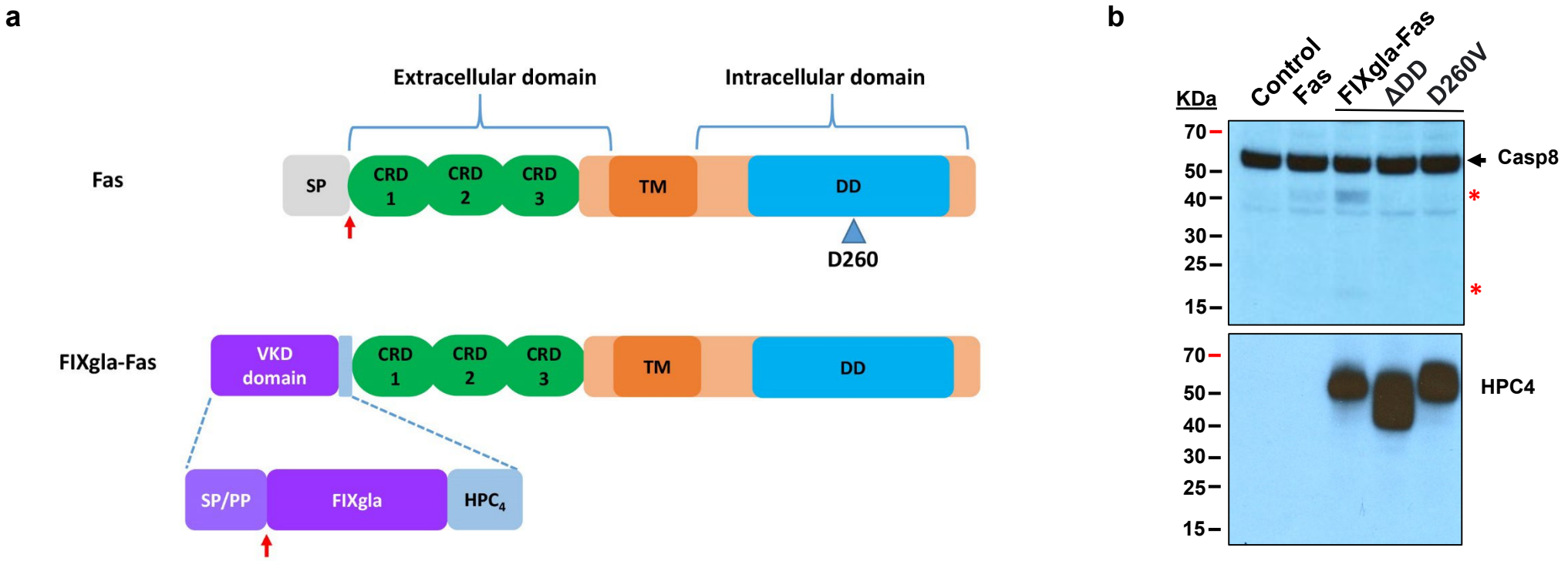
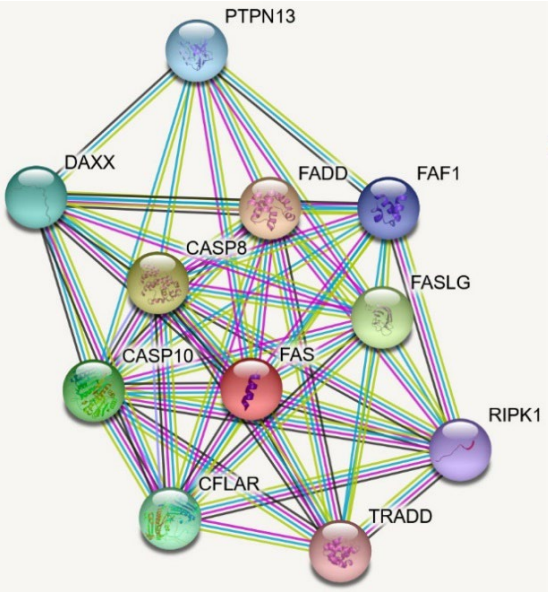


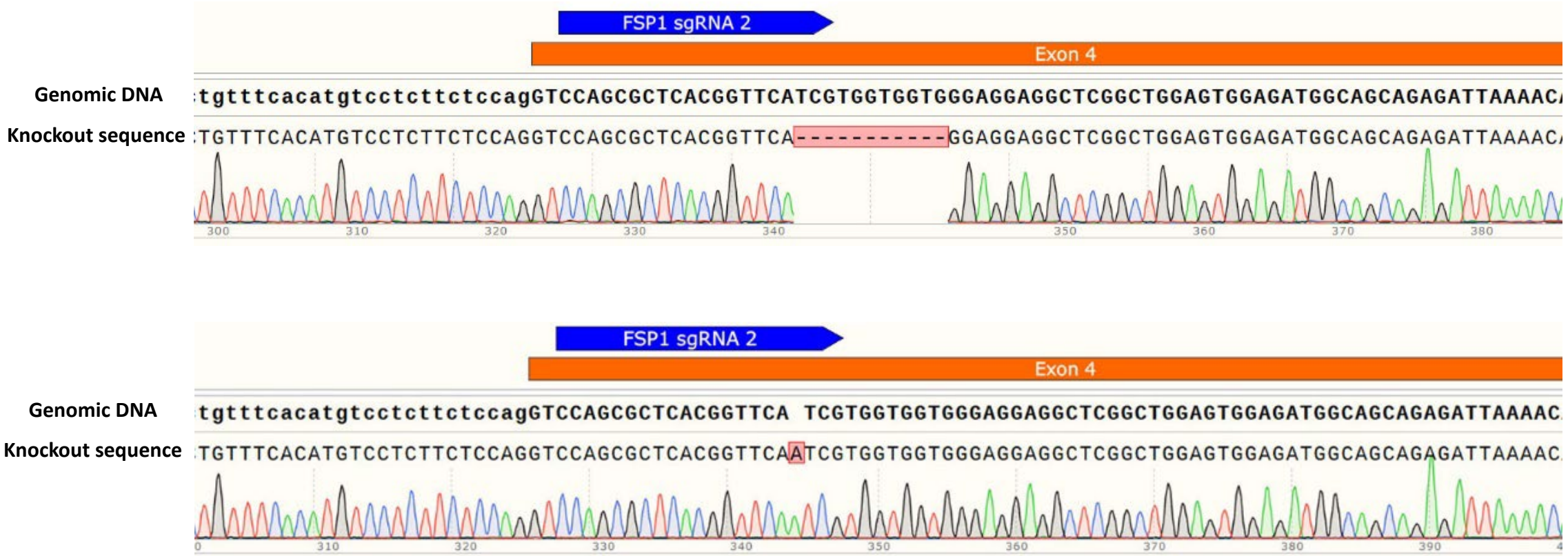
Supplementary Fig. 1 | Vitamin K-dependent apoptotic reporter protein. (**a**) Schematic diagram of the domain structure of the VKD apoptotic reporter protein FIXgla-Fas. The signal peptide (SP), propeptide (PP), and Gla domain of factor IX (FIXgla) is fused to the extracellular N-terminus of Fas with a HPC4 tag between the two fusion parts for detection purpose. Residue Asp260 within the death domain (DD), important for Fas-FADD interaction, is indicated by a triangle. CRD, cysteine-rich domain; TM, transmembrane domain. Arrowheads indicate positions of the cleavage of signal peptide or propeptide before mature protein is secreted. (**b**) Effect of FIXgla-Fas reporter protein and its mutants on caspase-dependent apoptosis detected by immunoblotting. The Fas, FIXgla-Fas, the D260V mutant, or FIXgla-Fas with its death domain (DD) deleted (ΔDD) constructs were transiently expressed in HEK293 cells. Transfected cells were incubated with 11 μM vitamin K for 48 hours before harvesting the cells for immunoblotting. Control: HEK293 cells only. Top: Activation of caspase-8 was probed using anti-caspase-8 antibody. Full-length caspase-8 was indicated by an arrowhead, activated caspase-8 was indicated by asterisks. Bottom: total reporter protein expression probed by anti-HPC4 tag antibody. Similar results were observed at least twice as shown in Fig. 1b.



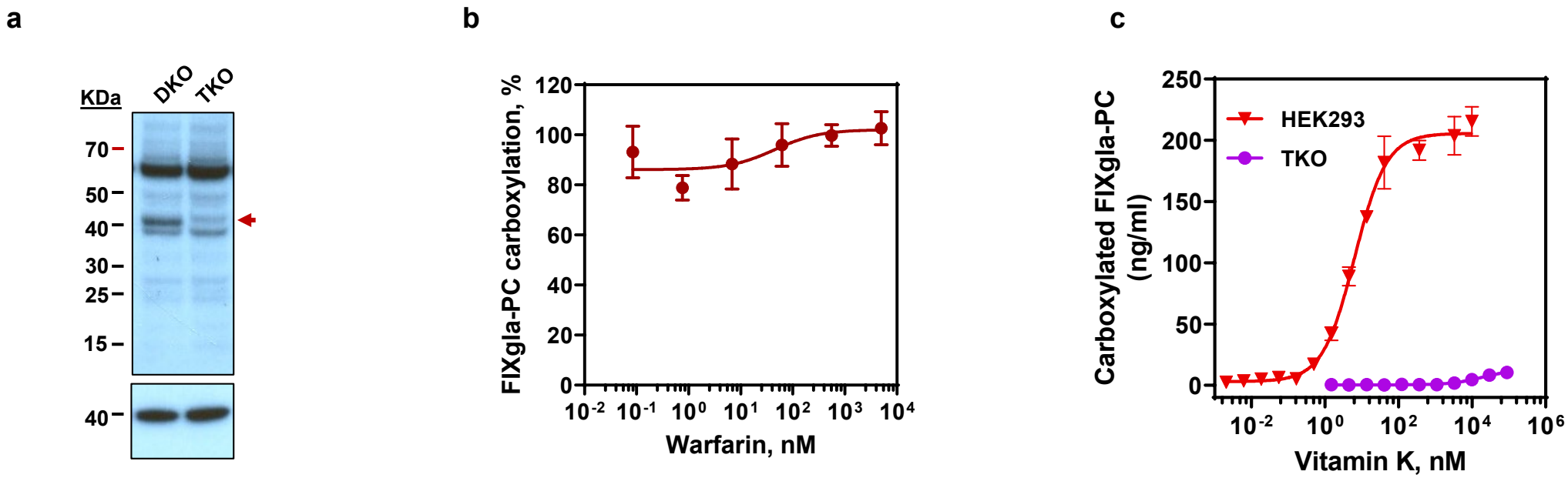
Predicted Functional Partners:

