EFFECTS OF CONFORMATIONAL ACTIVATION OF INTEGRIN α1I AND α2I DOMAINS ON SELECTIVE RECOGNITION OF LAMININ AND COLLAGEN SUBTYPES

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Abstract

Collagen receptor integrins α1β1 and α2β1 can selectively recognize different collagen subtypes. Here we show that their αI domains can discriminate between laminin isoforms as well: α1I and α2I recognized laminin-111, -211 and -511, whereas their binding to laminin-411 was negligible. Residue Arg-218 in α1 was found to be instrumental in high-avidity binding. The gain-of-function mutation E318W makes the α2I domain to adopt the "open" high-affinity conformation, while the wild-type α2I domain favors the "closed" low-affinity conformation. The E318W mutation markedly increased α2I domain binding to the laminins (-111, -211 and -511), leading us to propose that the activation state of the α2β1 integrin defines its role as a laminin receptor. However, neither wild-type nor α2IE318W domain could bind to laminin-411. α2IE318W also bound tighter to all collagens than α2I wild-type, but it showed reduced ability to discriminate between collagens I, IV and IX. The corresponding mutation, E317A, in the α1I domain transformed the domain into a high-avidity binder of collagens I and IV. Thus, our results indicate that conformational activation of integrin α1I and α2I domains leads to high-avidity binding to otherwise disfavoured collagen subtypes.

Keywords: integrin αI domain, integrin activation, ligand selectivity, collagen, laminin

Abrreviations GST, glutathione-S-transferase; IPTG, isopropyl β-D-1-thiogalactopyranoside; PDB, Protein Data Bank,

INTRODUCTION

Previous studies have indicated that the four collagen receptor integrins, α1β1, α2β1, α10β1 and α11β1, show distinct abilities to bind to different collagen subtypes and that similar properties can be seen when the corresponding αI domains are analyzed [1-4]. The best documented difference is seen in the action of α1β1 and α2β1 integrins. α1β1 favors basement membrane type IV collagen and α2β1 has higher affinity for fibril forming collagens, such as collagen I [1-2, 4-5]. The effect of integrin activation on the selective binding of collagen subtypes has been unclear.

Human basement membranes seem to contain 16 laminin isoforms, which have recently been renamed based on the presence of different α, β and γ chains in the trimeric laminin molecule [6]. Laminin-111 (originally named laminin-1) is the prototype laminin and the major laminin isoform expressed during early embryogenesis [7]. Another well known laminin isoform, laminin-211 (also known as merosin or laminin-2), is expressed in skeletal muscle and peripheral nerves [8]. In this paper we have also studied two other laminin isoforms, which only recently became available as recombinant proteins, enabling more detailed studies about their functions. Laminin-411 (previously laminin-8) is a component of vascular basement membranes, especially abundant in the heart [9]. Laminin-511 (previously laminin-10) is the most widespread laminin heterotrimer and is expressed for example in epidermis and dermis [10-11].

The major laminin receptors among integrins are α3β1, α6β1, α7β1 and α6β4 [12]. Integrins α1β1, α2β1 and α10β1 are better known as collagen receptors but can mediate laminin binding as well [2, 13-14]. Integrin α2β1 has been reported to function solely as a collagen receptor or both as a collagen and laminin receptor, depending on the cell type where it is expressed [13, 15]. The explanation for this cell type specific behavior is not known. The α1β1

receptor can, with an activating antibody, be switched from a collagen binding form to a form that accepts both collagen and laminin [16].

The affinity of integrin binding to ligands can be modulated through the regulation of the conformation of the integrin. Integrins remain inactive when the cytoplasmic part of the α subunit is associated with the cytoplasmic part of the β subunit [17], keeping the extracellular headpiece bent towards the plasma membrane where it is poorly accessible to large ligand proteins [18-20]. In integrin activation, the α and β intracellular parts separate from each other allowing the integrin to extend through a motion analogous to the opening of a switchblade [20-23].

The αI domain (also called αA domain), a 200 amino acid, independently folding domain found in 9 out of 18 human integrin α subunits, has been recognized as the ligand binding site [24-25]. In the published crystal structures, α2I domain is found in two conformations [26-27], which presumably represent two affinity states of the domain, the open high-affinity and the closed low-affinity conformation. The open conformation may be evoked by a "downward" pull of the C-terminal α7 helix of the αI domain [28], a function attributed to the β subunit I-like domain in the intact receptor [29]. The open conformation can also be created by mutating a function modulating residue E318 in the α2I domain. The mutation E318W in α2I leads to increased binding of both collagen and laminin [30]. For some integrins a third, intermediate-affinity, conformational state may exist, but based on molecular dynamics simulations this state does not seem probable for the α1I and α2I domains. In these simulations the closed conformation of the integrin α1I and α2I domains seemed to be more stable than that of the αLI and αMI domains [28]. Both the open and the closed conformational states of α2I have been shown to exist on the cell surface by selective antibodies [31].

Here we show that α1I and α2I can discriminate between laminin isoforms -111, -211 and -511, when compared to -411. Residue Arg-218 in α1 seems be essential for high-avidity binding, which may partially explain the relatively low avidity of α2I binding to laminins. However, the conformational activation of the α2I makes it a high-affinity laminin binder. Furthermore, our results indicate that conformational activation of integrin α1I and α2I domains leads to high-avidity binding to otherwise disfavoured collagen subtypes.

MATERIALS AND METHODS

Extracellular matrix proteins

Human collagens I, II, IV and VI were purchased from Biomarket, Turku, Finland. Rat collagen I was purchased from Sigma Aldrich, Helsinki, Finland and collagen IV from mouse Engelbreth-Holm-Swarm tumor was from Becton Dickinson, Helsinki, Finland. Recombinant human collagen IX was a kind gift from J. Jäälinoja and L. Ala-Kokko, University of Oulu, Finland.

Laminin-111 from mouse Engelbreth-Holm-Swarm tumor was purchased from Sigma Aldrich, Helsinki, Finland. Purified human merosin (laminin-211) of the placenta was purchased from Chemicon International, AH Diagnostics, Helsinki, Finland. Human recombinant laminins -411 [32] and -511 [33] were produced as described earlier.

Mutagenesis and production of the recombinant αI domains

The cloning of αI domains has been described previously: α1I domain "long" construct (amino acids 123-VSPT-LEATA-338; ref. 1), the α1I "short" construct (amino acids 138-ECS…LEATA-338; ref. 34] and α2I domain [35]. The point mutations α1IR218D, α2ID219R, α1IE317W, α1IE317A and α2IE318W were introduced into the corresponding cDNAs according

to Stratagene's Quickchange kit instructions (Stratagene). The mutant R218D was introduced into the "long" α1I construct and E317W and E317A were introduced into the "short" α1I domain construct. Proteins were expressed and purified as GST fusions in the vectors pGEX-2T, pGEX-4T-3 and pGEX-KT (Amersham Biosciences), essentially as described before [2-4] unless stated otherwise. The α1IE317A and E317W proteins were produced by using 15 ml of overnight (o/n) culture to inoculate 1 liter of LB_{AMP}-media and cultured at +37°C until the A_{600} reached 0.5-0.6. In order to induce expression of the protein, IPTG was added to a final concentration of 0.4 mM. Protein production was allowed to continue o/n at +17°C for α1E317W and at RT for α1IE317A. Cells were harvested by centrifugation and the protein was purified in the presence of PMSF, phenylmethylsulphonyl fluoride (1 mg/ml of PMSF in PBS for the cells of 1 L culture volume) otherwise as earlier described.

