STINGel: Controlled Release of a Cyclic Dinucleotide for Enhanced Cancer Immunotherapy

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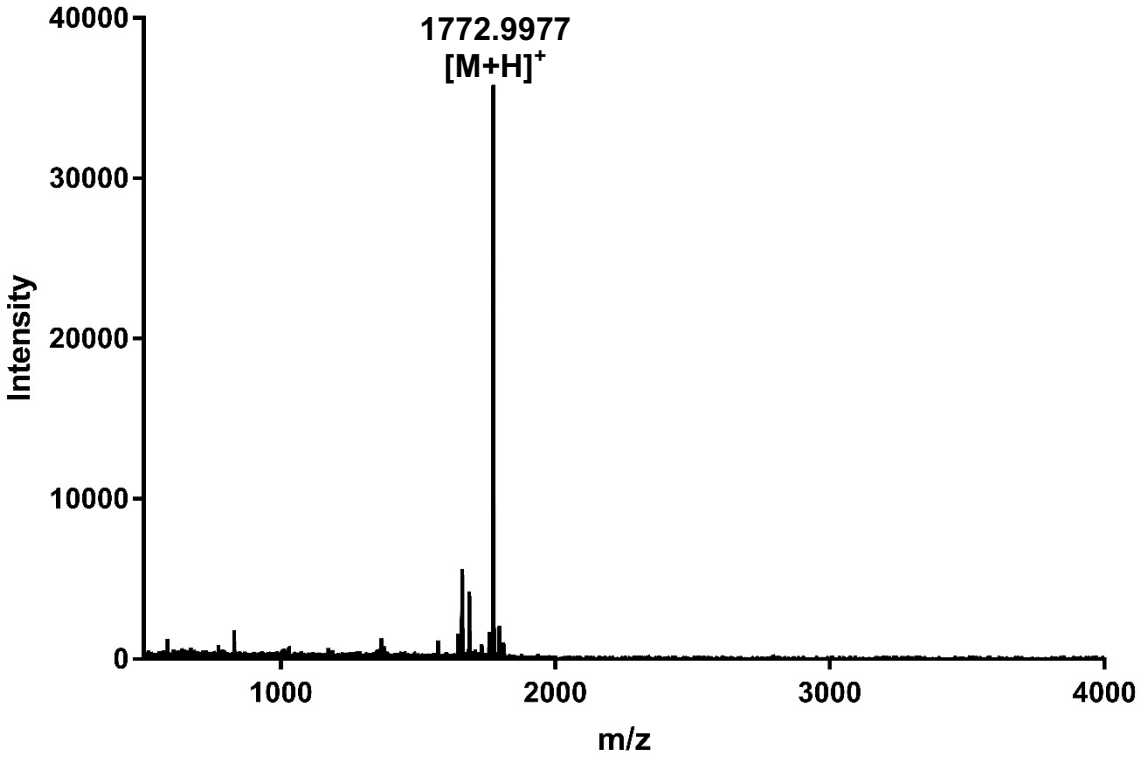


Figure S1. MALDI-TOF MS of MDP $K_2(SL)_6K_2$ purified peptide (amidated C-terminus, acetylated M-terminus). Expected mass is 1772.103 g/mol. The peak around 1660 g/mol is a small leucine deletion.

