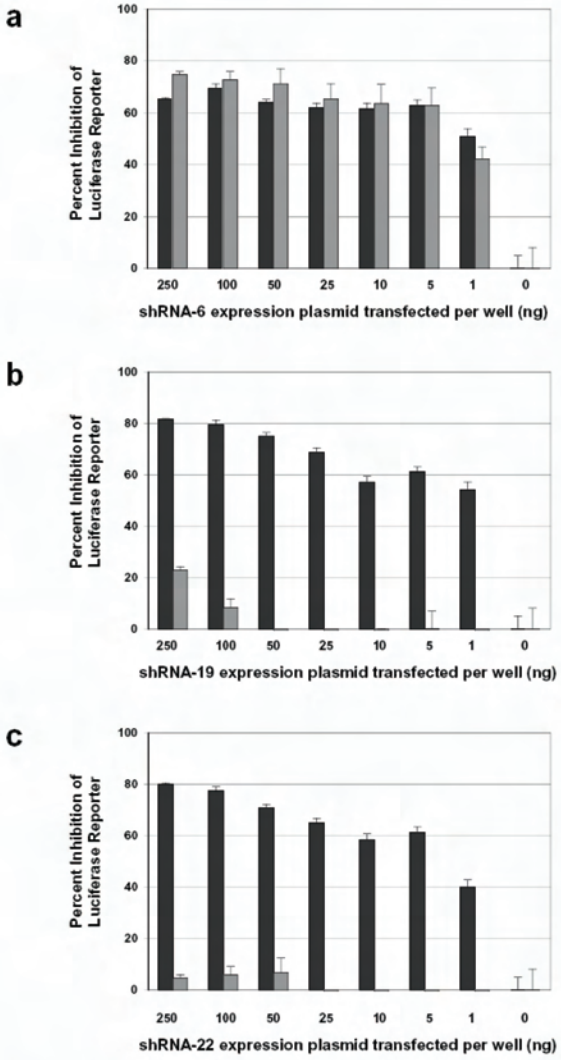
Figure S1 a-c



Supplementary Figure S1: ***In vitro*** **anti-HCV inhibitory activity of shRNAs against a surrogate model system.** Short stretches of HCV target genomic sequences, corresponding to either the (+) or (-) strand of RNA from the HCV genome were fused downstream of the sequences coding for the firefly luciferase protein but upstream of the poly A tail. These individual reporter constructs with target sequences against either shRNA-6 **(a)**, shRNA-19 **(b),** or shRNA-22 **(c)** were co-transfected into Huh-7 cells along with a plasmid expressing all three shRNA. A Renilla expression plasmid was also included in each transfection mixture to normalize for differences in transfection efficiency. A non-specific plasmid DNA was used to normalize the amount of nucleic acids used for each transfected condition. The percent inhibition was calculated by comparison of luciferase activity in cells transfected with an identical reporter and a U6-1 plasmid lacking an expressed shRNA sequence. Each analysis represents data collected from four replicate transfections ± standard deviation. Target sequences from the (+) strand are shown in black while target sequences from (-) strand are shown in grey.

