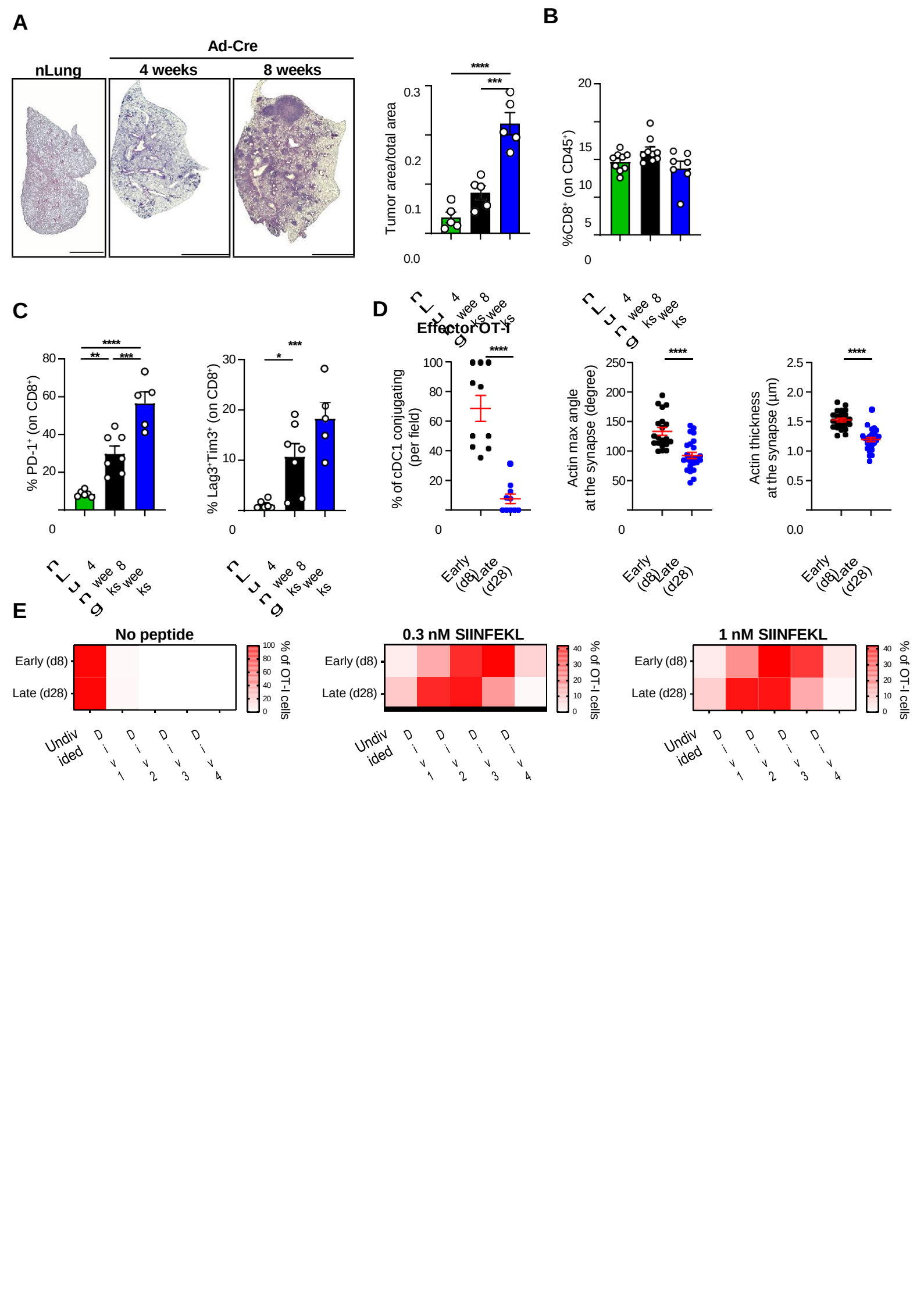
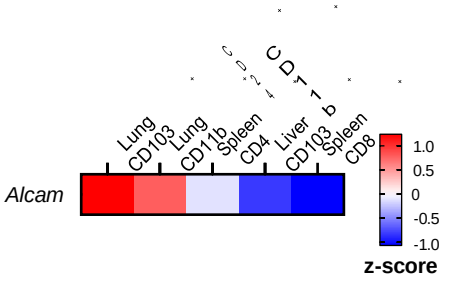

A
Ad-Cre
nLung
4 weeks
8 weeks
B
Tumor area/total area
%CD8+ (on CD45+)
C
% PD-1+ (on CD8+)
% Lag3+Tim3+ (on CD8+)
nLung
4 wee ks
8 wee ks
D
Effector OT-I
% of cDC1 conjugating (per field)
Actin max angle at the synapse (degree)
Actin thickness at the synapse (µm)
Early (d8)
Late (d28)
E
No peptide
0.3 nM SIINFEKL
1 nM SIINFEKL
Early (d8)
Late (d28)
% of OT-I cells
Undivided
Div 1
Div 2
Div 3
Div 4

