

SUPPLEMENTARY INFORMATION

Determinants of laminin polymerization revealed by the structure of the $\alpha 5$ chain amino-terminal region

Sadaf-Ahmahni Hussain¹, Federico Carafoli¹ & Erhard Hohenester¹

¹Department of Life Sciences, Imperial College London, London SW7 2AZ, UK

Supplementary Fig S1. Comparison of α 5LN to galactose binding domains. (A)

Superposition of laminin α 5LN (pale blue) and the GBD of *Clostridium perfringens* NanJ (PDB entry 2v72, magenta). The r.m.s. deviation of 130 matched C α atoms is 2.2 Å. A calcium ion in NanJ is shown as a sphere. The chain break in the α 5LN 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 α 5LN 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 α 5LN-LEa1-2 in the presence and absence of calcium. The solutions contained 4 μ M protein in 20 mM Na-HEPES pH 7.5, 150 mM NaCl, and either 2 mM CaCl₂ (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 α 5LN-LEa1 interface. (A) Details of the α 5LN-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 α 5LN-LEa1 interface rotated by $\sim 180^\circ$ 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 $\alpha 1$ and $\alpha 5$; mouse $\alpha 2$ and $\alpha 3B$; *Drosophila melanogaster* $\alpha 1/2$ (wing blister) and $\alpha 3/5$ (LamA); *Caenorhabditis elegans* $\alpha 1/2$ (lam-3) and $\alpha 3/5$ (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 $\alpha 5$ 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
α 1LN-LEa1-2	P19137	25-403	Yes	Yes
α 1LN-LEa1-4	P19137	25-509	Yes	Yes
α 2LN-LEa1-2	Q60675	29-409	Yes	Yes
α 2LN-LEa1-4	Q60675	29-513	Yes	Yes
α 5LN-LEa1-2	Q61001	44-433	Yes	Yes
α 5LN-LEa1-4	Q61001	44-546	Yes	Yes
β 1LN-LEa1-2	P02469	22-397	No	
β 1LN-LEa1-4	P02469	22-509	Yes	No
γ 1LN-LEa1-2	P02468	34-395	Yes	Yes
γ 1LN-LEa1-4	P02468	34-492	Yes	Yes

^aAll mature proteins contain a vector-derived APLA sequence at the N-terminus and a AAAHHHHHH tag at the C-terminus.

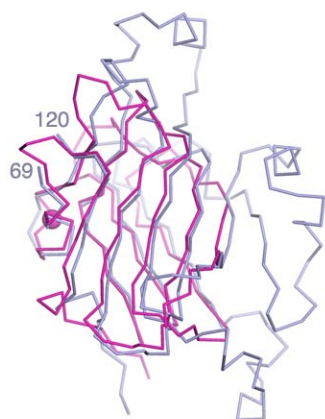
^bThe criterion used is a yield of > 0.5 mg from 1 litre of conditioned cell culture medium.

^cThe 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 CaCl₂.

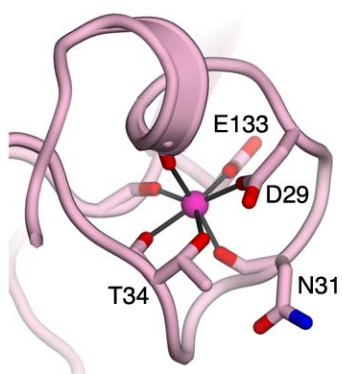
Supplementary Figure S1

Hussain et al.

