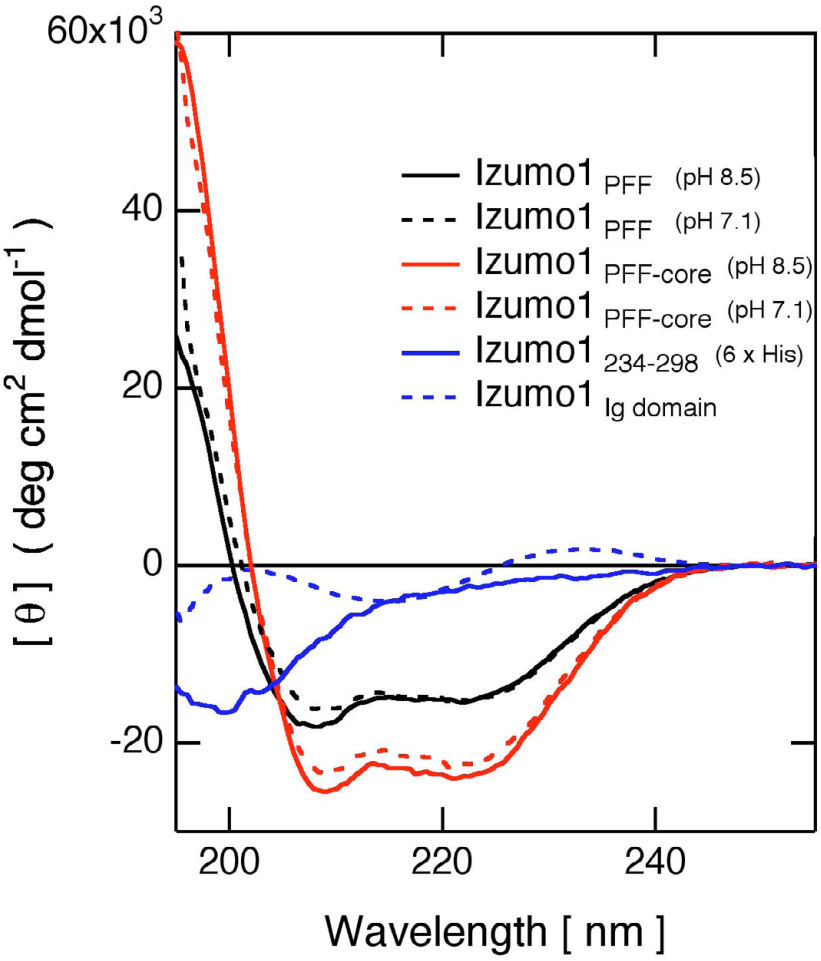

Fig. S1. Circular dichroism (CD) spectra of IZUMO1 fragments. CD spectra of $IZUMO1_{PFF}$ at pH 8.5 (black), $IZUMO1_{PFF}$ at pH 7.1 (broken black), $IZUMO1_{PFF\text{-}core}$ at pH 8.5 (red), $IZUMO1_{PFF\text{-}core}$ at pH 7.1 (broken red), $IZUMO1_{234\text{-}298}$ at pH 8.5 (blue) and $IZUMO1_{Ig\ domain}$ at pH 8.5 (broken blue). For CD measurements, the protein concentration was 10 μM, except $IZUMO1_{Ig\ domain}$ (20 μM).

