Supplemental Methods

Detection of surface EMR1 by flow cytometry. EMR1 expression in bone marrow aspirates and peripheral blood was assessed by multiparameter flow cytometry using directly-conjugated antibodies against CD45 (clone 2D1), CD34 (clone 8G12), IL-5Rα (CDw125; clone A14), CD16 (clone 3G8), CD9 (M-L13), CD14 (M5E2), CD49d (clone 9F10), CD117 (clone 104D2), CD19 (SJ25C1), CD15 (HI98), CD3 (SK7), CD69 (FN50) (BD Biosciences), CD203 (97A6) (Beckman Coulter, Indianapolis, In), CD10 (MEM78) and CD64 (10.1) (Invitrogen), Siglec-8 (7C9) (Biolegend) and anti-EMR1 (clone 1E7; Kalobios). After antibody staining, red blood cells were lysed and samples fixed as previously described (Maric et al. 2007 JACI). Eosinophils were identified in whole blood as CD45-positive, CD16-negative, CD9-positive cells with characteristic high side–scatter properties after doublet exclusion. Irrelevant, directly conjugated mouse IgG1 was used to ascertain background staining, and antibodies were titrated before use. Whole blood and bone marrow aspirates were analyzed on a FACSCanto II flow cytometer (BD Biosciences) using FCS Express 4 software (De Novo Software, Los Angeles, CA) for data analysis. Cell lines and purified cells were analyzed on an LSR II flow cytometer using Diva software (BD Biosciences). FlowJo software (Version 9.5.2, Tree Star, Ashland, Ore) was used for data analysis.

Primers used for real-time quantitiative PCR. EMR1 and Siglec-8 mRNA were amplified using previously published primer sets[1, 2] and 1X SYBR Green (SA Biosciences, Frederick, MD) in a final 20 μL reaction volume. Forward and reverse primer sequences for the 18S control were 3'- GCCCGAAGCGTTTACTTTGA-5' and 3'-

TCCATTATTCCTAGCTGCGGTATC-5', respectively. The cationic proteins, MBP, EPX, ECP, EDN, and IL5RA (membrane form) were amplified using the following commercially available TaqMan primers (Applied Biosystems) in a final volume of 10 μl according to the manufacturer's instructions: MBP, PRG2, Hs00794928_m1; EPX, Hs00946094_m1; ECP, RNAse2, Hs00795553_s1; EDN, RNAse3, Hs00795553_s1; IL-5RA, s00602482_m1; 18S, X03205.1.

Immunostaining. Immunohistochemical staining for eosinophil peroxidase (EPX) was performed on formalin-fixed paraffin sections of a skin biopsy using a mouse anti-mouse monoclonal antibody (1:500, provided by Dr. J.J. Lee) as previously described (Klion et al. Blood 2013). Slides were deparaffinized and heat-induced epitope retrieval was accomplished with Leica retrieval solution (Citric buffer) for 25 min. Immunostaining for EMR1 was performed on an adjacent frozen sample of the same biopsy using mouse 1E7 antibody at 10 μg/ml. Slides were prepared by Histoserv Inc. (Germantown, MD). Anti-EPX and anti-EMR1 antibodies were detected using the BondMax biotin-avidin free polymer-based detection system with Diaminobenzine as the chromogen. Samples were analyzed on a Leica BondMax autostainer (Leica Microsystems, Bannockburn, IL).

Immunofluorescence staining for EMR1, EPX and CD68 was performed on acetone-fixed nasal polyp frozen sections. Slides were washed twice with PBST (PBS with 0.1% Tween 20) prior to incubation with 700 μl of Blocking Solution (2% BSA, 1.5% goat serum (Abcam)), 1.5% donkey serum (Abcam) in PBS) at room temperature for 1 hour. After three washes with PBS, slides were incubated overnight at 4^{o}C with mouse 1E7-Alexa595

(10 μg/ml), or mouse anti-EPX antibody (Abcam; diluted 1:200 with 2% BSA with PBS), and/or mouse anti-CD68 (Ventana; clone KP-1, diluted 1:2). The slides treated with anti-EMR1, anti-EPX- and anti-CD68 were then washed and incubated with goat anti-mouse IgG-DyLight488 (Jackson) at 3 μg/mL in 2% BSA with PBS at room temperature for 1 hour. After three washes with PBST, 2-3 drops of mounting medium (VECTASHIELD Mounting Medium) were added prior to coverslipping. Images were taken on a Nikon fluorescent microscope.

