

Specific laminin binding to interleukins enhances diabetic wound healing

Author Information

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Supplementary Information

Supplementary Table 1 Sites filtrated from segments by combination of AI docking and physical bond computation. Orange marked sites were identified in previous studies ^{8,9}, to follow the same downstream characterisations with newly discovered ones to revise our state-of-the-art. This was for the first time LAMB & LAMC sites were discovered to bind cytokines.

Code	Chain	Site Window	AA Sequence	Length
A1-01	LAMA1	282-302	DETTKKLQCQCEHNTCGESC	20
A1-02	LAMA1	2210-2229	GNIGSLSVKEMSSNQKSPTA	20
A1-03	LAMA1	2516-2535	TTNSSGIILAAALGGDVEKRG	20
A1-04	LAMA1	2734-2745	RKRLQVQLSIRT	12
A1-05	LAMA1	2750-2760	GLIYYVAHQNQM	12
A2-01	LAMA2	1681-1700	FIKELARDAEAVNEKAIKLN	20
A2-02	LAMA2	2015-2034	TLNDTLGKLSAIPNDTAAKL	20
A2-03	LAMA2	2067-2086	KNYNKLADSVAKTNAVVKDP	20
A2-04	LAMA2	2341-3122	IQFDGEGYALVSRPIRWYPN	20
A2-05	LAMA2	2800-2811	GLLFYMARINHA	12
A3-01	LAMA3	196-215	KEFGREANMAVTRDDVLCV	20
A3-02	LAMA3	2589-2608	EPCRRRKEESDKNYFEGTGY	20
A3-03	LAMA3	2705-2727	DGEVDFDFSTYYLGGIPIAIRERF	23
A3-04	LAMA3	2932-2951	PPFLMLLKGSTRFNKTCTFR	20
A3-05	LAMA3	3031-3043	KNSFMALYLSKGR	13
A3-06	LAMA3	3043-3062	RLVFALGTDGKKLRIKSKEK	20
A4-01	LAMA4	494-518	QALNYVRDAEDMNRATAARQRDHEK	25
A4-02	LAMA4	976-995	DPEDTVFYVGGVPSNFKLPT	20
A4-03	LAMA4	1309-1328	DGLSHFVISSVSPTRYELIV	20
A4-04	LAMA4	1413-1432	HKKGKNLSKPKASQNKKGK	20
A4-05	LAMA4	1521-1540	TLFLAHGRLVYMFNVGHKKL	20
A4-06	LAMA4	1724-1743	QSLCDGRWHRITVIRDSNVV	20
A5-01	LAMA5	0378-397	RASQSLDGTYQGGGVCIDCQ	20
A5-02	LAMA5	2385-2404	REALNRAVDATREAQELNSR	20
A5-03	LAMA5	2834-2855	DRTLQFGHMSVTVRQMIQETK	22
A5-04	LAMA5	3300-3330	TPGLGPRGLQATARKASRRSRQPARHPA CML	31
A5-05	LAMA5	3312-3325	ARKASRRSRQPARH	14
A5-06	LAMA5	3345-3356	GPLPSYLQFVGI	12
A5-07	LAMA5	3417-3436	RQRSRPGRWHKVSVRWEKNR	20
A5-08	LAMA5	3539-3550	TLPDVGLELEVR	12
B1-01	LAMB1	68-87	SHLQEDKKCFICNSQDPYHE	20
B1-02	LAMB1	169-188	DCEASFPGISTGPMKKVDDI	20
B1-03	LAMB1	1567-1586	DIARAEMLLEEAKRASKSAT	20
B1-04	LAMB1	1738-1757	QLLKDLERKYEDNQRYLEDK	20
B2-01	LAMB2	60-79	DRLTASSTCGLNGPQPYCIV	20
B2-02	LAMB2	87-106	KCFLCDSRRPFSARDNPHSH	20
B2-03	LAMB2	394-413	YRDPTKDLRDPVCRSCDCD	20
B2-04	LAMB2	1779-1798	RMRSVLQAINLQVQIYNTCQ	20
B3-01	LAMB3	42-63	RASSTCGLTKPETYCTQYGGQ	20

B3-02	LAMB3	59-78	YGEWQMKCKCDSRQPHNY	20
B3-03	LAMB3	693-712	LTMYQRKREQFEKISSADPS	20
B3-04	LAMB3	1046-1065	LRHQARQQGAEAVQAQQLAE	20
C1-01	LAMC1	70-89	GTPPEEYCVQTGVTGVTKSC	20
C1-02	LAMC1	157-176	PESFAIYKRTREDGPWIPYQ	20
C1-03	LAMC1	1371-1390	KDFDRRVNDNKTAAEEALRK	20
C1-04	LAMC1	1444-1463	KAEAERTFAEVTDLNEVNN	20
C1-05	LAMC1	1466-1485	KQLQEAELKLRKQDDADQD	20
C1-06	LAMC1	1509-1527	NSVTSLLSIINDLLEQLGQL	20
C2-02	LAMC2	598-617	GGPNCEHGAFSCPACYNQVK	20
C2-03	LAMC2	971-990	EAMKRLSYISQKVSDASDKT	20
C2-04	LAMC2	1044-1063	KGLASLKSEMREVEGELERK	20