Figure S2 a-c

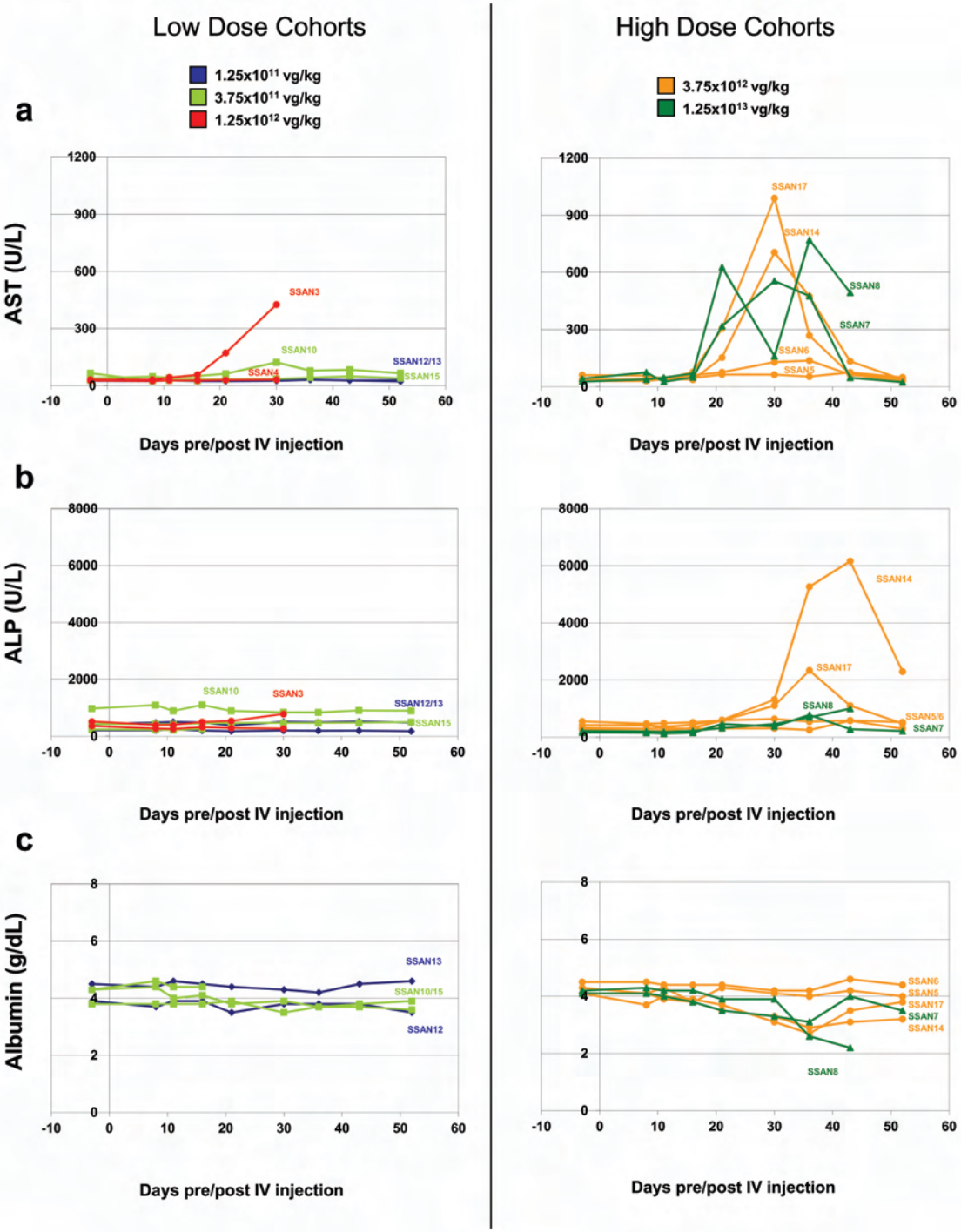
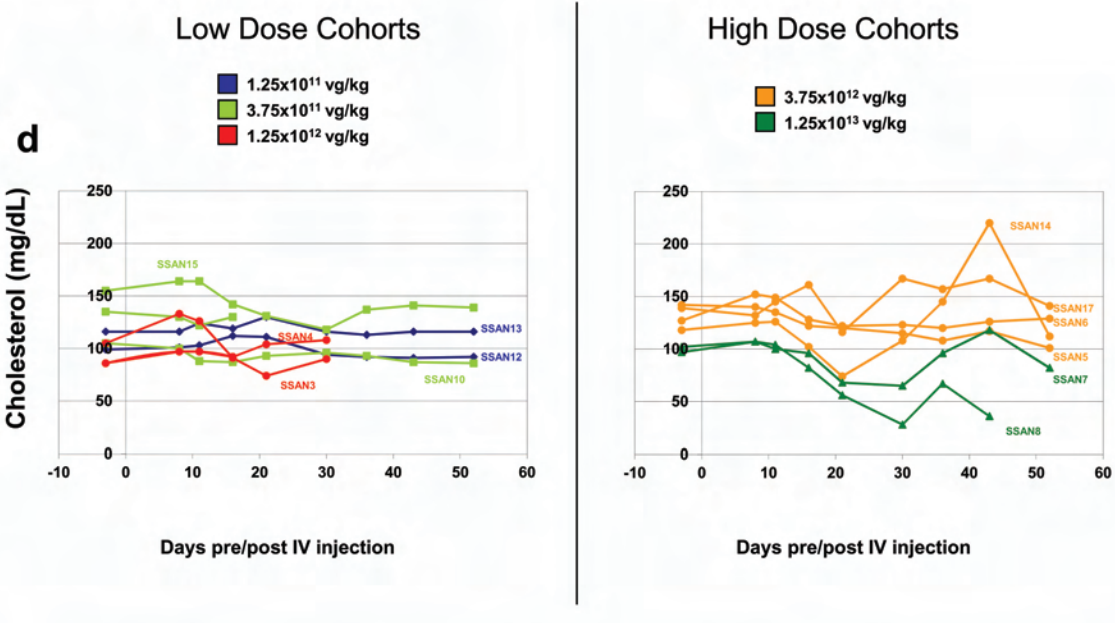

