

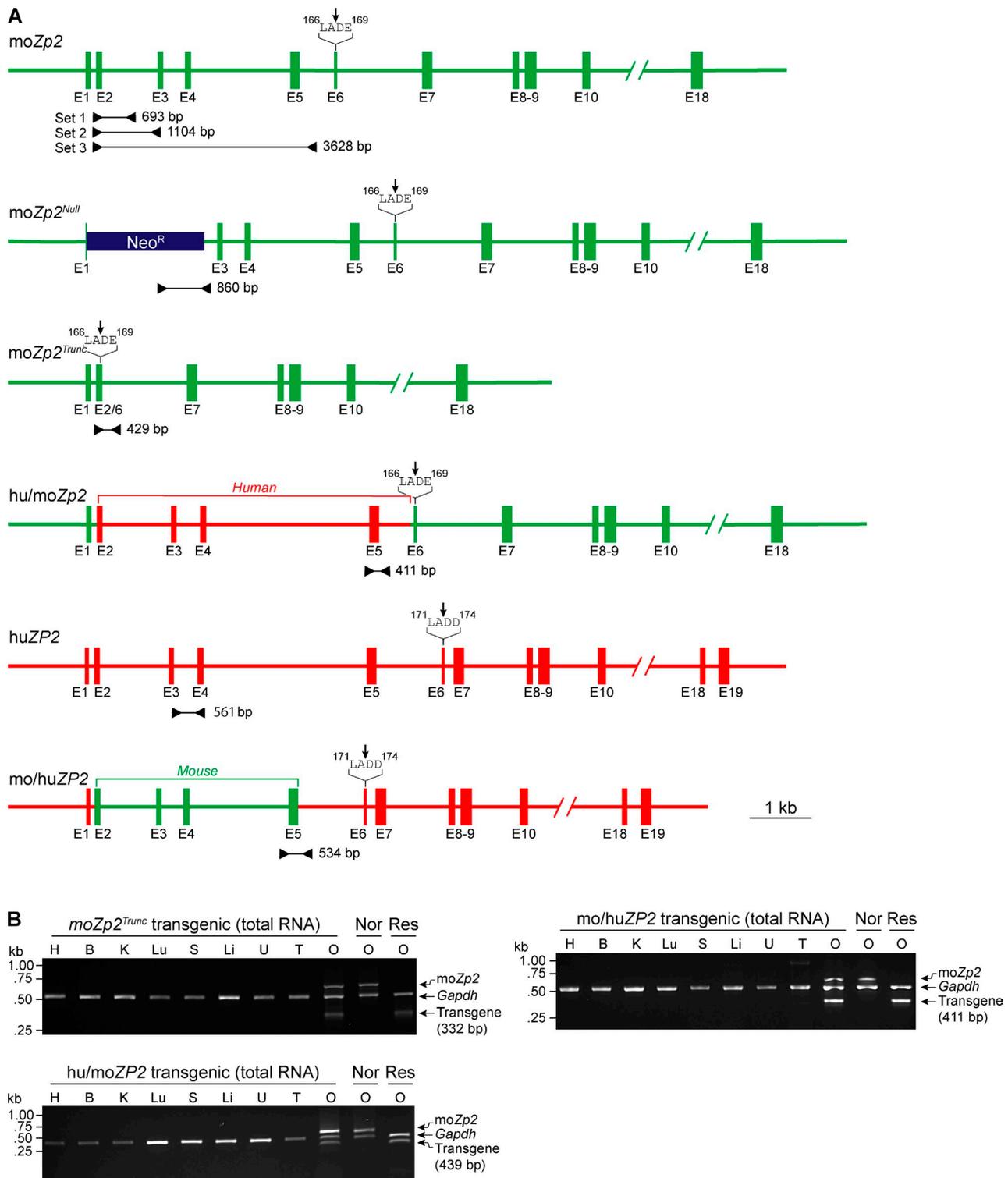
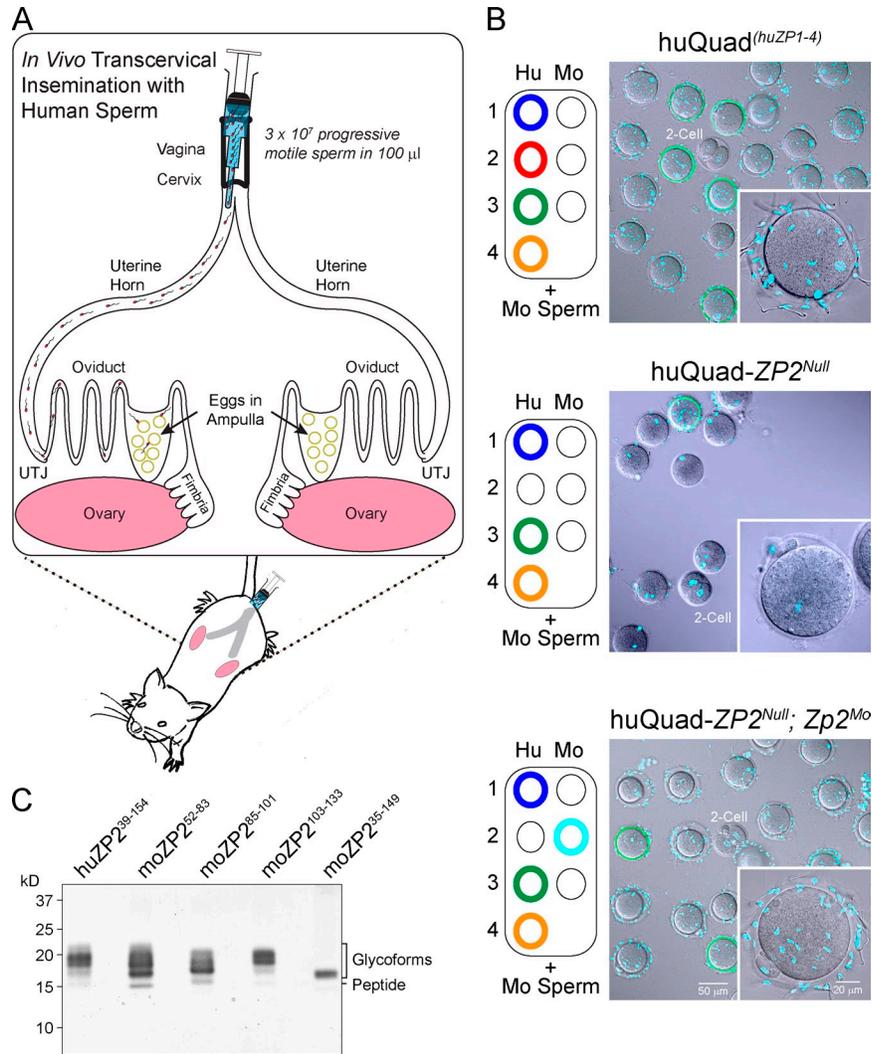
Avella et al., <http://www.jcb.org/cgi/content/full/jcb.201404025/DC1>

