

Supplementary Information

Environmentally relevant UV-light weathering of polystyrene micro- and nanoplastics promotes hepatotoxicity in a human cell line

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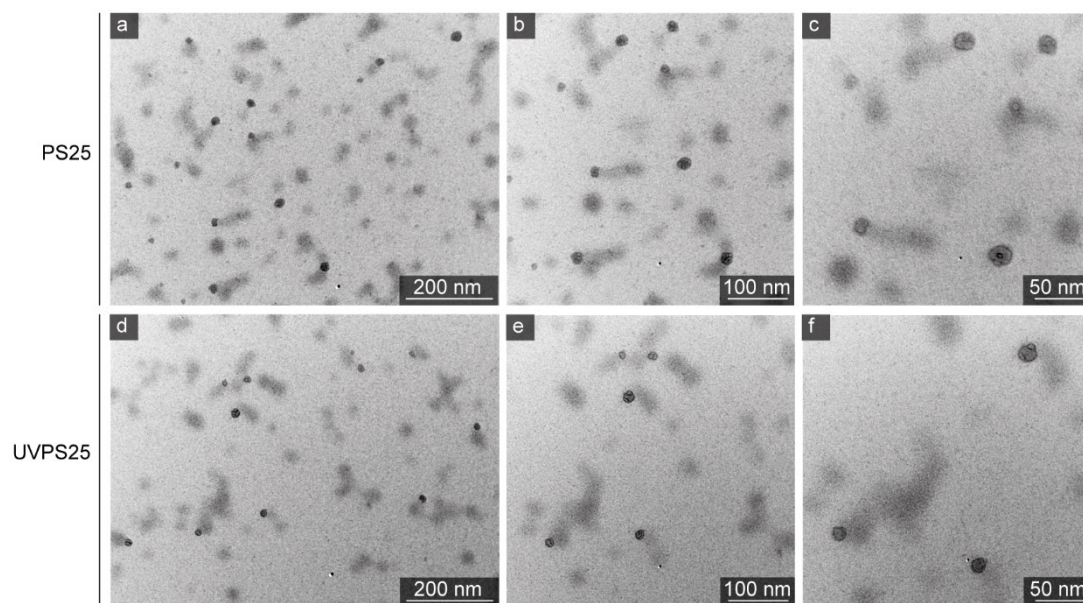


Fig. S1: Effects of UV exposure on the morphology of PS25 particles. Representative TEM images of 25 nm PS particles before (a and magnifications b,c) and after simulation of 2 months of sunlight exposure (d and magnifications e,f). No major morphological differences were apparent between PS25 and UVPS25.

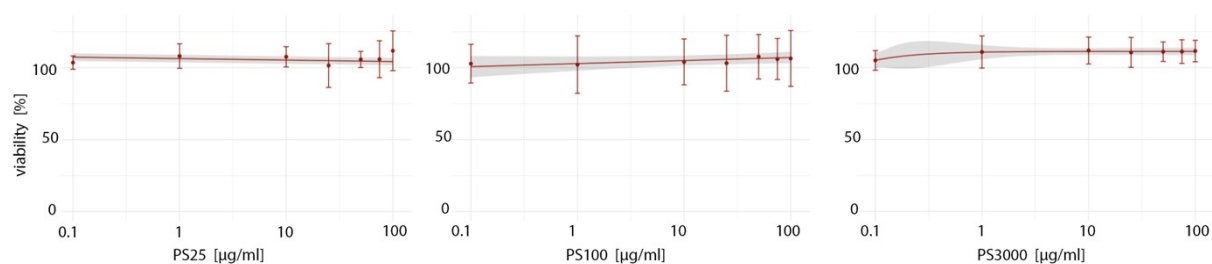


Fig. S2: Impact of primary PS particles on viability of HepaRG cells upon prolonged exposure. HepaRG cells were exposed to primary PS particles of 25 nm, 100 nm and 3000 nm for 10 days and viability was assessed by CellTiter-Glo® Luminescent Cell Viability assay. Solid red line represents mean values and 95% confidence intervals of the non-linear regression are shown in grey. Data represent mean \pm Stdev from three independent experiments with six technical replicates each. Values were normalized to untreated cells, which were defined as viability of 100%.

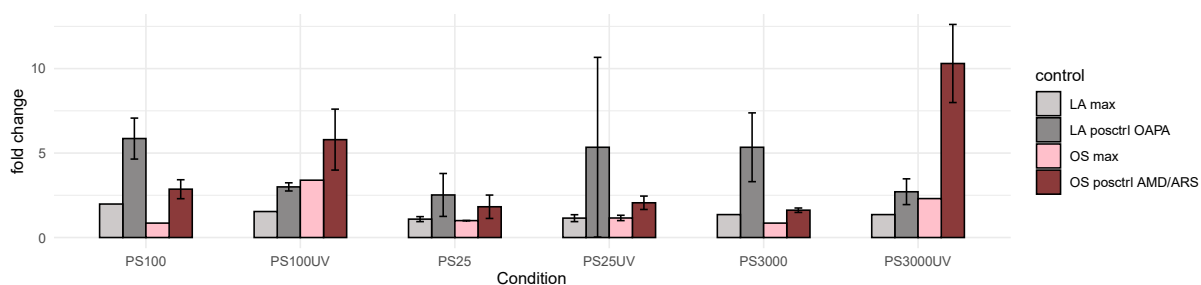


Fig. S3: Positive controls for high content imaging. Amiodarone (AMD) or arsenite (ARS) and oleic and palmitic acids (OAPA) were used as positive controls for oxidative stress (OS) and lipid accumulation (LA) in our high content imaging, respectively. Medium effects \pm Stdev of positive controls of three independent experiments are shown next to maximum effects seen in experimental conditions. Plates that underwent imaging but did not show induction of OS or LA in their positive control conditions were excluded.

