

Table S1. Soluble Cytokines/Chemokines (Mean +/- SEM, pg/ml)

	Placental Villous Explants					Amnion Explants				
	day 1	day 4	day 7	day 10	day 14	day 1	day 4	day 7	day 10	day 14
IL-1α	4.5 \pm 1.0	12.4 \pm 2.2	13.7 \pm 3.3	14.6 \pm 1.0	10.8 \pm 2.6	3.1 \pm 0.6	4.4 \pm 1.4	2.8 \pm 0.8	5.3 \pm 0.7	4.3 \pm 0.6
IL-1β	12.1 \pm 5.1	28.3 \pm 6.3	16.7 \pm 4.3	17.8 \pm 2.8	15.7 \pm 2.4	6.6 \pm 2.8	7.0 \pm 3.0	3.6 \pm 1.2	8.4 \pm 1.3	10.9 \pm 0.9
IL-2	5.2 \pm 1.5	10.0 \pm 2.2	10.0 \pm 2.4	11.2 \pm 2.9	5.9 \pm 1.1	3.9 \pm 1.3	5.2 \pm 1.9	3.1 \pm 1.0	3.9 \pm 0.9	3.4 \pm 0.6
IL-4	3.9 \pm 2.0	14.9 \pm 3.6	14.8 \pm 3.4	30.0 \pm 5.1	13.0 \pm 4.5	1.8 \pm 0.8	2.1 \pm 1.1	0.9 \pm 0.3	4.1 \pm 1.1	1.3 \pm 0.3
IL-6	23099.6 \pm 5394.0	135364.0 \pm 23217.9	101703.3 \pm 18987.4	39127.4 \pm 4854.3	23547.4 \pm 4465.6	871.1 \pm 568.2	21200.2 \pm 9960.1	13336.6 \pm 6870.3	13856.9 \pm 3288.7	7885.5 \pm 2706.7
IL-8	10062.0 \pm 1828.9	31942.9 \pm 3044.2	32938.8 \pm 3496.7	7955.2 \pm 638.1	17426.4 \pm 1845.5	4563.5 \pm 1957.2	21543.8 \pm 4439.9	13946.5 \pm 3824.5	6079.9 \pm 880.9	9423.5 \pm 2879.5
IL-10	8.2 \pm 1.6	28.0 \pm 5.5	28.2 \pm 3.6	46.0 \pm 9.3	40.0 \pm 7.3	13.6 \pm 8.0	45.6 \pm 25.4	37.6 \pm 12.7	113.2 \pm 37.0	138.4 \pm 39.5
IL-13	81.9 \pm 15.2	132.0 \pm 19.6	137.9 \pm 24.3	74.3 \pm 17.4	36.1 \pm 7.7	51.1 \pm 11.4	65.6 \pm 16.7	56.3 \pm 12.9	24.1 \pm 6.2	17.3 \pm 3.4
IL-15	9.0 \pm 1.7	36.9 \pm 6.5	30.7 \pm 5.3	28.9 \pm 4.9	32.0 \pm 3.2	0.3 \pm 0.1	3.4 \pm 1.4	0.0 \pm 0.0	3.5 \pm 1.6	0.6 \pm 0.3
IL-16	92.7 \pm 19.2	285.4 \pm 50.6	220.3 \pm 45.0	240.6 \pm 44.8	143.0 \pm 18.9	37.5 \pm 15.9	63.3 \pm 49.6	41.4 \pm 18.7	67.8 \pm 26.8	18.4 \pm 7.2
IL-18	3.8 \pm 0.7	12.2 \pm 2.6	6.7 \pm 0.9	5.5 \pm 0.7	5.8 \pm 1.0	0.3 \pm 0.1	1.8 \pm 1.5	0.4 \pm 0.2	2.1 \pm 1.1	0.8 \pm 0.2
IL-33	186.0 \pm 80.2	244.6 \pm 110.5	348.2 \pm 117.5	721.3 \pm 264.2	212.6 \pm 80.7	315.6 \pm 75.8	198.1 \pm 74.3	222.7 \pm 99.4	273.7 \pm 99.9	144.4 \pm 93.8
Calg A	96.8 \pm 18.0	271.4 \pm 44.8	337.6 \pm 69.9	241.8 \pm 68.4	69.0 \pm 20.3	69.6 \pm 24.6	59.1 \pm 41.5	48.8 \pm 18.7	68.5 \pm 26.8	0.0 \pm 0.0
Calg C	17694.8 \pm 4120.8	110497.9 \pm 24969.9	118456.1 \pm 29581.1	154215.6 \pm 30576.7	121067.0 \pm 14305.4	2639.3 \pm 496.6	6912.9 \pm 2568.1	3501.4 \pm 803.0	32843.0 \pm 20841.8	4973.8 \pm 1222.4
CRP	2954.3 \pm 593.3	3618.1 \pm 544.4	2793.5 \pm 545.1	1724.3 \pm 584.3	1164.9 \pm 321.6	370.9 \pm 156.7	893.2 \pm 382.4	351.0 \pm 119.4	392.6 \pm 160.3	152.9 \pm 43.3
CXCL6	0.0 \pm 0.0	19.2 \pm 8.9	11.9 \pm 6.1	74.2 \pm 42.5	87.8 \pm 56.4	1.3 \pm 1.3	373.1 \pm 3707.7	193.9 \pm 108.4	1006.4 \pm 463.1	983.7 \pm 283.2
CXCL13	0.0 \pm 0.0	2.7 \pm 2.7	10.2 \pm 6.7	13.4 \pm 10.2	31.6 \pm 28.1	0.0 \pm 0.0	1.9 \pm 1.9	0.0 \pm 0.0	2.4 \pm 1.9	4.6 \pm 4.1
Eotaxin	0.0 \pm 0.0	0.0 \pm 0.0	39.6 \pm 20.8	80.7 \pm 47.9	21.4 \pm 36.3	0.0 \pm 0.0	1.1 \pm 1.1	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
GM-CSF	22.9 \pm 11.8	338.2 \pm 146.7	260.2 \pm 124.0	363.2 \pm 182.8	350.0 \pm 187.3	2.8 \pm 1.3	7.9 \pm 3.3	4.1 \pm 1.1	7.8 \pm 1.8	5.2 \pm 2.5
