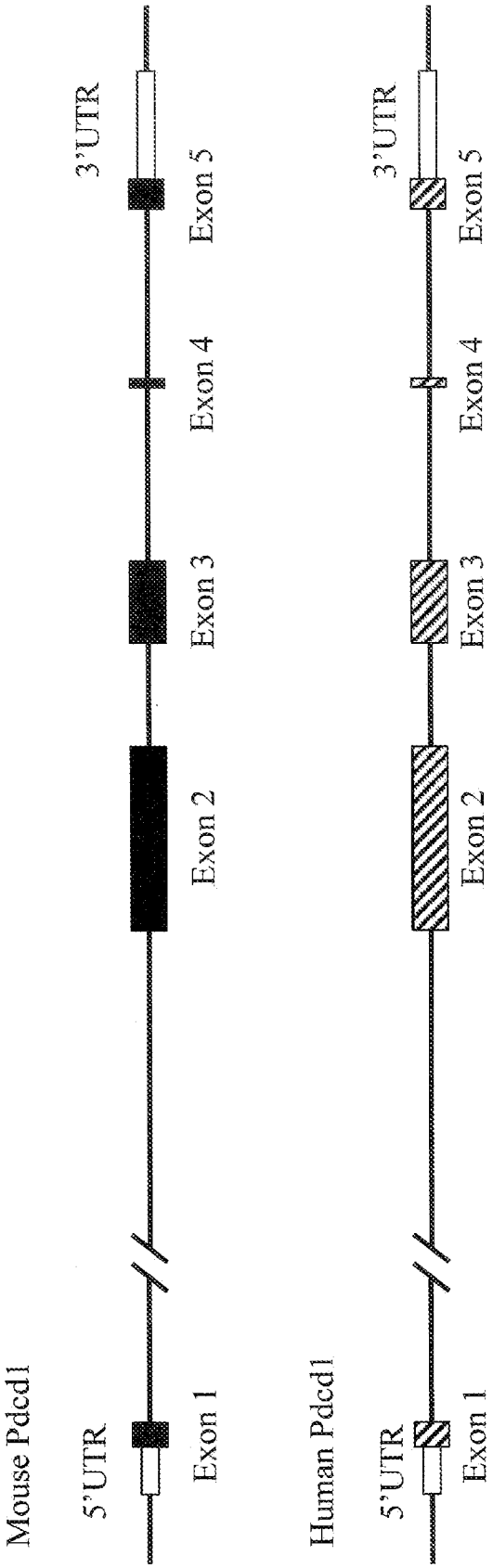
Mouse Pdcd1
5'UTR
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
3'UTR
Human Pdcd1
5'UTR
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
3'UTR

Figure 1

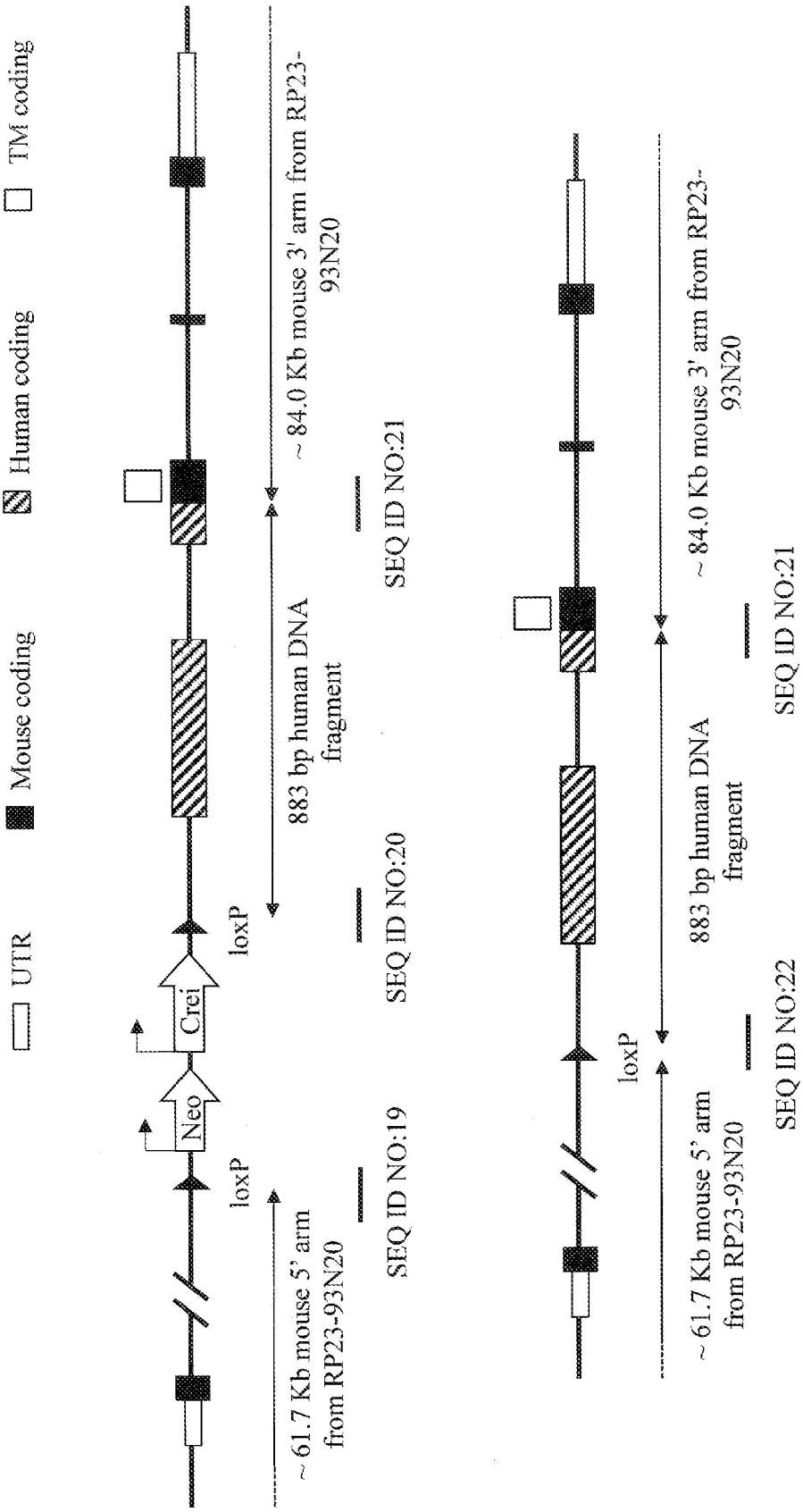
UTR
Mouse coding
Human coding
TM coding
Neo
Crei
loxP
loxP
~ 61.7 Kb mouse 5' arm from RP23-93N20
SEQ ID NO:19
883 bp human DNA fragment
SEQ ID NO:20
~ 84.0 Kb mouse 3' arm from RP23-93N20
SEQ ID NO:21
loxP
~ 61.7 Kb mouse 5' arm from RP23-93N20
SEQ ID NO:22
883 bp human DNA fragment
~ 84.0 Kb mouse 3' arm from RP23-93N20
SEQ ID NO:21