Solid phase binding assays

Binding assays were carried out essentially as previously described [4]. Shortly, 96-well plates were coated with matrix proteins 15 μg/ml o/n at +4°C unless stated otherwise. Wells were blocked with BSA containing Delfia® Diluent II, PerkinElmer Finland, and used to measure the background binding. GST-fusion αI domains were allowed to bind for one hour in the presence of 2 mM $MgCl_2$ or 10mM EDTA, as indicated. Wells were washed three times and signal was detected with Delfia® Europium labeled anti-GST antibody, PerkinElmer Finland, label was dissociated with Delfia® enhancement solution, PerkinElmer Finland and signal was determined using a time-resolved fluorescence spectrophotometer (Victor3 multilabel counter, Wallac Perkin-Elmer or Wallac Envision™ 2100 multilabel reader, PerkinElmer Finland). Estimates for

the dissociation constants were obtained using an equation: measured binding=maximal binding/(1+Kd/[αI]).

Small molecule integrin inhibitor L3008 (methyl 2-ethyl-4,5,7,12-tetrahydroxy-6,11-dioxonaphthacenecarboxylate, also known as anhydromaggiemycin) [36, 37] was diluted to DMSO and used as a concentration of 50μM.

Structural modeling

Coordinates of protein structures were obtained from the RCSB Protein Data Bank (PDB; 38) and sequence data is from the UniProt online database [39]. A structural model of the human integrin α1I domain in the active conformation was built based on the crystal structure of the α2I domain (27; PDB code: 1DZI). In the α2I domain, the mutation (E318W) [30] is assumed to increase the relative proportion of active versus inactive αI domain conformers; the corresponding residue in the α1I domain was identified for mutagenesis. The (E317W and E317A) mutants of α1I were expected to exert a similar effect as that observed for the (E318W) α2I domain. Sequences were aligned in Bodil v0.8 [40] and model structures were built using either Bodil v0.8 or Modeller v7.7 [41]. Molecular graphics were created using PyMOL v0.98 [42].

RESULTS

Integrin α1I binds to laminin-111, -211 and -511 in an Arg-218 dependent manner but cannot recognize laminin-411

The binding of human integrin α1I and α2I domain was tested with purified (-111, -211) and recombinant (-411, -511) laminins. The wild-type α1I domain bound to laminins-111, -211 and -

511, although weaker in comparison to collagen binding. Binding to laminin-411 was negligible, as was the case for α2I (Figure 1A,C). Integrin α2I domain bound weakly to laminins -111, -211 and -511. Importantly, the results indicate that laminin isoforms differ from each other as ligands for cell adhesion receptors. The ability of the two integrins to act as laminin receptors was clearly different, confirming an earlier report that integrin α1β1 binds laminin-111 better than α2β1 [5].

Earlier, we have shown that the amino acid residues Arg-218 (α1I) and Asp-219 (α2I) have important roles in determining the selective binding of the αI domains to collagen subtypes. A mutation that reverses the charge at this position in these αI domains, namely R218D in α1I and D219R in α2I, significantly weakens the binding of the αI domain to the collagen subtype that is preferred by the respective wild-type domain [2]. Here, we have tested the effect of these mutations for laminin recognition. The mutation R218D in α1I dramatically weakened the binding to laminins -111, -211 and -511 (Figure 1A). A similar debilitating effect was detected for collagen IV binding, while binding to collagen I remained unaffected (Figure 1B and reference 2). Binding to laminin-411 was insignificant for both the α1I wild-type and the variant α1IR218D domain. When compared to the α1I domain, the mutation in the corresponding amino acid in the α2I domain, D219R, did not exert a similar effect on laminin recognition: binding to laminins was either not affected or was slightly enhanced (Figure 1C). The same mutation reduced binding to collagen I but not to collagen IV (Figure 1D and reference 2). Taken together, these results indicate that laminin binding may take place using a similar basic mechanism as seen for type IV collagen binding to the αI domains, whereas type I collagen binding follows a different binding mechanism altogether. Especially, residue α1IR218 seems to be an important determinant of the binding specificity of the α1I domain.

Activation of integrin α2I domain decreases the selectivity for collagen subtypes

It has been unknown how integrin activation affects the ligand selectivity of collagen receptor integrin αI domains. E318W is a gain-of-function mutation for integrin α2I domain [30]. E318W prevents the formation of a salt bridge between residues Arg-288 and Glu-318 (α2I numbering) that in the wild-type integrin stabilizes the closed conformation. Consequently, the mutation removes this ionic lock, the closed conformation is destabilized, and the open form is favored creating a constitutively active domain (Figure 2). We tested the binding of α2IE318W to collagens I, IV, VI and IX and found that the binding was enhanced with all four collagen subtypes (Figure 3A). Concomitantly with the rise in the activity of the constantly open α2I domain, a decrease in selectivity was seen. The approximated K_d for collagen I binding to α2IE318W was repeatedly 2-6 times tighter (≈16±5 nM) than that for the wild-type (≈31±6 nM) (Figure 3B). The K_d estimate for collagen IV binding improved as well (≈13±2 nM) compared to the wild-type (≈68±25 nM) (Figure 3B). The data obey a simple model where the binding sites have about equal avidity independent of the number of them. The obtained K_d values of the open mutant for collagens I and IV were very close to each other (≈16±5 nM for collagen I and ≈13±2 nM for collagen IV) demonstrating a loss of selectivity. The increase of the maximal binding level of the mutated αI domain may indicate a change in the number of binding sites in the ligand in support of the idea of loss of selectivity. Thus, the closed conformation of the α2I domain discriminates between wild-type collagen subtypes in agreement with previous reports [1-2], whereas the activating mutation eradicates these differences in binding.

For the expected gain-of-function effect, the corresponding residue in α1I was identified as Glu-317 by sequence and structural comparisons with α2I domain. In gel filtration the α1IE317W protein was seen to form multimers, which affected the reproducibility of the results

(data not shown). Multimers were not detected with the α1IE317A mutant. The open α1I domain construct was also a bit shorter in sequence (consisting of amino acids 138-ECST-LEATA-338; ref. 34) than the construct we have used earlier (123-VSPT-LEATA-338; ref. 1). The correct folding of the "short" construct used here has been verified by its crystal structure [34]. The short wild-type α1I bound to collagen IV stronger than to collagen I (Figure 4A), exactly like the "long" construct [1-2], although the binding levels were lower and the estimates for the K_ds were somewhat higher for the short α1I wild-type construct (Figure 4B) when compared to the estimates what we have reported earlier with the long construct [2]. The mutation α1IE317A improved the collagen binding of the αI domain considerably (Figure 4A). Binding of both collagen types I and IV increased and the K_d approximates for the α1IE317A (≈23±3 nM for collagen I binding, ≈11±1.5 nM for collagen IV binding) were about ten times tighter than for the wild-type domain (≈268±38 nM for collagen I binding and ≈144±22 nM for collagen IV binding) (Figure 4B). The slightly higher binding level of α1IE317A for collagen I may indicate a larger number of binding sites in collagen I than in collagen IV for the activated integrin α1I domain. These results suggest that E317A represents a new gain-of-function mutation in the α1I domain, most probably inducing a switch from the closed to the open conformation. In agreement with our results with the α2I domain, the conformationally activated α1I domain was a high-avidity binder of both collagen I and IV.

