

Fig. S1. Micronuclei are retained in either the TE or ICM of blastocysts. To confirm that micronuclei can be present in the TE or ICM of bovine blastocysts, we examined an additional TE marker, Annexin A2 (ANXA2), by immunofluorescence. **(A)** The specificity of the ANXA2 antibody was first tested in a highly-pure Day 28 immortalized rhesus placental (iRP) first trimester trophoblast cell line, iRP-D28A (Rosenkrantz et al. 2021), by staining the nuclei with Hoechst (blue) and immunolabeling with Cytokeratin-7 (KRT7), a pan-trophoblast marker. **(B)** Robust ANXA2 (pink) expression was observed in the iRP-D28A cells that co-localized with KRT7 expression (yellow). **(C)** Maximum intensity projection (MIP) confocal images of bovine blastocysts with clear separation of the TE and ICM and stained with Hoechst revealed multiple nuclear structures resembling micronuclei (10X). **(D)** Several of these micronuclei were contained within the ICM and negative for ANXA2 expression, **(E)** which was more apparent at higher magnification (20X).

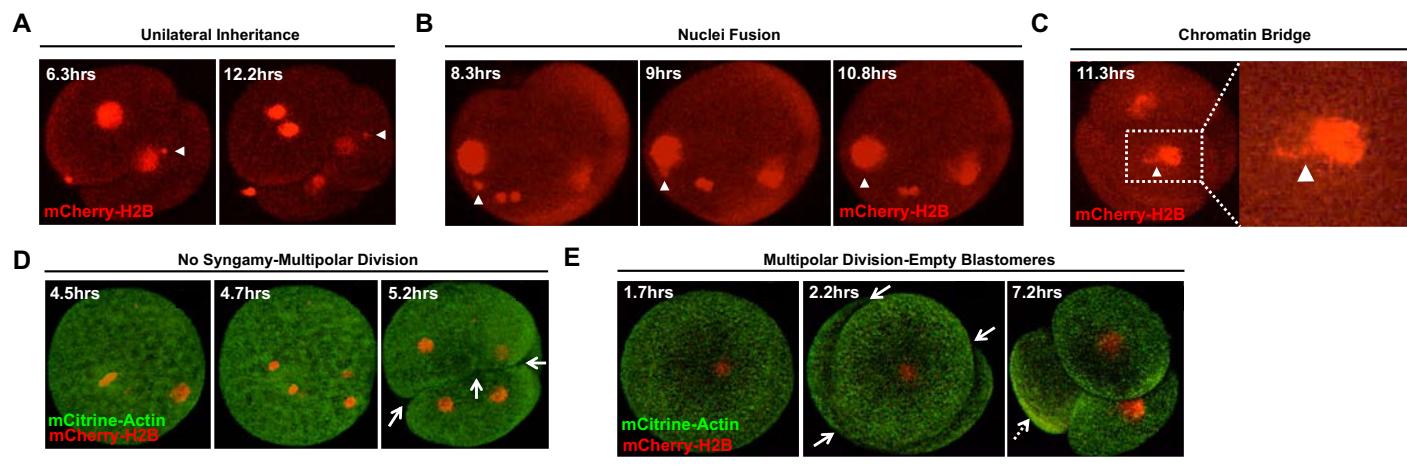


Fig. S2. Additional live-cell images representative of embryos with different phenotypes. Live-cell confocal microscopy of bovine zygotes microinjected with fluorescently labeled modified mRNAs to visualize DNA (Histone H2B-mCherry; red) and distinguish blastomeres (Actin-mCitrine; green) during the first three mitotic divisions. **(A)** Examples of other embryos with micronuclei that undergo unilateral inheritance, **(B)** fuse back with the primary nucleus, or **(C)** form a chromatin bridge (white arrowheads). **(D)** Images of additional embryos that bypassed pronuclear fusion (syngamy) prior to a multipolar division (white solid arrows) to produce blastomeres with asymmetric genome partitioning and/or **(E)** no apparent nuclear structure (white dashed arrows). Individual frames are represented in hours (hrs) from the start of imaging.

A

BUB1B targeting sequences: (BUB1B MAO #1; BUB1B MAO #2)

5'-GTTGCAGAAGGAGGCCAGG[**CGATCTGAGGCTCTGAAGAAAGGCC**]CGC...
...GGGAGGACGAGGCCCTGAGCCGGGAATGCAG[**G(ATG)GC GGCGATGCAGAAGGAAA**]GGG- 3'

