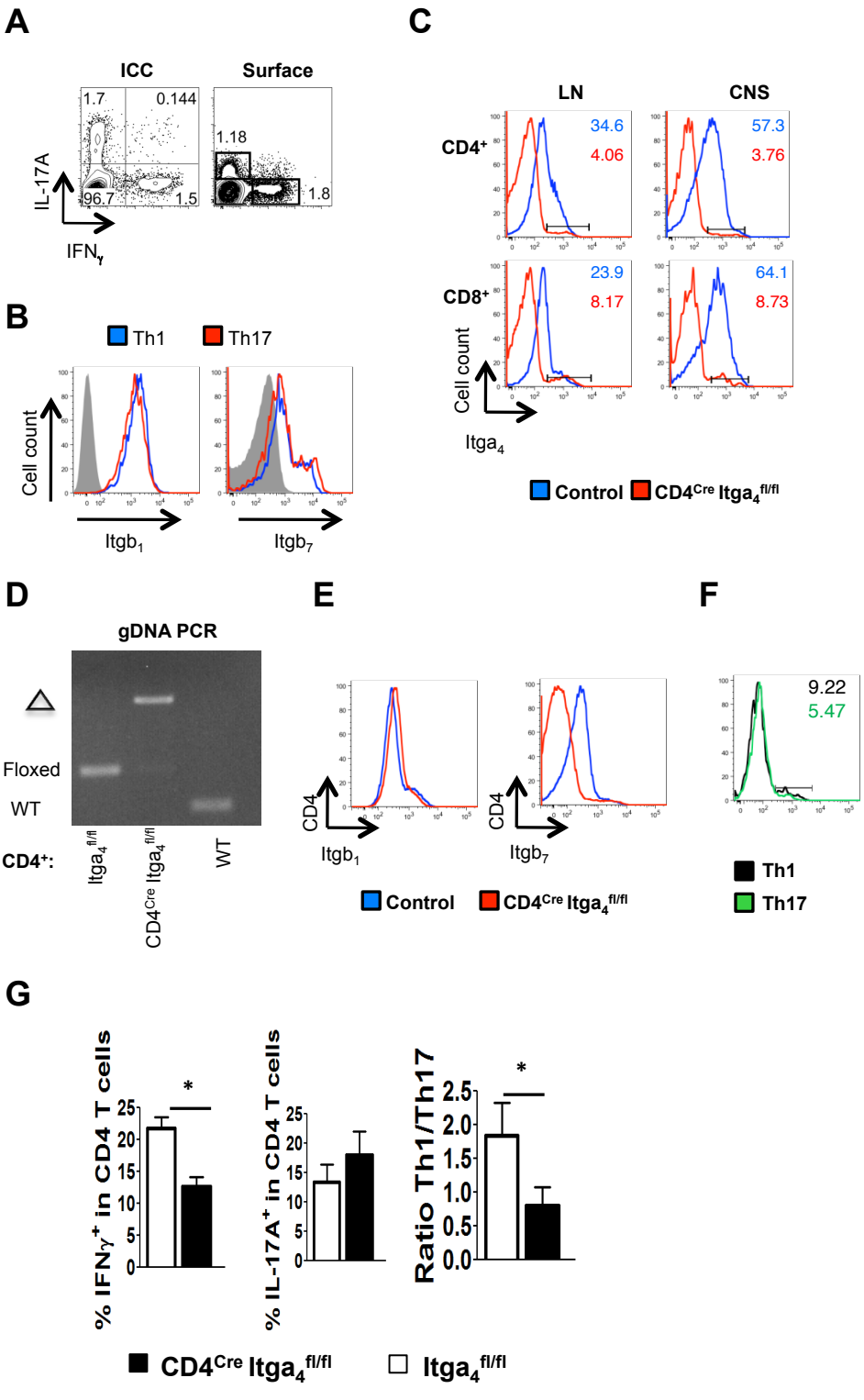
A
ICC
Surface
1.7
0.144
96.7
1.5
1.18
1.8
IL-17A
IFNγ
B
Th1
Th17
Cell count
Itgb1
Itgb7
C
LN
CNS
CD4+
34.6
4.06
57.3
3.76
CD8+
23.9
8.17
64.1
8.73
Cell count
Itga4
Control
CD4Cre Itga4fl/fl
D
gDNA PCR
Floxed
WT
CD4+:
Itga4fl/fl
CD4Cre Itga4fl/fl
WT
E
CD4
Itgb1
CD4
Itgb7
Control
CD4Cre Itga4fl/fl
F
9.22
5.47
Th1
Th17
G
% IFNγ+ in CD4 T cells
% IL-17A+ in CD4 T cells
Ratio Th1/Th17
CD4Cre Itga4fl/fl
Itga4fl/fl

Supplemental FIGURE 1. Characterization of Itg and cytokine expression on T cell subsets from $Itga_4^{fl/fl}$ $CD4^{Cre}$ and wild type mice. (*A, B*) WT mice were immunized with MOG_{35-55} in CFA. Ten days later, draining LNs (dLN) were collected and single-cell suspension prepared. Enriched $CD4^+$ T cells were stimulated with PMA and ionomycin for 3 hours and stained for IFNγ and IL17A by intracellular (*A*, left) or secretion assay (*A*, right). (*B*) $Itgb_1$ (left) and $Itgb_7$ expression (right) gated on Th1 ($IFN\gamma^+$, blue line) and Th17 subsets ($IL17A^+$, red line) stained by secretion assay. Filled histograms represent the isotype staining. Histograms are representative of three independent experiments. (*C*, *E* and *F*) Control and $CD4^{Cre}$ $Itga_4^{fl/fl}$ mice were immunized for EAE development with MOG_{35-55}/CFA. dLN and CNS were collected 8 and 20 days after immunization respectively. $Itga_4$ expression was evaluated on $CD4^+$ (top) and $CD8^+$ T cells (bottom) from control (blue line) and $CD4^{Cre}$ $Itga_4^{fl/fl}$ mice (red line) in dLN (left) and CNS (right). Overlays are representative of 4 different experiments. Note