

991 **Supplemental Figures and Tables:**

992 **Supplemental Figure 1:** IL-6 concentration in media from various control experiments

993 performed to confirm exosome specific activation of inflammatory mediators. Shown here is an
994 example of myometrial (A), decidual (B) and BeWo cells (C).

995 On the x axis are all the treatments included:

996 Control: negative control

997 Lipopolysaccharide (LPS) was used as a positive control

998 Cold – cold treatment of cells to prevent endocytosis of exosomes

999 Heat inactivation – to disrupt exosomal membrane and denature proteomic cargo

1000 Sonication - to disrupt exosomal membrane and denature proteomic cargo

1001 Control and oxidative stress exosomes treatments at a dose of 10^7

1002 A – Myometrial cells treated with LPS and control and oxidative stress exosomes show increased
1003 IL-6 compared to control. Cold, heat and sonication did not change IL-6 compared to negative
1004 control

1005 B - Decidual cells treated with LPS and control and oxidative stress exosomes show increased
1006 IL-6 compared to control. Cold treatment reduced IL-6 more than negative control settings
1007 whereas heat and sonication did not change IL-6 compared to negative control.

1008 C – BeWo cells increase IL-6 in response to LPS. No change was seen with any other conditions
1009 including exosome treatment.

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1011 **Supplemental Figure 2:** 3D reconstructions of confocal images of each target cell studied.
 1012 Blue is DAPI which shows the nucleus. Red is a cytoplasmic protein either α or β actin.
 1013 Exosomes are shown in green. Exosomes are identified within each cell type (myometrial,
 1014 decidual and BeWo) by yellow arrows.

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1016 **Supplemental Table 1A:** Cytokine and PGE₂ concentrations in myometrial cells treated with
 1017 control exosomes.

IL-6	Control	Exo 10 ⁵	Exo 10 ⁷	Exo 10 ⁹
Mean \pm SD	62.5 \pm 13.3	171.3 \pm 43.1	190.6 \pm 62.6	166.4 \pm 58.0
Median (IQR)	60.3 (21.5)	190.4 (47.8)	190.4 (107.9)	147.7 (78.6)
p - Untreated cells vs control exosomes		0.03	0.03	0.03

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IL-8	Control	Exo 10 ⁵	Exo 10 ⁷	Exo 10 ⁹
Mean \pm SD	19721 \pm 18210.7	99892.8 \pm 21968.2	90793.1 \pm 23044.6	114652.2 \pm 24259.5
Median (IQR)	11764.0 (200059)	103786.9 (31454)	88936.9 (37985.0)	24259.5 (119391.2)
p - Untreated cells vs control exosomes		0.03	0.03	0.03

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PGE₂	Control	Exo 10 ⁵	Exo 10 ⁷	Exo 10 ⁹
Mean \pm SD	1120.1 \pm 60.5	1301.5 \pm 62.9	1179.8 \pm 60.5	1064.1 \pm 47.2
Median (IQR)	1097.8 (83.5)	1300.6 (97.4)	1184.6 (99.2)	1073.4 (69.3)
p - Untreated cells vs control exosomes		0.03	0.19	0.31

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1027 **Supplemental Table 1B:** Cytokine and PGE₂ concentrations in myometrial cells treated with
 1028 exosomes derived from AEC exposed to cigarette smoke extract (Oxidative stress (OS)
 1029 exosomes).

IL-6	Control	OS Exo 10 ⁵	OS Exo 10 ⁷	OS Exo 10 ⁹
Mean ± SD	62.5±13.3	165.2±48.4	192.7±37.67	178.6±12.31
Median (IQR)	60.3 (21.5)	149.9 (58.2)	186.3 (56.0)	178.7 (21.0)
p - Untreated cells vs OS exosomes		0.03	0.03	0.03

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IL-8	Control	OS Exo 10 ⁵	OS Exo 10 ⁷	OS Exo 10 ⁹
Mean ± SD	19721.4±18210.7	99177.3±27778.8	130680.0±26657.2	131687±35418
Median (IQR)	11764.0 (200059.0)	107778.5 (37597.0)	119572.0(29365.0)	137049.3(51333.0)
p - Untreated cells vs OS exosomes		0.03	0.03	0.03

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PGE₂	Control	OS Exo 10 ⁵	OS Exo 10 ⁷	OS Exo 10 ⁹
Mean ± SD	1120±60.5	1650.0±174.4	1304.9±174.4	1360.0±101.5
Median (IQR)	1097.8 (83.5)	1705.6 (240.0)	1337.4(154.8)	1373.2 (153.3)
p - Untreated cells vs OS exosomes		0.03	0.06	0.03

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1045 **Supplemental Table 2A:** Cytokine and PGE₂ concentrations in decidual cells treated with
 1046 control exosomes.

