Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 365 (2008) 154–157
BBRC
www.elsevier.com/locate/ybbrc

# $PrP^C$ interacts with tetraspanin-7 through bovine $PrP_{154–182}$ containing alpha-helix 1

Mingxiong Guo [b], Tao Huang [b], Yejian Cui [a], Baiqun Pan [a], Ao Shen [b], Yuting Sun [b], Yourong Yi [a], Yan Wang [a], Gengfu Xiao [b,*], Guihong Sun [a,*]

[a] *Key Laboratory of Allergy and Immune-related Diseases and Centre for Medical Research, School of Medicine, Wuhan University, Wuhan 430071, PR China*
[b] *The State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan 430072, PR China*

Received 17 October 2007
Available online 5 November 2007

**Abstract**

The cellular prion protein ($PrP^C$) is highly conserved in the evolution of mammals, and therefore, thought to have important cellular functions. Despite decades of intensive research, the physiological function of $PrP^C$ remains enigmatic. We carried out a yeast two-hybrid screen on a bovine brain cDNA expression library and identified the transmembrane protein tetraspanin-7 (CD231), as a $PrP^C$ interacting protein. We confirmed the interaction between $PrP^C$ and tetraspanin-7 by yeast two-hybrid assay, immunofluorescent co-localization, and immunocoprecipitation. Our mutational studies further demonstrated that $PrP^C$ specifically binds tetraspanin-7 through the region corresponding to bovine $PrP_{154–182}$ containing alpha-helix 1.


*Keywords:* Prion; $PrP^C$; Tetraspanin; Transmembrane protein; CD231

The prion diseases or transmissible spongiform encephalopathies (TSEs) are invariably fatal neurodegenerative diseases that include bovine spongiform encephalopathy (BSE) in cattle, and Creutzfeldt–Jacob disease (CJD) in human, scrapie in sheep [1]. Prion diseases have been subjected to much recent attention due to the epidemic of BSE and the subsequent emergence of its human form, variant CJD (vCJD).

$PrP^C$ is a highly conserved GPI-anchored sialoglycoprotein and expressed in neurons, glia, and a variety of non-neuronal tissues [2,3]. It is also distributed widely in the immune system, in haematopoietic stem cells and mature lymphoid and myeloid compartments [4]. However, the physiological function of this widely expressed prion protein is unclear. It has been proposed that $PrP^C$ is part of a multiprotein complex that modulates various cellular functions, depending on both protein compositions in the complex and cell type. Moreover, a variety of molecules have been proposed to be $PrP^C$ binding protein, such as mouse STLI [5,6], Synapsin, Grb-2, and Pint 1 [7], and human 37-kDa/67-kDa LRP [8,9]. It is yet unknown whether $PrP^C$ functions through these binding proteins. Thus, attempts have been made in order to look for new $PrP^C$ binding proteins that may provide new insights into its physiological and pathological functions.

We employed a yeast two-hybrid approach to search for new potential cellular prion protein interacting partners by using a brain expression library. A novel $PrP^C$ interacting protein, tetraspanin-7 (also referred to as CD231, TALLA-1, A15, CCG-B7, or TM4SF2) [10–12], was identified. Our results showed $PrP^C$ interacts with tetraspanin-7 through the peptide region bovine $PrP_{154–182}$ containing alpha-helix 1.

*Abbreviations:* TSE, Transmissible spongiform encephalopathies; BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jacob disease.

* Corresponding authors. Fax: +86 27 68752897 (G. Xiao); +86 27 68759142 (G. Sun).
*E-mail addresses:* gxiao@whu.edu.cn (G. Xiao), ghsungsr@163.com (G. Sun).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.10.160

## Materials and methods

*Yeast two-hybrid screen. Saccharomyces cerevisiae* strain PJ69-4A cells, and vectors pGBKT7 and pGADT7, were obtained from Clontech. The bovine brain cDNA library (Uni-ZAP XR Library) was purchased from Stratagene and the cDNA fragments were ligated into pGADT7 at the EcoRI–XhoI sites, resulting in pGADT7-cDNA library as the prey. The gene encoding mature bovine $PrP^C$ (25–242) was amplified by polymerase chain reaction (PCR) using bovine genomic DNA as the template and then cloned into pGBDKT7 vector via NdeI–EcoRI restriction sites, yielding pGBDKT7-$PrP^C$ as the bait. The yeast two-hybrid Gal4 interaction trap assayed was done as previously described [13].

