Cancer and Metastasis Reviews (2020) 39:455–469
https://doi.org/10.1007/s10555-020-09892-9

Tyrosine phosphorylation of tumor cell caveolin-1: impact on cancer progression

Timothy H. Wong[1] · Fiona H. Dickson[1] · Logan R. Timmins[1] · Ivan R. Nabi[1]

Published online: 21 May 2020

Abstract
Caveolin-1 (CAV1) has long been implicated in cancer progression, and while widely accepted as an oncogenic protein, CAV1 also has tumor suppressor activity. CAV1 was first identified in an early study as the primary substrate of Src kinase, a potent oncoprotein, where its phosphorylation correlated with cellular transformation. Indeed, CAV1 phosphorylation on tyrosine-14 (Y14; pCAV1) has been associated with several cancer-associated processes such as focal adhesion dynamics, tumor cell migration and invasion, growth suppression, cancer cell metabolism, and mechanical and oxidative stress. Despite this, a clear understanding of the role of Y14-phosphorylated pCAV1 in cancer progression has not been thoroughly established. Here, we provide an overview of the role of Src-dependent phosphorylation of tumor cell CAV1 in cancer progression, focusing on pCAV1 in tumor cell migration, focal adhesion signaling and metabolism, and in the cancer cell response to stress pathways characteristic of the tumor microenvironment. We also discuss a model for Y14 phosphorylation regulation of CAV1 effector protein interactions via the caveolin scaffolding domain.

Keywords Tyrosine phosphorylation · Caveolin-1 (CAV1) · Cancer progression · Src kinase · Caveolae · Focal adhesions

1 Src kinase and the discovery of caveolin-1

Tyrosine kinases are key regulators of signaling events related to cell proliferation, differentiation, and apoptosis and as such, key instigators in cancer progression. Src kinase, a non-receptor tyrosine kinase, was first discovered as the protein responsible for transformation and maintenance of a neoplastic state in fibroblasts infected with avian sarcoma virus [1]. Excitingly at the time, the *v*-Src protein was discovered to have kinase activity suggesting a possible mechanism of transformation [2, 3], and shortly thereafter, targets of Src kinase were identified using anti-phosphotyrosine antibodies [4] (see [5] for historical review of the discovery of Src). Interestingly, caveolin-1 (CAV1) was identified as one of the primary tyrosine-phosphorylated substrates in Rous sarcoma virus-infected chick embryo fibroblasts; this 22 kDa target of *v*-Src kinase localized to puncta by immunofluorescence that were later identified as caveolae [6, 7]. This marked the discovery of CAV1. Moreover, CAV1 was the only Src kinase substrate identified whose phosphorylation, later identified at residue tyrosine 14 (Y14; pCAV1), correlated with cellular transformation [6, 8].

These early experiments were among the first to expose the role of tyrosine phosphorylation in cellular transformation and lead to what we know now as a complex signaling cascade that drives tumor formation and malignancy. Additionally, they placed CAV1 and, more specifically, CAV1 phosphorylation as potential regulators of cellular transformation and tumor progression for the first time. While CAV1 and Src kinase have since been widely implicated in tumorigenesis [9, 10], the specific role of pCAV1 in cancer has been difficult to determine. Here, we review the literature surrounding Src-dependent phosphorylation of tumor cell CAV1 focusing on pCAV1-dependent processes associated with cancer progression.

2 CAV1, caveolae, and scaffolds

CAV1 is an integral membrane protein that forms the coat of plasma membrane caveolae [11]; CAV1 associates with membranes via a small hairpin insert as well as three palmitoylation

Timothy H. Wong and Fiona H. Dickson contributed equally to this work.

✉ Ivan R. Nabi
irnabi@mail.ubc.ca

[1] Life Sciences Institute, Department of Cellular and Physiological Sciences, School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada

sites (Cys133/143/156) with its N- and C-termini exposed to the cytoplasm (Fig. 1) [12, 13]. Palmitoylation, the reversible post-translational addition of a 16-carbon lipid chain that enhances protein hydrophobicity, is not necessary for formation or localization of CAV1 to caveolae [13]. CAV1 palmitoylation is mediated by the DHHC7 and DHHC21 palmitoyl acyltransferases, inhibited by oxidative stress and reported to be irreversible as CAV1 embedding in the membrane prevents access and modification to the palmitoylation sites [14–16]. CAV1 was the first member discovered in a family of three caveolins and is phosphorylated by Src kinase at Y14 in response to a multitude of stimuli. Caveolin-2 (CAV2) is co-expressed with CAV1 and cannot form caveolae in the absence of CAV1 [17, 18]. CAV2 is also a Src kinase substrate, phosphorylated at Y19, and its phosphorylation results in the dissociation of CAV2 from CAV1 hetero-oligomers at the plasma membrane [19]. Caveolin-3 (CAV3) is predominantly expressed in muscle tissue and shares the highest sequence similarity with CAV1 but is not phosphorylated by Src kinase [20, 21]. Two isoforms of CAV1 exist with different methionine start sites, Met 1 (α-isoform) or an internal Met 32 (β-isoform) [22], such that only the α-isoform of CAV1 is Y14-phosphorylated. While Src kinase is the primary kinase studied that phosphorylates CAV1, Fyn and Abl are two other Src family kinases that also phosphorylate CAV1 at Y14 [23, 24].

CAV1 exists as monomers but also forms stable SDS-resistant oligomers that are composed of 14-16 CAV1 molecules [25]. CAV1 oligomerization is stabilized by interactions with fatty acids and cholesterol and mediated by the oligomerization domain (amino acids 61-101) [26–28]. This domain also mediates CAV1 interaction with cholesterol and overlaps the highly conserved membrane proximal caveolin scaffolding domain (CSD) (Fig. 1), a hydrophobic region from amino acids 82-101 [29, 30]. The CSD mediates CAV1 interaction with multiple signaling molecules, including Src family tyrosine kinases, growth factor receptors, endothelial nitric oxide synthase (eNOS), and G proteins [30–35].

CAV1 is the main structural component of caveolae, small plasma membrane invaginations of 50–100 nm [6, 7, 36]. Functional roles of caveolae include mechanoprotective membrane buffers, mechanosensors, signaling hubs, and endocytic transporters [11]. Caveolae formation also requires an adaptor protein CAVIN1 (also called PTRF) [37]. Other CAVINS include CAVIN2 and CAVIN3 that associate with and modify caveolae size and dynamics but are not required for caveolae formation, as well as muscle specific CAVIN4 [38, 39]. Other regulators of caveolae formation and stability include dynamin-2, PACSIN2, and EHD2. Dynamin-2 localizes to the neck of caveolae and mediates caveolae scission via GTP hydrolysis and Src-mediated phosphorylation of dynamin-2 [40–42]. EHD2 also localizes to the neck of caveolae where it interacts with PACSIN2, CAVIN1, and the actin cytoskeleton to stabilize and constrain caveolae to the plasma membrane [43–45]. The PACSIN2 F-BAR domain interacts directly with CAV1 to shape caveolae formation and recruit dynamin-2 [46, 47]. Protein kinase C (PKC) phosphorylation of PACSIN2 decreases its stabilizing effect on caveolae and triggers dynamin-mediated removal of caveolae [48]. Complex interactions between CAV1 and various effectors thereby impact the stability of caveolae at the plasma membrane.

CAV1 Y14 phosphorylation induces detachment of caveolae from the plasma membrane and accumulation of CAV1-

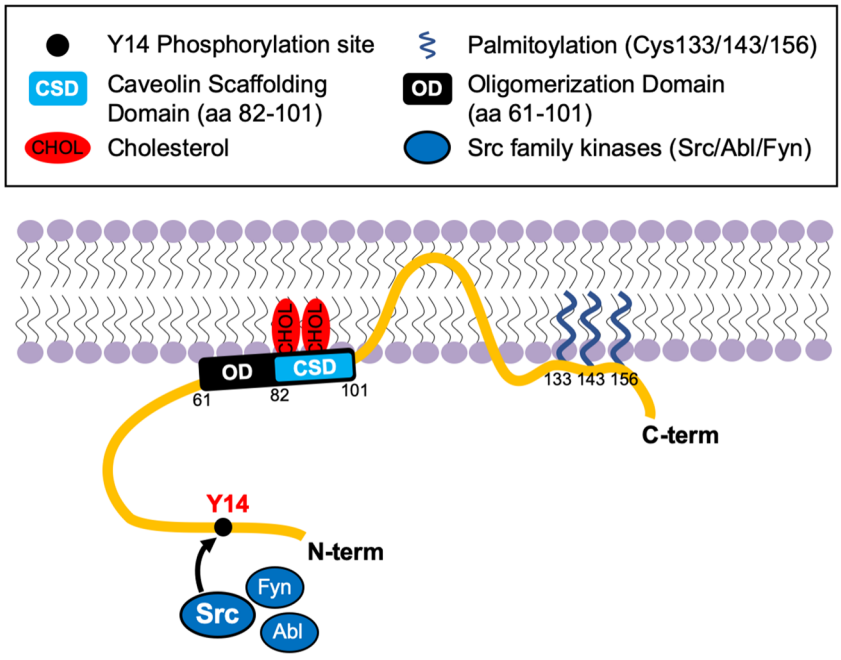

Fig. 1 Structure of a CAV1 monomer. Cav1 alpha isoform (178 amino acids) is an integral membrane protein embedded into the membrane via a small hairpin insert and three palmitoylation sites (Cys133/143/156) with both the N- and C-termini exposed to the cytoplasm. The oligomerization domain (aa 82-101) is necessary for homo- and hetero-oligomerization with other caveolin molecules and is stabilized by interactions with cholesterol. Within the oligomerization domain is a highly conserved caveolin scaffolding domain (CSD) (aa 61-101). CAV1 phosphorylation at residue tyrosine 14 (Y14) by Src, Abl, and Fyn tyrosine kinases

positive cytoplasmic vesicles [49]. More recently, expression of phosphomimetic CAV1Y14D was shown to increase vesicle number and mobility by total internal reflection fluorescence microscopy (TIRF) analysis and to induce swelling of caveolae vesicles [50]. Increased caveolae formation in response to epidermal growth factor (EGF) stimulation of PANC-1 human pancreatic tumor cells is pCAV1-dependent as it is prevented by expression of a non-phosphorylatable CAV1Y14F mutant [51]. However, mutation of the Y14 site to either a phosphomimetic Y14D or non-phosphorylatable Y14F did not prevent formation of caveolae upon expression of exogenous CAV1 in CAV1 null mouse embryo fibroblasts (MEFs) [52]. This study further showed that CAV1 phosphorylation in response to mechanical stress feeds back to regulate the plasma membrane abundance of caveolae *via* relief of Egr1 transcriptional inhibition of CAV1 and CAVIN1 [52]. Together, these studies suggest that while not a determinant of caveolae formation, CAV1 Y14 phosphorylation may alter CAV1 organization within caveolae, caveolae dynamics and also drive caveolae formation through transcriptional upregulation of the caveolae constituents, CAV1 and CAVIN1.

In the absence of CAVIN1, CAV1 is localized to non-caveolar homo-oligomerizations known as CAV1 scaffolds [53]. Non-caveolar CAV1 scaffolds lack a defined morphology, are difficult to identify by electron microscopy (EM), and cannot be distinguished from caveolae by diffraction-limited fluorescence microscopy, as both are smaller than the diffraction limit of visible light (~ 200–250 nm). Super-resolution single-molecule localization microscopy (SMLM) approaches (PALM, dSTORM) obtain resolutions of < 20 nm and have been used to study CAV1 [54–57]. SMLM network analysis [56, 57] showed that caveolae present a modular CAV1 coat, corresponding to that determined by cryoelectron microscopy [58, 59]. It also identified distinct scaffolds: small S1A scaffolds corresponding to SDS-resistant CAV1 oligomers of 10-15 CAV1 molecules [25, 60]; S1B scaffolds that correspond to S1A dimers and form modular units that compose the caveolae coat, and previously undescribed larger hemispherical S2 scaffolds [56, 57]. CAVIN1 has been reported to interact with both caveolae and scaffolds [61, 62]. This suggests that caveolae, the various scaffolds and, indeed, monomeric CAV1 may form part of a dynamic equilibrium impacted by expression of the various caveolae-associated proteins described previously. How pCAV1 impacts this equilibrium is not known.

