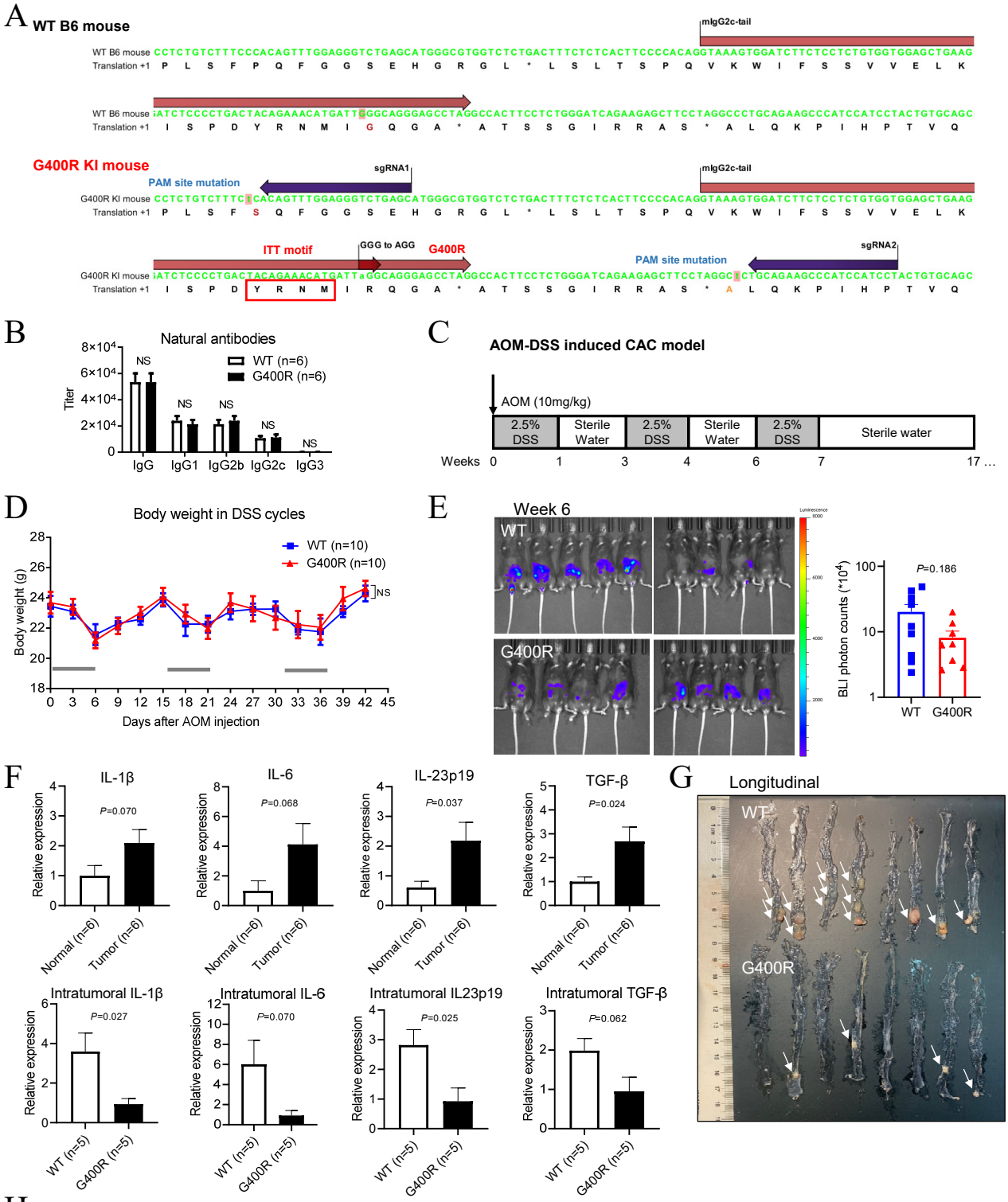
A
WT B6 mouse
mIgG2c-tail
WT B6 mouse CCTCTGTCTTTCCCACAGTTTGGAGGGTCTGAGCATGGGCGTGGTCTCTGACTTTCTCTCACTTCCCCACAGGTAAAGTGGATCTTCTCCTCTGTGGTGGAGCTGAAG
Translation +1 P L S F P Q F G G S E H G R G L * L S L T S P Q V K W I F S S V V E L K
WT B6 mouse ATCTCCCCTGACTACAGAAACATGATTGGGCAGGGAGCCTAGGCCACTTCCTCTGGGATCAGAAGAGCTTCCTAGGCCCTGCAGAAGCCCATCCATCCTACTGTGCAGC
Translation +1 I S P D Y R N M I G Q G A * A T S S G I R R A S * A L Q K P I H P T V Q
G400R KI mouse
PAM site mutation
sgRNA1
mIgG2c-tail
G400R KI mouse CCTCTGTCTTTCTCACAGTTTGGAGGGTCTGAGCATGGGCGTGGTCTCTGACTTTCTCTCACTTCCCCACAGGTAAAGTGGATCTTCTCCTCTGTGGTGGAGCTGAAG
Translation +1 P L S F S Q F G G S E H G R G L * L S L T S P Q V K W I F S S V V E L K
ITT motif
GGG to AGG
G400R
PAM site mutation
sgRNA2
G400R KI mouse ATCTCCCCTGACTACAGAAACATGATTaGGCAGGGAGCCTAGGCCACTTCCTCTGGGATCAGAAGAGCTTCCTAGGCTCTGCAGAAGCCCATCCATCCTACTGTGCAGC
Translation +1 I S P D Y R N M I R Q G A * A T S S G I R R A S * A L Q K P I H P T V Q
B
Natural antibodies
Titer
WT (n=6)
G400R (n=6)
NS
IgG
IgG1
IgG2b
IgG2c
IgG3
C
AOM-DSS induced CAC model
AOM (10mg/kg)
2.5% DSS
Sterile Water
2.5% DSS
Sterile Water
2.5% DSS
Sterile water
Weeks 0 1 3 4 6 7 17 …
D
Body weight in DSS cycles
WT (n=10)
G400R (n=10)
Body weight (g)
Days after AOM injection
NS
E
Week 6
WT
G400R
BLI photon counts (*10^4)
P=0.186
F
IL-1β
P=0.070
IL-6
P=0.068
IL-23p19
P=0.037
TGF-β
P=0.024
Relative expression
Normal (n=6)
Tumor (n=6)
Intratumoral IL-1β
P=0.027
Intratumoral IL-6
P=0.070
