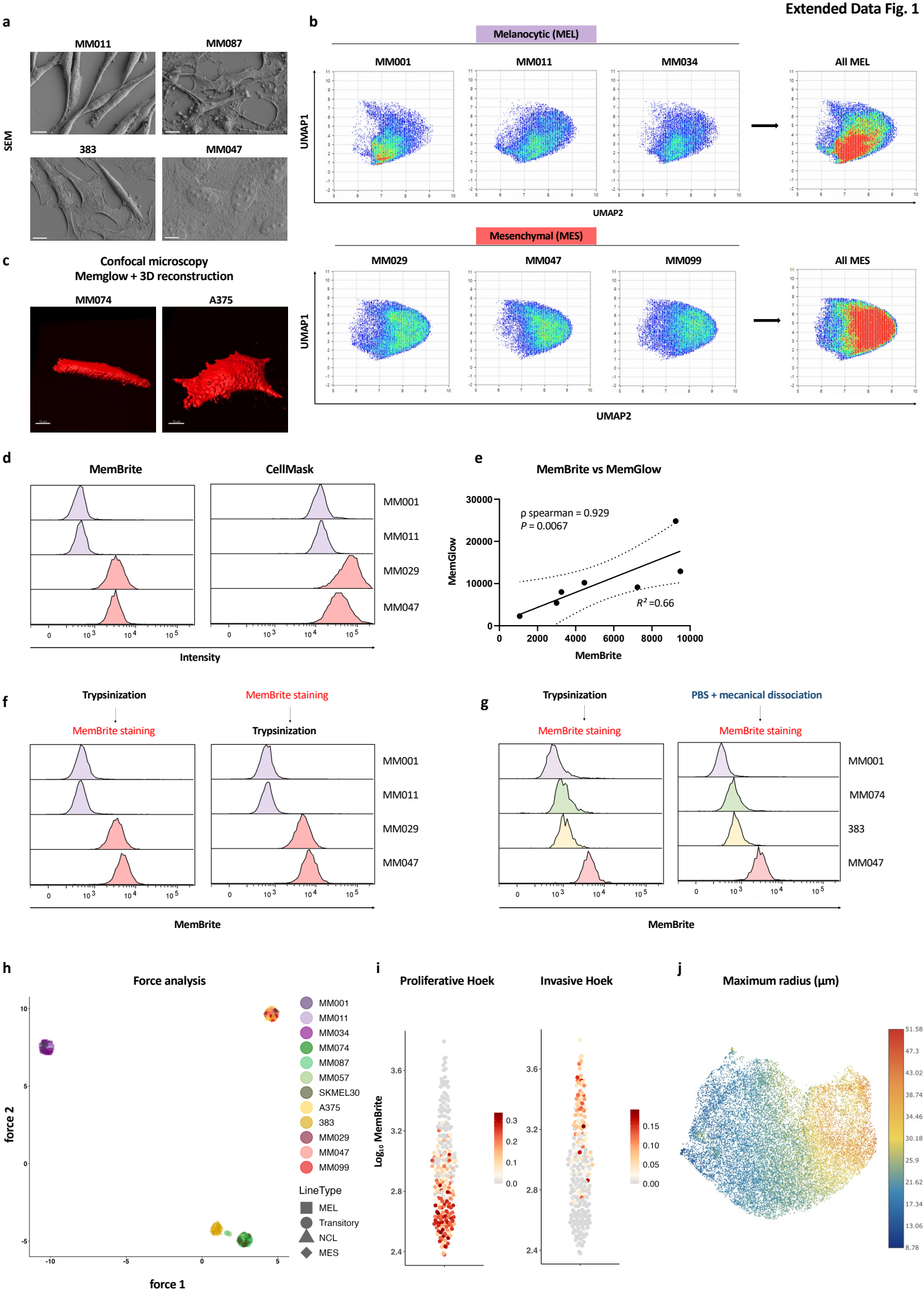

a
SEM
MM011
MM087
383
MM047
b
Melanocytic (MEL)
MM001
MM011
MM034
All MEL
UMAP1
UMAP2
Mesenchymal (MES)
MM029
MM047
MM099
All MES
c
Confocal microscopy
Memglow + 3D reconstruction
MM074
A375
d
MemBrite
CellMask
MM001
MM011
MM029
MM047
Intensity
e
MemBrite vs MemGlow
ρ spearman = 0.929
P = 0.0067
R² =0.66
MemGlow
MemBrite
f
Trypsinization
MemBrite staining
MemBrite staining
Trypsinization
MM001
MM011
MM029
MM047
MemBrite
g
Trypsinization
PBS + mecanical dissociation
MemBrite staining
MemBrite staining
MM001
MM074
383
MM047
MemBrite
h
Force analysis
force 2
force 1
MM001
MM011
MM034
MM074
MM087
MM057
SKMEL30
A375
383
MM029
MM047
MM099
LineType
MEL
Transitory
NCL
MES
i
Proliferative Hoek
Invasive Hoek
Log10 MemBrite
j
Maximum radius (µm)

Extended data Fig.1. Morphological and transcriptomic characterization of melanoma states

a, Scanning electron microscopy (SEM) images of additional melanoma cell lines corresponding to each of the four untreated transcriptomic states.

b, UMAP projection of ThinkCyte data showing distinct clustering of melanocytic (n = 3) and mesenchymal (n = 3) different melanoma lines, representative of two independent experiments.

c, Representative reconstructed 3D confocal Z-stacks used for single-cell surface quantification (MM074 and A375).

d, Comparison of MemBrite and CellMask fluorescence profiles by flow cytometry in melanocytic (MEL: MM001, MM011) and mesenchymal (MES: MM029, MM047) lines.

e, Correlation between MemBrite and MemGlow fluorescence intensities measured by flow cytometry (n = 7 lines; Spearman's correlation coefficient (ρ) and linear regression R^2 are shown).

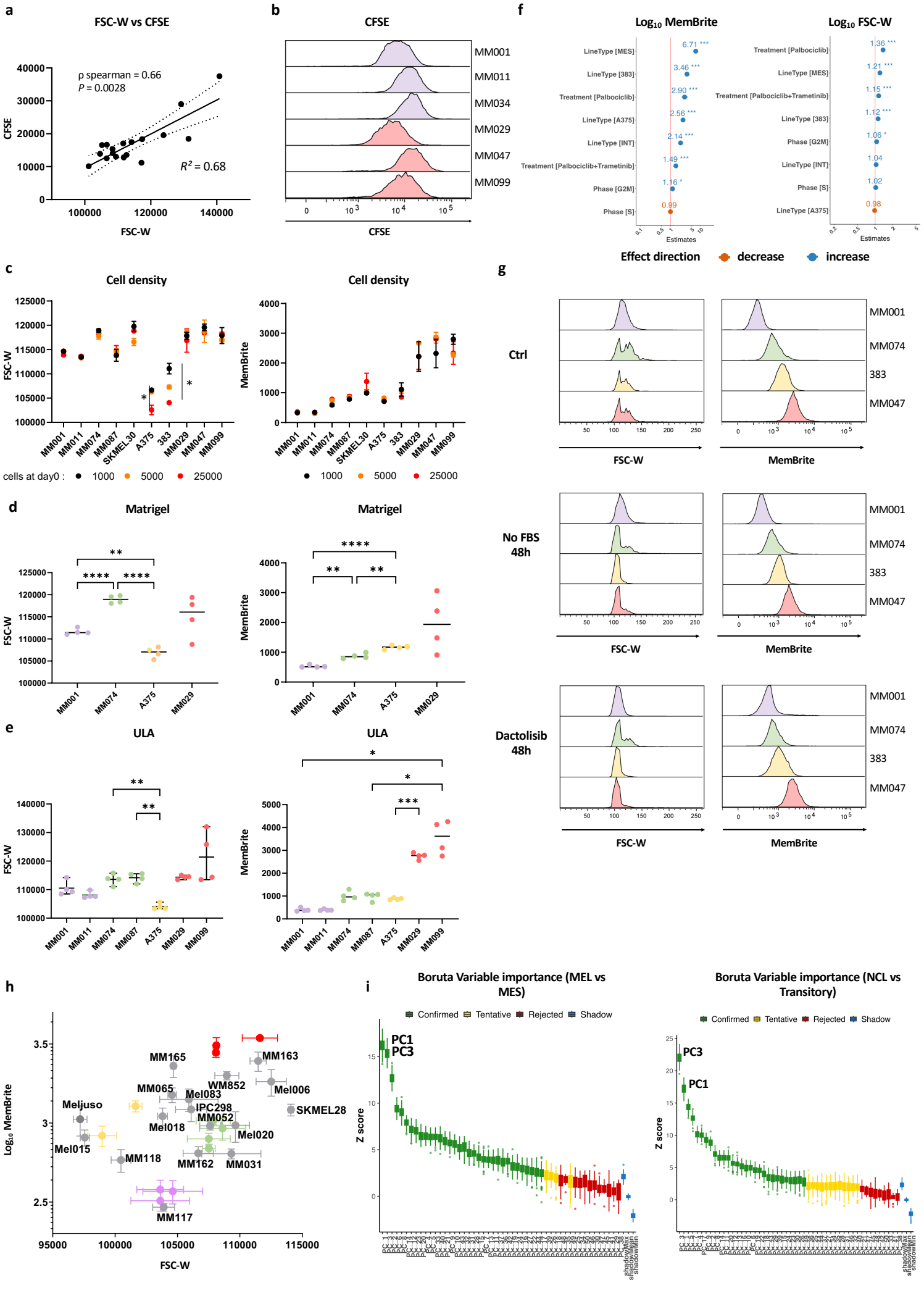
f, Comparison of MemBrite staining performed before versus after trypsinization, analyzed by flow cytometry.

