Supplementary Material

Table S1. Summary of mapping results

Sample	Total reads	Mapped (%)	Unique Mapped (%)	Properly mapped (%)	mapped to exon
HNE0-1	99687516	80786083 (81.0%)	74622780 (74.9%)	58462196 (58.6%)	87.3%
HNE0-2	94318720	77047792 (81.7%)	71232714 (75.5%)	54776864 (58.1%)	88.0%
HNE0-3	104033900	84348102 (81.1%)	78506570 (75.5%)	61718788 (59.30%)	88.3%
HNE15-1	94426598	77164128 (81.7%)	71472652 (75.7%)	55909976 (59.2%)	87.8%
HNE15-2	96157260	78457546 (81.6%)	72632204 (75.5%)	55825130 (58.1%)	87.3%
HNE15-3	108570254	88329318 (81.4%)	81886361 (75.4%)	61261632 (56.4%)	87.1%
HNE30-1	90984476	74380204 (81.8%)	68418655 (75.2%)	53203630 (58.4%)	86.3%
HNE30-2	139144958	114325242 (82.2%)	104880035 (75.4%)	85979950 (61.8%)	86.8%
HNE30-3	74187364	60273539 (81.2%)	55495650 (74.8%)	42519582 (57.3%)	87.7%
HNE45-1	97873448	80097638 (81.8%)	72820619 (74.4%)	61451506 (62.7%)	86.9%
HNE45-2	85010714	69249525 (81.5%)	64069477 (75.4%)	47829794 (56.2%)	85.9%
HNE45-3	111160152	90730095 (81.6%)	82753703 (74.4%)	67660182 (60.9%)	87.5%

Table S2. Differentially expressed genes detected from RNA-seq and microarray under 45 μM HNE treatment with the criteria abs($\log_2$FC)>1 & FDR<0.01.

	RNA-seq			**Microarray**		
Gene	$\log_2$FC	FDR	Criteria	$\log_2$FC	FDR	Criteria
PAK1IP1	-0.97014	0.0004	NO	-1.01235	0.000118	YES
POLR1B	-1.31517	0.120268	NO	-1.00851	1.92E-05	YES
RPP40	-1.5112	0.021626	NO	-1.23832	7.35E-05	YES
SLC39A10	0.394277	0.999992	NO	1.004566	0.00011	YES
TOB2	-0.13574	0.999992	NO	1.240623	9.17E-05	YES
TXNRD1	1.11741	0.564905	NO	1.202122	5.82E-09	YES
DOT1L	-1.65822	0.716448	NO	-1.12846	1.29E-08	YES
URB2	-1.4274	0.015881	NO	-1.13394	1.12E-05	YES
GCLM	1.05867	0.057677	NO	1.155502	4.69E-07	YES

EGR3	-1.87956	0.594447	NO	-1.57817	4.45E-06	YES
PPRC1	-1.2092	0.123428	NO	-1.04628	1.36E-05	YES
SEPT9	-1.02783	0.006052	YES	-0.37756	0.069757	NO
AKAP12	-1.65304	0	YES	-0.85003	9.69E-07	NO
ALDH1B1	-1.12244	0.001616	YES	-0.60484	0.033055	NO
AURKB	-1.12026	0.000727	YES	-0.65536	9.96E-05	NO
AXL	-1.34617	4.38E-11	YES	-0.32968	0.002317	NO
BCL2A1	-1.46144	4.26E-09	YES	-0.98468	5.43E-05	NO
C3orf52	-1.80099	0.003523	YES	-0.81917	0.000177	NO
CCDC86	-1.40385	1.99E-06	YES	-0.88293	0.000422	NO
CCNB1	-1.111	0.000344	YES	-0.71396	0.00014	NO
CLU	1.21162	0.007436	YES	0.798388	6.42E-06	NO
CSE1L	-1.03511	6.97E-06	YES	-0.36345	0.024872	NO
CYR61	-1.1846	5.18E-07	YES	-0.67495	0.000137	NO
DEPDC1B	-1.1586	9.98E-06	YES	-0.5746	0.00111	NO
ERCC1	-1.08378	0.000434	YES	-0.55601	1.77E-06	NO
FERMT1	-1.30449	0.004283	YES	-0.44572	0.063834	NO
GINS2	-1.09345	0.003098	YES	-0.68723	3.17E-05	NO
KIAA0020	-1.09857	0.007005	YES	-0.62611	0.013148	NO
KIF22	-1.03522	0.000837	YES	-0.65977	0.000281	NO
KLF10	-1.53113	0	YES	-0.97001	2.39E-06	NO
LMNB1	-1.1695	0.000422	YES	-0.54138	0.003965	NO
MAST4	-1.56621	2.03E-06	YES	-0.79445	2.93E-07	NO
MYBBP1A	-1.13179	2.67E-05	YES	-0.47795	0.005781	NO
MYBL2	-1.0629	0.000307	YES	-0.75639	6.94E-05	NO
NCAPH	-1.13729	0.008718	YES	-0.76061	2.12E-05	NO
NTSR1	-1.32196	3.50E-06	YES	-0.8147	2.20E-05	NO
NXT1	-1.16572	0.001753	YES	-0.48939	0.002271	NO
PHLDA1	-1.30851	3.21E-05	YES	-0.55906	2.72E-05	NO
PHLDA2	-1.18007	4.36E-06	YES	-0.53631	0.000373	NO
PLAUR	-1.20895	6.93E-07	YES	-0.7183	6.58E-06	NO
RCC2	-1.06411	1.98E-05	YES	-0.59732	8.72E-05	NO
RMRP	-2.79036	9.14E-08	YES	-0.04642	0.715176	NO
RUNX1	-1.50691	0.008183	YES	-0.75367	2.89E-06	NO
S100A16	-1.24604	6.95E-08	YES	-0.6623	3.68E-06	NO
SERPINE1	-1.40171	8.38E-07	YES	-0.56816	1.64E-05	NO
SH2B3	-1.61624	0	YES	-0.96093	2.93E-07	NO
SH3GL1	-1.0058	0.000464	YES	-0.54403	5.05E-05	NO
SLC35F2	-1.48649	1.25E-05	YES	-0.88975	8.96E-06	NO
SLC7A1	-1.44883	0.005529	YES	-0.78454	2.20E-05	NO
SMS	-1.03098	2.80E-05	YES	-0.28585	0.054275	NO
SNORD3A	-2.34242	0.001213	YES	0.717988	0.009885	NO
SNRPA	-1.01996	0.006338	YES	-0.54977	0.00107	NO

