Amyotrophic Lateral Sclerosis (ALS) and FUS

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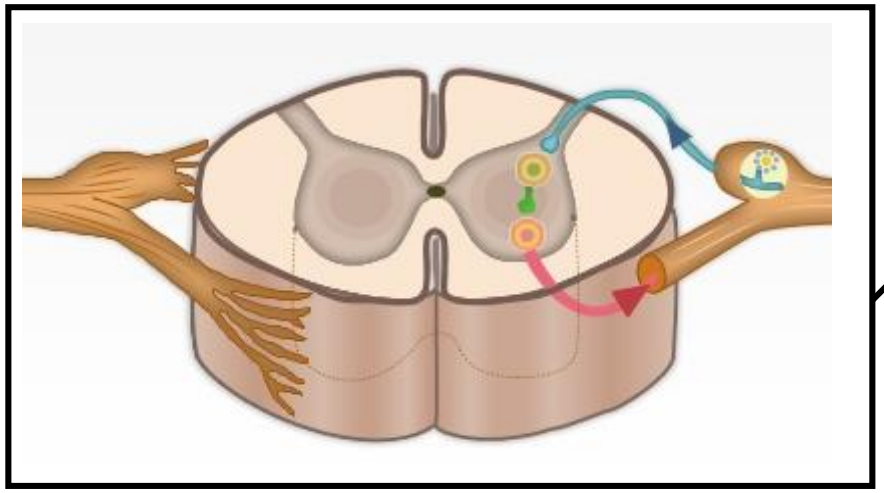
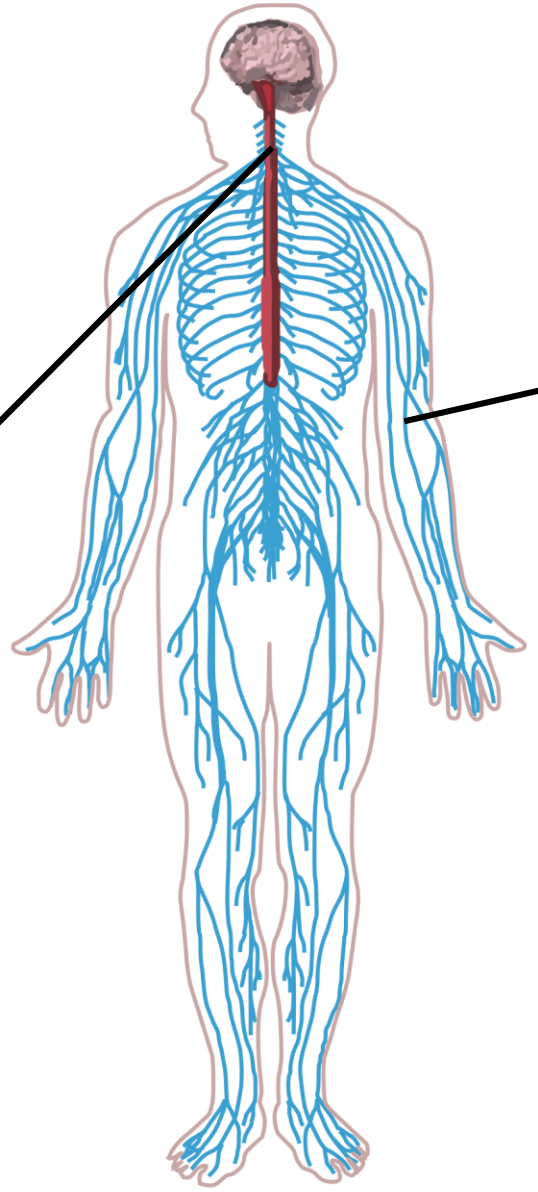
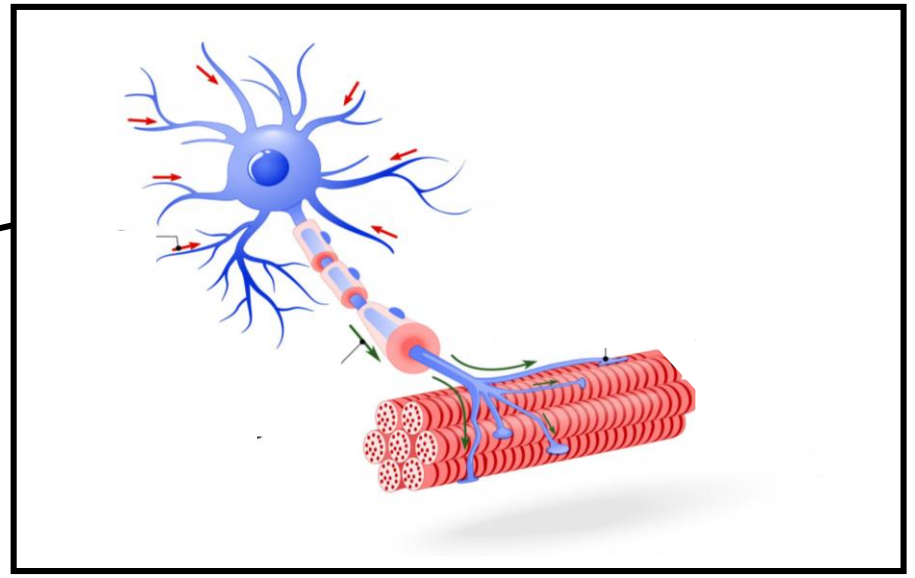
What is Amyotrophic Lateral Sclerosis?

