Supplemental Material

We could observe a clear tendency towards an enhanced homing of ^{64}Cu-OVA-Th1 cells in the lung and thymus/perithymic LNs of mice diseased from OVA-AHR confirming our *in vivo* acquired PET-data on ^{64}Cu-OVA-Th1 cell homing sites but the differences between the experimental groups didn´t reach statistical significance.
For the *ex vivo* biodistribution analysis the whole thymus including the perithymic LNs were measured in the γ-counter and the %ID/g was determined. The analysis of the *in vivo* data based on the mean activity of VOI drawn around the perithymic LNs in the PET images. These differences in the analysis could explain the differences between the *in vivo* and *ex vivo* data.

For *ex vivo* autoradiography analysis, the organ-to-muscle ratios of the lungs and the thymus were calculated. 24 h after ^{64}Cu-OVA-Th1 cell administration. A significantly higher activity in the thymus of OVA-immunized and OVA-challenged animals (thymus/muscle: 7.7±2.8) was detected compared with non-immunized PBS-challenged mice (thymus/muscle: 2.5±0.8; Fig. 6A). Despite failing statistical significance we found a clear tendency towards a higher activity in the lung of OVA-immunized and OVA-challenged animals (lung/muscle: 10.6±2.9) compared with the non-immunized OVA- (lung/muscle: 5.7±2.1) or PBS-challenged mice (lung/muscle: 5.3±0.9; thymus/muscle: 2.2±0.5; Fig. 6A). Despite failing statistical significance *in vitro* biodistribution measured by γ-counting analysis 48 h after ^{64}Cu-OVA-Th1 cell administration further yielded a clear tendency towards a higher activity in the lungs of OVA-immunized and OVA-challenged littermates (lung: 16.6±2.4 %ID/g; thymus: 48.3±36.1 %ID/g) compared with non-immunized OVA- (lung: 11.0±2.6 %ID/g;

thymus: 25.7±10.7 %ID/g) or PBS-challenged mice (lung: 9.7±1.5 %ID/g; thymus: 6.5±4.9 %ID/g; Fig. 6B). The differences between the *in vivo* and *ex vivo* analysis could be explained by the different data acquisition methods as we examined for the *ex vivo* biodistribution the whole thymus including the perithymic LNs (%ID/g) and in the *in vivo* PET images only the perithymic LNs (%ID/cm^3).

Supplemental Methods

Animals.

Female BALB/c mice were purchased from Charles River Laboratories (Sulzfeld, Germany) and were between 8 and 12 weeks of age. DO11.10 mice transgenic for the OVA peptide-specific TCR were originally kindly provided from the Max Planck Institute for Immunobiology, Freiburg, Germany and bred under specific pathogen-free and standardized environmental conditions (20±1 °C room temperature, 50±10 % relative humidity, and 12 h light-dark rhythm) with free access to food and water *ad libitum* at the animal care facility of the University of Tübingen, Tübingen, Germany. Animal experiments were performed according to the animal use and care protocols of the German Animal Protection Law and were approved by the Regierungspräsidium Tübingen (R11/08).

Induction of OVA-induced AHR

BALB/c mice were immunized by *i.p.* administration of 200 µL of an emulsion containing 150 µL of aluminum hydroxide gel (Alu Gel-S, Serva Electrophoresis GmbH, Heidelberg, Germany) and 10 µg of OVA protein (Grade V, Sigma Aldrich) dissolved in 50 µL of PBS. Two weeks later, the animals were anesthetized with 100 mg/kg ketamine (Ratiopharm, Ulm, Germany) and 5 mg/kg xylazine (Rompun, Bayer HealthCare, Leverkusen, Germany). The mice were intranasally challenged with 100 µg of OVA peptide dissolved in 50 µL of PBS on two consecutive days to induce lung inflammation (*1, 2*).

Reagents, Antibodies and T Cell Culture

T cells were cultured in Dulbecco´s modified Eagle`s Medium (DMEM; Biochrom, Berlin, Germany) supplemented with 10 % heat-inactivated fetal calf serum (FCS; Thermo Scientific, Hyclone, USA), 0.05 mM 2-mercaptoethanol (Sigma Aldrich, Munich, Germany), penicillin (100 U/mL)/streptomycin (100 µg/mL) liquid (Biochrom), 1 mM natrium-pyruvate (Biochrom), 1 % MEM amino acids (Invitrogen, Darmstadt, Germany) and 10 mM HEPES-buffer (Biochrom). Soluble CD4 monoclonal antibodies (mAb; Gk1.5), CD8 mAb (5367.2) and mouse anti-rat mAb (MAR 18.5) were isolated from hybridoma supernatants (ATCC, Manassas, VA, USA).

$CD4^+$ T cells were isolated from the LNs and spleens of DO11.10 OVA-TCR transgenic mice and sorted using anti-CD4 (L3T4) MACS magnetic beads and MACS columns according to the manufacturer´s protocol (Milteny Biotech, Bergisch Gladbach, Germany). $CD4^+$ T cells ($2x10^5$; 90-98 % purity) were cultured for 12-14 days with anti-IL4 mAb (10 µg/mL), OVA323-339 peptide (10 µg/mL EMC-microcollections GmbH, Tübingen, Germany), CpG-DNA 1668 (0.3 µM, Eurofins MWG Operon, Ebersberg, Germany) and with 5 U/mL IL-2 after day 3 and subsequently with 50 U/mL (Chiron Corporation, Emeryville, CA, USA). $CD4^+$ T cells were co-cultured with $5x10^5$ antigen-presenting cells (APCs) that had been irradiated with 30 Gy and were derived from T cell-depleted splenocytes. The APCs were prepared by incubating the total spleen cells with CD4, CD8, and MAR mAbs and subsequently with complement (Cedarlane, Burlington, Canada). The $CD4^+$ T cells and APCs were co-cultured in a total volume of 200 µL of medium in 96-well plates at 37 °C in a humidified atmosphere with 5 % CO_2. After 3 days, the T cells were cultured in 24-well plates before being transferred to 100 mL cell culture bottles after an additional 5 days (*3*).

