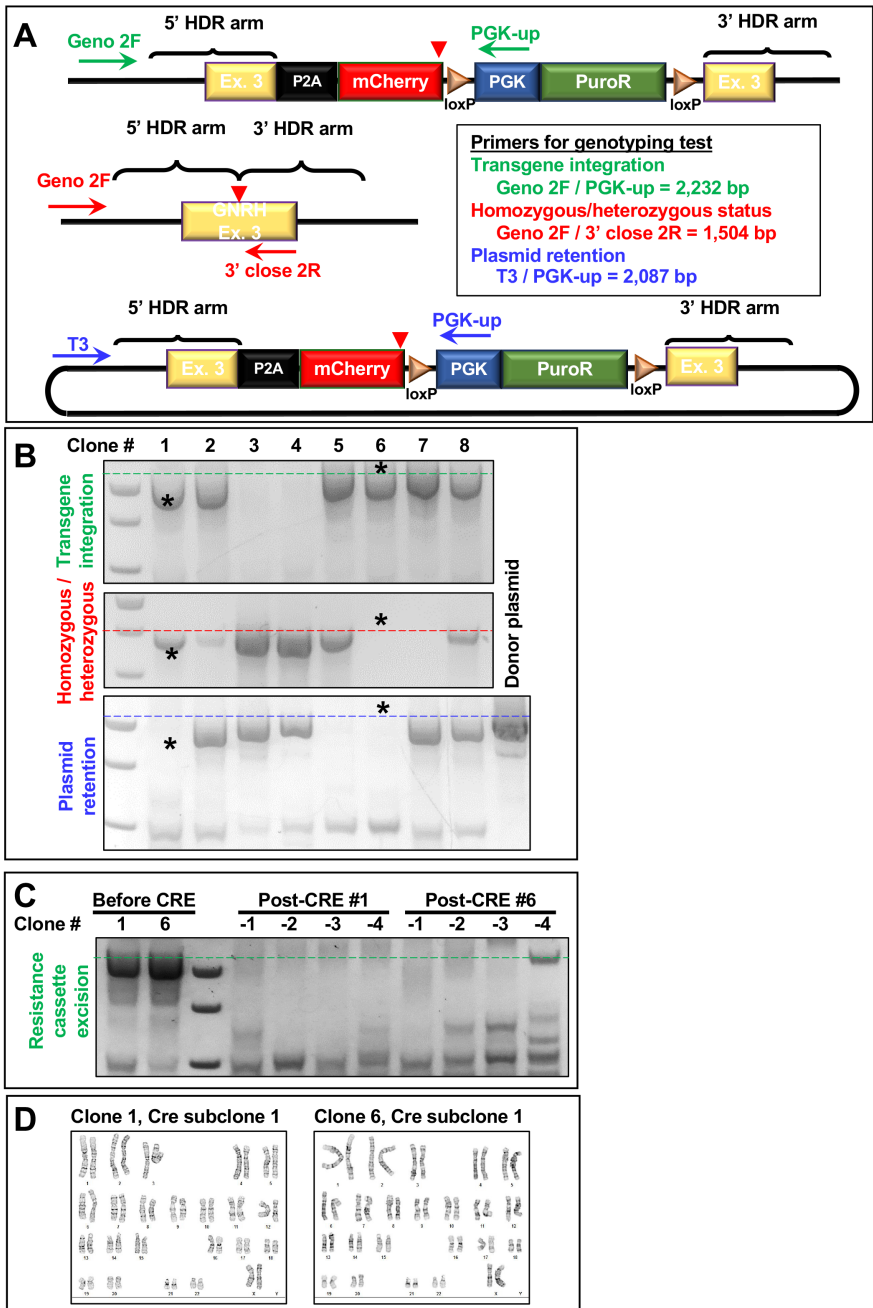
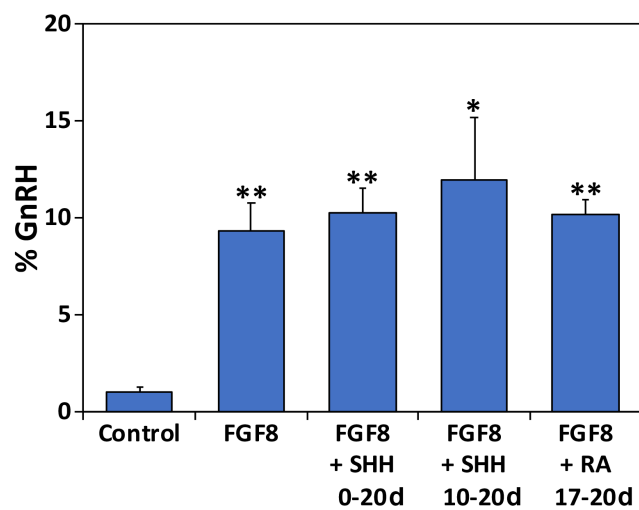


