SUPPLEMENTAL DATA

Supplemental Figure 1: Properties of the screen

A. GFP/RFP ratio at three different time points from one micro titer plate. The log of the GFP and RFP ratio at day, 0, 1, 2, and 3 is plotted. The data points representing pyrvinium pamoate are indicated.
B. Regression analysis of the screen. The slope of the compounds shown in panel A is shown. Substances outside the 3 fold standard deviations were selected for further analysis.
C. Regression slope of all compounds in the screen.

Supplemental Figure 2: Screen results

A. Schematic representation of the number of hits from the two stable cell lines.
B. List of 22 compounds that changes alternative splicing in both cell lines in the primary screen.

Supplemental Figure 3: CLUSTAL W alignment of HTR2c pre-RNAs.

The sequences corresponding to the regulated RNA shown in Figure 6 were aligned with CLUSTAL W. Changes are highlighted in yellow.

Supplemental Figure 4: RT-PCR validation of changes in alternative splicing caused by pyrvinium pamoate after six hours.

The highest-ranking changes caused by six hours treatment of cells with pyrvinium pamoate were tested by RT-PCR using primers in the flanking constitutive exons.

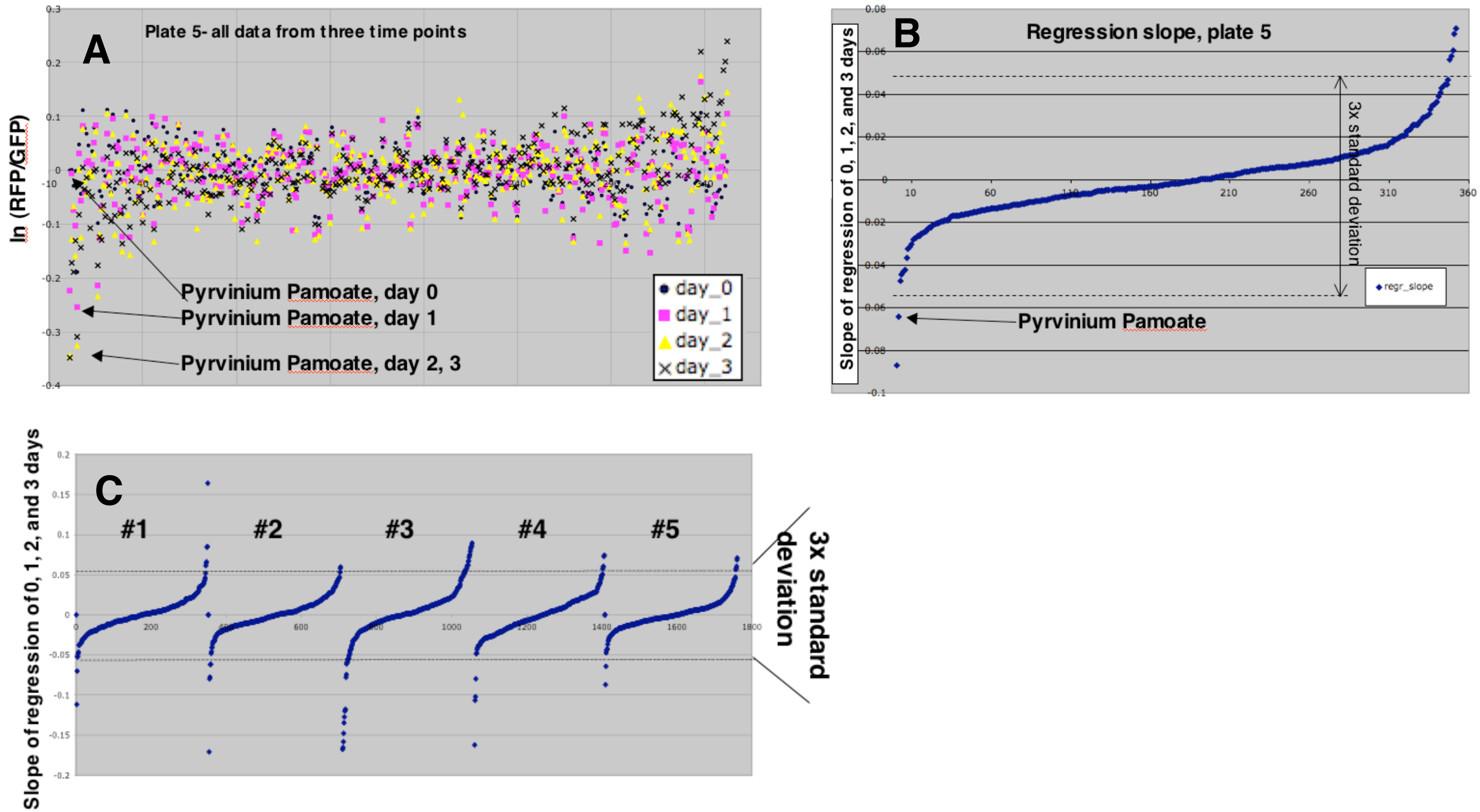
Supplemental Figure 5: RT-PCR validation of Changes in alternative splicing caused by pyrvinium pamoate after sixteen hours.

