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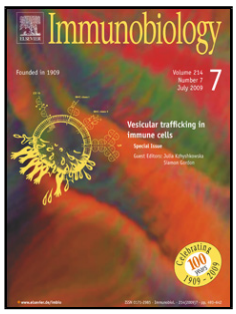

Title: Novel role for galectin-1 in T-cells under physiological and pathological conditions

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Title:

Novel role for galectin-1 in T-cells under physiological and pathological conditions

Short title:

Gal-1 induced apoptosis depends on intracellular Gal-1 level in T-cell

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Abbreviations: Gal-1 – Galectin-1; rGal-1 – Recombinant Gal-1; inGal-1/exGal-1 – Intracellular/extracellular Gal-1; SLE – Systemic Lupus Erythematosus; KO – Knock Out; BMMSC – Bone Marrow Mesenchymal Stem Cell

Abstract

Secreted, extracellular galectin-1 (exGal-1) but not intracellular Gal-1 (inGal-1) has been described as a strong immunosuppressive protein due to its major activity of inducing apoptosis of activated T-cells. It has previously been reported that T-cells express Gal-1 upon activation, however its participation in T-cell functions has remained largely elusive. To determine function of Gal-1 expressed by activated T-cells we have carried out a series of experiments. We have shown that Gal-1, expressed in Gal-1-transgenic Jurkat cells or in activated T-cells, remained intracellularly indicating that Gal-1-induced T-cell death was not a result of an autocrine effect of the *de novo* expressed Gal-1. Rather, a particular consequence of the inGal-1 expression was that T-cells became more sensitive to exGal-1 added either as a soluble protein or bound to the surface of a Gal-1-secreting effector cell. This was also verified when the susceptibility of activated T-cells from wild type or Gal-1 knockout mice to Gal-1-induced apoptosis were compared. Murine T-cells expressing Gal-1 were more sensitive to the cytotoxicity of the exGal-1 than their Gal-1 knockout counterparts. We also conducted a study with activated T-cells from patients with systemic lupus erythematosus (SLE), a disease in which dysregulated T-cell apoptosis has been well described. SLE T-cells expressed lower amounts of Gal-1 than healthy T-cells and were less sensitive to exGal-1. These results suggested a novel role of inGal-1 in T-cells as a regulator of T-cell response to exGal-1, and its likely contribution to the mechanism in T-cell apoptosis deficiency in lupus.

Key words: intracellular galectin-1; activated T-cells; systemic lupus erythematosus; apoptosis

Introduction

Galectin-1 (Gal-1) is a member of a family of β-galactoside binding lectin-type proteins. It has well-defined roles in maintaining immunohomeostasis [1,2] partly *via* the induction of apoptosis of activated T-cells, a function that has been attributed to its secreted form [3]. The mechanism of Gal-1-triggered cell death has extensively been studied *in vitro* and presented by our group and others [4,5]. As it has been shown, stimulation of apoptosis requires the presence of p56lck and ZAP70 kinases, release of ceramide, decrease of mitochondrial membrane potential and caspase activation [4,6,7] and requires the direct interaction between T-cells and the surrounding environment (cells or extracellular matrix) [4]. Immunoregulatory function of Gal-1 has also been confirmed by *in vivo* experiments [5]. Role of the endogenous Gal-1 in disease development and progression has recently been shown in the animal models of autoimmune or inflammatory diseases such as arthritis, colitis, hepatitis, nephritis, encephalomyelitis and SLE [8–13]. All parameters of experimentally induced arthritis, such as incidence, clinical score and paw edema being significantly higher in Gal-1 knockout mice than in wild type animals and Gal-1 therapy efficacious in the amelioration of the disease [14]. The therapeutical effect of exGal- 1 depends on its differential impact on the T-cell subpopulations. It inhibits cytokine production and triggers apoptosis of activated Th1 and Th17 cells while induces cytokine production of Th2 and Treg cells [15]. Hence this mechanism shifts the immune response from the inflammatory Th1 to the Th2 direction [16].

Systemic lupus erythematosus (SLE) is one of the most common and serious systemic autoimmune diseases. Very complex immune dysregulations have been explored, including T- and B-cell functional alterations that eventually lead to the loss

of peripheral tolerance [17,18]. Lupus is characterized by immune-mediated inflammation in multiple organs and by the production of various autoantibodies [19]. T-cell dysfunction, including the malfunction of apoptosis is a major factor in SLE pathophysiology [17]. Although Gal-1 participates in the maintenance of immunohomeostasis by regulating T-cell functions and survival, its role in the lupus T-cell pathology has not yet been revealed.

Intracellular roles of Gal-1 have also been studied; however these functions do not participate in immunoregulation. Two remarkable Gal-1 intracellular functions discovered so far are the membrane anchorage of the oncogene H-Ras [20] and the interaction with spliceosomes via Gemin4 [21]. Neither of these functions requires sugar binding activity of Gal-1 [22,23].

In addition to being targets of exGal-1, T-cells themselves express Gal-1 upon activation [24]. However the cellular localization and the function of the *de novo* expressed Gal-1 have not yet been appropriately clarified. Here we show that activated T-cells express Gal-1 and the protein remains intracellularly. As a result of the *de novo* production of Gal-1, T-cells become more sensitive to the apoptotic effect of exGal-1. Results of the analysis of SLE T-cells support this finding. Expression of Gal-1 in SLE T-cells is diminished compared to healthy control cells and, as a consequence, pathological T-cells are less sensitive to Gal-1-induced cell death.

Materials and methods

Ethics statement

The study was designed in accordance with the guidelines of the Declaration of Helsinki and was approved by the Human Investigation Review Board, University of Szeged.

Cells

The human Jurkat T-cell line was cultured in RPMI 1640 (Gibco, Carlsbad, CA, USA) medium supplemented with 5% heat inactivated fetal calf serum (FCS) (Gibco), 2 mM L-glutamine (Sigma, St. Louis, MO, USA), 100 IU penicillin (Biogal, Kibbutz Galed, Israel) and 100 μg/ml streptomycin (EGIS, Budapest, Hungary). Transgenic Jurkat cell lines, J^{mock} and J^{Gal} were established and cultured as described in Supplemental material.

HeLa human cervix adenocarcinoma cells were transfected as described previously [4]. Mock transfected ($HeLa^{mock}$) or Gal-1 transgenic ($HeLa^{Gal}$) human cervix adenocarcinoma cells were cultured in MEM (Gibco) supplemented with 100 IU penicillin, 100 μg/ml streptomycin, 2mM L-glutamine and 10% FCS.

Blood samples from SLE patients and healthy donors were separated using Ficoll (GE Healthcare, Chalfont St. Giles, UK) gradient centrifugation. Peripheral blood mononuclear cells (PBMC) were stimulated with 5 μg/ml Phytohaemagglutinin (PHA-M, Sigma-Aldrich) and were cultured for 72 hours at 37 °C in RPMI-1640 medium (Gibco) supplemented with 10% FCS, 2 mM L-glutamine, 100 IU penicillin and 100 μg/ml streptomycin.