Figure 2

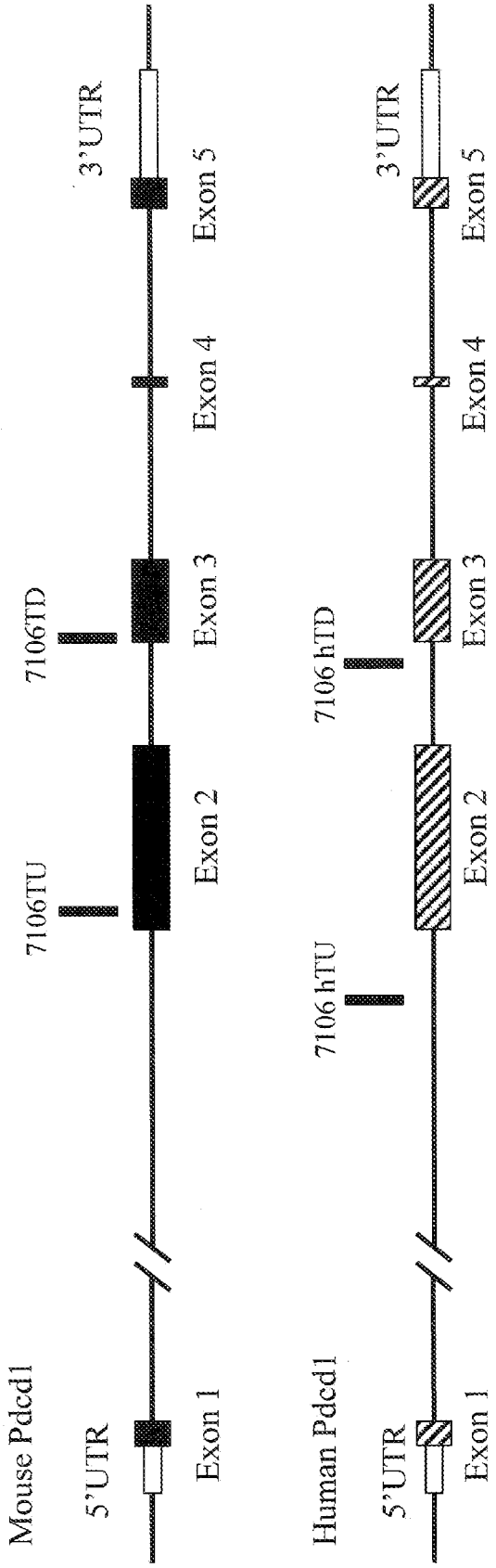
Mouse Pdcd1
5'UTR
Exon 1
7106TU
Exon 2
7106TD
Exon 3
Exon 4
3'UTR
Exon 5
Human Pdcd1
5'UTR
Exon 1
7106 hTU
Exon 2
7106 hTD
Exon 3
Exon 4
3'UTR
Exon 5

