**Supplemental information:**

**Genotypes**

Figure 2. A1-3) *yw*; *B2RT-GFP-Rab3 germline excision*/*+*; B1-3) *yw*; *B2RT-mCherry-Rab3 germline excision/+*; C1-3) *yw*; *FRT-F3-3XFLAG-Rab3 germline inversion*/*+*; D1-3) *yw*; *FRT-F3-2XHA-Rab3 germline inversion*/*+*.

Figure 3. A1-6) *yw*; *B2RT-STOP-B2RT-GFP-Rab3, UAS-DSCP-B2*/*+*; *N-syb-GAL4*/*+*; B1-6) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3, UAS-DSCP-B2*/*+*; *N-syb-GAL4*/*+*; C1-6) *yw*; *FRT-F3-3XFLAG-Rab3*/*+*; *N-syb-GAL4, UAS-FLP*/*+*; D1-6) *yw*; *FRT-F3-2XHA-Rab3*/*+*; *N-syb-GAL4, UAS-FLP*/*+*.

Figure 4. A1-3 + A5-7) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/*R72F11AD*; *UAS-CD8-mCherry*/*R57F07DBD*. A4) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/*+*; *UAS-CD8-mCherry*/*+*. B1-3 + B5-7) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/ *R72F11AD*; *FRT-STOP-FRT-HA-vAChT, UAS-DSCP-FLP*/ *R57F07DBD*. B4) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/*+*; *FRT-STOP-FRT-HA-vAChT, UAS-DSCP-FLP*/ *+*. C1-3 + C5-7) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*, *UAS-DSCP-B2*/ *R72F11AD*; *UAS-DSCP-6XGFP*/ *R57F07DBD*. C4) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*, *UAS-DSCP-B2*/*+*; *UAS-DSCP-6XGFP*/*+*. D1-3 + D5-7) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*, *UAS-DSCP-B2*/ *R72F11AD*; *FRT-STOP-FRT-HA-vAChT, UAS-DSCP-FLP*/ *R57F07DBD*. D4) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*, *UAS-DSCP-B2*/*+*; *FRT-STOP-FRT-HA-vAChT, UAS-DSCP-FLP*/ *+*. E1-3 + E5-7) *yw*; *FRT-F3-3XFLAG-Rab3*/ *R72F11AD*; *UAS-CD8-mCherry, UAS-DSCP-FLP*/ *R57F07DBD*. E4) *yw*; *FRT-F3-3XFLAG*/*+*. F1-3 + F5-7) *yw*; *FRT-F3-2XHA-Rab3*/ *R72F11AD*; *UAS-CD8-mCherry, UAS-DSCP-FLP*/ *R57F07DBD*. T) *yw*; *FRT-F3-2XHA-Rab3*/*+*.

Figure 5. A1-3) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/*R53G02AD*; *UAS-CD8-mCherry*/*R29G11DBD*. A4) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/*+*; *UAS-CD8-mCherry*/ *+*. B1-3) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/*R53G02AD*; *FRT-STOP-FRT-HA-vAChT, UAS-DSCP-FLP*/*R29G11DBD*. B4) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/*+*; *FRT-STOP-FRT-HA-vAChT, UAS-DSCP-FLP*/ *+*. C1-3) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*, *UAS-DSCP-B2*/*R53G02AD*; *UAS-DSCP-6XGFP*/*R29G11DBD*. C4) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*, *UAS-DSCP-B2*/*+*; *UAS-DSCP-6XGFP*/*+*. D1-3) *yw*; *FRT-F3-3XFLAG-Rab3*/*R53G02AD*; *UAS-CD8-mCherry, UAS-DSCP-FLP*/*R29G11DBD*. D4) *yw*; *FRT-F3-3XFLAG-Rab3*/*+*; *UAS-CD8-mCherry, UAS-DSCP-FLP*/ *+*. E1-3) *yw*; *FRT-F3-2XHA-Rab3*/*R53G02AD*; *UAS-CD8-mCherry, UAS-DSCP-FLP*/*R29G11DBD*. E4) *yw*; *FRT-F3-2XHA-Rab3*/*+*; *UAS-CD8-mCherry, UAS-DSCP-FLP*/ *+*. F1-3) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/*R13F02AD*; *UAS-CD8-mCherry*/*R89B01DBD*. F4) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/*+*; *UAS-CD8-mCherry*/ *+*. G1-3) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*, *UAS-DSCP-B2*/ *R13F02AD*; *UAS-DSCP-6XGFP*/ *R89B01DBD*. G4) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*, *UAS-DSCP-B2*/*+*; *UAS-DSCP-6XGFP*/*+*. H1-3) *yw*; *FRT-F3-3XFLAG-Rab3*/ *R13F02AD*; *UAS-CD8-mCherry, UAS-DSCP-FLP*/ *R89B01DBD*. H4) *yw*; *FRT-F3-3XFLAG-Rab3*/*+*. I1-3) *yw*; *FRT-F3-2XHA-Rab3*/ *R13F02AD*; *UAS-CD8-mCherry, UAS-DSCP-FLP*/ *R89B01DBD*. I4) *yw*; *FRT-F3-2XHA-Rab3*/*+*.

Figure 6. A1-6) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/*R82C10AD*; *UAS-CD8-mCherry*/*R72B05DBD*; B) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/*+*; *UAS-CD8-mCherry*/*+*; C1-6) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*, UAS-DSCP-B2/R82C10AD; *UAS-DSCP-6XGFP*/*R72B05DBD*; D) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*, *UAS-DSCP-B2*/*+*; *UAS-DSCP-6XGFP*/*+*.