Low Dose Cohorts
High Dose Cohorts
a
1.25x10^11 vg/kg
3.75x10^11 vg/kg
1.25x10^12 vg/kg
3.75x10^12 vg/kg
1.25x10^13 vg/kg
AST (U/L)
SSAN3
SSAN10
SSAN12/13
SSAN4
SSAN15
SSAN17
SSAN14
SSAN8
SSAN7
SSAN6
SSAN5
Days pre/post IV injection
b
ALP (U/L)
SSAN10
SSAN3
SSAN12/13
SSAN15
SSAN14
SSAN17
SSAN8
SSAN5/6
SSAN7
Days pre/post IV injection
c
Albumin (g/dL)
SSAN13
SSAN10/15
SSAN12
SSAN6
SSAN5
SSAN17
SSAN7
SSAN14
SSAN8
Days pre/post IV injection

Figure S2 d



Supplementary Figure S2: AST, ALP, albumin, and cholesterol activity in Cynomolgus monkeys dosed with TT-033. Serum was collected at various time points following intravenous administration of a single dose of TT-033 into either the cephalic or saphenous vein at low dose levels ranging from $1.25x10^{11}$ vg/kg (blue diamonds, n=2), $3.75x10^{11}$ vg/kg (light green squares) or $1.25x10^{12}$ vg/kg (red circles) and high doses of $3.75x10^{12}$ vg/kg (orange circles) or $1.25x10^{13}$ vg/kg (green triangles). Values representing the measurements in serum taken from individual animals are plotted at each data point. Blood chemistry analysis includes: **(a)** aspartate aminotransferase (AST), **(b)** alkaline phosphatase (ALP), **(c)** albumin or **(d)** cholesterol. Albumin was not measured in animals dosed with $1.25x10^{12}$ vg/kg.

