Supplementary figure legends

Supplementary Fig.1. Lgr4 facilitates macrophage M2 type polarization. (A) $Lgr4^{+/+}$ and $Lgr4^{-/-}$ bone marrow cells were induced *in vitro* with L929-conditioned medium for 7 days into matured macrophages, F4/80 was stained to mark matured BMMs. (B) Flow cytometry analysis of bone marrow $CD11b^{+}$ $Ly6C^{+}$ macrophage progenitor cells between $Lgr4^{+/+}$ and $Lgr4^{-/-}$ mice. (n=5). (C) Lgr4 interference was conducted in RAW 264.7 cells by short hairpin RNA (shRNA) and expression of Lgr4 was validated by Western Blotting. Control and Lgr4-knockdown RAW 264.7 cells were stimulated with or without IL-4 (50 ng/ml) for 2 h. mRNA expression of (D) CD206 and (E) Arg1 were quantified by quantitative PCR. (F) Lgr4 was overexpressed in RAW 264.7 cells by transfection with a pcDNA3.1 plasmid containing the Lgr4 cDNA (empty vectors as control). And Lgr4 overexpressing RAW 264.7 cells were stimulated with or without IL-4 (50 ng/ml) for 2 h. mRNA expression of (G) CD206 and (H) Arg1 markers was quantified. Expression was normalized to β-actin. I and J, LGR4 knock-down experiment was performed with RNAi in human monocytic THP-1 cells, LGR4 mRNA expression was validated by polymerase chain reaction. Expression of M2 specific genes (K) MRC1 and (L) ARG1 were investigated in the LGR4-knockdown THP-1 cells stimulated with or without recombinant human IL-4 (50ng/ml) for 2 h. Columns, means; bars, SD; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Supplementary Fig.2. Lgr4 impairs macrophage M1 type polarization. $Lgr4^{+/+}$ and $Lgr4^{-/-}$ BMMs were stimulated with 100 ng/ml mouse recombinant IFN-γ for 2h. Expression of (A) iNOS, (B) TNF-α, (C) CXCL10 and (D) IL-12β was measured by quantitative PCR. RAW264.7 cells were forced to express Lgr4 and expression of (E) iNOS, (F) TNF-α, (G) CXCL10 and (H) IL-12β

