

Sustar et al., 2026. Supplemental Figures.

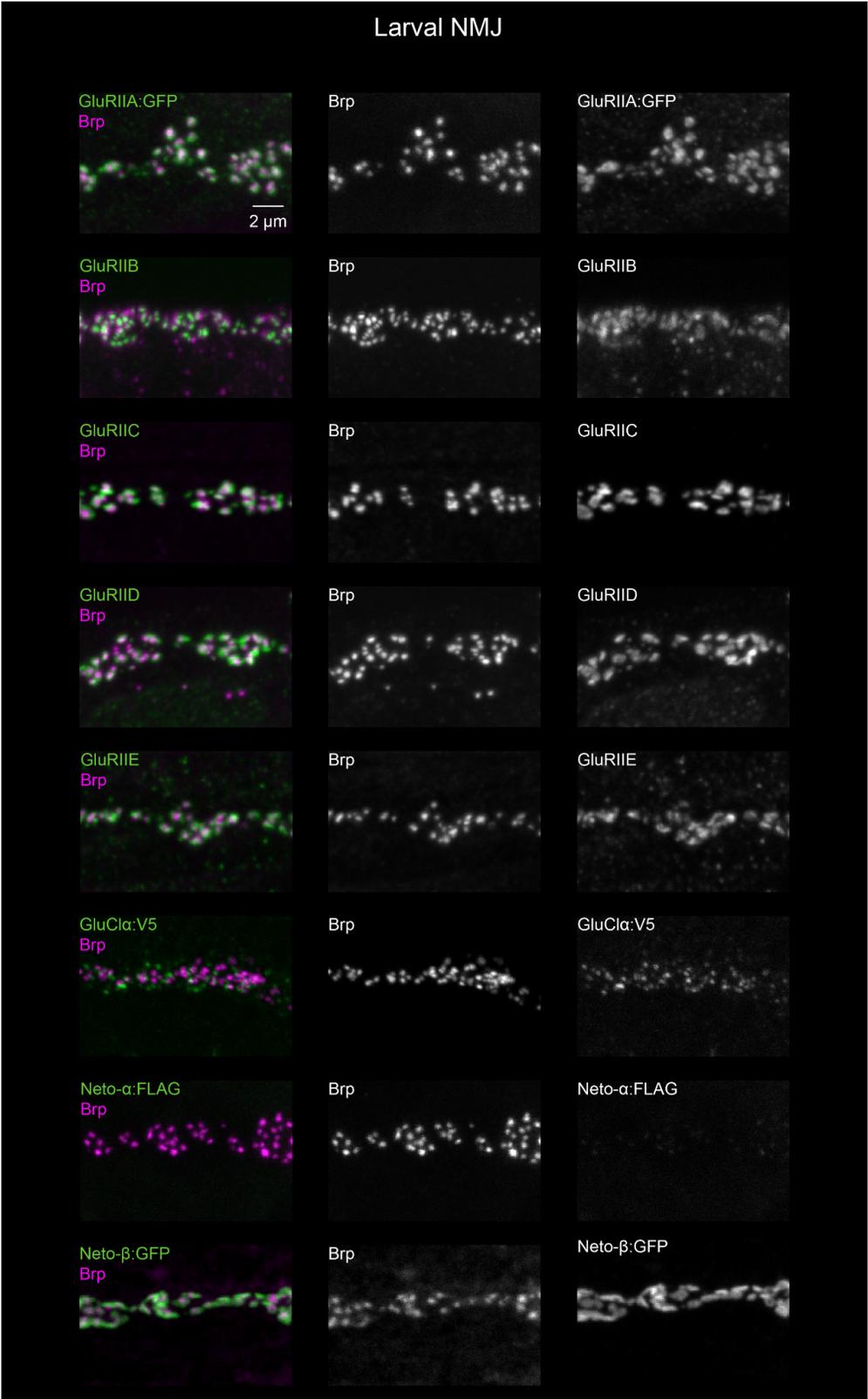


Figure s1. NMJ staining of larval body wall muscle.

