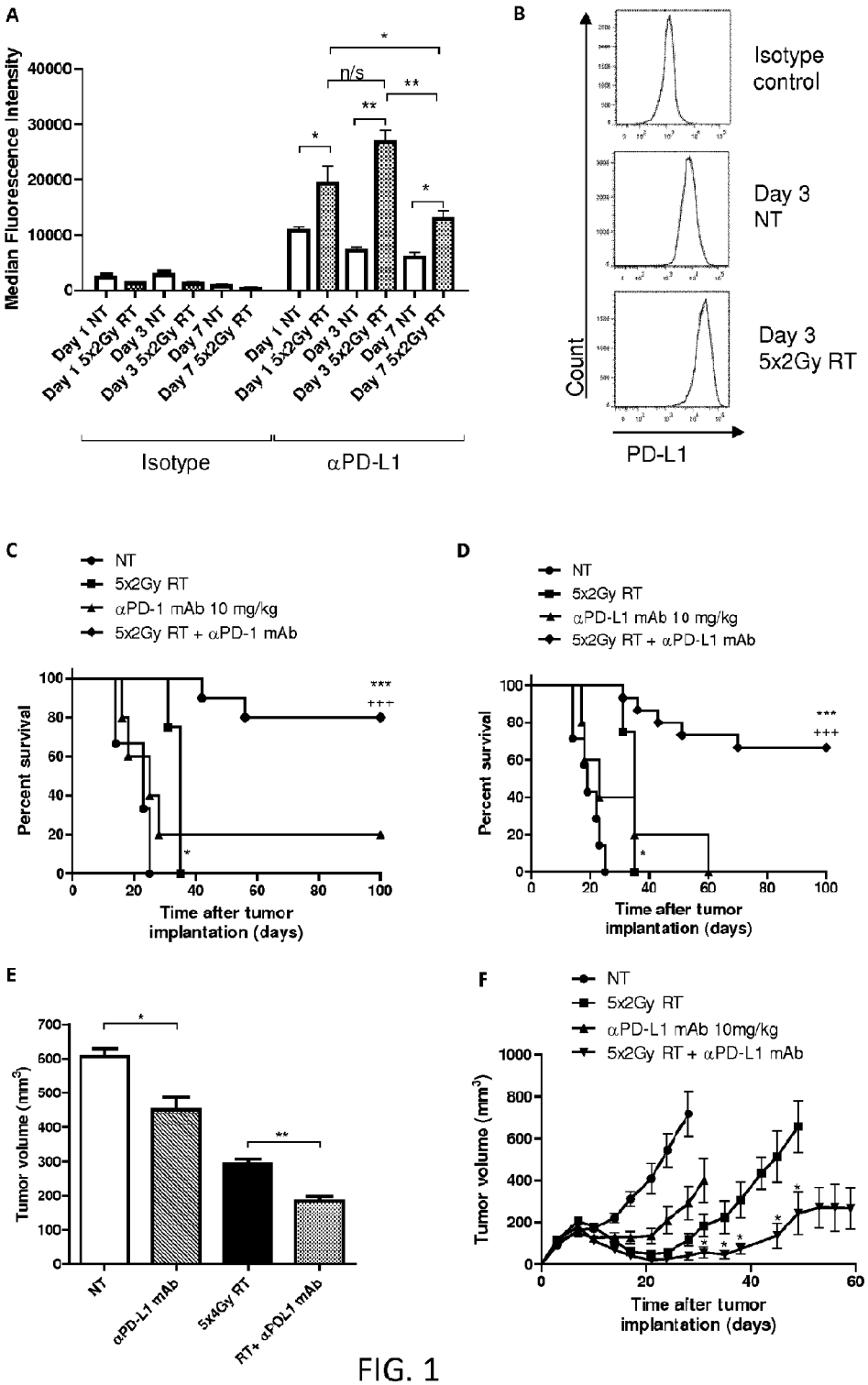
A
Median Fluorescence Intensity
40000
30000
20000
10000
0
n/s
*
**
**
*
*
Day 1 NT
Day 1 5x2Gy RT
Day 3 NT
Day 3 5x2Gy RT
Day 7 NT
Day 7 5x2Gy RT
Day 1 NT
Day 1 5x2Gy RT
Day 3 NT
Day 3 5x2Gy RT
Day 7 NT
Day 7 5x2Gy RT
Isotype
αPD-L1
B
Isotype control
Day 3 NT
Day 3 5x2Gy RT
Count
PD-L1
C
NT
5x2Gy RT
αPD-1 mAb 10 mg/kg
5x2Gy RT + αPD-1 mAb
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
***
+++
*
Time after tumor implantation (days)
D
NT
5x2Gy RT
αPD-L1 mAb 10 mg/kg
5x2Gy RT + αPD-L1 mAb
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
***
+++
*
Time after tumor implantation (days)
E
Tumor volume (mm3)
700
600
500
400
300
200
100
0
*
**
NT
αPD-L1 mAb
5x4Gy RT
RT+ αPDL1 mAb
F
NT
5x2Gy RT
αPD-L1 mAb 10mg/kg
5x2Gy RT + αPD-L1 mAb
Tumor volume (mm3)
1000
800
600
400
200
0
0
20
40
60
Time after tumor implantation (days)

