Supplementary Materials for

ZP2 peptide-beads that select human sperm *in vitro*, decoy mouse sperm *in vivo* and provide reversible contraception

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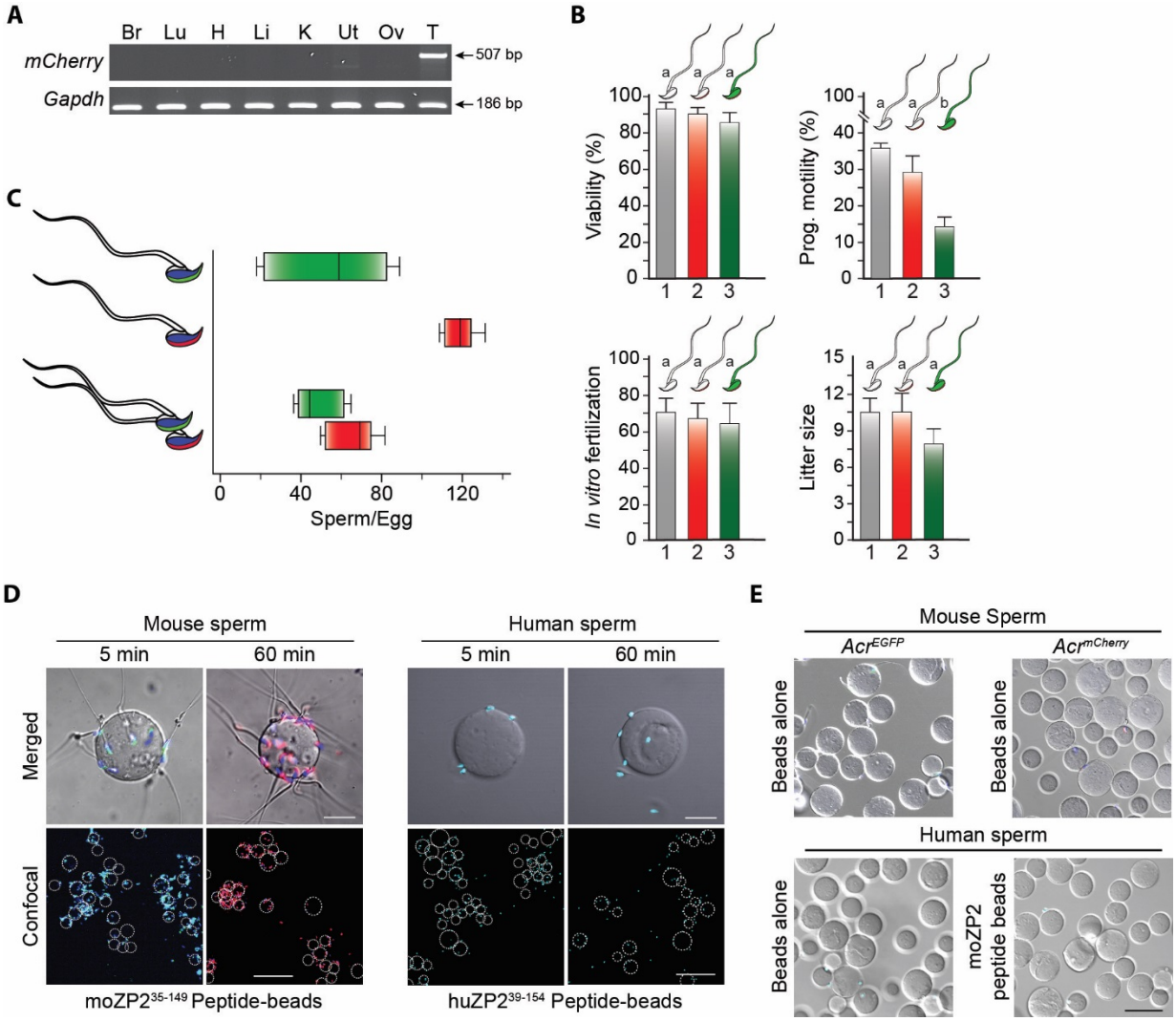

