**Supplementary Materials and Methods**

*UV-CLIP*

Hek293 cells were plated on 10 cm dishes and transfected with pscFv9 vectors. After 48h cells were irradiated with UV (15000 µjoule, Stratalinker, Stratagene), harvested, lysated in NT2 Buffer (Tris pH7.4 50mM, NaCl 150mM, MgCl2 1mM, 0.5%, protein solubilizer 40 (A.G. Scientific), RNasin (Promega), Superasin (Ambion) and proteases inhibitors (Sigma)) with 5-6 passages in 27G needle syringe and centrifuged at 14000 rpm for 10min. Supernatant (0.8 mg) were incubated overnight at 4°C with the precoated beads. Dynabeads-G (Invitrogen) were blocked overnight in NT2 - 5% BSA without inhibitors and then precoated with 2.6 µg anti-TDP-43 antibody (Proteintech) or normal rabbit IgGs (Santa Cruz) overnight at 4°C. After four washes with NT2 Buffer, beads were treated with 0.1mg of proteinase K (Promega) in Tris 100mM pH 7.4, NaCl 50mM, EDTA 10mM for 15min at 56°C. An aliquot was taken for western blot analyses before proteinase K digestion. RNAs were purified from beads using Trizol (Thermo Fisher Scientific) according to the manufacturer's instructions. The RNA quality and the absence of genomic DNA was verified by Bioanalyser (Agilent). Maxima H minus RT (Thermo Fisher Scientific) was used for the reverse transcription of RNA to cDNA with oligo(dT) and random primers. Human *TDP-43* was amplified by RT-PCR on 20ng cDNA with Taq polymerase (Feldan Therapeutics) and the following primers rev: 5' AACCCCTTTGAATGACCAGTCTTA, fwd: 5' GCTGGGGAAATCTGGTGTATG. A 240bp amplicon was visualized on an agarose gel with ethidium bromide (Sigma) on a GelDoc (Bio-Rad) apparatus.

*Cell survival assay*

Cell viability was assessed using MTS assay, as per the manufacturer's instructions (Promega). Absorbance was read at 490 nm using an EnSpire 2300 Multilabel reader (Perkin Elmer).

*Luciferase assay*

Assay was performed as previously published (1) using the Bright-Glo Luciferase assay system (Promega), according to the manufacturer's instructions. Results were expressed as luciferase activity (RLU)/μg cellular protein measured by BCA quantification (Bio-Rad).

*DCHFDA assay*

Assay was performed as previously published (2). Cells were incubated with dichlorofluorescin diacetate (Sigma) reagent for 1 hour in the dark and then lysated. Fluorescence (ex 485nm, em 535nm) was measured by in a TriStar2 (Berthold Technologies) plate reader. Results were expressed as Abs/μg cellular protein measured by BCA quantification (Bio-Rad).

*Western and Dot blot*

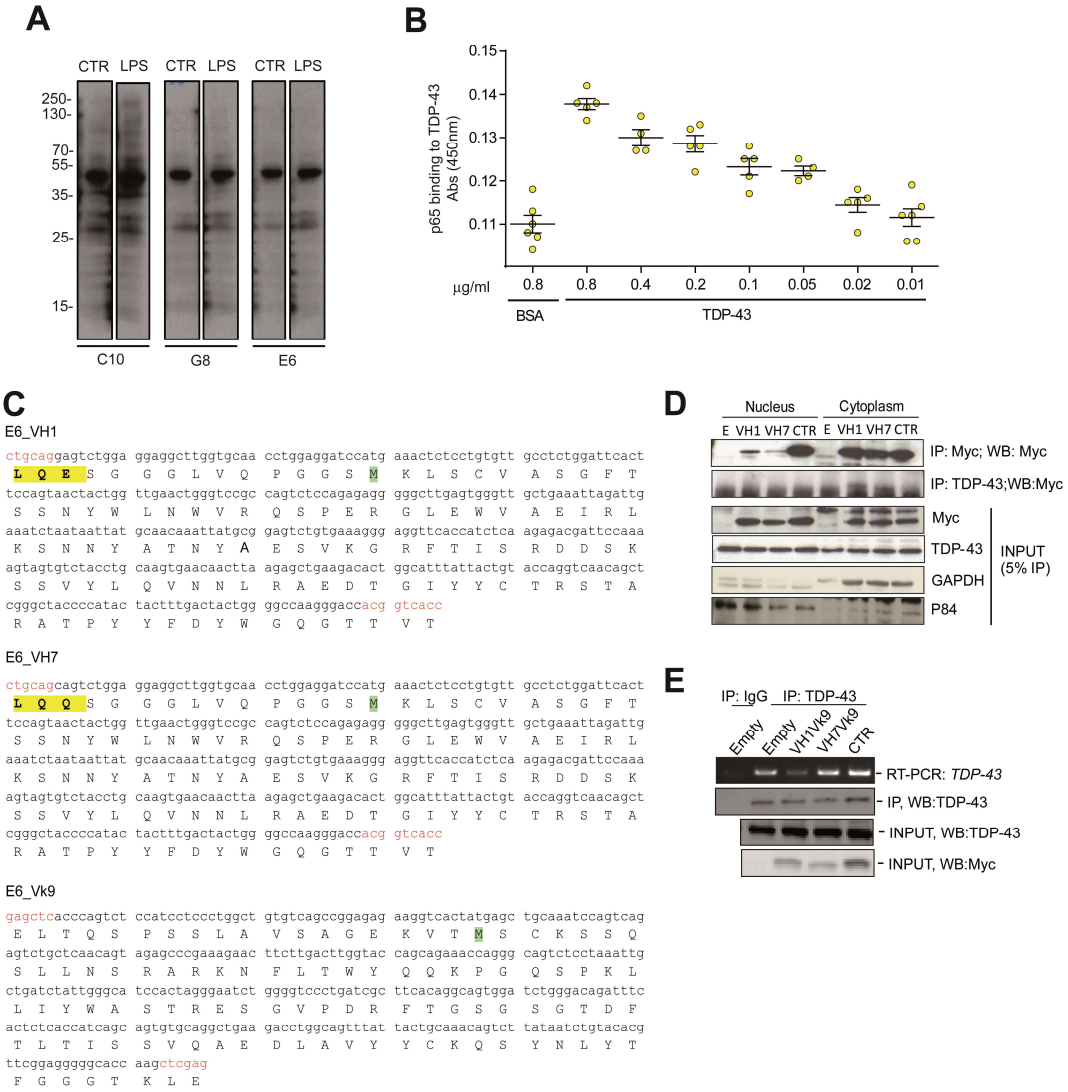
Equal amount of proteins (generally 20-30 μg) were resuspended in loading buffer supplemented with DTT (Sigma) 1mM, boiled 5 min at 100°C, loaded on 12% acrylamide home-made gels or precast gels (Bio-Rad) and transferred on PVDF membranes (Millipore). Dot blot were performed by loading equal amount of proteins or volumes of media on a PVDF membrane by vacuum filtration using the Dot-Blot apparatus (Bio-Rad). Membranes were incubated 1 h with blocking solution (BSA 3% or Milk 5% in TBS-Tween 0.1%), and then overnight in primary antibody diluted in blocking solutions (Supplementary Table 1), washed in TBS-Tween and incubated 2 h in secondary antibodies and finally washed in TBS-Tween. Goat anti-mouse HRP, goat anti-rabbit HRP or donkey anti-goat HRP (Jackson Immunoresearch) secondary antibodies were diluted 1:5000 in blocking, whereas IRDye-conjugated (Li-Cor) secondary antibodies were diluted 1:10000 in TBS-Tween and incubated in dark. Signal was acquired by exposing membranes to X-ray film (Biomax MR, Kodak) or using the Li-Cor sistem (Li-Cor Biotechnology). Total transfered proteins (TTP) were stained by incubation of membranes 5 min in Ponceau solution (Sigma) and washes in distilled water or by UV-acquisition of Stain Free staining (Bio-Rad) on membrane (3, 4).