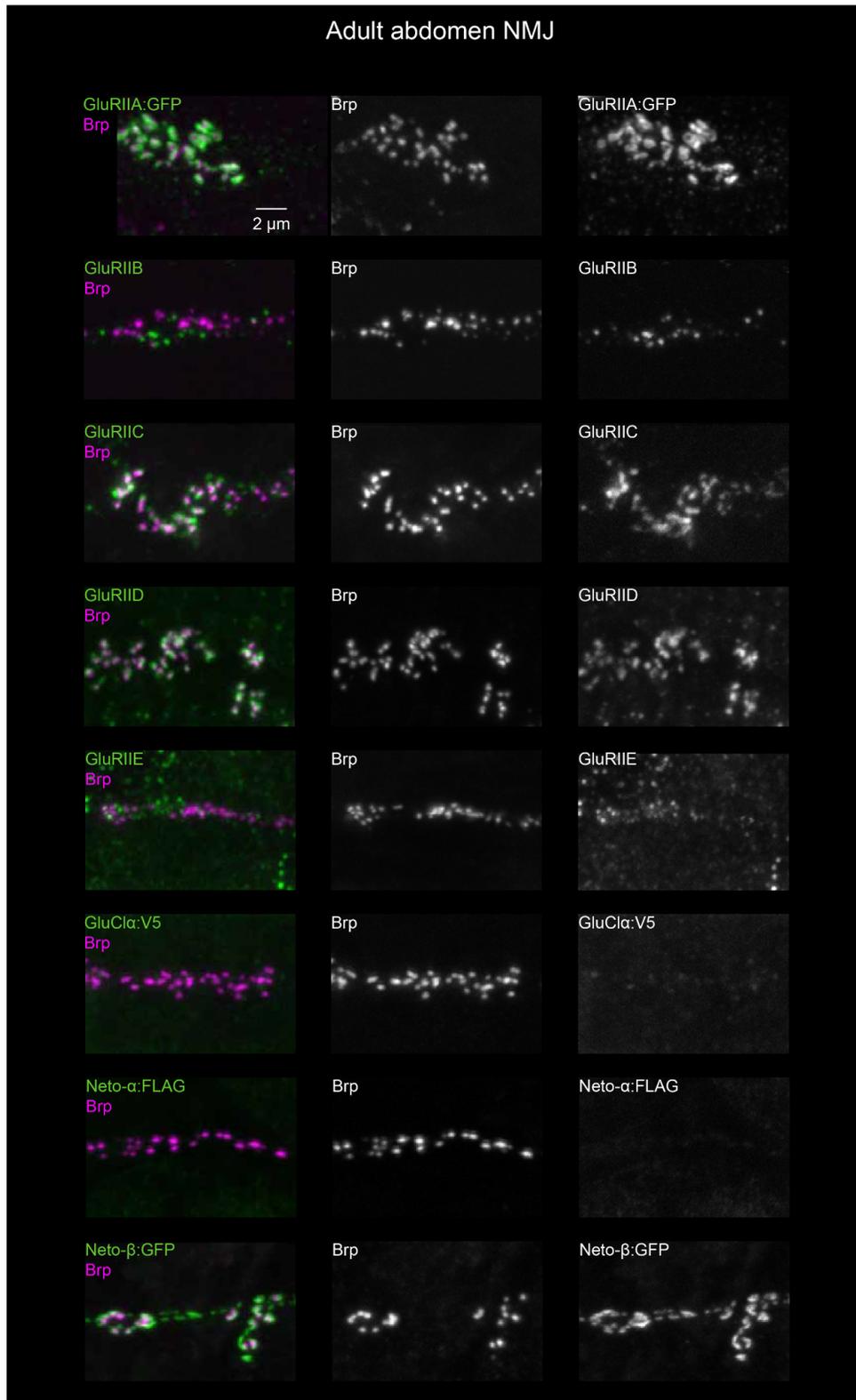


Figure s2. NMJ staining of adult abdomen muscle.

Indirect flight muscle NMJ

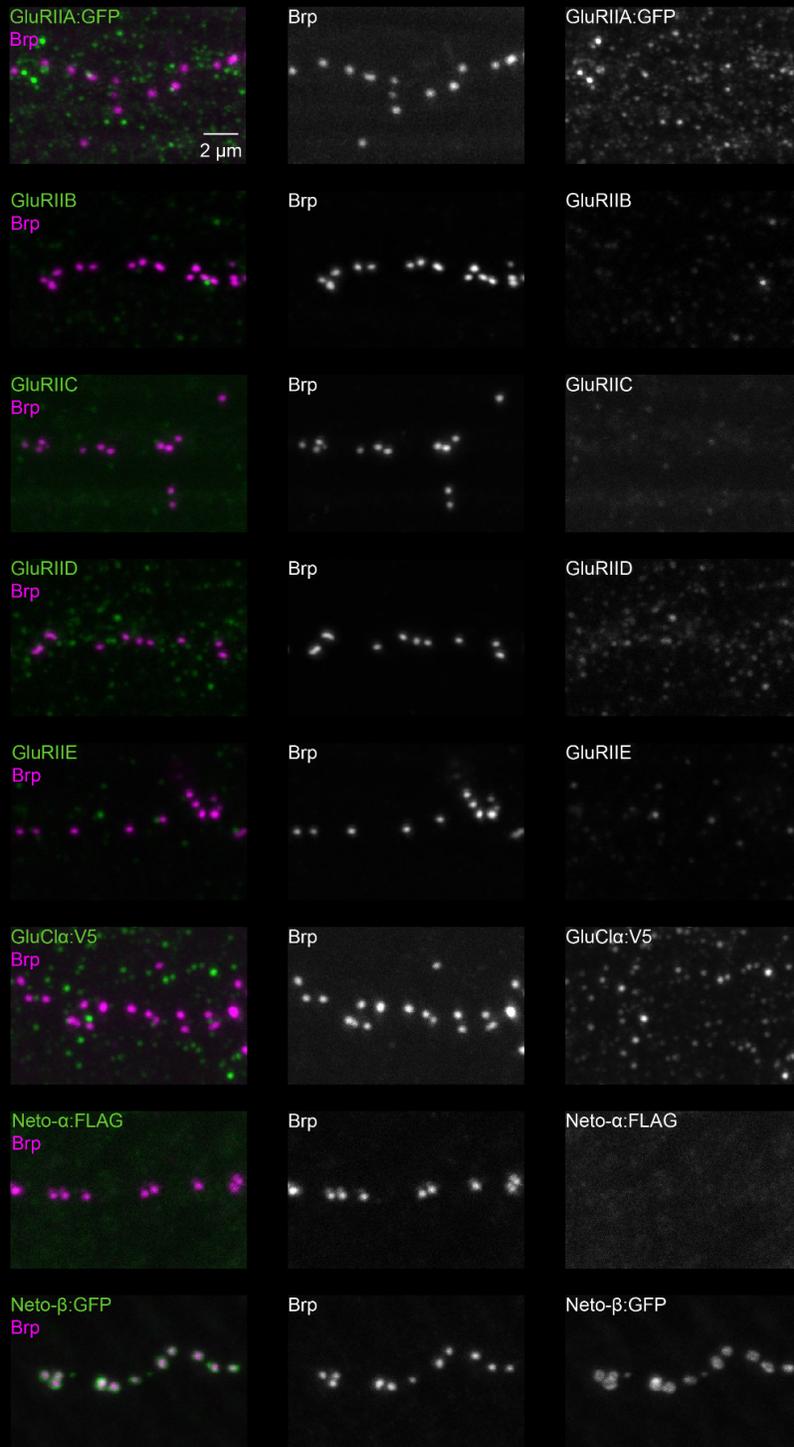


Figure s3. NMJ staining of adult indirect flight muscle.

