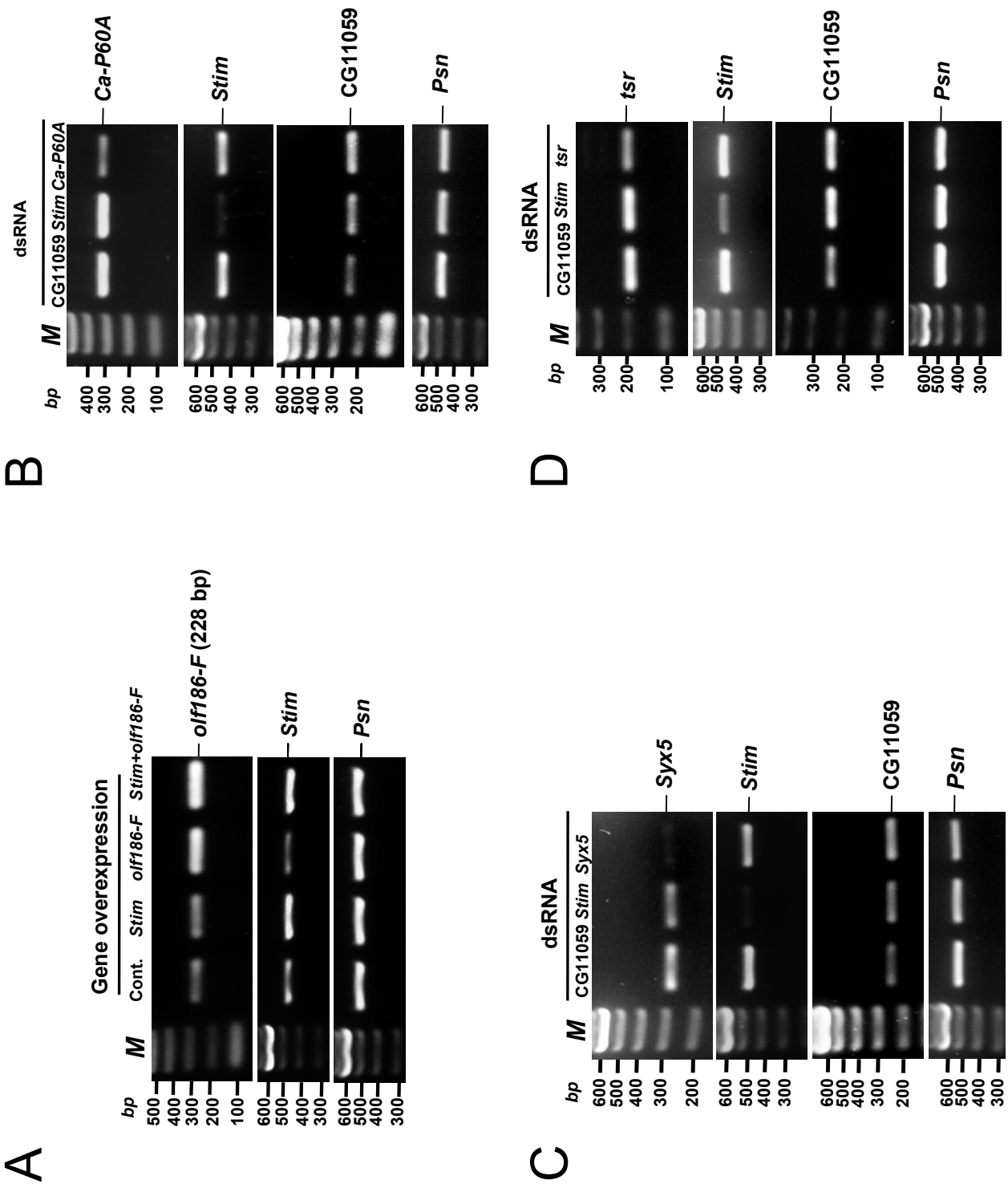


**Fig. S6.** Validation of effective mRNA overexpression or knockdown. RT-PCR analysis was performed as described in *Supporting Materials and Methods* using gene-specific primers (see Table 4). (*A*) Overexpression of *olf186-F*. (*B*) Suppression of *Ca-P60A*. (*C*) Suppression of *Syx5*. (*D*) Suppression of *tsr*.

