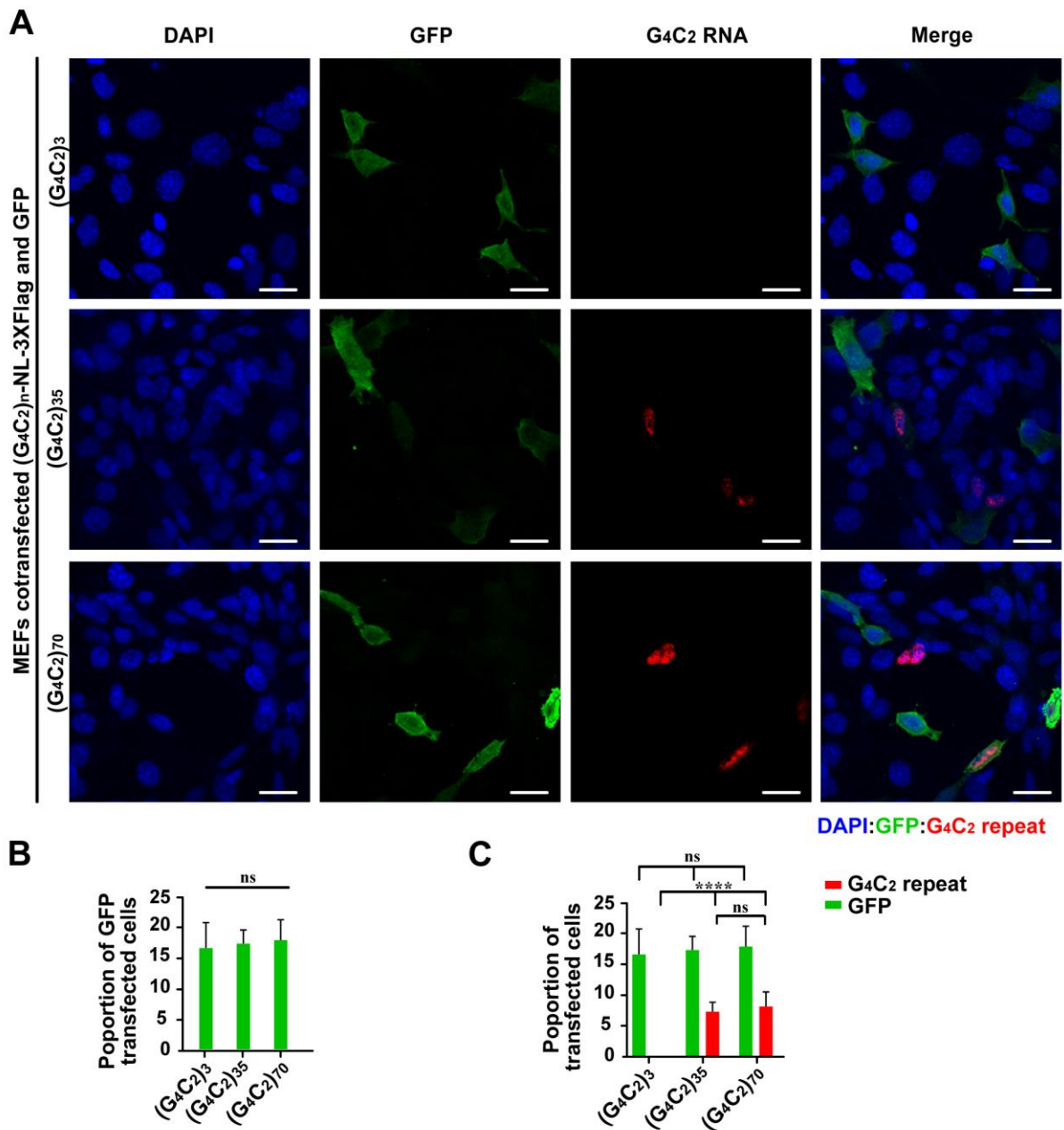
