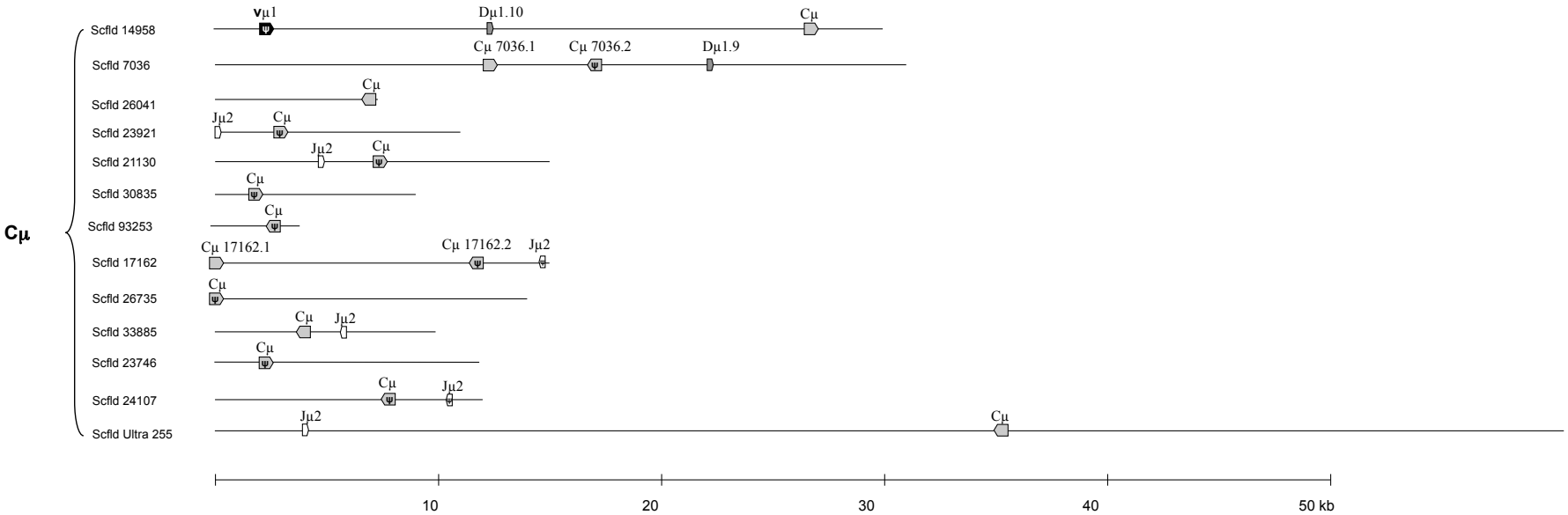
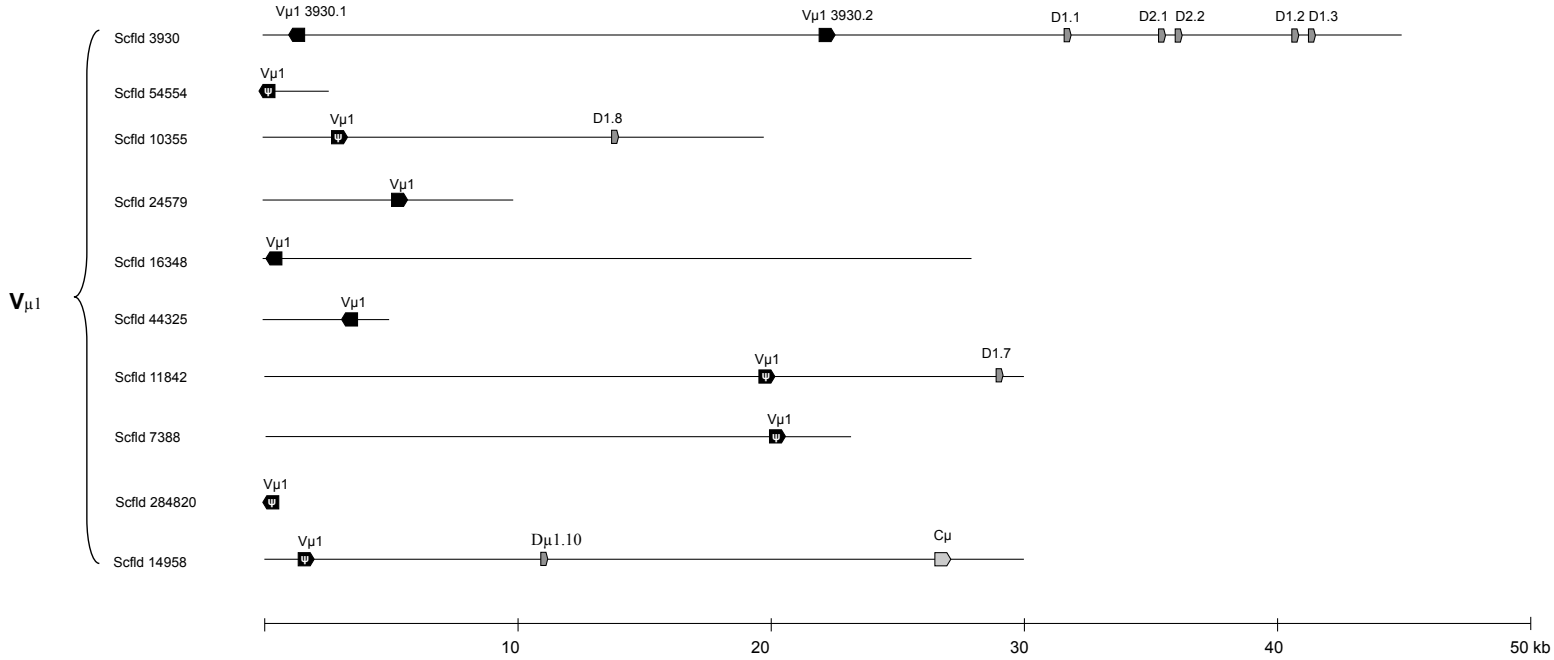
Supplementary Figure 1: Diagram of the scaffolds containing TCRμ genes from the platypus whole genome assembly version 5.0.1. Vμ (black), Dμ (dark grey) Jμ (white) and Cμ (light grey) gene segments are shown in their relative position and transcriptional. Presumptive pseudogenes are indicated with ψ.

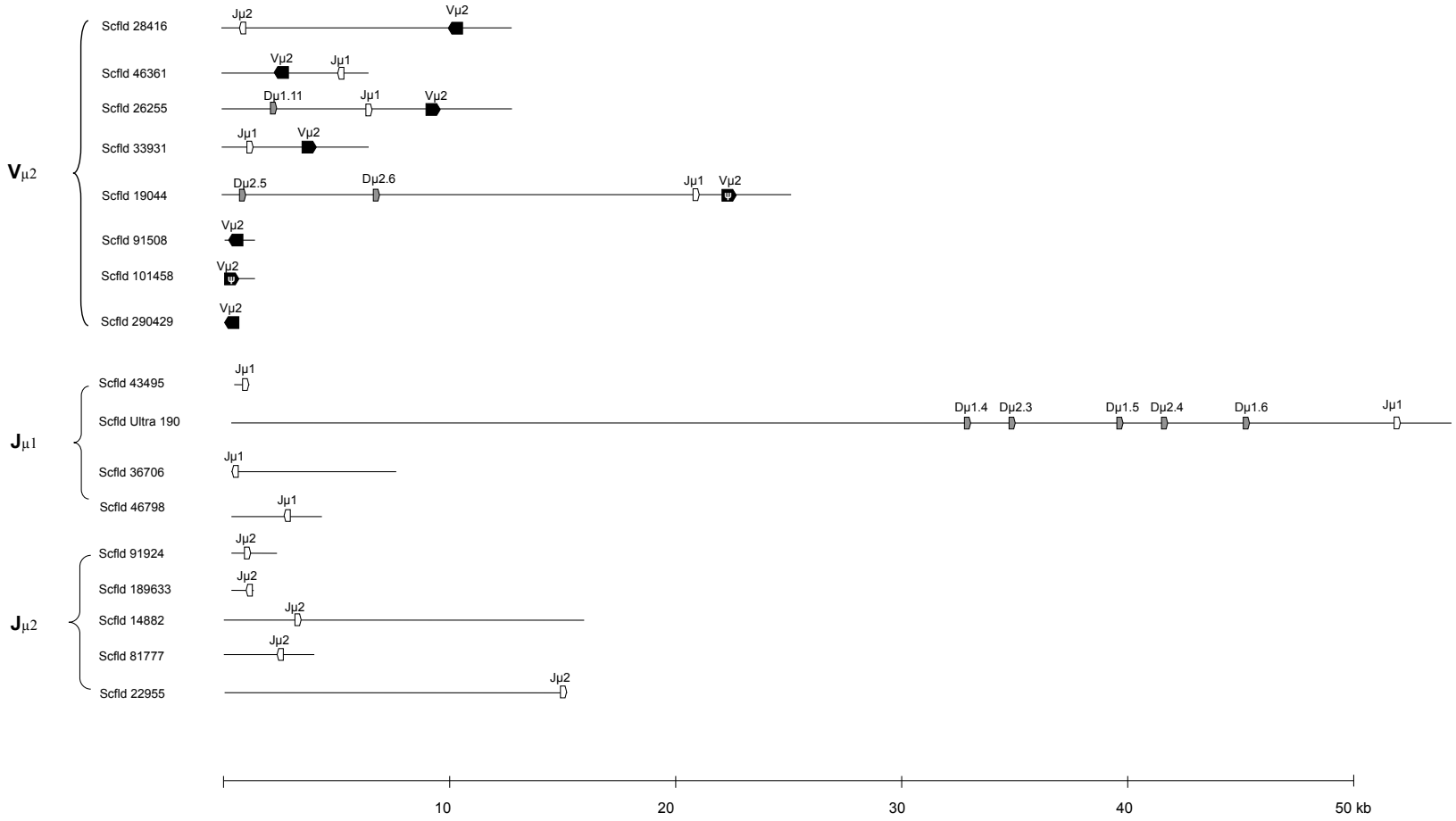
Supplementary Figure 2: Alignment of the predicted amino acid sequences of the six functional platypus Cμ identified in the whole genome assembly with their homologues from marsupials and