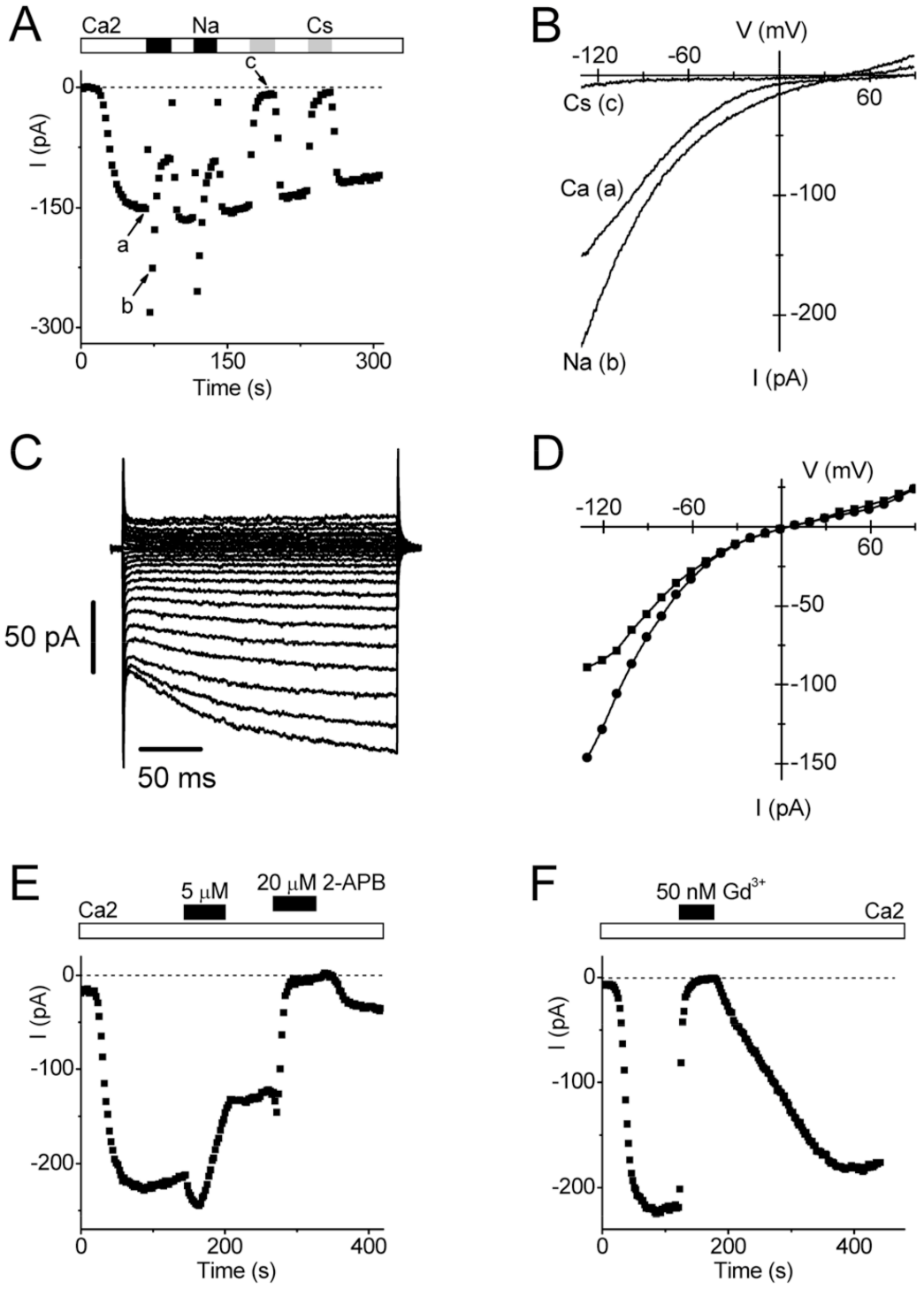


**Fig. S7.** Biophysical and pharmacological properties of enhanced CRAC current after *olf186-F* + *Stim* cotransfection. (*A*) Time course of currents with 2 mM external $Ca^{2+}$, and during subsequent exposure to divalent-free $Na^{+}$- or $Cs^{+}$-containing solution. Arrows indicate the time points for current–voltage curves presented in *B*. (*B*) Corresponding $Ca^{2+}$, $Na^{+}$, and $Cs^{+}$ current–voltage relations. (*C*) $Ca^{2+}$ currents in response to voltage pulses ranging from –130 to +90 mV in 10-mV increments from the holding potential of –10 mV. (*D*) Corresponding current–voltage curves (not leak subtracted) at beginning (squares) and end (circles) of pulses. (*E*) Effect of 2-APB at indicated concentrations. (*F*) $Gd^{3+}$ reversibly blocks the $Ca^{2+}$ current.

