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Supplemental Data

Deficiency of the Cytoskeletal Protein SPECC1L

Leads to Oblique Facial Clefting

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Fig. S1. *Specc1l* is expressed in the developing facial primordia, eyes and limbs. Whole mount (a, b) and section (a', b') *in situ* hybridizations with *Specc1l* probe at E9.5 (a, a') and E10.5 (b, b'). (c, c') Specc1l immunohistochemistry at E10.5. Arrow: eye expression; arrowhead: limb expression; brackets: branchial arch (ba) expression.

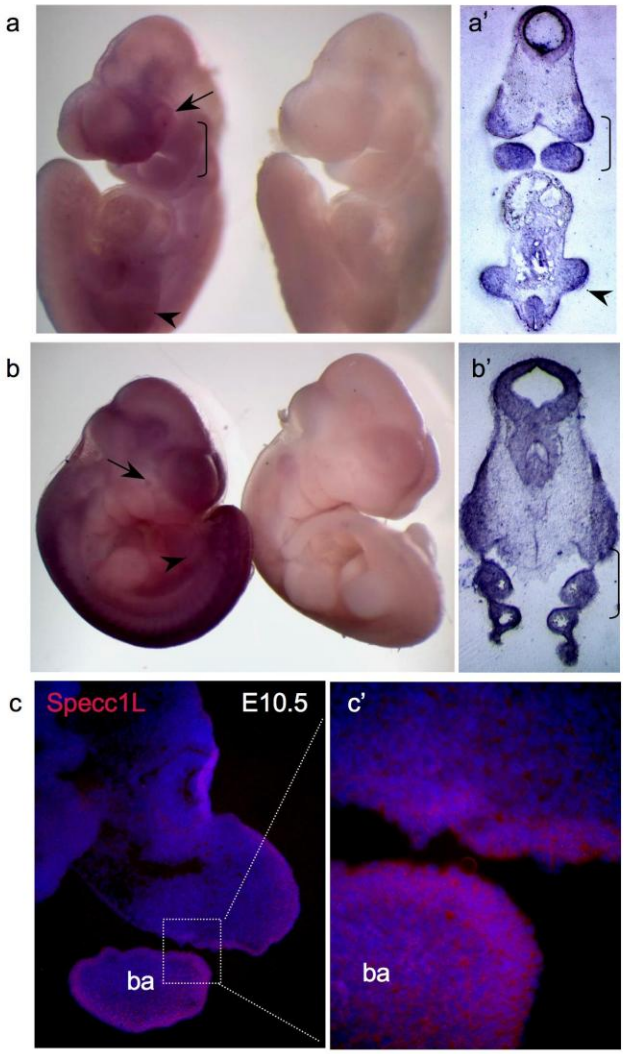

Fig. S2. Phylogenetic tree of SPECC1L orthologs in *Drosophila* and zebrafish. The phylogenetic tree was generated using the constrained-based multiple alignment tool (COBALT) available through NCBI. The protein sequences used were NP_569848.2 (*Drosophila CG13366*), NP_56145.2 (*SPECC1L*), ENSDARP00000047142 (zebrafish chr. 5 homolog), NP_001034905.1 (zebrafish chr. 8 homolog), and ENSDARP00000061911 (zebrafish chr. 21 homolog). Compared to human SPECC1L protein, the *Drosophila* ortholog is 54% similar, the zebrafish chr. 5 homolog is 55% similar, while the chr. 8 and 21 homologs are 75% similar.

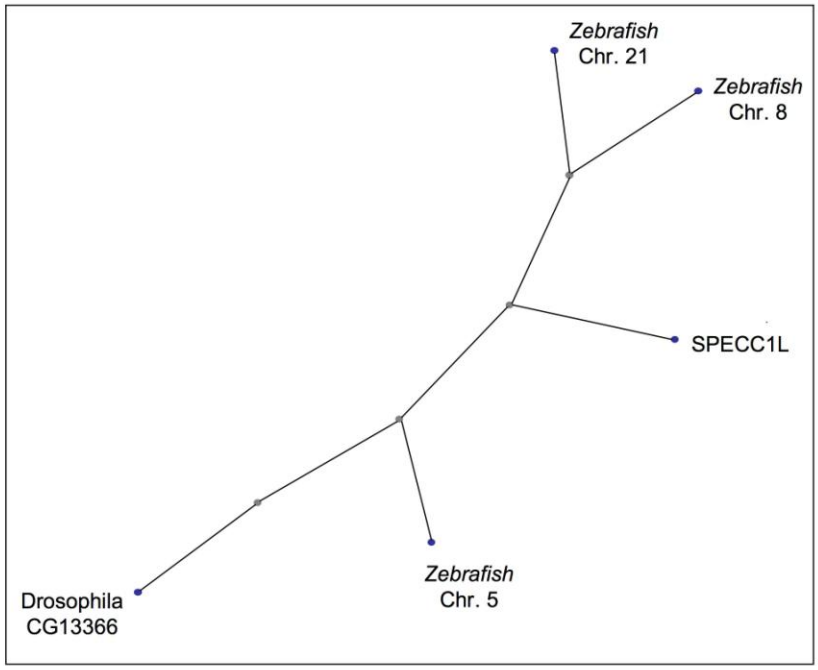

Fig. S3. Nocodazole prevents stabilization of microtubules following Specc1l-GFP expression. Specc1l-GFP expression shows stabilized microtubules (a,b; left panels), which are sensitive to nocodazole treatment (a, right panel) and are specifically recognized by the α-SPECC1L antibody (b, right panel).

