Supplementary Figures and Tables

The proteomic landscape of cysteine oxidation that underpins retinoic acid-induced neuronal differentiation

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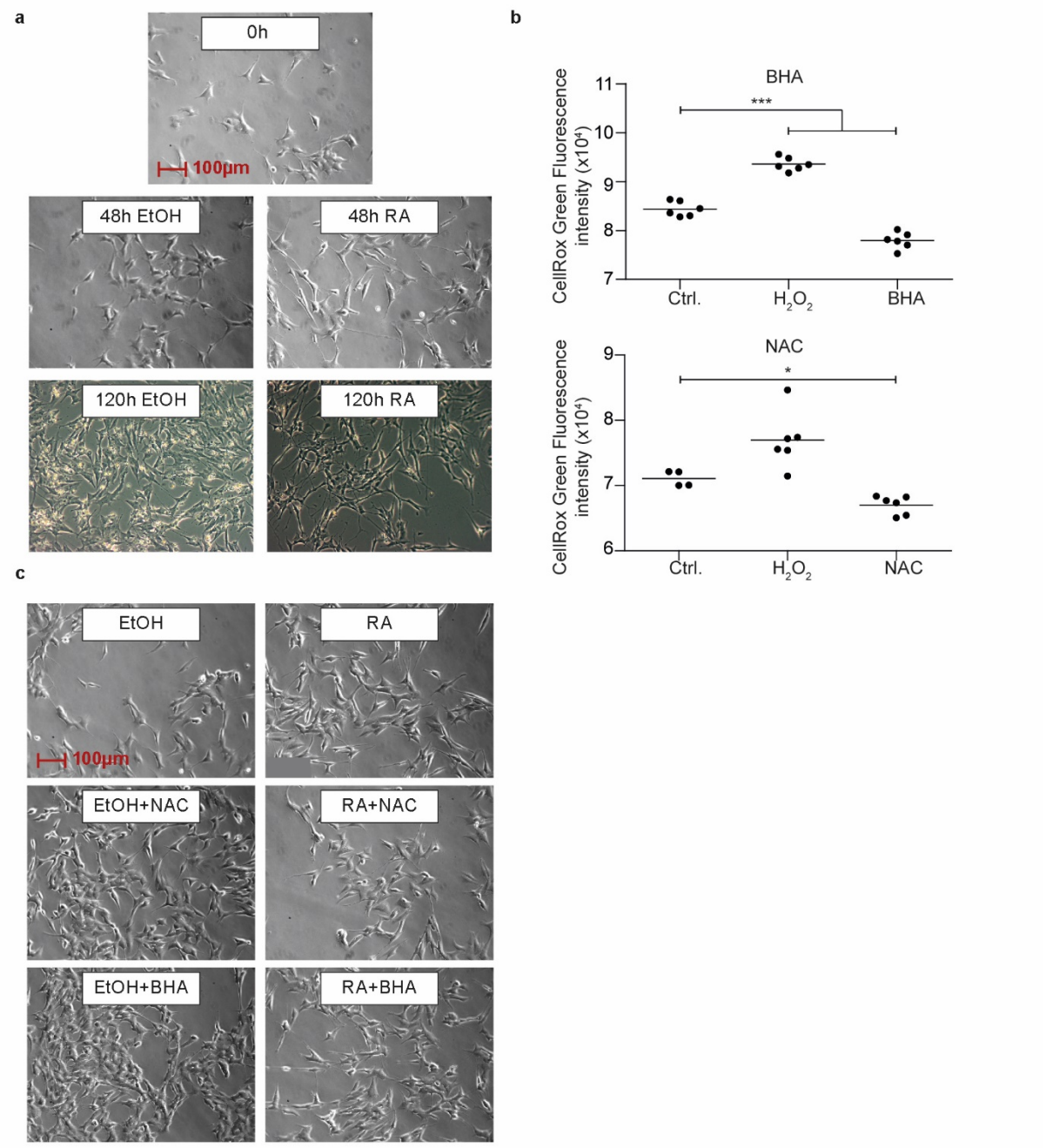
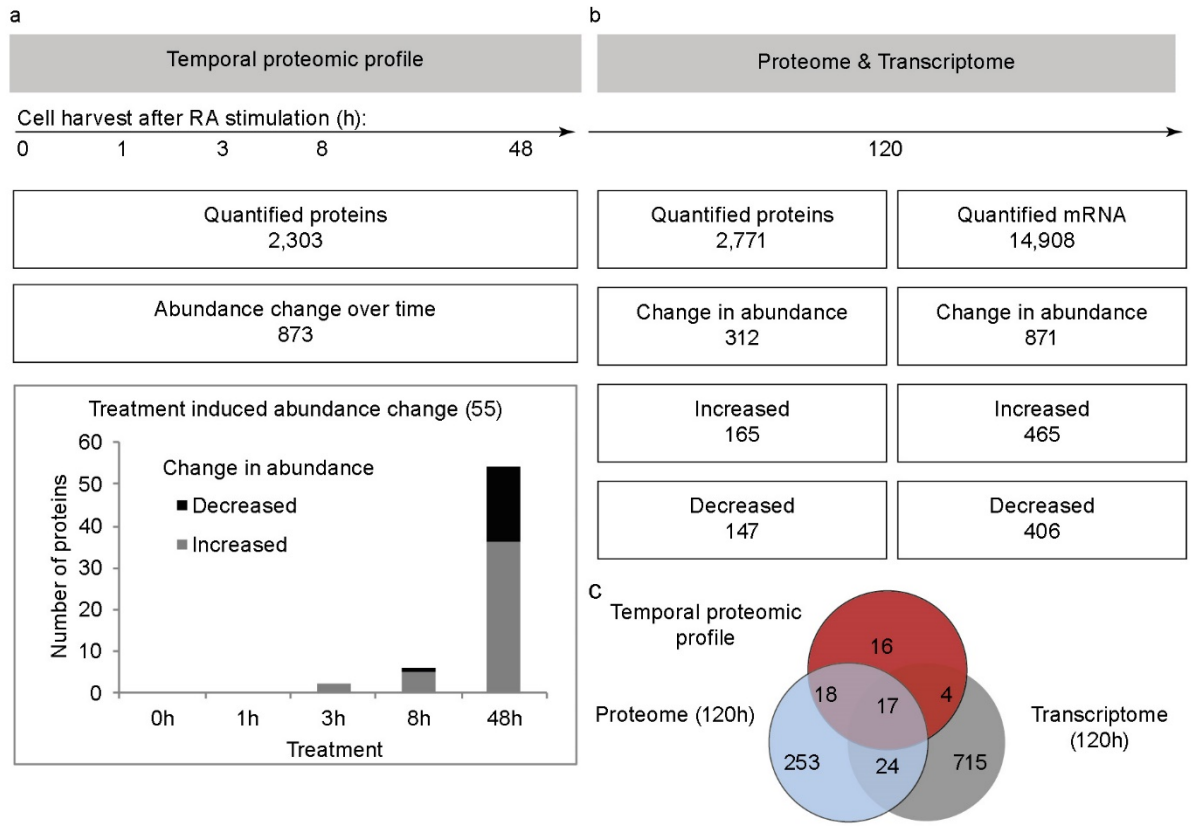
Addition supplemental files (separate Excel sheets)



