

**Supplemental Table 1.** Recognized functions of 11 genes identified in adult Mojave desert tortoises (*Gopherus agassizii*).

<b>Gene</b>	<b>Gene function</b>
CaM	Calmodulin (CaM) is a small acidic Ca <sup>2+</sup> -binding protein, with a structure and function that is highly conserved in all eukaryotes. CaM activates various Ca <sup>2+</sup> -dependent enzyme reactions, thereby modulating a wide range of cellular events, including metabolism control, muscle contraction, exocytosis of hormones and neurotransmitters, and cell division and differentiation (Chen et al. 2012). CaM has also been reported to be a pivotal calcium metabolism regulator in the shell formation (Li et al. 2004).
AHR	The arylhydrocarbon receptor (AHR) responds to classes of environmental toxicants including polycyclic aromatic hydrocarbons, polyhalogenated hydrocarbons, dibenzofurans, and dioxin (Oesch-Bartlomowicz et al. 2005). Depending upon the ligand, AHR signaling can modulate T-regulatory (T <sub>REG</sub> ) (immune-suppressive) or T-helper type 17 (T <sub>H</sub> 17) (pro-inflammatory) immunologic activity (Quintana et al. 2008, Veldhoen et al. 2008).
Mx1	The Mx1 gene responds to viral infection (Tumpey et al. 2007). Vertebrates have an early strong innate immune response against viral infection, characterized by the induction and secretion of cytokines that mediate an antiviral state, leading to the up-regulation of the MX-1 gene (Kibenge et al. 2005).
HSP 70	The heat shock protein 70 is produced in response to thermal or other stress (Iwama et al. 1999, Tsan and Gao 2004). In addition to being expressed in response to a wide array of stressors (including hyperthermia, oxygen radicals, heavy metals, and ethanol) heat shock proteins act as molecular chaperones (De Maio et al. 1999). For example, heat shock proteins aid the transport of the AHR/toxin complex in the initiation of detoxification (Tanabe et al. 1994).
SAA	Serum Amyloid A (SAA), an acute phase protein, serves as a core part of the innate immunity involving physical and molecular barriers and responses (Cray et al. 2009). Upon infection and inflammation or tissue damage and stress, SAA is induced by pro-inflammatory signals, and is a major indicator of bacterial infection, especially at early stage, in reptiles (Zhou et al. 2008, 2011).
MyD88	Myeloid differentiation factor 88 (MyD88) is one of the key adaptor proteins to signal transduction that triggers downstream cascades involved in innate immunity. MyD88 might possess an important role in defense against microbial infection in Chinese soft-shelled turtles similar to that in mammals (Li et al. 2011).
CD9	CD9 is a molecular facilitator, provides co-stimulation to naïve T lymphocytes, regulates the aggregation of MHC-II molecules, and triggers antigen presentation. Upregulation of turtle CD9 was

	shown in response to bacterial infection (Zhou et al. 2008, 2010).
SOD	Superoxide dismutase (SOD) – Superoxide dismutases are a class of enzymes that catalyze dismutation of superoxide into oxygen and hydrogen peroxide and function as important antioxidant defense molecules (Walsh et al. 2010). Oxidative stress itself can lead to or result from certain inflammatory conditions (Walsh et al. 2010).
ATF	Similar to inflammatory responses in mammals, ATF can mediate inflammatory responses in reptiles. The upregulation of ATF in reptiles indicates involvement in bacterial infection (Zhou et al. 2008).
CL	Cathepsin L, an acute phase protein, plays a major role in antigen processing, tumor invasion and metastasis, bone resorption, and turnover of intracellular and secreted proteins involved in growth regulation (Zhou et al. 2008). Turtle cathepsin L may be involved in anti-bacterial immune response (Zhou et al. 2008).
LEP	Leptin links nutritional status with neuroendocrine and immune functions. Initially thought to be a satiety factor that regulates body weight by inhibiting food intake and stimulating energy expenditure, leptin is a hormone whose multiple effects include regulation of endocrine function, reproduction, and immunity (Otero et al. 2005).