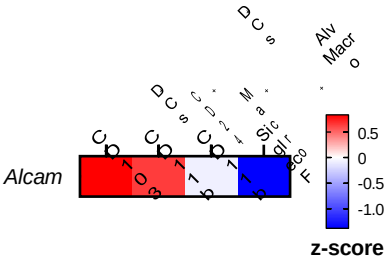
Figure S1

Figure S1. Late cDC1 fail to physically interact with CD8 T cells. (A) Representative H&E sections of healthy (nLung), or tumor-bearing lungs in the KP (*Kras*$^{LSL\text{-}G12D/+}$; *Trp53*$^{fl/fl}$) genetic model. Lungs were harvested after 4 or 8 weeks post Ad-Cre administration. Tumor burden was calculated as the fraction of total lung area occupied by tumor (n = 5 mice per group, pooled from two independent experiments). **(B)** Percentage of CD8 T cells was quantified by flow cytometry in lungs of normal or tumor bearing mice (n = 7-9 mice per group). **(C)** Percentage of PD-1+ (left) and LAG3+Tim3+ (right) CD8 T cells quantified by flow cytometry in normal and tumor bearing lungs n = 5-7 mice per group. One-way ANOVA followed by Tukey's post-test. **(D)** cDC1 were isolated from tumor-bearing lungs, loaded *ex vivo* with SIINFEKL and mixed with CFSE-labelled effector OT-I cells on fibronectin-coated coverslips. Quantification of the percentage of cDC1 engaged in conjugates (n = 10 planes per group), the surface of interaction (actin maximum angle) and actin thickness at the contact side are indicated (n = 20-32 DC1-OT-I conjugates per group). Unpaired Student t-test. **(E)** Isolated lung cDC1 from tumor-bearing mice were loaded *ex vivo* with different concentrations of SIINFEKL and mixed with CTV-labelled OT-I cells. Representative heatmaps showing the percentage of OT-I cells in each proliferation cycle after 72 hs of co-incubation at 0 (no peptide), 0.3 and 1 nM SIINFEKL. Data represent mean ± SEM, one representative out of three independent experiments is shown. *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001.

A


Steady-state DCs (ImmGen)
Alcam
Lung CD103
Lung CD11b
Spleen CD4
Liver CD103
Spleen CD8
1.0
0.5
0
-0.5
-1.0
z-score

B


Lung myeloid cells (ImmGen)
Alcam
Alv Macro
0.5
0
-0.5
-1.0
z-score

C

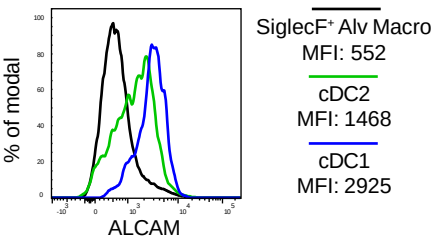

Lung myeloid cells (flow cytometry)
% of modal
ALCAM
SiglecF+ Alv Macro
MFI: 552
cDC2
MFI: 1468
cDC1
MFI: 2925

Figure S2

Figure S2. ALCAM is preferentially expressed by lung DC1. (A) Heatmap showing the expression of *Alcam* in steady-state DCs in lung, spleen and liver, obtained from the ImmGen Database. DCs were ordered based on *Alcam* z-score. **(B)** Heatmap of *Alcam* expression in steady-state lung myeloid cells, obtained from the ImmGen Database. cDC1 ($CD103^+$), cDC2 ($CD11b^+CD24^+$), interstitial ($CD11b^+$) and alveolar ($SiglecF^+$) macrophages were ordered based on *Alcam* z-score. **(C)** ALCAM expression in lung steady-state $SiglecF^+$ macrophages (black line), cDC1 (blue line) and cDC2 (green line) by flow cytometry, and quantification of the ALCAM Median Fluorescence Intensity (MFI) for the indicated populations.

