Supplementary Material and methods

RT-PCR analysis of TME

Six days after treatment start, tumors are harvested on ice and weighed. On ice, tumor is manually dissected and transferred to RNA later. For processing, samples are thawed on ice and Trizol was used to extract RNA as per manufacturers protocol. RNA is quantified on the Nanodrop with a 260nm/280nm ratio of around 2. cDNA synthesis is achieved using the Verso cDNA synthesis kit (Thermo Fisher) following the kit protocol. The Powerup SYBR green kit (Thermo Fisher) was used for the RT-PCR in a 384 well plate on an ABI 7900HT. Primers used are FOXP3 F-ACTCGCATGTTCGCCTACTTCAG and R-GGCGGATGGCATTCTTCCAGGT and β 2-microglobulin F-TTCTGGTGCTTGTCTCACTGA and R-CAGTATGTTCGGCTTCCCATTC. Delta CTs were calculated using SDS2.4 and compared against non treated controls.

PET imaging

Mice were fasted overnight and brought to the imaging facility. Mice were injected with 300-400 uCi (12-15 MBq) in 0.1 ml normal saline [^{18}F] fluoro-2-deoxyglucose (FDG) via tail vein and imaging begins 1 hour after injection. Mice were anestatized with isoflourane and imaged on an Inveon Multimodality scanner (Siemens). Analysis is performed using either ASIPRO and IRW (both Siemens) dedicated software.

India ink treated lungs

Treated 4T1 tumor bearing mice were sacrificed. Lungs were inflated with 10% India Ink by intra-tracheal injection. Lungs were harvested and washed in 1L of water before storage in Feket's solution (300 ml 70% EtOH, 30 ml 37% formaldehyde, 5 ml glacial

acetic acid) to allow for bleaching of macro-metastases. Metastatic lesions were enumerated by counting.