c1E7 ELISA. Plasma samples were analyzed for c1E7 using an antigen-based ELISA. Briefly, a microplate was coated with 100 ng/well of EMR1-Fc (Kalobios) in coating buffer (20 mM Tris; 150 mM NaCl, pH 8.0). After incubation for 1h at 37^{o}C with shaking, the plate was washed three times with wash buffer (PBS with 0.01% Tween 20; PBST) and blocked for 20 minutes at 37^{o}C with Superblock (Pierce). Diluted cynomolgus monkey plasma samples (1/300 and/or 1/600 for group 1 and 1/1500 and/or 1/3000 for group 2) and recombinant standards were incubated for 1h at room temperature. The plate was washed three times with wash buffer prior to addition of peroxidase-conjugated AffiniPure goat anti-human IgG Fab-specific antibody (JacksonImmunoResearch; 10 ng/ml in PBST with 1% BSA). After a final wash step, TMB substrate solution was added (Invitrogen) and the reaction was allowed to proceed for 7 minutes before the addition of stop solution (1N H_2SO_4). Absorbance was read at 450 nm using a Thermomax Molecular Device microplate reader. Data was plotted and fitted using a 4-paramter logistic regression using SoftMax Pro 4.0 software (Molecular Devices). The concentration in plasma samples was determined based on the values from the standard curve.

Measurement of eosinophil granule proteins. Levels of eosinophil granule proteins (major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil derived neurotoxin (EDN) and eosinophil peroxidase (EPO)) were measured in supernatants and primate plasma using a customized suspension array assay in multiplex (Makiya et al., J. Immunological Methods, submitted). Coupling of antibodies to the microspheres and assay conditions were performed according to the manufacturer's instructions (Luminex Corporation, Austin, TX). Briefly, supernatants and plasma were reduced and alkylated to remove MBP from complexes[3] before incubation at a final dilution of 1:8.8 (supernatants) or 1:44 (plasma) with magnetic microspheres coupled to mouse anti-MBP, ECP, EDN and EPO monoclonal antibodies[4]. The microspheres were subsequently incubated with rabbit anti-MBP, ECP, EDN and EPO[4], biotinylated goat anti-rabbit IgG and PE-streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA) and analyzed using a Bio-Plex 200 (Bio-Rad Laboratories, Hercules, CA) instrument. All assays were performed in duplicate and concentrations were calculated based on a standard curve using SoftMax Pro Software (Molecular Devices, Sunnyvale, CA).

Legend of Supplementary Figures

Figure E1: *In vitro* differentiation of CD34+ cells. **A.** Representative dot plots of EMR1/Siglec-8 and EMR1/IL-5Rα surface expression at Day 4, **B.** Overlay of EMR1 staining at Day 4 and Day 24, **C.** Representative dot plots of Siglec-8 and IL-5Rα surface expression on EMR1+ cells at Day 24.

Figure E2. *In vitro* modulation of EMR1 in response to IL-5 stimulation. **A, B**. Surface expression of EMR1 and CD69 on purified eosinophils after 2hr stimulation (n=14, 6 ND and 8 HES), **C**. EMR1 mRNA up-regulation by IL-5 after 6hr stimulation (n=13, 4 ND and 9 HES). CM=culture medium. * $P < .05$, ** $P < .01$ and *** $P < .001$.

Figure E3. Cytofluorimetric gating for the determination of eosinophil cell death induced by NK cells in the presence of afucosylated anti-EMR1 antibody (c1E7). Results shown are from one representative experiment. NK killing assays were performed with purified eosinophils at an E:T ratio of 5:1. The cells were incubated together for 4 hours and washed prior to the addition of Annexin-V-FITC. After exclusion of cell debris and doublets, the percentage of Annexin V+ cells were determined in the eosinophil (Eo) and effector NK (NK) cell gates.

Figure E4. Levels of eosinophil granule proteins (ECP, MBP, EPO and EDN) in plasma samples from the 4 monkeys at all time points.

Supplemental References.

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Table E1. Pharmacokinetic parameters following administration of chimeric afucosylated anti-EMR1 antibody (c1E7) to non-human primates.

	Dose 1mg/kg		**Dose 5 mg/kg**	
	CYNO 129-225c	**CYNO 444-93**	**CYNO 10-65c**	**CYNO 10-66c**
C_{max} (μg/mL)	33.3	48.8	130.5	162.8
AUC_{∞} (μg*hr/mL)	3226	2885	22132	27545
CL (mL/kg/hr)	310	347	226	182
V_{ss} (mL/kg)	42.7	37.0	68.7	58.4
$T_{1/2}$ (hr)	92.2	77.5	205	215

C_{max}, maximum concentration; T_{max}, time of peak concentration; AUC_{∞}, Area under the curve from time 0 extrapolated to infinite time; CL, systemic clearance; Vss, volume of distribution at steady state; $T_{1/2}$, elimination half life.