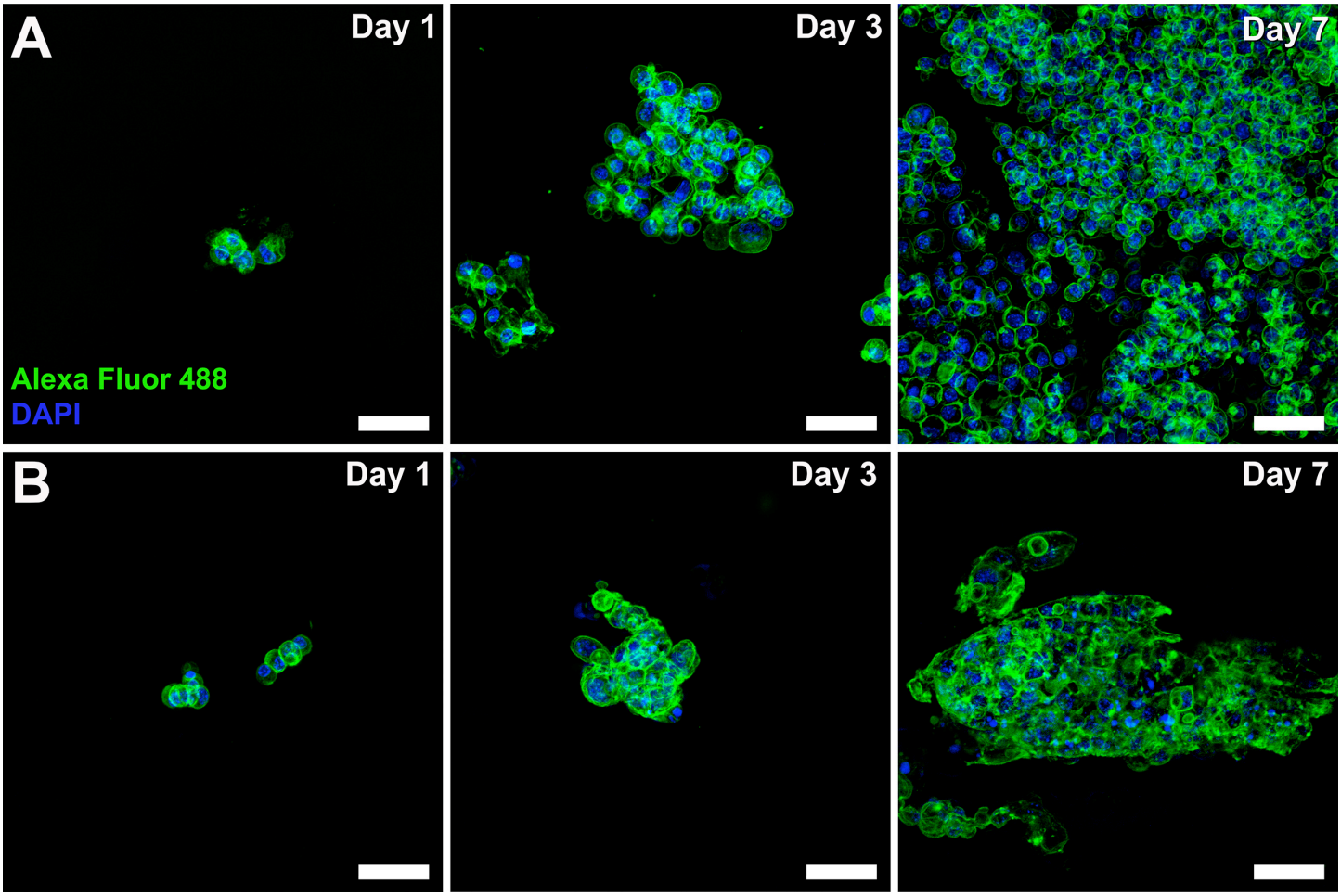


Figure S2. Cell proliferation study of MOC2-E6E7 cells seeded (A) in 2D on the surface of MDP unloaded hydrogel, (B) in 3D within MDP hydrogel (all in the absence of CDN). For 2-D, cells were seeded at a density of approximately 500 cells on the surface of a 70 µL gel puck, and started as small clumps that grew over time into wide-spread colonies over the whole surface of the gel. For 3-D, cells were seeded at a density of approximately 35,000 cells in 70 µL of gel, and started as small clumps that grew over time into large tumor-like colonies within the gel. Stains are Alexa Fluor 488 phalloidin for cytoskeleton, and DAPI for cell nuclei. Scale bars are 50 µm.

