

Electronic Supplementary Material
Induction of toxicity in human colon cells and organoids by size- and composition-dependent road dust

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	TOC		Elemental Analysis				XRF(wt%)																				py-GC-MS (peak area(unit: au))			
	TOC (proa d dust)	SD_TO C	C(wt %)	H(wt %)	N(wt %)	S(wt %)	Na	Mg	Al	Si	P	S	Cl	K	Ca	Ti	Cr	Mn	Fe	Ni	Cu	Zn	Rb	Sr	Zr	Ba	Pb	Caliphatic. HC	Caromatic. HC	Cphenol ic
Roa d dust 1	4.09	0.48	6.333	0.754	0.154	0.046	0	1.42	13.4	46.2	0.193	1.05	0.0714	6.53	14.6	1.37	0.0767	0.43	12.3		0.055	1.8	0.0635	0.112	0.055		0.148	0	186,143	0
Roa d dust 2	4.40	0.88	5.986	0.718	0.257	0.032	0.831	1.82	15	38.4	0.59	0.844	0.894	6.66	6.24	1.52	1.7	0.352	23.5	0.451	0.36	0.356	0.106	0.156	0.183			280,346	54,594	312,699
Roa d dust 3	4.44	0.81	4.717	0.592	0.256	0.000	0.88	1.12	14.7	47.4	0.44	0.578	0.0997	7.37	12.4	1.37	0.0599	0.426	12	0.0323	0.075	0.299	0.0817	0.145	0.134	0.328	0.0639	268,937	0	64,633
Roa d dust 4	2.81	0.28	4.918	0.423	0.000	0.000	0.871	1.1	11.3	42.1	0.258	0.963	0.173	5.3	13.8	1.28	0.179	0.637	20.4	0.0601	0.153	0.549	0.0596	0.145	0.161	0.302	0.0755	133,245	0	0

Table S1. Total elemental analysis data of road dust 1-4

Position	Symbol	Up-Down Regulation (comparing to control group)			
		SRM 1648a		1	
		Fold Regulation	Comments	Fold Regulation	Comments
A01	ABCB1	-1.26		-1.43	
A02	ALDOB	3.17		5.05	
A03	ATG16L1	-1.64		-1.54	
A04	C3	2.27		2.15	
A05	C4BPB	-1.04		-1.15	
A06	CASP1	-1.26		-1.20	
A07	CCL11	1.00		-1.18	
A08	CCL2	1.33		-1.45	
A09	CCL20	9.09		11.60	
A10	CCL25	1.17		1.15	
A11	CCL5	1.33		-1.15	
A12	CCR1	-1.03		-1.14	
B01	CCR2	1.07		-1.52	
B02	CCR5	1.11		-1.34	
B03	CCR9	1.35		-1.42	
B04	CD55	1.15		1.22	
B05	CHI3L1	-1.19		-1.54	
B06	CR2	-1.14		-1.11	
B07	CSTA	-1.12		-1.64	
B08	CX3CL1	-1.02		-1.13	
B09	CX3CR1	1.12		-1.46	
B10	CXCL1	6.56		9.62	
B11	CXCL10	-1.37		-1.44	
B12	CXCL11	-1.51		-1.33	
C01	CXCL12	1.56		-1.12	
C02	CXCL2	2.59		4.22	
C03	CXCL3	3.21		6.26	
C04	CXCL9	-1.44		-1.30	
C05	CXCR1	1.00		-1.40	
C06	CXCR3	1.42		-1.05	
C07	DEFA5	-1.01		-1.16	
C08	DEFA6	1.02		-1.40	
C09	EDN3	1.38		-1.04	
C10	EGR3	-1.43		-1.71	
C11	FPR1	1.00		-1.30	
C12	GCG	-1.26		-1.14	
D01	HLA-	-1.03		-1.13	

