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Supplemental information

Charting oncogenicity of genes and variants across lineages via multiplexed screens in teratomas

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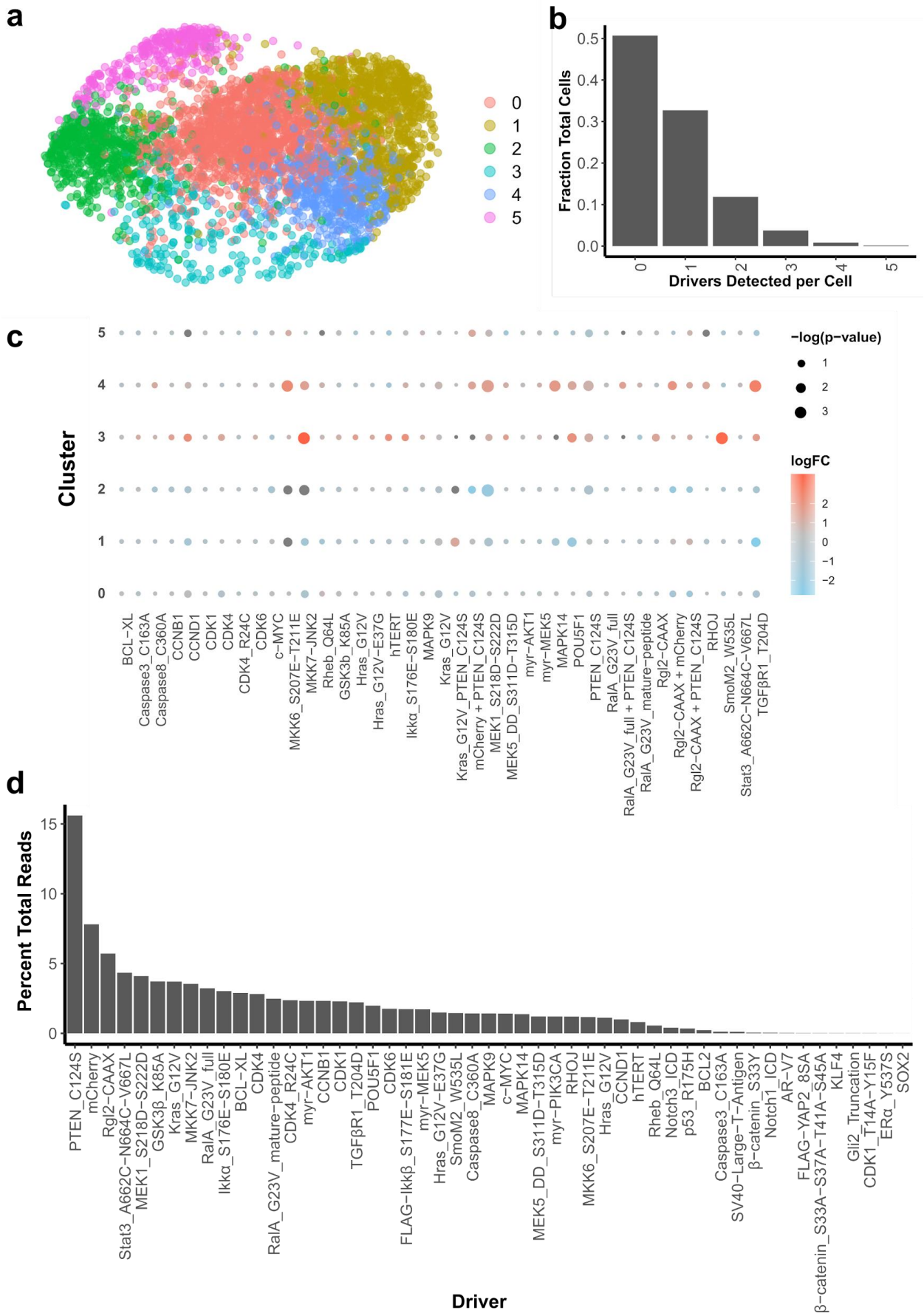
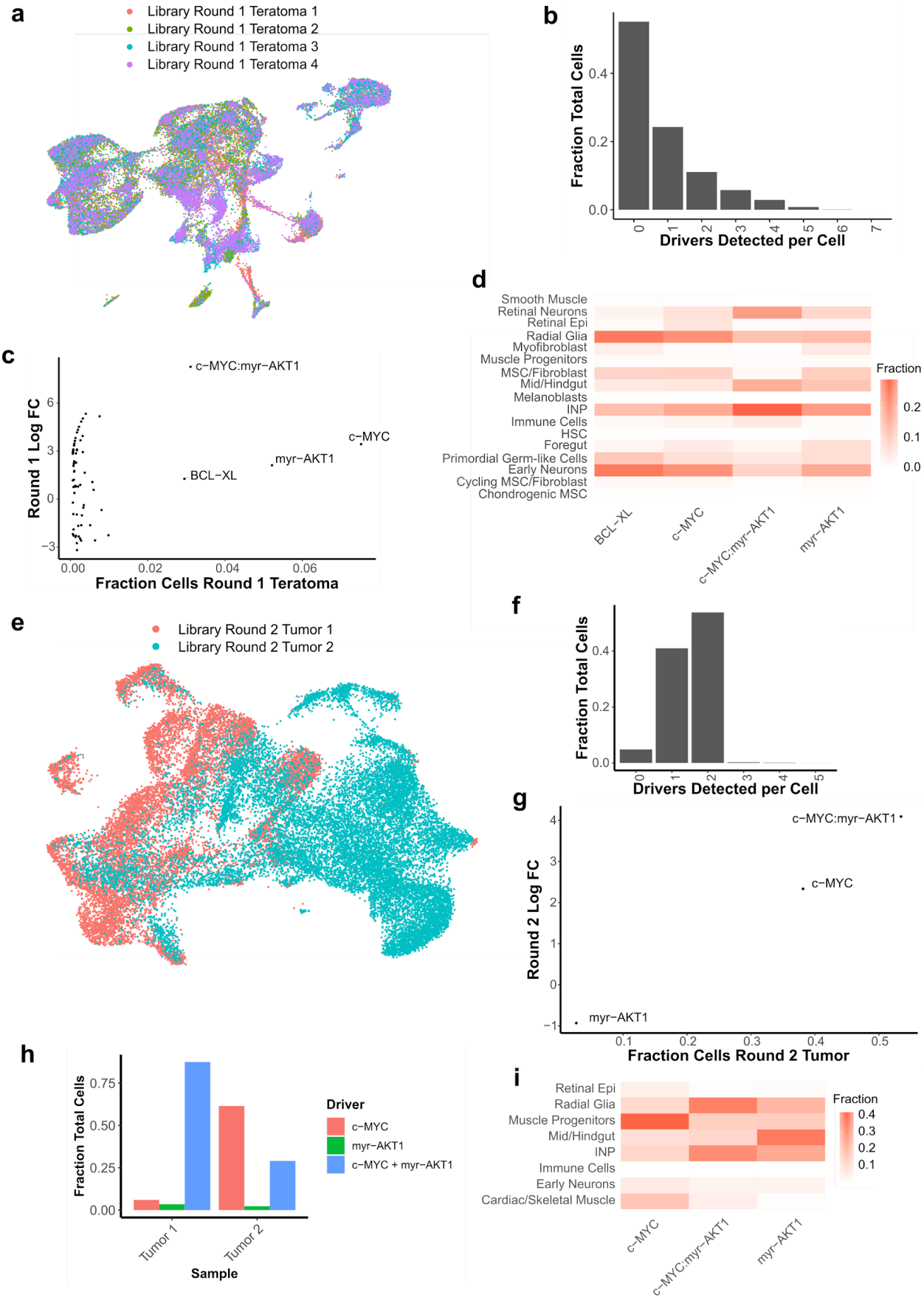
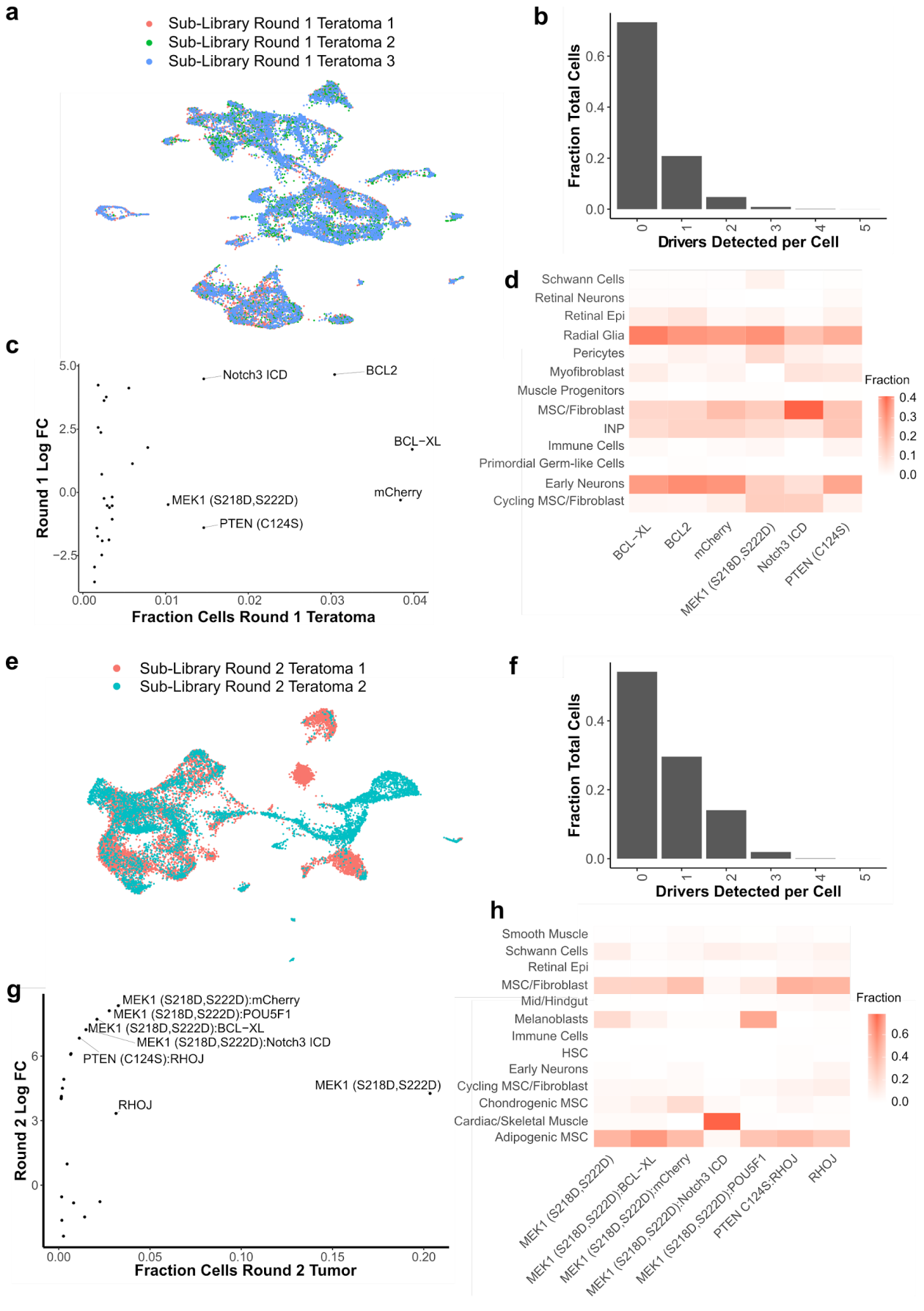

a
0
1
2
3
4
5
b
Fraction Total Cells
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
Drivers Detected per Cell
c
Cluster
5
4
3
2
1
0
−log(p−value)
1
2
3
logFC
2
1
0
−1
−2
BCL−XL
Caspase3_C163A
Caspase8_C360A
CCNB1
CCND1
CDK1
CDK4
CDK4_R24C
CDK6
c−MYC
MKK6_S207E−T211E
MKK7−JNK2
Rheb_Q64L
GSK3b_K85A
Hras_G12V
Hras_G12V−E37G
hTERT
Ikkα_S176E−S180E
MAPK9
Kras_G12V
Kras_G12V_PTEN_C124S
mCherry + PTEN_C124S
MEK1_S218D−S222D
MEK5_DD_S311D−T315D
myr−AKT1
myr−MEK5
MAPK14
POU5F1
PTEN_C124S
RalA_G23V_full
RalA_G23V_full + PTEN_C124S
RalA_G23V_mature−peptide
Rgl2−CAAX
Rgl2−CAAX + mCherry
Rgl2−CAAX + PTEN_C124S
RHOJ
SmoM2_W535L
