Integrin α11β1 is a receptor for collagen XIII

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Abstract
Collagen XIII is a conserved transmembrane collagen mainly expressed in mesenchymal tissues. Previously, we have shown that collagen XIII modulates tissue development and homeostasis. Integrins are a family of receptors that mediate signals from the environment into the cells and vice versa. Integrin α11β1 is a collagen receptor known to recognize the GFOGER (O=hydroxyproline) sequence in collagens. Interestingly, collagen XIII and integrin α11β1 both have a role in the regulation of bone homeostasis. To study whether α11β1 is a receptor for collagen XIII, we utilized C2C12 cells transfected to express α11β1 as their only collagen receptor. The interaction between collagen XIII and integrin α11β1 was also confirmed by surface plasmon resonance and pull-down assays. We discovered that integrin α11β1 mediates cell adhesion to two collagenous motifs, namely GPKGER and GF(S)QGEK, that were shown to act as the recognition sites for the integrin α11-I domain. Furthermore, we studied the in vivo significance of the α11β1-collagen XIII interaction by crossbreeding α11 null mice (Itga11−/−) with mice overexpressing Col13a1 (Col13a1oe). When we evaluated the bone morphology by microcomputed tomography, Col13a1oe mice had a drastic bone overgrowth followed by severe osteoporosis, whereas the double mutant mouse line showed a much milder bone phenotype. To conclude, our data identifies integrin α11β1 as a new collagen XIII receptor and demonstrates that this ligand-receptor pair has a role in the maintenance of bone homeostasis.

Keywords Collagen · Integrin · Cell adhesion · Bone homeostasis · ECM receptors

Introduction
The adhesion of cells to the neighboring cells and to the extracellular matrix (ECM) is critical for their survival, migration, differentiation, and signal transduction. Integrins are an important family of molecules involved in cell-ECM adhesion process. They are heterodimeric membrane glycoproteins consisting of non-covalently associated α and β subunits (Pandolfi et al. 2017; Barczyk et al. 2010). The ligand-binding specificity of most integrins is well known (Pandolfi et al. 2017; Barczyk et al. 2010). Four collagen-binding integrins, α1β1, α2β1, α10β1, and α11β1, are expressed in various tissues ( Hemler et al. 1986; Camper et al. 1998; Gullberg et al. 1995). The collagen receptor integrin α subunits are characterized by an inserted domain named as the I domain (often called as the A domain) that mediates interaction with native collagens (Hamaia and Farndale 2014; Zeltz and Gullberg 2016).

Collagen XIII is a type II transmembrane protein composed of a short cytosolic head, a transmembrane anchor, and a long extracellular domain with three collagenuous domains (COL1-3) and four non-collagenuous domains (NC1-4) (Hägg et al. 1998; Heikkinen et al. 2012). Our previous in vitro studies using solid-phase assays showed that the I domain of the integrin α1 subunit binds to collagen XIII with similar approximated avidity as to collagen IV.
(Nykvist et al. 2000), but the binding site in the collagen was not characterized. In another report, we suggested that collagen XIII mediates integrin α1β1-dependent transmigration of monocytes in renal fibrosis with a binding site different from other integrin I domain binding motifs (Dennis et al. 2010). These data prompted us to investigate the binding of collagen XIII to other collagen receptor integrins.

Collagen XIII is broadly distributed in mesenchymal tissues at low levels (Hågg et al. 2001; Sund et al. 2001; Sandberg et al. 1989; Juvenile et al. 1992; Sandberg-Lall et al. 2000). Many studies with genetically modified mice have indicated its physiological functions in musculoskeletal tissues (Ylönen et al. 2005; Härönen et al. 2017, 2019; Zainul et al. 2018; Koivunen et al. 2019) and congenital myasthenic syndrome type 19 (CMS19; OMIM #616,720) musculoskeletal phenotypes (Logan et al. 2015; Rodriguez Cruz et al. 2019).

Integrin α1β1 is a collagen receptor (Lu et al. 2010) recognizing the GFOGER (O=hydroxyproline) sequence in interstitial collagens (W. M. Zhang et al. 2003). We consider of interest that both collagen XIII and integrin α1β1 are induced in several tumor tissues (Väisänen et al. 2005, Härönen et al. 2017, 2019; Zainul et al. 2018; Koivunen et al. 2019) and congenital myasthenic syndrome type 19 (CMS19; OMIM #616,720) musculoskeletal phenotypes (Logan et al. 2015; Rodriguez Cruz et al. 2019). Therefore, we chose to analyze the interaction between these two molecules. We found that α1β1 mediates cell spreading on a collagen XIII-coated surface and that two collagenous motifs, GPKGER and GF(S)QGEK, in the collagen XIII ectodomain contribute in the in vitro binding to the integrin α11-I domain. Additionally, we show that collagen XIII-integrin α11 interaction participates in the maintenance of bone homeostasis in vivo.

Materials and methods

Cells

Murine C2C12 myoblast cells from the American Type Culture Collection (ATCC) were stably transfected with a human integrin α2 cDNA or a human integrin α11 cDNA (Tiger et al. 2001), the cDNA encoding the integrin α1 was tagged with enhanced green fluorescent protein (EGFP) C-terminally (Erusappan et al. 2019). Mouse embryonic fibroblast cells (MEFs) were isolated from wild-type mouse embryos as described previously (Popova et al. 2004, 2007) and cultured at 37 °C in DMEM (Biochrom AG, Berlin, Germany) supplemented with 10% FCS (Euroclone, UK) and antibiotics (Cambrex, USA). Immortalized cell lines were created by induction of simian virus 40 (SV40) as described previously (Barczyk et al. 2013).

Production of integrin I domains

The recombinant I domains of the human integrins α1 and α2 were produced as described previously (Nykvist et al. 2000). Briefly, the α1-I domain cDNA was generated by PCR using a human integrin α1 cDNA (Briesewitz et al. 1993) as a template, cloned into the pGEX-4T-3 vector with a GST tag (GE Healthcare, USA), and the protein was expressed in E. coli. The α2-I domain cDNA was produced by PCR using a human integrin α2 cDNA (Takada and Hemler 1989) as a template, cloned into the pGEX-2T vector (GE Healthcare, USA) and the GST- α2I fusion protein was expressed in E. coli. The α11-I domain cDNA was generated similarly by PCR using a human integrin α11 cDNA (Velling et al. 1999) as a template, and then cloned into the pAcSecG2T vector (Invitrogen, USA). The α11-I domain was expressed as a GST-tagged fusion protein and secreted into cell culture media in insect High-Five cells (Invitrogen) using a Baculovirus expression system (Invitrogen). All recombinant GST-I domains were purified using glutathione-Sepharose affinity chromatography (GE Healthcare) and their purities were analyzed by SDS-PAGE.

Production of the recombinant human collagen XIII ectodomain, its collagenous peptides and triple-helical motifs

The ectodomain of human collagen XIII was produced as described previously (Tu et al. 2002). High Five insect cells (Invitrogen) were cultured in a monolayer and co-infected with a virus encoding the human collagen XIII α1 chain lacking the cytosolic domain (Snellman et al. 2000) and another virus encoding both the α and β subunits of human prolyl 4-hydroxylase (Lambrech et al. 1996; Nokelainen et al. 1998). The ectodomain was purified from ~ 400 ml of 48-h infected culture media sequentially using a HiTrap™ Q 5 ml column (GE Healthcare), a HiTrap™ SP 5 ml column (GE Healthcare), and a HiLoad™ Superdex™ 200 column (GE Healthcare). Its purity was analyzed by native gel electrophoresis and SDS-PAGE.

The collagenous peptides of collagen XIII were produced by enzymatic digestion of 400 μg of the collagen XIII ectodomain with 0.4 μg of pepsin at pH 2–3 for 30 min at room temperature and then isolated by a Superdex-200 column in 20 mM HEPES buffer, pH 7.0, containing 0.3 M NaCl. The recombinant proteins composing the testing motifs were produced in E. coli or insect cells. The DNA fragmentencoding (GPP)2GFPGER(GPP)2 was produced by atwo-step PCR reaction. In the first step, the template for the reaction 1 was formed from annealing of two synthetic oligonucleotides 5′-GCCATGGGCTGGACCCCCCGGCCTCTCTGGACCTCCTGGTCCCCCTGGTCC-3′ and 5′-TGCTCC
CGTTGGACACGCTCCCGCAGGGAAAACCCGGAGGACC
AGGGGACC-3’. The only primer 5'-ACAAGGCCATGG
CTGGACCCCG-3’ was used for forward extension. The
template for the reaction 2 was from annealing of another
pair of oligos 5’-ACCGATCCGGTGGCCAGGTGG
TCCGGTTGAGGCCTCGGTTGGACC-3’ and 5’-GGTTCTGGCCTCGGCCTTTCTGGGAG
CTGGTCCACCGGAGGACC-3’ and a primer 5’-GTAACCC
GATCCCCGCTTGGGCAGT-3’ for the reverse extension.
After five cycles, the two reaction products were mixed
and 25 more PCR cycles continued.

The cloning of other motifs followed the same strategy
except changes in two oligonucleotides for annealing, one
in italics and underlined, and another in bold and underlined.

