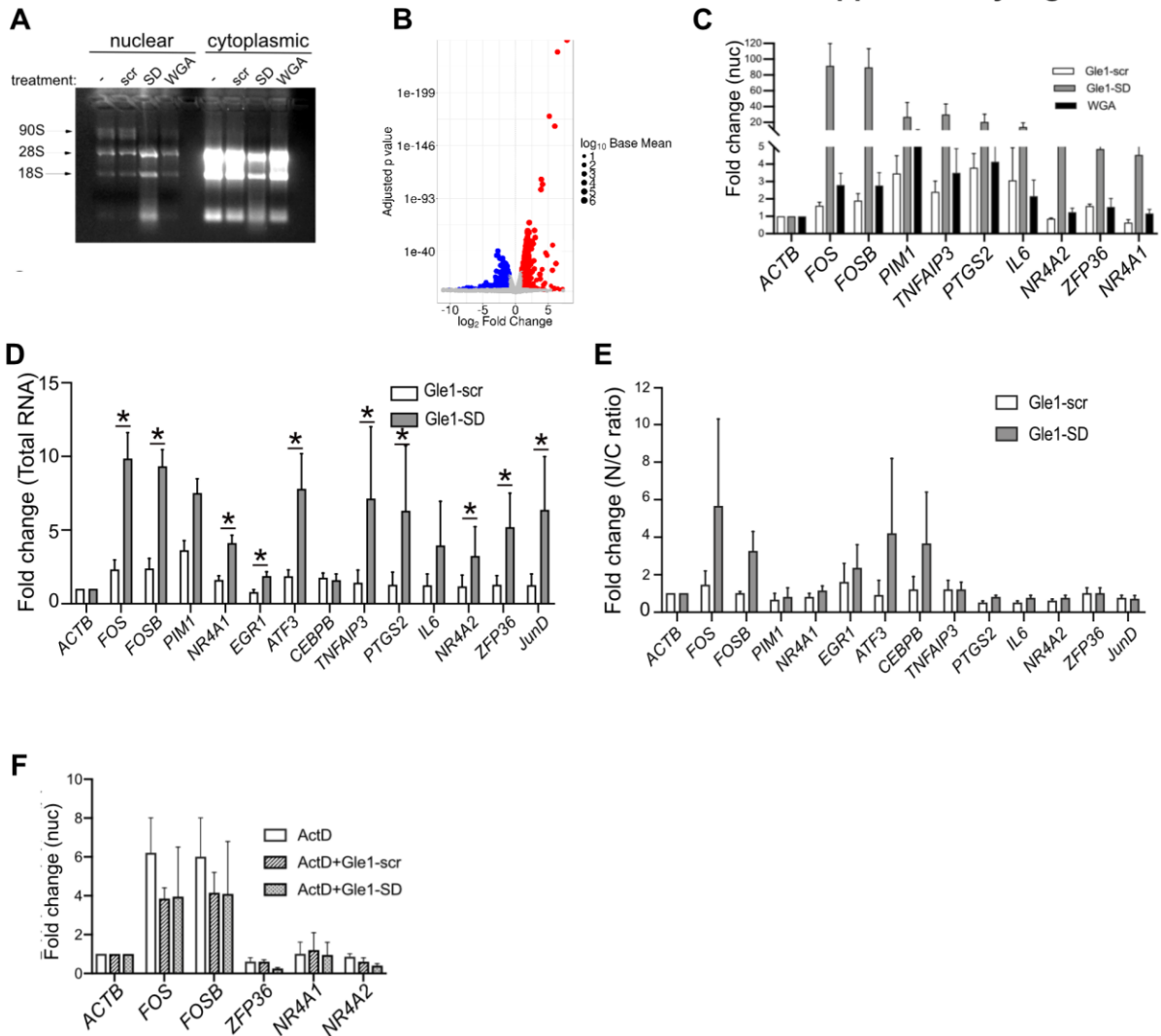


# Supplemental Materials

*Molecular Biology of the Cell*

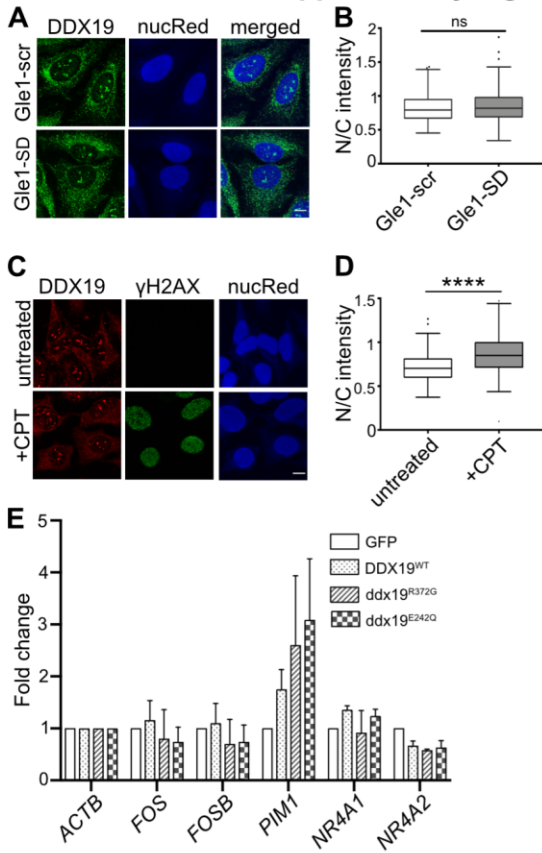
Sharma and Wente

## Supplementary Figure 1



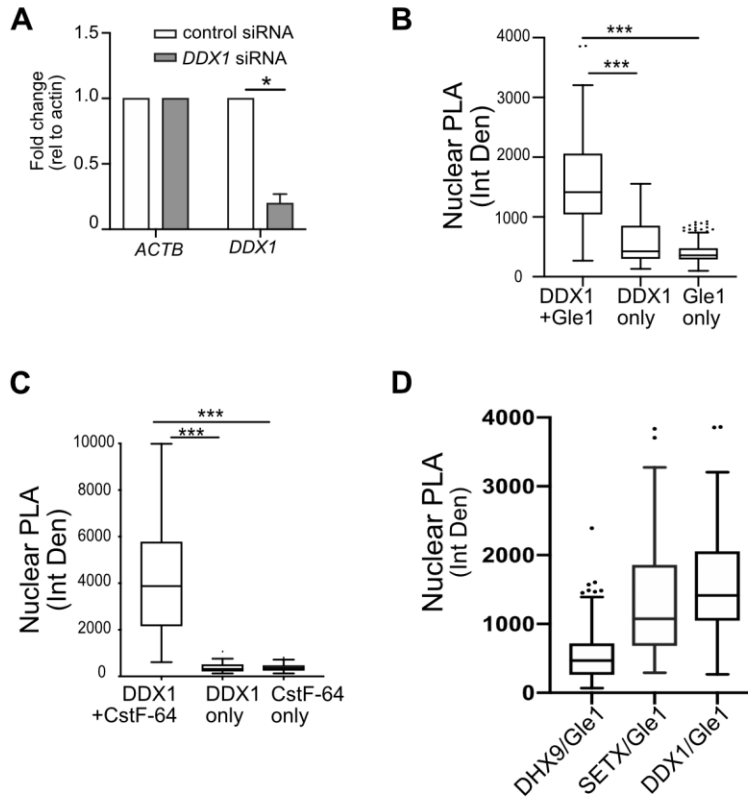
**Supplementary Figure 1:** (A) RNA gel showing equal volumes of nuclear and cytoplasmic RNA fractions under different treatments. Nuclear fraction is marked by the presence of 90s RNA, which is absent from cytoplasmic RNA. (B) Volcano plot showing differences in accumulation of all the transcripts as quantified using edgeR method (red and blue represents Gle1-SD and Gle1-scr treated samples). (C) HeLa cells were treated with 0.5mg/ml of Wheat Germ Agglutinin (WGA) or 5  $\mu$ M of Gle1-scr or Gle1-SD for 4 hours and tested for nuclear accumulation of selected target genes using RT-qPCR and normalized using  $\Delta\Delta$ CT values as detailed in Figure 1. The graph represents data of (mean  $\pm$  sem) from 3 biological replicates performed in triplicates. (D) Total RNA was extracted from Gle1-scr or Gle1-SD treated cells and targets were amplified using RT-qPCR. Fold change of twelve gene targets was calculated using  $\Delta\Delta$ CT method as in Figure 1. The graph represents mean  $\pm$  sem from 3 biological repeats performed in triplicates. (E) Amplicons from Gle1-scr or Gle1-SD treated cells were amplified from nuclear and cytoplasmic compartments of the cell. Fold change was calculated using  $\Delta\Delta$ CT method. The ratio of nuclear fold change to cytoplasmic fold change (mean  $\pm$  sem) is depicted in the graph. The graph represents data from 3 biological replicates performed in triplicates. (F) HeLa cells were pre-treated with 0.5mg/ml of Actinomycin D or DMSO for 1 hour prior to Gle1-SD or scr treatment (5 $\mu$ M) for 4 hours. RT-qPCR was performed on nuclear RNA as in Figure 1. The graph represents data from 2 biological repeats performed in triplicates.