The highest-ranking changes caused by six hours treatment of cells with pyrvinium pamoate were tested by RT-PCR using primers in the flanking constitutive exons.

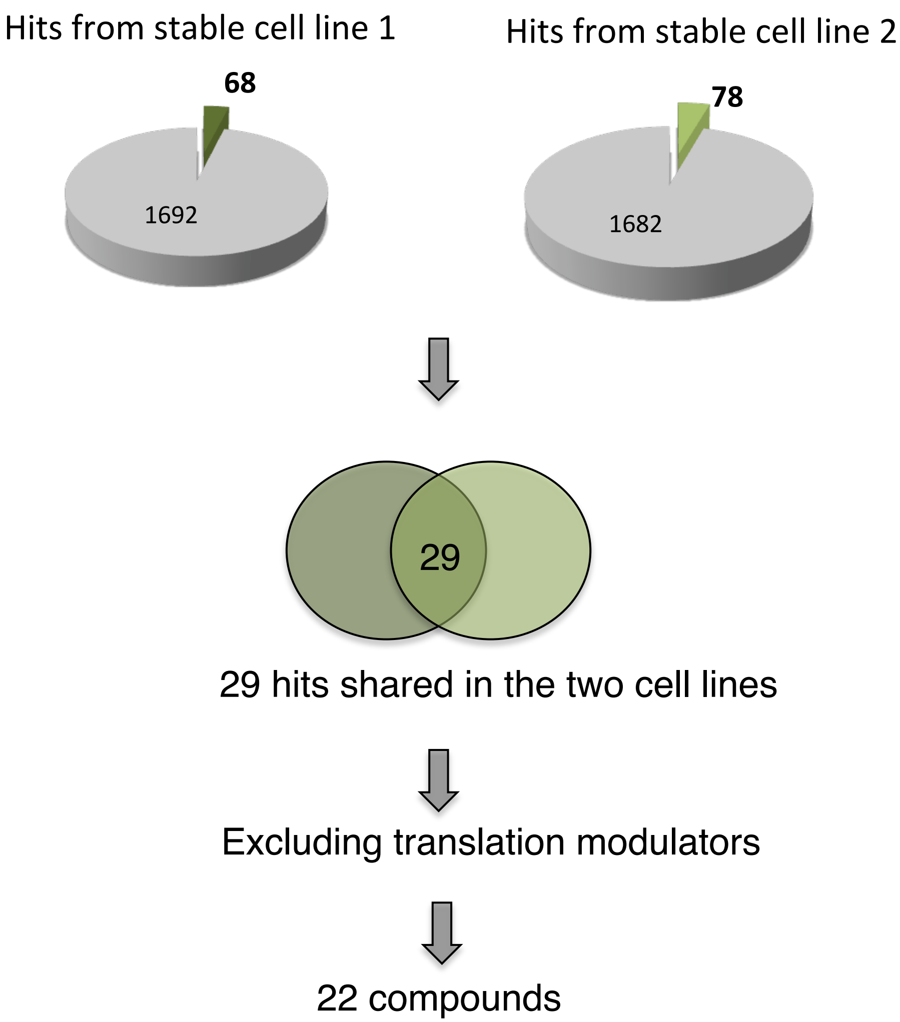
Supplemental Figure 6: Pathways changed by pyrvinium pamoate

