Supplementary information

Simulated microgravity perturbs mouse peri-implantation morphogenesis through epithelial mechanical disruption and associated metabolic adaptation

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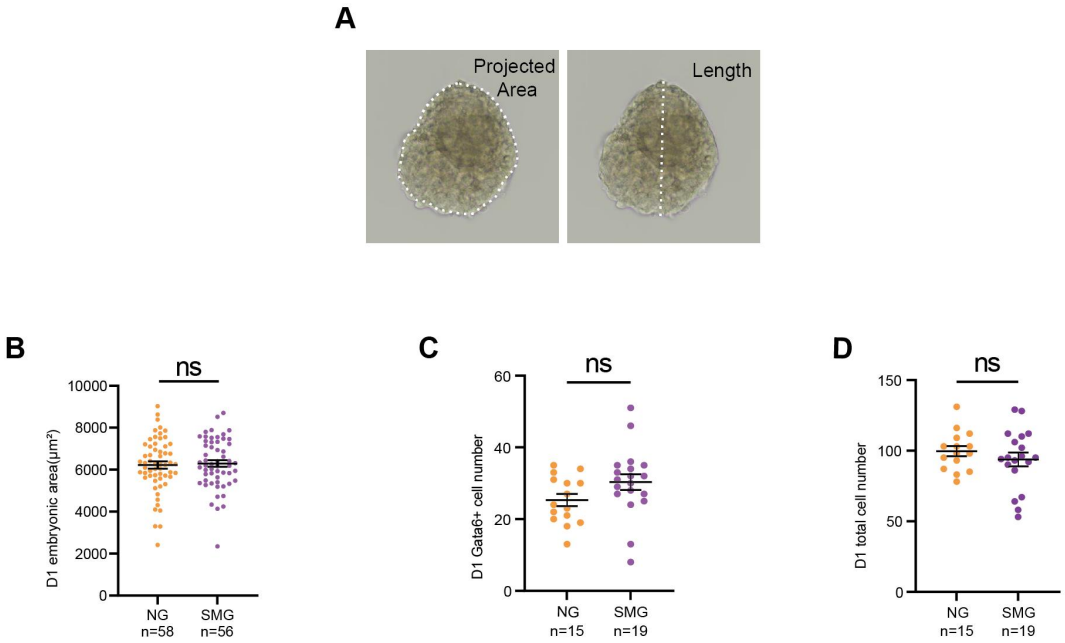
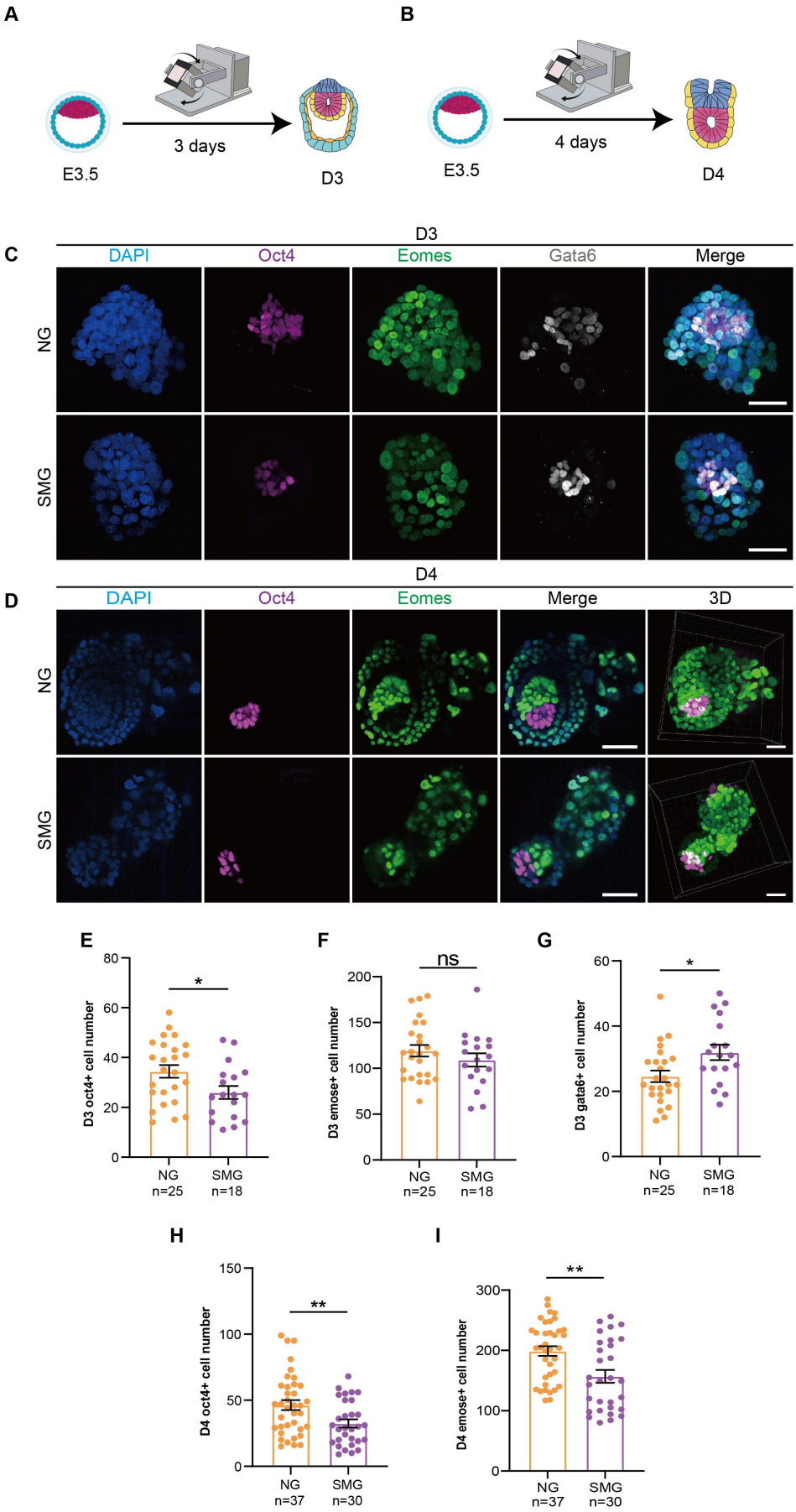


