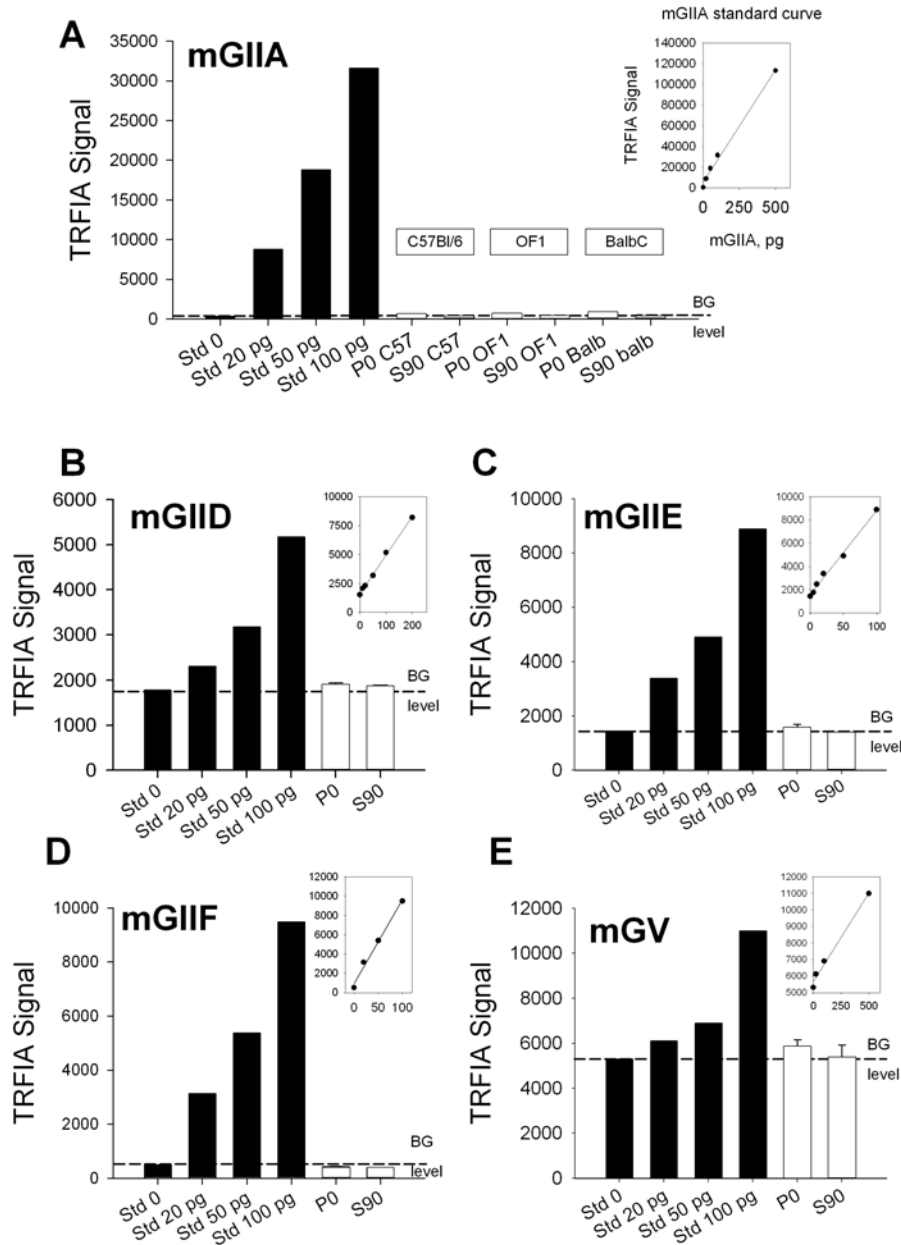


**Figure S1**

**Group X sPLA<sub>2</sub> is expressed in human spermatids.**

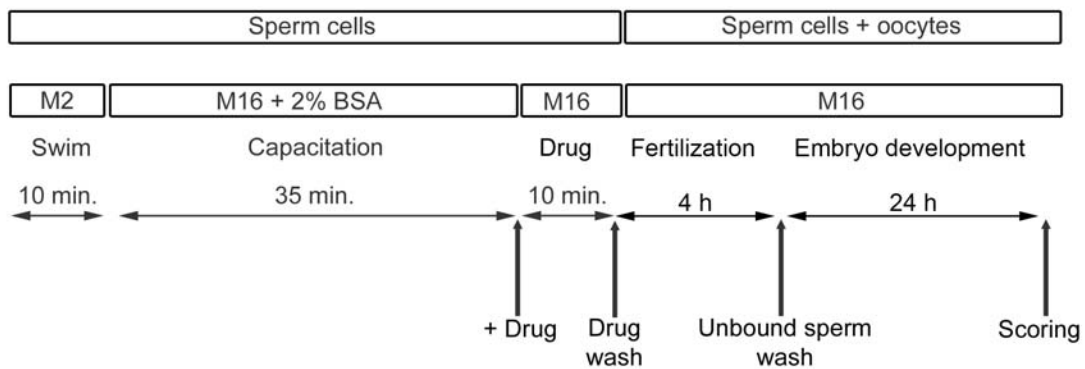
Immunohistochemistry of human testis performed with different antibodies raised against human sPLA<sub>2</sub>s. **(A)** hGX sPLA<sub>2</sub> is strongly expressed in spermatogenic cells except spermatogonia. Left: cross section of human seminiferous tubules at low magnification. Right: enlargement of the boxed region in the left panel: a strong staining surrounds spermatocytes (black arrows) and spermatids (red arrows). **(B)** Cross section of human seminiferous tubules at low magnification. Right: enlargement of the boxed region in the left panel: hGIIF sPLA<sub>2</sub> is strongly expressed in Leydig cells (purple arrows) and in testis boundary layers (green arrows) **(C)** hGV sPLA<sub>2</sub> is strongly expressed in Leydig cells (purple arrows) and spermatogonia (blue arrows). **(D)** Staining obtained with pre-immune serum.



