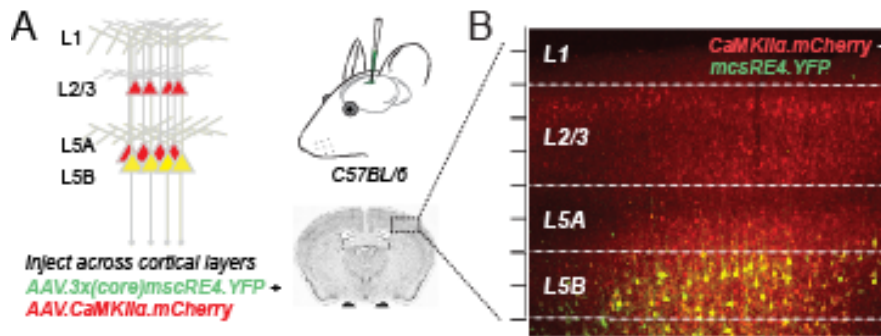


**Cell Reports Methods, Volume 4**

**Supplemental information**

**An enhancer-AAV approach selectively targeting  
dentate granule cells of the mouse hippocampus**

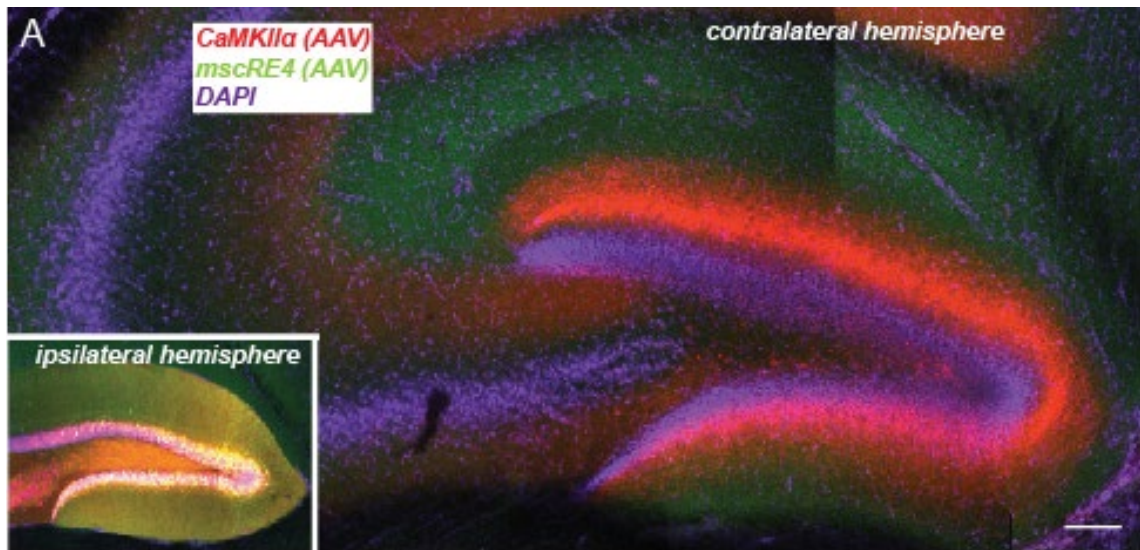
**Emmie Banks, Claire-Anne Gutekunst, Geoffrey A. Vargish, Anna Eaton, Kenneth A. Pelkey, Chris J. McBain, James Q. Zheng, Viktor Janos Oláh, and Matthew J.M. Rowan**