Gro-α	715.3 \pm 139.8	13143.0 \pm 2502.2	12568.6 \pm 2160.9	10049.5 \pm 2128.4	4731.8 \pm 730.2	1181.5 \pm 423.7	16369.5 \pm 4651.3	14927.1 \pm 3526.5	11259.7 \pm 3181.0	4774.7 \pm 889.2
HMGB-1	1140.8 \pm 383.4	4101.2 \pm 2691.0	1728.3 \pm 560.2	1556.1 \pm 281.1	1026.8 \pm 134.7	350.4 \pm 67.7	623.3 \pm 250.9	315.5 \pm 62.6	728.8 \pm 87.8	479.8 \pm 76.0
IFNβ	11.9 \pm 4.7	75.7 \pm 20.1	90.4 \pm 24.2	82.0 \pm 26.3	25.9 \pm 7.5	3.8 \pm 1.8	14.1 \pm 8.2	8.9 \pm 5.1	11.7 \pm 5.4	4.5 \pm 2.5
IFN-γ	3.2 \pm 3.2	23.9 \pm 11.4	10.9 \pm 4.3	109.3 \pm 40.8	31.1 \pm 11.6	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	10.2 \pm 6.5	1.3 \pm 1.1
IP-10	14721.9 \pm 4216.9	84756.4 \pm 26264.0	77190.7 \pm 22221.8	69809.3 \pm 10940.5	12435.2 \pm 1057.6	202.6 \pm 141.2	7325.4 \pm 4353.9	3648.0 \pm 2041.4	15535.1 \pm 7802.6	4535.5 \pm 632.7
I-TAC	258.6 \pm 50.5	1531.7 \pm 268.7	1278.8 \pm 189.1	1847.3 \pm 303.0	1997.9 \pm 276.6	75.7 \pm 20.4	171.3 \pm 70.3	68.9 \pm 18.6	514.4 \pm 310.4	128.9 \pm 27.2
Lacto	4117.4 \pm 829.9	35929.6 \pm 5350.7	38513.9 \pm 6354.9	37548.1 \pm 3752.6	25090.4 \pm 2393.6	813.0 \pm 235.6	3190.9 \pm 1828.0	2233.2 \pm 915.2	13554.5 \pm 4789.5	8908.1 \pm 2798.1
M-CSF	19.4 \pm 1.9	66.2 \pm 9.2	49.9 \pm 7.1	77.9 \pm 19.3	80.5 \pm 16.1	10.4 \pm 1.4	15.2 \pm 3.4	9.0 \pm 1.6	15.4 \pm 4.2	12.2 \pm 1.1
MCP-1	1162.3 \pm 237.9	12199.7 \pm 3885.4	13279.3 \pm 4008.6	13752.2 \pm 1763.1	45010.0 \pm 2112.0	207.7 \pm 89.5	1837.8 \pm 813.2	1468.3 \pm 498.0	3476.7 \pm 1592.7	18051.6 \pm 5081.9
MIF	59125.2 \pm 6939.6	72877.2 \pm 9357.0	73005.4 \pm 6572.2	90124.0 \pm 21839.8	64519.6 \pm 4581.3	28010.6 \pm 5176.4	52581.2 \pm 7895.0	25305.0 \pm 3897.3	29180.8 \pm 12188.0	29736.1 \pm 5712.5
MIG	93.0 \pm 48.4	471.2 \pm 242.1	942.7 \pm 449.0	1480.1 \pm 938.8	3413.6 \pm 2156.8	0.0 \pm 0.0	208.8 \pm 206.9	7.1 \pm 5.6	398.7 \pm 354.4	0.0 \pm 0.0
MIP-1α	563.1 \pm 196.3	830.0 \pm 247.3	354.1 \pm 61.4	255.8 \pm 34.6	193.0 \pm 25.1	1339.7 \pm 719.8	1249.1 \pm 510.9	351.6 \pm 93.9	198.4 \pm 22.8	121.7 \pm 16.2
MIP-1β	432.7 \pm 111.9	1168.0 \pm 285.1	827.2 \pm 148.8	753.7 \pm 131.3	698.5 \pm 137.6	712.7 \pm 372.1	1132.6 \pm 423.4	517.0 \pm 143.0	550.1 \pm 106.7	450.6 \pm 115.8
MIP-3α	225.3 \pm 80.3	6151.1 \pm 1828.5	4115.9 \pm 1062.4	6143.4 \pm 1515.3	5514.6 \pm 1575.6	12.0 \pm 12.0	369.6 \pm 268.1	47.8 \pm 34.0	1282.0 \pm 592.9	441.3 \pm 104.6
RANTES	219.8 \pm 39.1	538.2 \pm 153.4	149.5 \pm 39.2	249.9 \pm 49.3	205.5 \pm 44.6	0.0 \pm 0.0	21.2 \pm 13.0	1.2 \pm 1.2	37.2 \pm 6.6	59.4 \pm 28.9
TGF-β	1.0 \pm 0.6	4.9 \pm 2.0	5.9 \pm 2.9	12.7 \pm 6.4	0.5 \pm 0.4	0.6 \pm 0.4	2.4 \pm 2.0	0.6 \pm 0.4	0.8 \pm 0.7	0.0 \pm 0.0
TNF-α	4.4 \pm 1.3	10.0 \pm 1.1	10.1 \pm 2.1	16.9 \pm 3.9	10.3 \pm 1.4	5.0 \pm 2.5	7.2 \pm 2.8	3.2 \pm 1.1	6.3 \pm 1.8	2.2 \pm 0.7
TRAIL	5996.1 \pm 1083.0	8322.7 \pm 1057.2	5267.6 \pm 408.6	4459.5 \pm 701.0	2246.1 \pm 276.6	177.1 \pm 66.7	640.0 \pm 515.7	149.3 \pm 93.6	1011.1 \pm 449.1	522.0 \pm 175.8

