The cytotoxic effects of dimethyl sulfoxide in mouse preimplantation embryos: a mechanistic approach

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Supplementary Table 1. List of primer sets used for RT-qPCR.

Genes	Forward	Reverse
		<Oxidants and Anti-oxidants>
Gss	AAAAGGACGACTATACTGCC	TGTAATCTGAGCGATTCAGG
Nox1	GGCTTCTTCTGTAGCGTTCG	ACCTGCTCATTTTGCAACCG
Nox2	TACCTTACTGGCTGGGATGA	TCACTTGCAATGGTCTTGAA
Nox3	CTGGGATGAAAGTCTGGATG	GGGTGATTGTAGGCAATCTG
Nox4	TCCAATCATTCCAGTGGTTT	CCCATCTGTTTGACTGAGGT
Duox1	GCTGAGAAGTTCGACCTCAG	CAGACTCCTGTTCAGCACCT
Duox2	CTCTGGAAGAGCCGCTACTG	GGTAGCCAAAGAAGACCCCC
Ncf1	GCCTTAGCCAGGACACCTAT	GATTGTCTCTGCCCTCCAG
Ncf2	TCAGATGAGGATGTGGGACT	TTCTGTGTGACATGCAGCTT
Gsr	AGCTGTGAGGGTAAATTCAG	AGCTGTGAGGGTAAATTCAG
Sod1	GAAGAGAGGCATGTTGGAGA	CACGATCTTCAATGGACACA
Sod2	GAGTTGCTGGAGGCTATCAA	CGACCTTGCTCCTTATTGAA
Gpx1	CTCACCCGCTCTTTACCTTC	AAGTTCCAGGCAATGTCGTT
Gpx2	AATGTGGCGTCACTCTGAGG	GGGAAGCCGAGAACTACCAG
Gpx3	AGGCGAGAACTCGGAGATAC	GAGCTGGAAATTAGGCACAA
		<ER Stress>
Hspa5	TCTGGTGATCAGGATACAGGTG	TTCAGCTGTCACTCGGAGAATA
Hsp90b1	AAGAATGAAGGAAAAACAGGACAAAA	CAAATGGAGAAGATTCCGCC
Atf4	TCGATGCTCTGTTTCGAATG	AAGCAGCAGAGTCAGGCTTC
Ddit	GTCTTATGAGATGAGGAATG	AATGGCAGGATACTTCTT
Edem1	CTACCTGCGAAGAGGCCG	GTTCATGAGCTGCCCACTGA
Traf2	GATGGGGTCTTCATCTGGAA	CCACTGCAACAGAGCATCAT
Total *Xbp1*	TGGCCGGGTCTGCTGAGTCCG	GTCCATGGGAAGATGTTCTGG
Spliced *Xbp1*	CTGAGTCCGAATCAGGTGCAG	GTCCATGGGAAGATGTTCTGG
Unspliced *Xbp1*	GTCCATGGGAAGATGTTCTGG	CAGCACTCAGACTATGTGCA
		<Autophagy>
Itpr1	TTGTTGATACTCGTGTAAT	AGCATCACCATAGGATAA
Atg3	GCTTGTATTGTGTCAGTT	ACAGACTAATATGGAGACT
Atg4b	CCACAAGGTAGCAAGAGA	GAAGTCAAGGGACAAGATATG
Atg5	GGAGAGAAGAGGAGCCAGGT	TGTTGCCTCCACTGAACTTG
Atg6	ACAGTTCTATAACAAGTGA	GGGTTCTTTGCTATACAT
Atg7	CAGTAGCCTGTAGAATAAC	GTAGGTGTGTCTGTAATC
Map1lc3b	TTCTTCCTCCTGGTGAATGG	GTGGGTGCCTACGTTCTCAT
		<Mitophagy>
PINK1	GCCAACACTGAACTTTGCTT	GCTGGTTGCTGCTTACAAAT
Parkin	TCCGAAGATTCCTACCTTCC	AGGGGCTGCTTCTGTAATCT
		<Apoptosis>
Atf2	ACATCCAATGGAGTCAGTTC	GCCATGACAATCTGTGAAAG

Mapk8	CGTTGAGTAGTGTAAGAA	CTTGTTCCAGATAATACCA
Jun	TTAACATTGACCAAGAACT	TTAAGGAGCACTACAGAA
Casp3	GATAATGTCTTAGAACTTGAATCC	CTTCCATAAATCAGGTCCAA
Casp9	GACTTACAAGCAGATTCC	AGAATAATGAGGCAGAGA
Trp53	CAAACACGAACCTCAAAG	CTATCCTTACCATCATCACA
Bax	CGAGCTGATCAGAACCATCA	GAAAAATGCCTTTCCCCTTC
Bcl-2	TAAGCTGTCACAGAGGGGCT	TGAAGAGTTCCTCCACCACC

