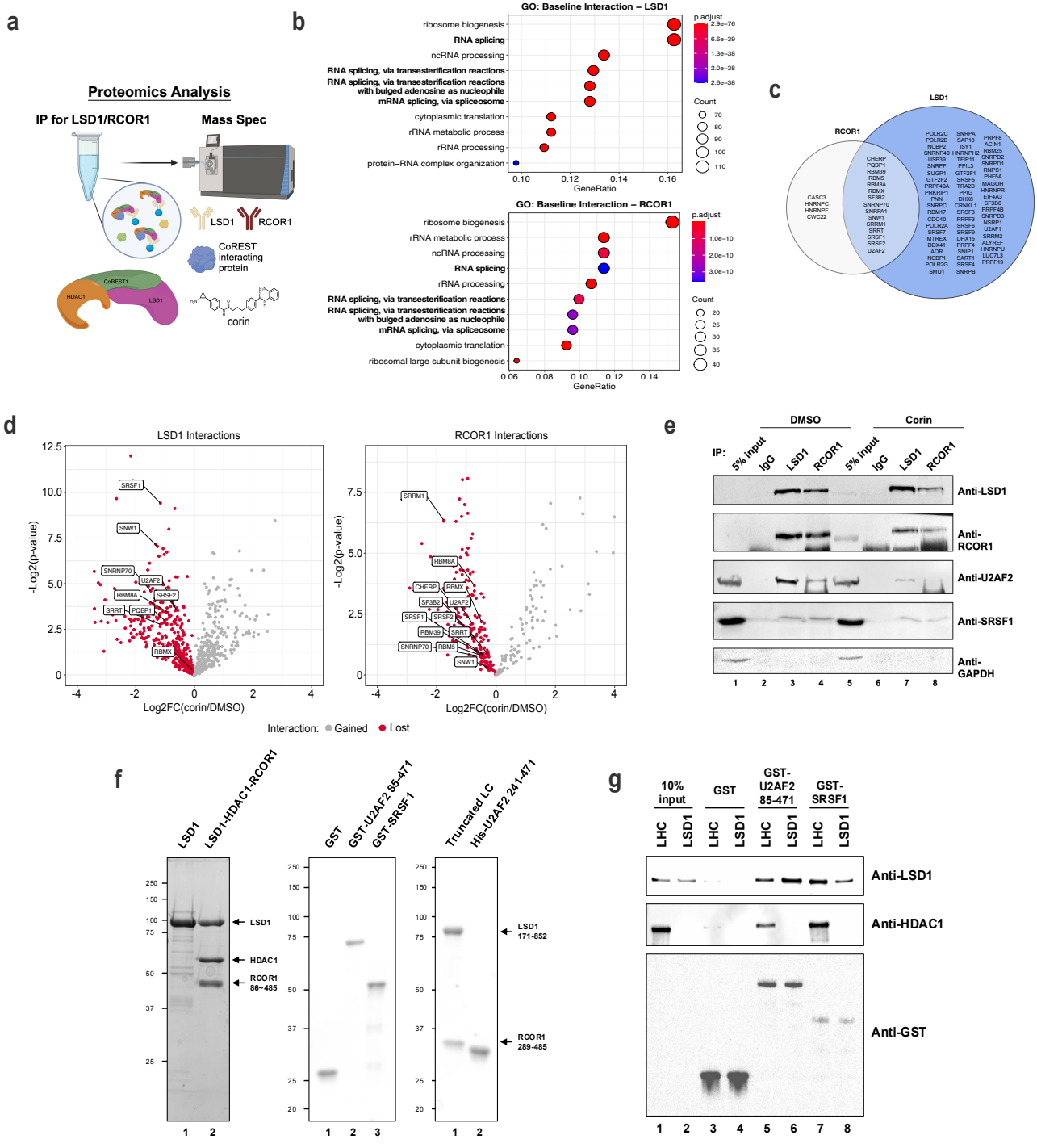
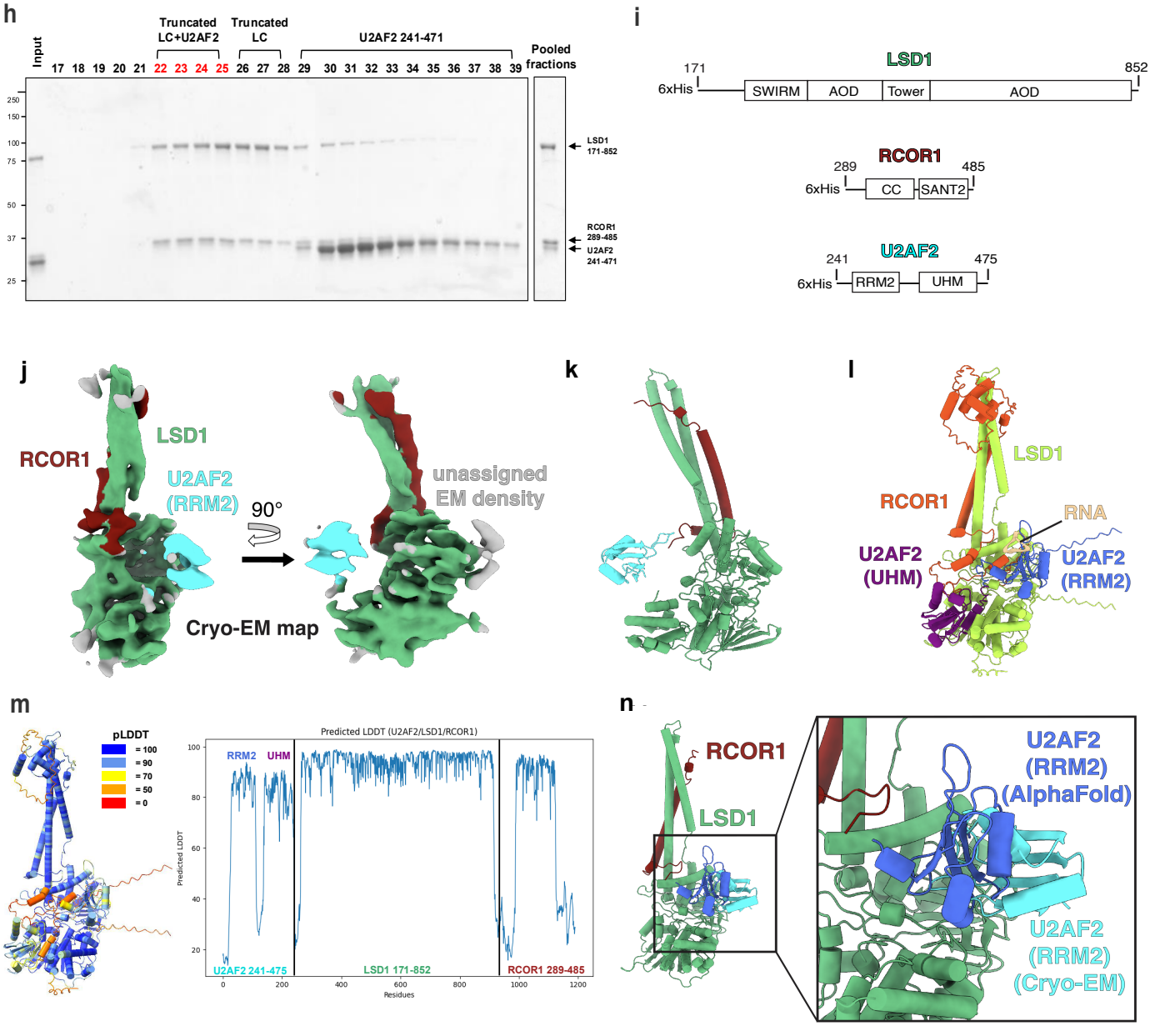
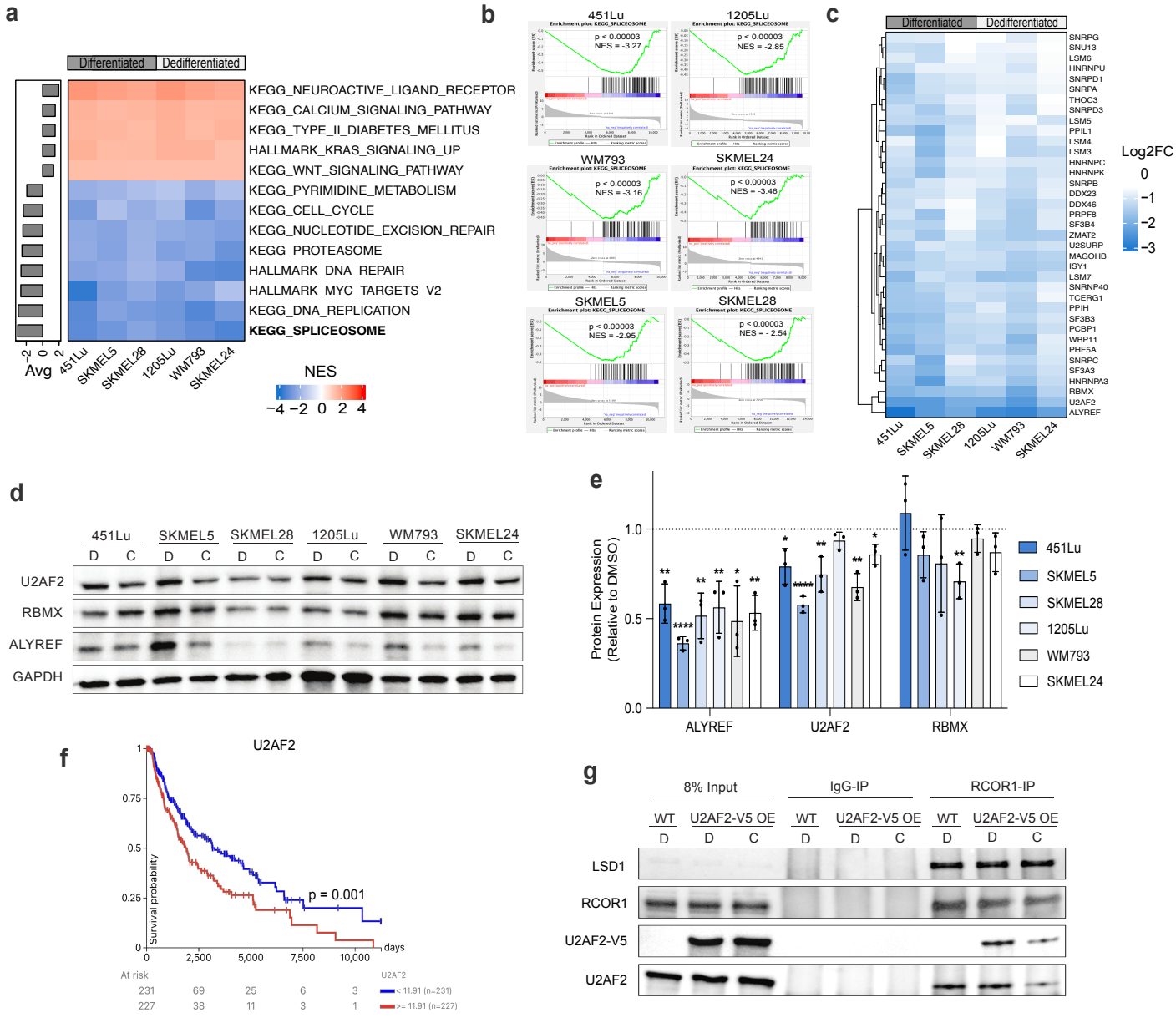
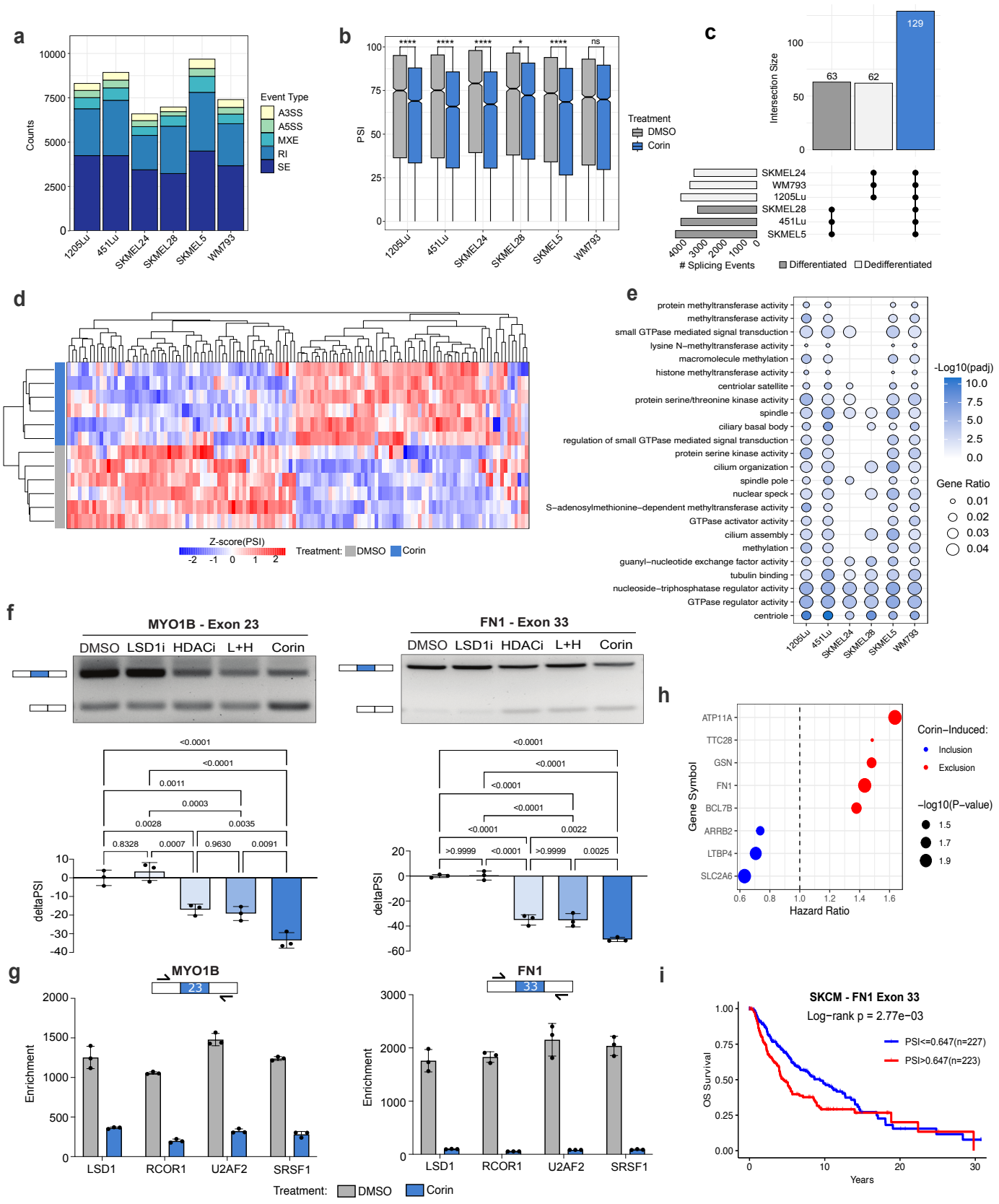
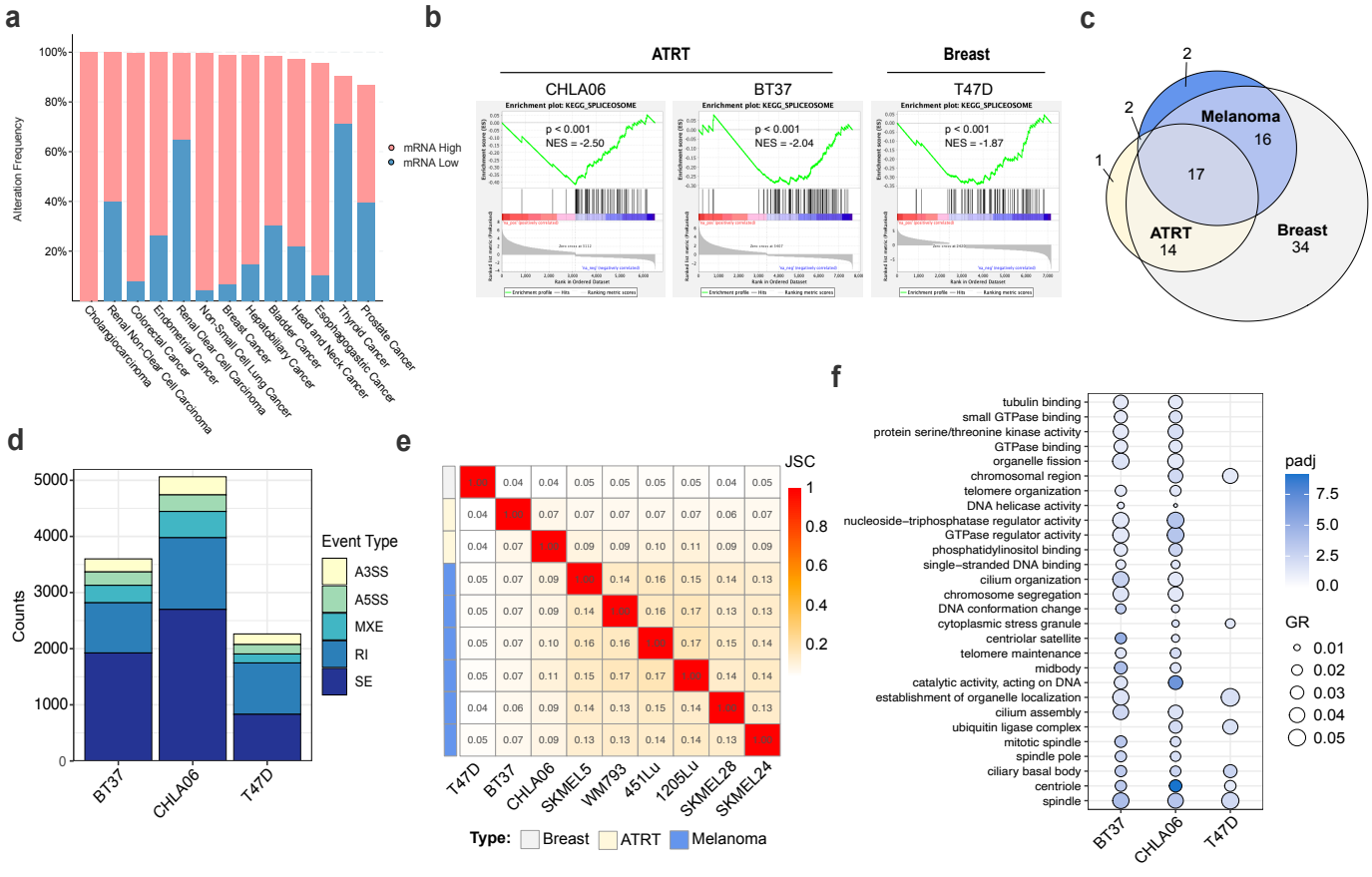
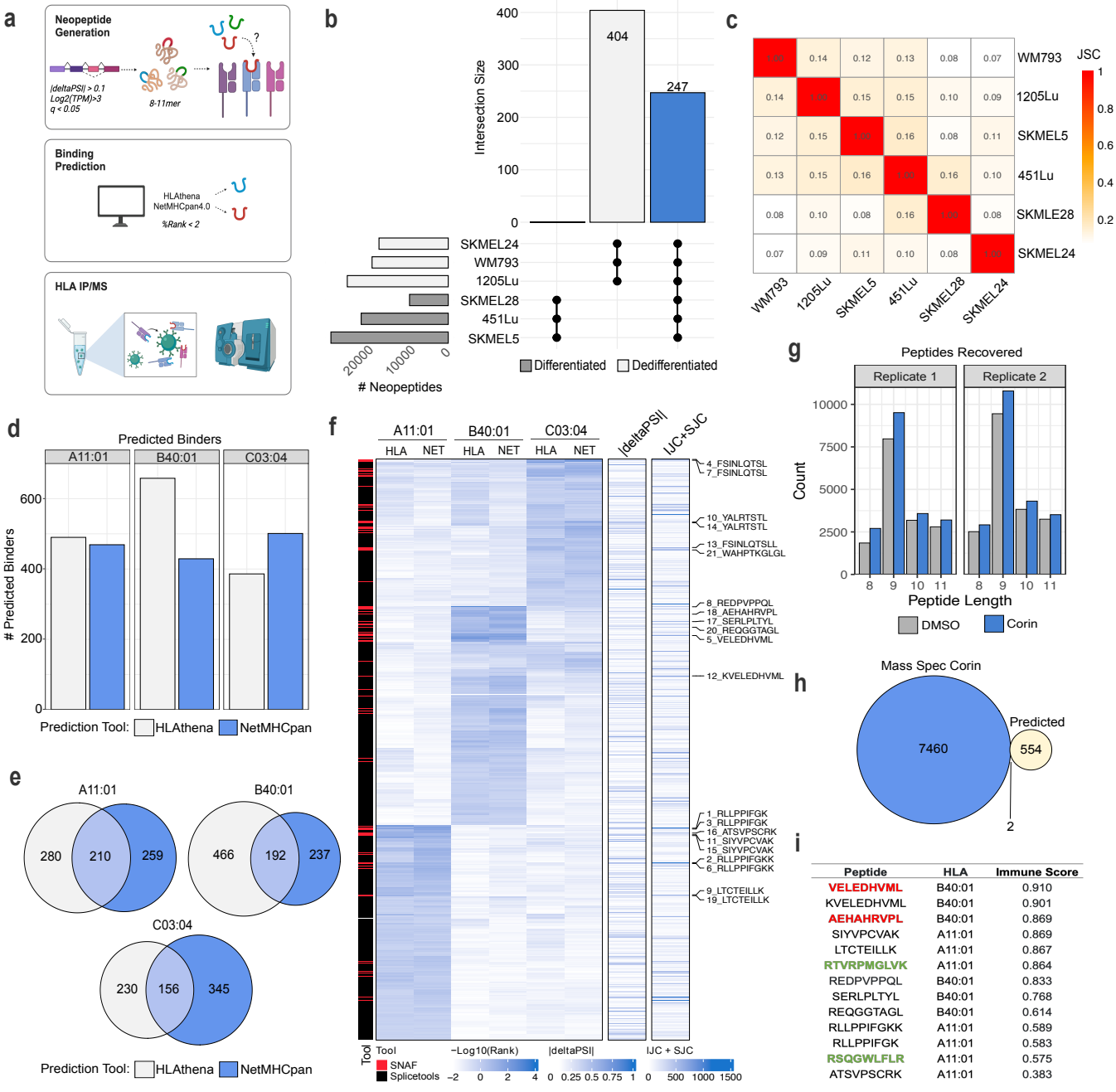

a
Proteomics Analysis
IP for LSD1/RCOR1
Mass Spec
LSD1
RCOR1
CoREST interacting protein
CoREST1
HDAC1
LSD1
corin
b
GO: Baseline Interaction – LSD1
ribosome biogenesis
RNA splicing
ncRNA processing
RNA splicing, via transesterification reactions
RNA splicing, via transesterification reactions with bulged adenosine as nucleophile
mRNA splicing, via spliceosome
cytoplasmic translation
rRNA metabolic process
rRNA processing
protein–RNA complex organization
GeneRatio
p.adjust
Count
GO: Baseline Interaction – RCOR1
ribosome biogenesis
rRNA metabolic process
ncRNA processing
RNA splicing
rRNA processing
RNA splicing, via transesterification reactions
RNA splicing, via transesterification reactions with bulged adenosine as nucleophile
mRNA splicing, via spliceosome
cytoplasmic translation
ribosomal large subunit biogenesis
c
LSD1
RCOR1
d
LSD1 Interactions
RCOR1 Interactions