## Supplementary Figure 2



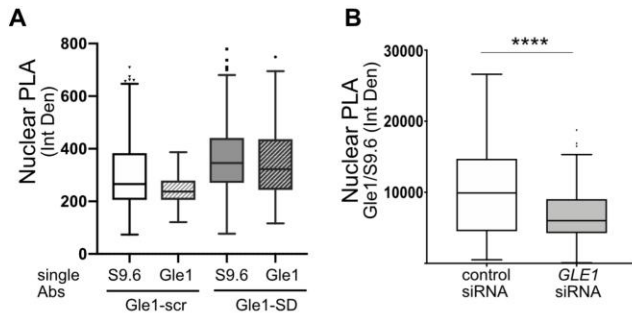
**Supplementary Figure 2: Gle1's effect is not mediated through DDX19.** (A) Gle1-scr or Gle1-SD peptide treated HeLa cells were immunostained with DDX19 antibody and imaged by confocal microscopy. Scale bar 10  $\mu$ m. (B) Nuclear-cytoplasmic intensity was quantified from three independent experiments and plotted on the graph. Unpaired two-tailed t-test was used to calculate significance (ns represents non-significant). (C) HeLa cells were treated with 250 nM camptothecin (CPT) for 18 hrs and immunostained with DDX19 and  $\gamma$ H2AX antibodies. NucRed is pseudo-colored as blue in the confocal images. Scale bar 10  $\mu$ m. (D) Nuclear-cytoplasmic intensity of DDX19 from two independent experiments was measured in ImageJ. Unpaired, two-tailed t-test was used to analyze statistical significance (ns represents non-significant, \*\*\*\* represents  $p < 0.0001$ ). (E) HeLa cells were transfected with *GFP*, *GFP-DDX19<sup>wt</sup>*, *GFP-DDX19<sup>R372G</sup>* or *GFP-DDX19<sup>E242Q</sup>* and flow sorted to isolate GFP-positive cells. Sorted cells were subjected to RT-qPCR from nuclear RNA. Fold change was calculated relative to actin in GFP transfected cells. Data represents mean  $\pm$  sem from at least 2 independent experiments and qPCR was performed in triplicates.