Pictures of developed X-Ray films and Ponceau stained membranes were acquired by GelDoc system (Bio-Rad). Densitometry was done by Image Lab (Bio-Rad) or Image Studio Lite 4.0 (Li-Cor) softwares.

*Cytokines array*

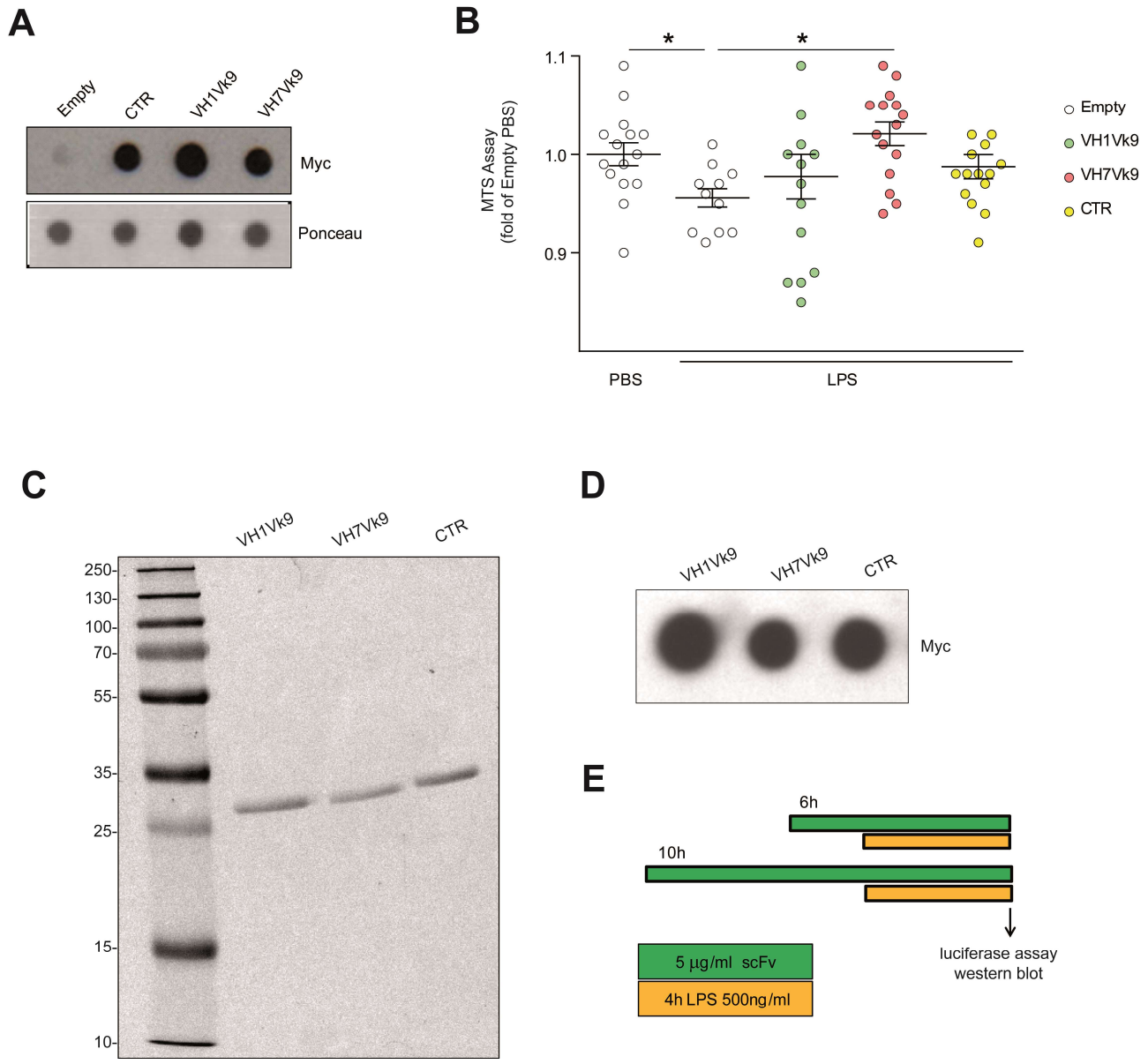
The cytokine array was performed as previously described (1). Briefly, samples, i.e. 300 μg of soluble cytoplasmic portion from mice tissues, were incubated with array membranes from mouse cytokine array kit (Raybio) and results were obtained according to manufacturer's instructions.

