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Supplemental Data

The Differentiation and Stress Response Factor

XBP-1 Drives Multiple Myeloma Pathogenesis

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Figure S1.

(A) *Eμ-xbp-1s* transgenic mice do not develop amyloid tissue deposition. Kidney tissue sections from a human patient with amyloidosis (left) and 40 week *Eμ-xbp-1s* transgenic mice (right) were stained with Congo Red and analyzed under light (upper) or dark field (lower) microscopy. Representative sections are shown. Note absence of apple green staining in *Eμ-xbp-1s* transgenic kidney. Scale bars, 20

μm.

(B) Increased numbers of plasma cells in *Eμ-xbp-1s* transgenic spleens. Spleens from 40-week-old control (WT) and *Eμ-xbp-1s* transgenic mice were analyzed by light microscopy (H&E) or immunostained with anti-CD138 antibodies, and by flow cytometric analysis on single cell suspensions with anti-B220 and anti-CD138 antibodies. Arrows indicate periaterial sheaths. Scale bars: H&E (left), 50 μm; H&E (right), 20 μm; CD138, 50 μm.

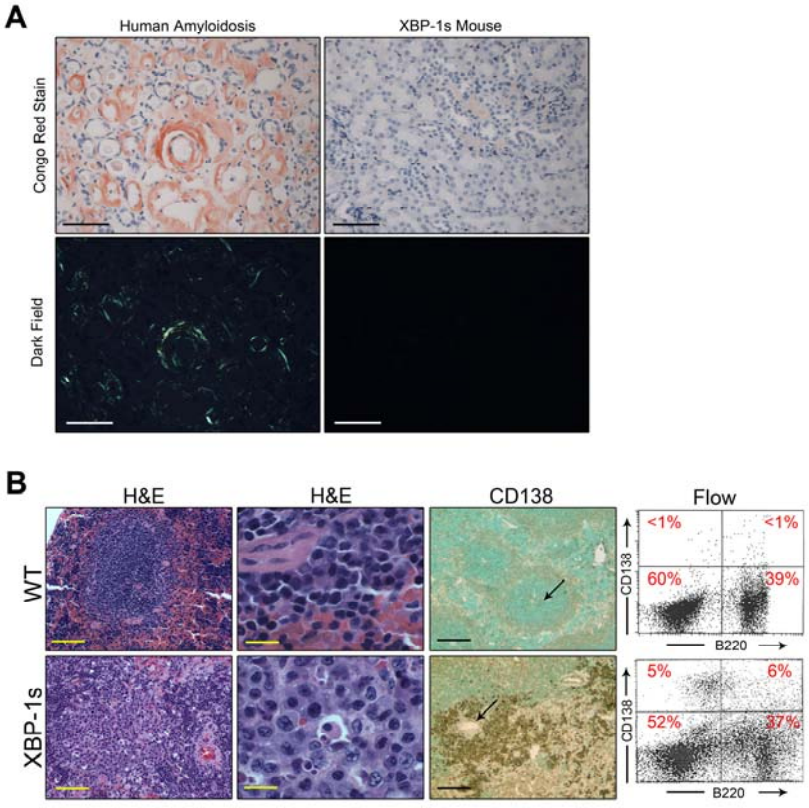

Figure S2.

(A) Unaltered total numbers of T-cell (CD3) and B cell (B220) populations in *Eμ-xbp-1s* transgenic spleens. Splenic B cells from 20-week-old control (WT) and *Eμ-xbp-1s* transgenic mice were double stained with anti-CD3 and B220 antibodies.

(B) Elevated splenic marginal zone B cells and mature follicular B cells and bone marrow mature B cells in *Eμ-xbp-1s* transgenic mice. Splenic B cells from 20-week-old nontransgenic (WT) and *Eμ-xbp-1s* transgenic mice were double stained with anti-CD3 and B220 antibodies or triple stained with anti-IgM, CD21, and CD23 before FACS analysis to distinguish MZ and T2/M populations after gating on $CD23^-$ and $CD23^+$ cells. Bone marrow cells from 20-week-old mice were labeled with anti-B220 and CD43 antibodies to characterize pro- and pre-B cells, as well as with anti-B220 and IgM to characterize mature B cells present in the bone marrow. The flow analysis were done for two independent animals and repeated twice. Representative selected profiles are shown.