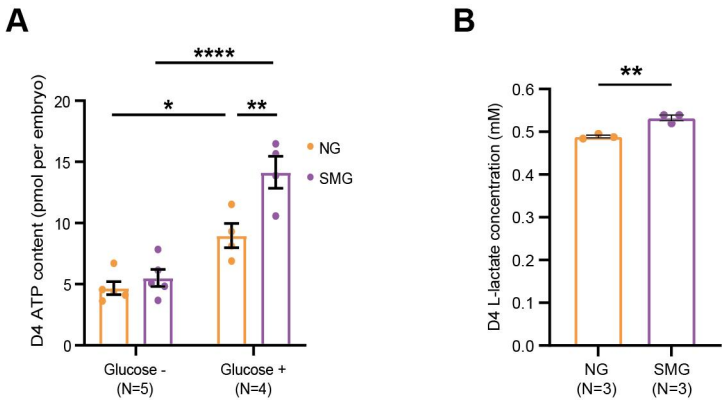
Supplementary Fig. S1: Early effects of SMG on blastocyst development.

(A) Representative images illustrating the measurement of embryo projected area and length. The dashed outline marks the embryo perimeter used to calculate projected area, and the dashed line indicates embryo length. **(B)** Projected area of D1 embryos (NG, n = 58; SMG, n = 56). **(C)** Gata6-positive cell number at D1 (NG, n = 15; SMG, n = 19). **(D)** Total cell number at D1 (NG, n = 15; SMG, n = 19). Data are presented as mean ± SEM. Groups were compared using two-sided unpaired Student's t-tests. n denotes the number of embryos. ns, not significant.


