Supplementary Materials

Generation of the mouse line iER-STOP-VCAM1.

The "induced ER-retention" (iER) transgenic mouse line iER-STOP-VCAM1 was generated by targeting the intrabody transgene to a defined location in the genome, the ROSA26 locus. The transgene consists of an expression cassette for the anti-VCAM1 ER intrabody scFv6C7.1 and a Cre-excisable STOP-cassette upstream to that. The STOP-cassette serves to prevent ER intrabody expression until mating with a Cre-expressing mouse line **(Fig. S1 A)**. The STOP-cassette contains the genes for RFP as a reporter and Neomycin as a selection marker. Expression of the ER intrabody after excision of the STOP-cassette is driven by the endogenous ROSA26 promoter. To generate the mouse line iER-STOP-VCAM1, the linearized transgenic construct was transfected via electroporation into HM-1 ES cells. Feeder free cultivation of HM1-ES cells was performed under selection pressure using G418 to obtain ES-cells containing the transgene. Surviving cells were then screened by PCR analysis for clones that had inserted the transgene into the ROSA26 locus (data not shown). From 96 clones 3 clones were found to be positive (clone 7, 14 and 67) and insertion of the transgene was further analyzed by Southern Blot. All three clones were positive for homologous recombination but clone 67 appeared to be a mixed clone as signals for wildtype and mutant alleles were different in intensity **(Fig S1 B and C)**. ES cells of the positive clone 7 were then injected into 3.5 day old blastocysts and morulae, which had been obtained after hormonal superovulation followed by uterus rinsing of C57BL/6 mice. A total of 81 embryos were then transferred to 6 pseudopregnant CD1 foster mothers. Four of the pups that had been identified as chimeric could be bred, which comprised a 25% chimeric female and three 100% chimeric males. The degree of chimerism was estimated from the colour of the fur where black originates from the acceptor blastocyst and brownish or whitish fur from ES cells. These four chimeric mice were mated with C57BL/6 animals in order to identify offspring with germline transmission of the transgene by inspection of the colour of the fur. One animal with germline transmission was found among the offspring of a male chimeric mouse, which became the founder animal of the mouse line after confirming the presence of the transgene by PCR and Southern Blot analysis **(Fig. S1 D-F)**. The founder animal was then continuously backcrossed to the C57BL/6 strain. Insertion of the transgene in ES cell clones and mice for generation of the iER-STOP-VCAM1 mouse line were confirmed by PCR and Southern Blot **(Fig. S1 B-F)**.

The iER-VCAM1 mouse line, which constitutively expresses the ER intrabody, was generated by mating with the K14 deleter line to delete the STOP cassette. Deletion of the STOP-cassette, presence of the transgene and absence of the gene for Cre was confirmed by PCR in the iER-VCAM1 mouse line **(Fig. S1 G-I)**. Expression of the ER-intrabody was confirmed by immunoblot using samples from spleen cells **(Fig. S2)**.

Evaluation of anti-VCAM1 detection antibodies. An antibody (clone mab6434, RnD Systems) which had been shown before to recognize a different epitope of VCAM1 than the ER intrabody (clone 6C7.1)[1] was used for detection of VCAM1 on the cell surface **(Fig. S3)**. This avoids potential false negative results due to masking of VCAM1 by ER intrabodies, since despite retention in the ER via the peptide motif KDEL is described to be very efficient[2], it cannot be excluded that some intrabodies may be released from lysed cells or by overwhelming the KDEL sorting apparatus. Compared to the cell surface expression of VCAM1 from bone marrow of a mouse that did not express the ER intrabody (iER-STOP-VCAM1, heterozygous), cell surface expression levels were strongly reduced in bone marrow cells from a heterozygous ER intrabody expressing mouse. However, there was a high background staining and the isotype control was positive. In order to avoid background signals due to the necessity to use an anti-Rat Fc secondary antibody, the detection method was changed to detection based on biotin and fluorescently labelled streptavidin. In order ensure independent binding of the anti-VCAM1 detection antibody and the ER intrabody, the biotinylated anti-VCAM1 antibody (clone 429) was evaluated by staining VCAM1 overexpressing HEK293T cells in competition with purified anti-VCAM1 6C7.1. The binding of the biotinylated anti-VCAM1 antibody clone 429 was not blocked in the presence of saturating concentrations of the purified ER intrabody clone 6C7.1, as the competition test showed **(Fig. S4)**. The cell surface staining of VCAM1 on bone marrow cells was therefore repeated with this antibody.

