Online Materials and Methods

Oligonucleotide Synthesis

Oligonucleotides were synthesized with a Mermade 48 (Bioautomation) using standard solid phase phosphoramidite methodology. Bases and reagents were purchased from Glen Research and Chemgenes. All oligonucleotides were purified by reverse-phase high performance liquid chromatography (HPLC) and molecular weights were measured using matrix-assisted laser desorption/ionization (MALDI) analysis.

Preparation of SNA Nanoconjugates (Supplementary Figure S1)

Liposomes were synthesized by extrusion of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) (NOF America) hydrated (100 g/L) in phosphate buffered saline solution (PBS) (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4, Hyclone) using polycarbonate membranes with 100 nm and 50 nm pores (Sterlitech). Liposome diameters were measured using dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments). Lipid concentration was determined using a phosphatidylcholine quantification kit (Sigma). Cholesterol conjugated oligonucleotides were attached to the surface of liposomes by mixing oligonucleotides to liposomes in a 100:1 ratio followed by incubation at room temperature for 4 h. L-SNAs were isolated from unreacted materials and concentrated using tangential flow filtration (TFF) using a MiniKros Pilot *i* System (Spectrum Labs) with a 30 kD molecular weight cutoff modified polyethersulfone (mPES) hollow fiber filter module. The purified solution containing L-SNAs were analyzed for lipid concentration using a phosphatidylcholine quantification kit. The liposome concentration was calculated using phospholipid concentration, liposome diameter, and phosphatidylcholine headgroup area (0.71 nm). Oligonucleotide concentration was measured with a Cary 100 Bio

UV/Vis spectrophotometer (Agilent) by dissolving the liposomes in 90% methanol to liberate the oligonucleotide. On average 100 oligonucleotides were measured per liposome. The oligonucleotide content of the L-SNAs after TFF was 99.9% of the added reactant oligonucleotides, demonstrating a nearly complete reaction of oligonucleotides with the liposomes in the self-assembly process. The L-SNA diameters were measured using dynamic light scattering using a Malvern Zetasizer Nano, which revealed a slight increase in diameter (2-3 nm) of the L-SNAs compared to the liposome diameter, which is indicating surface functionalization with the oligonucleotides.

Real-time quantitative PCR

To determine the knockdown capability in human keratinocytes and mouse cells, foreskin NHEKs were grown in Medium 154 supplemented with human keratinocyte growth factor (M154+HKGS; Invitrogen/Cascade Biologics, Portland, OR) and 0.07 mM calcium chloride (Ca^{2+}) and mouse J2 fibroblasts in J2-3T3 fibroblast growth media (DMEM High glucose, 10% heat inactivated Normal Calf Serum, 4 mM L-glutamine, 10 μg/ml gentamicin, 0.25 μg/ml amphotericin B). NHEKs and mouse J2 fibroblasts were treated with or without 10 ng/ml human or mouse TNFα for 24 h and then were treated once with 1.5 nM or 6 nM L-SNA nanoconjugates for 48 h. Total mRNA was isolated using the RNeasy Mini Kit (Qiagen). Using 1 μg of total RNA template, cDNA was prepared using SuperScript II reverse transcriptase (Invitrogen) and oligo(dT) and random hexamer primers. mRNA levels were measured by real- time quantitative PCR analysis using the Roche LightCycler 96. Sequences for the PCR primers are listed in Supplementary Table S1. Transcript levels were corrected for mouse *Gapdh* (mouse studies) or human *RPLP0* (human studies) mRNA levels to standardize for RNA amount. Total mRNA from 3D skin equivalents and the epidermis/dermis (fat removed) of back skin after sacrificing mice was extracted, mRNA quantified, and expression

measured as described for cultured cells (see Supplementary Table S1 for primer sequences).

Fluorescence Microscopy (Fig.1)

Foreskin NHEKs in 3rd passage were grown in Medium 154 supplemented with human keratinocyte growth factor (M154+HKGS; Invitrogen/Cascade Biologics, Portland, OR) and 0.07 mM calcium chloride (Ca^{2+}) to 70% confluence. NHEKs were incubated with 6 nM of fluorescently labeled Cy5-L-SNA and were fixed with 4% paraformaldehyde at indicated time points during 24 h. DAPI was used to label cell nuclei. Cells were imaged with a Zeiss Axio Imager.Z1 with ApoTome.