**Donor plasmid sequences**

**B2RT-STOP-B2RT-2XHA-Rab3**

**GAGGTCTGCCTCGTGAAGAAGGTGTTGCTGACTCATACCAGGCCTGAATCGCCCCATCATCCAGCCAGAAAGTGAGGGAGCCACGGTTGATGAGAGCTTTGTTGTAGGTGGACCAGTTGGTGATTTTGAACTTTTGCTTTGCCACGGAACGGTCTGCGTTGTCGGGAAGATGCGTGATCTGATCCTTCAACTCAGCAAAAGTTCGATTTATTCAACAAAGCCACGTTGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCTTGCTCGAAGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGGCGGCTTTGTTGAATAAATCGCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGACATCAGCGTAGTCATGCATATGCTAGTGTTGCTGCTACTAGTTATTATGGTCGTGGTATCTATGGTCTCAATTAGCTGACCTTCATCCTCGGAAGCAGCGATGGCCGCCTCGCTTGCTTGCTCCTGCTGAGCTTTCAAGTCGCTTTCCCTTTGCCGCTCCAGTTCGCGCTCTAGTTCCAATTGCTGCTCCTGCTCGATACGTTCCTCTTCGTGCTCCTCCTGAGCTTGAGCCTGGCATAGCTCCACATGCATTCTGGACAACTGGGCGGCATCGTATGGGCAGAAGCAGTTTAGGCTTACAACTCGATTGCCCATGTGCTGGACTGGTCGGCTGTTACGGGCTATGGGCGAGATCCCATCCGGACCTAGGAGCAAACGAAAGTGACGCAGCTGAAAGTCGCTAATGGCTAGGGATTCGGGAAAGATGAACCATTCGGTGCCCTGGTAACAGGGCGGATGGGTCAGCGAACCGCCGTAGGAATAGAATCCAGATCGAAATTGATACATCAAGTAGGATATGGGAAATGGAGAAATCTGGACACGCTTTCCAGGCTTCTGAACCAATCGCAAATTCTGCACCAGGGGATCCAGGAACGGATTGTGCGCGGATAGCTCAAAGACGTATCCGATCATCAGGAGATCATAGCTGCTGGTGCAAGTACGGGGAATACCCGATCCCGTTTTGTGCATCACCTGCATCTCAAGTGGGAACTTTCGGTGGTTGATCGTATGCTCGGATCCCTCGCTGTTGCACCAGCCCCAGTGGAAGCGCAGTTCCAGAAACGTATAGCTGGCCAGGAGATCAGCTCCACTGATAGTAGGTGCATTACCGTGGAACTGGGCACGGAGGATAAGTGTCTGACCCGTATTCTCCAGAGTGATGCTGGCGGGCAGATCATCATAGTGGTTCCAGCTCAGCAGCTCTCTTATGGCCATGCGTTGAATTTGAGATTCATCGATGTTGACTGGGCTCTGGAAGAAAGTCGCTTCCTCCACAGAACTGGAAGATGACCTGGACTTCGGCAACCAAGTATGCGGACCATGTTGCATGTCGTAACCGTATACTGGAGCAGAATTTCTTGAGCAAGATGTGTTCCCAAATTGAAGTGGAGTCCTAATCAAAAGGAGTACACGCTGATATATCTCCTTGAGTGTTTCCTGATCCGCGTGGTATGCAAATGCTCCCAACAGGCAAAGTCCAGTAGTAATCAATGGATGCTGGACCACTTGTCCCAGAATCTGACCAAATACTAGGATTATCAACTGCACCATAACGCTCAATACTGTTCCCAATGCGGTATCAAACATGTTGCCTTTTAGTAGACGCGGACTTGCATTAGAAAGCCAGTTATGGGATAAAAAGAATCTGCAAGTGCAGAAATGTCCAAACAAAAACGCGGAAACAAAATGCTCAACTCCTTTTAGATTTCCAAGTTATTTTACAAAACTTTCCGCTTCGTAGTTTTATGACTGTACGTTTTGAGAATGTGTTTATGTGACTGAGTTCTTAAAGGTTCTGTTGTCTTATATACCAGGTGACAGTCAAATCTGAGTTTCATTAAGGAATAACTAATTCCCTAATGAAACTCTAACGTAAGCTAGCTAGACCGGTGTCGACTAAAGCCAAATAGAAAATTATTCAGTTCCTGGCTTAAGTTTTTAAAAGTGATATTATTTATTTGGTTGTAACCAACCAAAAGAATGTAAATAACTAATACATAATTATGTTAGTTTTAAGTTAGCAACAAATTGATTTTAGCTATATTAGCTACTTGGTTAATAAATAGAATATATTTATTTAAAGATAATTGCGTTTTTATTGTCAGGGAGTGAGTTTGCTTAAAAACTCGTTTAGGTTTGTCCTCCCGAAATTATTTATTTAAATGCGATGGAGAGTTGGCGCCGAATCGAAAACTTTACGCGCTTAAAAGCACGAGTTGGCATCCCTAACGCGTAGGATCTTTGTGAAGGAACCTTACTTCTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATATAAAATTTTTAAGTGTATAATGTGTTAAACTACTGATTCTAATTGTTTGTGTATTTTAGATTCCAACCTATGGAACTGATGAATGGGAGCAGTGGTGGAATGCCTTTAATGAGGAAAACCTGTTTTGCTCAGAAGAAATGCCATCTAGTGATGATGAGGCTACTGCTGACTCTCAACATTCTACTCCTCCAAAAAAGAAGAGAAAGGTAGAAGACCCCAAGGACTTTCCTTCAGAATTGCTAAGTTTTTTGAGTCATGCTGTGTTTAGTAATAGAACTCTTGCTTGCTTTGCTATTTACACCACAAAGGAAAAAGCTGCACTGCTATACAAGAAAATTATGGAAAAATATTTGATGTATAGTGCCTTGACTAGAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCACTAGTGATCTGGCCGGGAGTTTCATTAAGGAATAACTAATTCCCTAATGAAACTCAGCCTGCTACTTTGTTAATCTCATCTTGATATCATGAGACGTTAAATAGATCATGTGGATTAGTTATAATAAAGTGTACAAAATTTAAATAAGTCCCAAACAAATGTCTTAGGGAGTAGTAAGATATTGCAAGACTACTAATCCTAATCGTTTCTAATCCTTGTTTCCATTCCCTCCGTGCAGCACCGCTCTAAACGGATAAAATGGGCTACCCCTACGACGTGCCCGACTACGCCGGCTCCGGCGGCTACCCCTACGACGTGCCCGACTACGCCGGCTCCGGCGGCGCGAGTGGCGGAGACCCCAAGTGGCAGAAGGATGCCGCCGACCAGAACTTTGACTACATGTTCAAGCTGCTCATCATTGGCAACTCCAGCGTGGGCAAGACCAGCTTCCTCTTCCGCTACGCCGACGATAGCTTCACATCCGCCTTCGTCTCCACGGTGGGCATTGACTTTAAGGTGAAGACCGTATTTCGGCACGATAAGCGCGTGAAACTCCAGATCTGGGTAAGTATCTATTGATGTTCTTTCTAAGACATATAATCGGGTCGCAGCATGTGCTGCCTTGCTGTTGAGATGTGTAGAACTTGTTTGACCTCGATTAGTTTTGTGGGGGGGAATGAATCGTCCGGAGAAGAGCATTAAACCGGAAAGCTGACACATTGATTTATTGATTGGCAATCGCTACGGACGAGAGCGCAGGTGAGCGTCCTCTCGAGAGTCCTTGCTGTGCGATGCCAGTCGTGTTTTCAGTCATCCATTAAGATAATATAATCAATGAGTGGAATACGGTAGAGGCTTGTGGTTTAAGCCGTTAATCTATGTTGGGGATTCATTCATTAGGGCTTAACTGCTATTTATTGAGTTATAAGTCAAGTATCGGCTCTAAAGGGATTTTAAACTCGTAGCCTCATTTCCCATTAAACTGTAATTTCGCTTTTATAGGACACTGCTGGACAGGAGCGGTACAGAACTATCACCACAGCCTACTACCGAGGAGCGATGGGTTTCATCCTAATGTACGATGTCACTAATGAGGACAGTTTTAACTCGGTTCAGGATTGGTAAGTGAATCTATTCCTTAAAACTGGATCACCTCTTATGGTGTTATATTTATTTCTCCAAAGGGATCCCCGGGTACCGAGCTCGAATTCGTAATCATGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGCGAACTTTTGCTGAGTTGAAGGATCAGATCACGCATCTTCCCGACAACGCAGACCGTTCCGTGGCAAAGCAAAAGTTCAAAATCAGTAACCGTCAGTGCCGATAAGTTCAAAGTTAAACCTGGTGTTGATACCAACATTGAAACGCTGATCGAAAACGCGCTGAAAAACGCTGCTGAATGTGCGAGCTTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGATCCGTCGA**

