

# Supplemental Materials

*Molecular Biology of the Cell*

Kunttas-Tatli et al.

**Supplemental Figure 1:** The APC Self-Association Domain (ASAD) is conserved across bilaterian phyla. A) Phylogenetic tree depicting the conservation of the ASAD (orange circles) and human oligomerization domain 1 (OD-1) (burgundy circles) across bilaterian phyla. OD-1 is primarily present in deuterostomes. The exception is the sea urchin APC, which does not have an OD-1 sequence, but instead contains an N-terminal insertion of a poly-glutamine repeat. All phyla examined contained the ASAD.

**Supplemental Figure 2:** A) Similar to Axin-GFP, HA-tagged Axin forms cytoplasmic puncta in S2 cells. B) APC2-FL promotes the formation of larger Axin puncta. Puncta size increases over time in cells expressing Axin-GFP alone, but appears to reach a plateau after 48hrs. Conversely, Axin-GFP/APC2-FL puncta are larger than Axin-GFP puncta at all time points and their size increased even after 48 hrs. Surprisingly, we frequently observed that Axin-GFP/APC2-FL puncta are abnormally shaped by 96 hrs. This was never observed for Axin-GFP puncta. Scale bar: 10 $\mu$ m

**Supplemental Figure 3:** FACS sorted cells were divided into three different groups based on the expression of Axin-GFP (high, medium and low) and puncta size and number was assessed. The striking alteration in destructosome morphology of the self-association mutants allowed quantification of their size only at low expression level. Scale bar: 10 $\mu$ m

**Supplemental Figure 4:** Disruption of APC2 self-association leads to the disruption of cortical localization in *Drosophila* S2 cells. APC2-FL is cortical while APC2- $\Delta$ ASAD is mostly cytoplasmic; actin is used to label the cortex. Scale bar: 10 $\mu$ m

**Supplemental Figure 5:** *Drosophila* embryos express a splice form of APC2 that disrupts the ASAD. A) Schematic representation of the APC2 protein and both APC2 splice forms APC2-A and APC2-B. B), Exon 1 of APC2 with the ASAD (dark green), the alternatively spliced intron (light green), potential 5' and 3' acceptor sites (line), and the branch site and polypyrimidine tract (dashed lines). C) PCR was used to specifically amplify the two splice variants from cDNA prepared from 4-8 hr wild type embryos. While the full-length version of APC2 (APC2-A) containing the ASAD appears to be expressed at significantly higher levels, we did identify a lower molecular weight product that corresponds in size to the expected product from APC2-B amplification. We extracted the putative APC2-B band, subjected it to 2 additional rounds of PCR (enriched PCR), and sequenced both the putative APC2-B and APC2-A bands to confirm their identity. The low expression of APC2-B relative to APC2-A might be due to various factors including tissue specificity. Splice site consensus sequences are YAG|G at the 3' splice site, preceded by a polypyrimidine tract, and MAG|GTRAGT (M= A or C, R= A or G) at the 5' splice site (Mount et al., 1992). The 5' and 3' splice sites in the APC2-B splice isoform generally follow the consensus albeit not perfectly. Although the 5' splice site

contains the most important GT dinucleotide at the first two positions of the intron, it only matches the overall consensus at 6 of 9 positions. Interestingly, while *Drosophila* APC1 does contain the ASAD (Fig. 1B), it does not appear to express a self-association incompetent isoform based on the modENCODE database.

Supplemental Movie 1

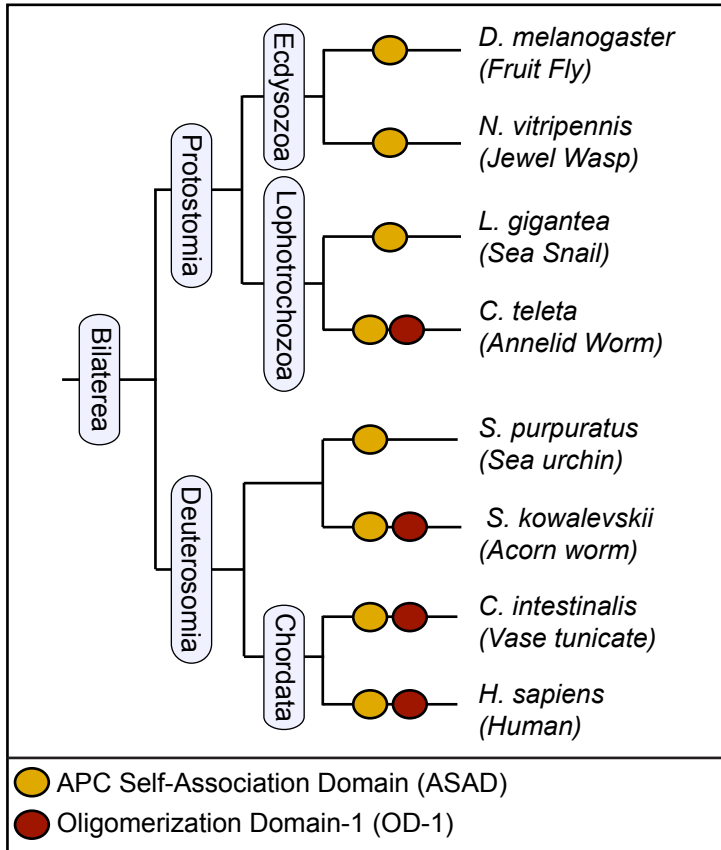
Axin-GFP puncta are stable and rarely split or merge with neighboring puncta.