g, Comparison of enzymatic versus mechanical dissociation prior to MemBrite labeling.

h, Force-directed graph layout identifying four main morphological states under untreated conditions.

i, Distribution of transcriptomic state-specific gene signature enrichment along MemBrite fluorescence intensity.

j, Maximum cell radius (μm) extracted from Deepcell morphological analysis.


a
FSC-W vs CFSE
ρ spearman = 0.66
P = 0.0028
R^2 = 0.68
CFSE
FSC-W
b
CFSE
MM001
MM011
MM034
MM029
MM047
MM099
f
Log10 MemBrite
Log10 FSC-W
Estimates
Effect direction
decrease
increase
c
Cell density
FSC-W
MemBrite
cells at day0 : 1000 5000 25000
g
Ctrl
No FBS 48h
Dactolisib 48h
d
Matrigel
e
ULA
h
Log10 MemBrite
FSC-W
i
Boruta Variable importance (MEL vs MES)
Boruta Variable importance (NCL vs Transitory)
Confirmed Tentative Rejected Shadow
Z score

Extended data Fig. 2. Technical parameters influencing cell surface and volume measurements

a, Correlation between FSC-W and CFSE fluorescence measured by flow cytometry. (n = 18 conditions including untreated and trametinib and/or palbociclib-treated lines).

b. CFSE fluorescence intensityies between MEL and MES lines.

c, Effect of initial plating density (time 0) on FSC-W and MemBrite intensity.

d, Influence of Matrigel-based culture versus standard polystyrene on FSC-W and MemBrite intensity.

e, Effect of ultra-low attachment (ULA) plates on FSC-W and MemBrite intensity.

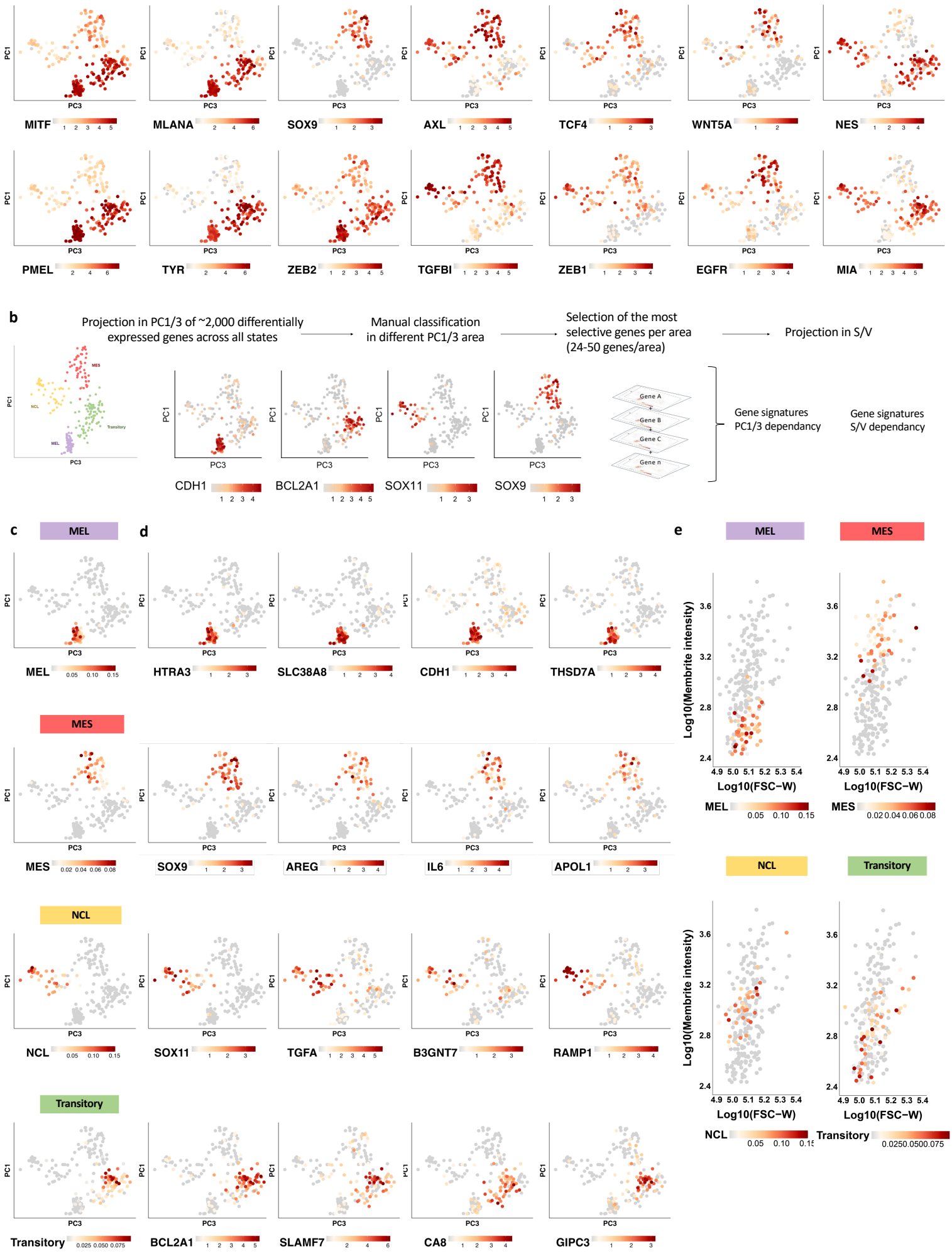
f, Model estimating the relative contribution of distinct feature classes to FSC-W and MemBrite readouts. Statistical significance for each term was determined using type II Wald tests.

g, Representative histograms showing the impact of cell cycle arrest (induced by FBS deprivation, dual PI3K/mTOR inhibition with dactolisib for 48 h, or palbociclib for 24 h) on FSC-W and MemBrite.

h, Median MemBrite and FSC-W values across additional melanoma cell lines (n = 12) and PDX-derived lines (n = 5); coloured dots represent reference lines (n = 12).

i, Boruta feature-selection analysis combined with principal component analysis (PCA) identifying PC1 and PC3 as the dominant axes discriminating MES/MEL (surface axis) and NCL/transitory (volume axis) states.

Statistical analyses: Welch's ANOVA followed by Dunnett's multiple comparison test was used for panels c-e (*P < 0.05; **P < 0.01; ***P < 0.001; **P < 0.0001). Spearman's correlation coefficient (ρ) and linear regression R^2 are reported in panel a.


a
MITF
MLANA
SOX9
AXL
TCF4
WNT5A
NES
PMEL
TYR
ZEB2
TGFBI
ZEB1
EGFR
MIA
b
Projection in PC1/3 of ~2,000 differentially expressed genes across all states
Manual classification in different PC1/3 area
Selection of the most selective genes per area (24-50 genes/area)
Projection in S/V
MES
NCL
Transitory
MEL
CDH1
BCL2A1
SOX11
SOX9
Gene A
Gene B
Gene C
Gene n
Gene signatures PC1/3 dependancy
Gene signatures S/V dependancy
c
MEL
HTRA3
SLC38A8
CDH1
THSD7A
MES
SOX9
AREG
IL6
APOL1
NCL
SOX11
TGFA
B3GNT7
RAMP1
Transitory
BCL2A1
SLAMF7
CA8
GIPC3
d
e
MEL
MES
Log10(Membrite intensity)
Log10(FSC-W)
NCL
Transitory

Extended data Fig. 3. Transcriptomic signatures projected onto the S/V landscape.