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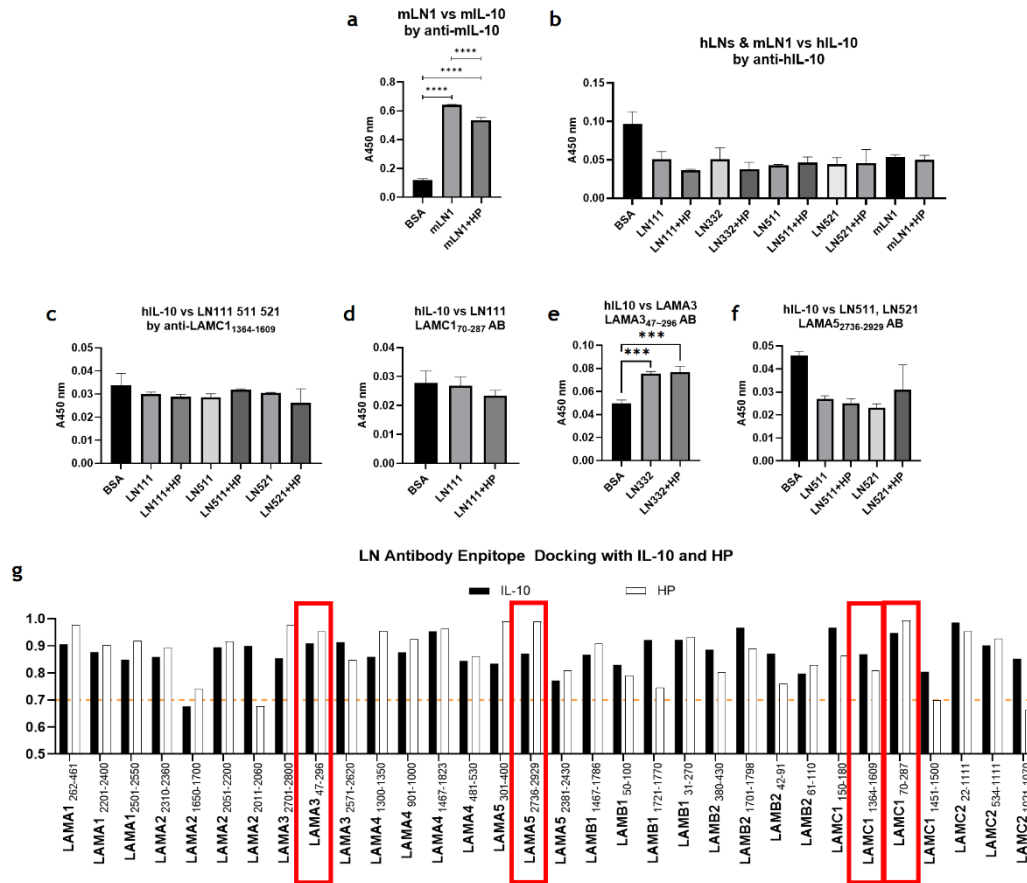
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Supplementary Table 2 SPR summary of association/disassociation constant resulting from LN332 and C1-01 versus ILs/GFs assays. Ka (or kon) is the association rate constant for the speed of binding, Kd (or koff) is the dissociation rate constant for the speed of unbinding, KD is the equilibrium dissociation constant, and KA is the equilibrium association constant. For IL-10 versus LN332 and C1-01 versus IL-10 fitting with the steady-state model, no Ka or Kd could be derived as association and disassociation were too fast. Yet KD and KA were still derived by steady state fitting to evaluate association/disassociation. For the pairs showing minor binding, all four constants were NA.