<Wnt Signalling>

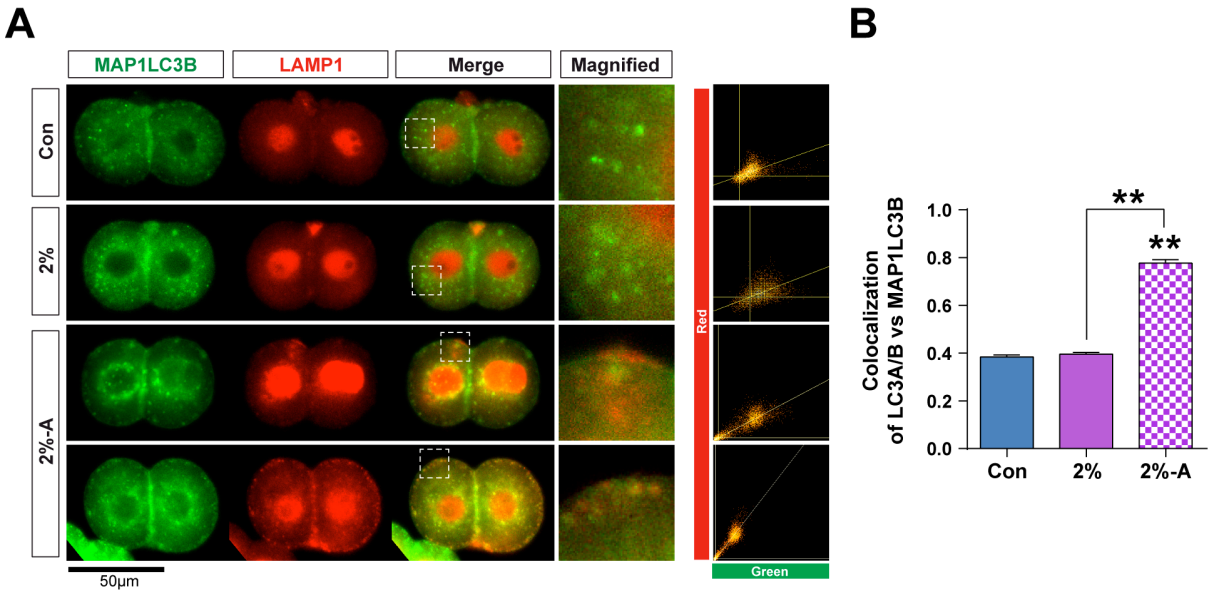
B3gnt5	ACGTGGGGCAATGAGAACTA	TTCAGTGGACCAGGAGTTCC
Eomes	GAGCTTCAACATAAACGGACTCAA	CGGCCAGAACCACTTCCA
Lrp5	GTGTGCAGTTGCAGGACAAT	CTCCAGGGGATCGTAGTCAA
Lrp6	GACAGACTGGGGAGAAGTGC	AACGTCAAGGCAAAAGGATG
Wnt1	CCGAGAAACAGCGTTCATCT	GGTTCATGAGGAAGCGTAGG
Wnt3a	GGTCTACTACGAGGCCTCAC	CATCTATGCCATGCGAGCTC