Activation is a prerequisite for integrin α2I domain to bind laminin

Since the activation of the integrin αI domain increased binding to all collagen subtypes, we wanted to see how the activation affects the binding to different laminin isoforms. When we tested the open conformation mutant α2IE318W with laminins -111, -211, -411 and -511, an

increase in binding was clearly seen, although the binding to laminin-411 remained insignificant (Figure 5A). Tests using a concentration series of wild-type α2I domain and the mutant α2IE318W were carried out on laminin-111 and -211 (Figure 5B). The gain-of-function mutation remarkably increased the binding of α2I towards the laminins, demonstrating the effect of a conformation particularly for laminin binding. Binding of the wild-type integrin α2I domain was so weak that no estimates for the strength of the binding could be obtained. For the open mutant the estimated K_ds were: 36±7 nM for laminin-111 binding and 97±16 nM for laminin-211 binding. The magnesium dependence of the α2IE318W ligand binding was tested using a metal chelator EDTA. The binding of α2IE318W was proven to be metal ion dependent similarly to α2I wild-type (Figure 5C). EDTA reduced the binding of α2IE318W significantly both on collagen and laminin-111.

Similarly, the open form of integrin α1I domain (α1IE317A) showed improved binding to laminins-111, -211 and -511 when compared to wild-type binding. Binding of laminin-411 was unaffected by the mutation, differing only slightly from the BSA control (Figure 6A). K_ds obtained for the open conformation mutant α1E317A were very similar to each other: ≈70±16 nM for laminin-111 binding and ≈78±11 nM for laminin-211 binding (Figure 6B). The Mg-dependence was tested using EDTA. Similarly to α2IE318W the binding of the open mutant α1IE317A was metal dependent (Figure 6C).

The binding of αI domain open mutants can be inhibited by a MIDAS-binding small molecule

To become fully confirmed that the binding of the open mutants remained "physiological" and included the MIDAS we inhibited the binding of α1I and α2I wild-types and the corresponding open mutants α1E317A and α2IE318W with a recently described small molecule inhibitor

L3008 [37]. The inhibitor binds to MIDAS in α1I and α2I domains at both the closed and open conformations. The inhibitor was seen to inhibit the binding more than 50% (Figure 7). The same effect was seen both on collagen I and laminin-111 (Figure 7).

DISCUSSION

The binding preferences of integrins for collagen subtypes have, in several publications, been shown to differ: for example, α2β1 is a better receptor for fibril forming collagens than α1β1 that prefers basement membrane collagen IV [1-2, 5]. The essential role of cell adhesion to laminins, the major constituents of basement membranes, is reflected by the presence of numerous cellular receptors that recognize laminins. Collagen receptors α1β1, α2β1 and α10β1 are also known to function in laminin binding. The laminin binding site has been located on the αI domains in α1β1 and α2β1 integrins and the binding mechanism has been shown to be cation dependent similarly to other ligands [25]. When tested here, the integrin α1I and α2I domains could recognize laminins, although the avidities were lower than for the collagens. Wild-type integrin α1I generally showed much better avidity for laminins, suggesting that α1β1 is the best basement membrane receptor among the collagen binding integrins.

This is the first report in which the ability of the collagen receptor integrins to bind to recombinant laminins -411 and -511 has been tested. The fact that integrin α1I and α2I domains did not significantly bind to laminin-411 indicates that the laminin isoforms have different cell adhesion properties and also that the collagen receptor integrins can discriminate between different laminin isoforms. The lack of binding by integrin α1I and α2I domains to laminin-411 may result from the structure of the α4 chain, which is truncated at the N-terminal end.

Recently, commercial preparations of minor laminin subtypes have been found to contain some impurities and to be partially degraded [43]. We used here human recombinant laminins -411 and -511 to avoid this problem. The laminin-211 preparations (Merosin, Chemicon) may contain minor fibronectin contamination [43], which should not affect the interpretation of our results, since fibronectin does not bind to collagen receptor integrins. No type IV collagen contamination has been reported in the commercial laminin preparations. Here, relatively weak binding of α1I domain to the laminin preparation (-211) indicates that it cannot contain significant amounts of type IV collagen.

A negatively charged amino acid residue in the α2I domain, aspartate-219, is directly involved in collagen binding [27]. The corresponding residue in the α1I domain, arginine-218, has a positive charge. We have earlier reported on the importance of these residues for the collagen binding preferences of the integrins [2]. Here, our results indicate that the positively charged arginine residue at position 218 in the α1I domain is critical for laminin binding as well. The laminin binding mechanism of the integrin α2I domain may be somewhat different and may not involve the amino acid residue in the corresponding position, Asp-219.

It has been detected that the affinity of the αI domain to ligands may increase due to conformational activation [44]. Activation of α2I domain may also lead to recognition of novel binding motifs [45]. It is notable that the responses in concentration dependence curves with open mutations increased significantly especially for collagens. This can be explained by the higher number of potential binding sites for the open αI domain. However, it is less well known what effect integrin activation has on ligand selectivity. Here, we have elucidated the effects of conformational activation of collagen receptor integrin αI domains on collagen and laminin binding. We tested the effects of glutamate (Glu-318) to tryptophan mutation, which makes the

α2I domain preferentially adopt an activated conformation. The binding mechanism of the open mutant remains MIDAS dependent shown both by chelating metal ions in the buffer and by a small molecule inhibitor designed to bind to the MIDAS in the α2 and α1 I domains. The results showed that the selectivity for collagen subtypes was reduced, since the binding to collagens I, IV and IX increased to equivalent levels and the K_ds for collagens I and IV became equal. Deletion of αC-helix (284-GYLNR-288) in α2I domain produces similarly behaving αI domain as α2IE318W (Käpylä et al., unpublished). Wild-type integrin α2I bound laminins only very weakly. However, α2I domain binding to laminin-111, -211 and -511 was remarkably enhanced by conformational activation. An earlier report has also suggested that α2I domain binding to laminin-111 may be enhanced after activation [30]. The open variant of the integrin α1I domain showed tight binding to collagens I and IV and to laminins -111, -211, and -511. Conformational activation appears to be a prerequisite for integrin αI domain binding to laminin, especially for integrin α2. As the conformational state of the integrin has been found to be dependent on the cell type in which the integrin is expressed [31], we propose here that the cell line dependent differences of the ligand binding pattern may be due to the different activation states of the integrins.

