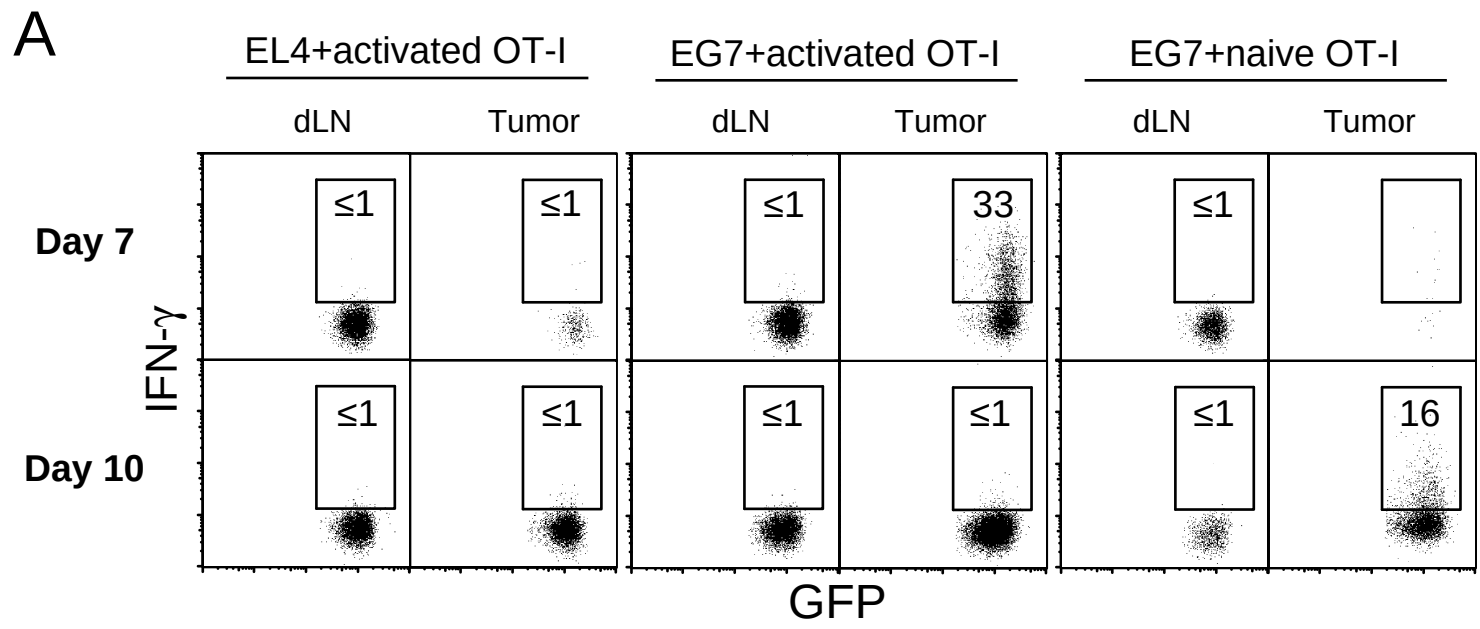
