

## **Supplementary Information**

### **Mitochondrial aquaporin-8-mediated hydrogen peroxide transport is essential for teleost spermatozoon motility**

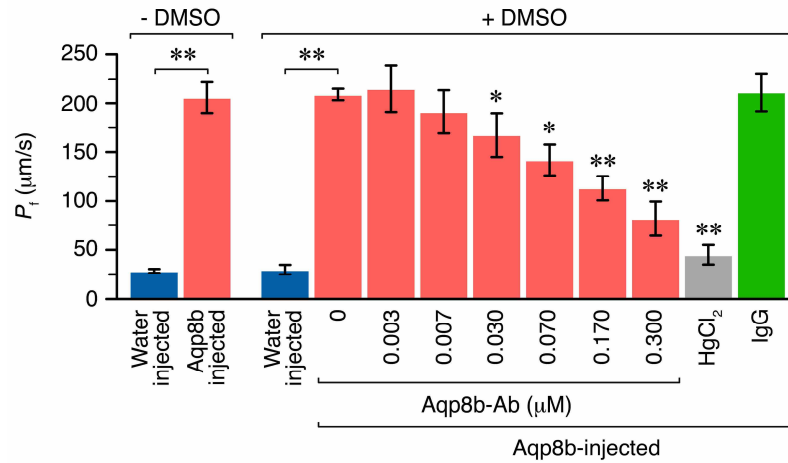
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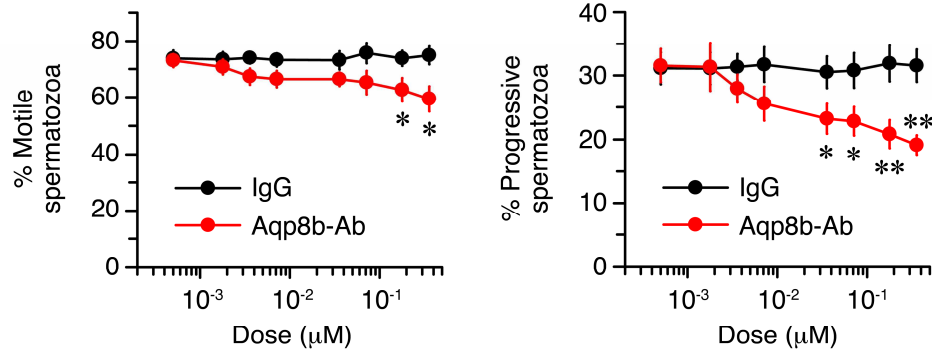
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**Figure S1 | Specific immunological inhibition of Aqp8b-mediated water transport in *X. laevis* oocytes.** Osmotic water permeability ( $P_f$ ) of water-injected (control) and Aqp8b-expressing oocytes, in the presence or absence of 0.5% DMSO, and increasing amounts of exogenously added Aqp8b antibody (Aqp8b-Ab), 0.3  $\mu\text{M}$  rabbit IgG or 100  $\mu\text{M}$  mercury. Data are the mean  $\pm$  SEM of three independent experiments using different oocyte batches ( $n = 12$  oocytes/treatment). \* $P < 0.05$ ; \*\* $P < 0.01$ , with respect to Aqp8b oocytes not exposed to Aqp8b-Ab, or between groups indicated in brackets.



**Figure S2 | Effect of increasing doses of IgG or Aqp8b-Ab on seabream spermatozoa motility and progressivity upon activation in SW for 5-10 s. Data are the mean  $\pm$  SEM ( $n = 8$  fish). \* $P < 0.05$ ; \*\* $P < 0.01$ , with respect to spermatozoa treated with IgG.**

