

1 **Supplementary Information for:**

2 **Title:** Fluorescence- and magnetic-activated cell sorting strategies to separate spermatozoa involving
3 plural contributors from biological mixtures for human identification

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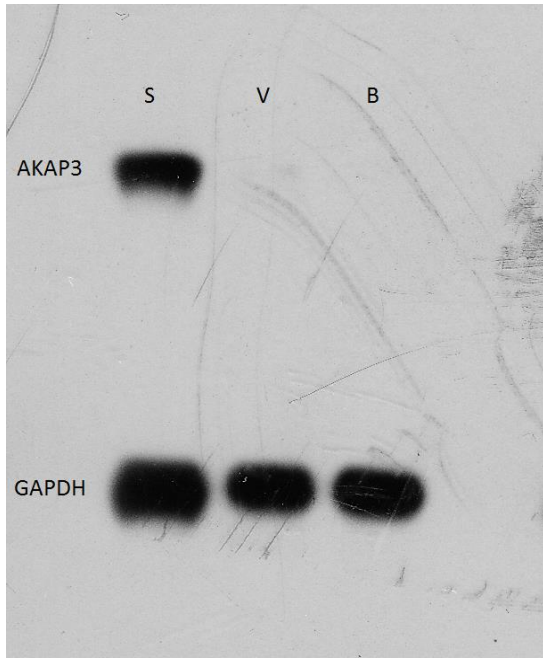
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2 **Figure S1. Partial sequence of FUT2 gene that contain 5 mutation sites.**

3 Green highlights represent primer sequence. Pink highlights represent mutations that lead to

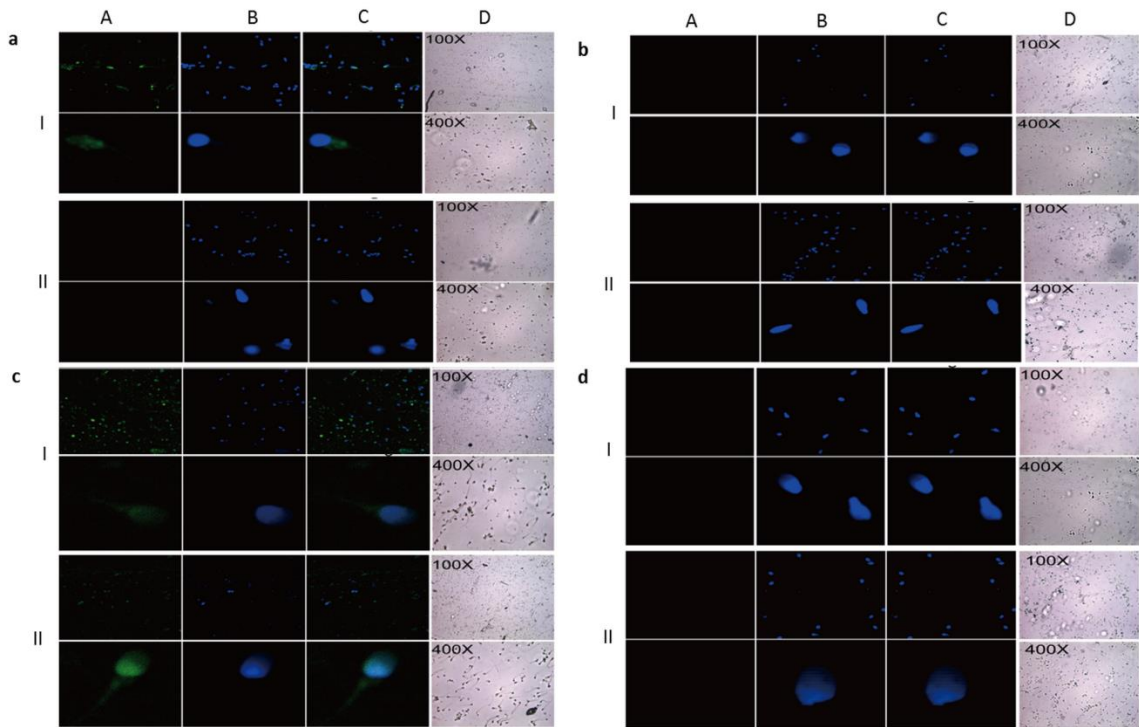
4 inactivation of Se enzyme.

