

SUPPLEMENTARY DATA

Supplementary Table 1. List of primers used for qRT-PCR transcript analysis and for cloning of V α 5 and V β 13 CDR3 sequences.

Primers for qRT-PCR	
TCR-V β 13-Fwd	TCGACACAGTTCTGTCTGAAAC
TCR-V β 13-Rev	TGTCCAGAAACAGGGTCACA
TCR-C β -Fwd	GGTCAGAGGACTCACCCAAA
TCR-C β -Rev	AGCACAGCATAACAGGGTGG
GAPDH-Fwd	GCACCACCAACTGCTTAGCC
GAPDH-Rev	CTGAGTGGCAGTGATGGCAT
Insulin-Fwd	CAAACAGCACCTTTGTGGT
Insulin-Rev	CCAGTTGGTAGAGGGAGCAG
Primers for TCR transcript cloning	
TCR-V β 13	TCGACACAGTTCTGTCTGAAAC
TCR-C β -Rev	GCTTCTGATGGCTCAAACAAGG
TCR-V α 5 Fwd (1)	ATGAGCCAAGGTGAGCAAGT
TCR-V α 5 Fwd (2)	CAGCTTCCTTCCATCCTGAG
TCR-C α Rev	GCTCTGGGACACATTGGTTT
Primers for TCR-Vα5 confirmation analysis	
TCR-V α 5-Fwd	AAAGAAAGCAGACCCAACGA
TCR-V α 68-Fwd	AACCCCATGGTTCAAGGATT
TCR-Ja28-Rev	TCCCAAAGTGAGCTGGTAAC
TCR-Ja53-Rev	CCAAATGTCAGTTTGTAGCTGG
TCR-Ja10-Rev	CAAAGACAAGGTTGTAGCCTGTT
TCR-Ja28-Fwd	GTACCAAACCTCTCAGTCATAACCGA
TCR-Ja53-Fwd	CAGGAAGTACCAGCTACAAACTGAC
TCR-Ja10-Fwd	GGGACAGGAACAAGACTGCT
TCR-C α -Rev	GCTCTGGGACACATTGGTTT

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Supplementary Table 2. This complete listing of all TCR-V β 13 CDR3-J β sequences and frequencies is being provided at the request of the reviewer.

TCR-Vβ13 Spleen Untreated (N=127 sequences)	
CDR3-Jβ Sequence	Frequency
CASSLGGLTDKIYFGSGTRLTVL	7.1%
CASQTGGVSYEQYFGPGTKLTVL	3.9%
CASRGTGEVFFGKGTRLTVV	2.4%
CASSLGGESYEQYFGPGTKLTVL	2.4%
CASSLETGALSIAEQFFGSGTRLTVL	2.4%
CASSLDQNTLFFGAGTRLSVL	1.6%
CASSLSDWGGQNTLFFGAGTRLSVL	1.6%
CASFQGPQLYFGEGSKLTVL	1.6%
CASSLGTGAGNVLYFGEGSRLLVV	1.6%
CASSRTGFNERLFFGHGTKLSVL	1.6%
CASSRTGFNERLFFGHGTKLSVL	1.6%
CASTWDNNERLFFGHGTKLSVL	1.6%
CASSLAGSSNTEVFFGKGTRLTVV	1.6%
CASSLGLLNTEVFFGKGTRLTVV	1.6%
CASSPSGGEVFFGKGTRLTVV	1.6%
CASRQTGGVSYEQYFGPGTKLTVL	1.6%
CASSLGTGSSYEQYFGPGTKLTVL	1.6%
CASSPFLNQETQYFGPGTRLLVL	1.6%
CASSLSLGGASDKIYFGSGTRLTVL	1.6%
CASLKQGNTLFFGAGTRLSVL	0.8%
CASSFGWGGVYDTLFFGAGTRLSVL	0.8%
CASSLASQNTLFFGAGTRLSVL	0.8%
CASSLKQGNTLFFGAGTRLSVL	0.8%
CASSPTGQNTLFFGAGTRLSVL	0.8%
CASSSSQNTLFFGAGTRLSVL	0.8%
CASRAGTHTGQLYFGEGSKLTVL	0.8%
CASSFQGPQLYFGEGSKLTVL	0.8%
CASSPETGGSNTGQLYFGEGSKLTVL	0.8%
CASLTGPSGNVLYFGEGSRLLVV	0.8%
CASRPTGESGNVLYFGEGSRLLVV	0.8%
CASSLGQISGNVLYFGEGSRLLVV	0.8%
CASSLHNSGNVLYFGEGSRLLVV	0.8%
CASSPGQGGNVLYFGEGSRLLVV	0.8%
CASSSTPSGNVLYFGEGSRLLVV	0.8%
CASSRDRGNQAQYFGEGTRLSVL	0.8%
CASRTNTGQGERLFFGHGTKLSVL	0.8%
CASSFGNSNERLFFGHGTKLSVL	0.8%
CASSLEGAINERLFFGHGTKLSVL	0.8%
CASSLPGPNERLFFGHGTKLSVL	0.8%
CASSPDRGRYERLFFGHGTKLSVL	0.8%
CASSPGTNERLFFGHGTKLSVL	0.8%
CASSPGAGGNERLFFGHGTKLSVL	0.8%