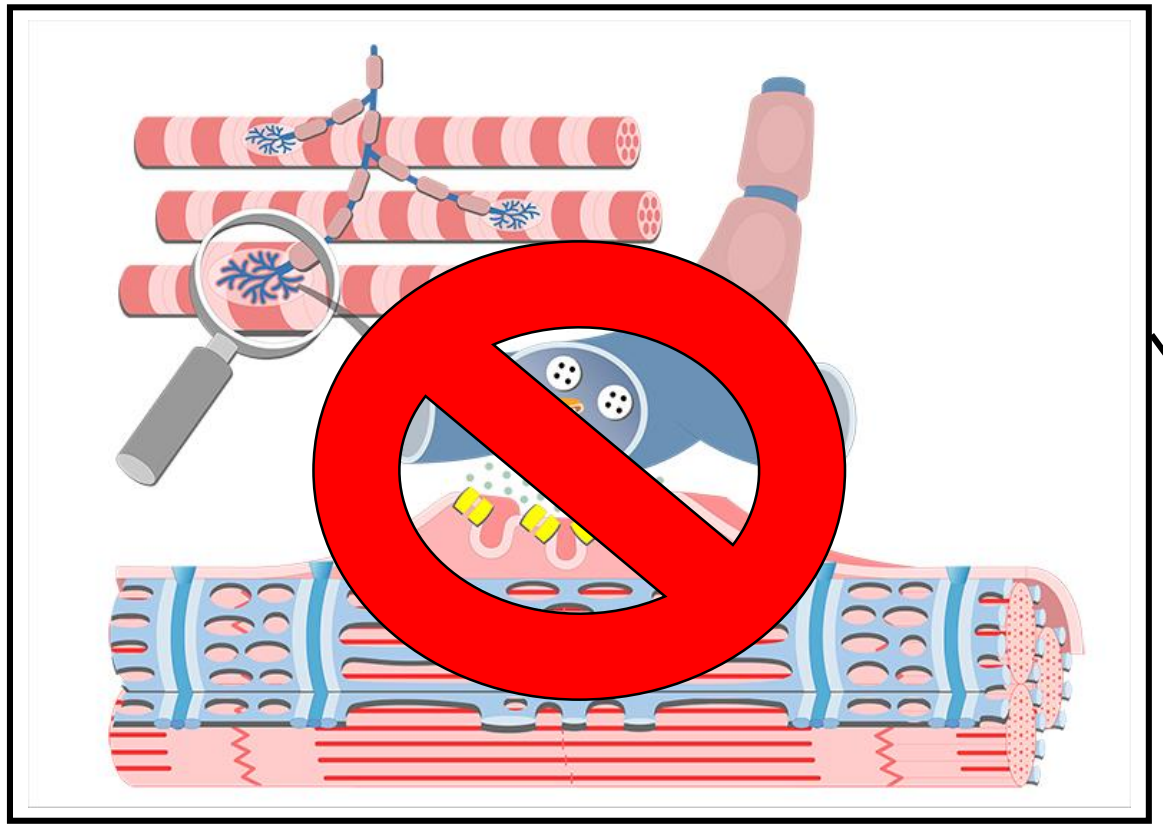


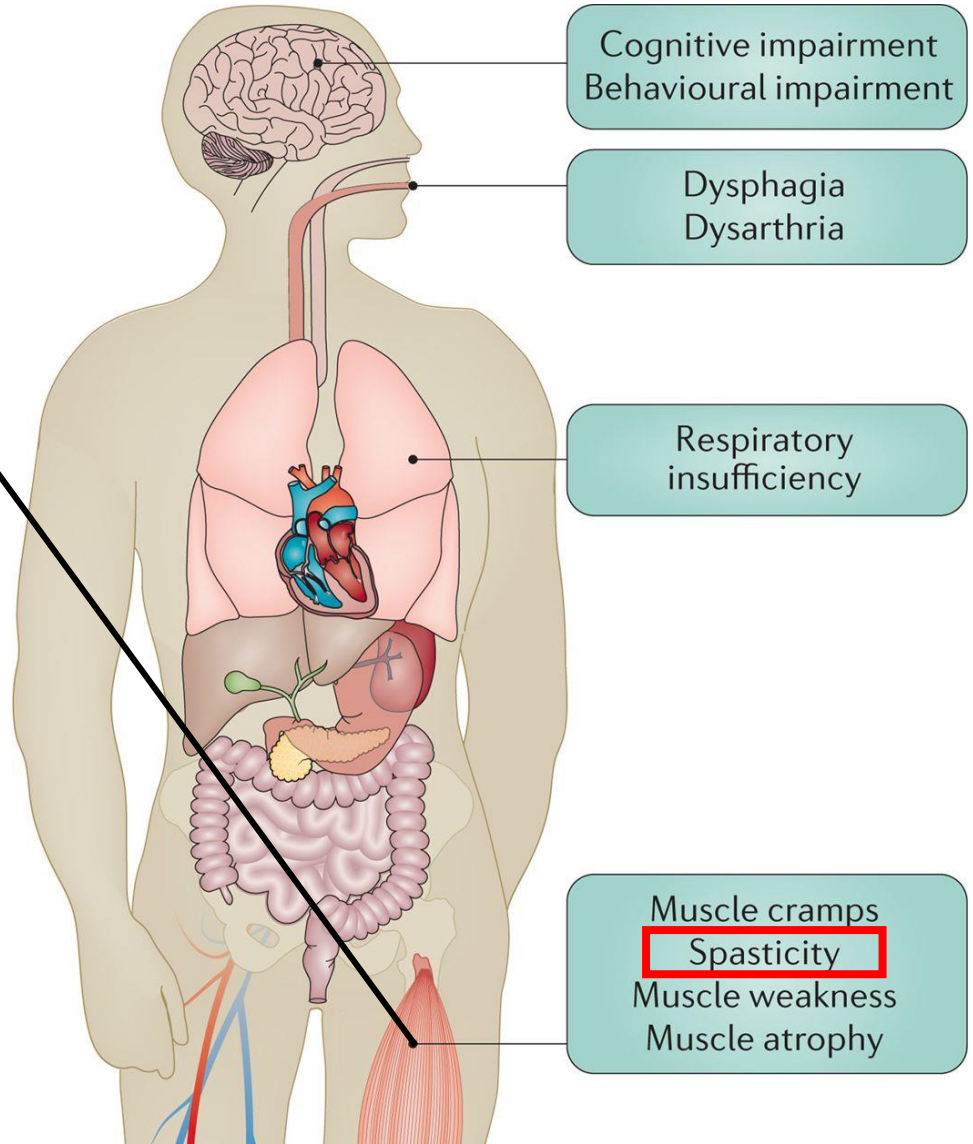




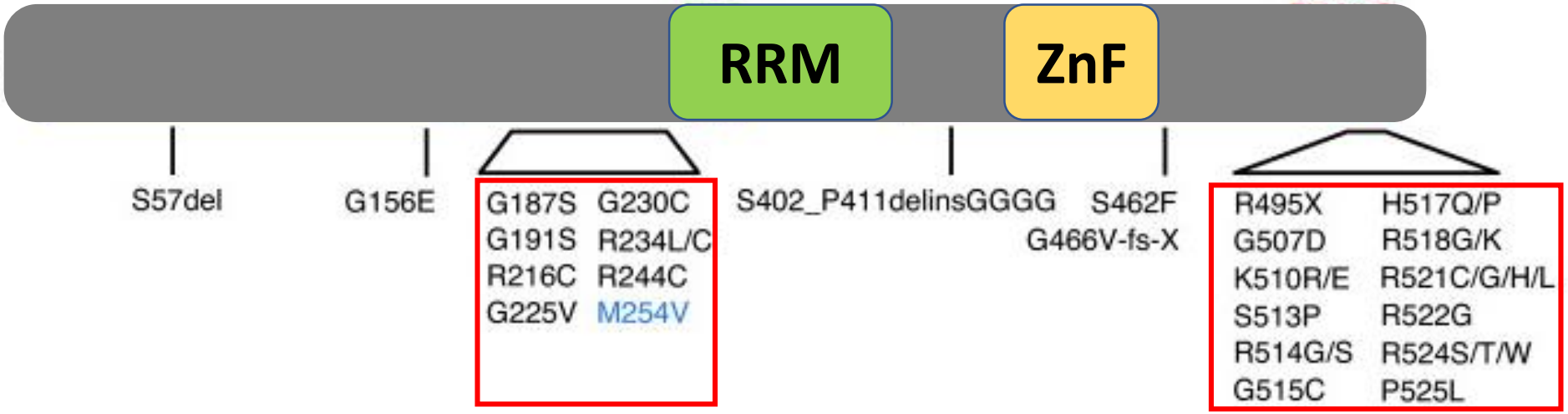
Motor neuron disease that results in nerve cell death in upper and lower motor neurons.

