Supplementary figures

Rapid activity-induced transcription of *arc* and other IEGs relies on poised RNA polymerase II

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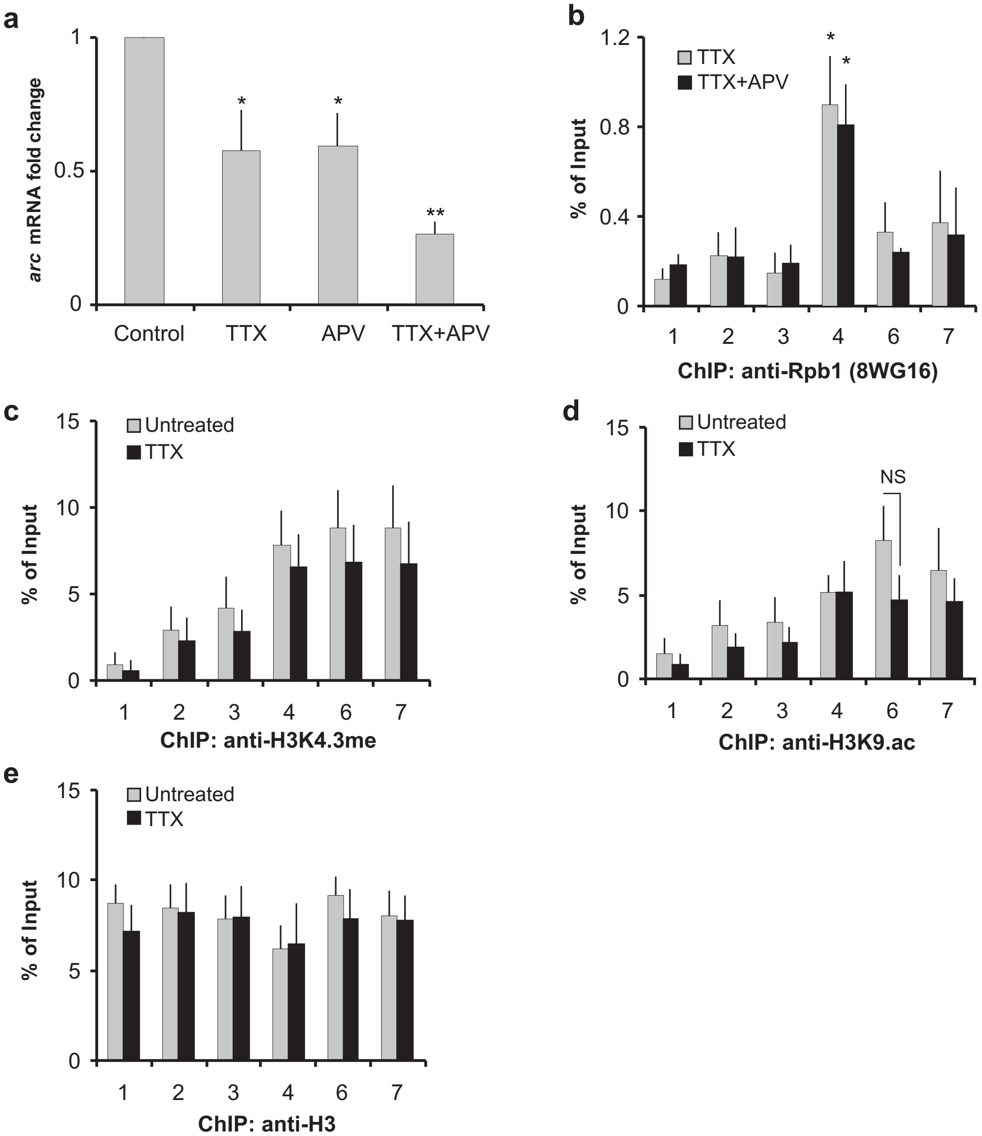
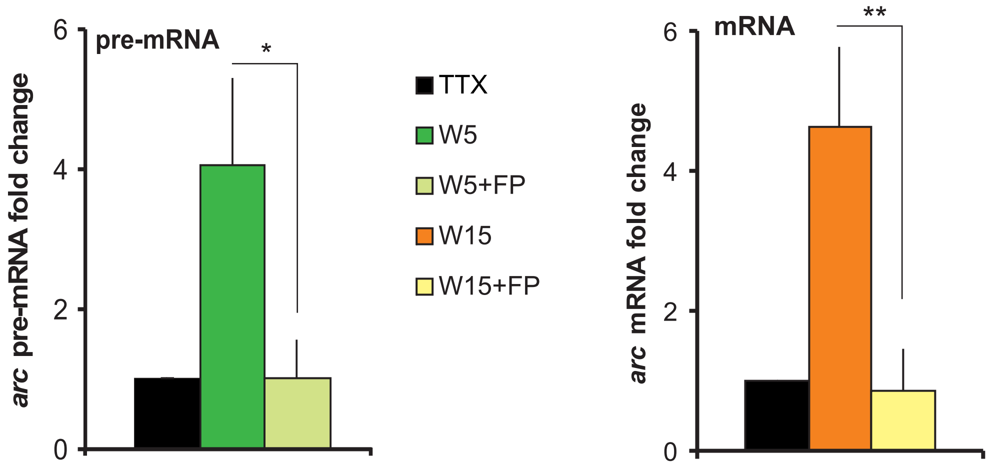
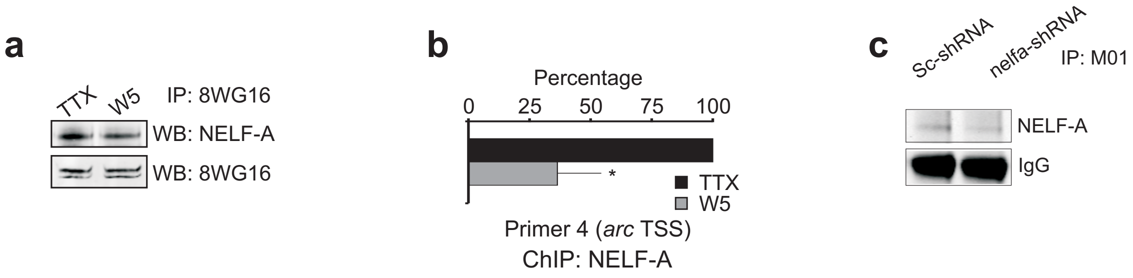


Supplementary Figure 1. Pol II stalling and histone markers remain unaltered in silenced neurons. Pol II enrichment near the *arc* TSS is maintained in neurons with diminished neuronal activity. (**a**) Level of *arc* mRNA after 48 hours treatment with TTX, 4 hours treatment with AP5, or both, as determined by qPCR and normalized to GAPDH. $n = 3$; error bars represent s.e.m. (**b**) Quantification of RNA Pol II binding to the promoter region and gene body of *arc* as estimated by ChIP with anti-Rpb1 antibody in TTX or TTX+APV treated neurons. $n = 3$; error bars represent s.d. $^*P < 0.05$. (**c, d, e**) Quantification of tri-methylated H3 lysine 4 (c), acetylated H3 lysine 9 (**d**), and total H3 (**e**) near the *arc* TSS in untreated or TTX treated neurons as estimated by ChIP. $n = 3$; error bars represent s.d. Note the relative abundance of the H3K4.3me and H3K9.ac near the TSS in both cases. NS = not significant.



