6. Materials:

6.1. Chemicals

Chemicals	Source
Acetic Acid	Roth
Acetonitrile (HPLC grade)	Fluka
Acrylamide	Roth
Acrylamide (Molecular biology grade)	Sigma
Agar	Oxoid
Agarose	Fermentos
Ampicillin	Sigma
Amylose resin	NEB
APS (Amonium persulphate)	Sigma
ATP	Sigma
Beta-Mercaptoethanol	Sigma
Bromophenol Blue	Sigma
BSA	Sigma/Pierce
Capsaicin	Sigma
Capsazepine	Sigma
Compleate protease inhibitor	Merck
Coomassie Brilliant Blue G250	Serva
Cytochalasin B	Sigma
Digitonin	Calbiochem
DMS	Sigma
dNTPs	NEB
DTT	Sigma
EGTA	Sigma
Epothelion	Merck
Ethidium Bromide	Biomol
EZ-linked Biotin	Pierce/Molecular Probe
Fluoromount G	Southern Biotechnology
Formaldehyde	Roth
Glycerol	Roth
Glycine	Roth
GTP	Sigma
Iodoacetamide	Sigma
Hydrogen Chloride	Roth
Maltose	Sigma
Methanol	Roth
$MgCl_2$	Sigma
Nitrocellulose membrane	BioScience

NGS	Sigma
Nocodazole	Sigma
PFA	Roth
Phalloidin	Sigma
Phosphocellulose	Whatman
PIPES	Sigma
PMSF	Sigma
Ponceau S	Sigma
Potassium Hydroxide	Sigma
Protein G agarose	Amarsham
RTX	Sigma
Silver nitrate	Roth
Sodium bicarbonate	Roth
Sodium Chloride	Roth
Sodium Dodecyl Sulphate	Roth
Sodium Hydroxide	Sigma
Sodium thiosulphate	Merck
Streptavidin agarose	Sigma
Taxol	Sigma
TEMED	Sigma
Tris base	Roth
Triton X100	Roth
Tryptone	ICN
Tween 20	Sigma
Vincristin	Sigma
Vinblastin	Sigma
Whatman paper	Whattman
Xylene cynol	Sigma
Yeast extracts	ICN

6.2. KITS and Markers.

Kits and markers	**Source**
BCA protein estimation kit	Pierce
ECL	Amarsham
Gel extraction kit	Quiagen
Plasmid DNA isolation (maxi prep) kit	Quiagen
Plasmid DNA isolation (mini prep) kit	Quiagen
Lipofectamine Cell transfection kit	Invitrogen
SDS-PAGE protein marker High range	Sigma

SDS-PAGE pre-stained protein marker	BioRad
1kb DNA ladder	MBI
100bp DNA ladder	MBI
λHindIII Digest	Sigma
ϕx174DNA Hae III digest	Sigma

6.3. Purified proteins and fractions.

Purified proteins and fractions.	**Source**
Actin	Sigma
Neurofilaments	Kind gift by O. Bogen. (Bogen et al. 2005)
Synaptosome	Kind gift by O. Bogen. (Bogen et al. 2005)

6.4. Software

Software
Adobe Photoshop
Biodoc
LSM

6.5. Mammalian cell lines:

Mammalian cell lines:	**Source**
F11	Research Group of Prof F. Hucho.
***TRPV1-F11**	***Dr. B. Schwappach***
HaCaT	Research Group of Prof F. Hucho.
HeLa	Research Group of Prof F. Hucho.
HEK	Research Group of Prof F. Hucho.

* This cell line was prepared in Heidelberg by B Schwappach.

6.6. Bacterial cell lines:

Bacterial cell lines:	**Source**
BL21DE3pLys	Research Group of Prof F. Hucho.
DH5α	Research Group of Prof F. Hucho.
XL1b	Research Group of Prof F. Hucho.

6.7. Primary antibodies

Primary antibodies used.

