Fig. S4

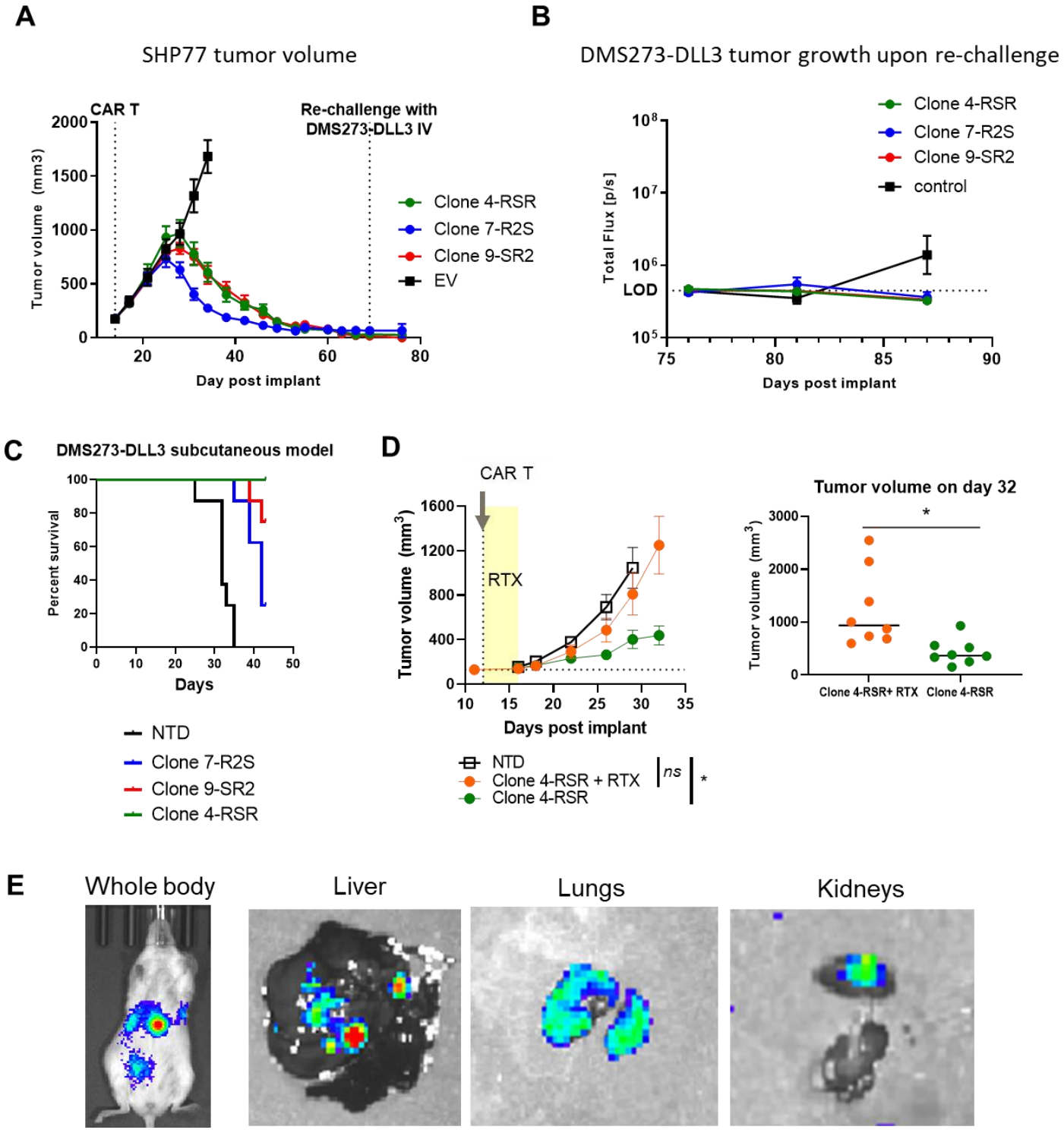
A
SHP77 tumor volume
CAR T
Re-challenge with DMS273-DLL3 IV
Tumor volume (mm3)
Day post implant
Clone 4-RSR
Clone 7-R2S
Clone 9-SR2
EV
B
DMS273-DLL3 tumor growth upon re-challenge
Total Flux [p/s]
LOD
Days post implant
Clone 4-RSR
Clone 7-R2S
Clone 9-SR2
control
C
DMS273-DLL3 subcutaneous model
Percent survival
Days
NTD
Clone 7-R2S
Clone 9-SR2
Clone 4-RSR
D
CAR T
RTX
Tumor volume (mm3)
Days post implant
NTD
Clone 4-RSR + RTX
Clone 4-RSR
ns
*
Tumor volume on day 32
Tumor volume (mm3)
Clone 4-RSR+ RTX
Clone 4-RSR
E
Whole body
Liver
Lungs
Kidneys

