Supplemental Information

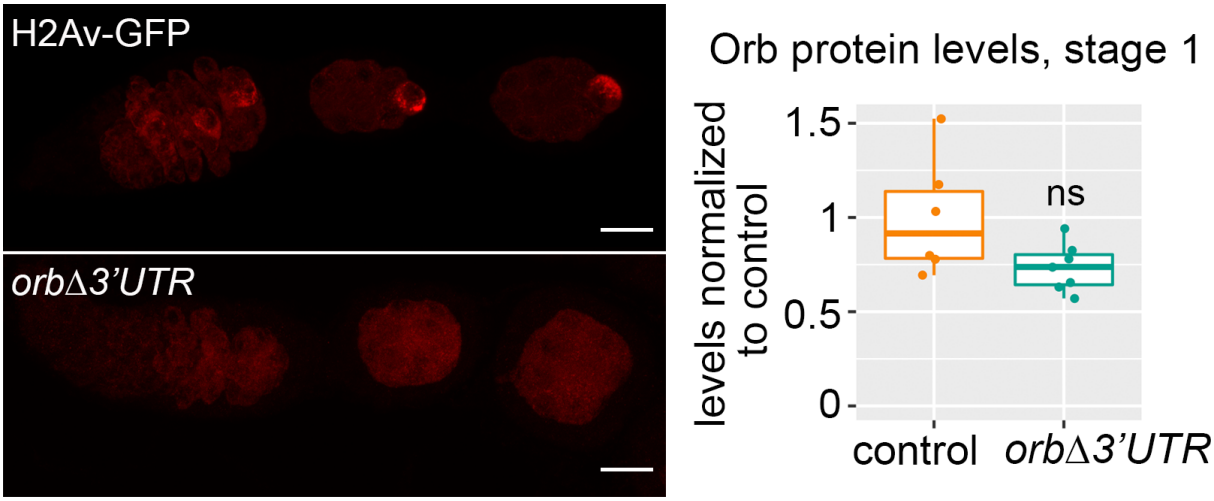

Figure S1. Orb protein levels in control compared with *orbΔ3'UTR*.
H2av-GFP (control, n=6) and *orbΔ3'UTR* (n=7) ovarioles were stained, mounted and imaged together. Maximum Intensity Projections were used to analyze Orb protein levels in the anterior nurse cells at stage 1. ns= not significant.