Antibodies	*Host*	*Source*	*Applications*	*Dilution*♠
Acetylated tubulin (clone 611-B-1)	∗Mo	Sigma	IHC, WB	1000
Actin (clone JLA20)	∗Mo	AbCam	WB	500
α-tubulin	∗Mo	Boehringer	WB, IP	1000
α-tubulin (clone DM1A)	∗Mo	Sigma	IHC, WB	1000
β-tubulin (clone D66)	∗Mo	Sigma	IHC, WB	1000
β-tubulin	∗Mo	Boehringer	WB, IP	1000
β-III tubulin (clone SDL.3D10)	∗Mo	Sigma	IHC, WB	1000
Detyrosinated tubulin	♣Rb	Chemicon	IHC, WB	1000
Gamma tubulin (clone GTU88)	∗Mo	Sigma	IHC, WB	1000
GFP	♣Rb	AbCam	WB, IP	1000
MAP2a/b (clone AP20)	∗Mo	Sigma	IHC, WB	200
MBP	∗Mo	NEB	WB	20000
Neurofilament 200kD (clone RT97)	∗Mo	Chemicon	IHC, WB	1000
Neurofilament 160kD (clone NN18)	∗Mo	Sigma	IHC, WB	1000
Neuromodulin /GAP43 (clone 31)	∗Mo	BD	IHC, WB	1000
Polyglutamylated tubulin (clone B3)	∗Mo	Sigma	IHC, WB	500
Polyglycylated tubulin (clone TAP)	∗Mo	Bre et al. 1996	WB	1000
Polyglycylated tubulin (clone AXO)	∗Mo	Bre et al. 1996	WB	1000
TAU (clone Tau-1 PC1C6)	∗Mo	Chemicon	WB	1000
TRPV1	♣Rb	ABR	IHC, WB, IP	1000
TRPV1	♣Gt	Santa Cruze	IHC, WB, IP	1000
TRPV1	♣Gp	Fermos	WB	1000
Tyrosinated tubulin (clone TUB1A2)	∗Mo	Sigma	IHC, WB	1000
Tyrosinated tubulin (clone YL1/2)	∗Rat	AbCam	IHC, WB	1000

gt, goat; **mo**, mouse; **rb**, rabbit; **Gp**, Guinea pig;
∗ Monoclonal antibody; ♣ Polyclonal antibody.
IHC, Immuno Histochemistry; **WB**, Western Blot; IP, Immuno precipitation.
♠ With respect to Western Blot analysis.

6.8. Secondary antibodies and related reagents

Secondary antibodies and reagents for immunofluorescence:

Description	**Host**	**Source**	**Dilution**♠
Alexa-594-labelled phalloidin	--	Molecular Probe	1000
Alexa-488-labelled phalloidin	--	Molecular Probe	1000
Alexa-594-labelled anti-rabbit	Chicken	Molecular Probe	600

Alexa-594-labelled anti-mouse	Chicken	Molecular Probe	600
Alexa-594-labelled anti-rat	Chicken	Molecular Probe	600
Alexa-594-labelled anti-guinea pig	Chicken	Molecular Probe	1000
Alexa-488-labelled anti-rabbit	Chicken	Molecular Probe	1000
Alexa-488-labelled anti-mouse	Chicken	Molecular Probe	1000
Alexa-488-labelled anti-rat	Chicken	Molecular Probe	1000
Alexa-488-labelled anti-guinea pig	Goat	Molecular Probe	1000
Alexa-488-labelled anti-goat	Chicken	Molecular Probe	1000
Cy2-labelled anti-goat	Rabbit	Dianova	500
Cy2-labelled anti-rabbit	Goat	Dianova	500
Cy2-labelled anti-mouse	Goat	Dianova	500
Cy2-labelled anti-rat	Goat	Dianova	500
Propidium iodide.	--	Molecular Probe	1000

Secondary antibodies and reagents for western blot analysis:

Description	**Host**	**Source**	**Dilution**
HRP-labelled anti goat	Mouse	Pierce	1000
HRP labelled anti mouse	Sheep	Amarsham	1000
HRP labelled anti rat	Goat	Dianova	1000
HRP labelled anti rabbit	Donkey	Pierce	1000
HRP labelled anti guinea pig	Goat	Biomol	1000
HRP labelled avidin	--	Sigma	1000

6.9. Peptides.

Peptides.