-Log2(p-value)
Log2FC(corin/DMSO)
Interaction: Gained Lost
e
DMSO
Corin
IP:
5% input
IgG
LSD1
RCOR1
Anti-LSD1
Anti-RCOR1
Anti-U2AF2
Anti-SRSF1
Anti-GAPDH
f
LSD1
LSD1-HDAC1-RCOR1
GST
GST-U2AF2 85-471
GST-SRSF1
Truncated LC
His-U2AF2 241-471
LSD1
HDAC1
RCOR1 86~485
LSD1 171-852
RCOR1 289-485
g
10% input
GST
GST-U2AF2 85-471
GST-SRSF1
LHC
LSD1
Anti-LSD1
Anti-HDAC1
Anti-GST

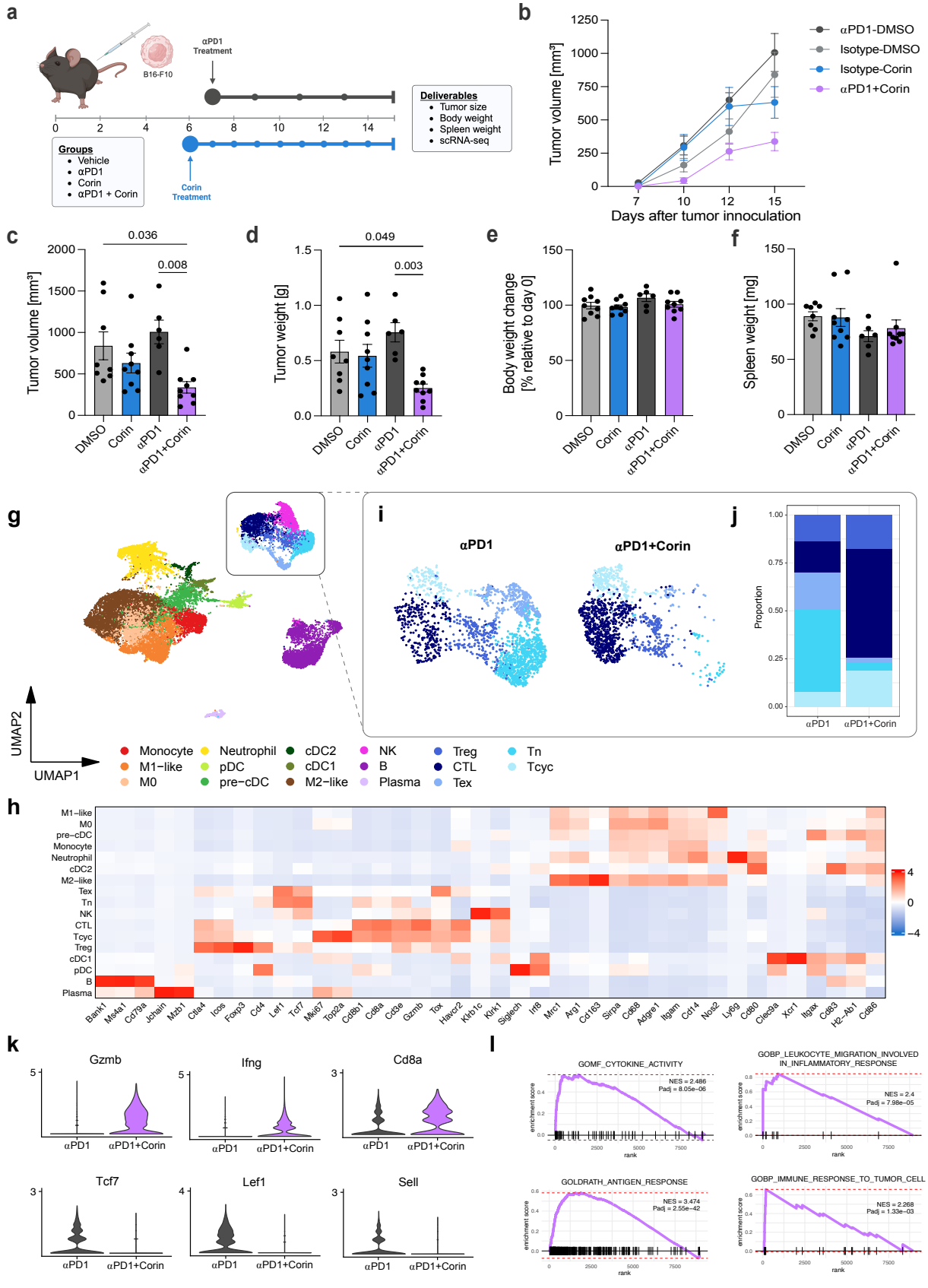
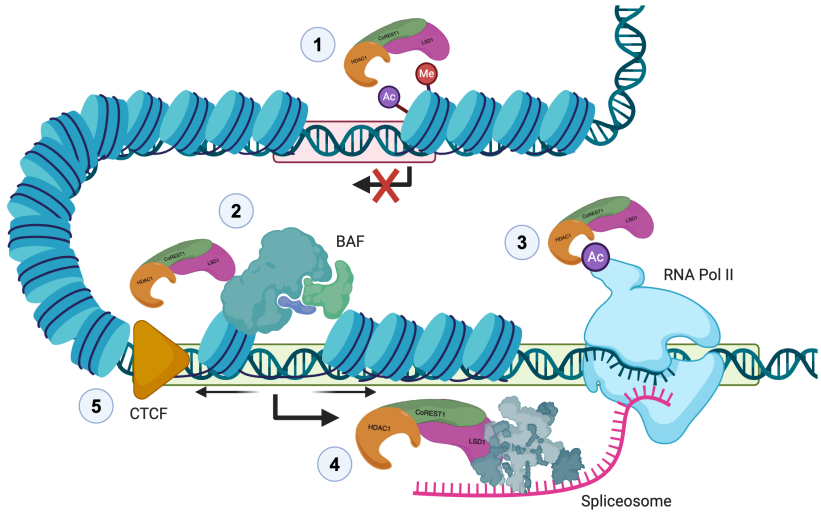

h
Input
Truncated LC+U2AF2
Truncated LC
U2AF2 241-471
Pooled fractions
17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
250
150
100
75
50
37
25
LSD1 171-852
RCOR1 289-485
U2AF2 241-471
i
LSD1
171
852
6xHis
SWIRM
AOD
Tower
AOD
RCOR1
289
485
6xHis
CC
SANT2
U2AF2
241
475
6xHis
RRM2
UHM
j
RCOR1
LSD1
U2AF2 (RRM2)
90°
unassigned EM density
Cryo-EM map
k
l
LSD1
RCOR1
RNA
U2AF2 (UHM)
U2AF2 (RRM2)
m
pLDDT
= 100
= 90
= 70
= 50
= 0
Predicted LDDT (U2AF2/LSD1/RCOR1)
RRM2
UHM
Predicted LDDT
100
80
60
40
20
U2AF2 241-475
LSD1 171-852
RCOR1 289-485
0
200
400
600
800
1000
1200
Residues
n
RCOR1
LSD1
U2AF2 (RRM2) (AlphaFold)
U2AF2 (RRM2) (Cryo-EM)


a
Differentiated
Dedifferentiated