**Key: PAM; Guide RNA, B2RT, STOP cassette, ATG, HA, Linker, pHSG298.**

**B2RT-STOP-B2RT-GFP-Rab3**

**GAGGTCTGCCTCGTGAAGAAGGTGTTGCTGACTCATACCAGGCCTGAATCGCCCCATCATCCAGCCAGAAAGTGAGGGAGCCACGGTTGATGAGAGCTTTGTTGTAGGTGGACCAGTTGGTGATTTTGAACTTTTGCTTTGCCACGGAACGGTCTGCGTTGTCGGGAAGATGCGTGATCTGATCCTTCAACTCAGCAAAAGTTCGATTTATTCAACAAAGCCACGTTGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCTTGCTCGAAGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGGCGGCTTTGTTGAATAAATCGCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGACATCAGCGTAGTCATGCATATGCTAGTGTTGCTGCTACTAGTTATTATGGTCGTGGTATCTATGGTCTCAATTAGCTGACCTTCATCCTCGGAAGCAGCGATGGCCGCCTCGCTTGCTTGCTCCTGCTGAGCTTTCAAGTCGCTTTCCCTTTGCCGCTCCAGTTCGCGCTCTAGTTCCAATTGCTGCTCCTGCTCGATACGTTCCTCTTCGTGCTCCTCCTGAGCTTGAGCCTGGCATAGCTCCACATGCATTCTGGACAACTGGGCGGCATCGTATGGGCAGAAGCAGTTTAGGCTTACAACTCGATTGCCCATGTGCTGGACTGGTCGGCTGTTACGGGCTATGGGCGAGATCCCATCCGGACCTAGGAGCAAACGAAAGTGACGCAGCTGAAAGTCGCTAATGGCTAGGGATTCGGGAAAGATGAACCATTCGGTGCCCTGGTAACAGGGCGGATGGGTCAGCGAACCGCCGTAGGAATAGAATCCAGATCGAAATTGATACATCAAGTAGGATATGGGAAATGGAGAAATCTGGACACGCTTTCCAGGCTTCTGAACCAATCGCAAATTCTGCACCAGGGGATCCAGGAACGGATTGTGCGCGGATAGCTCAAAGACGTATCCGATCATCAGGAGATCATAGCTGCTGGTGCAAGTACGGGGAATACCCGATCCCGTTTTGTGCATCACCTGCATCTCAAGTGGGAACTTTCGGTGGTTGATCGTATGCTCGGATCCCTCGCTGTTGCACCAGCCCCAGTGGAAGCGCAGTTCCAGAAACGTATAGCTGGCCAGGAGATCAGCTCCACTGATAGTAGGTGCATTACCGTGGAACTGGGCACGGAGGATAAGTGTCTGACCCGTATTCTCCAGAGTGATGCTGGCGGGCAGATCATCATAGTGGTTCCAGCTCAGCAGCTCTCTTATGGCCATGCGTTGAATTTGAGATTCATCGATGTTGACTGGGCTCTGGAAGAAAGTCGCTTCCTCCACAGAACTGGAAGATGACCTGGACTTCGGCAACCAAGTATGCGGACCATGTTGCATGTCGTAACCGTATACTGGAGCAGAATTTCTTGAGCAAGATGTGTTCCCAAATTGAAGTGGAGTCCTAATCAAAAGGAGTACACGCTGATATATCTCCTTGAGTGTTTCCTGATCCGCGTGGTATGCAAATGCTCCCAACAGGCAAAGTCCAGTAGTAATCAATGGATGCTGGACCACTTGTCCCAGAATCTGACCAAATACTAGGATTATCAACTGCACCATAACGCTCAATACTGTTCCCAATGCGGTATCAAACATGTTGCCTTTTAGTAGACGCGGACTTGCATTAGAAAGCCAGTTATGGGATAAAAAGAATCTGCAAGTGCAGAAATGTCCAAACAAAAACGCGGAAACAAAATGCTCAACTCCTTTTAGATTTCCAAGTTATTTTACAAAACTTTCCGCTTCGTAGTTTTATGACTGTACGTTTTGAGAATGTGTTTATGTGACTGAGTTCTTAAAGGTTCTGTTGTCTTATATACCAGGTGACAGTCAAATCTGAGTTTCATTAAGGAATAACTAATTCCCTAATGAAACTCTAACGTAAGCTAGCTAGACCGGTGTCGACTAAAGCCAAATAGAAAATTATTCAGTTCCTGGCTTAAGTTTTTAAAAGTGATATTATTTATTTGGTTGTAACCAACCAAAAGAATGTAAATAACTAATACATAATTATGTTAGTTTTAAGTTAGCAACAAATTGATTTTAGCTATATTAGCTACTTGGTTAATAAATAGAATATATTTATTTAAAGATAATTGCGTTTTTATTGTCAGGGAGTGAGTTTGCTTAAAAACTCGTTTAGGTTTGTCCTCCCGAAATTATTTATTTAAATGCGATGGAGAGTTGGCGCCGAATCGAAAACTTTACGCGCTTAAAAGCACGAGTTGGCATCCCTAACGCGTAGGATCTTTGTGAAGGAACCTTACTTCTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATATAAAATTTTTAAGTGTATAATGTGTTAAACTACTGATTCTAATTGTTTGTGTATTTTAGATTCCAACCTATGGAACTGATGAATGGGAGCAGTGGTGGAATGCCTTTAATGAGGAAAACCTGTTTTGCTCAGAAGAAATGCCATCTAGTGATGATGAGGCTACTGCTGACTCTCAACATTCTACTCCTCCAAAAAAGAAGAGAAAGGTAGAAGACCCCAAGGACTTTCCTTCAGAATTGCTAAGTTTTTTGAGTCATGCTGTGTTTAGTAATAGAACTCTTGCTTGCTTTGCTATTTACACCACAAAGGAAAAAGCTGCACTGCTATACAAGAAAATTATGGAAAAATATTTGATGTATAGTGCCTTGACTAGAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCACTAGTGATCTGGCCGGGAGTTTCATTAAGGAATAACTAATTCCCTAATGAAACTCAGCCTGCTACTTTGTTAATCTCATCTTGATATCATGAGACGTTAAATAGATCATGTGGATTAGTTATAATAAAGTGTACAAAATTTAAATAAGTCCCAAACAAATGTCTTAGGGAGTAGTAAGATATTGCAAGACTACTAATCCTAATCGTTTCTAATCCTTGTTTCCATTCCCTCCGTGCAGCACCGCTCTAAACGGATAAAATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGGGCCACGGCGGATCTGGCGGATCTGGCGGATCTGCGAGTGGCGGAGACCCCAAGTGGCAGAAGGATGCCGCCGACCAGAACTTTGACTACATGTTCAAGCTGCTCATCATTGGCAACTCCAGCGTGGGCAAGACCAGCTTCCTCTTCCGCTACGCCGACGATAGCTTCACATCCGCCTTCGTCTCCACGGTGGGCATTGACTTTAAGGTGAAGACCGTATTTCGGCACGATAAGCGCGTGAAACTCCAGATCTGGGTAAGTATCTATTGATGTTCTTTCTAAGACATATAATCGGGTCGCAGCATGTGCTGCCTTGCTGTTGAGATGTGTAGAACTTGTTTGACCTCGATTAGTTTTGTGGGGGGGAATGAATCGTCCGGAGAAGAGCATTAAACCGGAAAGCTGACACATTGATTTATTGATTGGCAATCGCTACGGACGAGAGCGCAGGTGAGCGTCCTCTCGAGAGTCCTTGCTGTGCGATGCCAGTCGTGTTTTCAGTCATCCATTAAGATAATATAATCAATGAGTGGAATACGGTAGAGGCTTGTGGTTTAAGCCGTTAATCTATGTTGGGGATTCATTCATTAGGGCTTAACTGCTATTTATTGAGTTATAAGTCAAGTATCGGCTCTAAAGGGATTTTAAACTCGTAGCCTCATTTCCCATTAAACTGTAATTTCGCTTTTATAGGACACTGCTGGACAGGAGCGGTACAGAACTATCACCACAGCCTACTACCGAGGAGCGATGGGTTTCATCCTAATGTACGATGTCACTAATGAGGACAGTTTTAACTCGGTTCAGGATTGGTAAGTGAATCTATTCCTTAAAACTGGATCACCTCTTATGGTGTTATATTTATTTCTCCAAAGGGATCCCCGGGTACCGAGCTCGAATTCGTAATCATGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGCGAACTTTTGCTGAGTTGAAGGATCAGATCACGCATCTTCCCGACAACGCAGACCGTTCCGTGGCAAAGCAAAAGTTCAAAATCAGTAACCGTCAGTGCCGATAAGTTCAAAGTTAAACCTGGTGTTGATACCAACATTGAAACGCTGATCGAAAACGCGCTGAAAAACGCTGCTGAATGTGCGAGCTTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGATCCGTCGA**