		Neighborhood	Gene Fusion	Cooccurence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
FADD	FAS-associated death domain protein; Apoptotic adaptor molecule that reci...					●	●	●		0.999
CASP8	Caspase-8; Most upstream protease of the activation cascade of caspases.					●	●	●		0.999
FASLG	Tumor necrosis factor ligand superfamily member 6; Cytokine that binds to.					●	●	●		0.999
CASP10	Caspase-10; Involved in the activation cascade of caspases responsible for...					●	●	●		0.997
CFLAR	CASP8 and FADD-like apoptosis regulator; Apoptosis regulator protein whic...					●	●	●		0.996
DAXX	Death domain-associated protein 6; Transcription corepressor known to rep...					●	●	●		0.995
PTPN13	Tyrosine-protein phosphatase non-receptor type 13; Tyrosine phosphatase ...					●	●	●		0.995
FAF1	FAS-associated factor 1; Potentiates but cannot initiate FAS-induced apopt...					●	●	●		0.992
RIPK1	Receptor-interacting serine/threonine-protein kinase 1; Serine-threonine kin...					●	●	●		0.985
TRADD	Tumor necrosis factor receptor type 1-associated DEATH domain protein; T...					●	●	●		0.983

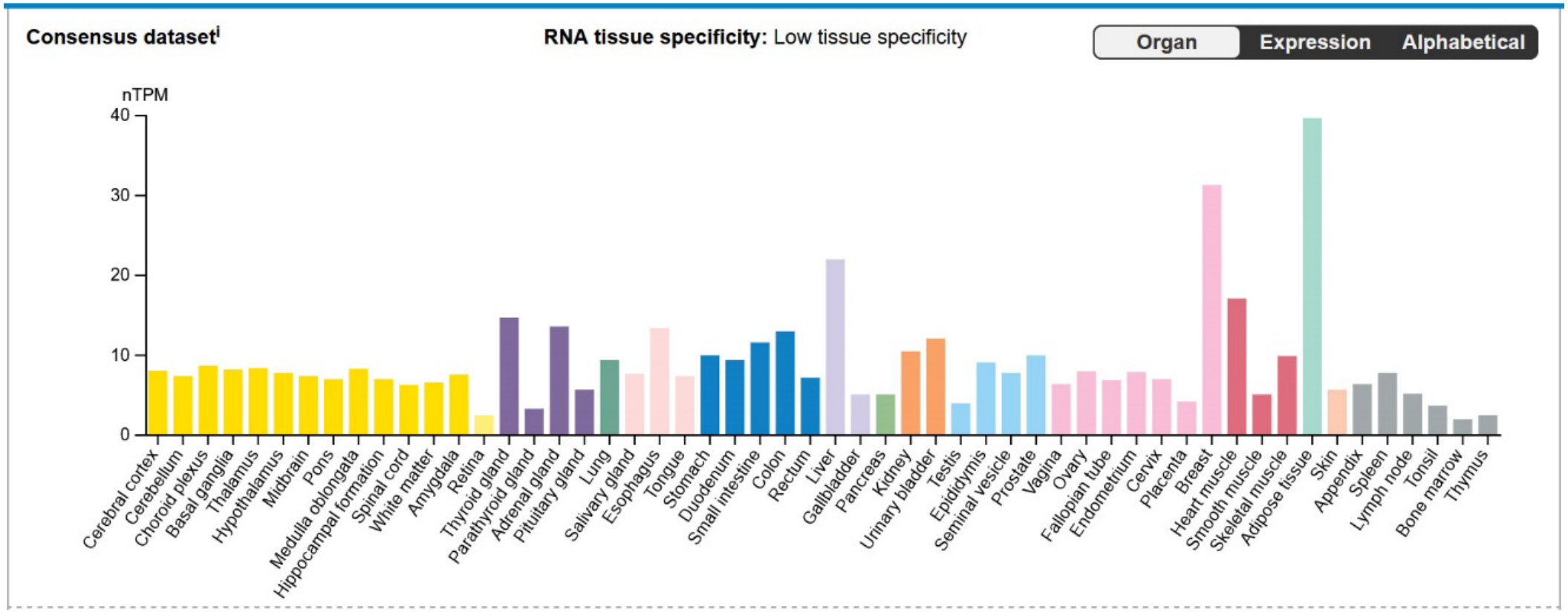
Supplementary Fig. 2 | Network analysis of Fas receptor using the STRING protein-protein interaction network database with default settings. Of the ten proteins that interact with Fas in this network, the sgRNA targeting eight of the proteins (CASP8, CASP10, CFLAR, FADD, FAF1, PTPN13, RIPK1, and TRADD) were enriched in the genome-wide CRISPR-Cas9 loss-of-function VKD screening. Detailed sgRNA enrichment of these genes can be found in Supplementary Table 1.



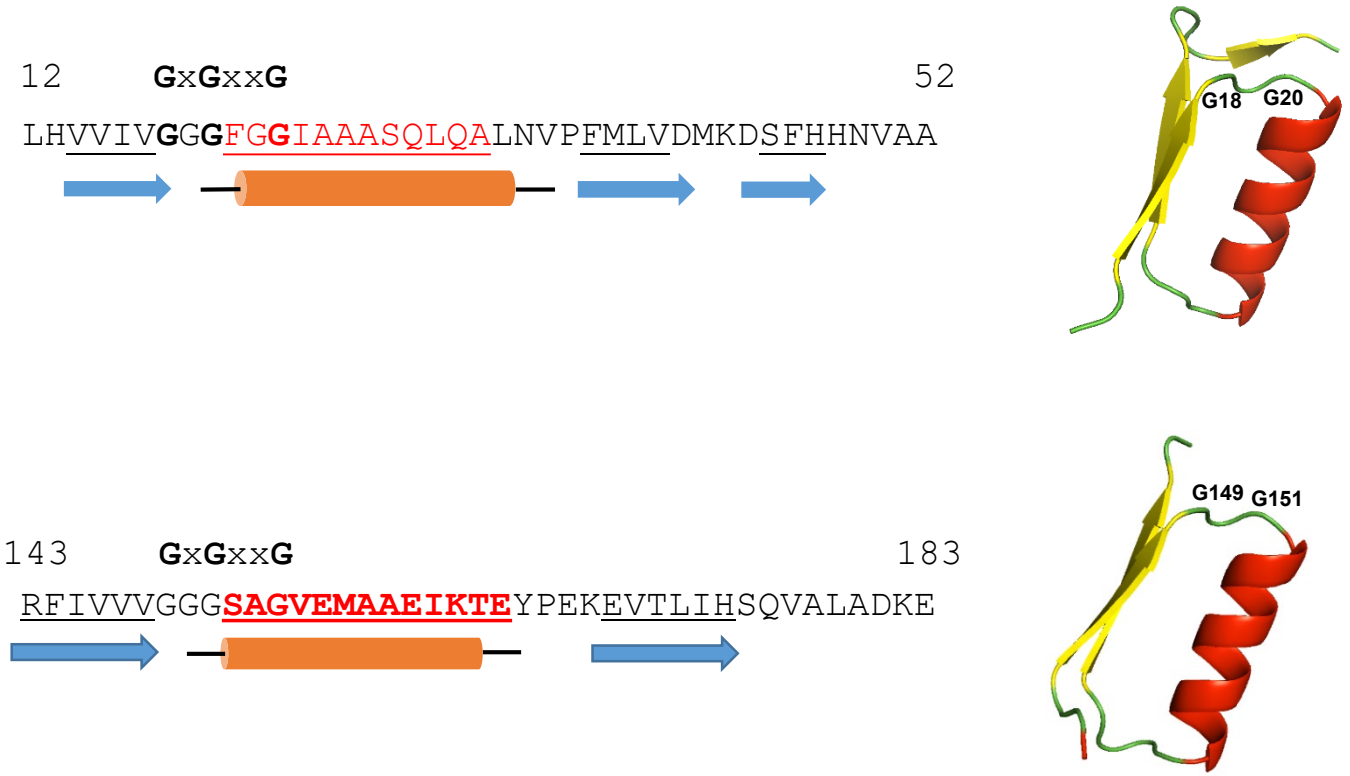
Supplementary Fig. 3 | Genomic DNA sequence analysis of FSP1 knockout HEK293 cells. The sgRNA of CCAGCGCTCACGGTTCATCG (FSP1-2) targeting exon 4 of *fsp1* was used to knock out the endogenous FSP1 in HEK293 cells. After single colonies were obtained, genomic DNA of the FSP1 knockout cells were extracted and used as a template for PCR amplification of the sgRNA targeted region. The sgRNA target sequence is indicated by blue arrows. Intron sequences are in lower case and exon sequences are in upper case. One of the single colonies has one allele has an 11-base deletion (top) while the other has a 1-base insertion (bottom) at the Cas9 cutting site.