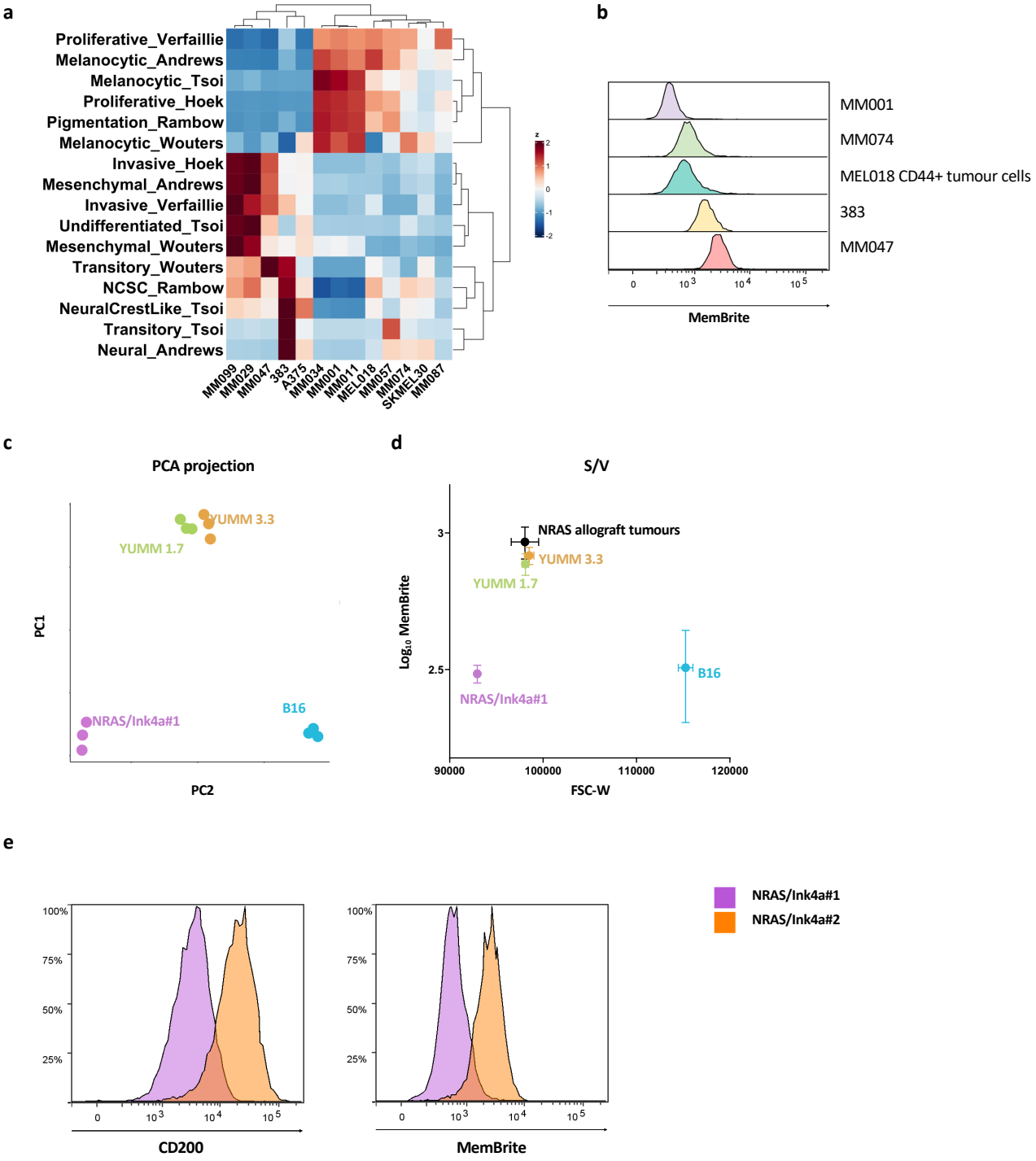
a, Projection of established melanoma state marker genes onto the PC1/PC3 space derived from scRNA-seq data.

b, Schematic illustrating the strategy used to define transcriptional signatures associated with specific PC1/PC3 regions, which correspond to distinct S/V positions.

c, Mapping of curated gene sets representative of melanocytic (MEL, n = 26 genes), mesenchymal (MES, n = 46), neural crest-like (NCL, n = 24) and transitory (n = 35) states onto the PC1/PC3 space.

d, PC1/PC3 projection of four representative genes from the newly defined transcriptional signatures.

e, Visualization of the resulting state-specific gene signatures projected across the S/V landscape.


a
Proliferative_Verfaillie
Melanocytic_Andrews
Melanocytic_Tsoi
Proliferative_Hoek
Pigmentation_Rambow
Melanocytic_Wouters
Invasive_Hoek
Mesenchymal_Andrews
Invasive_Verfaillie
Undifferentiated_Tsoi
Mesenchymal_Wouters
Transitory_Wouters
NCSC_Rambow
NeuralCrestLike_Tsoi
Transitory_Tsoi
Neural_Andrews
MM099
MM029
MM047
383
A375
MM034
MM001
MM011
MEL018
MM057
MM074
SKMEL30
MM087
z
2
1
0
-1
-2
b
MM001
MM074
MEL018 CD44+ tumour cells
383
MM047
0
10 3
10 4
10 5
MemBrite
c
PCA projection
YUMM 3.3
YUMM 1.7
NRAS/Ink4a#1
B16
PC1
PC2
d
S/V
NRAS allograft tumours
YUMM 3.3
YUMM 1.7
B16
NRAS/Ink4a#1
Log10 MemBrite
3
2.5
90000
100000
110000
120000
FSC-W
e
100%
75%
50%
25%
0
10 3
10 4
10 5
CD200
MemBrite
NRAS/Ink4a#1
NRAS/Ink4a#2

Extended data Fig. 4. Morphological and transcriptomic characterization of melanoma states

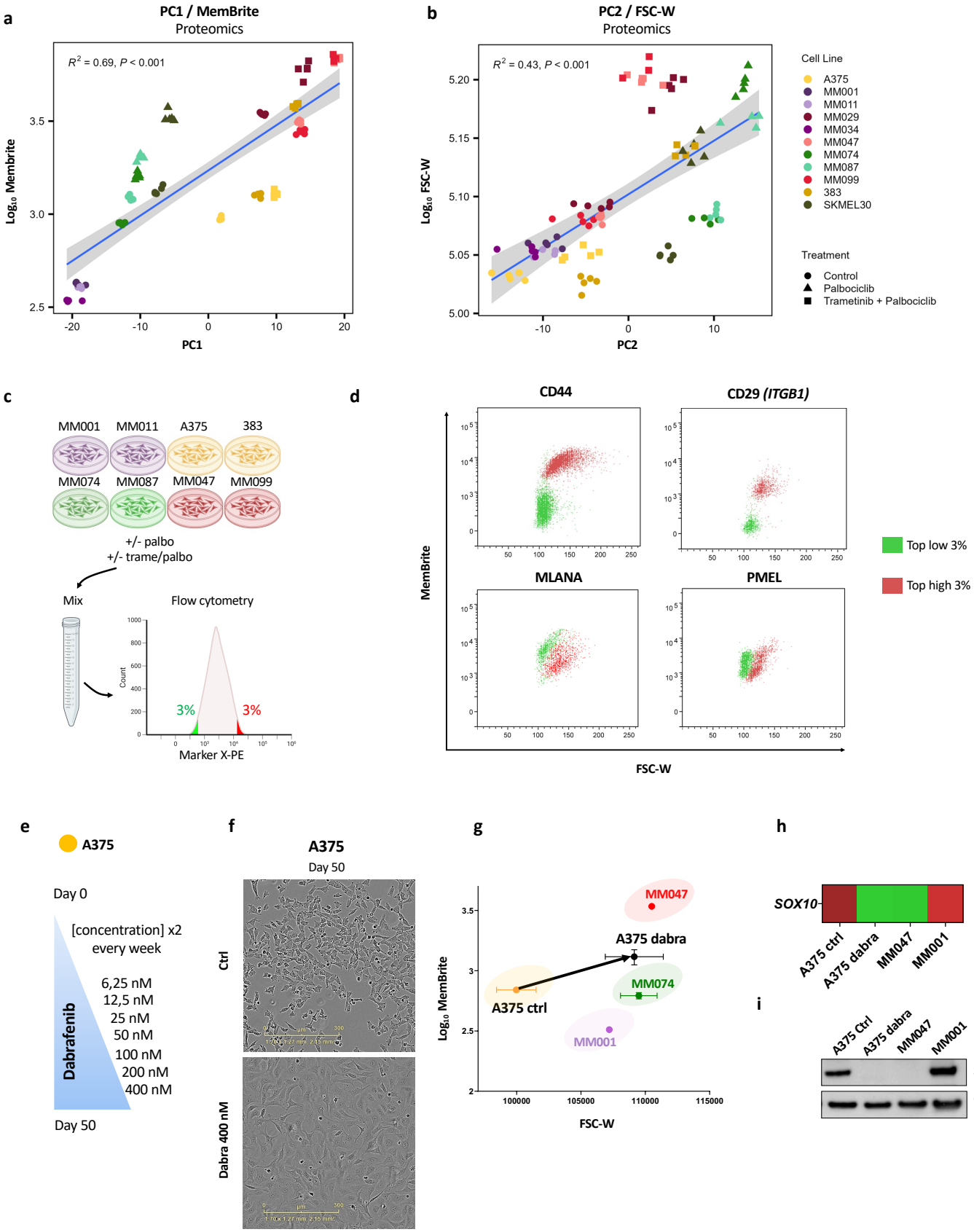
a, Heatmap from scRNA-seq data including the untreated Mel018 PDX sample and additional human melanoma cell lines.

