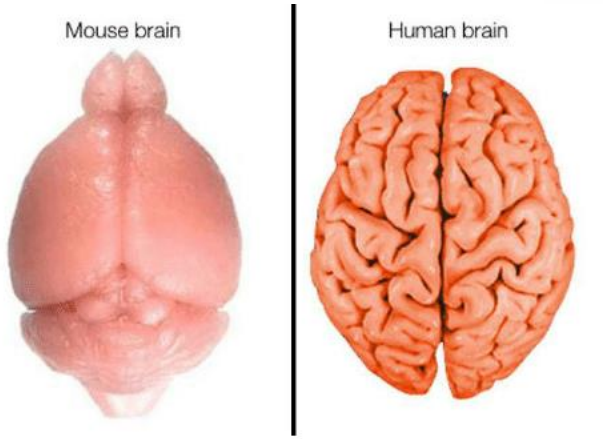
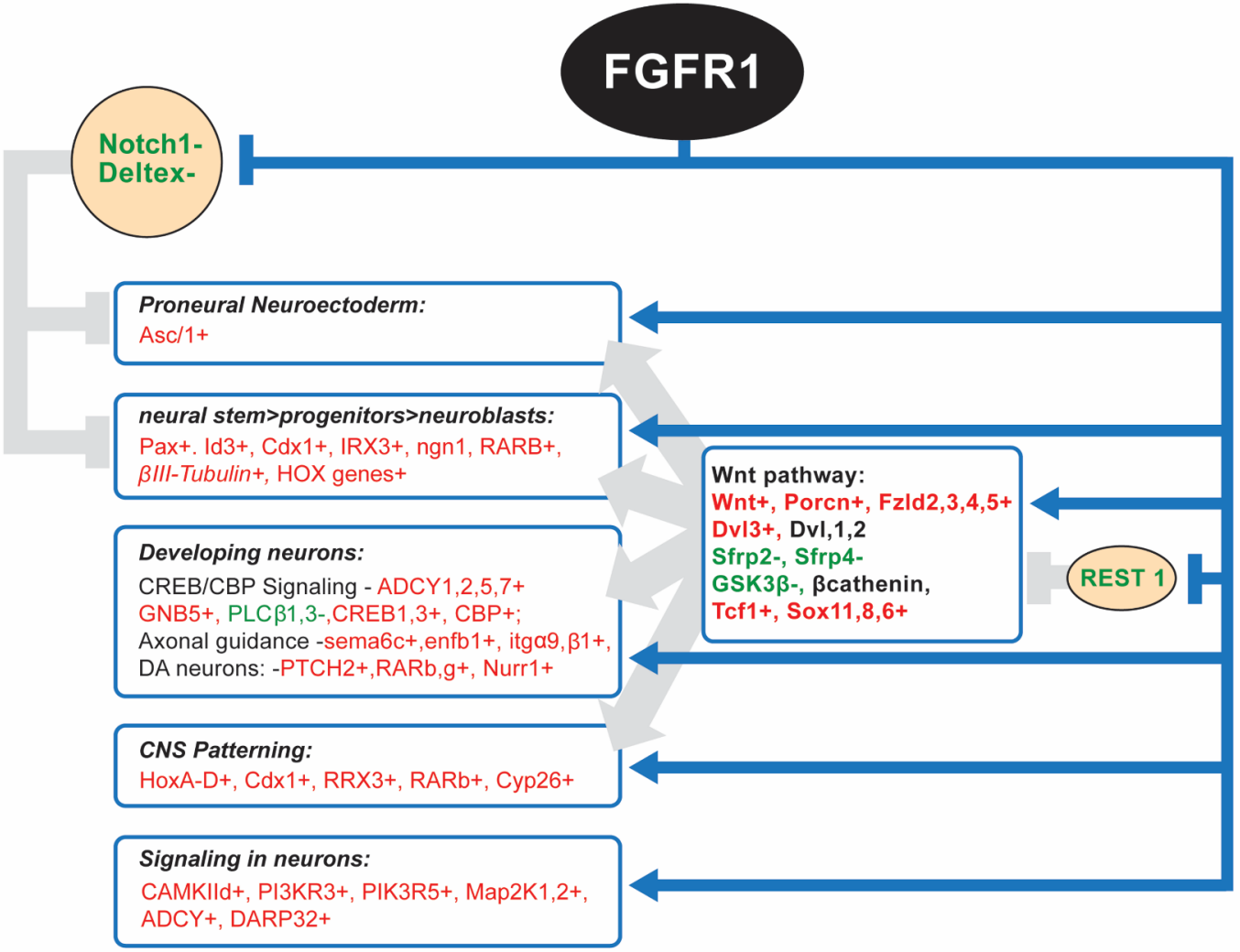
nFGFR1 controls multiple stages in neural development



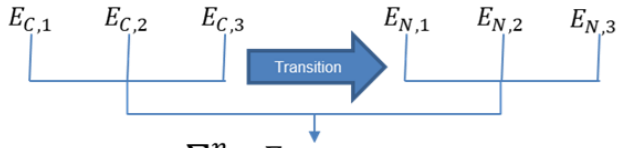


(Terranova et al., 2015, Narla et al., 2017 & linked databases).

COORDINATE GENOME – COMPUTATIONAL APPROACH

TRANSITION FROM NEURONAL PROGENITORS TO NEURONAL COMMITTED CELLS - FOLD CHANGE

Gene	C1	C2	C3		N1	N2	N3
KLHL17	-0.87744	-1.5863	0.253823		0.853696	0.698845	0.657376



$$\frac{\frac{\sum_{i=0}^{n} E_{N,i}}{n}}{\frac{\sum_{i=0}^{n} E_{c,i}}{n}} = \frac{Avg.E_N}{Avg.E_C}$$

Gene-Gene coordination: Correlation

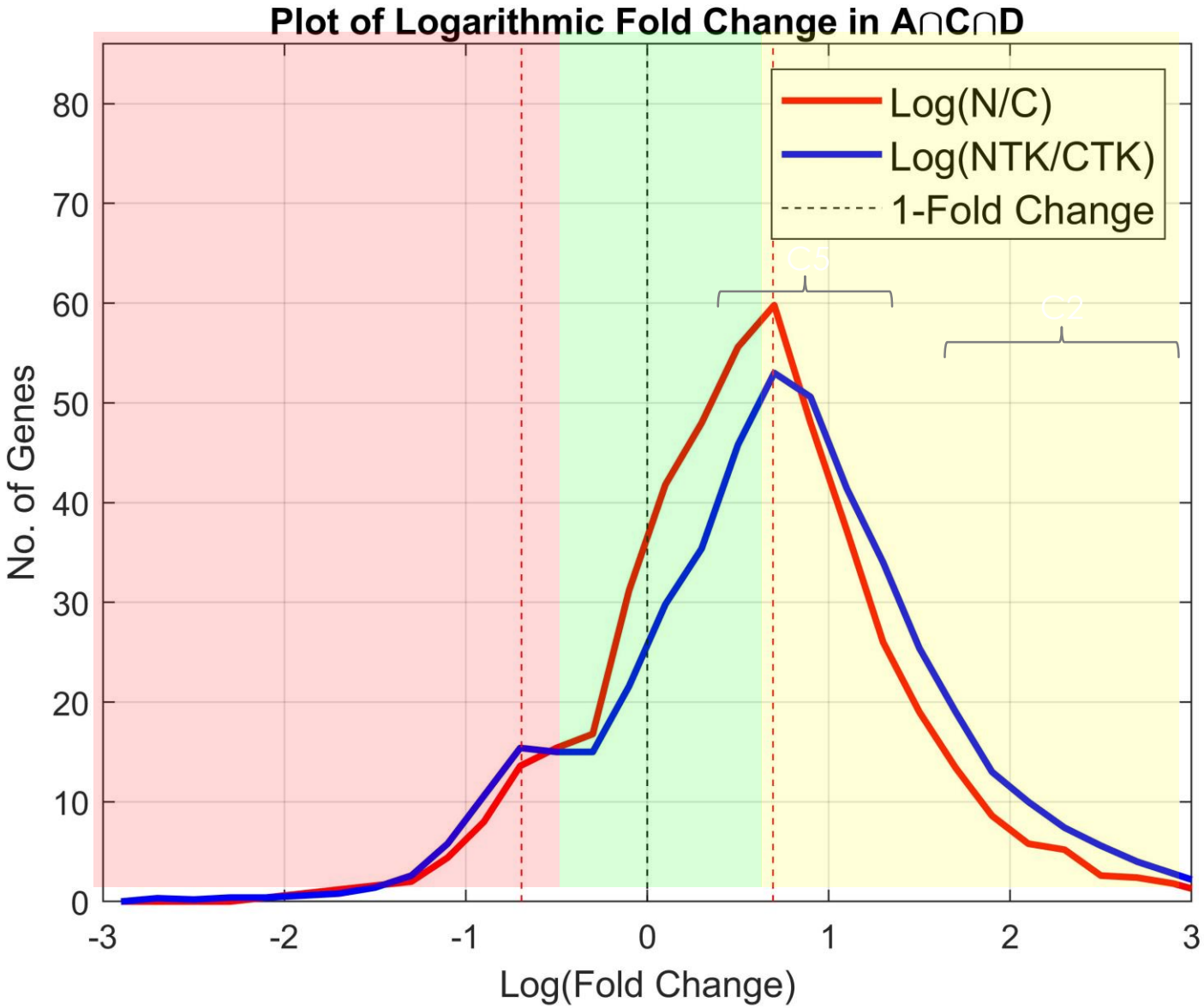
Standardized RNAseq Data			
Gene	C1	C2	C3
KLHL17	-0.87744	-1.5863	0.253823
ISG15	-0.25947	-1.08097	-0.86763
⋮	⋮	⋮	⋮
SCNN1D	-1.04985	-0.98428	-0.60987