Supplementary Figures

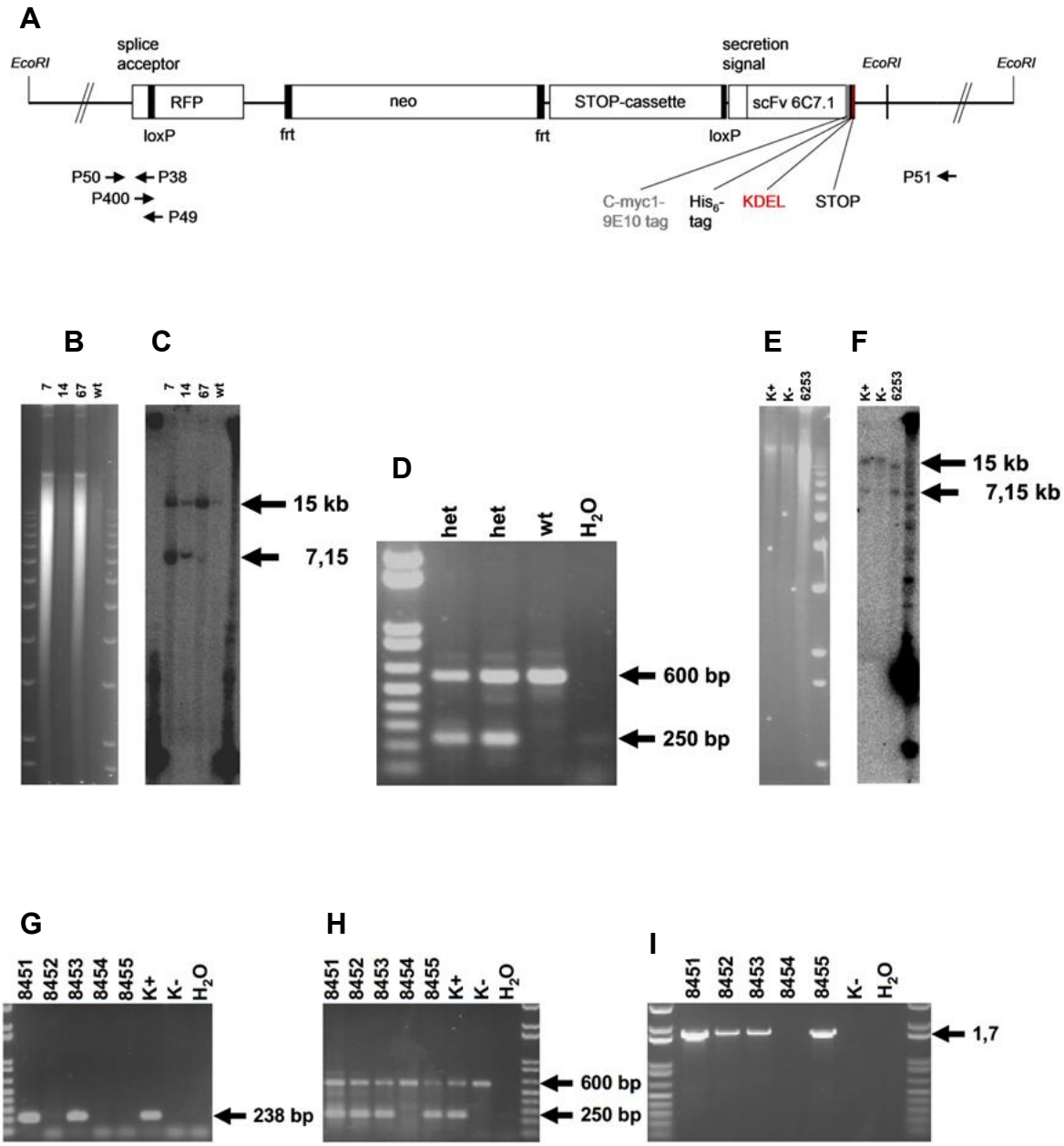

Figure S1: (A) Gene targeting strategy. A STOP-cassette consisting of the gene for red fluorescent protein (RFP) as a reporter and a neomycin resistance gene is flanked by loxP sites and followed by the transgene, the gene of the ER-intrabody. (B) ES clones analyzed by gel electrophoresis and (C) by southern Blot. A fragment sized 7.15 kb indicates the transgene. (D) Genotyping of the iER-STOP-VCAM1 mice by PCR, (E) by gel electrophoresis and (F) by Southern Blot. A PCR fragment sized 250 bp indicates the transgene whereas the wildtype allele is indicated by a 600 bp fragment. (G-I) Transgene analysis by PCR. Genotyping for (G) Cre, (H) ROSA26 and (I) Cre mediated deletion. The presence of the Cre allele in (G) is indicated by a 238 bp fragment. The transgene is indicated by a 250 bp and the wildtype allele by a 600 bp allele in (H). Successful deletion of the STOP-cassette by Cre is indicated by a 1.7 kb fragment in (I). Mice 8452 and 8455 were used as founder animals for the iER-STOP-VCAM1 mouse line.

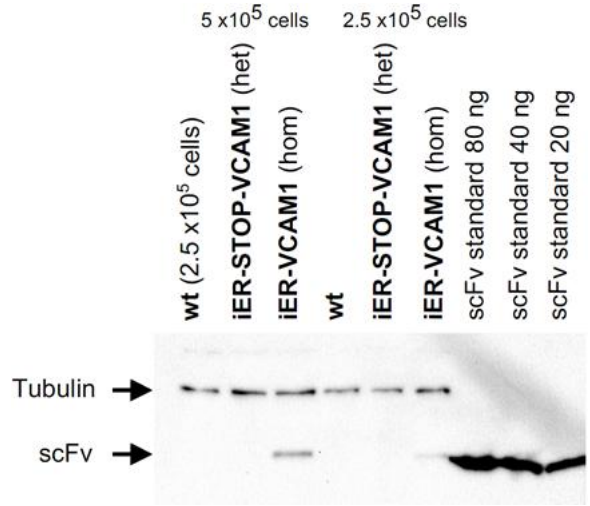

Figure S2: Immunoblot analysis of ER-intrabody expression in mice. The anti-VCAM1 ER-intrabody is expressed in spleen cells of iER-VCAM1 (hom) but not in wildtype or iER-STOP-VCAM1.

