

A

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Hsap-FKBP14  MRLFLWNAVLTFLVFTSLIGALIPPEVKIEVLQKPFICHRKTKGGDMLVHYEGYLEKDG 60
Mmus-Fkbp14  MRFFLWNAI LALWVTVLSGALIPPEVKIEVLQKPFICHRKTKGGDMLVHYEGYLEKDG 60
Ggal-FKBP14  -MAVLRVAVLLGALLGCAAAALI PAADVKEVLQKPFICHRRTKWDGMLVHYEGFLQSDG 59
Dmel-FKBP14  -MSKSNLVISCLLVAI NSLVRAQDLKVEVISTPEVCEQSKNGDSLTMHYTGLQADG 59
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      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Hsap-FKBP14  SLFHSTHKHNNQPIWFTLGLILEALKGWDQGLKGMVCVEKRRKIIPPALGYGKEGKG-KI 119
Mmus-Fkbp14  SLFHSTHKHNNQPIWFTLGLILEALKGWDQGLKGMVCVEKRRKIIPPALGYGKEGKG-KI 119
Ggal-FKBP14  SMFHSTHKHNNQPMWFTLGI REAIKGDWDLKGMVCVEKRRKIIPPALAYGKEGKG-KI 118
Dmel-FKBP14  KKFDSSFRD--QPFTFQLGAGQVIGKWDQGLLNMVCVEKRRKIIPPQLYGQDQAGNVI 117
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Hsap-FKBP14  PPESTLI FNIDLLEIRNGPRSHESFQEMDLNDDWKLKSKDEVKAYLKKFEKKGAVVNE-- 177
Mmus-Fkbp14  PPESTLI FNIDLLEIRNGPRSHESFQEMDLNDDWKLKSKDEVKAYLKKFEKKGAVVNE-- 177
Ggal-FKBP14  PPESTLI FNIDLLEIRNGPRSHESFQEMDLNDDWKLKSKDEVKAYLKKFEKKGAVVND-- 176
Dmel-FKBP14  PPKATLLFDVELINIGNAPPTTNVKEIDDNADKQLSREEVSEYLLKQMTAVEGQDSEEL 177
      ***.***.***.***.***.***.***.***.***.***.***.***.***.***.***.
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Hsap-FKBP14  ----SHHDALVEDIFDKEDEKDGFI SAREFT-YKHDEL 211
Mmus-Fkbp14  ----SHHDALVEDIFDKEDEKDGFI SAREFT-YVHDEL 211
Ggal-FKBP14  ----TQHDALVEDIFDKEDESDGFI SAREFT-YKHDEL 210
Dmel-FKBP14  KNMLAENDKLV EEIFQHEDKDKNGFISHDEFSGPKHDEL 216
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B

