

Fig. S1. The Buc antibody does not cross-react with GFP, *Xenopus* Velo or *Drosophila* Oskar.

Western blot showing anti-Buc (red in upper panel and black in lower panel) or anti-GFP (green in upper panel) antibody staining of *in vitro* translated GFP, sOsk-GFP, Velo-GFP and Buc-GFP. Unprogrammed lysate was used as negative control for protein translation. Buc-GFP is visualized by both anti-Buc and anti-GFP antibodies (yellow in merged panel and black in lower panel), whereas Velo-GFP, sOsk-GFP and GFP are only recognized by anti-GFP antibody, but not by anti-Buc antibody.

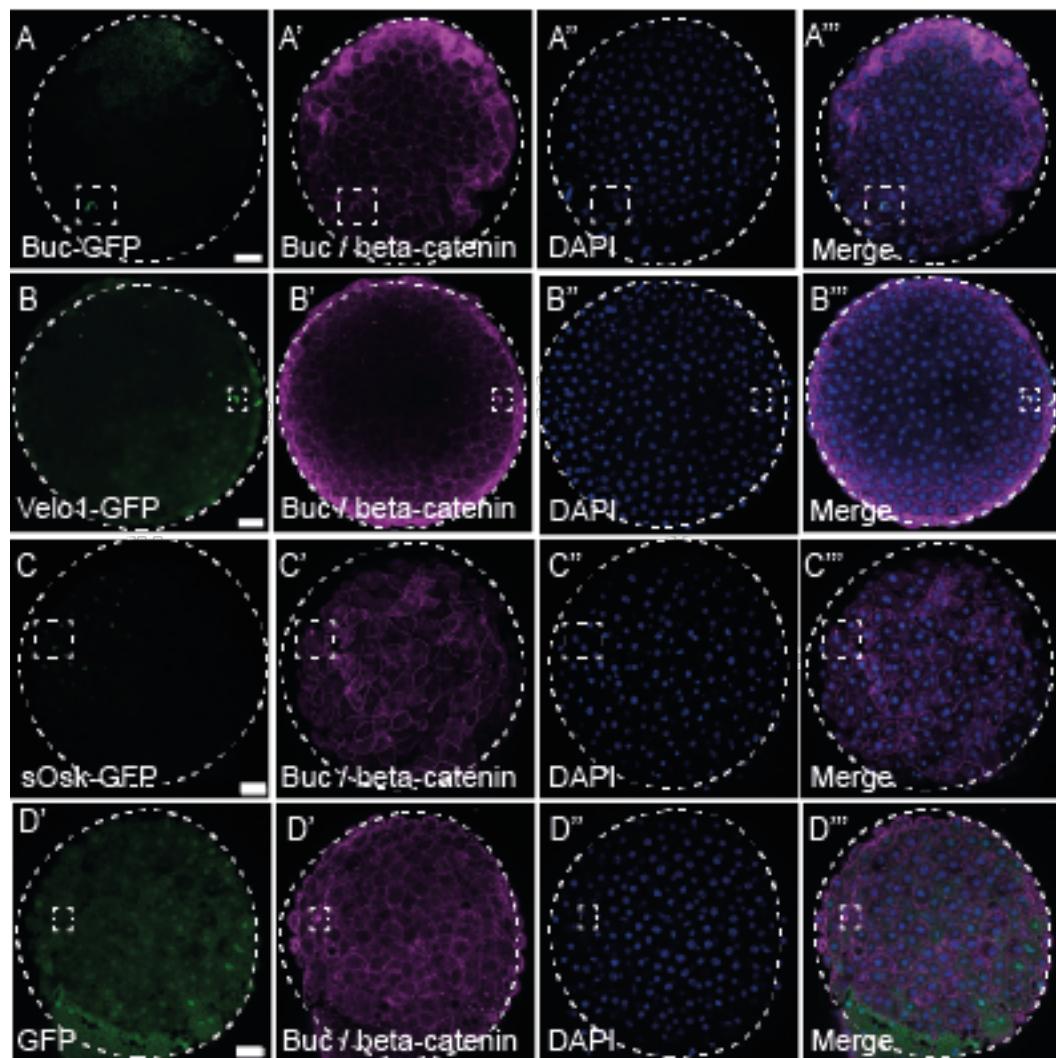


Fig. S2. Germ plasm localization is conserved in vertebrates.

Panels (A,B,C,D) show embryos at high stage from the animal view. Dotted circles outline the embryos. Dotted rectangles show magnified areas in figure 1. Colocalization of the GFP with endogenous Buc was determined by immunohistochemistry: 1st column – injected GFP fusions (green), 2nd column – endogenous Buc and beta-catenin (magenta), 3rd column – DAPI (blue) and 4th column – merge. Buc-GFP (A-A'') and *Xenopus* Velo1 (B-B'') colocalize with endogenous germ plasm, whereas *Drosophila* Osk(C-C'') shows nuclear localization. The GFP control shows ubiquitous low level fluorescence (D-D''). Scalebars: 50 μm.

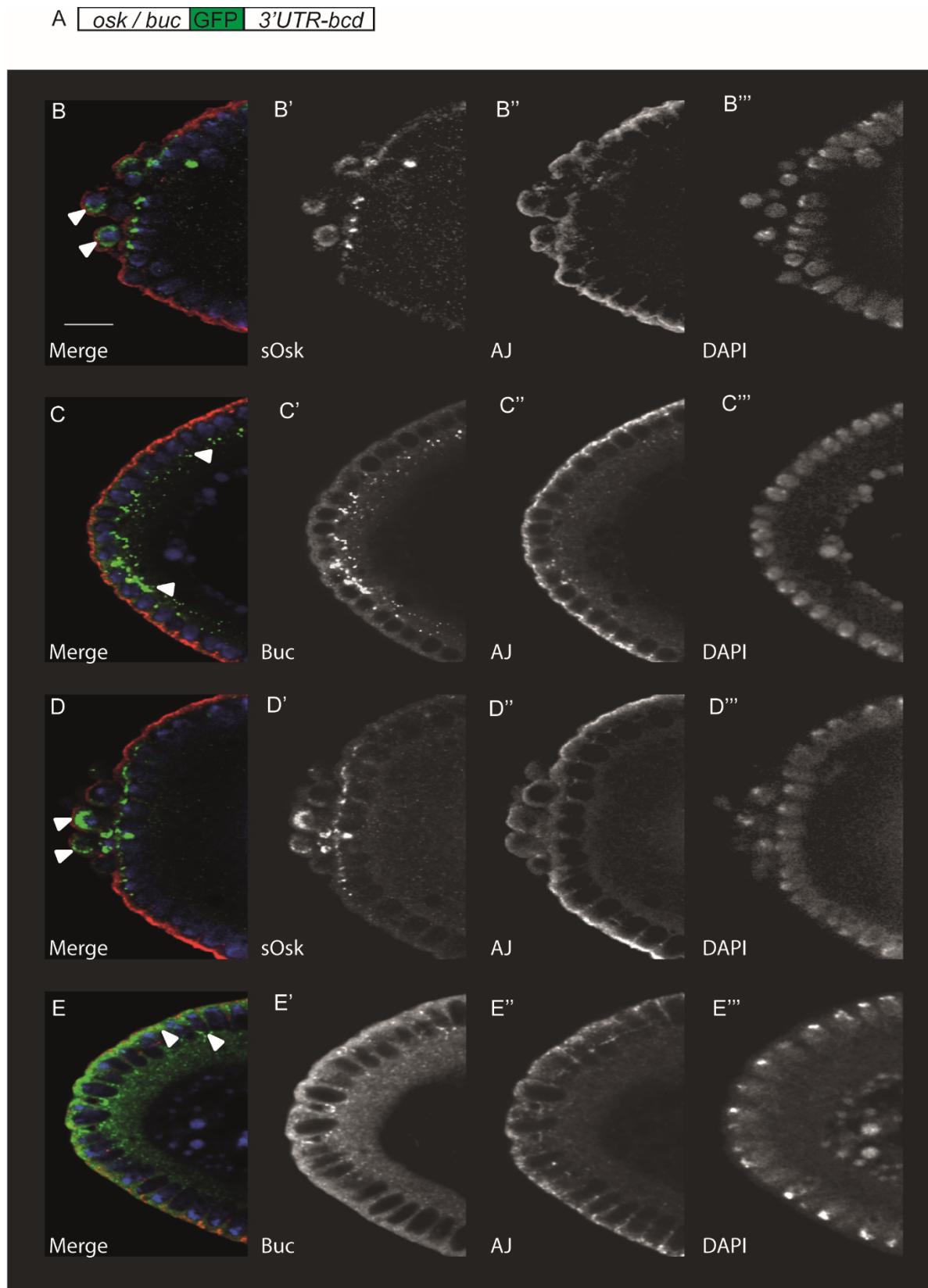


Fig. S3. Transgenic Buc- and Oskar-GFP *Drosophila* embryos show different localization patterns.

