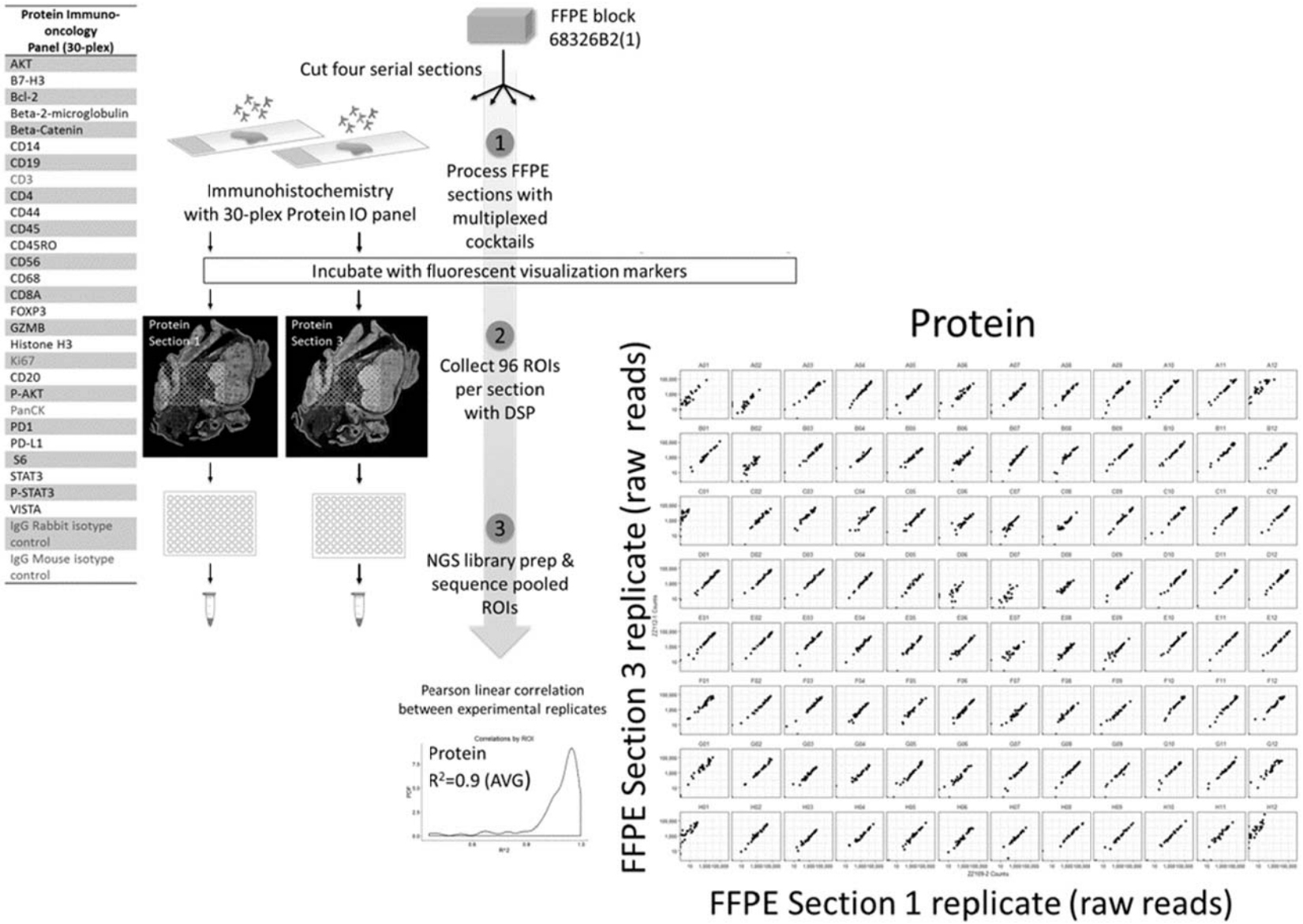
Protein Immuno-oncology Panel (30-plex)
AKT
B7-H3
Bcl-2
Beta-2-microglobulin
Beta-Catenin
CD14
CD19
CD3
CD4
CD44
CD45
CD45RO
CD56
CD68
CD8A
FOXP3
GZMB
Histone H3
Ki67
CD20
P-AKT
PanCK
PD1
PD-L1
S6
STAT3
P-STAT3
VISTA
IgG Rabbit isotype control
IgG Mouse isotype control
FFPE block
68326B2(1)
Cut four serial sections
1
Process FFPE sections with multiplexed cocktails
Immunohistochemistry with 30-plex Protein IO panel
Incubate with fluorescent visualization markers
Protein Section 1
Protein Section 3
2
Collect 96 ROIs per section with DSP
3
NGS library prep & sequence pooled ROIs
Pearson linear correlation between experimental replicates
Protein
R2=0.9 (AVG)
Protein
FFPE Section 3 replicate (raw reads)
FFPE Section 1 replicate (raw reads)