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Hsap-FKBP1A  -----MGVQVETIS-PGDGRTPFKRQGTQCVVHY 27
Mmus-Fkbp1a  -----MGVQVETIS-PGDGRTPFKRQGTQCVVHY 27
Ggal-FKBP1A  -----MGVHVETIA-PGDGRTPFKRQGTQCVVHY 27
Dmel-FK506-bp2  -----MGQVVPIA-PGDGSTYPKNGQKQVTVHY 27
Hsap-FKBP2  ----MRLSWFRVLTVLSICLSAVATATGAEGRKRLQIGVKKRV DHCPIKSRKGDV LHMHY 56
Mmus-Fkbp2  ----MRLSW--ILTILSICLSALAAATGAEGRKRLQIGVKKRV DHCPIKSRKGDV LHMHY 54
Dmel-CG14715  ----MKLTY----ILLICAFVAASAASDP--KVKTIGIKRVRNENCTRKAKGGDLVHVHY 48
Hsap-FKBP7  MPKTMHFLFRFIVFFYLWGLFTAQRQKKEESTEEVKIEVLRHPENC.SKTSRKGDL LNAHY 60
Mmus-Fkbp7  ----MNLFR LAVFLSLWCCSDAQGTKEESTEEVKIEVLRHPENC.SKTSRKGDL LNAHY 56
Ggal-FKBP7  ----MGRGLRLLLAALALLAAPARAEG-GAAEEVKIEVLRHPESCSPKSKGDL LNAHY 55
Hsap-FKBP14  -----MRLFLWNAVLTFLVFTSLIGALIPPEVKIEVLQKPFICHRKTKGGDMLVHY 52
Mmus-Fkbp14  -----MRFFLWNAI LALWVTVLSGALIPPEVKIEVLQKPFICHRKTKGGDMLVHY 52
Ggal-FKBP14  -----MAVLRVAVLLGALLGCAAAALI PAADVKEVLQKPFICHRRTKWDGMLVHY 51
Dmel-FKBP14  -----MSKSNL-----VISCLLVAI NSLVRAQDLKVEVISTPEVCEQSKNGDSLTMHY 51
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Hsap-FKBP1A  TGMLE-DGKKFDSRRDRN--KPFKFM LGKQEVIRGWEEGVAQMSV GQRAKLTISP DYAYG 84
Mmus-Fkbp1a  TGMLE-DGKKFDSRRDRN--KPFKFTL GKQEVIRGWEEGVAQMSV GQRAKLTISSDYAYG 84
Ggal-FKBP1A  TGMLE-DGKKFDSRRDRN--KPFKFM LGKQEVIRGWEEGVAQMSV GQRAKMTISP DYAYG 84
Dmel-FK506-bp2  TGTLD-DGTFKDSRRDRN--KPFKFTI GKQEVIRGWEDEGVAQL SVGQRAKLTICSPDYAYG 84
Hsap-FKBP2  TGKLE-DGTEFDSSSLPQN--QPFVFS LGTQVIGKWDQGLLGMCEGEKRRKLVIPSELGYG 113
Mmus-Fkbp2  TGKLE-DGTEFDSSSLPQN--QPFVFS LGTQVIGKWDQGLLGMCEGEKRRKLVIPSELGYG 111
Dmel-CG14715  RGALQ-DGTEFDS SRSRG--TPFSFTL GARQVIGKWDQGLLGMCEGEKRRKLTIPPELGYH 105
Hsap-FKBP7  DGYLAKDGSKFYCSRTQDEGH PKWFVLGVGVHVIKGLDIAMDMC PGEKRVVIPP SFAYG 120
Mmus-Fkbp7  DGYLAKDGSKFYCSRTQDEGH PKWFVLGVGVHVIKGLDIAMDMC PGEKRVVIPP SFAYG 116
Ggal-FKBP7  DGFVLSNGSKFYCSRTQNDGHPKWFVLGVGVHVIKGLDIAMNMCP GEKRVVIPP SLAYG 115
Hsap-FKBP14  EGYLEKDGSLFHSTHKHNNQPIWFTLGLILEALKGWDQGLKGMVCVEKRRKIIPPALGYG 112
Mmus-Fkbp14  EGYLEKDGSLFHSTHKHNNQPIWFTLGLILEALKGWDQGLKGMVCVEKRRKIIPPALGYG 112
Ggal-FKBP14  EGFLLQSDGSMFHSTHKHNNQPMWFTLGI REAIKGDWDLKGMVCVEKRRKIIPPALAYG 111
Dmel-FKBP14  TGTQLQADGKKFDSFRD--QPFTFQLGAGQVIGKWDQGLLNMVCVEKRRKIIPPQLYG 109
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      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Hsap-FKBP1A  ATGHPG-IIPPHATLVFDV ELLKLE----- 108
Mmus-Fkbp1a  ATGHPG-IIPPHATLVFDV ELLKLE----- 108
Ggal-FKBP1A  STGHPG-IIPPNATLIFDVELMKLE----- 108
Dmel-FK506-bp2  SRGHPG-VIPPNSTLTFDVELLKVE----- 108
Hsap-FKBP2  ERGAPP-KIPGGATLVFEV ELLKIERRT---EL----- 142
Mmus-Fkbp2  ERGAPP-KIPGGATLVFEV ELLKIERRS---EL----- 140
Dmel-CG14715  ASGAGGKIPPNAVLVFDTEL VKIEPRSGSEEL----- 138
Hsap-FKBP7  KEYG-EGKTPPDATLIFEI ELYAVTKGPRSIETFKQIDMDNDRQLSKAEINLYLQRF EK 178
Mmus-Fkbp7  KEYGAEKIPPNATLMFEI ELYAVTKGPRSIETFKQIDTNDNRQLSKAEI ELYLQKDFEK 175