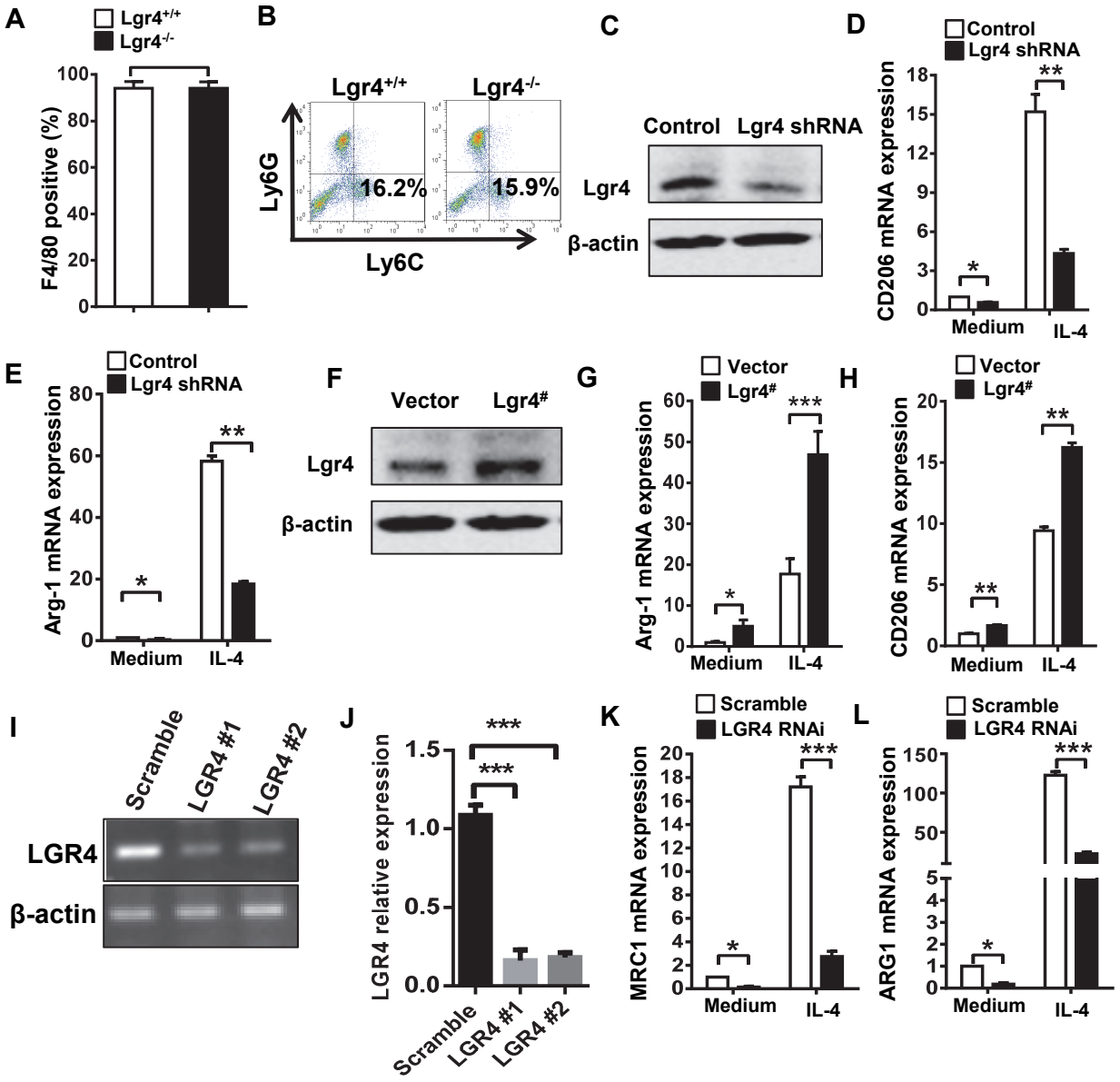
was measured by quantitative PCR. Columns, means; bars, SD; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Supplementary Fig.3. Lgr4 facilitates macrophage M2 polarization in tumors. (A) Co-localization of LGR4 and M2 macrophage marker CD68 in human breast cancer sample by immunofluorescence staining of LGR4 (Green) and CD68(Red). B, Infiltration of F4/80 and CD206 positive cells in LLC tumors from $Lyz2^{+/+}$ and $Lyz2^{cre/+}$ mice. Tumor specimens were immunohistochemical staining of F4/80 (upper panel) and CD206 (lower panel), respectively. Positive cells were calculated and showed (C). Columns, means; bars, SD; **, $p < 0.01$(n=3); n.s., no significant difference.

Supplementary Fig.4. LGR4 ECD, Rspo1 antibody and BLZ945 were of little toxicity to both tumor cells and macrophage. 1×10^4 LLC cells or wild type BMMs were seeded in 96 plate wells and were treated with indicated concentrations of LGR4 ECD, Rspo1 antibody and BLZ945 for different time periods. Proliferation tests of LLCs (A, B and C) and BMMs (D, E and F) were performed with the Cell Counting Kit-8 (CCK8). Columns, means; bars, SD; n.s., no significant difference.

Supplementary Fig.5. Rspo/Lgr4 blockade decreased M2 TAMs infiltration and promoted $CD8^+$ T cells activation in B16F10 melanoma. B16F10 tumors from mice received treatments were dissected and digested to obtain single cell suspensions, infiltrating immune cells were stained and analyzed by flow cytometry. Data shown are representative results of (A and B) M2

TAMs ($F4/80^+CD206^+$, indicated by red dots) and activated $CD8^+$ T cells (C and D) $CD8^+IFN\text{-}\gamma^+$ and (E and F) $CD8^+GzmB^+$, indicated by blue dots), n=6 each group. Columns, means; bars, SD; **, $p < 0.01$; ***, $p < 0.001$.


