PhD Program in Biology
Molecular, Cellular & Developmental Biology
First Examination 2019

Part 1: Essay Questions. Choice of 4 out of 6 questions.

Question 1

The questions below explore aspects of cytoskeleton assembly and dynamics.
A. (5 points) FtsZ and tubulin: compare and contrast. What are important similarities? Differences?

B. (6 points) In the study by Du and co-workers, the kinetic polarity of FtsZ protofilaments was determined to be the opposite of microtubules. Describe the logic of the genetic strategy employed by the authors in this study.

C. (4 points) The authors propose a polymerization-induced conformational switch model of FtsZ assembly. What implications does this model have for assembly of the αβ-tubulin heterodimer?

References:
Wagstaff and Löwe, 2018. Prokaryotic cytoskeletons: protein filaments organizing small cells. Nature Reviews, Vol. 16 p. 187- 201.

Shishen Du et al., 2018. FtsZ filaments have the opposite kinetic polarity of microtubules, Proc Natl Acad Sci U S A. 2018 Oct 16;115(42):10768-10773.

Question 2

Chromosome Replication in bacteria

A. (5 points) Describe the experiments that lead the authors to conclude that the crtS genetic element on *Vibrio cholerae* chromosome #1 regulates the copy number of chromosome #2?

B. (5 points) How was the minimal functional length of the crtS element identified?

C. (5 points) Provide a detailed description of the following techniques: “Tn7 transposition,” “the plasmid stability assay,” and “Quantification of the DNA loci.”

References:

de Lemos Martins, F. et al., 2018. “Vibrio Cholerae Chromosome 2 Copy Number Is Controlled by the Methylation-Independent Binding of Its Monomeric Initiator to the Chromosome 1 crtS Site.” *Nucleic Acids Research* 81 (September 3, 2018): e00019–17–12. doi:10.1093/nar/gky790.

Val, M E, et al., 2016. “A Checkpoint Control Orchestrates the Replication of the Two Chromosomes of Vibrio Cholerae.” *Science Advances* 2, no. 4 (April 1, 2016): e1501914–14. doi:10.1126/sciadv.1501914.

Question 3

Phosphorylation is a common way to regulate activity of enzymes and transporters.
A. (7 points) Discuss the ways in which phosphorylation and dephosphorylation can affect protein structure, alter enzyme activity and protein-protein interactions.

B. (8 points) Give **two** specific examples, discussing the specific mechanisms of phosphorylation and the direct and downstream effects of the modification.

References:

Biochem J. 2018 Nov 9;475(21):3331-3357. doi: 10.1042/BCJ20160819.
G protein subunit phosphorylation as a regulatory mechanism in heterotrimeric G protein signaling in mammals, yeast, and plants.
Chakravorty D[1], Assmann SM[1].

J Muscle Res Cell Motil. 2012 Dec;33(6):419-29. doi: 10.1007/s10974-012-9317-6.
Structural dynamics of muscle protein phosphorylation.
Colson BA[1], Gruber SJ, Thomas DD.

Question 4

Analysis of familial cases of melanoma documented in the articles by Horn et al. and Huang et al. has established that the causing mutation was a single nucleotide change in the promoter for the hTERT gene, coding for the catalytic subunit of telomerase.

A. (5 points) Describe the molecular cause of melanoma in this case as hypothesized in the articles. In doing so mention:

-the molecular effect of the mutation described, in general terms (we are not asking for the specific nucleotide change!).

-describe the proposed effect that the hTERT gene has in cellular transformation during oncogenesis as a result of this mutations. Does this mutation alone cause a fully transformed phenotype in cells? Explain.

B. (5 points) What is the pattern of inheritance followed by this disease? Provide a rationale that supports your answer in the context of your answer in A).

C. (5 points) -Explain why UV light would induce this particular tumor type (melanoma) in this particular cell type (melanocytes). Make sure to mention the specific cellular pathway involved.

References:
Horn, S. et al. (2013) TERT Promoter Mutations in Familial and Sporadic Melanoma Science 339:959

Huang et al. (2013) Highly Recurrent TERT Promoter Mutations in Human Melanoma *Science* 339:957

Question 5

The report by Zuryn et al., 2014 investigates the mechanisms for an epithelial to neuronal transdifferentiation in *C. elegans*.