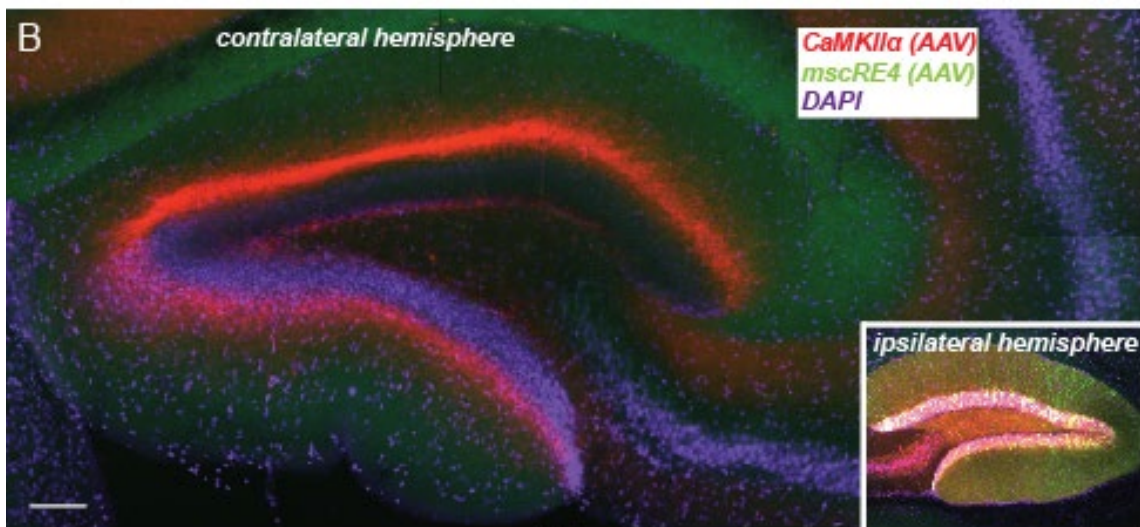
Supplemental Figure 1.

**AAV.3x(core)mscRE4.YFP labels layer 5B pyramidal neurons in primary motor cortex.**

(A) AAV.3x(core)mscRE4.YFP and AAV.CaMKII $\alpha$ .mCherry were co-injected into one hemisphere of the primary motor cortex (M1) in adult wild-type mice. (B) AAV.3x(core)mscRE4.YFP expression was directly mainly in layer 5B pyramidal neurons in M1, as indicated by co-expression of AAV.3x(core)mscRE4.YFP (green) and AAV.CaMKII $\alpha$ .mCherry (red). Scale between hash marks: 50  $\mu$ m. Related to Figure 1.



