Supplemental Figures

Germ cell-specific proteins AKAP4 and ASPX facilitate identification of rare spermatozoa in non-obstructive azoospermia

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List of Supplemental Figures

Figure S1

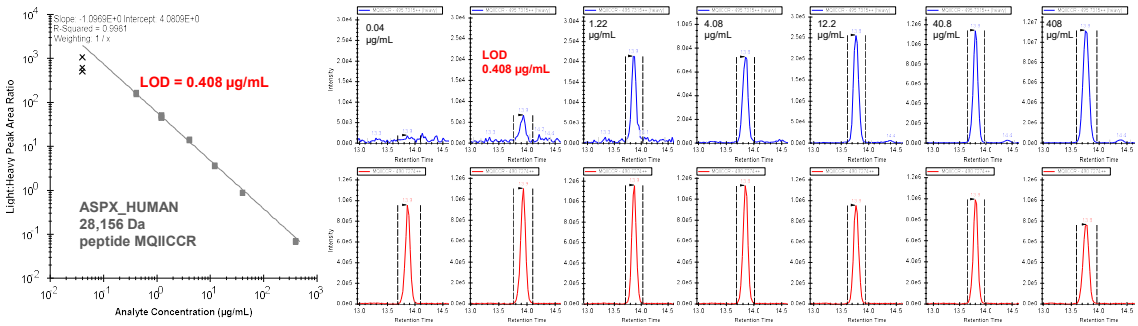

Fig. S1. Determination of a limit of detection of SRM assay for ASPX_HUMAN protein in SP. Serial dilutions of a heavy peptide MQIICC**R** were spiked into a digest of a pre-vasectomy SP sample with the high and constant levels of the endogenous "light peptide" MQIICCR.

(**Left panel**) The limit of detection (LOD=0.408 µg/mL) was calculated using the SP volume (accounted for dilutions), the UniProt molecular mass of ASPX_HUMAN (28,156 Da), and the lowest amount (moles) of MQIICCR detectable with the signal-to-noise ratio ≥3 and light-to-heavy ratio within the linear range. Three technical replicates were measured for each dilution.

(**Right panel)** Peaks correspond to the heavy and light peptides in one of the technical replicates.

