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Thesis

MAPPING THE BINDING OF PROTEIN HETERODIMERS CONTAINING HUMAN TRANSCRIPTION FACTORS AND VIRAL TRANSCRIPTION REGULATORS TO PROMOTERS OF IMMUNE RESPONSE GENES AND CANCER GENES

by

SAKSHI K. SHAH

B.A., Boston University, 2023

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

2023

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Approved by

First Reader ______________________________

Juan Fuxman-Bass, Ph.D.
Assistant Professor of Biology

Second Reader ______________________________

Trevor W. Siggers, Ph.D.
Associate Professor of Biology

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MAPPING THE BINDING OF PROTEIN HETERODIMERS CONTAINING HUMAN TRANSCRIPTION FACTORS AND VIRAL TRANSCRIPTION REGULATORS TO PROMOTERS OF IMMUNE RESPONSE GENES AND CANCER GENES

SAKSHI K. SHAH

Boston University Graduate School of Arts and Sciences, 2023

Major Professor: Juan Fuxman Bass, Ph.D., Assistant Professor of Biology

ABSTRACT

Viruses are infectious disease- and cancer-causing agents. Viral infection in humans leads to a variety of cytopathic effects. Viral transcription regulators (vTRs) play a central role in human biological processes by modulating host gene expression through direct and indirect methods of binding to nucleic acids making it important to study. Despite the important role of vTRs in human disease, our understanding of their molecular features and functions remains limited. The focus of this study looks at vTR and human transcription factor (hTF) pairs to determine how vTRs affect the binding of hTFs to cytokine and cancer gene promoters. In this study we are conducting a pY1H screen using 139 vTR-hTF pairs, 83 cancer promoters, and 41 cytokine promoters. A total of 108 interactions were seen with results highlighting information about viral genome, family, and species. Overall, this study has offered a revolutionary method to study hTFs

and vTRs as pairs in a variety of immune and cancer gene promoters to understand more about mechanisms of host gene regulation.

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LIST OF ABBREVIATIONS

C	Celsius
DNA	deoxyribonucleic acid
dsDNA	double-stranded DNA
EBV	Epstein-Barr virus
eY1H	enhanced yeast one hybrid assay
hCMV	human cytomegalovirus
HIV	human immunodeficiency virus
HPV	human papillomavirus
hTF	human transcription factor
L	liter
mL	milliliter
mM	millimolar
MYC	MYC proto-oncogene, bHLH transcription
PCR	factor polymerase chain reaction
pY1H	paired yeast one hybrid assay
RNA	ribonucleic acid
ssDNA	single-stranded DNA
TERT	telomerase reverse transcriptase
TF	transcription factor
uL	microliter
vTR	viral transcription regulator

YNB	yeast nitrogen base without amino acids and ammonium sulfate
Y1H	yeast one hybrid

CHAPTER ONE: INTRODUCTION

Section One: Viruses

Viruses are infectious disease- and cancer-causing agents (Risk Factors: Infectious Agents, 2019). They infect all forms of life, from bacteria and fungi to plants and animals, and are responsible for a variety of diseases and syndromes. Viral infection in humans leads to a variety of cytopathic effects. Globally they are attributed to numerous diseases and cancers. As a result of viral infection, viruses can affect several different cellular pathways which can lead to a range of symptoms and disease outcomes.

Viruses are composed of a small amount of genetic material either in the form of DNA or RNA, surrounded by a protective protein known as a capsid. Some types of viruses also have a lipid envelope derived from the host cell membrane, which may contain viral-specific proteins (Gelderblom, 2013). The genome of a virus is usually very small, containing only a few genes that are essential for its replication and survival. Viruses have been classified based on the type of genetic material they contain (ssDNA, dsDNA, positive RNA, negative RNA) and their morphological characteristics (Gelderblom, 2013). This simplicity, however, belies the complexity of the mechanisms by which viruses replicate, spread, and evade host defenses.

Section Two: Viral Transcriptional Regulators

Viruses can regulate their own genes using viral transcriptional regulators (vTRs). vTRs play a central role in human biological processes by modulating host gene expression through direct and indirect methods of binding to nucleic acids making it

important to study (Liu et al., 2020). They can have impacts on several key functions, including immune responses, cell cycle, transformation of cells, metabolism, and epigenetic regulation.

vTRs can affect central human biological processes and directly control cell growth and proliferation. Through their ability to modulate host gene expression, vTRs can affect cellular pathways, such as the transformation of cells and the expression of oncogenes and tumor suppressor genes. Different types and classes of vTRs coordinate different functions and they can exhibit high genetic diversity (Liu et al., 2020).

Despite the important role of vTRs in human disease, our understanding of their molecular features and functions remains limited. Studying vTRs is challenging because of their high mutation rates, difficulty in classification, and the impact of different assays on gene expression. Nonetheless, it is important to study them to better understand their binding interactions and the host-virus relationship. Until recently, there was no single resource with a comprehensive review of vTRs, and their high mutation rates and ability to evolve quickly make them difficult to study. Furthermore, different types and classes of vTRs control and coordinate different functions, making classification and characterization challenging.

Different types and classes of vTRs can regulate host genes through distinct mechanisms and studying the interactions between these different vTRs and human transcription factors (hTFs) can be significant for understanding more about host immune genes and the immune system as whole. One of the ways that the interactions between vTRs and hTFs can be studied is via protein-protein interactions (Davis et al., 2015).

Protein-protein interactions play a crucial role in the replication and pathogenesis of viruses. They have evolved in certain viruses to subvert and manipulate the host cell machinery, allowing the virus to evade host immune responses and promote its own replication (Zhou et al., 2018). To achieve this, vTRs interact with a diverse array of host proteins, including those involved in cancer-related signaling, immune signaling pathways, and regulation of cell growth and proliferation. These interactions can take place through both direct and indirect mechanisms, such as interactions with protein domains, binding to protein partners, or the formation of RNA-protein complexes (Liu et al., 2020).

One notable example of vTR-mediated protein-protein interactions involve the p53 signaling pathway, a crucial pathway involved in cell cycle regulation, DNA damage response, and programmed cell death. Various oncogenic vTRs have been shown to target p53, with some vTRs binding directly to p53 protein and inhibiting its transcriptional activity, while others interact indirectly through other cellular proteins, leading to its degradation (Hafner et al., 2019).

Section Three: Enhanced Yeast-One Hybrid Assay and Paired Yeast-One Hybrid Assay

These are several different protein-protein interactions between vTRs and hTFs, but what are other interactions that can provide more insight into the relationship between vTRs and hTFs? Some transcription factors (TFs) can bind DNA in a cooperative manner while other TFs can antagonize one another. This study wants to learn more

about the outcomes of protein-protein interactions and how they can affect hTF binding to DNA.

This will also provide insight into interactions between vTRs and hTFs on a genetic basis which would be done through the paired yeast one hybrid assay (pY1H). This method is an extension of the enhanced yeast one hybrid assay (eY1H). In this method interactions between TFs and DNA regions are tested in the milieu of the yeast nucleus using reporter genes. Y1H assays involve two components: a 'DNA-bait' which can include: promoters, enhancers, silencers and a 'TF-prey,' which can be screened for reporter gene activation (Shrestha et al., 2019).

