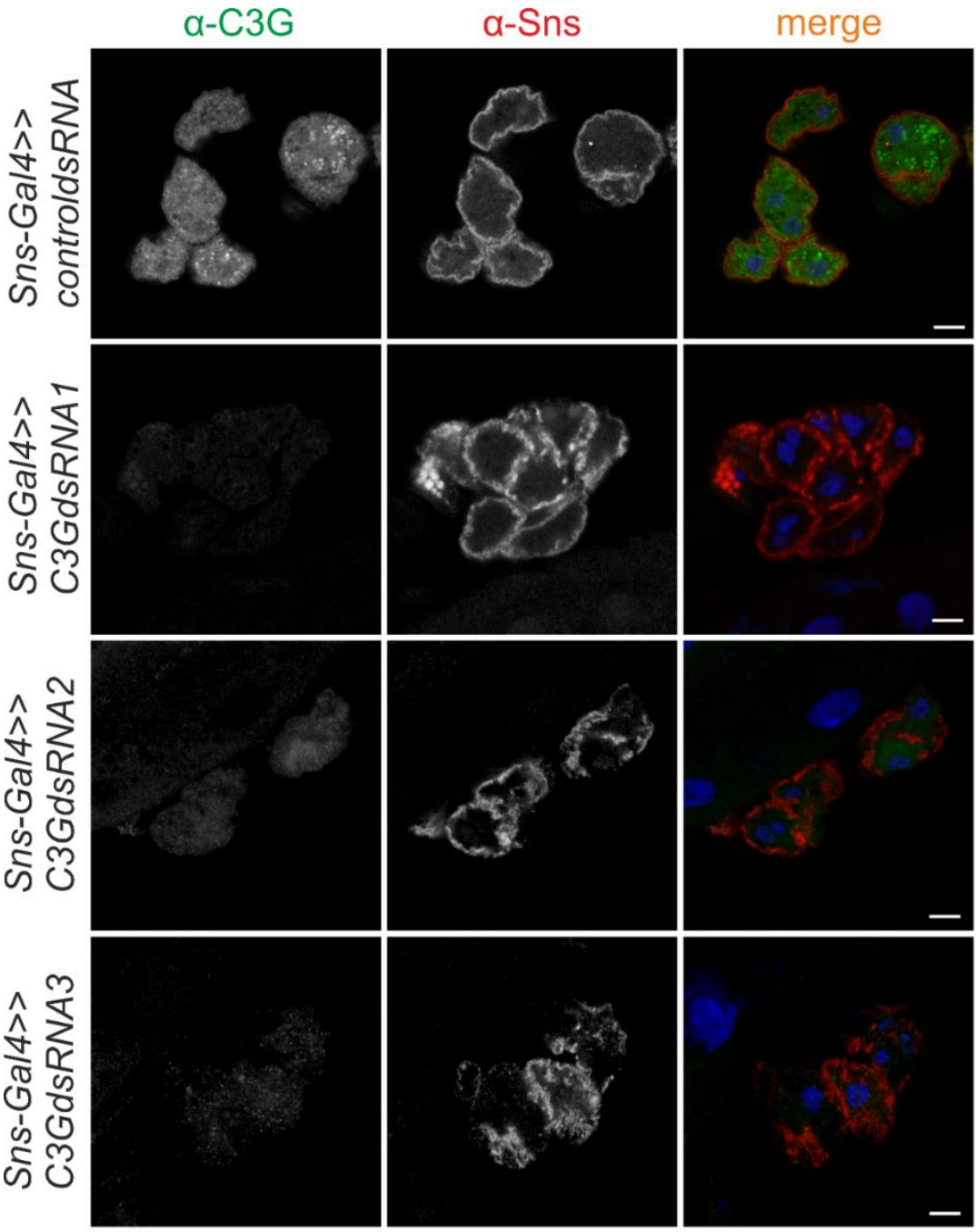
**Supplemental Material**

Supplemental Figures



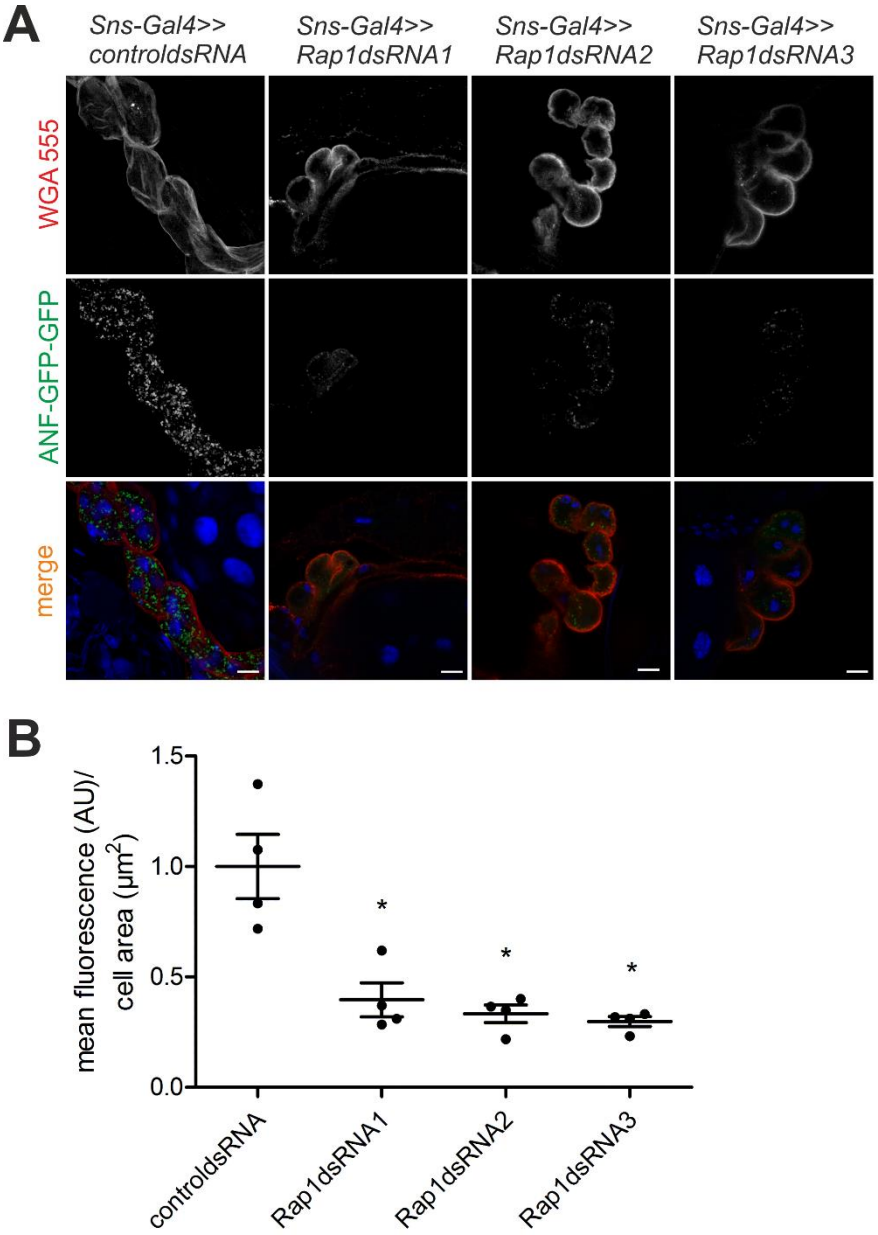
Supplemental Tables

**Supplemental Figures**



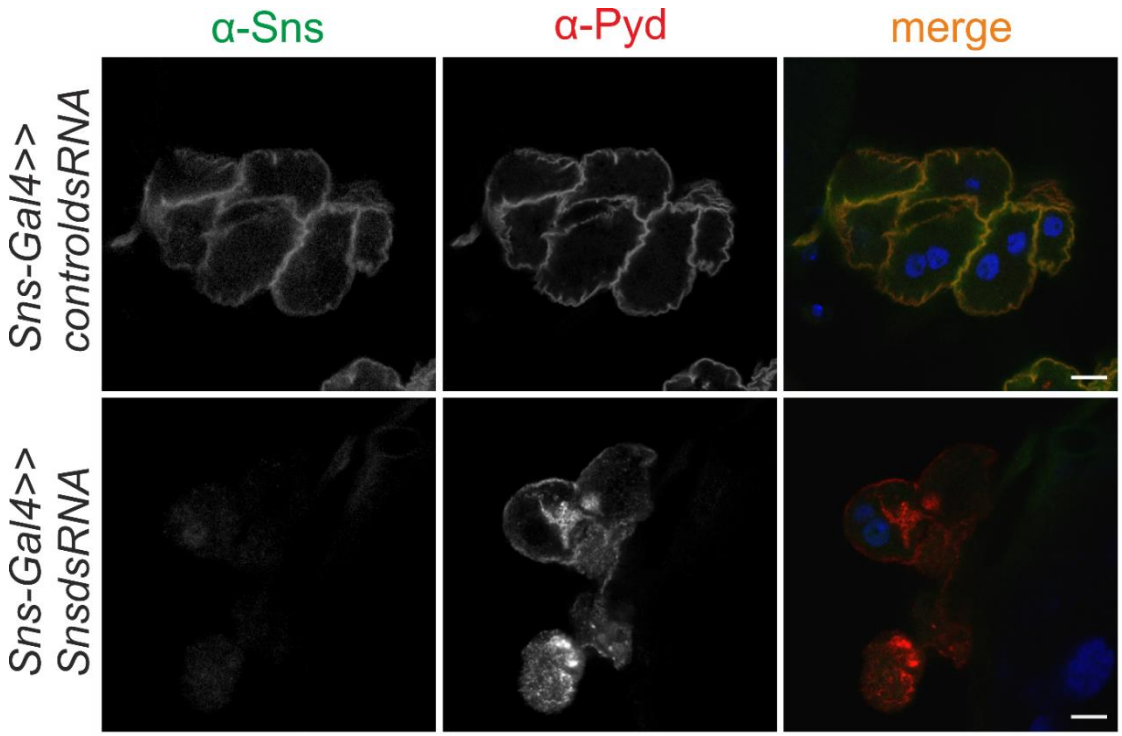

**Supp. Figure 1. Drosophila nephrocytes express C3G.**

Immunofluorescence analysis of control (*dsRNA* for *or83b*) or *c3g* knockdown nephrocytes are shown (*c3gdsRNA1-3*). Knockdown in nephrocytes was accomplished by employing *sns-GAL4*. Nephrocytes were prepared and immunofluorescence analysis was performed with antibody specific for C3G (green) and Sns (red). Scale bar: 10 µm. n=3.



