

# ELU535: A NOVEL BACTERIOSTATIC, TOPICAL, SPRAY-ON, SMALL MOLECULE WNT PATHWAY MODULATOR THAT STIMULATES ENDOGENOUS REGENERATION OF ELASTIC CARTILAGE IN NEW ZEALAND WHITE RABBIT EARS



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## ABSTRACT

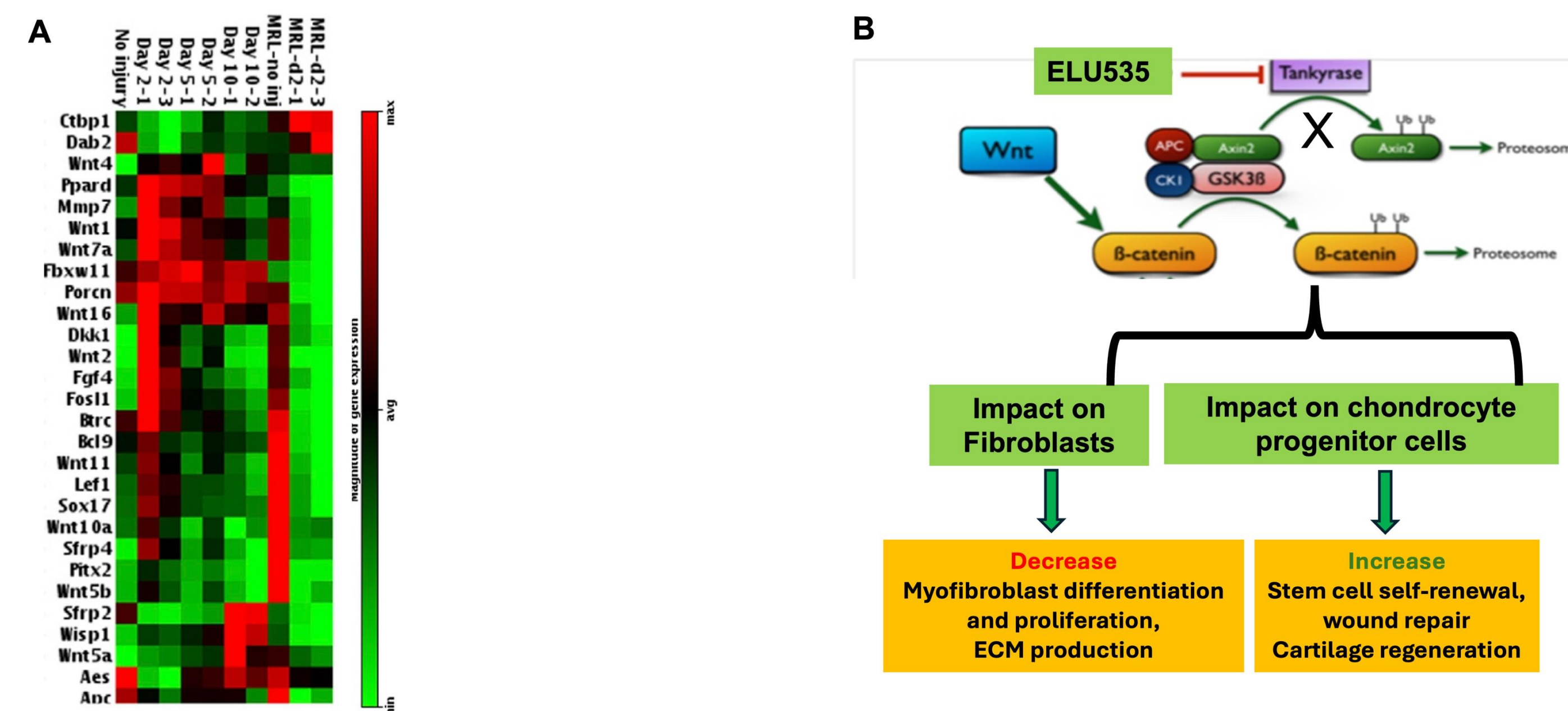
**BACKGROUND:** Auricular elastic cartilage injuries and post-surgical defects represent a significant clinical challenge. Resections of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) on the ear frequently result in partial cartilage loss, yielding structural defects that are difficult to repair due to the minimal intrinsic regenerative capacity of elastic cartilage (the main framework of the external ear). While trauma can lead to similar auricular cartilage defects, the high prevalence of BCC and SCC positions them at the forefront of unmet clinical need in this area, underscoring the urgency for regenerative therapies that can restore elastic cartilage *in situ* without grafts or prosthetics. Modulation of the Wnt signaling pathway has emerged as a promising strategy to promote true cartilage repair over fibrotic healing. Accordingly, ELU535—a novel Wnt-pathway modulator—is being investigated for its ability to activate the body's own reparative processes and induce *in situ* regeneration of auricular cartilage. This approach has the potential to transform the treatment paradigm for auricular cartilage defects by providing a regenerative solution to this longstanding clinical problem.