A
E3.5
3 days
D3
B
E3.5
4 days
D4
C
D3
DAPI
Oct4
Eomes
Gata6
Merge
NG
SMG
D
D4
DAPI
Oct4
Eomes
Merge
3D
NG
SMG
E
D3 oct4+ cell number
NG n=25
SMG n=18
F
D3 emose+ cell number
ns
NG n=25
SMG n=18
G
D3 gata6+ cell number
NG n=25
SMG n=18
H
D4 oct4+ cell number
NG n=37
SMG n=30
I
D4 emose+ cell number
NG n=37
SMG n=30

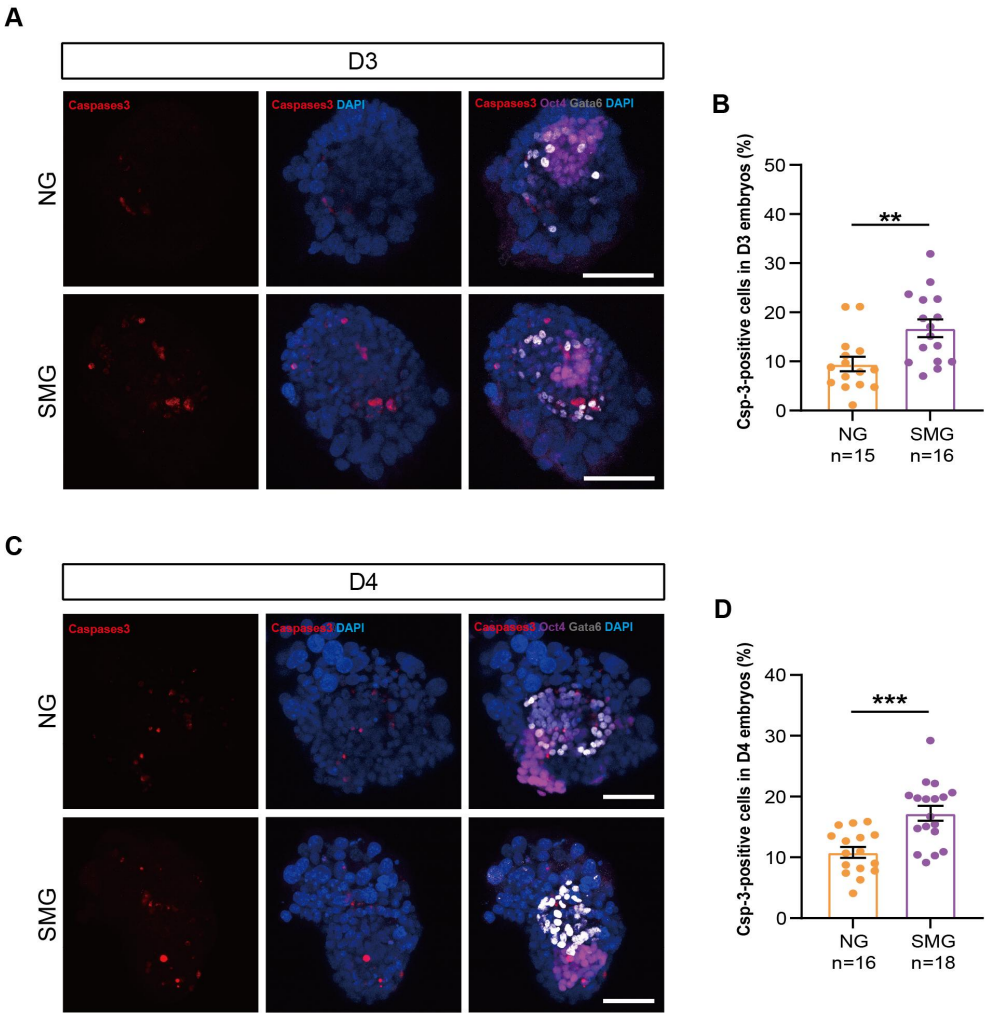
Supplementary Fig. S2: Lineage marker analysis of D3 and D4 embryos under SMG.