Table E2. Eosinophil counts measured at each time point in the 4 monkeys before and after receiving a single dose of afucosylated anti-EMR1 antibody

	Cynomologus	Timepoint	Eosinophils % (*)	White blood count (10^9/L)	Eosinophils Abs (/mm^3)
Group 1 1MPK	CYN 444-93	Predose	5.45	7.93	431.9
		5min	NA	NA	NA
		2HR	NA	NA	NA
		4HR	NA	NA	NA
		8HR	0.56	NA	NA
		Day 2	0.27	NA	NA
		Day 4	0.30	NA	NA
		Day 8	0.42	NA	NA
		Day 15	1.00	NA	NA
		Day 22	3.98	NA	NA
		Day 28	9.60	NA	NA
		Day 42	11.23	NA	NA
		Day 56	NA	NA	NA
	CYN 129-225c	Predose	7.18	13.80	990.3
		5min	NA	NA	NA
		2HR	NA	NA	NA
		4HR	NA	NA	NA
		8HR	0.00	NA	NA
		Day 2	0.00	NA	NA
		Day 4	0.00	NA	NA
		Day 8	0.42	NA	NA
		Day 15	0.55	NA	NA
		Day 22	2.95	NA	NA
		Day 28	8.96	NA	NA
		Day 42	9.43	NA	NA
		Day 56	NA	NA	NA
Group 2 5MPK	CYN 10-65c	Predose	3.32	12.66	420.1
		Predose	5.45	7.90	430.6
		5min	3.71	NA	NA
		2HR	0.29	NA	NA
		4HR	0.54	NA	NA
		8HR	0.00	NA	NA
		Day 2	0.00	NA	NA
		Day 4	0.32	NA	NA
		Day 8	0.00	13.11	0.0
		Day 15	0.92	NA	NA
		Day 22	0.60	NA	NA
		Day 28	0.74	12.07	88.8
		Day 42	7.55	11.81	891.3
		Day 56	4.72	13.16	621.3
	CYN 10-66c	Predose	6.21	10.82	672.4
		Predose	3.95	8.45	333.8
		5min	4.10	NA	NA

