Below are some suggested answers to Take-Home Exam 2. In most cases, there are many other answers that are equally correct (or even better).


1. The authors design, build and confirm an Iqgap2 knockout mouse as documented in Figure 1.

A. In the southern blots shown in Figure 1C, the genomic DNA was digested with ApaI before being separated by electrophoresis. What result would the authors have seen if they digested the DNA with XbaI instead of ApaI? What result would the authors have seen if they digested the DNA with BamHI instead of ApaI or XbaI? (3 points)

Had the DNA been digested with XbaI, a band of approximately 10kb would have been found for both alleles (and thus the two alleles couldn’t be differentiated).

If the DNA had been digested with BamHI, the null allele would have a band of about 7kb and the wildtype allele would have a band of about 40kb (which is hard to resolve on most agarose gels).

B. Four different tissues were analyzed by western blotting for IQGAP2 in Figure 1E. Which one tissue is most important to show here? Why? (3 points)

In my opinion, the western blot from liver tissue is the most important. Later in the paper, we find hepatocellular carcinomas in these animals. Since these tumors arose in the livers, it is important to show that IQGAP has been completely depleted in this tissue. Furthermore, it seems to be the cleanest of the four anti-IQGAP2 western blots.

C. What hypothesis were the authors evaluating when they ran western blots for IQGAP1 in Figure 1E? What conclusion did they reach about IQGAP1 from this experiment? (4 points)

The authors were exploring the hypothesis that the loss of IQGAP2 may affect the levels of IQGAP1 protein. Since the two genes are highly homologous, it’s reasonable to imagine that the loss of IQGAP2 may lead to the up-regulation of IQGAP1. From these blots, there is little evidence to suggest that IQGAP1 is more abundant with the loss of IQGAP2, except for a small increase in liver tissue.

2. In the abstract, the authors assert that they found “no evidence for direct IQGAP1-IQGAP2 interactions.” Where are the data that support this conclusion? When you analyze the available evidence, do you agree with this conclusion? (5 points)
Figure 6B is a coimmunoprecipitation experiment that shows no evidence for IQGAP1 and IQGAP2 binding \textit{in vivo}. The labels at the top of each of the six lanes indicates which gene had been transfected into the cells. The three on the left were immunoprecipitated with anti-IQGAP1 antibodies; the three on the right used IQGAP2 antibodies. In all cases, IQGAP2 was not detected in the anti-IQGAP1 immunoprecipitates and IQGAP1 was not detected in the anti-IQGAP2 immunoprecipitates. Since these proteins were overexpressed (and thus at a higher-than-typical concentration) it seems that we should have identified an interaction if it exists. Thus, I agree with their conclusion that we have no evidence for a IQGAP1- IQGAP2 interaction.

3. Figure 5D shows that E-cadherin levels are substantially lower in hepatocellular carcinomas than in untransformed liver tissues from \textit{Iqgap2}^- mice. Why didn’t they include livers from \textit{Iqgap2}^+/^- mice on this western blot? If they had included this wildtype tissue, what result would you expect to find? Or is it unclear what they might find? Why? (10 points)

The focus of this experiment is investigating the changes in the \textit{Iqgap2}^- cells as they form a tumor, not on how IQGAP2 affects cellular physiology in an untransformed tissue. Thus, they are asking a different question. (Although I would have liked to have seen these data anyway!)

If they had included liver tissue from a wildtype animal, it isn’t entirely clear to me what they would find. Figure 5E is the summary of a set of western blots that indicates that the livers from wildtype mice have just slightly more E-cadherin than the \textit{Iqgap2}^- animals. On the other hand the immunohistochemistry experiment shown on the bottom row of Figure 5A shows a very dark E-cadherin signal in the \textit{Iqgap2}^- tissue, but not the wildtype or the tumor tissue. In addition, the IP shown in Figure 6A indicates that there were equal levels of E-cadherin associated with $\beta$-catenin in both wildtype and mutant mice. Thus, I’m not sure how the loss of \textit{Iqgap2} would affect E-cadherin levels.

4A. The paper goes into detail about how the \textit{Iqgap2}^- mice were produced. In Figure 7, the authors look at double knockout mice, which have the genotype: \textit{Iqgap1}^- \textit{Iqgap2}^- . How were these double knockout mice produced? (5 points)

\textit{Iqgap1}^- mice were crossed with \textit{Iqgap2}^- mice to generate animals that were heterozygous at both loci (\textit{Iqgap1}^-\textit{Iqgap2}^+/^-). These mice were mated together and one-sixteenth of the resulting animals were \textit{Iqgap1}^-\textit{Iqgap2}^-.

4B. What can we conclude from the Kaplan-Meier curve shown in Figure 7C? (5 points)

The \textit{Iqgap2}^- mice have a shortened lifespan compared to wildtype mice. Deletion of \textit{Iqgap1} suppresses this phenotype, restoring a roughly normal lifespan.

4C. The data seem pretty clear that IQGAP2 acts as a tumor suppressor gene in these mice. Would you classify IQGAP1 as a putative tumor suppressor gene or a putative proto-oncogene for these mice? Or neither? Or is it unclear? Please explain your answer. (5 points)
The role of IQGAP1 in tumorigenesis is unclear. The data presented in Figure 7C and 7D indicate that the loss of IQGAP1 decreases the frequency of hepatocellular carcinoma and increases lifespan in mice lacking a functional IQGAP2. Thus, the presence of IQGAP1 is pro-tumorigenic in this genetic background. Yet there is no reason to think that it is a true oncogene, which would imply that it has undergone a somatic mutation or activation to increase tumor formation. In mice with intact IQGAP2, the loss of IQGAP1 has no impact on spontaneous cancer formation (Li et al., Mol. Cell Biol. 20:697).

5. 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 Schmidt 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. Be very clear about your dependent and independent variables. In other words, what will you alter and how you will alter it? What will you measure and how will you measure it? In your answer, cite at least one primary research paper other than the papers that we have read as a class. (10 points)

Obviously, there are a large number of excellent answers to this question.