

SUPPLEMENTAL MATERIAL

Hamouda et al., <http://www.jem.org/cgi/content/full/jem.20150983/DC1>

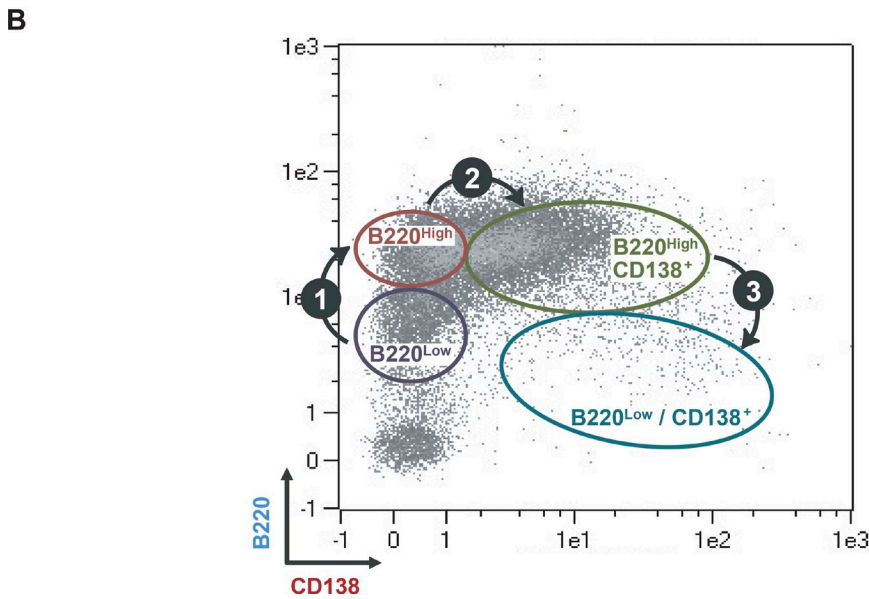
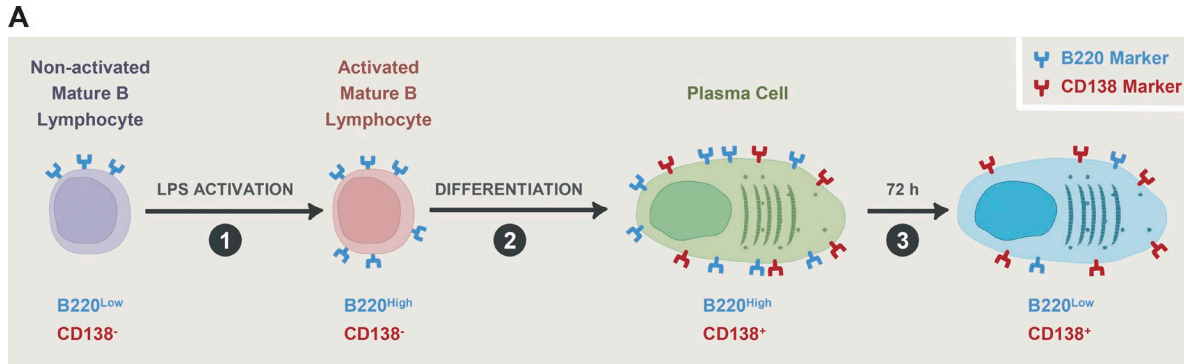


Figure S1. **Schematic representation of B cell activation and differentiation after LPS stimulation.** (A) During plasmacyte differentiation, B cells first exhibit increased expression of B220 (low to high; 1). Then, they differentiate into B220⁺/CD138⁺ plasmocytes (2). Finally, long-lived PCs lose the B220 marker (3). (B) Representation of the characteristic distribution of B cells after LPS treatment.

Table S1. List of antibodies used for flow cytometry, Western blot, and immunohistochemistry