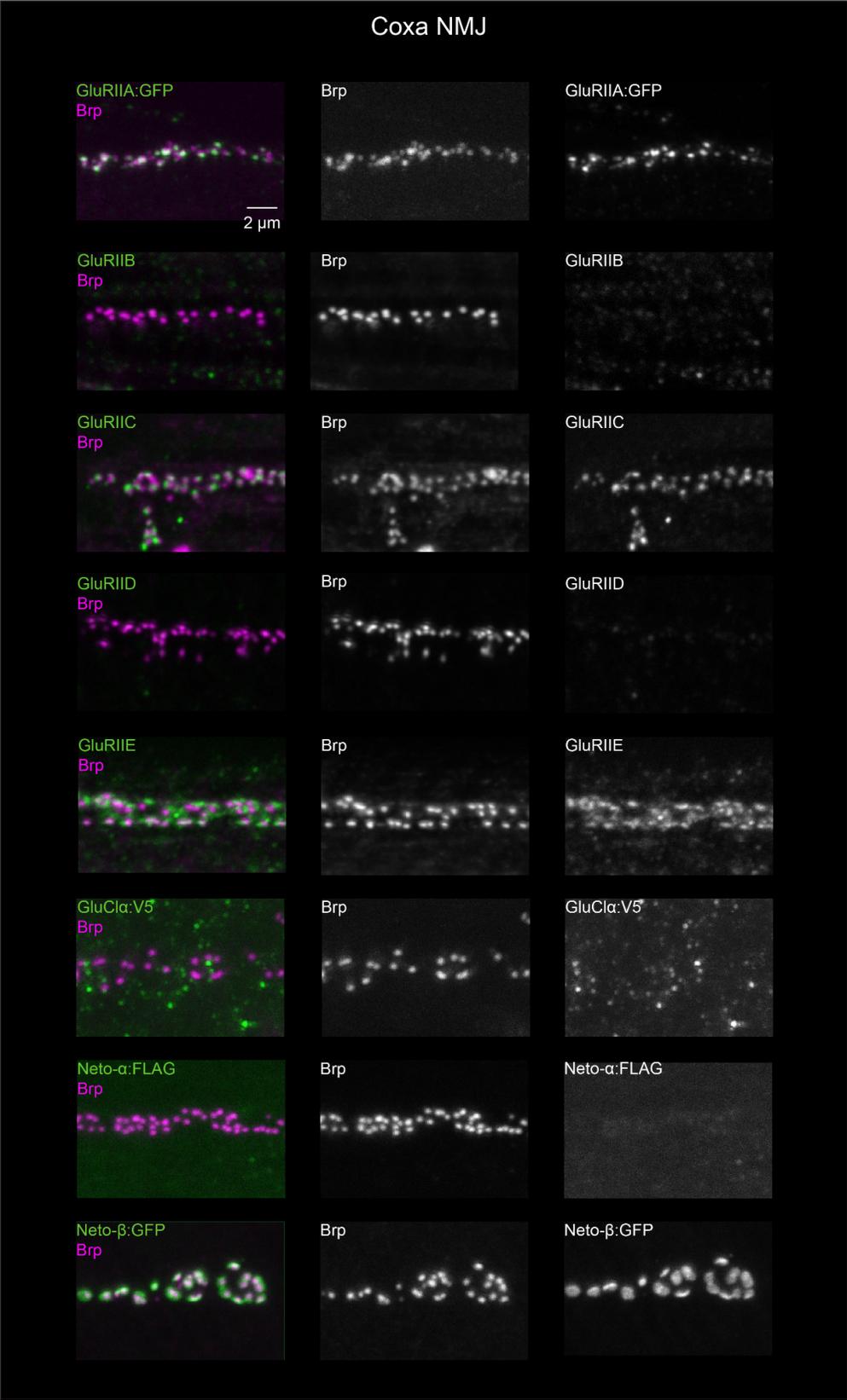


Figure s4. NMJ staining of adult leg muscle (coxa).

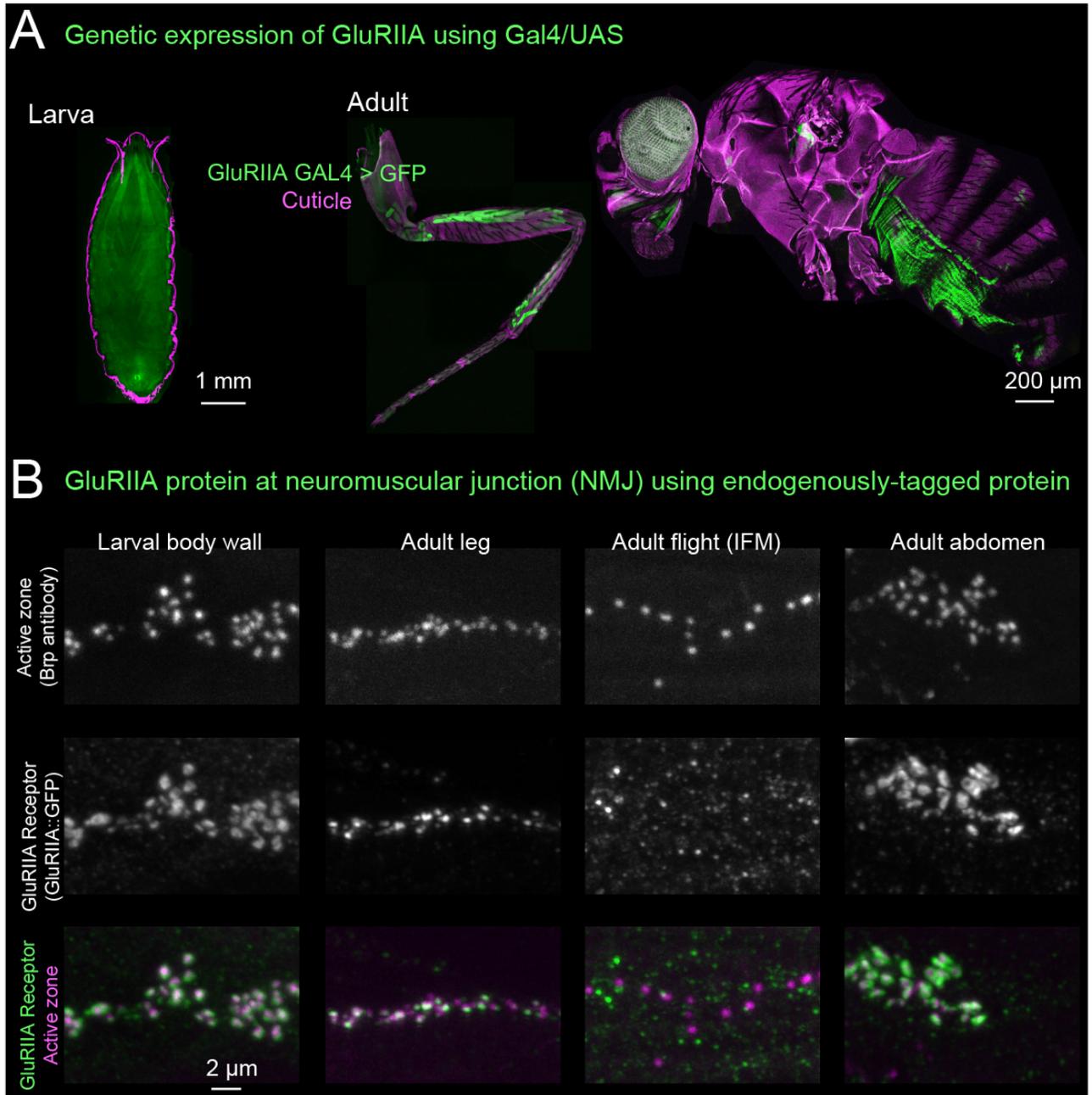


Figure s5. (A) Representative confocal images showing GFP expression driven by a GAL4 reporter line (GluRIIA-GAL4) in larval and adult muscles. (B) Representative images showing antibody staining of GluRIIA::GFP protein (green) at larval and adult neuromuscular junctions (magenta).

