Supporting Information

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SI Materials and Methods

Bacterial Strains. Virulent *M. tuberculosis* H37Rv (obtained from Trudeau Institute) strain has been described previously. Bacteria were grown in Middlebrook 7H9 broth supplemented with 10% (vol/vol) oleic acid–albumin-dextrose-catalase (OADC), 0.5% (vol/vol) glycerol, and 0.05% (vol/vol) Tween-80 supplemented with lysine and pantothenate. Middlebrook 7H10, supplemented with 10% (vol/vol) OADC, 0.5% (vol/vol) glycerol, and 0.05% (vol/vol) Tween-80, was used as solid media for CFU assessment.

Aerosol Challenge and Bacterial Loads in Tissues. All animal studies were approved by the institutional animal care and use committees of the AECOM. Aerogenic challenge was done using a Baker-Madison chamber to deliver a low dose of the *M. tuberculosis* H37Rv strain. A suspension of 10^6 CFU/mL in PBS containing 0.05% Tween-80 and 0.004% antifoam (Sigma Aldrich) was used as the inoculum. Four mice from each infection group were killed 24 h after aerosol challenge, and lung homogenates were plated on 7H9-agar plates to determine the efficiency of aerosol delivery of *M. tuberculosis*. The doses delivered were between 50 and 320 CFU per lung *M. tuberculosis* H37Rv. At different timepoints postchallenge, four to five mice from each challenged group were killed to determine organ bacterial loads in the lung, liver, and spleen. Organs of individual mice were aseptically removed and homogenized separately in 5 mL PBS plus 0.05% Tween-80, using a Seward Stomacher 80 blender (Seward Limited). The homogenates were diluted serially and plated on Middlebrook 7H9-agar plates supplemented with OADC, glycerol, and Tween-80, as described above. Agar plates were incubated at 37 °C for 3–4 wk before CFUs were assessed. Ten to 15 mice from each group were used to determine survival times of infected mice.

Histology. For histological analysis, mice were humanely euthanized at various timepoints after infection, and the lungs, liver, and spleen were removed and placed into 10% neutral buffered formalin (Fischer Scientific). Four to five mice were used for each timepoint for each group. Tissue samples were then routinely processed for paraffin embedding. Five-micrometer thickness tissue sections were cut, dewaxed, rehydrated through graded alcohols, and stained with either hematoxylin and eosin (HE) or acid fast bacillus (AFB) stain for identification of bacterial cells.

Immunohistochemistry. Paraffin sections were melted at 60 °C for 30 min and deparaffinized in xylene, then rehydrated through graded alcohols to water, and washed in TBS (Tris buffer saline). Slides were pretreated with 0.3% hydrogen peroxide for 10 min, then washed in TBS. Antigen retrieval was performed using 10 mM sodium citrate buffer pH 6.0 in a steamer for 20 min, followed by cooling at RT room temperature (RT) for 30 min. Slides were blocked in 5% normal donkey serum and 2% BSA for 1 h at RT before incubating with antibodies for 1 h at RT. To detect B cells, purified rat antibody to mouse B220 (BD Pharmingen) was used 1/50, diluted in blocking solution. T cells were detected by using polyclonal rabbit anti-CD3 ready to use (DAKO). Slides were washed four times, 3 min each with TBS before applying biotin-labeled secondary antibody (goat anti-rat or goat anti-rabbit, DAKO) at 1/300 for 1 h at RT. Slides were washed again and incubated for 20 min with the avidin-biotin-HRP complex, as directed by the supplier (VECTOR). Slides were washed in TBS and DAB applied (VECTOR) for 2 min before lightly counterstaining them with Harris Hematoxylin (Poly Scientific).

FACS Analysis. Mice were euthanized and lungs were removed after cardiac perfusion with 1× PBS. Lungs were cut into small pieces (~1 mm^3) and digested in DMEM containing 5% FCS, 1 mg/mL collagenase, and 0.5 mg/mL DNase at 37 °C for 45 min. The digest was further passed through a 0.45-mm strainer using the plunger of a 3-mL syringe. The lung cell suspension was washed and counted by trypan blue exclusion of dead cells. FACS staining was performed after blocking Fc receptors, by using the following antibodies: CD4-PE, CD8-FITC, CD11b-PE, and B220-FITC from BD Biosciences; purified anti-mouse CD16/CD32; and Gr-1-APC from eBioscience.

