Hematological disorder associated *Cxcr4*-gain-of-function mutation leads to uncontrolled extrafollicular immune response

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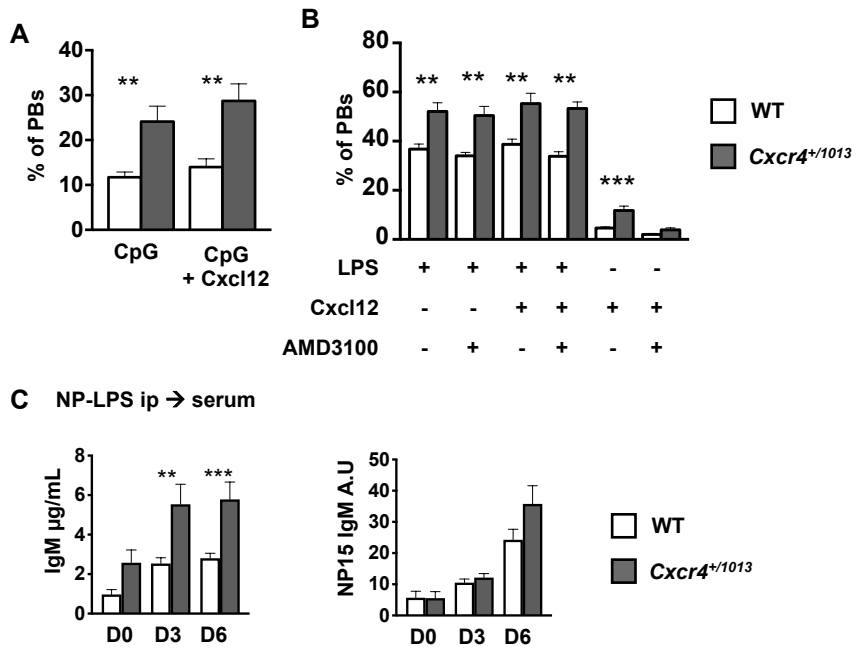
Supplementary Materials

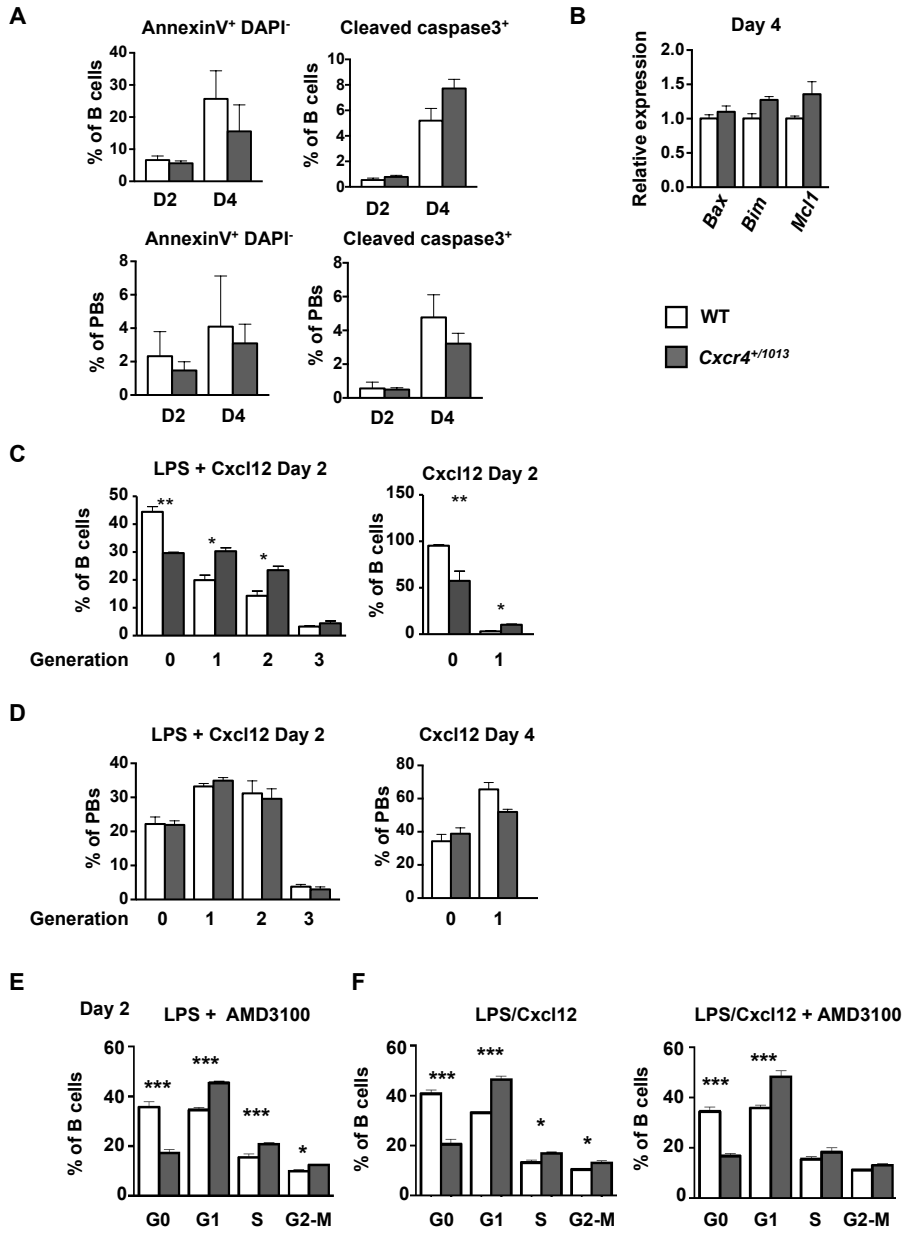
Supplementary methods:

qRT-PCR

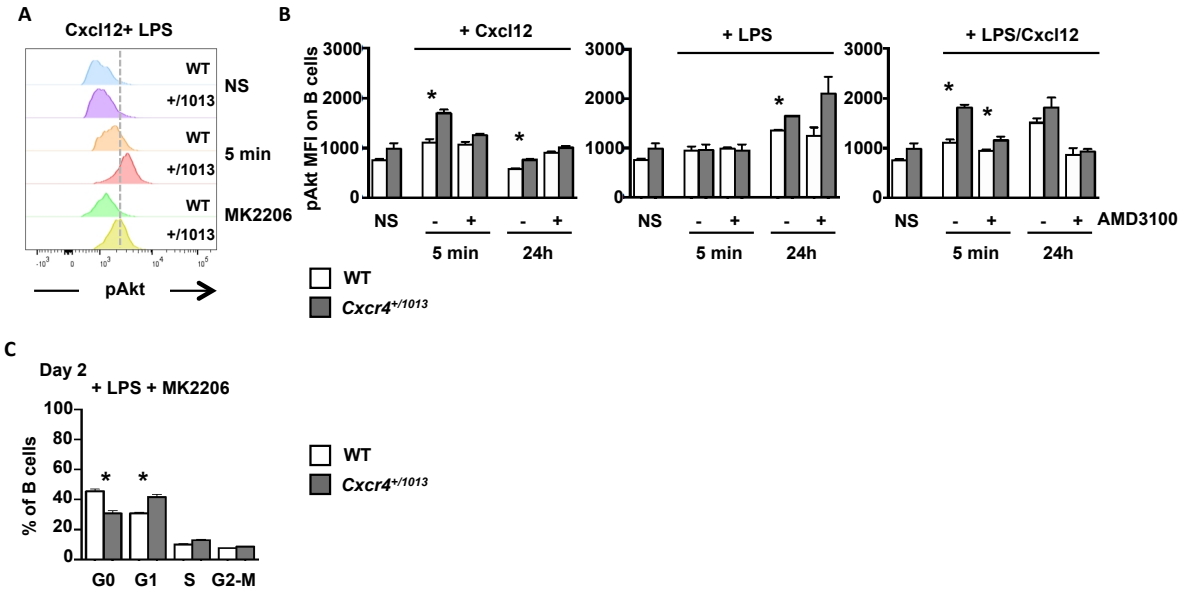
Total cellular RNA was extracted from samples using the RNeasy Plus Mini kit (Qiagen) and reverse transcribed with pd(T)-15 (Roche) and Moloney Murine Leukemia Virus reverse transcriptase (Invitrogen). Amplification of cDNAs was performed by quantitative real-time PCR reactions on a Light Cycler instrument (LC480, Roche Diagnostics) with the Light Cycler 480 SYBR Green detection kit (Roche Diagnostics) using the primers listed in Supplementary Table 2. *Actb* was used as the reference standard for normalization and relative quantification of fold differences in mRNA expression was determined by the comparative delta-delta-CT ($2^{-\Delta\Delta CT}$) method.