Fig. S1. Effect of incubation on sperm binding to ZP2 peptide-beads.
(**A**) To detect *Acr*^{mCherry} transcripts in transgenic mice, total RNA was extracted from brain (Br), lung (Lu), heart (H), liver (Li), kidney (K), uterus (U), ovary (Ov) and testis (T), and analyzed by RT-PCR using transgene specific primers (Table S1). GAPDH was used to as a load control and to ensure integrity of RNA. (**B**) Sperm viability, progressive-motility, *in vitro* fertilization and litter size of: control (grey); *Acr*^{mCherry} (red); and *Acr*^{mCherry}; *Prm1*^{EGFP}; *Figla*^{EGFP} (green) male mice (three biological replicates). Mean ± s.e.m. of >800 sperm. Statistical differences, P <0.05, are indicated by a letter different than a. (**C**) Quantification of *Acr*^{EGFP} sperm freshly released from the epididymis (green, top) and *Acr*^{mCherry} sperm after 1 hr in HTF/BSA incubation (red, middle) alone or mixed 1:1 (bottom) (three biological replicates) from Fig. 1C. Box plots reflect the median (vertical line) number of sperm binding to mouse eggs and data points within the 10th and 90th percentiles (error bars). Boxes include the middle two quartiles. (**D**) *Acr*^{EGFP} (incubated in HAT/BSA for 5 min) and *Acr*^{mCherry} (incubated for 60 min) mouse sperm (Hoechst-stained) binding to moZP2 peptide- beads (left). Human sperm (Hoechst-stained) incubated in HTF/BSA for 5 (left) or 60 (right) min binding to huZP2 peptide-beads (three biological replicates). DIC and confocal images were obtained after fixation and staining with Hoechst. Dashed circles in

lower panels represent beads. Scale bar of upper panels, 20 μm; of lower panels 100 μm. (**E**) As in D after 60 min incubation of mouse (*Acr*^{EGFP} or *Acr*^{mCherry}) sperm (upper) and human sperm (lower) with beads alone as well as human sperm with moZP2$^{35-149}$ peptide beads (three biological replicates). Scale bar, 50 μm.