(A) Scheme of transgenes to study Bucky ball (Buc) and short Oskar (sOsk) localization. Transgenic flies were generated expressing Buc-GFP or sOsk ectopically at the anterior pole of the embryo by fusion of the constructs to the *bicoid* 3'UTR. (B-E) Localization of sOsk and Buc-GFP was investigated by immunohistochemistry: 1st column – merge, 2nd column – expressed protein, 3rd column – apical junctions (AJ), 4th column – DAPI. Anterior pole of immunostained embryos expressing the indicated transgenic constructs at stage 4 (B, C) and 5 (D, E). sOsk (B, B', D, D') localizes in condensed aggregates at the most distal part of the anterior pole (white arrowheads), whereas Buc-GFP (C, C', E, E') distributes in a gradient along the cortex of the anterior pole (white arrowheads). Scale bar: 10 μ m.

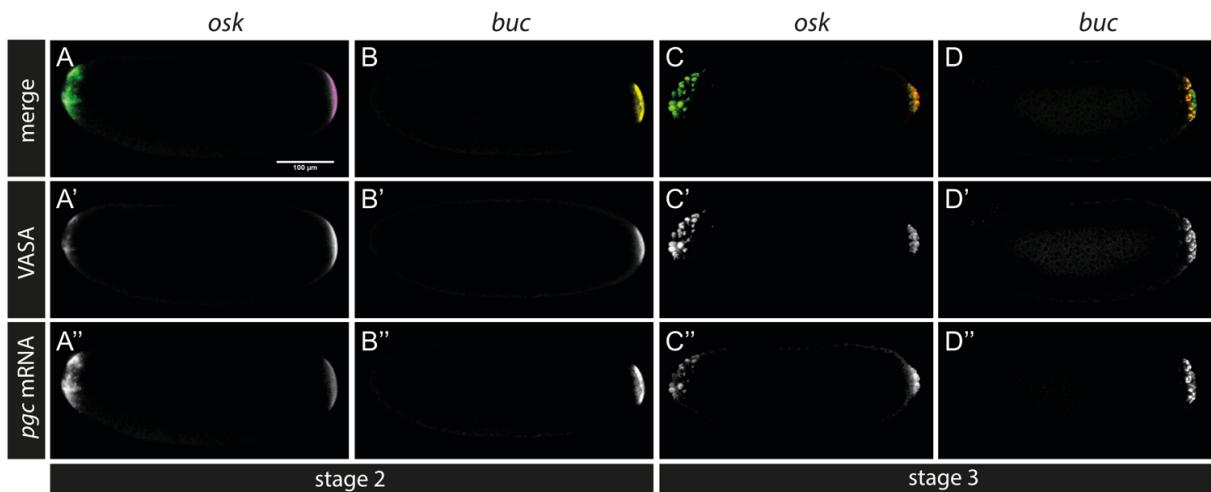


Fig. S4. Vasa protein and pgc mRNA recruitment in transgenic flies.

Vasa protein and *pgc* mRNA labeling of the transgenic embryos showed that sOsk specified ectopic PGCs, whereas Buc transgenics did not recruit Vasa protein or *pgc* mRNA at the anterior pole. (A-A'', B-B'') show VASA and *pgc* labeling of sOsk and Buc in stage 2 transgenic flies. (C-C'', D-D'') show VASA and *pgc* labeling of sOsk and Buc in stage 3 transgenic flies. Scale bar: 100 μm.

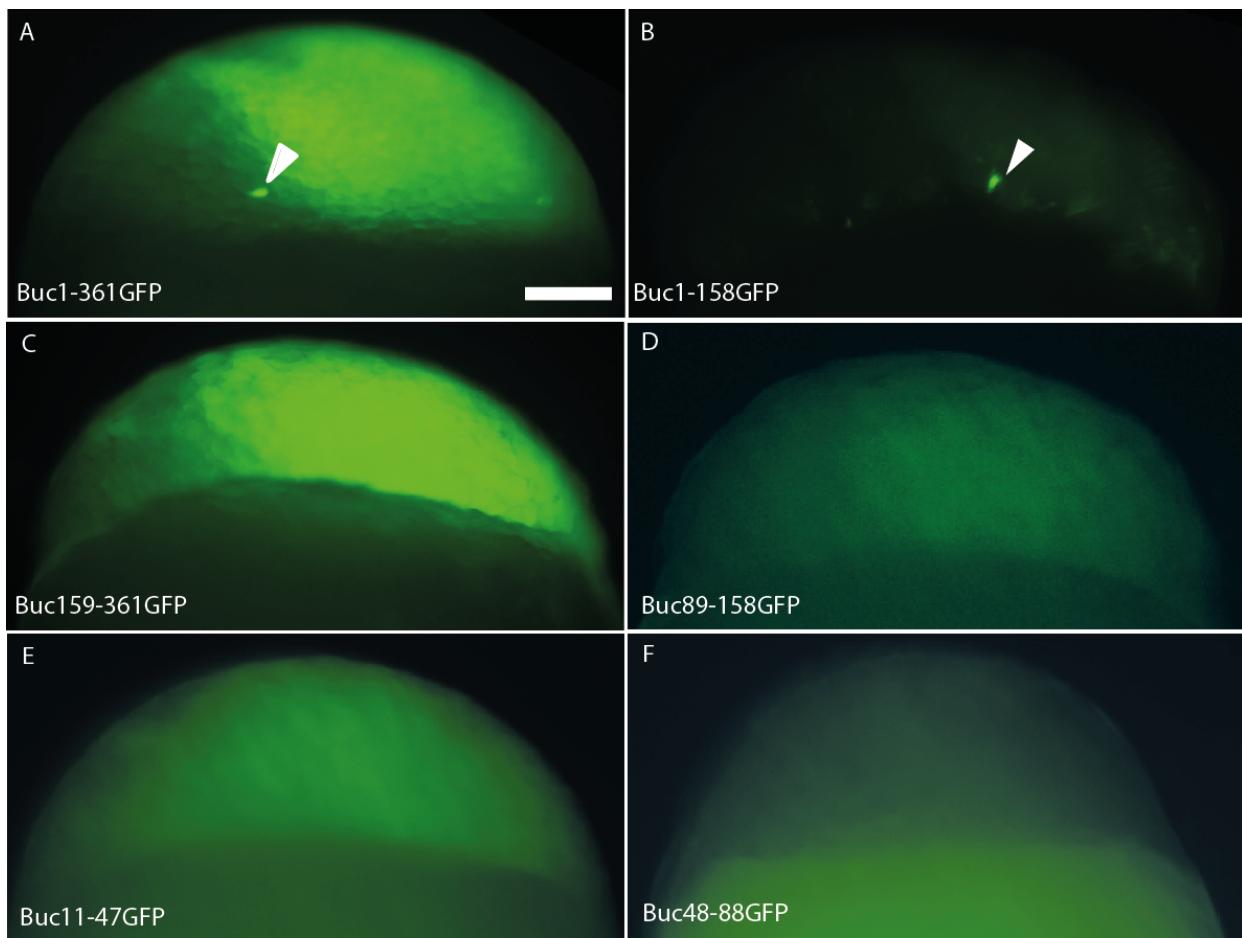


Fig. S5. Systematic mapping of Buc localization motif.

(A) N-terminal fragment of Buc (aa1-361) (white arrowhead) (100%). (B) Buc1-158 localizes (white arrowhead) (100%). (C) Buc159-361 is ubiquitous (0%). (D) Buc89-158 is ubiquitous (0%). (E) Buc11-47 is ubiquitous (0%). (F) Buc48-88 is ubiquitous (6.0±6.7%). Scale bar: 50 μm

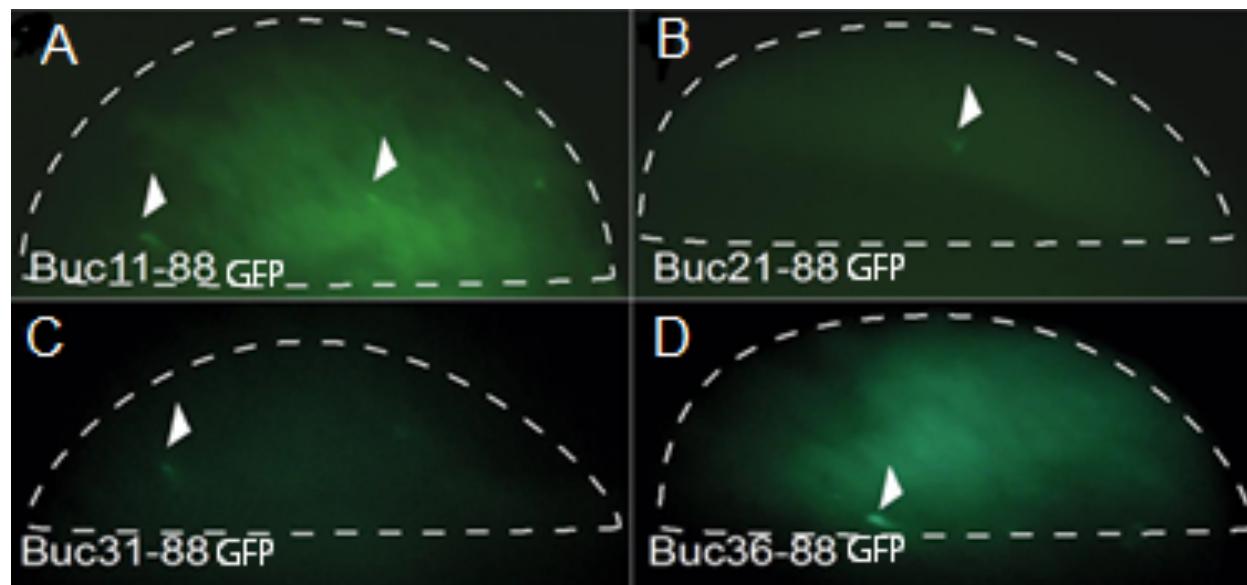


Fig. S6. Aggregation and localization of BucLoc are separate activities.