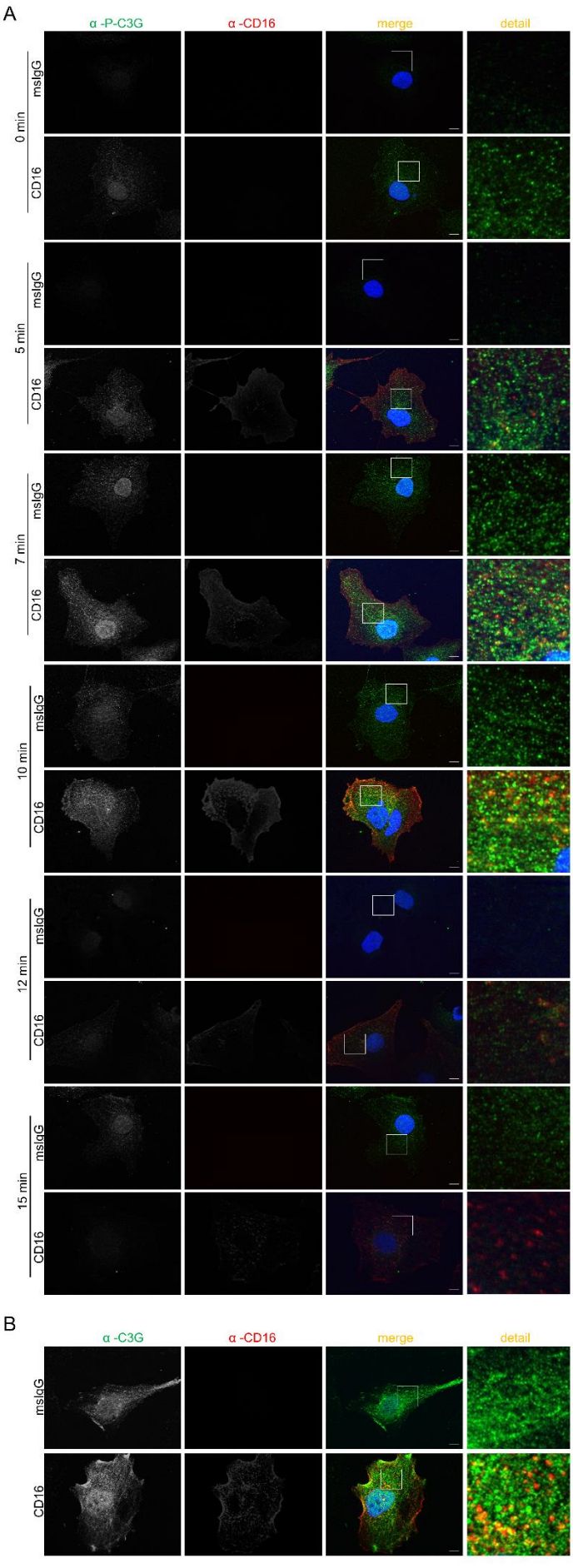

**Supp. Figure 2. Rap1 is necessary for nephrocyte function.**

(A) Uptake of secreted ANF-GFP-GFP into nephrocytes is shown in prepared control (RNAi for *or83b*) or *rap1* knockdown nephrocytes. Knockdown in nephrocytes was accomplished by employing *sns-Gal4*. Samples were stained with wheat germ agglutinin-Alexa$^{555}$ (WGA) to visualize membranes. Merged images are shown in the lower panel. Scale bar: 10 µm. (B) Accumulation of ANF-GFP-GFP into nephrocytes was quantified. Shown are means and SEM in AU (arbitrary units) per µm$^2$ cell area normalized to control condition. * $P < .05$ by unpaired two-tailed Mann-Whitney test. n=4.