## Supplementary Figure 1



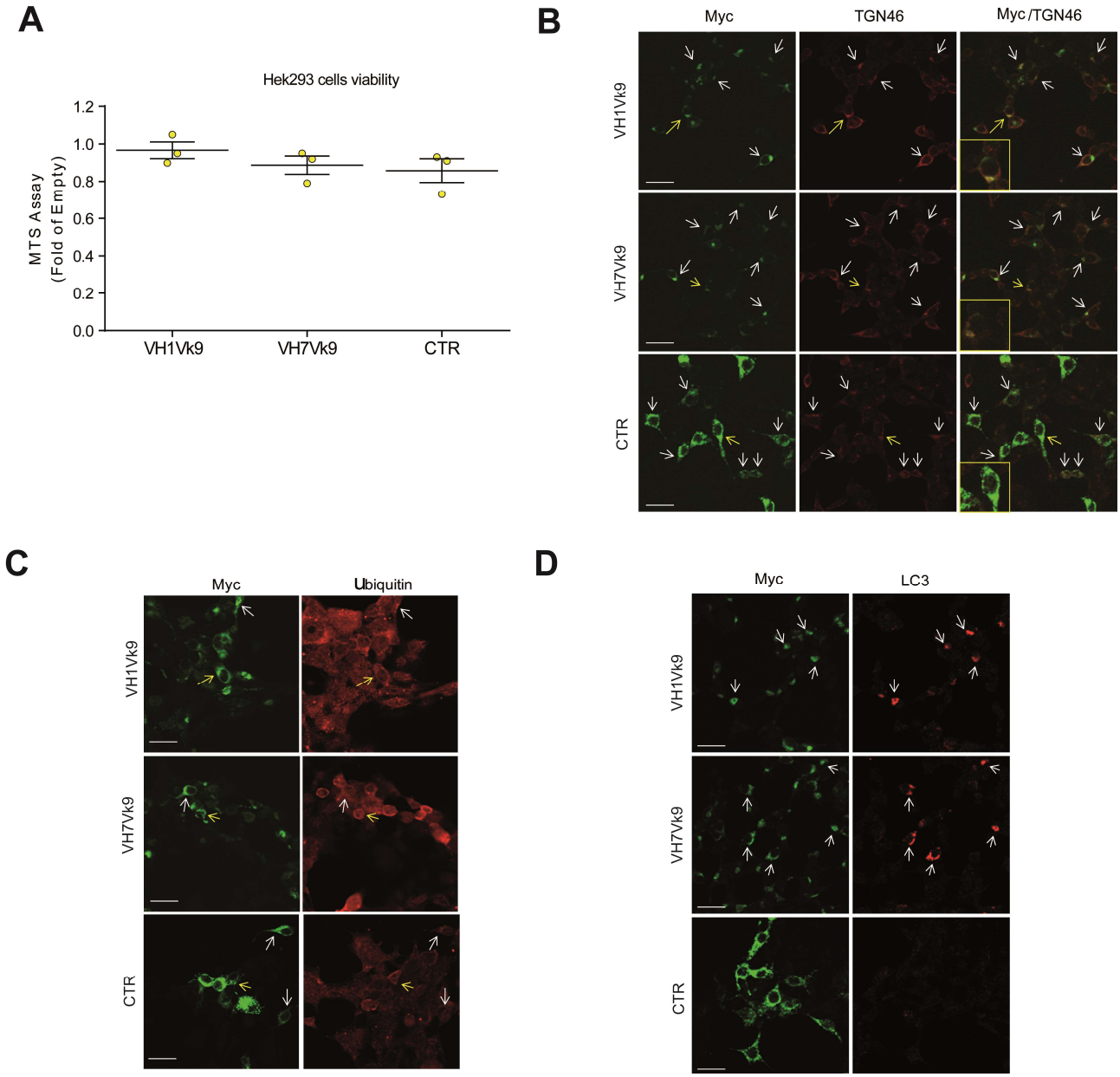

**Suppl. Fig. 1 Selection and characterization of monoclonal and scFv antibodies.** (**A**) Selection of monoclonal antibodies able to recognize TDP-43. Representative picture of western blot (replicated twice) of nuclear extracts of BV2 microglial cells treated with or without LPS and probed with monoclonal antibodies named C10, G8 and E6. (**B**) Elisa assay demonstrating the direct protein-protein interaction between TDP-43 and p65. Different concentrations of TDP-43 and BSA, as control, were incubated with p65 His-Tag, n=4-6 wells per condition, One-way Anova $p<0.0001$ and $p<0.0001$ by post test for linear trend. (**C**) Nucleic acid and amino acid sequence of E6 derived variable heavy chains (VH1 and VH7) and light chains (Vk9) used for the production of single chain antibodies. Digestion enzymes sequences are labelled in red, methionine in green and the amino acids difference between VH1 and VH7 are highlighted in yellow. (**D**) VH1Vk9 and VH7Vk9 specifically recognize TDP-43 in the cytoplasm. Representative picture of TDP-43 and scFv immunoprecipitation in nuclear and cytoplasmic fractions of Hek293 pscFv9-transfected cells. Experiment was replicated three times. Anti-myc antibody was used for scFv detection, GAPDH and p84 were used as fractions markers. (**E**) VH1Vk9 but not VH7Vk9 alters the interaction between TDP-43 and its RNA. UV-CLIP experiment performed in Hek293 transfected cells. Representative picture of RT-PCR and western blot for *TDP-43* performed on cDNA and proteins immunoprecipitated with anti-TDP-43 antibody- or normal IgG- coated beads. Presence of TDP-43 and scFv (myc) was confirmed by western blot on 20µg input. Experiment was replicated at least 3 times. E=Empty, no scFv; CTR, D1.3 scFv. Graph shows mean ± sem.

