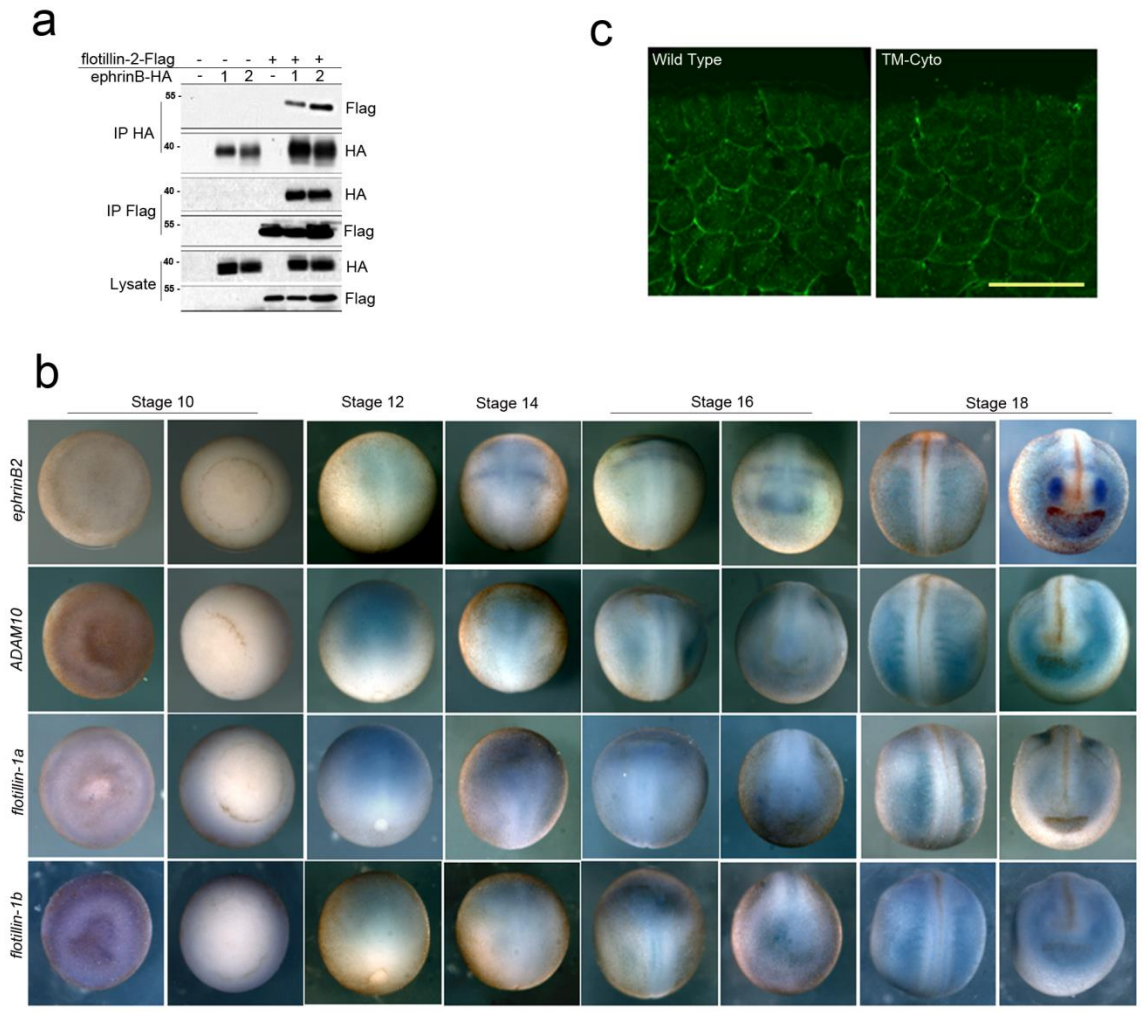
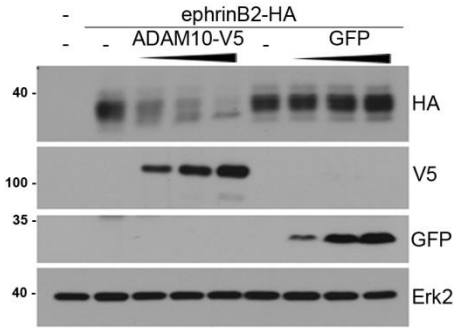
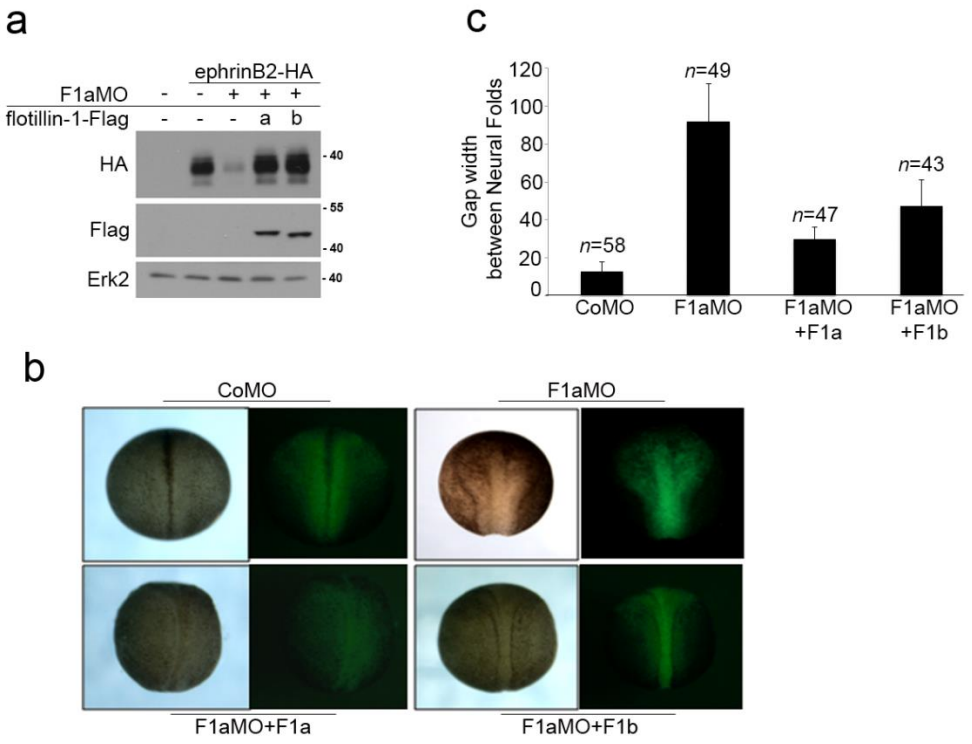


