

Supplementary Figure 1. The JSK1 mutant results from disruption of the lmbd2B gene. The *lmbd2B* knockout mutant generated using the recovered REMI plasmid does not produce an *lmbd2B* mRNA transcript.

In **A**, the pGEM3 plasmid used in REMI mutagenesis is shown. It contains a blasticidin resistant cassette (bsr). The BamHI site linearized for REMI is indicated. Also, the sites of the Sp6 and Bsr primers used for sequencing are labeled. In **B**, the *lmbd2B* nucleotide and amino acid sequence of a section of the coding region. A transcription start site is located at base number 1 and the stop site is at 2461. There is an intron at 278-366. DpnII sites are in red. The DpnII site of insertion is indicated with an arrow. The pink highlighted sequence (1-690) corresponds to the gene sequence obtained from sequencing the flanking DNA of the REMI plasmid insert from JSK1. In **C**, RNA from Wt and *lmbd2B*- cells was converted to cDNA and utilized in PCR. The first two lanes show cDNA from Wt cells. An Ig7 control gene transcript shows the presence of cDNA, and a 1.5Kb product of the expected size is produced with *lmbd2B* specific primers. The next two lanes show cDNA from *lmbd2B*- cells. No product is formed with the *lmbd2B* primers even though the Ig7 control gene transcript was made.

