Analysis of the subcellular localization of neurodevelopmental disease-associated proteins using Breasi CRISPR

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Many rare neurodevelopmental diseases are caused by loss of expression of a single protein. Knowing the subcellular localization of these proteins in neurons during cortical development is important in helping our understanding of their roles in normal physiology and disease states. We used Breasi-CRISPR, which combines in utero electroporation (IUE) and CRISPR/Cas9 genome editing, to insert epitope tags in genes of interest in the developing cortex. This enables visualization of endogenous proteins in a subset of developing neurons, facilitating analysis of subcellular localization in situ. Here we have tagged FMRP (C-terminus), an RNA binding protein silenced in Fragile X Syndrome. Preliminary data indicate that FMRP is expressed in the soma and neurites of migrating neurons. We also tagged the light chain of MAP1B, which is mutated in periventricular nodular heterotopia. The MAP1B protein is cleaved into a heavy chain (N-terminus) and light chain (C-terminus). Preliminary data indicate that the MAP1B light chain is preferentially expressed in axons in developing neurons, but is also expressed in neurites of migrating neurons. We plan to expand upon these findings by using Breasi-CRISPR to tag proteins such as FMRP and MAP1B with fluorophores, enabling us to examine their localization via live imaging of brain slices.