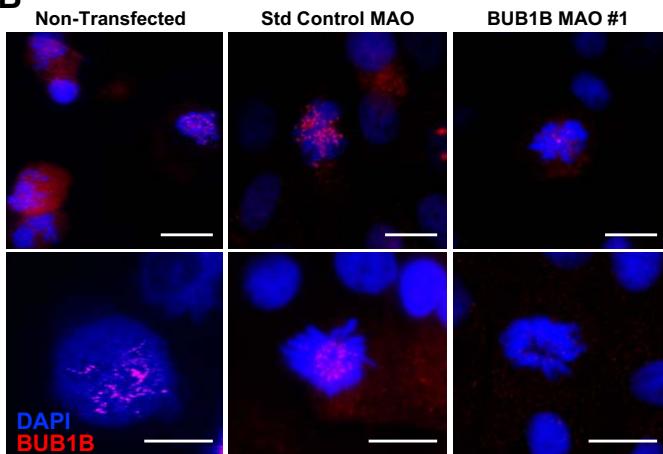
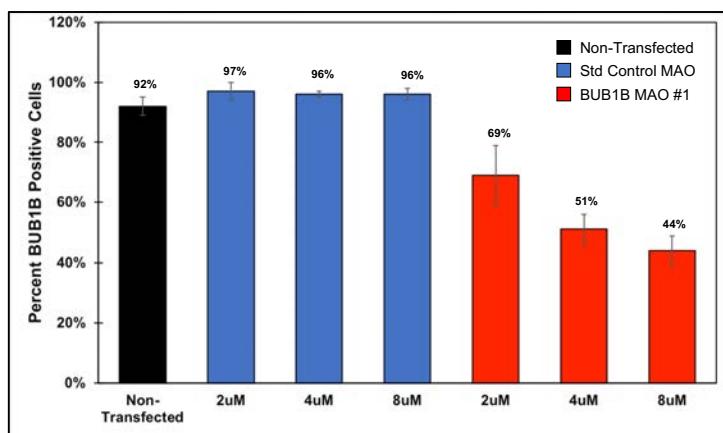
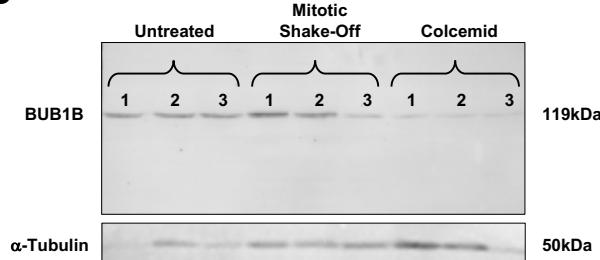
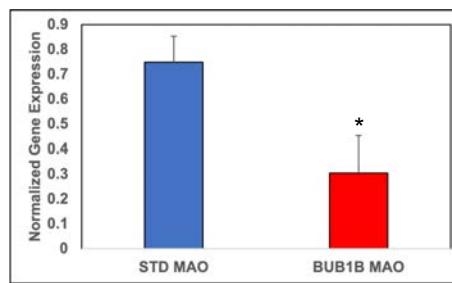
B**C****D****E**

Fig. S3. BUB1B MAO design and knockdown efficiency. (A) DNA sequences of two non-overlapping MAOs designed to target the ATG start site (shown in red, BUB1B MAO #1) and the 5' UTR (depicted in blue, BUB1B MAO #2) of BUB1B. (B) BUB1B knockdown efficiency was assessed in synchronized MDBK cells following 48 hours of treatment with 3 μ l/ml of colcemid alone (non-transfected), the Std control MAO, or BUB1B MAO #1 via immunofluorescence. BUB1B protein expression was analyzed in DAPI stained (blue) MDBK cells. Note the lack of or reduced number of BUB1B positive foci (red) in the BUB1B MAO #1 treated cells compared to the controls; Scale bars = 10 μ m (top) and = 20 μ m (bottom). (C) Bar graph showing the percentage of MDBK cells in metaphase with BUB1B expression after colcemid treatment (black) or transfection with different concentrations (2, 4, and 8 μ M) of the Std control MAO (blue) or BUB1B MAO #1 (red). While the number of cells exhibiting BUB1B positive foci was similar between the non-transfected and Std MAO controls, a dose-dependent significant decrease ($p<0.05$) in BUB1B expression was observed following BUB1B MAO #1 treatment using the Generalized Estimating Equations approach and Tukey's test for multiple comparisons. (D) Western Blot of BUB1B and α -Tubulin expression in untreated MDBK cells and following either mitotic shake-off or colcemid treatment all in triplicate to confirm reduced BUB1B expression at the protein level. (E) Quantitative RT-PCR (RT-qPCR) of normalized *BUB1B* expression in STD Control MAO versus BUB1B MAO injected bovine zygotes showing efficient BUB1B knockdown likely from negative feedback of inhibiting *BUB1B* mRNA translation. Mean CNRQ values \pm SEM were compared using the Mann-Whitney U-test; * $p=0.007$.