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CASSPRGWSERLFFGHGTKLSVL	0.8%
CASSRDALNERLFFGHGTKLSVL	0.8%
CASSSGTVNERLFFGHGTKLSVL	0.8%
CASSSGVSNERLFFGHGTKLSVL	0.8%
CASSSNERLFFGHGTKLSVL	0.8%
CASSSWGDERLFFGHGTKLSVL	0.8%
CASSFGPANTEVFFGKGTRLTVV	0.8%
CASSFGETEVFFGKGTRLTVV	0.8%
CASSFWGKDTEVFFGKGTRLTVV	0.8%
CASSGTGDTEVFFGKGTRLTVV	0.8%
CASSLAQGDTEVFFGKGTRLTVV	0.8%
CASSLDTNTEVFFGKGTRLTVV	0.8%
CASSLQGNTEVFFGKGTRLTVV	0.8%
CASSLTEINTEVFFGKGTRLTVV	0.8%
CASSPQGYTEVFFGKGTRLTVV	0.8%
CASSRDRGDTEVFFGKGTRLTVV	0.8%
CASSSSAGEGTEVFFGKGTRLTVV	0.8%
CASTGNTEVFFGKGTRLTVV	0.8%
CASSLDWGVSYEQYFGPGTKLTVL	0.8%
CASSLEDVYEQYFGPGTKLTAL	0.8%
CASSLLDGSYEQYFGPGTKLTVL	0.8%
CASSPLLGGSYEQYFGPGTKLTVL	0.8%
CASSRDSSYEQYFGPGTKLTVL	0.8%
CASSRQLVSYEQYFGPGTKLTVL	0.8%
CASSRTGNEQYFGPGTKLTVL	0.8%
CASSSGDSYEQYFGPGTKLTVL	0.8%
CASSSGQGLYEQYFGPGTKLTVL	0.8%
CASSXXYEQYFGPGTKLTVX	0.8%
CASVPEAYEQYFGPGTKLTVL	0.8%
CASXSGXGAYEXYFGPGTKLTVL	0.8%
CASIDREETQYFGPGTRLLVL	0.8%
CASRGTGMYDYTFGPGTRLLVI	0.8%
CASRKDRVQETQYFGPGTRLLVL	0.8%
CASSFTLNYDYTFGPGTRLLVI	0.8%
CASSLGQGLNYDYTFGPGTRLLVI	0.8%
CASSLLGGAQETQYFGPGTRLLVL	0.8%
CASSLPGADQETQYFGPGTRLLVL	0.8%
CASSLTQETQYFGPGTRLLVL	0.8%
CASSSGTEETQYFGPGTRLLVL	0.8%
CASSSGTEETQYFGPGTRLLVL	0.8%
CASSLXQITERLFFGQSKLSVL	0.8%
CASKEGNAEQFFGSGTRLTVL	0.8%
CASRSGTYTDKIYFGSGTRLTVL	0.8%
CASSADWGGADEQFFGSGTRLTVL	0.8%
CASSFNWGGKIYFGSGTRLTVL	0.8%
CASSLGQVYAEQFFGSGTRLTVL	0.8%
CASSLIGTDKIYFGSGTRLTVL	0.8%