**Supplementary Figure 2**



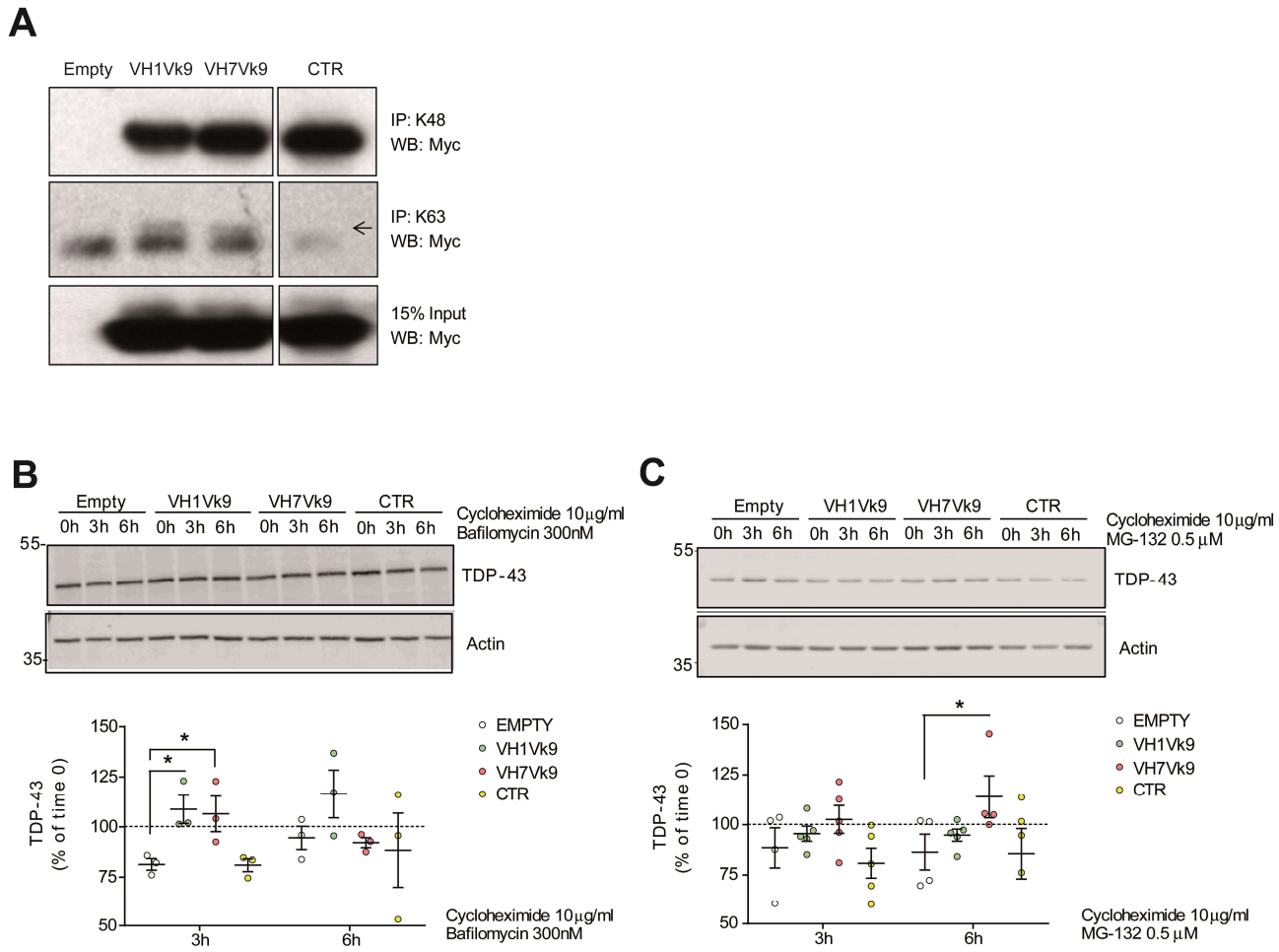

**Suppl. Fig. 2 Single chain antibodies purification and effect on microglial-mediated toxicity toward neurons**. (**A**) Representative picture of dot blot experiment showing the presence of scFv in the media of transfected cells. Western blot was performed by anti-myc antibody and Ponceau was used as loading reference. Experiment was repeated more than three times. (**B**) N2A cells were treated with media of transfected BV2 cells stimulated for 4 hours with LPS. Viability of N2A cells was assessed by MTS assay in n=12-15 samples (dots) from 3 independent experiments (4-5 wells each), One-way Anova $p=0.0451$, * $p<0.05$ by Sidak's multiple comparison test. (**C**) Purified scFv can be detected by Coomassie staining. 1 µg of purified scFv was loaded on a 12% acrylamide gel and subsequently stained with Coomassie. One unique band at 28kDa corresponding to scFv can be detectable. Experiment was repeated more than three times. (**D**) Representative dot blot experiment where 1 µg of purified scFv was loaded and blotted with anti-myc-HRP antibody. Experiment was repeated more than three times. (**E**) Representative schema of BV2 cells treatment. Cells were treated for 6 h or 10 h with scFv, or untreated with any scFv, together with 4h LPS, luciferase assay and western blot were performed soon after. Empty, no scFv; CTR, D1.3 scFv. Graph shows mean ± sem.

**Supplementary Figure 3**



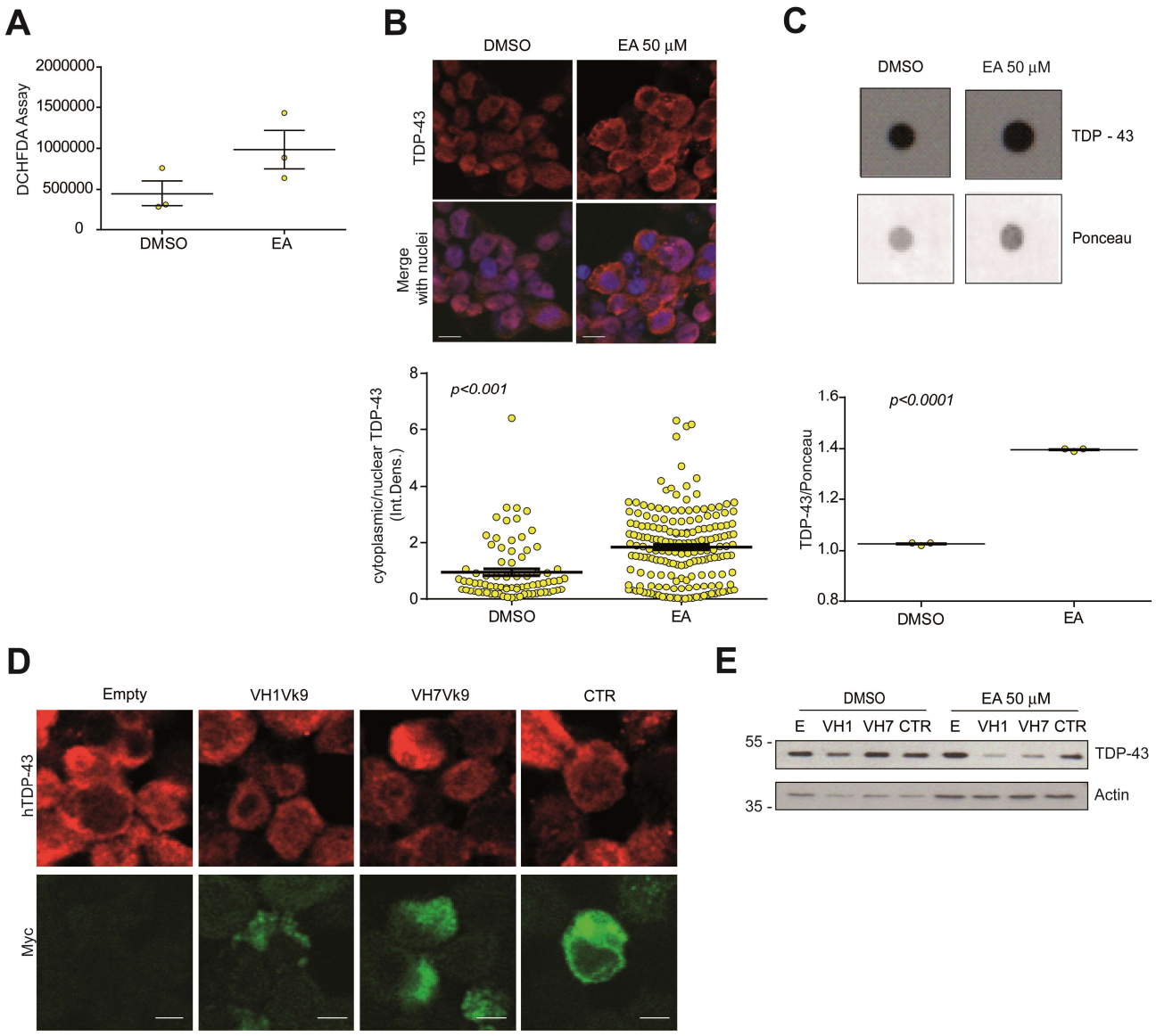

**Suppl. Fig. 3 ScFv did not alter cell viability of transfected cells.** (**A**) Survival assay on Hek293 pscFv9-transfected cells. Viability of cells was assessed by MTS assay on n=3 independent experiments (dots), One-way Anova p=0.174. Data are expressed and fold of Empty. (**B**) Representative images of immunofluorescence for scFv (myc, green) and the trans-Golgi network (TGN46, red) and merge. Arrows show cells with co-localization, yellow arrow shows cells enlarged 2.5 times in merge. Scale bar = 20 µm. (**C**) Representative images of single channel immunofluorescence for scFv (myc, green) and ubiquitin (red) corresponding to merged images in figure 3**B.** Arrows show cells with co-localization, yellow arrow shows cells enlarged 2.5 times in figure 3**B**. Scale bar = 20 µm. (**D**) Representative images of single channel immunofluorescence for scFv (myc, green) and LC3 (red) corresponding to merged images in figure 3**C.** Arrows show cells with co-localization, yellow arrow shows cells enlarged 2.5 times in figure 3**C**. Scale bar = 20 µm. Empty, no scFv; CTR, D1.3 scFv. Graph shows mean ± sem.