conventional TCRs from a variety of mammalian species. Dashes indicate identity with the first sequence and dots indicate gaps inserted to generate the alignment. Conserved cysteines involved in intra-domain disulfide bonds are indicated in shade.

Supplementary Figure 3: Sequences of Dμ including the flanking RSS and three alternative translations. Dμ coding sequences are shown lowercase and RSS are uppercase. Heptamers and nonamers are in bold. Stop codons are indicated by dashes. For those Dμ that were found used in cDNA sequences, the number of clones found is indicated to the right of the reading frame being used.

Supplementary Fig. 1






Vμ2
Scfld 28416
Jμ2
Vμ2
Scfld 46361
Vμ2
Jμ1
Scfld 26255
Dμ1.11
Jμ1
Vμ2
Scfld 33931
Jμ1
Vμ2
Scfld 19044
Dμ2.5
Dμ2.6
Jμ1
Vμ2
Scfld 91508
Vμ2
Scfld 101458
Vμ2
Scfld 290429
Vμ2
Jμ1
Scfld 43495
Jμ1
Scfld Ultra 190
Dμ1.4
Dμ2.3
Dμ1.5
Dμ2.4
Dμ1.6
Jμ1
Scfld 36706
Jμ1
Scfld 46798
Jμ1
Jμ2
Scfld 91924
Jμ2
Scfld 189633
Jμ2
Scfld 14882
Jμ2
Scfld 81777
Jμ2
Scfld 22955
10
20
30
40
50 kb

Supplementary Fig. 2

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                 . . . . .10 . . . .20 . . . .30 . . . .40 . . . .50 . . . .60 . . . .70 . . . .80 . . . .90 . . . .100. . . .110.