Antibody	Source	Reference	Supplier	Conjugate
Flow cytometry				
Mouse B220	Rat	103227	BioLegend	Pacific blue
Mouse IgD	Rat	560868	BD	APC
Mouse IgD	Rat	405706	BioLegend	PE
Mouse CD138	Rat	558626	BD	APC
Mouse CD4	Rat	553049	BD	PE
Mouse CD8	Rat	558106	BD	Vioblue
Human CD138	Mouse	130091250	Miltenyi Biotec	APC
Anti-Bcl-B	Rabbit	3869	Cell Signaling Technology	
Nonrelevant IgG	Rabbit	3888	Santa Cruz Biotechnology, Inc.	
Anti-rabbit IgG	Donkey	A21206	Invitrogen	Alexa 488
Western blot				
Anti-Bcl-B	Rabbit	3869	Cell Signaling Technology	
Anti-Bcl-2	Rabbit	2872	Cell Signaling Technology	
Anti-Mcl-1	Rabbit	4572	Cell Signaling Technology	
Anti-Xbp-1	Rabbit	7160	Santa Cruz Biotechnology, Inc.	
Anti-Myc-Tag	Rabbit	2278	Cell Signaling Technology	
Anti-Bim	Rabbit	2819	Cell Signaling Technology	
Anti-Actin	Goat	1616	Santa Cruz Biotechnology, Inc.	
Anti-Hsp60	Goat	1722	Santa Cruz Biotechnology, Inc.	
Anti-rabbit IgG-HRP	Goat	7074	Cell Signaling Technology	
Anti-goat IgG-HRP	Rabbit	P0449	Dako	
Immunohistochemistry				
Anti-mouse CD138	Rat	553712	BD	
Anti-rat IgG	Mouse	550325	BD	Biotin
Immunohistochemistry				
Anti-mouse CD138	Rat	60035	STEMCELL Technologies	
Anti-rat Alexa Fluor 647		112-606-003	Jackson ImmunoResearch Laboratories, Inc.	

Table S2. List of primers used for quantitative PCR

Target	Primer forward	Primer reverse
<i>bclb</i>	5'-GCGGCTAAAGGAGCAGGAGG-3'	5'-CTTTCACCTCAAGGAAGAGC-3'
<i>ccnd1</i>	5'-CGCCCTCCGTATCTTACTTCAA-3'	5'-AGCGGGAAGACCTCTCTTC-3'
<i>ccnd2</i>	5'-CCCAGTGTTCCTATTCAA-3'	5'-AATTCATGGCCAGAGGAAAGAC-3'
<i>bmp-6</i>	5'-CTACGCTGCCAACTACTGTGATG-3'	5'-GGATTCATAAGGTGGACCAAGGT-3'
<i>trap</i>	5'-TGGGCGGCTTACACAT-3'	5'-GGCTGGTCTTAAAGAGTGATTTTCC-3'
<i>rank</i>	5'-TGGACACCTGGAATGAAGAAGA-3'	5'-CAGCACTCGCAGTCTGAGTTC-3'
<i>rank-1</i>	5'-GTCTGCAGCATCGCTCTGTT-3'	5'-CAGGAGTCAGGTAGTGTCTTCA-3'
<i>il-7</i>	5'-CATCATCTGAGTGCCACATTAAGA-3'	5'-GGGCAATTAATATCAGTTCCTGTCA-3'
<i>igf-1</i>	5'-TCATGTCGTCTTACACCTCTTCT-3'	5'-CCACACACGAACTGAAGAGCAT-3'
<i>tgf-β2</i>	5'-CGTCCGCTTTGATGTCTCA-3'	5'-GCTGGTGGGAGATGTTAAGT-3'
<i>xbp-1</i>	5'-ACACGCTTGGGAATGGACAC-3'	5'-CCATGGGAAGATGTTCTGGG-3'
<i>myc</i>	5'-AGCCCTAGTGCTGCATGA-3'	5'-GTTTGCCTTCTCCACAGACA-3'
<i>maf</i>	5'-AGCAGTTGGTGACCATGTCG-3'	5'-TTTCAGGGTCCGCCTCTTCT-3'
<i>jun</i>	5'-AGTCCAGCAATGGGCACATC-3'	5'-AAGCGTGTCTGGCATGCA-3'
<i>il-6</i>	5'-ACAAGTCGGAGGCTTAATTACACAT-3'	5'-AAGTGCATCATCGTTGTTTCATACA-3'
<i>ubiquitin</i>	5'-AAGAATTCAGATCGGATGACACACT-3'	5'-GCCACTTGGAGGTTGACACTTT-3'
<i>beta actin</i>	5'-AAGATCTGGCACCACACCTTCT-3'	5'-TTTTACGGTTGGCCTTAGG-3'

Table S3. **IgH and IgK gene rearrangement and somatic mutations in mouse tumors**