SPOCD1	-1.29261	0.004687	YES	-0.59273	0.000306	NO
SRPRB	-1.11256	0.006268	YES	-0.57878	0.000422	NO
TAGLN2	-1.06624	0.002311	YES	-0.51506	0.008155	NO
TGIF1	-1.67345	7.35E-05	YES	-0.84841	0.000606	NO
TM4SF1	-1.17003	1.48E-06	YES	-0.63044	2.62E-05	NO
TOP2A	-1.00081	6.93E-07	YES	-0.5056	3.00E-05	NO
TPX2	-1.22191	2.04E-10	YES	-0.67708	6.61E-06	NO
TYMS	-1.3589	0.000212	YES	-0.58912	0.001679	NO
UBE2C	-1.19803	3.19E-06	YES	-0.592	0.002827	NO
UHRF1	-1.03948	2.41E-05	YES	-0.7672	0.000109	NO
URB1	-1.38841	8.10E-06	YES	-0.63253	0.001386	NO
VASP	-1.04796	0.000113	YES	-0.48527	0.002342	NO
WDR43	-1.40602	0.002439	YES	-0.89612	8.83E-06	NO
ZFP36L1	-1.41659	3.77E-12	YES	-0.7778	4.20E-06	NO
ZNF438	-1.61289	4.00E-11	YES	-0.94816	1.73E-06	NO
ANGPTL4	-3.00259	0	YES	-2.64975	5.42E-13	YES
BHLHE40	-2.57747	0	YES	-1.73652	7.19E-07	YES
CDCA7	-1.14369	0.00136	YES	-1.02284	4.56E-07	YES
CDCP1	-1.84593	2.91E-13	YES	-1.11176	9.65E-09	YES
DUSP5	-1.77486	0	YES	-1.26458	5.91E-09	YES
FHL2	-1.66195	8.29E-13	YES	-1.14685	9.91E-10	YES
G0S2	-2.28168	0	YES	-1.8494	4.10E-11	YES
GEM	-1.91191	7.15E-07	YES	-1.52413	5.29E-09	YES
GFPT2	-2.16498	4.52E-12	YES	-1.39221	2.46E-08	YES
HMOX1	2.77619	0	YES	2.791531	1.17E-13	YES
IER3	-2.03342	0	YES	-1.68872	5.08E-08	YES
JUN	-1.60032	0	YES	-1.18517	5.82E-09	YES
KIF23	-1.29826	0.002487	YES	-1.02632	1.60E-06	YES
LAMB3	-2.02213	8.29E-13	YES	-1.36648	3.15E-09	YES
MYC	-2.62035	0	YES	-2.11437	7.44E-08	YES
PDCD1LG2	-1.69406	1.16E-05	YES	-1.23795	1.84E-05	YES
PIR	1.37049	0.008218	YES	1.103485	2.93E-07	YES
RGS4	-2.53574	0	YES	-1.05942	0.000798	YES
SEMA7A	-3.22267	0	YES	-2.1345	3.22E-08	YES
SLC16A14	1.26122	1.28E-05	YES	1.247672	5.07E-06	YES
SLC43A2	1.38496	0.000577	YES	1.345411	2.23E-07	YES
SLC44A2	1.6754	4.75E-11	YES	1.911831	3.33E-12	YES
TNFSF9	1.52504	2.97E-07	YES	1.153288	7.19E-07	YES
TRIB1	-1.326	1.24E-09	YES	-1.00495	2.67E-07	YES

*YES: satisfy the criteria abs($\log_2$FC)>1 & FDR<0.01; NO: don’t satisfy the criteria.

Table S3. Overrepresented pathways at the gene level and the combined level under 15μM HNE treatment (FDR<0.05).

	Combined level		**Gene level**	
	Num. of genes	FDR	Num. of genes	FDR
Metabolic pathways	7	0.0037	4	0.0356
MAPK signaling pathway	*(2)	*(0.06)	2	0.0357
Pyrimidine metabolism	2	0.015	*	*
Cell cycle	2	0.019	*	*
Glutathione metabolism	3	0.0006	*	*

*: not significant (FDR>0.05).

Table S4. Overrepresented pathways at the gene level and the combined level under 30 μM HNE treatment (FDR<0.05).

