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Eukaryotic initiation factor 2α phosphorylation is required for B cell maturation and function in mice

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Running title: Phosphorylation of eIF2α in B cell development

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Abstract

Background

The control of translation initiation is a crucial component in the regulation of gene expression. The eukaryotic initiation factor 2α (eIF2α) mediates binding of the initiator transfer-messenger-RNA (tmRNA) to the AUG initiation codon, and thus controls a rate-limiting step in translation initiation. Phosphorylation of eIF2α at serine 51 (S51) is linked to cellular stress response and attenuates translation initiation. The biochemistry of translation inhibition mediated by eIF2α phosphorylation is well characterized, yet the physiological importance in hematopoiesis remains only partially known.

Design and Methods

Using hematopoietic stem cells carrying a non-phosphorylatable mutant form of eIF2α (eIF2αAA), we examined the efficiency of reconstitution in wildtype or B cell-deficient microMT C57BL/6 recipients in two independent models.

Results

We provide evidence that phosphorylation-deficient eIF2α mutant hematopoietic stem cells may repopulate lethally irradiated mice but have a defect in the development and maintenance of newly formed B cells in the bone marrow and of naïve follicular B cells in the periphery. The mature B cell compartment is markedly reduced in bone marrow, spleen and peripheral blood, and B cell receptor (BCR)-mediated proliferation *in vitro* and serum immunoglobulin secretion *in vivo* are impaired.

Conclusions

The data suggest that regulation of translation through eIF2α phosphorylation is dispensable in hematopoietic reconstitution but essential during late B cell development.

Keywords: hematopoietic stem cells, eIF2α phosphorylation, B cell development, mouse model, translation initiation.

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Introduction

The regulation of translation is an important mechanism that modulates gene expression during embryonic development, cell differentiation and metabolism.[1] There is increasing evidence that the regulated function of translation initiation factors is essential for hematopoietic differentiation and that their deregulation contributes to leukemogenesis.[2-6] Furthermore, the eukaryotic translation initiation factor 4E (eIF4E) acts as a promoter of nucleo-cytoplasmic transport of distinct transcripts and its deregulation is associated with acute and chronic myelogenous leukemias.[5] However, the importance of regulated functions of the key translation initiation factor 2 (eIF2) during hematopoietic differentiation is largely unknown.

The α subunit of eIF2 is subject to negative regulation by phosphorylation. Phosphorylation of eIF2α at serine 51 (S51) is required for cell survival in response to accumulation of unfolded proteins in the ER, translation attenuation and transcriptional induction.[7,8] Mice that carry the non-phosphorylatable homozygous eIF2αS51A mutation (eIF2αAA) die within 18 hours after birth due to hypoglycemia associated with defective gluconeogenesis.[7] The homozygous mutant embryos have a deficiency in pancreatic β cells.[7] Further analysis of the knock-in mutant eIF2α-AA mouse embryonic fibroblasts (MEFs) exhibited an increased basal translation rate and showed that phosphorylation of eIF2α is necessary to inhibit global protein synthesis under conditions of ER stress.[7] The biochemical mechanism of translation inhibition mediated by eIF2α phosphorylation is well characterized, however, little is known about the physiological implications in regeneration and in stem cell biology.[1] In its GTP-bound form eIF2 is part of a ternary complex that binds methionine-loaded initiator tRNA and recognizes the AUG start codon during initiation. After the initiator AUG has been recognized, GTP is hydrolyzed to GDP. The exchange of GDP for GTP is necessary for reconstitution of the ternary complex. This GTP-exchange reaction is blocked by phosphorylation of the α subunit of eIF2. Four kinases may phosphorylate eIF2α at S51: (1) the amino acid control kinase GCN2 (general control non-derepressible-2), (2) the heme-

regulated HRI (haem-regulated inhibitor), (3) the double-stranded RNA-activated protein kinase PKR and (4) PERK, which is activated in response to endoplasmic reticulum (ER) stress via a branch of the "unfolded protein response" (UPR) to prevent overload of the secretory pathway. Whether regulated eIF2α phosphorylation is important during hematopoietic recovery has been only partially addressed.[6]

During T helper cell differentiation and execution of effector functions such as cytokine secretion the regulated activity of eIF2α is needed to direct this process after T cell receptor priming in mice.[3] In line with these data a recent report showed that during human inflammatory T cell differentiation of T helper 17 cells, which are characterized by production of interleukin-17, phosphorylation of eIF2α after amino acid starvation is required for this inflammatory response.[4] However, the role of controlled eIF2α phosphorylation for B cell development and antibody secretion are not fully explored.[6] It has been shown that the proximal sensor of the UPR the inositol-requiring enzyme 1α (IRE1α) is required in B cell lymphopoiesis in a B and T cell-deficient rag2$^{-/-}$ BALB/c background. However, under these conditions the PERK/eIF2α UPR signaling was found to be dispensable for B cell development.[6]

By two independent strategies we examined the efficiency of reconstitution by hematopoietic stem cells carrying a non-phosphorylatable S51A mutant form of eIF2α (eIF2αAA) in wildtype (WT) or B cell-deficient microMT(μMT) C57BL/6 recipients. We show that eIF2α phosphorylation is dispensable during hematopoietic reconstitution and lineage commitment but is required for the development and maintenance of newly formed B cells in the bone marrow and of naïve follicular B (FOB) cells in the periphery. Hence, eIF2α phosphorylation may play a more important role in B cell development than was anticipated from previous studies.