KEGG_NEUROACTIVE_LIGAND_RECEPTOR
KEGG_CALCIUM_SIGNALING_PATHWAY
KEGG_TYPE_II_DIABETES_MELLITUS
HALLMARK_KRAS_SIGNALING_UP
KEGG_WNT_SIGNALING_PATHWAY
KEGG_PYRIMIDINE_METABOLISM
KEGG_CELL_CYCLE
KEGG_NUCLEOTIDE_EXCISION_REPAIR
KEGG_PROTEASOME
HALLMARK_DNA_REPAIR
HALLMARK_MYC_TARGETS_V2
KEGG_DNA_REPLICATION
KEGG_SPLICEOSOME
Avg
451Lu
SKMEL5
SKMEL28
1205Lu
WM793
SKMEL24
NES
−4 −2 0 2 4
b
451Lu
p < 0.00003
NES = -3.27
1205Lu
p < 0.00003
NES = -2.85
WM793
p < 0.00003
NES = -3.16
SKMEL24
p < 0.00003
NES = -3.46
SKMEL5
p < 0.00003
NES = -2.95
SKMEL28
p < 0.00003
NES = - 2.54
c
Differentiated
Dedifferentiated
SNRPG
SNU13
LSM6
HNRNPU
SNRPD1
SNRPA
THOC3
SNRPD3
LSM5
PPIL1
LSM4
LSM3
HNRNPC
HNRNPK
SNRPB
DDX23
DDX46
PRPF8
SF3B4
ZMAT2
U2SURP
MAGOHB
ISY1
LSM7
SNRNP40
TCERG1
PPIH
SF3B3
PCBP1
WBP11
PHF5A
SNRPC
SF3A3
HNRNPA3
RBMX
U2AF2
ALYREF
Log2FC
0
−1
−2
−3
451Lu
SKMEL5
SKMEL28
1205Lu
WM793
SKMEL24
d
451Lu
SKMEL5
SKMEL28
1205Lu
WM793
SKMEL24
D C D C D C D C D C D C
U2AF2
RBMX
ALYREF
GAPDH
e
Protein Expression
(Relative to DMSO)
1.0
0.5
0.0
ALYREF
U2AF2
RBMX
451Lu
SKMEL5
SKMEL28
1205Lu
WM793
SKMEL24
f
U2AF2
Survival probability