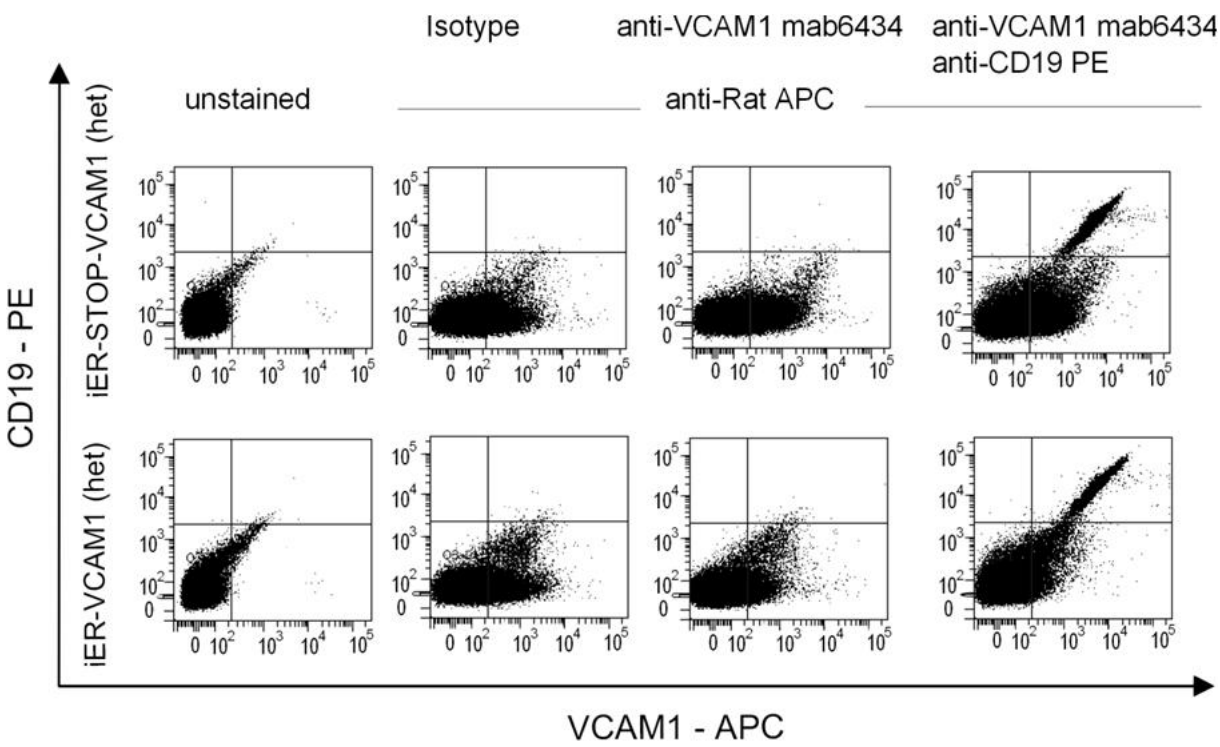

Figure S3: VCAM1 is downregulated in Bone marrow of iER-VCAM1 mice. Staining was performed with the anti-VCAM1 antibody mab6434 from RnD and cells were co-stained for the B-cell marker CD19.