Murine T-cells were isolated from lymph nodes of C57BL/6 wild type and Gal-1 knock out mice (strain B6.Cg-$Lgals1^{tm1Rob}$/J, 006337, Jackson Laboratory). The lymph nodes were crushed in RPMI 1640 medium until a homogenous cell suspension was achieved, then centrifuged at 300 x g for 10 min and washed twice in phosphate buffered saline (PBS). Afterwards, cells were centrifuged at 300 x g. The cells were

resuspended in RPMI 1640 medium supplemented with 10% FCS and 50μM β-mercaptoethanol (Sigma), followed by activation with 7.5 μg/ml Concanavalin A (ConA, Sigma) for 72 hours.

Isolation, characterization and maintenance of murine bone marrow mesenchymal stem cells (BMMSC) were carried out as described previously [25].

Western blotting

Cell lysates prepared with lysis buffer (10 mM Tris pH 7.5, 1% Triton X-100, 1 mM EDTA, 150 mM NaCl, 1 mM PMSF) from 2×10^5 cells were loaded onto a 12% SDS-polyacrylamide gel (SDS-PAGE). The separated proteins were electro-transferred onto nitrocellulose membrane (Whatman, GE Healthcare). After blocking with 3% gelatin (Sigma) in Tris buffered saline (TBS)/0.05% Tween 20, the membrane was incubated with rabbit anti-mouse Gal-1 antibody [25] or rabbit anti-actin antibody (Abcam, Cambridge, UK) and then with HRP-conjugated swine anti-rabbit IgG (Dako, Glostrup, Denmark). Immunoreactive proteins were visualized using the ECL Plus detection system (Amersham Pharmacia Biotech, GE Healthcare) on X-ray film (Agfa, Morstel, Belgium).

Indirect immunofluorescence and cytofluorimetry

Cells were suspended in cold PBS supplemented with 1% FCS and 0.1% NaN_3 (immunofluorescence buffer, IFB). Gal-1, presented on the cell surface, was detected using a mouse anti-human Gal-1 monoclonal antibody (clone 2c1/6, produced in our laboratory [4]). For detection of inGal-1, the cells were permeabilized in IFB containing 0.1% Triton-X 100 prior to adding the anti-Gal-1 antibody. After incubation for 45 min on ice, the samples were washed two times with IFB then treated with donkey anti-

mouse IgG conjugated with NorthernLights557 (R&D Systems, Minneapolis, MN, USA). For indirect immunofluorescence analysis, the cells were then mounted on microscopic slides with Fluoromount-G (Southern Biotech, Birmingham, AL, USA) and analyzed with laser scanning confocal microscope (Olympus FV 1000). For cytofluorimetric measurements the cells were incubated with biotinylated mAb to Gal-1 (2C1/6) followed with FITC labeled Streptavidin or with mAb to Gal-1 (2C1/6) and goat anti-mouse IgG – Atto 488 (Sigma), and then the samples were analyzed by FACSCalibur flow cytometer using the CellQuestTM software (Becton Dickinson, San Jose, CA, USA).

Apoptosis assays

T-cell apoptosis induced by soluble Gal-1: the cells were treated with human recombinant Gal-1 (produced in our laboratory [26]) for 24 hours and then subjected to DNA content analysis. Briefly, the cells were harvested, washed with PBS, then treated with PBS supplemented with 0.1% Triton X-100, 0.1% sodium-citrate, 10 μg/ml RNase and stained with 10 μg/ml propidium-iodide. After incubation in dark for 15 min at room temperature, the cells were analyzed with flow cytometry. The 'sub-G1' (hypodiploid) population was determined with DNA content analysis using CellQuest software (Becton Dickinson) and was considered as apoptotic cells.

T-cell apoptosis induced in co-culture: the apoptosis induced by cell-derived Gal-1 was detected as previously described [4]. Briefly, $HeLa^{mock}$ (control) or $HeLa^{Gal}$ cells (effector cells, 5×10^3 cells/sample) were plated on cover slips. Target T-cells (2×10^5 cells/sample) were labeled with Hoechst 33342 (100 ng/ml for 30 min at 37 °C) and co-cultured with HeLa cells for 16 hours. Co-culture experiments for murine T-cells were

carried out similarly as described for the human system, except for the following: effector cells were BMMSCs from wild type or Gal-1 knockout (KO) mice (used as control). T-cells derived from wild-type and Gal-1 KO mice were activated with Con-A for 72 hours and then co-cultured with the Gal-1 secreting and Gal-1 KO BMMSCs for 16 hours. The T-cells (human or mouse) were subsequently labeled for phosphatidylserine exposure on the outer cell membrane (early apoptotic signal) with Annexin V-Alexa Fluor 488 (Invitrogen) for 30 min and mounted with Fluoromount-G. Finally, the samples were analyzed with Carl Zeiss (Axioskop 2Mot) fluorescence microscope using AxioCam camera, AxioVision 3.1 software and 20 × objective magnifications. The contrasts of the images were adjusted using Adobe Photoshop CS4 Extended.

The degree of apoptosis was determined by counting at least 100 cells/sample and was calculated as follows: relative apoptotic ratio (RAR) = % of Annexin V positive cells on $HeLa^{Gal\text{-}1}$ - % of Annexin V positive cells on $HeLa^{mock}$.

Quantitative real-time PCR

Peripheral blood mononuclear cells (PBMC) ($1\text{-}3x10^6$ cells) were activated with PHA-M for 72 hours then washed twice with PBS. Total RNA was extracted applying Nucleospin RNA II isolation kit (MACHEREY-NAGEL GmbH, Düren, Germany) according to manufacturer's instructions. cDNA was synthesized using 2 μg of total RNA, in the presence of 50 pmol of oligo(dT_{18}) and of random hexamer primers, 0.5 mM dNTP, 20 U RiboLock RNase Inhibitor and 200 U RevertAid H Minus Reverse Transcriptase (Thermo Fisher Scientific Inc., Boston, MA, USA) for 60 min at 42 °C and then heated for 10 min at 70 °C. qPCR was performed using AccuPower Greenstar qPCR Master Mix (Bioneer, Daejeon, Korea) in RotoGene3000 instrument (Corbett

Life Science, Quiagen, Hilden, Germany). Relative quantification of gene expression was determined by comparison of threshold cycles (Ct) related to endogenous *RPL27*. Relative mRNA amounts (R) were calculated by the equation $R = 2^{Ct(RPL27)-Ct(Lgals-1)}$. The following primer sequences were used:

RPL27:

forward 5'-CGCAAAGCTGTCATCGTG-3'

reverse 5'-GTCACTTTGCGGGGGTAG-3'

LGALS1:

forward 5'-CGCCAGCAACCTGAATCT-3'

reverse 5'-CAGGTTCAGCACGAAGCTCT-3'.

Patients

Adult female SLE patients (age >18 years) were enrolled (the numbers of patients are indicated under the experiments) with the approval of the Local Ethics Committee. All patients fulfilled the American College of Rheumatology updated criteria for the classification of SLE [27]. The control group consisted of sex- and age-matched healthy volunteers.

The first blood samples were taken before starting an immunosuppressive therapy. These patients were in clinically active stage of the disease, and were either newly diagnosed or relapsing cases (active SLE). The second blood samples were taken when the enrolled patients' disease has become quiescent after treatment (inactive SLE). Treatments stably maintained for at least two months included low-dose (< 20 mg prednisolone) corticosteroid, azathioprin, methotrexate, cyclosporine or epratuzumab.