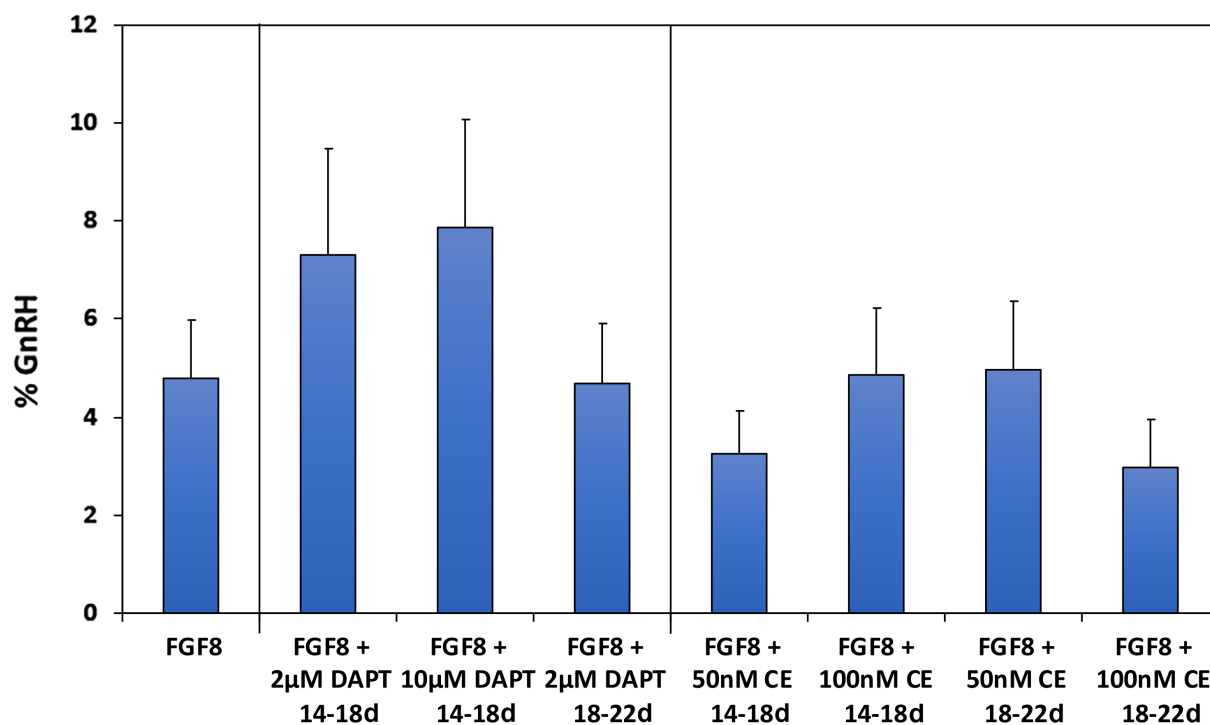
**SUPPLEMENTARY FIGURES:**



**Supplementary Figure 1:** Genotyping of CRISPR/Cas9 labeling of the *GnRH* gene with mCherry. **A.** A schematic representation of the *GnRH* locus and primer pairs used for genotyping the transgenic line. **B.** The agarose gel images used to identify the clone status. Predicted band sizes for the relevant PCR primer pairs are depicted with a dotted line matching the color of the pairs indicated in **A**. Clones 1 (heterozygous) and 6 (homozygous) were selected for Cre recombinase treatment. **C.