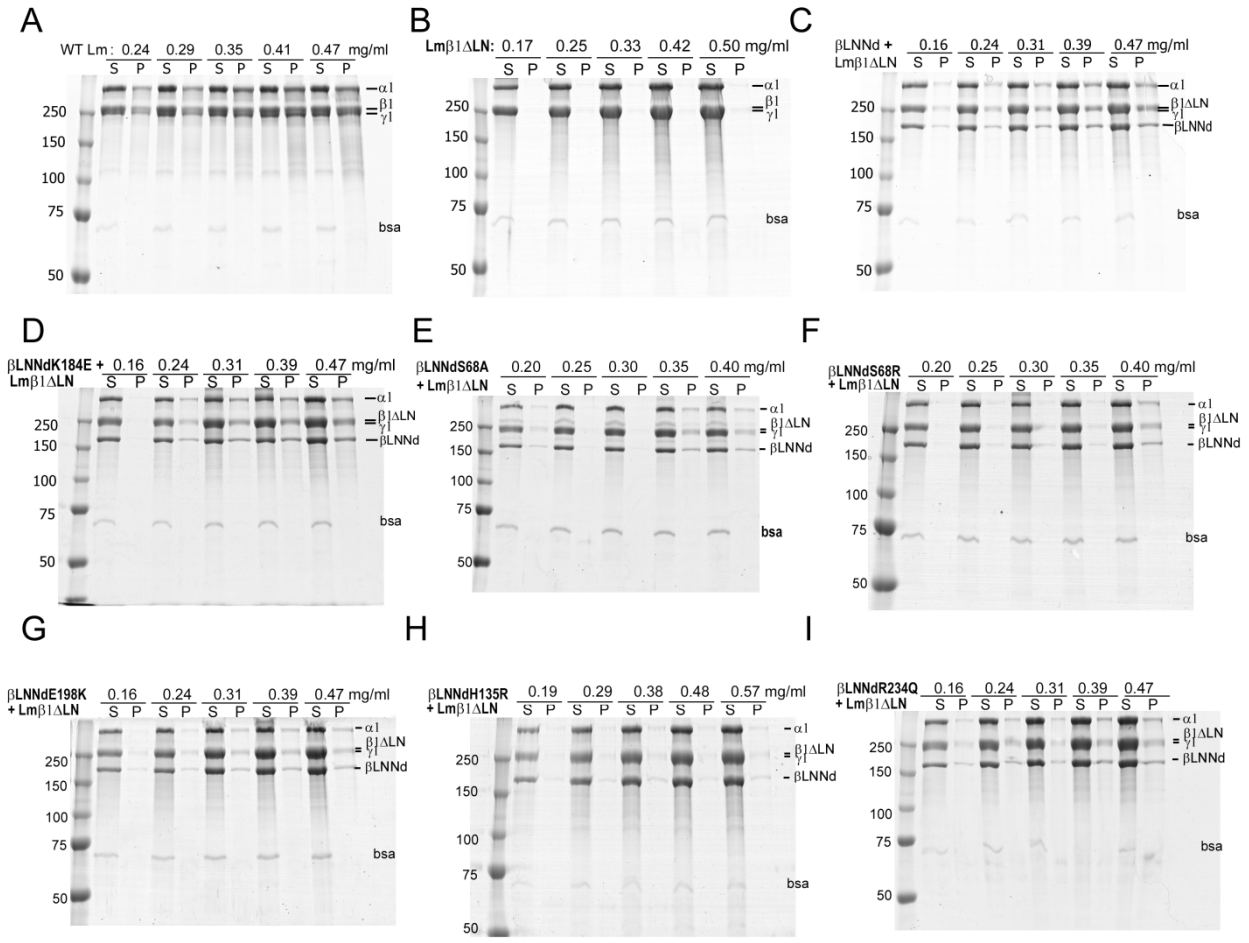
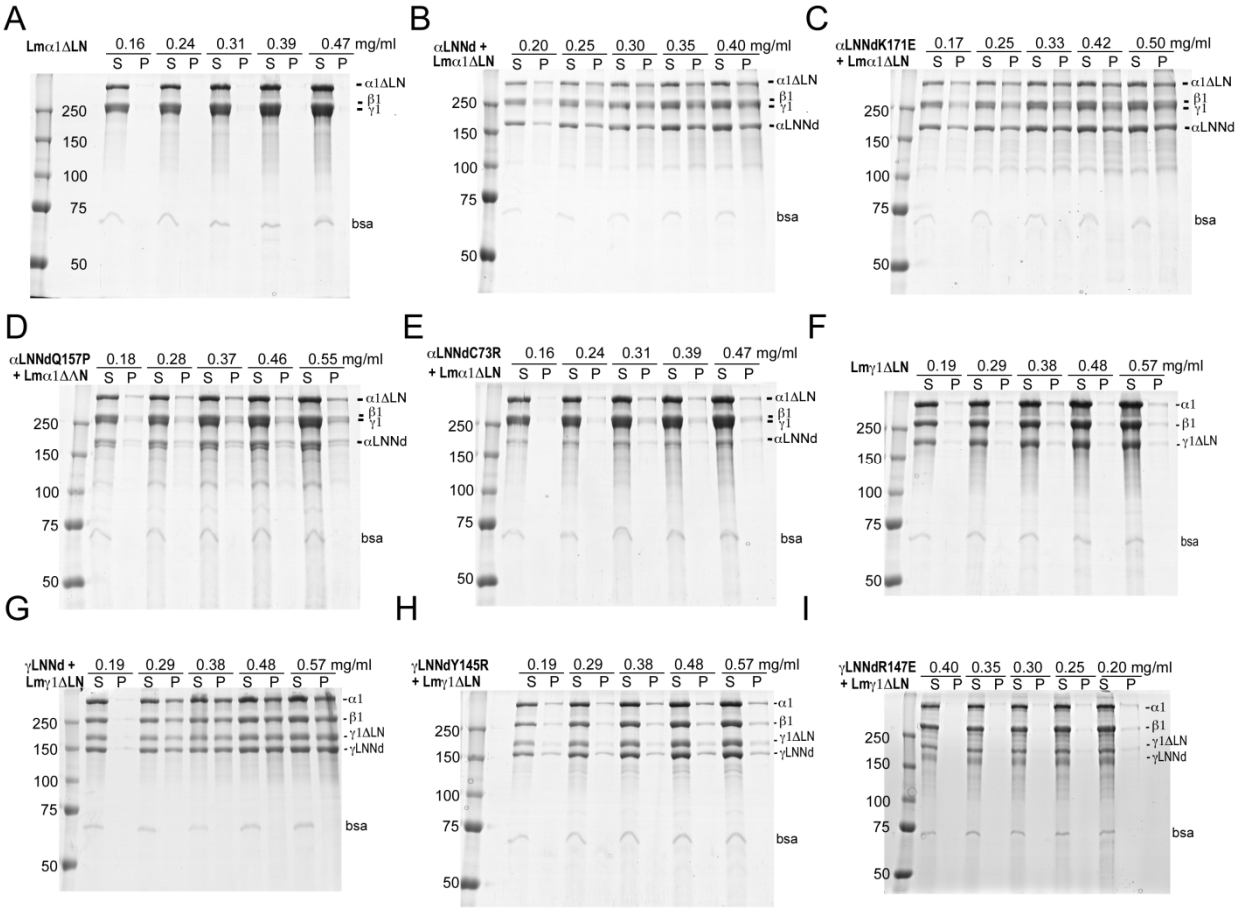
Supplemental Table 1. DNA constructs for xLNNd proteins

