Supplementary files

Table S1. Primary antibodies used for immunohistochemical staining in mouse lung sections

Antigen	Source	Manufacturer	Reference	Dilution	Unmasking
Mouse CD20	Goat polyclonal	Santa Cruz	Sc7735	1:100	Citrate microwave
Mouse CD3	Rabbit monoclonal	ThermoFisher	RM-9207-S	1:50	Citrate microwave
Mouse CD21	Rabbit monoclonal	Abcam	Ab 75985	1:200	Citrate microwave
Mouse PNAd	Rat monoclonal	BD Pharmingen	553863	1:50	Citrate microwave
Mouse PCNA	Rabbit polyclonal	Calbiochem	PC474	1:200	Protease

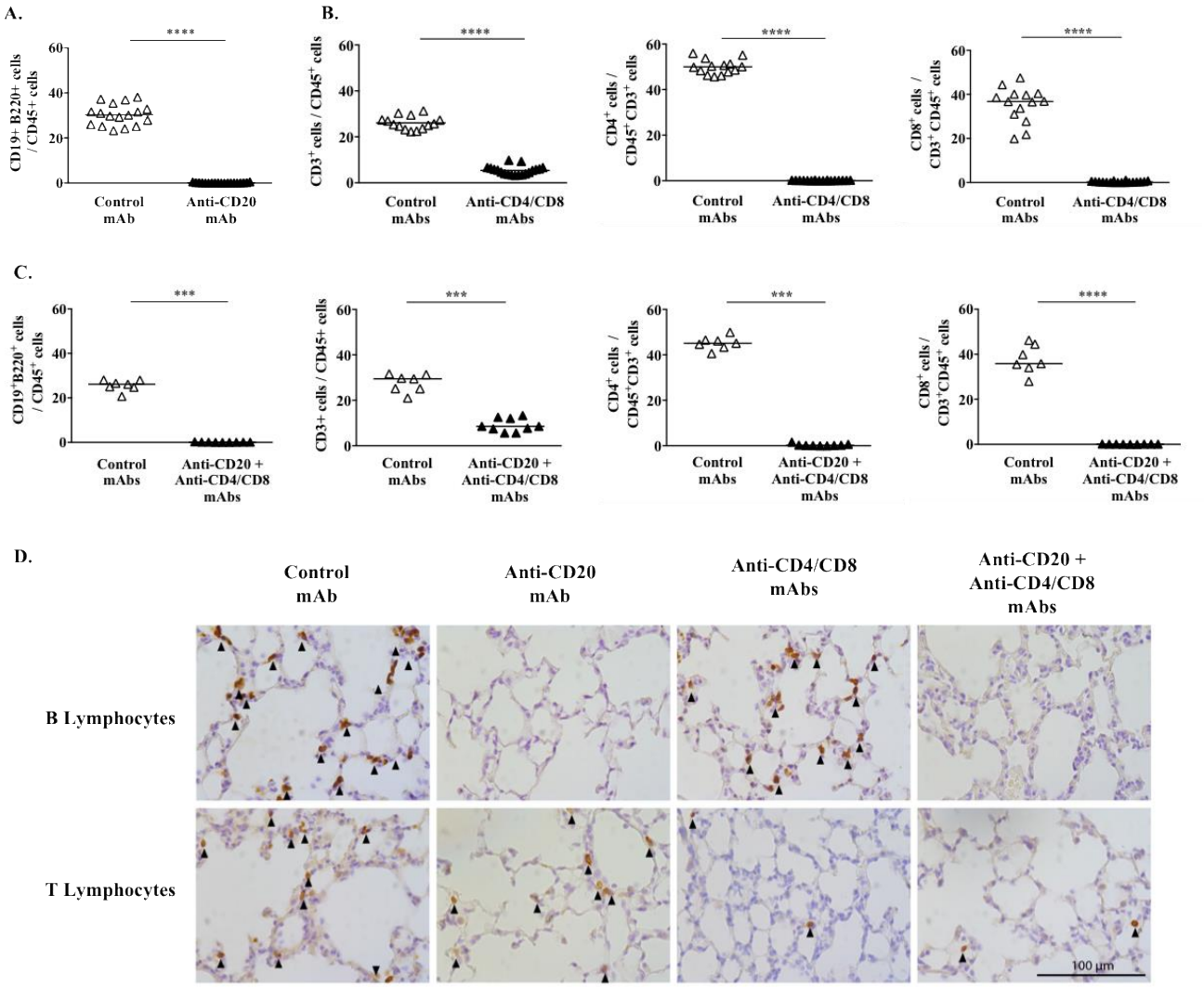
PNAd: peripheral nod adressin; PCNA: proliferating cell nuclear antigen

