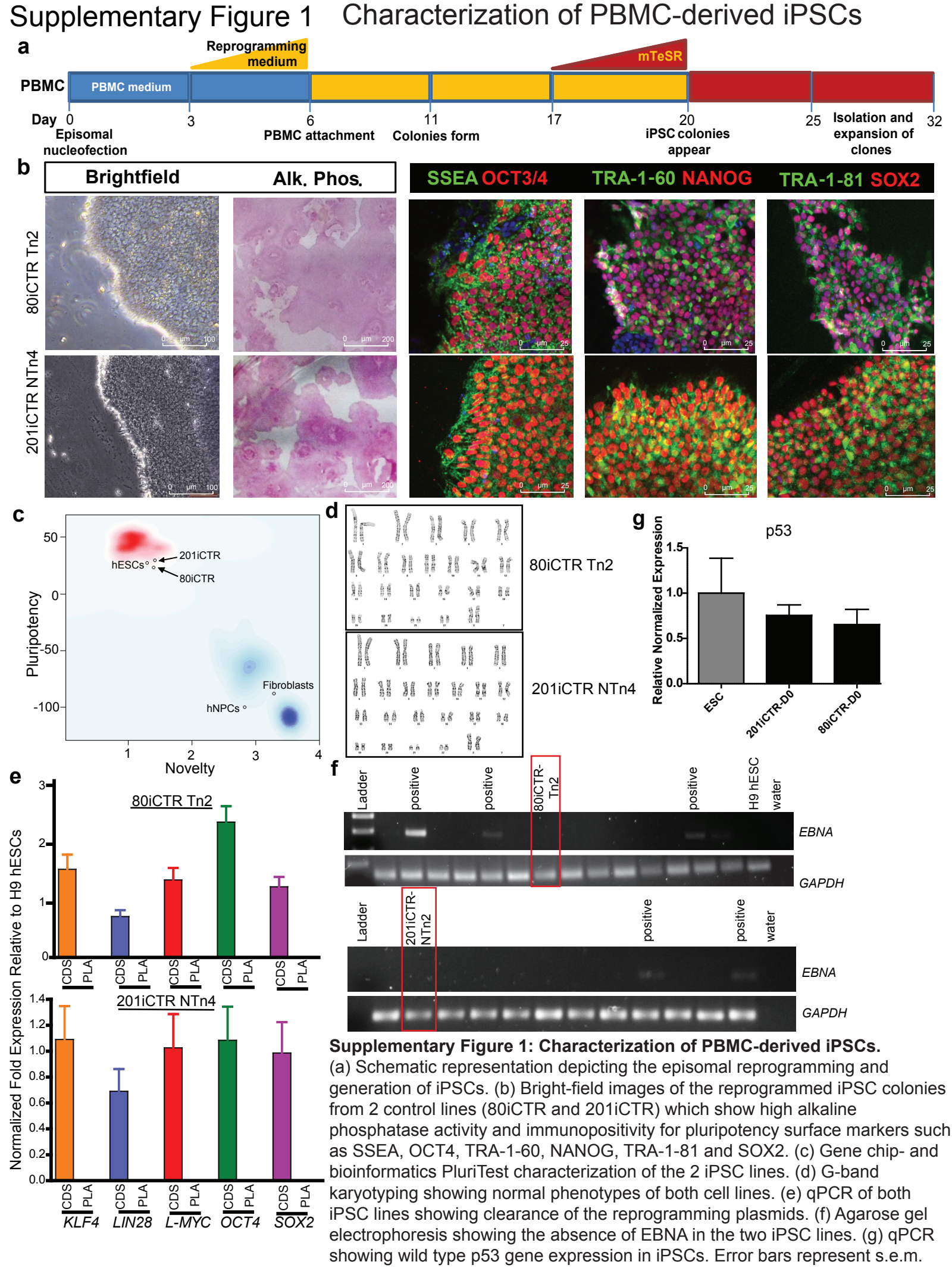
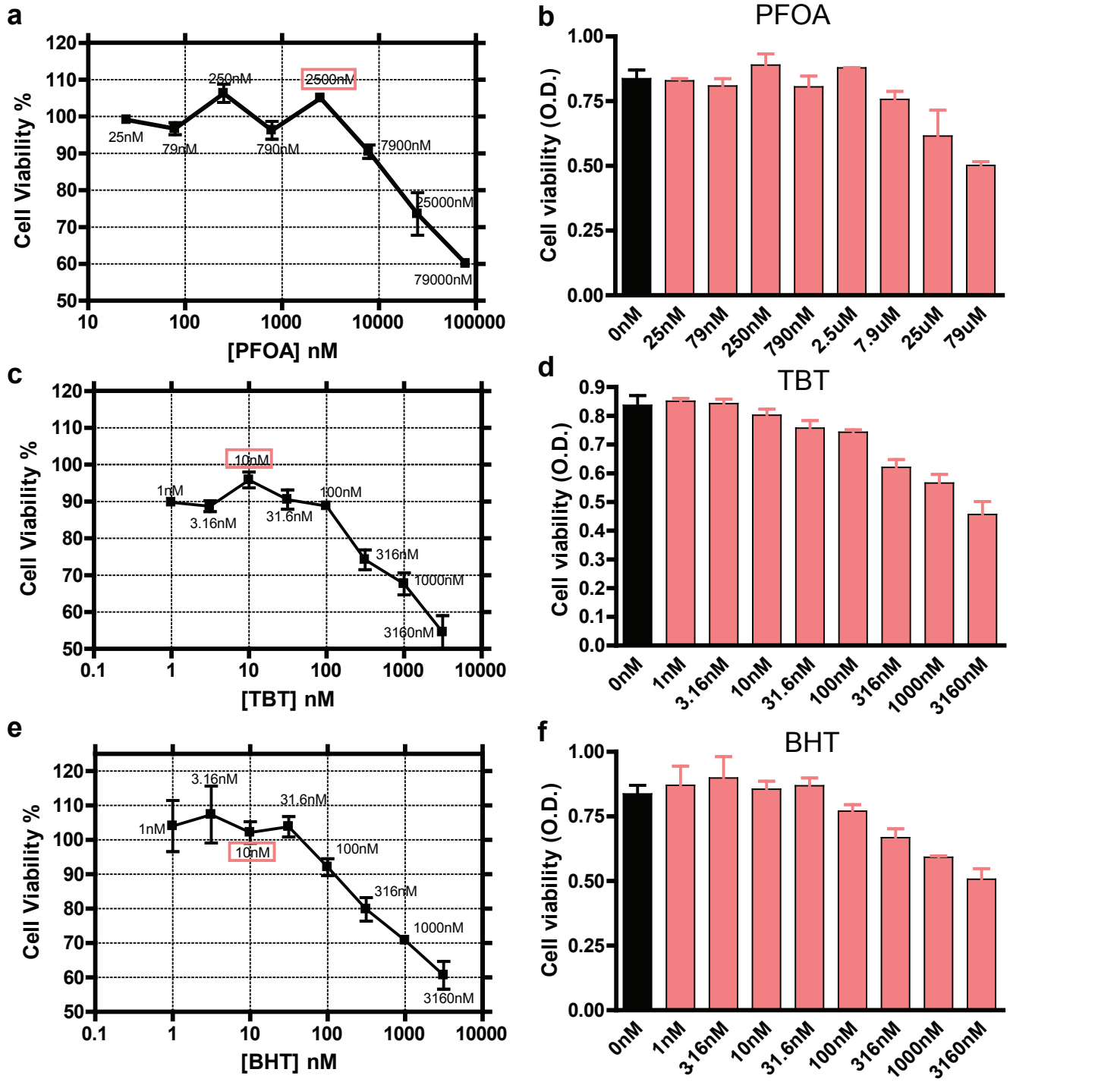
File name: Supplementary Information
Description: Supplementary Figures and Supplementary Tables