Stat3_A662C−N664C−V667L
TGFβR1_T204D
d
Percent Total Reads
15
10
5
0
PTEN_C124S
mCherry
Rgl2−CAAX
Stat3_A662C−N664C−V667L
MEK1_S218D−S222D
GSK3β_K85A
Kras_G12V
MKK7−JNK2
RalA_G23V_full
Ikkα_S176E−S180E
BCL−XL
CDK4
RalA_G23V_mature−peptide
CDK4_R24C
myr−AKT1
CCNB1
CDK1
TGFβR1_T204D
POU5F1
CDK6
FLAG−Ikkβ_S177E−S181E
myr−MEK5
Hras_G12V−E37G
SmoM2_W535L
Caspase8_C360A
MAPK9
c−MYC
MAPK14
MEK5_DD_S311D−T315D
myr−PIK3CA
RHOJ
MKK6_S207E−T211E
Hras_G12V
CCND1
hTERT
Rheb_Q64L
Notch3_ICD
p53_R175H
BCL2
Caspase3_C163A
SV40−Large−T−Antigen
β−catenin_S33Y
Notch1_ICD
AR−V7
FLAG−YAP2_8SA
β−catenin_S33A−S37A−T41A−S45A
KLF4
Gli2_Truncation
CDK1_T14A−Y15F
ERα_Y537S
SOX2
Driver

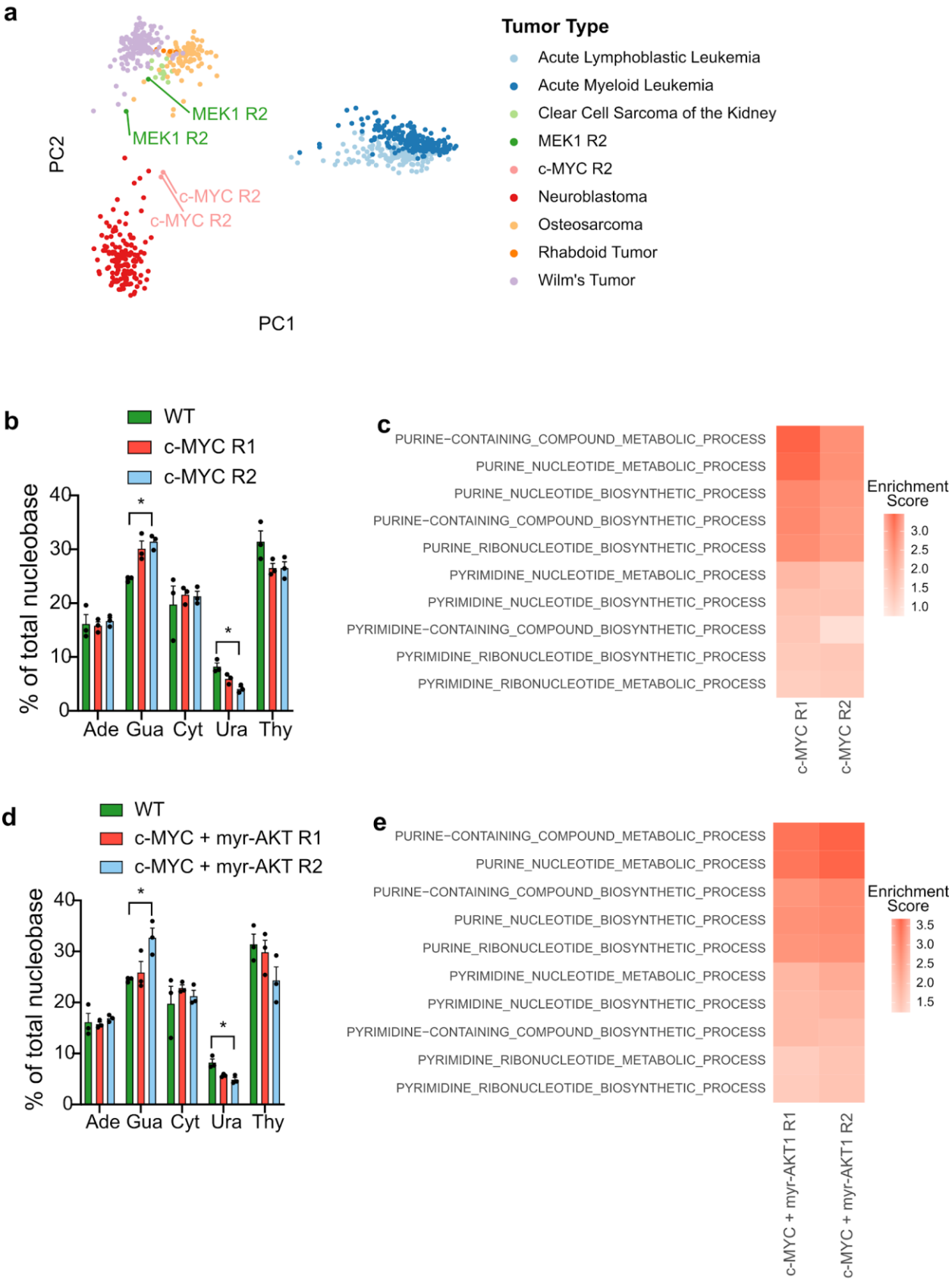
Supplementary Figure 1: Characterization of driver library transduced cells prior to injection, Related to Figure 1. (**a**) UMAP visualization of cells labelled by cluster identity. (**b**) Fraction of cells for each number of drivers detected in scRNA-seq for driver library transduced