Supplemental Movie 2

Similar to Axin-GFP alone, Axin-GFP/APC2-FL puncta are also stable and rarely split or merge with neighboring puncta.

Supplemental Movie 3

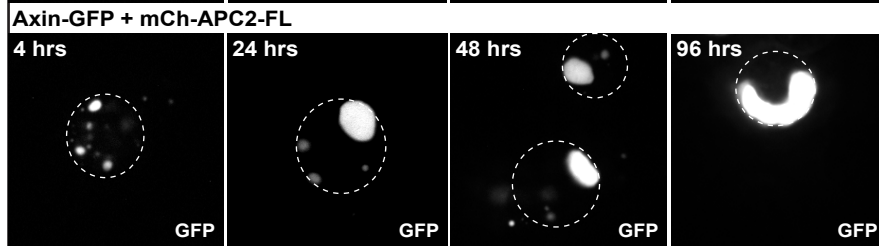
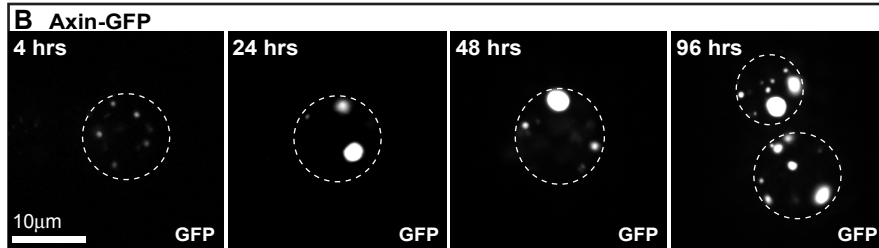
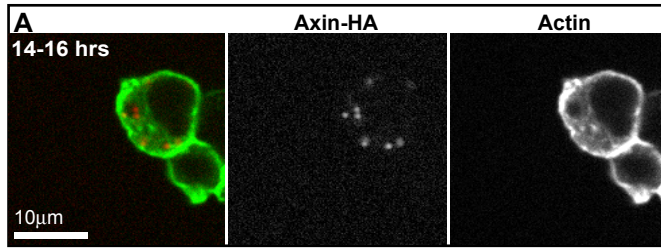
Axin-GFP/APC2- $\Delta$ ASAD puncta are highly dynamic and frequently split and merge with neighboring puncta.