Suppl. Table 1: Primers used in this study

Primers for qPCR-based analysis of gene level expression on cDNA			
Human gene and directionality	Sequence (5' - 3')	Respective Protein	Source
AHR <i>FW</i>	GTAAGTCTCCCTTCATACC	AhR	Moriguchi et al., 2003
AHR <i>REV</i>	AGGCACGAATTGGTTAGAG		
NR1I2 <i>FW</i>	TGCGAGATCACCCGGAAGAC	PXR	Takagi et al., 2008
NR1I2 <i>REV</i>	ATGGGAGAAGGTAGTGCAAAGG		
NR1H3 <i>FW</i>	AGCTCAAGGATCTGGTGGTG	LXR α	This study
NR1H3 <i>REV</i>	GACAGCAGTGCAGACTTA		
SREBF1 <i>FW</i>	GGCTCCTGCCTACAGCTTCT	SREBP1c	Higuchi et al., 2008
SREBF1 <i>REV</i>	CAGCCAGTGGATCACCACA		
PPARA <i>FW</i>	ACTTATCCTGTGGTCCCGG	PPAR α	Liu et al., 2004
PPARA <i>REV</i>	CCGACAGAAAGGCACTTGTGA		
IL6 <i>FW</i>	GCTGCAGGCACAGAACCA	IL-6	Forte et al., 2016
IL6 <i>REV</i>	GCTGCGCAGAATGAGATGAG		
CXCL8 <i>FW</i>	CTGGCCGTGGCTCTCTTG	IL-8	Forte et al., 2016
CXCL8 <i>REV</i>	CTTGGCAAAACCTGCACCTTCA		
CRP <i>FW</i>	GAAC TTCAGCCGAATACATCTTTT	CRP	Najar et al., 2012
CRP <i>REV</i>	CTTGGCAAAACCTGCACCTTCA		
IL1B <i>FW</i>	ACGATGCACCTGTACGATCACT	IL-1 β	Forte et al., 2016
IL1B <i>REV</i>	CACCAAGCTTTTTTGTGTGAGT		
TP53 <i>FW</i>	TCTGTCCCTTCCCAGAAAACC	p53	Forte et al., 2016
TP53 <i>REV</i>	CAAGAAGCCCAGACGGAAAC		
MYC <i>FW</i>	AGGGTCAAGTTGGACAGTGTC	c-Myc	Forte et al., 2016
MYC <i>REV</i>	TGGTGCATTTTCGGTTGTTG		

Suppl. Table 2: Raw mean Ct values for qPCR analysis

Gene	PS25		UVPS25		PS100		UVPS100		Untreated	
	mean Ct	stdev	mean Ct	stdev	mean Ct	stdev	mean Ct	stdev	mean Ct	stdev
Ahr	25.419	1.458	27.229	0.271	24.596	0.363	26.120	0.806	24.580	1.315
PXR	28.177	3.196	30.857	2.806	28.245	2.967	28.661	2.664	30.870	1.307
LXRalpha	26.226	2.175	29.657	2.127	28.313	3.197	28.400	2.473	28.513	1.135
SREBP1c	23.849	1.146	26.704	0.561	25.614	1.386	26.043	0.426	24.581	0.478
PPARalpha	26.827	0.904	31.596	4.056	29.426	1.965	28.945	0.269	27.164	0.674
Il6	30.547	2.997	34.394	1.998	32.748	0.576	31.635	2.720	31.154	1.039
Il8	28.002	1.040	30.613	3.384	29.043	2.042	27.416	0.194	26.708	0.713
CRP	27.964	0.759	32.158	3.673	29.187	1.826	28.389	0.719	26.130	1.071
IL1beta	27.306	1.027	26.247	2.360	27.605	0.315	27.684	0.916	25.301	0.922
TP53	26.889	1.578	27.753	3.788	35.746	8.843	29.028	0.185	27.156	1.346
C-myc	27.475	1.942	26.177	2.972	28.954	0.946	28.122	0.715	24.749	0.960
GAPDH	26.888	0.704	20.967	0.743	26.343	2.046	26.326	2.050	21.115	0.536

	PS3000		UVPS3000		Untreated	
	mean Ct	stdev	mean Ct	stdev	mean Ct	stdev
Ahr	27.926	0.638	28.463	0.776	27.982	2.254
PXR	32.101	0.294	32.490	1.011	29.949	0.895
LXRalpha	31.028	1.008	30.571	0.523	30.311	0.311
SREBP1c	28.546	0.574	29.121	0.385	28.356	0.441
PPARalpha	29.871	0.594	30.297	0.266	29.799	0.360
Il6	34.574	0.316	35.199	0.273	33.658	0.485
Il8	31.180	0.054	31.806	0.535	30.676	0.177
CRP	28.577	1.046	29.407	1.143	29.052	0.809
IL1beta	27.090	0.472	27.107	0.356	22.837	0.185
TP53	31.252	0.310	31.207	0.276	30.617	0.477
C-myc	29.776	0.474	29.743	0.249	26.908	0.026
GAPDH	24.564	0.405	24.629	0.336	24.134	0.424