Table S2. Antibody panels used in flow cytometry analysis.

Fluorochrome	Antigen (Antibody clone)
FITC	B220 (RA3-6B2)*
BV450	CD19 (1D3)*
PE-TexasRed	CD4 (MCD0417)£
PE-Cy7	CD45 (30-F11)*
APC	CD8 (53-6.7)*
AF700	CD3 (500A2)*
Amcyan	Viability marker (Live/dead cell dye)£

Conjugated antibodies or fluorescent dyes were purchased from *BD Bioscience, £ThermoFisher Scientific.

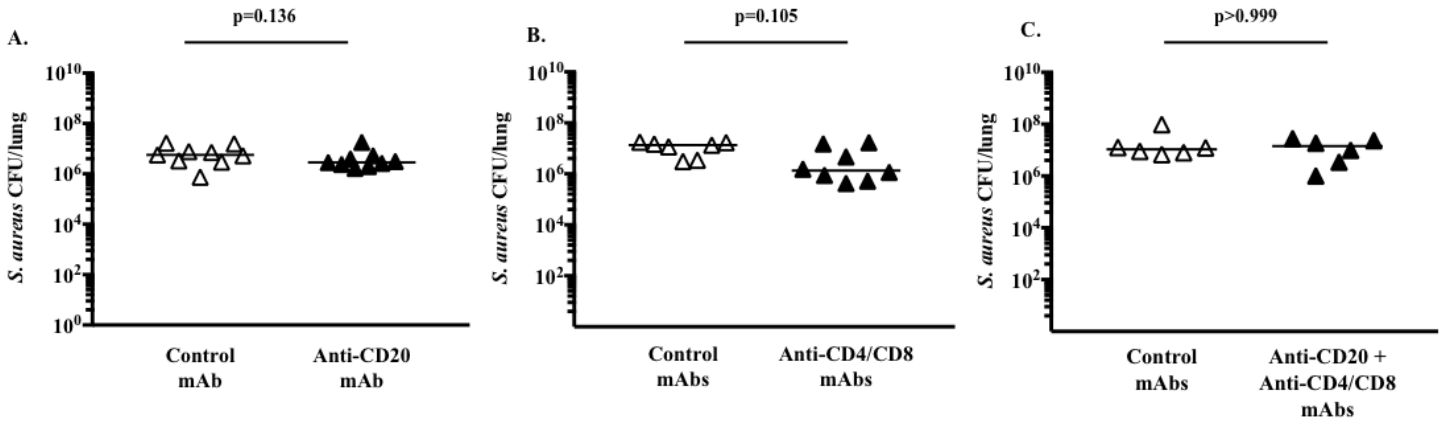
Figure S1. Treatment with anti-CD20 and/or anti-CD4/CD8 mAbs induces depletion in lung of B and /or T lymphocytes for at least 21 days.