Figure 3

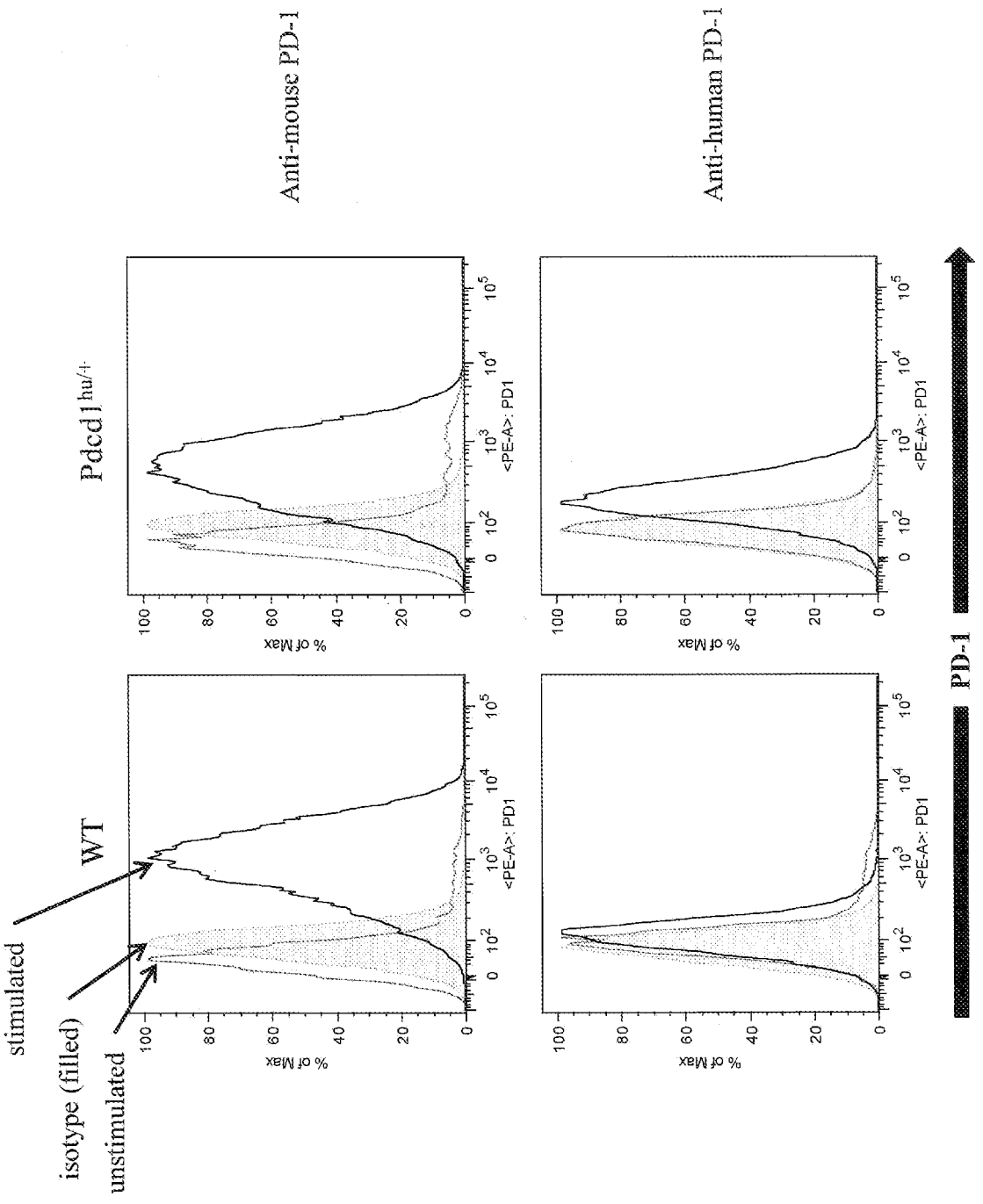
stimulated
isotype (filled)
unstimulated
WT
Pdcd1 hu/+
Anti-mouse PD-1
Anti-human PD-1
% of Max
<PE-A>: PD1
PD-1

Figure 4

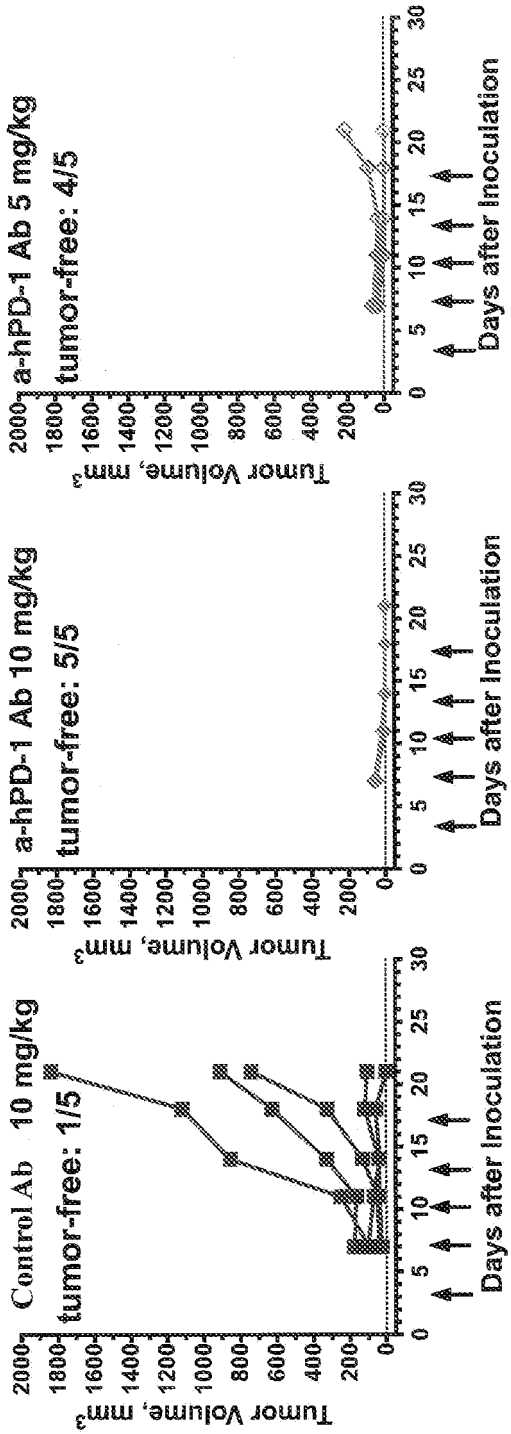
Control Ab 10 mg/kg
tumor-free: 1/5
a-hPD-1 Ab 10 mg/kg
tumor-free: 5/5
a-hPD-1 Ab 5 mg/kg
tumor-free: 4/5
Tumor Volume, mm3
Days after Inoculation
0
200
400
600
800
1000
1200
1400
1600
1800
2000
0
5
10
15
20
25
30

