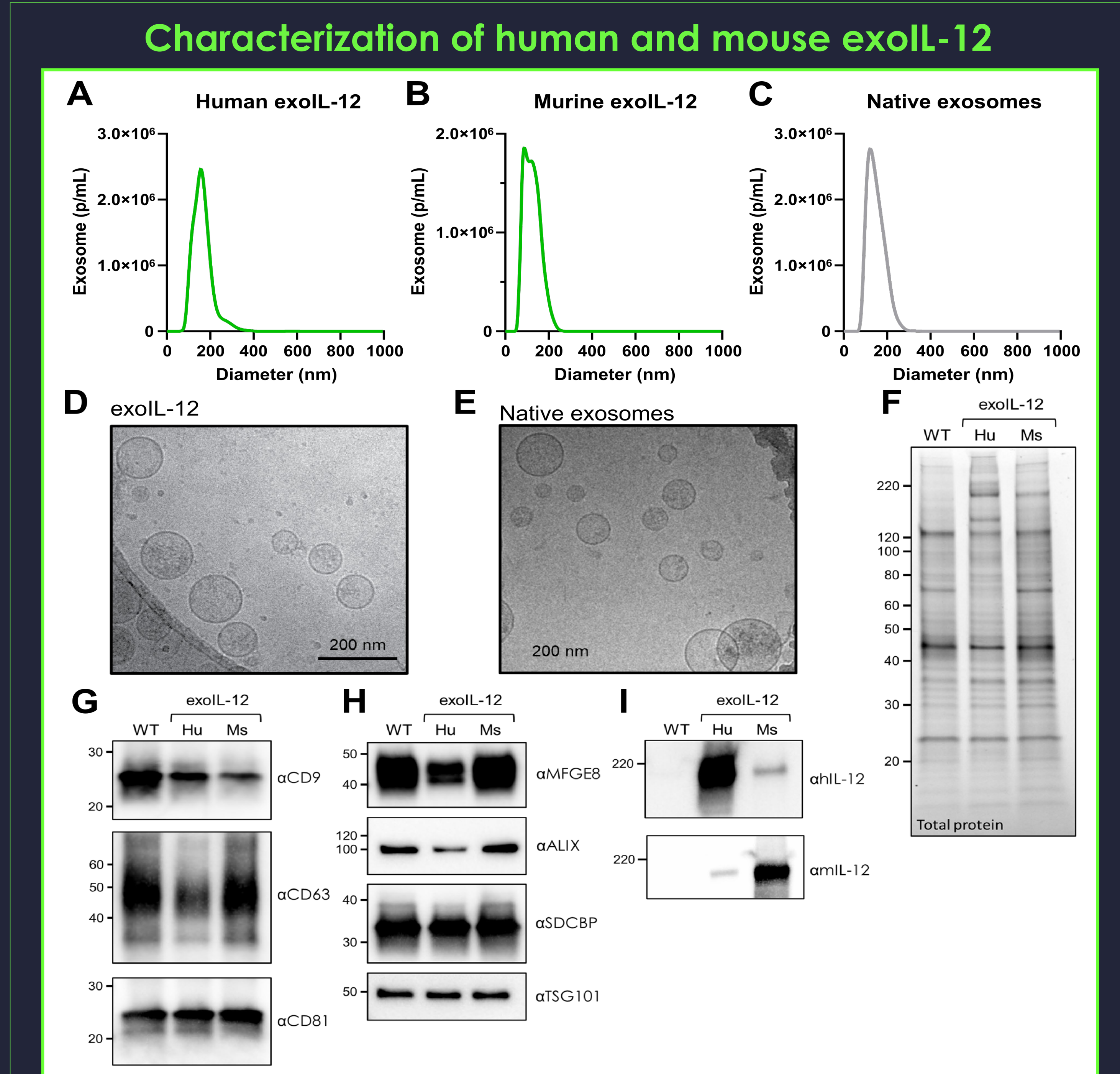
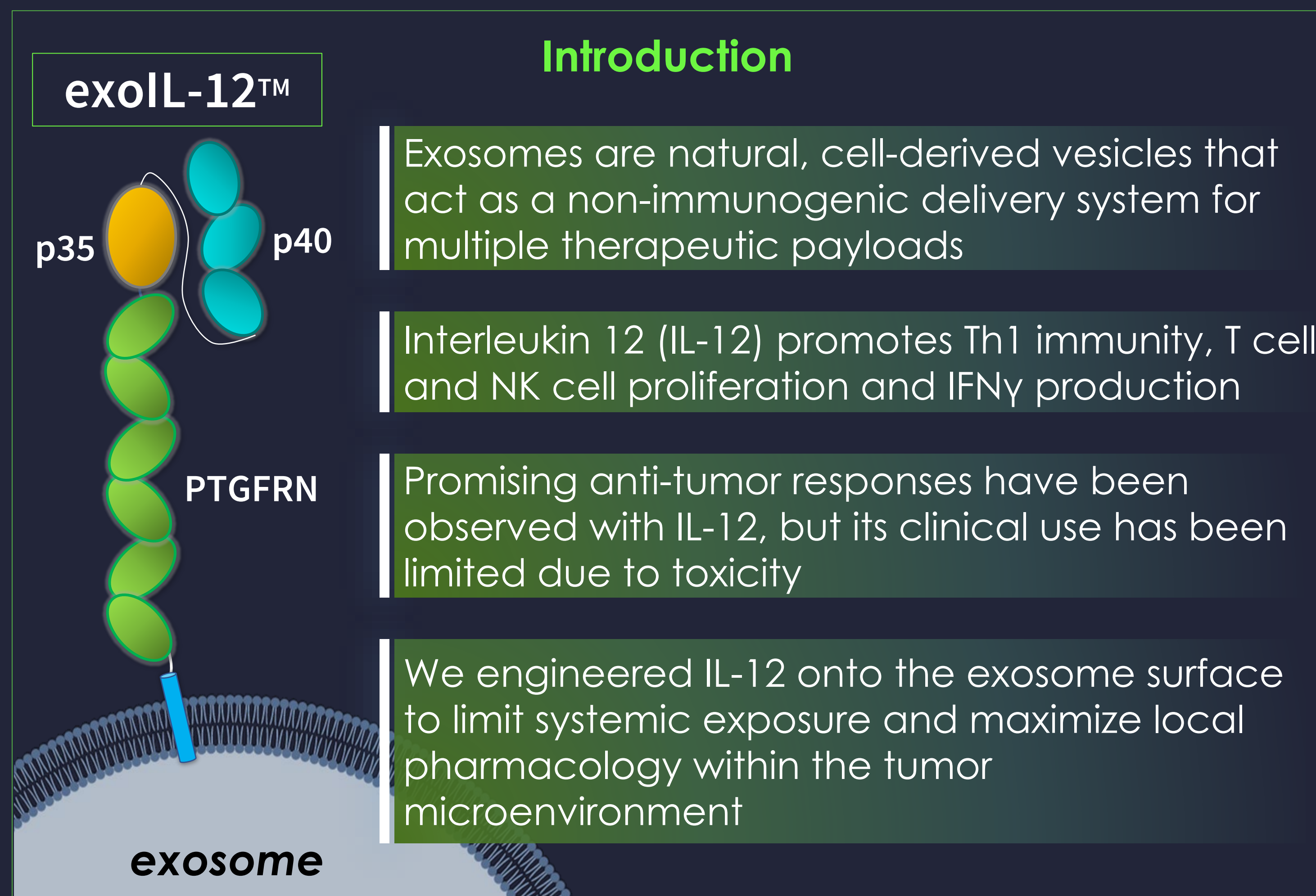


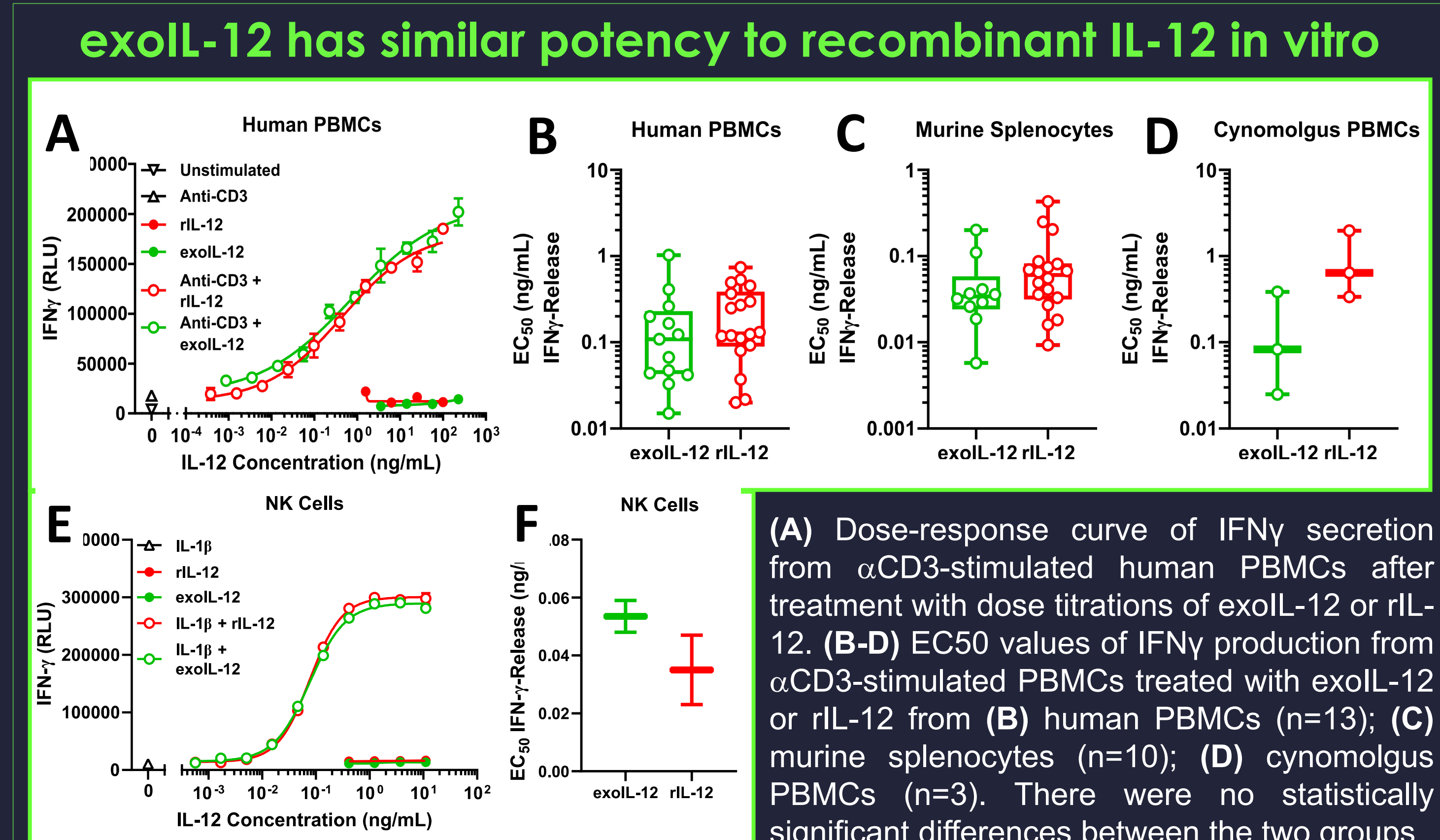
Exosome Surface Display of IL-12 Results