Mice were injected with anti-CD20 and/or anti-CD4/CD8 mAbs, or control mAbs and followed for 21 days (no infection). At 21 days after treatment with mAbs, lungs were harvested for flow cytometry (A, B, C) and histological analysis (D). A. Quantification of B cells obtained by flow cytometry analysis in the lungs of mice treated with control or anti-CD20 mAb. B. Quantification of T cell subsets (CD3+CD45+ [left panel], CD4+CD3+CD45+ [middle panel] and CD8+CD3+CD45+ [right panel]) obtained by flow cytometry analysis in the lungs of mice treated with control mAbs or anti-CD4/CD8 mAbs. C.

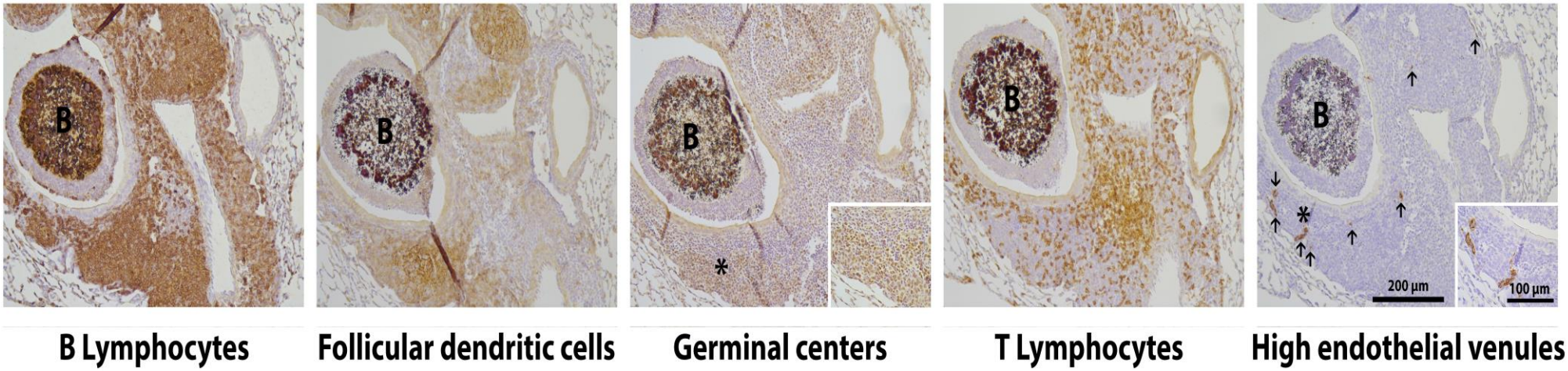
Quantification of B cells and T cell subsets in the lungs of mice treated with control mAbs or with both anti-CD20 and anti-CD4/CD8 mAbs. Each symbol represents data obtained from one animal (open symbol for Controls and solid symbols for depleted mice). Horizontal bars represent medians. The Mann-Withney test was used to compare depleted mice to Controls (Panel A, B and C). ****$p < 0.0001$ and ***$p<0.001$ compared to controls. D. Representative photomicrographs of immunostaining for B lymphocytes (CD20+, upper panel) or T lymphocytes (CD3+, lower panel) in the lungs of mice sacrificed 21 days after treatment with control mAb or anti-CD20 mAb, or anti-CD4/CD8 mAbs, or anti-CD20 and anti-CD4/CD8 mAbs. Positive staining appears in brown (arrowheads); sections were counterstained with hematoxylin. Original magnification, 400X.

Figure S2. Effects of anti-CD20 and/or anti-CD4/CD8 mAbs-induced depletion on lung bacterial load in mice persistently infected with *S. aureus*.



Mice were pre-treated with anti-CD20 mAb (A), anti-CD4 and anti-CD8 mAbs (B), or a combination of anti-CD20 and anti-CD4/CD8 mAbs (C) or with Control mAb prior to infection. Persistent infection was obtained by intratracheal instillation of agarose beads containing S. aureus (10^6 CFU per animal). Fourteen days after instillation, animals were sacrificed and lungs were harvested, homogenized and cultured for bacterial load assessment. Pretreatment with anti-CD20, anti-CD4/CD8 or a combination of anti-CD20 and anti-CD4/CD8 mAbs had no effect on lung bacterial load compared to control group. Each symbol represents data obtained from one animal. Horizontal bars correspond to medians. The Mann-Whitney test was used to compare depleted mice to Controls (Panel A, B and C).

