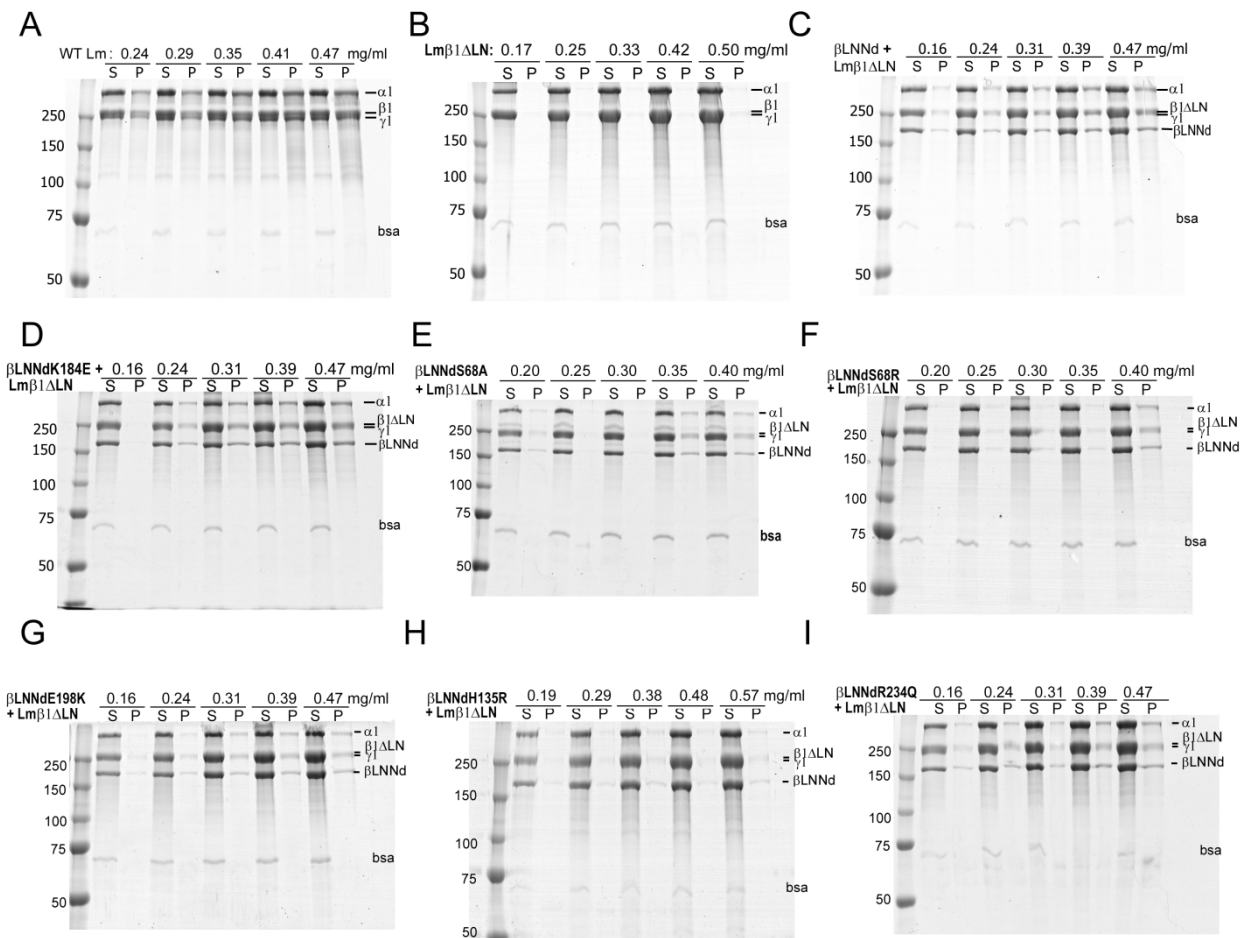
