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Supplementary Materials

Table S1. Primers used for cloning and sequencing

		PRIMERS SEQUENCE [1,2]
PGEX3		5′ GGGCTGCAAGCCACGTTTGGTG
		5′ CCGGGAGCTGCATGTGTCAGAGG
SPES	*speS*	5′ CTACAAAGGTGGTAGGATCG
		5’ CGATCCTACCACCTTTGTAG
	Y39I SDM*	5′ AAAAGTAAACTGCTTATTGCATACACTATAAC
		5′ GTTATAGTGTATGCAATAAGCAGTTTACTTTT
PRIMERS FOR RECOMBINANT FUSION PROTEINS		
START OF *SPES*		5′ GACGACGGATCCTTGTCAATAGTGAATCCCACAAAG
SEQ_0402	SEQ_0402	5′ GTCGTCGGATCCTTGCGACTACCCTAGCAGGACAAAC
	SEQ_0402^{ala}	5′ GATGTTTATCTTGAAAAAGCAGCGGCAGCAGCGACTACCCTAGCAGGACAAAC
		5′ CTAGGGTAGTCGCTGCTGCCGCTGCTTTTTCAAGATAAACATCAAAATGGC
	SEQ_0402^{GPGP}	5′ GATGTTTATCTTGAAAAAGGTCCTGGTCCTGCGACTACCCTAGCAGGACAAAC
		5′ CTAGGGTAGTCGCAGGACCAGGACCTTTTTCAAGATAAACATCAAAATGGC
	SEQ_0402 cloning primer	5′ GACGACGAATTCTGATGGAAGTGATTGCTCAGG
SEQ_1959	SEQ_1959	5′ GTCGTCGGATCCTTAATACCTATACGACTTATGCCAG
	SEQ_1959^{ala}	5′ GATGTTTATCTTGAAAAAGCAGCGGCAGCAAATACCTATACGACTTATGCCAG
		5′ CGTATAGGTATTTGCTGCCGCTGCTTTTTCAAGATAAACATCAAAATGGC
	SEQ_1959^{GPGP}	5′ GATGTTTATCTTGAAAAAGGTCCTGGTCCTAATACCTATACGACTTATGCCAG
		5′ CGTATAGGTATTAGGACCAGGACCTTTTTCAAGATAAACATCAAAATGGC
	SEQ_1959 cloning primer	5′ GTCGTCGAATTCAGATCTTGGCAAAGCCTTAGTC
SEQ_0933	SEQ_0933	5′ GTCGTCGGATCCTTGATTCTGTTGAGTCAGCTGG
	SEQ_0933^{ala}	5′ GATGTTTATCTTGAAAAAGCAGCGGCAGCAGATTCTGTTGAGTCAGCTGG
		5′ CTCAACAGAATCTGCTGCCGCTGCTTTTTCAAGATAAACATCAAAATGGC
	SEQ_0933^{GPGP}	5′ GATGTTTATCTTGAAAAAGGTCCTGGTCCTGATTCTGTTGAGTCAGCTGG
		5′ CTCAACAGAATCAGGACCAGGACCTTTTTCAAGATAAACATCAAAATGGC
	SEQ_0933 cloning primer	5′ GTCGTCGAATTCTGTTGATGGCAATTTGTTGGTG
SEQ_2190	SEQ_2190	5′ GTCGTCGGATCCTTGATATGAAAAGCGATGCCAAAAAGG
	SEQ_2190^{ala}	*5′* GATGTTTATCTTGAAAAAGCAGCGGCAGCAGATATGAAAAGCGATGCCAAAAAGG
		5′ GCTTTTCATATCTGCTGCCGCTGCTTTTTCAAGATAAACATCAAAATGGC

