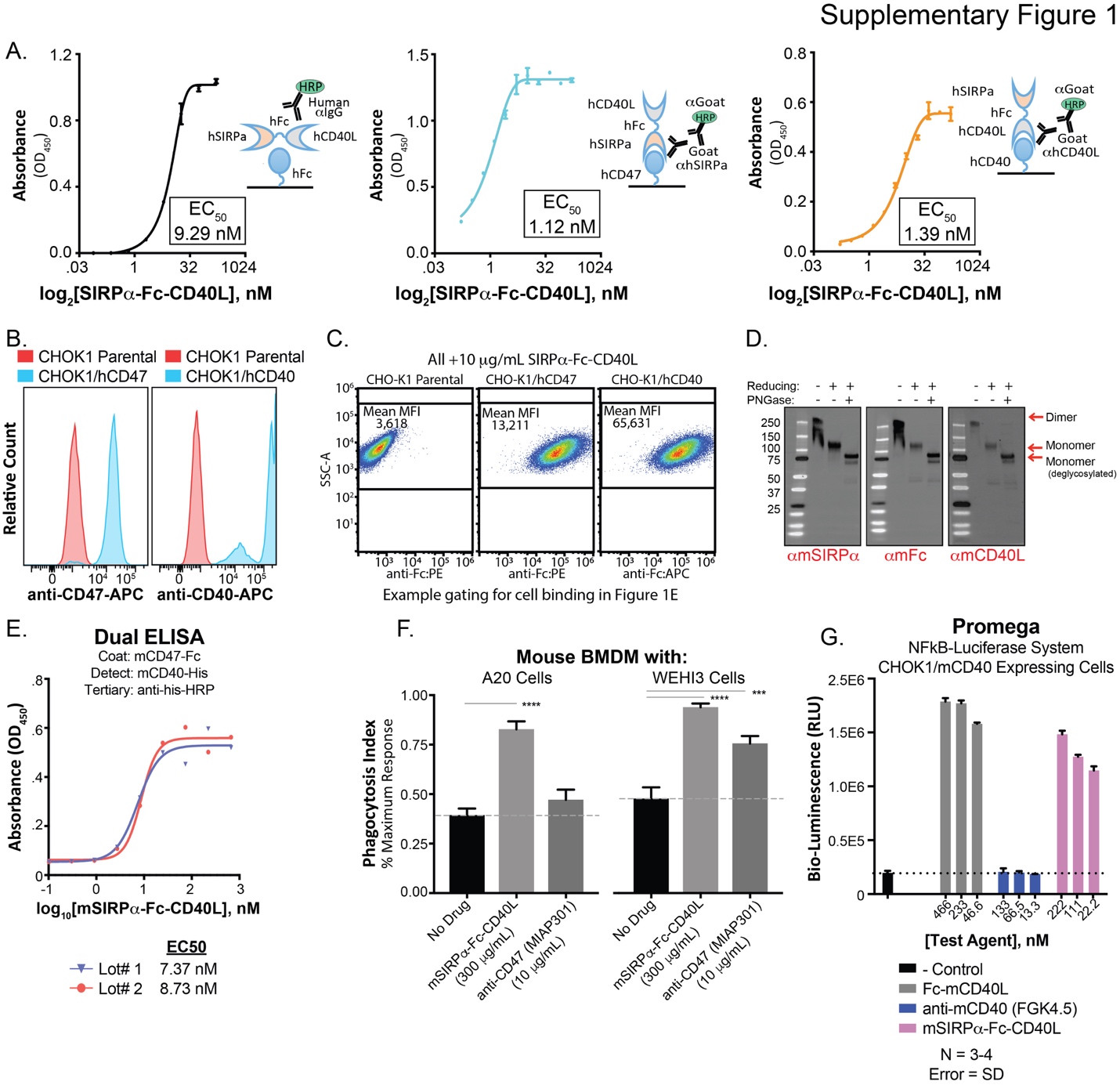
Supplementary Materials:



**Supplementary Figure 1. Further characterization of human SIRPα-Fc-CD40L and the murine mSIRPα-Fc-CD40L surrogate.**

(A) Single-sided ELISA detection of hSIRPα-Fc-CD40L using recombinant Fc, CD47, and CD40 capture.