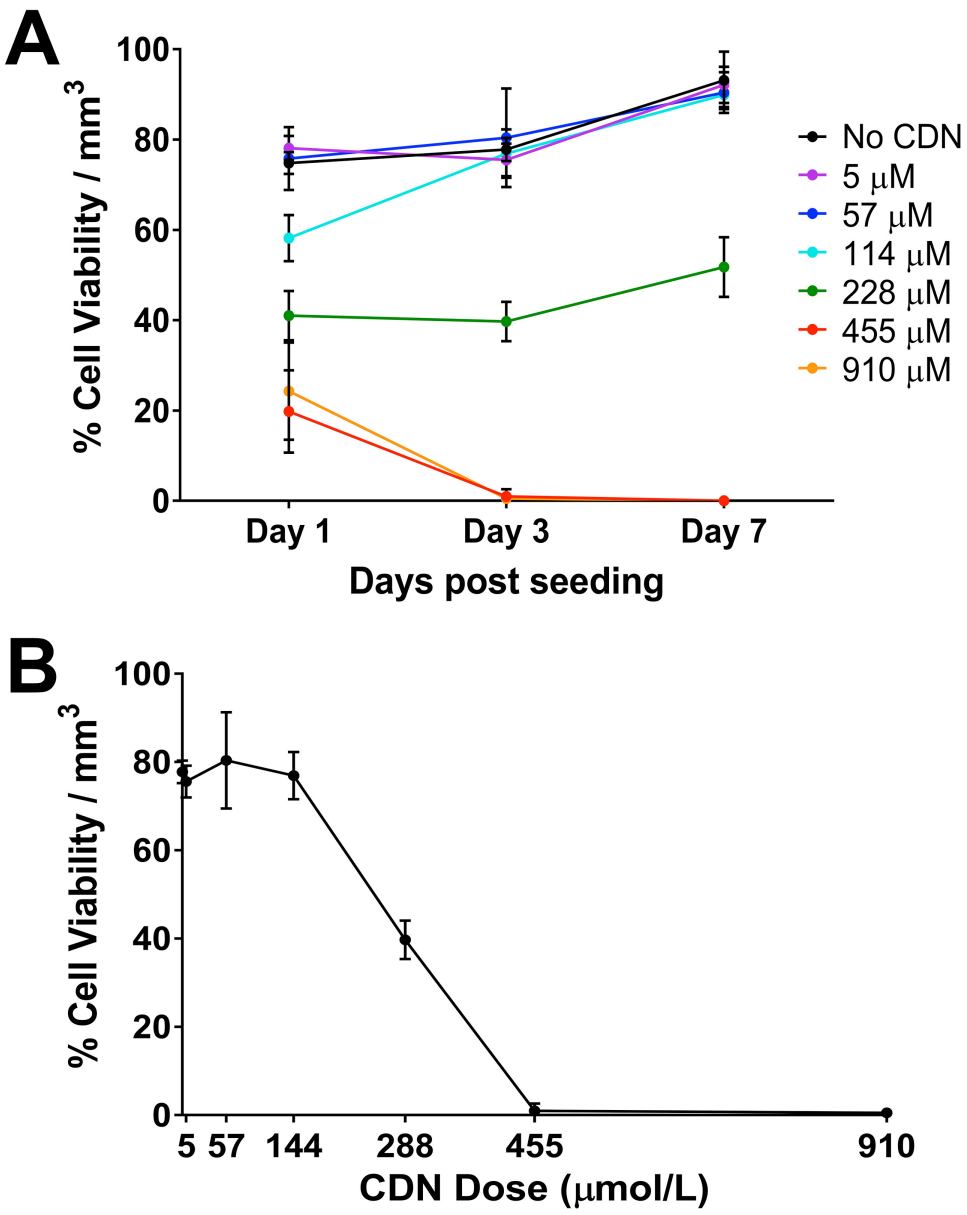


Figure S3. Live/dead viability assay quantification. (A) Percent cell viability per mm^3 of hydrogel over days 1-7 with increasing concentrations of CDN. (B) Dose response curve (day 3 timepoint) used to assess CDN toxicity to MOC2-E6E7 cells, showing percent viable cells per mm^3 of hydrogel at tested doses.

