

## Supporting Information

# Monitoring the Heavy Metal Lead Inside Living *Drosophila* with a FRET-Based Biosensor

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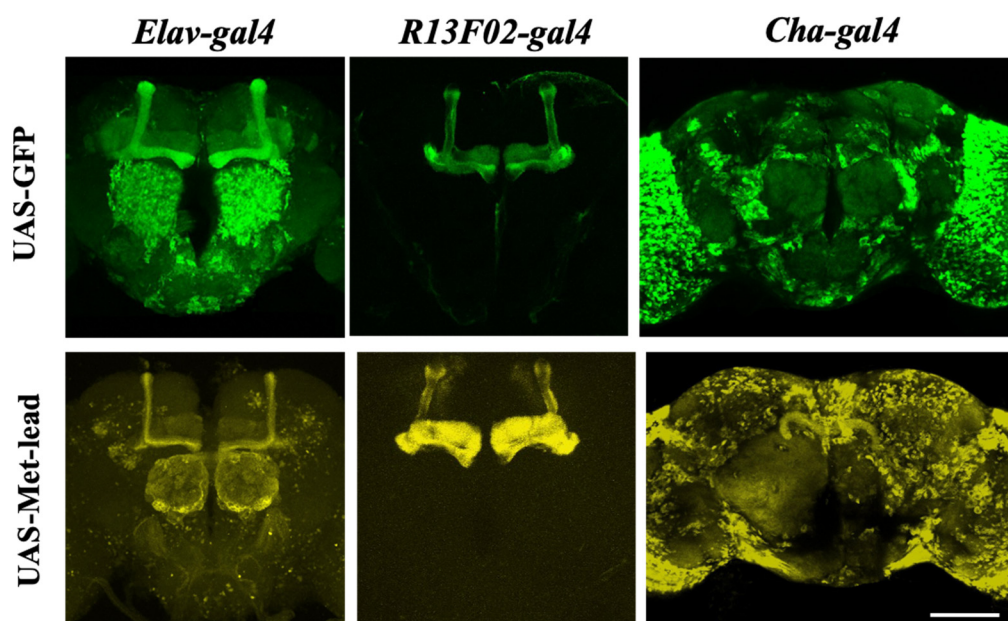
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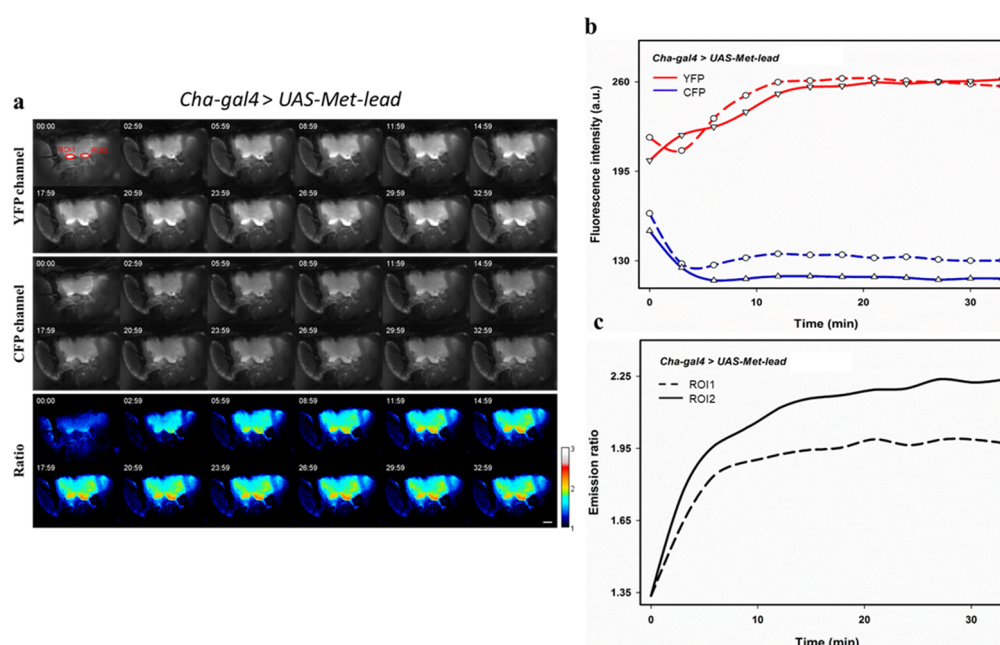
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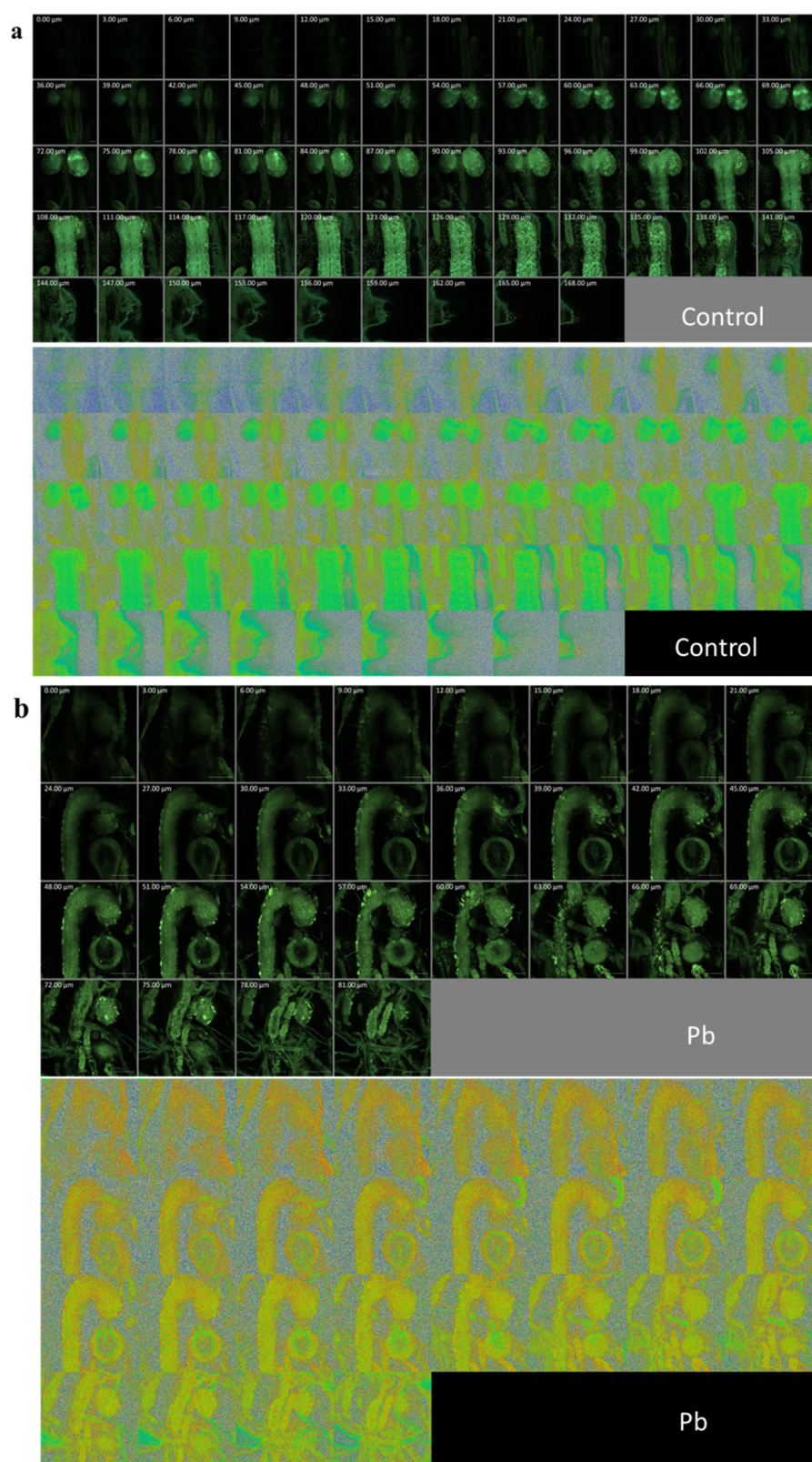
# Co-first author to this work.