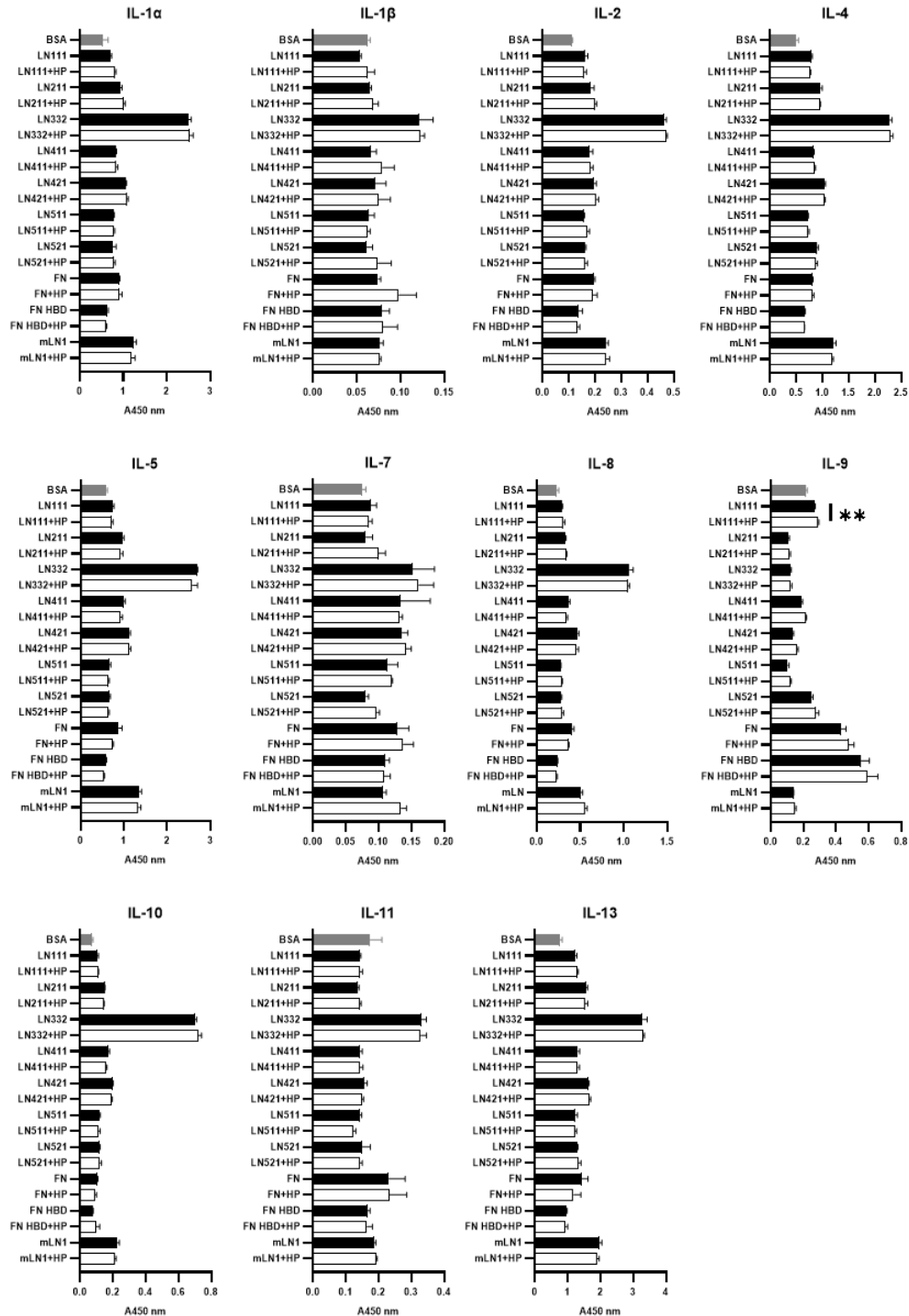
LN332 protein					
Receptor	Ligand	Ka	Kd	KD	KA
LN332	IFN- γ	1.082E5	0.010705	9.936e-8	1.01E+07
IL-10	LN332	NA	NA	1.672E-7	5.98E+06
LN332	IL-4	722.8	0.005937	8.213E-6	1.22E+05
LAMC-01 Site					
C1-01	IFN- γ	NA	NA	NA	NA
C1-01	IL-4	NA	NA	NA	NA
C1-01	IL-10	NA	NA	1.028E-6	9.73E+05
C1-01	VEGF-A165	NA	NA	NA	NA
C1-01	PDGF-BB	2.804E5	0.2422	8.63E-7	1.16E+06
C1-01	HB-EGF	NA	NA	NA	NA

Supplementary Table 3 Uniprot accession of Laminin chains and PDB accession of ILs employed in molecular docking

Laminin Chains	Uniprot ID	ILs	PDB ID	ILs	PDB ID
LAMA1	P25391	IL-1 α	2KKI	IL-12	1F45
LAMA2	P24043	IL-1 β	9ILB	IL-13	1IJZ
LAMA3	Q16787	IL-2	1M47	IL-17A	4HR9
LAMA4	Q16363	IL-4	2B8U	IL-17F	6HGO
LAMA5	O15230	IL-5	3VA2	IL-18	3WO2
LAMB1	P07942	IL-6	1ALU	IL-19	1N1F
LAMB2	P55268	IL-8	1IL8	IL-21	2OQP
LAMB3	Q13751	IL-9	7OX6	IL-22	1M4R
LAMC1	P11047	IL-10	1INR		
LAMC2	Q13753	IL-11	6O4O		