Symptoms of Amyotrophic Lateral Sclerosis

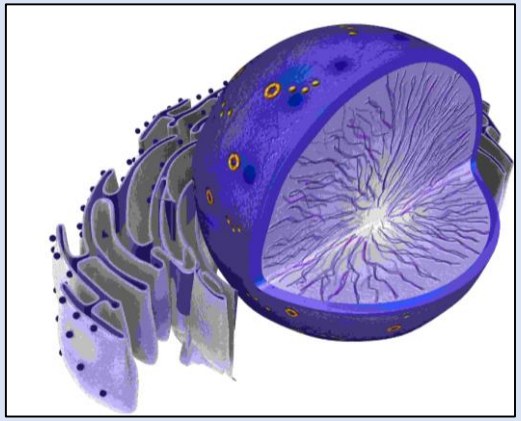




FUS is associated with Juvenile ALS



Cellular Localization

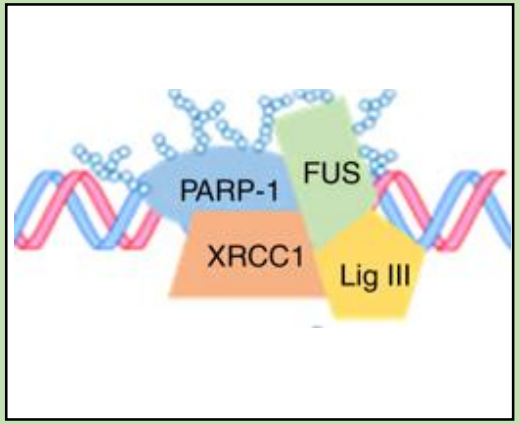


Nucleus

Molecular Function

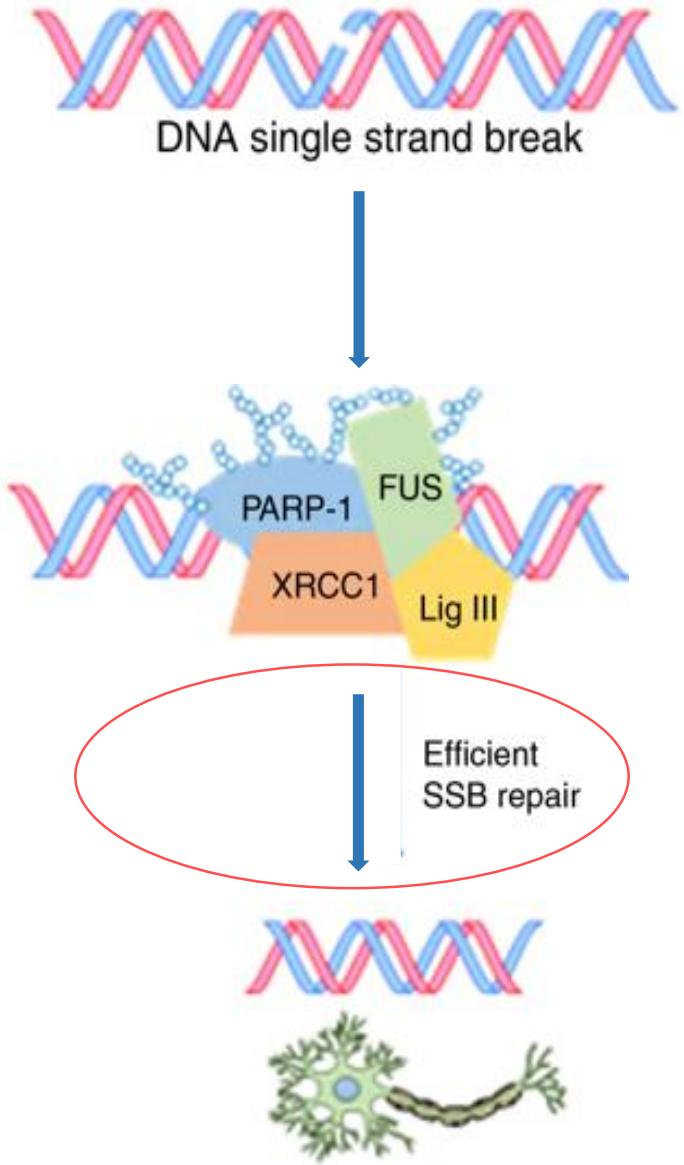
RNA/DNA Binding

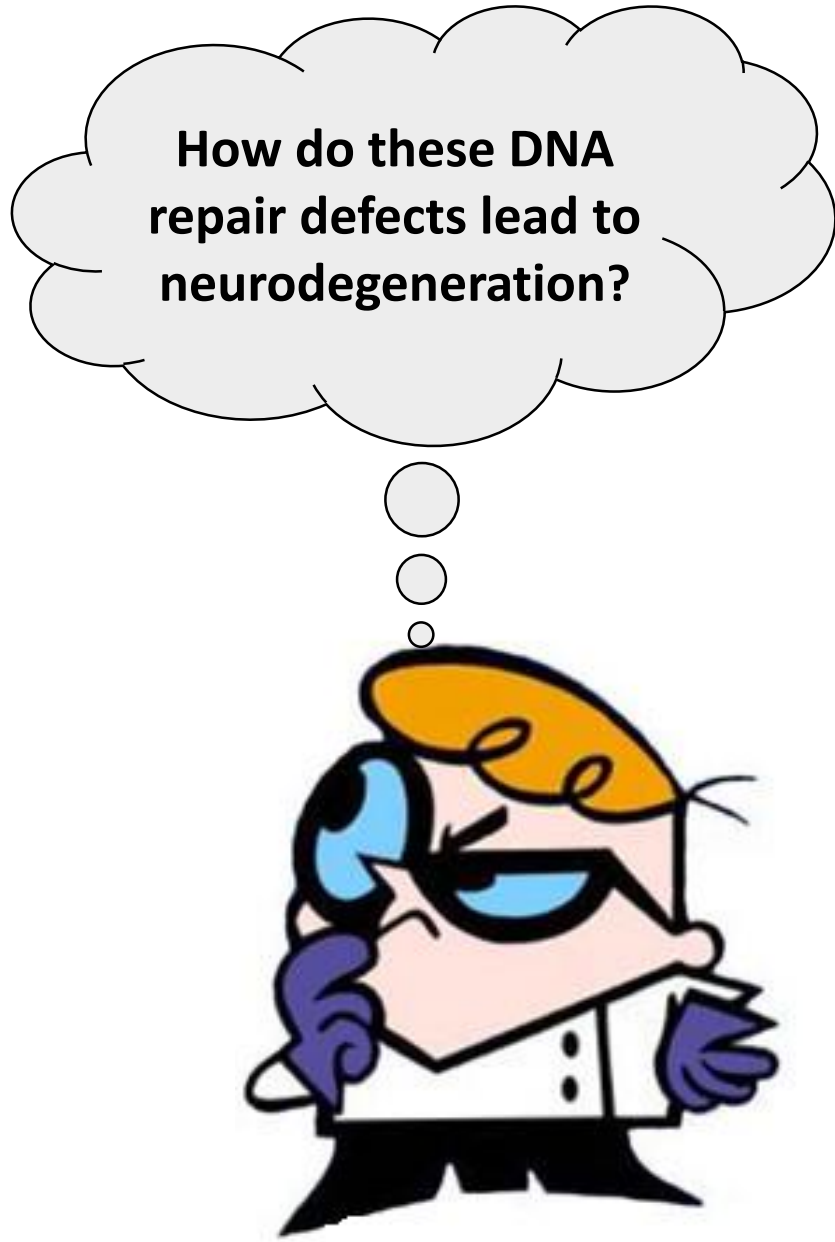
Biological Process



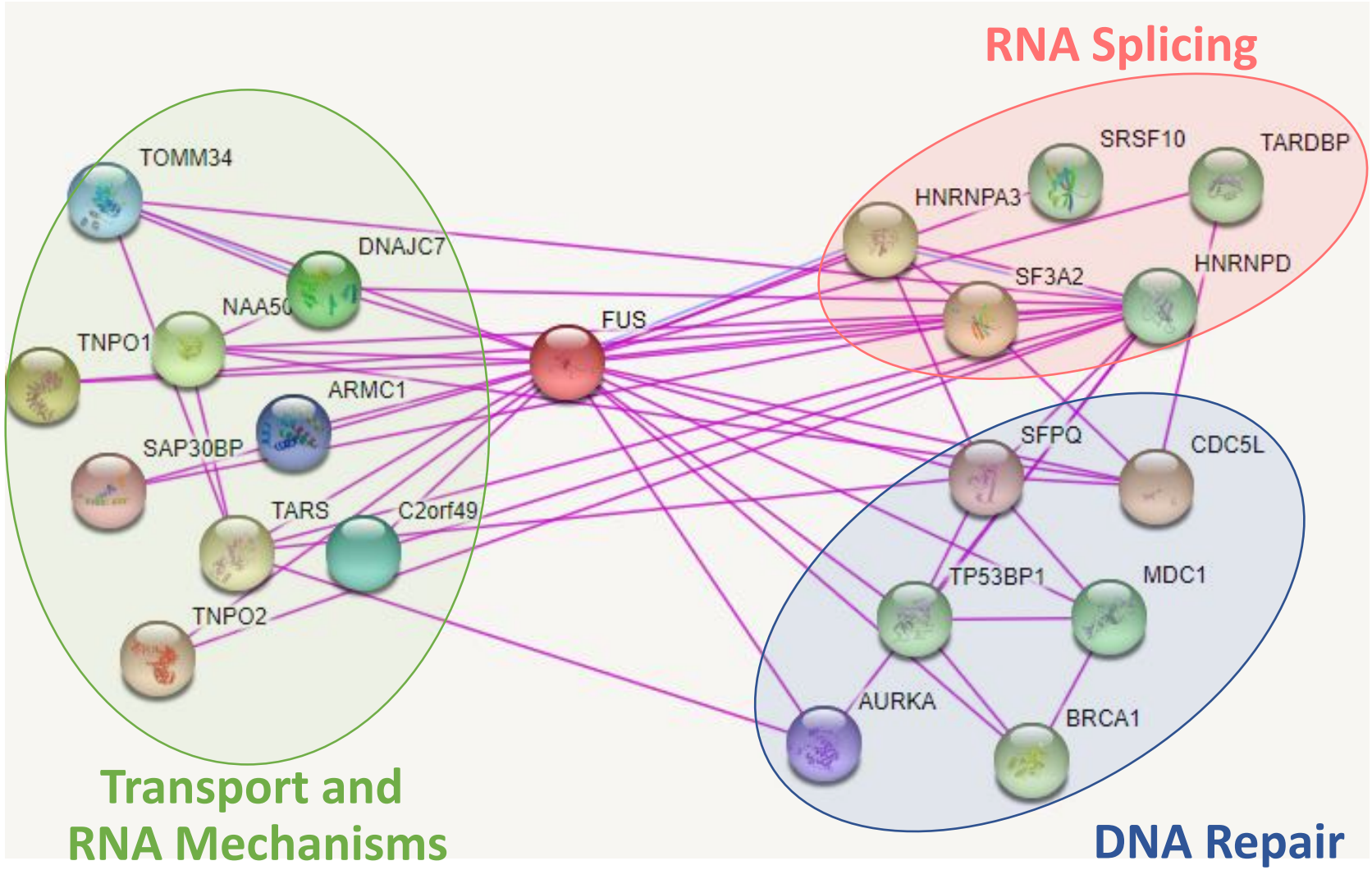
DNA Repair

FUS role in DNA Repair and Neurodegeneration



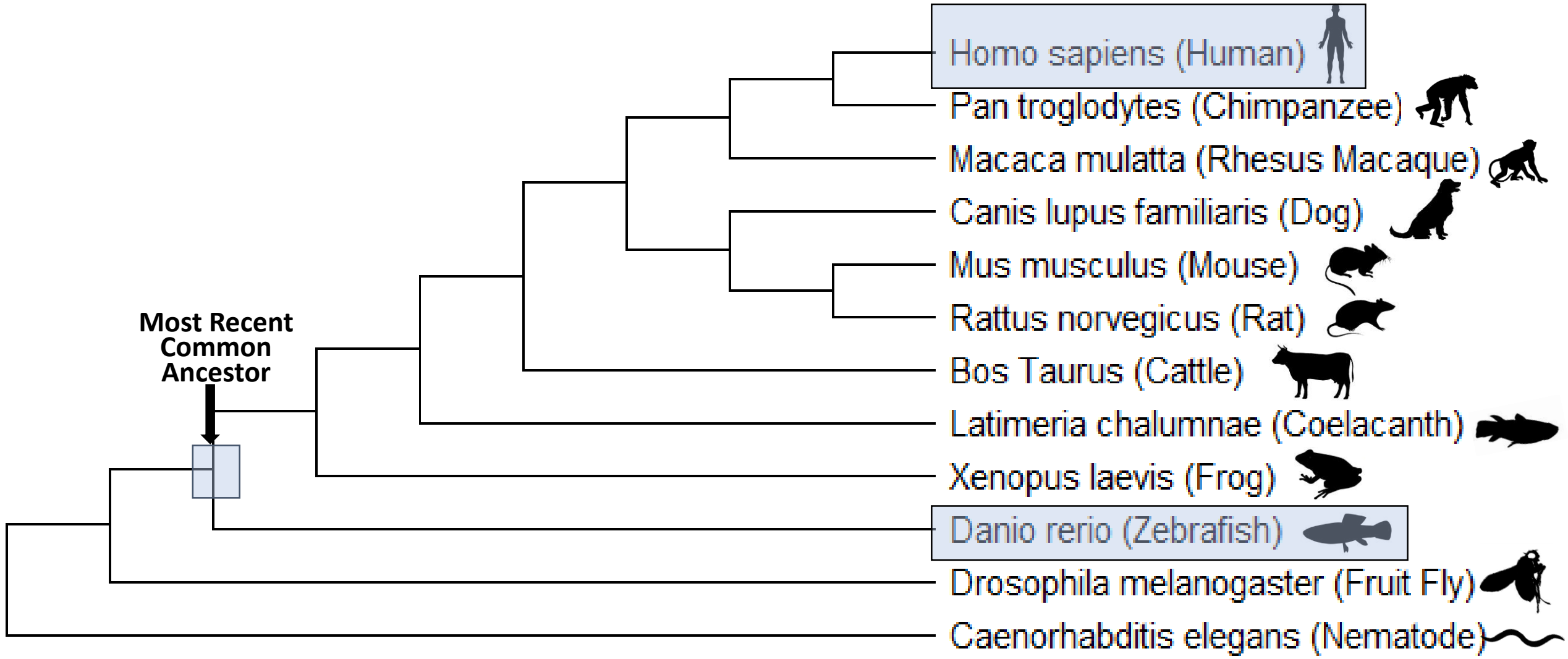


Known human FUS protein interactions



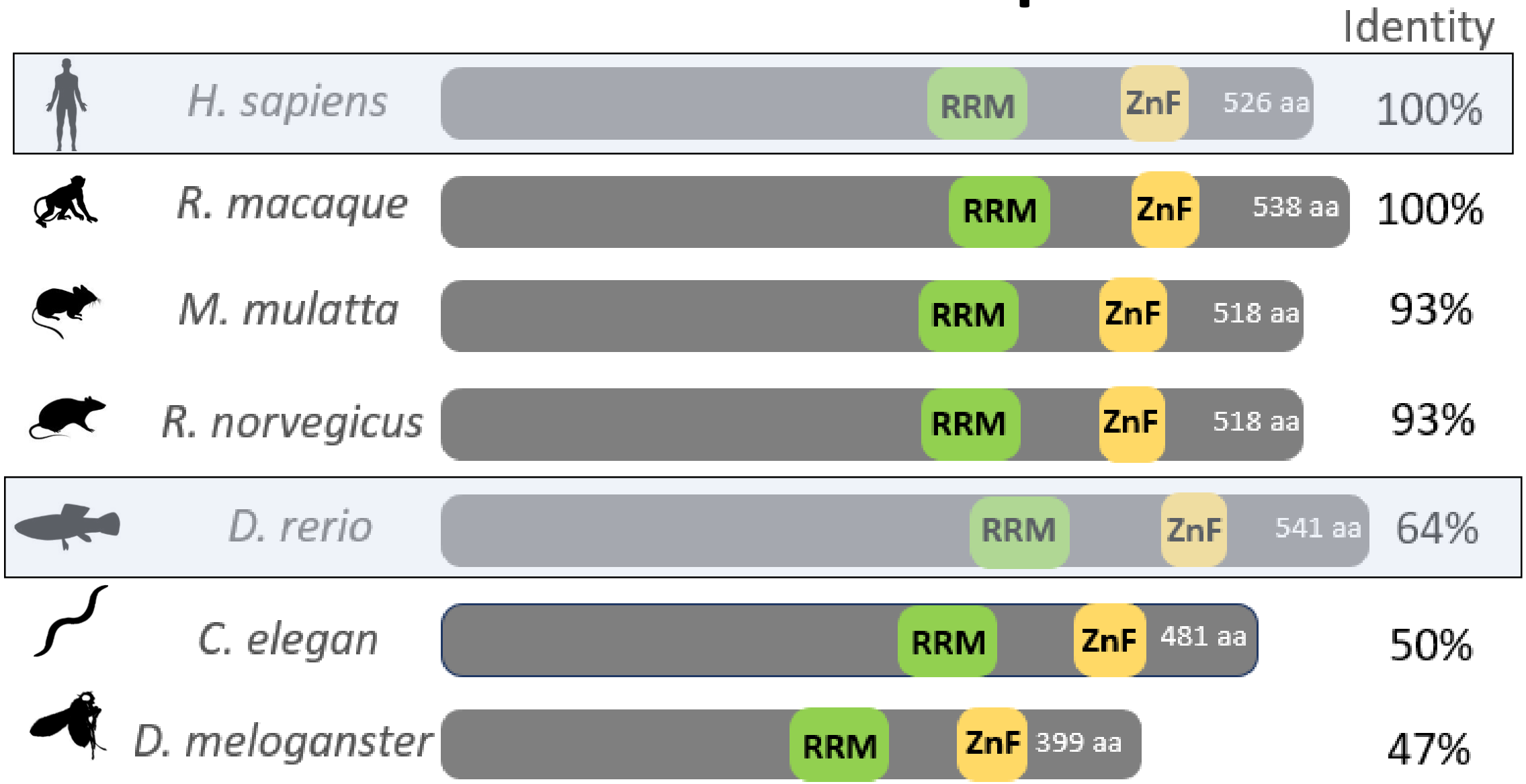
FUS has experimental interactions with genomic stability and DNA repair proteins.