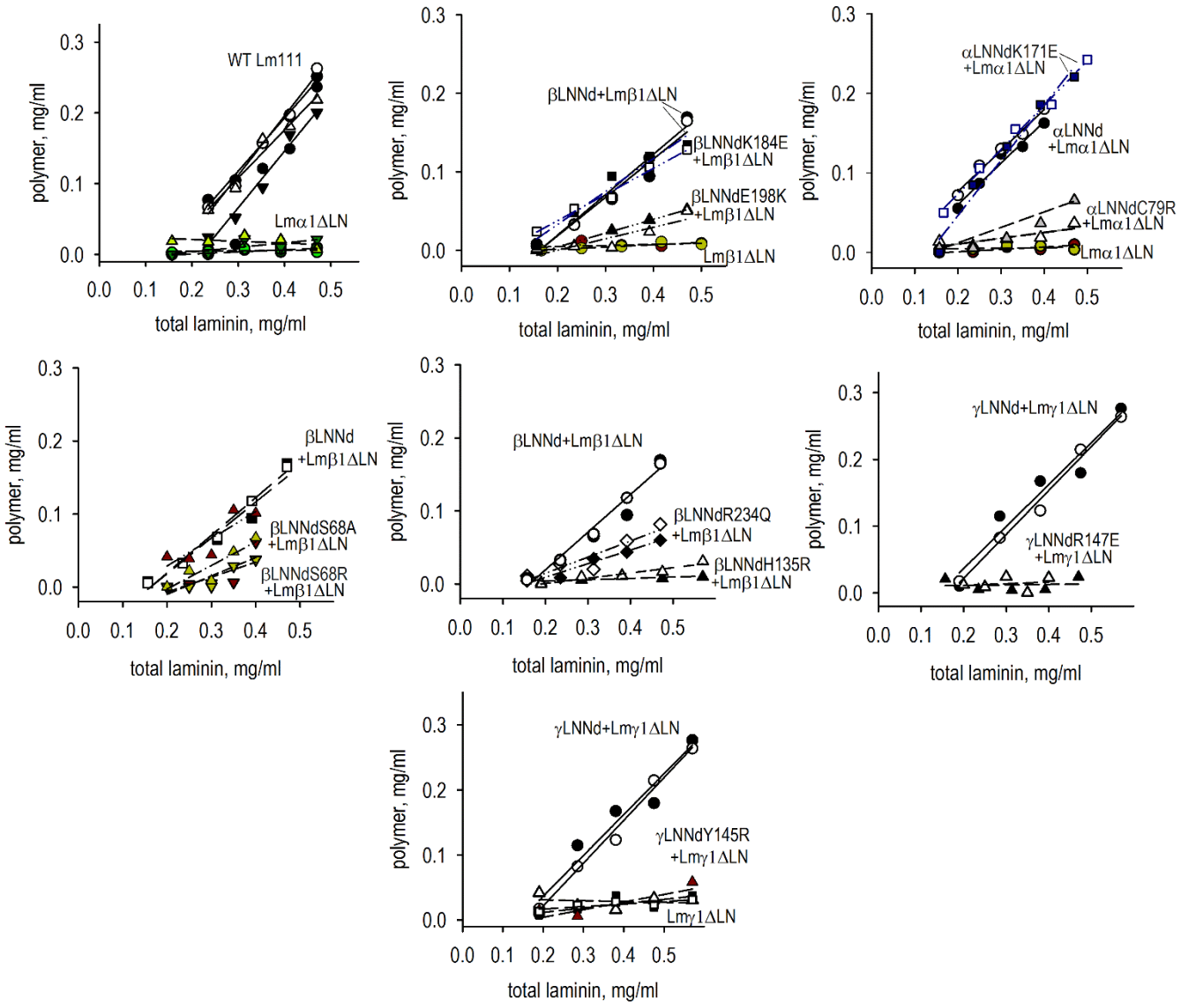
clone	*enzymes*	*5' 3' 1st pcr, 2nd pcr sizes*	*cut sites pcr*	*vector size*	*1st forward primer*	*2nd reverse primer*	*2nd forward primer*	*1st reverse primer*
αLNNd clones								
Q157P	HindIII-XagI	680bp, 1157bp, 1816bp	1510bp	7648bp	GTAGGCGTGTACGGTGGGAG	GACGGCATAGTACGGCCAGGG	CCCTGGCCGTACTATGCCGTC	CATGCAGAGTGCAGAGACTATGC
C73R	HindIII-Eco91I	430bp, 1780bp, 2180bp	2064bp	7094bp	GTAGGCGTGTACGGTGGGAG	CGTCACAGACCCGGCGTTGGGCGTGTCGA	TCGACACGCCCAACGCCGGGTCTGTGACG	TGGGTGCCCCAGGAAGGTCACCTCAGC
K171E	HindIII-XagI	710bp, 1109bp, 1816	1510bp	7648bp	GTAGGCGTGTACGGTGGGAG	GTGGAGTTATTTCGTAGCGGGTC	GACCCGCTACGAAATAACTCCAC	CATGCAGAGTGCAGAGACTATGC
βLNNd clones								
WT	HIndIII-BsrGI	1774bp, 965bp, 2738bp	2143bp	6897bp	GGCGTGGATAGCGGTTTGAC	CACAAGTCTGCTGTCGACATCCATC	GATGGATGTCGACAGCAGACTTGTG	CGAGGAGACGGGCAGACTTG
S68R	HIndIII-EcoNI	cut from 111S80R	826bp	8263bp				
S68A	HIndIII-EcoNI	512bp, 803bp, 1296bp	826bp	8263bp	GGCGTGGATAGCGGTTTGAC	CTGCAAGTGGGCGACGATACAG	CTGTATCGTCGCCCACTTGCAG	CCGAAACAGCAACGCCTGTA
R234Q	HIndIII-EcoNI	cut from 111R246Q	826bp	8263bp	GGCGTGGATAGCGGTTTGAC	CACAAACTTGATTTGCAAGTTGG	CCAACTTGCAAATCAAGTTTGTG	CCGAAACAGCAACGCCTGTA