Supplemental Figure 7: Excel files showing the results of the array experiments

Supplemental Figure 1



Supplemental Figure 2



List of compounds active in both cell lines

(-)-Isoproterenol hydrochloride
Ajmalicine
Corynanthine hydrochloride
Diltiazem
Doxorubicin hydrochloride
Ergocryptine-alpha
Fluvastatin
Harmaline
Hexetidine
Lysergol
Menadione
Mycophenolic acid
Nitrarine dihydrochloride
Phenazopyridine
Pyrvinium pamoate
Quinacrine
Rauwolscine hydrochloride
Reserpine
S(+)-Terguride
Scoulerine
Simvastatin
Yohimbinic acid monohydrate

Supplemental Figure 3: Alignment of the known serotonin receptor 2C pre-mRNAs

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human           TACGTAATCCTATTGAGCATAGCCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
chimp           TACGTAATCCTATTGAGCATAGCCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
gorilla         TACGTAATCCTATTGAGCATAGCCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
orangutan       TACGTAATCCTAT-GAGCATAGCCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 59
gibbon          TACGTAATCCTATTGAGCATAGCCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
rhesus          TACGTAATCCTATTGAGCATAGCCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
marmoset        TACGTAATCCTATTGAGCATAGCCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
mouse           TACGTAATCCTATTGAGCATAGCCGGTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
rat             TACGTAATCCTATTGAGCATAGCCGGTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
Nakedmole       TACGTAATCCTATTGAGCATAGTCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
rabbit          TACGTAATCCTATTGAGCATAGCCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
pig             TACGTAATCCTATTGAGCATAGCCGTTTCAATTCACGGACTAAGGCCATCATGAAGATTG 60
cow             TACGTAATCCTATTGAGCATAGCCGTTTCAATTCACGGACTAAGGCCATCATGAAGATTG 60
horse           TACGTAATCCTATTGAGCATAGCCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
dog             TACGTAATCCTATTGAGCATAGCCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
panda           TACGTAATCCTATTGAGCATAGCCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
microbat        TACGTAATCCTATTGAGCATAGCCGTTTCAATTCGCGGACTAAGGCCATCATGAAGATTG 60
                ************* ******** ** ******** *************************

human           CTATTGTTTGGGCAATTTCTATAGGTAAATAAAACTTTTTGGCCATAAGAATTGCAGCGG 120
chimp           CTATTGTTTGGGCAATTTCTATAGGTAAATAAAACTTTTTGGCCATAAGAATTGCAGCGG 120
gorilla         CTATTGTTTGGGCAATTTCTATAGGTAAATAAAACTTTTTGGCCATAAGAATTGCAGCGG 120
orangutan       CTATTGTTTGGGCAATTTCTATAGGTAAATGAAACTTTTTGGCCATAAGAATTGCAGCGG 119
gibbon          CTATTGTTTGGGCAATTTCTATAGGTAAATAAAACTTTTTGGCCATAAGAATTGCAGCGG 120
rhesus          CTATTGTTTGGGCAATTTCTATAGGTAAATAAAACTTTTTGGCCGTAAGAATTGCAGCGG 120
marmoset        CTATTGTTTGGGCAATTTCTATAGGTAAATAAAACTTTTTGGCCGTAAGAATTGCAGCGG 120
mouse           CCATCGTTTGGGCAATATCAATAGGTAATTA----TACCTGGCCA-TAGAATTGCAGCGG 115
rat             CCATCGTTTGGGCAATATCAATAGGTAAATA----TACCTGGCCA-TAGAATTGCAGCGG 115
Nakedmole       CTATTGTTTGGGCAATTTCTCTAGGTAAATAAAC-CTCTGGGCCATTAGAATTGCAACGG 119
rabbit          CTATCGTTTGGGCAATTTCTATAGGTAACTAAACTTTCTTGGCCATTAGAATTGCAGCGG 120
pig             CTATTGTTTGGGCAATTTCTTTAGGTAATGAACT-TTCTTGGCCAGTAGAATTGCAGCGG 119
cow             CTATTGTTTGGGCAATTTCTTTAGGTAATTAACT-TTCTTGGCCAGTAGAATTGCAGCGG 119
horse           CTATAGTTTGGGCAATTTCTATAGGTAATTAACT-TTCTTGGCCATTAGAATTGCAGCGG 119
dog             CTATTGTTTGGGCAATTTCGATAGGTAAATAACG-TTCTTGGCCATTAGAATTGCAGCGG 119
panda           CTATTGTTTGGGCAATTTCGATAGGTAAATAACT-TTCTTGGCCATTAGAATTGCAGCGG 119
microbat        CTATCGTTTGGGCAATTTCTATAGGTAAATGACT-TTCCTGGCCATTAGAATTGCAAGGG 119
                * ** *********** **  *******            ****   *********  **