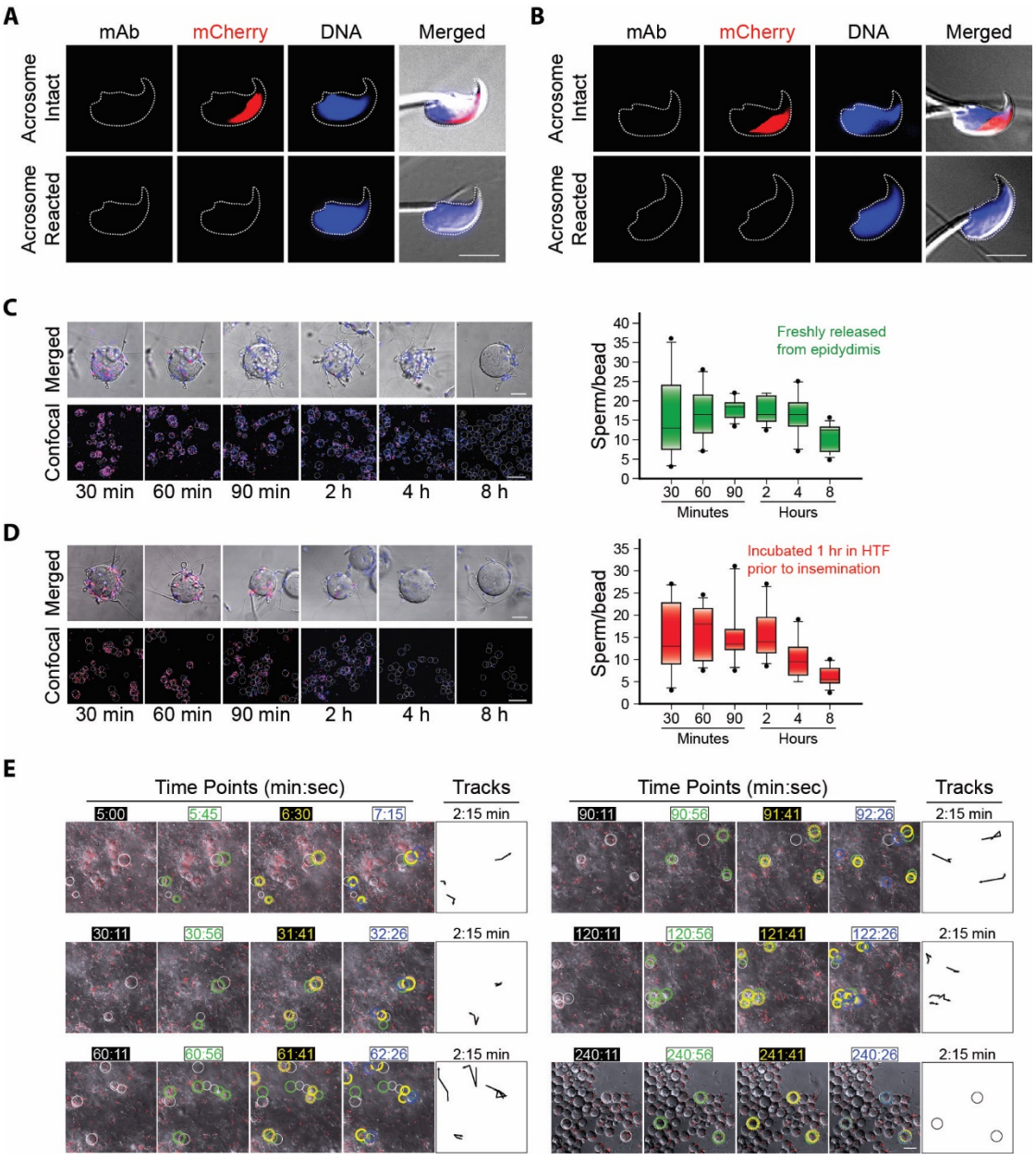

Fig. S2. Sperm binding and trans-location of moZP2 peptide-beads.
(**A**) After reduction and alkylation of disulfide bonds, moZP2$^{35\text{-}149}$ peptide isolated from Hi-Five cells was incubated with sperm from *Acr*^{mCherry} mice before (upper) or after (lower) acrosome exocytosis. The tissue was fixed with formaldehyde, stained with Hoechst, immunostained with a monoclonal antibody to the N-terminal ZP2-peptide and imaged by confocal microscopy and DIC. Asterisk, acrosome; Eq, equatorial segment; arrow, post-acrosomal region; scale bar, 5 μm. (**B**) Same as (**A**) but incubated with untransformed Hi-Five cell supernatant. (**C**) Epididymal *Acr*^{EGFP} mouse sperm (Hoechst-stained) binding to moZP2 peptide-beads (three biological replicates). Confocal images alone or merged with DIC were obtained at specified time points for 8 hr after insemination (left). Number of sperm bound per bead was quantified from Z projections (right). Box plots reflect the median (horizontal line) number of mouse sperm binding to moZP2 peptide-beads and data points within the 10th and 90th percentiles (error bars).

Boxes include the middle two quartiles and outliers are indicated by dots. Scale bar of upper panels, 20 μm; of lower panels 100 μm. (**D**) Same as **(C)**, but *Acr*^{mCherry} sperm were incubated in HAT/BSA for 60 min prior to insemination (three biological replicates). **(E)** Movie frames of *Acr*^{mCherry} sperm (incubated in HAT/BSA for 60 min prior to insemination) binding and trans-locating ZP2 peptide-coated beads. Each circle represents an individual bead tracked over 2:15 min and the color reflects the position of the bead at zero (white), 0:45 min (green), 1:30 min (yellow) and 2:15 min (blue) time points. The change from dashed to solid circles reflects the historic position of the bead in the new time frame. Trans-location tracks of individual beads are summarized at right, except at 4 hr where circles indicate no bead movement. Each series of four images spanning 2:15 min began at roughly 5 min (left, top), 30 min (left, middle), 60 min (left bottom), 90 min (right, top), 2 hr (right, middle) and 4 hr (right, bottom) after insemination. Experiment was performed in three biological replicates. Scale bar, 50 μm.