### Supplementary Fig 3



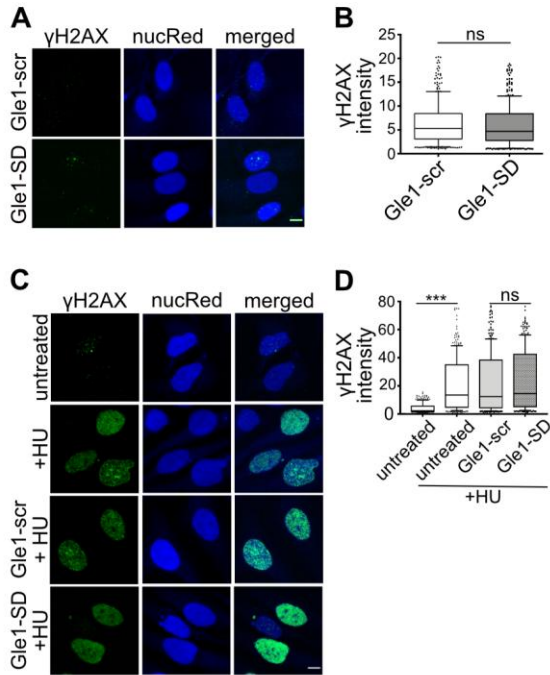
**Supplementary Figure 3:** (A) RT-qPCR data confirming reduced nuclear RNA levels of DDX1 upon silencing in Figure 4. Samples were normalized to actin and fold change was calculated using  $\Delta\Delta CT$  method (as detailed in Figure 1).  $\Delta CT$  values were used to calculate statistical significance using one-tailed, paired t-test (\* represents  $p < 0.05$ ). (B) Untreated HeLa cells were subjected to Proximity Ligation Assay (PLA) with either DDX1, Gle1 or both antibodies, counter stained with NucRed. Quantification of nuclear PLA signal measured as Integrated Density using ImageJ from maximum intensity projected cell images is depicted in the graph (\*\*\*) represents  $p < 0.0001$ ). (C) Nuclear PLA signal measured as Integrated Density using ImageJ in untreated cells stained with single antibody (DDX1, CstF-64 or both) is plotted in the graph. Data acquired from maximum intensity projected cell images. (D) Untreated HeLa cells were subjected to Proximity Ligation Assay (PLA) with either Gle1 and DHX9, SETX, DDX1 antibody (as indicated). Nuclear PLA signal measured as Integrated Density using ImageJ was quantified and depicted in the graph.

### Supplementary Fig 4



**Supplementary Figure 4:** (A) Single antibody controls for Proximity Ligation Assay (PLA) for Figure 7. Cells were counter stained with nucleolin antibody. Nuclear PLA signal (measured as Integrated Density from mid-plane sections) outside the nucleolus with individual antibodies was quantified for 200 cells from three independent experiments and depicted in the graph. (B) Specificity control for Proximity Ligation Assay for Figure 7. HeLa cells were transfected with control or *GLE1* siRNA, subjected to proximity ligation assay with Gle1 and S9.6 antibody and counter stained with nucleolin antibody. The graph shows nuclear PLA signal (measured as Integrated Density from mid-plane sections) outside the nucleolus for 150 randomly selected cells from three independent experiments. Statistical significance was calculated using unpaired two-tailed t-test \*\*\*\* represents  $p < 0.0001$ .

## Supplementary Figure 5



**Supplementary Figure 5: Gle1-SD treatment neither causes DNA damage nor pre-disposes cells to the damage.** (A) HeLa cells were treated with Gle1-scr or Gle1-SD peptide and immunostained with  $\gamma$ H2AX antibody to mark DNA damage and imaged on confocal microscope. NucRed is pseudo-colored as blue. Scale bar represents 10  $\mu$ m. (B) Nuclear intensity of  $\gamma$ H2AX was quantified from maximum intensity projected images and plotted on a box and whiskers graph from over 350 cells from at least 3 independent experiments. Unpaired, two-tailed t-test was used to analyze significance (ns represents non-significant). (C) HeLa cells were pre-treated with Gle1-scr or Gle1-SD peptide for 30 mins followed by 5mM hydroxyurea treatment in the presence of Gle1-scr or Gle1-SD peptide for 4 hrs, immunostained with  $\gamma$ H2AX antibody and imaged by confocal microscopy. Scale bar represents 10  $\mu$ m. (D) Nuclear intensity of  $\gamma$ H2AX was quantified from maximum intensity projected images and plotted on a box and whiskers graph from over 250 cells from at least 3 independent experiments. Unpaired, two-tailed t-test was used to analyze statistical significance (ns represents non-significant, \*\*\* represents  $p < 0.0005$ ).

