SUPPLEMENTARY INFORMATION

Determinants of laminin polymerization revealed by the structure of the α5 chain amino-terminal region

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Supplementary Fig S1. Comparison of α5LN to galactose binding domains. (**A**) Superposition of laminin α5LN (pale blue) and the GBD of *Clostridium perfringens* NanJ (PDB entry 2v72, magenta). The r.m.s. deviation of 130 matched Cα atoms is 2.2 Å. A calcium ion in NanJ is shown as a sphere. The chain break in the α5LN structure is labelled. (**B**) Structure of the calcium binding site in NanJ. Calcium ligands are shown in atomic detail and calcium-ligand bonds are indicated by black lines. (**C**) Region of α5LN corresponding to the NanJ calcium site. Selected residues are shown in atomic detail and hydrogen bonds are indicated by dashed lines. (**D**) Intrinsic fluorescence spectra of α5LN-LEa1-2 in the presence and absence of calcium. The solutions contained 4 μM protein in 20 mM Na-HEPES pH 7.5, 150 mM NaCl, and either 2 mM $CaCl_2$ (black line) or 5 mM EDTA (red line). The spectra were recorded using a Jobin Yvon Horiba FluoroMax-3 fluorimeter at an excitation wavelength of 290 nm.

Supplementary Fig S2. Laminin α5LN-LEa1 interface. (**A**) Details of the α5LN-LEa1 interface in a view direction similar to **Fig 1B**. The LN, LEa1 and LEa2 domains are in pale blue, green and pink, respectively. Disulphide bridges and *N*-linked glycans are in yellow and magenta, respectively. Selected residues are shown in atomic detail. The side chain of Pro52 in the N-terminal segment is in orange. Three phosphate ions are shown with phosphorus atoms in green. Selected hydrogen bonds are indicated by dashed lines. (**B**) Another view of the α5LN-LEa1 interface rotated by ~180° relative to the view in **A** showing the interactions made by the N-terminal segment. The LN domain is shown as a pale blue surface, and the LEa1 domain as a semi-transparent pale green surface. The N-terminal segment is shown in atomic detail, with Pro52 highlighted in orange.

Supplementary Fig S3. Sequence alignment of selected laminin α chain LN domains: mouse and zebrafish α1 and α5; mouse α2 and α3B; *Drosophila melanogaster* α1/2 (wing blister) and α3/5 (LamA); *Caenorhabditis elegans* α1/2 (lam-3) and α3/5 (epi-1). Identical residues are shaded yellow. Asparagine residues that are predicted to be modified by *N*-linked glycosylation are in magenta. The sequence numbering of the mouse laminin α5 chain is shown above the alignment.

Supplementary Table S1. Summary of laminin short arm constructs.

Construct	UniProt reference	Residues[a]	Expression[b]	Monodisperse protein[c]
α1LN-LEa1-2	P19137	25-403	Yes	Yes
α1LN-LEa1-4	P19137	25-509	Yes	Yes
α2LN-LEa1-2	Q60675	29-409	Yes	Yes
α2LN-LEa1-4	Q60675	29-513	Yes	Yes
α5LN-LEa1-2	Q61001	44-433	Yes	Yes
α5LN-LEa1-4	Q61001	44-546	Yes	Yes
β1LN-LEa1-2	P02469	22-397	No	
β1LN-LEa1-4	P02469	22-509	Yes	No
γ1LN-LEa1-2	P02468	34-395	Yes	Yes
γ1LN-LEa1-4	P02468	34-492	Yes	Yes

[a]All mature proteins contain a vector-derived APLA sequence at the N-terminus and a AAAHHHHHH tag at the C-terminus.

[b]The criterion used is a yield of > 0.5 mg from 1 litre of conditioned cell culture medium.

[c]The criterion used is > 90% of protein eluting as a single symmetric peak from a S200 size exclusion chromatography column run in 20 mM Na-HEPES pH 7.5, 150 mM NaCl, 2 mM $CaCl_2$.