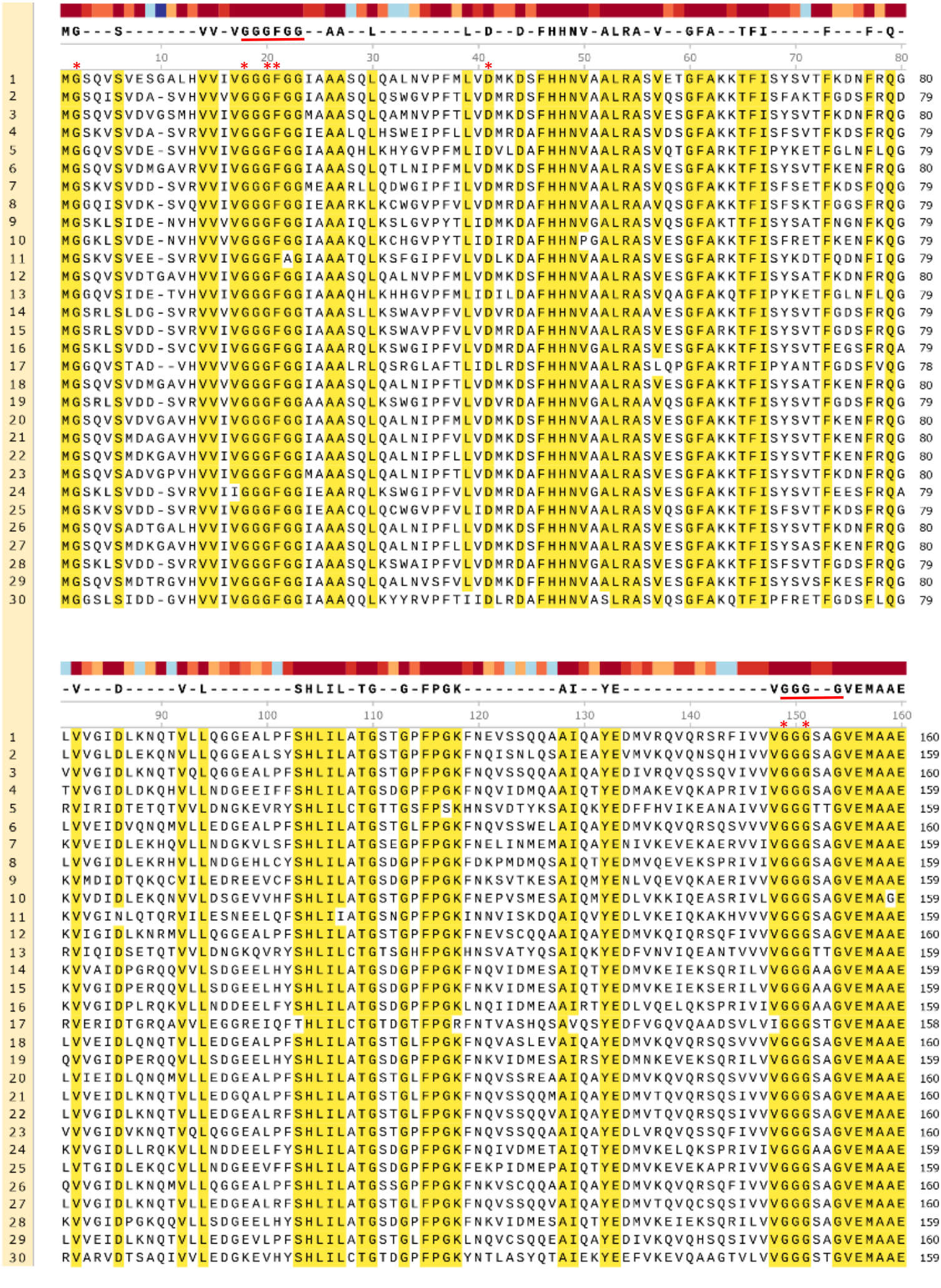
Supplementary Fig. 4 | Warfarin and vitamin K concentration titrations in HEK293 and TKO reporter cells. (**a**) Immunoblotting of DKO cells and these cells with their fsp1 gene knocked out (TKO). Full-length FSP1 is indicated by an arrowhead. Similar results were observed at least twice. Top: anti-FSP1, bottom: anti-GAPDH. (**b**) Inhibition of VKD carboxylation in TKO reporter cells. The TKO reporter cells were seeded in a 96-well plate and incubated with increasing concentrations of warfarin in the cell culture medium containing 11 µM vitamin K for the cell-based activity assay. The carboxylation activity of TKO cells without warfarin in the culture medium was normalized to 100%. Data are presented as mean ± SD of three independent experiments (n=3). (**c**) Vitamin K concentration titration of FIXgla-PC/HEK293 (HEK293) and TKO reporter cells. Reporter cells were seeded in 96-well plates and incubated with increasing concentrations of vitamin K for the cell-based activity assay. Carboxylated reporter protein FIXgla-PC in the cell culture medium were determined using ELISA without normalization. Data are presented as mean ± SD of three independent experiments (n=3).



Supplementary Fig. 5 | Tissue specificity of FSP1 RNA from The Human Protein Atlas Database. A low tissue specificity for the FSP1 gene was determined by The Human Protein Atlas Database (https://www.proteinatlas.org/ENSG00000042286-AIFM2/tissue). AIFM2 was the protein name in the database while searching this protein.



Supplementary Fig. 6 | Rossmann fold structural motifs identified in FSP1. Two Rossmann fold motifs were identified in FSP1 between residues 12 to 52 (top) and 143 to 183 (bottom). Rossmann fold motif structure typically contains an alpha helix flanked by two parallel beta strands. The loop connecting the first beta strand with the alpha helix contains the consensus sequence GxGxxG, which established direct interaction with the dinucleotide cofactor. Rossmann fold structures were obtain by Pymol using AlphaFold predicted model. Conserved glycine residues within the GxGxxG sequence that have been mutated in this study were indicated in the structure model.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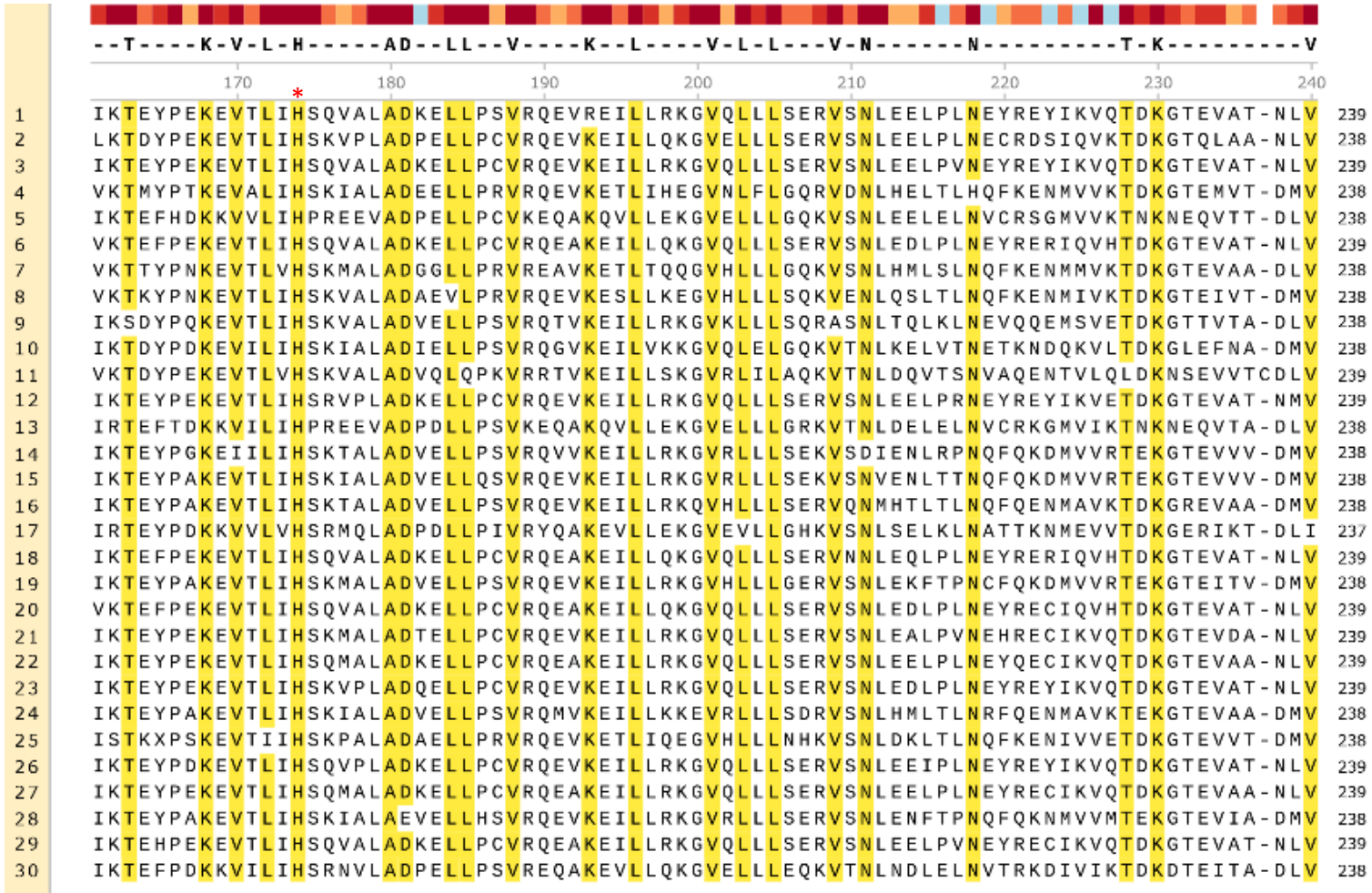
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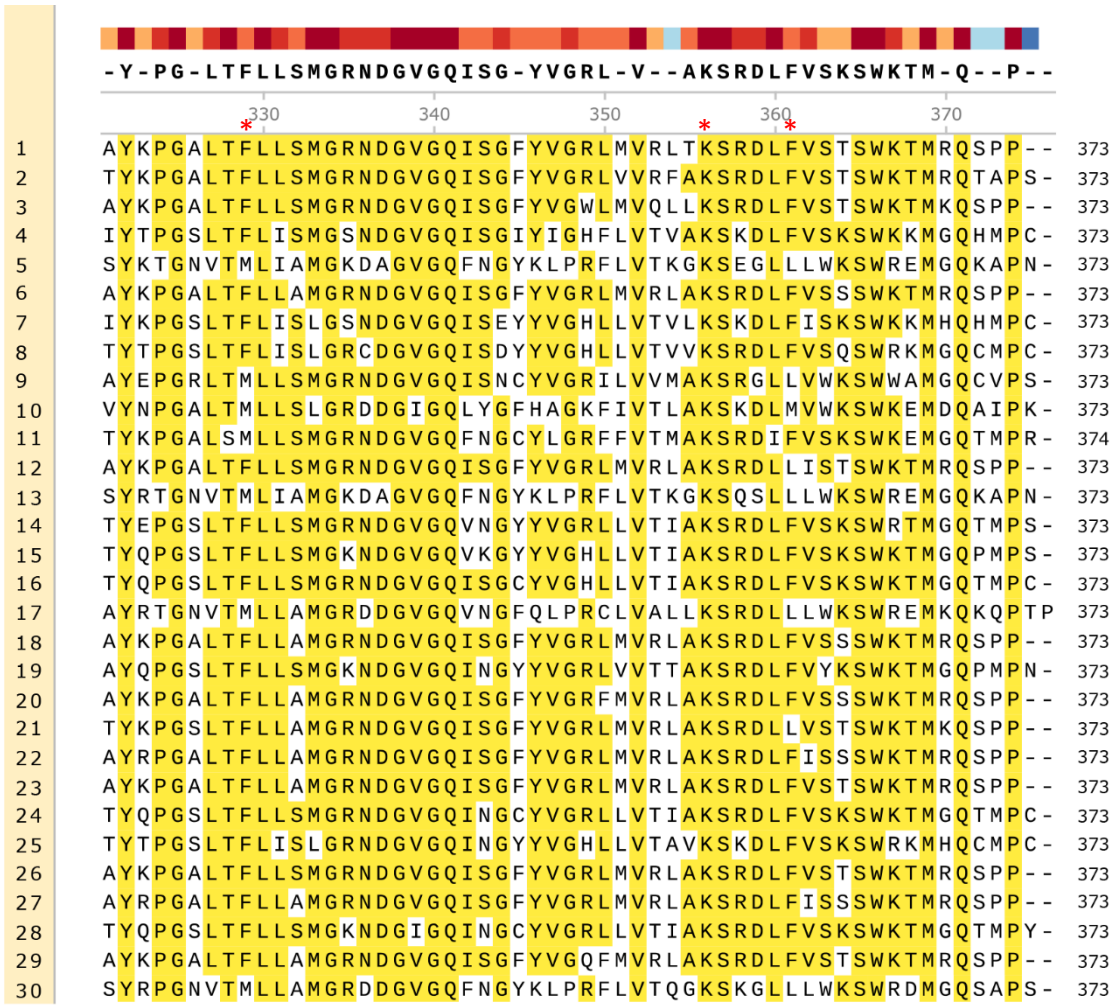