Supplementary Fig 1, inhibition by antibodies in detecting IL-10's binding with LNs. **a~b**: Commercial IL-10 Elisa kits (ThermoFisher) to detect IL-10 as ligand in solution when binding with LNs as receptor on surface. **c~f**: plot of some tested antibodies to detect LNs in solution as ligands when binding with IL-10 on surface as receptor. **g** Docking between IL-10 or Heparin with LNs' epitope for commercial available antibodies. In **a&b**, the binding between murine LN111 and IL-10 was positive while in human was negative. This was not sufficient to negate binding between LN111 and IL-10 in human, as human and murine IL-10 shared >90% common sequence. When applying Ishihara's method⁸ employing LAMC1 antibodies to detect LNs as ligands in solution to bind with IL-10 on surface as receptor, LAMC1 antibodies of 2 common epitopes offered negative signal. LAMA3 antibodies tested the binding while LAMA5 antibodies did not show binding. Molecular docking also illustrated that nearly all epitopes of current antibodies potentially interacted with IL-10 and heparin. An orthogonal method was required to identify whether there is binding between LNs and ILs. Error bars report mean $\pm$ SEM, n = 3.



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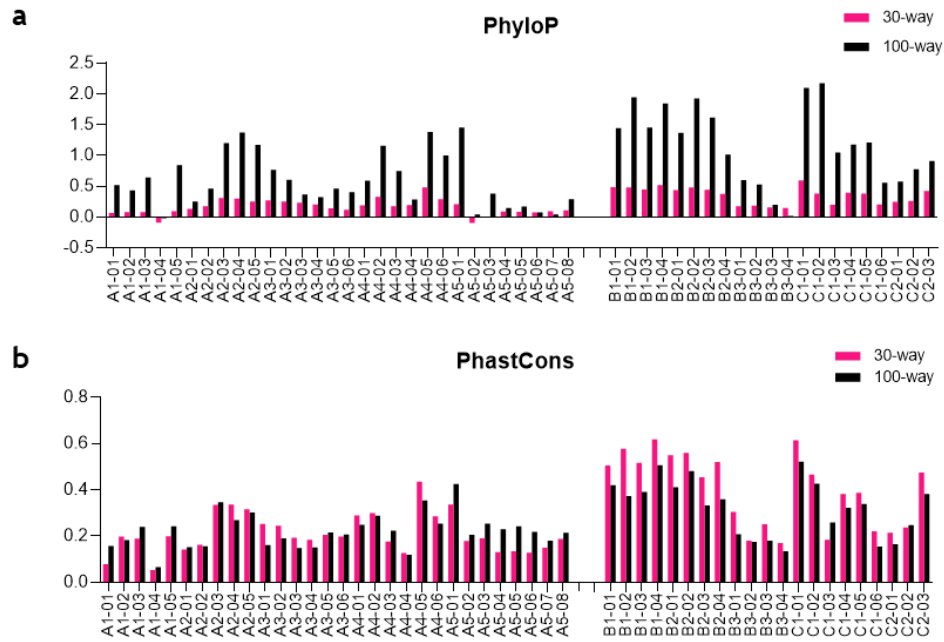
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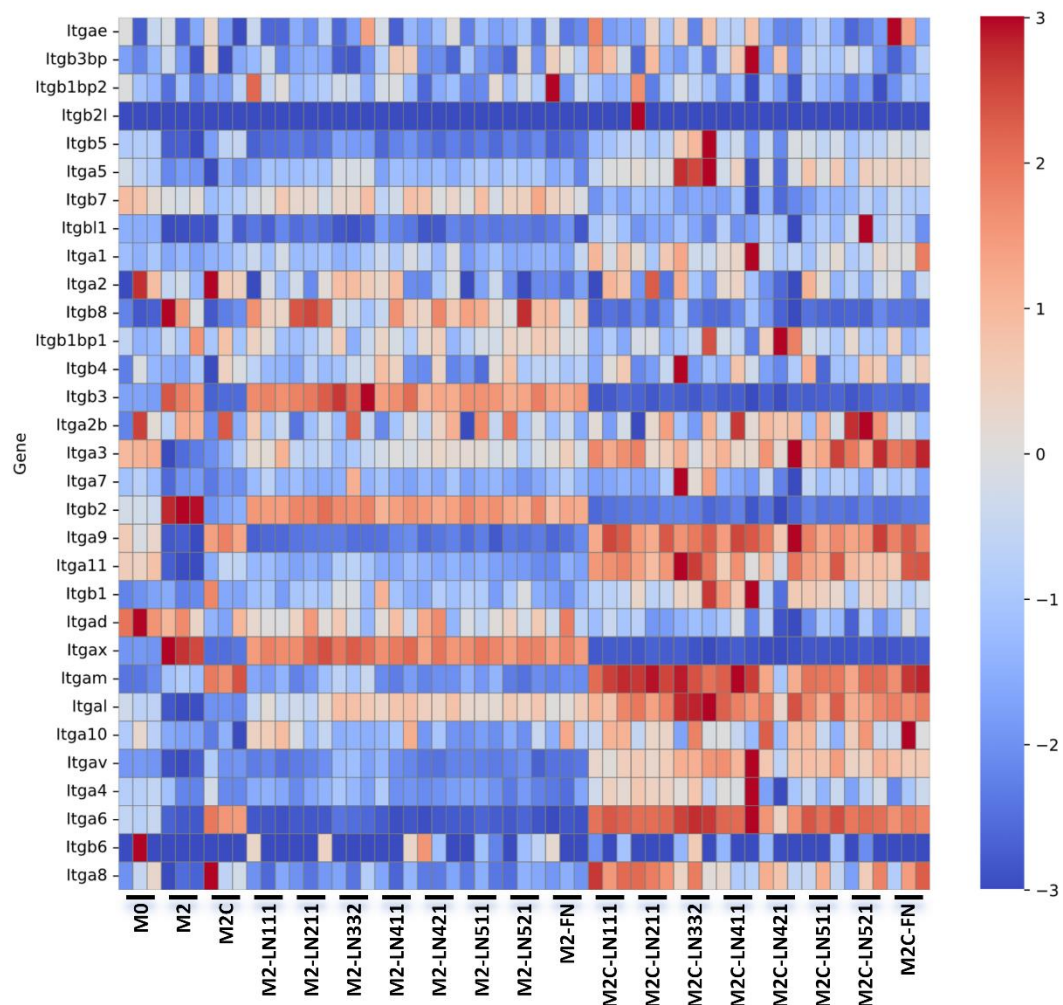
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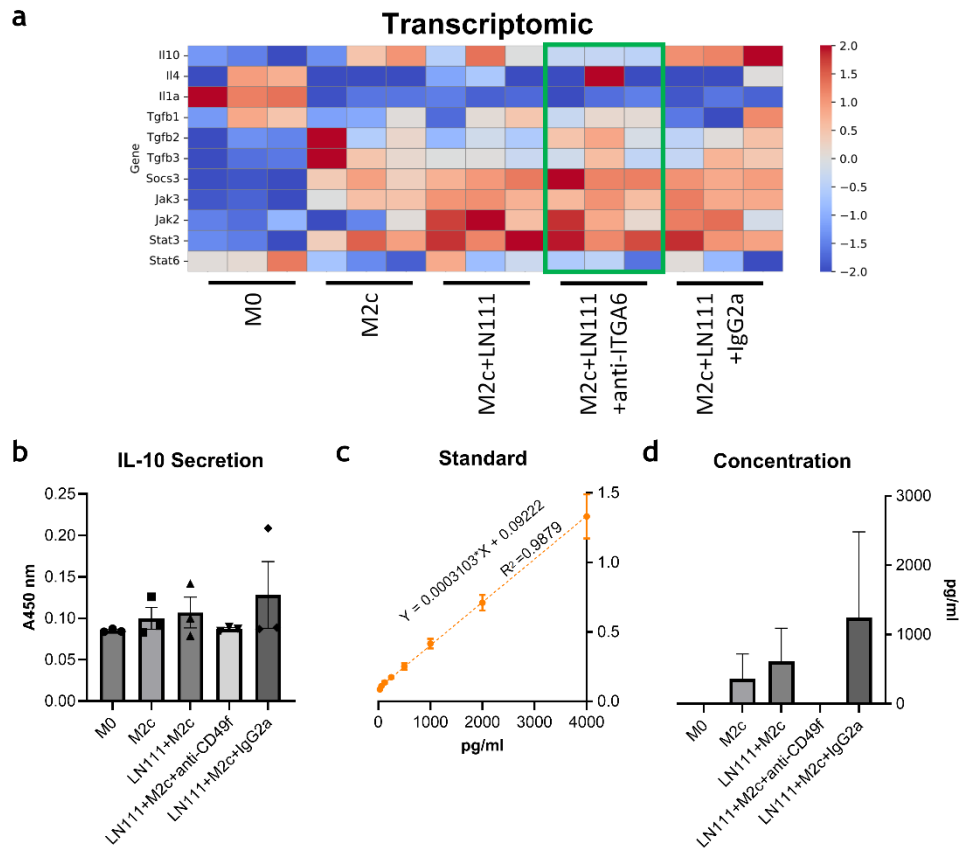
Supplementary Fig 2 Heparin did not significantly affect LN-IL binding. Attempt to inhibit IL-LN binding by excess heparin. ELISA plates were coated with 10nM LNs and then further treated with 10 μ M heparin or PBS. Bound of ILs were detected using avidin-HRP for each. Statistical analyses were performed using the Multiple T-test by comparing the HP+ vs HP- condition obtained from the LN/FN-coated wells. *** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Three experimental replicates.



