Development of a Robust Zinc Finger Activation Platform for Treatment of Neurological Disorders

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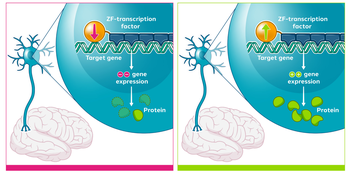
Poster #1609

Zinc finger (ZF) activation platform

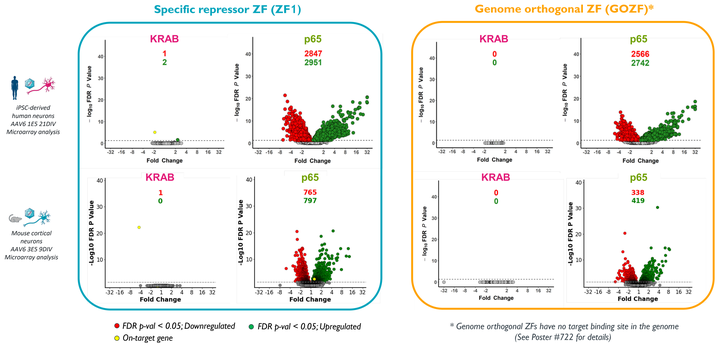
Modulation of target genes to controlled therapeutic levels in brain cells



- **ZF-repressors (ZFRs) and activators (ZFAs)** allow reversible, tunable modulation of target genes and are amenable to multiplexing.
- **ZFAs** enable treatment of neurological diseases that are caused by haploinsufficiency or gene silencing.
  - Sangamo's platform is focused on leveraging human-derived components to minimize potential immunological responses
  - The widely-used p65 was chosen as an initial transactivation domain (TAD)

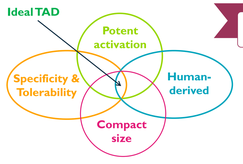
In contrast to ZF-KRAB, ZF-p65 candidates exhibited high numbers of differentially expressed genes (DEGs)

- In ZF-p65 activator screens, high numbers of DEGs were routinely observed in neurons.
- Expression of ZF-p65 in various neuronal cells indicates p65 has a suboptimal specificity profile when expressed long-term at high levels in neurons



* Genome orthogonal ZFs have no target binding site in the genome (See Poster #722 for details)

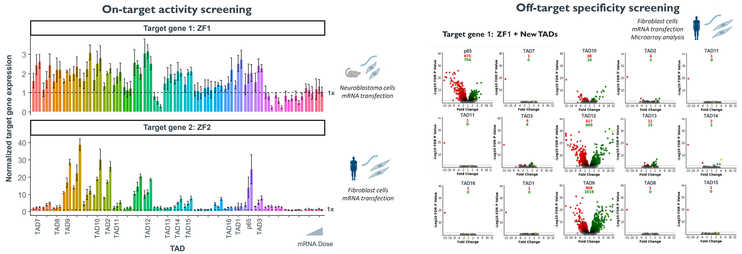
Identification of new TADs with improved specificity profiles



Design and assemble ZFAs → Activity screening → Specificity screening → Neuron validation → Mouse tolerability

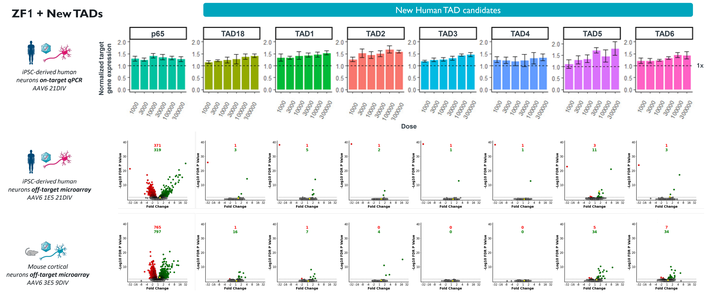
- New TADs fused to well-characterized ZFs were screened in high throughput in human and mouse cells
- Iterative process improving on identified candidates
- Activity measured by qPCR and specificity measured by microarray

TAD candidates displayed a wide range of activity and specificity



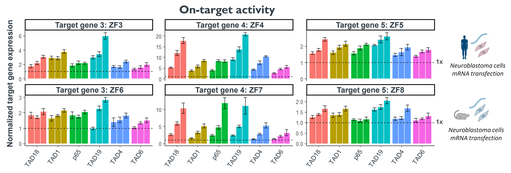
- A total of 110 TADs were screened for on-target activity and 50 TADs screened for off-target specificity

New TADs maintain on-target activity with greatly enhanced specificity profiles in both human and mouse neurons



- Some DEGs observed in TAD samples were common across TADs tested which suggests a ZFP off-target or a downstream effect of target gene upregulation

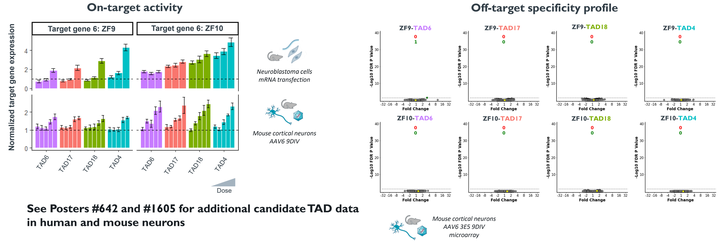
TADs are compatible with multiple ZFs across multiple targets



- Testing with additional ZFs and targets showed that TADs were active in a dose-dependent manner
- The data show the new TADs are interchangeable modules that may be used with our ZFs

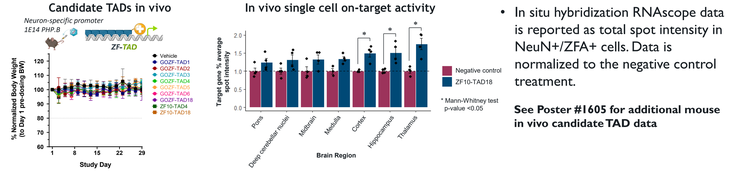
Validation of activity and specificity of additional ZFAs in neurons

- Additional ZFAs with new TADs were found to maintain activity and specificity in neurons



See Posters #642 and #1605 for additional candidate TAD data in human and mouse neurons

ZFAs with candidate TADs are active and well-tolerated in mice at high doses



- In situ hybridization RNAscope data is reported as total spot intensity in NeuN+/ZFA+ cells. Data is normalized to the negative control treatment.

See Poster #1605 for additional mouse in vivo candidate TAD data

- All candidate TAD mouse groups completed the study with no loss in body weight and clinical observations were normal
- qPCR of neuroinflammation markers did not show significant differences from control groups

Summary

- In early studies, high level, long-term expression of candidate ZF-p65 constructs resulted in high numbers of DEGs in neuronal cells
- We identified a panel of human-derived, active TADs with high specificity including engineered proprietary TADs
  - TADs have a range of activity, which provides a toolbox to fine tune levels of gene activation
- Candidate TADs maintained activity and specificity in mouse and human neuronal cells
- Candidate TADs were validated with additional activation targets demonstrating the modularity of Sangamo's ZFA platform
- Genome orthogonal ZFAs fused to new TADs were well-tolerated in vivo even at high AAV doses
- These data support further development of Sangamo's ZFA therapeutics to treat neurological disorders caused by haploinsufficiency and gene silencing

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