

Fig. S1. Localization and expression of Breasi-CRISPR tagged proteins. (A) Fraction of cells in electroporated region positive for MYC-LMNB1 lacking GFP signal over the total number of MYC-LMNB1 positive cells in brains electroporated at E13.5 and collected at either E14.5, E15.5, or E18.5. (B) Cartoon outlining approach for C-N. Breasi-CRISPR IUE was performed at E13.5 followed by sample collection at E18.5 and histological analysis of coronal sections. (C-G) Overlap of MYC-LMNB1 signal with signal detected using an anti-LMNB1 antibody. (H-M) LMNB1 expression in the electroporated region of scramble gRNA control Breasi and Myc-Lmnb1 Breasi brains. (N) Quantification of LMNB1 signal in GFP positive cells normalized to GFP negative cells in scramble gRNA control Breasi and Myc-Lmnb1 Breasi brains. $n > 5$ brains from 2 different experiments for each condition. Data represents average and SD, two-way Student t-test. *: $p < 0.05$. (O) Cartoon outlining approach for P-W. Breasi-CRISPR IUE was performed at E13.5 followed by sample collection at E14.5 and histological analysis of coronal sections. (P-W) Overlap of signal detected with (Q) FMRP antibody or (R) FMRP-HA detected with HA antibody. (S-W) Inset of area defined in (P) of FMRP and FMRP-HA signal. Scale bar is $20\mu\text{m}$ for C-G, H-M, S-W, $100\mu\text{m}$ for P-R.

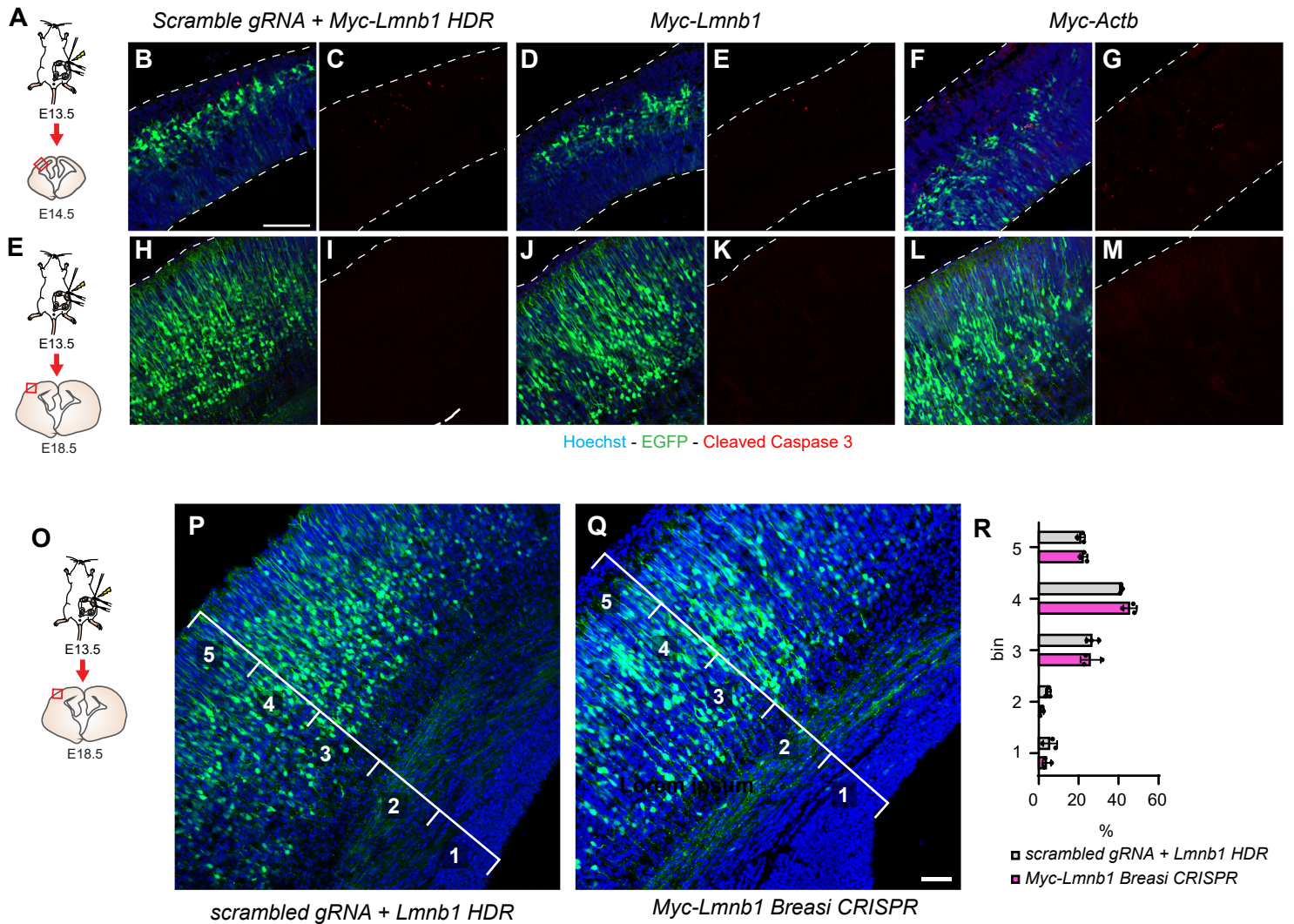
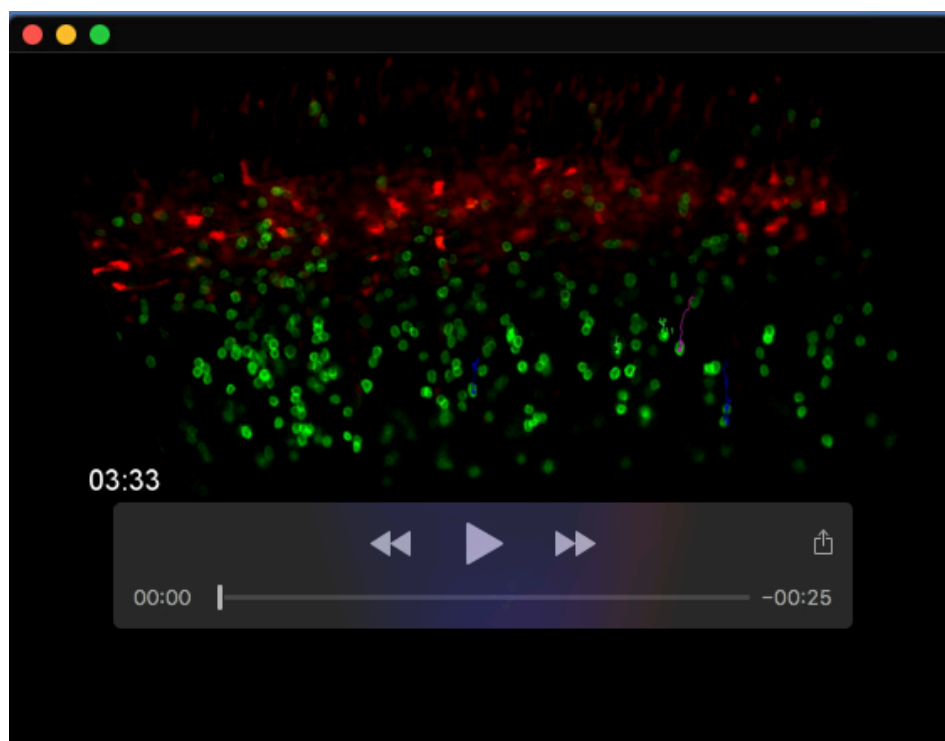