$x_1 \; x_2 \; x_3$
$y_1 \; y_2 \; y_3$

$$r = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2}\sqrt{\sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

- VARIABLES x AND y REFER TO RNA LEVEL OF A GENE
- $\bar{x}$ AND $\bar{y}$ ARE THE MEAN OF TRIPLICATES
- PAIRWISE LINEAR CORRELATION COEFFICIENTS COMPUTED FOR EVERY CONDITION IN A COMPARISON

Intersection of transitions: C→N and CTK→NTK genes affected by nFGFR1(TK-) only in N



This paints a picture of what **R1 suppression in N** does.

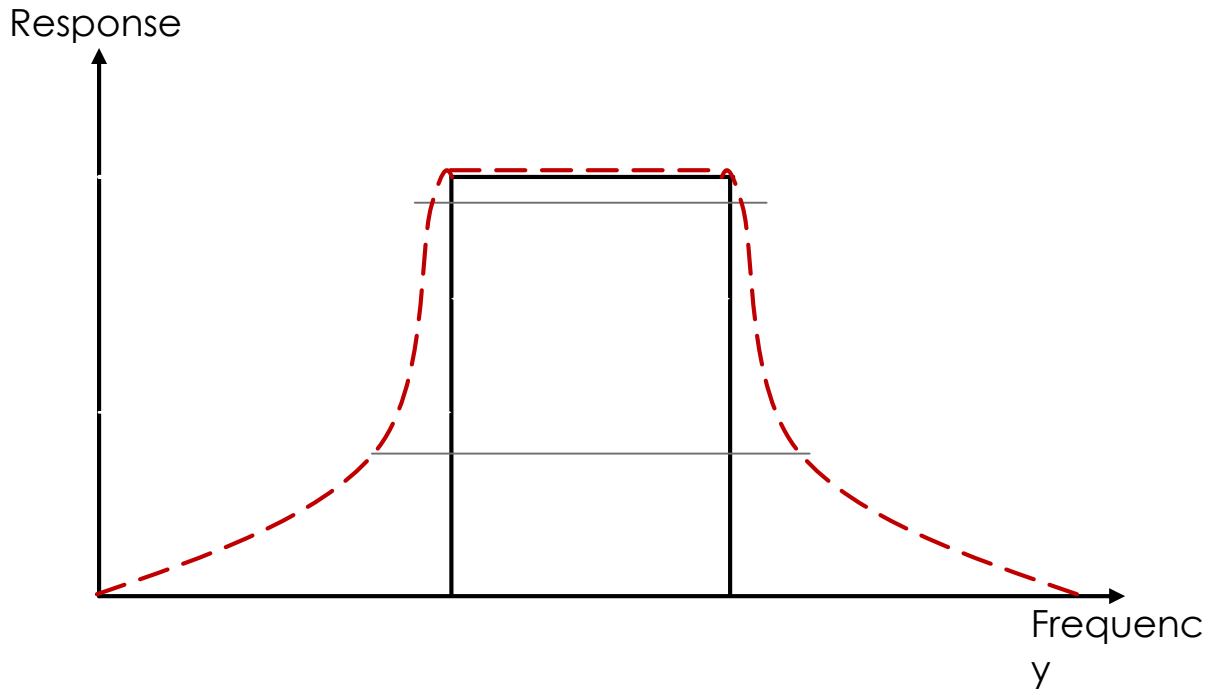
- Green zone: Decrease in genes that have low fold change in NTK/CTK
- Red & Yellow zones: Removal of R1 increases genes that have high-fold change in NTK/CTK

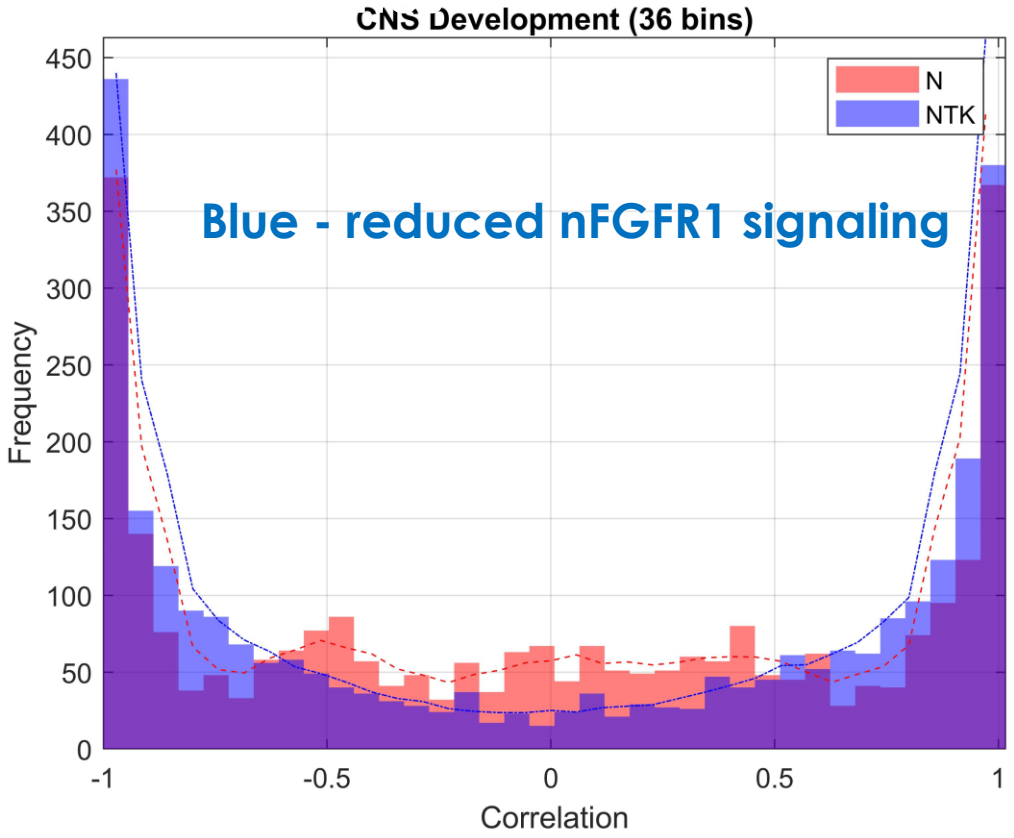
Small responses amplified by opening gate (for small activation and inhibition)
Large response suppressed by opening the gate smaller.

