Understanding the Cellular Metabolic Processes Regulated by Extracts of Hemp (*Cannabis sativa*)

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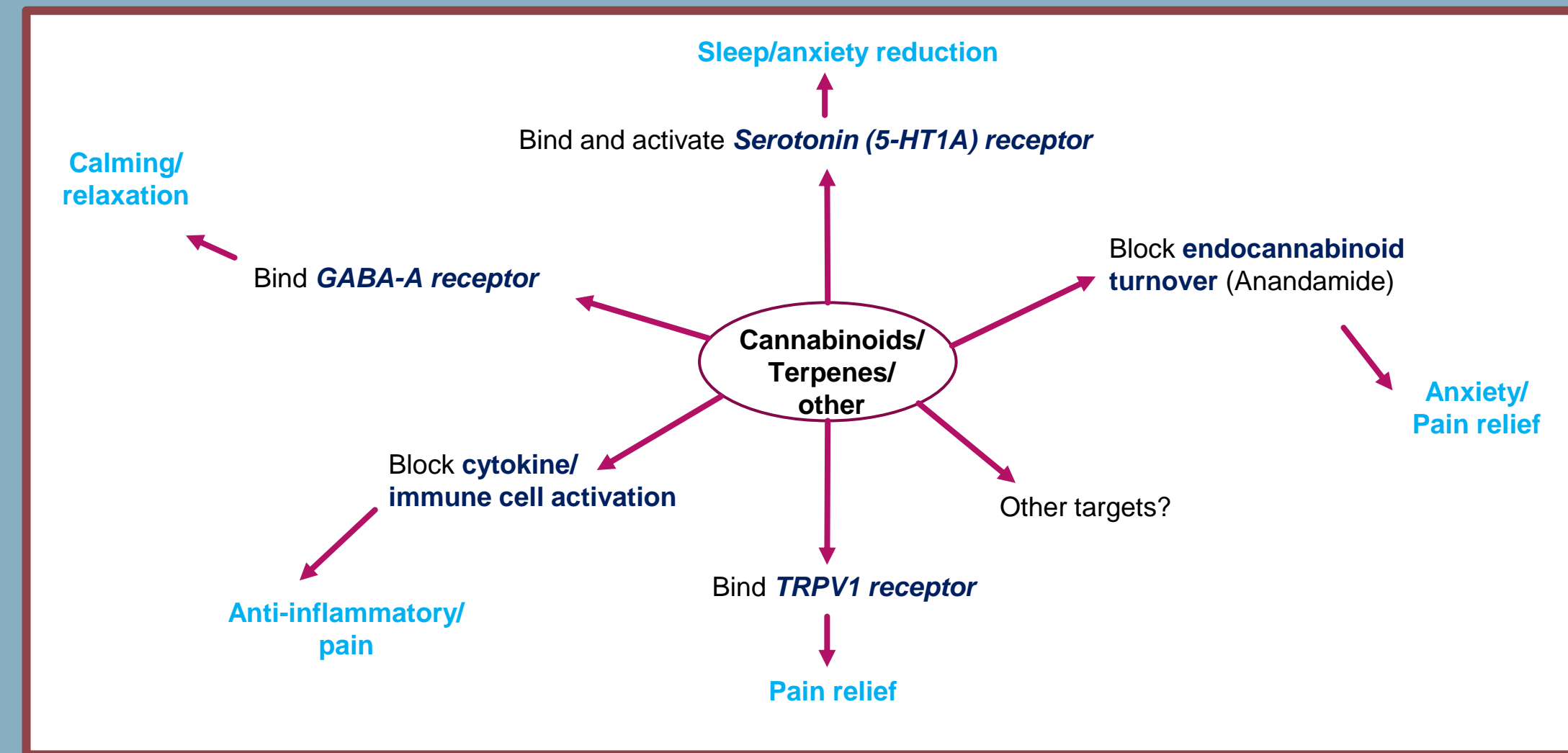
SUMMARY:

Optimal strain selection of *Cannabis* (hemp varieties) for medical treatments has been difficult to decipher due to variability and cannabinoid levels as well as other active constituents - terpenes and flavonoids. Aside from THC and cannabidiol (CBD), few of the cannabinoids have been characterized. It has been established that other molecules including terpenes and flavonoids contribute the effects of utilizing *Cannabis*, although therapeutically much less is known and even less with regard to synergy between molecules. Different strains of *Cannabis* produce differing profiles of chemical constituents. As a result, medical properties of *Cannabis* strains vary due to effects between the different compounds. Because of the number of constituents to be considered, it has been challenging to study the chemical composition of *Cannabis* strains as it relates to clinical effects.

GOAL:

To develop an *in vitro* cell culture system to screen *Cannabis* samples for their optimal therapeutic use for the conditions of **anxiety**, **pain**, **sleep**, and **anti-inflammatory** activity, which have been demonstrated to have clinical benefit.

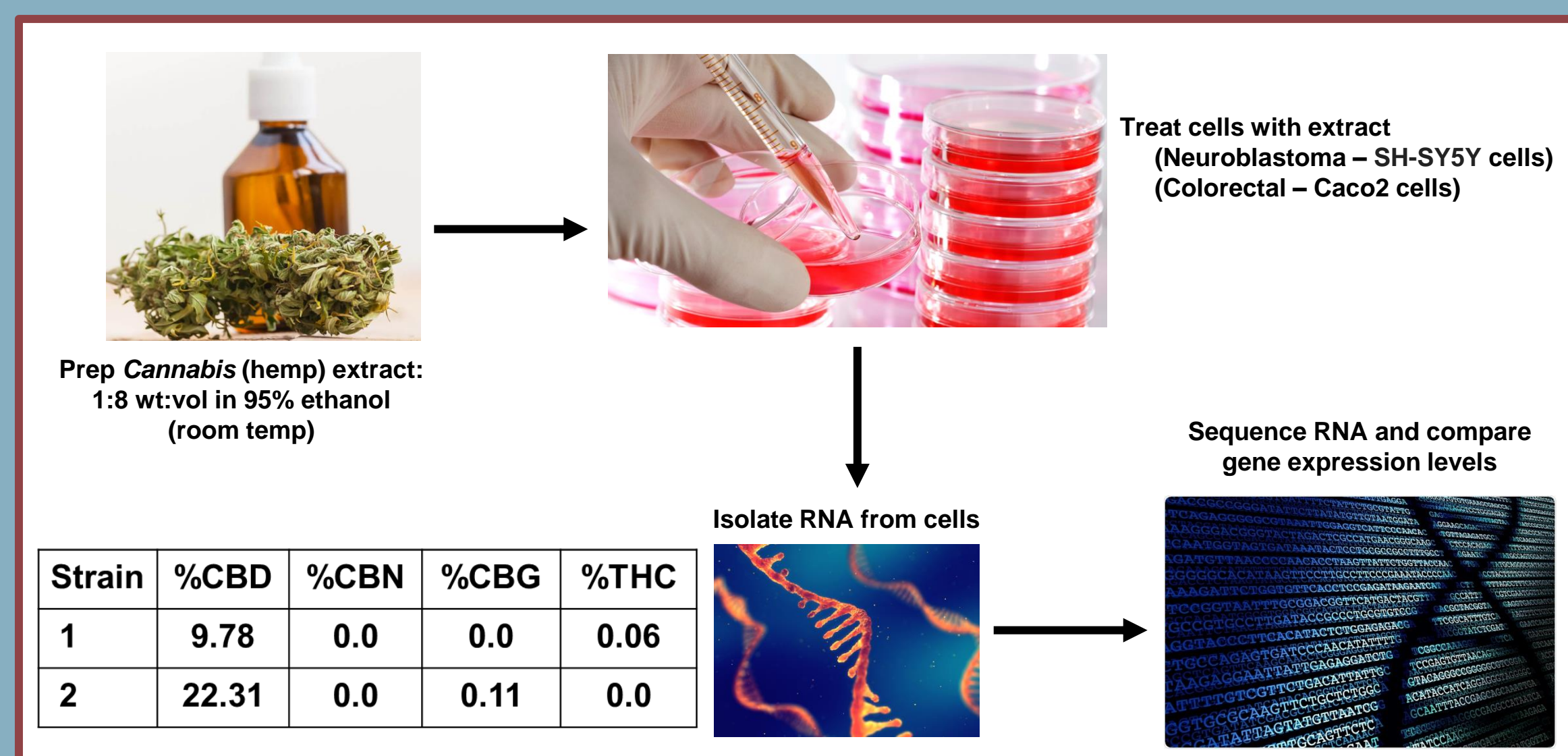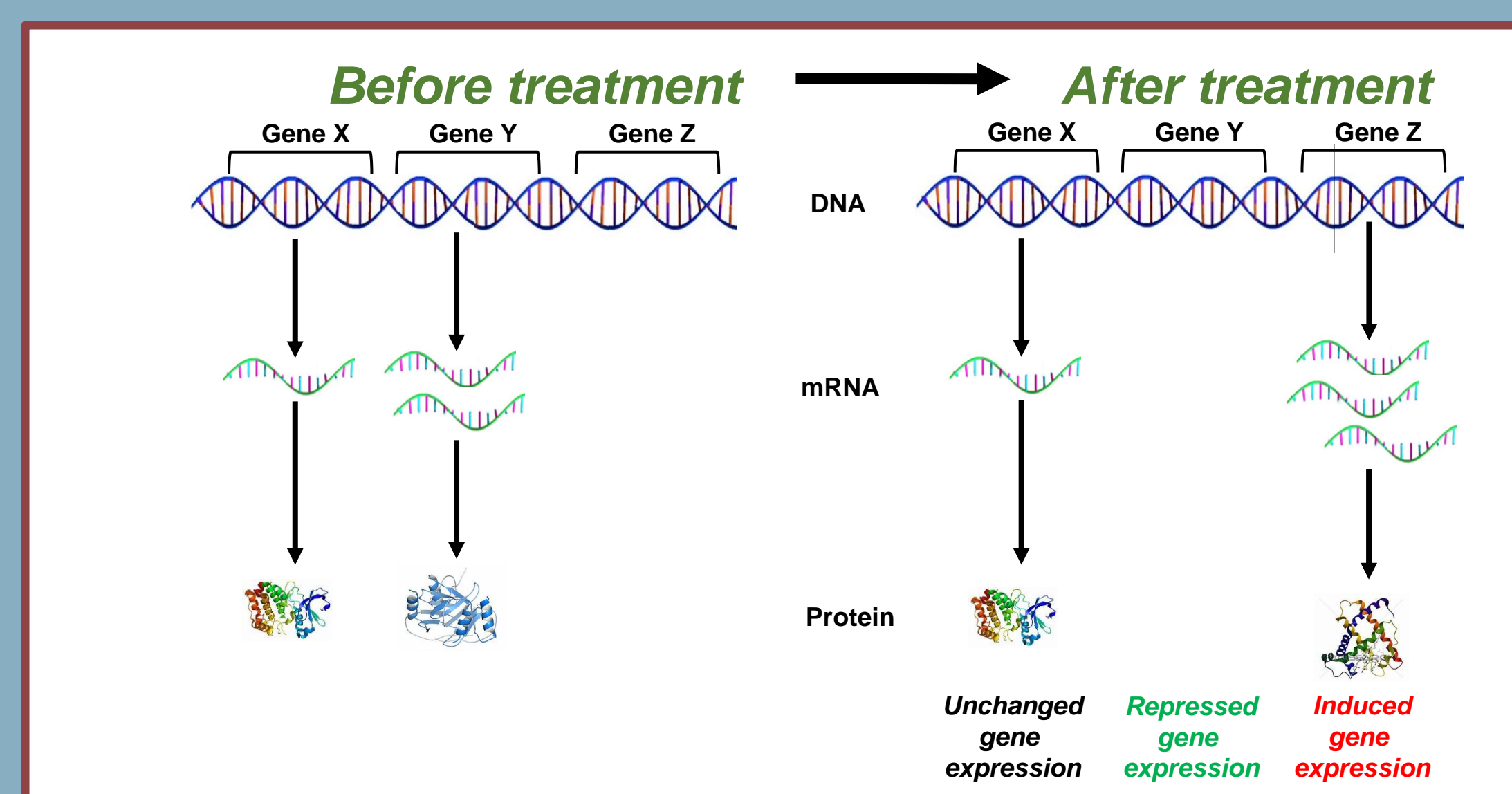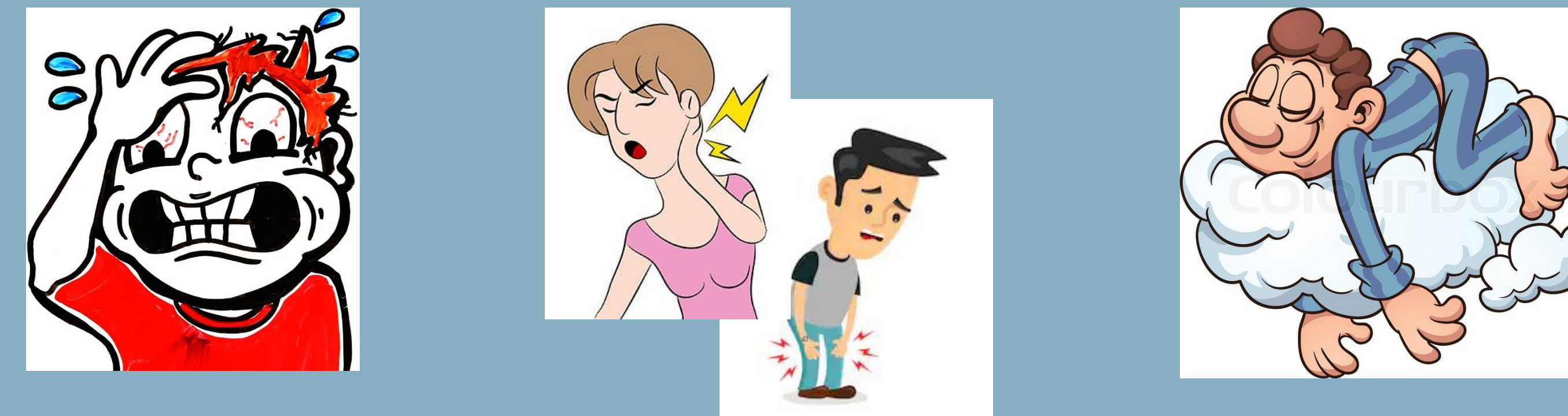**Figure 1.** Proposed theories on *Cannabis* activity.