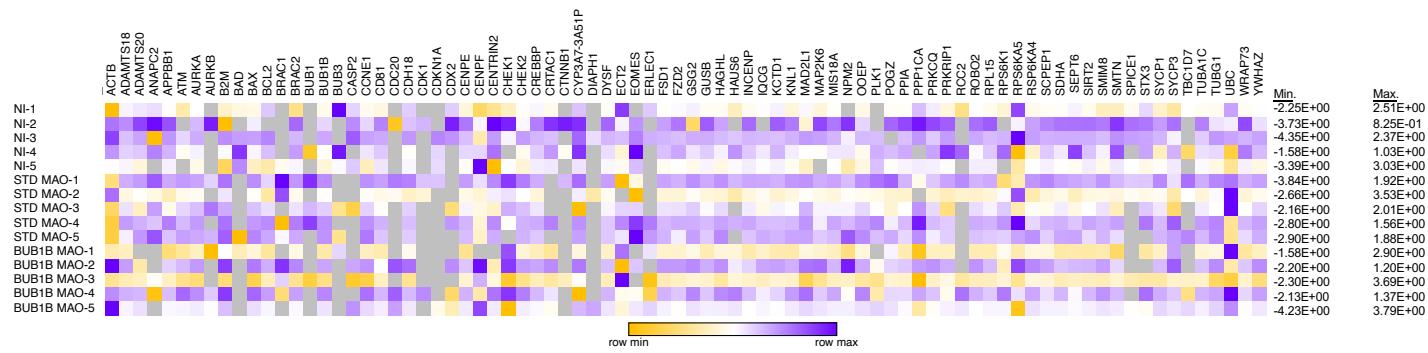


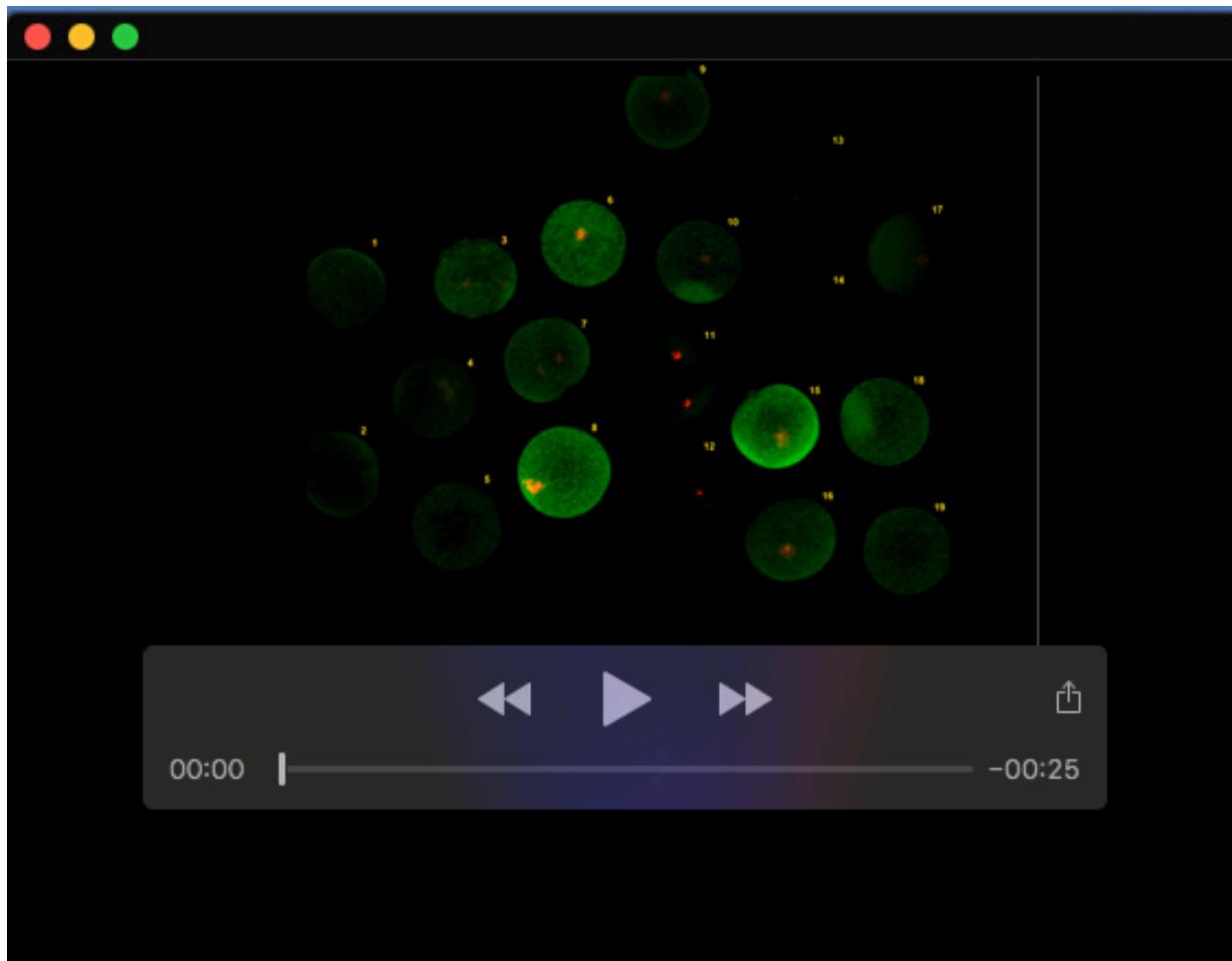
Fig. S4. Comprehensive assessment map of mitotic, cell cycle, developmentally-regulated, and cell survival genes assessed in individual BUB1B MAO #1 versus non-injected and Std Control-injected MAO bovine zygotes via single-cell microfluidic RT-qPCR. Cycle threshold (Ct) values were normalized to the most stable reference genes (*RPL15* and *GUSB*) across embryo groups and presented as the average. Gray squares indicated no expression, whereas yellow, white, and purple squares correspond to low, medium, and high expression, respectively. The range of expression levels for each gene with the minimum (Min.) and maximum (Max.) values is shown to the right of the heat map.