**Supplementary Figure 4**



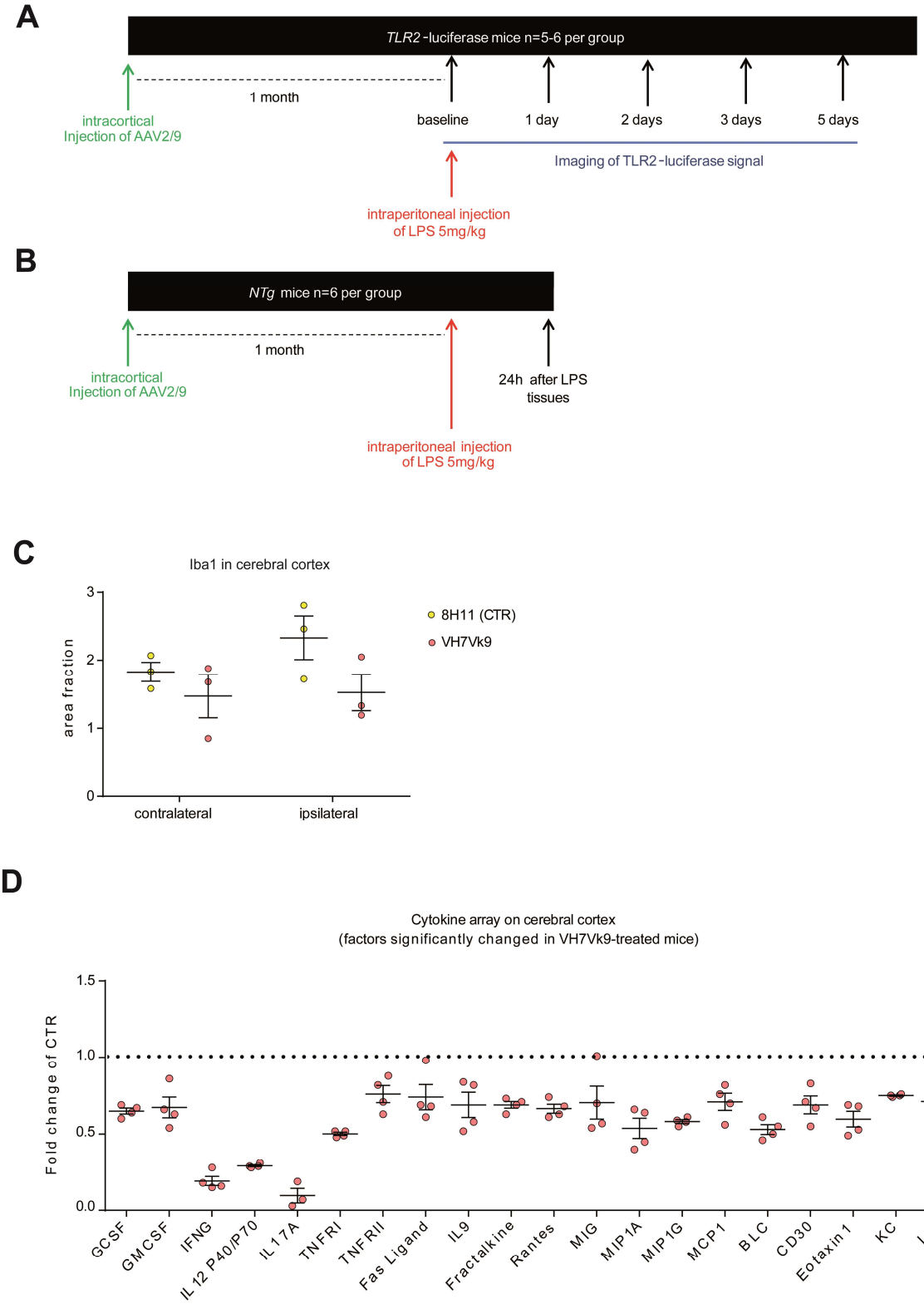

**Suppl. Fig. 4 ScFv mediated TDP-43 degradation through proteasome and autophagy pathways.** (**A**) Representative picture of immunoprecipitation for all K48- (proteasome) or K63-(autophagy) poly-ubiquitinated proteins and western blot for myc. Experiment was repeated three times. Lanes were run on the same gel but were noncontigous. (**B**,**C**) Representative picture of western blot for TDP-43 and quantification in Hek293 pscFv9-transfected cells treated with cycloheximide 10µg/ml and bafilomycin 300nM (**B**) or MG-132 0.5 µM (**C**). (**B**) Graph represents quantification of TDP-43 immunoreactivity and is expressed as percentage of time 0 (before treatment), n=3 independent experiments (dots). Two-way Anova interaction p=0.4706, time p=0.5997, scFv p=0.0306, *p<0.05 versus empty transfected cells by Dunnett's multiple comparison test. (**C**) Graph represents quantification of TDP-43 immunoreactivity and is expressed as percentage of time 0 (before treatment), n=4-5 independent experiments (dots). Two-way Anova interaction p=0.8520, time p=0.5777, scFv p=0.0272, *p<0.05 versus empty transfected cells by Dunnett's multiple comparison test. Empty, no scFv; CTR, 8H11 anti-GFP scFv. Graphs show mean ± sem.

**Supplementary Figure 5**




**Suppl. Fig. 5 TDP-43 mislocalization and aggregation obtained in cells by ethacrynic acid treatment.** (**A**) Empty transfected cells were treated with 50 µM ethacrynic acid (EA) or DMSO as vehicle. After 4 hours DCHFDA assay was performed to evaluate oxidative stress, p= 0.064 by Student T-Test, n=3 samples per condition (dots). (**B**) Empty transfected cells were treated with 50 µM ethacrynic acid (EA) or DMSO as vehicle. Representative enlarged picture and quantification of TDP-43 mislocalization determined by quantification of TDP-43 integrated density in nuclear and cytoplasmic compartements. Data are TDP-43 cytoplasmic to nuclear ratio quantified in 100-200 cells per conditions (dots) from 2 independent experiments, p<0.0001 by unpaired T-Test. Scale bar=20µm. (**C**) Representative picture and quantification of dot blot analysis of insoluble TDP-43 in transfected Hek293 cells treated with 50 µM ethacrynic acid (EA). TDP-43 immunoreactivity normalized on Ponceau from n=3 independent experiments (dots), p<0.0001 by unpaired T-Test. Spots were on the same membrane but were non-contiguous. (**D**) Representative images of single channels immunofluorescence for scFv (myc, green) and hTDP-43 (red) corresponding to merged images in figure 4**A**. Scale bar=5µm. (**E**) Representative western blot of insoluble TDP-43 in DMSO or EA treated cells. Western was performed on a pool of 3 experiments. Empty, no scFv; CTR, D1.3 scFv. Graph show mean ± sem.