** Successful Cre recombinase activity in clone 1 and clone 6 subclones. Left side of panel shows PCR product before Cre application. After Cre, resistance cassette PCR fails in all Clone 1 subclones and subclones 1, 2, and 3 from Clone 6, indicating successful Cre excision of the PGK-puromycin resistance cassette. **D.** Representative karyograms for Clone 1 Cre subclone 1 and Clone 6 subclone 1, both defined as normal

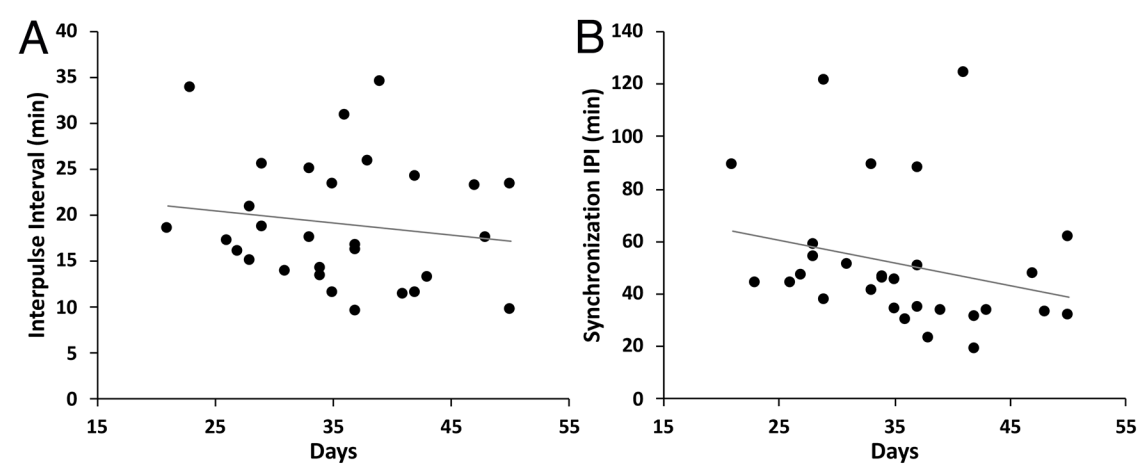


**Supplementary Figure 2.** Addition of the small molecules, SHH and RA, to FGF8 was not effective in increasing in GnRH generation over FGF8 alone. \* =  $p < 0.05$ , \*\* =  $p < 0.01$  vs. Control. All  $n=3$ .

