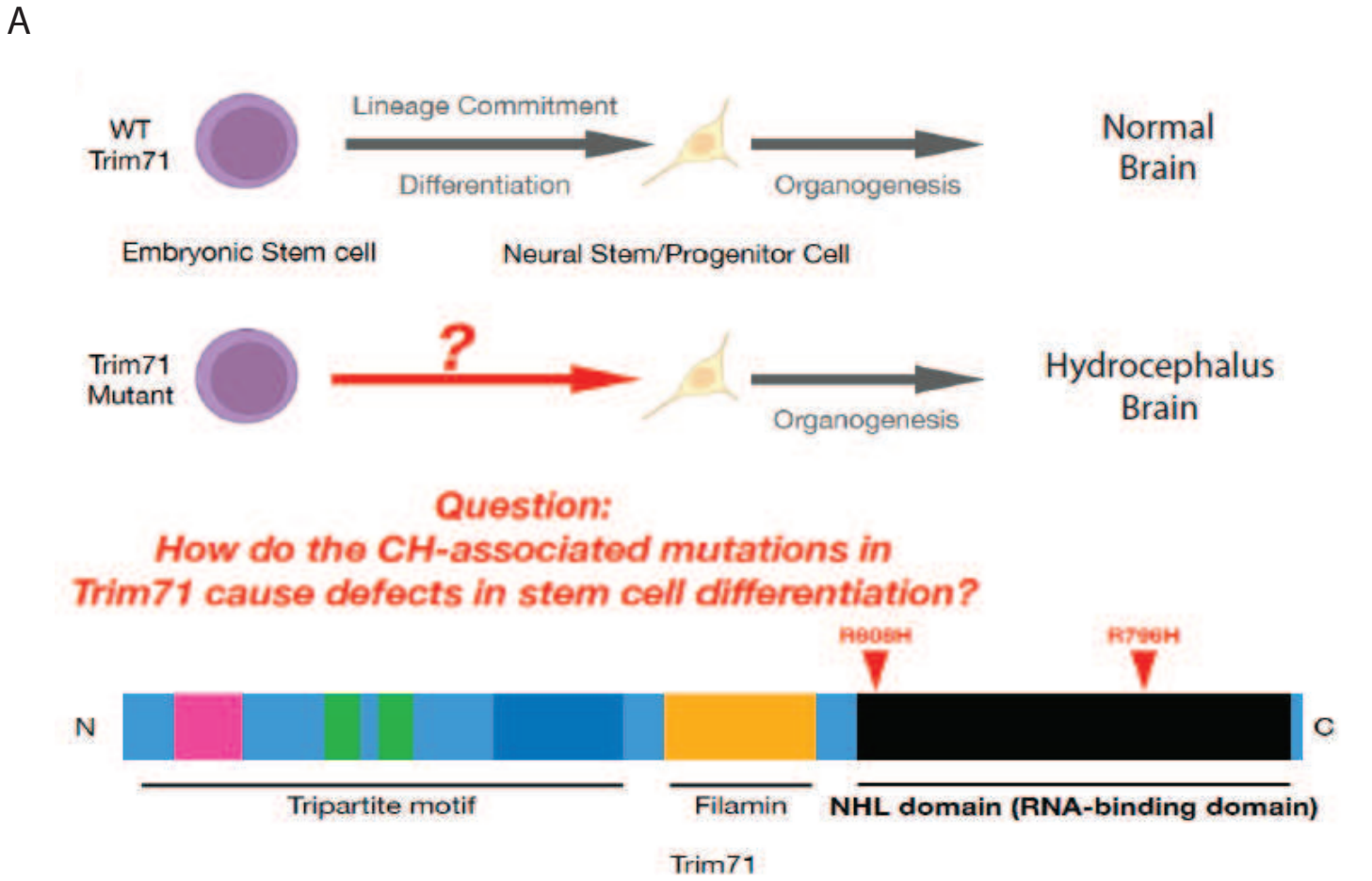
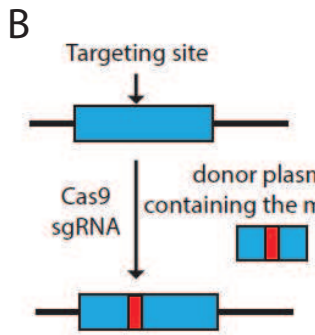
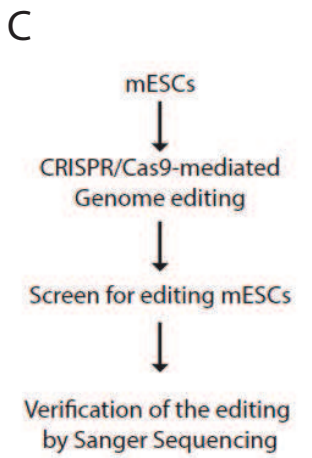
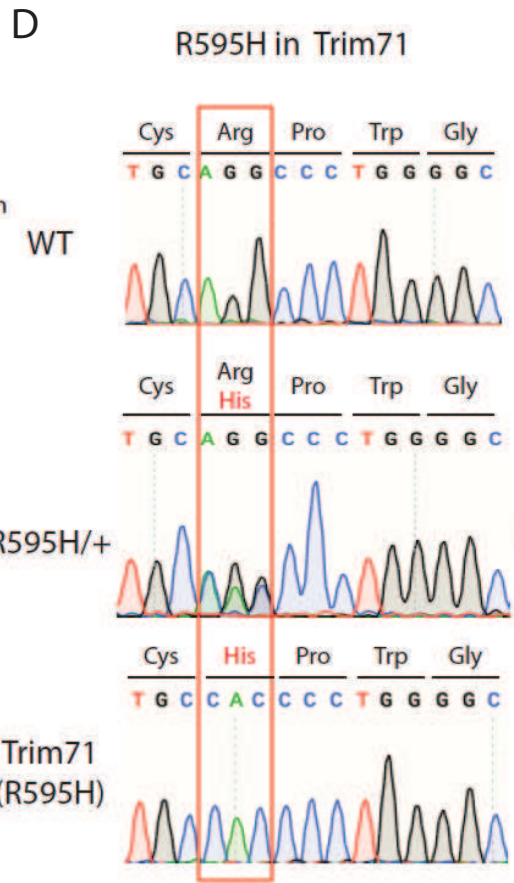
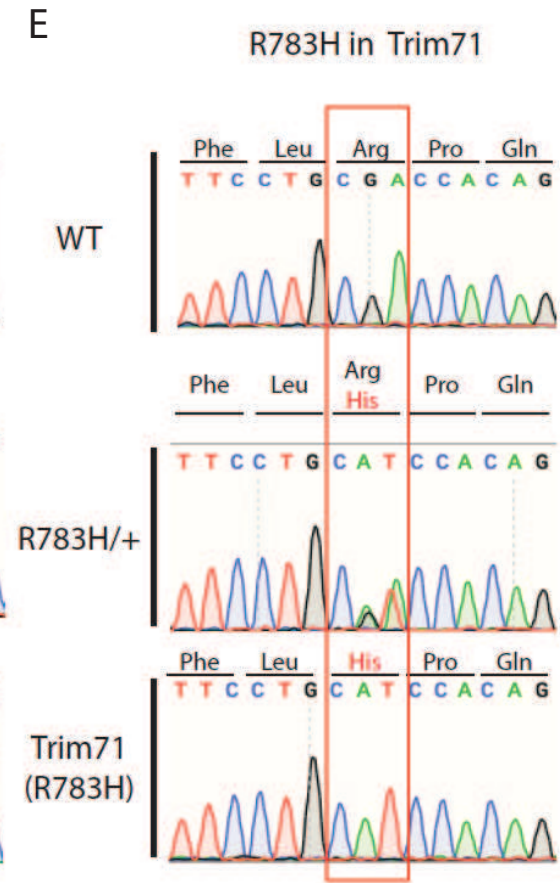