3 CAV1 and pCAV1 in cancer

CAV1 is known for its dual role in cancer acting as both a tumor suppressor and promoter depending on tumor type and context. Early studies showed that oncogene transformation of NIH-3T3 fibroblasts reduced CAV1 expression and correlated with increased cell growth; re-expression of CAV1 in the transformed lines prevented growth and induced apoptosis suggesting a tumor suppressor role for CAV1 [63, 64]. Subsequently, while CAV1 was shown to reduce xenograft tumor development of colon cancer cells in mice, increased CAV1 expression was found to correlate with drug resistance and metastatic potential [65]. This led to early consideration of the multifaceted role of CAV1 in cancer [66]. Indeed, CAV1 expression at later stages of cancer development is associated with a poor prognosis in breast and prostate cancers, and specifically with highly aggressive triple-negative basal-type breast cancers [67–69]. On the contrary, in some tumor types, CAV1 expression is associated with improved prognosis [9, 70–72]. Stromal CAV1 deficiency has also been associated with autophagy, hypoxia, and oxidative stress, processes in which pCAV1 also plays a role *in vitro,* and leads to tumor recurrence, metastasis, and a poor patient prognosis [73, 74].

Interestingly, a number of studies have shown that exogeneous CAVIN1 expression, which induces caveolae formation, neutralizes the pro-cancer effects of CAV1 in PC3 prostate cancer cells [75–77]. PC3 cells express neither CAVIN1 nor caveolae and only scaffolds [37, 57]. The pro-migratory role of CAV1 in these cells is therefore scaffold-mediated and suppressed by induction of caveolae through expression of CAVIN1 [75, 77]. Since CAV1 promotion of PC3 cell migration is Y14 phosphorylation-dependent, this argues that the pCAV1 functions in scaffolds to promote tumor cell migration and cancer progression [78]. Study of LNCaP prostate cancer cells that express neither CAV1 nor CAVIN1 showed that CAVIN1 expression only affected growth and metastasis of LNCaP cells upon overexpression of CAV1, indicating that the tumor suppressor function of CAVIN1 is mediated by CAV1 and acts through induction of caveolae [75]. Additionally, the receptor tyrosine kinase-like orphan receptor 1 (ROR1), a pro-survival signaling molecule in lung adenocarcinomas, is a scaffold protein that promotes the association between CAV1 and CAVIN1 and CAVIN3 maintaining caveolae at the plasma membrane [79, 80]. How CAVIN1, ROR1, and other regulators of caveolae stability, such as EHD2, and PACSIN2, interact to regulate CAV1 function in cancer progression through both caveolae and scaffolds remains to be determined.

Specific analysis of the contribution of CAV1 Y14 phosphorylation, as opposed to total CAV1 expression, to CAV1 function in cancer has been limited due to the lack of specific antibodies to Y14-phosphorylated CAV1. The CAV1 Y14 phosphorylation site shows high sequence homology with the Y118 site of phospho-paxillin, a phosphorylated focal adhesion protein, and the widely used monoclonal anti-pCAV1 antibody was found to bind phospho-paxillin [81]. The extensive labeling of focal adhesions with this antibody, presumed to reflect a role for pCAV1 in focal adhesions, was therefore not specific [81]. Nevertheless, use of CAV1Y14D

phosphomimetic and Y14F non-phosphorylatable mutants showed that CAV1 Y14 phosphorylation does indeed control focal adhesion dynamics and cell migration [82, 83] (see "pCAV1 and focal adhesion signaling" section). A second pCAV1-specific antibody did not show distinctive labeling in focal adhesions and had increased puncta labeling with the activation of a temperature-sensitive *v*-Src in NRK cells [49]. However, this polyclonal antibody has not been used extensively and a more recent test of the antibody found that the signal-to-noise ratio was not above non-immune purified IgG [81]. There are currently no pCAV1-specific antibody probes that can be used outside of Western blotting, limiting our ability to determine the specific contribution of Y14 phosphorylation to CAV1 function in cancer progression.

A key role for Y14 phosphorylation of CAV1 in tumor cell migration and invasion was defined by the demonstration that (1) wild-type (WT) CAV1 and phosphomimetic CAV1Y14D/Y14E but not non-phosphorylatable CAV1Y14F induced tumor cell migration and (2) that migration induced by WT CAV1 but not CAV1Y14D was sensitive to Src inhibition [67, 84–86]. Expression of phosphomimetic CAV1 Y14E results in an augmented metastatic phenotype in B16F10 melanoma cells promoting invasion and transendothelial cell migration (TEM) *in vivo* [85]. Comparable results were found for MDA-MB-231 breast cancer cells *in vitro* [86]. Further, enhanced extravasation from the bloodstream is exhibited in MDA-MB-435 cells expressing WT CAV1 and the Y14D mutant in comparison to control cells or cells expressing the non-phosphorylatable Y14F mutant [87]. Phosphorylation of CAV1 promotes the phosphorylation of either ERK or AKT kinase and contributes to enhanced tumor cell proliferation, migration, and invasion in rhabdomyosarcoma [88]. Finally, a recent study shows that CAV1 is a substrate for the phosphatase PTPN14 whose dephosphorylation of CAV1 prevents CAV1-induced metastasis, defining a critical role for CAV1 Y14 phosphorylation in tumor metastasis [89].

CAV1 has been associated with chemoresistance in various types of tumors [90–92]. Radiation sensitivity is enhanced by the disturbance of the association between CAV1 and ß1-integrin or focal adhesion kinase (FAK) in pancreatic carcinoma cell lines [93]. Cells overexpressing CAV1 were more resistant to treatment with the chemotherapeutic drugs cisplatin and doxorubicin, while CAV1 knockdown cells were respectively more sensitive to cell death upon treatment [88]. Interestingly, the Src kinase inhibitor PP2 reversed the drug-resistant phenotype of CAV1-overexpressing cells, suggesting a role for pCAV1 in chemoresistance of cancer cells [88]. However, pCAV1 enhanced paclitaxel-mediated cytotoxicity in MCF7 breast cancer cells [94].

At the same time, upregulation of CAV1 is associated with premature senescence [95]. MDM2 is an E3 ubiquitin ligase that normally targets p53 for degradation in the nucleus; in response to oxidative stress, CAV1 sequesters MDM2 out of the nucleus to the plasma membrane, resulting in increased expression of the tumor suppressor protein p53 [96, 97]. Indeed, while stable expression of phosphomimetic CAV1Y14D in MDA-MB-435 cancer cells induces invadopodia and promotes tumor cell extravasation, it also enhances p53 expression, which in turn, reverses the Warburg effect and restricts tumor growth *in vivo*, identifying a tumor suppressor role for pCAV1, albeit at supraphysiological levels [87].

These results highlight the multidimensional and conflicting roles of tumor cell CAV1 in cancer progression and underscore the importance of better understanding how Y14 phosphorylation impacts CAV1 functionality to better define its role in cancer.

4 pCAV1 in migrating tumor cells

In migrating fibroblast and endothelial cells, caveolae accumulate at the rear of the cell, and CAV1 expression is required to maintain cell polarity [83, 98–101]. Deletion of the N-terminal 60 amino acids of CAV1 prevented caveolae formation, trailing edge CAV1 polarization and polarized migration; the domain responsible for this phenotype mapped to amino acids 46-55 and was therefore distinct from the Y14 phosphorylation site [101]. Endothelial cells in 3D culture transmigrating through a pore present a redistribution of CAV1 from the trailing edge to leading edge pseudopodial protrusions, a redistribution that requires Y14 phosphorylation [99]. Further, in various cancer cell lines, CAV1 promotes cell migration and is not polarized to the trailing edge but also associates with the leading edge [67, 86].

Pseudopodia purified from an invasive variant of Moloney sarcoma virus-transformed MDCK (MSV-MDCK-INV) tumor cells are enriched for focal adhesion proteins, Src, CAV1, and pCAV1; similarly, pseudopodia of MDA-MB-231 breast carcinoma cells show enrichment for pCAV1 [67, 102]. A comparative proteomic analysis of pseudopodia from MDA-MB-435 cancer cells that express low CAV1 levels [103] and from CAV1-overexpressing MDA-MB-435 cells showed that CAV1 expression promoted pseudopodial enrichment of various glycolytic enzymes [87]. Similarly, CAV1 recruits glycolytic enzymes to the plasma membrane of smooth muscle cells [104]. Glycolysis promotes pseudopod protrusion and tumor cell migration [105, 106] and it is tempting to speculate that pCAV1 may act as a scaffolding molecule that recruits glycolytic enzymes to pseudopodia promoting local glycolysis and ATP production [87]. A role for CAV1 tumor cell metabolism is supported by CAV1 promotion of aerobic glycolysis in colorectal cancer cells via enhanced nuclear localization of HMGA1, which binds and activates the GLUT3 promoter [107]. In addition, loss of stromal CAV1 promotes a metabolic switch to aerobic glycolysis,

producing metabolites such as lactate and pyruvate that fuel neighbouring cancer cells [73].

CAV1 domains have a long-established role as signaling platforms that both suppress and promote signaling of multiple and varied receptors and activation of downstream pathways [108–110]. In addition, CAV1 is phosphorylated in response to a large variety of signaling molecules and pathways that are far too numerous to enumerate here. An example is the epidermal growth factor receptor (EGFR), whose binding to CAV1 via the CSD prevents EGFR autophosphorylation, effector protein binding, kinase activity, and downstream signaling [111–114]. CAV1 inhibition of EGFR signaling can be overridden by galectin-3, a glycan-binding protein with established roles in cancer progression that recruits EGFR to the galectin lattice and promotes EGFR signaling [114–116]. EGF also induces Src-dependent CAV1 phosphorylation [51, 117, 118]. EGF transactivation of integrins and downstream Rho signaling drives tumor cell migration and is dependent on both pCAV1 and galectin-3 signaling [67, 82, 118]. These studies define an interaction between two plasma membrane domains, the extracellular galectin lattice and cytoplasmic CAV1 domains, whose suppression or activation of EGFR signaling is critically dependent on CAV1 Y14 phosphorylation [53]. Curiously, CAV1 promotes pseudopodial recruitment of galectin-3 [87] and it is tempting to speculate that local pCAV1 interaction with pseudopodial galectin-3 induces the focal adhesion signaling, tension, and turnover responsible for pseudopod protrusion.

Finally, endocytosis of focal adhesion-associated integrins is mediated by pCAV1 and requires the CSD [119, 120]. These studies suggest that CAV1 Y14 phosphorylation promotes caveolae endocytosis and mediates integrin internalization at the leading edge. The clathrin-independent carrier (CLIC) raft endocytic pathway is also localized to the leading edge and internalizes β1-integrin [121, 122]. CLIC-mediated integrin endocytosis is promoted by extracellular galectin-3 [121], while CAV1 inhibits leading edge CLIC endocytosis [122]. CAV1 inhibition of leading edge CLIC endocytosis supports our early studies showing that CAV1 is a negative regulator of raft endocytosis [123, 124]; at the same time, they highlight the complex nature of CAV1, pCAV1, and galectin-3 interaction in endocytosis at the leading edge.

5 pCAV1 and focal adhesion signaling

The protrusion of pseudopodia and invadopodia that drives cancer cell migration and invasion is dependent on the dynamic formation and disassembly of focal adhesions. Coordination of RhoGTPases RhoA and Rac1 signaling regulates focal adhesion dynamics and actin remodeling that drive pseudopodial protrusion and migration [125]. CAV1 and pCAV1 have been proposed to participate in the regulation of various Src and RhoGTPase pathway signaling proteins [118]. CAV1 phosphorylation increased C-terminal Src kinase interaction with CAV1 by > 35-fold, resulting in the inactivation of Src [126]. Subsequent binding to pCAV1 of Src via its Src homology 2 (SH2) domain leads to the accumulation of activated Src in focal adhesions [127]. Directional migration is impaired in CAV1 knockout MEFs and can be rescued by WT CAV1 and p190RhoGAP knockdown, but not by CAV1Y14F [83]. Both Src inactivation and p190RhoGAP knockdown restore impaired migration in CAV1 knockout MEFs, suggesting that CAV1 stimulates normal Rho activity through inactivation of the Src–p190RhoGAP pathway [83]. In metastatic tumor cells, pCAV1 is associated with elevated GTP-RhoA levels and stable expression of CAV1Y14D in tumor cells stimulates ROCK- and Src-dependent Rho activation [67]. At the same time, activated Src and FAK stimulate Rac1 activity [128]. Rac1 binds CAV1 and promotes CAV1 accumulation at peripheral focal adhesions, while CAV1 controls Rac1 protein levels by regulating degradation of Rac1 [129].

pCAV1 stabilizes focal adhesion components within focal adhesions, promoting focal adhesion signaling and turnover to drive cancer cell migration [67, 82, 86]. Fluorescence recovery after photobleaching (FRAP) analysis showed that pCAV1 stabilized FAK in focal adhesions, reflecting reduced exchange of focal adhesion-associated FAK with cytosolic pools and increased focal adhesion signaling [82]. Using a fluorescent Laurdan probe that provides a measure of membrane order, CAV1 knockout MEFs were found to have both less abundant and less ordered focal adhesion membrane domains; transfection of CAV1 WT but not CAV1Y14F restored the ordered state of focal adhesion lipids in CAV1 knockout MEFs [130]. Consistent with FAK stabilization in focal adhesions, we observed that pCAV1 enhanced vinculin tension in focal adhesions, stabilizing high-tension cellular focal adhesions [78]. pCAV1-dependent focal adhesion tension and tumor cell migration were prevented by both mutation of the CSD (F92A/V94A in the essential F92TVT95 segment) and cell treatment with AP-CAV (Cavtratin), in which the CSD sequence of CAV1 is fused to the cell-permeable Antennapedia (AP) peptide [78]. These studies argue that pCAV1 acts via the CSD to promote engagement of the molecular clutch [131, 132] that links the extracellular matrix (ECM) to actin dynamics, driving membrane protrusion and migration of cancer cells [78].