H1 hESCs. (**c**) Log2 fold change and significance of proportion of cells in a cluster expressing a driver versus proportion expressing the internal control (mCherry), calculated by permutation testing. Only drivers expressed in 20 or more cells are included. (**d**) Percentage of reads detecting each library element for barcodes amplified from genomic DNA extracted from driver-library transduced H1 hESCs prior to injection.


a
Library Round 1 Teratoma 1
Library Round 1 Teratoma 2
Library Round 1 Teratoma 3
Library Round 1 Teratoma 4
b
Fraction Total Cells
0.0
0.2
0.4
0 1 2 3 4 5 6 7
Drivers Detected per Cell
c
c−MYC:myr−AKT1
c−MYC
myr−AKT1
BCL−XL
Round 1 Log FC
6
3
0
−3
0.00 0.02 0.04 0.06
Fraction Cells Round 1 Teratoma
d
Smooth Muscle
Retinal Neurons
Retinal Epi
Radial Glia
Myofibroblast
Muscle Progenitors
MSC/Fibroblast
Mid/Hindgut
Melanoblasts
INP
Immune Cells
HSC
Foregut
Primordial Germ-like Cells
Early Neurons
Cycling MSC/Fibroblast
Chondrogenic MSC
Fraction
0.2
0.1
0.0
BCL−XL
c−MYC
c−MYC:myr−AKT1
myr−AKT1
e
Library Round 2 Tumor 1
Library Round 2 Tumor 2
f
Fraction Total Cells
0.0
0.2
0.4
0 1 2 3 4 5
Drivers Detected per Cell
g
c−MYC:myr−AKT1
c−MYC
myr−AKT1
Round 2 Log FC
4
3
2
1
0
−1
0.1 0.2 0.3 0.4 0.5
Fraction Cells Round 2 Tumor
h
Fraction Total Cells
0.00
0.25
0.50
0.75
Tumor 1
Tumor 2
Sample
Driver
c−MYC
myr−AKT1
c−MYC + myr−AKT1
i
Retinal Epi
Radial Glia
Muscle Progenitors
Mid/Hindgut
INP
Immune Cells
Early Neurons
Cardiac/Skeletal Muscle
Fraction
0.4
0.3
0.2
0.1
c−MYC
c−MYC:myr−AKT1
myr−AKT1

Supplementary Figure 2: Characterization of driver library screen tumors, Related to Figure 2. (**a**) UMAP visualization of cells by sample from round 1 teratomas formed by driver library transduced hESCs. (**b**) Fraction of cells for each number of drivers detected in driver library round 1 teratomas. (**c**) Scatterplot of log fold change of drivers in round 1 teratomas over pre-injection cells, versus fraction of cells in round 1 teratomas for each driver. Only those drivers detected in at least 25 cells across all 4 round 1 teratomas are plotted. Those drivers detected in at least 1% of round 1 teratoma cells are annotated. (**d**) Heatmap of fraction of cells of each type detected for top detected drivers in round 1 teratomas. (**e**) UMAP visualization of cells by sample from round 1 teratomas formed by driver library transduced hESCs. (**f**) Fraction of cells for each number of drivers detected in driver library round 2 tumors. (**g**) Top enriched hits for each round 2 tumor sample. (**h**) Scatterplot of log fold change of drivers in round 2 tumors over round 1 teratomas, versus fraction of cells in round 2 tumors for each driver. Only those drivers detected in at least 25 cells across both round 2 tumors are plotted. Those drivers detected in at least 1% of round 2 tumor cells are annotated. (**i**) Heatmap of fraction of cells of each type detected for top detected drivers in round 2 tumors.


