Supporting Information

Matsuno *et al.* 10.1073/pnas.0807665106

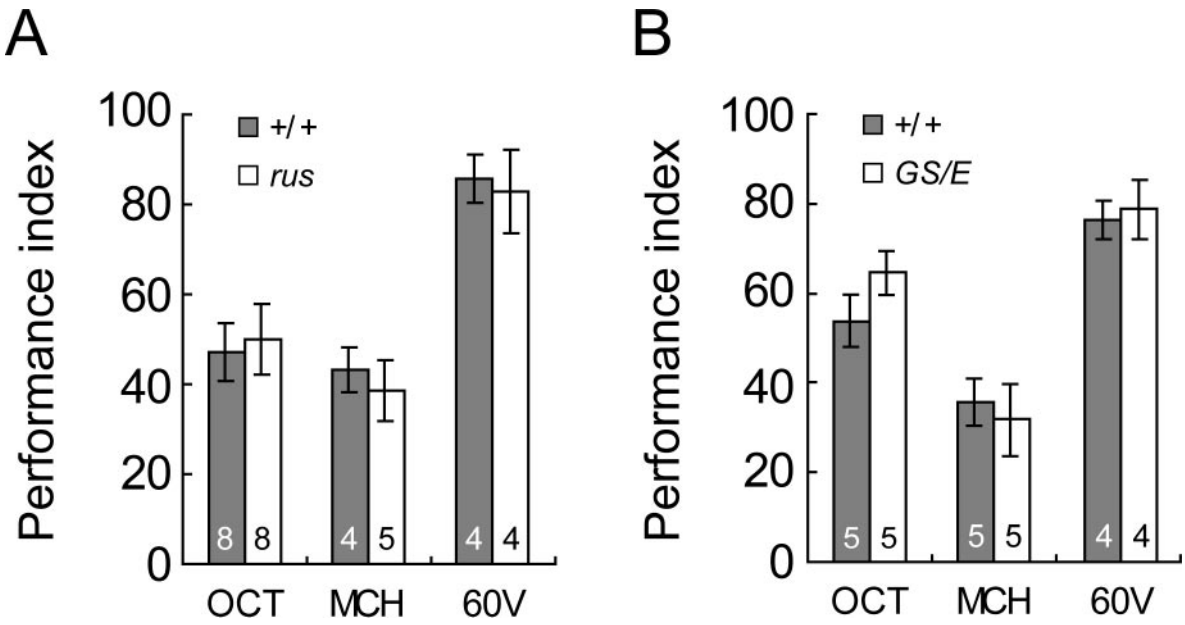

Fig. S1. Sensorimotor responses are not disrupted by *klg* mutations. Sensorimotor responses are not impaired in klg^{rus} (*A*) and in $klg^{GS10439}/klg^{E226}$ (*B*) flies. Avoidance of odors and 60V electrical shocks, which were used for olfactory conditioning, are indicated. OCT, octanol; MCH, 4-methylcyclohexanol.

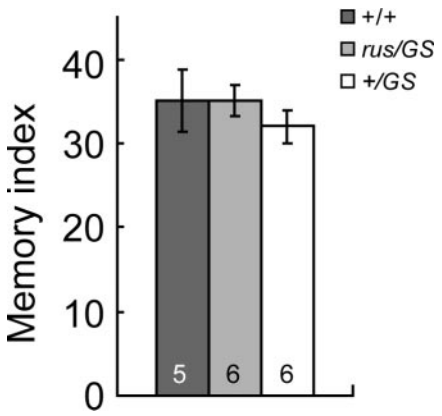

Fig. S2. The *klg*^{GS10439} mutation is recessive for memory defects. Both *klg*^{GS10439} and *klg*^{rus} (Fig. 1*E*) are recessive for memory defects. LTM is normal in *klg*^{rus}/*klg*^{GS10439} flies because the *klg*^{rus} mutation results from insertion of a P-GAL4 transposon, while the *klg*^{GS10439} mutation results from insertion of a P-UAS transposon. GAL4-dependent induction of *klg* expression rescues the LTM defects in *klg*^{rus}/*klg*^{GS10439} flies.

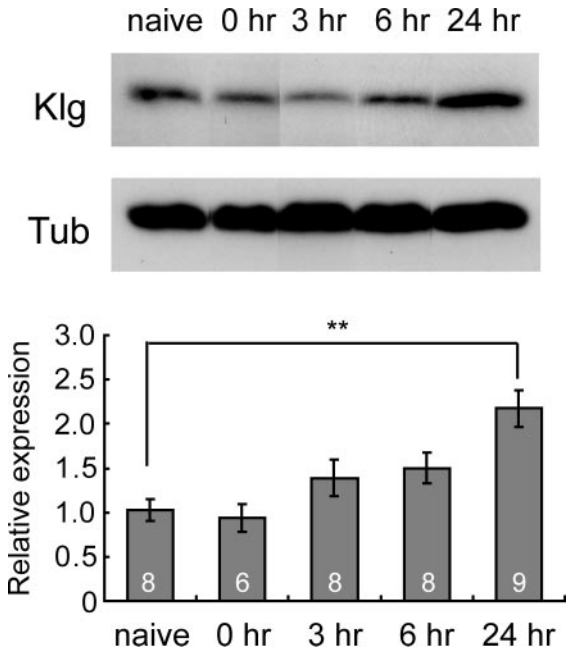

Fig. S3. Klg protein increases after spaced training. Klg protein amounts in head extracts were measured 0, 3, 6, and 24 h after spaced training. A gradual increase in Klg can be seen and this increase becomes significant at 24 h (**$P < 0.001$).