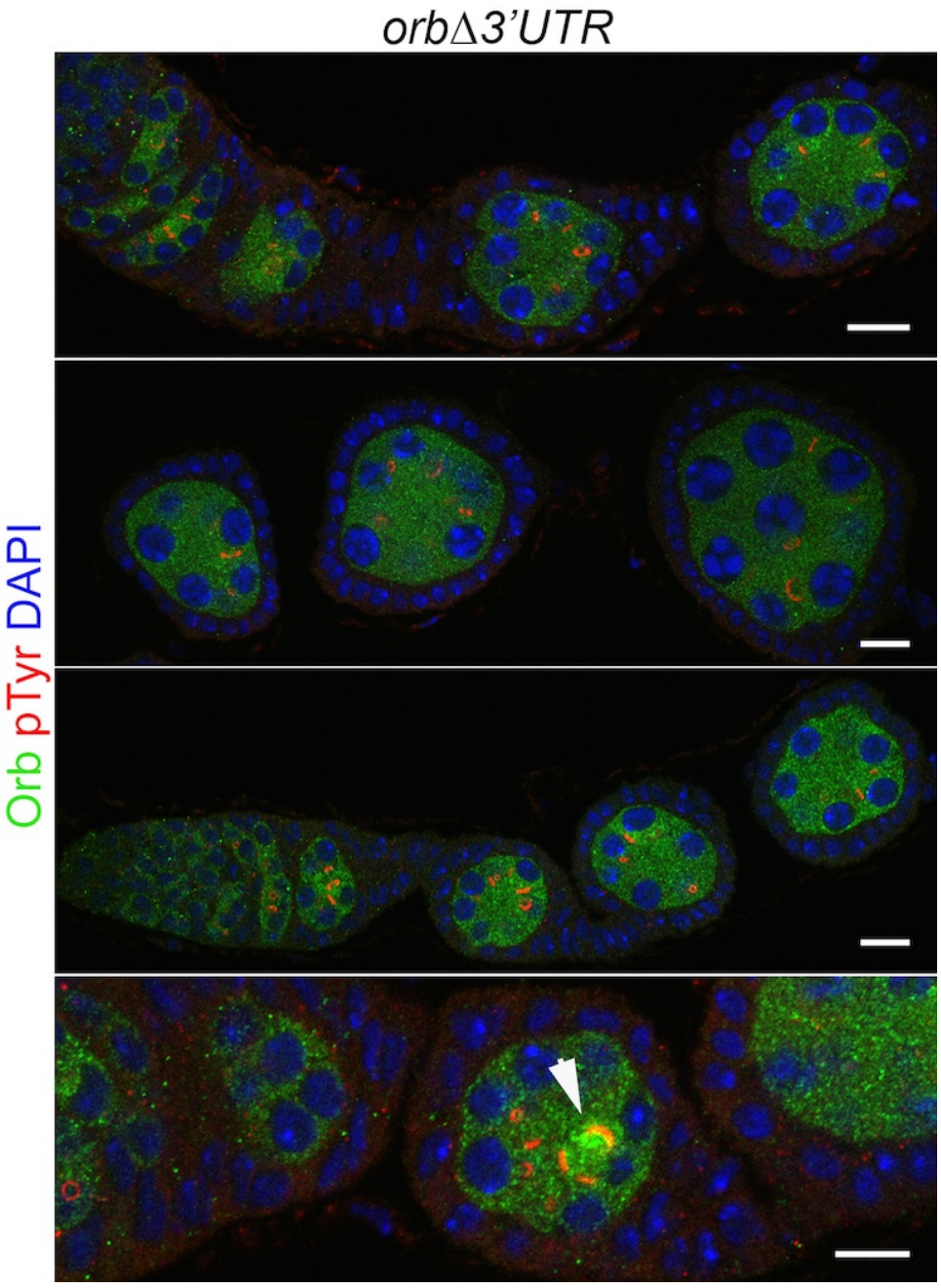

Figure S2. Orb protein in *orbΔ3'UTR*
In most *orbΔ3'UTR* chambers Orb protein appears to be evenly distributed. Infrequently, Orb is concentrated in a single cell (arrowhead). Scale bars 10 um.

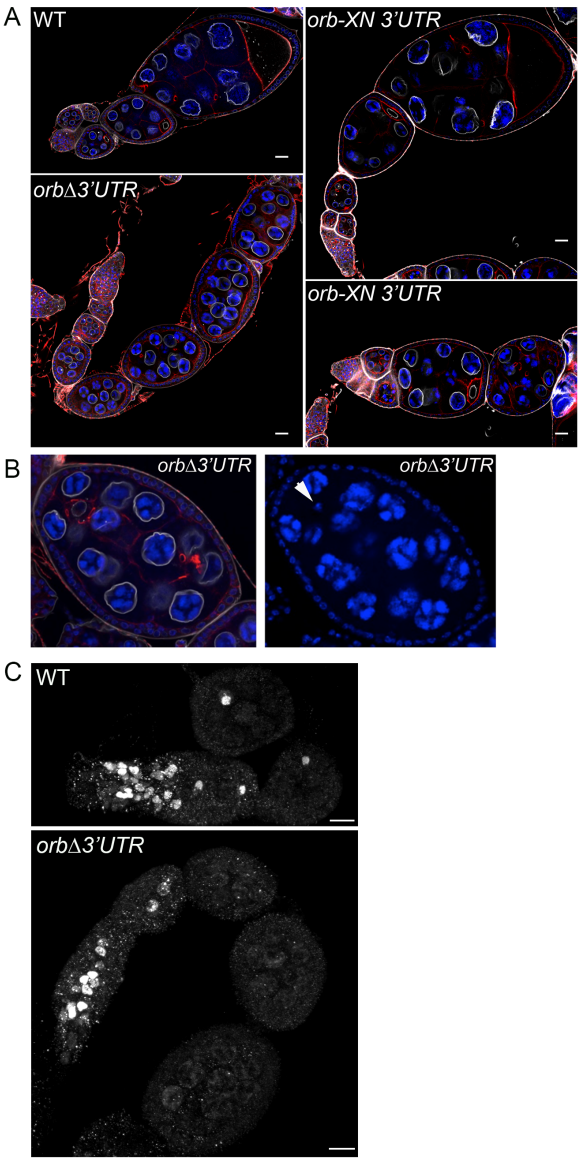

Figure S3. Ovarioles from *orbΔ3'UTR* and *orb-XN 3'UTR* demonstrate defects in oogenesis compared to WT.