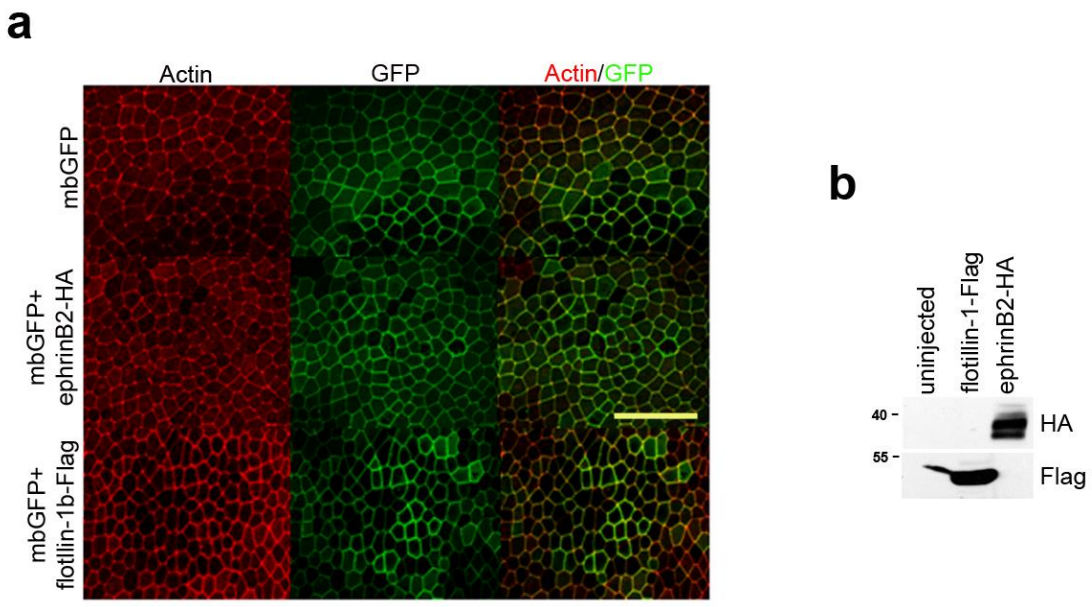
Supplementary Figure 1. Flotillin-2 Co-IPs with ephrin-B1 and ephrin-B2. (a) Immuno-precipitation and Western analysis of lysates from embryos co-injected with RNA encoding ephrin-B1-HA or ephrinB2-HA and flotillin2-Flag. Note that flotillin-2 is found in ephrin-B1 or B2 immunecopmlexes (top two panels), and both ephrinBs are found in flotillin-1 immunecomplexes (middle two panels) as indicated. The bottom two panels are direct westerns of lysates probed as indicated. **(b)** *RNA in situ* hybridization of embryos at the noted stages using the indicated probes. Stage 10 shows animal (left) and vegetal (right) views. All others are dorsal (left) and anterior (right) views. Note the overlap of expression among the various genes in the neural plate at early stages 14-16**. (c)** Immunofluorescent images of wild type ephrinB2-HA (left panel) exogenously expressed in embryos, and the truncated TM-Cyto ephrin-B2 construct. Scale bar represents 50 μm.



