

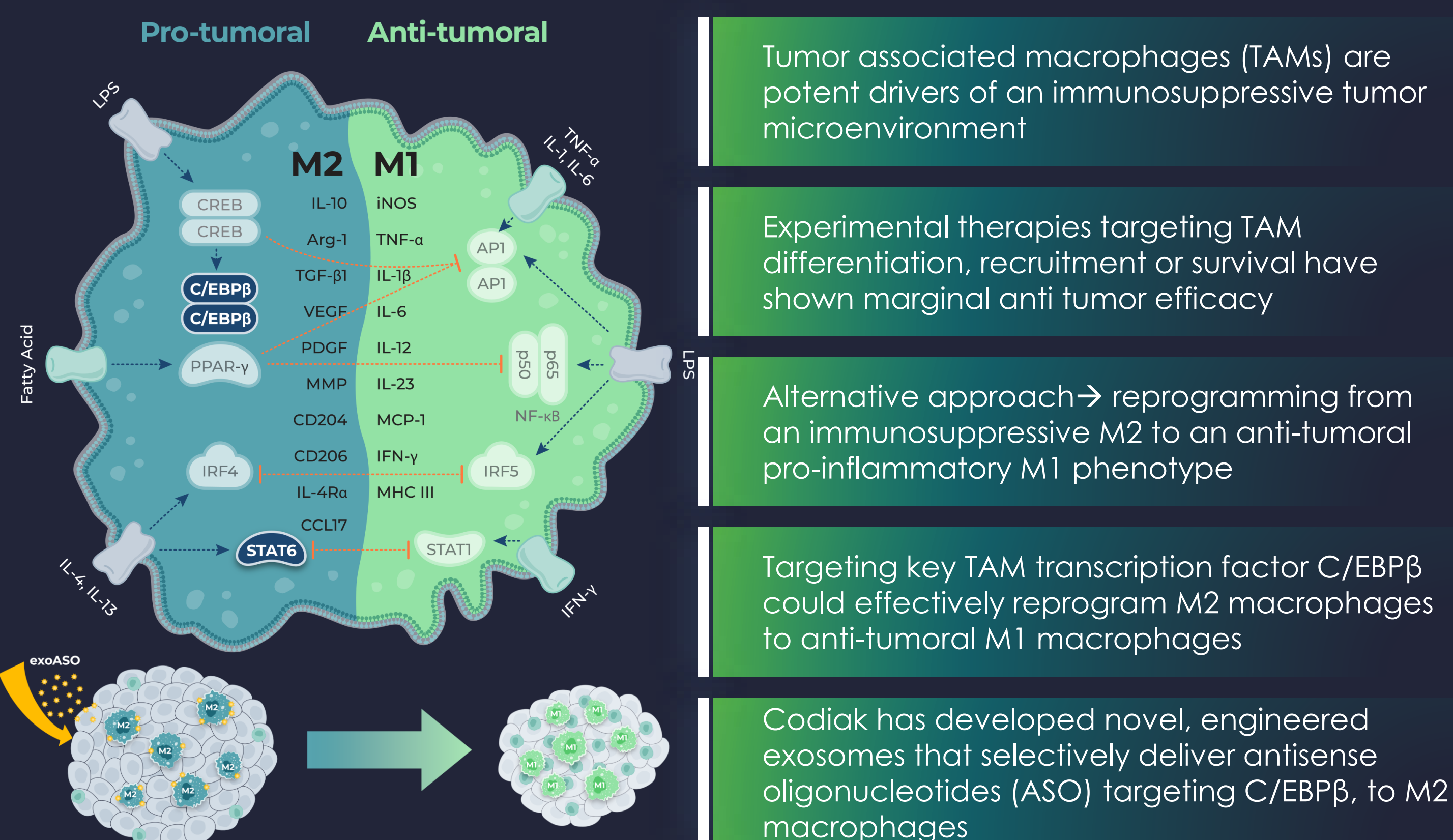
Exosome mediated delivery of antisense oligonucleotides reprograms tumor-associated macrophages and induces anti-tumor responses