Results

Galectin-1 is expressed de novo in activated T-cells and remains intracellularly

Expression of Gal-1 in activated T-cells has previously been reported [24] and suggested that it might act as an autocrine apoptotic factor. However it has not been decisively proven whether Gal-1 is secreted from the cells or remains intracellularly. This point is crucial to clarify since autocrine or paracrine mechanisms can exist if secreted, while its contribution to cell death as an intracellular protein has not been examined yet. To investigate this question we established a useful model of Gal-1 transgenic Jurkat cell line (Supplemental material). Western-blot analysis showed that untransfected Jurkat cells or cells transfected with empty vector (J^{mock}) do not express Gal-1, while cells transfected with Gal-1 cDNA (J^{Gal}) produce this protein (*Fig. 1 A*).

In order to determine whether Gal-1 was secreted to the extracellular space, permeabilized and non-permeabilized J^{Gal} cells were analyzed with confocal microscopy (*Fig. 1B.* upper*)* or flow cytometry (*Fig. 1B.* lower). Gal-1 was exclusively present intracellularly, and was not detectable on the cell surface (*Fig. 1B*). Failure of secretion of Gal-1 was also shown using J^{Gal} conditioned medium since no Gal-1 binding to Jurkat cells from J^{Gal} derived cell culture supernatant was detected (*Fig. 2*).

Gal-1 expression in human and mouse peripheral T-cells was also analyzed. As shown on *Fig. 3A*, both mouse (left) and human (right) activated but not resting T-cells expressed Gal-1. Cellular localization of Gal-1 in activated human T-cells was similar to that of Gal-1 transgenic Jurkat cells (*Fig. 3B, C and Fig. 1B, respectively*), as Gal-1 was only detected intracellularly (*Fig. 3B and C*).

Expression of Gal-1 in transgenic Jurkat or mouse activated T-cells modulates apoptotic response to extracellular Gal-1

To assess the effect of endogenous, intracellularly expressed Gal-1 in the course of exGal-1 induced apoptosis, J^{mock} and J^{Gal} Jurkat cell lines were treated with soluble, recombinant galectin-1 (rGal-1) (*Fig. 4A*) or were co-cultured with Gal-1-secreting HeLa cells (*Fig. 4B*). Gal-1 expressing Jurkat cells reacted significantly more vigorously to the cytotoxic effect of Gal-1 in either soluble or cell-bound form than the Gal-1 non-expressing Jurkat cells.

The role of inGal-1 in T-cells was further supported with studying murine T-cells. For this purpose, T-cells obtained from wild type and Gal-1 KO animals were activated and co-cultured with wild type, Gal-1 secreting or Gal-1 KO MSCs. As shown in *Fig. 5*, the apoptotic response of Gal-1 deficient T-cells was significantly weaker than that of the wild type, Gal-1-expressing counterparts.

Diminished Gal-1 expression coincides with decreased exGal-1 induced apoptosis in activated SLE T-cells

Previous studies have indicated that Gal-1 expression is diminished at the sites of severe chronic inflammation such as psoriatic skin ([28] and Krenacs L et al., unpublished data). However, Gal-1 expression in pathological T-cells has not yet been specifically investigated. Dysregulated T-cell apoptosis in SLE has been well documented [17,29] and has been determined as one of the crucial defects in SLE pathogenesis. Hence, it is a plausible question whether Gal-1, acting on T-cells as a pro-apoptotic protein, is dysregulated in activated SLE T-cells and apoptotic sensitivity of these cells to exGal-1 is down-regulated thereby contributing to the pathomechanism of lupus.

Fourteen active SLE, nine inactive SLE (in remission after therapy) patients and sixteen control individuals were enrolled into the study. SLE or control activated T-cells were used as the source of mRNA, and Gal-1 expression was analyzed by qPCR (*Fig. 6A*). Gal-1 expression declined significantly in active SLE patients compared to the controls, and elevated significantly after treatment, reaching a similar level to that of healthy controls. It must be noted that the individual values of the Gal-1 mRNA levels did not show correlation with other parameters of the disease activity such as SLEDAI-2K, anti-DNA antibodies or erythrocyte sedimentation rate (ESR) (data not shown).

Apoptotic response of activated SLE T-cells to exGal-1 in a co-culture experiment was also measured. Twenty active, ten inactive SLE and twenty control patients were included. As shown on *Fig. 6B*, activated T-cells of active SLE patients responded poorly to exGal-1 compared to control cells while the responsiveness significantly increased in T-cells of patients in remission.

Discussion

The apoptotic effect of extracellular Gal-1 on activated T-cells has been described in a number of papers [3,4,6,24,30]. It has also been shown that T-cells express Gal-1 upon activation. Blaser *et al*. have proposed that the apoptotic effect of exGal-1 on T-cells might stem from an autocrine loop that is created by the secretion of the protein, since they have found Gal-1 in the supernatant of an activated T-cell culture [24]. However, the resulting supernatant had to be concentrated 100-fold to be effective in inducing T-cell apoptosis which indicated a very low amount of secreted Gal-1. Moreover, the possibility could not be excluded that Gal-1 was released from necrotic cells in the culture. The result of these authors indicated that even if secreted, Gal-1 amount in unconcentrated supernatant could not be enough to exert cytotoxicity, since the concentration of soluble Gal-1 inducing T-cell death was shown to be between 25-400 μg/ml in all published experiments [4,7,30,31]. Moreover, Gal-1 is normally present in serum only in very low quantity (several ng/ml), which is far below the apoptosis inducing concentration of the soluble protein [32]. Accordingly, we showed here, that conditioned supernatant harvested from J^{Gal} or activated T-cell cultures did not contain detectable amount of Gal-1 and did not induce apoptosis (data not shown) confirming that the protein was not secreted but remained intracellularly. However the intracellular form of this protein has not been implicated in the process of apoptosis. Hence, we have examined whether the intracellular, *de novo* expressed Gal-1 in T-cells plays a role in the fate of T-cells by conducting studies in a Jurkat model system and on activated T-cells. Treatment of T-cells either with soluble rGal-1 or cell-bound Gal-1 in co-culture showed that the presence of endogenous inGal-1 in the target T-cells had an impact on the apoptotic response induced by exGal-1. T-cells expressing Gal-1 either

due to transfection with Gal-1 gene (J^{Gal}) or upon activation displayed higher sensitivity to exGal-1 induced apoptosis (see Fig. 7)

Since Gal-1 has been proposed as a possible candidate for immunosuppressive therapy in earlier studies [8,11,13,33], we turned to the prototypical inflammatory autoimmune disease, SLE as a pathological model. Our results showed that active SLE T-cells contained significantly lower levels of Gal-1 mRNA than healthy controls. These T-cells were also significantly less sensitive to the apoptotic signal of exGal-1. Although there was no clear correlation between disease activity index scores or particular symptoms and Gal-1 levels, we hypothesized that low expression and diminished responsiveness of activated SLE T-cells to Gal-1 might contribute to immune regulatory dysfunction and enhanced T-cell activity in SLE pathology [17,29,34,35]. This assumption was supported by the finding that successful immunosuppressive therapy resulted in restoration of the level of Gal-1 as well as of the apoptotic sensitivity of SLE T-cells.