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CASSPGTGGDTDKIYFGSGTRLTVL	0.8%
CASSQTGVAEQFFFGSGTRLTVL	0.8%
CASSRDWGVSYAEQFFFGSGTRLTVL	0.8%
CASSSGTSSYAEQFFFGSGTRLTVL	0.8%
CASSSTGTDKIYFGSGTRLTVL	0.8%
CASSRTGGSYAEQFFFGSGTRPTVL	0.8%
TCR-Vβ13 Islet-Day 4 (N=64 sequences)	
CDR3-Jβ Sequence	Frequency
CASIRTGGLKYFGPGTKLTVL	3.1%
CASSLDSSYEQYFGPGTKLTVL	3.1%
CASSLDWGGREYFGPGTKLTVL	3.1%
CASSLVWGGQNTLFFGAGTRLSVL	3.1%
CASSNWTGGGYEQYFGPGTKLTVL	3.1%
CASSPQGADKIYFGSGTRLTVL	3.1%
CASSRDISYEQYFGPGTKLTVL	3.1%
CASRARDGYEQYFGPGTKLTVL	1.6%
CASRHGLGGNAEQFFFGSGTRLTVL	1.6%
CASRSGGNTEVFFGKGTRLTVV	1.6%
CASSAPGRSDKIYFGSRTRLTVL	1.6%
CASSFWGKAEQFFFGSGTRLTVL	1.6%
CASSGQNTLFFGAGTRLSVL	1.6%
CASSGTEVFFGKGTRLTVV	1.6%
CASSKDWGGNSYAEQFFFGSGTRLTVL	1.6%
CASSKGQGAPNERLFFGHGTKLSVL	1.6%
CASSLAGRGETDKIYFGSGTRLTVL	1.6%
CASSLARGLSLYEQYFGPGTKLTVL	1.6%
CASSLARGQTRYILAQEPDPSRIKRLHPRS	1.6%
CASSLDRANTEVFFGKGTRLTVV	1.6%
CASSLDWGVNTLFFGAGTRLSVL	1.6%
CASSLEGVDNEILFFGHGTKLSVL	1.6%
CASSLFARGQETQYFGPGTRRLVL	1.6%
CASSLGAEQFFFGSGTRLTVL	1.6%
CASSLGDWGGREYFGPGTKLTVL	1.6%
CASSLGFNERLFFGHGTKLSVL	1.6%
CASSLGGLSNERLFFGHGTKLSVL	1.6%
CASSLGGPYEQYFGPGTKLTVL	1.6%
CASSLGGQYEQYFGPGTKLTVL	1.6%
CASSLGRDWGGRYAEQFFFGSGTRLKVL	1.6%
CASSLGIRNSPLYFAAGTRLTVT	1.6%
CASSLGLGGVQNTLFFGAGTRLSVL	1.6%
CASSLGQGRAEQFFFGSGTRLTVL	1.6%
CASSLGTLNERLFFGHGTKLSVL	1.6%
CASSLIGEERLFFGHGTKLSVL	1.6%
CASSLQGAYDYTFGPGTRLLVI	1.6%
CASSLTGDYAEQFFFGSGTRLTVL	1.6%
CASSLTGRDWGSYEQYFGPGTKLTVL	1.6%

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CASSLVGDTEVFFGKGTRLTVV	1.6%
CASSPDRDGNVLYFGEGSRLLVV	1.6%
CASSPGHNSPLYFAAGTRLTVT	1.6%
CASSPGQSGNVLYFGEGSRLLVV	1.6%
CASSPGTGGQAQYFGEGTRLSVL	1.6%
CASSPSSTGQLYFGEGSKLTVL	1.6%
CASSQTGRDTGQLYFGEGSKLTVL	1.6%
CASSRDSTGQLYFGEGSKLTVL	1.6%
CASSRDWGGRGDKIYFGSGTRLTVL	1.6%
CASSRRGGDTGQLYFGEGSKLTVL	1.6%
CASSRTGPWAEQFFGSGTRLTVL	1.6%
CASSSDSERLFFGHGTKLSVL	1.6%
CASSESYAEQFFGSGTRLTVL	1.6%
CASSSGAGGSYAEQFFGSGTRLTVL	1.6%
CASSSGSSSYEQYFGPGTKLTVL	1.6%
CASSSTGYAEQFFGSGTRLTVL	1.6%
CASSWAGGAYEQYFSPGTKLTVL	1.6%
CASSWDRENTLFFGAGTRLSVL	1.6%
CASSXGGGGDKIYFGSGTRLTVL	1.6%
TCR-Vβ13 Islet-Day 5 (Expt 1) (N=74 sequences)	
CDR3-Jβ Sequence	Frequency
CASSRAGSGNVLYFGEGSRLLVV	25.0%
CASSERDVQETQYFGPGTRLVL	15.6%
CASSWTGGDTLLFGAGTRLSVL	10.9%
CASSFGTGGTDKIYFGSGTRLTVL	9.4%
CASSQDKGGQLYFGEGSKLTVL	7.8%
CASSWTGGDTLFFGAGTRLSVL	6.3%
CASSLEXSGNVLYFGEGSRLLVV	6.3%
CASSFPGNTEVFFGKGTRLTVV	3.1%
CASSFGTGYEQYFGPGTKLTVL	3.1%
CASSSTGGTDKIYFGSGTRLTVL	3.1%
CASSSTGGDAEQFFGSGTRLTVL	3.1%
CASSWTGGDTLFFXASTLLSVL	1.6%
CASSRDREAQYFGEGARLSVL	1.6%
CASSRDREAQYFGEGTRLSVL	1.6%
CASSSTGGRHXIFFGCTLMTVL	1.6%
TCR-Vβ13 Islet-Day 5 (Expt 2) (N=71 sequences)	
CDR3-Jβ Sequence	Frequency
CASSRDRVSGNVLYFGEGSRLLVV	22.5%
CASSPDRDERLFFGHGTKLSVL	11.3%
CASSLDQNTLFFGAGTRLSVL	8.5%
CASSRQGERLFFGHGTKLSVL	5.6%
CASSWDRAFSNERLFFGHGTKLSVL	5.6%
CASSRAGSGNVLYFGEGSRLLVV	4.2%
CASSQLDKNQAQYFGEGTRLSVL	4.2%