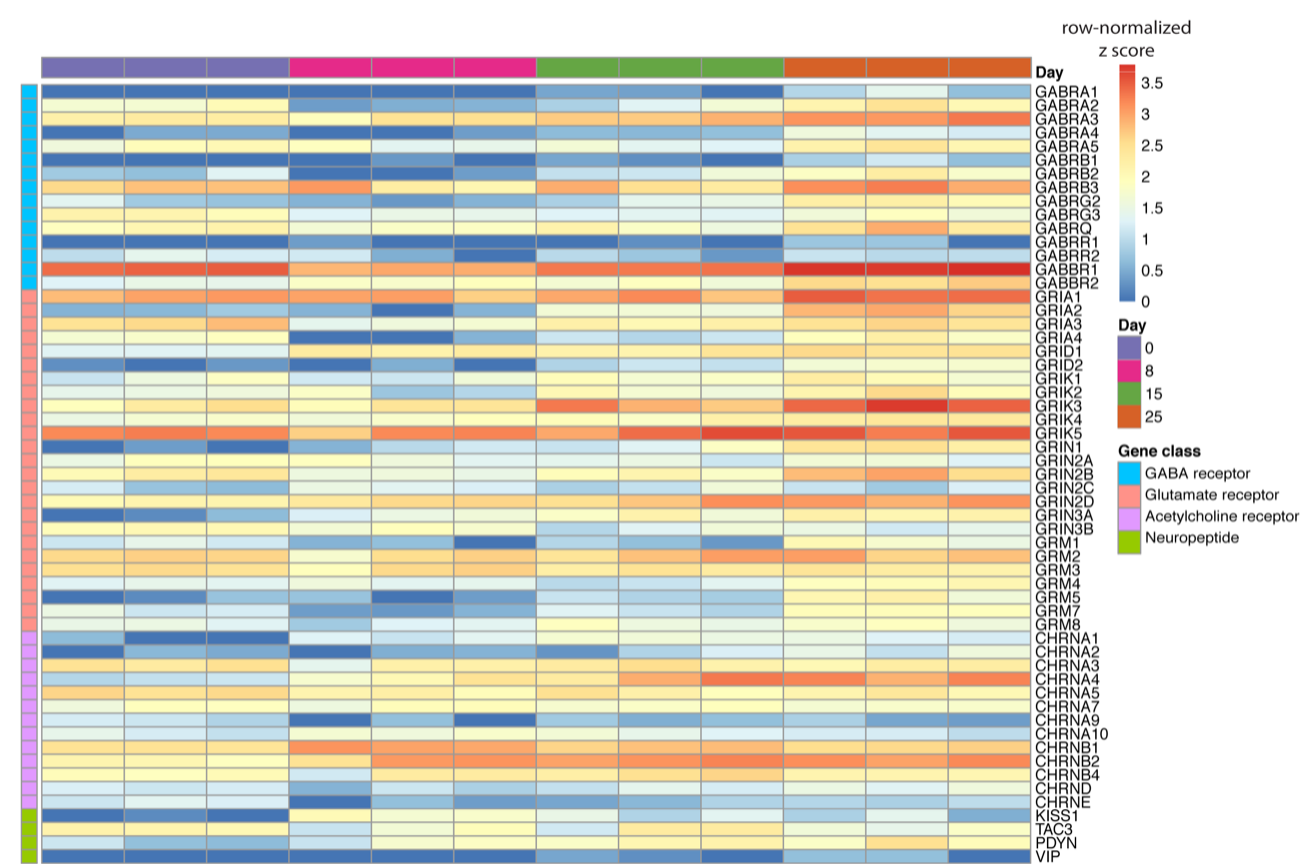


**Supplementary Figure 3:** Addition of the notch inhibitors, DAPT or CE, did not significantly increase the efficiency rate of GnRH neurogeneration. Note that FGF8 treatments at 2 or 10 µM on Day 14-18

had a trend to increase in the efficiency rate over FGF8 alone, they were not statistically significant. All n=6.



**Supplementary Figure 4:** Neither inter-pulse interval of  $[Ca^{2+}]_i$  oscillations (A, R-square = 0.021,  $F_{1,27} = 0.58$ ,  $p=0.451$ ) nor their periodical synchronization interval (B, R-square = 0.063,  $F_{1,27} = 1.811$ ,  $p=0.189$ ) was correlated with the age of GnRH neurons.



**Supplementary Figure 5:** Gene expression heatmap for 56 genes including major neurotransmitter (GABA, glutamate, acetylcholine) receptors, and neuropeptides (KISS1, TAC3, DYN, and VIP). The color scale of the heatmap represents row-scaled z-scored DESeq2 normalized counts.