Figure 21

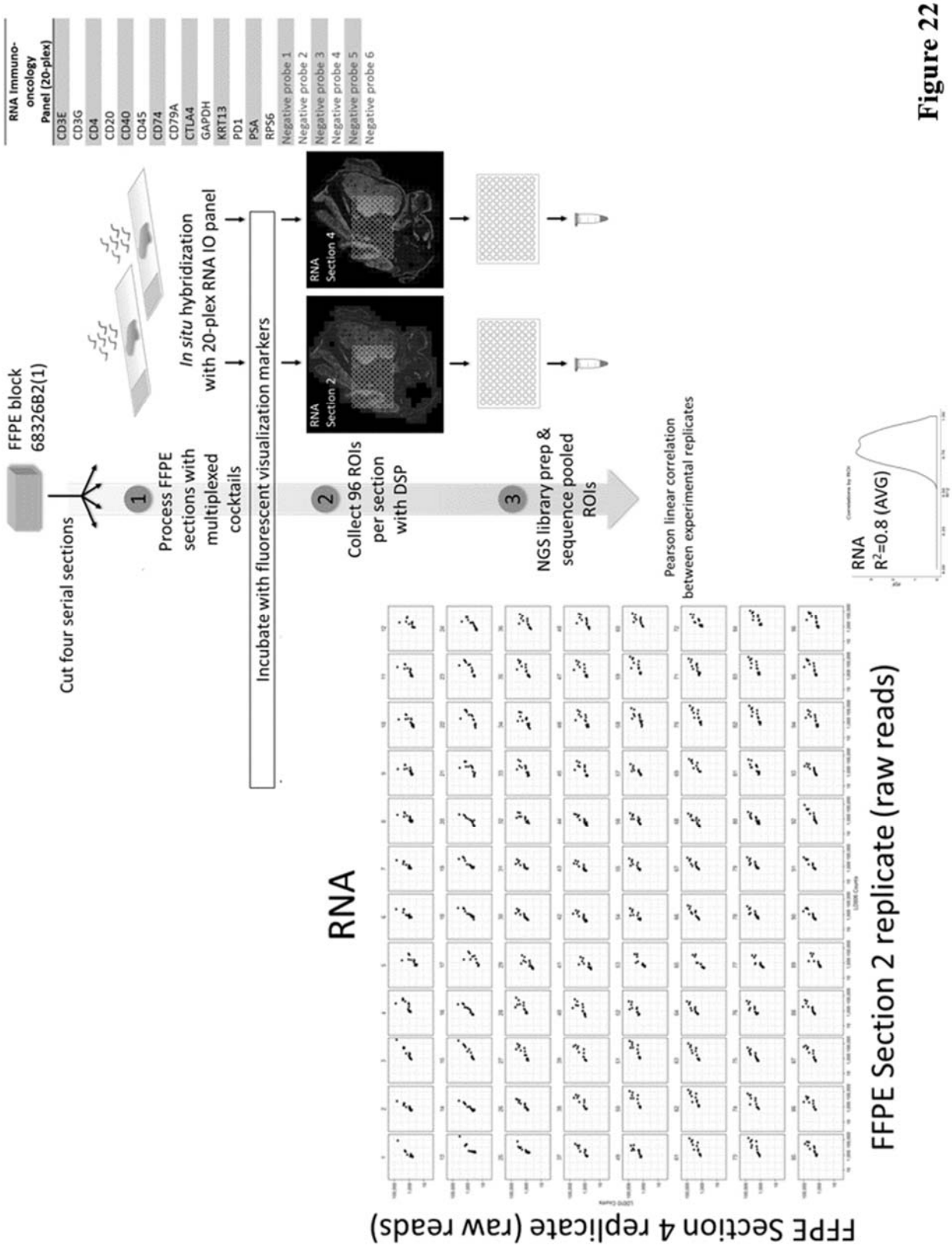
RNA Immuno-oncology Panel (20-plex)
CD3E
CD3G
CD4
CD20
CD40
CD45
CD74
CD79A
CTLA4
GAPDH
KRT13
PD1
PSA
RPS6
Negative probe 1
Negative probe 2
Negative probe 3
Negative probe 4
Negative probe 5
Negative probe 6
FFPE block 68326B2(1)
Cut four serial sections
1
Process FFPE sections with multiplexed cocktails
In situ hybridization with 20-plex RNA IO panel
Incubate with fluorescent visualization markers
2
Collect 96 ROIs per section with DSP
RNA Section 2
RNA Section 4
3
NGS library prep & sequence pooled ROIs
Pearson linear correlation between experimental replicates
RNA
R²=0.8 (AVG)
RNA
FFPE Section 2 replicate (raw reads)
FFPE Section 4 replicate (raw reads)