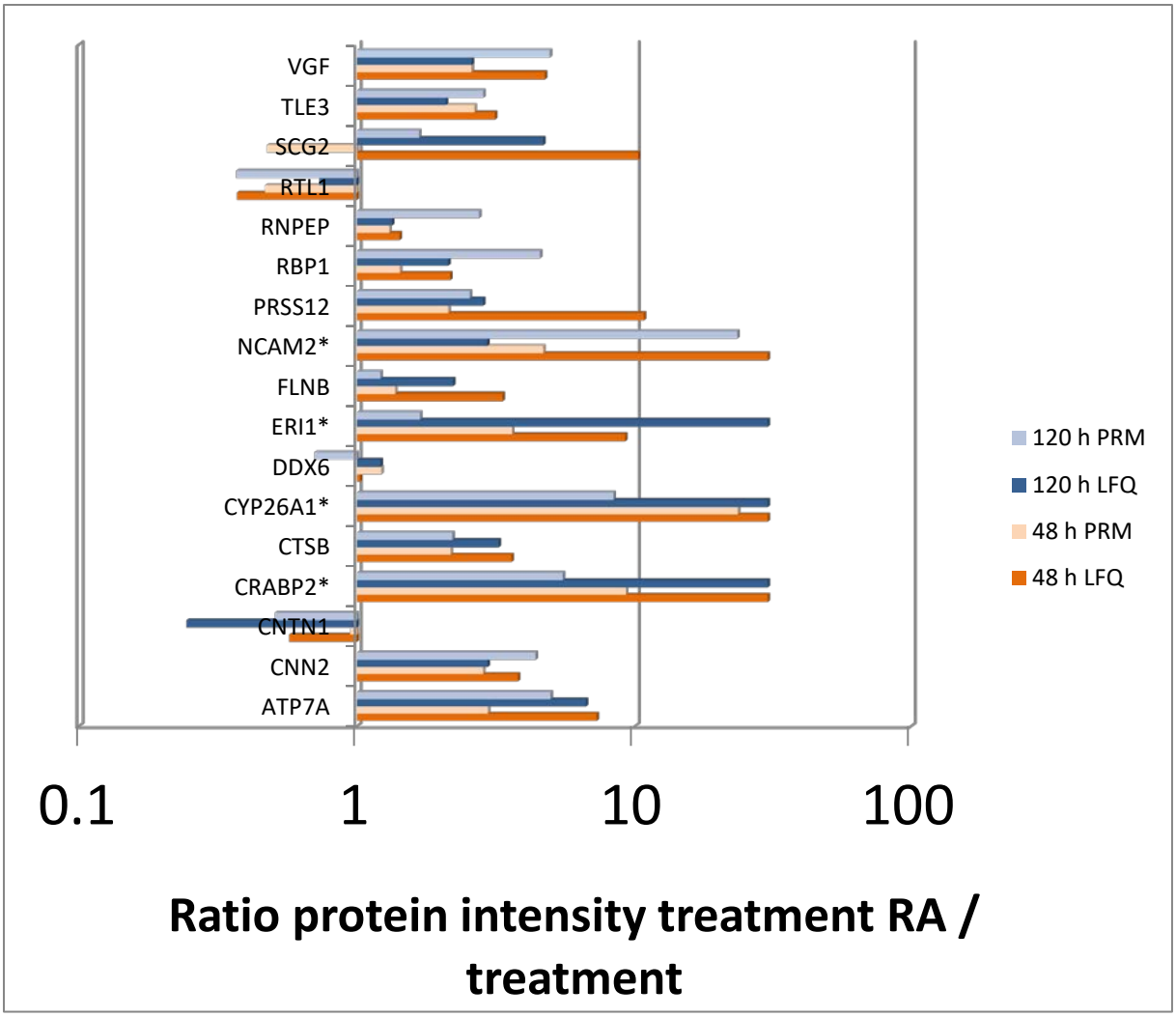
Supplementary Figure 1 Cell morphology and redox sensitivity of RA-treated SH-SY5Y cells (a) Microscopic images of the cells after RA and control (ethanol) treatment after 0h, 48h and 120h. (b) The addition of NAC and BHA reduced the fluorescence intensity of intracellular CellROX Green compared to the control, representing a more reduced state of the cells. Indicated are the signal intensities of each replicate (n=5). The horizontal line marks the mean value. (c) Microscopic images of the cells after 48h co-treatment with RA and BHA or NAC. Statistical significance between the groups was computed using one-way (b) or two-way (c) ANOVA followed by Tukey's multiple comparison tests. Asterisk indicate p-values *≤0.05 and ***≤0.001.