(B) Verification of human CD47 and human CD40 expression in CHO-K1 cells used to assess binding to hSIRPα-Fc-CD40L.

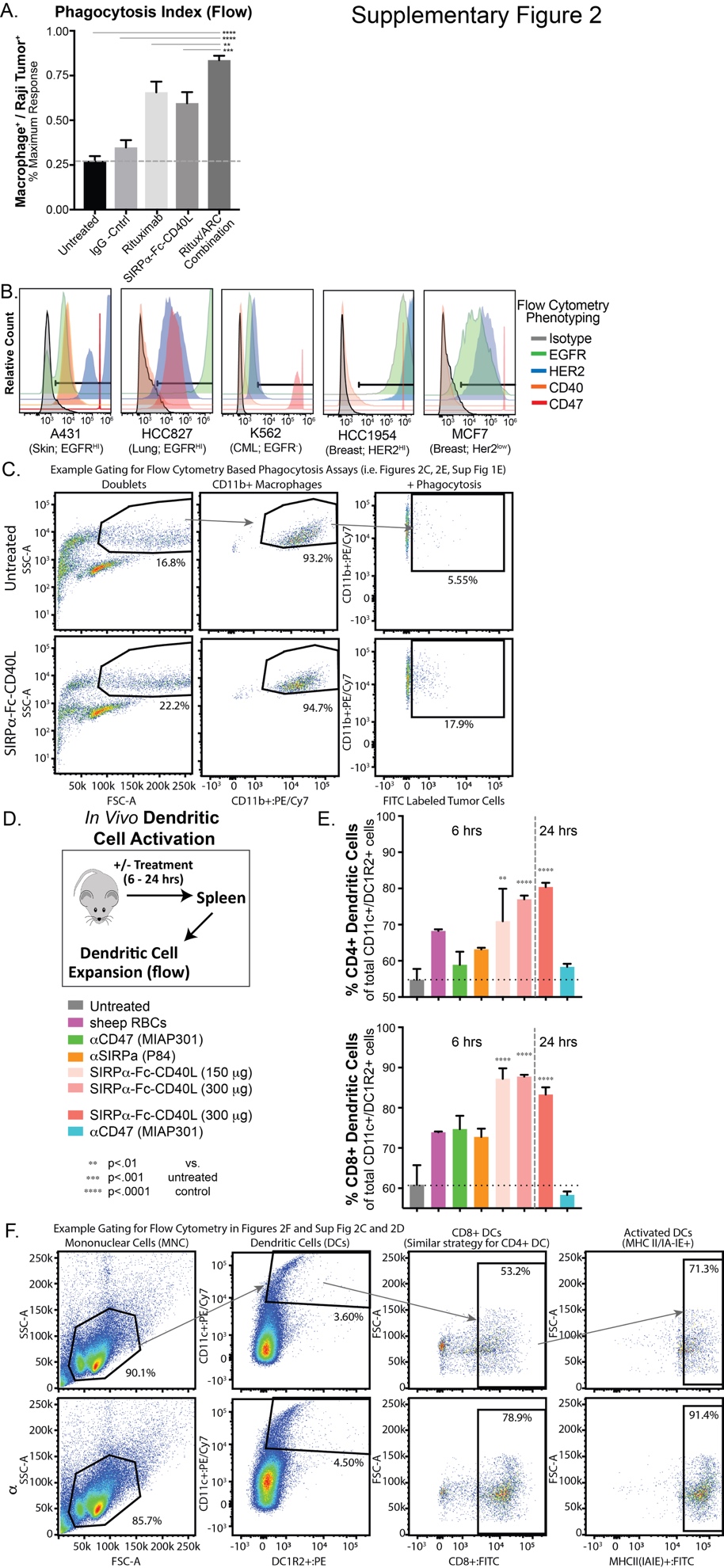
(C) Example flow cytometry gating from Figure 1E, depicting binding of SIRP-Fc-CD40L to CHO-K1 parental cells, or CHO-K1 cells engineered to overexpress human CD47 or CD40. In this example, cells were incubated with 10 g/mL of human SIRP-Fc-CD40L. To generate the binding curves in Figure 1E, cells were incubate with a dose titration of SIRP-Fc-CD40L.