*Plasmid construction.* For the construction of fusion proteins with fluorescent tags, commercial pEGFP-N1 and pDsRed-N1 (Clontech) vectors were used. For the pEGFP-N1/tetraspanin-7 construct, the tetraspanin-7 coding region was amplified from bovine brain cDNA library, and cloned upstream of the EGFP gene in the pEGFP-N1 plasmid by using EcoRI/BamHI restriction sites. For the construct pDsRed/$PrP^C$ and pcDNA3/$PrP^C$, $PrP^C$ (25–242) coding region was cloned into the pDsRed-N1 and pcDNA3/$PrP^C$, respectively, by using the EcoRI/BamHI sites.

*Cell cultures and transfections.* HeLa cells were cultured at 37 °C, 5% $CO_2$, in DMEM supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. Cell transfections were performed with Lipofectamine$^{TM}$ 2000 (Invitrogen) according to the manufacturer's instructions, and the cells were analyzed for 2 days after transfection.

*Immunofluorescent co-localization.* The fluorescent vectors pDsRed/$PrP^C$ and pEGFP-N1/tetraspanin-7 were transiently co-transfected in HeLa cells. After 48 h, the fluorescent signals were analyzed with the Leica TCS-AOBS-SP2-MP laser confocal microscope.

*Western blot and co-immunoprecipitation.* The expression vectors pcDNA3/$PrP^C$, pEGFP-N1/tetraspanin-7, and pEGFP were transiently transfected into HeLa cells either individually or in combination. The transfected cells were washed with PBS, and resuspended in an ice-cold lysis buffer (Beyotime institute of Biotechnology). The cells were then lysed on ice for 2 min. Subsequently they were centrifuged at 12,000*g* for 15 min at 4 °C. The supernatants from cells transfected with individual plasmid were analyzed by Western blotting with antibodies against PrP or GFP (Beyotime institute of Biotechnology). The co-transfected lysates (2–$3 \times 10^7$ cells/ml) were cleared by centrifugation and PrP-specific polyclonal anti-PrP antibody was added and incubated at 4 °C for 2–4 h. Protein G-Plus Agarose beads (Beyotime institute of Biotechnology) were added and incubated for 2 h at 4 °C. Proteins isolated from the beads were transferred onto NC membrane and EGFP-tetraspanin-7 fusion protein or EGFP protein was detected with EGFP antiserum and anti-rabbit HRP secondary antibody.

*Characterization of the interacting domains of the proteins by using yeast two-hybrid assay.* Different PrP deletion constructs were made with the pGBKT7 plasmid. These included pGBKT7/PrP(25–242), pGBKT7/PrP(51–242), pGBKT7/PrP(103–242), pGBKT7/PrP(154–242), pGBKT7/PrP(185–242), pGBKT7/PrP(212–242), pGBKT7/PrP(25–53), pGBKT7/PrP(25–103), pGBKT7/PrP(25–136), and pGBKT7/PrP(25–182). All were made by using primer pairs containing NdeI–EcoRI restriction sites (Fig. 4). The different deletion bait plasmids and the prey plasmids were co-transformed into yeast.

## Results and discussion

To identify proteins that physically interact with the bovine prion protein, we performed a yeast two-hybrid screen by using a bovine brain library. This revealed that several yeast clones had positive signals in the assay. The bait plasmid (pGBDKT7-$PrP^C$) and the empty vectors (pGBKT7 and pGADT7), when individually tested for self-activation of the reporter cassette, in the absence of interacting partners, produced no activation and thus confirmed the specificity of the interactions in the positive clones. The pGADT7-cDNA plasmids isolated from the positive clones were transformed into *Escherichia coli* for amplification and DNA purification. The purified DNA from the positive clones was then verified by yeast two-hybrid assay. Sequencing of the isolated positive plasmids showed that several of these positive clones encode the bovine tetraspanin-7 protein (NM_001076384, from nucleotides 72–552). Noticeably, tetraspanin-7 protein is well conserved between bovine and *Homo sapiens*, showing 99% of nucleotide sequence identity. Fig. 1D shows that tetraspanin-7 had no auto-activity in the yeast two-hybrid assay and Fig. 1E shows that tetraspanin-7 interacted with $PrP^C$.

