

Supplementary Information

S1- Materials and methods

Mapping 319 genes onto the apoptosis pathway

A transcriptome-wide scan for the co-occurrence of Alu exonization, editing and antisense at the single-exon level had revealed a set of 319 genes, enriched for apoptosis ([Mandal et al. 2013](#)). Because enrichment analyses are heavily confounded by background data used for normalization, we found it prudent to ask how many of these 319 genes are actually involved in apoptotic cell death and where in the apoptosis pathway they mapped. To begin with, we listed all the important steps in this pathway: arrest of division is usually the first cellular response to any apoptotic trigger, which is followed by cessation of DNA damage repair (DDR), initiation of p53 signaling and polymerase halt. At the later stages of apoptosis, endonucleases are activated and nuclear DNA fragmentation occurs, mitochondrial ROS may be generated and many proteins are ubiquitinated and marked for proteasomal degradation. The final stages are marked by blebbing and membrane flipping to attract phagocytes to engulf the dying cell. A flow diagram was constructed incorporating all the nodes and pathways involved in apoptosis. We performed an extensive, systematic manual curation of these 319 genes using published literature and other web resources with the intent of binning them in the major events of apoptosis listed above. We asked i.) which of these genes are involved in apoptosis, and ii.) for those that are involved, where in the pathway they occur. We mapped all those genes which interact with known apoptotic genes (like *BCL2* family members) or contain known motifs (e.g., WD repeats), or are a part of apoptotic signaling pathway, or have apoptosis (or any of its sub-readouts) as the major phenotype upon their perturbation. Many of the genes that we mapped are involved in cell division, DNA replication and ubiquitination; perturbations in these genes often lead to cell cycle arrest, impaired DDR and protein misfolding/degradation, respectively. Many genes are involved in multiple, interlinked functions but we had mapped them uniquely. Mapping was based on the major function of the gene, its mutant phenotype or the function affected by perturbation (knockdown, knock-out or over-expression), disease association and evolutionary conservation (wherever orthologues could be reliably ascertained). To check the accuracy of our curation and mapping, we used GeneMANIA (attributes: physical and genetic interactions, co-localization, same biological pathway) to fetch the top 100 interacting partners of our 91 apoptosis genes. There was no overlap between these interacting partners and the 228 genes that we have not mapped to apoptosis, reassuring us that our mapping stringency has been optimal.

Disparities in *CYP20A1* annotation across different portals

There exists a huge disparity in the annotation of *CYP20A1* transcript isoforms in different databases. There are 10, 9 and 4 annotated isoforms in Ensembl, NCBI and UCSC, respectively. All the four UCSC isoforms (GENCODE v24) are mapped onto a single RefSeq transcript NM_177538. Among the 10 isoforms listed in Ensembl, three are targeted for NMD and another three are processed transcripts. In NCBI, out of the 9 transcripts, two are miscellaneous RNAs, six are predicted protein-coding and only one is experimentally validated (NM_177538.2), which is the largest isoform and also annotated as the principal isoform (by APPRIS). It matches with the longest isoform on UCSC in terms of length (10.94kb) as well. Ensembl previously mapped it to ENST00000611416.4 (which has been removed from the current build - release 94) but currently maps this onto its transcript which is just 1949bp long. Notably, the size of the *CYP20A1* protein remains the same (462aa) on every portal. These annotations are based on microarray or RNA-seq data, both of which have their own limitations. Array imputes the expression based on one or few probes and cross hybridization is a potential confound. RNA-seq gives an FPKM value for a transcript regardless of the reads aligning over the entire length of the transcript. The scenario is further complicated because 65% of the long-3'UTR of *CYP20A1*(*CYP20A1*_Alu-LT) is made up of Alu repeats which is typically not captured by array probes

and where sequencing reads do not map uniquely. Also, from these data, one cannot discriminate if the full length 3'UTR containing isoform is expressed as a single transcript or as multiple short RNAs.

Confirmation of *CYP20A1*_Alu-LT by Sanger sequencing

The sequencing reaction was carried out using Big dye Terminator v3.1 cycle sequencing kit (ABI, 4337454) in 10 μ l volume (containing 2.5 μ l purified DNA, 0.8 μ l sequencing reaction mix, 2 μ l 5X dilution buffer and 0.6 μ l forward/ reverse primer) with the following cycling conditions - 3 mins at 95°C, 40 cycles of (10 sec at 95°C, 10 sec at 55°C, 4 mins at 60°C) and 10 mins at 4°C. Subsequently, the PCR product was purified by mixing with 12 μ l of 125mM EDTA (pH 8.0) and incubating at RT for 5 mins. 50 μ l of absolute ethanol and 2 μ l of 3M NaOAc (pH 4.8) were then added, incubated at RT for 10 mins and centrifuged at 3800rpm for 30 mins, followed by invert spin at <300rpm to discard the supernatant. The pellet was washed twice with 100 μ l of 70% ethanol at 4000rpm for 15 mins and supernatant was discarded by invert spin. The pellet was air dried, dissolved in 12 μ l of Hi-Di formamide (Thermo fisher, 4311320), denatured at 95°C for 5 mins followed by snapchill, and linked to ABI 3130xl sequencer. Base calling was carried out using sequencing analysis software (v5.3.1) (ABI, US) and sequence was analyzed using Chromas v2.6.5 (Technelysium, Australia).

miRNA expression in MCF-7 cells

We wanted to use publicly available expression data to find out which of the 994 miRNAs with predicted MREs on *CYP20A1*_Alu-LT 3'UTR, are expressed in our study system. In order to reduce false positives, we decided to take a consensus of miRNAs found to be expressed in MCF-7 in both microarray (doi:10.1371/journal.pone.0049067) as well as NGS (GSE68246) datasets. In the microarray data, 104 out of our 994 miRNAs of interest were expressed (averaged signal range 7.6 to 974.63). miRNome data of MCF-7 monolayer culture (using Illumina TruSeq small RNA on HiSeq2000) revealed the expression of 102 out of our 994 miRNAs.

Although we found around 100 (out of 994) miRNAs to be expressed in each of these data yet there was absolutely no overlap between the two sets.

Ka/Ks calculation

Protein sequence IDs of *CYP20A1* ortholog for each species was identified as the first hit of NCBI pBLAST using human NP-ID as the query term. The NM-IDs of corresponding mRNAs were also obtained from NCBI and sequences were fetched for these NP and NM-IDs. For a pair of orthologous genes, the protein-coding DNA alignment between human and each of the other species was constructed using ParaAT (Zhang et al. 2012). The protein sequence information was used to match each triplet codon with its respective amino acid. Aligned coding DNA between a pair (human vs. another species) was provided as an input to KaKs_Calculator (Zhang et al. 2006). Model averaging (MA), which takes an average of substitution rates of seven models, was used as a maximum likelihood method to calculate the Ka/Ks value.

RT-qPCR

RT-qPCR was performed in 10 μ l volume using 5 μ l 2X SYBR Green I master mix (Kappa; KK3605), 0.2 μ l each of 10 μ M forward and reverse primers and cDNA (stock: ~80ng/ μ l RNA equivalent, diluted 1:40 in the final reaction). The reaction was carried out in Roche Light Cycler 480 (USA) and the cycling conditions were: 95°C for 3', 45 cycles of (95°C for 30'', 58°C for 30'', 72°C for 40''), 72°C for 3'; followed by melting: 95°C for 5'', 65°C for 1'. Melting curves were confirmed to contain a single peak and the fold change was calculated by $\Delta\Delta$ Ct method. MIQE guidelines were followed for data analysis.

