**Supplementary Information for:**

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

**Authors:** Yan Xu[b,c], Jianhui Xie[c], Ronghua Chen[b], Yu Cao[b], Yuan Ping[b], Qingwen Xu[b], Wei Hu[b], Dan Wu[b], Lihua Gu[b], Huaigu Zhou[b], Xin Chen[b], ZiQin Zhao[c], Jiang Zhong[a], Rui Li[a*]

**Affiliations:**

[a]School of Life Sciences, Fudan University, 2005 Songhu Road, Shanghai 200438, China.

[b]Shanghai Key Laboratory of Crime Science Evidence, Key Laboratory of Forensic Evidence and Science Technology, Ministry of Public Security, Institute of Forensic Science, Shanghai Public Security Bureau, Shanghai 200083,China.

[c] Department of Forensic Medicine, Shanghai Medical College, Fudan University, 138 Yixueyuan Road, Shanghai 200032, China.

[*]**Corresponding author:** Tel.: +86 21 51630660; Fax: +86 21 51630661. E-mail address: rui_li@fudan.edu.cn (R. Li);


1

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.

5

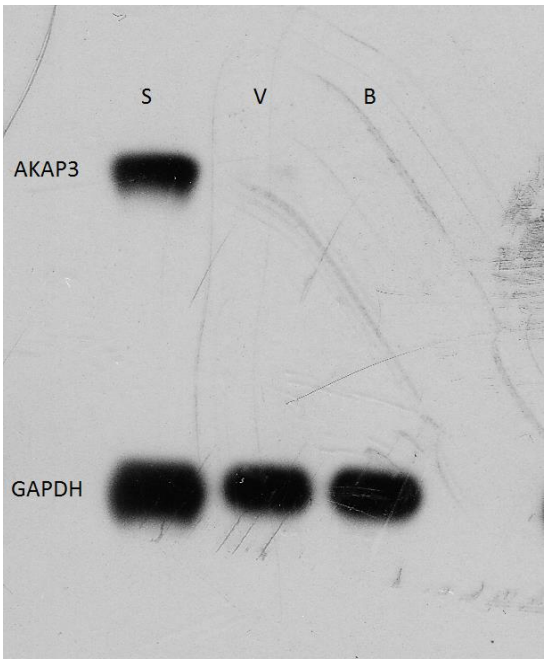


**Figure S2. Full-length gels and blots of Figure 1.**

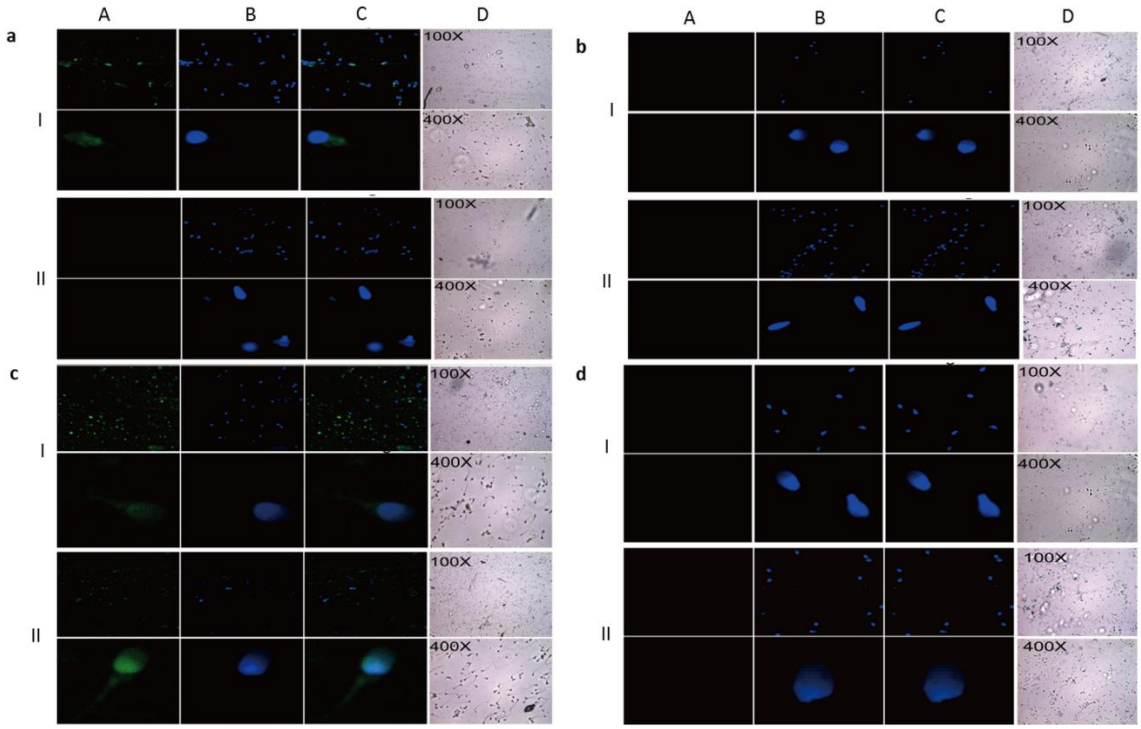


**Figure S3. Immunofluorescent localization of blood group antigens in A-type (a), O- type (b), AB-type (c) and non-secretors sperm cells (d).**

