Recombinant Human Z-FUS-5 scFv Antibody

Gene Name	FUS
Protein Name	RNA-binding protein FUS
Synonyms	ALS6, ETM4, FUS1, HNRNPP2, POMP75, TLS
Uniprot ID	P35637
Description	DNA/RNA-binding protein that plays a role in various cellular processes such as transcription regulation, RNA splicing, RNA transport, DNA repair and damage response [1]. Binds to nascent pre-mRNAs and acts as a molecular mediator between RNA polymerase II and U1 small nuclear ribonucleoprotein thereby coupling transcription and splicing [2]. Binds also its own pre-mRNA and autoregulates its expression; this autoregulation mechanism is mediated by non-sense-mediated decay [3]. Plays a role in DNA repair mechanisms by promoting D-loop formation and homologous recombination during DNA double-strand break repair [4]. In neuronal cells, plays crucial roles in dendritic spine formation and stability, RNA transport, mRNA stability and synaptic homeostasis.
Antigen Coverage	G266 – Y468 of 526
Antigen Sequence (Antigen - C-terminal linker and Avi tag)	SMGGPRDQGSRHDSEQDNSDNNTIFVQGLGENVTIESVADY FKQIGIIKTNKKTGQPMINLYTDRETGKLKGEATVSFDDPPSAK AAIDWFDGKEFSGNPIKVSFATRRADFNRGGGNGRGGRGRG GPMGRGGYGGGGSGGGGRGGFPSGGGGGGGQQRAGDW KCPNPTCENMNFSWRNECNQCKAPKPDGPGGGPGGSHMG GNYSSKGGYGLNDIFEAQKIEWHE
Z-FUS-5 scFv Sequence (V_H - linker - V_L)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYGMYWVRQAP GKGLEWVSSISGGGSSTYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCARESGSYGIDYWGQGTLVTVSSGGGGSG GGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYL NWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQGVALLTFGQGTKLEIK
Antibody Fragment Generation and Production	Z-FUS-5 was generated by phage display technology from a human synthetic scFv library. The scFv was produced by bacterial expression in *E. coli,* with secretion to the periplasm, and purified by protein A affinity. The final protein batch of Z-FUS-5 scFv also carries a C-terminal His_6 tag and a 3xFLAG tag.
Validated Applications	ELISA, HTRF, Luminex, SPR, and IP-MS.

Enzyme-Linked ImmunoSorbent Assay (ELISA):

Method Description	ELISA screens were performed to assess binding of selected single chain fragment variable (scFv) antibody clones. Each clone was tested in duplicate against its target antigen and an unrelated non-target control (Primary ELISA). Positive clones were selected and screened against its target antigen and a streptavidin control (Secondary ELISA). ELISA screens were performed in 384 well plates coated with [1ug/mL] streptavidin. Biotinylated antigens were diluted in PBT buffer (0.5% BSA, 0.05% Tween 20 in PBS) to [1ug/mL] and added prior to the