Figure S3 a-c

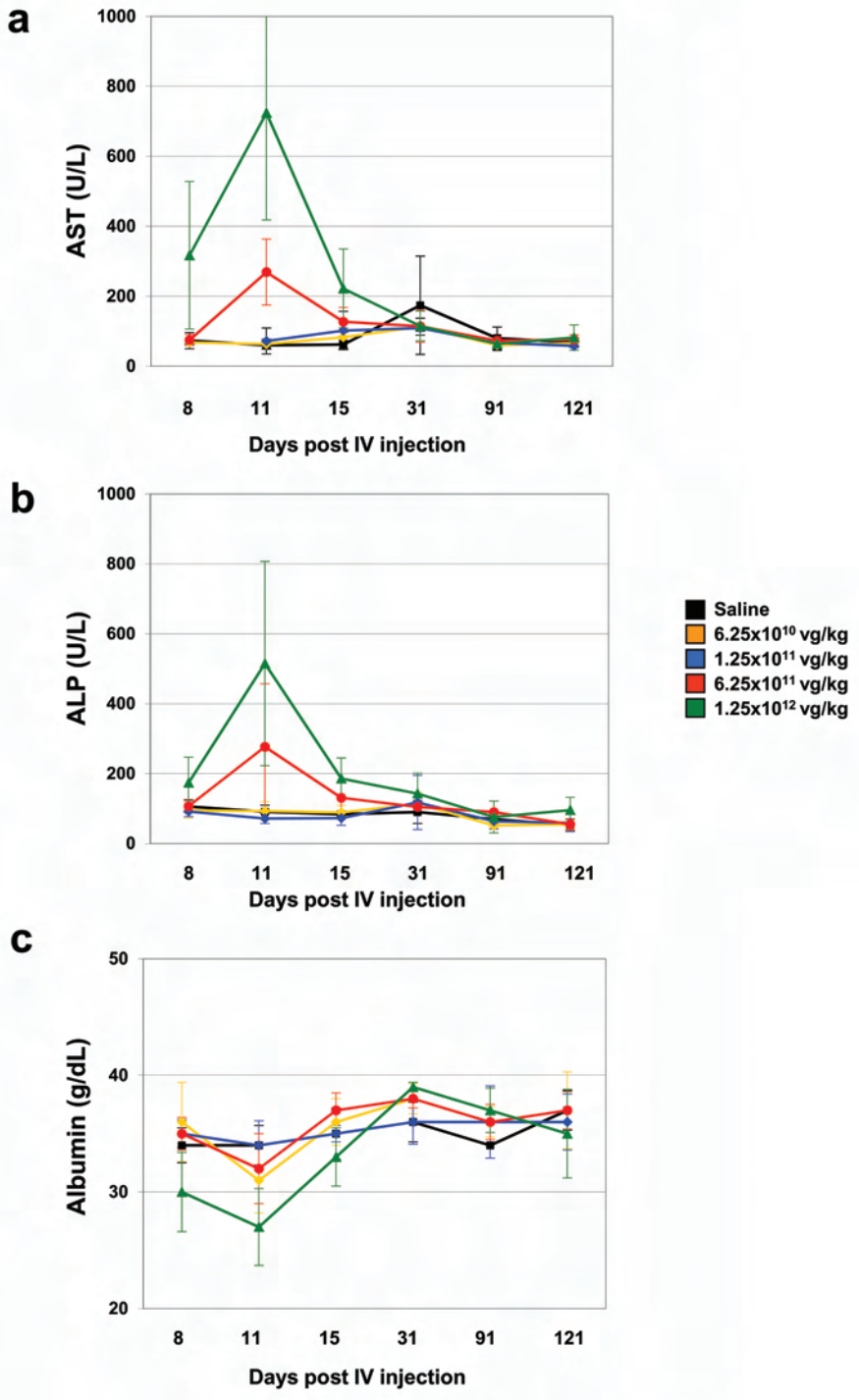
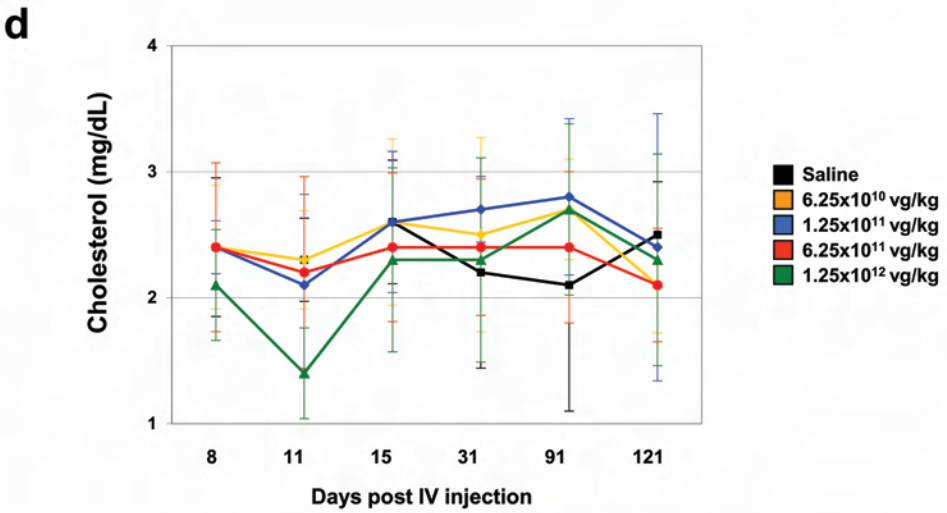