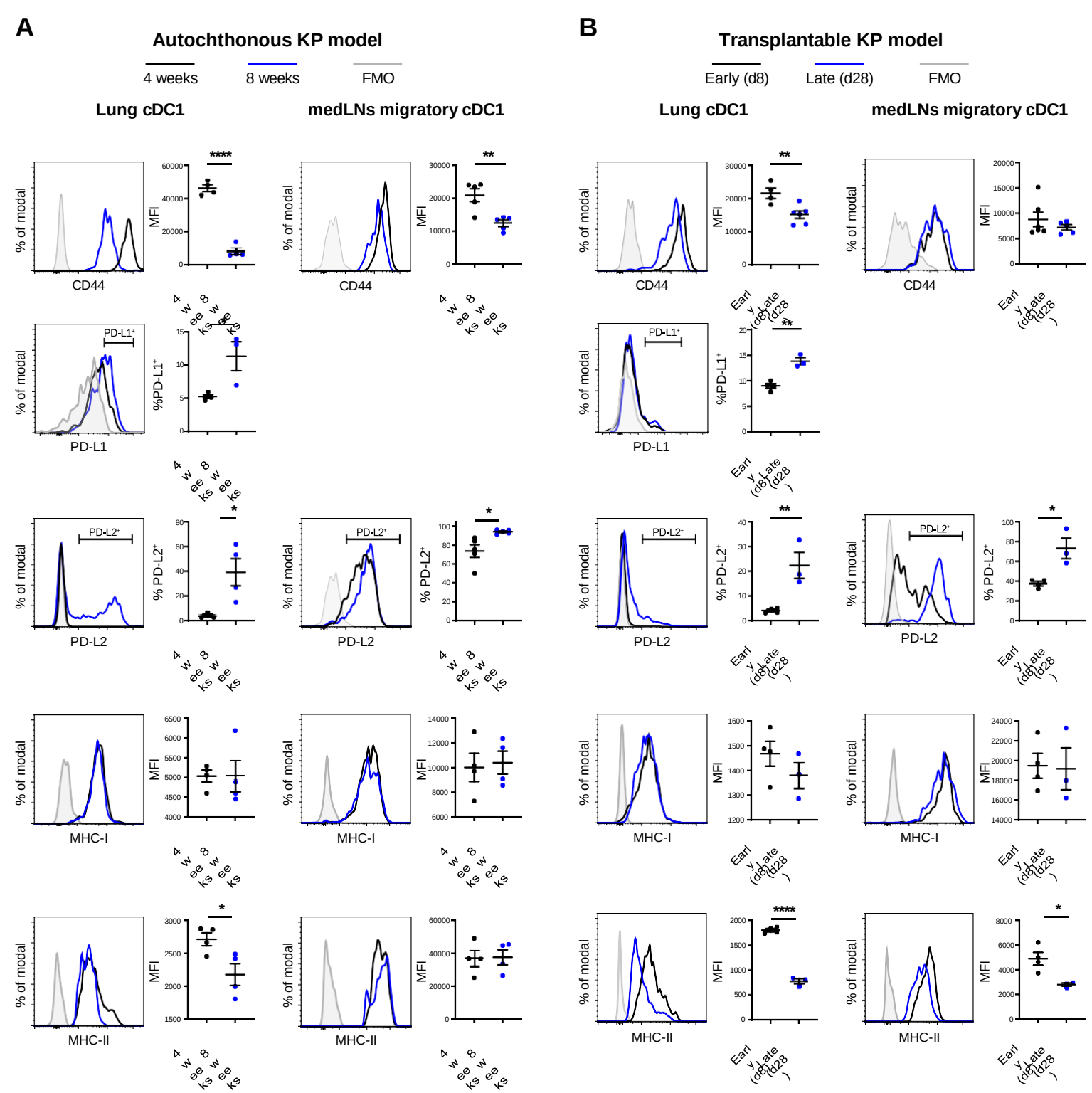

A
Autochthonous KP model
4 weeks
8 weeks
FMO
Lung cDC1
medLNs migratory cDC1
B
Transplantable KP model
Early (d8)
Late (d28)
FMO
Lung cDC1
medLNs migratory cDC1
% of modal
CD44
MFI
PD-L1
%PD-L1+
PD-L2
% PD-L2+
MHC-I
MHC-II
4 weeks
8 weeks
Early (d8)
Late (d28)

Figure S3

Figure S3. Adhesion molecules in lung and migratory DC1 are modulated in advanced KP tumors. (A-B) Lung and mediastinal lymph nodes (medLNs) from tumor-bearing mice were harvested and processed to analyze by flow cytometry the expression of CD44, PD-L1 PD-L2, MHC-I and MHC-II. In the autochthonous model **(A)**, tissues were harvested after 4 (black line) or 8 weeks (blue line) post Ad-Cre administration (n = 4-5 mice per group). In the transplantable model **(B)**, tissues were analyzed at day 8 (black line) or 28 (blue line) after intravenous KP tumors inoculation (n = 4-6 mice per group). Fluorescence minus one (FMO) control staining for each marker is shown in grey. Quantification of the Median Fluorescence Intensity (MFI) or percentage of positive cells in lung DC1 for the indicated markers is presented. Unpaired Student t-test. Data represent mean ± SEM, one representative out of three independent experiments. *$p<0.05$ **$p<0.01$ ***$p<0.001$ ****$p<0.0001$.