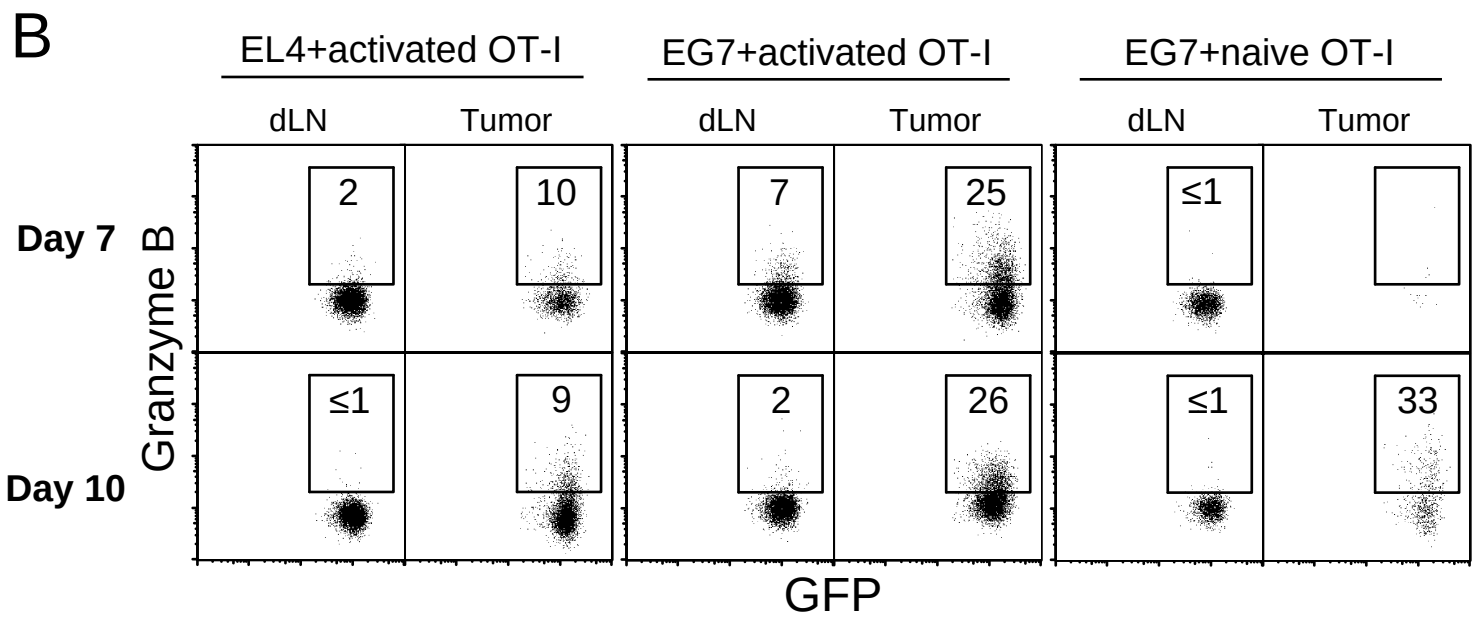
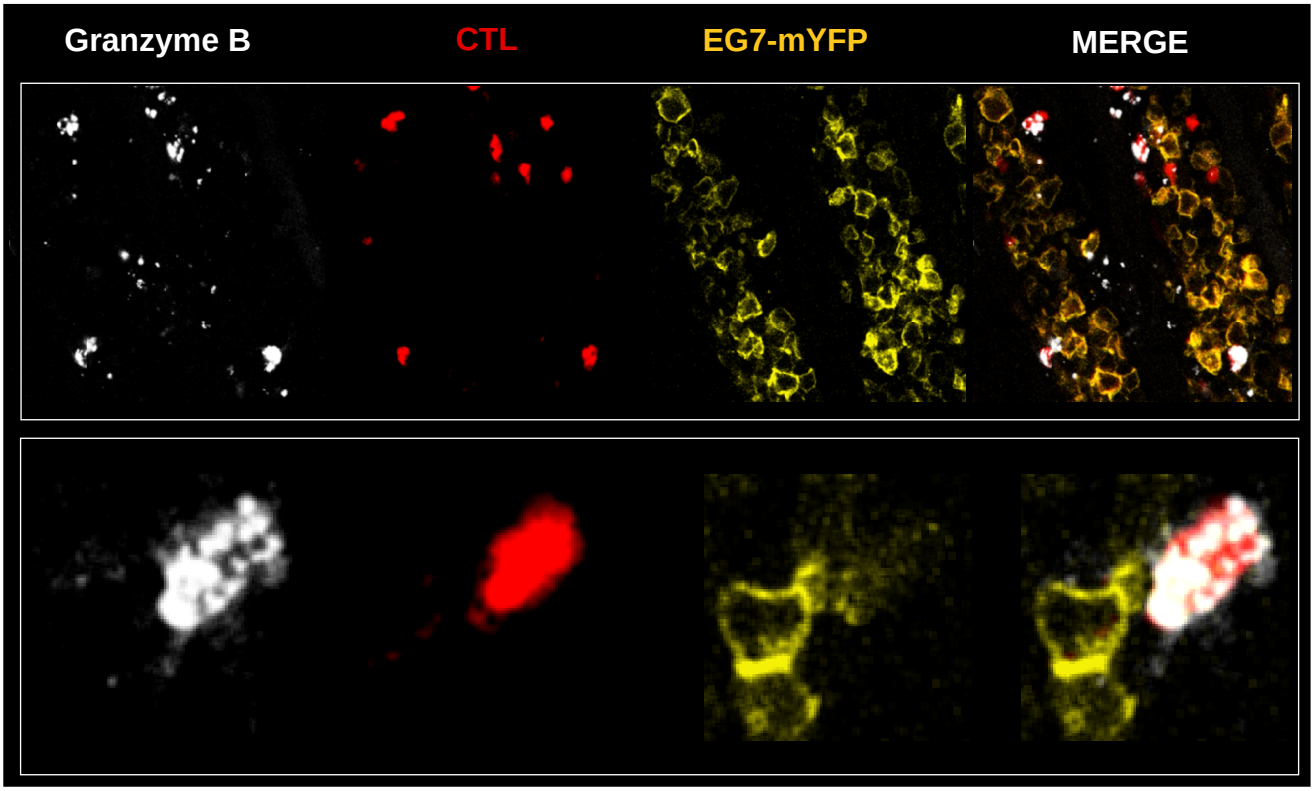
