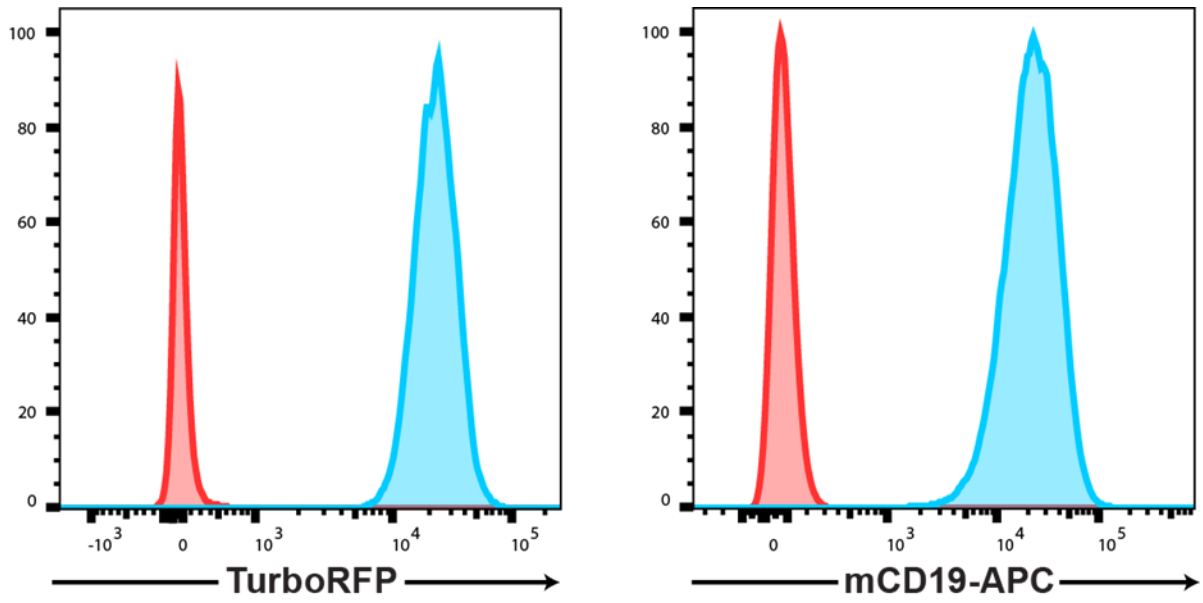
OMTO, Volume *17*

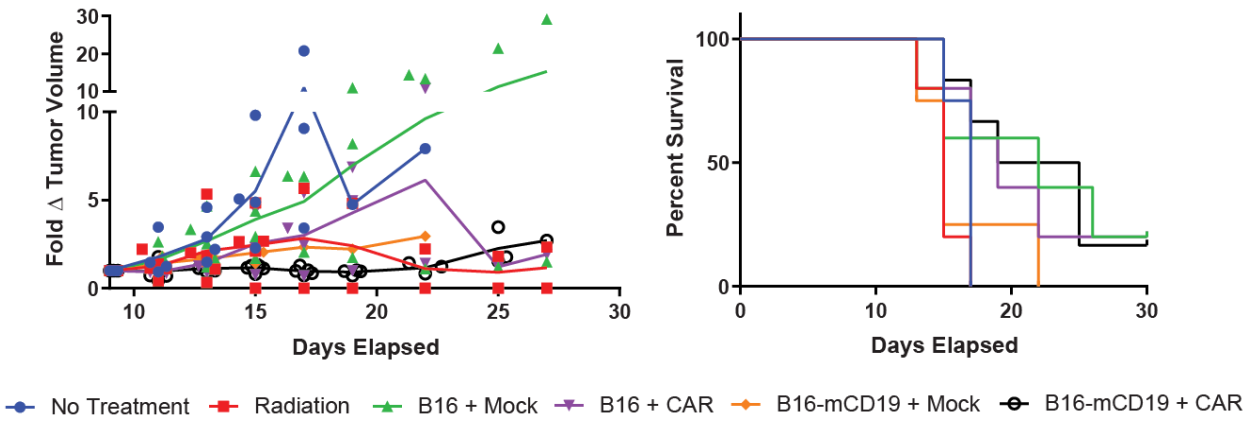
Supplemental Information

Viral Delivery of CAR Targets to Solid Tumors Enables Effective Cell Therapy

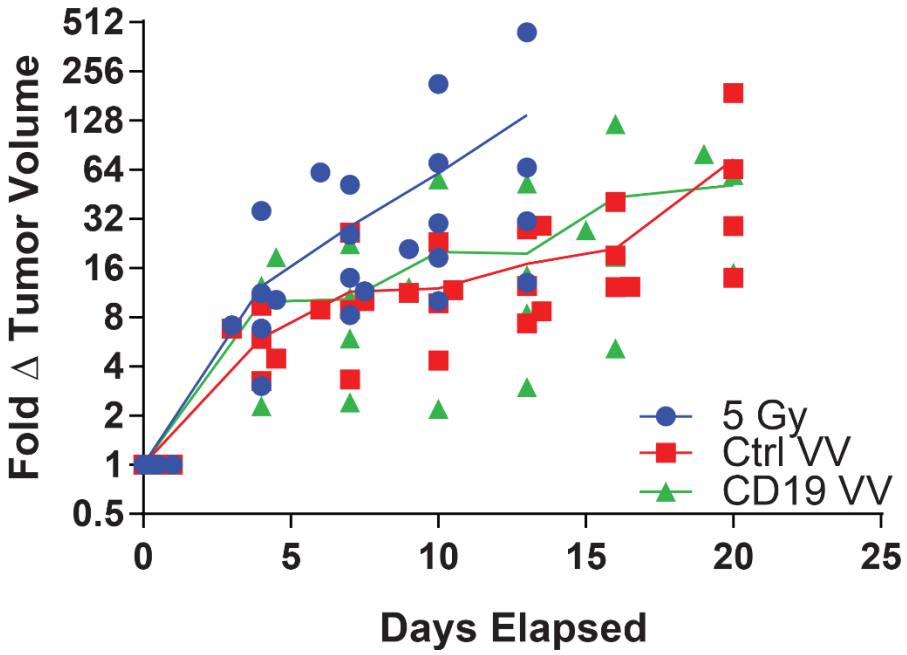
Amin Aalipour, Fabrice Le Boeuf, Matthew Tang, Surya Murty, Federico Simonetta, Alexander X. Lozano, Travis M. Shaffer, John C. Bell, and Sanjiv S. Gambhir



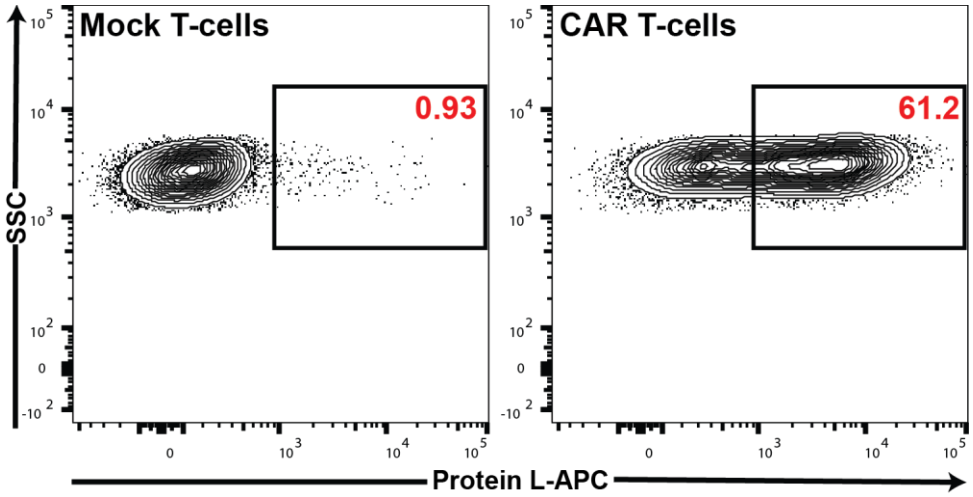
Supplementary Figure S1. Generation of engineered B16 cell lines.
Three cell lines were generated: B16-TurboRFP/RLuc8, B16-mCD19, and B16-TurboRFP/RLuc8-mCD19. Flow cytometry confirms uniform expression of TurboRFP (left) and mCD19 (right) transgenes. Data shown for B16-TurboRFP/RLuc8-mCD19 cell line (blue) relative to native B16 cell line (red).



Supplementary Figure S2. Intravenous delivery of mCD19 CAR T-cells is ineffective against B16 tumors constitutively expressing mCD19.
Intravenously delivered mCD19 CAR T-cells do not significantly delay B16-mCD19 tumor progression *in vivo* (left) or confer a survival benefit (right) relative to tumors treated with 5Gy TBI (radiation), mock