Targeting TRPV1 via epigenetic modulation: Implications for chronic orofacial pain management.

12th National Summit of Health and Population Scientist in Nepal

" Health Research Governance for evidence Informed Decision Making and Implementation in Nepal"

Dr Nisha Acharya, BDS, MDS, PHD (ongoing Psychosomatic Dentistry)

Assistant Professor;
Conservative Dentistry and Endodontics
Institute of Medicine (IOM), Maharajgunj Medical Campus, Tribhuvan University Teaching Hospital

Consultant Endodontist and Restorative Dentist
Braces and Faces Dental Clinic, Kathmandu, Nepal

Vice President of Conservative Dentistry and Endodontic Association of Nepal (CDEAN)

Executive Board Member of Nepal Health Research Council (NHRC)

Email Address: nishaacharya@iomcomedu.np,

menishaacharyanew@gmail.com

INTRODUCTION

TRPV1: Transient Receptor Potential Vanilloid 1.

Core Message:

"TRPV1 is epigenetically suppressed → becomes activated

→ leads to chronic pain"

Why orofacial pain becomes chronic??

Epigenetic modifications convert TRPV1 from a regulated pain sensor into a persistently overactive discharger of chronic pain.

Even after the original injury resolves, TRPV1 stays upregulated.

Background / Problem Statement

So the key question is not just what activates pain—but what keeps the pain system permanently switched ON?

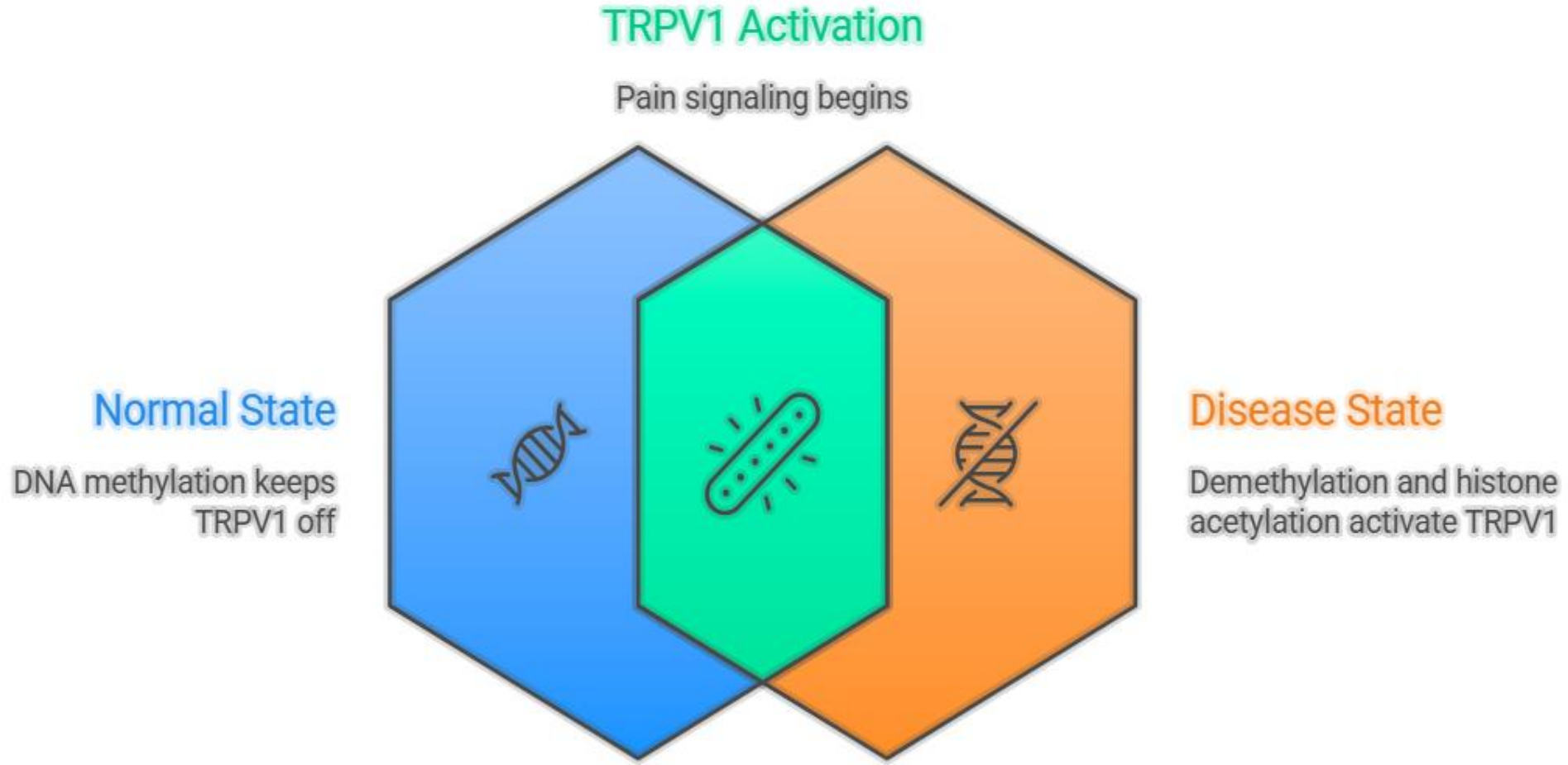
Rationale – Why Epigenetics?

