

# Negative regulators of integrin activity

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## Summary

Integrins are heterodimeric transmembrane adhesion receptors composed of  $\alpha$ - and  $\beta$ -subunits. They are ubiquitously expressed and have key roles in a number of important biological processes, such as development, maintenance of tissue homeostasis and immunological responses. The activity of integrins, which indicates their affinity towards their ligands, is tightly regulated such that signals inside the cell crucially regulate the switching between active and inactive states. An impaired ability to activate integrins is associated with many human diseases, including bleeding disorders and immune deficiencies, whereas inappropriate integrin activation has been linked to inflammatory disorders and cancer. In recent years, the molecular details of integrin ‘inside-out’ activation have been actively investigated. Binding of cytoplasmic proteins, such as talins and kindlins, to the cytoplasmic tail of  $\beta$ -integrins is widely accepted as being the crucial step in integrin activation. By contrast, much less is known with regard to the counteracting mechanism involved in switching integrins into an inactive conformation. In this Commentary, we aim to discuss the known mechanisms of integrin inactivation and the molecules involved.

**Key words:** Activation, Adhesion, Endocytosis, Integrin, SHARPIN, Talin

## Introduction

Integrins are heterodimeric transmembrane proteins composed of  $\alpha$ - and  $\beta$ -subunits. They are ubiquitously expressed, often in high numbers, and mediate cell–cell adhesion, as well as adhesion of cells to extracellular matrix (ECM) proteins (Hynes, 2002; Legate et al., 2009). The affinity of integrins for their ligands (integrin activation) is allosterically regulated such that the intracellular and extracellular domains of both subunits undergo conformational changes (Moser et al., 2009; Shattil et al., 2010). A controlled regulation of integrin activity is fundamentally important during embryogenesis and is central to many physiological processes in adults. Impaired integrin activation has been linked to diseases, including bleeding disorders, skin blistering and immune-deficiencies (Hogg and Bates, 2000; Legate et al., 2009). Conversely, increased integrin activity is associated with chronic inflammation, thrombosis and cancer (Moser et al., 2009; Shattil et al., 2010).

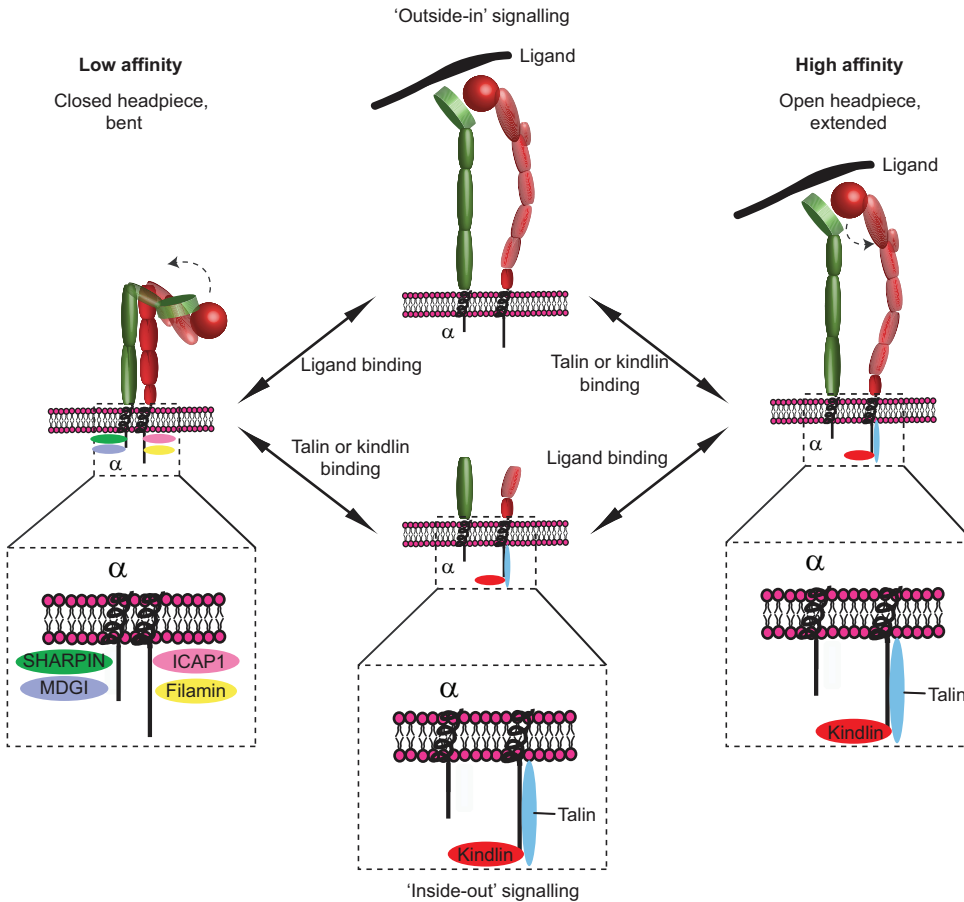
Integrins are unique in their ability to function as bidirectional signalling molecules. Binding of extracellular matrix (ECM) molecules or other ligands to the extracellular domain of integrins transmits a variety of signals into the cell. This ‘outside-in’ activation (Fig. 1) regulates many important cellular processes, including migration, survival, proliferation, gene expression and receptor tyrosine kinase signalling (Ivaska and Heino, 2010; Zaidel-Bar et al., 2007). Conversely, changes in the intracellular environment of the cell can alter the binding of integrins to their ligands through so-called ‘inside-out’ signalling (Fig. 1). Recently, the individual steps involved in integrin inside-out activation have been the focus of intense investigation. Despite some controversy over the details, it is now widely accepted that binding of the cytoplasmic proteins talin-1 and -2

(TLN1, TLN2) and of kindlins (the fermitin family members 1–3, FERMT1–FERMT3; also known as KIND1–KIND3) to the cytoplasmic tail of the integrin  $\beta$ -subunit are crucial for integrin activation (Calderwood et al., 2004; Moser et al., 2009).

Even though factors that keep integrins inactive or that are able to switch activated integrins back into an inactive conformation are likely to be as biologically important as integrin activators for regulating the dynamic nature of integrin function (Fig. 2), they have been studied less than the integrin activating proteins. On the basis of the existing data it appears that integrin activation can be prevented or diminished by at least three mechanisms: (1) interfering with the clasp interactions, (2) competition with talin and kindlin binding, and (3) decreasing the amount of integrins at the plasma membrane by altering integrin trafficking (Fig. 2). In this Commentary, we will describe new and previously described known negative regulators of integrin activity and their relevance to cell biology and human health.

## Mechanism of integrin activation

Recently, the molecules involved in integrin activation have been discussed extensively in many excellent reviews (Margadant et al., 2011; Shattil et al., 2010). Thus, only some details relevant for the discussion are presented here. The conformational changes of integrins are dynamic and we can assume that there is a constant equilibrium between their active and inactive conformations. The known mechanisms for integrin activation include the release of intramembrane interactions (Lau et al., 2009; Partridge and Marcantonio, 2006) and of juxtamembrane cytoplasmic interactions (also termed clasps) (Hughes et al., 1996; Lau et al., 2009) between the  $\alpha$ - and  $\beta$ -subunits.



**Fig. 1. A schematic representation of integrin conformation switching.** In the inactive conformation (i.e. low-affinity for ECM components; shown on the left), integrin  $\alpha$ - and  $\beta$ -subunits are in close proximity, with the headpiece bent towards the plasma membrane. Binding of, for example, SHARPIN or MDGI (to  $\alpha$ -subunits), or ICAP1 or filamin (to  $\beta$ -subunits) to the cytoplasmic domain of integrins stabilises the integrin heterodimer in this low-affinity conformation. The formation of the high-affinity conformation requires both the extension of the extracellular domains and the separation of the integrin  $\alpha$ - and  $\beta$ -subunits, resulting in the so-called open headpiece conformation (shown on the right). Formation of this open high-affinity conformation can be triggered by binding of ECM components to the extracellular domain of the integrin, termed 'outside-in' signalling, or by association of talin and kindlins with the cytoplasmic domain of integrin  $\beta$ -subunits in response to an intracellular signal, called 'inside-out' signalling as shown in the centre. In the schematic depicting 'inside-out' signalling, the extracellular domains of the integrins have been omitted for clarity.

Talin is a large cytoplasmic protein composed of an integrin-binding head domain and a rod domain, which links talin to vinculin (VCL) and the actin cytoskeleton (Critchley, 2009; Grashoff et al., 2010; Humphries et al., 2007). Talin binds to integrin- $\beta$  tails and induces conformational activation of the integrin by disrupting the integrin clasp (Anthis et al., 2009; Kalli et al., 2011; Wegener et al., 2007). For the platelet integrin  $\alpha$ IIb $\beta$ 3 and for the  $\alpha$ - $\beta$ 2 integrin heterodimers, expressed, for example, on leucocytes, there is compelling evidence that a clasp formed by a salt-bridge between the  $\alpha$ - and  $\beta$ -tails is crucial for maintaining these receptors in their inactive conformation (Springer and Dustin, 2012; Ye et al., 2012). For the  $\beta$ 1 integrins, which predominantly facilitate matrix binding of adherent cell types, the role of the juxtamembrane clasp is not that well established (Czuchra et al., 2006). This might reflect the fact that for most  $\alpha$ - $\beta$ 1-integrin-mediated biological processes the switching between integrin activation and inactivation is implicated in adhesion modulation rather than a complete transition between non-adherent and adherent states. Nevertheless, talin binding is also required for  $\beta$ 1-integrin activation (Calderwood, 2004). On the basis of in vitro and in vivo data, kindlins are also crucial for integrin activation; they bind to integrin- $\beta$  subunits and co-activate integrins together with talin (Czuchra et al., 2006; Karaköse et al., 2010; Montanez et al., 2008; Moser et al., 2008). Interestingly, talin is not important for the initial binding of  $\beta$ 1 integrins to the matrix, but it is absolutely required for subsequent cell spreading given that fibroblasts lacking both the talin-1 and -2 isoforms fail to spread fully (Zhang et al., 2008). In *Drosophila*, the talin-null phenotype has defects in the connections between integrin and the