a
AST (U/L)
1000
800
600
400
200
0
8
11
15
31
91
121
Days post IV injection
b
ALP (U/L)
1000
800
600
400
200
0
8
11
15
31
91
121
Days post IV injection
Saline
6.25x10^10 vg/kg
1.25x10^11 vg/kg
6.25x10^11 vg/kg
1.25x10^12 vg/kg
c
Albumin (g/dL)
50
40
30
20
8
11
15
31
91
121
Days post IV injection

Figure S3 d



Supplementary Figure S3: AST, ALP, albumin, and cholesterol activity in mice dosed with TT-033. TT-033 was injected intravenously into the tail vein at dose levels of $6.25x10^{10}$ vg/kg (orange diamonds), $1.25x10^{11}$ vg/kg (blue diamonds), $6.25x10^{11}$ vg/kg (red circles) and $1.25x10^{12}$ vg/kg (green triangles). Mice dosed with saline (black squares) served as the negative control. Murine serum was collected at terminal necropsy at each time point. The plotted data average and standard deviation at each point are based from n=5 mice per dosing cohort. Blood chemistry analysis includes: **(a)** aspartate aminotransferase (AST), **(b)** alkaline phosphatase (ALP), **(c)** albumin or **(d)** cholesterol.

Figure S4

SSAN	2	12	13	10	9	15	3	4	5	6	14	17	8	7
Dose (vg/kg)	saline	$1.25x10^{11}$	$1.25x10^{11}$	$3.75x10^{11}$	$3.75x10^{11}$	$3.75x10^{11}$	$1.25x10^{12}$	$1.25x10^{12}$	$3.75x10^{12}$	$3.75x10^{12}$	$3.75x10^{12}$	$3.75x10^{12}$	$1.25x10^{13}$	$1.25x10^{13}$
Necropsy Day	30	51	51	50	17*	51	30	30	52	52	51	52	42*	52
Apoptosis; hepatocytes	0	1	0	1	0	1	3	1	1	0	2	2	4	0
Deposition; pigment, brown	0	0	0	0	0	0	0	1	0	1	2	2	1	1
Hematopoieseis; extramedullary	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Hyperplasia; bile duct	0	0	0	1	0	1	2	0	2	1	3	2	2	2
Hyperplasia; Kupffer cells	0	0	0	3	0	1	2	0	2	1	2	2	2	1
Inflammation; mononuclear cells	0	0	0	3	0	2	2	0	2	0	2	2	4	0
Mitotic Figures; hepatocytes	0	0	0	1	0	0	2	1	1	0	0	1	3	0
Necrosis/Fibrosis; bridging	0	0	0	0	0	0	0	0	0	0	2	0	4	0
Regeneration; hepatocellular	0	0	0	0	0	0	0	0	0	0	1	0	4	0
Total Liver Lesion Score (TLLS)	**0**	**1**	**0**	**9**	**0**	**5**	**11**	**3**	**9**	**3**	**14**	**11**	**24**	**5**
TLLS Category	0	1	0	3	0	2	4	2	3	2	4	4	4	2