Tetraspanin-7 is a member of the tetraspanin family proteins which are highly expressed in the central nervous system, including the cerebral cortex and hippocampus [14]. Tetraspanin-7 is a membrane protein containing four transmembrane domains [15]. PrP is also a membrane protein with a glycosylphosphatidylinositol membrane anchor. These suggest that tetraspanin-7 and $PrP^C$ may interact with each other on the membrane. To further confirm whether $PrP^C$ and tetraspanin-7 interact with each other, we first carried out co-localization studies on $PrP^C$ and tetraspanin-7 in mammalian cells. We produced vectors

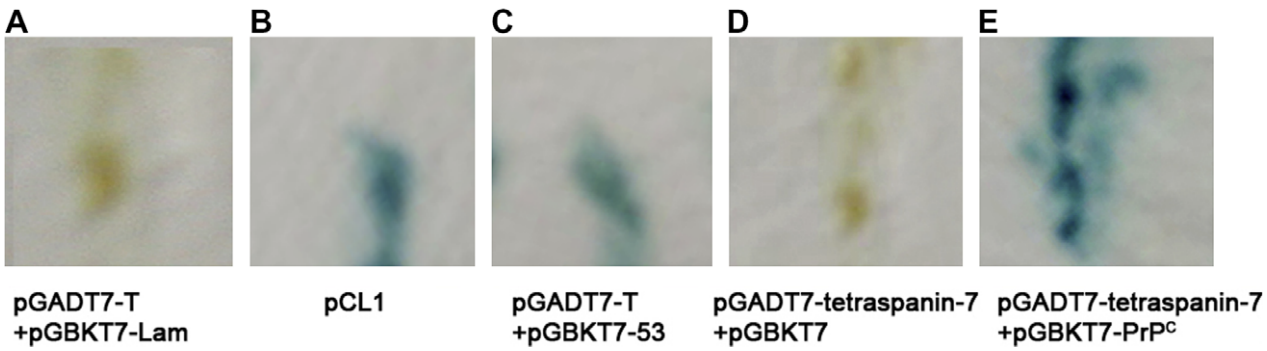


Fig. 1. $PrP^C$ and tetraspanin-7 interact with each other in the yeast two-hybrid assay as detected by the β-galactosidase activities. (A) Negative control, human lamin C did not interact with SV40 large T-antigen. (B) Positive control, pCL1 encoding full-length, wild-type GAL4 protein. (C) Positive control, murine P53 interacted with SV40 large T-antigen. (D) No auto-activity was found in pGADT7-tetraspanin-7. (E) $PrP^C$ interacted with tetraspanin-7.

for fluorescent fusion proteins of the two interacting proteins (pDsRed-N1/$PrP^C$ and pEGFP-N1/tetraspanin-7). The expression vectors were co-transfected transiently into HeLa cells. Confocal microscopic analysis revealed that $PrP^C$ and tetraspanin-7 proteins were co-localized in mammalian cells (Fig. 2).

Next, co-immunoprecipitation assays were carried out. As shown in Fig 3, GFP antiserum recognized EGFP (lane 1) and tetraspanin-7 fusion EGFP (lane 2); PrP antibody recognized PrP protein (lane 5) in Western blot using HeLa cell lysates. When the lysates were immunoprecipitated with the PrP antiserum followed by Western blot with the anti-EGFP antibody, which recognizes the EGFP-tetraspanin-7 fusion protein. The results showed that tetraspanin-7 was co-immunoprecipitated (the upper band in lane 3), indicating that tetraspanin-7-$PrP^C$ proteins interact with each other in vivo (Fig. 3).