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Introduction



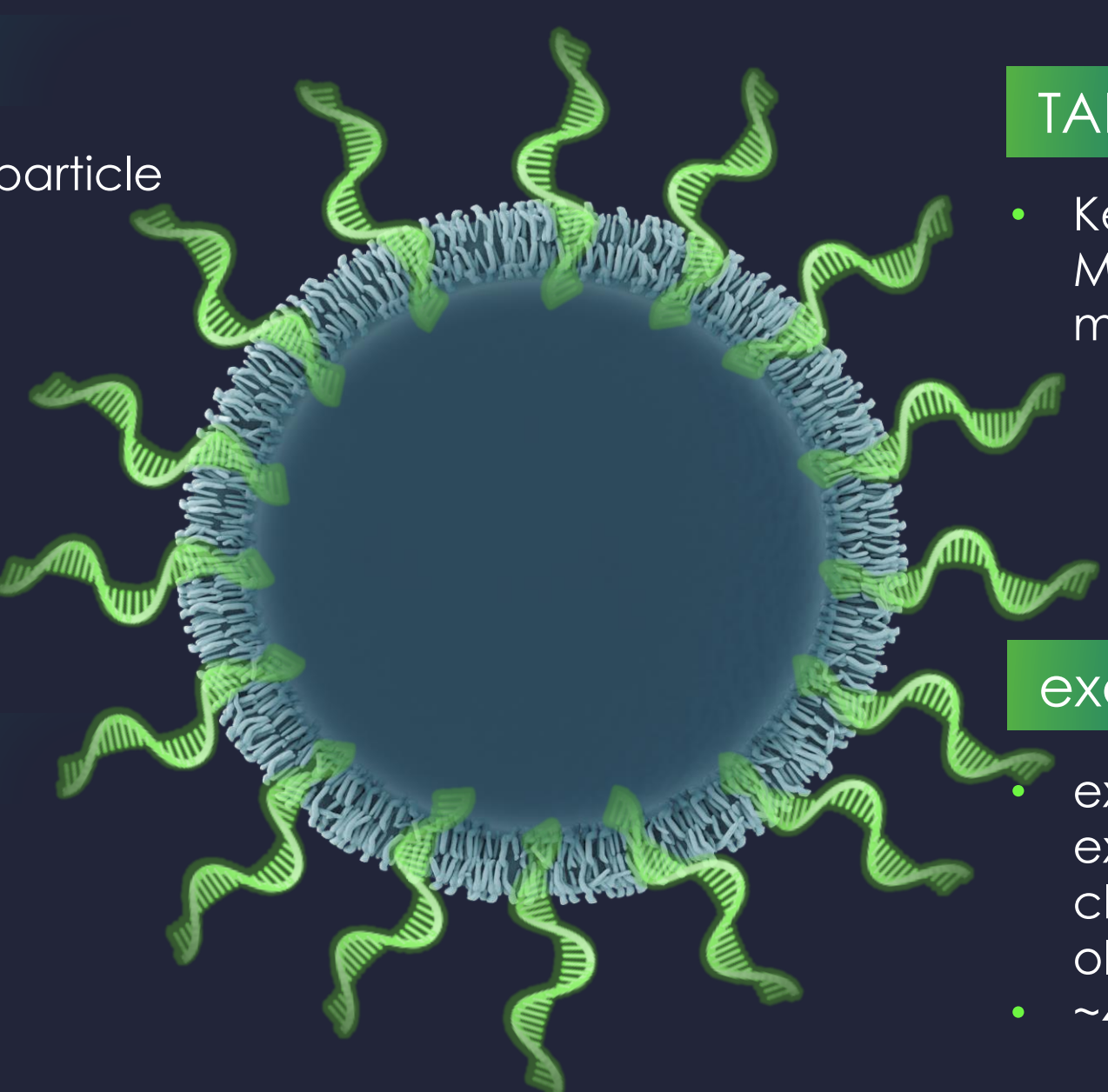
exoASO™: A novel engineered exosome

EXOSOMES

- 30-200nm lipid/protein nanoparticle
- Natural inter-cellular communication system for macromolecules
- Off-the-shelf immune silent drug delivery