in Tumor-Retained Pharmacology with Superior Potency and Limited Systemic Exposure

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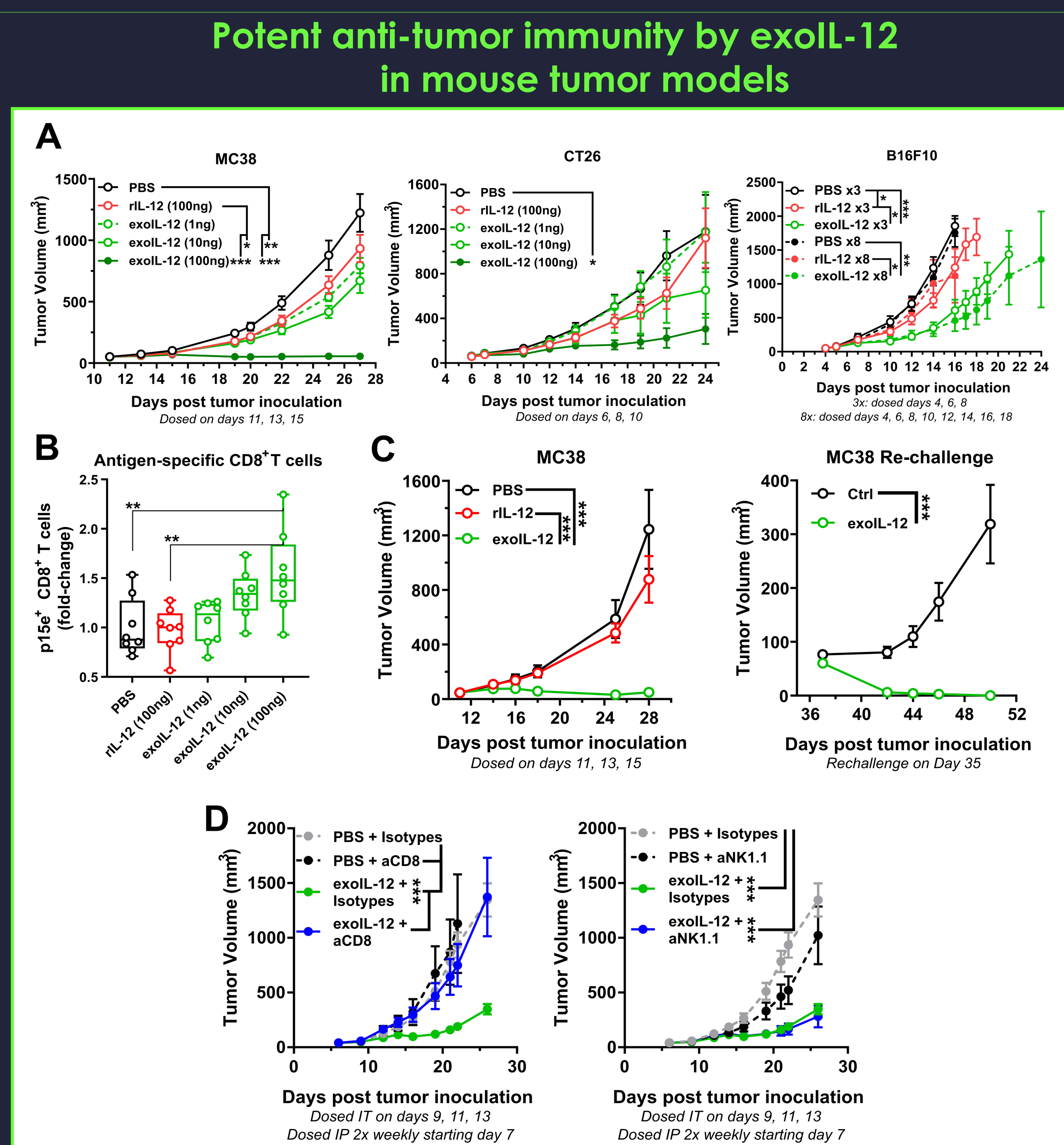