Many components of focal adhesions are found in invadopodia, and migratory phenotypes have been linked to the formation of invadopodia [133, 134]. Invadopodia are actin-based pseudopodial protrusions that attach to and degrade the ECM [133]. CAV1 has been reported to colocalize and cotraffic with MT1-MMP into pseudopodia [135–138]. Reduced ECM degradation due to CAV1 knockdown was restored by CAV1Y14F expression or addition of cholesterol,

but not CAV1Y14D, suggesting that pCAV1 inhibits invadopodia biogenesis via control of membrane cholesterol [139]. At the same time, conflicting findings have reported that the addition of cholesterol did not rescue ECM degradation in CAV1 knockdown MDA-MB-231 cells, suggesting a cholesterol-independent mechanism [135]. Further, low shear stress induced CAV1 phosphorylation and CAV1 colocalization with MT1-MMP into invadopodia [136]. As previously described, a study with MDA-MB-435 cells used an *in vivo* extravasation assay to show that phosphomimetic CAV1Y14D promoted invadopodia protrusion into the vasculature [87]. CAV1 and pCAV1 regulation of focal adhesions and pseudopod/invadopod protrusion highlights the need for more research into understanding their role in the promotion of migratory cancer phenotypes.

6 pCAV1 and the tumor cell response to stress

Within tumors, an irregular, leaky vasculature creates a microenvironment in which oxygen and nutrient levels are in constant flux, such that tumor cells encounter a local environment of oxidative stress and hypoxia [140]. Further, stiffness of the stromal ECM is closely associated with malignancy of various cancers, inducing mechanical stress on the tumor cell and promoting tumor cell migration and metastasis through focal adhesion signaling [141]. Both CAV1 and its Y14 phosphorylation are upregulated in response to these cellular stressors encountered in the tumor microenvironment that potentially play roles in tumor cell survival, metastasis, and drug resistance.

Mechanical stress induces both CAV1 expression and CAV1 Y14 phosphorylation [52, 142–144] while shear stress induces CAV1-dependent motility in MDA-MB-231 breast cancer cells [136, 145]. CAV1 has been implicated as a modulator of the activity of the YAP transcriptional regulator that mediates the mechanoresponsiveness of the cell and plays an important role in tumorigenesis [146]. Plating cells on soft matrix decreases CAV1 and β1-integrin levels and reduces cell spreading; expression of CAV1Y14D restored β1-integrin levels and induced cell spreading independently of matrix stiffness [147]. ECM-dependent pCAV1 activity was shown to promote melanoma metastasis [85]. Together with pCAV1 regulation of focal adhesion tension [78], these studies demonstrate that CAV1 Y14 phosphorylation plays an important role in the cellular response to mechanical stress. Moreover, pCAV1 mediates RhoA- and Akt-dependent matrix production in response to mechanical strain; expression of non-phosphorylatable CAVY14A reduced CAV1-RhoA association, RhoA activation, fibronectin production, and Akt-dependent type I collagen production [148, 149]. This supports a role for pCAV1 in matrix organization.

Caveolae flattening in response to mechanical stress acts as a membrane buffer to mediate membrane tension, protecting cells against membrane rupture and death. This role has been extensively validated in adipocytes, endothelial, and muscle cells [150–155]. Physical stress on the membrane flattens caveolae dissociating CAV1 from the CAVINS that are then released into the cytosol; incorporation of the caveolar membrane into the plasma membrane may expand the surface area of the membrane thereby acting to negate mechanical stress and prevent cell lysis [156]. The role of Y14 phosphorylation in caveolae membrane buffering remains unclear. CAV1 phosphorylation in response to mechanical stress feeds back to regulate, *via* relief of Egr1 transcriptional inhibition of CAV1 and CAVIN1, the plasma membrane abundance of caveolae [52]. Expression of CAV1 and CAVIN1 is also transcriptionally controlled in response to mechanical stress *via* the YAP/TAZ co-activator and caveolae-associated EHD2 ATPase [146, 157, 158]. Together, these studies suggest that complex signaling mechanisms control expression of caveolae through transcriptional control of CAV1 and CAVIN1 expression, including but not limited to Y14 phosphorylation of CAV1. Whether pCAV1 feedback control serves to promote cancer cell resistance and survival under conditions of mechanical stress remains to be determined.

In response to oxidative stress, Src kinase activity increases CAV1 Y14 phosphorylation [52, 142, 159, 160] and induces endocytosis and cytoplasmic relocalization of CAV1 [160, 161]. CAV1 Y14 phosphorylation mediates EGFR endocytosis and maintenance of EGFR activity in response to oxidative stress in A549 lung adenocarcinoma cells [162] and also mediate oxidative stress induced pulmonary vascular permeability [163]. CAV1 expression is upregulated in response to oxidative stress via enhanced binding of the SP1 transcription factor, and reduced binding of the EGR1 transcription factor that target similar GC-box promotor regions [52, 164]. EGR1 regulation of CAV1 gene transcription was shown to be pCAV1-mediated [52]. Studies using CAV1 knockout MEFs showed that CAV1 induced premature senescence in response to oxidative stress via CSD-dependent sequestration of MDM2 to the plasma membrane, thus preventing its interaction with and degradation of nuclear p53 [96]. Oxidative stress-induced senescence is not observed in MCF7 breast cancer cells lacking CAV1 [164]. Similarly, TP53 induction was minimal in MDA-MB-435 cancer cells that express low CAV1 levels and undetectable pCAV1 [103] and, interestingly, enhanced by expression of WT CAV1 but not CAV1Y14F [87]. Mouse tumor xenografts of CAV1Y14D-expressing MDA-MB-435 cells showed significantly higher TP53 staining and reduced tumor growth, suggesting CAV1 Y14 control of TP53-dependent cell growth *in vivo* [87].

Hypoxia is a result of abnormal tumor microvasculature and is a characteristic of the tumor microenvironment. Intriguingly, downregulation of endocytosis and cell surface

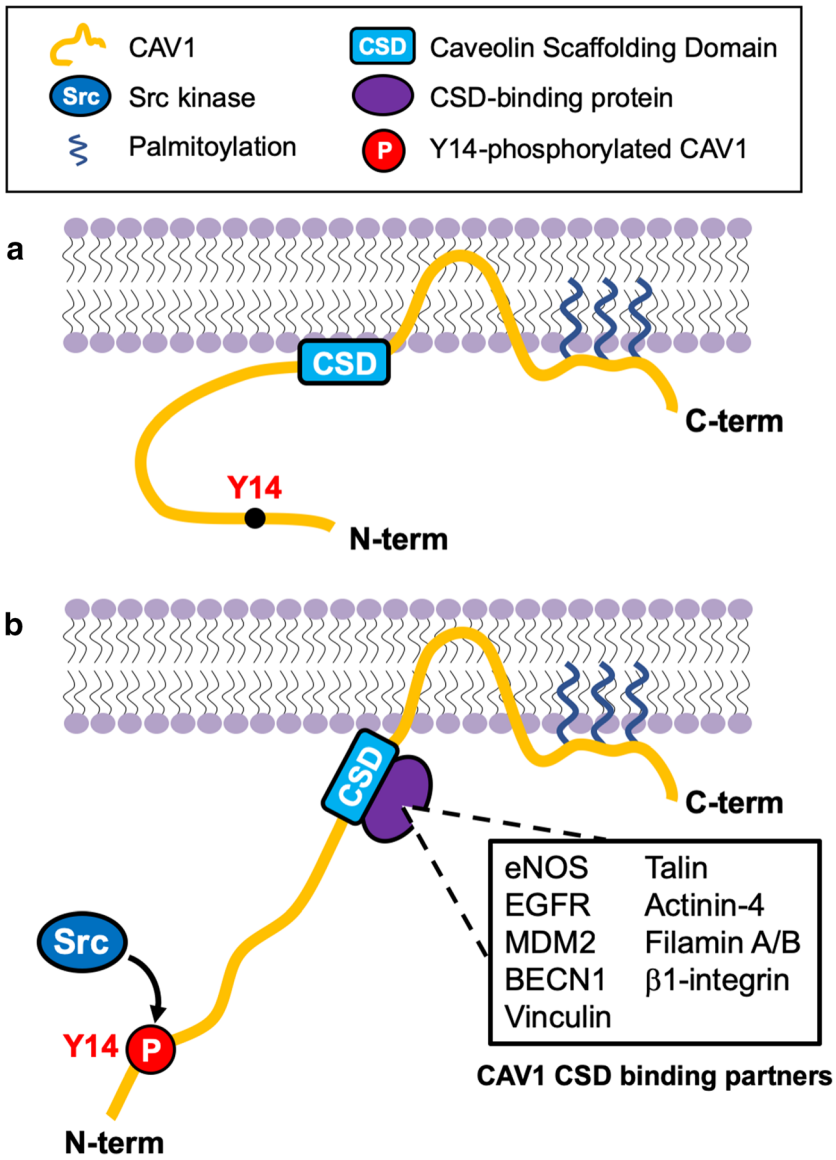

Fig. 2 CAV1 Y14 phosphorylation may induce conformational alterations in the N-termini that promote CSD interactions. **a** The CAV1 N-terminal domain is a flexible disordered domain in the cytoplasm. The CSD is a hydrophobic region that is speculated to be contacting or directly imbedded within the lipid bilayer membrane, but has also been shown to interact with many effector proteins. **b** CAV1 Y14 phosphorylation may induce spatial separation of CAV1 oligomers enabling CSD interaction with binding partners such as eNOS and focal adhesion proteins vinculin, talin, α-actinin-4, filamin A/B, and β1-integrin. Y14 phosphorylation may increase CSD accessibility through the N-termini uncoiling away from the CSD and/or by pulling the CSD away from the membrane

protein internalization in hypoxic conditions was shown to be regulated by CAV1 inhibition of dynamin-mediated endocytosis [165]. This is consistent with our early studies that documented CAV1 as a negative regulator of raft-dependent endocytosis [123, 124]. Indeed, CAVs and CAVINs also act to inhibit non-clathrin endocytic pathways [122]. Treatment of mouse melanoma cells with PP2, the Src kinase inhibitor, resulted in decreased hypoxia-induced migration, suggesting a potential role for CAV1 phosphorylation in the process [166]. Subsequently, uptake of the HER2-targeted antibody-drug conjugate trastuzumab-emtansine (T-DMI) was shown to be pCAV1-mediated; hypoxia inhibition of T-DMI uptake was related to disruption of pCAV1-dependent endocytosis [167]. These data are consistent with a role for pCAV1 in integrin and insulin receptor endocytosis [120, 168] and suggest that hypoxia induces T-DMI resistance by preventing pCAV1-dependent endocytosis.

7 pCAV1 and the CSD

Multiple studies on CAV1 function, including but not limited to those described previously, report on functional roles for the CAV1 CSD as a regulator of CAV1 function and interaction with effector proteins. However, close proximity of the CSD to the cell membrane and consequent low accessibility to binding partners led to questions as to whether the CSD could interact with CAV1-binding proteins [169].

Endothelial nitric oxide synthase (eNOS) is an important regulator of vascular health through its production of the signaling molecule nitric oxide (NO) [170]. eNOS binds directly to CAV1, an interaction that has been mapped to a 10-amino acid sequence within the CSD [32, 171, 172] and that has been shown to negatively regulate eNOS activity and NO production *in vitro* [171, 173, 174]. This interaction is Y14-dependent, with eNOS-derived NO production activating Src and

Y14 phosphorylation of CAV1 in a negative feedback loop to reduce eNOS activity [174]. eNOS is detected ubiquitously in tumors and eNOS-derived NO promotes vessel remodeling and angiogenesis and therefore promotes tumor progression [170, 175]. AP-CAV inhibits eNOS function and blocks NO release *in vitro* preventing eNOS remodeling of the tumor vasculature, blocking tumor angiogenesis, and delaying tumor progression *in vivo* [176]. pCAV1-dependent focal adhesion tension and tumor cell migration is prevented by mutation of the CSD and by cell treatment with AP-CAV [78]. The ability of AP-CAV to inhibit both tumor cell migration and tumor angiogenesis [78, 176] is intriguing and identifies critical roles for the CAV1 CSD in both stromal and tumor cells.