Cytokine Measurements. Blood samples were collected from groups of PD-$1^{-/-}$ and C57BL/6 mice infected with *M. tuberculosis H37Rv* (50 CFU per lung) at 2, 4, and 8 wk postinfection and from uninfected mice as well, by retro-orbital bleeding. Sera from five mice were collected in each group at each timepoint. Sera were separated by centrifugation using prepared microtubes (Sarstedt). Serum cytokines were measured using the mouse proinflammatory 7-plex kit (Meso Scale Discovery), according to the manufacturer's protocol. Minimum detection levels for the individual cytokines were: 4 pg/mL for TNF-α, 30 pg/mL for IL-6, 2 pg/mL for IFN-γ, 50 pg/mL for IL-12 p70, and 10 pg/mL for IL-10. IL-17 was measured using the Quantikine ELISA kit (R&D Systems and ELISA kit from eBioscience, respectively.).

For lung cytokine measurements, lungs were removed and homogenized immediately in 2.5 mL 1× PBS containing 0.05% Tween-80, using a Seward Stomacher 80 blender (Seward Limited). The homogenate was centrifuged for 10 min at 13,000 rpm, the supernatant was removed and filtered through 0.2-mm microfilters, and stored at −80 °C. Cytokines were measured as described above. Groups of four mice were analyzed at each timepoint.

RNA Extraction and Microarray Experiments. Whole lungs from PD-$1^{-/-}$ and C57BL/6 mice infected with *M. tuberculosis* H37Rv (173 CFU per lung), as well as from uninfected mice were removed at 4 wk postinfection, and immediately placed in RNAlater (Qiagen). Lungs from three mice per group were used for total RNA extraction using RNeasy Protect mini kit (Qiagen), based on the manufacturer's recommendation, followed by DNase digest (Ambion/Applied Biosystems). The integrity of the RNA was verified using the Agilent 2100 Bioanalyzer. A total of 100 ng of total RNA from each sample was labeled using standard protocols from the manufacturer (Affimetryx). Briefly, RNA was transcribed using T7-$(N)_6$ primers (250 ng/μL) followed by cDNA synthesis and amplification. The cDNA was then used directly in an in vitro transcription reaction, followed again by cDNA synthesis using random primers, in the presence of dUTP. After hydrolysis of cRNA, single-stranded DNA was fragmented by breaking at each incorporated dUTP residue, and applied for labeling in the presence of terminal deoxynucleotidyl transferase (TdT) and biotin-coupled labeling reagent. Each fragmented and terminally labeled cDNA sample was hybridized onto a GeneChip Mouse Gene 1.0 ST array (Affymetrix) for 17 h, followed by washing and staining steps, and scanned using an Affymetrix GeneChip fluidic station and scanner.

Microarray Data Analysis. The raw array data were imported into Expression Console v1.1 (Affymetrix), and normalized after quality control. The data were ranked using LIMMA (R package) analysis for comparison of different groups. There were three technical replicates for each group, which were averaged for fold-change study. Statistical significance was set to $P < 0.05$ by applying *t*-test analysis adjusted for multiple comparisons. Data were ex-

ported from R package to Excel and analyzed further. Gene intensity changes higher than twofold were accepted as biologically significant. Tables of genes were compiled on the basis of GO organizing principles such as biological process or molecular function. Hierarchical cluster analysis was performed using CLUSTER 3.0; the results are shown using TreeView software.