(A-D) Show embryos at high stage from the lateral view. Embryos are outlined by the dashed white line. Injected constructs showed fluorescent aggregates (white arrowheads). (A) Buc11-88 ($91.4\pm6.8\%$). (B) Buc21-88 ($67\pm4.0\%$). (C) Buc31-88 ($60.1\pm7.9\%$). (D) Buc36-88 (52.2 ± 13).

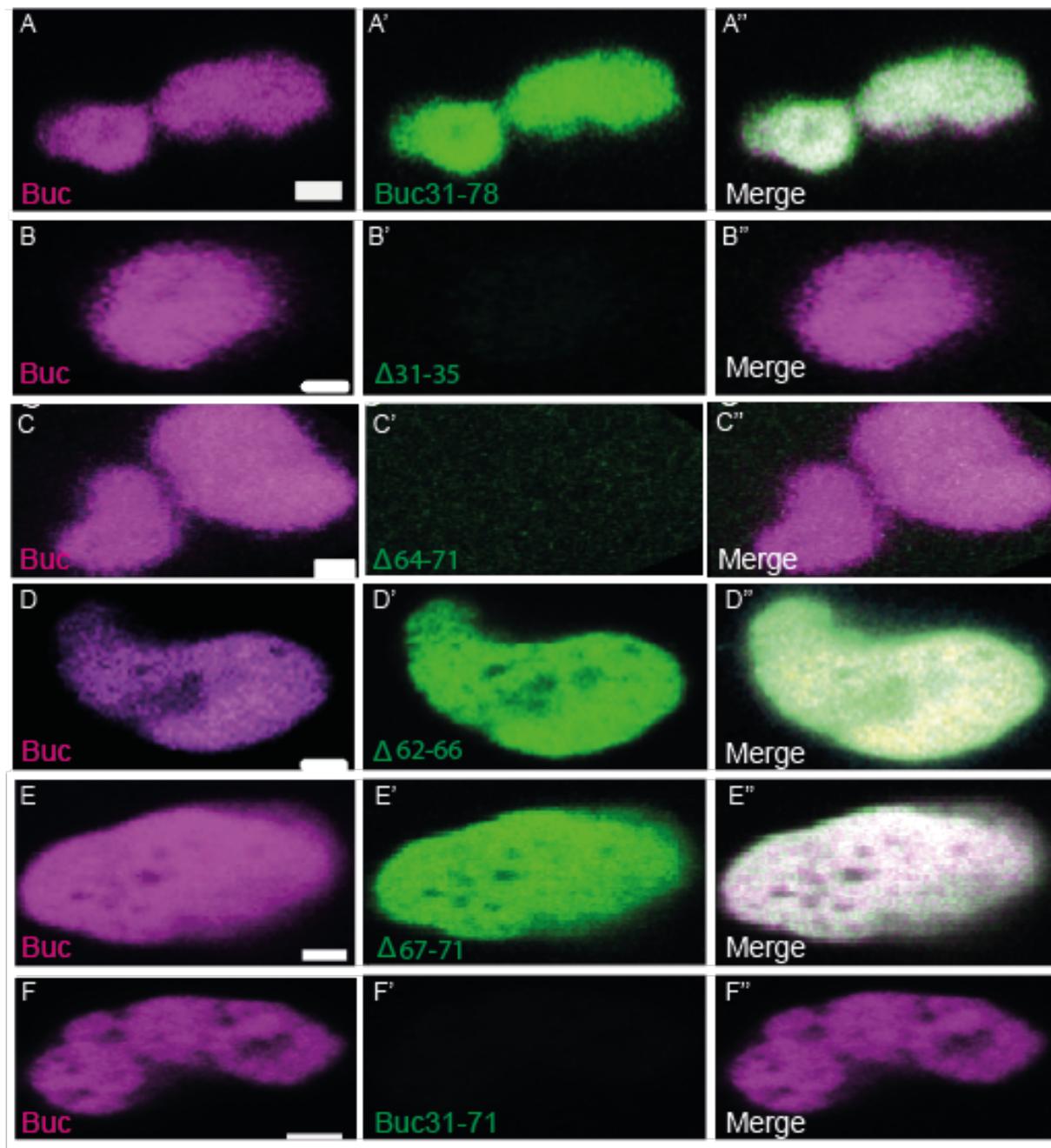


Fig. S7. Colocalization of deletion constructs of BucLoc mapping in Figure 5 with Buc-GFP.

(A-A'') shows colocalization of transgenic Buc-GFP (magenta) and BucLoc-m-cherry fusion (aa31-78) (green). (B-B'') Shows colocalization of transgenic Buc-GFP (magenta) and Buc31-78 (Δ 31-35)-m-cherry fusion (green). (C-C'') Shows colocalization of transgenic Buc-GFP (magenta) and Buc31-78 (Δ 64-71)-m-cherry fusion (green). (D-D'') Shows colocalization of transgenic Buc-GFP (magenta) and Buc31-78 (Δ 62-66)-m-cherry fusion (green). (E-E'') Shows colocalization of transgenic Buc-GFP (magenta) and Buc31-78 (Δ 67-71)-m-cherry fusion (green). (F-F'') Shows colocalization of transgenic Buc-GFP (magenta) and Buc31-71-m-cherry fusion (green). Embryos were injected at 1-cell stage with RNA encoding BucLoc-m-cherry fusions and imaged at high stage. The pictures are representing magnified germ plasm spots. Scale bars: 2 μ m.

