Definition of a consensus integrin adhesome and its dynamics during adhesion complex assembly and disassembly

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Integrin receptor activation initiates the formation of integrin adhesion complexes (IACs) at the cell membrane that transduce adhesion-dependent signals to control a multitude of cellular functions. Proteomic analyses of isolated IACs have revealed an unanticipated molecular complexity; however, a global view of the consensus composition and dynamics of IACs is lacking. Here, we have integrated several IAC proteomes and generated a 2,412-protein integrin adhesome. Analysis of this data set reveals the functional diversity of proteins in IACs and establishes a consensus adhesome of 60 proteins. The consensus adhesome is likely to represent a core cell adhesion machinery, centred around four axes comprising ILK–PINCH–kindlin, FAK–paxillin, talin–vinculin and α-actinin–zyxin–VASP, and includes underappreciated IAC components such as Rsu-1 and caldesmon. Proteomic quantification of IAC assembly and disassembly detailed the compositional dynamics of the core cell adhesion machinery. The definition of this consensus view of integrin adhesome components provides a resource for the research community.

Cellular adhesion to the extracellular matrix (ECM) is essential for a multicellular existence. Cell-surface integrin adhesion receptors engage the cytoskeleton and transduce signals that control cell morphology, migration, survival and differentiation in a wide range of developmental, homeostatic and disease processes1. The interactions of integrin cytoplasmic domains with cytoskeletal, adaptor and signalling molecules are central to regulation of integrin-mediated functions2,3. The complex multimolecular structures that form the connection between integrins and the actin cytoskeleton (termed IACs) contain over 200 reported components4–6. IAC proteins have been characterized as either ‘intrinsic’ components, which localize directly to IACs, or ‘associated’ components, which are effectors of intrinsic molecules8. Despite their apparent complexity, IACs are highly dynamic, turning over on a timescale of minutes.

IACs, like other membrane-receptor-associated signalling complexes, have been refractory to proteomic analysis owing to their lability and inaccessibility7. Recent approaches to isolate IACs and analyse their molecular composition using mass spectrometry have been performed in multiple cell types under various conditions8–17. These data sets are necessarily context-dependent (for example, cell-type- or integrin-heterodimer-specific) and generally represent steady-state cell adhesion. Consequently, an integrative, systems-wide description of IAC composition and dynamics is lacking.

To enable a systems-level analysis of IACs, we characterized their composition in mouse fibroblasts and computationally integrated these data with previously reported IAC proteomes from additional cell types11,13–16. Bioinformatic analyses allowed us to define the functional IAC landscape, from which we identified a robustly detected core ‘consensus’ adhesome, which consisted of both well-characterized and underappreciated components. Using this consensus adhesome in combination with proteomic quantification of IAC assembly and disassembly revealed distinct temporal profiles of protein recruitment. Together with identification of IAC components dependent on myosin-II-mediated tension, these results detailed the compositional dynamics and maturation of the core cell adhesion machinery.

RESULTS
An experimentally defined integrin meta-adhesome
To obtain a global overview of IAC composition, we constructed a database from multiple mass spectrometry (MS)-based proteomics...
reports. All six published quantitative proteomic data sets detailing the composition of IACs induced by the canonical ligand fibronectin (FN) were assembled. To distinguish and reduce nonspecific proteins from the data, we required the proteomic analyses to employ a negative-control ligand, which excluded several published data sets, and we included only those proteins that were at least twofold enriched over their respective controls (Supplementary Table 1; see Methods for details). The assembled data sets were generated in multiple laboratories using a variety of methodologies and from a range of cell types from different lineages: human malignant melanoma (A375), human foreskin fibroblast (HFF), human chronic myelogenous leukaemia (K562) and mouse kidney fibroblast (MKF) cells (Supplementary Table 1). In addition, to expand the number of controlled data sets in the database, we generated a seventh data set of FN-induced IACs purified from mouse embryonic fibroblast (MEF) cells using published protocols. A total of 1,461 proteins were identified (99% confidence) in IACs from MEF cells, of which 674 proteins were at least twofold enriched to FN-induced IACs over the negative control (transferrin; Supplementary Table 2), which is of a similar scale to other IAC proteomes (Supplementary Fig. 1 and Supplementary Table 1). FN-enriched MEF proteins were integrated with FN-enriched proteins from the six assembled IAC data sets. The resulting experimentally defined database contained 2,412 proteins observed in at least one IAC proteome and was termed the ‘meta-adhesome’ (Supplementary Table 3).

Comparative analyses identified cell-type-, negative-control- and biochemical-isolation-methodology-specific variations in IAC composition (Fig. 1a and Supplementary Fig. 1). Individual IAC proteomes contained hundreds of proteins (602 ± 250, mean ± s.d.; range, 314–1,023) and identified up to a third of literature-curated adhesome components (20.9 ± 7.1%, mean ± s.d.; range, 9.1–32.3%; Fig. 1a and Supplementary Fig. 1c). This variation is likely to result from the context under which the IACs were observed. Over half of the proteins in the meta-adhesome (1,359; 56.3%) were identified uniquely in a single data set (Fig. 1b). These proteins represent low-abundance or context-specific adhesome components, or those difficult to detect by MS. The number of proteins identified in the meta-adhesome decreased exponentially as the stringency in data set number increased (Fig. 1b,c). Four hundred and forty-eight proteins were detected in at least three data sets (Fig. 1c), more than the 63 proteins previously found in common between three published IAC proteomes. Only 10 proteins were enriched in all seven data sets (labelled in Fig. 1d). We reasoned that a restricted set of robustly detected proteins may represent a context-independent core of IAC components. Indeed, the proportion of identified proteins that were literature-curated adhesome components increased with data set occurrence (Fig. 1e and Supplementary Fig. 1a), suggesting that robustly detected proteins are more likely to represent canonical adhesion proteins.

To investigate the organization of proteins in the meta-adhesome, we performed interaction network analysis (Fig. 1d). Proteins detected in few IAC data sets exhibited lower network connectivity in general, whereas proteins detected in all seven data sets exhibited the potential to exert greater control over the interactions of other proteins in the complex, as determined by network topology (Fig. 1f and Supplementary Fig. 2). The high number of proteins identified in the meta-adhesome, together with their interconnected network of potential interactions, indicates that IACs, and the flow of information that they relay, are highly complex. Furthermore, it suggests that even the literature-curated adhesome understimates this complexity and that heterogeneity in IAC composition exists between experimental contexts even when cells are exposed to very similar extracellular microenvironments and ligands.