	Combined level		**Gene level**	
	Num. of genes	FDR	Num. of genes	FDR
programmed cell death	21	0.0438	*	*
Nucleotide excision repair	2	0.015	*	*
Base excision repair	2	0.012	*	*
p53 signaling pathway	4	0.0006	*	*
DNA replication	9	0.0375	*	*
spindle organization	4	0.0438	*	*
ATP binding	27	0.0051	*	*
Purine metabolism	5	0.0058	*	*
Cysteine and methionine metabolism	3	0.0009	2	0.0144
splicesome	3	0.015	3	0.0144
Endocytosis	4	0.0096	3	0.0244
Focal adhesion	5	0.0024	3	0.0244
Metabolic pathways	14	0.0004	7	0.0244
MAPK signaling pathway	7	0.0004	6	0.0007
Pyrimidine metabolism	6	3.62E-05	*	*
Cell cyle	5	0.006	2	0.03
Glutathione metabolism	3	0.0025	*	*

Table S5. Overrepresented pathways at the gene level and the combined level under 45μM HNE treatment (FDR<0.05).

	Combined level		**Gene level**	
	Num. of genes	FDR	Num. of genes	FDR
Ubiquitin mediated proteolysis	6	0.013	4	0.0257
DNA repair	29	2.75E-07	18	0.0001
microtubule-based process	34	1.48E-11	24	2.36E-09
Cell division	39	4.02E-12	25	3.97E-08
organelle organization	93	5.34E-13	61	6.7E-10
RNA transport	14	0.000042	10	0.0003
Mismatch repair	6	2.52E-06	5	3.51E-06
programmed cell death	59	0.000052	38	0.0007
Nucleotide excision repair	6	9.61E-05	6	3.51E-06
Base excision repair	5	0.0003	4	0.0005
p53 signaling pathway	7	9.61E-05	5	0.0005
DNA replication	9	4.83E-09	6	2.45E-06
spindle organization	13	1.9E-08	10	3.04E-07
ATP binding	84	2.88E-09	41	0.0047
Purine metabolism	8	0.0011	5	0.0081
Cysteine and methionine metabolism	5	0.0002	4	0.0005
Spliceosome	12	3.89E-07	9	2.45E-06
Endocytosis	9	0.0011	6	0.0046
Focal adhesion	7	0.018	*	*
Metabolic pathways	29	9.61E-05	18	0.0014
MAPK signaling pathway	13	9.61E-05	8	0.0015
Pyrimidine metabolism	8	9.61E-05	4	0.0095
Cell cycle	17	3.6E-12	11	3.63E-08
Glutathione metabolism	4	0.0084	*	*

Table S6. Potential miRNAs that target NEDD4

	miR-144	**miR-30a,b,c,d,e**	**miR-27a,b**	**miR-9**
Potential binding sites (relative to 3'UTR start site)	1112	959	381	1045
PhastCons score	0.5874	0.6127	0.6085	0.6397
MirSVR score	-0.9991	-0.7535	-1.0573	-1.1951
DIANAmT	1	1	1	1
miRanda	1	1	1	1
miRDB	0	1	1	1
miRWalk	1	1	1	1
RNAhybrid	1	1,0(30a,e)	1	1
PICTAR4	0	1,0(30a,e)	1(27b),0(27a)	1
PICTAR5	1	1	1	1
PITA	0	1,0(30a,d,e)	0	1
RNA22	0	0	0	0
Targetscan	1	1	1	1

isoform, CDS or promoter switching

We examined the 195 differentially expressions detected at the CDS level but not at the gene level to determine whether they were caused by isoform, CDS or promoter switching. Based on the results from Cuffdiff, ARHGEF2, IVNS1ABP, SEPT6, STK4, and XPO7 seemed to show differential splicing, whereas CNOT10, RND3 and TBRG4 appeared to have promoter switching. Further checking the transcript expression of each gene, we found only SEPT6 had true isoform switching between the transcript corresponding to the differentially expressed CDS and the other transcript. The CDS expression was significantly down-regulated, whereas the other transcript expression was significantly up-regulated, which led to the unchanged expression at the gene level (Figure S1). However, for IVNS1ABP and XPO7, the CDS expression was significantly down-regulated, whereas the other transcript abundance was not changed at all, which suggested differentially expressions detected at the CDS level but not at the gene level are mostly due to the noise added by the non-changed transcripts but not isoform switching (Figures S2 and S3). For ARHGEF2 and STK4, differentially expressed CDS and reported differential splicing were not from the same TSS group and the reported differential splicing occurred between two transcripts with very low expression value (FPKM<1), which was highly possible to be false positives.

We checked the expression of each TSS group for CNOT10, RND3 and TBRG4, which were reported to have promoter switching between different TSS groups. We found the expression of TSS group containing the differentially expressed CDS was changed, whereas other TSS groups didn't change at all, which suggested differentially expression detected at the CDS level but not at the gene level are mostly due to the noise added by the non-changed TSS group but not promoter switching (Figure S4, S5 and S6).

In summary, among 195 genes whose differential expression were detected only at the CDS level, only SEPT6 was found to have isoform switching between the differentially expressed CDS and other transcripts (Figure S1).

Figure S1. The expression change of transcripts for SEPT6 across conditions. Isoform switching occurs between the transcript corresponding to the differentially expressed CDS and the other transcript.

Figure S2. The expression change of transcripts for STK4 across conditions.

Figure S3. The expression change of transcripts for IVNS1ABP across conditions.

Figure S4. The expression change of TSS groups for CNOT10 across conditions.

Figure S5. The expression change of TSS groups for RND3 across conditions.

Figure S6. The expression change of TSS groups for TBRG4 across conditions.

