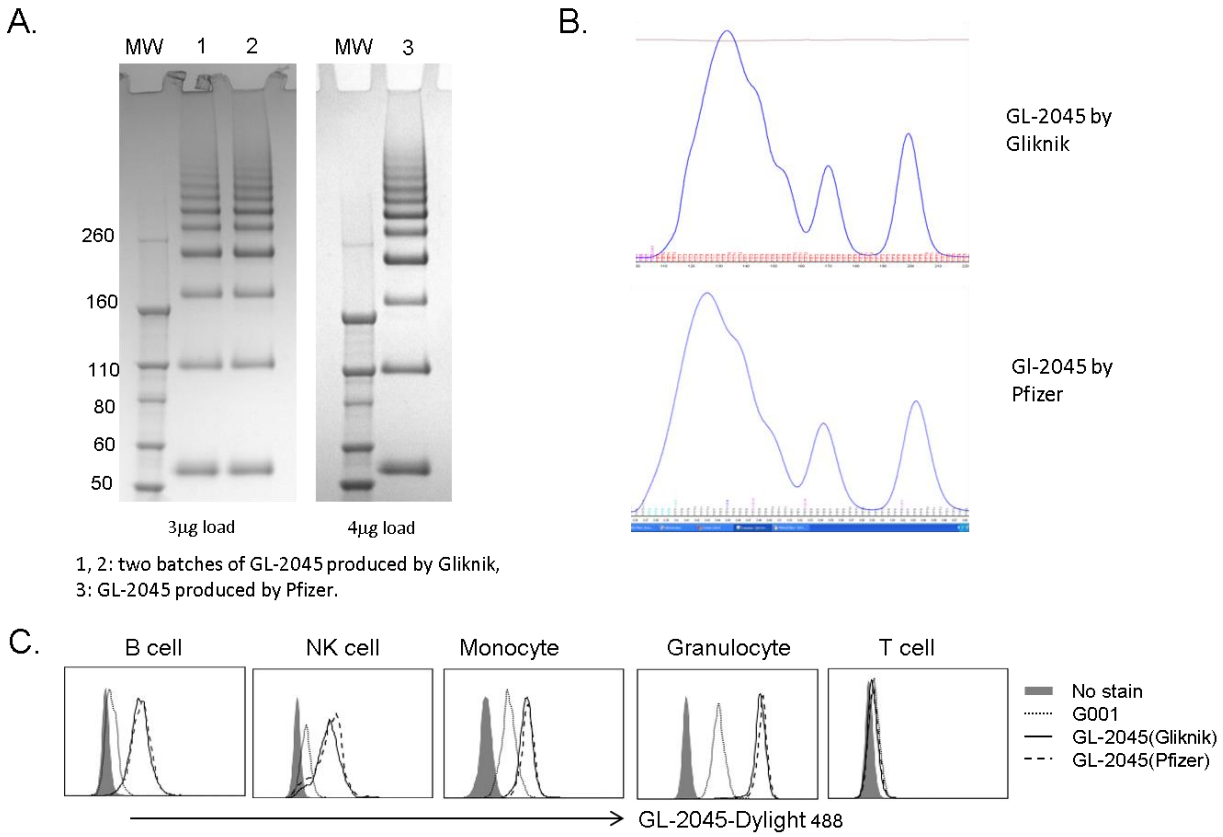
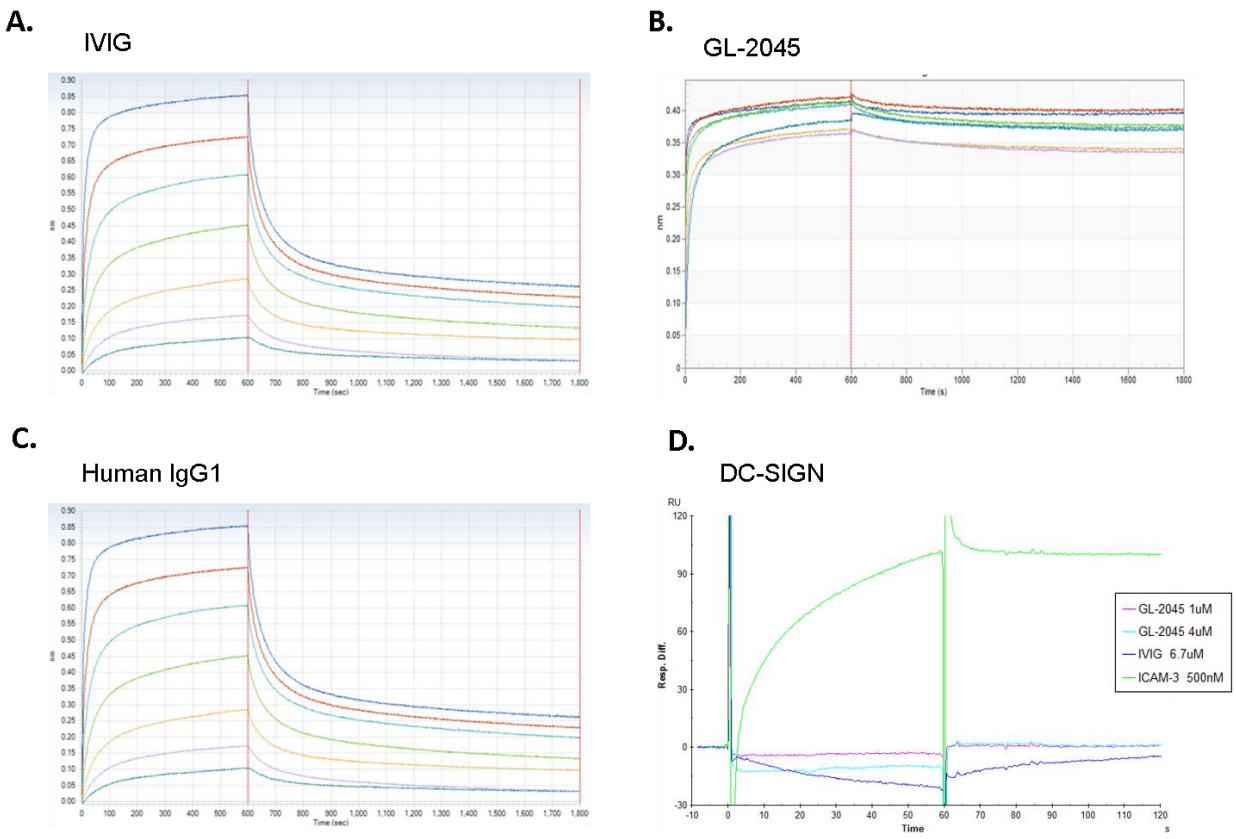
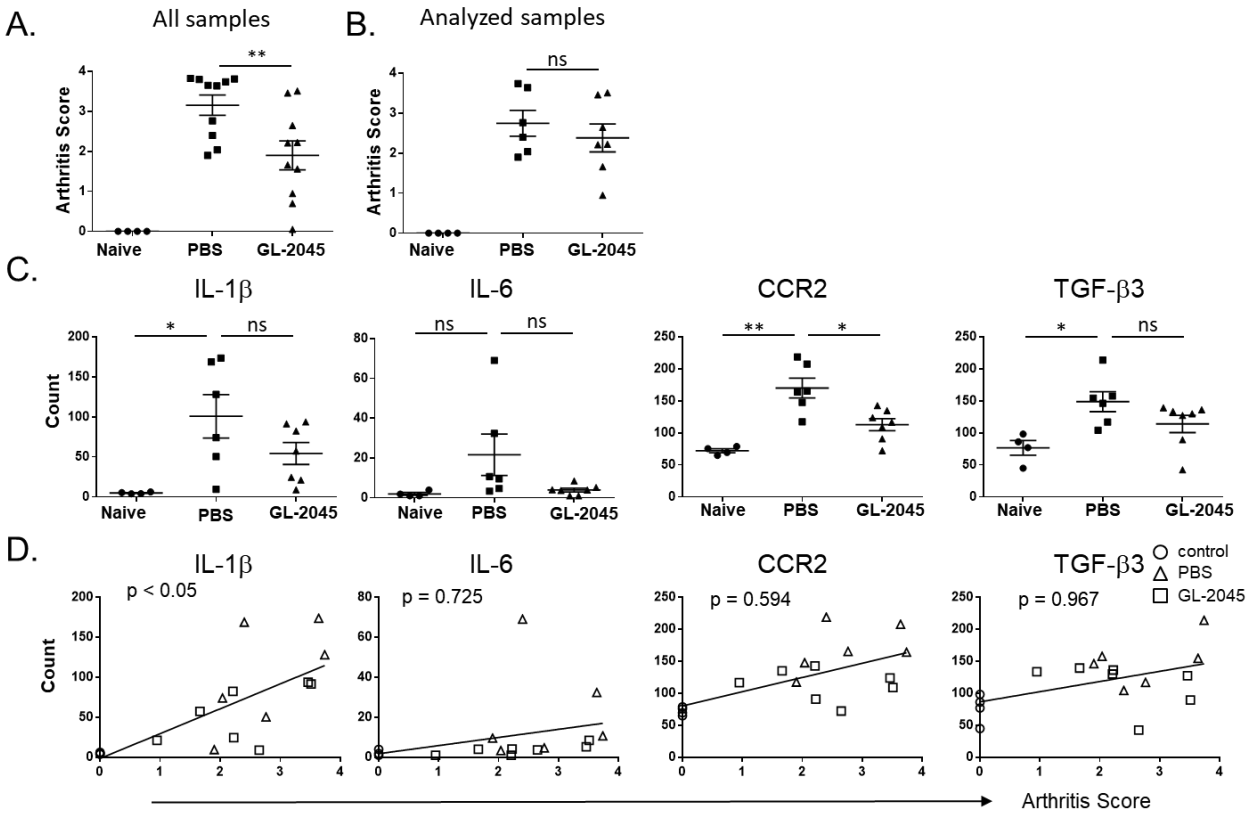


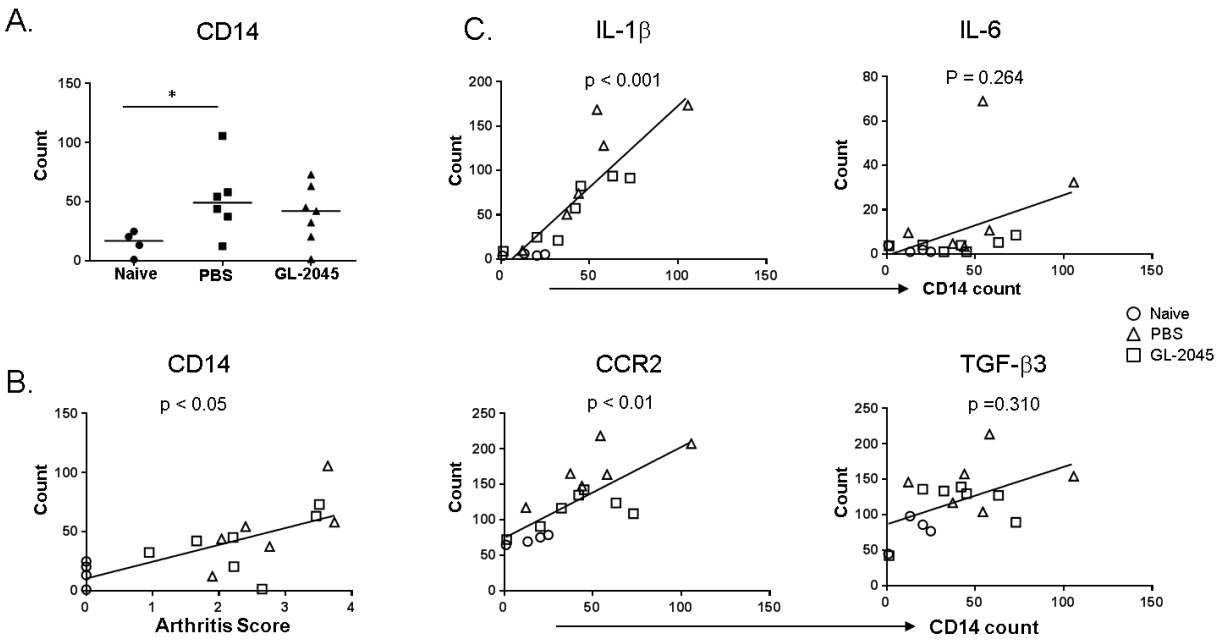
Supplemental Figure 1: Comparison of different preparations of GL-2045. Different batches of GL-2045 prepared by Gliknik or Pfizer were analyzed for the levels of multimerization by non-denatured SDS-PAGE (A) and gel filtration (B). (C) Two different GL-2045 preparations from Gliknik and Pfizer were labeled with Dylight 488. The binding of GL-2045 to immune cell populations from human peripheral blood were analyzed by FACS.



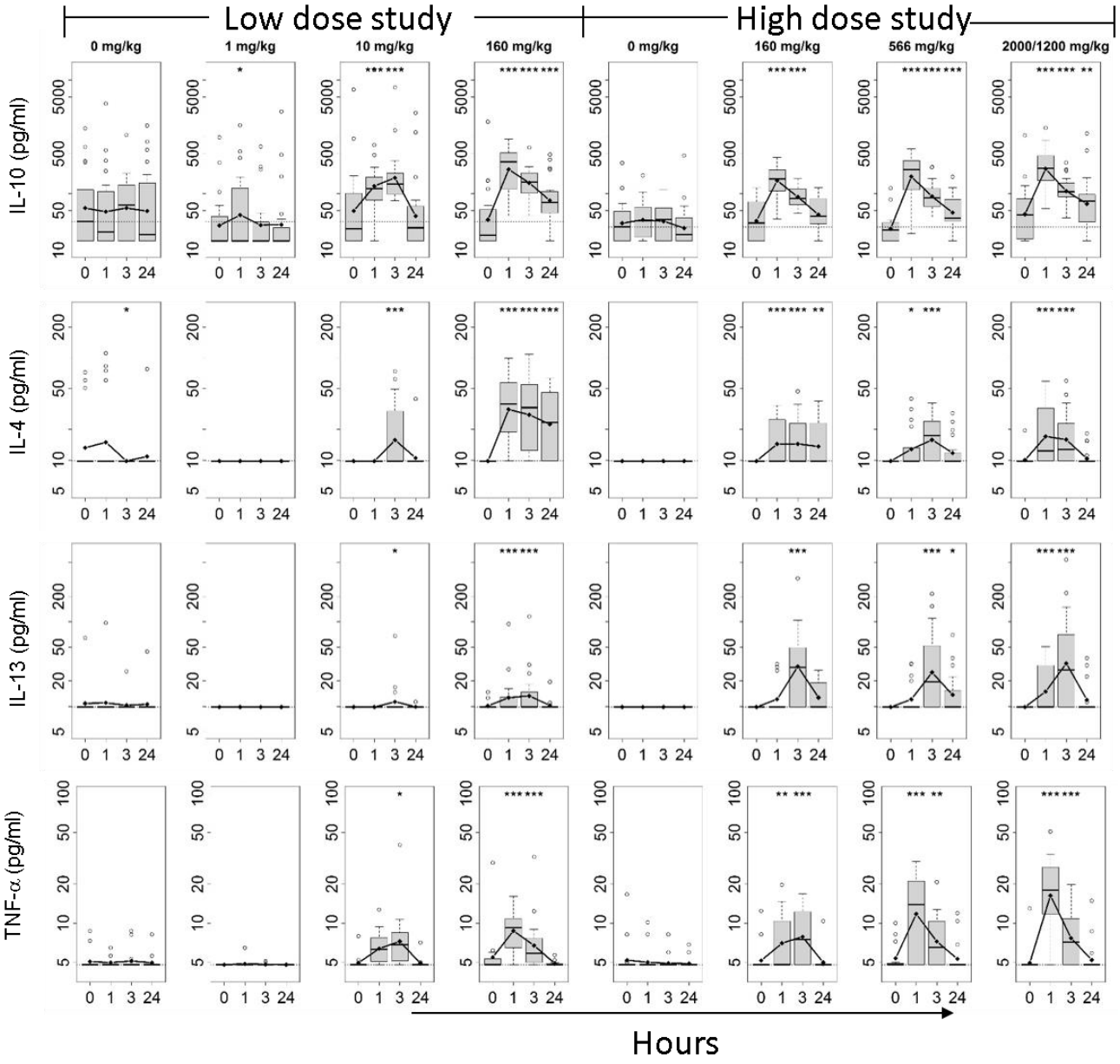