Figure 5

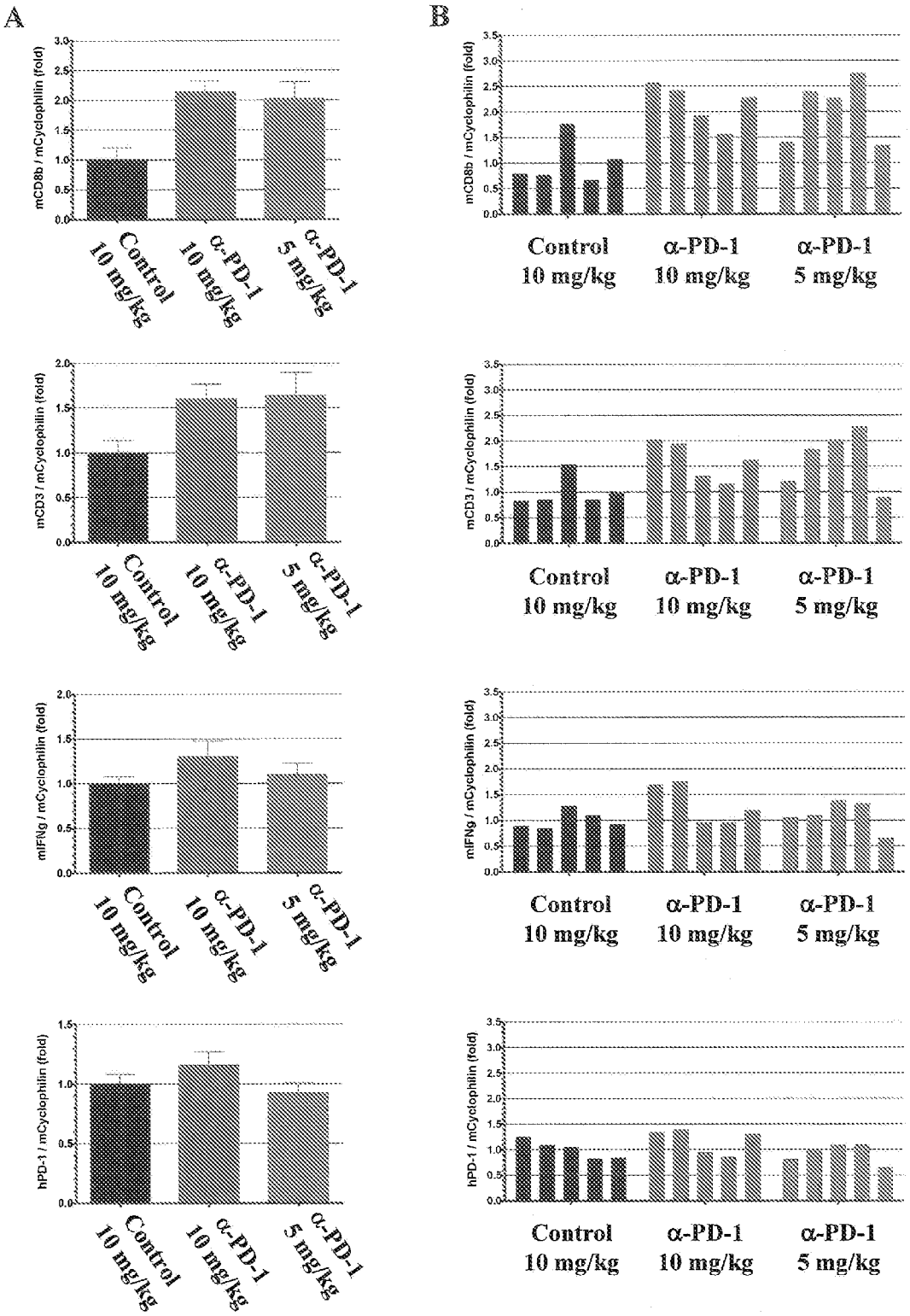
A
B
mCD8b / mCyclophilin (fold)
mCD3 / mCyclophilin (fold)
mIFNg / mCyclophilin (fold)
hPD-1 / mCyclophilin (fold)
Control 10 mg/kg
α-PD-1 10 mg/kg
α-PD-1 5 mg/kg