The eY1H takes the Y1H method a step further to study the TF-DNA interactions using an arrayed collection of TF preys and mating them with DNA baits in a high throughput screening manner (Reece-Hoyes et al., 2011). Compared to the original Y1H method, the eY1H method has a higher throughput with the fold increase being around 50-fold. It has been shown that eY1H assays incorporate several modifications that increase both throughput and coverage over previously reported Y1H methods. In eY1H assays, each DNA bait-prey combination is tested four times using "TF quad arrays" that contain each prey in quadruplicate (Reece-Hoyes et al., 2011). An advantage of this method is that eY1H assays test available TFs directly resulting in higher coverage and these TFs are compared directly to a negative control making it easier to detect weaker interactions. Also, some TFs have been detected in eY1H assays that were previously not detected in Y1H assays due to higher prey expression levels (Reece-Hoyes et al., 2011). eY1H assays have provided the ability for mapping on the gene level of genetic

regulatory networks in a variety of model organisms. This has been seen in both large-scale genome wide studies and small-scale in-depth studies on single promoter regions. (Reece-Hoyes et al., 2011).

An adaptation of the eY1H and the primary methodology of this study is the pY1H. The pY1H method allows for the ability to study two TFs on a singular bait at once to identify DNA-binding cooperativity and antagonism between several hundred TFs pairs at DNA regions of interest. This method reveals that several relationships can occur across both well and less-known TF-pairs in a DNA-sequence specific manner. Adapted from the eY1H, the pY1H also allows for a greater understanding of cooperative and antagonistic binding interactions as seen by TF-pairs. An aspect of this study will show how viral proteins for a great majority antagonize the binding of hTFs to their DNA targets which can provide insight into host transcriptional mechanisms and reprogramming by viruses.

The focus of this study looks at vTR-hTF pairs to determine how vTRs affect the binding of hTFs to cytokine and cancer gene promoters. vTRs and hTFs were selected based on known interactions in the literature and wanting to either re-evaluate them or compare them to other protein interactions, as well as in the attempt to find novel interactions between pairs and the promoter bait.

Thesis Rationale:

Despite an emerging role in human diseases, a thorough annotation of human viral transcriptional regulators (vTRs) is currently lacking, limiting our understanding of

their molecular features and functions (Liu et al., 2020). Learning more about interactions between vTRs and hTFs will be able to provide a range of insights from confirming interactions seen in literature to unveiling novel interactions. The data that is found and analyzed from this study can have larger implications in future research on both a molecular and clinical level in working towards revolutionizing our understanding of vTRs in relation to immune and cancer-causing genes.

CHAPTER TWO: MATERIALS AND METHODS

Gateway Cloning

Purpose: Cloning from PCR product into entry from destination vector to entry vector

BP Reaction + LR Reaction

Clones were amplified via PCR and a BP reaction was performed to get the clone into pDONR221 as that was the entry vector. An LR reaction was performed to put the hTFs in pgadt7 and the vTRs in AD2u as the destination vectors.

DH5a chemically competent cells were thawed on ice. 2.5 uL proteinase K (diluted in a 1:5 ratio in water) was added to each cloning reaction and incubated in a 37C water bath for 10 minutes. 20 uL DH5a cells were added to each cloning reaction and reactions were incubated on ice for 20 minutes. For exactly 45 seconds cells were heat shocked in a 42 C water bath and then incubated on ice for 3 minutes. 200 uL SOC media (a combination of SOB and 40% glucose in a 1:100 ratio) was added to each reaction and then cells were incubated in a 37C water bath for 1 hour. Cells were then plated on LB+ antibiotic plates for single-colony selection and were incubated overnight at 37C. Colonies were checked in pairs by clone via DreamTaq PCR. Successful colonies were grown in liquid cultures in 3 mL LB+ ampicillin at 37 C overnight in a shaking incubator.

MiniPrep

Bacteria samples were centrifuged at 3200 rpm for 10 minutes. The PureLink Quick Plasmid MiniPrep kit by Invitrogen from ThermoFisher was used for this part of the methodology. Samples were then resuspended in 250 ul of resuspension buffer. 250ul

of lysis buffer was added and kept at room temperature for 5 minutes. 350 ul of precipitation buffer was added and thoroughly mixed. The lysate was then centrifuged at 12000 g for 10 minutes. The supernatant was then loaded onto a spin column with a wash tube and spun down at 12000 g for 1 minute. Flowthough was discarded and 700 ul of wash buffer with ethanol was added to the column. The column was centrifuged at 12000 g for 1 minute and flow through was discarded. This step was repeated, the column was placed into an elution tube, and 50 ul of preheated TE buffer was added. The column was then incubated at room temperature for 1 minute and then centrifuged at 12000 g for 2 minutes where the elution tube contained purified plasmid DNA.

Yeast Transformation

High-Efficiency Transformation to generate TF prey Ya1867Δleu2

Ya1867Δleu2 yeast was extracted from glycerol stock, plated on YAPD-agar, and incubated at 30C for one day. The transformation process spanned 9 hours in total. 50 mL of 40% glucose was added to 1L YAPD liquid medium. 1mL of this was used as a blank solution for measuring OD600.Ya1867Δleu2 yeast was transferred from the YAPD plate to 1.5mL and 500uL of sterile water was added to resuspend the yeast. 300 ul of yeast suspension was added to the YAPD medium and mixed. A sample was used to measure the OD600 where it initially ideally should be 0.15 and after it should be incubated in a shaking incubator at 30C, 200pm until the culture reaches an OD600 of 0.5. This incubation time was found to be around 6 hours. Cells were then harvested by centrifugation at 700g for 5 minutes. Supernatant was discarded from the yeast and cells

were washed with sterile water, resuspended, and combined into 50mL yeast suspensions. Cells were centrifuged at the same conditions as before; the supernatant was discarded, and cells were resuspended in 25 mL of freshly made TE/LiAc. This was repeated one more time where this time cells were resuspended in 50 ul/sample of TE/LiAc solution. Salmon sperm ssDNA was added in a 1:10 concentration to the samples and then the TE/LiAc/ssDNA yeast suspensions were plated in a sterile 96-deep well plate with at least 2ul of each DNA sample to be transformed. 300 uL of freshly made TE/LiAc/Peg solution was added and the plate was incubated at 30 C between 30 minutes and 4 hours. This particular time frame optimizes the transformation efficiency. Cells were then heat shocked at 42C in a water bath for 20 minutes. Cells were centrifuged at 700g for 5 minutes and the supernatant was discarded. Cells were then resuspended in 5ul of sterile water and then 2ul of yeast was plated onto selective “-LT” 15 cm plates in 96 spot format and incubated at 30C for 1 week until yeast had grown.

Sequencing to Confirm Array

Zymolyase Reaction, PCR Check, Next Generation Sequencing

From yeast plates, touch and pick up yeast with a sterile toothpick and mix into 10ul of Zymolyase and then run the Zymolyase reaction (30 minutes on 37C and 10 minutes on 96C) on the thermocycler. After the completion of the zymolyase reaction a PCR check of the reaction was performed under the following conditions per reaction: 6.75 uL of water, 1ul of Dream Taq buffer, 0.2ul of DMSO, 0.2ul of dNTP, 0.05 uL of Dream taq, 0.4 ul of a reverse primer, and 0.4 ul of a forward primer. After preparing the

contents of the samples, they were subjected to a DreamTaq PCR reaction. After the PCR samples were checked for DNA bands via gel electrophoresis. Samples were also sent out for next generation sequencing to confirm products.

Screening

Spotting the TF Array:

Yeast glycerol stock plates with the TF array were thawed on ice. Yeast was resuspended in a volume of up to 50 uL. Stock plates were spotted in 96 spot-format to Sc -LT plates using the HDA Singer Robot. Plates were placed at room temperature 24 hours prior to usage. Spotted array plates were bagged and then incubated at 30 C for 2-3 days. The Robot was also used to generate 384 colony arrays from the 96 spot-format on the Sc -LT plates. These plates were bagged and incubated at 30 C for 2-3 days. 1,536 colony array plates were generated using the 384 colony array plates using the Robot on Sc -LT plates. The goal was to copy each colony into four colonies to obtain quadruplicates.

pY1H Screen:

Methods for the screen are spanned across 12-16 days. The first six days involved the creation of DNA-bait lawns by first spotting in 96 colony-format from glycerol stocks. From day six onwards goals included creating the bait lawn plates, mating with the TF array, and subsequent replications for diploid selection followed by a final transfer to X-gal plates for a final readout. Readout plates were photographed depending on bait activity. Autoactive baits were photographed 2, 3, and 4 days after transfer to X-gal

plates, while non-activate baits were photographed 4 and 7 days after transfer to X-gal plates.

