The goal of this take-home exam is to assess how well you are able to read and evaluate scientific literature because this is a central skill that you will need in whatever you do after NCC. For this to work, it is simply essential that you are the only one answering these questions. Please read the following statements. If you agree to these provisions, please sign and date below and turn this in with your completed take-home exam. As always, please ask if something is unclear.

- **You may not receive help from another person.** This is true for any student enrolled in this course or from any other person at North Central or elsewhere. The only exception is that you may ask Dr. Johnston for any clarification that is needed.

- You may not provide assistance to any other member of the class. If anyone approaches you for help with this exam, you are obligated to report that immediately to Dr. Johnston.

- You may use any published material or on-line resource to help you answer the questions, so long as you do not plagiarize that source. At the end of your answers to the exam questions, please provide a brief, informal list of all sources that you used.

- Seeking or providing help to another student on the take-home exam will result in your failing BIO360 and being reported to the Dean’s Office for further disciplinary action, including the possibility of permanent dismissal from the College.

*By signing below, I certify that I have neither received nor provided any help from another person in completing this assignment. I have read, understood and agree to abide by all of the above terms and conditions.*

*Printed Name: ________________________________

*Signed: ________________________________ Date: ________________*
Read the following paper and answer the questions below.


Try not to analyze what the authors of the paper say in the text – rather analyze what the data say. Please type your answers. You are welcome to augment your written answers with hand-drawings if that helps explain your ideas. Don’t quote this paper or any other. Be sure to refer to specific data contained in this paper frequently to support your answers. Please put your name only on the signature page only and not on the paper itself. All answers are due at noon on Wednesday October 3. Email submissions are fine, but still must meet the same deadline.

1. Why did Marschang et al. suspect that LRP1b might be a tumor suppressor gene (as the title indicates)? Based on the results presented in this paper, do you think LRP1b is or is not a tumor suppressor gene? Be sure to explain your reasoning, referring to data in this paper. (5 points)

2. The LRP1b gene is exceptionally large, covering more than half a megabase on chromosome 2, encoding 91 exons and a protein with an apparent molecular mass of around 600 kilodaltons (kDa). In building their knockout mouse, only exon 88 is removed. Since the promoter and enhancers are undisturbed, we would reasonably expect the recombinant gene to still be transcribed. What could be the structure of resulting protein? Do you expect it to be functional or nonfunctional? Based on the approached used, was the gene really effectively deleted or was this strategy flawed? Where in the cell would you expect this protein to be located? Do your expectations match the data presented in the paper? (10 points)

3. The authors provide solid evidence that LRP1b binds to the PSD-95 protein. Which domain of LRP1b binds PSD-95? How do you know? It is clear that the PDZ1 and PDZ3 domains of PSD-95 can bind to LRP1b, but it’s not clear if the SH3 domain of PSD-95 can bind to LRP1b. Why are we not sure about the SH3 domain? (10 points)

4. What question was Marschang et al. asking in Figure 9A? Figure 9A shows three western blots. Each of the blots started with HEK293 cells transfected with one of four plasmids. Those four plates of cells were lysed and then treated differently. How were the top, middle and bottom samples treated differently? What protein(s) is being detected in each of these western blots? Based on the data, what is the answer to the scientists’ question that you identified earlier? (10 points)

5. The authors present evidence in Figure 6 that there are at least two alternative splicing possibilities for LRP1b. In Figure 6A, why are there two bands in “panel a” but only a single band in “panel b”? Which spliced isoform (or isoforms) are present in mouse brain and in mouse testis? Why is β-actin included in Figure 6A? (5 points)

6. What else do you want to know? Good papers always tell us something new about the world which has the effect of raising new questions. Begin by putting forth a specific hypothesis or question. In your
answer, list your hypothesis or question in one sentence and underline that sentence. Design an additional experiment or two that will significantly extend the results of Marschang et al. In your answer, cite at least one primary research paper other than Marschang et al., Bäumer et al. or Aranda et al. Be original and don’t just re-state your answer to an earlier question on this exam. Be sure to explain the rationale behind your hypothesis, briefly outline an experiment or two and show why the new results will be important. For your experiment, clearly identify what variable you will be manipulating and what you will be measuring.