Ggal-FKBP7  QQGYAQGKIPPNATLIFEI ELYAVNKGPRSEAFRQIDKDNDDKLS ELSQLKEEFAR 174
Hsap-FKBP14  KEKG--GKIPPESTLI FNIDLLEIRNGPRSHESFQEMDLNDDWKLKSKDEVKAYLKKREFEK 169
Mmus-Fkbp14  KEKG--GKIPPESTLI FNIDLLEIRNGPRSHESFQEMDLNDDWKLKSKDEVKAYLKKREFEK 169
Ggal-FKBP14  KEKG--GKIPPESTLI FNIDLLEIRNGPRSHESFQEMDLNDDWKLKSKDEVKAYLKKREFEK 168
Dmel-FKBP14  DQGAGN-VIPPKATLLFDV ELINIGNAPPTTNVKEIDDNADKQLSREEVSEYLLKQMTA 168
      * * .: .: .: .: .: .: .: .: .: .: .: .: .: .: .: .: .: .: .: .:
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Hsap-FKBP1A  ----- 221
Mmus-Fkbp1a  ----- 218
Ggal-FKBP1A  -----SVHDEI LADI FKKNDHGDGFI SAKENYVYQHDEL 217
Dmel-FK506-bp2  -----SHRDALVEDIFDKEDEKDGFI SAREF-TYKHDEL 211
Hsap-FKBP2  -----SHHDALVEDIFDKEDEKDGFI SAREF-TYKHDEL 211
Mmus-Fkbp2  -----SHHDALVEDIFDKEDEKDGFI SAREF-TYKHDEL 211
Dmel-CG14715  -----TQHDALVEDIFDKEDESDGFI SAREF-TYKHDEL 210
Hsap-FKBP7  DEKPRDK-----SYQDAVLEDFK MNDHGDGFI SPKEYNVYQHDEL 221
Mmus-Fkbp7  DANPRDK-----SYQKAVLEDFK KNDHGDGFI SPKEYNVYQHDEL 218
Ggal-FKBP7  DGKRRHP-----SVHDEI LADI FKKNDHGDGFI SAKENYVYQHDEL 217
Hsap-FKBP14  HGAVVNE-----SHRDALVEDIFDKEDEKDGFI SAREF-TYKHDEL 211
Mmus-Fkbp14  HGAVVNE-----SHHDALVEDIFDKEDEKDGFI SAREF-TYKHDEL 211
Ggal-FKBP14  HGAVVND-----TQHDALVEDIFDKEDESDGFI SAREF-TYKHDEL 210
Dmel-FKBP14  VEGQDSEELKNMLAENDKLV EEIFQHEDKDKNGFISHDEFSGPKHDEL 216
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Fig. S1. A multiple sequence alignment of small molecular weight FKBP14 using NCBI accession sequences. (A) *Drosophila* FKBP14 (Dmel-FKBP14) contains an N-terminal signal peptide (residues underlined in yellow), a PPIase domain (residues underlined in black), an EF-hand calcium-binding motif (underlined in blue) and a C-terminal ER retention motif, HDEL (underlined in green). A multiple sequence alignment of FKBP14 orthologs from human (Hsap-FKBP14, NCBI accession NP_060416), mouse (Mmus-FKBP14, NCBI accession NP_705801), chicken (Ggal-FKBP14, NCBI accession XP_418735) and fly (Dmel-FKBP14, NCBI accession NP_476973) reveals highly conserved residues (asterisks), conserved substitutions (colons) and semiconserved substitutions (full-stops). (B) FKBP orthologs from human (Hsap-FKBP1A, accession NP_000792; Hsap-FKBP2, accession AAH03384; Hsap-FKBP7, accession AAQ57208; and Hsap-FKBP14, accession NP_060416), mouse (Mmus-Fkbp1a, accession NP_032045; Mmus-Fkbp2, accession NP_032046; Mmus-Fkbp7, accession NP_034352; and Mmus-Fkbp14, accession NP_705801), chicken (Ggal-FKBP1A, accession NP_989661; Ggal-FKBP7, accession XP_421981; and Ggal-FKBP14, accession XP_418735) and fly (Dmel-FK506-bp2, accession NP_523792; Dmel-CG14715, accession NP_650101; and Dmel-FKBP14, accession NP_476973) exhibit highly conserved residues (asterisks), conserved substitutions (colons) and semiconserved substitutions (full-stops). The residues in *Drosophila* FKBP14 that comprise the PPIase domain are in bold.

