

- **SUPPLEMENTARY DATA**

- **Antibodies** - Antibodies used for flow cytometry (clones and dilutions)
- **Stereotactic radiation with SARRP delivery system** - Each mouse was individually imaged with cone beam computed tomography (CBCT) using the SARRP with a 65 kVp and 0.7 mA beam. Using the treatment planning system from the SARRP system (Muriplan) and the CBCT, the target was exactly placed 3mm below the skull's burr hole. The planning system calculated the x-ray beam's (220kVp and 13mA) time of exposure according to the prescribed dose and moved the motorized couch to its target location, after which a 3-mm beam centered on the burr hole and underlying tumor was used to administer a total of 10 Gy radiation per animal at a rate of 1.9 Gy/min (18). The isodose distribution is shown on the figure provided. The dose to adjacent organs or rest of the brain is insignificant as the beam's penumbra demonstrates complete drop off on the edge of the field (17, 50, 51)
- **TIM-3 expression on lymphocytes** - Gating strategy to assess for surface expression of TIM-3 on (A) CD4+ and (B) CD8+ T cells isolated from peripheral lymph nodes, lungs, livers, spleens, and brains.
- **Gating strategy for APC characterization** - Cells were first gated on size and singularity by forward scatter and side scatter. Nonviable cells were excluded by live/dead gating. Live cells were gated on CD11c and CD11b, then on CD45 and F4/80 to identify macrophage and dendritic cell phenotypes. Finally myeloid cells were gated for expression of TIM-3. Final populations were (A) F4/80+CD45hiCD11c-CD11bhi (B) F4/80+CD45dimCD11bhi (C) F4/80+CD45hiCD11c+CD11b+ and (D) F4/80-CD45dimCD11c+CD11b-.
- **Lymphocyte gating strategy.** - Cells were first gated on size and singularity by forward scatter and side scatter. Nonviable cells were excluded by live/dead gating. CD3+ live cells were gated on CD4 and CD8. CD3+FoxP3+CD4+ cells were designated as Tregs. TIM-3 vs. PD-1 expression was assessed for each lymphocyte population.