Authentication of MCF- 7 Cell lines

We had authenticated the cell line identity by STR profiling. The cells were routinely screened for mycoplasma using MycoAlert Mycoplasma detection kit (Lonza, LT07-218.) as per manufacturer's protocol and confirmed to be free from contamination. Additionally, 16S rRNA PCR was performed using both genomic DNA from cells and cell-free DNA from spent media to confirm the absence of bacterial contamination.

Library preparation, RNA sequencing

Libraries were prepared following Illumina's TruSeq stranded total RNA kit (Illumina, RS-122-2202) protocol as per manufacturer's recommendations, using three biological replicates per experimental condition. Integrity of the RNA samples was checked on Agilent 2100 Bioanalyzer (Agilent RNA 6000 Nano Kit); RIN values ranged from 8.6-9.6 for our samples. 500ng of total RNA was taken in a volume of 10 μ l and ribosomal RNA was removed using Ribozero depletion kit. Briefly, total RNA was denatured at 68°C for 5', followed by removal of rRNA using magnetic beads, subsequent clean up with RNAClean XP beads (Beckman Coulter, A63987), two consecutive washes in freshly prepared 70% ethanol and then elution (by heating at 94°C for 8'; to fragment and prime the RNA for cDNA synthesis). First strand synthesis was carried out with random hexamers using the following program (25°C for 10', 42°C and 70°C for 15' in each). This was followed by second strand cDNA synthesis at 16°C for 1hr for elimination of the template RNA, replacement of dUTP by dTTP and to finally produce blunt-ended cDNA, which was then cleaned up using AMPure XP beads (Beckman Coulter, A63881) and two washes in 80% ethanol. Next, the 3' ends of these blunt cDNA molecules were adenylated to create a single 'A' overhang (incubation at 37°C for 30', followed by 70°C for 5') such that these can form complementary base pairs with the 'T's on the adapter ends and also do not concatenate with each other to form chimera during adapter ligation. Then, adapters were ligated (30°C for 10') at both the ends of these double-stranded cDNA molecules so that these can hybridize to the probes on the sequencing flow-cell. The fragments with adapter ligated on both ends (libraries) were enriched and amplified using a 15-cycle PCR (the PCR primer cocktail anneal to both ends of the adapters; PCR conditions: 98°C for 30", 15 cycles of (98°C for 10", 60°C for 30", 72°C for 30"), final extension 72°C for 5'). This was followed by their clean up using AMPure XP beads and two washes in 80% ethanol. The libraries were quantified using Qubit fluorometer (Invitrogen) and subsequently validated on Bioanalyzer (Agilent DNA 1000 kit) to confirm the insert size. The size of the libraries was in the range of 260-280bp. This was followed by normalization, dilution of the libraries to 10nM and pooling 3 samples together. The 10nM libraries were denatured using NaOH, diluted to 8pM and clusters were generated on a paired end flowcell (1 pool/lane) using TruSeq PE cluster kit v3-cBOT-HS (Illumina, PE-401-3001) on cBot system. Paired end sequencing was carried out on HiSeq 2000 using TruSeq SBS kit v3-HS (200 cycles) (Illumina, FC-401-3001).

Library preparation Small RNA sequencing s

cDNA libraries were prepared as per manufacturer's protocol using TruSeq small RNA library prep kit (Illumina, RS-200-0012, RS-200-0024) from 1 μ g total RNA (RIN \geq 8.5). Briefly, 3' and 5' adapters were ligated, followed by enrichment of RNA fragments with adapter molecules on both ends. Reverse transcription followed by amplification using primers that anneal to the adapter ends created the cDNA libraries which was then purified and size selected (~147nt) to enrich for small RNAs by running on 6% native PAGE. Subsequently, the libraries were concentrated by ethanol precipitation and quality checked on Agilent technologies 2100 Bioanalyzer (USA) using DNA high sensitivity

chips (Agilent, 5067-4626). Using 10mM Tris-HCl (pH 8.5) libraries were normalized to 2nM, denatured and applied to cBot for cluster generation (Illumina, GD-401-3001), which were then sequenced on HiSeq2000 (Illumina, California, USA) using TruSeq SBS kit (Illumina, FC-401-3002) for 50 cycles.

Immunostaining

Cells were seeded on coverslips and cultured in 4-well plates for this experiment. Spent media was discarded and cells were briefly washed with 1X PBS (to remove residual media completely). The cells were then fixed with 4% PFA (freshly prepared in 1X PBS) for 20 minutes, followed by 3 washes in 1X PBS (5 minutes each) to completely remove traces of PFA. Blocking was done with 4% BSA containing 0.5% Triton-X (to permeabilize the cells) for 1hr with gentle rocking. Incubation in primary antibody (MAP2, Tuj1, NeuN, Nestin, GFAP; all diluted 1:200 in 0.1% BSA) was done overnight at 4°C on a rocker, except GFAP (1hr at RT), followed by 3 washes in 1X PBS (5 minutes each). Incubation with fluorescently labeled secondary antibody (diluted 1:1000 in 0.1% BSA) was performed for 1hr in the dark with shaking. This was followed by five washes in 1X PBS (5 minutes per wash) on a rocker. The coverslips were mounted in DAPI hardset (Vectashield, USA) on clean glass slides. Images were captured from 7-10 random fields using AxioImager.Z1 microscope (Carl Zeiss, Germany).

MTT assay

~10,000 cells were seeded per well in 96 well plate. Post treatment, freshly prepared MTT (HiMedia, TC191) solution (in 1X PBS) was added to the cells at a final concentration of 0.5mg/ml. Cells were incubated for 3hrs at 37°C in dark and then checked for formazan crystals under the microscope. After removing the solution, the formazan crystals were dissolved in 100µl DMSO (Sigma, D2650) at RT for 5-10 minutes and then absorbance was acquired at 560nm on Tecan.

Supplementary Information S2

Primers used in this study

Oligo Name	Sequence (5'→3')	Length (nt)	Tm (°C)	GC%	Amplicon size (bp)	
CYP20A1_5'UTR_FP1	TGCTGAAATCGTGTGCAC	19	56.8	47.4	222	used as the "5'UTR primer"
CYP20A1_5'UTR_RP1	CCAATTTAAGGCATAGCGTG	20	55.7	45.0		
CYP20A1_5'UTR_FP2	ACAGAGCACTACTAATCCT	20	55.5	45.0	214	
CYP20A1_5'UTR_RP2	CAGAGGAAAGAGCAATGGAT	20	55.7	45.0		
CYP20A1_CDS_FP3a	TCACTGTTGATGAGAATGGG	20	55.6	45.0	655	
CYP20A1_CDS_FP3b	GTATTGGTGAAGAGACTGCA	20	55.7	45.0	470	
CYP20A1_3'UTR_RP3	ACAAACATGATCCCCAACT	20	55.7	40.0		
CYP20A1_3'UTR_FP4	TCCCTTCCCCTTATTTTCT	20	54.1	40.0	166	
CYP20A1_3'UTR_RP4	TTGCTTTAAACTCCACCTCT	20	55.1	40.0		
CYP20A1_3'UTR_FP5	ACCAAGTGCAGATCAGATG	20	55.4	45.0	177	
CYP20A1_3'UTR_RP5	GCTCTCTTTTCTTGATTC	20	54.6	45.0		
CYP20A1_3'UTR_FP6	GAGAGCATAGAGGAATCAGC	20	55.7	50.0	216	
CYP20A1_3'UTR_RP6	GTGAAGTGGCCAAAATTTGA	20	55.5	40.0		
CYP20A1_3'UTR_FP7	TTGCTACTCTGAATGTAGGC	20	55.7	45.0	207	
CYP20A1_3'UTR_RP7	AAAATGAGCTGGGTGTGATG	20	56.9	45.0		
CYP20A1_3'UTR_FP8	GCCACTACACTCAGCTAATT	20	55.7	45.0	238	
CYP20A1_3'UTR_RP8	GTTGATTGAGCCCTCCTTAA	20	55.6	45.0		
CYP20A1_3'UTR_FP9	TTTGTAAAGACTTCAGGGAA	20	54.8	40.0	209	used as the "3'UTR primer"
CYP20A1_3'UTR_RP9	CACCGAACTGTAAACCAATT	20	54.7	40.0		
CYP20A1_3'UTR_FP10	GCTTCAGGGAATAGGCTTTT	20	56.3	45.0	177	
CYP20A1_3'UTR_RP10	TTGGGAGTAGAACTGGAAGA	20	55.7	45.0		
CYP20A1_Exon5_FP	GGAAGTCATTCGCTTCCAGA	20	64.3	50.0	277 (including exon6) & 196 (excluding exon 6)	for discriminating the transcript isoforms
CYP20A1_Exon8_RP	TATGCAACTGGCCAGAGAAA	20	63.3	45.0		
MALAT1_FP	CTTCCTGTGGCAGGAGAGAC	20	64.1	60.0	217	
MALAT1_RP	CGCTTGAGATTTGGGCTTTA	20	64	45.0		
GAPDH_FP	CGACCACTTTGTCAAGCTCA	20	58.99	50.0	138	
GAPDH_RP	CTTCCTCCTTGCTCCTTGCTG	21	59.77	52.4		
Spike-in FP	CCTCTTGATCTCACAGCTCAA	22	54.1	45.5	141	zebrafish lncRNA durga IVT product
Spike-in RP	CTGGAGACAATAGAAAGGCAAT	21	52.2	42.9		
ACTB_FP	GTCTTCCCCTCCATCGTG	18	63.3	61.1	126	
ACTB_RP	GATGGGGTACTTCAGGGTGA	20	63.8	55.0		
18S rRNA_FP	GGCCTGTAATTGGAATGAGTC	22	59.63	50	146	
18S rRNA_RP	CCAAGATCCAACACTACGAGCTT	21	58.63	47.6		

