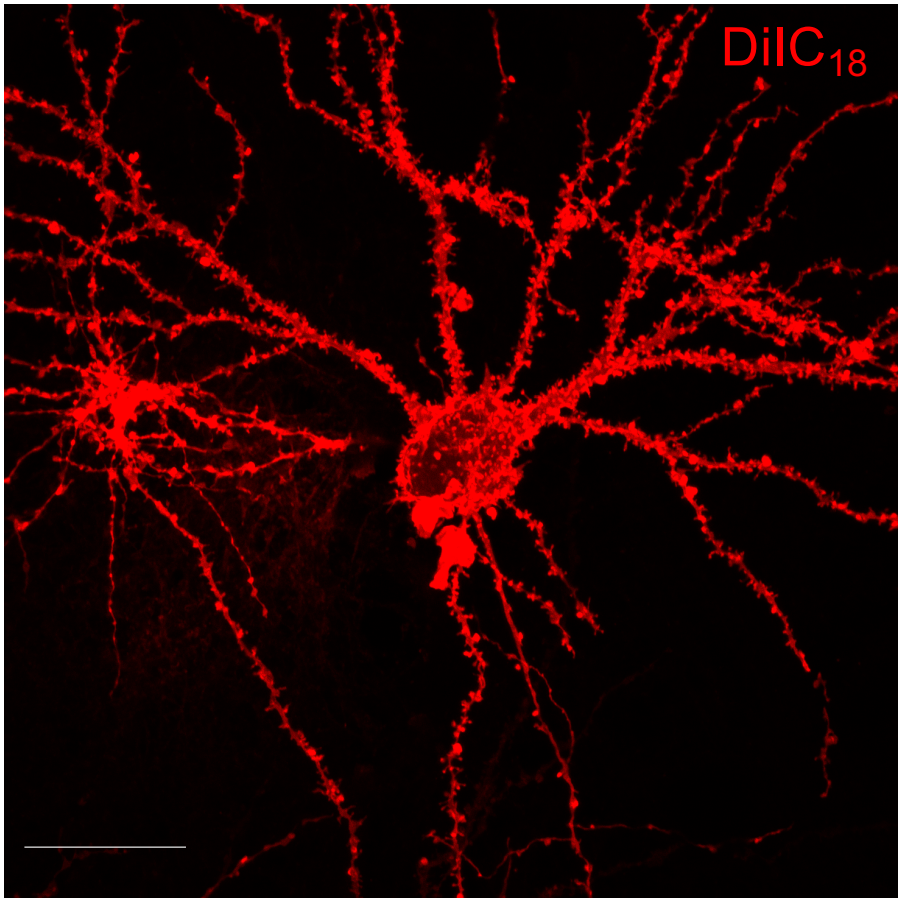

Figure S1. Representative confocal image of neurons in WT mouse hippocampus stained with $DiIC_{18}$; scale bar 25 μm.