(C) Increased serum levels of IL-6 and proliferation of $B220^+$ B cells in *Eμ-xbp-1s* transgenic mice. The plasma levels of IL-6 were analyzed in control (WT) and *Eμ-xbp-1s* transgenic mice using ELISA assay (left panel). Enriched spleen B cell populations obtained from control and *Eμ-xbp-1s* transgenic mice were cultured in the absence or presence of LPS, and proliferation was assayed by measuring incorporation of [^{3}H]thymidine. Each bar represents the mean of triplicates. Error bars show SD.

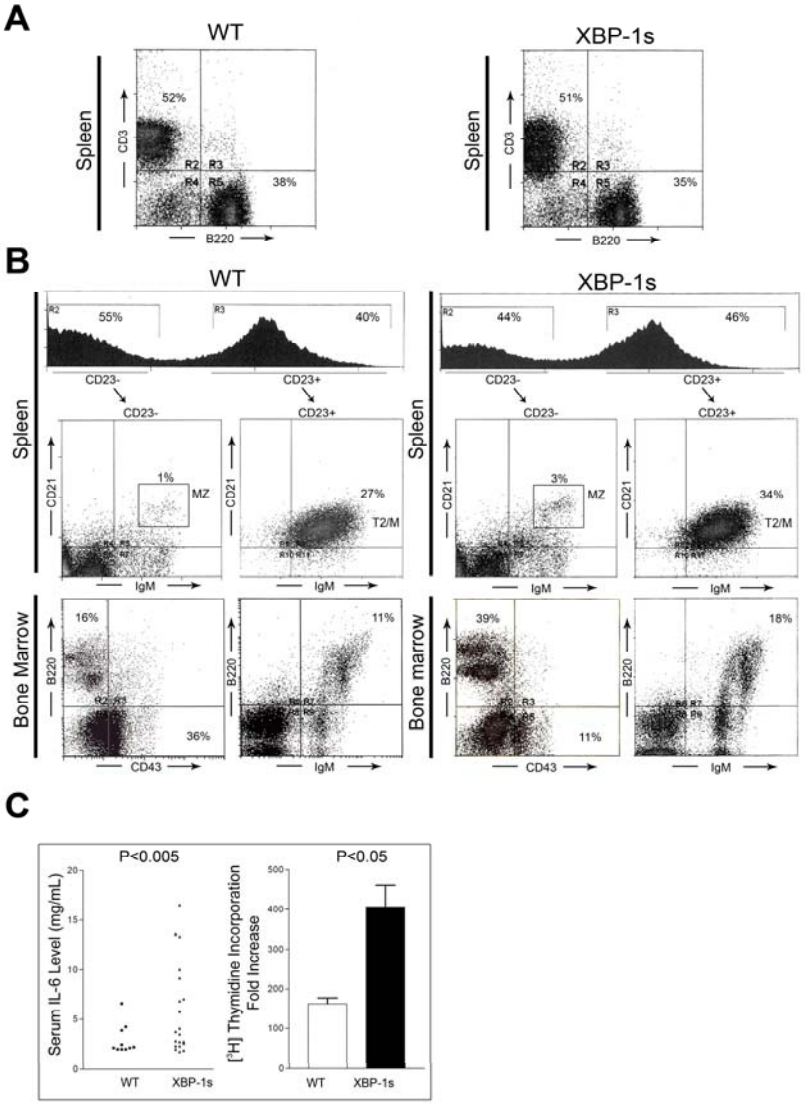

Figure S3. Altered Gene Expression of *Eμ-xbp-1s* B Cells and Tumor Plasma Cells

(A) Ingenuity analysis showing altered expression of CDBPA, CEBPB, CEBPD, and LITAF.

(B) Decreased MCL1 expression in *Eμ-xbp-1s* MM tumors based on the Affymetrix probe 1456381_x_at.