Design and Methods

Viral transduction of fetal liver cells and transplantation

The pcDNA3-eIF2α-SA plasmid was constructed as previously described.[9] The eIF2α-SA-HA DNA fragment was subcloned into the bicistronic GFP-expressing retroviral vector MIGR1.[10] We used the calcium phosphate-mediated transfection (Calcium Phosphate Transfection Kit, Invitrogen, Karlsruhe, Germany) of viral vector DNA into ecoPhoenix cells to produce viral supernatants. WT mice (C57BL/6) were crossed to produce embryos that were removed at E12-E14. The fetal livers were isolated, disrupted into a cell suspension and cultured in 50% DMEM, 50% IMDM, 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM l-alanyl-l-glutamin, 50 µM 2-mercaptoethanol, 50 ng/ml mouse stem cell factor, 10 ng/ml interleukin (IL)-3 and 10 ng/ml IL-6 for approximately 10 h. Fetal liver cells were infected three times by virus in the presence of RetroNectin® (TaKaRa, Cambrex Bio, Apen, Germany) according to the manufacturers protocol at intervals of 12 hours. 2 x 10^6 transfected cells were then transplanted into irradiated (800 rad) WT mice (C57BL/6) through tail vein injection.

Generation of eIF2α chimeric mice

eIF2α mice were generated as previously described.[7] Mice were genotyped by PCR using the following primers: eIF-2/3 5´-CAATGTTGTAGACCCTGACAATGAAGG-3´ and eIF-2/5 5´-CACACACCCATTCCATGATAGTAAATG-3´. The expected sizes of the PCR products are 500 bp for the mutant allele and 410 bp for the WT allele. All mouse experiments were approved by the local committee of the Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit Berlin (Berlin, Germany).

Isolation, transplantation, and culture of fetal liver cells

Heterozygous eIF2α-SA (CD45.2) mice were crossed to produce embryos that were removed at E12-E14 and the fetal livers were isolated. For each embryo, the head was collected for genotyping, and the fetal livers were disrupted into a cell suspension. Fetal liver cells (2×10^6) were transplanted into irradiated (800 rad) μMT (CD45.1) mice through tail vein injection.

Cell sorting and immunoblotting

For magnetic cell sorting of resting splenic B cells an isolation kit (Miltenyi Biotec) was used as previously described.[11] Whole cell extracts were prepared and quantitated by Bradford protein assay.[12] Proteins (30 μg) were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Protein load was normalized by Ponceau red staining and β-actin. Membranes were incubated with mouse monoclonal anti-HA (6E2, Cell Signaling Technology), rabbit monoclonal anti-eIF2α (D7D3, Cell Signaling Technology) and rabbit monoclonal anti-β-actin antibodies (13E5, Cell Signaling Technology), followed by HRP-conjugated secondary antibodies (Santa Cruz, Heidelberg, Germany) and detected by enhanced chemiluminescence (Amersham, Munich, Germany).

Flow cytometry analysis and antibodies

The following monoclonal antibodies were used: Flourescein isothiocyanate (FITC)-conjugated anti-CD45.2 (104), anti-Ly-51 (6C3/BP-1 antigen) and anti-CD43 (S7), phycoerythrin (PE)-conjugated anti-CD45.1 (A20), anti-CD43 (S7), anti-CD21 (7G6, all BD Pharmingen), anti-IgD (11-26, Southern Biotech), anti-CD90 (CT-TH1), and anti-IgM (M31504), PE-Cy5.5-conjugated anti-CD45R (B220, RA3-6B2), biotin-conjugated anti-CD24 (CT-HSA), anti-CD23 (B3B4) and anti-IgM (RMGM15, all Caltag, Hamburg, Germany), allophycocyanin (APC)-conjugated anti-CD19 (1D3, BD Pharmingen) and Streptavidin-

conjugated PerCP and APC. All analyses were performed with FACSCalibur and CellQuest software (BD Bioscience).

Blood counts

20 µl EDTA-anti-coagulated blood samples were used to obtain a complete blood count with a Sysmex, XE-2100 (Norderstedt, Germany).

Proliferation assay

For assaying proliferation of cells CellTiter-Glo Luminescent Cell Viability Assay (Promega, Mannheim, Germany) was used. Assay was carried out according to manufacturer's protocol. Briefly, cells were plated in 96-well plates at 10,000 cells per well in 100 µl medium and treated with indicated amounts of LPS (Sigma), or IgM $F(ab)_2$ (Jackson Immuno Research Laboratories; West Grove, USA). Experiments were performed in three independent replicates. 72 h after treatment 30 µl per well were transferred into an opaque-walled plate and lysed using CellTiter-Glo solution. Average values were calculated and normalized to the respective untreated sample.

ELISA

ELISA were conducted using affinity-purified anti-mouse IgM, IgG1, IgG2a, and IgG2b (all Becton Dickinson) to generate standard curves. To determine concentration of Ig 2 µg of rat anti-mouse isotype-specific antibodies (Becton Dickinson) were used as capture agents. Appropriate dilution of serum samples were loaded for 1 h, followed by addition of biotin-conjugated anti-mouse isotype-specific antibodies and streptavidin-peroxidase-conjugate (Sigma). o-Phenylenediamine (Sigma) was used as a substrate. Enzyme activities were measured at 450 nm in a microplate spectrophotometer (BioRad, Munich, Germany).

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Statistical analysis

Statistics were performed using the Mann-Whitney U test.