**Key: PAM; Guide RNA, B2RT, STOP cassette, ATG, GFP, Linker, pHSG298.**

**B2RT-STOP-B2RT-mCherry-Rab3**

**GAGGTCTGCCTCGTGAAGAAGGTGTTGCTGACTCATACCAGGCCTGAATCGCCCCATCATCCAGCCAGAAAGTGAGGGAGCCACGGTTGATGAGAGCTTTGTTGTAGGTGGACCAGTTGGTGATTTTGAACTTTTGCTTTGCCACGGAACGGTCTGCGTTGTCGGGAAGATGCGTGATCTGATCCTTCAACTCAGCAAAAGTTCGATTTATTCAACAAAGCCACGTTGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCTTGCTCGAAGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGGCGGCTTTGTTGAATAAATCGCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGACATCAGCGTAGTCATGCATATGCTAGTGTTGCTGCTACTAGTTATTATGGTCGTGGTATCTATGGTCTCAATTAGCTGACCTTCATCCTCGGAAGCAGCGATGGCCGCCTCGCTTGCTTGCTCCTGCTGAGCTTTCAAGTCGCTTTCCCTTTGCCGCTCCAGTTCGCGCTCTAGTTCCAATTGCTGCTCCTGCTCGATACGTTCCTCTTCGTGCTCCTCCTGAGCTTGAGCCTGGCATAGCTCCACATGCATTCTGGACAACTGGGCGGCATCGTATGGGCAGAAGCAGTTTAGGCTTACAACTCGATTGCCCATGTGCTGGACTGGTCGGCTGTTACGGGCTATGGGCGAGATCCCATCCGGACCTAGGAGCAAACGAAAGTGACGCAGCTGAAAGTCGCTAATGGCTAGGGATTCGGGAAAGATGAACCATTCGGTGCCCTGGTAACAGGGCGGATGGGTCAGCGAACCGCCGTAGGAATAGAATCCAGATCGAAATTGATACATCAAGTAGGATATGGGAAATGGAGAAATCTGGACACGCTTTCCAGGCTTCTGAACCAATCGCAAATTCTGCACCAGGGGATCCAGGAACGGATTGTGCGCGGATAGCTCAAAGACGTATCCGATCATCAGGAGATCATAGCTGCTGGTGCAAGTACGGGGAATACCCGATCCCGTTTTGTGCATCACCTGCATCTCAAGTGGGAACTTTCGGTGGTTGATCGTATGCTCGGATCCCTCGCTGTTGCACCAGCCCCAGTGGAAGCGCAGTTCCAGAAACGTATAGCTGGCCAGGAGATCAGCTCCACTGATAGTAGGTGCATTACCGTGGAACTGGGCACGGAGGATAAGTGTCTGACCCGTATTCTCCAGAGTGATGCTGGCGGGCAGATCATCATAGTGGTTCCAGCTCAGCAGCTCTCTTATGGCCATGCGTTGAATTTGAGATTCATCGATGTTGACTGGGCTCTGGAAGAAAGTCGCTTCCTCCACAGAACTGGAAGATGACCTGGACTTCGGCAACCAAGTATGCGGACCATGTTGCATGTCGTAACCGTATACTGGAGCAGAATTTCTTGAGCAAGATGTGTTCCCAAATTGAAGTGGAGTCCTAATCAAAAGGAGTACACGCTGATATATCTCCTTGAGTGTTTCCTGATCCGCGTGGTATGCAAATGCTCCCAACAGGCAAAGTCCAGTAGTAATCAATGGATGCTGGACCACTTGTCCCAGAATCTGACCAAATACTAGGATTATCAACTGCACCATAACGCTCAATACTGTTCCCAATGCGGTATCAAACATGTTGCCTTTTAGTAGACGCGGACTTGCATTAGAAAGCCAGTTATGGGATAAAAAGAATCTGCAAGTGCAGAAATGTCCAAACAAAAACGCGGAAACAAAATGCTCAACTCCTTTTAGATTTCCAAGTTATTTTACAAAACTTTCCGCTTCGTAGTTTTATGACTGTACGTTTTGAGAATGTGTTTATGTGACTGAGTTCTTAAAGGTTCTGTTGTCTTATATACCAGGTGACAGTCAAATCTGAGTTTCATTAAGGAATAACTAATTCCCTAATGAAACTCTAACGTAAGCTAGCTAGACCGGTGTCGACTAAAGCCAAATAGAAAATTATTCAGTTCCTGGCTTAAGTTTTTAAAAGTGATATTATTTATTTGGTTGTAACCAACCAAAAGAATGTAAATAACTAATACATAATTATGTTAGTTTTAAGTTAGCAACAAATTGATTTTAGCTATATTAGCTACTTGGTTAATAAATAGAATATATTTATTTAAAGATAATTGCGTTTTTATTGTCAGGGAGTGAGTTTGCTTAAAAACTCGTTTAGGTTTGTCCTCCCGAAATTATTTATTTAAATGCGATGGAGAGTTGGCGCCGAATCGAAAACTTTACGCGCTTAAAAGCACGAGTTGGCATCCCTAACGCGTAGGATCTTTGTGAAGGAACCTTACTTCTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAAATATAAAATTTTTAAGTGTATAATGTGTTAAACTACTGATTCTAATTGTTTGTGTATTTTAGATTCCAACCTATGGAACTGATGAATGGGAGCAGTGGTGGAATGCCTTTAATGAGGAAAACCTGTTTTGCTCAGAAGAAATGCCATCTAGTGATGATGAGGCTACTGCTGACTCTCAACATTCTACTCCTCCAAAAAAGAAGAGAAAGGTAGAAGACCCCAAGGACTTTCCTTCAGAATTGCTAAGTTTTTTGAGTCATGCTGTGTTTAGTAATAGAACTCTTGCTTGCTTTGCTATTTACACCACAAAGGAAAAAGCTGCACTGCTATACAAGAAAATTATGGAAAAATATTTGATGTATAGTGCCTTGACTAGAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCACTAGTGATCTGGCCGGGAGTTTCATTAAGGAATAACTAATTCCCTAATGAAACTCAGCCTGCTACTTTGTTAATCTCATCTTGATATCATGAGACGTTAAATAGATCATGTGGATTAGTTATAATAAAGTGTACAAAATTTAAATAAGTCCCAAACAAATGTCTTAGGGAGTAGTAAGATATTGCAAGACTACTAATCCTAATCGTTTCTAATCCTTGTTTCCATTCCCTCCGTGCAGCACCGCTCTAAACGGATAAAATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGGGCCACGGCGGATCTGGCGGATCTGGCGGATCTGCGAGTGGCGGAGACCCCAAGTGGCAGAAGGATGCCGCCGACCAGAACTTTGACTACATGTTCAAGCTGCTCATCATTGGCAACTCCAGCGTGGGCAAGACCAGCTTCCTCTTCCGCTACGCCGACGATAGCTTCACATCCGCCTTCGTCTCCACGGTGGGCATTGACTTTAAGGTGAAGACCGTATTTCGGCACGATAAGCGCGTGAAACTCCAGATCTGGGTAAGTATCTATTGATGTTCTTTCTAAGACATATAATCGGGTCGCAGCATGTGCTGCCTTGCTGTTGAGATGTGTAGAACTTGTTTGACCTCGATTAGTTTTGTGGGGGGGAATGAATCGTCCGGAGAAGAGCATTAAACCGGAAAGCTGACACATTGATTTATTGATTGGCAATCGCTACGGACGAGAGCGCAGGTGAGCGTCCTCTCGAGAGTCCTTGCTGTGCGATGCCAGTCGTGTTTTCAGTCATCCATTAAGATAATATAATCAATGAGTGGAATACGGTAGAGGCTTGTGGTTTAAGCCGTTAATCTATGTTGGGGATTCATTCATTAGGGCTTAACTGCTATTTATTGAGTTATAAGTCAAGTATCGGCTCTAAAGGGATTTTAAACTCGTAGCCTCATTTCCCATTAAACTGTAATTTCGCTTTTATAGGACACTGCTGGACAGGAGCGGTACAGAACTATCACCACAGCCTACTACCGAGGAGCGATGGGTTTCATCCTAATGTACGATGTCACTAATGAGGACAGTTTTAACTCGGTTCAGGATTGGTAAGTGAATCTATTCCTTAAAACTGGATCACCTCTTATGGTGTTATATTTATTTCTCCAAAGGGATCCCCGGGTACCGAGCTCGAATTCGTAATCATGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGCGAACTTTTGCTGAGTTGAAGGATCAGATCACGCATCTTCCCGACAACGCAGACCGTTCCGTGGCAAAGCAAAAGTTCAAAATCAGTAACCGTCAGTGCCGATAAGTTCAAAGTTAAACCTGGTGTTGATACCAACATTGAAACGCTGATCGAAAACGCGCTGAAAAACGCTGCTGAATGTGCGAGCTTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGATCCGTCGA**