FIG. 1

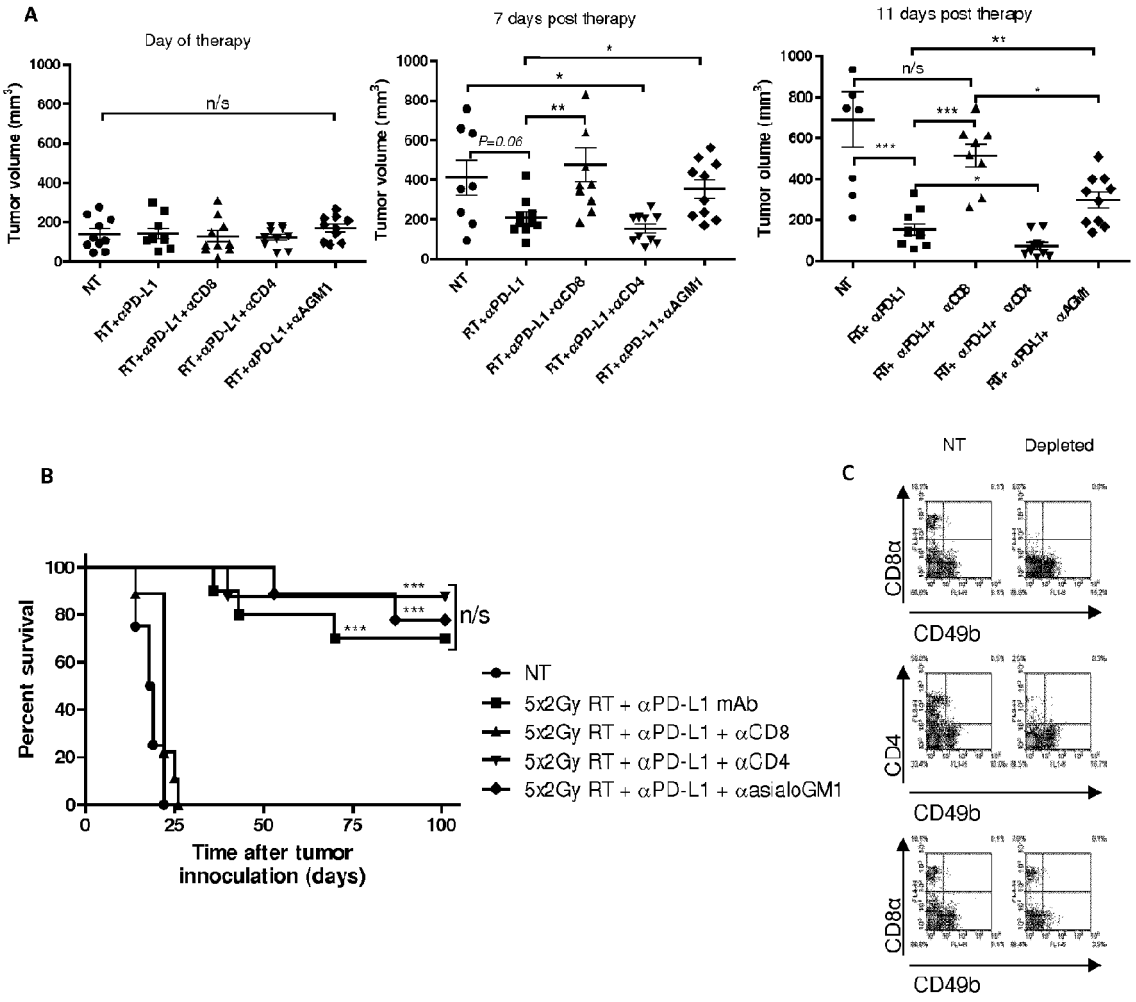
A
Day of therapy
7 days post therapy
11 days post therapy
Tumor volume (mm3)
n/s
P=0.06
NT
RT+αPD-L1
RT+αPD-L1+αCD8
RT+αPD-L1+αCD4
RT+αPD-L1+αAGM1
B
Percent survival
Time after tumor innoculation (days)
n/s
NT
5x2Gy RT + αPD-L1 mAb
5x2Gy RT + αPD-L1 + αCD8
5x2Gy RT + αPD-L1 + αCD4
5x2Gy RT + αPD-L1 + αasialoGM1
C
NT
Depleted
CD8α
CD49b
CD4
CD49b
CD8α
CD49b