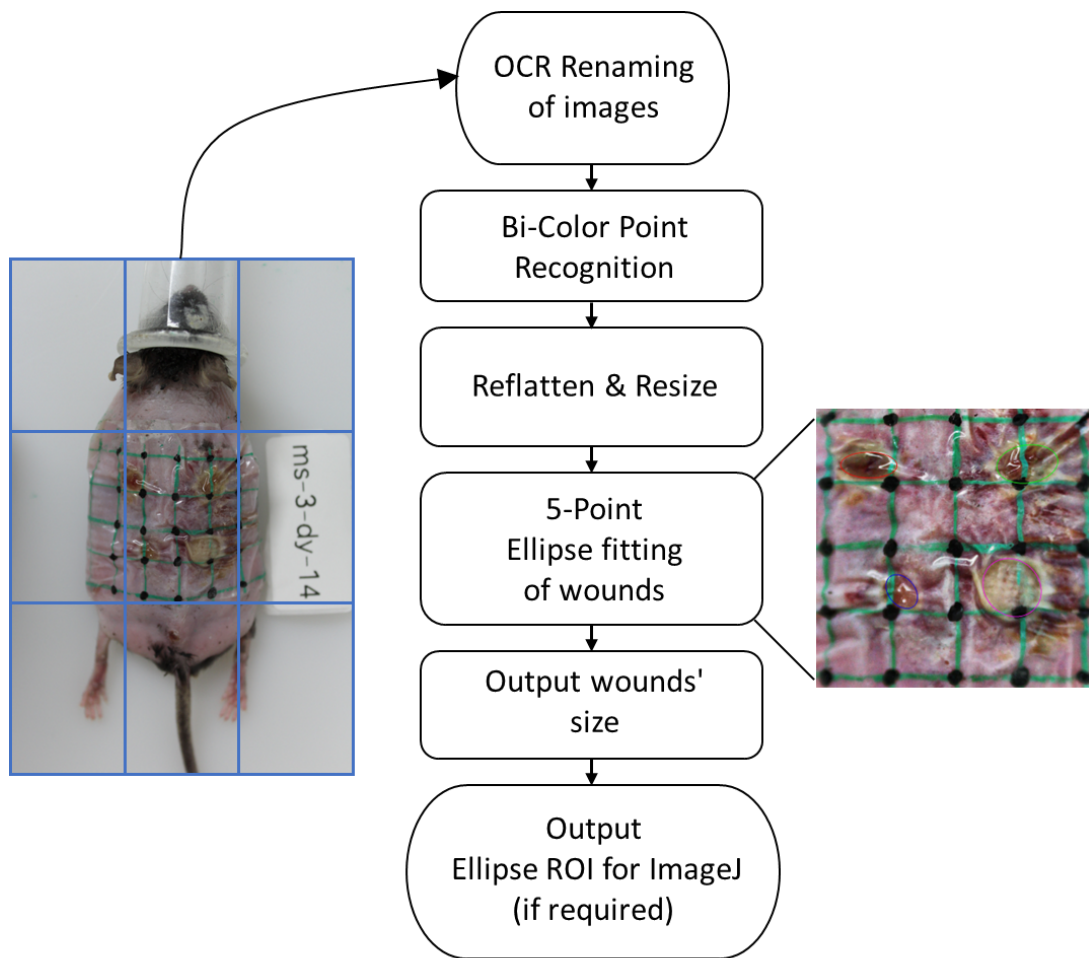
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 67 Supplementary Fig 3 Bioinformatics of the Laminin sites. Limited by plot area, A stands for
 68 LAMA, B for LAMB, and C for LAMC. **a** and **b** evaluated conservation of the sites among
 69 species. **a** PhyloP score of the sites, evaluating the evolving speed of the sites. Thirty-way
 70 represented 30 typical mammals while 100-way represented 100 typical vertebrates. **b**
 71 PhastCons score of the sites, evaluating to which degree can each site be considered as a
 72 conservative element.



Supplementary Fig 4 Heatmap of integrins' expression in M2 and M2c mBMDM derived on LN isoforms and FN.