**Key: PAM; Guide RNA, B2RT, STOP cassette, ATG, mCherry, Linker, pHSG298.**

**FRT-F3-3XFLAG-Rab3**

**GAGGTCTGCCTCGTGAAGAAGGTGTTGCTGACTCATACCAGGCCTGAATCGCCCCATCATCCAGCCAGAAAGTGAGGGAGCCACGGTTGATGAGAGCTTTGTTGTAGGTGGACCAGTTGGTGATTTTGAACTTTTGCTTTGCCACGGAACGGTCTGCGTTGTCGGGAAGATGCGTGATCTGATCCTTCAACTCAGCAAAAGTTCGATTTATTCAACAAAGCCACGTTGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCTTGCTCGAAGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGGCGGCTTTGTTGAATAAATCGCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGACATCAGCGTAGTCATGCATATGCTAGTGTTGCTGCTACTAGTTATTATGGTCGTGGTATCTATGGTCTCAATTAGCTGACCTTCATCCTCGGAAGCAGCGATGGCCGCCTCGCTTGCTTGCTCCTGCTGAGCTTTCAAGTCGCTTTCCCTTTGCCGCTCCAGTTCGCGCTCTAGTTCCAATTGCTGCTCCTGCTCGATACGTTCCTCTTCGTGCTCCTCCTGAGCTTGAGCCTGGCATAGCTCCACATGCATTCTGGACAACTGGGCGGCATCGTATGGGCAGAAGCAGTTTAGGCTTACAACTCGATTGCCCATGTGCTGGACTGGTCGGCTGTTACGGGCTATGGGCGAGATCCCATCCGGACCTAGGAGCAAACGAAAGTGACGCAGCTGAAAGTCGCTAATGGCTAGGGATTCGGGAAAGATGAACCATTCGGTGCCCTGGTAACAGGGCGGATGGGTCAGCGAACCGCCGTAGGAATAGAATCCAGATCGAAATTGATACATCAAGTAGGATATGGGAAATGGAGAAATCTGGACACGCTTTCCAGGCTTCTGAACCAATCGCAAATTCTGCACCAGGGGATCCAGGAACGGATTGTGCGCGGATAGCTCAAAGACGTATCCGATCATCAGGAGATCATAGCTGCTGGTGCAAGTACGGGGAATACCCGATCCCGTTTTGTGCATCACCTGCATCTCAAGTGGGAACTTTCGGTGGTTGATCGTATGCTCGGATCCCTCGCTGTTGCACCAGCCCCAGTGGAAGCGCAGTTCCAGAAACGTATAGCTGGCCAGGAGATCAGCTCCACTGATAGTAGGTGCATTACCGTGGAACTGGGCACGGAGGATAAGTGTCTGACCCGTATTCTCCAGAGTGATGCTGGCGGGCAGATCATCATAGTGGTTCCAGCTCAGCAGCTCTCTTATGGCCATGCGTTGAATTTGAGATTCATCGATGTTGACTGGGCTCTGGAAGAAAGTCGCTTCCTCCACAGAACTGGAAGATGACCTGGACTTCGGCAACCAAGTATGCGGACCATGTTGCATGTCGTAACCGTATACTGGAGCAGAATTTCTTGAGCAAGATGTGTTCCCAAATTGAAGTGGAGTCCTAATCAAAAGGAGTACACGCTGATATATCTCCTTGAGTGTTTCCTGATCCGCGTGGTATGCAAATGCTCCCAACAGGCAAAGTCCAGTAGTAATCAATGGATGCTGGACCACTTGTCCCAGAATCTGACCAAATACTAGGATTATCAACTGCACCATAACGCTCAATACTGTTCCCAATGCGGTATCAAACATGTTGCCTTTTAGTAGACGCGGACTTGCATTAGAAAGCCAGTTATGGGATAAAAAGAATCTGCAAGTGCAGAAATGTCCAAACAAAAACGCGGAAACAAAATGCTCAACTCCTTTTAGATTTCCAAGTTATTTTACAAAACTTTCCGCTTCGTAGTTTTATGACTGTACGTTTTGAGAATGTGTTTATGTGACTGAGTTCTTAAAGGTTCTGTTGTCTTATATACCAGGTGACAGTCAAATCTGAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTAGAAGTTCCTATTCCGAAGTTCCTATTCTTCAAATAGTATAGGAACTTCCAATAGATACTTACCCAGATCTGGAGTTTCACGCGCTTATCGTGCCGAAATACGGTCTTCACCTTAAAGTCAATGCCCACCGTGGAGACGAAGGCGGATGTGAAGCTATCGTCGGCGTAGCGGAAGAGGAAGCTGGTCTTGCCCACGCTGGAGTTGCCAATGATGAGCAGCTTGAACATGTAGTCAAAGTTCTGGTCGGCGGCATCCTTCTGCCACTTGGGGTCTCCGCCACTCGCTCCGGAGATTACAAGGACCACGACGGCGATTACAAGGACCACGATATCGACTACAAGGACGATGACGATAAGGGAGGCTCCGCCCATTTTATCCGTTTAGAGCGGTGCTGCACGGAGGGAATGGAAACAAGGATTAGAAACGATTAGGATTAGTAGTCTTGCAATATCTTACTACTCCCTAAGACATTTGTTTGGGACTTATTTAAATTTTGTACACTTTATTATAACTAATCCACATGATCTATTTAACGTCTCATGATATCAAGATGAGATTAACAAAGTAGCAGGCTGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGAATAGGAACTTCTTGCCTTAACCCAGAAATTATCACTGTTATTCTTTGAAATGGTGCAAAGAGAAGTTCCTATACTATTTGAAGAATAGGAACTTCGGAATAGGAACTTCATGTTCTTTCTAAGACATATAATCGGGTCGCAGCATGTGCTGCCTTGCTGTTGAGATGTGTAGAACTTGTTTGACCTCGATTAGTTTTGTGGGGGGGAATGAATCGTCCGGAGAAGAGCATTAAACCGGAAAGCTGACACATTGATTTATTGATTGGCAATCGCTACGGACGAGAGCGCAGGTGAGCGTCCTCTCGAGAGTCCTTGCTGTGCGATGCCAGTCGTGTTTTCAGTCATCCATTAAGATAATATAATCAATGAGTGGAATACGGTAGAGGCTTGTGGTTTAAGCCGTTAATCTATGTTGGGGATTCATTCATTAGGGCTTAACTGCTATTTATTGAGTTATAAGTCAAGTATCGGCTCTAAAGGGATTTTAAACTCGTAGCCTCATTTCCCATTAAACTGTAATTTCGCTTTTATAGGACACTGCTGGACAGGAGCGGTACAGAACTATCACCACAGCCTACTACCGAGGAGCGATGGGTTTCATCCTAATGTACGATGTCACTAATGAGGACAGTTTTAACTCGGTTCAGGATTGGTAAGTGAATCTATTCCTTAAAACTGGATCACCTCTTATGGTGTTATATTTATTTCTCCAAAGGGATCCCCGGGTACCGAGCTCGAATTCGTAATCATGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGCGAACTTTTGCTGAGTTGAAGGATCAGATCACGCATCTTCCCGACAACGCAGACCGTTCCGTGGCAAAGCAAAAGTTCAAAATCAGTAACCGTCAGTGCCGATAAGTTCAAAGTTAAACCTGGTGTTGATACCAACATTGAAACGCTGATCGAAAACGCGCTGAAAAACGCTGCTGAATGTGCGAGCTTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGATCCGTCGA**