Fig. S1

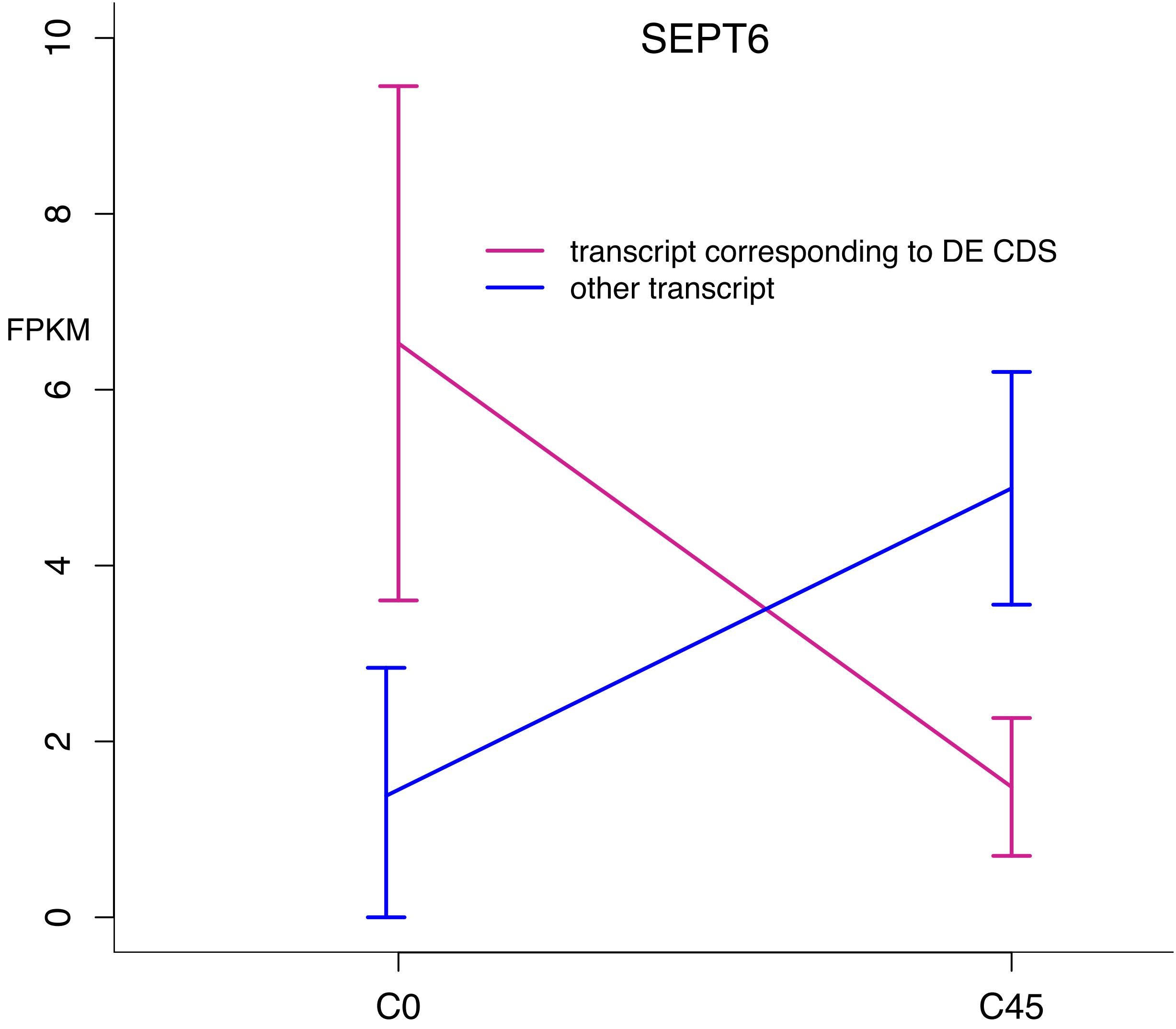

SEPT6
FPKM
0
2
4
6
8
10
C0
C45
transcript corresponding to DE CDS
other transcript