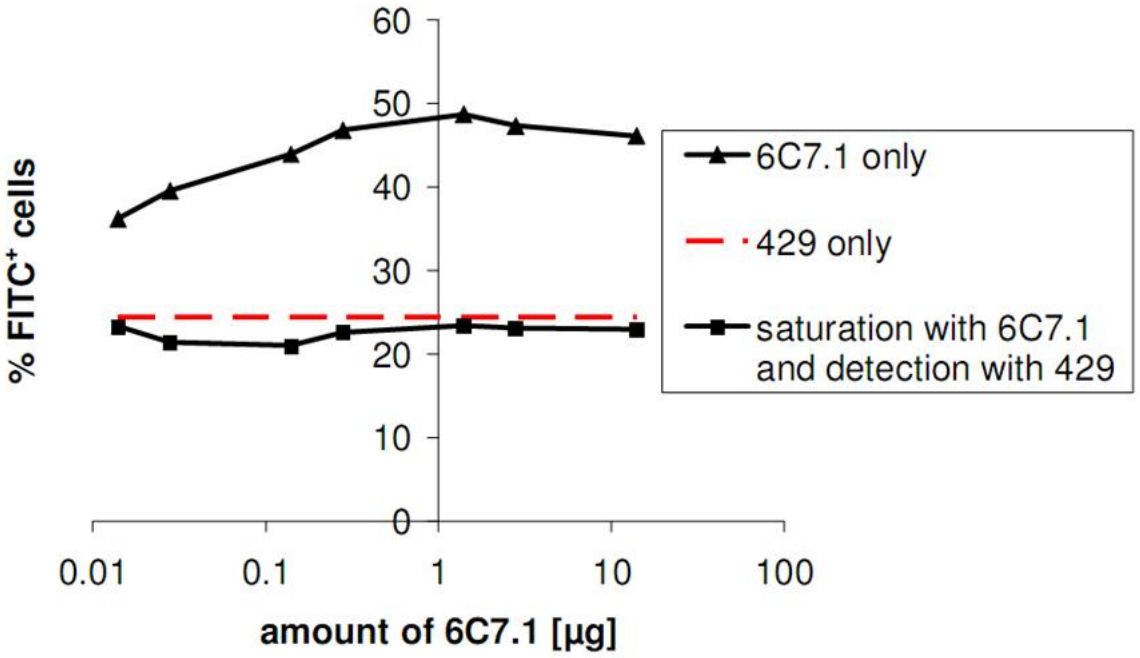

Figure S4: Binding of Anti-VCAM1 clone 429 to VCAM1 is not blocked by the intrabody 6C7.1. Detection of VCAM1 on transiently transfected HEK293T cells by clone 429 was possible even if binding sites had before been saturated by the anti-VCAM1 clone 6C7.1. Competition was performed by incubation of VCAM1-transfected HEK293T cells with 6C7.1 for 1h and the clone 429 was directly added afterwards without any wash step and incubated for 1h as well.