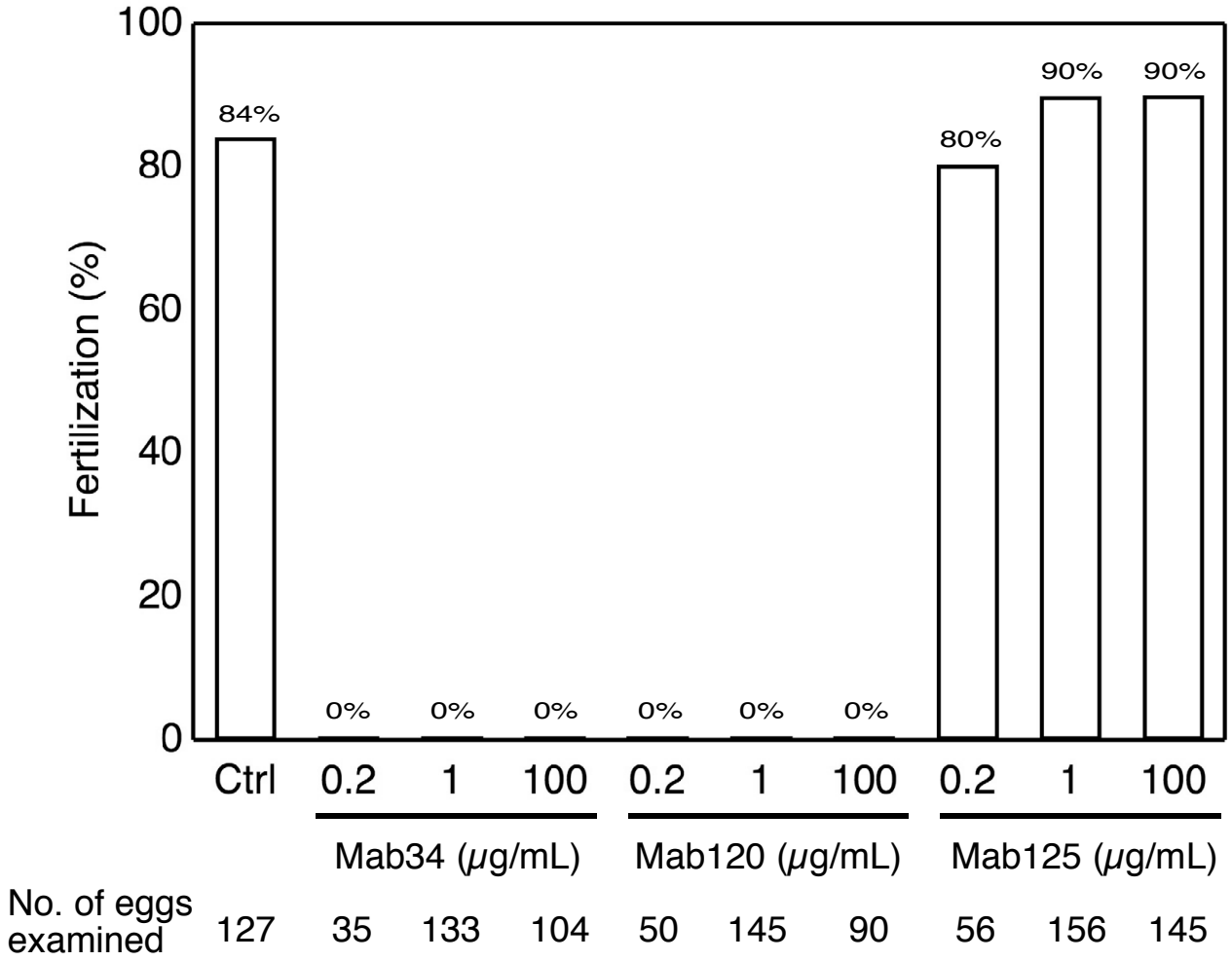

Fig. S2. Concentration dependence of anti-IZUMO1 antibody on the inhibition of *in vitro* fertilization. The average numbers of fused spermatozoa that were observed 30 minutes after insemination were calculated (*n*=3). Analyses were performed in the presence of 0.2, 1 and 100 μg/ml of control IgG, Mab34, Mab120 and Mab125 antibodies.