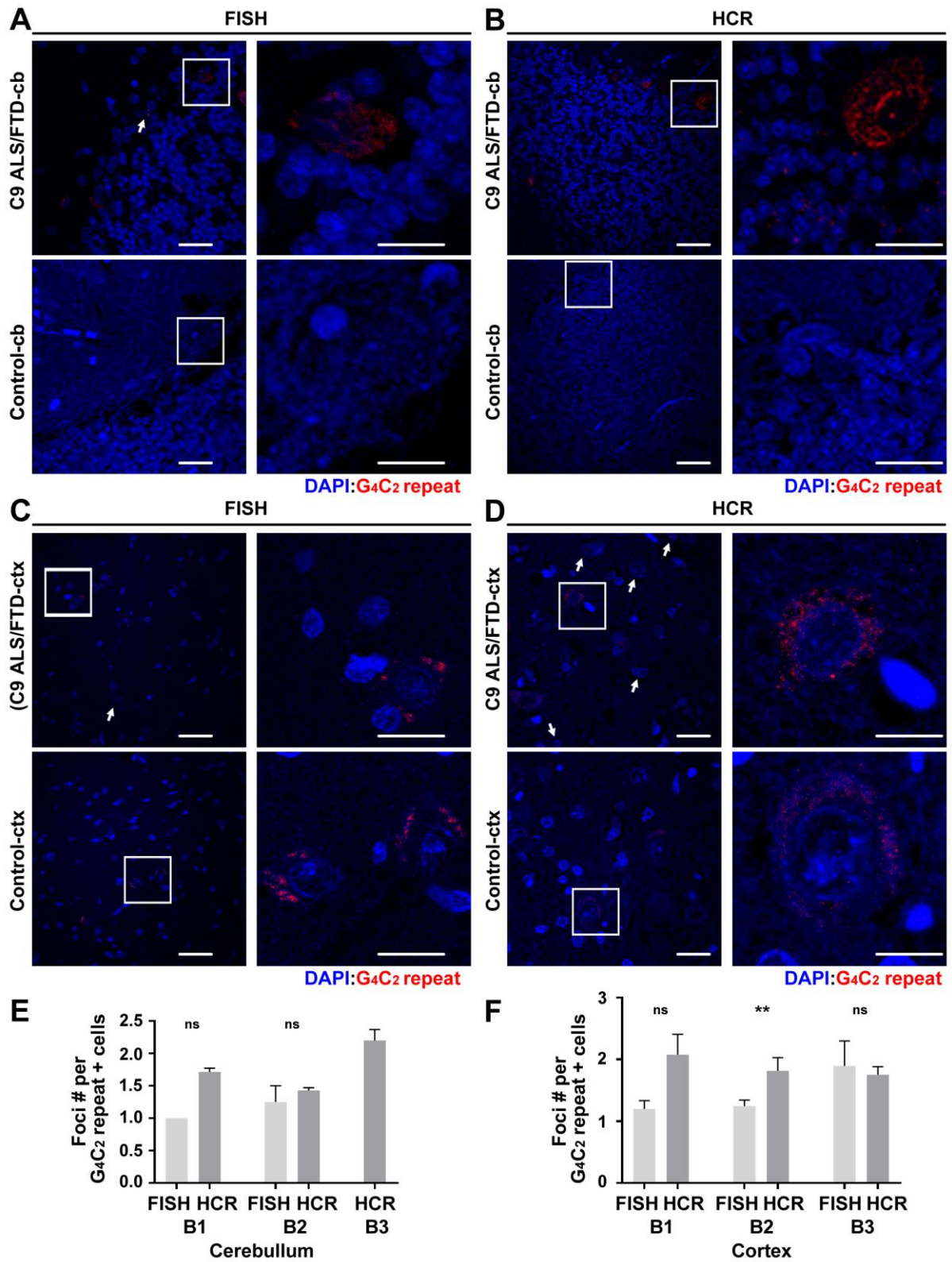