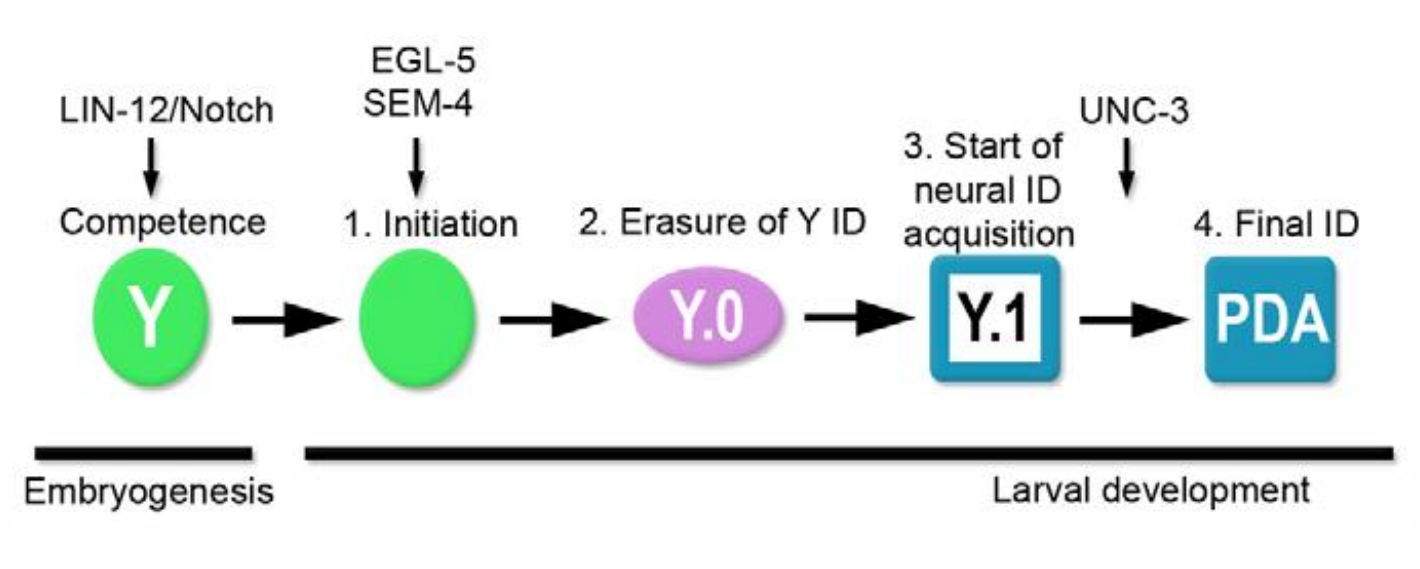


Figure 6 of Richard et al., 2011. Model for Y-to-PDA transdifferentiation.

A. (4 points) Describe how the steps of transdifferentiation occurs in *C. elegans*.

B. (2 points) Does transdifferentiation require cell division?

C. (3 points) What is the mutant phenotype and the wild type function of *jmjd-3.1*? Molecularly, what does *jmjd-3.1* encode and explain how that contributes to transdifferentiation?

D. (4 points) Explain the logic used by the authors to focus on genes that displayed an incompletely penetrant transdifferentiation defective phenotype, compared to genes that have a completely penetrant phenotype.

E. (2 points) The *jmjd-3.1* mutants have an incompletely penetrant phenotype. What arguments do the authors provide that the mutants with incompletely penetrant defects are null?

References:

Richard J.P. et al., 2011. Direct in vivo cellular reprogramming involves discrete, non-pluripotent steps. Development 138: p.1483-1492.

Zuryn S, Ahier A, Portoso M, White ER, Morin MC, Margueron R, Jarriault S., 2014. Sequential histone-modifying activities determine the robustness of transdifferentiation. *Science*. 2014. 345:826-9

Question 6

The nuclear pore proteins play crucial roles in controlling the nucleocytoplasmic transport and other cellular processes, including gene regulation and cell cycle progression. The paper “Age-Dependent Deterioration of Nuclear Pore Complexes Causes a Loss of Nuclear Integrity in Postmitotic Cells” by D’Angelo et al. demonstrated that the nuclear pores in aged differentiated cells lose their structural integrity, resulting in an increased nuclear permeability (or leaky nuclei). Based on their study, answer the following questions.