Codiak BioSciences, Cambridge, MA



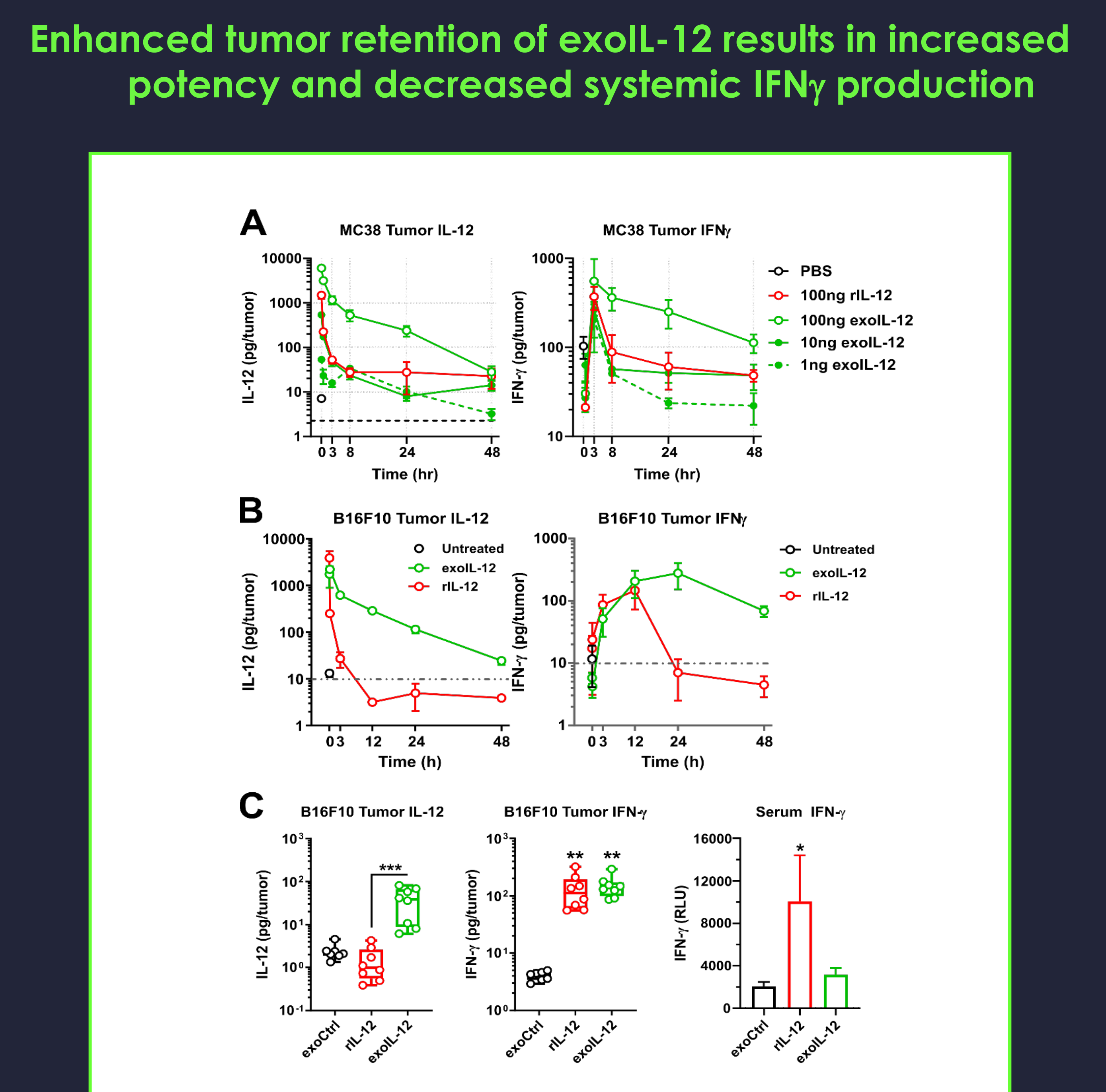
Nanoparticle tracking analysis showing consistent size distributions for human (A), mouse (B), and non-engineered native exosomes (C). (D-E) Cryo-EM analysis of exoIL-12 and native exosomes. (F) SDS-PAGE analysis of human and murine exoIL-12. The molecular weight markers are given in kDa. (G-I) Immunoblot analysis of the gel in (F) for CD9, CD63, CD81, MFGE8, ALIX, SDCBP, TSG101, human IL-12, and murine IL-12.



