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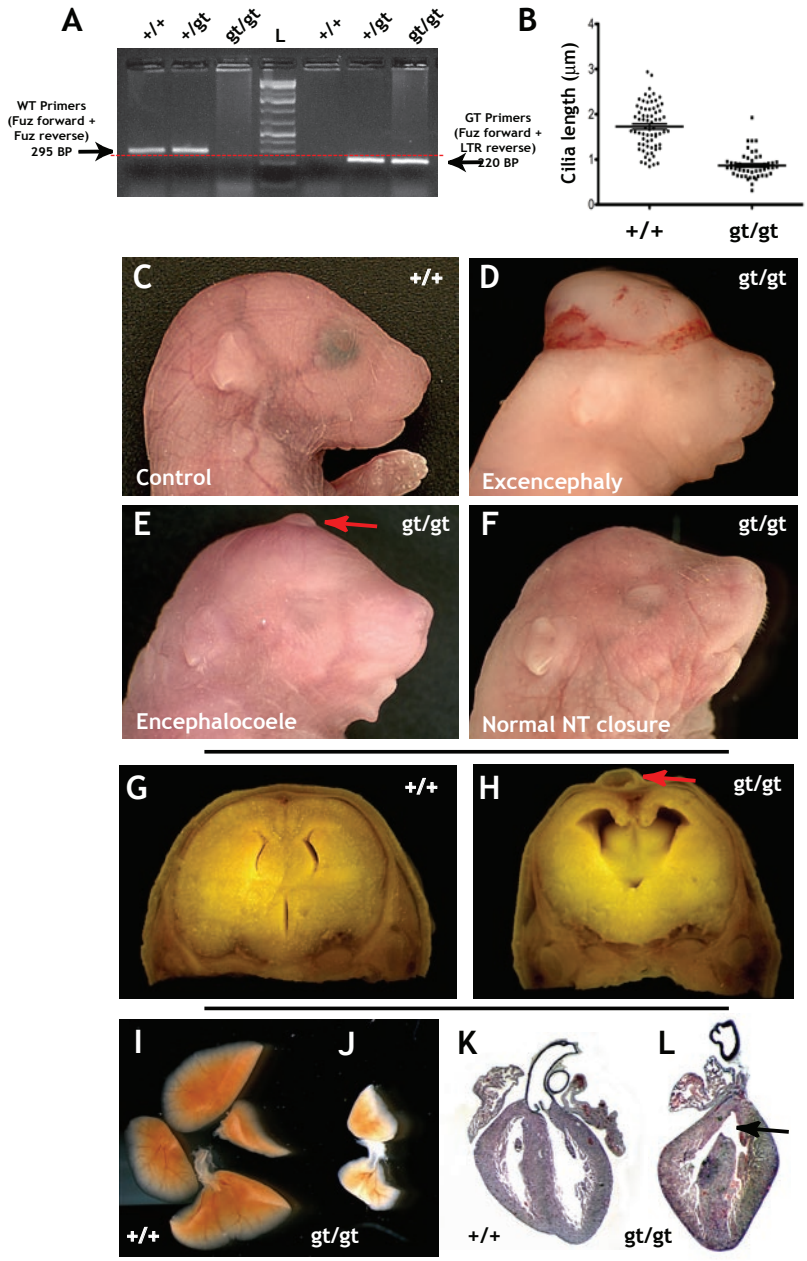

Figure S1 PCR genotyping, cilia length defects, and variably penetrant neural tube closure defects and organogenesis defects in Fuz mutant mice. **(a)** Agarose gel electrophoresis results of $Fuz^{Gt1(neo)}$ knockout mouse PCR genotyping (DNA extracted from tails of fetuses at E18). PCR with primers to detect the wild type allele (Fuz forward & Fuz reverse primers - see Supplemental Methods) produces a 295 bp product, which was detectable in both +/+ and +/gt mice (left). PCR with primers detecting the mutant allele (Fuz forward & LTR reverse - see Supplemental Methods) produces a 220 bp product, which was detected in +/gt and gt/gt mice (right). **(b)** Graph of primary cilia length in chondrocytes of Meckel's cartilage in wild type and Fuz mutant mice, as determined by the length of the acetylated tubulin signal following immunostaining (See Fig. 1G, H). E18.5 mice $Fuz^{gt/gt}$ showing variable NTDs. **(c)** Control mouse. **(d)** $Fuz^{gt/gt}$ mouse displaying excencephaly. **(e)** $Fuz^{gt/gt}$ mouse displaying encephalocoele (red arrow). **(f)** $Fuz^{gt/gt}$ mouse displaying normal neural tube closure (note reduced eyes and jaw). **(g)** Thick section of control brain. **(h)** Thick section of $Fuz^{gt/gt}$ brain from a fetus with an encephalocele (red arrow). **(i, j)** $Fuz^{gt/gt}$ mice display severely hypoplastic lungs. **(k)** Section through control heart. **(l)** Section through $Fuz^{gt/gt}$ heart with ventriculoseptal defect (arrow).