Figure 22

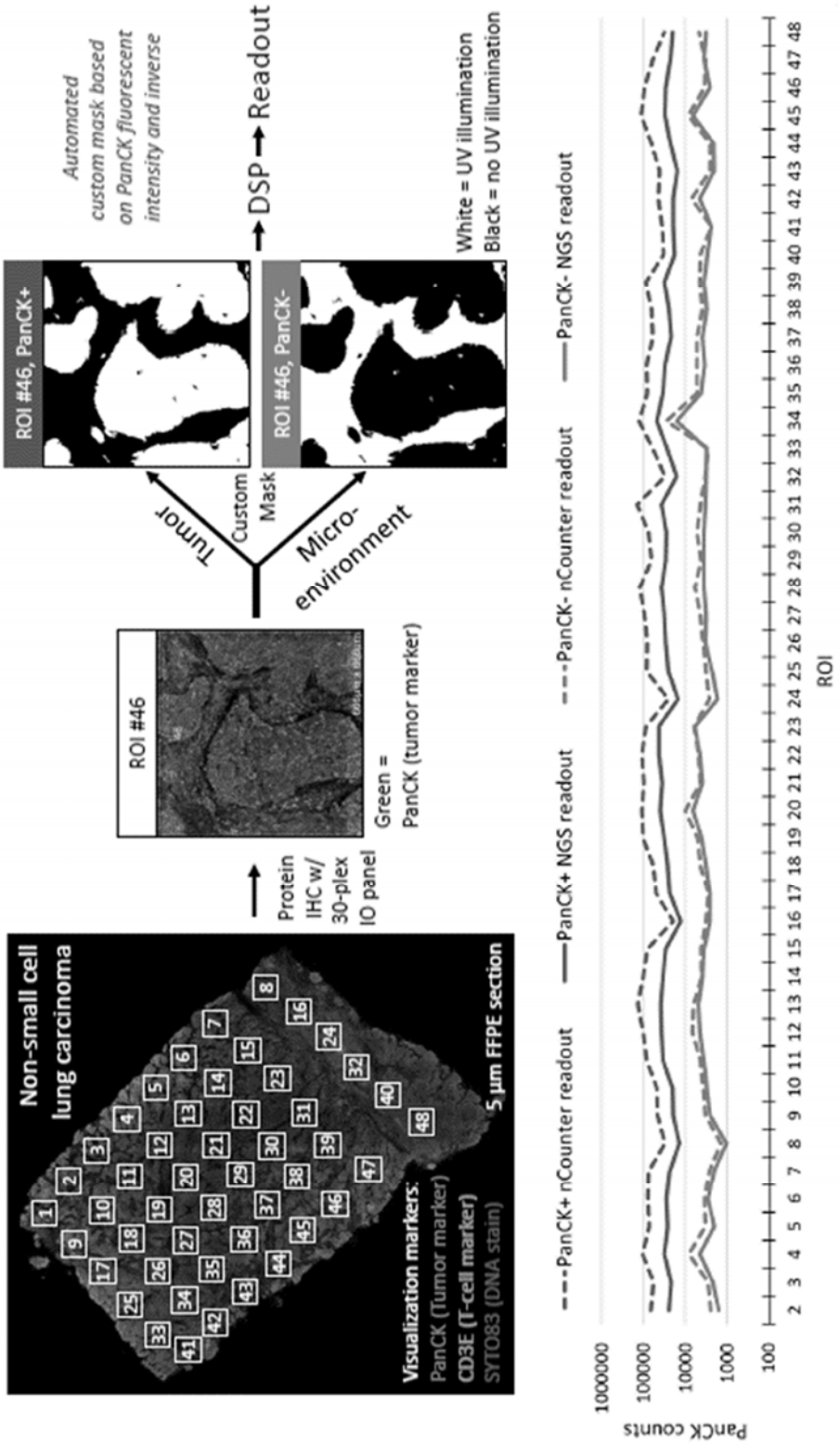
Non-small cell lung carcinoma
5 µm FFPE section
Visualization markers:
PanCK (Tumor marker)
CD3E (T-cell marker)
SYTO83 (DNA stain)
Protein IHC w/ 30-plex IO panel
ROI #46
Green = PanCK (tumor marker)
Tumor
Custom Mask
Micro-environment
ROI #46, PanCK+
ROI #46, PanCK-
Automated custom mask based on PanCK fluorescent intensity and inverse
DSP → Readout
White = UV illumination
Black = no UV illumination
PanCK+ nCounter readout
PanCK+ NGS readout
PanCK- nCounter readout
PanCK- NGS readout
PanCK counts
1000000
100000
10000
1000
100
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48
ROI