^{64}Cu-PTSM Production

^{64}Cu was produced at the PETtrace cyclotron (GE Healthcare, Uppsala, Sweden) using a proton energy of 12.5 MeV according to the protocol of McCarthy *et al.* (*4*). Briefly, ^{64}Ni was electroplated on a platinum/iridium plate (90/10) (20 mg, >98 % enrichment; Campro Scientific, Berlin, Germany). ^{64}Ni was irradiated, and subsequently ^{64}Cu was separated from ^{64}Ni and other metallic impurities after dissolution using ion exchange chromatography. For ^{64}Cu-PTSM synthesis, 600 MBq of freshly prepared ^{64}Cu (in 140 µL of 0.1 N HCl) and 20 µg of H_2PTSM (*5*) in 10 µL of DMSO were added to 500 µL of 0.4 N ammonium acetate (pH 5.5) and incubated for 1-2 min at room temperature. ^{64}Cu-PTSM was purified using a C18 light cartridge (Waters, Milford, MA, USA), and the identity and quality were assured using thin-layer chromatography.

In vitro Evaluation of the ^{64}Cu Uptake and Efflux

To evaluate the ^{64}Cu uptake and efflux, OVA-Th1 cells ($1x10^6$) were labeled with 0.7 or 1.4 MBq of ^{64}Cu-PTSM for 3 h in a volume of 0.5 mL cell culture medium. The OVA-Th1 cells were then washed twice immediately (0 h) or at 5 or 24 h after the labeling procedure. The supernatants (incubation solution and each washing solution) and the $1x10^6$ OVA-Th1 cells were analyzed in a γ-counter (Perkin Elmer, Waltham, USA) to determine the amount of ^{64}Cu efflux and trapped ^{64}Cu in the OVA-Th1 cells.

Analysis of OVA-Th1 Cell Viability

The impact of ^{64}Cu-PTSM and Cu-PTSM-labeling on OVA-Th1 cell viability was evaluated by trypan blue staining (Biochrom) using a hemocytometer (Assistent, Sondheim, Germany) 3, 24 or 48 h after the various ^{64}Cu-PTSM-labeling conditions

were applied. Trypan blue positive (dead) and negative (alive) OVA-Th1 cells were counted in a Neubauer chamber, and the mean proportion of trypan blue-negative versus trypan blue-positive cells was calculated for each sample.

Cytokine and Proliferation Assays

To investigate the impact of ^{64}Cu-PTSM-labeling on OVA-Th1 cell function, we analyzed OVA-specific IFN-γ production and T cell proliferation 3, 24 and 48 h after the varying ^{64}Cu-PTSM-labeling conditions. We additionally analyzed the impact of Cu-PTSM or PTSM on OVA-Th1 cell function. For the analysis of OVA-specific IFN-γ production, $1x10^5$ OVA-Th1 cells were cultured with or without OVA-peptide (10 µg/mL) in presence of $5x10^5$ irradiated APCs in a total volume of 200 µL of cell culture medium (with 5 U/mL IL-2) in 96-well flat bottom plates at 37 °C in a humidified atmosphere with 7.5 % CO_2 for 24 h before the supernatants were harvested. We determined the IFN-γ concentration in the supernatants using BD-Opt-EIA™ IFN-γ ELISA-Set (Becton Dickinson Bioscience, Heidelberg, Germany) according to the manufacturer's protocol. To investigate the impact of ^{64}Cu-PTSM-labeling on specific OVA-Th1 cell proliferation, $1x10^5$ OVA-Th1 cells were cultured with or without OVA-peptide (10 µg/mL) in presence of $5x10^5$ irradiated APCs in 100 µL of medium (with 5 U/mL IL-2) in 96-well round bottom plates for 24 h. OVA-Th1 cells were pulsed with 10 µL (9.25 kBq) of ^{3}H-thymidine (Perkin Elmer) for the final 12 h at 37 °C in a humidified atmosphere with 7.5 % CO_2. ^{3}H-thymidine incorporation into the OVA-Th1 cells was detected using a scintillation counter (Microbeta TriLux 1450 LSC & Luminescence Counter, Perkin Elmer).

Analysis of DNA Double Strand Breaks by Detection of the Phosphorylated Histone H2AX

The radiolabeling-induced deoxyribonucleic acid (DNA) double strand breaks of OVA-Th1 cells were quantified by the detection of phosphorylated histone H2AX (γ-H2AX) using FACSort flow cytometry (Becton Dickinson Bioscience) and immunofluorescence microscopy. OVA-Th1 cells were investigated immediately (0 h) after the described ^{64}Cu-PTSM-labeling conditions were applied. OVA-Th1 cells were also investigated for γ-H2AX expression after labeling with 0.7 MBq ^{64}Cu-PTSM for different incubation times. OVA-Th1 cells were washed with D-PBS (Invitrogen), fixed in 1 mL of 70 % ethanol and stored at -20 °C. For γ-H2AX-analysis, 1 mL of cold D-PBS was added to each tube before the tubes were centrifuged (200xg, 5 min, 4 °C) and decanted. The OVA-Th1 cells were resuspended in 1 mL of cold D-PBS containing 4 % FCS and 0.1 % Triton X-100 (Sigma Aldrich) and stored on ice for 10 min. After an additional centrifugation, the OVA-Th1 cells were resuspended in a 200 µL of a solution containing a mouse monoclonal anti-phospho-histone H2AX antibody (Upstate, Charlottesville, VA, USA; dilution 1:500 in PBS-T (Invitrogen)). The tubes were incubated on a shaker for 2 h at room temperature, rinsed with PBS-T and centrifuged. The pellets were subsequently resuspended in a 200 µL solution containing Alexa-Fluor 488 secondary antibody (Molecular Probes; dilution 1:200 in PBS-T) for 1 h at room temperature under light protection. Subsequently, the OVA-Th1 cells were rinsed with D-PBS plus 2 % FCS and resuspended in 400 µL D-PBS containing 10 µg/mL propidium iodide (Sigma Aldrich). A FACS analysis was performed using a FACSort flow cytometer (Becton Dickinson Bioscience) and BD CellQuest Pro software.