	DQA1				
D02	HLA-DRA	-1.12		-1.19	
D03	HLA-DRB1	-1.32		-1.77	
D04	HSP90B1	-1.77		-1.61	
D05	HSPA5	-1.84		-1.43	
D06	IFNG	-1.01		-1.13	
D07	IL13	-1.20		-1.43	
D08	IL17A	-1.17		-1.44	
D09	IL1B	1.06		-1.05	
D10	IL1RN	1.05		1.24	
D11	IL23A	-1.05		-1.37	
D12	IL2RA	-1.15		-1.38	
E01	IL5	-1.52		-1.33	
E02	IL6	1.03		-1.05	
E03	CXCL8	5.37		8.73	
E04	IRF5	1.02		-1.35	
E05	ISG15	-1.99		-1.30	
E06	ITGB2	1.42		-1.17	
E07	LCN2	3.08		5.08	
E08	LTB	3.74		6.30	
E09	LYZ	-1.39		-1.47	
E10	MMP1	1.09		1.24	
E11	MMP10	-1.48		-1.35	
E12	MMP3	-1.20		-1.13	
F01	MMP7	1.88		1.31	
F02	MUC1	2.02		2.37	
F03	NOD2	1.06		-1.31	
F04	NOS2	-1.25		-1.55	
F05	NR3C2	-1.15		-1.52	
F06	PCK1	-2.31		-2.61	
F07	PECAM1	-1.04		-1.24	
F08	REG1A	-1.24		-1.46	
F09	REG1B	-1.00		1.00	
F10	S100A8	-1.13		-1.72	
F11	S100A9	1.03		-1.02	
F12	SAA1	2.90		4.81	
G01	SELE	1.23		1.14	
G02	SELL	1.05		-1.10	
G03	SOD2	1.90		3.24	

G04	STAT1	-1.19		-1.12	
G05	STAT3	-1.19		-1.49	
G06	TDO2	-1.03		-1.27	
G07	TFF1	1.24		1.62	
G08	TIMP1	-1.30		1.11	
G09	TNF	1.09		-1.05	
G10	TYK2	-1.70		-1.88	
G11	UBD	1.23		1.29	
G12	VWF	1.30		-1.14	
H01	ACTB	1.42		1.94	
H02	B2M	1.18		1.39	
H03	GAPDH	-1.24		-1.39	
H04	HPRT1	1.03		-1.43	
H05	RPLP0	-1.34		-1.45	

<5 Housekeeping genes>

ACTB - Actin, beta

B2M - Beta-2-microglobulin

GAPDH - Glyceraldehyde-3-phosphate dehydrogenase

HPRT1 - Hypoxanthine phosphoribosyltransferase 1

RPLP0 - Ribosomal protein, large, P0

Table S2. Expression level of 84 colon inflammatory-related genes evaluated against SRM1648a and road dust 1 (2 mg/mL) treated colon organoids.

ALDOB (NM_000035)

Forward	AGCCTCGCTATCCAGGAAAACG
Reverse	TGGCAGTGTTCCAGGTCATGGT

C3 (NM_000064)

Forward	GTGGAAATCCGAGCCGTTCTCT
Reverse	GATGGTTACGGTCTGCTGGTGA

CCL20 (NM_004591)

Forward	AAGTTGTCTGTGTGCGCAAATCC
Reverse	CCATTCCAGAAAAGCCACAGTTTT

CXCL1 (NM_001511)

Forward	AGCTTGCCTCAATCCTGCATCC
Reverse	TCCTTCAGGAACAGCCACCAGT

CXCL2 (NM_002089)

Forward	GGCAGAAAGCTTGTCTCAACCC
Reverse	CTCCTTCAGGAACAGCCACCAA

CXCL3 (NM_002090)

Forward	TTCACCTCAAGAACATCCAAAGTG
Reverse	TTCTTCCCATTCTTGAGTGTGGC

CXCL8 (NM_000584)

Forward	GAGAGTGATTGAGAGTGGACCAC
Reverse	CACAACCCTCTGCACCCAGTTT

LCN2 (NM_005564)

Forward	GTGAGCACCAACTACAACCAGC
Reverse	GTTCCGAAGTCAGCTCCTTGGT

LTB (NM_002341)

Forward	GGTTTCAGAAGCTGCCAGAGGA
Reverse	CGTCAGAAACGCCTGTTCCTTC

MUC1 (NM_002456)

Forward	CCTACCATCCTATGAGCGAGTAC
Reverse	GCTGGGTTTGTGTAAGAGAGGC

PCK1 (NM_002591)

Forward	CATTGCCTGGATGAAGTTTGACG
Reverse	GGGTTGGTCTTCACTGAAGTCC

SAA1 (NM_000331)

Forward	TCGTTCCCTTGGCGAGGCTTTTG
Reverse	AGGTCCCCTTTTGGCAGCATCA

SOD2 (NM_000636)

Forward	CTGGACAAACCTCAGCCCTAAC
Reverse	AACCTGAGCCTTGGACACCAAC

Table S3. Forward and reverse primer sequence used for qPCR.