Cμ               platypus 62     APAESQPT.VFILRNQSFGGCLVKDFYPKELSLTLSAPRPPLTEPLQATASSS........QGTYTTIRIG...QFS................EEDSVTCSVRHGSVQINVTE
                 platypus 58     -R------.--V----N-AT-----Y---K---S--TS----------M-PTA.........----SAV---...--R...................---T----AW----------
                 platypus 36     -------M.--V--K---AS--------EK--------STL--K----M-PTA.........----SAVG--...--G................-GET-I-------M---M--
                 platypus 41     -R-----K.--V------AA---------K--------SS----------PTA.........----SAVG--...--G................KVET-----Q---M---M--
                 platypus 85     -------M.--VM-----AA---------S------T-H-R-M-----M-PT-........W---SAVG--...--G................-A-M-----Q----------
                 platypus 55     --------.--V--------------H----------------------------.......----------...---.................------------A------
                  opossum 08     PRDPLS-S.--VV-S-DAVA---S------IDAS--SLGASVSAQALTV-PII........H-V-SA-Q--...KAG................DN-------K-LGKETHESH
                  opossum 06     PRDPLS-S.--VV-S-DAVA---S------IDAS--SLGASVSAQALTV-PII........H-V-SA-Q--...KAG................DN-------K-LGKETHESH
                  opossum 04     PRDPLS-S.--VV-S-DAVA---S------IDAS--SLGASVSAQALTV-PTI........H-V-SA-Q--...KAG................DN-------K-LGKETHESH
                  opossum 02     PRDPLS-S.--VV-S-DAVA---S------IDAS--SLGASVSAQAETV-PTI........H-V-SA-Q--...KAG................DN-------K-LGKETHESH
                  opossum 01     PR-PLT-S.--L--S-DAVA---S-----D-NVS-ASS-ASIPGQVLTV-PTA........R---SA-Q--...RVD................-N-A-I---Q-LGKE-HMSH
                  opossum 07     PR-PLS-S.--LV-D-DAVA--IRN------HVS-TSSGTLISAQNLSL-PTA........S---SA-H--...RVG................-N-AII---K-LGKE-HIFH
                  opossum 03     PQ-PLS-S.--LV-D-NAVA--IRN------HVS-TSSGTLISAQNLTL-PMA........T---SA-H--...RVG................-N-AI----K-LEKE-HMSH
                  opossum 05     PA-PLS-S.--LV-D-DAVA--IRN------HVS-TSSGTLISAQSLSL-PTA........S---SA-H--...RVG................-N-AI----K-LGKE-HMSH
                 bandicoot93     SQ-PLY-S.--LV-S-NAVA--IRN------HVS-VSS-TLILAKNISLVPMP........N---SAVQ--...KIG................-N-P---L-N-LGKETH-SY
                  wallaby 50     LKPPYL-S.--MM-D-DSVA--IRN------NVS-DS-GALISAQPLTLI-MA........N---SAVQT-...KVG................-N-T---L-K-LGKV-H-SH
Cδ                 human 24     .QPHTK-S.--VMK-GTNVA----E----DIRIN-V.SSKKI--FDP-IVI-P........S-K-NAVKL-...KYE................DSN------Q-DNKTVHS-D
                   mouse 94     SQPPAK-S.---MK-GTNVA----------VTIS-R.SSKKIV-FDP-IVI-P........S-K-SAVKL-...-YG................DSN------Q-N-ETVHS-D
                  opossum 71     FQET-V-S.--VMK-GTNVA---------TVEIQ-H.-DNNPS-...GVVATA........D-KFSAVKL-...-YKK................DL-QIN-T-W-NNKVVVASY
                 bandicoot95     .QET-L-SKI-VMK-GTNAA---------SIAIN-Y.-ENN.-R...QVVTIV........N-KFSAVKF-...-YTQ................DLENI--T-QYKNQT-MTSY
                  wallaby 47     NQNP-T-S.--VMK-GTNVA---------SVDIH-P.-EGKVI-...GVVTTA........N-KFSAVKL-...-YKQ................DLEQ-K-T-K-NNNTVEAFY
                 platypus 59     NQDYRE-S.--L-K-E-TYA-VANN----NAKIHMKL-GKKTITNIEHK-VT-........D-K-SMVQ-T...E-E................SDGA-N-T-E-EGKYVTPQQ