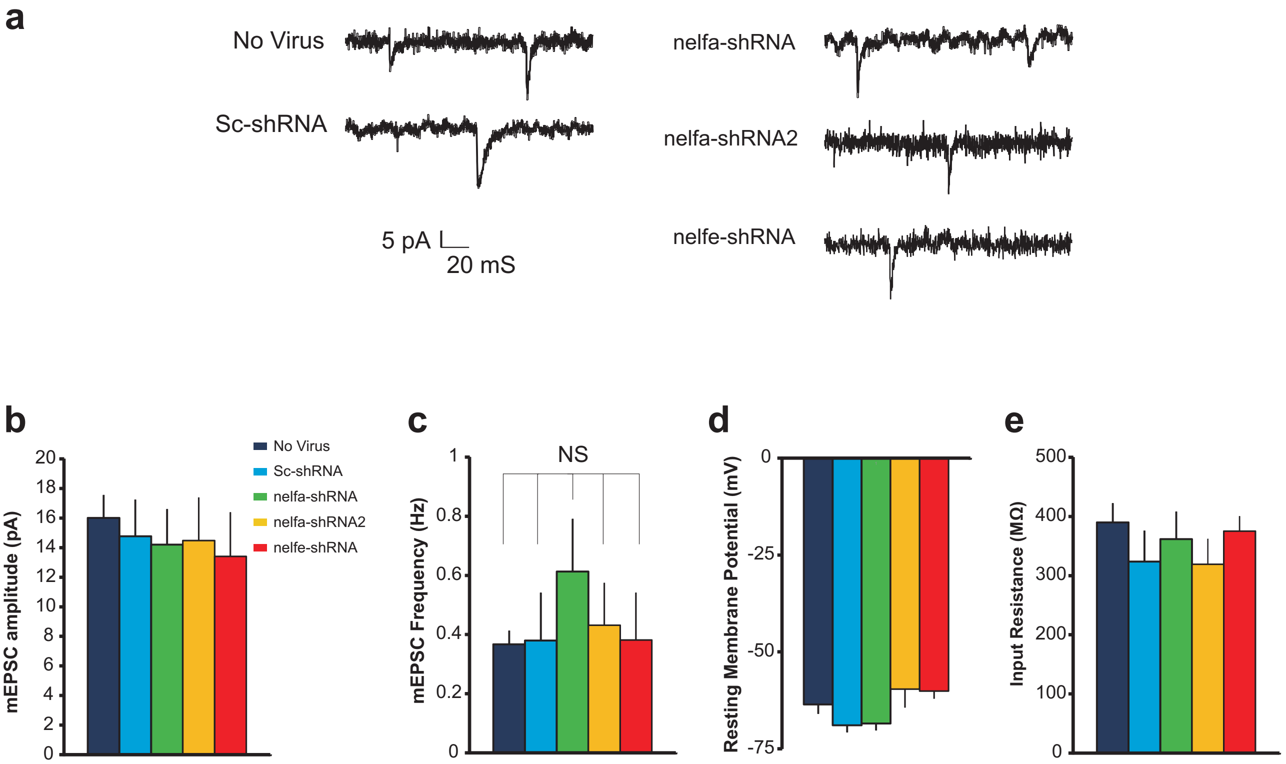
Supplementary Figure 2. FP blocks upregulation of TTX withdrawal-induced *arc* pre-mRNA and mRNA.

Graph representing the levels of *arc* pre-mRNA and mRNA in neurons treated as in Fig. 4(a), but harvested fifteen minutes after the washout (W15). The qPCR data was normalized to GAPDH. *n* = 3; error bars represent s.e.m.



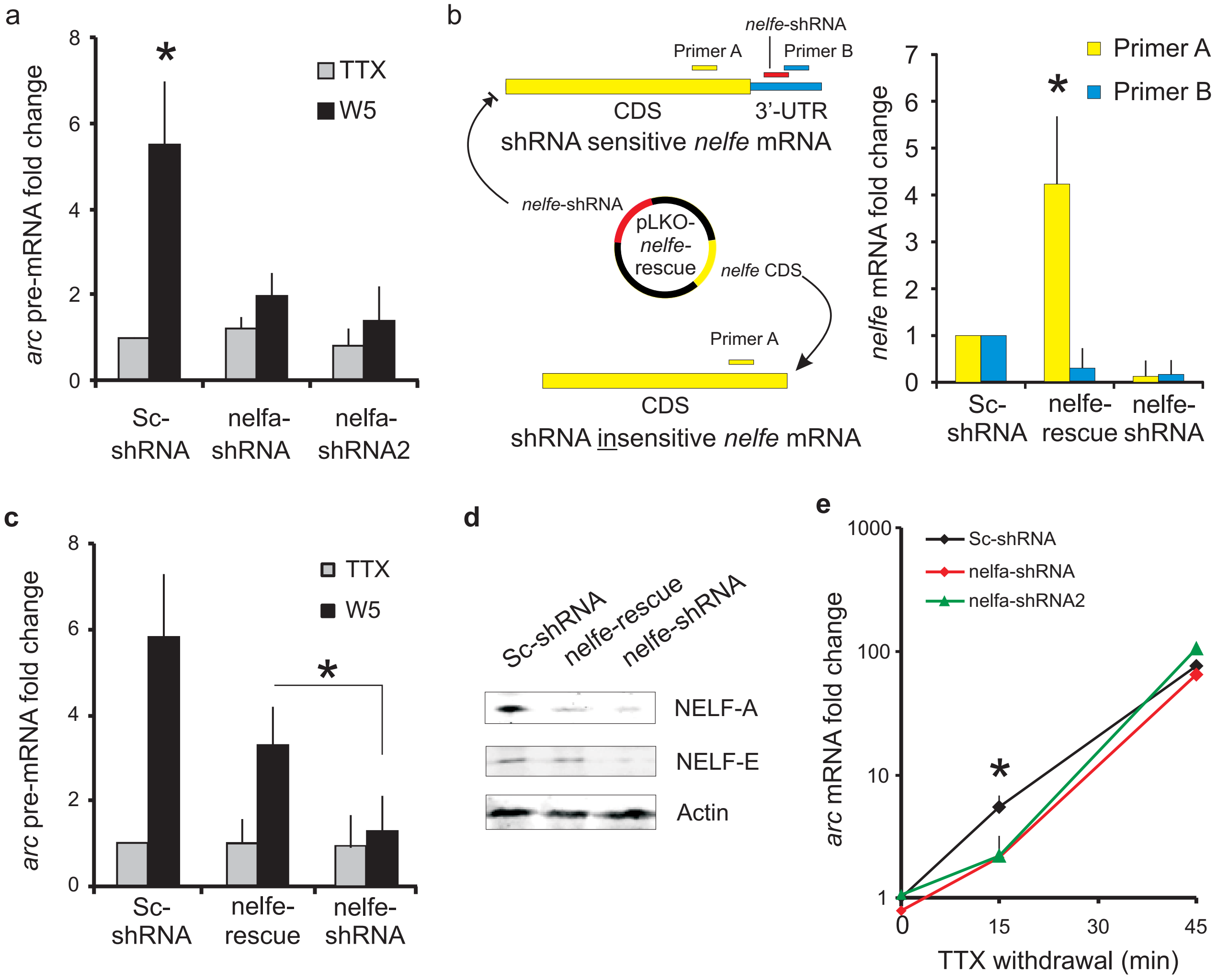
Supplementary Figure 3. Association of NELF-A and Pol II.