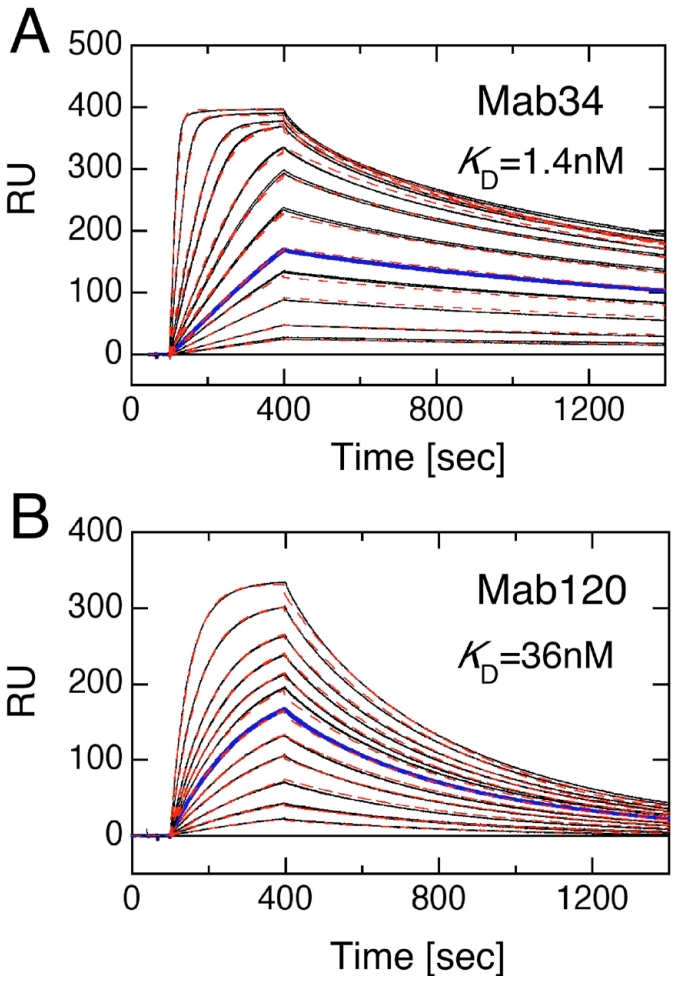

Fig. S3. The surface plasmon resonance (SPR) spectra of immobilized antibodies with various concentrations of IZUMO1$_{PFF}$. (**A**,**B**) To measure Mab34 (A) and Mab120 (B), 11 ng/ml-2 μg/ml and 63 ng/ml-4 μg/ml of IZUMO1$_{PFF}$ were applied to the sensor chips, respectively. The blue lines indicate the empirical sensorgram of 90 ng/ml (7 nM monomer; A) and 640 ng/ml (50 nM monomer; B) of IZUMO1$_{PFF}$, and the black lines show experimental curves at other concentrations of IZUMO1$_{PFF}$. The sensorgrams were analyzed by the global fitting program that was supplied by the manufacturer, and the theoretical curves are shown as broken red lines.