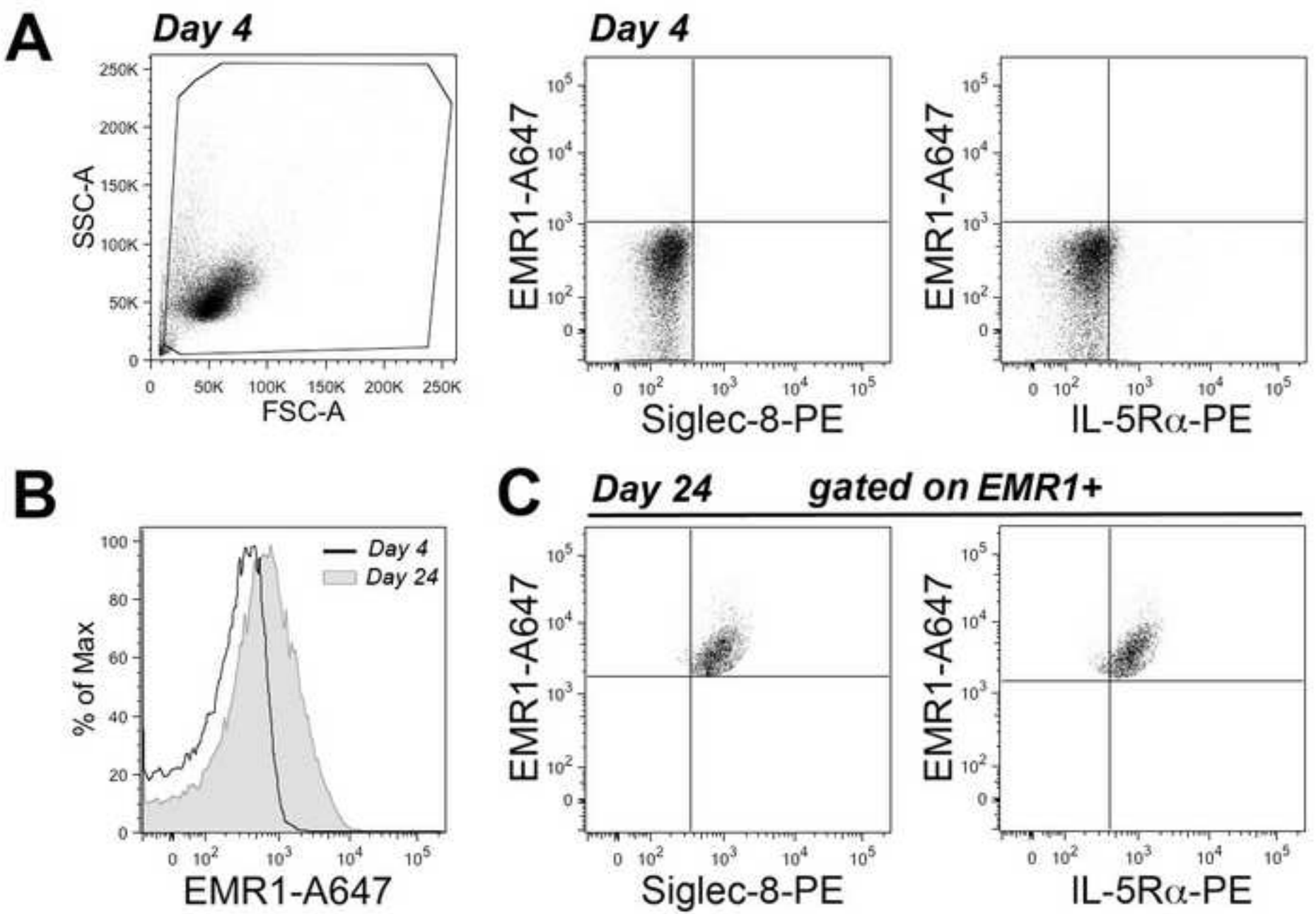
		2HR	0.00	NA	NA
		4HR	0.00	NA	NA
		8HR	0.31	NA	NA
		Day 2	0.00	NA	NA
		Day 4	0.00	NA	NA
		Day 8	0.48	7.07	33.7
		Day 15	0.96	NA	NA
		Day 22	1.26	NA	NA
		Day 28	0.47	7.17	33.8
		Day 42	0.88	9.65	84.6
		Day 56	2.53	9.09	229.9

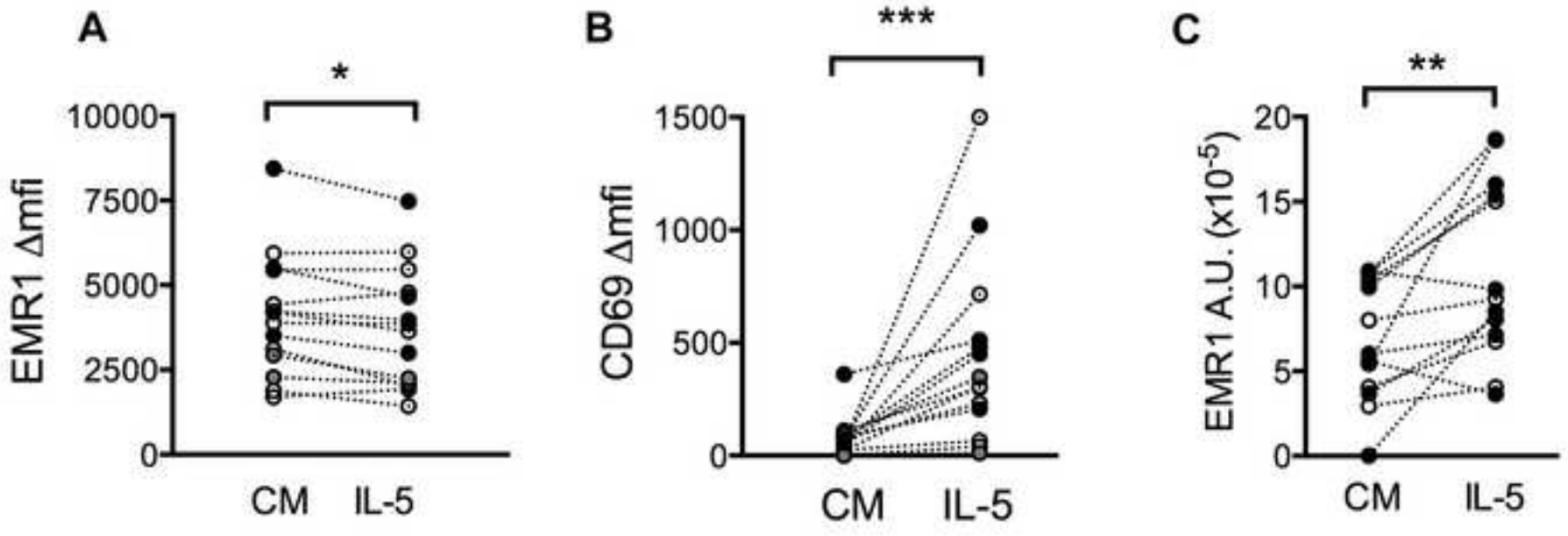
NA: Not available
(*): % based on cytospin counting on 200-500 cells per slides