R1 is acting as a **gating control** which prevents large changes from taking place. Gate **opens** for **small responses** and **partially open** for **big responses**. Big changes that may be detrimental are not allowed, but smaller changes are encouraged.
More changes seen in genes that are activated instead of inhibition.

NFGFR1 ACTS AS BANDPASS FILTER

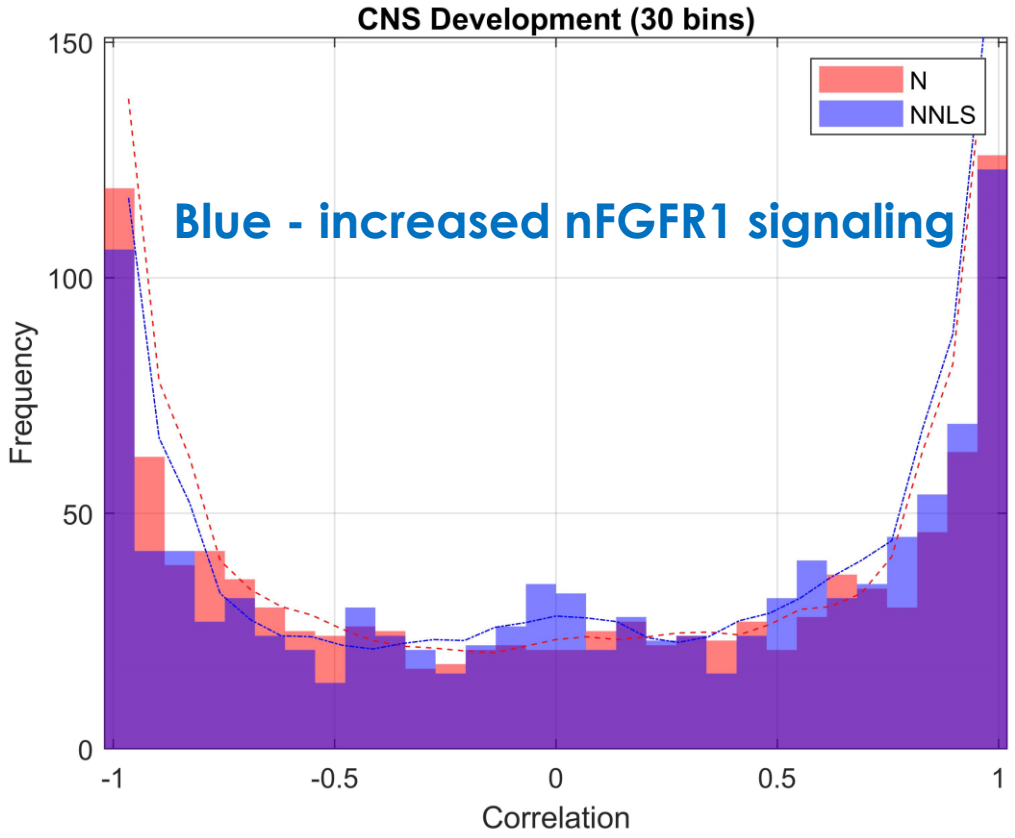
- In practice, bandpass filters are not ideal
  - Not all frequencies outside the passband are attenuated (red dashed line)
  - There are several forms of bandpass filters that are practical in nature
    - Gaussian
    - Butterworth
    - Chebyshev





	Mean	Std. Error
N	0.004209	0.013672
NTK	0.005955	0.014998

	Decision	p-value
T-Test	0	0.93143
KS-Test	1	0.04929



	Mean	Std. Error
N	0.011443	0.02203
NNLS	0.085798	0.02143

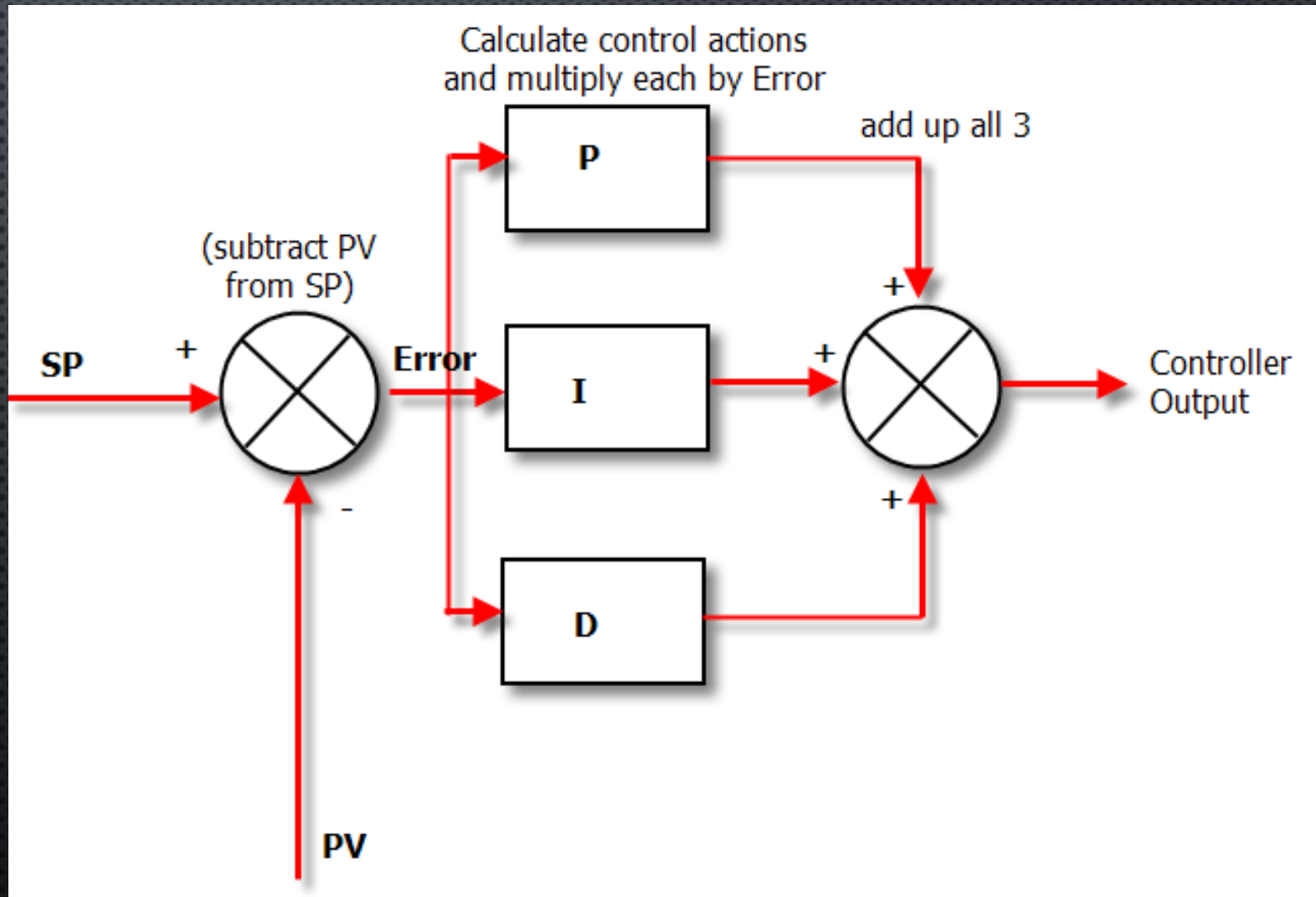
	Decision	p-value
T-Test	1	0.015632
KS-Test	0	0.927009

