Supplemental information of the manuscript:

**Molecular and morphological configuration for 2-arachidonoylglycerol-mediated retrograde signaling at mossy cell-granule cell synapses in the dentate gyrus**

**(Abbreviated title: 2-AG signaling in the dentate gyrus)**

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Figure legends

Supplemental Figure S1. Production of MGL knockout mice by the Cre/loxP recombination system. A, Scheme of the knockout strategy. Homologous recombination of the targeting vector with the endogenous Mgll locus results in the insertion of the neo cassette (Neo) and loxP (black triangles) sequences into introns 2 and 3 of the Mgll gene. After homologous recombination in ES cells followed by germ-line transmission (Targeted genome), the floxed mice were bred to “Cre-deleter” mice to delete exon 3 (Ex3) and neo cassette from germ-line (Mgll). The residues (S122, D239, H269) predicted to form the catalytic triad are marked with arrowheads. Frt sites are indicated by open half-circles. E, EcoRI; A, Afl II; Ex4, exon 4; DT, diphtheria toxin gene. B, Southern blot analysis using the outside probes (5’ and 3’) of the targeting vector and neo probe. Proper targeting is represented by EcoR I-digested genomic DNA hybridized with the 5’ or 3’ probe, and Afl II-digested genomic DNA hybridized with the neo probe. WT, wild-type. C, Southern blot analysis using the 5’ probes shows that after Cre-mediated excision of exon 3, the 7.7 kb WT band (WT allele) in Afl II-digested genomic DNA shifts to 6.9 kb (KO allele). Het, heterozygous; KO, knockout. D, Genotyping PCR using tail samples from WT and Het and MGL-KO mice. E, Isotopic in situ hybridization for MGL mRNA in WT and MGL-KO mice. Note complete loss of hybridizing signals in MGL-KO mice. F, Immunoblot using protein samples from the brain in WT and MGL-KO mice. MGL signal is completely abolished in MGL-KO mice, while actin expression is comparable in each genotype.

Supplemental Figure S2. Specificity of fluorescent in situ hybridization (FISH) for DGLα and MGL mRNAs. A, C, E, G, parasagittal sections of whole brains: B, D, F,
H, magnified images of the hippocampus formation. A-D, FISH for DGLα mRNA in wild-type mice (WT) with two non-overlapping riboprobes antisense to 2176-3220 bp (A, B) and 331-1020 bp (C, D) of DGLα cDNA. Note identical signal patterns with use of the two antisense riboprobes. E-H, FISH for MGL mRNA in WT (E, F) and MGL-knockout (KO, G, H) mice. Substantial reduction of FISH signals are noted in MGL-KO mice. Residual signals in MGL-KO mice may reflect the expression and detection of truncated MGL mRNA lacking nucleotide residues encoded by the exon 3.

Scale bars: A, C, E, G, 1 mm; B, D, F, H, 200 µm.
Uchigashima et al., Supplemental Figure S2