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Results

Reconstitution of fetal liver chimera

Two approaches were chosen to investigate the role of eIF2α phosphorylation in hematopoiesis of the mouse. First, we generated retroviral vectors that express the HA-tagged mutant form of eIF2α (MIGR1-eIF2α-SA). The S51 phosphorylation site of the eIF2α coding sequence was mutated to a non-phosphorylatable alanine residue (S51A). We transduced WT fetal liver cells from C57BL/6 embryos at E14 with control MIGR1 and MIGR1-eIF2α-SA vectors and transplanted these cells into irradiated C57BL/6 mice (Figure 1A). Two and four months after transplantation, peripheral blood from transplanted C57BL/6 WT mice was analyzed by flow cytometry analysis for GFP expression (Figure 1B). Whereas almost 80% of mononuclear cells in the peripheral blood derived from WT (MIGR1-transduced) fetal liver cells were GFP positive, only 10% of cells from mutant eIF2α (MIGR1-eIF2α-SA transduced) fetal liver cells showed GFP positivity two months after transplantation (Figure 1B). However, immunoblotting of whole cell extracts from splenic B and non-B cells of mice transplanted with MIGR1-eIF2α-SA transduced fetal liver cells revealed substantial expression of the HA-tagged eIF2α mutant protein (eIF2α-SA-HA; Figure 1A,C). Previously, it has been described that for IRES-dependent gene expression the phosphorylation of eIF2α is indispensable.[13-15] These data suggest that in mice transplanted with MIGR1-eIF2α-SA transduced fetal liver cells hematopoietic cells highly express the HA-tagged mutant form of eIF2α (MIGR1-eIF2α-SA) that competes for WT eIF2α, which is necessary for IRES-mediated GFP expression. Therefore, we suggest that GFP expression does not correlate with the transduction efficiency in MIGR1-eIF2α-SA transduced hematopoietic cells.

Second, fetal liver cells isolated from the homozygous eIF2α knock-in embryos (Ser51/Ala; eIF2α-AA;[7]) and WT embryos (eIF2α-SS) at E14 were transplanted into irradiated B cell-deficient μMT mice to reconstitute the hematopoietic system (Figure 1D). Due to the phosphorylation defect of eIF2α the basal translation rate is increased in mutant (eIF2α-AA) as compared to WT (eIF2α-SS) cells.[7] The irradiated C57BL/6 and μMT mice died within 12

days after irradiation without transplantation. In contrast, mice reconstituted with retrovirally transduced (MIGR1; MIGR1-eIF2α-SA), knock-in mutant (eIF2α-AA) or WT (eIF2α-SS) fetal liver cells survived 10 months and longer. Flow cytometry analysis of the bone marrow (Figure 1E) and peripheral blood (Figure 1F) of fetal liver chimera revealed 90% donor cells (CD45.2 positive) in mice reconstituted with WT (eIF2α-SS) and mutant (eIF2α-AA) cells over a 10-month period. Remaining hematopoietic cells of the recipient μMT mice were CD45.1 positive (Figure 1E). In this study we generated two different mouse models where hematopoietic recovery of stem cells occurs, which express the phosphorylation-deficient mutant form of eIF2α.

B cell development is impaired in phosphorylation-deficient eIF2α stem cells

To analyze whether eIF2α phosphorylation is implicated in hematopoiesis, we performed differential blood counts in reconstituted (1) C57BL/6 WT and (2) B cell-deficient μMT recipients as described above (Figure 1A,D). Differential blood counts in mice transplanted with mutant (MIGR1-eIF2α-SA and eIF2α-AA) as compared to WT (MIGR1, eIF2α-SS) fetal liver cells revealed a 50% reduction of the number of white blood cells (WBC; Table 1). Interestingly, this reduction was due to a decrease in the number of lymphocytes (Table1 and Figure 2A,B), whereas the number of neutrophils (NEU) and all other WBC which were analyzed remained unchanged. Numbers of red blood cells (RBC) and platelets (PLT) were also not different between the groups (Table 1). Flow cytometry analysis with antibodies against the B cell antigen CD19 revealed that the reduction of lymphocytes in mutant eIF2α mononuclear cells in the peripheral blood was due to diminished B cell numbers rather than T cell numbers (CD90) in both models (Figure 2C,D). Accordingly, T cell subset analysis with the markers CD3, CD4, CD8 and analysis of the myeloid compartment using Gr-1, CD11b did not reveal differences between mice transplanted with mutant as compared to WT fetal liver cells (data not shown). Although we were unable to exactly quantify the degree of reconstitution in C57/BL6 WT mice transplanted with retrovirally transduced mutant eIF2α

(MIGR1-eIF2α-SA) fetal liver cells (Figure 1B,C) by GFP expression, we received remarkably similar results in the two different models (C57BL/6 WT and B cell-deficient μMT).

To determine B lineage maturation in the bone marrow as previously described,[16] we analyzed mononuclear cells of μMT mice reconstituted with WT (eIF2α-SS) and mutant (eIF2α-AA) fetal liver cells by flow cytometry analysis (Figure 3). Within the $CD43^+B220^+$ B cell compartment, pre-pro B cells (A), pro B cells (B) and large pre-B cells (C) can be distinguished by antibodies against CD24 and BP1. In the $CD43^-B220^+$ fraction we analyzed small pre-B cells (D) as well as immature (E) and mature (F) B cells using the B cell surface immunoglobulin markers IgM and IgD. A marked reduction in the number of IgM^+IgD^+ mature B cells was found in mice with mutant eIF2α (eIF2α-AA) as compared to WT controls (eIF2α-SS), whereas all other B cell compartments remained unchanged (Figure 3A-F). These data suggest that eIF2α phosphorylation is dispensable for the hematopoietic reconstitution of all hematopoietic lineages analyzed except of $B220^+IgM^+IgD^+$ mature B cells.

