

## Supplementary Materials

### Advanced maternal age differentially affects embryonic tissues with the most severe impact on the developing brain

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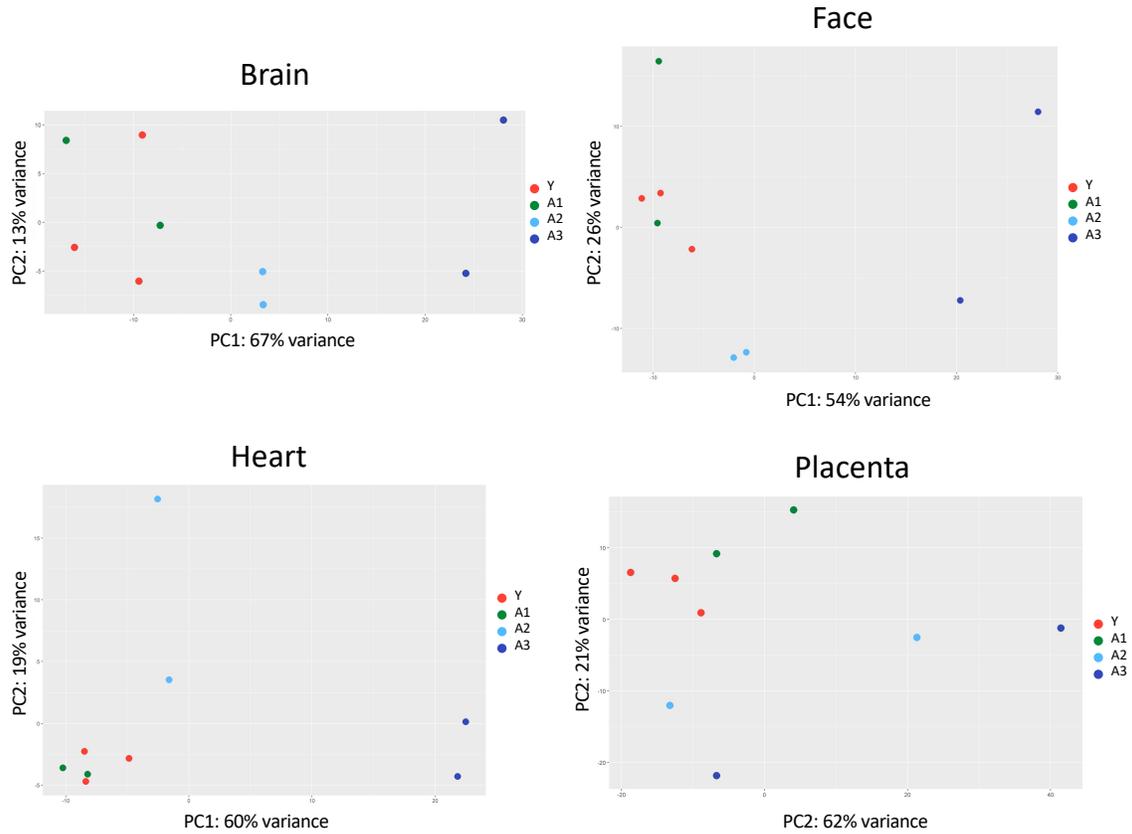
## Figure S1

