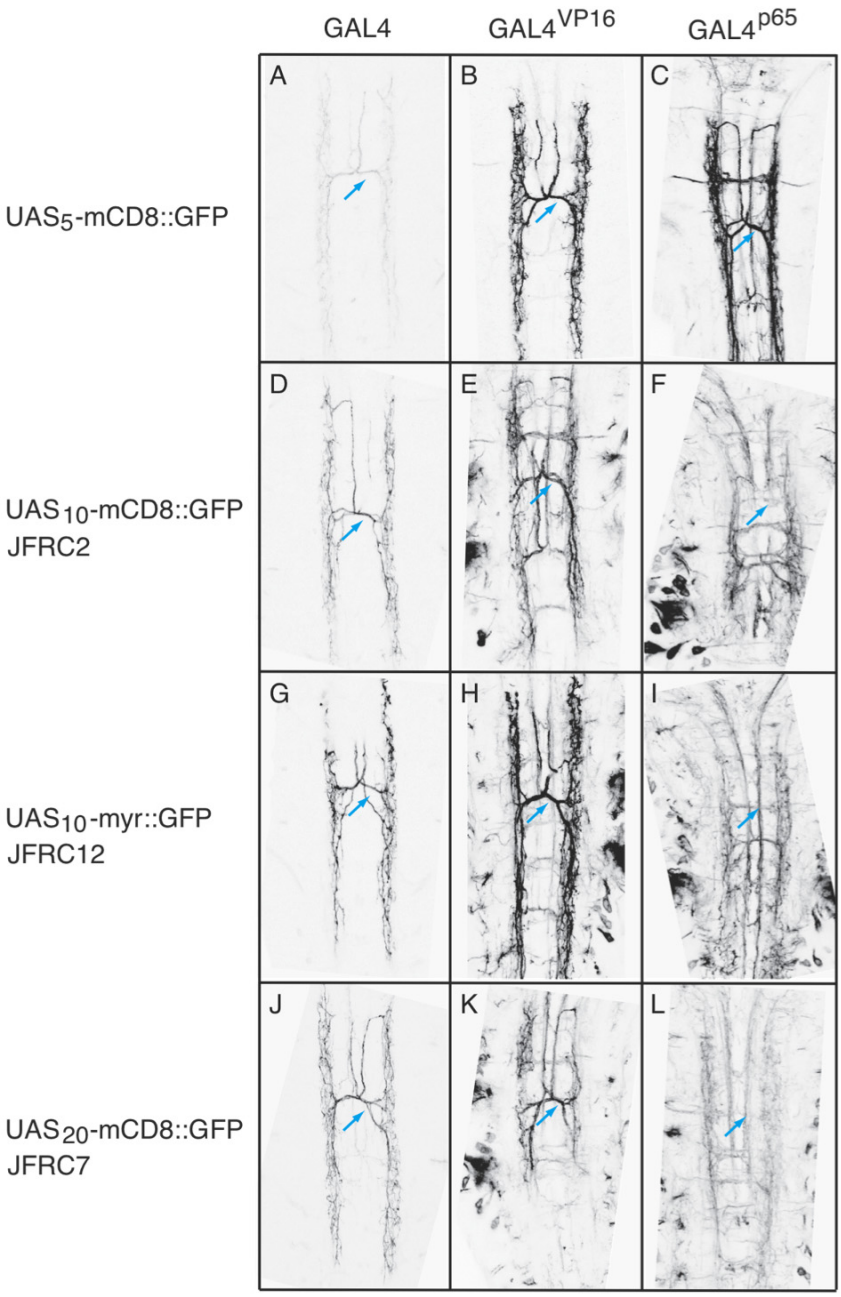

FIGURE S2.—Cell damage caused from excessive expression of responder proteins. Drosophila third instar larvae were immunostained for GFP and a dorsal view of the thoracic VNC is shown. With the exception of the 5XUAS-mCD8::GFP (LEE and LUO 1999) construct all transgenes are integrated into *attP2*. CRM R9C11 was used to direct expression of three GAL4 variants: standard GAL4 (as used in the constructs described by PFEIFFER *et al.* 2008), GAL4.2::VP16, or GAL4.2::p65. These three GAL4 drivers were crossed to different responders as indicated, which vary in number of UAS sites and localization tag: (A-C) 5XUAS-mCD8::GFP of LEE and LUO (1999). (D-F) 10XUAS-mCD8::GFP (pJFRC2). (G-I) 10XUAS-myr::GFP (pJFRC12): myristoylated, codon-optimized GFP. (J-L) 20XUAS-mCD8::GFP (pJFRC7). CRM R9C11 drives strong expression in a pair of medial thoracic interneurons; each sends a primary neurite across the midline that then bifurcates (arrow) to produce prominent anterior- and posterior-directed arbors. These arbors look normal in all cases when the driver is GAL4. With the VP16 activation domain, neurons that were apparently below our detection threshold with the GAL4 driver begin to become obvious using the pJFRC responders and expression in the medial interneurons is diminished in the 20XUAS relative to the 10XUAS responder. With the p65 activation domain, the line with the 5XUAS driver appears normal, but with higher UAS copy number (F,I,L) the large medial interneuron is no longer evident [arrows point to its expected location] and the expression in the formerly weak neurons is quite prominent.