Dual regulation of focal adhesion tension [78], eNOS binding [174], and stress-induced mitochondrial recruitment of beclin-1 (BECN1) [177] by both CAV1 Y14 phosphorylation and the CSD is suggestive of a functional interaction between these two CAV1 domains. pCAV1-dependent focal adhesion tension and tumor cell migration is prevented by mutation of the CSD and by cell treatment with AP-CAV [78]. To study the impact of Y14 phosphorylation on CAV1 interaction with its binding partners, CAV1 polypeptides from amino acids 1-101, encompassing both Y14 and the CSD, were conjugated to glutathione-S-transferase (GST) for GST pulldown analysis [78]. Phosphomimetic Y14D GST-CAV1(1-101) showed significantly elevated binding to a large number of proteins relative to non-phosphorylated Y14F GST-CAV1(1-101), such as focal adhesion proteins (vinculin, talin, α-actinin-4, filamin A/B, and β1-integrin [78]). Interestingly, pCAV1 activity at focal adhesions is mimicked not only by a negatively charged phosphomimetic Y14D mutation but also by a positively charged Y14R mutation [82]; this suggests that introduction of a charged phosphate group at the CAV1 N-terminal may induce conformational alterations that impact CSD interaction with effector proteins (Fig. 2). The CAV1 N-terminal domain is a disordered domain, lacking any distinct structural features, and is therefore quite flexible [178]. Consistent with this model, intermolecular fluorescent resonance energy transfer (FRET) analysis of CAV1 oligomers showed that CAV1 Y14 phosphorylation induced spatial separation of CAV1 oligomers [50]. Together, these studies suggest that Y14 phosphorylation may promote CSD-mediated CAV1 binding not only to eNOS and focal adhesion proteins, but also to multiple other CAV1 effectors.

8 Conclusion

CAV1 plays an important yet complex role in cancer progression. In spite of being initially identified as a major Src kinase substrate, understanding the contribution of CAV1 Y14 phosphorylation remains limited. This is in part due to the lack of specific probes and limited tools to study pCAV1, particularly *in vivo*. It also relates to the ongoing challenges of considering and distinguishing the function of CAV1 and pCAV1 in the context of both caveolae and scaffolds. As outlined in this review, pCAV1 is upregulated in response to stressors encountered in the tumor microenvironment and is critically involved in the promotion of tumor cell migration, invasion, and metastasis (Fig. 3). Further, regulation of CSD function by Y14 phosphorylation places this post-translational modification as a key regulator of CAV1 interaction with effector

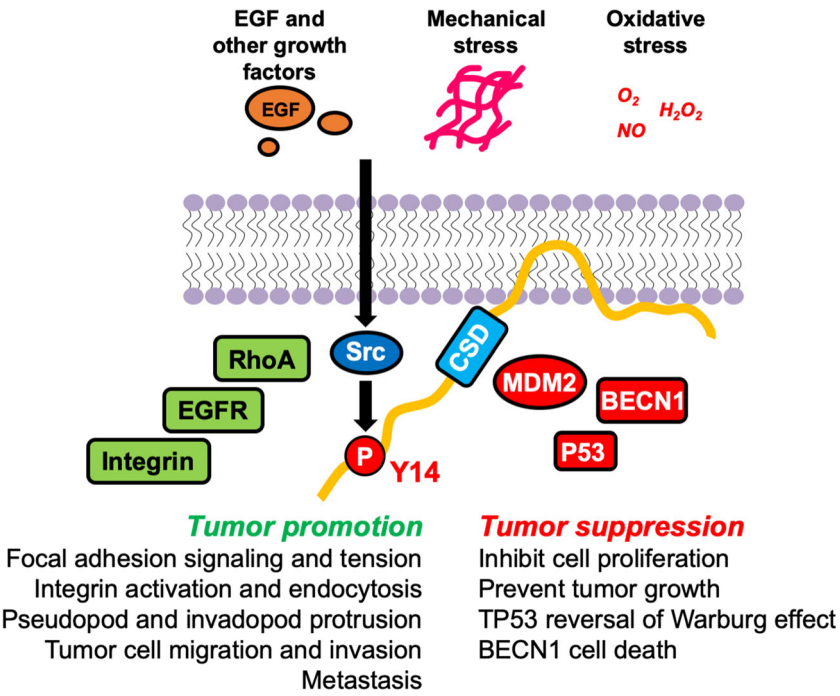

Fig. 3 CAV1 phosphorylation in the tumor microenvironment. Src-dependent CAV1 phosphorylation has both tumor promoting and suppressing functions. pCAV1 inhibits cell proliferation and tumor growth and induces TP53; in neurons, pCAV1 induces cell death through CSD interaction with BECN1. Simultaneously, pCAV1 is upregulated in highly aggressive breast and prostate cancers, promoting focal adhesion signaling and tension, integrin activation, and endocytosis and producing pseudopodia and invadopodia protrusions, placing pCAV1 as a critical proponent of tumor cell migration, invasion, and metastasis in aggressive cancers

proteins and thereby CAV1 function. Further definition of how pCAV1 promotes cancer may enable the development of pCAV1-specific therapeutics that selectively target the pro-cancer functions of this complex and functionally diverse protein.

Funding information Work on CAV1 in the Nabi lab is supported by grants from the Canadian Institutes for Health Research (CIHR PJT-156424, PJT-159845). Timothy Wong is supported by British Columbia Graduate Scholarship #6768 and UBC Faculty of Medicine Graduate Award #6442, Fiona Dickson by UBC Faculty of Medicine Graduate Award #6442 and NSERC Canada Graduate Scholarship Master's Award #6563, and Logan Timmins by British Columbia Graduate Scholarship #6768.

References

1. Brugge, J. S., & Erikson, R. L. (1977). Identification of a transformation-specific antigen induced by an avian sarcoma virus. *Nature, 269*(5626), 346–348. https://doi.org/10.1038/269346a0.
2. Collett, M. S., & Erikson, R. L. (1978). Protein kinase activity associated with the avian sarcoma virus src gene product. *Proc Natl Acad Sci U S A, 75*(4), 2021–2024. https://doi.org/10.1073/pnas.75.4.2021.
3. Levinson, A. D., Oppermann, H., Levintow, L., Varmus, H. E., & Bishop, J. M. (1978). Evidence that the transforming gene of avian sarcoma virus encodes a protein kinase associated with a phosphoprotein. *Cell, 15*(2), 561–572. https://doi.org/10.1016/0092-8674(78)90024-7.
4. Sefton, B. M., Hunter, T., Beemon, K., & Eckhart, W. (1980). Evidence that the phosphorylation of tyrosine is essential for cellular transformation by Rous sarcoma virus. *Cell, 20*(3), 807–816. https://doi.org/10.1016/0092-8674(80)90327-x.
5. Lipsick, J. (2019). A history of cancer research: tyrosine kinases. *Cold Spring Harbor perspectives in biology, 11*(2). https://doi.org/10.1101/cshperspect.a035592.
6. Glenney Jr., J. R., & Zokas, L. (1989). Novel tyrosine kinase substrates from Rous sarcoma virus-transformed cells are present in the membrane skeleton. *J Cell Biol, 108*(6), 2401–2408. https://doi.org/10.1083/jcb.108.6.2401.
7. Rothberg, K. G., Heuser, J. E., Donzell, W. C., Ying, Y.-S., Glenney, J. R., & Anderson, R. G. W. (1992). Caveolin, a protein component of caveolae membrane coats. *Cell, 68*, 673–682.
8. Li, S., Seitz, R., & Lisanti, M. P. (1996). Phosphorylation of caveolin by src tyrosine kinases. The alpha-isoform of caveolin is selectively phosphorylated by v-Src in vivo. *J Biol Chem, 271*(7), 3863–3868.
9. Ketteler, J., & Klein, D. (2018). Caveolin-1, cancer and therapy resistance. *Int J Cancer, 143*(9), 2092–2104. https://doi.org/10.1002/ijc.31369.
10. Espada, J., & Martín-Pérez, J. (2017). Chapter three—an update on Src family of nonreceptor tyrosine kinases biology. In L. Galluzzi (Ed.), *Int Rev Cell Molec Biol* (Vol. 331, pp. 83-122): Academic Press.
11. Parton, R. G. (2018). Caveolae: structure, function, and relationship to disease. *Ann Rev Cell Dev Biol, 34*(1), 111–136. https://doi.org/10.1146/annurev-cellbio-100617-062737.
12. Dupree, P., Parton, R. G., Raposo, G., Kurzchalia, T. V., & Simons, K. (1993). Caveolae and sorting in the trans-Golgi network of epithelial cells. *The EMBO Journal, 12*(4), 1597–1605.
13. Dietzen, D. J., Hastings, W. R., & Lublin, D. M. (1995). Caveolin is palmitoylated on multiple cysteine residues. Palmitoylation is not necessary for localization of caveolin to caveolae. *J Biol Chem, 270*(12), 6838–6842.
14. Tonn Eisinger, K. R., Woolfrey, K. M., Swanson, S. P., Schnell, S. A., Meitzen, J., Dell'Acqua, M., & Mermelstein, P. G. (2018). Palmitoylation of caveolin-1 is regulated by the same DHHC acyltransferases that modify steroid hormone receptors. *J Biol Chem, 293*(41), 15901–15911. https://doi.org/10.1074/jbc.RA118.004167.
15. Parat, M. O., & Fox, P. L. (2001). Palmitoylation of caveolin-1 in endothelial cells is post-translational but irreversible. *J Biol Chem, 276*(19), 15776–15782.
16. Parat, M. O., Stachowicz, R. Z., & Fox, P. L. (2002). Oxidative stress inhibits caveolin-1 palmitoylation and trafficking in endothelial cells. *Biochem J, 361*(Pt 3), 681–688. https://doi.org/10.1042/0264-6021:3610681.
17. Li, S., Galbiati, F., Volonte, D., Sargiacomo, M., Engelman, J. A., Das, K., et al. (1998). Mutational analysis of caveolin-induced vesicle formation. Expression of caveolin-1 recruits caveolin-2 to caveolae membranes. *FEBS Lett, 434*(1-2), 127–134. https://doi.org/10.1016/s0014-5793(98)00945-4.
18. Parolini, I., Sargiacomo, M., Galbiati, F., Rizzo, G., Grignani, F., Engelman, J. A., Okamoto, T., Ikezu, T., Scherer, P. E., Mora, R., Rodriguez-Boulan, E., Peschle, C., & Lisanti, M. P. (1999). Expression of caveolin-1 is required for the transport of caveolin-2 to the plasma membrane. Retention of caveolin-2 at the level of the golgi complex. *J Biol Chem, 274*(36), 25718–25725. https://doi.org/10.1074/jbc.274.36.25718.
19. Lee, H., Park, D. S., Razani, B., Russell, R. G., Pestell, R. G., & Lisanti, M. P. (2002). Caveolin-1 mutations (P132L and null) and the pathogenesis of breast cancer: caveolin-1 (P132L) behaves in a dominant-negative manner and caveolin-1 (-/-) null mice show mammary epithelial cell hyperplasia. *Am J Pathol, 161*(4), 1357–1369. https://doi.org/10.1016/S0002-9440(10)64412-4.
20. Scherer, P. E., Okamoto, T., Chun, M., Nishimoto, I., Lodish, H. F., & Lisanti, M. P. (1996). Identification, sequence, and expression of caveolin-2 defines a caveolin gene family. *Proc Natl Acad Sci U S A, 93*(1), 131–135. https://doi.org/10.1073/pnas.93.1.131.
21. Tang, Z., Scherer, P. E., Okamoto, T., Song, K., Chu, C., Kohtz, D. S., Nishimoto, I., Lodish, H. F., & Lisanti, M. P. (1996). Molecular cloning of caveolin-3, a novel member of the caveolin gene family expressed predominantly in muscle. *J Biol Chem, 271*(4), 2255–2261. https://doi.org/10.1074/jbc.271.4.2255.
22. Scherer, P. E., Tang, Z., Chun, M., Sargiacomo, M., Lodish, H. F., & Lisanti, M. P. (1995). Caveolin isoforms differ in their N-terminal protein sequence and subcellular distribution. Identification and epitope mapping of an isoform-specific monoclonal antibody probe. *J Biol Chem, 270*(27), 16395–16401. https://doi.org/10.1074/jbc.270.27.16395.
23. Mastick, C., Sanguinetti, A. R., Knesek, J. H., Mastick, G. S., & Newcomb, L. F. (2001). Caveolin-1 and a 29-kDa caveolin-associated protein are phosphorylated on tyrosine in cells expressing a temperature-sensitive v-Abl kinase. *Exp Cell Res, 266*(1), 142–154. https://doi.org/10.1006/excr.2001.5205.
24. Mastick, C. C., & Saltiel, A. R. (1997). Insulin-stimulated tyrosine phosphorylation of caveolin is specific for the differentiated adipocyte phenotype in 3T3-L1 cells. *J Biol Chem, 272*(33), 20706–20714. https://doi.org/10.1074/jbc.272.33.20706.
25. Monier, S., Parton, R. G., Vogel, F., Behlke, J., Henske, A., & Kurzchalia, T. V. (1995). VIP21-caveolin, a membrane protein constituent of the caveolar coat, oligomerizes in vivo and in vitro. *Mol Biol Cell, 6*(7), 911–927.
26. Sargiacomo, M., Scherer, P. E., Tang, Z., Kubler, E., Song, K. S., Sanders, M. C., & Lisanti, M. P. (1995). Oligomeric structure of