	SEQ_2190^{GPGP}	5′ GATGTTTATCTTGAAAAAGGTCCTGGTCCTGATATGAAAAGCGATGCCAAAAAGG
		5′ GCTTTTCATATCAGGACCAGGACCTTTTTCAAGATAAACATCAAAATGGC
	SEQ_2190 cloning primer	5′ GTCGCTGAATTCAGTTGCTGGTAATTGTTTTTTAGG
SEM	SeM	5′ GTCGTCGGATCCTTAACTCTGAGGTTAGTCGTACGGGATGCTAAGGTAGCAGAGCTTG
	SeM^{ala}	5′ GATGTTTATCTTGAAAAAGCAGCGGCAGCAAACTCTGAGGTTAGTCGTACGG
		5′ AACCTCAGAGTTTGCTGCCGCTGCTTTTTCAAGATAAACATCAAAATGGC
	SeM^{GPGP}	5′ GATGTTTATCTTGAAAAAGGTCCTGGTCCTAACTCTGAGGTTAGTCGTACGG
		5′ AACCTCAGAGTTAGGACCAGGACCTTTTTCAAGATAAACATCAAAATGGC
	SeM cloning primer	5′ GACGACGAATTCAGATGGCAATTGACCTGCTTTAGC
SEQ_0232	SEQ_0232	5′ GTCGTCGGATCCTTTTGAAAGCAGAAACAACTCAATTG
	SEQ_0232^{ala}	5′ GATGTTTATCTTGAAAAAGCAGCGGCAGCATTGAAAGCAGAAACAACTCAATTG
		5′ GTTTCTGCTTTCAATGCTGCCGCTGCTTTTTCAAGATAAACATCAAAATGGC
	SEQ_0232^{GPGP}	5′ GATGTTTATCTTGAAAAAGGTCCTGGTCCTTTGAAAGCAGAAACAACTCAATTG
		5′ GTTTCTGCTTTCAAAGGACCAGGACCTTTTTCAAGATAAACATCAAAATGGC
	SEQ_0232 cloning primer	5′ GTCGCTGAATTCTGATGGTAGCTGGCCTGCTG

[1] BamHI (GGATCC) and EcoRI (GAATTC) restriction sites are underlined in the sequences.[2] Sequences of the peptide linkers are shown in red (tetra alanine) and blue (glycine-proline-glycine-proline). * SDM = Site directed mutagenesis.

Table S2. RT-qPCR reaction primers.

	Primers Sequence
ACTB	5′ CCAGCACGATGAAGATCAAG 5′ GTGGACAATGAGGCCAGAAT
GAPDH	5′ CAGAACATCATCCCTGCTTC 5′ ATGCCTGCTTCACCACCTTC
IFNγ	5′ CTACCTATTACTGCCAGGCCG 5′ TCCAGGAAAAGAGGCCCAC
TNFα	5′ AAAGGACATCATGAGCACTGAAAG 5′ GGGCCCCCTGCCTTCT
il-10	5′ GCCTTGTCGGAGATGATCCA 5′ TTTTCCCCCAGGGAGTTCAC
il-6	5′ TGCTGGCTAAGCTGCATTCA 5′ GGAAATCCTCAAGGCTTCGAA

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SpeS    MKRLTFILIIL---MVMSTTNLNAVNSESHKDISTIKSKLLYAYTITPYDYKNCDVIFIT  57
        MK++  I I+    +++ +T    + S+S KDIS +KS LLYAYTITPYDYK+C V F T
SpeC    MKKINIIKIVFTITVILISTISPIIKSDSKKDISNVKSDLLYAYTITPYDYKDCRVNFST  60
                                              YAY

                                                                PS00277
SpeS    THTLNIDTQKYKGQCYYINSEVDSEAAKKFKPGDKVDVFGLFYVLNSHTGEYIYGGITPS  117
        THTLNIDTQKY+G+ YYI+SE+  EA++KFK  D VDVFGLFY+LNSHTGEYIYGGITP+
SpeC    THTLNIDTQKYRGKDYYISSEMSYEASQKFKRDDHVDVFGLFYILNSHTGEYIYGGITPA  120
                                                      YGG*Txx
                                                       (LIV)
                                      PS00278
SpeS    QKNKVSKKLLGTLFVSGEPQKSLNNEITLEKDLITIQEFDFKIRNYLMKKYNLYSTTSPY  177
        Q NKV+ KLLG LF+SGE Q++LNN+I LEKD++T QE DFKIR YLM  Y +Y  TSPY
SpeC    QNNKVNHKLLGNLFISGESQQNLNNKIILEKDIVTFQEIDFKIRKYLMDNYKIYDATSPY  180
        xxN                           Kxx*xxxx*DxxxRxxLxxxxx*Y
                                      (LIVF)(LIVF)        (LIV)

SpeS    KGGRIEIGMKDGKHEKIDLFFFPNKGTREDIFKKYKDNKTLDMKLFSHFDVYLEK  232
          GRIEIG KDGKHE+IDLF  PN+GTR DIF KYKDN+ ++MK FSHFD+YLEK
SpeC    VSGRIEIGTKDGKHEQIDLFDSPNEGTRSDIFAKYKDNRIINMKNFSHFDIYLEK  235
                                                    HFD
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Figure S1. Alignment of the predicted amino acid sequence of SpeS and SpeC. The conserved T cell receptor ß-binding motif YAY of SpeC [19,20], the consensus sequence Y-G-G-(LIV)-T-x_4-N and K-x_2-(LIVF)-x_4-(LIVF)-D-x_3-R-x_2-L-x_5-(LIV)-Y of the superantigen Prosite domains PS00277 and PS00278, respectively, and the zinc binding domain, HxD, are highlighted. Consensus matching residues are indicated in red. * represents one of the 3-4 possible amino acids indicated in brackets on the line directly below.