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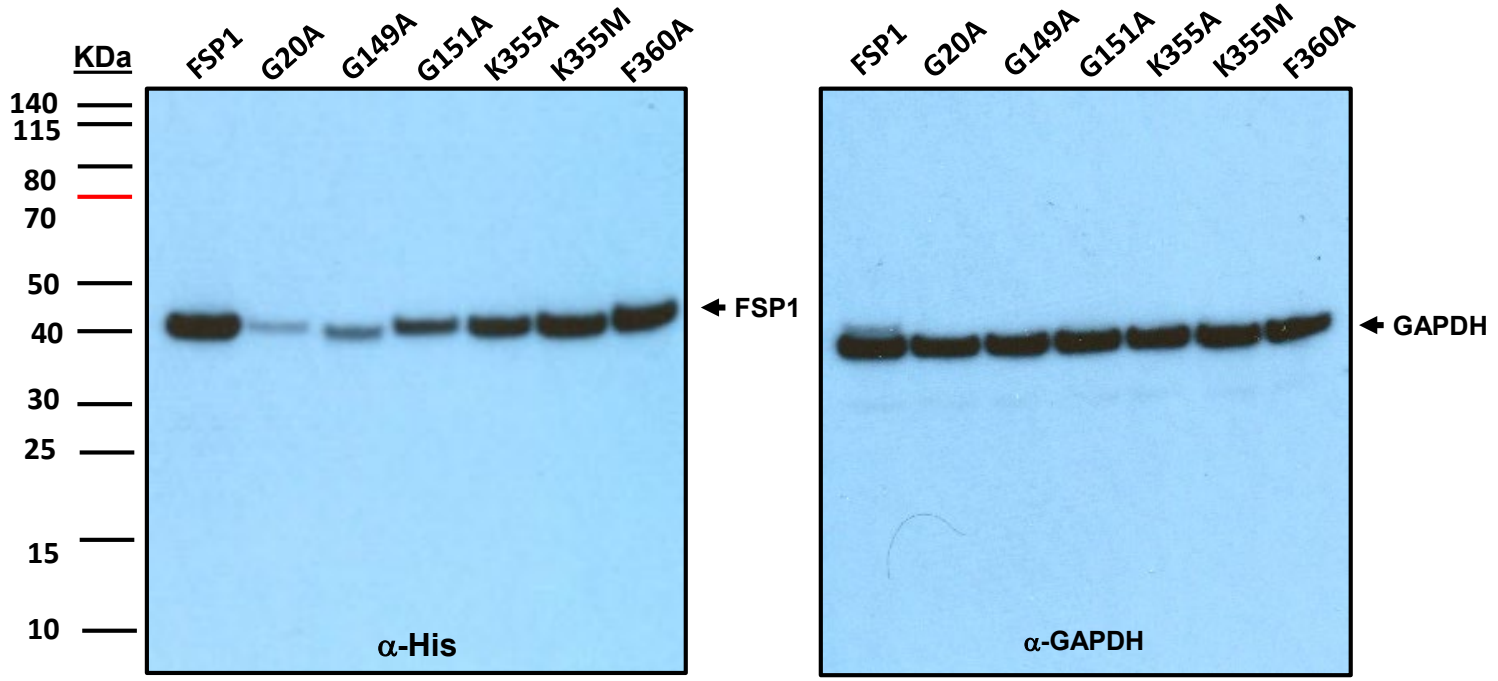
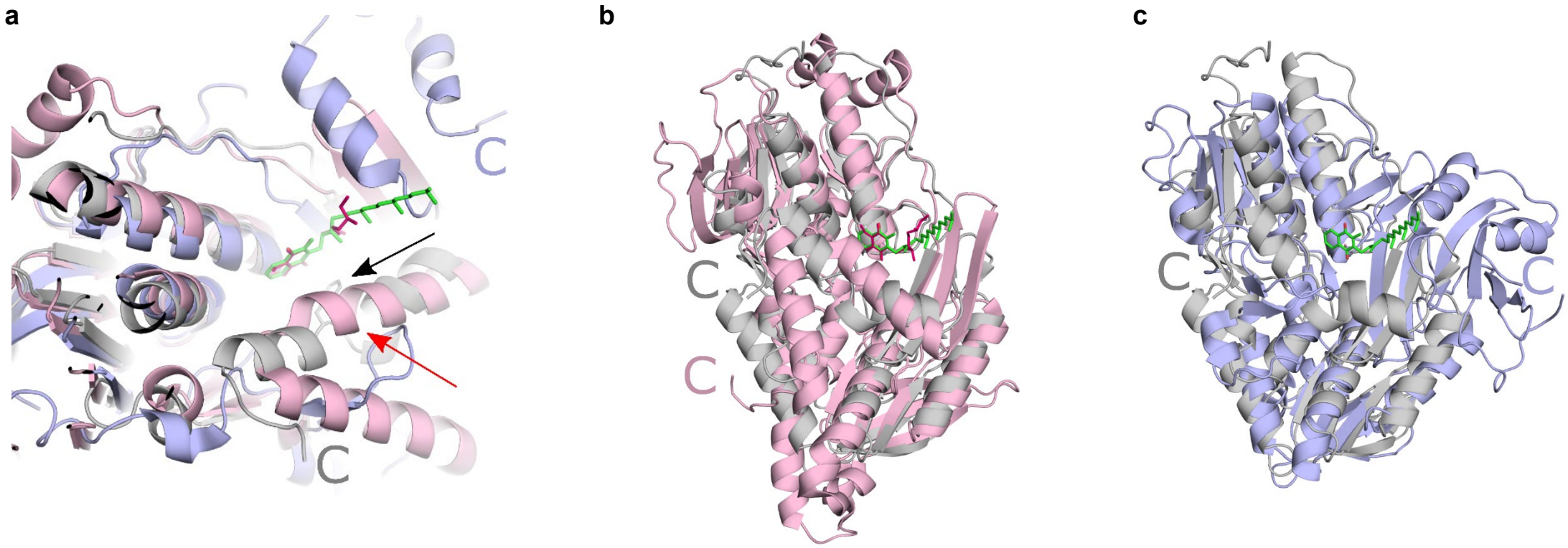
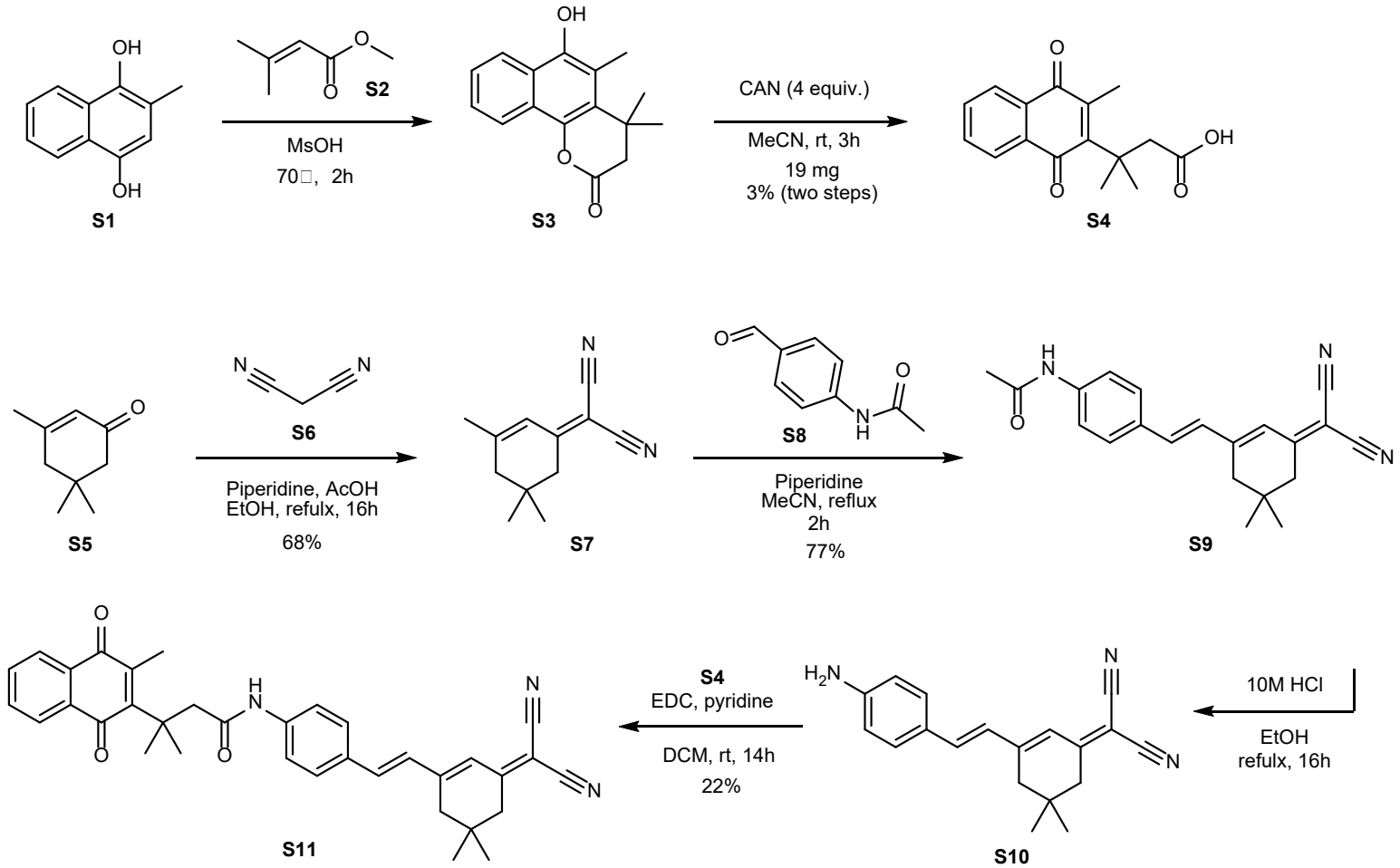
Supplementary Fig. 7 | FSP1 sequence alignment from 30 diverse species using SnapGene. Conserved sequences are highlighted in yellow. Consensus threshold was set as 95%. Residues mutated for functional study in this paper are indicated by asterisks. The GxGxxG conserved consensus sequences within the Rossmann fold are underlined.