The modulating effect of inGal-1 content on the apoptotic reaction of cells to exGal-1 is a novel finding. Even though the signaling pathways responsible for exGal-1 driven T-cell apoptosis have largely been mapped [6,7,36–38], many questions about the exact mechanism by which the cells were sensitized by inGal-1 to exGal-1 mediated apoptosis remain to be elucidated. While the anti-apoptotic effect of intracellular Gal-3 can be explained by the Bcl-2 homolog motif of the protein [39], Gal-1 does not have a homologous segment with any known apoptotic protein, and thus its function in apoptosis must stem from a yet unknown mechanism.

In this study we have demonstrated that the *de novo* expressed Gal-1 remains intracellularly and regulates responsiveness of activated T-cells to the apoptosis

inducing effect of extracellular Gal-1. The analysis of activated T-cells from SLE patients shows a clear diminution in Gal-1 expression and concomitant resistance to exGal-1 triggered apoptosis. These latter findings serve as potential novel markers to SLE pathogenesis.

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Authors declare no conflict of interest.

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Figure legends

Figure 1. Gal-1 transgenic Jurkat cells express but do not secrete Gal-1. A) Cell lysates prepared from 2×10^5 cells were loaded onto a 12% SDS-polyacrylamide gel. The separated proteins were electro-transferred onto nitrocellulose membrane. After blocking with 3% gelatin, the membrane was incubated with rabbit anti-human Gal-1 and rabbit anti-human actin antibody followed by HRP-conjugated swine anti-rabbit IgG. Immunoreactive proteins were visualized using the ECL Plus detection system.

B) Cells were suspended in cold IFB or IFB supplemented with 0.1% Triton-X 100 for non-permeabilized or permeabilized samples, respectively. Cellular Gal-1 was detected using mouse anti-human Gal-1 monoclonal antibody followed by donkey anti-mouse IgG conjugated with NorthernLights557. Finally, the cells were mounted on microscopic slides with Fluoromount-G and analyzed with laser scanning confocal microscope (upper). Alternatively, the cells were incubated with mouse mAb to Gal-1 (2C1/6) followed by goat anti-mouse IgG – Atto 488 (Sigma). The samples were analyzed by FACSCalibur flow cytometer using the CellQuestTM software or subjected to flow cytometry (lower).

Figure 2. Supernatant of J^{Gal} cells does not contain galectin-1. Jurkat cells were incubated with fresh (dashed line), J^{Gal} -conditioned (thin line) medium or 50μg/ml rGal-1 (thick line). Then the samples were stained with biotinylated mAb to Gal-1 (2C1/6) followed by FITC labeled Streptavidin and subjected to cytofluorimetric analysis.

Figure 3. Human and mouse peripheral T-cells express Gal-1 after activation. Western blotting of resting and activated mouse and human T-cells (A) and analysis of cellular

localization of Gal-1 in activated human T-cells (B) was performed as described under Fig. 1A and B, respectively. Expression of intracellular (left) and extracellular (right) Gal-1 in human activated T-cells was analyzed also with flow cytometry as described under Fig. 1B lower.

Figure 4. Gal-1 expression sensitizes Jurkat cells to the apoptotic effect of extracellular Gal-1. (A) J^{mock} or J^{Gal} cells were treated with 1.8 μM rGal-1 for 24 hours or left untreated. The percentage of apoptotic cells was determined by detecting the sub-G1 cell population by cytofluorimetry. n=3, * $p<0.05$. (B) J^{mock} or J^{Gal} cells were co-cultured with $HeLa^{mock}$ or $HeLa^{Gal-1}$ cells for 16 hours. Jurkat cells were then stained with Annexin V-AlexaFluor 488 and analyzed with fluorescence microscopy. The Annexin V positive cells were counted in every sample (at least 100 cells/sample) and the percentage of apoptotic cells was calculated. Apoptosis calculated as relative apoptotic ratio, RAR as follows: RAR = % of Annexin V positive cells on $HeLa^{Gal-1}$ - % of Annexin V positive cells on $HeLa^{mock}$. n=3, ** $p<0.01$.

Figure 5. Gal-1 deficient T- cells respond weakly to the cytotoxic effect of exGal-1. T-cells from wild type and Gal-1 knockout mice were activated with Con-A and co-cultured with $BMMSC^{wt}$ or $BMMSC^{Gal1-/-}$ cells for 16 hours. Apoptotic T-cells were then stained and analyzed as described under Fig. 4 B. n=3, ** $p< 0.01$

Figure 6. Decreased Gal-1 expression in activated SLE T-cells coincides with the poor apoptotic response of these cells to exGal-1. A) Gal-1 mRNA level was measured in activated T-cells by qPCR. HC: healthy control, n= 16, Active SLE, n= 14, Inactive (treated) SLE, patients in remission after treatment, n=9, ** $p<0.01$. Relative mRNA level was calculated as described in Materials and Methods. B) Apoptosis assay was

carried out in co-culture as described under Fig. 4B. HC n=20, active SLE n=20, inactive SLE n=10. *** $p<0.001$, * $p<0.05$

Figure 7. A proposed mechanism of the interaction of inGal-1 and exGal-1 in apoptosis induction: Gal-1 produced by activated T- cells sensitizes the cells to the apoptotic effect of exogenous Gal-1. Activated T-cells expressing inGal-1 meet Gal-1 secreting activated B-cells and macrophages in an inflammatory environment and upon direct cell contact T-cells may undergo Gal-1 induced apoptosis (route A). The autocrine induction of apoptosis can be excluded since activated T-cells do not secrete Gal-1 (route B).

