Supplementary Figure Legend.

Supplementary Figure-1. Immunostaining negative control. Human spermatozoa sample was treated as detailed in material and methods section, but the step of adding of the primary antibody was omitted. A) Panel in blue depicts the nuclei of 2 different human spermatozoa stained with DAPI. B) Panel shows the FITC signal (not staining is appreciated). C) Panel shows the images obtained using the phase contrast objective. D) Panel shows the merge of all images. Scale bar in white: 5 μm.

Supplementary Figure-2. Effect of YK 3-237 on human sperm functional parameters. Human spermatozoa were incubated up to 6 hours at 37°C in capacitating conditions (BWW-modified medium supplemented with HCO_3^- 25 mM and BSA 26 mg/mL) in presence or absence of YK 3-237 (10μM). Measurement was performed after 1 and 6 hours (A-D) or for 6 hours (E-F). A) Histograms represent the percentage of live spermatozoa ($SYBR^+/PI^-$). B) Histograms represent the percentage of live spermatozoa with acrosome reacted or damaged (PNA^+/PI). C) Histograms represent the percentage of the sperm population exhibiting high mitochondrial membrane potential (MMP). D) Histograms represent the percentage of spermatozoa with high mitochondrial superoxide anion (MitoSOX) production. Light blue bars represent control conditions whereas dark blue bar represent YK 3-237 treatment. Values represent the mean ± SEM of at least 6 independent experiments. Data were analyzed statistically by one-way analysis of variance (ANOVA). No statistical differences were found. E) A representative western blot using anti-4-hydroxynonenal (HNE4) antibody of human spermatozoa incubated for 6 hours at 37°C in in presence or absence of YK 3-237 (10μM). F) Western blots were analyzed using Image Lab (n=3). Histograms represent the average ± SEM. For comparison between blots, pixels for each lane were quantified and normalized using the control lane as reference. Data were analyzed statistically by one-way analysis of variance (ANOVA). No statistical differences were found.