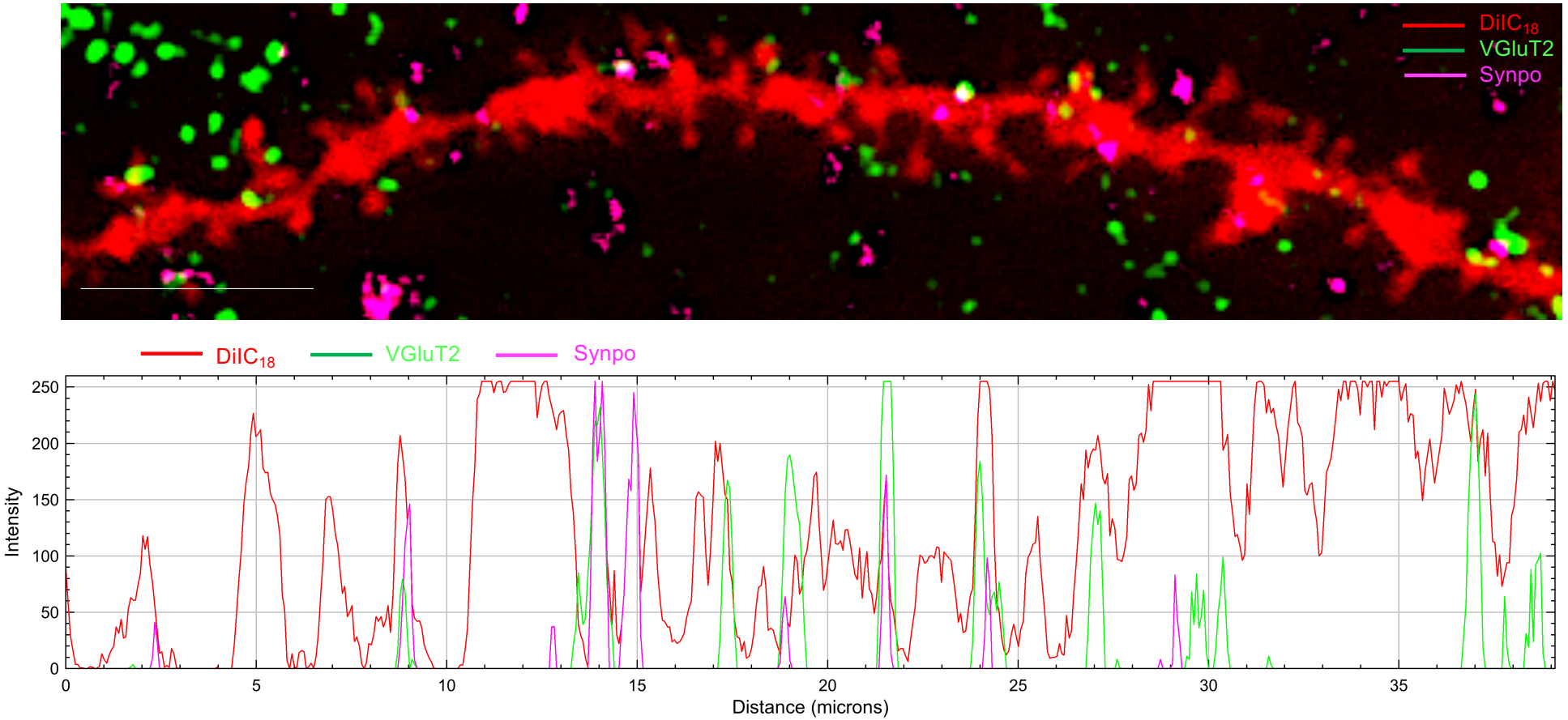

Figure S2. Representative line scan illustrating signal intensity for $DiIC_{18}$ (red), VGluT2 (green), and Synpo (Magenta) in function of distance. Scale bar, 5µm.

