

Methods

Generation of Spi-C mutant mice. To delete exons 2 through 5 of Spi-C (containing the entire coding region), a targeting vector was constructed in pLNTK¹. A 2 kb genomic fragment upstream of exon 2 was generated by PCR from genomic DNA using the following oligonucleotides: CCACTCGAGTGCTGAGCTTTGAGTTTGATTCT and CTGTCTCATGTCTCGAGGTTACA. The PCR product was digested with XhoI and ligated into the XhoI site of pLNTK. A 4 kb genomic fragment downstream of exon 5 was generated by PCR with oligonucleotides ACGCGTCGACTGTCTCTTGCTATGCAAACA and ACGCGTCGACTTGGTGAGTTACTGGAAGATGGC, digested with Sal I and ligated into the Sal I site of pLNTK. MC50 (a gift from R. Schreiber) and EDJ22 embryonic stem cells, both 129 SvEv background, were transfected and targeted clones were identified by Southern blot analysis with 5' and 3' probes. Blastocyst injections were performed on 2 distinct recombinant clones from each cell line. Male chimeras were bred to female C57BL/6 or 129SvEv mice. Experimental animals were generated from pure 129SvEv background Spi-C^{+/-} breeders and age and sex-matched Spi-C^{+/+} and Spi-C^{-/-} littermates were used. Mice were also backcrossed to C57BL/6, and intercrossed to obtain F1, F2, F4 and F5 generations. When pure 129 SvEv background mice were used, this is mentioned specifically in the text.

Gene expression analysis. The following populations were purified from C57BL/6 mice: splenic B cells, peritoneal macrophages, alveolar macrophages, bone marrow monocytes, conventional splenic dendritic cells and red pulp macrophages. Splenic B cells were purified by MACS (Miltenyi Biotec) using CD43 MicroBeads and negative selection on an LD column, resulting in a purity of >95% B220⁺ cells. Peritoneal macrophages were purified by MACS using negative selection with a combination of CD19⁻, CD4⁻, CD8⁻, DX5⁻, and CD11c-MicroBeads on an LS column (Miltenyi Biotec), followed by positive selection with CD11b-MicroBeads. The purity of

peritoneal macrophages was >98% CD11b⁺F4/80⁺ cells. Alveolar macrophages were collected by bronchoalveolar lavage and were >96% pure by differential counting of a modified Wright-Giemsa stained cytospin (Hema 3 Staining Kit, Fisher Scientific). Bone marrow monocytes were purified by cell sorting for CD11b⁺Ly6C^{hi} cells using CD11b-PE and Ly6C-FITC, excluding CD11b⁺Ly6C^{int} neutrophils by gating. The final purity was >92% CD11b⁺Ly6C^{hi} cells. Conventional splenic DCs were purified by cell sorting for CD11c^{hi}F4/80^{lo/-} cells using CD11c-PE and F4/80-FITC, excluding F4/80^{hi} red pulp macrophages by gating. The final purity was >95% CD11c^{hi}F4/80^{lo/-} cells. Red pulp macrophages were purified by cell sorting for F4/80^{hi} autofluorescent cells using F4/80-FITC, excluding CD11c^{hi} DCs by gating. The final purity was >90% F4/80^{hi} autofluorescent cells. RNA was isolated using RNeasy kits (Qiagen) and biotinylated cRNA probes were prepared using one or two cycle target preparation kits (Affymetrix). Global gene expression analysis was performed using mouse expression 430 2.0 arrays (Affymetrix). Data were normalized and expression values were modeled using DNA-Chip Analyzer (dChip)².

Quantitative real-time PCR. For gene expression analysis, total RNA and cDNA were prepared from various cell types with the RNeasy Mini Kit (Qiagen) and Superscript III reverse transcriptase (Invitrogen). For real-time PCR, the relative standard curve method, SYBR Green PCR master mix and an ABI7000 machine (Applied Biosystems) were used according to the manufacturer's instructions. PCR conditions were 2 min at 50 °C and 10 min at 95 °C, followed by 40 two-step cycles consisting of 15 s at 95 °C and 1 min at 60 °C. The values were normalized to that of HPRT by dividing the average copy number of the respective transcript sample.

