Exome sequenced patients

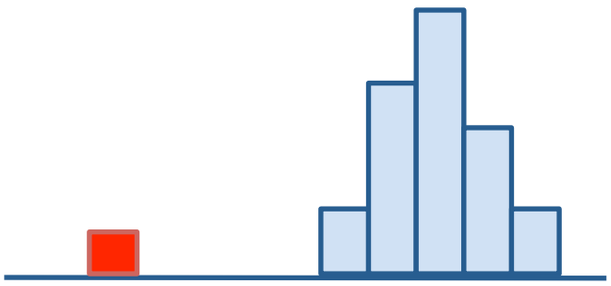
Genetic diagnosis

No genetic diagnosis

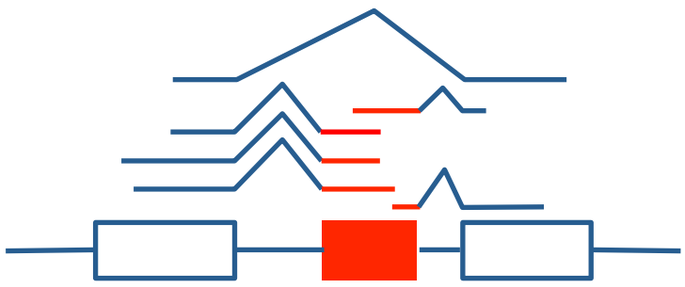
1. Patient fibroblasts

2. RNA sequencing

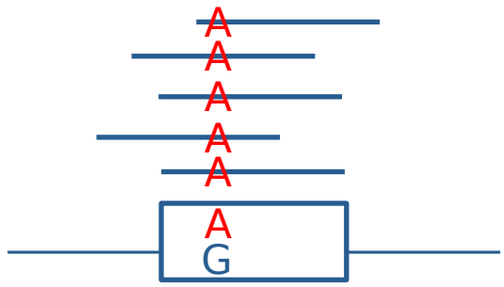
Aberrant expression



Aberrant splicing

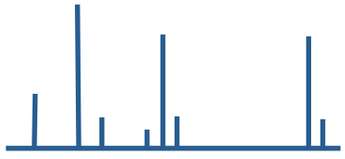


Mono-allelic expression



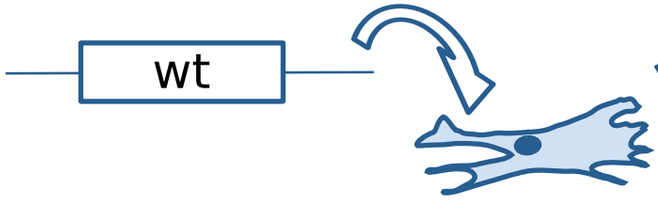
3. Functional and biochemical validation