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CASSPTISNERLFFGHGKLSVL	4.2%
CASSFAQNTEVFFGKGTRLTVV	4.2%
CASSLGGARTGQLYFGEGSKLTVL	2.8%
CASSLGTGAGNVLYFGEGSRLLVV	2.8%
CASSLGTGAGNVLYFGEGSRLLVV	2.8%
CASSPRDRDQAQYFGEGTRLSVL	2.8%
CASSLGTGAGNVLYFGERSRLLVV	2.8%
CASSLEGTSSYEQYFGPGTKLTVL	2.8%
CASSPDWGGRGNTGQLYFGEGSKLTVL	1.4%
CASSPRDRDQAQYFGEGTWLSVL	1.4%
CASSLRPHMGVFFGKGTRLTVV	1.4%
CASSLTGTEVFFGKGTRLTVV	1.4%
CASSSDRVDTEVFFGKGTRLTVV	1.4%
CASSFGGRSYEQYFGPGTKLTVL	1.4%
CASKEGNAEQFFGSGTRLTVL	1.4%
CASSLGQVYAEQFFGSGTRLTVL	1.4%
CASSLSGRYAEQFFGSGTRLTVL	1.4%
TCR-Vβ13 Islet-Day 7 (N=66 sequences)	
CDR3-Jβ Sequence	Frequency
CASSFAGGYEQYFGPGTKLTVL	43.9%
CASSERGNVDYTFGPGTRLLVI	6.1%
CASSSGRYNSPLYFAAGTRLTVT	3.0%
CASSQGGFGNVLYFGEGSRLLVV	3.0%
CASSSGTGWERLFFGHGKLSVL	3.0%
CASSRDSSYEQYFGPGTKLTVL	3.0%
CASSSGGASYAEQFFGSGTRLTVL	3.0%
CASSLGGRNDLFFGAGTQLSVL	1.5%
CASSLAGNTLFFGAGTRLSVL	1.5%
CASSLAPGEDTLFFGAGTRLSVL	1.5%
CASSSTGGGEVTLFFGAGTRLSVL	1.5%
CASSEGAGNSPLYFAAGTRLTVT	1.5%
CASKGGNTGQLYFGEGSKLTVL	1.5%
CASSFEQGGISNERLFFGHGKLSVL	1.5%
CASSLSQDRAPERLFFGHGKLSVL	1.5%
CASSLRASTEVFFGKGRTLTVV	1.5%
CASSFSGLTNTEVFFGKGTRLTVV	1.5%
CASSRGPTEVFFGKGTRLTVV	1.5%
CASSFAGGYEQYFGTGTKLTVL	1.5%
CASASGGTYEQYFGPGTKLTVL	1.5%
CASRLLGGLDXDYFPGTKLTDQ	1.5%
CASSLGARTEVFFGPGTRLTVV	1.5%
CASSLAGGVNQETQYFGPGTRLLVL	1.5%
CASSLGLGGETQYFGPGTRLLVL	1.5%
CASSLWDWGGEETQYFGPGTRLLVL	1.5%
CASRSGGGQETQYFGPGTRLLVL	1.5%
CASSSTPNQETQYFGPGTRLLVL	1.5%

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CASSLAGGYEQXFGSGTKLTVL	1.5%
CASSLGGGADKIYFGSGTRLTVL	1.5%
CASSSGDWGTDKIYFGSGTRLTVL	1.5%
TCR-Vβ13 Cultured Islet T-Cells (N=117 sequences)	
CDR3-Jβ Sequence	Frequency
CASSFAGGVYEQYFGPGTKLTVL	50.4%
CASSLGLGGETQYFGPGTRLLVL	18.8%
CASSLEGGERLFFGHGTKLSVL	5.1%
CASRAGLGGDYEQYFGPGTKLTVL	3.4%
CASSLWTGDGNTEVFFGKGTRLTVV	2.6%
CASREAGTGSDKIYFGSGTRLTVL	2.6%
CASSRTGGYAEQFFGSGTRLTVL	2.6%
CASSFRGGRENTLFFGAGTRLSVL	1.7%
CASSFYLGLSNERLFFGHGTKLSVL	1.7%
CASSPRLGGALAEQFFGSGTRLTVL	1.7%
CASSPRGGGAIQNTLFFGAGTRLSVL	0.9%
CASSQGDQNTLFFGAGTRLSVL	0.9%
CASSLGTGAGNVLYFGEGSRLLVV	0.9%
CASSFRGSNERLFFGHGTKLSVL	0.9%
CASSFYLGLXNERMFFGHGTKLSVL	0.9%
CASSSGTGGPNERLFFGHGTKLSVL	0.9%
CASSFAVGVYNQYFGPGTHLLVL	0.9%
CASSLAGGVYEQYFGPGTKLTVL	0.9%
CASSLFGGTSYEQYFGPGTKLTVL	0.9%
CASSLGGGGETQYFGPGTRLLVL	0.9%
CASSPSGKETQYFGPGTRLLVL	0.9%

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Supplementary Table 3. This complete listing of all TCR-V α 5 CDR3-J α sequences and frequencies is being provided at the request of the reviewer.