**Functional analysis of the integrin meta-adhesome**

To visualize proteins identified in the meta-adhesome in the context of the literature-curated adhesome, meta-adhesome proteins were mapped onto adhesome functional categories. In total, 114 (49%) adhesome components were detected across all data sets (Fig. 2a), with almost half (56) detected in three or more data sets (Fig. 2b). The functional categories with the highest coverage in the meta-adhesome were adaptors (46; 65%), actin regulators (14; 82%) and chaperones (3; 100%; Fig. 2c). GTPases, phosphatases, kinases, channels and adhesion receptors were less well represented. Notably, the receptors most robustly detected were the prominent FN-binding α5β1 and αvβ3 integrins, which confirms the specificity of FN-induced IACs incorporated in the meta-adhesome. The 114 FN-specific adhesome components comprised 87 ‘intrinsic’ and 27 ‘associated’ proteins (Fig. 2a). These data probably reflect the ability of IAC isolation methods to stabilize and identify structural adhesome molecules, such as adaptors and actin regulators. Associated proteins were generally enriched in fewer data sets compared with intrinsic proteins (Fig. 2b), which may be due to the low stoichiometry, context specificity or highly dynamic and labile nature of associated proteins, such as adhesion-related enzymes, within IACs.

**Characterization of a consensus integrin adhesome**

The meta-adhesome provides a resource detailing global IAC composition from multiple cell types and experimental designs. Proteins with diverse cellular functions were detected in the meta-adhesome, but the most robustly detected proteins were over-represented for numerous adhesion-related functions (Supplementary Fig. 3). To identify the core set of IAC components, and thereby aid the identification of key nodes controlling adhesion functions, we examined proteins identified in at least five data sets (excluding ECM components), which resulted in a consensus integrin adhesome comprising 60 proteins (Supplementary Table 4). Pathways regulating adhesion-related functions were the most significantly enriched in the consensus adhesome (Fig. 3 and Supplementary Table 5), and there was over-representation of actin-binding domains and, most significantly, LIM domains, which have been shown previously to be involved in force recognition at adhesion sites (Supplementary Tables 4 and 5). Nine consensus adhesome genes (15%) had links to inherited diseases (Supplementary Table 4), including seven also identified in a recent report and two others (α-actinin-4 and cyclophilin B) associated with glomerular disease and bone disorders, which have previously been linked to adhesome genes.

To validate further the consensus integrin adhesome network, interactions between proteins were scored according to the level of supporting experimental evidence (Supplementary Table 6). The resulting interaction network contained many known IAC interactions of proteins and their context-dependent adhesomes, establishing a foundational resource for integrin adhesome research.
Figure 1 Overlap and comparison of IAC proteomes in the meta-adhesome. (a) Pairwise overlaps of FN-enriched proteins identified in the seven proteomic data sets and the literature-curated adhesome are shown as a hierarchically clustered heatmap. K562, human chronic myelogenous leukaemia cells; MEF, mouse embryonic fibroblast cells (this study); A375, human malignant melanoma cells; HFF, human foreskin fibroblast cells; MKF1, MKF2, and MKF3, mouse kidney fibroblast cells. Details of the proteomic data sets are provided in Supplementary Table 1. (b) The number of proteomic data sets in which proteins in the meta-adhesome are identified (data set occurrence) is shown as a pie chart. Numbers of proteins identified are indicated for each segment (proportions of the meta-adhesome are shown in parentheses). (c) Line graph showing the cumulative proportion of the meta-adhesome in at least x proteomic data sets, where x is the minimum (min.) data set occurrence category. Numbers of proteins identified are indicated for each data point. (d) Protein–protein interaction network model of the meta-adhesome. The 2,412 meta-adhesome proteins were mapped onto a curated database of reported protein–protein interactions. The largest connected graph component is displayed, comprising 11,430 interactions (grey lines, edges) between 2,035 proteins (circles, nodes). Node size and colour are proportional to the number of proteomic data sets in which a protein was identified. Proteins identified in all seven data sets are named. (e) Line graph showing the proportion of identified proteins that are in the literature-curated adhesome. Numbers of literature-curated adhesome proteins identified are indicated for each data point. (f) The number of reported protein–protein interactions (degree) for each protein is plotted according to the number of proteomic data sets in which it was identified. Box-and-whisker plot shows the median (line), mean (plus sign), 25th and 75th percentiles (box) and 5th and 95th percentiles (whiskers) (n = 1,117, 518, 238, 102, 33, 25 and 10 mapped proteins identified in 1–7 data sets, respectively, with degree ≥1). *P < 0.05, **P < 0.01, ***P < 0.0001; Kruskal–Wallis test with Dunn’s post hoc correction (see Supplementary Table 15 for statistics source data).
Figure 2 Meta-adhesome coverage of the literature-curated adhesome. (a) The proportion of the literature-curated adhesome identified in the meta-adhesome is plotted as a percentage bar chart. Proportions of the total literature-curated adhesome (black), intrinsic adhesome components (blue) and associated adhesome components (red) are shown. Numbers of identified proteins are indicated. (b) Line graph showing the cumulative number of literature-curated adhesome proteins identified in at least x proteomic data sets, where x is the minimum (min.) data set occurrence category. Data for intrinsic (blue) and associated (red) adhesome components are shown. (c) Protein–protein interaction network of the literature-curated adhesome proteins identified in the meta-adhesome. Node size and colour are proportional to the number of proteomic data sets in which a protein was identified; ND, not detected (grey node). Nodes are clustered according to literature-curated adhesome functions; numbers (meta-adhesome/literature-curated adhesome total) and proportions of each functional category identified in the meta-adhesome are indicated in parentheses. Nodes are labelled with protein symbols for clarity (see Supplementary Table 3 for details).
Functional enrichment map of the consensus integrin adhesome. 
(a, b) Over-represented biological process (a) and cellular component (b) terms from proteins identified in the consensus adhesome were hierarchically clustered according to proteomic data set occurrence.

This identified clusters of similarly detected proteins associated with a similar set of functional terms. Related terms are summarized (black bars). Protein symbols are shown for clarity (see Supplementary Table 4 for details).

Confidence in their involvement in the core adhesion machinery, but their contributions to adhesive functions remain to be elucidated, and it remains possible that their association is nonspecific.

To verify that underappreciated proteins identified in the consensus adhesome localize to IACs, we visualized by immunofluorescence two consensus adhesome proteins that are not literature-curated adhesome members (Fig. 5). Caldesmon, localized to actin within vinculin-positive areas (Fig. 5a; Mander’s overlap coefficient (MOC) = 0.51 ± 0.19, mean ± s.d.). Rsu-1, which has been reported to associate with IACs in other cell types, co-localized with vinculin (Fig. 5b; MOC = 0.98 ± 0.03, mean ± s.d.). These data confirm IAC localization of caldesmon and Rsu-1 and suggest that underappreciated consensus adhesome proteins may participate in regulating the integrin–actin connection.