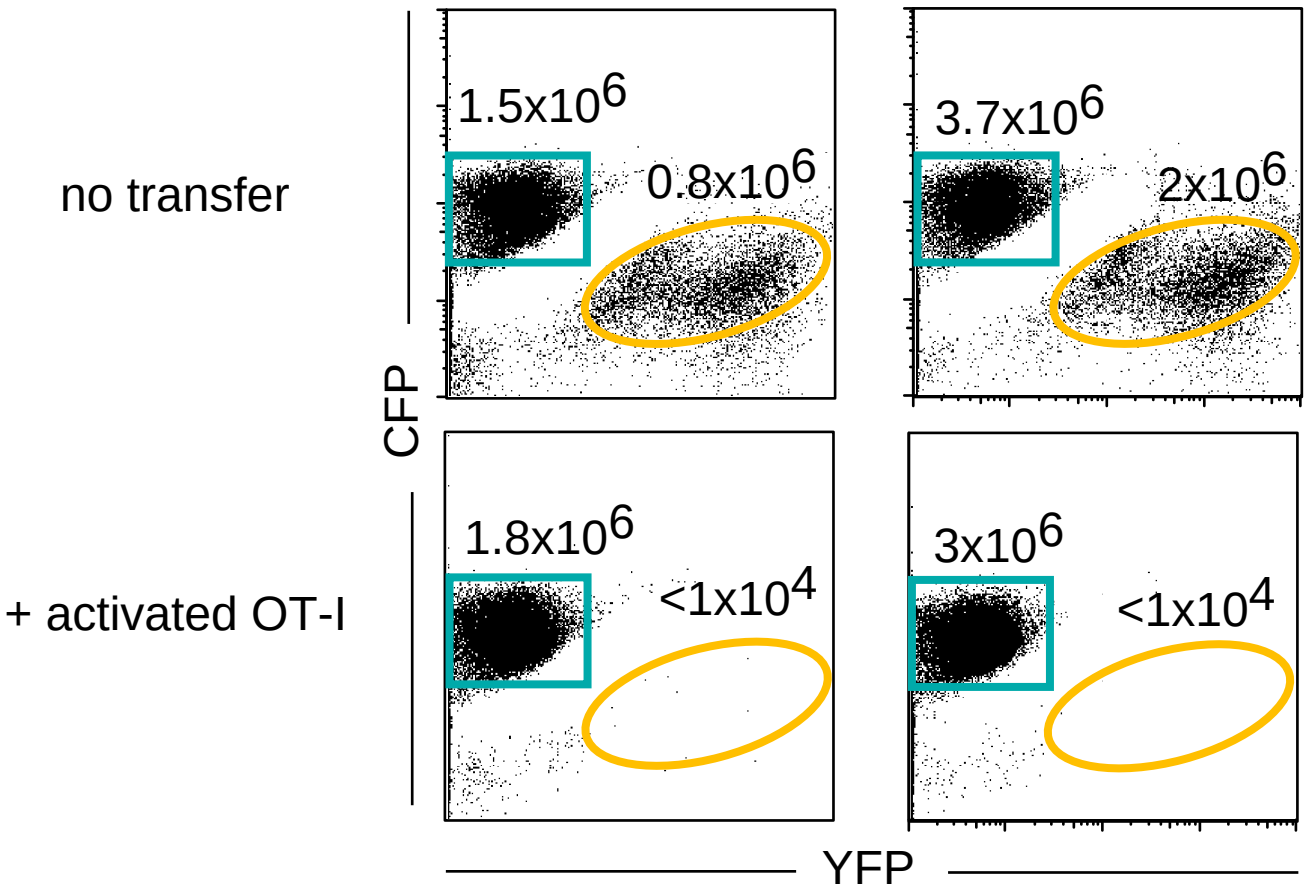