Why Look Beyond Conventional Regulation?

So to test this, we designed an experimental model to directly manipulate epigenetic pathways.

If TRPV1 is the pain switch, epigenetics might be the mechanism that keeps that switch permanently ON.

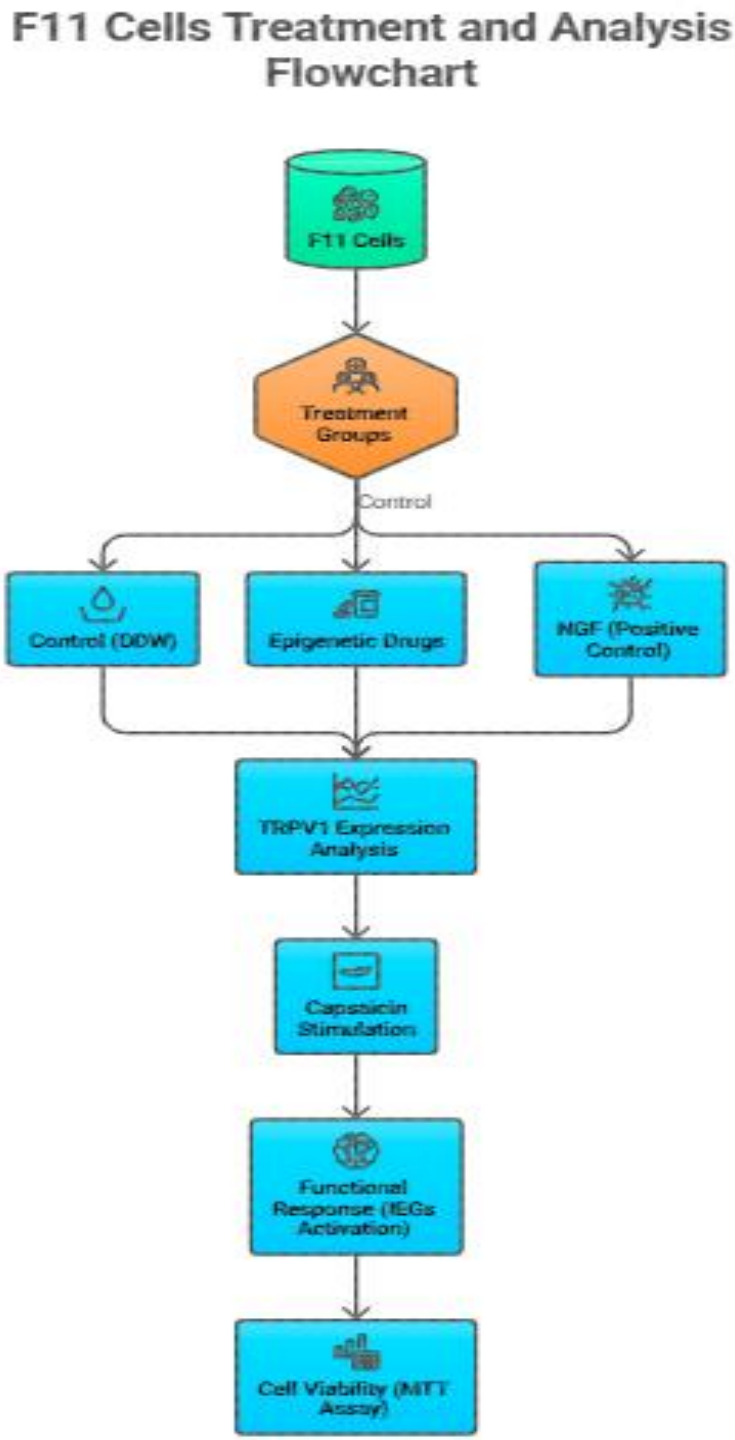
Epigenetic Control of TRPV1 and Pain



DNA methylation → TRPV1 OFF → Epigenetic changes → TRPV1 ON → Pain signaling ↑ → Chronic pain

So we are not only increasing TRPV1, but also testing whether it is functionally active and biologically relevant!!