OVERVIEW OF PID CONTROLLER

A PID IS ESSENTIALLY A CONTROL LOOP FEEDBACK MECHANISM THAT STRIVES TO KEEP MAINTAIN A SYSTEM IN THE SAME STATE

THERE ARE THREE IMPORTANT COMPONENTS OF A PID CONTROLLER:

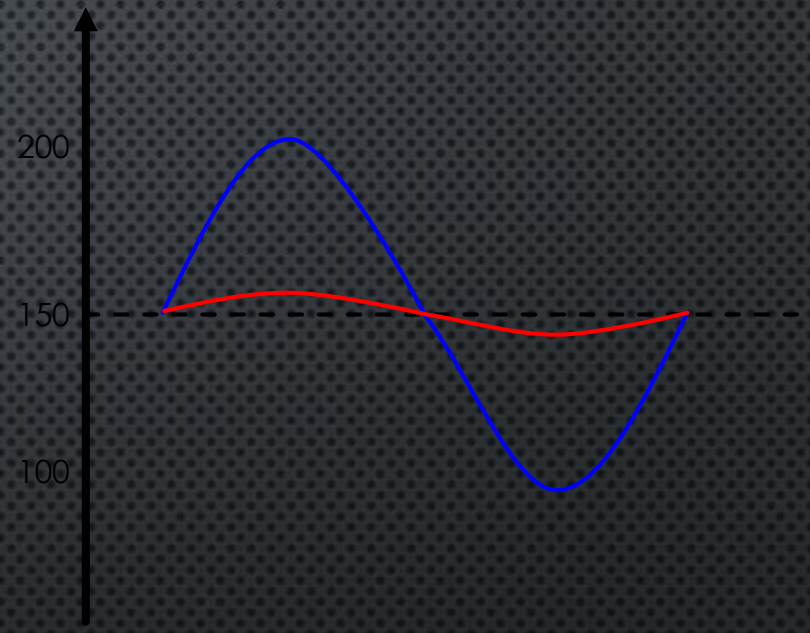
1. A DESIRED SETPOINT (SP), E.G. YOUR CRUISE SPEED
2. A MEASURED PROCESS VARIABLE (PV), E.G. YOUR ACTUAL SPEED
3. ERROR VALUE $e(t)$, E.G. DIFFERENCE BETWEEN PV AND SP
4. CORRECTION OR RESPONSE, E.G. STEPPING ON THE GAS PEDAL

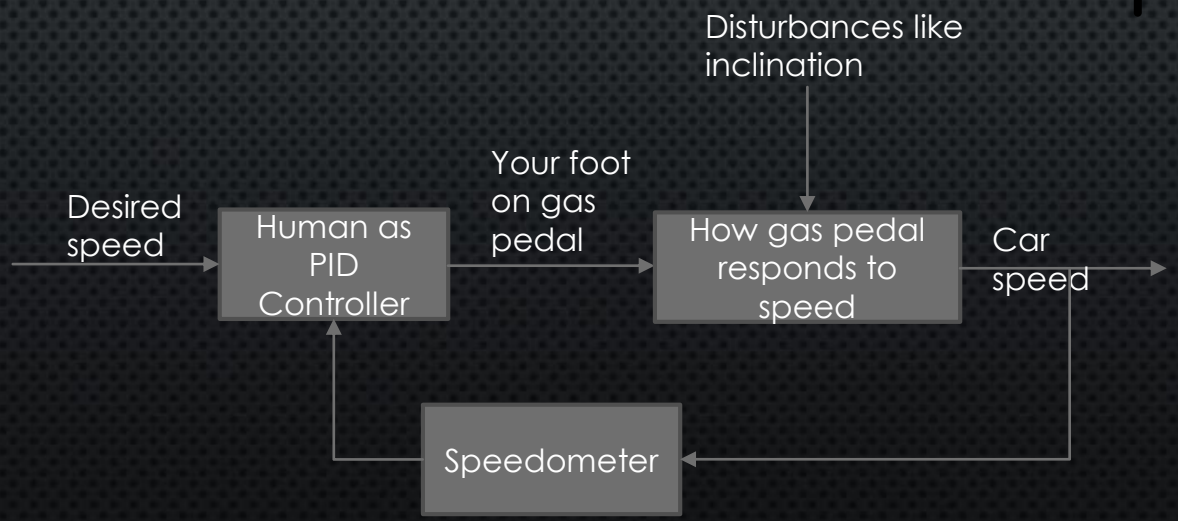


OVERVIEW OF PID (Proportional-Integral-Derivative) CONTROLLER

- YOUR DESIRED SPEED: 150 KM/H
- YOUR ACTUAL SPEED: 100 KM/H
- $e(t)$ = 150 KM/H-100 KM/H = 50 KM/H

YOU CAN EITHER STEP ON THE PEDAL HARD AND OVERSHOOT BEYOND 150 KM/H, OR YOU CAN GO EASY ON THE PEDAL AND TAKE LONGER TO REACH YOUR DESIRED SPEED ACCURATELY



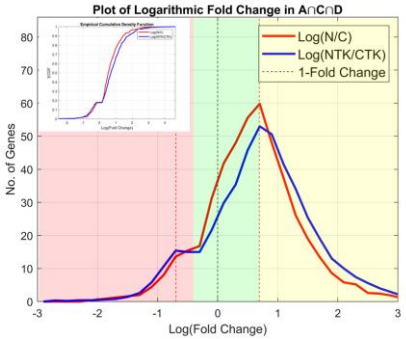


A proportional–integral–derivative

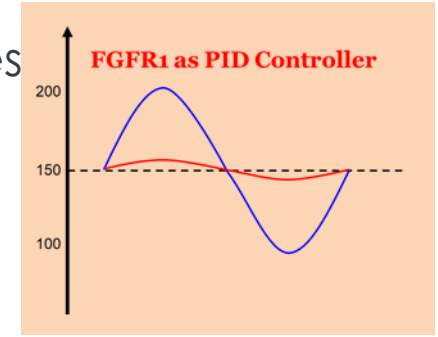
FGFR1 a master orchestrator of genes

gene responses:

(i) First, fold changes are discriminated via a bandpass filter. Number of genes with lar fold changes (detrimental to organism's ontogeny) are suppressed. Meanwhile ge with smaller fold changes are encouraged.



(ii) nFGFR1acts as a PID (Proportional-Integral-Derivative) controller, regulates gene-to-gene coordination. By identifying a 'normal' setpoint and controls the number of genes that display moderate or high coordination to fit within the setpoint.



Understanding Developmental Disorders

Schizophrenia is most severe multifaceted mental illness that features hallucinations, delusions, depression, anxiety,…

Common developmental genome deprogramming in schizophrenia – role of Integrative Nuclear FGFR1 Signaling (INFS)