Figure 25

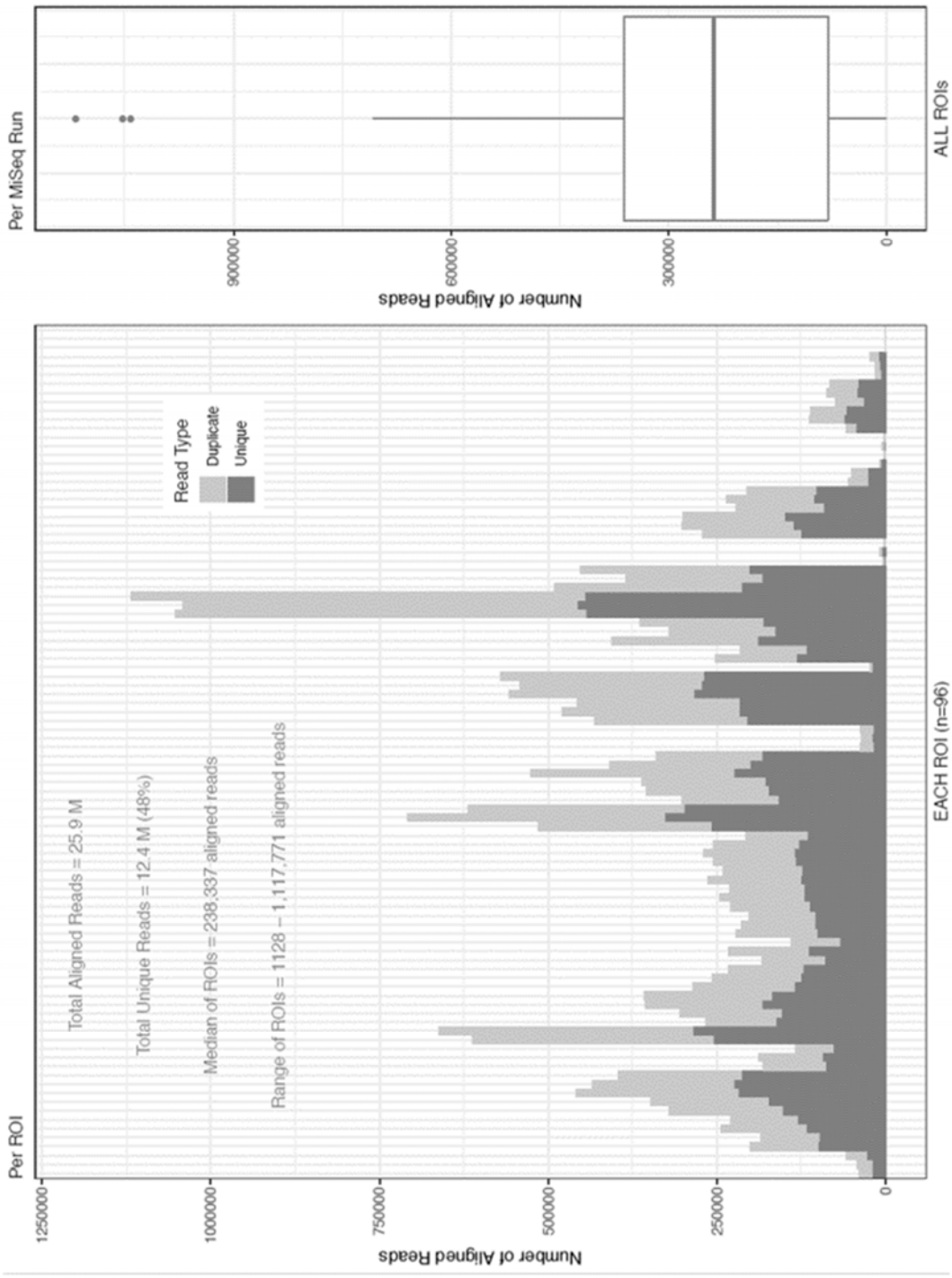
Per MiSeq Run
Number of Aligned Reads
900000
600000
300000
0
ALL ROIs
Per ROI
Number of Aligned Reads
1250000
1000000
750000
500000
250000
0
Total Aligned Reads = 25.9 M
Total Unique Reads = 12.4 M (48%)
Median of ROIs = 238,337 aligned reads
Range of ROIs = 1128 – 1,117,771 aligned reads
Read Type
Duplicate
Unique
EACH ROI (n=96)