Supplementary Figure S4: Histopathology of liver tissues from cynomolgus monkeys treated with TT-033. Liver tissues were collected and preserved in 10% neutral buffered formalin. Following adequate fixation, the tissues were trimmed and routinely processed to paraffin block, sectioned, and the slides were stained with hematoxylin and eosin (H&E) and examined microscopically. Apoptosis in hepatocytes involved single cells or occasionally clusters of cells that appeared as round to oval masses with intensely eosinophilic cytoplasm. The nuclear chromatin was condensed, fragmented and often no longer visible. Bile duct hyperplasia was characterized by increase in the size and number of bile ducts and bile ductules in the portal triads and surrounding hepatocytes, often accompanied by periductular fibrosis. Mononuclear cell infiltration was often present. Deposition of brown to brownish black pigment was present primarily in the cytoplasm of Kupffer cells. This pigment was not usually birefringent. Extramedullary hematopoiesis was characterized by the presence of small aggregates of hematopoietic cells, usually erythroid precursors, in hepatic sinusoids. Kupffer cell hyperplasia was characterized multifocal or diffuse increase in size and number of these cells within the sinusoids. Kupffer cells were larger than other sinusoidal cells and were sometimes present in small oval clusters. Mononuclear cell inflammation was characterized by multifocal or diffuse infiltration of mononuclear cells in portal triads and sinusoids, often accompanied by hepatocellular degeneration. Mitotic figures were occasionally observed in hepatocytes throughout the tissue, especially in areas adjacent to hepatocellular apoptosis. Bridging necrosis/fibrosis was characterized by confluence of areas of necrosis, fibrosis, congestion/hemorrhage, and/or hepatocellular loss extending from one hepatic lobule to another in a centrolobular distribution. Hepatocellular regeneration was characterized by an increase in size and/or number of hepatocytes. Affected hepatocytes had enlarged nuclei, multiple nuclei and/or increased amphophilia or basophilia of the cytoplasm. Mitotic figures were often present, and in severe examples the regenerating hepatocytes often formed irregular clusters instead of hepatic plates. Hepatocellular regeneration was present in areas adjacent to bridging necrosis/fibrosis.

The severity score of individual findings is as follows : 0 = Not Remarkable, 1 = Minimal, 2 = Mild, 3 = Moderate, 4 = Marked. A total liver lesion score (TLLS) was calculated for each animal by adding the severity scores of each lesion considered test article-related. TLLS of 0 were considered within normal limits, 1 – 2 were considered minimal, 3 – 5 were considered mild, 6-9 were considered moderate, and >9 were considered marked. The TLLS category was graded according to the same scale as the individual findings.