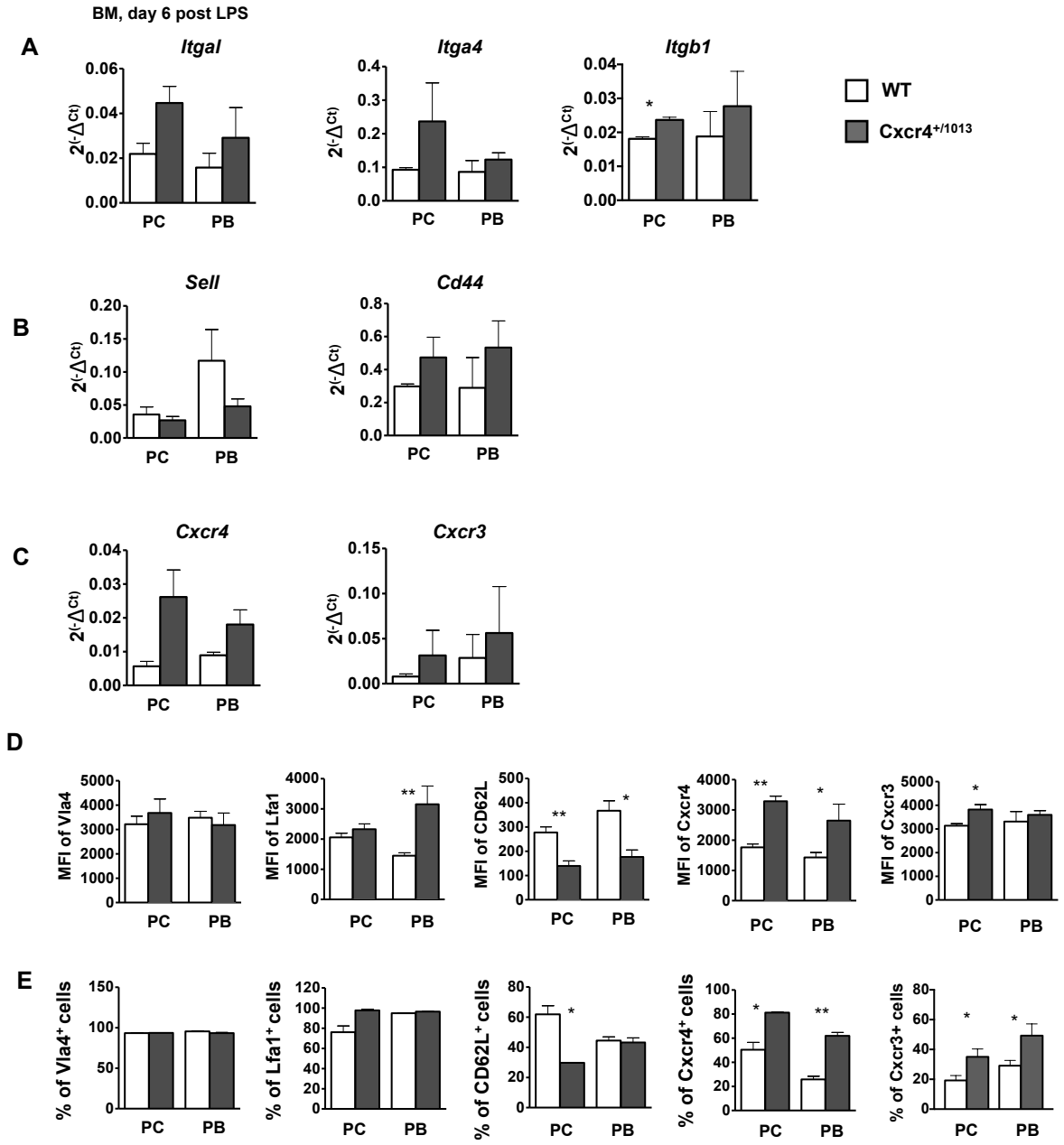
Supplementary figure 1: The gain of function of Cxcr4 enhances TLR-dependent plasmablast differentiation: (A) Splenic B cells were cultured in presence of CpG or CpG+Cxcl12 for 4 days, the percentage of generated PBs was assessed by FACS **(B)** Splenocytes were cultured in presence of LPS and/or Cxcl12 and/or AMD3100. The frequency of PBs generated was assessed by FACS. **(C)** Serum titers of both total IgM^+ and NP15-IgM^+ from both genotypes were measured by ELISA at day 0, 3 and 6 post immunization with NP-LPS. Results are from 2 (A-B) or 3 (C) independent experiments (Mean ± SEM, n=3-4 for A-B; n=5-13 for C-G). Mann–Whitney U test was used to assess statistical significance (*P<0.05, ** P<0.01, *** P<0.001).