H135R	HIndIII-EcoNI	713bp, 604bp,1296bp	826bp	8263bp	GGCGTGGATAGCGGTTTGAC	GTCATTACGAGATGAGTAAAATGG	CCATTTTACTCATCTCGTAATGAC	CCGAAACAGCAACGCCTGTA
E198K	HIndIII-EcoNI	893bp, 420bp, 1296bp	826bp	8263bp	GGCGTGGATAGCGGTTTGAC	GTTGAGGGTTCAATCTTAGAATATC	GATATTCTAAGATTGAACCCTCAAC	CCGAAACAGCAACGCCTGTA
K184E	HIndIII-EcoNI	859bp, 458bp, 1296bp	826bp	8263bp	GGCGTGGATAGCGGTTTGAC	GTCATCGACTTCTTTCATGGGGCC	GGCCCCATGAAAGAAGTCGATGAC	CCGAAACAGCAACGCCTGTA
γLNNd clones								
WT	NheI-BsrGI	2642bp	2188bp	2188bp	GGCGTGGATAGCGGTTTGACT	GGCACAAGTCTGCTGTGGCCTGCATCCTGC	GCAGGATGCAGGCCACAGCAGACTTGTGCC	CGAGGAGACGGGCAGACTTG
Y145R	NheI-BsrGI	795bp,1866bp, 2642bp	2188bp	2188bp	GGCGTGGATAGCGGTTTGACT	GGAACTTGAGACGCACACGGGTGATG	CATCACCCGTGTGCGTCTCAAGTTCC	CGAGGAGACGGGCAGACTTG
R147E	NheI-BsrGI	801bp,1860bp, 2642bp	2188bp	2188bp	GGCGTGGATAGCGGTTTGACT	GAACTTGAGCTCCACATAGGTGATGTC	GACATCACCTATGTGGAGCTCAAGTTC	CGAGGAGACGGGCAGACTTG
Laminin								
hLm111β 1R234Q	HindIII-ClaI	1012bp, 1875bp, 2865bp	1957bp	8446bp	GGCGTGGATAGCGGTTTGAC	CACAAACTTGATTTGCAAGTTGG	CCAACTTGCAAATCAAGTTTGTG	GCAGTGTGATCGGTGCTTAC