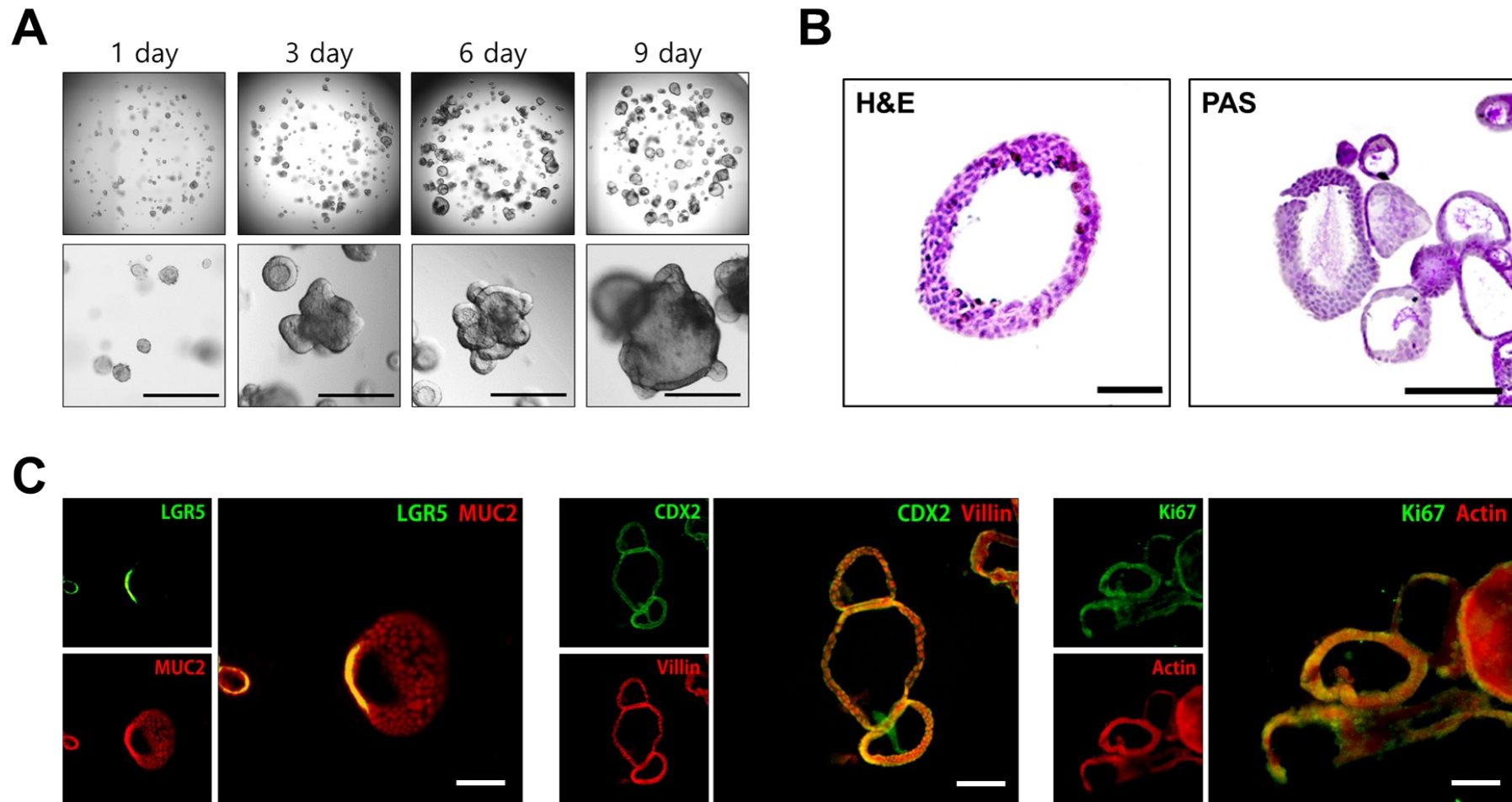


Figure S1. (A) BF images (B) H&E and PAS staining (C) Immunofluorescence staining of colon organoids.