Supplementary Figure 2 Proteome and Transcriptome analysis of SH-SY5Y cells stimulated with RA
Quantification of (a) proteins within 0-48 h and (b) proteins and RNA at timepoint 0 h and 120 h. (c) Number of proteins showing abundance changes in different experiments.

Supplementary Table 1 Early marker proteins Seventeen proteins show significantly altered abundance in the temporal proteome profile as well as proteome and transcriptome analysis of 120 h differentiated cells and are here considered as early marker proteins. The ratio was determined from the mean LFQ intensities and the mean RPKM values, respectively. If no intensity was available for members of the ethanol group, the respective protein ratio was marked with 'only RA'.

		Ratio (RA/ethanol)	
Gen name	Temporal proteomic profile (48 h)	120 h proteome	120 h RNA
ATP7A	7.28	6.66	5.05
CNN2	3.79	2.95	2.07
CNTN1	0.57	0.24	0.30
CRABP2	357.24	only RA	18.63
CTSB	3.60	3.24	3.67
CYP26A1	only RA	only RA	1616.68
DDX6	1.03	1.22	1.49
ERI1	9.21	only RA	3.07
FLNB	3.34	2.22	2.24
NCAM2	only RA	2.94	24.87
PRSS12	10.77	2.84	1.71
RBP1	2.16	2.13	2.24
RNPEP	1.42	1.34	1.50
RTL1	0.37	0.73	0.17
SCG2	10.27	4.68	8.09
TLE3	3.14	2.09	2.58
VGF	4.74	2.59	1.95



Supplementary Figure 3 Validation of early marker proteins – comparison with parallel reaction monitoring based quantification The chart displays the ratios of mean values from either label-free quantification based experiments (LFQ) or parallel reaction monitoring (PRM) based quantification. Ratios > 30 or protein quantifications where no intensities in the ethanoltreated sample were available were set to 30 (marked by asterisk). Nearly all protein ratios point in the same direction. Exceptions are DDX6 as well as SCG2. For DDX6 an elevated abundance at early time points was found at 8 h after RA stimulation and additionally larger abundance changes at 120 h were predominantly previously found on RNA level. For SCG2 only one peptide (amino acid 182-207) was chosen for PRM quantification. This peptide is included in secretoneurin (amino acid 182-214) – a proteolytically processed variant of SCG2 and known to be secreted. This might explain the discrepancy between the LFQ and PRM based quantification.

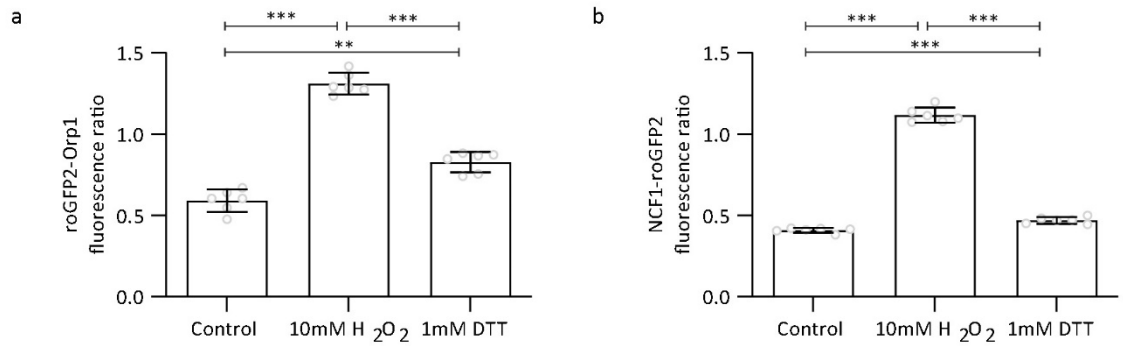
Supplementary Table 2 Proteins associated to cellular redox processes Proteins with a statistically significant change in abundance over time, based on the Benjamini-Hochberg (BH) corrected p-value of the two-way ANOVA are grayed out. The ratio was determined from the average LFQ intensities. Abbreviations: RA: retinoic acid, LFQ: label-free quantification.