CD138 ⁺ DNA from	V gene	D gene	JH gene	No. of bp analyzed	No. of mutations (median)	% of mutations	Status ^a	Presence of intracлонаl variation	No. of identical clones/total clones sequenced
IgH rearrangement									
Tumor #1	IGHV1-63*02	IGHD2-1*01	IGHJ4*01	677	16	2.36%	M, C	yes	5 out of 5
	IGHV1-63*02 ^b	IGHD2-1*01	IGHJ4*01	677	33	4.87%			
Tumor #2	IGHV1-39*01	not assigned	IGHJ4*01	666	14	2.10%	M, O	no	3 out of 6
Tumor #3 ^c	IGHV1S36*01	IGHD3-13*01	IGHJ2*01	774	32	4.13%	M, P	no	2 out of 10
	IGHV1-56*01	IGHD3-2*01	IGHJ2*01	774	21	2.71%	M, P	no	2 out of 10
	IGHV1-53*01	IGHD1-1*01	IGHJ3*01	720	11	1.53%	M, P	no	2 out of 10
	IGHV1S126*01 or IGHV1S35*01	IGHD1-3*01	IGHJ3*01	720	8	1.11%	M, P	no	3 out of 10
IgH rearrangement									
WT_PC_clone 1	IGHV1-39*01 or IGHV1-34*01	IGHD1-1*01	IGHJ4*01	681	13 ^d	1.91%	M	no	2
WT_PC_clone 2	IGHV1S126*01 or IGHV1S35*01	IGHD2-5*01	IGHJ4*01	672	8 ^d	1.19%	M	no	2
WT_PC_clone 3	IGHV1-82*01	IGHD2-1*01	IGHJ4*01	672	12 ^d	1.79%	M	no	1
WT_PC_clone 4	IGHV1-56*01	IGHD2-4*01	IGHJ3*01	179	3 ^d	1.68%	M	no	1
WT_PC_clone 5	IGHV1-53 or IGHV1-56	IGHD4-1*02	IGHJ3*01	538	18 ^d	3.35%	M	no	1
WT_PC_clone 6	IGHV1S126*01	IGHD2-9*01	IGHJ2*01	664	1 ^d	0.15%	UM	no	1
WT_PC_clone 7	IGHV1-63*01	IGHD1-1*01	IGHJ2*01	669	6 ^d	0.90%	M	no	1
WT_PC_clone 8	IGHV14-3*01, IGHV1-63	IGHD4-1*02	IGHJ2*01	652	1 ^d	0.15%	M	no	1
WT_PC_clone 9	IGHV1-77*01	IGHD2-5*01	IGHJ2*01	659	0 ^d	0%	UM	no	1
IgK rearrangement									
Tumor #1	IGKV4-79*01		IGKJ4*01	594	14	2.36%	M, C	yes	5 out of 5
Tumor #2	IGKV4-59*01		IGKJ5*01	261	5	1.92%	M, O	yes	4 out of 5
Tumor #3	IGKV4-59*01		IGKJ2*01	815	23	2.82%	M, O	no	4 out of 8

^aC, clonal; M, mutated; O, oligoclonal; P, polyclonal; UM, unmutated.

^bSubclone of the tumor #1 accumulating mutations in the VDJ junction indicative of ongoing SHM.

^cThe tumor #3 is polyclonal with five distinct rearrangements among 10 sequenced clones (4 clones are shown).

^dTotal number of mutations is shown rather than the median.

Table S4. **Comparison of the biological and clinical features of available MM mouse models; overlap with clinical features of human MM**

Parameter	Human MM	3 ⁺ KE/ <i>bcl-X_L</i>	<i>bcl-X_L</i> × <i>imyc</i>	<i>VK*myc</i>	<i>Εμ-xbp1</i>	<i>Εμ-c-maf</i>	<i>Εμ-bcl-b</i>
		C57BL/6	C57BL/6x129SvJ	C57BL/6	C57BL/6	C57BL/6	C57BL/6
Shorter life span	Yes	No	Yes	Yes	Yes	Yes	Yes
Plasmacytosis localization	BM	Spleen	BM, EM	BM	BM, EM	BM, EM	BM
Indolent	Yes	Yes	No	Yes	Yes	Yes	Yes
Most common Ig isotype	IgG	IgM, IgG1, IgG2, IgA, IgE	IgG > IgM	IgG	IgM >> IgG	IgG = IgM	IgG2b
Age-related M-spike IgG [g/liter], × control	Yes, >35, 5X control	No, ND, 1–2.5× control	Yes, ND, 5–10× control	Yes, 80% at 50 wk, 15 at 70 wk, 5× control	Yes, 30% at 50 wk, ND, 1.5× control	Yes, 50% at 120 wk, 17 at 120 wk, 2× control	Yes, 50% at 50 wk, 30 at 70 wk, 3× control
Transplantability between mice	Yes	ND	Yes	Yes	No	No	Yes
Anemia	Yes	ND	ND	Yes	ND	ND	Yes
Lytic bone disease	Yes	No	Yes	Yes	Yes	No	Yes
Kidney disease	Yes	Yes	ND	Yes	Yes	Yes	Yes
Splenomegaly	Rare	No	Yes, 100% at 6 wk	Yes, 30% at 70 wk	No	No	Yes, 53% at 70 wk