Figure S5 a-c

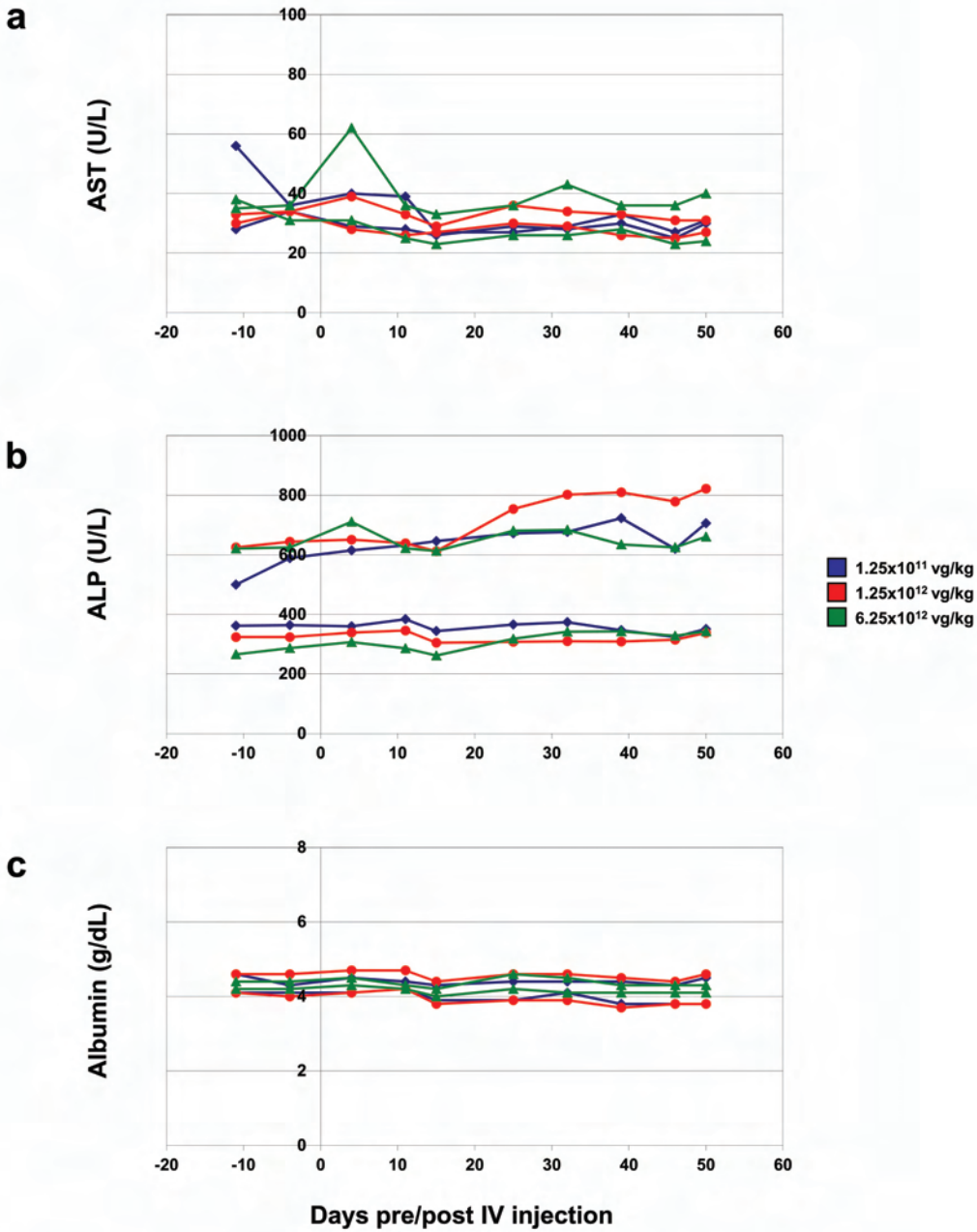
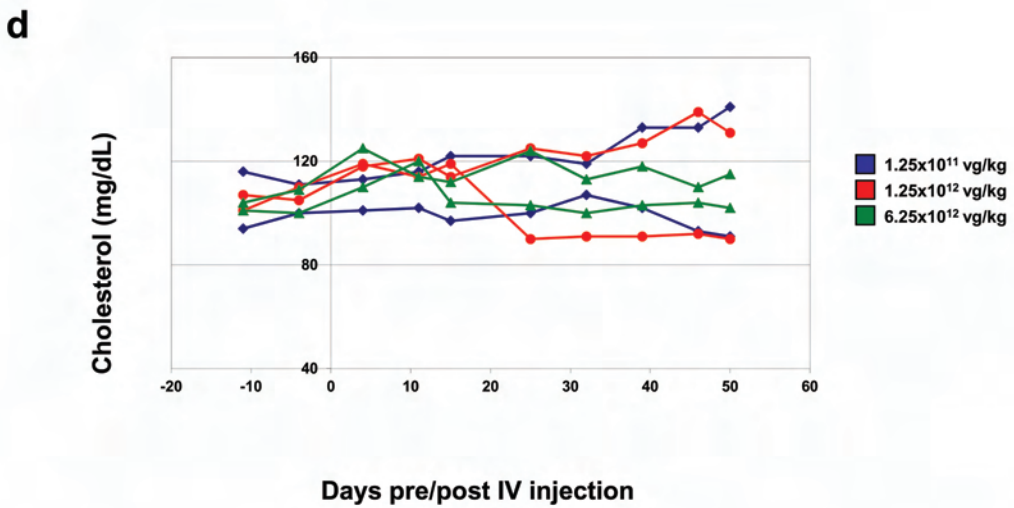
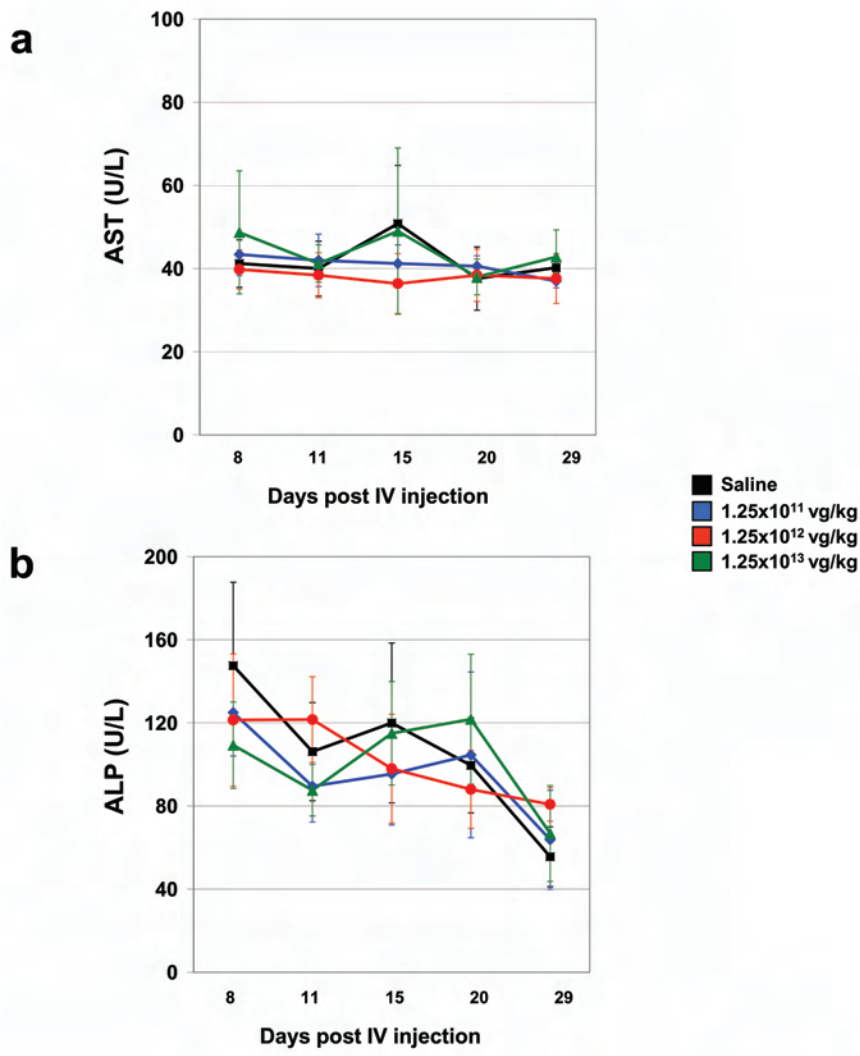