To map the tetraspanin-7 interaction domain, deletion mutants of $PrP^C$ were made and analyzed by using the yeast two-hybrid assay. The results indicate that tetra-

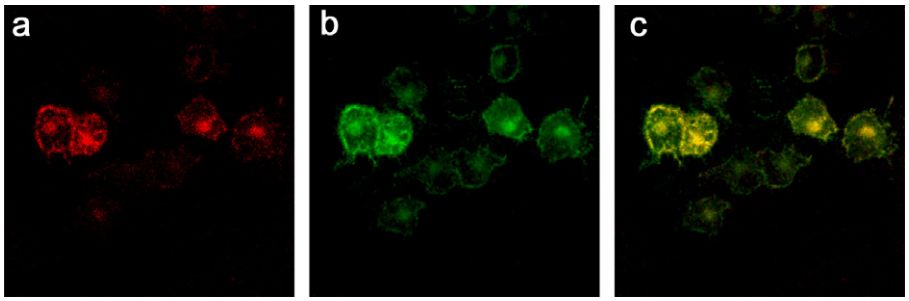


Fig. 2. Co-localization of $PrP^C$ and tetraspanin-7 fusion proteins. $PrP^C$ and tetraspanin-7 fusion proteins had red (a) and green (b) colors, respectively, and were co-localizated as demonstrated by yellow color in the overlaid image (c). (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

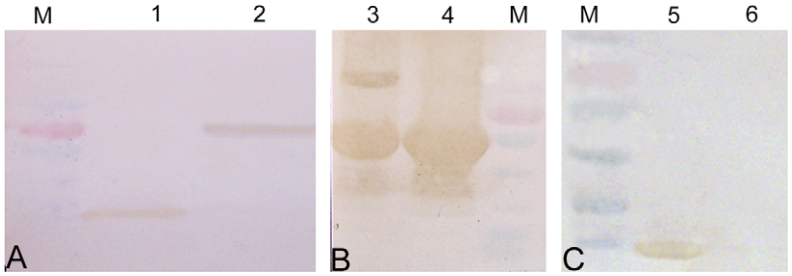


Fig. 3. Co-immunoprecipitation of $PrP^C$ and tetraspanin-7 from transfected HeLa cells. (A) Western blot analyses were performed with anti-GFP. EGFP (lane 1) and EGFP-tetraspanin-7 (lane 2) were specifically detected. (B) HeLa cells were co-transfected with pcDNA3/$PrP^C$ and pEGFP-N1/tetraspanin-7 or pcDNA3/$PrP^C$ and pEGFP. The extract was immunoprecipitated with anti-PrP antibody followed by Western blot with anti-EGFP antibody. Note that EGFP-tetraspanin-7 was co-immunoprecipitated (the upper band in lane 3, the lower band is PrP antibody heavy chain) while EGFP was not (lane 4, the lower band matches the lower band in lane 3). (C) Western blot analyses were performed with anti-PrP antibody, showing the specific recognition of $PrP^C$ (lane 5). The negative control is shown in lane 6.

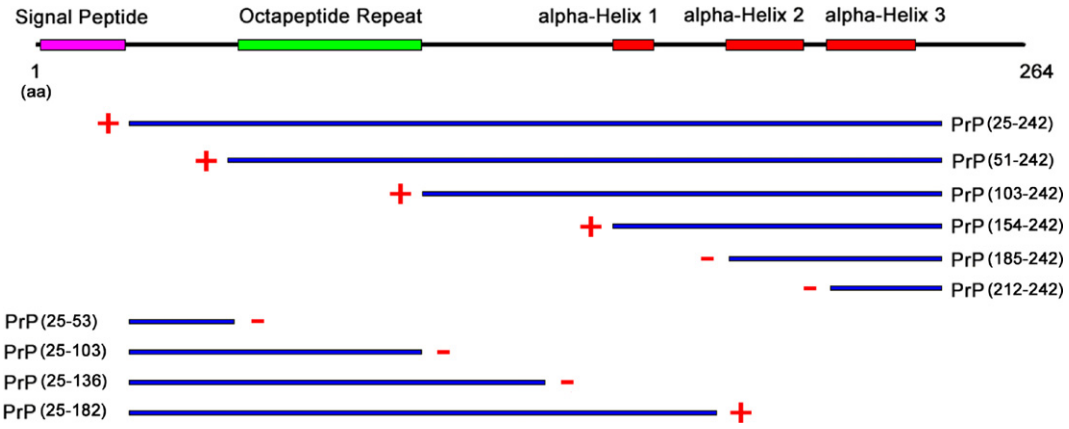


Fig. 4. Schematic representation of the $PrP^C$ deletion mutants and their interaction with tetraspanin-7 as detected by using yeast two-hybrid assay. +, positive interaction; −, no interaction. The result shows that $PrP^C$ binds tetraspanin-7 through the peptide region $PrP_{154–182}$ containing the alpha-helix 1.

spanin-7 binds the peptide region $PrP_{154–182}$ in bovine $PrP^{C}$ that contains the alpha-helix 1 domain (Fig. 4). The alpha-helix 1 is known to play a important role in prion aggregation [16,17]. Thus, it will be of interest in the future to determine whether the interaction between tetraspanin-7 and $PrP^{C}$ affects prion aggregation process.