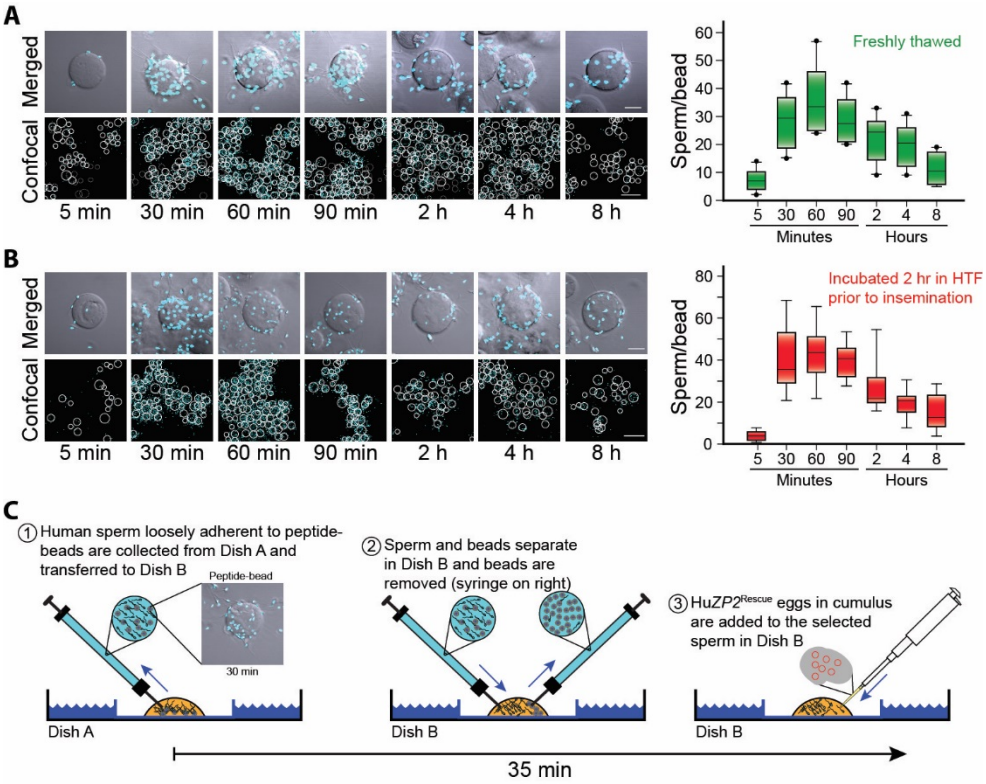

Fig. S3. Selection of human sperm with huZP2 peptide-beads.
(**A**) Same as fig. S2C, but with human sperm. Scale bar of upper panels, 20 μm; of lower panels 100 μm. (**B**) Same as fig. S2D, but with human sperm. (**C**) After 30 min of incubation of human sperm and huZP2 peptide-beads, the peptide-beads with loosely adherent sperm were transferred from Dish A to Dish B (1). The human sperm dissociated from the peptide beads in Dish B and after removal of beads, only unbound sperm remained (2). Eggs in cumulus mass were added (3) to the unbound human sperm in Dish B and assayed for binding to and penetration of the zona pellucida. Isolation of the human sperm takes 35 min.