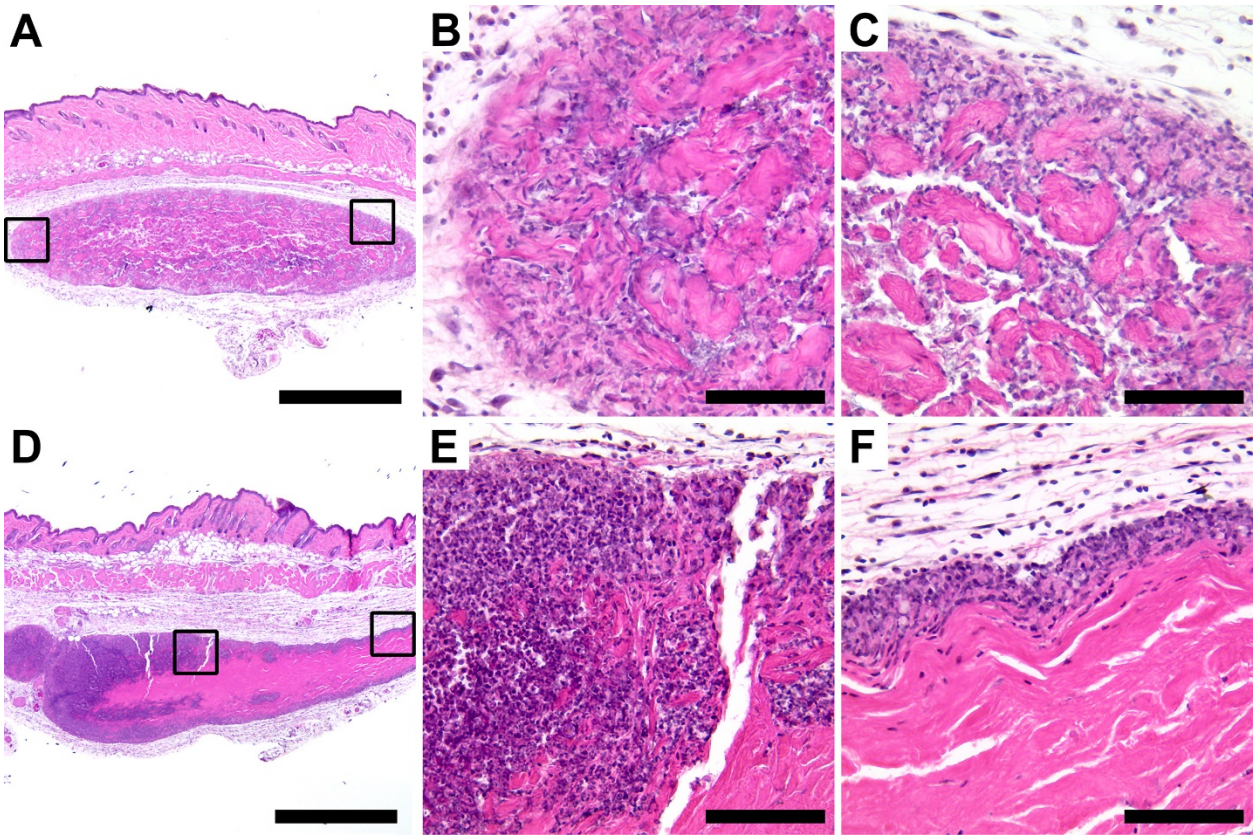


Figure S4. H&E stained MDP peptide hydrogel implant unloaded and loaded with CDN, injected subcutaneously in mice. Time point shown is 3 days post injection, at which time hydrogel implant was removed and processed for histology. 4X scale bars in panels A and D are 1 mm; 40X scale bars in panels B, C, E, and F are 0.1 mm. (A-C) MDP control implant at 4X magnification showing even infiltration of cells, with boxes drawn around relevant areas whose 40X counterparts are shown in panels B and C, respectively. (D-F) MDP hydrogel implant loaded with 910 μM CDN (STINGel) at 4X magnification showing uneven infiltration of cells across the implant. Boxes drawn around relevant areas in panel D again have 40X counterparts shown in panels E and F, respectively.