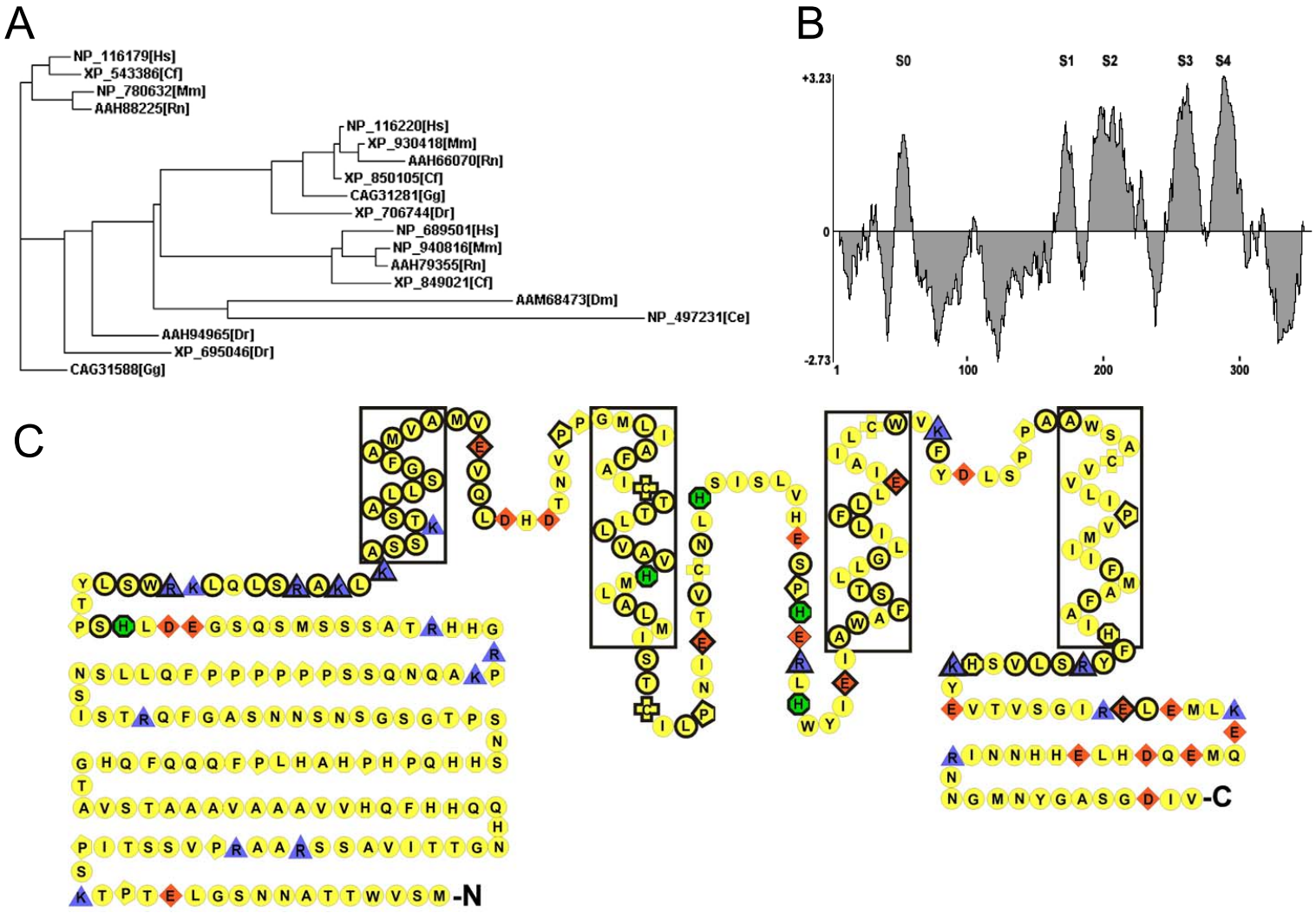


**Fig. S8.** *olf186-F* is a member of a conserved gene family. (*A*) Phylogram of *olf186-F* family. Homologous proteins of the *Drosophila olf186-F* gene product were searched with PHI-BLAST. Hs, human; Mm, mouse; Rn, rat; Cf, dog; Gg, chicken; Dr, zebrafish; Dm, fly; Ce, worm. There are three conserved gene subfamilies within mammals. (*B*) Kyte–Doolittle hydropathy plot (window = 11 residues) of the *Drosophila olf186-F* gene product. The *y* axis represents hydrophobicity, and the *x* axis represents the 351-aa linear polypeptide sequence. The four putative transmembrane segments represented in fly and human homologs are designated S1–S4; S0 indicates an additional predicted a-helical structure found uniquely in the fly sequence. (*C*) Diagram of predicted transmembrane topology and sequence of *Drosophila olf186-F*. Positively charged residues are shown in blue, negatively charged residues are red, and conserved histidines are green. Residues that are identical in fly and three human homologs are shown enlarged and bold.