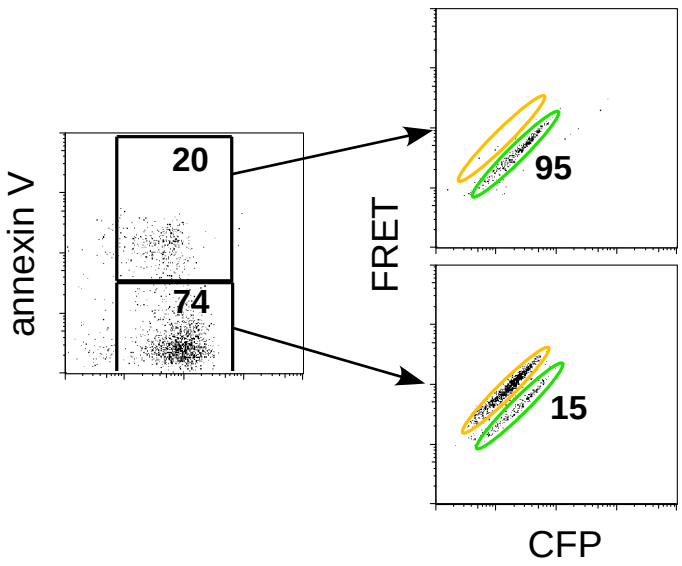
Breart et al. Supplemental Figure 1



Breart et al. Supplemental Figure 2



Breart et al. Supplemental Figure 3



Breart et al. Supplemental Figure 4

Supplemental materials

Supplementary Figure 1. CTLs enhance their effector functions upon Ag recognition in the tumor environment.

Naïve or in vitro activated GFP-expressing OT-I T cells were adoptively transferred 3 or 5 days after EL4 or EG7 tumor injection, respectively. Seven and 10 days after tumor injection, OT-I CD8 T cells present in the draining lymph node or in the tumor were assayed for IFN-γ (**A**) and granzyme B (**B**) expression by intracellular flow cytometry, in the absence of any restimulation. Data are gated on GFP positive cells.

Supplementary Figure 2. Intratumoral CTLs displayed high granzyme B content.

Granzyme B staining was performed 2 days after adoptive transfer of in vitro activated OT-I T cells on tumor frozen sections. Confocal images showing CTLs (red) displaying high granzyme B content (white) found in close contact with EG7-mYFP tumor cells (yellow).