Experimental Design and Methodology



F11 Cells

↓

Treatment Groups

→ Control (DDW)

→ Epigenetic Drugs

a. Zebularine
b. VPA
c. Combination
d. NGF (Positive Control)

↓

TRPV1 Expression Analysis

↓

Capsaicin Stimulation

↓

Functional Response (IEGs Activation)

↓

Cell Viability (MTT Assay)

Treatment and Analysis

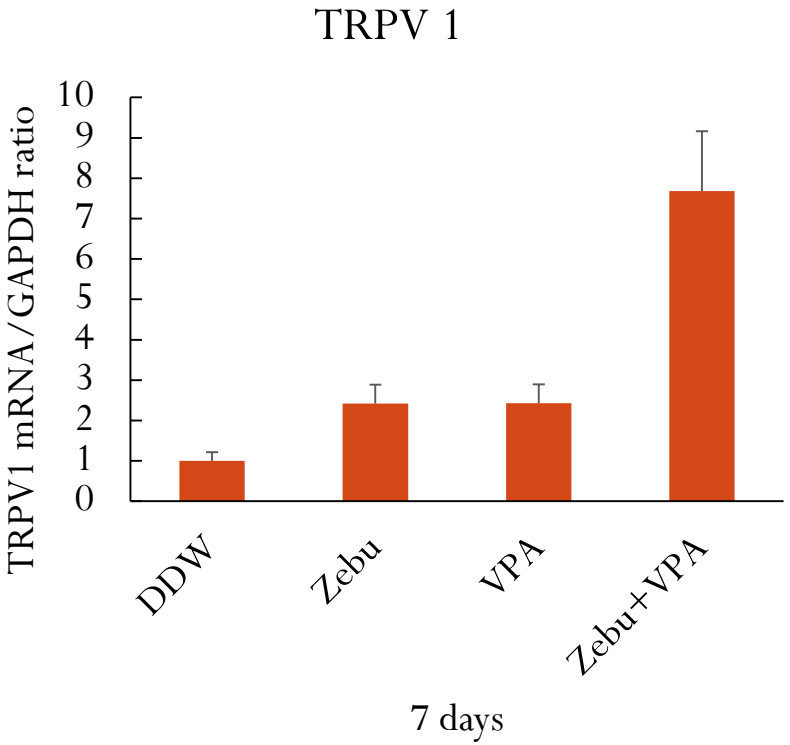
Protocol

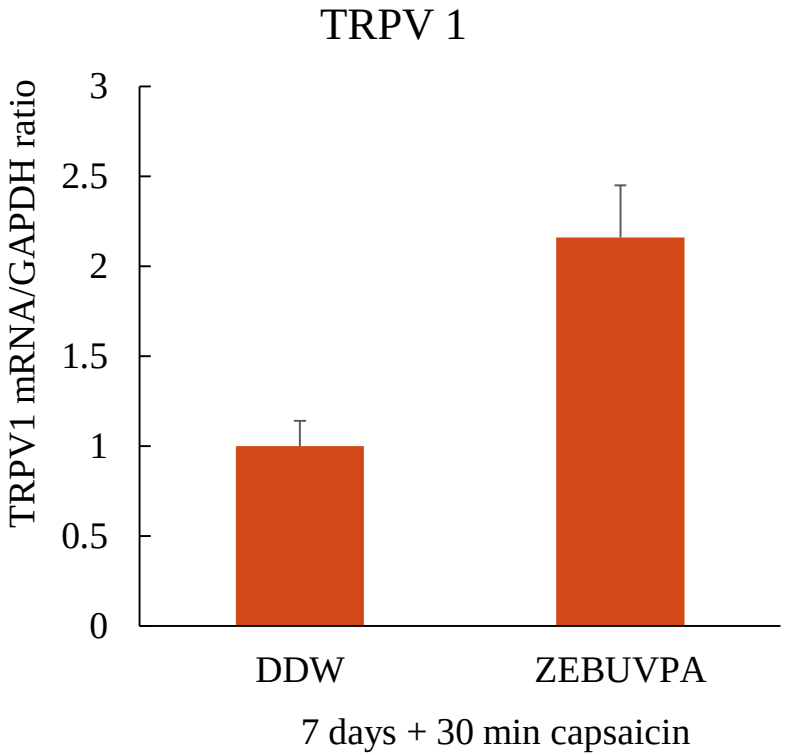
Treatment: **3–7 days**

Followed by **Capsaicin stimulation**

Category	Method
Gene Expression	TRPV1, FOS, JUN, EGR1, ARC (qRT-PCR)
Protein Expression	TRPV1 (Western blot / Flow cytometry)
Functional Assay	Capsaicin-induced IEG activation
Cell Viability	MTT assay