Antibodies used in WB

Sl.no.	Company	Catalogue no.	Antibody name	Dilution	Incubation
1	abcam	136094	Rabbit polyclonal to CYP20A1	1:500	Overnight at 4°, without shaking
2	Santa cruz	sc32233	Mouse monodonal anti GAPDH	1:2000	RT for 2 hrs, with shaking
3	abcam	176842	Rabbit monoclonal anti H3	1:1000	Overnight at 4°, with shaking
4	Santa cruz	sc 2004	anti rabbit IgG- HRP conjugated raised in goat	1:10,000	RT for 1 hour, with shaking
5	Santa cruz	sc 2005	anti mouse IgG- HRP conjugated raised in goat	1:10,000	RT for 1 hour, with shaking

S3- Results

Details of 3'RACE amplicons

Sl. No.	Band size (bp)	Primer	Seq. length (nt)	NCBI BLAST (hg38)			UCSC BLAT (hg38)
				Top hit	Identity (%)	Query cover (%)	
1	~1500	FP10	546	<i>CYP20A1</i> mRNA (NM_177538.2)	99	34	Mapped to <i>CYP20A1</i> 3'UTR with high score
3	>800	FP10	181	<i>CYP20A1</i> mRNA (NM_177538.2)	98	100	Mapped to <i>CYP20A1</i> 3'UTR with high score
5	>700	FP10	656	<i>CYP20A1</i> mRNA (NM_177538.2)	99	28	Mapped to <i>CYP20A1</i> 3'UTR with high score
7	>600	FP10	592	<i>CYP20A1</i> mRNA (NM_177538.2)	98	32	Mapped to Chr.6(-) with low score
9	~500	FP10	362	<i>MFSO1</i>	95	99	Mapped to <i>MFSO1</i> exon 16(3'UTR) with very high score
* no significant similarity was found with inner primer for any of the bands							

Sample-wise data generated in primary neurons RNA-seq experiment

Sample Name	Read 1	Read 2	Total data (in GB)
Untreated Set 1	7.8	7.9	15.7
Untreated Set 2	5.2	5.2	10.4
Untreated Set 3	11.8	11.2	23
HS treated + 1hr recovery Set 1	7.5	7.6	15.1
HS treated + 1hr recovery Set 2	8.8	8.8	17.6
HS treated + 1hr recovery Set 3	9.8	9.9	19.7
Tat treated + with recovery Set 1	7.2	7.3	14.5
Tat treated + with recovery Set 2	7.8	7.9	15.7
Tat treated + with recovery Set 3	6.8	6.9	13.7

Details of small RNA sequencing data

Sample	Total# of reads	% of 5'adapter +ve reads	% of 3'adapter +ve reads	# of reads post length filtering	# of reads post quality filtering	# of reads mapped
Pr. neuron Set 1	58507953	3.52	96.48	45873165	44297019	36653535
Pr. neuron Set 2	65588157	4.07	95.93	52787771	51364925	40148127
Pr. neuron Set 3	35409327	3.57	96.43	31974327	31426458	25742827
MCF-7 Set 1	49174023	3.76	96.24	43824299	41480248	36657502
MCF-7 Set 2	94370695	4.27	95.73	83124005	78450081	68786702
MCF-7 Set 3	59179178	8.05	91.95	50024126	46832593	46832593
MCF-7 Set 4	75156581	7.77	92.23	62389991	57937708	49243044
MCF-7 Set 5	62465203	8.56	91.44	54622720	51028477	42745201
MCF-7 Set 6	73825714	3.73	96.27	64923259	60749505	53921524

Details of nuclear cytosolic RT-qPCR

Experiment	Lowest(%) Ct difference between +RT and -RT					Ct value in NTC					Ct variation in Spike-in +RT(%)
	3'UTR primer	5'UTR primer	MALAT1	GAPDH	Spike-In	3'UTR primer	5'UTR primer	MALAT1	GAPDH	Spike-In	
1	ND	7.19	9.34	16.44	ND	ND	35.12	33.35*	39.5	35.39*	0.20
2	7.31	6.45	9.04	11.77	6.66	40.93*	33.99	33.3*	33.55	31.67*	0.06
3	2.87	7.40	8.93	13.03	4.88	32.03	32.56	33.49*	34.57	34.99*	0.58
4	3.81	6.71	9.56	14.13	4.85						1.09

Noise band was set at 1.00 in all experiments

among the total, nuclear and cytosolic fractions

* Ct value detected in one technical replicate only

ND = Ct Not detected in -RT; NTC = no template control

RT-qPCR for both experiment 3 and 4 were put in the same plate, therefore single NTC value

Supplementary Table Legends

Table S1: 91 genes (out of 319 containing the cooccurrence of Alu exonization, A-to-I editing and cis-antisense at single exon level) were mapped onto the apoptosis network. Out of these, 68 clustered around three central hubs (termed 'mito.', 'p53' and 'ubi').

Table S2: RNA-seq reads from *CYP20A1* different transcript isoforms mapping to 15928 single nuclei from different regions of human cerebral cortex.

Table S3: Expression of *CYP20A1* in human specific Rosehip neurons.

Table S4: 46 out of the 169 miRNAs with MREs on *CYP20A1* 3'UTR were present in miRDB as FuncMir. 19 of them occur exclusively in one or more primate species; some of them are also stress responsive.

Table S5: List of 994 miRNAs with predicted MREs on *CYP20A1*_ALu-LT. Out of these 994, 140 miRNAs have ≥ 10 MREs.

Table S6: 380 genes that show concordant directionality of expression as *CYP20A1*_Alu-LT in heat shock and Tat response.

Table S7: Major biological pathways enriched in toppfun analysis of 380 genes using Benjamini-Hochberg corrected FDR (q- value 0.05).