caveolin: implications for caveolae membrane organization. *Proc Natl Acad Sci U S A, 92*(20), 9407–9411.
27. Song, K. S., Tang, Z., Li, S., & Lisanti, M. P. (1997). Mutational analysis of the properties of caveolin-1. A novel role for the C-terminal domain in mediating homo-typic caveolin-caveolin interactions. *J Biol Chem, 272*(7), 4398–4403.
28. Monier, S., Dietzen, D. J., Hastings, W. R., Lublin, D. M., & Kurzchalia, T. V. (1996). Oligomerization of VIP21-caveolin in vitro is stabilized by long chain fatty acylation or cholesterol. *FEBS Lett, 388*(2-3), 143–149.
29. Epand, R. M., Sayer, B. G., & Epand, R. F. (2005). Caveolin scaffolding region and cholesterol-rich domains in membranes. *J Mol Biol, 345*(2), 339–350.
30. Li, S., Couet, J., & Lisanti, M. P. (1996). Src tyrosine kinases, Galpha subunits, and H-Ras share a common membrane-anchored scaffolding protein, caveolin. *Caveolin binding negatively regulates the auto-activation of Src tyrosine kinases. J Biol Chem, 271*(46), 29182–29190.
31. Couet, J., Li, S., Okamoto, T., Ikezu, T., & Lisanti, M. P. (1997). Identification of peptide and protein ligands for the caveolin- scaffolding domain. Implications for the interaction of caveolin with caveolae-associated proteins. *J Biol Chem, 272*(10), 6525–6533.
32. Garcia-Cardena, G., Martasek, P., Masters, B. S., Skidd, P. M., Couet, J., Li, S., et al. (1997). Dissecting the interaction between nitric oxide synthase (NOS) and caveolin. Functional significance of the nos caveolin binding domain in vivo. *J Biol Chem, 272*(41), 25437–25440. https://doi.org/10.1074/jbc.272.41.25437.
33. Li, S., Okamoto, T., Chun, M., Sargiacomo, M., Casanova, J. E., Hansen, S. H., Nishimoto, I., & Lisanti, M. P. (1995). Evidence for a regulated interaction between heterotrimeric G proteins and caveolin. *J Biol Chem, 270*, 15693–15701.
34. Nystrom, F. H., Chen, H., Cong, L. N., Li, Y., & Quon, M. J. (1999). Caveolin-1 interacts with the insulin receptor and can differentially modulate insulin signaling in transfected Cos-7 cells and rat adipose cells. *Mol Endocrinol, 13*(12), 2013–2024.
35. Hoop, C. L., Sivanandam, V. N., Kodali, R., Srnec, M. N., & van der Wel, P. C. (2012). Structural characterization of the caveolin scaffolding domain in association with cholesterol-rich membranes. *Biochemistry, 51*(1), 90–99. https://doi.org/10.1021/bi201356v.
36. Kurzchalia, T. V., Dupree, P., Parton, R. G., Kellner, R., Virta, H., Lehnert, M., & Simons, K. (1992). VIP21, a 21-kD membrane protein is an integral component of trans-Golgi-network-derived transport vesicles. *J Cell Biol, 118*(5), 1003–1014. https://doi.org/10.1083/jcb.118.5.1003.
37. Hill, M. M., Bastiani, M., Luetterforst, R., Kirkham, M., Kirkham, A., Nixon, S. J., Walser, P., Abankwa, D., Oorschot, V. M. J., Martin, S., Hancock, J. F., & Parton, R. G. (2008). PTRF-Cavin, a conserved cytoplasmic protein required for caveola formation and function. *Cell, 132*(1), 113–124.
38. Parton, R. G., McMahon, K.-A., & Wu, Y. (2020). Caveolae: formation, dynamics, and function. *Current Opin Cell Biol, 65*, 8–16. https://doi.org/10.1016/j.ceb.2020.02.001.
39. Nabi, I. R. (2009). Cavin fever: regulating caveolae. *Nat Cell Biol, 11*(7), 789–791.
40. Oh, P., McIntosh, D. P., & Schnitzer, J. E. (1998). Dynamin at the neck of caveolae mediates their budding to form transport vesicles by GTP-driven fission from the plasma membrane of endothelium. *J Cell Biol, 141*(1), 101–114. https://doi.org/10.1083/jcb.141.1.101.
41. Henley, J. R., Krueger, E. W., Oswald, B. J., & McNiven, M. A. (1998). Dynamin-mediated internalization of caveolae. *J Cell Biol, 141*(1), 85–99. https://doi.org/10.1083/jcb.141.1.85.
42. Shajahan, A. N., Timblin, B. K., Sandoval, R., Tiruppathi, C., Malik, A. B., & Minshall, R. D. (2004). Role of Src-induced dynamin-2 phosphorylation in caveolae-mediated endocytosis in endothelial cells. *J Biol Chem, 279*(19), 20392–20400. https://doi.org/10.1074/jbc.M308710200.
43. Morén, B., Shah, C., Howes, M. T., Schieber, N. L., McMahon, H. T., Parton, R. G., Daumke, O., & Lundmark, R. (2012). EHD2 regulates caveolar dynamics via ATP-driven targeting and oligomerization. *Mol Biol Cell, 23*(7), 1316–1329. https://doi.org/10.1091/mbc.E11-09-0787.
44. Shah, C., Hegde, B. G., Morén, B., Behrmann, E., Mielke, T., Moenke, G., Spahn, C. M. T., Lundmark, R., Daumke, O., & Langen, R. (2014). Structural insights into membrane interaction and caveolar targeting of dynamin-like EHD2. *Structure (London, England : 1993), 22*(3), 409–420. https://doi.org/10.1016/j.str.2013.12.015.
45. Stoeber, M., Stoeck, I. K., Hänni, C., Bleck, C. K. E., Balistreri, G., & Helenius, A. (2012). Oligomers of the ATPase EHD2 confine caveolae to the plasma membrane through association with actin. *The EMBO Journal, 31*(10), 2350–2364. https://doi.org/10.1038/emboj.2012.98.
46. Hansen, C. G., Howard, G., & Nichols, B. J. (2011). Pacsin 2 is recruited to caveolae and functions in caveolar biogenesis. *J Cell Sci, 124*(16), 2777–2785. https://doi.org/10.1242/jcs.084319.
47. Senju, Y., Itoh, Y., Takano, K., Hamada, S., & Suetsugu, S. (2011). Essential role of PACSIN2/syndapin-II in caveolae membrane sculpting. *J Cell Sci, 124*(12), 2032–2040. https://doi.org/10.1242/jcs.086264.
48. Senju, Y., Rosenbaum, E., Shah, C., Hamada-Nakahara, S., Itoh, Y., Yamamoto, K., Hanawa-Suetsugu, K., Daumke, O., & Suetsugu, S. (2015). Phosphorylation of PACSIN2 by protein kinase C triggers the removal of caveolae from the plasma membrane. *J Cell Sci, 128*(15), 2766–2780. https://doi.org/10.1242/jcs.167775.
49. Nomura, R., & Fujimoto, T. (1999). Tyrosine-phosphorylated caveolin-1: immunolocalization and molecular characterization. *Mol Biol Cell, 10*(4), 975–986. https://doi.org/10.1091/mbc.10.4.975.
50. Zimnicka, A. M., Husain, Y. S., Shajahan, A. N., Sverdlov, M., Chaga, O., Chen, Z., Toth, P. T., Klomp, J., Karginov, A. V., Tiruppathi, C., Malik, A. B., & Minshall, R. D. (2016). Src-dependent phosphorylation of caveolin-1 Tyr14 promotes swelling and release of caveolae. *Mol Biol Cell, 27*, 2090–2106. https://doi.org/10.1091/mbc.E15-11-0756.
51. Orlichenko, L., Huang, B., Krueger, E., & McNiven, M. A. (2006). Epithelial growth factor-induced phosphorylation of caveolin 1 at tyrosine 14 stimulates caveolae formation in epithelial cells. *J Biol Chem, 281*(8), 4570–4579. https://doi.org/10.1074/jbc.M512088200.
52. Joshi, B., Bastiani, M., Strugnell, S. S., Boscher, C., Parton, R. G., & Nabi, I. R. (2012). Phosphocaveolin-1 is a mechanotransducer that induces caveola biogenesis via Egr1 transcriptional regulation. *J Cell Biol, 199*(3), 425–435. https://doi.org/10.1083/jcb.201207089.
53. Lajoie, P., Goetz, J. G., Dennis, J. W., & Nabi, I. R. (2009). Lattices, rafts, and scaffolds: domain regulation of receptor signaling at the plasma membrane. *J Cell Biol, 185*(3), 381–385.
54. Gabor, K. A., Kim, D., Kim, C. H., & Hess, S. T. (2015). Nanoscale imaging of caveolin-1 membrane domains in vivo. *PLoS One, 10*(2), e0117225. https://doi.org/10.1371/journal.pone.0117225.
55. Tachikawa, M., Morone, N., Senju, Y., Sugiura, T., Hanawa-Suetsugu, K., Mochizuki, A., & Suetsugu, S. (2017). Measurement of caveolin-1 densities in the cell membrane for quantification of caveolar deformation after exposure to hypotonic membrane tension. *Scientific Reports, 7*(1), 7794. https://doi.org/10.1038/s41598-017-08259-5.
56. Khater, I. M., Liu, Q., Chou, K. C., Hamarneh, G., & Nabi, I. R. (2019). Super-resolution modularity analysis shows polyhedral