**Supplementary Table S1: List of top 70 Gle1 target genes with 5 fold change - Gle1 SD vs Gle1-scrambled peptide**

	Feature_gene_biot	Feature_gen	Gle1-scr	Gle1-scr	Gle1-SD	Gle1-SD	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	FoldChange
ENSG00000169429	protein_coding	IL8	48.00	87.00	9291.00	7278.00	5617.73	7.87	0.18	43.66	0.00	0.00	233.29
ENSG00000197632	protein_coding	SERPINB2	0.00	0.00	15.00	14.00	9.86	7.21	2.09	3.45	0.00	0.00	147.93
ENSG00000197887	protein_coding	OR152	0.00	0.00	0.00	24.00	8.87	7.05	2.49	2.83	0.00	0.01	132.58
ENSG00000255073	protein_coding	ZFP91-CNTF	0.00	0.00	25.00	2.00	8.56	7.01	2.29	3.06	0.00	0.01	128.83
ENSG00000176798	protein_coding	OR51L1	0.00	0.00	25.00	0.00	7.82	6.88	2.56	2.69	0.01	0.02	117.68
ENSG00000234485	pseudogene	OR7E46P	0.00	0.00	24.00	0.00	7.51	6.82	2.58	2.64	0.01	0.02	112.97
ENSG00000255292	protein_coding	SDHD	0.00	0.00	11.00	10.00	7.13	6.74	2.29	2.94	0.00	0.01	107.01
ENSG00000226537	pseudogene	OR7E33P	0.00	0.00	0.00	19.00	7.02	6.71	2.64	2.54	0.01	0.03	104.94
ENSG00000068985	protein_coding	PAGE1	0.00	0.00	7.00	13.00	6.99	6.71	2.32	2.89	0.00	0.01	104.69
ENSG00000159713	protein_coding	TPPP3	1.00	0.00	31.00	18.00	16.56	6.47	1.79	3.61	0.00	0.00	88.73
ENSG00000163739	protein_coding	CXCL1	51.00	90.00	3488.00	2881.00	2179.80	6.43	0.19	33.39	0.00	0.00	86.11
ENSG00000170345	protein_coding	FOS	59.00	130.00	4990.00	2230.00	2416.91	6.19	0.54	11.48	0.00	0.00	72.87
ENSG00000081041	protein_coding	CXCL2	65.00	70.00	2797.00	1875.00	1591.96	6.01	0.22	27.77	0.00	0.00	64.28
ENSG00000205449	pseudogene	NBPF22P	1.00	0.00	6.00	27.00	12.06	5.99	1.98	3.02	0.00	0.01	63.39
ENSG00000260765	pseudogene	CES1P2	0.00	2.00	41.00	22.00	21.26	5.94	1.46	4.06	0.00	0.00	61.19
ENSG00000178734	protein_coding	C13orf45	0.00	1.00	22.00	9.00	10.36	5.85	2.00	2.92	0.00	0.01	57.79
ENSG00000115009	protein_coding	CCL20	13.00	19.00	483.00	383.00	298.23	5.68	0.38	14.83	0.00	0.00	51.19
ENSG00000125740	protein_coding	FOSB	71.00	81.00	3004.00	1191.00	1406.99	5.65	0.56	10.01	0.00	0.00	50.05
ENSG00000167390	pseudogene	POM121L3P	1.00	2.00	77.00	6.00	26.82	5.64	1.37	4.10	0.00	0.00	49.76
ENSG00000143536	protein_coding	CRNN	0.00	1.00	26.00	1.00	8.65	5.60	2.24	2.49	0.01	0.04	48.45
ENSG00000197124	protein_coding	ZNF682	0.00	1.00	13.00	11.00	8.28	5.52	2.11	2.62	0.01	0.03	46.04
ENSG00000166592	protein_coding	RRAD	2.00	2.00	48.00	44.00	32.00	5.44	1.08	5.03	0.00	0.00	43.27
ENSG00000073756	protein_coding	PTGS2	159.00	158.00	3424.00	2699.00	2125.99	5.16	0.18	28.60	0.00	0.00	35.85