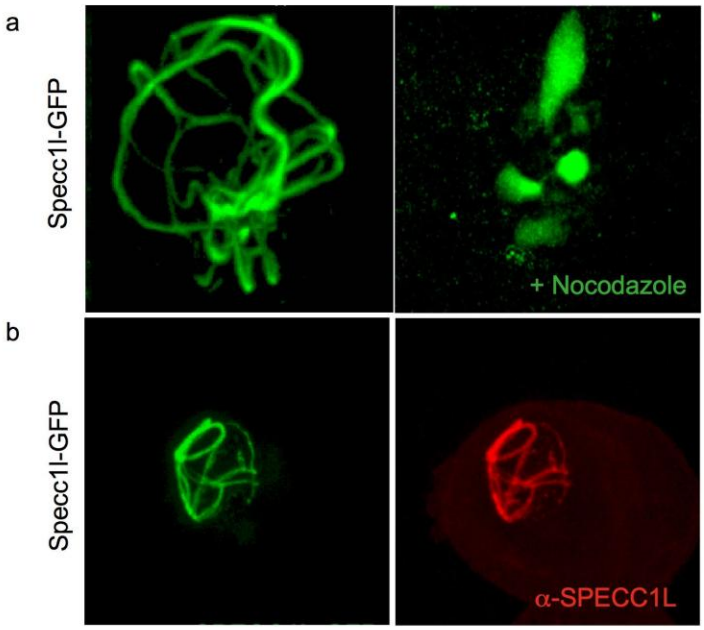

Fig. S4. Validation of *SPECC1L* knockdown in 293T and U2OS clonal cells. (a) *SPECC1L* knockdown in 293T cells (C1-1, B8-2), compared to control knockdown cells (GFP_RNAi), is confirmed by Western blot analysis using the α-SPECC1L antibody. SPECC1L protein is detected at approximately 120 kDa. (b) qRT-PCR data showing percent *SPECC1L* transcripts in clonal cell lines (uB8-5, uB8-9, uC1-5, uC1-9) compared to control GFP knockdown cells (uGFPi). The 293T control (293T-GFPi) and knockdown clones (B8-2, C1-1) are shown for comparison.

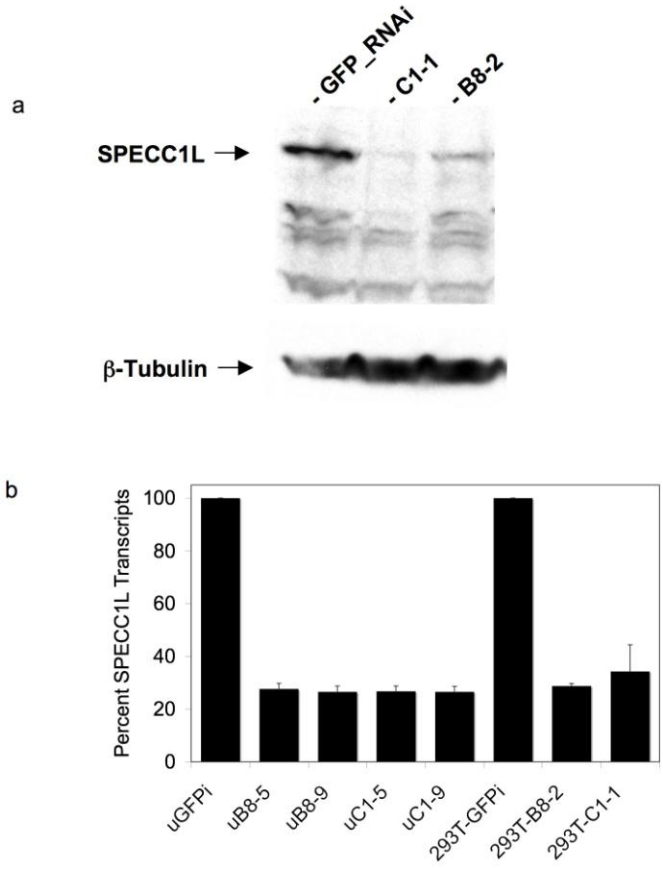

Table S1. Primer sequences used in RT-PCR, *in situ* probe and morpholino synthesis

Primer Name	Experiment	Sequence
SPECC1L Exon 8 Fwd	RT-PCR	5'-TCTGCGGAATGGCGGCAGTTT-3'
SPECC1L Exon 14 Rev	RT-PCR	5'-CACCACTTCAGCAAGGCGTTCCT-3'
Specc1l probe1 SP6 Fwd	*In situ* hybridization	5'-GCTATTTAGGTGACACTATAGtgtgatcctcatggcagtgt-3'
Specc1l probe1 T7 Rev	*In situ* hybridization	5'-TTGTAATACGACTCACTATAGGGaaggtagctggccctttgt-3'
Specc1l probe2 SP6 Fwd	*In situ* hybridization	5'-GCTATTTAGGTGACACTATAGccaggagaagtggctctacg-3'
Specc1l probe2 T7 Rev	*In situ* hybridization	5'-TTGTAATACGACTCACTATAGGGggcctcagtgaaggcaaata-3'
Specc1l-chr5	Morpholino	5'-ACTTCTACTTCACTGCATACCTGCT-3'
Specc1l-chr8	Morpholino	5'-GTAGTTATTTCTAACCTCAGGCAGT-3'
Specc1l-chr21	Morpholino	5'-ATTTTCTGAATATGTTTCACCTTGC-3'

Note: SP6 and T7 sequences are in uppercase for *in situ* hybridization probe primers.