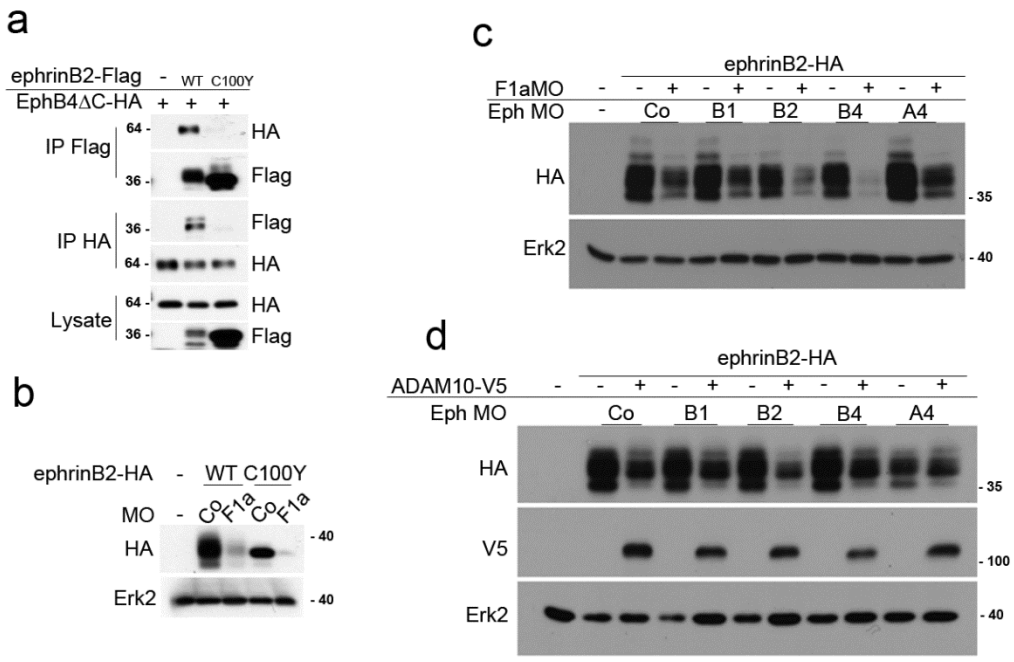
Supplementary Figure 2. Control for non-specific competitive inhibition of ephrinB2 translation. Western analysis of lysates from embryos injected with ephrinB2-HA RNA and increasing amounts of ADAM10-V5 RNA or control GFP RNA, and probed with the indicated antibodies. Erk2 was used as a loading control.