Results

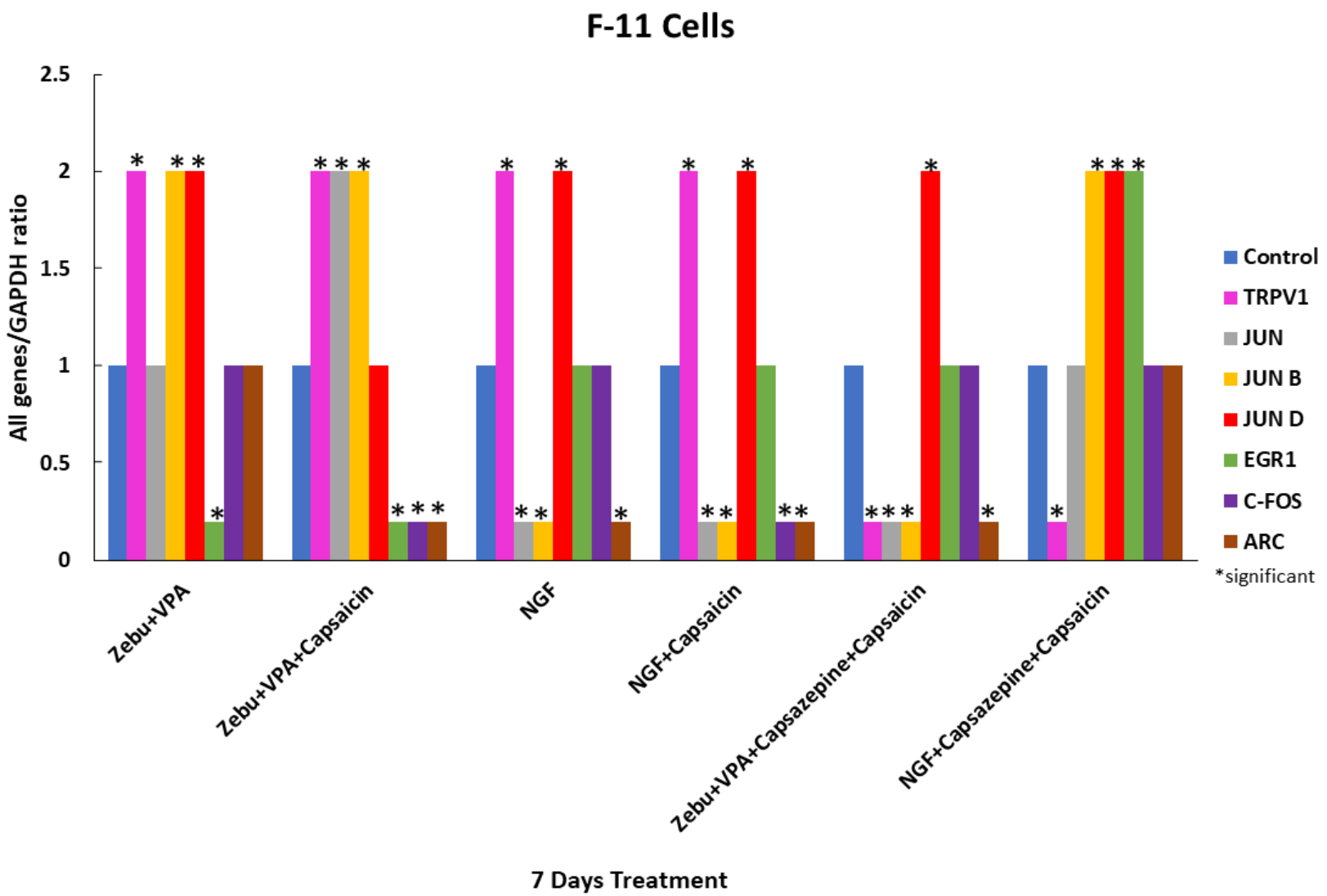




☞ ***Epigenetic treatment significantly increased TRPV1 expression, with the combination group showing the highest upregulation, both at baseline and after capsaicin stimulation.***

			Genes/protein						
No	Methods	Control	TRPV1	JUN	JUN B	JUN D	EGR1	C-FOS	ARC
1	Zebu + Vpa	DDW	Up*	Not sig	Up*	Up*	Down *	Not sig	Not sig
2	Zebu + Vpa + 30 mins capsaicin	DDW+Capsaicin	Up*	Up*	Up*	Not sig	Down *	Down *	Down *
3	Zebu + Vpa + 20 min Capsazepine+ 30 mins capsaicin	DDW+Capsaicin	Down *	Down*	Down*	Up*	Not sig	Not sig	Down *
4	NGF	DDW	Up*	Down*	Down*	Up*	Not sig	Not sig	Down *
5	NGF+30 mins capsaicin	DDW+Capsaicin	Up*	Down*	Down*	Up*	Not sig	Down*	Down *
6	NGF+20 min Capsazepine+ 30 mins capsaicin	DDW+Capsaicin	Down *	Not sig	Up*	Up*	Up*	Not sig	Not sig

Epigenetic modulation and NGF both upregulated TRPV1, and upon capsaicin stimulation, this led to significant activation of immediate early genes, confirming functional receptor activity, while inhibition reduced this response.