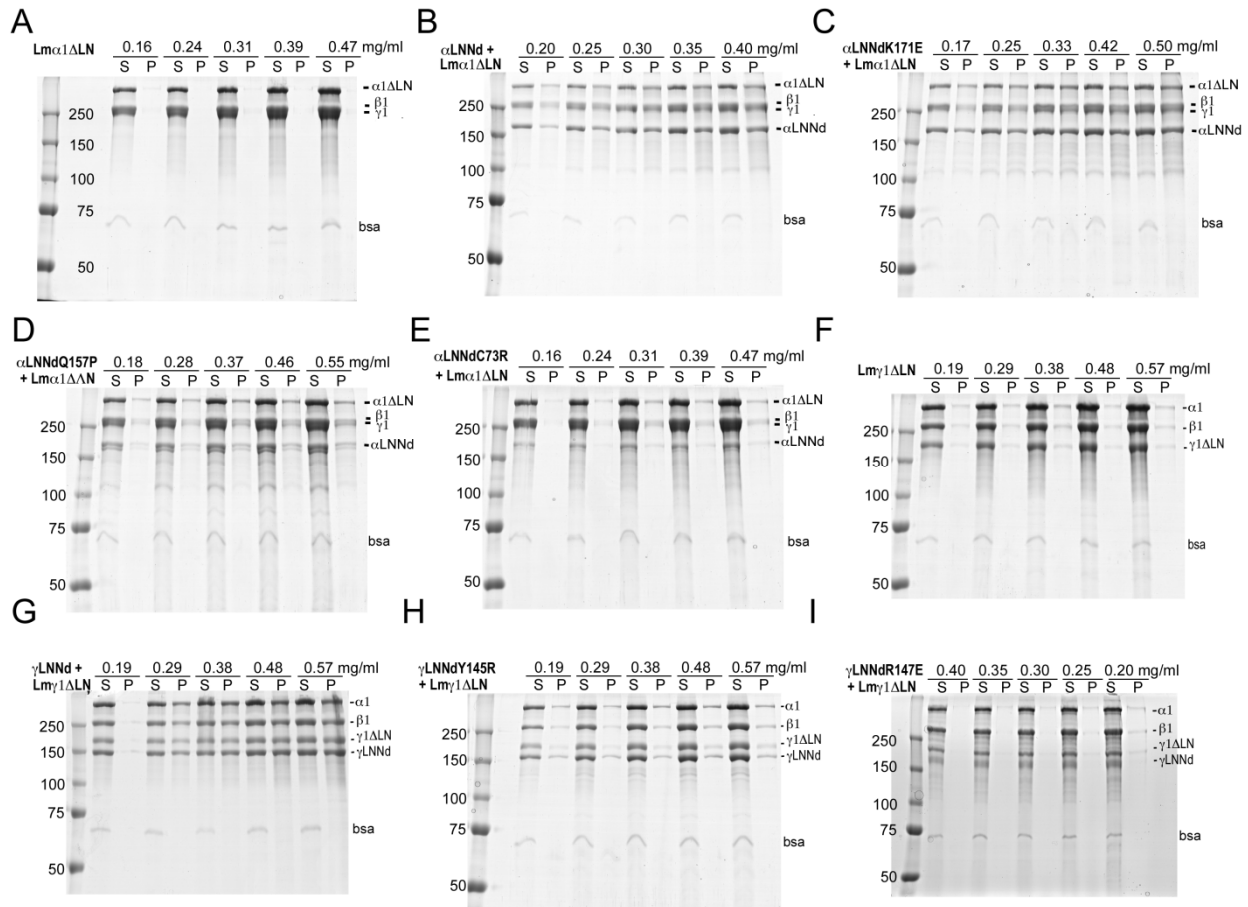