(**a**) Coimmunoprecipitation was performed in nuclear extracts of indicated samples with 8WG16 antibody. Blots were probed with NELF-A antibody and re-probed with 8WG16. Representative figure of two independent trials. (**b**) Quantification of NELF-A at the *arc* TSS in indicated samples as determined by ChIP before and 5 minutes after TTX washout. *n* = 3 biological trials; error bars represent S.D. of the mean. Note the drop in NELF binding to the *arc* promoter. (**c**) Co-Immunoprecipitation was performed in nuclear extracts of indicated samples with Pol II (M01) antibody. The blot was probed with NELF-A antibody. Representative figure of two independent trials. Note the diminished interaction of Pol II with NELF-A after the later was knocked down.



Supplementary Figure 4. NELF-A knockdown does not interfere with neuronal activity.

Representative mEPSC traces (**a**) and summary graphs of mEPSC amplitude (**b**), mEPSC frequencies (**c**), resting membrane potentials (**d**), and input resistances (**e**) recorded from uninfected neurons (n = 9) and neurons infected with lentivirus expressing a scrambled sequence (n = 7), NELF-A shRNA (n = 10), NELF-A shRNA2 (n = 6) and NELF-E shRNA (n = 8).
No significant effect of NELF-A or NELF-E knockdown was observed. Error bars represent s.e.m.



Supplementary Figure 5. Studies with an alternate NELF-A shRNA and NELF-E rescue.

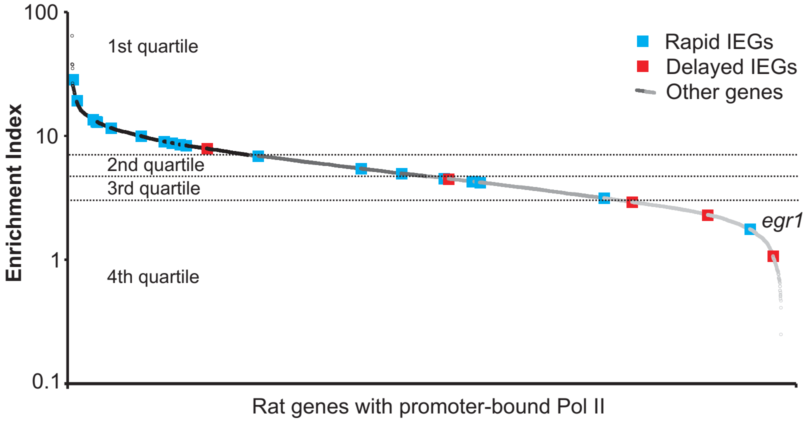
(**a**) Graphical representation of *arc* pre-mRNA levels in neurons infected with scrambled, nelfa-shRNA or nelfa-shRNA2 producing lentiviruses for indicated treatment (*n* = 3, *P* < 0.01).

(**b**) Illustration showing the cloning strategy to create shRNA insensitive *nelfe* (left) and *nelfe* mRNA levels in neurons infected with scrambled, nelfe-shRNA+rescue, or nelfe-shRNA producing lentiviruses (*n* = 3, *P* < 0.05). Note that the *nelfe* mRNA was rescued from the exogenous construct (right) after loss of endogenous mRNA (as reported by Primer B).

(**c**) Graphical representation of *arc* pre-mRNA levels in neurons infected with scrambled, nelfe-shRNA or nelfe-shRNA+rescue producing lentiviruses for indicated treatment (*n* = 5, *P* < 0.05).

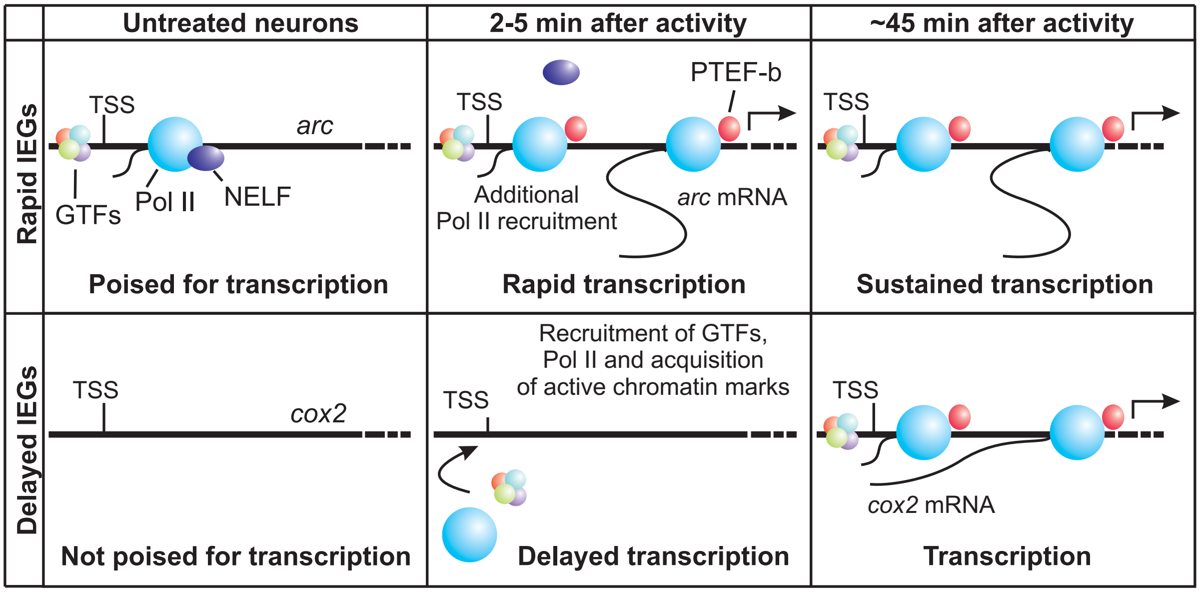
(**d**) Neurons were infected with indicated viruses and nuclear extract was prepared from them after seven days. These extracts were separated by electrophoresis and were western blotted for the indicated proteins. Note the loss of NELF-A protein despite rescue of NELF-E.

(**e**) *arc* mRNA levels in neurons infected with scrambled, nelfa-shRNA or nelfa-shRNA2 producing lentiviruses for indicated lengths of treatment (*n* = 3, *P* < 0.05).



Supplementary Figure 6. Distribution of the stalling indices for all bound genes.

After size exclusion (see Methods), stalling indices were computed for 7324 bound genes and are represented above. Note that the majority of rapid-IEGs have high indices and reside in the top three quartile except *egr1* (marked), which had a significant Pol II enrichment as detected using the 8WG16 antibody (See **Fig. 6e**).



Supplementary Figure 7. Synopsis.

The NELF-mediated Pol II stalling near promoters of rapid IEGs (e.g. *arc*) poise them for rapid transcription within 2-5 minutes of activity. The presence of P-TEFb promotes the entry of stalled Pol II into the productive elongation phase. Moreover, the stalling of the pioneer Pol II facilitates the recruitment of additional Pol II to these promoters pre-loaded with general transcription factors (GTFs) and active histone marks. Freshly recruited Pol II can then re-initiate successive rounds of transcription resulting in a quick burst of the gene product. In contrast, delayed IEGs (e.g. *cox2*) lack Pol II stalling near their promoter and are therefore not poised for rapid expression. Instead, they must undergo transcription initiation, which includes recruitment of GTFs, Pol II and acquisition of histone modifications. Thus, delayed IEGs manifest slower expression kinetics.