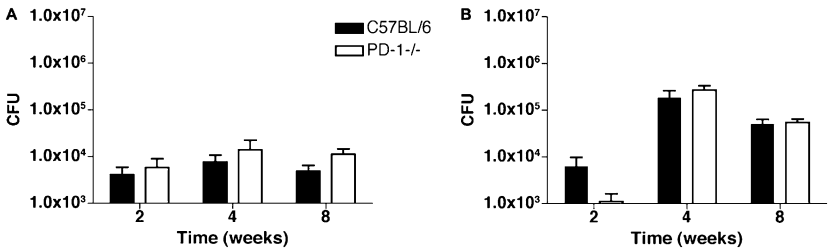

Fig. S1. Bacterial loads in the liver (*A*) and spleen (*B*) of PD-1$^{-/-}$ and control mice infected with *M. tuberculosis* aerosol. CFU values were determined at 2, 4, and 8 wk after low-dose H37Rv aerosol infection (50 CFU). Mean values of five mice at each timepoint are shown ± SD.

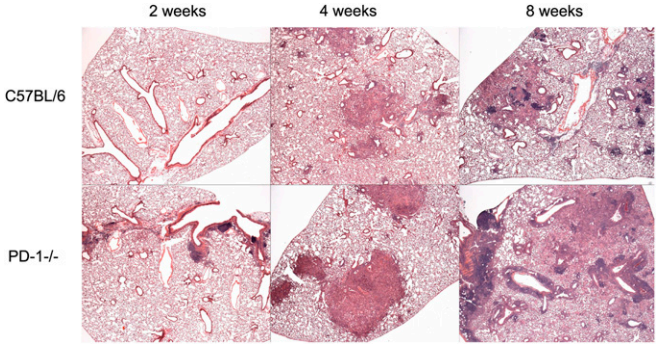

Fig. S2. Necrotic pneumonia develops in the lungs of PD-1$^{-/-}$ mice infected with *M. tuberculosis H37Rv* aerosol, by 4 wk postinfection. HE stains of lungs from mice infected with 50 CFU/mouse are shown at 2, 4, and 8 wk postinfection. (*Upper*) C57BL/6 mice. (*Lower*) PD-1$^{-/-}$ mice. At 2 wk postinfection, two out of five PD-1$^{-/-}$ mice had prominent bronchiolar-associated lymphoid tissue (BALT) as shown here, which was not observed in the wild-type lungs, 25× magnification.

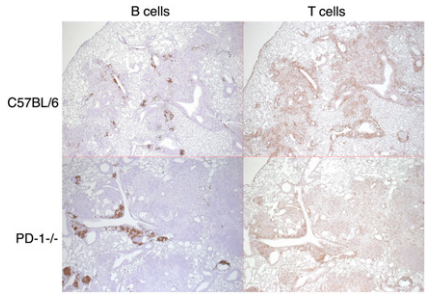

Fig. S3. Immunohistochemical staining of the lungs 4 wk postinfection with *M. tuberculosis* H37Rv aerosol. B cells formed prominent clusters in the lungs of PD-1$^{-/-}$ mice (*Left*), but they were largely absent from the necrotic nodules. Similarly, lower numbers of T cells were infiltrating the pyogranulomatous nodules in the PD-1$^{-/-}$ mice, compared with the wild-type mice (*Right*), ×40 magnification.