Figure S2

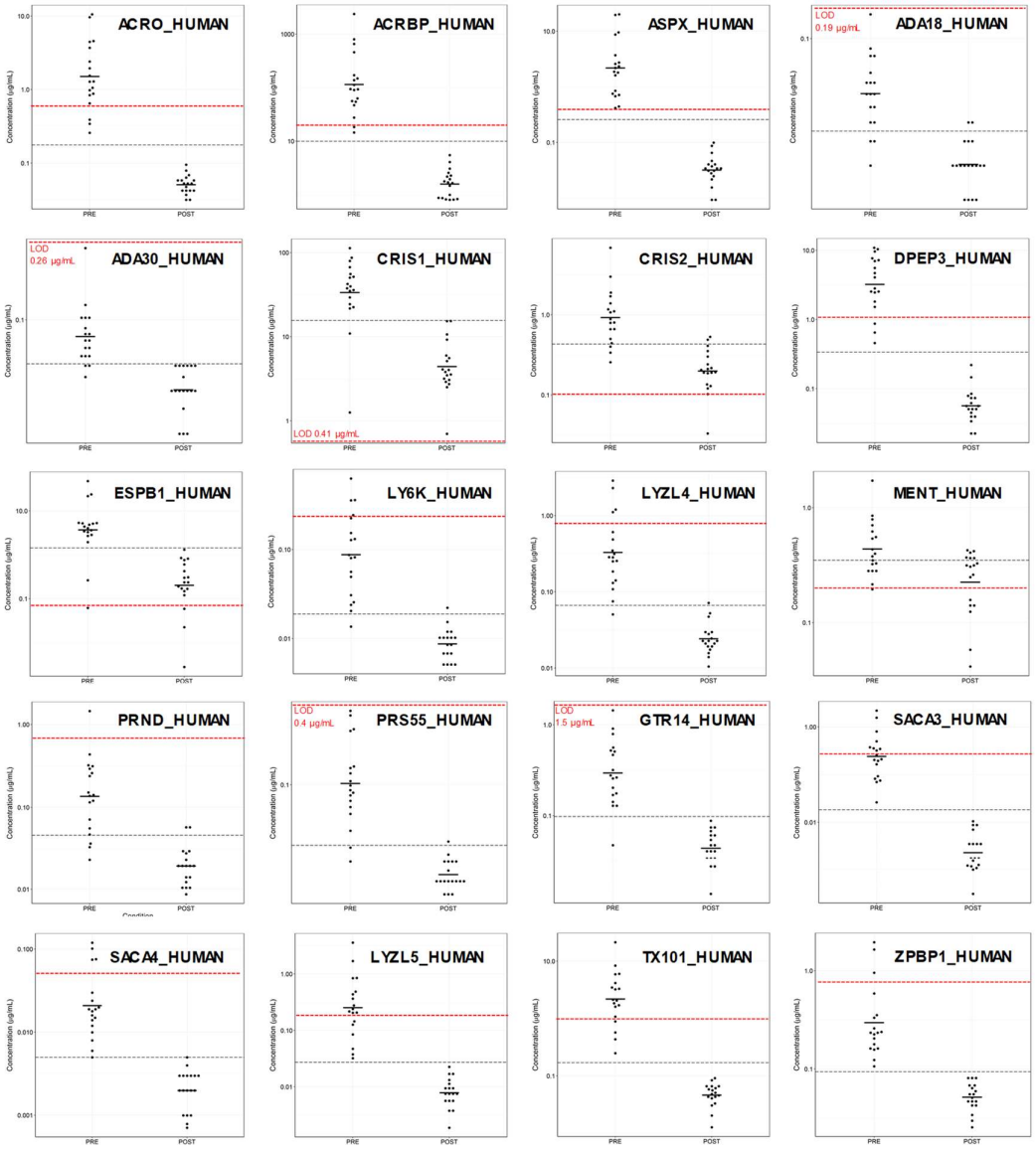

Fig. S2. SRM analysis of the matched pre-vasectomy (N=18) and post-vasectomy (N=18) SP to access testis/epididymis specificity of candidate proteins. Grey dashed lines represent apparent cutoffs for the unadjusted raw levels calculated using SRM L/H ratios, while red dashed lines represent SRM assay LODs. In post-vasectomy SP, levels of testis- and epididymis-specific proteins decreased below LODs. Proteins with the residual expression in the prostate or seminal vesicles would still be detectable in some post-vasectomy SP samples (for example, CRIS2_HUMAN and MENT_HUMAN proteins). ASPX_HUMAN at its limit of detection (0.4 ug/mL) revealed ultimate specificity and sensitivity (100%) to detect post-vasectomy.