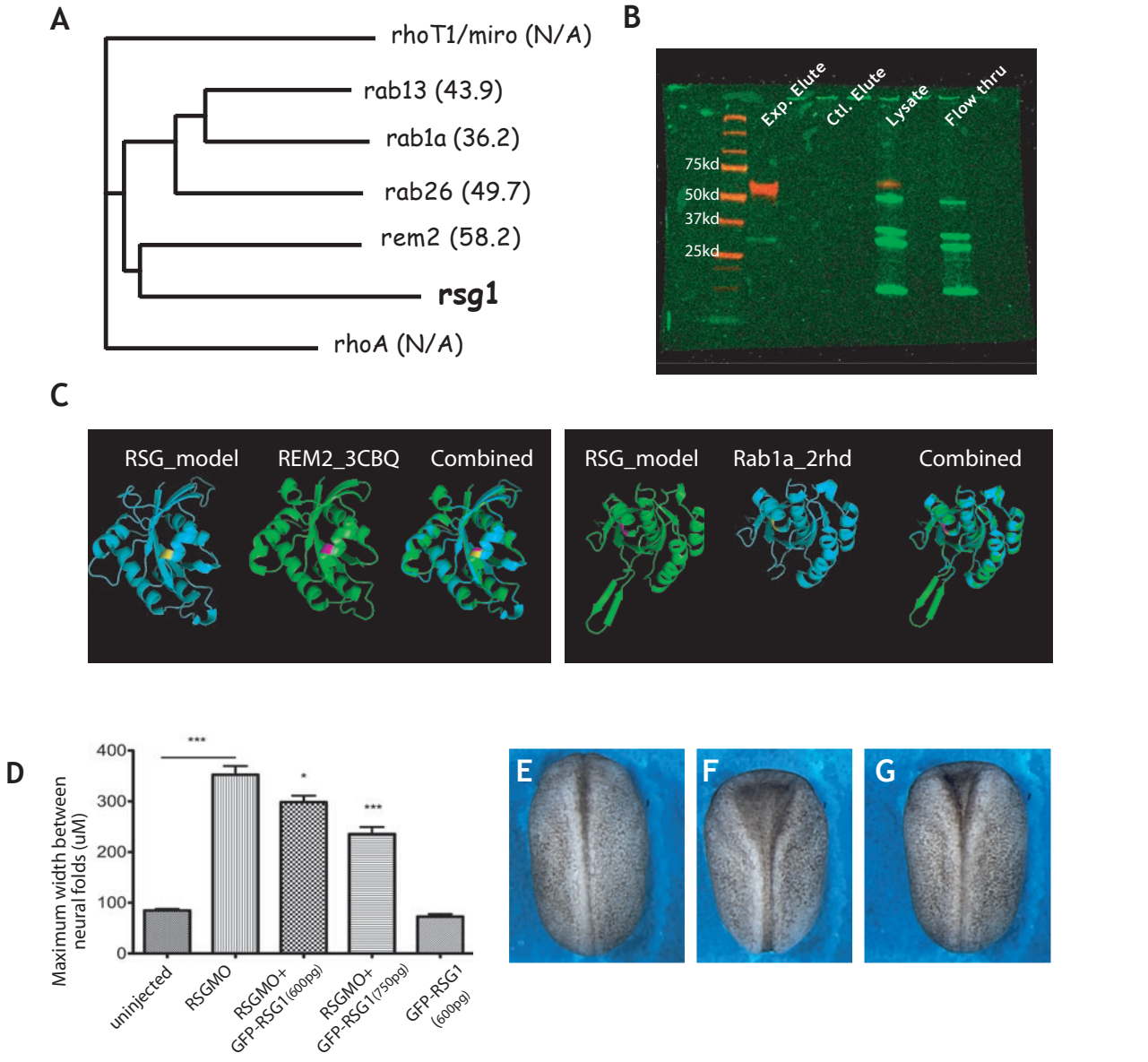

Figure S2 Human chromosome 1 open-reading frame 89 encodes a novel Rab-Similar GTPase (RSG) that is a Fuz interacting protein and dorsally targeted RSG1 MO results in anterior neural tube closure defects that are rescued by co-injection of a GFP-RSG mRNA. **(a)** Neighbor joining tree of human GTPase proteins with RhoT1 and RhoA serving as outgroups. RSG1 forms a clade with REM2 as its closest protein homolog. Parentheses indicate percent amino acid identities to RSG1. **(b)** Co-immunoprecipitation of FLAG-RSG protein (green band at ~27kD in Exp. Elute lane), by pull-down of MYC-FUZ with anti-MYC beads (red band at ~57kD in Exp. Elute lane). Whereas embryo lysates expressing only FLAG-RSG protein exhibit no interactions with anti-MYC beads (Ctl. Elute). Both products are present in raw lysates. **(c, d)** Rendered protein models (Open-Source PyMOL 0.99rc6 software). **(c)** Predicted model of RSG1 (cyan) threaded on the REM2 structure (green) (pdb:3CBQ). **(d)** Predicted model of RSG1 (green) threaded on the Rab1a structure (cyan) (pdb:2RHD). Contrasting colored amino acid (i.e. yellow or magenta) in each structure reflects the location of the conserved threonine residue mutated in our study (T65 in RSG, mutated to N; see main text for discussion). **(d)** RSG morphants exhibit significant defects in anterior neural tube closure compared to uninjected or GFP-RSG1 injected sibling embryos ($P < 0.001$). **(e)** Representative uninjected stage 20 embryo. **(f)** Representative RSG morphant embryo displaying a severe anterior neural tube closure defect. **(g)** Representative GFP-RSG1 (750pg) rescue embryo displaying a subtle but