For immunofluorescence microscopy, aliquots of the samples prepared for flow cytometry were taken after secondary antibody incubation, spun down on poly-l-

lysine-coated slides with a cytospin 3 (Shandon, Astmoor, Great Britain) and mounted with DAPI-containing mounting medium (Vectashild Vector Laboratories, Burlingame, CA, USA) for nuclear counterstaining. Fluorescence microscopy was performed with a Zeiss Axiophot Microscope (Carl Zeiss, Göttingen Microimaging, Germany), and images were collected using analySIS software (Soft Imaging System, Münster, Germany).

Real-time Polymerase Chain Reaction

Non-labeled or 0.7 MBq ^{64}Cu-PTSM-labeled OVA-Th1 cells (10^7) were homogenized in lysis buffer (peqGOLD Total RNA Kit, Peqlab, Erlangen, Germany) 3 and 24 h after incubation. The genomic DNA was digested (peqGOLD DNase I digest kit; Peqlab), and 2 μg of RNA was subsequently reverse transcribed (Superskript II Reverse Transcriptase; Invitrogen). For relative quantification by reverse-transcribed polymerase chain reaction, 20 ng of cDNA was used for analysis in a LightCycler Real Time PCR System (Roche Diagnostics). For each primer pair, a standard curve was developed. The relative mRNA expression level of IFN-γ was normalized against the mRNA expression level of the housekeeping gene aldolase. The following primers were used: aldolase (241 bp amplificate), 5'-TGGG-CCTTGACTTTCTCCTAT and 3'-TGTTGATGGAGCAGCCT-TAGT; IFN-γ (237 bp), 5'-ACTGGCAAAAGGATGGTGAC and 3'-TGAGCTCA-TTGAATGCTTGG.

Analytical Electron Microscopy Analyses and Energy Dispersive X-Ray Spectroscopy

We analyzed the Cu concentration and distribution in OVA-Th1 cells by electron microscopy and energy disperses X-ray (EDX) spectroscopy. For each labeling condition, three single OVA-Th1 cells were analyzed. In each cell, we selected 2-3

areas within the nucleus and one area within the cytoplasm and analyzed these areas by EDX spectroscopy. Non-labeled OVA-Th1 cells or OVA-Th1 cells labeled with 0.7 MBq ^{64}Cu-PTSM or 0.7 MBq ^{64}Cu-PTSM plus 2 μg Cu-PTSM were fixed with Karnovsky's fixative for 24 h at 4 °C. For electron microscopic analyses and EDX spectra acquisition, the cell pellets were embedded in 3.5 % agarose at 37 °C and coagulated at room temperature. Small parts of the agarose blocks were fixed again in Karnovsky's solution. After dehydration in a graded series of alcohol, the blocks were embedded in glycide ether and cut using an ultra-microtome (Ultracut, Reichert, Vienna, Austria). Ultra-thin sections (30-80 nm) were mounted on aluminum grids. The EDX spectroscopy method yields high lateral (< 100 nm) and spectral resolution; however, it cannot distinguish among different Cu isotopes. Note that the EDX measurements for Cu were close to the minimum detectable mole fraction of 0.02 at%. Therefore, aluminum grids were essential because they do not contain Cu and because aluminum is a light element that produces reduced background intensities of Bremsstrahlung to the EDX spectra. The samples were analyzed in a Zeiss 912 electron microscope (Carl Zeiss, Oberkochen, Germany) equipped with an Omega energy filter and an EDX detector with an ultrathin window (Oxford Instruments, Oxfordshire, UK). The microscope was operated at 120 kV, and the details of the EDX spectrum acquisition are explained in Eibl *et al.*(*6*) A spot size of 100 nm was used to yield adequate counting statistics. Quantitative EDX analysis was performed using the Cliff Lorimer k factor method. The k factors used were those specified in Eibl *et al.* (*6*). A detailed discussion of the minimum detectable mole fraction of Cu is provided in Biesemeier *et al.* (*7*).

Flow Cytometry Analysis of the OVA-Th1 cells

To determine whether ^{64}Cu-PTSM impairs the homing patterns of OVA-Th1 cells *in vivo*, naïve BALB/c mice (n = 4) were injected with 10^7 ^{64}Cu-OVA-Th1 cells or unlabeled OVA-Th1 cells *i.p..* The upper part of the thymus was removed including the perithymic LNs and the spleen were isolated 21 h after OVA-Th1 cell administration and prepared as a single cell suspension, and the absolute cell numbers were assessed by trypan blue staining to enable absolute quantification of the OVA-Th1 cells within the isolated organs. To identify the transferred OVA-Th1 cells, surface staining was performed according to the manufacturer´s protocol using an allophycocyanin-CD4 antibody (Biolegend GmbH, Fell, Germany) and a phycoerythrin-KJ1-26 antibody (Becton Dickinson Bioscience), which was specific for the OVA-TCR. The OVA-Th1 cells were analyzed with a BD™ LSRII flow cytometer (Becton Dickinson Bioscience). The FACS data analysis was performed using FACS Xpress software (DeNovo Software, Los Angeles, USA).