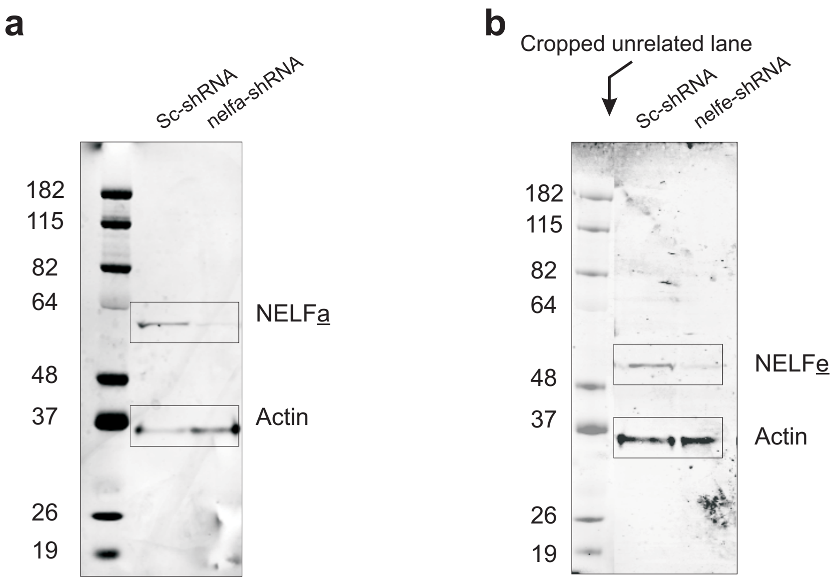


Figure 4d
in main text

Figure 4e
in main text

Supplementary Figure 8. Full length blots.

Gene name	Accession	Ensmbl Gene ID	Chr	Orientation	Gene Start	Gene Stop	Category	Validation
Npas4	NM_153626	SRNOG00000020	1	Minus	207,597,736	207,602,499	Rapid	MicArr, qRT-PCR, 1-step qPCR
Fos	XM_234422	ENSRNOG00000	6	Plus	109,559,154	109,562,546	Rapid	MicArr, qRT-PCR, 1-step qPCR
Junb	NM_021836	SRNOG00000042	19	Plus	24,831,535	24,833,318	Rapid	MicArr
Egr4	NM_019137	ENSRNOG00000	4	Minus	119,767,612	119,765,288	Rapid	MicArr, qRT-PCR
ania-5	AF030090	?	2	Plus	23,582,120	23,585,777	Rapid	MicArr
Egr1	NM_012551	SRNOG00000019	18	Plus	27,342,193	27,347,352	Rapid	MicArr, qRT-PCR, 1-step qPCR
Mbnl2	AA875457	ENSRNOG00000	15	Plus	105,356,456	105,514,135	Rapid	MicArr
NR4a3	NM_017352	SRNOG00000005	5	Plus	64,713,468	64,753,304	Rapid	MicArr
Arc	NM_019361	SRNOG00000043	7	Minus	112,775,381	112,771,938	Rapid	MicArr, qRT-PCR, 1-step qPCR
Arf4	AA956116	SRNOG00000012	16	Plus	1,955,745	1,972,321	Rapid	MicArr
Gadd45g	XM_237999	ENSRNOG00000	17	Minus	19,230,896	19,232,641	Rapid	MicArr, qRT-PCR
Egr2	NM_053633	SRNOG00000000	20	Minus	21,883,889	21,888,177	Rapid	MicArr, qRT-PCR
Arih1	AW144003	ENSRNOG00000	8	Minus	63,201,872	63,305,302	Rapid	MicArr
Ier2	NM_001009541	SRNOG00000002	19	Plus	25,159,276	25,160,796	Rapid	MicArr, qRT-PCR
Olr670	NM_001000633	ENSRNOG00000	3	Minus	73,204,873	73,203,940	Delayed	MicArr, 1-step qPCR
Cyr61	NM_031327	SRNOG00000014	2	Minus	243,827,262	243,824,303	Rapid	MicArr, 1-step qPCR
Gadd45b	NM_001008321	ENSRNOG00000	7	Minus	10,291,102	10,289,073	Rapid	MicArr, 1-step qPCR
Nup98	AI176079	SRNOG00000020	1	Minus	159,696,720	159,598,049	Rapid	MicArr, 1-step qPCR
Egr3	NM_017086	ENSRNOG00000	15	Plus	50,477,453	50,479,908	Delayed	MicArr, qRT-PCR, 1-step qPCR
Fosl2	NM_012954	ENSRNOG00000	6	Minus	23,832,427	23,749,170	Delayed	MicArr, 1-step qPCR
Rasl11a	NM_001002829	SRNOG00000000	12	Minus	8,710,040	8,705,994	Delayed	MicArr, 1-step qPCR
Nur77	NM_024388	ENSRNOG00000	7	Plus	140,012,807	140,020,587	Delayed	MicArr, qRT-PCR, 1-step qPCR
Jun	BC078738	ENSRNOG00000	5	Minus	115,360,401	115,359,397	Delayed	MicArr, 1-step qPCR
Ccno	BF403931	ENSRNOG00000	2	Plus	44,377,953	44,381,020	Delayed	MicArr, 1-step qPCR
Nfkbid	XM_218484	SRNOG00000025	1	Plus	85,377,148	85,383,179	Delayed	MicArr, 1 step-qPCR
Dusp1	NM_053769	ENSRNOG00000	10	Plus	16,942,927	16,945,694	Rapid	MicArr, 1-step qPCR
Dusp6	U42627	ENSRNOG00000	7	Plus	36,893,264	36,897,493	Rapid	MicArr, 1-step qPCR
Trib1	AI230116	ENSRNOG00000	7	Plus	96,442,239	96,448,785	Delayed	MicArr, 1-step qPCR
Kcnf1	XM_216678	ENSRNOG00000	6	Minus	40,913,068	40,910,396	Delayed	MicArr, 1-step qPCR
PPP1r15a	NM_133546	ENSRNOG00000	1	Minus	95,997,080	95,994,025	Rapid	MicArr, 1-step qPCR
Nurr1	NM_019328	ENSRNOG00000	3	Minus	38,871,678	38,866,212	Delayed	MicArr, qRT-PCR, 1-step qPCR
Fam46b	XM_232748	SRNOG00000007	5	Minus	151,612,602	151,605,299	Delayed	MicArr, 1-step qPCR
Ch25h	XM_220063	ENSRNOG00000	1	Minus	238,458,335	238,457,020	Delayed	MicArr, 1-step qPCR
Btg2	NM_017259	ENSRNOG00000	13	Minus	47,030,704	47,026,983	Rapid	MicArr, 1-step qPCR