human           CTATGCTCAATACTTTCGGATTATGTA 147
chimp           CTATGCTCAATACTTTCGGATTATGTA 147
gorilla         CTATGCTCAATACTTTCGGATTATGTA 147
orangutan       CTATGCTCAATACTTTCGGATTATGTA 146
gibbon          CTATGCTCAATACTTTCGGATTATGTA 147
rhesus          CTATGCTCAATACTTTCGGATTATGTA 147
marmoset        CTATGCTCAATACTTTCGGATTATGTA 147
mouse           CTATGCTCAATACCTTCGGATTATGTA 142
rat             CTATGCTCAATACCTTCGGATTATGTA 142
Nakedmole       CTATGCTCAATACCTTCGGATTATGTA 146
rabbit          CTATGCTCAATACCTTCGGATTATGTA 147
pig             CTATGCTCAATACTTTCGGATTATGTA 146
cow             CTATGCTCAATACTTTCGGATTATGTA 146
horse           CTATGCTCAATACTTTCGGATTATGTA 146
dog             CTATGCTCAATACTTTCGGATTATGTA 146
panda           CTATGCTCAATACTTTCGGATTATGTA 146
microbat        CTATGCTCAATACCTTCGGATTATGTA 146
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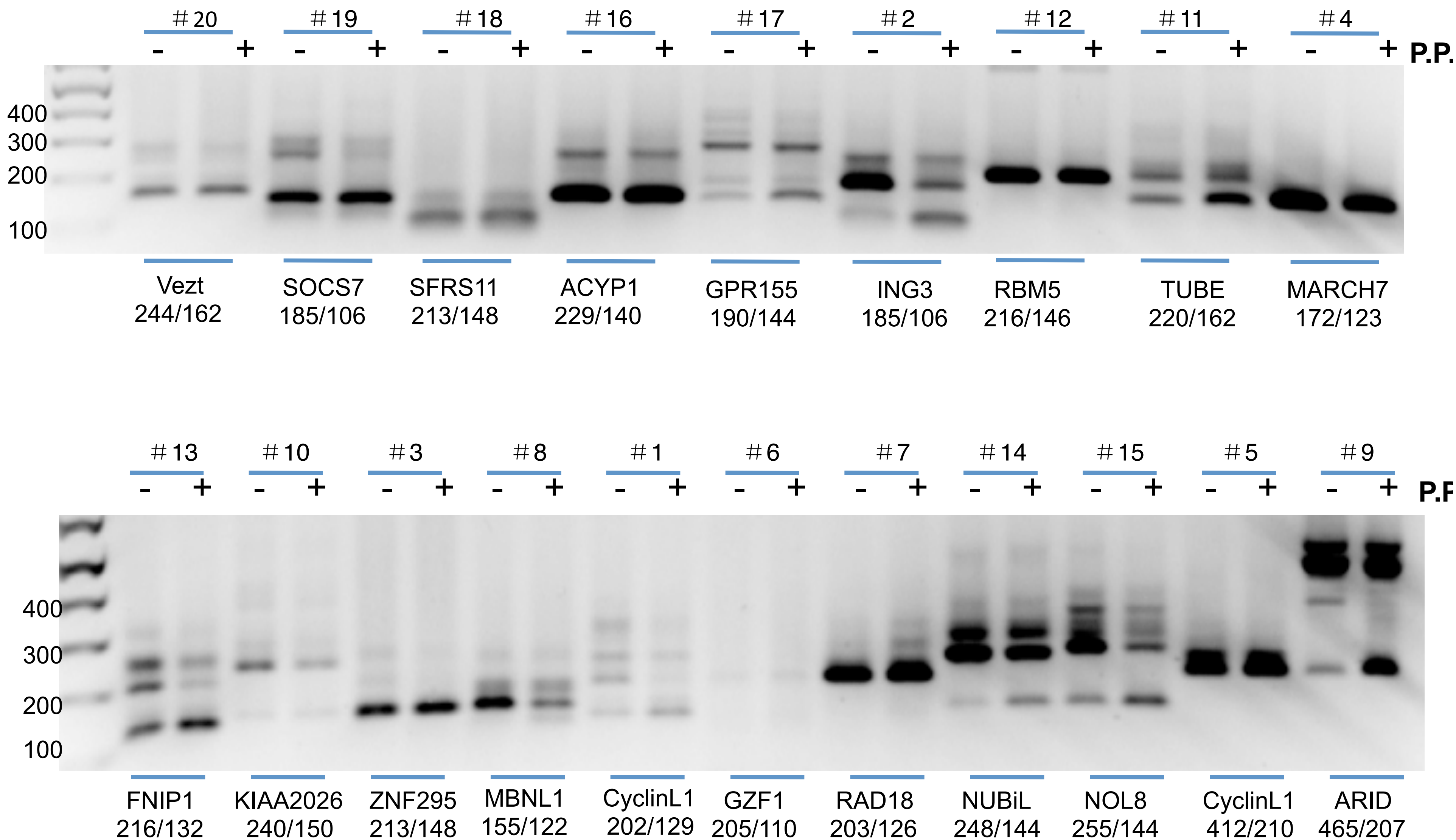
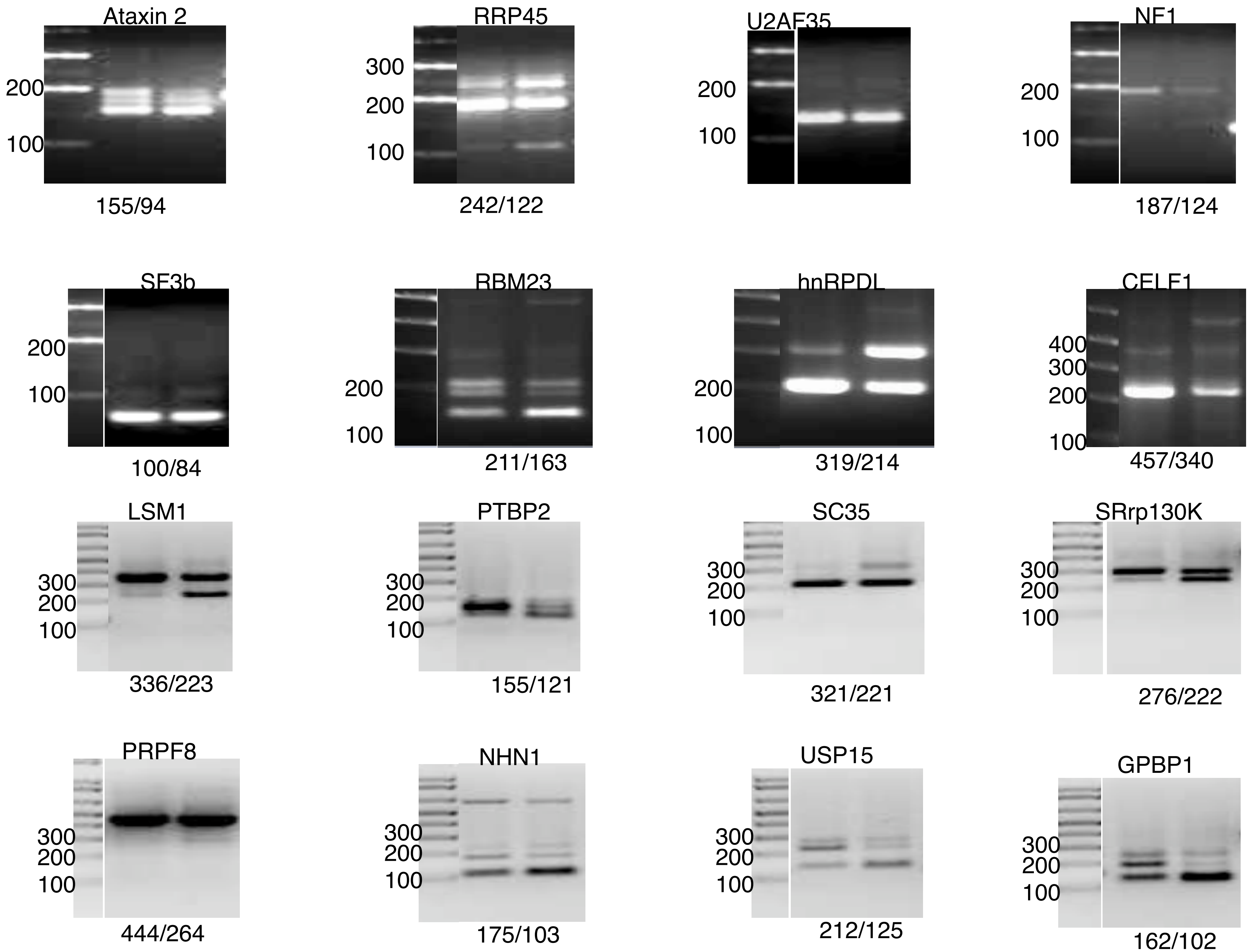
Supplemental Figure 4