A. (4 points) What are the transcription states of scaffold and peripheral nucleoporin genes in dividing and nondividing cells?

B. (4 points) How did the authors analyze the transcription levels of nucleoporin genes in dividing and nondividing cells?

C. (4 points) Explain why the nuclei in the aged differentiated cells become leaky. Please consider both the biochemical change of the nuclear pore proteins and the structural change of the nuclear pore complexes.

D. (3 points) The authors used different approaches to examine the permeability barrier of the nuclear pores. Use one example to explain how they were convinced that the nuclei of aged differentiated cells were leaky.

References:

D'Angelo MA, Raices M, Panowski SH, Hetzer MW. Age-dependent deterioration of nuclear pore complexes causes a loss of nuclear integrity in postmitotic cells. Cell. 2009, 136(2): 284-95.

Sakuma, Stephen; D'Angelo, Maximiliano A. The roles of the nuclear pore complex in cellular dysfunction, aging and disease. Seminars in Cell & Developmental Biology 2017. 68 SI: 72-84.

Part 2A: Experimental Design

Question 7

You are following up on the study of Keleman et al. and have designed a genetic strategy in which a specific single commissural mutant (*comm*-) neuron is formed in the context of a wild-type Drosophila embryo. This particular neuron (the EP neuron) is normally a contralateral (crosses the midline) projecting neuron in a wild-type embryo. With this genetic strategy, you also have the ability to express any protein specifically in wildtype or *comm*- EP neurons.

A. **(3 points)** Will the axon of the *comm*- EP neuron cross the midline or not cross the midline of the embryo? Why or why not?

B. **(3 points)** Assuming that you can perform high resolution live imaging of the EP neurons using the same assay that Keleman used, describe what, if any, differences you expect in this assay between wildtype and comm- EP neurons as their axons begin to form. Explain your reasoning.

C. You identify a tyrosine kinase called TyrC that is able to phosphorylate the comm protein on tyrosine residue 42 in an in vitro assay. You hypothesize that this phosphorylation results in the comm protein being completely nonfunctional and unable to interact with any other proteins. Furthermore, you hypothesize that this phosphorylation controls the switch in comm activity during axon outgrowth and that the TyrC gene is transcriptionally regulated such that its mRNA is not present during axon outgrowth towards the midline, but is rapidly induced after an axon crosses the midline.

i. **(7 points)** Design a controlled in vitro experiment to test your hypothesis about the role of this phosphorylation event and the TyrC kinase in terms of Comm's proposed protein interaction.

ii. **(7 points)** Design a controlled in vivo experiment to test if this phosphorylation event and this kinase are important in midline guidance.

References:

Keleman et al., 2005. Comm function in commissural axon guidance: cell-autonomous sorting of Robo *in vivo, Nature Neurosciences* 8(2):156-63.

Dickson and Gilestro, 2006. Regulation of commissural axon pathfinding by slit and its Robo receptors. *Annu Rev Cell Dev Biol.* 2006; 22:651-75.

Question 8

Different mechanisms underlie sex determination in animals. In humans sex determination is a balance of opposing pathways: those promoting maleness versus those promoting femaleness.
A. (2 points) In bullet form, give the experimental data that indicate that sex is determined by a specific chromosome in humans.

B. (4 points) In humans, the embryonic gonadal tissue is bipotential, which means that it can form either male or female sex tissues. The *sry* gene encodes a transcription factor that is proposed to be the master regulatory gene that determines maleness by activating male-specific pathways (some of which are seen in the figure) to form male tissues. What experiments would you propose to show that *sry* determines maleness in mice? What results would you expect in terms of sex organ differentiation?