FIG. 2

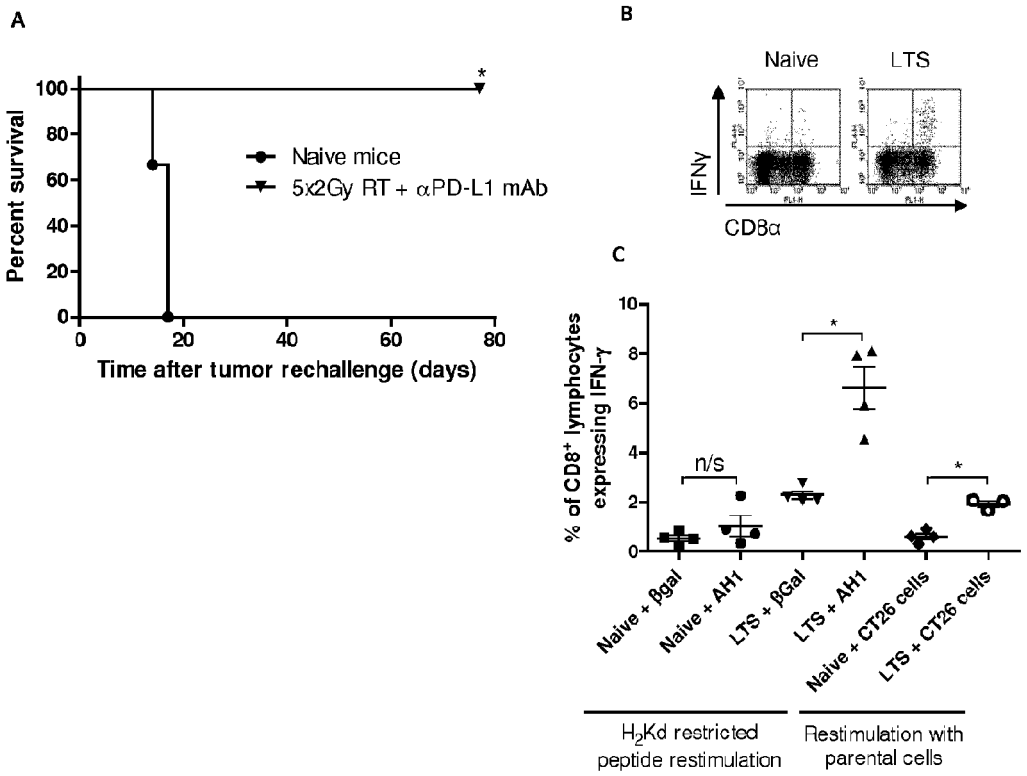
A
Percent survival
100
80
60
40
20
0
0
20
40
60
80
Time after tumor rechallenge (days)
Naive mice
5x2Gy RT + αPD-L1 mAb
*
B
Naive
LTS
IFNγ
CD8α
C
% of CD8+ lymphocytes expressing IFN-γ
10
8
6
4
2
0
n/s
*
*
Naive + βgal
Naive + AH1
LTS + βGal
LTS + AH1
Naive + CT26 cells
LTS + CT26 cells
H2Kd restricted peptide restimulation
Restimulation with parental cells