	10	20	30	40	50	60	70	80	90	100	110	120
Hs-LMBD 2		·	ا []	MS GA AL GL H	I EI VF VF <mark>F</mark> L AI	I L FLLHRYGD FKKO	HRLVIIGTLL	AWYLCFLIVF	ILPLDVSTTI	I I <mark>YNRC</mark> KHAAA	INSSPPE-NSN	IT GL YAT
X1-LMED 2]	MS GV AL GIH	EIVSVFFLA	LFLLHRYGDFKKO	HKLVIVGTLL	AWYLCFLIVF	IIPLDVSTTI	YNRCVARHA	VTPAPSN	ITVL SPT
NV-LMED 2					-MILCFVLT	LCLLHYFGNWRKO	HILVILSVLI HILVTIAVFV	AWYFSFVIVE	VLPLDVISII	YRKCLLENK	(PIPAPNV (PIQNTTRLFQ	LVTLSNS
Ce-LMBD 2]	MGTI SLAV(QL FIVFLLT	SYLLNKYSTIRKQ	NPIVTISTFI	GWYFSLIIVF	VLPLDVAITH	FHKCENDRQ	RILNT	-TTS-TP
D d- LMBD 2B	MS S <mark>NTTT</mark> P TP TS TP	TP TS SP SI G	PVPIPYE SASF	GNLL FFAIS	SL VL CG VV VI	LIGMKQYISLKRT	PUIVATFFSFL	GWFMCFSIVI	LVPLDILUTI	HLQCVIENN	IETLTG	
	1 30	140	1 50	160	:: 170	180	190	.* :.: *. 200	:*:*: * 210	. :*	230	240
W- WED 0	1	1		I	I	I	I		l			1
HS-LMED 2 X1-LMED 2	AN PV P PG IV SN TT AO NH LP	SADKLRSSD	-SQHPCFKPWS VSLEECSKPWS	YIPD GIMP J YIPR GIMP J	IFWRVVYWT: IFWRVVYWT:	SOFLTWILLPFMU	SYARSGEFSI	TGKIKTALIE	NAIYYGTYLI NAIYYGTYLI	IFGALLIYV IFGALLIYV	AVNPHLHLEW AVNPNLHLEW	NULUTIG YOLOTIG
Dm-LMED 2	VG PP P		QCQEPWG	MV PA SV FP1	NLWRIIYWS	SOFLTWLIMPLMO	SYLKAGDFTV	KGKLKSALIE	NAIYYGSYLI	ICGVLLIYI	AVK-GESLDW	OKLKAIA
Ce-LMBD2	AP VV P			YU PROALFI YV PD DV LFI	NL WR VV YW SA NL WR VV YW SA	AOLLTWLILPLLO	SYVTAGNETI	LGKIRAAVIN	NALWYGSYVI NALYYAIYSI	CFLAILIYA	MFK-GVSINI	ENLKVIV
D d- LMBD 2A D d- LMBD 2B	NSGSNNNNNNNEI	IN	- KYDS CEEP WAY	YI SNDKLEY	YI YQ TFYFG. Oo MD FI MFG	FLLLTWLVYPLMG	SEVLAGDENL	SGRITRSIKE	NAYLYLIFG	IGLVVMIWL	LAVKQLDW	NS MV GFA
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	2 50 I	260 I	270 I	280 I	290 I	300 I	310 I	320 I	330 I	340 I	350 I	360 I
HS-LMED 2	IA AA NT UG LF LL VL	LL GY GL VE I	PRS YWNG AKRG	YL LMKTYFI	KA AK LMTE KA	ADAEENLEDAMEE	VRKVNESIKY	N-HPLRKCVD	TILKKCPTEN	QEKMGRNMD	DYEDFDE	KHS
Dm-LMBD2	SSAS NTWG LF LL IL	LL GY AL VE V	PRS LUNNAK PG	FA LQ YA YFI	KAAK LSTEK	AEAEEHVDDILES	LQGLSRVIPN	N-HELRPCLE	TILRKVPIEI	QERASRNFA	RTGGSGMGA-	
NV-LMED 2	TTAS NT WG LL LL VL	LM GY GL VE I	PRIVUNAAS LD	YQ LAHTYFI YD ID MTYFI	KV SK LS VE R	EEAEEQLADVLEE	VRQASENIRY	N-DPLRECID	TIIKKCPEMS	ESMFSRGTD	DYVDYNDYLK	ETGNKIK
D d- LMBD 2A	MAAANTWGLCLVII	LM GY GL VE T	PRS INVS SQRS	LV LKHL QFI	KAVE LLNSK	KKANEELIATMKV	IRRIQEKTKK	Y-DPYEKYIK	IIVDQCPPEQ	YALVQRGEG	DGEATYS	
D d- LMBD 2B	ML LANVYGVI LITI	TMGYGLIDV	PRNLLRKGSHY.	AI LRNYRVI	EAVV LKTE LI	EDVKRQLIDHLKL	IKTISDRAGQ	Y-DPFRIYLD	VIISKCPTEY	TOVLIQEYHA	EPLPAGM	EA
	370	380	390	400	410	420	430	440	450	460	470	480
Hs-LMBD 2	IY PSEKSL VKLHKQ	VIYSVORHR	RTQ VQ WQ IL LE (OA FY LE DV	AKNETSAT	HQFVHTFQSPE	PENRFIQYFY	NPTFEWYWEC	LLRPWFYKII	AVVLSIFSV	/IVVWSECTFF	STT-PVL
X1-LMED 2 Dm-LMED 2	SY PSEKTL VK LHKQ	VIYAVQRHR	RTQ VQ USILLE (RTQ VQ USILLE)	QAFHLEDVA	AKNETSAA AKNEHSSD	KQFVHTFPHQE	PESWIMERLY	TPTIEWYWEC SASLOUVWEC	LLRPWCSRII	AVILALEST	VVVWSECTFF	SAK-PVL
NV-LMED 2	EP FSEKNL IK LHOR	VIVIVQTAR	RTQ CQ YN GQ LDI	KAFGLEDIV	VRNAGN LD	RCFKSSFKKPI	GCCKSIS	-PILEWYWKI	WLCPIFLRLI	AAILVLLSL	ALVUSEVTFF	TTK-PIL