Cα                opossum 04     N-QPALYQ.LRSPKSSNTSV--LT--G.FYNGSIKNET.VTGS-ATVLEMMTME.......SKS-GAVTW-SKSN-TCTDAFR...KDMFDFNQFSGSK-NSS-AEQGFETDR
                   human 57     N-DPAVYQ.LRDSKSSDKSV--FT--DSQTNVSQSKDSDVYI-DKTVLDMR-MD.......FKSNSAVAWSNKSD-ACANAFNNSIIPEDTFFPSPESS-D-KLVEKSFETDT
                   mouse 79     N-EPAVYQ.LKDP-S-DSTL--FT--DSQINVPKTMESGTFI-DKTVLDMKAMD.......SKSNGA-AWSNQTS-TCQDIFK....ETNATYPSSD-P-DATLTEKSFETDM
                 echidna 36     N-QPRMYR.LKKPQVNDLSI--FT--GND-VNM-GIRN..IMRA-SVVDVKRLE.......SKSLGIVAWDNSLDWDCQAQAS...EAVYSLSNSSGKV-NAKVVNENFSSDP
                 platypus 99     N-QPRMYH.LKKP-VNDLSV--FT--GNE-VNMMGINN..IKRT-SMVAEKRLA.......SKSLGIVAWNNNLDWKCQAKIS...NITYSLSNSSGKV-NTTAVTENFSSDP
Cβ                opossum 07     ..QP-EEE..IGEKGKATLV--ATG---DLVE-SWWVNGQETKIGVSTDPEP-.KEHPKEEHSS-SLSSRL...RI-APFWRN..........PKNNFR-Q-QFYGIAE-E-W
                   human 78     ..EP-EAE..ISHTQKATLV--ATG---DHVE-SWWVNGKEVHSGVSTDPQPL.KEQPALNDSR-CLSSRL...RV-ATFWQN..........PRNHFR-Q-QFYGLSE-DEW
                   mouse 08     ..EP-KAE..IANKQKATLV--ARG-F-DHVE-SWWVNGKEVHSGVSTDPQAY.KES...NYS.-CLSSRL...RV-ATFWHN..........PRNHFR-Q-QFHGLSEEDKW
                 echidna 35     ..DP-EEE..INELGKATLV--ATG-F-DHVEMSWWVDGREMQDGVSTD-QPLNETEPEASNR--ALSSRL...RV-AGFWQN..........PSRRFR-Q--FYGLKDSD-W
                 platypus 80     ..DP-EEE..INELGKATLV--ATG-F-DLVEISWWVDGQETK-GVSTD-QPLNYTETKSSNS--ALSSRL...RV-AGFWQN..........PWRRFR-Q-HFYGLSDSD-W
Cγ                opossum 32     FFLPTSEE..IKQKQSGTYI--LE--F-NVVKTYWKEDGNSQPLDA-FGPITG.......GGNS-SQVSWL...TVKE...DV..........LRKNL-YFYQ-EDLGMEPKA
                   human 19     ...P-IAE..TK-QKAGTYL--LEK-F-DIIKIHWQEKKSNTILGS-EGNNMK.......TND--MKFSWL...TVPE...ES..........LDKEHR-I---ENNKNGIDQ
                   mouse 02     ...LLLAE..TN-HKAGTYL--LEK-F--VIRVYWKEKDGEKILES-EGNTIK.......TNDR-MKFSWL...TVTE...DS..........MAKEHS-I-K-ENNKRG-DQ
                 platypus 95     KFFP-SLE..QEKQ-TESYI--F-----DVIRMHWKEEGSDKILES-QSDPFR.......VKDK-WQMSWL...TVKK...SS..........PGKIYRLIYK-EKTGR.-EK
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Supplementary Fig. 3

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Dμ1.1   GTTTTAGTGTTGGGTACGCATCACTGTG gtaaatatagaac CACAGCGAAACATCCTCATCAGAGCCCTGTGCAAAAACC
                                      V  N  I  E     2
                                       -  I  -  N    1
                                         K  Y  R
Dμ1.2   AGTTTAGTGTTGGCTGTGTATCACTGTG  atgactggagc  CACAGTGAAACTTCCTCGGCAAGACACCGTACAAAAACC
                                       M  T  G       4
                                         -  L  E
                                          D  W  S    3
Dμ1.3   AGTTTAGTGTTGGCTGTGTATCACTGTG  atgactggagc  CACAGTGAAACTTCCTCGGCAAGGCACCGTACAAAAACC
                                       M  T  G       4
                                         -  L  E
                                          D  W  S    3
Dμ1.4   GGTTTAGTGTTTACTGTGTATCACTGTG  acgactacgac  CACAGTGAAACCTCCTTGGCAGGGCCCTGTGCAAAAACC
                                       T  T  T
                                         R  L  R     1