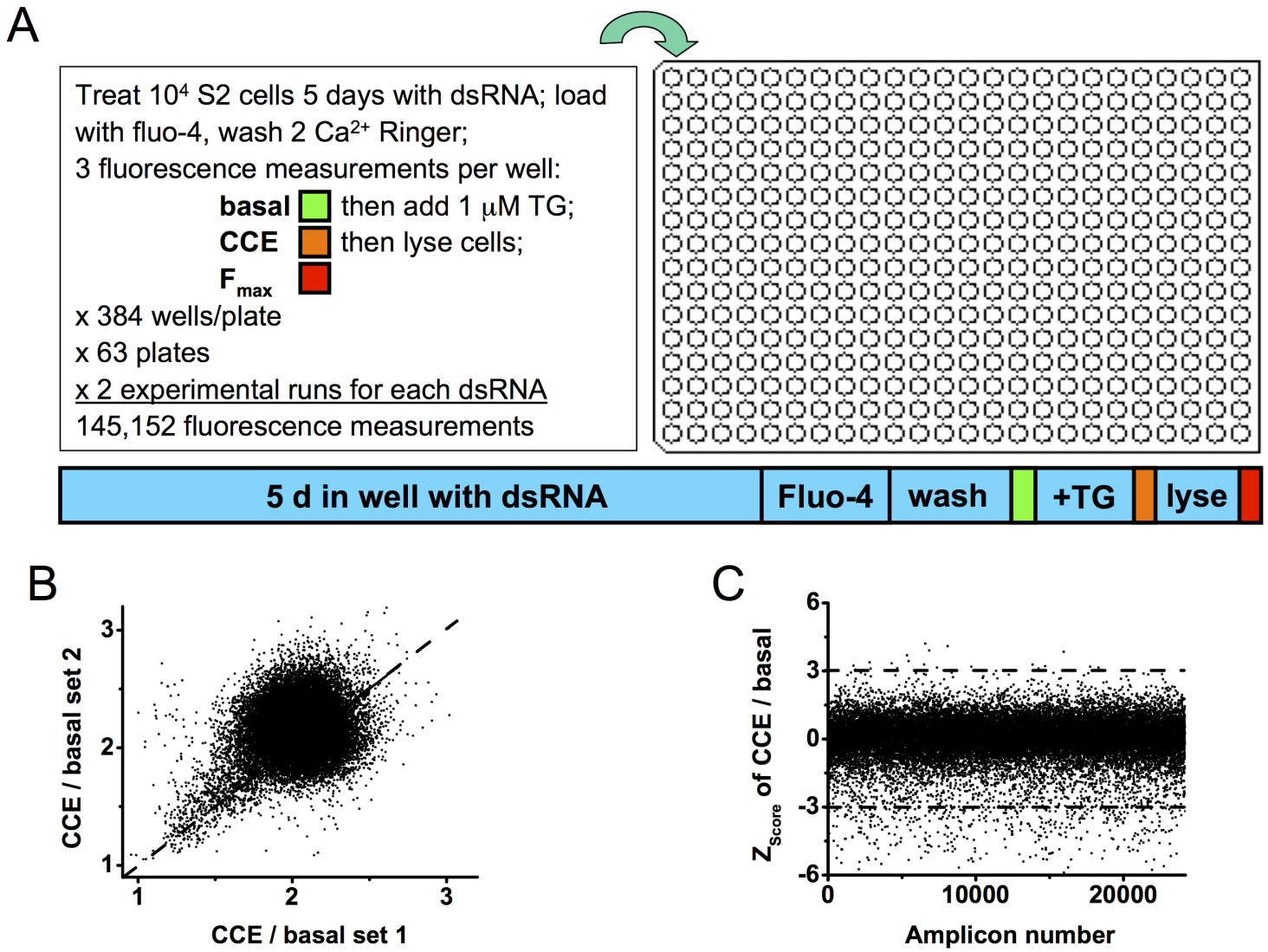


**Fig. S9.** Genome-wide RNAi screen for SOC influx. (*A*) Schematic diagram showing the screening protocol and timeline. (*B*) Scatter plot for the duplicate genome-wide screens. The two "CCE/basal" values derived for each amplicon are plotted on the *x* and *y* axes to show the overall reproducibility. (*C*) The *z*-score was derived from the averaged CCE/basal value of each well. All dsRNAs that inhibited TG-evoked calcium entry with a *z*-score of < –3 (lower dashed line) were selected as hits for further analysis.

**Table 1. Top 10 hits involved in store-operated $Ca^{2+}$ entry**

| DRSC amplicon | Target gene | CCE/ basal | basal/$F_{max}$ | *Z of* $F_{max}$ | Predicted TM segments | Putative function | Potential off-targets |
|---|---|---|---|---|---|---|---|
| DRSC11164 | *Ets65A* | 1.16 | 0.23 | –0.35 | 0 | Transcription factor | 0 |