Figure S1. **Transgenes encoding truncated isoforms and human/mouse chimeric ZP2.** (A) Exon maps of endogenous mouse *Zp2* (*moZp2*) and transgenes of truncated mouse *Zp2* (*moZp2<sup>Trunc</sup>*), chimeric *hu/moZp2*, human *ZP2* (*huZP2*), and chimeric *mo/huZP2* assembled from BAC clones by DNA recombining. Exons are indicated by the numbers below; red and green exons encode human mouse proteins, respectively. The postfertilization cleavage site (arrow) of *ZP2* is encoded by exon 6. PCR products for genotyping are indicated below each construct. (B) Tissue-specific expression of transgenes was determined by reverse transcription followed by RT-PCR of total RNA isolated from tissues from transgenic, normal (Nor), and rescue (Res, transgenic line crossed into the *Zp2<sup>Null</sup>* background) mouse lines. H, heart; B, brain; K, kidney; Lu, lung; S, spleen; Li, liver; U, uterus; T, testis; O, ovary. RT-PCR products from primers (Table S5): mouse *Zp2*, 703 bp; *hu/moZp2*, 439 bp; *mo/huZP2*, 411 bp; and *moZp2<sup>Trunc</sup>*, 332 bp. Detection of *Gapdh* (510 bp) was used to ensure the integrity of isolated RNA.



**Figure S2. Transcervical insemination and sperm binding in transgenic mice.** (A) Schematic representation of transcervical insemination. Transgenic female mice were stimulated to ovulate with gonadotrophins, inseminated with human sperm ( $3 \times 10^7$  in  $100 \mu\text{l}$ ), and mated with vasectomized males to mimic physiological copulations. Eggs were collected 2 h later and fixed, then z projections from confocal microscopy were used to determine the number of sperm that had accumulated in the perivitelline space. (B) Mouse sperm binding to huQuad<sup>(huZP1-4)</sup>, huQuad-ZP2<sup>Null</sup>, and huQuad-ZP2<sup>Null</sup>; Zp2<sup>Mo</sup> eggs using mouse Zp3<sup>EGFP</sup> oocytes (green zona) and mouse two-cell embryos as positive and negative controls, respectively. The inset is a 2.5x magnification of a single egg. The schematic to the left of each panel reflects the protein composition of the zona pellucida surrounding the eggs being assayed after insemination with mouse sperm. (C) Coomassie blue-stained SDS-PAGE of recombinant ZP2 peptides expressed in High Five cells after purification of IMAC beads. Molecular mass is shown on the left.