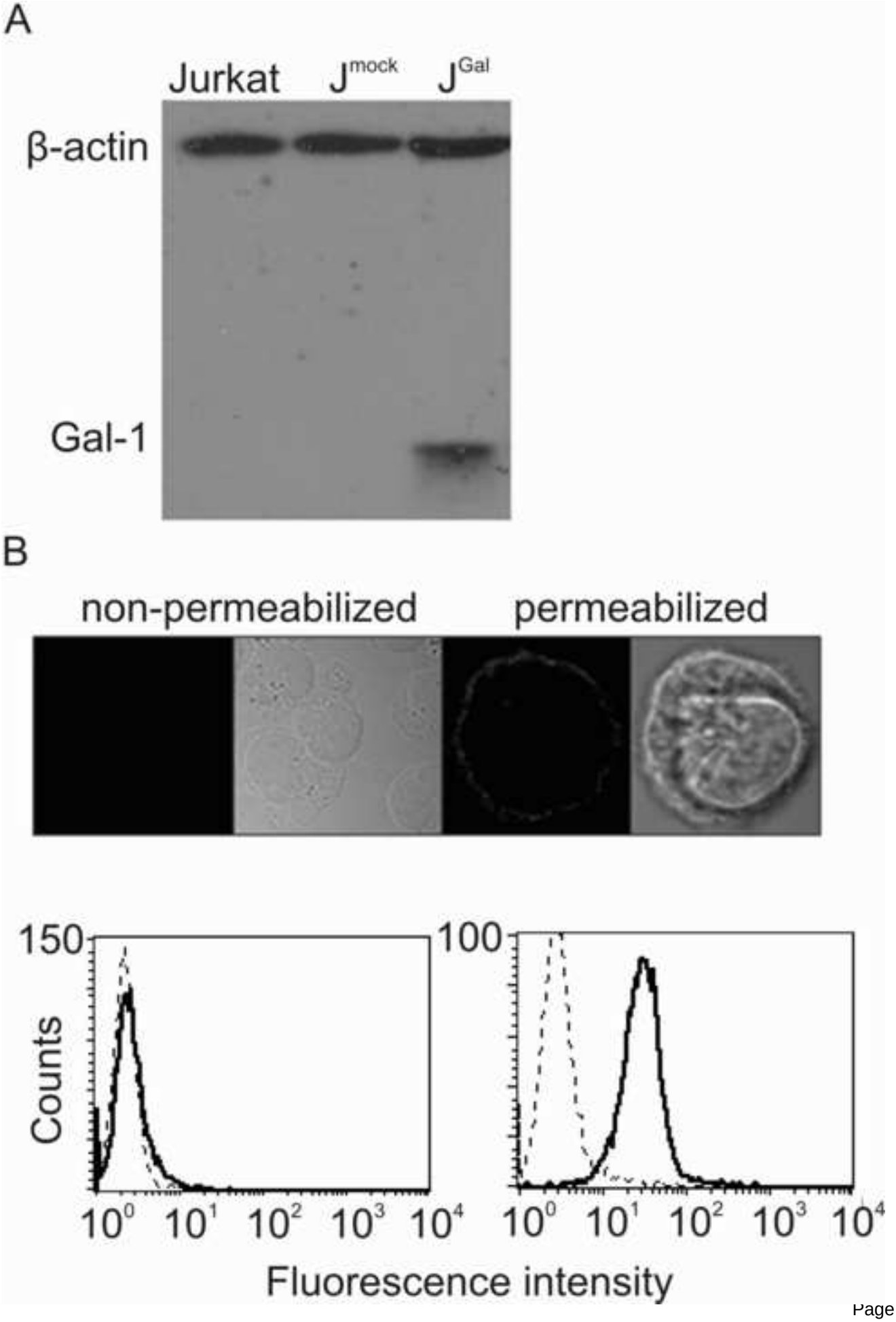
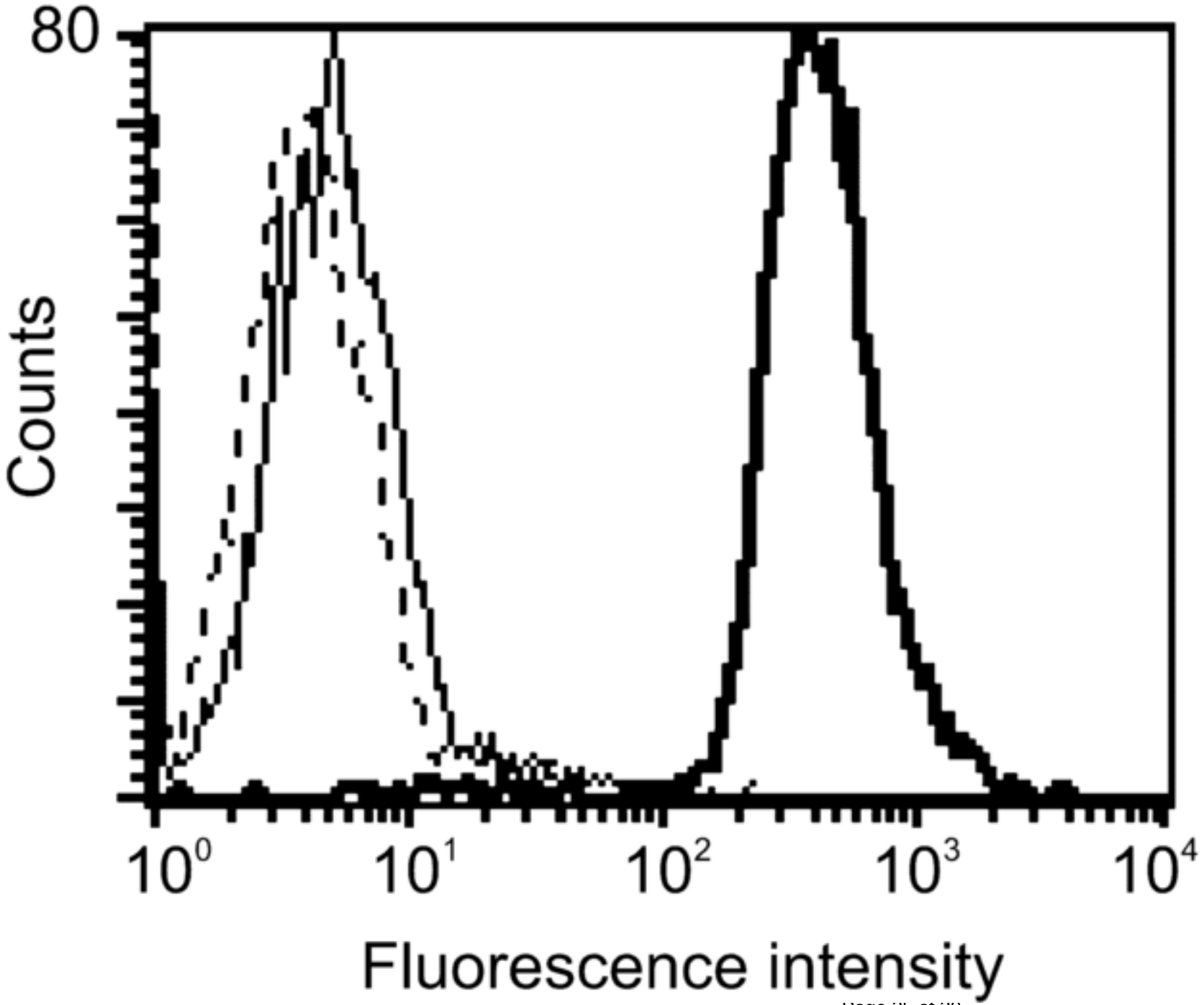