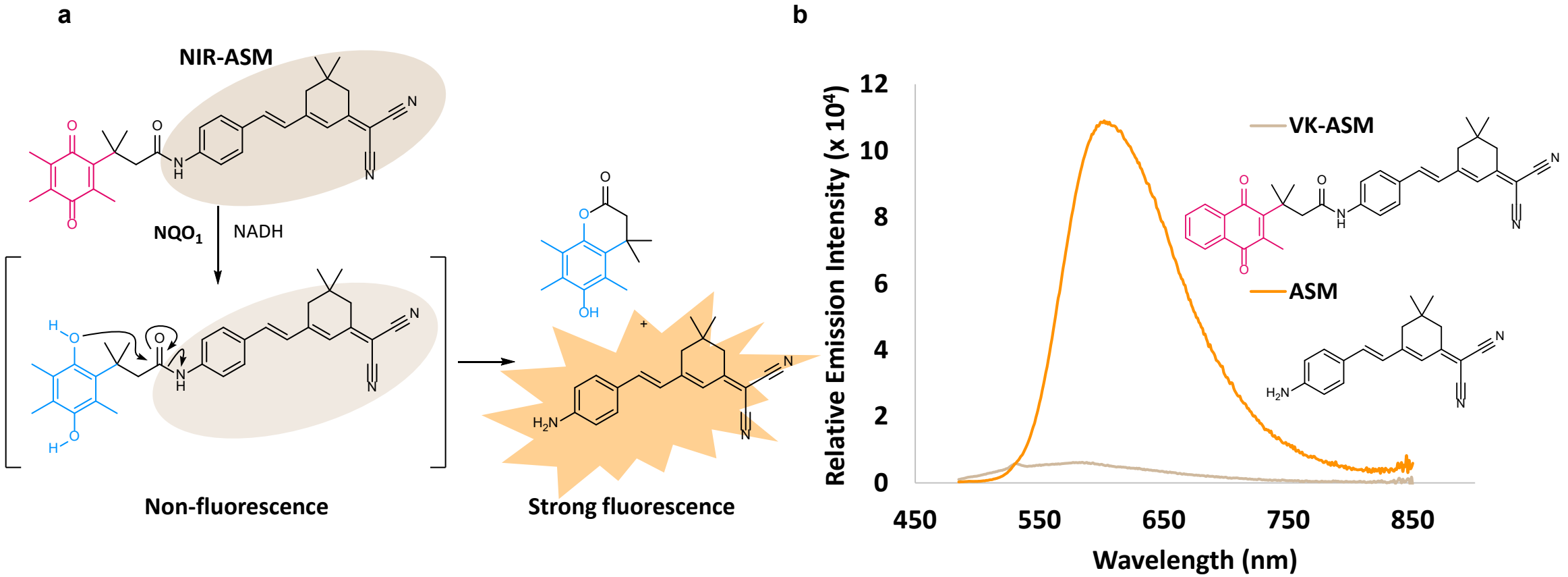
Supplementary Fig. 8 | Immunoblotting of FSP1 and its mutants with a His-tag at the C-terminus. His-Tagged FSP1 and its mutants were transiently expressed in HEK293 cells. Forty-eight hours post-transfection, transfected cells were harvested and the cell lysates were used for SDS-PAGE electrophoresis. Protein bands were transferred to PVDF membrane and probed by anti-His antibody (left) or anti-GADPH antibody as described in the Methods. Similar results were observed at least twice times as shown in these blots.