(A) WT ovarioles have an array of egg chambers that increase in size and developmental stage from anterior to posterior. In *orbΔ3'UTR* ovarioles, most egg chambers fail to develop an oocyte and egg chambers do not grow past a certain size, nor do they develop properly. In *orb-XN 3'UTR*, some ovarioles contain egg chambers that follow a more or less normal developmental progression (top right panel), while other ovarioles contain egg chambers that arrest development (bottom right panel). Scale bars 50 um (B) Some *orbΔ3'UTR* egg chambers do develop an oocyte-like cell with a karyosome (arrowhead, bottom panels, ~1/75). Scale bars 50 um. (C) In WT, Corolla protein is visible in the oocyte nucleus in egg chambers after stage 1, while in *orbΔ3'UTR* ovarioles Corolla is present at stage 1 in multiple cells and is missing from later stage egg chambers. Scale bar = 10 microns.

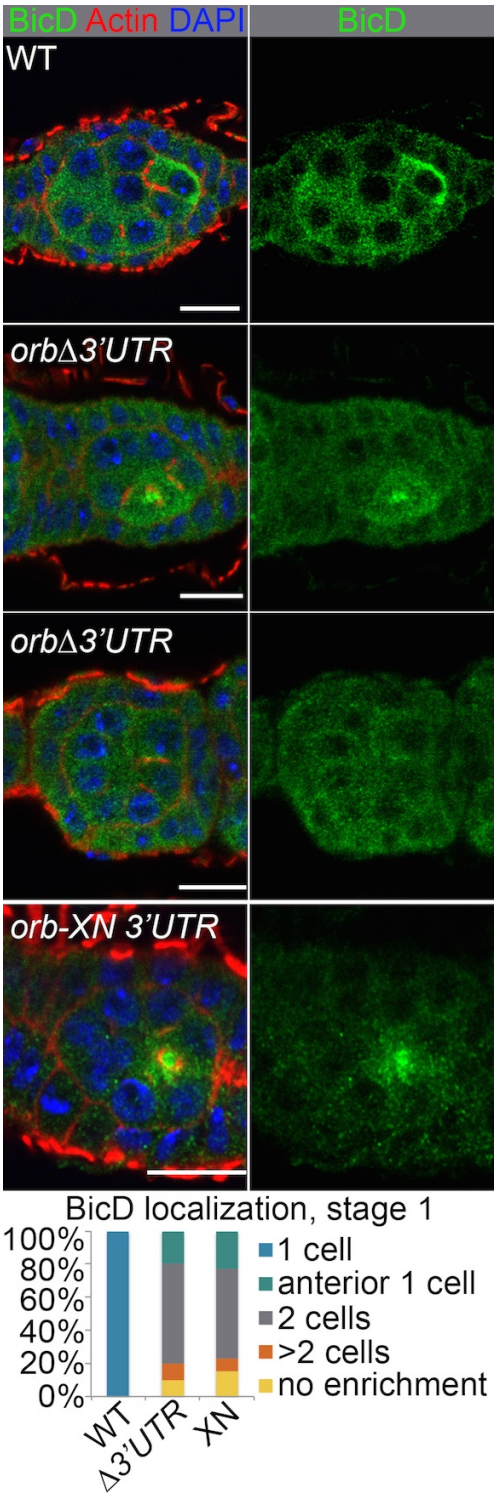

Figure S4. BicD protein is not properly enriched at the oocyte posterior at stage 1 in *orbΔ3'UTR* or *orb-XN 3'UTR*.