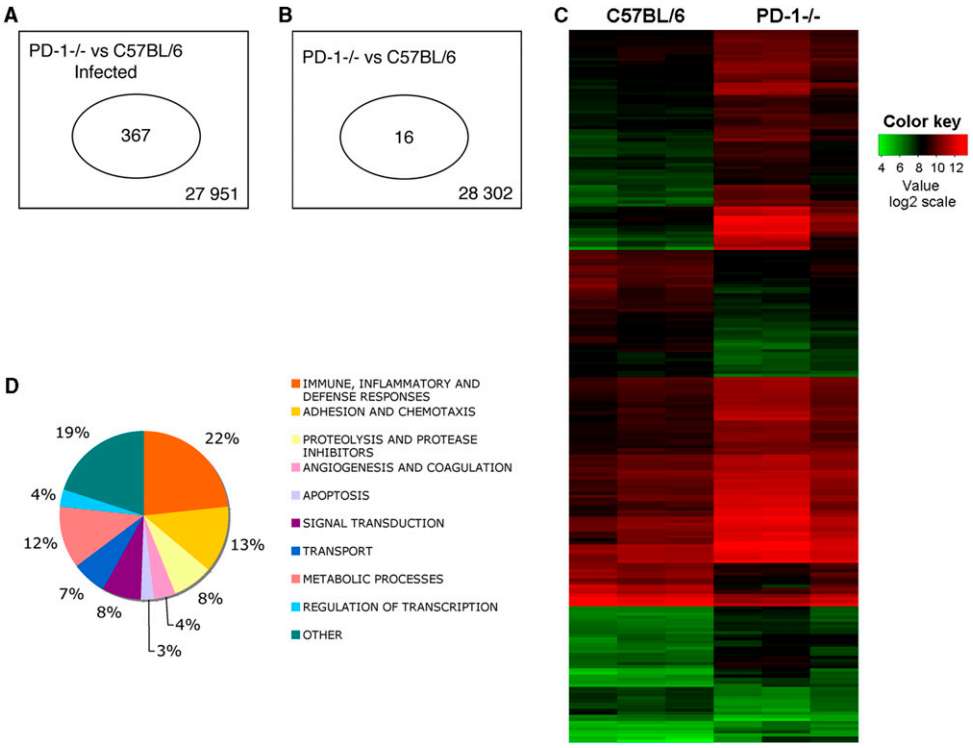

Fig. S4. Gene expression profiles of the lungs of *M. tuberculosis* infected PD-1$^{-/-}$ and wild-type mice. Affymetrix Mouse Gene 1.0 ST array was used to compare gene expression profiles in the lungs of infected PD-1$^{-/-}$ and wild-type mice. (*A*) A total of 367 transcripts of genes showed significantly different expression levels between infected PD-1$^{-/-}$ versus infected wild-type mice, whereas only 16 of them were different between uninfected PD-1$^{-/-}$ versus uninfected wild-type mice (*B*). (*C*) Hierarchical clustering analysis for the selected panel of genes from infected wild-type (*Left* lanes) and PD-1$^{-/-}$ mice (*Right* lanes), three replicates per group are shown. (*D*) Distribution of the functional categories of genes that were significantly different between infected PD-1$^{-/-}$ and control mice.

Table S1. Differential expression of genes regulating immune, inflammatory, and defense responses in *M. tuberculosis*-infected PD-1$^{-/-}$ versus control mice

Function	Fold change (log 2)	Adj. *P*	Description	GeneBank accession no.
Immune and inflammatory responses				
S100a9	3.56	0.024	S100 calcium binding protein A9 (calgranulin B)	NM_009114
S100a8	3.43	0.024	S100 calcium binding protein A8 (calgranulin A)	NM_013650
Orm1	2.42	0.022	Orosomucoid 1	NM_008768
A2m	2.41	0.011	Alpha-2-macroglobulin	NM_175628
Orm3	2.30	0.033	Orosomucoid 3	NM_013623
Selp	1.94	0.032	Selectin, platelet	NM_011347
Fpr1	1.87	0.046	Formyl peptide receptor 1	NM_013521
Spp1	1.86	0.049	Secreted phosphoprotein 1	NM_009263
Clec4d	1.71	0.044	C-type lectin domain family 4, member d	NM_010819
C5ar1	1.62	0.032	Complement component 5a receptor 1	NM_007577