Supplementary Figure Legends

Figure S1: Out of 38300 genes, 512 have their longest 3'UTR greater than 6kb but only 98 of these contain Alu exonization events.

Figure S2: Snapshot (1-60aa) of multiple sequence alignment of CYP20A1 proteins depicts high degree of sequence conservation across vertebrates. As expected, the primates show the highest similarity to the human protein, which reduces as we move from the mammals to other non-mammalian vertebrates. The similarity is least for zebrafish which is also the most evolutionarily distant vertebrate from humans. (* exact aa conserved, : replaced by aa containing similar functional group)

Figure S3: UCSC track representing DNA conservation analysis of 10kb upstream of 5'UTR and 10 kb downstream of 3'UTR region among 20 mammals.

Figure S4: Expression of *CYP20A1* isoform in MCF-7 cells was checked by RT-qPCR using two different primer pairs and normalized to the geometric mean of β -actin, *GAPDH* and 18S rRNA. The error bars represent the SD of four biological replicates. Average of three technical replicates was taken for each biological replicate.

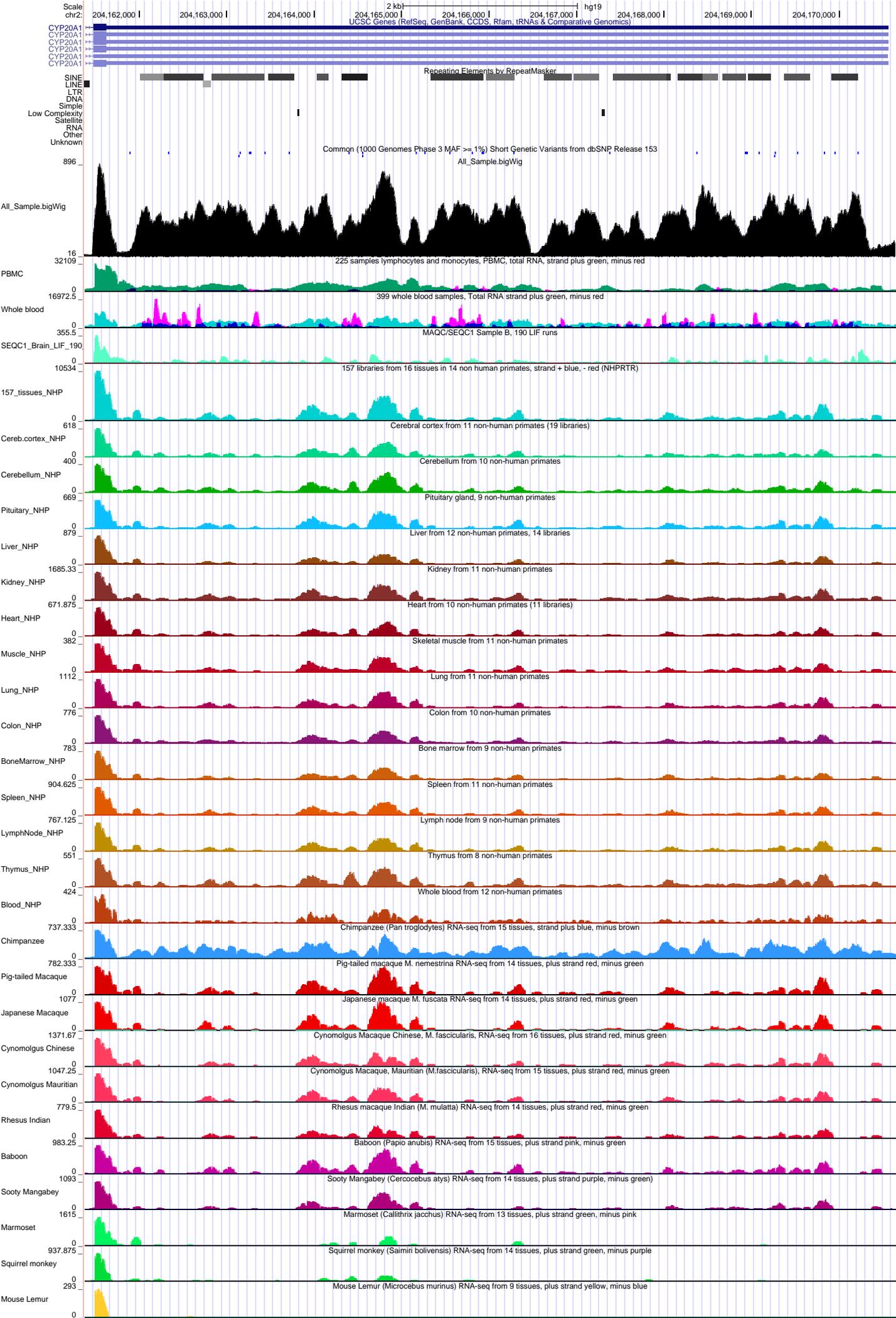
In contrast to the low expression of our transcript of interest, CYP20A1 protein is highly expressed in MCF-7 cells (~50% of GAPDH level).

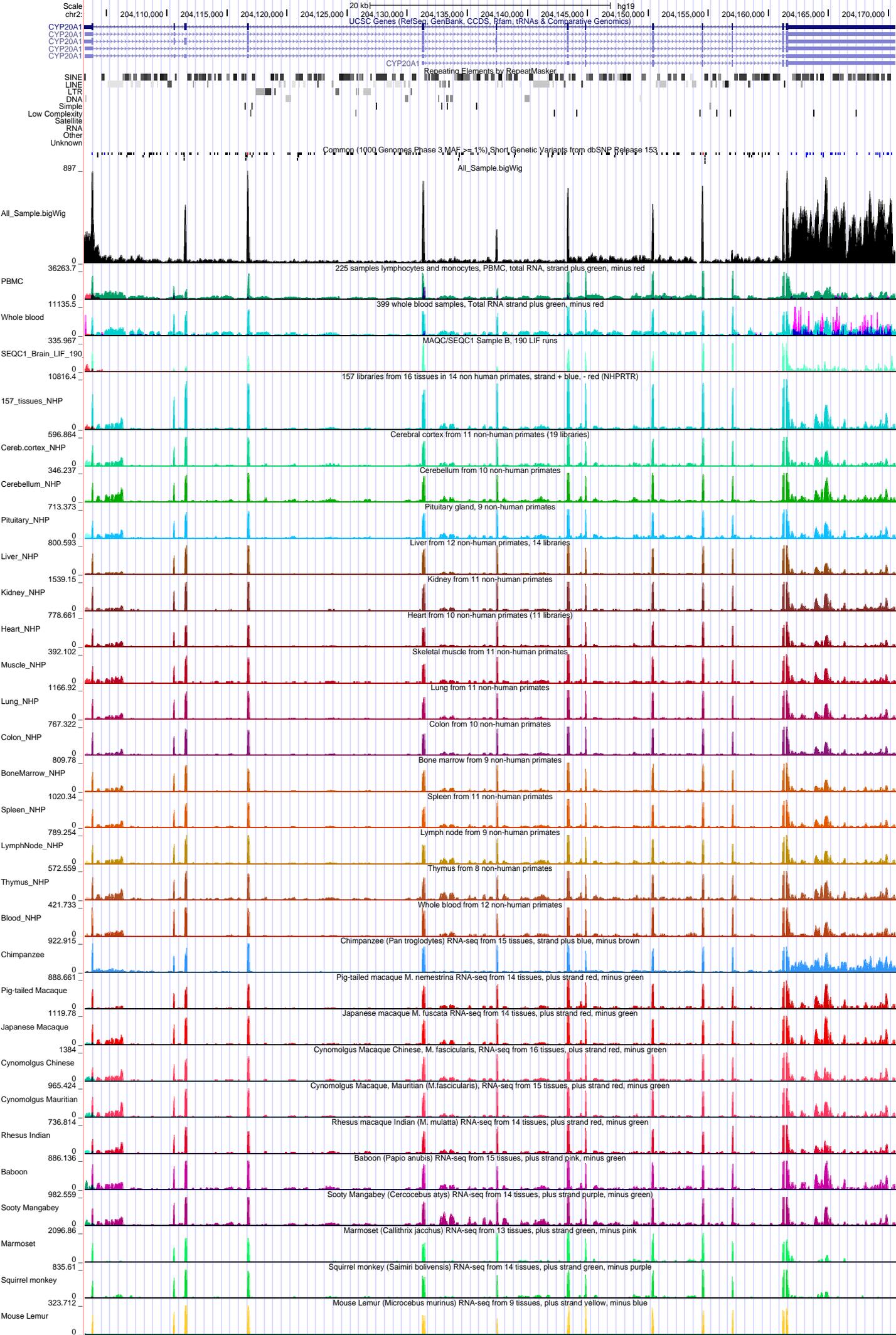
Figure S5: Transcript models built by Cufflinks based on RNA-seq data suggest that 3'UTRs longer than 9kb, occur for *CYP20A1*. TCONS_00101353 denotes the isoform studied by us.