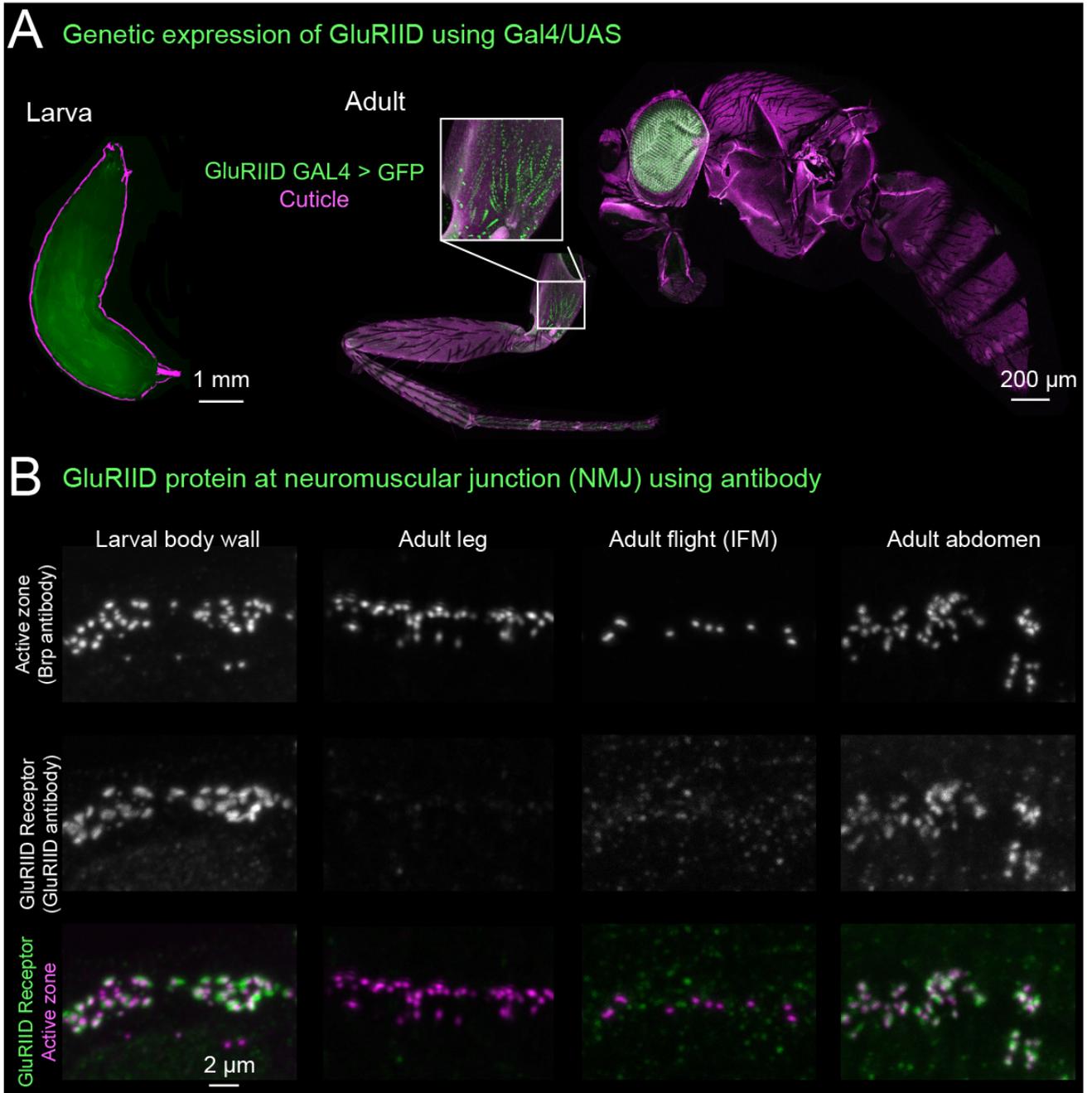


Figure s7. (A) Representative confocal images showing GFP expression driven by a GAL4 reporter line (GluRIID-GAL4, green) in larval and adult muscles. (B) Representative images showing antibody staining of GluRIID protein (green) at larval and adult neuromuscular junctions (magenta).