BicD protein localization at the oocyte posterior in WT. Defects in BicD protein localization in *orbΔ3'UTR* and *orb-XN* 3'UTR. Quantification of BicD protein localization for WT (n=10), *orbΔ3'UTR* (n=10) and *orb-XN* 3'UTR (n=12). Scale bars 10 um

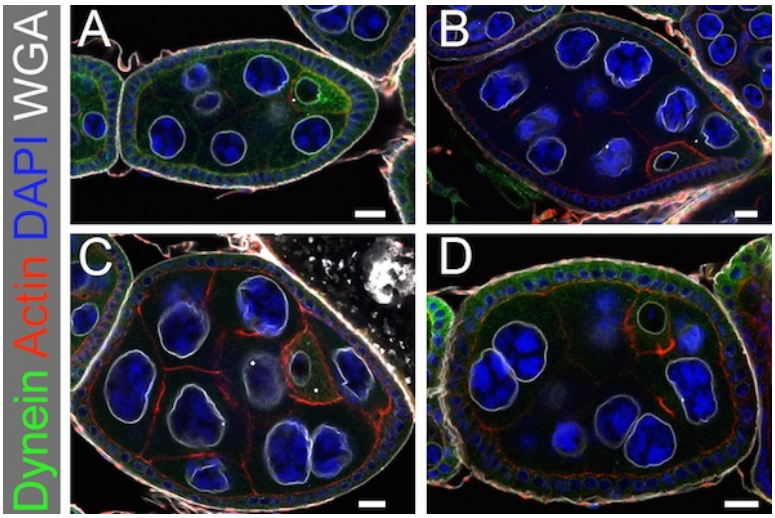

Figure S5. The oocyte is not correctly positioned in *orb-XN 3'UTR*.
(A-D) Mispositioned oocytes in *orb-XN 3'UTR* egg chambers occur frequently. Of the *orb-XN 3'UTR* egg chambers that successfully specify an oocyte, 40% fail to position the oocyte at the posterior in stage 3-7 chambers (n=80).
(A-B) Examples of oocytes which are offset from the posterior end of the egg chamber (63% of the mispositioned oocytes).
(C-D) Examples in which the oocyte is positioned away from the posterior end of the egg chamber (38% of the mispositioned oocytes).
Scale bars 10 um.