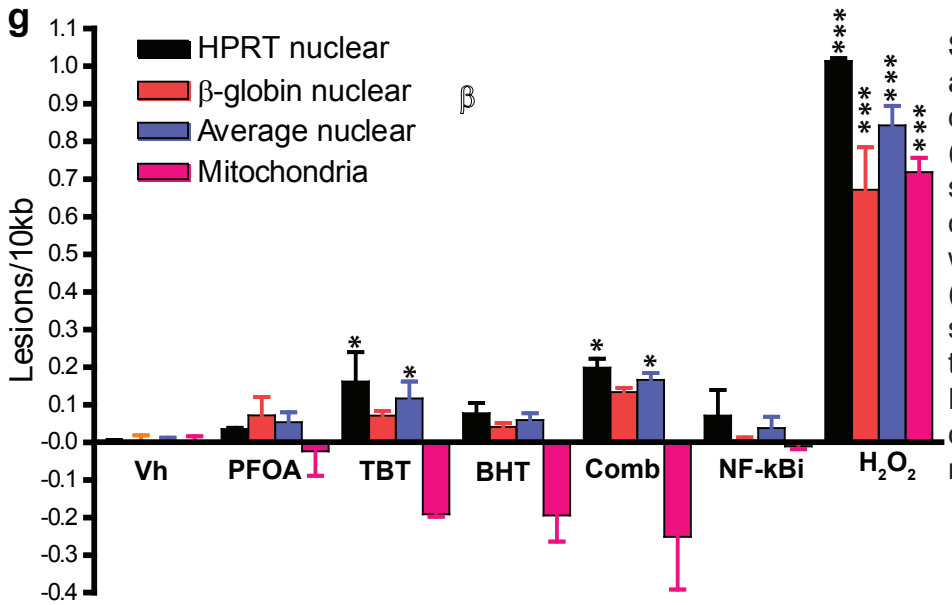
Supplementary Figure 1 Characterization of PBMC-derived iPSCs



Supplementary Figure 1: Characterization of PBMC-derived iPSCs. (a) Schematic representation depicting the episomal reprogramming and generation of iPSCs. (b) Bright-field images of the reprogrammed iPSC colonies from 2 control lines (80iCTR and 201iCTR) which show high alkaline phosphatase activity and immunopositivity for pluripotency surface markers such as SSEA, OCT4, TRA-1-60, NANOG, TRA-1-81 and SOX2. (c) Gene chip- and bioinformatics PluriTest characterization of the 2 iPSC lines. (d) G-band karyotyping showing normal phenotypes of both cell lines. (e) qPCR of both iPSC lines showing clearance of the reprogramming plasmids. (f) Agarose gel electrophoresis showing the absence of EBNA in the two iPSC lines. (g) qPCR showing wild type p53 gene expression in iPSCs. Error bars represent s.e.m.

Supplementary Figure 2 Cell viability (MTT assay) and DNA damage

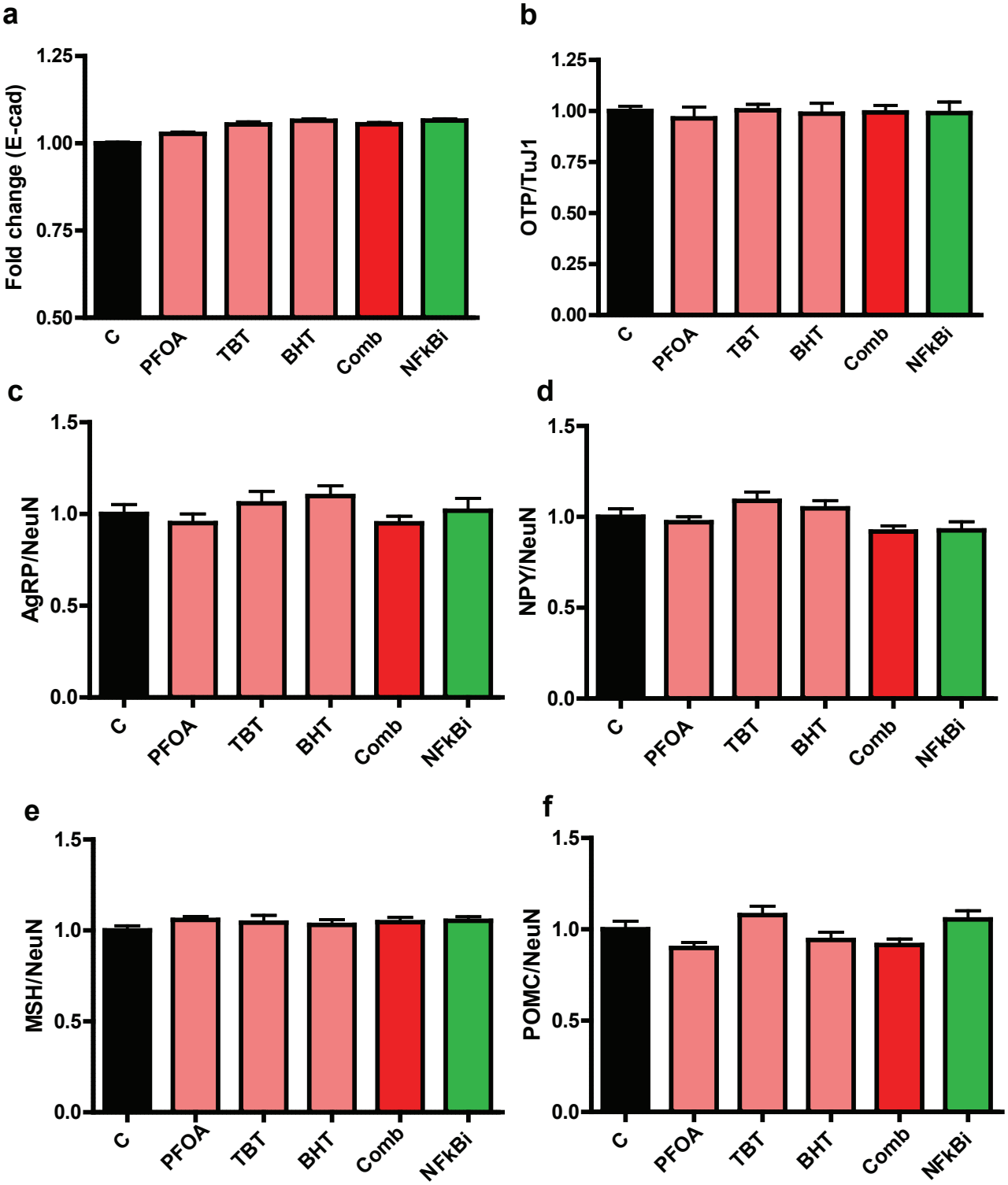




Supplementary Figure 2: Cell viability (MTT assay) and DNA damage. Cell viability semi-log dose response curves of (a) PFOA, (c) TBT and (e) BHT. The highlighted dose was used in this study. Bar graphs representing the optical density values of MTT assay on iHTNs treated with increasing doses of (b) PFOA, (d) TBT and (f) BHT. (g) Long-range PCR DNA damage showing lack of DNA lesions with EDC treatment. Note: A slight increase in nuclear HPRT lesions was observed with TBT and combination treatments. * $p<0.05$; ***$p<0.001$. n = 3. Error bars are defined as s.e.m.