a
Sub-Library Round 1 Teratoma 1
Sub-Library Round 1 Teratoma 2
Sub-Library Round 1 Teratoma 3
b
Fraction Total Cells
0.0
0.2
0.4
0.6
0 1 2 3 4 5
Drivers Detected per Cell
c
Round 1 Log FC
5.0
2.5
0.0
−2.5
Notch3 ICD
BCL2
BCL−XL
mCherry
MEK1 (S218D,S222D)
PTEN (C124S)
0.00 0.01 0.02 0.03 0.04
Fraction Cells Round 1 Teratoma
d
Schwann Cells
Retinal Neurons
Retinal Epi
Radial Glia
Pericytes
Myofibroblast
Muscle Progenitors
MSC/Fibroblast
INP
Immune Cells
Primordial Germ-like Cells
Early Neurons
Cycling MSC/Fibroblast
Fraction
0.0
0.1
0.2
0.3
0.4
BCL−XL
BCL2
mCherry
MEK1 (S218D,S222D)
Notch3 ICD
PTEN (C124S)
e
Sub-Library Round 2 Teratoma 1
Sub-Library Round 2 Teratoma 2
f
Fraction Total Cells
0.0
0.2
0.4
0 1 2 3 4 5
Drivers Detected per Cell
g
Round 2 Log FC
6
3
0
MEK1 (S218D,S222D):mCherry
MEK1 (S218D,S222D):POU5F1
MEK1 (S218D,S222D):BCL−XL
MEK1 (S218D,S222D):Notch3 ICD
PTEN (C124S):RHOJ
MEK1 (S218D,S222D)
RHOJ
0.00 0.05 0.10 0.15 0.20
Fraction Cells Round 2 Tumor
h
Smooth Muscle
Schwann Cells
Retinal Epi
MSC/Fibroblast
Mid/Hindgut
Melanoblasts
Immune Cells
HSC
Early Neurons
Cycling MSC/Fibroblast
Chondrogenic MSC
Cardiac/Skeletal Muscle
Adipogenic MSC
Fraction
0.0
0.2
0.4
0.6
MEK1 (S218D,S222D)
MEK1 (S218D,S222D):BCL−XL
MEK1 (S218D,S222D):mCherry
MEK1 (S218D,S222D):Notch3 ICD
MEK1 (S218D,S222D):POU5F1
PTEN C124S:RHOJ
RHOJ

Supplementary Figure 3: **Characterization of driver sub-library screen tumors, Related to Figure 3.** (**a**) UMAP visualization of cells by sample from round 1 teratomas formed by driver sub-library transduced hESCs. (**b**) Fraction of cells for each number of drivers detected in driver sub-library round 1 teratomas. (**c**) Scatterplot of log fold change of drivers in round 1 sub-library teratomas over pre-injection cells, versus fraction of cells in round 1 teratomas for each driver. Only those drivers detected in at least 25 cells across all 3 round 1 sub-library teratomas are plotted. Those drivers detected in at least 1% of round 1 teratoma cells are annotated. (**d**) Heatmap of fraction of cells of each type detected for top detected drivers in round 1 teratomas. (**e**) UMAP visualization of cells by sample from round 1 teratomas formed by driver sub-library transduced hESCs. (**f**) Fraction of cells for each number of drivers detected in driver sub-library round 2 tumors. (**g**) Scatterplot of log fold change of drivers in round 2 tumors over round 1 teratomas, versus fraction of cells in round 2 tumors for each driver. Only those drivers detected in at least 25 cells across both round 2 tumors are plotted. Those drivers detected in at least 1% of round 2 tumor cells are annotated. (**h**) Heatmap of fraction of cells of each type detected for top detected drivers in round 2 tumors.