Fam46a	XM_217218	ENSRNOG00000	8	Minus	90,506,129	90,502,441	Delayed	MicArr, 1-step qPCR
Nfil3	NM_053727	ENSRNOG00000	17	Plus	18,092,489	18,107,705	Delayed	MicArr, 1-step qPCR
Ifng	NM_138880	ENSRNOG00000	7	Plus	57,621,756	57,625,792	Delayed	MicArr, 1-step qPCR
Klf4	NM_053713	ENSRNOG00000	5	Minus	73,451,286	73,446,930	Delayed	MicArr, 1-step qPCR
Tiparp/RM1	AB032087	ENSRNOG00000	2	Plus	155,312,341	155,339,357	Delayed	MicArr, 1-step qPCR
Dusp5	NM_133578	SRNOG00000014	1	Plus	259,754,234	259,767,645	Delayed	MicArr, 1-step qPCR
Fbxo33	XM_234205	ENSRNOG00000	6	Minus	79,934,591	79,902,508	Rapid	MicArr, 1-step qPCR
Fosl1	NM_012953	ENSRNOG00000	1	Plus	208,090,612	208,099,118	Delayed	MicArr, 1-step qPCR
Cartpt	NM_017110	ENSRNOG00000	2	Minus	30,891,837	30,889,817	Delayed	MicArr, 1-step qPCR
Maff	XM_345857	ENSRNOG00000	7	Plus	117,328,924	117,340,276	Delayed	MicArr, 1-step qPCR
Cox2/Ptgs2	NM_017232	ENSRNOG00000	13	Plus	64,427,288	64,432,982	Delayed	MicArr, 1-step qPCR
Klf14	AW534376	ENSRNOG00000	4	Minus	58,013,460	58,010,538	Delayed	MicArr, 1-step qPCR
Vgf	NM_030997	ENSRNOG00000	12	Plus	20,903,304	20,906,327	Delayed	MicArr, qRT-PCR, 1-step qPCR
Bdnf	NM_012513	?	3	Plus	949,646	979,996	Delayed	MicArr, 1-step qPCR
Edn1	NM_012548	ENSRNOG00000	17	Minus	28,309,775	28,303,886	Delayed	MicArr, 1-step qPCR

Gene name	TTX/W45 (Microarray)	W45 (qRT-PCR)	TTX/W15 (Microarray)	W15 (qRT-PCR)	W5 (1-step qRT-PCR)	Pol II bound?	Enrichment Index
Npas4	100	289.27 ± 14.43	43.416	49.75 ± 3.36	35.39 ± 22.95	Yes	4.288673478
Fos	81.67533	61.73 ± 5.48	26.34377	16.21 ± 2.07	37.42 ± 3.21	Yes	8.526184539
Junb	30.52895	Not tested	8.8431	Not tested	Not tested	Yes	12.99797382
Egr4	36.69477	35.30 ± 4.27	4.13121	5.44 ± 1.54	5.29 ± 0.12	Untestable	Not Applicable
ania-5	5.05034	Not tested	4.13121	Not tested	Not tested	Yes	4.567680798
Egr1	17.55808	23.63 ± 2.86	4.08613	6.64 ± 2.01	16.73 ± 7.68	Yes	1.767444135
Mbnl2	1.93784	Not tested	3.66576	Not tested	Not tested	Yes	8.771948221
NR4a3	2.6527	Not tested	3.00364	Not tested	2.66 ± 0.98	Yes	4.224707796
Arc	37.57973	38.97 ± 3.70	2.99679	4.34 ± 1.20	6.57 ± 0.73	Yes	4.981831136
Arf4	2.0532	Not tested	2.78191	Not tested	0.94 ± 0.05	Yes	4.408050554
Gadd45g	6.46392	8.74 ± 2.25	2.3584	2.84 ± 0.80	4.41 ± 0.98	Yes	9.039220131
Egr2	17.88106	27.08 ± 3.47	2.12732	4.06 ± 1.22	9.52 ± 1.39	No	Not Applicable
Arih1	-1.19927	Not tested	2.10271	Not tested	Not tested	Yes	13.65576914
Ier2	4.51723	7.61 ± 1.68	1.95318	2.71 ± 0.76	1.13 ± 0.54	Yes	28.73494834
Olr670	2.14754	Not tested	1.88824	Not tested	0.95 ± 0.65	No	Not Applicable
Cyr61	18.48776	Not tested	1.86446	Not tested	4.11 ± 1.31	Yes	4.26759189
Gadd45b	5.68986	Not tested	1.68739	Not tested	2.00 ± 0.22	Yes	19.31577307
Nup98	2.00291	Not tested	1.59909	Not tested	2.00 ± 0.61	Yes	3.154277339
Egr3	2.23405	4.53 ± 0.91	1.59169	1.72 ± 0.04	1.23 ± 0.36	No	Not Applicable
Fosl2	3.20344	Not tested	1.42321	Not tested	0.96 ± 0.21	No	Not Applicable
Rasl11a	5.26561	Not tested	1.41099	Not tested	1.13 ± 0.27	No	Not Applicable
Nur77	17.19182	22.56 ± 3.09	1.32137	1.38 ± 0.64	1.63 ± 0.27	No	Not Applicable
Jun	2.02566	Not tested	1.31136	Not tested	0.92 ± 0.54	Yes	1.079444247
Ccno	2.04537	Not tested	1.31025	Not tested	1.06 ± 0.26	No	Not Applicable
Nfkbid	3.1718	Not tested	1.28987	Not tested	1.10 ± 0.32	No	Not Applicable
Dusp1	6.10034	Not tested	1.2608	Not tested	2.59 ± 0.66	Yes	6.885184997
Dusp6	3.20723	Not tested	1.26074	Not tested	2.84 ± 0.37	Yes	8.384672123
Trib1	2.12475	Not tested	1.25472	Not tested	1.35 ± 0.27	Yes	7.916895262
Kcnf1	2.5827	Not tested	1.24604	Not tested	0.89 ± 0.48	No	Not Applicable
PPP1r15a	2.47382	Not tested	1.21186	Not tested	2.84 ± 0.23	Yes	9.978221114
Nurr1	3.25785	5.25 ± 1.62	1.18654	1.05 ± 0.33	1.50 ± 0.22	Yes	2.928315575
Fam46b	2.97462	Not tested	1.17824	Not tested	0.78 ± 0.44	No	Not Applicable
Ch25h	9.21442	Not tested	1.17386	Not tested	0.97 ± 0.26	No	Not Applicable
Btg2	4.96648	Not tested	1.14577	Not tested	2.52 ± 0.35	Yes	11.63384642

Fam46a	2.55448	Not tested	1.12997	Not tested	0.96 ± 0.41	No	Not Applicable
Nfil3	3.06302	Not tested	1.12444	Not tested	1.57 ± 0.55	No	Not Applicable
Ifng	2.09574	Not tested	1.09689	Not tested	1.49 ± 0.42	No	Not Applicable
Klf4	4.59757	Not tested	1.06465	Not tested	1.17 ± 0.21	No	Not Applicable
Tiparp/RM1	3.04924	Not tested	1.01633	Not tested	1.53 ± 0.54	Yes	13.12251472
Dusp5	2.92218	Not tested	1.01407	Not tested	1.68 ± 0.30	Yes	2.310284289
Fbxo33	2.0987	Not tested	1.00735	Not tested	2.13 ± 0.30	Yes	5.460038024
Fosl1	6.26492	Not tested	1.00247	Not tested	0.91 ± 0.35	No	Not Applicable
Cartpt	2.03459	Not tested	-1.01111	Not tested	1.26 ± 0.25	No	Not Applicable
Maff	2.63431	Not tested	-1.02721	Not tested	1.17 ± 0.25	No	Not Applicable
Cox2/Ptgs2	2.1927	Not tested	-1.03256	Not tested	1.39 ± 0.23	No	Not Applicable
Klf14	2.0284	Not tested	-1.06298	Not tested	0.89 ± 0.41	No	Not Applicable
Vgf	2.32727	3.62 ± 1.03	-1.1278	1.27 ± 0.45	1.21 ± 0.29	Yes	4.518959013
Bdnf	5.19267	Not tested	-1.14391	Not tested	0.80 ± 0.38	No	Not Applicable
Edn1	7.33345	Not tested	-1.24776	Not tested	1.10 ± 0.22	No	Not Applicable