Autoradiography, Histology and Biodistribution

For autoradiography analysis, the mice were sacrificed 24 h after the administration of ^{64}Cu-OVA-Th1 cells and immediately after the final *in vivo* PET scan to isolate the lung, thymus (including the perithymic LNs) and muscle tissue. The organs were embedded, snap-frozen (-18 °C) in OCT TissueTek Compound (Sakura Finetek, Torrance, CA, USA) and cut into 20 µm slices using a cryostat (Leica Microsystems, Wetzlar, Germany). Every 10th slice was placed on a phosphor screen for 48 h. The phosphor screen was read using a STORM Phosphor-Imager (Molecular Dynamics, Sunnyvale, CA, USA). For the image analysis of the autoradiography scans, we used the open source software Image J (National Institute of Health, Bethesda, MD, USA).

Standardized regions of interest (ROIs) were drawn for the lung, thymus or muscle tissue and the mean count intensity was obtained to calculate the organ–to-muscle ratios.

The 20 μm autoradiography sections of organs were stained with hematoxylin and eosin (H&E). The H&E-stained slides were analyzed using a DM5500B microscope (Leica Microsystems, Wetzlar, Germany; magnification x10). Images were acquired with a C8484-03G digital camera (Hamamatsu Photonics, Herrsching am Ammersee, Germany) using MM AF Imaging Systems Software (Leica Microsystems).

For the biodistribution analysis, diseased or healthy mice were sacrificed immediately after the final *in vivo* PET scan, i.e., 24 h after the administration of ^{64}Cu-OVA-Th1 cells, to isolate the organs of interest [lung, thymus (including the perithymic LNs) and muscle tissue]. As the thymus and the perithymic LNs are associated close to each other and a differentiation to the surrounding fat tissue can be ensured only by histological analysis, both structures were isolated in total for *ex vivo* γ-counting. Organ radioactivity was measured using a γ-counter (Perkin Elmer) with an energy window between 350 and 650 keV. The fraction of the injected dose per gram (%ID/g) was calculated by normalizing the acquired counts to the injected dose and the weight of the organs.

PET Data Analysis

The data analysis was performed using Inveon Research Workplace (IRW Siemens). The reconstructed images were corrected for radioactive decay and normalized to the injected activity. The acquired PET images were co-registered to the corresponding CT scan using IRW. Based on anatomical CT scans, volumes of interest (VOIs) were placed on the lung. The spleen, pulmonary and perithymic LN

signals were assessed by placing VOIs based on the normalized PET-images. All data were presented as the %-injected dose per cm^3 (%ID/cm^3). No corrections of the partial volume effects were applied since quantification errors are dependent on organ size and the target-to-background uptake ratios and thus adequate corrections are challenging (*8*). We decided to apply standardized imaging protocols and analysis procedures to ensure the same partial volume effects and therefore allowing the comparison of the different scans. The acquired PET images of ^{64}Cu-PTSM injected experimental mice were analyzed by using a template high resolution CT-scan providing detailed anatomical information.

Calculation of the absolute number of ^{64}Cu-PTSM-labeled OVA-Th1 cells

To calculate the absolute number of ^{64}Cu-PTSM-labeled OVA-Th1 cells within the perithymic LNs the amount of activity within the perithymic LNs was calculated by multiplication of the mean activity/cm^3 within the VOI with the size of the corresponding VOI. The amount of activity was then divided by the total injected amount of activity and multiplied with 10^7 (the amount of injected Th1 cells). The efflux of ^{64}Cu-PTSM from OVA-Th1 cells was not considered in this calculation.

Statistical Analysis

Dunnet`s test was used to compare differences in viability, functionality and proliferation between the non-labeled and 0.7, 1.5 or 2.2 MBq ^{64}Cu-PTSM-labeled OVA-Th1 cells as well as to compare the differences in the accumulation of ^{64}Cu-OVA-Th1 cells in the pulmonary LNs of non-treated and OVA-immunized/unimmunized and PBS- or OVA-challenged mice. A two-sampled student´s t test was applied to compare differences in copper concentration (at%) between the unlabeled and ^{64}Cu-PTSM-labeled (0.7 MBq) OVA-Th1 cells as well as

the differences in the accumulation of ^{64}Cu-OVA-Th1 cells in the perithymic LNs, lung or spleen between the *i.p.*- or *i.v.*-injected mice. The Tukey-Kramer test was used to analyze differences in the ^{64}Cu-PTSM uptake or function among the OVA-Th1 cells with different ^{64}Cu-PTSM incubation times. $P < 0.05$ was regarded as significant (* P<0.05, ** P<0.01, *** P<0.001). All *in vitro* results are presented as the mean ± standard deviation (SD), and all *in vivo* results are presented as the mean ± standard error of the mean (SEM).