The final PCR products were digested with NcoI and
BamHI, and the purified DNA was inserted into a plasmid
pHisTRX2-Foldon (a gift kindly from J. Engel). The DNA
sequence was confirmed by DNA sequencing. The DNAs
encoding (GPP)5motif(GPP)5-foldon were further cloned by
PCR using primers containing KpnI and HindIII cleavage
sites (forward primer AGCTC
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Cell attachment assay

The cells attached to the culture plates were monitored
directly by microscopy or quantitatively analyzed using a
β-hexosaminidase release assay (Landegren 1984). The
ligands used for the cell attachment assays were human
plasma fibronectin (Invitrogen), rat tail collagen I (Collab-
orative Biomedical Products, USA), collagen XIII ecto-
domain, and its motif-foldon fusion proteins, at a concen-
tration of 10 μg/ml in PBS or in 20 mM HEPES, 0.15 M
NaCl, pH 7.0, 0.2% heat-inactivated BSA (crystallized,
Serva, Germany) in PBS was used as a blank control. The
24-well cell culture plates (Greiner Bio-one, Germany)
were coated with 300 μl of the ligands at 4 °C overnight.
The plates were rinsed 3 times in PBS, and the uncoated
surface was then blocked with 0.2% heat-inactivated BSA
in PBS at 37 °C for 1 h and washed with Buck’s saline
(137 mM NaCl, 5 mM KCl, 4 mM NaHCO3, 5.5 mM
D-glucose, and 2 mg/L phenol red equilibrated with 5%
CO2 at 37 °C) containing 2 mM MgCl2 and 20 μM CaCl2.
For the attachment testing, C2C12 cells at 60–80% con-
fluence were detached by trypsin-EDTA treatment and
washed once with DMEM containing FCS to inactivate
the trypsin and then 3 times with Buck’s saline. The cells
were added to the wells at a density of 100,000 cells/0.5 ml/well.
They were allowed to attach for 1 h at 37 °C in Buck’s
saline, and non-attached cells were removed by washing for
3 times with Buck’s saline. For the β-hexosaminidase assay,
the attached cells were lysed for 2 h at 37 °C in 200 μl
0.05 M citrate, pH 5.0, containing 0.25% Triton X-100
and 3.75 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide
(Sigma, USA). Fifty microliters of the cell lysates were
loaded onto a 96-well microtiter plate (Nunc, Denmark)
and the reaction was stopped, and color developed by add-
ing 75 μl of 45 mM glycine solution, pH 10.4, containing
4.5 mM EDTA. The absorbance at 405 nm was measured
using a Victor microplate reader (Perkin Elmer, USA).
A cell number calibration curve was made by incubating
known numbers of cells under equivalent conditions. Three
repeated experiments were performed in the tests. The data
were normalized by taking adhesion to a fibronectin-coated
surface as 100% cell attachment and that to wells coated
with BSA only as 0%. In the inhibition assay, motif-fusion
proteins, with the molar ratios of 1:1, 5:1, and 10:1 to the
coating collagen XIII, were mixed with the cells first and
then added to the collagen XIII-coated plate wells.

Immunofluorescence staining and EGFP imaging

The glass coverslips were coated and blocked in the same
way as for the 24-well plates in the cell attachment assay.
The cells were allowed to adhere to a density of 9000 cells/30 μl/
coverslip in DMEM containing 10% FCS or in serum-free
DMEM for 1.5–2 h at 37 °C complemented with 5% CO2.
After washing the coverslips with PBS, the attached cells
were fixed in acetone at −20 °C for 8 min or in 4% PFA
at room temperature for 10 min and then permeabilized in
acetone at −20 °C for 5 min. Non-specific binding sites
were blocked by incubating with 10% goat serum (Zymed
Laboratories, Inc., USA) diluted in PBS. The fixed cells
were incubated with the antibody against integrin α11 cyto-
plasmic domain (Popova et al. 2004) for 1–2 h at 37 °C or
at 4 °C overnight. Cy2-, Cy3- (Jackson ImmunoResearch
Laboratories, USA), Alexa Fluor 488-, and Alexa Fluor
568-conjugated secondary antibodies (Invitrogen) were used
for detection. The stained cells were mounted in Immu-
Mount mounting solution (Thermo Shandon, USA), visu-
alized and photographed under a Leitz Aristoplan micro-
scope (Leica Microsystems, Germany) or an Olympus BX51

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(Olympus, Japan) equipped with optics for observing fluorescence. To visualize C2C12-α11+, cells were fixed in 2% PFA + 0.1% Triton X-100 and mounted as described above.

**Western blotting**

To verify the expression of integrin α11, C2C12 cells in one culture plate (Φ = 100 mm) at 80–90% confluence were washed once with 10 ml of PBS and then detached with a cell scraper into 1 ml of PBS. The cells were harvested by centrifuging at 340×g for 10 min and then homogenized for 1 min with 0.5 ml of 70 mM Tris/0.3 M NaCl/0.2% Triton X-100, pH 7.4, containing Protease Inhibitor cocktail (Roche Applied Science, Germany), followed by incubation on ice for 30 min. After centrifuging at 8000×g for 10 min, the supernatant of the cell lysate was applied to SDS-PAGE followed by western blotting. The expression of recombinant human integrin α11 in C2C12 cells was detected with an affinity-purified antibody against the human α11 cytoplasmic domain (Tiger et al. 2001).

**Solid-phase binding and surface plasmon resonance assays**

Fifty microliters of collagen solutions, collagen I (Collaborative Biomedical Products, USA), collagen IV (BD Biosciences), collagen XIII ectodomain, its pepsin-digested fragments, and the recombinant motif-fusion proteins, were coated on the plastic surfaces of MaxiSorp™ 96-well microplates (Nunc) at 4 °C overnight. All the other steps were performed at room temperature using a buffer containing 20 mM Tris, 0.15 M NaCl, 2 mM MgCl₂, 20 μM CaCl₂, 0.05% Tween-20, or 10 mM HEPES, 0.15 M NaCl, 2 mM MgCl₂, 20 μM CaCl₂, pH 7.0. Three parallel samples were analyzed. The wells were blocked with 5% fat-free milk in Tris buffer or in HEPES buffer for 1 h and the samples incubated for 1.5 h with α1-, α2-, and α11-I domains diluted in the buffer containing 30 μg/ml BSA (crystallized, Serva). After thorough washing, the binding ligands were detected with a goat polyclonal anti-GST antibody (Rockland Immunochemicals, USA) and then with an anti-goat IgG secondary antibody conjugated with horseradish peroxidase (Dako, Denmark). TMB-Xtra (Kem-En-Tec Diagnostics A/S, Denmark) was used as the substrate for detection.

Pull-down assay

Insect High-Five cells were infected with viruses encoding GST and GST-α11-I domain respectively with MOI of 10 in TNM-FH media (Sigma) containing 10% FCS. Eighty percent of the recombinant proteins were secreted into the media due to a signaling peptide in the expression vector. Forty-eight postinfection 5 ml media from each cell culture plate were collected and dialyzed in PBS containing 0.1 mM DTT and 0.001% Triton. The media were then mixed with the glutathione-Sepharose pre-equilibrated with the dialysis buffer at 4 °C overnight. After washing out the unbound proteins, the purified collagen XIII ectodomain was incubated with the Sepharose resin in 10 mM HEPES, pH 7.0, containing 0.15 M NaCl and 2 mM MgCl₂ at room temperature for 2 h. The unbound collagen XIII was removed by extensive washing steps and the proteins absorbed on the glutathione-Sepharose were eluted by incubating with either 10 mM glutathione or one cleavage unit of thrombin at room temperature overnight. The unbound collagen XIII was incubated at room temperature overnight as a control. The elution fractions were analyzed with a western blotting using a monoclonal antibody recognizing the recombinant human collagen XIII (Tu et al. 2002).

**Sequence retrieval and alignment**

The full-length human integrin α11 sequence is 1188 residues in length. We retrieved only the I domain residues ranging from 164 to 345 which comprises 182 residues in total (later renumbered from 1 to 182) from the UniProtKB (Q9UKX5). The crystal structure of the human integrin α2-I domain bound with the collagen peptide (PDB code: 1DZI) as well as the sequence information was obtained from the PDB (Berman et al. 2000). Sequence alignment between the integrin domains of α2 and α11 was carried out using ClustalW2 (Larkin et al. 2007) with default settings.

**Homology modeling of the human integrin α11-I domain**

The final sequence alignment obtained from the CLUSTALW2 program was submitted to the MODELER9v1 software package (Eswar et al. 2006; Sali and Blundell 1993) for constructing the homology model of the human integrin α11-I domain. Twenty different models were generated and the model with the lowest energy was considered as the ideal model.
Energy minimization and structure validation

Energy minimization of the human integrin α11-I domain model was carried out using GROMACS version 4.0.7. (Hess et al. 2008; Van Der Spoel et al. 2005). Initially, the protein was placed in a cubic box and a SPC water model was used for solvation. After solvating, suitable ions were added to neutralize the whole system. The final system was composed of 13,073 molecules, which includes the protein (182 residues), water (12,882 molecules), and ions (9 Na⁺).

The whole system was then subjected to 1000 steps of steepest descent minimization using the GROMOS96 53a6 force field until the maximum force converged to <1000.0 kJ/mol/nm with PME (Particle Mesh Ewald) as the Coulomb term, the rCoulomb cutoff of 1.0 and van der Waals cutoff of 1.0. For validation purposes, the minimized final model was submitted to various structure validation programs (PROCHECK, VERIFY3D and ERRAT) available through SAVes server (https://servicesn.mbi.ucla.edu/SAVES/).