Table S1. Sequencing statistics of all embryonic and control samples. A table depicting the number or percentage of reads following de-multiplexing of embryonic (with embryo stage) and fibroblast samples at each step of the post-sequencing process, including adaptor removal, repeat masking, genome mapping, and quality assessment. The sequencing kit used and whether single- or paired-end is also included.

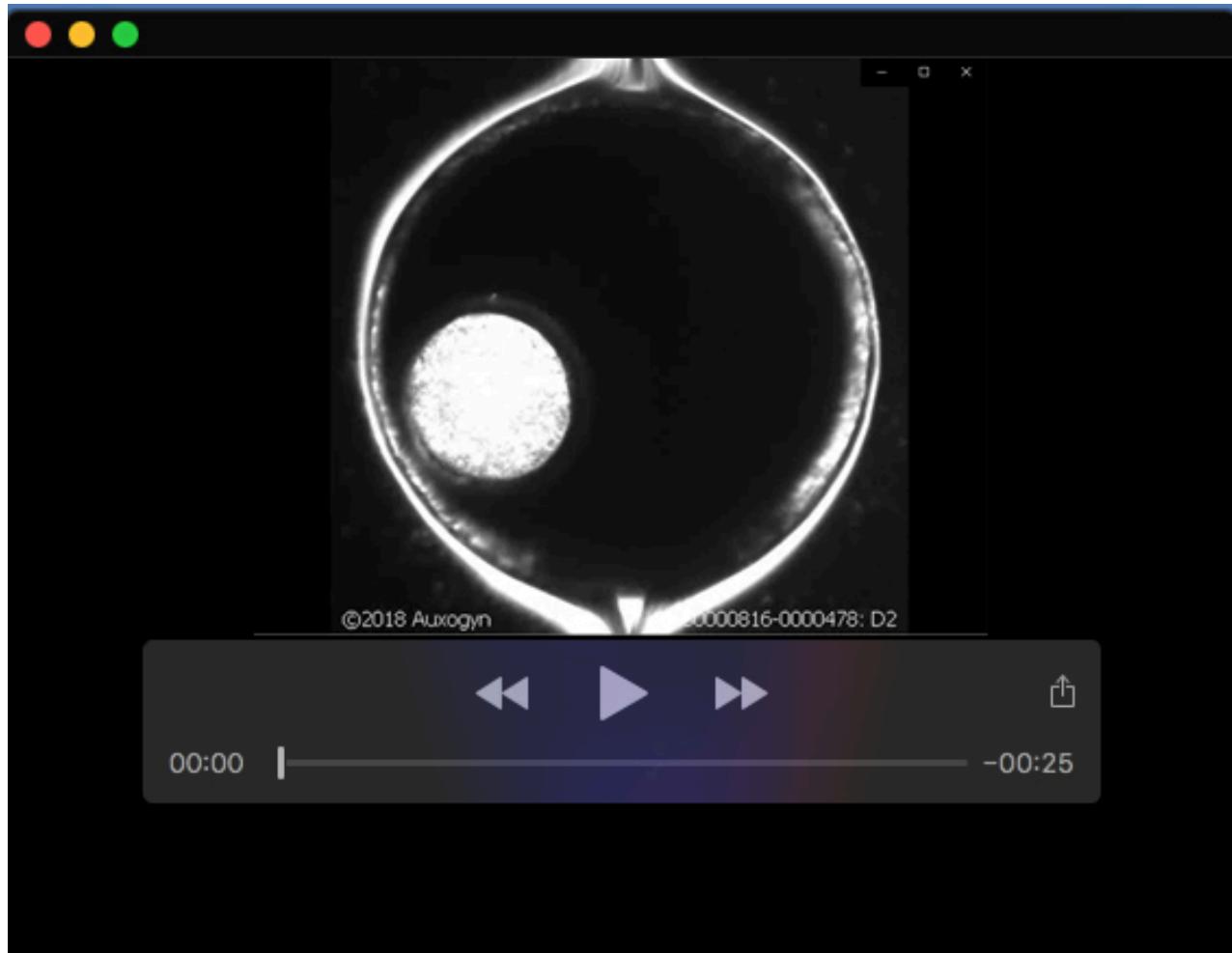
[Click here to download Table S1](#)

Table S2. List of all genes with primers analyzed by RT-qPCR in zygotes. A table of the genes analyzed by microfluidic qRT-PCR in non -injected bovine zygotes and following Std Control MAO versus BUB1B MAO #1 microinjection. Included is the sequence of the forward and reverse primer used for amplification as well as the NCBI accession number of each gene.