Although the prion protein is expressed widely in the immune system, including haematopoietic stem cells, mature lymphoid and myeloid compartments [4], $PrP^{-/-}$ mice have been reported to have only minor abnormality in immune function. For this reason, the field of TSEs has not attracted much attention [18]. On the other hand, recently, it has been suggested that the lack of a clear immune response in prion disease is likely be due to tolerance to $PrP^{Sc}$. Furthermore, the immune system contributes to pathogenesis by amplifying prion in lymphoid compartments, thereby facilitating efficient neuroinvasion [4,19]. In addition, incubation of T cells with anti-PrP monoclonal antibodies for >10 min results in internalization of a large proportion of surface PrP into Limp-2 positive endosomes[20]. Our discovery here that PrP binds to the immune molecule tetraspanin-7 may help to understand how immune cells respond to PrP. The tetraspanin-family proteins are evolutionally conserved. They regulate a variety of normal and pathological processes such as immunological responses, protein trafficking, cell adhesion, virus invasion, and cancer metastasis through formation of a network of multi-molecule complexes via interactions among tetraspanins and between tetraspanins and other proteins [21,22]. Of interest, tetraspanin-7(CD231) is associated with X-linked mental retardation and neuropsychiatric diseases such as Huntington's chorea [14]. The finding that PrP interacts with tetraspanin raises the possibility that the interaction may have a role in TSEs immune response or efficient transportation of prion protein by tetraspanin. Whether the association between $PrP^{C}$ and tetraspanin can affect immunological responses to influence neuroinvasion awaits further study.

## Acknowledgments

We thank Dr. Yun-Bo Shi (NICHD, NIH), Dr. Wenwu Cui (NCBI, NIH) for critical reading of the manuscript. This work was supported by grants from National Natural Science Foundation of China (Grant No. 30770078) and grants from Health Department of Hubei Province Foundation of China (Grant No. 301140471).