Maturation state of the consensus adhesome
To evaluate further the function of the consensus adhesome, we compared consensus adhesome proteins identified in individual IAC proteomes, demonstrating that they were distributed relatively evenly between data sets (range, 26–59 proteins; Supplementary Fig. 4). The data set generated from K562 cells using FN-coated beads identified the lowest number of consensus components, with a high proportion of the absent proteins containing LIM domains. As it has been shown previously that LIM-domain proteins are force-sensitive,

Temporal dynamics of the consensus integrin adhesome
The consensus adhesome provides a comprehensive view of commonly identified, steady-state IAC composition. To identify the temporal dynamics of IACs, we characterized their composition during assembly and disassembly (Supplementary Tables 11 and 12). Analysis of meta-adhesome proteins identified in the temporal IAC profiles revealed distinct dynamics of proteins involved in specific processes.
To examine the core adhesion machinery, hierarchical clustering revealed that different consensus adhesome components exhibit distinct dynamics (Figs 6 and 7). $\beta_1$, $\alpha_5$ and $\alpha_V$ integrins reached maximum abundance by 30 min in this system. Integrins were relatively stable throughout IAC disassembly, and this was also the case for other cell-surface molecules (for example, annexin A1, transglutaminase-2 and the CD98 heavy chain (SLC3A2)). Most consensus components, although distributed in different clusters (Fig. 6), were detected in high abundance late in IAC assembly here, indicating distinct dynamics of protein recruitment. Integrin-binding proteins decreased during IAC disassembly but with different kinetics (clusters D1, D4; Fig. 7). Most of the adaptors in the consensus adhesome were almost completely absent from IACs after 15 min (cluster D1, Fig. 7), whereas 13 of the 17 actin-binding proteins, five of which were integrin-binding, decreased in abundance less rapidly (cluster D4, Fig. 7). These data suggest that adaptor proteins located between actin and integrins are lost earlier and at a faster rate than actin-binding proteins and that the integrin–actin linkage is disrupted late during IAC disassembly.

To confirm the temporal differences in IAC components revealed by MS, IAC proteins were visualized during nocodazole washout. On nocodazole washout, the area of the ventral cell surface covered by $\alpha_5$ or $\beta_1$ integrin did not change (Fig. 8 and Supplementary Fig. 7). In support of the different rates of loss of IAC components, the decrease in vinculin (30 min; Fig. 8) was delayed compared with the loss of zyxin (10 min; Fig. 8) and other adhesion molecules (phospho-FAK, 10 min; parvin and phospho-parvin, 15 min; Supplementary Fig. 7). These data validate the findings obtained using MS that indicate that different adhesion molecules exhibit distinct temporal profiles during IAC disassembly.

**DISCUSSION**

Here, we performed extensive analyses of IAC proteomes, resulting in an experimentally defined meta-adhesome of 2,412 proteins. An emergent property of the meta-adhesome was the identification functional processes (Supplementary Figs 5 and 6 and Supplementary Tables 13 and 14). Proteins involved in membrane organization, which may localize to the plasma membrane to coordinate morphological changes during cell spreading, increased during IAC assembly. Proteins involved in cytoskeletal or adhesive functions were generally more abundant later in IAC assembly and decreased during IAC disassembly. Both consensus and non-consensus adhesome components co-clustered in different groups, which suggests that their combined contributions are involved in IAC dynamics. For example, vimentin and myosin II co-clustered with consensus adhesome molecules that bind actin during IAC disassembly (Supplementary Fig. 6). Moreover, Rac1 and Lyn co-clustered with other consensus proteins and were abundant early and late in IAC assembly (Supplementary Fig. 5). In contrast, proteins involved in RNA processing and translation peaked early during IAC assembly and increased during IAC disassembly, suggesting a reciprocal temporal relationship between these cellular processes at IACs.

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**DISCUSSION**

Here, we performed extensive analyses of IAC proteomes, resulting in an experimentally defined meta-adhesome of 2,412 proteins. An emergent property of the meta-adhesome was the identification
of a consensus adhesome comprising core adhesion machinery robustly detected in IAC proteomes. The proteomic data sets used here provide a global description of IACs in the context of FN-mediated adhesion. Analysis of the meta-adhesome overcomes the heterogeneity encountered when studying individual data sets from different laboratories and cell types. The heterogeneity between IAC proteomes collected from cells exposed to similar microenvironments and the increased number of proteins identified in the meta-adhesome compared with the literature-curated adhesome5 highlight an unanticipated complexity in IAC composition. Evidence for IAC localization of 118 adhesome proteins (51%; 64 intrinsic, 54 associated) that were not detected in the meta-adhesome may be context-dependent or may need re-examining. One outstanding question is how the consensus adhesome differs for other ECM ligands, such as laminin or collagen, or integrin heterodimers or cell types. Increasing the numbers of proteomic data sets of IACs induced by alternative ECM ligands or adhesive function. Some of these proteins have functional roles related to RNA processing and translation (Supplementary Table 4) and therefore may be involved in localized protein synthesis, which is supported by IAC localization of translation machinery and β-actin messenger RNA (refs 38–42). They may be co-purifying contaminants from the IAC isolation process, which is supported by their identification in the contaminant repository for affinity purification–MS data (CRApome; ref. 43). However, comparative analysis of IAC proteomes with the CRApome is risky, as many canonical IAC proteins and cytoskeletal components (for example, talin and β, integrin) occur in the CRApome. Conversely, some well-characterized IAC components were not enriched in all seven data

IACs at the ends of actin fibres, which was shown to be the case for caldesmon. Importantly, not all cellular actin-binding proteins were identified by these analyses, indicating that the IAC isolation strategies allow the separation and characterization of a functionally distinct pool of actin and associated proteins. Most (46; 90%) candidate IAC proteins common to three proteomic data sets highlighted in a recent analysis21 were not present in the consensus adhesome, but some protein isoforms exhibit cell-type-specific expression33–35 and related isoforms, and additional non-canonical IAC components, were identified. With the exception of signal-induced proliferation-associated 1 (SIPA1; ref. 36) and LIM domains containing 1 (LIMD1; ref. 37), we found no evidence supporting the involvement of the unconnected consensus adhesome proteins (Fig. 4, legend) in IACs or adhesive function. Some of these proteins have functional roles related to RNA processing and translation (Supplementary Table 4) and therefore may be involved in localized protein synthesis, which is supported by IAC localization of translation machinery and β-actin messenger RNA (refs 38–42). They may be co-purifying contaminants from the IAC isolation process, which is supported by their identification in the contaminant repository for affinity purification–MS data (CRApome; ref. 43). However, comparative analysis of IAC proteomes with the CRApome is risky, as many canonical IAC proteins and cytoskeletal components (for example, talin and β, integrin) occur in the CRApome. Conversely, some well-characterized IAC components were not enriched in all seven data