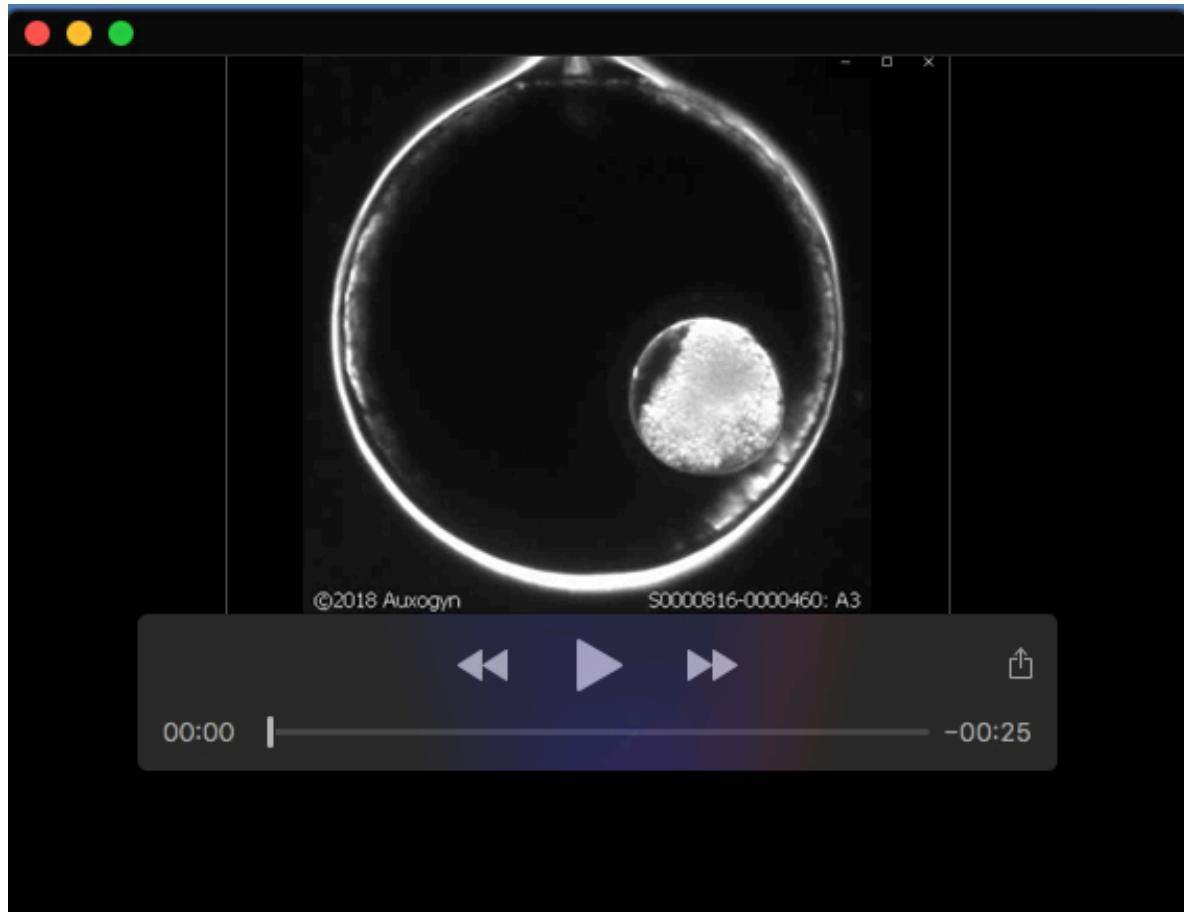
Gene Symbol	Forward primer sequence (5'->3')	Reverse primer sequence (5'->3')	NCBI Accession #
ACTB	CCTTCCTGGCATGGAATCCT	GGCTTTGGAAAGGCAAGG	NM_17397.3
ADAMTS18	GCAGCGATTAAACACGATTA	ATCGTAAATCAGGGAGCTG	NM_001192486
ADAMTS20	CAGGCAGGAAGCTTAGTGA	TCTGTGGAAATCTTCGCG	NM_001206093
ANAPC10	AACAGATCCCCCCTTCGCGAG	CCACCAATTCAGGTTCCGA	NM_001080357.2
ANAPC2	GTATTTCCAGGACCAAGCCAGC	GCGGCTCACGCCAACCTCT	XM_003584964.2
APPBB1	GATGAGACGCTGAAGCTGGT	ACGTAGGCAAAGTCCCTTC	NM_001075186
ATM	GGCCAATGTCGAAACACC	AGCCAAAGACACCCACCAA	NM_001205935.1
AURKA	AGCATGGATGAGCTGGTAAAT	TCTGTCCATGATGCCGTAGCT	NM_001038028.1
AURKB	TCCGACCCCTACTCTCTC	AGGAACGCTTGGGATGTTG	NM_183084.2
B2M	GCACCATCGAGATTGAAACATT	GCAGAAGACAGGAGATGTTG	NM_173893
BAD	TCAGGGCCCTTATTATCGGG	GGAAAGCCCCCTTGAGGAGACG	NM_001035459.1
BAX	TAACATGGAGCTTCAGAGGATGA	CAGCAGGCCCTCTCGAA	NM_173894.1
BCL2	GAGGCTGGGACGCCCTTGT	GGCCTCACTTATGGCCAGAT	NM_001166486.1
BRCA1	CTTACCTTCAGGAAACCGT	AATTGGTCTTGGCCTGGCT	NM_178573.1
BRCA2	AGTTTCCCCTGCTTCTCC	GGTTTCTGTCGCTTGTGAG	XM_002684277.2
BUB1	GCAGCTGGTATAAGGGGAA	AAAACCTCGATTCTCCGCA	NM_001102011.2
BUB1B	AGCTACAAAGGGGATGACC	CTTGTCTCCCTTATCACCAGC	NM_001145173.1
BUB3	ATGGGACACCGCTTCATAA	TGGTTAGGTGCACTTGGTT	NM_001076177.1
CASP2	CTGTAGCTCCGGCTGTGAG	CATCGCTCTCCGCATTG	NM_001144104.1
CASP3	ACGAAAATACTGGCATGCC	TCCGCTTGTGGCATTTGCC	NM_001077840.1
CCNA1	CTTCACCTCTCCAGGAGA	GCTACTCTGCTCTGGTGGAGT	XM_005194120.1
CCND1	AGATGTGACCCGGACTGCC	GGAAAACACCCAGGACAGTGTGAG	NM_001046273.2
CCNE1	TTGCTGCTCCGCTTGTAT	TTGCTGGCTTGTGTCAGC	NM_001192776.1
CD81	ATTCGTTCTCTGGCTGC	CGATAAGCTGTAGATGCCACA	NM_001035099
CDC20	TGGAGGGCCGAGTTAAAGT	CCATGGGAACCTCGTCAGT	NM_001082436.2
CDH18	AATGAAGATAACACAGCCAGCA	TGCTGAGAGAGGGGATTCCA	NM_001076837
CDK1	GGCGATAAAAGCCGGGCT	GCTCTGGCAAGGCAAAATC	NM_174016.2
CDK2	ATACACTCGTTCCATCCG	TACACAGAGTCACCCCTG	NM_001014934.1
CDKN1A	GGAGACCGTGGGGAGA	CGTTGAGGTGGTAGAAATCTG	NM_001098958.2
CDX2	ACGTGAGCATGTATCCAGC	TTCTGTTCTGCTGGCTTCT	NM_001202699.1