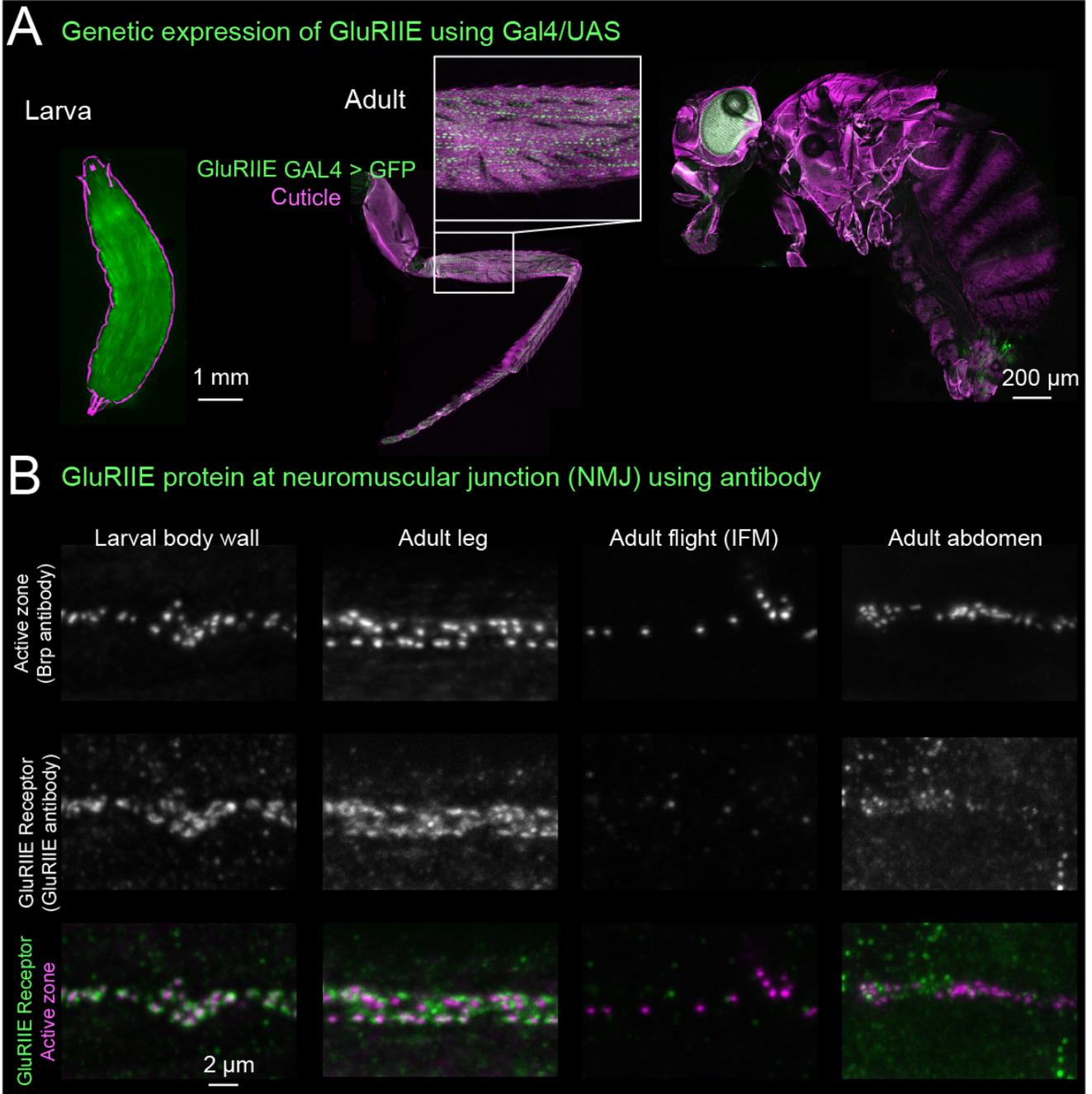


Figure s8. (A) Representative confocal images showing GFP expression driven by a GAL4 reporter line (GluRIIE-GAL4, green) in larval and adult muscles. (B) Representative images showing antibody staining of GluRIIE protein (green) at larval and adult neuromuscular junctions (magenta).

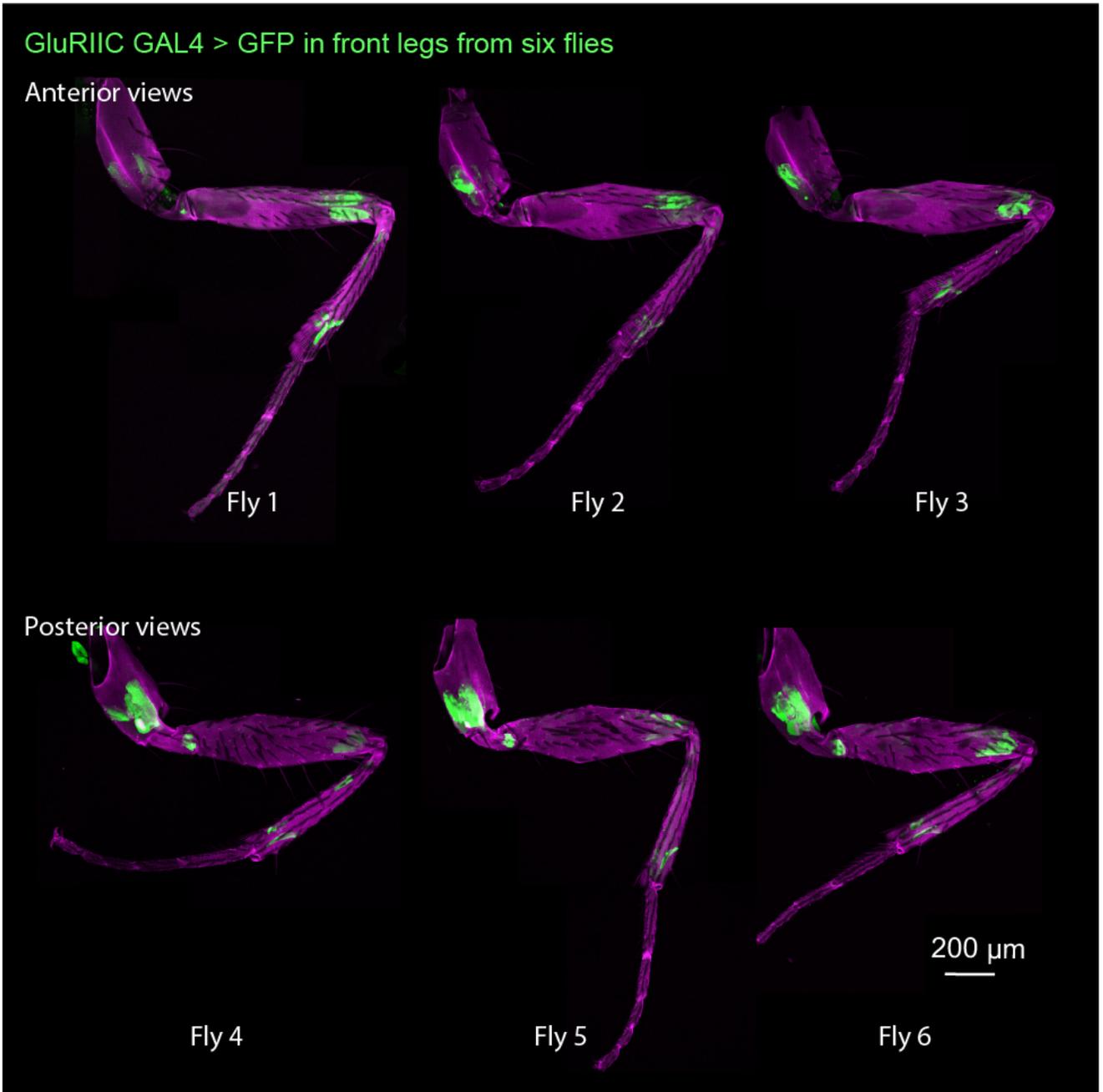


Figure s9. Representative confocal images showing GFP expression driven by a GAL4 reporter line (GluRIIC-GAL4, green) in six front legs from six flies. Anterior views (top), posterior views (bottom).