Supplemental Table 1. DNA constructs for xLNND proteins

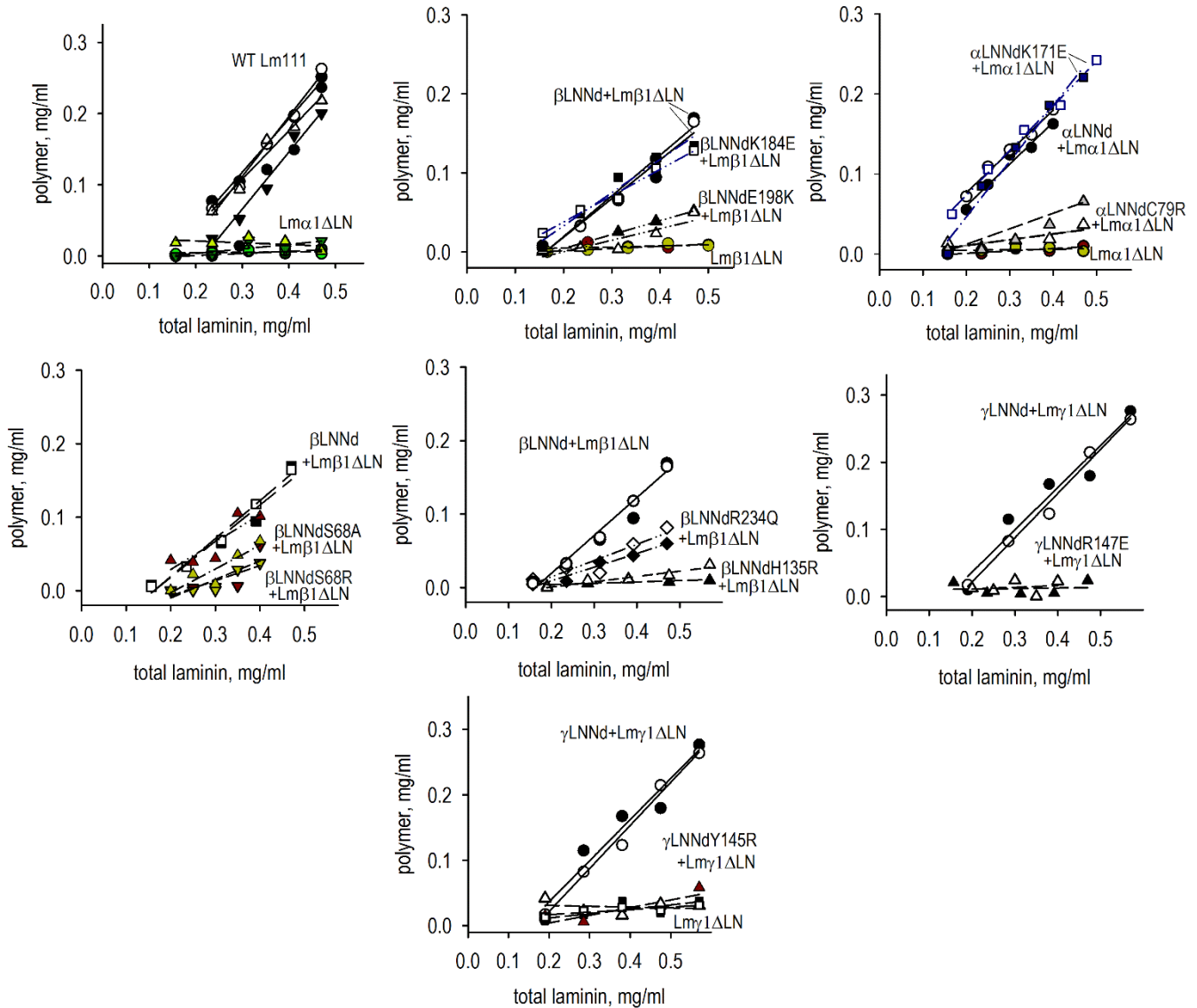
<i>clone</i>	<i>enzymes</i>	<i>5' 3' 1st pcr, 2nd pcr sizes</i>	<i>cut sites</i>	<i>vector size</i>	<i>1st forward primer</i>	<i>2nd reverse primer</i>	<i>2nd forward primer</i>	<i>1st reverse primer</i>
αLNND clones								
Q157P	HindIII-XagI	680bp, 1157bp, 1816bp	1510bp	7648bp	GTAGCGGTGACGGT GGGAG	GACGGCATAGTACGG CCAGGG	CC2TGGCCGTACTATGC CGTC	CATGCAGAGTGCAGA GACTATGC
C73R	HindIII-Eco91I	430bp, 1780bp, 2180bp	2064bp	7094bp	GTAGCGGTGACGGT GGGAG	CGTACACAGACCCGGCG TTGGCGGTGTCGA	TCGACACGCCCAACGC CGGGTCTGTGACG	TGGGTGCCCCAGGAA GGTCACCTCAGC
K171E	HindIII-XagI	710bp, 1109bp, 1816bp	1510bp	7648bp	GTAGCGGTGACGGT GGGAG	GTGGAGTTATTTTCGTA GCGGGTTC	GACCCGCTACGAAATA ACTCCAC	CATGCAGAGTGCAGA GACTATGC
βLNND clones								
WT	HindIII-BsrGI	1774bp, 965bp, 2738bp	2143bp	6897bp	GGCGTGGATAGCGGT TTGAC	CACAAGTCTGCTGTCTCG ACATCCATC	GATGGATGTCGACAGC AGACTTGTG	CGAGGAGACGGGCA GACTTTC
S68R	HindIII-EcoNI	cut from 111580R	826bp	8263bp				
S68A	HindIII-EcoNI	512bp, 803bp, 1296bp	826bp	8263bp	GGCGTGGATAGCGGT TTGAC	CTGCAAGTGGCGGAC GATACAG	CTGTATCGTGCCTTACT TGCAG	CCGAAACAGCAACCGC CTGTA