Ce-LMBD2 Dd-LMBD24	GPCSEAKL IS LHKK	TI YA VQ TL N LK NA TT NS O	VATAQ MKVLVDI PAFFLVD OCLVI	RALFLENLA FAFELODIO	AFSESNG MISATS LD	YNLELSRNI 	CV	PIGVRRFWYT LDIMENIIWYN	RLQTPFCRII	GIVTVFMTF ATVFAVLSL	FVLFSECTFF	VVS-YTL FTS-FDT
D d- LMBD 2B	EI LSYKYL VG IH ST	LLDLVDRNH	SAEVLYERLLGI	KA FA IE DI I	IE TRERNKO	TAGNN QI AGEERS	IQUSFKSTKS	NGKFEYLWHM	YI <mark>HPWYFIIA</mark>	GLVCVCLSG	FILUSEIVLA	LV SN PD Y
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	490 I	500 I	510	520 I	5 30 I	540 I	550 I	560 I	570 I	580 I	590 I	60 O
Hs-LMED 2	SL FAVE IQ LAEKTY	NY IY IE LAC	FLSIFFLSICV	YSTVFRIR	VFNY YY LA SI	HHQ TD AY SLLFSC	MLFCRLTPPL	CLNFLGLTHM	DSSISHKN	TOPTAYTS	MGSMKVLS	FIADGFY
XI-LMBD2 Dm-LMBD2	SL FAVF IQ QAEQ TH SI FANV IY VAKE SY	DFFTIEVFS	IVV LCYFFYCT	YS TV FRIR YS TI LRIRI	FLNL YY LA SI FLNL YY LA PI	HHQTDAYSLLFSC HHQTNEHSLIFSC	MLFCRLTPPL MLLCRLTPPM	CLNFLGLIHM	DVSISHQN DTHIIPNF	RIMETVYTQI	MGSLRVLP MGHMDVIG	LIAD VEY II SNGEN
NV-LMED 2	SL FAIL VN AAGKNY	YY FY VE LI C	FFV LAYMEVES	YYTV FKMRI	LFNY YY FA PI	NHQ TO PYSLLFSG	LLLCRLTYAL	SLNFLAMIHL CLNFL CMTHM	DGHVTG-AVE	RVETSFIK	MGHMDVLS	FV SKGFN
D d-LMBD 2A	SV LS NI VKHS NV SN	I IFVQ FIL	F FP LG YE AL TC	YSTLFKIRI	IFNY YRLI P	HQHSD SNSIIFSA	AYLCRLGAPL	CYNFIQFINM	NSGIE	-DNRTSFSVV	MGTMNVAP	FL GTYFY
D d- LMBD 2B	SP FYRA IVRMEP GI	: G L QI FC F.	P-MIYMCVCS	YSTL FKLRI	ISNY YR LV P	- QQ SNTFSIMFSA	NYLCRLAAPL	AYNFIQICHV	NQDNS	IVSPFSKI	MGDMNAFGDN	AL GKRFT
	610	620	630	640	6 50	660	670	680	690	700	710	720
Hs-LMBD 2	IY YP ML VV IL CI AT	YF SL GTRC LI	VLL GF QQ FM GD I	DDMTSDLVI	NE GKEL IRK			QRQEEGEN	RRREWKERYC	HINRED	strnrni	нтр
X1-LMED 2 Dm-LMED 2	IY YP ML VL IL CI AT	YF SL GTRC LI WF SL GS PA LI	VLL GE QQ FM GD I VAL GE OO FL OMI	ND MT SD LTI FT TA TF LV (DE GKEL IKR	EKRKR		QRLEDGET	RRREWKERYF RRRD FMRTDO	TNRED	TSRNRSV	NSD CDCCLTS
NV-LMED 2	LY YP MC VL LV CL AT	YFSLGTRC L	IYL GFOT FI IEI	DD MS IE YVI	NE GKDL VRR	E		RAEAAKS	RLSDRRKDRS	SSSLS	RDHAT	S
Ce-LMED2 Dd-LMED2A	IY LP IC II LL CA IH IY FP LL IV IV CL ST	IYYRVGA YVL LFNVYSRIM	HNI GFDQ FVEAI NCLNI SK FR FD'	DE MIND MIN VD FSHE OII	NSGRSLVQI DEGKFLIDS	ERNSI ERRKU		KRSNDRSQ TONNIKPL	RTQ <mark>N</mark> WTNSFO SSK <mark>S</mark> PPPSLD	GSSNAG STSNNP	·NGSTTS ·KOIFKSG	K S-TTISK
D d- LMBD 2B	LFFPIFMIVVCVIS	FFNL HKRLA	GSC CIRS LR IV	TD TSEGAVI	DHGL KI LK Q	EREERSLTGGVAP	VKTRMSIVKE	MFTKKKGAGI	LVTDNSQLDO	GASGAHREPS	IDSSNRYKPT	PTKTSIN
	730	740	7 50	760	770	780	790	800	810	820	830	840
Hs-LMED 2	PKES NF SD VN TN RS	AFKY TRANN	RTERDRIELL-	ا 	I 	I D FN AE TETDD P LE	SESGRYOPG-	ا 	ا 	ا GRY	LSMSRSDI	FNDV
X1-LMED 2	OKEP TY TE MT TNRS	S-KYTRASN	RTERDRIEL L-			D FN AD TFNDD PLD	SESGRYQPG-			GRY	LSMSQSNSRI	FD DV
NV-LMBD 2	QT NL TG SD VK	VV KY SR FD N	5 5ALG VPR5 L 2 GG			- FGRS PTTK	DPDIEAEID-			GRI	PPKNL	FDDV
Ce-LMED 2	FKR SNKN	DE ER PM LE DI	DEE			RISMMSPTEHP	OTLIFDESMO	FDDDDDTFSC	CACDETVNAL	H SSSENCCA	SSSGF	FD DM
D d- LMBD 2B	IPKL SRTYTS AA GN	IVY SS TO DGDI	ENSING GI KP IN:	SF FS SI LGH	EKGNASNNN	NNNNNNNNN	NKNSNNNNS	ILTSNYESYS	TPRTKDKQGI	LSSALDKMD	FSFQDDDDHT	FDDIEMG
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Supplementary Figure 2. A ClustalW alignment of LMBD2 sequences.