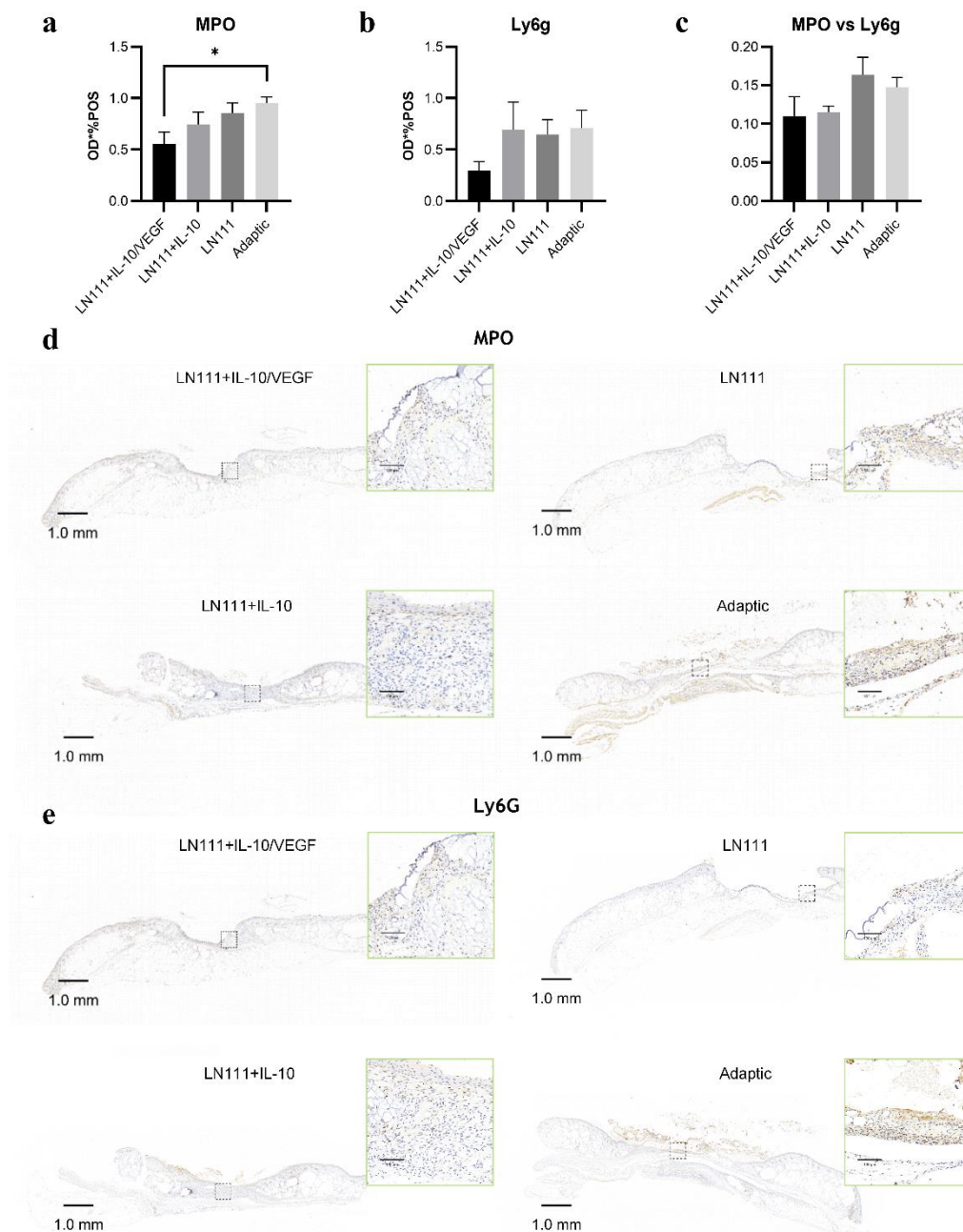
Supplementary Fig 5 mBMDM's M2c polarization under ITGA-6 neutralization. **a** Transcriptomic heatmap of most focused M2&M2c distinguishable markers; **b~d** Elisa characterization of IL-10 secretion post-stimulation. **b** channel signal; **c** standard concentration curve; **d** scaled concentration. ITGA6 neutralization induced significant silence in IL-10 secretion. Interestingly, with existence of isotype control antibody a higher level of IL-10 was derived, compared to the M2c control and with LN111. This also happened in cytometric markers. Error bars report mean $\pm$ SEM, n = 3, experiments repeated for twice.