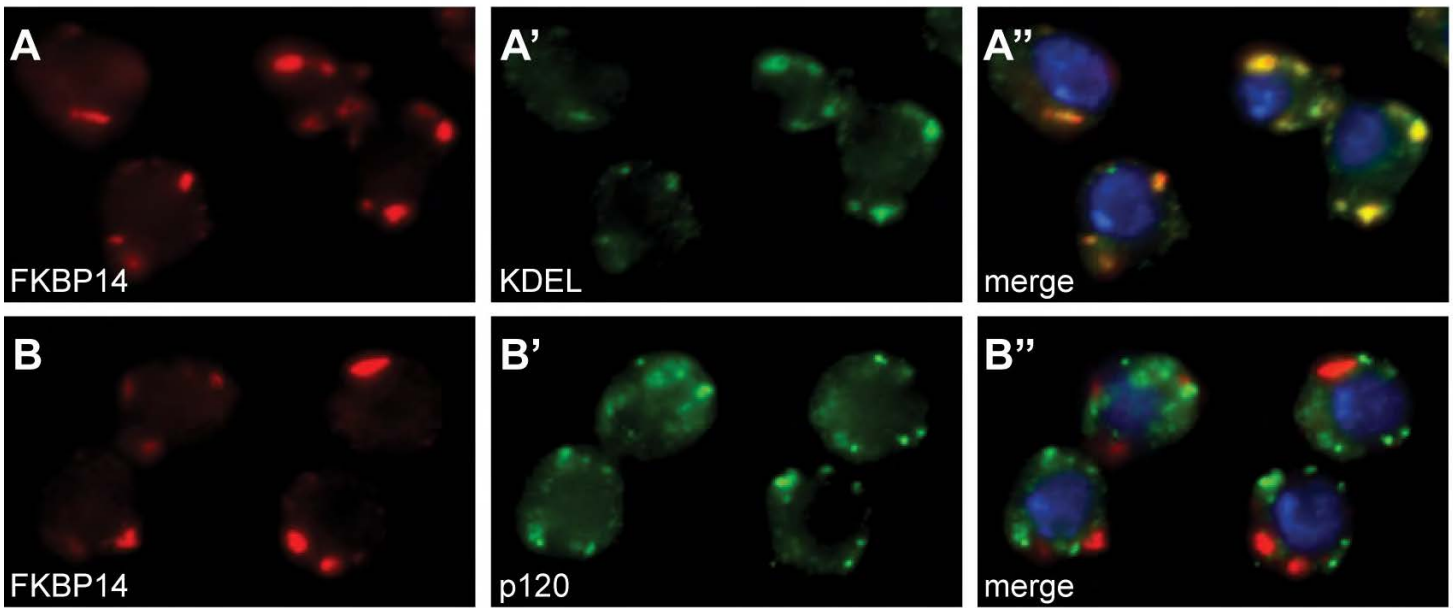


Fig. S2. FKBP14 localizes to the ER in *Drosophila* cells. (A-A'') FKBP14 (red) is expressed in a punctate pattern in S2 cells and colocalizes with anti-KDEL (green). Colocalization (yellow) is indicated in the merge. (B-B'') FKBP14 (red) does not colocalize with anti-p120 (green) in S2 cells (lack of yellow in the merge). As a control, S2 cells were stained using preimmune sera, and we failed to detect similar localization patterns (data not shown). Cell stains represent single plane images.

