

Figure S1. Multiple sequence alignment of Shisa proteins. This alignment includes the aligned regions of the cysteine-rich domains and predicted transmembrane segments (labeled TM at the bottom). The divergent C-terminal low complexity regions after the predicted transmembrane segments are not aligned. The sequences are denoted by NCBI accession numbers. Genome- or EST-derived proteins, some of which are incomplete, have *italic and underlined* accession numbers. Sequence names for the two *Brachionus plicatilis* EST-derived proteins with two cysteine-rich domains (labeled domain1 and domain2) are colored in **green**. Starting residue numbers for sequences with protein accession numbers are shown before the sequences. Conserved cysteines are shaded in **yellow** (for those that are also conserved in Shisa-like proteins) or **red** (for the additional two cysteine positions compared to Shisa-like proteins). Mutations in conserved cysteines are shaded in **black**. Cysteines near the C-terminal ends of the predicted transmembrane segments are shaded in **gray**. Arginines and lysines near the C-terminal ends of predicted transmembrane segments are shown as **blue** letters. PY motifs (PPxY) in the C-terminal regions after the predicted transmembrane segments are colored in **cyan**. C-terminal PDZ-binding motifs ([TSVYF]x[VIL]) are shaded in **red**. Species abbreviations are as follows: Ail.mel, *Ailuropoda melanoleuca*; Bos.tau, *Bos taurus*; Bra.pli, *Brachionus plicatilis*; Bra.flo, *Branchiostoma floridae*; Cal.jac, *Callithrix jacchus*; Cal.mil, *Callorhynchus milii*; Can.lup, *Canis lupus*; Dan.rer, *Danio rerio*; Equ.cab, *Equus caballus*; Gal.gal, *Gallus gallus*; Hom.sap, *Homo sapiens*; Leu.eri, *Leucoraja erinacea*; Mac.mul, *Macaca mulatta*; Mel.gal, *Meleagris gallopavo*; Mon.dom, *Monodelphis domestica*; Mus.mus, *Mus musculus*; Onc.myk, *Oncorhynchus mykiss*; Orn.ana, *Ornithorhynchus anatinus*; Ory.cun, *Oryctolagus cuniculus*; Pan.tro, *Pan troglodytes*; Pet.mar, *Petromyzon marinus*; Pon.abe, *Pongo abelii*; Rat.nor, *Rattus norvegicus*; Squ.aca, *Squalus acanthias*; Sus.scr, *Sus scrofa*; Tae.gut, *Taeniopygia guttata*; Tet.nig, *Tetraodon nigroviridis*; Xen.tro, *Xenopus tropicalis*; and Xen.lae, *Xenopus laevis*. Background coloring of species names are as follows: mammal, **red**; bird, **grey**; amphibian, **magenta**; bony fish, **cyan**; cartilaginous fish, **blue**; lamprey, **green**; amphioxus, **brown** and rotifera: **black**.