Cd300lf	1.61	0.049	CD300 antigen like family member F	NM_145634
Orm2	1.56	0.017	Orosomucoid 2	NM_011016
Hdc	1.55	0.039	Histidine decarboxylase	NM_008230
Il11	1.27	0.033	Interleukin 11	NM_008350
Ptafr	1.25	0.035	Platelet-activating factor receptor	NM_001081211
Clec4e	1.20	0.041	C-type lectin domain family 4, member e	NM_019948
Il7r	1.18	0.036	Interleukin 7 receptor	NM_008372
Pram1	1.18	0.046	PML-RAR alpha-regulated adaptor molecule 1	NM_001002842
Il1rap	1.17	0.032	Interleukin 1 receptor accessory protein, transcript variant 1	NM_008364
Clec4n	1.17	0.034	C-type lectin domain family 4, member n	NM_020001
Cd38	1.05	0.023	CD38 antigen	NM_007646
Sh2d1b1	−1.06	0.048	SH2 domain protein 1B1	NM_012009
Trat1	−1.08	0.026	T cell receptor associated transmembrane adaptor 1	NM_198297
Klrg1	−2.09	0.032	Killer cell lectin-like receptor subfamily G, member 1	NM_016970
Hc	−3.07	0.039	Hemolytic complement	NM_010406
Defense response				
Cd14	2.28	0.031	CD14 antigen	NM_009841
Irg1	1.85	0.032	Immunoresponsive gene 1	NM_008392
Ifitm1	1.60	0.032	IFN induced transmembrane protein 1, transcript variant 1	NM_026820
Ptx3	1.45	0.043	Pentraxin related gene	NM_008987
Ifitm1	1.29	0.031	IFN induced transmembrane protein 1, transcript variant 1	NM_026820
Pglyrp1	1.26	0.032	Peptidoglycan recognition protein 1	NM_009402
Tlr13	1.21	0.023	Toll-like receptor 13	NM_205820
Rsad2	1.15	0.042	Radical S-adenosyl methionine domain containing 2	NM_021384
Adm	1.13	0.032	Adrenomedullin	NM_009627
Tlr11	−1.02	0.035	Toll-like receptor 11	NM_205819
Ncr1	−1.15	0.035	Natural cytotoxicity triggering receptor 1	NM_010746
Lyz1	−1.20	0.041	Lysozyme 1	NM_013590
Klra8	−1.32	0.035	Killer cell lectin-like receptor, subfamily A, member 8	NM_010650