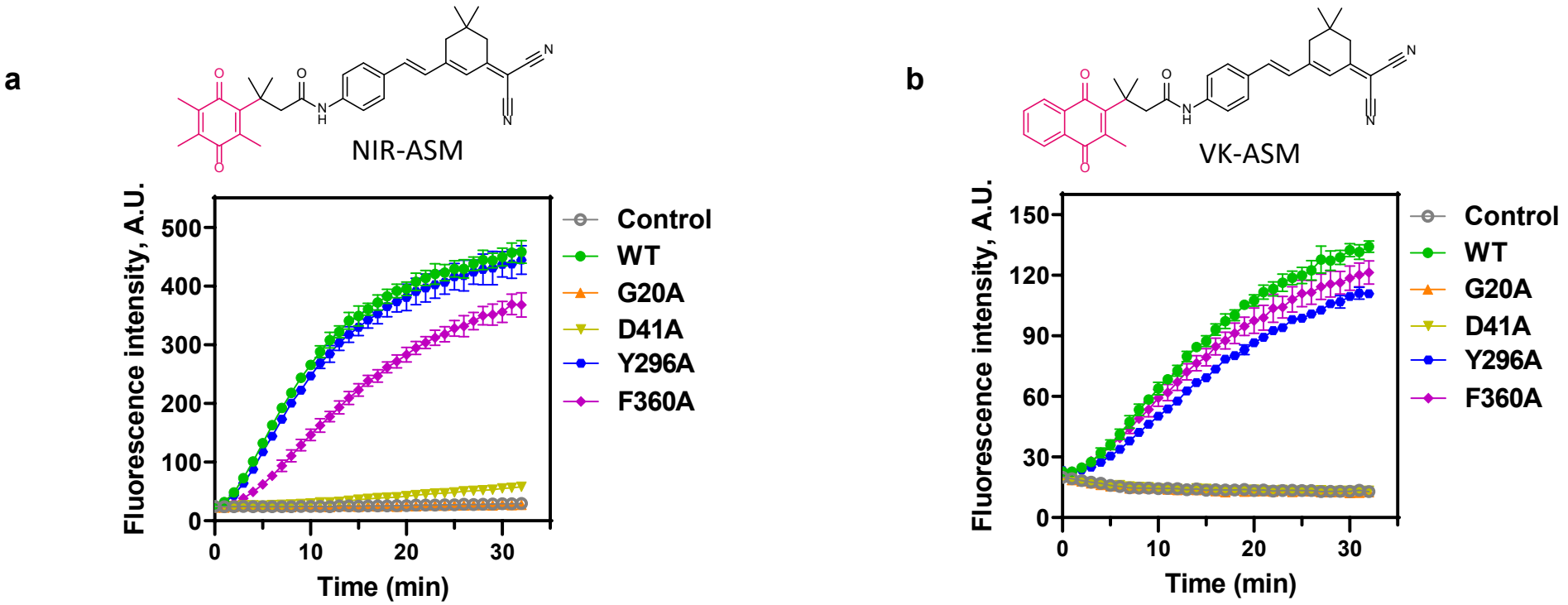
Supplementary Fig. 9 | Structure comparison of FSP1 and yeast Ndi1 and AIF. (a) Superpositions of Ndi1 (pink, PDBcode 4G73) with a bound quinone (raspberry) and apoptosis inducing factor (AIF, PDBcode 4BUR, cornflower blue) onto the AlphaFold model of FSP1 (grey) display differences in the C-termini's surrounding the putative vitamin K (green) binding site. Red arrow denotes deviations between the kinked C-terminal helix of the FSP1 model and the equivalent penultimate helix of Ndi1. The C-terminus of AIF is located away from this position. Black arrow denotes the position of residue F360 located near the vitamin K quinone moiety in the FSP1 model. (b) Global superposition of Nid1 structure with Alphafold model of FSP1. (c) Global superposition of 4BUR with Alphafold model of FSP1.



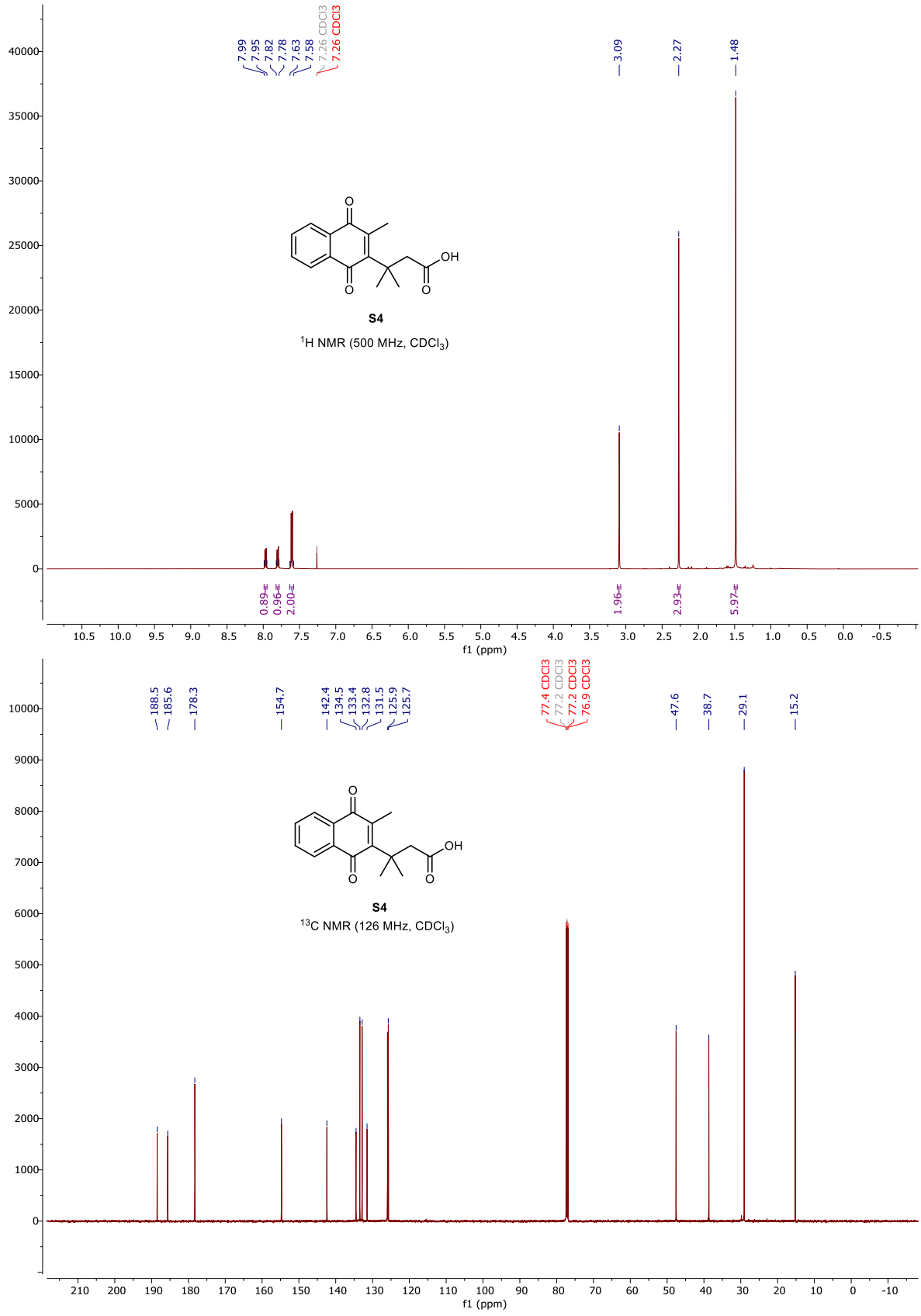
Supplementary Fig. 10 |Synthetic route for VK-ASM (S11).



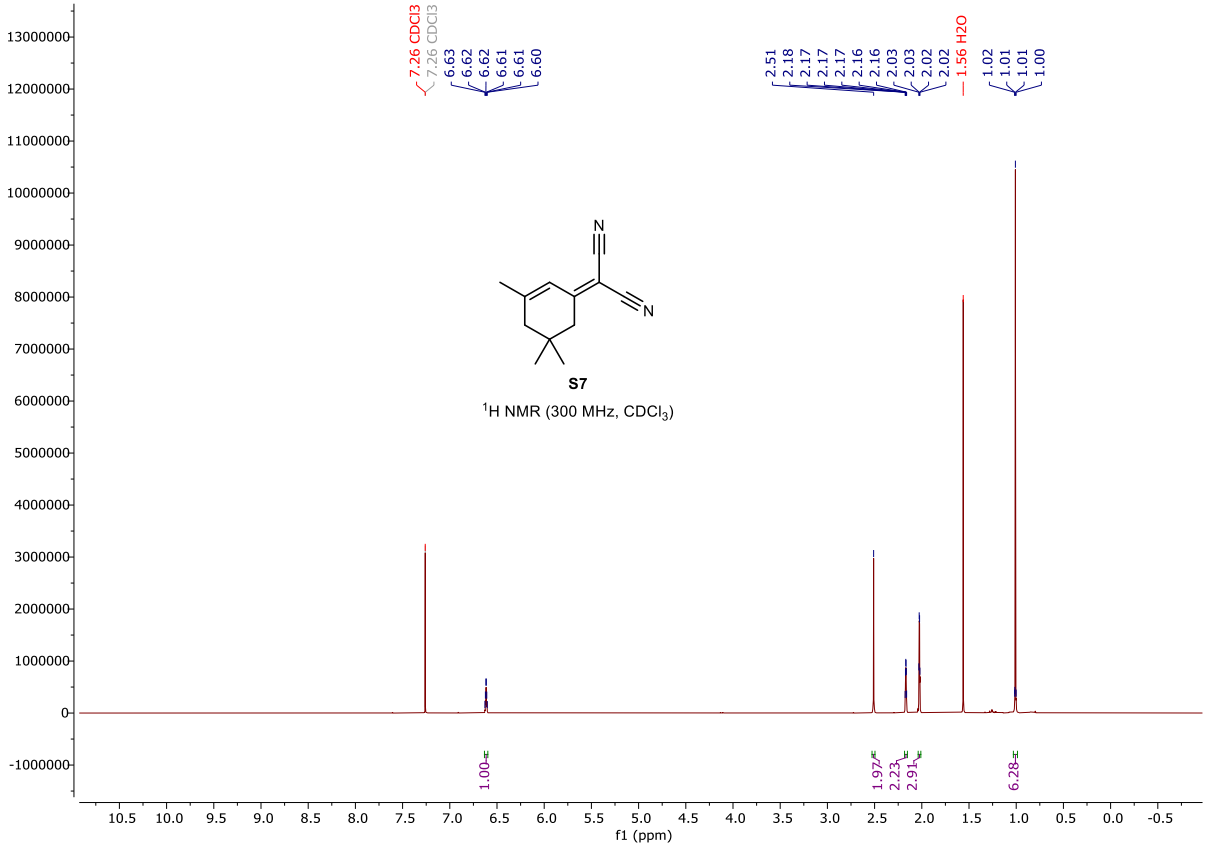
Supplementary Fig. 11 | Activity-based fluorescence probe of vitamin K and ubiquinone for FSP1. (**a**) Proposed activation mechanism of the activity-based fluorescent probe of ubiquinone (NIR-ASM) towards NQO1. The probe is inactive until the ubiquinone moiety is reduced to hydroquinone, and the rearrangement of the reduced intermediated releases the strong fluorescent tag ASM. (**b**) Fluorescence emission spectra of the fluorescence tag ASM (blue) and the activity-based probe of vitamin K before activation (orange) excited at 460 nm. Solution measurements were performed in 10×10 mm^2 quartz cuvettes. Photoluminescence spectra (PL) were measured using Edinburgh Instruments FS5 fluorescence spectrometer in steady-state mode with xenon lamp as the excitation source, exciting at 460 nm.