b, Representative flow cytometry histogram of MemBrite fluorescence in $CD44^+$ Mel018 tumour cells compared with reference human melanoma lines.

c, Principal component analysis (PC1-PC2) of bulk RNA-seq data from murine melanoma lines (Nras;Ink4a#1, B16, YUMM1.7, and YUMM3.3). Each dot represents a biological replicate.

d, Median $\log_{10}$ MemBrite intensity and FSC-W values in Nras;Ink4a allograft tumours (n=4 biological replicates) compared with reference murine melanoma lines.

e, Differential expression of CD200, a neural crest-like-enriched marker[18], across Nras;Ink4a allograft-derived murine cell lines (Nras;Ink4a #1 and #2).


a
PC1 / MemBrite
Proteomics
R^2 = 0.69, P < 0.001
Log_{10} Membrite
PC1
b
PC2 / FSC-W
Proteomics
R^2 = 0.43, P < 0.001
Log_{10} FSC-W
PC2
Cell Line
A375
MM001
MM011
MM029
MM034
MM047
MM074
MM087
MM099
383
SKMEL30
Treatment
Control
Palbociclib
Trametinib + Palbociclib
c
MM001
MM011
A375
383
MM074
MM087
MM047
MM099
+/- palbo
+/- trame/palbo
Mix
Flow cytometry
Count
3%
3%
Marker X-PE
d
CD44
CD29 (ITGB1)
MLANA
PMEL
MemBrite
FSC-W
Top low 3%
Top high 3%
e
A375
Day 0
[concentration] x2
every week
Dabrafenib
6,25 nM
12,5 nM
25 nM
50 nM
100 nM
200 nM
400 nM
Day 50
f
A375
Day 50
Ctrl
Dabra 400 nM
g
MM047
A375 dabra
MM074
A375 ctrl
MM001
Log_{10} MemBrite
FSC-W
h
SOX10
A375 ctrl
A375 dabra
MM047
MM001
i
A375 Ctrl
A375 dabra
MM047
MM001
SOX10
GAPDH

Extended data Fig. 5. Proteomic profiling reveals patterns consistent with transcriptomic and S/V-based classifications.

a, Linear regression between PC1 from proteomic analysis and $\log_{10}$ MemBrite fluorescence intensity measured from index-sorted cell samples.

b, Linear regression between PC2 from proteomic analysis and FSC-W from index-sorted cell samples.

c, Schematic representation of the workflow used to identify protein markers enriched in specific regions along the S/V continuum.

d, Projection of the top 3% highest and lowest expressing cell populations for selected protein markers across the S/V landscape.

e, Experimental design of a long-term treatment assay (day 0-50) in A375 cells exposed to escalating doses of dabrafenib, with concentrations doubled weekly (adapted from [58]).

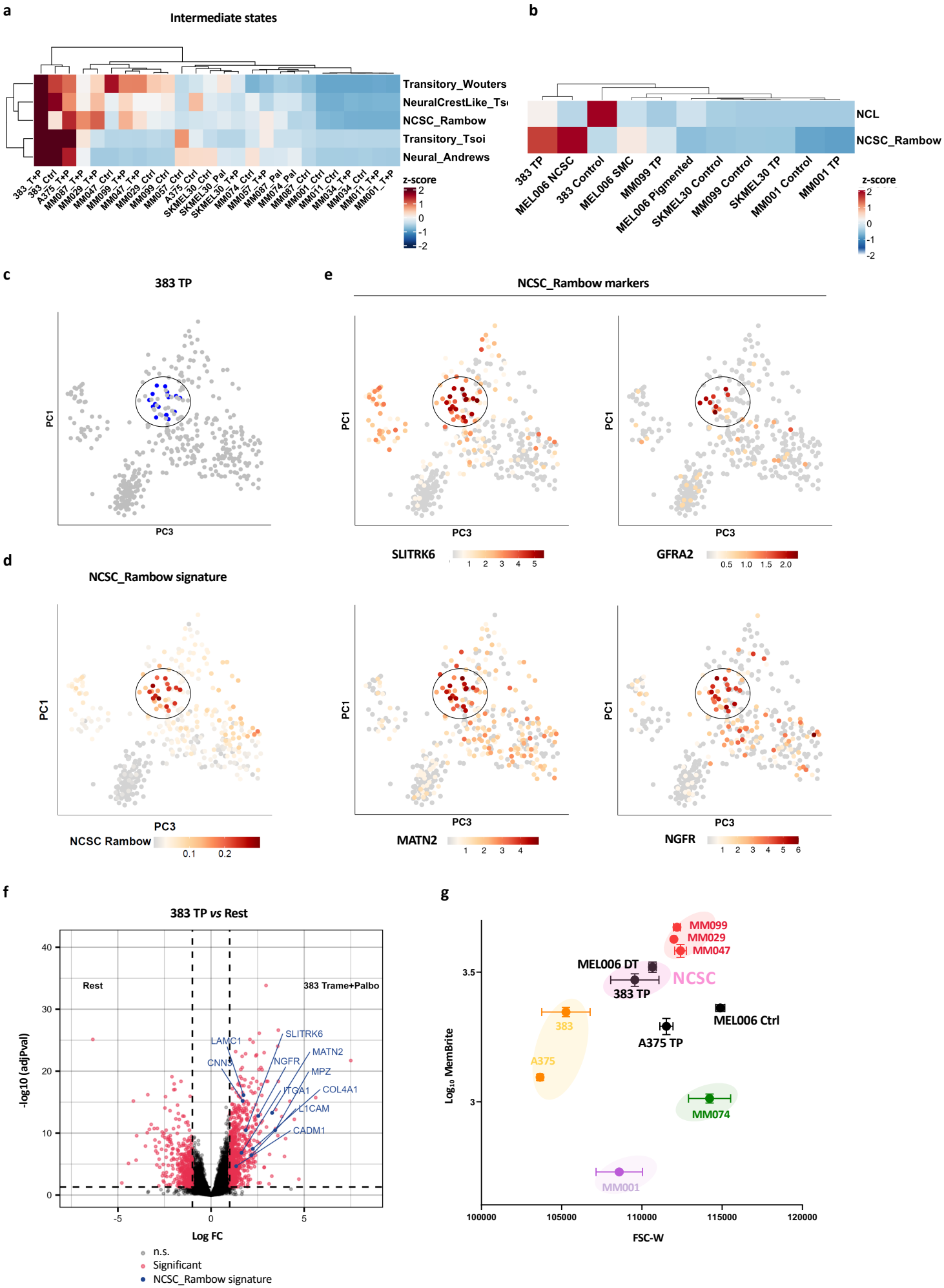
f, Representative incucyte images of A375 cells at day 50 under control and dabrafenib-treated conditions (dabra 400 nM).

g, Median $\log_{10}$ MemBrite intensity and FSC-W values for A375 cells treated with dabrafenib for 50 days (n = 6 biological replicates), compared with reference melanoma lines.

h, Quantitative PCR analysis of A375 control and dabrafenib-treated cells compared to MEL and MES reference lines. n = 3 biological replicates per condition, each performed in technical triplicate; median values are shown.

i, Western blot analysis of A375 control and dabrafenib-treated cells alongside MEL and MES reference lines, representative of two independent sample preparations.