Figure 6

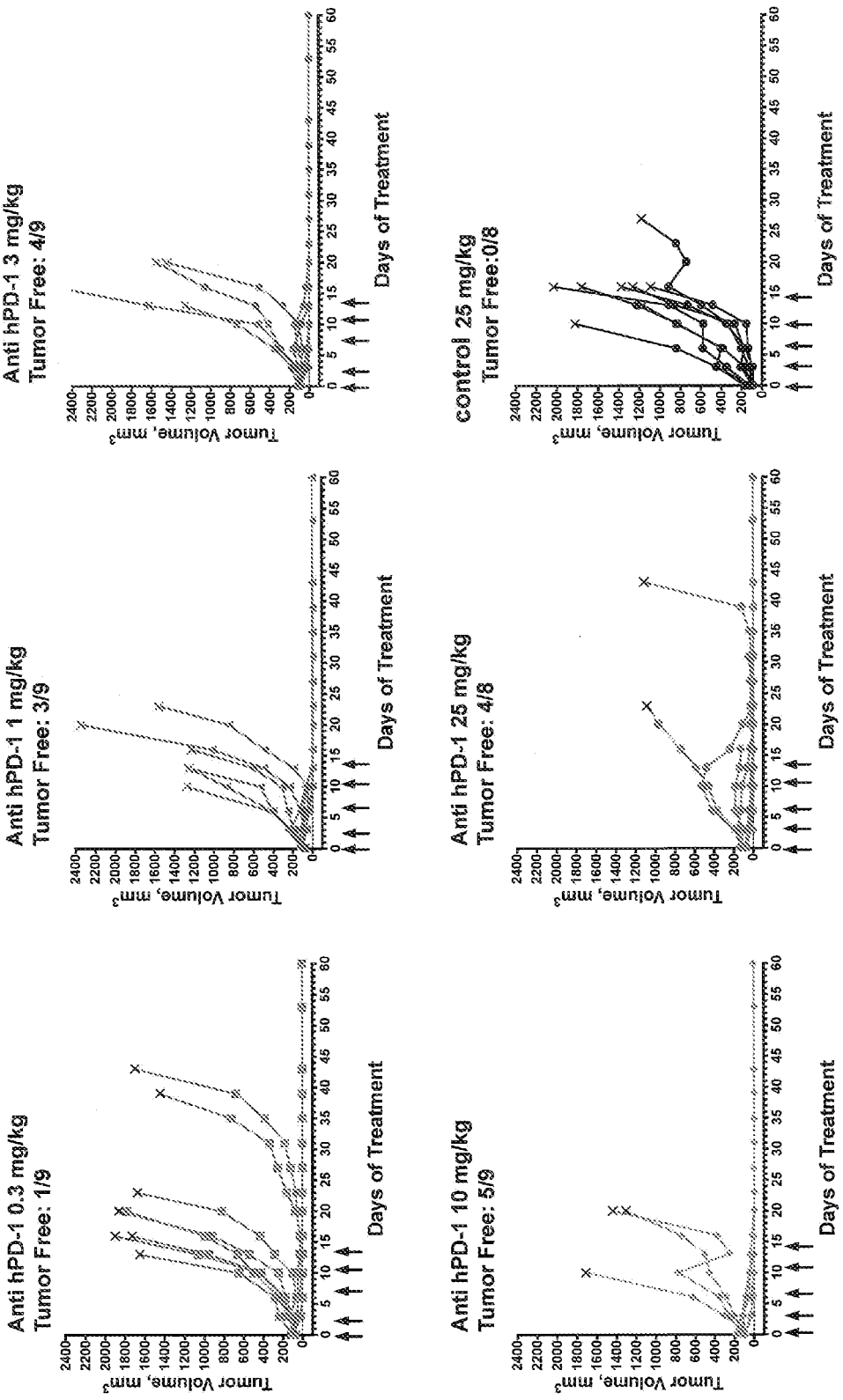
Anti hPD-1 0.3 mg/kg
Tumor Free: 1/9
Anti hPD-1 1 mg/kg
Tumor Free: 3/9
Anti hPD-1 3 mg/kg
Tumor Free: 4/9
Anti hPD-1 10 mg/kg
Tumor Free: 5/9
Anti hPD-1 25 mg/kg
Tumor Free: 4/8
control 25 mg/kg
Tumor Free:0/8
Tumor Volume, mm3
Days of Treatment

Figure 7

Figure 8

Mouse Pdcd1 mRNA (NM_008798.2)