**Key: PAM; Guide RNA, FRT, F3 ATG, 3XFLAG, Linker, pHSG298**

**FRT-F3-2XHA-Rab3**

**GAGGTCTGCCTCGTGAAGAAGGTGTTGCTGACTCATACCAGGCCTGAATCGCCCCATCATCCAGCCAGAAAGTGAGGGAGCCACGGTTGATGAGAGCTTTGTTGTAGGTGGACCAGTTGGTGATTTTGAACTTTTGCTTTGCCACGGAACGGTCTGCGTTGTCGGGAAGATGCGTGATCTGATCCTTCAACTCAGCAAAAGTTCGATTTATTCAACAAAGCCACGTTGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCTTGCTCGAAGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGGCGGCTTTGTTGAATAAATCGCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGACATCAGCGTAGTCATGCATATGCTAGTGTTGCTGCTACTAGTTATTATGGTCGTGGTATCTATGGTCTCAATTAGCTGACCTTCATCCTCGGAAGCAGCGATGGCCGCCTCGCTTGCTTGCTCCTGCTGAGCTTTCAAGTCGCTTTCCCTTTGCCGCTCCAGTTCGCGCTCTAGTTCCAATTGCTGCTCCTGCTCGATACGTTCCTCTTCGTGCTCCTCCTGAGCTTGAGCCTGGCATAGCTCCACATGCATTCTGGACAACTGGGCGGCATCGTATGGGCAGAAGCAGTTTAGGCTTACAACTCGATTGCCCATGTGCTGGACTGGTCGGCTGTTACGGGCTATGGGCGAGATCCCATCCGGACCTAGGAGCAAACGAAAGTGACGCAGCTGAAAGTCGCTAATGGCTAGGGATTCGGGAAAGATGAACCATTCGGTGCCCTGGTAACAGGGCGGATGGGTCAGCGAACCGCCGTAGGAATAGAATCCAGATCGAAATTGATACATCAAGTAGGATATGGGAAATGGAGAAATCTGGACACGCTTTCCAGGCTTCTGAACCAATCGCAAATTCTGCACCAGGGGATCCAGGAACGGATTGTGCGCGGATAGCTCAAAGACGTATCCGATCATCAGGAGATCATAGCTGCTGGTGCAAGTACGGGGAATACCCGATCCCGTTTTGTGCATCACCTGCATCTCAAGTGGGAACTTTCGGTGGTTGATCGTATGCTCGGATCCCTCGCTGTTGCACCAGCCCCAGTGGAAGCGCAGTTCCAGAAACGTATAGCTGGCCAGGAGATCAGCTCCACTGATAGTAGGTGCATTACCGTGGAACTGGGCACGGAGGATAAGTGTCTGACCCGTATTCTCCAGAGTGATGCTGGCGGGCAGATCATCATAGTGGTTCCAGCTCAGCAGCTCTCTTATGGCCATGCGTTGAATTTGAGATTCATCGATGTTGACTGGGCTCTGGAAGAAAGTCGCTTCCTCCACAGAACTGGAAGATGACCTGGACTTCGGCAACCAAGTATGCGGACCATGTTGCATGTCGTAACCGTATACTGGAGCAGAATTTCTTGAGCAAGATGTGTTCCCAAATTGAAGTGGAGTCCTAATCAAAAGGAGTACACGCTGATATATCTCCTTGAGTGTTTCCTGATCCGCGTGGTATGCAAATGCTCCCAACAGGCAAAGTCCAGTAGTAATCAATGGATGCTGGACCACTTGTCCCAGAATCTGACCAAATACTAGGATTATCAACTGCACCATAACGCTCAATACTGTTCCCAATGCGGTATCAAACATGTTGCCTTTTAGTAGACGCGGACTTGCATTAGAAAGCCAGTTATGGGATAAAAAGAATCTGCAAGTGCAGAAATGTCCAAACAAAAACGCGGAAACAAAATGCTCAACTCCTTTTAGATTTCCAAGTTATTTTACAAAACTTTCCGCTTCGTAGTTTTATGACTGTACGTTTTGAGAATGTGTTTATGTGACTGAGTTCTTAAAGGTTCTGTTGTCTTATATACCAGGTGACAGTCAAATCTGAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGCAGAATGGTAGCTGGATTGTAGCTGCTATTAGCAATATGAAACCTCTTAGAAGTTCCTATTCCGAAGTTCCTATTCTTCAAATAGTATAGGAACTTCCAATAGATACTTACCCAGATCTGGAGTTTCACGCGCTTATCGTGCCGAAATACGGTCTTCACCTTAAAGTCAATGCCCACCGTGGAGACGAAGGCGGATGTGAAGCTATCGTCGGCGTAGCGGAAGAGGAAGCTGGTCTTGCCCACGCTGGAGTTGCCAATGATGAGCAGCTTGAACATGTAGTCAAAGTTCTGGTCGGCGGCATCCTTCTGCCACTTGGGGTCTCCGCCACTCGCGCCGCCGGAGCCGGCGTAGTCGGGCACGTCGTAGGGGTAGCCGCCGGAGCCGGCGTAGTCGGGCACGTCGTAGGGGTAGCCCATTTTATCCGTTTAGAGCGGTGCTGCACGGAGGGAATGGAAACAAGGATTAGAAACGATTAGGATTAGTAGTCTTGCAATATCTTACTACTCCCTAAGACATTTGTTTGGGACTTATTTAAATTTTGTACACTTTATTATAACTAATCCACATGATCTATTTAACGTCTCATGATATCAAGATGAGATTAACAAAGTAGCAGGCTGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGAATAGGAACTTCTTGCCTTAACCCAGAAATTATCACTGTTATTCTTTGAAATGGTGCAAAGAGAAGTTCCTATACTATTTGAAGAATAGGAACTTCGGAATAGGAACTTCATGTTCTTTCTAAGACATATAATCGGGTCGCAGCATGTGCTGCCTTGCTGTTGAGATGTGTAGAACTTGTTTGACCTCGATTAGTTTTGTGGGGGGGAATGAATCGTCCGGAGAAGAGCATTAAACCGGAAAGCTGACACATTGATTTATTGATTGGCAATCGCTACGGACGAGAGCGCAGGTGAGCGTCCTCTCGAGAGTCCTTGCTGTGCGATGCCAGTCGTGTTTTCAGTCATCCATTAAGATAATATAATCAATGAGTGGAATACGGTAGAGGCTTGTGGTTTAAGCCGTTAATCTATGTTGGGGATTCATTCATTAGGGCTTAACTGCTATTTATTGAGTTATAAGTCAAGTATCGGCTCTAAAGGGATTTTAAACTCGTAGCCTCATTTCCCATTAAACTGTAATTTCGCTTTTATAGGACACTGCTGGACAGGAGCGGTACAGAACTATCACCACAGCCTACTACCGAGGAGCGATGGGTTTCATCCTAATGTACGATGTCACTAATGAGGACAGTTTTAACTCGGTTCAGGATTGGTAAGTGAATCTATTCCTTAAAACTGGATCACCTCTTATGGTGTTATATTTATTTCTCCAAAGGGATCCCCGGGTACCGAGCTCGAATTCGTAATCATGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGCGAACTTTTGCTGAGTTGAAGGATCAGATCACGCATCTTCCCGACAACGCAGACCGTTCCGTGGCAAAGCAAAAGTTCAAAATCAGTAACCGTCAGTGCCGATAAGTTCAAAGTTAAACCTGGTGTTGATACCAACATTGAAACGCTGATCGAAAACGCGCTGAAAAACGCTGCTGAATGTGCGAGCTTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGATCCGTCGA**

**Key: PAM; Guide RNA, FRT, F3 ATG, HA, Linker, pHSG298.**

Note that although the PAM sequences are indicated in the sequences shown, they have been deleted in the donor plasmids to prevent cleavage by the Cas9 endonuclease.

**Supplemental Figure Legends**

Figure S1. Assessment of conditional expression in tagged variants of Rab3 genome-edited flies. A1-3) anti-HA staining in negative control (A1) and B2RT-STOP-B2RT-2XHA-Rab3 flies without excision (A2) and after pan-neuronal excision (A3) of the STOP cassette. Without stop cassette excision B2RT-STOP-B2RT-2XHA-Rab3 is constitutively expressed at a level approaching that observed with pan-neuronal excision. B1-3) anti-GFP staining in negative control (B1) and B2RT-STOP-B2RT-GFP-Rab3 flies without excision (B2) and after pan-neuronal excision (B3) of the STOP cassette. C1-3) anti-mCherry staining in negative control (C1) and B2RT-STOP-B2RT-mCherry-Rab3 flies without excision (C2) and after pan-neuronal excision (C3) of the STOP cassette. D1-3) anti-FLAG staining in negative control (D1) and FRT-F3-3XFLAG-Rab3 flies without inversion (D2) and after pan-neuronal inversion (D3) of the STOP cassette. E1-3) anti-HA staining in negative control (E1) and FRT-F3-2XHA-Rab3 flies without inversion (E2) and after pan-neuronal inversion (E3) of the STOP cassette. B2RT-STOP-B2RT-GFP-Rab3, B2RT-STOP-B2RT-mCherry-Rab3, FRT-F3 3XFLAG-Rab3, and FRT-F3 2XHA-Rab3 all show little or no expression prior to excision or inversion, but high-level expression after excision or inversion. Scale bar: 100μm (E1).