Collagen triple-helical peptide construction

The crystal structure of the α2-I domain-collagen peptide complex was downloaded from the PDB databank, and then collagen triple-helical peptides (strands B, C, D) were manually separated from the integrin domain. Each peptide sequence comprises of 21 amino acid residues (the residues in each peptide strands were later renumbered from 1 to 21). Here, we constructed three different collagen triple-helical peptides namely, (GPP)₂GFQGEK(GPP)₃, and (GPP)₂GPKGER(GPP)₃. These collagen triple-helical peptides were constructed using COOT software package (P. Emsley et al. 2010). All these constructed triple-helical peptides were then subjected to 500 steps of steepest descent minimization using GROMACS version 4.0.7 (Hess et al. 2008; Van Der Spoel et al. 2005). The final energy minimized triple-helical peptides were then used for docking with the human integrin α11-I domain.

Protein-protein docking

Docking of modeled human α11-I domain and the constructed triple-helical peptides (α11-(GPP)₂GFQGEK(GPP)₃ and α11-(GPP)₂GPKGER(GPP)₃) were carried out using the HADDOCK program (Dominguez et al. 2003), a Web-based tool to perform protein-protein docking. The HADDOCK docking program is driven based on the Ambiguous Interaction Restraints derived from the experimental information of the intermolecular interacting residues provided by the user. We used the easy interface for all the docking calculations. However, before carrying out the docking experiments between the modeled human α11-I domain and collagen XIII peptides, we first performed a control docking experiment using the crystal structure of ligand-binding α2-I domain to validate the HADDOCK docking program. Upon docking of α2-I domain and its collagen triple-helical peptide (GFOGER), the program had predicted the key interacting residues as seen in the crystal structure suggesting that HADDOCK can be used for the docking calculations using the triple-helical peptides. The constructed peptides were then docked to the modeled α11-I domain. The best representative docked complex was chosen based on the lowest energy cluster and HADDOCK score obtained through diverse energy terms such as van der Waals energy, electrostatic energy, desolvation energy, restraints violation energy and buried surface area. PDBsum database was used to analyze the protein-peptide interactions while the binding energy (ΔG) was calculated using the PRODIGY server (Xue et al. 2016).

Mouse line

Generation of the transgenic Col13a1oe mice has been described previously (Ylönen et al. 2005), and the mouse line is publicly available in the Infratro EMMA repository with the strain name B6.Cg-Tg(Col13a1)2Pih/Oulu (EM:09885). Generation of Itga11−/− mice has been described previously (Popova et al. 2007). Here, Itga11−/− mice in C57BL/6J background were bred with Col13a1oe to generate a Col13a1oe:Itga11−/− double mutant mouse line, overexpressing Col13a1 and lacking Itga11. Col13a1oe littermates were used as controls. Permission for the maintenance of mice (license ID ESAVI/4220/04.10.07/2013) was obtained from the Finnish Animal Care and Use Committee of the State Provincial Office of Southern Finland (Hämeenlinna, Finland) and the European Community Council Directive on the protection of animals used for scientific purposes (September 22, 2010; 2010/63/EUC), national legislation and the regulations for the care and use of laboratory animals were followed.

µCT

Tubular bone microarchitecture was assessed from left femurs of Col13a1oe:Itga11−/− mice, and they were compared to Col13a1oe mice. Number of mice analyzed in order of increasing age: female Col13a1oe (7, 4, 6, 6, 6), female Col13a1oe:Itga11−/− (3, 3, 4, 3, 2), male Col13a1oe (5, 4, 7, 6), male Col13a1oe:Itga11−/− (3, 3, 3, 3). The most recent guidelines for bone microstructure assessment were used (Bouxsein et al. 2010). Bones were imaged using a Skyscan 1176 scanner (Bruker microCT, Kontich, Belgium) using the following parameters: X-ray tube voltage of 50 kV, current of 500 μA, exposure time of 4000 ms, and a 0.5 mm aluminum filter. Projection
images were collected every 0.3° over a 360° rotation. Image processing was performed with the manufacturer’s software NRecon (v. 1.6.5.2), Dataviewer (v. 1.5.6.2), and CTAn (v. 1.11.10.0). Femur length was measured from reconstructed datasets from the superior top of trochanter major to the distal patellofemoral groove in mid-frontal plane. The average value of three separate measurements was used as the result for the femur length. Measurements were conducted using the manufacturer’s software (Skyscan, Dataviewer v. 1.5.6.2). Besides the femur length, cortical bone morphology was assessed. Diaphyseal cortical bone was assessed using the VOI starting 0.9 mm distally from the femoral neck and extending further distally. Two different ROI lengths were used for cortical bone analysis: 3.6 mm was used for 4-week time point, whereas 5.4 mm was used for other time points. 2D structural analysis was performed to define cortical bone area (Ct.Ar) and average cortical thickness (Ct.Th). 2D volumetric analyses of datasets were conducted using CTAn software (Skyscan, v. 1.11.10.0).

Statistics

Statistical significance was determined by two-way ANOVA followed by the Benjamini, Krieger, and Yekutieli FDR post hoc test (Benjamini et al. 2006). Values of FDR-corrected p (q) < 0.05 values were considered statistically significant. No statistical methods were used to predetermine sample size.

Results

Integrin α11β1 is localized at focal contacts in cells spreading on a collagen XIII-coated surface

To determine whether α11β1 mediates the spreading of cells on collagen XIII, α11-EGFP transfected C2C12 mouse myoblasts (C2C12-α11+), and mouse embryonic fibroblasts (MEFs) derived from a wild-type mouse embryo were tested. Direct fluorescence imaging of C2C12-α11+ cells showed that α11-EGFP localized at cell-matrix adhesion structures during cell spreading on collagen XIII (Fig. 1a) and on collagen I (Fig. 1c), but not on fibronectin (Fig. 1e). The cellular localization of integrin α11β1 on primary MEFs on collagen XIII was studied by immunofluorescence staining using an antibody against the cytoplasmic domain of the mouse integrin α11 subunit (Fig. 1b). Spreading on collagen I was analyzed as a control (Fig. 1d). These data indicate that both collagen XIII and collagen I, but not fibronectin, are functional ligands for integrin α11β1.

C2C12 cells expressing integrin α11β1 adhere to collagen I and collagen XIII-coated surfaces

The interaction between collagen XIII and integrin α11β1 was further studied by using the transfected C2C12 cells that expressed human α11β1 as their only collagen receptor (Tiger et al. 2001). C2C12 cells transfected with the vector plasmid (MOCK) were tested in parallel. The expression of the α11 subunit was verified by western blotting using an antibody specific for the cytoplasmic domain of the human integrin α11 (Fig. 1e). C2C12 control cells did not adhere to native collagen I or to native collagen XIII, but the cells attached well to fibronectin due to the endogenous expression of fibronectin receptor integrins (Fig. 2a). Cell attachment assays utilizing C2C12 cells that were stably transfected with the full-length human integrin α11 cDNA (C2C12-α11+ cells) indicated a remarkable increase in adhesion to collagens I and XIII when compared to control C2C12 cells (Fig. 2a). The attached C2C12-α11+ cells started to spread on the collagen XIII-coated surfaces after 2 h (Fig. 2b). For control purposes, C2C12-α2+ cells were tested with similar results as in our previously published study (Fig. S1), i.e., integrin α2 can mediate cell spreading on collagen I but not on collagen XIII (Nykivist et al. 2000). Integrin α2β1 and α11β1 have both been suggested to be the cellular receptors for fibrillar collagens (Zeltz and Gullberg 2016), but the present results demonstrate a remarkable difference in their preference for collagen XIII.

Collagen XIII binds to the I domain of integrin α11 subunit with high avidity in vitro

A GST-α11 I-fusion protein has previously been produced in E. coli (W. M. Zhang et al. 2003), but the protein yield and binding activity varied from batch to batch (Käpylä J. et al., unpublished). To facilitate studies on the interaction between integrin α11β1 and collagen XIII in more detail, we set up an expression system of the α11-I domain as a secreted protein in insect cells. The recombinant protein contains a GST-tag and a thrombin cleavage site between GST and the I domain. The α11-I domain was purified from the medium by a one-step GST-affinity purification method. The unpurified media containing GST-tagged α11-I domain or GST as a blank control was used for binding specificity test in vitro. The human collagen XIII ectodomain was expressed as a homotrimer using a baculovirus system in insect cells (Tu et al. 2002). Furthermore, collagen types I and IV were compared to collagen XIII in the binding assays. The integrin α1- and α2-I domains obtained from E. coli (Nykivist et al. 2000) which also contained the GST-tags, were included for selected interaction studies. The binding tests were performed using an enzyme-linked immunosorbent assay (ELISA) in which collagens I, IV, and XIII were coated onto...
the plastic surface as solid ligands and the I domains were used as soluble analytes. The interaction was detected using a GST antibody. The α11-I domain interacted with collagen types I and XIII in a dose-dependent manner (Fig. 3a). Collagen XIII was identified to bind α11-I domain with similar affinity as collagen I and stronger than collagen IV (Fig. 3b). Consistently with the previously published results (W. M. Zhang et al. 2003; Tiger et al. 2001; Nykvist et al. 2000), collagen IV showed preferential binding to the α1-I domain, but no binding to the α11-I domain (Fig. 3b). In contrast to collagens IV and XIII, collagen I bound well to all the tested integrin I domains (Fig. 3b).

The specific interaction between collagen XIII and integrin α11β1 was further confirmed by surface plasmon resonance (SPR) and pull-down assays. In SPR, collagen XIII showed a typical sensorgram of binding to the immobilized fusion protein α11-I domain (Fig. 3c, black curve) unlike in the blank control in which no protein was immobilized (Fig. 3c, gray curve). In a pull-down assay, collagen XIII was trapped only by the GST-α11-I domain coupled to the glutathione-Sepharose but not when GST was used alone.