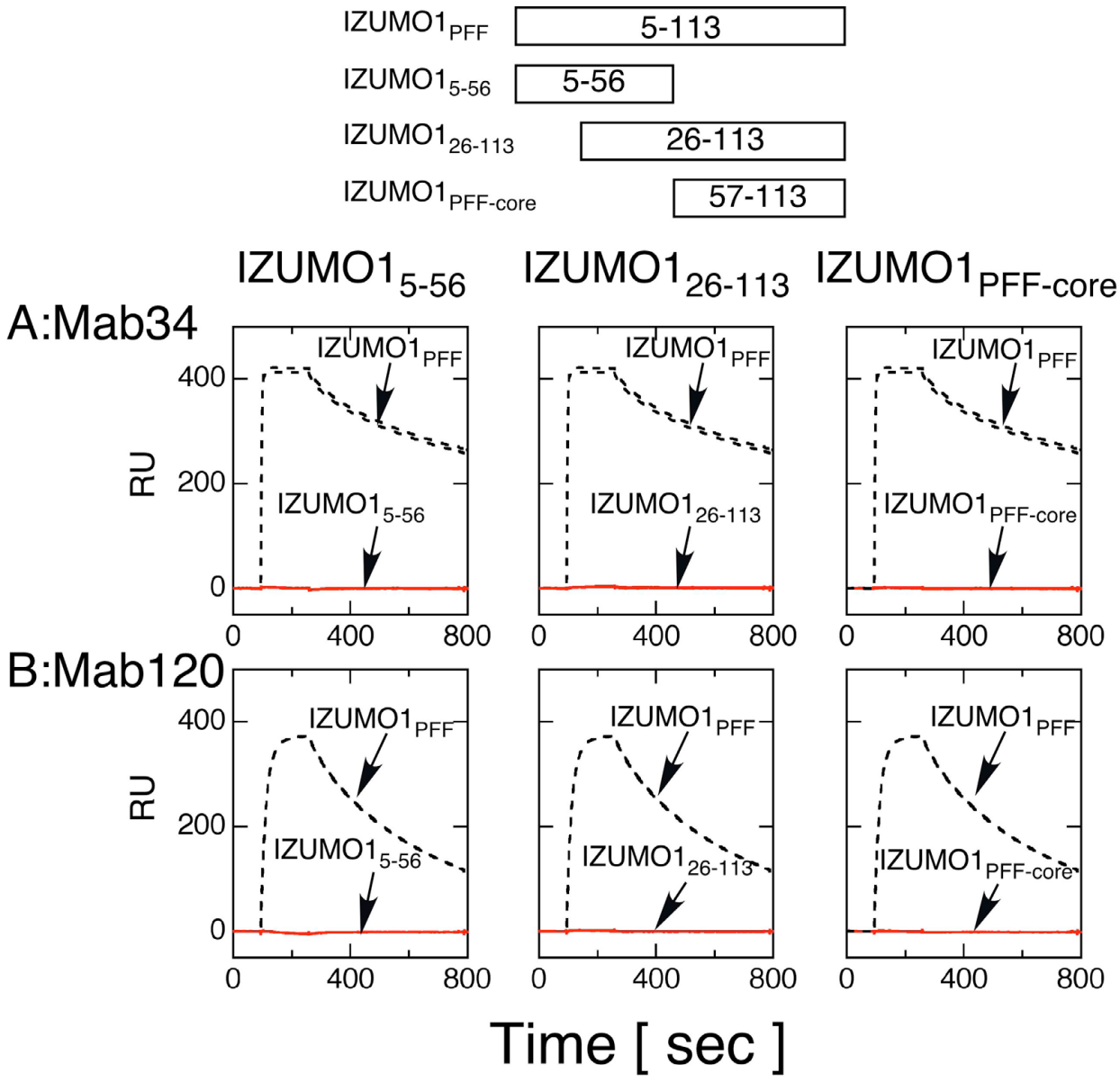

Fig. S4. Interaction of antibodies against IZUMO1 fragments. (**A**,**B**) The SPR spectra of immobilized Mab34 (A) and Mab120 (B) with 20 μg/ml of $IZUMO1_{5\text{-}56}$ (molar concentration is 2.8 μM, assuming the monomer in solution; left panels), $IZUMO1_{26\text{-}113}$ (1.7 μM; middle) and $IZUMO1_{PFF\text{-}core}$ (2.9 μM; right) (red lines). From all of the fragments, only negligible signals were observed compared with $IZUMO1_{PFF}$ (broken black lines).