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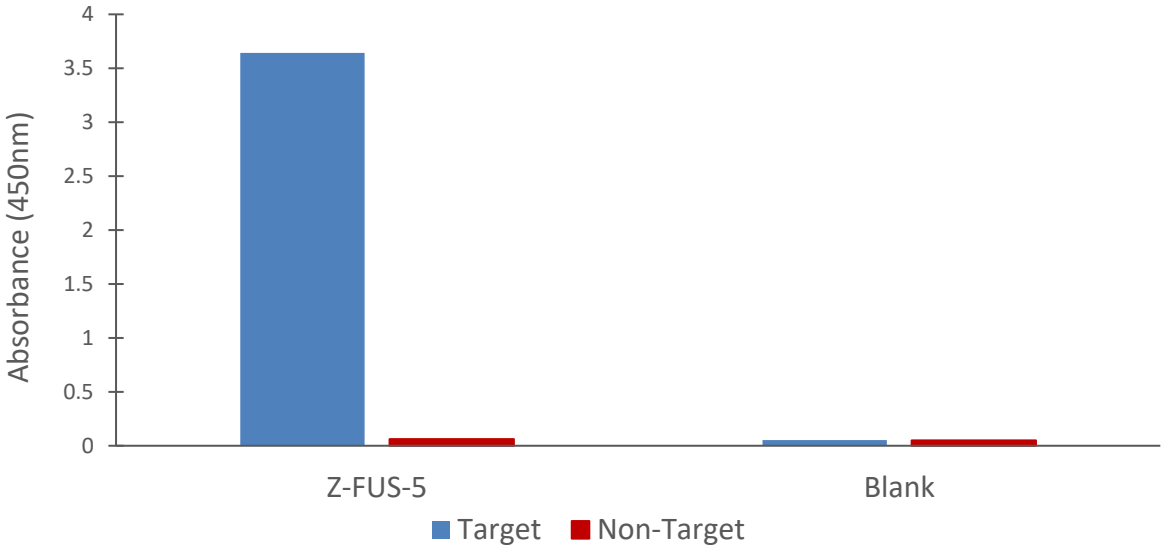
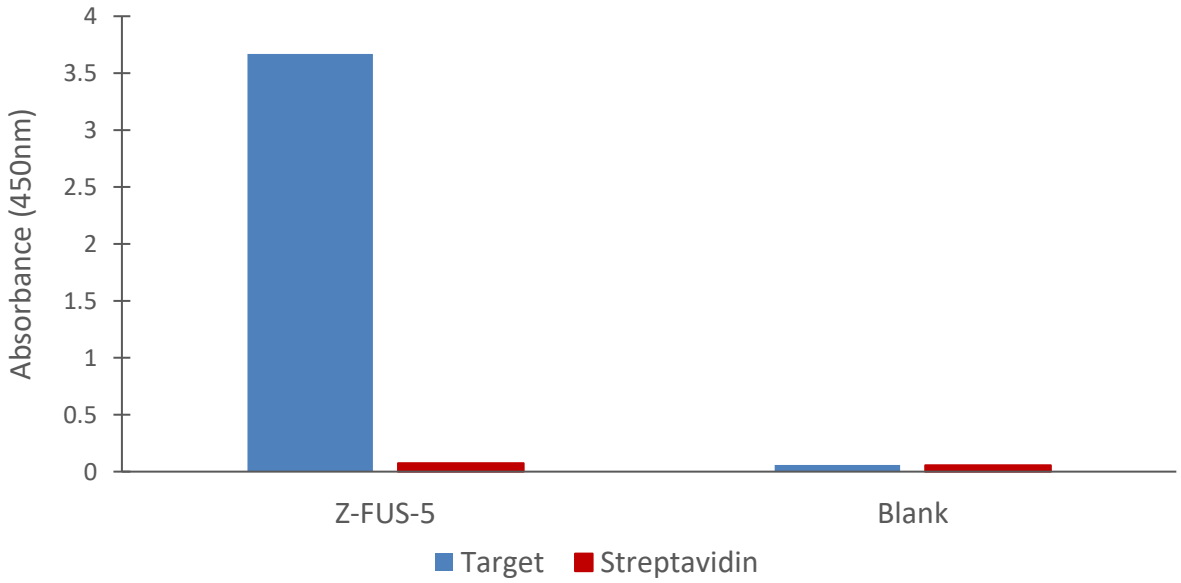
	addition of scFv bacterial culture supernatants. Bound scFvs, which contain a FLAG-tag, were detected using an HRP conjugated anti-FLAG M2 antibody (Sigma, [1:10,000]). TMB substrate solution (Thermo Scientific) was used as a chromogenic substrate, before the final addition of 1M H_2SO_4 stop solution. Plates were washed four times in between each step listed above with PBS-T (0.05% Tween 20 in PBS). Absorbance was measured at 450nm. Only scFv clones with a high enough absorbance ratios (Target / Non-Target and Target / Streptavidin) were selected for further analysis. ELISA results for Z-FUS-5 are shown below (blank indicates no scFv was added).