FUS homologs across species



The human FUS protein has homologs across many species.

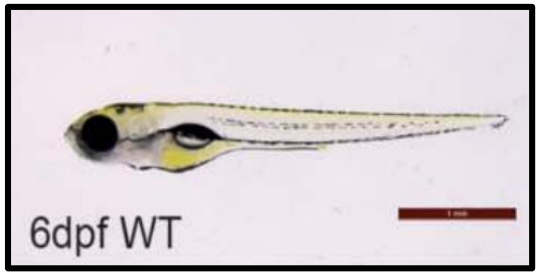
FUS domains across species

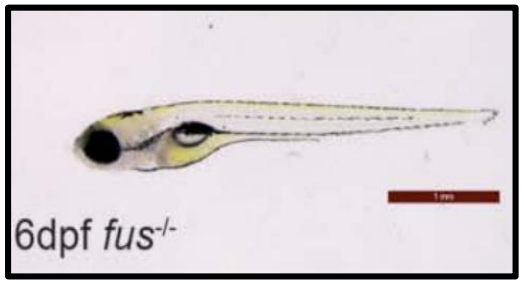


The human FUS protein has conserved domains across many species.

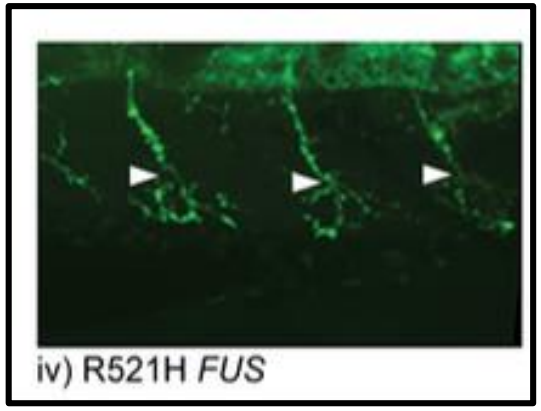
Zebrafish as a model organism

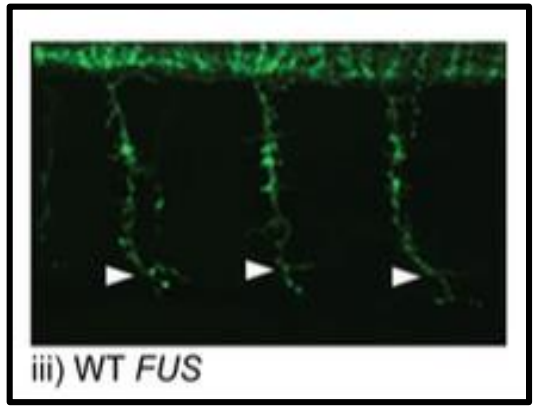
Transparency



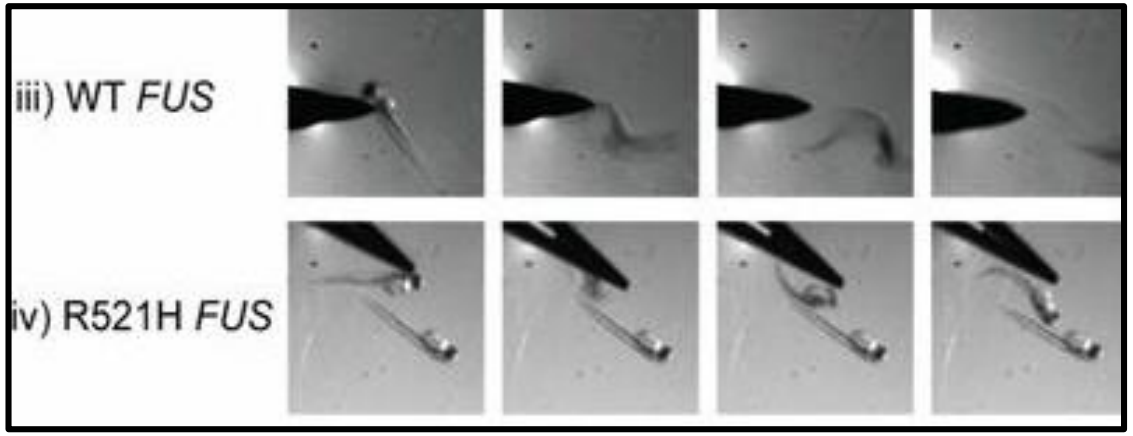


Neuron Similarity





Motor Movement Screen

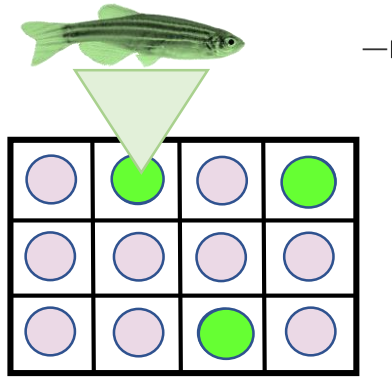


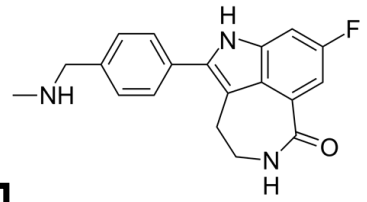
Zebrafish have desirable phenotypes that are useful for studying ALS.