Figure S3

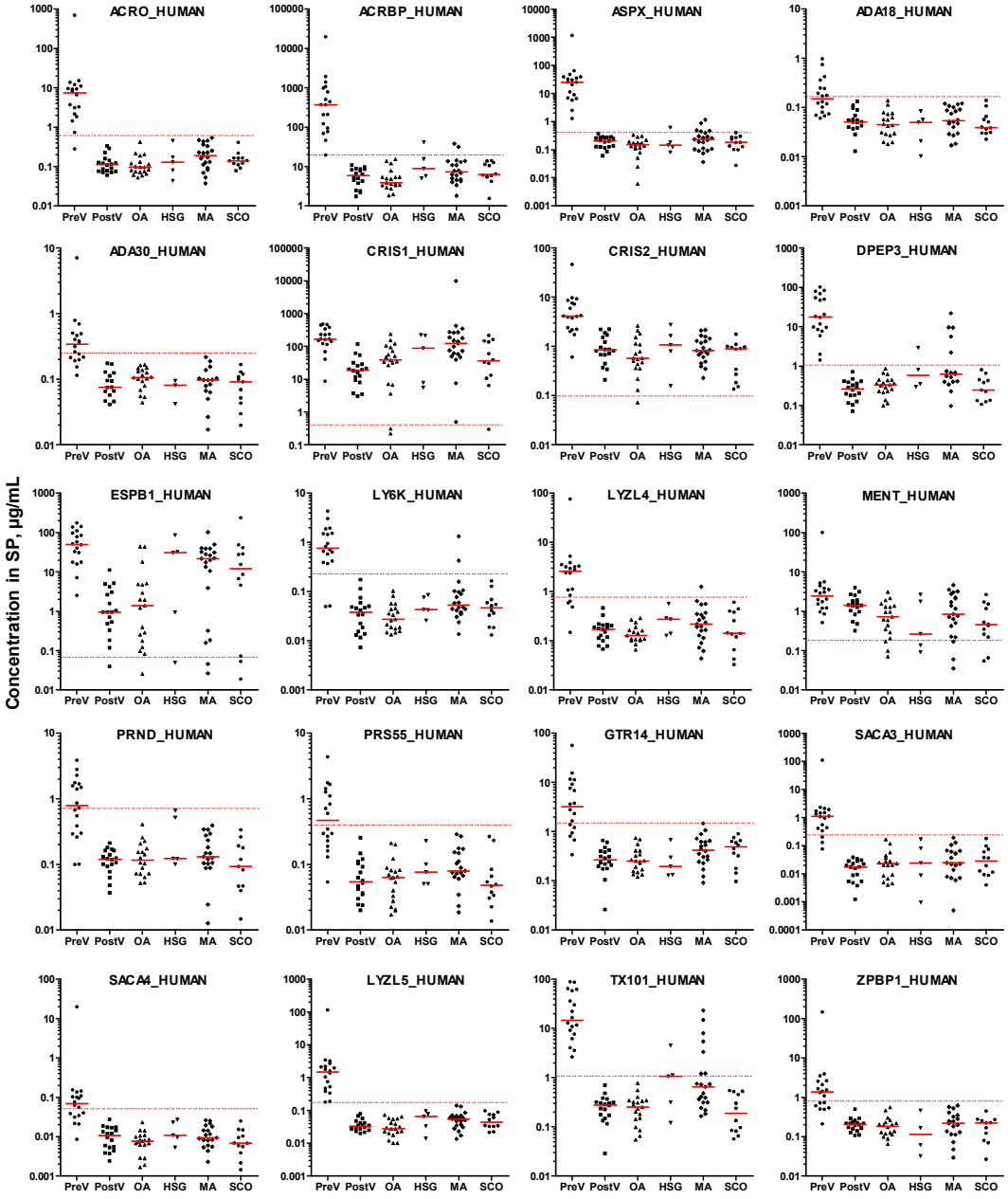

Fig. S3. SRM quantification of candidate proteins in SP of pre-vasectomy, post-vasectomy, and NOA patients. Testis-specific proteins and two epididymal proteins CRIS1_HUMAN and ESPB1_HUMAN were measured in the independent set of unmatched pre-vasectomy (PreV; N=18) and post-vasectomy (PostV; N=18) SP, as well as SP of OA patients (N=19) and NOA patients with the histological subtypes of hypospermatogenesis (HGS; N=5), maturation arrest (MA; N=21) and Sertoli cell-only (SCO; N=12). Dashed lines represent SRM assay limits of detection.