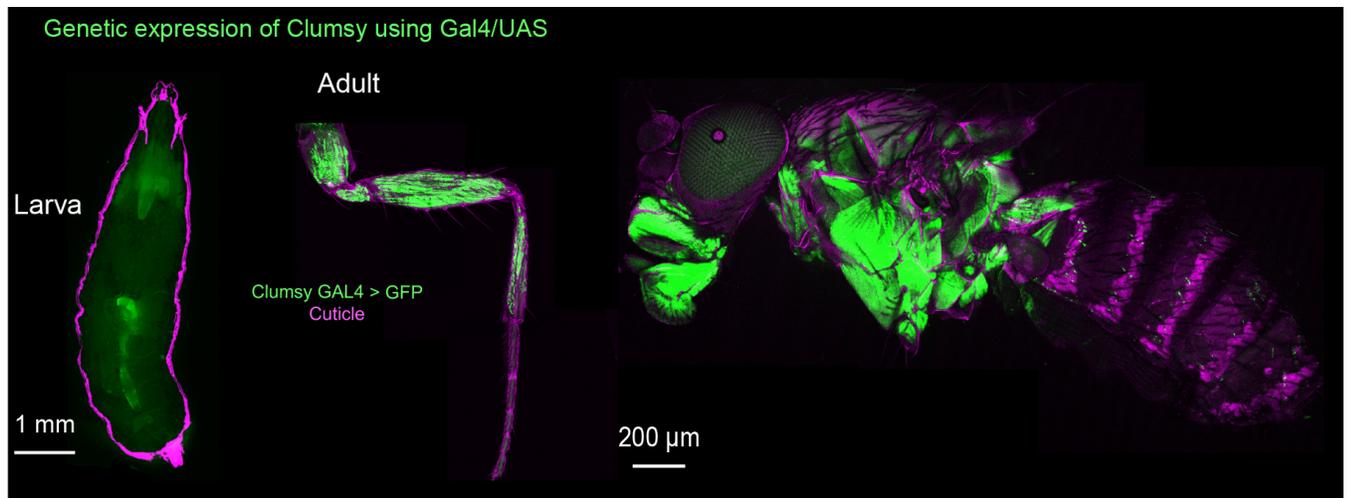


Figure s10. Representative confocal images showing GFP expression driven by a GAL4 reporter line (Clumsy-GAL4, green) in adult but not larval muscles. Note that the Clumsy trojan GAL4 has a 3xP3-GFP marker left from its construction that gives faint glial GFP expression in the brain, vnc, and gut that can be seen in the larva. Larval expression is absent when we screen the line with a UAS-mCherry reporter. Adult muscle expression is consistent with both fluorescent reporters.

Adult Leg RNA-seq

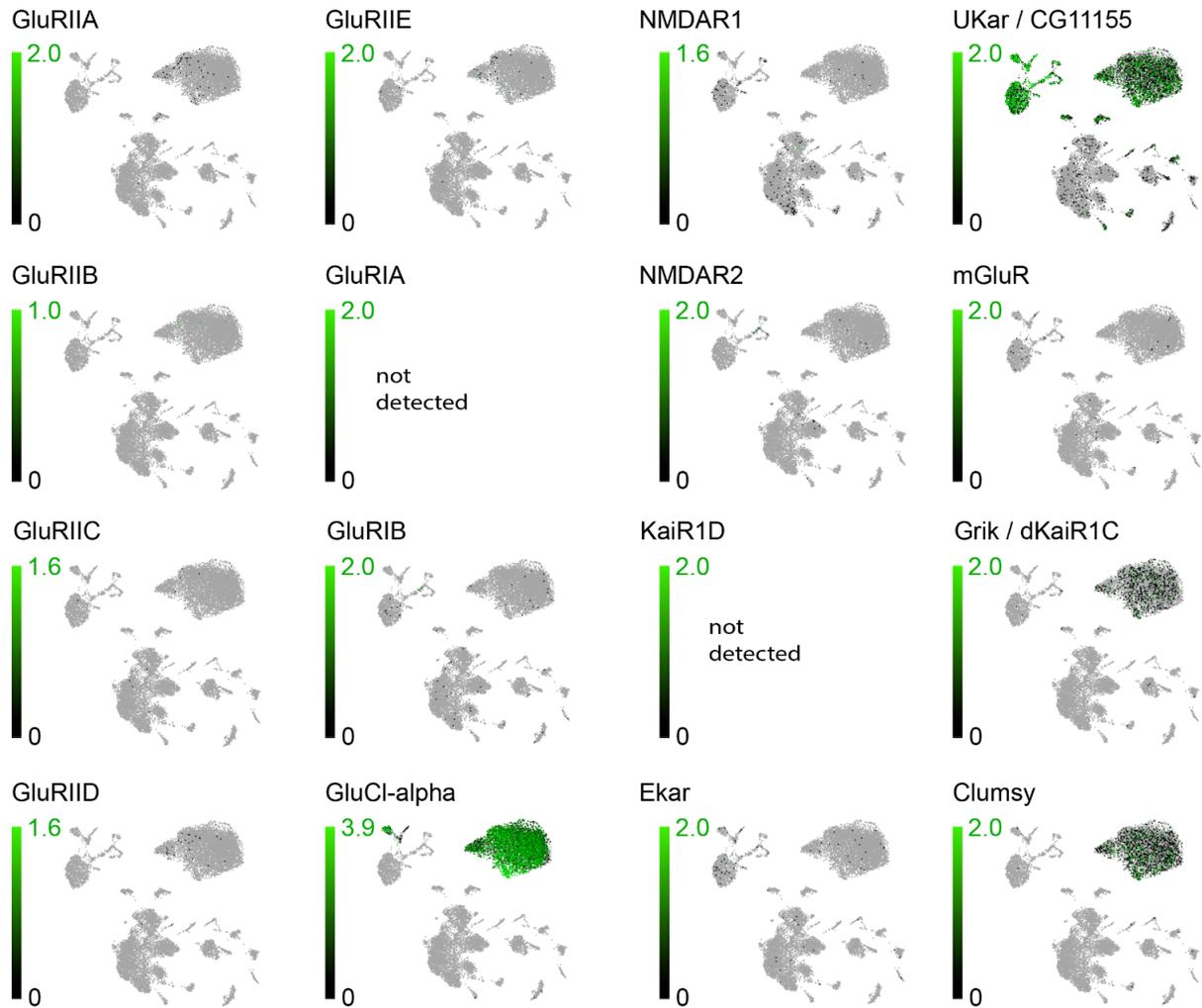
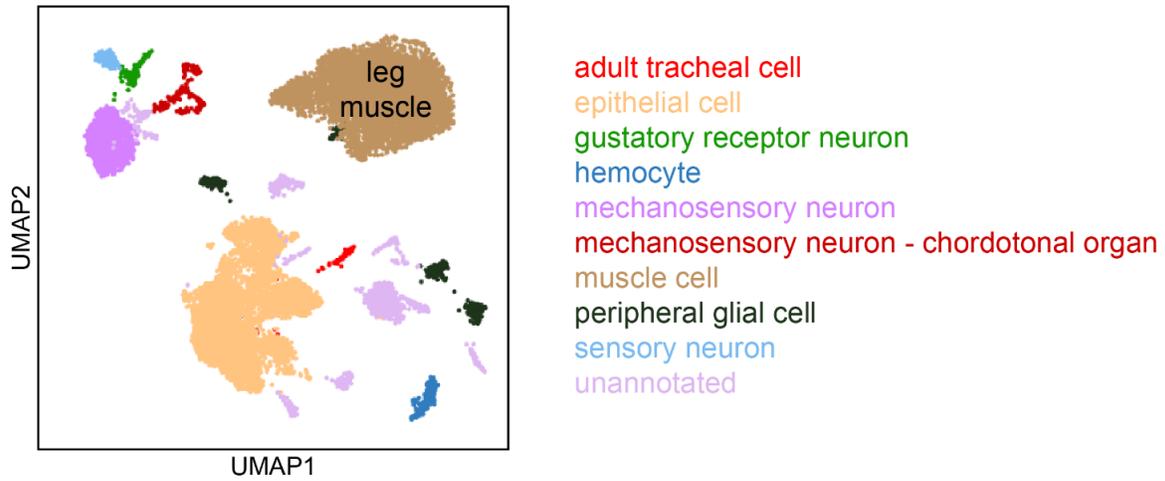


Figure s11. RNA-Seq analysis of glutamate receptors in all tissues of the adult leg (Li et al., 2022).