Supplementary figure 2: Cxcr4 desensitization controls LPS-mediated B cell cycling but not apoptosis: (A-B) Splenocytes from WT and *Cxcr4*$^{+/1013}$ mice were cultured in presence of LPS for 4 days**. (A)** Apoptosis was assessed by measuring the frequency of Annexin V and cleaved caspase 3 positive B cells (top) and PBs (bottom) from both genotypes at the indicated time points. **(B)** Expression of *Bax, Bim* and *Mcl1* was measured by qPCR on cDNA from cells from both genotypes cultured for 4 days. Expression levels were normalized to the level of *Actb* transcripts. The fold change compared to WT B cell expression is shown. **(C-D)** Splenocytes from WT and *Cxcr4*$^{+/1013}$ mice were loaded with CTV and cultured in presence of Cxcl12 +/- LPS for up to 4 days. The frequency of B cells (C) and PBs (D) present in each generation based on the CTV-dilution at day 2 or 4 is shown. **(E-F)** Frequency of splenic B cells in each cell cycle phase at day 2 post LPS+/-Cxcl12 stimulation in presence or in absence of AMD3100. Results are from one representative experiment out of 2 (A-D) or from two independent experiments (E-F) (Mean ± SEM, n=2-4 for (A-D); n=8 for (E-F)). Mann–Whitney U test was used to assess statistical significance (*P<0.05, ** P<0.01, *** P<0.001).



Supplementary figure 3: Exacerbated Akt signaling in *Cxcr4*$^{+/1013}$ mutant B cells: (A) Representative histograms for pAkt (Ser473) in splenic B cells non-stimulated (NS) or stimulated for 5 minutes with LPS and Cxcl12 in presence or in absence of the MK-2206 inhibitor. **(B)** Quantification of the MFI of pAkt staining on B cells non-stimulated, stimulated for 5 minutes or 24 hours with Cxcl12, LPS or a combination of both in presence or in absence of AMD3100. **(C)** Frequency of splenic B cells in each cell cycle phase at day 2 post LPS stimulation in presence of MK-2206. Results are from one representative experiment out of 2-3 (Mean ± SEM, n=4). Mann–Whitney U test was used to assess statistical significance (*P<0.05).



Supplementary figure 4: Cxcr4 desensitization modulates the migratory/adhesive properties of BM plasmablasts and plasma cells: (A-C) Relative expression of selected transcripts in BM PBs and PCs from both *Cxcr4*$^{+/1013}$ and WT mice at day 6 post immunization. Data are presented as ($2^{-\Delta Ct}$). **(D-E)** Flow cytometry-based quantification of the surface expression of selected adhesion molecules and chemokine receptors on BM PBs and PCs from both WT and C*xcr4*$^{+/1013}$ mice. Data are presented as MFI (geometrical mean) **(D)** and as a percentage of positive cells **(E)**. Results are from one experiment (A-C) or from 2-3 independent experiments (D-E) (Mean ± SEM, n=2 for A-C and n=5-10 for D-E). Two-tailed Student's T test (A-C) and Mann–Whitney U test (D-E) were used to assess statistical significance (*P<0.05).

Table S1. List of antibodies used in flow cytometry and immunofluorescence

Antibody	Clone	Host/Isotype	Supplier
Mouse flow cytometry			
Anti-CD138	281-2	Rat IgG2a, κ	BD Biosciences
Anti-CD45R/B220	RA3-6B2	Rat / IgG2a, kappa	BD Biosciences
Anti-CD19	1D3	Rat IgG2a, κ	BD Biosciences
Anti-CD21/35	7G6	Rat gG2b, κ	BD Biosciences
Anti-CD23	B3B4	Rat IgG2a, κ	BD Biosciences
Anit-Vla-4 (CD49d)	R1-2	Rat IgG2b, κ	BD Biosciences
Anti-Lfa-1 (CD11a)	2D7	Rat IgG_{2a}, κ	BD Biosciences
Anti-CD62L	MEL 14	Rat IgG_{2a}, κ	BD Biosciences
Anti-Ki-67	B56	Mouse IgG_1, κ	BD Biosciences
Anti-Cxcr4	2B11	Rat / IgG2b, kappa	BD Biosciences
Anti-Cxcr3	HTK888	Armenian Hamster IgG	BioLegend
PE Annexin-V Apoptosis Detection Kit I			BD Pharmingen
V450-anti-cleaved caspase-3			BD Biosciences
Anti-KI67 Alexa fluor 700	B56	mouse IgG1	BD Biosciences
Anti-Akt (pS473)	M89-61	Mouse IgG1, κ	BD Biosciences
Anti-S6 (p 235/236)	D57.2.2E	Rabbit IgG	Cell signaling
Mouse immunofluorescence			
Anti-Laminin		Rabbit IgG	Sigma Aldrich
anti-mouse IgM Alexa fluor 594		Goat IgG	Thermofisher
anti-Rabbit IgG Alexa fluor 488		Goat IgG	Thermofisher
Human flow cytometry			
Anti-CD3 FITC	OKT3		BD Biosciences
Anti-CD19 APC-Cy7	SJ25C1		BD Biosciences
Anti-CD38 BV786	HIT2		BD Biosciences
Anti-CD27 BV650	L128		BD Biosciences
Anti-CD138 BV605	MI15		BD Biosciences
Viability dye e506			eBioscience

