SUPPLEMENTARY METHODS

Antibodies

Mouse anti-MAGE-A (Santa Cruz Biotech, Heidelberg, Germany, clone 6C1, dilution 1:500), mouse anti-GAGE (produced in house (23), clone C12, dilution 1:100), anti-MAGE-C1 (Santa Cruz Biotech, clone CT7-33, dilution 1:500), anti-NY-ESO-1 (Santa Cruz Biotech, clone E978, dilution 1:100) and anti-PRAME (Abcam, Cambridge, UK, clone EPR20330, dilution 1:4000) antibodies.

Lung cancer tumors

Fresh tumor tissue was collected from lung adenocarcinoma (LUAD) primary tumors. The tissue was stored in MACS Tissue Storage Solution (Miltenyi Biotec, Lund, Sweden) at 5°C for up to 24 hours and subsequently dissociated with the Human Tumor Dissociation Kit (Miltenyi Biotec) using the gentleMACS Octo Dissociator with Heaters (Miltenyi Biotec). Live tumor cells were isolated by magnetic separation with LS columns and a MACS separator (Miltenyi Biotec) using the human Tumor Cell Isolation Kit followed by the Dead Cell Removal Kit (Miltenyi Biotec) according to the manufacturer's instructions.

scRNAseq analysis of cancer/testis antigen expression in melanoma and lung cancer

Initially, we analyzed melanoma and lung cancer gene expression data available in The Cancer Genome Atlas (TCGA) repository, enabling the identification of CTAs expressed in tumor cells from melanoma and LUADs. Bulk RNA-sequencing expression data from 471 melanomas and 529 LUADs were downloaded from the TCGA repository and processed using Qlucore (Qlucore, Lund, Sweden). CTA genes were identified from previous publications (30, 31) and the CTA database (CTdatabase; http://www.cta.lncc.br/) (Supplementary Table 1; "CTAs") and final lists of CTA genes expressed in at least 5% of melanomas or LUADs (TPM>5(log2)), and with testis-specific expression in healthy somatic tissues, were compiled. The final lists contained 81 genes for melanoma (Supplementary Table 1; "CTAs melanoma") and 78 genes for LUAD (Supplementary Table 1; "CTAs lung adenocarcinoma"). Next, we used targeted scRNAseq to profile the expression of CTAs in low-passage melanoma primary cell cultures or isolated cancer cells from surgical specimens of LUAD tumors. Cells were counted using the BD Rhapsody Scanner (BD Biosciences, Lyngby, Denmark) and single cell capture and reverse transcription was performed using the BD

Rhapsody Single-Cell Analysis System (BD Biosciences) according to the manufacturer's recommendations. Final mRNA libraries were prepared using the BD Rhapsody Targeted mRNA and AbSeq Amplification Kit (BD Biosciences) combined with custom primer libraries for amplification of CTA target genes (prepared by BD Biosciences). For analysis of melanoma primary cell cultures, the CTA primary library was combined with the BD Rhapsody Onco-BC targeted panel (BD Biosciences) and a panel of melanoma marker genes (Supplementary Table 1) to identify CTA-negative subpopulations of cancer cells. For the LUAD samples, the CTA primer library was supplemented with a panel of housekeeping genes to identify CTA-negative subpopulations of cancer cells and genes encoding specific markers for various cell types of the human lung (Supplementary Table 1; "lung marker genes") to exclude non-cancer cells (32). Libraries were sequenced in paired-end mode (60 R1, 8 i7, 8 i5, 42 R2) with 20% PhiX Spike-in on an Illumina NovaSeq 6000 System, aiming for a coverage of approximately 10,000 raw reads per cell. Basecalling and demultiplexing were performed using bcl2fastq 2.20. Quality filtering, mapping, putative cell calling, multiplet removal and distribution-based UMI error correction were performed using the BD Rhapsody Targeted Analysis Pipeline v1.11.1 on the SevenBridges platform. Sequencing saturation for all libraries varied between 82-100% (primary melanomas 86.53%, metastatic melanomas 82.34%, LUAD1 82.15%, LUAD2 99.4%, LUAD3 97.97%) and the total numbers of detected cells for further processing varied between 1213-5449 (Yudoso: 1948, Yupeet: 1907, Yutogs: 1420, WW165: 1213, Yusit: 3671, Yusit: 2479, Yuksi: 2938, LUAD1: 2688, LUAD2: 2240, LUAD3: 5449). For visualization and clustering, the expression matrices generated from the BD Rhapsody Targeted Analysis Pipeline were further analyzed using the Seurat 4.3.0.1 package in R (version 4.3.1) (33). The data was normalized using "LogNormalize" and genes exhibiting high cell-to-cell variation were identified. Next, the data was scaled and the dimensionality of the datasets were reduced by PCA using the previously determined variable genes. The resulting PCs were used as input for UMAP embedding and clusters were identified using Louvain clustering (resolution = 0.1) based on the UMAP embedding.