Table E3. Laboratory parameters before and after administration of a single 5 mg/kg intravenous dose of chimeric afucosylated anti-EMR1 antibody to two cynomolgus monkeys.

Laboratory Parameter	Normal value	Unit	PREDOSE		Day 8		Day 28		Day 29		Day 42		Day 56	
			CYNO 10-65c	CYNO 10-66c	CYNO 10-65c	CYNO 10-66c	CYNO 10-65c	CYNO 10-66c	CYNO 10-65c	CYNO 10-66c	CYNO 10-65c	CYNO 10-66c	CYNO 10-65c	CYNO 10-66c
Hematology														
White blood cell count	**5.7-21**	**10^9/ L**	7.9	10.82	13.11	7.07	12.07	7.17	nd	nd	11.81	9.65	13.16	9.09
Absolute lymphocyte count	**4.3-10**	**10^9/ L**	4.65	5.69	6.5	5.05	6.79	5.24	nd	nd	5.59	4.38	6.79	5.49
Absolute monocyte count	**0-1**	**10^9/ L**	0.05	0.47	0.4	0.13	0.52	0.04	nd	nd	0.39	0.24	0.33	0.3
Absolute neutrophil count	**1.9-6.24**	**10^9/ L**	3.21	4.66	6.21	1.9	4.76	1.9	nd	nd	5.84	5.04	6.04	3.3
Red blood cell count	**4.8-6.3**	**10^{12} / L**	5.79	6.26	5.74	4.93	5.92	5.71	nd	nd	5.64	5.21	5.73	5.74
Platelets	**130-934**	**10^9/ L**	334	438	507	378	525	394	nd	nd	461	376	422	336
Hemoglobin	**8-15**	**g/dl**	13.2	12.6	11.8	12.6	12.6	12.5	nd	nd	12	12.3	12	13
Clinical Chemistry														
Total Protein	**5.9 - 7.6**	**g/dl**	7.1	7.2	7.2	7	7.4	7	7	7	7.3	7.2	6.6	6.9
Albumin	**2.8 - 4.4**	**g/dl**	3.5	3.9	3.5	3.9	3.8	3.8	3.8	4	4.1	4.4	3.4	3.8
Globulin	**2 - 3.9**	**g/dl**	3.6	3.3	3.7	3.1	3.5	3.2	3.2	3	3.2	2.8	3.2	3.1
Alkaline phosphatase	**73- 210**	**U/L**	353	298	347	345	292	273	305	258	361	286	411	386
Alanine Aminotransferase	**20 - 120**	**U/L**	40	45	27	35	vnr	vnr	30	55	28	24	57	41
Amylase	**149 - 500**	**U/L**	156	232	146	231	171	255	178	273	184	284	149	273
Aspartate Aminotransferase	**23 - 94**	**U/L**	17	36	8	18	vnr	vnr	48	22	36	21	24	11
Urea	**7 -25**	**mg/dl**	16	13	16	14	19	15	21	18	16	12	19	14
Calcium	**8.3 - 10.1**	**mg/dl**	9.1	10.2	9.6	9.6	9.9	10.4	10.2	10.5	10.2	10.1	9.7	10.2
Cholesterol	**73 - 179**	**mg/dl**	173	175	182	199	186	180	185	186	165	185	162	178
Creatinine	**0.4 - 1.2**	**mg/dl**	0.8	0.7	0.7	0.7	0.8	0.6	0.8	0.6	0.7	0.7	0.7	0.6
Glucose	**50 - 100**	**mg/dl**	83	87	74	98	86	79	78	71	99	103	73	82
Inorganic Phosphate	**2.4 - 6.5**	**mg/dl**	5.1	4.1	5.5	6.6	4.3	3.8	5	3.6	4.1	2.5	4.7	4.4
Total Bilirubin	**0.1 - 0.6**	**mg/dl**	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Values above or below the normal range are indicated by gray shading. vnr = value not returned due to sample or machine error, nd= not done





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