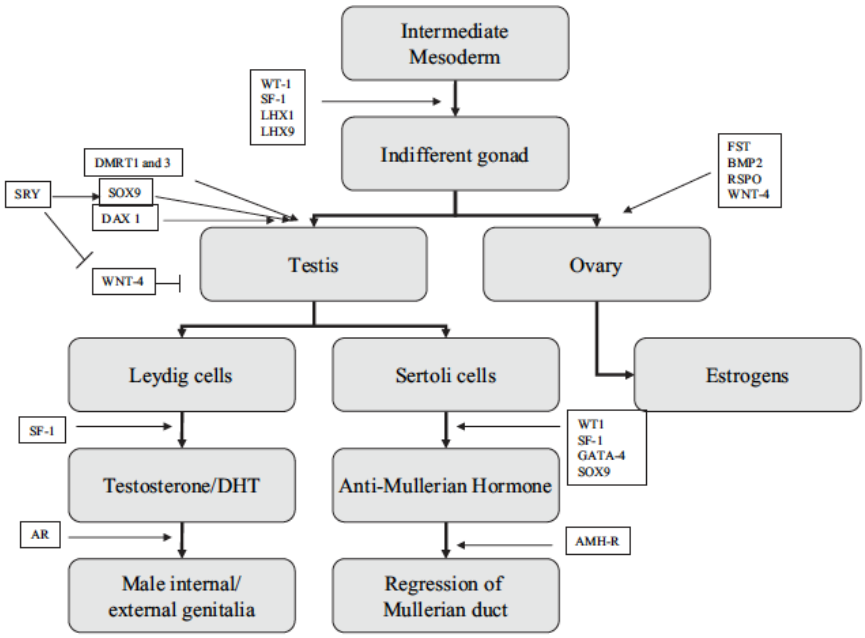


FIG. 4. Genes involved in sexual differentiation: *WT-1*, Wilms' tumor 1; *SF-1*, steroidogenic factor 1; *LHX1*, LIM homeobox 1; *LHX9*, LIM homeobox 9; *DMRT1* and *-3*, doublesex and Mab3-related transcription factors 1 and 3 (located on chromosome 9p24); *SRY*, sex-determining region of Y chromosome; *SOX9*, SRY-box-related 9; *DAX1*, dosage-sensitive sex reversal locus-adrenal hypoplasia congenita-critical region on the X, gene 1; *WNT-4*, wingless-type MMTV integration site family, member 4 (member of Wnt family of locally secreted growth factors); *RSPO*, R-spondins; *FST*, follistatin; *BMP2*, bone morphogenetic protein 2; *GATA-4*, GATA-binding protein 4 (codes for a zinc finger transcription factor); *AR*, androgen receptor; *AMH-R*, anti-Mullerian hormone receptor.

C. A 17 year-old female comes into a clinic because she has not begun menstruating. She is genotyped and found to be XY in all her tissues. Her mother was examined and similarly found to be XY in all her tissues. The mother was examined for mutations in the coding sequences of *sry*, *DMRT1, DMRT3, Sox9, DHH, WNT4,* and *SF1* genes, and all coding regions of these genes were wild type.

i) (4 points) Propose **<u>two</u>** hypotheses as to why the mother is not male. What experiments would you perform to test your model while the mother is alive?

ii) (3 points) From whom did the female daughter inherit the Y chromosome, the mother or father? How was this determined?

iii) (3 points) Suppose that the daughter inherited the Y chromosome from the father. Can the mother have sons?

D. (4 points) A 46 XX male with no trace of the Y chromosome (and any genes therein) showed normal external and internal male genitalia, but was infertile. No mutations in the coding regions of the *DMRT1, DMRT3, Sox9, DHH, WNT4,* and *SF1* genes were found. Propose a model for why this person appears phenotypically male. What experiments could you perform while the male is alive to test this model?

References:

Sakashita A, Wakai T, Kawabata Y, Nishimura C, Sotomaru Y, Alavattam KG, Namekawa SH Kono T (2018) XY oocytes of sex-reversed females with a *Sry* mutation deviate from the normal developmental process beyond the mitotic stage. Biol Repro https://doi.org/10.1093/biolre/ioy214.

Rotgers E, Jorgensen A, Yao HH-C (2018) At the crossroads of fate-somatic cell lineage specification in the fetal gonad. Endocrine Reviews 39:739-759.