T-cells, or antigen negative tumors treated with either mock or CAR T-cells. Kaplan-Meier survival curve p = 0.0902, df = 5, Chi square = 9.514 by Mantel-Cox test. Number of independent mice in each group as follows: n = 4 (No Treatment), n = 5 (Radiation), n = 5 (B16 + Mock), n = 5 (B16 + CAR), n = 4 (B16-mCD19 + Mock), and n = 6 (B16-mCD19 + CAR).



Supplementary Figure S3. Therapeutic index of vaccinia viruses as single agents. Intratumoral Control VV (n = 5 independent mice) and mCD19 VV (n = 4 independent mice) combined with TBI can yield modest delays in B16 tumor progression relative to mice only receiving TBI (n = 6 independent mice). TBI Only vs. Control VV: day 11 tumor volume p = 0.0040, t = 3.836, df = 9. TBI Only vs. CD19 VV: day 11 tumor volume p = 0.0246, t = 2.762, df = 8. The two viruses also have a similar therapeutic index *in vivo* Control VV vs. CD19 VV: p = 0.7293, t = 0.3602, df = 7.



Supplementary Figure S4. Generation of murine mCD19 CAR T-cells.
Retroviral transduction of primary murine T-cell achieved efficiencies of ~50-65% and can be measured by staining chimeric antigen receptors with Protein L.