Concentration of cytokines measured by multiplexed bead assays in culture supernatants at various time intervals of culture; medium is changed and replenished at each time point, n=10. Cultures maintain production of these factors over the entire culture length.

Table S2. Soluble Growth Factors/Angiogenic Factors (Mean +/- SEM, pg/ml)

	Placental Villous Explants					Amnion Explants				
	day 1	day 4	day 7	day 10	day 14	day 1	day 4	day 7	day 10	day 14
Activin A	397.8 86.1	249.0 ±87.4	361.0 ±83.1	796.2 ±163.8	1022.2 ±132.0	330.4 ±84.1	408.8 ±78.7	150.5 ±77.5	436.5 ±158.3	747.2 ±154.9
ADAM-12	2762.1 ±390.6	3625.3 ±472.2	3765.3 ±367.9	3309.5 ±471.4	4122.6 ±234.5	32.8 ±9.8	511.8 ±424.6	80.7 ±21.8	703.3 ±399.1	288.2 ±68.2
Adiponectin	2512.4 ±629.4	10571.9 ±3923.4	4040.6 ±809.1	1539.2 ±333.5	896.6 ±109.1	76.9 ±17.0	1917.3 ±817.0	82.4 ±22.8	271.8 ±147.6	58.5 ±21.9
Angiogenin	2279.7 ±614.8	3925.4 ±922.5	3539.2 ±592.7	5996.4 ±1536.9	5668.0 ±781.9	460.9 ±166.9	1438.0 ±573.9	964.7 ±252.6	2681.4 ±569.0	1966.3 ±372.7
CD40L	73.2±20.6	74.3±11.1	69.8±15.5	48.8±11.9	58.3±7.5	32.1±11.9	44.7±19.1	26.6±10.4	40.4±5.8	24.5±7.5
EGF	2.9±0.9	0.8±0.4	0.4±0.2	1.1±0.5	4.2±1.1	1.9±0.6	4.0±1.7	2.1±0.7	2.2±1.1	1.7±0.9
Endoglin	118.6±32.3	174.1±36.8	112.9±17.1	140.6±21.8	204.0±16.4	1.4±0.2	8.8±7.2	1.4±0.3	27.4±19.6	4.4±1.0
FasL	8.5±5.4	9.0±3.0	8.4±3.8	6.0±2.2	11.9±3.1	3.6±2.9	5.1±4.4	1.7±1.7	1.9±1.1	0.9±0.8
Fibronectin	6913.3 ±2141.7	23761.9 ±3225.3	35511.2 ±4964.6	302861.3 ±49256.4	312236.3 ±36257.5	14090.8 ±1762.0	19308.3 ±1820.4	25296.5 ±2880.9	20255.1 ±2962.8	27961.8 ±3405.0
Galectin-1	9909.2 ±1948.0	27419.3 ±3379.2	26310.1 ±4109.0	7358.8 ±1171.7	40032.3 ±3495.4	2683.7 ±703.1	7377.6 ±1436.3	2684.3 ±727.5	2061.5 ±602.8	8387.1 ±1789.4
hCG	570.9 ±158.9	2215.2 ±775.7	8445.3 ±4147.4	41295.8 ±28562.0	29859.8 ±16447.2	43.0 ±23.1	179.9 ±89.4	181.4 ±75.4	952.7 ±482.8	233.8 ±114.9
ICAM-1	873.7 ±181.8	3752.2 ±534.7	3691.3 ±316.3	3763.5 ±515.2	4801.1 ±419.3	117.7 ±30.3	492.5 ±317.0	212.9 ±34.9	1069.4 ±475.7	409.7 ±60.6
IGFBP1	613.9 ±271.1	5337.3 ±3029.8	5630.1 ±3415.5	5266.6 ±3187.5	3029.6 ±2437.2	2443.7 ±747.8	4401.5 ±1639.0	2770.9 ±1087.5	575.9 ±369.4	513.2 ±161.2
IL-1Ra	940.5 ±187.1	893.1 ±66.9	767.3 ±133.8	797.7 ±178.6	1261.4 ±154.5	496.8 ±80.7	579.7 ±147.7	258.0 ±67.2	477.8 ±99.7	246.4 ±104.2
IL-27	572.8 ±163.6	4410.5 ±1107.7	4543.2 ±810.1	6631.5 ±1024.2	8805.4 ±1526.7	123.4 ±43.0	705.0 ±241.2	724.5 ±185.7	2865.2 ±1138.2	2788.2 ±743.3
Leptin	23.6±10.9	130.1±36.9	171.1±33.2	222.7±50.6	273.7±43.4	2.1±1.5	33.2±12.7	41.7±18.1	201.1±71.3	237.7±78.6
MMP-7	34.1±11.2	34.3±6.8	144.7±42.0	413.3±118.9	1534.4±359.6	4.4±2.0	6.7±1.9	4.2±2.0	90.0±75.9	11.1±4.3
MMP-9	110.0±22.6	141.8±77.4	121.4±53.3	65.4±27.3	117.7±46.9	0.0±0.0	9.7±7.6	4.1±2.8	64.9±48.4	6.3±5.6
PAPP-A	6004.3 ±1431.1	3128.6 ±506.9	866.3 ±150.2	6642.5 ±1312.8	6896.7 ±875.3	104.3 ±56.1	189.1 ±94.5	46.1 ±23.0	630.9 ±311.4	172.6 ±61.2
PGE2	1274.0 ±423.4	4633.2 ±782.2	4468.2 ±710.1	7843.2 ±1602.1	12187.1 ±2402.6	705.2 ±146.5	582.1 ±178.7	163.6 ±11.2	2440.3 ±265.4	7336.3 ±147.8
PIGF	14.3±2.0	9.1±1.2	6.6±0.8	13.5±1.4	22.1±6.0	0.7±0.4	3.2±1.1	2.0±0.7	7.8±1.3	4.9±0.8
Resistin	46.0±8.4	298.0±49.9	351.4±64.3	247.6±52.7	284.2±57.4	6.1±1.7	30.0±18.2	11.2±2.4	51.2±32.9	11.1±1.7
Serpin E	4309.4 ±523.1	31951.4 ±4884.9	39441.4 ±4599.4	49559.4 ±7181.0	64146.4 ±6836.8	430.8 ±114.5	6045.1 ±2192.2	6675.8 ±1350.2	26931.4 ±5825.6	46612.0 ±11342.6
TFPI	908.6 ±201.8	27271.5 ±8842.0	35727.4 ±6254.7	47198.0 ±9823.7	6383.3 ±377.5	121.1 ±52.0	3093.9 ±2845.8	292.0 ±108.2	4076.7 ±3052.2	1801.6 ±783.9
TGFβ3	15.9±6.5	8.2±3.0	5.4±2.3	23.0±7.3	35.9±11.7	7.4±4.4	7.1±3.0	4.3±2.3	20.0±7.6	15.8±4.5
Tie-2	86.5±15.1	298.7±48.5	145.6±27.4	165.0±23.3	135.1±10.5	52.2±7.3	61.0±11.4	40.5±5.2	73.2±14.2	59.7±11.1
TIMP-1	14927.0 ±4687.2	125048.5 ±19490.5	140392.5 ±22651.5	68954.6 ±3723.2	1271340.1 ±256430.5	4496.2 ±1361.7	66343.4 ±17065.2	89762.0 ±20250.0	50733.9 ±6090.2	1448916.0 ±183185.8
Tissue Factor	48.5±10.9	126.3±22.0	96.6±15.5	83.9±12.5	78.5±5.9	7.2±1.5	43.6±27.7	13.0±1.8	24.9±4.8	20.8±2.6
TLR2	38.0±13.5	129.5±31.6	132.2±34.3	114.8±18.6	169.9±24.0	11.7±7.0	34.0±17.7	18.4±5.9	55.5±20.1	47.2±13.3
TREM-1	17.8±4.3	327.1±61.6	401.4±69.8	459.8±80.7	660.9±82.2	0.0±0.0	108.5±29.7	86.9±29.8	130.4±39.6	60.3±23.1
uPA	1868.2 ±388.3	7952.0 ±1530.1	7017.2 ±878.6	8069.8 ±1248.9	10067.4 ±963.8	64.6 ±27.3	1057.7 ±592.8	632.3 ±174.7	1735.7 ±452.5	1668.7 ±368.5
uPAR	2067.5 ±443.9	28640.7 ±2309.0	39007.3 ±3052.3	49659.5 ±4995.8	78355.4 ±5625.0	727.7 ±144.6	13000.4 ±3898.6	19487.1 ±3937.0	40398.4 ±7532.4	37964.0 ±5880.3
VEGF	22.2±10.6	42.2±8.8	40.7±9.6	33.1±8.8	59.4±9.2	6.7±6.7	19.2±10.1	4.5±3.6	9.1±5.1	19.3±9.0
VEGFR1	3353.1 ±523.3	15402.8 ±2023.0	17780.1 ±2672.1	21244.5 ±2699.6	28962.0 ±1968.8	2171.1 ±439.9	4623.4 ±1116.0	3996.1 ±603.1	8862.8 ±2587.7	8532.1 ±1607.8
VEGFR2	474.3 ±78.0	2093.5 ±388.5	1332.4 ±237.6	530.3 ±87.5	458.5 ±44.1	8.9 ±3.7	161.5 ±156.4	8.4 ±3.4	93.6 ±58.5	25.8 ±7.7