TARGETING C/EBPβ

- Key transcriptional regulator of M2 immune-suppressive macrophages



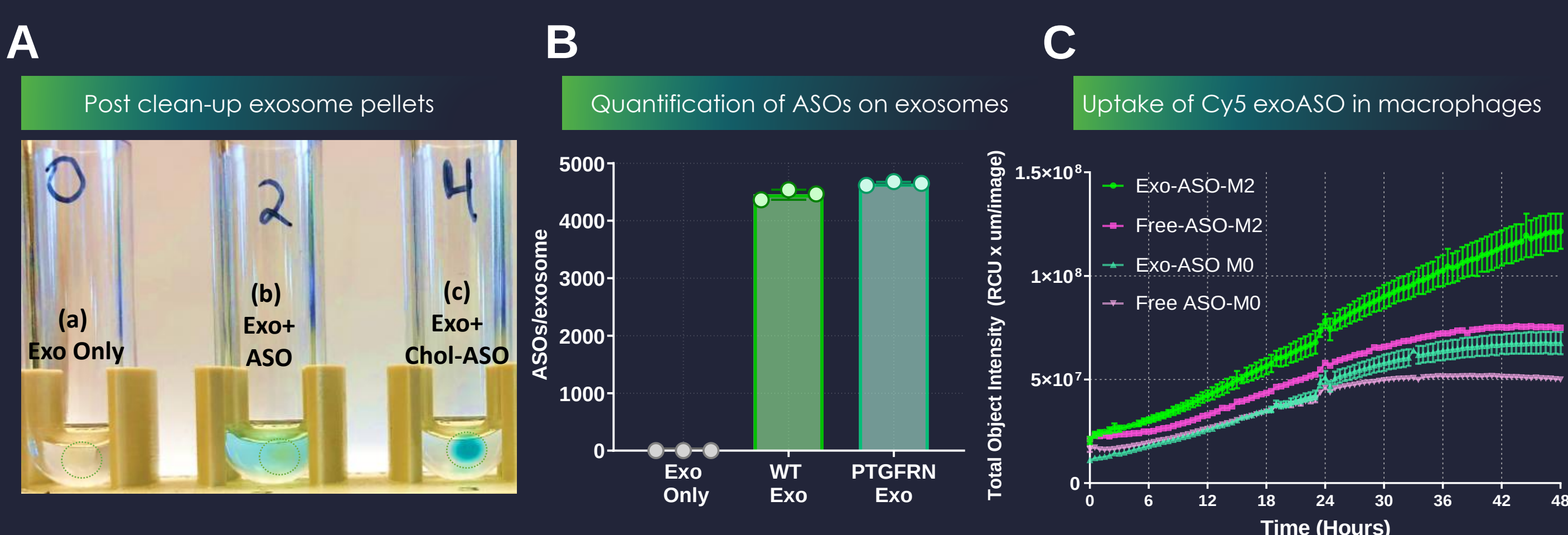
DELIVERY TO TAMs

- Selective targeting of M2 macrophages in the tumor microenvironment (TME)