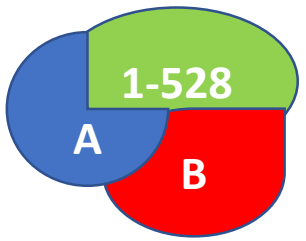
Primary goal of Specific Aims

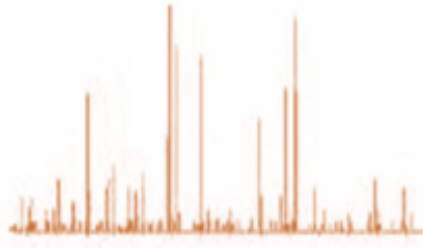
To determine how the role of FUS related DNA repair leads to neurodegeneration

Aim 1:	Aim 2:	Aim 3:
Identify conserved amino acids associated with DNA repair and ALS	**Identify small molecules that restore DNA repair defects**	**Identify new FUS protein-protein interactions in DNA repair**



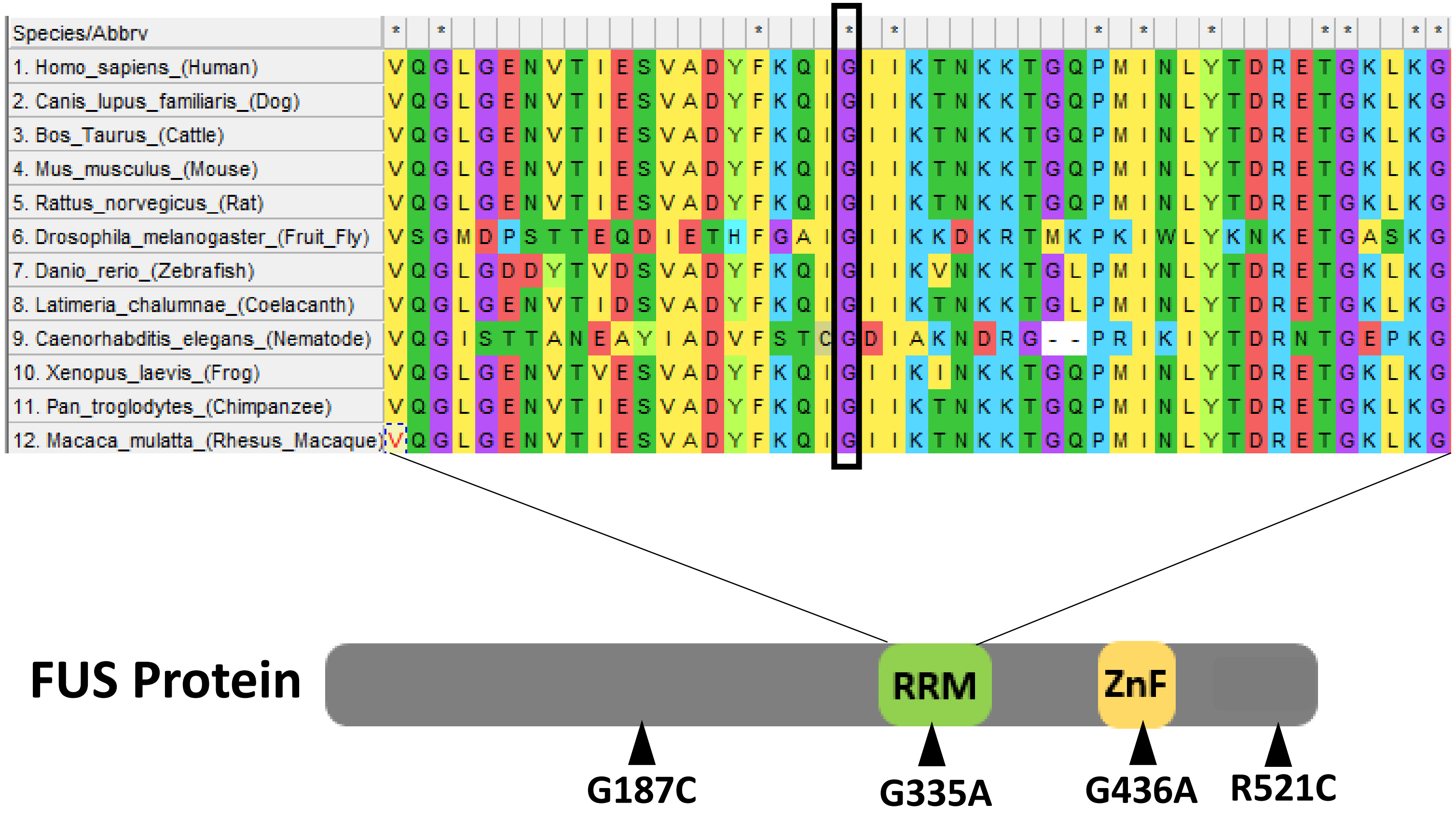


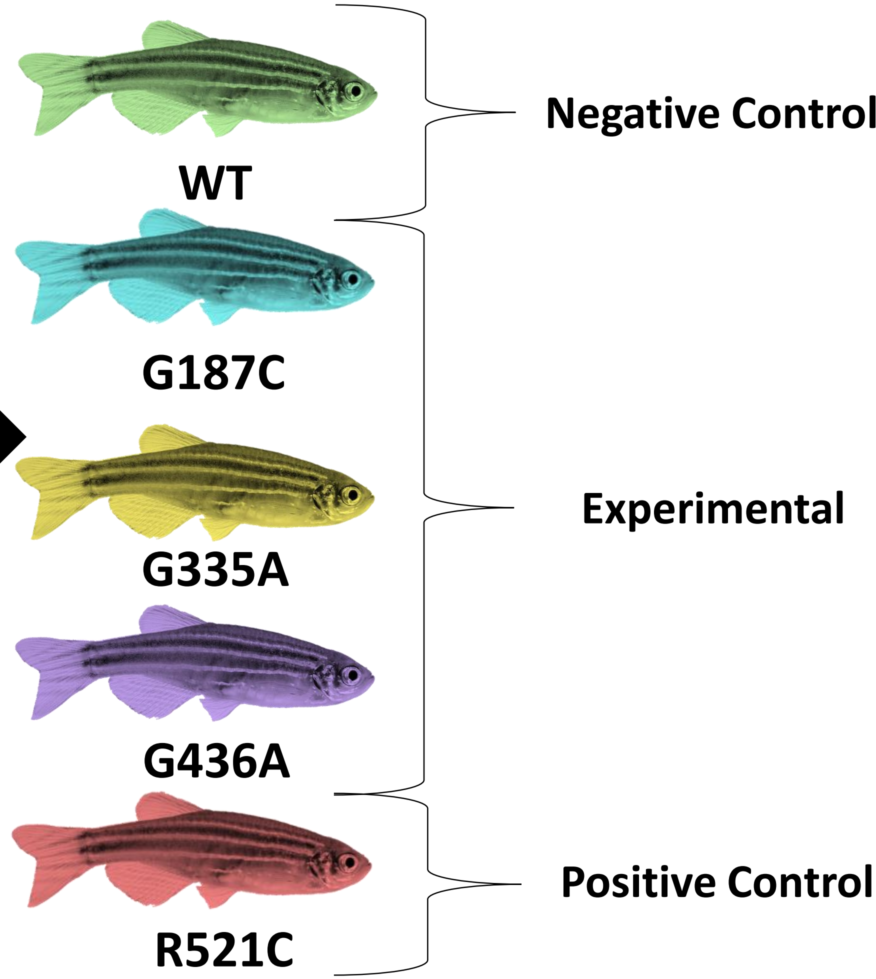




Aim 1: Identify amino acids associated with DNA repair and ALS

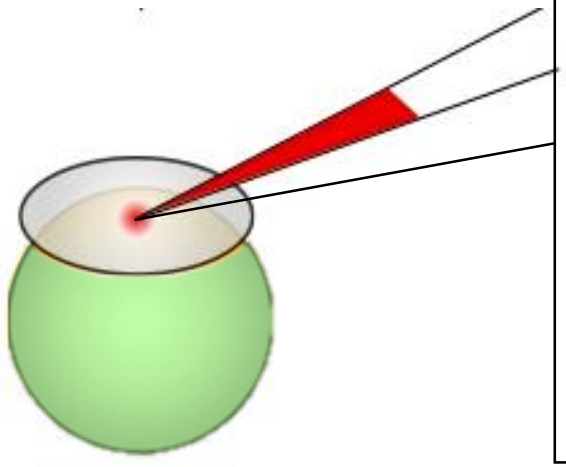
MEGA Molecular Evolutionary Genetics Analysis