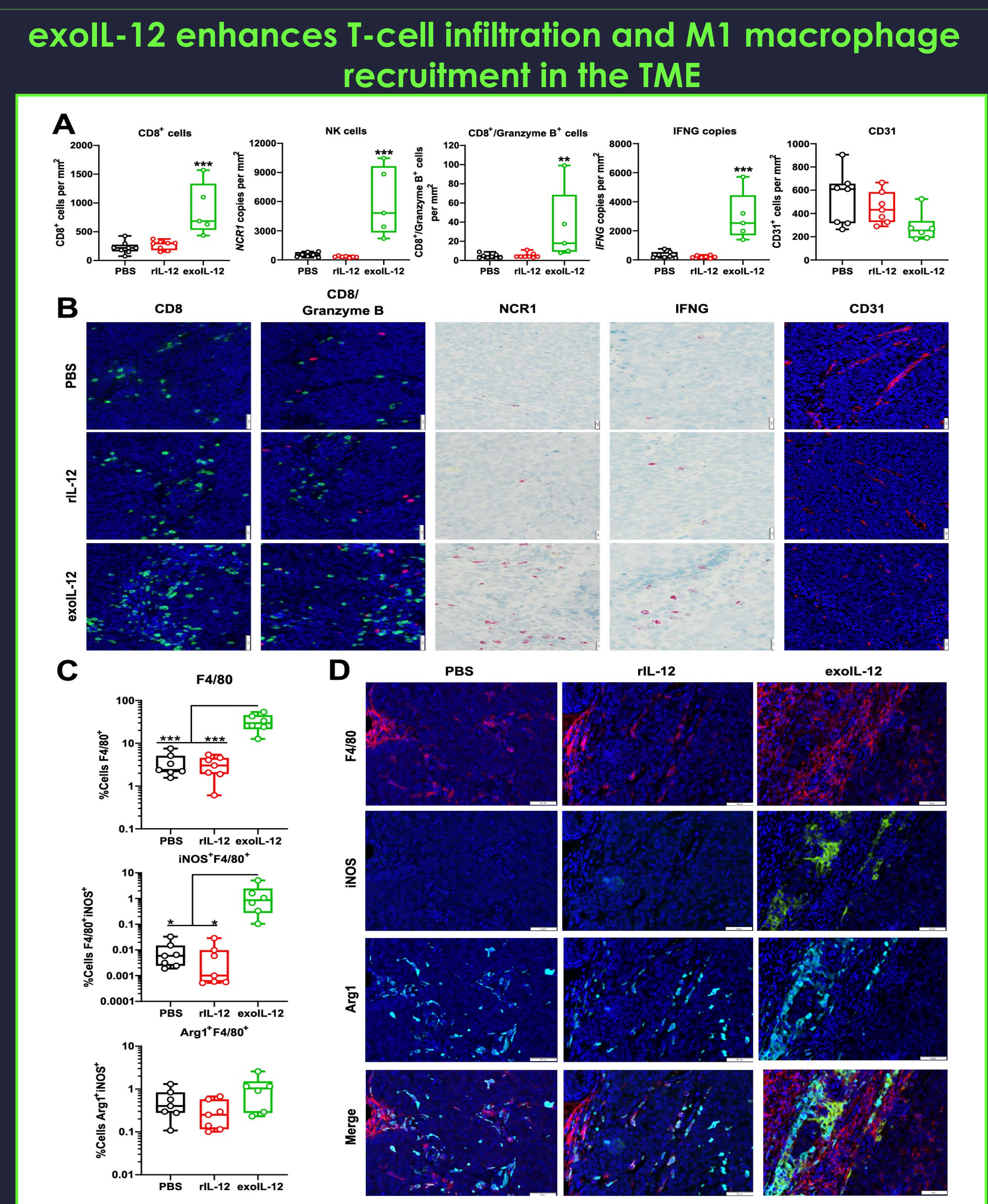
(A) Dose-response curve of IFN γ secretion from α CD3-stimulated human PBMCs after treatment with dose titrations of exoIL-12 or rIL-12. (B-D) EC50 values of IFN γ production from α CD3-stimulated PBMCs treated with exoIL-12 or rIL-12 from (B) human PBMCs (n=13); (C) murine splenocytes (n=10); (D) cynomolgus PBMCs (n=3). There were no statistically significant differences between the two groups in all three species using an unpaired t-test (B&C) or paired t-test (D). (E) Representative dose-response curves of IFN γ secretion from IL-1 β -stimulated human NK cells after treatment with dose titrations of exoIL-12 or rIL-12. (F) EC50 values derived from the NK cell dose-response curves (n=2). Differences were not statistically different using a paired t-test.