| DRSC04600 | *Ca-P60A* | 1.23 | 0.37 | 0.43 | 8 | SERCA pump | 0 |
| DRSC20158 | *Stim* | 1.26 | 0.28 | –1.03 | 1 | Putative ER $Ca^{2+}$ sensor for SOC activation | 0 |
| DRSC04718 | *tsr* | 1.28 | 0.37 | 0.56 | 0 | Actin binding protein | 0 |
| DRSC02708 | *cdc23* | 1.30 | 0.35 | –1.69 | 0 | Component of anaphase-promoting complex for mitotic anaphase | 1 |
| DRSC22061 | *olf186-F* | 1.31 | 0.29 | –1.11 | 4 | *Drosophila CRAC candidate* | 0 |
| DRSC04558 | *dom* | 1.32 | 0.35 | 0.38 | 0 | Component of chromatin remodeling complex for DNA recombination | 0 |
| DRSC03256 | *Sec61alpha* | 1.32 | 0.41 | 1.40 | 10 | Component of translocon complex for protein trafficking | 0 |
| DRSC03432 | *Syx5** | 1.33 | 0.33 | –2.21 | 1 | t-SNARE protein for vesicle fusion | 0 |
| DRSC18760 | *deltaCOP* | 1.34 | 0.32 | –1.39 | 0 | Component of COPI complex for protein trafficking | 0 |

DRSC, *Drosophila* RNAi Screening Center at Harvard University.