The αC-helix in αI domain is unique for collagen receptor integrins and upon the activation the αC-helix unwinds and moves away from the proximity of the metal binding site [27]. We hypothesize that the αC-helix acts in "preselection" for ligands before αI domain activation. Especially tyrosine residues being the largest residue in αC-helices could have a major impact for preselection. If the αI domain is activated before ligand binding no preselection may take place, since the metal ion and residues involved in ligand recognition are exposed for immediate binding. Consequently a broader selection of motifs is accepted.

In general, the high-avidity binding to otherwise disfavoured ligands after receptor activation may reflect the basic mechanism of integrin mediated cell adhesion. After primary recognition of the ligand, integrins can be seen to aggregate to form focal binding sites. This receptor clustering may be dependent on signals generated after initial ligand binding, which then activate the surrounding integrins through an inside-out mechanism. Our results support the idea that cell adhesion is initiated by specific recognition of a limited number of motifs in matrix proteins and later in the process the strengthening of the adhesion site is best gained by allowing integrin binding to a larger number of potential ligands.

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FIGURE LEGENDS

Figure 1. ***Amino acid Arg-218 in α1I domain is important for high-avidity laminin binding*** Microtiter plates were coated with laminins or collagens 15μg/ml in PBS over night. BSA containing Delfia® Diluent in PBS was used as a background control and to block the wells. GST-fusion α1I WT and α1I R218D (A, B), α2I WT and α2I D219R domains (C, D) (A, B and C 500 nM, in D 400nM) were allowed to bind to the wells for one hour at RT in the presence of 2 mM $MgCl_2$. Wells were washed three times. Bound αI domains were detected with Eu^{3+}-labeled GST antibody and the signal was measured using time-resolved fluorophotometry. Data are means of three parallel determinations (±S.D.). The BSA background was subtracted.

Figure 2. ***The two conformations of integrin αI domains.*** The I domains of integrins α1 and α2 are shown as cartoon representations, highlighted residues are drawn as sticks and a MIDAS coordinated divalent magnesium ion is drawn as a sphere. The structural model of the human integrin α1I domain in the open conformation was built based on the crystal structure of the α2I domain (27; PDB code: 1DZI). Mutation of residues Glu-317/Glu-318 into tryptophan residues affects the dynamics of the closed to open conformational reorganization of integrin α1I and α2I domains: the mutation disrupts the Glu-317/Glu-318 to Arg-287/Arg-288 salt bridges present in the closed forms of the I domains, subsequently promoting the adoption of the open forms. Residue Arg-218 in α1I and the corresponding residue in α2I, Asp-219, are the predominant factors responsible for the distinct collagen subtype specificity seen for these integrins.

Figure 3. ***Integrin activation decreases the selectivity of α2I for collagen subtypes*** Microtiter plates were coated with human collagens I, IV, VI and IX (A) or with rat collagen I and mouse collagen IV (B) 16,4μg/ml in PBS over night. BSA containing Delfia® Diluent in PBS was used as a background control and to block the wells. GST-fusion α2IWT and α2IE318W (400 nM) (A) and concentration series of α2I WT and α2I E318W (3,125-400 nM) (B) were allowed to bind to wells for one hour at RT in the presence of 2 mM $MgCl_2$. Wells were washed three times. Bound αI domains were detected with Eu^{3+}-labeled GST antibody and the signal was measured using time-resolved fluorophotometry. Data are means of three parallel determinations (±S.D.).

Figure 4. ***Integrin activation increases the binding of collagen subtypes by α1I domain.*** Microtiter plates were coated with human collagens I, II, IV and VI (A) or with rat collagen I and mouse collagen IV (B) 15 μg/ml in PBS over night. BSA containing Delfia® Diluent in PBS was used as a background control and to block the wells. GST-fusion α1IWT and α1IE317W domains 400 nM (A) or 3,125-400 nM (B) were allowed to bind to wells for one hour at RT in the presence of 2 mM $MgCl_2$. Wells were washed three times. Bound αI domains were detected with Eu^{3+}-labeled GST antibody and the signal was measured using time-resolved fluorophotometry. Data are means of three parallel determinations (±S.D.).

Figure 5. ***The open variant of α2I domain shows enhanced binding to laminin-111, -211 and -511.*** Microtiter plates were coated with laminins -111, -211, -411, and -511 or collagen I 15 μg/ml in PBS over night. BSA containing Delfia® Diluent in PBS was used as a background control and to block the wells. GST-fusion α2IWT and α2IE318W domains (A, 500 nM) (C, 400nM) or concentration series (3.9-500 nM) (B) were allowed to bind to wells for one hour at

RT in the presence of 2 mM $MgCl_2$ (A-C) or 10mM EDTA (C, where indicated). Wells were washed three times. Bound αI domains were detected with Eu^{3+}-labeled GST antibody and the signal was measured using time-resolved fluorophotometry. Data are means of three parallel determinations (±S.D.).

Figure 6. ***The open variant of α1I domain shows enhanced binding to laminin-111, -211 and -511.*** Microtiter plates were coated with laminins -111, -211 -411 and -511 or collagen I 15μg/ml in PBS over night. BSA containing Delfia® Diluent in PBS was used as a background control and to block the wells. GST-fusion α1IWT and α1IE317W domains (A, 500 nM) (C, 400nM) or concentration series (3.9-500 nM) (B) were allowed to bind to wells for one hour at RT in the presence of 2 mM $MgCl_2$ (A-C) or 10mM EDTA (C, where indicated). Wells were washed three times. Bound αI domains were detected with Eu^{3+}-labeled GST antibody and the signal was measured using time-resolved fluorophotometry. Data are means of three parallel determinations (±S.D.).

Figure 7. ***The small molecule L3008 inhibits the binding of open αI domain variants to collagen and laminin.*** Microtiter plates were coated with collagen I or laminin-111 15μg/ml in PBS over night. BSA containing Delfia® Diluent in PBS was used as a background control and to block the wells. The GST fusion α1IWT, α1IE317A, α2IWT, α2IE318W (500nM) were allowed to bind to wells for one hour at RT in the presence of 2 mM $MgCl_2$ and either with the small molecule inhibitor L3008 (50μM, diluted in DMSO) or a equal amount of DMSO (control). Wells were washed three times. Bound αI domains were detected with Eu^{3+}-labeled

GST antibody and the signal was measured using time-resolved fluorophotometry. Data are means of three parallel determinations (±S.D.).