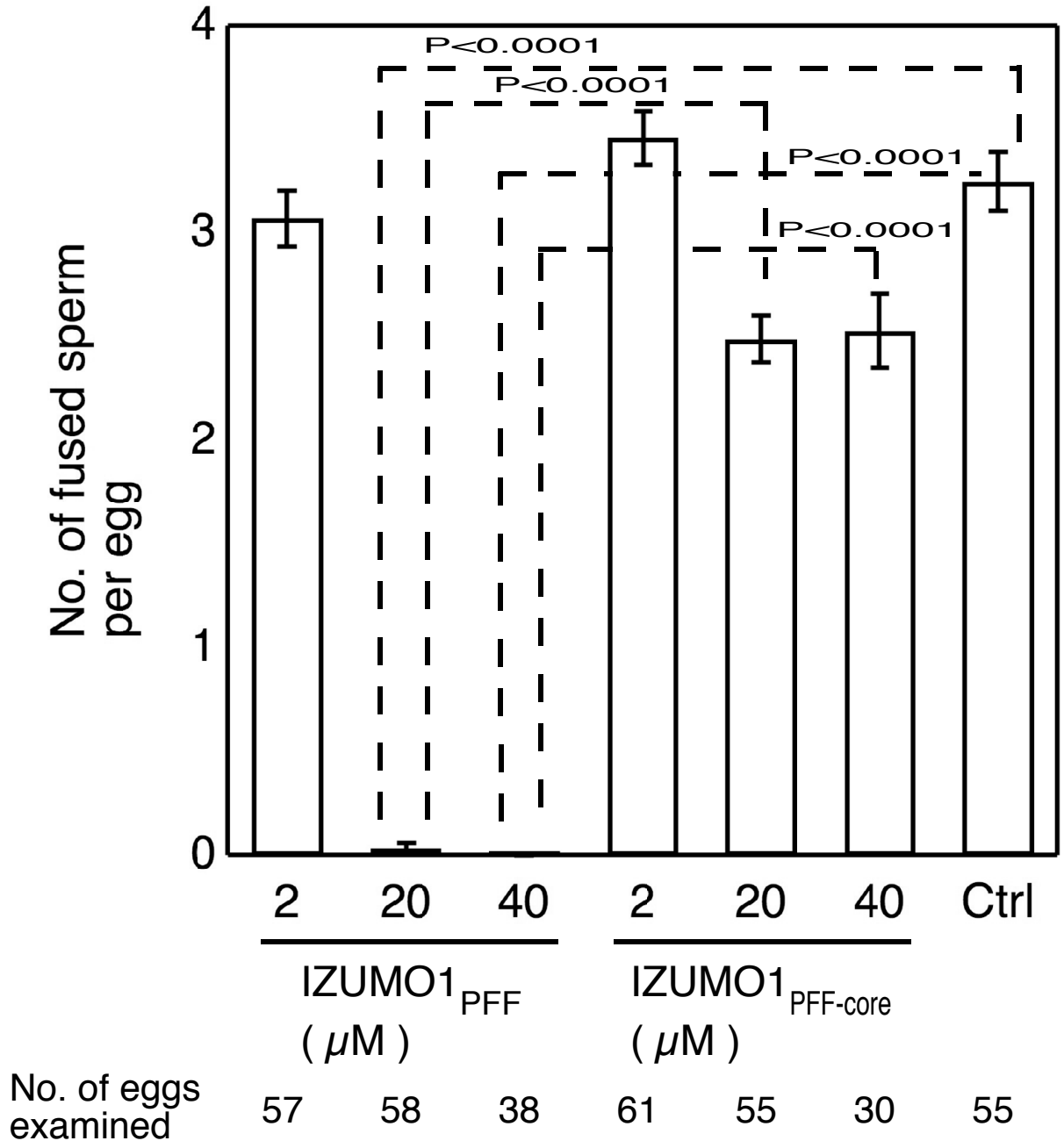

Fig. S5. Concentration dependence of IZUMO1$_{PFF}$ on the inhibition of sperm-egg fusion. The average number of fused spermatozoa in the presence (2, 20 and 40 μM) and absence of IZUMO1$_{PFF}$ was calculated. The number of spermatozoa that fused to an egg was counted using ~50 eggs in each group (n=2). Values are presented as mean±s.e.m.