**Table 2. Group 3 hits, decreased CCE**

| DRSC amplicon | Target gene | CCE/ basal | Basal/$F_{max}$ | *Z of* $F_{max}$ | Potential off-targets |
|---|---|---|---|---|---|
| DRSC00777 | *Rab5* | 1.41 | 0.40 | 2.98 | 1 |
| DRSC02278 | *CG13773* | 1.45 | 0.40 | –1.48 | 0 |
| DRSC03611 | *smt3* | 1.36 | 0.37 | –1.69 | 0 |
| DRSC03342 | *Hel25E* | 1.40 | 0.30 | –1.08 | 0 |
| DRSC03574 | *mts* | 1.36 | 0.28 | 0.43 | 0 |
| DRSC03080 | *Pvr* | 1.37 | 0.39 | –1.58 | 0 |
| DRSC03256 | *Sec61alpha* | 1.32 | 0.41 | 1.40 | 0 |
| DRSC02179 | *CG12750* | 1.35 | 0.31 | –1.91 | 0 |
| DRSC02708 | *cdc23* | 1.30 | 0.35 | –1.69 | 1 |
| DRSC04600 | *Ca-P60A* | 1.23 | 0.37 | 0.43 | 0 |
| DRSC04558 | *dom* | 1.32 | 0.35 | 0.38 | 0 |
| DRSC08370 | *CG13900* | 1.47 | 0.29 | –1.74 | 0 |
| DRSC07000 | *Bap55* | 1.54 | 0.28 | 2.89 | 0 |
| DRSC07659 | *pAbp* | 1.38 | 0.34 | –1.75 | 0 |
| DRSC06044 | *DMAP1* | 1.54 | 0.31 | 1.42 | 0 |
| DRSC11164 | *Ets65A* | 1.16 | 0.23 | –0.35 | 0 |
| DRSC11032 | *CG8743* | 1.50 | 0.34 | 2.95 | 0 |
| DRSC11257 | *Prosbeta2* | 1.52 | 0.33 | –1.55 | 0 |
| DRSC11124 | *CycT* | 1.47 | 0.33 | –1.66 | 4 |
| DRSC12536 | *CG1249* | 1.54 | 0.27 | –1.65 | 0 |
| DRSC15625 | *CG4699* | 1.55 | 0.32 | –0.17 | 0 |
| DRSC15948 | *CG6015* | 1.55 | 0.30 | –1.53 | 0 |
| DRSC15166 | *CG16941* | 1.53 | 0.28 | –1.67 | 0 |
| DRSC16034 | *Dis3* | 1.42 | 0.30 | –1.52 | 0 |
| DRSC16839 | *Rpn2* | 1.41 | 0.33 | –1.87 | 0 |
| DRSC18760 | *deltaCOP* | 1.34 | 0.32 | –1.39 | 0 |
| DRSC18360 | *APC4* | 1.54 | 0.36 | –0.27 | 0 |
| DRSC20158 | *Stim* | 1.26 | 0.28 | –1.03 | 0 |
| DRSC00782 | *RpL40* | 1.58 | 0.31 | –1.28 | 0 |
| DRSC03261 | *CG9548* | 1.58 | 0.30 | –1.55 | 0 |
| DRSC02680 | *CG18591* | 1.61 | 0.28 | –1.78 | 0 |
| DRSC02721 | *Vha68-2* | 1.64 | 0.32 | 0.31 | 0 |
| DRSC02868 | *Pect* | 1.65 | 0.28 | 1.74 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| DRSC04718 | *tsr* | 1.28 | 0.37 | 0.56 | 0 |
| DRSC04884 | *Nipped-A* | 1.54 | 0.36 | –1.17 | 0 |
| DRSC04838 | *Bub1* | 1.59 | 0.36 | –1.38 | 0 |