**Figure 5** Caldesmon and Rsu-1 localization in IACs. (a,b) U2OS cells were spread on FN for 2 h and visualized using antibodies against caldesmon (green) (a) and Rsu-1 (green) (b). IACs were visualized by immunofluorescence staining for vinculin (red) and the actin cytoskeleton was visualized by staining with fluorophore-conjugated phalloidin (blue). Graphs show fluorescence intensity values for each channel across line segments in corresponding zoomed areas above each graph. In addition, co-localization with vinculin-positive areas was quantified for caldesmon (MOC (ref. 27) = 0.51 ± 0.19) and Rsu-1 (MOC = 0.98 ± 0.03). Values are mean ± s.d. (n = 20 cells from one independent experiment; see Supplementary Table 15 for source data). Scale bars, 20 µm.
**Figure 6** Temporal profiling of the consensus adhesome during IAC assembly. IACs were isolated from K562 cells in biological duplicate after 3, 9 and 32 min incubation with FN-coated beads and analysed by MS (data are from 2 independent experiments; see Supplementary Table 11). Throughout IAC assembly, 39 of the 60 consensus adhesome proteins were identified and were analysed by unsupervised hierarchical clustering, revealing distinct temporal profiles of protein recruitment to IACs. Six clusters, labelled A1–6, were chosen on the basis of a Pearson correlation threshold greater than 0.9 and are indicated by blue and green bars. Clusters are shown alongside corresponding profile plots, with the mean temporal profile for each cluster indicated by a red line. Quantitative heat map shows mean spectral counts as a proportion of the maximum spectral count for each given protein. Protein symbols are shown for clarity. Proteins also identified during IAC disassembly (Fig. 7 and Supplementary Table 12) are indicated by an asterisk. Literature-curated adhesome proteins and their isoforms are in bold. Proteins able to bind actin or integrin are indicated by black bars.

sets (for example, β3 integrin, FAK, kindlin, paxillin and talin) or were observed in the meta-adhesome but not the consensus adhesome (for example, p130Cas and Src family kinases). These omissions may be due to cell-type-specific expression, cell-type-specific IAC maturation, protein abundance at IACs, preferential use of β3 integrin or nonspecific detection in negative controls. Additional examination of the phosphoproteome and stoichiometry of IACs will provide deeper coverage of IAC composition and further insights into their relative functions in adhesion signalling.

The consensus adhesome contained many evolutionarily conserved proteins across multiple species and whose genetic depletion causes marked defects in integrin-mediated adhesion, indicating that consensus adhesome proteins form an essential contribution to integrin function. Analysis of interactions between consensus adhesome molecules resulted in the identification of four interconnected axes that link integrins to actin. Proteomics methods are complementary to super-resolution microscopy approaches used to study IACs (ref. 20). Indeed, the four axes that form the integrin–actin structural connection defined in this study support the vertical Z-plane model of IACs (ref. 50) where talin spans IACs, FAK and paxillin are in an integrin-proximal signalling layer and α-actinin, zyxin and VASP are localized distal to integrins near actin.
The association of α-actinin with β1 integrin occurs in early adhesions and is lost during maturation. Applied cytoskeletal force could induce α-actinin–integrin dissociation, allowing distal localization of α-actinin and potentially associated actin-binding and LIM-domain proteins from the membrane. Interactions with other consensus adhesome proteins may maintain the localization of α-actinin and associated molecules in IACs. Important next steps will be to determine the dynamics and nanoscale localization of other consensus adhesome proteins using super-resolution microscopy.

IACs are highly dynamic structures that can be characterized depending on their size, localization and maturation state. Current MS-based approaches to analyse IACs result in the combined analysis of these heterogeneous IAC structures (which are compositionally different from podosomes and invadopodia) from a cell population to give a compositional snapshot at a particular time point. To demonstrate how the meta-adhesome and consensus adhesome can be used by the research community, we generated time-course data sets during IAC assembly and disassembly. By filtering the acquired data sets using these adhesomes, we found that adhesion molecules are recruited to, and disassembled from, IACs with distinct kinetics, suggesting that these processes are differentially regulated and not simply reciprocal events. In support of studies showing hierarchical IAC formation, α-actinin was abundant early during IAC assembly, whereas zyxin was most abundant later. Most IAC molecules decreased in abundance during disassembly with different kinetic rates, and adaptor proteins were lost from IACs on the basis of a Pearson correlation threshold greater than 0.9 and are indicated by blue and green bars. Clusters are shown alongside corresponding profile plots, with the mean temporal profile for each cluster indicated by a red line. Quantitative heat map shows mean spectral counts as a proportion of the maximum spectral count for each given protein. Protein symbols are shown for clarity. Proteins also identified during IAC assembly (Fig. 6 and Supplementary Table 11) are indicated by an asterisk. Literature-curated adhesome proteins and their isoforms are in bold. Proteins able to bind actin or integrin are indicated by black bars.

Figure 7 Temporal profiling of the consensus adhesome during IAC disassembly. IACs were isolated from U2OS cells in biological triplicate on nocodazole removal and 5, 10 and 15 min after nocodazole washout to examine changes in IAC composition throughout IAC disruption. Isolated IACs at each time point were analysed by MS (data are from 3 independent experiments; see Supplementary Table 12). Throughout IAC disassembly, 43 of the 60 consensus adhesome proteins were identified and were analysed by unsupervised hierarchical clustering, revealing distinct temporal profiles of protein dissociation from IACs. Four clusters, labelled D1–4, were chosen on the basis of a Pearson correlation threshold greater than 0.9 and are indicated by blue and green bars. Clusters are shown alongside corresponding profile plots, with the mean temporal profile for each cluster indicated by a red line. Quantitative heat map shows mean spectral counts as a proportion of the maximum spectral count for each given protein. Protein symbols are shown for clarity. Proteins also identified during IAC assembly (Fig. 6 and Supplementary Table 11) are indicated by an asterisk. Literature-curated adhesome proteins and their isoforms are in bold. Proteins able to bind actin or integrin are indicated by black bars.

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earlier than actin-binding proteins, suggesting that adaptor proteins may be primary targets for disassembly. Therefore, as demonstrated here, we propose that the meta-adhesome and consensus adhesome can be used for removal of nonspecific components from future analyses of IAC composition by MS, thus contextualizing and streamlining identification of candidate adhesion molecules for follow-up studies.

In summary, the data presented in this study provide a systems-wide analysis of FN-induced IAC composition, detail a comprehensive reductionist view of an experimentally defined integrin adhesome and catalogue the first global characterization of IAC dynamics during the initial phases of assembly and disassembly.