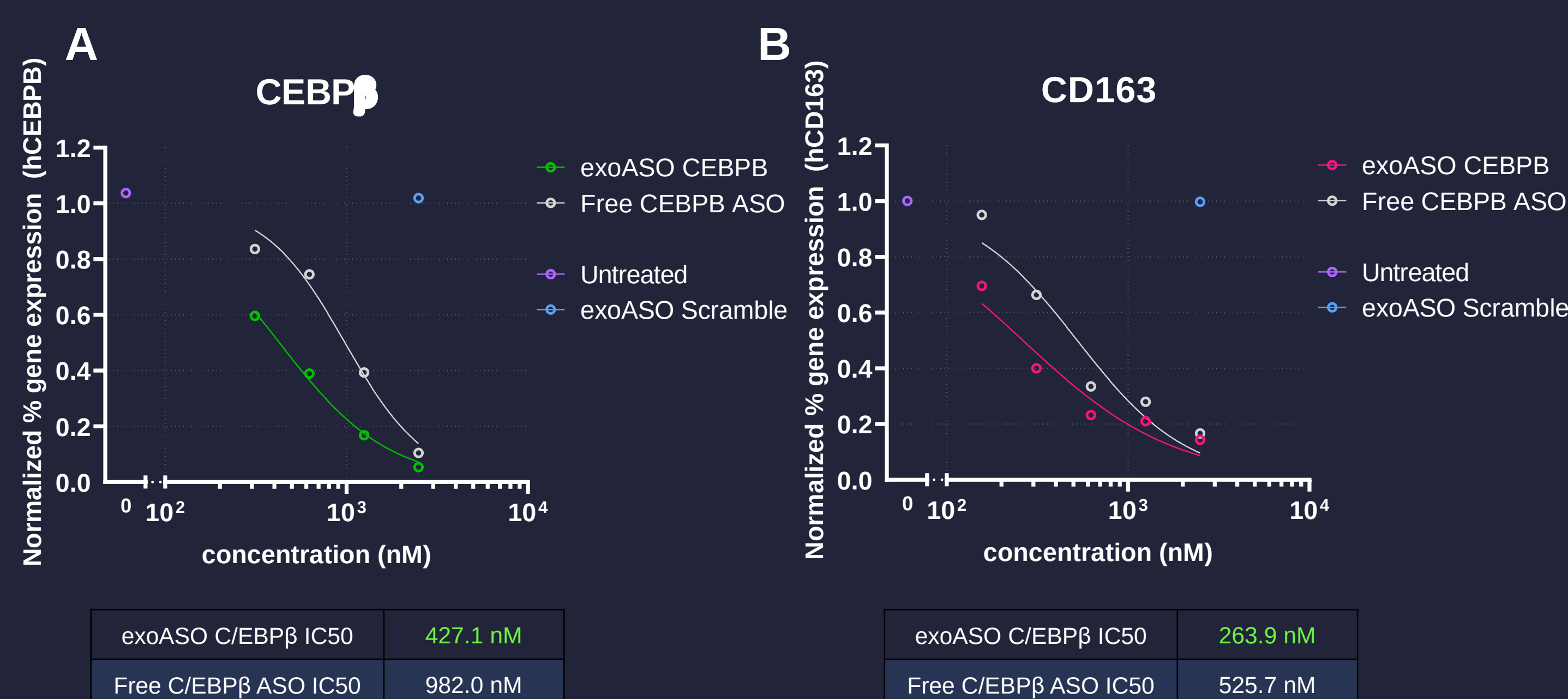
exoASO

- exoASO is a novel engineered exosome, stably loaded with cholesterol tagged antisense oligos
- ~4000 copies/exosome

Cholesterol-tagged ASOs load efficiently on exosomes



Potent target gene silencing *in vitro* by C/EBPβ exoASO



Effective knockdown of C/EBPβ and reduction of CD163 expression *in vitro* by exoASO. **A:** Human primary M2 macrophages were incubated for 48 hours with equivalent doses of exoASO and free ASO targeting C/EBPβ, along with an exoASO scramble control. Gene expression levels of C/EBPβ (**A**) and the M2 marker CD163 (**B**) were analyzed by qPCR, post treatment. One representative donor of five is shown

