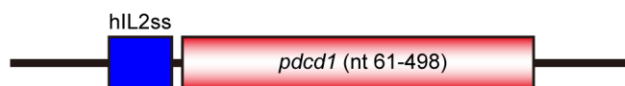


A. mPD1 fragment DNA (pVAX)



B. mCTLA4-PD-L1 DNA (pVAC1)



C

	Mus musculus <i>pdcd1</i> nt 61-498 (GeneID: 18566)	Mus musculus <i>cd274</i> nt 55-381 (GeneID: 60533)	Mus musculus <i>ctla4</i> nt 106-483 (GeneID: 12477)
Rattus norvegicus <i>pdcd1</i> nt 61-498 (GeneID: 301626)	93%		
Rattus norvegicus <i>cd274</i> nt 55-381 (GeneID: 499342)		93%	
Rattus norvegicus <i>ctla4</i> nt 106-483 (Gene ID: 63835)			94%

Figure S1. Schema for experimental design for the construct of mPD-1 DNA vaccine. (A) Schema for experimental design for the construct of mCTLA4-PD-L1 DNA vaccine, (B) The similarities of PD1, PD-L1, and CTLA4 nucleotide sequences between mice (*Mus musculus*) and rats (*Rattus norvegicus*).

Identities:409/438(93%), Gaps:0/449(0%), Strand: Plus/Plus

Mus musculus	1	TCAGGTGGTTGCTAGAGGTCCCCAATGGGCCCTGGAGGTCCCTCACCTTCTACCCAGCC	60
Rattus norvegicus	1	TCAGGTGGCTTCTAGAGGTCTCAATAAGCCCTGGAGGCCCTCACCTTCTCCCAACC	60
Mus musculus	61	TGGCTCACAGTGTGAGAGGGAGCAAATGCCACCTTCACCTGCAGCTTGTCCAACCTGGTCG	120
Rattus norvegicus	61	TGGCTCACAGTGTGAGAGGGAGCAAATGCCACCTTCACCTGCAGTTTCTCCAACCTGGTCG	120
Mus musculus	121	GAGGATCTTATGTCTGAAGTGAACCGCTGAGTCCCAGCAACCAGACTGAAAAACAGGCC	180
Rattus norvegicus	121	GAGGATCTTAAGCTGAAGTGGTACCGTCTGAGTCCCAGCAACCAGACTGAAAAACAGGCC	180
Mus musculus	181	GCCTTCTGTAATGGTTTGAGCCAACCCGTCAGGATGCCCGCTTCCAGATCATACAGCTG	240
Rattus norvegicus	181	GCCTTCTGCAATGGTTACAGCCAGCCCGTCGGGATGCCCGCTTCCAGATCGTACAACTG	240
Mus musculus	241	CCCAACAGGCATGACTTCCACATGAACATCCTTGACACACGGCGCAATGACAGTGGCATC	300
Rattus norvegicus	241	CCCAACGGACATGACTTCCACATGAACATCCTCGATGCACGGCGCAATGACAGTGGCATC	300
Mus musculus	301	TACCTCTGTGGGGCCATCTCCCTGCACCCCAAGGCAAAAATCGAGGAGAGCCCTGGAGCA	360
Rattus norvegicus	301	TACCTCTGTGGGGCCATCTCCCTGCCTCCCAAGGCACAAATCAAAGAGAGTCCTGGAGCA	360
Mus musculus	361	GAGCTCGTGGTAACAGAGAGAATCCTGGAGACCTCAACAAGATATCCCAGCCCCTCGCC	420
Rattus norvegicus	361	GAGCTTGTGGTAACAGAGAGAATCCTGGAGACCCCAACAAGATATCCCAGACCTCACCC	420
Mus musculus	421	AAACCAGAAGCCGGTTT	438
Rattus norvegicus	421	AAGCCAGAAGGCCAGTTT	438

Figure S2. A nucleotide sequence alignment of mouse *pcdcl* and rat *pcdcl*.

Mus musculus cd274 (nt 55-381) and Rattus norvegicus cd274 (nt 55-381)

Score: 486 bits(263), Expect: 3e-142,

Identities: 305/327(93%), Gaps:0/326(0%), Strand: Plus/Plus

Mus musculus	1	TTTACTATCACGGCTCCAAAGGACTTGTACGTGGTGGAGTATGGCAGCAACGTCACGATG	60
Rattus norvegicus	1	TTTACTATCACGGCTCCAAAGGACCTGTACGTGGTGGAGTATGGCAGCAACGTCACGATG	60
Mus musculus	61	GAGTGCAGATTCCCTGTAGAACGGGAGCTGGACCTGCTTGCCTTAGTGGTGTACTGGGAA	120
Rattus norvegicus	61	GAGTGCAGATTCCCTGTAGAACAGGAGCTGGACCTGCATGCATTAGTGGTGTACTGGGAA	120
Mus musculus	121	AAGGAAGATGAGCAAGTGATTGAGTTTGTGGCAGGAGAGGAGACCTTAAGCCTCAGCAC	180
Rattus norvegicus	121	AAGGAAGACAAGCAAGTGATTGAGCTTGTGGCGGAGAGGAGACATAAAGCCTCAGCAC	180
Mus musculus	181	AGCAACTTCAGGGGAGAGCCTCGCTGCCAAAGGACCAGCTTTTGAAGGAAATGCTGCC	240
Rattus norvegicus	181	AGTAGCTTCAGGGGAGAGCCTCCTTGCCAAAGGACCAGCTTCTGAAGGAAATGCTGCA	240
Mus musculus	241	CTTCAGATCACAGACGTCAAGCTGCAGGACGAGGCGTTTACTGCTGCATAATCAGCTAC	300
Rattus norvegicus	241	CTTCAGATCACGGATGTCAAGCTGCAGGACGAGGCGTCTACTGCTGCATGATCAGCTAC	300
Mus musculus	301	GGTGGTGGGACTACAAGCGAATCACG	327
Rattus norvegicus	301	GGCGGTGGGACTACAAGCGAATCACA	327