Adult Muscle Cross-Tissue RNA-seq

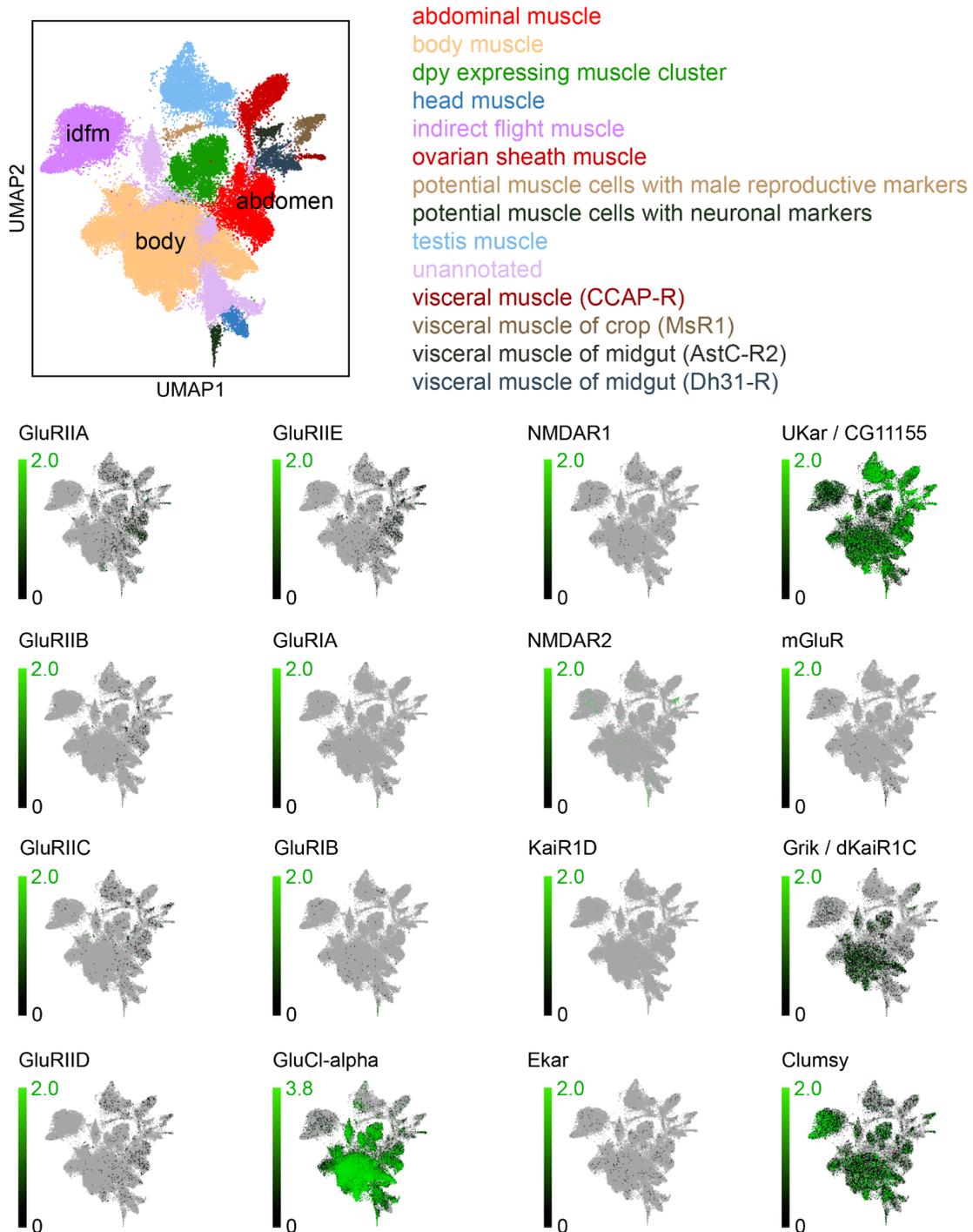


Figure s12. RNA-Seq analysis of glutamate receptors in all adult muscles (Li et al., 2022).