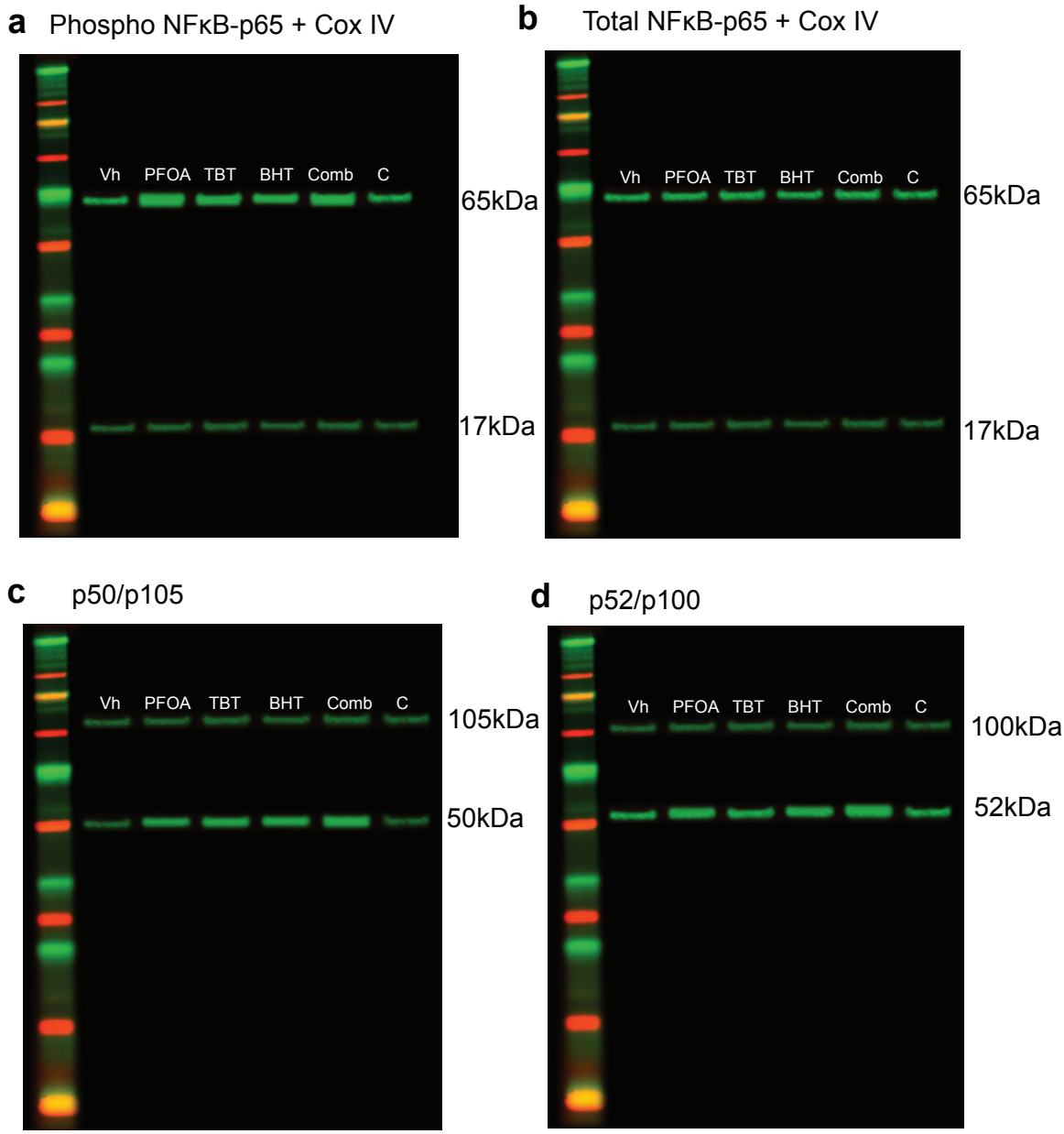
Supplementary Figure 3

EDC treatment does not affect differentiation efficiencies in iFGE and iHTN.



Supplementary Figure 3: EDC treatment does not affect differentiation efficiencies in iFGE and iHTN. Quantification of (a) E-cadherin positive cells in iFGE cultures and (b) OTP, (c) AgRP, (d) NPY, (e) MSH and (f) POMC positive neurons in iHTN cultures observed by immunocytochemistry shows no differences in the immune-positive cell numbers in control vs. EDC-treated cultures. The hypothalamic neuronal markers are normalized to pan-neuron markers, βIII-tubulin (Tuj1) or neuronal nuclei (NeuN). n = 3. Error bars are defined as s.e.m.

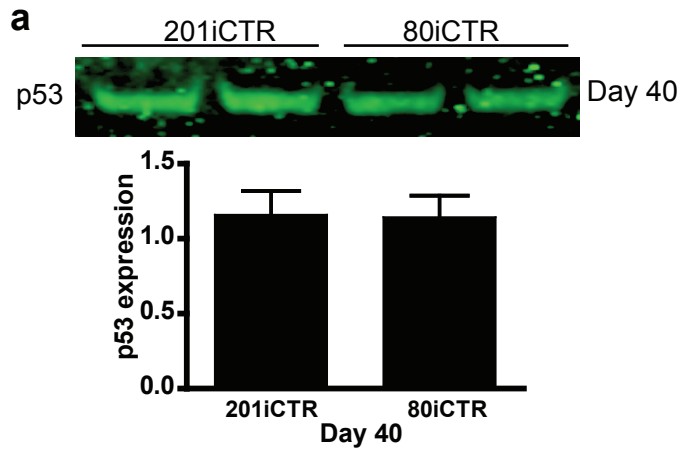
Supplementary Figure 4 iFGE full immunoblots



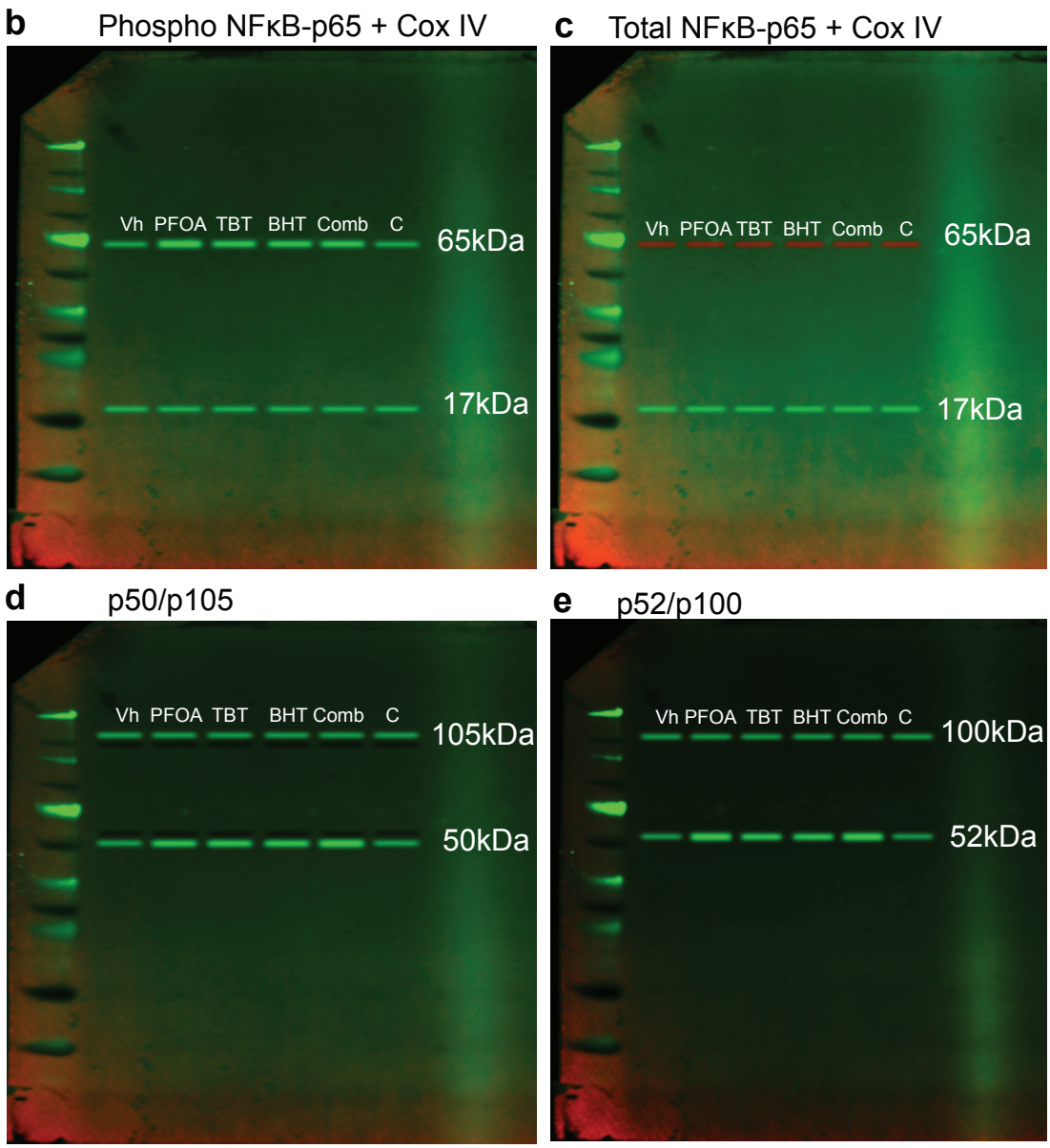
Supplementary Figure 4: iFGE full immunoblots (a-d) Full immunoblots of iFGE samples represented in Figure 4