Non-identified normal human female abdominoplasty skin and 5 mm fresh biopsy samples from female psoriatic upper arm skin were obtained after signed informed consent through the Northwestern Skin Disease Research Center. Within 2 h of acquisition, the skin was placed into culture at the air-media interface in DMEM with 10% FBS at 37ºC, 5% CO_2 and treated on the surface with 30 μM Cy5-labelled L-SNA mixed in Aquaphor®:PBS 1:1; a small piece of sterile parafilm was gently placed at the center of the skin specimen to retain the L-SNAs at the biopsy surface. After 24 h, organ cultures were embedded in OCT media, and sectioned (4 μm) for fluorescence imaging. Nuclei were stained with DAPI and the sections were viewed by confocal microscopy on a Nikon A1R+ Confocal Laser Microscope.

3D Culture Model of Psoriasis (Fig.1 and Supplementary Figure S2)

For raft cultures, primary foreskin NHEKs were expanded and grown in Medium 154 supplemented with human keratinocyte growth factor (M154+HKGS; Invitrogen/Cascade Biologics, Portland, OR) and 0.07 mM calcium chloride (Ca^{2+}). 3T3-J2 cells were expanded in J2 media (see above) and resuspended in ice-cold 10X Collagen Plug Resuspension Buffer. Ice-cold 10X DMEM and 5 mg/ml rat tail collagen I (final concentration; 4 mg/ml) was added and the collagen-fibroblast slurry was pipetted into a 12-well tissue culture plate. The culture was allowed to incubate at 37^{o}C for 20 min to allow for polymerization, E-medium (DMEM F12; 18 μM adenine; 0.5 μg/ml human recombinant insulin; 0.5 μg/ml human apotransferrin; 0.5 μg/ml triiodothyronine; 10 μg/ml gentamicin; 0.25 μg/ml amphotericin B; 4 mM L-glutamine; 0.4 μg/ml hydrocortisone; 10 ng/ml cholera toxin; and 5% FBS) was added to each well and cultures were incubated for at least 24 h prior to seeding keratinocytes. Organotypic cultures were lifted onto wire mesh and cultured at the liquid-air interface 48 h after seeding.

A model of psoriasis was induced by adding mixed human TNF, IL-17A, and IL-22 (each 10 ng/mL) to culture medium beginning 6 days post-organotypic culture lifting, and 50 nM L-SNAs (Scr and TNF) were applied to the raft center every other day, beginning 3 days after cytokine initiation. Rafts were harvested 7 days after cytokine initiation (13 days post lifting), and either lysed for mRNA/protein analysis or fixed in 10% neutral-buffered formalin for histological analysis.

Histological (H&E) and Immunohistological Analyses (IHC)

Formalin-fixed and paraffin-embedded human 3D organotypic cultures and 2 cm long strips of mouse skin were sectioned (4 μm) and stained with hematoxylin and eosin (H&E). Mouse skin

sections were also processed for immunohistological analysis (CD3 and Ki67) using 3,3'-diaminobenzidine (DAB) as a chromogen and a hematoxylin counterstain. All H&E and IHC processing was performed by our Skin Disease Reasearch Center core facility. To measure acanthosis in the 3D rafts, two non-overlapping photographs were taken at 0.5 cm from the ends of each sample and 3 equally-spaced measurements (length from the base of the stratum corneum to the base of the keratinocytes) were made across the specimens with a mean epidermal thickness generated for each raft. As a second measurement, the area of the subcorneal epidermis was measured and divided by the length of epidermis at the stratum corneum for each sample (area (μm^2)/length (μm) (Supplementary Figure S3). To measure mouse skin thickness, two non-overlapping photographs were taken at 0.5 cm from the ends of each 2 cm skin strip for analysis and 5 equally-spaced measurements were made across the specimens with a mean skin thickness generated for each mouse. ImageJ software (NIH) was used to quantify epidermal thickness, area and length for all samples. Cells staining $CD3^+$ and $Ki67^+$ immunohistochemically were quantified by counting positively stained cells in the epidermis in 5 non-overlapping fields per sample and plotted as Mean$\pm$SEM for each experimental group. Measurements and cell counts were performed by two reviewers blinded to specimen identification and results were pooled.