The protein complex could be eluted by glutathione or thrombin, indicating that the interaction site locates in the α11-I domain (Fig. 3d). Due to the long incubation time at room temperature, collagen XIII eluted from the interaction complex was partly degraded, resulting in a reduced molecular mass of 60 kDa. The unbound fraction contained both this 60 kDa fragment as well as the full-length 72 kDa collagen XIII ectodomain, and after overnight incubation, mimicking the enzyme digested fractions, equal degradation of the unbound fraction was evidenced (Fig. 3d).

**Integrin α11-I domain binds specifically to the C-terminal collagenous domain of collagen XIII**

To identify the potential integrin α11β1 binding sites in collagen XIII, the purified collagen XIII ectodomain was digested by pepsin and the cleaved fragments were separated by gel-filtration chromatography (Tu et al. 2002). The peptide representing the COL3 domain showed similar integrin-binding capability as the intact ectodomain...
Collagen XIII does not contain the GFOGER or other previously identified integrin-binding motifs (UniProt Consortium 2019). However, the COL3 domain in collagen XIII harbors two GER and seven GEK sequences that are typically found as parts of the known integrin-binding motifs. To test these putative binding sequences, two GER motifs, namely GPKGER and GNRGER, and one GEK motif, namely GF(S)QGEK (“S” in mouse), were expressed in $E. coli$ using an expressing vector encoding two (GPP)×5 sequences flanking the tested fragments, a foldon sequence for triple-helical formation (Frank et al. 2001), and a His-tag for the protein purification (Fig. 4c). GFPGER was chosen as a positive control and GEKGARGSPGLP, a peptide located on the COL2 domain and binding to integrin α1β1 (Dennis et al. 2010), was produced for a negative control. Other GEK motifs were considered less interesting based on the knowledge gained from the peptide ToolKits of collagen-receptor interactions (Farnadle et al. 2008). The motifs GPKGER and GF(S)QGEK showed positive binding to the integrin α11-I domain, albeit at a 50–60% lower approximated avidity when compared to GFPGER (Fig. 4b). GEKGARGSPGLP and GNRGER showed no binding (Fig. 4b). The protein-protein interaction was further confirmed by a cell attachment assay utilizing collagen I, the ectodomain of collagen XIII, and the recombinant proteins containing the putative integrin-binding motifs. The binding of cells was markedly stronger to the GPKGER than to the GF(S)QGEK motif (Fig. 4d). Furthermore, the attachment of C2C12-α11+ cells to the collagen XIII-coated surface could be partially inhibited by GPKGER (Fig. 4e). These data suggested that both GPKGER and GF(S)QGEK contributed to the binding of collagen XIII to the α11-I domain. The two binding sites identified in the COL3 domain of collagen XIII lie within a highly conserved region (Fig. 4f).

To study the molecular interaction between the human α11-I domain and the triple-helical peptides, a homology model of the human α11-I domain was constructed using the crystal structure of the human integrin α2-I domain (PDB code: 1DZI) (J. Emsley et al. 2000) as a template. Prior to the homology model construction, the sequence alignment of α11 and α2 integrin I domains were subjected to manual inspection as a quality check; this identified a shared sequence identity and similarity of 44% and 63.1%, respectively (Fig. 5), being amendable for the further modeling studies. The energy minimized model of the human α11-I domain is shown in Fig. 6a. The root mean square deviation between the final energy minimized model of the α11-I domain and the α2-I domain was 0.237 (Fig. 6b), suggesting that the initial backbone structure was intact and there was not much deviation from its original conformation. Ramachandran plot analysis using PROCHECK (Laskowski et al. 1993) showed that 90.1% of the residues fall in the most favored regions and 9.3% of the residues in the additionally allowed regions (Fig. S2). Also, the assessment of the constructed model by its 3D profile using VERIFY3D (Eisenberg et al. 1997) showed that 100% of the residues are within the allowed regions (Fig. S3). Further, the reliability and the quality of the model were validated using the ERRAT program (Colovos and Yeates 1993). The comparison between the initial model (before minimization) and the final model (after minimization) showed that the quality of the modeled protein was significantly increased after minimization with the overall quality factor score of 97.093 (Fig. S4). The structure validation programs suggest that the constructed homology model of the human α11-I domain structure is of high quality and reliable, thus, can be used for our docking studies.
Docking of α11-I domain and triple-helical collagen peptides

To study the collagen XIII binding mode to the α11-I domain, we performed docking studies using our homology model of α11-I domain and the HADDOCK program (Domínguez et al. 2003). First, the interface residues that directly interact with the triple-helical collagen were identified from the crystal structure of the α2-I domain. Then, the identified residues were mapped correspondingly to the α11-I domain in the sequence alignment. These residues were then supplied as active residues of the α11-I domain while the passive residues were set to define automatically by the HADDOCK program (Domínguez et al. 2003) to perform the docking calculations.

The HADDOCK server produced 176 different structures of the α11-(GPP)GFQGEK(GPP)3 complex classified in 8 clusters which represents 88.0% of the water-refined models. For the α11-(GPP)GFKGER(GPP)3 complex, the HADDOCK server resulted in 136 structures classified in 10 clusters representing 68% of the water-refined models. Analysis of the α11-(GPP)GFQGEK(GPP)3 complex revealed that the residues from all the three strands (strands B, C, and D) of the collagen peptide contributed to the interactions with the interface residues of the α11-I domain (Fig. 7a). Lys12 of the strand B, Glu11 and Lys12 of the strand C, and Glu11 and Lys12 of the strand D were involved in the formation of multiple hydrogen bonds with the α11-I domain. Apart from the hydrogen bonds, Glu11 and Lys12 of the strand

Fig. 3 Selective binding of collagen XIII to the integrin α11-I domain; a 0.25 μg of collagens I, IV, and XIII in 50 μl of TBS were coated on microtiter plates. The GST-α11-I domain was diluted in series in TBS containing 30 μg/ml BSA, 2 mM MgCl₂, and 0.05% Tween-20 and used for binding tests employing a solid-phase assay with an antibody against GST. Five percent of fat-free milk in TBS was used for blocking the uncoated surface and for a blank control. b A summary of relative binding comparison of I domains to the coated collagen types I, IV, and XIII (Col I, Col IV, and Col XIII, respectively). c SPR response of the binding of collagen XIII to the immobilized integrin α11-I domain (black curve). The gray curve shows the background of the sensogram. d A pull-down assay was performed by incubation of collagen XIII with the GST-α11-I domain or GST protein coupled glutathione-Sepharose. The interaction complex was eluted by 10 mM of reduced glutathione or cleaved by thrombin. The eluted, unbound, and unbound incubated overnight (o.n.) collagen XIII was detected by a collagen XIII monoclonal antibody.
C, Lys12 of the strand D also participated in the salt bridge formation with the α11-I domain residues. The energy values of the docked complexes are shown in Table 1 and detailed list of hydrogen bonds formed between the atoms of α11-I domain and GFQGEK is shown in Table 2.

The interaction between the α11-I domain and (GPP)_{2}GFQGEK(GPP)_{3} interaction with the α11-I domain. Here, much of the interactions come from the Lys9 residue of the strand C collagen peptide (Fig. 7b). Further, Glu76 of the α11-I domain and Lys9 and Arg12 of the collagen peptide from the strand C participated in the H-bond and salt bridge formation. Another salt bridge is formed between Glu110 of α11-I domain and Lys9 of the
The femoral cortical bone morphology of middiaphyseal femur is enlarged (Koivunen et al. 2019). Itga11−/− mice (α11β1 interaction, we crossbred integrin α11 null mice with mice overexpressing Col13a1 (Col13a1oe;Itga11−/−) (Ylönen et al. 2005, Koivunen et al. 2019)) and created a double mutant mouse line (Col13a1oe;Itga11−/−). The Col13a1oe mice possess a severe bone phenotype, in which especially the circumference of the bone length between these genotypes (females in Fig. S5b) was significantly diminished when compared to Col13a1oe mice. No differences were observed in the bone length between these genotypes (females in Fig. 8c and males in Fig. S5c). These results indicate that collagen XIII-integrin α11β1 interaction has a role in cortical bone homeostasis, and this role is evident in both genders.