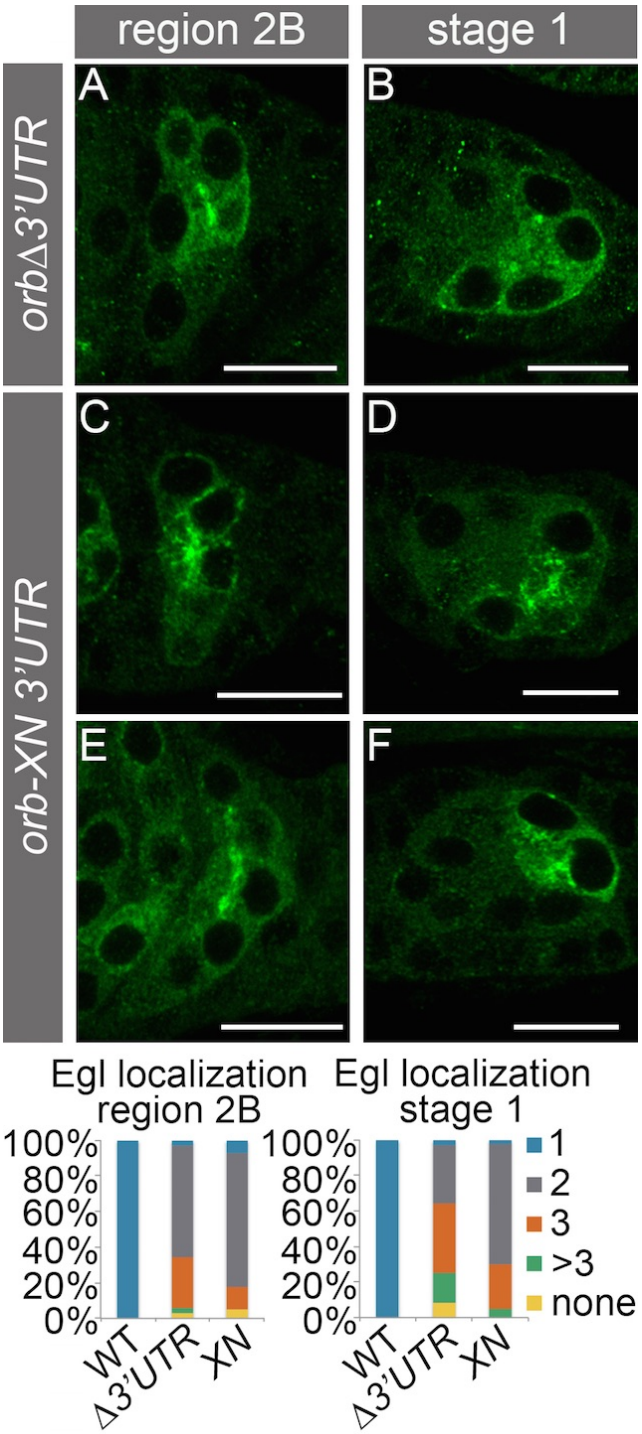

Figure S6. Similar defects in Egl localization in *orbΔ3'UTR* and *orb-XN 3'UTR.*
(A-B) Egl fails to localized to a single in *orbΔ3'UTR* cysts in region 2b (A) and stage 1 (B). Another example of defects in Egl localization in *orbΔ3'UTR* is shown in Fig 3.
(C-F) Egl does not properly localize to one cell during region 2b or stage 1 in *orb-XN 3'UTR*.
Bottom: Quantification of Egl localization defects in region 2b and stage 1 cysts for WT, *orbΔ3'UTR* (also see Fig 3 legend) and *orb-XN 3'UTR* (region 2b n=30; stage 1 n=29).
Scale bars 10 um.