**Figure S2**

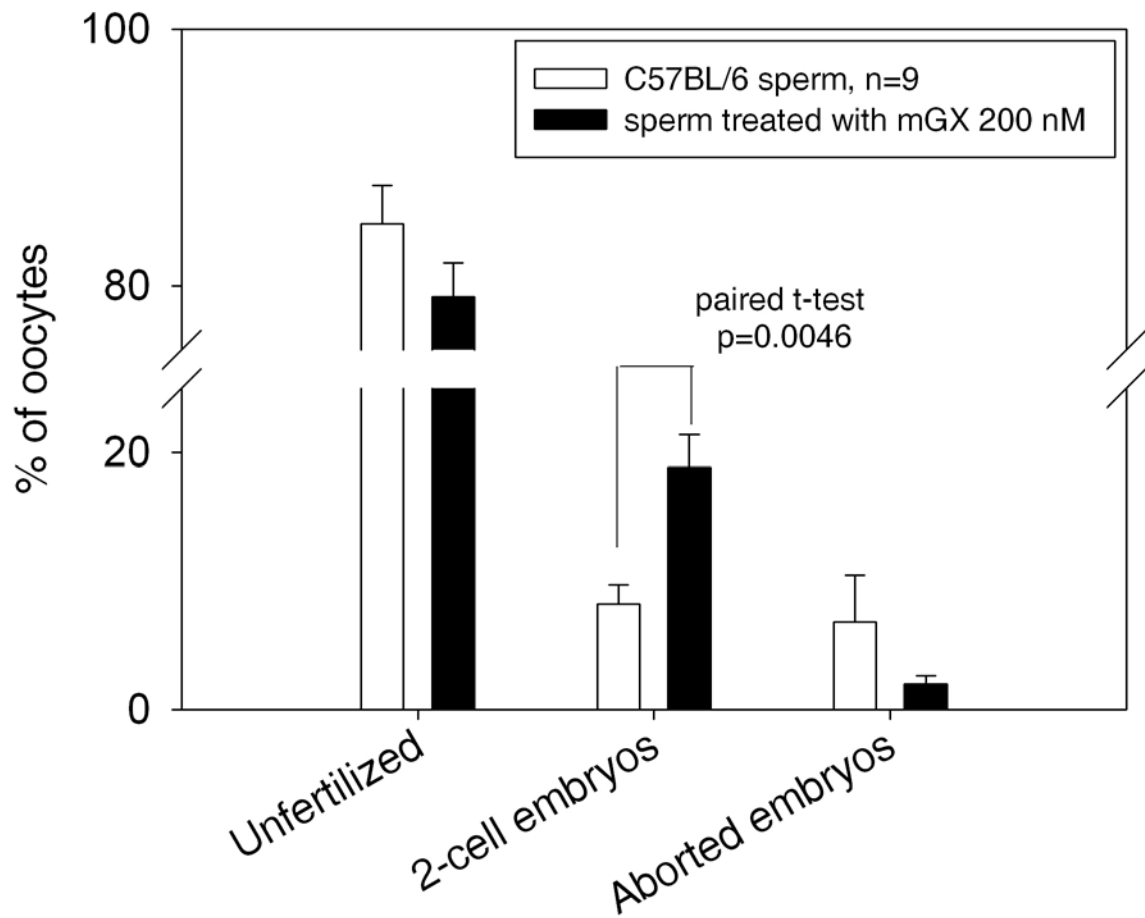
**mGIIA, mGIID, mGIIE, mGIIF and mGV are not detected by TR-FIA in mouse sperm cells.**

(A) TR-FIA signals measured with different concentrations of recombinant mGIIA, and in the pellets at 0 min (P0) and the supernatants at 90 min + A23187 (S90) obtained with capacitated sperm from C57BL/6J, OF1 and BALB/c males (n=3). No specific signal was measured above background level (BG level), as indicated by the dashed line. The inset shows the calibration curve with recombinant mGIIA, validating the sensitivity of the TR-FIA assay. (B, C, D and E) TR-FIA signals obtained with different concentrations of the indicated recombinant sPLA<sub>2</sub>s (n=2) and with the pellets at 0 min (P0) and the supernatants at 90 min + A23187 (S90) obtained with capacitated sperm from C57BL/6J (n=6). Insets show the calibration curves obtained with recombinant enzymes.



**Figure S3**  
**Schematic drawing of IVF experiments.**

Sperm were first capacitated for 35 min in M16-2% BSA and then incubated for the last 10 min with the different effectors as specified in Figure legends, *i.e.* mGX recombinant protein, sPLA<sub>2</sub> inhibitors or a combination of both. After treatment, sperm were washed by centrifugation to remove unbound effectors, putative mGX catalytic products and all acrosomal compounds released during sPLA<sub>2</sub>-induced AR. Finally, washed sperm were introduced into droplets containing oocytes (20-109 oocytes per experiment). After 4 h of gamete mixing, unbound sperm were washed away and IVF outcomes were scored at 24 h.



**Figure S4**

**Recombinant mGX doubles the yield of viable mouse embryos in IVF experiments performed with sperm and oocytes obtained from C57BL/6J males and females, respectively.**

IVF was performed either with control sperm (untreated, white bars) or the same sperm briefly treated with 200 nM mGX 10 min before the end of the capacitation period (black bars, n=9 males, p=0.004). Unfertilized oocytes, 2-cell embryos and aborted embryos were scored.