Supplementary Figure S1
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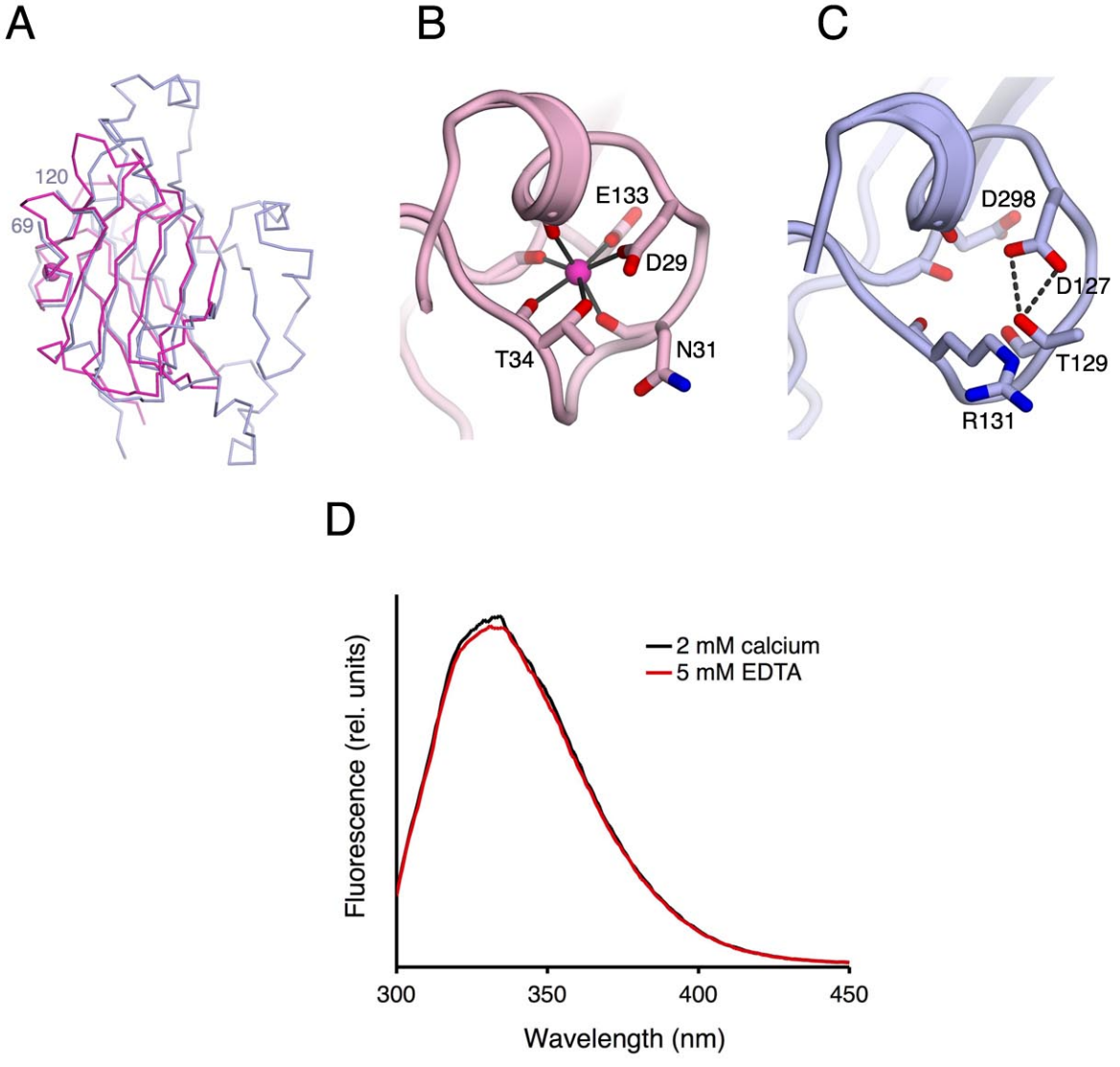

Supplementary Figure S2
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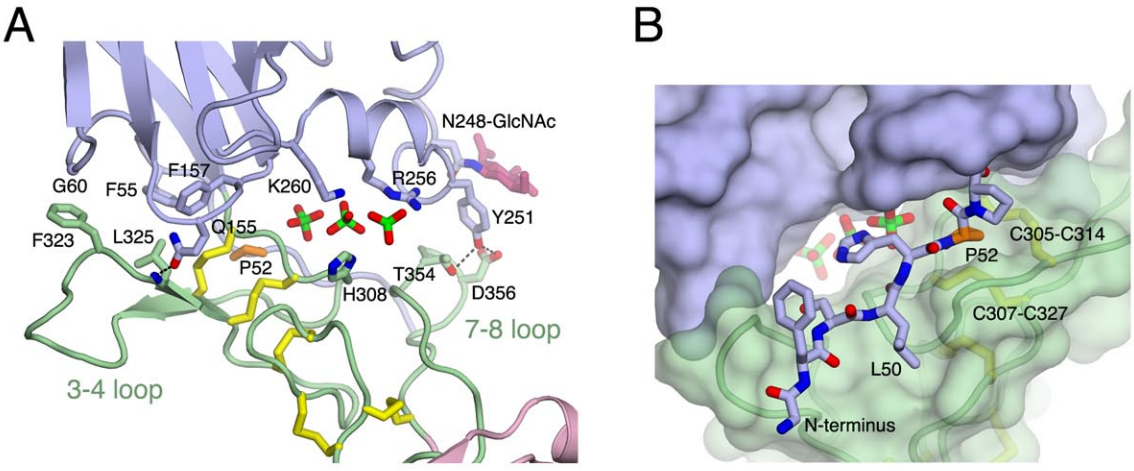

Supplementary Figure S3
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