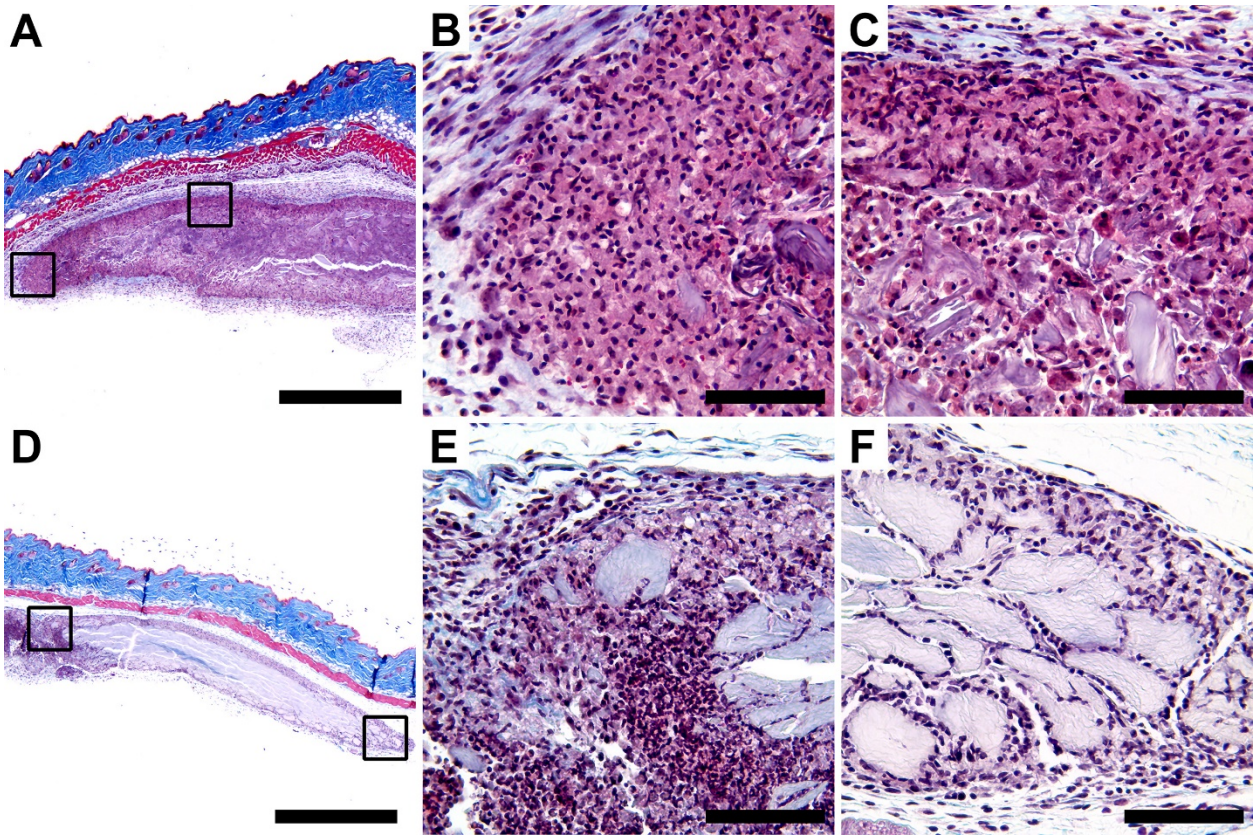


Figure S5. Masson's trichrome stained MDP peptide hydrogel implant unloaded and loaded with CDN, injected subcutaneously in mice. Time point shown is 7 days post injection, at which time hydrogel implant was removed and processed for histology. 4X scale bars in panels A and D are 1 mm; 40X scale bars in panels B, C, E, and F are 0.1 mm. (A-C) MDP control implant at 4X magnification showing even infiltration of cells, with boxes drawn around relevant areas whose 40X counterparts are shown in panels B and C, respectively. (D-F) $K_2(SL)_6K_2$ hydrogel implant loaded with 910 μM CDN (STINGel) at 4X magnification showing uneven infiltration of cells across the implant even at day 7 post injection. Boxes drawn around relevant areas in panel D again have 40X counterparts shown in panels E and F, respectively.

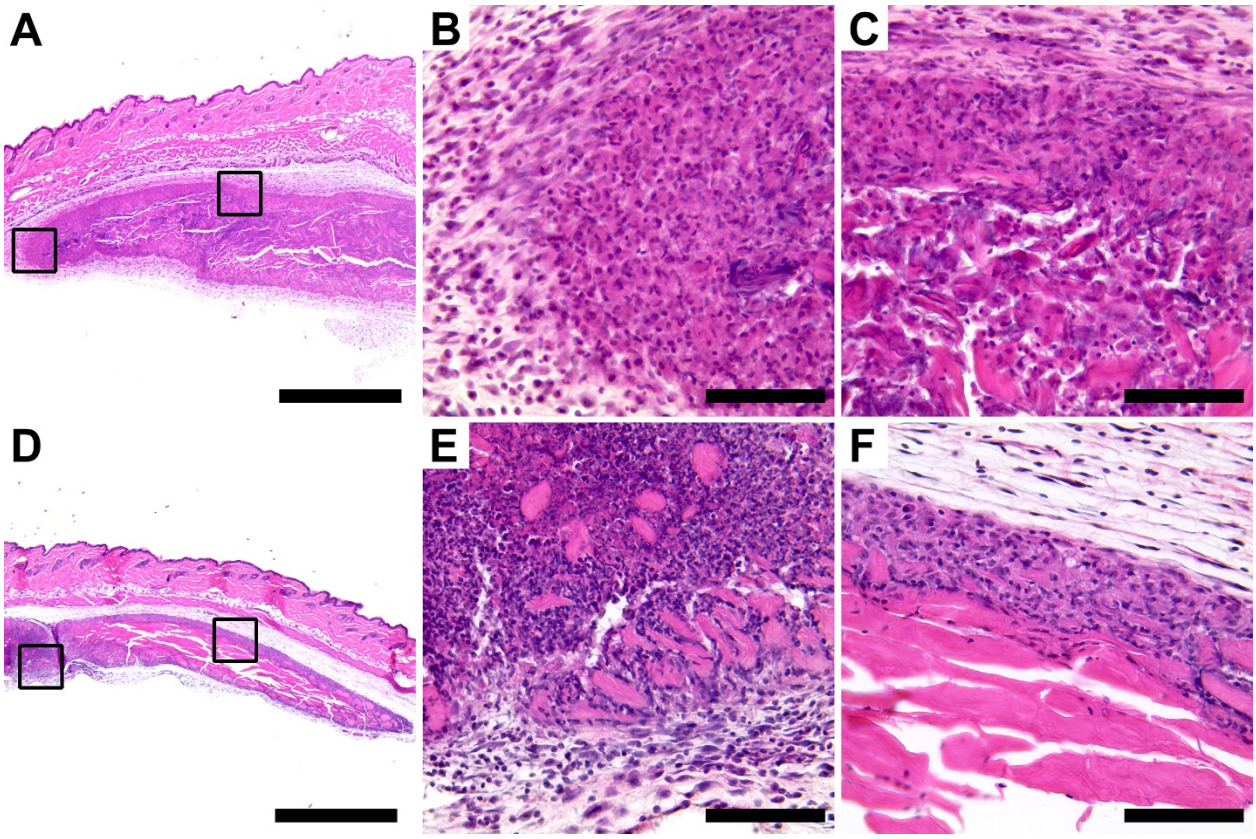
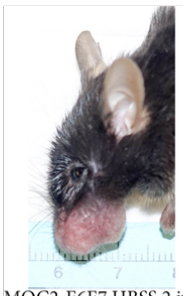