Antibodies and flow cytometry. All flow cytometry data was collected on a BD FACSCalibur instrument (BD Biosciences) and analyzed using FlowJo analysis software (Tree Star, Inc.). The following were purchased from BD Biosciences; anti-CD3- fluorescein isothiocyanate (FITC) (clone 145-2C11), anti CD11c-

allophycocyanin (APC) (clone HL-3), anti NK1.1- phycoerythrin (PE) (clone NKR-P1B, NKR-P1C), anti-B220-phycoerythrin PE/Cy7 (clone RA3-6B2), anti-B220-APC (clone RA3-6B2), anti-CD11b-PE /Cy7 (clone M1/70), anti-Gr1-APC (clone RB6-8C5), anti-CD43-biotin (clone S7), anti-CD24-FITC (clone M1/69), anti-IgM- FITC (clone II/41), anti-CD23- PE (clone B3B4), and streptavidin (SA)-APC and SA-PE/Cy7. The following were purchased from eBioscience;

anti-M-CSF-R-biotin (clone AFS98), anti-VCAM-1-biotin (429), anti-AA4.1- APC (clone AA4.1) and anti-IgD-PE (clone 11-26c). Anti-F4/80-PE (A3-1) was purchased from Caltag. Anti-CD68-biotin (clone FA-11) was purchased from AbD Serotec.

Serum immunoglobulins. Basal serum immunoglobulin levels were quantified by ELISA using the SBA Clonotyping System/HRP (SouthernBiotech).

Retroviral constructs and infection. Murine Spi-C cDNA was amplified from C57BL/6 splenocyte RNA using Superscript III (Invitrogen) and oligonucleotides (5'-Spi-C-Bgl II, AGAGATCTGCAACCCAAGACTCTTCAATTC; and 3'-Spi-C-XhoI, CCACTCGAGAGGCAAGAAGCTGGGGTCA). To generate the Spi-C-RV retroviral plasmid, the Spi-C PCR product was digested with BglII and XhoI, and cloned into BglII/XhoI-digested GFP-RV³. Retroviral vectors were packaged using Phoenix E cells as described³. RAW264.7 macrophages were infected with viral supernatants and GFP positive cells enriched by cell sorting. Cells were treated with M-CSF (1 or 10 ng/ml) for 24 hours and used for RT-PCR analysis.

RBC uptake by F4/80 positive splenocytes *in vivo*. RBCs from CD47^{-/-} mice were labeled with CFSE (Sigma-Aldrich). CFSE labeling was performed by incubating 1.5 ml of blood in 30 ml PBS with 2 μ M CFSE for 10 min at 25 °C. The cells were incubated with 2 ml of fetal calf serum for 1 min, washed three times with PBS and resuspended in 1.5 ml of PBS. Mice were injected intravenously with 200 μ l of these cells and the clearance of labeled RBCs was measured by flow cytometry at various time points. Spleens were removed 24 hours after injection of RBCs, and portions of

spleen were embedded in O.C.T compound (Sakura Finetek USA). Splenocytes were also analyzed by flow cytometry, after staining for F4/80 and CD68. The data presented were gated on leukocytes by FSC and SSC to eliminate analysis of non-engulfed CFSE-labeled RBCs.