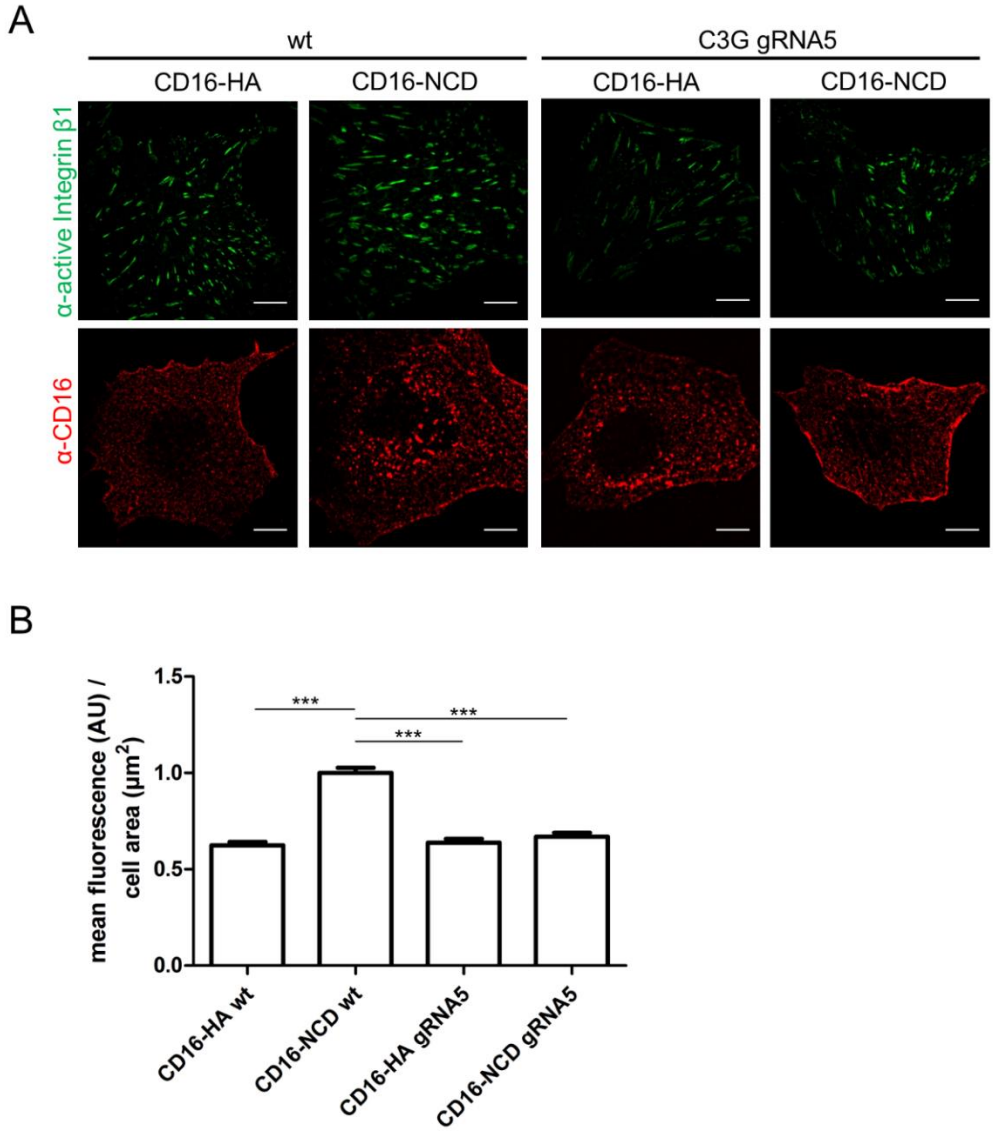

**Supp. Figure 3. Anti-Sns antibody specifically recognizes Sns.**

Immunofluorescence analysis of control (RNAi for *or83b*) or *sns* knockdown nephrocytes is shown. Knockdown in nephrocytes was accomplished by employing *sns-GAL4*. Nephrocytes were prepared and immunofluorescence analysis was performed to test Sns antibody specificity (purified large rabbit bleed). Scale bar: 10 µm. n=3.


A
α -P-C3G
α -CD16
merge
detail
0 min
5 min
7 min
10 min
12 min
15 min
mslgG
CD16
B
α -C3G
α -CD16
merge
detail
mslgG
CD16

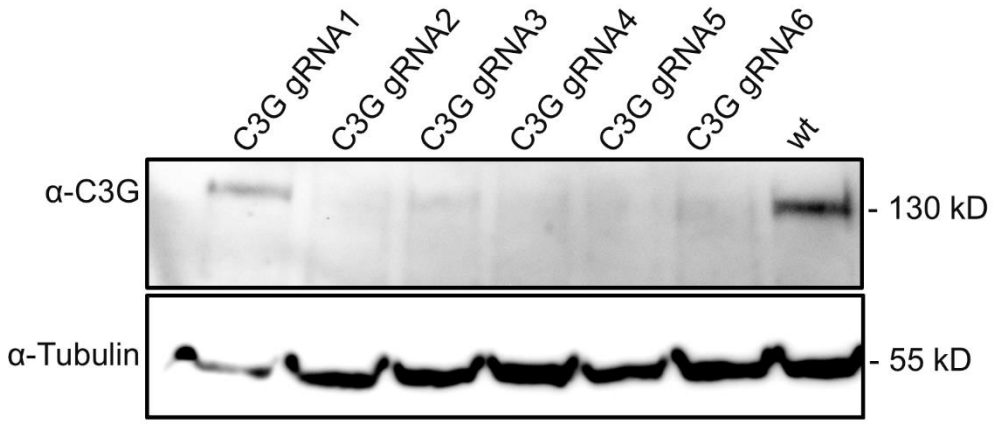
**Supp. Figure 4. Activated Nephrin recruits C3G.**