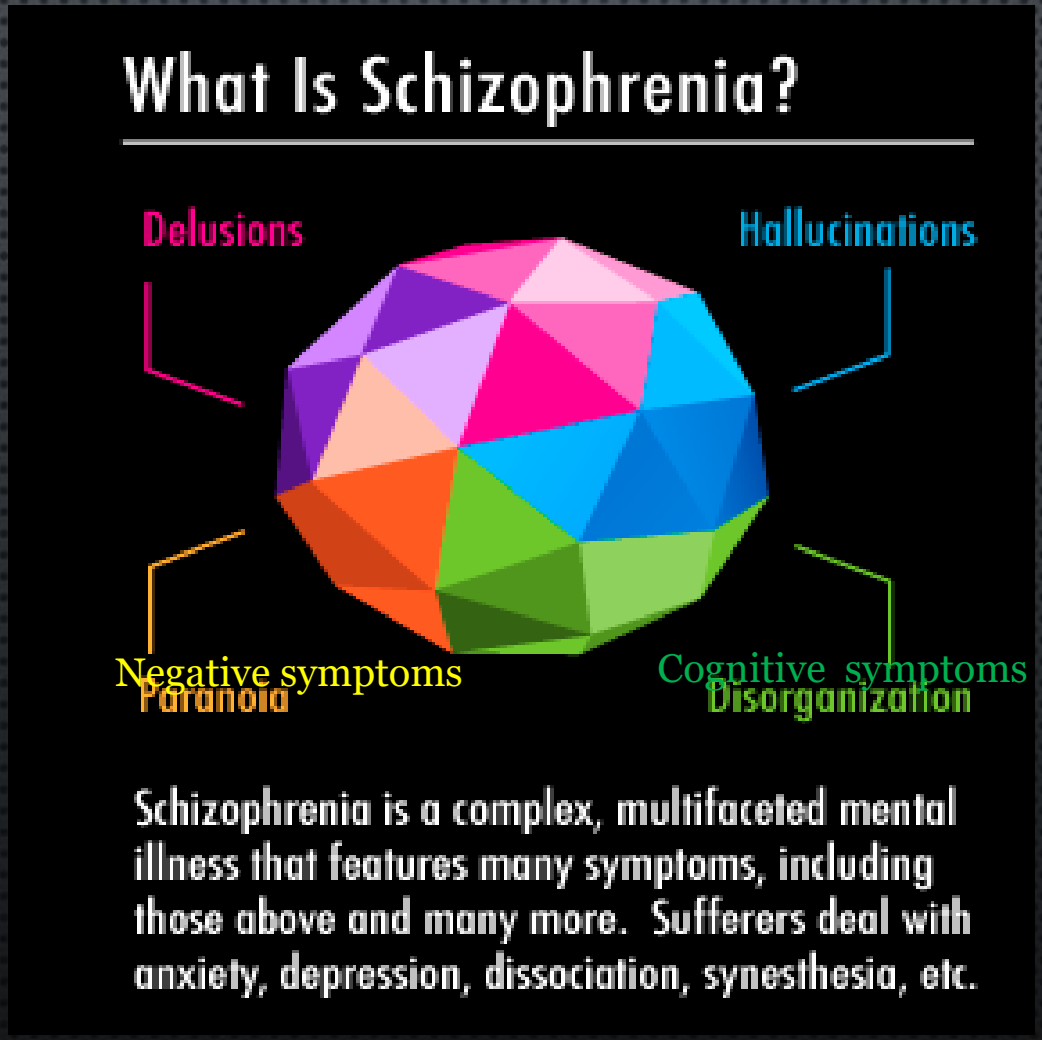
S. T. Narla[1,2], Y-W. Lee[1], C.A. Benson[1,2], P. Sarder[1], K. Brennand[3], E.K. Stachowiak[1,2], M.K. Stachowiak[1,2*]

[1] Department of Pathology and Anatomical Sciences, State University of New York at Buffalo, Buffalo, NY, USA. [2] Western New York Stem Cell Culture and Analysis Center, State University of New York at Buffalo, Buffalo, NY, USA. [3] Icahn School of Medicine at Mount Sinai, Departments of Psychiatry and Neuroscience, New York, NY, USA

Schizophr. Res. (2016),

http://dx.doi.org/10.1016/j.schres.2016.12.012

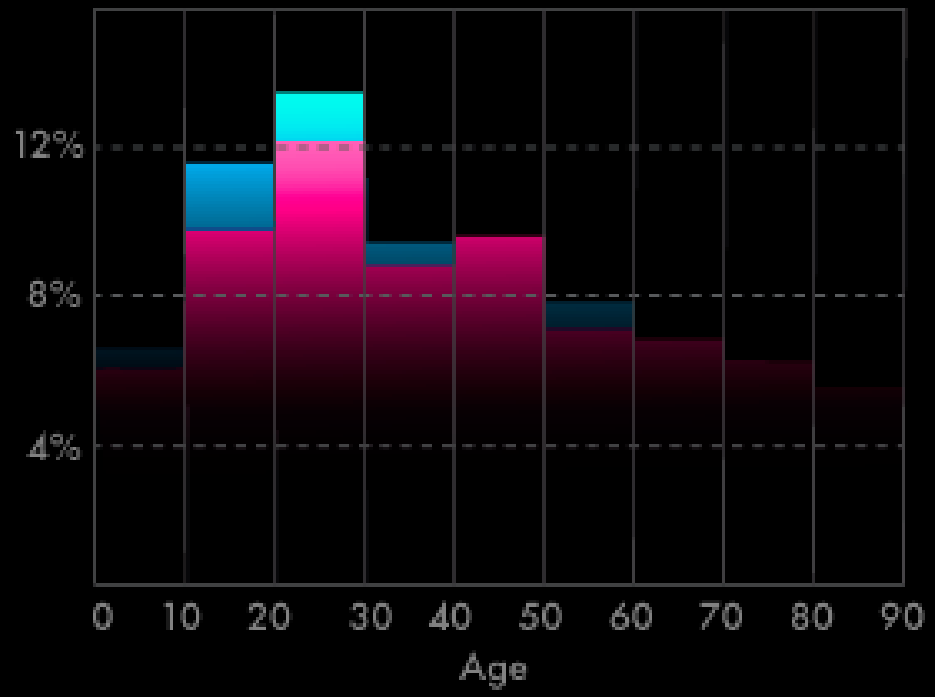
What is Schizophrenia?



Age of Onset

What is the Age of Onset?

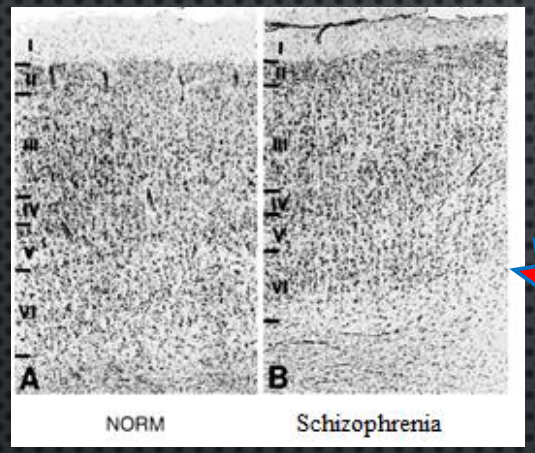
Symptoms begin for males and females at varying times through life. The graph below shows the typical age of onset.