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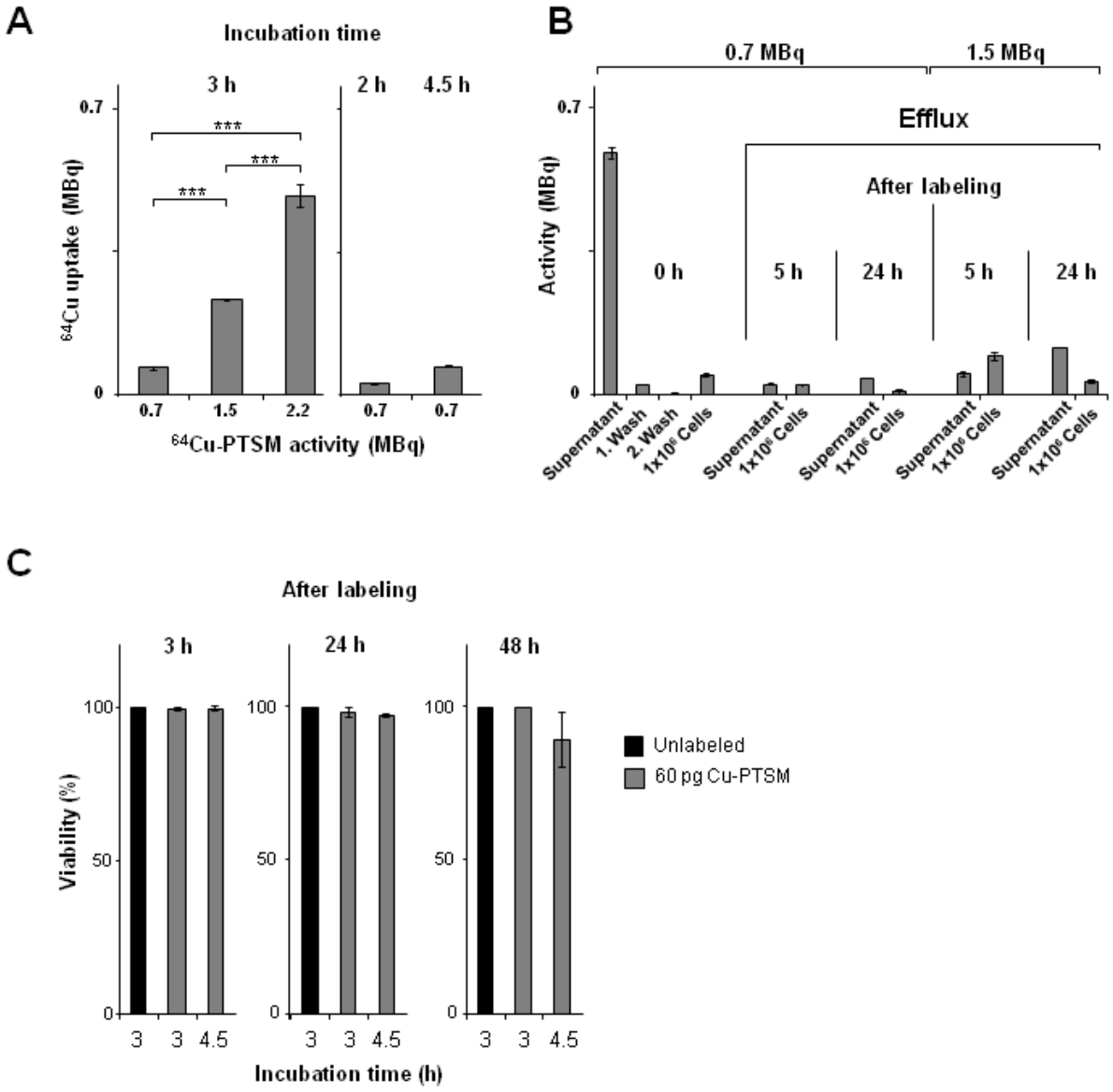
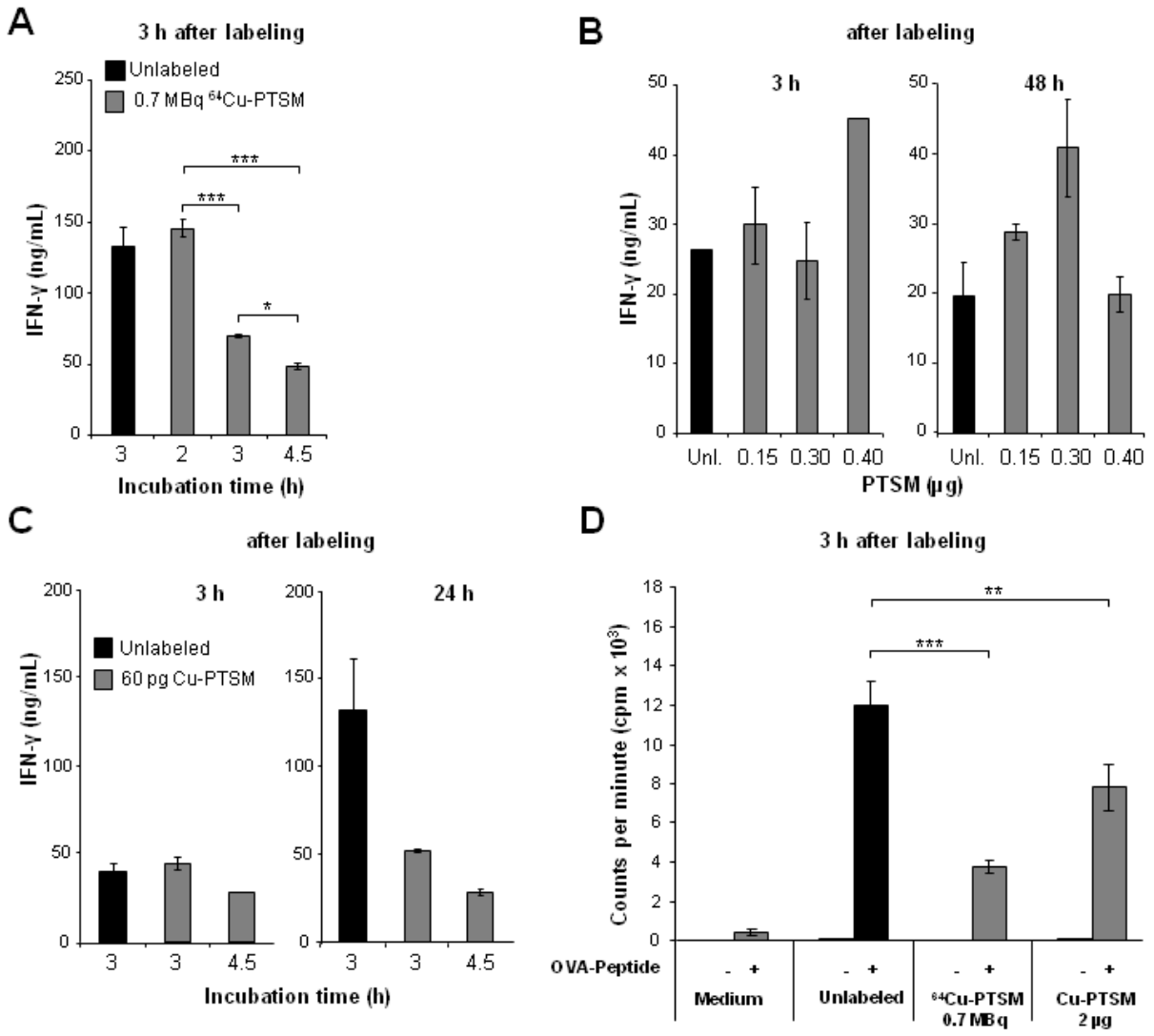
Supplemental Table 1: Increased appearance of phosphorylated γ-H2AX in OVA-Th1 cells caused by ^{64}Cu-PTSM-labeling. Differences in the relative appearance of phosphorylated γ-H2AX among unlabeled or 0.7, 1.5 or 2.2 MBq ^{64}Cu-PTSM-labeled OVA-Th1 cells 3 h after the labeling procedure were assessed by Dunnet´s test. (1.5 MBq vs. unlabeled: *P*=0.0048, 2.2 MBq vs. unlabeled: *P*<0.0001).