FIG. 3

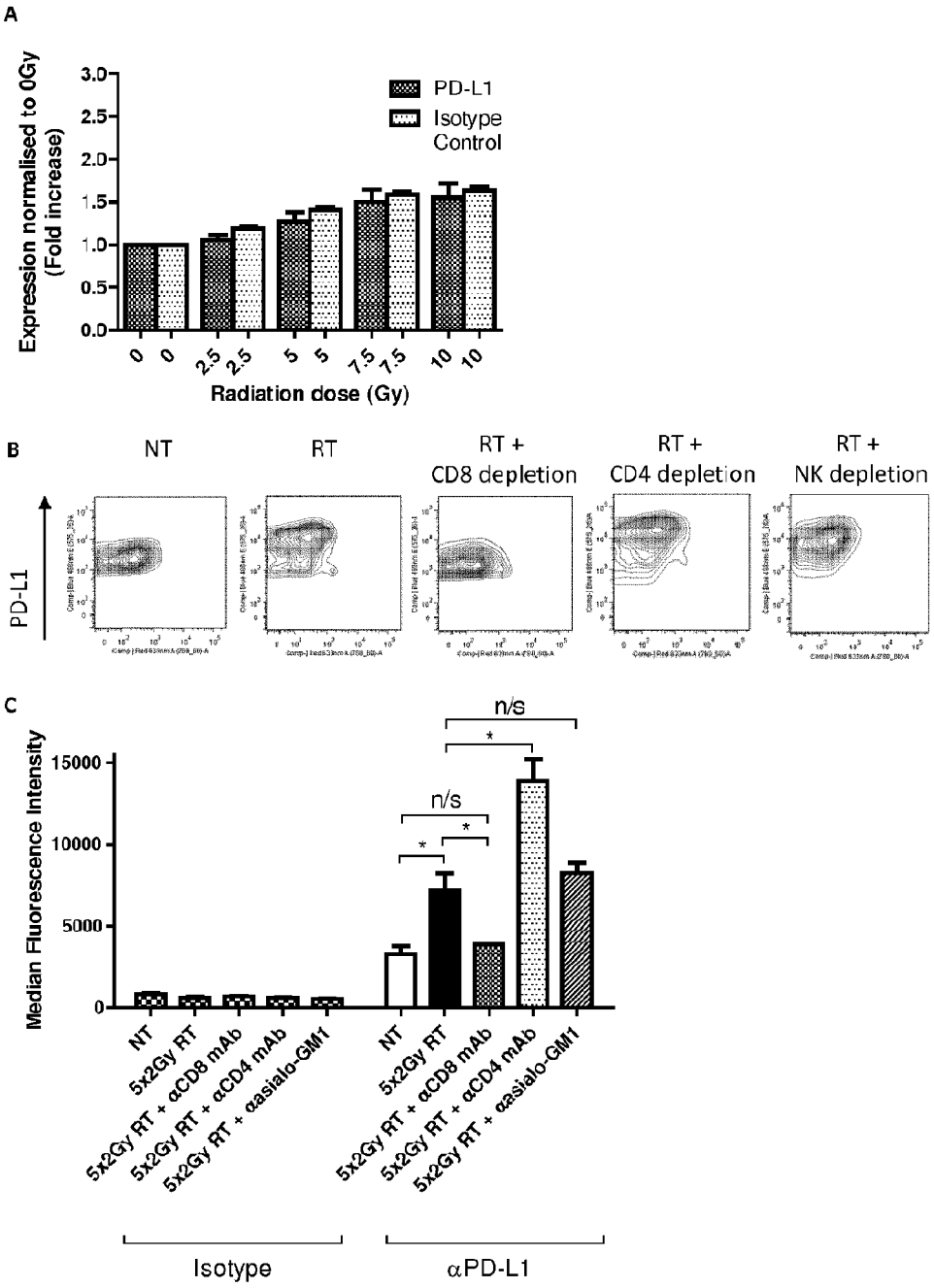
A
Expression normalised to 0Gy (Fold increase)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
PD-L1
Isotype Control
0 0 2.5 2.5 5 5 7.5 7.5 10 10
Radiation dose (Gy)
B
NT
RT
RT + CD8 depletion
RT + CD4 depletion
RT + NK depletion
PD-L1
C
Median Fluorescence Intensity
15000
10000
5000
0
n/s
n/s
*
*
*
NT
5x2Gy RT
5x2Gy RT + αCD8 mAb
5x2Gy RT + αCD4 mAb
5x2Gy RT + αasialo-GM1
NT
5x2Gy RT
5x2Gy RT + αCD8 mAb
5x2Gy RT + αCD4 mAb
5x2Gy RT + αasialo-GM1
Isotype
αPD-L1