Proteomics

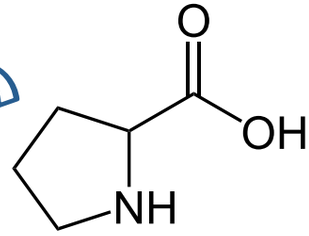


m/z

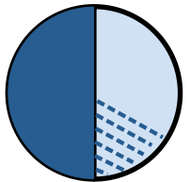
Complementation



Supplementation

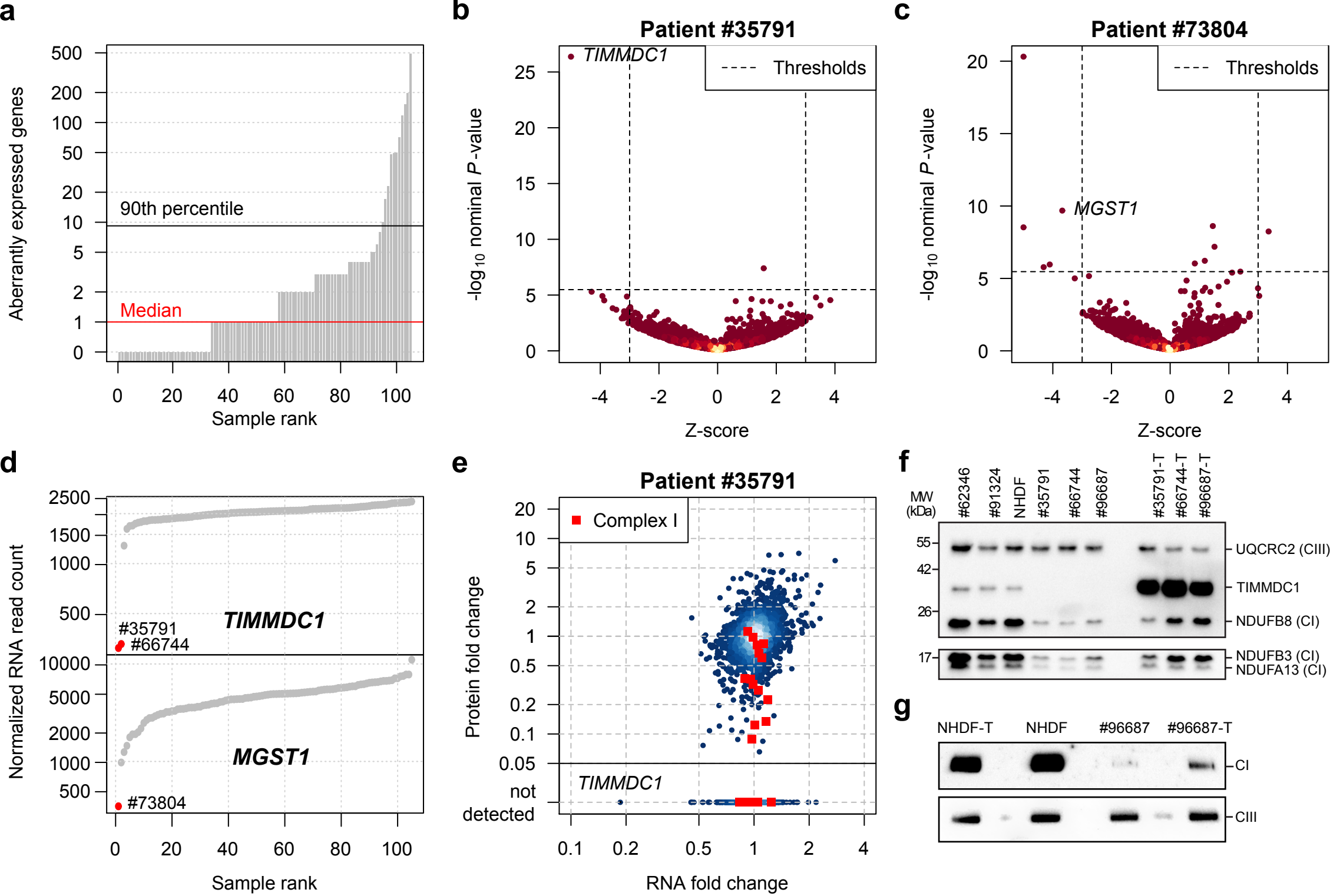


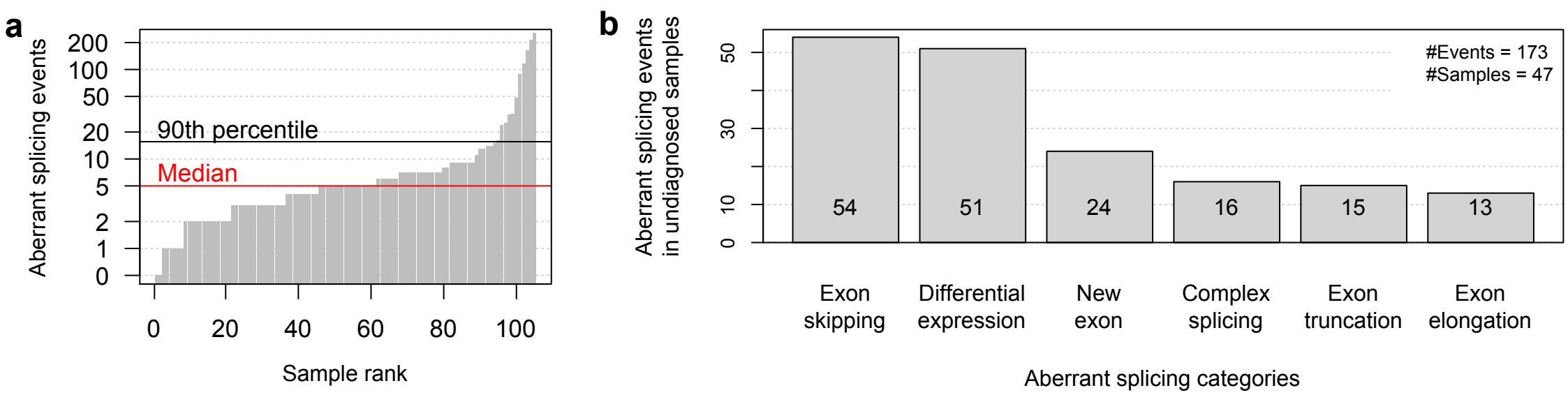
Genetic diagnosis