VCAM-1 reporter constructs. The murine VCAM-1 reporter⁴ spanning -1889 to -1 (VCAM-1-Luc) was generated by PCR spanning the regions from -1895 to +19 first using the following oligonucleotides; -1895, GCCGGTACCGATCTACATAGCCACGGAGAG; and 19, CCACCATCTTCACAGGCATTT; followed by amplification with the following oligonucleotides; -1889, CGGGGTACCATAGCCACGGAGAGTTCTT, and -1, GCCCTCGAGTTCAAGTCTCTGCTTCAAAGCC. The VCAM Ets element was deleted using the oligonucleotide -1019, GAAAAGCTTATTCCGGTTTCTTTCTGATG and inner primers, spanning -1019 to -1, to produce the Δ Ets-Luc reporter. The products were digested with Hind III and Xho I and inserted in to the HindIII/XhoI-digested pBS-Luc⁵.

Transient transfection. J774 cells (6×10^6) were suspended in 400 μ l of Iscove's Modified Dulbecco's Media supplemented with 20% fetal calf serum and transfected with 15.6 μ g of total DNA containing 12 μ g of the indicated luciferase plasmid, 0.6 μ g of pRL-CMV (Promega), 3 μ g of the pEF4 expression vector for Spi-C or PU.1, or empty pEF4 expression vector (Invitrogen). Cells were electroporated at room temperature in 0.4-mm cuvettes (Bio-Rad) in a Bio-Rad Gene Pulser at 300V and 960 μ F, divided equally among three wells, and harvested 20 h later and assayed for Luciferase activity measured and normalized using CMV-Renilla as described³.

Electrophoretic mobility shift assay. 293 F/T cells (Invitrogen) were transfected using calcium phosphate precipitation with empty vector GFP-RV³, PU.1-MIG-R1 (gift from Harinder Singh), or Spi-C-RV. 20 hours after transfection, whole cell extracts were prepared as described⁶. EMSA were performed at room temperature as

described⁷ using 20 µg of extract with probes FcγR2b⁸ or VCAM Ets residing at -1091 to -1079 (AAGGAAGTGGCT)⁴. Supershifts used rabbit anti-PU.1 (Santa Cruz) or polyclonal hamster anti-Spi-C generated against recombinant Spi-C protein. Competitions used 50-fold excess unlabeled annealed probes added 15 min prior to labeled probe. No complexes were found to form using the ECR1 Ets probe taken from another potential Ets GGAA consensus located upstream in the VCAM-1 promoter located at -1490 to -1479. Oligonucleotide sequences used were:

FcγR2b-T: 5' tcgaTTCTTTTCACTTCCCCATTTGGA;
FcγR2b-B: 5' gactTCCAAATGGGGAAGTGAAAAGAA;
VCAM Ets- T: 5' tcgaATAATACAAGGAAGTGGCTAT;
VCAM Ets-Bot: 5' ggaATAGCCACTTCCTTGTATTA;
Mut Vcam-T: 5' tcgaATAATACAAGGACGTGGCTAT;
Mut Vcam-B: 5' ggaATAGCCACGTCCTTGTATTAT;
ECR1 Ets-T: 5' tcgaTGTATTCTCAAGAGGGAATTCAGAAG;
ECR1 Ets-B: 5' gagaCTTCTGAATTCCTCTTGAGAATTCT.

Supplementary Figure 1. Spi-C is highly expressed in red pulp macrophages.

Expression of Spi-family transcription factors was assessed by expression microarrays in the indicated cell types. Normalized and modeled expression values for PU.1, Spi-B and Spi-C are shown.