	p-value (two-way ANOVA_BH corrected)	p-value RA vs. ethanol (two-way ANOVA Tukey HSD)				Ratio RA/ethanol (based on LFQ intensities)				
Gen name	Time	1h	3h	8h	48h	1h	3h	8h	48h	Cluster
Decomposition of superoxide anion and peroxides										
SOD1	4.73E-01	1.00E+00	4.92E-01	9.97E-01	9.49E-01	1.06	1.25	1.09	1.13	3
SOD2;ATF7IP;GRM3	8.66E-01	1.00E+00	1.00E+00	9.98E-01	6.75E-01	1.06	1.02	1.15	0.99	5
CAT	3.07E-02	1.00E+00	1.00E+00	1.00E+00	4.34E-01	1.02	0.97	0.98	0.93	2
GPX1	2.06E-05	9.78E-01	1.00E+00	2.02E-01	1.00E+00	0.76	1.16	1.73	0.90	5
TXNDC17	3.89E-02	9.89E-01	9.25E-01	5.12E-01	1.00E+00	0.90	1.14	1.23	0.61	8
PRDX1	2.44E-01	9.94E-01	9.49E-01	9.93E-01	1.00E+00	0.92	1.11	1.09	0.81	5
PRDX2	7.95E-01	9.99E-01	1.00E+00	1.00E+00	9.99E-01	1.06	0.98	1.04	0.74	2
PRDX4	3.79E-04	9.76E-01	9.62E-01	9.87E-01	9.99E-01	0.94	0.93	0.94	0.37	7
PRDX6	1.92E-01	9.74E-01	7.80E-01	1.00E+00	9.52E-01	0.94	0.92	1.03	0.30	5
GSTK1	1.01E-01	1.00E+00	6.77E-01	1.00E+00	9.26E-01	0.94	1.21	0.95	0.82	4
GMPR;GPX4	2.77E-03	9.99E-01	5.70E-01	1.00E+00	9.41E-01	1.14	1.59	1.14	0.88	4
MGST3	1.74E-02	9.96E-01	1.20E-01	1.00E+00	9.98E-01	1.13	0.68	1.07	0.43	5
NXN	5.71E-01	9.36E-01	9.98E-01	1.00E+00	9.99E-01	1.31	1.18	0.77	0.51	3
Oxidative folding (endoplasmic reticulum)										
PDIA4	1.10E-02	3.99E-02	7.74E-01	9.85E-01	9.36E-02	0.82	0.91	0.94	0.18	9
PDIA6	6.41E-01	1.00E+00	1.00E+00	8.45E-01	1.00E+00	1.01	0.98	0.84	0.70	2
ERO1L	9.45E-01	9.85E-01	1.00E+00	1.00E+00	1.00E+00	0.93	0.97	1.00	0.80	9
Cellular response to oxidative stress										
KSR2	2.42E-01	8.15E-01	9.84E-01	1.00E+00	1.00E+00	1.25	1.06	0.95	0.63	7
RELA	2.29E-01	1.00E+00	1.00E+00	9.55E-01	1.00E+00	0.95	0.98	1.11	0.95	5
ATP5B	6.28E-01	1.00E+00	9.87E-01	1.00E+00	1.00E+00	1.08	0.82	1.08	0.86	9
TXNL1	2.04E-02	8.97E-01	9.96E-01	7.15E-01	9.82E-01	0.87	1.09	1.20	0.98	5
PRKRA	9.89E-03	9.89E-01	1.00E+00	1.00E+00	3.11E-01	0.92	1.00	0.96	0.55	1
CDK1	6.91E-01	1.00E+00	1.00E+00	1.00E+00	9.99E-01	0.97	1.07	0.92	0.87	4
ATP5B	6.28E-01	1.00E+00	9.87E-01	1.00E+00	1.00E+00	1.08	0.82	1.08	0.86	9
PARP1	2.74E-02	1.00E+00	9.86E-01	1.00E+00	6.97E-01	1.01	1.11	1.02	0.99	6
G6PD	2.72E-03	6.56E-01	9.99E-01	4.26E-01	2.82E-04	0.92	1.03	1.11	0.08	1
APEX1	8.24E-02	1.00E+00	1.00E+00	7.41E-01	9.97E-01	0.97	0.96	0.88	0.43	9
PPIF	7.38E-01	1.00E+00	9.27E-01	1.00E+00	8.24E-01	0.91	0.88	1.00	0.34	5
MAPK8	8.07E-01	1.00E+00	9.35E-01	9.89E-01	1.00E+00	0.89	1.44	1.17	0.92	5
ATP7A	1.81E-08	9.76E-01	1.00E+00	1.00E+00	5.00E-12	0.83	1.01	1.05	0.00	2
SLC25A24	1.32E-02	6.84E-01	2.96E-01	1.00E+00	5.15E-01	0.87	0.82	0.95	0.63	1
SIN3A	1.73E-01	9.99E-01	1.00E+00	4.62E-02	1.00E+00	0.89	1.07	0.56	0.49	2
PARK7	2.83E-03	1.00E+00	9.91E-01	9.99E-01	1.00E+00	1.01	1.04	1.03	0.72	8
ADPRHL2	5.40E-01	9.73E-01	1.00E+00	7.87E-01	9.65E-01	1.32	1.09	1.42	0.40	4
Lipid oxidation										
HADHB	2.64E-02	9.98E-01	1.00E+00	1.00E+00	3.15E-01	0.95	1.02	1.00	0.64	2
ABCD3	8.24E-04	9.99E-01	9.84E-01	9.98E-01	9.90E-01	1.13	0.87	0.92	0.95	2

