Figure S1

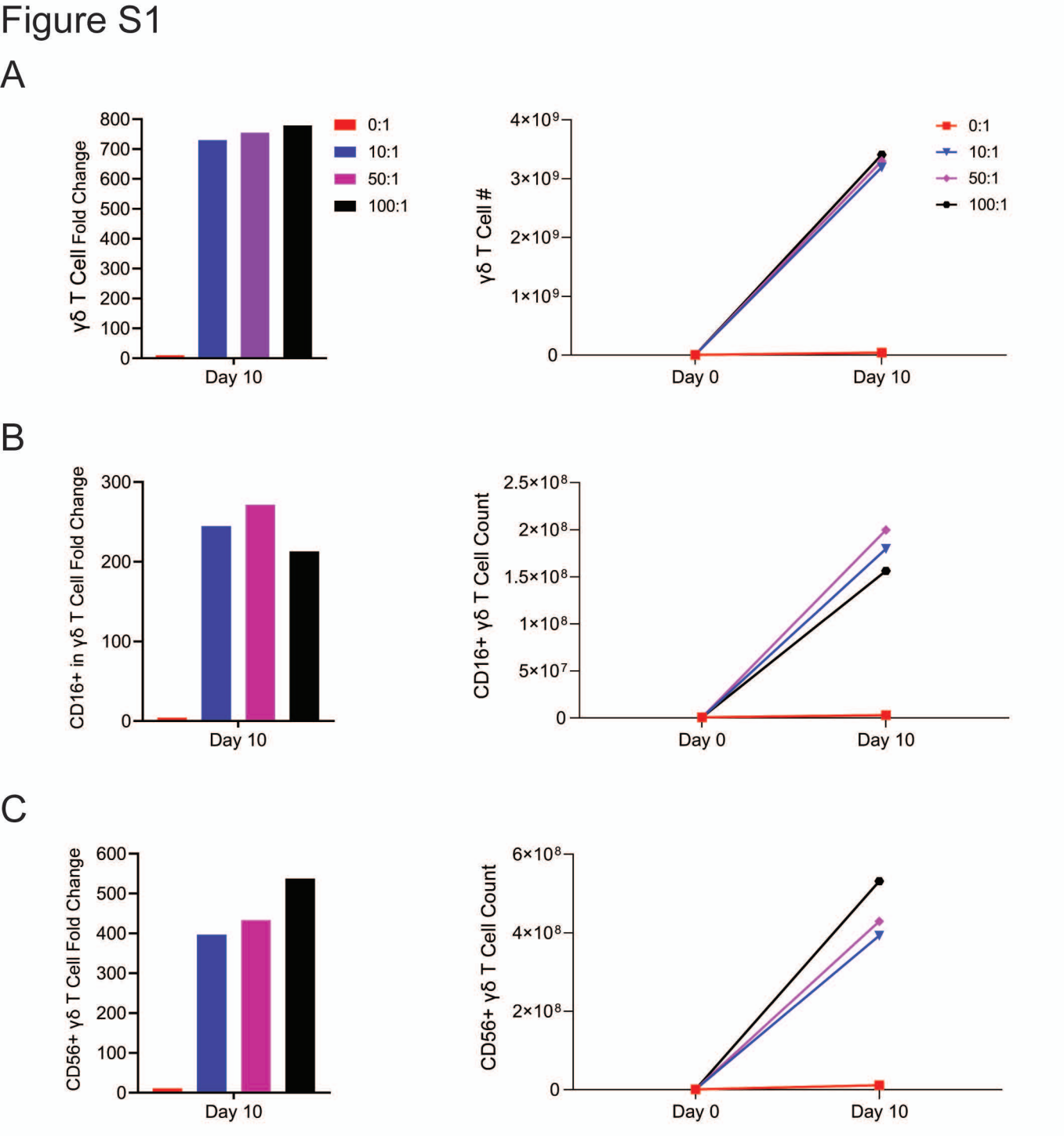
A
γδ T Cell Fold Change
800
700
600
500
400
300
200
100
0
Day 10
0:1
10:1
50:1
100:1
γδ T Cell #
4×10⁹
3×10⁹
2×10⁹
1×10⁹
0
Day 0
Day 10
0:1
10:1
50:1
100:1
B
CD16+ in γδ T Cell Fold Change
300
200
100
0
Day 10
CD16+ γδ T Cell Count
2.5×10⁸
2×10⁸
1.5×10⁸
1×10⁸
5×10⁷
0
Day 0
Day 10
C
CD56+ γδ T Cell Fold Change
600
500
400
300
200
100
0
Day 10
CD56+ γδ T Cell Count
6×10⁸
4×10⁸
2×10⁸
0
Day 0
Day 10