Z-FUS-5 binding in Primary ELISA screen:



Z-FUS-5 binding in Secondary ELISA screen:



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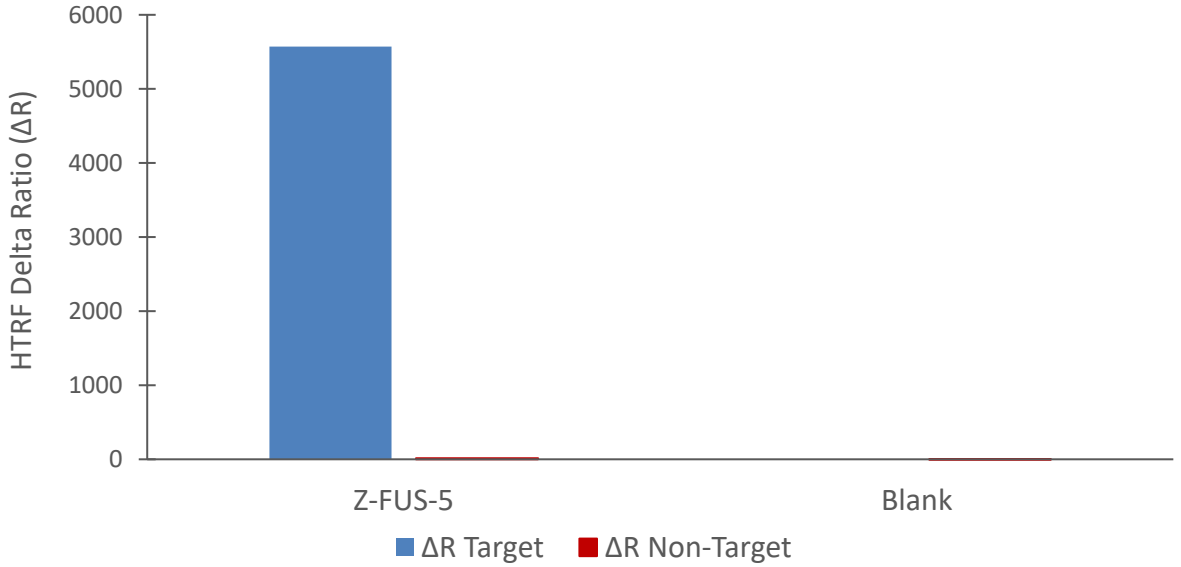
Homogenous Time Resolved Fluorescence (HTRF):

Method Description	HTRF technology was employed to robustly and reliably determine, in solution, the binding of each scFv antibody to its target antigen. Sequence unique clones, that tested positive in ELISA, were evaluated by HTRF. Supernatants from bacterial cultures containing scFvs were diluted in assay buffer (0.1% BSA in PBS), then mixed with biotinylated target antigens to a final concentration of 50nM. Donor anti-FLAG M2 antibody labeled with Terbium (Cisbio) and acceptor Streptavidin labeled with XL665 (Cisbio) were added to each scFv in 384 well plates. Samples were incubated for 2 hours at RT. The binding signal (665nm) and the background/noise signal (615nm) were measured using a EnVision instrument (PerkinElmer). HTRF ratios (665nm Acceptor / 620nm Donor) were calculated. Delta ratios (ΔR) were determined by subtracting the background from the mean emission fluorescence ratio for each sample. Delta ratios were plotted to compare the energy transfer signal for each scFv to its non-target control. Results for Z-FUS-5 are shown in the graph below (blank indicates no scFv was added).

HTRF results for Z-FUS-5:



Luminex Assay:

Method Description	The specificity of the anti-FUS scFv antibodies were evaluated against 48 different antigens (including FUS) using a bead based multiplex Luminex assay. Color-coded magnetic beads (Magplex Luminex Corp.) were activated with [5mg/mL] NHS and EDC in activation buffer (0.1M NaH_2PO_4, pH 6.2), then added to 96 well plates and incubated with shake at RT for 20min. Beads were washed twice with MES buffer (0.05M 2-(N-morpholino) ethane sulfonic acid, pH 5.0) before adding neutravidin and incubating for 2 hours at RT with shake. Beads were then washed twice with PBS-T before storage buffer (Blocking Reagent for ELISA (Roche) with 0.1% ProClin300 (Sigma) was added and incubated