                                          D  Y  D
Dμ1.5   GGTTTAGTGTTGGCTGTGAATCACTGTG atacttacacgag CACGATGAAACTTTCTCGGCAGGACCCTGTGCAAAAACC
                                      I  L  T  R     1
                                        Y  L  H  E
                                         T  Y  T     1
Dμ1.6   GGTTTAGTGTTGGATGTGTATCGCTGTG   atgatggaaa  CACAATGAAACTTCCTTGGCAGAGTCCTGAGCAAAAACC
                                        M  M  E      1
                                         -  W  K
                                           D  G
Dμ1.7   AGTTTAGTGTTCATTGTGTATCACTGTG ctggctgtagaac CACAGTGAAACTTCCTCTGCAAGGCCGTGTTCAAAAACC
                                      L  A  V  E
                                        W  L  -  N   1
                                         G  C  R
Dμ1.8   AATTTAGTGTTGGTCGTGTATCACTGTG gtgggagtggaac CACAGTGAAATTTCCTTTGCTGGGCCCTGGGCAAAACCC
                                      V  G  V  E     7
                                        W  E  W  N   4
                                         G  S  G     1
Dμ1.9   GGTTTAGGGTTGGCCATGCACCACTGTG gtggacataggac CACAGTGAAACATCCTCTGCAAGGCCCTGTGCAAAAATC
                                      V  D  I  G
                                       W  T  -  D
                                        G  H  R      2
Dμ1.10  GTTTTAGTGTTGGCCATACATCACTGTG gtagatagaggac CACAATGAAACGTCCTCGGCAGGGCCTTGTGCAAAAACC
                                      V  D  R  G     1
                                        -  I  E  D   4
                                         R  -  R
Dμ1.11  GGTTTAGTGTTGATGGTGTATCACTGTG attcctggaac   CACAGTGAATCTTCCTCAGCAAGGCTCTGTGCAAAAACC
                                      I  P  G        1
                                        F  L  E      4
                                         S  W  N     1
Dμ1.12  GGTTTAGGGTTGGCCATGCATCACTGTG gtggatataggac CACAGTGAAACGTCCTCTGCAAGGCCCTGTGCAAAAACC
                                      V  D  I  G     1
                                        W  I  -  D   1
                                         G  Y  R     1
Dμ1.13  GGTTTAGTGTTAGCTGTGTATCACTGTG aaggatggagc   CACAGTGAAAGCTCCTCGGCAGGGCCCTGTGTTAAAAAC
                                      K  D  G
                                        R  M  E
                                         G  W  S     2
Dμ1.14  AGTTTAGTGTTCATTGTGTATCACTGTG atgcatgctgtac CACAGTGAAACTTCCTCTGCAAGGAACTGTGCAAAACCC
                                      M  H  A  V
                                      C  M  L  Y
                                       A  C  C
Dμ1.15  AGTTTAGTGTTGGCTGTGTATCACTGTG atgcctggagc   CACAGAGAAACTTCCTCGGCAAGGCACCATGCAAAAACC
                                      M  P  G        2
                                        C  L  E
                                         A  W  S
Dμ1.16  GACTTAGTGTTAGCTGTGTATCACTGTG aaatatggagc   CACGGTGAAACCTCCTTGGCAGGGCCCTGTGTAACAACA
                                      K  Y  G
                                        N  M  E
                                         I  W  S
Dμ1.17  AGTTTAGTGTTCATTGTGTATCACTGTG ctggctgtagtac CACAGTGAAACTTCCTCTGCAAGGCCGTGTTCAAAAACC
                                      L  A  V  V
                                        W  L  -  Y