p = 0.001
days
0
2,500
5,000
7,500
10,000
At risk
U2AF2
231 69 25 6 3 < 11.91 (n=231)
227 38 11 3 1 >= 11.91 (n=227)
g
8% Input
IgG-IP
RCOR1-IP
WT
U2AF2-V5 OE
D D C
LSD1
RCOR1
U2AF2-V5
U2AF2


a
Counts
Event Type
A3SS
A5SS
MXE
RI
SE
1205Lu
451Lu
SKMEL24
SKMEL28
SKMEL5
WM793
b
PSI
Treatment
DMSO
Corin
c
Intersection Size
63
62
129
SKMEL24
WM793
1205Lu
SKMEL28
451Lu
SKMEL5
Splicing Events
Differentiated
Dedifferentiated
d
Z-score(PSI)
Treatment: DMSO Corin
e
protein methyltransferase activity
methyltransferase activity
small GTPase mediated signal transduction
lysine N−methyltransferase activity
macromolecule methylation
histone methyltransferase activity
centriolar satellite
protein serine/threonine kinase activity
spindle
ciliary basal body
regulation of small GTPase mediated signal transduction
protein serine kinase activity
cilium organization
spindle pole
nuclear speck
S−adenosylmethionine−dependent methyltransferase activity
GTPase activator activity
cilium assembly
methylation
guanyl−nucleotide exchange factor activity
tubulin binding
nucleoside−triphosphatase regulator activity
GTPase regulator activity