Fig. S4 continued

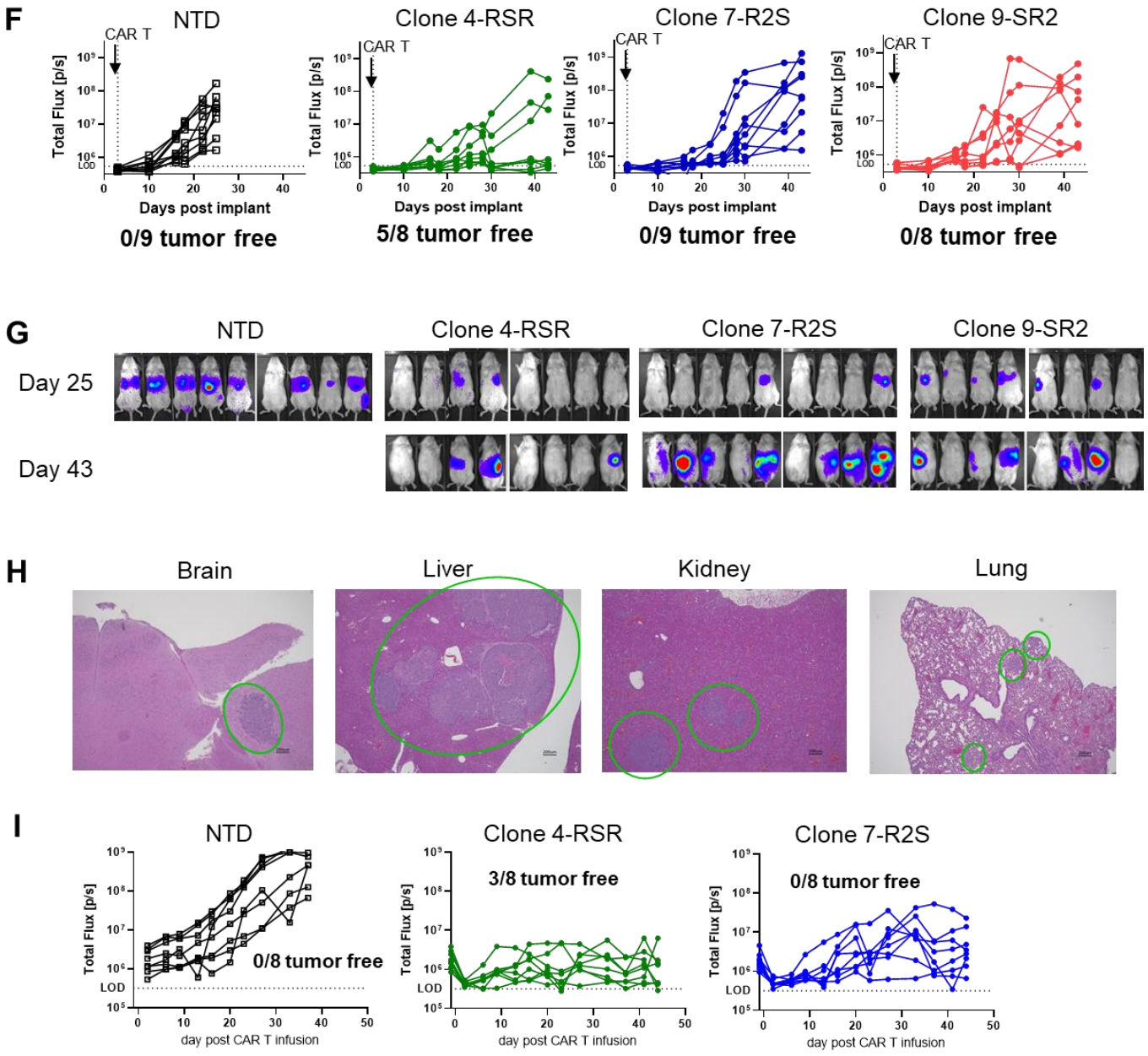

Fig. S4. In vivo activity of DLL3 CAR T cells. (**A**) The study shown in Fig. 4A was monitored for tumor growth out to 87 days. Most mice remained tumor free of SHP77 SC tumors and were re-challenge with luciferase-labeled DMS273-DLL3 tumors injected intravenously at $1x10^5$ cells/mouse on day 69. (**B**) The DMS273-DLL3 tumor burden in the study shown in Fig. S4A

was tracked by luminesce imaging and compared to tumor burden in naïve non-CAR treated mice. No detectable tumor was observed in DLL3 CAR-treated mice, while tumors did begin to grow out in CAR naïve control mice. (**C**) Survival curve of DMS273-DLL3 subcutaneous model demonstrating that animals who received clone 4-RSR had the best survival at the end of the study. (**D**) Rituximab treatment efficiently suppressed in vivo activity of DLL3 CAR T cells. DMS273-DLL3 cells were implanted subcutaneously and a single dose of CAR T cells ($3x10^6$ CAR+ cells per animal) were injected on day 12. Starting from the day of CAR T dosing, 10 mg/kg rituximab (RTX) was dosed for 5 consecutive days via IP injection. RM One Way ANOVA was performed on tumor volume on days 16-29 with Dunnett's multiple comparisons test, $p<0.05$. Paired t-test was performed on tumor volume on day 32. *, $p < 0.05$. Error bars represent SEM, n=8. (**E**) Representative bioluminescence image of mouse, liver, lungs, and kidneys after IV implantation of DMS273-DLL3 tumor cells. (**F**) Tumor burden of individual animals in DMS273-DLL3 systemic model demonstrating only the clone 4-RSR-treated group had tumor-free animals. (**G**) Bioluminescence images of animals in DMS273-DLL3 systemic model. (**H**) H&E images demonstrating tumor cells in SHP-77 systemic model were detected in brain, liver, kidney and lung. Green circles indicate tumor cells. (**I**) Tumor burden of individual animals in SHP-77 systemic model demonstrating only clone 4-RSR-treated group had tumor-free animals.