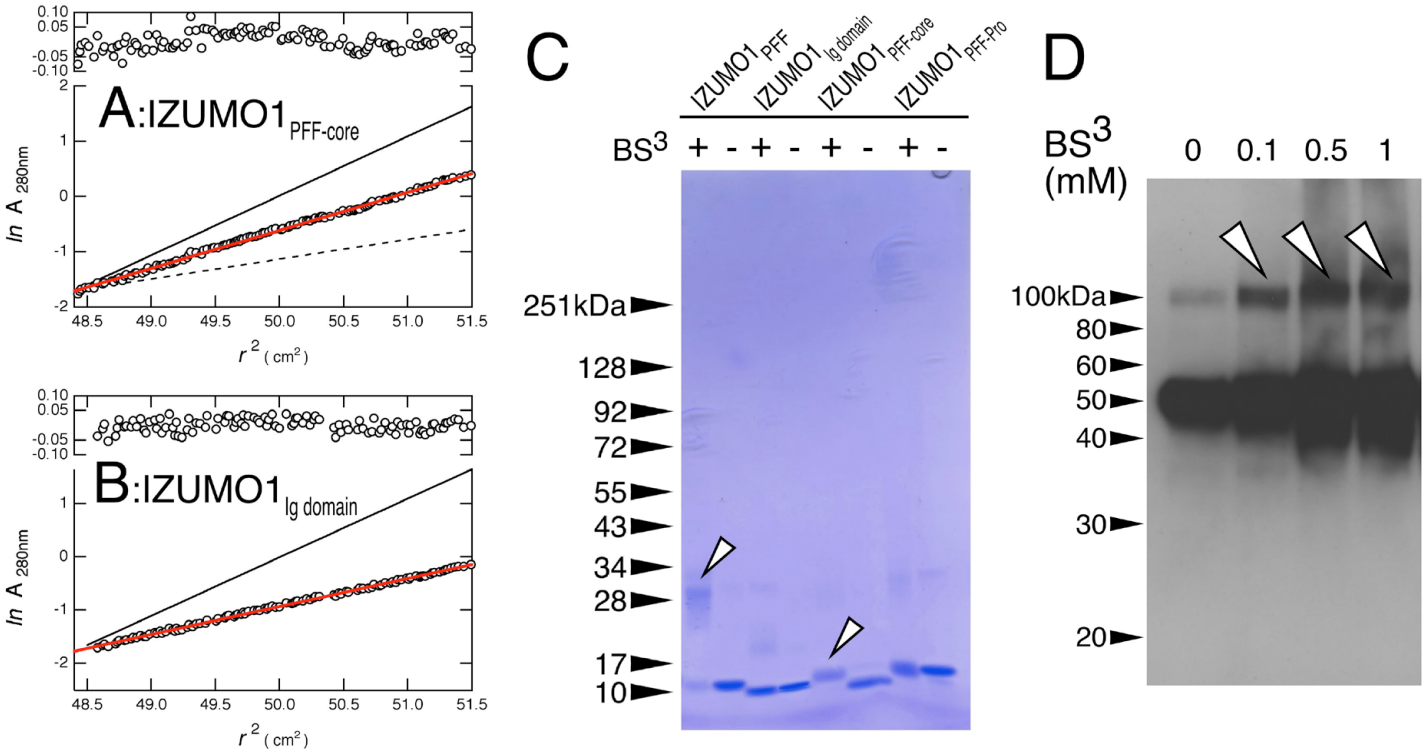

Fig. S6. Sedimentation equilibrium experiments. (**A-D**) IZUMO1$_{PFF\text{-}core}$ (A) and IZUMO1$_{Ig\ domain}$ (B), and chemical crosslinking of IZUMO1 fragments (C) and intact IZUMO1 on sperm (D). (A,B) Red lines are the linear fitting results of the data, indicating that the apparent molecular weights were 13,200 for IZUMO1$_{PFF\text{-}core}$ and 9673 for IZUMO1$_{Ig\ domain}$. The deviation between the empirical data and fitted lines was plotted in the upper panel. The calculated molecular masses of monomeric IZUMO1$_{PFF\text{-}core}$ and IZUMO1$_{Ig\ domain}$ are 6908 and 10,129, respectively. The theoretical lines for the monomer (broken black line in A), dimer (solid black line in B) and trimer (solid black line in A) were shown for comparison. The deviations between molecular weights that were calculated by linear fitting and nonlinear fitting were 1% in both IZUMO1$_{PFF\text{-}core}$ and IZUMO1$_{Ig\ domain}$. (C) IZUMO1$_{PFF}$, IZUMO1$_{Ig\ domain}$, IZUMO1$_{PFF\text{-}core}$ and IZUMO1$_{PFF\text{-}Pro}$ were reacted with a 25 times excess molar amount of a chemical crosslinker, bis(sulfosuccinimidyl) suberate (BS^3), at room temperature and were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reduced conditions. Arrowheads indicate bands that correspond to the supposed crosslinked dimer molecule. (D) Solubilized spermatozoa were incubated with a given concentration of BS^3 at 4°C. The samples were analyzed by SDS-PAGE under non-reduced conditions and western blotting carried out using anti-IZUMO1 antibody. The possible crosslinked dimer fractions were shown by arrowheads.