Supplementary Figure 3. Flotillin-1a and -1b paralogues rescue ephrinB2 protein levels and neural tube closure in the presence of F1aMO. **(a)** Western analysis of lysates from embryos injected with ephrinB2-HA RNA and F1aMO along with either flotillin-1a-Flag or flotillin-1b-Flag RNA, and probed with indicated antibodies. Note that either paralogue will rescue ephrin-B2 protein levels. **(b)** Light and fluorescent dorsal view images of st.18 embryos previously injected with Alexa-488 conjugated dextran (fluorescent marker) and the F1aMO or control MO alone or with flotillin-1a-Flag RNA or flotillin-1b-Flag RNA as indicated**. (c)** Histogram of the gap width differences in the neural folds among the embryos injected with the indicated reagents. The data represents three independent experiments, and the error bars represent sd. Note that paralogue 1b partially rescues neural tube closure.

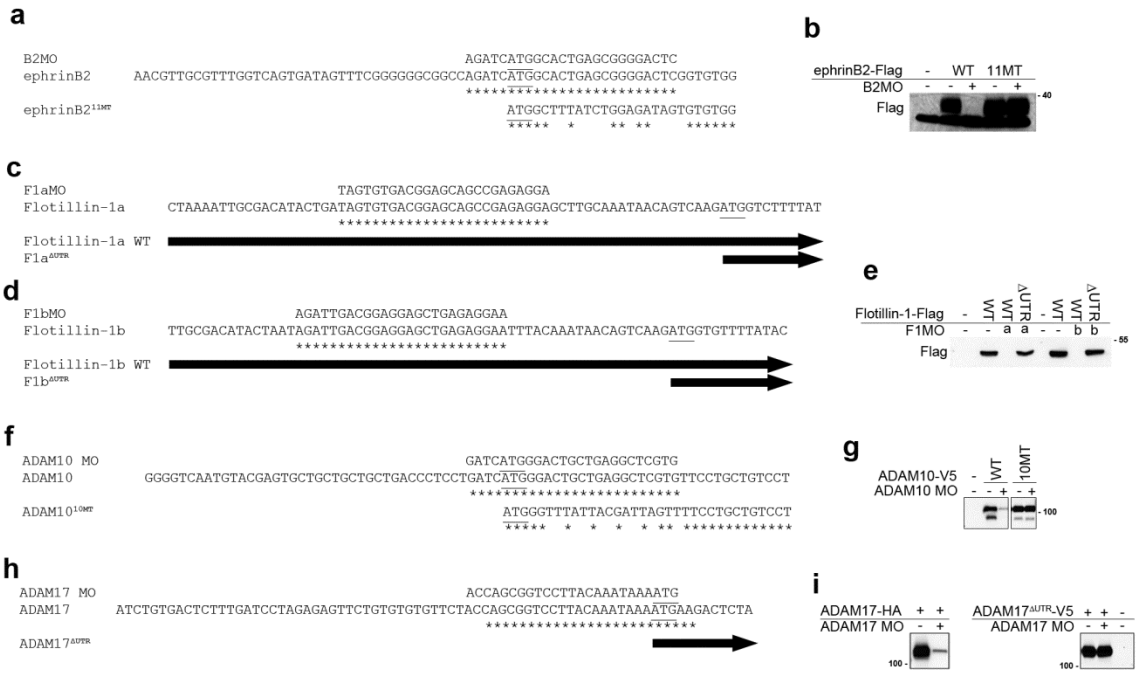


Supplementary Figure 4. Expression of ephrin-B2 or flotillin-1 does not induce apical constriction in ectodermal explants. (a) Embryos were injected with RNA encoding membrane bound-GFP (mbGFP) as a tracer alone or with either ephrin-B2 or flotillin-1 RNA. Ectodermal explants were excised at stage 10 and stained for actin (phalloidin) and viewed by confocal microscopy. No observable constriction occurs. Scale bar represents 100 μm. **(b)** Western analysis of embryonic lysates in (a) with indicated antibodies.