Figure S4

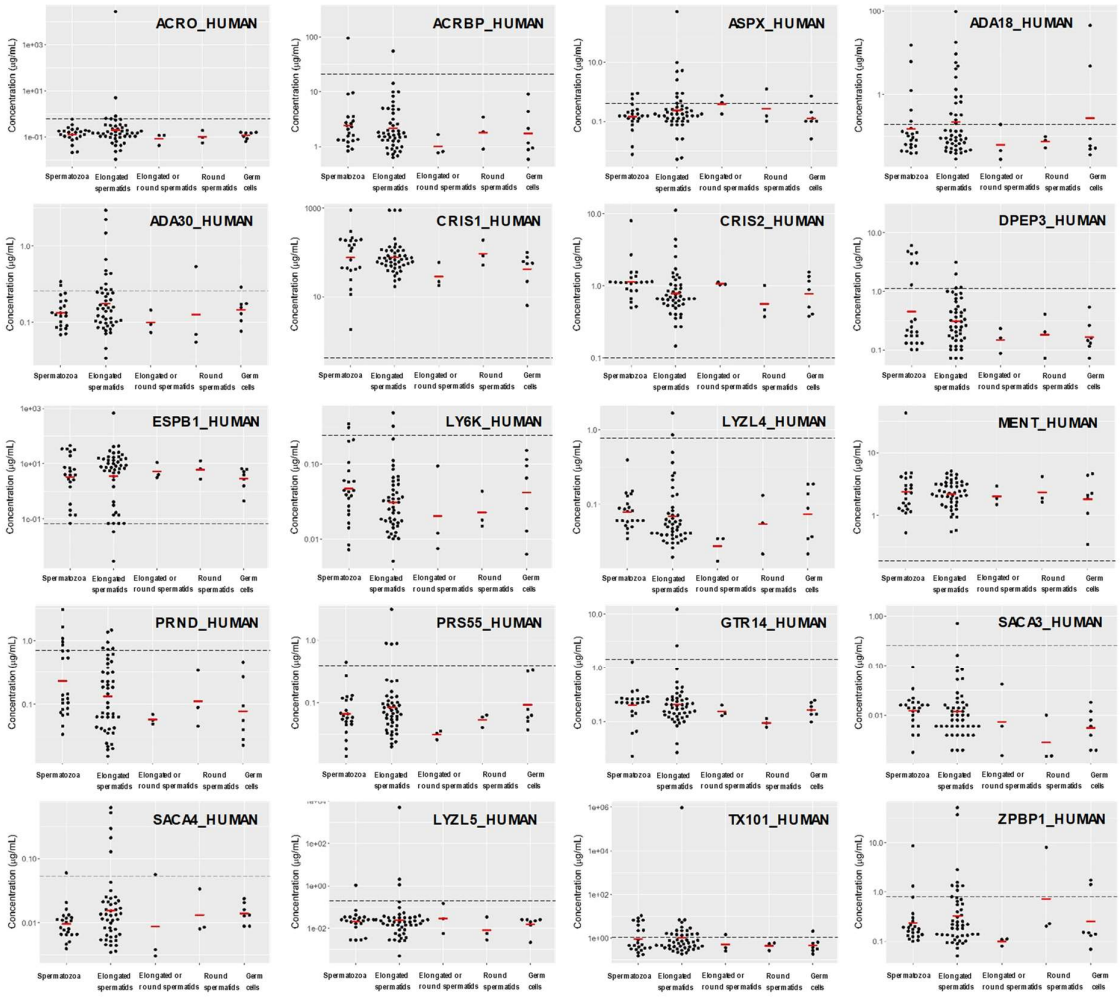

Fig. S4. SRM quantification of candidate proteins in SP of NOA patients with the known mTESE outcomes. Testis-specific proteins and two epididymal proteins CRIS1_HUMAN and ESPB1_HUMAN were measured in 84 serial SP samples of 27 NOA patients (obtained at baseline and at 1-3 months, before mTESE). The following cells types were retrieved with mTESE: spermatozoa (N=25 SP samples), elongated spermatids (N=46), elongated+round spermatids (N=3), round spermatids only (N=3), and immature germ cells (N=7). Dashed lines represent SRM assay limits of detection.

Figure S5

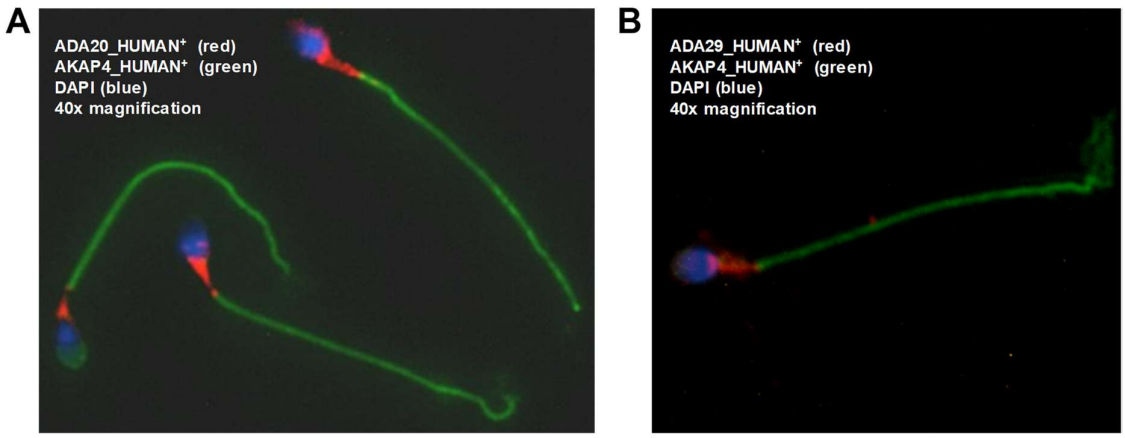

Fig. S5. Localization of ADA20_HUMAN and ADA29_HUMAN proteins in motile spermatozoa. Immunofluorescent microscopy analysis of ADA20_HUMAN (ADAM20 gene; red) (**A**) and ADA29_HUMAN (ADAM29 gene; red) (**B**) revealed their expression in the post-acrosomal region of spermatozoa, similar to the testis-specific proteins TX101_HUMAN, DPEP3_HUMAN and LY6K_HUMAN, as we previously reported in Schiza et al. *Mol Cell Proteomics* 2019, 18, 338-351 and *Mol Cell Proteomics* 2018, 17, 2480-2495. The tail and nucleus were stained with AKAP4_HUMAN (green) and DAPI (blue).