Part 2B: Data Analysis

Question 9

Question on the human microbiome

The human microbiome is the collection of microorganisms that live in or on the human body. In a healthy adult the human microbiome is home to over 35 trillion microorganisms, that is there is ~1 microbial cell for every human cell. Moreover, while the human genome consists of just over 20 thousand genes, the human microbiome contributes as many as eight million unique genes. Recent advances in DNA sequencing technology, and culture-independent microbial community analysis, have allowed researchers to explore how the human microbiome and its associated metagenome contribute to human development and health. The wealth of new data has uncovered important roles for the human microbiome related to immune system development, nutrition, drug metabolism, protection from pathogenic invaders, and behavior. An understanding of the composition of an individual's microbiome will become increasingly important in determining treatments for a variety of diseases and illnesses. Recent research has shown that manipulating the composition of the microbiome can effectively treat some acute and chronic diseases. In the article by Leclercq et al. (2017), administering antibiotics in early life can increase the risk of immune and metabolic diseases.

A. (6 points) From the data presented in this article, what changes are observed in gut microbiota during and after low-dose penicillin therapy?

B. (8 points) What sort of behavioral changes are observed in the mice treated with antibiotics? Based on your knowledge of this subject, discuss possible mechanisms that could link antibiotic treatment with changes in the behavior of the mice. Support your answer with data from the Leclercq article.

C. (4 points) Can the effect of these changes caused by antibiotics be reduced or reversed? Support your answer with data from the Leclercq article.

D. (2 points) Studying Fig. 4, graphs A and B, what conclusions can be drawn from inflammation between males and females? Support your answer with data from the Leclercq article.

