Phosphoproteomics and functional analyses reveal sperm-specific protein changes downstream of kappa opioid receptor in human spermatozoa

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Supplemental Figures:

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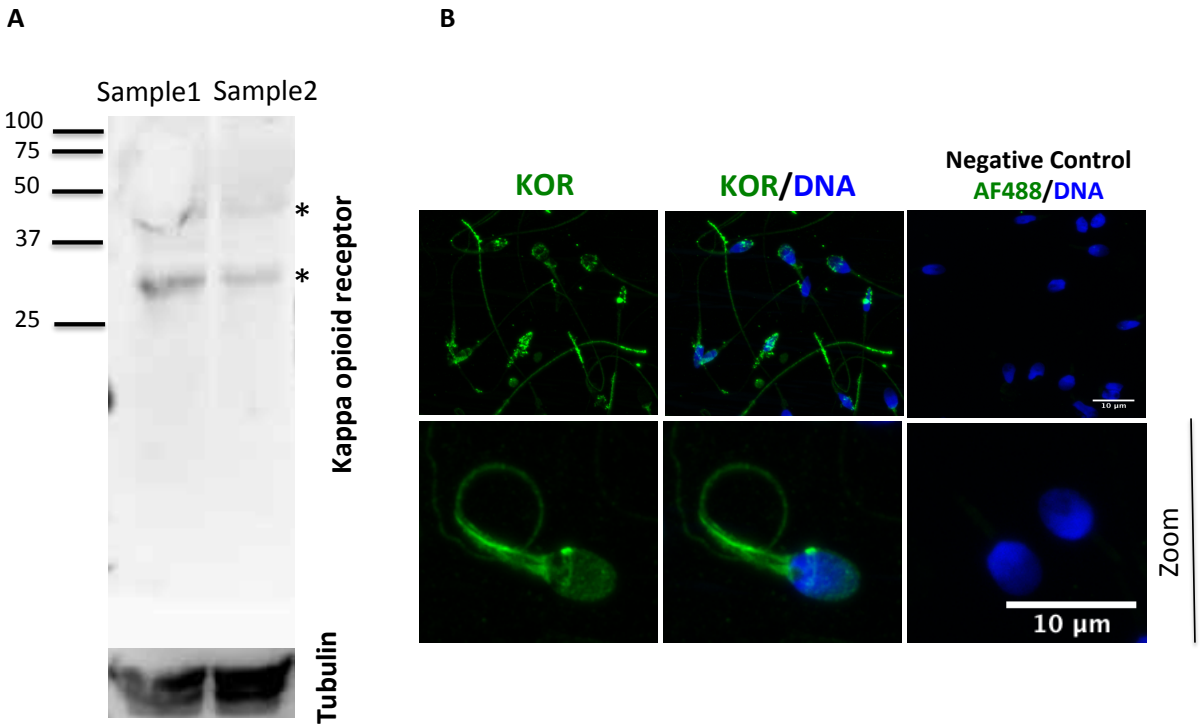
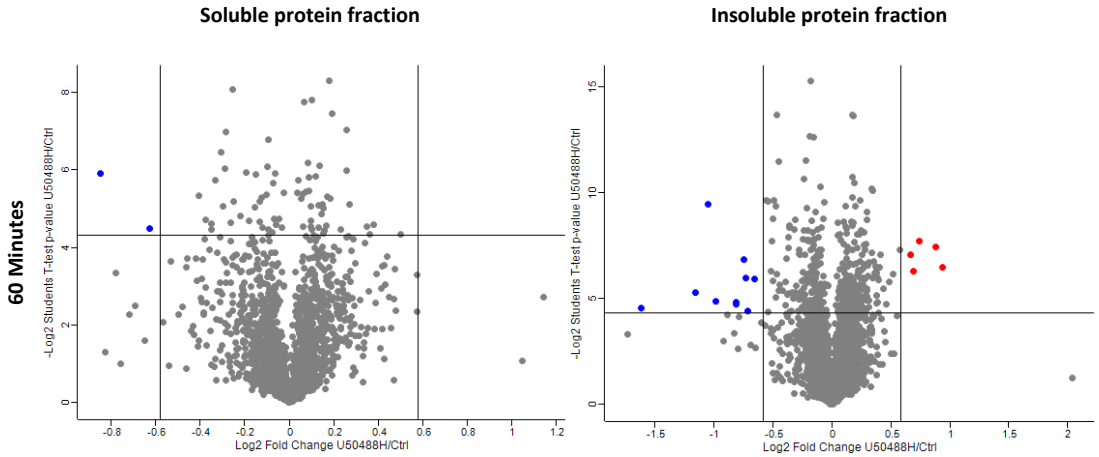
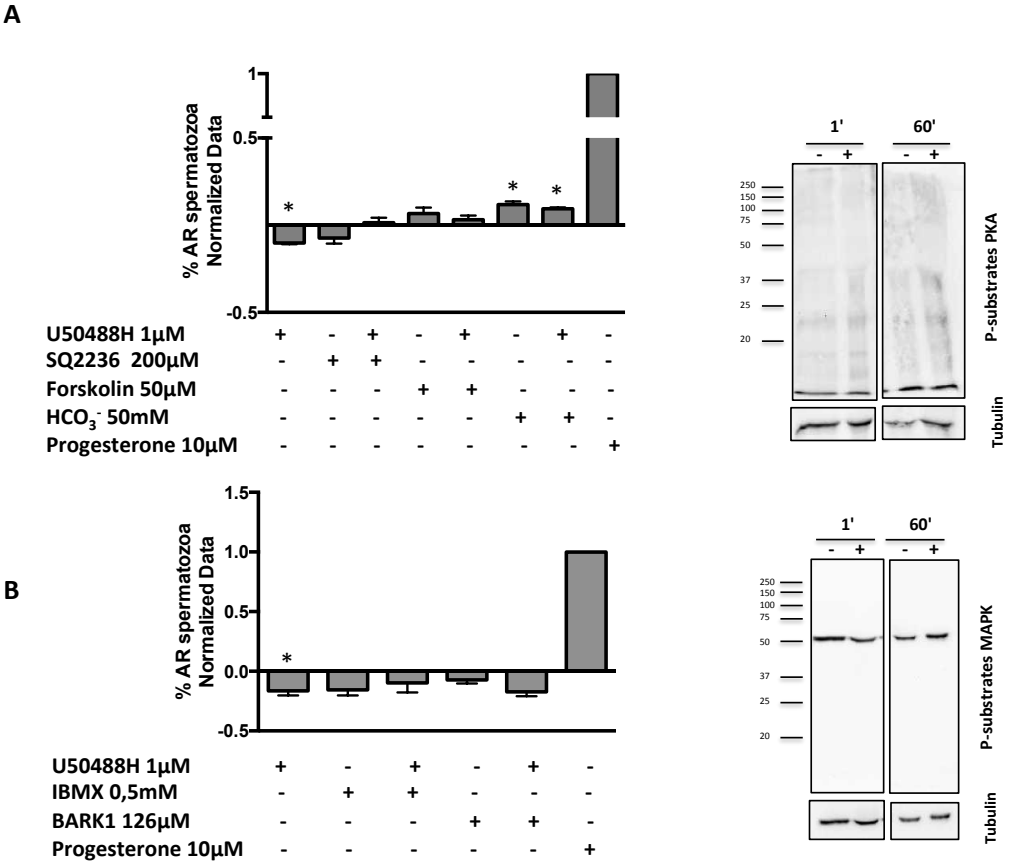