Supplementary Figure 2. Targeting the Spi-C locus by homologous

recombination. **a**, The endogenous Spi-C locus, targeting construct, and targeted loci are shown. Exons are represented by black boxes, and are numbered. BamHI digestion of the germline locus generates a restriction fragment of 11 kb detected by the 5' probe. In correctly targeted clones still containing the neomycin resistance cassette, this probe detects a 4 kb fragment. B, BamHI; S, Sall; X, XhoI; TK, thymidine kinase gene; neo, neomycin resistance cassette. The neomycin resistance cassette was deleted from the neo-containing Spi-C⁻ allele by intercrossing Spi-C^{+/-} mice with the CMV-Cre deleter strain (BALB/c-Tg(CMV-cre)1Cgn/J, Jackson Labs) to produce the Neo-deleted Spi-C allele, referred to as Spi-C^{null}. **b**, Southern analysis of targeted Spi-C alleles. The 5' probe in **a** was used to hybridize to BamHI-digested genomic DNA. DNA was obtained from intercrossing heterozygous Spi-C^{+/-} mice. Shown are examples of DNA produced from tail biopsies of Spi-C^{+/+} (+/+), Spi-C^{+/-} (+/-) and Spi-C^{-/-} (-/-) mice. Intercrosses of heterozygous Spi-C^{+/-} mice was found to generate genotypes at the following frequencies (30%, +/+; 60%, +/-; 9%, -/-; n=551), indicating that some loss of Spi-C^{-/-} embryos is occurring before birth.

Supplementary Figure 3. Spi-C^{null/null} mice have a selective loss of red pulp

macrophages, but normal B cell development. **a**, Spleen cells from Spi-C^{+/+} and neo-deleted Spi-C^{null/null} mice were stained with antibodies to F4/80 and analyzed by flow cytometry. Numbers shown are the percentage of live cells in the indicated gate. **b**, Bone marrow cells from Spi-C^{+/+} or Spi-C^{null/null} mice were stained for B220, CD43,

and either BP-1 and CD24, or IgM and IgD, to identify B cell development subsets as described⁹. No differences were seen in the percentages of B220⁺CD43⁺ (A-C) or B220⁺CD43⁻ (D-F) fractions between Spi-C^{+/+} or Neo-deleted-Spi-C^{null/null} bone marrow cells. The percentages were similar between the subsets of B220⁺CD43⁺ cells; BP-1⁻CD24⁻ (Hardy fraction A), BP-1⁻CD24⁺ (Hardy fraction B), and BP-1⁺CD24⁺ (Hardy fraction C), between Spi-C^{+/+} and neo-deleted Spi-C^{-/-} bone marrow. The percentages were also similar between B220⁺CD43⁻ cells; IgM⁻IgD⁻ (Hardy fraction D), IgM⁺IgD^{lo} (Hardy fraction E), and IgM^{lo}IgD^{hi} (Hardy fraction F), between Spi-C^{+/+} and Spi-C^{null/null} bone marrow. **c**, Spleen cells were stained for B220 and IgM to identify B-1 B cells (IgM⁺ B220^{lo/-}) and B-2 B cells (IgM⁺ B220^{hi}).

Supplementary Figure 4. B cell and DC development are normal in Spi-C^{-/-} mice.

a, Spleen cells were stained for B220 and IgM to identify B-1 B cells (IgM⁺ B220^{lo/-}) and B-2 B cells (IgM⁺ B220^{hi}). **b**, Spleen cells were stained for CD11b and CD11c to identify conventional DC (CD11c^{hi}). **c**, CD11c^{low}CD11b⁻ cells in **b** were gated and examined for expression of B220 and Gr-1 to identify plasmacytoid DC (CD11c^{lo} CD11b⁻ B220⁺ Gr1⁺). Numbers shown are the percentage of live cells within the indicated gate. **d**, Spleen cells from Spi-C^{+/+} or Spi-C^{-/-} mice were stained with antibodies to B220, CD23 and AA4.1. Similar development of mature (B220⁺ AA4.1⁻) B cells and immature (B220⁺, AA4.1⁺) B cells is evident between Spi-C^{+/+} and Spi-C^{-/-} mice (upper panels). Within AA4.1⁺B220⁺ immature cells, profiles of IgM and CD23 expression showed a normal progression of transitional B cells (middle panels). Within AA4.1⁻B220⁺ mature B cells, profiles of IgM and CD23 expression were also normal (lower panels). **e**, Bone marrow cells from Spi-C^{+/+} or Spi-C^{-/-} mice were stained for B220, CD43, and either BP-1 and CD24, or IgM and IgD, to identify B cell development subsets. No