Figure 1

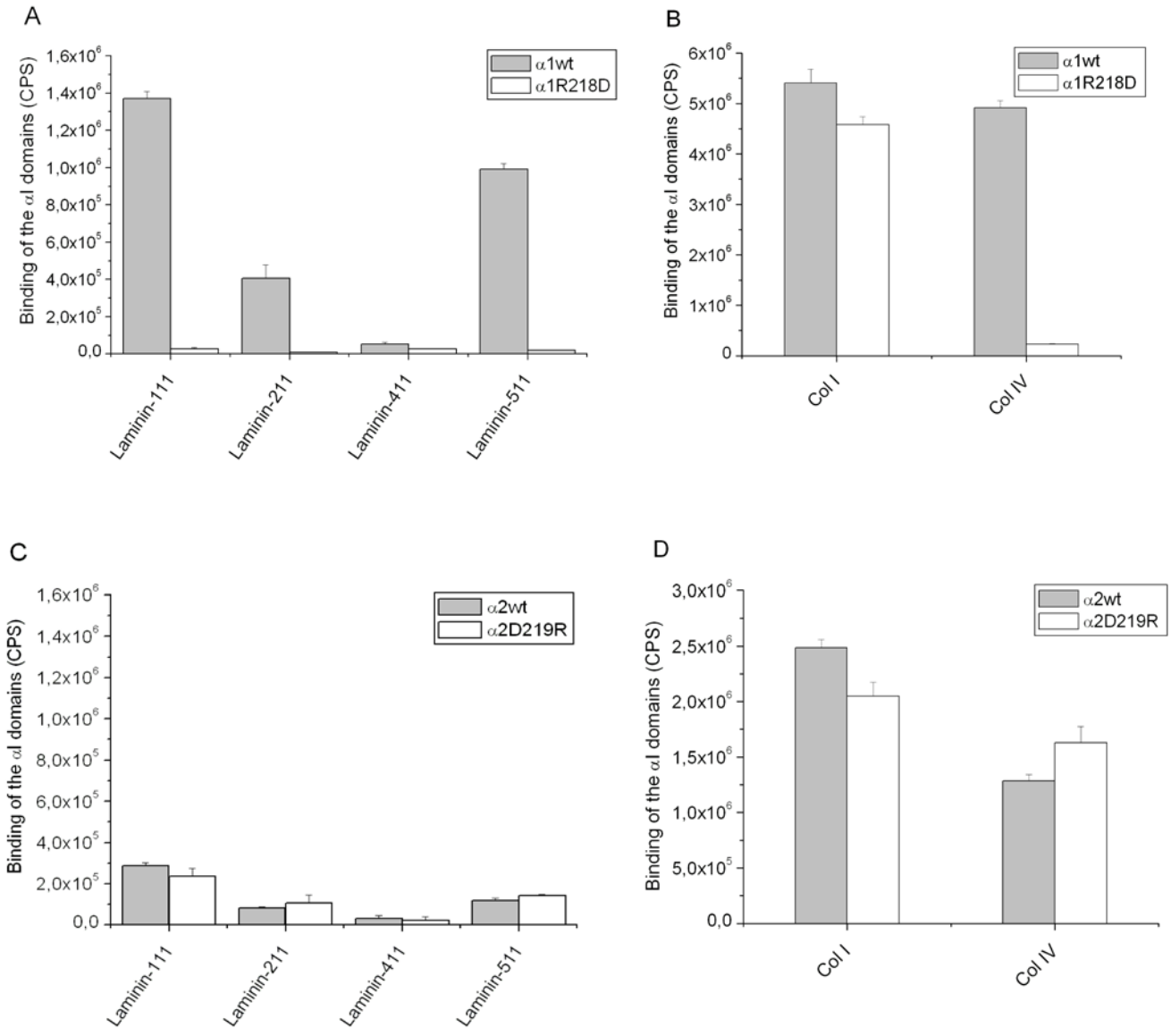

A
Binding of the αI domains (CPS)
1,6x10^6
1,4x10^6
1,2x10^6
1,0x10^6
8,0x10^5
6,0x10^5
4,0x10^5
2,0x10^5
0,0
α1wt
α1R218D
Laminin-111
Laminin-211
Laminin-411
Laminin-511
B
Binding of the αI domains (CPS)
6x10^6
5x10^6
4x10^6
3x10^6
2x10^6
1x10^6
0
α1wt
α1R218D
Col I
Col IV
C
Binding of the αI domains (CPS)
1,6x10^6
1,4x10^6
1,2x10^6
1,0x10^6
8,0x10^5
6,0x10^5
4,0x10^5
2,0x10^5
0,0
α2wt
α2D219R
Laminin-111
Laminin-211
Laminin-411
Laminin-511
D
Binding of the αI domains (CPS)
3,0x10^6
2,5x10^6
2,0x10^6
1,5x10^6
1,0x10^6
5,0x10^5
0,0
α2wt
α2D219R
Col I
Col IV

Figure 2

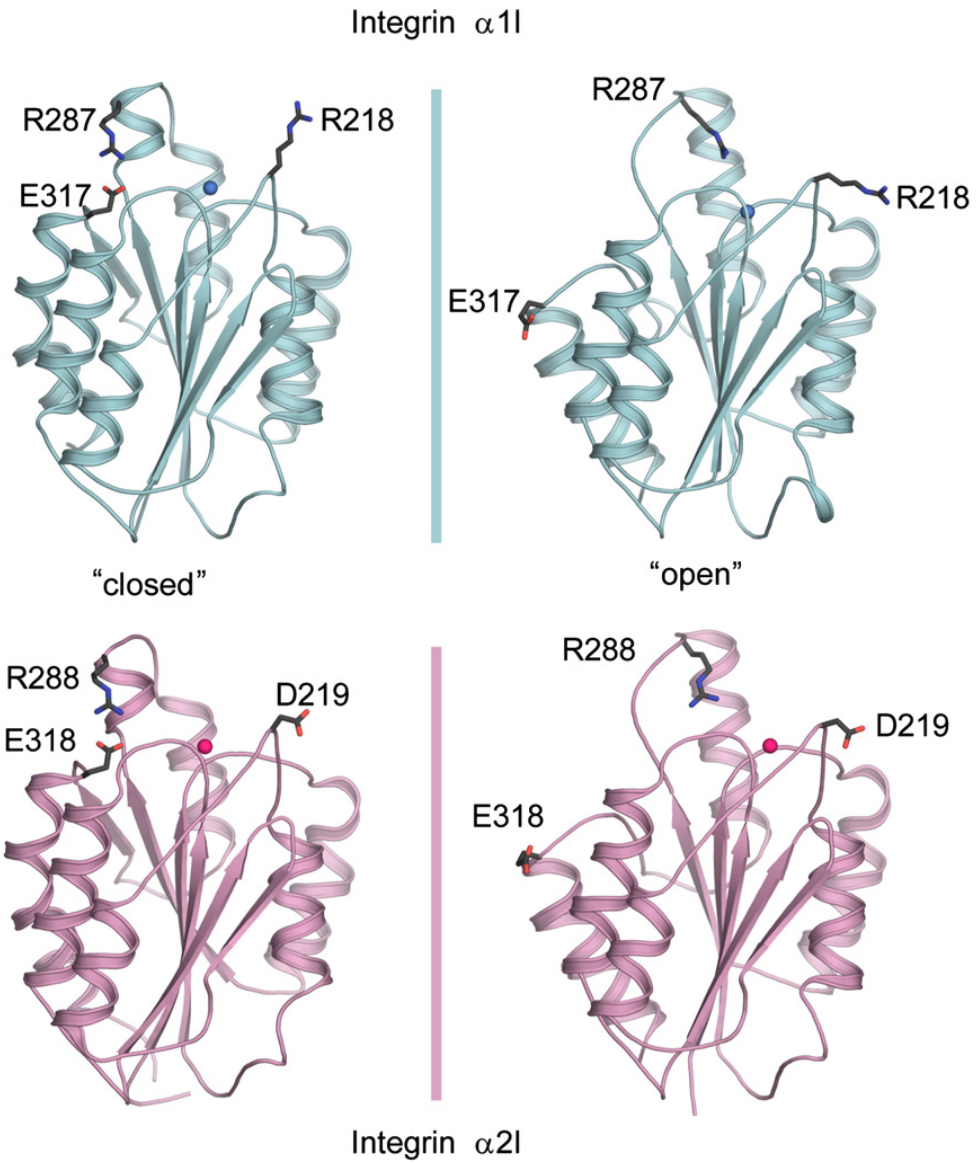

Integrin α1I
R287
R218
E317
"closed"
R287
R218
E317
"open"
R288
D219
E318
R288
D219
E318
Integrin α2I

