Supplementary methods

Flow Cytometry Analysis. After TRYPLE incubation, PDOs were dissociated into single-cell suspension. Cells were counted and aliquots of 1×10^6 cells per condition were prepared. The samples were incubated for 5 minutes with 1 μL of Zombie Aqua™ fixable viability dye in PBS 1X, then washed with flow cytometry buffer (FCB, 0.5% BSA, 0.02% NaN_3 and 2mM EDTA in PBS 1X) and centrifuged (500 × g, 5 minutes) before adding PFA at a final concentration of 1.6% for 10 minutes at room temperature in FCB. Cells were then centrifuged at 500 × g for 5 minutes at 4°C to pellet cells, PFA was removed, and cells were washed again with FCB. Cells were either long-term stored at -80°C in 500 μL of FCB or permeabilised with 100 μL of eBioscience™ Permeabilisation Buffer diluted at 1X concentration for 30 minutes on ice with a master mix of primary antibodies (see Key Resource Table). After the incubation with primary antibodies, cells were washed with FCB and spun down at 500 × g for 5 minutes at 4°C (2X). Then, cells were resuspended in 500 µl of FCB, strained and analysed using a BD LSRFortessa™ X-20. Results were analysed using Cytobank single-cell analysis software with the gating strategies described in Extended Data Fig. 6.

EMT analysis with PhenoSTAMP. PHENOSTAMP was downloaded from GitHub under https://github.com/anchangben/PHENOSTAMP. Briefly, FCS files previously gated in Cytobank according to singlets and Live/Dead (Extended Data Fig. 6), were uploaded into R, and the PHENOSTAMP algorithm was used to project the PDOs on the 2D EMT–MET state map, as previously described here[1].

Supplementary references

1. Karacosta LG, Anchang B, Ignatiadis N, et al. Mapping Lung Cancer Epithelial-Mesenchymal Transition States and Trajectories with Single-Cell Resolution. *Nat Commun*. 2019;10:5587.