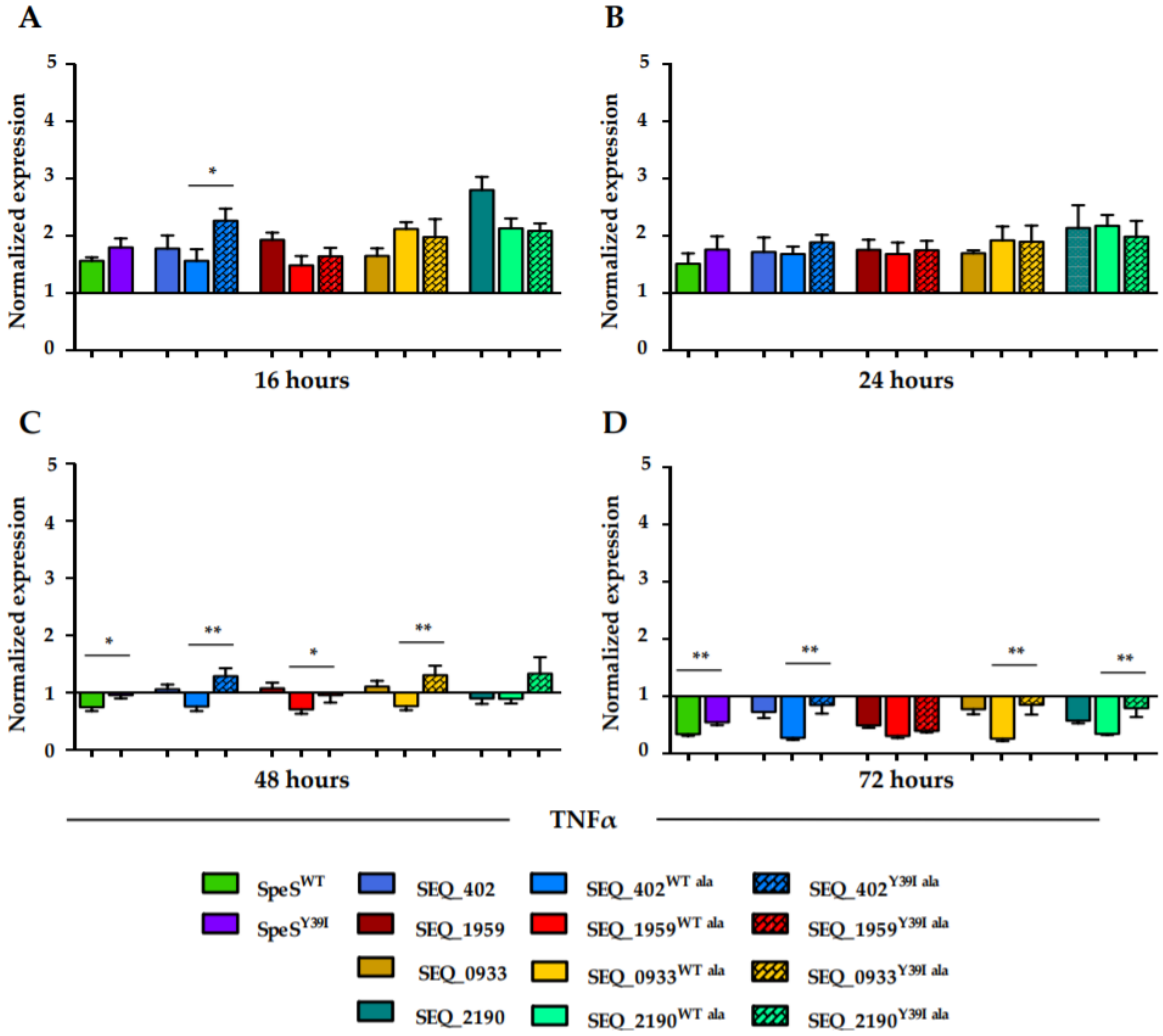

Figure S2. Expression of TNFα by qPCR. A total of 4×10^6 equine PBMCs were stimulated in duplicate with 1 µg/ml of PBS (control), SpeS^{WT}, SpeS^{Y39I} or the fusion proteins for (**A**) 16 h, (**B**) 24 h, (**C**) 48 h and (**D**) 72 h. Samples were normalized to the reference genes ß-actin and GAPDH and differences between the experimental samples and controls calculated. Data is from 3 independent experiments using PBMCs from 2 different donors. Error bars represent SEM. * $p \leq 0.05$, ** $p \leq 0.01$.