Figure 3

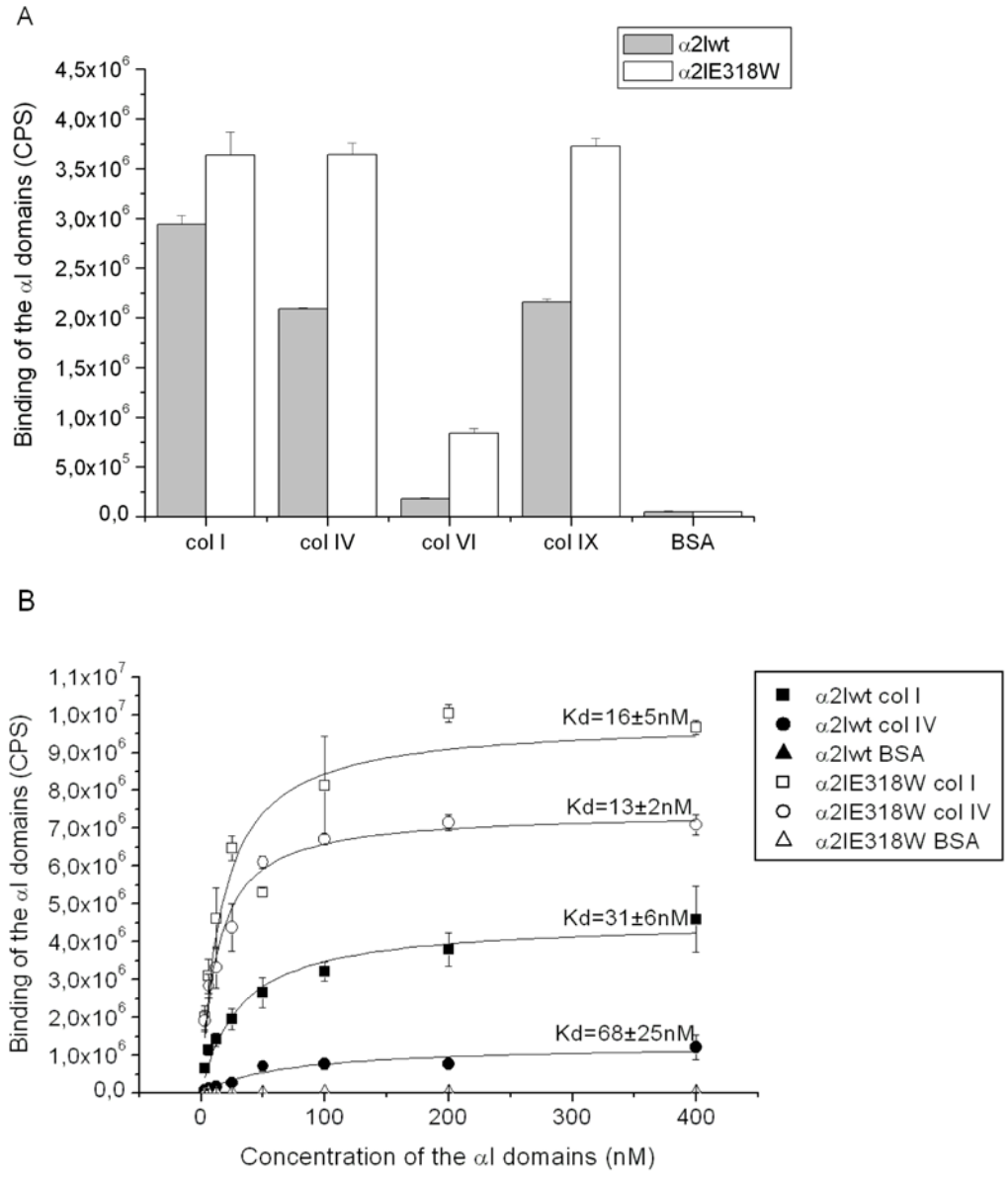

A
α2Iwt
α2IE318W
Binding of the αI domains (CPS)
4,5x10^6
4,0x10^6
3,5x10^6
3,0x10^6
2,5x10^6
2,0x10^6
1,5x10^6
1,0x10^6
5,0x10^5
0,0
col I
col IV
col VI
col IX
BSA
B
Binding of the αI domains (CPS)
1,1x10^7
1,0x10^7
9,0x10^6
8,0x10^6
7,0x10^6
6,0x10^6
5,0x10^6
4,0x10^6
3,0x10^6
2,0x10^6
1,0x10^6
0,0
Kd=16±5nM
Kd=13±2nM
Kd=31±6nM
Kd=68±25nM
0
100
200
300
400
Concentration of the αI domains (nM)
α2Iwt col I
α2Iwt col IV
α2Iwt BSA
α2IE318W col I
α2IE318W col IV
α2IE318W BSA