Figure S6

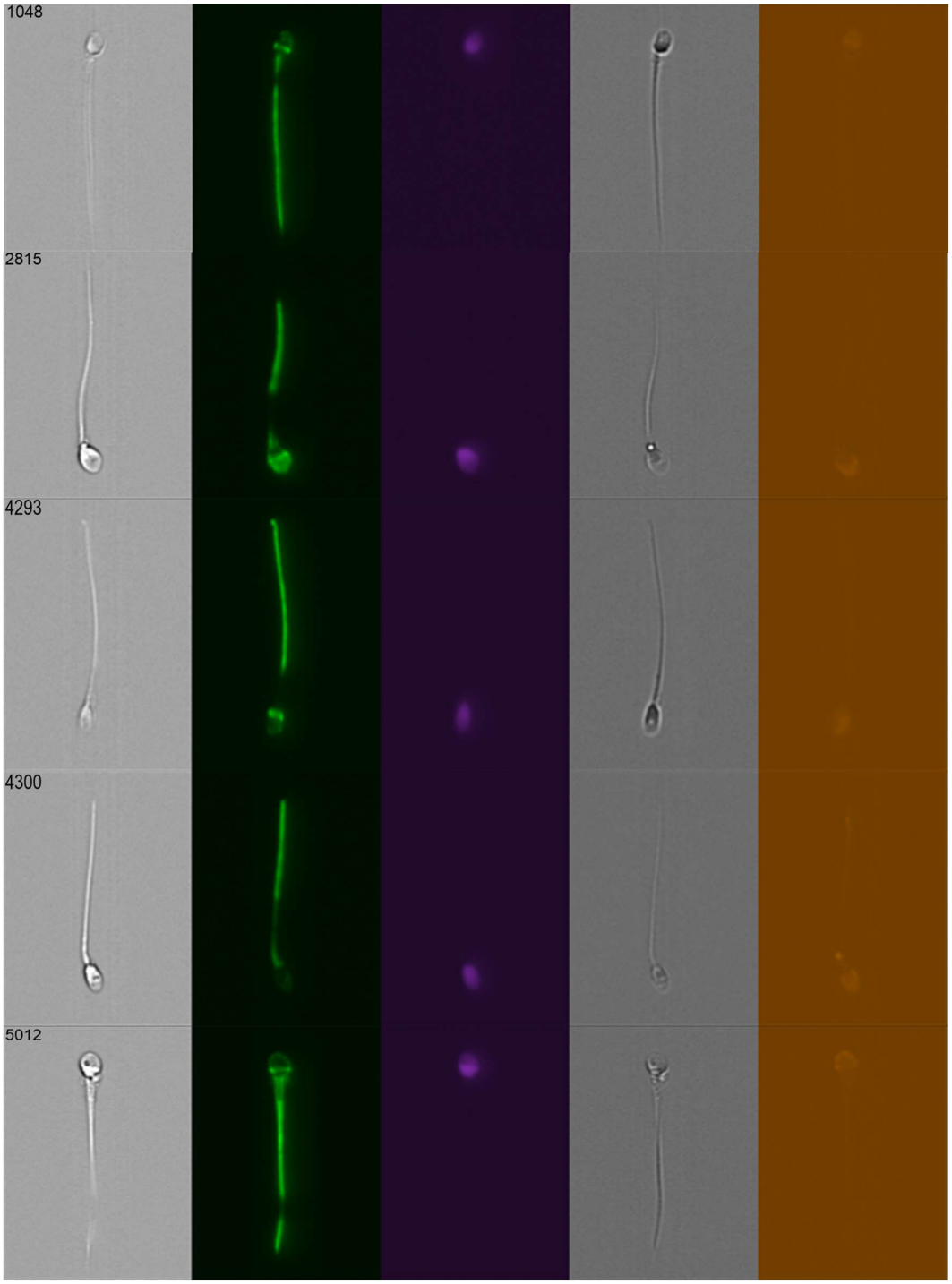

Fig. S6. Imaging flow cytometry identification and visualization of the morphologically normal and intact AKAP4^{+}/ASPX^{+}/Hoechst^{+} spermatozoa in semen pellet of a normozoospermic patient.