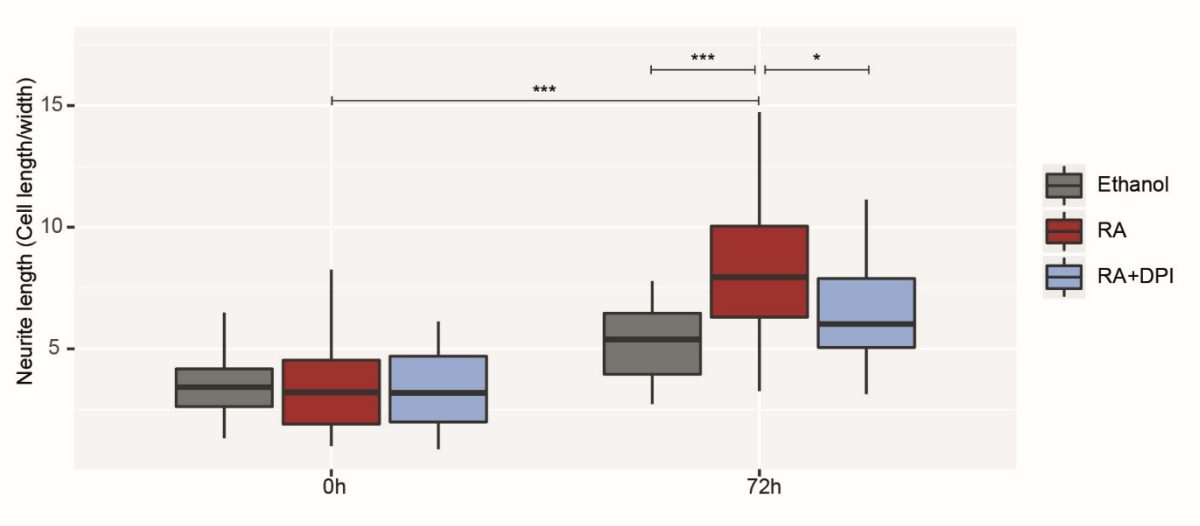
POR	3.24E-04	7.22E-01	8.16E-01	1.00E+00	1.00E+00	1.12	1.13	1.04	0.28	2
SCP2	8.18E-02	4.75E-01	9.75E-01	1.00E+00	1.00E+00	0.81	1.13	0.99	0.77	4
HSD17B4	2.83E-03	9.95E-01	9.97E-01	1.00E+00	1.00E+00	0.96	1.03	0.98	0.95	2
ACOX1	7.67E-01	5.33E-01	1.00E+00	8.84E-01	1.00E+00	1.40	1.04	1.23	0.25	3
DNA repair mechanism										
LIG1	1.17E-02	1.00E+00	9.99E-01	1.00E+00	8.26E-01	1.01	0.86	1.10	0.64	9
POLD1	3.58E-01	7.59E-01	9.98E-01	9.59E-01	9.89E-01	1.24	1.08	1.18	0.46	8
HMGB1;HMGB1P1	3.20E-01	1.00E+00	9.95E-01	1.00E+00	9.99E-01	1.04	1.24	1.03	0.64	6
PCNA	4.43E-01	1.00E+00	1.00E+00	1.00E+00	4.89E-02	1.02	1.01	1.03	0.26	1
RPA2	2.74E-01	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.01	1.06	1.01	0.72	7
XRCC1	5.98E-01	1.00E+00	8.89E-01	9.81E-01	1.00E+00	0.95	1.34	1.23	0.65	3
FEN1	4.26E-01	7.73E-01	5.39E-01	1.00E+00	1.00E+00	0.87	1.19	1.02	0.68	7
POLD2	5.09E-05	1.00E+00	9.93E-01	7.35E-01	9.86E-01	0.95	1.10	1.19	0.81	4
LIG3	2.03E-02	1.00E+00	9.99E-01	9.98E-01	1.00E+00	0.99	1.27	1.23	0.28	3
DDB1	7.56E-02	9.99E-01	1.00E+00	9.98E-01	9.99E-01	0.96	1.01	1.05	0.94	5