ENSG00000163734	protein_coding	CXCL3	20.00	30.00	443.00	307.00	260.83	4.82	0.37	13.09	0.00	0.00	28.27
ENSG00000187479	protein_coding	C11orf96	42.00	33.00	611.00	394.00	350.64	4.61	0.34	13.52	0.00	0.00	24.43
ENSG00000164400	protein_coding	CSF2	1.00	21.00	103.00	138.00	86.65	4.52	0.72	6.28	0.00	0.00	22.94
ENSG00000215749	protein_coding	GOLGA6L18	12.00	14.00	10.00	256.00	102.41	4.37	1.78	2.46	0.01	0.04	20.74
ENSG00000120738	protein_coding	EGR1	94.00	100.00	1682.00	466.00	733.57	4.30	0.78	5.50	0.00	0.00	19.73
ENSG00000153234	protein_coding	NR4A2	489.00	650.00	6904.00	3816.00	3773.04	4.13	0.18	22.43	0.00	0.00	17.47
ENSG00000145283	protein_coding	SLC10A6	2.00	5.00	41.00	22.00	22.15	4.10	1.11	3.68	0.00	0.00	17.19
ENSG00000164949	protein_coding	GEM	23.00	34.00	273.00	232.00	181.22	4.07	0.40	10.22	0.00	0.00	16.83
ENSG00000213885	pseudogene	RPL13AP7	0.00	4.00	17.00	14.00	11.11	4.00	1.62	2.48	0.01	0.04	16.02
ENSG00000162891	protein_coding	IL20	2.00	3.00	20.00	20.00	14.53	3.94	1.33	2.96	0.00	0.01	15.30
ENSG00000136244	protein_coding	IL6	470.00	862.00	5026.00	5425.00	3808.88	3.94	0.17	22.94	0.00	0.00	15.30
ENSG00000162772	protein_coding	ATF3	968.00	1062.00	10387.00	5993.00	5831.86	3.89	0.18	21.87	0.00	0.00	14.79
ENSG00000123358	protein_coding	NR4A1	347.00	356.00	3703.00	1639.00	1892.20	3.78	0.49	7.67	0.00	0.00	13.71
ENSG00000118503	protein_coding	TNFAIP3	1407.00	1958.00	16457.00	7680.00	8585.05	3.73	0.42	8.97	0.00	0.00	13.27
ENSG00000164651	protein_coding	SP8	2.00	3.00	12.00	21.00	12.40	3.69	1.42	2.59	0.01	0.03	12.89
ENSG00000219773	pseudogene	RP11-506B6	2.00	3.00	7.00	23.00	11.57	3.58	1.50	2.39	0.02	0.04	11.96
ENSG00000105808	protein_coding	RASA4	7.00	5.00	61.00	16.00	27.25	3.49	1.02	3.41	0.00	0.00	11.25
ENSG00000144785	protein_coding	RP11-977G1	0.00	11.00	47.00	7.00	18.99	3.30	1.39	2.38	0.02	0.05	9.87
ENSG00000168334	protein_coding	XIRP1	3.00	8.00	37.00	19.00	20.46	3.29	1.10	3.00	0.00	0.01	9.79
ENSG00000127540	protein_coding	UQCR11	5.00	8.00	32.00	33.00	24.50	3.27	0.99	3.31	0.00	0.00	9.62
ENSG00000177910	pseudogene	SPATA31C2	9.00	8.00	78.00	12.00	31.97	3.21	1.00	3.23	0.00	0.00	9.26
ENSG00000163661	protein_coding	PTX3	26.00	49.00	193.00	164.00	134.04	3.20	0.44	7.27	0.00	0.00	9.17
ENSG00000172602	protein_coding	RND1	4.00	9.00	31.00	27.00	21.91	3.12	1.04	3.01	0.00	0.01	8.68
ENSG00000221869	protein_coding	CEBPD	11.00	14.00	61.00	53.00	43.16	3.10	0.74	4.19	0.00	0.00	8.60
ENSG00000197576	protein_coding	HOXA4	1.00	10.00	27.00	17.00	16.48	3.03	1.26	2.40	0.02	0.04	8.15