Supplementary Figure 5

Eph receptor MOs do not prevent the decrease in ephrinB2 observed in the absence of flotillin-1, but EphA4 MO has an effect with over-expression of ADAM10. (a) EphrinB2^{C100Y} is an EphB4 binding mutant. Co-immunoprecipitation analysis was performed on lysates from embryos expressing wild-type ephrinB2-Flag or the ephrinB2^{C100Y} mutant and EphB4$^{\Delta C}$, an EphB4 receptor carboxyl terminal deletion mutant. The ephrinB2^{C100Y} mutant was not present in EphB4$^{\Delta C}$ immune complexes, and nor was Eph4$^{\Delta C}$ present in the reciprocal immune complexes. **(b)** Eph receptor engagement with ephrinB2 may not be involved in the reduction of ephrinB2 expression in the absence of flotillin-1a. Wild-type ephrinB2 RNA or RNA encoding an Eph receptor-binding mutant, ephrinB2^{C100Y}, were injected along with F1aMO into embryos. When flotillin-1a is knocked down, a decrease in ephrinB2^{C100Y} mutant expression is observed, similar to wild-type ephrinB2. **(c)** Embryos were injected with ephrinB2-HA RNA along with F1aMO and the indicated Eph receptor MO. Western analysis was performed on the lysates using anti-HA antibodies to visualize ephrinB2, and anti-Erk2 as a loading control. **(d)** Embryos were injected with ephrinB2-HA RNA along with ADAM10-V5 RNA and the indicated Eph receptor MO. Western analysis was performed on the lysates using anti-HA antibodies to visualize ephrinB2, and anti-Erk2 as a loading control.



Supplementary Figure 6. EphrinB2, flotillin-1, ADAM10 and ADAM17 MOs. (a) The B2 MO sequence is the reverse compliment of that shown here. The ephrinB2 5'-UTR and a part of the ORF sequence are aligned with the B2 MO sequence. The first nine codons of the MO-resistant ephrinB2^{11MT}-HA construct, which differs in eleven bases from the B2 MO target sequence, are shown. Underline indicates the start codon, and asterisks indicate matched bases. **(b)** The efficiency of the B2 MO knockdown of wild type and MO resistant ephrinB211^{MT}-Flag is shown by Western analysis of embryonic lysates as indicated. Flag-tagged wild-type ephrinB2 expression is decreased by the B2 MO, while ephrinB2^{11MT}-Flag is resistant to the B2 MO. **(c)** F1aMO sequence. The reverse compliment of the F1aMO sequence is aligned with the flotillin-1a sequence. The MO targets the 5'-UTR. The Flotillin-1a WT (wild-type) construct contains the 5'-UTR sequence, including the sequence that matches the F1aMO, and the whole ORF. F1a$^{\Delta UTR}$-Flag has only the ORF and lacks the sequence recognized by the F1aMO. **(d)** F1bMO sequence. The reverse compliment of the F1bMO sequence is aligned with the flotillin-1b sequence. The MO targets the 5'-UTR.The Flotillin-1b WT construct contains the 5'-UTR sequence and includes the whole ORF. F1b$^{\Delta UTR}$-Flag has only the ORF and lacks sequence recognized by F1bMO. **(e)** F1MO knockdown efficiency and F1$^{\Delta UTR}$-Flag resistance to the F1MO. Flotillin-1-Flag expression was detected by Western blotting using anti-Flag antibody. F1a or b MO blocked Flotillin-1a or -1b WT-Flag expression, while the F1a or b$^{\Delta UTR}$-Flag was resistant to F1a or bMO. **(f)** ADAM10 MO sequence. The reverse compliment of the ADAM10 MO sequence is aligned with the ADAM10 sequence. The MO-resistant ADAM10^{10MT} construct contains ten mismatched bases which made silent mutations. **(g)** The efficiency of the ADAM10 MO knockdown of wild type and MO-resistant ADAM10^{10MT} is shown by Western analysis of embryonic lysates as indicated. Wild-type ADAM10 expression is decreased by the ADAM10 MO, while ADAM10^{10MT} is resistant to the ADAM10 MO. **(h)** ADMA-17 MO sequence. The reverse compliment of the ADAM17 MO sequence is aligned with the ADMA-17 sequence. The MO targets the 5'-UTR and the start codon. The ADAM17 WT construct contains 5'-UTR sequence which is matched to the ADAM17 MO, and the whole ORF. ADAM17$^{\Delta UTR}$-V5 has only the ORF. **(i)** ADAM17 MO knockdown efficiency and ADAM17$^{\Delta UTR}$-V5 resistance to the ADAM17 MO.