Illustrations in c were created using BioRender (https://biorender.com).


a
Intermediate states
Transitory_Wouters
NeuralCrestLike_Ts
NCSC_Rambow
Transitory_Tsoi
Neural_Andrews
z-score
b
NCL
NCSC_Rambow
383 TP
MEL006 NCSC
383 Control
MEL006 SMC
MM099 TP
MEL006 Pigmented
SKMEL30 Control
MM099 Control
SKMEL30 TP
MM001 Control
MM001 TP
c
383 TP
PC1
PC3
e
NCSC_Rambow markers
SLITRK6
GFRA2
d
NCSC_Rambow signature
NCSC Rambow
MATN2
NGFR
f
383 TP vs Rest
Rest
383 Trame+Palbo
-log10 (adjPval)
Log FC
SLITRK6
LAMC1
MATN2
CNN3
NGFR
MPZ
ITGA1
COL4A1
L1CAM
CADM1
n.s.
Significant
NCSC_Rambow signature
g
MM099
MM029
MM047
MEL006 DT
NCSC
383 TP
383
MEL006 Ctrl
A375 TP
A375
MM074
MM001
Log10 MemBrite
FSC-W

Extended data Fig. 6. Therapy-induced emergence of a NCSC morphostate

a, Heatmap showing enrichment of transitory, neural crest-like (NCL), and neural crest stem-like (NCSC) transcriptional signatures in 383 cells treated with TP.

b, Integrated scRNA-seq analysis combining 383 TP cells, additional melanoma lines, and Mel006 minimal residual disease (MRD) samples collected in vivo at day 20, along with previously defined subpopulations (NCSC, SMC, pigmented)[6].

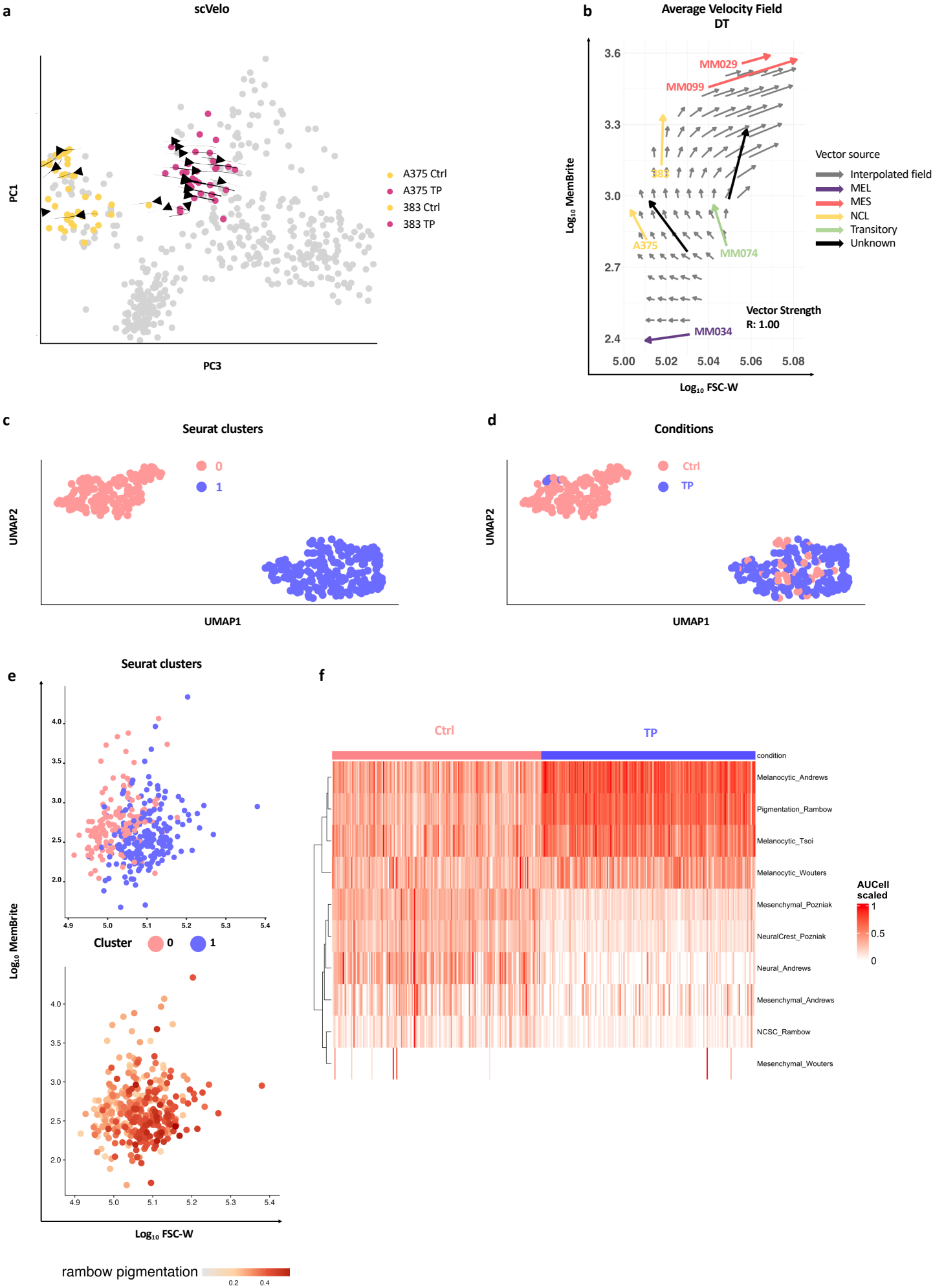
c, PC1-PC3 projection of 383 TP cells across untreated and treated melanoma scRNA-seq datasets.

d, Projection of the NCSC_Rambow signature[6].

e, PC1-PC3 projection of representative genes defining the NCSC transcriptional program as previously described[6].

f, Proteomic analysis showing enrichment of NCSC_Rambow associated markers in 383 TP cells compared to all other clusters.

g, S/V distributions of 383 TP and Mel006 cells treated with dabrafenib (40 nM) and trametinib (8 nM) (DT) for 4 days. N = 3 biological replicates.


a
scVelo
PC1
PC3
A375 Ctrl
A375 TP
383 Ctrl
383 TP
b
Average Velocity Field
DT
MM029
MM099
383
A375
MM074
MM034
Vector Strength
R: 1.00
Log10 MemBrite
Log10 FSC-W
Vector source
Interpolated field
MEL
MES
NCL
Transitory
Unknown
c
Seurat clusters
UMAP2
UMAP1
0
1
d
Conditions
Ctrl
TP
e
Seurat clusters
Cluster
rambow pigmentation
f
condition
Melanocytic_Andrews
Pigmentation_Rambow
Melanocytic_Tsoi
Melanocytic_Wouters
Mesenchymal_Pozniak
NeuralCrest_Pozniak
Neural_Andrews
Mesenchymal_Andrews
NCSC_Rambow
Mesenchymal_Wouters
AUCell scaled

Extended data Fig. 7. Predicting therapy-induced trajectories

a, scVelo analysis based on scRNA-seq data from A375 and 383 cell lines cultured under control or trametinib/palbociclib (TP) conditions for 7 days, projected onto PC1-PC3 space.