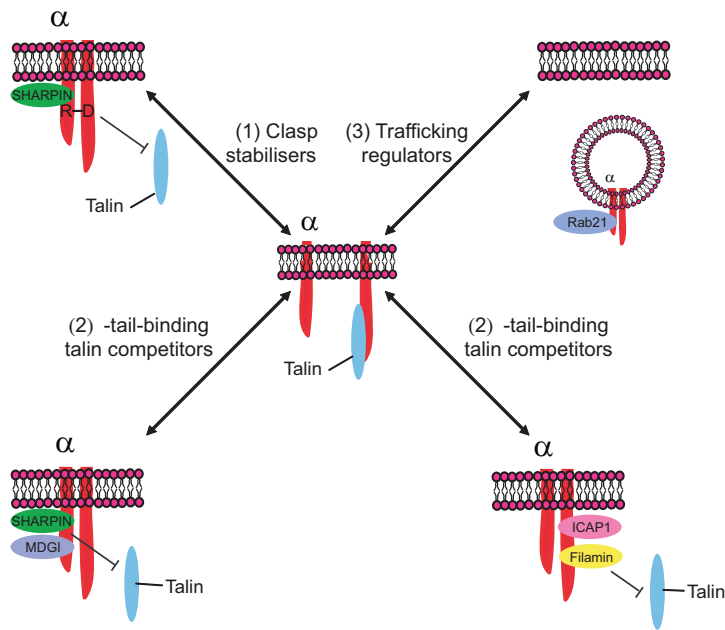
cytoskeleton, but the binding of integrin to the extracellular matrix is not abolished (Brown et al., 2002). However, subsequent work has also demonstrated a role for talin binding to the *Drosophila* integrins in their activation (Tanentzapf and Brown, 2006). These data indicate that integrins can bind the ECM and become activated in the absence of talin, but talin binding to integrin- $\beta$  tails is crucial for the ability of integrins to fulfil their role as integrators between the ECM and the cytoskeleton.

### Inhibitors interacting with integrin- $\beta$ tails

The role of the integrin  $\beta$ -subunit in the regulation of integrin activity has been the main focus in the field (reviewed by Harburger and Calderwood, 2009; Kim and McCulloch, 2011; Shattil et al., 2010) and important inhibitors interacting with the  $\beta$ -tails, such as filamin and integrin cytoplasmic domain-associated protein 1 (ICAP1; encoded by *ITGB1BP1*) (Liu et al., 2000), have been identified (Fig. 3; Table 1). The main focus of our Commentary is the role of the integrin- $\alpha$  tail in the modulation of integrin activity. However, in the following sections we will also describe the  $\beta$ -tail-binding integrin inhibitors. As we are not aware of clasp-stabilising  $\beta$ -subunit-binding proteins, we will divide the inhibitors in two groups; those that compete with talin and kindlin binding, and those that affect integrin trafficking (as described in the Introduction).

### $\beta$ -tail-binding inhibitors that compete with the binding of talins and kindlins

Different integrin  $\beta$ -subunit cytoplasmic domains share two highly conserved NxxY motifs, which are referred to as the



**Fig. 2. Schematic illustration of three possible mechanisms of integrin inactivation.** Negative integrin regulators can inactivate integrins by using three different mechanisms. In the first, clasp stabilisers shield the salt bridge between an arginine residue in the  $\alpha$ -integrin cytoplasmic domain and an aspartate residue in the  $\beta$ -integrin cytoplasmic domain (1). The presence of this salt bridge prevents separation of the cytoplasmic domains of integrin  $\alpha$ - and  $\beta$ -subunits and therefore stabilises the integrin in its inactive form. Second, binding of talin competitors to integrin cytoplasmic domains prevent talin, which is an essential integrin activator, from associating with the cytoplasmic domain of the integrin  $\beta$ -subunit (2). Finally, integrin binding by proteins involved in receptor endocytosis (such as the Rab21 small GTPase) can reduce the amount of active receptor on the cell surface and thus influence integrin-dependent biological functions (3). Some of the integrin-inactivating proteins that have been shown or are suspected (see text for details) to inhibit integrin activity according to these mechanisms are shown here. Inactivators binding to the cytoplasmic domains of integrin  $\alpha$ - and  $\beta$ -subunits are shown separately.

membrane-proximal and membrane-distal motifs. Mutational analyses of these sequences have suggested that they have a crucial role both in integrin ‘inside-out’ and ‘outside-in’ signalling (Liu et al., 2000). Talin binds to the membrane-proximal NPxY motif in the integrin  $\beta$ -subunits through its FERM domain (Goldmann, 2000; Horwitz et al., 1986; Knezevic et al., 1996; Pfaff et al., 1998) and there are at least two classes of

proteins that inhibit integrin activity by competing with talin for binding to this motif.

The first class of talin inhibitors are proteins that contain a phosphotyrosine-binding (PTB) domain (Calderwood et al., 2003). The F3 subdomain of the talin FERM domain is similar to PTB domains, and talin interacts with integrins in a similar way to that of PTB domains binding to phosphorylated tyrosine motifs

**Table 1. Overview of all integrin inhibitors described in this Commentary**

| Integrin inhibitor | $\alpha$ -tail binding | $\beta$ -tail binding | Actin-binding | Type of inhibition   | Reference(s)   |
|--------------------|------------------------|-----------------------|---------------|--|--|
| ICAP1              | –                      | +                     | –             | Competition with talin   | (Bouvard et al., 2003)                                     |
| Filamin A          | –                      | +                     | +             | Competition with talin   | (Kiema et al., 2006)                                       |
| Numb               | –                      | +                     | ?             | Competition with talin?, endocytosis                               | (Calderwood et al., 2003; Nishimura and Kaibuchi, 2007)    |
| DAB2               | –                      | +                     | ?             | Endocytosis  | (Teckchandani et al., 2009)                                |
| PKC $\alpha$       | –                      | +                     | –             |  | (Ng et al., 1999; Parsons et al., 2002; Upla et al., 2004) |
| Nischarin          | +                      | –                     | –             | $\alpha$ -Subunit-specific inhibitor                               | (Alahari and Nasrallah, 2004)                              |
| CIB1               | +                      | –                     | –             | $\alpha$ -Subunit-specific inhibitor                               | (Gushiken et al., 2008; Naik et al., 1997)                 |
| PP2A               | +                      | +                     | –             | Other  | (Gushiken et al., 2008; Kim et al., 2004)                  |
| SHARPIN            | +                      | –                     | ?             | Clasp stabiliser or competition with talin and/or kindlin?         | (Rantala et al., 2011)                                     |
| MDGI               | +                      | –                     | –             | Competition with kindlin   | (Nevo et al., 2010)  |
| Rab21              | +                      | –                     | –             | Endocytosis  | (Pellinen et al., 2006)                                    |
| p120RasGAP         | +                      | –                     | –             | $\beta$ 1 integrin recycling to the plasma membrane                | (Mai et al., 2011)   |
| ACAP1              | –                      | +                     | +             | $\beta$ 1 integrin recycling to the plasma membrane                | (Li et al., 2005)  |
| PKD1               | –                      | +                     | +             | $\beta$ 3 integrin recycling to the plasma membrane                | (Woods et al., 2004)                                       |
| GIPC1              | ?                      | ?                     | –             | Active $\alpha$ 5 $\beta$ 1 integrin; endocytosis                  | (Valdembri et al., 2009)                                   |
| MMP8               | ?                      | ?                     | –             | Inhibits $\beta$ 1 integrin by binding to the extracellular domain | (Pellinen et al., 2012)                                    |

+ , positive interaction; – , no interaction; ? , not determined

(Forman-Kay and Pawson, 1999). However, unlike most PTB-mediated protein–protein interactions, the binding of talin F3 to integrin does not require tyrosine phosphorylation of the integrin NPxY motif. By contrast, the talin–integrin interaction is inhibited by tyrosine phosphorylation (Anthis et al., 2009; Oxley et al., 2008).

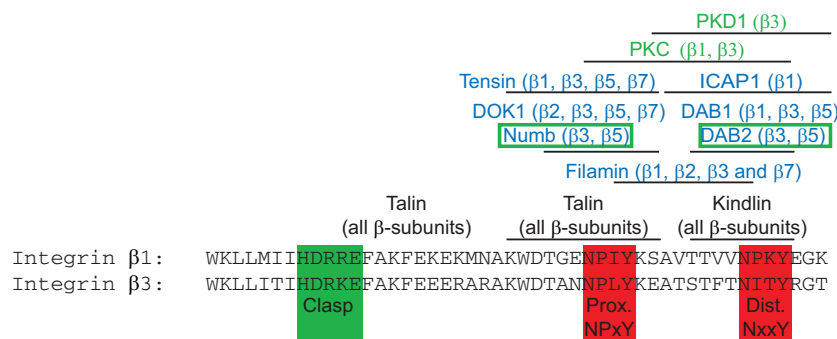
At least 17 PTB domain proteins interact with integrin- $\beta$  tails (Calderwood et al., 2003). The PTB domains of tensins 1–4, numb and docking protein 1 (DOK1) bind to the same membrane-proximal NPxY motif in integrins as talin and are thus expected to compete with talin for integrin binding (Fig. 3). However, the relative binding affinities of these interactions are not known. Kindlins interact with the distal NxxY sequence of integrin tails (Moser et al., 2009) and have been shown to activate integrins, possibly by stabilising talin–integrin interactions. However, in the case of  $\beta$ 1 integrins, overexpression of kindlin-2 (FERMT2) has been shown to interfere with talin binding (Harburger et al., 2009), suggesting that in some cases the ability of kindlins to activate integrins is cell type and integrin heterodimer specific. Some PTB domain proteins preferentially bind to the distal NxxY motif of the  $\beta$ -tails (Calderwood et al., 2003). Of these, Shc binding to integrin is phosphorylation dependent, whereas disabled homolog 1 and 2 (DAB1 and DAB2) and ICAP1 do not require integrin tyrosine phosphorylation. Of these, the role of ICAP1 as a negative regulator of  $\beta$ 1 integrin activity (ICAP1 does not bind  $\beta$ 3 integrins) is well-characterized in vitro and in vivo. ICAP1 competes for talin binding in vitro (Bouvard et al., 2003) and regulates focal adhesion (FA) formation (Millon-Frémillon et al., 2008) such that increased integrin activity in the absence of ICAP1 slows down FA dynamics. In addition, mice lacking ICAP1 have defective osteoclast proliferation (Bouvard et al., 2007), which could be integrin dependent. Interestingly, mice null for SHARPIN (see below) show a similar defect in osteoclast proliferation (Xia et al., 2011).