**Supplemental Table 2.** Morphological diagnoses of tissues evaluated for adult Agassiz's desert tortoises (*Gopherus agassizii*) classified as "clinically abnormal" within 24 hrs post euthanasia.

Animal		Morphological Diagnoses of Tissue(s)
ID	Sex	
A1	F	Lung (pneumonia, severe, granulomatous and fibrosing), nasal cavity (rhinitis, mild to moderate, heterophilic), trachea (tracheitis, mild, heterophilic), conjunctiva (conjunctivitis, mild, heterophilic), large intestine (colitis, mild, heterophilic).
A2	F	Nasal cavity (rhinitis, moderate, heterophilic with marked fibrosis), tongue (glossitis, minimal, heterophilic), stomach (gastritis, mild, lymphocytic).
A3	M	Morphology not assessed.
A4	M	Morphology not assessed.
A5	M	Kidney (renal gout; nephritis, moderate, granulomatous), nasal cavity (rhinitis, moderate, heterophilic), esophagus (esophagitis, moderate, lymphocytic and heterophilic), tongue (glossitis, mild, lymphocytic and heterophilic), trachea (tracheitis, mild, lymphocytic and heterophilic), lung (pneumonia, mild heterophilic), liver (vacuolar change, mild), large intestine (colitis, mild, heterophilic), urinary bladder (urolithiasis).
A6	F	Liver (hematoma, chronic; hepatic lipodosis, severe), nasal cavity (rhinitis, moderate to severe, heterophilic with fibrosis), lung (pneumonia, mold, lymphocytic with fibrosis), tongue (glossitis, minimal, lymphocytic), trachea (tracheitis, mild, lymphocytic and heterophilic), conjunctiva (conjunctivitis, mild to moderate, lymphocytic), small intestine (enteritis, mild, lymphocytic with fibrosis); adrenal gland (adrenal fibrosis).
A7	F	Nasal cavity (rhinitis, severe, heterophilic to granulomatous, with squamous metaplasia and fibrosis), lung (pneumonia, moderate, lymphocytic and heterophilic), tongue (glossitis, mild, lymphocytic), esophagus (esophagitis, mild, lymphocytic), kidney (multifocal cystic urinary spaces), urinary bladder (urolithiasis).
A8	F	Nasal cavity (rhinitis, moderate, heterophilic and lymphocytic), tongue (glossitis, mild, lymphocytic), trachea (tracheitis, moderate, lymphocytic and heterophilic), lung (pneumonia, mild to moderate, heterophilic and lymphocytic), esophagus (esophagitis, mild, lymphocytic and heterophilic), stomach (gastritis, mild, lymphohistiocytic), tongue (glossitis, mild, lymphocytic).
A9	M	Lung (pneumonia, moderate to severe, heterophilic to granulomatous), nasal cavity (rhinitis, mild, heterophilic), tongue (glossitis, mild, heterophilic), kidney (glomerular atrophy), cloaca (proctitis, moderate, heterophilic and lymphocytic), esophagus (esophagitis, mild, heterophilic and lymphocytic), large intestine (colitis, mild, lymphocytic), shell and limb (partial traumatic amputation), skeletal muscle (sarcozystiasis).
A10	M	Nasal cavity (rhinitis, moderate, heterophilic), tongue (glossitis, mild, lymphocytic and heterophilic), liver (hepatic lipodosis, marked), small intestine (enteritis, mild, lymphocytic).

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A11	F	Nasal cavity (rhinitis, moderate, heterophilic), liver (hepatic lipidosis, moderate), esophagus (esophagitis, mild, lymphocytic), urinary bladder (urolithiasis).
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**Supplemental Table 3.** Spearman’s rank-order correlation was used to measure the strength and direction of association between evaluated gene transcript, hematology, plasma biochemistry, and trace element blood variables for 21 adult Agassiz’s desert tortoises (*Gopherus agassizii*) classified as clinically abnormal and clinically normal in Clark County, NV, USA. Correlated variables listed had a statistical significance based on p values  $\leq 0.01$ .