**METHODS**

Methods and any associated references are available in the online version of the paper.

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Reagents. FN, PDL, transferrin, blebbistatin and nocodazole were from Sigma-Aldrich. Monoclonal antibodies used for immunofluorescence were mouse anti-vinculin (hVIN-1, V9131, Sigma-Aldrich; 1:400), rabbit anti-phospho-FAK (Tyr925/926) (44-725G, Invitrogen; 1:200), mouse anti-paxillin (439/4G10, BD Biosciences; 1:400), mouse anti-zyxin (ZO011, 39-6000, Thermo Fisher Scientific; 1:400), rat anti-α-tubulin (mAb11, provided by K. M. Yamada, National Institutes of Health, Bethesda, Maryland, USA; 1:200), rat anti-β-tubulin (9EG7, provided by D. Vestweber, University of Münster, Münster, Germany; 1:200) and rabbit anti-Rac-1 (provided by M. L. Cutler, University of Manitoba, Winnipeg, Manitoba, Canada; 1:500). Secondary antibodies were from Jackson Immunoresearch and Alexa Fluor 647-conjugated phallolidin was from Invitrogen.

Cell culture. K562 cells (provided by M. E. Hemler, Dana-Farber Cancer Institute, Boston, Massachusetts, USA) were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum (FCS; Lonza Bioscience) and 2 mM l-glutamine. Telomerase-immortalized HFF (provided by K. Clark, University of Leicester, Leicester, UK), conditionally immortalized MEF (generated in-house, see ref. 57), A375-SM (provided by I. J. Fidler, MD Anderson Cancer Center, Houston, Texas, USA) and osteosarcoma (U2OS; purchased from Sigma-Aldrich, 92022711) cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (v/v) FCS and 2 mM l-glutamine. MEF cells were supplemented with interferon-γ (Sigma-Aldrich). All cells were maintained at 37 °C in a humidified 5% (v/v) CO₂ atmosphere, except for MEF cells, which were maintained at 33 °C. All cell lines were frequently tested for mycoplasma and were negative. Cell lines were not authenticated and are not listed in the database of commonly misidentified cell lines maintained by ICLAC (http://iclac.org) and NCBI Biosample (http://www.ncbi.nlm.nih.gov/biosample).

IAC isolation. IACs were isolated using a similar approach to the ligand affinity purification method described previously52. For isolation of IACs from MEF cells, cells were resuspended in DMEM supplemented with 25 mM HEPES (Sigma-Aldrich) and incubated in suspension for 20 min at 37 °C to downregulate ECM adhesion signalling events. Cells were spread on tissue culture dishes coated with 10 μg ml⁻¹ FN or transferrin for 120 min at 37 °C, 8% (v/v) CO₂, in the presence or absence of 50 μM blebbistatin. Cells were incubated with the membrane-permeable crosslinker dimethyl-3, 30 min), washed twice with PBS, and DTBP was quenched using 1 M Tris-HCl (pH 8.5), five times with PBS, recovered by scraping in 100 μl recovery solution (125 mM Tris-VO₄, VO₃, NaCl, KCl, and 1 mM MnCl₂), and were lysed in CSK buffer (110 mM NaCl, 5 mM MgCl₂, 0.5 mM EGTA, 1% (v/v) Triton X-100) and sonicated for 3 min with DTBP (6 mM, 3 min), and DTBP was quenched with 1 M Tris-HCl (pH 8.5), followed by sonication for 2.5 min (VibraCell VCX 500) to lyse cells. Isolated IACs were washed and collected using the same method used to generate the MEF data set. IACs isolated at each time point were analysed by quantitative MS (3 biological replicates).

Immunoblotting. Proteins were resolved by SDS–PAGE and transferred to nitrocellular membrane (Whatman). Membranes were washed and incubated with antibodies as described previously55. Briefly, membranes were blocked with blocking buffer (Sigma-Aldrich) for 1 h and were incubated with appropriate primary antibodies diluted in blocking buffer overnight at 4 °C. After three 5-min washes, membranes were incubated with secondary antibodies diluted in blocking buffer for 45 min in the dark. Secondary antibodies used were donkey Alexa Fluor 680-conjugated anti-goat IgG or anti-mouse IgG (Life Technologies) and donkey IRDye 800-conjugated anti-mouse IgG (Rockland Immunocchemicals). Membranes were washed in the dark and scanned using the Odyssey infrared imaging system (LI-COR).

MS data acquisition. Following SDS–PAGE, gel lanes were sliced and subjected to in-gel digestion with trypsin53 with modifications54. Peptide samples were analysed by liquid chromatography (LC)-tandem MS using a nanoACQUITY UltraPerformance LC system (Waters) coupled online to an LTQ Velos mass spectrometer (Thermo Fisher Scientific) or an Ultimate 3000 Rapid Separation LC system (Thermo Fisher Scientific). Peptides were concentrated and desalted on a Symmetry C₁₈ preparative column (20 mm × 180 μm, 5-μm particle size; Waters) and separated on a Symmetry C₁₈ analytical column (250 mm × 75 μm, 1.7-μm particle size; Waters) using a 45-min linear gradient from 1% to 25% or 8% to 33% (v/v) acetonitrile in 0.1% (v/v) formic acid at a flow rate of 200 nl min⁻¹. Peptides were selected for fragmentation automatically by data-dependent analysis.

MS data analysis. MS data were searched using an in-house Mascot server (version 2.2.03; Matrix Science)55 as described previously. Mass tolerances for precursor and fragment ions were 0.4 Da and 0.5 Da, respectively, for LTQ Velos data or 5 ppm and 0.5 Da, respectively, for Orbitrap Elite data. Data were validated in Scaffold (version 3.00.06; Proteome Software) using a threshold of identification of at least 90% probability at the peptide level, assignment of at least two unique, validated peptides, and at least 99% probability at the protein level. These acceptance criteria resulted in an estimated protein false discovery rate of ≤0.1% for all data sets. MS data were quantified as described previously55. Briefly, relative protein abundance was calculated using the unweighted spectral count of a given protein normalized to the total number of spectra observed in that sample and to the molecular weight of that protein (termed normalized spectral count). Final results were reported as mean normalized spectral counts of biological replicate isolations. Only proteins with a spectral count of at least four were used for further analysis. For the IAC assembly and disassembly data sets, data were reported as mean spectral counts as a proportion of the maximum spectral count for each given protein.