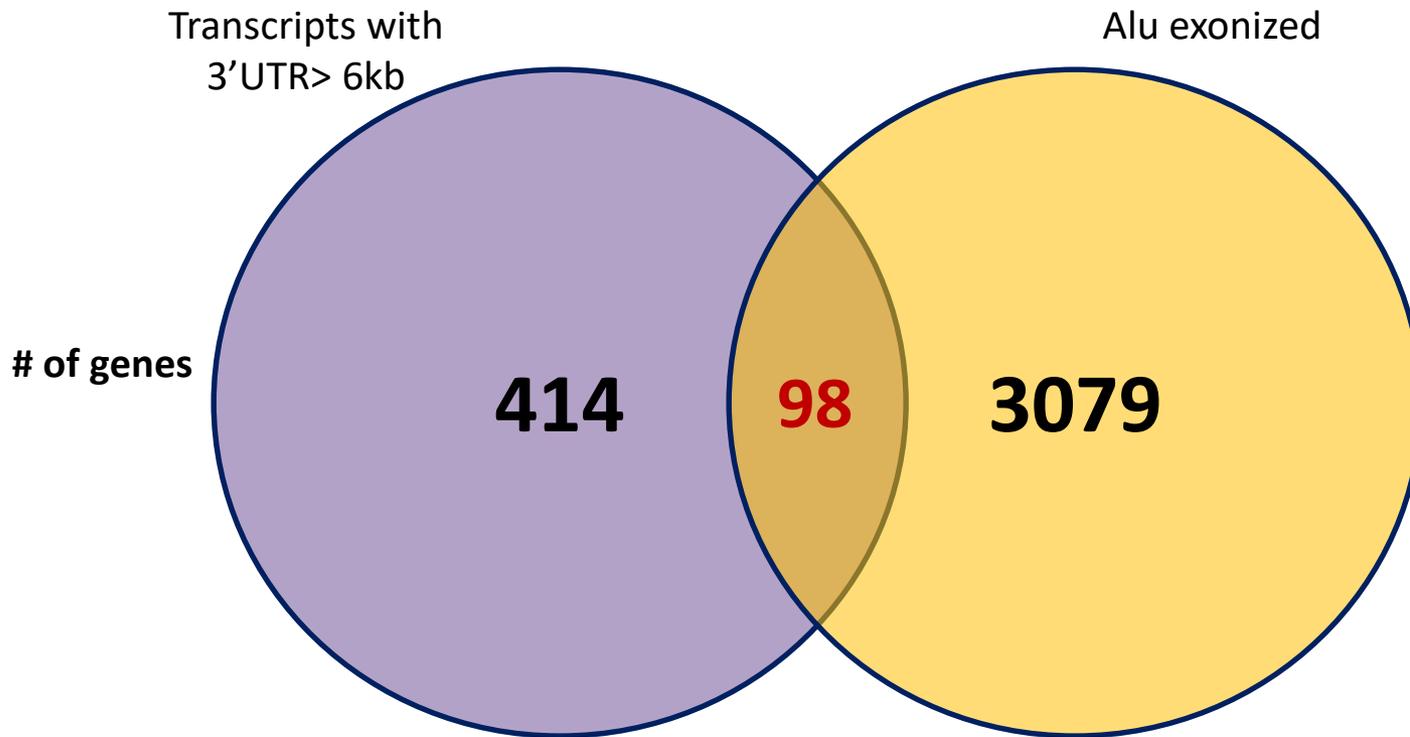
Figure S6: >95% of the cells are immuno-positive for neuronal markers like MAP2, Tuj1 and NeuN, while no cells are stained with GFAP, a glial cell marker, ensuring it is a pure neuron culture.

Figure S7: A. ‘Kill-curve’ of different doses of HIV1-Tat on primary neurons was prepared using viability and cytotoxicity parameters (ApoToxGlo assay; Promega) after 24hrs of treatment.

B. Subsequently, the protocol was standardized for 6hrs treatment. ~40% cell death occurs in primary neurons upon 100ng/ml Tat treatment for 6hrs. The data represent the mean (\pm S.D.) of two independent experiments.







- Average # of exonized Alus/3'UTR= 5.42
- 3'UTR length is not correlated to density of exonized Alus ($r= 0.25$)