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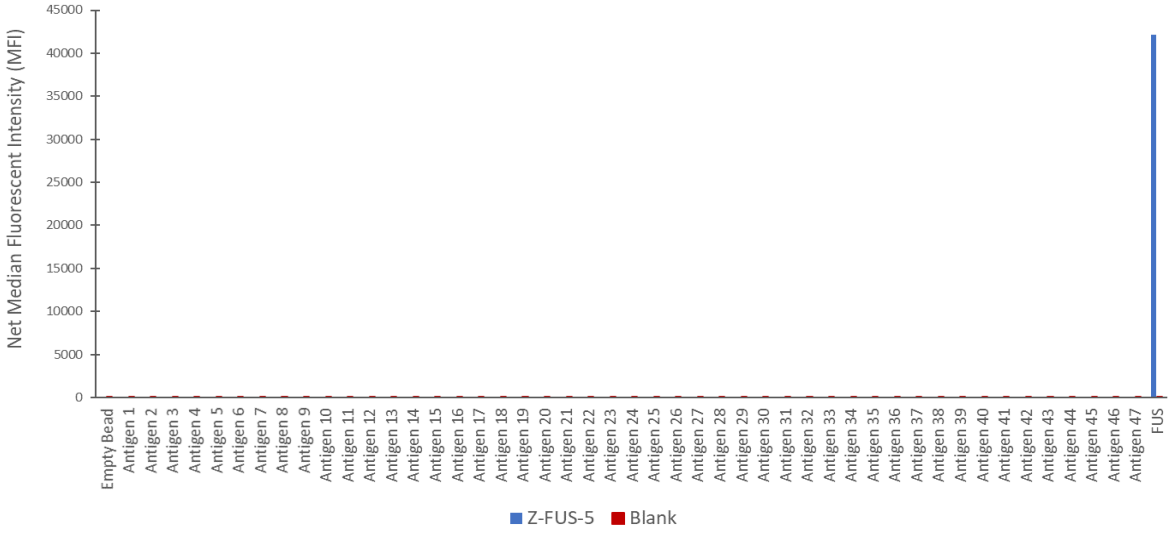
	overnight at 4°C. Storage buffer was removed and biotinylated proteins (1μM in PBS) were added to neutravidin coupled beads then incubated for 1 hour at RT with shake. Plates were washed 3x with PBS-T, storage buffer was added, and incubated at 4°C overnight. scFv antibodies were diluted in assay buffer (3% BSA, 0.05% Tween 20, and [10μL/mL] neutravidin in PBS) and incubated for 1 hour at 4°C. A beadstock was prepared with coupled beads in storage buffer at a concentration of 100beads/ID/μL. The beadstock solution (5μLs) was mixed with each scFv (45μL) in 384 well plates and incubated for 1 hour at RT with shake. Plates were washed 3x with PBS-T and anti-FLAG RPE (Prozyme, [1:1000]) was added for 30min at RT with shake. Plates were washed 3x in PBS-T and samples were analyzed on a FLEXMAP3D (Luminex Corp). Luminex results for Z-FUS-5 are plotted below. Graph illustrates the degree of specificity of Z-FUS-5 to bind to its respective antigen (FUS) and none of the other 47 antigens (blank indicates no scFv was added).

Luminex results for Z-FUS-5:



Surface Plasmon Resonance (SPR) Affinity Measurements:

Method Description	Kinetic measurements were performed using SPR on a Biacore T200 (GE Healthcare). The anti-FLAG M2 antibody (Sigma) was used to capture the FLAG-tagged scFv before antigen injection. Single Cycle Kinetics was performed. Accordingly, five concentrations of antigen are sequentially injected in increasing order within the same cycle before regeneration with Glycine-HCl, pH 2.5. Antigen concentrations tested against Z-FUS-5 include: 0.16, 0.8, 4, 20, and 100nM with a flowrate of 30μL/min. The kinetic constants were calculated using the Biacore T200 Evaluation Software 3.1 and the 1:1 Langmuir binding model, after removal of the reference cycle (running buffer instead of antigen) and the reference channel. Transient spikes from *e.g.* air bubbles were also removed from the obtained sensorgrams. The Single Cycle Kinetic sensorgram for Z-FUS-5 is shown below.