**METHODS:** In this study, we utilized a rabbit model to analyze the regeneration of elastic cartilage following a full-thickness ear punch hole. Eight holes (8 mm) were created in the ears of New Zealand White Rabbits. ELU535 (0.1%, 200  $\mu$ L) was applied topically three times per week to Day 21. The animals were sacrificed on Day 46, and histopathological analyses were performed on excised tissues.

**RESULTS:** Topical application of ELU535 significantly enhanced chondrogenitor cells and promoted the formation of elastic cartilage, as measured by the distance between opposing cartilage endplates as compared to saline; results were assessed using Safranin-O and Trichrome Blue staining. Wounds treated with ELU535 demonstrated a 68.47% improvement in regeneration of elastic cartilage as compared to saline-treated (23.03%) wounds ( $P \leq 0.001$  using T-test with  $N \geq 7$  per group). There was a significant rate of wound closure with ELU535 treatment (82.6%) as compared to saline (69.2%) by Day 14 ( $P \leq 0.0001$  using ANOVA ( $N = 8$ )) with complete closure observed by Day 25 with ELU535 and Day 39 with saline treatment. Remarkably, ELU535 demonstrated robust bacteriostasis against 14 different strains of bacteria (anaerobic, aerobic, gram-positive, and gram-negative), including MRSA, *P. aeruginosa*, and *S. aureus*.

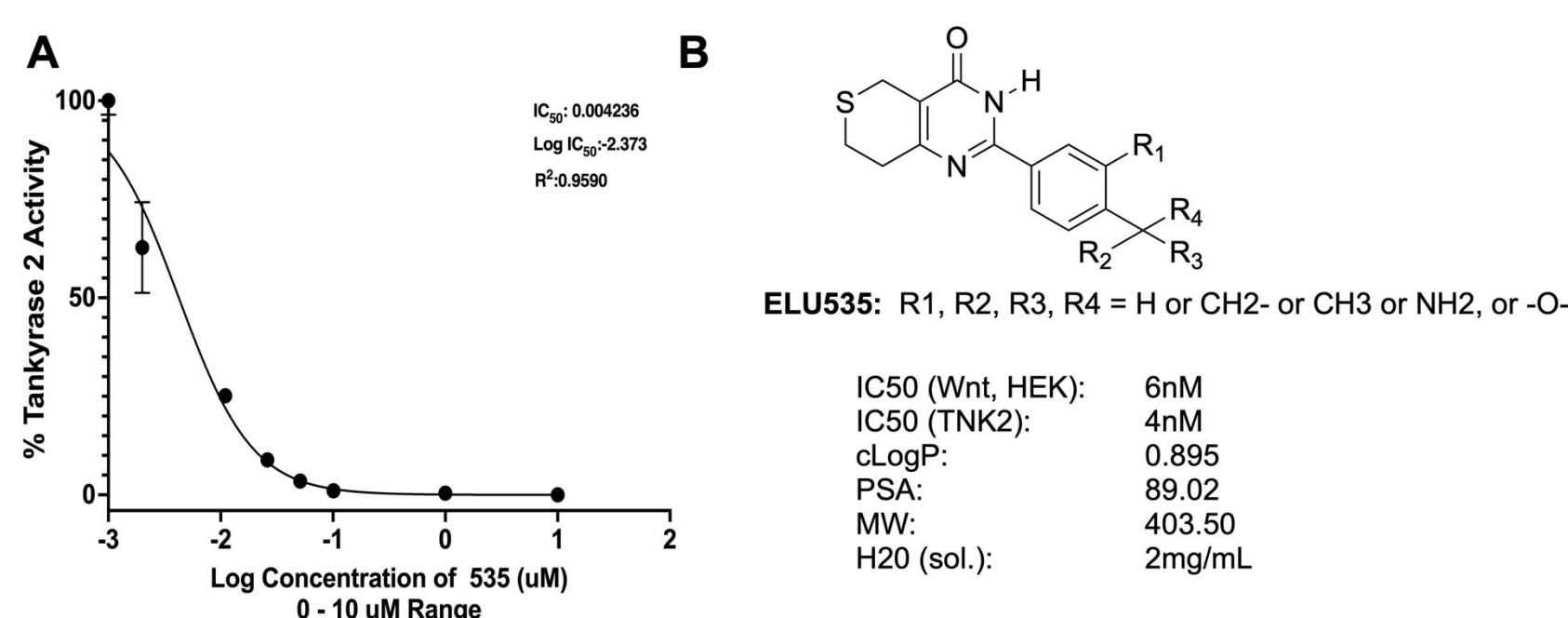
**IMPACT:** ELU535 is the first compound ever to demonstrate elastic cartilage regenerative properties. It shows strong promise as a treatment to facilitate regenerative healing following surgeries to remove BCC- and SCC-damaged tissue, as well as following traumatic injuries. ELU535 represents a paradigm-shifting regenerative approach that has the potential to redefine the Standard of Care for a tissue long considered beyond repair.