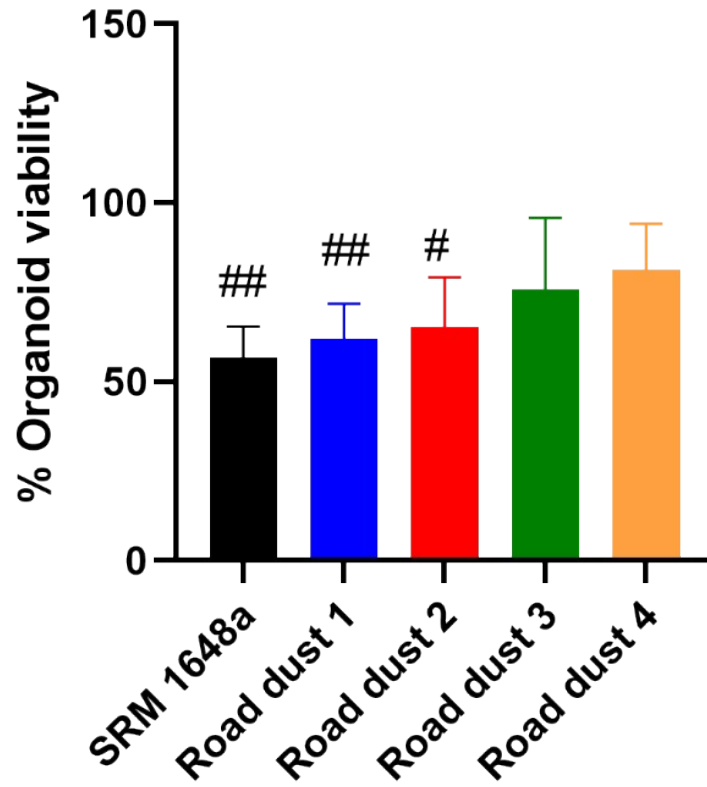


Figure S2. % colon organoid viability upon addition of 2 mg/mL road dust. Error bars represent the standard deviation from three independent experiments. Statistical significance is denoted as # for $P < 0.05$ and ## for $P < 0.01$

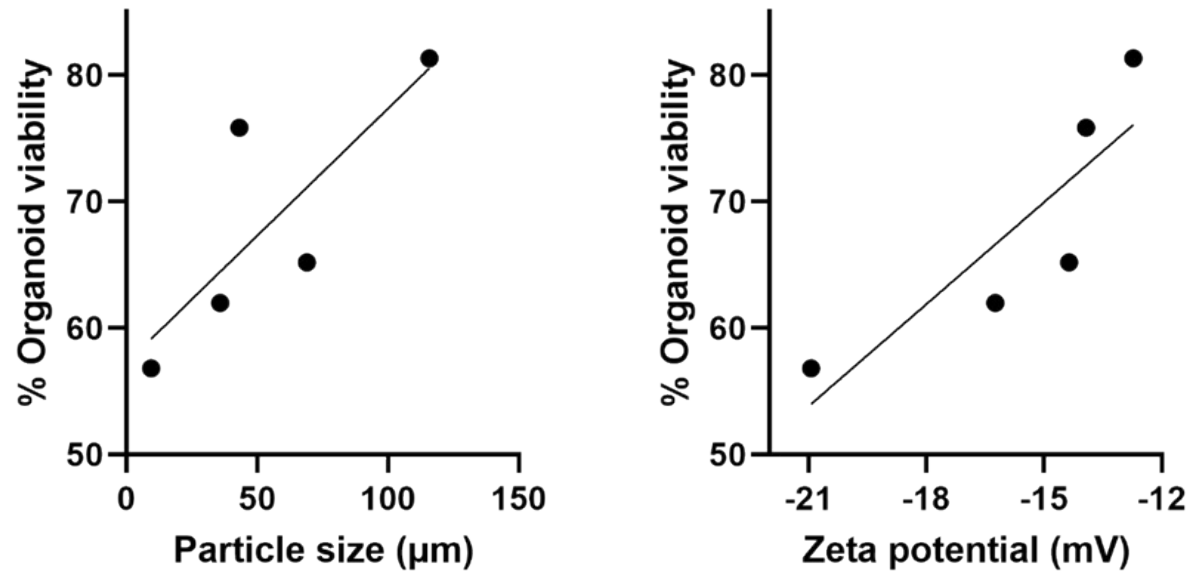


Figure S3. Road dust particle size (left) and surface charge (right) dependent human colon organoid toxicity.