Functional impairment of antibody-secreting B cells through deficient eIF2α phosphorylation

Immature B cells in the bone marrow receive signals to home to the spleen to complete maturation.[17] Therefore, we investigated B cell maturation of splenic B cells in μMT mice reconstituted with WT (eIF2α-SS) and mutant (eIF2α-AA) fetal liver cells. Spleen weight and size in mice with mutant eIF2α hematopoietic cells were significantly reduced compared to WT controls (Figure 4A). Flow cytometry analysis of the splenic T and B cell compartments revealed that the number of mature $CD19^+IgM^+IgD^+$ B cells was diminished (Figure 4B,C). In the spleen, B cells mature into either follicular (FOB) or marginal zone (MZB) B cells. Figure 4C shows that the FOB cell pool with $IgD^{hi}IgM^{hi}CD21^{mid}CD23^+$ cells is reduced in mice with eIF2α phosphorylation-deficient hematopoietic cells.

To assess the capacity of eIF2α mutant B cells to respond to mitogenic signals, purified splenic mutant (eIF2α-AA) and WT (eIF2α-SS) B cells were treated with different

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concentrations of LPS and IgM $F(ab)_2$. *In vitro* stimulation of sorted mutant splenic B cells revealed significantly decreased proliferation rates with both mitogens as compared to WT B cells (Figure 5A). Moreover, basal serum Ig levels of IgG1, IgG2a, IgG2b in mutant eIF2α µMT transplanted chimera were hardly detectable and IgM levels were strongly reduced as compared to WT controls (Figure 5B), indicating that development and/or function of antibody-secreting cells is impaired upon deficient eIF2α phosphorylation.

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Discussion

The role of translation initiation factors and their regulation in hematopoiesis is not well understood. Here we show by two independent types of hematopoietic reconstitution protocols that eIF2α phosphorylation at serine 51 is required for the BCR-mediated development and maintenance of newly formed B cells in the bone marrow and of naïve FOB cells in the periphery. BCR stimulation induces a short lived physiologic UPR, similar to the LPS-triggered UPR during the development of antibody-secreting plasma cells.[18] Data presented here show that BCR-mediated proliferation of splenic B cells following *in vitro* stimulation through IgM $F(ab)_2$ is markedly reduced in eIF2αAA mutant cells compared to WT cells. Although it remains to be addressed whether eIF2α phosphorylation is involved in the BCR-mediated UPR, presumably the inability to induce eIF2α phosphorylation during antigen stimulation results in hampered expression of components of the BCR-associated regulatory network. Our data thus complement a previous study showing that in a B and T cell-deficient BALB/c *rag2*$^{-/-}$ background the eIF2αAA mutant does not affect B cell maturation and plasma cell differentiation.[6] In this study, the C57BL/6 WT and B cell-deficient but T cell unaffected μMT genetic background were used for reconstitution by eIF2αAA mutant fetal liver cells. After reconstitution the developing mutant B cells encounter a mixture of WT (from the recipients) and mutant (from the donor) T cells in both of our models. In contrary, in the model of Zhang and colleagues the mutant B cells encounter only mutant donor T cells. In addition, it has been shown that during T helper type 2 priming the regulated phosphorylation of eIF2α is required for activation of the integrated stress response and for cytokine secretion such as interleukin 4.[3] Therefore, we suggest that in the model of Zhang and colleagues the developing mutant T cells might have a defect after priming and are not able to cause the B cell phenotype which we observe in our models.

Although phosphorylation of eIF2α regulates translation initiation in all eukaryotic cells, eIF2α phosphorylation is apparently not essential for the development and regeneration of erythroid, megakaryocytic, myelo-monocytic, and myeloid lineages following stem cell

transplantation (Table 1 and data not shown). This is in accordance with the finding of Zhang and co-workers that deficiency of either IRE1α or phosphorylation of eIF2α does not affect hematopoietic reconstitution. Nevertheless, other studies have revealed that regulated phosphorylation of eIF2α probably plays distinct roles during maturation of different hematopoietic lineages. For example, persistent hyper-phosphorylation of eIF2α through knockout of the eIF2α-phosphatase PPP1R15b gene results in severe growth retardation and impaired erythropoiesis.[2] The impaired erythropoiesis in Ppp1r15b$^{-/-}$ mice can be rescued by homozygous phosphorylation-deficient eIF2αAA alleles, indicating the importance of eIF2α de-phosphorylation in erythropoiesis.[2] We did not observe differences in erythropoiesis between mice reconstituted with the eIF2αAA mutant or WT fetal liver cells, which is in agreement with the conclusion derived from the Ppp1r15b$^{-/-}$ study that de-phosphorylation of eIF2α is an important process during erythropoiesis.

There is increasing evidence that in cell types that have metabolic and immune functions cross-talk between the UPR pathway and immune responsive pathway accumulate at the level of eIF2α phosphorylation.[3,4,19,20] Further studies using experimental inflammatory conditions or interference with the nutrient availability in combination with eIF2α mutations may further elucidate the role of UPR/eIF2α in hematopoietic cell function.

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Authorship and Disclosures

NM designed and performed experiments and interpreted data; RS and CC interpreted data and contributed to writing of the ms; RJK generated the eIF2α knock-in mice, interpreted data and contributed to writing of the ms; BD interpreted data and contributed to writing of the ms; AL and FJ designed research, interpreted data and wrote the manuscript.

