Supplementary Note 1. Genetic demultiplexing of single-cell RNA-seq data using demuxlet

In four of the 13 experiments we performed, we pooled melanoma cultures into a single 10x Chromium lane (**Supplementary Table 1)**. To determine the identity of the cells in these runs, we used sample-specific genetic mutations and demuxlet (Kang et al. 2018). Demuxlet predicts the likelihood of observing a set of sample-specific SNPs in each cell given the set of uncorrelated SNPs with genotype probabilities. We used high-coverage bulk RNA-seq data on the same melanoma cultures to identify sample-specific SNPs. Genotype calling was performed on ten cultures with the samtools mpileup command (version 1.3.1 with options -q 1 -Q 13 -d 8000) on the bulk RNA-seq reads that were mapped and post-processed as described previously (Verfaillie et al. 2015). We filtered the resulting vcf file based on the genotype fields to obtain an uncorrelated set of SNPs.

First, to assess the accuracy of SNP-based demultiplexing, we combined the bam files of three single-sample scRNA-seq runs (72h after SOX10-KD in MM057, MM074 and MM087) to obtain a pseudo-pooled scRNA-seq run. We analysed the resulting bam file with demuxlet and compared the predictions based on sample-specific SNPs with known cell identities. demuxlet classified 13543 of the 13545 cells correctly (**Figure 1**).
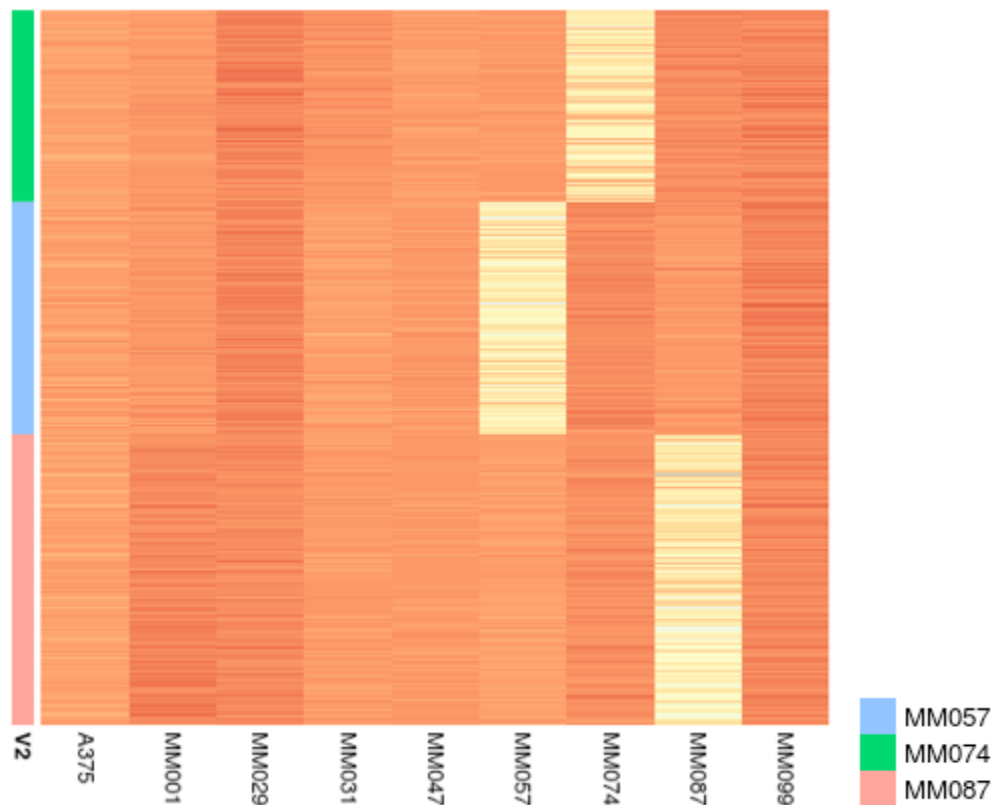

Figure 1. Heatmap showing log-likelihood ratios (row median-scaled) of cell identity predictions from demuxlet. On the left hand side of the heatmap, true sample identities are indicated with colored blocks (green for MM074, blue for MM057 and red for MM087).

Next, we used demuxlet on the four pooled experiments (with --alpha 0 --alpha 0.5 option to include doublet predictions as well) (**Table 1**) . Predicted doublet percentages ranged from two to five percent, with the pooled sample of ten cultures having the highest doublet percentage (5.2%). Predicted singlets were used in the downstream analyses.

Sequencing Run	Number of demultiplexed cells	Sample Ratios	Number of predicted doublets
Baselines (10 mixed)	4,334	566(A375):428(MM001):319(MM011):457(MM029):461(MM031):322(MM047):478(MM057):385(MM074):453(MM087):465(MM099)	240
48h after SOX10-KD (3 mixed)	3,364	932(MM057):901(MM074):1531(MM087)	82
24h after SOX10-KD (3 mixed)	3,699	1398 (MM057):1280 (MM074):1021(MM087)	89
72h after NTC-KD (MM074-MM057 mix)	4,072	2161(MM057):1910(MM074)	84

Table 1. Demuxlet statistics

References

Kang, Hyun Min, Meena Subramaniam, Sasha Targ, Michelle Nguyen, Lenka Maliskova, Elizabeth McCarthy, Eunice Wan, et al. 2018. "Multiplexed Droplet Single-Cell RNA-Sequencing Using Natural Genetic Variation." *Nature Biotechnology* 36 (1): 89–94. https://doi.org/10.1038/nbt.4042.

Verfaillie, Annelien, Hana Imrichova, Zeynep Kalender Atak, Michael Dewaele, Florian Rambow, Gert Hulselmans, Valerie Christiaens, et al. 2015. "Decoding the Regulatory Landscape of Melanoma Reveals TEADS as Regulators of the Invasive Cell State." *Nature Communications* 6 (April): 6683. https://doi.org/10.1038/ncomms7683.