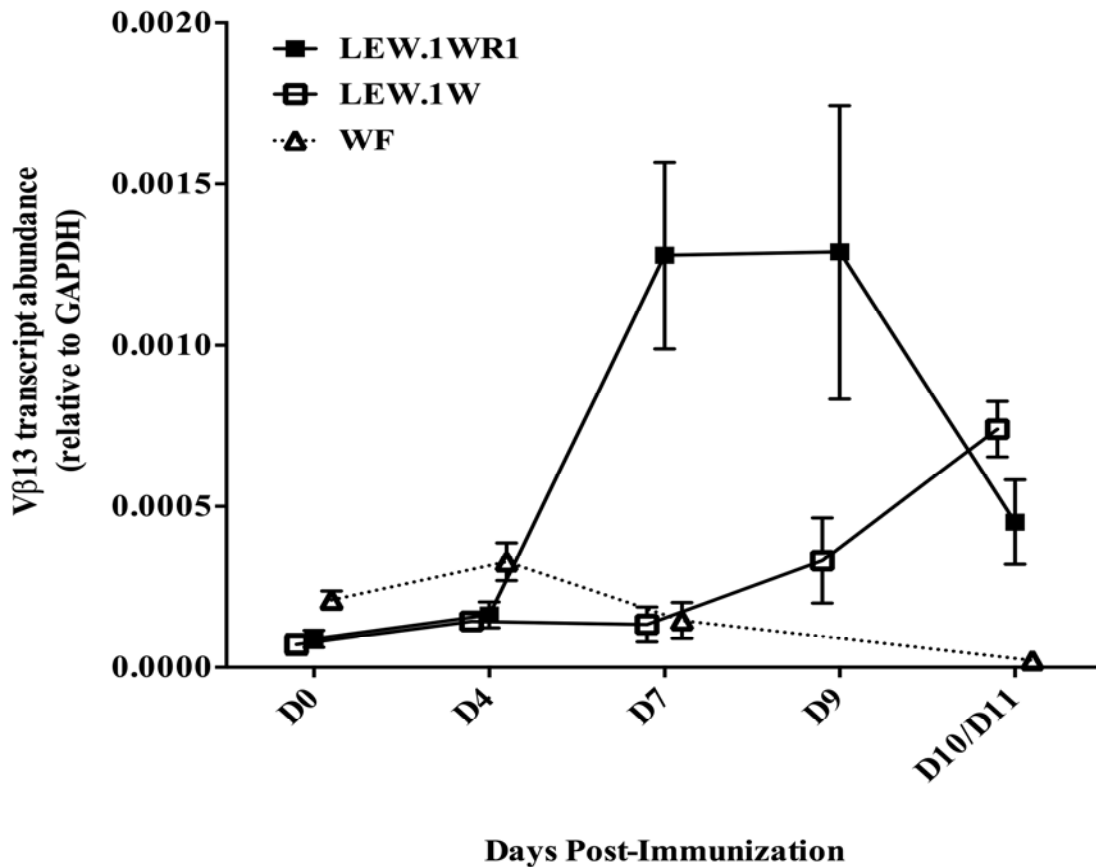
TCR-Vα5 Spleen Untreated (N=62 sequences)	
CDR3-Jα Sequence	Frequency
CAASDNSNYQLIWGSGTKLIIKPD	3.9%
CAASRGNNNAPRFGAGTRLTVKPN	2.6%
CAATPYNSNAGKLTFGDGTRLTVKPN	2.6%
CAASEGGGSFAKPTFGKGTKLSVKS	2.6%
CAAKGYNQGKLIFGQGTKLSIKPN	2.6%
CANRAGNRLTFGRGTRVLIRPD	2.6%
CAASNNNAPRFGAGTRLTVKPN	1.3%
CAARAHNNNAPRFGAGTRLTVKPN	1.3%
CAASAGNNNAPRFGAGTRLTVKPN	1.3%
CAARANNNNAPRFGAGTRLTVKPN	1.3%
CAASAGNNAPRFGAGTRLTVKPN	1.3%
CAASRHTGNKYVFGAGTRLKVIPH	1.3%
CAARYNNNAPRFGAGTRLTVKPN	1.3%
CAASASNNNNRLFFGDGTQLVVKPN	1.3%
CAPRGNRLSFGDGTQLVVKPN	1.3%
CAASNSNAGKLTFGDGTRLTVKPN	1.3%
CAASPNSNAGKLTFGDGTRLTVKPN	1.3%
CAASAGGGSQKLIFGEGTKLTVSLY	1.3%
CAASGVAGGLSGKLTFGEGTQVTVISD	1.3%
CAASAGSALGKLYFGIGTQLTVTPD	1.3%
CTASIRGSALGKLYFGIGTQLTVTPD	1.3%
CTARPHRGSALGKLYFGIGTQLTVTPD	1.3%
CTASRGSALGKLYFGIGTQLTVTPD	1.3%
CAASNANKVIFGKGTRLHVLPN	1.3%
CAASAGTGSYQLTFGKGTKLSVIPN	1.3%
CAASEGSGGSFAKPTFGKGTKLSVKS	1.3%
CAASGGGSFAKPTFGKGTKLSVKS	1.3%
CAAGGSFAKPTFGKGTKLSVKS	1.3%
CAAVGAGSTSYKLTFGKGTLTVNPN	1.3%
CAAINSGGSFAKPTFGKGTKLSVKS	1.3%
CAAPPGTGSYQLTFGKGTKLSVIPN	1.3%
CAASRESGRTLIFGKGTLVLPSPN	1.3%
CAAVSGGSFAKPTFGKGTKLSVKS	1.3%
CAAGTGSTSYKLTFGKGTLTVNPN	1.3%
CAASVRDGANNKLTFGKGTTLSVIPD	1.3%
CAASGKGNTGKLIFGLGTTLQVQPD	1.3%
CTASNDVGVNSKLIWGLGTS LAVNPN	1.3%
CAALTAMQANPLGMGPGSQSQMS	1.3%