The authors declare no financial or commercial conflict of interests.

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Table 1. Differential blood counts.

	MIGR1	MIGR1-eIF2α-SA	eIF2α-SS	eIF2α-AA
WBC (10e9/L)	15 ± 2,8	8 ± 2,3	14,1 ± 3,2	6,5 ± 2,4
NEU (10e9/L)	1,2 ± 0,5	1,2 ± 0,4	0,98 ± 0,16	0,94 ± 0,34
LYM (10e9/L)	13,2 ± 2,2	6,2 ± 1,9	11,7 ± 2,4	4,4 ± 1,8
MONO (10e9/L)	0,51 ± 0,29	0,52 ± 0,15	0,49 ± 0,34	0,54 ± 0,26
EOS (10e9/L)	0,24 ± 0,12	0,18 ± 0,05	0,10 ± 0,07	0,09 ± 0,05
BASO (10e9/L)	0,02 ± 0,01	0,03 ± 0,01	0,017 ± 0,008	0,015 ± 0,011
RBC (10e12/L)	9 ± 1,1	9,7 ± 0,4	9,8 ± 0,5	9,4 ± 0,5
HGB (g/dL)	11,43 ± 4,15	11,92 ± 4,96	14 ± 0,5	14 ± 0,7
HCT (L/L)	0,41 ± 0,06	0,35 ± 0,13	0,47 ± 0,02	0,45 ± 0,02
PLT (10e9/L)	978 ± 184	1161 ± 67	657 ± 142	558 ± 140

WBC, white blood cells; NEU, neutrophils; LYM, lymphocytes; MONO, monocytes; EOS, eosinophils; BASO, basophils; RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; PLT, platelets; MIGR1 (n=7); MIGR1-eIF2α-SA (n=7); eIF2α-SS (n=13), eIF2α-AA (n=13); numbers represent the mean ± SD.

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Figure Legends

Figure 1

Reconstitution of fetal liver chimeras. (A) Scheme of retroviral transduction (MIGR1; MIGR1-eIF2α-SA) of fetal liver cells and transplantation into C57BL/6 WT mice. (B) Percentages of GFP-positive cells (flow cytometry analysis) in the peripheral blood of C57BL/6 mice two and four months after transplantation of fetal liver cells transduced with MIGR1 and MIGR1-eIF2α-SA retroviral vectors (MIGR1, n=7; MIGR1-eIF2α-SA, n=6; data are shown as mean + SEM). (C) Immunoblotting of splenic B and non-B cells for expression of total levels of eIF2α and the HA tag in WT (MIGR1) and eIF2α-SA-transduced cells (MIGR1-eIF2α-SA). (D) Genotyping of WT (eIF2α-SS) and mutant (eIF2α-AA) fetal liver cells and transplantation into B cell-deficient μMT mice. (E) Flow cytometry analysis of bone marrow cells of chimeras reconstituted with WT (eIF2α-SS) and mutant (eIF2α-AA) fetal liver cells. CD45.2 is the marker of the donor cells and CD45.1 is the marker of the remaining recipient cells. (F) Percentages of CD45.2 positive donor cells (flow cytometry analysis) in the peripheral blood of chimeras one, four and ten months after transplantation (n=13 each group; data are shown as mean + SEM).

Figure 2

Decrease of $CD19^+$ B cells in the peripheral blood of transplanted mice. (A) Number of white blood cells and lymphocytes (in 109/L) as determined by differential blood counts of mice after transplantation of retrovirally transduced fetal liver cells and (B) after transplantation of WT (eIF2α-SS) and mutant (eIF2α-AA) fetal liver cells. (C, D) Flow cytometry analysis of B (CD19 positive) and T (CD 90 positive) cells after transplantation at times indicated. MIGR1, n=7; MIGR1-eIF2α-SA, n=6; eIF2α-SS, n=14; eIF2α-AA, n=14. Data are shown as mean + SEM. *P-value < 0,05; **P-value < 0,001; ***P-value < 0,0001, n.s., not significant.

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Figure 3

Diminished mature B cells in the bone marrow of mice transplanted with eIF2α-AA (n=13) mutant fetal liver cells as compared to WT controls (eIF2α-SS, n=17). Flow cytometry analysis of pre-pro-B (A), pro-B (B), pre-B (large, small, C, D), immature (E) and mature (F) B cells in the bone marrow of mice transplanted with WT (eIF2α-SS; black boxes) and mutant (eIF2α-AA; white triangles) fetal liver cells. For discrimination of different B cell subsets antibodies against B220, CD43, IgM, IgD, CD24 and BP1 were used. **P-value < 0,001.

Figure 4

Follicular B cells are reduced in mice transplanted with eIF2α-AA mutant fetal liver cells. (A) Spleen size and weight of mice transplanted with eIF2α-AA or eIF2α-SS fetal liver cells (n=10 each group). (B) Flow cytometry analysis of mononuclear splenic cells with antibodies against CD45.2 (donor cells), CD90 (T cells) and CD19 (B cells). Data are shown as mean + SEM. (C) Flow cytometry analysis of splenic B cells with antibodies against IgM, IgD, CD21 and CD23 to discriminate between follicular ($CD21^{hi}CD23^{lo}IgM^{hi}IgD^{lo}$, FOB) and marginal zone ($CD21^{hi}CD23^{hi}IgM^{lo}IgD^{hi}$, MZB) B cells. Absolute numbers of FOB and MZB cells were calculated by multiplying the fraction of each B cell subset analyzed by the total number of $CD19^{+}$ cells (data are shown as mean + SD). *P-value < 0,05; ***P-value < 0,0001; n.s., not significant. For B and C, n=12 each group.