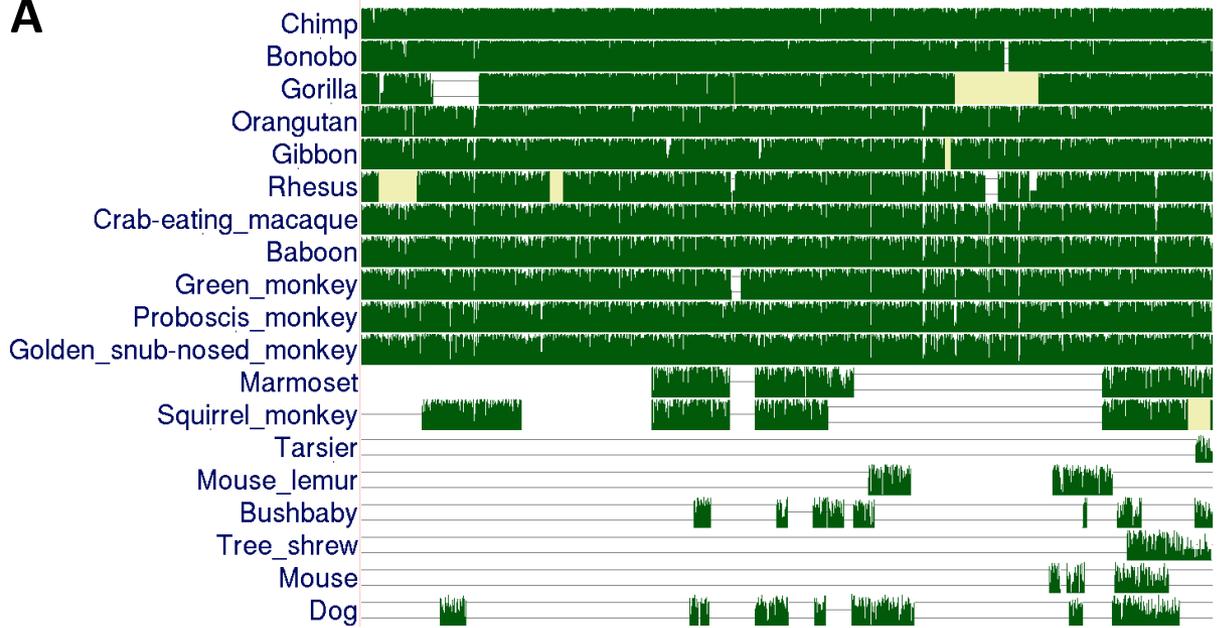
Figure S1

Homo_sapiens	MLDFAIFAVTFLLALVGAVLYLYPASRQAAGIPGITPTEEKDGNLPDIVNSGSLHEFLVN
Pan_troglodytes	MLDFAIFAVTFLLALVGAVLYLYPASRQAAGIPGITPTEEKDGNLPDIVNSGSLHEFLVN
Pan_paniscus	MLDFAIFAVTFLLALVGAVLYLYPASRQAAGIPGITPTEEKDGNLPDIVNSGSLHEFLVN
Gorilla_gorilla	MLDFAIFAVTFLLALVGAVLYLYPASRQAAGIPGITPTEEKDGNLPDIVNSGSLHEFLVN
Pongo_abelii	MLDFAIFAVTFLLALVGAVLYLYPASRQAAGIPGITPTEEKDGNLPDIVNSGSLHEFLVN
Macaca_mulatta	MLDFAIFAVTFLLALVGAVLYLYPAFRQAAGIPGITPTEEKDGNLPDIVNSGSLHEFLVN
Mus_musculus	MLDFAIFAVTFLLALVGAVLYLYPASRQASGIPGLTPTEEKDGNLPDIVNSGSLHEFLVN
Rattus_norvegicus	MLDFAIFAVTFLLALVGAVLYLYPASRQASGIPGLTPTEEKDGNLPDIVNSGSLHEFLVN
Bos_taurus	MLDFAIFAVTFLLALVGAVLYLYPASRQAAGIPGITPTEEKDGNLPDIVNSGSLHEFLVN
Gallus_gallus	MLDFAIFAVTFLLILVGAVLYLYPASRQASGIPGLAPTDKDGSLPDIIASRSLHEFLVN
Xenopus_tropicalis	MLDFAIFAITFLLILVGAVLYLYPSSRQACGIPGLAPTEEKDGNLQDIVNSGSLHEFLVN
Danio_rerio	MLDFAIFAVTFVIIIGAVLYLYPSSRRASGVPGLNPTEEKDGNLQDIVNKGSLHEFLVG
	*****: **:: *;*****: *; *; **; **::***** **; , *****

Figure S2

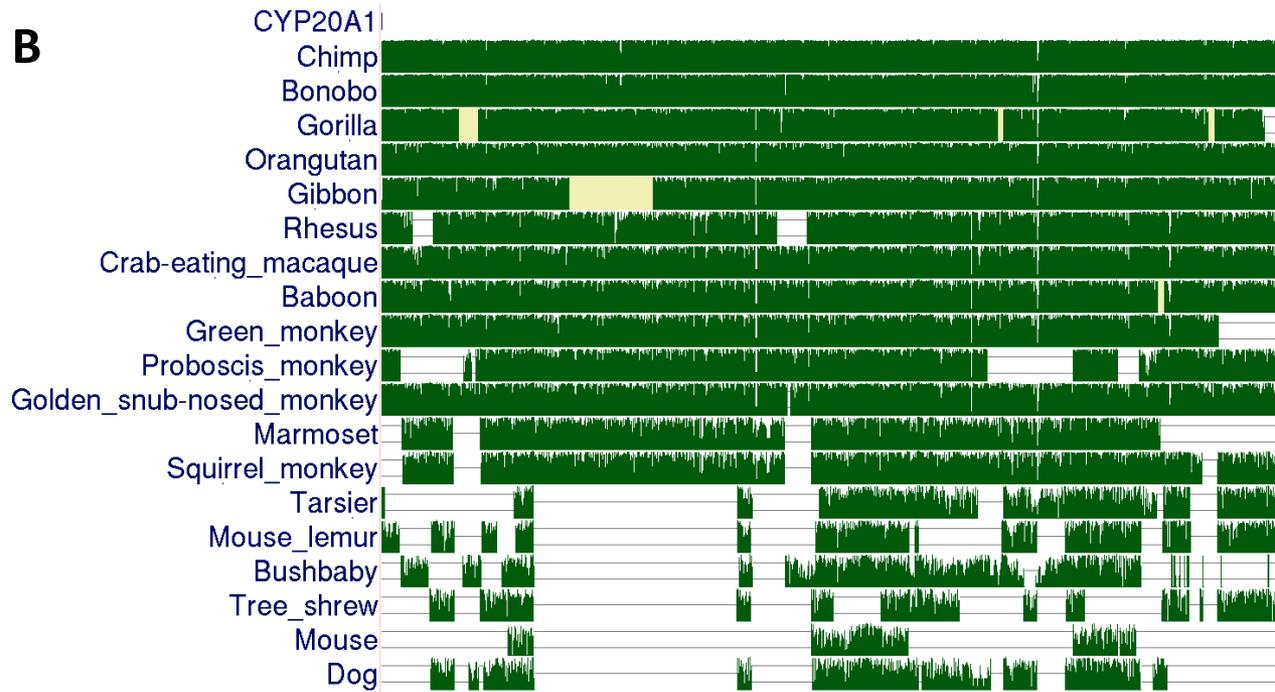
CYP20A1

A



10kb upstream of 5'UTR

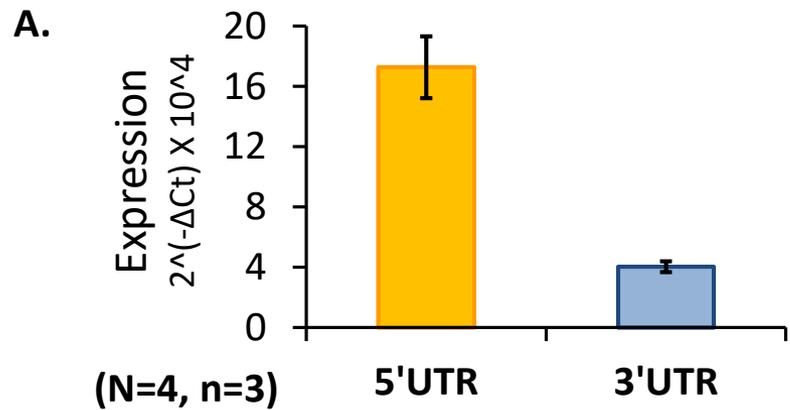
B



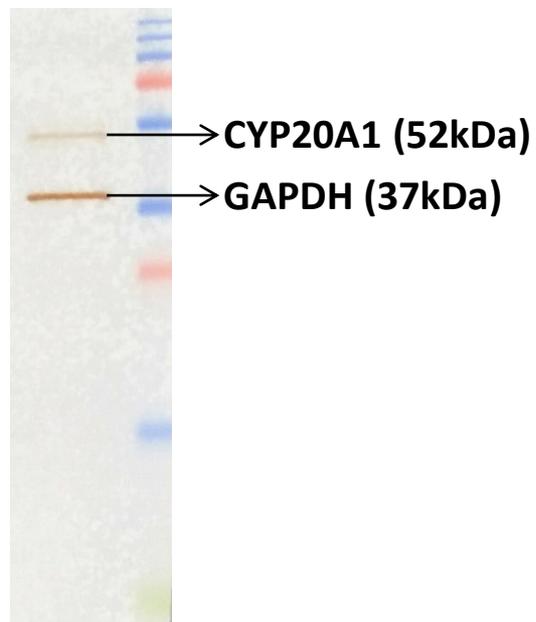
10kb downstream of 3'UTR

MRPL50P2

Figure S3



B.