Figure S2.

Assessment of Brp distribution and intensity at the third instar larval neuromuscular junction in germline excision or germline inversion alleles of tagged Rab3 variants. A) Brp distribution in *yw* control. B) The Brp distribution phenotype in a *rab3* homozygous loss-of-function mutant includes a reduced number of larger, higher intensity puncta. C1, D1, E1, F1) The distribution of Brp is not affected in *GFP-Rab3* or *mCherry-Rab3* heterozygous or homozygous germline excision alleles but levels of Brp expression are severely reduced. C2, D2, E2, F2) Expression of GFP-Rab3 or mCherry-Rab3 in the germline excision alleles. G1, H1, I1, J1) The distribution of Brp is not affected in *3XFLAG-Rab3* or *2XHA-Rab3* heterozygous or homozygous germline inversion alleles, although there is a minor reduction in the levels of Brp expression. G2, H2, I2, J2) Expression of 3XFLAG-Rab3 or 2XHA-Rab3 in the germline inversion alleles. These results demonstrate amino-terminal fusions onto the Rab3 protein result in the dominant phenotype of reduced levels of Brp. GE-germline excision. GI-germline inversion. Scale Bar 50μm.

Figure S3.

Comparison of SV specificity between conditional GFP-Rab3 and mChry-Rab3 variants with existing UAS-N-syb-GFP and UAS-Syt-GFP Drosophila SV markers in third larval instar ventral nerve cord. Pan-neuronal excision of the STOP cassette in GFP-Rab3 (A) or mChry-Rab3 (B) variants results in a distribution pattern in which GFP-Rab3 and mCherry-Rab3 localize tightly to the neuropil where SVs are located with little or no detectable signal in the surrounding cortex where neuronal cell bodies are located. In contrast, upon pan-neuronal expression, a substantial fraction of N-syb-GFP (C) and Syt-GFP (D) localize outside the neuropil in the cell body region of neurons (arrowheads). Control larva showing the endogenous distributions of the SV-specific proteins Syt (E) or Syn (F) exhibit a tight distribution to the neuropil with little or no detectable signal in the surrounding cortex. Orthogonal cross-section images of each VNC are shown directly below the dorsal views. Arrows to the left of each dorsal view indicate the location of the orthogonal cross-section. The substantial cell body signal observed with the existing UAS-N-syb-GFP and UAS-Syt-GFP SV markers is not detected with endogenous SV markers, suggesting this cell body signal is artifact and these markers do not reliably track SVs. The nearly indistinguishable distribution patterns of GFP- and mChry-Rab3 as compared the endogenous SV-specific proteins Syt and Syn suggest they are reliable indicators of SV localization. Scale Bar 100μm.

Figure S4. Assessment of co-localization of the cholinergic synaptic vesicle marker HA-vAChT with GFP-Rab3 and mCherry-Rab3 upon conditional expression in MB131B adult γ-lobe mushroom body neurons. A1-3) Distribution of HA-vAChT (A1), GFP-Rab3 (A2), and overlay (A3) upon co-conditional expression in MB131B neurons. A4) Control showing background levels of HA-vAChT in the absence of a GAL4 driver. B1-3) Distribution of HA-vAChT (B1), mCherry-Rab3 (B2), and overlay (B3) upon co-conditional expression in MB131B neurons. B4) Control showing background levels of signal in HA-vAChT brains in the absence of a GAL4 driver. Although HA-vAChT expression levels are low, HA-vAChT distribution in the mushroom body can be discerned above background and strongly correlates with the distribution of GFP-Rab3 and mCherry-Rab3 in the mushroom body neuropil. Scale bar: 50μm (B1).

**Supplemental Figure Genotypes**

Figure S1. A1) *yw*; A2) *yw*; *B2RT-STOP-B2RT-HA-Rab3*/*+*; A3) *yw*; *B2RT-STOP-B2RT-HA-Rab3*/ *UAS-DSCP-B2*; *N-syb-GAL4*/*+*. B1) *yw*; B2) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*/*+*; B3) yw; *B2RT-STOP-B2RT-GFP-Rab3*/*UAS-DSCP-B2*; *N-syb-GAL4*/*+*. C1) *yw*; C2) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*/*+*; C3) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*/*UAS-DSCP-B2*; *N-syb-GAL4*/*+*. D1) *yw*; D2) yw; *FRT-F3-3XFLAG-Rab3*/*+*; D3) *yw*; *FRT-F3-3X-FLAG-Rab3*/*+*; *N-syb-GAL4*, *UAS-FLP*/+. E1) *yw*; E2) *yw*; *FRT-F3-2XHA-Rab3*/*+*; E3) *yw*; *FRT-F3-2XHA-Rab3*/*+*; *N-syb-GAL4, UAS-FLP*.

Figure S2. A) *yw*; B) *rab3rup*/*rab3rup*; C1-2) *yw*; *B2RT-GFP-Rab3 GE*/*+*; D1-2) *yw*; *B2RT-GFP-Rab3* *GE*/ *B2RT-GFP-Rab3 GE*; E1-2) *yw*; *B2RT-mChry-Rab3 GE*/*+*; F1-2) *yw*; *B2RT-mChry-Rab3 GE*/ *B2RT-mChry-Rab3 GE*; G1-2) *yw*; *FRT-F3-3XFLAG-Rab3 GI*/*+*; H1-2) *yw*; *FRT-F3-3XFLAG-Rab3 GI*/FRT-F3-3XFLAG-Rab3 GI; I1-2) yw; *FRT-F3-2XHA-Rab3 GI*/*+*; J1-2) *yw*; *FRT-F3-2XHA-Rab3 GI*/*FRT-F3-2XHA-Rab3 GI*. GE-germline excision; GI-germline inversion.

Figure S3. A) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/*N-syb-GAL4*; B) *yw*; *B2RT-STOP-B2RT-mChry-Rab3*, *UAS-DSCP-B2*/*N-syb-GAL4*; C) *yw*; *N-syb-GAL4*/*+*; *UAS-N-syb-GFP*/*+*; D) *yw*; *N-syb-GAL4*/*+*; *UAS-Syt-GFP*/*+*; E-F) *yw*.

Fig S4. A1-3) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/ *R13F02AD*; *FRT-STOP-FRT-HA-vAChT, UAS-DSCP-FLP*/ *R89B01DBD*. A4) *yw*; *B2RT-STOP-B2RT-GFP-Rab3*, *UAS-DSCP-B2*/*+*; *FRT-STOP-FRT-HA-vAChT, UAS-DSCP-FLP*/ *+*. B1-3) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*, *UAS-DSCP-B2*/ *R13F02AD*; *FRT-STOP-FRT-HA-vAChT, UAS-DSCP-FLP*/ *R89B01DBD*. B4) *yw*; *B2RT-STOP-B2RT-mCherry-Rab3*, *UAS-DSCP-B2*/*+*; *FRT-STOP-FRT-HA-vAChT, UAS-DSCP-FLP*/ *+*.