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

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

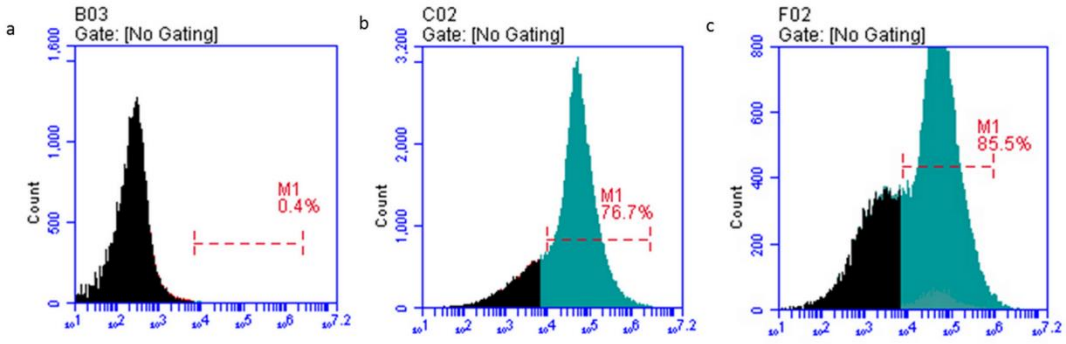


**Figure S4. Cytofluorimetric analysis of mock sample mixture S2 involving female vaginal epithelial cells, AB-type sperm cells and O-type sperm cells using FITC-labeled anti- blood group A antigen antibody.**

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

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

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

Numbers indicate the percentage of positive cells.

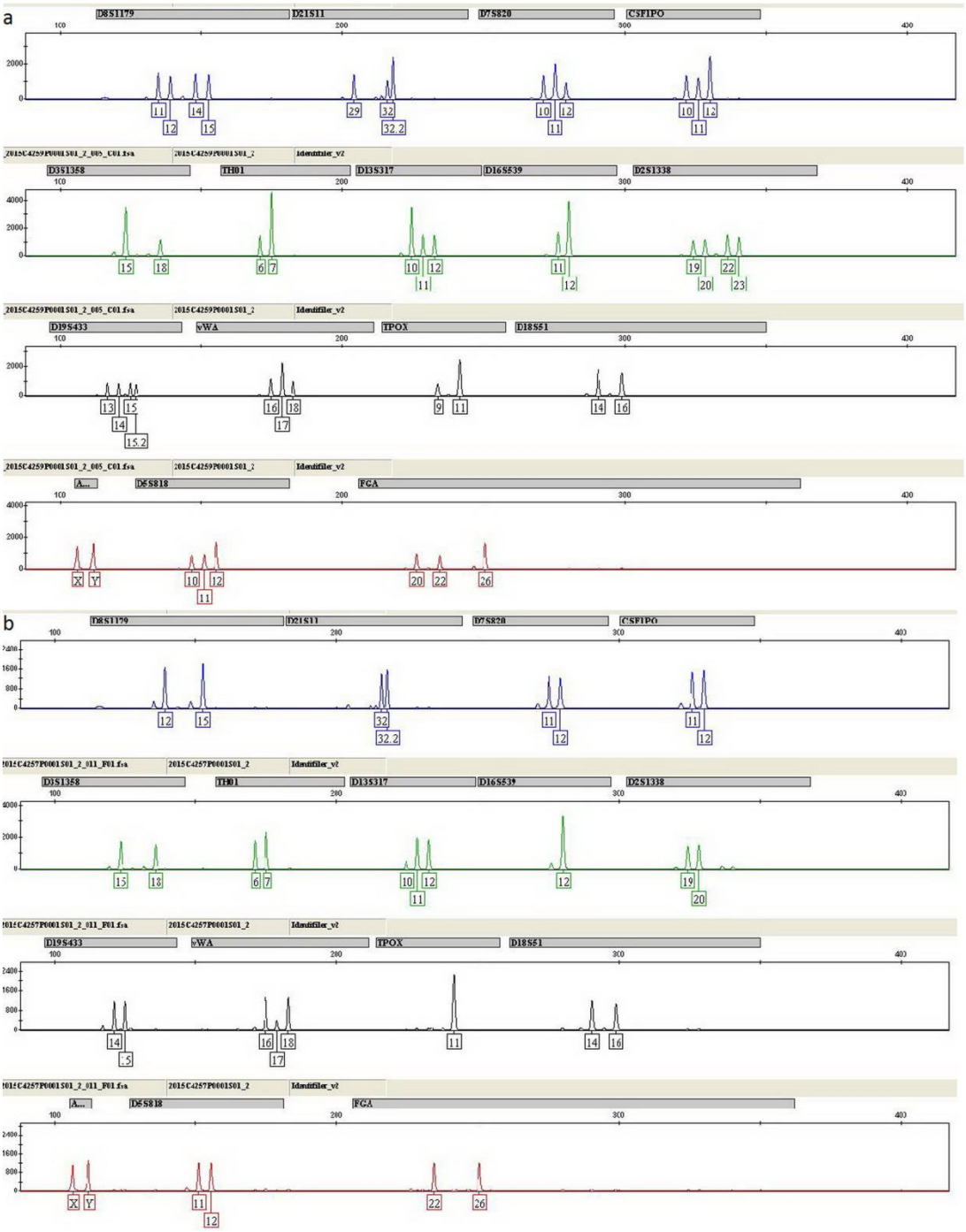


**Figure S5. CE of the STR typing with ID-plus by FACS of mock sample mixture S2 involving female vaginal epithelial cells , AB-type sperm cells and O-type sperm cells.**

(a) STR typing of AB-type sperm and O-type sperm cell mixture.

(b) STR typing of single-source AB-type sperm cells, using blood group A antigen antibody after cell sorting.

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

| Antibody (cell mixture type) | Success rate of STR loci amplification (RFU≥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 |

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

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