CYP20A1 level relative
to GAPDH = 0.46

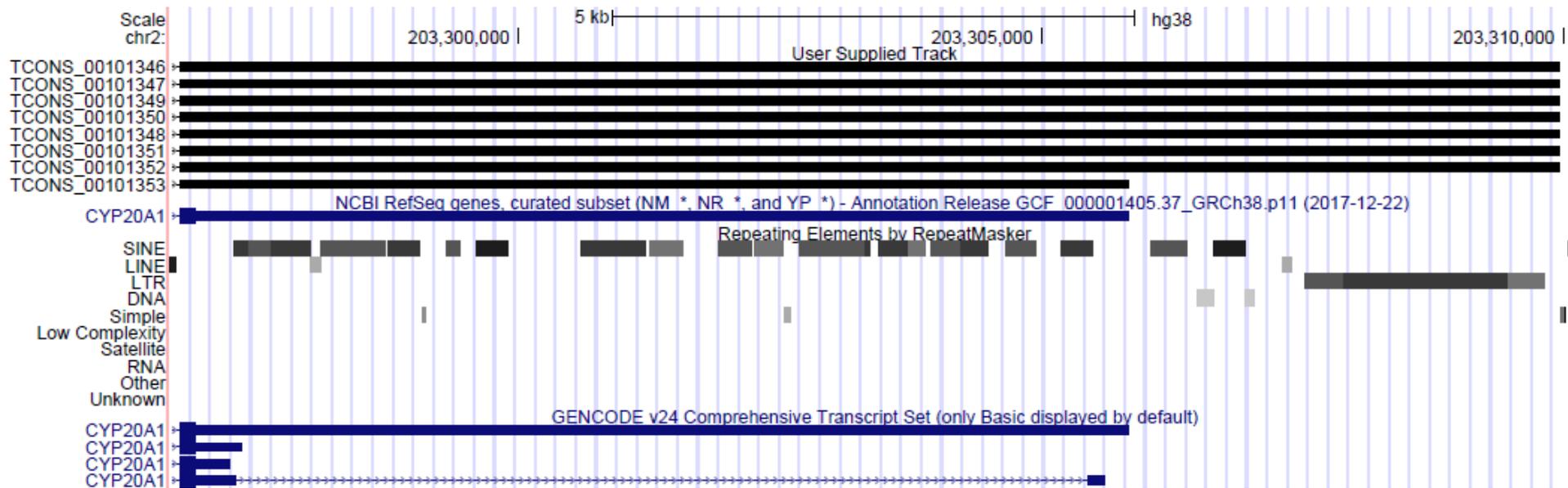
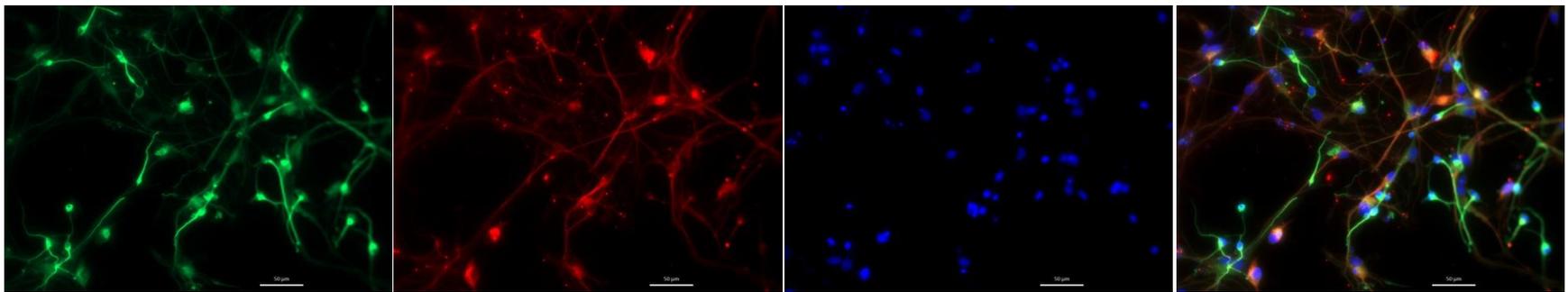