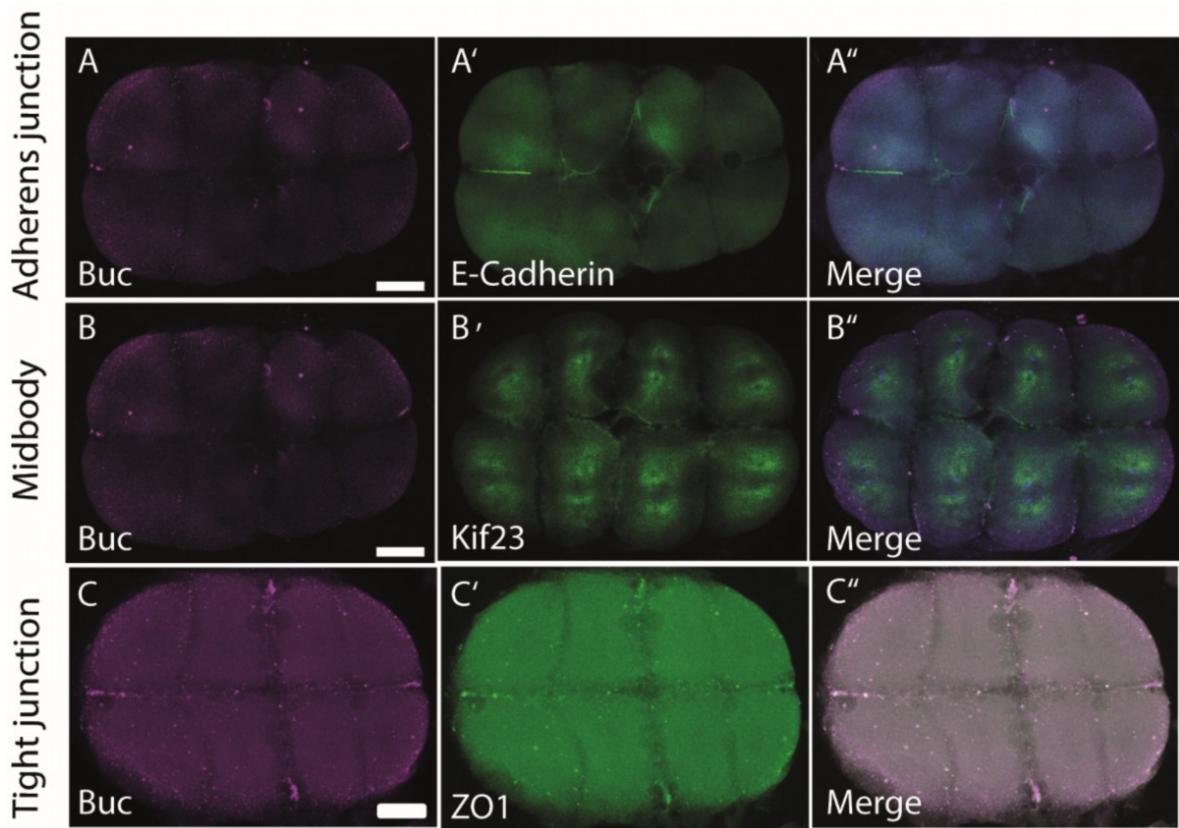
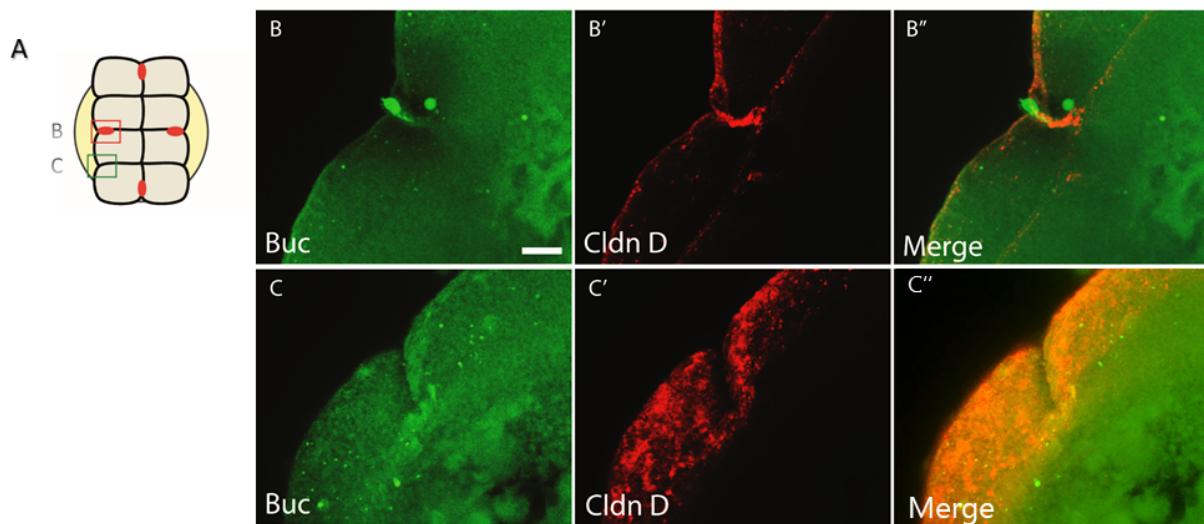
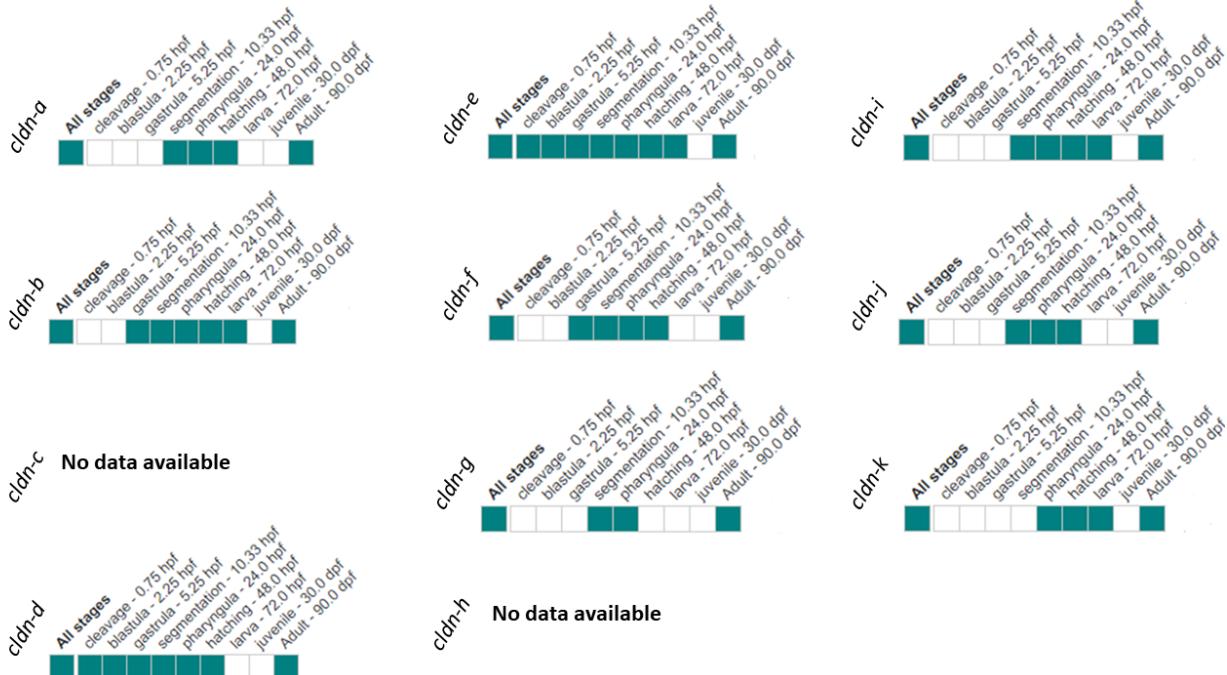


Fig. S8. Tight junction protein ZO1 colocalizes with Buc.

Colocalization analysis of Buc with different cellular structure markers. (A, B, C) Show animal view of immunostained 8-cell stage embryos 1st column - Buc (magenta), 2nd column – respective cellular structure marker (green), 3rd column – merge. (A-A‘‘) Immunostaining for Buc and adherens junction marker E-cadherin; (B-B‘‘) Buc and midbody marker Kif23; (C-C‘‘) Buc and tight junction marker Zonula occludens 1 (ZO1). Scale bars: 50 μm.

**Fig. S9. Immunostaining shows overlap of Buc and *cldn-d*.**

Zebrafish embryo at 8-cell stage was double stained with Buc and Cldn-d antibodies and their colocalization was analysed using confocal microscopy. A) A representative cartoon showing an embryo at 8-cell stage. (B-B'') Colocalization of Buc and Cldn-d at the middle cleavage furrow (the upper red rectangle in A). (C-C'') Localization of Buc and Cldn-d at the lower cleavage furrow (the lower green rectangle in A). Scale bar: 10 μ m.

**Fig. S10. Expression data of zebrafish cldns available on <https://zfin.org/>.**

Expression of cldns at different developmental stages is shown in zebrafish. The green boxes represent annotated expression and the white boxes show no expression. Note only *cldn-d* and *cldn-e* showed expression at early developmental stages. The data is updated as on (date February 28th, 2021).

Table S1. 213 BucLoc interaction candidates selected from 3464 proteins identified by mass spectrometry analysis.

Identified Protein	Accession Number	GFP Co-IP	Buc-GFP Co-IP	BucLoc-GFP Co-IP
PREDICTED: protein PRRC2C [Danio rerio]	gi 528511146	79.6	1278.6	817.9
PREDICTED: zinc finger protein 318-like isoform X1 [Danio rerio]	gi 528497145	12.4	666.7	512.8
Cluster of PREDICTED: protein PRRC2B isoform X4 [Danio rerio] (gi 528481247)	gi 528481247 [2]	22.6	480.1	320.0
large proline-rich protein BAT2 [Danio rerio]	gi 319738640 (+2)	71.6	362.1	280.0
PREDICTED: msx2-interacting protein isoform X1 [Danio rerio]	gi 326678004 (+2)	63.3	434.0	257.4
PREDICTED: microtubule-actin cross-linking factor 1, isoforms 1/2/3/5 isoform X1 [Danio rerio]	gi 528510265	0.0	546.5	218.8
uncharacterized protein LOC792544 [Danio rerio]	gi 194353937	15.8	83.1	201.9
Cluster of PREDICTED: OTU domain-containing protein 4 isoformX1 [Danio rerio] (gi 528518829)	gi 528518829 [2]	83.8	417.6	189.9
PREDICTED: YLP motif-containing protein 1 isoform X2 [Danio rerio]	gi 528511017	36.9	232.6	185.5
PREDICTED: symplekin isoform X2 [Danio rerio]	gi 326668188 (+1)	52.9	218.7	184.5
PREDICTED: microtubule-associated protein futsch-like [Danio rerio]	gi 528497151	1.7	123.8	181.0
retinoblastoma-binding protein 6 isoform 1 [Danio rerio]	gi 302632528 (+4)	13.0	75.0	159.1
Cluster of PREDICTED: membrane-associated guanylate kinase, WW and PDZ domain-containing protein 1 [Danio rerio] (gi 528488944)	gi 528488944 [4]	62.6	191.7	157.7

