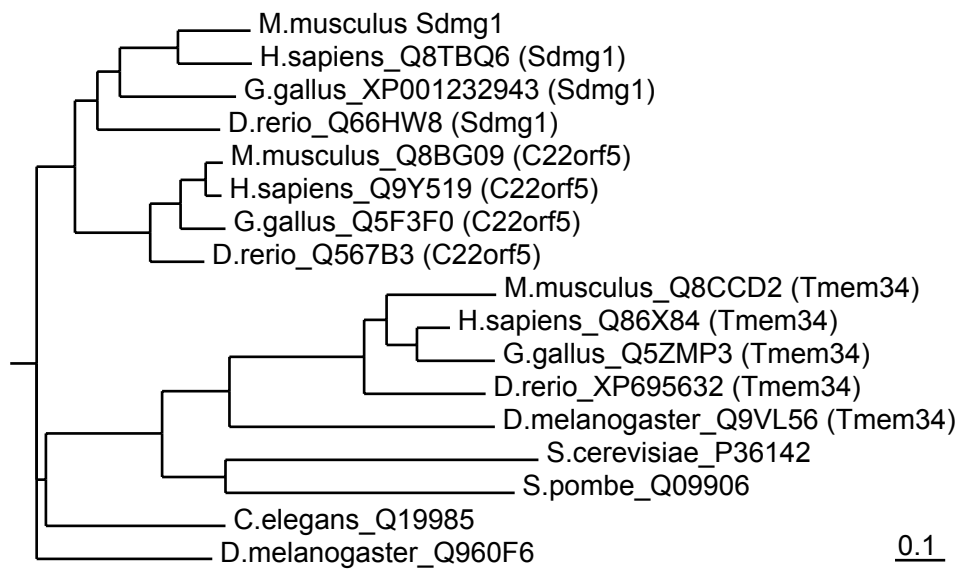


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S Q G A P Q L F L T S A L A R G V S G V F V W T A L L L T G 98
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H Q I Y S H L R S Y T A P R E Q R F V I R L L F I V P I Y A 128
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F D S W L S L L L L G G H P Y Y V Y F D S V R D C Y E A F V 158
CCTTCGACTCCTGGCTCAGCCTCCTCCTTGGGGGCCATCCTTACTACGTCTACTTCGACTCTGTGCGAGACTGCTACGAAGCATTTG 540
I Y S F L T L C F Q Y L G G E S A I M A E I R G K P I R S S 188
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C F Y G T C C L R G M S Y S I T F L R F C K Q A T L Q F C I 218
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V K P V M A L I T I I L Q A F D K Y H D G D F N I H S G Y L 248
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F E P V L K F L T I K A I I F L S F W Q G M L L A I L E R C 308
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Supplementary Figure S1. Sequence of Sdmg1

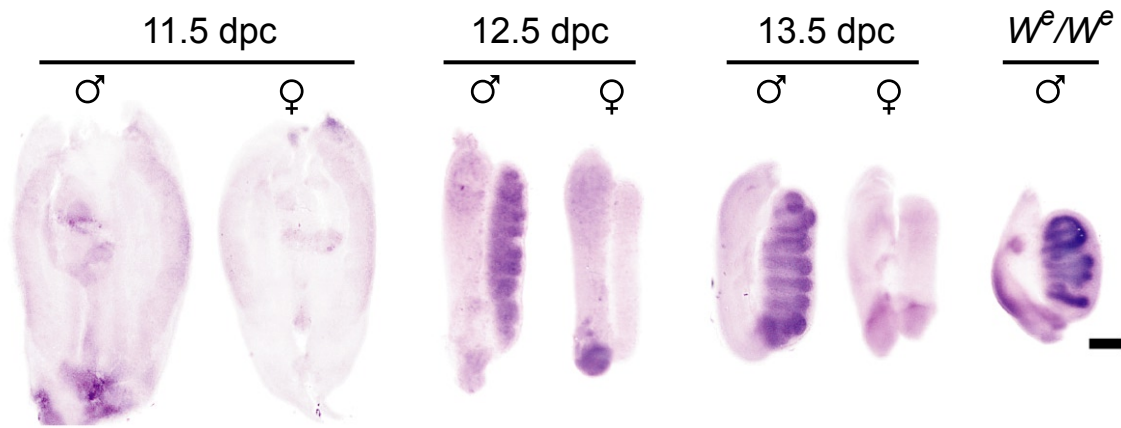
Predicted transmembrane domains are underlined and the potential C-terminal dileucine targeting motif is highlighted in blue.