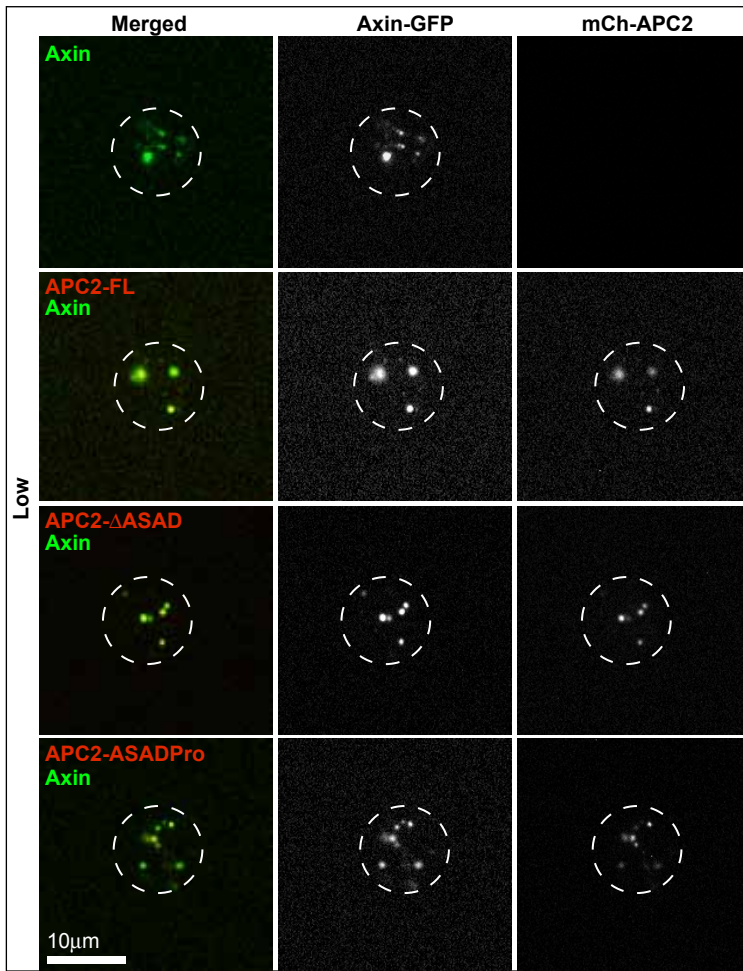


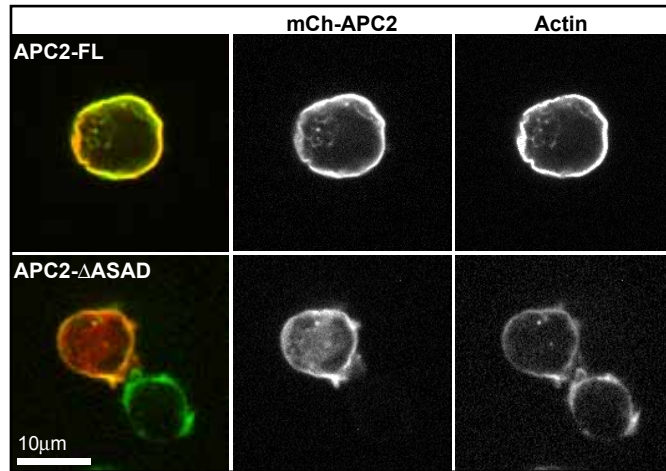
**Percent Sequence Similarity to hAPC(AAA03586.1)**

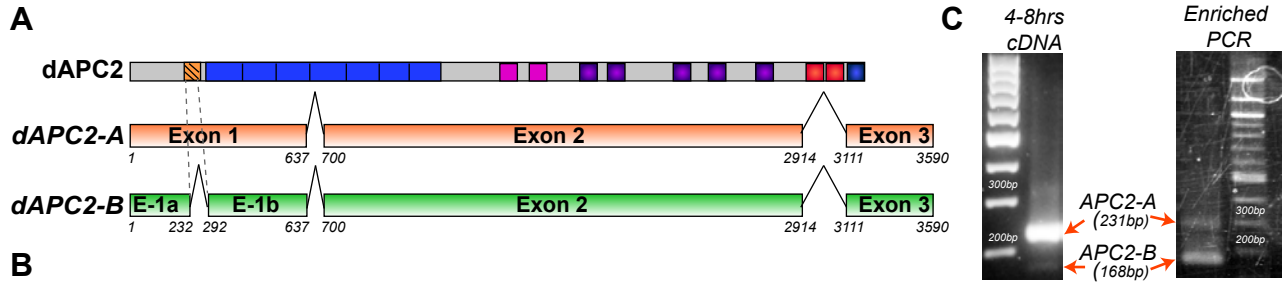
Species Name & Accession #	hAPC-OD-1	hAPC-ASAD
<i>C. intestinalis</i> (XP002124987.2)	83%	55%
<i>S. kowalevskii</i> (XP002738523.1)	96%	78%
<i>S. purpuratus</i> (XP783363.3)	NA	78%
<i>L. gigantea</i> (ESO95067.1)	NA	81%
<i>C. teleta</i> (ELU12449.1)	93%	74%
<i>N. vitripennis</i> (XP001602839.2)	NA	81%
<i>D. melanogaster</i> (AAF56249.1)	NA	68%

NA: Not Applicable









**APC2 Exon 1**

5' - ATGACGCTGAACGAGAGCGACGCTACTCGCCTGGAGCTGACGCGCAACTTCCTGGAAGTGTCTCGAAATCCGGAACATGCACCGCCCTGCGCAGCTCGGACTGTATCCAGCTTCTGGTGCAGATTCTGCACGCCAACGACGAAGGCCTCTCCACGGCGAAAAAGTACGCCAGCCAGGCGCTGCACAACATCGTCCACAATAATCCG **GAGGAGAAGGAGCGCCAGCGGGAG** **GTGAAGATGCTGCGCCTGCTGGACCAGATCCTCGACTACTGTAACTTTCTGCACACCCAGTTGCAG** AGCGGCGGTGAGGCTATCGCAGATGATGAAGATCGTCATCCGCTGGCGGCTATGAAGCTCCTGATGAAAGCCAGTTTCGACGAGGAGCACCGCCAGACTATGTGCGAACTGGGAGCCCTCAAGGCGATTCCCAATTTGGTCCACCTTGATCATGCGGTCCATGGACCGGCTGCCGGTAGGGAACAGTGCAACGCCCTCAGGAGCTACGGCCTCATGGCCCTCACGAATCTCACCTTCGGAGACGAGAACGTCCATAACAAATCGTATCTGTGCGGT CAGCGACAGTTCATGGAAGTGGTCATTGCTCAATTGAACACGGCTCCGGATGAACTGCTACAG - 3'

- APC Self Association Domain (ASAD)
- Alternatively spliced intron in APC2-B
- Forward and reverse primers
- Potential 5' and 3' splice sites
- Potential branch site
- Poly-pyrimidine tract