guanine nucleotide-binding protein subunit beta-2-like 1 [Danio rerio]	gi 18859301	53.1	208.3	149.1
PREDICTED: uncharacterized protein LOC767754 isoform X1 [Danio rerio]	gi 528467168 (+2)	45.1	104.3	134.7
Cluster of eukaryotic translation initiation factor 4A, isoform 1A [Danio rerio] (gi 38198643)	gi 38198643 [3]	43.2	230.2	127.8
PERQ amino acid-rich with GYF domain-containing protein 2 [Danio rerio]	gi 71834468	55.0	261.2	123.5
Cluster of zinc finger CCH domain-containing protein 13 [Danio rerio] (gi 319738618)	gi 319738618	39.2	118.6	120.6
Cluster of PREDICTED: cell cycle associated protein 1b isoform X1 [Danio rerio] (gi 528508316)	gi 528508316 [3]	39.1	262.3	117.6
Cluster of PREDICTED: eukaryotic translation initiation factor 4E transporter isoform X1 [Danio rerio] (gi 528482894)	gi 528482894 [2]	6.5	204.5	114.7
PREDICTED: cytoskeleton-associated protein 5 isoform X1 [Danio rerio]	gi 528520895	2.9	71.0	110.6
regulation of nuclear pre-mRNA domain containing 2a [Danio rerio]	gi 41053979	0.0	65.7	96.8
Cluster of LIM domain only 7b [Danio rerio] (gi 319996634)	gi 319996634 [2]	5.5	182.2	89.9
GPI-anchored membrane protein 1 [Danio rerio]	gi 51011059	17.0	206.9	89.8
PREDICTED: trinucleotide repeat-containing gene 6B protein [Danio rerio]	gi 528495941	0.0	130.1	84.6
Cluster of glutathione S-transferase pi [Danio rerio] (gi 18858197)	gi 18858197	7.1	32.3	82.7
Cluster of PREDICTED: pyrroline-5-carboxylate reductase isoform X1 [Danio rerio] (gi 528495079)	gi 528495079 [2]	28.1	108.1	81.0
ATP synthase subunit O, mitochondrial [Danio rerio]	gi 51467909	4.7	21.0	76.8
pre-mRNA cleavage complex 2 protein Pcf11 [Danio rerio]	gi 55925534	0.9	100.4	75.3

PREDICTED: DNA-directed RNA polymerase II subunit RPB1 isoform X2 [Danio rerio]	gi 528496057 (+1)	0.0	58.5	72.8
Cluster of ataxin-2 [Danio rerio] (gi 190358425)	gi 190358425 [2]	30.9	149.0	70.6
5'-3' exoribonuclease 1 [Danio rerio]	gi 289577074 (+1)	1.4	114.6	65.5
voltage-dependent anion-selective channel protein 2 [Danio rerio]	gi 41054601 (+1)	16.5	33.7	65.5
Cluster of splicing factor 45 [Danio rerio] (gi 41055474)	gi 41055474 [2]	13.5	132.2	57.6
voltage-dependent anion-selective channel protein 1 [Danio rerio]	gi 47777306	17.5	42.1	57.0
PREDICTED: protein FAM208A [Danio rerio]	gi 528517763	1.9	70.5	54.5
single-stranded DNA-binding protein, mitochondrial [Danio rerio]	gi 62955585	11.5	24.0	54.4
PREDICTED: tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide isoform X1 [Danio rerio]	gi 528509453	3.6	13.6	53.7
non-POU domain-containing octamer-binding protein [Danio rerio]	gi 42415509	23.4	96.3	52.9
Cluster of PREDICTED: fish-egg lectin isoform X1 [Danio rerio] (gi 528491480)	gi 528491480 [5]	7.2	64.2	52.2
alpha-2-macroglobulin-like precursor [Danio rerio]	gi 320118891	0.0	17.3	50.3
Cluster of PREDICTED: chromodomain helicase DNA binding protein 4 isoform X1 [Danio rerio] (gi 528509046)	gi 528509046 [4]	1.7	30.1	49.5
14-3-3 protein epsilon [Danio rerio]	gi 47086819	4.6	12.4	47.8
Cluster of uncharacterized protein LOC100141336 precursor [Danio rerio] (gi 168823478)	gi 168823478 [5]	11.2	41.2	47.5
PREDICTED: protein SCAF11 isoform X1 [Danio rerio]	gi 528475676 (+2)	0.9	52.1	45.5
PREDICTED: cytoplasmic dynein 2 heavy chain 1-like, partial [Danio rerio]	gi 528502710	20.8	42.4	44.2

Cluster of PREDICTED: pericentriolar material 1 protein isoform X7 [Danio rerio] (gi 528467744)	gi 528467744 [3]	1.7	12.9	44.1
alpha-2-macroglobulin-like [Danio rerio]	gi 319655740 (+2)	2.9	18.7	43.1
PREDICTED: ubiquitin carboxyl-terminal hydrolase 10 isoformX1 [Danio rerio]	gi 326669691	1.0	138.2	42.9
PREDICTED: cleavage stimulation factor subunit 2-like isoform X1 [Danio rerio]	gi 326673799	11.3	86.7	40.3
Cluster of PREDICTED: alpha-2-macroglobulin isoformX1 [Danio rerio] (gi 326665588)	gi 326665588 [2]	8.9	60.0	39.8
PREDICTED: eukaryotic translation initiation factor 4 gamma 3 [Danio rerio]	gi 528517986	3.0	151.5	39.8
Ndufa9 protein [Danio rerio]	gi 157423514 (+2)	9.6	22.4	39.6
Zgc:158157 protein [Danio rerio]	gi 55250357	0.0	63.4	39.5
Cluster of myosin light chain alkali, smooth-muscle isoform [Danio rerio] (gi 47174755)	gi 47174755 [6]	8.7	17.3	39.1
Cluster of PREDICTED: uncharacterized protein LOC100000125 isoform X2 [Danio rerio] (gi 528510415)	gi 528510415 [3]	6.1	103.3	38.8
signal-induced proliferation-associated 1-like protein 1 [Danio rerio]	gi 529250122	2.8	31.4	38.7
eukaryotic translation initiation factor 4E-1B [Danio rerio]	gi 18858611	11.8	92.6	37.4
Cyc1 protein [Danio rerio]	gi 51327354 (+1)	11.5	32.9	35.8
complement component 1 Q subcomponent-binding protein, mitochondrial [Danio rerio]	gi 324021711 (+1)	9.0	19.2	35.8
LOC449616 protein [Danio rerio]	gi 213624848 (+2)	0.0	92.5	35.8
PREDICTED: cyclin-dependent kinase 13 [Danio rerio]	gi 326679472	5.8	67.7	33.7
Cluster of CWF19-like protein 2 [Danio rerio] (gi 76253886)	gi 76253886 [2]	0.0	28.5	32.6
nanog homeobox [Danio rerio]	gi 528505177 (+1)	3.9	60.8	32.4

PREDICTED: zinc finger CCCH domain-containing protein 4-like isoform X1 [Danio rerio]	gi 528501822	0.0	18.3	31.6
Cluster of PREDICTED: unconventional myosin-Va [Danio rerio] (gi 326680074)	gi 326680074 [4]	3.7	20.9	31.4
PREDICTED: protein FAM208A-like [Danio rerio]	gi 528516938	0.0	23.0	31.2
glutamate dehydrogenase 1b [Danio rerio]	gi 41282194	0.0	27.4	31.0
S-phase kinase-associated protein 1 [Danio rerio]	gi 41152201	14.5	29.8	30.8
PREDICTED: tyrosine-protein phosphatase non-receptor type 13 isoform X1 [Danio rerio]	gi 528513092 (+4)	6.4	107.4	30.7
Cluster of ADP-ribosylation factor 1 like [Danio rerio] (gi 41393117)	gi 41393117 [2]	0.0	11.2	30.1
Si:dkey-16k6.1 protein [Danio rerio]	gi 45767805 (+1)	2.4	7.8	29.8
Cluster of ras homolog gene family, member Ad [Danio rerio] (gi 50539958)	gi 50539958 [3]	1.2	16.1	29.4
tight junction protein ZO-2 isoform 1 [Danio rerio]	gi 320118869 (+2)	0.0	13.5	29.2
Cluster of uncharacterized protein LOC569235 precursor [Danio rerio] (gi 350536793)	gi 350536793 [3]	13.0	42.7	29.2
ATP-dependent RNA helicase DDX42 [Danio rerio]	gi 302318882	0.0	73.3	27.9
Cluster of PREDICTED: hypothetical protein LOC565404 [Danio rerio] (gi 189517232)	gi 189517232 [5]	6.7	58.2	27.5
PREDICTED: telomerase-binding protein EST1A-like isoform X1 [Danio rerio]	gi 326671361 (+1)	0.0	90.3	27.4
PREDICTED: ATP synthase subunit b, mitochondrial isoform X1 [Danio rerio]	gi 528487650 (+1)	3.5	9.1	27.1
mitochondrial import inner membrane translocase subunit Tim13 [Danio rerio]	gi 50539998	0.0	10.2	26.9
PREDICTED: RNA-binding protein 27 isoform X2 [Danio rerio]	gi 528491090 (+1)	5.8	32.9	26.7
PREDICTED: nudC domain-containing protein 1 isoform X1 [Danio rerio]	gi 528509803	2.7	11.2	26.6