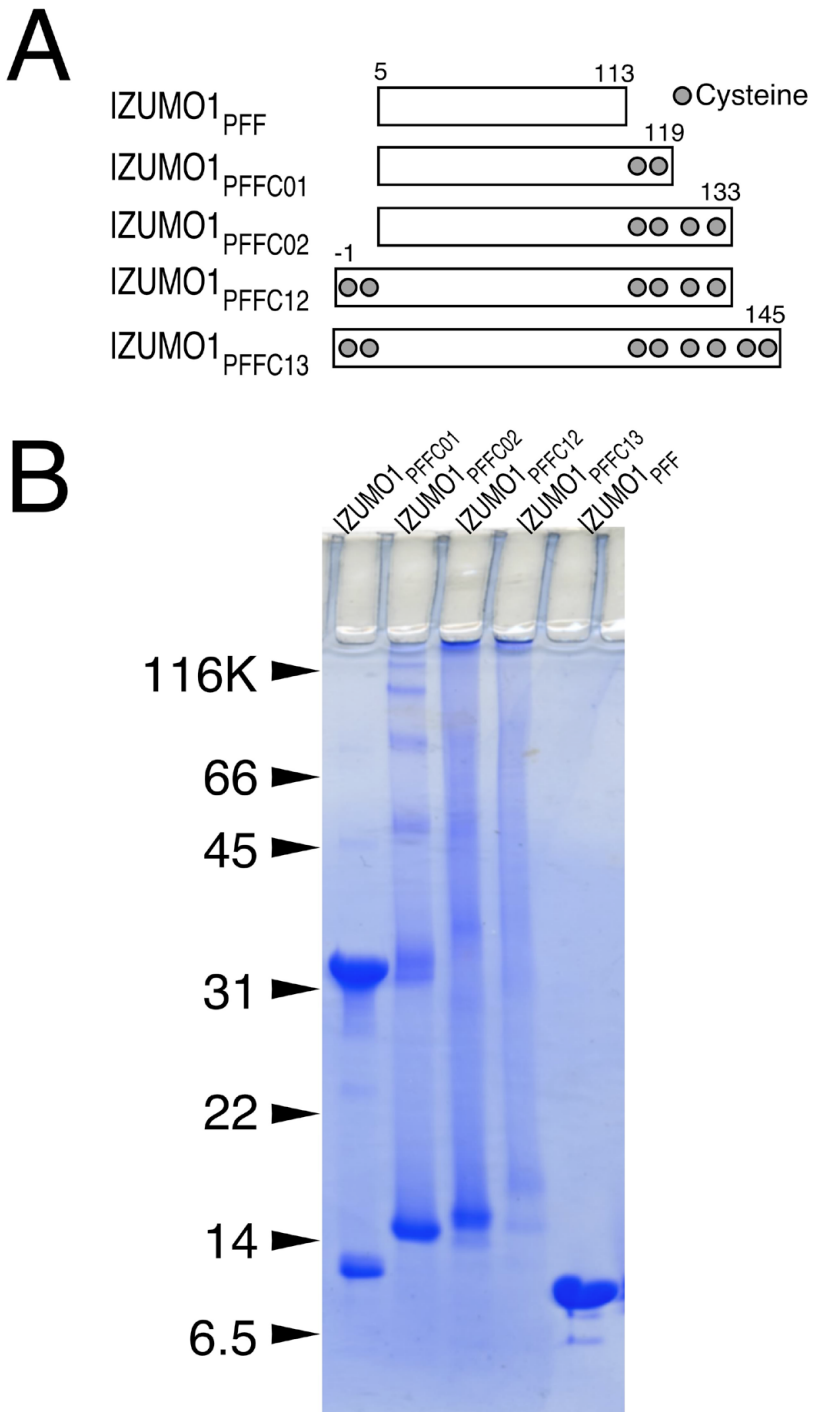

Fig. S7. Aberrant oligomer formation and aggregation of recombinant IZUMO1 fragments with cysteine residues produced by an *Escherichia coli* expression system. (**A**) Schematic representations of IZUMO1 fragments with cysteine clusters found in the IZUMO domain. The positions of cysteines in this cluster are 1, 4, 114, 118, 128, 131, 138 and 144 in mature mouse IZUMO1. (**B**) The refolding and oxidation of IZUMO1 fragments. The samples were subjected to SDS-PAGE under non-reduced conditions.

Table S1. Molar extinction coefficients calculated using the Edelhoch spectral parameters*

Protein	e_{280nm}
$IZUMO1_{Ig\ domain}$	18,020
$IZUMO1_{PFF}$	13,940
$IZUMO1_{234-298}$	2560
$IZUMO1_{PFF-core}$	11,380
$IZUMO1_{5-56}$	2560
$IZUMO1_{26-113}$	12,660
$IZUMO1_{PFF-Pro}$	13,940

*Extinction coefficients of amino acid at 280 nm: Trp, 5690; Tyr, 1280; half cysteine, 120 (Edelhoch, 1967).