CENPE	CCCTGGAGGTTCTGACGTA	CAGGGCTCTTCTCTGTGA	XM_010805939.3
CENPF	CTTATTGGGGAAAAAGAGCA	CTCTTAACTTGTCTTTCAG	NM_001256586.1
CENTRIN2	CGTCCGGATGCGCTTAA	AATGGCAGGCACTAAACCGA	NM_001038515.1
CHEK1	CAACTTATGGCAGGGTGT	ATGTAAGAGCTAGAGGAGC	NM_001098023.1
CHEK2	GGGTTTATGCCACTCCGCT	ACCCATTCTCTGAAGATCCGAA	NM_001034531.1
CREBBP	CAAACTGGAGGGCAGCAGAT	CATCTGAGGAGCTTGGCA	NM_001164022.1
CRTAC1	GACAAGCGCTGTGTCAA	AAGGAGTGGAGGGAGGCCACA	NM_001205325
CSPP1	TCCCTCCATTGGTAGAGAGT	GTCTGTTCCCGTACATCTGTT	NM_001193015.2
CTNNB1	AGAACACAAATGACGTGAGA	GACCTTCCATCCTCTCTGT	NM_001076141.1
CYP3A7-3A51P	GGCCATGGAGCTATCTGA	TCCATATAGATAGAGGAGCACCAGA	NM_001099367
DIAPH1	CACTAGCAACGAAACCTGG	TTGAGGGAGACACCCAGGGA	XM_001787599.3
DYSF	ATGTTGGTCGACCTGTITCC	CGCAGGAAAACCTCTGG	NM_001102490
ECT2	ACGAGAGACAGAAATTGCCA	GAGTATGTGAAACAAAGACCA	NM_001097573.1
EOMES	GACAACATGATCATCCATCAGA	TGTTGGTAGGGGTGTCTT	NM_001191188.1
ERLEC1	GCCAGTCATCCAGGATCG	CCACCAACCAACCCCTT	NM_00119407.1
FSD1	AAGCTCAAGTGGAACGGCT	CCAGCGCTTGAACCCATTAC	NM_001081518
FZD2	TCCACGGAGAGAAAGGCTA	CCCGAGAGCTTGGGAGT	XM_003587455.5
GSG2	ACAACAACTGTGGGGTAA	CTTCAGGGCCGGGGTTT	NM_001076544
GUSB	TCGGCAGGGACAAAGATCAC	TGGGCAATCAGCTTGA	NM_001083436
HAGHL	CTGCCCCCTGAGACAAAGG	TGGTGTGTTAGGGCTTAC	NM_001075540
HAU6	AGGTATCAATGGTGTGGAT	ATGCCACTGTGCTAGGACT	XM_002689566.6
INCENP	AGAACGCTTCGAGAAGAA	GTCTTCTGCGGACAAACCT	XM_584352.7
ICG	CGACCTACCTGGCTGAGTAC	GGCTTCCAGGCTTCTTCCA	NM_001038195
KAT2A	TGTGAGCACCTTGGCTGA	AACCGCCCTTACTTGGGAAG	XM_001788901.3
KAT2B	TTCGGGTGGAAAGGTTCTC	TTCTGTTGACAGGCTTGA	XM_613744.7
KCTD1	AATGGGCACAGAACGAGCA	ATATGGGGCAGACTGTCTG	NM_001080360
KNL1	CGGGAGTAACCTCTGCT	AAACTTCTGAGGCCAGCG	XM_02690821.6
MAD2L1	GAGAGGCTCTGAAAGATGCCA	AGACTTTCTCTGGGTGACTAT	NM_001191513.1
MAP2K6	TTGCATGAAGATTCGACCC	TCGCTTCTGCTTCTGACT	NM_001034045
MCL1	CGGTATGGCGGAAGCG	AACCCATCCAGGCCCTTGT	NM_00109206.1
MIS18A	TGCATCTGCTACGCTGT	GTGAGGGCACATCTGTC	NM_001098010
MYH2	AAAGAGCCCTTGAATGAGGC	GCTGAACTCAGGGCTTGT	NM_001166227
NANOG	CGGACACTCTCTCTCTTC	CCATTGCTTCTCTGGCCA	NM_001025344.1