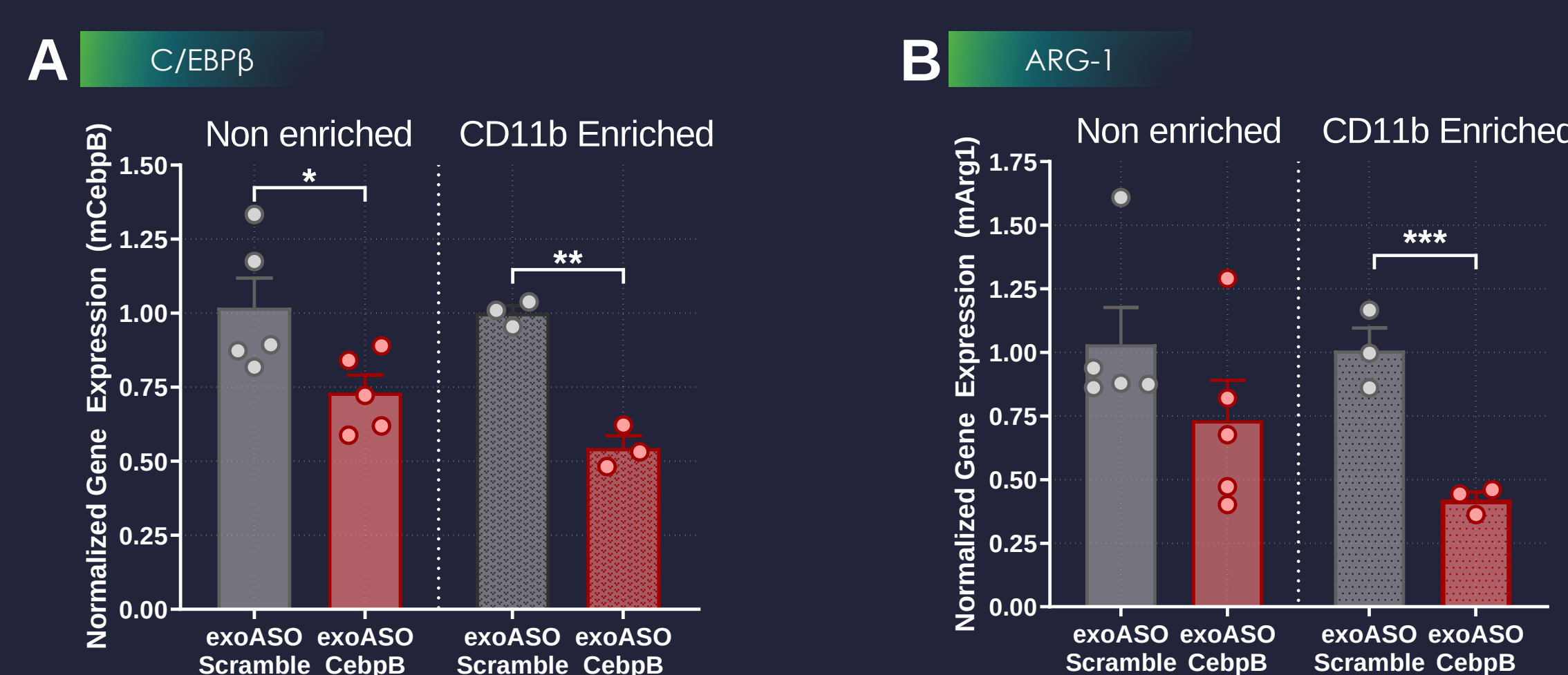
C/EBPβ mRNA silencing by exoASO effectively repolarizes human M2 macrophages