(D) Western blot analysis of the murine SIRPα-Fc-CD40L surrogate with antibodies detecting mSIRPα, mFc, and mCD40L under non-reducing, reducing, and PNGase F/reducing conditions.

(E) Dual functional ELISA of the murine SIRPα-Fc-CD40L surrogate, demonstrating simultaneous binding to recombinant mouse CD47 and CD40.

(F) Murine version of the phagocytosis assay using bone marrow derived macrophages co-cultured with A20 lymphoma or WEHI3 leukemia cells, in the presence of mSIRPα-Fc-CD40L or anti-CD47.

(G) Murine version of the NFB-luciferase reporter assay in CHO-K1 cells developed to express murine CD40 and an NFB-luciferase reporter.



**Supplementary Figure 2. Supportive phagocytosis data and SIRP- driven activation of dendritic cells *in vivo*.**

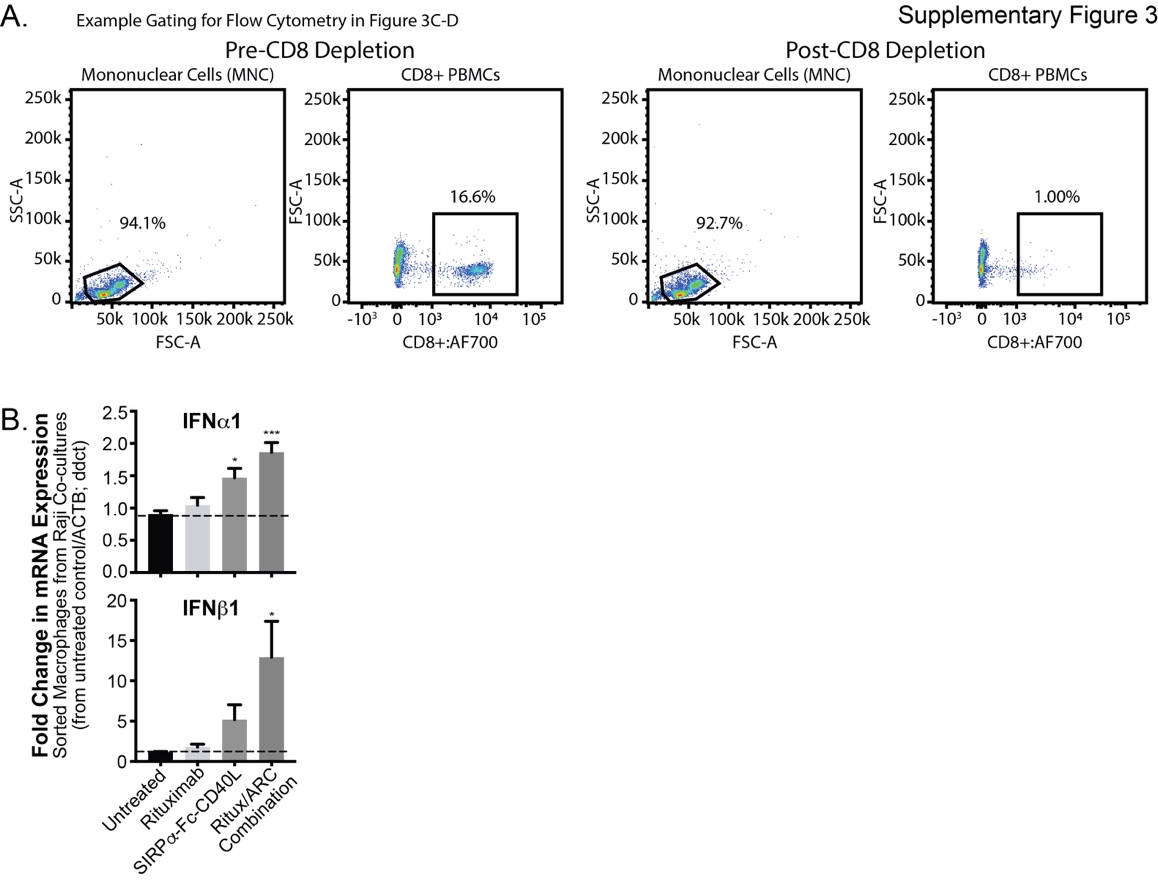
(A) Phagocytosis quantitation of Raji cells by human macrophages using flow cytometry cytometry in the presence of hSIRP-Fc-CD40L+/-Rituximab.

