

SUPPLEMENTARY DATA

STable 1. Amino acid sequences of peptides selected from 10 promising vaccine candidates.

Peptide ID	1st aa	Peptide ID	Last aa
p0660-42	329	DIRAILSVDGLFDSKA	344
p1150-18	137	NGINDEDLGGMYGLLL	152
p1150-19	145	GGMYGLLLLGGFFSVM	160
p2540-p6	41	KNRTINIGVEFRIQDG	56
p2540-p16	121	HDNLNTKELSISLRIK	136
p2540-p19	145	IPQEKVILNRFLQDY	160
p2540-p20	153	LNRFLQDYVNQENLGL	168
p2540-p21	161	VNQENLGLINFWKKK	176
p5420-13	97	TSNNIFHSNVYKDIVM	112
p5420-14	105	NVYKDIVMFPISFALK	120
p7140-p6	41	ELDKVELPKTRARETS	56
p7140-p7	49	KTRARETSSDITVISD	64
p7140-p12	89	QQCCECSSSFGVKWSH	104
p7140-p13	97	SFGVKWSHNDTSDKSD	112
p7140-p20	153	KAEDKVVKAAQIQDVP	168
p7320-p8	57	VYNITRVSSTSSSSLS	72
p7320-p9	65	STSSSSLSTLSPTTII	80
p7320-p21	161	IVSSDTSNNGSVAEEN	176
p7350-9	65	MVSICCQGTSLGGFSE	80
p7360-8	57	VVIVMDLCCQIAGLLC	72
p7620-p2	9	HPKKDSLIFVQDGFSL	24
p7620-p12	89	TKLKRMGYKIYNVIFA	104
p8010-p8	57	LGSSIMAIFGKLPWPA	72

STable 2. The top 23 Th1 immune response inducing peptides. Cytokine production analysis of PBMC from immune sheep (s6821, s6822, and s6823) stimulated with peptides at a final concentration of 50 µg/ml. Values in bold indicate the best average and significant responses induced by peptides in PBMC.

Peptides	Average number of spots/million PBMC for sheep ± the standard deviation for the experimental repeats			
	s6821	s6822	s6823	Ave±Stdev
p0660-42	253±8	53±4	0	102±133
p1150-18	0	118±12	58±4	59±59
p1150-19	0	115±20	88±4	68±60
p2540-6	123±4	73±4	88±4	95±25
p2540-16	270±49	215±7	88±32	191±93
p2540-19	135±71	90±35	53±11	93±41
p2540-20	150±14	68±25	50±0	89±53
p2540-21	148±67	135±49	68±11	117±42
p5420-13	115±10	40±3	20±8	58±50
p5420-14	83±21	60±17	3±1	49±41
p7140-6	135±35	113±18	60±7	103±38
p7140-7	220±21	180±18	35±14	145±97
p7140-12	93±32	298±124	93±32	161±118
p7140-13	133±74	118±11	45±14	99±47
p7140-20	240±21	165±42	70±14	158±85
p7320-8	263±60	113±4	110±78	162±87
p7320-9	245±7	118±18	108±53	157±76
p7320-21	203±88	113±11	48±11	121±77
p7350-9	260±163	195±11	183±14	213±41
p7360-8	253±25	237±28	387±0	292±82
p7620-2	178±95	360±7	93±35	210±136
p7620-12	128±81	150±35	118±14	132±16
p8010-8	250±99	83±25	113±37	149±89
NC	0	0	0	0
Ag+	499±50	481±44	613±77	531±71
ConA	303±45	407±90	178±25	296±114

^a Average number of spots/million PBMC for sheep ± the standard deviation for the experimental repeats. Bold values were statistically significant, p value ≤ 0.05

STable 3. Cytokine profiles induced by recombinant proteins (rproteins) Erum1150, Erum7320, Erum8010 and their overlapping peptides in PBMC from immune sheep as determined by RT-qPCR assays. The average relative mRNA FI was normalised to β -actin and GAPDH after stimulation with antigen. Peptides in red induced the best cytokine specific response in PBMC from all sheep tested and are conserved.