Exosome mediated selective delivery of ASOs to TAMs *in vivo*

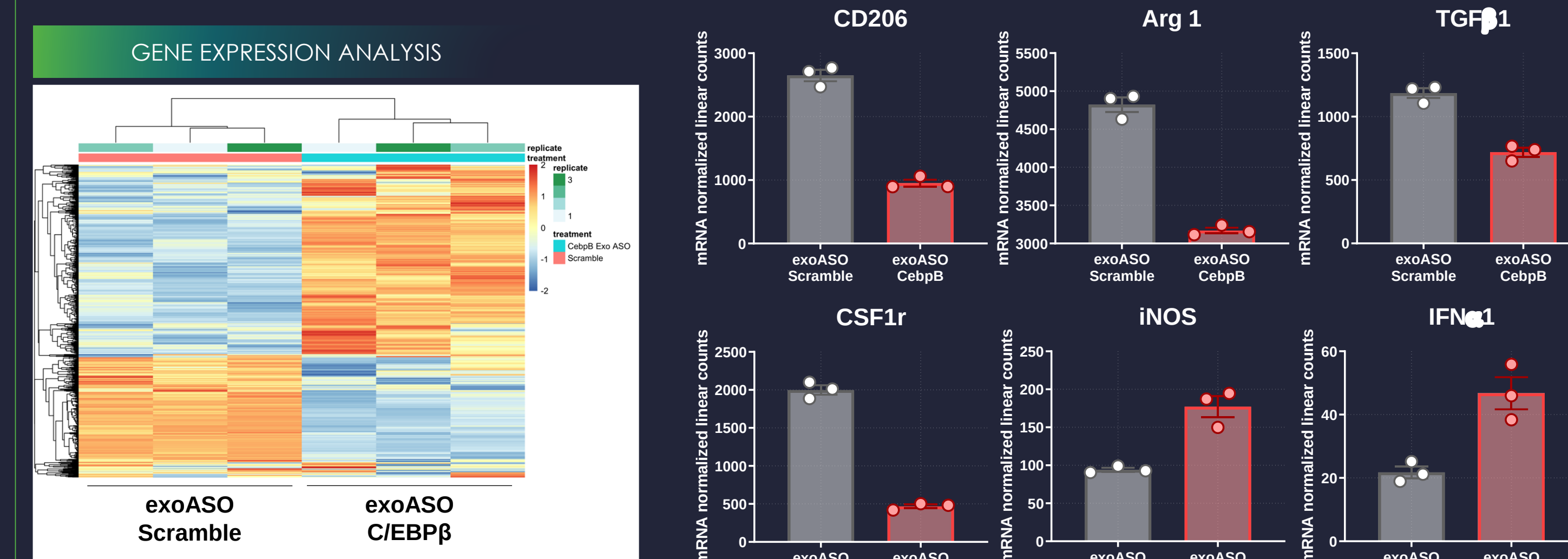


Effective silencing of C/EBPβ *in vivo* in TAMs by exoASO



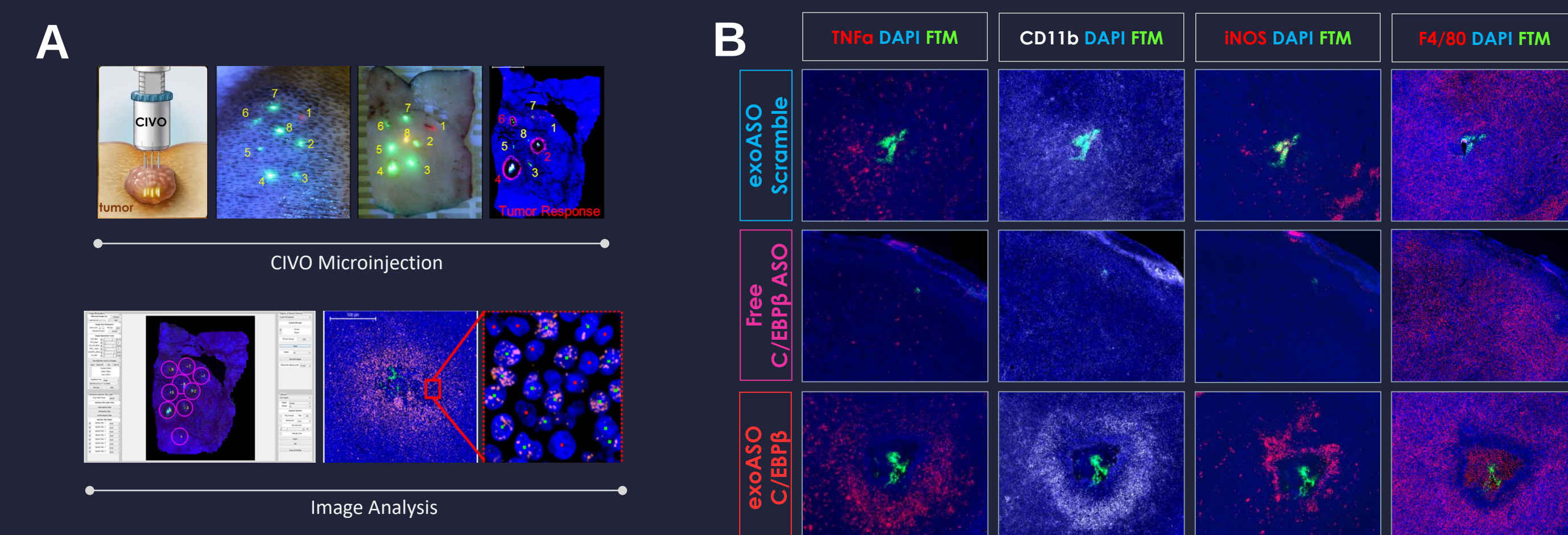
***In vivo* knockdown by exoASO.** CT26 tumors were treated IT with 4μg of exoASO C/EBPβ or exoASO Scramble, 3 injections (q.o.d.). After treatment, tumor-associated myeloid cells were isolated using CD11b-positive selection magnetic bead isolation. **A-B:** C/EBPβ (**A**) and Arg-1 (**B**) knockdown in pre- and post-enrichment samples were analyzed by qPCR in whole tumor and in tumor CD11b+ cells. *, P < 0.05, ** P < 0.01 and ***, P < 0.001 by unpaired two-tailed t-test comparing exoASO C/EBPβ and exoASO Scramble