(A) Schematic of the experimental design for SMG treatment during D3 development. (**B**) Schematic of the experimental design for SMG treatment during D4 development. (**C**) Representative immunofluorescence images of lineage markers in D3 embryos. Oct4 (magenta), Eomes (green), and Gata6 (white). Nuclei were counterstained with DAPI (blue). Scale bar, 50 μm. (**D**) Representative immunofluorescence images and 3D reconstruction of D4 embryos stained for Oct4 (magenta) and Eomes (green). Scale bar, 50 μm. (**E**) Quantification of Oct4+ cells in D3 embryos(NG, n = 25; SMG, n = 18). (**F**) Quantification of Eomes+ cells in D3 embryos (NG, n = 25; SMG, n = 18). (**G**) Quantification of Gata6+ cells in D3 embryos (NG, n = 25; SMG, n = 18). (**H**) Quantification of Oct4+ cells in D4 embryos (NG, n = 37; SMG, n = 30). (**I**) Quantification of Eomes+ cells in D4 embryos (NG, n = 37; SMG, n = 30). Data are expressed as mean ± SEM. *$P < 0.05$; **$P < 0.01$; ns, not significant.



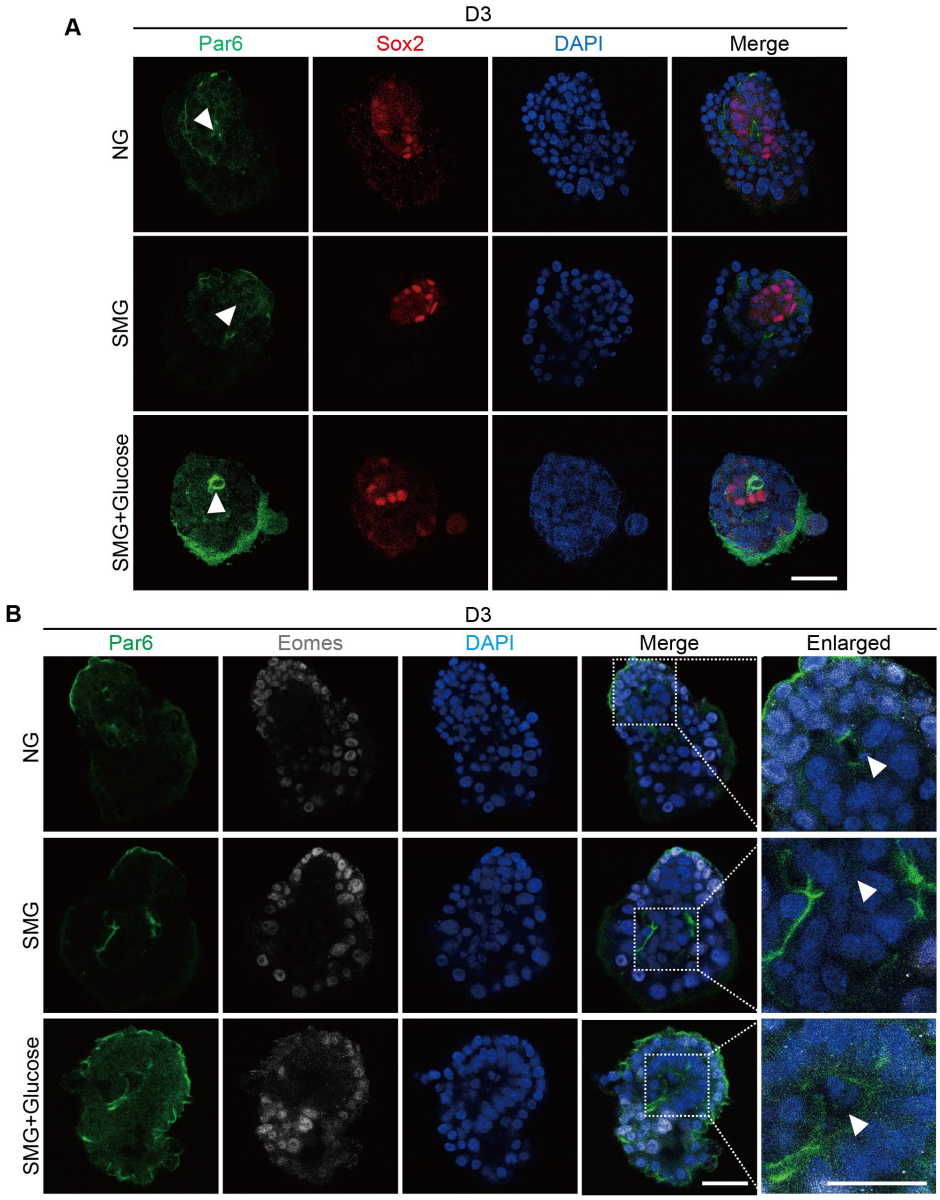
Supplementary Fig. S3: ATP content and extracellular L-lactate concentration at D4