Figure 4

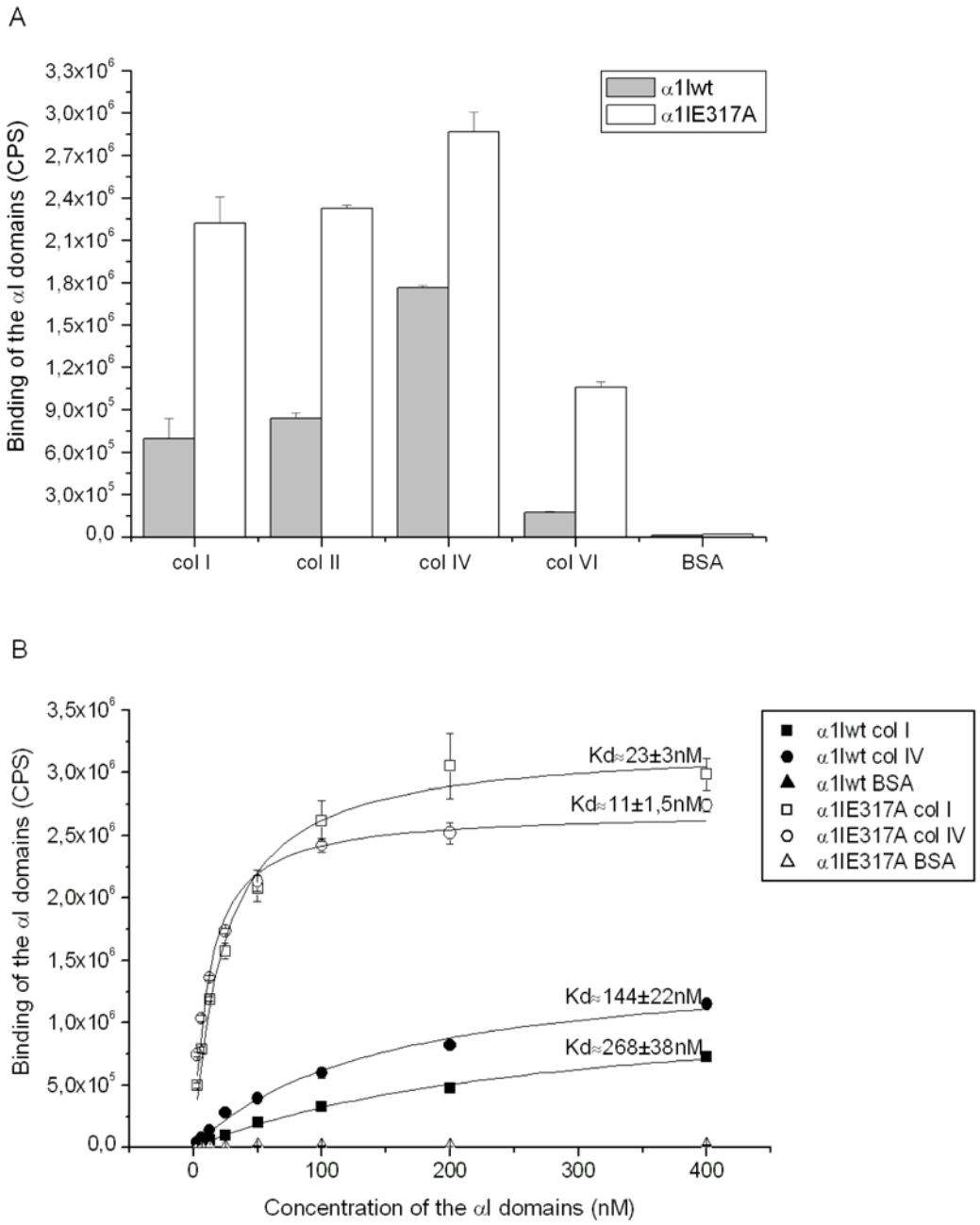

A
Binding of the αI domains (CPS)
3,3x10^6
3,0x10^6
2,7x10^6
2,4x10^6
2,1x10^6
1,8x10^6
1,5x10^6
1,2x10^6
9,0x10^5
6,0x10^5
3,0x10^5
0,0
α1Iwt
α1IE317A
col I
col II
col IV
col VI
BSA
B
Binding of the αI domains (CPS)
3,5x10^6
3,0x10^6
2,5x10^6
2,0x10^6
1,5x10^6
1,0x10^6
5,0x10^5
0,0
Kd≈23±3nM
Kd≈11±1,5nM
Kd≈144±22nM
Kd≈268±38nM
α1Iwt col I
α1Iwt col IV
α1Iwt BSA
α1IE317A col I
α1IE317A col IV
α1IE317A BSA
0
100
200
300
400
Concentration of the αI domains (nM)

Figure 5

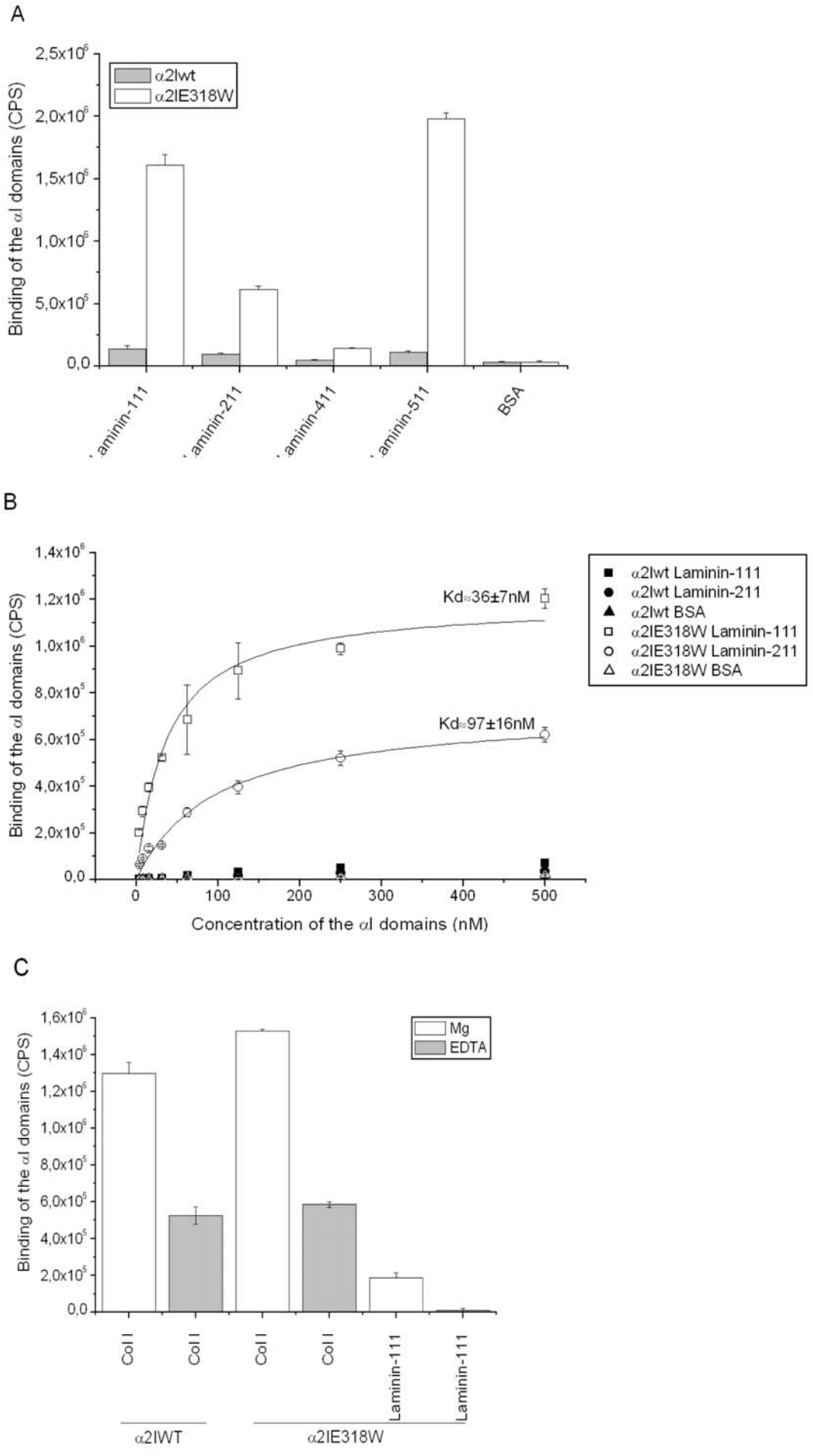

A
Binding of the αI domains (CPS)
α2Iwt
α2IE318W
BSA
B
Binding of the αI domains (CPS)
Concentration of the αI domains (nM)
Kd≈36±7nM
Kd≈97±16nM
α2Iwt Laminin-111
α2Iwt Laminin-211
α2Iwt BSA
α2IE318W Laminin-111
α2IE318W Laminin-211
α2IE318W BSA
C
Binding of the αI domains (CPS)
Mg
EDTA
Col I
Laminin-111
α2IWT
α2IE318W