Mice and treatments

Male C57BL/6 mice were purchased from Jackson Laboratory. Mice were kept under specific pathogen-free conditions and provided with food and water ad libitum. All experiments were approved by the Northwestern University Institutional Animal Care and Use Committee (IACUC). Mice at 6 wks of age received a daily topical dose of 62.5 mg of commercially available IMQ cream (5%) (Taro) on the shaved/depilated back for 6 consecutive days (van der

Fits, 2009). In preliminary studies, we confirmed that serial application of L-SNAs in Aquaphor®: PBS 1:1 vehicle and IMQ cream had no effect on the ability of the L-SNA to knock down *Tnf*, regardless of order applied (Supplementary Figure S4a) or in generating a psoriasis-like model (Fig. 2). In addition, preliminary investigations with application of Scr L-SNA vs *TNF* L-SNA (0, 10, 50, and 100 μM) determined 50 μM to be the optimal L-SNA dose for *Tnf* knockdown (Supplementary Figure S4b). As a result, during a course of 6 days, mouse skin was treated with daily IMQ, but was pretreated every other day 10 mins before the daily IMQ with vehicle, 50 μM Scr L- SNA, or 50 μM *TNF* L-SNA (Supplementary Fig. S4b).

Every other day, Aquaphor®: PBS 1:1 vehicle (Veh), 50 μM Scr L-SNA, or 50 μM *TNF* L-SNA was applied to a templated area on the back of the mice treated daily with IMQ (Fig. 2b). IMQ alone and mice without IMQ served as additional controls. The treated area was covered with a plastic window to prevent displacement of the treatment, kept in place with a Tegaderm adhesive dressing.

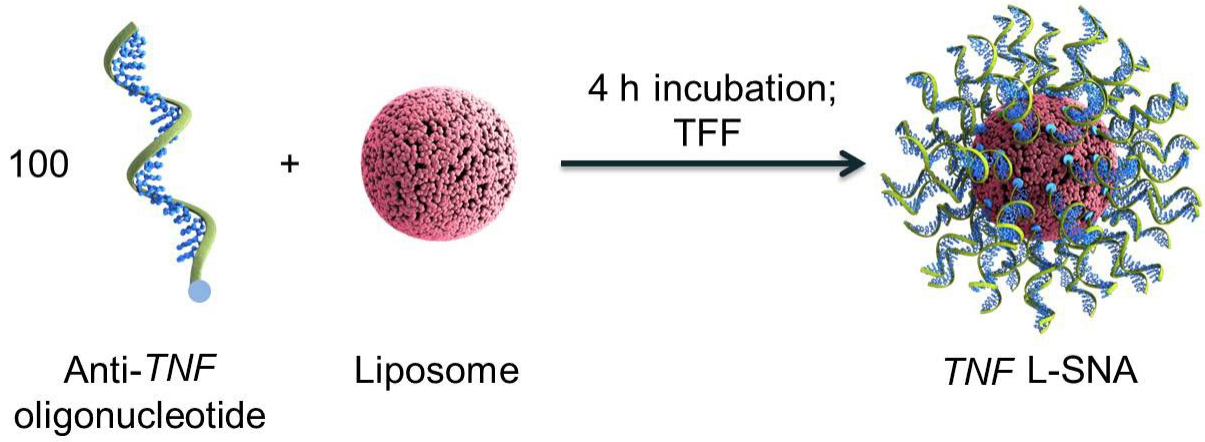
Scoring severity of skin inflammation (Supplementary Figure S5)

A modified Psoriasis Area and Severity Index (mPASI) was used to assess severity. Erythema, scaling, and thickness were scored independently by two blinded reviewers on a scale from 0 to 4 as: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked. Scores were combined from both reviewers, averaged, and the Mean±SEM plotted. Scores for each feature were added to generate the mPASI score.