R234Q	HindIII-EcoNI	cut from 111R246Q	826bp	8263bp				
H135R	HindIII-EcoNI	713bp, 604bp, 1296bp	826bp	8263bp	GGCGTGGATAGCGGT TTGAC	GTCATTACGAGATGAG TAAATGG	CCATTTACTCATCTCGT AATGAC	CCGAAACAGCAACCGC CTGTA
E198K	HindIII-EcoNI	893bp, 420bp, 1296bp	826bp	8263bp	GGCGTGGATAGCGGT TTGAC	GTTGAGGGTTCAATCT TAGAATATC	GATATTTAAGATTGAA CCCTCAAC	CCGAAACAGCAACCGC CTGTA
K184E	HindIII-EcoNI	859bp, 458bp, 1296bp	826bp	8263bp	GGCGTGGATAGCGGT TTGAC	GTCAATCGACTTCTTTCA TGGGGCC	GGCCCATGAAAAGAAG TCGATGAC	CCGAAACAGCAACCGC CTGTA
γLNND clones								
WT	NheI-BsrGI	2642bp	2188bp	2188bp	GGCGTGGATAGCGGT TTGACT	GGCACAAAGTCTGCTGT GGCCTGCATCCTGC	GCAAGGATGCAGGCCAC AGCAGACTTGTGCC	CGAGGAGACGGGCA GACTTTC
Y145R	NheI-BsrGI	795bp, 1866bp, 2642bp	2188bp	2188bp	GGCGTGGATAGCGGT TTGACT	GGAACCTTGAGACGCA CACGGGTGATG	CATCACCCGTGTGCGTC TCAAAGTTCC	CGAGGAGACGGGCA GACTTTC
R147E	NheI-BsrGI	801bp, 1860bp, 2642bp	2188bp	2188bp	GGCGTGGATAGCGGT TTGACT	GAACTTGAGCTCCACA TAGGTGATGTC	GACATCACCTATGTGG AGCTCAAAGTTTC	CGAGGAGACGGGCA GACTTTC
Laminin								
hLm111β	HindIII-	1012bp, 1875bp,	1957bp	8446bp	GGCGTGGATAGCGGT	CACAAACTTGATTTTC	CAAACCTTGCAAAATCAAG	GCAGTGTGATCGGTG
1R234Q	ClaI	2865bp			TTGAC	AAGTTGG	TTTGTG	CTTAC