Figure 1d: Aqp8b in sperm

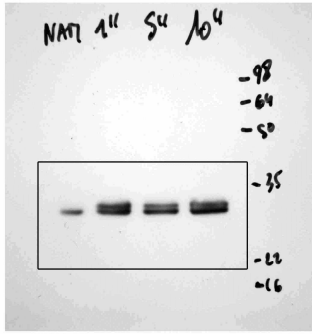


Figure 1d: Tubulin in sperm

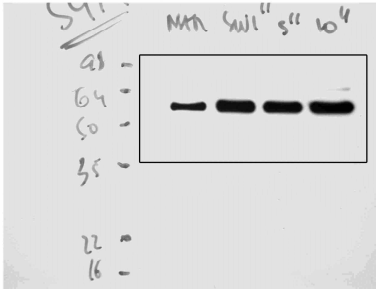


Figure 1e: Aqp8b in Mc and IMM

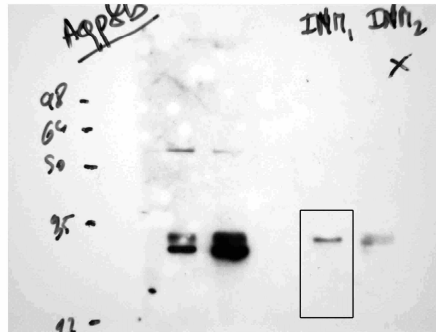
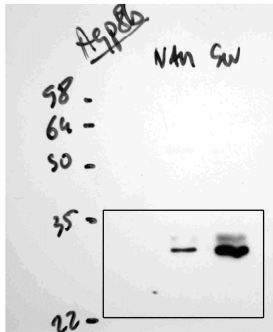


Figure 1e: PhB in Mc and IMM

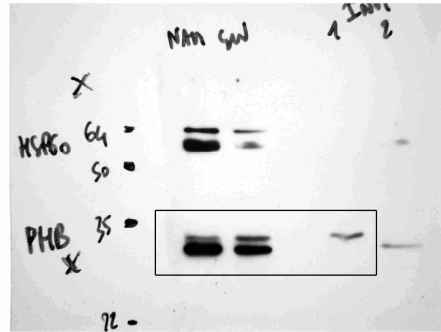


Figure 1e: Hsp60 in Mc and IMM

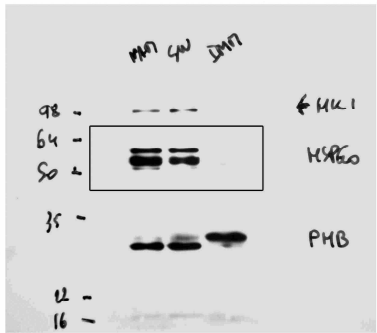


Figure 1e: Hk1 in Mc and IMM

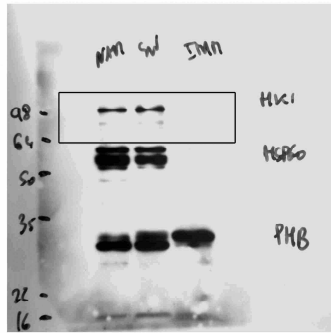


Figure 1f: Aqp8b

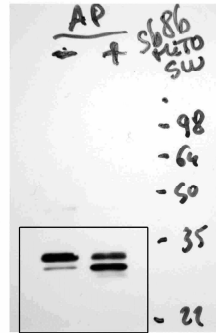
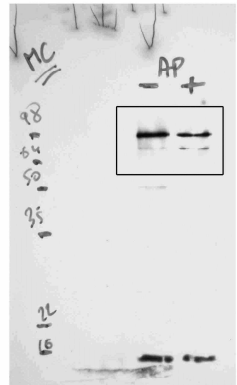