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CTASITGFGSALKFGPGTKVIVIPY	1.3%
CAATSSNSKLVFGQGTSLVIPN	1.3%
CAANRAGNRLTFGRGTRVLIRPD	1.3%
CTASEFYDKLYFGSGTKLIVKPN	1.3%
CAAIESGGYKLVFGSGTRLLVSAD	1.3%
CTARGQVVGQLTFGSGTRLQVYAN	1.3%
CTASISSGNWQLVFGSGTQLTVLPD	1.3%
CTAVRGGGYKLVFGSGTRLLVSAD	1.3%
CAASAGGSANXLIFGTGTLISVKPN	1.3%
CAAPGDSGTYSFSGTGKLSVIPN	1.3%
CAARGSSGTDKVIFGTGTRLQVSPN	1.3%
CTASIGTGFGKLFQGTGTQVVVTPD	1.3%
CTASKSGTYQSFSGTGKLSVIPN	1.3%
CTASPSSGTDKVIFGTGTRLQVSPN	1.3%
CAARSYGGSGNKLIFGTGTLISVKPN	1.3%
CAARSGTDKVIFGTGTRLQVSPN	1.3%
CAASSGTDKVIFGTGTRLQVSPN	1.3%
TCR-Vα5 islet Day 5 (N=24 sequences)	
CDR3-Jα Sequence	Frequency
CAASLYGNEKMTFGAGTRLTIKPN	45.8%
CAAGNSNAGKLTFGDGTRLTVKPN	29.2%
CAASPGGNYKLTFGKGTSLVVHPH	8.3%
CAASAWSSGGSFAKPTFGKGTKLSVKS	8.3%
CAASATGTDKVIFGTGTRLQVSPN	4.2%
CAASNISSGNWQLVFGSGTQLTVLPD	4.2%
TCR-Vα5 Cultured Islet T-Cells (N=113 sequences)	
CDR3-Jα Sequence	Frequency
CAASPGTGSYQLTFGKGTKLSVIPN	59.3%
CAASSYGANNKLTFGKGTTLVIPD	14.2%
CAASALRGSGNKLIFGTGTLISVKPN	9.7%
CAASAGDSGTYSFSGTGKLSVIPN	4.4%
CAASPGTGSYQLTFGKD	3.5%
CAVESSGNWQLVFGSGTQLTVLPD	2.7%
CAGQGGSQKLIFGEGTKLTVSLY	1.8%
CAASALHGNEKMTFGAGTRLTIKPN	0.9%
CAGQGGXQKLIFGEGTKLTVSLY	0.9%
CAASSYGANNKLTFGEGTTLVIPD	0.9%
CAASALRGSGNKLIFGTGTLISVKPN	0.9%
CAAVTTTGFGKLFQGTGTQVVVTPD	0.9%