Supplemental Fig. 1. Polymerization of β LNNd--Lm β 1 Δ LN complexes. WT Lm111, Lm β 1 Δ LN, and WT and modified β LNNd proteins bound to and co-purified with Lm β 1 Δ LN were incubated at 37°C for 3 h in 50 μ l aliquots at different concentrations that were then centrifuged to sediment the polymer (P) fraction and separate it from the non-polymerized supernatant (S) fraction. These were then analyzed under reducing conditions by SDS-PAGE and stained with Coomassie blue. WT Lm111, β LNNd complex, and β LNNdK184E (chosen as likely “control” with non-conserved residue) polymerized whereas complexes with β LNNd containing conserved residue mutations showed little or no polymerization. Plots shown in Fig. 3.








Supplemental Fig 2. Polymerization of α LNNd--Lm α 1 Δ LN and γ LNNd-Lm γ 1 Δ LN complexes. Panels **A-E**. Lm α 1 Δ LN, and WT and modified α LNNd proteins bound to and co-purified with Lm α 1 Δ LN were incubated at 37°C for 3 h in 50 μ l aliquots at different concentrations that were then centrifuged to sediment the polymer (P) fraction and separate it from the non-polymerized supernatant (S) fraction. These were then analyzed under reducing conditions by SDS-PAGE and stained with Coomassie blue. WT Lm111, α LNNd complex, and α LNNdK171E (containing a poorly conserved residue with a charge change) polymerized whereas complexes with α LNNd containing conserved residue mutations showed low levels of polymerization. Panels **F-I**. Lm γ 1 Δ LN and WT and modified γ LNNd proteins co-purified with Lm γ 1 Δ LN were treated and analyzed as above. The two mutations substantially reduced polymerization. Plots shown in Fig. 3.



Supplemental Fig. 3. Comparisons of polymerization of laminins and laminin/linker protein preparations. Different preparations of laminins and linker protein bound to laminins were incubated at 37°C, centrifuged to separate supernatant (S) from polymer (P), and analyzed by SDS-PAGE as described. Scans of the Coomassie blue stained gels were quantitated by densitometry and plotted as total concentration against polymer concentration. Comparison of pairs (and 4 WT laminin preparations) evaluated reveal limited experimental variability.

Laminin beta LN domain:

mus Net4	GANSRCEKACNPRMGNLALGR--KLRADTMCGQNATELFCFYSE-NADLTCRQPKCD-----KCNAH-HSH	86
Dro Lmβ1	PC---ERSSCYPATGNLLIGRENRLTASSTCGLHSPERFCILSHLQ-DKKCF--LCDTREETKHDPYK---	109
hum Lmβ2	GC---SRGSCYPATGDLVGRADRLTASSTCGLNGPQPYCIVSHLQDEKKCF--LCDSR-----RPF-SAR	100
mus Lmβ2	GC---SRGSCYPATGDLVGRADRLTASSTCGLHSPQPYCIVSHLQDEKKCF--LCDSR-----RPF-SAR	103
mus Lmβ1	GC---AEGSCYPATGDLVGRADRLTASSTCGLHSPQPYCIVSHLQDEKKCF--ICDSR-----DPY-HET	88
		