Sequence	*Blocking activity against*	*Source*
$M^{1}EQRASLDSEESESPPQENSC^{21}$	Rabbit polyclonal anti-TRPV1antibody	Alexis
***P**^{823}EDAEVFKDSMV^{834}	Goat polyclonal anti-TRPV1 antibody	Santa Cruz Biotechnology

***** The sequence was determined by MALDI-MS analysis

6.10. Vectors.

Vectors	**Source**
pMALc2x	NEB
pCDNA3.1	
pDSRed-Monomer	Invitrogen

6.11. Enzymes

Enzymes.	Source
Restriction endonucleases	NEB
Taq DNA polymerase	NEB
Pfu DNA polymerase	MBI
High Fidelity DNA polymerase	Invitrogen
DNA ligase	NEB
Factor Xa protease	NEB
Lysozyme	Sigma
Benzonase	Merck

6.12. Constructs

Constructs used	Vector	Expression system	Source
MBP-TRPV1-Ct	pMALc2x	*E.Coli*	Jahnel R 2005
MBP-TRPV1-Nt	pMALc2x	*E.Coli*	Jahnel R 2005
MBP-TRPV1-CtΔ1	pMALc2x	*E.Coli*	This study
MBP-TRPV1-CtΔ2	pMALc2x	*E.Coli*	This study
MBP-TRPV1-CtΔ3	pMALc2x	*E.Coli*	This study
MBP-TRPV1-CtF1	pMALc2x	*E.Coli*	This study
MBP-TRPV1-CtF2	pMALc2x	*E.Coli*	This study
MBP-TRPV1-CtF3	pMALc2x	*E.Coli*	This study
MBP-TRPV1-CtF4	pMALc2x	*E.Coli*	This study
MBP-TRPV1-CtF5	pMALc2x	*E.Coli*	This study
MBP-TRPV1-CtF6	pMALc2x	*E.Coli*	This study
MBP-TRPV1-CtF7	pMALc2x	*E.Coli*	This study
MBP-TRPV1-CtF8	pMALc2x	*E.Coli*	This study
MBP-LacZ	pMALc2x	*E.Coli*	NEB
MBP	pMALc2x	*E.Coli*	This study
MBP-TRPV2-Ct	pMALc2x	*E.Coli*	Jahnel R 2005
MBP-TRPV4-Ct	pMALc2x	*E.Coli*	This study*
TRPV1	pCDNA3.1	Mammalian	Jahnel et al. 2001
TPRV1-Ct	pCDNA3.1	Mammalian	Jahnel R 2005
TRPV1-ΔNt (TRPV1-Ct+TM)	pCDNA3.1	Mammalian	Jahnel R 2005
TRPV1-Nt	pCDNA3.1	Mammalian	Jahnel R 2005
TRPV1ΔCt (TRPV1-Nt+TM)	pCDNA3.1	Mammalian	Jahnel R 2005
GFP-TRPV1	pCDNA3.1	Mammalian	Jahnel R 2005

TRPV1-GFP	pCDNA3.1	Mammalian	Jahnel R 2005
Tubulin-CFP	pCFP	Mammalian	Kai Simons
Tubulin-YFP	pYFP	Mammalian	Kai Simons
Actin-RFP	pDSRed-Monomer	Mammalian	BD-Clontech
Tubulin-RFP	pDSRed-Monomer	Mammalian	This study

∗ Prepared by Alejandra Perez Sastre in the research group of Prof. F. Hucho.
Other few constructs used in this study were prepared by Mark Hartman, Shu Liu and Linda Stewani.