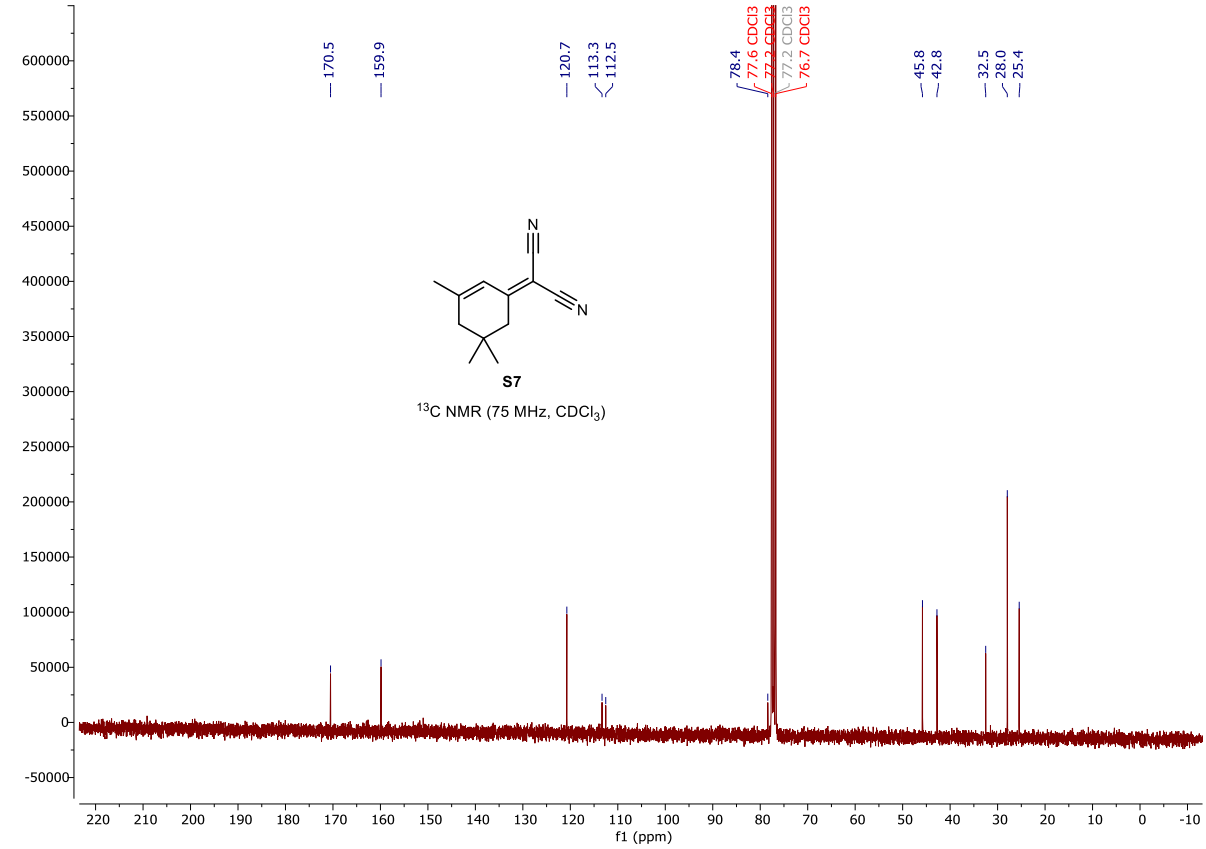


Supplementary Fig. 12 | Comparison of the effect of FSP1 mutations on activity-based fluorescence probes of NIR-ASM and VK-ASM. Wild-type FSP1 or its mutants were transiently expressed in TKO cells and the transfected cells were harvested 48 hours after transfection. Cell lysate was used for activity assays using the fluorescence probe NIR-ASM (**a**) and VK-ASM (**b**) for ubiquinone and vitamin K reduction, respectively, as described in the method section. Control: TKO cells without transfection. Data are presented as mean ± SD of three independent experiments (n=3).

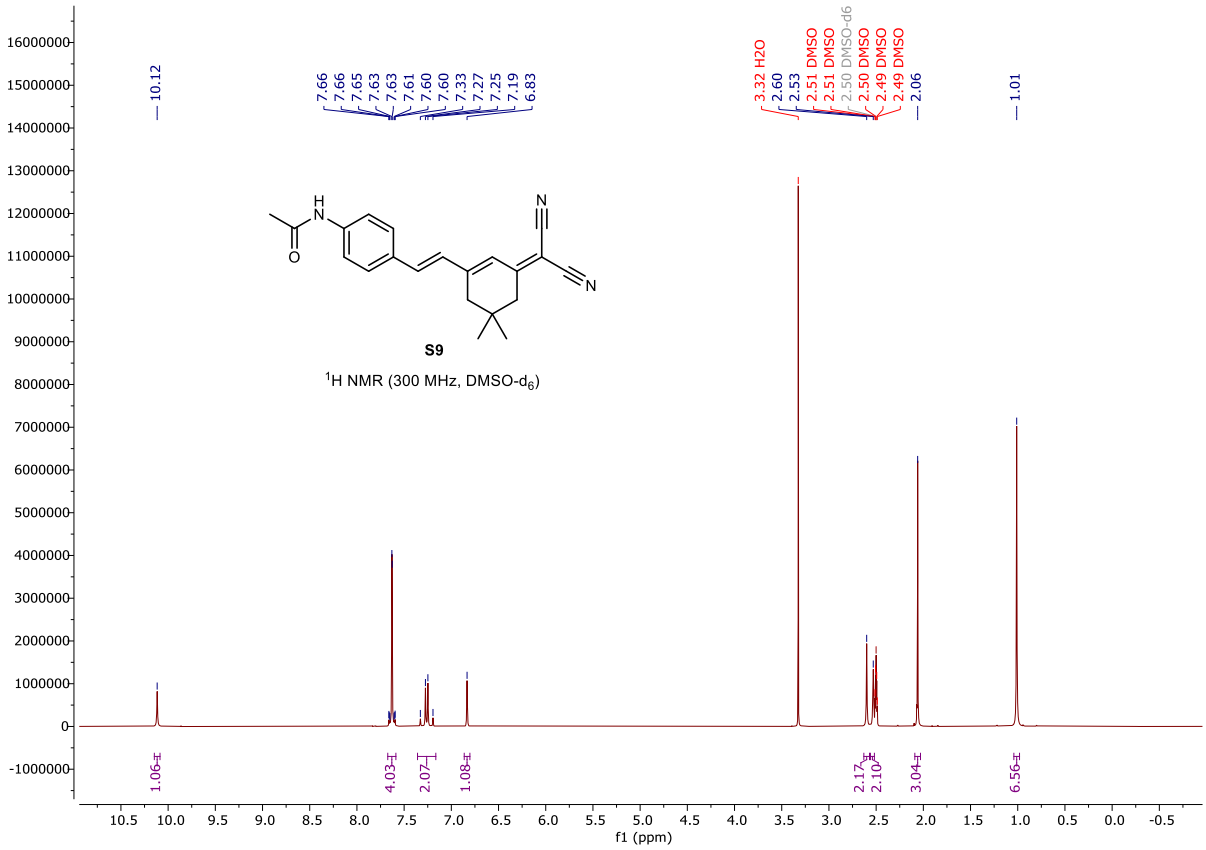


Supplementary Fig. 13 ^{1}H and ^{13}C NMR spectra of synthetic intermediates (S4, S7, S9) and VK-ASM (S11)