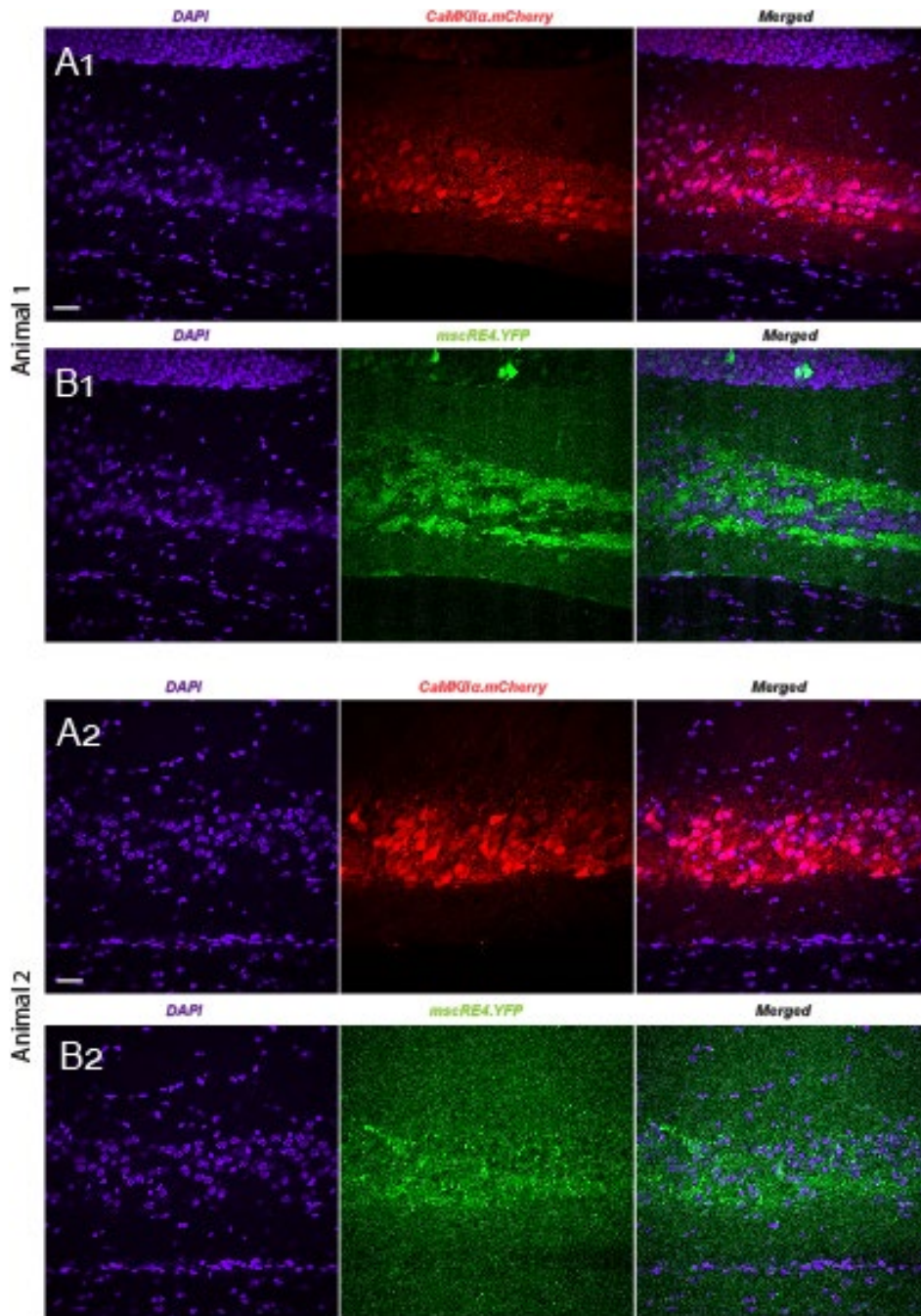
Animal 1



Animal 2

Supplemental Figure 2.

**AAV.3x(core)mscRE4.YFP and AAV.CaMKII $\alpha$ .mCherry labeling in contralateral DG hemispheres.**  
 (A, B) Representative confocal images (10x objective) of the contralateral (non-injected) dentate gyrus from two wild-type mice injected with AAV.3x(core)mscRE4.YFP and AAV.CaMKII $\alpha$ .mCherry in the ipsilateral dentate gyrus (insets). Scale bars: 100  $\mu$ m. Related to Figure 2.