Protein/ Peptides	average relative mRNA FI ^a		
	s6821	s6822	s6823
	β -actin; GAPDH	β -actin; GAPDH	β -actin; GAPDH
	GM-CSF	GM-CSF	GM-CSF
Erum7320	1; 12	12; 32	4; 3
p7320-8 ^b	107; 254	1; 3	30; 2272
p7320-9 ^b	40; 32	9; 7	1; 605
p7320-21 ^b	498; 360	1; 3	14; 7886
Neg. peptide	-	-	-
Ag+	1;7	2;18	3;-
	TNF-α	TNF-α	TNF-α
Erum7620	3;-	5; 11	4;-
p7620-2 ^b	13; 1	1; 1	135; 1013
p7620-12 ^b	6; 3	3; 2	55; 43
Erum0660	-;9	3;8	-
p0660-42 ^b	-;8	-;8	-
Neg. peptide	-	-	1;-
Ag+	-;8	1;4	-
	IFN-γ	IFN-γ	IFN-γ
Erum2540	2;1	-	6;2
p2540-6 ^b	3;1	5;2	2;1
p2540-16 ^b	1;-	-	1;1
p2540-19 ^b	1;-	2;2	1;1
p2540-20 ^b	2;1	3;1	1;2
p2540-21 ^b	3;1	2;3	1;3
Erum7350	2;1	1;1	2;3
p7350-9 ^b	2;-	1;1	2;2
NC	-	-	-
Ag+	1;4	2;1	1;2
	iNOS	iNOS	iNOS
Erum8010	4;4	6;3	-
p8010-8 ^b	1; 3	49; 87	1; 1
Erum1150	2; 2	6; 16	32; 7
p1150-18 ^b ,19 ^b	3;2	-;3	-
NC	-	-	1;1
Ag+	9;5	-;6	-
	IL-1α	IL-1α	IL-1α
Erum7140	3; 3	135; 37	4; 5
p7140-6 ^b	236; 282	2; 1	2; 130
p7140-7 ^b	35; 42	2; 1	2; 1789
p7140-12	6; 4	9; 1	1; 1
p7140-13	7; 5	3; 487	1; 1
p7140-20	12; 8	2; 1	1; 5
Neg. peptide	-	-	1;1
Ag+	1;2	1;16	1;9

STable 3. continued.

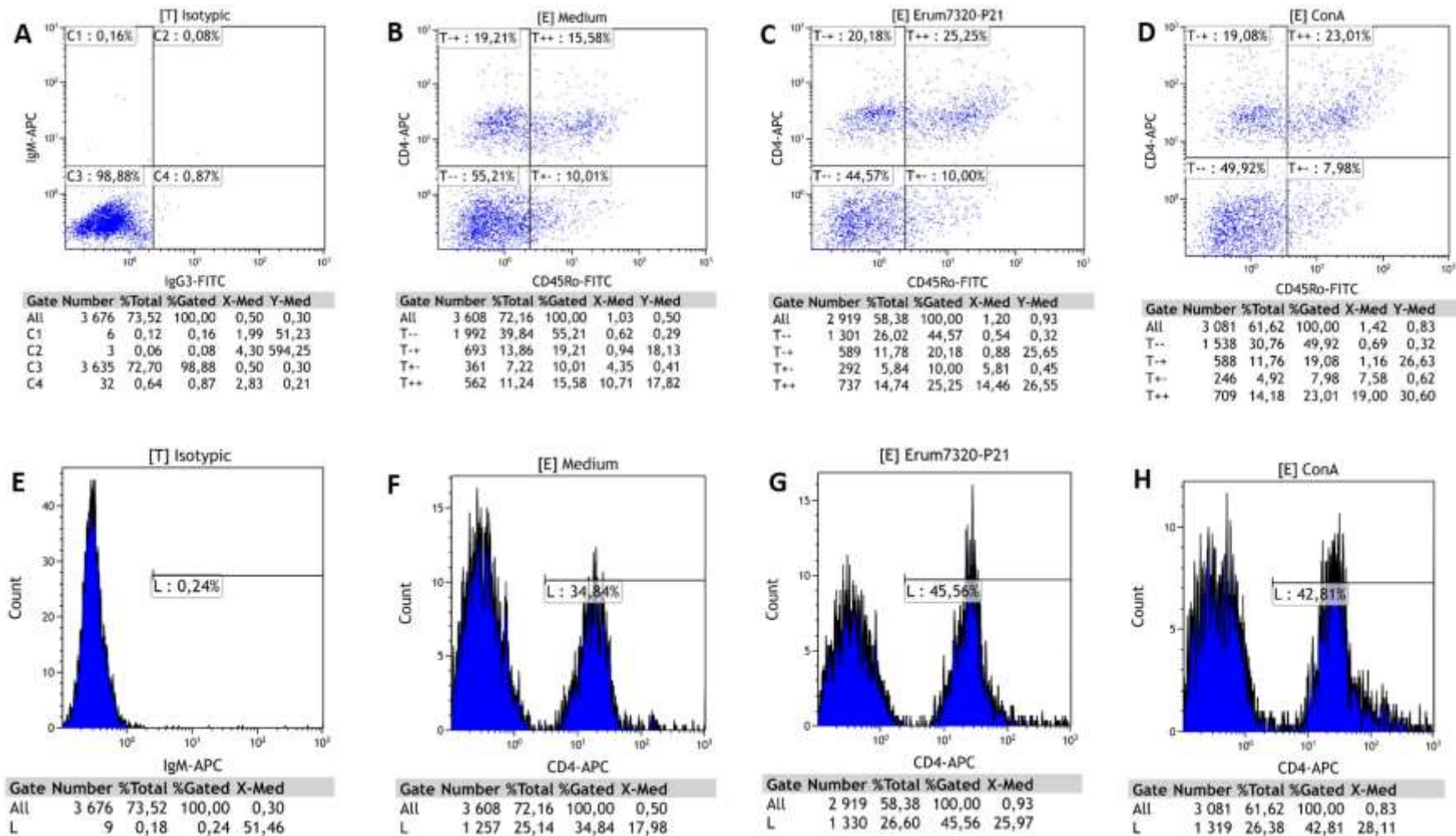
	IL-12	IL-12	IL-12
Erum5420	2;6	2;4	-
p5420-13 ^b , 14 ^b	5;2	1;-	-
Neg.peptide	-	1;1	-
Ag+	7;2	6;3	-
	IL-2	IL-2	IL-2
Erum7360	8;2	4;9	-
p7360-8 ^b	1;5	2;6	-
Neg.peptide-	-	2;2	-
Ag+-	1;5	2;1	-