Thus, proteins that bind to either the proximal NPxY or the distal NxxY motifs of the integrin tail can negatively regulate integrin activation by competing with talin or kindlin binding. However, elucidating the details of how these interactions are regulated in time and space still requires more research.

Filamins form the second class of inhibitors of talin binding. The filamin-binding site is located close to the talin-binding site on the  $\beta$ -integrin tails and filamin and talin compete with each other, whereby filamin inhibits integrin activity, as opposed to talin (Kiema et al., 2006). Filamin depletion increases the amount of active integrins on the cell surface, but is it unclear whether any of the developmental defects caused by mutations in filamin genes is the consequence of increased integrin activation (reviewed by Zhou et al., 2010). The genetic analysis of filamin function is complicated by the existence of multiple genes that have partially overlapping expression patterns. In addition to this, migfilin, a filamin-binding protein that is enriched at cell–cell and cell–ECM contact sites, can displace filamin from  $\beta$ 1 and  $\beta$ 3 integrins and promote integrin activation (Das et al., 2011; Ithychanda et al., 2009; Lad et al., 2008). Therefore, the balance between filamin and migfilin expression will influence the extent to which filamin inhibits integrin activity, and, thus, the filamin–migfilin interaction provides an additional regulatory layer for filamin-induced inhibition of integrins.

### Integrin trafficking regulators that bind the integrin- $\beta$ tail

In addition to the regulation of integrin receptor activity on the cell surface by ‘inside-out’ signalling as described above, selective integrin endocytosis can affect the availability of active integrin receptors on the cell surface. Most integrins are constantly endocytosed and recycled back to the plasma membrane in adherent cells and this is regulated in part by proteins interacting with the integrin cytoplasmic tails (Margadant et al., 2011; Pellinen and Ivaska, 2006). However, to date the relationship between integrin activity and integrin endocytic trafficking remains unclear. As mentioned above, many PTB-domain-containing proteins have been shown to interact with integrin- $\beta$  tails in vitro (Calderwood et al., 2003) and, interestingly, several of these are clathrin adaptor proteins that are involved in endocytosis. According to one report, DAB2 is important for the endocytosis of active integrins from disassembling FAs (Ezratty et al., 2009), whereas another study suggests that there is a role for DAB2 in the endocytosis of



**Fig. 3. Alignment of activator and inhibitor binding sites on  $\beta$ 1 and  $\beta$ 3 integrin cytoplasmic regions.** The green box represents the ‘clasp’, which forms between the  $\alpha$ - and  $\beta$ -integrin cytoplasmic regions and which is required to keep an integrin in its inactive conformation. The red boxes indicate the membrane-proximal (Prox.) NPxY and membrane-distal (Dist.) NxxY motifs. The binding sites for regulators of integrin activity are indicated with lines. Integrin-activating proteins are written in black, proteins that affect integrin trafficking are in green, and proteins that compete with talin or kindlin for binding to the  $\beta$ -integrin cytoplasmic region are in blue (a green rectangle around the name indicates that that protein has also been shown to be involved in integrin trafficking). The  $\beta$ -integrins that the respective integrin activator or inactivator has been shown to bind is given in parentheses. The  $\beta$ -integrin-interacting proteins are talin (Calderwood et al., 1999), kindlins (FERMT1–3) (Moser et al., 2009), filamins (Kiema et al., 2006), tensin (Calderwood et al., 2003; Katz et al., 2007), DAB1, DAB2, numb (Calderwood et al., 2003), DOK1 (Anthis et al., 2009), (Chang et al., 1997; Zhang and Hemler, 1999), PKC $\alpha$  (Parsons et al., 2002) and PKD1 (Woods et al., 2004).

inactive integrins from the dorsal surface of cells (Teckchandani et al., 2009). On the basis of the available structural information, it is conceivable that DAB2 could interact with both active and inactive integrins, as DAB2 binding to the  $\beta$ 3-tail involves the membrane distal NPxY motif, which is not bound by talin in active integrins. The PTB-containing clathrin adaptor numb has also been shown to regulate integrin endocytosis and cell migration (Nishimura and Kaibuchi, 2007). Interestingly, the binding sites of talin and numb on the integrin  $\beta$ 3 tail overlap (Calderwood et al., 2003), which suggests that numb could specifically regulate endocytosis of inactive integrin receptors by specifically recruiting non-talin-binding inactive receptors for endocytosis. This possibility, however, has not been investigated.

In addition to these clathrin adaptors, other  $\beta$ -tail-interacting proteins can also trigger integrin endocytosis. For example, protein kinase C alpha (PKC $\alpha$ ) binds directly to the integrin  $\beta$ 1 tail and its binding sequence spans both NPxY motifs. PKC $\alpha$  induces endocytosis of  $\beta$ 1 integrin in migrating cancer cells (Ng et al., 1999; Parsons et al., 2002) and in response to echovirus-1 (EV-1) binding to  $\alpha$ 2 $\beta$ 1 integrin or integrin clustering (Upla et al., 2004). As talin and PKC $\alpha$  probably cannot bind integrin simultaneously due to the overlapping binding sites on the  $\beta$ -integrin cytoplasmic tail (Fig. 3) (Parsons et al., 2002), it is not surprising that PKC $\alpha$ -dependent EV-1-induced integrin endocytosis is specific for the inactive  $\alpha$ 2 $\beta$ 1 conformer (Jokinen et al., 2010). Recently, it has also been shown that PKC $\alpha$  affects integrin endocytosis through another pathway. Binding of transmembrane proteoglycan syndecan-4 to fibronectin triggers PKC $\alpha$ -dependent RhoG binding to  $\alpha$ 5 $\beta$ 1 integrins (the fibronectin-binding integrin) and subsequent clearance of the integrin from the membrane by accelerated endocytosis (Bass et al., 2011). This transient reduction of integrin levels at the cell surface is important to regulate the adhesive strength of the cells to favour migration (Bass and Humphries, 2002).

The cell surface integrin levels are also influenced by the rate of recycling of the endocytosed receptors to the plasma membrane. For  $\beta$ 3 integrins, which predominantly recycle through a Rab4-dependent pathway, the direct binding of protein kinase D1 (PKD1) is required for return of the endocytosed receptor to the plasma membrane (Woods et al., 2004). The recycling of the endocytosed  $\beta$ 1 integrins, by contrast, is regulated by binding of ACAP1 (coiled-coil, ANK repeat and PH domain-containing protein 1) to the integrin  $\beta$ 1 cytoplasmic domain (Li et al., 2005). Therefore, these proteins contribute to the levels of active integrin present on the cell surface through their effect on receptor recycling.

### Inhibitors interacting with integrin $\alpha$ -tails

For years, the main focus of the integrin field has been on the role of the  $\beta$ -subunits in regulating integrin activity. As several of the  $\beta$ -subunits (especially  $\beta$ 1) can pair with many different  $\alpha$ -subunits, proteins that are involved in regulating  $\beta$ -subunits are expected to have important functions in triggering various cellular functions regulated by all  $\beta$ 1 integrins. However, in principle, the  $\alpha$ -subunits offer two distinct layers of regulating integrin activity. First, they share conserved sequence elements in the membrane-proximal regions of their cytoplasmic tails, which allows for proteins to interact with and regulate all of the different  $\alpha$ -subunits at once (Liu et al., 2000; Rantala et al., 2011). In addition, the poorly conserved specific membrane-distal parts of the  $\alpha$ -subunit cytoplasmic tails facilitate protein

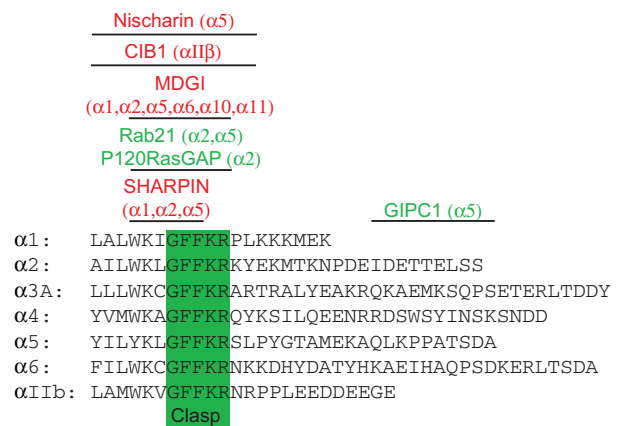
interactions and could regulate integrin activity in a heterodimer-specific manner. At present, our insight into the molecular mechanisms of  $\alpha$ -tail-mediated integrin activity regulation is far from that of the  $\beta$ -tail-binding activators or inactivators. Therefore it is not possible to categorise  $\alpha$ -tail-interacting activity regulators into the three mechanistic classes used above to describe different  $\beta$ -tail-binding regulators. Thus, we have opted to group them into common  $\alpha$ -tail-binding inhibitors,  $\alpha$ -tail-interacting regulators of integrin trafficking and  $\alpha$ -subunit-specific inhibitors (Fig. 4; Table 1), as discussed below.

### Integrin inhibitors binding to several or all $\alpha$ -subunits

#### SHARPIN

Until recently very little was known about SHARPIN, except that it localises in the postsynaptic density of excitatory synapses in the brain, where it binds Shank proteins (Lim et al., 2001) and that a spontaneous mutation in the Sharpin gene results in eosinophilic proliferative dermatitis and multiorgan inflammation (Liang et al., 2011; Seymour et al., 2007). During the last few years, however, new roles of SHARPIN have emerged as it was shown to bind to and inhibit the lipid-phosphatase activity of phosphatase and tensin homolog (PTEN) (He et al., 2010), act as a transcriptional co-activator of eyes absent homolog 1 (EYA1) (Landgraf et al., 2010), and be part of the linear ubiquitin chain assembly complex [LUBAC, composed of heme-oxidised IRP2 ubiquitin ligase 1 (HOIL1)-interacting protein (HOIP), HOIL1 and SHARPIN proteins], regulating nuclear factor kappa B (NF $\kappa$ B) activity and apoptosis (Gerlach et al., 2011; Ikeda et al., 2011; Tokunaga et al., 2011).