caveolin-1 oligomers combine to form scaffolds and caveolae. *Scientific Reports, 9*(1), 9888. https://doi.org/10.1038/s41598-019-46174-z.
57. Khater, I. M., Meng, F., Wong, T. H., Nabi, I. R., & Hamarneh, G. (2018). Super resolution network analysis defines the molecular architecture of caveolae and caveolin-1 scaffolds. *Scientific Reports, 8*(1), 9009. https://doi.org/10.1038/s41598-018-27216-4.
58. Stoeber, M., Schellenberger, P., Siebert, C. A., Leyrat, C., Helenius, A., & Grunewald, K. (2016). Model for the architecture of caveolae based on a flexible, net-like assembly of Cavin1 and Caveolin discs. *Proc Natl Acad Sci U S A, 113*(50), E8069–E8078. https://doi.org/10.1073/pnas.1616838113.
59. Ludwig, A., Nichols, B. J., & Sandin, S. (2016). Architecture of the caveolar coat complex. *J Cell Sci, 129*(16), 3077–3083. https://doi.org/10.1242/jcs.191262.
60. Hayer, A., Stoeber, M., Bissig, C., & Helenius, A. (2010). Biogenesis of caveolae: stepwise assembly of large caveolin and cavin complexes. *Traffic, 11*(3), 361–382. https://doi.org/10.1111/j.1600-0854.2009.01023.x.
61. Khater, I., Aroca-Ouellette, S., Meng, F., Nabi, I., & Hamarneh, G. (2019). Caveolae and scaffold detection from single molecule localization microscopy data using deep learning. *PLoS One, 14*(8), e0211659.
62. Khater, I. M., Meng, F., Nabi, I. R., & Hamarneh, G. (2019). Identification of caveolin-1 domain signatures via graphlet analysis of single molecule super-resolution data. *Bioinformatics, 35*(18), 3468–3475.
63. Engelman, J. A., Wykoff, C. C., Yasuhara, S., Song, K. S., Okamoto, T., & Lisanti, M. P. (1997). Recombinant expression of caveolin-1 in oncogenically transformed cells abrogates anchorage-independent growth. *J Biol Chem, 272*(26), 16374–16381.
64. Koleske, A. J., Baltimore, D., & Lisanti, M. P. (1995). Reduction of caveolin and caveolae in oncogenically transformed cells. *Proc Natl Acad Sci U S A, 92*, 1381–1385.
65. Bender, F. C., Reymond, M. A., Bron, C., & Quest, A. F. (2000). Caveolin-1 levels are down-regulated in human colon tumors, and ectopic expression of caveolin-1 in colon carcinoma cell lines reduces cell tumorigenicity. *Cancer Research, 60*(20), 5870–5878.
66. Quest, A. F., Leyton, L., & Parraga, M. (2004). Caveolins, caveolae, and lipid rafts in cellular transport, signaling, and disease. *Biochem Cell Biol, 82*(1), 129–144. https://doi.org/10.1139/o03-071.
67. Joshi, B., Strugnell, S. S., Goetz, J. G., Kojic, L. D., Cox, M. E., Griffith, O. L., Chan, S. K., Jones, S. J., Leung, S. P., Masoudi, H., Leung, S., Wiseman, S. M., & Nabi, I. R. (2008). Phosphorylated caveolin-1 regulates Rho/ROCK-dependent focal adhesion dynamics and tumor cell migration and invasion. *Cancer Res, 68*(20), 8210–8220. https://doi.org/10.1158/0008-5472.CAN-08-0343.
68. Savage, K., Lambros, M. B., Robertson, D., Jones, R. L., Jones, C., Mackay, A., et al. (2007). Caveolin 1 is overexpressed and amplified in a subset of basal-like and metaplastic breast carcinomas: a morphologic, ultrastructural, immunohistochemical, and in situ hybridization analysis. *Clin Cancer Res, 13*(1), 90–101.
69. Xu, K., Usary, J., Kousis, P. C., Prat, A., Wang, D.-Y., Adams, J. R., Wang, W., Loch, A. J., Deng, T., Zhao, W., Cardiff, R. D., Yoon, K., Gaiano, N., Ling, V., Beyene, J., Zacksenhaus, E., Gridley, T., Leong, W. L., Guidos, C. J., Perou, C. M., & Egan, S. E. (2012). Lunatic fringe deficiency cooperates with the Met/caveolin gene amplicon to induce basal-like breast cancer. *Cancer Cell, 21*(5), 626–641.
70. Williams, T. M., & Lisanti, M. P. (2005). Caveolin-1 in oncogenic transformation, cancer, and metastasis. *Am J Physiol Cell Physiol, 288*(3), C494–C506.
71. Goetz, J. G., Lajoie, P., Wiseman, S. M., & Nabi, I. R. (2008). Caveolin-1 in tumor progression: the good, the bad and the ugly. *Cancer Metastasis Rev, 27*(4), 715–735.
72. Nunez-Wehinger, S., Ortiz, R. J., Diaz, N., Diaz, J., Lobos-Gonzalez, L., & Quest, A. F. (2014). Caveolin-1 in cell migration and metastasis. *Curr Mol Med, 14*(2), 255–274.
73. Sotgia, F., Martinez-Outschoorn, U. E., Pavlides, S., Howell, A., Pestell, R. G., & Lisanti, M. P. (2011). Understanding the Warburg effect and the prognostic value of stromal caveolin-1 as a marker of a lethal tumor microenvironment. *Breast Cancer Res, 13*(4), 213. https://doi.org/10.1186/bcr2892.
74. Witkiewicz, A. K., Dasgupta, A., Sotgia, F., Mercier, I., Pestell, R. G., Sabel, M., Kleer, C. G., Brody, J. R., & Lisanti, M. P. (2009). An absence of stromal caveolin-1 expression predicts early tumor recurrence and poor clinical outcome in human breast cancers. *Am J Pathol, 174*(6), 2023–2034.
75. Moon, H., Lee, C. S., Inder, K. L., Sharma, S., Choi, E., Black, D. M., et al. (2014). PTRF/cavin-1 neutralizes non-caveolar caveolin-1 microdomains in prostate cancer. [Original Article]. *Oncogene, 33*(27), 3561–3570. https://doi.org/10.1038/onc.2013.315.
76. Aung, C. S., Hill, M. M., Bastiani, M., Parton, R. G., & Parat, M. O. (2011). PTRF-cavin-1 expression decreases the migration of PC3 prostate cancer cells: role of matrix metalloprotease 9. *Eur J Cell Biol, 90*(2-3), 136–142.
77. Meng, F., Joshi, B., & Nabi, I. R. (2015). Galectin-3 overrides PTRF/Cavin-1 reduction of PC3 prostate cancer cell migration. *PLoS One, 10*(5), e0126056. https://doi.org/10.1371/journal.pone.0126056.
78. Meng, F., Saxena, S., Liu, Y., Joshi, B., Wong, T. H., Shankar, J., Foster, L. J., Bernatchez, P., & Nabi, I. R. (2017). The phospho-caveolin-1 scaffolding domain dampens force fluctuations in focal adhesions and promotes cancer cell migration. *Mol Biol Cell, 28*(16), 2190–2201. https://doi.org/10.1091/mbc.E17-05-0278.
79. Yamaguchi, T., Hayashi, M., Ida, L., Yamamoto, M., Lu, C., Kajino, T., Cheng, J., Nakatochi, M., Isomura, H., Yamazaki, M., Suzuki, M., Fujimoto, T., & Takahashi, T. (2019). ROR1-CAVIN3 interaction required for caveolae-dependent endocytosis and pro-survival signaling in lung adenocarcinoma. *Oncogene, 38*(26), 5142–5157. https://doi.org/10.1038/s41388-019-0785-7.
80. Yamaguchi, T., Lu, C., Ida, L., Yanagisawa, K., Usukura, J., Cheng, J., Hotta, N., Shimada, Y., Isomura, H., Suzuki, M., Fujimoto, T., & Takahashi, T. (2016). ROR1 sustains caveolae and survival signalling as a scaffold of cavin-1 and caveolin-1. *Nat Commun, 7*, 10060. https://doi.org/10.1038/ncomms10060.
81. Hill, M. M., Scherbakov, N., Schiefermeier, N., Baran, J., Hancock, J. F., Huber, L. A., Parton, R. G., & Parat, M. O. (2007). Reassessing the role of phosphocaveolin-1 in cell adhesion and migration. *Traffic, 8*(12), 1695–1705. https://doi.org/10.1111/j.1600-0854.2007.00653.x.
82. Goetz, J. G., Joshi, B., Lajoie, P., Strugnell, S. S., Scudamore, T., Kojic, L. D., & Nabi, I. R. (2008). Concerted regulation of focal adhesion dynamics by galectin-3 and tyrosine-phosphorylated caveolin-1. *J Cell Biol, 180*(6), 1261–1275. https://doi.org/10.1083/jcb.200709019.
83. Grande-García, A., Echarri, A., de Rooij, J., Alderson, N. B., Waterman-Storer, C. M., Valdivielso, J. M., et al. (2007). Caveolin-1 regulates cell polarization and directional migration through Src kinase and Rho GTPases. *J Cell Biol, 177*(4), 683–694. https://doi.org/10.1083/jcb.200701006.
84. Shatz, M., Lustig, G., Reich, R., & Liscovitch, M. (2010). Caveolin-1 mutants P132L and Y14F are dominant negative regulators of invasion, migration and aggregation in H1299 lung cancer cells. *Exp Cell Res, 316*(10), 1748–1762. https://doi.org/10.1016/j.yexcr.2010.02.006.
85. Ortiz, R., Diaz, J., Diaz, N., Lobos-Gonzalez, L., Cardenas, A., Contreras, P., et al. (2016). Extracellular matrix-specific Caveolin-

1 phosphorylation on tyrosine 14 is linked to augmented melanoma metastasis but not tumorigenesis. *Oncotarget, 7*(26), 40571–40593. https://doi.org/10.18632/oncotarget.9738.
86. Urra, H., Torres, V. A., Ortiz, R. J., Lobos, L., Diaz, M. I., Diaz, N., et al. (2012). Caveolin-1-enhanced motility and focal adhesion turnover require tyrosine-14 but not accumulation to the rear in metastatic cancer cells. *PLoS One, 7*(4), e33085. https://doi.org/10.1371/journal.pone.0033085.
87. Joshi, B., Pawling, J., Shankar, J., Pacholczyk, K., Kim, Y., Tran, W., et al. (2019). Caveolin-1 Y14 phosphorylation suppresses tumor growth while promoting invasion. *Oncotarget, 10*(62), 6668–6677. https://doi.org/10.18632/oncotarget.27313.
88. Faggi, F., Mitola, S., Sorci, G., Riuzzi, F., Donato, R., Codenotti, S., Poliani, P. L., Cominelli, M., Vescovi, R., Rossi, S., Calza, S., Colombi, M., Penna, F., Costelli, P., Perini, I., Sampaolesi, M., Monti, E., & Fanzani, A. (2014). Phosphocaveolin-1 enforces tumor growth and chemoresistance in rhabdomyosarcoma. *PLoS One, 9*(1), e84618. https://doi.org/10.1371/journal.pone.0084618.
89. Díaz-Valdivia, N. I., Díaz, J., Contreras, P., Campos, A., Rojas-Celis, V., Burgos-Ravanal, R. A., Lobos-González, L., Torres, V. A., Perez, V. I., Frei, B., Leyton, L., & Quest, A. F. G. (2020). The non-receptor tyrosine phosphatase type 14 blocks caveolin-1-enhanced cancer cell metastasis. *Oncogene, Epub, 39*, 3693–3709. https://doi.org/10.1038/s41388-020-1242-3.
90. Lavie, Y., Fiucci, G., & Liscovitch, M. (2001). Upregulation of caveolin in multidrug resistant cancer cells: functional implications. *Adv Drug Deliv Rev, 49*(3), 317–323.
91. Shatz, M., & Liscovitch, M. (2004). Caveolin-1 and cancer multidrug resistance: coordinate regulation of pro-survival proteins? *Leuk Res, 28*(9), 907–908.
92. Hehlgans, S., & Cordes, N. (2011). Caveolin-1: an essential modulator of cancer cell radio-and chemoresistance. *Am J Cancer Res, 1*(4), 521–530.
93. Cordes, N., Frick, S., Brunner, T. B., Pilarsky, C., Grutzmann, R., Sipos, B., et al. (2007). Human pancreatic tumor cells are sensitized to ionizing radiation by knockdown of caveolin-1. *Oncogene, 26*(48), 6851–6862. https://doi.org/10.1038/sj.onc.1210498.
94. Shajahan, A. N., Wang, A., Decker, M., Minshall, R. D., Liu, M. C., & Clarke, R. (2007). Caveolin-1 tyrosine phosphorylation enhances paclitaxel-mediated cytotoxicity. *J. Biol. Chem., 282*(8), 5934–5943. https://doi.org/10.1074/jbc.M608857200.
95. Volonte, D., & Galbiati, F. (2020). Caveolin-1, a master regulator of cellular senescence *Cancer Met Rev, In press*.
96. Bartholomew, J. N., Volonte, D., & Galbiati, F. (2009). Caveolin-1 regulates the antagonistic pleiotropic properties of cellular senescence through a novel Mdm2/p53-mediated pathway. *Cancer Res, 69*(7), 2878–2886. https://doi.org/10.1158/0008-5472.CAN-08-2857.
97. Volonte, D., Zhang, K., Lisanti, M. P., & Galbiati, F. (2002). Expression of caveolin-1 induces premature cellular senescence in primary cultures of murine fibroblasts. *Mol Biol Cell, 13*(7), 2502–2517. https://doi.org/10.1091/mbc.01-11-0529.
98. Beardsley, A., Fang, K., Mertz, H., Castranova, V., Friend, S., & Liu, J. (2005). Loss of caveolin-1 polarity impedes endothelial cell polarization and directional movement. *J Biol Chem, 280*(5), 3541–3547. https://doi.org/10.1074/jbc.M409040200.
99. Parat, M. O., Anand-Apte, B., & Fox, P. L. (2003). Differential caveolin-1 polarization in endothelial cells during migration in two and three dimensions. *Mol Biol Cell, 14*(8), 3156–3168. https://doi.org/10.1091/mbc.e02-11-0761.
100. Podar, K., Shringarpure, R., Tai, Y. T., Simoncini, M., Sattler, M., Ishitsuka, K., Richardson, P. G., Hideshima, T., Chauhan, D., & Anderson, K. C. (2004). Caveolin-1 is required for vascular endothelial growth factor-triggered multiple myeloma cell migration and is targeted by bortezomib. *Cancer Res, 64*(20), 7500–7506. https://doi.org/10.1158/0008-5472.CAN-04-0124.
101. Sun, X. H., Flynn, D. C., Castranova, V., Millecchia, L. L., Beardsley, A. R., & Liu, J. (2007). Identification of a novel domain at the N terminus of caveolin-1 that controls rear polarization of the protein and caveolae formation. *J Biol Chem, 282*(10), 7232–7241.
102. Jia, Z., Barbier, L., Stuart, H., Amraei, M., Pelech, S., Dennis, J. W., Metalnikov, P., O'Donnell, P., & Nabi, I. R. (2005). Tumor cell pseudopodial protrusions. Localized signaling domains coordinating cytoskeleton remodeling, cell adhesion, glycolysis, RNA translocation, and protein translation. *J Biol Chem, 280*(34), 30564–30573.
103. Kojic, L. D., Joshi, B., Lajoie, P., Le, P. U., Leung, S., Cox, M. E., et al. (2007). Raft-dependent endocytosis of autocrine motility factor is phosphatidylinositol-3-kinase-dependent in breast carcinoma cells. *J Biol Chem, 282*(40), 29305–29313.
104. Raikar, L. S., Vallejo, J., Lloyd, P. G., & Hardin, C. D. (2006). Overexpression of caveolin-1 results in increased plasma membrane targeting of glycolytic enzymes: the structural basis for a membrane associated metabolic compartment. *J Cell Biochem, 98*(4), 861–871. https://doi.org/10.1002/jcb.20732.
105. Beckner, M. E., Stracke, M. L., Liotta, L. A., & Schiffmanm, E. (1990). Glycolysis as primary energy source in tumor cell chemotaxis. *J Natl Canc Inst, 82*, 1836–1840.
106. Nguyen, T. N., Wang, H. J., Zalzal, S., Nanci, A., & Nabi, I. R. (2000). Purification and characterization of beta-actin-rich tumor cell pseudopodia: role of glycolysis. *Exp. Cell Res., 258*(1), 171–183.
107. Ha, T. K., Her, N. G., Lee, M. G., Ryu, B. K., Lee, J. H., Han, J., Jeong, S. I., Kang, M. J., Kim, N. H., Kim, H. J., & Chi, S. G. (2012). Caveolin-1 increases aerobic glycolysis in colorectal cancers by stimulating HMGA1-mediated GLUT3 transcription. *Cancer Res, 72*(16), 4097–4109. https://doi.org/10.1158/0008-5472.CAN-12-0448.
108. Fiala, G. J., & Minguet, S. (2018). Chapter three—caveolin-1: the unnoticed player in TCR and BCR signaling. In F. Alt (Ed.), *Adv Immunol* (Vol. 137, pp. 83-133): Academic Press.
109. Boscher, C., & Nabi, I. R. (2012). Caveolin-1: role in cell signaling. *Adv Exp Med Biol, 729*, 29–50.
110. Lisanti, M. P., Scherer, P. E., Tang, Z., & Sargiacomo, M. (1994). Caveolae, caveolin and caveolin-rich membrane domains: a signalling hypothesis. *Trends Cell Biol, 4*, 231–235.
111. Couet, J., Sargiacomo, M., & Lisanti, M. P. (1997). Interaction of a receptor tyrosine kinase, EGF-R, with caveolins. Caveolin binding negatively regulates tyrosine and serine/threonine kinase activities. *J Biol Chem, 272*(48), 30429–30438. https://doi.org/10.1074/jbc.272.48.30429.
112. Agelaki, S., Spiliotaki, M., Markomanolaki, H., Kallergi, G., Mavroudis, D., Georgoulias, V., & Stournaras, C. (2009). Caveolin-1 regulates EGFR signaling in MCF-7 breast cancer cells and enhances gefitinib-induced tumor cell inhibition. *Cancer Biol Ther, 8*(15), 1470–1477. https://doi.org/10.4161/cbt.8.15.8939.
113. Mineo, C., James, G. L., Smart, E. J., & Anderson, R. G. (1996). Localization of epidermal growth factor-stimulated Ras/Raf-1 interaction to caveolae membrane. *J Biol Chem, 271*(20), 11930–11935. https://doi.org/10.1074/jbc.271.20.11930.
114. Lajoie, P., Partridge, E. A., Guay, G., Goetz, J. G., Pawling, J., Lagana, A., Joshi, B., Dennis, J. W., & Nabi, I. R. (2007). Plasma membrane domain organization regulates EGFR signaling in tumor cells. *J Cell Biol, 179*(2), 341–356. https://doi.org/10.1083/jcb.200611106.
115. Nabi, I. R., Shankar, J., & Dennis, J. W. (2015). The galectin lattice at a glance. *J Cell Sci, 128*(13), 2213–2219. https://doi.org/10.1242/jcs.151159.

