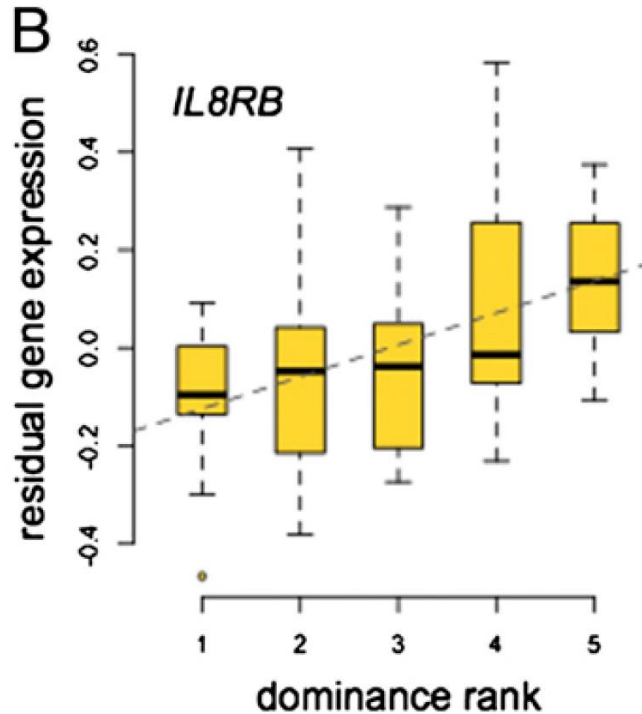## HOW TO USE THIS RESOURCE

Show the figure below to your students along with the caption and background information. The "Interpreting the Graph" and "Discussion Questions" sections provide additional information and suggested questions that you can use to guide a class discussion about the characteristics of the graph and what it shows.

Caption: Expression levels of IL8RB, *a gene involved in the inflammatory immune response, in rhesus macaque monkeys of different dominance rankings (1 is the highest and 5 is the lowest). For each dominance rank,* IL8RB *expression levels are shown for 9 or 10 monkeys. Each monkey within a dominance rank was from a different social group. Scientists created social groups with one monkey from each rank. The gene expression levels are shown relative to the mean (shown as 0.0) of* IL8RB *expression for all 49 monkeys across all 10 groups. The trend line has a* p-*value of 0.003.*

BACKGROUND INFORMATION

Social status in primates is known to impact many physiological conditions including disease susceptibility, fertility, and survival, with lower social status generally being a predictor of negative impacts on each of these conditions. To determine the genes underlying such physiological changes in primates of different social statuses, a team of scientists manipulated the dominance ranking of female rhesus macaques living in social groups. They did this by controlling the order in which individuals were introduced to the social group. The monkeys introduced first were perceived and treated as having the highest social status by the monkeys introduced later. The scientists set up 10 replicate social groups, each with four or five monkeys with dominance rankings of 1 through 4 or 5, with 1 being the highest rank and 5 the lowest.

The scientists collected blood samples from the monkeys and examined gene expression levels across the entire genome. Gene expression was quantified by measuring amounts of mRNA. They found that the expression levels of genes involved in immune system processes differed the most between monkeys of different ranks. This figure shows data for one of these genes, *IL8RB* (Interleukin 8 receptor, beta). The IL8RB protein (more commonly called

CXCR2) is a receptor found on white blood cells. When a protein called cytokine IL8 binds to the IL8RB receptor, the white blood cell migrates to the point of infection. The migration of immune cells results in inflammation. While inflammation is a necessary part of healing, chronic inflammation has been associated with increased risk of cancer, heart disease, depression, and obesity.

INTERPRETING THE GRAPH

The figure shows the expression of the *IL8RB* gene for monkeys at five social rankings using box-and-whisker plots. The mean *IL8RB* expression level for all 49 monkeys across all five dominance ranks (social statuses) was used to normalize the data. In the figure, this mean gene expression level is expressed as 0.0 on the *y*-axis, and expression levels for the five ranks are plotted relative to the mean.

In the box-and-whisker plots, the dark horizontal lines represent the median *IL8RB* gene expression level for the 9-10 animals in each dominance rank. The upper and lower sides of the box include the data that fall within the middle 50% of the expression levels. The whiskers extend to the minimum and maximum expression levels, with the exception of any outliers. The dot located near the *x*-axis for dominance rank 1 shows that one of the individuals was an outlier in terms of its *IL8RB* expression level.

This graph shows a significant (p = 0.003) linear relationship (indicated by the dashed trend line) between the expression of *IL8RB* and social status. The lowest status group (5) experienced a modest increase in *IL8RB*, with a shift in its median expression level of about 0.1 (10%). In contrast, the highest status group (1) experienced a modest decrease in *IL8RB* expression, with a shift in its median expression level of about -0.1 (-10%). This suggests that more highly stressed individuals (those who were low status in their groups) experience an increase in inflammation, which can result in myriad negative physiological effects.

It is worth noting that the scientists observed similar patterns when they looked at other genes associated with inflammation. In another paper by this same group, published four years later, the scientists found evidence that an entire proinflammatory signaling pathway is upregulated in lower-ranked individuals, connecting the three isolated genes to a larger story. Since the figure shows changes in gene expression, not changes in the genetic makeup of the individuals, the responses to stress are epigenetic changes.

Teacher Tip: Prompt your students to explain the following:
- <u>Graph Type</u>: Box-and-whisker plot with linear regression trend line
- <u>X-axis</u>: Dominance rank of the animals in each group (1 = highest, 5 = lowest social status)
- <u>Y-axis</u>: *IL8RB* gene expression levels relative to the mean (shown here as 0.0) of *IL8RB* expression for all 49 monkeys
- <u>Trend Line</u>: A line of best fit, in this case showing a positive correlation between gene expression and decreasing dominance rank
- <u>p-value</u>: An indication of the significance of the trend line

DISCUSSION QUESTIONS
- What trends do you see in the data shown in the figure?
- How would you describe the relationship between *IL8RB* gene expression and dominance rank? Provide at least two pieces of evidence from the graph.
- Does the trend line help you to make sense of the box-and-whisker plots? In what way?
- What does the *p*-value tell us about the data?
- How would you interpret the negative and positive numbers on the *y*-axis? What about zero on the *y*-axis?

- Explain what the boxes and whiskers represent. What is the difference between short and tall boxes? Long and short whiskers?
- Explain the meaning of the dot shown below the box-and-whisker plot for dominance rank 1.
- Why do you think the authors chose to use a box-and-whisker plot rather than a scatter plot?
- Do the changes in gene expression illustrated in this figure represent genetic or epigenetic changes? How do you know?
- Predict the gene expression levels for the offspring of one of the females in the lowest social status group. What information would you need to make a better prediction?
- Why do you think monkeys were used in this study? Why only female monkeys?
- If humans could be studied in the same way, do you think the results would be similar? Why or why not?
- What implications does this study have in terms of social status and/or bullying in a school environment?

KEY TERMS

dominance rank, gene expression, immune system, inflammation, social status, white blood cells

SOURCE

Figure 2B:

J. Tung *et al*. (2012) Social environment is associated with gene regulatory variation in the rhesus macaque immune system. *PNAS*, 109(17), 6490–6495. http://doi.org/10.1073/pnas.1202734109

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