^aValues represent the $\Delta\Delta C_t$ values, calculated as the mean of duplicate samples each normalised to its own reference genes. Values ≥ 1 were considered as positive.

^bAmino acid sequence of peptide is conserved between 13 different strains.

^c(-) value less than one fold.

NC = Negative control peptide



SFigure 1. Representative dot plots and linear histograms showing phenotype analysis for sheep 6823 immune PBMC, stained with markers for CD4⁺ and CD45R0⁺, after incubation with A) isotypic controls for IgG3 (CD45R0) and IgM (CD4), B) medium (unstimulated PBMC), C) Erum7320-P21 or D) ConA after 72 h incubation. Linear CD4 plots were also included in order to indicate the separation of the CD4 population with E) isotypic control for IgM (CD4), F) medium (unstimulated PBMC), G) Erum7320-P21 or H) ConA.

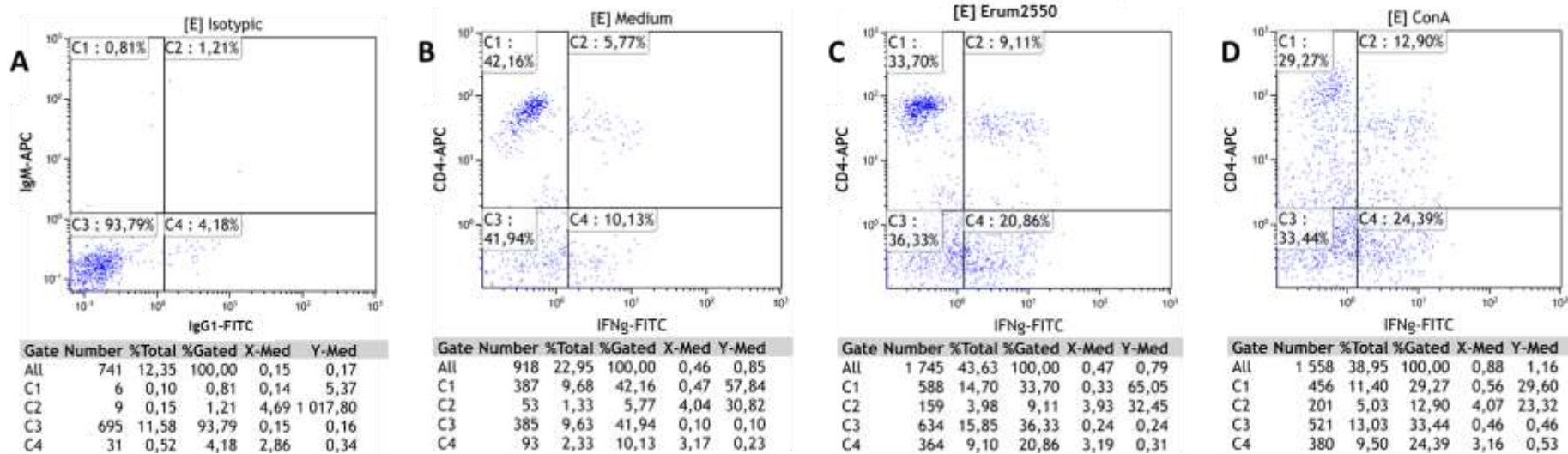


Figure 2. Representative intracellular cytokine staining (ICS) data for sheep 6823 immune PBMC cells, stained intracellularly for IFN- γ , after incubation with B) medium (unstimulated PBMC), C) recombinant protein (rErum2550) and D) ConA for 72 h.