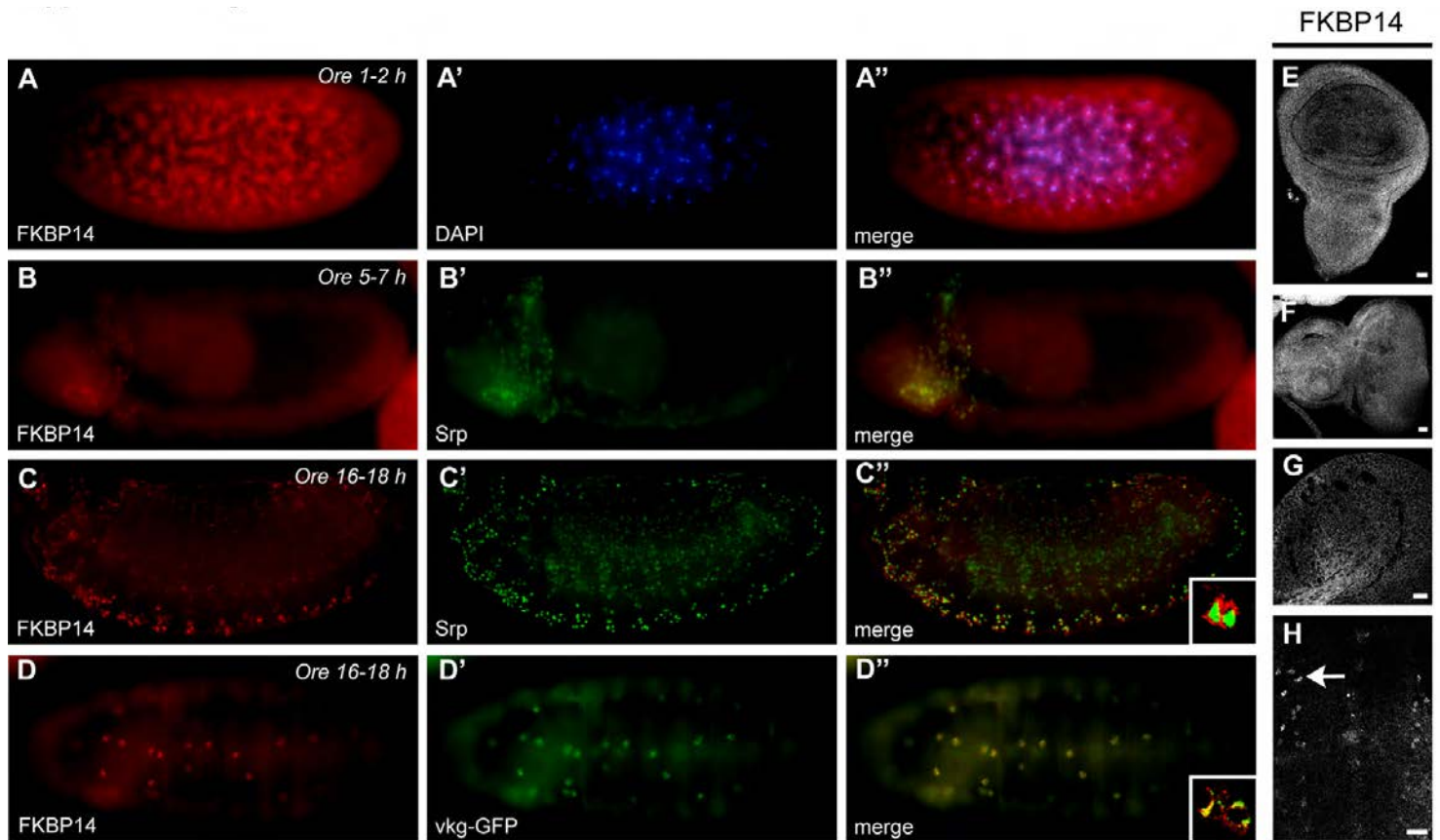


Fig. S3. FKBP14 is broadly expressed throughout development, and co-localizes with hemocyte and plasmatocyte markers in mid- and late-stage embryos. (A-A'') Oregon-R syncytial blastoderm embryos, 1-2 hours after egg laying. FKBP14 expression (red) surrounds nuclei (stained with DAPI, blue). (B-D'') Lateral views of Oregon-R embryos, anterior is to the left. (B-B'') In 5- to 7-hour embryos, FKBP14 (red) stains cells migrating from the head mesoderm that also express Srp (green). (C-C'') In 16- to 18-hour embryos, FKBP14 (red) is expressed in hemocytes, which also express Srp (green). A subset of cells express Srp alone, located in the interior region of the embryo. The pattern of Srp staining is consistent with that described in a previous study (Sam et al., 1996). Srp functions in the nucleus as a transcription factor, thus its subcellular localization is distinct from that of FKBP14 (inset). (D-D'') Ventral view of a 16- to 18-hour embryo, anterior is to the left. FKBP14 staining (red) is detected in a subset of cells that express the collagen type IV protein, Viking-GFP (Vkg-GFP; green). An insertion in the *viking* locus causes expression of Vkg-GFP fusion proteins in hemocytes (Yasothornsrikul et al., 1997; Olofsson and Page, 2005). FKBP14 localization partially overlaps with Viking-GFP (yellow; inset). (E-H) Endogenous FKBP14 is expressed in a punctate pattern in control third instar larval wing discs (E), eye discs (F), CNS optic lobes (G) and in the ventral nerve cord (H, arrow). Scale bars: 10 μ m. As a staining control, we labeled third instar larval *FKBP14* mutant discs using anti-FKBP14 antisera, and failed to detect similar expression patterns (data not shown). Single plane images were captured for ovary and embryo stains, and embryo inset images show projections of two sections, 1 μ m apart.

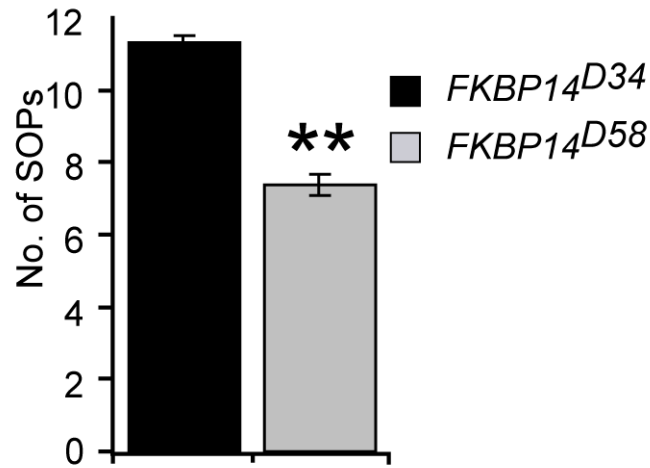


Fig. S4. Quantitation of SOP loss in *FKBP14* mutant presumptive notum tissue. Wild-type larvae exhibit 11 SOPs (Bryant, 1975), similar to *FKBP14^{D34}* third instar presumptive nota (11.3±0.2, $n=20$). *FKBP14^{D58}* mutants exhibit 7.4±0.3 SOPs in presumptive nota tissues, which is a significant reduction compared with control (** $P<0.0001$, $n=20$). Error bars indicate s.e.m.