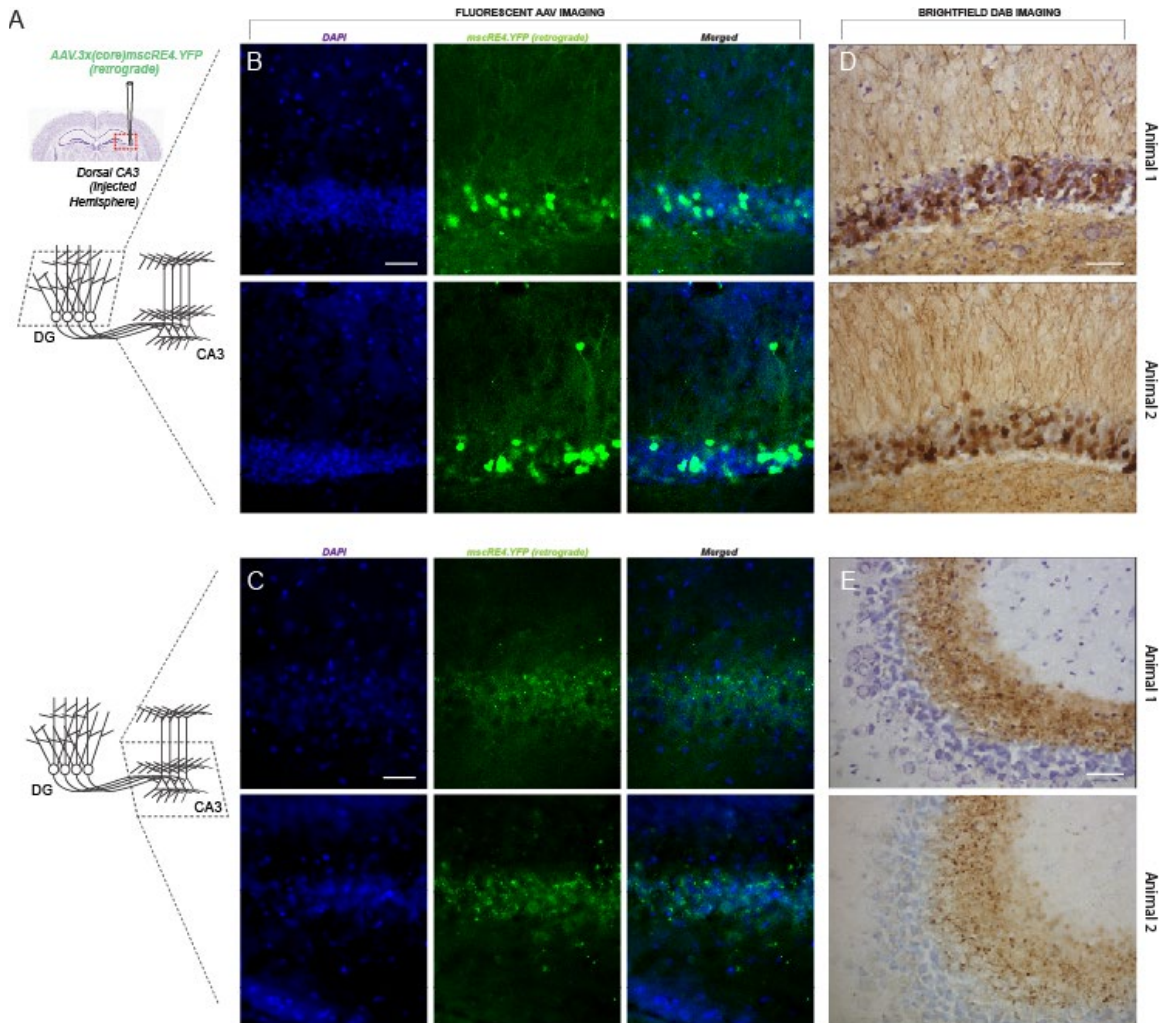


Supplemental Figure 3.

**AAV.3x(core)mScRE4.YFP expression in mossy fibers but not in CA3 pyramidal neurons.**

(A1, A2) Representative confocal images (40x objective, oil) of the CA3 region from two wild-type mice injected in one hemisphere dentate gyrus with AAV.3x(core)mScRE4.YFP and AAV.CaMKII $\alpha$ .mCherry, notable for the overlap between the DAPI (blue) and AAV.CaMKII $\alpha$ .mCherry (red) signals compared to (B1, B2) the lack of overlap between the signals for DAPI and AAV.3x(core)mScRE4.YFP (green). Scale bars: 50  $\mu$ m. Related to Figure 3.