<b>Variable 1 (unit)</b>	<b>Variable 2 (unit)</b>	<b>Variable 1 Type</b>	<b>Variable 2 Type</b>	<b>Spearman’s Rank Correlation Coefficient</b>
Chloride (mmol/L)	Sodium (mmol/L)	biochemistry	biochemistry	0.97
Globulins (g/dl)	Total Protein (g/dl)	biochemistry	biochemistry	0.93
Albumin (g/dl)	Total Protein (g/dl)	biochemistry	biochemistry	0.92
ATF (C <sub>T</sub> )	CL (C <sub>T</sub> )	gene transcript	gene transcript	0.91
Albumin (g/dl)	Globulin (g/dl)	biochemistry	biochemistry	0.84
CL (C <sub>T</sub> )	Leptin (C <sub>T</sub> )	gene transcript	gene transcript	0.82
MyD88 (C <sub>T</sub> )	SOD (C <sub>T</sub> )	gene transcript	gene transcript	0.82
Mx1 (C <sub>T</sub> )	SAA (C <sub>T</sub> )	gene transcript	gene transcript	0.81
Albumin (g/dl)	Cholesterol (mg/dl)	biochemistry	biochemistry	0.77
CaM (C <sub>T</sub> )	HSP70 (C <sub>T</sub> )	gene transcript	gene transcript	0.77
Albumin (g/dl)	Plasma Protein (mg/dl)	biochemistry	biochemistry	0.75
Blood Urea Nitrogen (mg/dl)	Sodium (mmol/L)	biochemistry	biochemistry	0.75
AHR (C <sub>T</sub> )	Leptin (C <sub>T</sub> )	gene transcript	gene transcript	0.74
CD9 (C <sub>T</sub> )	SOD (C <sub>T</sub> )	gene transcript	gene transcript	0.74
Azurophils (/μl)	Leptin (C <sub>T</sub> )	hematology	gene transcript	0.73
Calcium (ppm)	Phosphorus (ppm)	trace element	trace element	0.73
Potassium (mEq/l)	MyD88 (C <sub>T</sub> )	trace element	gene transcript	0.73
Leptin (C <sub>T</sub> )	MyD88 (C <sub>T</sub> )	gene transcript	gene transcript	0.73
Calcium (ppm)	Magnesium (ppm)	trace element	trace element	0.71
Magnesium (ppm)	Sodium (mEq/l)	trace element	trace element	0.71
Albumin (g/dl)	Uric Acid (mg/dl)	biochemistry	biochemistry	0.70
Copper (ppm)	MyD88 (C <sub>T</sub> )	trace element	gene transcript	0.70
Azurophils (/μl)	Potassium (mmol/L)	hematology	trace element	0.69
Alkaline Phosphatase (U/l)	Vitamin A (ppm)	biochemistry	biochemistry	0.68