In addition to these functions, a small interfering RNA (siRNA) screen for integrin inhibitors identified SHARPIN as a ubiquitously expressed integrin-inactivating protein (Rantala et al., 2011). Depletion of SHARPIN increases active  $\beta$ 1



**Fig. 4. Alignment of integrin-inhibitor-binding sites on  $\alpha$ -integrin cytoplasmic regions.** As in Fig. 3, the green box represents the 'clasp', and binding sites for regulators of integrin activity are indicated with lines.

Proteins that affect integrin trafficking are in green and in red are proteins for which the mechanism of integrin inactivation requires further study. The  $\alpha$ -integrins that the respective integrin activator or inactivator has been shown to bind is given in parentheses. The  $\alpha$ -integrin-interacting proteins are SHARPIN (Rantala et al., 2011), Rab21 and p120RasGAP (also known as RAS p21 protein activator, RASA1) (Mai et al., 2011; Pellinen et al., 2006), GIPC1 (Valdembri et al., 2009), MDGI (Nevo et al., 2010), (Barry et al., 2002) and nischarin (Alahari and Nasrallah, 2004).

integrin at the cell surface without affecting the total amount of integrins. SHARPIN binds to the highly conserved WKxGFFKR (Fig. 4) sequence present in the cytoplasmic tail of  $\alpha$ -integrins (Rantala et al., 2011) and this interaction is required for SHARPIN-mediated integrin inactivation. The essential amino acids within the integrin cytoplasmic tail are WKxGFF (amino acids 1154–1159 in  $\alpha 2$  integrin), whereas the KR residues (the lysine and arginine residues immediately adjacent to the WKxGFF sequence) are not required for SHARPIN binding (Rantala et al., 2011). This observation is compatible with a function of SHARPIN as an integrin inhibitor, as the arginine residue (R) within the  $\alpha$ -tail WKxGFFKR sequence has been suggested to form a salt bridge (clasp) with the cytoplasmic tail of the  $\beta$ -integrin subunit, keeping the integrin in an inactive state (O'Toole et al., 1991).

Mechanistically, SHARPIN inhibits the binding of talin and kindlin to the cytoplasmic domain of  $\beta 1$  integrin (Rantala et al., 2011). Thus, on the one hand, the 'inside-out' integrin activation is controlled by the balance of counteracting forces that are exerted by SHARPIN (and possibly other integrin inhibitors), and on the other hand and talins and kindlins. It remains to be determined whether this inhibition is direct (through steric hindrance or by stabilising the integrin inactivating clasp) or indirect (for example by recruiting inhibiting kinases). In cells, SHARPIN colocalises with inactive, but not active, integrins in detached ruffles (Rantala et al., 2011), consistent with SHARPIN acting as an integrin-inactivating protein. SHARPIN binding to integrin  $\alpha$ -subunits also inhibits  $\alpha$ - $\beta 1$ -integrin-dependent functions, such as cell spreading and cell migration.

Epidermal homeostasis is regulated by integrin expression, and downregulation of  $\beta 1$  integrin expression in the suprabasal keratinocytes is a prerequisite for keratinocyte differentiation (Watt, 2002). Interestingly, the proliferative dermatitis phenotype of mice null for SHARPIN resembles that of the transgenic mouse models with forced suprabasal  $\beta 1$  integrin expression under the control of the involucrin promoter (Carroll et al., 1995). Thus, loss of the  $\beta 1$  integrin inactivator SHARPIN and overexpression of  $\beta 1$  integrin results in very similar physiological outcomes *in vivo*. In addition, the function of SHARPIN as a  $\beta 1$ -integrin inhibitor *in vivo* is supported by the observation that keratin-14-positive keratinocytes from *Sharpin*-null mice contain higher active  $\beta 1$ -integrin levels than keratinocytes from wild-type mice (Rantala et al., 2011).

Importantly, the integrin inhibitory effect of SHARPIN is independent of the aforementioned other functions of SHARPIN. SHARPIN silencing induces integrin activation in PC3 cells, which are null for PTEN (Gustin et al., 2001) and lack EYA1 expression (Kilpinen et al., 2008). Furthermore, silencing of HOIP (the catalytic subunit of LUBAC) does not affect integrin activity in cells (Rantala et al., 2011). In addition, mice lacking HOIL1 (another member of the LUBAC complex) (i.e. after knockout of the HOIL1-encoding gene *Rbck*) do not have any obvious integrin-related phenotype (Tokunaga et al., 2009), indicating that SHARPIN has important, LUBAC-independent, functions *in vivo*.

The identification of SHARPIN opens a new paradigm in integrin regulation as it demonstrates that the dynamic switching between inactive and active integrin conformations is physiologically controlled *in vivo* by a protein that interacts with the  $\alpha$ -subunit cytoplasmic domain. As SHARPIN is expressed in most human tissues and several cancer cell types (Rantala et al., 2011), and it binds several (and potentially all)  $\alpha$ -integrins,

SHARPIN might represent a general means for cells to suppress integrin activity.

#### MDGI

Mammary-derived growth inhibitor (MDGI, also known as FABP3) has been named after its origin from lactating bovine mammary glands and its growth inhibitory properties in human mammary carcinoma cell cultures (Böhmer et al., 1987). MDGI is almost ubiquitously expressed, and is particularly abundant in muscle and mammary gland (Hauerland and Spener, 2004). Recently, MDGI has been shown to interact with several different integrin  $\alpha$ -subunits ( $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 5$ ,  $\alpha 6$ ,  $\alpha 10$  and  $\alpha 11$ ), probably through the highly conserved WKxGFFKR sequence that is present in most, if not all,  $\alpha$ -subunits (Nevo et al., 2010). MDGI overexpression reduces adhesion of cells to type I collagen and fibronectin and impairs both migration and invasion specifically in human breast cancer cell lines but not in other cell types (Nevo et al., 2010). These effects are due to an MDGI-induced significant reduction in the active  $\beta 1$  integrin conformation on the cell surface as determined with conformation-sensitive antibodies against  $\beta 1$  integrin (Nevo et al., 2010). The exact molecular details of the function of MDGI as a negative regulator of integrin activity remain unsolved, but MDGI overexpression in MDA-MB-231 breast cancer cells reduces the association of kindlin with active  $\beta 1$  integrin, suggesting that MDGI binding to cytoplasmic integrin- $\alpha$  tails could indirectly hamper the binding of integrin agonists to integrin- $\beta$  tails (Nevo et al., 2010). It remains unclear why these effects of MDGI on integrin activity are specific to breast cancer cells. One possibility is that they are linked to epidermal growth factor receptor (EGFR) overexpression, a common genetic alteration for breast cancer cells. MDGI influences EGFR trafficking so that the receptor is increasingly localised on endosomes (Nevo et al., 2009). As EGFR and  $\beta 1$  integrins participate in extensive crosstalk (Ivaska and Heino, 2011), the altered EGFR dynamics could also influence  $\beta 1$  integrin conformation in breast cancer cells. Regardless of the molecular details, the ability of MDGI to inhibit breast cancer cell invasion *in vitro* appears to be clinically relevant. Analysis of a tissue microarray of 1331 breast carcinomas revealed that patients with MDGI-positive tumours have a more favourable 10-year disease-free survival prognosis compared with that of patients with MDGI-negative tumours (Nevo et al., 2010).

#### Integrin trafficking regulators that bind the integrin $\alpha$ -tail

As discussed above, the conserved membrane-proximal segment of integrin  $\alpha$ -tails is important for SHARPIN and MDGI binding and integrin inactivation. Interestingly, this binding site overlaps with the site on the integrin  $\alpha$ -tails, at which the small GTPase Rab21 binds many integrin  $\alpha$ - $\beta 1$  heterodimers and subsequently induces their endocytosis (Pellinen et al., 2006). Furthermore, on endosomes, the GTPase-activating protein (GAP) p120RasGAP (also known as RASA1) competes with Rab21 for integrin binding and regulates the recycling of the receptor to the plasma membrane (Mai et al., 2011). The possible selectivity of Rab21 (and p120RasGAP) for active integrins has not been investigated but, as in the case of the clathrin adaptor Numb, one could envision a scenario, in which integrin activity regulators (for example talin and SHARPIN) compete with endocytosis regulators (Numb and Rab21) for integrin binding. This would provide a mechanism to control the relative amounts of active and inactive integrin receptors on the cell surface.

In endothelial cells, endocytosis of the active  $\alpha 5 \beta 1$  integrin is also specifically regulated by the  $\alpha$ -tail interacting homomultimeric endocytic adaptor GAIIP interacting protein C terminus member 1 (GIPC1). In addition to the  $\alpha 5 \beta 1$  integrin, GIPC1 also binds to Neuropilin-1 (Nrp-1) and Myosin-6, and formation of this complex triggers endocytosis of the active  $\alpha 5 \beta 1$ -integrin into Rab5-positive endosomes (Valdembri et al., 2009). This results in increased adhesion of endothelial cells to fibronectin, in line with the previously established link between increased integrin traffic and faster cell spreading and adhesion (Pellinen et al., 2006). At present it is not clear how stimulation of endothelial cells with Nrp-1 ligands triggers active-conformer specific endocytosis. However, the selective uptake of the active receptor from the cell surface is absolutely dependent on Neuropilin-1 as endocytosis of the inactive  $\alpha 5 \beta 1$  is Neuropilin-1 independent. This suggests that in addition to the ability of cells to modulate their adhesion receptor activity on the plasma membrane via 'inside-out' signalling, selective endocytosis of a specific integrin conformer may be an important mechanism to regulate the availability of active integrins at the cell surface in a spatially controlled or polarised manner. In addition, very recent data indicate that active and inactive  $\beta 1$ -integrins also use distinct recycling pathways to return to the plasma membrane (Arjonen et al., 2012).

#### Integrin inhibitors binding to specific $\alpha$ -subunits

There are a few proteins that have been described to bind to a specific integrin  $\alpha$ -subunit and inhibit integrin function. However, the negative regulatory role of these proteins is controversial, and data demonstrating their actual regulation of integrin activity on the cell surface is lacking. Nevertheless, these examples are discussed here briefly as integrin-heterodimer-specific regulation of integrin activity is an understudied area and at present better examples are lacking.