CHAPTER THREE: RESULTS

Summary of pY1H Results:

From a total of 418 vTR-hTF pairs, the final array contained 139 vTR-hTF pairs, 83 cancer promoters and 41 cytokine promoters on which a pY1H screen was performed where a total of 108 interactions (both cooperative and antagonistic between vTR and hTF) were seen ranging from qualitative descriptions of very weak to very strong. In summary from these 108 interactions, a total of 59 unique baits, 15 unique hTFs, and 12 unique vTRs were found to be involved in the interactions. (Figure 1, Figure 2, Table S1)

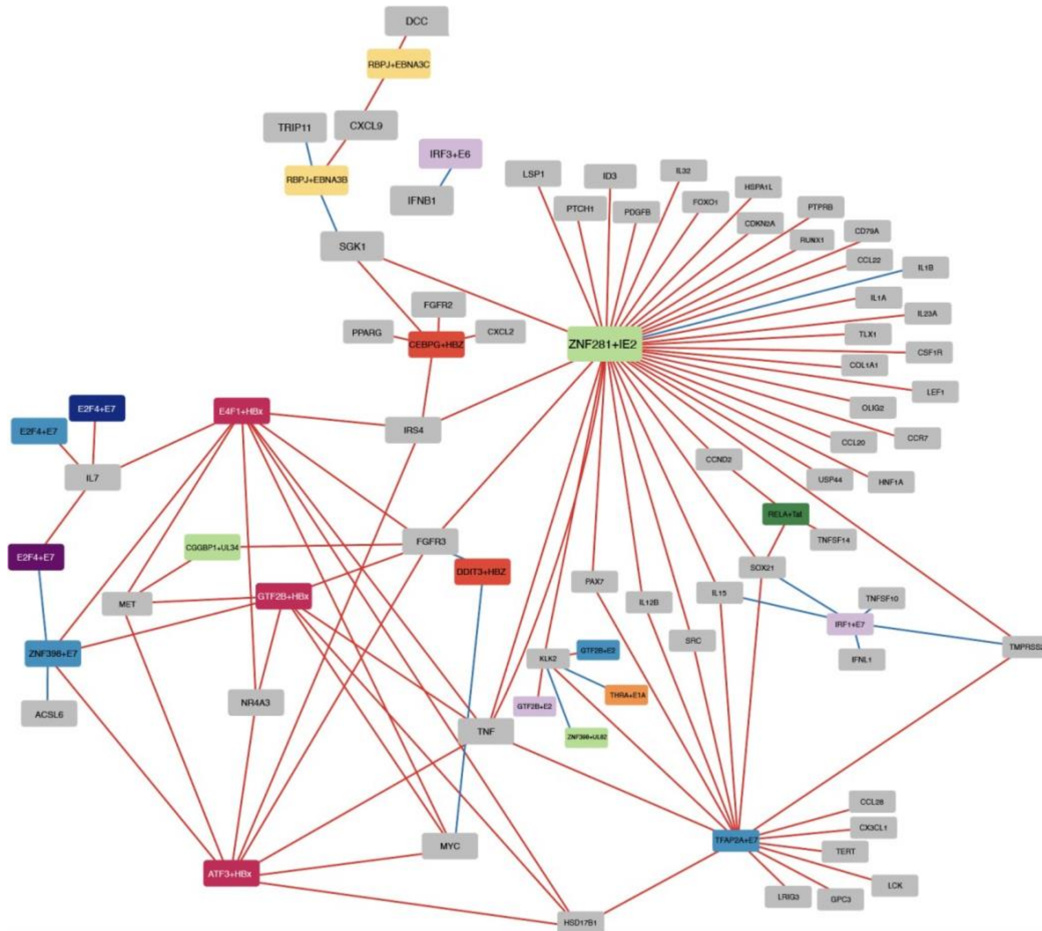

Figure 1. Genetic Network of Interactions between vTRs and promoter baits

Red lines indicate antagonistic interactions, while blue lines represent cooperative interactions.

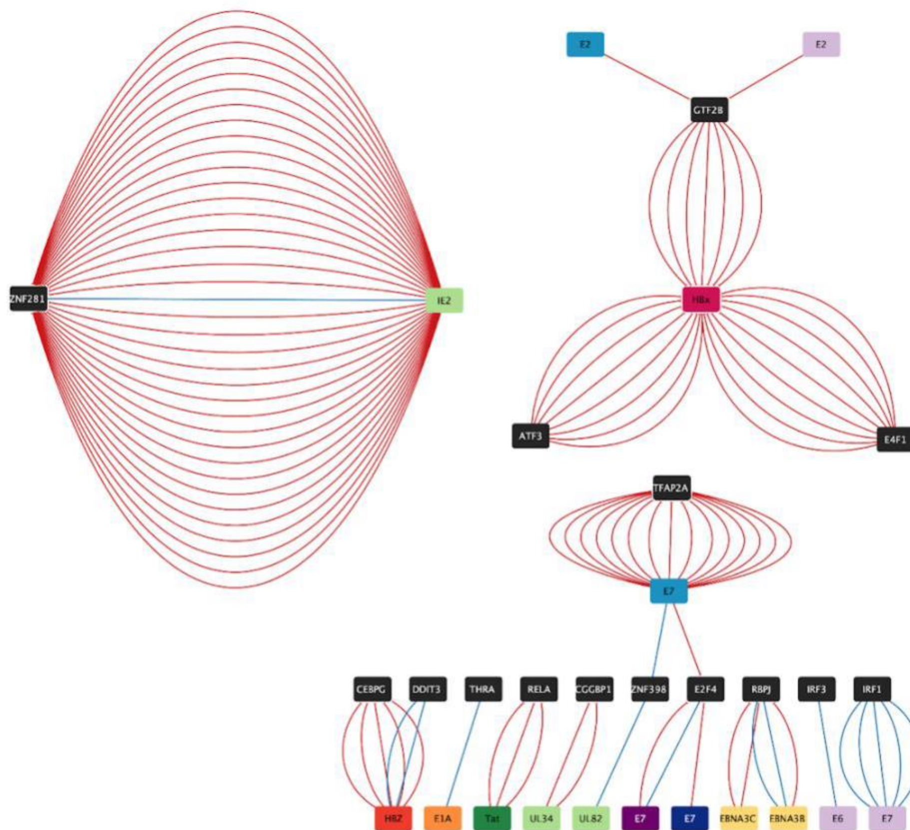

Figure 2. Functional Relationships between vTRS and hTFs

Red lines indicate antagonistic interactions while blue lines represent cooperative interactions. The number of lines between each vTR and hTF indicate the number of interactions that were found between the two under different baits.

Interactions based Genome Type

Starting with the finalized list of vTR-hTF pairs in the array, there were 29 unique vTRs from which 90% of them were DNA based and 10% were Retroviral (Figure 3A) From the total 108 interactions performed in the screen, 91% of these interactions had a DNA based genome while 9% were Retroviral (Figure 3B). From the screen, a total of 13 unique vTRs were identified from which 87% had a DNA based genome while 13% were

Retroviral (Figure 3C). Overall, the percent of the type of genome seen across vTRs was relatively consistent meaning that neither DNA viruses nor Retroviruses participated in more interactions than expected.