116. Nangia-Makker, P., Hogan, V., & Raz, A. (2018). Galectin-3 and cancer stemness. *Glycobiology, 28*(4), 172–181. https://doi.org/10.1093/glycob/cwy001.
117. Kim, Y. N., Wiepz, G. J., Guadarrama, A. G., & Bertics, P. J. (2000). Epidermal growth factor-stimulated tyrosine phosphorylation of caveolin-1. Enhanced caveolin-1 tyrosine phosphorylation following aberrant epidermal growth factor receptor status. *J Biol Chem, 275*(11), 7481–7491. https://doi.org/10.1074/jbc.275.11.7481.
118. Boscher, C., & Nabi, I. R. (2013). Galectin-3- and phospho-caveolin-1-dependent outside-in integrin signaling mediates the EGF motogenic response in mammary cancer cells. *Mol Biol Cell, 24*(13), 2134–2145. https://doi.org/10.1091/mbc.E13-02-0095.
119. Hoffmann, C., Berking, A., Agerer, F., Buntru, A., Neske, F., Chhatwal, G. S., Ohlsen, K., & Hauck, C. R. (2010). Caveolin limits membrane microdomain mobility and integrin-mediated uptake of fibronectin-binding pathogens. *J Cell Sci, 123*(Pt 24), 4280–4291. https://doi.org/10.1242/jcs.064006.
120. del Pozo, M. A., Balasubramanian, N., Alderson, N. B., Kiosses, W. B., Grande-Garcia, A., Anderson, R. G., et al. (2005). Phospho-caveolin-1 mediates integrin-regulated membrane domain internalization. *Nature cell biology, 7*(9), 901–908.
121. Lakshminarayan, R., Wunder, C., Becken, U., Howes, M. T., Benzing, C., Arumugam, S., Sales, S., Ariotti, N., Chambon, V., Lamaze, C., Loew, D., Shevchenko, A., Gaus, K., Parton, R. G., & Johannes, L. (2014). Galectin-3 drives glycosphingolipid-dependent biogenesis of clathrin-independent carriers. *Nature cell biology, 16*(6), 595–606.
122. Chaudhary, N., Gomez, G. A., Howes, M. T., Lo, H. P., McMahon, K. A., Rae, J. A., Schieber, N. L., Hill, M. M., Gaus, K., Yap, A. S., & Parton, R. G. (2014). Endocytic crosstalk: cavins, caveolins, and caveolae regulate clathrin-independent endocytosis. *PLoS Biol, 12*(4), e1001832. https://doi.org/10.1371/journal.pbio.1001832.
123. Le, P. U., Guay, G., Altschuler, Y., & Nabi, I. R. (2002). Caveolin-1 is a negative regulator of caveolae-mediated endocytosis to the endoplasmic reticulum. *J Biol Chem, 277*(5), 3371–3379.
124. Nabi, I. R., & Le, P. U. (2003). Caveolae/raft-dependent endocytosis. *J Cell Biol, 161*(4), 673–677.
125. Devreotes, P., & Horwitz, A. R. (2015). Signaling networks that regulate cell migration. *Cold Spring Harbor perspectives in biology, 7*(8), a005959–a005959. https://doi.org/10.1101/cshperspect.a005959.
126. Cao, H., Courchesne, W. E., & Mastick, C. C. (2002). A phosphotyrosine-dependent protein interaction screen reveals a role for phosphorylation of caveolin-1 on tyrosine 14: recruitment of C-terminal Src kinase. *J Biol Chem, 277*(11), 8771–8774. https://doi.org/10.1074/jbc.C100661200.
127. Gottlieb-Abraham, E., Shvartsman, D. E., Donaldson, J. C., Ehrlich, M., Gutman, O., Martin, G. S., & Henis, Y. I. (2013). Src-mediated caveolin-1 phosphorylation affects the targeting of active Src to specific membrane sites. *Mol Biol Cell, 24*(24), 3881–3895. https://doi.org/10.1091/mbc.E13-03-0163.
128. Chodniewicz, D., & Klemke, R. L. (2004). Regulation of integrin-mediated cellular responses through assembly of a CAS/Crk scaffold. *Biochim Biophys Acta, 1692*(2-3), 63–76. https://doi.org/10.1016/j.bbamcr.2004.03.006.
129. Nethe, M., Anthony, E. C., Fernandez-Borja, M., Dee, R., Geerts, D., Hensbergen, P. J., Deelder, A. M., Schmidt, G., & Hordijk, P. L. (2010). Focal-adhesion targeting links caveolin-1 to a Rac1-degradation pathway. *J Cell Sci, 123*(Pt 11), 1948–1958. https://doi.org/10.1242/jcs.062919.
130. Gaus, K., Le Lay, S., Balasubramanian, N., & Schwartz, M. A. (2006). Integrin-mediated adhesion regulates membrane order. *J Cell Biol, 174*(5), 725–734. https://doi.org/10.1083/jcb.200603034.
131. Mitchison, T., & Kirschner, M. (1988). Cytoskeletal dynamics and nerve growth. *Neuron, 1*(9), 761–772.
132. Case, L. B., & Waterman, C. M. (2015). Integration of actin dynamics and cell adhesion by a three-dimensional, mechanosensitive molecular clutch. [Review]. *Nature cell biology, 17*(8), 955–963. https://doi.org/10.1038/ncb3191.
133. Murphy, D. A., & Courtneidge, S. A. (2011). The 'ins' and 'outs' of podosomes and invadopodia: characteristics, formation and function. *Nature reviews. Molecular cell biology, 12*(7), 413–426. https://doi.org/10.1038/nrm3141.
134. Hoshino, D., Jourquin, J., Emmons, S. W., Miller, T., Goldgof, M., Costello, K., et al. (2012). Network analysis of the focal adhesion to invadopodia transition identifies a PI3K-PKCα invasive signaling axis. *Science Signaling, 5*(241), ra66. https://doi.org/10.1126/scisignal.2002964.
135. Yamaguchi, H., Takeo, Y., Yoshida, S., Kouchi, Z., Nakamura, Y., & Fukami, K. (2009). Lipid rafts and caveolin-1 are required for invadopodia formation and extracellular matrix degradation by human breast cancer cells. *Cancer Research, 69*(22), 8594–8602. https://doi.org/10.1158/0008-5472.CAN-09-2305.
136. Yang, H., Guan, L., Li, S., Jiang, Y., Xiong, N., Li, L., et al. (2016). Mechanosensitive caveolin-1 activation-induced PI3K/Akt/mTOR signaling pathway promotes breast cancer motility, invadopodia formation and metastasis in vivo. *Oncotarget, 7*(13), 16227–16247. https://doi.org/10.18632/oncotarget.7583.
137. Brisson, L., Driffort, V., Benoist, L., Poet, M., Counillon, L., Antelmi, E., et al. (2013). NaV1.5 Na + channels allosterically regulate the NHE-1 exchanger and promote the activity of breast cancer cell invadopodia. *J Cell Sci, 126*(21), 4835. https://doi.org/10.1242/jcs.123901.
138. Albrechtsen, R., Stautz, D., Sanjay, A., Kveiborg, M., & Wewer, U. M. (2011). Extracellular engagement of ADAM12 induces clusters of invadopodia with localized ectodomain shedding activity. *Exp Cell Res, 317*(2), 195–209. https://doi.org/10.1016/j.yexcr.2010.10.003.
139. Caldieri, G., Giacchetti, G., Beznoussenko, G., Attanasio, F., Ayala, I., & Buccione, R. (2009). Invadopodia biogenesis is regulated by caveolin-mediated modulation of membrane cholesterol levels. *J Cell Molec Medicine, 13*(8B), 1728–1740. https://doi.org/10.1111/j.1582-4934.2008.00568.x.
140. Fiedler, E. C., & Hemann, M. T. (2019). Aiding and abetting: how the tumor microenvironment protects cancer from chemotherapy. *Ann Rev Cancer Biol, 3*(1), 409–428. https://doi.org/10.1146/annurev-cancerbio-030518-055524.
141. Papalazarou, V., Salmeron-Sanchez, M., & Machesky, L. M. (2018). Tissue engineering the cancer microenvironment-challenges and opportunities. *Biophys Rev, 10*(6), 1695–1711. https://doi.org/10.1007/s12551-018-0466-8.
142. Volonte, D., Galbiati, F., Pestell, R. G., & Lisanti, M. P. (2001). Cellular stress induces the tyrosine phosphorylation of caveolin-1 (Tyr(14)) via activation of p38 mitogen-activated protein kinase and c-Src kinase. Evidence for caveolae, the actin cytoskeleton, and focal adhesions as mechanical sensors of osmotic stress. *J Biol Chem, 276*(11), 8094–8103. https://doi.org/10.1074/jbc.M009245200.
143. Radel, C., Carlile-Klusacek, M., & Rizzo, V. (2007). Participation of caveolae in beta1 integrin-mediated mechanotransduction. *Biochem Biophys Res Commun, 358*(2), 626–631.
144. Radel, C., & Rizzo, V. (2005). Integrin mechanotransduction stimulates caveolin-1 phosphorylation and recruitment of Csk to mediate actin reorganization. *Am J Physiol Heart Circ Physiol, 288*(2), H936–H945.
145. Xiong, N., Li, S., Tang, K., Bai, H., Peng, Y., Yang, H., Wu, C., & Liu, Y. (2017). Involvement of caveolin-1 in low shear stress-

induced breast cancer cell motility and adhesion: roles of FAK/Src and ROCK/p-MLC pathways. *Biochim Biophys Acta, 1864*(1), 12–22. https://doi.org/10.1016/j.bbamcr.2016.10.013.