significant decrease in severity of the anterior neural tube defect. * $P < 0.05$ and *** $P < 0.001$ versus RSG morpholino injection embryos or control as indicated by the line. $n = 3$ independent replicate experiments. All P values were analyzed by one-way ANOVA with Bonferoni correction. Data are shown as means ±SEM.

A

GO id	Description	Type	Combined Score	B	C	C*	D	E	F	G	H
GO:0031988	membrane-bound vesicle	CC	0.226	0.00512	0.81	0.897	1	0.0334	0.128	0.00447	0.612
GO:0031982	vesicle	CC	0.22	0.00547	0.798	0.838	1	0.0334	0.183	0.00315	0.603
GO:0031410	cytoplasmic vesicle	CC	0.214	0.00549	0.792	0.867	1	0.000473	0.171	0.00301	0.614
GO:0016023	cytoplasmic membrane-bound vesicle	CC	0.204	0.0133	0.778	0.893	1	0.102	0.14	0.00387	0.624
GO:0006886	intracellular protein transport	BP	0.13	0.0188	0.601	0.735	1	0.0353	0.0407	0.00897	0.56
GO:0008104	protein localization	BP	0.129	0.0123	0.6	0.686	1	0.000426	0.13	0.01	0.559
GO:0016192	vesicle-mediated transport	BP	0.129	0.00592	0.597	0.85	1	0.000457	0.109	0.00676	0.552
GO:0046907	intracellular transport	BP	0.127	0.011	0.595	0.741	1	0.0163	0.0965	0.0105	0.537
GO:0045184	establishment of protein localization	BP	0.126	0.0328	0.593	0.739	1	0.00441	0.0684	0.00913	0.563
GO:0051641	cellular localization	BP	0.125	0.0394	0.591	0.738	1	0.000356	0.168	0.0106	0.543
GO:0015031	protein transport	BP	0.124	0.024	0.587	0.723	1	0.000874	0.0611	0.00901	0.552
GO:0051649	establishment of cellular localization	BP	0.124	0.0403	0.588	0.74	1	0.349	0.0978	0.0106	0.54
GO:0009628	response to abiotic stimulus	BP	0.00729	0.0207	0.0706	0.0171	0.122	0.0352	0.109	0.00847	0.293
GO:0006952	defense response	BP	0.00708	0.0147	0.0687	0.0215	0.00211	0.00137	0.306	0.0101	0.304