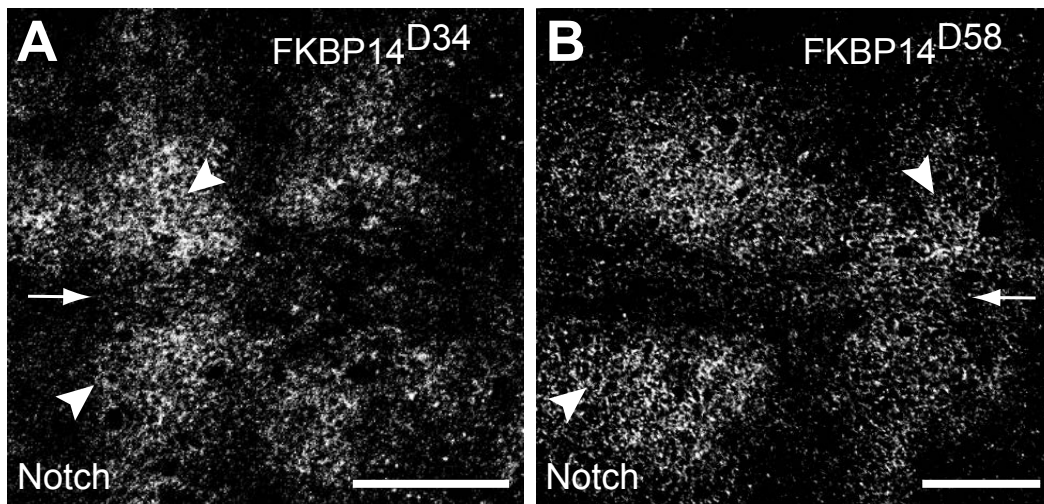


Fig. S5. Intact Notch expression at the membrane. Notch expression at the membrane is intact in cells (arrowheads) that border third instar larval presumptive wing margins (arrows) in control (A) and *FKBP14^{D58}* mutant (B) tissues. Scale bars: 20 μm .