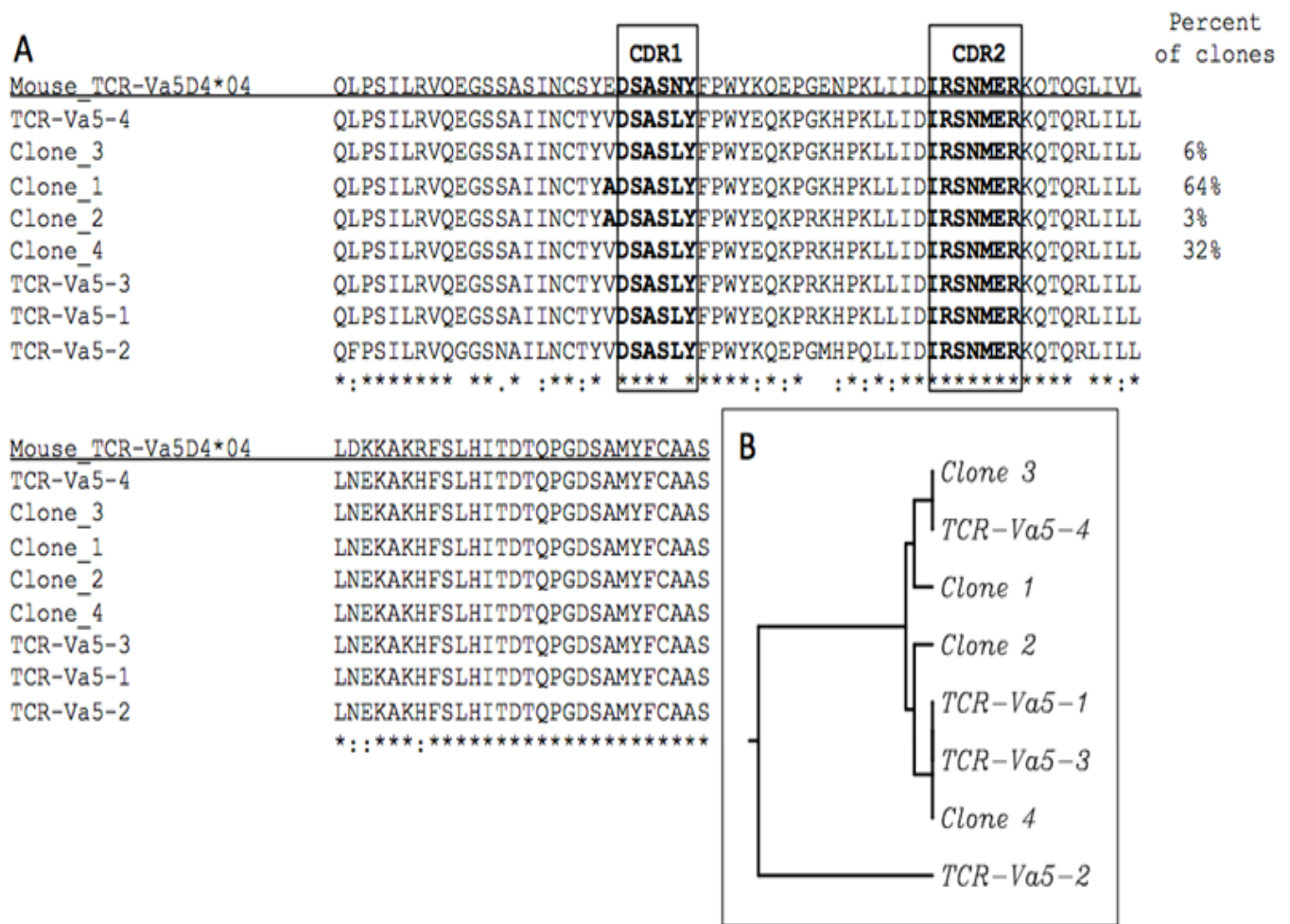
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Supplementary Figure 1. V β 13+ T-cells in the islets of diabetes resistant rats. We analyzed the abundance of V β 13+ transcripts in the islets of LEW.1WR1, LEW.1W and WF rats to determine if we could compare the clonality of V β 13+ transcripts between susceptible and resistant rats at a given time. While V β 13+ transcripts increase significantly in LEW.1WR1 islets on day 7, the abundance in resistant islets is not above the baseline level of untreated samples. Of interest, this is a slight elevation of V β 13+ transcripts between day 9 and 11 in the LEW.1W rat. This delayed accumulation of potentially diabetogenic T-cells corresponds to the later onset of diabetes in a limited subset of LEW.1W rats.