(B) Flow cytometry phenotyping of surface expressed EGFR, HER2, CD40, and CD47 in the human tumor cell lines used for phagocytosis assays in Figure 2F.

(C) Example gating for flow cytometry based phagocytosis assays in Figures 2C, 2E, and Supplementary Figure 1E.

(D) Schematic and (E) quantitation of *in vivo* dendritic cell activation; corresponding to Figure 2E. Shown is the absolute percentage of CD4+ and CD8+ dendritic cells; also gated on CD11c and DC1R2.

(F) Example gating for flow cytometry in Figures 2F, and Supplementary Figures 2C and 2D. The same gating strategy was used for CD4+ DCs as is shown for CD8+ DCs.­

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**Supplementary Figure 3. Type I interferon expression in macrophages co-cultured with Raji cells.**

(A) Example gating for CD8 depletion flow cytometry from human PBMCs presented in Figure 3C-D.

(B) Supportive data for Figure 3E. Fold-change in gene expression relative to ACTB and the untreated control was assessed in CD11b+ sorted macrophages following 2 h co-culture with Raji cells treated with hSIRP-Fc-CD40L +/- Rituximab (Ritux).

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**Supplementary Figure 4. Blockade of CD4, CD8, and IFNAR1.**

(A) Example gating for flow cytometry in Figure 4B.

Peripheral blood analysis by flow cytometry of (B) CD4 (left), CD8 (middle), and IFNAR1 (right) following depleting antibody treatment corresponding to Figure 4C-E. Samples were normalized to untreated animals.

(C) Example flow cytometry gating for CD4, CD8, and IFNAR1 depletion shown in Supplementary Figure 4B.

(D) CD4 and CD8 cells were depleted from CT26 tumor bearing mice on days 9, 11, and 15 of the time-course; after treatment with SIRPα-Fc-CD40L began (treatment on days 7, 9, and 11). This strategy is referred to as ‘late’ depletion and correlates with the ‘early’ CD4 / CD8 depletion shown in Figure 4C. IFNAR1+ cells were depleted from (E) WEHI3 (left) and A20 (right) bearing mice on days 9, 11, and 13 in the WEHI3 animals and on days 12, 14, and 16 in the A20 animals after treatment with SIRPα-Fc-CD40L began. As in Figure 4D-E, mSIRPα-Fc-CD40L was given on days 7, 9, and 11 in WEHI3 bearing mice and on days 10, 12, and 14 in A20 bearing mice. This strategy is referred to as ‘late’ depletion and correlates with the ‘early’ IFNAR1 depletion shown in Figure 4D-E.