regulation of nuclear pre-mRNA domain-containing protein 1B [Danio rerio]	gi 41054665 (+3)	0.0	63.3	25.4
nuclear pore complex protein Nup133 [Danio rerio]	gi 47087231	0.0	8.9	25.1
PREDICTED: RNA-binding protein 6 isoform X1 [Danio rerio]	gi 528483145	0.0	25.4	24.6
Cluster of PREDICTED: RNA-binding protein 26 [Danio rerio] (gi 528481486)	gi 528481486 [2]	2.5	30.2	24.6
glutamate dehydrogenase 1a [Danio rerio]	gi 47086875	0.0	11.1	24.5
Cluster of Bub3 protein [Danio rerio] (gi 53734038)	gi 53734038 [2]	3.2	16.2	23.8
Cluster of PREDICTED: cat eye syndrome critical region protein 2 isoform X2 [Danio rerio] (gi 528521383)	gi 528521383 [2]	0.0	46.3	23.6
LYR motif-containing protein 4 [Danio rerio]	gi 256000753	0.0	7.7	23.4
mitochondrial inner membrane protein [Danio rerio]	gi 47777298 (+1)	3.5	15.4	22.5
Abcf2 protein [Danio rerio]	gi 42542861 (+1)	7.3	27.2	22.5
DNA-directed RNA polymerase II subunit RPB2 [Danio rerio]	gi 302488402	0.0	33.2	22.5
Cluster of SNW domain-containing protein 1 [Danio rerio] (gi 50838798)	gi 50838798	7.9	39.3	22.4
PREDICTED: NFX1-type zinc finger-containing protein 1-like [Danio rerio]	gi 528483584	5.8	39.3	22.4
periphilin-1 [Danio rerio]	gi 121583944 (+1)	1.4	33.7	22.3
PREDICTED: unconventional myosin-IXa isoform X1 [Danio rerio]	gi 528486090 (+1)	0.0	56.0	22.2
PREDICTED: histone-lysine N-methyltransferase SETD1A isoform X1 [Danio rerio]	gi 326666050	0.0	38.1	21.9
PREDICTED: death-inducer obliterator 1-like [Danio rerio]	gi 528516988	0.0	59.7	21.9
Cluster of LOC100000597 protein [Danio rerio] (gi 66910514)	gi 66910514 [5]	0.0	10.2	21.7
dolichyl-diphosphooligosaccharide--	gi 154426290	0.0	10.2	21.1

protein glycosyltransferase subunit DAD1 [Danio rerio]				
protein QIL1 [Danio rerio]	gi 113678245	4.2	10.2	21.1
PREDICTED: E3 ubiquitin-protein ligase TTC3 isoform X2 [Danio rerio]	gi 528491184	2.8	70.6	21.0
serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit delta isoform [Danio rerio]	gi 50726892	0.0	5.5	20.9
cytochrome b-c1 complex subunit Rieske, mitochondrial [Danio rerio]	gi 157073897	0.0	6.1	20.7
Cluster of Protein regulator of cytokinesis 1 [Danio rerio] (gi 28279644)	gi 28279644 [2]	7.5	61.7	20.4
PREDICTED: E3 ubiquitin-protein ligase HERC2, partial [Danio rerio]	gi 528483182	0.0	8.5	20.0
Cluster of LOC563225 protein [Danio rerio] (gi 115292012)	gi 115292012 [2]	7.9	17.8	19.7
Cluster of eukaryotic translation initiation factor 4B [Danio rerio] (gi 47550837)	gi 47550837 [6]	0.0	41.9	19.6
high mobility group protein B2 [Danio rerio]	gi 82658290	0.9	5.7	19.4
Icn protein [Danio rerio]	gi 44890532 (+2)	7.6	23.6	19.3
nuclear pore complex protein Nup160 [Danio rerio]	gi 41054908	0.0	5.3	19.3
Cluster of TNF receptor-associated protein 1 [Danio rerio] (gi 165972373)	gi 165972373 [2]	0.0	71.3	18.8
Cluster of PREDICTED: microtubule-associated serine/threonine-protein kinase 3-like [Danio rerio] (gi 528470977)	gi 528470977 [2]	0.0	25.5	18.8
M-phase phosphoprotein 8 [Danio rerio]	gi 187607764	2.4	42.2	18.7
PREDICTED: PHD finger protein 3 isoform X1 [Danio rerio]	gi 528498598 (+1)	0.0	18.4	18.5
PREDICTED: sideroflexin-3 isoform X1 [Danio rerio]	gi 528497698	1.9	7.2	18.2
Cluster of Epithelial cell adhesion molecule [Danio rerio] (gi 44890710)	gi 44890710 [2]	1.8	13.9	18.0
Sept2 protein [Danio rerio]	gi 115313325 (+3)	0.0	13.3	17.6

Cluster of PREDICTED: regulation of nuclear pre-mRNA domain-containing protein 2 isoform X1 [Danio rerio] (gi 528502856)	gi 528502856 [2]	0.0	12.6	17.5
Zgc:77560 protein [Danio rerio]	gi 42542976 (+1)	2.4	66.6	17.4
Cluster of PREDICTED: nucleolar pre-ribosomal-associated protein 1-like [Danio rerio] (gi 528501168)	gi 528501168 [2]	2.9	20.9	17.3
ras-related protein Rab-14 [Danio rerio]	gi 41393147	0.0	5.1	16.8
NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10 [Danio rerio]	gi 41152268	3.5	17.2	16.8
Cluster of oxoglutarate (alpha-ketoglutarate) dehydrogenase (lipoamide) [Danio rerio] (gi 254028264)	gi 254028264 [2]	0.0	5.6	16.6
Pard3 protein [Danio rerio]	gi 190339230 (+4)	0.9	32.3	16.6
DNA-directed RNA polymerase II subunit RPB3 [Danio rerio]	gi 269784633	0.0	22.8	16.3
RecName: Full=Protein CASC3; AltName: Full=Cancer susceptibility candidate gene 3 protein homolog; AltName: Full=Metastatic lymph node protein 51 homolog; Short=DrMLN51; Short=Protein MLN 51 homolog	gi 123886565 (+1)	6.3	28.9	16.1
Cluster of serine/threonine kinase 36 (fused homolog, Drosophila) [Danio rerio] (gi 320043268)	gi 320043268	6.0	13.9	16.0
cytotoxic granule-associated RNA binding protein 1 [Danio rerio]	gi 47086779	6.4	16.9	16.0
Cluster of PREDICTED: uncharacterized protein LOC393431 isoform X1 [Danio rerio] (gi 528486792)	gi 528486792	0.0	9.1	16.0
Cluster of nuclear receptor corepressor 2 [Danio rerio] (gi 380420327)	gi 380420327 [4]	0.0	57.7	15.9
Cas-Br-M (murine) ecotropic retroviral transforming sequence-like 1 [Danio rerio]	gi 41055074	3.3	20.8	15.7

PREDICTED: polyribonucleotide 5'-hydroxyl-kinase Clp1-like [Danio rerio]	gi 189528302	0.0	53.2	15.4
uncharacterized protein LOC556124 [Danio rerio]	gi 157909776	1.4	30.5	15.2
succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial precursor [Danio rerio]	gi 148922926	5.4	11.3	14.9
Cluster of cAMP-dependent protein kinase catalytic subunit alpha [Danio rerio] (gi 130493522)	gi 130493522 [3]	0.0	8.8	14.9
serine/threonine-protein kinase 3 [Danio rerio]	gi 41054445 (+1)	3.6	49.0	14.8
uncharacterized protein LOC541537 [Danio rerio]	gi 62122901	0.0	7.4	14.6
chromodomain-helicase-DNA-binding protein 8 [Danio rerio]	gi 320461545 (+2)	0.0	22.7	14.6
PREDICTED: splicing factor, arginine-serine-rich 15 [Danio rerio]	gi 528492333	0.0	20.4	14.4
zinc finger HIT domain-containing protein 3 [Danio rerio]	gi 41053670	3.5	33.1	14.0
eukaryotic translation initiation factor 6 [Danio rerio]	gi 41055624 (+2)	0.0	9.1	13.9
PREDICTED: nuclear pore complex protein Nup153 isoform X1 [Danio rerio]	gi 528510621	0.0	17.5	13.7
PREDICTED: uncharacterized protein LOC436879 isoform X4 [Danio rerio]	gi 528517594	0.0	11.1	13.3
Zgc:111960 protein [Danio rerio]	gi 166796880	0.0	11.6	13.2
very low-density lipoprotein receptor precursor [Danio rerio]	gi 169646705 (+4)	0.0	5.1	13.0
C-terminal binding protein 1 [Danio rerio]	gi 40254690	3.3	11.6	12.9
claudin-like protein ZF-A89 [Danio rerio]	gi 30725822	0.0	5.2	12.8
Cluster of PREDICTED: C2 domain-containing protein 3 [Danio rerio] (gi 528501432)	gi 528501432	0.0	9.2	12.7
PREDICTED: histone deacetylase 1 isoform X1 [Danio rerio]	gi 528510099	2.6	29.6	12.0
peptidyl-prolyl cis-trans isomerase-like 1 [Danio rerio]	gi 77683061	1.9	18.6	11.9