Intratumoral IL23p19
P=0.025
Intratumoral TGF-β
P=0.062
WT (n=5)
G400R (n=5)
G
Longitudinal
WT
G400R
H
WT
G400R

Extended Data Fig. 4. The mIgG2c-G400R knock-in mice, manipulated by CRISPR-Cas9 system, have significantly ameliorated colon tumorigenesis

(**A**) Alignment of the *IGHG1* DNA sequence from NCBI database with the sequencing data of the mIgG2c-G400R knock-in mouse. Annotated are the sgRNA target sequences, mIgG2c-tail and mIgG2c-G400R variant site. (**B**) Basal levels of natural IgG, IgG1, IgG2b, IgG2c and IgG3 antibodies in the serum samples of untreated 6-week-old WT (n=6) and mIgG2c-G400R (n=6) mice. (**C**) A schematic overview of the AOM-DSS induced CAC model. (**D**) Body weights of AOM-DSS induced mice during three rounds of DSS treatment. (**E**) Bioluminescent images obtained at week 6 after AOM-DSS treatment following intraperitoneal injection of L-012 solution. (**F**) RT-qPCR analyses of intratumoral cytokine mRNAs from colon tumors and matched normal colons of CAC-induced WT mice and mIgG2c-G400R mice. (**G**) Representative longitudinal images of tumor burden in the colon specimens from CAC induced mice. Tumors indicated by white arrows. (**H**) Representative images of H&E-stained colon cross-sections from CAC induced WT mice (n=5) and mIgG2c-G400R mice (n=5). Scale bar, 1000 μm. One of three representative experiments is shown (B, E, F). Statistical significance was determined using an unpaired two-tailed t-test (B, E, F) and two-way ANOVA (D). Mean± SEM. NS, not significant.