Supplemental Figure 2: GL-2045 binds FcγRIIIa with high avidity, but does not bind DC-SIGN. Representative BLI sensograms of binding of (A) IVIG, (B) GL-2045 and (C) Human IgG1 to FcγRIIIa. (D) GL-2045 and IVIG do not bind DC-SIGN using similar binding conditions and analyte concentrations.



Supplemental Figure 3. mRNA expression analysis of GL-2045-treated joints reveals reduced levels of cytokine and chemokine receptors. (A) All samples: there were 4, 10, and 10 animals for naive, diseased-vehicle treated (PBS), and diseased-GL-2045 treated (GL-2045) groups, respectively. GL-2045 treatment significantly reduced the arthritis score ($p=0.007$, two tailed Mann-Whitney test) compared to the vehicle treated group. (B) Samples analyzed with NanoString: after NanoString quality control checks, a total of 4 samples from the naive group, 6 from PBS- treated group and 7 from GL-2045 treated group were suitable for analysis. With the reduced sample size, statistically significant differences in arthritis score between the vehicle-treated, and GL-2045-treated groups were not evident. (C) mRNA expression of IL-1β, IL-6, CCR2, and TGF-β3 in the joints of naïve, diseased vehicle-treated and diseased GL-2045-treated mice were analyzed by NanoString. Absolute gene expression counts are shown. *$p<0.05$, **$p<0.01$, ns denotes not significant. One-way ANOVA with Tukey correction. (D) Association of mRNA expression of IL-1β, IL-6, CCR2, and TGF-β3 with arthritis scores in individual animals. Linear models adjusted for group were used to test the significance of the linear association.