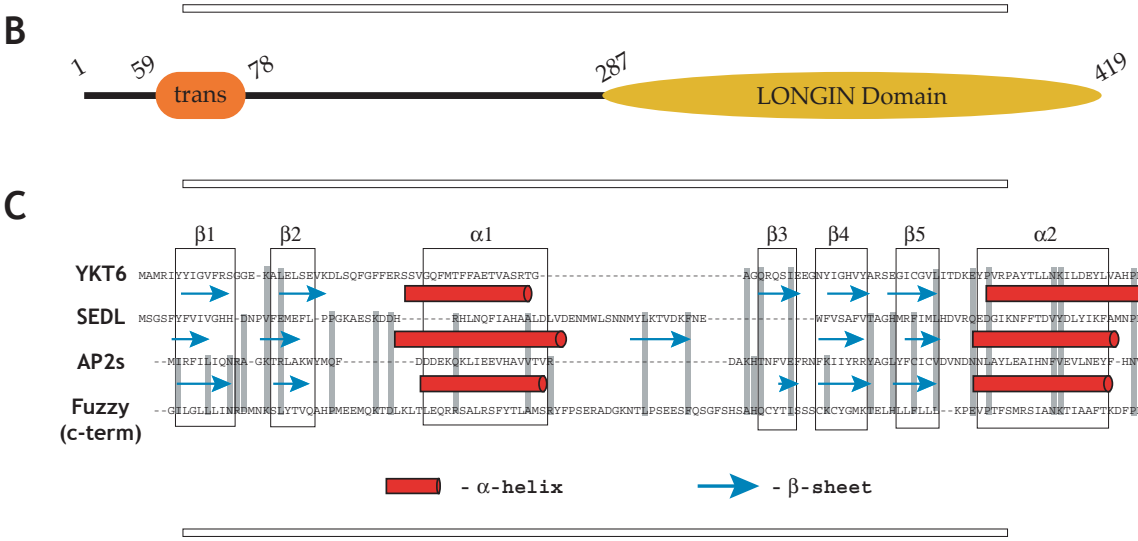

Figure S3 Structure modeling of the Fuz protein. **(a)** MouseFUNC predicts a vesicle trafficking function for Fuz. The description column defines the Gene Ontology descriptors for Fuz function ranked in order of combined score (blue column). Specific Gene Ontology identifiers (GO id's) are listed in the leftmost column. The combined score represents the overall prediction of GO id by all algorithms generated in the MouseFUNC competetion[19]. The columns at right (B-H) are the relative scores for each GO id that were predicted by individual algorithms. The Type column indicates the parent GO hierarchy for the annotations (cc, cellular compartment; bp, biological process). **(b)** The primary sequence of Fuz is predicted to contain a single transmembrane-spanning domain in the N-terminus (MEMSAT3) and a putative longin-domain in the C-terminus (mGENTHREADER). **(c)** Comparison of secondary structures for *Xenopus* Fuz and three longin-domain-containing proteins, Ykt6 (3bw6A0), SEDL (1hgA0), and AP2σ(1vg1S0). The β-sheets and α-helices predicted for *Xenopus* Fuz are indicated by the boxes (see labels above each box). The sheets and helices of the other three proteins are indicated by blue arrows and red barrels, respectively. Critical residues in the Fuz C-terminus, which are conserved in the other proteins, are indicated by the vertical grey bars. **(d)** Rendered protein models of AP-2 (left), and homology threaded model of the C-terminus of *Xenopus* Fuz (middle) and homology-threaded model of the C-terminus of human Fuz (right).