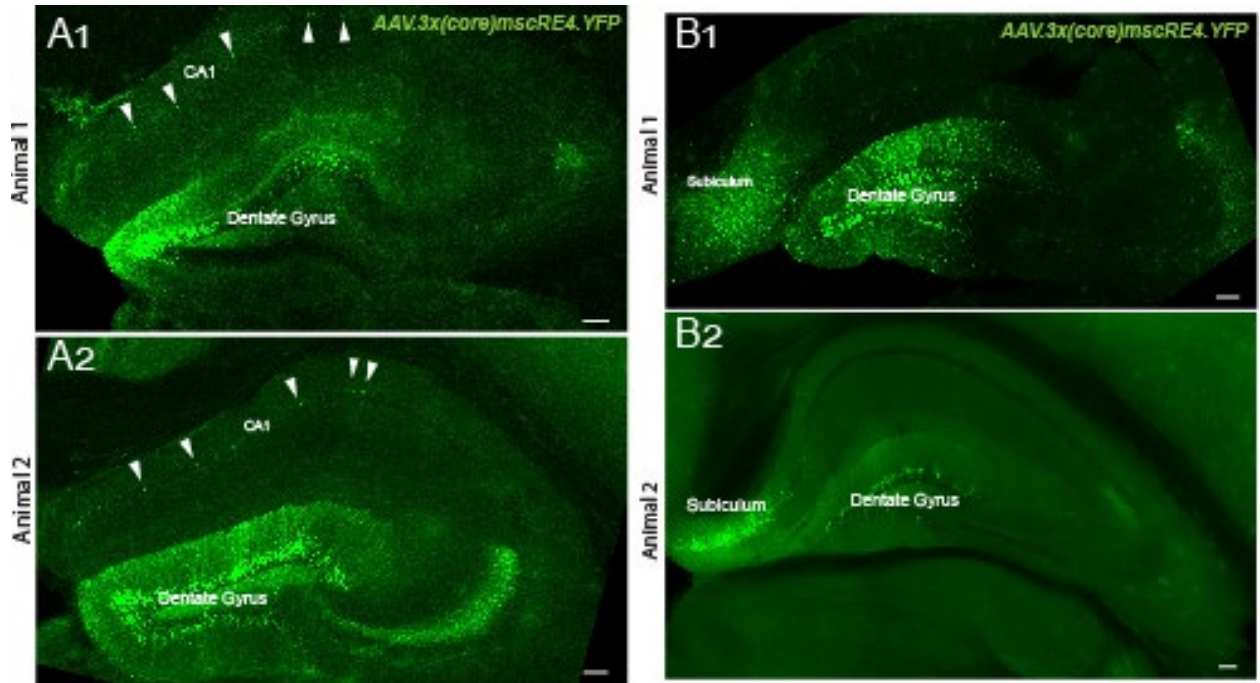




Supplemental Figure 4.

**AAV.3x(core)mscRE4.YFP (retrograde) produces a similar expression pattern to anterograde AAV.3x(core)mscRE4.YFP.**

(A) The YFP-expressing mscRE4 construct was packaged into a retrograde capsid and injected at the CA3b/CA3a border in adult wild-type mice. After 1-2 weeks, fluorescent images (40x objective) (B) in the DG showed YFP expression in granular layer cell somas and dendrites in the molecular layer, while (C) CA3 showed punctate YFP expression surrounding, but not overlapping with, stratum pyramidale cell somas. Brightfield images (40x objective) of slices which received immunostaining against GFP show abundant dark brown reaction products in (D) DG granular layer somas and molecular layer dendrites, as well as (E) punctate expression in CA3. Scale bars: 50  $\mu$ m. Related to Figure 2.



Supplemental Figure 5.

**AAV.3x(core)mscRE4.YFP expression in CA1 and the subiculum.**

(A1, A2) Representative confocal images (10x objective, dry) from two wild-type mice injected in the dentate gyrus in both hemispheres with AAV.3x(core)mscRE4.YFP, showing sparse YFP expression in putative pyramidal neurons (white arrows) in the CA1 region, as well as (B1, B2) dense YFP expression in the subiculum. Scale bars: 100  $\mu$ m. Related to Figure 2.