A
WT
Trim71
Lineage Commitment
Differentiation
Organogenesis
Normal
Brain
Embryonic Stem cell
Neural Stem/Progenitor Cell
Trim71
Mutant
?
Organogenesis
Hydrocephalus
Brain
Question:
How do the CH-associated mutations in
Trim71 cause defects in stem cell differentiation?
R808H
R796H
N
C
Tripartite motif
Filamin
NHL domain (RNA-binding domain)
Trim71


B
Targeting site
Cas9
sgRNA
donor plasmid
containing the mutation


C
mESCs
CRISPR/Cas9-mediated
Genome editing
Screen for editing mESCs
Verification of the editing
by Sanger Sequencing


D
R595H in Trim71
WT
Cys Arg Pro Trp Gly
T G C A G G C C C T G G G G C
R595H/+
Cys Arg His Pro Trp Gly
T G C A G G C C C T G G G G C
Trim71
(R595H)
Cys His Pro Trp Gly
T G C C A C C C C T G G G G C


E
R783H in Trim71
WT
Phe Leu Arg Pro Gln
T T C C T G C G A C C A C A G
R783H/+
Phe Leu Arg His Pro Gln
T T C C T G C A T C C A C A G
Trim71
(R783H)
Phe Leu His Pro Gln
T T C C T G C A T C C A C A G