Epigenetic treatment and NGF increased TRPV1 expression, and capsaicin further enhanced immediate early gene activation, while capsazepine reduced this response, confirming TRPV1 -mediated signaling.

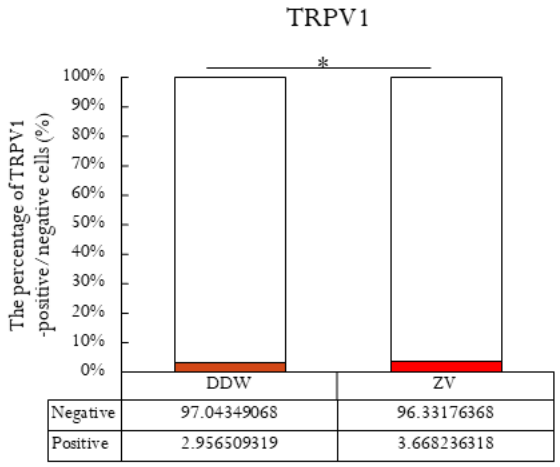
FLOW CYTOMETRY EXPERIMENT

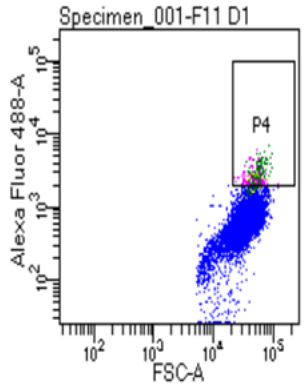
- To check the protein TRPV1

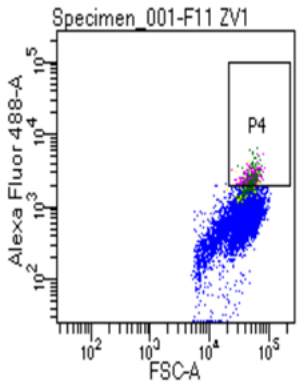
			Protein
No	**Methods**	**Control**	**TRPV1**
1	Zebu + Vpa	DDW	Up*
2	Zebu + Vpa + 30 mins capsaicin	DDW+Capsaicin	Up*
4	NGF	DDW	Up*
5	NGF+30 mins capsaicin	DDW+Capsaicin	Up*

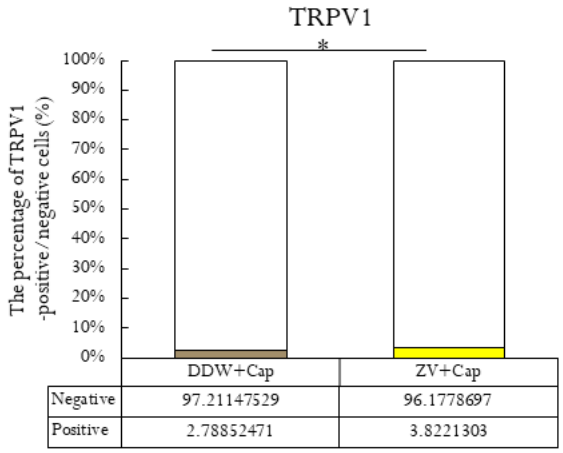
*Significant different using chi-square statistical analysist N=3

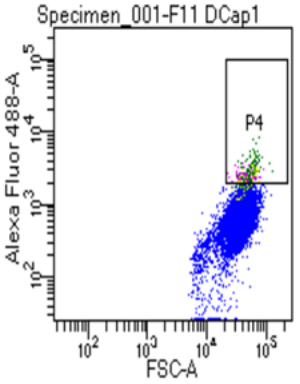
Flow cytometry confirmed that both epigenetic treatment and NGF significantly increased TRPV1 protein expression, regardless of capsaicin stimulation.