Fig. S2. Impact of Breasi-CRISPR directed endonuclease activity on apoptosis and the distribution of electroporated cells. (A) Cartoons outlining approach for B-G and H-M. Breasi-CRISPR IUE was performed at E13.5 followed by sample collection at E14.5 for B-G and E18.5 for H-M with histological analysis of coronal sections. (B-G) Representative confocal images of Cleaved Caspase 3 (red) indicating apoptotic cells in the electroporated region of (C) Scramble gRNA, (E) Myc-Lmnb1, and (G) Myc-Actb Breasi-CRISPR brains. (H-M) Representative confocal images of Cleaved Caspase 3 (red) indicating apoptotic cells in the electroporated region of (I) Scramble gRNA, (K) Myc-Lmnb1, and (M) Myc-Actb Breasi-CRISPR brains. (N) Cartoon outlining approach for O-Q. Breasi-CRISPR IUE was performed at E13.5 followed by sample collection at E18.5 and histological analysis of coronal sections. (P,Q) Visualization of bins used to assess migration of GFP positive cells in scrambled gRNA control and Myc-Lmnb1 Breasi-CRISPR brains. (R) Quantification of GFP+ cells distribution in embryonic cortices. n=3 brains from 2 different experiments for each condition. Data represents average and SD. A two-way ANOVA analysis showed no significant differences between conditions. Scale bar is 100µm for B-M and 50µm for O-P.

Table S1. Table providing the sequences of crRNAs and HDR ssODNs, together with antibodies used in this study.

[Click here to download Table S1](#)



Movie 1. Overnight live imaging of endogenous EGFP-tagged LMNB1 in an E15.5 organotypic brain slice, following Breasi-CRISPR IUE at E13.5. Time in hh:mm.