Fig. S2

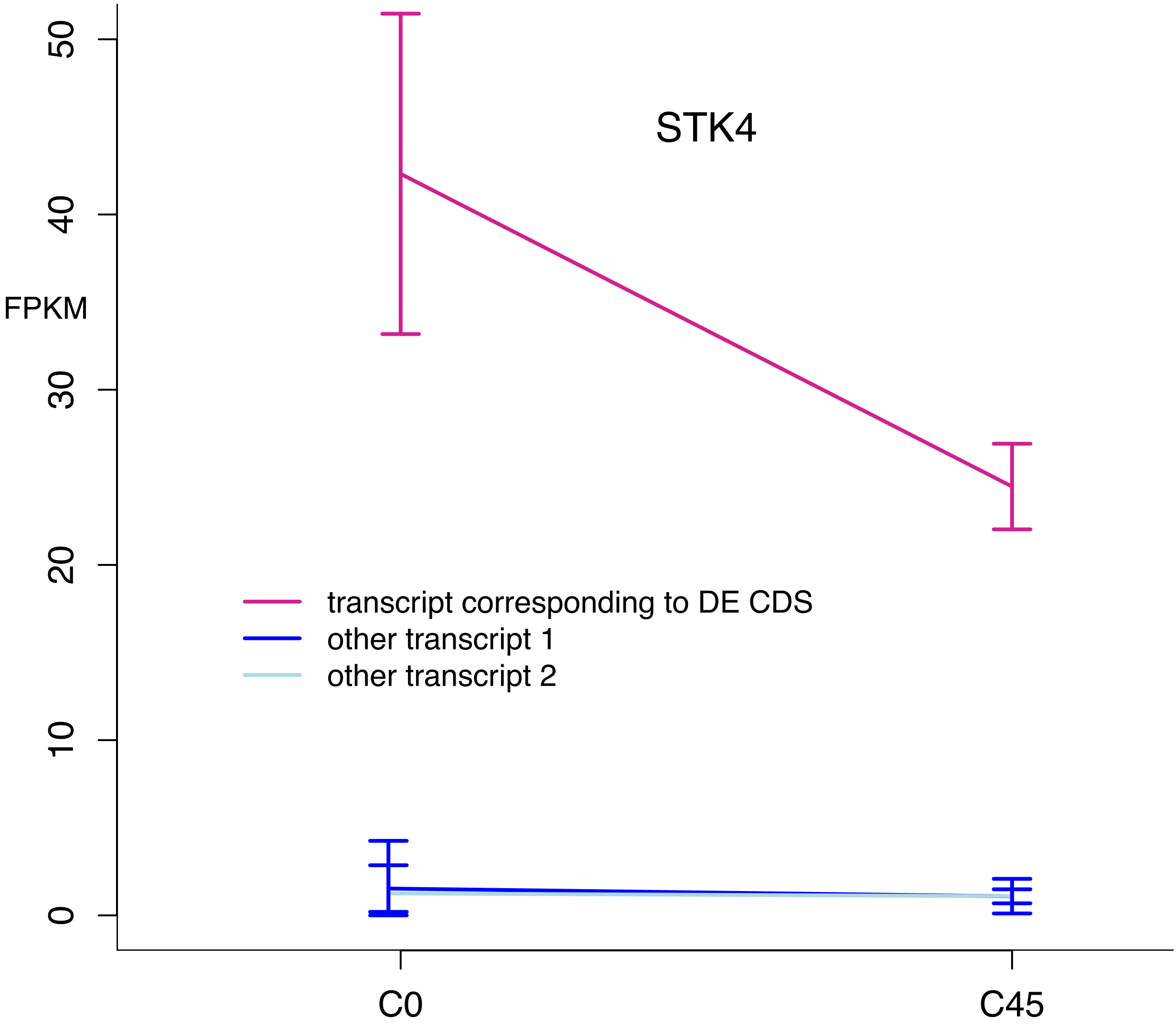

STK4
FPKM
0
10
20
30
40
50
C0
C45
transcript corresponding to DE CDS
other transcript 1
other transcript 2

Fig. S3

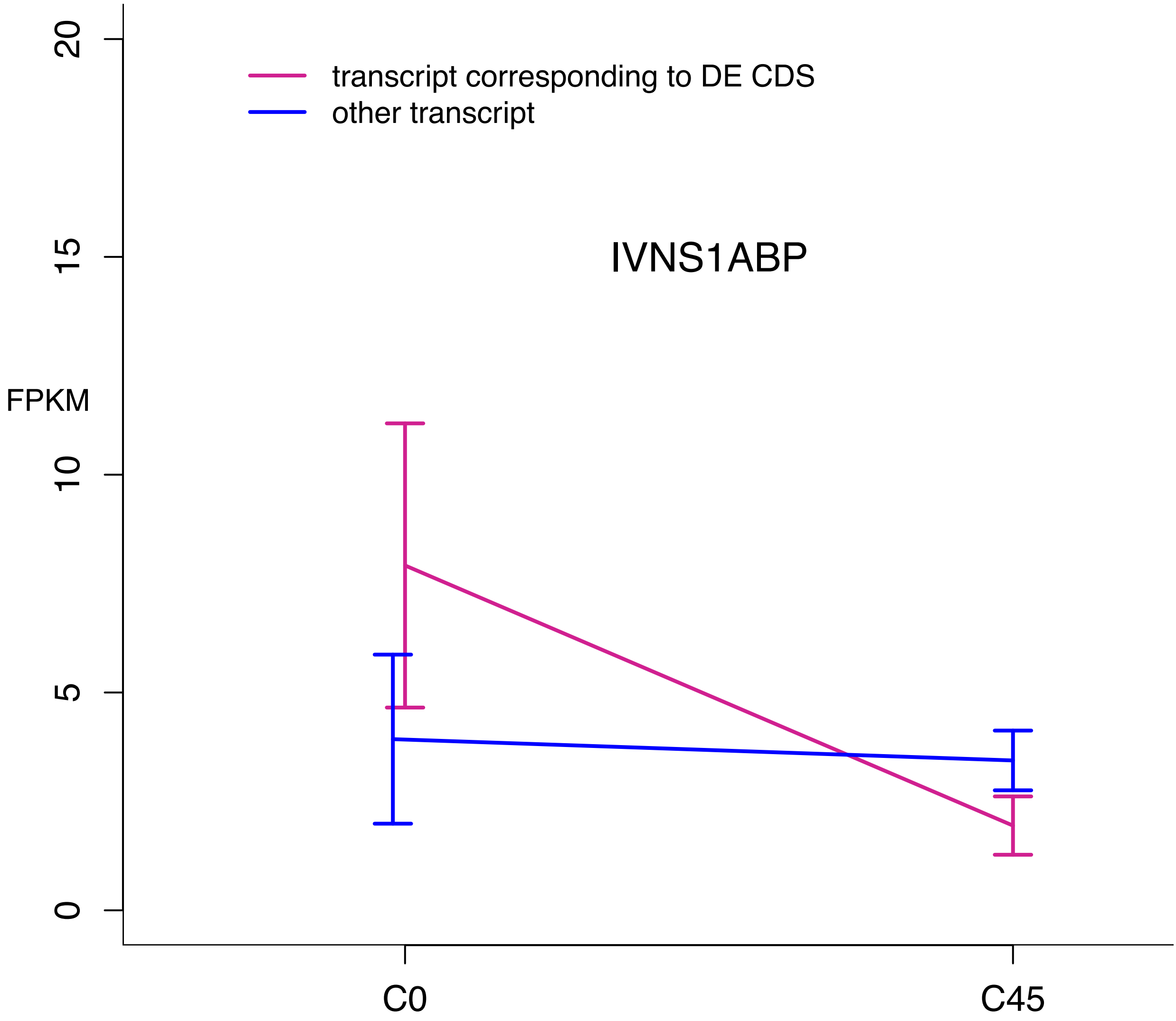

transcript corresponding to DE CDS
other transcript
IVNS1ABP
FPKM
0
5
10
15
20
C0
C45