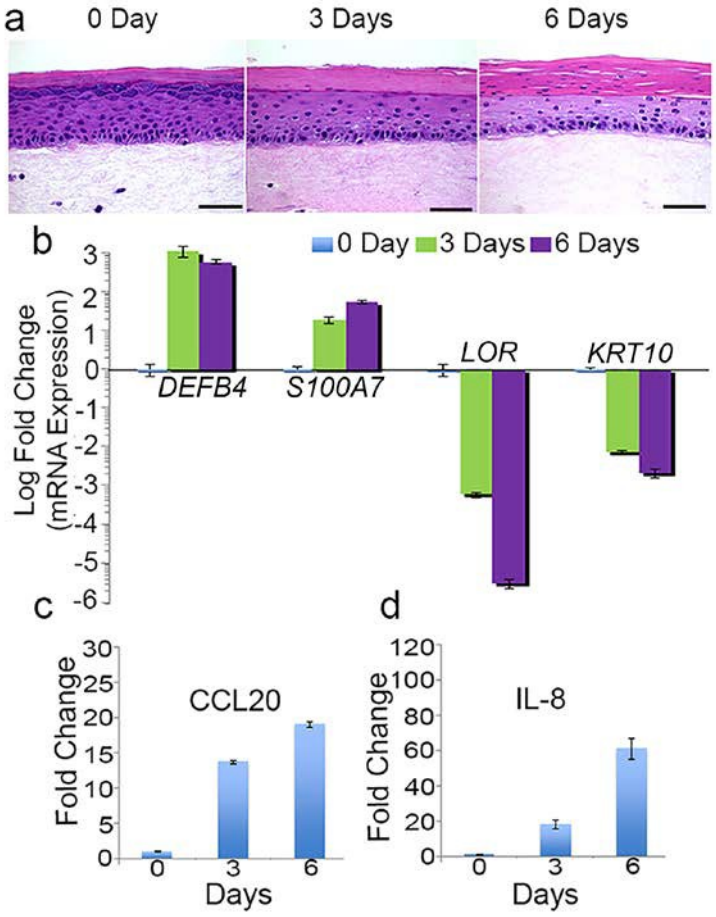
Supplementary Table Sl. **Primer sequences used for qPCR analysis of mRNA expression.**

Primer name	**Forward Sequence**	**Reverse Sequence**
Mouse *Tnf*	5-TCT TTG AGA TCC ATG CCG TTG	5-AGA CCC TCA CAC TCA GAT CA
Mouse *Lor*	5-TCT TTC CAC AAC CCA CAG G	5-CAC ATC AGC ATC ACC TCC TTC
Mouse *Defb4*	5-TGG CTC CAT TGG TCA TGC	5-TTC AGT CAT GAG GAT CCA TTA CC
Mouse *S100a7a*	5-GCT CTG TGA TGT AGT ATG GCT	5-ATC ATA CAC TGC TTC CAT CAC T
Mouse *Flg*	5-CTT CAG CGA TGT CTT GGT CAT	5-AAG GAG GAA GAA ACA CTG AGC
Mouse *Krt16*	5-CAG CTC ATT CTC GTA CTT GGT C	5-AGA CTA CAG CCC CTA CTT CA
Mouse *Ivl*	5-GCT TTG CTT GTT CCT GCT	5-CTT CTC CCT CCT GTG AGT TTG
Mouse *Gapdh*	5- CAT GGC CTT CCG TGT TCC TA	5- CCT GCT TCA CCA CCT TCT TGA T
human *TNF*	5-TCA GCT TGA GGG TTT GCT AC	5-TGC ACT TTG GAG TGA TCG G
human *DEFB4*	5-CGC CTA TAC CAC CAA AAA CAC	5-TCC TGG TGA AGC TCC CA
human *S100A7*	5-GGT GGG AGA AGA CAT TTT ATT GTT C	5-TCT GAG TTT CTG TCC TTG CTG
human *RPLP0*	5-TGT CTG CTC CCA CAA TGA AAC	5-TCG TCT TTA AAC CCTGCG TG

Supplemental Figures

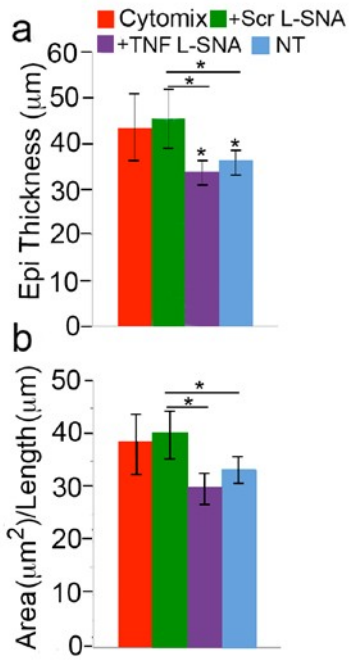


Supplementary Figure S1. **Schematic of *TNF* L-SNA preparation.** Liposomes and oligonucleotides are mixed in a 1:100 ratio to produce crude *TNF* L-SNAs. After incubation the mixture is subjected to tangential flow filtration (TFF) to isolate purified *TNF* L-SNAs.