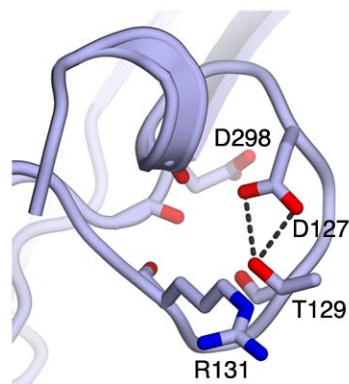
A



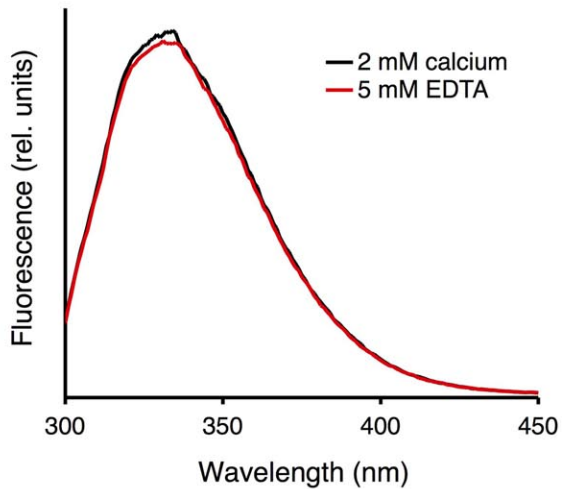
B



C

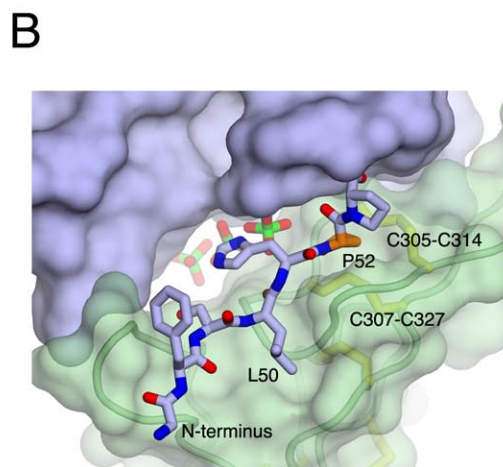
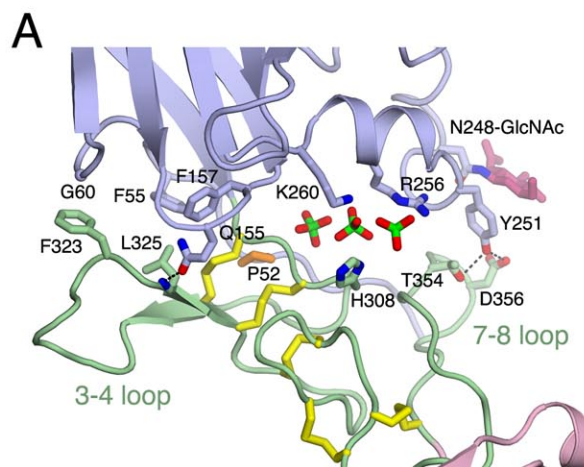


D



Supplementary Figure S2

Hussain et al.