Supplementary Figure 5

Intact p53 protein expression in differentiated iHTNs and full immunoblots

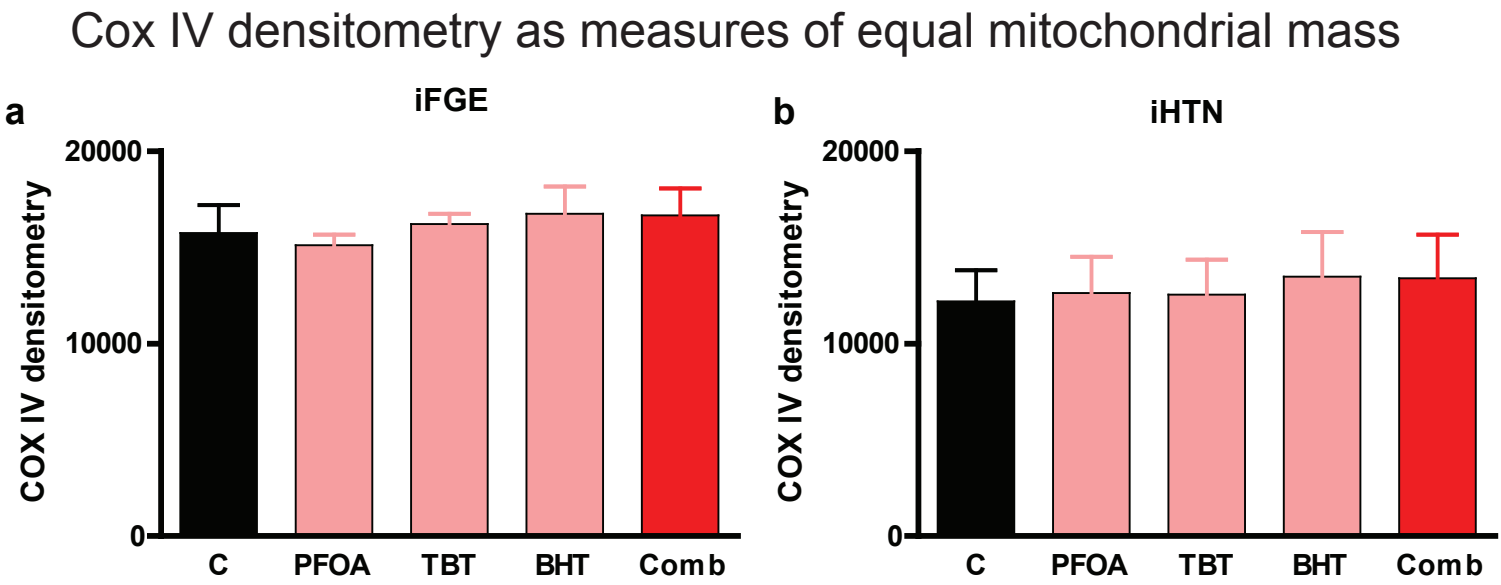


Full blots - iHTN; Figure 4



Supplementary Figure 5: Intact p53 protein expression in differentiated iHTNs, and full iHTN immuno blots. (a) Day 40 iHTNs showing expression of total p53 protein in 201iCTR and 80iCTR. (b-e) Original images of iHTN immunoblots represented in Figure 4

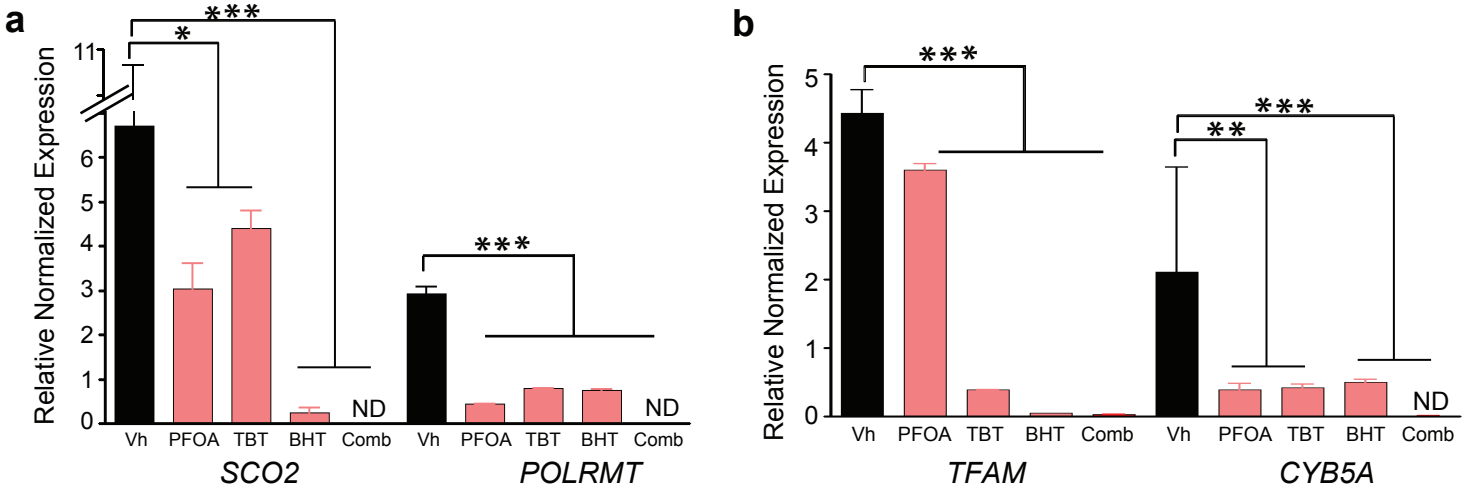
Supplementary Figure 6



Supplementary Figure 6: Cox IV densitometry as measures of equal mitochondrial mass. Cox IV densitometry revealing equal amounts of cytochrome C oxidase 4 in (a) iFGEs and (b) iHTNs used as loading controls and as measures of mitochondrial mass in the samples employed. Error bars are defined as s.e.m.