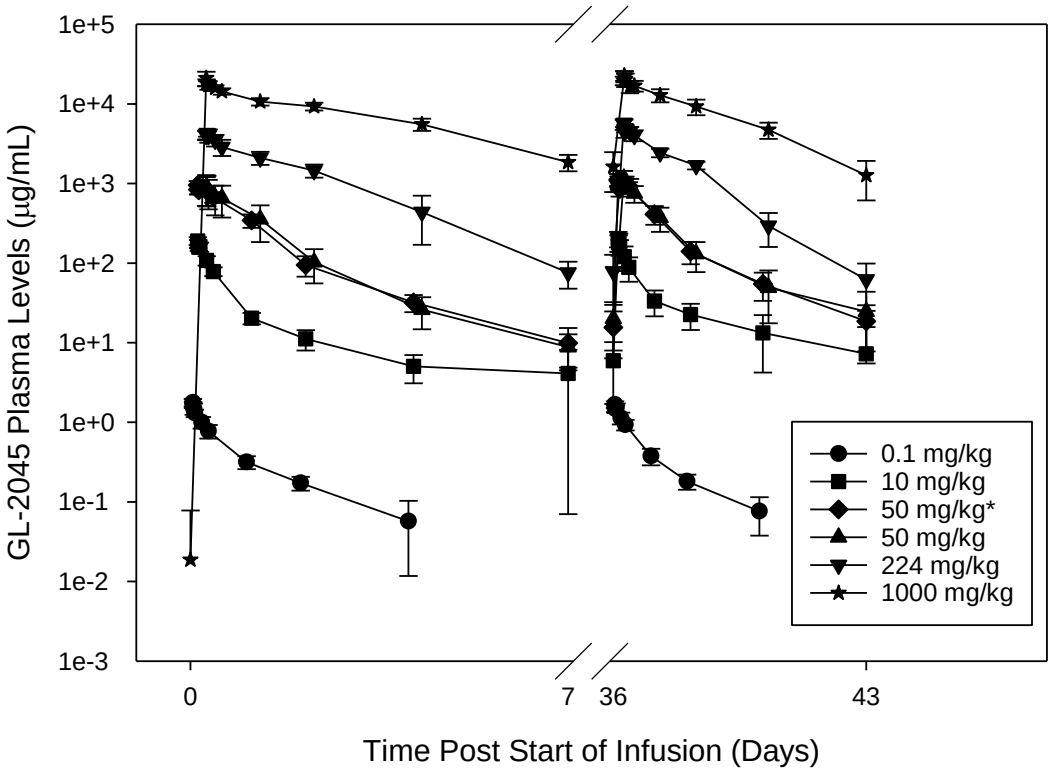


Supplemental Figure 4. **CD14 mRNA expression correlates with arthritis score and the expression of IL-1β and CCR2 in the joints of CIA mice.** (A) mRNA expression of CD14 in the joints of naïve (n=4), diseased vehicle-treated (PBS)(n=6), and diseased GL-2045-treated (GL-2045) mice (n=7) were assessed by Nanostring. CD14 mRNA expression is shown as absolute gene expression counts. * $p<0.05$, One-way ANOVA with Tukey correction. Association of CD14 mRNA expression with arthritis score (B) and with mRNA expression of IL-1β, IL-6, CCR2, and TGF-β3 in the joints (C). Linear models adjusted for group were used to test significance of the linear association.