	Intron-based primers		
Rat Gene		**Amplicon**	**Notes**
Arc			
Forward	GAATTTGCTATGCCAACTCACGGG	92 bp	This amplicon spans arc intron 1 and exon 2.
Reverse	AGTCATGGAGCCGAAGTCTGCTTT		
Arf4			
Forward	CACAGTTTGGGATGTTGGTGGTCA	87 bp	This amplicon spans exon 3 and intron 3.
Reverse	TGTACGGCATTTCTCACCTGGGTA		
BDNF			
Forward	TGTCTCTGCTTCCTTCCCACAGTT	123 bp	This amplicon spans the last intron and the last exon.
Reverse	TGGACGTTTGCTTCTTTCATGGGC		
Btg2			
Forward	CTCTCTCTCTTGTTTCCTCCACAG	99 bp	This amplicon spansntron 1 and exon 2.
Reverse	TGTGGTTGATGCGGATACAGCGAT		
Cartpt			
Forward	TGCAGGAAGTCCTGAAGAAGCTCA	111 bp	This amplicon spans exon 2 and intron 2.
Reverse	TCCTGCCAAAGTAAGGGAAGAGGA		
Ccno			
Forward	TCGGATTTAAGGCTAGGCGGGTTT	86 bp	This amplicon spans intron 1 and exon 2.
Reverse	TGCACTTCTACCTGCAACGCAAGA		
cFos			
Forward	ACAGCCTTTCCTACTACCATTCCC	88 bp	This amplicon spans arc intron 1 and exon 1.
Reverse	CTGCACAAAGCCAAACTCACCTGT		
Cox2			
Forward	TACATCCTGACCCACTTCAAGGGA	125 bp	This amplicon spans exon 3 and intron 3.
Reverse	TGTTCGATGCTGGAGAACAAGGGA		
Cyr61			
Forward	ATGTATGAGTTTCAGCGTGTGGCG	85 bp	This amplicon spans intron 2 and exon 3.

Reverse	GTCTGCCTTCTGACTGAGCTGTAA		
Dusp1			
Forward	CTCTACGACCAGGTTAGTAGGAGT	108 bp	This amplicon spans exon 2 and intron 2.
Reverse	ACAGCCGCTTTCTCTATTCTCCCT		
Dusp5			
Forward	GACCAGCTTATGACCAGGTATGTG	68 bp	This amplicon spans exon 2 and intron 2.
Reverse	AATGGGATGTGAGGAAGGAGCAGA		
Dusp6			
Forward	TCCTGTGCCTCTCACAAGCTGAAA	89 bp	This amplicon spans exon 2 and intron 1.
Reverse	AACTTACTGAAGCCACCTGCCAGA		
Edn1			
Forward	TGTCTACTTCTGCCACCTGGACAT	79 bp	This amplicon spans exon 2 and intron 2.
Reverse	ATGGCTAGGACTACAATGCCCTCT		
Egr2			
Forward	TCCACGTGCCTGTATTCTCATCGT	123 bp	This amplicon spans intron 1 and exon 2.
Reverse	AGCTACTCGGATATGGGAGATCCA		
Egr3			
Forward	TGTGAGTTCTGTGGGCGCAAGTTT	188 bp	This amplicon spans exon 2 and intron 2.
Reverse	AAAGAAGGGATCTGAGAGGCGGAT		
Egr4			
Forward	TCCTTTGGCAGGCGACTTCTTGA	80 bp	This amplicon spans intron 1 and exon 2.
Reverse	CCAGGAAGCAGGAGTCTGTTAAGT		
Fam46a			
Forward	TCGTGCTGGATTGCCTGTTAGACT	95 bp	This amplicon spans exon 2 and intron 2.
Reverse	AAGCCCATCAGGTGGCTTTACCTT		

Fam46b			
Forward	ATTAATCCCGGAGGTTGTTGGTGC	105 bp	This amplicon spans exon 2 and intron 1.
Reverse	TGCAGTCCCTGTTCTTCCAAGCTA		
Fbxo33			
Forward	GCATCTACTTGGAGCTGGTGTTGT	67 bp	This amplicon spans exon 2 and intron 2.
Reverse	TCCACGCAAGCCTACCTGTTGTT		
Fosl1			
Forward	CCTGTGAGCAGGTAAGGAACAGAGAT	90 bp	This amplicon spans exon 2 and intron 2.
Reverse	ATTCCCTCATAGCAAGATCCAGCC		
Fosl2			
Forward	TGCCTTCGGATTGTGGTATGTCCT	47 bp	This amplicon spans exon 3 and intron 3.
Reverse	TGACCAGTTCACAGACAGCAGACA		
Gadd45b			
Forward	TCCCTCTGACACTCCTTCTTTCCT	128 bp	This amplicon spans intron 1 and exon 2.
Reverse	GCAGAACGATTGGATCAGGGTGAA		
Gadd45g			
Forward	ACTCACGGCGCTTGTTCTTTCACA	125 bp	This amplicon spans intron 1 and exon 2.
Reverse	ATTCAGGACTTTGGCGGACTCGTA		
GAPDH			
Forward	AACATGCACAGGGTACTTCGAGGA	120 bp	This amplicon spans GAPDH intron 2 and exon 3.
Reverse	ACGACATACTCAGCACCAGCATCA		
Ifng			
Forward	TGTTTCCCAAGGACGGTAACACGA	99 bp	This amplicon spans exon 2 and intron 1.
Reverse	TGCTGATGGCCTGGTTGTCTTTCA		
Klf4			
Forward	GTGTGCTTCACCCAGCAAGTCAAT	121 bp	This amplicon spans exon 2 and intron 1.
Reverse	AGTCGCTTCATGTGAGAGAGTTCC		

Maff			
Forward	ATCCCTTATCCAGCAAAGCCCTGA	90 bp	This amplicon spans exon 2 and intron 2.
Reverse	GGGCTTCCAGTTAGGAAAGGTGGAAA		
Nfil3			
Forward	AGTCGAAAGAACAGCTTCGTGGGT	87 bp	This amplicon spans exon 1 and intron 1.
Reverse	AAGCCAACTGAACTTGCCTGACTC		
Npas4			
Forward	GTTGCATCAACTCCAGAGCCAAGT	63 bp	This amplicon spans intron 7 and exon 7.
Reverse	ACATTTGGGCTGGACCTACCTTCA		
NR4a3			
Forward	AAGATACCCTCCAGGTAGGTCCGAA	70 bp	This amplicon spans exon 2 and intron 2.
Reverse	TGTTGGTTTCTCCACCCATTTCCC		
Nup98			
Forward	GGGCTTTGGTACAACGTCAACA	149 bp	This amplicon spans exon 2 and intron 1.
Reverse	GCTACACTGAGTTAAGCCAGCTAC		
Nur77			
Forward	ACCAACTCTTCTGGCTTCCCTTAC	118bp	This amplicon spans intron 1 and exon 2.
Reverse	GGCTGGTTGCTGGTGTTCCATATT		
Nurr1			
Forward	CGACATTTCTGCCTTCTCCTGCAT	124 bp	This amplicon spans exon 6 and intron 6.
Reverse	TGTGATTAGGGAAAGCCATGACCG		
Ppp1r15a			
Forward	ACAATGACTCAGTGCTGTGACCTG	135 bp	This amplicon spans exon 2 and intron 1.
Reverse	AGAAAGAGTGGGCTTCCTTCCAGT		
RasL11a			
Forward	ACCAGTGATGTGAAGGGAGTGGTT	90 bp	This amplicon spans exon 2 and intron 2.
Reverse	ATGCCAATGAATGTGCTTCCTCCC		
Sgk1			