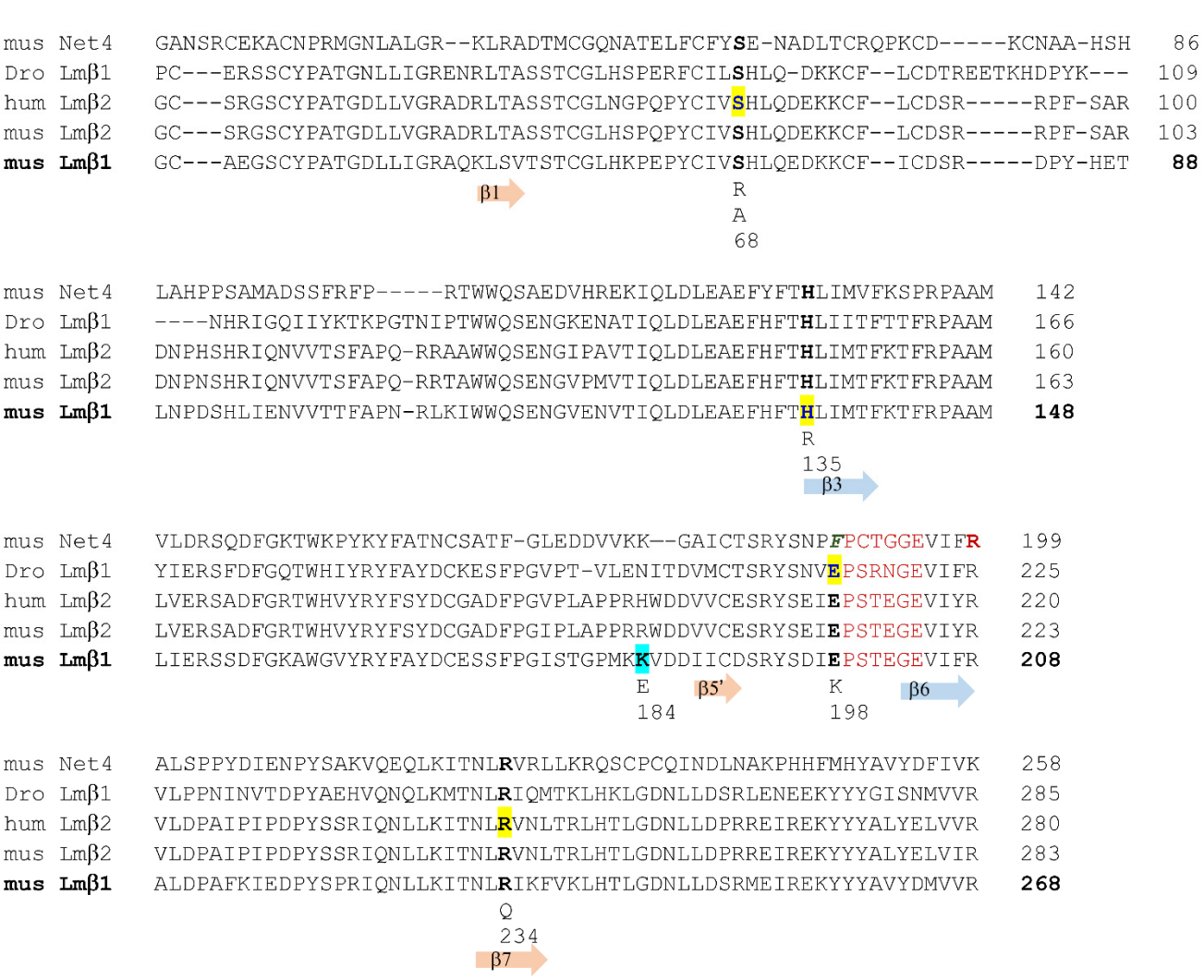
Supplemental Fig. 1. *Polymerization of βLNNd--Lmβ1ΔLN complexes*. WT Lm111, Lmβ1ΔLN, and WT and modified βLNNd proteins bound to and co-purified with Lmβ1ΔLN were incubated at 37°C for 3 h in 50 μl aliquots at different concentrations that were then centrifuged to sediment the polymer (P) fraction and 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, βLNNd complex, and βLNNdK184E (chosen as likely "control" with non-conserved residue) polymerized whereas complexes with βLNNd containing conserved residue mutations showed little or no polymerization. Plots shown in Fig. 3.