Supplementary Figure 7

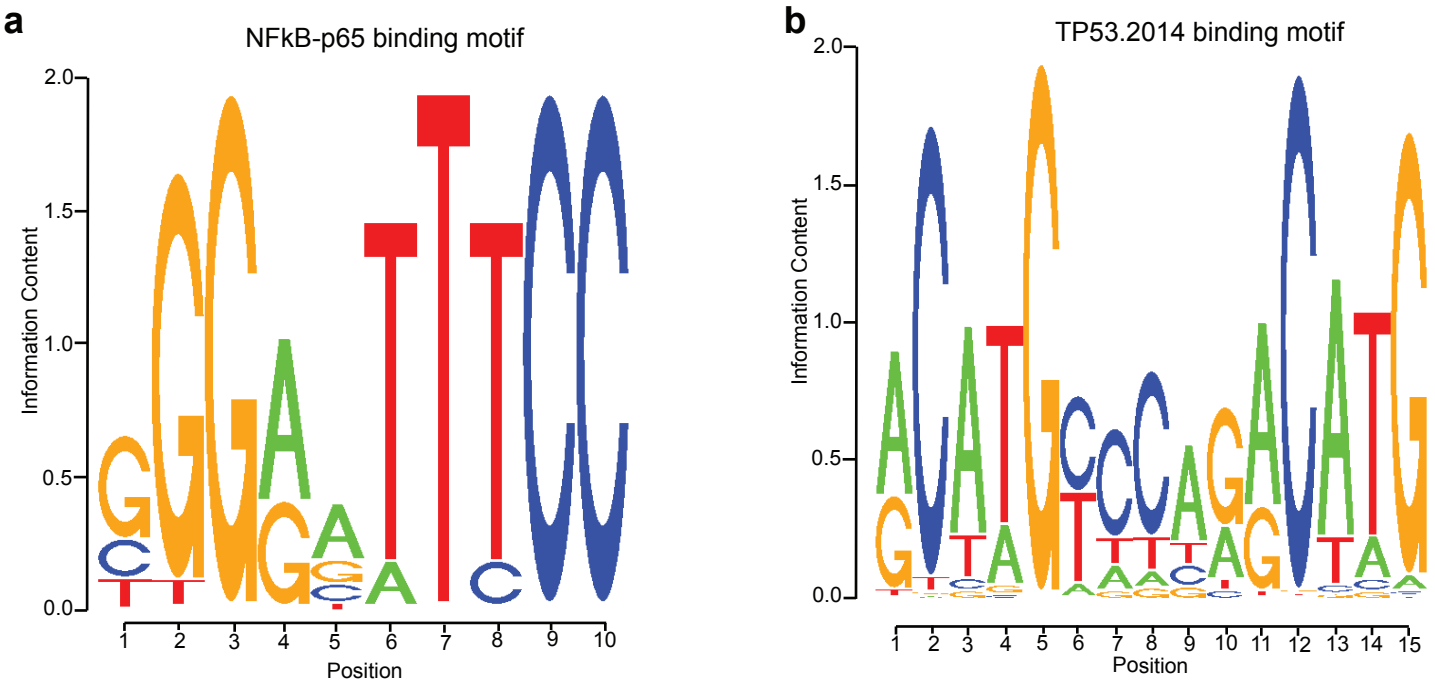
EDCs decrease expression of nuclear and mitochondrially-encoded genes in iFGEs



Supplementary Figure 7: EDCs decrease expression of both nuclear and mitochondrially-encoded genes in iFGEs. (a) RT-qPCR showing mRNA levels of nuclear-encoded genes, SCO2 and POLRMT, involved in mitochondrial respiration after vehicle or EDC treatment in iFGEs. (b) mRNA levels of nuclear-encoded mitochondrial gene TFAM and mitochondrially-encoded gene, CYB5A, also decreased upon EDC treatment of iFGEs. *p<0.05, **p<0.01, ***p<0.001. n = 3. Error bars are defined as s.e.m.

Supplementary Figure 8

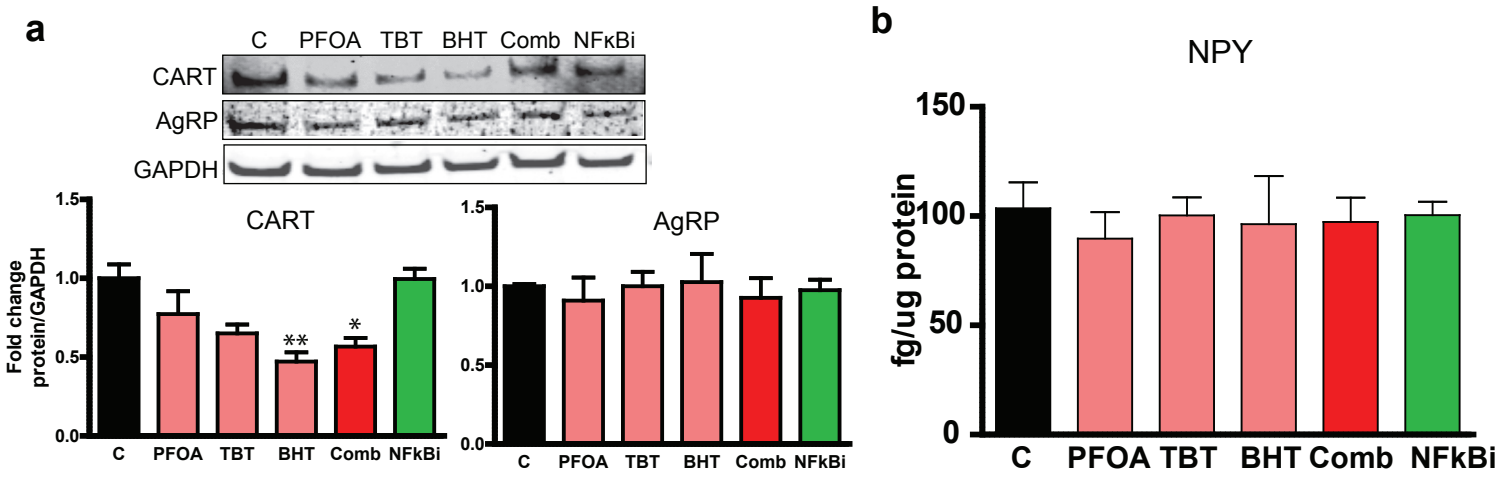
Bioinformatic determination of putative DNA binding sites for NFκB-p65 (RELA) and TP53



Supplementary Figure 8: Bioinformatic determination of putative DNA binding sites for NFκB-p65 (RELA) and TP53. DNA binding motif of (a) NFκB-p65 and (b) TP53 used in the bioinformatics analyses.

Supplementary Figure 9

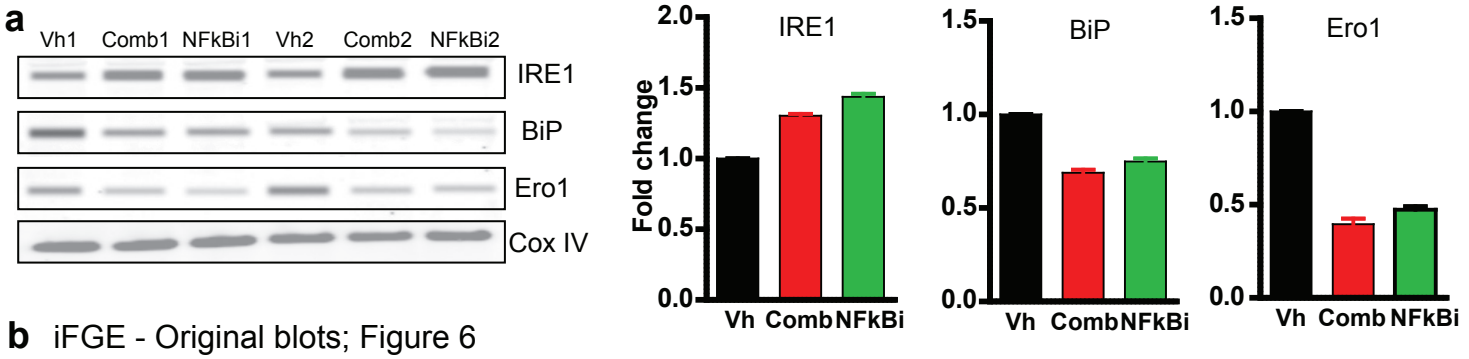
EDC treatment does not affect orexigenic neurons but decreases anorexigenic proteins

