

## **Involvement of Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchanger SLC26A3 and SLC26A6 in preimplantation embryo cleavage**

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## Supplementary figure legend

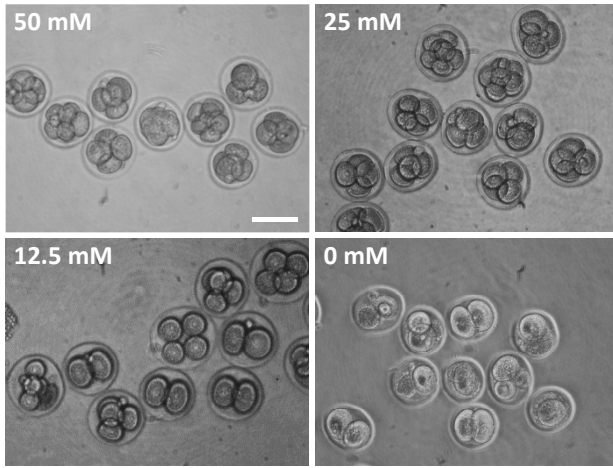
**Supplemental Figure S1. HCO<sub>3</sub><sup>-</sup> ion is required for embryo cleavage.** **A**, Four-cell embryo formation after 12 hours of 2-cell embryo culture in TALP medium with varying concentrations of HCO<sub>3</sub><sup>-</sup> and constant Cl<sup>-</sup> concentration (115 mM) (50 mM: 27/35 embryos; 25 mM: 35/40 embryos; 12.5 mM: 15/34 embryos; 0 mM: 3/34). Scale bar: 100 μm. **B**, Summary of the results from **C**. ns indicates  $P > 0.05$ , \*\*\* indicates  $P < 0.001$  (by one-way ANOVA,  $n = 4$ ).

**Supplemental Figure S2. Expression of SLC channels mRNA in 2-cell to 8-cell embryos.** Quantitative real-time PCR results showing the mRNA expression level of SLC channel family members. The expression was normalized to rRNA expression in 2-cell to 8-cell embryos.

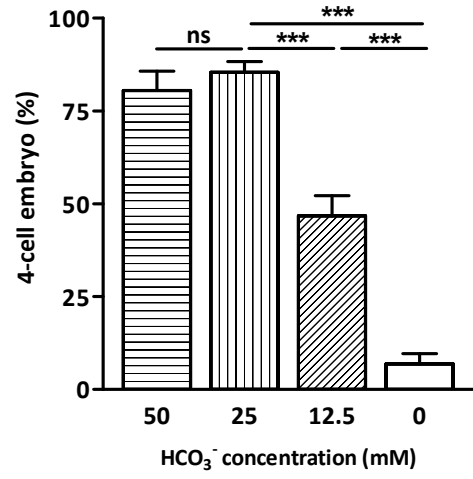
**Supplemental Figure S3. Dose-dependent effects of niflumate and DIDS on inhibiting embryo cleavage.** Embryos were treated with indicated concentration of niflumate or DIDS in complete TALP. a, Compared with DIDS control,  $P < 0.05-0.01$ ; b, compared with niflumate control,  $P < 0.05-0.001$ ; c, compared with corresponding DIDS treatment,  $P < 0.05-0.01$ . ( $n = 4$ ).