	R	
	A	
	68	
mus Net4	LAHPPSAMADSSFRFP-----RTWWQSAEDVHREKIQLDLEAEFYFTHLIMVFKSPRPAAM	142
Dro Lmβ1	----NHRIGQIIYKTKPGTNIPTWWQSENGKENATIQLDLEAEFHFTHLIIITFTFRPAAM	166
hum Lmβ2	DNPNSHRIQNVVTSFAPQ-RRAAWQSENGI PAVTIQLDLEAEFHFTHLIMTFKTFRPAAM	160
mus Lmβ2	DNPNSHRIQNVVTSFAPQ-RRTAWWQSENGVPMVTIQLDLEAEFHFTHLIMTFKTFRPAAM	163
mus Lmβ1	LNPDSHLIENVVTTFAPN-RLKIWWQSENGVENVTIQLDLEAEFHFTHLIMTFKTFRPAAM	148
	R	
	135	
		
mus Net4	VLDRSQDFGKTWKPKYKGFATNCSATF-GLEDDVVKK--GAICTSRYSNPFPTGGGEVIFR	199
Dro Lmβ1	YIERSDFGQTDWHIYRYFAYDCKESFPGVPT-VLENITDVMCTSRYSNVEPSRNGEVIFR	225
hum Lmβ2	LVERSADFGRTWHVYRYFSDYDCGADFPVPLAPPRHWDVVCESRYSEIEPSTEGEVYIR	220
mus Lmβ2	LVERSADFGRTWHVYRYFSDYDCGADFPVPLAPPRHWDVVCESRYSEIEPSTEGEVYIR	223
mus Lmβ1	LIERSDFGKAWGVYRYFAYDCESSFPGISTGPMKVVDDIICDSRYSDIEPSTEGEVIFR	208
	E	
	184	
		
	K	
	198	
		
mus Net4	ALSPPYDIENPYSAKVQEQLKITNLRVRLLLKRQSCPCQINDLNKPHHFMHYAVYDFIVK	258
Dro Lmβ1	VLPPNINVTDPYAEHVQNQLKMTNLRIQMTKLHKLGDNLLDSRLENEEKYYYGISNMVVR	285
hum Lmβ2	VLDPAIPIPDYSSRIQNLKLTNLRVNLRLHRTLGDNLLDPRREIREKYYYALYELVVR	280
mus Lmβ2	VLDPAIPIPDYSSRIQNLKLTNLRVNLRLHRTLGDNLLDPRREIREKYYYALYELVIR	283
mus Lmβ1	ALDPAFKIEDPYSRIQNLKLTNLRKIFVKLHRTLGDNLLDSRMEIREKYYYAVYDMVVR	268
	Q	
	234	
		

Supplemental Fig.4. Laminin βLN mutations and sequence homologies. Comparison among mouse (mus), human (hum) and Drosophila (Dro) LN domain sequences including that of the laminin polymer-inhibitor netrin-4. Mutated residues indicated below mouse β1 sequence (mouse sequence number designations used for experimental residue labels). Colored arrows indicate secondary structure near affected residues [36]. Selected human Pierson syndrome –associated and Drosophila heart-associated mutations are indicated in yellow for affected subunit isoform and species. The K184 residue (blue) was chosen for comparison. Patch (red) corresponds in location to the PLENGE polymerization patch described in Lmα5 and located on the β6-β3-β8 “front” face of the LN domain [35, 36].

Laminin alpha LN domain:

Dro Lm α 5 (A)	-QAE L TPPYFN L ATGRKIYATATCGQD T DGP-----ELYCKLVGANTE-HDH--IDYSVIQG	73
mus Lm α 5	----LHPPYFN L AEGARITASATCGEE--AP T RSVSRPT--DLYCKLVGGPVA-GGDPN---QTIQ G	105
hum Lm α 3B	----LHPT Y FN L AEAAARIWATATCGER--GPGE G --RPQP-ELYCKLVGGP T A--GS-G---HTIQ G	99
hum Lm α 2	QQRGLFP A VNLN L ASNALIT T NATCGEK--GP-----EMYCKLV-----EHVPG---QPVRN	80
mus Lm α 2	QQRGLFP A VNLN L ASNALIT T NATCGEK--GP-----EMYCKLV-----EHVPG---QPVRN	76
mus Lmα1	QQRGLFP A ILN L ATNAHISANATCGEK--GP-----EMFCKLV-----EHVPG---RPVRH	70
Dro Lm α 5 (A)	QVC D YCD---PTVPERNHPPENAIDGTEAWWQSPPLSRGMKFNEVNLTINFEQEFHVAYL F FIRMGNSPRPGLWTL	145
mus Lm α 5	QY C DI C T---AANSNK A HPVSN A IDGTERWWQSPPLSRGLE Y NEVNNTLDLGGV F HVAYVLIK F FANSRPPDLWVL	177
hum Lm α 3B	QF C DYCN---SEDPRKAHPVTNAIDGSERWWQSPPLSSGTQY N RVNLTLDLGGV F HVAYILIK F FANSRPPDLWVL	171
hum Lm α 2	PQ C RICN-QNSSNPYQRHPITNAIDGKNTWWQSPSIKNGIEYHYVTITLDLQ V FQIAYVIVKAANSRPPGNWIL	154
mus Lm α 2	PQ C RICN-QNSSNPYQRHPITNAIDGKNTWWQSPSIKNGVEYHYVTITLDLQ V FQIAYVIVKAANSRPPGNWIL	150
mus Lmα1	AQC R VCD-GNSTNPRERHPI S H A IDGTNNWWQSPSIQNGREYHWVTITL D LRQV F QVAYII I KAANAPRPGNWIL	144
	R 73	
Dro Lm α 5 (A)	EKSTDY G KTWTPWQ H FS D TPAD C ET Y F-----GKDTYKPI T RDD D VICTTEYSKIV	196
mus Lm α 5	ERSTDFGHTYQ P WQ F F A SSKRD C L E R F -----GPRTLERITQDD D VICTTEYSRIV	228
hum Lm α 3B	ERSVDFG S TYS P WQ Y F A HSKVD C L K E F G---REANMAV T ---RDD D VLCVTEYSRIV	222
hum Lm α 2	ERSLDDVE-YK P WQ Y HAVT D TECLTLYNIY P R T GP P SYA---KDDEVICTSFY S KI H	207
mus Lm α 2	ERSLDDVE-YK P WQ Y HAVT D TECLTLYNIY P R T GP P SYA---KDDEVICTSFY S KI H	203
mus Lmα1	ERSVDG V K-FK P WQ Y YAVS D TECL T R Y K I T P RRG P P T YR---ADNEVICTSYYSK L V	197
	P 157	E 171
		
Dro Lm α 5 (A)	PLENGEIPV L LLNERPSSTNYFNSTVLQEWTRATNVRIRLLR T KNLLGHLMSVARQ-----DPTVTRRY	260
mus Lm α 5	PLENGEIVVSLVNGRPGALNFSYSP L LRDFTKATNIRL R FLRTNTLLGHLMGKALR-----DPTVTRRY	292
hum Lm α 3B	PLENGEVV V SLINGRPGAKNFTFSHTLREFTKATNIRL R FLRTNTLLGHLISKAQR-----DPTVTRRY	286
hum Lm α 2	PLENGEIHISLINGRPSADDP--SPELLE F TSARYIRL R RFQRI R TNADLMMFAHKDPREIDPIVTRRY	274
mus Lm α 2	PLENGEIHISLINGRPSADDP--SPELLE F TSARYIRL R RFQRI R TNADLMMFAHKDPREIDPIVTRRY	270
mus Lmα1	PLEHGEIHTSLINGRPSADDP--SPQ L LE F TSARYIRL R LRQRI R TNADLMTLSHRDLRDLDPPIVTRRY	264
		

Supplemental Fig. 5. Laminin α LN mutations and sequence homologies. Comparison among mouse (mus), human (hum) and Drosophila (Dro) LN domain sequences. Mutated residues indicated below mouse α 1 sequence. Colored arrows and bar indicate secondary structure near affected residues. Human LAMA2-deficient muscular dystrophy-associated mutations indicated in yellow for affected subunit isoform. Lm α 5 residues within PLENGE found to affect LN trimerization indicated in grey [36]. The affected and flanking residues show considerable sequence identity. The non-conserved E171 residue (blue) was chosen for comparison. Patch (red) corresponds in location to the PLENGE polymerization patch described in Lm α 5 and located on the “front” face of the LN domain [36]. Italics for Lm α 5 indicate disordered residues in the α 5LN-LEa1-2 structural analysis [36].