Figure S7

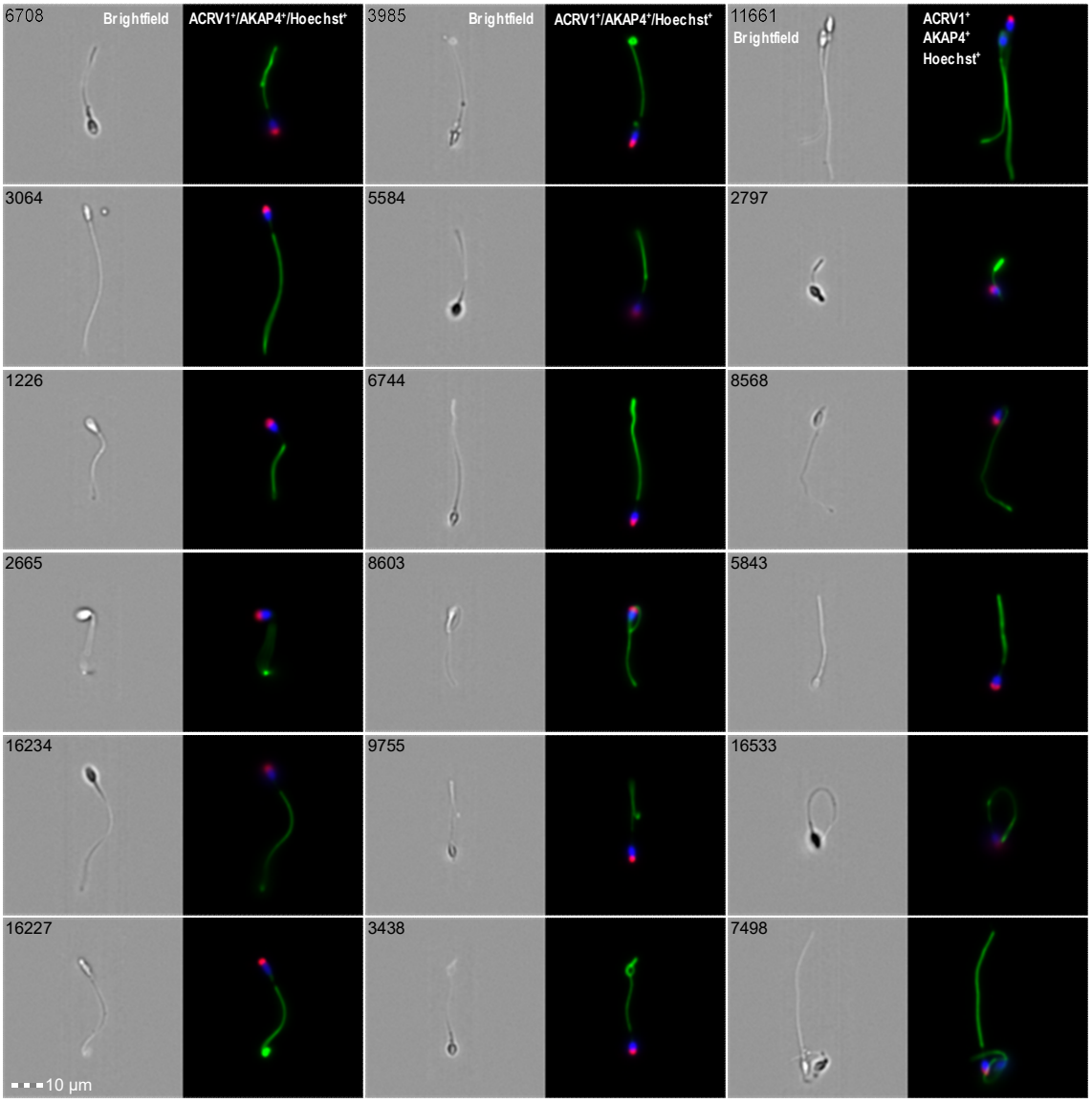

Fig. S7. Imaging flow cytometry identification and visualization of intact AKAP4^{+}/ASPX^{+}/Hoechst^{+} spermatozoa in semen pellet of a patient diagnosed with oligospermia.