Supplementary Figure S3

Hussain et al.

```

50      60      70      80      90      100      110
LAMA5_MOUSE  FSLHPPYFNLAEGARITASATCGEEAPTRSVSRPPTEDLYCKLVGGPVAGGDPQTQCG----OYCDIC
LAMA3B_MOUSE RSLHPPYFNLAQAARIWATATCGERDPE--VSRPRPELFCKLVGGPAAQGS--GHTTQG----QFCDYC
LAMA5_DANRE  FSLHPPYFNLAEGTKITATATCGVDENE---QPIQDLYCKLVGGPVSG--DPSQTQG----OYCDIC
EPI1_CAEEEL  QVLTPTSQITTSRRKPIATATSCGEIQGQ----PVTEIYGLTGSTQYTPLNSYSYQDDEI17JGGHC
LAMA_DROME   AELTPPYFNLATGRKIIYATATCGQDQTDG-----PELYGKLVGANTEHDHIDYSVIQG----QVCDYC
LAMA1_MOUSE  RGLFPAILNLATNAHISANATCGEKGPE-----MFCKLVEHVPGRPVPR--HAQ-----CRVC
LAMA2_MOUSE  RGLFPFAVLNLSANALITTNATCGEKGPE-----MYCKLVEHVPGQPVR--NPO-----CRIC
LAMA1_DANRE  RGLFPAILNLSANAEISTTNATCGDPPDE-----MFCKLVEHVPGRRIR--NPO-----CRIC
LAMA3_CAEEEL RGLFPNIFNLATNSLITATDTCGQYTAE-----EYCKLVEHVLLRKTNTQSPQ-----CDIC
WB_DROME     GGLYPLPLFNVPVRAQISVNAATCGQNGAE-----EYCKQVQAKP-----CGIC

120      130      140      150      160      170
LAMA5_MOUSE  TAANS--NKAHPVSNADGTERWQWQSPPLSRGLSEYNEVVTLDLQGVFHVAYVLIKAFNSPRPDLWVLE
LAMA3B_MOUSE NSEDS--RKAHPASHAIDGSERWQWQSPPLSSGTQYNOVVTLDLQGLFHVAYVLIKAFNSPRPDLWVLE
LAMA5_DANRE  SSQDT--NRAHPIISNAIDGTERWQWQSPPLSRSAKHNOVVTLDLQGLFHVAYVLIKAFNSPRPDLWVLE
EPI1_CAEEEL  NAGN---ENSHFPAANMVDGNSWWMSPPLSRGLQHNEVVTITDLEQEFHVAYVVIQMANSPRPGSWVLE
LAMA_DROME   DPTVP--ERNHFPENAIIDGTEAWQWQSPPLSRGKHFNEVVTITNFEQEFHVAYLFIKMGNSPRPGLWVLE
LAMA1_MOUSE  DCHSTNPRRERHPIISHAIDCTNNWQWQSPSIQNGREYHVVTVTLDLQGVFOVAYIILKANAAPRGNWVLE
LAMA2_MOUSE  NQSSNPNYQRHPIITNAIDCKNTWQWQSPSIKNGRQPHWVTVTLDLQGVFOVAYIILKANAAPRGNWVLE
LAMA1_DANRE  DANSONPKQHPITNAIDCTNLWQWQSPSIKNGRQPHWVTVTLDLQGVFOVAYIILKANAAPRGNWVLE
LAMA3_CAEEEL DANN--VHKRHPITNAIDCTRRWQWQSPSLANGLRFEKVTITIDLRQYQVAYIILKMGNSPRPGLWVLE
WB_DROME     NAHS[14]SGSGSGSGSGFEEGWQWQSPTLQGGRQFEYVTILLDLKQTFQIFSVWLKSANSPRPAWVLE

180      190      200      210      220      230      240
LAMA5_MOUSE  RSTDFGHTYQPWFQFASSKRDCLERFGPRTLERIT--QDDVICTTEYSRIVPLENGEIVVSLVNGRPF
LAMA3B_MOUSE RSVDFGSTYSPWFQFAHSRRDCVEQFGQENMAIT--QDDQMLCVTEYSRIVPLENGEIVVSLVNGRPF
LAMA5_DANRE  RSIDFGKTYQPWFQFASSKRDCIERFGQRTIERIY--HDDVICTTEYSRIVPLENGEIVVSLVNGRPF
EPI1_CAEEEL  RSTDHGKTYQPWFNFAENAAECMRRFGMESLSPIS--EDDSVICTRTDMSASLPLENAEMVIRILEHRPF
LAMA_DROME   KSTDYGKWTWPWFQHSFSDTPADCETYFGKDTYKPIIT--RDDVICTTEYSKIVPLENGEIVVSLVNGRPF
LAMA1_MOUSE  RSLDD--VEYKWPQYHAVTDTECLTRYNIYPRTGPPSYAKDDEVICTSFYSKIHPLENGEIHHSLINGRPF
LAMA2_MOUSE  RSLDG--VNFQWPQFYAISDTECLTRYITPRIGPPTYKRDEVICTSYYSRLVPLENGEIHHSLINGRPF
LAMA1_DANRE  RSLDG--EYEPWPQYAMQDAECMRQFGIPATTGVPRFQKEDEVHCTSEYSKITPLENGEIHHSLINGRPF
LAMA3_CAEEEL RSLDG--INFEPWPQYGLSDADCQRWNLGQNGKVFVQDTEIICSTQFSKPGLENGVLSLKNRPF
WB_DROME     KSLDG--INFEPWPQYGLSDADCQRWNLGQNGKVFVQDTEIICSTQFSKPGLENGVLSLKNRPF

250      260      270      280      290      300
LAMA5_MOUSE  GALFYSYSPILRDFTKATNIRLRLRFLRNTLLGHLMGKALR----DPTVTRRYYSIKDISIGGR
LAMA3B_MOUSE GAKKFAFSDTLREFTKATNIRLRLRFLRNTLLGHLISKAER----DPTVTRRYYSIKDISVGGGR
LAMA5_DANRE  GAMFYSYSPVLRFTKATNIRLRLRFLRNTLLGHLMGKTLR----DPTVTRRYYSIKDISIGGR
EPI1_CAEEEL  SSRQFATSEALNFRATNVRRLRLRTRTLQGHLMMDNEWR----DPTVTRRYYSIAKIMIGGR
LAMA_DROME   SSTNYFISTVLOEWTRATNVRRLRLRTRTLQGHLMMSVARQ----DPTVTRRYYSIKDISIGGR
LAMA1_MOUSE  SAD--DPSPELLEFTSARYIRLRLRQIRTLNADLMTLSHRDLRDLDPVTRRYYSIKDISVGGM
LAMA2_MOUSE  SAD--DPSPELLEFTSARYIRLRLRQIRTLNADLMTLFAHRKDPREIDPVTTRRYYSVVKDISVGGM
LAMA1_DANRE  SAD--DLTPELLEFTSARYIRLRLRQIRTLNADLMTLSYRDKPVDVPTTRRYYSIKDISVGGM
LAMA3_CAEEEL GAE--NLSLELQKFTRARFVRLRLISPRTLNADLMIINKKS--DSLKDSVTMRYFYSISDISIGGQ
WB_DROME     GAT--DQSPLEMKFITTRYIRIRLQGMHSTANQDNSLDWLLD--SPSLKHSFYSLSQVLSVAR

```