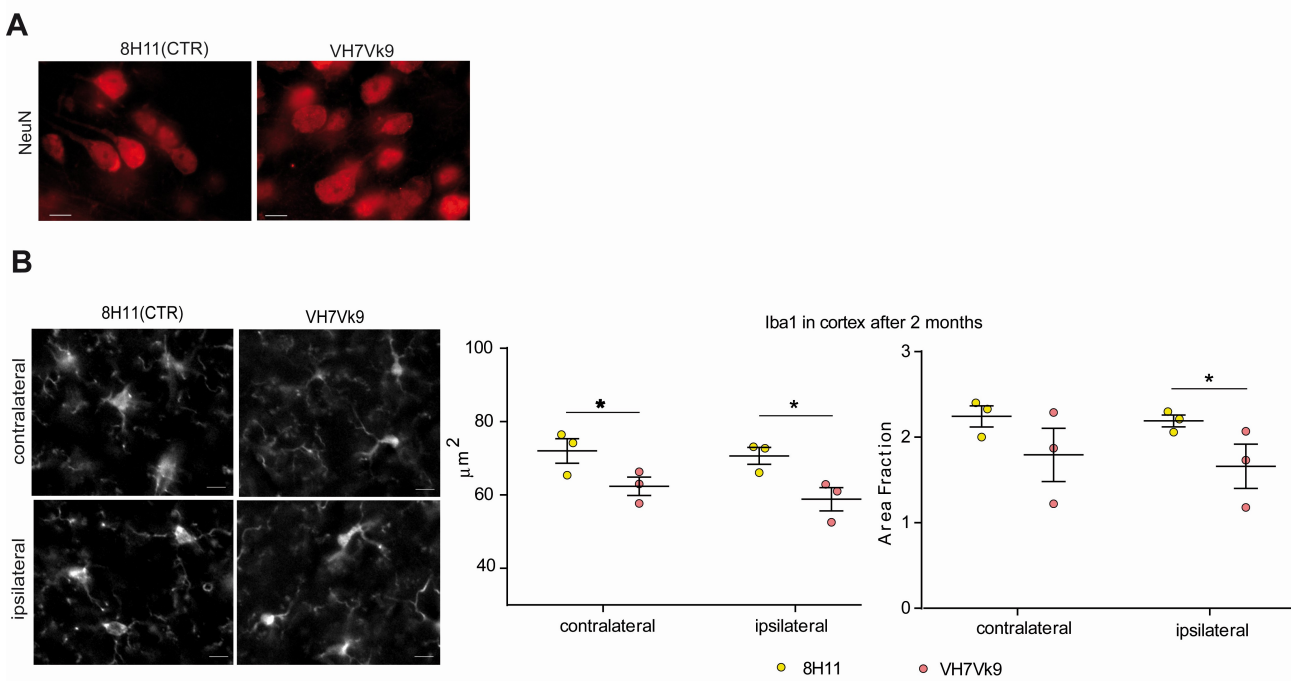
**Supplementary Figure 6**




**Suppl. Fig. 6 Single chain antibody reduced LPS-induced inflammation in vivo**. (**A**, **B**) Schematic representation of the experiment in *TLR2*-luciferase mice (**A**) and non-transgenic (NTg) mice (**B**). (**A**) After 1 month from intracortical injection of scAAV2/9 virus, mice were imaged before (baseline) or after 1 to 5 days from intraperitoneal injection of LPS (5mg/Kg). (**B**) Ntg mouse tissues were collected after 24h from LPS injection. (**C**) Quantification of Iba1 area fraction in contra- and ipsilateral cortex of mice injected with scAAV2/9 and challenged with LPS. Graph represents quantification of total Iba1 staining (area fraction), n=3 independent mice (dots), Two-way Anova p=0.430. (**D**) Cytokine array was performed on ipsilateral cerebral cortices. Graph

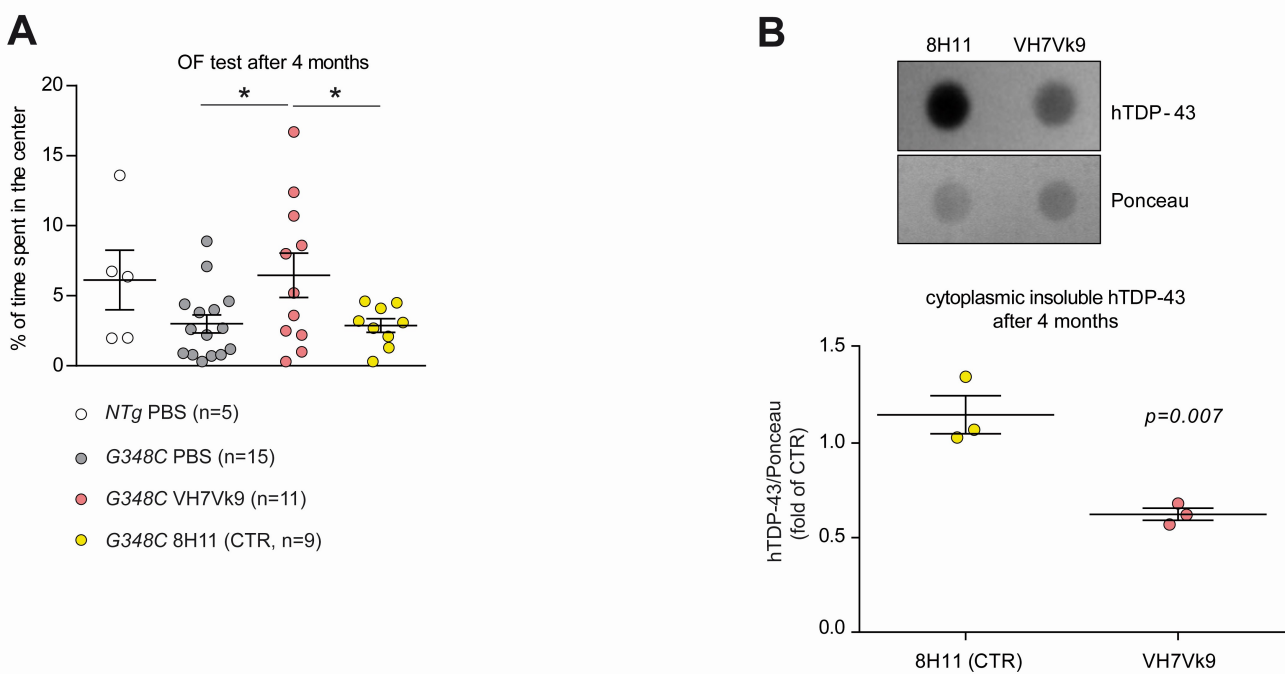
represents quantification of significantly changed factors in VH7Vk9-injected compared to 8H11-injected mice, n=3 independent mice pooled (array was run twice, dots) and expressed as fold of control (CTR=1, dotted line). $p<0.05$ for all shown factors in VH7Vk9 versus control scFv by unpaired T-Test. CTR, 8H11 anti-GFP scFv. Graphs show mean ± sem.