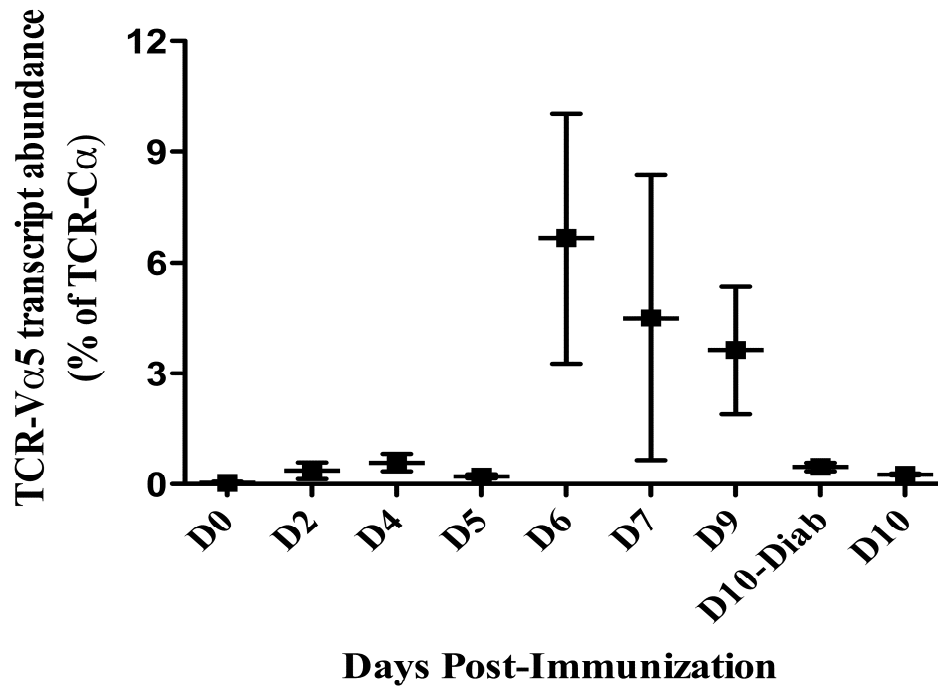
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Supplementary Figure 2. TCR-V α 5 paralogs: novel identification and alignments A. Amino acid sequences from mouse TCR-V α 5D4*04 and the four rat TCR-V α 5 paralogs identified in the rat reference sequence with cDNA clones (Clone 1-4) from our experiments. Clones 1 and 2 are novel sequences and differ from the reference sequences at the residues indicated in bold to the left of CDR1. Interestingly, the CDR2 nucleotide sequence of TCR-V α 5-2 differs from the other paralogs at a single base but retains the same amino acid sequence indicating a concerted evolutionary selection to retain this particular CDR2 peptide sequence B. Branch-length dendrogram display of rat sequences in A. Clones 1 and 2 are likely to be novel paralogous variants derived from TCR-V α 5-4 and TCR-V α 5-1 (or 5-3) respectively with the difference of a single nucleotide polymorphism. This identification of two novel paralogs suggests there may be an additional TCR α locus duplication adding further complexity to our understanding of TCR genetics.



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Supplementary Figure 3. TCR-V α 5+ Transcripts have a tight temporal accumulation in the LEW.1WR1 pre-diabetic islet. TCR-V α 5+ Transcripts from LEW.1WR1 islets were analyzed by qRT-PCR analysis and normalized to TCR-C α to determine the percent of V α 5+ T-cells. There is a trend toward V α 5+ transcript accumulation by day 6 in the LEW.1WR1 islet but means are not significantly different. Latency is variable even among littermates so within each day (6,7,9) biological replicates may represent different staging of diabetes progression. If V α 5+ T-cells infiltrate the islet only briefly during initiation of diabetes this explains the high variability between animals on days 6-9. N= 3-5 rats per time point, 29 rats total.



SUPPLEMENTARY DATA

Supplementary Figure 4. Islet specific antigen focusing of V α 5+ transcripts. TCR-V α 5+J α 28+ sequences represent about 60% of cultured islet T-cell transcripts analyzed above (Figure 4.B, sequence 3). To confirm the islet-specific skewing of V α 5-J α 28 transcripts we analyzed the abundance of specific CDR3-J α regions among V α 5 and V α 68 transcripts (V α forward and J α reverse primers) by qRT-PCR. V α 68 was chosen as an additional V α gene comparator as it was frequent on T-cells infiltrating cardiac allografts in Lewis rats and thus expected to be in low abundance in infiltrated islet (35). The abundance of each V α -J α amplicon was normalized to total J α abundance (J α forward and C α reverse primers). qRT-PCR of V α -J α and J α -C α transcripts was performed as described in methods and primers are listed in supplemental table 1. We confirmed a ~100 fold enrichment of V α 5-J α 28 in islet T-cells relative to the periphery. We also show that other J α segments were not over represented in V α 5 transcripts (J α 53 was frequent on insulin-reactive, NOD V α 5+ T-cells while J α 10 was rarely observed in our sequence analysis). Lastly, V α 68 joined to any J α analyzed was infrequent in islet or peripheral T-cells.