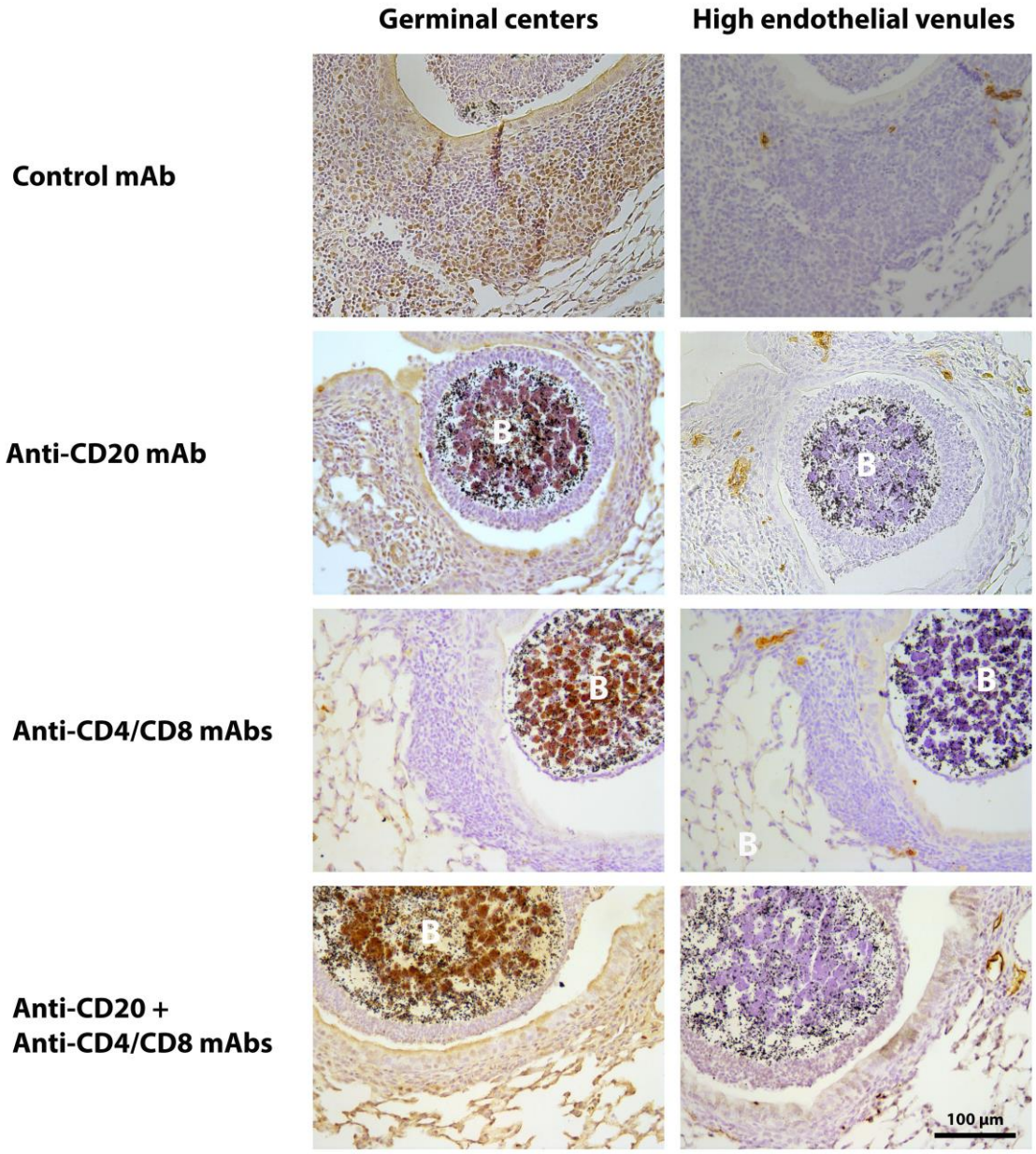
Figure S3. Representative photomicrographs of *S. aureus*-induced peribronchial lymphoid neogenesis in mice treated with control mAb.