Supplemental Figure S1. KOR expression in human spermatozoa (A) by Western blotting showing immunoreactivity at around 45 and 35 kDa. * indicate the different bands of KOR. (B) by immunofluorescence, in the sperm head, middle/postacrosomal region and tail. The nuclei are stained with Hoechst and are represented in blue (N=3). Scale bar 10 µm.

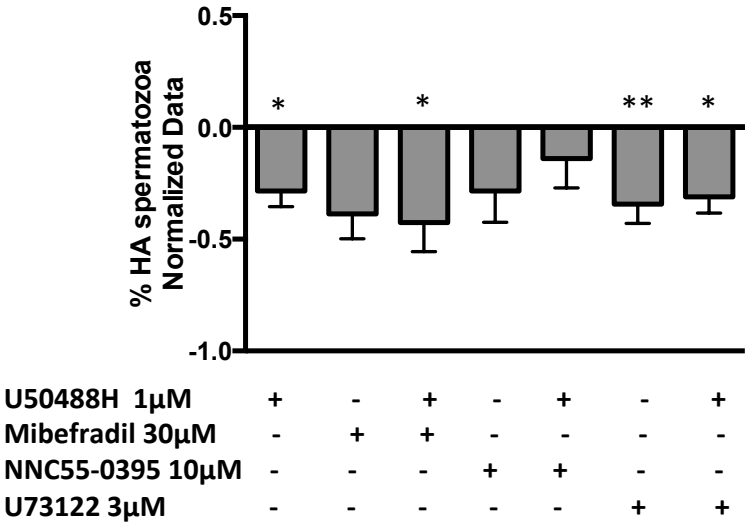


60 Minutes	
Soluble protein fraction	
Up-regulated proteins	---
Down-regulated proteins	KRT18 PPP6R3
Insoluble protein fraction	
Up-regulated proteins	GPRIN1 SEMA3F IPO11 USP6NL FGL2
Down-regulated proteins	ENTPD3 C4orf17 DDX50 C5orf49 TTC37 ARL14EP FBLN2 TBC1D1 SOGA1 HAX1

Supplemental Figure S2. Effect of U50488H on the human sperm proteome. (A) Overall Log_2 U50488H/Control TMT fold change as a functional of –Log statistical significance of U50488H/Control (p value< 0.05) of the soluble and insoluble protein fractions after 60 minutes U50488H treatment. In blue are indicated the down-regulated proteins by U50488H. In red are represented the U50488H-up-regulated proteins. Table containing the U50488H-regulated proteins following 60 minutes treatment in the soluble and insoluble protein fractions. U50488H/Ctrl > 1.5 in red and U50488H/Ctrl < 0.67 in blue.



Supplemental Figure S3. Study of the acrosome reaction downstream KOR in human spermatozoa. (A) Study of the acrosome reaction by 60 minutes co-incubation of U50488H (1 µM) and: SQ2236 (200 µM), the tmAC inhibitor; Forskolin (50 µM), the tmAC activator, and HCO3-(50 mM), the SACY activator. X axis shows the different treatments used for this study and the Y axis represents the normalized data of the % of acrosome reacted spermatozoa. The normalization was performed using the untreated samples and the acrosome reacted samples. N=6. (*p<0.05 vs Control). Immunoblotting assays showing the expression of the phosphorylated substrates of protein kinase A following 1 and 60 minutes U50488H treatment **(B)** Study of the acrosome reaction by 60 minutes co-incubation of U50488H (1 µM) and: IBMX (0,5 mM), the phosphodiesterases inhibitor, and BARK1 (126 µM), the GRK2 inhibitor. X axis shows the different treatments used for this study and the Y axis represents the normalized data of the % of acrosome reacted spermatozoa. The normalization was performed using the untreated samples and the acrosome reacted samples. (*p<0.05 vs Control). N=6. Immunoblotting assays showing the expression of the phosphorylated substrates of MAP kinases following 1 and 60 minutes U50488H treatment. tmAC: Transmembrane Adenylate cyclase. SACY: soluble adenylate cyclase.



Supplemental Figure S4. Role of KOR in human sperm hyperactive motility at 60 minutes. Study of the hyperactive motility by 60 minutes co-incubation of U50488H (1 µM) and: NNC-55-0395 (10 µM), the Catsper inhibitor; Mibefradil (30 µM), a calcium channel activator, and U73122 (3 µM), the PLC inhibitor. X axis shows the different treatments used for this study and the Y axis represents the normalized data of the % of hyperactive spermatozoa. The normalization was performed using the untreated samples and the acrosome reacted samples. N=8. (*p<0.05 and **p<0.01 vs Control).