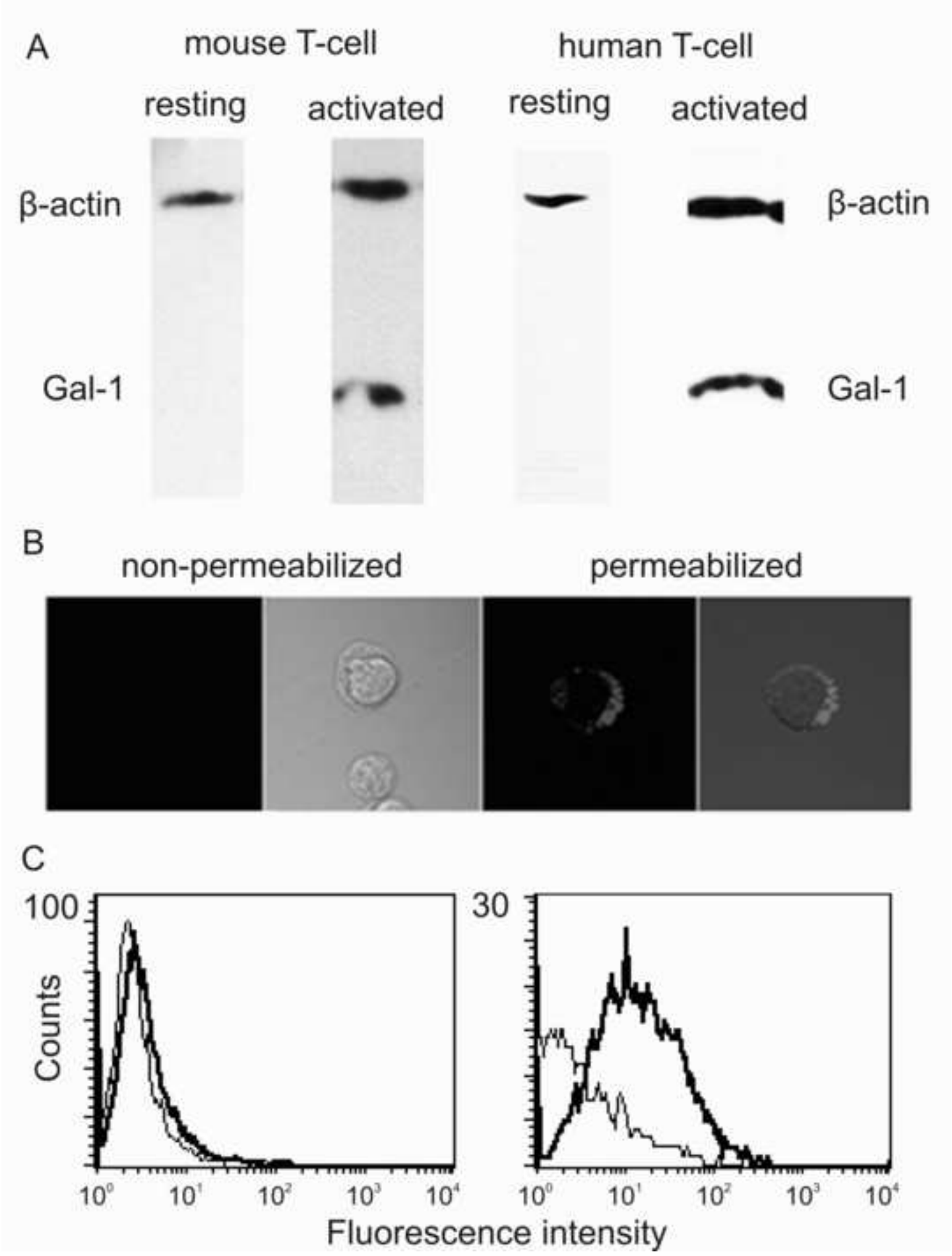