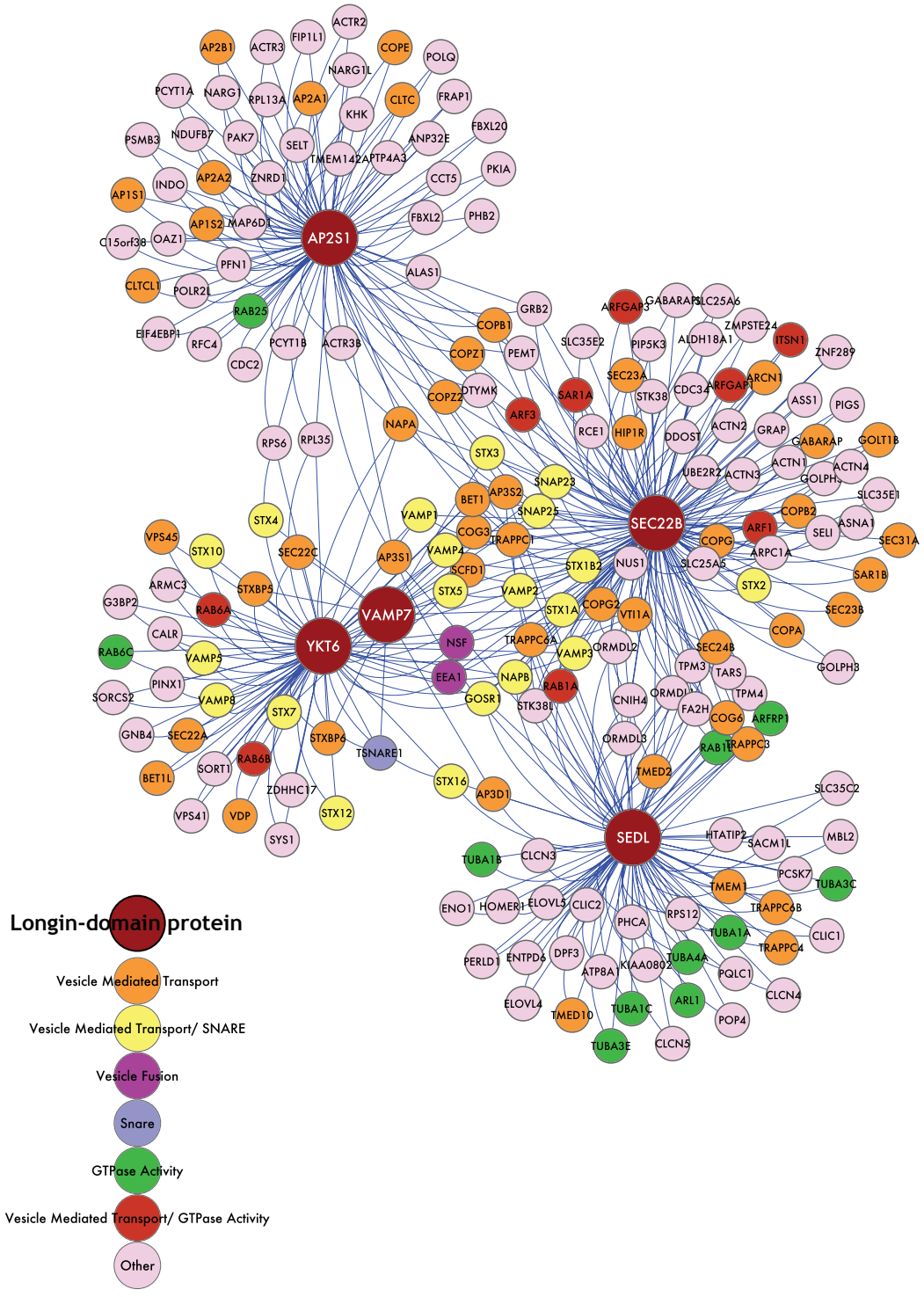

Figure S4 Network diagram of functional interactions between other structurally related Fuz like longin-domain containing proteins SEDL, YKT6, SEC22B, VAMP7 and AP2(PDB id: 1H3Q, 3BW6, 1IFQ, 2VX8 and 1VGL respectively).