FIG. 4

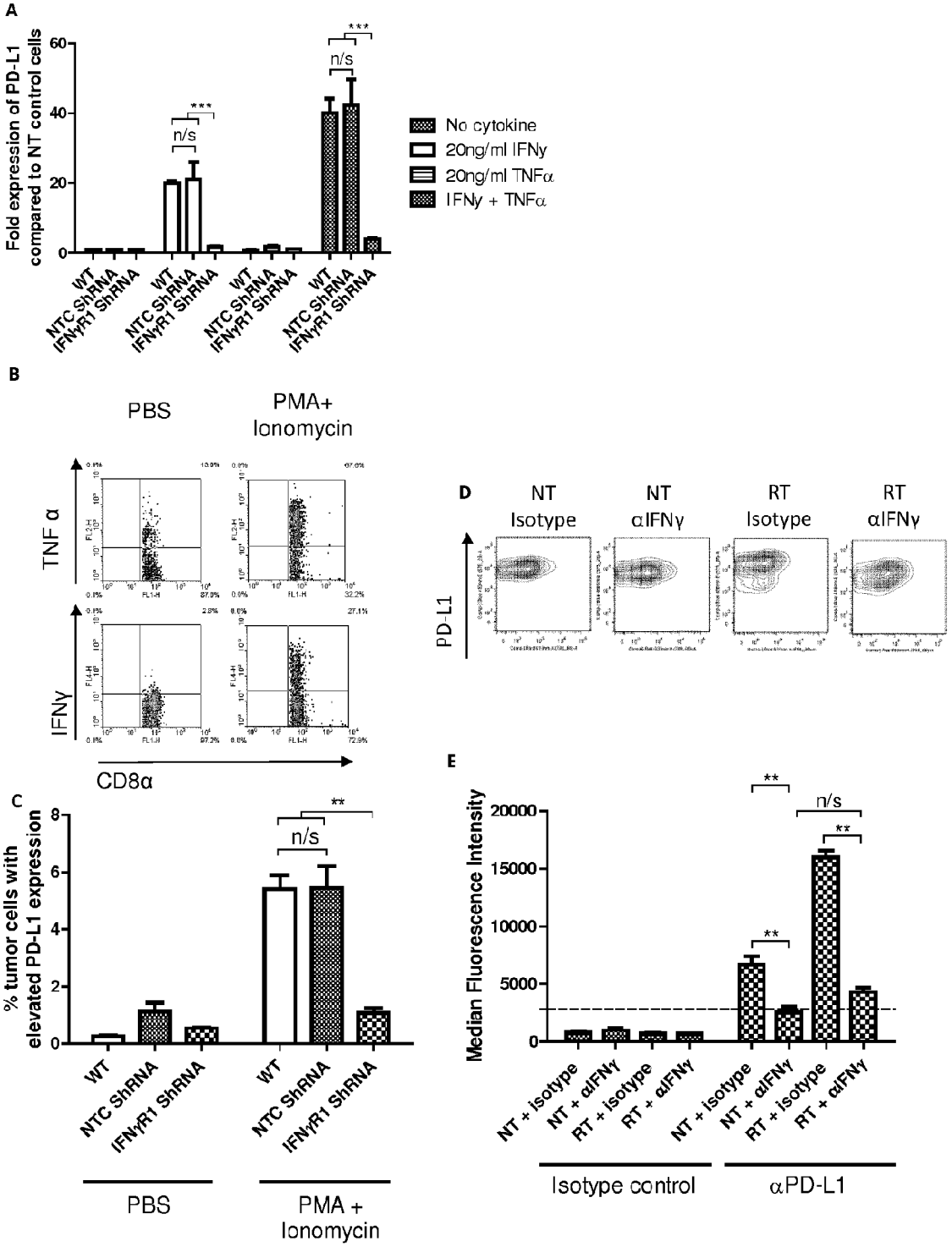
A
Fold expression of PD-L1 compared to NT control cells
60
40
20
0
n/s
***
n/s
***
No cytokine
20ng/ml IFNγ
20ng/ml TNFα
IFNγ + TNFα
WT
NTC ShRNA
IFNγR1 ShRNA
B
PBS
PMA+ Ionomycin
TNF α
IFNγ
CD8α
C
% tumor cells with elevated PD-L1 expression
8
6
4
2
0
n/s
**
WT
NTC ShRNA
IFNγR1 ShRNA
PBS
PMA + Ionomycin
D
NT Isotype
NT αIFNγ
RT Isotype
RT αIFNγ
PD-L1
E
Median Fluorescence Intensity
20000
15000
10000
5000
0
**
n/s
**
**
NT + isotype
NT + αIFNγ
RT + isotype
RT + αIFNγ
Isotype control
αPD-L1

FIG. 5

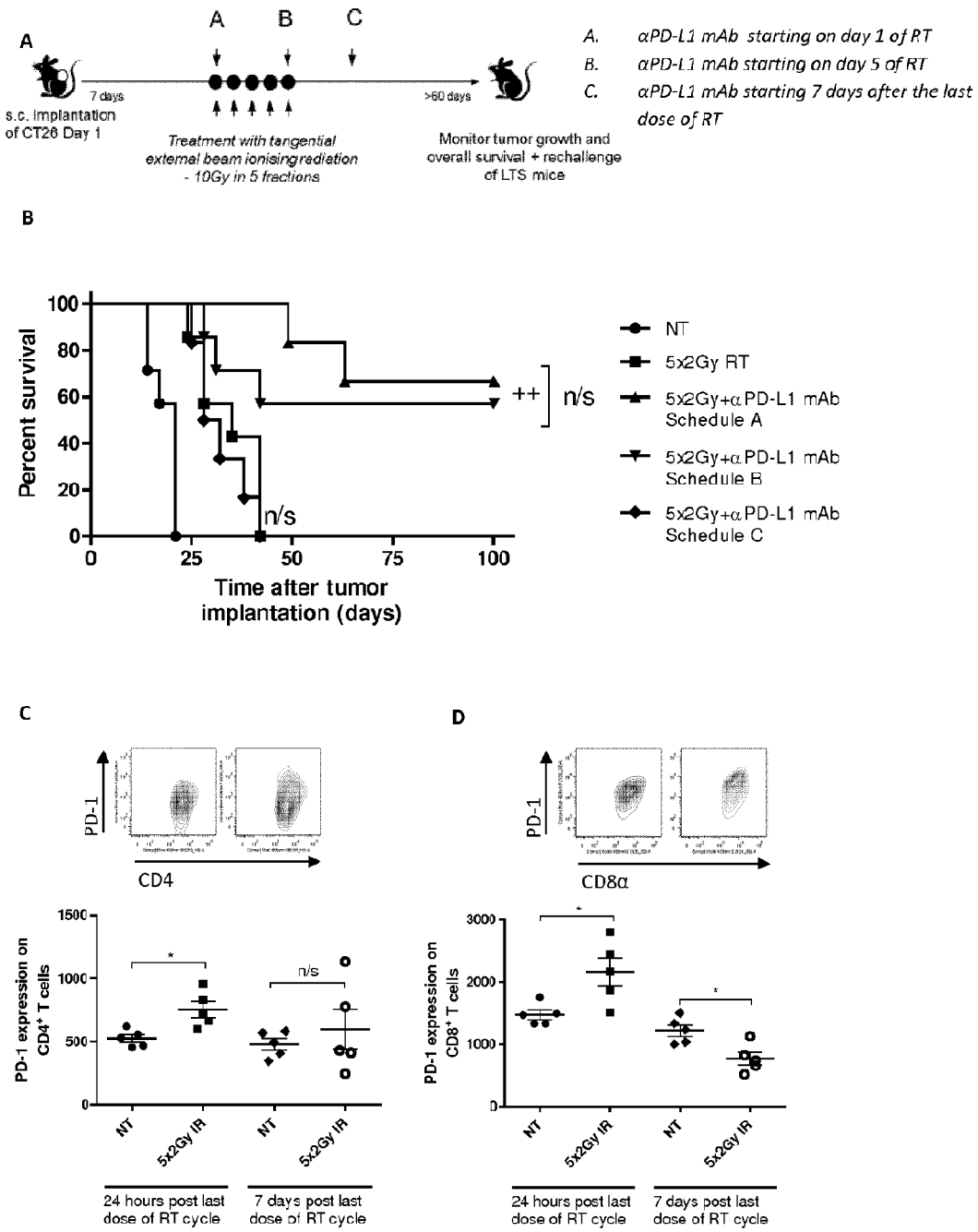
A
A
B
C
7 days
>60 days
s.c. implantation of CT26 Day 1
Treatment with tangential external beam ionising radiation - 10Gy in 5 fractions
Monitor tumor growth and overall survival + rechallenge of LTS mice
A. αPD-L1 mAb starting on day 1 of RT
B. αPD-L1 mAb starting on day 5 of RT
C. αPD-L1 mAb starting 7 days after the last dose of RT
B
Percent survival
Time after tumor implantation (days)
++
n/s
n/s
NT
5x2Gy RT
5x2Gy+αPD-L1 mAb Schedule A
5x2Gy+αPD-L1 mAb Schedule B
5x2Gy+αPD-L1 mAb Schedule C
C
PD-1
CD4
PD-1 expression on CD4+ T cells
n/s
NT
5x2Gy IR
24 hours post last dose of RT cycle
7 days post last dose of RT cycle
D
PD-1
CD8α
PD-1 expression on CD8+ T cells
24 hours post last dose of RT cycle
7 days post last dose of RT cycle