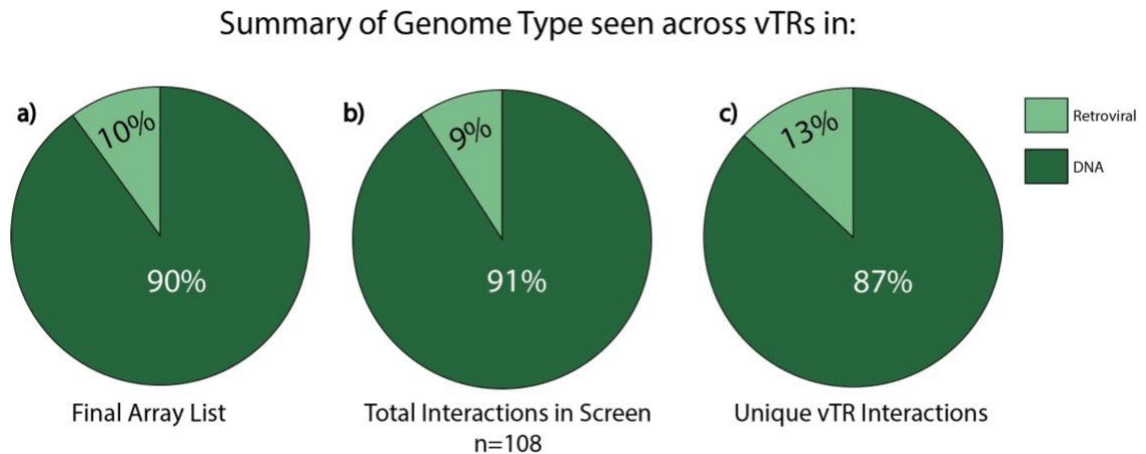

Figure 3. Summary of genome type (DNA vs Retroviral) seen across vTRs

A) finalized list of unique hTF-vTR pairs B) the 108 different interactions seen in the pY1H screen and C) the unique vTRs that had interactions found in the screen.

Interactions based on Viral Families

Five different viral families were found to be involved in the different interactions between vTRs and hTFs. Those 5 families were: *Herpesviridae, Papillomaviridae, Hepadnaviridae,* and *Adenoviridae.* From the 29 unique vTRs in the final vTR-hTF array list, 38% belonged to *Papillomavirdae*, 28% to *Herpesviridae*, 21% to *Adenoviridae*, 10% to *Retroviridae* and 3% to *Hepadnaviridae* (Figure 4A). In order of decreasing abundance, the percent at which each viral family was seen across the total interactions of the screen was *Herpesviridae* (42%), *Papillomaviridae* (26%), *Hepadnaviridae* (22%), *Retroviridae* (9%), and *Adenoviridae* (1%) (Figure 4B). Across the unique vTR

interactions, 44% of the vTRs belonged to *Papillomaviridae*, 31% to *Herpesviridae*, 13% to *Retroviridae*, 6% to *Hepadnaviridae* and *Adenoviridae* respectively (Figure 4C). Overall, it was seen that there were differences across the abundance of viral families from the initial list to the conduction of the screen with *Adenovirdae* participating far less than expected and *Hepadnaviridae* and *Herpesviridae* participating in more interactions than expected.

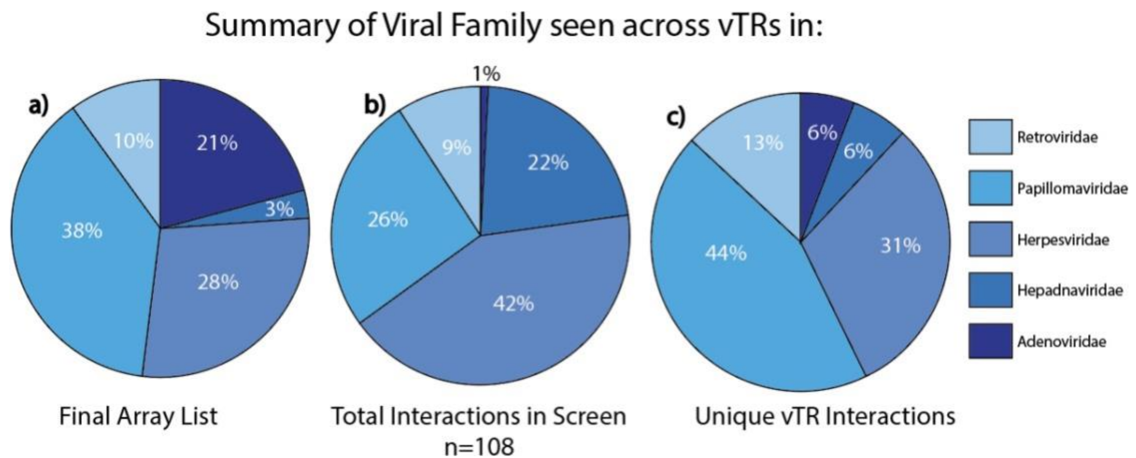

Figure 4. Summary of different viral families seen across vTRs

A) finalized list of unique hTF-vTR pairs B) the 108 different interactions seen in the pY1H screen and C) the unique vTRs that had interactions found in the screen. In total 5 different viral families were identified to be present in the finalized vTR-hTF pair list, across all 108 interactions, and across unique vTR interaction.

Interactions base Viral Species

Further analysis has also shown that a total of 14 different vTR viral species were in the finalized list of vTR-hTF pair array. These species included: *Human adenoviruses A, B, C and D, Human papillomavirus (HPV) types 2, 5, 16, and 18, Hepatitis B virus, Human herpesvirus 4, 5 and 8 which are EBV, Human cytomegalovirus (hCMV),* and *Kaposi sarcoma-associated herpesvirus respectively, HIV 1, and Human T-cell leukemia*

virus 1 or Human T-lymphotropic virus 1. The most abundant species in the finalized array list was *hCMV* at 14% followed by *HPV* types 2, 5, and 18 each at 10%, then *Human T-lymphotropic virus 1*, *Human adenoviruses A* and *C*, *EBV, Human herpesvirus 8,* and *HPV 16* each at 7%, followed by *Hepatitis B virus* and *Human adenovirus B* each at 4% with *Human adenovirus D* and *HIV* with only 3% abundance (Figure 5A).

Across the total interactions in the screen the number of unique viral species dropped from 14 to 10. These vTR viral species included: *Hepatitis B Virus, Human T-Cell Leukemia Virus 1; Human T-lymphotropic virus 1, Human herpesvirus 5; Human cytomegalovirus, HPV type 2, HPV type 16, HPV type 18, HPV type 5, Human herpesvirus 4; EBV, HIV 1, Human adenovirus B.* The most abundant vTR virus type was *hCMV (*37%), and the least abundant type was *Human adenovirus B* (1%)(Figure 5B).

Across the unique vTR interactions the number of viral species was again 10 with the most abundant being *HPV type 5* and *hCMV* each at 19% followed by *HPV16* and *EBV* at 13% with *Hepatitis B, Human T-lymphotropic virus 1, HPV2* and *HPV18*, *HIV1*, and *Human adenovirus* B each at 6% being the least abundant (Figure 5C).