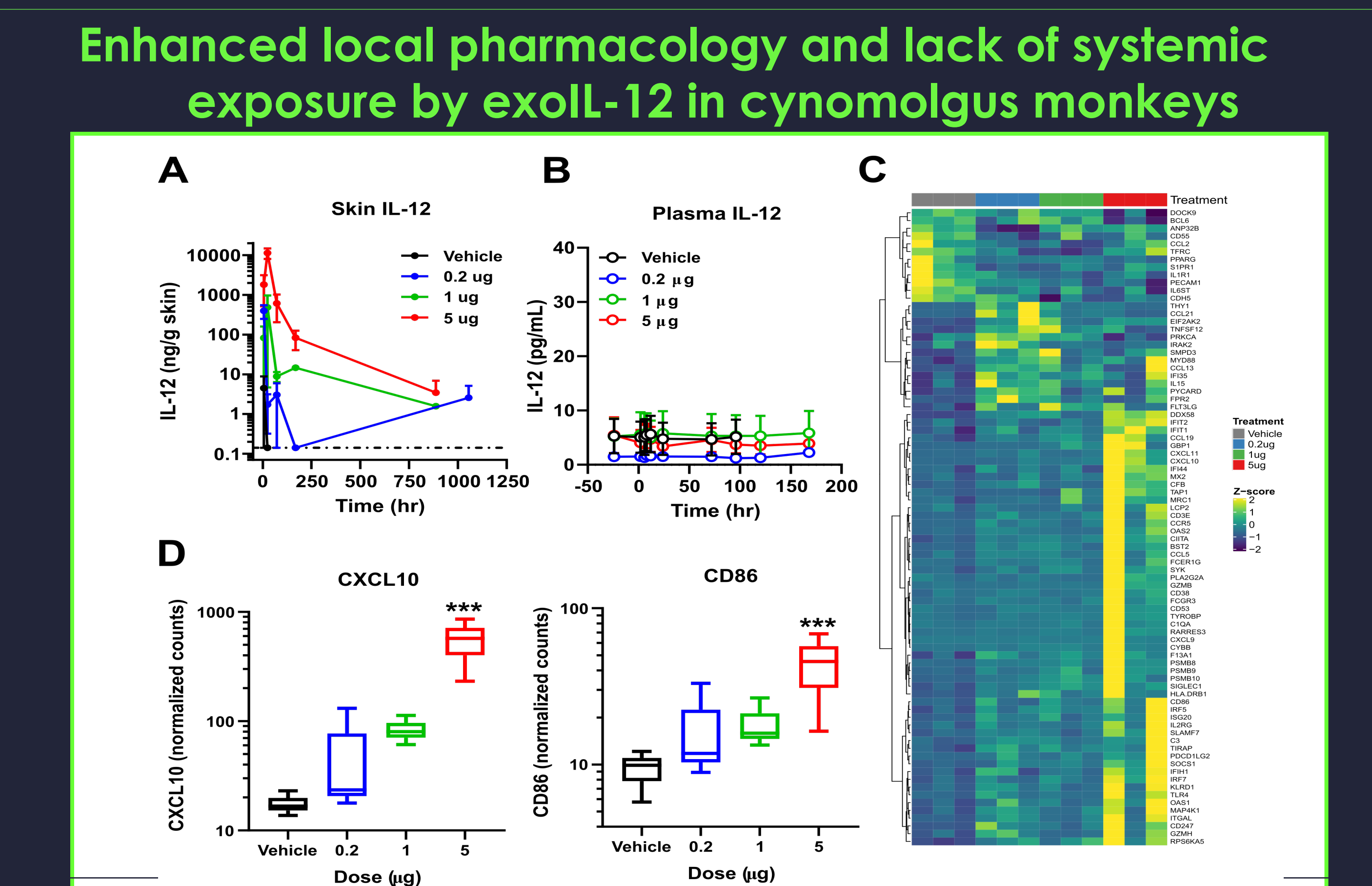
(A) Mean tumor volume from different treatment groups in different tumor models. Mice were dosed intratumorally (IT) three times for MC38 (n=8) and CT26 (n=10) and either three or eight times for B16F10 (n=5). (B) Spleens from the MC38 study were assessed for p15e+ MC38-specific CD8⁺ T cells using tetramers (n=8). (C) Mean tumor volume from different IT treatment groups in the MC38 model (left). Mice with complete responses (n=4) were re-challenged with MC38 cells in the opposite flank (right). (D) Mean MC38 tumor volume in mice depleted of CD8⁺ T cells (left) or NK cells (right) and dosed three times IT with PBS or exoIL-12. *** p<0.001, ** p<0.01, *p<0.05 by one-way ANOVA with Tukey's multiple comparison test.