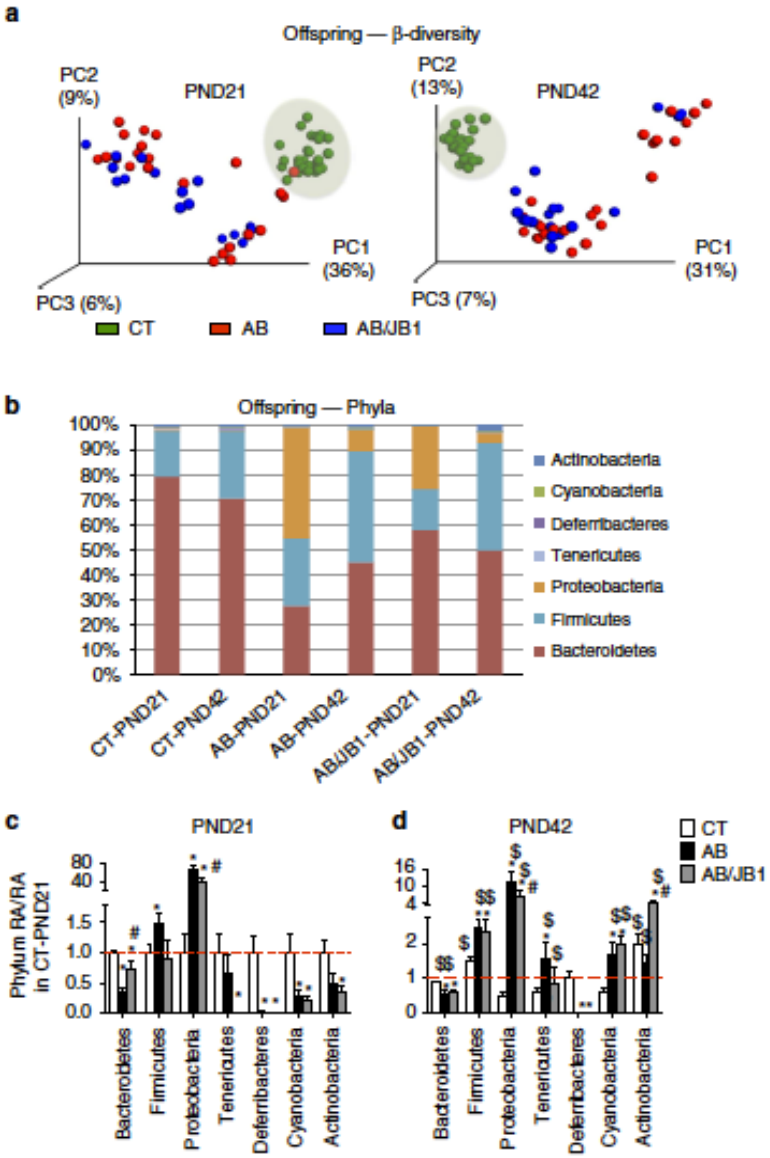


Figure 3 | Gut microbiota composition in offspring at 3 and 6 weeks old. (**a**) β-Diversity calculated with unweighted UniFrac matrix showing significant differences ($P = 0.01$) at both time points (PND21, 3 weeks old; PND42, 6 weeks old). (**b**) Relative abundance of bacteria phyla, expressed in percentage. (**c**,**d**) To better visualize the change induced by AB treatment in low-abundance phyla, the relative abundance of each phylum has been divided by the mean of relative abundance obtained in the CT group at PND21. The red line indicates the mean relative abundance of the phylum in the CT group at PND21, which corresponds to the value 1. Results are means ± s.e.m., $n = 68$ (26 CT, 24 AB, 18 AB/JB1) (mixed ANOVA). *$P < 0.05$ compared to CT group after Bonferroni adjustment for multiple comparisons (within the same study time point). #$P < 0.05$ compared to AB group after Bonferroni adjustment for...

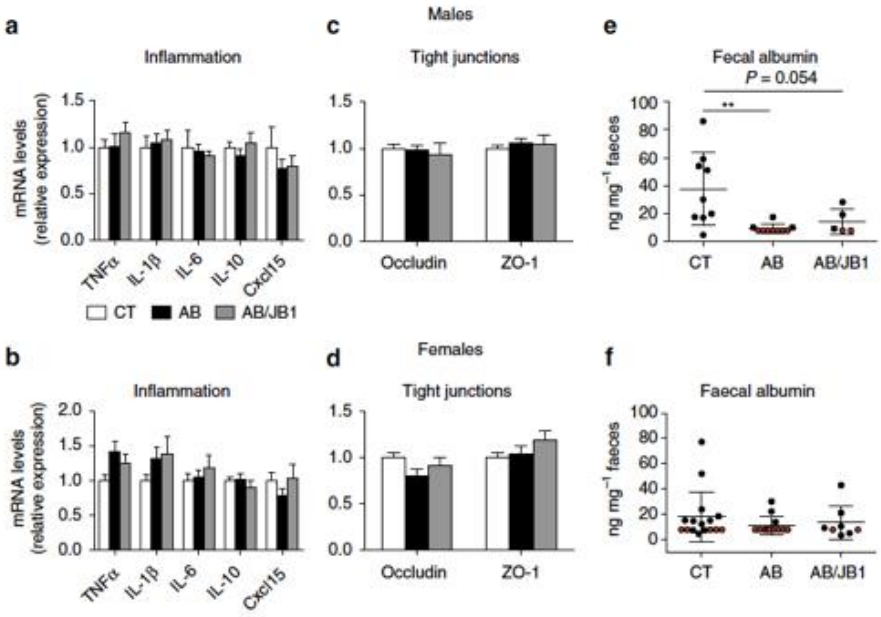


Figure 4 | Assessment of inflammation and intestinal permeability in colon. (**a**,**b**) Intestinal inflammation was evaluated by the mRNA expression of cytokines and chemokine. (**c**–**f**) Evaluation of intestinal barrier integrity was performed by measuring mRNA expression of tight junctions and fecal albumin concentration. Results are means ± s.e.m. (s.d. for **e**,**f**), $n = 28$ males (11 CT, 12 AB, 5 AB/JB1) and 43 females (17 CT, 13 AB, 13 AB/JB1) (one-way ANOVA). Red dot in graphs (**e**,**f**) indicates that the level of faecal albumin was below the detection limit. $^{**}P < 0.01$. AB, antibiotic; AB/JB1, antibiotic and *L. rhamnosus* JB-1; CT, control.