Supplementary Figure 7. Full scans of Western blot data in Figures 1a, b, 2d-h, 3a-c, 4a-d, f, g, 5a-h, 6a, c, 9b, f, 10e, h.

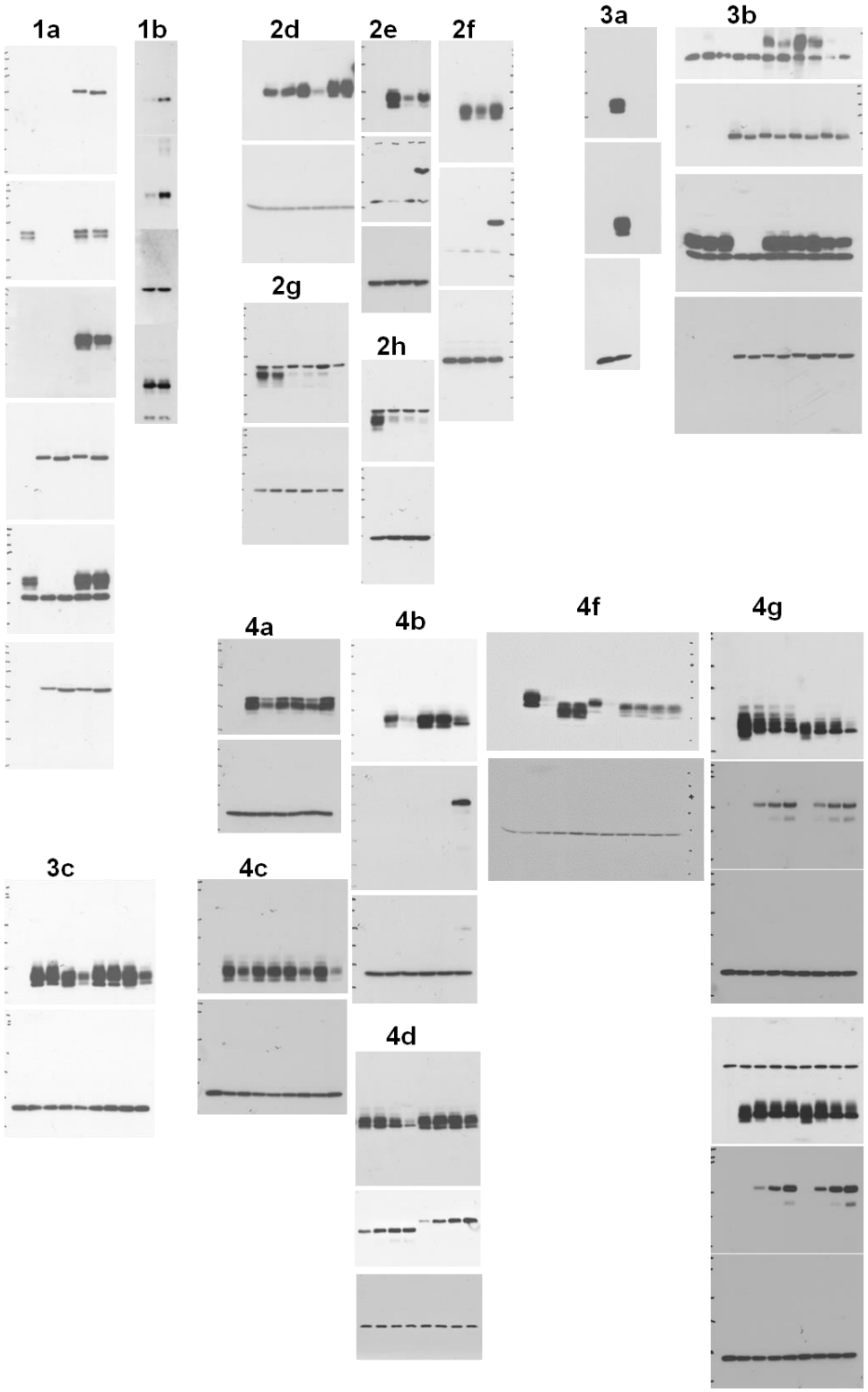


Fig.S7 continued