

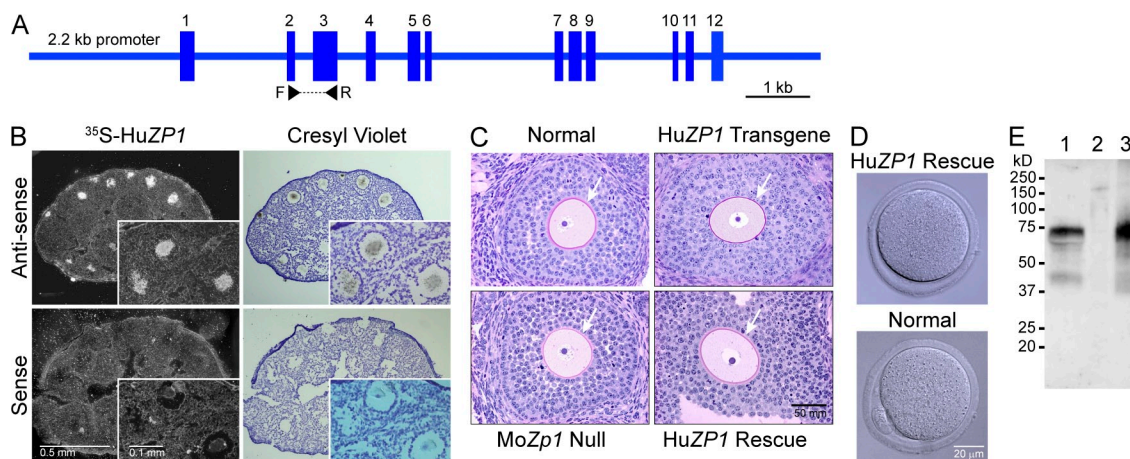
Baibakov et al., <http://www.jcb.org/cgi/content/full/jcb.201203062/DC1>

Figure S1. Transgenic mice expressing human *ZP1*. (A) Schematic of a 11.9-kbp human *ZP1* transgene composed of a 2.2-kbp promoter, 8.2-kbp coding region, and 1.5-kbp 3' of the last exon. Exons are indicated by numbers. Arrowheads represent exon-spanning forward (F) and reverse (R) PCR primers used for genotyping. (B) In situ hybridization of human *ZP1* transgenic mouse ovaries. Specific ^{35}S -labeled sense and antisense human *ZP1* cRNA probes were hybridized to Tissue-Tek OCT-embedded ovarian sections from 15-d-old human *ZP1* transgenic females. Sections were viewed with bright- and dark-field microscopy. Insets are magnifications. (C) Plastic-embedded ovarian sections (5 μm) from 3–4-wk-old normal, human *ZP1* transgenic, mouse *Zp1*-null, and human *ZP1* rescue female mice were stained with periodic acid Schiff's reagent to highlight the zona pellucida (arrows) and counterstained with hematoxylin. (D) DIC microscopy of human *ZP1* rescue and normal ovulated eggs. (E) Immunoblot of human and mouse eggs. Lane 1, noninseminated immature human oocytes ($n = 6$); lane 2, huZP4 transgenic eggs ($n = 30$); lane 3, huZP1 transgenic eggs ($n = 30$). The blot was probed with a monoclonal antibody specific to human *ZP1*, which was detected with HRP-conjugated secondary antibodies and chemiluminescence. Molecular masses are indicated to the left.