Figure S1. aAPC:γδ T cell ratios of 10:1 or higher facilitate similar expansion of γδ T cells. Irradiated aAPCs were co-cultured with zol enriched γδ T cells at 0:1, 10:1, 50:1 and 100:1 aAPC:γδ T cell ratios. At day 10 γδ T cells were enumerated and phenotyped by flow cytometry. (A) γδ T cell fold change and cell counts are similar at all aAPC:γδ T cell ratios. (B) CD16+ γδ T cell fold change and counts are similar at all aAPC:γδ T cell ratios. (C) CD56+ γδ T cells have similar fold change and count at all aAPC:γδ T cell ratios. Data is from a healthy donor.

Table S1

humanCD3 scFv-P2A-CD137L	GCGGCCGCCACCACAACACCCGCTCCCAGACCTCCCACACCCGCTCCTACCATCGCTAGCCAGCCTCT CTCTTTAAGACCCGAGGCTTGTAGACCCGCTGCCGGAGGAGCCGTGCACACCAGAGGTTTAGACTTCG CTTGCGACATCTATATCTGGGCTCCTCTCGCCGGAACTTGTGGAGTGCTGCTGCTCTCTTTAGTGATTA CTTTATACTGCAACCATCGTAATCGTAGGAGGGTGTGCAAATGCCCCAGACCCGTTGTCAAGAGCGGC GACAAGCCCTCTTTATCCGCTAGATACGTGGGAAGCGGAGCCACCAATTTCTCTTTACTGAAGCAAGCT GGCGACGTGGAGGAGAATCCCGGCCCCATGGAGTACGCCAGCGATGCCTCTTTAGACCCCGAAGCTC CTTGGCCTCCCGCTCCCAGAGCTAGGGCTTGTAGGGTGCTGCCTTGGGCTTTAGTGGCTGGATTACTG CTGCTGTTACTGCTGGCCGCCGCTTGTGCCGTGTTTCTGGCTTGTCCTTGGGCTGTCAGCGGAGCTCG TGCTAGCCCCGGTAGCGCTGCCTCCCCTAGACTGAGAGAGGGACCCGAACTGAGCCCCGATGATCCC GCTGGTTTACTGGATTTAAGGCAAGGTATGTTCGCCCAGCTGGTGGCCCAGAACGTTTTACTGATTGAT GGCCCTTTATCTTGGTACAGCGATCCCGGACTGGCCGGAGTGAGCCTCACCGGCGGCCTCAGCTACAA GGAGGATACCAAGGAGCTGGTGGTGGCTAAGGCCGGCGTCTACTACGTCTTCTTTCAGCTGGAGCTGA GGAGGGTGGTGGCCGGAGAGGGATCCGGAAGCGTGAGCCTCGCTTTACATCTGCAACCTTTAAGATCC GCTGCTGGCGCTGCTGCTCTGGCTCTGACCGTGGATCTGCCTCCCGCTAGCAGCGAGGCTCGTAACTC CGCCTTCGGATTCCAAGGTAGACTGCTGCATTTAAGCGCTGGCCAGAGACTGGGCGTGCATTTACACA CAGAGGCTCGTGCTAGGCACGCTTGGCAACTGACCCAAGGTGCCACCGTGCTGGGTTTATTTCGTGTC ACCCCCGAAATTCCCGCCGGCCTCCCCAGCCCTAGGAGCGAGTGACAGCCACTCGAGATCCGGATTA GTCCAATTTGTTAAAGACAGGATATCAGTGGTCCAGGCTCTAGTTTTGACTCAACAATATCACCAGCTGA AGCCTATAGAGTACGAGCCATAGATAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGGAATGAA AGACCCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATACATA ACTGAGAATAGAGAAGTTCAGATCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGA TATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCA AACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCG GTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGAC CCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCGCGCGCTTCTGCTCCCCGAG CTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAGTCCTCCGATTGACTGAGTCGCCCGGGTA CCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCCTTGGGAGGGTCT CCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCACACATGCAGCATGTATCAAAATTAATTTGG TTTTTTTTCTTAAGTATTTACATTAAATGGCCATAGTACTTAAAGTTACATTGGCTTCCTTGAAATAAACAT GGAGTATTCAGAATGTGTCATAAATATTTCTAATTTTAAGATAGTATCTCCATTGGCTTTCTACTTTTTCTT TTATTTTTTTTTGTCCTCTGTCTTCCATTTGTTGTTGTTGTTTGTTTGTTTGTTTGTTGGTTGGTTGGT TAATTTTTTTTTAAAGATCCTACACTATAGTTCAAGCTAGACTATTAGCTACTCTGTAACCCAGGGTGACC TTGAAGTCATGGGTAGCCTGCTGTTTTAGCCTTCCCACATCTAAGATTACAGGTATGAGCTATCATTTTT GGTATATTGATTGATTGATTGATTGATGTGTGTGTGTGTGATTGTGTTTGTGTGTGTGACTGTGAAAATG TGTGTATGGGTGTGTGTGAATGTGTGTATGTATGTGTGTGTGTGAGTGTGTGTGTGTGTGTGTGCATGT GTGTGTGTGTGACTGTGTCTATGTGTATGACTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG TGTGTGTGTTGTGAAAAAATATTCTATGGTAGTGAGAGCCAACGCTCCGGCTCAGGTGTCAGGTTGGTT TTTGAGACAGAGTCTTTCACTTAGCTTGGAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAA CCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGA GGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCCTGATGCGGTATT TTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATATGGTGCACTCTCAGTACAATCTGCTCTGATGC CGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTC CCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACCGTC ATCACCGAAACGCGCGATGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAA TAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTAACTTACTCTAGC TTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCC TTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCA GCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTAT GGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCA AGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCT TTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAA AAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCAC CGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCA GCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTG TAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGT GTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGG TTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCATT GAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAAC AGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCC ACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGC AACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCC CTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACC GAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGC GTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAAC GCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGT TGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTT

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humanCD28scFv-P2A-humanIL15RA	CCGGTGGTACCTCACCCTTACCGAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAAGAACCT AGAACCTCGCTGGAAAGGACCTTACACAGTCCTGCTGACCACCCCCACCGCCCTCAAAGTAGACGGCA TCGCAGCTTGGATACACGCCGCCCACCATGGCCTCCCCCCTCACCAGGTTCCTGAGCCTGAACCTGCT GCTGCTGGGCGAGAGCATCATCCTGGGAAGCGGCGAGGCTCAGGTGCAGCTGCAGCAGAGCGGCAC CGAACTGGTGAAGCCTGCCTCCAGCGTGAAGATCAGCTGCAAGGCCAGCGGCTACACCTCCACCAGC AACTACATGCACTGGATCAGGCAGCAGCCCGGCAATGGCCTGGAGTGGATCGGCAGGATTTACCCCG GCAACGGCAACACCAAATACAATCAGAAGTTCGATGGCAAGGCCACCCCCACCGCTGACAAGTCCTCC TCCACCGCCTACATGCAGCTGAGCAGACTGACCTTCGAGGATAGCGCCGTCTACTTCTGCGCTAGCGC CCCTCTGGACTACGGAGGCCACATCATGGACGCCTGGGGCCAGGGCACCACAGTGACAGTGAGCTCC GGAGGCGGCGGCAGCGGCGGCGGCGGCAGCGGCGGAGGCGGCAGCGATATCCAGCTCACCCAGAG CCCTGCCTTTCTGAGCGCCAGCCTGGGCGAGACCGTGAGCATTGAGTGCCTCGGCTCCGAGGACATC TACGGCTACCTGGCCTGGTATCAGCAGAAGCCCGGCAAGAGCCCCCAGCTGCTGATCTACGTGGCCAA CAGGCTGCAGGATGGCGTGCCTAGCAGATTTAGCGGCAGCGGCAGCGGAACCCAGTATTCCCTCAAG ATCAGCGGCATGCAGCCTGAGGACGAGGGCGACTACTACTGCCTCCAGGGCAGCAAGTTTCCCCTCAC CTTCGGCAGCGGCACCAAGCTGGAGATCAAAAGGGCGGCCGCCACAACCACCCCCGCTCCCAGACCT