equivalent deletion of $Itga_4$ on $CD4^+$ and $CD8^+$ T cells isolated from LN and CNS. (*D*) $CD4^+$ T cells from $CD4^{Cre}$ $Itga_4^{fl/fl}$, $Itga_4^{fl/fl}$ and WT mice were isolated by MACS positive purification (overall purity of $CD4^+$ T cells: 90%) and stimulated with plate bound anti-CD3/CD28 antibodies (both at 1µg/ml) for 48 hours. Genomic DNA was isolated and deletion of $Itga_4$ at the genomic level was determined using previously described primers (Priestley GV. and al, Blood 2007, 109(1):109-11). Upper band detects $Itga_4$ deleted allele (Δ), middle band detects floxed allele (floxed) and lower band detects wild type allele (WT). (*E*) $Itgb_1$ (left) and $Itgb_7$ (right) expression gated on $CD4^+$ T cells from control (blue line) and $CD4^{Cre}$ $Itga_4^{fl/fl}$ mice (red line). (*F*) $Itga_4$ expression was evaluated on Th1 cells (dark line) and Th17 cells (green line) from dLN of $CD4^{Cre}$ $Itga_4^{fl/fl}$ mice using secretion assay. (*G*) $CD4^{Cre}$ $Itga_4^{fl/fl}$ and $Itga_4^{fl/fl}$ mice were immunized for EAE development with MOG_{35-55} in CFA and pertussis toxin. Eight days later, CNS were collected and single cell suspensions prepared. Cells were stimulated with PMA/ionomycin and analyzed for cytokine secretion by intracellular cytokines staining. Percentages of IFNγ and IL17A infiltrating $CD4^+$ T cells recovered from the CNS of non sick mice were determined and used to calculate the ratio of Th1/Th17 cells in the CNS of $CD4^{Cre}$ $Itga_4^{fl/fl}$ (dark) and $Itga_4^{fl/fl}$ mice (white)(*$p<0.01$). Data representative of 2 independent experiments with 6 mice per group.