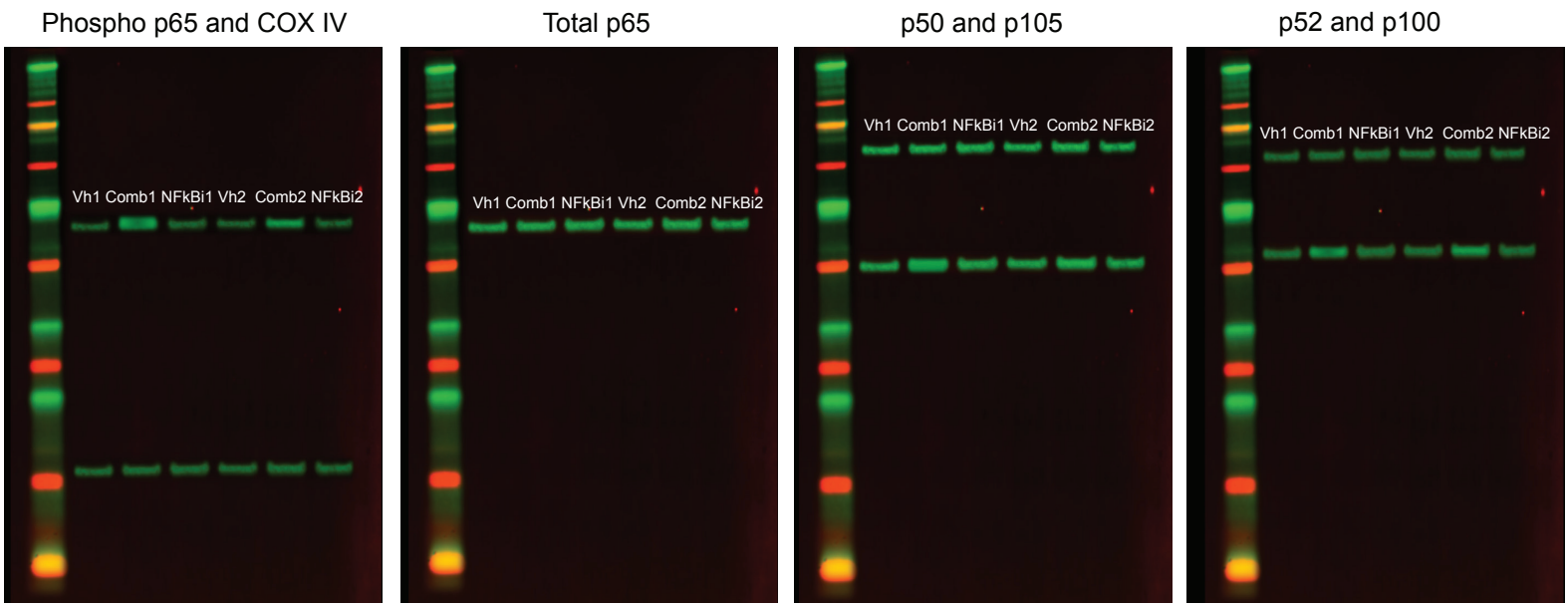


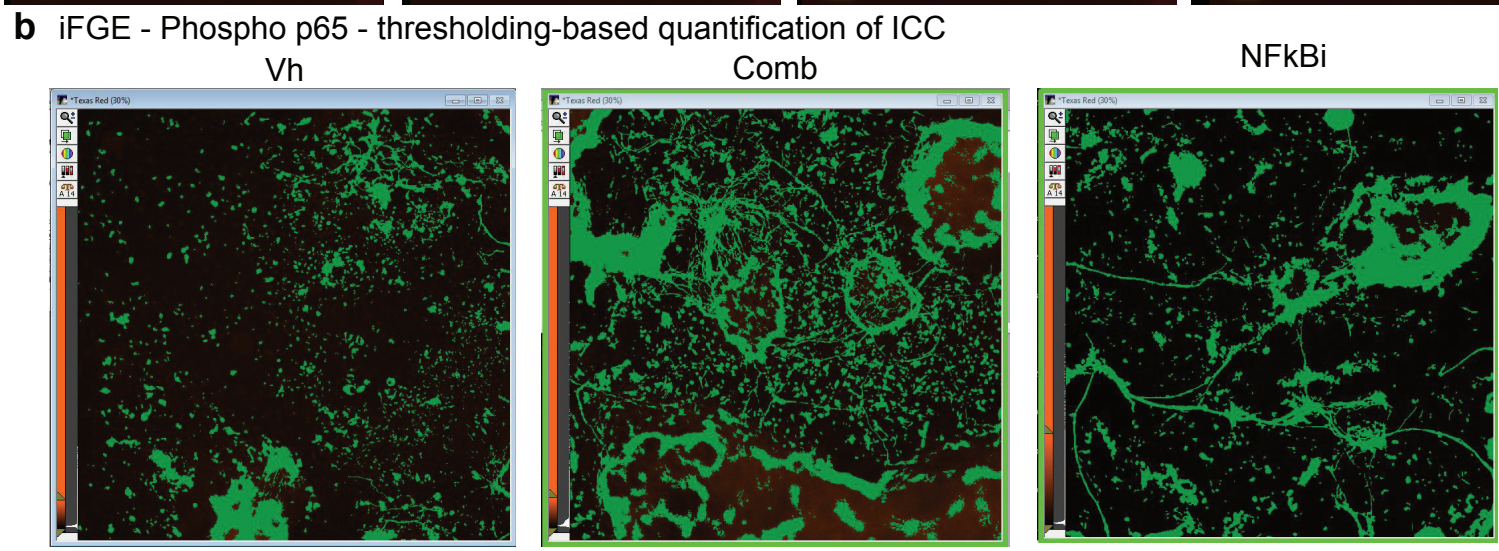
Supplementary Figure 9: EDC treatment does not affect orexigenic neurons but decreases anorexigenic proteins. (a) Western blots from iHTN shows decreases in CART peptide levels with EDC treatment and no changes in AgRP peptide levels, (b) no significant changes in NPY peptide secretion was observed as measured by ELISA; * $p<0.05$, ** $p<0.01$. n = 3. Error bars are defined as s.e.m.

Supplementary Figure 10

NFκBi treatment does not rescue ER stress but attenuates phospho NFκB-p65 in iFGE