Mice were pre-treated with control mAb. Persistent infection was obtained by intratracheal instillation of agarose beads containing *S. aureus* (10^6 CFU per animal). Beads (referred as "B" in the figure) are found in the lumen of the mice bronchi. Fourteen days after instillation, animals were euthanized and lungs were harvested for histological analysis**.** Sections were immunostained (brown color) with antibodies directed against B lymphocytes ($CD20^+$), follicular dendritic cells ($CD21^+$), germinal centers (proliferating cell nuclear antigen, $PCNA^+$), T lymphocytes ($CD3^+$), or high endothelial venules (HEVs, peripheral node adressin, $PNAd^+$), and counterstained with hematoxylin. Peribronchial lymphoid aggregates were found around bead-containing bronchi; these aggregates contained B-cell areas ($CD20^+$) with follicular dendritic cells ($CD21^+$) and germinal centers ($PCNA^+$) and were surrounded by T cell aggregates ($CD3^+$) containing high endothelial venules ($PNAd^+$) and were consistent

with tertiary lymphoid structures. Symbols (*) identify areas represented in the inserts. Arrows identify HEVs. Original magnification, 100X; inserts, 400X. Bar= 200 micrometers; insert bar = 100 micrometres.

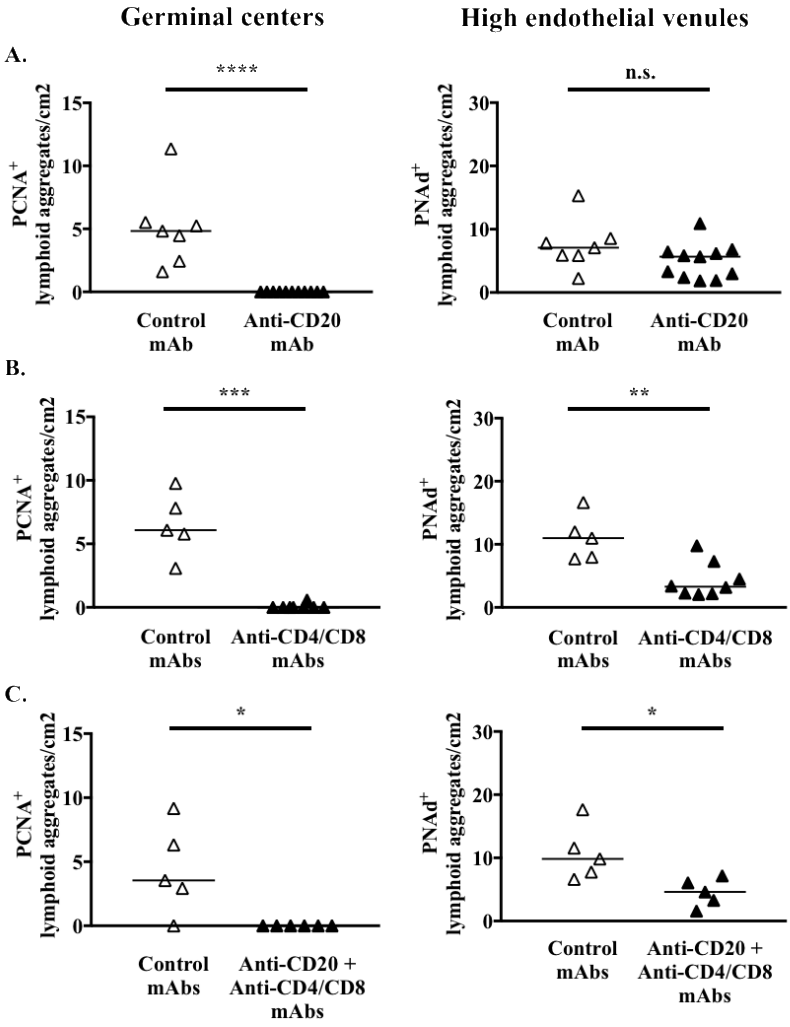
Figure S4. Representative photomicrographs of germinal centers and HEV in the lungs of mice persistently infected with *S. aureus* and treated with anti-CD20, anti-CD4 plus CD8 mAbs or all three monoclonal antibodies.