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	TTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGG ATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAG TCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTC ATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTG AGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTG TGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTTTGCTCTTAGGAGT TTCCTAATACATCCCAAACTCAAATATATAAAGCATTTGACTTGTTCTATGCCCTAGGGGGCGGGGGGAA GCTAAGCCAGCTTTTTTTAACATTTAAAATGTTAATTCCATTTTAAATGCACAGATGTTTTTATTTCATAAG GGTTTCAATGTGCATGAATGCTGCAATATTCCTGTTACCAAAGCTAGTATAAATAAAAATAGATAAACGT GGAAATTACTTAGAGTTTCTGTCATTAACGTTTCCTTCCTCAGTTGACAACATAAATGCGCTGCTGAGCA AGCCAGTTTGCATCTGTCAGGATCAATTTCCCATTATGCCAGTCATATTAATTACTAGTCAATTAGTTGAT TTTTATTTTTGACATATACATGTGAATGAAAGACCCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACG CCATTTTGCAAGGCATGGAAAAATACATAACTGAGAATAGAAAAGTTCAGATCAAGGTCAGGAACAGAT GGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAG AACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAG GGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTT CCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGC TTCTGTTCGCGCGCTTATGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGGCGCCAG TCCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAACCCTCTTGCAGTTGCATCCGACTT GTGGTCTCGCTGTTCCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCATT TGGGGGCTCGTCCGGGATCGGGAGACCCCTGCCCAGGGACCACCGACCCACCACCGGGAGGTAAGC TGGCCAGCAACTTATCTGTGTCTGTCCGATTGTCTAGTGTCTATGACTGATTTTATGCGCCTGCGTCGGT ACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGGTGGAACTGACGAGTTCGGAACACCCGGC CGCAACCCTGGGAGACGTCCCAGGGACTTCGGGGGCCGTTTTTGTGGCCCGACCTGAGTCCTAAAATC CCGATCGTTTAGGACTCTTTGGTGCACCCCCCTTAGAGGAGGGATATGTGGTTCTGGTAGGAGACGAG AACCTAAAACAGTTCCCGCCTCCGTCTGAATTTTTGCTTTCGGTTTGGGACCGAAGCCGCGCCGCGCGT CTTGTCTGCTGCAGCATCGTTCTGTGTTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATATGG GCCCGGGCTAGACTGTTACCACTCCCTTAAGTTTGACCTTAGGTCACTGGAAAGATGTCGAGCGGATC GCTCACAACCAGTCGGTAGATGTCAAGAAGAGACGTTGGGTTACCTTCTGCTCTGCAGAATGGCCAAC CTTTAACGTCGGATGGCCGCGAGACGGCACCTTTAACCGAGACCTCATCACCCAGGTTAAGATCAAGG TCTTTTCACCTGGCCCGCATGGACACCCAGACCAGGTCCCCTACATCGTGACCTGGGAAGCCTTGGCT TTTGACCCCCCTCCCTGGGTCAAGCCCTTTGTACACCCTAAGCCTCCGCCTCCTCTTCCTCCATCCGCC CCGTCTCTCCCCCTTGAACCTCCTCGTTCGACCCCGCCTCGATCCTCCCTTTATCCAGCCCTCACTCCT TCTCTAGGCGCCCCCATATGGCCATATGAGATCTTATATGGGGCACCCCCGCCCCTTGTAAACTTCCCT GACCCTGACATGACAAGAGTTACTAACAGCCCCTCTCTCCAAGCTCACTTACAGGCTCTCTACTTAGTC CAGCACGAAGTCTGGAGACCTCTGGC GGCAGCCTACCAAGAACAACTGGACCGA

Table S1. Nucleotide sequences of constructs

Table S2

	Day 7: Seeding density of Total number of cells per G-REX100 MCS Flask	Day 7: Number of γδ T cells per Flask	# of G-REX 100MCS flasks used	Day 17: Total number of γδ T cells at harvest
Donor 1	$5x10^6$ cells/flask	$4.68x10^6$ cells/flask	1	$3.3x10^9$ cells
Donor 2	$5x10^6$ cells/flask	$2.22x10^6$ cells/flask	1	$2.65x10^8$ cells
Donor 3	$5x10^6$ cells/flask	$4.38x10^6$ cells/flask	1	$2.58x10^9$ cells

Table S2. Total viable counts of γδ T cells after 10 days expansion in the presence of aAPCs.