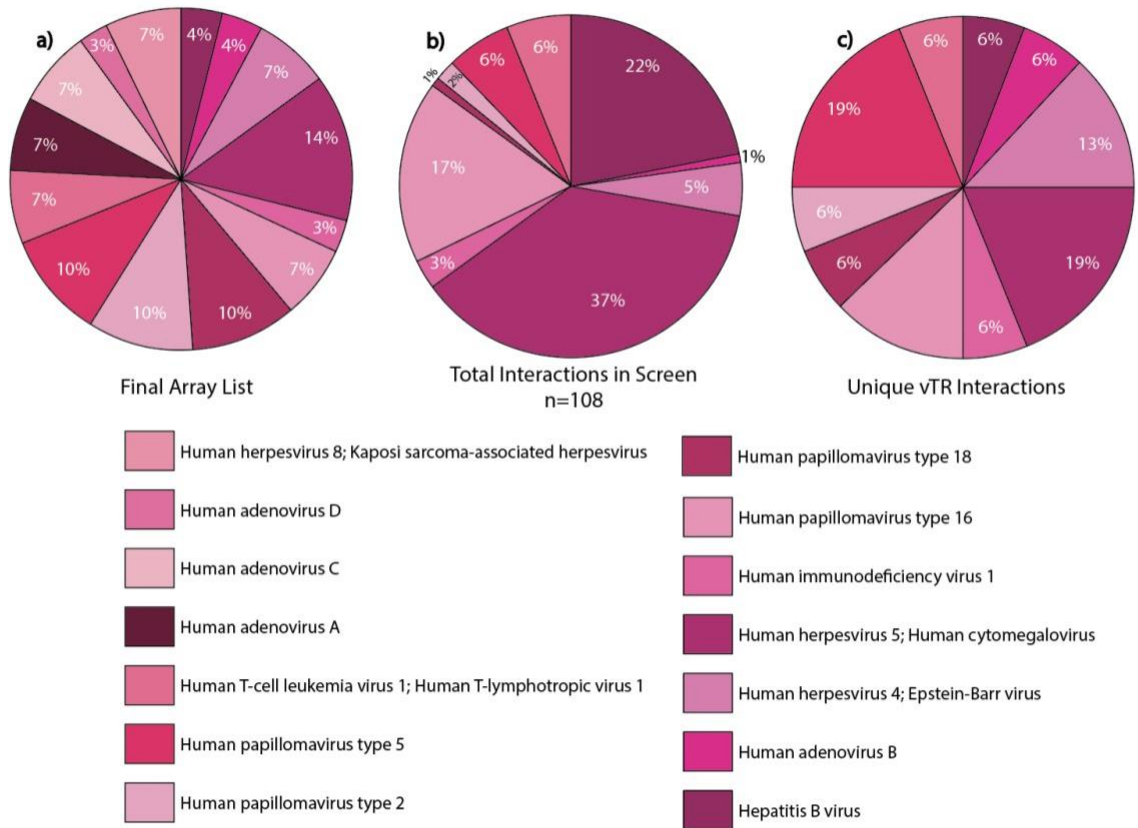

Figure 5. Summary of different viral species seen across vTRs

A) finalized list of unique hTF-vTR pairs B) the 108 different interactions seen in the pY1H screen and C) the unique vTRs that had interactions found in the screen. A total of 14 different viral species were found to be unique in the finalized vTR-hTF pair list. Across the 108 interactions in the pY1H screen and the unique vTR interactions, only 10 different viral species were present.

Overall, there were 14 vTRs that were expected to participate in different interactions within the screen, but only 10 were seen with *Human adenoviruses A, C,* and *D,* and *Human herpesvirus 8* being the 4 vTRs to not appear in the screen. Consistent

with initial thoughts, *hCMV* was seen to participate in a high number of interactions. Also, the general percentage of each type of vTR was consistent indicating that none of the viruses participated in more interactions than expected.

Cooperativity + Antagonism

Interactions between vTRs and hTFs were also categorized based on cooperative or antagonistic binding properties seen qualitatively. Many of the interactions found were antagonistic (92%) while only 8% of the interactions found displayed cooperative binding (Figure 6). Within the antagonistic interactions found, we further classified them based on which TF was antagonized, defining antagonism 1 as the hTF being antagonized and antagonism 2 as the vTR being antagonized. From the 93 antagonistic interactions there were, 69 interactions were found where the hTF was antagonized and 24 interactions were found where the vTR was antagonized.

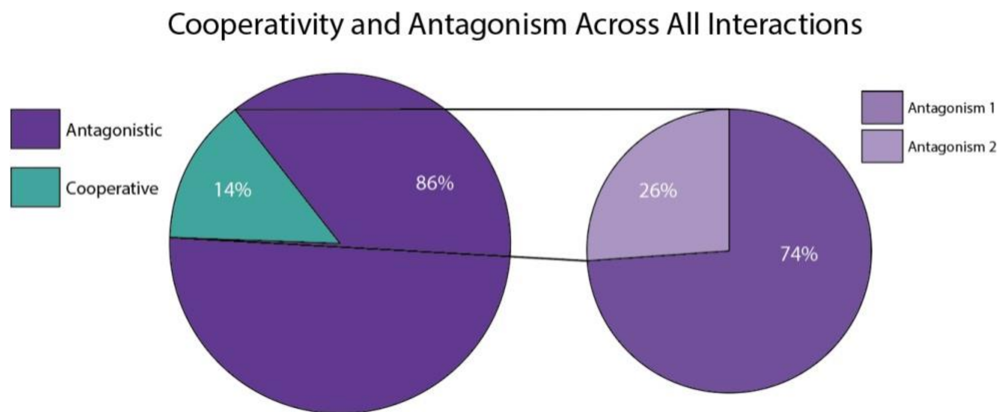

Figure 6: Cooperativity and Antagonism across all interactions

Out of a total of 108 interactions seen in the pY1H screen, distinguishing between cooperative and antagonistic interactions, and then further distinguishing between the antagonistic reactions as to whether the hTF was antagonized or the vTR was

antagonized. Antagonism 1 is defined as the vTR antagonizing the hTF and antagonism 2 is defined as the hTF antagonizing the vTR.

Targeted promoters

Analysis done on the various vTRs highlights the importance of the targeted promoters in the screen. Screening started with 83 cancer gene promoters and 41 cytokine promoters. At least one interaction was seen in the baits which included 41 cancer gene promoters and 18 cytokine gene promoters. From the 41 cancer gene promoters it was found that some interactions included well-known cancer genes such as TERT and MYC.

E7, LRIG3, and TFAP27 as a highlighted model

Previously conducted studies by members of the lab looked at C-33A cells expressing HPV16-E7 in which E7 levels were overexpressed. RNA was extracted from cells and RNA-seq was performed to look for differentially expressed genes with E7 overexpression.

TFAP2A is an hTF that was antagonized by the HPV16 E7 vTR. A total of 14 different interactions were seen between these two TFs across different baits with confidence levels of the interaction ranging from very weak to strong on a qualitative scale (Figure 6). The LRIG3 promoter bait is an example of an interaction between TFAP27 and E7 that had strong confidence in the antagonism of E7 over TFAP2A. Figure 7 demonstrated the significant downregulation of LRIG3 with the expression of E7 based on the fold-change from RNA-seq data.

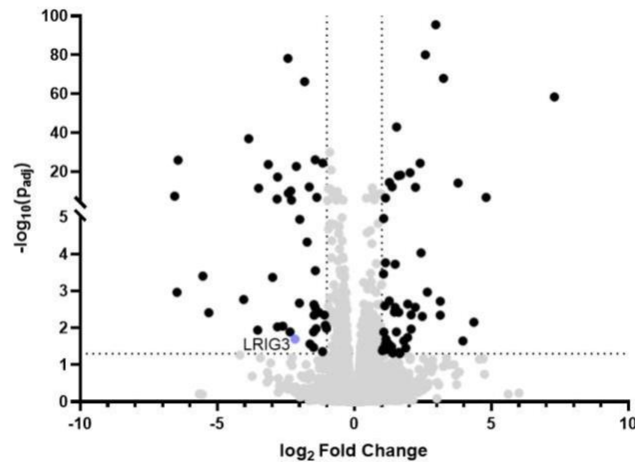

Figure 7. Upregulation or Downregulation of Genes determined by E7 expression

A volcano plot to summarize the effects of E7 expression on different genes. Changes in expression are relative to the control and based on fold change values shown using a log2 scale. Dotted lines represent cut-offs for significance, vertical cut-offs were fold changes greater than 1 or less than -1 and horizontal cut-offs were based on the p-value of $p < 0.05$. All points highlighted in black represent either significant up or downregulation of the gene with the expression of E7. Highlighted in purple is the LRIG3 gene which is downregulated by the expression of E7 with a fold change between 1 and 2.