Fig. S4

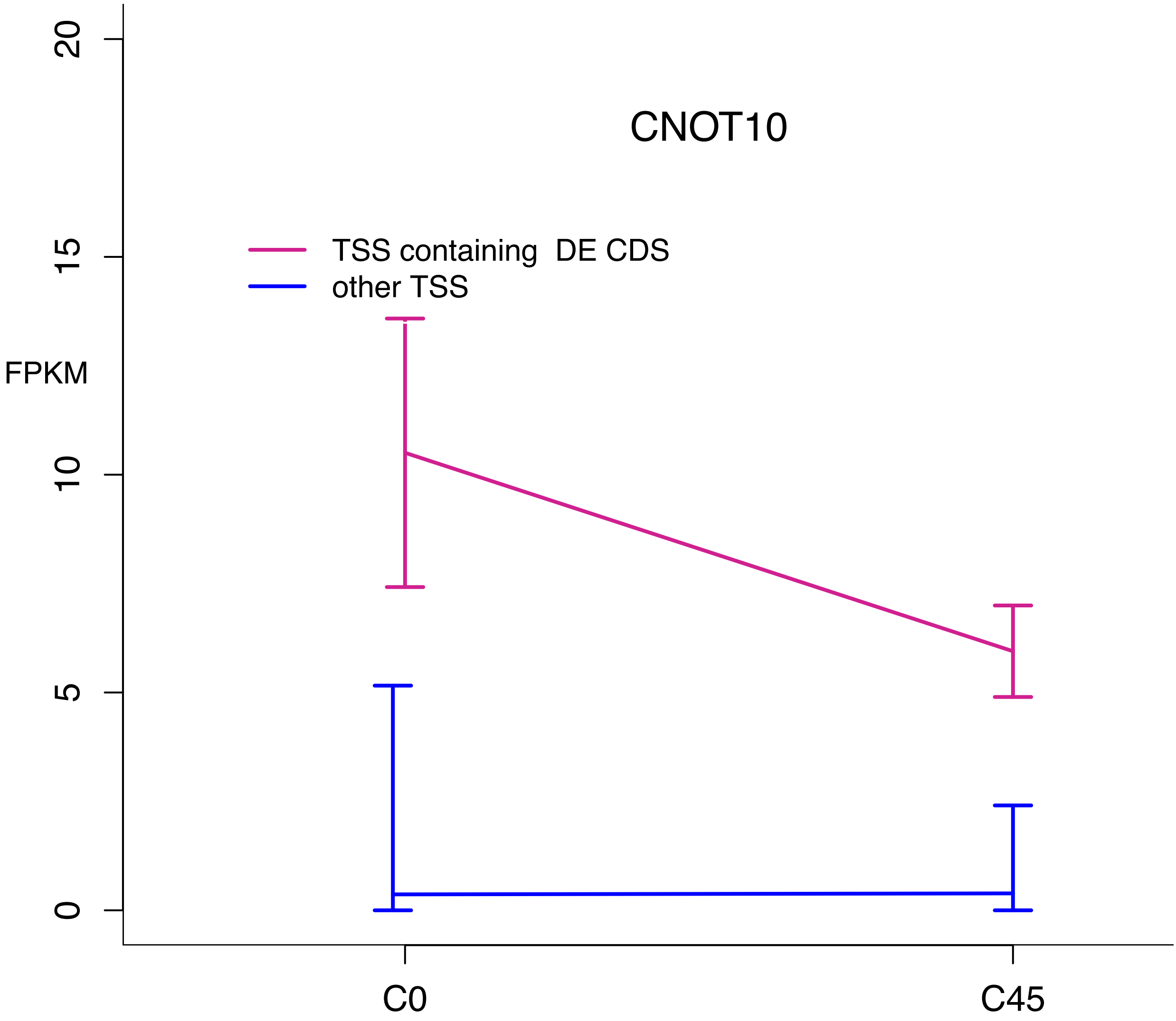


Fig. S5

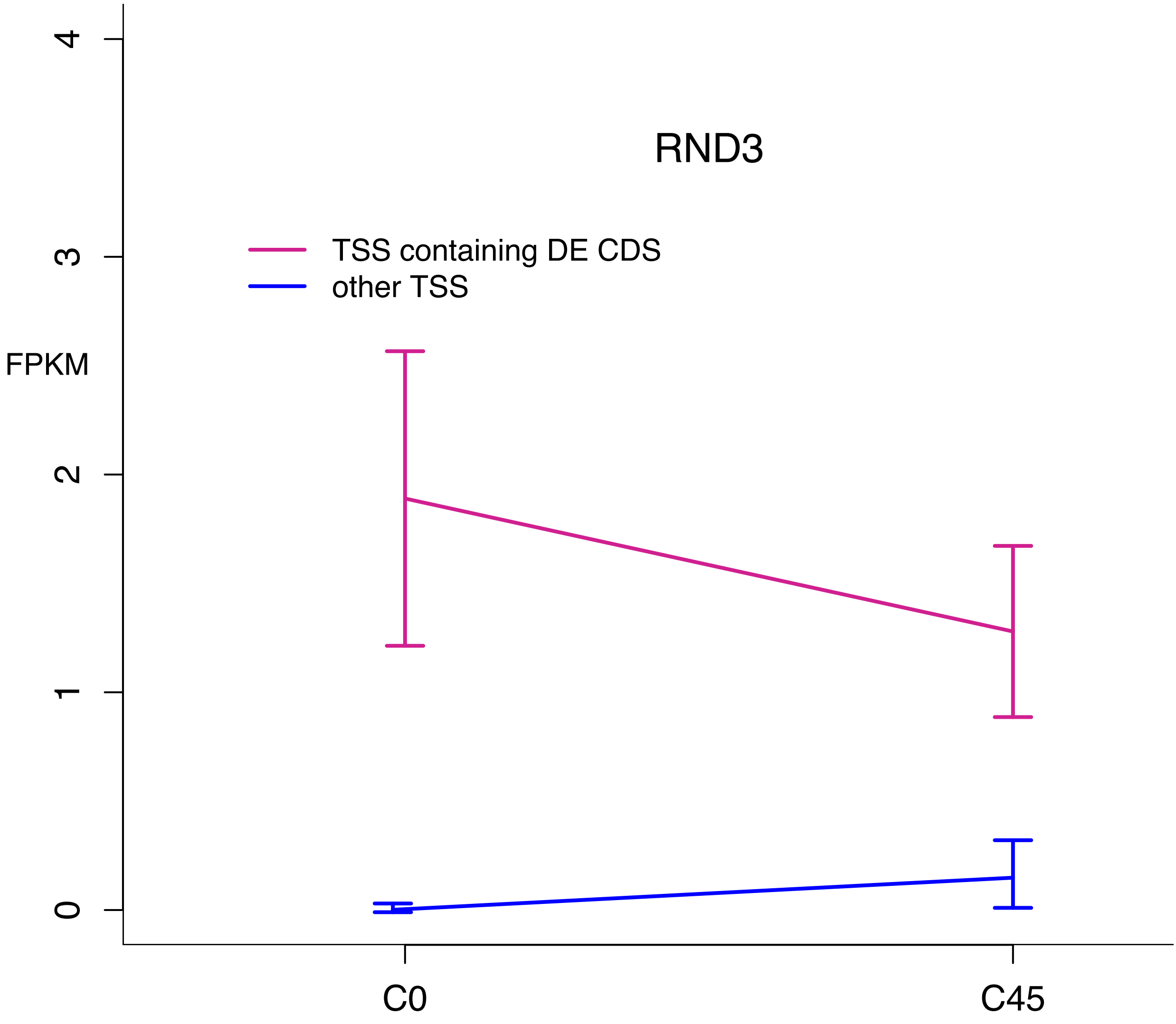


Fig. S6

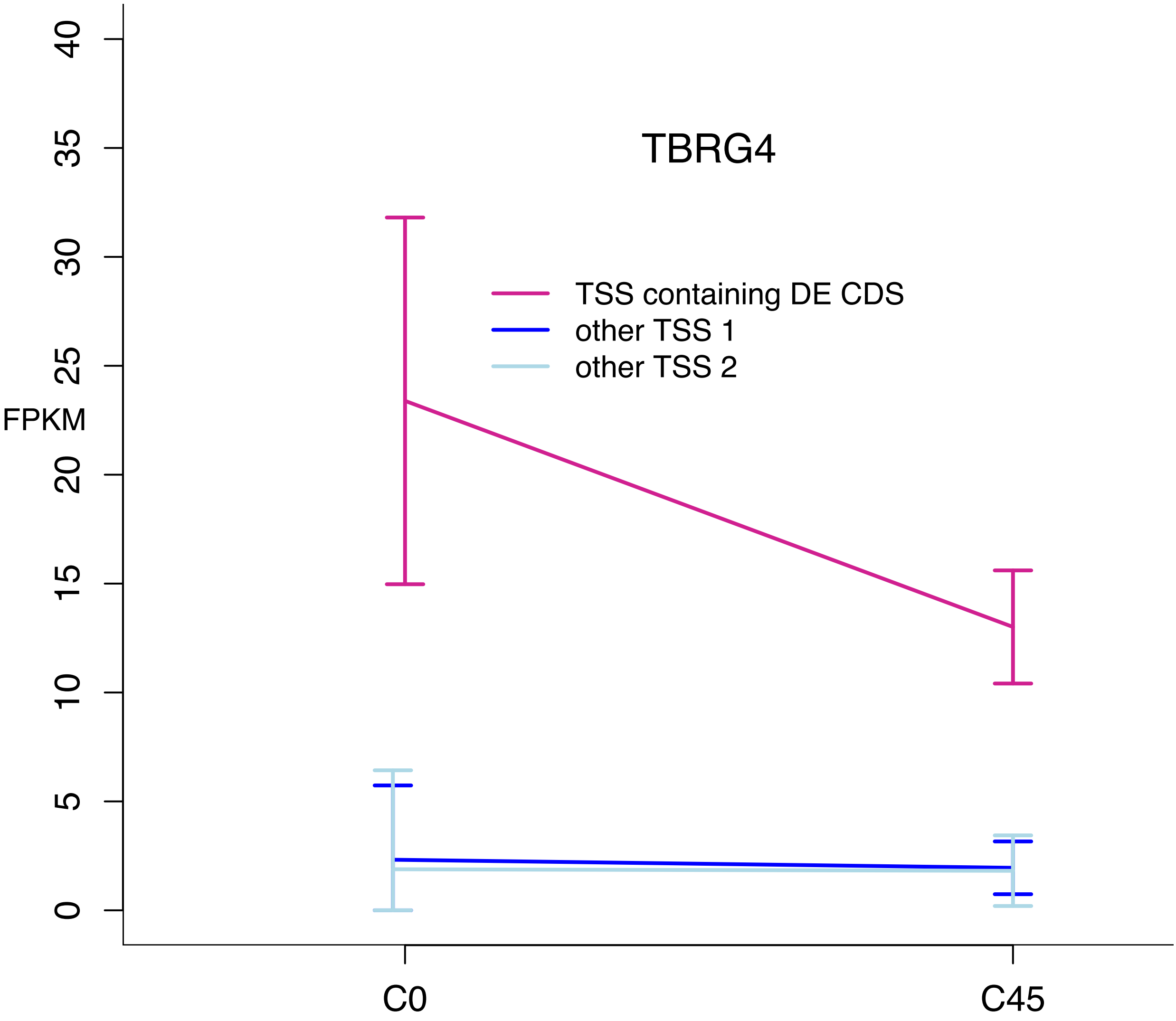