	Incubation ^{64}Cu-PTSM-activity			
Time after incubation	**Unlabeled**	**0.7 MBq**	**1.5 MBq**	**2.2 MBq**
3 h	1.0	8.9±2.8	24.3±5.5	45.8±10.8



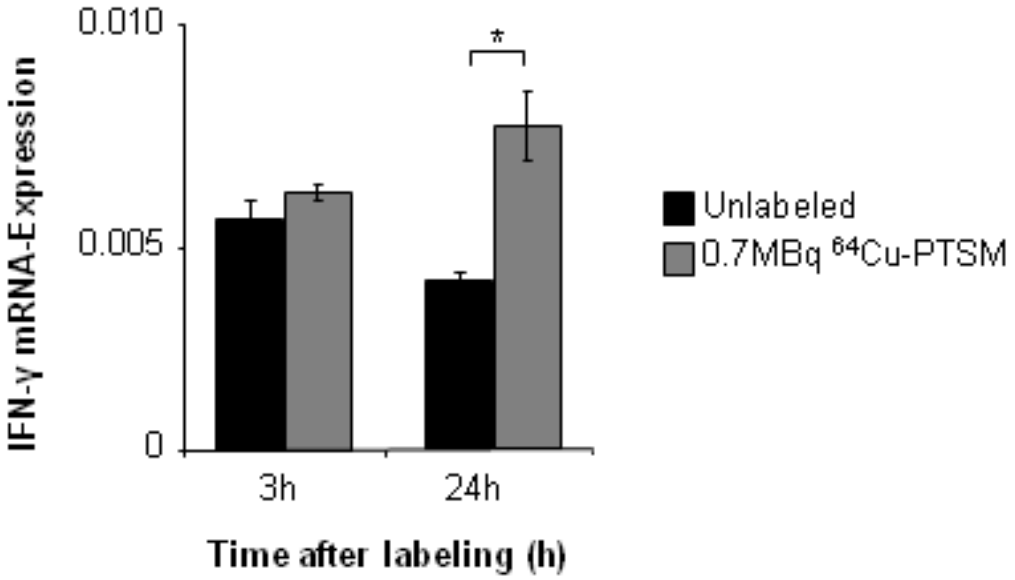
Supplemental Figure 1. Increased applied activity and incubation time enhanced the ^{64}Cu-PTSM uptake in OVA-Th1 cells. (A) ^{64}Cu-PTSM uptake (MBq) in OVA-Th1 cells labeled with 0.7, 1.4 or 2.2 MBq for 2, 3 or 4.5 h. A Tukey-Kramer test was used to determine significant differences among the experimental groups (n = 3); *** P<0.001. Data are presented as the mean ± SD. (B) ^{64}Cu in 10^6 OVA-Th1 cells, supernatants and the washing solution immediately, 5 and 24 h after labeling with 0.7 or 1.5 MBq of ^{64}Cu-PTSM. Data are presented as the mean ± SD (n = 3) of three independent experiments. (C) Impact of 3 and 4.5 h labeling with 60 pg Cu-PTSM on

OVA-Th1 cell viability 3, 24 and 48 h after the labeling procedure. The number of viable OVA-Th1 cells was normalized against that of the non-labeled control cells and presented as a percentage. Data are presented as the mean ± SD (n = 3).