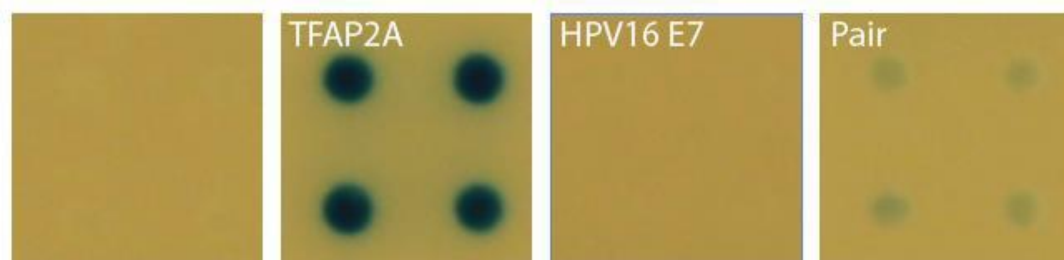

Figure 8. Qualitative example of vTR antagonizing the hTF.

Example highlighted is the HPV 16 E7 vTR antagonizing the TFAP2A hTF. Identified as a strong confidence interaction, this occurred on the LRIG3 promoter bait.

CHAPTER FOUR: DISCUSSION AND FUTURE PERSPECTIVES

pY1H analysis

To reiterate the purpose of this investigation is to complete a viral protein screen to determine if the interactions between vTRs and hTFs are cooperative or antagonistic with each one another at specific promoters for the regulation of host immune genes and cancer genes. Several different comparisons were shown and described including differences across finalized vTR-hTF pairs list, the total number of interactions in the pY1H screen and the unique vTRs found in the screen as well as differences across genome type, viral family and species, and cooperative vs antagonistic interactions. For its strong confidence based on the results of the screen, a model to highlight a feature of the study and specifically how the methodology can unveil potential mechanisms for vTRs to alter host gene expression was the antagonistic effect that the HPV16 E7 vTR had on the TFAP2A hTF at the LRIG3 promoter bait.

It has been consistently seen that a vast majority of the vTRs in this screen had a DNA based genome over being a Retrovirus as expected based on representation in the vTR-hTF array. Of the 5 viral families, 3 have dsDNA genome, 1 has a ssDNA, and 1 has a positive sense ssRNA reverse transcriptase genome (Liu et al., 2020) The family viral families seen throughout this screen in alphabetical order were: *Adenoviridae, Hepadnaviridae, Herpesviridae, Papillomaviridae,* and *Retroviridae. Adenoviridae, Herpesviridae and Papillomavirdae* are each viral families with dsDNA-based genomes, *Hepadnaviridae* is a viral family with a ssDNA-based genome, and *Retroviridae* belongs to a positive sense ssRNA reverse transcriptase viral family.

In the initial list of unique vTRs in the finalized list of vTR-hTF pairs, about a ⅕ of the vTRs belonged to the *Adenoviridae* family, however, after the screen only 1% of all total interactions belonged to the *Adenoviridae* family and further analysis based on unique vTRs in the screen yielded 6% of unique vTRs in the screen belonging to *Adenoviridae.* The two families that have consistently been represented as the major family seen across vTRs in this screen include *Herpesviridae* and *Papillomaviridae*. While only 3% of the unique vTRs in the finalized list of vTR-hTF pairs belonged to *Hepadnaviridae,* almost ¼ of the total interactions involved vTRs that belonged to *Hepadnaviridae.* When comparing the viral families, the unique vTRs in the screen belonged to, the percentage of vTRs belonging to *Hepadnaviridae* dropped back to 6% which was equal to that of *Adenoviridae.* Given the different properties of each of these viral families, a consensus as to why in particular these were the dominant families for interactions with hTFs could be due to their ability to manipulate host gene expression in order to replicate and cause disease.

Prior to starting the screen, the finalized list of vTR-hTF pairs contained 14 unique viral species based on vTR ID which included: *Human adenoviruses A, B, C and D, HPV types 2, 5, 16, and 18, Hepatitis B virus, Human herpesvirus 4, 5 and 8 which are EBV, hCMV,* and *Kaposi sarcoma-associated herpesvirus* respectively*, HIV 1, and Human T-cell leukemia virus 1 or Human T-lymphotropic virus 1.* Upon conducting the screen, only 10 of the 14 viral species were found to have interactions with the promoter baits. The 10 viral species seen across all the interactions were: *Hepatitis B Virus, Human T-Cell Leukemia Virus 1; Human T-lymphotropic virus 1, Human herpesvirus 5; hCMV,*

HPV type 2, HPV type 16, HPV type 18, HPV type 5, Human herpesvirus 4; EBV, HIV 1, Human adenovirus B. The viral species seen to have dropped out during the screen included: *Human adenoviruses A, C,* and *D, Human herpesvirus 8*. Comparing the list of viral species seen in the screen versus the ones not seen there could be some reasons behind this.

One thing that could be seen compiling all the data together is the significant drop in *Adenoviridae* abundance between the finalized vTR-hTF pair list and the interactions in the screen. Three of the four viral species seen to drop out of the screen belonged to the *Adenoviridae* family. *Human adenovirus A, C,* and *D* are associated with mild respiratory infections (Kajon & Lynch, 2016) and had not been previously studied extensively for their interactions with hTFs. These species of *Adenoviridae* may not have the necessary protein-protein interactions or DNA-binding properties to bind to cytokine promoters or cancer baits. These species adenovirus could also be amongst those that have been found to have immune evasion mechanisms (Mahr & Gooding, 1999) that may limit their interactions with hTFs involved in the immune response.

As expected, with the large proportion of vTRs belonging to *Papillomaviridae*, over ¼ of all interactions in the screen belonged to that family with almost half of the unique vTR interactions in the screen being *Papillomaviridae*. This aligns with the viral species seen with some of the relatively higher abundance of interactions which included *HPV types 2, 5, 16* and *18.* The viral species seen to be involved in the most interactions in the overall screen were *hCMV* with the most unique interactions being split between *hCMV* and *HPV 16.*

HPV16 was also one of the viral species that was highlighted to unveil potential mechanisms by which vTRs can alter host gene expression. This was seen through the interactions between *HPV16 E7* and *TFAP2A* at the LRIG3 promoter bait. E7 significantly antagonized TFAP2A at the promoter which in turn also showed a significant downregulation of the LRIG3 promoter with E7 expression.

Across the total interactions seen in the screen, 92% of the interactions were antagonistic reactions from which a majority of those interactions involved the vTR antagonizing the hTF when both were bound together at the promoter bait. An example of this was highlighted with *HPV16 E7* and *TFAP2A* at the LRIG3 promoter bait as previously described. From the fraction of the interactions that involved the hTF antagonizing the vTR at the promoter bait, all these interactions involved the viral species *Hepatitis B*. Every interaction of *Hepatitis B*, saw it being antagonized by every hTF it was paired with. This also correlates to the notion of *Hepadnaviridae* being the only viral family seen in this type of interaction. *Adenoviridae* was seen to only be involved in cooperative interactions while the other viral families were distributed amongst cooperative interactions and antagonistic interactions involving the vTR antagonizing the hTF.

