Characterization of human cancer xenografts in humanized mice

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Additional File 1
Supplemental Materials and Methods

Immunohistochemistry

Immunohistochemistry (IHC) was performed by Indivumed (Hamburg, Germany). IHC was implemented on the Discovery XT staining platform (Roche Diagnostics/Ventana Medical Systems, Tucson, AZ), using the rabbit monoclonal anti-CD4 antibody clone SP35 (Roche/Ventana) and the rabbit monoclonal anti-CD8 antibody clone SP16. Three tumors per model were evaluated. As run control, IHC of CD4⁺ tonsil tissue and CD8⁺ colon cancer and breast cancer tissues was performed in each respective run. Isotype controls were prepared for each sample. Formalin-Fixed Paraffin-Embedded tissue samples were sliced into 3–5 μm serial sections and mounted on SuperFrost Ultra Plus glass slides (Carl Roth, Karlsruhe, Germany). Hematoxylin & eosin (H&E)–stained sections were prepared according to Indivumed’s standard operating procedure. For IHC, slides were deparaffinized within the staining instrument and immunostained using the Discovery ChromoMap DAB Kit (Roche Diagnostics, Indianapolis, IN). After staining, the slides were manually washed using hot tap water supplemented with detergent, followed by tap water only and dH2O in a final step. For dehydration, the slides were transferred to an ascending ethanol series (2×80%, 2×96%, 2×abs. EtOH; 1 min each). After dehydration, the slides were transferred to xylene (2×1 min) and automatically coverslipped in Pertex. Scans were generated with the Axio Scan.Z1 automated slide scanner (Zeiss, Oberkochen, Germany) using ZEN 2 (blue edition) slidescan software (Zeiss).

Digital quantification of IHC images was performed by OracleBio (Scotland, UK). Whole slide images were evaluated using Indica Labs HALO platform v2.3 (Albuquerque, NM). A single annotated region defining ‘tumor cells + intertumoral stroma’ as viable tissue was created by a certified pathologist on H&E whole slide images, which were transcribed onto the
corresponding IHC and isotype stained WSI by OracleBio. A customized classifier algorithm was initially developed to detect the viable tissue area and large areas of white space within annotated region of interest. Following this, a cellular analysis algorithm was developed to detect CD4⁺ and CD8⁺ cell staining. Data are reported as number of positive cells per square millimeter.