Figure S3. A nucleotide sequence alignment of mouse *cd274* and rat *cd274*.

Mus musculus *ctla4* (nt 106-483) and Rattus norvegicus *ctla4* (nt 106-483)

Score: 577 bits(312), Expect:2e-169,

Identities: 356/378(94%), Gaps: 0/378(0%), Strand: Plus/Plus

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Mus musculus      1 GAAGCCATACAGGTGACCCAACCTTCAGTGGTGTGGCTAGCAGCCATGGTGTGCCAGC 60
                   |||
Rattus norvegicus 1 GAAGCCATACAAGTGACCCAACCTTCAGTGGTGTGGCCAGCAGCCACGGTGTGCCAGC 60

Mus musculus      61 TTTCCATGTGAATATTACCGTCACACAACACTGATGAGGTCCGGGTGACTGTGCTGCCG 120
                   |||
Rattus norvegicus 61 TTTCCATGTGAATATGCATCTTCACACAACACTGATGAGGTCCGGGTGACGGTGTGCGG 120

Mus musculus      121 CAGACAAATGACCAAATGACTGAGGTCTGTGCCACGACATTCACAGAGAAGAATACAGTG 180
                   |||
Rattus norvegicus 121 CAGACAAATGACCAAGTGACAGAGGTCTGTGCCACGACATTCACAGTGAAGAACACGTTG 180

Mus musculus      181 GGCTTCCTAGATTACCCCTTCTGCAGTGGTACCTTTAATGAAAGCAGAGTGAACCTCACC 240
                   |||
Rattus norvegicus 181 GGCTTCCTAGATGACCCCTTCTGCAGTGGTACCTTTAATGAAAGCAGAGTGAACCTCACC 240

Mus musculus      241 ATCCAAGGACTGAGAGCTGTTGACACGGGACTGTACCTCTGCAAGGTGGAACATCATGTAC 300
                   |||
Rattus norvegicus 241 ATCCAAGGACTGAGGGCTGCTGACACCGGACTGTACTTCTGCAAGGTGGAACATCATGTAC 300

Mus musculus      301 CCACCGCCATACTTTGTGGGCATGGGCAACGGGACGCAGATTTATGTCATTGATCCAGAA 360
                   |||
Rattus norvegicus 301 CCACCGCCATACTTTGTGGGCATGGGCAACGGGACCCAGATTTATGTCATCGATCCAGAA 360

Mus musculus      361 CCATGCCCGGATTCTGAC 378
                   |||
Rattus norvegicus 361 CCATGCCCAGATTCAGAC 378

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Figure S4. A nucleotide sequence alignment of mouse *ctla4* and rat *ctla4*.

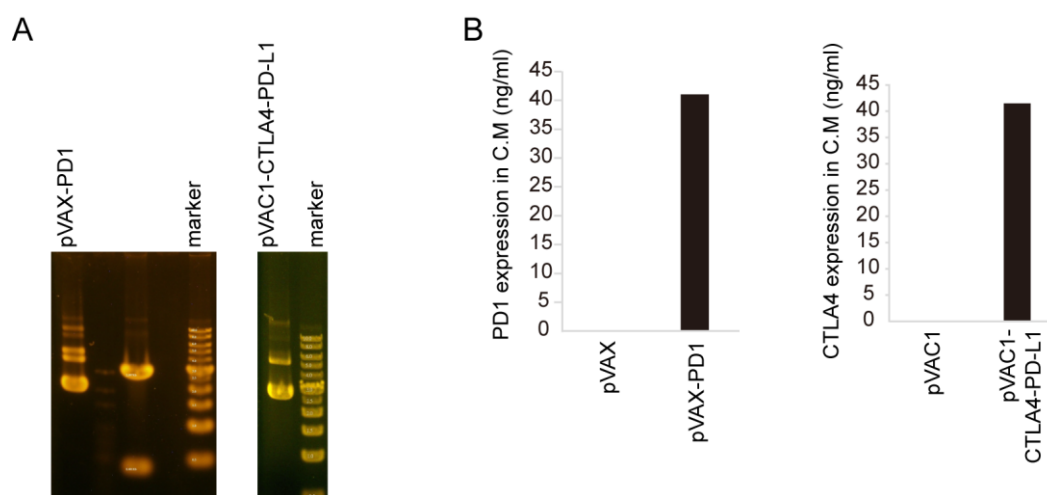


Figure S5. The quality control of DNA vaccines. (A) The agarose gel electrophoresis of two plasmids, (B) The expression of PD1 or CTLA4 in conditioned medium (C.M.) from CHO cells transiently expressing CTLA4-PDL1 or PD1 for 48 hours.