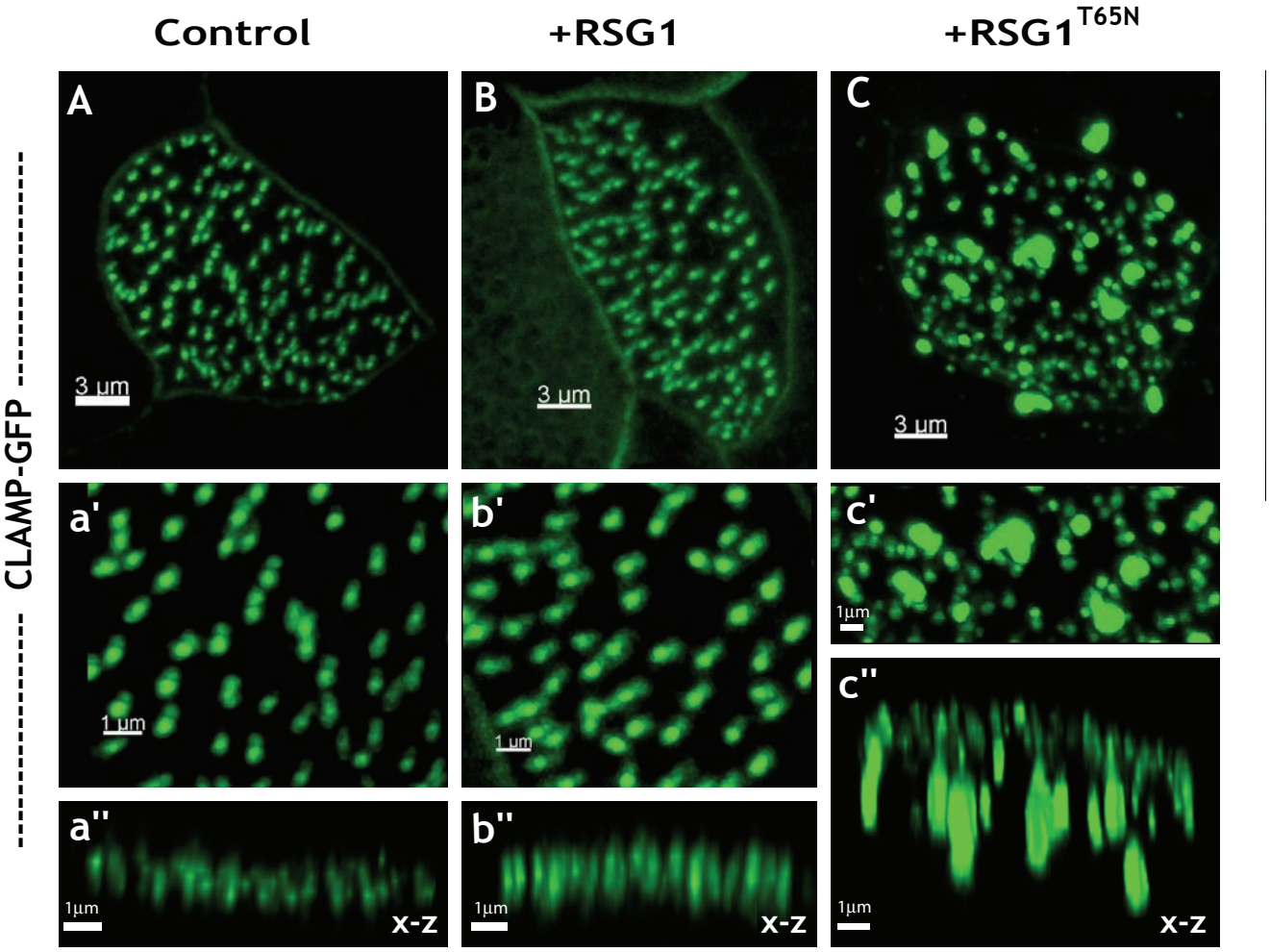

Figure S5 Localization and function of RSG1 in formaldehyde fixed multi-ciliated epidermal cells. **(c)** Multi-ciliated cell view (x-y) of uninjected control embryo exhibits elongated CLAMP-GFP signal. **(c')** Thin x-y section from **[c]**. **(c")** Z-projection of section **[c']** displays apical alignment of CLAMP-GFP. **(d)** Multi-ciliated cell view (x-y) of wild type RSG1 mRNA injected embryo exhibits subtle defects in the elongation of the CLAMP-GFP signal. **(d')** Thin x-y section from **[d]**. **(d")** Z-projection of section **[d']** displays apical alignment of CLAMP-GFP with a subtle defect in the resolution of apical punctae. **(e)** Multi-ciliated cell view (x-y) of RSG1^{T65N} mRNA injected embryo exhibits dramatic defects in the elongation of the CLAMP-GFP signal. **(e')** Thin x-y section from **[e]**. **(e")** Z-projection of section **[e']** displays dramatic loss of the apical alignment as well as aberrant cytoplasmic punctae of CLAMP-GFP signal.