                                         G  C  S
Dμ1.18  AGTTTAGTGTTCATTGTGTATCACTGTG atgcatggagtac CACAATGAAACTTCCTCTGCAAGGCACTGTGCAAAAACC
                                      M  H  G  V
                                        C  M  E  Y
                                         A  W  S
Dμ1.19  AGTTTAGTGTTCATTGTGTATCACTGTG atgcatgcagtac CACAATGAAACTTCCTCTGCAAGGCACTGTGCAAAAACC
                                      M  H  A  V
                                      C  M  Q  Y
                                       A  C  S
Dμ1.20  GGTTTAGTGTTGGCTATGTATCACTGTG cagactggtat   CACAGTGAAATTTCCTCTGCAGGGCCCCATGCAAAAACC
        nonamer  12bp spacer heptamer Q  T  G      heptamer    23bp spacer        nonamer
                                        R  L  V      1
                                         D  W  Y     1
```

```
Dμ2.1    AAAAGATAGTGTTTGTGTGTGCACAGTG tgggcaggacagggagaca CACACTGGACTTTTCAGCCACATAAGCACTCATAAGTGA
                                      W  A  G  Q  G  D      3
                                       G  Q  D  R  E  T     4
                                        G  R  T  G  R
Dμ2.2    AAAAGATAGTGTTTGTGTGTGCACAGTG  tgggcaggacagggagaca CACACTGGACTTTTCAGCCACACAAGCACTCATAAGTGA
                                       W  A  G  Q  G  D     3
                                        G  Q  D  R  E  T    4
                                         G  R  T  G  R
Dμ2.3    AAAGTATGGCTTTTGTGTGTGCACAGTG  tggtcataacaggaggata CATTTTGGCCTTTTCAGCCACACAAGCACCTCCTTCAAA
                                       W  S  -  Q  E  D     7
                                        G  H  N  R  R  I
                                         V  I  T  G  G
Dμ2.4    AAAATATGGCTTTTGTGTGTGCACAGTG  tggtcaggacaggagaca  CACATTGGCCTTTTCAGCCACACAAGCACTCACAAGTGA
                                       W  S  G  Q  E  T     3
                                        G  Q  D  R  R
                                         V  R  T  G  D      1
Dμ2.5    AAAAGATGGCCTTTGTGTGTGCACAGTG  tggttaggttaggggaca CACATTGGACTTTTCAGCCACACGAGCACTCATAAGGGA
                                       W  L  G  -  G  D
                                        G  -  V  R  G  T    1
                                         V  R  L  G  G
Dμ2.6    AAAAGATGGCCTTTGTGTATGCACAGTG  tggtcaggttagggga    CACACGTTGCACTTTTCAGCCACACAAGCACTCATAAGGGA
                                       W  S  G  -  G        1
                                        G  Q  V  R  G
                                         V  R  L  G         1
Dμ2.7    AAAAGATGGCCTTTGTGTGTGCACAGTG  tggtcaggacaggggaca CACATTGGCCTTTTCAGCCACACAAGCAATCAACAGTGA
                                       W  S  G  Q  G  D     1
                                        G  Q  D  R  G  T
                                         V  R  T  G  G
Dμ2.8    AAAAGATGGCCTTTGTGTGTGCACAGTG  tggtcagattagggga    CACACGTTGGATTTTTCAGCCACACAAGCACTCATAAGGGA
                                       W  S  D  -  G        1
                                         G  Q  I  R  G      2
                                          V  R  L  G
Dμ2.9    AAAAGATGACTTTTGTGTGTGCACAGTG  tggtcaggacaggggaca  CATATTGGCCTTTTCAGCCACATAAGCAATCAACAGTGA
                                       W  S  G  Q  G  D     1