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Figure 5

Impaired proliferative responses of splenic B cells of mice transplanted with eIF2α-AA mutant fetal liver cells. (A) Relative cell counts of sorted splenic B cells (eIF2α-SS, n=3, eIF2α-AA, n=4) after stimulation with amounts of LPS and IgM $F(ab)_2$ as indicated (data are shown as mean + SEM). (B) Serum Ig concentrations in non-immunized WT (eIF2α-SS, black boxes; n=6) and mutant (eIF2α-AA, white triangles, n=6) mice 3 and 10 months after transplantation. *P-value < 0,05.

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Figure 1

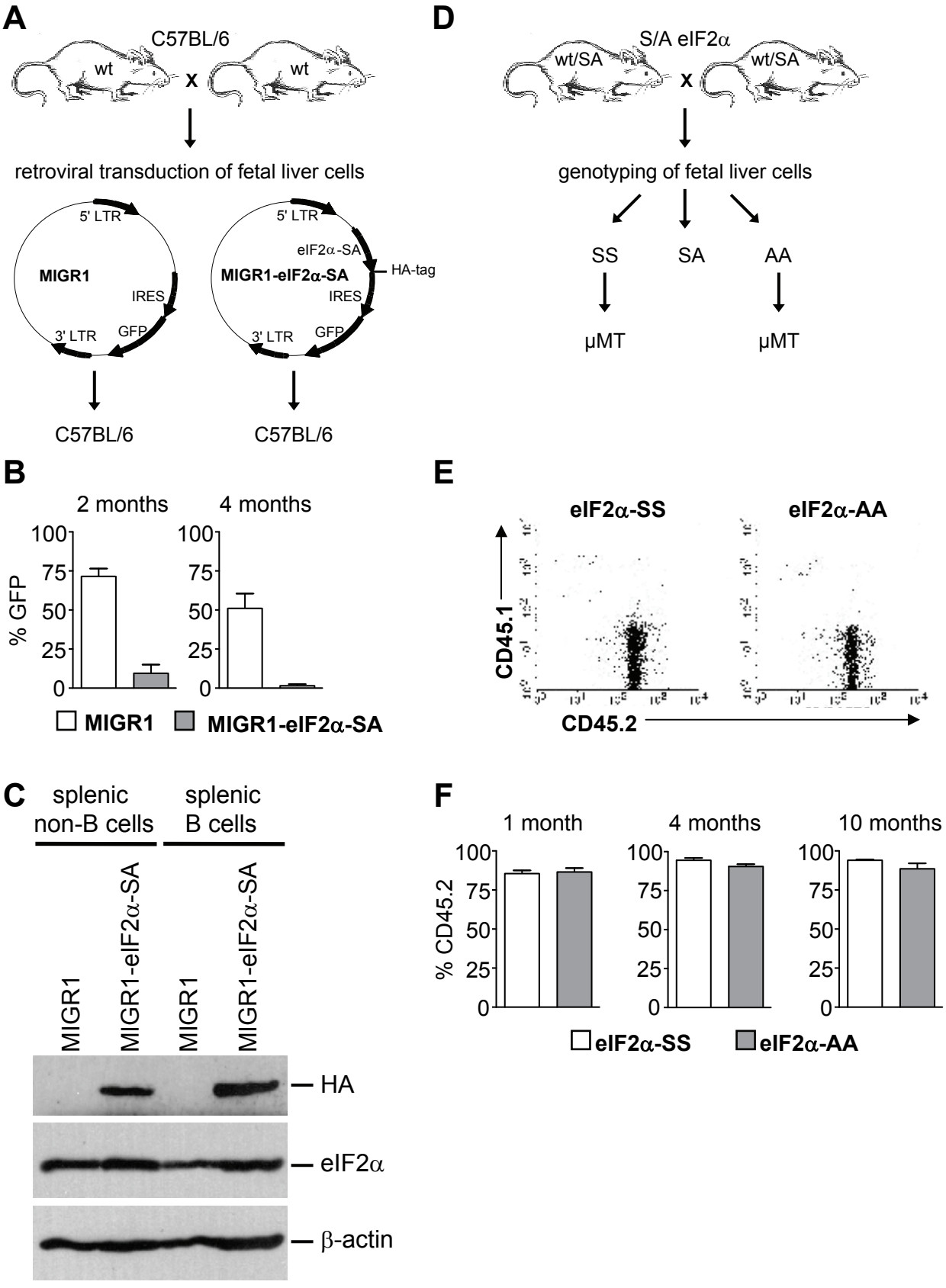

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Figure 2