Figure S8

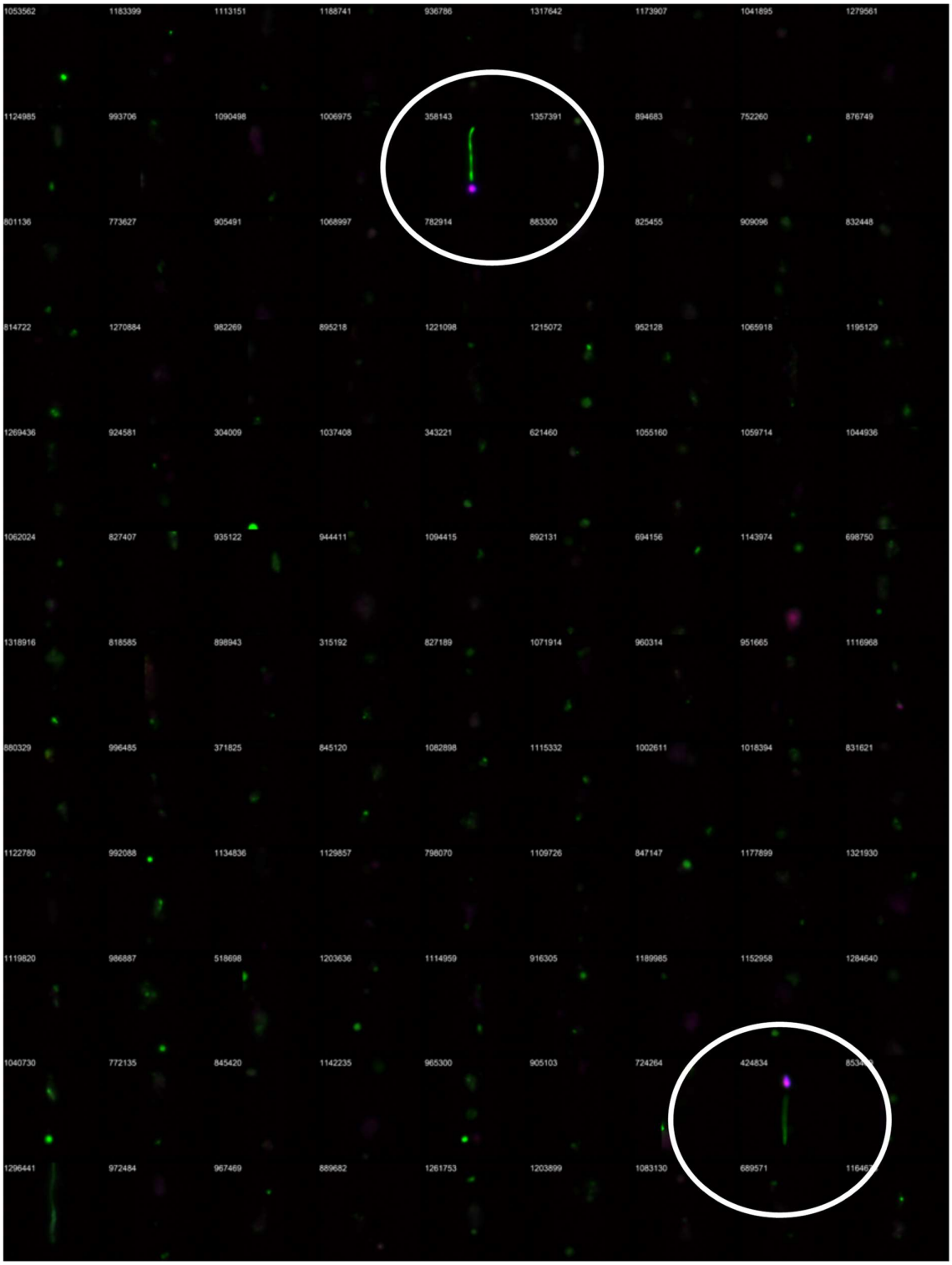

Fig. S8. Visualization of two intact AKAP4^{+}/ASPX^{+}/Hoechst^{+} spermatozoa in NOA semen pellet. The figure includes 108 ImageStream images obtained for patient #88.