Concentration of growth and angiogenic factors measured by multiplexed bead assays in culture supernatants at various time intervals of culture; medium is changed and replenished at each time point, n=10. Cultures maintain production of these factors over the entire length of culture.

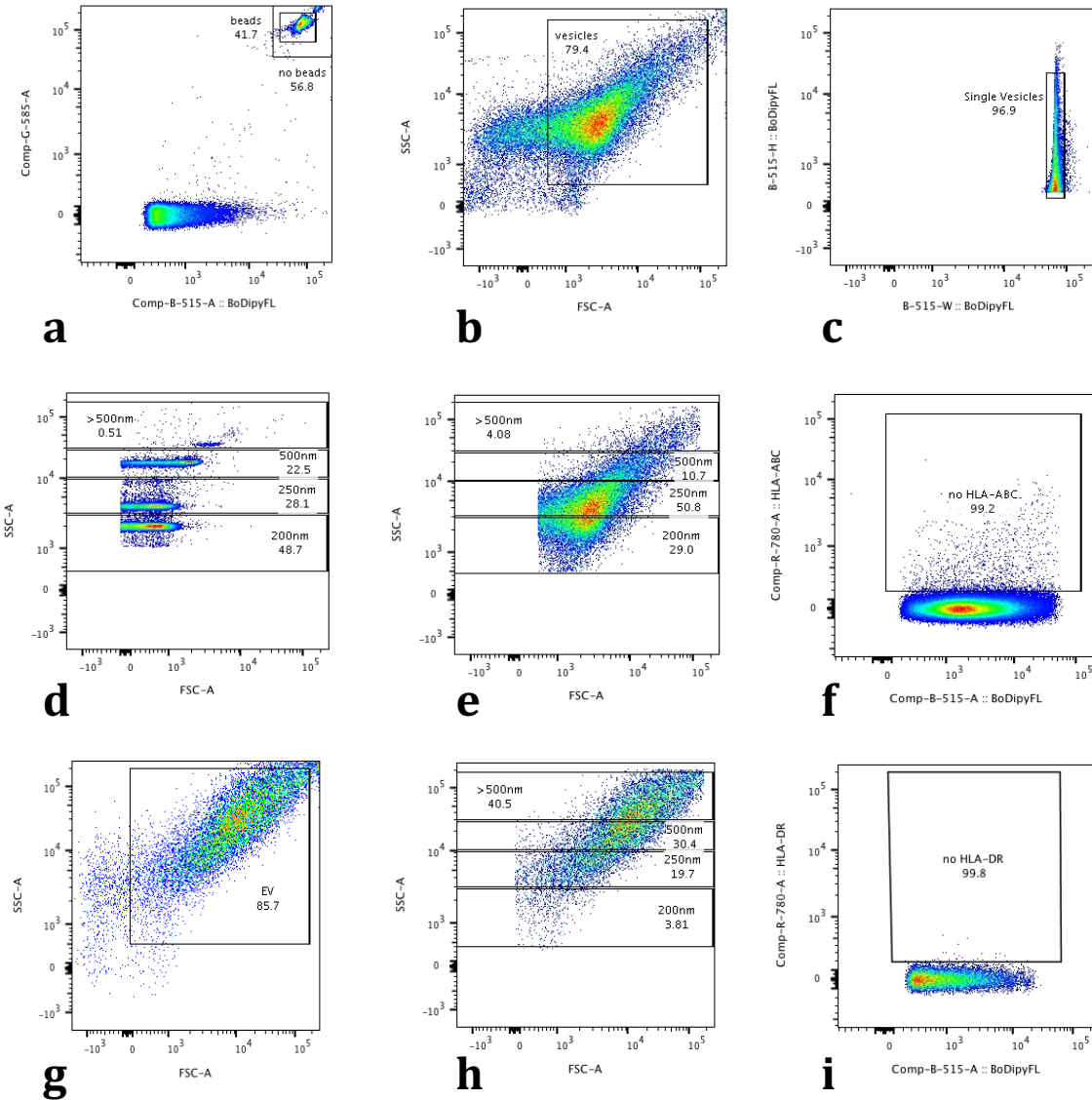


Fig. S1. Flow cytometry analysis of EVs in culture supernatants of placental villous and amnion tissues

Bodipy-FL labeled EVs are captured from culture supernatants using anti-PLAP MNPs for villous tissue and anti-CD90 MNPs for amnion tissue and acquired on a flow cytometer set to threshold on fluorescence of EVs. **(a)** gates are established to enumerate count beads, and exclude the beads from the rest of the analysis, **(b)** general EV gate in FSC vs SSC plot for placental villous EVs, **(c)** single particles are selected in a Bodipy-FL width versus height plot, **(d)** size ranges are defined by Megamix SSC reference beads and **(e)** applied to placental villous EVs. **(f)** EVs carrying HLA-ABC are excluded from analysis **(g)** EV gate established for amnion EVs, **(h)** size gates applied to amnion EVs, **(i)** EVs carrying HLA-DR are excluded from analysis. One representative experiment of 10.