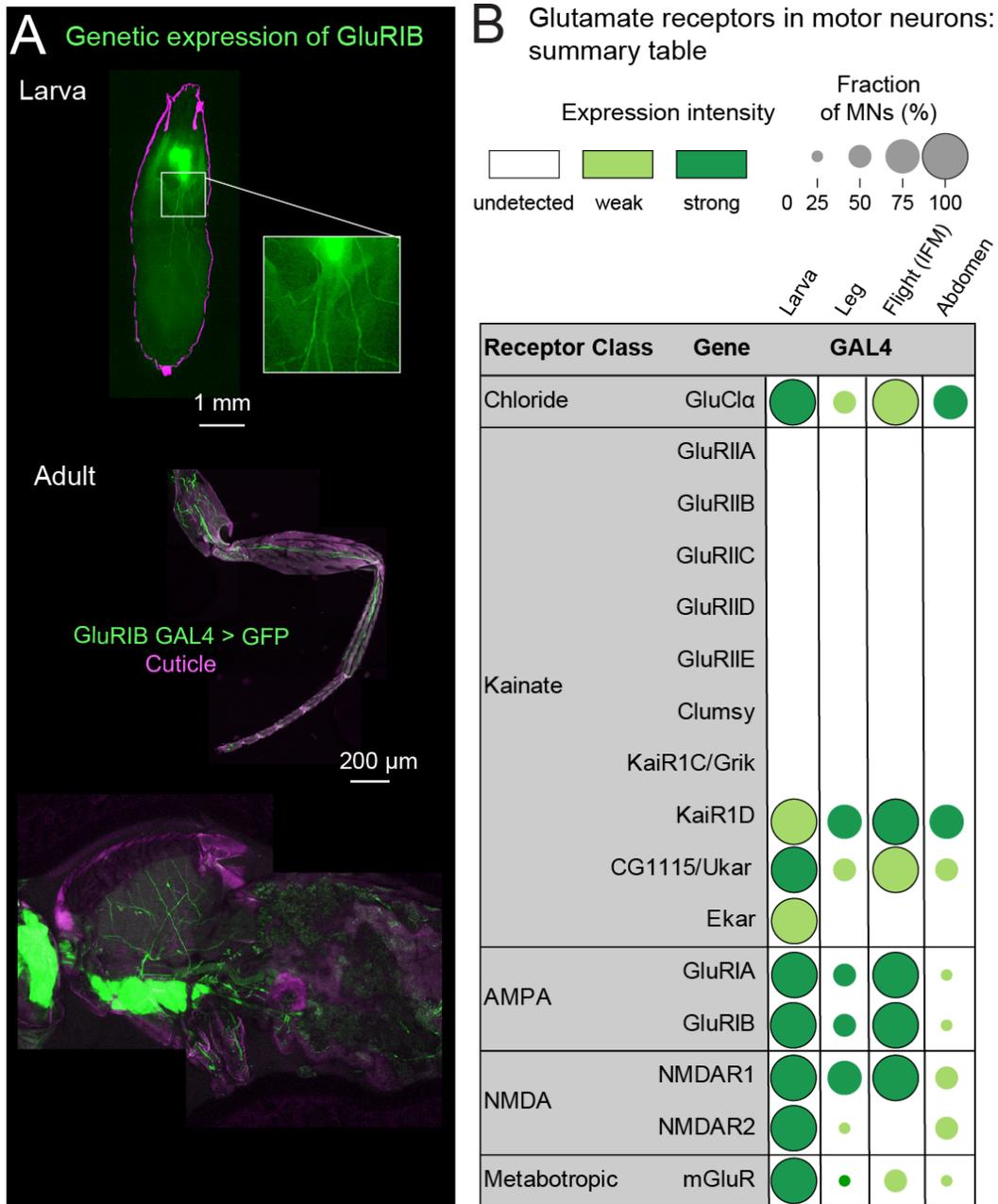


Figure s13. (A) Representative images showing expression of one example glutamate receptor subunit (GluRIB-GAL4, green) in larval and adult fly neurons. Motor neurons are visible as axons leaving the central nervous system. (B) Summary table of Gal4 expression in motor neurons.

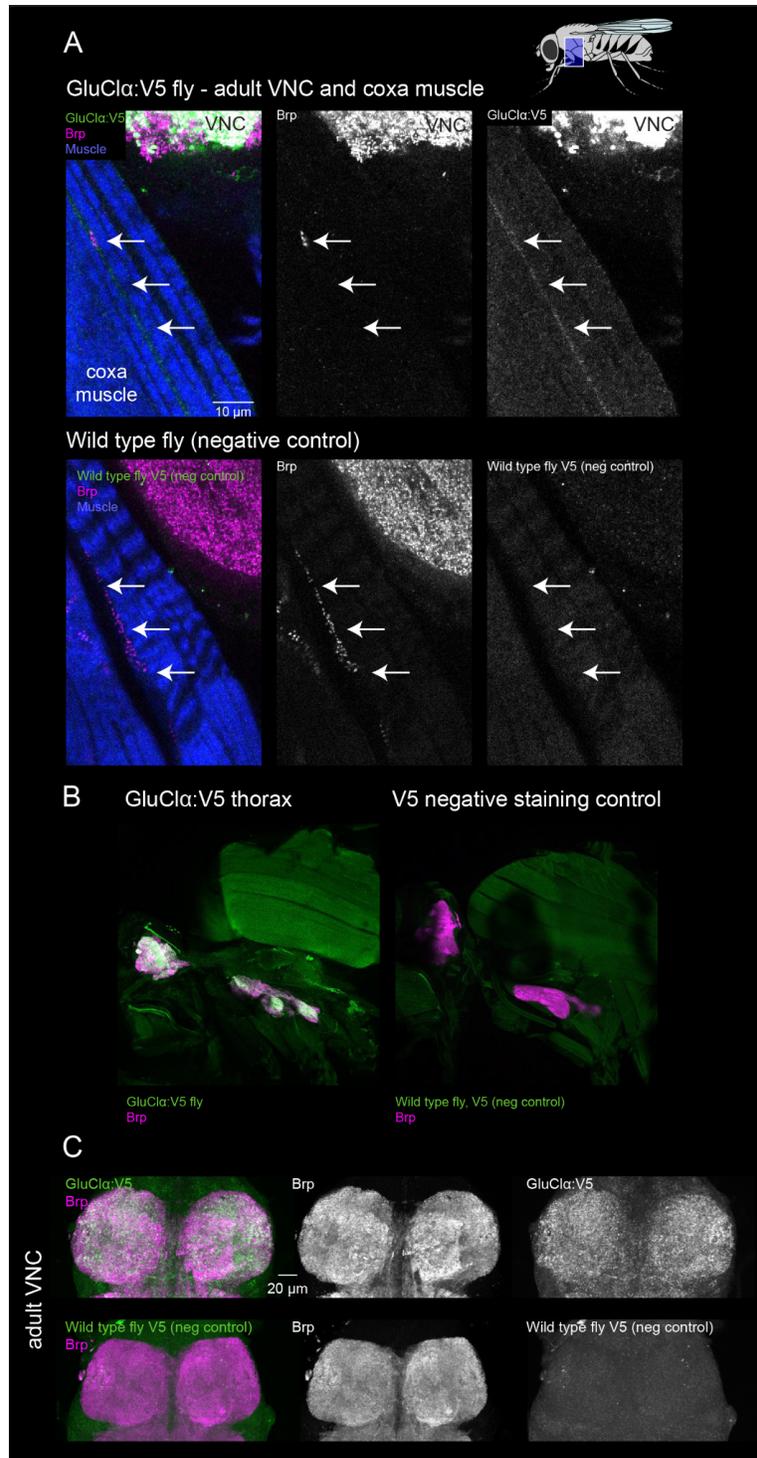


Figure s14. Validation of GluClα:V5 staining, showing positive controls (V5 staining in GluClα:V5 tagged flies) and negative controls (V5 staining in wild type flies, no tag). (A) Ventral nerve cord with nearby leg coxa muscle. Anti-V5 (green) localizes to the extrasynaptic muscle space (arrows) in the top fly (GluClα:V5) but not the bottom fly (wild type) (B) V5 staining positive and negative controls in the fly thorax. (C) V5 staining positive and negative controls in

central nervous system. Antibody against V5 (green), antibody against Brp (magenta), and muscle (phalloidin, blue).