

## Reduced cell invasion may be a characteristic of placental defects in pregnant women of advanced maternal age at single-cell level

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**Table S1 Clinical characteristics of the subjects in present study**

Cases	Age (years)	BMI	Gestational age of delivery (weeks)	Nature conceived	Singleton pregnancy	Ethnicity	Cesarean section	Newborn gender	Birthweight	For used
A1	39	26.9	37+3	Y	Y	Han	Y	Male	3120	Sing-cell seq
A2	38	21.2	38+1	Y	Y	Han	Y	Male	3270	Sing-cell seq
A3	40	21.2	37+5	Y	Y	Han	Y	Male	3330	Cell culture
A4	39	19.8	39+3	Y	Y	Han	Y	Male	3040	Cell culture
A5	38	20.6	39+2	Y	Y	Han	Y	Female	3230	Cell culture
A6~A20	37.5±3.2	23.8±2.6	38.3±1.4	Y	Y	Han	-	Male (53.3%)	3080±23.5	Validated experiments
C1	29	24.4	40+4	Y	Y	Han	Y	Male	3260	Sing-cell seq
C2	28	22.9	38+3	Y	Y	Han	Y	Female	3050	Sing-cell seq
C3	24	17.4	39+0	Y	Y	Han	Y	Female	3430	Cell culture
C4	26	21.2	40+4	Y	Y	Han	Y	Male	3420	Cell culture
C5	28	21.3	39+1	Y	Y	Han	Y	Female	3130	Cell culture
C6	25	21.0	6	Y	Y	Han	-	-	-	First trimester
C7	26	21.8	6	Y	Y	Han	-	-	-	First trimester
C8	22	20.5	6	Y	Y	Han	-	-	-	First trimester
C9~C23	26.8±4.6	21.3±2.8	38.7±1.2	Y	Y	Han	-	Male (53.3%)	3100±34.6	Validated experiments

**Table S2 Key resources of the study**

Reagent or resource	Source	Product number
<b>Single-cell RNA-sequencing</b>		
Cell Ranger software pipeline (version 3.0)	10× Genomics	
R package Seurat (version 2.3.4)		
<b>Immunofluorometric assay</b>		
HLA-G (1:100)	Bioss	bs-0752R-AF647
PARP1(1:100)	Bioss	bs-20764R-AF555
ERVFRD-1 (1:100)	Bioss	bs-15466R-AF488
KCNK12 (1:100)	Bioss	
NREP (1:100)	Bioss	bs-0427R-AF647
HOPX (1:100)	Bioss	bs-17356R-AF647
ENFB2 (1:50)	Abcam	ab241556
NRP2 (1:100)	Bioss	bs-10241R-AF350
MK167 (1:100)	Bioss	bs-23101R-AF647
SPC25 (1:100)	Bioss	bs-7716R-AF488
<b>Immunohistochemistry</b>		
Anti- PAI-1 ( <u>1:1000</u> )	Santa	sc-5297
MaxVision Kit	Manxing	KIT-5010
DAB Kit	Manxing	DAB-1031
<b>Western blot</b>		
SERPINE1 ( <u>1:1000</u> )	Abcam	ab222754
GAPDH	Abcam	ab181602
Goat anti rabbit IgG HRP	Kaiji	KGAA35
<b>Cell culture</b>		
HTR-8	Kaiji	
CK-7	Abcam	ab68459
VIM	Abcam	ab92547
<b>Transwell assay</b>		
Transwell	Corning Incorporated	3422
Matrigel	BD	356234
Crystal Violet	Sigma	C3886
<b>Transcriptome sequence</b>		

NEBNext Ultra RNA Library Prep Kit for Illumina	NEB	E7530S
NEBNext Poly(A) mRNA Magnetic Isolation Module	NEB	E7490S
NEBNext Multiplex Oligos for Illumina (Index Primers Set1)	NEB	E7335S
NEBNext Multiplex Oligos for Illumina (Index Primers Set2)	NEB	E7500S
RNA 6000 Pico chip	Agilent	5067-1524
High Sensitivity DNA Assay Kit	Agilent	5067-4626
RNA 6000 Nano chip	Agilent	5067-1511
QUBIT RNA BR ASSAY KIT	Invitrogen	Q10211
QUBIT DNA BR ASSAY KIT	Invitrogen	Q32855
QUBIT DNA HS ASSAY KIT	Invitrogen	Q32854
KAPA SYBR FAST Master Mix Universal 2X qPCR Master Mix	KAPA Biosystem	KK4602
DNA Quantification Standards and Primer Premix Kit	KAPA Biosystem	KK4808

**Table S3 Sequencing quality assessment of the study**

Index	AMA 1	AMA 2	Control 1	Control 2
Estimated number of cells	7568	7410	7829	4800
Mean reads per cell	71804	73821	67825	130068
Median genes per cell	1786	1081	1524	1054
Median UMI counts per cell	6161	3424	5202	2654
Sequencing saturation (%)	60.6	69.9	60.7	86.4
Q30 bases in RNA read (%)	90.7	90.4	90.2	88.6
Reads mapped confidently to transcriptome (%)	56.4	57.9	60.2	52.9
Fraction reads in cells (%)	89.5	91.3	91.0	87.7

Note: Cell Ranger, a software of 10x Genomics, was used to control the quality of sequencing.