A
Lgr4+/+
Lgr4-/-
F4/80 positive (%)
B
Lgr4+/+
Lgr4-/-
Ly6G
Ly6C
16.2%
15.9%
C
Control
Lgr4 shRNA
Lgr4
β-actin
D
Control
Lgr4 shRNA
CD206 mRNA expression
Medium
IL-4
E
Control
Lgr4 shRNA
Arg-1 mRNA expression
Medium
IL-4
F
Vector
Lgr4#
Lgr4
β-actin
G
Vector
Lgr4#
Arg-1 mRNA expression
Medium
IL-4
H
Vector
Lgr4#
CD206 mRNA expression
Medium
IL-4
I
Scramble
LGR4 #1
LGR4 #2
LGR4
β-actin
J
LGR4 relative expression
Scramble
LGR4 #1
LGR4 #2
K
Scramble
LGR4 RNAi
MRC1 mRNA expression
Medium
IL-4
L
Scramble
LGR4 RNAi
ARG1 mRNA expression
Medium
IL-4

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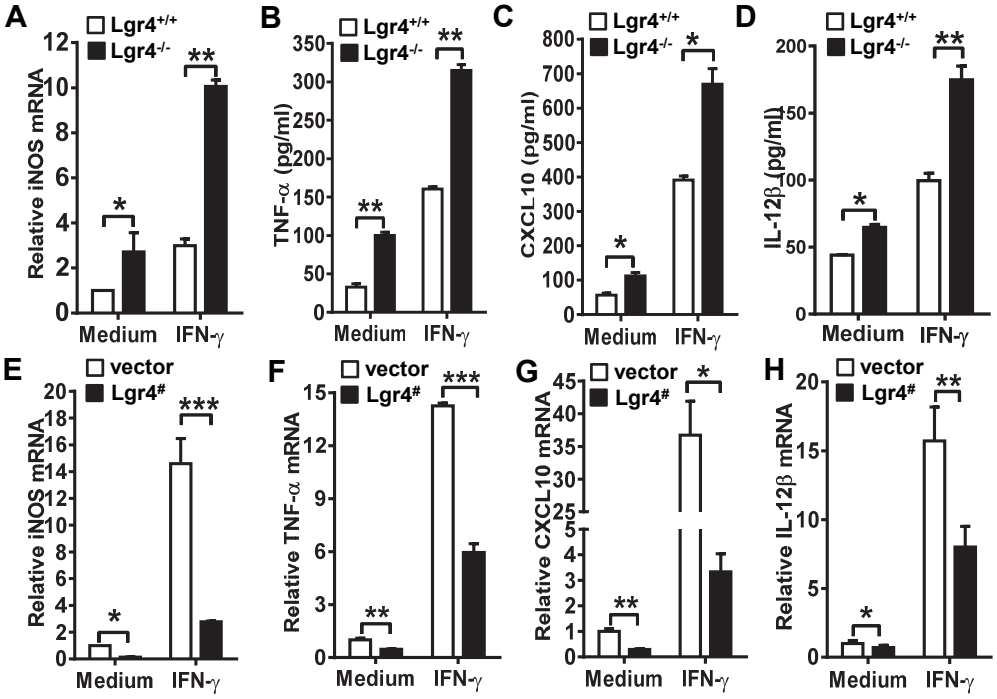

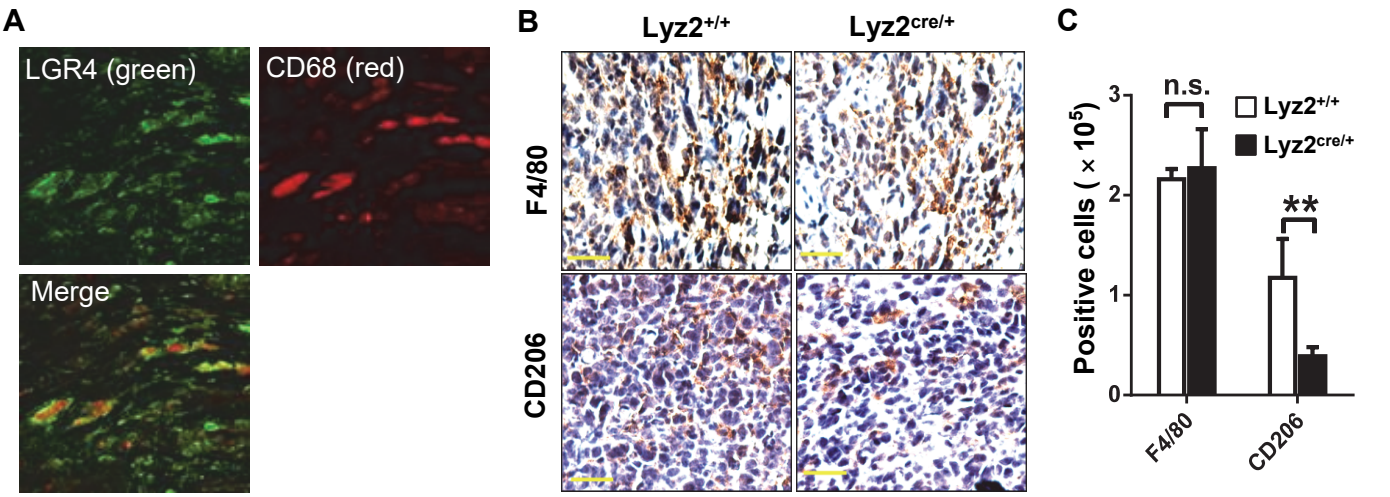
A
LGR4 (green)
CD68 (red)
Merge
B
Lyz2+/+
Lyz2cre/+
F4/80
CD206
C
n.s.
**
Lyz2+/+
Lyz2cre/+
Positive cells (× 10^5)
3
2
1
0
F4/80
CD206

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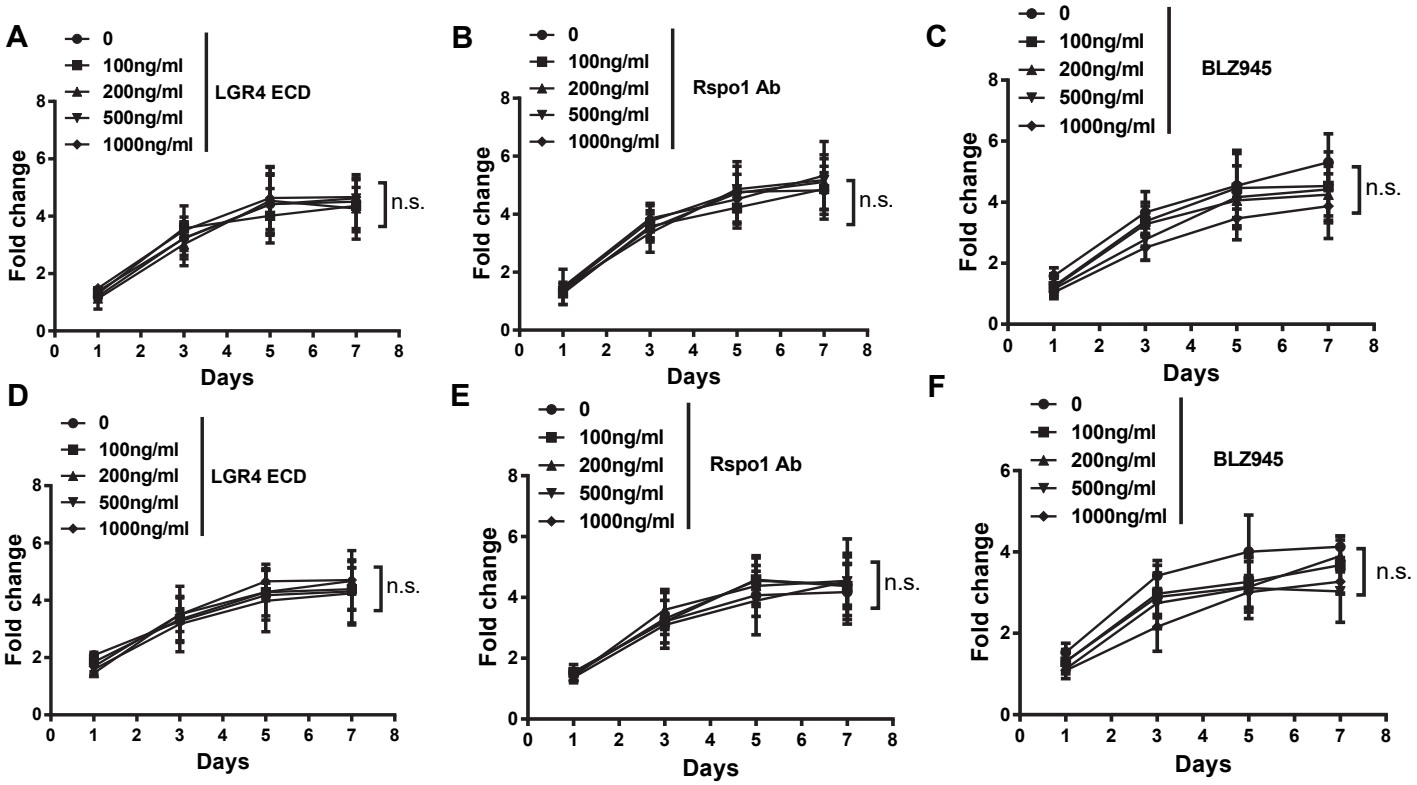