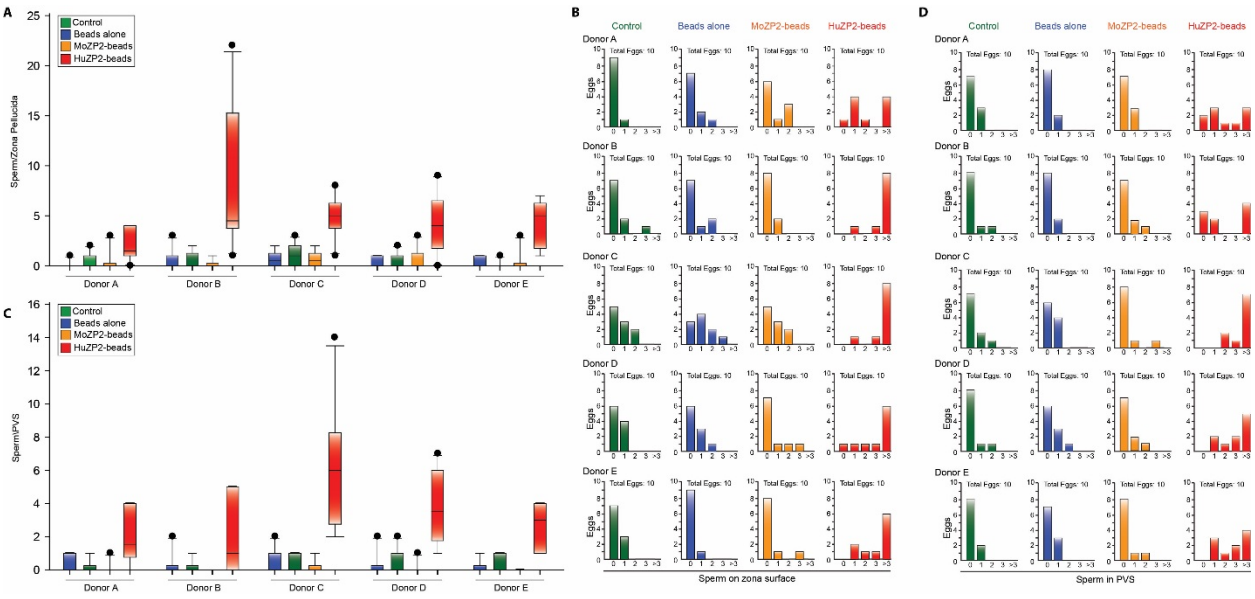

Fig. S4. Selection of superior human sperm from individual fertile donors.
(**A**) Human sperm from five different fertile donors (Donors A-E) were unselected or selected on the basis of binding to beads alone, to moZP2 peptide beads, or to huZP2 peptide-beads and tested for their ability to bind to the surface of the zona pellucida surrounding hu*ZP2*^{Rescue} eggs (see fig. S3C). Box plots reflect the median (horizontal line) number of human sperm binding (3 replicates from 5 sperm donors) and data points within the 10th and 90th percentiles (error bars). Boxes include the middle two quartiles and outliers are indicated by dots. (**B**) The number of hu*ZP2*^{Rescue} eggs with 0, 1, 2, 3 or >3 sperm bound to the zona surface. Sperm were selected under the conditions in (**A**) from each of five donors. (**C**) Same as (**A**), but assayed for sperm penetration into the perivitelline space. (**D**) Same as (**B**), but for sperm present in the perivitelline space.