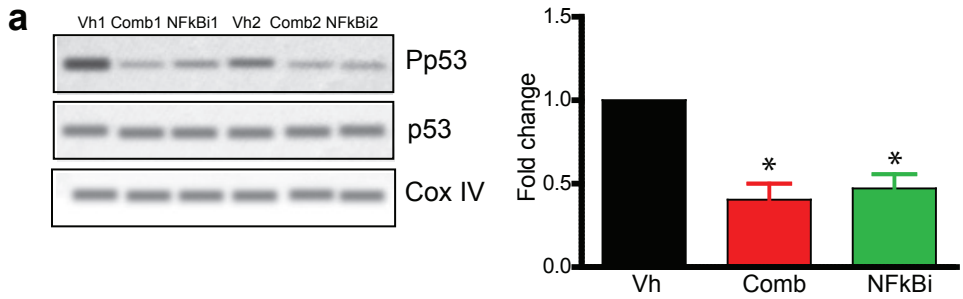


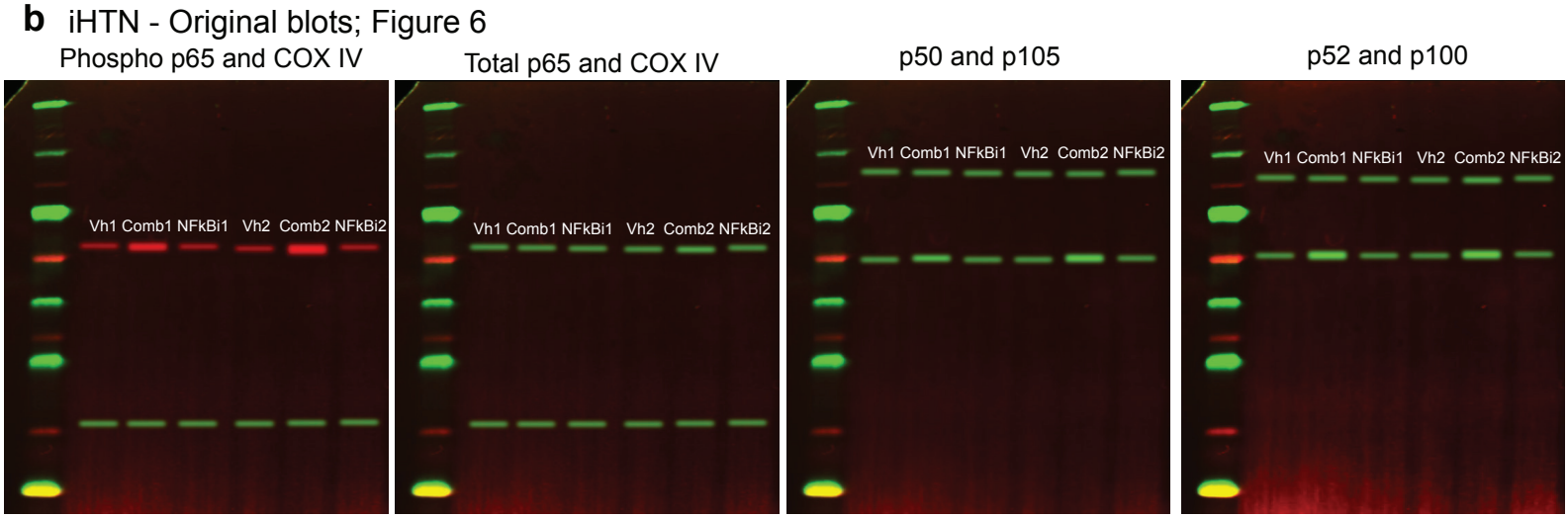


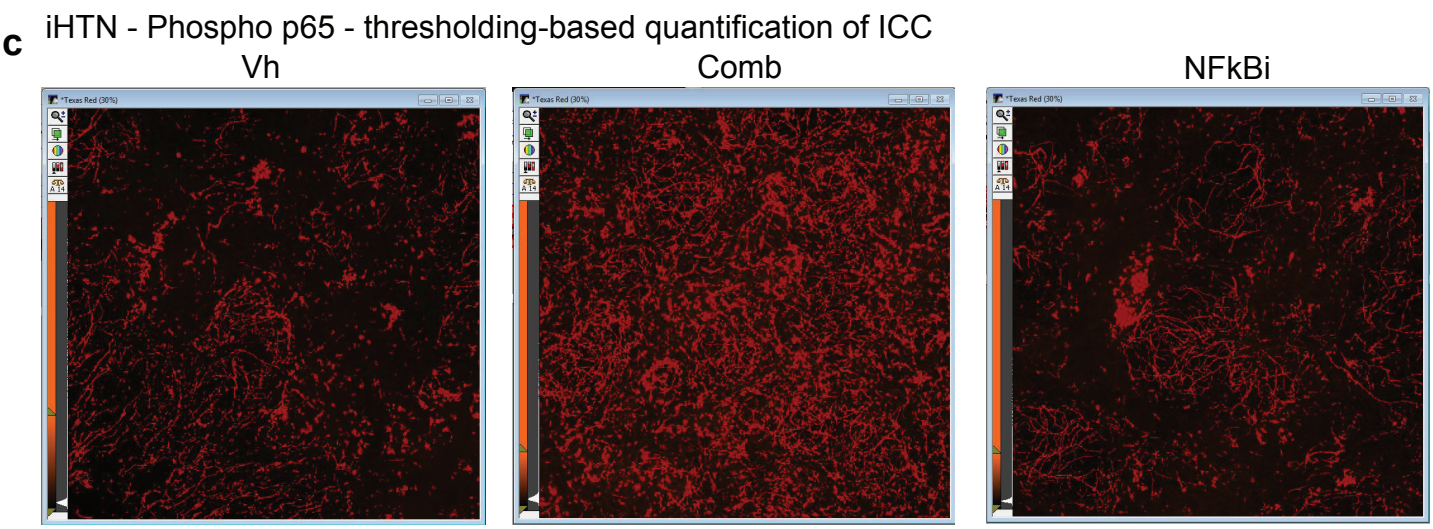
Supplementary Figure 10: NFκBi treatment does not rescue ER stress but attenuates phospho NFκB-p65 in iFGE. (a) Western blots in iFGEs showing no rescue of ER stress markers upon NF-κBi treatment compared to EDC-treated conditions, (b) Original images of iFGE blots represented in figure 6. 2 different cell lines were loaded in 6 lanes as Lane 1, 2 and 3 belonging to 80iCTR (Vh1, Comb1 and NFκBi1) and lanes 4 ,5 and 6 from 201iCTR (Vh2, Comb2 and NFκBi2). (c) Quantification of immunocytochemistry staining of phospho NF-κB p65 in iFGEs using MetaXpress with the threshold tool to measure specific Phospho p65 signals. The panel represents images post thresholding in each of the treatments. n = 3. Error bars are defined as s.e.m.

Supplementary Figure 11

NFκBi treatment does not lower phospho p53 but attenuates phospho NFκB-p65 in iHTN







Supplementary Figure 11: NFκBi treatment does not lower phospho p53 but attenuates phospho NFκB-p65 in iHTN. (a) Western blot showing no rescue in phospho p53 (Ser15) levels upon NF-κBi treatment compared to EDC-treated conditions. *p<0.05. (b) Original images of iHTN blots represented in figure 6. 2 different cell lines were loaded in 6 lanes as Lane 1, 2 and 3 belonging to 80iCTR (Vh1, Comb1 and NFκBi1) and lanes 4 ,5 and 6 from 201iCTR (Vh2, Comb2 and NFκBi2). (c) Quantification of immunocytochemistry staining of phospho NF-κB p65 in iHTNs using MetaXpress with the threshold tool to measure specific Phospho p65 signals. The panel represents images post thresholding in each of the treatments. n = 3. Error bars are defined as s.e.m.

Supplementary Table 1: Number putative of binding sites

Minimum Distance		60%	70%	80%	85%
RELA	SCO2	82	94	350	-
	TFAM	36	38	77	-
	POLRMT	79	157	1384	1947
	CYB5A	131	696	1202	1202
	RELA	**18**	**426**	**958**	**1674**
	TP53	**81**	**958**	**1317**	**-**
	Neg, min	3	4	4	4
	Neg, lower quartile	25	93.25	760.3	1889
	Neg, median	39.5	208.5	1404	-
	HOX, min	1	14	45	246
	HOX, lower quartile	12.25	85.25	515.5	1399
	HOX, median	40.5	153.5	1276	-
	IL1A	10	47	364	1981
	IL1B	27	228	1980	-
	TNF	67	243	-	-
	IL6	124	596	-	-
TP53.2014	SCO2	34	822	-	-
	TFAM	115	1863	-	-
	POLRMT	104	1985	-	-
	CYB5A	86	322	-	-
	RELA	**44**	**349**	**-**	**-**
	TP53	**77**	**423**	**-**	**-**
	Neg, min	6	21	62	-
	Neg, lower quartile	37	427.3	-	-
	Neg, median	76	825.5	-	-
	HOX, min	1	5	389	-
	HOX, lower quartile	27.25	253	-	-
	HOX, median	79	837.5	-	-
	CDKN1A	13	188	-	-
	GADD45A	24	1173	-	-

	GADD45B	63	1235	-	-
	GADD45G	64	-	-	-
	PERP	67	-	-	-
	BAX	102	-	-	-

Supplementary Table 1: Identification of the number of putative binding sites of NFκB-p65 and TP53 binding motifs on genes of interest such as *SCO2, POLRMT, TFAM, CYB5A* and respective known genes regulated by NFκB-p65 (RELA) such as *IL1A, IL1B, TNF, IL6* or regulated by TP53 such as *GADD45A, GADD45B, GADD45G, PERP, BAX*.