(**A**) ATP content per embryo at D4 under basal conditions (NG, N = 5; SMG, N = 5) and glucose-supplemented conditions (NG, N = 4; SMG, N = 4). **(B)** Endpoint L-lactate concentration in D4 embryo-conditioned medium(NG, N = 3; SMG, N = 3 independent biological replicates). Data are presented as mean ± SEM. Technical replicates were averaged before statistical analysis, and N denotes the number of independent biological replicates. *P < 0.05; **P < 0.01; ****P < 0.0001; ns, not significant.



Supplementary Fig. S4: SMG increases apoptosis during peri-implantation development.

(A) Representative immunofluorescence images of D3 embryos cultured under NG or SMG and stained for cleaved Caspase-3 (red), Oct4 (magenta), and Gata6 (white). Nuclei were counterstained with DAPI (blue). Scale bar, 50 µm. **(B)** Percentage of cleaved Caspase-3-positive cells in D3 embryos (NG, n = 15; SMG, n = 16). **(C)** Representative images of D4 embryos stained as in (A). Scale bar, 50 µm. **(D)** Percentage of cleaved Caspase-3-positive cells in D4 embryos (NG, n = 16; SMG, n = 18). Data are presented as mean ± SEM. n denotes the number of embryos. **P < 0.01; ***P < 0.001.



Supplementary Fig. S5: Effects of glucose supplementation on epithelial polarity under SMG

(A) Representative immunofluorescence images of D3 embryos stained for the apical polarity protein Par6 (green) and the Epi marker Sox2 (red). Scale bar, 50 μm. (**B**) Representative immunofluorescence images of D3 embryos stained for Par6 (green) and the extraembryonic ectoderm marker Eomes (white). Boxed regions show higher-magnification views. White arrows indicate the apical localization of Par6. Scale bar, 50 μm.

Supplementary Table S1. Antibodies and fluorescent reagents used in this study

Antibodies	Ratio	Source	Identifier
SOX2	1:200	Abcam	AB_92494
OCT3/4	1:50	Santa Cruz Biotechnology	sc-5279
Eomes	1:200	Abcam	AB_23345
Gata6	1:200	R&D Systems	AF1700
Pard6B	1:200	Santa Cruz Biotechnology	sc-166405
Laminin	1:200	Sigma	L9393
F-actin	1:500	invitrogen	R37110
YAP	1:200	Cell Signaling	14074S
Integrin β1	1:200	Sigma	MAB1997
ZO-1	1:200	Abcam	AB_216880
E-cadherin	1:300	Cell Signaling	3195S
Cleaved Caspase-3	1:200	Cell Signaling	9664S
DAPI	1:500	invitrogen	R37606
Alexa Fluor 488 Donkey Anti-Goat IgG	1:500	invitrogen	AB_2534102
Alexa Fluor 488 Donkey Anti-Mouse IgG	1:500	invitrogen	AB_141607
Alexa Fluor 568 Donkey Anti-Mouse IgG	1:500	invitrogen	A10037
Alexa Fluor 568 Donkey Anti-Rabbit IgG	1:500	invitrogen	A10042
Alexa Fluor 647 Donkey Anti-Mouse IgG	1:500	invitrogen	AB_162542
Alexa Fluor 647 Donkey Anti-Goat IgG	1:500	invitrogen	AB_2535864
Alexa Fluor 647 Donkey Anti-Mouse IgG	1:500	invitrogen	A32787
Alexa Fluor 647 Donkey Anti-Rabbit IgG	1:500	invitrogen	A31573