**Supplemental Table 1. List of primers used.**

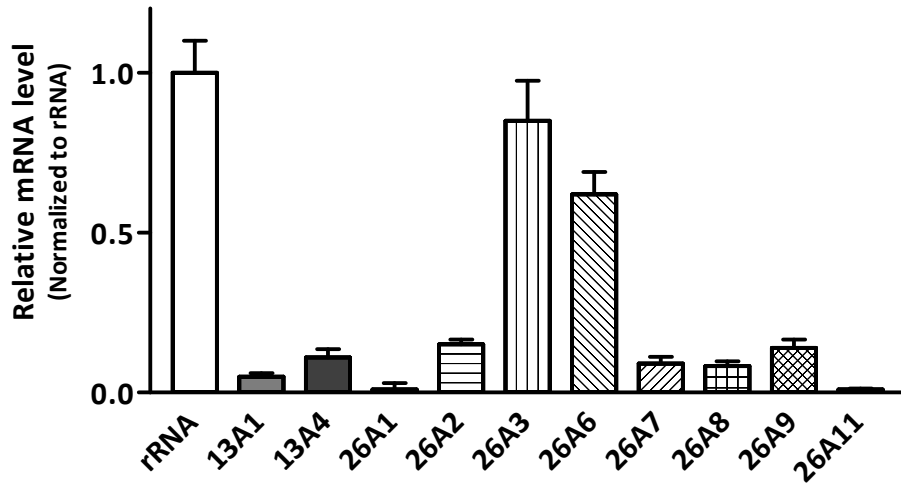
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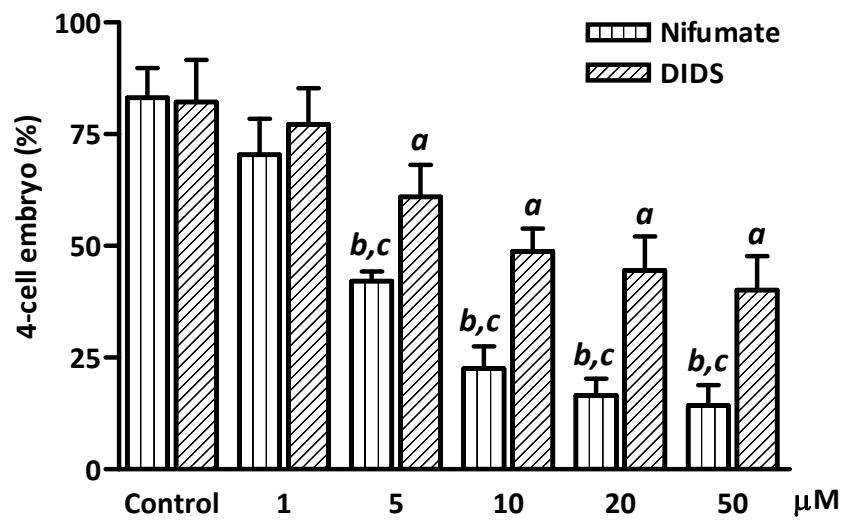


B



SLC mRNA expression in embryo (2-8-cell)





**Supplementary Table 1: Table Nucleotide sequences of primers used for quantitative real-time PCR (qPCR) (SYBR Green)**

Target RNA	Forward (5'- 3')	Reverse (5'- 3')
Mouse SLC13A1	CTATCGCCGCTTTCTCCTTGT	GGCAGTAGAGCTGTGATTGACA
Mouse SLC13A4	GGAAGCTGCTATTGGTCATCTG	GGTCACAAGCAACACGTAAGC
Mouse SLC26A1	CAGAAGGGTGGGACACTGGTA	ACATCACCTGCTAGGTATTCTT
Mouse SLC26A2	AAGAGCAGCATGACCTCTCAC	CTGCCTCAAGTCAGTGCCT
Mouse SLC26A3	CTGAAGCGCCACACTGAATA	CTGGTGACAGTGGCTAGTGG
Mouse SLC26A6	TGCGGAGCCTTAGTTTACCG	ACTCCTCGGTAAGCAGCAAC
Mouse SLC26A7	CCCCACCGAGAAGACATTAAGC	TGAACTGCCAACATTATCCCAG
Mouse SLC26A8	TTTTGTGTACGATGTTAAGCGGA	GTGGTGCTTGAAAGTGGTGA
Mouse SLC26A9	CCCCGCTACGTGGTAGACA	AGCACCTGAAAGTGTTCGA
Mouse SLC26A11	AGTGTATGCGGGAACACATGC	CGAAGGCGTAAGCAATCAGAG
Mouse rRNA	GTAACCCGTTGAACCCATT	CCATCCAATCGGTAGTAGCG
Mouse p53	GTCAAGAAAGTGGGGCCTGA	TGAGTGGAATCTGGGATTGTG
Mouse p21	CCAGATCTCCGGAGCCAGG	GAGGGGCGTCATCAAAGGAA
Mouse FGF2	ACTGTCCTGTCAGTGTGGAT	AGTGCCACATACCAACTGGAG
Mouse GAPDH	CCCCAGCAAGGACACTGAGCAAGAG	GCCCTCCTGTTATTATGGGGGTC
Human SLC26A3	CAGTGGCCTTTTCAGTTGCC	GGGCAGTACTCCCAGCAAAT
Human SLC26A6	TACAGTGATGCGCTGAAGCA	CCAGACATGCAGACAAAAGGG
Human GAPDH	CTTCTTTTGCGTCGCCAGCC	TCCGTTGACTCCGACCTTCA