Table S3. Quantification of EVs by Flow Cytometry (EVs/ml)				
Placental Villous Explants - Total EVs by size				
	Day 1	Day 4	Day 7	Day 14
Total EV	1.9 ± 0.3 × 10 ⁶	1.6 ± 0.2 × 10 ⁶	3.2 ± 0.6 × 10 ⁵	9.4 ± 1.7 × 10 ⁴
≤ 200nm	1.1 ± 0.2 × 10 ⁶	7.1 ± 1.0 × 10 ⁵	1.4 ± 0.3 × 10 ⁵	2.6 ± 0.6 × 10 ⁴
~ 250nm	5.3 ± 0.9 × 10 ⁵	4.7 ± 0.7 × 10 ⁵	1.0 ± 0.2 × 10 ⁵	3.3 ± 0.6 × 10 ⁴
~ 500nm	1.5 ± 0.3 × 10 ⁵	1.6 ± 0.3 × 10 ⁵	3.8 ± 0.5 × 10 ⁴	1.6 ± 0.2 × 10 ⁴
> 500nm	4.1 ± 0.8 × 10 ⁴	6.6 ± 0.9 × 10 ⁴	2.1 ± 0.5 × 10 ⁴	1.4 ± 0.4 × 10 ⁴
Placental Villous Explants - Total EVs for each marker				
CD51	7.3 ± 1.7 × 10 ⁵	5.5 ± 1.2 × 10 ⁵	9.4 ± 2.2 × 10 ⁴	8.8 ± 2.6 × 10 ⁴
CD63	4.9 ± 0.9 × 10 ⁵	4.4 ± 0.9 × 10 ⁵	6.1 ± 1.5 × 10 ⁴	7.3 ± 1.7 × 10 ⁴
CD105	4.5 ± 0.9 × 10 ⁵	4.8 ± 1.3 × 10 ⁵	7.5 ± 1.2 × 10 ⁴	1.9 ± 0.3 × 10 ⁵
CD200	1.3 ± 0.2 × 10 ⁶	1.0 ± 0.1 × 10 ⁶	1.7 ± 0.3 × 10 ⁵	1.9 ± 0.5 × 10 ⁵
CD274	5.2 ± 1.0 × 10 ⁵	5.0 ± 1.0 × 10 ⁵	7.2 ± 1.3 × 10 ⁴	8.5 ± 1.5 × 10 ⁴
Syncytin 1	2.8 ± 0.3 × 10 ⁴	3.0 ± 0.4 × 10 ⁴	1.2 ± 0.1 × 10 ⁴	8.6 ± 1.5 × 10 ⁴
Amnion Explants - Total EVs by size				
	Day 1	Day 4	Day 7	Day 14
Total EV	9.5 ± 1.3 × 10 ⁴	8.8 ± 1.1 × 10 ⁴	9.1 ± 1.4 × 10 ⁴	10.2 ± 1.6 × 10 ⁴
≤ 200nm	4.8 ± 1.0 × 10 ³	4.6 ± 0.8 × 10 ³	4.6 ± 0.9 × 10 ³	5.0 ± 1.3 × 10 ³
~ 250nm	2.7 ± 0.4 × 10 ⁴	2.6 ± 0.4 × 10 ⁴	2.6 ± 0.5 × 10 ⁴	2.6 ± 0.5 × 10 ⁴
~ 500nm	3.0 ± 0.4 × 10 ⁴	2.7 ± 0.3 × 10 ⁴	2.8 ± 0.4 × 10 ⁴	3.3 ± 0.5 × 10 ⁴
> 500nm	2.8 ± 0.3 × 10 ⁴	2.5 ± 0.3 × 10 ⁴	2.7 ± 0.4 × 10 ⁴	3.3 ± 0.5 × 10 ⁴
Amnion Explants - Total EVs for each marker				
CD29	9.1 ± 1.7 × 10 ³	8.5 ± 1.8 × 10 ³	8.2 ± 1.8 × 10 ³	10.9 ± 3.0 × 10 ³
CD44	1.7 ± 0.4 × 10 ⁴	1.7 ± 0.4 × 10 ⁴	1.8 ± 0.5 × 10 ⁴	1.7 ± 0.4 × 10 ⁴
CD105	1.8 ± 0.3 × 10 ⁴	1.7 ± 0.2 × 10 ⁴	1.6 ± 0.3 × 10 ⁴	1.6 ± 0.2 × 10 ⁴
CD140b	3.3 ± 0.5 × 10 ³	3.2 ± 0.3 × 10 ³	4.6 ± 0.5 × 10 ³	5.6 ± 1.2 × 10 ³
CD324	1.0 ± 0.2 × 10 ⁴	1.2 ± 0.3 × 10 ⁴	1.2 ± 0.3 × 10 ⁴	8.1 ± 2.9 × 10 ³
CD326	1.3 ± 0.3 × 10 ⁴	1.2 ± 0.2 × 10 ⁴	1.3 ± 0.3 × 10 ⁴	1.5 ± 0.4 × 10 ⁴

Table S3. EVs from placental villous and amnion cells are produced throughout the culture period

EVs were analyzed on a flow cytometer according to size and expression of various phenotypic markers and were quantified using 123 eCount beads. Counts are expressed as average EVs/ml ± SEM, n=10.