### Discussion

Collagens are known to be recognized by the α-I domains in collagen receptor integrins α1β1, α2β1, α10β1, and α11β1 (Gullberg and Lundgren-Akerlund 2002; Zeltz and Gullberg 2016), and it has been shown that α1β1 and α10β1 interact preferentially with the basement membrane collagen IV and the beaded filament forming collagen VI (Tulla et al. 2001; Nykvist et al. 2000), while α11β1 shows similar features as α2β1 and selects the interstitial collagens (W. M. Zhang et al. 2003). However, collagen IX, a cartilage matrix component, shows exceptionally high binding capacity to all four integrin I domains, suggesting a more complicated mechanism for cell adhesion to collagens (Käpylä et al. 2004). Here, we show the firm interaction of collagen XIII with integrin α11β1. The motif GFOGER in a triple-helical context has been identified as a target sequence for α1β1, α2β1, α10β1, and α11β1 integrins (Knight et al. 1998, Xu et al. 2000, W. M. Zhang et al. 2003, Sipilä et al. 2014), but these integrins also bind to other ECM proteins that do not contain this particular sequence (Pandolfi et al. 2017). On the other hand, collagen IV does not bind to α2β1 or α11β1 as strongly as collagens I–III, although it contains the GFOGER sequence in the α1(IV), α3(IV), α4(IV), and α5(IV) chains. Additionally, collagen IX, which binds avidly to all integrin I domains, lacks the GFOGER motif. Hence, it has been proposed that GFOGER is not the only target sequence for collagen-binding integrins. Indeed, studies on the integrin-recognition motifs in collagen III have identified GROGER, GLKGEN, and GLOGR as integrin-binding sites (Raynal et al. 2006; Kim et al. 2005). Moreover, a prokaryotic collagen sequence GLPGER has been reported to bind to integrins α2β1 and α11β1 (Caswell et al. 2008). A study using the collagen peptide ToolKits, which consist of sets of overlapping triple-helical peptides, identified two motifs GLOGR and GVOGEA that are specific for α1β1, α2β1, α10β1, and α5β1 integrins (Knight et al. 1998, Xu et al. 2000, W. M. Zhang et al. 2003). However, collagen IX, which binds avidly to all integrin I domains, lacks the GFOGER motif. Hence, it has been proposed that GFOGER is not the only target sequence for collagen-binding integrins. Indeed, studies on the integrin-recognition motifs in collagen III have identified GROGER, GLKGEN, and GLOGR as integrin-binding sites (Raynal et al. 2006; Kim et al. 2005). Moreover, a prokaryotic collagen sequence GLPGER has been reported to bind to integrins α2β1 and α11β1 (Caswell et al. 2008). A study using the collagen peptide ToolKits, which consist of sets of overlapping triple-helical peptides, identified two motifs GLOGR and GVOGEA that are specific for α1β1, α2β1, α10β1, and α5β1 integrins (Hamaia et al. 2012). Additional binding motifs identified for collagen-binding integrins are GPKGER, GLQGER, GLOGR, and GASGER (Zwolanek et al. 2014; Xu et al. 2000).

Our previous study has shown that the ectodomain of the transmembrane collagen XIII binds avidly to the α1-I domain (Nykvist et al. 2000). Here, we show that C2C12 cells expressing α11β1 spread on a collagen XIII-coated surface in the same manner as on a collagen I-coated surface. Among the integrin α1-, α2-, and α11-I domains, the α11-I domain showed strongest binding to collagen XIII, and...
the collagen XIII showed more specific interaction with the α11-I domain when compared to other collagen types. These data suggest differences in the interaction of collagen XIII with α11 integrin compared to other α subunits.

Collagen XIII is reported to be a ligand mediating integrin α1β1-dependent transmigration of monocytes in Alport renal fibrosis (Dennis et al. 2010). In that study, α1β1-positive monocytes were shown to bind to a GEK- GAEGSPGLP sequence located in the COL2 domain of the collagen XIII. In the present study, the α11β1 binding site in collagen XIII was localized in the COL3 domain. Some of the known integrin-binding collagenous motifs, such as GFOGER, GLOGER, and GVPGEA (see above), do not exist in collagen XIII. Furthermore, we did not consider GXXGEN-like sequences (X = any of the amino acid residues) since these motifs were not preferred in a previous study on the α11-I domain utilizing the collagen peptide ToolKits (W. M. Zhang et al. 2003). In the present study, four motifs, GPKGER, GFQGER, GSQGER, and GNRGER from the COL3 domain, and GFPGER, used as a positive control, were chosen for the binding tests. GNRGER showed no binding to the α11, a finding consistent with the ToolKit data. GPKGER, GFQGER, and GSQGER bound to α11, but the interaction was more modest than that with GFPGER. Proline hydroxylation is known to be important in collagen-integrin interaction (Sipilä et al. 2018; Rappu et al. 2019). However, in this study, coexpression of prolyl 4-hydroxylase (P4H) in the insect cells did not catalyze the proline hydroxylation probably due to the short (GPP)5 sequence, which was not sufficient for binding of the enzyme P4H to the collagen substrate. Thus, here all prolines are non-hydroxylated. While many previously reported integrin-binding motifs contain hydroxyproline (Rappu et al. 2019), there are also several exceptions, such as GLSGER and GQRGER (Siljander et al. 2004), GFKGER (Zwolanek et al. 2014), GLQGER (Zwolanek et al. 2014), and GASGER (Xu et al. 2000). Moreover, in the hydroxyproline containing functional domains hydroxylation of proline residues is not absolutely required for integrin binding, but it increases the avidity of the interaction. For example, the α2-I domain can bind to GFPGER containing peptides, but more weakly than to GFOGER (Knight et al. 2000).

To model the interaction between the α11-I domain and collagen motifs, two different collagen peptides ((GPP)2GFQGER(GPP)2 and (GPP)2GPKGER(GPP)2) were constructed through silico approaches. The docking analysis showed that both constructed triple-helical

Fig. 5  Sequence alignment. Human integrin α2-I (ITGA2) and α11-I (ITGA11) domains share sequence identity of 43.9% and 63.1%, respectively

Fig. 6  Molecular model of the integrin α11-I domain. a An energy minimized structure of human integrin α11-I domain. b A superimposed model of the α11-I domain (blue) and the crystal structure of human integrin α2-I domain (pink, PDB: 1DZI) is shown. The root mean square deviation between these two structures is 0.237
Docked complexes of collagen tripeptides and the α11-I domain. 

**a** The collagen XIII tripeptide (GPP)$_2$GFQGEK(GPP)$_3$ bound to the α11-I domain, sidechains of the interacting residues are labelled and shown on the right side. 

**b** A complex of α11-(GPP)$_2$GPKGER(GPP)$_3$ along with the interacting residues.

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**Table 1** The best representative docked complex was chosen based on the HADDOCK score and the cluster size. Binding energy ($\Delta$G) for each of the docked complexes was calculated using the PRODIGY server. Van der Waals, electrostatic energy, and buried surface area obtained from HADDOCK server are shown.

| Docked Complex | HADDOCK score | Binding energy ($\Delta$G) (kcal/mol) | Van der Waals energy (kcal/mol) | Electrostatic energy (kcal/mol) | Buried Surface (Å) |
|----------------|---------------|---------------------------------------|---------------------------------|---------------------------------|-------------------|
| α11-I domain-(GPP)$_2$GFQGEK(GPP)$_3$ | −105.99 ± 9.9 | −6.7 | −41.0 ± 6.1 | −391.4 ± 45.7 | 1505.4 ± 129.6 |
| α11-I domain-(GPP)$_2$GPKGER(GPP)$_3$ | −60.3 ± 3.4 | −7.1 | −30.199 ± 4.8 | −290.0 ± 37.0 | 1109.4 ± 109.2 |

**Table 2** Residues contributing to hydrogen bonds between the α11-I domain and triple-helical peptide (GPP)$_2$GFQGEK(GPP)$_3$ (chains B, C, and D) is shown. The atom names and the interacting distance (in Angstroms) are shown in square brackets.

| α11 | (GPP)$_2$GFQGEK(GPP)$_3$ |
|-----|--------------------------|
|     | Chain B                  | Chain C                  | Chain D                  |
| TYR139[O] | GLN9[NE2] [2.84] |                     |                      |
| ASN141[O] | LYS12[NZ] [2.91] |                     |                      |
| ASN141[OD1] | LYS12[NZ] [3.05] |                     |                      |
| ASN10[OD1] | GLN9[NE2] [3.03] |                     |                      |
| THR77[OG1] | LYS12[NZ] [2.91] |                     |                      |
| ASP108[OD2]* | LYS12[NZ]* [2.67] |                     |                      |
| GLU110[O] | LYS12[NZ] [2.76] |                     |                      |
| ASN14[N] | GLU11[OE2] [3.06] |                     |                      |
| ARG142[NE] | GLU11[OE2] [2.63] |                     |                      |
| ARG142[NE]* | GLU11[OE1]* [2.63] |                     |                      |
| ASN10[ND2] | GLU11[OE2] [2.78] |                     |                      |
| THR75[OG1] | GLU11[OE1] [2.62] |                     |                      |
| GLU76[OE1]* | LYS12[NZ] [2.69]* |                     |                      |
| GLU76[OE2] | LYS12[NZ] [2.69] |                     |                      |
| HIS112[NE2] | LYS12[O] [2.79] |                     |                      |

*Involved in salt bridge formation
peptides indeed bind to the interface of α11-I domain. In the crystal structure of the integrin α2-I domain bound to the triple-helical GFOGER peptide (J. Emsley et al. 2000), the strand C makes most of the interactions with the α2-I domain whereas the strand B contributes fewer interactions and the strand D makes no interactions as it is exposed to the solvent. Consistently, this trend was observed in our docking experiments for the GPKGER peptide, where most of the hydrogen bonds came from the strand C, less hydrogen bonds from the strand B and no interaction from the strand D. It can be hypothesized that the presence of an arginine (Arg12, the 12th residue in the strand C) residue might influence the orientation of the triple-helical peptide with the integrin domain such that the strand C makes many contacts with the integrin I domain similar as seen in the template crystal structure. However, for the GFQGEK, most of the hydrogen bonds came from the strand C and multiple hydrogen bonds were observed from both the strands B and D. It is conceivable that the change of arginine (Arg12) to lysine (Lys12) may disrupt the orientation exposing all the three strands contacting the I domain. Consequently, our experimental data supports the molecular modeling results as C2C12-α11+ cells attach much stronger on GPKGER than on GFQGEK (Fig. 4d). Homology modeling and docking can be considered a powerful strategy to predict protein-protein interactions if there is no crystal structure of the complexes available (Quignot et al. 2018). Accuracy of the docking depends on the quality of the homology model (Quignot et al. 2018; Rodrigues et al. 2013); thus, we have validated our model at different stages both manually as well as using various online servers to obtain a reliable prediction of the α11-I domain structure and the docked complex (see “Materials and methods”). However, despite striving for high-quality predictions in our study, it should be noted that the crystal structures of the α11-I domain in an unbound and a collagen-bound state remain to be solved, allowing further confirmation of our modeling results.