Figure S6. H&E stained MDP peptide hydrogel implant unloaded and loaded with CDN, injected subcutaneously in mice. Time point shown is 7 days post injection, at which time hydrogel implant was removed and processed for histology. 4X scale bars in panels A and D are 1 mm; 40X scale bars in panels B, C, E, and F are 0.1 mm. (A-C) MDP control implant at 4X magnification showing even infiltration of cells, with boxes drawn around relevant areas whose 40X counterparts are shown in panels B and C, respectively. (D-F) MDP hydrogel implant loaded with 910 μM CDN (STINGel) at 4X magnification showing uneven infiltration of cells across the implant even at day 7 post injection. Boxes drawn around relevant areas in panel D again have 40X counterparts shown in panels E and F, respectively.

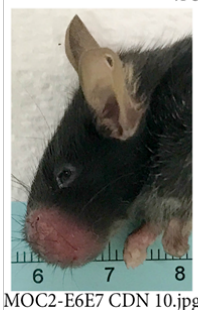
A

HBSS

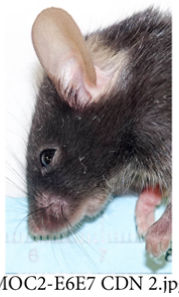


MOC2-E6E7 HBSS 2.jpg

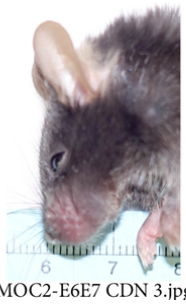
CDN



MOC2-E6E7 CDN 10.jpg



MOC2-E6E7 CDN 2.jpg



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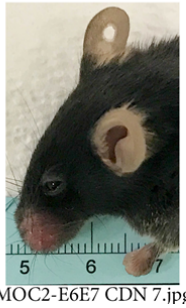
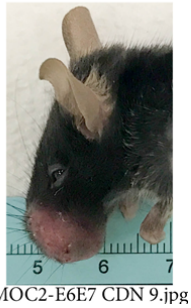


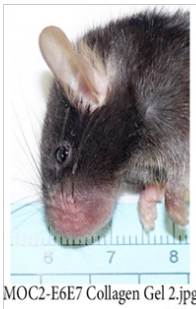
MOC2-E6E7 CDN 7.jpg



MOC2-E6E7 CDN 9.jpg

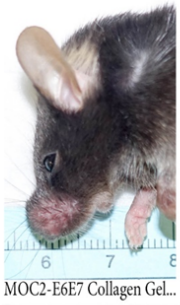
B

Collagen

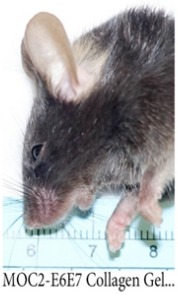


MOC2-E6E7 Collagen Gel 2.jpg

Col + CDN



MOC2-E6E7 Collagen Gel...