Figure 29

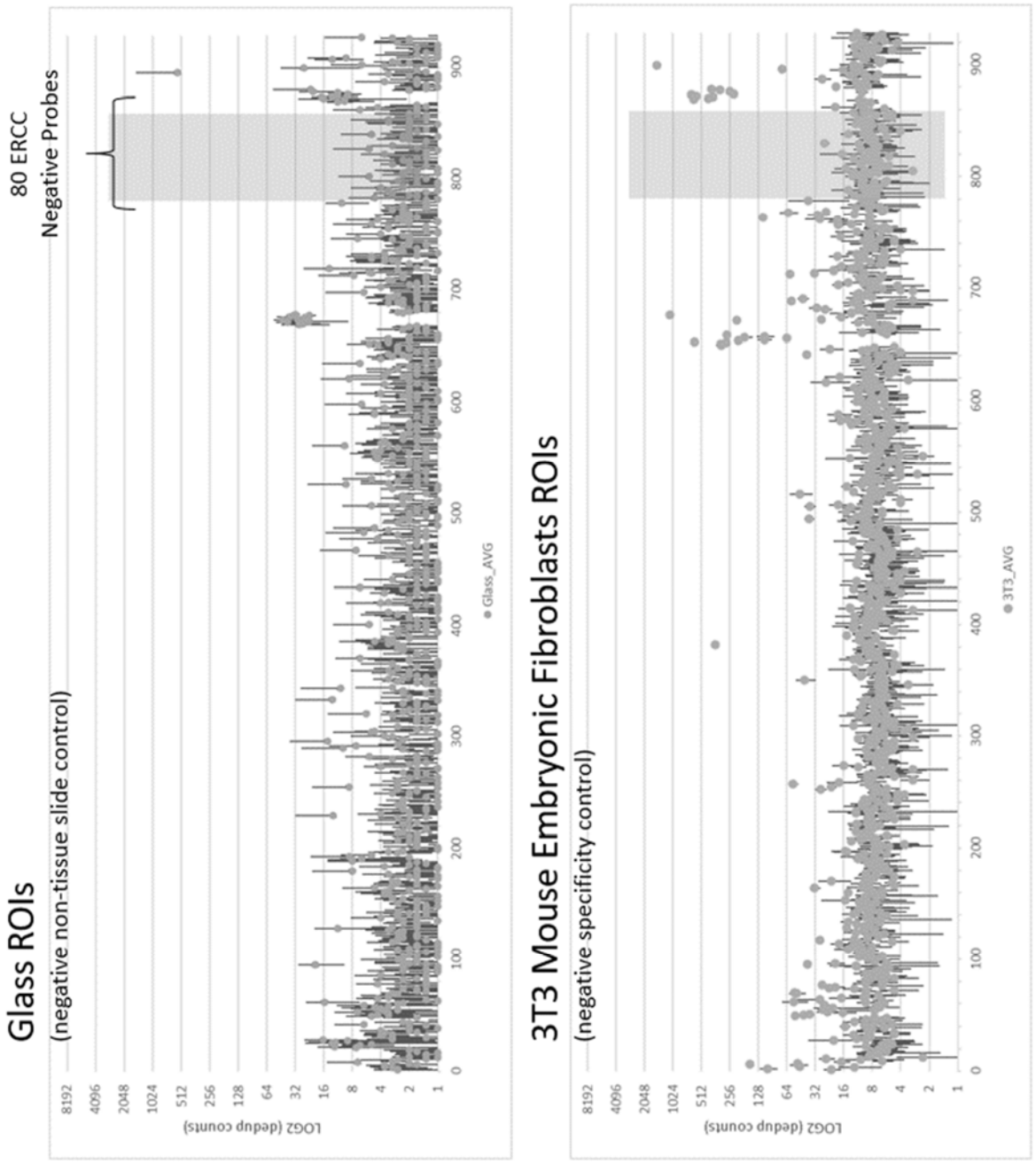
Glass ROIs
(negative non-tissue slide control)
80 ERCC
Negative Probes
LOG2 (dedup counts)
8192
4096
2048
1024
512
256
128
64
32
16
8
4
2
1
0
100
200
300
400
500
600
700
800
900
Glass_AVG
3T3 Mouse Embryonic Fibroblasts ROIs
(negative specificity control)
3T3_AVG

Figure 30

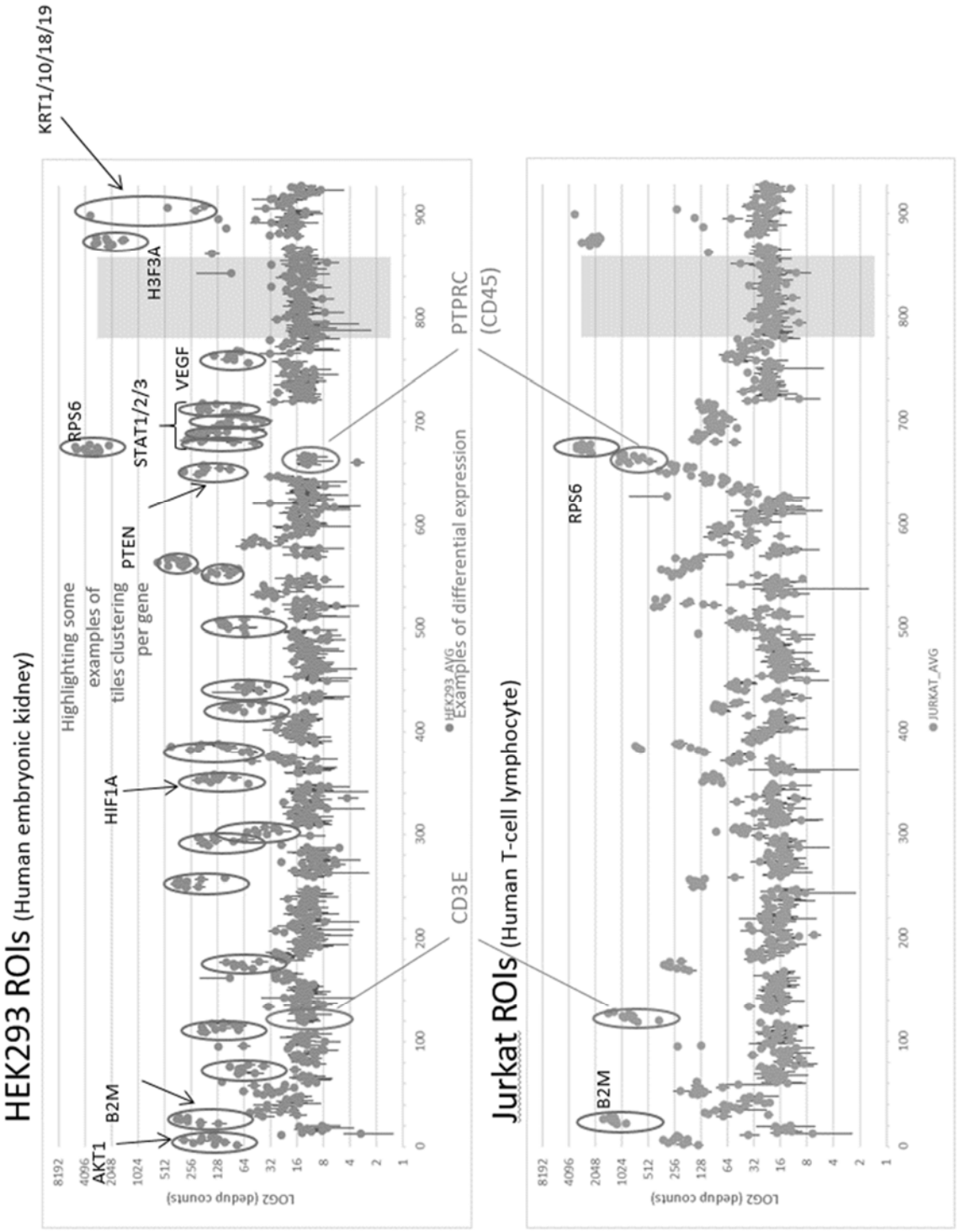
HEK293 ROIs (Human embryonic kidney)
KRT1/10/18/19
Highlighting some examples of tiles clustering per gene
HIF1A
B2M
AKT1
PTEN
STAT1/2/3
VEGF
RPS6
H3F3A
CD3E
PTPRC (CD45)
Examples of differential expression
HEK293_AVG
LOG2 (dedup counts)
Jurkat ROIs (Human T-cell lymphocyte)
RPS6
B2M
JURKAT_AVG