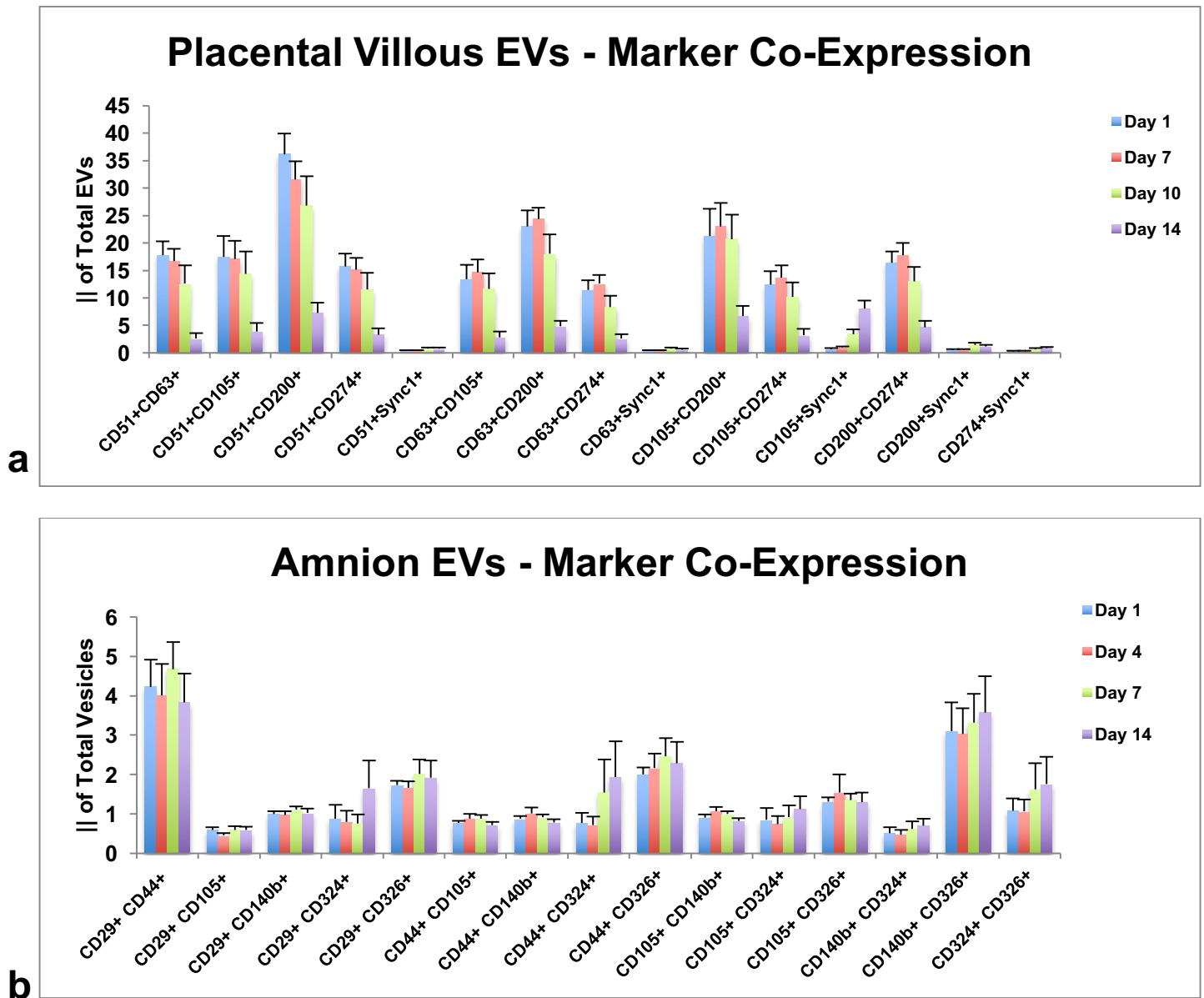


Figure S2. Co-expression of different surface markers on EVs from placental villous and amnion tissues

(a) Placental villous explants release EVs that carry more than one marker (average || of total EVs \pm SEM, n=10), further confirming their cellular source. (b) amnion explants release EVs that carry more than one marker (average || of total EVs \pm SEM, n=10), further confirming their cellular source.

Table S4. Placental Villous Explants - EV-Associated Cytokines (Mean, pg/ml)

	EV Surface				EV Internal			
	Exoquick (total)	PLAP Capture	CD31 capture	HLA-G capture	Exoquick (total)	PLAP Capture	CD31 capture	HLA-G capture
IL-1 α	0.0	0.2	0.0	4.1* [†]	1.2	2.2 [‡]	0.8	3.3 [†]
IL-1 β	2.5	1.0	0.7	1.0	0.9	1.3 [§]	0.8	0.0
IL-2	0.0	0.0	0.0	0.0	1.1	1.7	0.5	3.8* [†]
IL-4	320.5	0.0	0.0	72.0	773.5	546.1 ^{‡§}	182.2	0.0
IL-6	50.2	43.0	19.7	20.4	0.3	1.9	2.1	0.6
IL-8	844.9	723.8	327.2	773.6 [†]	75.6	75.9	96.5	96.4
IL-10	0.0	0.0	0.0	0.0	1409.1	469.1 [§]	586.5	0.0
IL-13	127.0	0.0	0.0	824.6	368.1	196.8	0.0	0.0
IL-15	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
IL-16	0.0	0.0	0.0	0.0	92.7	119.0 ^{‡§}	63.1	49.1
IL-18	2.6	0.5	0.1	2.2	0.7	2.2 [§]	0.9	0.0
IL-33	313.4	319.6	322.2	0.0	1602.9	1388.6	1191.1	1729.0
Calg A	65.9	23.8 [‡]	3.2	65.3 [†]	10.1	16.6 [§]	5.5	0.0
Calg C	9498.5	0.0	0.0	270.0	22006.5	5195.7	1376.1	12293.7 [†]
CRP	3684.0	54.1	24.4	22.7	0.0	17.4	4.1	6.8
CXCL6	0.0	0.0	0.0	0.0	183.9	145.1 [§]	185.7	25.6
CXCL13	0.0	0.0	0.0	18.5	207.1	242.4 [§]	188.7	139.7
Eotaxin	116.3	0.0	0.0	222.2	0.0	0.0	0.0	0.0
GM-CSF	0.0	11.7 [‡]	0.0	38.4* [†]	56.1	79.7	52.4	79.4
Gro- α	51.5	6.5	1.7	9.0	98.2	58.6	19.8	54.0
HMGB-1	0.0	0.0	0.0	0.0	336.9	0.0	0.0	207.9
IFN- γ	0.0	0.0	0.0	42.2	929.7	631.0	195.7	850.9
IP-10	944.9	428.9	284.8	660.9* [†]	1439.3	1331.7	1201.6	1794.6* [†]
I-TAC	0.0	0.0	0.0	0.0	88.8	0.0	41.5	0.0
M-CSF	3.3	1.4	0.0	7.2	38.5	30.2 [‡]	17.1	23.2
MCP-1	175.5	27.4	8.2	9.7	104.8	26.0	26.3	30.0
MIF	22378.6	4799.8	1947.3	18282.7* [†]	10885.0	8701.0	5609.5	13925.5* [†]
MIG	324.2	32.0	0.0	393.2	1185.6	1798.4 ^{‡§}	296.6	0.0
MIP-1 α	0.4	0.0	0.0	0.0	3.5	2.9	0.8	3.9 [†]
MIP-1 β	6.7	0.1	0.0	0.7	8.8	6.3	2.0	7.6 [†]
MIP-3 α	2291.1	0.0	0.0	298.2	9544.7	10454.2 [§]	8478.8	2841.6
RANTES	21.9	0.0	0.0	0.0	46.8	2.9	0.0	44.2
TGF- β	3.8	0.0	0.0	2.2	48.2	115.8 ^{‡§}	18.8	13.9
TNF- α	0.0	0.0	0.0	0.6	6.7	6.1 [§]	2.9	0.5
TRAIL	157.7	0.0	0.0	302.2	2451.8	1752.4 [‡]	29.1	1759.9 [†]