FIG. 6

Supplemental

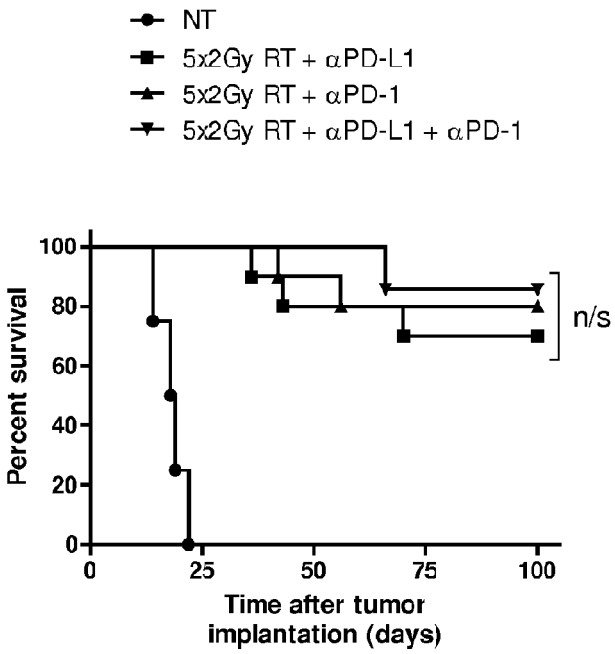

FIG. 7

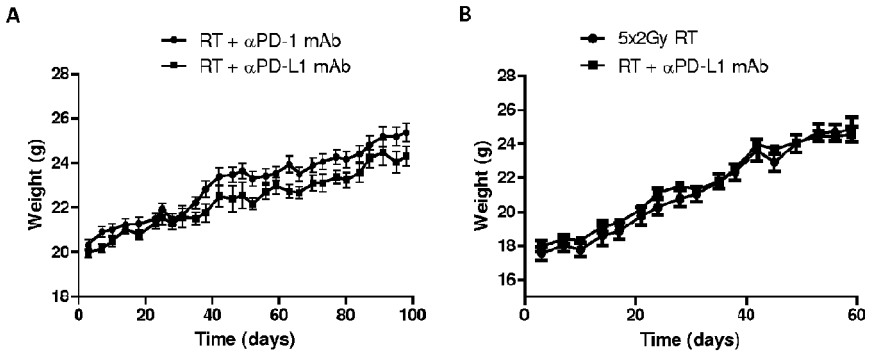
A
RT + αPD-1 mAb
RT + αPD-L1 mAb
Weight (g)
Time (days)
B
5x2Gy RT
RT + αPD-L1 mAb
Weight (g)
Time (days)