METHODS:

Figure 2. Methods. *Cannabis sativa* strains were extracted in 95% ethanol at RT. Cells were treated with *Cannabis* extracts; total RNA extracted (Quigen RNAeasy) and gene expression levels compared (RNAseq by Novogene). Herbalite provide cannabis strains.

Figure 3. Hypothetical example of gene expression study before and after treatment to cells

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In vitro characterization of Cannabis/ Hemp strains and /or specific compounds can assist in differentiating your brands marketing goals by identifying potential targets for cannabis on:

ANXIETY/STRESS, PAIN /INFLAMMATION, & SLEEP activity and validating BioFitness of your product.

BioFit standardization: each batch of product is tested for biological activity (fitness) rather than a marker compound.

The Competitive Advantage of BioFit Standarization

The science-based understanding of the biological activity of different strains or compounds helps to validate claims and provides scientific support for marketing.

CONCLUSIONS:

	Anxiety/ Stress	Pain	Inflammation	Sleep
Strain 1: 9.78% CBD	+++	++	+	+++
Strain 2: 22.31% CBD	+	+	+++	+

TABLE 1: BioFit comparison of *Cannabis* strains with mid (strain 1) and high (strain2) levels of CBD concentration. Strain 1 has a higher BioFit score than strain 2 for anxiety (3), pain (2), and sleep (3). Strain 2 has a higher score for anti-inflammation than strain 1, (3) versus (1), respectively.

Toolbox of experimental assays for BioFit standardization

- **Global gene expression** (*Transcriptome profiling*)
- **Specific gene expression level testing** (*RT-PCR*)
- **Protein gene expression and activation**
- **Cell receptor activation**
- **Signal transduction pathways analysis**
- **Anti-microbial activity validation**

RESULTS:

Herein we report results from a preliminary *in vitro* study on 2 cells lines, neuroblastoma (SH-SY5Y) and colorectal (caco2), treated with 2 *Cannabis sativa* strain extracts (strain 1: mid-range CBD concentration (9.8%); and strain 2: high CBD (22%). Comparative gene expression was performed between strain 1 and strain 2 to identified and validated biomarkers of interest for each therapeutic condition – anxiety/stress, pain, inflammation and sleep.
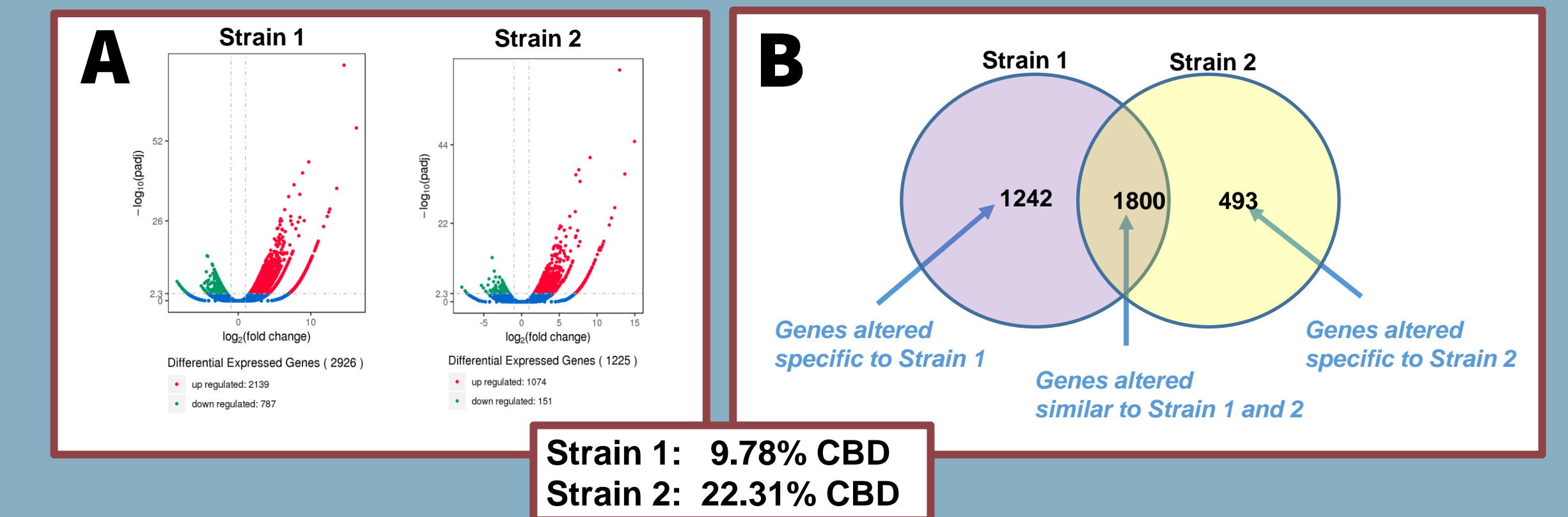