NPM2	GTGCTTGTGCTAGCTGATT	ATGTTGTTCTACTGCTCTTC	NM_001168706.1
OOP1	CGCCCGAGCTGAGAAATGG	GGTGGGGAAAGGCGAGATT	NM_001077869.2
PLK1	GTATGGCTCGGGTATCAGC	TCGGCTGCTGATGTACTGTAG	NM_001038173.2
POGZ	ACTACTACAGCTGGCAATTCT	ATGGGCAGGTCACTAGTTG	NM_001163190.1
PP1A	GGATTATGTGCGAGGGTGA	CCAGGACCTTGTATGCCAAATG	NM_178320.2
PPP1CA	TGCCAAGAGACAGTTGTGA	TGCCCATACCTGCCCTTACT	NM_001035316.2
PRKCQ	CCCCAACCTTCTGAGGACT	CATTCCGACCATGGCTG	NM_001192077
PRKRIP1	AGAACCTGGCTGACTCCCA	GCAGTCAGCTCTCCACATC	NM_001079641
RCC2	CTCCTCATCACCAAGGAAGG	CAGGACCAAGCTGTGGTTAG	NM_001101911.2
ROBO2	ACAGATGATCTTCACCAACAC	AAGTTGGCTCTTGGCTGTCT	XM_024993907.1
RPL15	GGCAGCCATCAGGGTAG	CATCACGCTGGACTGCTTCT	NM_00107866.1
RPS6K1	GTTCAGACACAGCAAGGACC	ACAGAGCCCTTGTAGTGC	NM_001083722.1
RPS6K5	ACCCCTTCTCAGGGCTG	CAGGCTTCACTGGGTAAT	NM_001192023.1
RSP6K4A	CACTCTCACTACCGCTGCC	TTGTTGAAGGGCTGGAAAGTG	NM_001191400.1
SCPEP1	ACACATGGTCTCCGACCC	CAGCCCAGGCCATCTTATC	NM_001045909
SDHA	TCTCTGAGACCCGGAGATAA	TCTGCTGATGGTGGCTGAGT	NM_174178
SEPT6	CCGGATATAGCTGGCAGGTG	CCAAACCTGCTCTCCACG	NM_001035430
SIRT2	GTCAAGGATAGAGCAGTCG	TCTGAGTCTGAGCTCTCTG	NM_001113531.1
SMIM8	GCTCTTAAAAGGAGCCGCC	AAGGCCATTACAGGTTTGTAGGT	NM_001081531
SMNT	TTTGTACCGCTGTGGTCC	CAGTCACCCAGCATCCGTC	NM_001076879
SPICE1	GCTATCGGGAAACGACAAGATGT	CGCCCTGGAGGAAACCAAC	NM_001038117.2
STX3	TTTAGCAACTGAGCAGAACGG	CATACCTCTCATCCCTCTG	NM_001101971
SYCP1	CCGGCCCTTCTGGAGTAGAT	TCTCTGGAAAGTCTGAGGTT	XM_003581953.2
SYCP3	CCAACAAAGGCAAAAGGCAAG	TGCTGCTGTACATGAGAGAAGAT	NM_001040588.2
SYT1	GACCATGAAAGATCAGGCC	CAGGACCTGGTTATCTGGA	NM_174192
SYT2	CTTGGGCAAAAGACACTC	CAGAGGGACAGCGGGGT	XM_024976596.1
TBC1D7	CGGACTTGGCTAGGACT	CAACTCCACGAAACCCCACT	NM_001015643
TEX14	ACGAAGTCTGAAGGCCAAC	GATGCTCTTACGAGTTCTCG	NM_001192568.1
TUBA1C	TTCTCCCGGAGACTCTTAG	ATGCACTCACGCATAACGGA	NM_001034204
TUBG1	ACCAAGCATTCTGGCTCTT	CAGTAAAGGAGATGAGGGTCC	XM_001790429.3
UBC	GTCCGGACCCGGAGGTTC	TCACAAAGATCTGCTCATTTA	NM_001206307.1
WRAP73	GTACCTGGCTTCTGATCC	CACTCGAGGTGCTGATCTG	NM_001193006
YWHAZ	ACCTACTCCGGACACAGAAC	ATCATATCCCTCACGGCTC	NM_174814