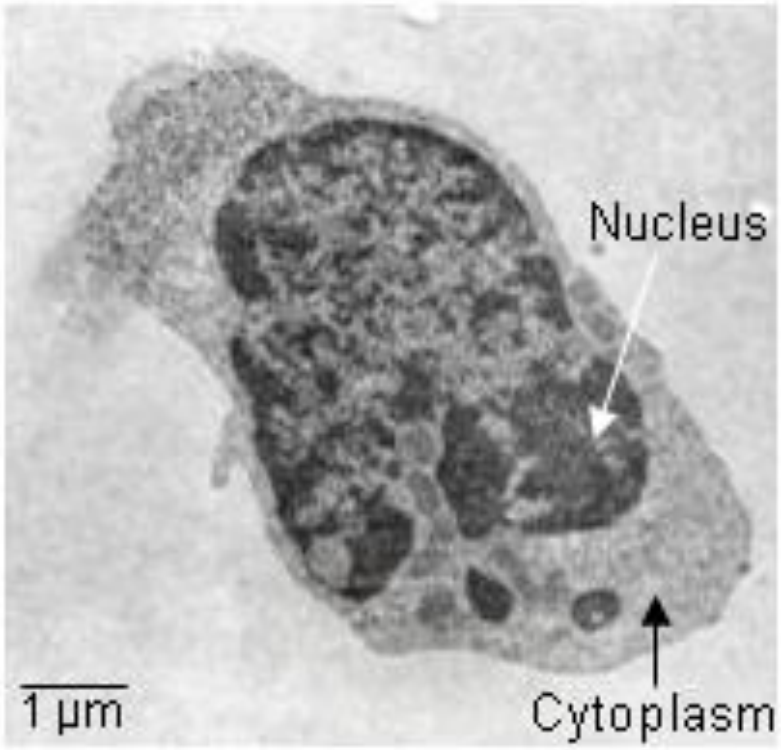


Supplemental Figure 2. Reduced IFN-γ production of OVA-Th1 cells is proportional to increased ^{64}Cu-PTSM incubation time. (A) The impact of different times of incubation with 0.7 MBq of ^{64}Cu-PTSM on OVA-Th1 cell specific IFN-γ production 3 h after the labeling procedure. OVA-specific IFN-γ concentration (ng/mL) in the supernatants of OVA-Th1 cells ($1x10^5$) cultured for 24 h with irradiated APCs ($5x10^5$) and 20 µg/mL OVA-peptide 3 h after 2, 3 or 4.5 h of incubation with or without 0.7 MBq of ^{64}Cu-PTSM. A Tukey-Kramer test was used to determine significant differences in IFN-γ concentrations between the unlabeled and ^{64}Cu-OVA-Th1 experimental groups * P<0.05; *** P<0.001. Data are presented as the mean ± SD (n

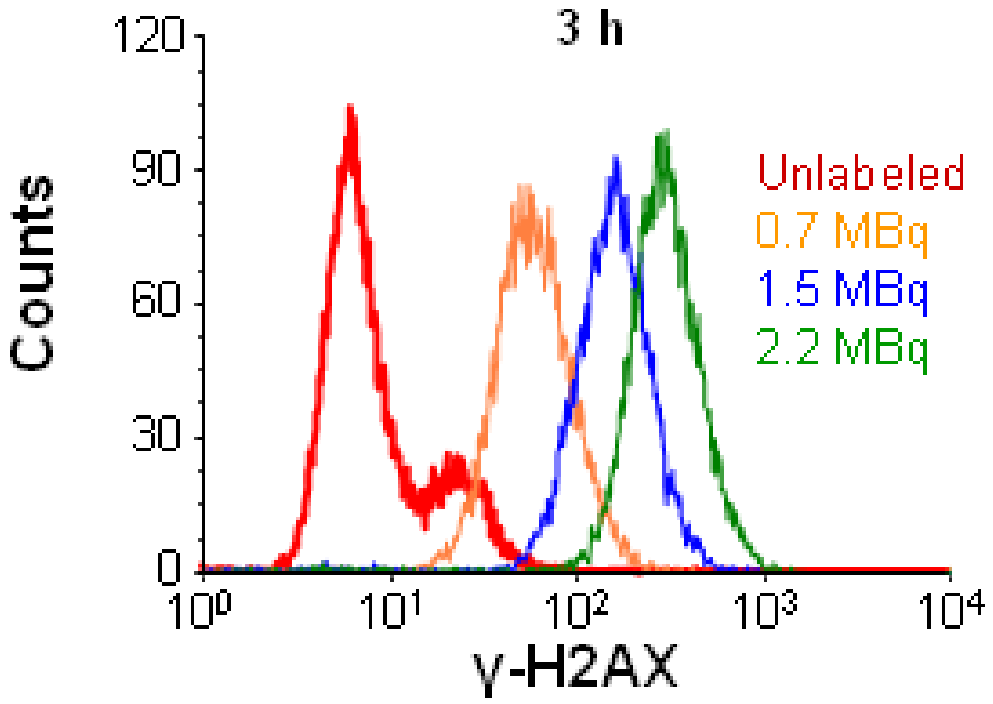
= 2). (B) PTSM does not impair the specific IFN-γ secretion of OVA-Th1 cells. OVA-specific IFN-γ production of OVA-Th1 cells 3 and 48 h after labeling with or without 0.15, 0.30 and 0.40 μg of pure PTSM. Data are presented as the mean ± SD (n = 2). (C) High concentrations of non-radioactive Cu-PTSM suppress the OVA-specific IFN-γ secretion of OVA-Th1 cells. OVA-specific IFN-γ production of OVA-Th1 cells 3 and 24 h after 3 or 4.5 h of incubation with or without 60 pg of non-radioactive Cu-PTSM. Data are presented as the mean ± SD (n = 2). (D) ^{64}Cu-PTSM-labeling and Cu-PTSM-labeling causes reduced proliferation in OVA-Th1 cells. OVA-Th1 cells (10^5) were labeled with 0.7 MBq of ^{64}Cu-PTSM or 60 pg of Cu-PTSM and cultured 3 h after the labeling procedure for 24 h with irradiated APCs ($5x10^5$) and 20 μg/mL OVA-peptide. OVA-Th1 cells were then pulsed with ^{3}H-thymidine for the final 12 h, and the proliferation rate (counts per minute) was measured. Dunnet's test was used to reveal significant differences in cell proliferation rates among unlabeled and ^{64}Cu-PTSM- and Cu-PTSM-labeled OVA-Th1 cells; ** P<0.01; *** P<0.001. Data are presented as the mean ± SD (n = 2).