Meta-adhesome construction. The assembled IAC data sets (Supplementary Table 1) were filtered to include only proteins from cells spread on FN and the corresponding negative control in the absence of perturbation. To reduce the identification of nonspecific IAC components, only those proteins enriched in FN-induced IACs compared with the negative control, with relative abundance satisfying log(FN/control) ≥ 1, were assembled into the meta-adhesome database (Supplementary Table 3). Two data sets were included from ref. 16 for cells expressing either β₁ or β₃ integrins. Membranes were washed in the dark and scanned using the Odyssey infrared imaging system (LI-COR).

Consensus adhesome construction. Proteins enriched in at least five proteomic data sets in the meta-adhesome database were incorporated into the consensus adhesome. ECM or secreted proteins (COL1A1, COL1A2, COL5A2, COL6A1, COL6A2, FGF, FN1, PCOLCE, PRSS23, SERPINE1) were excluded because, although relevant to adhesion biology, we sought to focus on intracellular components of IACs. All isoforms of literature-curated adhesome members were classified as adhesome molecules. Functional information was adapted from the HUGO Gene Nomenclature Committee (HGNC) database64, protein domain information was assigned from InterPro65 and disease annotations were extracted from the Online Mendelian Inheritance in Man database (http://www.omim.org).

Hierarchical clustering and principal component analyses. Proteins or data sets were hierarchically clustered on the basis of uncentred Pearson correlation using Cluster 3.0 (C Clustering Library, version 1.50; ref. 66) and visualized using Java TreeView (version 1.1.5; ref. 67). Binary data were clustered on the basis of Jaccard distance and visualized using R (version 3.1.0). Distances between hits
were computed using a complete-linkage matrix in all cases. Additional heatmaps were visualized using MultiExperiment Viewer (version 4.8.1; ref. 64). Principal component analysis was performed using MATLAB (version R2012a; MathWorks).

Interaction network analyses. Interaction network analysis was performed using Cytoscape (version 3.0.2; ref. 65). Enriched proteins were mapped onto a merged human interactome consisting of physical protein–protein interactions as described previously6. Graph clustering was performed using the yFiles Organic algorithm implemented in Cytoscape. Topological parameters were computed from undirected graphs, excluding self-interactions, using NetworkAnalyzer27. For the consensus adhesion, evidence for protein–protein interactions was manually verified and scored. Low-evidence interactions included those based on a single publication or on co-precipitation or yeast two-hybrid studies. Medium-evidence interactions were based on data from multiple sources, or a single source if there were phosphorylation or peptide binding data. High-evidence interactions were based on structural evidence of direct binding between two proteins, such as X-ray crystallography or nuclear magnetic resonance, or confirmation using a wide variety of techniques. Experimental evidence and source publications are detailed in Supplementary Table 6.

Functional enrichment analyses. Functional enrichment analysis was performed using DAVID (version 6.7; ref. 67). Keywords with fold enrichment ≥ 1.5, Bonferroni-corrected P value < 0.05, EASE score (modified Fisher’s exact test) < 0.05 and at least two proteins per keyword were considered significantly over-represented.

For generation of functional enrichment maps, over-representation of gene ontology terms was calculated using High-Throughput GoMiner4. One thousand randomizations were performed and data were thresholded for a 5% false discovery rate. Over-represented terms with ≥ 5 and ≤ 500 assigned proteins were reported. Data set occurrence was mapped onto proteins assigned to each over-represented term, and the data matrix was subjected to hierarchical clustering analysis as described above.

Immunofluorescence microscopy. To confirm localization of Rsu-1 and caldesmon at IACs, U2OS cells were spread on FN-coated dishes (MatTek) for 2 h at 37°C, 8% (v/v) CO₂. To validate MS data of IAC disassembly, HFF cells were treated with nocodazole and nocodazole was washed out as described previously27. Cells were washed with PBS, fixed with −20°C methanol or 4% (v/v) paraformaldehyde for 7 min at room temperature and permeabilized with 0.5% (w/v) Triton X-100 for 10 min. Permeabilized cells were washed three times with PBS before incubation with appropriate primary antibodies diluted in 2% (w/v) BSA in PBS for 1 h. Vinculin, zyxin and αi integrin were analysed, as each protein exhibited different IAC disassembly dynamics (Fig. 7). In addition, phospho-paxillin (paxillin, phospho-FAK ster and βi integrin were visualized to test whether proteins within the same cluster exhibited similar disassembly dynamics (Supplementary Fig. 7). Cells were washed three times with PBS and incubated with appropriate secondary antibodies diluted in 2% (w/v) BSA in PBS for 30 min in the dark. Stained cells were washed once in PBS, twice in water and stored in water at 4°C until imaging. Images were acquired on a Delta Vision RT (Applied Precision) restoration microscope using a ×60/1.4 Plan Apo objective and the Sedet filter set (Chroma 90000). Images were collected with a Z optical spacing of 0.2 μm, five images per stack, using a CoolSnap HQ camera (Photometrics) and Softworx software (Applied Precision). To assess co-localization of Rsu-1 and caldesmon with vinculin, cells were also imaged using a spinning-disc confocal inverted microscope (Marianas; 3i). Images were collected with a Z optical spacing of 0.2 μm, three images per stack, using a 63×/1.4 Plan Apochromat objective and SlideBook 6.0 software (3i).

Image analysis and quantification. Maximum intensity projections of raw images were generated and background filtered (rolling ball, 10-pixel radius) using ImageJ (version 1.48c; ref. 69). Areas containing positive staining of IAC proteins were measured and normalized to total cell area. Box-and-whisker plots were generated using Prism (version 6.04; GraphPad). To quantify Rsu-1 and caldesmon co-localization with vinculin, images were individually band-pass filtered (A trous wavelet, linear 3 × 3 filter, keeping scales 2–8) using custom software written in Python and NumPy to create a mask of vinculin-positive adhesion structures. Co-localization analysis was performed using the Imagl plugins Coloc 2, with the mask as a region of interest to calculate MOC (ref. 27), and Plot, Multicolor (version 4.3) to plot line profiles. Figures were assembled using Illustrator (Adobe).

Statistics and reproducibility of experiments. Statistical significance was calculated using Kruskal–Wallis tests (non-parametric, one-way ANOVA) with Dunn’s post hoc correction as indicated in the figure legends, with P < 0.05 considered statistically significant (*P < 0.05, **P < 0.01, ***P < 0.001). No statistical method was used to predetermine sample size. Statistical analyses were carried out using Prism software. MS data were obtained from two independent experiments for the MEF and IAC assembly data sets and from three independent experiments for the IAC disassembly data set. Immunofluorescence quantification is based on data obtained from one independent experiment from at least 10 cells, and representative images are shown.