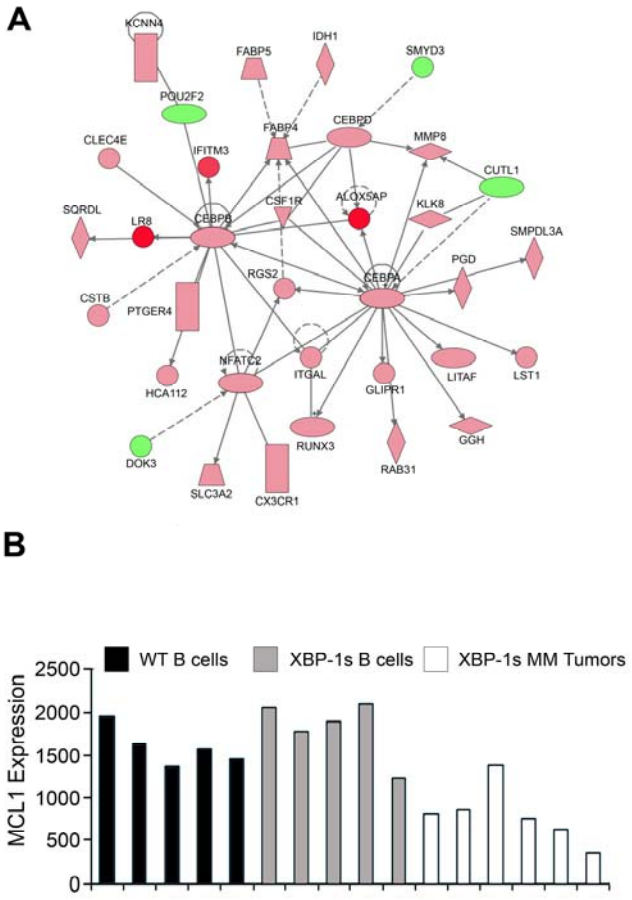

Table S1. Characterization of *Eu-xbp-1s* Transgenic Mice

No.	Age (Wks)	Strain	Age (Months)	Skin Alterations	Bone Lytic Lesions	M Spike	MGUS	MM	Isotype
1	30	S.7	8	+	-	-	-	-	
2	32	S.7	8	-	-	-	-	-	
3	32	S.7	8	+	ND	ND	-	-	
4	32	S.9	8	+	-	-	-	-	
5	32	S.9	8	-	-	-	-	-	
6	34	S.7	9	+	-	+	+	-	IgG,λ
7	34	S.7	9	+	ND	ND	-	-	
8	35	S.9	9	+	-	-	-	-	
9	37	S.7	9	+	-	-	-	-	
10	37	S.9	9	+	ND	ND	-	-	
11	38	S.7	10	-	-	-	-	-	
12	45	S.9	11	+	-	-	-	-	
13	45	S.9	11	+	ND	ND	+	-	polyclonal
14	45	S.7	11	+	ND	ND	-	-	
15	47	S.7	12	+	-	-	-	-	
16	47	S.9	12	+	-	+	+	-	polyclonal
17	48	S.9	12	+	-	-	-	-	
18	52	S.9	13	-	-	+	+	-	IgG,κ
19	56	S.7	14	+	-	-	-	-	IgM,λ
20	57	S.9	14	+	-	+	+	-	IgG,κ
21	58	S.9	15	-	-	-	-	-	
22	58	S.7	15	+	-	+	+	-	IgM,λ
23	59	S.7	15	-	+	+	-	+	IgM,κ
24	59	S.9	15	+	ND	ND	-	-	
25	60	S.7	15	-	-	-	-	-	
26	60	S.7	15	+	ND	ND	+	-	IgG,κ
27	60	S.7	15	+	+	+	-	+	IgM,κ
28	78	S.9	20	-	-	ND	+	-	IgM,κ
29	86	S.9	22	+	-	ND	+	-	IgG,κ
30	87	S.7	22	-	ND	ND	-	+	ND
31	90	S.7	23	-	+	ND	-	+	IgM,κ
32	91	S.9	23	-	ND	ND	-	+	IgG,κ
33	101	S.7	24	+	+	ND	-	+	IgG,κ
34	101	S.9	24	+	ND	ND	-	+	ND
35	102	S.7	24	-	+	ND	-	+	IgM,κ
Total				24/35	5/25	7/20	9/35	8/35	
Percentage				65%	20%	35%	26%	26%	

Clinical and histopathologic characterization of *Eμ-XBP-1s* transgenic mice. The disease manifestations presented in the table are of the propagated lines S7 and S9; however, similar disease was also documented in all transgenic founders.

Diagnostic criteria: MGUS: Presence of clonal M spike in the plasma, ≥10% clonal plasma cells in the bone marrow (BM), and absence of bone lytic lesions. MM: Presence of clonal M spike in the plasma, >10% of clonal plasma cells in the bone marrow, and presence of bone lytic lesions. Polyclonal: polyclonal serum hypergammaglobulinemia.