centriole
-Log10(padj)
Gene Ratio
f
MYO1B - Exon 23
FN1 - Exon 33
DMSO LSD1i HDACi L+H Corin
deltaPSI
h
Gene Symbol
ATP11A
TTC28
GSN
FN1
BCL7B
ARRB2
LTBP4
SLC2A6
Hazard Ratio
Corin−Induced:
Inclusion
Exclusion
−log10(P−value)
g
MYO1B
FN1
Enrichment
LSD1
RCOR1
U2AF2
SRSF1
Treatment: DMSO Corin
i
SKCM - FN1 Exon 33
Log−rank p = 2.77e−03
PSI<=0.647(n=227)
PSI>0.647(n=223)
OS Survival
Years


a
Alteration Frequency
100%
80%
60%
40%
20%
mRNA High
mRNA Low
Cholangiocarcinoma
Renal Non-Clear Cell Carcinoma
Colorectal Cancer
Endometrial Cancer
Renal Clear Cell Carcinoma
Non-Small Cell Lung Cancer
Breast Cancer
Hepatobiliary Cancer
Bladder Cancer
Head and Neck Cancer
Esophagogastric Cancer
Thyroid Cancer
Prostate Cancer
b
ATRT
Breast
CHLA06
BT37
T47D
Enrichment plot: KEGG_SPLICEOSOME
p < 0.001
NES = -2.50
p < 0.001
NES = -2.04
p < 0.001
NES = -1.87
c
Melanoma
16
17
ATRT
14
Breast
34
2
2
1
d
Counts
5000
4000
3000
2000
1000
0
BT37
CHLA06
T47D
Event Type
A3SS
A5SS
MXE
RI
SE
e
JSC
1
0.8
0.6
0.4
0.2
T47D
BT37
CHLA06
SKMEL5
WM793
451Lu
1205Lu
SKMEL28
SKMEL24
Type:
Breast
ATRT
Melanoma
f
tubulin binding
small GTPase binding