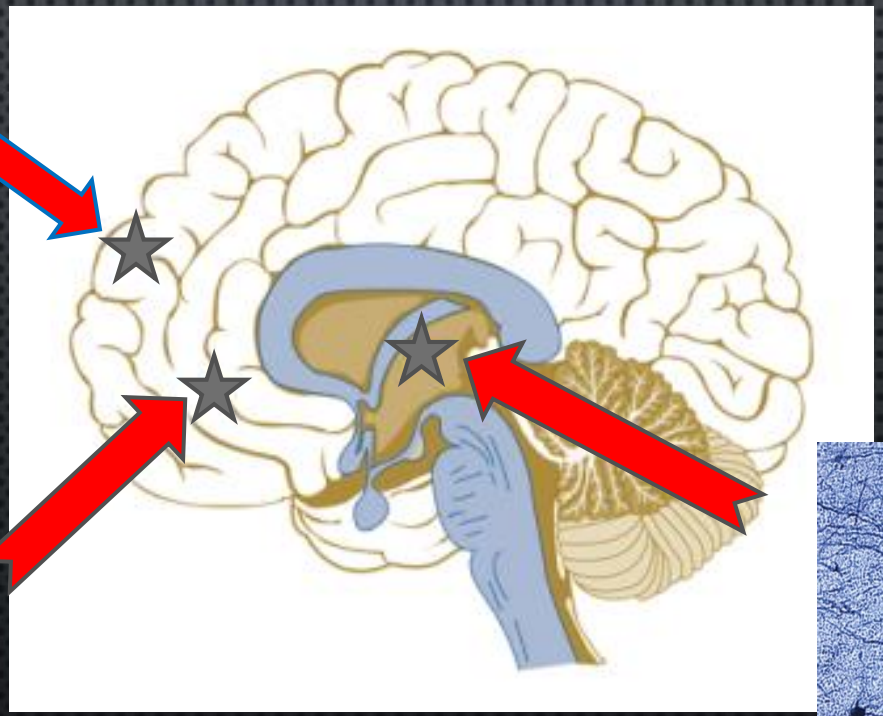


Causes of Schizophrenia

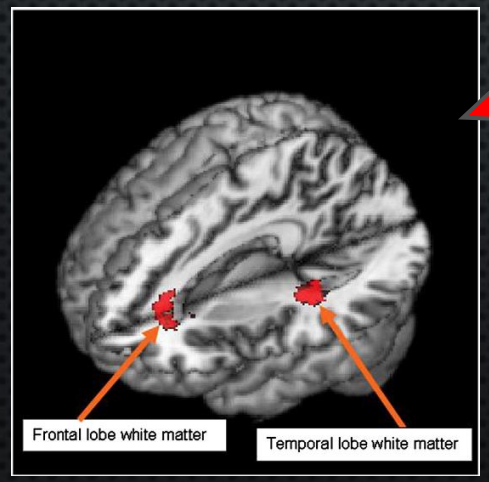
Schizophrenia - disease that affects brain development