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Figure S2. Full-length gels and blots of Figure 1.



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2 **Figure S3. Immunofluorescent localization of blood group antigens in A-type (a), O- type (b),**

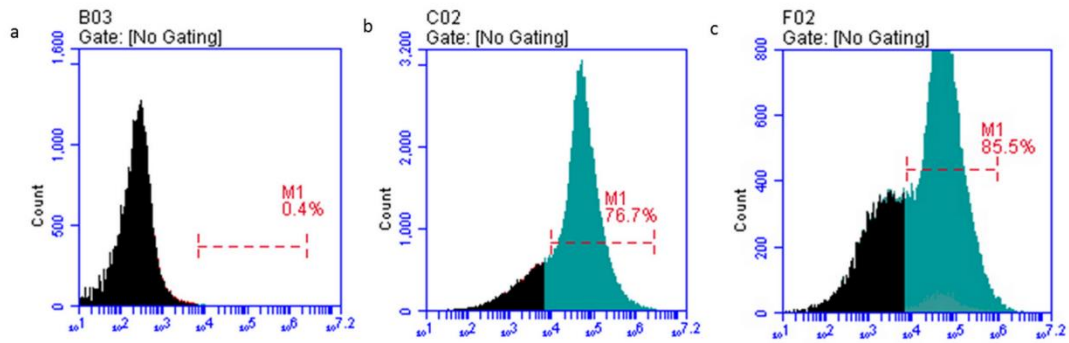
3 **AB-type (c) and non-secretors sperm cells (d).**

4 **I. (A)** Blood group A-stained sperm cells. **(B)** DAPI-stained sperm nuclei. **(C)** Merged image (A,
5 **B).** **(D)** Sperm cells under a light microscope ($100\times$ and $400\times$).

6 **II. (A)** Blood group B-stained sperm cells. **(B)** DAPI-stained sperm nuclei. **(C)** Merged image (A,
7 **B).** **(D)** Sperm cells under a light microscope ($100\times$ and $400\times$).

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2 **Figure S4. Cytofluorimetric analysis of mock sample mixture S2 involving female vaginal**
 3 **epithelial cells, AB-type sperm cells and O-type sperm cells using FITC-labeled anti- blood group**
 4 **A antigen antibody.**