protein serine/threonine kinase activity
GTPase binding
organelle fission
chromosomal region
telomere organization
DNA helicase activity
nucleoside−triphosphatase regulator activity
GTPase regulator activity
phosphatidylinositol binding
single−stranded DNA binding
cilium organization
chromosome segregation
DNA conformation change
cytoplasmic stress granule
centriolar satellite
telomere maintenance
midbody
catalytic activity, acting on DNA
establishment of organelle localization
cilium assembly
ubiquitin ligase complex
mitotic spindle
spindle pole
ciliary basal body
centriole
spindle
BT37
CHLA06
T47D
padj
7.5
5.0
2.5
0.0
GR
0.01
0.02
0.03
0.04
0.05


a
Neopeptide Generation
|deltaPSI| > 0.1
Log2(TPM)>3
q < 0.05
8-11mer
Binding Prediction
HLAthena
NetMHCpan4.0
%Rank < 2
HLA IP/MS
b
Intersection Size
404
247
SKMEL24
WM793
1205Lu
SKMEL28
451Lu
SKMEL5
Neopeptides
Differentiated
Dedifferentiated
c
JSC
WM793
1205Lu
SKMEL5
451Lu
SKMLE28
SKMEL24
d
Predicted Binders
A11:01
B40:01
C03:04
Predicted Binders
Prediction Tool: HLAthena NetMHCpan
e
A11:01
280
210
259
B40:01
466
192
237
C03:04
230
156
345
Prediction Tool: HLAthena NetMHCpan