Strain 1: 9.78% CBD
Strain 2: 22.31% CBD

Figure 4. Overview of global gene expression of neurblastoma cells treated with *Cannabis*. A) Volcano plot to visualize all genes that are upregulated (red) and down regulated (green), B) Venn diagram to assist in identify subsector of genes of interest for analysis.
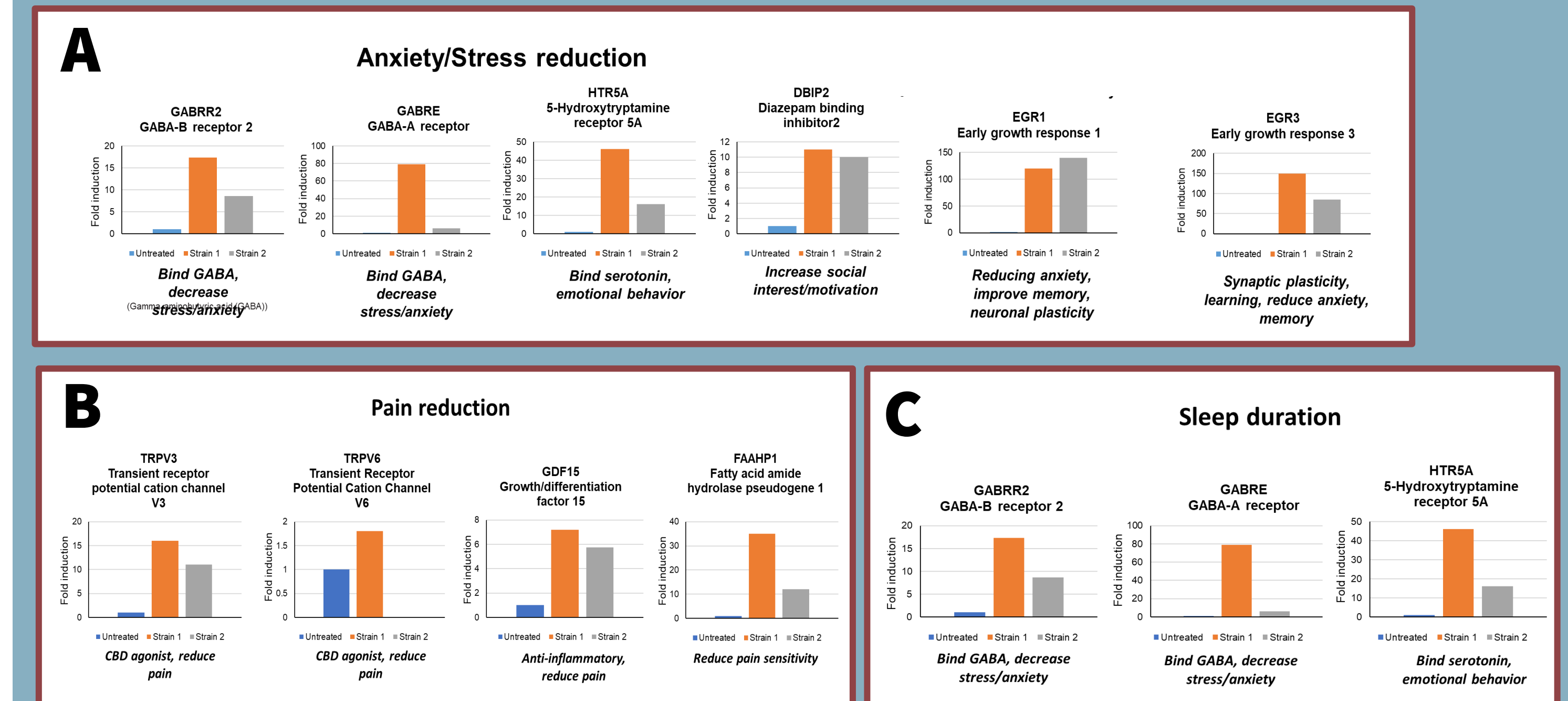