FIG. 8

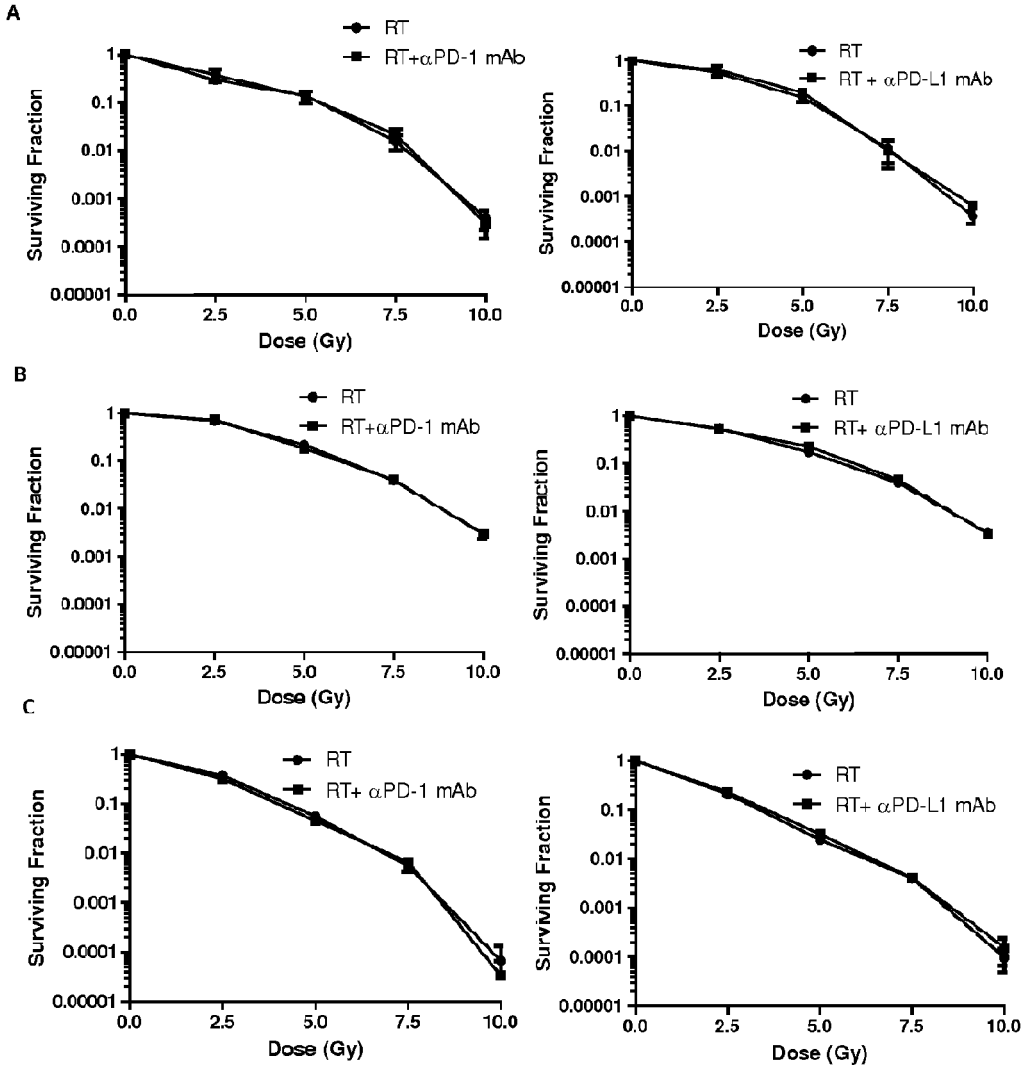
A
RT
RT+αPD-1 mAb
RT + αPD-L1 mAb
Surviving Fraction
1
0.1
0.01
0.001
0.0001
0.00001
0.0
2.5
5.0
7.5
10.0
Dose (Gy)
B
RT
RT+αPD-1 mAb
RT+ αPD-L1 mAb
C
RT
RT+ αPD-1 mAb
RT+ αPD-L1 mAb