ENSG00000108691	protein_coding	CCL2	1153.00	1464.00	4664.00	6350.00	4275.46	3.01	0.19	16.01	0.00	0.00	8.07
ENSG00000179388	protein_coding	EGR3	14.00	2.00	47.00	29.00	28.69	2.99	1.04	2.88	0.00	0.01	7.96
ENSG00000232810	protein_coding	TNF	9.00	12.00	47.00	41.00	33.61	2.99	0.83	3.60	0.00	0.00	7.93
ENSG00000184779	protein_coding	RPS17	10.00	35.00	47.00	123.00	67.67	2.98	0.72	4.14	0.00	0.00	7.87
ENSG00000166920	protein_coding	C15orf48	0.00	19.00	38.00	29.00	25.53	2.91	1.18	2.46	0.01	0.04	7.49
ENSG00000169242	protein_coding	EFNA1	882.00	969.00	4537.00	2979.00	2856.38	2.90	0.17	17.02	0.00	0.00	7.49
ENSG00000128016	protein_coding	ZFP36	341.00	262.00	1629.00	852.00	937.07	2.87	0.28	10.33	0.00	0.00	7.33
ENSG00000164266	protein_coding	SPINK1	18.00	19.00	62.00	78.00	54.96	2.84	0.67	4.26	0.00	0.00	7.16
ENSG00000137193	protein_coding	PIM1	230.00	321.00	1318.00	754.00	789.17	2.81	0.23	12.05	0.00	0.00	7.02
ENSG00000163331	protein_coding	DAPL1	5.00	13.00	29.00	32.00	23.96	2.75	0.99	2.79	0.01	0.02	6.72
ENSG00000198930	protein_coding	CSAG1	34.00	55.00	159.00	149.00	120.49	2.73	0.45	6.09	0.00	0.00	6.64
ENSG00000139318	protein_coding	DUSP6	23.00	53.00	151.00	109.00	100.57	2.73	0.51	5.38	0.00	0.00	6.64
ENSG00000112096	protein_coding	SOD2	897.00	1657.00	3981.00	4504.00	3355.44	2.70	0.18	15.04	0.00	0.00	6.50
ENSG00000011083	protein_coding	SLC6A7	25.00	22.00	118.00	44.00	61.86	2.62	0.67	3.91	0.00	0.00	6.15
ENSG00000146678	protein_coding	IGFBP1	8.00	44.00	74.00	81.00	61.57	2.62	0.71	3.70	0.00	0.00	6.14
ENSG00000113212	protein_coding	PCDHB7	2.00	20.00	32.00	32.00	25.35	2.60	1.05	2.48	0.01	0.04	6.07
ENSG00000124882	protein_coding	EREG	314.00	378.00	1403.00	858.00	880.82	2.60	0.22	11.87	0.00	0.00	6.05
ENSG00000172216	protein_coding	CEBPB	161.00	180.00	650.00	445.00	429.67	2.57	0.27	9.67	0.00	0.00	5.95
ENSG00000130066	protein_coding	SAT1	455.00	644.00	1894.00	1528.00	1352.94	2.56	0.17	15.48	0.00	0.00	5.90
ENSG00000182774	protein_coding	RPS17L	39.00	176.00	259.00	343.00	243.20	2.54	0.46	5.54	0.00	0.00	5.80
ENSG00000133134	protein_coding	BEX2	5.00	16.00	33.00	28.00	24.20	2.53	0.98	2.57	0.01	0.03	5.77
ENSG00000214016	pseudogene	RP6-105D16	8.00	77.00	129.00	106.00	93.11	2.53	0.68	3.73	0.00	0.00	5.76
ENSG00000179776	protein_coding	CDH5	11.00	36.00	98.00	35.00	51.48	2.45	0.75	3.27	0.00	0.00	5.47
ENSG00000156711	protein_coding	MAPK13	5.00	19.00	20.00	43.00	26.14	2.45	1.00	2.45	0.01	0.04	5.45
ENSG00000138166	protein_coding	DUSP5	163.00	264.00	716.00	476.00	475.18	2.40	0.26	9.40	0.00	0.00	5.29
ENSG00000100906	protein_coding	NFKBIA	5656.00	5545.00	19787.00	12654.00	12920.55	2.40	0.17	14.25	0.00	0.00	5.28
ENSG00000141682	protein_coding	PMAIP1	101.00	163.00	424.00	305.00	291.91	2.39	0.30	7.93	0.00	0.00	5.25
ENSG00000188293	protein_coding	IGFL1	49.00	101.00	159.00	237.00	163.29	2.39	0.43	5.50	0.00	0.00	5.24
ENSG00000138670	protein_coding	RASGEF1B	27.00	10.00	69.00	42.00	44.37	2.37	0.80	2.97	0.00	0.01	5.18
ENSG00000163993	protein_coding	S100P	58.00	66.00	213.00	134.00	138.63	2.37	0.43	5.57	0.00	0.00	5.17
ENSG00000184497	protein_coding	TMEM255B	7.00	22.00	22.00	49.00	29.87	2.33	0.94	2.50	0.01	0.03	5.05
ENSG00000197061	protein_coding	HIST1H4C	503.00	608.00	1324.00	1589.00	1201.84	2.32	0.20	11.31	0.00	0.00	4.99