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**Supplementary Figure 5. CT26 combination experiment with various sequencing of mSIRPα-Fc-CD40L and anti-CTLA4 or anti-PD1.**

(A)(C) Combinations with anti-CTLA4 or anti-PD1 respectively; number of mice in each treatment group, number of mice that rejected the primary CT26 tumor, number of mice that rejected the secondary CT26 tumor re-challenge (without subsequent retreatment), and (B)(D)

Mantle Cox survival and statistical analysis between monotherapy and combination groups. ‘d’ represents ‘day’ on which the therapies were administered.

(E) Example flow cytometry gating for Figure 5C.

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**Supplementary Figure 6. Hemolysis assessment and pharmacodynamic decrease in peripheral B cells following treatment with SIRPα-Fc-CD40L.**

(A) Example flow cytometry gating for Figure 6B.

(B) *In vitro* hemolysis assay using human donor RBCs treated with a titration of the positive control Triton X-100, a CD47 blocking antibody previously shown to induce RBC lysis (clone CC2C6), and a titration of 3 separate lots of SIRPα-Fc-CD40L.

(C) A decrease in overall CD45+ peripheral lymphocytes was observed 24 h following a single IP injection of mSIRP-Fc-CD40L (300 g).

(D) Peripheral blood was isolated from mice receiving 3 IP doses (300 g) of the murine SIRPα-Fc-CD40L surrogate (arrows). Cell populations were assessed by flow cytometry and included CD20+ B cells, CD11C+, CD4+/CD11c+, and CD8+/CD11c+ dendritic cells. No significant differences were observed in mice treated with an interferon alpha receptor 1 depleting antibody (anti-IFNAR1).

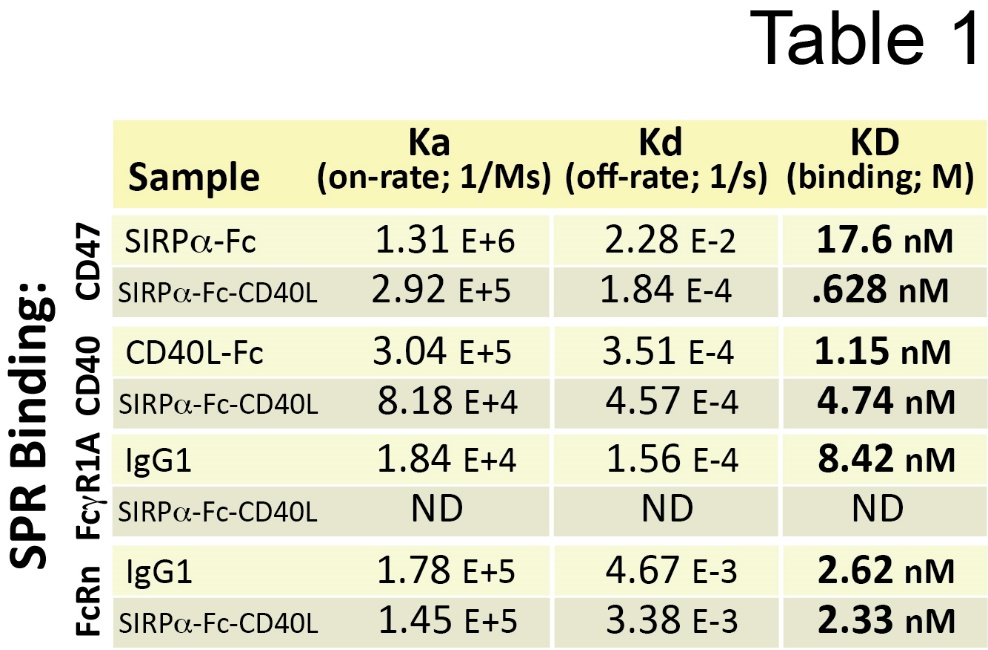
(E) A decrease in peripheral CD20+ B cells was observed 24 h following a single IP injection of a dose range of mSIRP-Fc-CD40L.