#: Rank in the cassette exon array data

Supplemental Figure 5

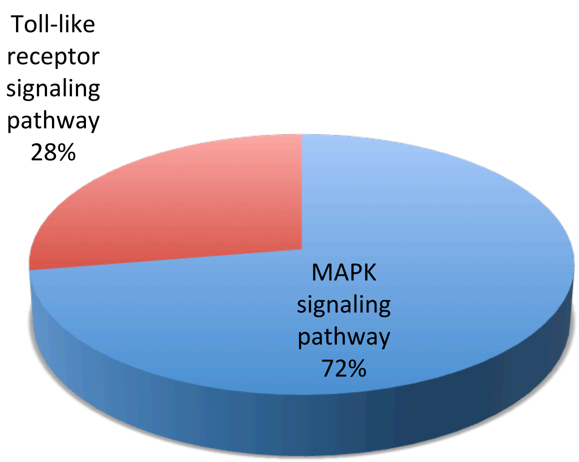


Supplemental Figure 6

After 6 hours

176 gene changes

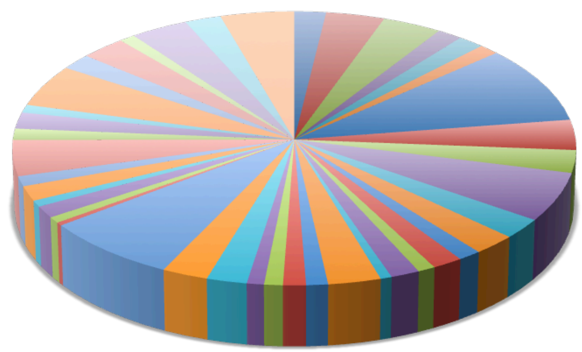
379 changes in splicing



After 18 hours

1120 cassette exons

5284 regulated genes



Regulated genes

p53 signaling pathway
Spliceosome
Axon guidance
Basal cell carcinoma
Inositol phosphate metabolism
Valine, leucine and isoleucine degradation
Pathways in cancer
Cell cycle
Fc gamma R-mediated phagocytosis
Endocytosis
ECM-receptor interaction
Small cell lung cancer
Amino sugar and nucleotide sugar metabolism
RNA degradation
Fructose and mannose metabolism
Hedgehog signaling pathway
Glycosaminoglycan biosynthesis - chondroitin sulfate
Melanogenesis
Fatty acid metabolism
Lysine degradation
Basal transcription factors
Propanoate metabolism
Phosphatidylinosit
ol signaling system
Prostate cancer
MAPK signaling pathway
Sulfur metabolism
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis
Glycerolipid metabolism
Glycosaminoglycan biosynthesis - heparan sulfate
NOD-like receptor signaling pathway
Bladder cancer
Wnt signaling pathway
N-Glycan biosynthesis
PPAR signaling pathway
Base excision repair
Calcium signaling pathway
Adipocytokine signaling pathway
Pyrimidine metabolism
Ether lipid metabolism
Neurotrophin signaling pathway
Glycerophospholipid metabolism
Purine metabolism