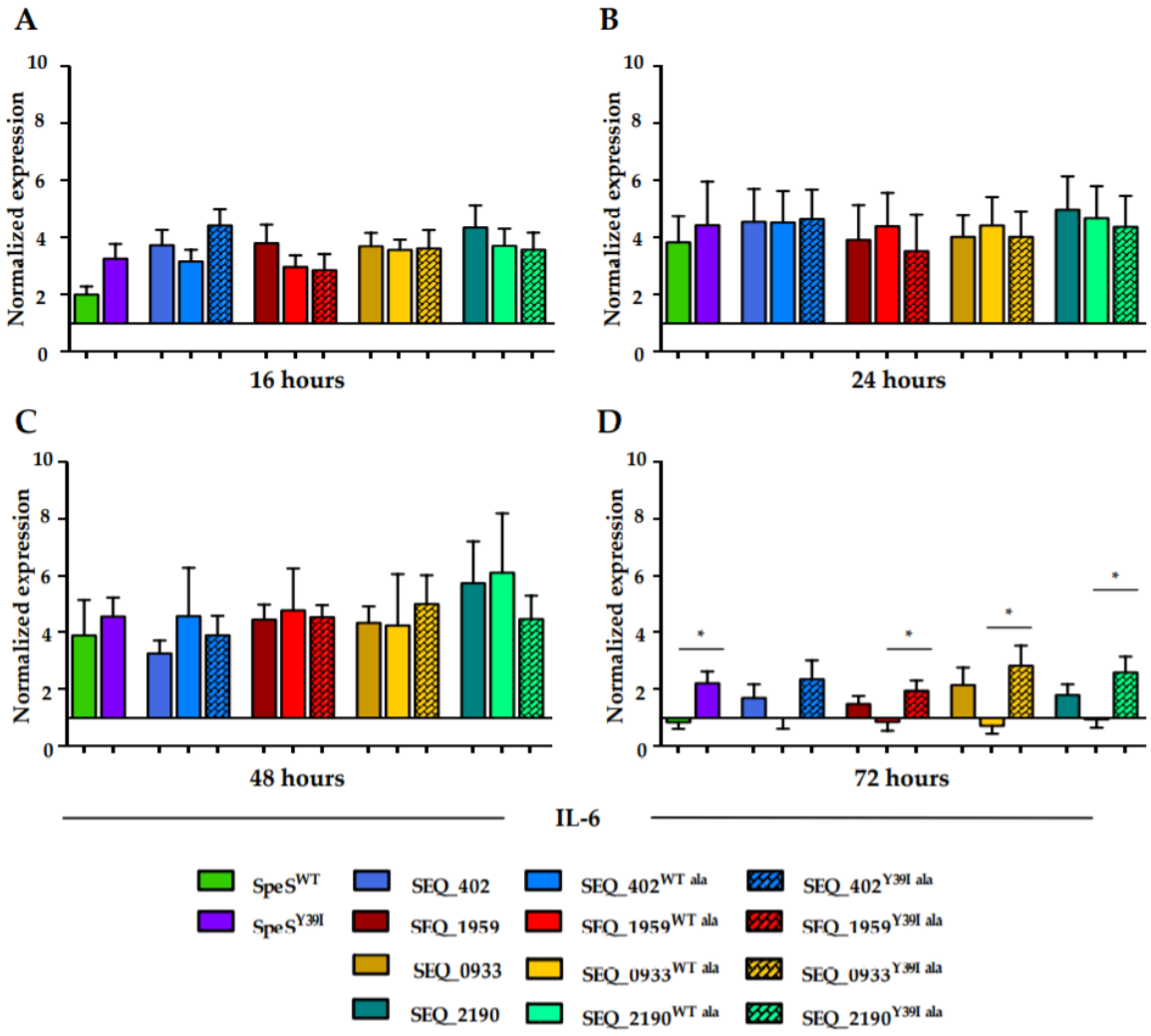

Figure S3. Expression of IL-6 by qPCR. Equine PBMCs (4 × 10^6 cells/ml) were incubated in duplicate for (**A**) 16 h, (**B**) 24 h, (**C**) 48 h and (**D**) 72 h with 1 µg/mL of PBS (control), $SpeS^{WT}$, $SpeS^{Y39I}$ or the fusion proteins. The housekeeping genes, ß-actin and GAPDH, were used to normalize samples and differences between the experimental samples and the control samples were adjusted. Results are from 3 different experiments where PBMCs from 2 different donors were used. Error bars correspond to SEM. * $p \leq 0.05$.