EVs were captured from aliquots of placental villous culture supernatants at day 4 using MNPs with different capture antibodies, or isolated using Exoquick. EV surface bound and encapsulated cytokines were quantified by multiplexed bead assays, n=5. p<0.05 *HLA-G higher than PLAP, [†]HLA-G higher than CD31, [‡]PLAP higher than CD31, [§]PLAP higher than HLA-G, ^{||}CD31 higher than HLA-G

Table S5. Amnion Explants - EV-Associated Cytokines (Mean, pg/ml)

	EV Surface			EV Internal		
	Exoquick (total)	CD90 Capture	HLA-G capture	Exoquick (total)	CD90 Capture	HLA-G capture
IL-1 α	0.0	0.0	5.7 [†]	0.9	2.6	7.2 [†]
IL-1 β	0.0	0.0	0.0	0.0	0.0	0.0
IL-2	0.0	0.0	0.0	1.8	2.6	7.3 [†]
IL-4	31.0	87.2*	0.0	750.8	760.5*	0.0
IL-6	2.7	1.2	1.1	1.8	1.3	0.4
IL-8	358.2	163.1	545.5	255.4	146.2	321.1
IL-10	70.4	1337.4*	0.0	4039.2	3759.4*	260.0
IL-13	0.0	924.5	578.4	1129.9	903.5*	0.0
IL-15	0.0	0.0	0.0	0.0	0.0	0.0
IL-16	0.0	0.0	0.0	121.4	165.1	130.8
IL-18	0.0	0.0	0.0	0.9	0.9*	0.0
IL-33	744.3	862.5*	190.3	937.2	1064.5	1071.2
Calg A	0.0	0.0	11.3	33.7	11.5	9.5
Calg C	1588.3	0.0	2463.6 [†]	13284.0	11355.5	16190.8 [†]
CRP	157.2	0.0	1.1	0.3	4.0	7.0
CXCL6	0.0	0.0	0.0	321.5	426.3*	130.9
CXCL13	0.0	0.0	0.0	312.6	227.4	150.6
Eotaxin	0.0	0.0	0.0	22.8	129.1*	0.0
GM-CSF	0.0	0.0	86.5 [†]	100.9	82.2	157.6 [†]
Gro- α	652.1	74.8	948.5	1098.9	275.1	994.3
HMGB-1	183.2	0.0	0.0	0.0	431.5	389.2
IFN- γ	0.0	0.0	0.0	788.5	911.7	897.0
IP-10	0.0	0.0	0.0	182.3	260.0	146.3
I-TAC	0.0	0.0	0.0	303.8	322.3*	0.0
M-CSF	0.0	0.0	0.0	28.9	35.9	39.9
MCP-1	5.5	0.0	7.7	20.6	13.7	29.6
MIF	2063.3	111.9	2360.5 [†]	4606.4	608.8	5561.2 [†]
MIG	59.7	174.8	247.1	496.3	567.1*	240.0
MIP-1 α	0.0	0.0	0.0	7.0	6.0	8.0
MIP-1 β	5.7	0.0	5.0	8.9	10.1	16.0 [†]
MIP-3 α	0.0	0.0	0.0	1326.6	1365.7*	133.7
RANTES	0.0	0.0	0.0	0.0	3.0	19.3
TGF- β	0.0	0.0	0.0	148.6	167.0*	39.9
TNF- α	0.0	0.0	0.0	4.7	8.7*	0.8
TRAIL	0.0	410.6	1371.0 [†]	3751.3	4562.2	4733.4

EVs were captured from aliquots of amnion culture supernatants at day 4 using MNPs with different capture antibodies, or isolated using Exoquick. EV surface bound and encapsulated cytokines were quantified by multiplexed bead assays, n=5. p<0.05 *CD90 higher than HLA-G, [†]HLA-G higher than CD90

Table S6. Placental Villous Explants - EV-Associated Growth/Angiogenic Factors (Mean, pg/ml)								
	EV Surface				EV Internal			
	Exoquick (total)	PLAP Capture	CD31 capture	HLA-G capture	Exoquick (total)	PLAP Capture	CD31 capture	HLA-G capture
Activin A	1162.7	1306.1 [†]	1203.5	1339.3 [†]	858.6	249.5	249.9	180.9
ADAM-12	409.1	33.9 [§]	14.9	12.4	296.5	189.7 ^{‡§}	39.0	31.1
Adiponectin	6060.4	713.8	473.8	1145.3 ^{*†}	4990.4	565.8	445.6	1627.6 ^{*†}
Angiogenin	45.4	0.0	0.0	0.0	151.8	0.0	0.0	2.7
CD40L	49.9	73.4 [‡]	36.2	204.6 ^{*†}	279.0	145.1	89.0	155.4 [†]
EGF	5.6	10.3 [‡]	4.2	32.6 ^{*†}	54.6	35.3 [‡]	20.3	34.7 [†]
Endoglin	16.0	15.3	3.6	7.8 [†]	985.4	1060.6 ^{‡§}	103.0	286.8
FasL	0.0	14.2	0.0	145.0 ^{*†}	478.3	197.0 [‡]	105.2	380.4 ^{*†}
Fibronectin	5649.9	1648.1 [‡]	919.6	3795.2 ^{*†}	5501.1	2969.8	2006.6	4863.4 ^{*†}
Galectin-1	8131.0	5769.7	3917.4	10339.0 ^{*†}	26236.4	10057.1 [‡]	6155.6	11370.9
hCG	1371.4	0.0	0.0	0.0	1389.5	536.8	250.5	0.0
ICAM-1	1559.5	1199.8 [‡]	820.3	1478.4 [†]	1378.1	1048.3 [‡]	601.0	1148.6 [†]
IGFBP1	127.1	115.1 [‡]	50.7	153.6 [†]	204.2	116.3 [‡]	81.3	153.2 [†]
IL-1Ra	10794.0	10041.4 [‡]	4808.0	15293.6 ^{*†}	30299.5	13796.1	8903.5	15982.7 [†]
IL-27	642.3	164.9 [§]	126.8	0.0	1227.5	612.6 [§]	455.7	16.4
Leptin	35.2	0.0	0.0	0.0	22.1	16.4 [§]	3.6	0.0
MMP-7	13.9	120.4	24.0	109.8 [†]	381.2	289.6 [‡]	167.5	246.5
MMP-9	2788.8	80.9 [§]	52.9	41.8	1108.5	125.6 [§]	108.0	73.6
PAPP-A	6213.9	3173.9 [‡]	1472.2	1569.4	3920.8	2947.9	1607.2	1995.2
PGE2	25909.2	0.0	0.0	27098.1 ^{*†}	8806.0	6024.3	0.0	9552.3 ^{*†}
PIGF	73.9	27.7 ^{‡§}	16.9	10.4	7.0	0.0	0.1	5.5 ^{*†}
Resistin	49.5	14.3 [‡]	7.6	41.2 ^{*†}	51.5	31.6	23.1	77.9 ^{*†}
Serpin E	18229.8	19039.5 [‡]	11246.5	27468.9 [†]	52263.0	39389.6	32198.0	39096.7
TFPI	10535.0	14021.9 [‡]	5002.5	38769.6 ^{*†}	57791.7	27633.4	22265.2	56184.4 ^{*†}
TGFβ3	51.3	37.4	29.8	273.5 ^{*†}	638.9	316.3 [‡]	226.1	281.9
Tie-2	152.3	20.8	7.9	97.5 ^{*†}	340.0	55.7	51.8	109.9 ^{*†}
TIMP-1	7616.8	2340.8	1280.2	2117.8	1719.6	190.1	294.6 [§]	333.4
Tissue Factor	20.8	33.4 [‡]	12.6	63.2 ^{*†}	121.2	24.1	25.2	62.7 ^{*†}
TLR2	8.0	0.0	0.0	22.9 ^{*†}	383.1	160.1 ^{‡§}	56.1	34.1
TREM-1	61.8	92.0 [‡]	24.4	1129.0 ^{*†}	4599.9	5547.9 [§]	5822.6	1585.0
uPA	597.4	104.1	26.6	755.4 ^{*†}	2068.3	601.9 [‡]	442.3	1094.9 ^{*†}
uPAR	2203.5	1452.1 [‡]	885.9	4500.4 ^{*†}	9760.4	2629.9	2383.5	3802.0
VEGF	0.0	0.0	0.0	0.0	400.1	32.0	0.0	35.8
VEGFR1	22453.9	3813.5 [‡]	1973.0	6815.3 ^{*†}	13533.6	4368.6 [‡]	2677.6	5591.5
VEGFR2	0.0	10.8	0.0	305.7 ^{*†}	865.3	445.9 [‡]	222.6	488.4