Effective reprogramming of M2 macrophages *in vivo* by knockdown of C/EBPβ



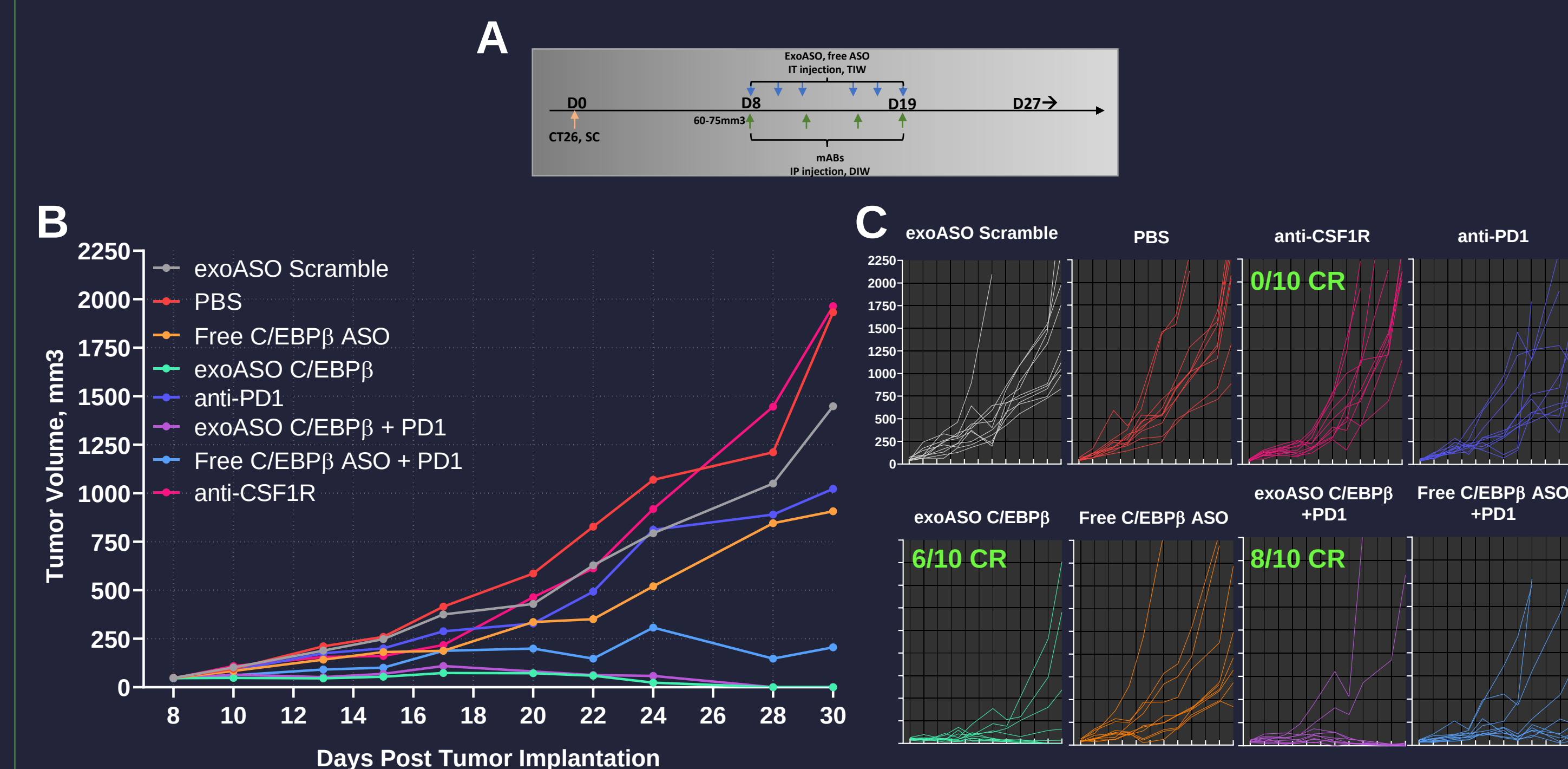
***In vivo* reprogramming of TAMs by exoASO.** CT26 tumors were treated IT with 4μg of exoASO C/EBPβ or exoASO Scramble, 3 injections (q.o.d.). After treatment, tumor-associated myeloid cells were isolated using CD11b-positive selection magnetic bead isolation. Gene expression analysis in the enriched myeloid fraction was performed by Nanostring using the *nCounter Human Myeloid Innate Immunity Panel v2*.