| DRSC06417 | *MrgBP* | 1.56 | 0.34 | –1.42 | 0 |
| DRSC06421 | *CG30349* | 1.59 | 0.32 | –1.73 | 0 |
| DRSC07501 | *Pabp2* | 1.42 | 0.31 | –1.50 | 0 |
| DRSC07408 | *E(Pc)* | 1.48 | 0.34 | 2.04 | 0 |
| DRSC07575 | *RacGAP50C* | 1.62 | 0.26 | 2.70 | 0 |
| DRSC07583 | *betaTub56D* | 1.55 | 0.34 | –1.91 | 2 |
| DRSC07502 | *hrg* | 1.53 | 0.36 | 0.77 | 0 |
| DRSC08730 | *pav* | 1.55 | 0.34 | 1.31 | 1 |
| DRSC10696 | *CG6694* | 1.58 | 0.31 | –1.59 | 0 |
| DRSC09740 | *sti* | 1.50 | 0.27 | 0.48 | 0 |
| DRSC11079 | *CG9598* | 1.69 | 0.34 | 1.36 | 0 |
| DRSC11330 | *brm* | 1.54 | 0.33 | –1.38 | 0 |
| DRSC11663 | *CG11451* | 1.52 | 0.34 | –1.10 | 0 |
| DRSC12351 | *Gnf1* | 1.57 | 0.35 | –1.49 | 0 |
| DRSC12623 | *alphaTub84D* | 1.45 | 0.35 | –1.52 | 2 |
| DRSC14371 | *CG31258* | 1.53 | 0.32 | –1.50 | 0 |
| DRSC16555 | *bel* | 1.56 | 0.30 | 3.39 | 3 |
| DRSC16899 | *alphaTub85E* | 1.39 | 0.37 | –0.46 | 3 |
| DRSC16940 | *eff* | 1.41 | 0.33 | –1.60 | 0 |
| DRSC16808 | *Rab1* | 1.40 | 0.34 | –1.50 | 0 |
| DRSC16938 | *eIF-3p66* | 1.41 | 0.36 | –1.65 | 0 |
| DRSC16704 | *Hmgcr* | 1.44 | 0.36 | –1.26 | 0 |
| DRSC16920 | *cdc16* | 1.46 | 0.38 | –0.89 | 0 |
| DRSC18483 | *Roc1a* | 1.64 | 0.31 | –1.32 | 0 |
| DRSC18713 | *Rpt4* | 1.37 | 0.34 | –0.97 | 0 |
| DRSC19385 | *CG11138* | 1.50 | 0.30 | –0.21 | 3 |
| DRSC19570 | *CG14214* | 1.51 | 0.33 | –0.69 | 1 |
| DRSC21306 | *xmas-2* | 1.63 | 0.35 | –1.55 | 0 |
| DRSC05281 | *E(Pc)* | 1.56 | 0.34 | 3.86 | 0 |
| DRSC09005 | *dpr6* | 1.47 | 0.29 | –1.54 | 2 |
| DRSC09132 | *CycA* | 1.57 | 0.29 | 1.24 | 0 |
| DRSC04725 | *zip* | 1.59 | 0.26 | 1.58 | 0 |
| DRSC18419 | *dalao* | 1.66 | 0.28 | 0.49 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| DRSC21641 | *CG40127* | 1.52 | 0.28 | 1.71 | 0 |
| DRSC21554 | *Syx1A* | 1.59 | 0.30 | 0.04 | 0 |
| DRSC21831 | *swm* | 1.66 | 0.29 | –1.12 | 0 |
| DRSC22061 | *olf186-F* | 1.31 | 0.29 | –1.11 | 0 |
| DRSC22489 | *zip* | 1.64 | 0.26 | 3.28 | 0 |
| DRSC23010 | *Atx2* | 1.49 | 0.33 | 0.63 | 0 |

DRSC, *Drosophila* RNAi Screening Center at Harvard University.