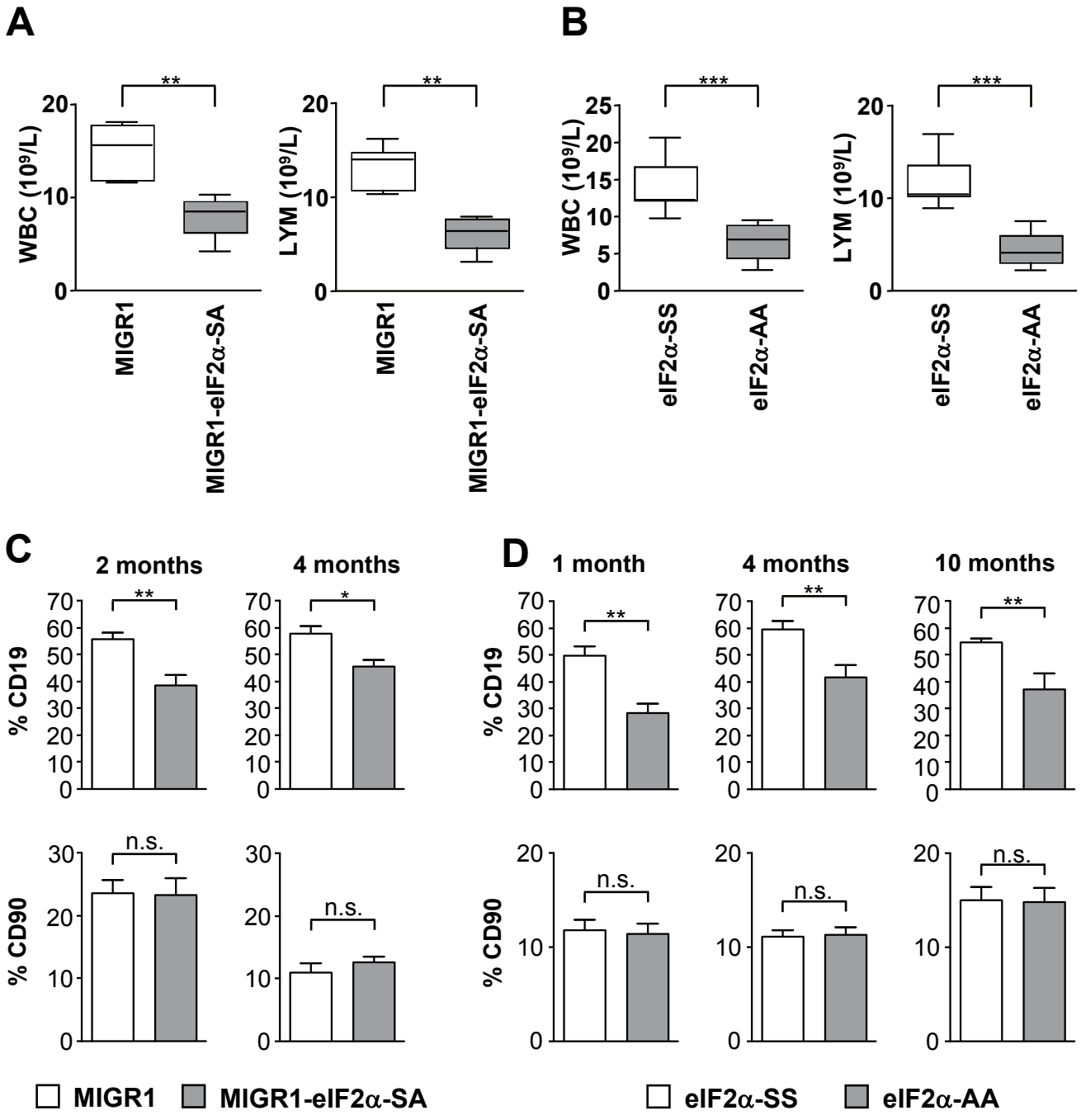

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Figure 3

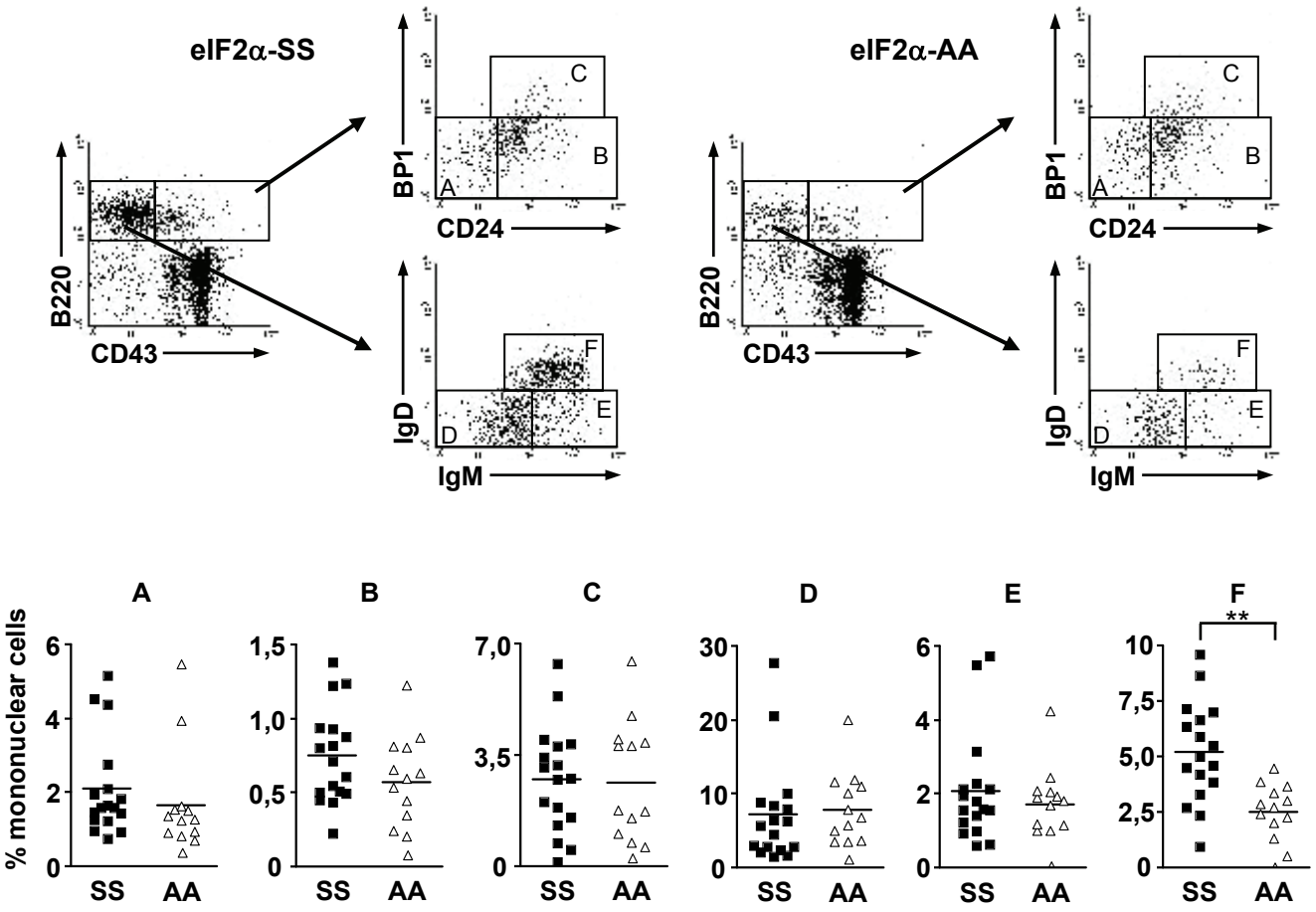

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Figure 4

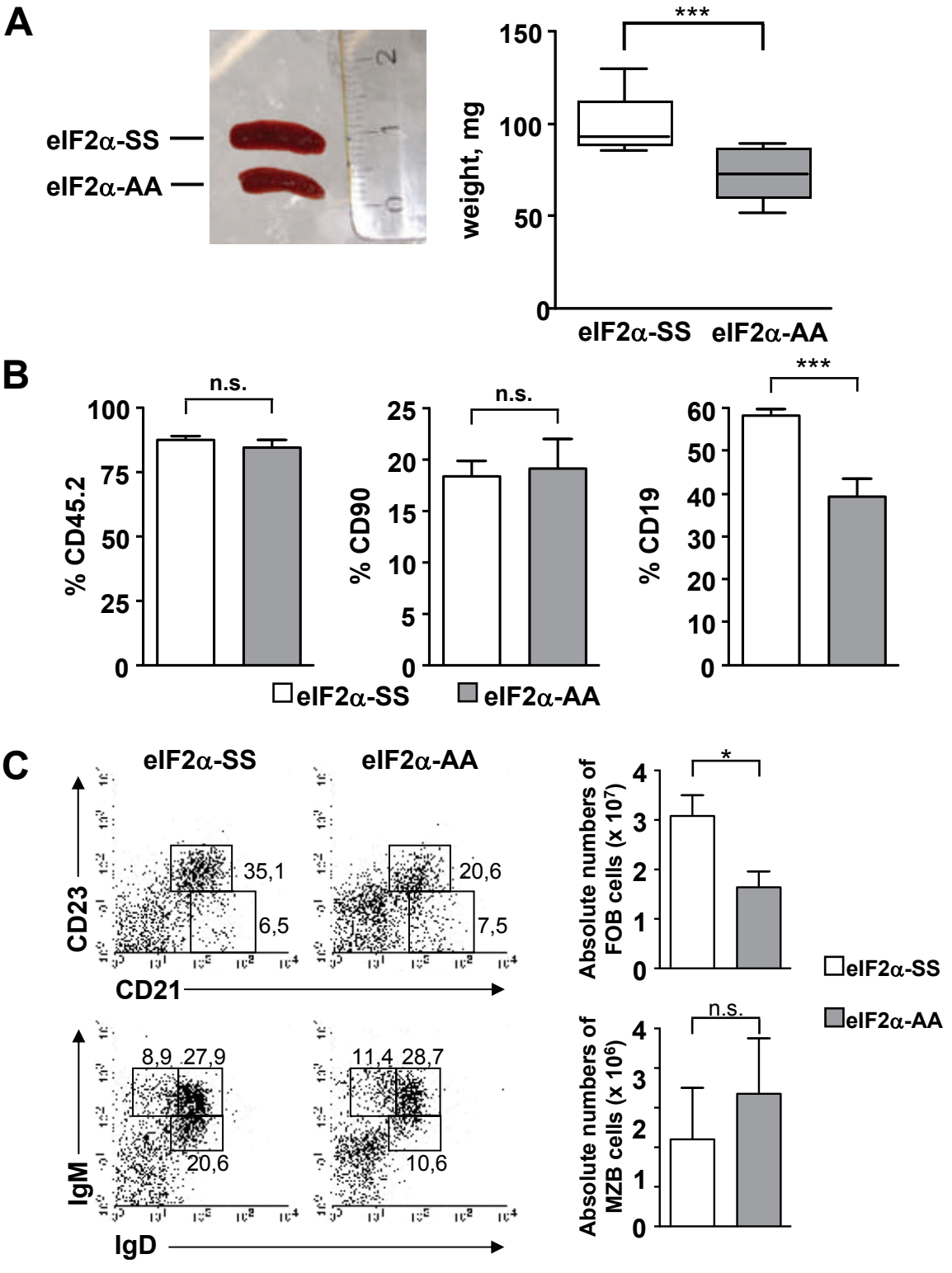

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Figure 5