Figure 31

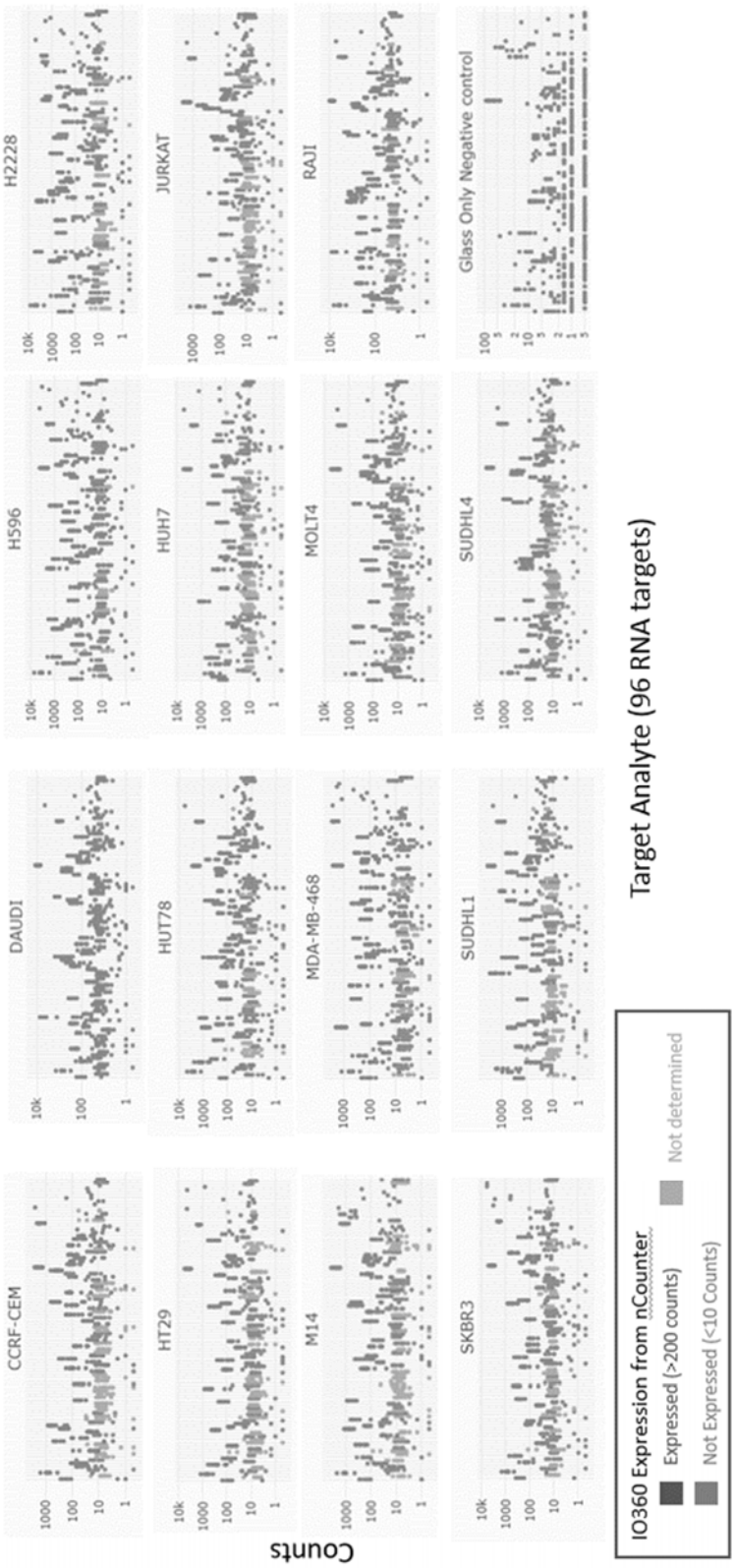
CCRF-CEM
DAUDI
H596
H2228
HT29
HUT78
HUH7
JURKAT
M14
MDA-MB-468
MOLT4
RAJI
SKBR3
SUDHL1
SUDHL4
Glass Only Negative control
Counts
Target Analyte (96 RNA targets)
IO360 Expression from nCounter
Expressed (>200 counts)
Not Expressed (<10 Counts)
Not determined