Disrupted cortical layers, Immature neurons



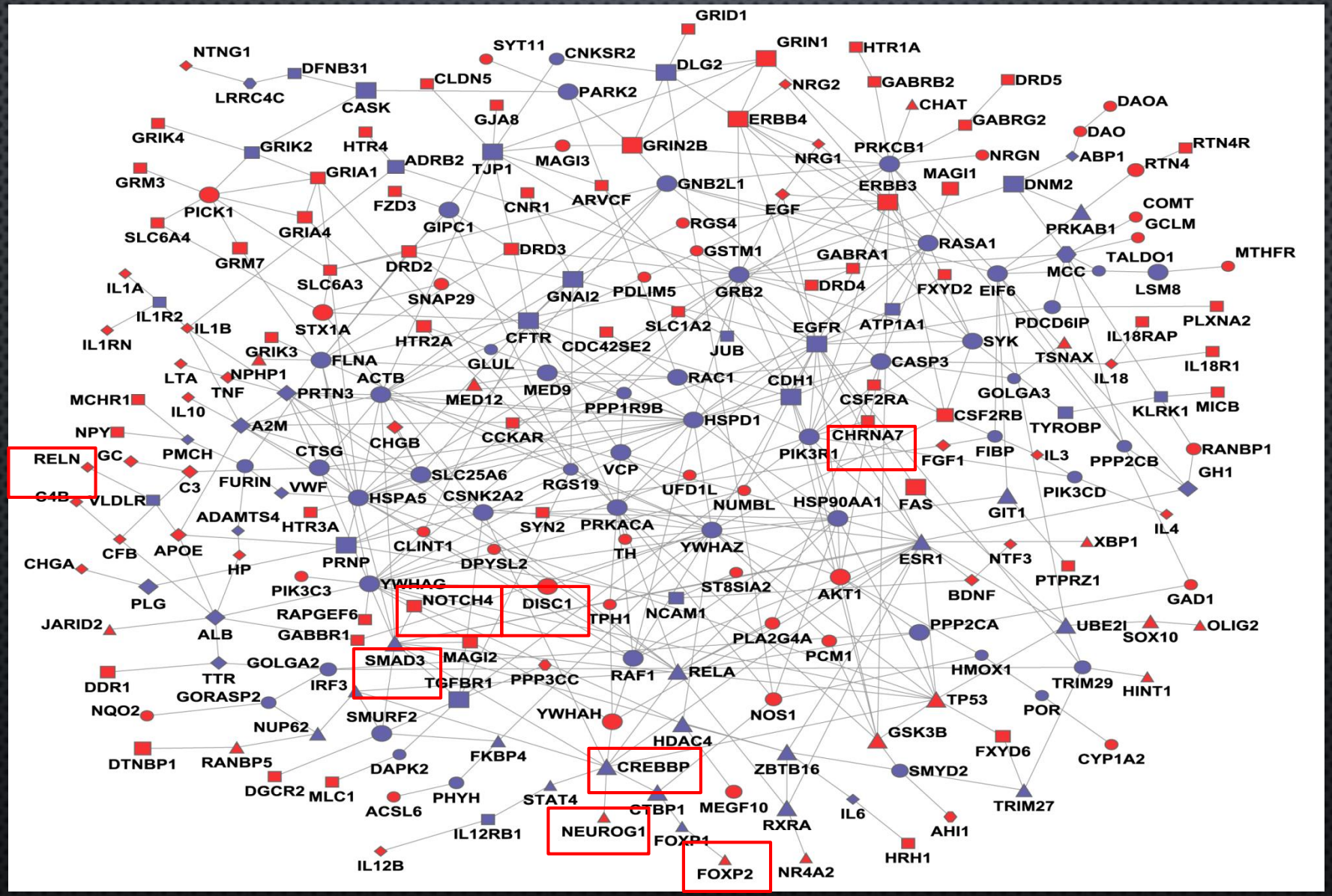


Disorganized subcortical fibers

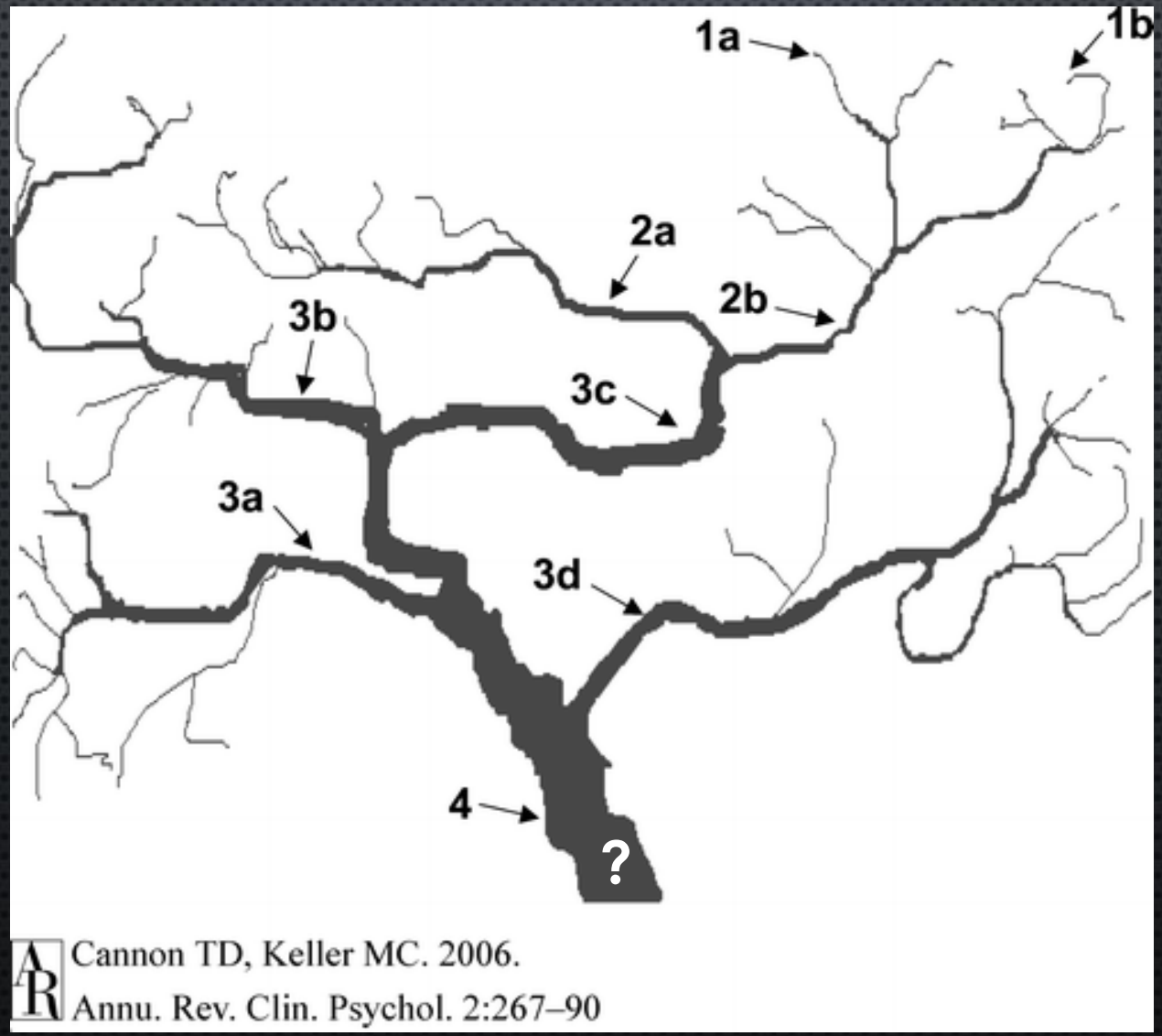


Hypoplastic Dopamine neurons

MUTATIONS IN SCHIZOPHRENIA

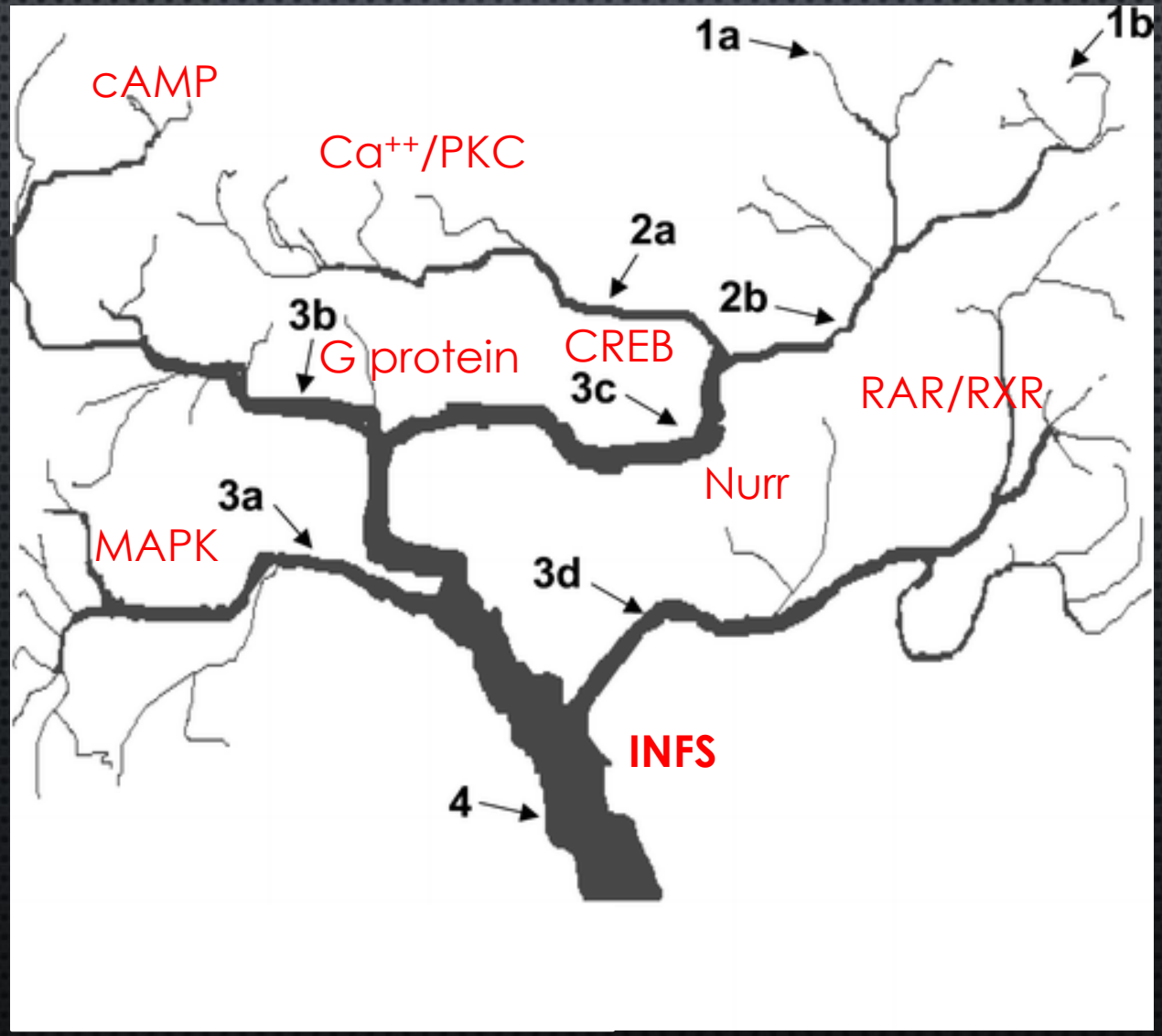


WATERSHED HYPOTHESIS – CANNON & KELLER, 2006



Cannon TD, Keller MC. 2006.
Annu. Rev. Clin. Psychol. 2:267–90

INFS – A COMMON MECHANISM IN SCHIZOPHRENIA?



FGFR1 and FGF-2 mutations in Schizophrenia and related Kallmann syndrome.

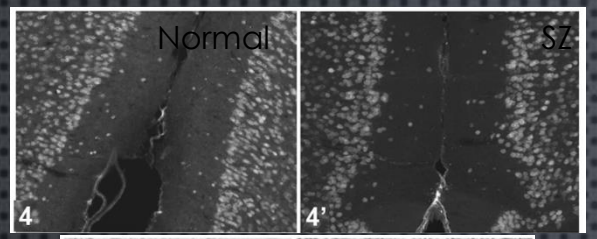
SZ – schizophrenia (*(Jungerius et al., 2008);***(O'Donovan et al., 2009); KS – Kallmann Syndrome with multiple FGFR1 mutations co-segregates with schizophrenia; ** (Albuisson et al., 2005; Cowen and Green, 1993; Vagenakis et al., 2004).

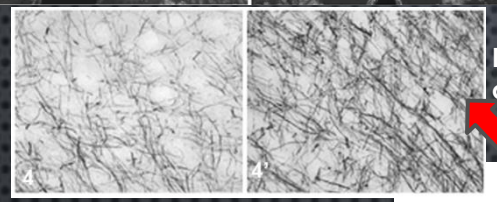
Gene	Polymorphism/Mutation	P-value	Disease	
FGFR1	**SNP rs3925**	**0.0049**	SZ*	
	SNP rs6987534	**0.0079**	SZ*	
	SNP rs7012413	0.105	SZ*	
	Multiple Mutations	-	KS**	
FGF2	**SNP rs12506776**	**0.0048**	SZ***	
	SNP rs7700205	0.0699	SZ***	

Modeling schizophrenia in transgenic mouse

INFS *mutation*

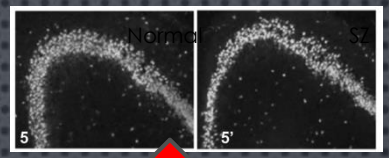
Disrupted cortical layers,
Immature neurons