Figure 2





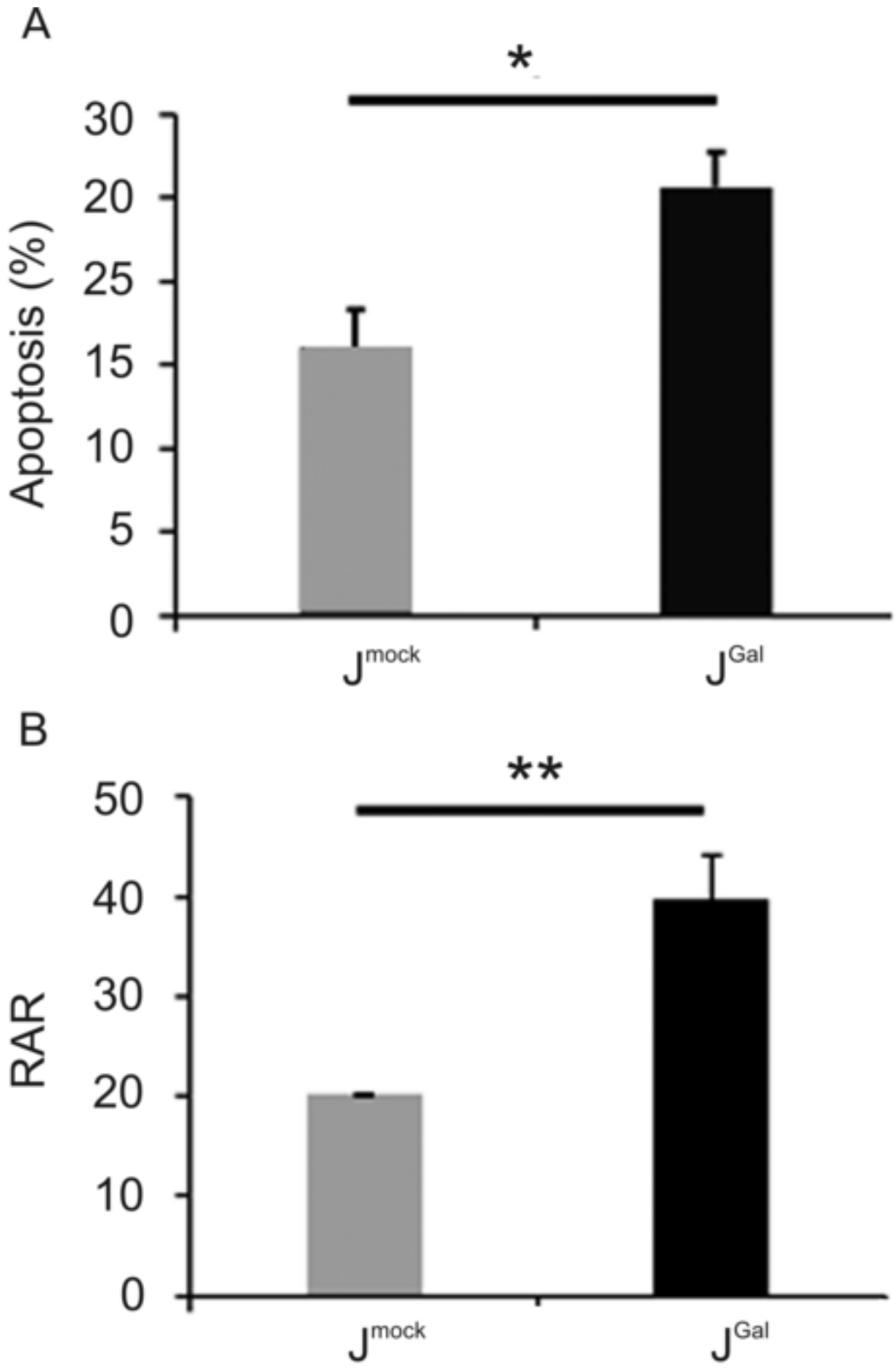
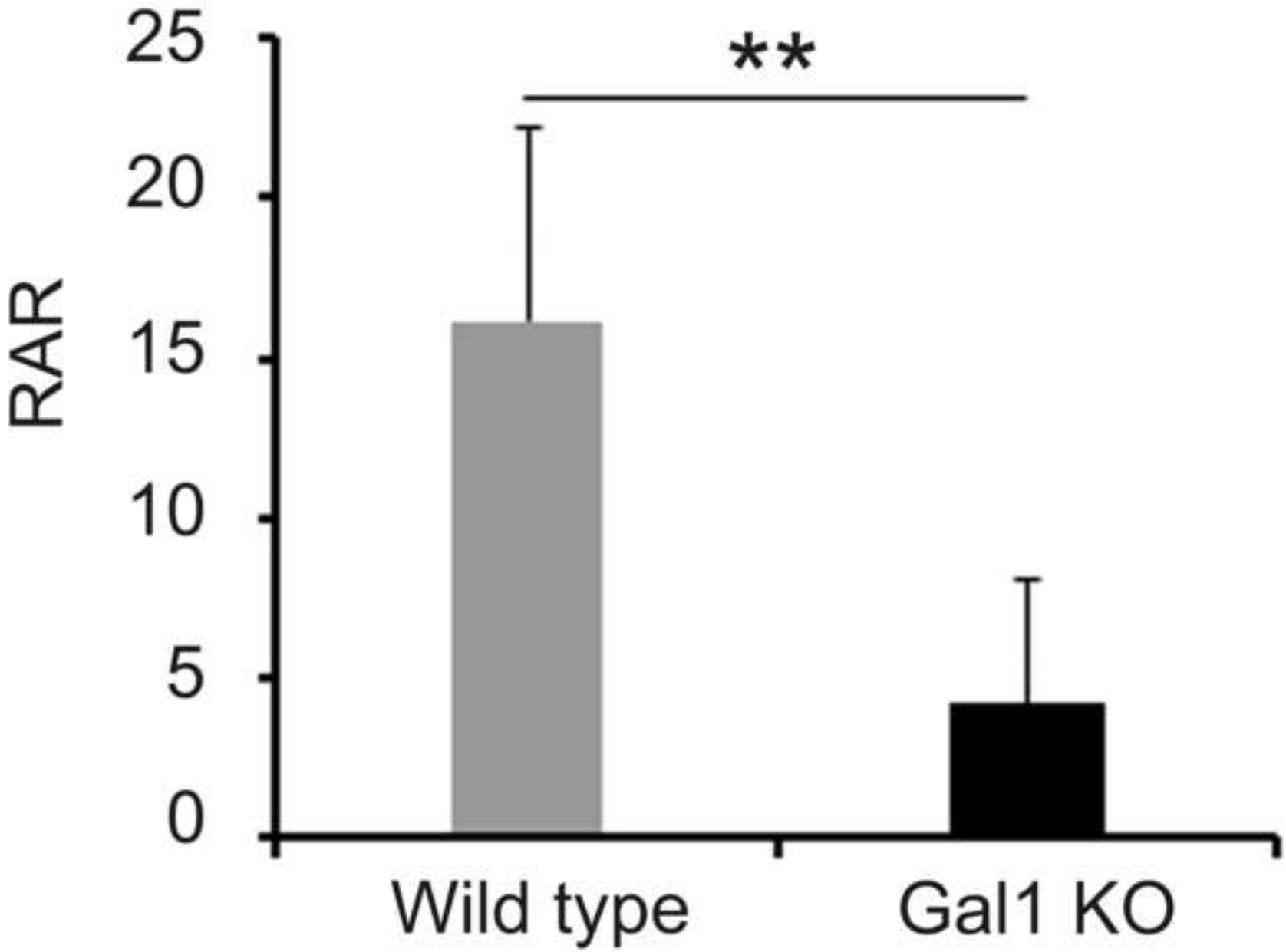
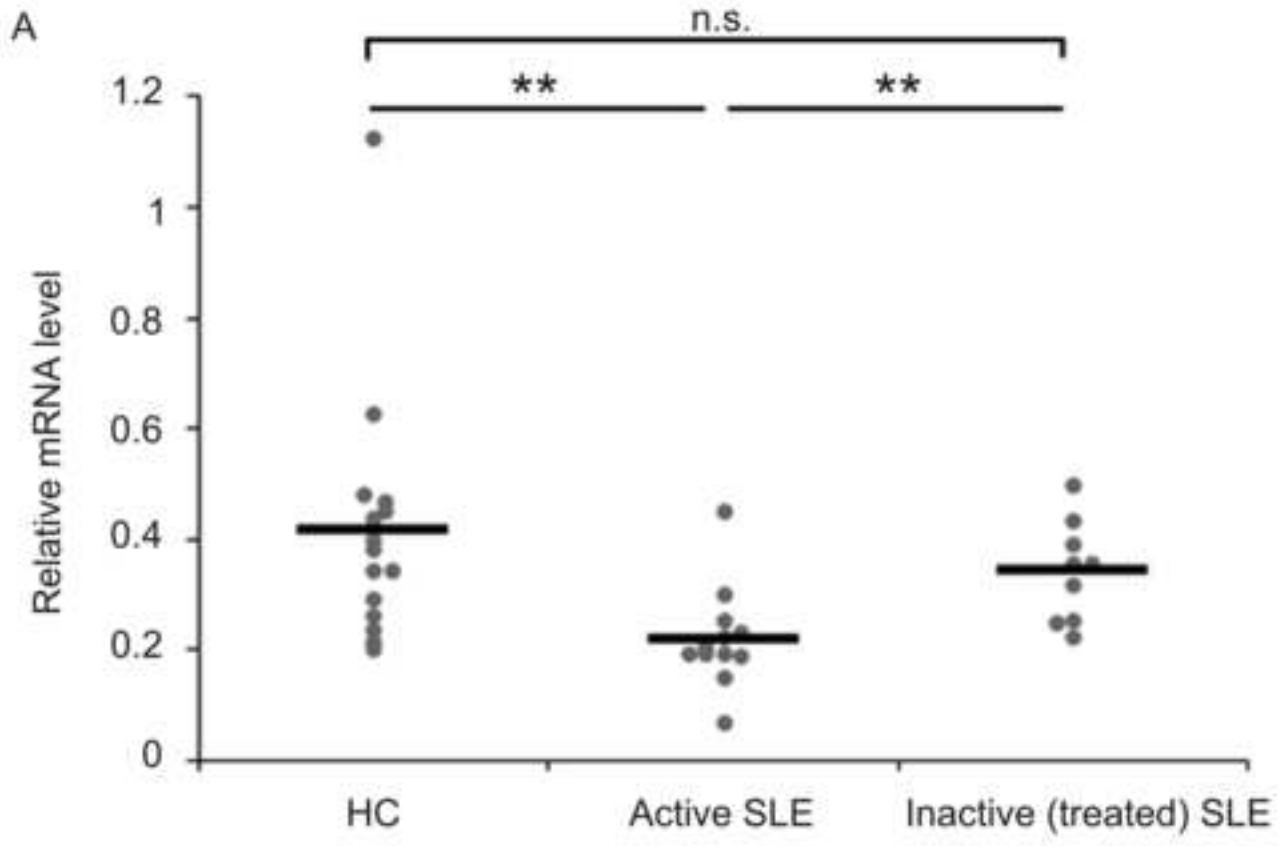