For example, you might decide to knockout the \textit{NCCI} gene in mice and monitor the expression of the Ncc2 protein in the liver by western blotting. For this experiment, you don’t need to report your \textit{NCCI} targeting vector or how to run a western blot but you should explain why you expect \textit{NCCI} to affect Ncc2 (or why you’d expect \textit{no} effect!) and what important controls you would run. (10 points)
Read the following paper and answer the questions below.


Please type your answers. You are welcome to augment your written answers with hand-drawings if that helps explain your ideas. Don’t quote this paper or any other. Be sure to refer to specific data contained in this paper frequently to support your answers – in other words, say the bottom western blot in Figure 8B, not just Figure 8. Please put your name only on the signature page only and not on the paper itself. All answers are due at noon on Wednesday October 17. Email submissions are fine, but still must meet the same deadline.

1. In the abstract, the authors assert that Dyrk1A can phosphorylate p53 on Serine-15 in neurons. Which one part of a figure supports this claim? How strong is this claim? (5 points)

2. Figures 1B and 1D both show coimmunoprecipitations. Is there any value in showing Figure 1D? Be sure to briefly explain why Figure 1D is or is not useful. (5 points)

3. In Figure 5D, the authors are showing precisely the same cells in the first row and the second row. All cells have been stained with DAPI but only some of the cells have been stained with BrdU. How do these two stains work? Why are some cells DAPI+ but BrdU? (5 points)

4. What is the hypothesis and rationale behind the experiment shown in Supplementary Figure 2C? (5 points)

5. Figure 4A shows that excess Dyrk1A levels decreases the number of living cells. There are many reasonable hypotheses to explain the decrease the number of viable cells on a dish, such as a) Dyrk1A is inducing apoptosis, b) Dyrk1A is decreasing cell proliferation by leading to an increase in Cdk inhibitors, c) Dyrk1A is keeping cells in G0 by blocking the expression of cyclin D. Evaluate each of those hypotheses using data from the paper. (10 points)

6. How do we know that Dyrk1A directly phosphorylates p53? Is it possible that Dyrk1A really phosphorylates another kinase which then phosphorylates p53? Naturally, use data from the paper to support your answer (5 points)

7. Based on these data, do you think that Dyrk1A is more likely to be an oncogene or a tumor suppressor gene? Support your answer with data from the paper. (5 points)

8. What else do you want to know? Good papers always tell us something new about the world which has the effect of raising new questions. Begin by putting forth a specific hypothesis or question. In your answer, list your hypothesis or question in one sentence and underline that sentence. Design an
additional experiment or two that will significantly extend the results of this paper. In your answer, cite at least one primary research paper other than those that we’ve read as a class. Be original and don’t just re-state your answer to an earlier question on this exam. Be sure to explain the rationale behind your hypothesis, briefly outline an experiment or two and show why the new results will be important. (10 points)
Read the following paper and answer the questions below.


Please type your answers. You are welcome to augment your written answers with hand-drawings if that helps explain your ideas. Don’t quote this paper or any other. Be sure to refer to specific data contained in this paper frequently to support your answers. Please put your name only on the signature page only and not on the paper itself. All answers are due at noon on Monday October 29. Email submissions are fine, but still must meet the same deadline.

1. Figure 1a presents the results of several genechip (or microarray) experiments. Each of the eight columns shows data from a single genechip experiment. What is indicated in each row? What is the treatment that was done in the experiment shown in the first column on the left? None of the eight experiments seems to show a ‘normal’ or a ‘no drug’ condition as a negative control. How is the negative control is already built into this experiment? (5 points)

2. This paper shows that some genes like CHK1 and E2F1 are downregulated when cells are treated with either valproic acid or vorinostat. If we downloaded the microarray data from the Yamaguchi et al. paper that we discussed in class, do you expect higher or lower levels of mRNA for CHK1 and E2F1 in the HDAC1/2 KO/Kd MEFs? Why? (5 points)

3. In the absence of radiation, treating cells with valproic acid causes only small changes in some key proteins like DNAPK (shown in Fig. 5a). How do we know that the concentration of valproic acid used by these scientists was sufficient to inhibit the HDACs? (5 points)

4. What hypothesis is being tested in Figure 6b? (5 points)

5. Figure 7a shows a ChIP assay. Six different lanes are shown on four different gels (for convenience, you can number them 1 through 6 from left to right). When we see bands, what molecule are we seeing? For each of the six lanes, state in one sentence whether this is a positive control, a negative control or an experimental sample and explain why. (10 points)