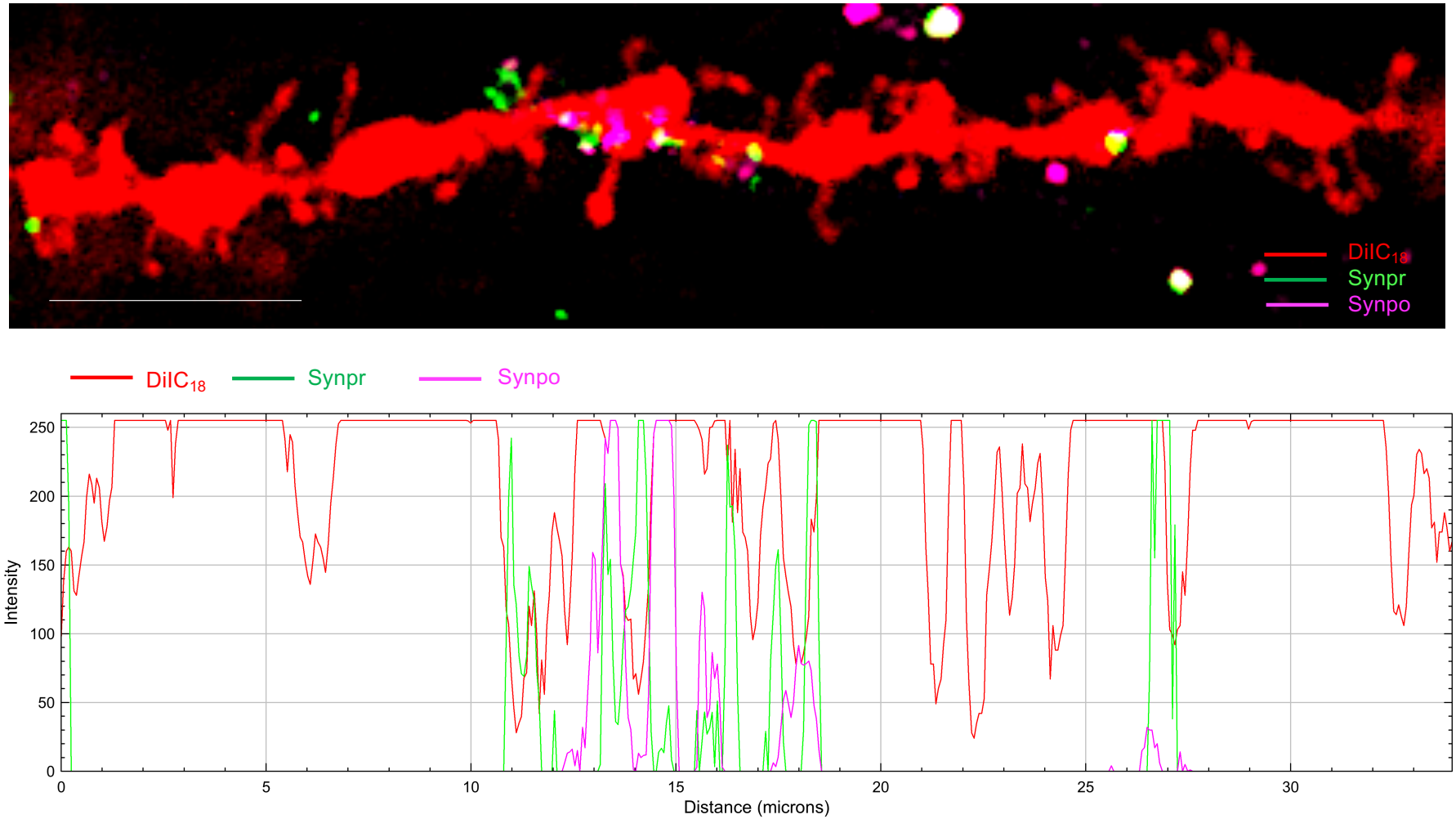

Figure S3. Representative line scan illustrating signal intensity for $DiIC_{18}$ (red), Synpr (green), and Synpo (Magenta) in function of distance. Scale bar, 5µm.