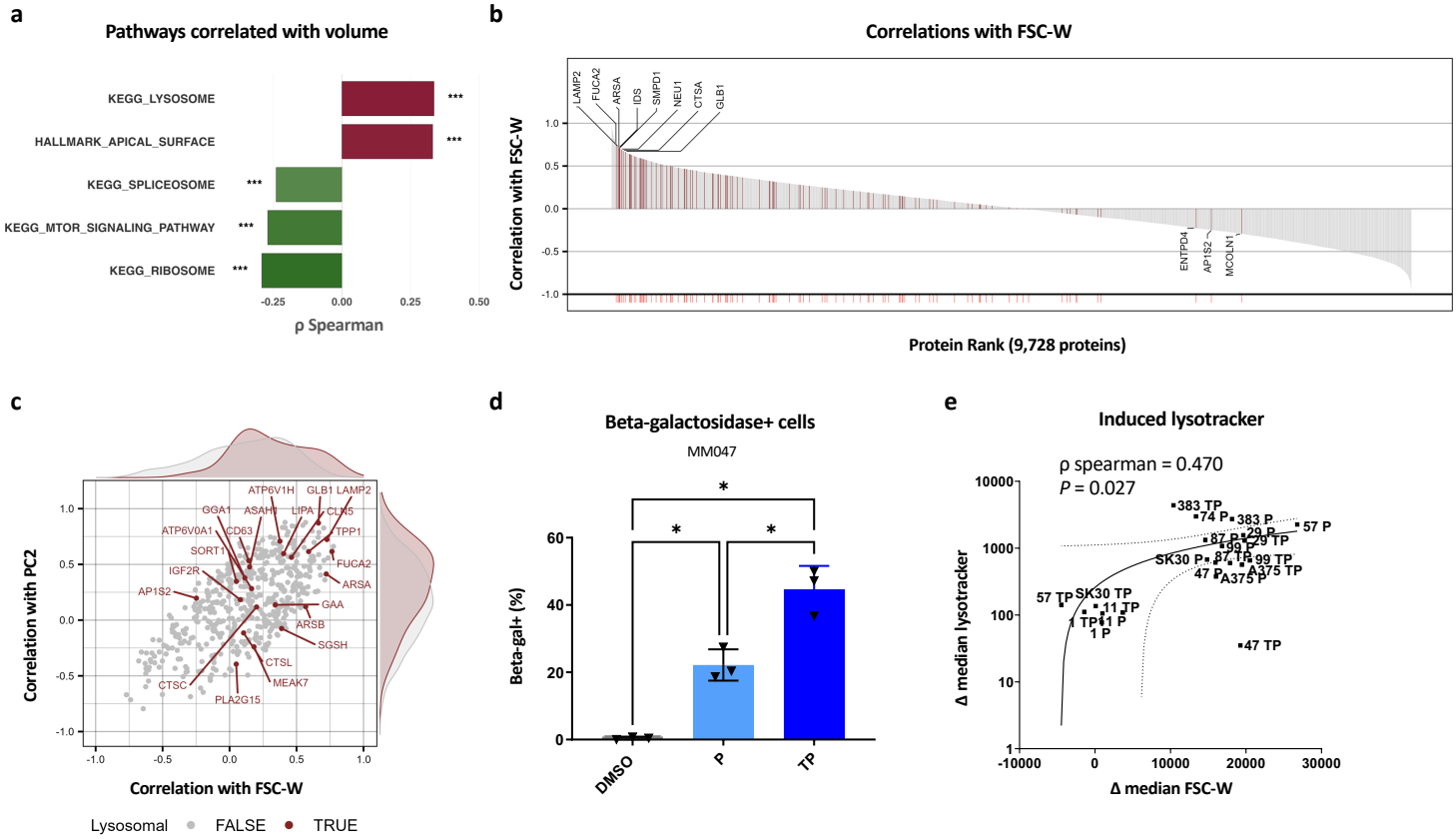
b, Average velocity field under dabrafenib/trametinib (DT; 20/100 nM) treatment for 3 days. Colored arrows represent reference cell lines, while grey arrows indicate interpolated trajectories inferred from reference S/V dynamics.

c, Seurat clustering analysis (clusters 0 and 1) projected onto UMAP1-UMAP2 space.

d, UMAP1-UMAP2 projection comparing control and TP-treated conditions.

e, Projection of the Rambow pigmentation signature within S/V space and across Seurat clusters.

f, Heatmap showing transcriptional signatures differentially activated or repressed between control and TP-treated cells.


a
Pathways correlated with volume
KEGG_LYSOSOME
HALLMARK_APICAL_SURFACE
KEGG_SPLICEOSOME
KEGG_MTOR_SIGNALING_PATHWAY
KEGG_RIBOSOME
***
-0.25
0.00
0.25
0.50
ρ Spearman
b
Correlations with FSC-W
Correlation with FSC-W
LAMP2
FUCA2
ARSA
IDS
SMPD1
NEU1
CTSA
GLB1
ENTPD4
AP1S2
MCOLN1
Protein Rank (9,728 proteins)
c
Correlation with PC2
Correlation with FSC-W
ATP6V1H
GLB1
LAMP2
GGA1
ASAH1
LIPA
CD45
ATP6V0A1
CD63
TPP1
SORT1
IGF2R
FUCA2
ARSA
AP1S2
GAA
ARSB
SGSH
CTSL
CTSC
MEAK7
PLA2G15
Lysosomal
FALSE
TRUE
d
Beta-galactosidase+ cells
MM047
Beta-gal+ (%)
DMSO
P
TP
e
Induced lysotracker
ρ spearman = 0.470
P = 0.027
Δ median lysotracker
Δ median FSC-W
f
MLSA1
% DAPI-neg
Ctrl
MLSA1
Nige
Nige MLSA1
g
MK6-83
% DAPI-neg
Ctrl
MK6-83
Nige
Nige MK6-83
h
SN-2
% DAPI-neg
Ctrl
SN-2
Nige
Nige SN-2

Extended Data Fig. 8. Targeting therapy-induced high-volume cells through modulation of lysosomal activity

a, ScRNA-seq data identifying top biological processes positively correlated with FSC-W.

b, Proteomic ranking of proteins correlated with FSC-W values; lysosomal proteins are highlighted in red.

c, Representation of lysosomal proteins showing correlation with both PC2 and FSC-W dimensions.

d, β-galactosidase activity in MM047 cells treated with palbociclib or trametinib/palbociclib for 7 days.

e, Correlation between changes in Lysotracker signal (Δ median Lysotracker) and corresponding changes in median FSC-W (Δ median FSC-W) under P or TP treatment.

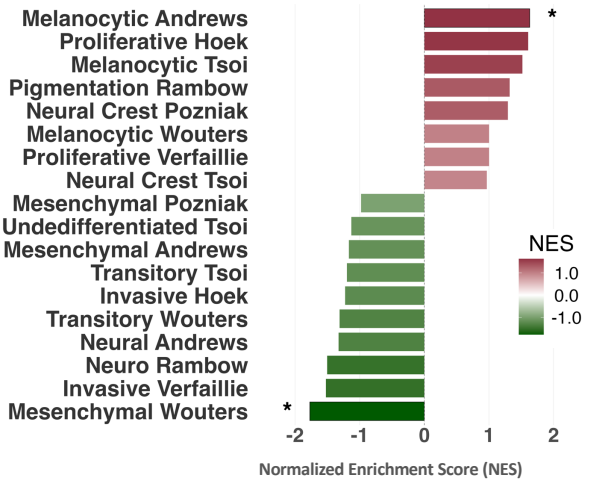
f, Rescue of nigericin-induced cell death (% DAP-negative cells) after 12 days of treatment with MLSA1, a TRPML1 agonist.

g, Rescue of nigericin-induced cell death (% DAP-negative cells) after 12 days of treatment with MK6-83, a TRPML1 agonist.

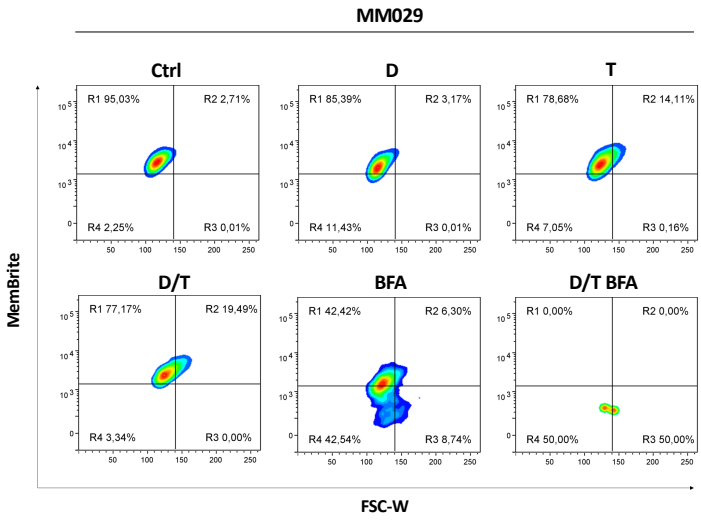
h, Rescue of nigericin-induced cell death (% DAP-negative cells) after 12 days of treatment with SN-2, a dual TRPML1/TRPML3 agonist.