The peptide sequence GPKGER exists in 15 different collagens (collagens III, IV, V, VII, IX, XI, XIII, XIV, XV, XVII, XIX, XX, XXII, XXV, XXVII), while GFQGEK exists only in collagens XIII and IV, and GSQGER only in mouse collagen XIII and in rat XVII (UniProt Consortium 2019). It is not clear why collagen IV does not bind to α11 although it contains both GFOGER and GFKGER sequences (Farndale et al. 2008, W. M. Zhang et al. 2003). Collagen IX, which binds to all the four I domains, contains the GFKGER sequence also. We propose GFKGER as a new general integrin-binding motif, albeit with modest affinity.

In the light of data obtained with cells and mutant mice (Hägg et al. 1998, 2001; Kvist et al. 2001; Sund et al. 2001; Tahkola et al. 2010; Härönen et al. 2017; Zainul et al. 2019), collagen XIII appears to play a role in cell-matrix adhesion. Recent in vivo studies highlight involvement of collagen XIII in the development, differentiation, and maturation of musculoskeletal tissues and vessels and in maintaining tissue integrity (Latvanlehto et al. 2010; Härönen et al. 2017; Zainul et al. 2018; Heikkinen et al. 2019; Koivunen et al. 2019). The 150-nm flexible ectodomain of collagen XIII can potentially cross the basement membrane around myofibrils or under endothelium and can penetrate into the surrounding stroma. Thus, the in vitro properties of collagen XIII in binding to the basement membrane collagen receptor α1β1 and to the matrix interstitial collagen receptor α1β1 may be of physiological relevance. Moreover, COL3, the furthest collagenous domain from the plasma membrane, appeared largely responsible for the binding to the α11-I domain, suggesting that collagen XIII molecules could interact with integrin α1β1 molecules occurring in neighboring cells, but this aspect was not studied in the present study.

Under physiological conditions, integrin α1β1 is expressed in specific subsets of fibroblasts and mesenchymal stem cells (MSCs) (Popov et al. 2011; Shen et al. 2019) and it has previously been observed to be up-regulated in carcinoma-associated fibroblasts (CAFs) in the tumor stroma (Zhu et al. 2007; Smeland et al. 2019). Collagen XIII is shown to be up-regulated by TGF-β in the reactive stromal cells of epithelial tumors and throughout mesenchymal tumors. The induction of collagen XIII expression occurs at an early stage in tumor progression in response to malignant transformation (Väisänen et al. 2005).

Integrin α1β1 is a regulator of MSC survival on collagen I (Popov et al. 2011). Moreover, α1β1 is found in leptin receptor positive MSCs in the bone marrow where α11β1 has shown to

### Table 3

| α11  | (GPP)_2GPKGER(GPP)_3 |
|------|---------------------|
|      | Chain B | Chain C |
| GLU110[OE1] | LYS9[NZ] [2.66] |
| GLU110[OE2]* | LYS9[NZ]* [2.66] |
| TYR140[OH]* | PRO6[O] [3.06] |
| ASN10[ND2] | PRO6[O] [2.88] |
| GLU45[OE2]* | LYS9[NZ]* [2.68] |
| THR75[O] | LYS9[N] [2.75] |
| THR75[OG1] | GLY7[O] [2.77] |
| GLU76[OE1]* | LYS9[NZ]* [2.67] |
| GLU76[OE2] | ARG12[NH2] [3.34] |
| GLU76[OE2]* | ARG12[NH2]* [2.75] |
| HIS112[NE2] | LYS9[O] [2.67] |

*Involved in salt bridge formation
be an osteolectin receptor and a regulator of osteogenesis and bone homeostasis (Shen et al. 2019). Conditional deletion of the α11 subunit from leptin receptor positive bone marrow cells accelerates trabecular bone loss in aged mice without alterations to body size, femoral length or cortical bone area. However, the mineral apposition rate is significantly reduced in cortical bone in these mice compared to littermate controls. We have previously shown that collagen XIII regulates bone remodeling and angiogenesis through β1 integrins (Koivunen et al. 2019). The novel double mutant mouse line presented here, Col13a1oe;I

\textit{Col13a1oe};Itga11−/−, has a milder high bone mass phenotype compared to Col13a1oe mice. More specifically, double mutants have significantly less bone overgrowth and milder osteoporosis compared to Col13a1oe mice. A comparable phenotype was seen in both sexes up to 35 weeks of age. We were not, however, able to complete a full cohort of the male mice to the final time point on account of insufficient amount of mice available for the experiment. These data suggest that collagen XIII-triggered effects in
bone homeostasis may be mediated by α11β1. However, Itga11 deficiency does not lead to full rescue of the bone phenotype indicating existence of other, unknown, mechanisms triggered by collagen XIII.

In conclusion, we identify integrin α11 as a new collagen XIII receptor and show that this ligand/receptor pair may have a role in bone homeostasis. Additionally, we propose GFKGER as a new general integrin-binding motif with moderate affinity.

Supplementary Information The online version contains supplementary material available at (https://doi.org/10.1007/s00441-020-03300-y).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the European Community Council Directive on the protection of animals used for scientific purposes (September 22, 2010; 2010/63/EEC), national legislation and the regulations for the care and use of laboratory animals.