A Young				B Aged			
Sample	# of reads	# of mappable reads	# mappable reads/tissue	Sample	# of reads	# of mappable reads	# mappable reads/tissue
B5-1 L001	18115368	16405809 (90.56%)	16736193	B_A1-1 L001	25085236	22426812 (89.40%)	18930423
B5-1 L002	18202445	16489012 (90.59%)		B_A1-1 L002	24665766	22034455 (89.33%)	
B6-2 L001	15498559	13939001 (89.94%)		B_A1-4 L001	24658959	21793710 (88.38%)	
B6-2 L002	15607380	14046066 (90.00%)		B_A1-4 L002	24310882	21473392 (88.33%)	
B7-5 L001	21867857	19699874 (90.09%)		B_A2-1 L001	22118628	19428881 (87.84%)	
B7-5 L002	22010132	19837401 (90.13%)	B_A2-1 L002	21659873	19013685 (87.78%)	16731799	
F5-1 L001	18269895	16462042 (90.10%)	B_A2-2 L001	18342874	15939254 (86.90%)		
F5-1 L002	18355323	16545200 (90.14%)	B_A2-2 L002	18034015	15658297 (86.83%)		
F6-2 L001	19816443	17705112 (89.35%)	B_A3-1 L001	23250012	20152905 (86.68%)		
F6-2 L002	19916812	17801554 (89.38%)	B_A3-1 L002	22927608	19861151 (86.63%)		
F7-5 L001	21843315	19663937 (90.02%)	B_A3-2 L001	18375294	14848125 (80.80%)	16545717	
F7-5 L002	21947617	19770277 (90.08%)	B_A3-2 L002	17990672	14534403 (80.79%)		
H5-1 L001	21937313	20033574 (91.32%)	F_A1-1 L001	19561818	17426617 (89.08%)		
H5-1 L002	22065962	20160279 (91.36%)	F_A1-1 L002	19096899	17001401 (89.03%)		
H6-2 L001	14553119	13009485 (89.39%)	F_A1-4 L001	21502939	19039307 (88.54%)		
H6-2 L002	14627585	13085492 (89.46%)	F_A1-4 L002	21100549	18670659 (88.48%)	18973167	
H7-5 L001	19003397	17203426 (90.53%)	F_A2-1 L001	19251902	16925613 (87.92%)		
H7-5 L002	19164522	17362964 (90.60%)	F_A2-1 L002	18931199	16636105 (87.88%)		
P5-1 L001	21667487	19390523 (89.49%)	F_A2-2 L001	19570215	17287715 (88.34%)		
P5-1 L002	21812585	19535173 (89.56%)	F_A2-2 L002	19243009	16988142 (88.28%)		
P6-2 L001	17140098	15062724 (87.88%)	F_A3-1 L001	17463910	15162001 (86.82%)	17234359	
P6-2 L002	17220401	15145847 (87.95%)	F_A3-1 L002	17109047	14841656 (86.75%)		
P7-5 L001	19003152	17072871 (89.84%)	F_A3-2 L001	17954439	15571576 (86.73%)		
P7-5 L002	19134723	17199016 (89.88%)	F_A3-2 L002	17572531	15230797 (86.67%)		
			H_A1-1 L001	20640736	17832644 (86.40%)		
			H_A1-1 L002	20312012	17537905 (86.34%)	16731799	
			H_A1-4 L001	22410551	19911031 (88.85%)		
			H_A1-4 L002	21981520	19514867 (88.78%)		
			H_A2-1 L001	17963091	15958566 (88.84%)		
			H_A2-1 L002	17571961	15596072 (88.76%)		
			H_A2-2 L001	17311214	15575568 (89.97%)	16545717	
			H_A2-2 L002	16758893	15063762 (89.89%)		
			H_A3-1 L001	17565877	15389820 (87.61%)		
			H_A3-1 L002	17173086	15037790 (87.57%)		
			H_A3-2 L001	18065976	15871622 (87.85%)		
			H_A3-2 L002	17655000	15498961 (87.79%)	18973167	
			P_A1-1 L001	21530883	18858378 (87.59%)		
			P_A1-1 L002	21057867	18429102 (87.52%)		
			P_A1-4 L001	23323075	20084292 (86.11%)		
			P_A1-4 L002	22873048	19678823 (86.03%)		
			P_A2-1 L001	16119215	14339185 (88.96%)	17234359	
			P_A2-1 L002	15796336	14038858 (88.87%)		
			P_A2-2 L001	18205932	16068119 (88.26%)		
			P_A2-2 L002	17817190	15712057 (88.18%)		
			P_A3-1 L001	18314309	14548534 (79.44%)		
			P_A3-1 L002	17888546	14193807 (79.35%)	16731799	
			P_A3-2 L001	34604859	31198764 (90.16%)		
			P_A3-2 L002	33879666	30528083 (90.11%)		

**Figure S1. RNA-seq metrics.**

(A) Number of mappable reads per tissue for all young tissues samples RNA-sequenced. B, F, H, and P stand for brain, face, face, and placenta, respectively. L001 and L002 correspond to lanes 1 and 2, respectively. The percentage of mappable reads per sample is bolded. (B) Number of mappable reads per tissue for all aged tissues samples RNA-sequenced. B, F, H, and P stand for brain, face, face, and placenta, respectively. L001 and L002 correspond to lanes 1 and 2, respectively. The percentage of mappable reads per sample is bolded.