Forward	CAAAGCACATGTTTGCTGTGCGGA	95 bp	This amplicon spans intron 8 and exon 9.
Reverse	TTATGGAGGACCTCAGGAGCCAGATA		
Tiparp			
Forward	CACCTTTCTTCCTTTCCTGGTAGG	83 bp	This amplicon spans exon 2 and intron 1.
Reverse	CGGTGGTTTCCATGTCCATGATGT		
Trib1			
Forward	TAAACCGGTGACTTTCTCTCCGCA	117 bp	This amplicon spans exon 2 and intron 1.
Reverse	AGGATCACTTCCACGATGCCAGTA		
Vgf			
Forward	TTGCGAGCGTTCTCTGACCATTTG	80 bp	This amplicon spans intron 2 and exon 2.
Reverse	CCCGAAGAGAAACGTTTAGCAGAG		
Zif268			
Forward	TTCGGCTCTCATCGTCCAGTGATT	141 bp	This amplicon spans intron 1 and exon 2.
Reverse	AACCGGGTAGTTTGGCTGGGATAA		
Zscan10			
Forward	TTCCAAGGATCCCTCAACATCTCG	181 bp	This amplicon spans exon 1 and intron 1.
Reverse	ACCAGTAGGTTCCCAAACACCCTT		

Exon-based primers	Primers and corresponding NCBI database entry #	Amplicon size
NELF-A	NM_001008339	
Forward	TTCCCTGACACAGGCTCCTTGAAT	142 bp
Reverse	TCTTGTTCAGGTACTGGCACTCCA	
GAPDH	NM_017008	
Forward	AGAGACAGCCGCATCTTCTTG	99 bp
Reverse	GGTAACCAGGCGTCCGATAC	
Arc	NM_019361	
Forward	ATCCTGCAGATTGGTAAGTGCCGA	157 bp
Reverse	GCACATAGCCGTCCAAGTTGTTCT	

All of the following additional genes were tested for induction after TTX withdrawal.

cFos	NM_022197	
Forward	ACGGAGAATCCGAAGGGAAAGGAA	125 bp
Reverse	TCTGCAACGCAGACTTCTCGTCTT	
c-Jun	NM_021835	
Forward	GGAACAGGTGGCACAGCTTAAACA	82 bp
Reverse	TTGCAACTGCTGCGTTAGCATGAG	
Egr1/Zif268	NM_012551	
Forward	TCTGAATAACGAGAAGGCGCTGGT	122 bp
Reverse	ACAAAGTGTTGCCACTGTTGGGTG	
Nurr1	NM_019328	
Forward	AGGTTCCAGGCAAACCCTGACTAT	117 bp
Reverse	AATCTTCTCTGCCCACCCTCTGAT	
Nur77/NGFI-b	NM_024388	
Forward	GACAACGCTTCGTGCCAGCATTAT	71 bp
Reverse	ACTGTGCGCTTGAAGAAGCCTTTG	
Ier2	NM_001009541	
Forward	ACTGTCCCTTCCTTGGTTTGGAGA	114 bp

Reverse	GCCGAAACGCGAATGCTAGTGAAA	
Egr2	NM_053633	
Forward	TGTGGCCCTAGAACAGTGAATGGA	113 bp
Reverse	TTGCTTTGTCCTTCCCGACATTGC	
Ch25h	NM_001025415	
Forward	ATGTGTTTCTGCAGCCCGACAAAG	102 bp
Reverse	AAGGACACTGGGACGAGTTGGAAT	
Olr670	NM_001000633	
Forward	GTTGCCCTTCTGTGGACCCAATTT	122 bp
Reverse	TAGACCCACTGTTGGCAAAGACCA	
Gadd45g	AB020978	
Forward	CGGGAAAGCATTGCACGAACTTCT	88 bp
Reverse	ATTCAGGACTTTGGCGGACTCGTA	
Vgf	NM_030997	
Forward	ACACCCTTATCCAAGGCGTACCAA	125 bp
Reverse	ACCTGAGCTAACCCTTGTTGAAGC	
Dusp6	NM_053883	
Forward	ACCAGGCTGCTTTCTTTGTGTGTG	117 bp
Reverse	AATGGAGCAAATCTCTCCCTCCGT	

Primers for ChIP

Promoter	Primer #	Primer sequence	Distance from TSS	Amplicon size
Arc	Primer set #1			
	Forward	TGTAGGTGTGTGAACAAAGGTGGG	Minus 1468	109bp
	Reverse	ATGCCTCCACTCACTCACAGTCTT		
Arc	Primer set #2		Minus 489	119bp
	Forward	GGCACTTCTGAGGTTGCATACTGT		
	Reverse	ATTTGCCCTCAGGGTCTGATCTCT		
Arc	Primer set #3		Minus 312	122 bp
	Forward	ATAACCTGCCTTAGCTTCACCCTC		
	Reverse	TTCACAGCCCGGAGTGACTAATGT		
Arc	Primer set #4		Minus 8	64 bp
	Forward	TTTAGTGCAGTGCTCTGGCGAGTAGT		
	Reverse	TCAAGCTGAAGAGGCCCAGAGA		
Arc	Primer set #5		Plus 180	56bp
	Forward	TTCTTAGCCTGTCCCGAACCGTAA		
	Reverse	TCATATGGTCCAGCTCCATCTGCT		
Arc	Primer set #6		Plus 214	93bp
	Forward	AGATGGAGCTGGACCATATGACGA		
	Reverse	TCTGCAGGATCACATTGGGTTTGG		
Arc	Primer set #7		Plus 297	83bp
	Forward	ATCCTGCAGATTGGTAAGTGCCGA		
	Reverse	TTGGACACTTCGGTCAACAGATGC		
Arc	Primer set #8		Plus 2195	92bp
	Forward	GAATTTGCTATGCCAACTCACGGG		
	Reverse	AGTCATGGAGCCGAAGTCTGCTTT		
Arc	Primer set #9		Plus 2749	115 bp
	Forward	CTGCACAGAGGTTCCAAGACACTT		
	Reverse	TGGCTTGTCTTCACCTTCAGCTCT		