## References

[1] S.B. Prusiner, Prions, Proc. Natl. Acad. Sci. USA 95 (1998) 13363–13383.
[2] H.A. Kretzschmar, S.B. Prusiner, L.E. Stowring, S.J. DeArmond, Scrapie prion proteins are synthesized in neurons, Am. J. Pathol. 122 (1986) 1–5.
[3] T. van Rheede, M.M. Smolenaars, O. Madsen, W.W. de Jong, Molecular evolution of the mammalian prion protein, Mol. Biol. Evol. 20 (2003) 111–121.
[4] J.D. Isaacs, G.S. Jackson, D.M. Altmann, The role of the cellular prion protein in the immune system, Clin. Exp. Immunol. 146 (2006) 1–8.
[5] L.B. Chiarini, A.R. Freitas, S.M. Zanata, R.R. Brentani, V.R. Martins, R. Linden, Cellular prion protein transduces neuroprotective signals, EMBO J. 21 (2002) 3317–3326.
[6] S.M. Zanata, M.H. Lopes, A.F. Mercadante, G.N. Hajj, L.B. Chiarini, R. Nomizo, A.R. Freitas, A.L. Cabral, K.S. Lee, M.A. Juliano, E. de Oliveira, S.G. Jachieri, A. Burlingame, L. Huang, R. Linden, R.R. Brentani, V.R. Martins, Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection, EMBO J. 21 (2002) 3307–3316.
[7] C. Spielhaupter, H.M. Schatzl, PrPC directly interacts with proteins involved in signaling pathways, J. Biol. Chem. 276 (2001) 44604–44612.
[8] S. Gauczynski, J.M. Peyrin, S. Haik, C. Leucht, C. Hundt, R. Rieger, S. Krasemann, J.P. Deslys, D. Dormont, C.I. Lasmezas, S. Weiss, The 37-kDa/67-kDa laminin receptor acts as the cell-surface receptor for the cellular prion protein, EMBO J. 20 (2001) 5863–5875.
[9] C. Hundt, J.M. Peyrin, S. Haik, S. Gauczynski, C. Leucht, R. Rieger, M.L. Riley, J.P. Deslys, D. Dormont, C.I. Lasmezas, S. Weiss, Identification of interaction domains of the prion protein with its 37-kDa/67-kDa laminin receptor, EMBO J. 20 (2001) 5876–5886.
[10] N. Emi, K. Kitaori, M. Seto, R. Ueda, H. Saito, T. Takahashi, Isolation of a novel cDNA clone showing marked similarity to ME491/CD63 superfamily, Immunogenetics 37 (1993) 193–198.
[11] E. Ito, R. Honma, J. Imai, S. Azuma, T. Kanno, S. Mori, O. Yoshie, J. Nishio, H. Iwasaki, K. Yoshida, J. Gohda, J. Inoue, S. Watanabe, K. Semba, A tetraspanin-family protein, T-cell acute lymphoblastic leukemia-associated antigen 1, is induced by the Ewing's sarcoma-Wilms' tumor 1 fusion protein of desmoplastic small round-cell tumor, Am. J. Pathol. 163 (2003) 2165–2172.
[12] S.H. Li, M.G. McInnis, R.L. Margolis, S.E. Antonarakis, C.A. Ross, Novel triplet repeat containing genes in human brain: cloning, expression, and length polymorphisms, Genomics 16 (1993) 572–579.
[13] G. Sun, M. Guo, A. Shen, F. Mei, X. Peng, R. Gong, D. Guo, J. Wu, P. Tien, G. Xiao, Bovine PrPC directly interacts with alphaB-crystalline, FEBS Lett. 579 (2005) 5419–5424.
[14] R. Zemni, T. Bienvenu, M.C. Vinet, A. Sefiani, A. Carrie, P. Billuart, N. McDonell, P. Couvert, F. Francis, P. Chafey, F. Fauchereau, G. Friocourt, V. des Portes, A. Cardona, S. Frints, A. Meindl, O. Brandau, N. Ronce, C. Moraine, H. van Bokhoven, H.H. Ropers, R. Sudbrak, A. Kahn, J.P. Fryns, C. Beldjord, J. Chelly, A new gene involved in X-linked mental retardation identified by analysis of an X-2 balanced translocation, Nat. Genet. 24 (2000) 167–170.
[15] C.S. Stipp, T.V. Kolesnikova, M.E. Hemler, Functional domains in tetraspanin proteins, Trends Biochem. Sci. 28 (2003) 106–112.
[16] M.P. Morrissey, E.I. Shakhnovich, Evidence for the role of PrP(C) helix 1 in the hydrophilic seeding of prion aggregates, Proc. Natl. Acad. Sci. USA 96 (1999) 11293–11298.
[17] J. Watzlawik, L. Skora, D. Frense, C. Griesinger, M. Zweckstetter, W.J. Schulz-Schaeffer, M.L. Kramer, Prion protein helix1 promotes aggregation but is not converted into beta-sheet, J. Biol. Chem. 281 (2006) 30242–30250.
[18] L.J. Berg, Insights into the role of the immune system in prion diseases, Proc. Natl. Acad. Sci. USA 91 (1994) 429–432.
[19] A. Aguzzi, Prions and the immune system: a journey through gut, spleen, and nerves, Adv. Immunol. 81 (2003) 123–171.
[20] C.A. Stuermer, M.F. Langhorst, M.F. Wiechers, D.F. Legler, S.H. Von Hanwehr, A.H. Guse, H. Plattner, PrPc capping in T cells promotes its association with the lipid raft proteins reggie-1 and reggie-2 and leads to signal transduction, FASEB J. 18 (2004) 1731–1733.
[21] F. Berditchevski, Complexes of tetraspanins with integrins: more than meets the eye, J. Cell. Sci. 114 (2001) 4143–4151.
[22] S. Levy, T. Shoham, Protein–protein interactions in the tetraspanin web, Physiology (Bethesda) 20 (2005) 218–224.