Table S2. Differential gene expression in the lungs of *M. tuberculosis*-infected PD-1$^{-/-}$ mice, compared with control mice

Function/gene name	Fold change (log 2)	Adj. *P*	Description	GeneBank accession no.
Proteolysis				
Asprv1	4.26	0.027	Aspartic peptidase, retroviral-like 1	NM_026414
Mmp8	3.75	0.025	Matrix metallopeptidase 8	NM_008611
Ctse	2.89	0.002	Cathepsin E	NM_007799
Mmp9	2.39	0.033	Matrix metallopeptidase 9	NM_013599
Mmp10	1.91	0.016	Matrix metallopeptidase 10	NM_019471
Mmp12	1.37	0.044	Matrix metallopeptidase 12	NM_008605
Adam8	1.34	0.037	A disintegrin and metallopeptidase domain 8	NM_007403
Pappa	1.21	0.029	Pregnancy-associated plasma protein A	NM_021362
Mmp13	1.16	0.036	Matrix metallopeptidase 13	NM_008607
Adamts10	−1.01	0.031	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 10	NM_172619
Mme	−1.21	0.024	Membrane metallo endopeptidase	NM_008604
Tinag	−1.89	0.033	Tubulointerstitial nephritis antigen	NM_012033
Protease inhibitors				
Serpina3m	1.80	0.018	Serine (or cysteine) peptidase inhibitor, clade A, member 3M	NM_009253
Timp1	1.58	0.032	Tissue inhibitor of metalloproteinase 1 (Timp1), transcript variant 1	NM_001044384
Serpina3n	1.52	0.022	Serine (or cysteine) peptidase inhibitor, clade A, member 3N	NM_009252
Cell adhesion				
Chl1	1.85	0.024	Cell adhesion molecule with homology to L1CAM	NM_007697
Itgam	1.55	0.035	Integrin alpha M, transcript variant 1	NM_001082960
Tnc	1.53	0.033	Tenascin C	NM_011607
Emilin2	1.34	0.035	Elastin microfibril interfacer 2	NM_145158
Cd33	1.28	0.047	CD33 antigen (Cd33), transcript variant 1	NM_001111058
Csf3r	1.11	0.025	Colony stimulating factor 3 receptor (granulocyte)	NM_007782
Ninj1	1.05	0.032	Ninjurin 1	NM_013610
Postn	−1.10	0.036	Periostin, osteoblast specific factor	NM_015784
Lama3	−1.22	0.031	Isoform B of Laminin subunit alpha-3 precursor gene	ENSMUST00000092070
Dpt	−1.24	0.033	Dermatopontin	NM_019759
Thbs3	−1.27	0.018	Thrombospondin 3	NM_013691
Tnxb	−1.54	0.018	Tenascin XB	NM_031176
Itga8	−1.88	0.034	Integrin alpha 8	NM_001001309
Blood coagulation				
F13a1	1.81	0.035	Coagulation factor XIII, A1 subunit	NM_028784
F3	1.20	0.034	Coagulation factor III	NM_010171
Signal transduction				
Mrgpra2	3.03	0.031	MAS-related GPR, member A2	NM_153101
Mrgpra2	2.89	0.032	MAS-related GPR, member A2	NM_153101
Gpr109a	2.78	0.031	G protein-coupled receptor 109A	NM_030701
Rhov	1.73	0.041	Ras homolog gene family, member V	NM_145530
Plaur	1.60	0.046	Plasminogen activator, urokinase receptor	NM_011113
Rnd1	1.46	0.040	Rho family GTPase 1	NM_172612
Angptl4	1.20	0.022	Angiopoietin-like 4	NM_020581
P2ry13	1.11	0.022	Purinergic receptor P2Y, G protein coupled 13	NM_028808
Ms4a6d	1.05	0.034	Membrane-spanning 4-domains, subfamily A, member 6D	NM_026835
Rasd1	1.04	0.042	RAS, dexamethasone-induced 1	NM_009026
Glp1r	−1.04	0.035	Glucagon-like peptide 1 receptor	NM_021332
Gfra2	−1.05	0.027	Glial cell line derived neurotrophic factor family receptor alpha 2	NM_008115
Gpr33	−1.07	0.033	G protein-coupled receptor 33	NM_008159
Agtrl1	−1.32	0.032	Angiotensin receptor-like 1	NM_011784
Ltbp4	−1.59	0.031	Latent transforming growth factor beta binding protein 4, transcript variant 1	NM_175641
Angiogenesis				
Serpine1	1.83	0.032	Serine (or cysteine) peptidase inhibitor, clade E, member 1	NM_008871
Ereg	1.66	0.036	Epiregulin	NM_007950
Edn1	1.56	0.042	Endothelin 1	NM_010104
Lox	1.22	0.011	Lysyl oxidase	NM_010728
Enpep	−1.36	0.034	Glutamyl aminopeptidase	NM_007934
Nppa	−1.57	0.037	Natriuretic peptide precursor type A	NM_008725
Apoptosis				
Egln3	1.84	0.041	EGL nine homolog 3 (*C. elegans*)	NM_028133