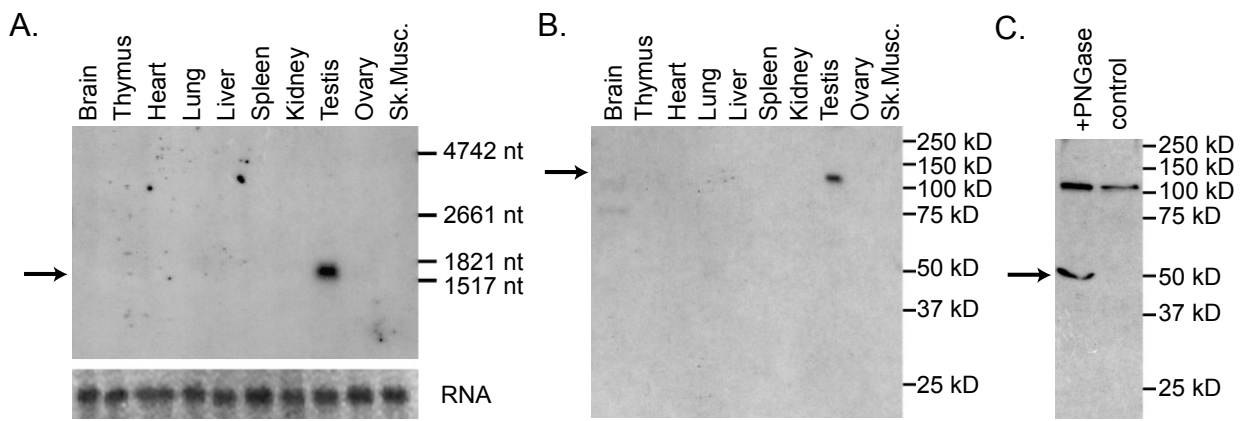
Supplementary Figure S2. Phylogeny of DUF300 protein family members from selected model organisms

Scale bar indicates 0.1 substitutions per site. Uniprot or Genbank accession numbers are indicated.

Multiple alignments and phylogenetic analysis were performed using CLUSTAL W (Thompson et al., 1994)



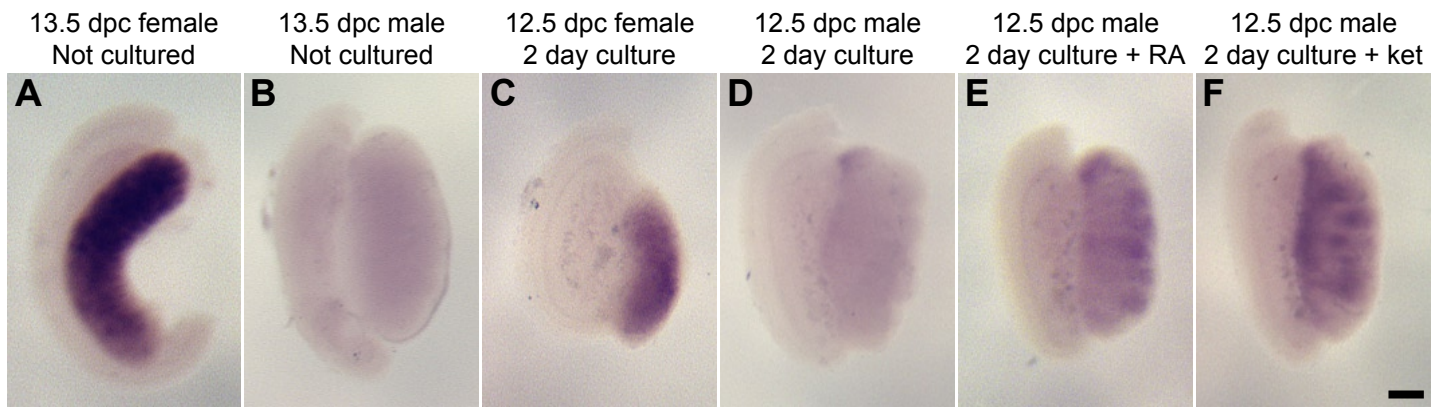
Supplementary Figure S3. In situ hybridisation for *Sdmg1* in male and female embryonic gonads
Sdmg1 exhibits male-specific expression in the testis cords at 12.5 dpc and 13.5 dpc, and expression in 14.5 dpc male *W^e/W^e* gonads. In situ hybridisation was performed as described by Henrique et al., 1995. Scale bar 0.2 mm.