TGAGCAGCGGGGAGGAGGAAGAGGAGACTGCTACTGAAGGCGACACTGCCAGGGGCT
CTGGGC**ATGTGGGTCCGGCAGGTACCCTGGTCATTCACTTGGGCTGTGCTGCAGT**
TGAGCTGGCAATCAGGGTGGCTTCTAGAGGTCCCCAATGGGCCCTGGAGGTCCCT
CACCTTCTACCCAGCCTGGCTCACAGTGTCAGAGGGAGCAAATGCCACCTTCACC
TGCAGCTTGTCCAACTGGTCGGAGGATCTTATGCTGAACTGGAACCGCCTGAGTC
CCAGCAACCAGACTGAAAAACAGGCCGCCTTCTGTAATGGTTTGAGCCAACCCGT
CCAGGATGCCCGCTTCCAGATCATACAGCTGCCCAACAGGCATGACTTCCACATG
AACATCCTTGACACACGGCGCAATGACAGTGGCATCTACCTCTGTGGGGCCATCT
CCCTGCACCCCAAGGCAAAAATCGAGGAGAGCCCTGGAGCAGAGCTCGTGGTAA
CAGAGAGAATCCTGGAGACCTCAACAAGATATCCCAGCCCCTCGCCCAAACCAGA
AGGCCGGTTTCAAGGCATGGTCATTGGTATCATGAGTGCCCTAGTGGGTATCCCT
GTATTGCTGCTGCTGGCCTGGGCCCTAGCTGTCTTCTGCTCAACAAGTATGTCAG
AGGCCAGAGGAGCTGGAAGCAAGGACGACACTCTGAAGGAGGAGCCTTCAGCAG
CACCTGTCCCTAGTGTGGCCTATGAGGAGCTGGACTTCCAGGGACGAGAGAAGAC
ACCAGAGCTCCCTACCGCCTGTGTGCACACAGAATATGCCACCATTGTCTTCACT
GAAGGGCTGGGTGCCTCGGCCATGGGACGTAGGGGCTCAGCTGATGGCCTGCAG
GGTCCTCGGCCTCCAAGACATGAGGATGGACATTGTTCTTGGCCTCTTTGACCAG
ATTCTTCAGCCATTAGCATGCTGCAGACCCTCCACAGAGAGCACCGGTCCGTCCCTCAG
TCAAGAGGAGCATGCAGGCTACAGTTCAGCCAAGGCTCCCAGGGTCTGAGCTAGCTGG
AGTGACAGCCCAGCGCCTGCACCAATTCCAGCACATGCACTGTTGAGTGAGAGCTCAC
TTCAGGTTTACCACAAGCTGGGAGCAGCAGGCTTCCCGGTTTCCTATTGTCACAAGGTG
CAGAGCTGGGGCCTAAGCCTATGTCTCCTGAATCCTACTGTTGGGCACTTCTAGGGACT
TGAGACACTATAGCCAATGGCCTCTGTGGGTTCTGTGCCTGGAAATGGAGAGATCTGA
GTACAGCCTGCTTTGAATGGCCCTGTGAGGCAACCCCAAAGCAAGGGGGTCCAGGTAT
ACTATGGGCCCAGCACCTAAAGCCACCCTTGGGAGATGATACTCAGGTGGGAAATTCG
TAGACTGGGGGACTGAACCAATCCCAAGATCTGGAAAAGTTTTGATGAAGACTTGAAA
AGCTCCTAGCTTCGGGGGTCTGGGAAGCATGAGCACTTACCAGGCAAAAGCTCCGTGA
GCGTATCTGCTGTCCTTCTGCATGCCCAGGTACCTCAGTTTTTTTCAACAGCAAGGAAA
CTAGGGCAATAAAGGGAACCAGCAGAGCTAGAGCCACCCACACATCCAGGGGGCACT
TGACTCTCCCTACTCCTCCTAGGAACCAAAAGGACAAAGTCCATGTTGACAGCAGGGA
AGGAAAGGGGGATATAACCTTGACGCAAACCAACACTGGGGTGTTAGAATCTCCTCAT
TCACTCTGTCCTGGAGTTGGGTTCTGGCTCTCCTTCACACCTAGGACTCTGAAATGAGC
AAGCACTTCAGACAGTCAGGGTAGCAAGAGTCTAGCTGTCTGGTGGGCACCCAAAATG
ACCAGGGCTTAAGTCCCTTTCCTTTGGTTTAAGCCCGTTATAATTAAATGGTACCAAAA
GCTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO:1)

Mouse PD-1 amino acid (Q02242)

MWVRQVPWSFTWAVLQLSWQ**SGWLLEVPNG(PWRSLTFYPAWLTVSEGANATFTCSL**
SNWSEDLMLNWNRLSPSNQTEKQAAFCNGLSQPVQDARFQIIQLPNRHDFHMNILDT
RRNDSGIYLCGAISLHPKAKIEESPG)AELVVTERILETSTRYPSPSPKPEGRFQGMVIGI
MSALVGIPVLLLLAWAL*AVFCSTSMSEARGAGSKDDTLKEEPSAAPVPSVAYEELDFQGREKT*
PELPTACVHTEYATIVFTEGLGASAMGRRGSADGLQGPRPPRHEDGHCSWPL (SEQ ID NO:2)

Figure 8, continued…

Human Pdcd1 mRNA (NM_005018.2)