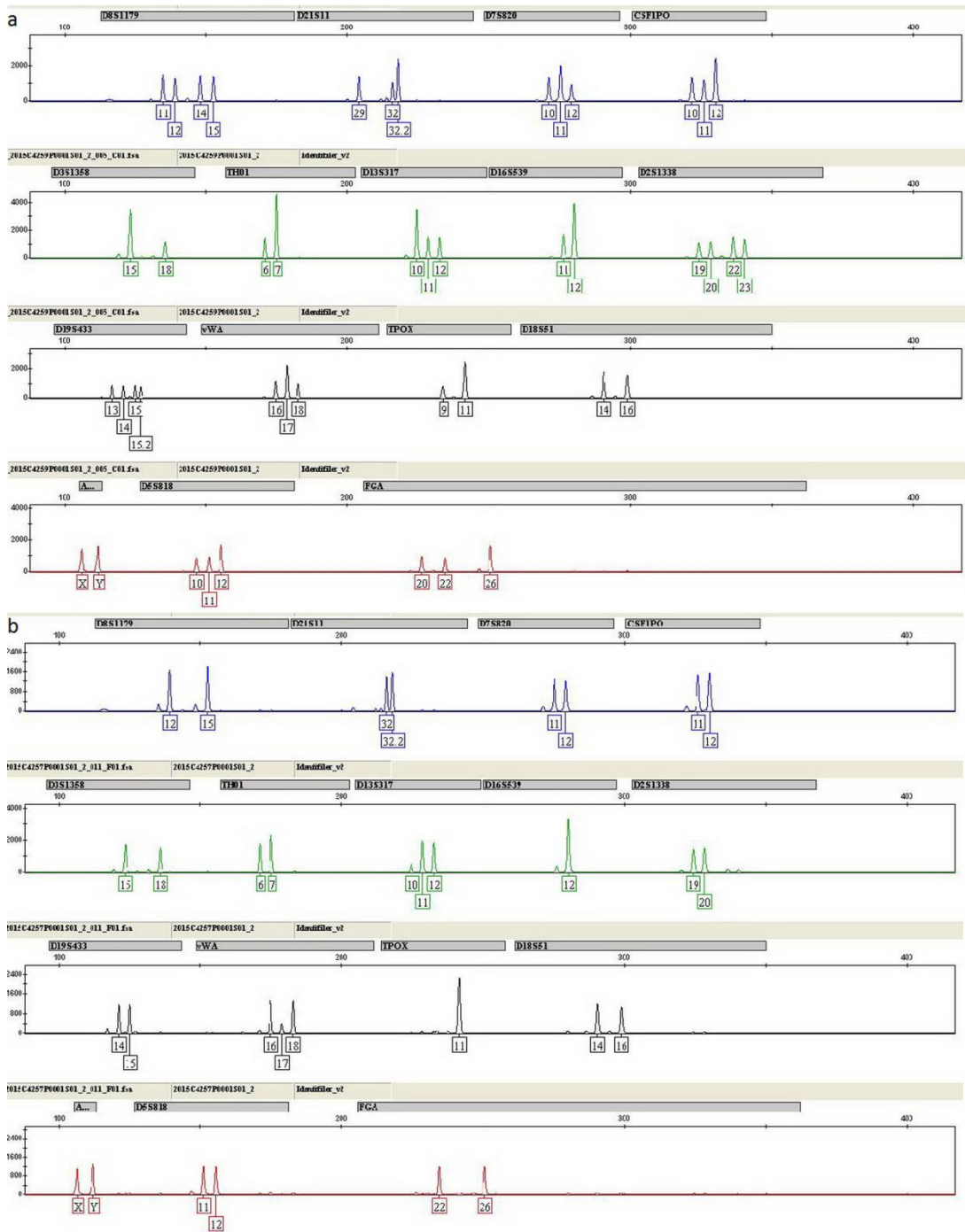
5 (a) Negative control (Female vaginal swab sample suspension).

6 (b) Detection of sperm cells in AB-type and O-type sperm mixture with FITC-labeled blood group A
 7 antigen antibody.

8 (c) Flow cytometry analysis of A-type sperm cells after sorting.

9 Numbers indicate the percentage of positive cells.

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 2 **Figure S5. CE of the STR typing with ID-plus by FACS of mock sample mixture S2 involving**
 3 **female vaginal epithelial cells ,AB-type sperm cells and O-type sperm cells.**
 4 (a) STR typing of AB-type sperm and O-type sperm cell mixture.
 5 (b) STR typing of single-source AB-type sperm cells, using blood group A antigen antibody after cell
 6 sorting.

1 **Table S1. Success rate of STR loci amplification after cell mixtures incubated with different**
 2 **antibodies (mixtures ratio at 1:1)**

Antibody (cell mixture type)	Success rate of STR loci amplification (RFU \geq 100)			
	14-16 (full)	9-13 (partial)	6-8 (low partial)	<6 (none)
AKAP3 (s:v) (n=30)	30	0	0	0
ABO (A:B) (n=6)	4	2	0	0
ABO (A:O) (n=6)	4	2	0	0
ABO (A:AB) (n=6)	4	2	0	0
ABO (B:O) (n=6)	4	2	0	0
ABO (B:AB) (n=6)	3	3	0	0
ABO (O:AB) (n=6)	4	2	0	0
ABO (A:O:AB) (n=6)	0	6	0	0
ABO (B:O:AB) (n=6)	0	6	0	0
ABO (A:B:AB) (n=6)	0	6	0	0
ABO (A:O:B) (n=6)	0	6	0	0

3 s:v (sperm and vaginal epithelial cell); A:B (sperm of A type and sperm of B type)

4 A:O:B (sperm of A type, sperm of O type and sperm of B type)