Cluster of ras-related C3 botulinum toxin substrate 1 [Danio rerio] (gi 54792776)	gi 54792776 [2]	3.5	11.7	11.9
PREDICTED: uncharacterized protein KIAA0556-like [Danio rerio]	gi 528520519	0.0	14.9	11.8
PREDICTED: trinucleotide repeat-containing gene 6B protein-like isoform X2 [Danio rerio]	gi 528472879 (+1)	0.0	23.4	11.7
nuclear pore complex protein Nup107 [Danio rerio]	gi 71834480	1.8	6.9	11.3
Cluster of PREDICTED: tight junction protein ZO-1-like [Danio rerio] (gi 528521995)	gi 528521995	0.0	12.5	11.3
DIS3-like exonuclease 1 [Danio rerio]	gi 160333118	0.0	32.6	11.3
PREDICTED: PAB-dependent poly(A)-specific ribonuclease subunit 2-like isoform X2 [Danio rerio]	gi 528518391 (+1)	0.0	13.3	11.2
Cluster of PREDICTED: PERQ amino acid-rich with GYF domain-containing protein 1-like isoform X1 [Danio rerio] (gi 528492127)	gi 528492127 [2]	0.0	13.3	11.2
uncharacterized protein LOC550263 [Danio rerio]	gi 62955177	0.0	14.9	11.2
Cluster of PREDICTED: serine/threonine-protein kinase TAO2-like [Danio rerio] (gi 125812164)	gi 125812164	0.0	17.9	10.7
PREDICTED: uncharacterized protein LOC503771 isoform X3 [Danio rerio]	gi 528519733	0.0	17.1	10.6
Cluster of LOC559853 protein, partial [Danio rerio] (gi 79151969)	gi 79151969 [2]	0.0	38.9	10.4
PREDICTED: serine/threonine-protein kinase LATS1 isoform X1 [Danio rerio]	gi 528510786	0.0	20.4	10.2
PREDICTED: cyclin-dependent kinase 12 [Danio rerio]	gi 528509066	0.9	29.5	10.0
poly(rC)-binding protein 2 [Danio rerio]	gi 41055221	0.0	12.7	9.9
nanog homeobox [Danio rerio]	gi 148357118	0.0	28.5	9.9
Cluster of TIA1 cytotoxic granule-associated RNA binding protein [Danio rerio] (gi 37681959)	gi 37681959 [4]	4.8	33.1	9.9

protein mago nashi homolog [Danio rerio]	gi 62955377	3.8	15.8	9.8
nucleoporin 98 [Danio rerio]	gi 320118905 (+1)	0.0	7.5	9.7
uncharacterized protein LOC100126100 precursor [Danio rerio]	gi 157954446 (+1)	2.9	22.5	9.7
N-acetyltransferase 10 [Danio rerio]	gi 41055301	4.7	15.0	9.7
OCIA domain-containing protein 1 [Danio rerio]	gi 41053513 (+1)	0.0	5.8	9.5
cytochrome c oxidase subunit II [Danio rerio]	gi 8395615	2.4	5.1	9.5
PREDICTED: transcription factor 19 [Danio rerio]	gi 292624089	0.0	17.6	9.4
signal recognition particle 9 [Danio rerio]	gi 41055367	0.0	5.1	9.4
actin related protein 2/3 complex subunit 4 [Danio rerio]	gi 45387521	0.0	7.4	9.1
PREDICTED: lysine-specific demethylase 6A isoform X1 [Danio rerio]	gi 528490350 (+1)	0.0	26.6	8.8
exosome complex exonuclease RRP4 [Danio rerio]	gi 339717151	2.9	22.8	8.8
PREDICTED: bromodomain adjacent to zinc finger domain, 2A isoform X1 [Danio rerio]	gi 528517226 (+1)	0.0	8.0	8.7
PREDICTED: AF4/FMR2 family member 4 isoform X1 [Danio rerio]	gi 528514500 (+3)	2.6	13.3	8.6
Cluster of PREDICTED: kinesin family member 13A [Danio rerio] (gi 528505240)	gi 528505240 [5]	0.0	5.4	8.4
exosome complex exonuclease RRP45 [Danio rerio]	gi 54400656	0.0	20.2	8.4
exosome complex component MTR3 [Danio rerio]	gi 66472734	1.0	14.4	8.1
MGC174638 protein [Danio rerio]	gi 156230391 (+2)	0.0	7.8	8.0
Cluster of cyclin-L1 [Danio rerio] (gi 41054323)	gi 41054323	3.6	13.3	7.7
Xrcc5 protein [Danio rerio]	gi 133777834	0.0	12.9	7.7
sorting and assembly machinery component 50 homolog B [Danio rerio]	gi 55925219	0.0	5.2	7.4

Cluster of PREDICTED: rho GTPase-activating protein 21 isoform X1 [Danio rerio] (gi 528470502)	gi 528470502 [2]	0.0	10.9	7.4
60S ribosomal protein L29 [Danio rerio]	gi 51010951	2.9	11.6	7.1
immediate early response 3-interacting protein 1 precursor [Danio rerio]	gi 356991159	0.0	5.1	7.0
transmembrane and coiled-coil domains 1 [Danio rerio]	gi 50540216 (+1)	1.9	8.7	7.0
PREDICTED: wu:fc48e01 [Danio rerio]	gi 125820176	0.0	18.4	6.8
Zgc:123096 protein, partial [Danio rerio]	gi 50417024 (+1)	0.0	6.1	6.6
PREDICTED: protein TANC1-like isoform X2 [Danio rerio]	gi 528481904 (+1)	0.0	9.1	6.4
Centrin2 [Danio rerio]	gi 161213715 (+1)	1.9	14.9	6.3
PREDICTED: dehydrogenase/reductase SDR family member 7B isoform X1 [Danio rerio]	gi 528473478	0.0	10.8	6.3
cyclin T2b [Danio rerio]	gi 47086855	2.7	16.8	6.3
U4/U6.U5 tri-snRNP-associated protein 1 [Danio rerio]	gi 50540414	0.0	15.6	5.9
PREDICTED: uveal autoantigen with coiled-coil domains and ankyrin repeats [Danio rerio]	gi 292616084 (+1)	0.0	6.6	5.9
C-Myc-binding protein [Danio rerio]	gi 91176306	0.0	15.4	5.9
transcription elongation factor B polypeptide 1 [Danio rerio]	gi 52219182 (+1)	0.0	7.7	5.9
Cluster of cytochrome c oxidase assembly factor 5 [Danio rerio] (gi 238859533)	gi 238859533	0.0	5.1	5.9
PREDICTED: uncharacterized protein DKFZp762I1415-like [Danio rerio]	gi 68399071	0.0	5.1	5.9
Ku70 autoantigen [Danio rerio]	gi 114215700 (+2)	1.6	7.4	5.7
uncharacterized protein LOC100216070 [Xenopus (Silurana) tropicalis]	gi 213983243 (+1)	0.0	11.3	5.6
RNA-binding protein 8A [Danio rerio]	gi 61651846	2.4	9.0	5.6

G kinase anchoring protein 1 [Danio rerio]	gi 32451811 (+3)	0.0	17.8	5.6
PREDICTED: nuclear receptor coactivator 3 [Danio rerio]	gi 326671802	0.0	14.8	5.3
Cluster of PREDICTED: mps one binder kinase activator-like 1B-like [Danio rerio] (gi 292614396)	gi 292614396 [5]	0.0	9.1	5.1
coiled-coil-helix-coiled-coil-helix domain-containing protein 2, mitochondrial [Danio rerio]	gi 41152140	0.0	8.1	5.1
store-operated calcium entry-associated regulatory factor precursor [Danio rerio]	gi 115495791 (+2)	0.0	13.2	5.0

Table S2. Data for 16-cell injection assay.

<i>Cldn-dΔYV</i>	R1	R2	R3	R4
<i>total</i>	38	20	20	16
<i>same</i>	21	8	12	9
<i>down</i>	10	10	6	7
 <i>uninjected</i>	 R1	 R2	 R3	 R4
<i>total</i>		20	21	16
<i>same</i>		18	20	16
<i>down</i>		0	0	0
 <i>Cldn-d</i>	 R1	 R2	 R3	 R4
<i>total</i>	20	16	13	
<i>same</i>	19	8	13	
<i>down</i>	0	3		0

R: Replicate total: total number of embryos same: embryos did not lose germ plasm spot down: embryos lost a germ plasm spot

Note: Buc spots were counted twice in each embryo, right after injection (16 cell stage) and at 2 hpf.

Table S3. Cloned constructs.