Future Directions

The results of the screen have been beneficial in understanding if and how interactions between the pairs of vTRs and hTFs can potentially regulate the human immune responses. Seeing whether these interactions were cooperative or antagonistic

can help provide a basis for further research in this field. DNA binding of vTR-hTF pairs had not been studied very well until recently, and while previous literature has shown DNA binding in a variety of other ways, this experiment was an attempt to look for a novel approach in studying the function of transcription factor pairs to understand DNA binding and interactions that occur on a genomic level. Creating a novel method of understanding DNA binding can revolutionize the field of genetics and genomics allowing for a much greater understanding of the biological processes in the world.

Further scientific research can be built upon the results of this project.
In this screen, the use of the pY1H over a standard Y1H or even eY1H has allowed for the ability to study a variety of interaction types within the realm of cooperative and antagonistic interactions between the different hTFs and vTRs at varying promoter baits. The ability to qualitatively depict interactions and then further quantify them in different ways has been a major advancement to further learning more about genetic regulation with hTFs and vTRs.

The applications of the pY1H can further be expanded from the results of this study and be put towards gaining a larger understanding of cancer baits and genes. Another ongoing study is working towards screening pairs of hTFs to see if they bind cooperatively or antagonistically to cancer gene promoters. Also, further applications of pY1H assays include studying different designs of activation domains for DNA binding, as well as looking further into more TF-pairs either isoforms or mutations and seeing the effect they have on DNA binding. Overall, pY1H assays have the ability to provide

evidence of serval complex relationship between TFs. These assays also further allow for a method of studying pairs of proteins at DNA regions of interest.

Finally, on a broader spectrum this viral assay can help better understand how viruses really regulate host genes, how they are able to evade the host immune response and disrupt mechanisms that naturally occur with its own genome. A better understanding of viral gene regulation and expression can be a key factor in understanding how a variety of diseases work. By understanding the mechanism of infection methods can be designed to combat these particular issues.

Alongside applications of the pY1H and gaining a better understanding of viral mechanisms in gene regulation having done this study in a yeast model, one of the next future steps could be to see if the interactions seen in this screen can be replicated in human cell lines. Interactions that were seen in specific hTFs and vTRs at specific bait promoters highlighting the different examples of cooperativity between pairs, the hTF being antagonized by the vTR, and the vTR being antagonized by the hTF can potentially be modeled in these cell lines. Also given the information and data collected surrounding viral family and species this information can further give more contextualization into understanding the results of this screen and further push its applications closer towards a human model.

In conclusion, this study has offered a better understanding of a method to study hTFs and vTRs as pairs in a variety of immune and cancer gene promoters to understand more about mechanisms of host gene regulation. There have been several proposed applications to this study, first acting as an advancement to the Y1H and secondly adding

more data to a recently written comprehensive review of vTRs. The pairs of hTFs and vTRs found in this pY1H screen have shown some novel interactions that were previously not seen in literature as well as further verified or even refuted known literature-based interactions. Future studies hope to build on the initial findings of this experiment and include applications to a variety of genes in different models to increase our overall understanding of genetic mechanisms.

APPENDIX

Recipes for Plates:

Sc-Ura-His (-"UH") plates used for spotting and growing the DNA-bait strains.

To make 2L of media, dissolve 2.6g of Drop-out mix synthetic minus histidine, leucine, tryptophan, and uracil, adenine rich (2g) w/o yeast nitrogen base (US Biological), 3.4g of yeast nitrogen base without amino acids and ammonium sulfate (YNB), 160 mg of Adenine hemisulfate, and 10 g of ammonium sulfate in 920mL of water and pH to 5.9 with NaOH 5M in a 2L flask and add a stir bar. In a second flask, 35 g of agar was combined with 950 mL of water. Flasks were autoclaved under the P15 cycle. After removal from the autoclave contents of the flask containing the stir bar were directly poured into the flask with agar and placed into the water bath for 1 hour at 55 C. After while on a stir plate100ml 40% glucose, 16 ml Leucine (100mM), and 16mL Tryptophan (100mL) were added and mixed together. Media was poured into 150-mm sterile petri dishes and dried at room temperature for 3-5 days.

YAPD Singer Plates

Purpose: make the DNA-bait lawn for mating

To make 2L of media, 40 g Peptone, 20 g yeast extract, and 0.32 g adenine hemisulfate were dissolved in 950mL of water and a stir bar was added to the flask. In a separate flask, 950 mL of water was combined with 35g of agar. The flasks were autoclaved using the P15 cycle. After removal from the autoclave contents of the flask containing the stir bar were directly poured into the flask with agar and placed into the

water bath for 1 hour at 55 C. The flask was transferred to a stir plate and 50 mL of 40% glucose was added and mixed together. Media was poured into Singer plates (60mL per plate) using the Omnispence and dried at room temperature for 1 day before use.

Sc -Leu -Trp (-"LT") Singer Plates

Purpose: grow the TF array

To make 2L of media, dissolve 2.6g of Drop-out mix synthetic minus histidine, leucine, tryptophan, and uracil, adenine rich (2g) w/o yeast nitrogen base (US Biological), 3.4g of YNB, 160 mg of Adenine hemisulfate, and 10 g of ammonium sulfate in 936mL of water and pH to 5.9 with NaOH 5M in a 2L flask and add a stir bar. In a second flask, 35 g of agar was combined with 950 mL of water. Flasks were autoclaved under the P15 cycle. After removal from the autoclave contents of the flask containing the stir bar were directly poured into the flask with agar and placed into the water bath for 1 hour at 55 C. The flask was transferred to a stir plate and 100 mL of 40% glucose, 16 mL of 100mM Histidine, and 16 mL of 20mM Uracil was added and mixed together. Media was poured into Singer plates (60mL per plate) using the Omnispence and dried at room temperature for 1 day before use.

Sc -U-Leu-Trp ("-ULT") Singer Plates

Purpose: selection of diploid yeast after mating

To make 2L of media, 2.6g of Drop-out mix synthetic minus histidine, leucine, tryptophan, and uracil, adenine rich (2g) w/o yeast nitrogen base (US Biological), 3.4g of

YNB, 160 mg of Adenine hemisulfate, and 10 g of ammonium sulfate were dissolved in 936mL of water and pH to 5.9 with NaOH 5M in a 2L flask and a stir bar was added. In a second flask, 35 g of agar was combined with 950 mL of water. Flasks were autoclaved under the P15 cycle. After removal from the autoclave contents of the flask containing the stir bar were directly poured into the flask with agar and placed into the water bath for 1 hour at 55 C. The flask was transferred to a stir plate and 100 mL of 40% glucose and 16 mL of 100 mM Histidine were added and mixed together. Media was poured into Singer plates (60mL per plate) using the Omnispence and dried at room temperature for 1 day before use.

Sc -U-H-Leu-Trp+3AT+X-gal "-UHLT X-gal" Singer Plates

Purpose: readout plates for the eY1H assays

To prepare 2L of media, 2.6g of Drop-out mix synthetic minus histidine, leucine, tryptophan, and uracil, adenine rich (2g) w/o yeast nitrogen base (US Biological), 3.4g of YNB, 160 mg of Adenine hemisulfate, and 10 g of ammonium sulfate were dissolved in 866 of water and a stir bar was added. In a second flask, 35 g of agar was combined with 950 mL of water. Flasks were autoclaved under the P15 cycle. After removal from the autoclave contents of the flask containing the stir bar were directly poured into the flask with agar and placed into the water bath for 1 hour at 55 C. The flask was transferred to a stir plate and 100 mL of 40% glucose, 200 mL of 10X BU salts, 5 mL of 2 mM 3AT, and 4 mL of 160 mg/mL X-gal in DMF were added and mixed together. Media was poured

into Singer plates (60mL per plate) using the Omnispence and dried at room temperature for 1 day before use.