FIG. 9

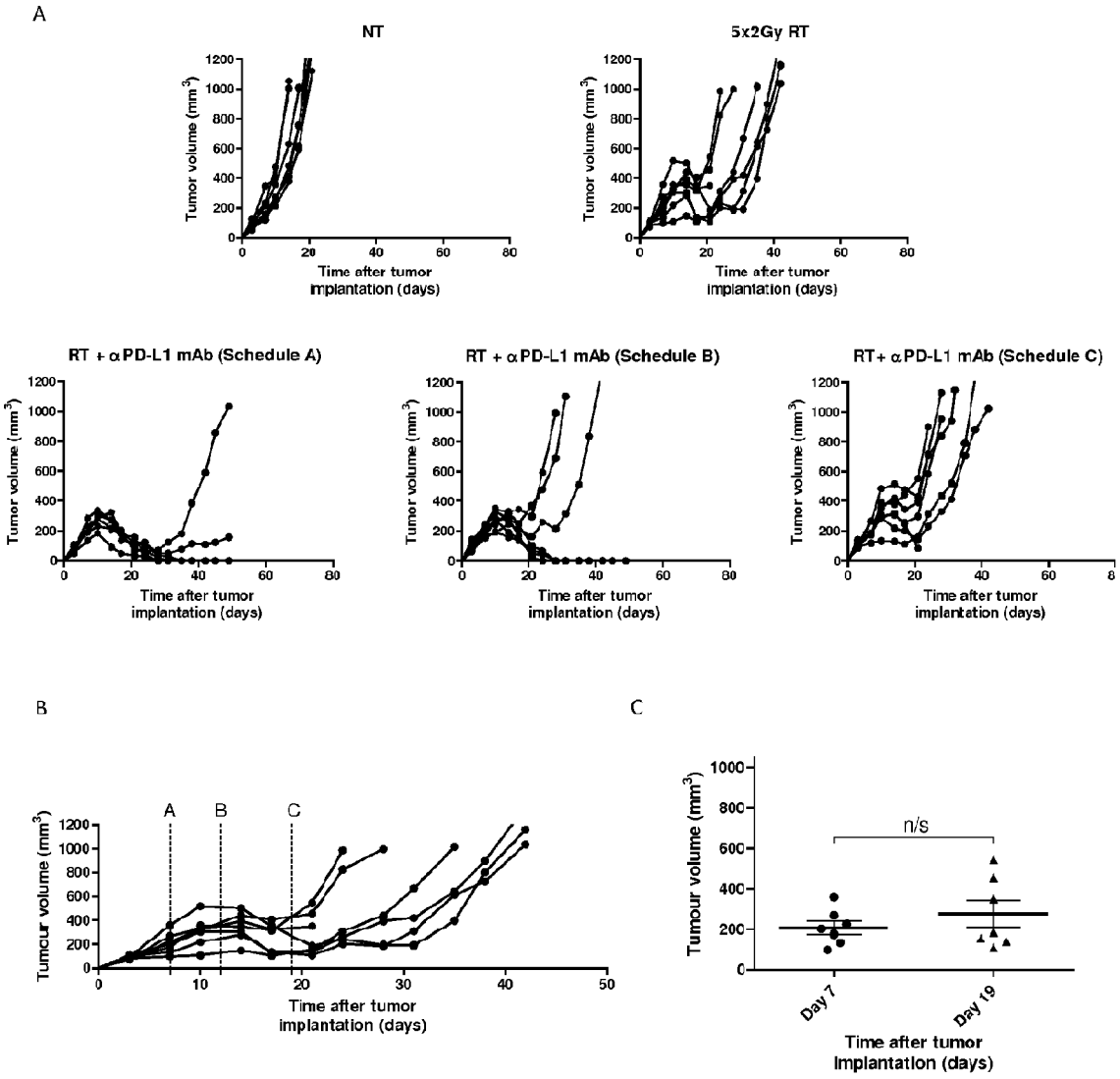
A
NT
5x2Gy RT
RT + αPD-L1 mAb (Schedule A)
RT + αPD-L1 mAb (Schedule B)
RT+ αPD-L1 mAb (Schedule C)
Tumor volume (mm3)
Time after tumor implantation (days)
B
C
A
B
C
Tumour volume (mm3)
Time after tumor implantation (days)
n/s
Day 7
Day 19

FIG. 10

A

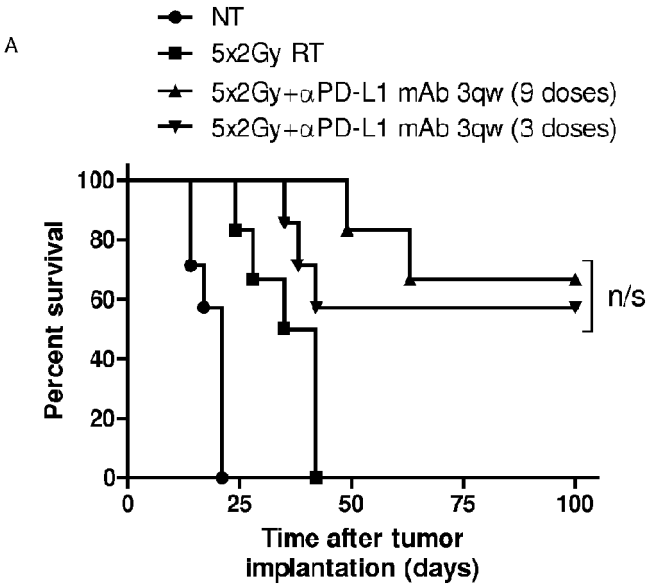
NT
5x2Gy RT
5x2Gy+αPD-L1 mAb 3qw (9 doses)
5x2Gy+αPD-L1 mAb 3qw (3 doses)
Percent survival
100
80
60
40
20
0
0
25
50
75
100
Time after tumor implantation (days)
n/s

FIG. 11