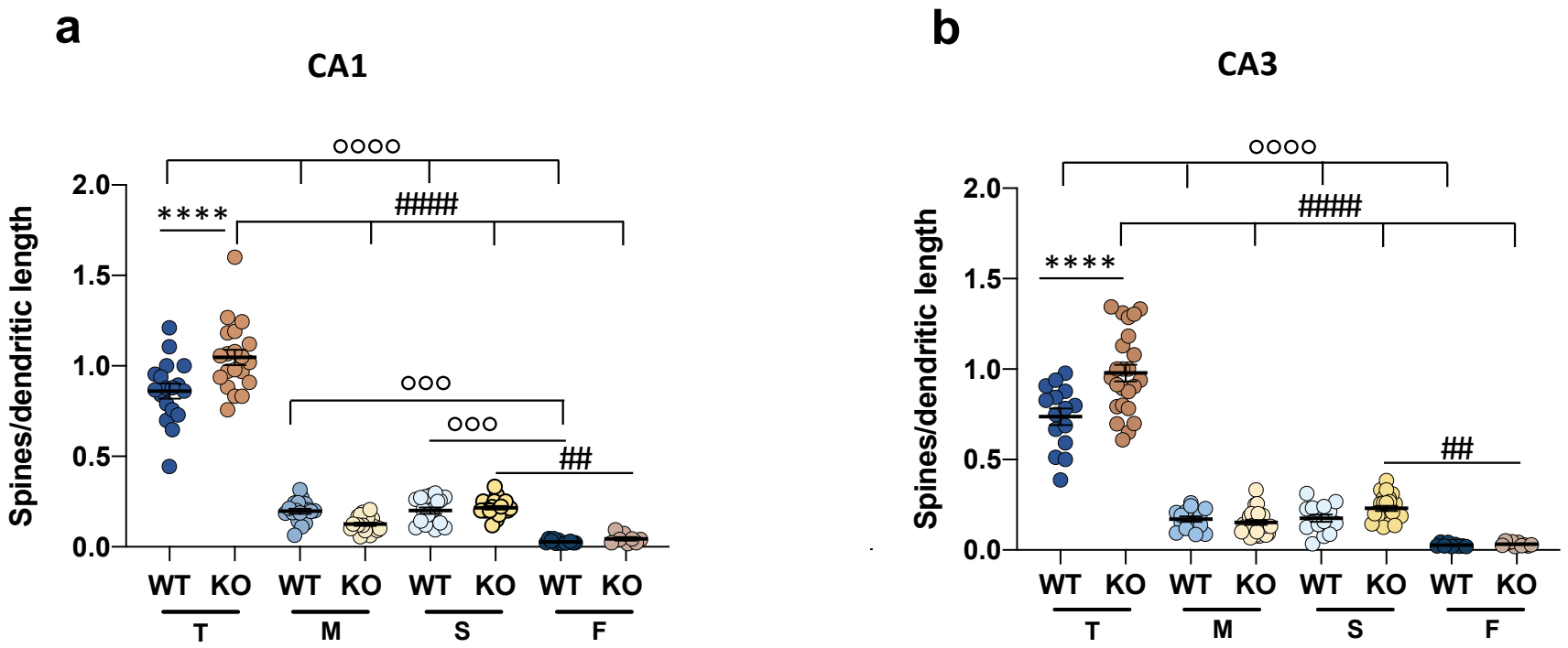

Figure S4. DiIC$_{18}$ staining reveals dysgenesis of hippocampal spines in juvenile Fmr1 KO mice. (**a**) Quantification of thin (T), mushroom (M), stubby (S), and filopodia (F) spines per dendritic length (μm) in CA1 of WT and Fmr1 KO mice. Differences were evaluated by two-way ANOVA followed by Tukey's post-hoc multiple comparisons test **** $p < 0.0001$ KO vs WT, °°°°$p < 0.0001$ WT vs WT, °°°$p \leq 0.0007$ WT vs WT ####$p < 0.0001$ KO vs KO, ##$p = 0.0044$ KO vs KO. A two-factor ANOVA demonstrated a significant effect of spine type ($p < 0.0001$) but a non-significant effect of genotype ($p = 0.057$), and the interaction of genotype by spine's type is significant ($p < 0.0001$). **(b)** Quantification of spines per dendritic length (μm) in CA3 of WT and Fmr1 KO mice. Differences were evaluated by two-way ANOVA followed by Tukey's post-hoc multiple comparisons test ****$p < 0.0001$, °°°°$p < 0.0001$ WT vs WT, ##$p < 0.0011$ KO vs KO. A two-factor ANOVA demonstrated a significant effect of genotype ($p = 0.0022$) and spine's type ($p < 0.0001$), and the interaction of genotype by spine's type is significant ($p < 0.0001$).