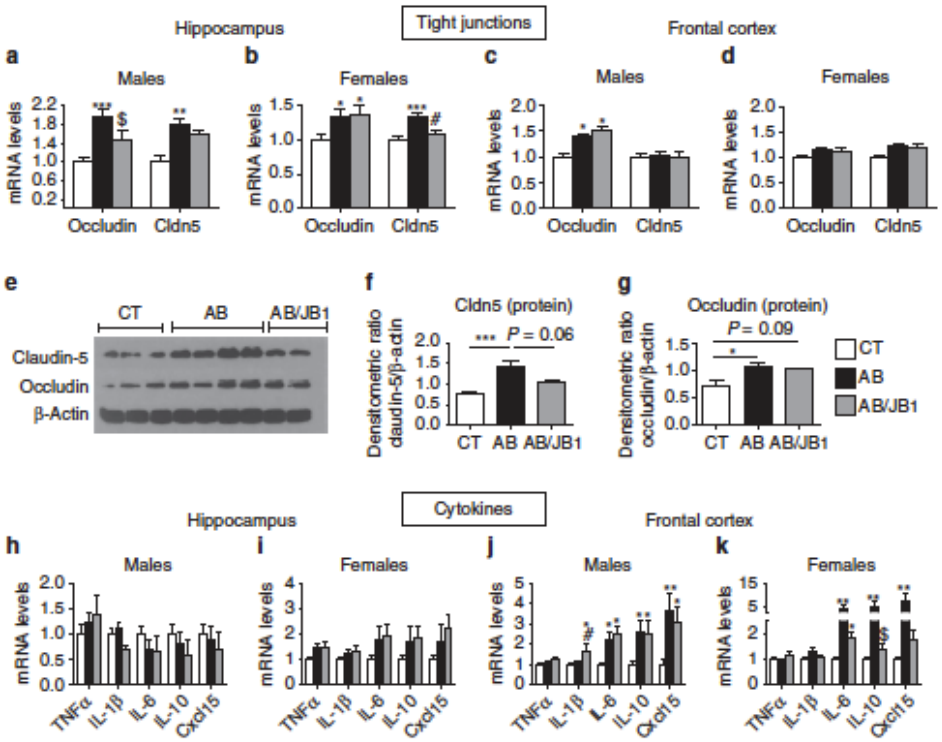


Figure 6 | Brain tight junctions and cytokine expression. (**a–d**) mRNA expression of tight junctions measured in the hippocampus and the frontal cortex of male and female mice. (**e–g**) Representative western blot of tight junctions proteins claudin-5 and occludin performed in hippocampus of male mice and quantification by densitometry normalized to the loading control β-actin (in total: $n=5$ CT, 8 AB and 4 AB/JB1 mice). Full blots are shown in Supplementary Fig. 22. (**h–k**) mRNA expression of cytokines and chemokine in hippocampus and frontal cortex of male and female mice. Results are means ± s.e.m. (Hippocampus $n=24$ males (9 CT, 11 AB, 4 AB/JB1) and 39 females (15 CT, 11 AB, 13 AB/JB1); frontal cortex $n=28$ males (11 CT, 12 AB, 5 AB/JB1) and 42 females (17 CT, 13 AB, 12 AB/JB1)) (one-way ANOVA) $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus CT. $^{\#}P<0.05$ versus AB and $^{\$}P<0.10$ versus AB. AB, antibiotic; AB/JB1, antibiotic and *L. rhamnosus* JB-1; CT, control.