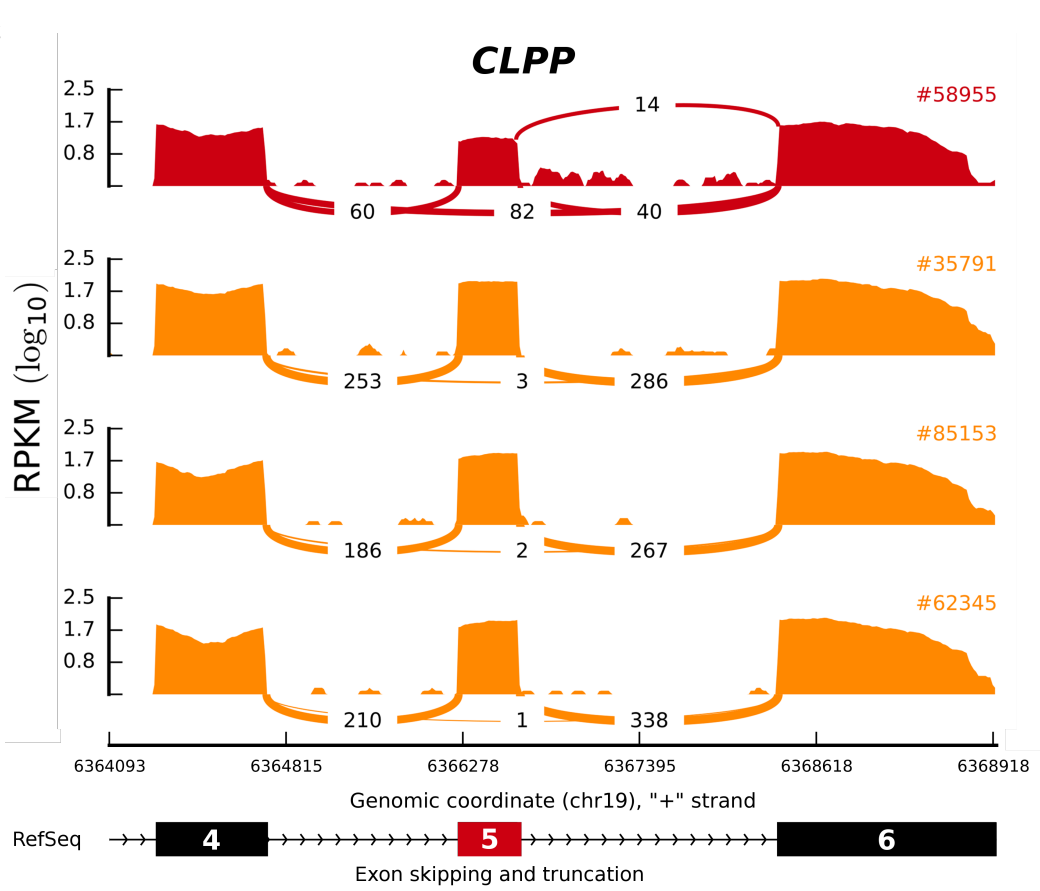


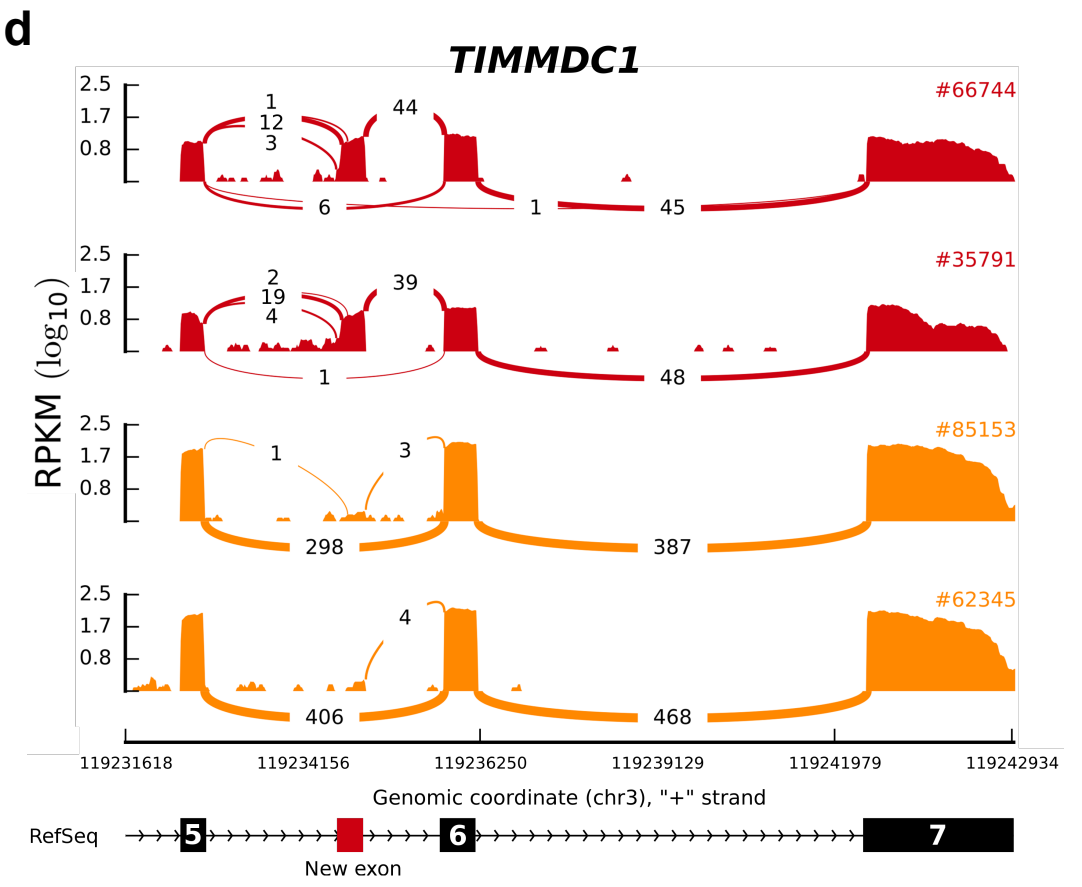
No genetic diagnosis

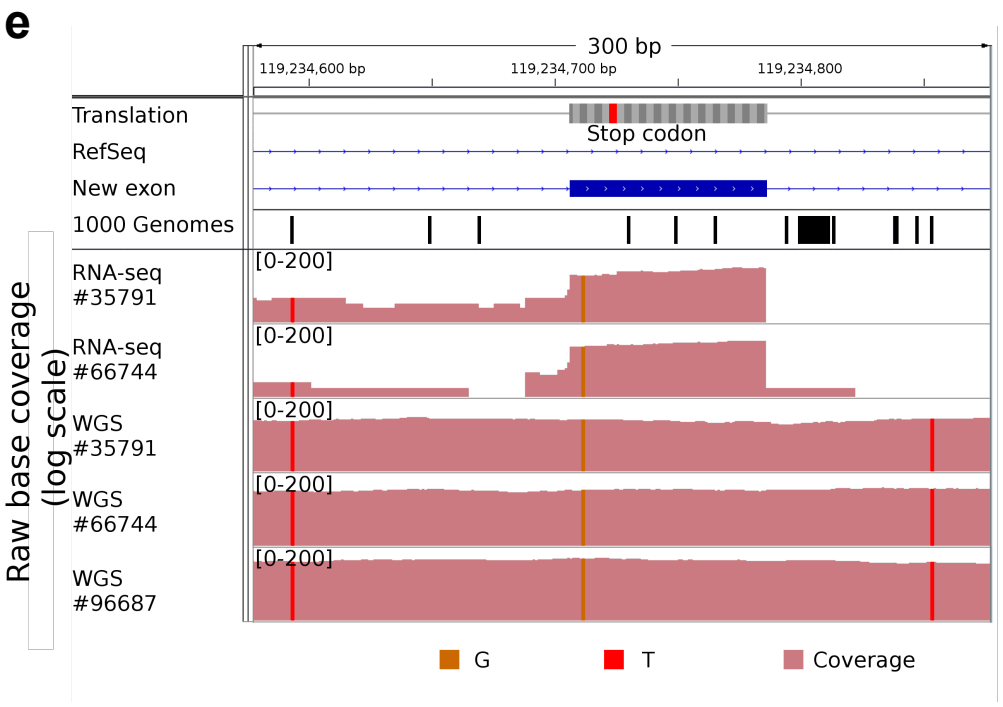
New genetic diagnosis

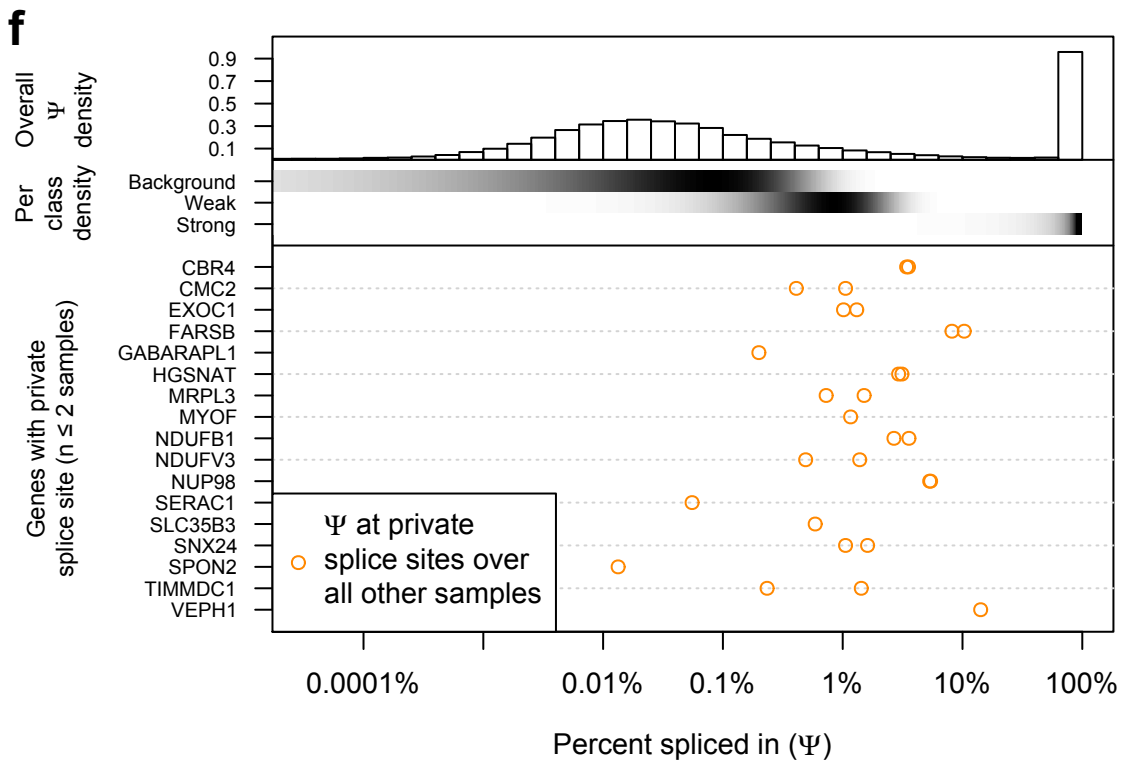

a
Aberrantly expressed genes
90th percentile
Median
Sample rank
b
Patient #35791
TIMMDC1
Thresholds
-log10 nominal P-value
Z-score
c
Patient #73804
MGST1
Thresholds
-log10 nominal P-value
Z-score
d
Normalized RNA read count
TIMMDC1
#35791
#66744
MGST1
#73804
Sample rank
e
Patient #35791
Complex I
TIMMDC1
not detected
Protein fold change
RNA fold change
f
MW (kDa)
#62346
#91324
NHDF
#35791
#66744
#96687
#35791-T
#66744-T
#96687-T
UQCRC2 (CIII)
TIMMDC1
NDUFB8 (CI)
NDUFB3 (CI)
NDUFA13 (CI)
g
NHDF-T
NHDF
#96687
#96687-T
CI
CIII