Supplemental Figure 5. GL-2045 increased levels of circulating cytokines in rats. Male and female Wistar rats were assigned to 8 groups in two separate studies. GL-2045 was infused IV weekly at different doses for 6 weeks. The highest dose (2000 mg/kg) was reduced to 1200 mg/kg beginning on Day 8 of the dosing phase. Blood samples were collected at different time points and serum cytokine levels were analyzed. Only the day 1 data is shown. Cytokines exhibiting increased concentrations post start of infusion on Day 1 of the dosing phase were IL-4, IL-10, IL-13, and TNF-α; the increases affected both

sexes at all doses at 1 and/or 3 hours post start of infusion. The number of animals in the low dose study was 20 per group. The number of animals in high dose study: n=30, Vehicle control; n=19, 160 mg/kg; n=20, 566 mg/kg; n=26, 2000/1200 mg/kg. *p<0.05, **p<0.01, ***p<0.001, compared with pre-treatment levels (0 hours).



Supplemental Figure 6. GL-2045 time-concentration profiles in monkeys after the first and sixth weekly IV infusion of GL-2045. GL-2045 was infused IV weekly at different doses for 6 weeks to male and female cynomolgus monkeys. Doses were 0.1, 10 or 50 mg/kg (Low dose study) and 50, 224 or 1000 mg/kg (High dose study). There were no apparent sex-related differences in systemic exposure as assessed by C_{max} and AUC_{168}. Systemic exposure increased with increasing dose, with C_{max} approximately increasing in proportion to dose and AUC_{168} increasing slightly greater than in proportion to dose. Exposures after the sixth dose were similar to those after the first. *Denotes 50 mg/kg group from the Low dose study. Data points represent group means ± standard deviation.

Biomarker	Low dose study 0 mg/kg	Low dose study 1 mg/kg	Low dose study 10 mg/kg	Low dose study 160 mg/kg	High dose study 0 mg/kg	High dose study 160 mg/kg	High dose study 566 mg/kg	High dose study 2000/1200 mg/kg
IL-2 (pg/mL)								
0 hours	34.4 (27.6, 42.7)	29.9 (24.2, 37.0)	39.4 (31.9, 48.7)	35.8 (28.9, 44.2)	24.4 (23.6, 25.3)	24.4 (23.4, 25.4)	24.4 (23.4, 25.4)	24.4 (23.6, 25.2)
1 hours	36.8 (29.7, 45.4)	31.7 (25.6, 39.2)	35.3 (28.5, 43.6)	43.7 (35.3, 54.0)	24.4 (23.6, 25.2)	24.5 (23.5, 25.5)	24.4 (23.4, 25.4)	24.4 (23.6, 25.2)
3 hours	34.2 (27.7, 42.3)	31.3 (25.3, 38.7)	46.8 (37.9, 57.8)	42.3 (34.2, 52.3)	24.4 (23.6, 25.2)	24.8 (23.8, 25.8)	24.4 (23.4, 25.4)	24.4 (23.6, 25.2)
24 hours	31.1 (25.2, 38.4)	29.1 (23.5, 35.9)	27.9 (22.5, 34.4)	37.1 (30.0, 45.9)	24.4 (23.6, 25.2)	26.7 (25.7, 27.8)	24.4 (23.4, 25.4)	24.4 (23.6, 25.2)
IL-6 (pg/mL)								
0 hours	192.7 (158.4, 234.5)	146.4 (121.4, 176.6)	146.4 (121.4, 176.6)	146.4 (121.4, 176.6)	146.4 (138.0, 155.3)	146.4 (136.2, 157.4)	146.4 (136.2, 157.4)	146.4 (138.0, 155.3)