Supplemental Data:

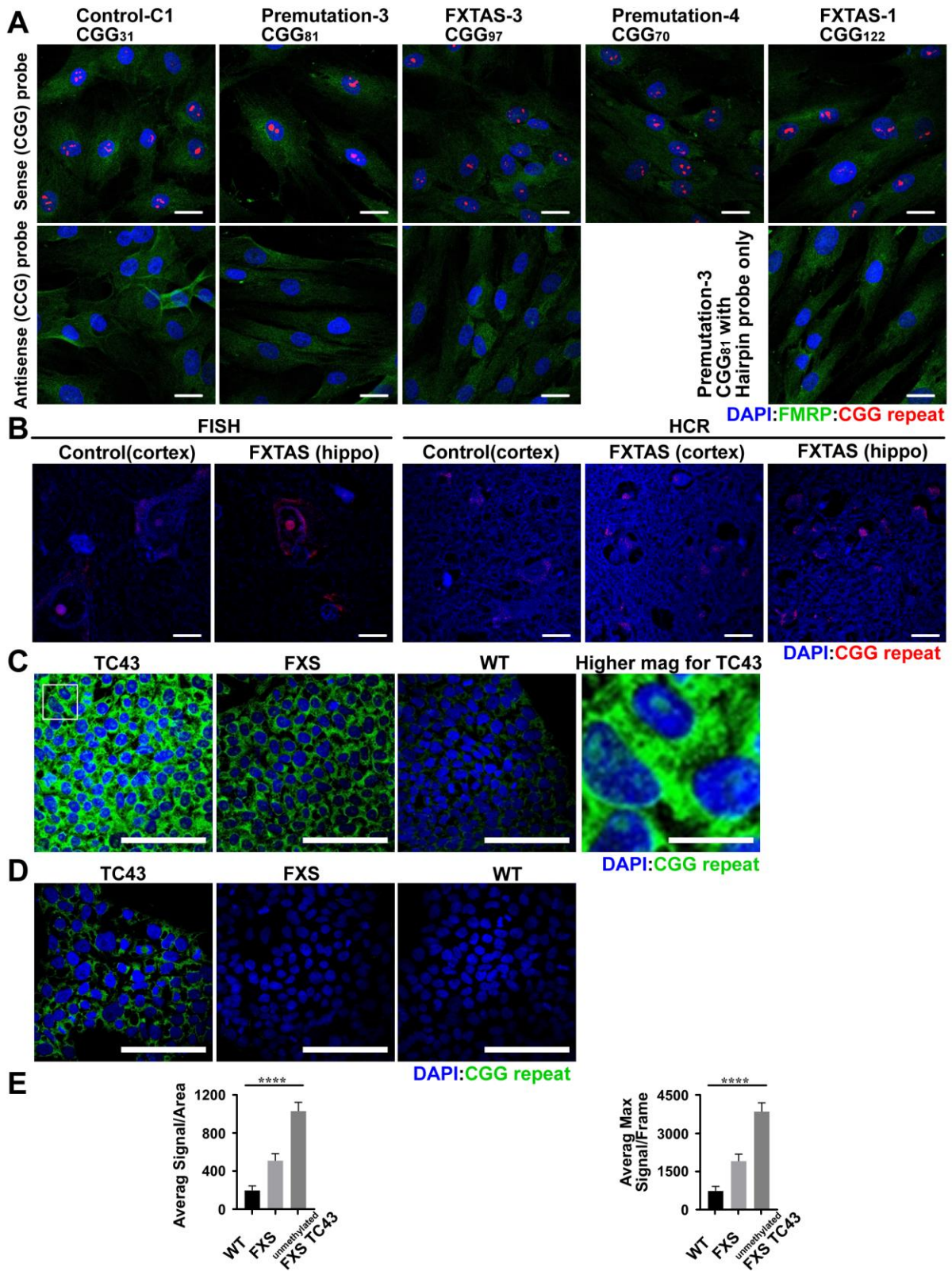


Supplemental Figure 1. Transfection efficiencies for MEFs co-transfected with GFP and G₄C₂ repeats **A)** R-HCR-ICC of MEFs co-transfected with GFP and G₄C₂ repeat constructs with increasing repeat size. **B-C)** Quantification of cells with detectable GFP and G₄C₂ repeat RNA foci signal in total cells. (G₄C₂)₃: N=313; (G₄C₂)₃₅ N=575; (G₄C₂)₇₀ N=514. Statistics: Chi-square test and Fisher's exact test (**B and C**). *****p*<0.0001, ns: not significant. Scale bar=50 μm in **A**.



Supplemental Figure 2.

Supplemental Figure 2 legend. Diffuse R-HCR and FISH staining in *C9orf72* ALS-FTD patient brains. A-D) FISH (A, C) and R-HCR (B, D) of *C9orf72* ALS-FTD patient and control cerebellum (A, B) and frontal cortex (C, D). White arrows indicate RNA foci positive cells in some images. Higher magnification images of the boxed areas are shown on the right. E-F) Quantification of RNA foci number per RNA foci + cells \pm SEM in cerebellum (E) and cortex (F). Statistics: unpaired t test for E and F. ** $p < 0.01$, ns: not significant. Scale bar = 50 μ m in left for A-D; 20 μ m in right for A-D.



Supplemental Figure 3.

Supplemental Figure 3 legend: Endogenous CGG repeat is highly expressed in human cells. A) R-HCR of FXTAS, permutation carrier and control fibroblasts. **B)** FISH and R-HCR of FXTAS patient and control brains. **C-D)** CGG R-HCR of iPSCs/hESCs derived from an unmethylated CGG expansion carrier (TC-43; 270 CGG repeats, transcriptionally active), fully methylated embryonic cells (FXS, 800 CGG repeats, transcriptionally silent) and a control patient (30 CGG repeats). Panel **C** is without background subtraction, demonstrating signal in all cells. Panel **D** is after subtraction of signal detected in FXS line, revealing significant signal above background only in the actively transcribed expanded CGG repeat line. **E)** Quantification of CGG repeat RNA foci signal in each cell line of figure **(C)** *left*: average signal/frame, *right*: maximum signal/frame. One-way ANOVA (**E**). $n=15$, **** $p<0.0001$. Scale bar=50 μm in A; 20 μm in B; 100 μm in **C** and **D** except for the higher mag picture of **C** (20 μm).