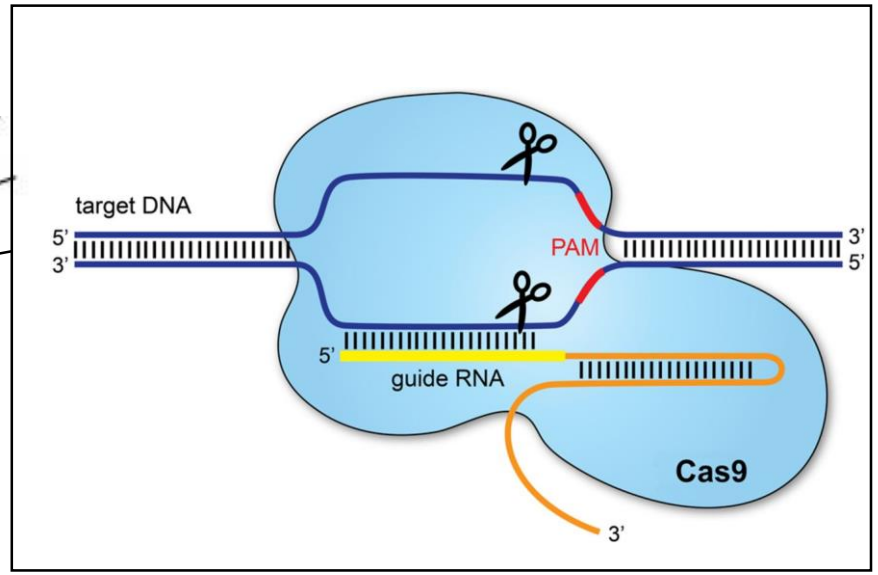


Identify Variants → CRISPR/Cas9 → Phenotype Screen → Analyze Results

Aim 1: Identify amino acids associated with DNA repair and ALS



WT Embryo

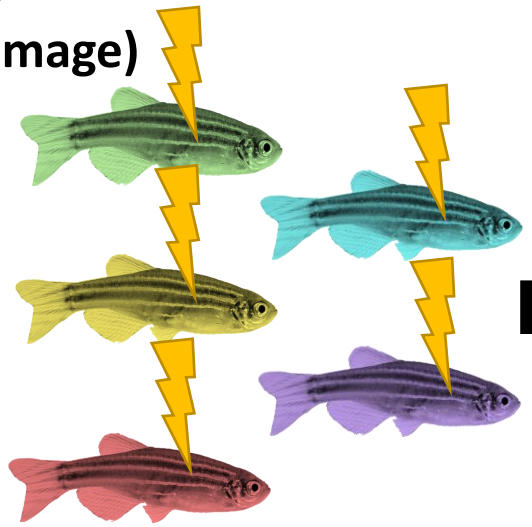


CRISP/Cas9 Protocol

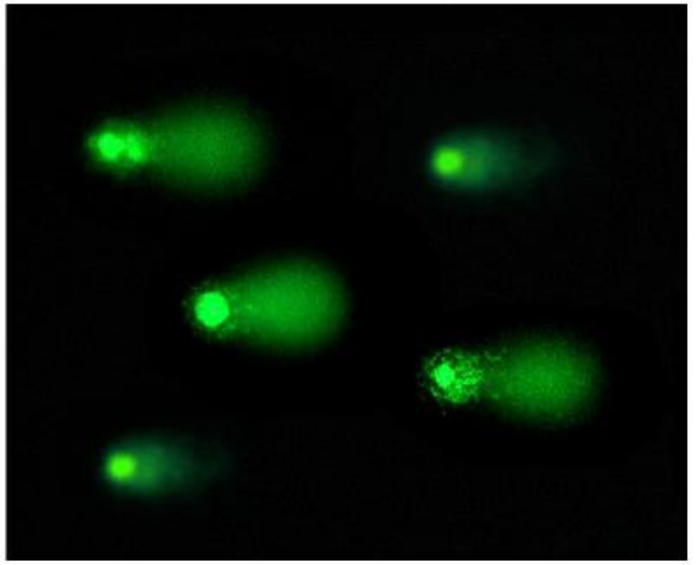
Identify Variants | CRISPR/Cas9 | Phenotype Screen | Analyze Results

Aim 1: Identify amino acids associated with DNA repair and ALS

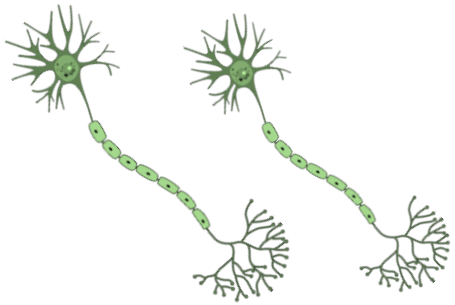
UV
(DNA Damage)

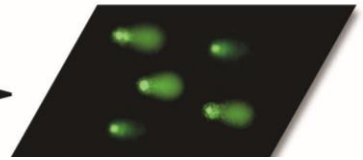


Motor Movement Screen



Comet Assay



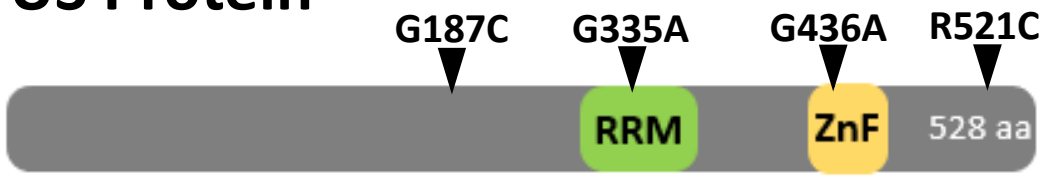


Comet Assay IV

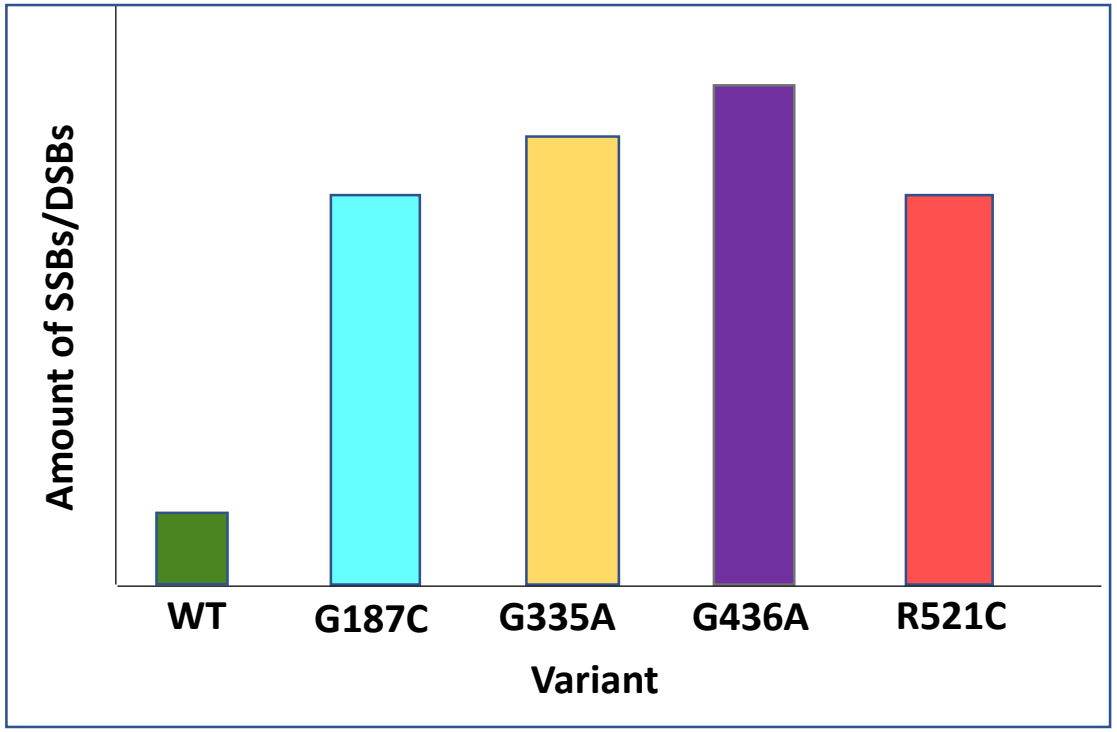
Identify Variants → CRISPR/Cas9 → Phenotype Screen → Analyze Results

Aim 1: Identify amino acids associated with DNA repair and ALS

FUS Protein



Expected Results



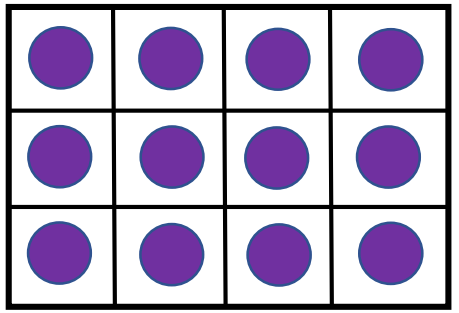
Hypothesis: Conserved regions will result in more SSBs/DSBs and a more severe phenotype.

Identify Variants → CRISPR/Cas9 → Phenotype Screen → Analyze Results

Aim 2: Identify small molecules that restore DNA repair defects

Variants of Interest

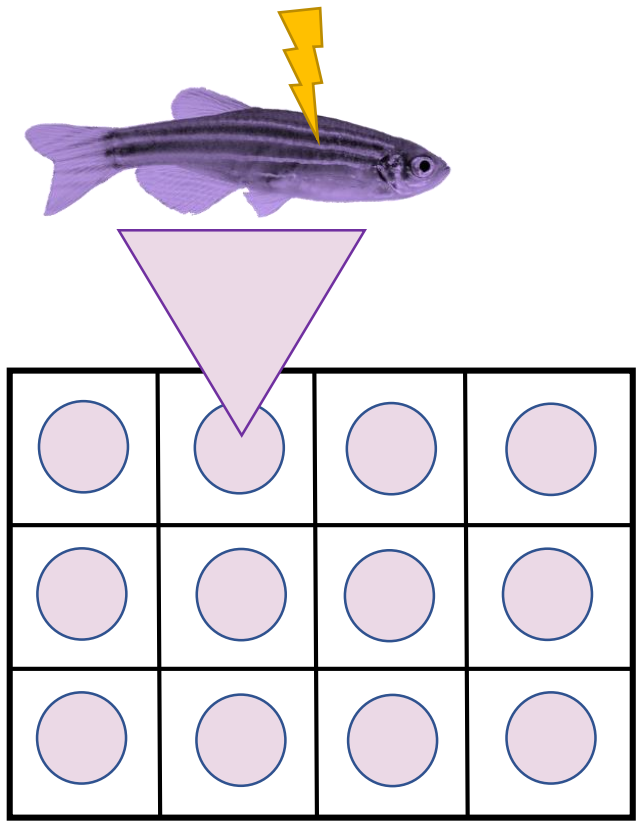
Breeding



Collection

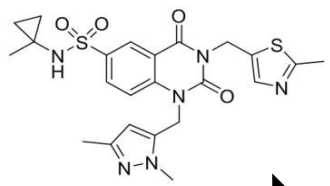
Select Variants → Chemical Library → Phenotype Screen

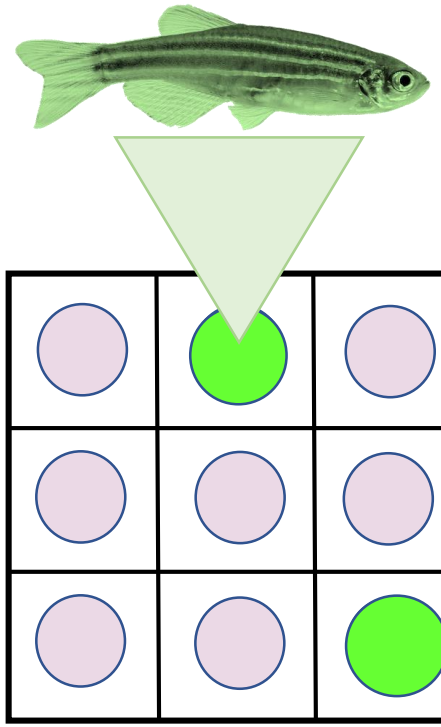
Aim 2: Identify small molecules that restore DNA repair defects