1 hours	210.5 (174.5, 253.8)	146.4 (121.4, 176.6)	146.4 (121.4, 176.6)	377.1 (312.7, 454.9)	146.4 (138.0, 155.3)	146.4 (136.2, 157.4)	149.8 (139.0, 161.3)	156.7 (147.6, 166.4)
3 hours	146.4 (121.4, 176.6)	146.4 (121.4, 176.6)	277.2 (229.8, 334.4)	440.2 (365.0, 530.9)	146.4 (138.0, 155.3)	153.8 (143.1, 165.3)	173.2 (161.1, 186.2)	150.2 (141.6, 159.3)
24 hours	172.7 (143.2, 208.3)	146.4 (121.4, 176.6)	152.2 (126.2, 183.6)	212.4 (176.1, 256.1)	146.4 (138.0, 155.3)	146.4 (136.2, 157.4)	146.4 (136.2, 157.4)	146.4 (138.0, 155.3)
IL-12p70 (pg/mL)								
0 hours	80.9 (57.1, 114.8)	75.7 (54.0, 106.0)	67.9 (48.5, 95.2)	81.7 (58.3, 114.4)	57.3 (45.7, 71.9)	49.7 (37.7, 65.6)	87.0 (65.9, 114.7)	94.2 (75.1, 118.1)
1 hours	77.2 (55.1, 108.2)	74.3 (53.0, 104.1)	80.5 (57.5, 112.8)	107.3 (76.6, 150.3)	64.8 (51.6, 81.2)	90.6 (68.7, 119.5)	108.2 (81.4, 143.8)	143.0 (113.6, 179.9)
3 hours	102.0 (72.8, 143.0)	51.0 (36.4, 71.4)	99.8 (71.2, 139.8)	113.3 (80.8, 158.7)	115.2 (91.9, 144.4)	148.0 (112.2, 195.3)	131.8 (99.9, 173.8)	130.5 (104.0, 163.6)
24 hours	70.5 (50.3, 98.8)	41.6 (29.7, 58.3)	55.4 (39.5, 77.6)	87.3 (62.3, 122.3)	55.5 (44.3, 69.6)	66.4 (50.3, 87.6)	77.3 (58.6, 102.0)	73.3 (58.5, 91.9)
IL-17 (pg/mL)								
0 hours	15.5 (14.0, 17.3)	14.6 (13.2, 16.2)	14.6 (13.2, 16.2)	16.0 (14.4, 17.7)	14.6 (13.9, 15.4)	14.6 (13.7, 15.6)	14.6 (13.7, 15.6)	14.6 (13.9, 15.4)
1 hours	16.1 (14.5, 17.8)	14.6 (13.2, 16.2)	14.8 (13.4, 16.4)	19.3 (17.4, 21.4)	14.6 (13.9, 15.4)	14.6 (13.7, 15.6)	14.9 (13.9, 15.9)	15.5 (14.7, 16.3)
3 hours	15.6 (14.1, 17.3)	14.6 (13.2, 16.2)	19.0 (17.2, 21.1)	19.6 (17.7, 21.7)	14.6 (13.9, 15.4)	15.1 (14.1, 16.1)	15.6 (14.6, 16.6)	15.2 (14.4, 16.0)
24 hours	15.5 (14.0, 17.2)	14.6 (13.2, 16.2)	14.6 (13.2, 16.2)	16.5 (14.9, 18.3)	14.6 (13.9, 15.4)	14.7 (13.8, 15.7)	15.8 (14.8, 16.8)	15.6 (14.8, 16.4)
IFN-γ (pg/mL)								
0 hours	46.7 (38.5, 56.6)	29.2 (24.3, 35.1)	29.2 (24.3, 35.1)	29.2 (24.3, 35.1)	29.2 (27.7, 30.8)	29.2 (27.4, 31.1)	29.2 (27.4, 31.1)	29.2 (27.7, 30.8)
1 hours	50.5 (42.0, 60.7)	29.2 (24.3, 35.1)	29.2 (24.3, 35.1)	29.2 (24.3, 35.1)	29.2 (27.7, 30.8)	29.2 (27.4, 31.1)	29.2 (27.4, 31.2)	29.5 (27.9, 31.1)
3 hours	29.2 (24.3, 35.1)	29.2 (24.3, 35.1)	29.2 (24.3, 35.1)	29.2 (24.3, 35.1)	29.2 (27.7, 30.8)	29.2 (27.4, 31.1)	29.2 (27.4, 31.1)	29.2 (27.7, 30.8)
24 hours	32.9 (27.4, 39.6)	29.2 (24.3, 35.1)	34.1 (28.4, 41.0)	29.2 (24.3, 35.1)	29.2 (27.7, 30.8)	29.2 (27.4, 31.1)	32.7 (30.7, 34.8)	33.3 (31.6, 35.0)

Supplemental Table 1. Least Square Means for GL-2045-induced Cytokine Modulation in rats.

Values represent mean concentrations with 95% Confidence Intervals in brackets for each cytokine measured, in a high dose and low dose study, before and within the first day of dosing.