Data deposition and accession numbers. MS data were deposited in ProteomeXchange (http://proteomecentral.proteomexchange.org) through the PRIDE partner repository49 with the primary accession identifiers PXD000018 (DOI: http://dx.doi.org/10.6019/PXD000018; MEF data set), PXD002159 (DOI: http://dx.doi.org/10.6019/PXD002159; IAC assembly data set) and PXD002129 (DOI: http://dx.doi.org/10.6019/PXD002129; IAC disassembly data set). Details of all identified proteins are provided in Supplementary Tables 2 and 8 for the MEF data set, Supplementary Table 11 for the IAC assembly data set and Supplementary Table 12 for the IAC disassembly data set.

Previously published MS data sets that were reanalysed here are available in the PRIDE database (http://www.ebi.ac.uk/pride) with the referenced accession identifiers 9985–9987 (K562 data set)1 and in ProteomeXchange with the identifiers PXD001578 (DOI: http://dx.doi.org/10.6019/PXD001578; A375 data set)50 and PXD001183 (DOI: http://dx.doi.org/10.6019/PXD001183; HFF data set)49. MS data from the MKF data set15 and MKF data sets49 are not available in an online repository.
Supplementary Figure 1 Comparison of FN-enriched IAC proteomes. (a) Seven proteomic datasets of FN-enriched IACs were analysed by unsupervised hierarchical clustering. The binary heat map shows proteins at least two-fold enriched to FN over the negative control (red). Dataset occurrence is plotted for each protein (rainbow), and literature-curated adhesome components are indicated by purple bars. Details of the proteomic datasets are provided in Supplementary Table 1. (b) Dendogram illustrating the clustering of the FN-enriched IAC proteomes shown in a. Dataset dissimilarity is measured by Jaccard distance. (c) Pairwise overlaps of FN-enriched proteins identified in the seven proteomic datasets and the literature-curated adhesome were measured by Jaccard coefficient and are displayed as a hierarchically clustered heatmap (lower diagonal matrix; blue). Numbers of proteins in each overlap set are indicated (upper diagonal matrix). (d) FN-enriched proteins identified in the seven proteomic datasets were analysed by principal component analysis. A plot of the first two principal components is shown. K562, human chronic myelogenous leukaemia cells; MEF, mouse embryonic fibroblast cells (this study); A375, human malignant melanoma cells; HFF, human foreskin fibroblast cells; MKF1, mouse kidney fibroblast cells; MKF2 and MKF3, mouse kidney fibroblast cells.
Supplementary Figure 2: Topological analysis of the meta-adhesome interaction network. (a) Clustered protein-protein interaction network model of the meta-adhesome. The largest connected graph component is displayed, comprising 11,430 interactions (black lines; edges) between 2,035 proteins (circles; nodes). Node size is proportional to degree and node colour is proportional to betweenness centrality. Black node borders indicate literature-curated adhesome components, which are labelled with gene names. (b) Betweenness centrality (a measure of the control a node exerts over the interactions of other nodes in the network) for each protein is plotted according to the number of datasets in which it was identified. Box-and-whisker plot shows the median (line), mean (plus sign), 25th and 75th percentiles (box) and 5th and 95th percentiles (whiskers) (*n = 1,117, 518, 238, 102, 33, 25 and 10 mapped proteins identified in 1–7 datasets, respectively, with degree ≥ 1). *P < 0.05, **P < 0.01, ***P < 0.001; Kruskal–Wallis test with Dunn’s post hoc correction (see Supplementary Table 15 for statistics source data).
**Supplementary Figure 3** Functional enrichment map of the meta-adhesome. (a) Overrepresented biological process terms from proteins identified in the meta-adhesome were hierarchically clustered according to proteomic dataset occurrence. This identified clusters of similarly detected proteins associated with a similar set of functional terms. (b) The two clusters containing proteins detected in the most datasets (grey boxes in a; 1, 2) are shown in detail. Proteins are labelled with gene names for clarity (see Supplementary Table 3 for details).
**Supplementary Figure 4** Comparison of IAC proteomes in the consensus adhesome. Proteins identified in the consensus adhesome were analysed by unsupervised hierarchical clustering. The binary heat map shows proteins at least two-fold enriched to FN over the negative control (red). Dataset occurrence is plotted for each protein (rainbow), literature-curated adhesome components are indicated by purple bars, and the presence of a LIM domain is indicated by grey bars. Dataset dissimilarity is measured by Pearson correlation. The numbers of consensus adhesome proteins identified in each IAC proteome are displayed below the heat map. Details of the proteomic datasets are provided in Supplementary Table 1, and details of proteins identified in the consensus adhesome are provided in Supplementary Table 4. K562, human chronic myelogenous leukaemia cells; MEF, mouse embryonic fibroblast cells (this study); A375, human malignant melanoma cells; HFF, human foreskin fibroblast cells; MKF1, mouse kidney fibroblast cells; MKF2 and MKF3, mouse kidney fibroblast cells.
Supplementary Figure 5 Hierarchical clustering analysis of meta-adhesome proteins identified during IAC assembly. IACs were isolated from K562 cells in biological duplicate after 3, 9 and 32 min incubation with FN-coated beads and analysed by MS (data are from 2 independent experiments; see Supplementary Table 11). Throughout IAC maturation, 1,266 of the 2,412 meta-adhesome proteins were identified and were analysed by unsupervised hierarchical clustering, revealing distinct temporal profiles of protein recruitment to IACs. Quantitative heat map displays mean spectral counts as a proportion of the maximum spectral count for each given protein. Twelve clusters were chosen on the basis of a Pearson correlation threshold greater than 0.8, labelled SA1–12, and are indicated by blue and green bars. Literature-curated adhesome and consensus adhesome proteins identified in each cluster are indicated by gene name (italic, literature-curated adhesome; regular, consensus adhesome; bold, literature-curated adhesome and consensus adhesome). Literature-curated adhesome proteins that interact with consensus adhesome molecules in interaction network analyses are indicated by an asterisk (see Supplementary Table 7 for details). Clusters are shown alongside corresponding profile plots, with the mean temporal profile for each cluster indicated by a red line. The most significantly overrepresented functional annotations for selected clusters are listed. Full details of enriched functional terms are provided in Supplementary Table 13.
Supplementary Figure 6 Hierarchical clustering analysis of meta-adhesome proteins identified during IAC disassembly. (a) IACs were isolated from adherent U2OS cells in biological triplicate upon nocodazole removal and 5, 10 and 15 min after nocodazole washout to examine changes in IAC composition throughout IAC disruption. Isolated IACs at each time point were analysed by MS (data are from 3 independent experiments; see Supplementary Table 12). Throughout IAC disassembly, 455 of the 2,412 meta-adhesome proteins were identified and were analysed by unsupervised hierarchical clustering, revealing distinct temporal profiles of protein dissociation from IACs. Quantitative heat map displays mean spectral counts as a proportion of the maximum spectral count for each given protein. Seventeen clusters were chosen on the basis of a Pearson correlation threshold greater than 0.8, labelled SD1–17, and are indicated by blue and green bars. Literature-curated adhesome and consensus adhesome proteins identified in each cluster are indicated by gene name (italic, literature-curated adhesome; regular, consensus adhesome; bold, literature-curated adhesome and consensus adhesome). Literature-curated adhesome proteins that interact with consensus adhesome molecules in interaction network analyses are indicated by an asterisk (see Supplementary Table 7 for details). Clusters are shown alongside corresponding profile plots, with the mean temporal profile for each cluster indicated by a red line. The most significantly overrepresented functional annotations for selected clusters are listed. Full details of enriched functional terms are provided in Supplementary Table 14.