Figure 5. Potential gene targets for *Cannabis* on anxiety/stress (A) and pain (B), and sleep (C). Fold induction (or fold change) is a measure describing how much a quantity changes (increases) between the original (untreated) and subsequent measurements (treatments). Comparison between upregulated targeted genes know to be induced for anxiety, pain, or sleep show that strain 1 are overall more highly induced.
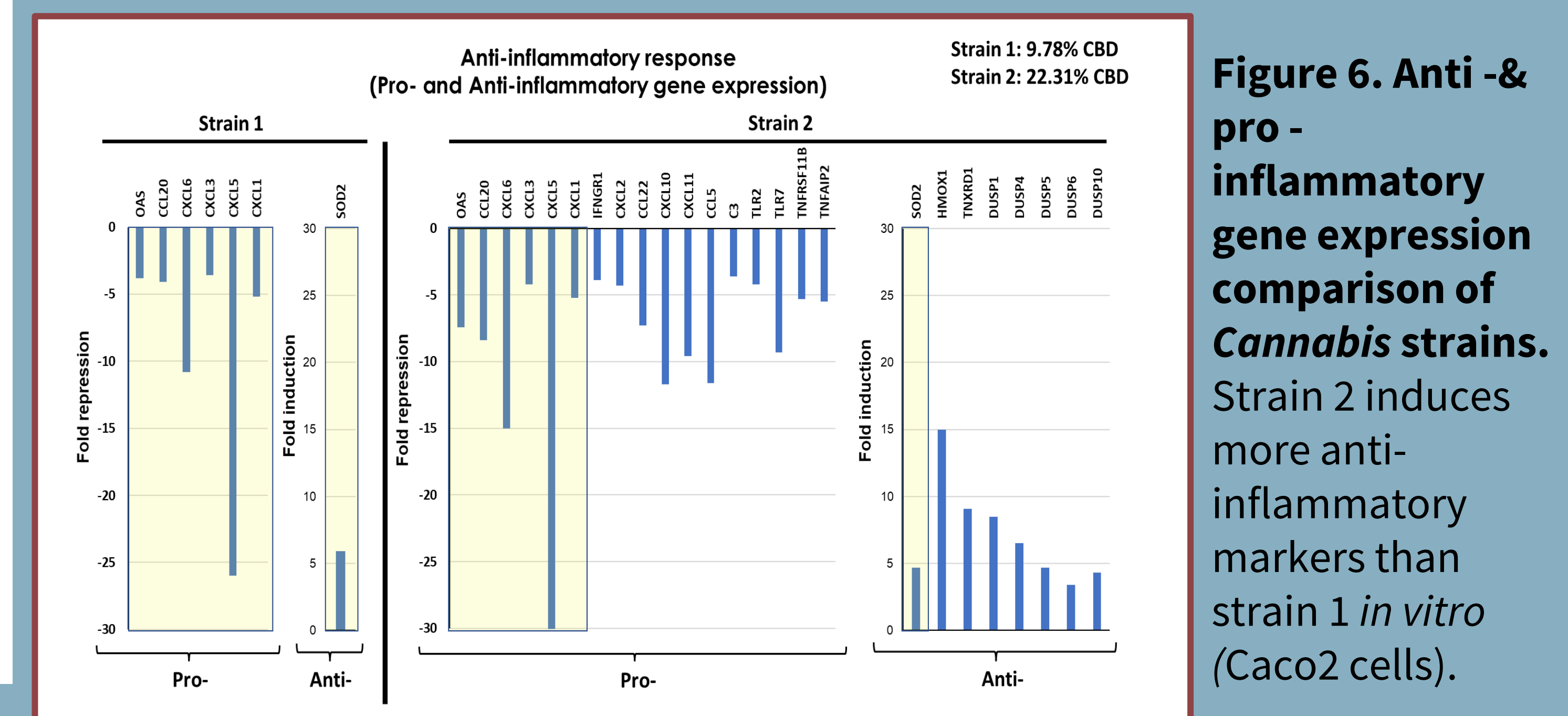

Figure 6. Anti -& pro - inflammatory gene expression comparison of *Cannabis* strains. Strain 2 induces more anti-inflammatory markers than strain 1 *in vitro* (Caco2 cells).

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