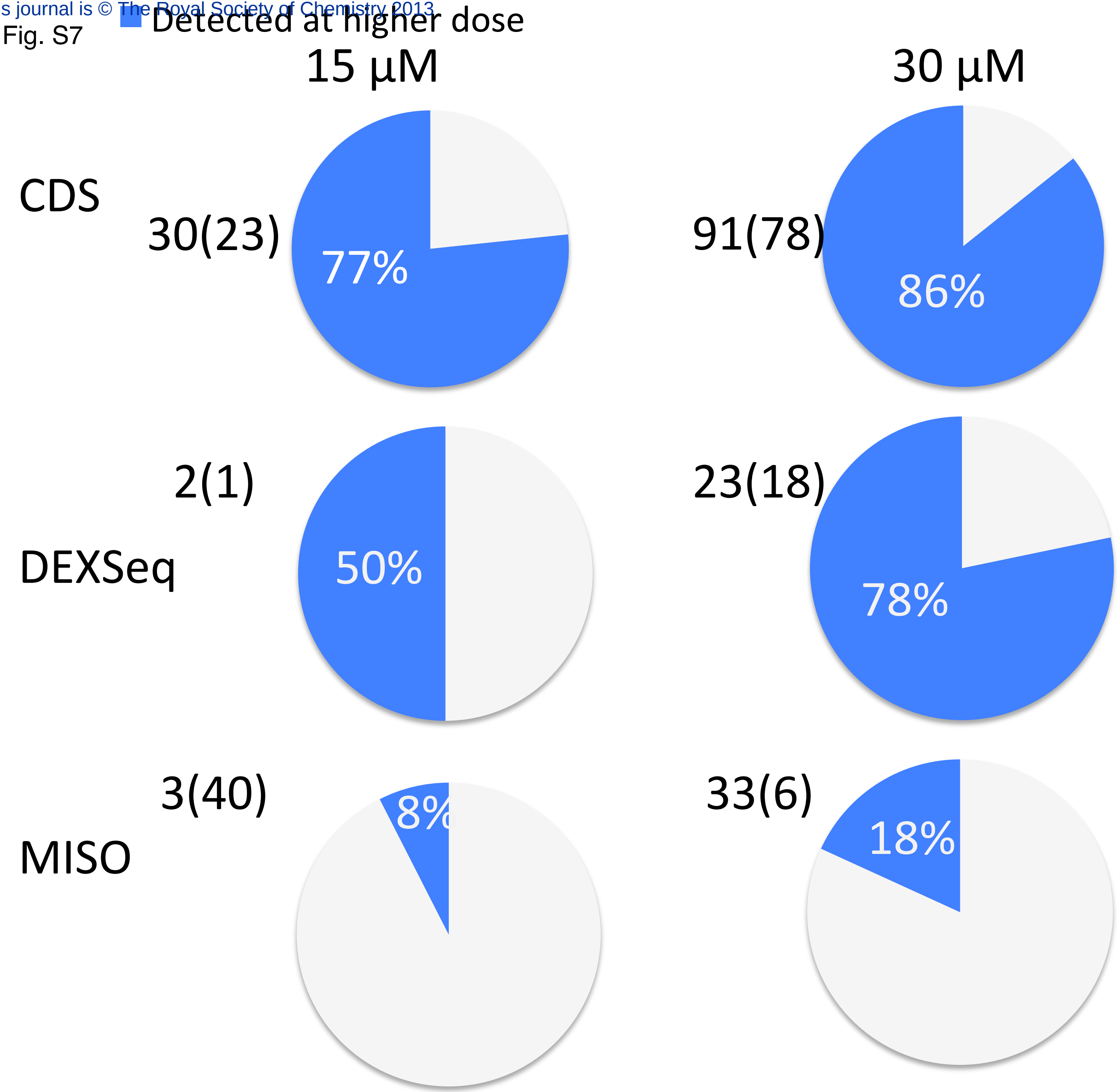

TBRG4
FPKM
0
5
10
15
20
25
30
35
40
C0
C45
TSS containing DE CDS
other TSS 1
other TSS 2

Fig. S7


🟦 Detected at higher dose
15 µM
30 µM
CDS
30(23)
77%
91(78)
86%
2(1)
DEXSeq
50%
23(18)
78%
3(40)
MISO
8%
33(6)
18%