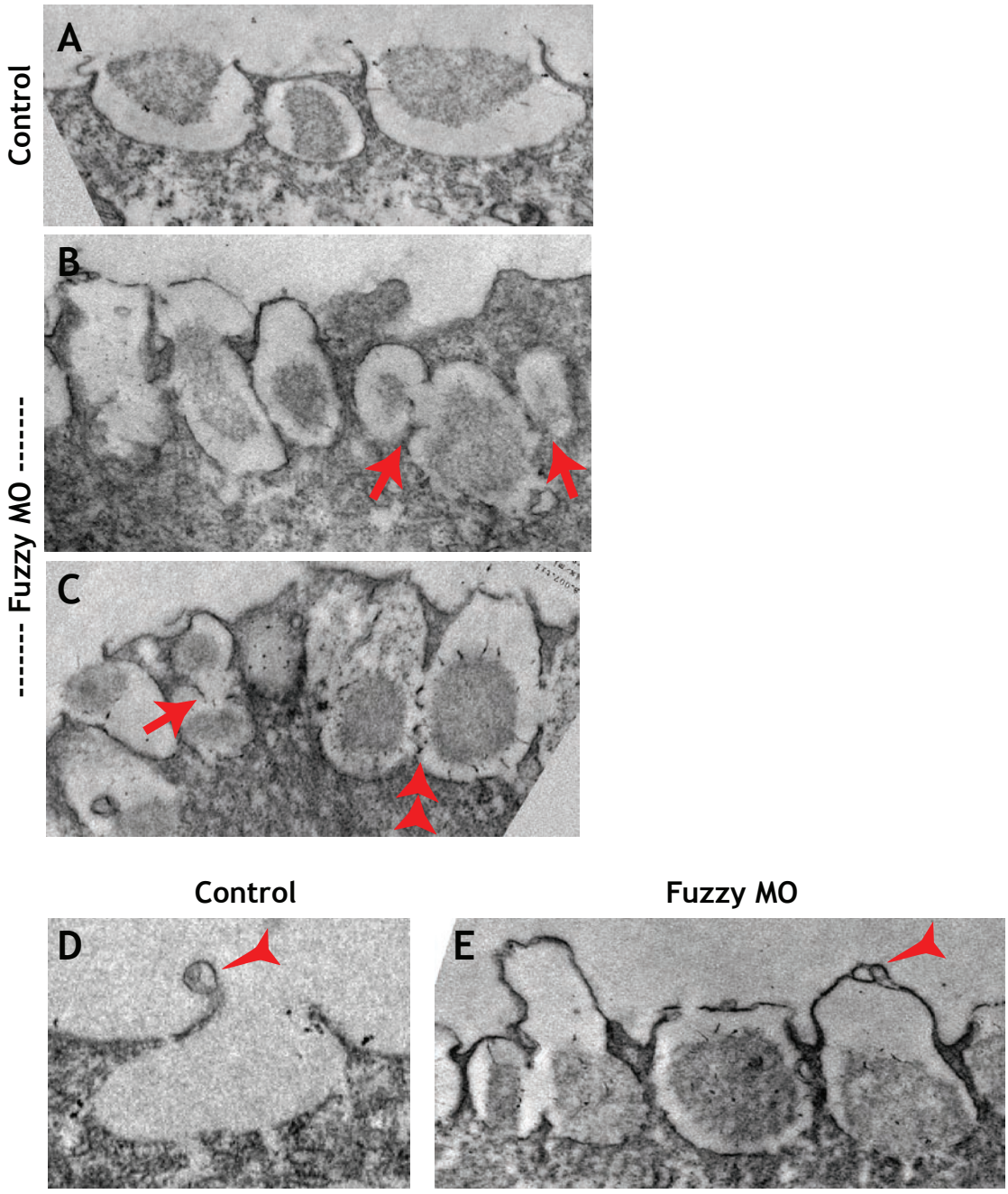

Figure S6 Additional TEM analysis of mucus secreting cells in Fuz morphants. **(a)** Control image showing representative *Xenopus* thin section epidermis. Generally the vesicles display even spacing of vesicles with no lateral mixing. **(b, c)** Fuz morphants display multiple lateral mixing events (red arrows and double red arrowheads **[b, c]**) as well as uneven spacing of the vesicles. **(d)** Wild-type secretory granule. Red arrowhead indicates a membrane signature that maybe indicative of hemifusion. **(e)** Fuz morphant secretory granule. Plasma membrane blebs out significantly, which may indicate a lack of complete fusion. A membrane signature (possibly hemifusion) similar to that observed in in controls cells is present (red arrowhead).