IL-6	Control	Exo 10 ⁵	Exo 10 ⁷	Exo 10 ⁹
Mean ± SD	22.8±14.1	101.8±12.1	113.6±31.8	75.1±3.290
Median (IQR)	18.4 (16.2)	104.8 (18.6)	119.3(45.6)	75.8 (4.6)
p - Untreated cells vs control exosomes		0.03	0.03	0.03

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IL-8	Control	Exo 10 ⁵	Exo 10 ⁷	Exo 10 ⁹
Mean ± SD	148.1±109.1	687.4±312.3	747.6±464.9	1546±217.8
Median (IQR)	150.4 (186.5)	800.5 (363.3)	818.1 (715.2)	1589.2 (274.2)
p - Untreated cells vs control exosomes		0.11	0.11	0.03

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PGE₂	Control	Exo 10 ⁵	Exo 10 ⁷	Exo 10 ⁹
Mean ± SD	18.0±1.5	23.5±2.2	19.2±3.1	24.8±4.4
Median (IQR)	18.3 (2.4)	23.3 (3.5)	18.7 (4.7)	22.7 (4.5)
p - Untreated cells vs control exosomes		0.03	0.66	0.03

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1050 **Supplemental Table 2B:** Cytokine and PGE₂ concentrations in decidual cells treated with
 1051 exosomes derived from AEC exposed to cigarette smoke extract (Oxidative stress (OS)
 1052 exosomes).

IL-6	Control	OS Exo 10 ⁵	OS Exo 10 ⁷	OS Exo 10 ⁹
Mean ± SD	22.8±14.1	94.2±17.4	100.7±27.5	150.8±12.4
Median (IQR)	18.4 (16.2)	92.8 (27.2)	104.9 (39.7)	148.6 (18.3)
p - Untreated cells vs OS exosomes		0.03	0.03	0.03

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IL-8	Control	OS Exo 10 ⁵	OS Exo 10 ⁷	OS Exo 10 ⁹
Mean ± SD	148.1±109.1	672.8±351.7	673.1±334.7	946.9±478.3
Median (IQR)	150.4 (186.5)	676.9(505.8)	772.6 (482.6)	936.1 (703.4)
p - Untreated cells vs OS exosomes		0.06	0.11	0.03

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PGE₂	Control	OS Exo 10 ⁵	OS Exo 10 ⁷	OS Exo 10 ⁹
Mean ± SD	18.0±1.5	30.4±4.2	32.1±6.1	34.24±3.1
Median (IQR)	18.3 (2.4)	29.2(6.4)	32.9 (8.5)	33.4 (3.9)
p - Untreated cells vs OS exosomes		0.03	0.03	0.03

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1060 **Supplemental Table 3A:** Cytokine and PGE₂ concentrations in BeWo cells treated with control
 1061 exosomes.

IL-6	Control	Exo 10 ⁵	Exo 10 ⁷	Exo 10 ⁹
Mean ±SD	57.0±6.0	52.6±9.4	48.6±7.2	47.4±11.6
Median (IQR)	55.4 (8.3)	50.6 (11.5)	46.5 (10.3)	47.1 (14.8)
p - Untreated cells vs control exosomes		0.31	0.19	0.19

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PGE₂	Control	Exo 10 ⁵	Exo 10 ⁷	Exo 10 ⁹
Mean ±SD	0.09± 0.01	0.12± 0.02	0.12± .01	0.09 ± 0.01
Median (IQR)	0.10 (0.01)	0.12 (0.03)	0.12 (0.01)	0.09 (0.02)
p - Untreated cells vs control exosomes		0.03	0.03	0.89

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1070 **Supplemental Table 3B:** Cytokine and PGE₂ concentrations in BeWo cells treated with
 1071 exosomes derived from AEC exposed to cigarette smoke extract (Oxidative stress (OS)
 1072 exosomes).

IL-6	Control	OS Exo 10 ⁵	OS Exo 10 ⁷	OS Exo 10 ⁹
Mean ±SD	57.0±6.0	47.1±9.1	50.5±10.1	54.7±16.1
Median (IQR)	55.4 (8.3)	46.0 (14.9)	54.3 (13.1)	50.2 (22.7)
p - Untreated cells vs OS exosomes		0.19	0.67	0.67

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PGE₂	Control	OS Exo 10 ⁵	OS Exo 10 ⁷	OS Exo 10 ⁹
Mean ±SD	0.09±0.01	0.10±0.01	0.10±0.01	0.10±0.02
Median (IQR)	0.10 (0.01)	0.10 (0.02)	0.10 (0.02)	0.10 (0.02)
p - Untreated cells vs OS exosomes		0.56	0.67	0.19

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1076 **Supplemental Table 4** – Control experiments used to show exosome mediated immune
 1077 activation effects in myometrial, decidual and BeWo cells.