EVs were captured from aliquots of placental villous culture supernatants at day 4 using MNPs with different capture antibodies, or isolated using Exoquick. EV surface bound and encapsulated growth and angiogenic factors were quantified by multiplexed bead assays, n=5. p<0.05 *HLA-G higher than PLAP, [†]HLA-G higher than CD31, [‡]PLAP higher than CD31, [§]PLAP higher than HLA-G, ^{||}CD31 higher than HLA-G, [§] CD31 higher than PLAP

Table S7. Amnion Explants - EV-Associated Growth/Angiogenic Factors (Mean, pg/ml)

	EV Surface			EV Internal		
	Exoquick (total)	CD90 Capture	HLA-G capture	Exoquick (total)	CD90 Capture	HLA-G capture
Activin A	1040.2	1076.5	1212.5 [†]	713.1	315.4*	159.3
ADAM-12	0.0	0.0	0.0	24.8	0.9	0.0
Adiponectin	158.9	13.6	350.1 [†]	2180.0	614.2	1812.4 [†]
Angiogenin	0.0	0.0	0.0	0.0	0.0	0.0
CD40L	21.0	1.3	119.8 [†]	241.3	114.4	159.4 [†]
EGF	1.4	0.1	16.1 [†]	37.0	14.9	26.6 [†]
Endoglin	0.0	0.5	5.3 [†]	3.1	0.5	2.4
FasL	0.0	0.0	26.3	332.3	64.3	341.8 [†]
Fibronectin	11497.4	515.9	5423.6 [†]	20666.2	3459.1	9164.7 [†]
Galectin-1	1794.3	386.0	5547.9 [†]	33164.4	13385.3	17107.9
hCG	0.0	0.0	0.0	0.0	0.0	0.0
ICAM-1	308.8	222.6	407.6 [†]	836.7	440.1	490.6
IGFBP1	87.3	16.7	67.7 [†]	175.2	65.6	105.2 [†]
IL-1Ra	2883.2	593.5	8589.4 [†]	24941.6	7142.1	15428.6 [†]
IL-27	0.0	0.0	0.0	106.7	0.0	0.0
Leptin	0.0	0.0	0.0	35.8	0.0	0.0
MMP-7	0.0	0.0	0.0	177.1	24.0	75.7
MMP-9	146.9	0.0	10.3	351.7	80.1	60.8
PAPP-A	325.2	0.0	0.0	4183.5	1443.1*	101.7
PGE2	0.0	0.0	1623.6	0.0	684.1	1223.2 [†]
PIGF	1.1	0.0	0.0	1.9	0.0	0.0
Resistin	2.5	0.0	21.4 [†]	51.6	21.4	37.4 [†]
Serpin E	6857.3	4182.8	12739.0 [†]	55291.3	50997.4*	31252.7
TFPI	4342.0	1233.1	13584.3 [†]	83521.3	17338.4	60121.0 [†]
TGFβ3	22.7	0.0	85.2 [†]	533.2	208.3	313.4 [†]
Tie-2	25.2	0.0	33.9 [†]	250.2	45.9	112.1 [†]
TIMP-1	923.3	204.0	914.9	360.5	156.8	95.0
Tissue Factor	11.1	0.9	35.8 [†]	97.1	32.5	55.9 [†]
TLR2	0.0	0.0	0.0	89.4	0.0	20.7
TREM-1	0.8	5.5	981.5 [†]	3983.6	5174.4*	1631.3
uPA	0.0	0.0	84.5 [†]	1283.4	346.3	957.9 [†]
uPAR	251.7	0.0	3065.3 [†]	10609.7	3835.6	4662.7
VEGF	0.0	0.0	0.0	125.5	0.0	4.3
VEGFR1	813.2	191.9	2673.7 [†]	7272.7	2236.7	4825.9 [†]
VEGFR2	0.0	0.0	43.5	702.4	122.9	483.8 [†]

EVs were captured from aliquots of amnion culture supernatants at day 4 using MNPs with different capture antibodies, or isolated using Exoquick. EV surface bound and encapsulated growth and angiogenic factors were quantified by multiplexed bead assays, n=5. p<0.05 *CD90 higher than HLA-G, [†]HLA-G higher than CD90