Figure 6

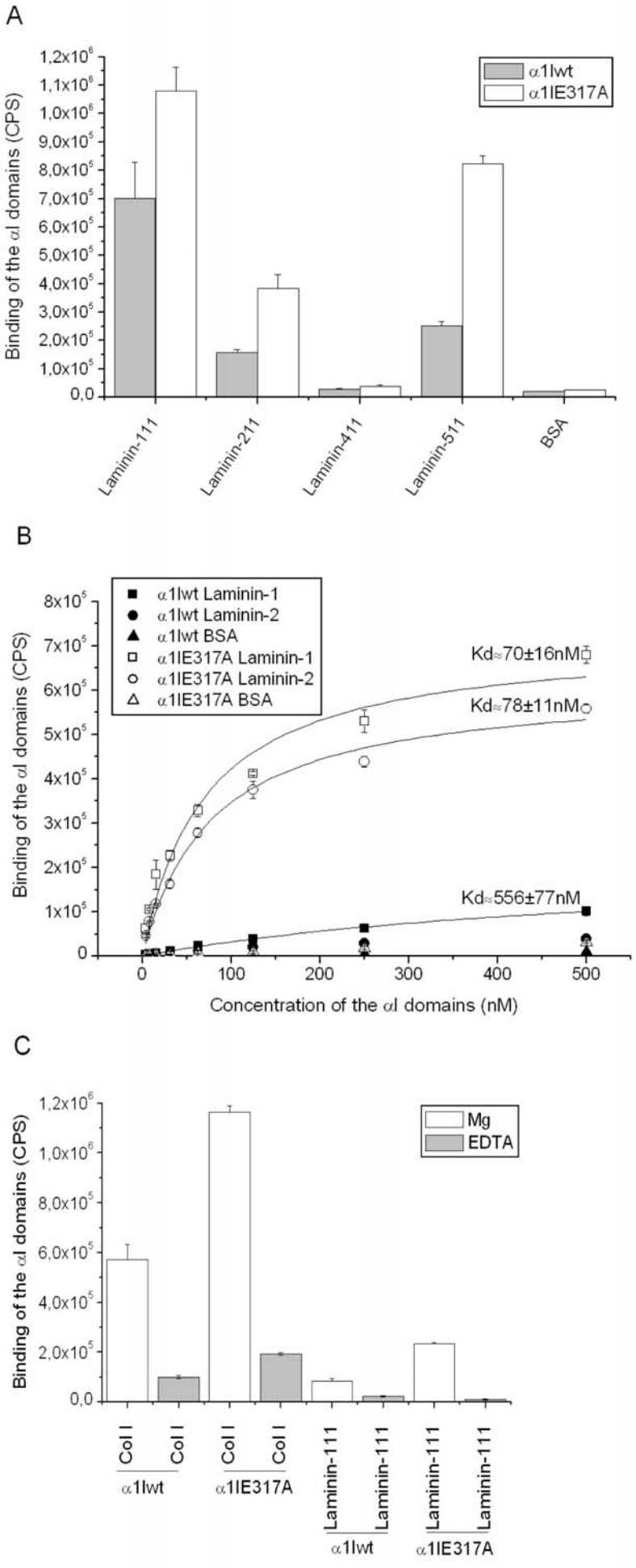
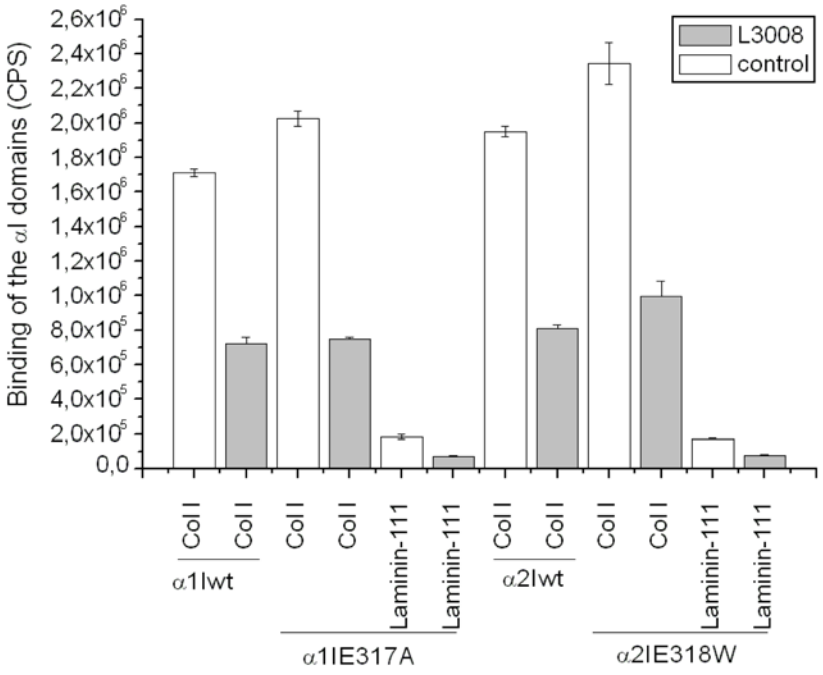

A
Binding of the αI domains (CPS)
α1Iwt
α1IE317A
Laminin-111
Laminin-211
Laminin-411
Laminin-511
BSA
B
Binding of the αI domains (CPS)
α1Iwt Laminin-1
α1Iwt Laminin-2
α1Iwt BSA
α1IE317A Laminin-1
α1IE317A Laminin-2
α1IE317A BSA
Kd≈70±16nM
Kd≈78±11nM
Kd≈556±77nM
Concentration of the αI domains (nM)
C
Binding of the αI domains (CPS)
Mg
EDTA
Col I
α1Iwt
α1IE317A
Laminin-111

Figure 7


Binding of the αI domains (CPS)
2,6x10^6
2,4x10^6
2,2x10^6
2,0x10^6
1,8x10^6
1,6x10^6
1,4x10^6
1,2x10^6
1,0x10^6
8,0x10^5
6,0x10^5
4,0x10^5
2,0x10^5
0,0
L3008
control
Coll
Coll
Coll
Coll
Laminin-111
Laminin-111
Coll
Coll
Coll
Coll
Laminin-111
Laminin-111
α1Iwt
α2Iwt
α1IE317A
α2IE318W