Nischarin is a ubiquitously expressed cytosolic protein that interacts with the cytoplasmic domain of integrin  $\alpha 5$  (Alahari et al., 2000). The interaction involves the  $\alpha 5$  membrane proximal sequence IYLYKLGFFKRSLS (residues 1017–1030), for which residues Tyr1018 and Lys1022 are crucial (Alahari and Nasrallah, 2004). As these residues are relatively conserved in other integrin  $\alpha$ -subunits it is possible that nischarin could also interact with other integrin- $\alpha$  tails; however, this has not been studied. Overexpression of nischarin inhibits both cell migration and invasion, but not adhesion to fibronectin (Alahari et al., 2000; Alahari, 2003). This is owing to alterations in Rac activation and the organisation of peripheral actin filaments in nischarin-overexpressing cells. Overexpression of the integrin  $\alpha 5$  subunit enhances coprecipitation of the serine/threonine-protein kinase PAK1 [p21 protein (Cdc42/Rac)-activated kinase 1] with nischarin, which inhibits PAK1 kinase activity (Alahari and Nasrallah, 2004). This suggests that  $\alpha 5$  has a role in the localised control of PAK1 function. Although it appears that nischarin inhibits integrin-dependent processes, data that formally address the effect of nischarin expression on integrin activation are missing. Interestingly, recently nischarin expression has been shown to correlate with low-grade less-aggressive tumours in breast cancer and with reduced tumour growth and lung metastasis in a nude mouse model (Baranwal et al., 2011), which could be linked to reduced integrin activity.

Fine-tuned platelet aggregation is responsible for physiological homeostasis and aberrant platelet function can lead to pathological

thrombosis. The ubiquitously expressed calcium- and integrin-binding protein 1 (CIB1) has a key role in the regulation of the platelet integrin  $\alpha \text{IIb} \beta 3$ . CIB1 interacts directly with the cytoplasmic domain of  $\alpha \text{IIb}$  (Naik et al., 1997), of which the  $\alpha \text{IIb}$  membrane-proximal sequence LVLAMWKVGFKRNRR (residues 983 to 997) forms a minimal-binding domain for CIB1 (Barry et al., 2002). Residues Leu983, Trp988, Phe992 and Phe993 and  $\text{Ca}^{2+}$ -binding are crucial for the complex formation (Barry et al., 2002; Shock et al., 1999). CIB1 has been reported to function as an endogenous inhibitor of agonist-induced  $\alpha \text{IIb} \beta 3$  integrin activation (Yuan et al., 2006). However, CIB1 has several other binding partners apart from integrins and they might explain the variable roles that have been described for the CIB1- $\alpha \text{IIb}$  complex. For example, in contrast to the proposed role as an integrin inactivator, CIB1 has been shown to activate  $\alpha \text{IIb} \beta 3$  integrin 'inside-out' signalling by converting the integrin from the resting state into its active conformation (Tsuboi, 2002) and to have an important role in platelet spreading on immobilised fibrinogen (Naik and Naik, 2003). Finally, the physiological consequences of loss of CIB1 (in knockout mice) are impaired thrombosis and increased mouse tail-bleeding time (Naik et al., 2009), suggesting that in vivo CIB1 functions as a positive regulator of  $\alpha \text{IIb} \beta 3$ .

Serine-threonine phosphatase 2A (PP2A) has been shown to interact directly with the membrane-proximal KVGFFKR sequence of  $\alpha \text{IIb}$  integrin (Gushiken et al., 2008). In human embryonal kidney (HEK) 293 cells, PP2A overexpression decreases  $\alpha \text{IIb} \beta 3$ -mediated adhesion to immobilised fibrinogen (Gushiken et al., 2008). By contrast, PP2A association with  $\beta 1$  integrin followed by dephosphorylation of crucial residues within the  $\beta 1$  integrin cytoplasmic tail is required for integrin localisation to focal contacts and subsequent activation of downstream signalling (Kim et al., 2004; Mulrooney et al., 2000).

Taken together, at present there are no clear examples of proteins binding to specific  $\alpha$ -subunits as bona fide integrin inhibitors. For all the examples discussed above there are data indicating context dependent roles for these proteins in both integrin inactivation and activation.

#### Matrix metalloproteinase 8 (MMP8)

As described here, integrin activity can also be regulated by extracellular proteins. Recently, we reported an siRNA screen that revealed new genes that were involved in regulating  $\beta 1$  integrin activity (Pellinen et al., 2012). One particularly interesting finding is that the secreted collagenase MMP8 is a negative regulator of  $\beta 1$  integrin activity in several prostate cancer and one breast cancer cell line. MMP8 has been previously associated with tumour suppression owing to its anti-inflammatory or anti-metastatic role in several cancers (Dejonckheere et al., 2011). The increase in integrin activity caused by MMP8 silencing is counteracted by the addition of recombinant MMP8 to the growth medium and correlates with increased in vitro invasion through Matrigel (Pellinen et al., 2012). In a mouse metastasis assay, MMP8-silenced cancer cells extravasate to the lungs to a much greater extent than do control cells. In addition, MMP8 co-precipitates with  $\beta 1$  integrin, implying that binding of MMP8 to the extracellular part of the  $\beta 1$  integrin could modulate integrin activity, although we cannot rule out that MMP8 binds to integrin indirectly through its interactions with collagen. It is also possible that the collagenase activity of MMP8 modifies the integrin ligand collagen in a way

**Table 2. Overview of some of the phenotypes observed in knockout mice for integrin activators and inactivators**

| Protein   | Phenotype   | Reference(s)   |
|-----------|---|--|
| ICAP1     | Reduced osteoblast proliferation and delayed bone mineralisation  | (Bouvard et al., 2007)                                       |
| Filamin A | Incomplete septation of the heart during gestation  | (Feng et al., 2006; Hart et al., 2006)                       |
| Filamin B | Boomerang Dysplasia; lethal early embryonic or postnatal  | (Bicknell et al., 2007)                                      |
| Filamin C | Reduced birth weight and respiratory failure shortly after birth  | (Dalkilic et al., 2006)                                      |
| MDGI      | No overt phenotype in the mammary gland (only tissue examined in knockout or overexpressing mice)   | (Binas et al., 1995; Clark et al., 2000)                     |
| Talin1    | Arrest mouse development at gastrulation stage  | (Monkley et al., 2000)                                       |
| Talin2    | Myopathy with centrally nucleated fibres  | (Conti et al., 2009)   |
| Kindlin1  | Skin atrophy and lethal neonatal intestinal epithelial dysfunction  | (Ussara et al., 2008)  |
| Kindlin2  | Impaired angiogenesis   | (Pluskota et al., 2011)                                      |
| Kindlin3  | Impaired leukocyte adhesion to endothelial cells  | (Moser et al., 2008)   |
| SHARPIN   | Multiorgan inflammation, eosinophilic proliferative dermatitis, epidermal hyperplasia, dermal granulocytic infiltration, reduced osteoblast proliferation | (Liang et al., 2011; Seymour et al., 2007; Xia et al., 2011) |

that abrogates integrin binding. In the future, it will be interesting to study the exact mechanism underlying the effect of MMP8 on integrin activity and how it might be related to the anti-inflammatory role of MMP8.

### In vivo roles of integrin activators and inactivators

Above we have discussed several integrin activity inhibitors which all have the ability to induce the inactive integrin conformation on the cell surface, albeit the mechanisms involved differ between the proteins. In cells, these inactivators are counteracting the integrin-activating talin and kindlin proteins such that switching of the integrins between active and inactive states is inhibited. However, a more complex picture emerges when the phenotypes of mice lacking these activators or inactivators are compared (Table 2). In general, mice lacking the integrin activators (especially talins) appear to have a much stronger phenotype than mice lacking integrin inactivators. This might be explained by the relatively large number of integrin inactivators compared with that of activators (talins and kindlins). In addition, this could indicate that integrin inactivators have more redundant roles than activators *in vivo*. Furthermore, differences in the expression profiles and tissue distribution could account for some of the relatively mild phenotypes, such as for example in *Icap1*-knockout mice, in which it is mostly bone formation that appears to be affected (Bouvard et al., 2007), or in the kindlin-3 (*Fermt3*)-knockout mouse, in which only leukocytes are affected (Moser et al., 2008). Another complication is that several integrin inhibitors also have integrin-independent functions, such as SHARPIN (Gerlach et al., 2011; He et al., 2010; Ikeda et al., 2011; Landgraf et al., 2010; Tokunaga et al., 2011) and filamins (for a review, see Zhou et al., 2010), which will also contribute to the overall phenotype observed. Finally, mice lacking one integrin inactivator might compensate by increasing the expression or activity of other inactivators, albeit examples of this remain to be identified. Clearly, unravelling the *in vivo* roles of integrin inhibitors is an important challenge for the future.

### Concluding remarks

In this Commentary, we have aimed to present the known mechanisms of integrin activation and inactivation with a main focus on the less-well understood role of integrin inactivating proteins. Many of the molecular details regarding the mechanism of integrin inactivation remain to be investigated for proteins

such as SHARPIN, MDGI and others. However, one feature appears to be shared by many of these inactivating proteins. Unlike the best-studied integrin activator talin, which provides the integrins with a link to the actin cytoskeleton, integrin inactivators appear to uncouple integrin receptors from the cytoskeleton. Rapid actin-based movement has been shown to transport active but unengaged integrins (i.e. integrins binding talin but not ECM ligands) along the leading edge to newly formed protrusions (Galbraith et al., 2007), suggesting that the final targeting of the active integrin receptor to adhesion sites is driven by actin. In cells, inactive integrins are often found located in detached ruffles (Rantala et al., 2011) or dorsally (Teckchandani et al., 2009), and uncoupling from the actin cytoskeleton would probably allow for a more rapid and free diffusion of integrins along the membrane to areas in which activation of integrins and cell adhesion are triggered. Surprisingly little is known about the switch between active and inactive integrin conformations, and examples of heterodimer-specific regulation of integrin activity are particularly sparse. Specific integrin heterodimers have very different biological functions *in vitro* and *in vivo* (Hynes, 2002), with the consequence that animals lacking a specific integrin display diverse phenotypes that span from early embryonic lethal to very mild defects. On the basis of this finding, it is plausible to assume that mechanisms that specifically inactivate a particular  $\alpha$ - $\beta$ -heterodimer exist. The identification of these mechanisms, or of regulators of specific integrins, will in the future allow the elucidation of additional layers of complexity to the fine-tuned regulation of integrin function in man.