Mutant Zebrafish

DNA Repair Protein Regulators





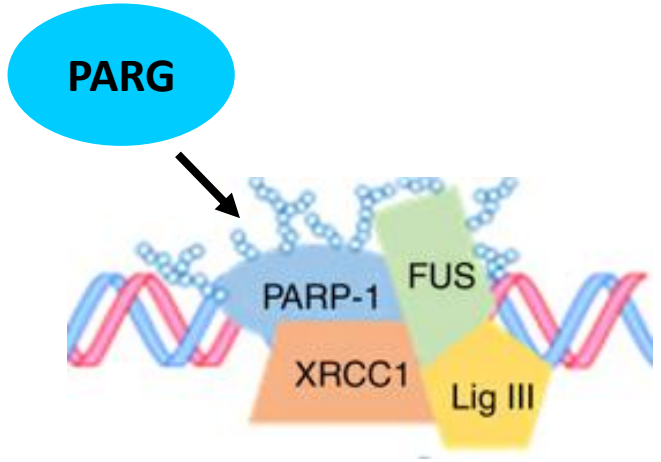
Wild Type Phenotype

Potential Drugs:

PARG Inhibitor

XRCC1 Promoter

Ligase III Promoter

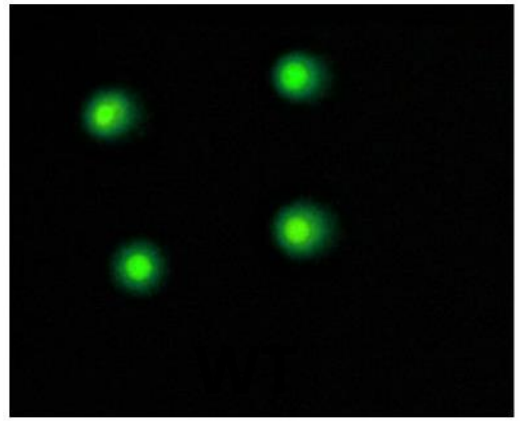


Select Variants → Chemical Library → Phenotype Screen

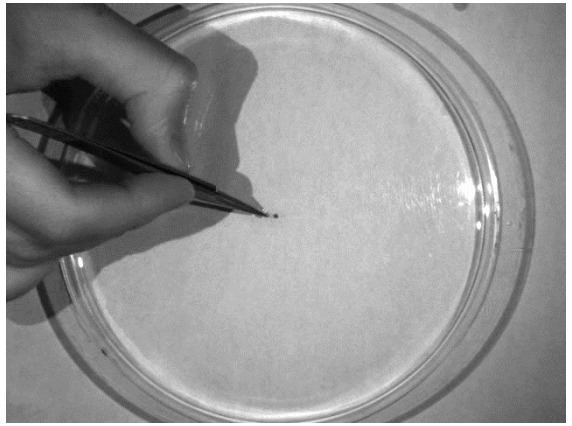
Aim 2: Identify small molecules that restore DNA repair defects

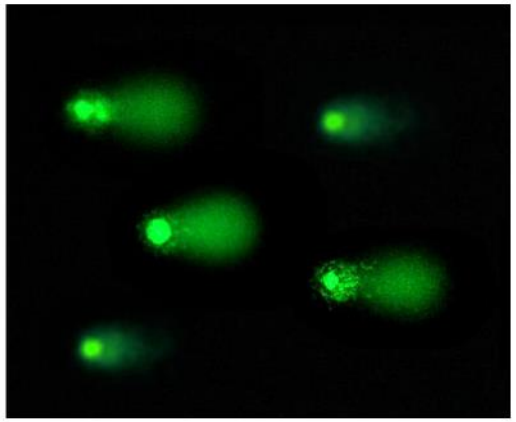
Motor Movement Screen **Comet Assay**

Rescued Phenotype:



Mutant Phenotype:



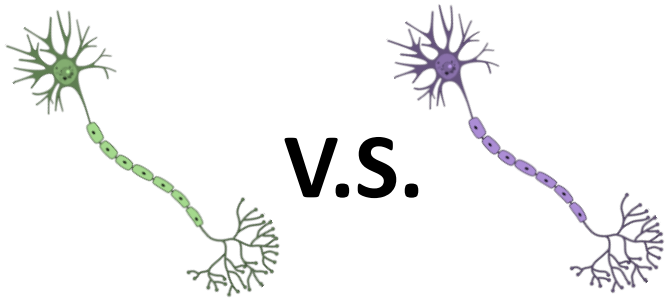


Hypothesis: Small molecules associated with DNA repair will rescue the FUS mutant phenotype.

Select Variants → Chemical Library → Phenotype Screen

Aim 3: Identify new FUS protein-protein interactions in DNA repair

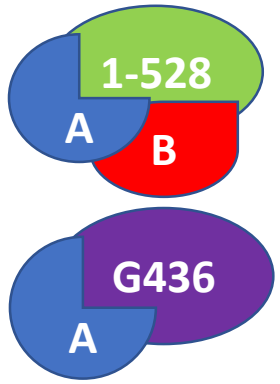
Tandem Affinity Purification:



V.S.

Neuronal Samples

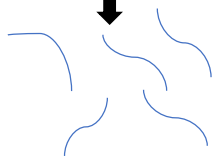
FUS Fragment Baits

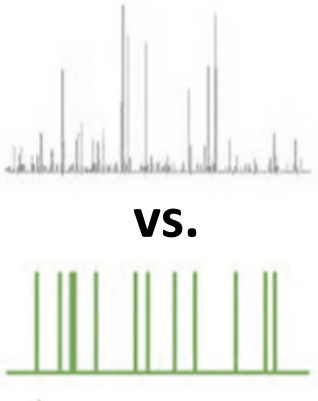


Protein Complex

SDS-Page

Trypsin Digest





Mass Spectrometry

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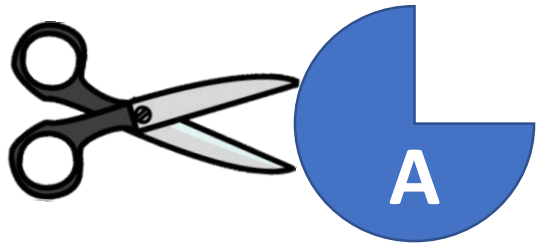
GQQDRGGRGRGGSGGGGGGGGGGGYNRSSGGYEPRGRGGGRGGRGGMGGSDRG
GFNKFGGPRDQGSRHDSEQDNSDNNTIFVQGLGENVTIESVADYFKQIGIIK
TNKKTGQPMINLYTDRETGKLKGEATVSFDDPPSAKAAIDWFDGKEFSGNPI
KVSFATRRADFNRGGGNGRGGRGRGGPMGRGGYGGGGSGGGGRGGFPSGGGG
GGGQQRAGDWKCPNPTCENMNFSWRNECNQCKAPKPDGPGGGPGGSHMGGNY
GDDRRGGRGGYDRGGYRGRGGDRGGFRGGRGGGDRGGFGPGKMDSRGEHRQD
RRERPY

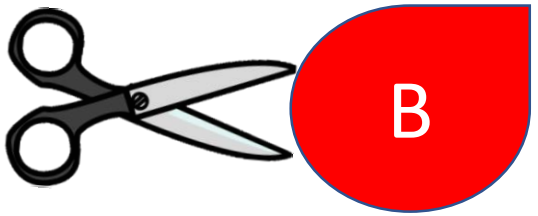
Protein Scoring Algorithm

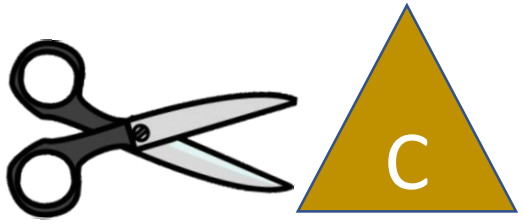
Mass Spectrometry | CRISPR/Cas9 | Gene Ontology