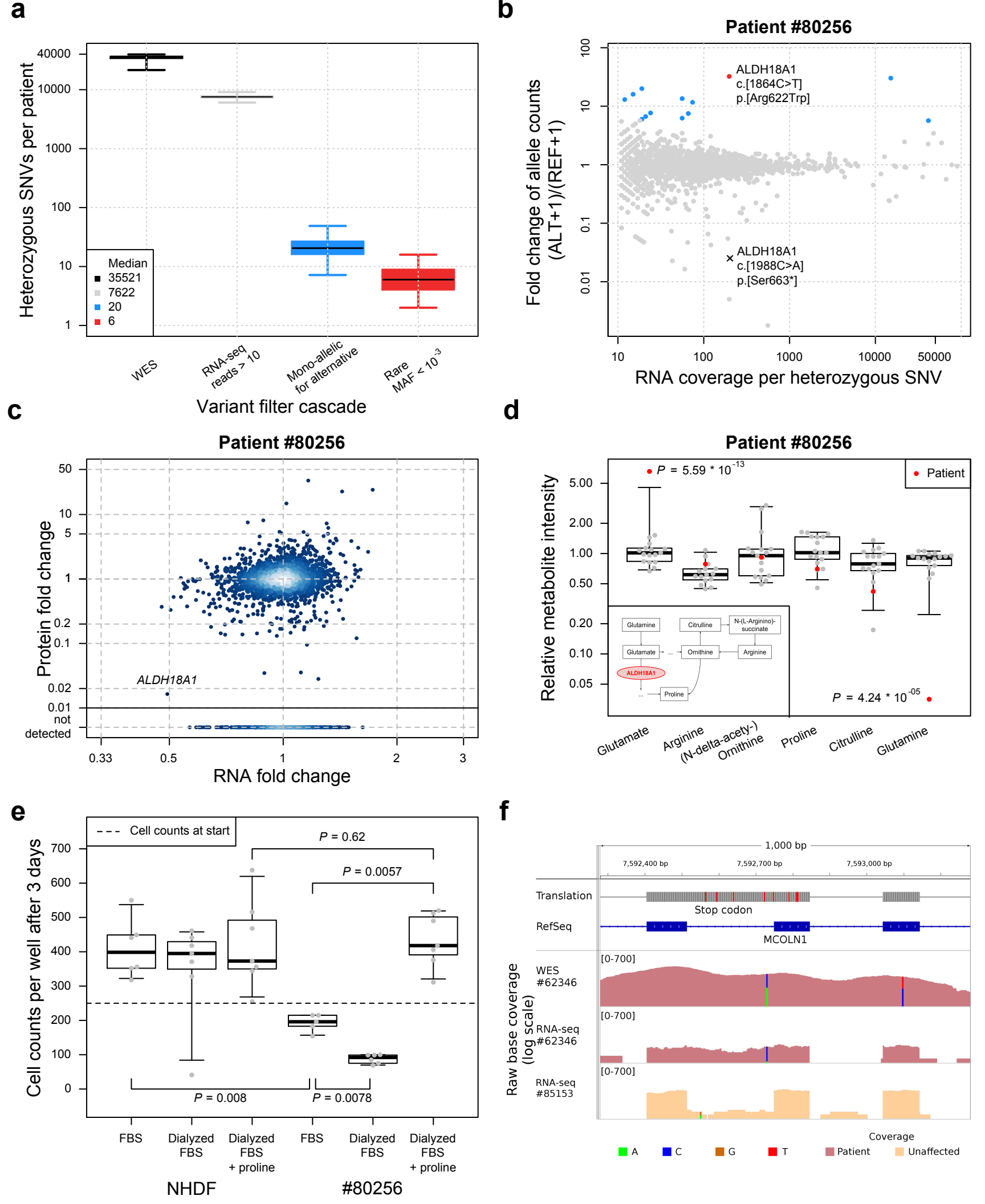

a
Aberrant splicing events
200
100
50
20
10
5
2
1
0
90th percentile
Median
0
20
40
60
80
100
Sample rank
b
Aberrant splicing events in undiagnosed samples
0
10
30
50
#Events = 173
#Samples = 47
54
51
24
16
15
13
Exon skipping
Differential expression
New exon
Complex splicing
Exon truncation
Exon elongation
Aberrant splicing categories