Supplementary Figure S4. *Sdmg1* Has a Restricted Expression Profile in Adult Mouse Tissues

A. Northern blot showing expression of *Sdmg1* RNA in testis (arrows). Methylene blue staining of ribosomal RNA is shown as a loading control. B. Western blot showing expression of *Sdmg1* protein in testis (arrow). The anti-*Sdmg1* band migrates at around 110 kD. C. Western blot showing an anti-*Sdmg1* band can be detected at around 50 kD after deglycosylation of testis extracts with PNGase. Northern and Western blotting was performed as described by Adams and McLaren, 2002. PNGase treatment of testis extracts was performed as recommended by the supplier (New England Biolabs).

Abbreviations: Sk.Musc., skeletal muscle; PNGase, Peptide: N-glycosylase F.



Supplementary Figure S5. Retinoic acid or ketoconazole can induce *Stra8* expression in cultured male gonads

A,B. In situ hybridisation showing that *Stra8* is normally expressed in 13.5 dpc female, but not male gonads.

C,D. Sexually dimorphic expression of *Stra8* is maintained in our culture system when 12.5 dpc male or female

gonads are cultured for 2 days on agar blocks. E,F. Treatment of 12.5 dpc male gonads with either 0.7 μ M retinoic acid (RA) or 0.7 μ M ketoconazole (ket) for 2 days in culture induces ectopic expression of *Stra8* in our culture system consistent with previous reports (Bowles et al., 2006, Koubova et al., 2006).

In situ hybridisation was performed as described by Henrique et al., 1995

Scale bar 0.1 mm.

Antibody	Source	Concentration for immunostaining
mouse anti-Amh	Abcam	1:40
mouse anti-GFP/YFP	Roche Applied Sciences	1 µg/mL
mouse anti-Sycp3	Abcam	1:1000
mouse anti-Tfrc	Invitrogen	1 µg/mL
mouse anti-Vti1b	Becton Dickinson	1:50
Oregon Green - phalloidin	Invitrogen	0.15 µM
rabbit anti-HA	Sigma-Aldrich	10 µg/mL
rabbit anti-Mvh	Abcam	0.1 µg/mL
rabbit anti-Sdmg1	This study	1 µg/mL
rabbit anti-Stx2	Calbiochem	1 µg/mL
rabbit anti-Stx3	Calbiochem	1:100
rabbit anti-Stx7	Borner et al., 2006	1:400
mouse anti-Stx16	Borner et al., 2006	1:300
rabbit anti-Vamp3	Borner et al., 2006	1:100
rabbit anti-Vamp4	Steggmaier et al., 1999	1:500
rabbit anti-Vamp7	Borner et al., 2006	1:50
rabbit anti-Vamp8	Borner et al., 2006	1:200
rabbit IgG (non-specific control)	Sigma-Aldrich	1 µg/mL
rat anti-GCNA	Enders and May, 1994	1:10
rat anti-Lamp1	Developmental Studies Hybridoma Bank	1 µg/mL

Supplementary Table S1. Sources and Concentrations of Primary Antibodies

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