	Promega
Freezer (-20 °C) Liebherr Premium Biofresh	Liebherr GmbH
Freezer Liebherr MEDline	Liebherr GmbH
Freezer (-80 °C) FORMA 900S-RIFS	Thermo Fisher Scientific
Gel documentation system E-Box VX2	Vilber Lourmat Deutschland GmbH
Safety clean bench HERAsafe	Thermo Fisher Scientific
Clean bench UV sterilizing PCR workstation	PeqLab Biotechnologie GmbH
Nanodrop Spectrophotometer ND-1000	PeqLab Biotechnologie GmbH
Microwave 800	Severin
Precision scales CP124S	Sartorius
Scales 440-21A	KERN®
Scales 440-47N	KERN®
Safety clean bench NuAire	Integra Biosciences GmbH
Thermocycler TProfessional standard gradient 96	Biometra GmbH
Agarose gel comb 12 well 1,5 mm	PeqLab Biotechnologie GmbH
Agarose gel comb 6 well 1,5 mm	PeqLab Biotechnologie GmbH
Tracable® Calibration Control	VWR®
Printer P95	Mitsubishi
Vortex Genie 2	Scientific Industries™
Mini centrifuge SPROUT™	Heathrow Scientific® LLC
Vacuum pump PC500 LAN NT	Vacuum brand
Water bath	GFC
Water bath ED	Julabo
Vornado™	Benchmark Scientific Inc.
Secuflow Airflow Controller AC3	Waldner
Magnetic stirrer RCT standard	IKA®
pH211 Microprocessor pH metre	Hanna Instruments
Pipettor pipetus®	Hirschmann Laborgeräte

2.11 Software

Software which was used in the present work is listed below.

Table 12 List of software.

Software	Provider
Canvas 11™	ACD Systems
Cell Profiler™	Broad Institute
ChemSketch 2012	Advanced Chemistry Development (ACD)
Clustal Omega	EMBL-EBI
ExPaSy	Swiss Institute of Bioinfromatics
Gene construction kit	Textco Biosoftware Inc.
GraphPad Prism 5	GraphPad Software
Instinct®	Promega
LAS X	Leica Microsystems CMS GmbH
Mendeley Desktop reference manager	Mendeley Ltd.
Office 2013	Microsoft Cooperation
PyMol	Schrödinger, LLC

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