**Table S1. Transgenic mouse lines**

Mouse line	moZP1	moZP2	moZP3	huZP1	huZP2	huZP3	huZP4	Zona
Normal	√	√	√	—	—	—	—	Yes
moZp2 <sup>Null</sup>	√	×	√	—	—	—	—	No
moQuad <sup>(huZP4)</sup>	√	√	√	—	—	—	√	Yes
moQuad-Zp2 <sup>Null</sup>	√	×	√	—	—	—	√	Yes
huQuad <sup>(huZP1-4)</sup>	×	×	×	√	√	√	√	Yes
huQuad-ZP2 <sup>Null</sup>	×	×	×	√	—	√	√	Yes
huQuad-ZP2 <sup>Null</sup> ; Zp2 <sup>Mo</sup>	×	√	×	√	—	√	√	Yes
huZP2 <sup>Rescue</sup>	√	×	√	—	√	—	—	Yes
huZP3 <sup>Rescue</sup>	√	√	×	—	—	√	—	Yes

√, human protein from transgene or endogenous mouse protein; ×, absence of mouse protein due to genetic ablation; —, absence of human protein in transgenic mouse

Table S2. **Fertility of mutant female mice**

Mouse line	Zona genes	Ovulated eggs <sup>a</sup>	2C embryos <sup>b</sup>	Pups <sup>c</sup>	Controls <sup>d</sup>
Normal	moZp1, moZp2, moZp3	20.6 ± 1.0 (11)	9.8 ± 2.7 (5)	8.1 ± 0.8 (45)	–
moQuad <sup>(huZP4)</sup>	moZp1, moZp2, moZp3, huZP4	26.4 ± 2.5 (11)	14.5 ± 0.9 (6)	8.8 ± 0.8 (15)	10.0 ± 0.4 (15)
moQuad-Zp2 <sup>Null</sup>	moZp1, moZp3, huZP4	13.0 ± 1.3 (9)	0 (5) <sup>e</sup>	Sterile <sup>f</sup>	9.4 ± 0.2 (15)
moZp2 <sup>Trunc</sup>	moZp1, moZp <sup>Trunc</sup> , moZp3	4.8 ± 0.8 (6)	0 (5) <sup>g</sup>	–	–
moZp2 <sup>Trunc</sup> ; huZP4	moZp1, moZp <sup>Trunc</sup> , moZp3, huZP4	27.0 ± 5.7 (6)	0 (5) <sup>e</sup>	Sterile <sup>f</sup>	8.8 ± 0.8 (18)
huQuad <sup>(huZP1-4)</sup>	huZP1, huZP2, huZP3, huZP4	17.5 ± 0.7 (11)	7.0 ± 1.4 (6)	5.2 ± 0.6 (15)	8.6 ± 0.9 (15)
huQuad-ZP2 <sup>Null</sup>	huZP1, huZP3, huZP4	13.3 ± 0.5 (9)	0 (5) <sup>e</sup>	Sterile <sup>f</sup>	8.8 ± 1.1 (15)
huQuad-ZP2 <sup>Null</sup> ; Zp2 <sup>Mo</sup>	huZP1, moZp2, huZP3, huZP4	18.8 ± 1.1 (10)	14.2 ± 1.9 (6)	7.8 ± 0.9 (15)	8.1 ± 0.7 (15)
huZP2 <sup>Rescue</sup>	moZp1, huZP2, moZp3	10.1 ± 5.5 (5)	5.4 ± 0.4 (5)	5.0 ± 0.6 (30)	8.8 ± 0.7 (15)
hu/moZp2 <sup>Rescue</sup>	moZp1, hu/moZp2, moZp3	12.6 ± 4.3 (5)	14.5 ± 3.7 (5)	–	–
mo/huZP2 <sup>Rescue</sup>	moZp1, mo/huZP2, moZp3	14.2 ± 3.1 (5)	11.0 ± 1.1 (5)	7.0 ± 0.7 (15)	10.4 ± 0.7 (15)

<sup>a</sup>Mean ± SEM eggs/animal (number of ovulations).

<sup>b</sup>Mean ± SEM 2-cell embryos/animal (number of plugged female mice).

<sup>c</sup>Mean ± SEM pups/litter (litters of ≥5 female mice).

<sup>d</sup>Mean ± SEM pups/litter (litters of ≥5 co-caged normal female mice).

<sup>e</sup>≥9 (mean) nonfertilized eggs/animal (number of plugged female mice).