Increased tumor retention of exoIL-12 and enhanced PD after IT dosing. (A) MC38 subcutaneous (SC) tumors were injected IT with exoIL-12 (100 ng, 10 ng, or 1 ng) or rIL-12 (100 ng) and assayed for IL-12p70 and IFN γ levels over time (N=3 mice / group / timepoint). Total amount of cytokine (pg) in entire tumor shown. (B) B16F10 SC tumors were injected IT with exoIL-12 (100 ng) or rIL-12 (100 ng) and assayed for IL-12p70 and IFN γ levels over time (N=3 mice / group / timepoint). Total amount of cytokine (pg) in entire tumor shown. (C) Mice bearing B16F10 SC tumors and B16F10 lung metastases were dosed IT into flank tumor once daily for nine total doses (n=8). IT IL-12p70, IT IFN γ , and serum IFN γ assessed on day 17 post-inoculation are shown (n=8). *** p<0.001, ** p<0.01, *p<0.05 by one-way ANOVA with Tukey's multiple comparison test.



exoIL-12 induces tumor infiltration of CD8⁺ cells, NK cells and M1 macrophages. (A) Quantitation of CD8⁺ cells, NK cells, CD8⁺/Granzyme B⁺, IFN γ mRNA copies, and CD31⁺ cells performed by IHC and ISH in MC38 tumors after treatment with exoIL-12 or rIL-12. (B) Representative images for the quantitation shown in (A). (C) Quantitation of tumor-infiltrating macrophages (F4/80⁺) and assessment of M1 (iNOS⁺) or M2 (Arg1⁺) polarization performed by IHC in MC38 tumors. (D) Representative images for the quantitation shown in C. *** p<0.001, ** p<0.01, *p<0.05 by one-way ANOVA with Tukey's multiple comparison test.



Lack of systemic exposure with local exoIL-12 administration. Cynomolgus monkeys were dosed SC with vehicle or 0.2, 1 or 5 μ g of exoIL-12. IL-12 levels in skin biopsies (A) at 6, 24 hrs, day 4, 7, 37 & 44 hrs post-dosing, and (B) in plasma at pre-dosing, 2, 6, 8, 12, 24 hrs, day 3, 4, 5 & 7 post-dosing. (C) Nanostring heatmap of gene analysis of skin biopsies on day 7 post-dosing. All significantly modulated genes vs Vehicle group using a one-way ANOVA and Dunnett's test are shown. (D) CXCL10 and CD86 gene expression were significantly upregulated with exoIL-12.

- Summary**
- The enhanced PK/PD profile of exoIL-12 leads to >100-fold improvement in tumor growth inhibition and superior tumor antigen-specific T cell responses
 - exoIL-12 addresses the limitations of rIL-12 via increased retention in the tumor, improved local IFN γ production, enhanced anti-tumor activity and reduced systemic cytokine expression
 - The safety profile of exoIL-12 is currently being investigated in a phase 1 clinical study