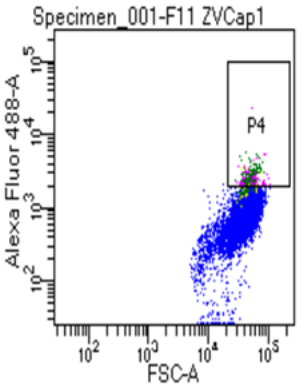






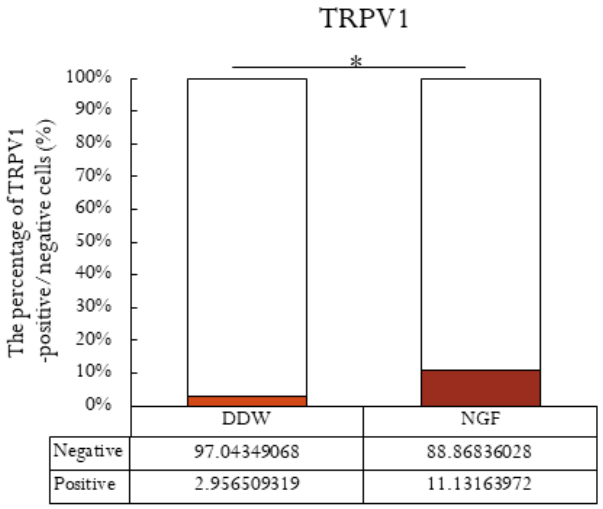




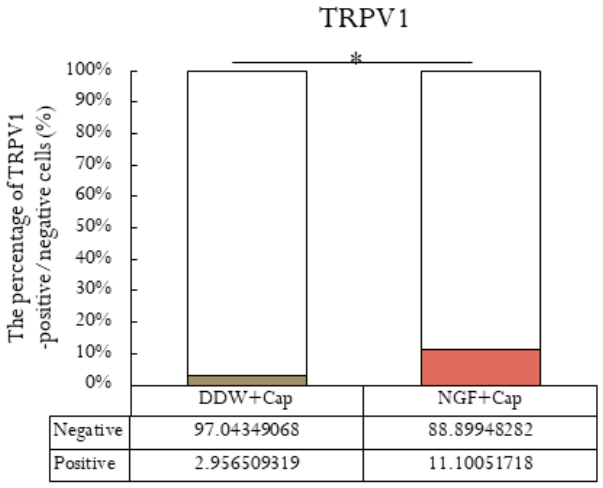


Possitive cells significantly different upregulated beetween control (DDW,DDW+Cap) and experiments group (ZV, ZV+Cap) (chi-square, N=3)

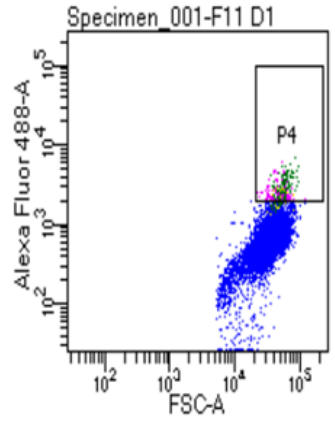
Flow cytometry shows a significant increase in TRPV1-positive cells in epigenetic and NGF- treated groups compared to control, confirming protein-level upregulation.

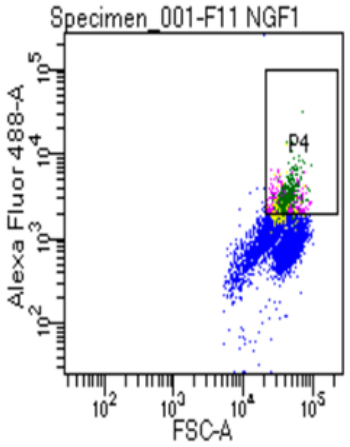


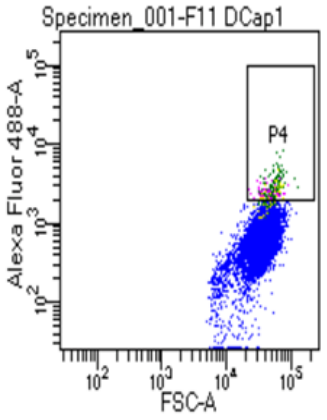
	DDW	NGF
Negative	97.04349068	88.86836028
Positive	2.956509319	11.13163972

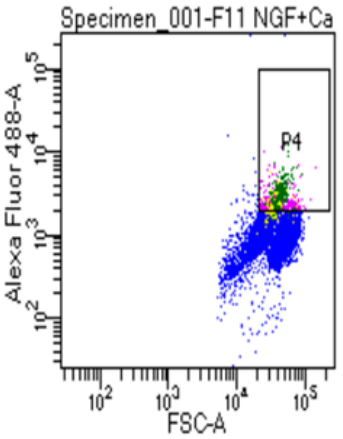


	DDW+Cap	NGF+Cap
Negative	97.04349068	88.89948282
Positive	2.956509319	11.10051718









Flow cytometry demonstrates a Significant increase in TRPV1 -positive cells in NGF-treated groups compared to control, confirming protein-level upregulation.