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### References

- Alahari, S. K. (2003). Nischarin inhibits Rac induced migration and invasion of epithelial cells by affecting signaling cascades involving PAK. *Exp. Cell Res.* **288**, 415-424.
- Alahari, S. K. and Nasrallah, H. (2004). A membrane proximal region of the integrin  $\alpha 5$  subunit is important for its interaction with nischarin. *Biochem. J.* **377**, 449-457.
- Alahari, S. K., Lee, J. W. and Juliano, R. L. (2000). Nischarin, a novel protein that interacts with the integrin  $\alpha 5$  subunit and inhibits cell migration. *J. Cell Biol.* **151**, 1141-1154.
- Anthis, N. J., Haling, J. R., Oxley, C. L., Memo, M., Wegener, K. L., Lim, C. J., Ginsberg, M. H. and Campbell, I. D. (2009).  $\beta$  integrin tyrosine phosphorylation is a conserved mechanism for regulating talin-induced integrin activation. *J. Biol. Chem.* **284**, 36700-36710.



- Arjonen, A., Alanko, J., Veltel, S. and Ivaska, J. (2012). Distinct recycling of active and inactive  $\beta 1$  integrins. *Traffic* **13**, 610-625.
- Baranwal, S., Wang, Y., Rathinam, R., Lee, J., Jin, L., McGoey, R., Pylayeva, Y., Giancotti, F., Blobe, G. C. and Alahari, S. K. (2011). Molecular characterization of the tumor-suppressive function of nischarin in breast cancer. *J. Natl. Cancer Inst.* **103**, 1513-1528.
- Barry, W. T., Boudignon-Proudhon, C., Shock, D. D., McFadden, A., Weiss, J. M., Sondek, J. and Parise, L. V. (2002). Molecular basis of CIB binding to the integrin  $\alpha 1 \text{Ib}$  cytoplasmic domain. *J. Biol. Chem.* **277**, 28877-28883.
- Bass, M. D. and Humphries, M. J. (2002). Cytoplasmic interactions of syndecan-4 orchestrate adhesion receptor and growth factor receptor signalling. *Biochem. J.* **368**, 1-15.
- Bass, M. D., Williamson, R. C., Nunan, R. D., Humphries, J. D., Byron, A., Morgan, M. R., Martin, P. and Humphries, M. J. (2011). A syndecan-4 hair trigger initiates wound healing through caveolin- and RhoG-regulated integrin endocytosis. *Dev. Cell* **21**, 681-693.
- Bicknell, L. S., Farrington-Rock, C., Shafeghati, Y., Rump, P., Alanay, Y., Alembik, Y., Al-Madani, N., Firth, H., Karimi-Nejad, M. H., Kim, C. A. et al. (2007). A molecular and clinical study of Larsen syndrome caused by mutations in FLNB. *J. Med. Genet.* **44**, 89-98.
- Binas, B., Gusterson, B., Wallace, R. and Clark, A. J. (1995). Epithelial proliferation and differentiation in the mammary gland do not correlate with cFABP gene expression during early pregnancy. *Dev. Genet.* **17**, 167-175.
- Böhmer, F. D., Sun, Q., Pepperle, M., Müller, T., Eriksson, U., Wang, J. L. and Grosse, R. (1987). Antibodies against mammary derived growth inhibitor (MDGI) react with a fibroblast growth inhibitor and with heart fatty acid binding protein. *Biochem. Biophys. Res. Commun.* **148**, 1425-1431.
- Bouvard, D., Vignoud, L., Dupé-Manet, S., Abed, N., Fournier, H. N., Vincent-Mongat, C., Retta, S. F., Fassler, R. and Block, M. R. (2003). Disruption of focal adhesions by integrin cytoplasmic domain-associated protein-1 $\alpha$ . *J. Biol. Chem.* **278**, 6567-6574.
- Bouvard, D., Aszodi, A., Kostka, G., Block, M. R., Albigès-Rizo, C. and Fassler, R. (2007). Defective osteoblast function in ICAP-1-deficient mice. *Development* **134**, 2615-2625.
- Brown, N. H., Gregory, S. L., Rickoll, W. L., Fessler, L. I., Prout, M., White, R. A. and Fristrom, J. W. (2002). Talin is essential for integrin function in *Drosophila*. *Dev. Cell* **3**, 569-579.
- Calderwood, D. A. (2004). Integrin activation. *J. Cell Sci.* **117**, 657-666.
- Calderwood, D. A., Zent, R., Grant, R., Rees, D. J., Hynes, R. O. and Ginsberg, M. H. (1999). The Talin head domain binds to integrin  $\beta$  subunit cytoplasmic tails and regulates integrin activation. *J. Biol. Chem.* **274**, 28071-28074.
- Calderwood, D. A., Fujioka, Y., de Pereda, J. M., García-Alvarez, B., Nakamoto, T., Margolis, B., McGlade, C. J., Liddington, R. C. and Ginsberg, M. H. (2003). Integrin  $\beta$  cytoplasmic domain interactions with phosphotyrosine-binding domains: a structural prototype for diversity in integrin signaling. *Proc. Natl. Acad. Sci. USA* **100**, 2272-2277.
- Calderwood, D. A., Tai, V., Di Paolo, G., De Camilli, P. and Ginsberg, M. H. (2004). Competition for talin results in trans-dominant inhibition of integrin activation. *J. Biol. Chem.* **279**, 28889-28895.
- Carroll, J. M., Romero, M. R. and Watt, F. M. (1995). Suprabasal integrin expression in the epidermis of transgenic mice results in developmental defects and a phenotype resembling psoriasis. *Cell* **83**, 957-968.
- Chang, D. D., Wong, C., Smith, H. and Liu, J. (1997). ICAP-1, a novel  $\beta 1$  integrin cytoplasmic domain-associated protein, binds to a conserved and functionally important NPXY sequence motif of  $\beta 1$  integrin. *J. Cell Biol.* **138**, 1149-1157.
- Clark, A. J., Neil, C., Gusterson, B., McWhir, J. and Binas, B. (2000). Deletion of the gene encoding H-FABP/MDGI has no overt effects in the mammary gland. *Transgenic Res.* **9**, 439-444.
- Conti, F. J., Monkley, S. J., Wood, M. R., Critchley, D. R. and Müller, U. (2009). Talin 1 and 2 are required for myoblast fusion, sarcomere assembly and the maintenance of myotendinous junctions. *Development* **136**, 3597-3606.
- Critchley, D. R. (2009). Biochemical and structural properties of the integrin-associated cytoskeletal protein talin. *Annu. Rev. Biophys.* **38**, 235-254.
- Czuchra, A., Meyer, H., Legate, K. R., Brakebusch, C. and Fassler, R. (2006). Genetic analysis of  $\beta 1$  integrin "activation motifs" in mice. *J. Cell Biol.* **174**, 889-899.
- Dalkilic, I., Schienda, J., Thompson, T. G. and Kunkel, L. M. (2006). Loss of FilaminC (FLNc) results in severe defects in myogenesis and myotube structure. *Mol. Cell Biol.* **26**, 6522-6534.
- Das, M., Ithychanda, S. S., Qin, J. and Plow, E. F. (2011). Migfilin and filamin as regulators of integrin activation in endothelial cells and neutrophils. *PLoS ONE* **6**, e26355.
- Dejonckheere, E., Vandenbroucke, R. E. and Libert, C. (2011). Matrix metalloproteinase8 has a central role in inflammatory disorders and cancer progression. *Cytokine Growth Factor Rev.* **22**, 73-81.
- Ezratty, E. J., Bertaux, C., Marcantonio, E. E. and Gundersen, G. G. (2009). Clathrin mediates integrin endocytosis for focal adhesion disassembly in migrating cells. *J. Cell Biol.* **187**, 733-747.
- Feng, Y., Chen, M. H., Moskowitz, I. P., Mendonza, A. M., Vidali, L., Nakamura, F., Kwiatkowski, D. J. and Walsh, C. A. (2006). Filamin A (FLNA) is required for cell-cell contact in vascular development and cardiac morphogenesis. *Proc. Natl. Acad. Sci. USA* **103**, 19836-19841.
- Forman-Kay, J. D. and Pawson, T. (1999). Diversity in protein recognition by PTB domains. *Curr. Opin. Struct. Biol.* **9**, 690-695.
- Galbraith, C. G., Yamada, K. M. and Galbraith, J. A. (2007). Polymerizing actin fibers position integrins primed to probe for adhesion sites. *Science* **315**, 992-995.
- Gerlach, B., Cordier, S. M., Schmukle, A. C., Emmerich, C. H., Rieser, E., Haas, T. L., Webb, A. L., Rickard, J. A., Anderton, H., Wong, W. W. et al. (2011). Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* **471**, 591-596.
- Goldmann, W. H. (2000). Kinetic determination of focal adhesion protein formation. *Biochem. Biophys. Res. Commun.* **271**, 553-557.
- Grashoff, C., Hoffman, B. D., Brenner, M. D., Zhou, R., Parsons, M., Yang, M. T., McLean, M. A., Sligar, S. G., Chen, C. S., Ha, T. et al. (2010). Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. *Nature* **466**, 263-266.
- Gushiken, F. C., Patel, V., Liu, Y., Pradhan, S., Bergeron, A. L., Peng, Y. and Vijayan, K. V. (2008). Protein phosphatase 2A negatively regulates integrin  $\alpha 1 \text{Ib}$  signaling. *J. Biol. Chem.* **283**, 12862-12869.
- Gustin, J. A., Maehama, T., Dixon, J. E. and Donner, D. B. (2001). The PTEN tumor suppressor protein inhibits tumor necrosis factor-induced nuclear factor  $\kappa \text{B}$  activity. *J. Biol. Chem.* **276**, 27740-27744.
- Harburger, D. S. and Calderwood, D. A. (2009). Integrin signalling at a glance. *J. Cell Sci.* **122**, 159-163.
- Harburger, D. S., Bouaouina, M. and Calderwood, D. A. (2009). Kindlin-1 and -2 directly bind the C-terminal region of  $\beta$  integrin cytoplasmic tails and exert integrin-specific activation effects. *J. Biol. Chem.* **284**, 11485-11497.
- Hart, A. W., Morgan, J. E., Schneider, J., West, K., McKie, L., Bhattacharya, S., Jackson, I. J. and Cross, S. H. (2006). Cardiac malformations and midline skeletal defects in mice lacking filamin A. *Hum. Mol. Genet.* **15**, 2457-2467.
- Hauenerland, N. H. and Spener, F. (2004). Fatty acid-binding proteins—insights from genetic manipulations. *Prog. Lipid Res.* **43**, 328-349.
- He, L., Ingram, A., Rybak, A. P. and Tang, D. (2010). Shank-interacting protein-like 1 promotes tumorigenesis via PTEN inhibition in human tumor cells. *J. Clin. Invest.* **120**, 2094-2108.
- Hogg, N. and Bates, P. A. (2000). Genetic analysis of integrin function in man: LAD-1 and other syndromes. *Matrix Biol.* **19**, 211-222.
- Horwitz, A., Duggan, K., Buck, C., Beckerle, M. C. and Burridge, K. (1986). Interaction of plasma membrane fibronectin receptor with talin—a transmembrane linkage. *Nature* **320**, 531-533.
- Hughes, P. E., Diaz-Gonzalez, F., Leong, L., Wu, C., McDonald, J. A., Shattil, S. J. and Ginsberg, M. H. (1996). Breaking the integrin hinge. A defined structural constraint regulates integrin signaling. *J. Biol. Chem.* **271**, 6571-6574.
- Humphries, J. D., Wang, P., Streuli, C., Geiger, B., Humphries, M. J. and Ballestrem, C. (2007). Vinculin controls focal adhesion formation by direct interactions with talin and actin. *J. Cell Biol.* **179**, 1043-1057.
- Hynes, R. O. (2002). Integrins: bidirectional, allosteric signaling machines. *Cell* **110**, 637-687.
- Ikeda, F., Deribe, Y. L., Skånland, S. S., Stieglitz, B., Grabbe, C., Franz-Wachtel, M., van Wijk, S. J., Goswami, P., Nagy, V., Terzic, J. et al. (2011). SHARPIN forms a linear ubiquitin ligase complex regulating NF- $\kappa \text{B}$  activity and apoptosis. *Nature* **471**, 637-641.
- Ithychanda, S. S., Das, M., Ma, Y. Q., Ding, K., Wang, X., Gupta, S., Wu, C., Plow, E. F. and Qin, J. (2009). Migfilin, a molecular switch in regulation of integrin activation. *J. Biol. Chem.* **284**, 4713-4722.
- Ivaska, J. and Heino, J. (2010). Interplay between cell adhesion and growth factor receptors: from the plasma membrane to the endosomes. *Cell Tissue Res.* **339**, 111-120.
- Ivaska, J. and Heino, J. (2011). Cooperation between integrins and growth factor receptors in signaling and endocytosis. *Annu. Rev. Cell Dev. Biol.* **27**, 291-320.
- Jokinen, J., White, D. J., Salmela, M., Huhtala, M., Käpylä, J., Sipilä, K., Puranen, J. S., Nissinen, L., Kankaanpää, P., Marjomäki, V. et al. (2010). Molecular mechanism of  $\alpha 2 \text{Ib}$  integrin interaction with human echovirus 1. *EMBO J.* **29**, 196-208.
- Kalli, A. C., Campbell, I. D. and Sansom, M. S. (2011). Multiscale simulations suggest a mechanism for integrin inside-out activation. *Proc. Natl. Acad. Sci. USA* **108**, 11890-11895.
- Karaköse, E., Schiller, H. B. and Fassler, R. (2010). The kindlins at a glance. *J. Cell Sci.* **123**, 2353-2356.
- Katz, M., Amit, I., Citri, A., Shay, T., Carvalho, S., Lavi, S., Milanezi, F., Lyass, L., Amariglio, N., Jacob-Hirsch, J. et al. (2007). A reciprocal tensin-3-cten switch mediates EGF-driven mammary cell migration. *Nat. Cell Biol.* **9**, 961-969.
- Kiema, T., Lad, Y., Jiang, P., Oxley, C. L., Baldassarre, M., Wegener, K. L., Campbell, I. D., Yläanne, J. and Calderwood, D. A. (2006). The molecular basis of filamin binding to integrins and competition with talin. *Mol. Cell* **21**, 337-347.
- Kilpinen, S., Autio, R., Ojala, K., Iljin, K., Bucher, E., Sara, H., Pisto, T., Saarela, M., Skotheim, R. L., Björkman, M. et al. (2008). Systematic bioinformatic analysis of expression levels of 17,330 human genes across 9,783 samples from 175 types of healthy and pathological tissues. *Genome Biol.* **9**, R139.
- Kim, H. and McCulloch, C. A. (2011). Filamin A mediates interactions between cytoskeletal proteins that control cell adhesion. *FEBS Lett.* **585**, 18-22.
- Kim, S. M., Kwon, M. S., Park, C. S., Choi, K. R., Chun, J. S., Ahn, J. and Song, W. K. (2004). Modulation of Thr phosphorylation of integrin  $\beta 1$  during muscle differentiation. *J. Biol. Chem.* **279**, 7082-7090.