Figure 32

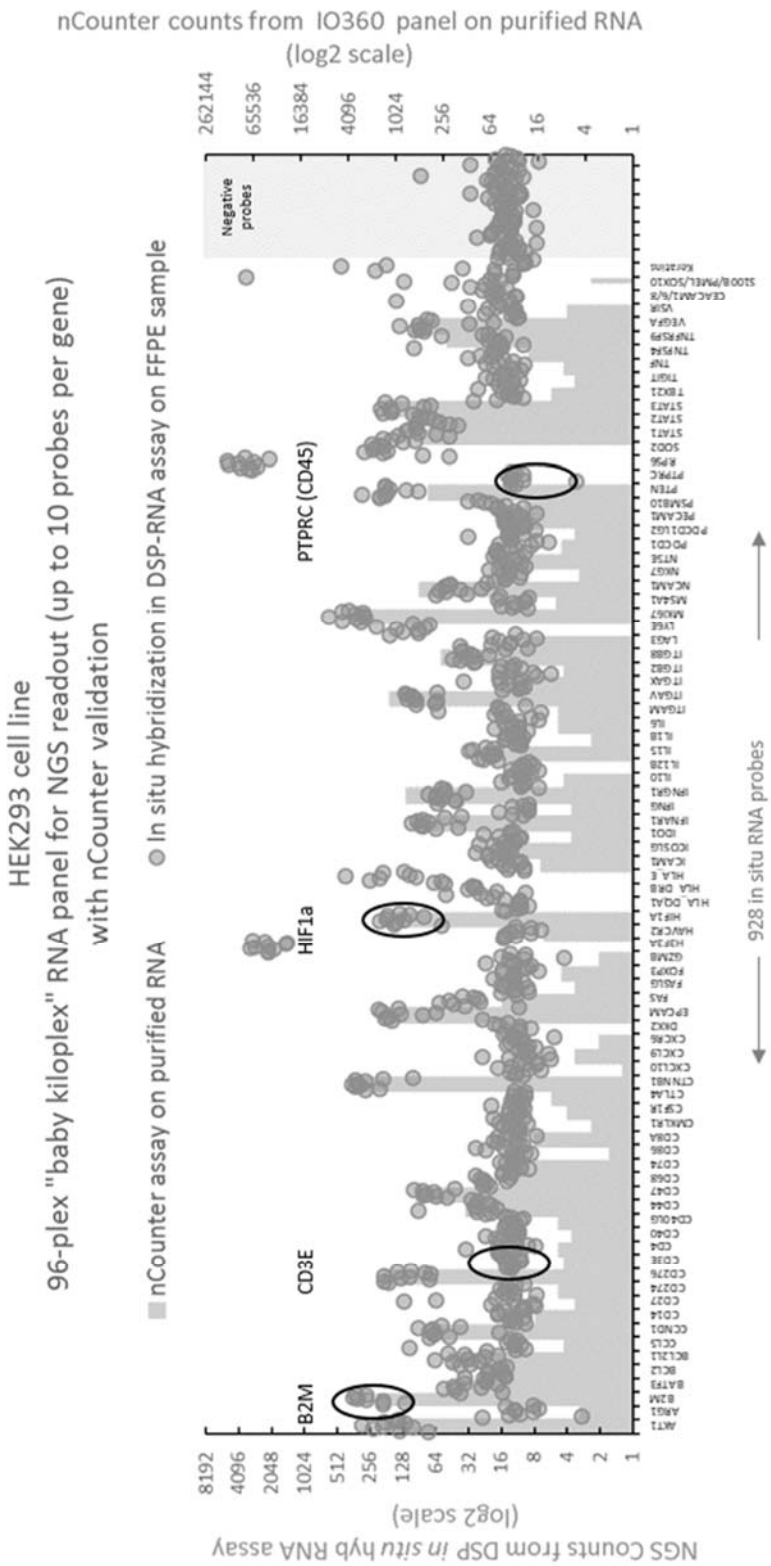
nCounter counts from IO360 panel on purified RNA
(log2 scale)
HEK293 cell line
96-plex "baby kiloplex" RNA panel for NGS readout (up to 10 probes per gene)
with nCounter validation
nCounter assay on purified RNA
In situ hybridization in DSP-RNA assay on FFPE sample
B2M
CD3E
HIF1a
PTPRC (CD45)
Negative probes
928 in situ RNA probes
NGS Counts from DSP in situ hyb RNA assay
(log2 scale)

