



Supplementary Figure 1. Expression of *SAD-ΔG-mCherry-EnvA* in recorded and non-recorded mouse brains. **a,b** Maximum intensity projections (red channel only) of 5 consecutive 100 μm slices from two brains in which a single layer 5 cell was recorded. mCherry signal was amplified using DsRed antibody (see methods) in panel a, while panel b shows native fluorescence. Mice were sacrificed 13 and 15 days (panel a), and 6 and 8 days (panel b) after virus injection and recording respectively. **c,d** Maximum intensity projections of consecutive slices (spanning 500 μm of tissue) from two mice that received injection of *SAD-ΔG-mCherry-EnvA* only. In these animals the volume of virus injected (500 nl) was more than double of that used for retrograde labeling. Control mice were sacrifice 4 days following virus injection. Scale bars = 250 μm.