**Table 3. Solutions for $Ca^{2+}$ imaging and whole-cell recording**

| Name | $Na^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $Cl^-$ | HEPES | pH | Osmolality |
|---|---|---|---|---|---|---|---|---|
| S2 Ringer (**Ca2**) | 150 | 5 | 2 | 4 | 167 | 10 | 7.2 | 328 |
| $Ca^{2+}$-free S2 Ringer (**Ca0**) | 150 | 5 | – | 6 | 167 | 10 | 7.2 | 332 |
| S2 external (**Ca2**) | 160 | – | 2 | – | 164 | 10 | 6.6 | 325 |
| High-$Ca^{2+}$ S2 external (**Ca20**) | 124 | – | 20 | – | 164 | 10 | 6.6 | 324 |
| Divalent free $Na^+$ (**Na**) | 152 | – | – | – | 152 | 10 | 6.6 | 328 |
| Divalent free $Cs^+$ (**Cs**) | 160 | – | – | – | 164 | 10 | 6.6 | 324 |
| Name | $Cs^+$ aspartate | | CsCl | $Mg^{2+}$ gluconate | | HEPES | pH | Osmolality |
| S2 internal | 133 | | 2 | 8 | | 15 | 7.2 | 320 |

Solution names used in figures are indicated in bold. Ringer solutions were used for $[Ca^{2+}]_i$ imaging; external solutions were used in patch-clamp experiments. Concentrations are in mM, and osmolality is in mOsm/kg. S2 Ringer solutions contained 2.5 mM probenecid. $Ca^{2+}$-free Ringer and external solutions contained 1 mM EGTA. All Ringer and external solutions contained 10 mM d-glucose. High-$Ca^{2+}$ external solution contained 10 mM sucrose. Internal solutions contained 12 mM BAPTA. pH was adjusted with the appropriate hydroxide.

**Table 4. Primers**

| Gene | Primer | Primer sequence 5' to 3' |
|---|---|---|
| *Drosophila dsRNA primers (T7 sequence underlined)* | | |
| *olf186-F* | olf186-F-RNAi F1 | GAATTAATACGACTCACTATAGGGAGAATACGAATGTACCACCGGG |
| | olf186-F-RNAi R1 | GAATTAATACGACTCACTATAGGGAGACCAAGTGATGCTAGACAATGT |
| Cloning primers | | |
| *olf186-F* | olf186-F-clone F1 | CTGAACATGAAGCGGCCGCATCATGTCTGTGTGGACCAC |
| | olf186-F-clone R1 | GCTGAACTCGAGCTAGACAATGTCCCCGGATG |
| RT-PCR primers | | |
| *olf186-F* | olf186-F-RT F1 | GAATTAATACGACTCACTATAGGGAGAATACGAATGTACCACCGGG |
| | olf186-F-RT R1 | GAAAGAGTATGAGTCCCAGC |
| | olf186-F-RT F2 | CCAACAATTCGGGCCTAGAGAC |
| | olf186-F-RT R2 | GTAGGTGGGCGAGTGGAGATC |
| *Stim* | Stim-RT F1 | CAGTGGAAGTGTTCAGGATCGC |
| | Stim-RT R1 | CCACATCCATTGCCTTCAATGAG |
| CG11059 | CG11059-RT F1 | CTCGCCTAGACTTATGTGAC |
| | CG11059-RT R1 | CCAGTAGACCCATCAAAGTG |
| *Presenilin (Psn)* | *PSN-RT F1* | CTACGGAGGCGAACGAACG |
| | PSN-RT R1 | GGCGATTGTTCATGGAAAGG |
| *Ca-P60A* | CaP60A-RT F1 | CGATATCCGTATCACCCACA |
| | CaP60A-RT R1 | CTCACCGAACTCGTCCAGTT |
| *Syntaxin 5 (Syx5)* | Syx5-RT F1 | CGCTTCCATTCCGACTAGTT |
| | Syx5-RT R1 | GCTTCTCCAGTTTTGCGTAG |
| *tsr* | Tsr-RT F1 | GAAATGCGGACCTGGAGAGT |
| | Tsr-RT R1 | CGACTTCTTGAGAGCATCGA |