a
Tumor Type
Acute Lymphoblastic Leukemia
Acute Myeloid Leukemia
Clear Cell Sarcoma of the Kidney
MEK1 R2
c-MYC R2
Neuroblastoma
Osteosarcoma
Rhabdoid Tumor
Wilm's Tumor
MEK1 R2
MEK1 R2
c-MYC R2
c-MYC R2
PC1
PC2
b
WT
c-MYC R1
c-MYC R2
% of total nucleobase
Ade Gua Cyt Ura Thy
c
PURINE−CONTAINING_COMPOUND_METABOLIC_PROCESS
PURINE_NUCLEOTIDE_METABOLIC_PROCESS
PURINE_NUCLEOTIDE_BIOSYNTHETIC_PROCESS
PURINE−CONTAINING_COMPOUND_BIOSYNTHETIC_PROCESS
PURINE_RIBONUCLEOTIDE_BIOSYNTHETIC_PROCESS
PYRIMIDINE_NUCLEOTIDE_METABOLIC_PROCESS
PYRIMIDINE_NUCLEOTIDE_BIOSYNTHETIC_PROCESS
PYRIMIDINE−CONTAINING_COMPOUND_BIOSYNTHETIC_PROCESS
PYRIMIDINE_RIBONUCLEOTIDE_BIOSYNTHETIC_PROCESS
PYRIMIDINE_RIBONUCLEOTIDE_METABOLIC_PROCESS
c-MYC R1
c-MYC R2
Enrichment Score
d
WT
c-MYC + myr-AKT R1
c-MYC + myr-AKT R2
% of total nucleobase
Ade Gua Cyt Ura Thy
e
PURINE−CONTAINING_COMPOUND_METABOLIC_PROCESS
PURINE_NUCLEOTIDE_METABOLIC_PROCESS
PURINE−CONTAINING_COMPOUND_BIOSYNTHETIC_PROCESS
PURINE_NUCLEOTIDE_BIOSYNTHETIC_PROCESS
PURINE_RIBONUCLEOTIDE_BIOSYNTHETIC_PROCESS
PYRIMIDINE_NUCLEOTIDE_METABOLIC_PROCESS
PYRIMIDINE_NUCLEOTIDE_BIOSYNTHETIC_PROCESS
PYRIMIDINE−CONTAINING_COMPOUND_BIOSYNTHETIC_PROCESS
PYRIMIDINE_RIBONUCLEOTIDE_METABOLIC_PROCESS
PYRIMIDINE_RIBONUCLEOTIDE_BIOSYNTHETIC_PROCESS
c-MYC + myr-AKT1 R1
c-MYC + myr-AKT1 R2
Enrichment Score

Supplementary Figure 4: **Metabolic Effects of *c-MYC* and *c-MYC + myr-AKT1*, Related to Figure 4.** (**a**) PC1 vs PC2 plot of *c-MYC* and *MEK1*$^{S218/S222D}$ driven tumors with pediatric tumors from the TARGET initiative. (**b**) Nucleobase abundance in Control (Wild Type) teratomas, *c-MYC* driven Round 1 tumors and *c-MYC* driven Round 2 tumors. Data represent the mean ± s.d (*p ≤ 0.05) (**c**) Heatmap of enrichment scores for Gene Ontology genesets related to purine and pyrimidine nucleotide metabolism and synthesis for *c-MYC* driven Round 1 and Round 2 tumors compared to wild type teratomas. (**d**) Nucleobase abundance in Control (Wild Type) teratomas, *c-MYC* + *myr-AKT1* driven Round 1 tumors and *c-MYC* + *myr-AKT1* driven Round 2 tumors. Data represent the mean ± s.d (*p ≤ 0.05) (**e**) Heatmap of enrichment scores for Gene Ontology genesets related to purine and pyrimidine nucleotide metabolism and synthesis for *c-MYC* + *myr-AKT1* driven Round 1 and Round 2 tumors compared to wild type teratomas.