**Supplementary Table S2**

**Primer list:**

<b>Primer Name</b>	<b>Forward primer (5'-3')</b>	<b>Reverse primer (5'-3')</b>
<i>ACTB</i>	CACCATTGGCAATGAGCGGTTC	AGGTCTTTGCGGATGTCCACGT
<i>FOS</i>	GCCTCTCTTACTACCACTCACC	AGATGGCAGTGACCGTGGGAAT
<i>FOSB</i>	GCCTCTCTTACTACCACTCACC	AGATGGCAGTGACCGTGGGAAT
<i>PIMI</i>	TCTACTCAGGCATCCGCGTCTC	CTTCAGCAGGACCACTTCCATG
<i>TNFAIP3</i>	CTCAACTGGTGTCGAGAAGTCC	TTCCTTGAGCGTGCTGAACAGC
<i>PTGS2</i>	CGGTGAAACTCTGGCTAGACAG	GCAAACCGTAGATGCTCAGGGA
<i>IL6</i>	AGACAGCCACTCACCTCTTCAG	TTCTGCCAGTGCCTCTTTGCTG
<i>ZFP36</i>	GCTATGTTCGGACCTTCTCAGAG	CCTGGAGGTAGAACTTGTGACAG
<i>NR4A1</i>	GGACAACGCTTCATGCCAGCAT	CCTTGTTAGCCAGGCAGATGTAC
<i>NR4A2</i>	AAACTGCCAGTGGACAAGCGT	GCTCTTCGGTTTCGAGGGCAA
<i>EGR1</i>	AGCAGCACCTTCAACCCTCAGG	GAGTGGTTTGGCTGGGGTAACT
<i>JUND</i>	ATCGACATGGACACGCAGGAGC	CTCCGTGTTCTGACTCTTGAGG
<i>CEBPB</i>	AGAAGACCGTGGACAAGCACAG	CTCCAGGACCTTGTGCTGCGT
<i>ACTB In1</i>	CGGGGTCTTTGTCTGAGC	CAGTTAGCGCCCAAAGGAC
<i>FOSB primer A</i>	CCTGCCAACCACAATTCAATG	TGCGTTTTTAAGAGCTTTTTTATT
<i>FOSB primer B</i>	CAGTGCCTCCTACTAGAAAAAGTAAG	GGATTACAGGTGTGCACCACC
<i>FOSB primer C</i>	CTATACACAGAAGAAGCTTAATG	CTTTTTTTTTTTTTTCTCAACATAATG
<i>FOS primer A</i>	GAGGAGAAACACATCTTC	GCCTGGTGTGTTTCAC
<i>FOS primer D</i>	GATCCCTTATCAAAGAGGAC	GAAGCAGTAGAAAATCTTAGC
<i>FOS primer E</i>	TCACGTGGAGGGTTATTC	TGGATACGATAACGAGCAG