(b, c) Area-proportional Venn diagrams showing the overlap between the meta-adhesome and proteins identified by MS during IAC assembly (b) or IAC disassembly (c). For each set, the total number of proteins (black text) and the number of proteins identified in the consensus adhesome (bold red text) is indicated.
Supplementary Figure 7 Changes in additional consensus adhesome components during IAC disassembly. (a) To examine IAC dynamics during microtubule-induced IAC disassembly32, HFF cells treated with DMSO, 10 µM nocodazole or after nocodazole removal at different times were stained for phospho-paxillinY118, paxillin, phospho-FAKY397 and β1 integrin. Representative images are shown. Scale bars, 20 µm. (b–e) Quantification of images in a. Phospho-paxillinY118 (b), paxillin (c), phospho-FAKY397 (d) and β1 integrin (e) levels were quantified as a proportion of total cell area. Box-and-whisker plots show median (line), mean (plus sign), 25th and 75th percentiles (box) and 5th and 95th percentiles (whiskers) (n = 10 cells per condition from one independent experiment). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; Kruskal–Wallis test with Dunn’s post hoc correction (see Supplementary Table 15 for statistics source data). Noc, nocodazole.
**Supplementary Table Legends**

**Supplementary Table 1** Proteomic datasets assembled in the meta-adhesome. Seven datasets detailing the composition of FN-induced IACs were assembled to create the meta-adhesome database. Metadata for each dataset are provided.

**Supplementary Table 2** Proteins identified in purified mouse embryonic fibroblast integrin adhesion complexes by mass spectrometry. IACs were isolated from MEF cells spread on FN or transferrin (as a control) for 2 h. All proteins identified by mass spectrometry are detailed.

**Supplementary Table 3** The meta-adhesome. Proteins at least two-fold enriched to FN-induced IACs over a corresponding negative control ligand condition in at least one of seven datasets were incorporated into the meta-adhesome database. The database contains 2,412 proteins identified in at least one dataset.

**Supplementary Table 4** The consensus adhesome. Proteins enriched in at least five proteomic datasets in the meta-adhesome database were incorporated into the consensus adhesome, excluding ECM or secreted proteins. The consensus adhesome contains 60 proteins commonly identified in IAC proteomic datasets, and functional information for each protein is provided.

**Supplementary Table 5** Functional enrichment analysis of the consensus adhesome. Consensus adhesome proteins were subjected to functional enrichment analysis against terms from the Gene Ontology (biological process, cellular component, molecular function), KEGG pathways and InterPro protein domains. Significantly overrepresented terms are indicated.

**Supplementary Table 6** Reported interactions between consensus adhesome proteins. Evidence for protein-protein interactions between proteins in the consensus adhesome was manually verified and scored. A list of all reported interactions and corresponding source publications is provided.

**Supplementary Table 7** Consensus adhesome protein binding partners of non-consensus meta-adhesome proteins. Proteins identified from the meta-adhesome that interact with consensus adhesome proteins in network analyses (Fig. 1d), termed consensus interactors, are indicated.

**Supplementary Table 8** Proteins identified in purified mouse embryonic fibroblast integrin adhesion complexes, in the presence or absence of myosin II inhibition, by mass spectrometry. IACs were isolated from MEF cells spread on FN or transferrin (as a control) for 2 h, in the presence or absence of 50 μM blebbistatin, in biological duplicate. All proteins identified by mass spectrometry are detailed.

**Supplementary Table 9** Effects of myosin II inhibition on integrin adhesion complex composition. (a,b) To analyse effects of myosin II inhibition on IAC composition, proteins from the consensus adhesome (a) and the literature-curated adhesome (b) that were identified in at least one of three studies that analysed IAC proteomes upon blebbistatin treatment are indicated.

**Supplementary Table 10** Adhesive components identified in other adhesion protein datasets. (a,b) Proteins from the consensus adhesome (a) or the literature-curated adhesome (b) that were also identified in a dataset of proteins that co-immunoprecipitated with paxillin, vinculin or talin31, a dataset of invadopodia proteins29 or a dataset of podosome proteins30 are indicated.

**Supplementary Table 11** Proteins identified during integrin adhesion complex assembly by mass spectrometry. IACs were isolated from K562 cells in biological duplicate after 3, 9 and 32 min incubation with FN-coated beads. All proteins identified by mass spectrometry are detailed.

**Supplementary Table 12** Proteins identified during integrin adhesion complex disassembly by mass spectrometry. IACs were isolated from U2OS cells in biological triplicate upon nocodazole removal and 5, 10 and 15 min after nocodazole washout to examine changes in IAC composition throughout IAC disruption32. All proteins identified by mass spectrometry are detailed.

**Supplementary Table 13** Functional enrichment analysis of meta-adhesome proteins co-clustered during integrin adhesion complex assembly. Meta-adhesome proteins that co-clustered in hierarchical clustering analyses of IAC assembly (Supplementary Fig. 5) were subjected to functional enrichment analysis against terms from the Gene Ontology (biological process, cellular component, molecular function), KEGG pathways and InterPro protein domains. Significantly overrepresented terms are indicated.

**Supplementary Table 14** Functional enrichment analysis of meta-adhesome proteins co-clustered during integrin adhesion complex disassembly. Meta-adhesome proteins that co-clustered in hierarchical clustering analyses of IAC disassembly (Supplementary Fig. 6) were subjected to functional enrichment analysis against terms from the Gene Ontology (biological process, cellular component, molecular function), KEGG pathways and InterPro protein domains. Significantly overrepresented terms are indicated.

**Supplementary Table 15** Statistics source data. (a–j) Statistics source data are provided for topological analysis of the meta-adhesome interaction network (Fig. 1f, Supplementary Fig. 2b) (a,b), quantification of adhesion protein colocalisation for Rsu-1 and caldesmon (Fig. 5) (c) and quantification of cell adhesion area during nocodazole washout (Fig. 8, Supplementary Fig. 7) (d–j).