differences were seen in the percentages of B220⁺CD43⁺ (A-C) or B220⁺CD43⁻ (D-F) fractions between Spi-C^{+/+} or Spi-C^{-/-} bone marrow cells. The percentages were similar between the subsets of B220⁺CD43⁺ cells; BP-1⁻CD24⁻ (Hardy fraction A), BP-1⁻CD24⁺ (Hardy fraction B), and BP-1⁺CD24⁺ (Hardy fraction C), between Spi-C^{+/+} and Spi-C^{-/-} bone marrow. The percentages were also similar between B220⁺CD43⁻ cells; IgM⁻IgD⁻ (Hardy fraction D), IgM⁺IgD^{lo} (Hardy fraction E), and IgM^{lo}IgD^{hi} (Hardy fraction F), between Spi-C^{+/+} and Spi-C^{-/-} bone marrow. Data are the mean \pm S.D. of four mice per group. **f**, Basal serum immunoglobulin levels in 3 to 8 month old Spi-C^{+/+} (white bars) and Spi-C^{-/-} (black bars) mice were quantitated by ELISA. Shown is the serum concentration (mean \pm SD, n=5) of the indicated isotype of antibody.

Supplementary Figure 5. Normal Th1 and Th2 development in Spi-C^{-/-} CD4 T cells.

a, Spleen cells from Spi-C^{+/+} or Spi-C^{-/-} mice were stained with antibodies against CD4 and CD8. Shown are the percentage of live cells inside the indicated gates. CD4⁺ and CD8⁺ T cell percentages were similar between Spi-C^{+/+} and Spi-C^{-/-} mice. **b**, Naïve CD4⁺ T cells from Spi-C^{+/+} and Spi-C^{-/-} mice were stimulated with α -CD3 and α -CD28 mAb using Th1 and Th2 skewing conditions. After 7 days, T cells were restimulated with α -CD3 and α -CD28 mAb for 24 hours, and cells analyzed for intracellular expression of IFN- γ and IL-4.

Supplementary Figure 6. Normal hematopoietic stem cell (HSC), macrophage dendritic precursor cell (MDP) and monocyte development in Spi-C^{-/-} mice.

a, Normal F4/80⁺ macrophages in the peritoneum and liver of Spi-C^{-/-} mice. Cells from peritoneum and liver were stained for F4/80 and CD11b and analyzed by flow cytometry. Numbers are the percentage of live cells within the indicated gate. **b**, Bone marrow cells from Spi-C^{+/+} and Spi-C^{-/-} mice were stained with lineage-specific (Lin)

antibodies (CD3, CD19, B220, Gr1 and TER-119), Sca-1, and c-kit or M-CSF receptor (M-CSF-R). Shown are the percentages of live cells within the indicated gates. Similar percentages of HSC (Lin^- , c-kit^+ , Sca-1^+) and MDP (Lin^- , M-CSF-R^+ , Sca-1^+) were seen between $\text{Spi-C}^{+/+}$ and $\text{Spi-C}^{-/-}$ bone marrow. **c and d**, Bone marrow cells (c) and blood cells (d) from $\text{Spi-C}^{+/+}$ and $\text{Spi-C}^{-/-}$ mice were stained with antibodies to M-CSF-R and CD11b, or F4/80, CD11b and Gr1. Shown are the percentages of live cells within the indicated gates. Similar percentages of monocytes were seen between $\text{Spi-C}^{+/+}$ and $\text{Spi-C}^{-/-}$ bone marrow and blood. **e**, Bone marrow cells from CD45.2^+ C57BL/6 background $\text{Spi-C}^{+/+}$ or $\text{Spi-C}^{-/-}$ mice were transferred into irradiated CD45.1^+ B6.SJL mice. After 10 weeks, spleen cells were stained for CD45.1 and CD45.2. The majority of spleen cells (>97%) at this time were donor derived $\text{CD45.2}^+\text{CD45.1}^-$ cells. Cells were stained for CD45.2, CD45.1, CD11b and CD11c, or NK1.1 and CD3 expression as indicated. Shown are two color histograms of donor-derived ($\text{CD45.2}^+\text{CD45.1}^-$) cells for the indicated surface proteins or autofluorescence (auto). Numbers are the percentage of live $\text{CD45.2}^+\text{CD45.1}^-$ cells present in the indicated gates.