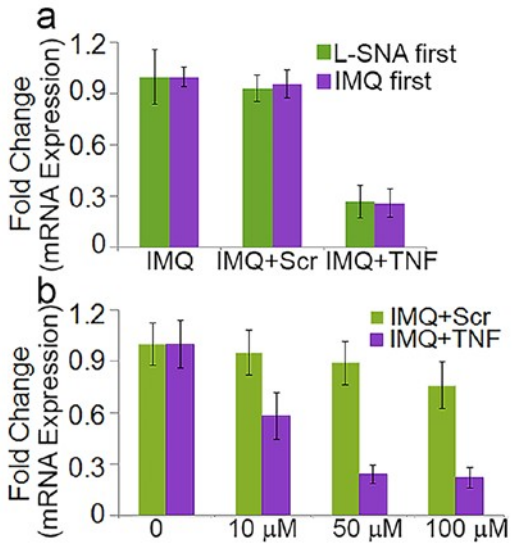
Supplementary Figure S2. **Establishing a human skin equivalent model of psoriasis.** 3D skin equivalents were established with NHEKs and, 6 days after lifting, were treated with a cocktail of 10 ng/mL each of TNF, IL-17 and IL-22 in the medium. Rafts were harvested for analysis at baseline, 3 days and 6 days after cytokine exposure. Psoriasis-like 3D rafts showed histological, mRNA expression, and protein expression changes consistent with the features of psoriasis by 3 days after cytokine exposure with maximal effect at 6 days (all data points at 3 and 6 days $p<0.001$).

(**a**) H&E-stained histological images of raft sections. Bar = 50 μm. (**b**) mRNA expression. (**c, d**) ELISA analysis of 3D rafts for CCL20 (**c**) and IL-8 (**d**). Mean±SEM, n=3 3D rafts in each group, studies performed at least 3 times.



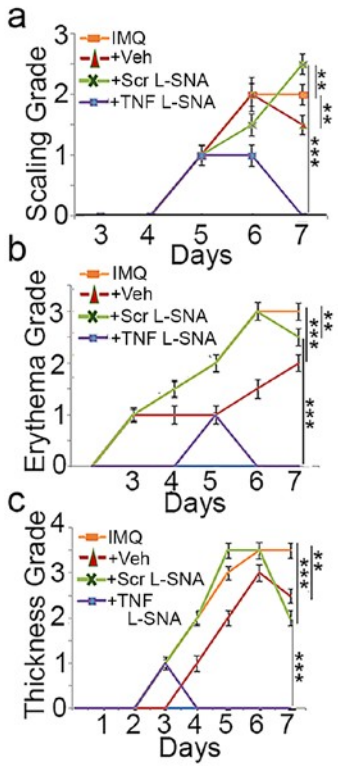
Supplementary Figure S3. **TNF L-SNA treatment improves epidermal acanthosis in the psoriasis raft model.** The extent of epidermal acanthosis was quantified in hematoxylin/eosin-stained untreated (NT) and cytokine-treated (Cytomix) psoriasis-like rafts, treated topically twice with 50 nM *TNF* L-SNAs or controls. Acanthosis was measured as: (**a**) length from the base of the stratum corneum to the base of the keratinocytes at 3 evenly spaced sites; and (**b**) area of the subcorneal epidermis/length of the stratum granulosum. Mean±SEM, (n=6 rafts in each group,

$p<0.05$, *)



Supplementary Figure S4. **Effect of serial application of L-SNAs and IMQ and optimal L-SNA dosing in the psoriatic mouse model**. (**a**) Impact on *Tnf* mRNA expression of the serial application of IMQ and then Scr or *TNF* L-SNAs 10 mins later or vice versa, in contrast to IMQ alone. (**b**) Dose-effect studies led to the selection of 50 μM *TNF* L-SNAs for studies. Studies with vehicle control were similar to those with Scr SNAs in the Aquaphor:PBS vehicle (not shown).

Mean+SEM, 6 mice in each group. Asterisks over columns are vs. IMQ+Scr-treated mice; others as indicated ($p<0.001$,***; $p<0.01$, **).



Supplementary Figure S5. **Individual components of the modified PASI (mPASI) score shown in Fig. 2d.**

(**a**) Scaling. (**b**) Erythema. (**c**) Thickness. The composite mPASI was the sum of the 3 scores (maximum=12). Mice without IMQ treatment had scores of 0 for all components. Veh = vehicle; Scr = scrambled L-SNA 50 μM; TNF = *TNF* L-SNA 50 μM. ** p<0.01; ***p<0.001.