Possitive cells significantly different upregulated beetween control (DDW,DDW+Cap) and experiments group (NGF, NGF+Cap) (chi-square, N=3)

Epigenetic Modulation Upregulates TRPV1 Expression

Interpretation

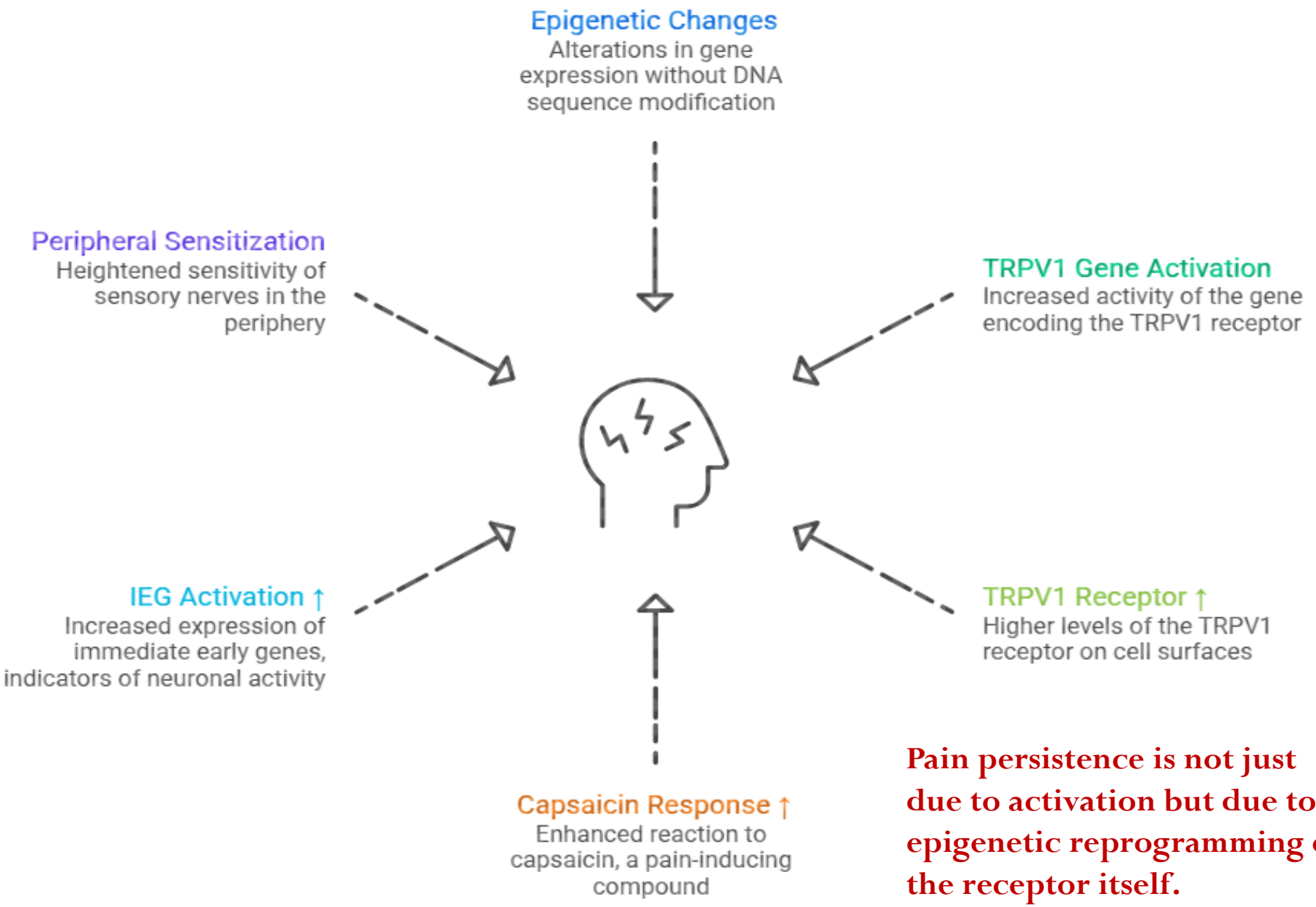
- TRPV1 is **not constitutively active**
- It is **epigenetically suppressed under normal conditions**
- Epigenetic modulation **"unlocks"TRPV1 gene expression**