6. The authors’ model is that treating cells with valproic acid leads to less E2F1 protein being in the cell, which is needed to bind to the promoters and activate transcription of key repair genes like RAD51 and CHK1, thus making the cells highly sensitive to DNA damage from radiation. Where are the data that support each of these four ideas:
   a. treating cells with valproic acid leads to less E2F1 protein
   b. E2F1 binds to the promoters of key repair genes like RAD51
c. E2F1 protein is needed to activate transcription of key repair genes like RAD51
d. lower levels of RAD51 and related genes make the cells sensitive to DNA damage
Be aware that they have very clear and direct data to support some of these ideas and only indirect data
to support others. (10 points)

7. What else do you want to know? Good papers always tell us something new about the world which
has the effect of raising new questions. Begin by putting forth a specific hypothesis or question. In your
answer, list your hypothesis or question in one sentence and underline that sentence. Design an
additional experiment or two that will significantly extend the results that were presented here. In your
answer, cite at least one primary research paper other than those that we’ve read as a class. Be original
and don’t just re-state your answer to an earlier question on this exam. Be sure to explain the rationale
behind your hypothesis, briefly outline an experiment or two and show why the new results will be
important. (10 points)
Read the following paper and answer the questions below.


Please type your answers. You are welcome to augment your written answers with hand-drawings if that helps explain your ideas. Don’t quote this paper or any other. Be sure to refer to specific data contained in this paper frequently to support your answers. Please put your name only on the signature page only and not on the paper itself. All answers are due at noon on Friday November 16. Email submissions are fine, but still must meet the same deadline.

1. Zhang et al. are investigating p53 and its two homologous proteins, p63 and p73. They begin with p63. This protein has several different isoforms. Two are designated at TAp63 or ΔN-p63 and others are designated with a suffix of α, β or γ. How do these isoforms differ from each other? (Be sure to cite the source of your answer). In some experiments (like Figure 1B), they use the ΔN-p63 protein as a negative control – why does this protein make a good negative control? (5 points)

2. Figure 3F shows four western blots and one qRT-PCR measurement for cells incubated with and without etoposide. What is the hypothesis and rationale behind this experiment? Why did the authors choose to look use etoposide? For each western or qRT-PCR measurement, identify if it serves as a control or as an experiment and briefly explain why. (10 points)

3. Zhang et al. make the four claims listed below in the abstract. For each one, identify the specific part of a figure that supports the claim. For one of these claims, the supporting data are fairly weak. Identify which one has the weakest supporting data and design one very simple experiment that would more fully support (or refute!) the claim. (10 points)
   a. Overexpression of miR-1246 reduces DYRK1A levels
   b. Overexpression of miR-1246 leads to the induction of apoptosis
   c. Inhibiting miR-1246 prevents NFATc1 from entering the nucleus
   d. p53 induces the expression of mi-1246

4. Figure 4C shows how miR-1246 influences the movement of NFATc1 in and out of the nucleus. What phenotype would you expect if we used these same cells and treated them with etoposide instead of a miR-1246 inhibitor or mimic? Please briefly explain the logic behind your answer. (5 points)

5. The authors clearly show that increasing levels of p53 (or another family member) leads to less DYRK1A protein (Fig. 3F) and that decreasing p53 levels leads to more DYRK1A protein (Fig. 3E). The authors assert that this is because of miR-1246. How do we know that miR-1246 is necessary for p53 to lead to less DYRK1A protein? Is activation of miR-1246 by p53 sufficient to lead to less
DYRK1A protein? How do we know? As always, be sure that your answer relies on data from the paper (10 points)

6. What else do you want to know? Good papers always tell us something new about the world which has the effect of raising new questions. Begin by putting forth a specific hypothesis or question that grows out of this paper. In your answer, list your hypothesis or question in one sentence and underline that sentence. Design an additional experiment or two that will significantly extend the results that were presented here. In your answer, cite at least one primary research paper other than those that we’ve read as a class. Be original and don’t just re-state your answer to an earlier question on this exam. Be sure to explain the rationale behind your hypothesis, briefly outline an experiment or two and show why the new results will be important. Please explicitly state what variable you will be manipulating and what you will be measuring. (10 points)