AGTTTCCCTTCCGCTCACCTCCGCCTGAGCAGTGGAGAAGGCGGCACTCTGGTGGGGCT
GCTCCAGGC**ATGCAGATCCCACAGGCGCCCTGGCCAGTCGTCTGGGCGGTGCTAC**
AACTGGGCTGGCGGCCAGGATGGTTCTTAGACTCCCCAGACAGGCCCTGGAACCC
CCCCACCTTCTCCCCAGCCCTGCTCGTGGTGACCGAAGGGGACAACGCCACCTTC
ACCTGCAGCTTCTCCAACACATCGGAGAGCTTCGTGCTAAACTGGTACCGCATGA
GCCCCAGCAACCAGACGGACAAGCTGGCCGCCTTCCCCGAGGACCGCAGCCAGC
CCGGCCAGGACTGCCGCTTCCGTGTCACACAACTGCCCAACGGGCGTGACTTCCA
CATGAGCGTGGTCAGGGCCCGGCGCAATGACAGCGGCACCTACCTCTGTGGGGC
CATCTCCCTGGCCCCCAAGGCGCAGATCAAAGAGAGCCTGCGGGCAGAGCTCAG
GGTGACAGAGAGAAGGGCAGAAGTGCCCACAGCCCACCCCAGCCCCTCACCCAG
GCCAGCCGGCCAGTTCCAAACCCTGGTGGTTGGTGTCGTGGGCGGCCTGCTGGG
CAGCCTGGTGCTGCTAGTCTGGGTCCTGGCCGTCATCTGCTCCCGGGCCGCACGA
GGGACAATAGGAGCCAGGCGCACCGGCCAGCCCCTG**AAGGAGGACCCCTCAGCC**
GTGCCTGTGTTCTCTGTGGACTATGGGGAGCTGGATTTCCAGTGGCGAGAGAAGA
CCCCGGAGCCCCCCGTGCCCTGTGTCCCTGAGCAGACGGAGTATGCCACCATTGT
CTTTCCTAGCGGAATGGGCACCTCATCCCCCGCCCGCAGGGGCTCAGCTGACGGC
CCTCGGAGTGCCCAGCCACTGAGGCCTGAGGATGGACACTGCTCTTGGCCCCTCT
GACCGGCTTCCTTGGCCACCAGTGTTCTGCAGACCCTCCACCATGAGCCCGGGTCAGCG
CATTTCCTCAGGAGAAGCAGGCAGGGTGCAGGCCATTGCAGGCCGTCCAGGGGCTGAG
CTGCCTGGGGGCGACCGGGGCTCCAGCCTGCACCTGCACCAGGCACAGCCCCACCACA
GGACTCATGTCTCAATGCCCACAGTGAGCCCAGGCAGCAGGTGTCACCGTCCCCTACA
GGGAGGGCCAGATGCAGTCACTGCTTCAGGTCCTGCCAGCACAGAGCTGCCTGCGTCC
AGCTCCCTGAATCTCTGCTGCTGCTGCTGCTGCTGCTGCTGCCTGCGGCCCGGGGC
TGAAGGCGCCGTGGCCCTGCCTGACGCCCCGGAGCCTCCTGCCTGAACTTGGGGGCTG
GTTGGAGATGGCCTTGGAGCAGCCAAGGTGCCCCTGGCAGTGGCATCCCGAAACGCCC
TGGACGCAGGGCCCAAGACTGGGCACAGGAGTGGGAGGTACATGGGGCTGGGGACTC
CCCAGGAGTTATCTGCTCCCTGCAGGCCTAGAGAAGTTTCAGGGAAGGTCAGAAGAGC
TCCTGGCTGTGGTGGGCAGGGCAGGAAACCCCTCCACCTTTACACATGCCCAGGCAGC
ACCTCAGGCCCTTTGTGGGGCAGGGAAGCTGAGGCAGTAAGCGGGCAGGCAGAGCTG
GAGGCCTTTCAGGCCCAGCCAGCACTCTGGCCTCCTGCCGCCGCATTCCACCCCAGCCC
CTCACACCACTCGGGAGAGGGACATCCTACGGTCCCAAGGTCAGGAGGGCAGGGCTGG
GGTTGACTCAGGCCCCTCCCAGCTGTGGCCACCTGGGTGTTGGGAGGGCAGAAGTGCA
GGCACCTAGGGCCCCCCATGTGCCCACCCTGGGAGCTCTCCTTGGAACCCATTCCTGAA
ATTATTTAAAGGGGTTGGCCGGGCTCCCACCAGGGCCTGGGTGGGAAGGTACAGGCGT
TCCCCCGGGGCCTAGTACCCCCGCCGTGGCCTATCCACTCCTCACATCCACACACTGCA
CCCCCACTCCTGGGGCAGGGCCACCAGCATCCAGGCGGCCAGCAGGCACCTGAGTGGC
TGGGACAAGGGATCCCCCTTCCCTGTGGTTCTATTATATTATAATTATAATTAAATATG
AGAGCATGCTAAGGAAAA (SEQ ID NO:3)

Figure 8, continued…

Human PD-1 amino acid (Q15116)
MQIPQAPWPVVWAVLQLGWR**PGWFLDSPDRPWNP(PTFSPALLVVTEGDNATFTCSFS NTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR RNDSGTYLCGAISLAPKAQIKESLRAELRVT)ERRAEVPTAHPSPSPRPAGQFQTLV**VG VVGGLLGSLVLLVWVLAVI*CSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTP EPPVPCVPEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL* (SEQ ID NO:4)