Supplementary Table 3 Influence of BHA and NAC on proteins involved in the control of cellular redox homeostasis Selected proteins that control ROS within the cell or contribute to the cellular redox balance through the conversion of NAD(P)+/H are shown. The ratios were calculated from the mean LFQ intensities. The statistical evaluation was carried out using a two-way ANOVA and a correction according to Benjamini-Hochberg, followed by a comparative test according to Tukey. Entries highlighted in grey indicate a significant change in abundance (p≤0,05). RA: retinoic acid, BHA: butylated hydroxyanisole, NAC: N-acetylcysteine, Mito: mitochondrion, Cyt.: cytosol

		Ratio RA/ethanol (based on LFQ intensities)			Adjusted p-value (two-way ANOVA, Benjamini-Hochberg)	p-value RA-treatment * redox-active agent (Tukey post-hoc test)		
Function	Gen name	RA/ ethanol	[RA+ BHA] /RA	[RA+ NAC] /RA	RA vs. ethanol	[RA+BHA] vs. RA	[RA+NAC] vs. RA	Effect of redox-active agent
Decomposition of superoxide anion:								
	SOD1	0.73	0.78	0.99	8.34E-01	9.74E-01	1.00E+00	-
	SOD2	1.34	0.92	0.83	5.44E-01	9.99E-01	9.13E-01	-
Decomposition of H_2O_2:								
	PRDX4	1.04	1.06	0.91	1.00E+00	9.96E-01	9.05E-01	-
	PRDX5	0.99	1.03	0.91	1.00E+00	1.00E+00	9.23E-01	-
	PRDX6	0.91	1.11	1.04	7.90E-01	7.47E-01	9.93E-01	-
TXN- and GSH-system:								
	TXN	0.67	0.57	0.82	5.51E-01	4.53E-02	8.78E-01	
	TXNDC5	0.96	1.26	0.95	9.91E-01	4.35E-02	9.80E-01	Indirect BHA
	TXNRD1	1.88	1.17	0.82	1.42E-01	9.96E-01	9.81E-01	
	GLRX3	1.01	1.27	1.33	9.91E-01	7.73E-01	6.24E-01	
	GPX1	1.01	0.94	1.08	1.00E+00	9.99E-01	9.83E-01	
	GSR	0.91	1.95	1.61	1.00E+00	3.01E-01	6.86E-01	
NADPH delivery:								
	ALDOA	0.91	1.31	1.13	7.86E-01	1.09E-02	5.53E-01	Indirect BHA
	G6PD	0.92	1.24	1.03	7.46E-01	1.58E-02	9.99E-01	Indirect BHA
	NDUFV2	1.04	0.45	0.68	1.00E+00	4.84E-02	6.00E-01	



Supplementary Figure 4 Dynamic range of roGFP2 redox sensor constructs (a) The difference between fluorescence intensity ratios of fully oxidized and fully reduced (a) roGFP2-Orp1 and (b) NCF1-roGFP2 indicates the maximal dynamic range of the sensor constructs.



Supplementary Figure 5 DPI co-treatment suppresses RA-induced prolongation of neurites within 72 h (a) Quantified length of SH-SY5Y neurites after 0 h and 72 h RA and ethanol (control) treatment, represented as a ratio of length-to-width of the cell body (n=40 cells/group). Statistical significance between the groups was computed using two-way ANOVA followed by Tukey's multiple comparison test. Asterisk indicate *p*-values *≤ 0.05, ***≤0.001.