Biomarker	Low dose study 0 mg/kg	Low dose study 0.1 mg/kg	Low dose study 10 mg/kg	Low dose study 50 mg/kg	High dose study 0 mg/kg	High dose study 50 mg/kg	High dose study 224 mg/kg	High dose study: 1000 mg/kg
IL-2 (pg/mL)								
0 hours	3.2 (2.2, 4.8)	5.1 (3.4, 7.6)	3.8 (2.6, 5.7)	3.9 (2.6, 5.9)	9.6 (5.6, 16.5)	10.1 (5.0, 20.4)	5.0 (2.5, 10.1)	5.3 (3.1, 9.0)
1 hours	3.2 (2.2, 4.8)	3.7 (2.5, 5.5)	3.2 (2.2, 4.8)	3.8 (2.5, 5.6)	8.5 (4.9, 14.6)	3.5 (1.8, 7.1)	3.2 (1.6, 6.4)	3.2 (1.9, 5.5)
3 hours	3.2 (2.2, 4.8)	3.8 (2.6, 5.6)	3.2 (2.2, 4.8)	3.9 (2.6, 5.8)	10.2 (5.9, 17.5)	3.2 (1.6, 6.4)	3.2 (1.6, 6.4)	3.2 (1.9, 5.5)
24 hours	3.2 (2.2, 4.8)	4.6 (3.1, 6.8)	3.2 (2.2, 4.8)	3.9 (2.6, 5.9)	11.3 (6.6, 19.5)	3.2 (1.6, 6.4)	3.2 (1.6, 6.4)	3.2 (1.9, 5.5)
IL-4 (pg/mL)								
0 hours	6.8 (3.2, 14.5)	4.9 (2.3, 10.4)	7.6 (3.6, 16.2)	4.9 (2.3, 10.4)	4.9 (3.2, 7.5)	4.9 (2.8, 8.5)	4.9 (2.8, 8.5)	8.0 (5.2, 12.2)
1 hours	6.5 (3.0, 13.7)	4.9 (2.3, 10.4)	7.2 (3.4, 15.3)	4.9 (2.3, 10.4)	4.9 (3.2, 7.5)	4.9 (2.8, 8.5)	4.9 (2.8, 8.5)	7.4 (4.9, 11.3)
3 hours	6.7 (3.2, 14.2)	4.9 (2.3, 10.4)	9.9 (4.7, 21.1)	6.4 (3.0, 13.5)	4.9 (3.2, 7.5)	4.9 (2.8, 8.5)	4.9 (2.8, 8.5)	7.1 (4.7, 10.9)
24 hours	6.0 (2.8, 12.8)	4.9 (2.3, 10.4)	11.5 (5.4, 24.4)	7.4 (3.5, 15.7)	4.9 (3.2, 7.5)	4.9 (2.8, 8.5)	4.9 (2.8, 8.5)	4.9 (3.2, 7.5)
IL-6 (pg/mL)								
0 hours	3.2 (1.9, 5.5)	5.8 (3.3, 9.9)	3.6 (2.1, 6.2)	3.2 (1.9, 5.5)	8.2 (4.2, 16.1)	9.6 (4.0, 23.0)	6.2 (2.6, 14.9)	5.3 (2.7, 10.3)
1 hours	3.2 (1.9, 5.5)	3.9 (2.3, 6.7)	3.9 (2.2, 6.7)	7.9 (4.5, 13.6)	8.9 (4.5, 17.6)	3.2 (1.3, 7.7)	16.6 (6.9, 39.7)	12.4 (6.3, 24.5)
3 hours	3.2 (1.9, 5.5)	5.4 (3.1, 9.3)	9.2 (5.3, 15.9)	5.4 (3.1, 9.3)	17.8 (9.0, 34.9)	12.2 (5.1, 29.2)	12.0 (5.0, 28.7)	10.7 (5.5, 21.1)
24 hours	4.5 (2.6, 7.8)	4.9 (2.8, 8.4)	3.6 (2.1, 6.2)	3.2 (1.9, 5.5)	15.5 (7.9, 30.4)	9.1 (3.8, 21.7)	6.4 (2.7, 15.2)	5.8 (2.9, 11.4)
IL-12/23p40 (pg/mL)								
0 hours	3.2 (1.1, 9.7)	15.1 (5.0, 45.7)	6.7 (2.2, 20.3)	5.0 (1.7, 15.1)	28.1 (10.6, 74.5)	178.4 (50.5, 629.3)	14.8 (4.2, 52.1)	14.4 (5.4, 38.2)
1 hours	3.2 (1.1, 9.7)	10.1 (3.3, 30.5)	4.1 (1.4, 12.5)	4.8 (1.6, 14.6)	17.9 (6.7, 47.5)	13.5 (3.8, 47.5)	3.7 (1.0, 12.9)	5.1 (1.9, 13.4)
3 hours	3.2 (1.1, 9.7)	8.3 (2.7, 25.0)	4.0 (1.3, 12.2)	4.8 (1.6, 14.6)	24.0 (9.1, 63.8)	14.3 (4.1, 50.5)	4.0 (1.1, 14.1)	4.4 (1.7, 11.7)
24 hours	3.2 (1.1, 9.7)	10.9 (3.6, 32.8)	3.9 (1.3, 11.7)	4.0 (1.3, 12.0)	33.2 (12.5, 88.1)	15.5 (4.4, 54.7)	4.0 (1.1, 14.3)	5.0 (1.9, 13.3)
IL-13 (pg/mL)								