Table S2. Cont.

Function/gene name	Fold change (log 2)	Adj. *P*	Description	GeneBank accession no.
Nlrp3	1.67	0.044	NLR family, pyrin domain containing 3	NM_145827
Fbxl5	1.65	0.029	F-box and leucine-rich repeat protein 5	NM_178729
Tnfaip3	1.19	0.035	Tumor necrosis factor, alpha-induced protein 3	NM_009397
Actc1	−1.64	0.033	Actin, alpha, cardiac	NM_009608
Transport				
Slc7a11	1.99	0.046	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 11	NM_011990
Clca4	1.67	0.033	Chloride channel calcium activated 4	NM_139148
Marco	1.61	0.046	Macrophage receptor with collagenous structure	NM_010766
Slc40a1	1.61	0.008	Solute carrier family 40 (iron-regulated transporter), member 1	NM_016917
Slc16a3	1.31	0.040	Solute carrier family 16 (monocarboxylic acid transporters), member 3	NM_001038653
Msr1	1.26	0.036	Macrophage scavenger receptor 1, transcript variant 1	NM_031195
Ltf	1.25	0.037	Lactotransferrin	NM_008522
Slc39a14	1.25	0.026	Solute carrier family 39 (zinc transporter), member 14	NM_144808
Glrx	1.20	0.034	Glutaredoxin	NM_053108
Slc2a1	1.14	0.043	Solute carrier family 2 (facilitated glucose transporter), member 1	NM_011400
Slc16a1	1.06	0.011	Solute carrier family 16 (monocarboxylic acid transporters), member 1	NM_009196
Scn7a	−1.67	0.032	Sodium channel, voltage-gated, type VII, alpha	NM_009135
Abca8a	−1.68	0.042	ATP-binding cassette, subfamily A (ABC1), member 8a	NM_153145
Regulation of transcription				
Padi4	1.58	0.040	Peptidyl arginine deiminase, type IV	NM_011061
Mxd1	1.57	0.027	MAX dimerization protein 1	NM_010751
Basp1	1.10	0.033	Brain abundant, membrane attached signal protein 1	NM_027395
Ankrd22	1.09	0.043	Ankyrin repeat domain 22	NM_024204
Nfil3	1.05	0.032	Nuclear factor, interleukin 3, regulated	NM_017373
Rbpjl	−1.03	0.018	Recombination signal binding protein for Ig kappa J region-like	NM_009036
Nr1d1	−1.15	0.025	Nuclear receptor subfamily 1, group D, member 1	NM_145434
Metabolic processes				
Arg1	3.30	0.006	Arginase 1, liver	NM_007482
Dhrs9	2.20	0.025	Dehydrogenase/reductase (SDR family) member 9	NM_175512
Upp1	1.93	0.033	Uridine phosphorylase 1	NM_009477
Mgam	1.89	0.044	similar to maltase-glucoamylase gene:ENSMUSG00000068587	ENSMUST00000071535
Arg2	1.86	0.033	Arginase type II (Arg2)	NM_009705
Hmox1	1.71	0.036	Heme oxygenase (decycling) 1	NM_010442
Smox	1.66	0.032	Spermine oxidase	NM_145533
Agpat9	1.34	0.043	1-acylglycerol-3-phosphate O-acyltransferase 9, transcript variant 1	NM_172715
Pygl	1.22	0.039	Liver glycogen phosphorylase	NM_133198
Ptges	1.21	0.040	Prostaglandin E synthase	NM_022415
Pdk4	1.16	0.018	Pyruvate dehydrogenase kinase, isoenzyme 4	NM_013743
Gla	1.08	0.036	Galactosidase, alpha	NM_013463
Il4i1	1.07	0.034	Interleukin 4 induced 1	NM_010215
Pdzd2	−1.10	0.036	PDZ domain containing 2	NM_001081064
Lrp2	−1.20	0.031	Low density lipoprotein receptor-related protein 2	NM_001081088
Car3	−1.69	0.010	Carbonic anhydrase 3	NM_007606
Adh1	−1.81	0.032	Alcohol dehydrogenase 1 (class I)	NM_007409
Lipid metabolism				
Pla1a	1.12	0.028	Phospholipase A1 member A	NM_134102
Cyp7b1	1.09	0.029	Cytochrome P450, family 7, subfamily b, polypeptide 1	NM_007825
Apoc2	1.07	0.029	Apolipoprotein C-II	NM_009695
Adfp	1.03	0.024	Adipose differentiation related protein	NM_007408
Acoxl	−1.55	0.033	Acyl-CoA oxidase-like	NM_028765
Metal ion homeostasis				
Mt2	2.32	0.049	Metallothionein 2	NM_008630
Mt1	1.41	0.044	Metallothionein 1	NM_013602
Regulation of cell growth				
Socs3	1.29	0.033	Suppressor of cytokine signaling 3 (Socs3)	NM_007707
Socs3	1.06	0.032	EF-10 mRNA	U72673
Igfbp2	−1.53	0.025	Insulin-like growth factor binding protein 2	NM_008342

Table S3. Gene expression differences in the lungs of uninfected PD-1$^{-/-}$ and C57BL/6 mice

Gene	Fold change (log 2)	Adj. *P*	Description	GenBank accession no.
Ctse	1.98	0.005	Cathepsin E	NM_007799
Snrpe	1.17	0.018	Small nuclear ribonucleoprotein E	NM_009227
Nnt	1.05	0.002	Nicotinamide nucleotide transhydrogenase, transcript variant 2	NR_003544
Erdr1	−1.97	0.028	Erythroid differentiation regulator 1	NM_133362.1
Hspa1b	−1.62	0.005	Heat shock protein 1B	NM_010478
Hspa1a	−1.50	0.012	Heat shock protein 1A	NM_010479