Laminin gamma LN domain:

Dro Lmy1		ECYDPYGRPQKCLPEFINAAYQ	77
mus Lmy3		RVLSLLATVASMA-LVIQETHFAAGADMGSCYDGVGRAQRCLPEFENAAFG	54
hum Lmy1		MRGSHRAAPALRPRGRLWPLAVLAAAAAAGC-----AQAAMDECTDEGGRPQRCMPEFVNAAFN	60
mus Lmy1		MTGGGRAALALQPRGRLWPLAVLAAVA--GCVR-----AAMDECADEGGRPQRCMPEFVNAAFN	58
Dro Lmy1		LQIESTNTCGEQNDNHFCIQT-MNQNHKNCEFC----KYNDHNPSFLTDLHDPQSPTWWQS	133
mus Lmy3		RRAEASHTCGRPPE-DFCPHVGAAGLQCQRCDADPGRRHDAASYLTDHFSPDDSTWWQS	114
hum Lmy1		VTVVATNTCGTPPE-EYCVQGTGVTGVTKSCHLCDAGQPHLQHGA AFLTDYNNQADTTWWQS	120
mus Lmy1		VTVVATNTCGTPPE-EYCVQGTGVTGVTKSCHLCDAGQQHLQHGA AFLTDYNNQADTTWWQS	118
Dro Lmy1		ETMFEGIQHPNYVNLTLHLGKSYDITY YV RILFRSPRPESFTIYKRTSESGPWIPYQFYSA	193
mus Lmy3		PSMAFGVQYPTSVNLTLSLGKAYEITY YV RLKFHTSRPESFAIYKRTYASGPWEPYQYYS	174
hum Lmy1		QTMLAGVQYPSINLTLHLGKAFDITY YV RLKFHTSRPESFAIYKRTREDGPWIPYQYYS	180
mus Lmy1		QTMLAGVQYPSINLTLHLGKAFDITY YV RLKFHTSRPESFAIYKRTREDGPWIPYQYYS	178
		R E	
		145 147	
			
Dro Lmy1		TCRDYSLPDSRAIRKGEGEHAHALCTSEYSDIS PLRDGE IAFSTLEGRPSGINFERSGEL	253
mus Lmy3		SCQKTYGRPEGHYLRPGEDERVAFTSEFSDIS PLNGGN VAFSTLEGRPSAYNFEESPVL	234
hum Lmy1		SCENTYSKANRGFIRTTGGDEQQALCTDEFSDIS PLTGGN VAFSTLEGRPSAYNFNPNPVL	240
mus Lmy1		SCENTYSKANRGFIRTTGGDEQQALCTDEFSDIS PLTGGN VAFSTLEGRPSAYNFNPNPVL	238
Dro Lmy1		QEWVTATDIRITLDRLNTFGDELFGDSQVLKSYFYAI	290
mus Lmy3		QEWVTSTDILISLDRLNTFGDDIFK DPRVLQSYYYAV	271
hum Lmy1		QEWVTATDIRVTLNRLNTFGDEVFNPKVLKSYYYAI	277
mus Lmy1		QEWVTATDIRVTLNRLNTFGDEVFNPKVLKSYYYAI	275

Supplemental Fig. 6. Laminin γ LN mutations and sequence homologies. Comparison among mouse (mus), human (hum) and Drosophila (Dro) LN domain sequences. Mutated residues indicated below mouse γ 1 sequence (mouse sequence number designations used in experimental labels). Patch (red) corresponds in location to the PLENGE polymerization patch described in Lm α 5 and located on the “front” face of the LN domain [35, 36].