**Supplementary Figure S1.** Expression pattern of GFP and Met-lead on adult *Drosophila* brain. Different types of neurons expressing GFP (upper part, e.g., *Elav-gal4* > *UAS-GFP*; *R13F02-gal4* > *UAS-GFP*; and *Cha-gal4* > *UAS-GFP*) or expressing Met-lead (lower part, e.g., *Elav-gal4* > *Met-lead*; *R13F02-gal4* > *UAS-Met-lead*; and *Cha-gal4* > *UAS-Met-lead*) were taken under confocal microscope for 3D imaging. The scale bar is 50  $\mu\text{m}$ .



**Supplementary Figure S2.** Practical applications of Met-lead on adult *Drosophila* brain with lower magnification (4x, compared with Figure 4 in 20x). (a) The in vivo lead bio-sensing of *Drosophila* (*Cha-gal4* > *UAS-Met-lead*) was carried out under an upright FRET image platform. During a 30 sec recording, a buffer containing lead ions (10  $\mu\text{M}$ ) and ionomycin (5  $\mu\text{M}$ ) was added. The representative fly brain (cholinergic neurons) images of YFP channel (12 graphs numbered with the time point in min, upper), CFP channel (middle), and ratio (YFP/CFP, bottom) are shown. The rainbow color images were transformed through a ratio image process. (b,c) The time-lapse plots from selected regions are displayed as fluorescent intensities, FIs (YFP in red lines; CFP in blue lines (b)) and emission ratios (c). In panel (a), the scale bar is 50  $\mu\text{m}$  and the rainbow color ratio bar is from 1 to 3.



**Supplementary Figure S3.** The CY montage images of Met-lead within larva CNS of *Drosophila*. Control (upper) and lead (lower) CNS are shown in different optical sections in RG merged manner or in ratio color one (ratio color bar from  $-0.5$  to  $2.8$ ).