## WNT SIGNALING PATHWAY IS ACTIVATED FOLLOWING EAR PUNCH INJURY



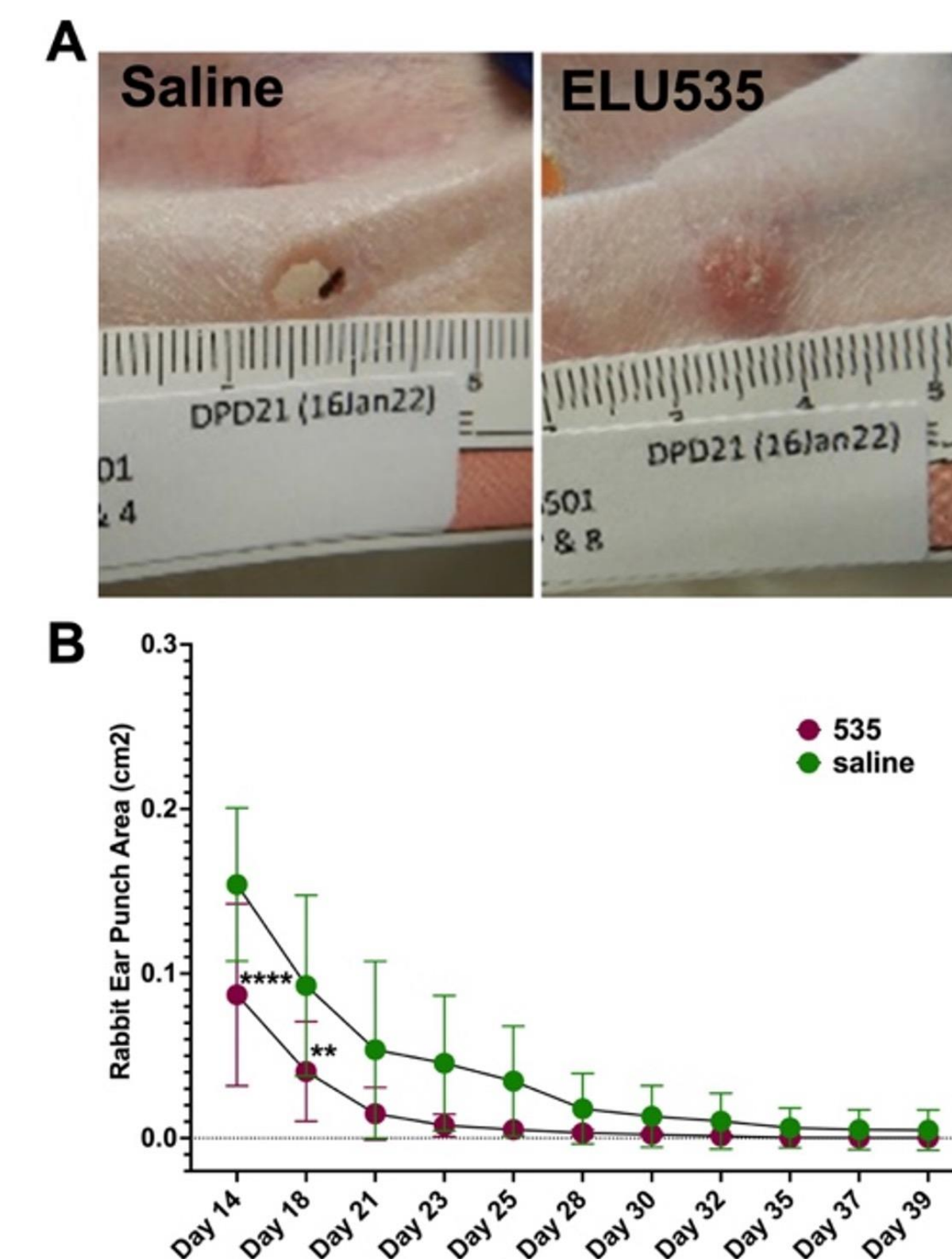
**Figure 1. Wnt signaling is activated in mouse cutaneous wounds after ear punch injury.** A) Heat map generated from gene expression PCR array of Wnt pathway-associated genes at different time points following a full-thickness ear punch hole in C57Bl6 and superhealer MRL mice. B) Proposed mechanism of action of Wnt signaling modulator ELU535.