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References

Barczyk MM, Carracedo S, Gullberg D (2010) Integrins. Cell Tissue Res 339:269–280
Barczyk MM, Lu N, Popova SN, Bolstad AD, Gullberg D (2013) alpha11beta1 integrin-mediated MMP-13-dependent collagen lattice contraction by fibroblasts: evidence for integrin-coordinated collagen proteolysis. J Cell Physiol 228:1108–1119
Benjamini Y, Krieger AM, Yekutieli D (2006) Adaptive linear step-up procedures that control the false discovery rate. Biometrika 93:491–507
Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000) The Protein Data Bank. Nucleic Acids Res 28:235–242
Blumberg K, Niehoff A, Belgardt BF, Ehlen HW, Schmitz M, Hallinger R, Schulz JN, Bruning JC, Krieg T, Schubert M, Gullberg D, Eckes B (2012) Dwarfism in mice lacking collagen-binding integrins alpha2beta1 and alpha1beta1 is caused by severely diminished IGF-1 levels. J Biol Chem 287:6431–6440
Bouxein ML, Boyd SK, Christiansen BA, Gullberg RE, Jepsen KJ, Muller R (2010) Guidelines for assessment of bone micro-structure in rodents using micro-computed tomography. J Bone Miner Res 25:1468–1486
Briesewitz R, Epstein MR, Marcantonio EE (1993) Expression of native and truncated forms of the human integrin alpha 1 subunit. J Biol Chem 268:2989–2996
Camper L, Hellman U, Lundgren-Akerlund E (1998) Isolation, cloning, and sequence analysis of the integrin subunit alpha10, a beta1-associated collagen binding integrin expressed on chondrocytes. J Biol Chem 273:20383–20389
Caswell CC, Barczyk M, Keene DR, Lukomska E, Gullberg DE, Lukomski S (2008) Identification of the first prokaryotic collagen sequence motif that mediates binding to human collagen receptors, integrins alpha2beta1 and alpha1beta1. J Biol Chem 283:36168–36175
Colovos C, Yeates TO (1993) Verification of protein structures: patterns of nonbonded atomic interactions. Protein Sci 2:1511–1519
Dennis J, Meehan DT, Delimont D, Zalocchi M, Perry GA, O’Brien S, Tu H, Pihlajaniemi T, Cosgrove D (2010) Collagen XIII induced in vascular endothelium mediates alpha1beta1 integrin-dependent transmigration of monocytes in renal fibrosis. Am J Pathol 177:2527–2540
Dominguez C, Boelens R, Bonvin AM (2003) HADDOCK: a protein-protein docking approach based on biochemical or biophysical information. J Am Chem Soc 125:1731–1737
Eisenberg D, Luthy R, Bowie JU (1997) VERIFY3D: assessment of protein models with three-dimensional profiles. Methods Enzymol 277:396–404
Emsley J, Knight CG, Farndale RW, Barnes MJ, Liddington RC (2000) Structural basis of collagen recognition by integrin alpha2beta1. J Cell Sci 101:47–56
Emsley P, Lohkamp B, Scott WG, Cowtan K (2010) Features and development of Coot. Acta Crystallogr D Biol Crystallogr 66:486–501
Erusappan P, Alam J, Lu N, Zeltz C, Gullberg D (2019) Integrin alpha11 cytoplasmic tail is required for FAK activation to initiate 3D cell invasion and ERK-mediated cell proliferation. Sci Rep 9:15283–15286
Eswar N, Webb B, Marti-Renom MA, Madhusudhan MS, Eramian D, Shen MY, Pietter U, Salì A (2006) Comparative protein structure modeling using Modeller. Curr Protoc Bioinformatics Chapter 5:Unit-5.6
Farndale RW, Lisman T, Bihan D, Hamaia S, Smerling CS, Pugh N, Konitsiotis A, Leitinger B, de Groot PG, Jarvis GE, Raynal N (2008) Cell-collagen interactions: the use of peptide Toolkits to
investigate collagen-receptor interactions. Biochem Soc Trans 36:241–250
Frank S, Kammerer RA, Mechling D, Schulthess T, Landwehr R, Bann J, Guo Y, Lustig A, Bachinger HP, Engel J (2001) Stabilization of short collagen-like triple helices by protein engineering. J Mol Biol 308:1081–1089
Gullberg D, Lundgren-Akerlund E (2002) Collagen-binding I domain integrins–what do they do? Prog Histochem Cytochem 37:3–54
Gullberg D, Velling T, Sjoberg G, Sejerensen T (1995) Up-regulation of a novel integrin alpha-chain (alpha mt) on human fetal myotubes. Dev Dyn 204:57–65
Hägg P, Rehn M, Huhtala P, Väisäinen T, Tamminen M, Pihlajaniemi T (1998) Type XIII collagen is identified as a plasma membrane protein. J Biol Chem 273:15590–15597
Hägg P, Väisäinen T, Tuomisto A, Rehn M, Tu H, Huhtala P, Eskelinen S, Pihlajaniemi T (2001) Type XIII collagen: a novel cell adhesion component present in a range of cell-matrix adhesions and in the intercalated discs between cardiac muscle cells. Matrix Biol 19:727–742
Hamaia SW, Farndale RW (2014) Integrin recognition motifs in the human collagens. Exp Adv Med Biol 819:127–142
Hamaia SW, Pugh N, Raynal N, Nemoz B, Stone R, Gullberg D, Bihan D, Farndale RW (2012) Mapping of potent and specific binding motifs, GLOGEN and GVGOEA, for integrin alpha beta 1 using collagen toolkits II and III. J Biol Chem 287:26019–26028
Häronen H, Zainul Z, Tu H, Helaakoski T, Myllyharju J, Peteltonen S, Notbohm H, Pihlajaniemi T, Kivirikko KI (1996) Characterization of human type III collagen expressed in a baculovirus system. Production of a protein with a stable triple helix requires coexpression with the two types of recombinant prolyl 4-hydroxylase subunit. J Biol Chem 271:11988–11995
Landegren U (1984) Measurement of cell numbers by means of the endogenous enzyme hexosaminidase. Applications to detection of lymphokines and cell surface antigens. J Immunol Methods 67:379–388.
Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948
Laskowski RA, MacArthur MW, Moss DS, Thornton JM (1993) PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Cryst 26:283–291
Latanlehto A, Fox MA, Sormunen R, Tu H, Oikarinen T, Koski A, Naumenko N, Shakirzynova A, Kallio M, Ilves M, Giniatullin R, Sanes JR, Pihlajaniemi T (2010) Muscle-derived collagen XIII regulates maturation of the skeletal neuromuscular junction. Hum Mol Genet 29:166–176
Knight CG, Morton LF, Onley DJ, Peachey AR, Messent AJ, Smethurst PA, Tuckwell DS, Farndale RW, Barnes MJ (1998) Identification in collagen type I of an integrin alpha2 beta1-binding site containing an essential GER sequence. J Biol Chem 273:33287–33294
Knight CG, Morton LF, Peachey AR, Tuckwell DS, Farndale RW, Barnes MJ (2000) The collagen-binding A-domains of integrins alpha(1)beta(1) and alpha(2)beta(1) recognize the same specific amino acid sequence, GFOGER, in native (triple-helical) collagens. J Biol Chem 275:35–40
Koivunen J, Kemppainen AV, Finnila MA, Keskil-Filppula R, Haronen H, Tu H, Pellikka H, Heikkinen A, Kylmajoa E, Sormunen R, Miinalainen I, Saarakkala S, I兹 Z, Pihlajaniemi T (2019) Collagen XIII-derived ectodomain regulates bone angiogenesis and intracortical remodeling. Matrix Biol 83:6–25
Kivist AP, Latvanlehto A, Sund M, Eklund L, Väisänen T, Hägg P, Sormunen R, Komulainen J, Fässler R, Pihlajaniemi T (2001) Lack of cytosolic and transmembrane domains of type XIII collagen results in progressive myopathy. Am J Pathol 159:1581–1592
Lambert A, Helaakoski T, Myllyharju J, Peteltonen S, Notbohm H, Pihlajaniemi T, Kivirikko KI (1996) Characterization of human type III collagen expressed in a baculovirus system. Production of a protein with a stable triple helix requires coexpression with the two types of recombinant prolyl 4-hydroxylase subunit. J Biol Chem 271:11988–11995
Logan CV, Cossins J, Rodriguez Cruz PM, Parry DA, Maxwell S, Martinez-Martinez P, Riepsaame J, Abdelhamed ZA, Lake AV, Moran M, Robb S, Chow G, Sewry C, Hopkins PM, Sheridan E, Jayawant S, Palace J, Johnson CA, Beeson D (2015) Congenital myasthenic syndrome type 19 is caused by mutations in COL13A1, encoding collagen XIII. Dev Biol 402:207–217
Lindahl E (2008) GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. J Chem Theory Comput 4:435–447
Lipton SA, Birk DE, Ala-Kokko L, Heino J (2004) The fibril-associated collagen IX provides a novel mechanism for cell adhesion to cartilage. J Cell Biol 166:714–717
Marti R, Lanyon L, Lehtonen J, Hiekkanne H, Nissinen L, Kallajoki M, Alanen K, Gullberg D, Heino J (2006) Regulation of prostate cancer development by collagen XIII. Cancer Res 66:44:714–717
Miinalainen I, Saarakkala S, I兹 Z, Pihlajaniemi T (2019) Pan-Cancer analysis of the expression of short collagen-like triple helices by protein engineering. J Mol Biol 308:1081–1089
Mori T, Nakajima T, Obata K, Shikuma N, Kuzuya N, Fujioka Y, Kiyohara Y, Kaneda Y (2000) The collagen-binding A-domains of integrins alpha(1)beta(1) and alpha(2)beta(1) recognize the same specific amino acid sequence, GFOGER, in native (triple-helical) collagens. J Biol Chem 275:35–40
Nokelainen M, Helaakoski T, Myllyharju J, Notbohm H, Pihlajaniemi T, Fietzek PP, Kivirikko KI (1998) Expression and characterization of recombinant human type II collagens with low and high contents of hydroxylysine and its glycosylated forms. Matrix Biol 16:329–338
Nykvist P, Tu H, Ivaska J, Käpylä J, Pihlajaniemi T, Heino J (2000) Identification in collagen type I of an integrin alpha2 beta1-binding site containing an essential GER sequence. J Biol Chem 273:33287–33294
Nykvist P, Tu H, Ivaska J, Käpylä J, Pihlajaniemi T, Heino J (2000) Identification in collagen type I of an integrin alpha2 beta1-binding site containing an essential GER sequence. J Biol Chem 273:33287–33294
Shakirzyanova A, Oikarainen T, Abdullin A, Martin P, Santoleri S, Kemppainen AV, Giniatullin R, Pihlajaniemi T, Kivirikko KI (1996) Characterization of human type III collagen expressed in a baculovirus system. Production of a protein with a stable triple helix requires coexpression with the two types of recombinant prolyl 4-hydroxylase subunit. J Biol Chem 271:11988–11995
Sp1-dependent manner. Matrix Biol 29:166–176
Wood MT, Birk DE, Ala-Kokko L, Heino J (2004) The fibril-associated collagen IX provides a novel mechanism for cell adhesion to cartilage. J Cell Biol 166:714–717
Yang L, Li W, Zuo Q, Su W, Ji L, Sun P, Wang Y, Cao W, Zhang L, Hu L, Xu Y, Tu H, Zhao J, Chen J, Yang Z, Li Y, Liu X, Zhang Y, Li Q, Liu X, Yang L, Li W, Zuo Q, Su W, Ji L, Sun P, Wang Y, Cao W, Zhang L, Hu L, Xu Y, Tu H, Zhao J, Chen J, Yang Z, Li Y, Liu X, Zhang Y, Li Q, Liu X, Zhang Y, Li Q, Liu X, Zhang Y, Li Q, Liu X

[Note: The rest of the text contains references to various scientific articles and studies related to collagen and its interactions, but the specific content is not transcribed here.]
Sandberg-Lall M, Hagg PO, Wahlstrom I, Pihlajaniemi T (2000) Sandberg M, Tamminen M, Hirvonen H, Vuorio E, Pihlajaniemi Sali A, Blundell TL (1993) Comparative protein modeling by satisfaction of spatial restraints. J Mol Biol 234:779–815

Pandolfi F, Franza L, Altamura S, Mandolini C, Cianci R, Ansari A, Kurnick JT (2017) Integrins: integrating the biology and therapy of cell-cell interactions. Clin Ther 39:2420–2436

Popov C, Radic T, Haasters F, Prall WC, Aszodi A, Gullberg D, Schieker M, Docheva D (2011) Integrins alpha2beta1 and alpha11beta1 regulate the survival of mesenchymal stem cells on collagen I. Cell Death Dis 2:e186

Popova SN, Barczyk M, Tiger CF, Beertsen W, Zigrino P, Aszodi A, Miosge N, Forsberg E, Gullberg D (2007) Alpha1 beta1 integrin-dependent regulation of periodontal ligament function in the erupting mouse incisor. Mol Cell Biol 27:4306–4316

Popova SN, Rodriguez-Sanchez B, Liden A, Betsholtz C, Van Den Bos T, Gullberg D (2004) The mesenchymal alpha1beta1 integrin attenuates PDGF-BB-stimulated chemotaxis of embryonic fibroblasts on collagens. Dev Biol 270:427–442