**Supplementary Figure 7**



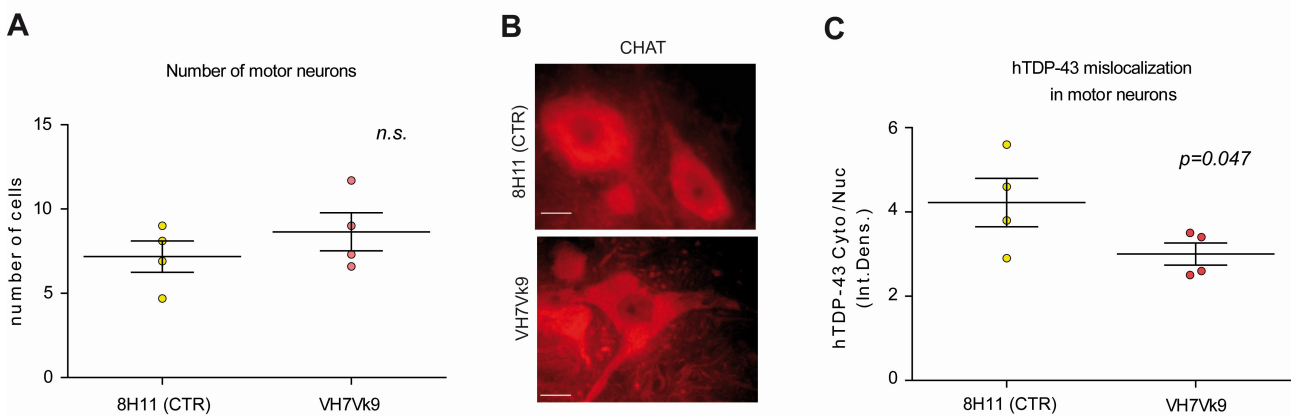

**Suppl. Fig. 7 scFv-mediated reduction of inflammation in TDP-43$^{G348C}$ mice after 2 months from injection.** (**A**) Representative picture of the single channel immunofluorescence for NeuN (red) in brain cortex corresponding to merged images in figure 6**C**, scale bar=20µm. (**B**) Representative enlarged picture of Iba1 staining in cortex and relative quantification of cell body size and area fraction, n=3 independent mice (dots). Two-way Anova for cell body size interaction p=0.7145, hemisphere p=0.4211, scFv p=0.0057 and for area fraction: interaction p=0.8568, hemisphere p=0.675, scFv p=0.0518, *p<0.05 versus control scFv by uncorrected Fisher's LSD. Scale bar=10µm. CTR, 8H11 anti-GFP scFv. Graphs show mean ± sem.

**Supplementary Figure 8**



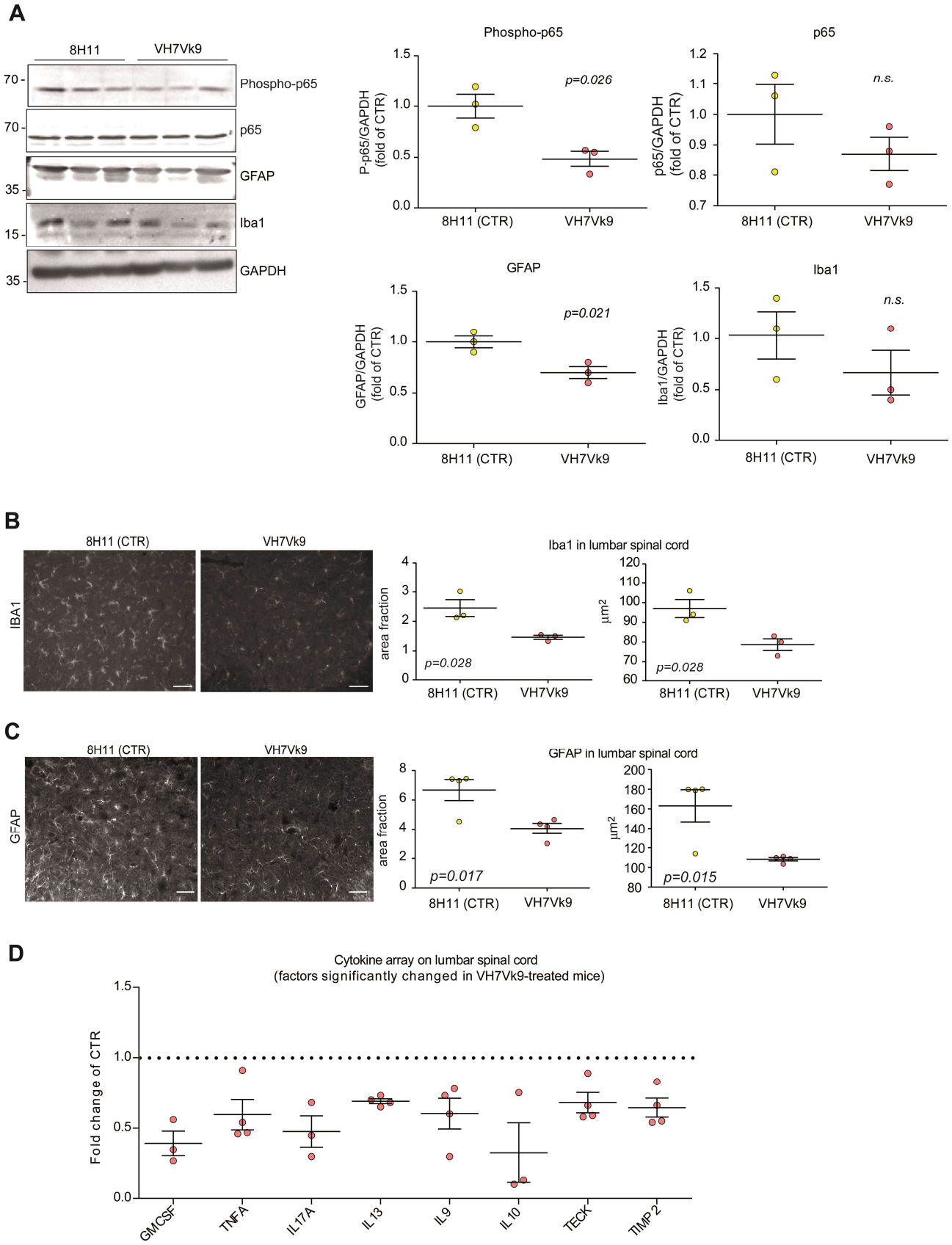

**Suppl. Fig. 8 scAAV2/9-mediated delivery of scFv reduced anxiety and TDP-43 aggregation in TDP-43$^{G348C}$ mice after 4 months from injection.** (**A**) Open field (OF) test after 4 months from injection. Graph show percentage of time spent in the center of the field and number of independent mice (dots) tested. One-way Anova p=0.0508, *p<0.05 by uncorrected Fisher's LSD. (**B**) Representative picture and quantification of insoluble cytoplasmic human TDP-43 in ipsilateral cortices of n=3 independent mice (dots). TDP-43 immunoreactivity was normalized on Ponceau and values were expressed as fold of control scFv. Statistical analysis was made by unpaired T-test. CTR, 8H11 anti-GFP scFv. Graphs show mean ± sem.