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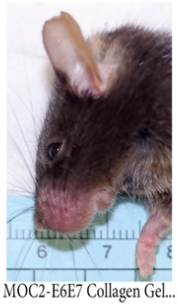

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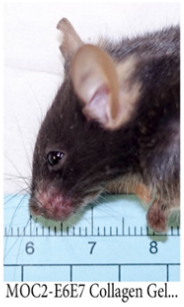

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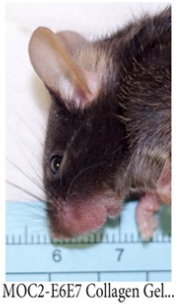

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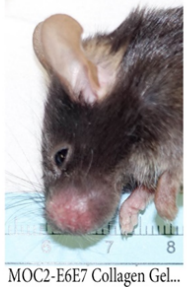

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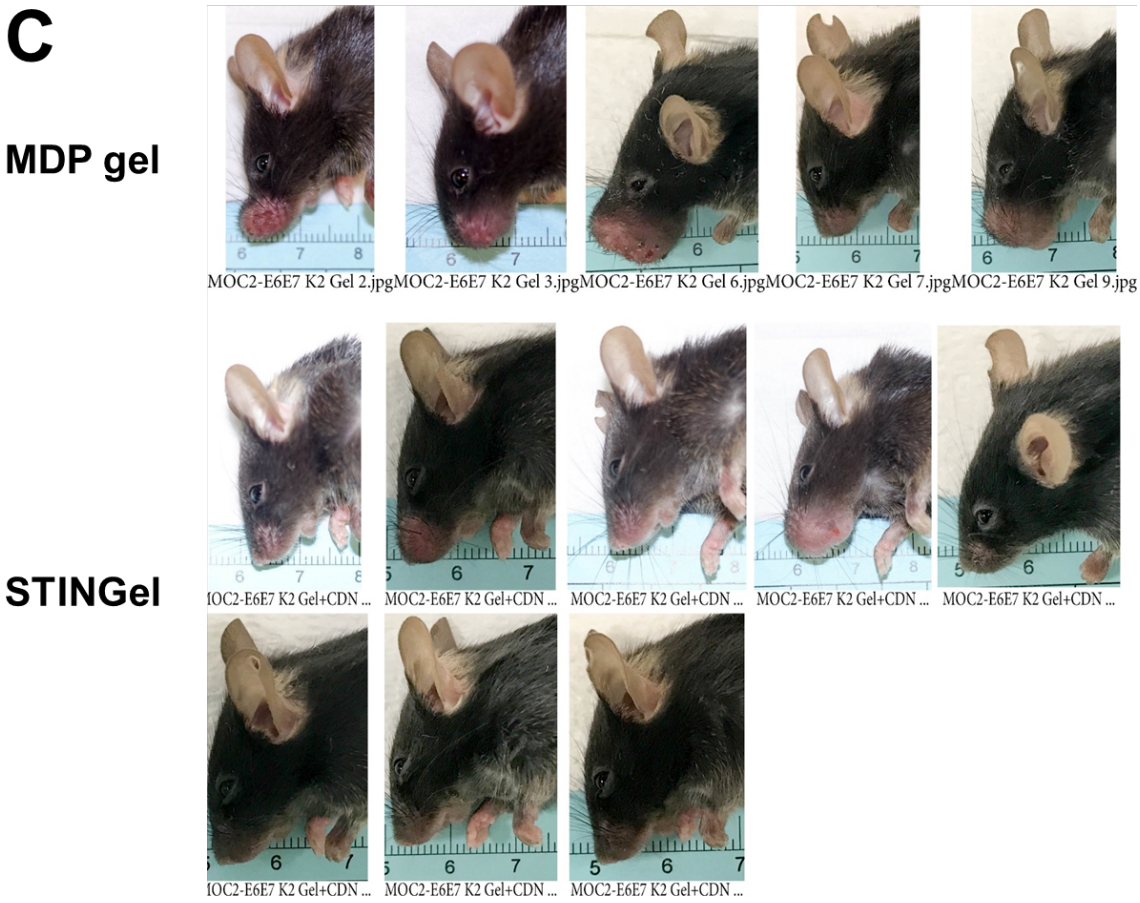

Figure S7. Representative time point midway through *in vivo* survival experiment, showing all groups at day 37 post inoculation with MOC2-E6E7 tumor cells. All surviving mice from each group (n=10) are shown (whether growing tumors or not). (A) HBSS control group and CDN alone treatment group. (B) Collagen control group and collagen + CDN treatment group. (C) MDP control group and STINGel treatment group.