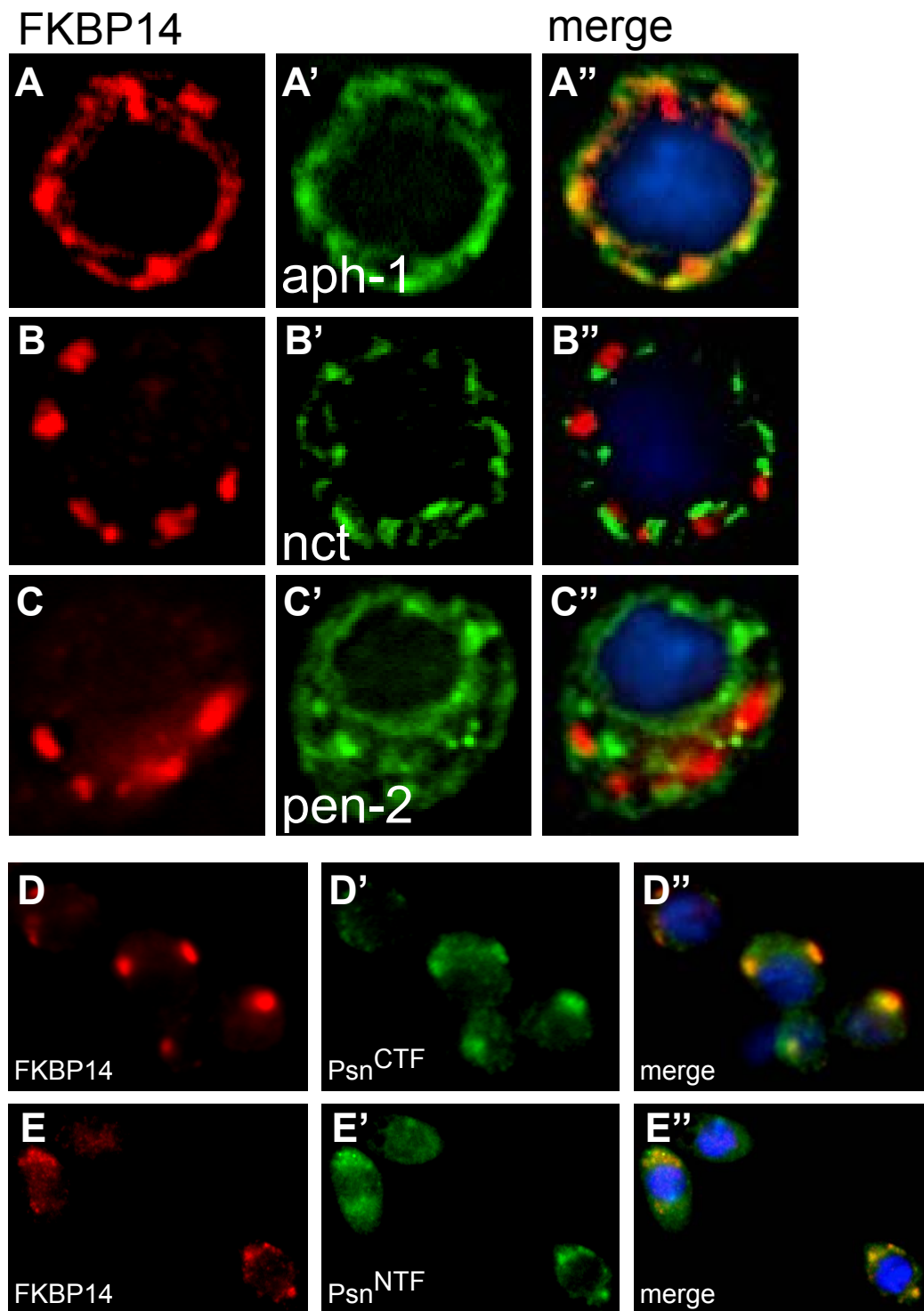


Fig. S6. FKBP14 partially co-localizes with Aph-1 and Psn in *Drosophila* cells. (A-C'') FKBP14 partially co-localizes with Aph-1 (A-A'') but not Nct (B-B'') or Pen-2 (C-C'') in transfected *Drosophila* S2 cells. (D-D'') FKBP14 (red) and Psn^{CTF} (green) partially colocalize (yellow; merge). (E-E'') FKBP14 (red) and Psn^{NTF} (green) partially colocalize (yellow; merge). DAPI is in blue.