Table S2. List of primers used for RT-qPCR

	Gene	Reference/ Primers
Taqman assays	*Gapdh*	Mm99999915_g1
	Actb	Mm01205647_g1
	Prdm1	Mm00476128_m1
	Xbp1	Mm00457357_m1
	Irf4	Mm00516431_m1
	Pax5	Mm00435501_m1
	Cd3e	Mm00599684_g1
	Cxcr3	Mm00438259_m1
	Cxcr4	Mm01292123_m1
	Cxcr5	Mm00432086_m1
	Ccr7	Mm01301785_m1
	Ccr10	Mm01292449_m1
	Cd62l / sell	Mm00441291_m1
	Cd11a/Itgal/LFA-1	Mm00801807_m1
	Cd49d/Itga4	Mm01277951_m1
	Cd29/Itgb1	Mm01253230_m1
	Cd44	Mm01277161_m1
	Klf2	Mm00500486_g1
	Bach2	Mm00464379_m1
	Ackr4	Mm02620636_s1
	Tnfrsf13c	Mm00840578_g1
	Tnfrsf17	Mm00495682_m1
	Tnfrsf13b	Mm00495682_m1
	Cxcl9	Mm03047441_m1
	Cxcl10	Mm00445235_m1
	Cxcl12	Mm00445553_m1
	Tnfsf13b	Mm00446347_m1
	Il12p35	Mm00434169_m1
	Il10	Mm01288386_m1
	TNFa	Mm00443258_m1
	Glg1	Mm00486029_m1
	F11r	Mm00554113_m1
	Gja1	Mm01179639_s1
	CD11b	Mm00434455_m1
	ICAM1	Mm00516023_m1
	Itga5	Mm00439797_m1
	Itgb7	Mm00442916_m1
	Cd61	Mm00443980_m1
	MKi67	Mm01278617_m1
	Bcl2	Mm00477631_m1
	Cdt1	Mm00466006_m1
	Gmnn	Mm00517463_m1

	Ccnd1	Mm00432359_m1
	Ccnd2	Mm00438070_m1
	Ccnd3	Mm01612362_m1
	Ccne1	Mm01266311_m1
	Ccne2	Mm00438077_m1
	Ccna2	Mm00438063_m1
	Ccnb1	Mm02015429_g1
	Ccnb2	Mm01171453_m1
	Fh	Mm01321349_m1
	Slc2a1	Mm00441480_m1
	Ldha	Mm01612132_g1
	Ldhb	Mm00493146_m1
	Nr4a1	Mm01300401_m1
	Hk1	Mm00439344_m1
	Hk2	Mm00443385_m1
	CMyc	Mm00487804_m1
	Slc1a5	Mm00436603_m1
	Slc3a2	Mm00500521_m1
	Dgat1	Mm00515643_m1
	Cpt1a	Mm01231183_m1
	Hif1	Mm00468869_m1
SYBR Green assays	*Mcl1*	Forward: 5' GATCATCTCGCGCTACTTGC 3'
		Reverse: 5' CTGATGCCGCCTTCTAGGTC 3'
	Bax	Forward: 5' ACACTGGACTTCCTCCGTGA 3'
		Reverse: 5' TCCTAATGCCAACCTGTGAAGT 3'
	Bim	Forward: 5' GAGTTGTGACAAGTCAACACAAACC 3'
		Reverse: 5' GAAGATAAAGCGTAACAGTTGTAAGATAACC 3'