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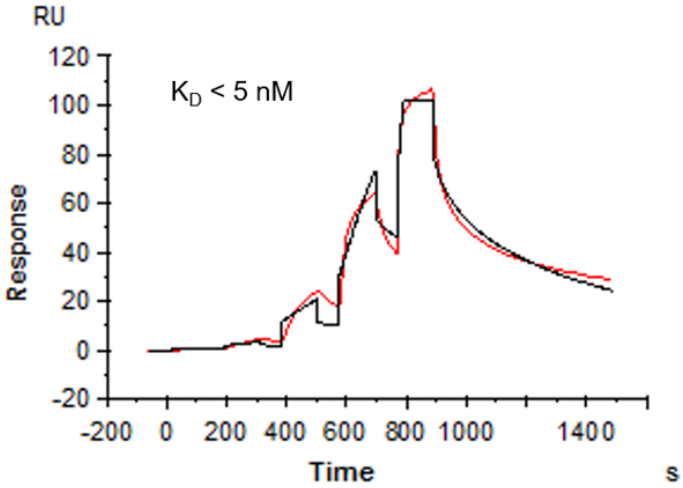
Single Cycle Kinetics curve for Z-FUS-5:



Preliminary data - optimization on-going

Immunoprecipitation – Mass Spectrometry (IP-MS):

Method Description	Immunoprecipitation followed by mass spectrometry was employed to verify the interaction specificity of each scFv antibody with its intended target. Immunoprecipitation was performed on HEK293 cell lysates expressing endogenous FUS protein as previously described [5]. Obtained samples were loaded onto a Dionex Ultimate 3000 HPLC system (Thermo Fisher Scientific) coupled to an o-QTOF impact II™ (Bruker Daltonics) mass spectrometer with a Captive Spray ion source (Bruker Daltonics). Data was analyzed with Protein Scape software (Bruker Daltonics) using Mascot search engine (Matrix Science Ltd.). Normalized spectral abundance factor (NSAF) values were calculated as previously described [5].

Z-FUS-5 was able to capture endogenous FUS from HEK293 cell lysates. The target protein was at the top of the list of immunoprecipitated proteins with an NSAF value of 99 and 7 unique peptides detected. No other specific interactors were found. More detailed data can be shared upon request.

Comments and Contact:

Three additional scFv antibodies against FUS, from the same selection campaign, passed all validation criteria, but with slightly lower values. Sequences for those can be provided upon request. For all inquiries, please contact Dr. Susanne Gräslund (susanne.graslund@ki.se) at SGC Karolinska.

References:

1. Yamaguchi, A. and K. Takanashi, *FUS interacts with nuclear matrix-associated protein SAFB1 as well as Matrin3 to regulate splicing and ligand-mediated transcription.* Sci Rep, 2016. **6**: p. 35195.
2. Yu, Y. and R. Reed, *FUS functions in coupling transcription to splicing by mediating an interaction between RNAP II and U1 snRNP.* Proc Natl Acad Sci U S A, 2015. **112**(28): p. 8608-13.
3. Zhou, Y., et al., *ALS-Associated FUS Mutations Result in Compromised FUS Alternative Splicing and Autoregulation.* PLOS Genetics, 2013. **9**(10): p. e1003895.
4. Baechtold, H., et al., *Human 75-kDa DNA-pairing Protein Is Identical to the Pro-oncoprotein TLS/FUS and Is Able to Promote D-loop Formation.* J. Biol. Chem, 1999. **274**(48): p. 34337-42.
5. Persson, H., et al., *Antibody Validation by Immunoprecipitation Followed by Mass Spectrometry Analysis*, in *Synthetic Antibodies: Methods and Protocols*, T. Tiller, Editor. 2017, Springer New York: New York, NY. p. 175-187.