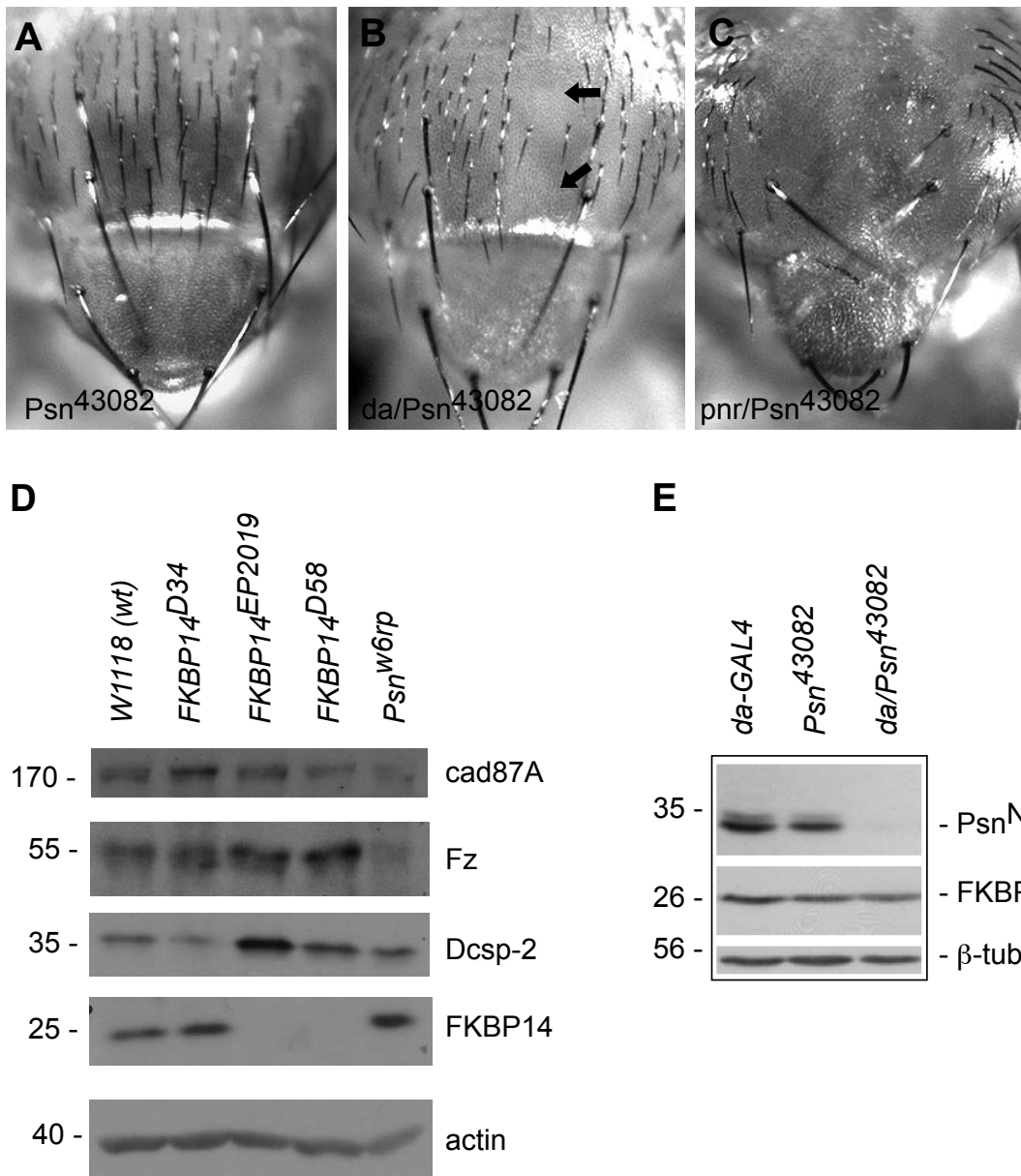


Fig. S7. Loss of Psn results in phenotypes similar to FKBP14 mutants, and the loss of Psn in FKBP14 mutants appears specific. (A-C) *UAS-Psn-RNAi*⁴³⁰⁸² flies (*Psn*⁴³⁰⁸²) exhibit bristle patterns in adult nota similar to wild type (not shown). (B) Overexpression of *UAS-Psn*⁴³⁰⁸² using *da-GAL4* (*da/Psn*⁴³⁰⁸²) results in a mild loss of microchaetae (arrows point to two bald patches) in adult nota. (C) Overexpression of *UAS-Psn*⁴³⁰⁸² using *pnr-GAL4* (*pnr/Psn*⁴³⁰⁸²) results in a significant loss of microchaetae and a mild loss of macrochaetae in adult nota. (D) Loss of FKBP14 does not result in significant loss of other single or multipass transmembrane proteins. (E) *Psn*^{NTF} protein expression is significantly reduced in *da/Psn*⁴³⁰⁸² adult flies compared with control extracts. FKBP14 protein expression is normal in *Psn* RNAi knockdown flies. β -tubulin is used as a loading control.

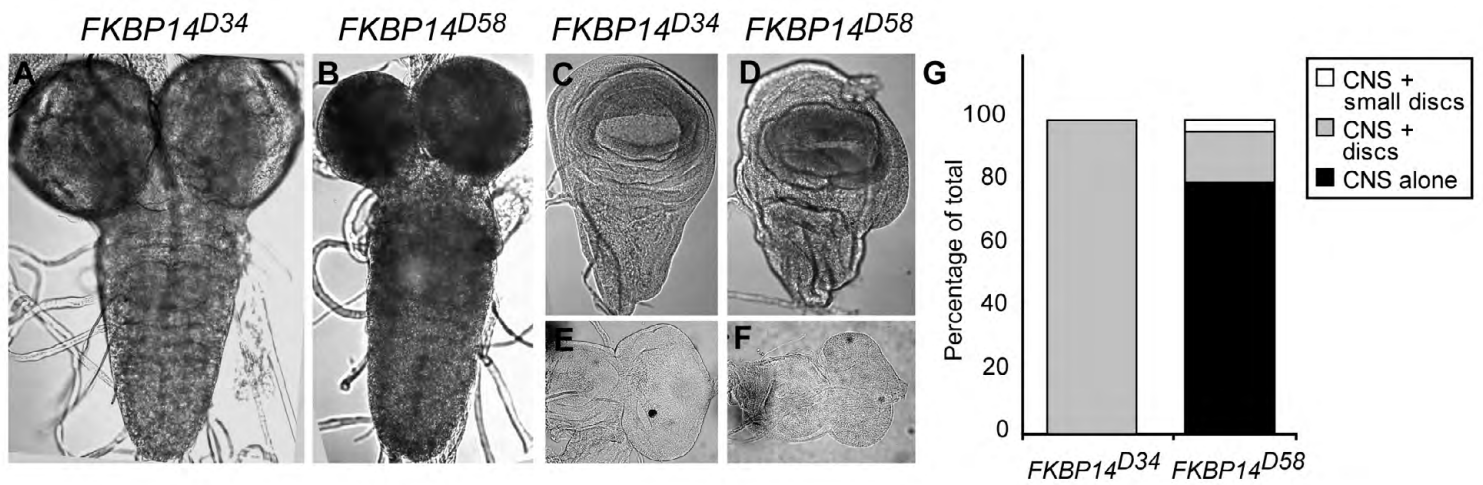


Fig. S8. FKBP14 mutants display missing or disordered imaginal discs. (A-F) Brightfield images of third instar larval CNS and discs. (A) *FKBP14^{D34}* larval CNS, (C) wing disc and (E) eye disc appear larger compared with (B) *FKBP14^{D58}* mutant CNS, (D) wing disc and (F) eye disc. Single plane images are shown. (G) Quantitation of third instar larval CNS and discs reveals a significant loss of discs in *FKBP14* mutants compared with controls ($n=250$).