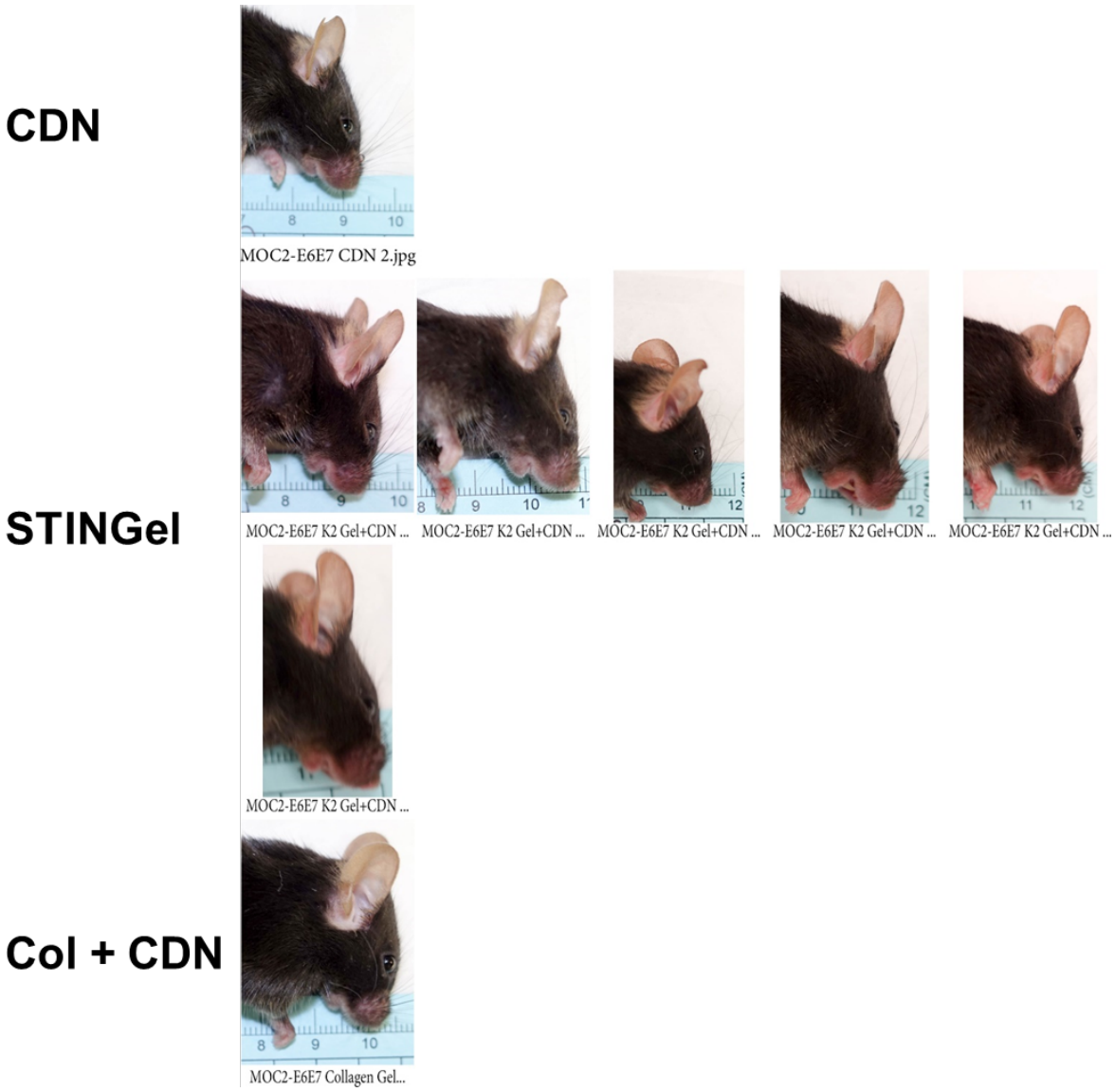

Figure S8. Day 105 time point near the end of the *in vivo* survival experiment (n=10 for each group), showing all mice that survived the initial test and were rechallenged on day 105 with a secondary inoculation of MOC2-E6E7 tumor cells on the opposite cheek (thus mice are pictured facing the opposite direction to show relevant side). All mice pictured here showed acquire immunity, surviving rechallenge with no tumor growth to day 140 when the study was concluded. No mice from controls (HBSS, MDP gel, or collagen gel) survived to this point.