(F) Example flow cytometry gating for Supplementary Figures 6C-E.

**Supplementary Table 1:** Murine SIRPα-Fc-CD40L Sequences

|  |  |
| --- | --- |
| mSIRPα ECD | KELKVTQPEKSVSVAAGDSTVLNCTLTSLLPVGPIRWYRGVGPSRLLIYSFAGEYVPRIRNVSDTTKRNNMDFSIRISNVTPADAGIYYCVKFQKGSSEPDTEIQSGGGTEVYVLAKPSPPEVSGPADRGIPDQKVNFTCKSHGFSPRNITLKWFKDGQELHPLETTVNPSGKNVSYNISSTVRVVLNSMDVNSKVICEVAHITLDRSPLRGIANLSNFIRVSPTVKVTQQSPTSMNQVNLTCRAERFYPEDLQLIWLENGNVSRNDTPKNLTKNTDGTYNYTSLFLVNSSAHREDVVFTCQVKHDQQPAITRNHTVLGFAHSSDQGSMQTFPDNNATHNWN |
| mhinge-CD2-CD3 | VPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMNTNGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK |
| mCD40L ECD | HRRLDKVEEEVNLHEDFVFIKKLKRCNKGEGSLSLLNCEEMRRQFEDLVKDITLNKEEKKENSFEMQRGDEDPQIAAHVVSEANSNAASVLQWAKKGYYTMKSNLVMLENGKQLTVKREGLYYVYTQVTFCSNREPSSQRPFIVGLWLKPSSGSERILLKAANTHSSSQLCEQQSVHLGGVFELQAGASVFVNVTEASQVIHRVGFSSFGLLKL |
| hSIRPα ECD | EEELQVIQPDKSVLVAAGETATLRCTATSLIPVGPIQWFRGAGPGRELIYNQKEGHFPRVTTVSDLTKRNNMDFSIRIGNITPADAGTYYCVKFRKGSPDDVEFKSGAGTELSVRAKPSAPVVSGPAARATPQHTVSFTCESHGFSPRDITLKWFKNGNELSDFQTNVDPVGESVSYSIHSTAKVVLTREDVHSQVICEVAHVTLQGDPLRGTANLSETIRVPPTLEVTQQPVRAENQVNVTCQVRKFYPQRLQLTWLENGNVSRTETASTVTENKDGTYNWMSWLLVNVSAHRDDVKLTCQVEHDGQPAVSKSHDLKVSAHPKEQGSNTAAENTGSNERNIY |
| hIgG4 hinge-CH2-CH3 | ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK |
| hCD40L ECD | HRRLDKIEDERNLHEDFVFMKTIQRCNTGERSLSLLNCEEIKSQFEGFVKDIMLNKEETKKENSFEMQKGDQNPQIAAHVISEASSKTTSVLQWAEKGYYTMSNNLVTLENGKQLTVKRQGLYYIYAQVTFCSNREASSQAPFIASLCLKSPGRFERILLRAANTHSSAKPCGQQSIHLGGVFELQPGASVFVNVTDPSQVSHGTGFTSFGLLKL |

**Supplemental Table 2:** Binding affinity as measured by surface plasmon resonance

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**Supplemental Table 3:** Maximum fold-change for each indicated cytokine in NHP serum post-infusion as compared to pre-infusion

|  |  |  |
| --- | --- | --- |
|  | **Vehicle** | **hSIRPα-Fc-CD40L** |
| CCL2 | 2.89 | 31.04 |
| CXCL9 | 8.33 | 1482.36 |
| CXCL10 | 34.57 | 1830.25 |
| IFNα | Not Detected | 723.74 |
| IL-6 | 21.7 | 617.35 |
| IL-15 | Not Detected | 2381.65 |
| IL-23 | 20.67 | 420 |