f
A11:01
B40:01
C03:04
HLA
NET
|deltaPSI|
IJC+SJC
4_FSINLQTSL
7_FSINLQTSL
10_YALRTSTL
14_YALRTSTL
13_FSINLQTSLL
21_WAHPTKGLGL
8_REDPVPPQL
18_AEHAHRVPL
17_SERLPLTYL
20_REQGGTAGL
5_VELEDHVML
12_KVELEDHVML
1_RLLPPIFGK
3_RLLPPIFGK
16_ATSVPSCRK
11_SIYVPCVAK
15_SIYVPCVAK
2_RLLPPIFGKK
6_RLLPPIFGKK
9_LTCTEILLK
19_LTCTEILLK
Tool
SNAF
Splicetools
−Log10(Rank)
|deltaPSI|
IJC + SJC
g
Peptides Recovered
Replicate 1
Replicate 2
Count
Peptide Length
DMSO
Corin
h
Mass Spec Corin
Predicted
7460
554
2
i
Peptide | HLA | Immune Score
VELEDHVML | B40:01 | 0.910
KVELEDHVML | B40:01 | 0.901
AEHAHRVPL | B40:01 | 0.869
SIYVPCVAK | A11:01 | 0.869
LTCTEILLK | A11:01 | 0.867
RTVRPMGLVK | A11:01 | 0.864
REDPVPPQL | B40:01 | 0.833
SERLPLTYL | B40:01 | 0.768
REQGGTAGL | B40:01 | 0.614
RLLPPIFGKK | A11:01 | 0.589
RLLPPIFGK | A11:01 | 0.583
RSQGWLFLR | A11:01 | 0.575
ATSVPSCRK | A11:01 | 0.383
j
A11:01
RTVRPMGLVK
RSQGWLFLR
VELEDHVML
AEHAHRVPL
DMSO
Peptide
CEF
PHA
k
Spot Number / 9.0 x 10^4 APCs
0.005
0.003
RTVRPMGLVK
RSQGWLFLR
VELEDHVML
AEHAHRVPL
DMSO (-)
Peptide
CEF (+)
PHA (+)


a
B16-F10
αPD1 Treatment
Corin Treatment
Groups
Vehicle