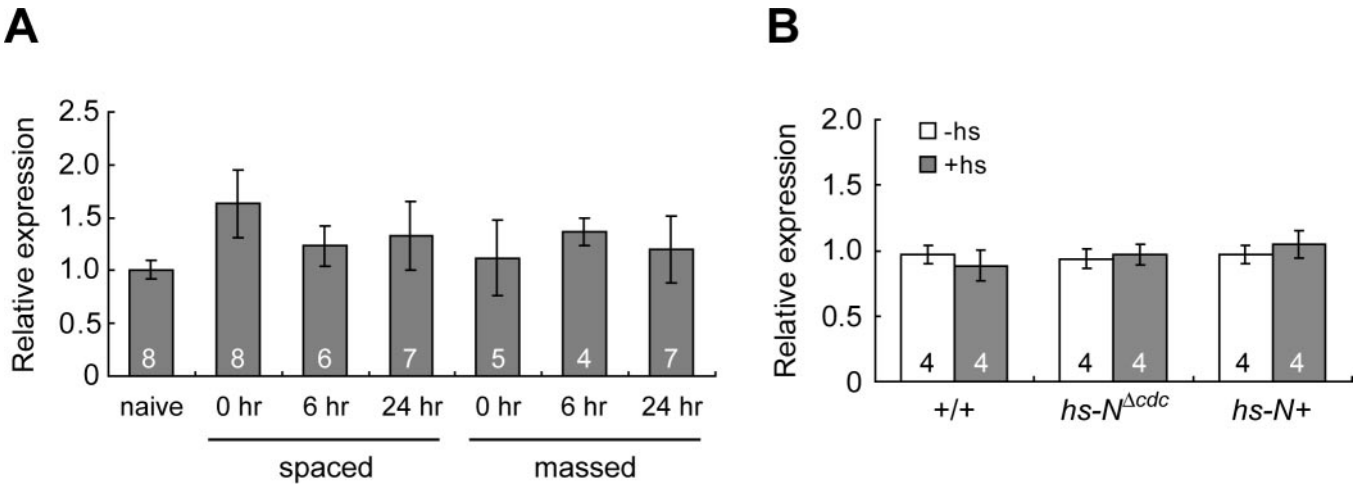

Fig. S4. *klg* mRNA levels do not increase upon LTM induction or elevation Notch activity. (*A*) Relative amounts of *klg* mRNA from fly heads harvested at 0, 6, and 24 h after spaced or massed training. In contrast to Klg protein, *klg* mRNA amounts were indistinguishable after spaced and massed training. Relative expression refers to the *klg* to *GAPDH1* ratio at each time-point. This ratio was normalized to 1.0 for naïve flies. (*B*) *klg* mRNA expression in fly heads did not change after decreasing (+hs in *hs-N*$^{\Delta cdc}$ flies) or increasing (+hs in *hs-N*$^{+}$ flies) Notch activity.

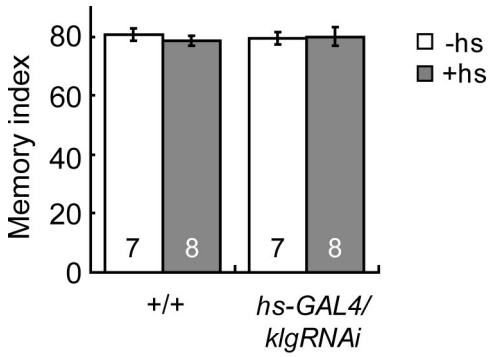

Fig. S5. Normal short-term memory in *hs-GAL4/klgRNAi* flies. RNAi mediated silencing of *klg* did not affect short-term memory (memory quantified 30 min after a single training session).

Table S1. Primer sequences used for semi-quantitative and q-PCR

	Upstream	Downstream
Semi-qPCR		
GPDH	5′-CCACTGCCGAGGAGGTCAACTA-3′	5′-GCTCAGGGTGATTGCGTATGCA-3′
GAL4	5′-GCCAATTTTAATCAAAGTGGGAATA-3′	5′-GTTTGGTGGGGTATCTTCATCATC-3′
CG31281	5′-GTCACCCACTTCACTCAATCAA-3′	5′-GCGAGCTAATGCTTTGCTATTT-3′
klg	5′-AACTCCGGAACCTAGAAACTCC-3′	5′-GATTACCAGATCCCTTGCACTC-3′
CG6660	5′-TTGGCTACCTCACCTTTCATTT-3′	5′-TTGCGTATGTAGGTCTTGCAGT-3′
rp49	5′-CACCGGATTCAAGAAGTTCC-3′	5′-GACAATCTCCTTGCGCTTCT-3′
q-PCR		
GAPDH1	5′-TCCTGGGCTACACCGATGA-3′	5′-GGCGTCGAACACAGACGAA-3′
klg	5′-GGAGTGCAAGGGATCTGGTA-3′	5′-GCTCCAGCTTCTCCAGTGTC-3′