Figure 5





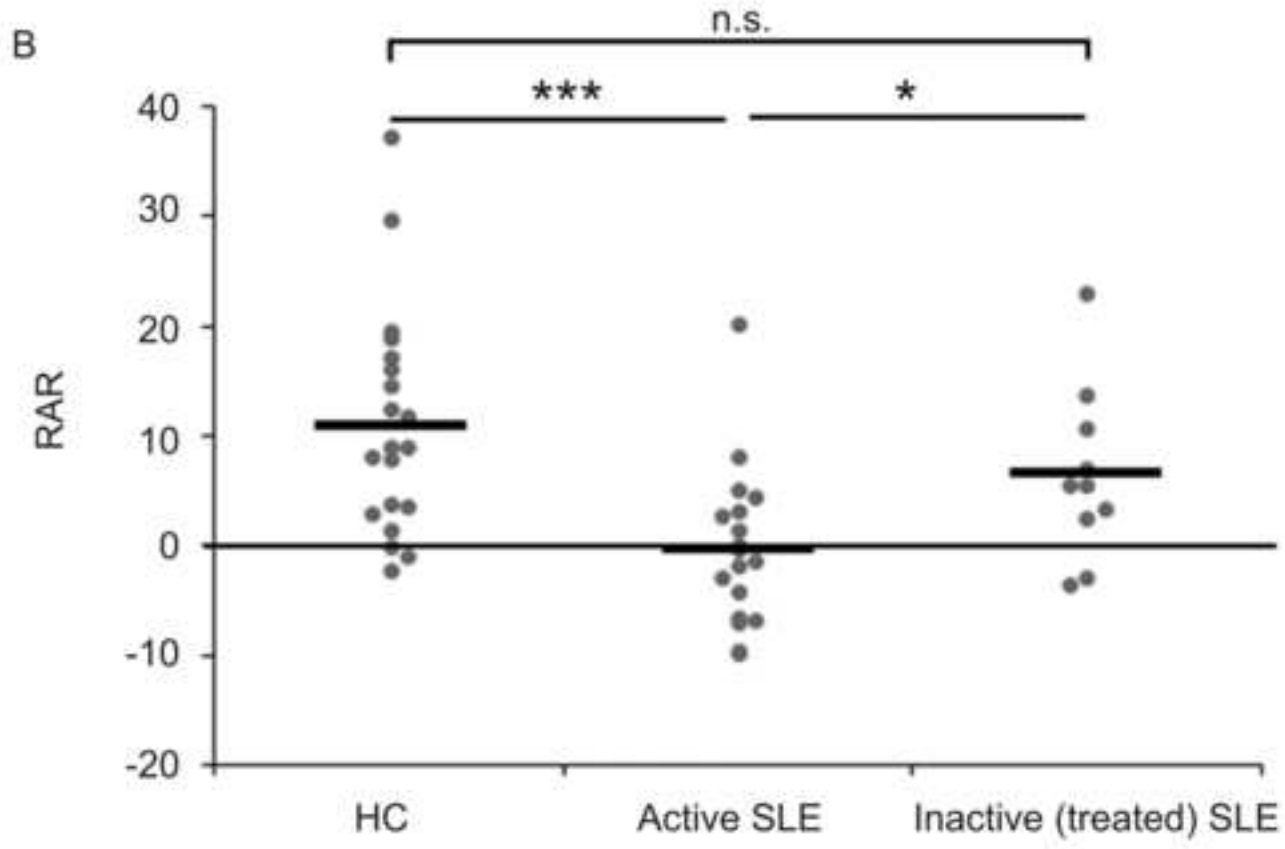
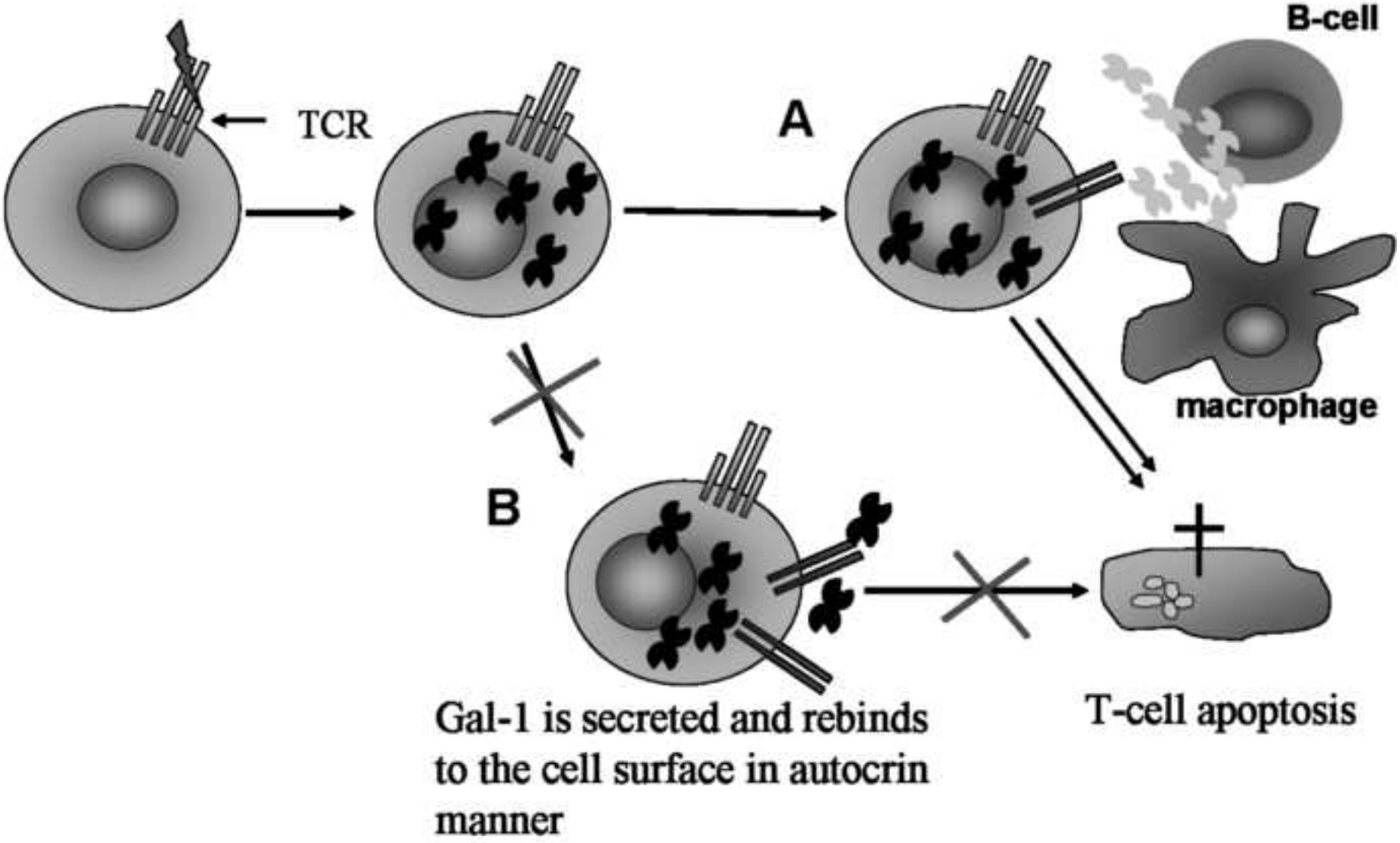


Figure 7


B-cell
TCR
A
macrophage
B
Gal-1 is secreted and rebinds to the cell surface in autocrin manner
T-cell apoptosis
Gal-1 produced by T-cells (endogenous)
Gal-1 binding membranestructures
Gal-1 produced and secreted by other cells (exogenous).