Robust induction of pro-inflammatory M1 markers *in vivo* with C/EBPβ exoASO



Induction of pro-inflammatory M1 markers following exoASO treatment, by CIVO®. YUMM1.7 tumors were dosed with IT microinjections of exoASO C/EBPβ, Free C/EBPβ ASO, or exoASO Scramble. Mice were euthanized 24 hours after one single dose, (n=6 mice per group). **A:** Schematic for the Comparative In Vivo Oncology (CIVO®) Platform by Presage Biosciences. **B:** Expression of TNFα, CD11b, iNOS and F4/80 by In situ hybridization. Each panel row is a different injection site on the same tumor. FTM: fluorescent tracking marker denoting the injection site.

Potent monotherapy and combination anti-tumor activity with C/EBPβ exoASO



Anti-tumor activity of exoASO C/EBPβ. CT26 tumor cells were implanted SC in the flanks of mice (n = 10 per group). ExoASO and free ASO were dosed IT and antibodies intraperitoneally following dosing regimen in (**A**). **A:** Dosing scheme. **B-C:** Tumor volumes of testing agents. Geometric means of tumor volumes are depicted in (**B**). Individual tumor growth curves are shown in (**C**). CR: Number of complete responses.

Summary

- exoASO is a novel, engineered exosome that can selectively deliver antisense oligonucleotides to tumor associated M2 macrophages.
- exoASO enables selective silencing of C/EBPβ, a key transcription factor that controls the immunosuppressive program.
- Effective silencing of C/EBPβ both *in vitro* and *in vivo*, lead to the modulation of key factors involved in the M2 → M1 transition
- Intratumoral administration of exoASO C/EBPβ resulted in potent monotherapy anti-tumor responses, resulting in 60% complete responses via IT route of administration.
- Reprogramming of tumor associated myeloid cells by targeting C/EBPβ resulted in vastly improved anti-tumor outcomes, as compared to anti-CSF1R, a macrophage-depleting therapy
- Collectively, exoASOs against C/EBPβ represent a first-in-class strategy to target tumor-associated myeloid cells in a highly selective manner.