## ELU535: FIRST-IN-CLASS, NOVEL, POTENT, AQUEOUSLY SOLUBLE, TOPICAL SMALL MOLECULE WNT SIGNALING MODULATOR FOR CARTILAGE REGENERATION



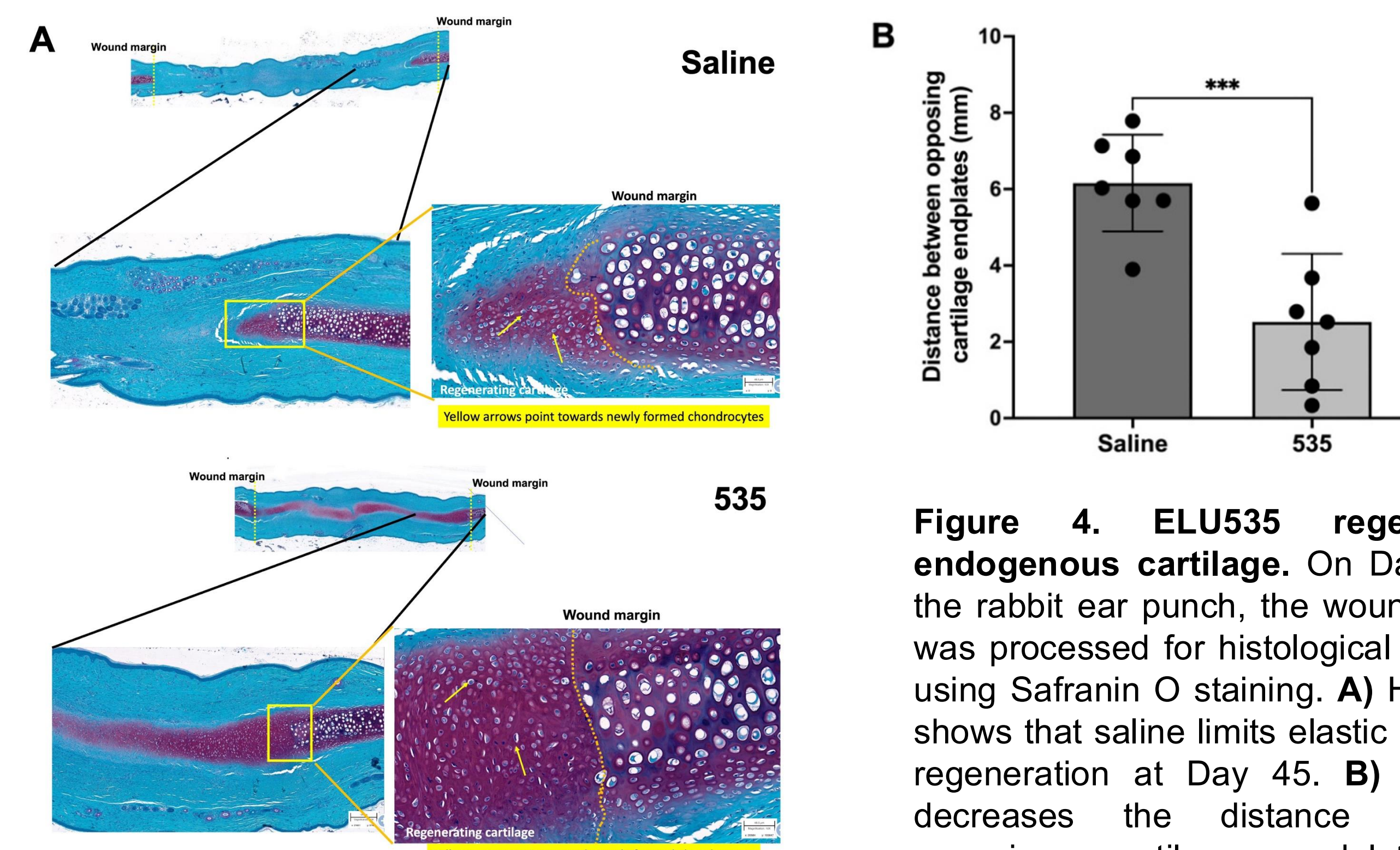
**Figure 2. ELU535: A novel potent Wnt signaling modulator.** ELU535 is a novel, potent, tankyrase inhibitor that can effectively modulate the Wnt signaling pathway. ELU535 is aqueously soluble for convenient formulation in an 81% saline spray solution. Physicochemical parameters are shown.

## ELU535 SIGNIFICANTLY IMPROVES RATE OF CLOSURE IN A RABBIT EAR PUNCH WOUND INJURY MODEL



**Figure 3. ELU535 recapitulates the superhealer phenotype and significantly improves rate of closure in a rabbit ear punch wound injury model.** To assess the effect of ELU535 in a rabbit ear punch model, eight circular, 8 mm diameter through-and-through holes were created in rabbit ears (four per ear). ELU535 (1 mg/mL solution) and saline were sprayed (200  $\mu$ L) on the holes three days per week (Mon, Wed, Fri) for 21 days. A) Rabbit ear punch wound on Day 21 treated with ELU535 or saline. ELU535 closed the hole/wound within 21 days, while saline-treated wounds remained open. B) The rate of healing of the rabbit ear wounds with either ELU535 topical administration or saline. ELU535 closed the wounds by Day 21. Saline closed the wounds in ~39 days. \*\* $P \leq 0.01$ , and \*\*\*\* $P \leq 0.0001$  were calculated using ANOVA with  $n = 8$  per group.

## ELU535 SUBSTANTIALLY REGENERATES ENDOGENOUS CARTILAGE AND REDUCES SCAR FORMATION / FIBROSIS



**Figure 4. ELU535 regenerates endogenous cartilage.** On Day 45 of the rabbit ear punch, the wound tissue was processed for histological analysis using Safranin O staining. A) Histology shows that saline limits elastic cartilage regeneration at Day 45. B) ELU535 decreases the distance between opposing cartilage endplates as compared to saline, signifying an increased degree of regeneration. \*\*\* $P \leq 0.001$  were calculated using T test with  $n = 7$  per group.