Supplemental Fig 2. *Polymerization of αLNNd--Lmα1ΔLN and γLNNd-Lmγ1ΔLN complexes*. Panels **A-E.** Lmα1ΔLN, and WT and modified αLNNd proteins bound to and co-purified with Lmα1ΔLN were incubated at 37°C for 3 h in 50 μl 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, αLNNd complex, and αLNNdK171E (containing a poorly conserved residue with a charge change) polymerized whereas complexes with αLNNd containing conserved residue mutations showed low levels of polymerization. Panels **F-I**. Lmγ1ΔLN and WT and modified γLNNd proteins co-purified with Lmγ1ΔLN 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.



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 indicted 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].

Supplemental Fig. 5. *Laminin αLN mutations and sequence homologies.* Comparison among mouse (mus), human (hum) and Drosophila (Dro) LN domain sequences. Mutated residues indicated below mouse α1 sequence. Colored arrows and bar indicate secondary structure near affected residues. Human LAMA2-deficient muscular dystrophy-associated mutations indicted in yellow for affected subunit isoform. Lmα5 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 Lmα5 and located on the "front" face of the LN domain [36]. Italics for Lmα5 indicate disordered residues in the α5LN-LEa1-2 structural analysis [36].

Laminin gamma LN domain:

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Dro Lmγ1                                                ECYDPYGRPQKCLPEFINAAYQ  77
mus Lmγ3                    RVLSLLATVASMA-LVIQETHFAAGADMGSCYDGVGRAQRCLPEFENAAFG  54
hum Lmγ1     MRGSHRAAPALRPRGRLWPVLAVLAAAAAAGC------AQAAMDECTDEGGRPQRCMPEFVNAAFN  60
mus Lmγ1     MTGGGRAALALQPRGRLWPLLAVLAAVA--GCVR------AAMDECADEGGRPQRCMPEFVNAAFN  58

Dro Lmγ1     LQIESTNTCGEQNDNHFCIQT-MNQNHKNCEFC----KYNDHNPSFLTDLHDPQSPTWWQS  133
mus Lmγ3     RRAEASHTCGRPPE-DFCPHVGAPGAGLQCQRCDDADPGRRHDASYLTDFHSPDDSTWWQS  114
hum Lmγ1     VTVVATNTCGTPPE-EYCVQTGVTGVTKSCHLCDAGQPHLQHGAAFLTDYNNQADTTWWQS  120
mus Lmγ1     VTVVATNTCGTPPE-EYCVQTGVTGVTKSCHLCDAGQQHLQHGAAFLTDYNNQADTTWWQS  118

Dro Lmγ1     ETMFEGIQHPNYVNLTLHLGKSYDITYVRILFRSPRPESFTIYKRTSESGPWIPYQFYSA  193
mus Lmγ3     PSMAFGVQYPTSVNLTLSLGKAYEITYVRLKFHTSRPESFAIYKRTYASGPWEPYQYYSA  174
hum Lmγ1     QTMLAGVQYPSSINLTLHLGKAFDITYVRLKFHTSRPESFAIYKRTREDGPWIPYQYYSG  180
mus Lmγ1     QTMLAGVQYPNSINLTLHLGKAFDITYVRLKFHTSRPESFAIYKRTREDGPWIPYQYYSG  178
                                          R E
                                        145 147
                                          β3

Dro Lmγ1     TCRDTYSLPDSRAIRKGEGEAHALCTSEYSDISPLRDGEIAFSTLEGRPSGINFERSGEL  253
mus Lmγ3     SCQKTYGRPEGHYLRPGEDERVAFCTSEFSDISPLNGGNVAFSTLEGRPSAYNFEESPVL  234
hum Lmγ1     SCENTYSKANRGFIRTGGDEQQALCTDEFSDISPLTGGNVAFSTLEGRPSAYNFDNSPVL  240
mus Lmγ1     SCENTYSKANRGFIRTGGDEQQALCTDEFSDISPLTGGNVAFSTLEGRPSAYNFDNSPVL  238

Dro Lmγ1     QEWVTATDIRITLDRLNTFGDELFGDSQVLKSYFYAI  290
mus Lmγ3     QEWVTSTDILISLDRLNTFGDDIFKDPRVLQSYYYAV  271
hum Lmγ1     QEWVTATDIRVTLNRLNTFGDEVFNDPKVLKSYYYAI  277
mus Lmγ1     QEWVTATDIRVTLNRLNTFGDEVFNEPKVLKSYYYAI  275
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Supplemental Fig. 6. *Laminin γLN mutations and sequence homologies*. Comparison among mouse (mus), human (hum) and Drosophila (Dro) LN domain sequences. Mutated residues indicated below mouse γ1 sequence (mouse sequence number designations used in experimental labels). Patch (red) corresponds in location to the PLENGE polymerization patch described in Lmα5 and located on the "front" face of the LN domain [35, 36].