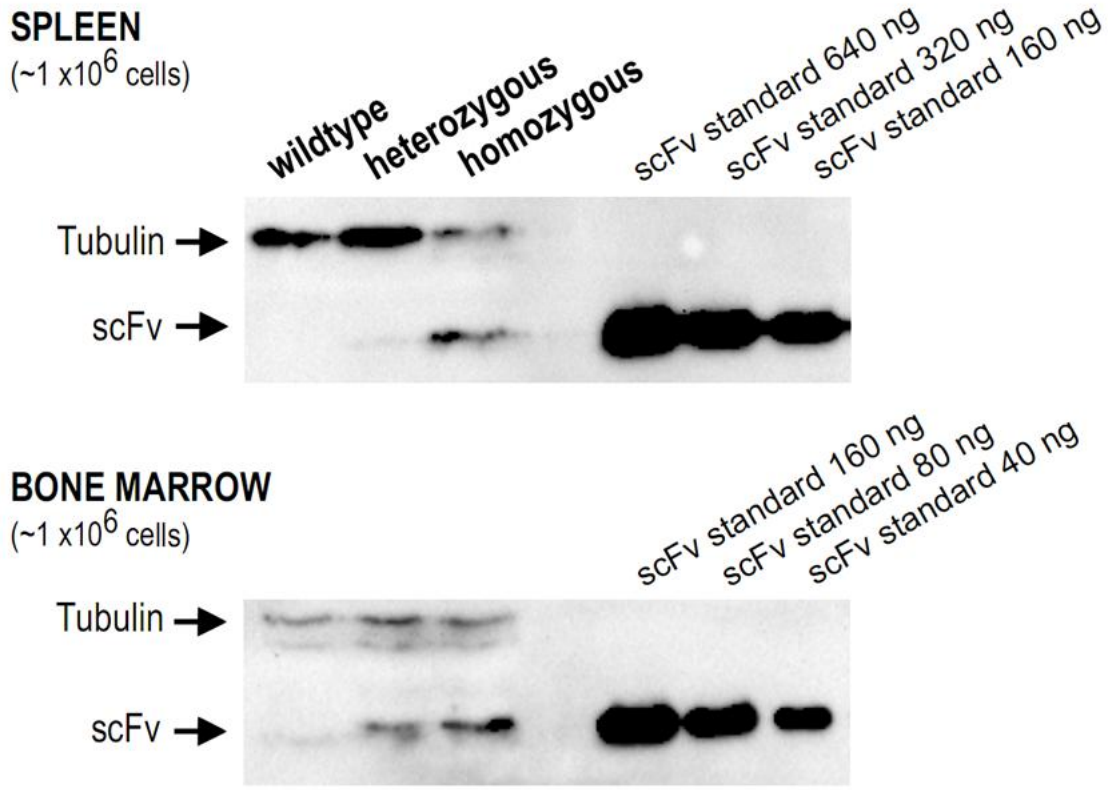

Figure S5: Comparison of expression levels of the ER-intrabody in spleen and bone marrow of iER-VCAM1 mice analysed by Immunoblot.

Literature

1. Strebe N, Guse A, Schüngel M, Schirrmann T, Hafner M, Jostock T, Hust M, Müller W, Dübel S. Functional knockdown of VCAM-1 at the posttranslational level with ER retained antibodies. J Immunol Methods 2009; 341:30-40.
2. Pelham HR. The dynamic organisation of the secretory pathway. Cell Struct Funct 1996; 21:413-9.