Figure S5

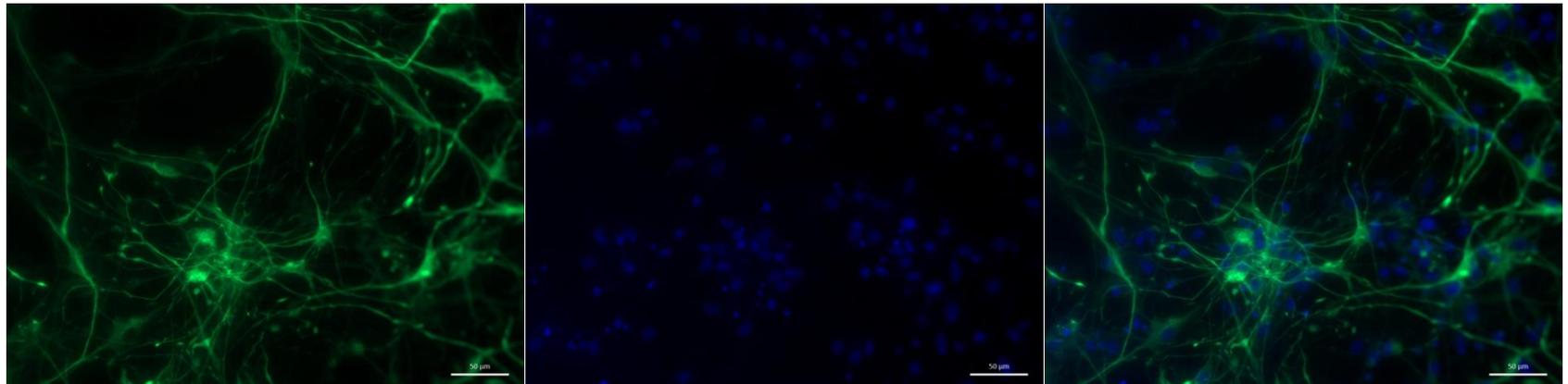


TUJ1

MAP2

DAPI

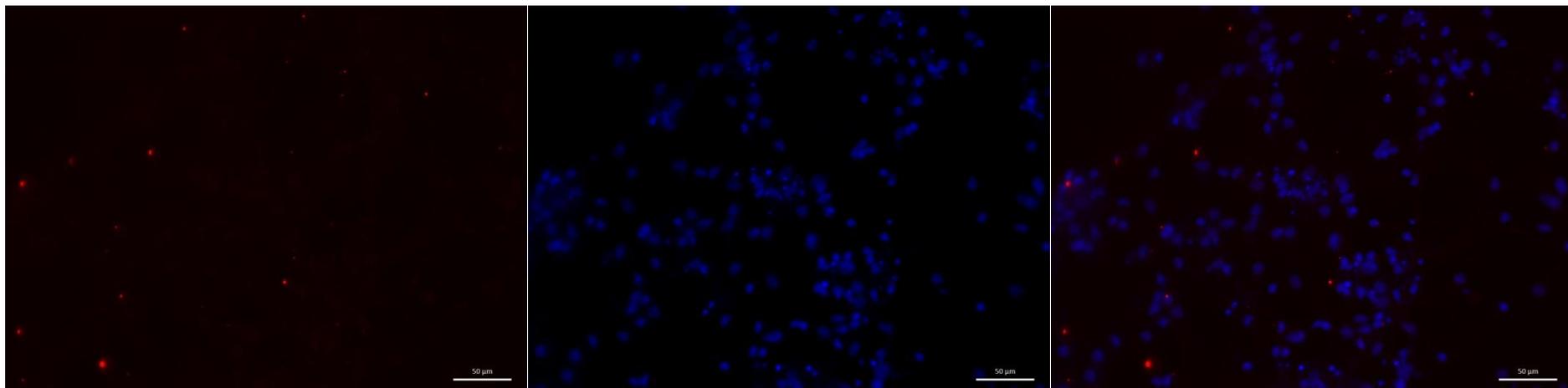
Merged



NeuN

DAPI

Merged



GFAP

DAPI

Merged

Figure S6

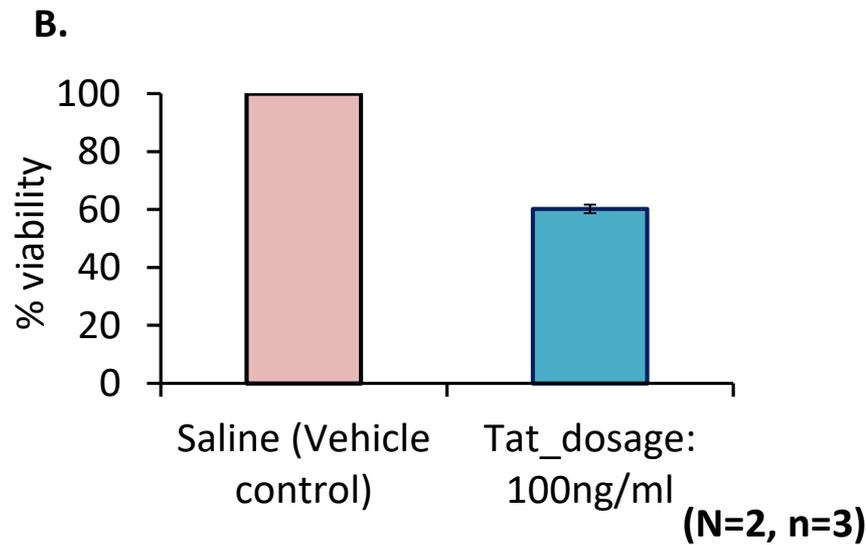
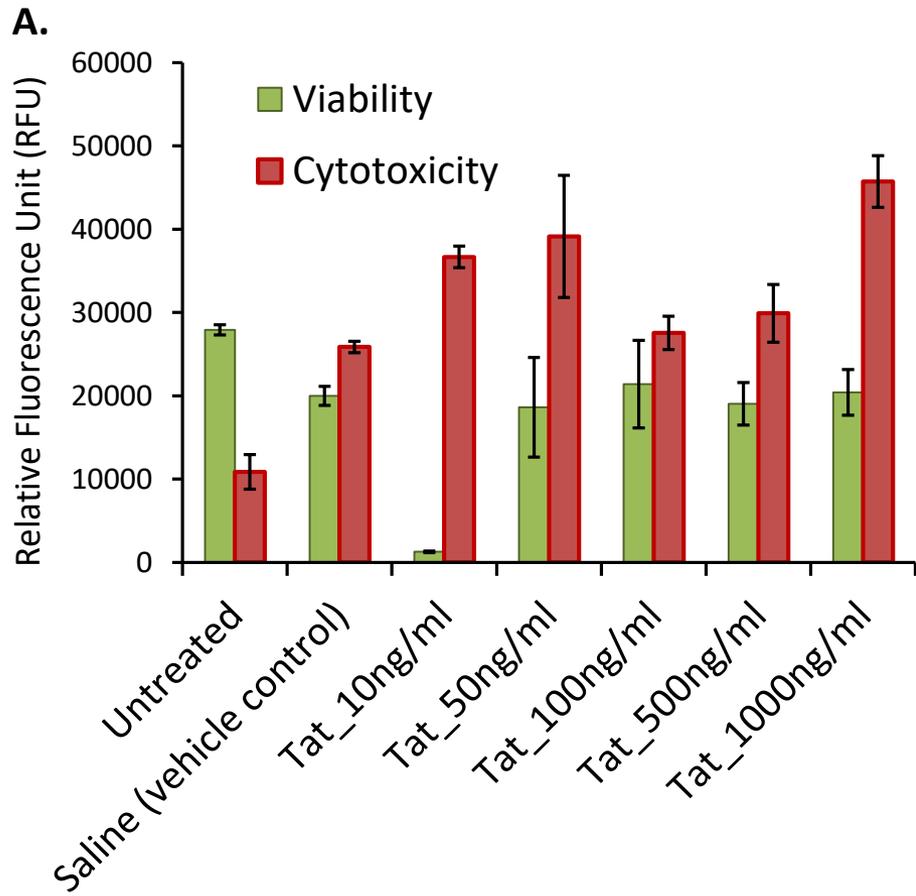
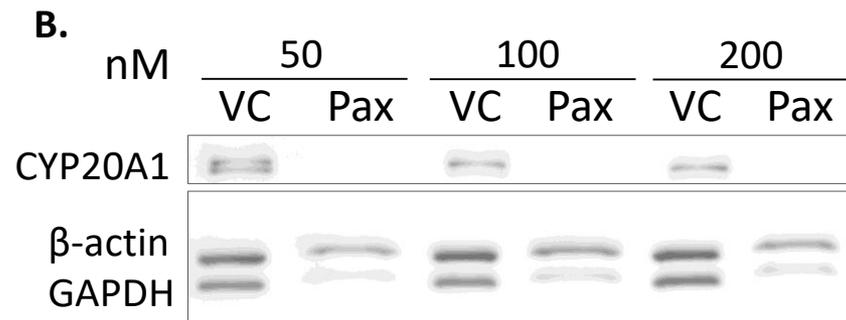
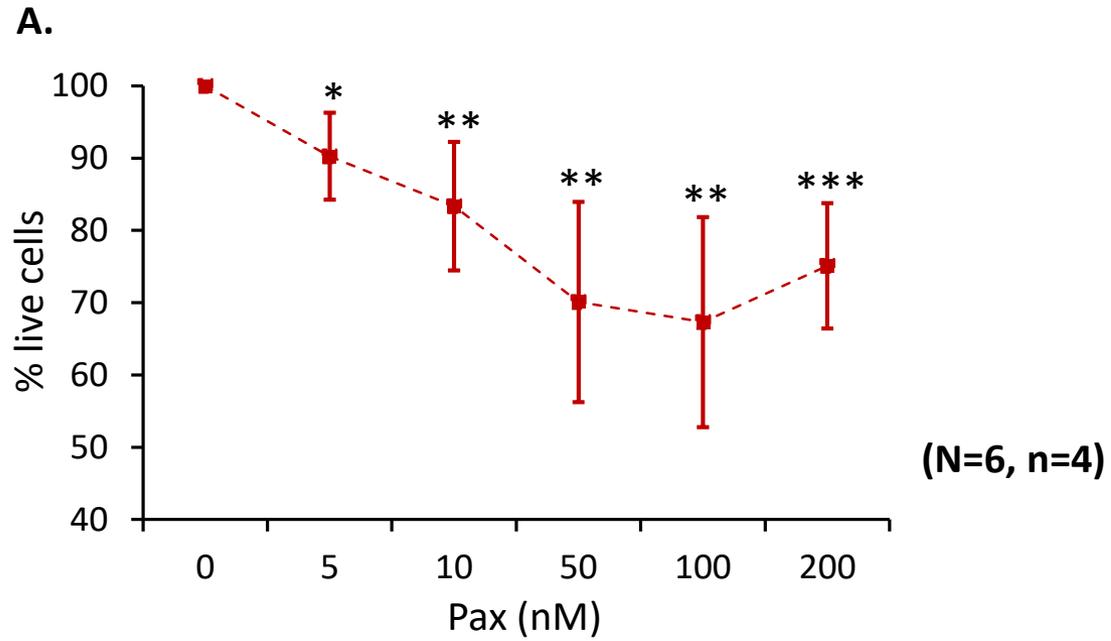


Figure S7



VC: vehicle control
Pax: paclitaxel