<Mitochondrial Formation, Function and Copy Number>

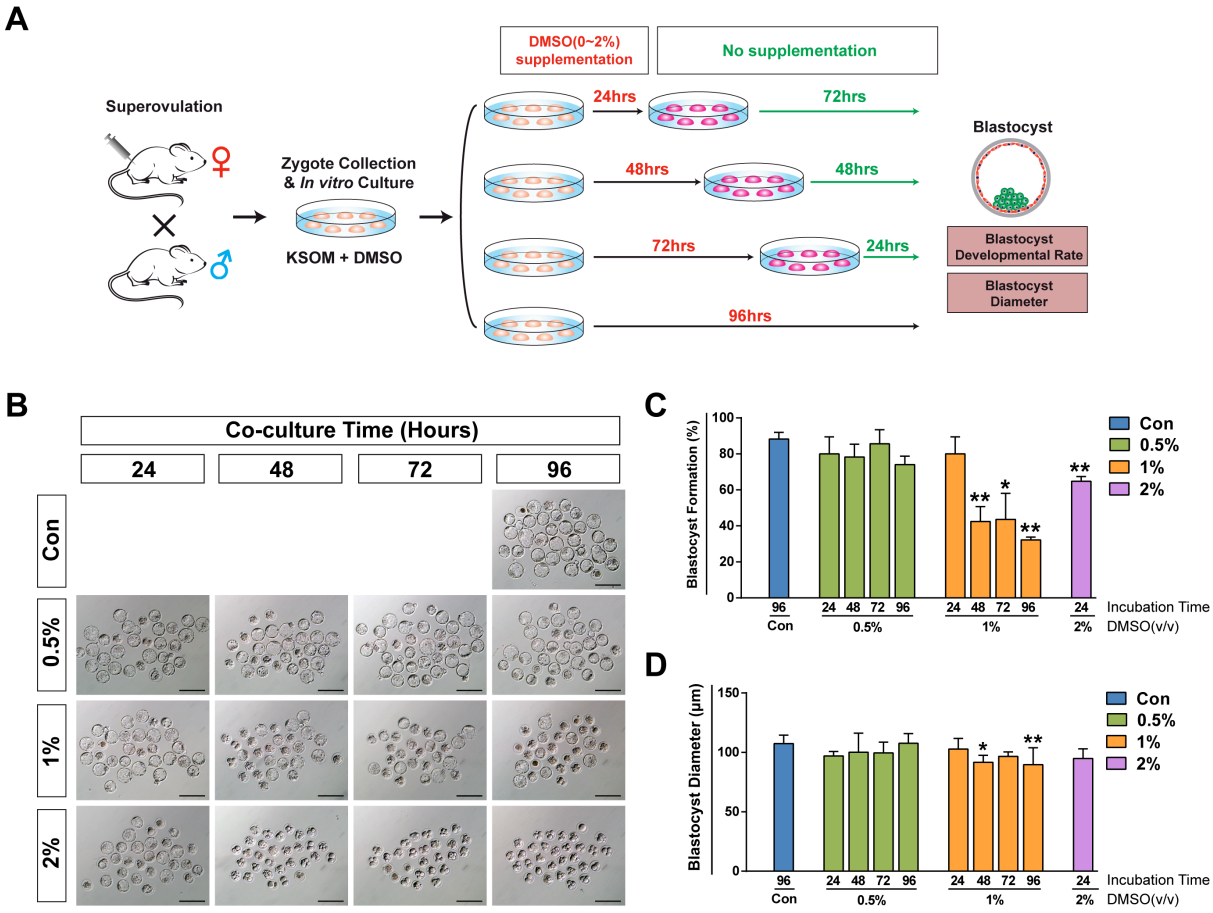
Mpv17	TCGGAGGCTGGTACAAAGTT	ATTGTCCTGGGCTGACATTC
Cbr1	AGTGGTGAATGTGTCCAGCA	CAGGACTGTCACCCCAATCT
Immp1l	GGCATCCAAAGAGGTGACAT	ACATGACCTGTTGGCACGTA
Mtfp1	GCTGTGGTGTGGTTGAGCTA	ACACAGACGGTTGATGGTGA
Rhot2	CAGTTACCCGCGAGAAGAAG	GGCTGTCAGCATTCACTTCA
Cycs	TTCCACAACCCTCATGTGAA	TAAGGGTCCAAAACCAGTGC
Atp5a1	GCCCTCGGTAATGCTATTGA	CACAGAGATTCGGGGGATAA
Atp5e	TCAGCTACATCCGGTTTTCC	GGAGGTGAGGTTGATTTCCA
Atp5d	CGGACAGATGTCCTTCACCT	ACTTAGTCGTGGTGCCGTCT
Atp5k	CGGTTCAGGTCTCTCCACTC	TGACGCCTCACTTGAGAATG
Atp5f1	TCAGAAGCGCCATTACCTCT	TTGGCAATGGTCTCCTTTTC
Cytb	ATTGACCTACCTGCCCCATC	CTCGTCCGACATGAAGGAAT
Acta2	TCGCCATGGATGACGATA	CACGATGGAGGGGAATACAG

Supplementary Table 2. Antibodies used for immunostaining and blotting.

Antibody	Cat#	Company
PDI	ab31811	Abcam
p-p53	9286s	Cell Signalling
CYCS/Cytochrome C	ADI-AAP-170	Enzo Life Sciences
Bcl-2	ab59348	Abcam
BAX	ab10813	Abcam
PINK1	ab23707	Abcam
Parkin	ab15954	Abcam
MAP1LC3B	4108s	Cell Signalling
LAMP1	ab24170	Abcam
GRP78/BiP	ab21685	Abcam
p38	ab27986	Abcam
p-p38	sc-7973	Santa Cruz
TOM20	sc-17764	Santa Cruz
ATG5/12	NB110-53818	Novus Biologicals
Beclin1	ab16998	Abcam
SQSTM1/p62	ab56416	Abcam
OCT3/4	sc-9081	Santa Cruz
CDX2	MU392A-UC	BioGenex
ACTB	ab8227	Abcam



Supplementary Figure 1. (A) MAP1LC3B and LAMP1 were co-immunostained in 2-cell embryos treated with DMSO for 24 and 36 h (2%-Arrested). **(B)** Fluorescence intensities of both of MAP1LC2B and LAMP1 were analyzed for co-localization. **: p<0.01.



Supplementary Figure 2. (A) Schematic experimental diagram for rescuing embryos from DMSO toxicity. Zygotes are cultured with different concentration (0%, 0.5%, 1%, and 2% v/v) of DMSO during 24, 48, and 72 h and then moved to fresh KSOM medium without DMSO for further culture, respectively. **(B)** A representative embryo picture cultured according to (A)'s schedule. **(C, D)** Developmental rate and diameter of blastocysts developed after exposure to 0%, 0.5%, 1%, and 2% DMSO for 24, 48, 72, and 96 h. Scale bar = 200 μm. *: $p<0.05$, **: $p<0.01$. Even though any blastocyst stage of embryos could not be obtained from 2% of DMSO supplementation, approximately 70% of zygotes, which are exposed 2% of DMSO for 24h and then move to fresh KSOM medium, can develop to fully hatched blastocyst stage of embryos.

Supplementary Movie 1. Time lapse observation of preimplantation embryo development in KSOM supplemented with 0%, 0.5%, 1%, and 2% DMSO.