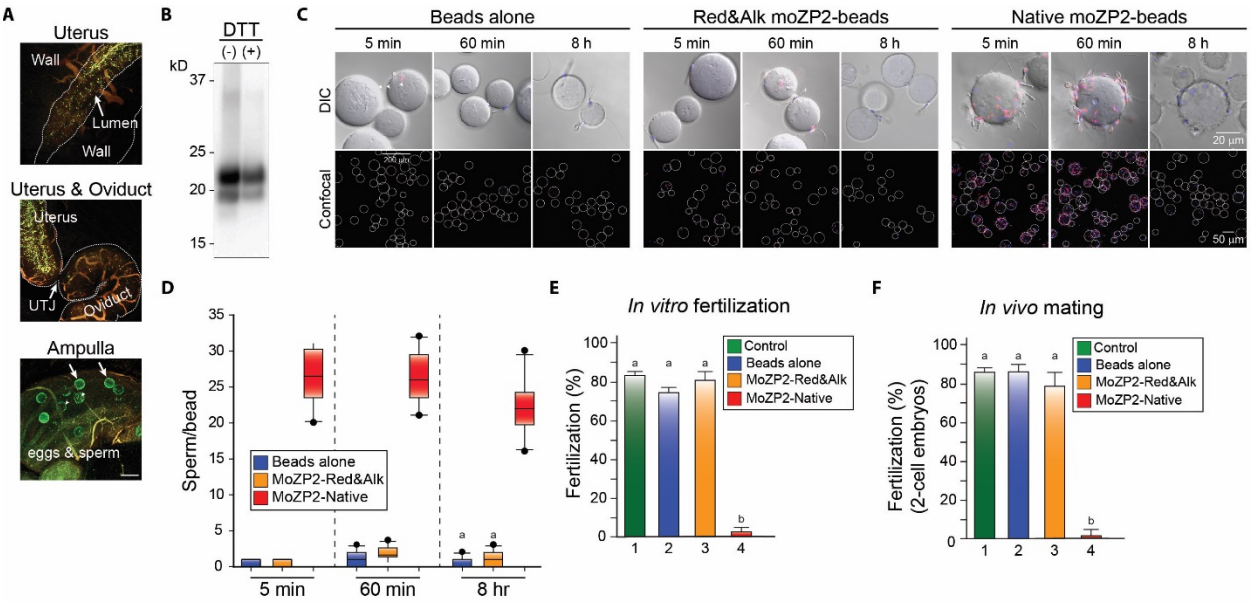

Fig. S5. Sperm binding to native mouse ZP2 peptide beads and inhibition of fertilization. (**A**) Control for Fig. 5F in which *Zp3*^{EGFP} female mice were hormonally stimulated and mated with *Acr*^{mCherry}; *Prm1*^{EGFP}; *Figla*^{EGFP} male mice after transcervical administration of media. Following clarification, confocal images were obtained to assay migration of fluorescently-tagged sperm in the uterus (top, acrosome-intact sperm) oviduct (middle, mixture of acrosome-intact and –reacted sperm) and interaction with eggs in the ampulla of the oviduct (bottom, most acrosome-reacted sperm). In the middle panel: arrow indicates UTJ, uterotubal junction. In the bottom panel: arrows, *Zp3*^{EGFP} eggs; arrowheads, *Acr*^{mCherry}; *Prm1*^{EGFP}; *Figla*^{EGFP} sperm. Orange auto fluorescence. Scale bar, 200 µm. (**B**) Immunoblot of moZP2$^{35-149}$ eluted from 1 x 10^{6} beads before (-) and after (+) reduction and alkylation of disulfide bonds using IE-3, a monoclonal antibody that binds to the N-terminus of ZP2. Molecular masses, kDa, indicated at left. (**C**) Confocal and DIC images of capacitated epididymal *Acr*^{mCherry} mouse sperm (Hoechst-stained) binding to beads alone (left), reduced and alkylated (middle) and native moZP2$^{35-149}$ peptide-beads at 5 min, 60 min or 8 hr after insemination. Scale bar of upper panels, 20 µm; of lower panels 100 µm. (**D**) Quantification of capacitated mouse sperm binding to beads alone (blue) and moZP2$^{35-149}$ peptide beads before (red) and after (yellow) reduction and alkylation to disrupt disulfide bonds. Box plots reflect the median (line) and data points within the 10th and 90th percentiles (error bars). Boxes include the middle two quartiles and outliers are indicated by dots. (**E**) Eggs in cumulus mass were inseminated with capacitated sperm in the presence of: (1) media; (2) beads alone; (3) reduced and alkylated moZP2$^{35-149}$ peptide beads; or (4) native moZP2$^{35-149}$ peptide beads. Fertilization (%) was determined by the number of 2-cell embryos divided by the number of eggs plus 2-cell embryos 40 hr after insemination. Statistical differences, P <0.05, are indicated by a letter different than a. (**F**) Gonadotrophin-stimulated female mice were mated after transcervical administration of: (1) media; (2) beads alone; (3) reduced and alkylated moZP2$^{35-149}$ peptide beads; or (4) native moZP2$^{35-149}$ peptide beads. Fertilization (%) was determined by the number of 2-cell embryos divided by the number of eggs plus 2-cell embryos recovered from the oviduct 40 hr later. Statistical differences, P <0.05, are indicated by a letter different than a.

Table S1. Primers

Primer Set	**F/R**	**DNA sequence (5' to 3')**	**PCR product (bp)**
Acr^{mCherry}	F	TTTGTGAGGTCACAGCTTGC	507
	R	GTAGATGAACTCGCCGTCCT	
Gapdh	F	GGTTGTCTCCTGCGACTTCA	186
	R	GGGTGGTCCAGGGTTTCTTA	