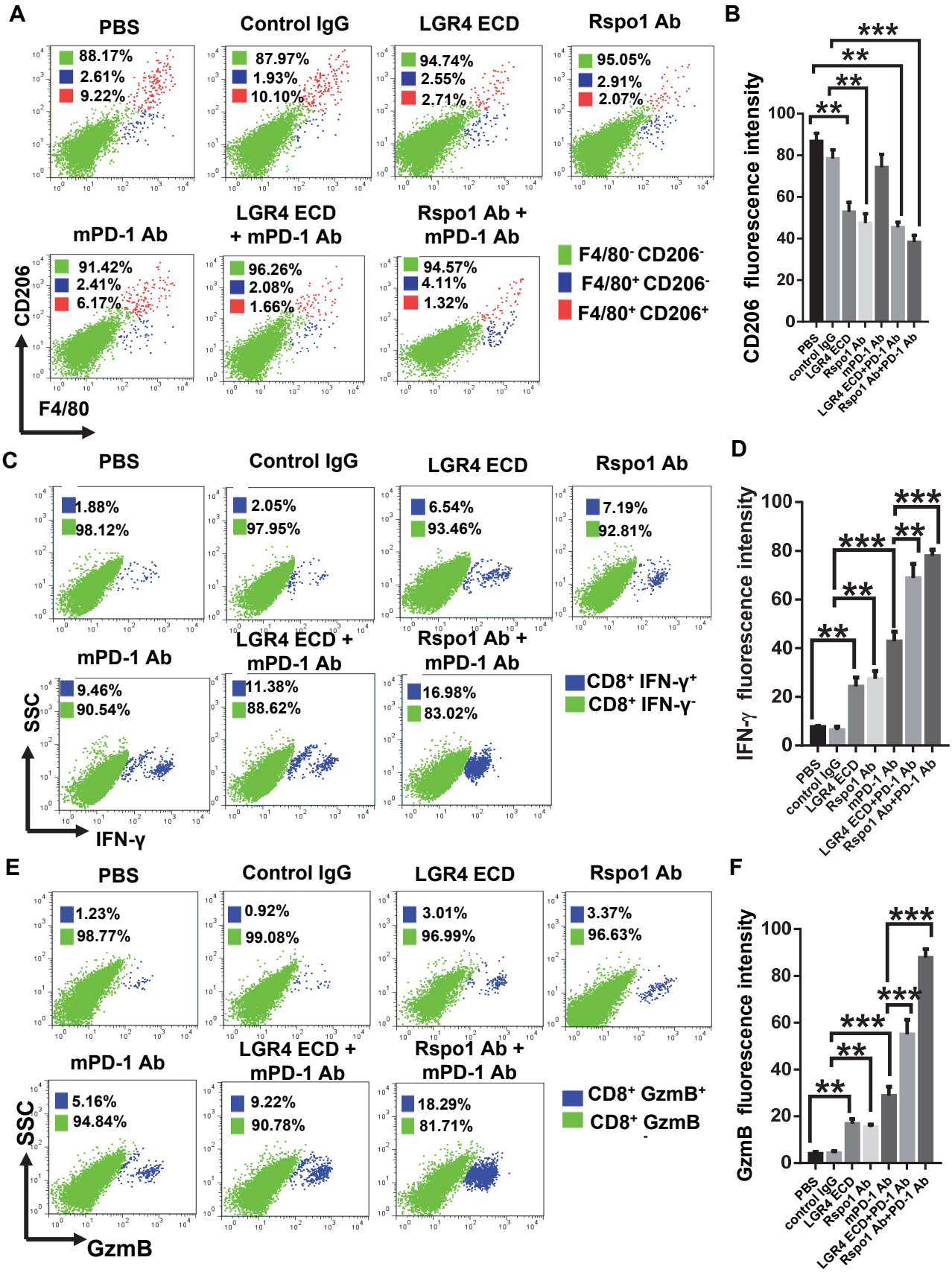
A
PBS
Control IgG
LGR4 ECD
Rspo1 Ab
88.17%
2.61%
9.22%
87.97%
1.93%
10.10%
94.74%
2.55%
2.71%
95.05%
2.91%
2.07%
mPD-1 Ab
LGR4 ECD + mPD-1 Ab
Rspo1 Ab + mPD-1 Ab
91.42%
2.41%
6.17%
96.26%
2.08%
1.66%
94.57%
4.11%
1.32%
F4/80- CD206-
F4/80+ CD206-
F4/80+ CD206+
CD206
F4/80
B
CD206 fluorescence intensity
PBS
control IgG
LGR4 ECD
Rspo1 Ab
mPD-1 Ab
LGR4 ECD+PD-1 Ab
Rspo1 Ab+PD-1 Ab
C
PBS
Control IgG
LGR4 ECD
Rspo1 Ab
1.88%
98.12%
2.05%
97.95%
6.54%
93.46%
7.19%
92.81%
mPD-1 Ab
LGR4 ECD + mPD-1 Ab
Rspo1 Ab + mPD-1 Ab
9.46%
90.54%
11.38%
88.62%
16.98%
83.02%
CD8+ IFN-γ+
CD8+ IFN-γ-
SSC
IFN-γ
D
IFN-γ fluorescence intensity
E
PBS
Control IgG
LGR4 ECD
Rspo1 Ab
1.23%
98.77%
0.92%
99.08%
3.01%
96.99%
3.37%
96.63%
mPD-1 Ab
LGR4 ECD + mPD-1 Ab
Rspo1 Ab + mPD-1 Ab
5.16%
94.84%
9.22%
90.78%
18.29%
81.71%
CD8+ GzmB+
CD8+ GzmB-
SSC
GzmB
F
GzmB fluorescence intensity