0 hours	3.2 (1.8, 5.8)	6.1 (3.4, 11.0)	4.8 (2.7, 8.7)	3.2 (1.8, 5.8)	22.8 (11.2, 46.4)	20.5 (8.2, 51.2)	9.9 (4.0, 24.8)	11.1 (5.5, 22.7)
1 hours	3.2 (1.8, 5.8)	5.0 (2.8, 9.0)	3.2 (1.8, 5.8)	3.2 (1.8, 5.8)	16.1 (7.9, 32.8)	3.2 (1.3, 8.0)	3.2 (1.3, 8.0)	3.6 (1.8, 7.3)
3 hours	3.2 (1.8, 5.8)	5.3 (2.9, 9.5)	3.2 (1.8, 5.8)	3.2 (1.8, 5.8)	22.2 (10.9, 45.2)	3.2 (1.3, 8.0)	3.2 (1.3, 8.0)	3.2 (1.6, 6.5)
24 hours	3.2 (1.8, 5.8)	5.5 (3.0, 9.9)	3.2 (1.8, 5.8)	3.2 (1.8, 5.8)	28.1 (13.8, 57.2)	3.2 (1.3, 8.0)	3.2 (1.3, 8.0)	3.2 (1.6, 6.5)
IL-17 (pg/mL)								
0 hours	2.4 (1.4, 4.1)	6.6 (3.8, 11.4)	3.8 (2.2, 6.5)	2.4 (1.4, 4.1)	30.2 (16.3, 56.1)	24.6 (11.0, 54.6)	21.0 (9.5, 46.8)	15.5 (8.3, 28.8)
1 hours	2.4 (1.4, 4.1)	3.9 (2.3, 6.8)	2.4 (1.4, 4.1)	2.4 (1.4, 4.1)	25.0 (13.5, 46.5)	7.0 (3.2, 15.7)	7.8 (3.5, 17.4)	5.3 (2.8, 9.8)
3 hours	2.4 (1.4, 4.1)	3.6 (2.1, 6.3)	2.8 (1.6, 4.9)	2.4 (1.4, 4.1)	29.7 (16.0, 55.2)	10.3 (4.6, 22.9)	10.0 (4.5, 22.3)	4.9 (2.7, 9.2)
24 hours	2.4 (1.4, 4.1)	4.0 (2.3, 6.9)	2.4 (1.4, 4.1)	2.4 (1.4, 4.1)	32.8 (17.7, 61.0)	7.8 (3.5, 17.4)	7.2 (3.2, 15.9)	4.8 (2.6, 8.9)
IFN-γ (pg/mL)								
0 hours	3.2 (2.4, 4.3)	5.0 (3.7, 6.6)	4.1 (3.1, 5.4)	3.7 (2.8, 4.9)	3.9 (3.0, 5.1)	9.5 (6.8, 13.3)	3.8 (2.7, 5.4)	3.6 (2.8, 4.7)
1 hours	3.2 (2.4, 4.3)	3.2 (2.4, 4.3)	3.2 (2.4, 4.3)	3.6 (2.7, 4.8)	3.6 (2.8, 4.7)	3.9 (2.8, 5.4)	3.2 (2.3, 4.5)	3.2 (2.5, 4.2)
3 hours	3.2 (2.4, 4.3)	3.7 (2.8, 4.9)	3.2 (2.4, 4.3)	3.3 (2.5, 4.4)	4.0 (3.1, 5.2)	4.0 (2.8, 5.5)	3.3 (2.3, 4.6)	3.2 (2.5, 4.2)
24 hours	3.2 (2.4, 4.3)	3.9 (2.9, 5.2)	3.2 (2.4, 4.3)	3.2 (2.4, 4.3)	5.6 (4.3, 7.2)	3.2 (2.3, 4.5)	3.5 (2.5, 5.0)	3.3 (2.6, 4.3)
TNF-α (pg/mL)								
0 hours	16.0 (7.7, 33.1)	31.8 (15.4, 65.8)	31.4 (15.2, 64.9)	17.8 (8.6, 36.8)	25.4 (15.9, 40.7)	44.9 (24.4, 82.4)	19.1 (10.4, 35.1)	20.8 (13.0, 33.3)
1 hours	16.0 (7.7, 33.1)	22.8 (11.0, 47.2)	19.7 (9.5, 40.8)	26.4 (12.8, 54.6)	25.3 (15.8, 40.6)	16.0 (8.7, 29.4)	16.0 (8.7, 29.4)	17.1 (10.7, 27.4)
3 hours	16.0 (7.7, 33.1)	29.4 (14.2, 60.8)	22.3 (10.8, 46.2)	19.9 (9.6, 41.2)	26.9 (16.8, 43.0)	16.0 (8.7, 29.4)	16.0 (8.7, 29.4)	16.0 (10.0, 25.6)
24 hours	16.0 (7.7, 33.1)	30.5 (14.7, 63.0)	21.3 (10.3, 43.9)	16.0 (7.7, 33.1)	27.5 (17.2, 44.0)	16.0 (8.7, 29.4)	16.0 (8.7, 29.4)	16.0 (10.0, 25.6)

Supplemental Table 2. Least Square Means for GL-2045-induced Cytokine Modulation in NHPs.

Values represent mean concentrations with 95% Confidence Intervals in brackets for each cytokine measured, in a high dose and low dose study, before and within the first day of dosing.