- Knezevic, I., Leisner, T. M. and Lam, S. C.-T. (1996). Direct binding of the platelet integrin  $\alpha$ IIb $\beta$ 3 (GPIIb-IIIa) to talin. Evidence that interaction is mediated through the cytoplasmic domains of both  $\alpha$ IIb and  $\beta$ 3. *J. Biol. Chem.* **271**, 16416-16421.
- Lad, Y., Jiang, P., Ruskamo, S., Harburger, D. S., Ylänne, J., Campbell, I. D. and Calderwood, D. A. (2008). Structural basis of the migfilin-filamin interaction and competition with integrin  $\beta$  tails. *J. Biol. Chem.* **283**, 35154-35163.
- Landgraf, K., Bollig, F., Trowe, M. O., Besenbeck, B., Ebert, C., Kruspe, D., Kispert, A., Hänel, F. and Englert, C. (2010). Sipl1 and Rbck1 are novel Eyal-binding proteins with a role in craniofacial development. *Mol. Cell Biol.* **30**, 5764-5775.
- Lau, T. L., Kim, C., Ginsberg, M. H. and Ulmer, T. S. (2009). The structure of the integrin  $\alpha$ IIb $\beta$ 3 transmembrane complex explains integrin transmembrane signalling. *EMBO J.* **28**, 1351-1361.
- Legate, K. R., Wickström, S. A. and Fässler, R. (2009). Genetic and cell biological analysis of integrin outside-in signaling. *Genes Dev.* **23**, 397-418.
- Li, J., Ballif, B. A., Powelka, A. M., Dai, J., Gygi, S. P. and Hsu, V. W. (2005). Phosphorylation of ACAP1 by Akt regulates the stimulation-dependent recycling of integrin  $\beta$ 1 to control cell migration. *Dev. Cell* **9**, 663-673.
- Liang, Y., Seymour, R. E. and Sundberg, J. P. (2011). Inhibition of NF- $\kappa$ B signaling retards eosinophilic dermatitis in SHARPIN-deficient mice. *J. Invest. Dermatol.* **131**, 141-149.
- Lim, S., Sala, C., Yoon, J., Park, S., Kuroda, S., Sheng, M. and Kim, E. (2001). Sharpin, a novel postsynaptic density protein that directly interacts with the shank family of proteins. *Mol. Cell Neurosci.* **17**, 385-397.
- Liu, S., Calderwood, D. A. and Ginsberg, M. H. (2000). Integrin cytoplasmic domain-binding proteins. *J. Cell Sci.* **113**, 3563-3571.
- Mai, A., Veltel, S., Pellinen, T., Padzik, A., Coffey, E., Marjomäki, V. and Ivaska, J. (2011). Competitive binding of Rab21 and p120RasGAP to integrins regulates receptor traffic and migration. *J. Cell Biol.* **194**, 291-306.
- Margadant, C., Monsuur, H. N., Norman, J. C. and Sonnenberg, A. (2011). Mechanisms of integrin activation and trafficking. *Curr. Opin. Cell Biol.* **23**, 607-614.
- Millon-Frémillon, A., Bouvard, D., Grichine, A., Manet-Dupé, S., Block, M. R. and Albiges-Rizo, C. (2008). Cell adaptive response to extracellular matrix density is controlled by ICAP-1-dependent  $\beta$ 1-integrin affinity. *J. Cell Biol.* **180**, 427-441.
- Monkley, S. J., Zhou, X.-H., Kinston, S. J., Giblett, S. M., Hemmings, L., Priddle, H., Brown, J. E., Pritchard, C. A., Critchley, D. R. and Fässler, R. (2000). Disruption of the *talin* gene arrests mouse development at the gastrulation stage. *Dev. Dyn.* **219**, 560-574.
- Montanez, E., Ussar, S., Schifferer, M., Bösl, M., Zent, R., Moser, M. and Fässler, R. (2008). Kindlin-2 controls bidirectional signaling of integrins. *Genes Dev.* **22**, 1325-1330.
- Moser, M., Nieswandt, B., Ussar, S., Pozgajova, M. and Fässler, R. (2008). Kindlin-3 is essential for integrin activation and platelet aggregation. *Nat. Med.* **14**, 325-330.
- Moser, M., Legate, K. R., Zent, R. and Fässler, R. (2009). The tail of integrins, talin, and kindlins. *Science* **324**, 895-899.
- Mulrooney, J., Foley, K., Vineberg, S., Barreuther, M. and Grabel, L. (2000). Phosphorylation of the  $\beta$ 1 integrin cytoplasmic domain: toward an understanding of function and mechanism. *Exp. Cell Res.* **258**, 332-341.
- Naik, M. U., Nigam, A., Manrai, P., Millili, P., Czymmek, K., Sullivan, M. and Naik, U. P. (2009). C1B1 deficiency results in impaired thrombosis: the potential role of C1B1 in outside-in signaling through integrin  $\alpha$ IIb $\beta$ 3. *J. Thromb. Haemost.* **7**, 1906-1914.
- Naik, U. P. and Naik, M. U. (2003). Association of C1B with GPIIb/IIIa during outside-in signaling is required for platelet spreading on fibrinogen. *Blood* **102**, 1355-1362.
- Naik, U. P., Patel, P. M. and Parise, L. V. (1997). Identification of a novel calcium-binding protein that interacts with the integrin  $\alpha$ IIb cytoplasmic domain. *J. Biol. Chem.* **272**, 4651-4654.
- Nevo, J., Mattila, E., Pellinen, T., Yamamoto, D. L., Sara, H., Iljin, K., Kallioniemi, O., Bono, P., Heikkilä, P., Joensuu, H. et al. (2009). Mammary-derived growth inhibitor alters traffic of EGFR and induces a novel form of cetuximab resistance. *Clin. Cancer Res.* **15**, 6570-6581.
- Nevo, J., Mai, A., Tuomi, S., Pellinen, T., Pentikäinen, O. T., Heikkilä, P., Lundin, J., Joensuu, H., Bono, P. and Ivaska, J. (2010). Mammary-derived growth inhibitor (MDGI) interacts with integrin  $\alpha$ -subunits and suppresses integrin activity and invasion. *Oncogene* **29**, 6452-6463.
- Ng, T., Shima, D., Squire, A., Bastiaens, P. I., Gschmeissner, S., Humphries, M. J. and Parker, P. J. (1999). PKC $\alpha$  regulates  $\beta$ 1 integrin-dependent cell motility through association and control of integrin traffic. *EMBO J.* **18**, 3909-3923.
- Nishimura, T. and Kaibuchi, K. (2007). Numb controls integrin endocytosis for directional cell migration via aPKC and PAR-3. *Dev. Cell* **13**, 15-28.
- O'Toole, T. E., Mandelman, D., Forsyth, J., Shattil, S. J., Plow, E. F. and Ginsberg, M. H. (1991). Modulation of the affinity of integrin alpha IIb beta 3 (GPIIb-IIIa) by the cytoplasmic domain of alpha IIb. *Science* **254**, 845-847.
- Oxley, C. L., Anthis, N. J., Lowe, E. D., Vakonakis, I., Campbell, I. D. and Wegener, K. L. (2008). An integrin phosphorylation switch: the effect of  $\beta$ 3 integrin tail phosphorylation on Dok1 and talin binding. *J. Biol. Chem.* **283**, 5420-5426.
- Parsons, M., Keppler, M. D., Kline, A., Messent, A., Humphries, M. J., Gilchrist, R., Hart, I. R., Quittau-Prevostel, C., Hughes, W. E., Parker, P. J. et al. (2002). Site-directed perturbation of protein kinase C-integrin interaction blocks carcinoma cell chemotaxis. *Mol. Cell Biol.* **22**, 5897-5911.