Aim 3: Identify new FUS protein-protein interactions in DNA repair

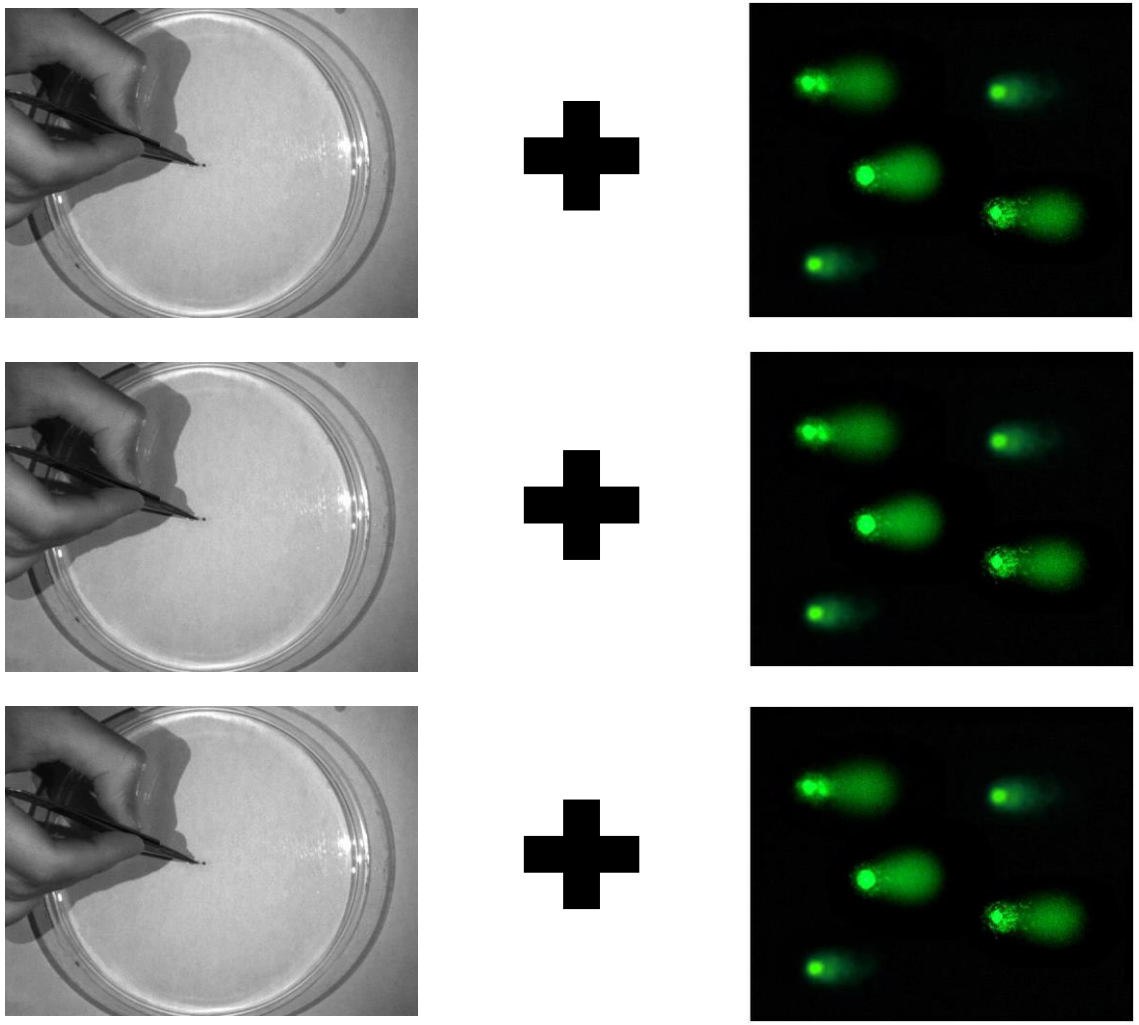
CRISPR





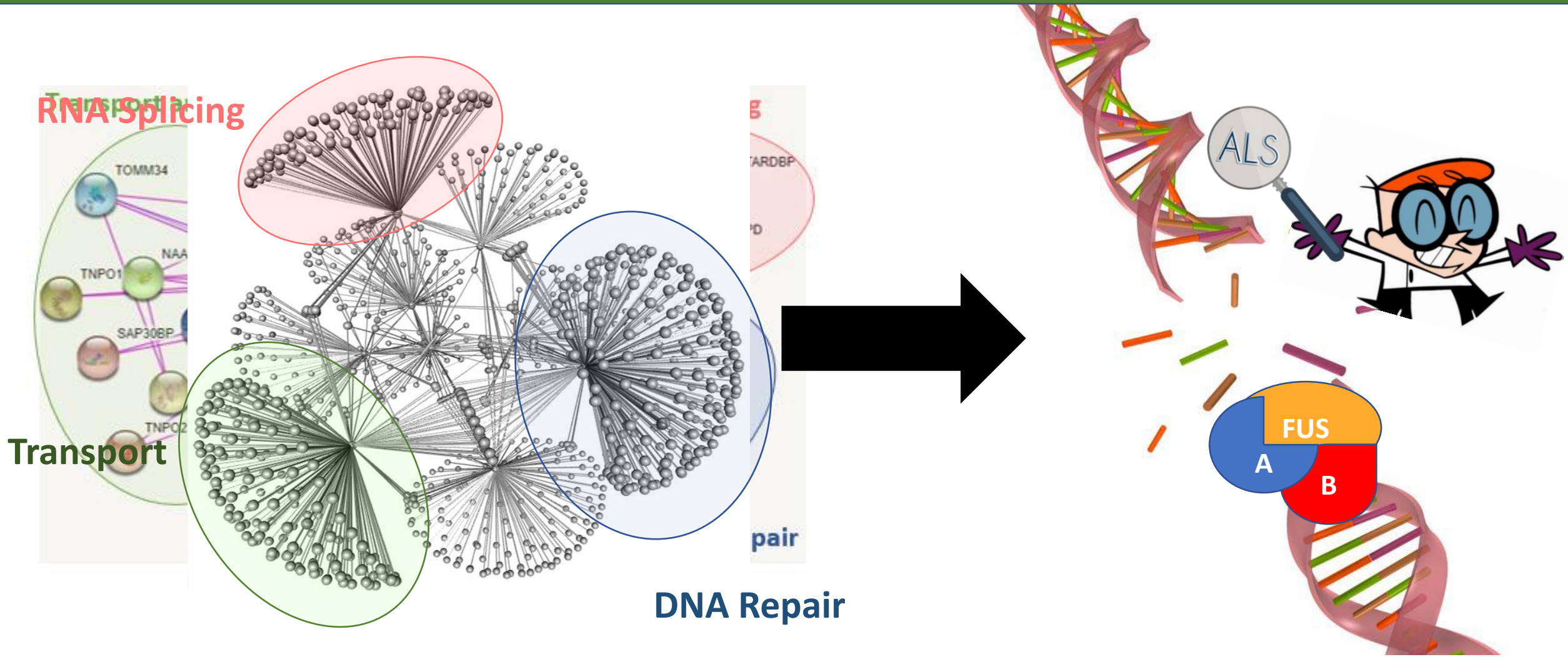


Mutant Phenotype



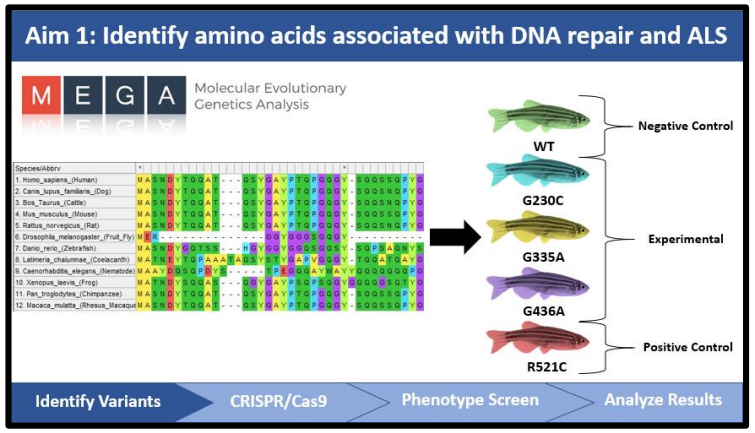
Mass Spectrometry → CRISPR/Cas9 → Gene Ontology

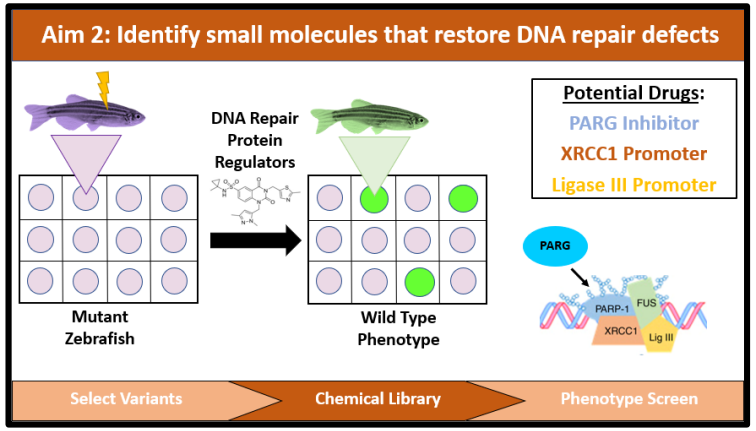
Aim 3: Identify new FUS protein-protein interactions in DNA repair

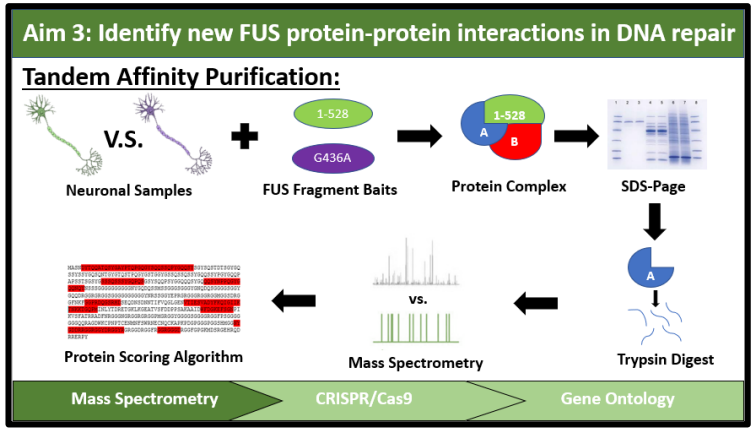


Hypothesis: Mass Spectrometry will highlight new DNA repair proteins associated with FUS.

Mass Spectrometry → CRISPR/Cas9 → Gene Ontology







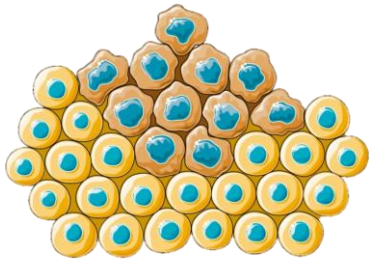
Conclusion:

Sequence alignment and CRISPR/Cas9 will allow identification of conserved protein sequences associated with DNA repair.

Small molecules associated with the inhibition or promotion of DNA repair proteins could rescue DNA repair defects.

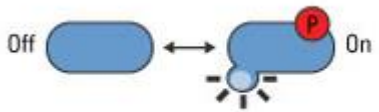
Mass Spectrometry will highlight new protein-protein interactions of FUS normally associated with DNA repair.

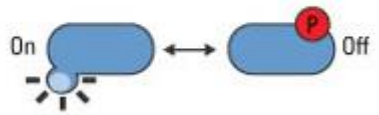
Future Directions



Why do FUS-ALS individuals fail to develop cancer?

Drug administration at different time points





Phosphorylation of partially conserved human variants

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Note: All images not created by myself are hyperlinked to their original source.