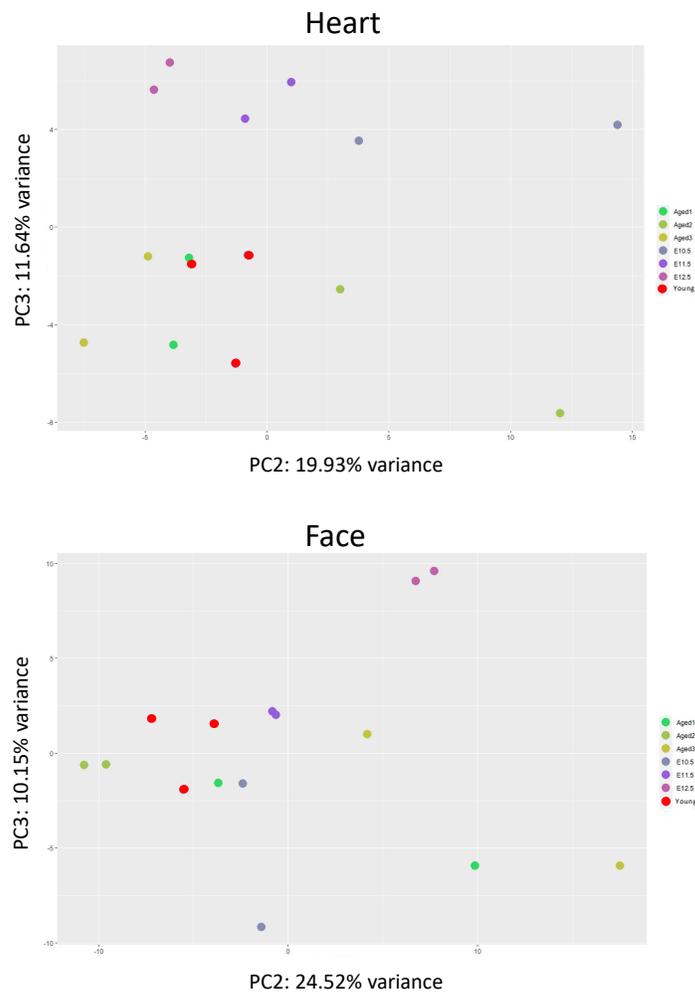
**Figure S2**



**Figure S2. Increased transcriptional variability as a functions of AMA by tissue type.**

PCA plots of RNA-seq data clustering samples by tissue type. Top left to bottom right: brain, face, heart, and placental PCA plots. Red, green, light blue, and dark blue dots correspond to tissue samples from young litters, aged litter 1, aged litter 2, aged litter 3, respectively.

**Figure S3**

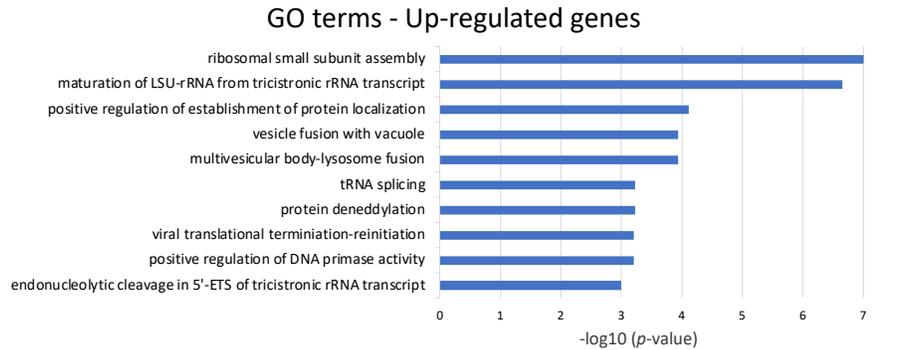


**Figure S3. Developmental time course integration of heart and brain samples.**

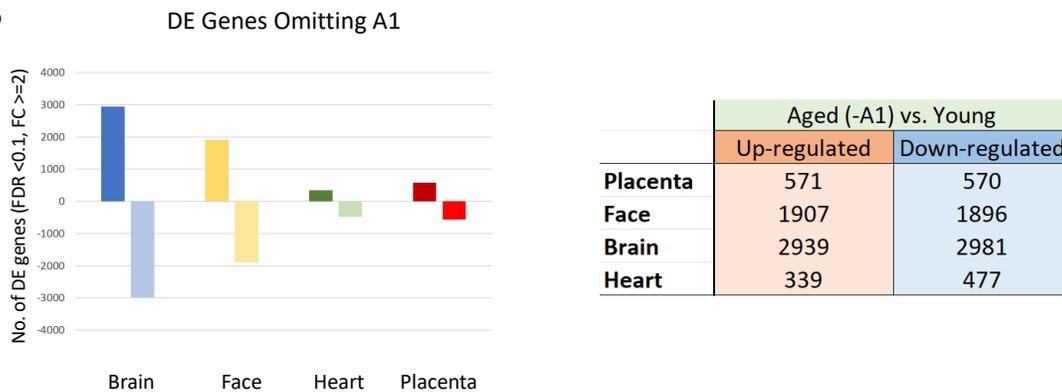
PCAs plot of embryonic heart (top) and brain (bottom) data from the young and aged cohorts, mapped onto a developmental time course of these tissues from E10.5, E11.5, and E12.5. Samples from aged litter 1, 2, and 3 are bright green, green, and yellow, respectively. Samples from the young cohort are red.