Insert	Vector	Cloning method	FW Primer	RV Primer	Note
<i>buc-egfp</i>	pCS2+	-	-	-	Bontems et al., 2009.
<i>sosk</i>	pCS2+	restriction enzyme cloning	TTGTTCTTTGCAGGATCATGA CCATCATCGAGAGAAC	TCGAATCGATGGGATCGTGG TATGTTCTCCAGGGACGG	
<i>sOsk</i>	pDONR2 21	Gateway	GGGGACAAGTTGTACAAAAAA GAGGCTATGACCATCATCGA G AGCAAC	GGGGACCACTTGTACAAGAAAG AAAG CTGGGTAGGGTATGTTCTCAG GGACGG	pENTR221 was recombined with pCSDest2 and p3EeGFP to produce pEXPpCSDest2.
<i>buc1-361</i>	pDONR2 21	Gateway	GGGGACAAGTTGTACAAAAAA GCAGGCTCTATGGAGGAATAA ATAACAATTCA	GGGGACCACTTGTACAAGAAAG CTGGGTAGGGTAGGAATAAGCAC TGCC	pENTR221 was recombined with pCSDest2 and p3EeGFP to produce pEXPpCSDest2.
<i>buc1-158</i>	pDONR2 21	Gateway	GGGGACAAGTTGTACAAAAAA GCAGGCTCTATGGAGGAATAA ATAACAATTCA	GGGGACCACTTGTACAAGAAAG CTGGGTAGGGTAGAGAGACT ACATTGTT	pENTR221 was recombined with pCSDest2 and p3EeGFP to produce pEXPpCSDest2.
<i>buc159-361</i>	pDONR2 21	Gateway	GGGGACAAGTTGTACAAAAAA GCAGGCTCTATGGATGTGGTC AGGGAGAGAA	GGGGACCACTTGTACAAGAAAG CTGGGTAGGGTAGGAATAAGCAC TGCC	pENTR221 was recombined with pCSDest2 and p3EeGFP to produce pEXPpCSDest2.
<i>buc11-88</i>	pDONR2 21	Gateway	GGGGACAAGTTGTACAA AAAAGCAGGCTATATGGG AGTTGGCAACCTCA	GGGGACCACTTGTACAAGAAAG AAAG CTGGGTATCTCTGTAATCAATT GGCTG	pENTR221 was recombined with pCSDest2 and p3EeGFP or p3EmCherry to produce pEXPpCSDest2.
<i>buc89-158</i>	pDONR2 21	Gateway	GGGGACAAGTTGTACAAAAAA GCAGGCTTAATGATTAACCCCA CTACCCCTC	GGGGACCACTTGTACAAGAAAG CTGGGTAGGGTAGAGAGACT ACATTGTT	pENTR221 was recombined with pCSDest2 and p3EeGFP to produce pEXPpCSDest2.
<i>buc11-47</i>	pDONR2 21	Gateway	GGGGACAAGTTGTACAAAAAA GCAGGCTTAATGGAGTTGGG CAACCTCA	GGGGACCACTTGTACAAGAAAG CTGGGTAGCCATATGGATTGATGG GCC	pENTR221 was recombined with pCSDest2 and p3EeGFP to produce pEXPpCSDest2.
<i>buc48-88-</i>	pDONR2 21	Gateway	GGGGACAAGTTGTACAAAAAA GCAGGCTTAATGGAGTGCTG TGAAACTG	GGGGACCACTTGTACAAGAAAG CTGGGTATCTCTGTAATCAATT GCT	pENTR221 was recombined with pCSDest2 and p3EeGFP to produce pEXPpCSDest2.
<i>bucΔ11-88-egfp</i>	pCS2 ⁺		buc_265bp_fw: ATTAACCCCCACTACCCCTCAGT buc_1bp_wo-loc_fw: ATGGAAGGAATAAACAAATT CACACCCAATTAAACCCCACTAC CCCTCAGTTGCATCC Buc_1bp_ClaI_fw: GGGATCGATATGGAGGAATA ATAACAATTCAAC	eGFP_end_XbaI_rv: GGGTCTAGATTACTGTACAGCTC GTCCATGC	<i>bucΔ11-88-egfp</i> sequence was amplified using a 3-step PCR from pCS2+ <i>buc-eGFP</i> , using the primers buc_265bp_fw, buc_1bp_wo-loc_fw, Buc_1bp_ClaI_fw, eGFP_end_XbaI_rv. The PCR product was cut with ClaI, XbaI and ligated into pCS2+.
<i>buc21-88-GFP</i>		Gateway	GGGGACAAGTTGTACAA AAAAG CAGGCTATATGCCACCATCTC AGCCTTATT	GGGGACCACTTGTACAAG AAAG CTGGGTATCTCTGTAATCAATT GGCTG	pENTR221 was recombined with pCSDest2 and p3EmCherry to produce pEXPpCSDest2.
<i>Buc36-88-GFP</i>				GGGGACCACTTGTACAAG AAAG CTGGGTATCTCTGTAATCAATT GGCTG	pENTR221 was recombined with pCSDest2 and p3EmCherry to produce pEXPpCSDest2.

<i>buc31-78-mCherry</i>		Gateway	GGGGACAAGTTGTACAA AAAAGCAGGCTATGCCACC ATCTAG CCT	GGGGACCACTTGTACAAG AAAG CTGGGTAATGTGGAATCACATA GCC	pENTR221 was recombined with pCSDest2 and p3EmCherry to produce pEXPpCSDest2.
<i>buc31-78(Δ31-35)mCherry</i>		Gateway	GGGGACAAGTTGTACAA AAAAGCAGGCTATGTATT CATGTATC AGTGG	GGGGACCACTTGTACAAG AAAG CTGGGTAATGTGGAATCACATA GCC	pENTR221 was recombined with pCSDest2 and p3EmCherry to produce pEXPpCSDest2.
<i>Buc31-78(Δ31-35)mCherry</i>		Oligo + Gateway	Oligo: ATGTATTTCATGTATCGT GGCCCATGAATCCAATGG CCATTACGGTTTCCCGG GCCGGCTTGACTTGGCC GTCCCTAT		buc31-78-Δ31-35 was amplified using the primers Buc31-78_Δ31-35_fw_gateway, Buc31-78_rev_gateway from the extended overlapping oligos: Buc31-78_Δ31-35_fw and Buc_57-78_rev. The PCR product was recombined into pDONR221.
<i>Buc31-78(62-66)mCherry</i>		Oligo + Gateway		Oligo: ATGTGGAATCACATAGCCAG GATACTGCATAAAACTGGGG ACGGCCAAGTGCAAAGCC GGCCCGGGAAAACCGTAATGGC CATATGG	buc31-78-Δ62-66 was amplified using the primers Buc31-78_fw_gateway, Buc31-78_rev_gateway from the extended overlapping oligos: BucLoc_Δ62-66_rev, Buc_31-51_fw. The PCR product was recombined into pDONR221
<i>Buc31-78(67-71)mCherry</i>		Oligo + Gateway		Oligo: ATGTGGAATCACATAGCCAG GATAAGGGGCCATATAGGG ACGGCCAAGTGCAAAGCC GGCCCGGGAAAACCGTAATGGC CATATGG	buc31-78-Δ67-71 was amplified using the primers Buc31-78_fw_gateway, Buc31-78_rev_gateway from the extended overlapping oligos: BucLoc_Δ67-71_rev, Buc_31-51_fw. The PCR product was recombined into pDONR221
<i>Cldn-d</i>		Gateway	GGGGACAAGTTGTACAAAAAA GCAGGGCTTAATGGCATCTGTTG GGCTTCAG	GGGGACCACTTGTACAAGAAAAG CTGGGTTTCACACATAAGCTCCCTG GAGCAG	pENTR221 was recombined with pCSDest2 to produce pEXPpCSDest2.
<i>Cldn-a</i>		Gateway	GGGGACAAGTTGTACAAAAAA GCAGGGCTTAATGGTATCAGCAG GGCTGCAG	GGGGACCACTTGTACAAGAAAAG CTGGGTTTCAGACATAACCCCTTGG TTCCTC	pENTR221 was recombined with pCSDest2 and p3EmCherry to produce pEXPpCSDest2.
<i>Cldn-dΔYV</i>		Gateway	GGGGACAAGTTGTACAAAAAA GCAGGGCTTAATGGCATCTGTTG GGCTTCAG	GGGGACCACTTGTACAAGAAAAG CTGGGTTTAAGCTCCTGGAGCAG ACCT	pENTR221 was recombined with pCSDest2 and p3EmCherry to produce pEXPpCSDest2.

Table S4. A full list of the 3464 proteins identified by mass spectrometry

[Click here to download Table S4](#)

Table S5. Antibodies used for immunostaining

Antibody	Dilution
Guinea pig- α -Buc (Biogenes, Berlin)	1:5000
Mouse- α -B-catenin (Merck, Kenilworth, USA)	1:1000
Mouse- α -E-cadherin (BD Transduction Laboratories, Franklin Lakes, New Jersey, USA)	1:50
Rabbit- α -p-NMII (Cell Signaling Technology, Danvers, USA)	1:50
Mouse- α -Kif23 (Gene Tex, Irvine, California, USA)	1:50
Rat- α -ZO1 (Santa Cruz Biotechnology, Dallas, Texas, USA)	1:100
Rabbit - α -DDX4 (Bioss, USA)	1:300
Goat- α -guinea pig Alexa Fluor 488 (Life Technologies, Carlsbad, USA)	1:500
Goat- α -rabbit Alexa Fluor 594 (Life Technologies, Carlsbad, USA)	1500
Cldn-d	1:25

Table S6. Antibodies used for western blotting

Antibody	Dilution
Guinea pig- α -Buc (BioGenes, Berlin)	1:5000
Mouse- α -GFP (Merck, Kenilworth, USA)	1:2500
Goat- α -guinea pig 800CW (IRDye, Li-Cor)	1:20,000
Goat- α -mouse 680CW(IRDye, Li-Cor)	1:20,000