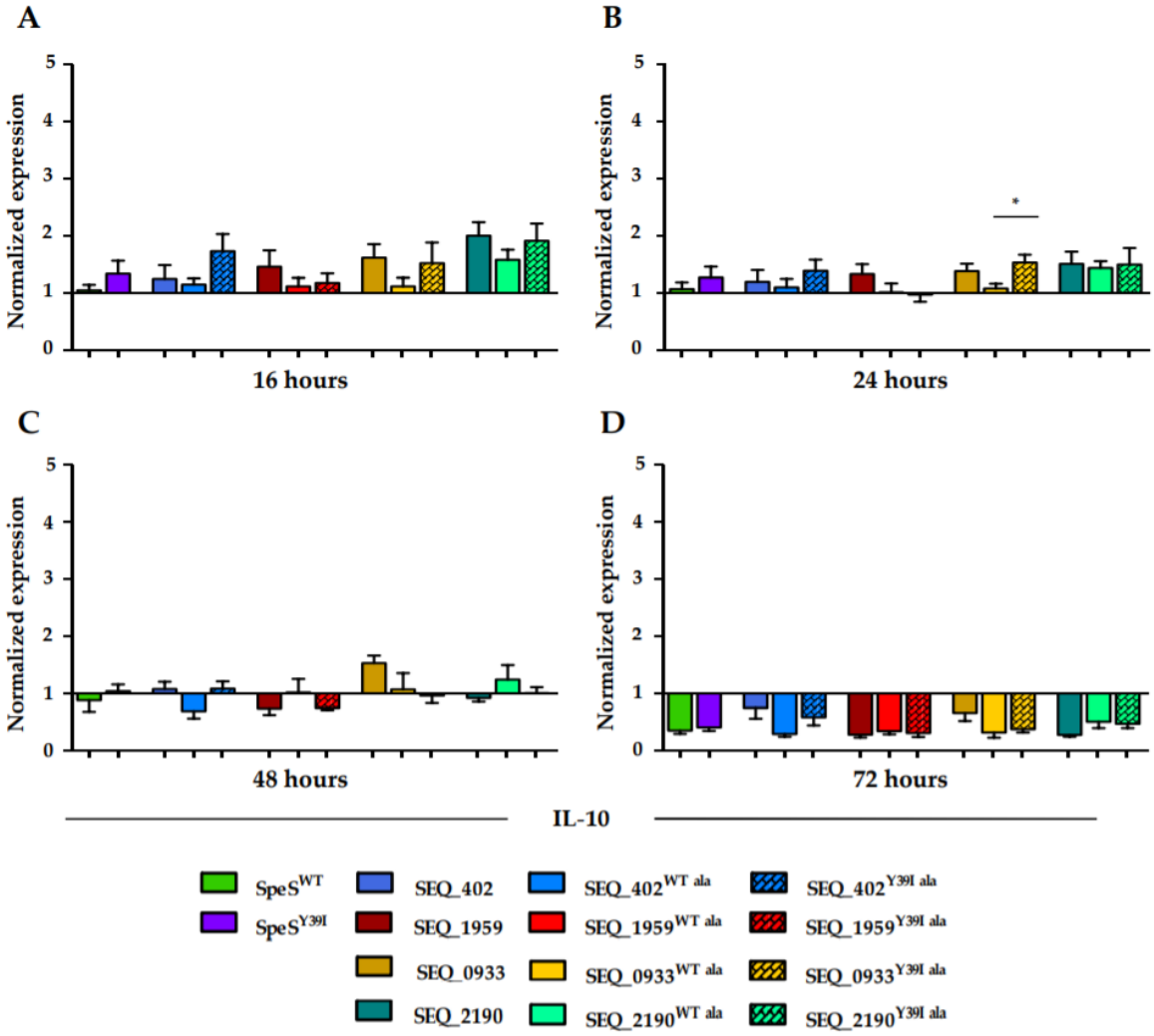

Figure S4. Expression of IL-10 by qPCR. A concentration of 4 × 10^6 equine PBMCs were cultured in duplicate with 1 µg/mL of PBS (control), $SpeS^{WT}$, $SpeS^{Y39I}$ or the fusion proteins for (**A**) 16 h, (**B**) 24 hours, (**C**) 48 h and (**D**) 72 h. Samples were normalized against the reference genes β-actin and GAPDH and differences between the experimental samples and controls calculated. Data is from 3 independent experiments using PBMCs from 2 different donors. Error bars represent SEM. * $p \leq 0.05$.