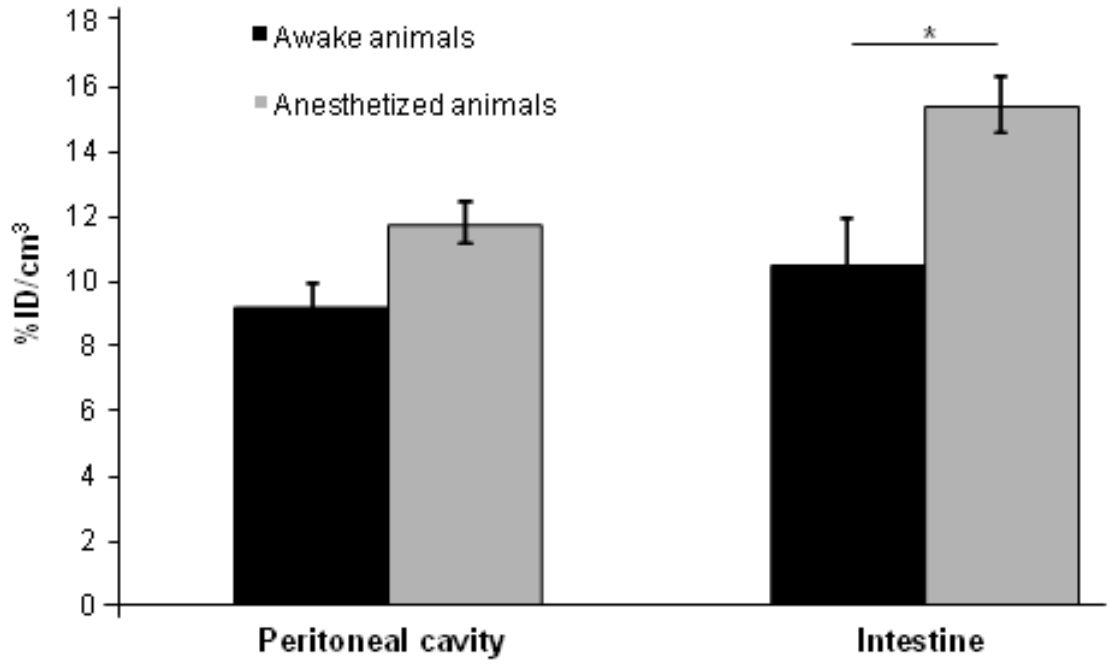
Supplemental Figure 3. Analysis of IFN-γ mRNA-expression patterns 3 and 24 h after labeling without or with 0.7 MBq ^{64}Cu-PTSM. mRNA-expression was normalized to the housekeeping gene aldolase and presented as mean ± SEM (n=3). We performed a two-sampled t-test to reveal statistical differences between unlabeled and ^{64}Cu-PTSM-labeled OVA-Th1; * P<0.05.



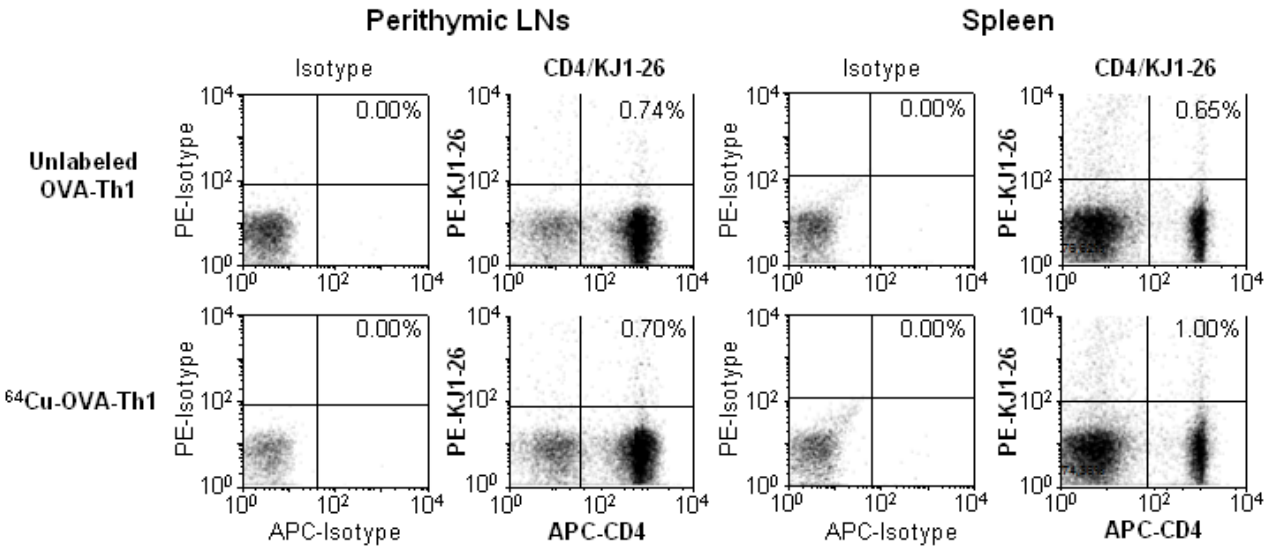
Supplemental Figure 4. A representative electron microscopy image (magnification $12x10^3$) of a ^{64}Cu-PTSM-labeled OVA-Th1 cell indicates the locations of the acquired EDX-spectra within the cell nucleus (white arrow) and cytoplasm (black arrow).



Supplemental Figure 5. Representative phosphorylated γ-H2AX-FACS-histograms of ^{64}Cu-OVA-Th1 cells indicate an increase in γ-H2AX that was proportional to the initially applied labeling intensity (for statistics, please see supplemental Table 1).



Supplemental Figure 6. Quantification of *i.p.* transferred ^{64}Cu-OVA-Th1 cells (%ID/cm^3) in the peritoneal cavity and intestine after 3 h in awake (static PET-scans; n = 6) and anesthetized animals (dynamic PET-scans; n = 3); two-sampled student's t-test; * P<0.05; mean ± SEM.



Supplemental Figure 7. FACS-analysis of the perithymic LNs and spleen 21 h after *i.p.* injection of 10^7 unlabeled (upper row) or ^{64}Cu-PTSM-labeled OVA-Th1 cells (lower row). Representative FACS-diagrams display APC-CD4- and PE-KJ1-26-stained leukocytes (right column) and corresponding isotype controls (left column) derived from the perithymic LNs and spleens.