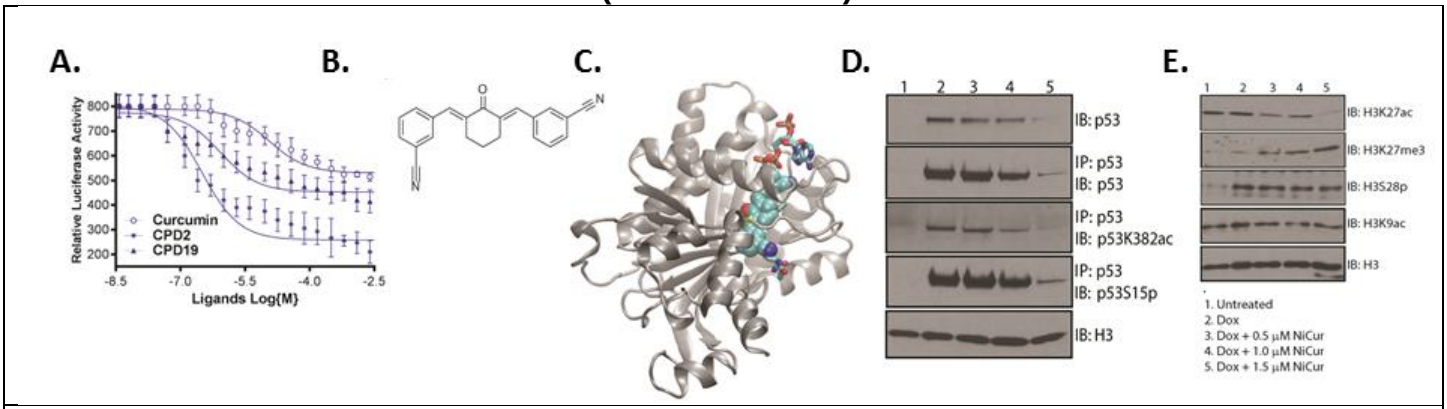
References:

Leclercq S, Mian FM, Stanisz AM, Bindels LB, Cambier E, Ben-Amram H, Koren O, Forsythe P, Bienenstock J. Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. *NATURE COMMUNICATIONS 2017| 8:15062 | DOI: 10.1038/ncomms15062 |www.nature.com/naturecommunications*

Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol*. 2015;14(1):20-32.

Question 10

One of the most effective approaches to discover a small molecule that could have a therapeutic value or could be used as a tool to dissect molecular mechanisms is through: i) Structure-guided approach or; ii) cell-based approach. In a cell-based approach, a cellular assay developed to screen a library of small molecules, and subsequently most potent ligand, is identified by determining the effective IC50. This lead compound is further validated for its target selectivity and its biochemical and cellular effects. **Figure 1** below shows a library of curcumin analogues that were screened by a luciferase assay to identify compounds that would block the acetylation-based function of the ubiquitous transcriptional co-activator CREB-binding protein (CBP), mediated by its histone acetyl transferase (HAT) domain **(A)**. In this luciferase assay, the p53 reporter gene was cloned adjacent to the luciferase gene (Promega). Panel **B** shows that compound 2 is most effective as determined by IC50. Subsequently, docking experiments showed that compound 2 completely docked into the active site of CBP HAT. To test the biochemical effects of compound 2, U2OS cells were treated with doxorubicin and increasing concentrations of compound 2. The effect was determined by Western blot on the nuclear extracts of U2OS cells **(Panels D and E)**.



Working Platform for drug discovery program: **A** and **B)** screening of library of compounds by luciferase assay and identification of most potent ligand; **C)** Target validation by docking of lead compound on model structure of CBP HAT; **D** and **E)** biochemical effect of lead on protein level of p53.

A. (3 points) What is the role of CBP in activating p53?

B. (3 points) In Panel D, why the p53 level declines with increasing amount of compound 2?

C. (3 points) In Panel E, what is the effect of the compound on the levels of acetylation and methylation on lysine 27 of histone H3 (H3K27ac/H3K27me)?

D. (2 points) Which enzyme mediates the methylation H3K27 site?

E. (3 points) What is the effect of compound 2 on the level H3S28p?

F. (6 points) What do you envision the effect of this compound are on growth arrest and the apoptosis function of p53?

References:

Wang, X.; Wei, L.; Cramer, J.M.; Leibowitz, B.J.; Judge, C.; Epperly, M.; Greenberger, J.; Wang, F.; Li, L.; Stelzner, M.G.; et al. Pharmacologically blocking p53-dependent apoptosis protects intestinal stem cells and mice from radiation. *Sci. Rep.* **2015**, *5*, 8566, doi:10.1038/srep08566.

Gudkov, A.V.; Komarova, E.A. Prospective therapeutic applications of p53 inhibitors. *Biochem. Biophys.res. Commun*. **2005**, *331*, 726–736, doi:10.1016/j.bbrc.2005.03.153.