a
AST (U/L)
100
80
60
40
20
0
-20
-10
0
10
20
30
40
50
60
b
ALP (U/L)
1000
800
600
400
200
0
1.25x10^11 vg/kg
1.25x10^12 vg/kg
6.25x10^12 vg/kg
c
Albumin (g/dL)
8
6
4
2
0
Days pre/post IV injection

Figure S5 d



Supplementary Figure S5: AST, ALP, albumin, and cholesterol activity in Cynomolgus monkeys dosed with TT-034. Serum was collected at various time points following intravenous administration of a single dose of TT-034 into either the cephalic or saphenous vein at doses ranging from $1.25x10^{11}$ vg/kg (blue diamonds), $1.25x10^{12}$ vg/kg (red circles) or $6.25x10^{12}$ vg/kg (green triangles). Values representing the measurements in serum taken from individual animals are plotted at each data point. Blood chemistry analysis includes: **(a)** aspartate aminotransferase (AST), **(b)** alkaline phosphatase (ALP), **(c)** albumin or **(d)** cholesterol.

Figure S6 a,b



Supplementary Figure S6: AST and ALP in mice dosed with TT-034. TT-034 was injected intravenously into the tail vein at dose levels of $1.25x10^{11}$ vg/kg (blue diamonds), $1.25x10^{12}$ vg/kg (red circles) and $1.25x10^{12}$ vg/kg (green triangles). Mice dosed with saline (black squares) served as the negative control. Murine serum was collected at terminal necropsy at each time point. The plotted data average and standard deviation at each point are based from n=5 mice per dosing cohort. Blood chemistry analysis includes either **(a)** aspartate aminotransferase (AST) or **(b)** alkaline phosphatase (ALP).