**Figure S4**

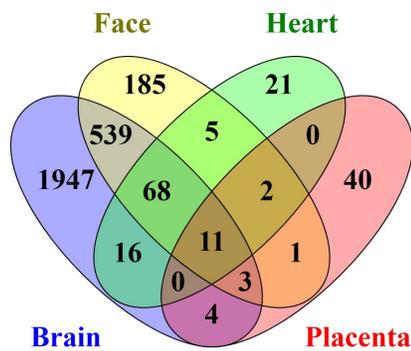
**A**



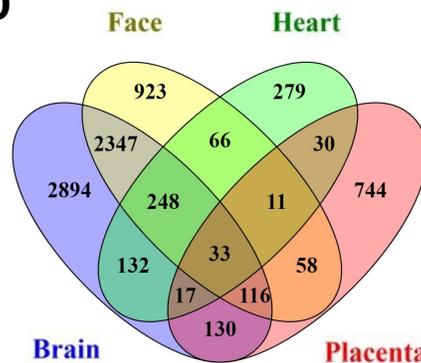
**B**



**C**



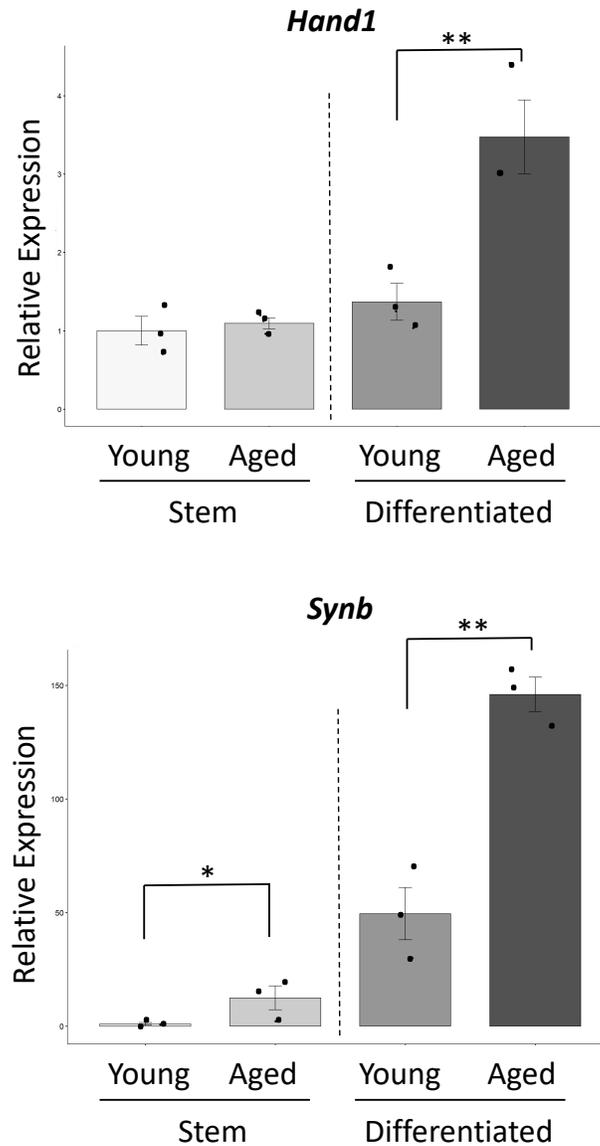
**D**



**Figure S4. Differential gene expression analysis.**

(A) Gene ontology (GO) terms associated with the top up-regulated genes in aged brain samples using Panther GO analysis. (B) Number of differentially expressed genes per tissue comparing young vs. aged samples when aged litter 1 is omitted shown in a bar graph (left) and in a table (right). Left: Bars above and below zero indicate up- and down-regulated genes, respectively. (C) Number of overlapping up- or down-regulated genes between the face, heart, brain, and placenta. (D) Number of overlapping up- or down-regulated genes between the face, heart, brain, and placenta when aged litter 1 is omitted.

Figure S5



**Figure S5. Impact on trophoblast cell types-specific gene expression as a consequence of uterine stromal cell conditioned medium from young and aged females.**

RT-qPCR data of trophoblast cell-type specific marker genes on exposure to young vs. aged decidualizing uterine stromal-cell conditioned medium. Cells were assessed in stem cell conditions and after three days of differentiation. *Hand1* is a giant cell marker, while *Synb* is a syncytiotrophoblast marker. Data are normalized to the stem cell conditions exposed to young conditioned media and plotted as mean +/- SEM. Data are representative of three independent biological replicates. \* $p < 0.05$ , \*\* $p < 0.01$ .