c
CLPP
#58955
14
60
82
40
#35791
253
3
286
#85153
186
2
267
#62345
210
1
338
RPKM (log10)
2.5
1.7
0.8
6364093
6364815
6366278
6367395
6368618
6368918
Genomic coordinate (chr19), "+" strand
RefSeq
4
5
6
Exon skipping and truncation


d
TIMMDC1
#66744
1
12
3
44
6
1
45
#35791
2
19
4
39
1
48
#85153
1
3
298
387
#62345
4
406
468
RPKM (log10)
2.5
1.7
0.8
119231618
119234156
119236250
119239129
119241979
119242934
Genomic coordinate (chr3), "+" strand
RefSeq
5
6
7
New exon


e
300 bp
119,234,600 bp
119,234,700 bp
119,234,800
Translation
Stop codon
RefSeq
New exon
1000 Genomes
RNA-seq #35791
RNA-seq #66744
WGS #35791
WGS #66744
WGS #96687
[0-200]
Raw base coverage (log scale)
G
T
Coverage


f
Overall Ψ density
0.9
0.7
0.5
0.3
0.1
Per class density
Background
Weak
Strong
Genes with private splice site (n ≤ 2 samples)
CBR4
CMC2
EXOC1
FARSB
GABARAPL1
HGSNAT
MRPL3
MYOF
NDUFB1
NDUFV3
NUP98
SERAC1
SLC35B3
SNX24
SPON2
TIMMDC1
VEPH1
Ψ at private splice sites over all other samples
0.0001%
0.01%
0.1%
1%
10%
100%
Percent spliced in (Ψ)


a
Heterozygous SNVs per patient
40000
10000
1000
100
10
1
Median
35521
7622
20
6
WES
RNA-seq reads > 10
Mono-allelic for alternative
Rare MAF < 10^-3
Variant filter cascade
b
Patient #80256
Fold change of allele counts (ALT+1)/(REF+1)
100
10
1
0.1
0.01
ALDH18A1
c.[1864C>T]
p.[Arg622Trp]
ALDH18A1
c.[1988C>A]
p.[Ser663*]
10
100
1000
10000
50000
RNA coverage per heterozygous SNV
c
Patient #80256
Protein fold change
50
10
5
1
0.2
0.1
0.02
0.01
not detected
ALDH18A1
0.33
0.5
1
2
3
RNA fold change
d
Patient #80256
Relative metabolite intensity
P = 5.59 * 10^-13
Patient
5.00
2.00
1.00
0.50
0.20
0.10
0.05
Glutamine
Citrulline
N-(L-Arginino)-succinate
Glutamate
Ornithine
Arginine
ALDH18A1
Proline
P = 4.24 * 10^-05
Glutamate
Arginine (N-delta-acety-) Ornithine
Proline
Citrulline
Glutamine
e
Cell counts per well after 3 days
Cell counts at start
P = 0.62
P = 0.0057
700
600
500
400
300
200
100
0
P = 0.008
P = 0.0078
FBS
Dialyzed FBS
Dialyzed FBS + proline
FBS
Dialyzed FBS
Dialyzed FBS + proline
NHDF
#80256
f
1,000 bp
7,592,400 bp
7,592,700 bp
7,593,000 bp
Translation
Stop codon
RefSeq
MCOLN1
Raw base coverage (log scale)
WES #62346
[0-700]
RNA-seq #62346
[0-700]
RNA-seq #85153
[0-700]
Coverage
A
C
G
T
Patient
Unaffected

a

		TIMMDC1	MGST1	CLPP	ALDH18A1	MCOLN1	Candidates per sample
Detected by	Aberrant expression	✓	✓	-	✓	⋯✓	1± 1
	Aberrant splicing	✓	-	✓	-	✓	5± 3
	Mono-allelic expression	-	-	-	✓	⋯✓	6± 3
Validated by	Proteomics/Western blot	✓	✓	✓	✓	-	
	Complementation	✓	-	-	-	-	
	Supplementation	-	-	-	✓	-	
	Disease associated variant detecetd	✓	-	✓	✓	✓	

b