- Partridge, M. A. and Marcantonio, E. E. (2006). Initiation of attachment and generation of mature focal adhesions by integrin-containing filopodia in cell spreading. *Mol. Biol. Cell* **17**, 4237-4248.
- Pellinen, T. and Ivaska, J. (2006). Integrin traffic. *J. Cell Sci.* **119**, 3723-3731.
- Pellinen, T., Arjonen, A., Vuoriluoto, K., Kallio, K., Fransén, J. A. and Ivaska, J. (2006). Small GTPase Rab21 regulates cell adhesion and controls endosomal traffic of  $\beta$ 1-integrins. *J. Cell Biol.* **173**, 767-780.
- Pellinen, T., Rantala, J. K., Arjonen, A., Mpindi, J. P., Kallioniemi, O. and Ivaska, J. (2012). A functional genetic screen reveals new regulators of  $\beta$ 1-integrin activity. *J. Cell Sci.* **125**, 649-661.
- Pfaff, M., Liu, S., Erle, D. J. and Ginsberg, M. H. (1998). Integrin  $\beta$  cytoplasmic domains differentially bind to cytoskeletal proteins. *J. Biol. Chem.* **273**, 6104-6109.
- Pluskota, E., Dowling, J. J., Gordon, N., Golden, J. A., Szpak, D., West, X. Z., Nestor, C., Ma, Y. Q., Bialkowska, K., Byzova, T. et al. (2011). The integrin coactivator kindlin-2 plays a critical role in angiogenesis in mice and zebrafish. *Blood* **117**, 4978-4987.
- Rantala, J. K., Pouwels, J., Pellinen, T., Veltel, S., Laasola, P., Mattila, E., Potter, C. S., Duffy, T., Sundberg, J. P., Kallioniemi, O. et al. (2011). SHARPIN is an endogenous inhibitor of  $\beta$ 1-integrin activation. *Nat. Cell Biol.* **13**, 1315-1324.
- Seymour, R. E., Hasham, M. G., Cox, G. A., Shultz, L. D., Hogenesch, H., Ruppenian, D. C. and Sundberg, J. P. (2007). Spontaneous mutations in the mouse Sharpin gene result in multiorgan inflammation, immune system dysregulation and dermatitis. *Genes Immun.* **8**, 416-421.
- Shattil, S. J., Kim, C. and Ginsberg, M. H. (2010). The final steps of integrin activation: the end game. *Nat. Rev. Mol. Cell Biol.* **11**, 288-300.
- Shock, D. D., Naik, U. P., Brittain, J. E., Alahari, S. K., Sondek, J. and Parise, L. V. (1999). Calcium-dependent properties of C1B binding to the integrin  $\alpha$ IIb cytoplasmic domain and translocation to the platelet cytoskeleton. *Biochem. J.* **342**, 729-735.
- Springer, T. A. and Dustin, M. L. (2012). Integrin inside-out signaling and the immunological synapse. *Curr. Opin. Cell Biol.* **24**, 107-115.
- Tanentzapf, G. and Brown, N. H. (2006). An interaction between integrin and the talin FERM domain mediates integrin activation but not linkage to the cytoskeleton. *Nat. Cell Biol.* **8**, 601-606.
- Teckchandani, A., Toida, N., Goodchild, J., Henderson, C., Watts, J., Wollscheid, B. and Cooper, J. A. (2009). Quantitative proteomics identifies a Dab2/integrin module regulating cell migration. *J. Cell Biol.* **186**, 99-111.
- Tokunaga, F., Sakata, S., Saeki, Y., Satomi, Y., Kirisako, T., Kamei, K., Nakagawa, T., Kato, M., Murata, S., Yamaoka, S. et al. (2009). Involvement of linear polyubiquitylation of NEMO in NF- $\kappa$ B activation. *Nat. Cell Biol.* **11**, 123-132.
- Tokunaga, F., Nakagawa, T., Nakahara, M., Saeki, Y., Taniguchi, M., Sakata, S., Tanaka, K., Nakano, H. and Iwai, K. (2011). SHARPIN is a component of the NF- $\kappa$ B-activating linear ubiquitin chain assembly complex. *Nature* **471**, 633-636.
- Tsuboi, S. (2002). Calcium integrin-binding protein activates platelet integrin  $\alpha$ IIb $\beta$ 3. *J. Biol. Chem.* **277**, 1919-1923.
- Upla, P., Marjomäki, V., Kankaanpää, P., Ivaska, J., Hyypiä, T., Van Der Goot, F. G. and Heino, J. (2004). Clustering induces a lateral redistribution of  $\alpha$ 2 $\beta$ 1 integrin from membrane rafts to caveolae and subsequent protein kinase C-dependent internalization. *Mol. Biol. Cell* **15**, 625-636.
- Ussar, S., Moser, M., Widmaier, M., Roggoni, E., Harrer, C., Genzel-Boroviczeny, O. and Fässler, R. (2008). Loss of Kindlin-1 causes skin atrophy and lethal neonatal intestinal epithelial dysfunction. *PLoS Genet.* **4**, e1000289.
- Valdembri, D., Caswell, P. T., Anderson, K. I., Schwarz, J. P., König, I., Astanina, E., Caccavari, F., Norman, J. C., Humphries, M. J., Bussolino, F. et al. (2009). Neuropilin-1/GIPC1 signaling regulates  $\alpha$ 5 $\beta$ 1 integrin traffic and function in endothelial cells. *PLoS Biol.* **7**, e25.
- Watt, F. M. (2002). Role of integrins in regulating epidermal adhesion, growth and differentiation. *EMBO J.* **21**, 3919-3926.
- Wegener, K. L., Partridge, A. W., Han, J., Pickford, A. R., Liddington, R. C., Ginsberg, M. H. and Campbell, I. D. (2007). Structural basis of integrin activation by talin. *Cell* **128**, 171-182.
- Woods, A. J., White, D. P., Caswell, P. T. and Norman, J. C. (2004). PKD1/PKC $\mu$  promotes  $\alpha$ v $\beta$ 3 integrin recycling and delivery to nascent focal adhesions. *EMBO J.* **23**, 2531-2543.
- Xia, T., Liang, Y., Ma, J., Li, M., Gong, M. and Yu, X. (2011). Loss-of-function of SHARPIN causes an osteopenic phenotype in mice. *Endocrine* **39**, 104-112.
- Ye, F., Kim, C. and Ginsberg, M. H. (2012). Reconstruction of integrin activation. *Blood* **119**, 26-33.
- Yuan, W., Leisner, T. M., McFadden, A. W., Wang, Z., Larson, M. K., Clark, S., Boudignon-Proudhon, C., Lam, S. C.-T. and Parise, L. V. (2006). C1B1 is an endogenous inhibitor of agonist-induced integrin  $\alpha$ IIb $\beta$ 3 activation. *J. Cell Biol.* **172**, 169-175.
- Zaidel-Bar, R., Itzkovitz, S., Ma'ayan, A., Iyengar, R. and Geiger, B. (2007). Functional atlas of the integrin adhesome. *Nat. Cell Biol.* **9**, 858-867.
- Zhang, X. A. and Hemler, M. E. (1999). Interaction of the integrin  $\beta$ 1 cytoplasmic domain with ICAP-1 protein. *J. Biol. Chem.* **274**, 11-19.
- Zhang, X., Jiang, G., Cai, Y., Monkley, S. J., Critchley, D. R. and Sheetz, M. P. (2008). Talin depletion reveals independence of initial cell spreading from integrin activation and traction. *Nat. Cell Biol.* **10**, 1062-1068.
- Zhou, A.-X., Hartwig, J. H. and Akyürek, L. M. (2010). Filamins in cell signaling, transcription and organ development. *Trends Cell Biol.* **20**, 113-123.