Figure 1f: Hk1



**Figure S3 | Full-length blot images for Figure 1.** The boxes indicate the part of the blot shown in the main figures.

Figure 2a

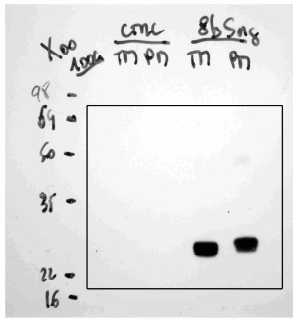


Figure 2g

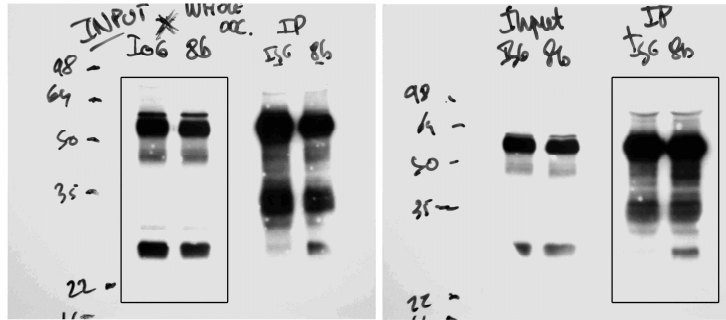
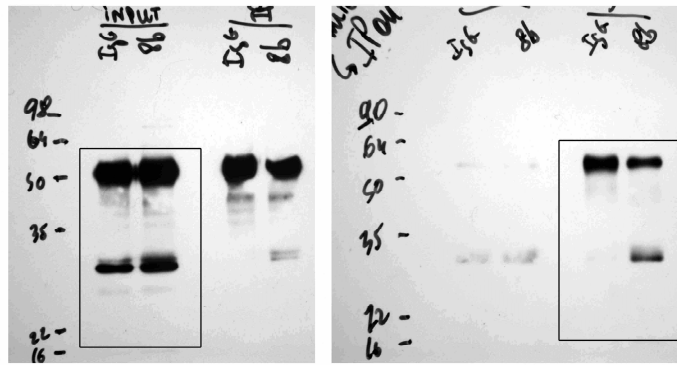


Figure 2h



**Figure S4 | Full-length blot images for Figure 2.** The boxes indicate the part of the blot shown in the main figures.

Figure 3d



**Figure S5 | Full-length blot images for Figure 3.** The boxes indicate the part of the blot shown in the main figures.

Figure 4h: sperm

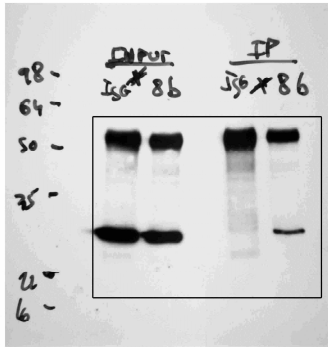


Figure 4h: Mc



Figure 4i: Aqp8b

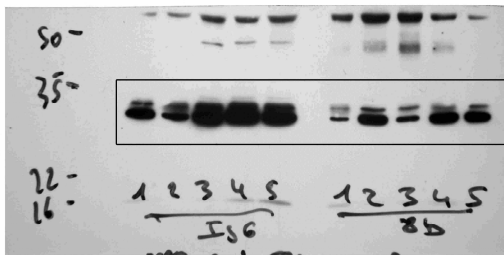
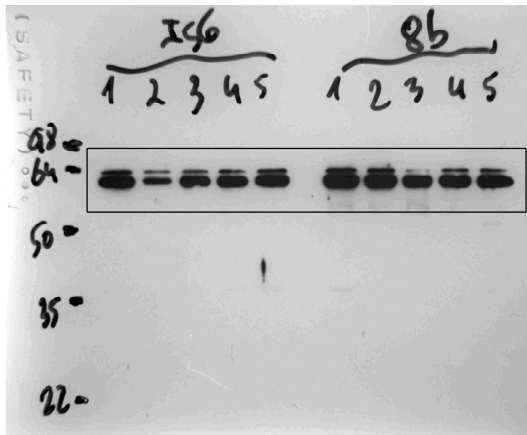


Figure 4i: Hsp60



**Figure S6 | Full-length blot images for Figure 4.** The boxes indicate the part of the blot shown in the main figures. Note that in Figure 4h (Mc blot), the lines IgG and Aqp8b-Ab in the original blot were inverted.