	LPS			Cold treatment of cells			Heat inactivation of exosomes			Sonication of exosomes		
	IL-6	IL-8	PGE ₂	IL-6	IL-8	PGE ₂	IL-6	IL-8	PGE ₂	IL-6	IL-8	PGE ₂
Myometrium	↑	↑	↑	↓	↓	↔	↔	↔	↔	↓	↔	↔
Decidua	↑	↑	↑	↓	↓	↓	↔	↔	↔	↔	↔	↔
BeWo	↑	↔	X	↓	↔	↓	↔	↔	↔	↔	↔	↔

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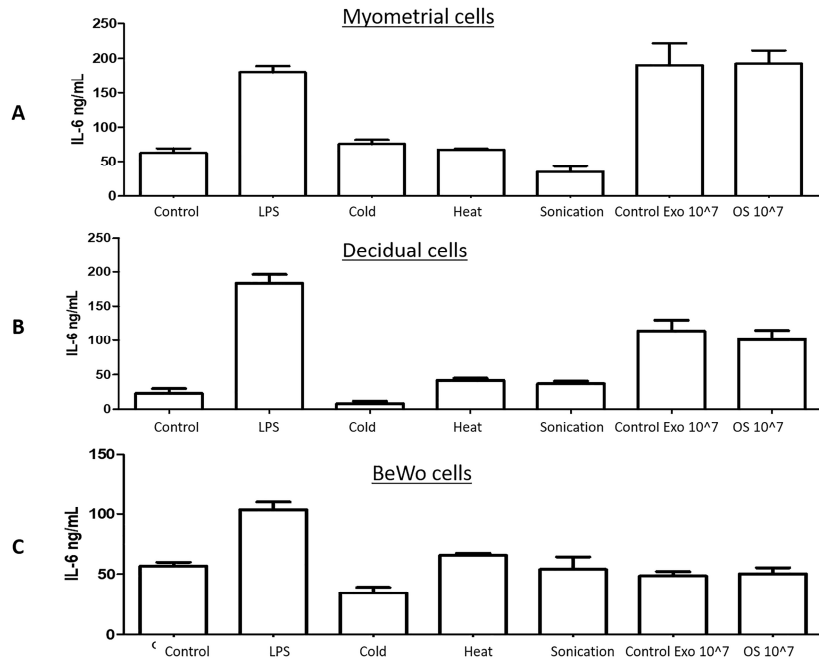
1080 ↑ **Increase**
 1081 ↓ **Decrease**
 1082 ↔ **No change**
 1083 **X not detectable**

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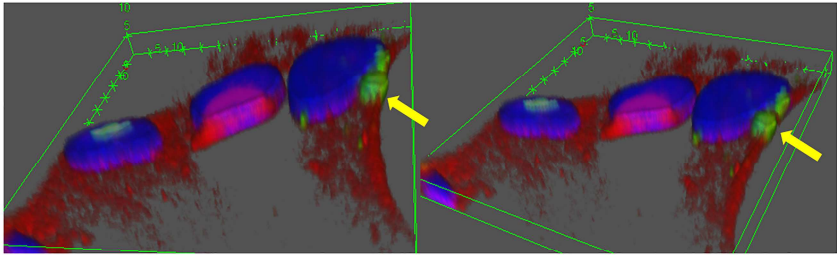
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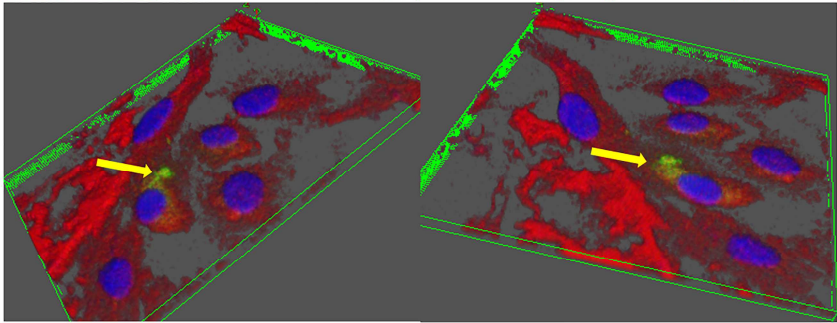
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Myometrial



Decidual



BeWo