αPD1
Corin
αPD1 + Corin
Deliverables
Tumor size
Body weight
Spleen weight
scRNA-seq
b
Tumor volume [mm³]
Days after tumor innoculation
αPD1-DMSO
Isotype-DMSO
Isotype-Corin
αPD1+Corin
c
Tumor volume [mm³]
0.036
0.008
d
Tumor weight [g]
0.049
0.003
e
Body weight change [% relative to day 0]
f
Spleen weight [mg]
DMSO
Corin
αPD1
αPD1+Corin
g
UMAP2
UMAP1
Monocyte
M1−like
M0
Neutrophil
pDC
pre−cDC
cDC2
cDC1
M2−like
NK
B
Plasma
Treg
CTL
Tex
Tn
Tcyc
i
αPD1
αPD1+Corin
j
Proportion
h
M1−like
M0
pre−cDC
Monocyte
Neutrophil
cDC2
M2−like
Tex
Tn
NK
CTL
Tcyc
Treg
cDC1
pDC
B
Plasma
Bank1 Ms4a1 Cd79a Jchain Mzb1 Ctla4 Icos Foxp3 Cd4 Lef1 Tcf7 Mki67 Top2a Cd8b1 Cd8a Cd3e Gzmb Tox Havcr2 Klrb1c Klrk1 Siglech Irf8 Mrc1 Arg1 Cd163 Sirpa Cd68 Adgre1 Itgam Cd14 Nos2 Ly6g Cd80 Ciec9a Xcr1 Itgax Cd83 H2−Ab1 Cd86
k
Gzmb
Ifng
Cd8a
Tcf7
Lef1
Sell
αPD1
αPD1+Corin
l
GOMF_CYTOKINE_ACTIVITY
NES = 2.486
Padj = 8.05e−06
GOBP_LEUKOCYTE_MIGRATION_INVOLVED IN_INFLAMMATORY_RESPONSE
NES = 2.4
Padj = 7.98e−05
GOLDRATH_ANTIGEN_RESPONSE
NES = 3.474
Padj = 2.55e−42
GOBP_IMMUNE_RESPONSE_TO_TUMOR_CELL
NES = 2.268
Padj = 1.33e−03
enrichment score
rank

m