(A) To assess the time of maximum co-localization of CD16-NCD and p-C3G, podocytes expressing CD16-NCD were incubated with CD16-Alexa$^{647}$ or mouse (ms) IgG$^{647}$ antibody for the indicated time to induce Nephrin clustering. Cells were fixed, and immunofluorescence analysis was performed with antibody specific for p-C3G. (B) Shown are immunofluorescence analyses of cultured podocytes expressing CD16-NCD that were incubated with anti-CD16 Alexa$^{647}$ or ms IgG$^{647}$ antibody for 10 minutes to activate Nephrin signaling. After fixing, cells were stained with antibody specific for C3G. Please note that activated Nephrin partly co-localizes with total C3G. Scale bar: 10 µm. n=3.




**Supp. Figure 5. Nephrin activation results in Integrin β1 activation (additional data).**

(A) Control podocytes or C3G KO podocytes (gRNA 5) transiently expressing CD16-NCD or CD16-HA were incubated with anti-CD16-Alexa$^{647}$ antibody to initiate Nephrin clustering. Podocytes were fixed, and immunofluorescence analysis was performed employing antibody specific for active Integrin β1. Scale bar: 10 µm. (B) Statistical analysis of mean fluorescence intensity of active Integrin β1 in AU (arbitrary unis) per µm$^2$ cell area normalized to control condition. *** P < .001 by unpaired two-tailed Student's t-test. n=3, 30 cells per condition were evaluated.




**Supp. Figure 6. C3G knockout in human podocytes.**

Knockout of C3G by *CRISPR/Cas9* was performed in podocytes employing 6 different guide RNAs (gRNA). Immunoblots with lysates of respective podocyte lines are shown using antibody specific for C3G or α-Tubulin as loading control. Wild-type (wt) podocyte lysate was used as a control.

**Supplemental Tables**

**Supp. Table 1. *Guide RNA* (*gRNA*) sequences employed for generation of C3G knockout podocytes by *CRISPR/Cas9* technology.**

| | |
|---|---|
| *gRNA1* | GCCATGGACACAGGTGAGAG |
| *gRNA2* | TGCTCCAGATCCAAGGGGAG |
| *gRNA3* | GCGCTACTTTAAGACCATTG |
| *gRNA4* | GTCAGCCAGCAAGGTGCTGG |
| *gRNA5* | GATGGTGACGACTGTGAAGG |
| *gRNA6* | GCTCCCCCTGACAGACCGCG |

**Supp. Table 2. Genes identified to be necessary for nephrocyte function.**

Flies of the genotype *MHC::ANF-RFP; Hand::GFP*; *dot-Gal4* were crossed with *UAS-dsRNA* strains. ANF-RFP uptake was assessed by eye and divided into two categories: no effect (-); decreased uptake (+) compared to the control *dsRNA*. Genes are listed with Drosophila name, CG number, *dsRNA* reference, name of human ortholog or protein class, and uptake effect. *For Drosophila proteins without a defined human ortholog the related protein class was listed. Genes are assigned to groups but may not always belong uniquely to this group.