Humanized Pdcd1 mRNA
TGAGCAGCGGGGAGGAGGAAGAGGAGACTGCTACTGAAGGCGACACTGCCAGGGGCT CTGGGC**ATGTGGGTCCGGCAGGTACCCTGGTCATTCACTTGGGCTGTGCTGCAGT TGAGCTGGCAATCAGGGTGGCTTCTAG(ACTCCCCAGACAGGCCCTGGAACCCCC CCACCTTCTCCCCAGCCCTGCTCGTGGTGACCGAAGGGGACAACGCCACCTTCAC CTGCAGCTTCTCCAACACATCGGAGAGCTTCGTGCTAAACTGGTACCGCATGAGC CCCAGCAACCAGACGGACAAGCTGGCCGCCTTCCCCGAGGACCGCAGCCAGCCC GGCCAGGACTGCCGCTTCCGTGTCACACAACTGCCCAACGGGCGTGACTTCCACA TGAGCGTGGTCAGGGCCCGGCGCAATGACAGCGGCACCTACCTCTGTGGGGCCA TCTCCCTGGCCCCCAAGGCGCAGATCAAAGAGAGCCTGCGGGCAGAGCTCAGGG TGACAG**AGAGAAGGGCAGAAGTGCCCACAGCCCACCCCAGCCCCTCACCCAGGC **CAGCCGGCCAGTTCCAAACCCTG)GTCATTGGTATCATGAGTGCCCTAGTGGGTAT CCCTGTATTGCTGCTGCTGGCCTGGGCCCTAGCTGTCTTCTGCTCAACAAGTATG TCAG**AGGCCAGAGGAGCTGGAAGCAAGGACGACACTCTG**AAGGAGGAGCCTTCA GCAGCACCTGTCCCTAGTGTGGCCTATGAGGAGCTGGACTTCCAGGGACGAGAG AAGACACCAGAGCTCCCTACCGCCTGTGTGCACACAGAATATGCCACCATTGTCT TCACTGAAGGGCTGGGTGCCTCGGCCATGGGACGTAGGGGCTCAGCTGATGGCC TGCAGGGTCCTCGGCCTCCAAGACATGAGGATGGACATTGTTCTTGGCCTCTTTG** ACCAGATTCTTCAGCCATTAGCATGCTGCAGACCCTCCACAGAGAGCACCGGTCCGTCC CTCAGTCAAGAGGAGCATGCAGGCTACAGTTCAGCCAAGGCTCCCAGGGTCTGAGCTA GCTGGAGTGACAGCCCAGCGCCTGCACCAATTCCAGCACATGCACTGTTGAGTGAGAG CTCACTTCAGGTTTACCACAAGCTGGGAGCAGCAGGCTTCCCGGTTTCCTATTGTCACA AGGTGCAGAGCTGGGGCCTAAGCCTATGTCTCCTGAATCCTACTGTTGGGCACTTCTAG GGACTTGAGACACTATAGCCAATGGCCTCTGTGGGTTCTGTGCCTGGAAATGGAGAGA TCTGAGTACAGCCTGCTTTGAATGGCCCTGTGAGGCAACCCCAAAGCAAGGGGGTCCA GGTATACTATGGGCCCAGCACCTAAAGCCACCCTTGGGAGATGATACTCAGGTGGGAA ATTCGTAGACTGGGGGACTGAACCAATCCCAAGATCTGGAAAAGTTTTGATGAAGACT TGAAAAGCTCCTAGCTTCGGGGGTCTGGGAAGCATGAGCACTTACCAGGCAAAAGCTC CGTGAGCGTATCTGCTGTCCTTCTGCATGCCCAGGTACCTCAGTTTTTTTCAACAGCAA GGAAACTAGGGCAATAAAGGGAACCAGCAGAGCTAGAGCCACCCACACATCCAGGGG GCACTTGACTCTCCCTACTCCTCCTAGGAACCAAAAGGACAAAGTCCATGTTGACAGC AGGGAAGGAAAGGGGGATATAACCTTGACGCAAACCAACACTGGGGTGTTAGAATCT CCTCATTCACTCTGTCCTGGAGTTGGGTTCTGGCTCTCCTTCACACCTAGGACTCTGAAA TGAGCAAGCACTTCAGACAGTCAGGGTAGCAAGAGTCTAGCTGTCTGGTGGGCACCCA AAATGACCAGGGCTTAAGTCCCTTTCCTTTGGTTTAAGCCCGTTATAATTAAATGGTAC CAAAAGCTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO:5)

Figure 8, continued…

Humanized PD-1 amino acid
MWVRQVPWSFTWAVLQLSWQSGWLL**DSPDRPWNP(PTFSPALLVVTEGDNATFTCSFS NTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR RNDSGTYLCGAISLAPKAQIKESLRAELRVT)ERRAEVPTAHPSPSPRPAGQFQTL**VIGI MSALVGIPVLLLLAWAL*AVFCSTSMSEARGAGSKDDTLKEEPSAAPVPSVAYEELDFQGREKT PELPTACVHTEYATIVFTEGLGASAMGRRGSADGLQGPRPPRHEDGHCSWPL* (SEQ ID NO:6)

Human 883 bp DNA fragment
AAGAGGCTCTGCAGTGGAGGCCAGTGCCCATCCCCGGGTGGCAGAGGCCCCAGCAGA
GACTTCTCAATGACATTCCAGCTGGGGTGGCCCTTCCAGAGCCCTTGCTGCCCGAGGGA
TGTGAGCAGGTGGCCGGGGAGGCTTTGTGGGGCCACCCAGCCCCTTCCTCACCTCTCTC
CATCTCTCAGACTCCCCAGACAGGCCCTGGAACCCCCCCACCTTCTCCCCAGCCCTGCT
CGTGGTGACCGAAGGGGACAACGCCACCTTCACCTGCAGCTTCTCCAACACATCGGAG
AGCTTCGTGCTAAACTGGTACCGCATGAGCCCCAGCAACCAGACGGACAAGCTGGCCG
CCTTCCCCGAGGACCGCAGCCAGCCCGGCCAGGACTGCCGCTTCCGTGTCACACAACT
GCCCAACGGGCGTGACTTCCACATGAGCGTGGTCAGGGCCCGGCGCAATGACAGCGGC
ACCTACCTCTGTGGGGCCATCTCCCTGGCCCCCAAGGCGCAGATCAAAGAGAGCCTGC
GGGCAGAGCTCAGGGTGACAGGTGCGGCCTCGGAGGCCCCGGGGCAGGGGTGAGCTG
AGCCGGTCCTGGGGTGGGTGTCCCCTCCTGCACAGGATCAGGAGCTCCAGGGTCGTAG
GGCAGGGACCCCCCAGCTCCAGTCCAGGGCTCTGTCCTGCACCTGGGGAATGGTGACC
GGCATCTCTGTCCTCTAGCTCTGGAAGCACCCCAGCCCCTCTAGTCTGCCCTCACCCCT
GACCCTGACCCTCCACCCTGACCCCGTCCTAACCCCTGACCTTTGTGCCCTTCCAGAGA
GAAGGGCAGAAGTGCCCACAGCCCACCCCAGCCCCTCACCCAGGCCAGCCGGCCAGTT
CCAAACCCTG (SEQ ID NO:23)