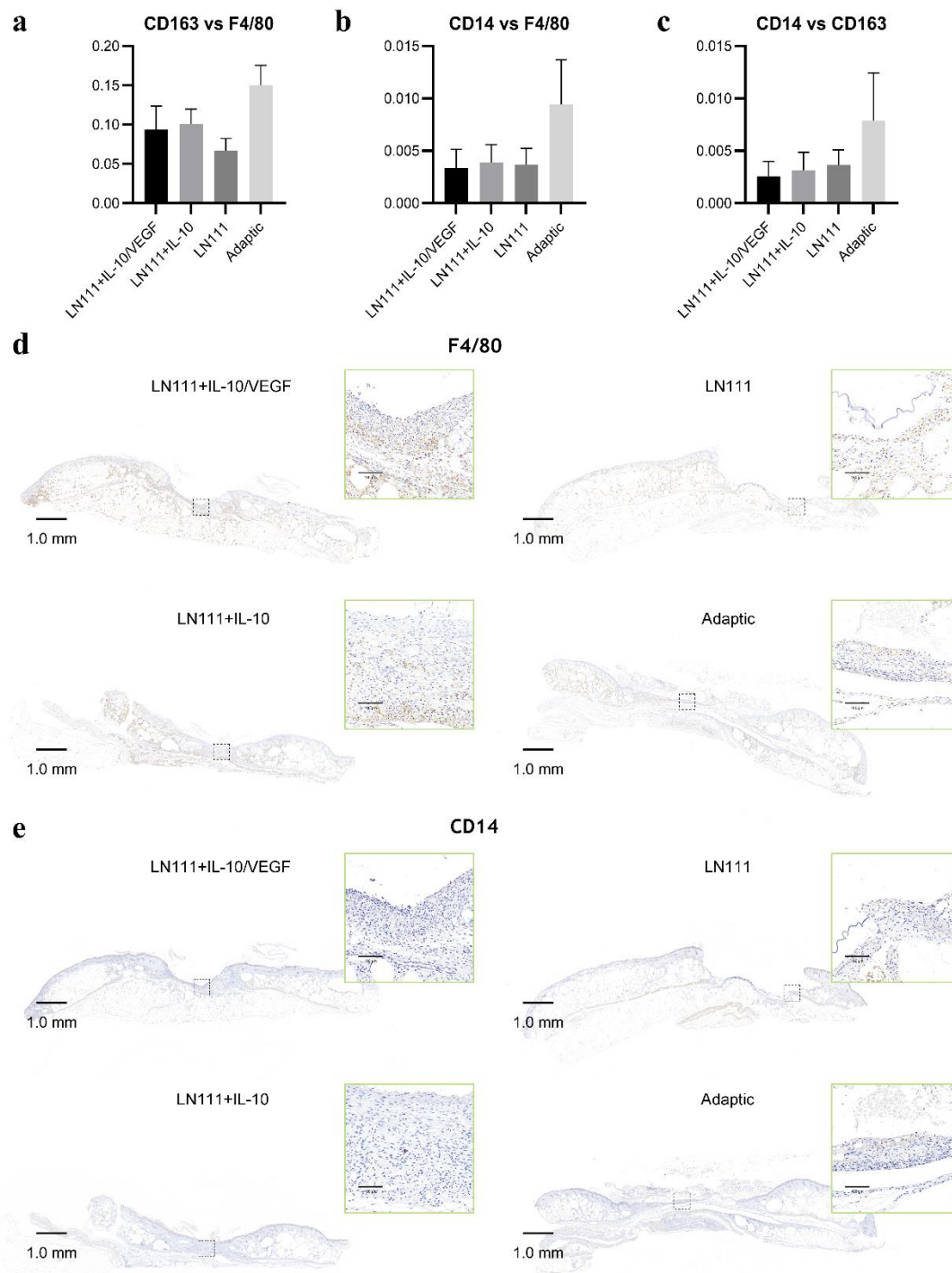
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87 Supplementary Fig 6 Workflow of image analysis with the customized Wound-Shape software.
 88 Images were taken at consistent position utilizing the rule of thirds grid. The lattice, marked on
 89 the envelope Tegaderm membrane with known size, enabled the double-color point tracking,
 90 which was latter used to flatten and resize the wounds. Five points on wounds' edge were
 91 manually labelled and fitted with ellipse function, which was exported to individual .json file
 92 and can be imported into ImageJ for downstream analysis with another customized script.

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 95 Supplementary Fig 7, Histoimmunostaining of wound samples **a** Quantification of Neutrophilic
 96 marker MPO. **b** Quantification of Neutrophilic marker Ly6G. **c** Co-localization ratio of MPO
 97 vs Ly6G, showing neutrophils' NETosis activity. **a~c**: Error bars report mean $\pm$ SEM, $n = 10$.
 98 Statistical analysis was performed using the one-way ANOVA test with Tukey
 99 multicomparison. **d** MPO. **e** Ly6G. Scale bar of full vision = 1 mm. Amplification in cyan
 100 frame, Scale bar = 100 μ m.



Supplementary Fig 8, Histoimmunostaining of wound samples **a** Co-localization ratio of CD163 vs F4/80. **b** Co-localization of CD14 vs F4/80. **c** Co-localization ratio of CD14 vs CD163. **a~c:** Error bars report mean $\pm$ SEM, $n = 10$. Statistical analysis was performed using the one-way ANOVA test with Tukey multicomparision. **d.** Macrophagic marker F4/80. **e** CD14. Scale bar of full vision = 1 mm. Amplification in cyan frame, Scale bar = 100 μ m.