Key finding—TRPV1 is not just regulated, it is epigenetically controlled

Upregulated TRPV1 is Functionally Active

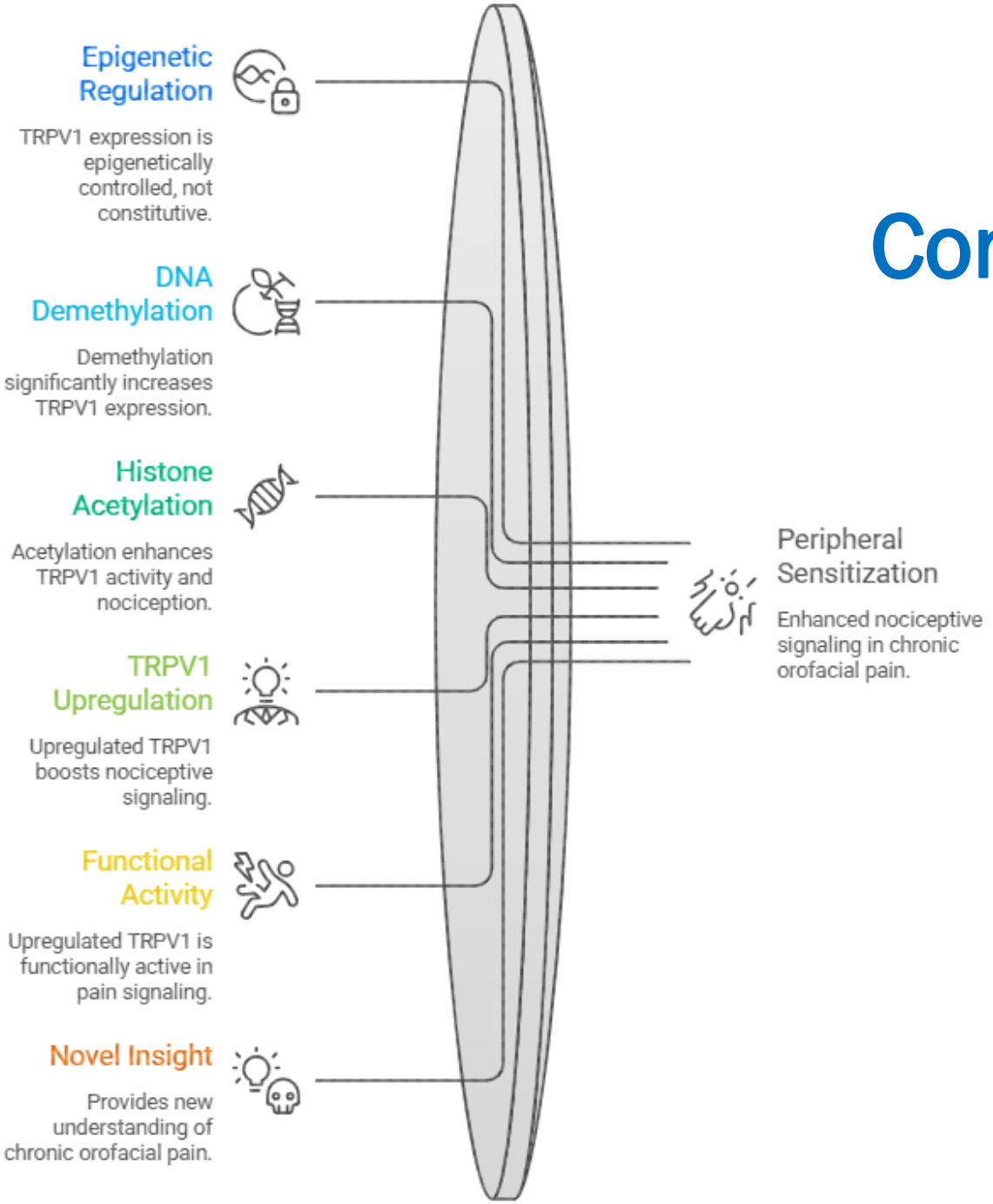
- **Capsaicin alone does NOT increase TRPV1 expression**
- **It only activates already expressed TRPV1 receptors**
- **Epigenetic modulation leads to enhanced neuronal responsiveness (sensitization)**
- **Increased TRPV1 is not just present but it is functionally active**
- ***So we are not just increasing receptor numbers but we are increasing pain signaling capacity.***

Mechanistic Link to Chronic Pain



Pain persistence is not just due to activation but due to epigenetic reprogramming of the receptor itself.

Conclusion


Epigenetic Regulation
TRPV1 expression is epigenetically controlled, not constitutive.
DNA Demethylation
Demethylation significantly increases TRPV1 expression.
Histone Acetylation
Acetylation enhances TRPV1 activity and nociception.
TRPV1 Upregulation
Upregulated TRPV1 boosts nociceptive signaling.
Functional Activity
Upregulated TRPV1 is functionally active in pain signaling.
Novel Insight
Provides new understanding of chronic orofacial pain.
Peripheral Sensitization
Enhanced nociceptive signaling in chronic orofacial pain.

Take-Home Message

👉 **Epigenetics transforms TRPV1 from a silent receptor into a driver of chronic pain.**

Clinical Relevance

Targeting epigenetics may help control pain at its source, allowing more precise and effective treatment in chronic orofacial pain patients like BMS.

So instead of only blocking pain, we can think about preventing its molecular programming

Therapeutic Implications

- Epigenetic modulators as **novel targets for pain management**
- Potential to develop **TRPV1-specific epigenetic therapies**
- Opportunity for **personalized medicine in chronic orofacial pain**
- Shift from **symptom control → disease mechanism targeting**

THANK YOU