Mice were pre-treated with control mAb or with anti-CD20 and/or anti-CD4/CD8 mAbs prior to infection. Persistent infection was obtained by intratracheal instillation of agarose beads containing *S. aureus* (10^6 CFU per animal). Beads (referred as "B" in the figure) are found in the lumen of the mice bronchi. Fourteen days after instillation, animals were euthanized and lungs were harvested for histological analysis**.** Sections were immunostained (brown color) with antibodies directed against germinal centers (proliferating cell nuclear antigen, $PCNA^+$)

or high endothelial venules (HEVs, peripheral node adressin, $PNAd^+$), and counterstained with hematoxylin. *Control mAbs*: in animal pretreated with Control mAb, 14-days infection with *S. aureus*- induced peribronchial tertiary lymphoid structures containing germinal centers, as well as HEV. *$CD20^+$ B cell depletion*: pretreatment with anti-CD20 mAb prevented germinal center formation. HEV recruitment was unaffected. *$CD4^+$ and/or $CD8^+$ T cell depletion*: pretreatment with anti-CD4/CD8 mAbs reduced HEVs formation and germinal centers were absent. *$CD20^+$ B and $CD4^+$/$CD8^+$ T cell depletion*: pretreatment with anti-C20 and anti-CD4/CD8 mAbs prevented the formation of germinal centers. HEVs were reduced. Arrows identify HEVs. Original magnification, 100X. Bar= 100 micrometers.

Figure S5. Quantitative morphometric analyses of germinal center- and HEV-containing lymphoid aggregates in the lungs of mice persistently infected with *S. aureus* and treated with anti-CD20, anti-CD4 plus CD8 mAbs or all three monoclonal antibodies.



Mice were pretreated with Control mAb or with anti-CD20 and/or anti-CD4/CD8 mAbs prior to infection. Persistent infection was obtained by intratracheal instillation of agarose beads containing *S. aureus* (10^6 CFU per animal). Fourteen days after instillation, animals were euthanized and lungs were harvested for histological analysis**.** Sections were immunostained with antibodies directed against germinal center (proliferating cell nuclear antigen, $PCNA^+$), or high endothelial venules (peripheral node adressin, $PNAd^+$). Lymphoid aggregates were counted using morphometric analysis as described in the methods section. A. *$CD20^+$ B cell*

depletion: pretreatment with anti-CD20 mAb significantly reduced the number of germinal center-containing lymphoid aggregates. HEV^+ lymphoid aggregate number was unaffected. B. *$CD4^+$ and $CD8^+$ T cell depletion*: pretreatment with anti-CD4/CD8 mAbs significantly reduced the number of germinal center-containing lymphoid aggregate; HEV-containing lymphoid aggregates number was also significantly reduced. C. *$CD20^+$ B- and $CD4^+$/$CD8^+$ T cell depletion*: pretreatment with anti-CD20 and anti-CD4/CD8 mAbs significantly reduced the number of germinal center-containing lymphoid aggregates, as well as the number of HEV^+ lymphoid aggregates. Each symbol represents data obtained from one animal. Horizontal bars correspond to median values. The Mann-Whitney test was used to compare depleted groups to Controls. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, ****: $P<0.0001$ compared to controls.