Supplementary Figure 7. Efficient phagocytosis of red blood cells by F4/80^{hi} red pulp macrophages in $\text{Spi-C}^{+/+}$ mice. **a**, CFSE-labeled $\text{CD47}^{-/-}$ RBCs were injected intravenously into $\text{Spi-C}^{+/+}$ or $\text{Spi-C}^{-/-}$ mice. At the indicated times, 5 μl of blood was sampled from the tail vein of recipient mice and analyzed by flow cytometry to determine the fraction of CFSE-labeled RBCs in the circulation. Data were normalized to the level determined at 30 min after injection. **b**, Spleens were prepared from recipient mice 24 hours after injection and frozen sections were examined histologically for the localization of CFSE-labeled cells (green). A lower intensity of CFSE-label was evident in $\text{Spi-C}^{-/-}$ spleens compared to $\text{Spi-C}^{+/+}$ spleens. **c**, Spleen cells from recipient mice were prepared 24 hours after injection of CFSE-labeled RBCs as in **b**, and stained for F4/80 and CD68. Shown is a two color histogram of F4/80 and

CD68 staining to identify F4/80^{hi}CD68⁺ cells (red pulp macrophages), F4/80^{lo} CD68⁺ cells (monocytes and DCs) and F4/80⁻ CD68⁺ cells. Numbers are the percentage of live cells that are present within the indicated gate. **d**, Cells in **c** from the indicated F4/80 CD68 gates are shown in single color histograms for the intensity of their cell-associated CFSE-label. F4/80^{hi}CD68⁺ red pulp macrophages showed the highest level of CFSE labeling, indicating that this population of macrophages ingested the largest numbers of CD47^{-/-} RBCs. F4/80^{hi}CD68⁺ red pulp macrophages are absent in the Spi-C^{-/-} spleen sample. There was no compensatory increase in the phagocytosis of CFSE-labeled RBCs by other populations of macrophages present in the Spi-C^{-/-} spleen, indicating the absence of any population capable of efficient RBC phagocytosis. **e**, Liver cells from recipient mice as described above in **b** were prepared 24 hours after injection of CFSE-labeled RBCs, and stained for F4/80 and CD11b. Shown are single color histograms for the intensity of cell-associated CFSE that is gated on F4/80⁺ CD11b⁺ cells.

Supplementary Figure 8. Spi-C^{-/-} mice have increased splenic iron stores. **a**, Spi-C^{-/-} mice show iron accumulation in spleen. Tissue iron levels in spleen (left panel) and liver (right panel) of Spi-C^{+/+} (white bars) and Spi-C^{-/-} (black bars) maintained on a standard diet (mean ± SD, n=4) (d). **b**, Serum iron levels were determined for Spi-C^{+/+} (white) and Spi-C^{-/-} (black) female mice maintained on a standard diet. **c and d**, Perl's Prussian blue stain for ferric iron in the spleens(c) and liver (d) of 32-week old Spi-C^{+/+} and Spi-C^{-/-} female mice fed a standard diet.

Supplementary Figure 9. Spleen histology in Spi-C^{-/-} mice. **a**, Spleen of Spi-C^{+/+} and Spi-C^{-/-} mice were stained with hematoxylin and eosin. Increased hemosiderin is evident in the red pulp regions of Spi-C^{-/-} spleens. **b**, Electron microscopy of spleen

from Spi-C^{+/+} and Spi-C^{-/-} mice. **c**, Peripheral blood smear of 16 week old 129 SvEv Spi-C^{+/+} and Spi-C^{-/-} mice stained with modified Wright-Giemsa stain.