cFos	Primer set **#1**		Minus 1480bp	91 bp
	Forward	AGGTTTGTGGTTCTGCACCTCTTG		
	Reverse	AACTAACTGGTGGGCATTCCGAGA		
cFos	Primer set **#2**		Minus 53 bp	78 bp
	Forward	TTCTATAAAGCGGCCAGCTGAGG		
	Reverse	GGCTCTATCCAGTCTTCTCAGTTGCT		
cFos	Primer set **#3**		Plus 437 bp	79 bp
	Forward	AGCGCGTGCACACTTGTCATACTA		
	Reverse	AGAGCGCAGGACACTCAGAGTAAA		
cFos	Primer set **#4**		Plus 857 bp	99 bp
	Forward	TGAACAGAGGTGACACTAGACCAC		
	Reverse	TCCTTTCCCTTCGGATTCTCCGTT		
Zif268	Primer set **#1**		Minus 1138 bp	112 bp
	Forward	TCACCAGACTGGCCAAAGCAAAGT		
	Reverse	GTTGGTTCTTGTGATGGTTGGGCA		
Zif268	Primer set **#2**		Minus 62 bp	72 bp
	Forward	CCCGTGCTGTTTCAGACCCTTGAAAT		
	Reverse	AAGTTCTGCGCGCTGGGATCTCT		
Zif268	Primer set **#3**		Plus 291 bp	100 bp
	Forward	GCAATTGATGTCTCCGCTGCAGAT		
	Reverse	CAGCAGCATCATCTCCTCCAGTTT		
Zif268	Primer set **#4**		Plus 1182	141 bp
	Forward	TTCGGCTCTCATCGTCCAGTGATT		
	Reverse	AACCGGGTAGTTTGGCTGGGATAA		
Egr3	Primer set **#1**		Minus 1360 bp	84 bp
	Forward	TTCAACTGAGAGTCAGTGCAGGGT		
	Reverse	TCGAGCCGACTTCATTCCGATTCA		

Egr3	Primer set **#2**		Minus 8 bp	60 bp
	Forward	GGAGTGCTATGACCGGCAAACT		
	Reverse	TTAGCAAACTGCTCATGGTCACCG		
Egr3	Primer set **#3**		Plus 212 bp	73 bp
	Forward	AGGGAGAGAGCTAGCGATGTATGC		
	Reverse	TCTTTCCATCCGTCTCTCCATCCA		
Egr3	Primer set **#4**		Plus 2531 bp	188bp
	Forward	TGTGAGTTCTGTGGGCGCAAGTTT		
	Reverse	AAAGAAGGGATCTGAGAGGCGGAT		
Gadd45g	Primer set **#1**		Minus 1706bp	64 bp
	Forward	TTCACCACCCACCTAGAGAACACT		
	Reverse	AGACAGGCAGAGAGAGACACAGAA		
Gadd45g	Primer set **#2**		0 bp	91 bp
	Forward	CGCGGATCGTCTTTGGGAATCTTT		
	Reverse	CATTGTGCGATCCACGAACAGCAA		
Gadd45g	Primer set **#3**		Plus 634 bp	88 bp
	Forward	CGGGAAAGCATTGCACGAACTTCT		
	Reverse	ATTCAGGACTTTGGCGGACTCGTA		
Gadd45g	Primer set **#4**		Plus 1178 bp	118 bp
	Forward	TGGAGAAGCTCAGTTTGTTCTGCG		
	Reverse	AGTGTCACCAAGTCGATCAGACCA		
Cox2	Primer set **#1**		Minus 807	114 bp
	Forward	AATGCGGTGGACACTTAGCATTCC		
	Reverse	AGTCTTTCACCGAACTGTCCTCCA		
Cox2	Primer set **#2**		Minus 6 bp	73 bp
	Forward	TCTGCGATGCTCTTCCGAGCTGT		
	Reverse	AAGCCACTCACTCACCTGCAT		
Cox2	Primer set **#3**		Plus 791 bp	87 bp

	Forward	CCTTGCTGTTCCAACCCATGTCAA		
	Reverse	TCCAGTCCGGGTACAGTCACATTT		
Cox2	Primer set **#4**		Plus 1081 bp	125 bp
	Forward	TACATCCTGACCCACTTCAAGGGA		
	Reverse	TGTTCGATGCTGGAGAACAAGGGA		
Ier2	Primer set **#1**		Minus 1706 bp	105 bp
	Forward	AATTGGAGAGGTGTGGTCCACGAT		
	Reverse	ACCCACTTCCGGATGCCCATATAA		
Ier2	Primer set **#2**		Minus 45 bp	102 bp
	Forward	TAAAGGACTCCAGCTTGCTTTCCC		
	Reverse	ACAATGGATTAAGACCGGCGTTGC		
Ier2	Primer set **#3**		Plus 618 bp	116 bp
	Forward	AAGGCGCTTCTCCAGTCTCTTGAA		
	Reverse	GCACCAGCACGTTGAGCATATTGT		
Ier2	Primer set **#4**			
	Forward	ACTGTCCCTTCCTTGGTTTGGAGA	Plus 933	114 bp
	Reverse	GCCGAAACGCGAATGCTAGTGAAA		
Dusp6	Primer set **#1**		Minus 629 bp	127 bp
	Forward	GCATTTAACACTTCGTTGGGTCGC		
	Reverse	TCGTGTGTGGCATTTGTTTGGCTC		
Dusp6	Primer set **#2**		Minus 41 bp	116 bp
	Forward	TGTATCCATTGAGACGCTGGCTGT		
	Reverse	TCGCCGGATTATTCAAGACTGGGT		
Dusp6	Primer set **#3**		Plus 335 bp	125 bp
	Forward	TTGGACGTGTTGGAAGAGTTTGGC		
	Reverse	TGGCTCCAGTGATCAGAGATAGGA		
Dusp6	Primer set **#4**		Plus 1230 bp	89 bp
	Forward	TCCTGTGCCTCTCACAAGCTGAAA		

	Reverse	AACTTACTGAAGCCACCTGCCAGA		
Nurr1	Primer set **#1**		Minus 1375 bp	64 bp
	Forward	GCGGAGATGTGCTCAAAGTTTGCT		
	Reverse	AGATGGAGCCTACTCAAGCCACAA		
Nurr1	Primer set **#2**		Minus 23 bp	95 bp
	Forward	TATTTCAGGGAGATCTGACGGGCT		
	Reverse	GGTGGACAGTGTCGTAATTCAGCA		
Nurr1	Primer set **#3**		Plus 664bp	75 bp
	Forward	TCTTCGGGAGAATACAGCTCCGAT		
	Reverse	TTCAGTGTTGGTGAGGTCCATGCT		
Nurr1	Primer set **#4**		Plus 4358 bp	124 bp
	Forward	CGACATTTCTGCCTTCTCCTGCAT		
	Reverse	TGTGATTAGGGAAAGCCATGACCG		
Egr2	Primer set **#1**		Minus 896 bp	90 bp
	Forward	AGCAGCTCTTCTGCTCCATTCTCT		
	Reverse	GGAACAGGTCTGGGCTGTTTGTTT		
Egr2	Primer set **#2**		Plus 2 bp	66 bp
	Forward	GCAGGAGAGAGTCAGTGGCAGATA		
	Reverse	GCAACCAAGTTGTTGGCTTCT		
Egr2	Primer set **#3**		Plus 1842 bp	106 bp
	Forward	ACCAGACCTTCACTTACATGGGCA		
	Reverse	TGCAAGATGCCCGCACTCACAATA		
Egr2	Primer set **#4**		Plus 3514 bp	113 bp
	Forward	TGTGGCCCTAGAACAGTGAATGGA		
	Reverse	TTGCTTTGTCCTTCCCGACATTGC		