**Supplementary Figure 9**




**Suppl. Fig. 9 scAAV2/9-mediated delivery of scFv reduced TDP-43 mislocalization in motor neurons of TDP-43$^{A315T}$ mice after 2 months from injection.** (**A**) Quantification of motor neurons in lumbar spinal cord of mice injected with VH7Vk9 or CTR scFv. CHAT+ve cells larger than 250 μm$^2$ were counted in n=4 independent mice (dots). Statistical analysis was made by unpaired T-test, n.s. not significant. (**B**) Representative picture of the single channel immunofluorescence for CHAT (red) in lumbar spinal cord corresponding to merged images in figure 7**C**, scale bar=20μm. (**C**) Quantification of human TDP-43 in motor neurons, stained with CHAT, of lumbar spinal cord. Cytoplasmic to nuclear ratio of TDP-43 in CHAT+ve cells (bigger than 250 μm$^2$) quantified in 4 independent mice (dots). Statistical analysis was made by unpaired T-Test. CTR, 8H11 anti-GFP scFv. Graphs show mean ± sem.

**Supplementary Figure 10**




**Suppl. Fig. 10 scAAV2/9-mediated delivery of scFv reduced neuroinflammation in TDP-43$^{A315T}$ mice after 2 months from injection.** (**A**) Representative picture and quantification of cytoplasmic phospho-p65, p65, GFAP and Iba1 in n=3 independent mice (dots) lumbar spinal

cords. Proteins immunoreactivity was normalized on GAPDH and values were expressed as fold of control scFv. Statistical analysis was made by unpaired T-test. (**B**, **C**) Representative picture of Iba1 (**B**) or GFAP (**C**) staining in lumbar spinal cord and relative quantification of area fraction and cell body size in n=3 independent mice (dots). Statistical analysis was made by unpaired T-test, scale bar=50μm. (**D**) VH7Vk9 reduces NF-κB activity. Cytokine array was performed in lumbar spinal cords. Graph represents quantification of significantly changed factors in VH7Vk9-injected compared to 8H11-injected mice, n=3 independent mice pooled (array was run twice, dots) and expressed as fold of control (CTR=1, dotted line). $p<0.05$ for all shown factors in VH7Vk9 versus control scFv by unpaired T-Test. CTR, 8H11 anti-GFP scFv. Graphs show mean ± sem. n.s. not significant.

**Supplementary Table 1.** Antibodies used for experiments

| Antigen | Antibody/clone | WB dilution | IF dilution | Company | Catalog number |
|---|---|---|---|---|---|
| Actin | mouse monoclonal/ clone C4 | 1 :10000 | | Millipore (Temecula CA, USA) | MAB1501 |
| Choline acetyltransferase (CHAT) | rabbit polyclonal | | 1 :500 | Millipore | AB143 |
| C-myc | mouse monoclonal/ clone 9E10 | 1 :1000 | | Santa Cruz | sc-40 |
| C-myc-HRP conjugated | mouse monoclonal/ clone 9E10 | 1 :1000 | | Abcam | ab62928 |
| FUS/TLS | mouse monoclonal | 1 :1000 | | Abcam | ab84078 |
| Glial fibrillary acidic protein (GFAP) | mouse monoclonal/ clone GA5 | 1:5000 | 1:500 | Cell Signaling Technologies (Danvers, MA, USA) | 3670 |
| Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) | mouse monoclonal/ clone 6C5 | 1:2000 | | Santa Cruz (Santa Cruz, CA, USA) | sc-32233 |
| Human TDP-43 | mouse monoclonal/ clone 2E2-D3 | 1:1000 | 1:500 | Abnova (Taipei City, Taiwan) | H00023435-M01 |

| | | | | | |
|---|---|---|---|---|---|
| Iba1 | rabbit polyclonal | 1:500 | 1:500 | Wako Chemicals (Richmond, VA, USA) | 019-19741 |
| Lamin A/C | goat polyclonal | 1:500 | | Santa Cruz | sc-6215 |
| LC3 | rabbit polyclonal | | 1:500 | Novus Biologicals (Littleton, CO, USA) | NB100-2220 |
| Myc-Tag-488 conjugated | mouse monoclonal/ clone 9E10 | | 1:500 | Millipore | 16-308 |
| Myc-Tag-biotin conjugated | mouse monoclonal/ clone 9E10 | | 1:500 | Millipore | 16-170 |
| Myc-Tag | rabbit polyclonal | 1:1000 | | Abcam | ab9106 |
| NeuN | rabbit monoclonal/ clone D3S3I | | 1:500 | Cell Signaling Technologies | D3531 |
| Neurofilament H | mouse monoclonal/ clone N52 | | 1:250 | Millipore | MAB5266 |
| Neurofilament L | mouse monoclonal/ clone NR4 | 1 :1000 | | Sigma | N5139 |
| NFKB p65 subunit | rabbit polyclonal | 1:1000 | | Santa Cruz | sc-372 |
| p84 nuclear membrane | mouse monoclonal/ clone 5E10 | 1:500 | | Abcam (Cambridge, UK) | ab487 |

| | | | | | |
|---|---|---|---|---|---|
| Pan-TDP-43 N-Term | rabbit polyclonal | 1:5000 | | Proteintech (Chicago, IL, USA) | 10782-2-AP |
| Pan-TDP-43 C-Term | rabbit polyclonal | 1:5000 | | Proteintech (Chicago, IL, USA) | 12892-2-AP |
| Peripherin | mouse monoclonal | 1 :500 | | MAB1527 | Millipore |
| POLDIP3 | rabbit monoclonal/ clone D65E8 | 1:1000 | | Cell Signaling Technologies | 5439 |
| phospho (Ser 536) NFKB p65 subunit | mouse monoclonal/ clone 7F1 | 1:1000 | | Cell Signaling Technologies | 3036S |
| RRM1 TDP-43 (C10, G8 and E6) | mouse monoclonal/ clones 5C10, 55G8, 52E6 | | 0.4 μg/ml | Medimabs (Montreal, QC, CA) | |
| Synaptic vesicle protein 2 (SV-2) | mouse monoclonal/ clone unknown | | 1:25 | Developmental Studies Hybridoma Bank (Mt.Prospect, IA, USA) | AB_231587 |
| Trans-Golgi network protein 46 (TGN46) | rabbit polyclonal | | 1:1000 | Abcam | ab50595 |
| Ubiquitin | rabbit polyclonal | | 1:1000 | Dako (Glostrup, Denmark) | Z0458 |

## Supplementary References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