Blood Urea Nitrogen (mg/dl)	Chloride (mmol/L)	biochemistry	biochemistry	0.68
ATF (C <sub>T</sub> )	Leptin (C <sub>T</sub> )	gene transcript	gene transcript	0.67
Plasma Protein (mg/dl)	Total Protein (g/dl)	biochemistry	biochemistry	0.67
Blood Urea Nitrogen (mg/dl)	Cholesterol (mg/dl)	biochemistry	biochemistry	0.66
CD9 (C <sub>T</sub> )	MyD88 (C <sub>T</sub> )	gene transcript	gene transcript	0.66
Globulins (g/dl)	Plasma Protein (mg/dl)	biochemistry	biochemistry	0.66
Basophils (/μl)	White Blood Cells (/μl)	hematology	hematology	0.64
Blood Urea Nitrogen (mg/dl)	Uric Acid (mg/dl)	biochemistry	biochemistry	0.64
Leptin (C <sub>T</sub> )	Potassium (mEq/l)	gene transcript	trace element	0.64
Albumin (g/dl)	Glucose (mg/dl)	biochemistry	biochemistry	0.63
Albumin:Globulins	MyD88 (C <sub>T</sub> )	biochemistry	gene transcript	0.63
Azurophils (/μl)	Heterophils (/μl)	hematology	hematology	0.63
Leptin (C <sub>T</sub> )	SOD (C <sub>T</sub> )	gene transcript	gene transcript	0.63
Magnesium (ppm)	Phosphorus (ppm)	trace element	trace element	0.63
AHR (C <sub>T</sub> )	CL (C <sub>T</sub> )	gene transcript	gene transcript	0.62
Alkaline Phosphatase (U/l)	MyD88 (C <sub>T</sub> )	biochemistry	gene transcript	0.62
Aspartate Aminotransferase (U/l)	Glucose (mg/dl)	biochemistry	gene transcript	0.61
Azurophils (/μl)	CL (C <sub>T</sub> )	hematology	gene transcript	0.61
Eosinophils (/μl)	MyD88 (C <sub>T</sub> )	hematology	gene transcript	0.61
Cholesterol (mg/dl)	Lymphocytes (/μl)	biochemistry	hematology	0.61
CD9 (C <sub>T</sub> )	Zinc (ppm)	gene transcript	trace element	0.60
Azurophils (/μl)	White Blood Cells (/μl)	hematology	hematology	0.59
Glucose (mg/dl)	Uric Acid (mg/dl)	biochemistry	biochemistry	0.59
Heterophils (/μl)	White Blood Cells (/μl)	hematology	hematology	0.59
AHR (C <sub>T</sub> )	Mx1 (C <sub>T</sub> )	gene transcript	gene transcript	0.58
Eosinophils (/μl)	Leptin (C <sub>T</sub> )	hematology	gene transcript	0.57
Calcium (ppm)	Sodium (mEq/l)	trace element	trace element	0.56
CD9 (C <sub>T</sub> )	Mx1 (C <sub>T</sub> )	gene transcript	gene transcript	0.56
AHR (C <sub>T</sub> )	CD9 (C <sub>T</sub> )	gene transcript	gene transcript	0.56
Glucose (mg/dl)	Plasma Protein (mg/dl)	biochemistry	biochemistry	0.56
Mx1 (C <sub>T</sub> )	Zinc (ppm)	gene transcript	trace element	0.56
Blood Urea Nitrogen (mg/dl)	SOD (C <sub>T</sub> )	biochemistry	gene transcript	-0.55
Globulins (g/dl)	MyD88 (C <sub>T</sub> )	biochemistry	gene transcript	-0.55

ATF (C <sub>T</sub> )	Cholesterol (mg/dl)	gene transcript	biochemistry	-0.56
Blood Urea Nitrogen (mg/dl)	MyD88 (C <sub>T</sub> )	biochemistry	gene transcript	-0.57
Cholesterol (g/dl)	SOD (C <sub>T</sub> )	biochemistry	gene transcript	-0.57
ATF (C <sub>T</sub> )	Total Protein (g/dl)	gene transcript	biochemistry	-0.58
Azuophils (/μl)	Albumin (g/dl)	hematology	biochemistry	-0.58
Azuophils (/μl)	Globulins (g/dl)	hematology	biochemistry	-0.58
Cholesterol (mg/dl)	Leptin (C <sub>T</sub> )	biochemistry	gene transcript	-0.59
Albumin (g/dl)	CL (C <sub>T</sub> )	biochemistry	gene transcript	-0.60
CL (C <sub>T</sub> )	Total Protein (g/dl)	gene transcript	biochemistry	-0.60
Globulins (g/dl)	Leptin (C <sub>T</sub> )	biochemistry	gene transcript	-0.60
Albumin (g/dl)	ATF (C <sub>T</sub> )	biochemistry	gene transcript	-0.63
Aspartate Amino transferase (U/l)	Eosinophils (/μl)	biochemistry	hematology	-0.63
Cholesterol (mg/dl)	CL (C <sub>T</sub> )	biochemistry	gene transcript	-0.63
Copper (ppm)	SOD (C <sub>T</sub> )	trace element	gene transcript	-0.63
Copper (ppm)	Potassium (mEq/l)	trace element	trace element	-0.65
Azuophils (/μl)	Copper (ppm)	hematology	trace element	-0.75

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