Supplementary Figure 10. Spi-C regulates VCAM-1 expression.

a, Splenocytes, resident peritoneal cells, and lymph node cells from Spi-C^{+/+} or Spi-C^{-/-} mice were stained for F4/80 and VCAM-1 and were analyzed by flow cytometry.

b, Splenocytes and resident peritoneal cells from Spi-C^{+/+} or Spi-C^{-/-} mice were stained for VCAM-1 and either B220 or IgM and analyzed by flow cytometry.

c, Bone marrow monocytes from C57BL/6 mice were treated for 0, 6 or 12 hrs with M-CSF (20 ng/ml) or GM-CSF (20 ng/ml) as indicated and RNA were prepared as described in the Methods. Global gene expression was assessed by DNA microarray. Normalized gene expression values are shown for Spi-C and VCAM-1.

d, VCAM-1 expression in bone marrow cells from Spi-C^{+/+} and Spi-C^{-/-} mice cultured with M-CSF (20 ng/ml) for 7 days. RNA was prepared and analyzed by RT-PCR for the expression of the indicated genes. **e**, Spi-C induces VCAM-1 expression in macrophages.

RAW264.7 cells transduced with Spi-C-GFP-RV or control RV were purified by cell sorting for GFP. Cells were treated with the indicated concentration of M-CSF for 20 hr, and RNA were prepared and analyzed by RT-PCR for the expression of the indicated genes. **f**, Normalized gene expression values for the hemetransporter FLVCR is shown for B cells, alveolar macrophages, peritoneal macrophages and red pulp macrophages as described in Fig. 4a.

References

1. Gorman, J.R. *et al.* The Ig(kappa) enhancer influences the ratio of Ig(kappa) versus Ig(lambda) B lymphocytes. *Immunity*. **5**, 241-252 (1996).
2. Li, C. & Wong, W.H. Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. *Proc. Natl. Acad. Sci U. S. A.* **98**, 31-36

(2001).

3. Ranganath,S. *et al.* GATA-3-dependent enhancer activity in IL-4 gene regulation. *J. Immunol.* **161**, 3822-3826 (1998).
4. Hosking,B.M., Wang,S.C.M., Downes,M., Koopman,P. & Muscat,G.E.O. The VCAM-1 gene that encodes the vascular cell adhesion molecule is a target of the Sry-related high mobility group box gene, Sox18. *J. Biol. Chem.* **279**, 5314-5322 (2004).
5. Murphy,T.L., Cleveland,M.G., Kulesza,P., Magram,J. & Murphy,K.M. Regulation of interleukin 12 p40 expression through an NF-kappa B half-site. *Mol. Cell Biol.* **15**, 5258-5267 (1995).
6. Nakshatri,H. & Currie,R.A. Differential whole-cell extract preparation and electrophoretic mobility shift assay to evaluate the effect of tyrosine phosphatases on DNA binding activity of transcription factors. *Anal. Biochem.* **236**, 178-181 (1996).
7. Szabo,S.J., Gold,J.S., Murphy,T.L. & Murphy,K.M. Identification of cis-acting regulatory elements controlling interleukin-4 gene expression in T cells: roles for NF-Y and NF-ATc [published erratum appears in Mol Cell Biol 1993 Sep;13(9):5928]. *Mol. Cell Biol.* **13**, 4793-4805 (1993).
8. Schweitzer,B.L. *et al.* Spi-C has opposing effects to PU.1 on gene expression in progenitor B cells. *J. Immunol.* **177**, 2195-2207 (2006).
9. Hardy,R.R. & Hayakawa,K. B cell development pathways. *Annu. Rev. Immunol.* **19**, 595-621 (2001).