                                         G  Q  D  R  G  T
                                           V  R  T  G  G
Dμ2.10   AAAATATGGCCTTTGTGTGTGCACAGTG  tggtcaggacaagggaca  TACATTGGCCTTTTCAGCCACACAAGCACTCACAAGTGA
                                       W  S  G  Q  G  T
                                         G  Q  D  K  G      1
                                          V  R  T  R  D
Dμ2.11   AAAAGATGGCCTTTGTGAGCGCACAGTG  tggccaggttagagggaca CACATTGGACTTTTCAGCCACACGAGCACTCATAAGGGA
                                       W  P  G  -  R  D
                                         G  Q  V  R  G  T
                                          A  R  L  E  G     1
Dμ2.12   AAAAGATGGCCTTTGTGTGTGCACAGTG  tgatcaggttagggga    CACACGTTGGACTTTTCAGCCACACAAGCACTCAGAAGGGA
                                       -  S  G  -  G        1
                                         D  Q  V  R  G
                                          I  R  L  G
Dμ2.13   AAAAGATGGCCTTTGTGAGTGCACAGTG  tggccaggtaagagggaca CACAATGGACTTTTCAGCCACCCGAGCACTCATAAGGGA
                                       W  P  G  K  R  D
                                         G  Q  V  R  G  T
                                          A  R  -  E  G     1
Dμ2.14   AAAAGATGGCTTTTCTGTGTACACAGTG  tggtcaggttaggggaca CACATTGGACTTGTCATCCACACAAGCACTCATAAGGAG
                                       W  S  G  -  G  D
                                         G  Q  V  R  G  T   2
                                          V  R  L  G  G
Dμ 2.15  AAAAGATGGCCTTTGTGTGTGCACAGTG  tggacaggacaggggaca CACATTGGCCTTTTCAGCCACACAAACACTCCTAGATG
         nonamer  12bp spacer heptamer W  T  G  Q  G  D   1 heptamer    23bp spacer        nonamer
                                         G  Q  D  R  G  T 2
                                          D  R  T  G  G
```

Supplementary Table 1: *The V, D, J and C usage in platypus TCRμ cDNA sequences*

Clone name	Vμ1[#]	Dμ	Dμ	Dμ	Dμ	Jμ1	Vμ2	Dμ?	Jμ2	Cμ
21F	16348	2.3	1.2/1.3	1.9/1.12	1.20	46361	46361		91924	17162.1
26R	16348	1.4	1.8/1.11	1.8/2.1/2.2	1.8/1.11	46361	46361	2.8	91924	17162.1
2.22	16348	1.13	1.8/2.1/2.2	1.8/1.11	1.15	46361	46361		91924	17162.1
3815	16348	1.20	1.9	1.1/1.7/1.8/1.11		46361	46361	2.8	91924	17162.1
1915	16348	1.2/1.3	1.8/1.11	1.8/2.1/2.2		46361	46361		91924	17162.1
1953	16348	1.6/1.8/1.11	1.20	1.8/2.1/2.2	1.10/2.3	46361	46361		91924	17162.1
1954	16348	1.2/1.3	1.5	1.10/2.3		46361	46361		91924	17162.1
1955	16348	1.10	1.2/1.3	2.5	1.8/2.1/2.2	46361	46361		91924	17162.1
4951	16348	1.1	1.2	2.1/2.2	1.12	46361	46361		91924	21130
4942	16348	2.11/2.13	1.2/1.3			46361	46361		91924	17162.1
786*	16348	1.12	1.1			46361				
6	16348	2.12	2.2/2.3			46361	46361		91924	17162.1
17	16348	1.8/2.1/2.2	2.6/2.14	1.8/2.1/2.2		46361	46361		91924	17162.1
2.34	24579	1.8/2.1/2.2	1.8/2.1/2.2	1.8/1.11		46361	46361		91924	17162.1
10	24579	1.2/1.3	2.1/2.2	1.13		36706	28416		28416	26041
36F*	3930.2	1.8/2.1/2.2	1.1/1.7/1.8/1.11	2.3/2.4		46798				
4966	3930	1.2/1.3	1.5			46798	33931		21130	21130
1.22	3930	2.4/2.6	1.15			46798	33931		21130	21130

* 3' partial sequence lacking V2 domain.
[#] The number in the column designates the scaffold on which germ-line gene segment is identified.