Supplementary Table 2: Minimum distance from transcription start sites (TSS)

Minimum Distance		60%	70%	80%	85%
RELA	SCO2	82	94	350	-
	TFAM	36	38	77	-
	POLRMT	79	157	1384	1947
	CYB5A	131	696	1202	1202
	RELA	**18**	**426**	**958**	**1674**
	TP53	**81**	**958**	**1317**	**-**
	Neg, min	3	4	4	4
	Neg, lower quartile	25	93.25	760.3	1889
	Neg, median	39.5	208.5	1404	-
	HOX, min	1	14	45	246
	HOX, lower quartile	12.25	85.25	515.5	1399
	HOX, median	40.5	153.5	1276	-
	IL1A	10	47	364	1981
	IL1B	27	228	1980	-
	TNF	67	243	-	-
	IL6	124	596	-	-
TP53.2014	SCO2	34	822	-	-
	TFAM	115	1863	-	-
	POLRMT	104	1985	-	-
	CYB5A	86	322	-	-
	RELA	**44**	**349**	**-**	**-**
	TP53	**77**	**423**	**-**	**-**
	Neg, min	6	21	62	-
	Neg, lower quartile	37	427.3	-	-
	Neg, median	76	825.5	-	-
	HOX, min	1	5	389	-
	HOX, lower quartile	27.25	253	-	-
	HOX, median	79	837.5	-	-
	CDKN1A	13	188	-	-
	GADD45A	24	1173	-	-

	GADD45B	63	1235	-	-
	GADD45G	64	-	-	-
	PERP	67	-	-	-
	BAX	102	-	-	-

Supplementary Table 2: Identification of minimum distance in base pairs upstream of the transcription start sites of the DNA binding motifs of NFκB-p65 and TP53 on the indicated genes of interest. *HOX* genes were employed as neutral genes or genes that are not well-known in the literature to be controlled either by NFκB-p65 and TP53.

Supplementary Table 3. List of antibodies

Immunocytochemistry				
Antibody	**Source**	**Company**	**Catalog #**	**Dilution**
α-MSH	Rabbit	Phoenix Pharma	H-43-01	1:250
β-catenin	Rabbit	Santa Cruz	sc7199	1:500
CART	Goat	Santa Cruz	sc18068	1:250
CPE	Goat	R&D Systems	AF3587	1:250
E-cadherin	Goat	R&D Systems	AF648	1:500
GABA	Rabbit	Sigma-Aldrich	A2025	1:250
Gastrin	Rabbit	Dako	A056801-2	1:250
Ghrelin	Goat	Santa Cruz	sc10368	1:250
NF-κB (pSer-311)	Mouse	Santa Cruz	sc166748	1:250
NP-II	Goat	Santa Cruz	sc27093	1:250
NPY	Rabbit	MerckMillipore	AB9608	1:250
OTP	Rabbit	Genetex	GTX119601	1:250
Peptide YY	Rabbit	Abcam	ab22663	1:250
Serotonin	Rabbit	Immunostar	20080	1:250
Somatostatin	Rabbit	Santa Cruz	sc13099	1:250
Sox17	Mouse	Novus	47996	1:250
Sox2	Rabbit	Stemgent	09-0024	1:500
Synaptophysin	Mouse	Santa Cruz	sc17750	1:250
TH	Mouse	Immunostar	22941	1:250
AgRP	Mouse	Neuromics	15105	1:250
POMC	Rabbit	Santa Cruz	Sc20148	1:250
βIII-tubulin	Rabbit	Biolegend	845501	1:250
NeuN	Mouse	EMD Millipore	MAB377	1:250
Secondary (1:200): AlexaFluor (AF) 488 donkey anti-rabbit, AF 555 donkey anti-mouse, AF 594 donkey anti-mouse, AF 568 donkey anti-goat, AF 647 donkey anti-goat.				
Immunoblotting				
COX IV	Rabbit	Cell Signaling	4850	1:2000
NF-κB p65 (pSer-311)	Mouse	Santa Cruz	sc166748	1:1000
NF-κB p65 (RelA)	Rabbit	Cell Signaling	8242	1:1000
NF-κB1 (p105/p50)	Rabbit	Cell Signaling	12540	1:1000
NF-κB2 (p100/p52)	Rabbit	Cell Signaling	4882	1:1000
AgRP	Mouse	Neuromics	15105	1:1000

CART	Goat	Santa Cruz	Sc18068	1:500
Phospho p53 (Ser15)	Rabbit	Cell Signaling	9284T	1:500
p53	Rabbit	Cell Signaling	9282T	1:500
IRE1α	Rabbit	Cell Signaling	3294	1:500
Ero1	Rabbit	Cell Signaling	3264	1:500
BiP	Rabbit	Cell Signaling	3177	1:500
Secondary (1:2000): IRDye 800CW, donkey anti-rabbit, Li-Cor, 926-32213; IRDye 680LT, donkey anti-mouse, Li-Cor, 926-68022.				

Supplementary Table 4. List of PCR Primers.

Gene	**Primers**
AGRP	Forward 5' – GGATCTGTTGCAGGAGGCTCAG – 3' Reverse 5' – TGAAGAAGCGGCAGTAGCACGT – 3'
CDX2	Forward 5' – CTGGAGCTGGAGAAGGAGTTTC – 3' Reverse 5' – ATTTTAACCTGCCTCTCAGAGAGC – 3'
GKN1	Forward 5' – CTTTCTAGCTCCTGCCCTAGC – 3' Reverse 5' – GTTGCAGCAAAGCCATTTCC – 3'
MC4R	Forward 5' – CTTATGATGATCCCAACCCG – 3' Reverse 5' – GTAGCTCCTTGCTTGCATCC – 3'
NKX2-1	Forward 5' – AACCAAGCGCATCCAATCTCAAGG – 3' Reverse 5' – TGTGCCCAGAGTGAAGTTTGGTCT – 3'
NPY	Forward 5' – GGTCTTCAAGCCGAGTTCTG – 3' Reverse 5' – AACCTCATCACCAGGCAGAG – 3'
OPRM1	Forward 5' – TGGTGGCAGTCTTCATCTTG – 3' Reverse 5' – GATCATGGCCCTCTACTCCA – 3'
PDX1	Forward 5' – CGTCCGCTTGTTCTCCTC – 3' Reverse 5' – CCTTTCCCATGGATGAAGTC – 3'
PGA5	Forward 5' – CCATCTTGCCTTCTCCCTCG – 3' Reverse 5' – TCTGATGAGGGGGACCTTGT – 3'
SOX2	Forward 5' – TTC ACA TGT CCC AGC ACT ACC AGA – 3' Reverse 5' – TCA CAT GTG TGA GAG GGG CAG TGT GC – 3'
TAS1R3	Forward 5' – ACGTCTGACAACCAGAAGCC – 3' Reverse 5' – CAGTCCACACAGTCGTAGCA – 3'
TFF1	Forward 5' – TGGAGGGACGTCGATGGTAT – 3' Reverse 5' – TGGAGGGACGTCGATGGTAT – 3'
TFF2	Forward 5' – CTGAGCCCCCATAACAGGAC – 3' Reverse 5' – ACGCACTGATCCGACTCTTG – 3'
Large mito	Forward 5' - TCTAAGCCTCCTTATTCGAGCCGA – 3' Reverse 5' - TTTCATCATGCGGAGATGTTGGATGG – 3'
Small mito	Forward 5' - CCC CAC AAA CCC CAT TAC TAA ACC CA – 3' Reverse 5' - TTTCATCATGCGGAGATGTTGGATGG – 3'
β-globin	Forward 5' - CGA GTA AGA GAC CAT TGT GGC AG – 3' Reverse 5' - GCA CTG GCT TAG GAG TTG GAC T – 3'
HPRT	Forward 5' - TGG GAT TAC ACG TGT GAA CCA ACC – 3' Reverse 5' - GCT CTA CCC TCT CCT CTA CCG TCC – 3'