The LMBD2 protein sequences from *Homo sapiens* (Hs), *Xenopus laevis* (Xl), *Drosophila melanogaster* (Dm), *Nematostella vectensis* (Nv), *Caenorhabditis elegans* (Ce), and *Dictyostelium discoideum* (Dd) are aligned. Accession numbers are the same as in figure 2B. Grey shaded areas are predicted transmembrane domains and yellow shaded areas are predicted glycosylation sites.



Supplementary figure 3. Creating the *lmbd2BmRFP* cell strain

A, a map of the *lmbd2B-mRFPmars* fusion construct is shown. The construct has 3' coding and non coding regions of the *lmbd2B* gene. A Ser-Ala linker immediately follows the coding region. After the linker mRFPmars is translated in frame with the coding region and a terminator sequence directly following the mRFPmars sequence. A bsr cassette with flanking lox p sites (for future removal by Cre recombinase) is present for selection. **Proper** *lmbd2BmRFP* **integration and protein production**

B, genomic PCR analysis showing correct insertion of the mRFP plasmid is shown. Primers flanking the expected mRFP insertions site were utilized. The mRFP construct is 2343 bps in length. In the *lmbd2BmRFP* cells there is about a 2300 bp shift in product size from the 2700 bp band found in the wild type strain to a 5000 bp band in the knock-in strain, indicating the correct insertion.

C shows a western blot using anti-mRFP primary antibody indicating a fusion protein of the correct size. The first lane is a low MW ladder, the second is 1×10^6 LMBD2BmRFP cells, the

third lane is a high MW ladder, and the fourth is Wt control cells. The mRFP protein is 27 KDa, the LMBD2B protein is 89 KDa so the LMBD2BmRFP fusion product is expected to be about 116 KDa and is highlighted by a red arrow.

Results indicating normal function of the *lmbd2B-mRFP* fusion protein

D, cells were plated over *E.coli B/r*, and after 4 days images were taken and plaques measured. Wt and *lmbd2BmRFP* cells produce plaques that are similar in size and are not significantly different. The plaque sizes of *lmbd2BmRFP* are significantly larger than *lmbd2B*- cell plaques. These results suggest that the *lmbd2BmRFP* retains the Wt phenotype. **E** displays images of plaque sizes after 4 days (top row), and development on nitrocellulose filters after 24 hours (bottom row). The *Lmbd2BmRFP* knock in strain appears very similar to Wt cells in plaque formation and development. This is in contrast to *lmbd2b* null cells which show delayed and sparse development. The black scale bar represents 1000 μ m.



Supplementary figure 4. LMBD2B knockout effect on endocytosis, phagocytosis and growth.

In **A**, rates of endocytosis in the different cells types were measured based on the amount of FITC dextran uptake. The procedure was adapted from Brazill et al., 2001 (5). The rate of endocytosis of Wt and LMBD2B null cells were compared. There was an increase in endocytosis rates between Wt and LMBD2B null cells (N =at least 3 rounds). In **B**, phagocytosis was assayed by following the uptake of Carboxylated fluorescent latex beads (FITC#15702) from PolyScience, Warrenton Pa. (1um in diameter) as described by Witke et al., 1992 (63)._The amount of phagocytosis was determined by the amount of fluorescence the cell ingested compared to the amount available. 100 percent relative phagocytosis is equal to complete ingestion of all beads in the solution. There was an increase in phagocytosis in

LMBD2B null cells (N = at least 6 measures for each time point). In C, cells were grown axenically and counts were taken starting at a low density (20×10^4) and continuing every 24 hours for 96 hours. The graph is exponential, each time point represents an additional 24 hours. There was no difference between the growth curve of LMBD2B null cells compared to Wt cells (N = at least 3 experiments).



Supplementary figure 5. LMBD2B null cells show Wt levels of substrate adhesion and actin polymerization.

In **A**, substrate adhesion results are shown. Cells were placed on Petri dishes and allowed to adhere overnight. The cells were shaken at 50 RPM the next day for 45 minutes. The number of cells that detached after shaking was counted. The percentage of cells that remained attached after 45 minutes are depicted in the bar graph. N = At least 3 repeats.

B displays F-actin levels. Growing cells in log phase were placed on a coverslip to adhere. The cells were then fixed and stained with Alexa Fluor 488 phalloidin, which binds polymerized F-actin. The graph shows the intensity of LMBD2B null fluorescence compared to Wt intensity, which was calculated using ImageJ. The F actin levels are the same. N = 15+ cells from 3 rounds of actin staining.

Cytoplasm Side (top)



Supplementary Figure 6. Predicted topography of LMBD2B. The predicted topography of LMBD2B is shown. The program TopPred 0.01 was used to display the likely topography of LMBR1 receptors (9, 59). The transmembrane proteins are oriented such that cytoplasmic regions are on top and extracellular regions are on the bottom. The transmembrane segments are numbered. Since the N-terminus could be modeled as a transmembrane domain but with lower probability, the high probability transmembrane domain is number 2.



Supplementary Figure 7. LMBD2B rescue plasmid

The rescue plasmid was constructed from the pTIKL-MyD plasmid. It contains a G418 resistance cassette for selection and is driven by an actin 15 promoter. The full length LMBD2B cDNA has been inserted.