Statistical analyses: Panel d,f-h: Welch's ANOVA followed by Dunnett's multiple comparison test.

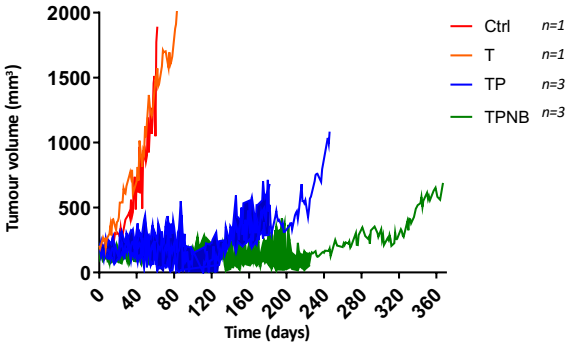
a


Pathways altered by tunicamycin in MES lines
Melanocytic Andrews
Proliferative Hoek
Melanocytic Tsoi
Pigmentation Rambow
Neural Crest Pozniak
Melanocytic Wouters
Proliferative Verfaillie
Neural Crest Tsoi
Mesenchymal Pozniak
Undedifferentiated Tsoi
Mesenchymal Andrews
Transitory Tsoi
Invasive Hoek
Transitory Wouters
Neural Andrews
Neuro Rambow
Invasive Verfaillie
Mesenchymal Wouters
*
*
NES
1.0
0.0
-1.0
-2
-1
0
1
2
Normalized Enrichment Score (NES)

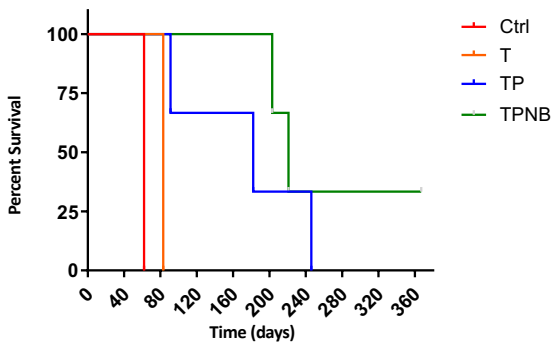
b


MM029
Ctrl
D
T
D/T
BFA
D/T BFA
R1 95,03%
R2 2,71%
R4 2,25%
R3 0,01%
R1 85,39%
R2 3,17%
R4 11,43%
R3 0,01%
R1 78,68%
R2 14,11%
R4 7,05%
R3 0,16%
R1 77,17%
R2 19,49%
R4 3,34%
R3 0,00%
R1 42,42%
R2 6,30%
R4 42,54%
R3 8,74%
R1 0,00%
R2 0,00%
R4 50,00%
R3 50,00%
MemBrite
FSC-W

c


MEL083 - Tumour volume
Tumour volume (mm³)
2000
1500
1000
500
0
0
40
80
120
160
200
240
280
320
360
Time (days)
Ctrl n=1
T n=1
TP n=3
TPNB n=3

d


MEL083 - Overall Survival
Percent Survival
100
75
50
25
0
0
40
80
120
160
200
240
280
320
360
Time (days)
Ctrl
T
TP
TPNB

Extended data Fig. 9. Exacerbating therapy responses by decreasing cell surface

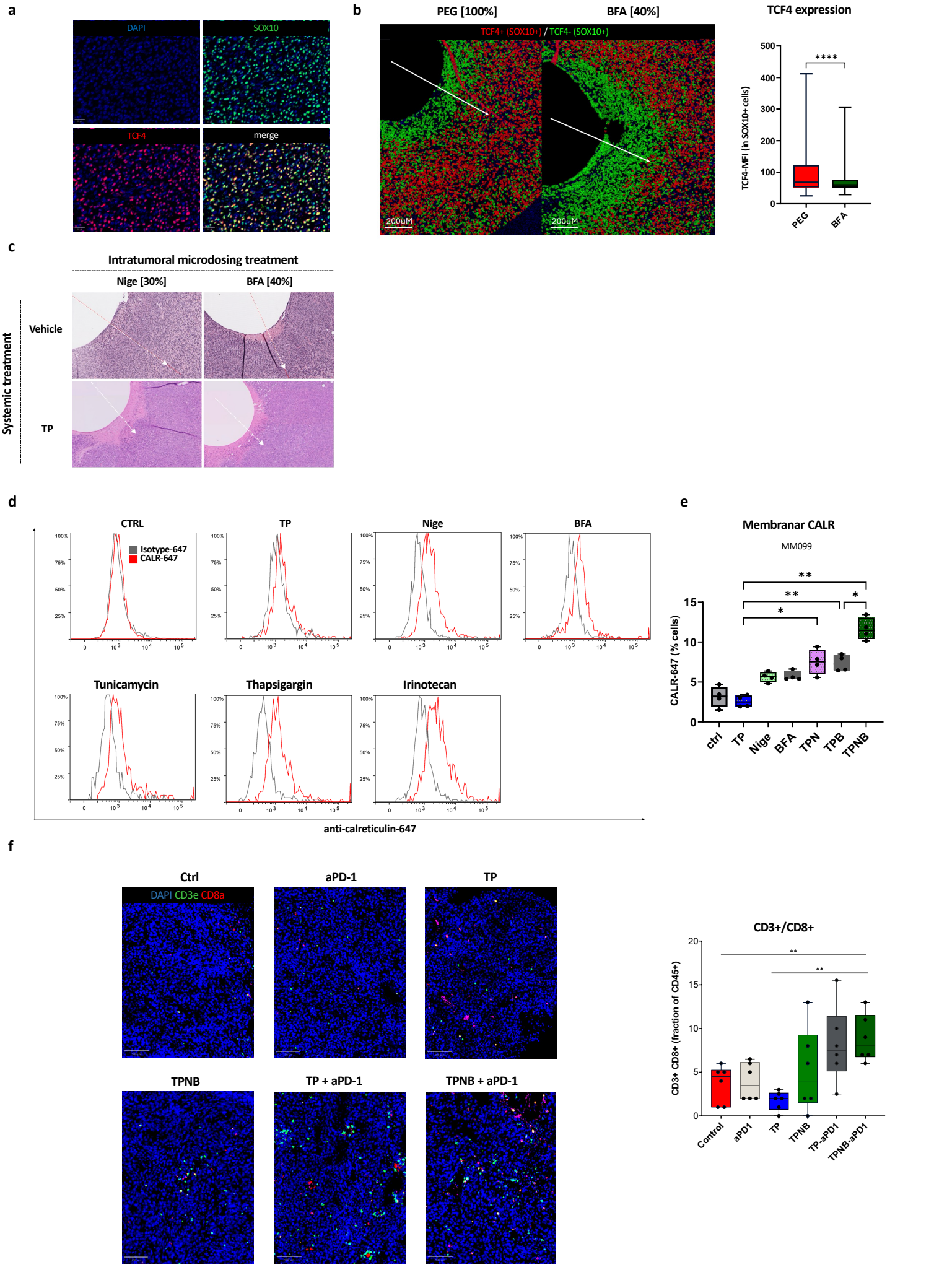
a, Bulk RNA-seq analysis showing up- and downregulated pathways across MES lines (MM029, MM047, MM099) following tunicamycin treatment (250 nM, 5 days).

b, Representative S/V flow cytometry plots of MM029 cells treated with dabrafenib (100 nM), trametinib (20 nM), BFA (25 nM), and in combinations at day 21.

c, Tumour growth kinetics of Mel083 PDX models treated with T, TP, or the TPNB combination (trametinib + palbociclib + nigericin + BFA). The experiment was terminated at day 367.

d, Overall survival of Mel083 PDX-bearing mice treated as in c (Log-rank test).