<sup>f</sup>≥ 5 female mice

<sup>g</sup>Thin zona pellucida around ovulated eggs; no eggs/embryos recovered at E1.5

Table S3. **Primers to produce and evaluate transgenes**

Primer Set	F/R <sup>a</sup>	DNA sequence (5' to 3') <sup>b</sup>
moZp2-GalK	F	<u>GGTTTCTTTCCCTCTTATTACCCTTGTGACTTCAGTGAAGCCCTGTTGACAATTAATCATCGGCA</u>
	R	<u>gaagacagaacaattgtattcttactCTGGTTTCATCAGCAAGCTAGATCAGACTGCTCTGCTCCTT</u>
moZp2-huZP2	F	<u>caagttcactctttcttaacgTTTTctctctctctccatccagCATCTACAGGTTTCTTCCCTCTTATTACCCTTGTGACTTCAGT-</u> <u>GAACTCAGTAAGCGTTTCTCAGTTGGTAAATCC</u>
	R	<u>caggTggggggggggacacagagagagagggTgggggggggacagaggaagaagacagaacaattgtattcttactCTGGTTTTTCATCAG-</u> <u>CAAGCCTAGAGAAGACCCGTGGCAAGGAAAActgg</u>
huZP2-GalK	F	<u>TTGTTTGTAAAGTCCATTCTTCCCTGACTATCTCCCTGTCTCTTTCCAGCCTGTTGACAATTAATCATCGGCA</u>
	R	<u>TTCCCCCTGCAGTAGCCATATACCCCGAGCAGTCAGCCCGTTTCACTCACTCAGCACTGCTCTGCTCCTT</u>
huZP2-moZp2	F	<u>CAGGCTGGAGGTGAGTAATTCTGGAAGTGGAGGGGGGTATGGTAGCTTTGTTTGTAAAGTCCATTCTTCCCTTACTCTCCCTGTCT</u> <u>CTTCCAGCATCTACAGGTTTCTTCCCTCTTA</u>
	R	<u>TAAATCATGGTCTGATTTCTAAGCCCCCGCTGGTATAGATCATCATATTTCCCCCTGCAGTAGCCATATACCCCGAGCAGTCAGCCCGTT</u> <u>TCACTCACAGATATTAGATCTCTCTGCAGACA</u>
mo/huZP2	F	<u>GGTAGTGTCTTCTGCTATGGC</u>
5' junction	R	<u>CTTGACTACCTGGGGATGGA</u>
mo/huZP2	F	<u>TTTCCCCTGAGGAATCGAC</u>
3' junction	R	<u>ATCTAGGGTAGGGCCTGGAA</u>
hu/moZp2	F	<u>GGTGTACCTTCCAACATGG</u>
5' junction	R	<u>GAGCAGTCCAGTTTTGCTT</u>
hu/moZp2	F	<u>AATCGCTTGAACCCAGGAG</u>
3' junction	R	<u>GGACAGAGGAAGACAGAACAA</u>
Zp2 <sup>Trunc</sup>	F	<u>TTTGACGCTTCTCTGTAGC</u>
	R	<u>GGGACAGGATATGGGATGTC</u>

<sup>a</sup>F, forward primer; R, reverse primer.

<sup>b</sup>Fonts: moZp2 (normal); GalK (bold, underline); huZP2 (italic, underline).

Table S4. Primers used for genotyping transgenic mice

Primer set	F/R <sup>a</sup>	DNA sequence (5' to 3')	PCR product (bp)
Zp2 (Set 1)	F	TCCTCAGTCCGAGAATCCTG	693
	R	CTTGACTACCTGGGATGGA	
Zp2 (Set 2)	F	TCCTCAGTCCGAGAATCCTG	3,478
	R	GCTAGACACAGTGTACTCAACATGG	
Zp2 (Set 3)	F	TCCTCAGTCCGAGAATCCTG	1,104
	R	CCAAGGGTTGGACTCTGTGT	
Zp2 <sup>Null</sup>	F	GCCTGAAGAACGAGATCAGC	860
	R	CTTGACTACCTGGGATGGA	
hu/moZp2	F	AATCGCTTGAAGCCAGGAG	411
	R	GGACAGAGGAAGAAGACAGAACAA	
mo/huZP2	F	TTTCCCACTGAGGAATCGAC	534
	R	ATCTAGGGTAGGGCCTGGAA	
moZp2 <sup>Trunc</sup>	F	TTTGCACGCTTTCCTGTAGC	429
	R	GGGACAGGATATGGGATGTG	

<sup>a</sup>F, forward primer; R, reverse primer.

Table S5. Primers used for RT-PCR of RNA

Primer Set	F/R <sup>a</sup>	DNA sequence (5' to 3')	PCR product (bp)
hu/moZp2	F	TTTCCAGGCACTGTCACTTG	439
	R	TGTGGCTCTTGATACCATTG	
mo/huZP2	F	TCCTCAGTCCGAGAATCCTG	411
	R	TTGGTCCCCTTACTGTCGTC	
moZp2 <sup>Trunc</sup>	F	GGCAGGAGCATCTACAGGTT	406
	R	AGACGATCTTCTGCCCACTG	

<sup>a</sup>F, forward primer; R, reverse primer.