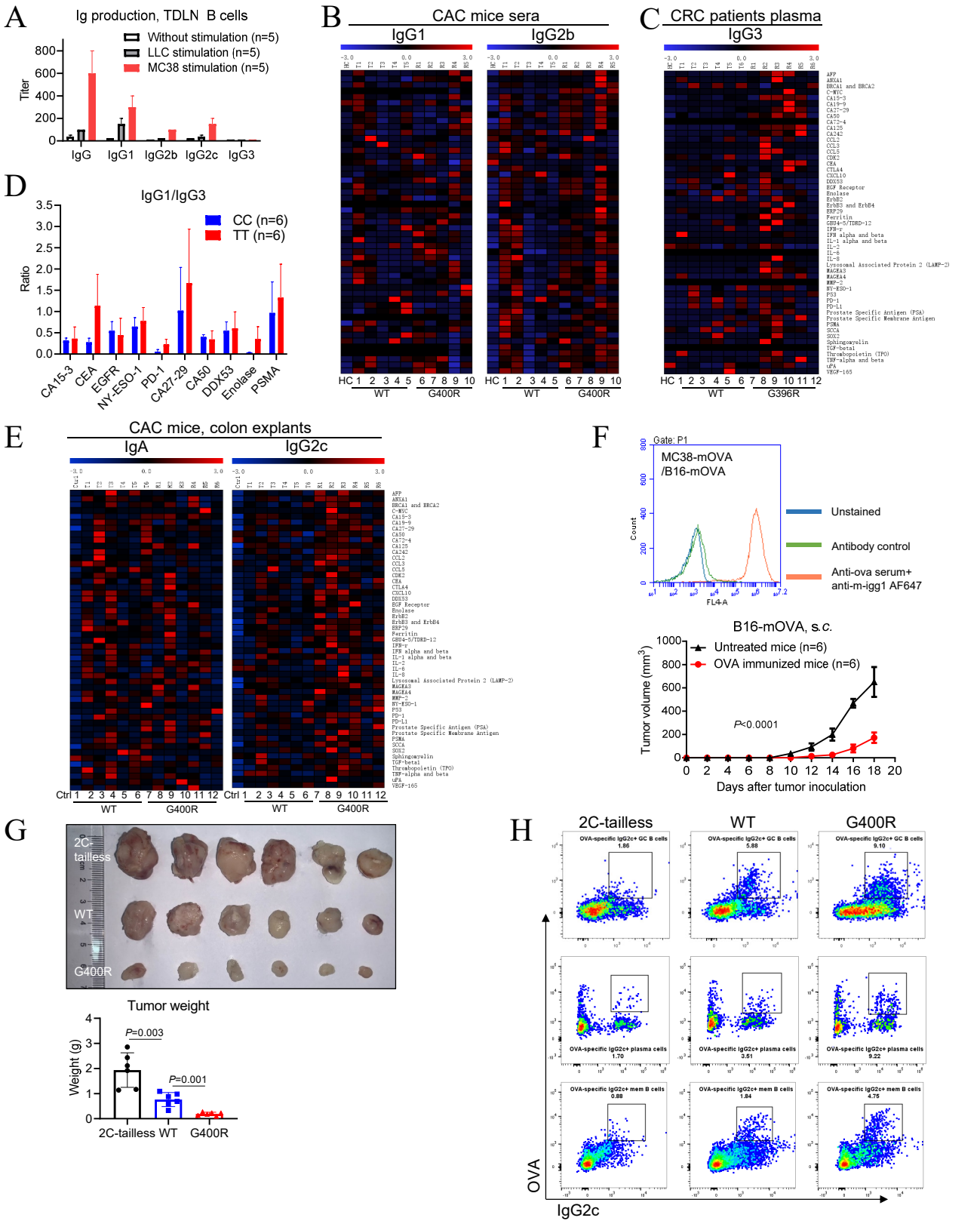
A
Ig production, TDLN B cells
Without stimulation (n=5)
LLC stimulation (n=5)
MC38 stimulation (n=5)
Titer
IgG
IgG1
IgG2b
IgG2c
IgG3
B
CAC mice sera
IgG1
IgG2b
HC
WT
G400R
C
CRC patients plasma
IgG3
G396R
D
IgG1/IgG3
CC (n=6)
TT (n=6)
Ratio
CA15-3
CEA
EGFR
NY-ESO-1
PD-1
CA27-29
CA50
DDX53
Enolase
PSMA
E
CAC mice, colon explants
IgA
IgG2c
Ctrl
F
Gate: P1
MC38-mOVA
/B16-mOVA
Count
FL4-A
Unstained
Antibody control
Anti-ova serum+
anti-m-igg1 AF647
B16-mOVA, s.c.
Untreated mice (n=6)
OVA immunized mice (n=6)
Tumor volume (mm3)
P<0.0001
Days after tumor inoculation
G
2C-tailless
WT
G400R
Tumor weight
P=0.003
P=0.001
Weight (g)
2C-tailless
WT
G400R
H
2C-tailless
WT
G400R
OVA-specific IgG2c+ GC B cells
1.86
5.88
9.10
OVA-specific IgG2c+ plasma cells
1.70
3.51
9.22
OVA-specific IgG2c+ mem B cells
0.88
1.84
4.75
OVA
IgG2c

Extended Data Fig. 5. Altered levels of tumor-specific antibody production and plasma cell differentiation occurring with both hIgG1-G396R and mIgG2c-G400R variant

(**A**) Secretion of IgG subclasses by activated TDLN B cells in stimulation with cell medium, irradiated LLC cells or MC38 cells. (**B**) TAA microarray analyses of IgG1 and IgG2b in the sera from untreated mouse (n=1), CAC-induced WT mice (n=5) and mIgG2c-G400R mice (n=5). (**C**) TAA microarray analyses of IgG3 in the plasma samples of healthy donor (n=1), WT CRC patients (n=6) and hIgG1-G396R homozygous CRC patients (n=6). (**D**) The ratios of IgG1/IgG3 were evaluated based on the TAA microarray results. (**E**) TAA microarray analyses of IgA and IgG2c subclasses in the colon explants from WT CAC mice (n=6), mIgG2c-G400R CAC mice (n=6) and cell medium. (**F**) OVA expression in MC38-mOVA cells and B16-mOVA cells detected by flow cytometry. The growth curves of B16-mOVA tumor cells in untreated mice and OVA-immunized mice are shown. (**G**) The MC38-mOVA tumor size in mIgG2c-tailless, WT and mIgG2c-G400R mice. Tumor tissues were isolated and weighed after euthanasia. (**H**) Representative flow cytometry plots of OVA-specific $IgG2c^+$ GC B cells, plasma cells and memory B cells. OVA-specific $IgG2c^+$ GC B cells were gated from total $B220^+$ $GL\text{-}7^+$ GC B cells, OVA-specific $IgG2c^+$ memory B cells were gated from total $B220^+$ $CD38^+$ IgD^- memory B cells, and OVA-specific $IgG2c^+$ plasma cells were gated from total $B220^{low}$ $CD138^{high}$ plasma cells. One of three representative experiments is shown (A, F, G, H). Statistical significance was determined using two-way ANOVA (F) and an unpaired two-tailed t-test (G). Mean± SEM.