## ELU535 IS BACTERIOSTATIC AGAINST A BROAD SPECTRUM OF AEROBIC AND ANAEROBIC, GNB (GRAM-NEGATIVE), AND GPB (GRAM-POSITIVE) BACTERIA

Strain	24-Hours			72-Hours		
	Avg Result	Log Value	Log Reduction	Avg Result	Log Value	Log Reduction
<i>A. baumannii</i> (ATCC 19606)	<200	<2.3	>3.0	<200	<2.3	>3.0
<i>C. acnes</i> (ATCC 11827)	$1.7 \times 10^4$	4.2	0.7	$6.5 \times 10^3$	3.8	1.1
<i>E. coli</i> (ATCC 8739)	$5.3 \times 10^2$	2.7	1.8	<200	<2.3	>2.2
<i>E. coli</i> (ATCC 25922)	<200	<2.3	>2.3	<200	<2.3	>2.3
<i>E. coli</i> 0157:H7 (ATCC 12900)	<200	<2.3	>1.9	<200	<2.3	>1.9
<i>E. faecalis</i> (ATCC 19433)	$2.7 \times 10^5$	5.4	No Reduction	$1.9 \times 10^5$	5.3	No Reduction
<i>K. pneumoniae</i> (ATCC 10031)	<200	<2.3	>2.5	<200	<2.3	>2.5
<i>P. aeruginosa</i> (ATCC 9027)	<200	<2.3	>2.5	<200	<2.3	>2.5
<i>P. aeruginosa</i> (ATCC 15442)	<200	<2.3	>2.9	<200	<2.3	>2.9
<i>P. vulgaris</i> (ATCC 8427)	<200	<2.3	>3.6	<200	<2.3	>3.6
<i>S. aureus</i> (ATCC 6538)	$4.7 \times 10^4$	4.7	0.5	$9.3 \times 10^2$	3.0	2.2
<i>S. aureus</i> (MRSA) (ATCC 33593)	$1.5 \times 10^3$	3.2	1.6	<200	<2.3	>2.5
<i>S. epidermidis</i> (ATCC 14990)	$8.0 \times 10^2$	2.9	2.0	<200	<2.3	>2.6
<i>S. maltophilia</i> (ATCC 13637)	<200	<2.3	>2.8	<200	<2.3	>2.8
<i>S. pyogenes</i> (ATCC 19615)	<200	<2.3	>3.2	<200	<2.3	>3.2

**Table 5. ELU535 shows robust bacteriostasis against 15 different strains of bacteria (anaerobic, aerobic, GNB, and GPB).** The bacteriostatic properties of the ELU535 serum (0.25 mg/mL) were tested against a panel of bacterial strains comprised of aerobic and anaerobic, GNB and GPB. ELU535 and a control sample were spiked with 0.5 mL of the appropriate organism, such that the final concentration in the tube was  $\sim 1 \times 10^6$  CFU/mL. Negative sample controls, containing neat sample with no organism spike (one for the sample and one for the vehicle), and positive organism controls, containing 1 mL saline and 1 mL of the  $1 \times 10^6$  CFU/mL organism, were used. ELU535 exhibited robust bacteriostasis across all tested bacterial strains.

## CONCLUSIONS

- Wnt signaling pathway is upregulated following full-thickness ear punch wound.
- Eluciderm, Inc. has developed ELU535, a first-in-class, novel, potent, bacteriostatic, **topical, small molecule** Wnt signaling modulator.
- ELU535 promotes significant wound closure and endogenous cartilage regeneration.
- ELU535 exhibits **bacteriostatic activity** against both aerobic and anaerobic Gram-negative bacteria (GNB) and Gram-positive bacteria (GPB).
- ELU535 represents a significant advancement in regenerative medicine because it shifts the reconstructive repair paradigm from a reliance solely on grafts and implants to **true functional healing** via a novel topical small molecule that stimulates regeneration of endogenous elastic cartilage.
- ELU535 shows strong promise as a treatment to facilitate regenerative healing following surgeries to remove BCC- and SCC-damaged tissue, as well as following traumatic injuries.

## ACKNOWLEDGMENTS

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