6.13. Primers:

Number	Sequence	Use	Construct
1.	5’ GAATTCGGTACCTGAAAGCTT 3’	**F/L**	**MBP**
	5’ CTTAAGCCATGGACTTTCGAA 3’	**R/L**	**MBP**
2.	5’ CCG*GAATTC*CTCATGGGTGAGACCGTCAAC 3’	**F**	**MBP-TRPV1-Ct-Δ1**
	5’ CCC*AAGCTT*TTAGCTTGCATCCCTCAGAAGGGG 3’	**R**	
3.	5’ CCG*GAATTC*CTCATGGGTGAGACCGTCAAC 3’	**F**	**MBP-TRPV1-Ct-Δ2**
	5’ CCC*AAGCTT*TTAGTTGATGATACCCACATTGGT 3’	**R**	
4.	5’ CCG*GAATTC*CTCATGGGTGAGACCGTCAAC 3’	**F**	**MBP-TRPV1-Ct-Δ3**
	5’ CCC*AAGCTT*TTAGAACCCCACCTGCAGCAGCTT 3’	**R**	
5.	5’ CCG*GAATTC*CTCATGGGTGAGACCGTCAAC 3’	**F**	**MBP-TRPV1-Ct-F1**
	5’ CCC*AAGCTT*TTACTCTGTATCCAGGATGGTGAT 3’	**R**	
6.	5’ CCG*GAATTC*AAGAGCTTCCTGAAGTGCATG 3’	**F**	**MBP-TRPV1-Ct-F2**
	5’ CCC*AAGCTT*TTAGAACCCCACCTGCAGCAGCTT 3’	**R**	
7.	5’ CCG*GAATTC*ACTCCTGACGGCAAGGATGAC 3’	**F**	**MBP-TRPV1-Ct-F3**
	5’ CCC*AAGCTT* TTAGACGCCCTCACAGTTGCCTGG 3’	**R**	
8.	5’ CCG*GAATTC*AAGCGCACCCTGAGCTTCTCC 3’	**F**	**MBP-TRPV1-Ct-F4**
	5’ CCC*AAGCTT*TTACCTCAGAAGGGGAACCAGGGC 3’	**R**	
9.	5’ CCGGAATTCACTCGAGATAGACATGCCACC 3’	**F**	**MBP-TRPV1-Ct-F5**
	5’ CCCAAGCTTTTATTTCTCCCCTGGGACCATGGA 3’	**R**	
10.	5’ CCG*GAATTC*GAGGACCCAGGCAACTGTGAG 3’	**F**	**MBP-TRPV1-Ct-F6**

	5’ CCCAAGCTTTTATTTCTCCCCTGGGACCATGGA 3’	**R**	
11.	5’ CCG*GAATTC*ACTCCTGACGGCAAGGATGAC 3’	**F**	**MBP-TRPV1-Ct-F7**
	5’ CCCAAGCTTTTATTTCTCCCCTGGGACCATGGA 3’	**R**	
12.	5’ CCG*GAATTC*AAGAGCTTCCTGAAGTGCATG 3’	**F**	**MBP-TRPV1-Ct-F8**
	5’ CCC*AAGCTT*TTACCTCAGAAGGGGAACCAGGGC 3’	**R**	
13.	5’ TGACGAATTCATGGGTGAGACCGTGGGCCA 3’	**F**	**MBP-TRPV4-Ct∗**
	5’ TGACAAGCTTCTACAGTGGTGCGTCCTCCG 3’	**R**	

F, Forward primer; **R**, Reverse primer; **L**, Used as a linker; **S**, Used for sequencing of constructs sub-cloned in pMALc2X vector, Underlines indicate presence of stop codon. Sequence written in *Italic* font indicates the presence of a restriction site. All primers were purchased from MWG.

∗ Prepared by Alejandra Perez Sastre in the research group of Prof. F. Hucho.