YUMM3.3 R^{TT}


a
DAPI
SOX10
TCF4
merge
b
PEG [100%]
BFA [40%]
TCF4+ (SOX10+) / TCF4- (SOX10+)
200uM
200uM
TCF4 expression
TCF4-MFI (in SOX10+ cells)
****
PEG
BFA
c
Intratumoral microdosing treatment
Nige [30%]
BFA [40%]
Systemic treatment
Vehicle
TP
d
CTRL
TP
Nige
BFA
Isotype-647
CALR-647
Tunicamycin
Thapsigargin
Irinotecan
anti-calreticulin-647
e
Membranar CALR
MM099
CALR-647 (% cells)
ctrl
TP
Nige
BFA
TPN
TPB
TPNB
f
Ctrl
aPD-1
TP
DAPI CD3e CD8a
TPNB
TP + aPD-1
TPNB + aPD-1
CD3+/CD8+
CD3+ CD8+ (fraction of CD45+)
Control
aPD1
TP
TPNB
TP-aPD1
TPNB-aPD1

Extended Data Fig. 10. TPNB promotes Immunogenic cell death

a, Immunofluorescence staining for SOX10 and TCF4 in the YUMM3.3 R^{TT} model.

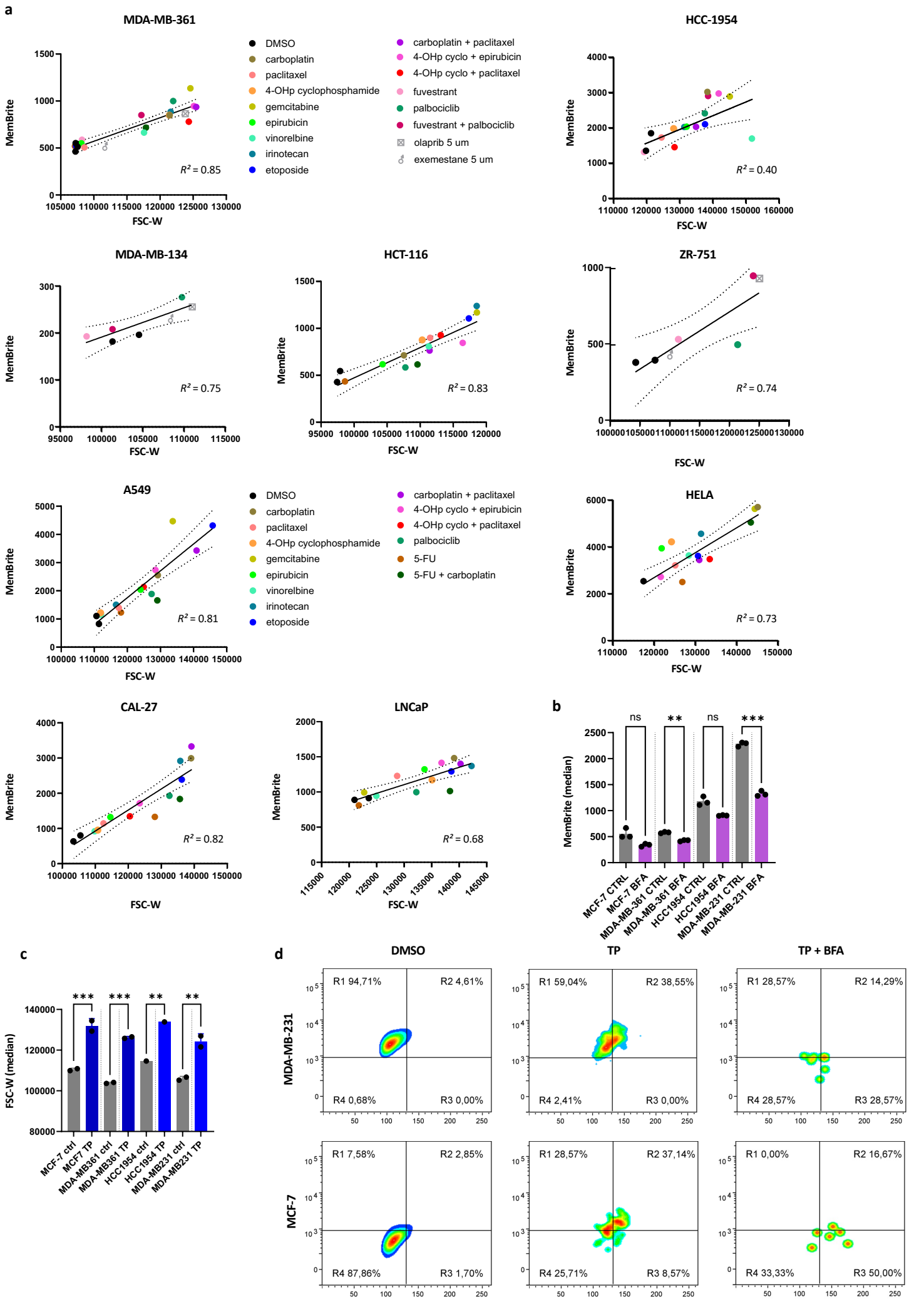
b,Quantification of TCF4 expression within $SOX10^+$ cells ($SOX10^+/TCF4^+$ and $SOX10^+/TCF4^-$) in the YUMM3.3 model treated or not with BFA. (two-tailed Welch's t-test).

c, Representative H&E staining images of YUMM3.3 R^{TT} tumours treated systemically with vehicule or TP and the indicated compounds via the IMD, and quantification of necrotic area (n = 2-6 reservoirs per condition).

d, Representative flow cytometry histograms showing membranar calreticulin exposure after 3 days of treatment with TP, nige, BFA, additional UPR inducers (tunicamycin, thapsigargin) and the positive immunogenic cell-death control irinotecan.

e, Quantification of calreticulin exposure at day 6 under the different drug treatments.

f, Representative immunofluorescence images showing $CD3^+/CD8^+$ T-cell infiltration under the indicated treatment conditions, analysed at humane endpoint (≈1000 mm^3), with corresponding quantifications.


a
MDA-MB-361
HCC-1954
MDA-MB-134
HCT-116
ZR-751
A549
HELA
CAL-27
LNCaP
MemBrite
FSC-W
R^2 = 0.85
R^2 = 0.40
R^2 = 0.75
R^2 = 0.83
R^2 = 0.74
R^2 = 0.81
R^2 = 0.73
R^2 = 0.82
R^2 = 0.68
DMSO
carboplatin
paclitaxel
4-OHp cyclophosphamide
gemcitabine
epirubicin
vinorelbine
irinotecan
etoposide
carboplatin + paclitaxel
4-OHp cyclo + epirubicin
4-OHp cyclo + paclitaxel
fuvestrant
palbociclib
fuvestrant + palbociclib
olaprib 5 um
exemestane 5 um
5-FU
5-FU + carboplatin
b
MemBrite (median)
ns
**
ns
***
MCF-7 CTRL
MCF-7 BFA
MDA-MB-361 CTRL
MDA-MB-361 BFA
HCC1954 CTRL
HCC1954 BFA
MDA-MB-231 CTRL
MDA-MB-231 BFA
c
FSC-W (median)
***
***
**
**
MCF-7 ctrl
MCF7 TP
MDA-MB361 ctrl
MDA-MB361 TP
HCC1954 ctrl
HCC1954 TP
MDA-MB231 ctrl
MDA-MB231 TP
d
DMSO
TP
TP + BFA
MDA-MB-231
MCF-7
R1 94,71%
R2 4,61%
R4 0,68%
R3 0,00%
R1 59,04%
R2 38,55%
R4 2,41%
R3 0,00%
R1 28,57%
R2 14,29%
R4 28,57%
R3 28,57%
R1 7,58%
R2 2,85%
R4 87,86%
R3 1,70%
R1 28,57%
R2 37,14%
R4 25,71%
R3 8,57%
R1 0,00%
R2 16,67%
R4 33,33%
R3 50,00%

Extended data Fig. 11. Chemotherapy rewires S/V in a panel of cancer cell lines

a, Modulation of S/V by standard-of-care chemotherapies across multiple human cancer cell lines, including MDA-MB-361, HCC-1954, MDA-MB-134, HCT116, ZR-751, A549, HeLa, CAL27, and LNCaP. Spearman's ρ and linear regressions are shown.

b, Changes in MemBrite intensity following chemotherapy treatment in MCF-7, MDA-MB-361, HCC-1954, and MDA-MB-231 cell lines.

c, Corresponding modulation of FSC-W under the same conditions as in b.

d, Representative S/V plots of MDA-MB-231 and MCF-7 cells treated with TP (10 nM/375 nM) and BFA (25 nM)

Statistical analyses: Panels b-c: One-way ANOVA followed by multiple-comparison tests (****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$).