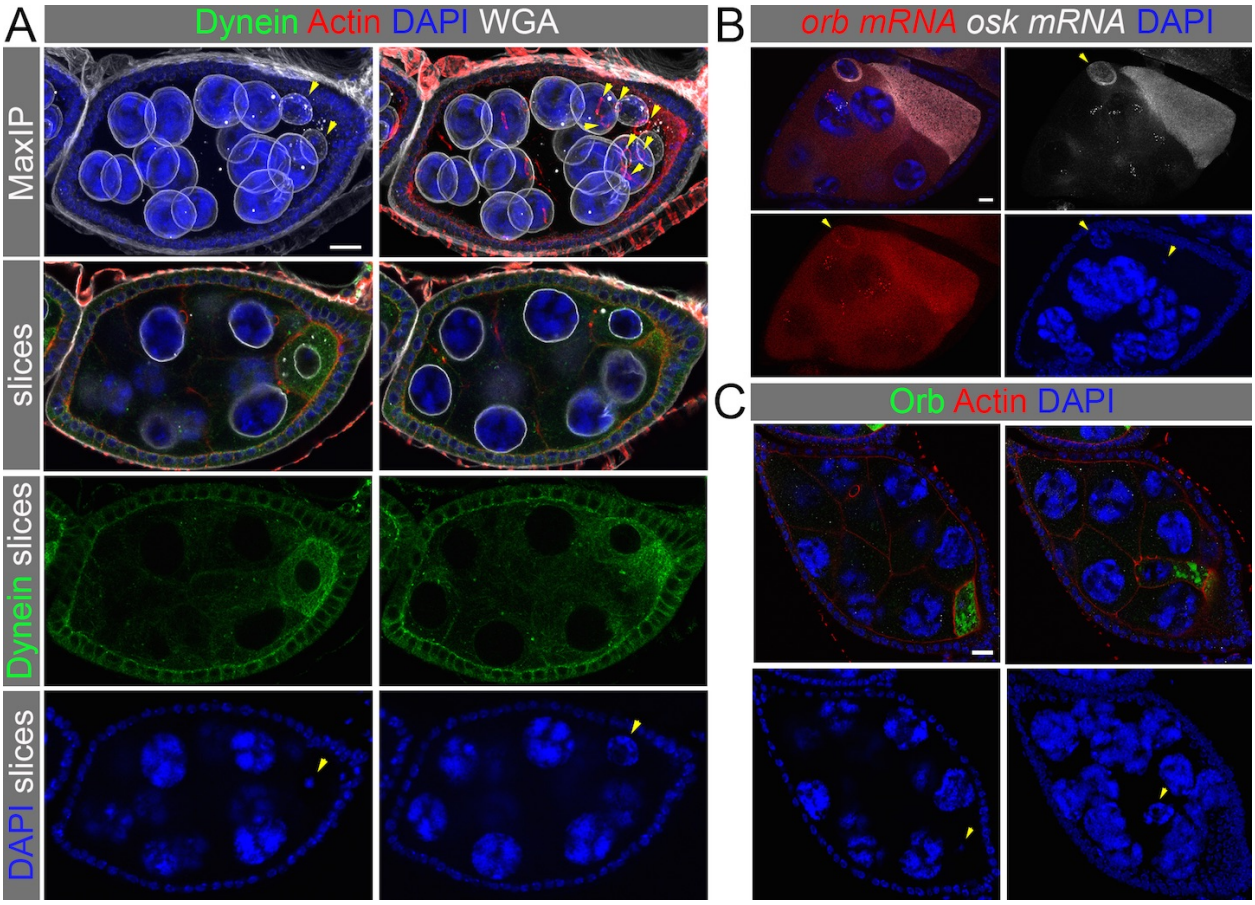

Figure S7.The delay in oocyte specification in *orb-XN 3'UTR* chambers can disrupt the development of both "pro-oocytes."

(A) Maximum intensity projection shows an *orb-XN 3'UTR* egg chamber with 2 small nuclei at the posterior (arrowheads), and both of these cells have 4 ring canals marked by Actin (arrowheads). Image slices show Dynein is enriched in both of the cells with four ring canals and small nuclei. DAPI indicates that one of these cells (bottom) has condensed DNA (arrowhead) as expected for an oocyte, while the neighboring cell is polyploid. However, this cell appears to have much less DNA than other nurse cells in the egg chamber.

(B-C) Other examples of *orb-XN 3'UTR* egg chambers in which a nurse cell neighboring the oocyte appears to have less DNA content than the other nurse cells in the egg chamber, and is enriched with markers of oocyte fate.

Scale bars 10 ums.

Supplemental Methods 1. Sequence of *orb 3'UTR* deletion and *XN-3'UTR*

Wild type orb 3'UTR
CE fragment
XN fragment

ACTGTTACGGCTTTTATCCACACCGTTTTAACGGATGTTTCCGCAATATAATGTGTGAGA
CTTTGGACTTGTAGGCGACGTAGAAATATGGCGGAGATAGTTTTCTTGCAGCCGATGGA
GACCCGGGCTCATCATCGTCATTATCATTGTAAACACAGTTTTATTGATGTGTATATAATA
TCGGTACAATATGAAGCTTACACTTTGAGTTTCATTTAAGATAATTATTGTTAGACGACTC
CCCCAAAAACGAACACCCTCGAATCGAAACAGAAGAGAGACCCCCATTCATACTATTTTT
TGATAATCATGCTTACATATCAGCATTTGGAGCTGGCATTCGAATGCTAAATGAATGATA
CATGAAATGAGCAATTTCATTTACTCATACCCCTCATACAAATACTACTCAAGTTACTTTTT
GGCTAAGAAAGCAGTTATTAATTTCTAATTTGTATGTTTAATTTTAAGGAAACGAAACC**GC**
GCGGCAAATGGCTACTTGAAATGTCTCGACCAATTTGCCGCGCTGCGAGATTAGAAAC
ACCATTTTTGTTATGTTTTGTGCTATTACTGAGGAATTTTATGTAGTTTCTTTTTACATAG
CCAAGCCCCCGACTCGAGTTAATATGATATTATATTATTTGGATTGTCCGCTAAGCGTT
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CCAGTTTTATAGCTCATGGGCAATAAGCATATAATTTATGTCTACTTTATTTTTCATGTATT
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CACAGAATAGGAAAAAAATACTTAAGTCTATATCTTAAACACGAATTGATCACTAAACGAT
AAAATAACGCACACACACCTATTCACCAACCAAACAAATATACAAAATCGCTATTG

orbΔ3'UTR
orb 3'UTR
attP
loxP

ACTGTTACGGCTTTTATCCACACGCGGCCGCGGACATATGCACACCTGCGATC**GTAGTG**
CCCCAACTGGGGTAACCTTTGAGTTCTCTCAGTTGGGGGCGTAGATAACTTCGTATAA
TGTATGCTATACGAAGTTATAGAAGAGCACTAGTAAAGATCTCAGAATAGGAAAAAAATA
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Supplemental Methods 2. oligoFISH probe sequences

***orb* mRNA oligo probes**	***BicD* mRNA oligo probes**
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