10x BU Salts:

To prepare 1L of 10x BU Salts, in a flask 900 mL of water, 70 g $Na2PO4.7H2O$ and 34.5 g $NaH2PO4.H2O$ were combined and a stir bar was added. The mixture was pHed to 7.0 using 5M NaOH and then brought to 1L and autoclaved.

X-gal solution:

In a 50 mL plastic tube, 42.5 mL dimethyl formamide and 3.25g X-gal powder were combined. The mixture was allowed to combine and dissolve for 30 minutes. The solution was stored at -20C in a dark environment.

Supplemental Tables

Table 1: List of all 108 Interactions seen in pY1H screen

Bait gene	tf1	tf2	vTR	vTR virus	Viral family	genome type	event type	confidence level
TNF	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	moderate
TNF	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	moderate
CCL20	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	weak
IL7	E2F4	A3F906	E7	Human papillomavirus type 2	Papillomaviridae	DNA	ant1	moderate
IL7	E2F4	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	moderate
IL7	E2F4	P06788	E7	Human papillomavirus type 18	Papillomaviridae	DNA	ant1	moderate
IL32	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	moderate
IL1A	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	very weak
IL12B	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	strong
IL12B	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	moderate
CXCL9	RBPJ	P03203	EBNA3B	Human herpesvirus 4; Epstein-Barr virus	Herpesviridae	DNA	ant1	weak
CXCL9	RBPJ	P03204	EBNA3C	Human herpesvirus 4; Epstein-Barr virus	Herpesviridae	DNA	ant1	weak
CXCL2	CEBPG	P0C746	HBZ	Human T-cell leukemia virus 1; Human T-lymphotropic virus 1	Retroviridae	Retroviral	ant1	very weak
CCL28	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	moderate
CCL22	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
TNFSF14	RELA	P04608	Tat	Human Immunodeficiency virus 1	Retroviridae	Retroviral	ant1	weak
IL23A	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
IL15	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	very weak
IL15	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	moderate
CX3CL1	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	weak
USP44	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	moderate
SGK1	CEBPG	P0C746	HBZ	Human T-cell leukemia virus 1; Human T-lymphotropic virus 1	Retroviridae	Retroviral	ant1	strong
SGK1	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
TERT	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	weak
SOX21	RELA	P04608	Tat	Human Immunodeficiency virus 1	Retroviridae	Retroviral	ant1	very weak
SOX21	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	very weak
SOX21	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	weak (strong > moderate)
LEF1	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	weak
PTPRB	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	moderate
TLX1	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
PAX7	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	moderate
PAX7	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	weak (strong > moderate)
OLIG2	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
MET	CGGBP1	P16812	UL34	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	weak
LCK	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	strong
DCC	RBPJ	P03204	EBNA3C	Human herpesvirus 4; Epstein-Barr virus	Herpesviridae	DNA	ant1	moderate
ID3	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
CSF1R	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	weak
PTCH1	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	weak
PDGFB	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	moderate
TNF	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	moderate
GPC3	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	moderate
FOXO1	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	weak
FGFR3	CGGBP1	P16812	UL34	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	weak
FGFR3	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	weak
CCND2	RELA	P04608	Tat	Human Immunodeficiency virus 1	Retroviridae	Retroviral	ant1	weak
CCND2	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	weak
TMPRSS2	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	strong
TMPRSS2	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
CDKN2A	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
LRIG3	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	strong
HSD17B1	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	moderate
LSP1	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	weak
RUNX1	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
IRS4	CEBPG	P0C746	HBZ	Human T-cell leukemia virus 1; Human T-lymphotropic virus 1	Retroviridae	Retroviral	ant1	weak
IRS4	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	weak
HSPA1L	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
COL1A1	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	moderate
CCR7	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
SRC	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	moderate
SRC	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
PPARG	CEBPG	P0C746	HBZ	Human T-cell leukemia virus 1; Human T-lymphotropic virus 1	Retroviridae	Retroviral	ant1	strong
CD79A	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
KLK2	GTF2B	P03120	E2	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	weak
KLK2	GTF2B	P06921	E2	Human papillomavirus type 5	Papillomaviridae	DNA	ant1	weak
KLK2	TFAP2A	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	ant1	moderate
KLK2	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
HNF1A	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	ant1	strong
FGFR2	CEBPG	P0C746	HBZ	Human T-cell leukemia virus 1; Human T-lymphotropic virus 1	Retroviridae	Retroviral	ant1	moderate
IL7	E4F1	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	moderate
MET	ATF3	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
MET	E4F1	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
MET	GTF2B	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
TNF	ATF3	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
TNF	E4F1	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
TNF	GTF2B	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
NR4A3	ATF3	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	moderate
NR4A3	E4F1	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	moderate
NR4A3	GTF2B	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	moderate
FGFR3	ATF3	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
FGFR3	E4F1	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
FGFR3	GTF2B	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
HSD17B1	ATF3	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	strong
HSD17B1	E4F1	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	strong
HSD17B1	GTF2B	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	strong
IRS4	ATF3	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
IRS4	E4F1	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
MYC	ATF3	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
MYC	E4F1	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
MYC	GTF2B	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	weak
ACSL6	ATF3	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	moderate
ACSL6	E4F1	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	moderate
ACSL6	GTF2B	P0C686	HBx	Hepatitis B virus	Hepadnaviridae	DNA	ant2	moderate
IL1B	ZNF281	P19893	IE2	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	coop	weak
TNFSF10	IRF1	P06932	E7	Human papillomavirus type 5	Papillomaviridae	DNA	coop	moderate
IFNB1	IRF3	P06930	E6	Human papillomavirus type 5	Papillomaviridae	DNA	coop	weak
IL15	IRF1	P06932	E7	Human papillomavirus type 5	Papillomaviridae	DNA	coop	moderate
IFNL1	IRF1	P06932	E7	Human papillomavirus type 5	Papillomaviridae	DNA	coop	strong
SGK1	RBPJ	P03203	EBNA3B	Human herpesvirus 4; Epstein-Barr virus	Herpesviridae	DNA	coop	strong
TRIP11	RBPJ	P03203	EBNA3B	Human herpesvirus 4; Epstein-Barr virus	Herpesviridae	DNA	coop	very weak
SOX21	IRF1	P06932	E7	Human papillomavirus type 5	Papillomaviridae	DNA	coop	weak
FGFR3	DDIT3	P0C746	HBZ	Human T-cell leukemia virus 1; Human T-lymphotropic virus 1	Retroviridae	Retroviral	coop	very weak
TMPRSS2	IRF1	P06932	E7	Human papillomavirus type 5	Papillomaviridae	DNA	coop	very weak
MYC	DDIT3	P0C746	HBZ	Human T-cell leukemia virus 1; Human T-lymphotropic virus 1	Retroviridae	Retroviral	coop	weak
ACSL6	E2F4	A3F906	E7	Human papillomavirus type 2	Papillomaviridae	DNA	coop	very weak
ACSL6	ZNF398	P03129	E7	Human papillomavirus type 16	Papillomaviridae	DNA	coop	very weak
KLK2	THRA	P03256	E1A	Human adenovirus B	Adenoviridae	DNA	coop	strong
KLK2	ZNF398	P06726	UL82	Human herpesvirus 5; Human cytomegalovirus	Herpesviridae	DNA	coop	moderate

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