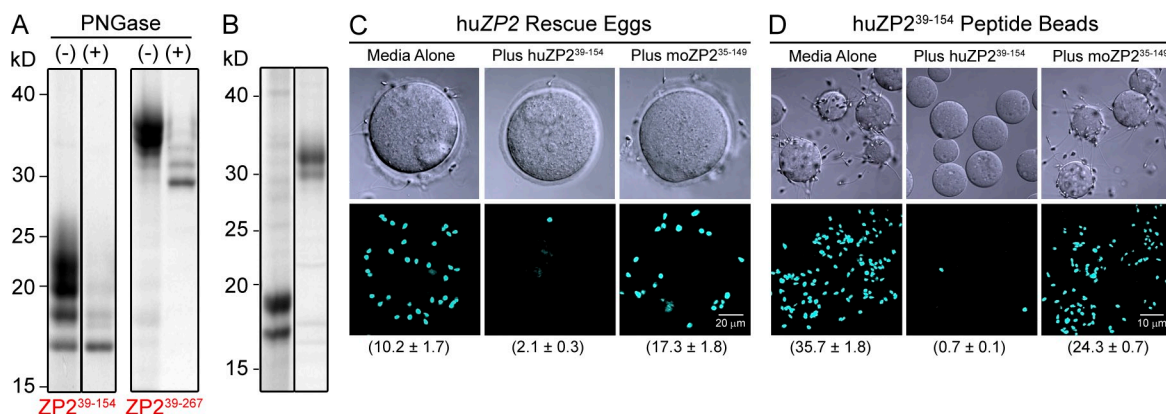


Figure S2. Human and mouse *ZP2* peptides. (A) Coomassie blue-stained SDS-PAGE of recombinant huZP2³⁹⁻¹⁵⁴ and huZP2³⁹⁻²⁶⁷ peptides expressed in High Five insect cells before (–) and after (+) deglycosylation with PNGase F to release three (N⁸⁹, N¹⁰⁵, and N¹²²) or four (N⁸⁹, N¹⁰⁵, N¹²², and N²²³) N-glycans, respectively. (B) Coomassie blue-stained SDS-PAGE of recombinant moZP2³⁵⁻¹⁴⁹ (left) and moZP2³⁵⁻²⁶² (right) peptides after purification on IMAC beads. (C) DIC (top) and confocal (bottom) images after staining with Hoechst. Capacitated human sperm binding to huZP2 rescue eggs (left) was inhibited by coinubation with huZP2³⁹⁻¹⁵⁴ (middle), but not moZP2³⁵⁻¹⁴⁹ (right), peptides. Number (mean \pm SEM) of bound sperm from three independent experiments, each with seven to eight eggs. (D) Same as C but with 18–27 huZP2³⁹⁻¹⁵⁴ peptide beads.

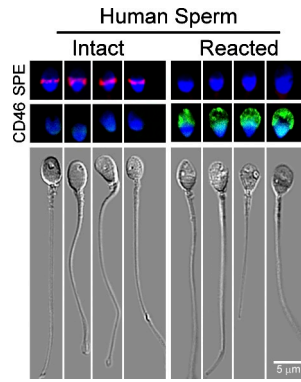
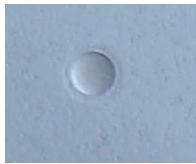


Figure S3. **Acrosome status of human sperm.** Acrosome-intact and -reacted human sperm bound antibodies to SPESP1 (SPE; top) and CD46 (middle), respectively. Sperm, stained with Hoechst and antibody, were imaged by confocal microscopy (top and middle) and DIC (bottom). Bar, 5 μ m.

Table S1. **Fertility of human *ZP1* transgenic and rescue mice**

Genotype	Ovulated eggs	Litter size
Human <i>ZP1</i> transgenic	29.0 \pm 7.5	7.8 \pm 0.7
Human <i>ZP1</i> rescue	34.3 \pm 2.8	6.3 \pm 1.5
Normal	26.5 \pm 3.7	6.7 \pm 1.0

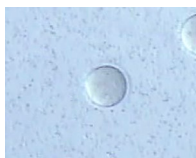
Values are given as means \pm SEM.



Video 1. **Human sperm interacting with huZP1 rescue eggs.** Human sperm were incubated with huZP1 rescue eggs for 4 h at 37°C in HTF and imaged for 1 min at 30 frames/s with a charge-coupled device (CCD) camera (KP-D20A/B) mounted on a stereomicroscope (SMZ-U; Nikon) using Studio II Plus software (Pinnacle) and exported as MPEG files.



Video 2. **Human sperm interacting with huZP2 rescue eggs.** Human sperm were incubated with huZP2 rescue eggs for 4 h at 37°C in HTF and imaged for 1 min at 30 frames/s with a CCD camera (KP-D20A/B) mounted on a stereomicroscope (SMZ-U) using Studio II Plus software and exported as MPEG files.



Video 3. **Human sperm interacting with huZP3 rescue eggs.** Human sperm were incubated with huZP3 rescue eggs for 4 h at 37°C in HTF and imaged for 1 min at 30 frames/s with a CCD camera (KP-D20A/B) mounted on a stereomicroscope (SMZ-U) using Studio II Plus software and exported as MPEG files.



Video 4. **Human sperm interacting with huZP4 transgenic eggs.** Human sperm were incubated with huZP4 transgenic eggs for 4 h at 37°C in HTF and imaged for 1 min at 30 frames/s with a CCD camera (KP-D20A/B) mounted on a stereomicroscope (SMZ-U) using Studio II Plus software and exported as MPEG files.