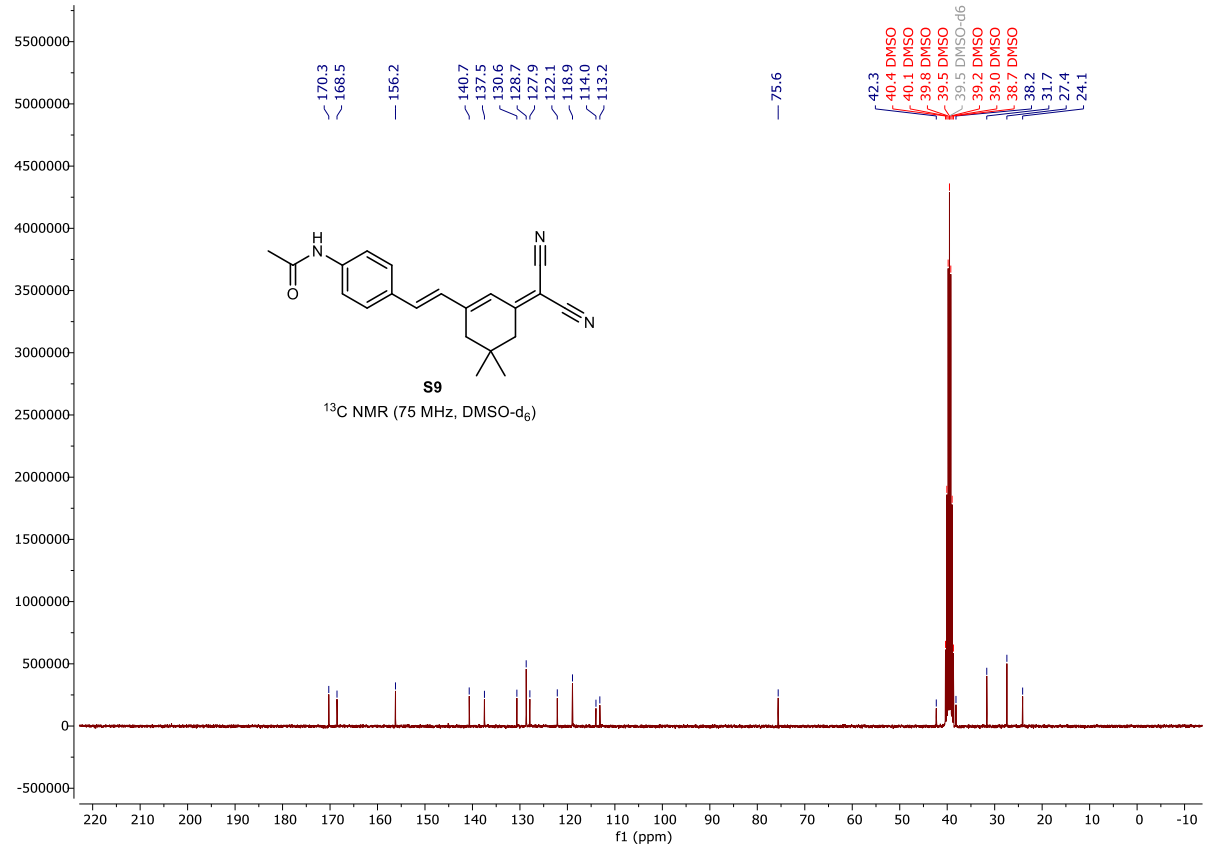


Supplementary Fig. 13 (continued)



S9

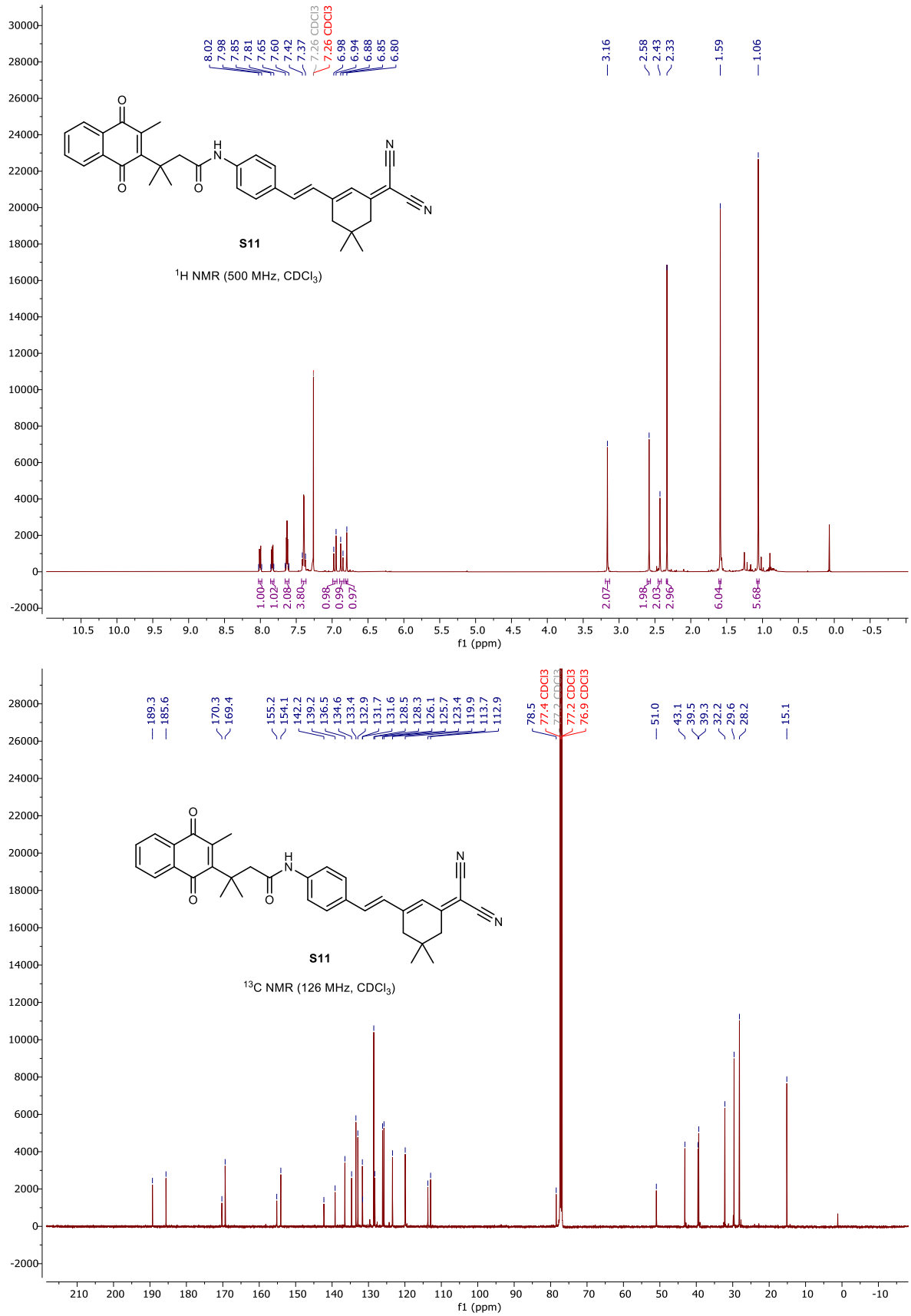
^{1}H NMR (300 MHz, DMSO-d_6)



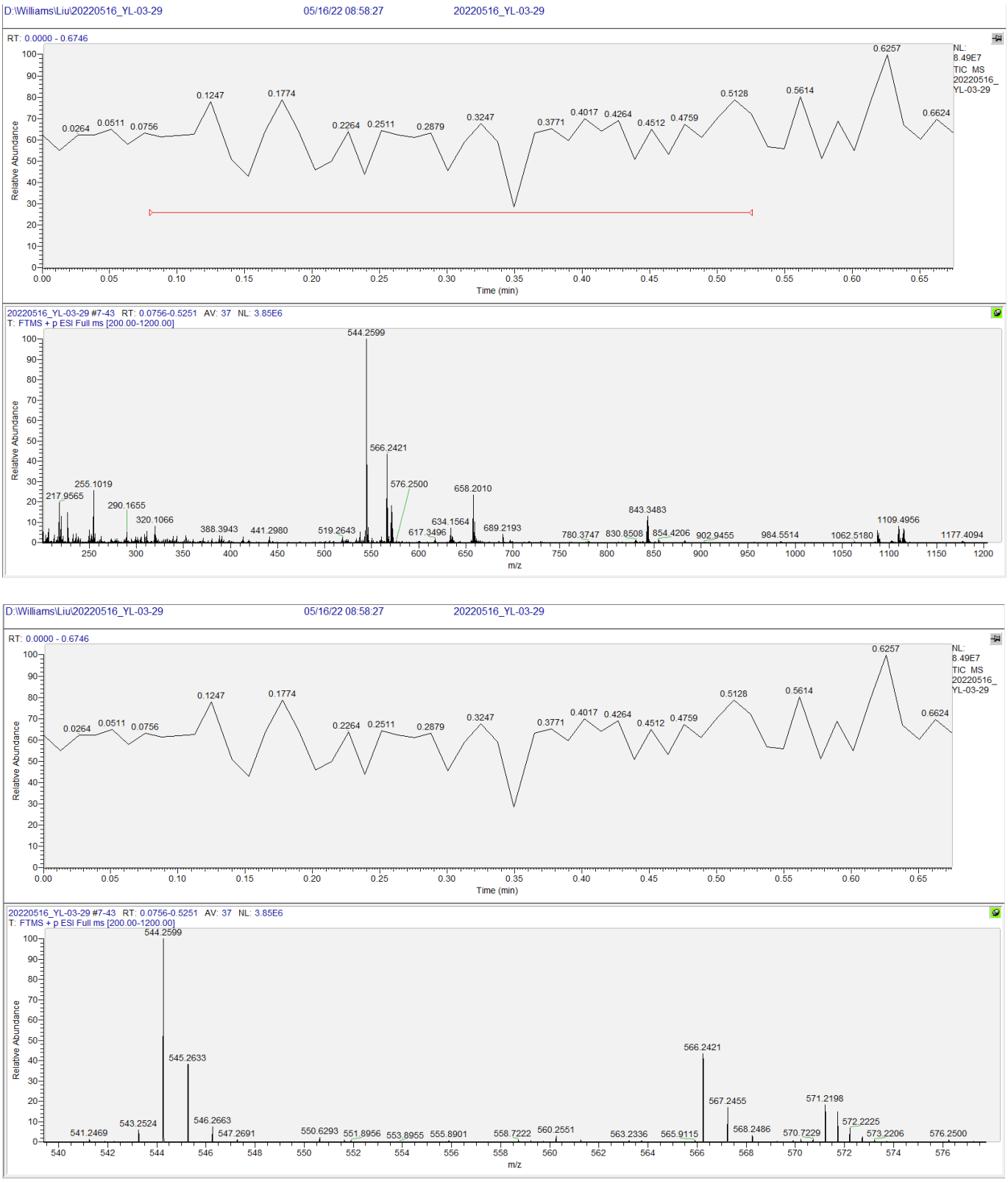
S9

^{13}C NMR (75 MHz, DMSO-d_6)

Supplementary Fig. 13 (continued)



Supplementary Fig. 13 (continued)



Supplementary Fig. 14 ESI MS Spectra of VK-ASM (S11)