| **Drosophila name** | **CG Number** | **Human ortholog or protein class*** | **Origin** | **Effect on ANF-GFP-GFP Uptake** |
|---|---|---|---|---|
| **Control** | | | | |
| *Or83b* | CG10609 | | VDRC #100825 | - |
| | | | | |
| **Slit diaphragm-associated** | | | | |
| *Actinin* | CG4376 | α-ACTININ2 | VDRC #7762 | + |
| *Actinin* | CG4376 | α-ACTININ2 | VDRC #7761 | + |
| *cindr* | CG31012 | CD2AP | VDRC #38854 | + |
| *kirre* | CG3653 | NEPH1 | VDRC #6695 | + |
| *sns* | CG33141 | NEPHRIN | VDRC #109442 | + |
| | | | | |
| **GTPases/GAPs/GEFs** | | | | |
| *Asap1* | CG30372 | ASAP1-related proteins | VDRC #19284 | + |
| *Arf51F* | CG8156 | ARF6 | VDRC #100726 | - |
| *Cdc42* | CG12530 | CDC42 | VDRC #100794 | - |
| *C3G* | CG42328 | RAPGEF1/C3G | VDRC #105664 | + |
| *C3G* | CG42328 | RAPGEF1/C3G | VDRC #29829 | + |
| *C3G* | CG42328 | RAPGEF1/C3G | VDRC #29828 | + |
| *C3G* | CG42328 | RAPGEF1/C3G | VDRC #21306 | + |
| *Mig-2-like* | CG5588 | RHOG | VDRC #102528 | + |
| *Rac1* | CG2248 | RAC1 | VDRC #2248R-1 | - |
| *Rac1* | CG2248 | RAC1 | VDRC #2248R-2 | - |
| *Rac1* | CG2248 | RAC1 | VDRC #49246 | - |
| *Rac2* | CG8556 | RAC2 | VDRC #28926 | + |
| *Rac2* | CG8556 | RAC2 | VDRC #50349 | - |
| *Rac2* | CG8556 | RAC2 | VDRC #50350 | - |
| *Rap1* | CG1956 | RAP1 | VDRC #33437 | + |
| *Rap1* | CG1956 | RAP1 | VDRC #110757 | + |
| *Rap1* | CG1956 | RAP1 | VDRC #20761 | + |
| *RhoGAPp190* | CG32555 | ARHGAP35 | VDRC #110213 | - |
| *Rho1* | CG8416 | RHOA | VDRC #3793 | - |
| *RhoL* | CG9366 | RHOB-related proteins | VDRC #102461 | - |
| *pebble* | CG8114 | ARHGEF-related proteins | VDRC #35349 | - |