146. Moreno-Vicente, R., Pavon, D. M., Martin-Padura, I., Catala-Montoro, M., Diez-Sanchez, A., Quilez-Alvarez, A., et al. (2018). Caveolin-1 modulates mechanotransduction responses to substrate stiffness through actin-dependent control of YAP. *Cell Rep, 25*(6), 1622–1635 e1626. https://doi.org/10.1016/j.celrep.2018.10.024.
147. Yeh, Y. C., Ling, J. Y., Chen, W. C., Lin, H. H., & Tang, M. J. (2017). Mechanotransduction of matrix stiffness in regulation of focal adhesion size and number: reciprocal regulation of caveolin-1 and beta1 integrin. *Scientific reports, 7*(1), 15008. https://doi.org/10.1038/s41598-017-14932-6.
148. Peng, F., Wu, D., Ingram, A. J., Zhang, B., Gao, B., & Krepinsky, J. C. (2007). RhoA activation in mesangial cells by mechanical strain depends on caveolae and caveolin-1 interaction. *J Am Soc Nephrol, 18*(1), 189–198. https://doi.org/10.1681/ASN.2006050498.
149. Zhang, B., Peng, F., Wu, D., Ingram, A. J., Gao, B., & Krepinsky, J. C. (2007). Caveolin-1 phosphorylation is required for stretch-induced EGFR and Akt activation in mesangial cells. *Cell Signal, 19*(8), 1690–1700.
150. Cheng, J. P., Mendoza-Topaz, C., Howard, G., Chadwick, J., Shvets, E., Cowburn, A. S., et al. (2015). Caveolae protect endothelial cells from membrane rupture during increased cardiac output. *J Cell Biol, 211*(1), 53–61. https://doi.org/10.1083/jcb.201504042.
151. Gervasio, O. L., Phillips, W. D., Cole, L., & Allen, D. G. (2011). Caveolae respond to cell stretch and contribute to stretch-induced signaling. *J Cell Sci, 124*(Pt 21), 3581–3590. https://doi.org/10.1242/jcs.084376.
152. Lim, Y. W., Lo, H. P., Ferguson, C., Martel, N., Giacomotto, J., Gomez, G. A., Yap, A. S., Hall, T. E., & Parton, R. G. (2017). Caveolae protect notochord cells against catastrophic mechanical failure during development. *Curr Biol, 27*(13), 1968–1981 e1967. https://doi.org/10.1016/j.cub.2017.05.067.
153. Lo, H. P., Nixon, S. J., Hall, T. E., Cowling, B. S., Ferguson, C., Morgan, G. P., Schieber, N. L., Fernandez-Rojo, M. A., Bastiani, M., Floetenmeyer, M., Martel, N., Laporte, J., Pilch, P. F., & Parton, R. G. (2015). The caveolin-cavin system plays a conserved and critical role in mechanoprotection of skeletal muscle. *J Cell Biol, 210*(5), 833–849. https://doi.org/10.1083/jcb.201501046.
154. Sinha, B., Koster, D., Ruez, R., Gonnord, P., Bastiani, M., Abankwa, D., et al. (2010). Cells respond to mechanical stress by rapid disassembly of caveolae. *Cell, 144*(3), 402–413.
155. Garcia, J., Bagwell, J., Njaine, B., Norman, J., Levic, D. S., Wopat, S., Miller, S. E., Liu, X., Locasale, J. W., Stainier, D. Y. R., & Bagnat, M. (2017). Sheath cell invasion and trans-differentiation repair mechanical damage caused by loss of caveolae in the zebrafish notochord. *Curr Biol, 27*(13), 1982–1989 e1983. https://doi.org/10.1016/j.cub.2017.05.035.
156. Parton, R. G., & del Pozo, M. A. (2013). Caveolae as plasma membrane sensors, protectors and organizers. *Nat Rev Mol Cell Biol, 14*(2), 98–112.
157. Rausch, V., Bostrom, J. R., Park, J., Bravo, I. R., Feng, Y., Hay, D. C., Link, B. A., & Hansen, C. G. (2019). The hippo pathway regulates caveolae expression and mediates flow response via caveolae. *Curr Biol, 29*(2), 242–255 e246. https://doi.org/10.1016/j.cub.2018.11.066.
158. Torrino, S., Shen, W. W., Blouin, C. M., Mani, S. K., Viaris de Lesegno, C., Bost, P., Grassart, A., Köster, D., Valades-Cruz, C. A., Chambon, V., Johannes, L., Pierobon, P., Soumelis, V., Coirault, C., Vassilopoulos, S., & Lamaze, C. (2018). EHD2 is a mechanotransducer connecting caveolae dynamics with gene transcription. *J Cell Biol, 217*, 4029–4015. https://doi.org/10.1083/jcb.201801122.
159. Sanguinetti, A. R., & Mastick, C. C. (2003). c-Abl is required for oxidative stress-induced phosphorylation of caveolin-1 on tyrosine 14. *Cell Signal, 15*(3), 289–298. https://doi.org/10.1016/s0898-6568(02)00090-6.
160. Aoki, T., Nomura, R., & Fujimoto, T. (1999). Tyrosine phosphorylation of caveolin-1 in the endothelium. *Exp Cell Res, 253*(2), 629–636. https://doi.org/10.1006/excr.1999.4652.
161. Percy, C. J., Pat, B. K., Healy, H., Johnson, D. W., & Gobe, G. C. (2008). Phosphorylation of caveolin-1 is anti-apoptotic and promotes cell attachment during oxidative stress of kidney cells. *Pathology, 40*(7), 694–701. https://doi.org/10.1080/00313020802436402.
162. Khan, E. M., Heidinger, J. M., Levy, M., Lisanti, M. P., Ravid, T., & Goldkorn, T. (2006). Epidermal growth factor receptor exposed to oxidative stress undergoes Src- and caveolin-1-dependent perinuclear trafficking. *J. Biol. Chem., 281*(20), 14486–14493. https://doi.org/10.1074/jbc.M509332200.
163. Sun, Y., Hu, G., Zhang, X., & Minshall, R. D. (2009). Phosphorylation of caveolin-1 regulates oxidant-induced pulmonary vascular permeability via paracellular and transcellular pathways. *Circ Res, 105*(7), 676-685, 615 p following 685, doi:https://doi.org/10.1161/CIRCRESAHA.109.201673.
164. Dasari, A., Bartholomew, J. N., Volonte, D., & Galbiati, F. (2006). Oxidative stress induces premature senescence by stimulating caveolin-1 gene transcription through p38 mitogen-activated protein kinase/Sp1-mediated activation of two GC-rich promoter elements. *Cancer Res, 66*(22), 10805–10814.
165. Bourseau-Guilmain, E., Menard, J. A., Lindqvist, E., Indira Chandran, V., Christianson, H. C., Cerezo Magana, M., et al. (2016). Hypoxia regulates global membrane protein endocytosis through caveolin-1 in cancer cells. *Nat Commun, 7*, 11371. https://doi.org/10.1038/ncomms11371.
166. Castillo Bennett, J., Silva, P., Martinez, S., Torres, V. A., & Quest, A. F. G. (2018). Hypoxia-Induced caveolin-1 expression promotes migration and invasion of tumor cells. *Curr Mol Med, 18*(4), 199–206. https://doi.org/10.2174/1566524018666180926163218.
167. Chandran, V. I., Mansson, A. S., Barbachowska, M., Cerezo-Magana, M., Nodin, B., Joshi, B., et al. (2020). Hypoxia attenuates trastuzumab uptake and trastuzumab-emtansine (T-DM1) cytotoxicity through redistribution of phosphorylated caveolin-1. *Mol Cancer Res, Epub*, doi:https://doi.org/10.1158/1541-7786.MCR-19-0856, 18, 644, 656.
168. Boothe, T., Lim, G. E., Cen, H., Skovso, S., Piske, M., Li, S. N., et al. (2016). Inter-domain tagging implicates caveolin-1 in insulin receptor trafficking and Erk signaling bias in pancreatic beta-cells. *Mol Metab, 5*(5), 366–378. https://doi.org/10.1016/j.molmet.2016.01.009.
169. Collins, B. M., Davis, M. J., Hancock, J. F., & Parton, R. G. (2012). Structure-based reassessment of the caveolin signaling model: do caveolae regulate signaling through caveolin-protein interactions? *Dev Cell, 23*(1), 11–20. https://doi.org/10.1016/j.devcel.2012.06.012.
170. Sessa, W. C. (2004). eNOS at a glance. *J Cell Sci, 117*(Pt 12), 2427–2429. https://doi.org/10.1242/jcs.01165.
171. Ju, H., Zou, R., Venema, V. J., & Venema, R. C. (1997). Direct interaction of endothelial nitric-oxide synthase and caveolin-1 inhibits synthase activity. *J Biol Chem, 272*(30), 18522–18525. https://doi.org/10.1074/jbc.272.30.18522.
172. Trane, A. E., Pavlov, D., Sharma, A., Saqib, U., Lau, K., van Petegem, F., Minshall, R. D., Roman, L. J., & Bernatchez, P. N. (2014). Deciphering the binding of caveolin-1 to client protein endothelial nitric-oxide synthase (eNOS): scaffolding subdomain identification, interaction modeling, and biological significance. *J*

Biol Chem, 289(19), 13273–13283. https://doi.org/10.1074/jbc.M113.528695.

173. Michel, J. B., Feron, O., Sacks, D., & Michel, T. (1997). Reciprocal regulation of endothelial nitric-oxide synthase by Ca2+-calmodulin and caveolin. *J Biol Chem, 272*(25), 15583–15586. https://doi.org/10.1074/jbc.272.25.15583.
174. Chen, Z., Bakhshi, F. R., Shajahan, A. N., Sharma, T., Mao, M., Trane, A., Bernatchez, P., van Nieuw Amerongen, G. P., Bonini, M. G., Skidgel, R. A., Malik, A. B., & Minshall, R. D. (2012). Nitric oxide-dependent Src activation and resultant caveolin-1 phosphorylation promote eNOS/caveolin-1 binding and eNOS inhibition. *Mol Biol Cell, 23*(7), 1388–1398. https://doi.org/10.1091/mbc.E11-09-0811.
175. Fukumura, D., Kashiwagi, S., & Jain, R. K. (2006). The role of nitric oxide in tumour progression. *Nature Reviews Cancer, 6*(7), 521–534. https://doi.org/10.1038/nrc1910.
176. Gratton, J. P., Lin, M. I., Yu, J., Weiss, E. D., Jiang, Z. L., Fairchild, T. A., Iwakiri, Y., Groszmann, R., Claffey, K. P., Cheng, Y. C., & Sessa, W. C. (2003). Selective inhibition of tumor microvascular permeability by cavtratin blocks tumor progression in mice. *Cancer Cell, 4*(1), 31–39.
177. Nah, J., Yoo, S. M., Jung, S., Jeong, E. I., Park, M., Kaang, B. K., & Jung, Y. K. (2017). Phosphorylated CAV1 activates autophagy through an interaction with BECN1 under oxidative stress. *Cell Death Dis, 8*(5), e2822. https://doi.org/10.1038/cddis.2017.71.
178. Root, K. T., Julien, J. A., & Glover, K. J. (2019). Secondary structure of caveolins: a mini review. *Biochem Soc Trans, 47*(5), 1489–1498. https://doi.org/10.1042/bst20190375.