Supplementary Figure 3. Killing of tumor cells by OT-I CTLs in EL4/EG7 mixed tumors is highly Ag specific

Mice were injected with a mixture of EG7 and EL4 tumors cells. In vitro activated OT-I CTLs ($5x10^6$) were transferred on day 5. On day 10, the absolute number of EL4 and EG7 tumor cells recovered from collagenase digested tumors is shown for two mice transferred with activated OT-I CTLs and two mice left untrated. Note that the absolute number of EL4 cells recovered is minimally (if at all) affected by OT-I cell transfer.

Supplementary Figure 4. FRET loss in EG7-DEVD tumors killed by CTLs in vitro correlated with Annexin V staining

In vitro activated OT-I T cells were incubated with EG7-DEVD tumors for 4h. Flowcytometric analysis of FRET loss is shown within tumors cells displaying either positive or negative Annexin V staining. Numbers indicate the percentage of cells with FRET loss.

Supplemental Movie. 1. Intravital two photon imaging on tumor bearing mice 2-3 days after CTL adoptive transfer.

Mice bearing EG7-mYFP tumors and adoptively transferred with activated GFP-expressing OT-I CD8 T cells were subjected to intravital two photon imaging. Time-lapse video revealed that CTLs located within regions in which tumor cells had been eliminated (delineated by a blue dashed line) were very motile. Other CTLs were sequestered at the border of these regions, being engaged in long-lasting interactions with tumor cells. This movie illustrates that intratumoral CTL dissemination occurs concomitantly with tumor cell elimination.

Supplemental Movie. 2. Intravital two photon imaging of tumor cell apoptosis during adoptive T cell therapy.

Mice bearing EG7-DEVD tumors and adoptively transferred with activated GFP-expressing OT-I CD8 T cells were subjected to intravital imaging. Time-lapse video showed that apoptotic tumor cells (green) were closely associated with CTLs (red) for prolonged period of time. The vast majority of tumor cells that were not contacting CTLs were alive (yellow).

Supplemental Movie. 3 Tumor cells that are not contacted by CTLs remain alive.

Mice bearing EG7-DEVD tumors and adoptively transferred with activated GFP-expressing OT-I CD8 T cells were subjected to intravital two photon imaging. Time-lapse video illustrating the absence of detectable caspase 3 activity in tumor cells that are not engaged by CTLs.

Supplemental Movie. 4. Tumor cells undergo apoptosis during their interactions with CTLs.

Mice bearing EG7-DEVD tumors and adoptively transferred with activated GFP-expressing OT-I CD8 T cells were subjected to intravital two photon imaging. Time lapse video showing 3 examples of tumor cells undergoing apoptosis while being engaged by antitumor CTLs (red). Note that the color of these tumor cells shifts from yellow to green indicative of increasing caspase 3 activity.

Supplemental Movie 5. Intratumoral CTLs remain sequestered near apoptotic tumor cells.

Mice bearing EG7-DEVD tumors and adoptively transferred with activated GFP-expressing OT-I CD8 T cells were subjected to intravital imaging. Time lapse video showing an example of a CTLs (red) closely associated with an apoptotic tumor cell (green) during the entire period of the movie.

Online supplemental material. Video 1-5 show time-lapse imaging of intratumoral CTL in live anesthetized mice. Video 1 illustrates that intratumoral CTL dissemination occurs concomitantly with tumor cell elimination. Video 2 shows that apoptotic tumor cells were closely associated with CTLs for prolonged period of time. Video 3 illustrates that Tumor cells that are not contacted by CTLs remain alive. Video 4 shows 3 examples of tumor cells undergoing apoptosis while being engaged by antitumor CTLs. Video 5 shows an example of a CTL closely associated with an apoptotic tumor cell during the entire period of the movie.

FigS1 demonstrates that CTLs enhance their effector functions upon Ag recognition in the tumor environment. FigS2 shows that intratumoral CTLs displayed high granzyme B content.