Primac I, Maquoi E, Blacher S, Heljasvaara R, Van Deun J, Smeland HY, Canale A, Louis T, Stuhr L, Soumi N, Cataldo D, Pihlajaniemi T, Pequeux C, De Weyer O, Gullberg D, Noel A (1999) Stromal integrin alpha1 regulates PDGF-beta signaling and promotes breast cancer progression. J Clin Invest 130:4609–4628

Quignot C, Rey J, Yu J, Tuffery P, Guerois R, Andreani J (2018) InterEvDock2: an expanded server for protein docking using evolutionary and biological information from homology models and multimeric inputs. Nucleic Acids Res 46:W408–W416

Rappu P, Salo AM, Myllýrharju J, Heino J (2019) Role of prolyl hydroxylation in the molecular interactions of collagens. Essays Biochem 63:325–335

Raynal N, Hamaia SW, Siljander PR, Maddox B, Peachey AR, Fernandez R, Foley LJ, Slatter DA, Jarvis GE, Farndale RW (2006) Use of synthetic peptides to locate novel integrin alpha2beta1-binding motifs in human collagen III. J Biol Chem 281:3832–3833

Rodrigues J, Melquiond ASJ, Karaca E, Trellet M, Mv D, van Zundert GCP, Schmitz C, Vries SJd, Bordogna A, Bonati L, Kastritis PL, Bonvin AMJJ (2013) Defining the limits of homology modeling in information-driven protein docking. Proteins: Struct, Funct, Bioinf 81:2119–2128

Rodriguez Cruz PM, Cossins J, Estephan EP, Munell F, Selby K, Raynal N, Hamaia SW, Siljander PR, Maddox B, Peachey AR, Fernandez R, Foley LJ, Slatter DA, Jarvis GE, Farndale RW (2006) Use of synthetic peptides to locate novel integrin alpha2beta1-binding motifs in human collagen III. J Biol Chem 281:3832–3833

Sali A, Blundell TL (1993) Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol 234:779–815

Sandberg M, Tamminen M, Hirvonen H, Vuorio E, Pihlajaniemi T (1989) Expression of mRNAs coding for the alpha 1 chain of type XIII collagen in human fetal tissues: comparison with type X collagen. J Cell Biol 109:10I domains. J Biol Chem 276:48206–48212

Sandberg M, Tamminen M, Hirvonen H, Vuorio E, Pihlajaniemi T (2000) Type XIII collagen is widely expressed in the adult and developing human eye and accentuated in the ciliary muscle, the optic nerve and the neural retina. Exp Eye Res 70:401–410

Shen B, Vardy K, Hughes P, Tasdogan A, Zhao Z, Yue R, Crane GM, Morrison SJ (2019) Integrin alpha11 is an osteoelastin receptor and is required for the maintenance of adult skeletal bone mass. Elife 8[https://doi.org/10.7554/eLife.42274]

Siljander PR, Hamaia S, Peachey AR, Slatter DA, Smethurst PA, Ouwehand WH, Knight CG, Farndale RW (2004) Integrin activation state determines selectivity for novel recognition sites in fibrillar collagens. J Biol Chem 279:47763–47772

Sipilä KH, Drushinin K, Rappu P, Jokinen J, Salminen TA, Salo AM, Kääpylä J, Myllýrharju J, Heino J (2018) Proline hydroxylation in collagen supports integrin binding by two distinct mechanisms. J Biol Chem 293:7645–7658

Sipilä KH, Haag S, Denessiouk K, Kääpylä J, Peters EC, Denesuyk A, Hansen U, Konttinen Y, Johnson MS, Holmdahl R, Heino J (2014) Citrullination of collagen II affects integrin-mediated cell adhesion in a receptor-specific manner. FASEB J 28:3758–3768

Smeland HY, Askeland C, Wik E, Knutsvik G, Molven A, Edelman RJ, Reed RR, Warren DJ, Gullberg D, Stuhr L, Akslen LA (2019) Integrin alpha1beta1 is expressed in breast cancer stroma and associates with aggressive tumor phenotypes. J Pathol Clin Res

Snellman A, Tu H, Väisäinen T, Kivist P, Huhtala P, Pihlajaniemi T (2000) A short sequence in the N-terminal region is required for the trimerization of type XIII collagen and is conserved in other collagenous transmembrane proteins. EMBO J 19:5051–5059

Sund M, Väisänen T, Kaukinen S, Ilves M, Tu H, Autio-Harmanen H, Rauvala H, Pihlajaniemi T (2001) Distinct expression of type XIII collagen in neuronal structures and other tissues during mouse development. Matrix Biol 20:215–231

Tahkola J, Räisänen J, Sund M, Mäkikallio K, Autio-Harmanen H, Pihlajaniemi T (2008) Cardiac dysfunction in transgenic mouse fetuses overexpressing shortened type XII collagen. Cell Tissue Res 333:61–9

Takada Y, Hemler ME (1989) The primary structure of the VLA-2/alpha 2 integrin receptor alpha 2 subunit (platelet GPIa): homology to other integrins and the presence of a possible collagen-binding domain. J Cell Biol 109:397–407

Tiger CF, Fougouresse F, Grundstrom G, Velling T, Gullberg D (2001) Alpha1beta1 integrin is a receptor for interstitial collagens involved in cell migration and collagen reorganization on mesenchymal nonmuscle cells. Dev Biol 237:116–129

Tu H, Sasaki T, Snellman A, Gohring W, Pirlil P, Timpl R, Pihlajaniemi T (2002) The type XIII collagen ectodomain is a 150-nm rod and capable of binding to fibronectin, nidogen-2, perlecain, and heparin. J Cell Biol 277:23092–23099

Tulla M, Pentikäinen OT, Vittasalo T, Kääpylä J, Impola U, Nykvist P, Nissinen L, Johnson MS, Heino J (2001) Selective binding of collagen subtypes by integrin alpha 11, alpha 21, and alpha 10f domains. J Biol Chem 276:48206–48212

Tuomisto A, Sund M, Tahkola J, Latvanlehto A, Savolainen ER, Autio-Harmanen H, Liakka A, Sormunen R, Vuoristo J, West A, Lahesmaa R, Morse HC, Pihlajaniemi T (2008) A mutant collagen XIII alters intestinal expression of immune response genes and predisposes transgenic mice to develop B-cell lymphomas. Cancer Res 68:10324–10332

UniProt Consortium (2019) UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res 47:D506–D515

Väisänen T, Väisänen MR, Autio-Harmanen H, Pihlajaniemi T (2005) Type XIII collagen expression is induced during malignant transformation in various epithelial and mesenchymal tumours. J Pathol 207:324–335

Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJ (2005) GROMACS: fast, flexible, and free. J Comput Chem 26:1725–1731

Velling T, Kusche-Gullberg M, Sejersen T, Gullberg D (1999) cDNA cloning and chromosomal localization of human alpha11 integrin. A collagen-binding, I domain-containing, beta(1)-associated integrin alpha chain present in muscle tissues. J Biol Chem 274:25735–25742

Xu Y, Gurusiddappa S, Rich RL, Owens RT, Keene DR, Mayne R, Hook A, Hook M (2000) Multiple binding sites in collagen type...
I for the integrins alpha1beta1 and alpha2beta1. J Biol Chem 275:38981–38989
Xue LC, Rodrigues JP, Kastritis PL, Bonvin AM, Vangone A (2016) PRODIGY: a web server for predicting the binding affinity of protein-protein complexes. Bioinformatics 32:3676–3678
Ylönen R, Kyrölahti T, Sund M, Ilves M, Lehenkari P, Tuukkanen J, Pihlajaniemi T (2005) Type XIII collagen strongly affects bone formation in transgenic mice. J Bone Miner Res 20:1381–1393
Zainul Z, Heikkinen A, Koivisto H, Rautalahti I, Kallio M, Lin S, Härönen H, Norman O, Ruegg MA, Tanila H, Pihlajaniemi T (2018) Collagen XIII is required for neuromuscular synapse regeneration and functional recovery after peripheral nerve injury. J Neurosci 38:4243–4258
Zeltz C, Gullberg D (2016) The integrin-collagen connection - a glue for tissue repair? J Cell Sci 129:1284
Zhang H, Fredericks T, Xiong G, Qi Y, Rychahou PG, Li JD, Pihlajaniemi T, Xu W, Xu R (2018) Membrane associated collagen XIII promotes cancer metastasis and enhances anoikis resistance. Breast Cancer Res 20:116-y
Zhang WM, Käpylä J, Puranen JS, Knight CG, Tiger CF, Pentikäinen OT, Johnson MS, Farndale RW, Heino J, Gullberg D (2003) alpha 1beta 1 integrin recognizes the GFGER sequence in interstitial collagens. J Biol Chem 278:7270–7277
Zhu CQ, Popova SN, Brown ER, Barsyte-Lovejoy D, Navab R, Shih W, Li M, Lu M, Jurisica I, Penn LZ, Gullberg D, Tsao MS (2007) Integrin alpha 11 regulates IGF2 expression in fibroblasts to enhance tumorigenicity of human non-small-cell lung cancer cells. Proc Natl Acad Sci U S A 104:11754–11759
Zwolanek D, Veit G, Eble JA, Gullberg D, Ruggiero F, Heino J, Meier M, Stetefeld J, Koch M (2014) Collagen XXII binds to collagen-binding integrins via the novel motifs GLQGER and GFKGER. Biochem J 459:217–227