| | | | | |
|---|---|---|---|---|
| *schizo* | CG32434 | ARF6GEF | VDRC #36625 | - |
| *schizo* | CG32434 | ARF6GEF | VDRC #36627 | - |
| *Trio* | CG18214 | ARHGEF-related proteins | VDRC #40138 | + |
| | | | | |
| **Integrin-associated** | | | | |
| *Fermitin 1* | CG14991 | FERMT1 | VDRC #46495 | - |
| *Fermitin 1* | CG14991 | FERMT1 | VDRC #46494 | - |
| *Fermitin 2* | CG7729 | FERMT2 | VDRC #37010 | - |
| *Fermitin 2* | CG7729 | FERMT2 | VDRC #37009 | - |
| *inflated* | CG9623 | INTEGRIN α | VDRC #T0530 | - |
| *inflated* | CG9623 | INTEGRIN α | VDRC #T0531 | - |
| *inflated* | CG9623 | INTEGRIN α | VDRC #9623R-2 | - |
| *Integrin linked kinase* | CG10504 | ILK | VDRC #43170 | + |
| *Laminin A* | CG10236 | LAMININ3 | VDRC #18873 | + |
| *myospheroid* | CG1560 | INTEGRIN β | VDRC #1560R-1 | + |
| *myospheroid* | CG1560 | INTEGRIN β | VDRC #1560R-2 | + |
| *myospheroid* | CG1560 | INTEGRIN β | VDRC #K026 | - |
| *myospheroid* | CG1560 | INTEGRIN β | VDRC #K027 | - |
| *p130Cas* | CG1212 | P130CAS | VDRC #41479 | - |
| *Parvin* | CG32528 | PARVIN | VDRC #11670 | + |
| *Parvin* | CG32528 | PARVIN | VDRC #105356 | - |
| *Paxillin* | CG31794 | PAXILLIN | VDRC #25853 | - |
| *rhea* | CG6831 | TALIN | VDRC #6831R-2 | - |
| *Vinculin* | CG3299 | VINCULIN | VDRC #34586 | + |
| *Vinculin* | CG3299 | VINCULIN | VDRC #3299R-1 | - |
| *Vinculin* | CG3299 | VINCULIN | VDRC #3299R-2 | - |
| *wech* | CG42396 | TRIM71 | VDRC #41623 | + |
| *wech* | CG42396 | TRIM71 | VDRC #106390 | - |
| | | | | |
| **Kinases/Phosphatases** | | | | |
| *Akt1* | CG4006 | AKT1 | VDRC #103703 | - |
| *csk* | CG42317 | CSK | VDRC #32877 | + |
| *csk* | CG42317 | CSK | VDRC #48281 | + |
| *csk* | CG42317 | CSK | VDRC #102313 | - |
| *Fak56D* | CG10023 | FAK | VDRC #17957 | + |
| *Fak56D* | CG10023 | FAK | VDRC #108608 | - |
| *Pak* | CG10295 | PAK | VDRC #12553 | - |
| *Pak* | CG10295 | PAK | VDRC #12553 | - |
| *Pi3K 21B* | CG4141 | PI3K-related proteins | VDRC #104179 | + |
| *Pi3K 68D* | CG11621 | PI3K-related proteins | VDRC #109582 | - |
| *Pi3K 68D* | CG11621 | PI3K-related proteins | VDRC #16233 | - |
| *Pi3K 92E* | CG4141 | PI3K-related proteins | VDRC #107390 | - |
| *Pi3K 92E* | CG4141 | PI3K-related proteins | VDRC #38985 | - |
| *Pten* | CG5671 | PTEN | VDRC #35731 | - |
| *rolled* | CG12559 | MAPK-related proteins | VDRC #109108 | - |
| *rolled* | CG12559 | MAPK-related proteins | VDRC #432123 | - |
| *rolled* | CG12559 | MAPK-related proteins | BDSC #34855 | - |
| *slingshot* | CG6238 | SSH2, SSH3 | VDRC #30136 | + |
| *Src42A* | CG44128 | FRK | VDRC #17643 | + |
| *Src42A* | CG44128 | FRK | VDRC #17644 | + |

| | | | | |
|---|---|---|---|---|
| *Src64B* | CG7524 | SRC | VDRC #35352 | + |
| *Twinstar* | CG4254 | COFILIN | VDRC #110599 | + |
| | | | | |
| **Actin-associated** | | | | |
| *capulet* | CG33979 | CAP1, CAP2 | VDRC #21995 | - |
| *capulet* | CG33979 | CAP1, CAP2 | VDRC #101588 | - |
| *Ced-12* | CG5336 | ELMO | VDRC #10455 | + |
| *diaphanous* | CG1768 | DIAPH | VDRC #20518 | - |
| *enabled* | CG15112 | ENAH | VDRC #43058 | - |
| *enabled* | CG15112 | ENAH | VDRC #43056 | - |
| *formin 3* | CG33556 | INF2 | VDRC #45594 | - |
| *nervous wreck* | CG43479 | FCHSD2 | VDRC #44282 | - |
| *shroom* | CG34379 | SHROOM2 | VDRC #107966 | - |
| *shroom* | CG34379 | SHROOM2 | VDRC #100672 | - |
| *spaghetti squash* | CG3595 | MYL9 | VDRC #7916 | + |
| *spaghetti squash* | CG3595 | MYL9 | VDRC #7917 | + |
| *zipper* | CG15792 | MYH10 | VDRC #7819 | - |

**Supp. Table 3. Nomenclature of mouse Nephrin tyrosine (Y) residues.**

| Y# | Tyrosine # |
|---|---|
| Y1 | Y1128 |
| Y2 | Y1153 |
| Y3 | Y1154 |
| Y4 | Y1172 |
| Y5 | Y1191 |
| Y6 | Y1198 |
| Y7 | Y1208 |
| Y8 | Y1216 |
| Y9 | Y1225 |
| Y10 | Y1232 |