Figure 33

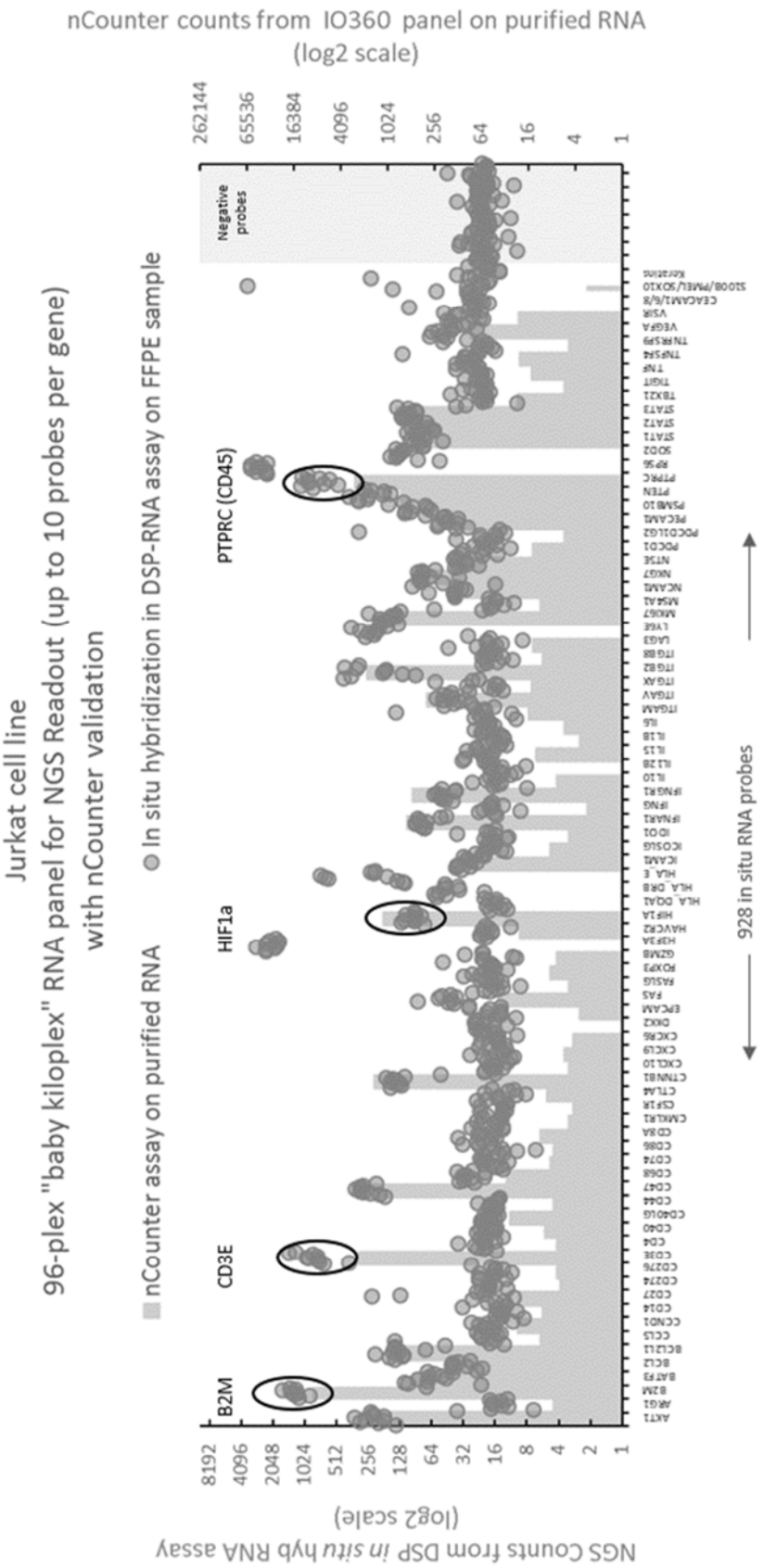
nCounter counts from IO360 panel on purified RNA
(log2 scale)
Jurkat cell line
96-plex "baby kiloplex" RNA panel for NGS Readout (up to 10 probes per gene)
with nCounter validation
nCounter assay on purified RNA
In situ hybridization in DSP-RNA assay on FFPE sample
NGS Counts from DSP in situ hyb RNA assay
(log2 scale)
B2M
CD3E
HIF1a
PTPRC (CD45)
Negative probes
928 in situ RNA probes

Figure 34