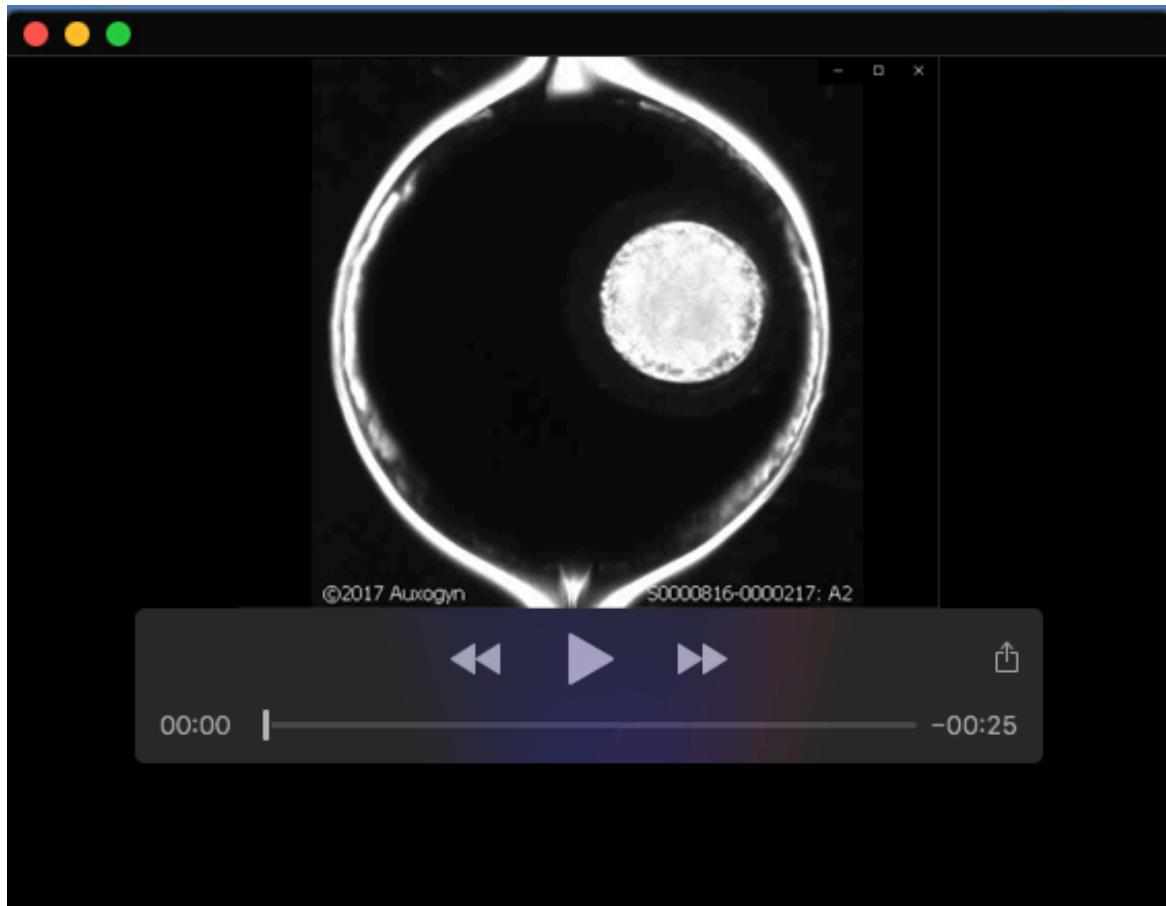
Movie 1. Live-cell fluorescent imaging of early cleavage divisions. Bovine zygotes were microinjected with fluorescently labeled modified mRNAs to mCitrine-Actin (green) and mCherry-Histone H2B (red) to distinguish blastomeres and DNA, respectively, and early mitotic divisions visualized by live-cell confocal microscopy. Note the micro-/multi-nuclei in embryos #3, #4, and #11, chromatin bridge in embryo #1, lack of syngamy in embryos #3 and #11, multipolar divisions in embryos #1, #3-6, #11, and #15, and production of empty blastomeres in embryos #5 and #15.



Movie 2. MCC-deficient embryos struggle to divide. A bovine zygote following BUB1B MAO microinjection attempted to divide by forming multiple cleavage furrows, but never successfully completed cytokinesis.



Movie 3. Multipolar divisions are observed in MCC-deficient embryos. Certain bovine zygotes were able to undergo cytokinesis even with BUB1B knockdown, but these divisions were abnormal with multipolar cleavage.



Movie 4. MCC deficiency causes blastomere asymmetry. Besides abnormal divisions, BUB1B-injected bovine embryos often exhibited blastomere asymmetry following the multipolar cleavage.