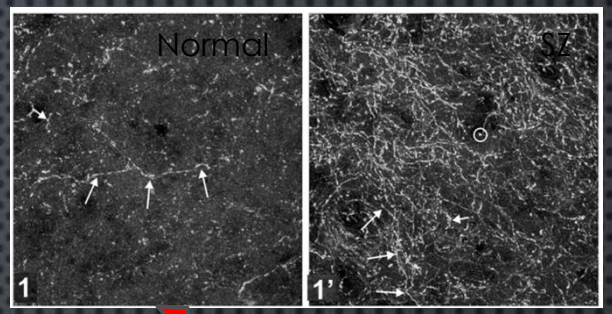


Disorganized
cortical fibers

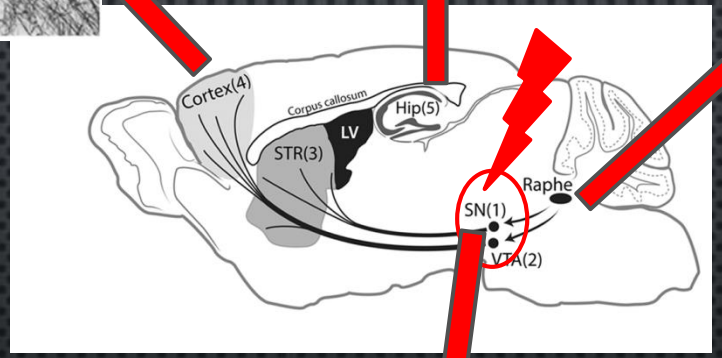
Disrupted
hippocampal
layers

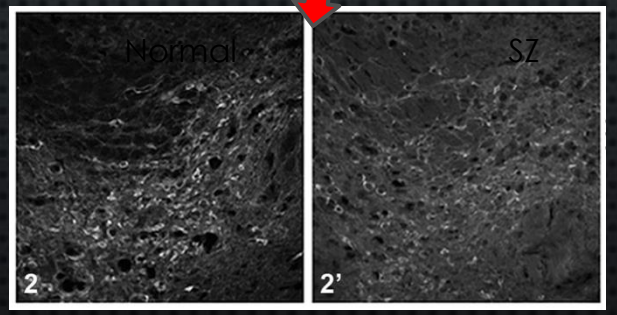


Overgrown Serotonin
Neurons



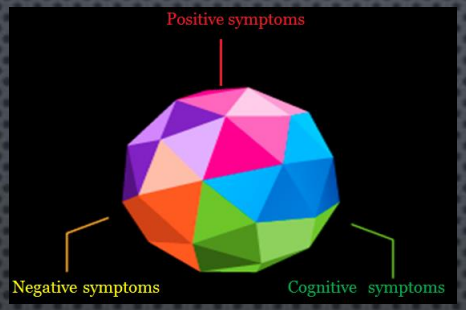
INFS *mutation*





Hypoplastic Dopamine
Neurons

Modeling Schizophrenic Symptoms in Mice

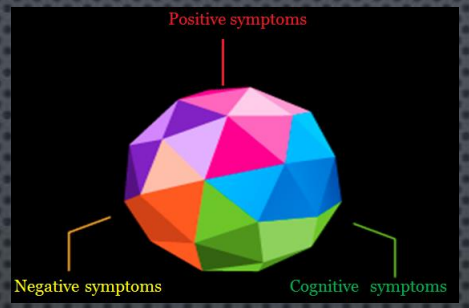


Positive: Sensory processing

Negative: associality

Cognitive: working memory

Testing Established Human Therapy in Mice



Typical neuroleptics

Atypical neuroleptics

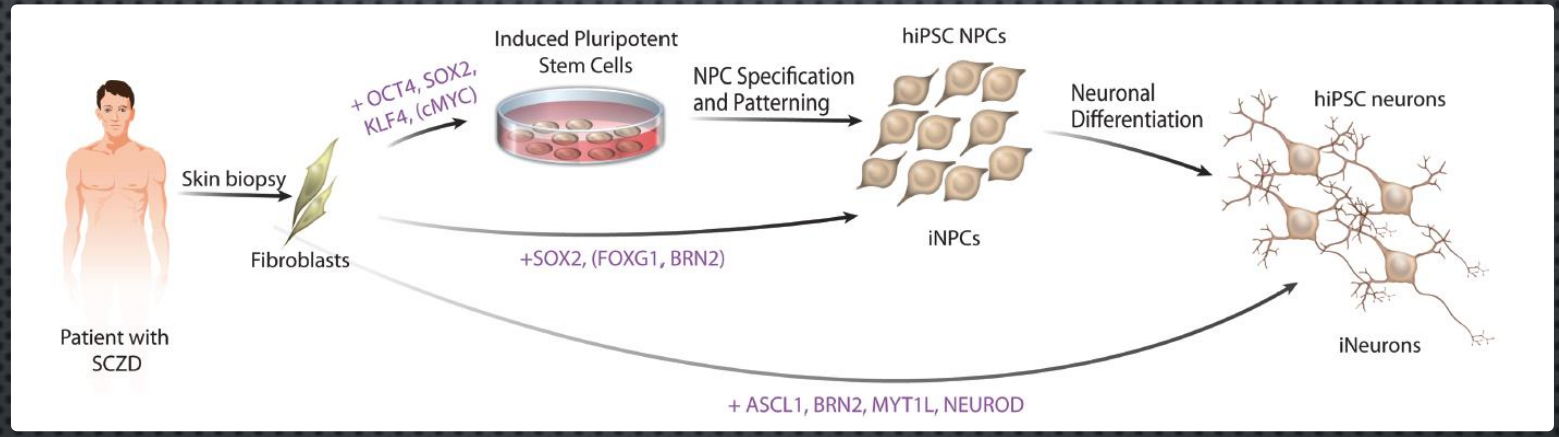
a7 Nic R agonists)

Positive: Sensory processing
Dopamine Release

Negative: associality

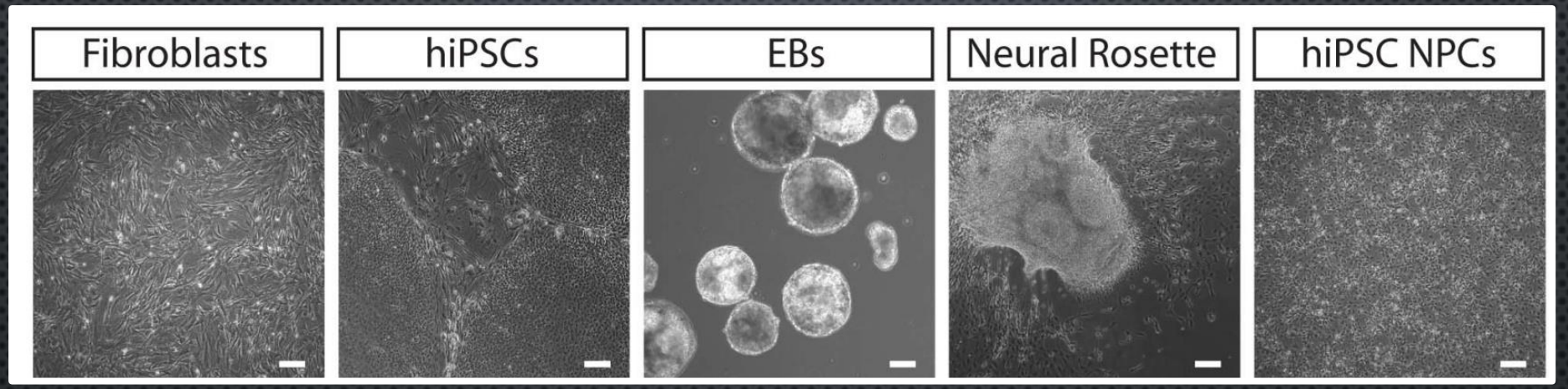
Cognitive: working memory

Model for Schizophrenia



- Preform skin biopsy on patients
- Grow Fibroblasts
- Transform fibroblasts into iPSC
- Differentiate iPSC into NPCs

IPSC > NPC



> NCC (Neuronal Committed Cells):

- NPCs placed in neuronal media (DMEM/F12 plus N2, B27, 1mg/ml laminin, 20ng/ml BDNF and GDNF, 500ug cAMP, 200nm L-Ascorbic acid) for 2 day

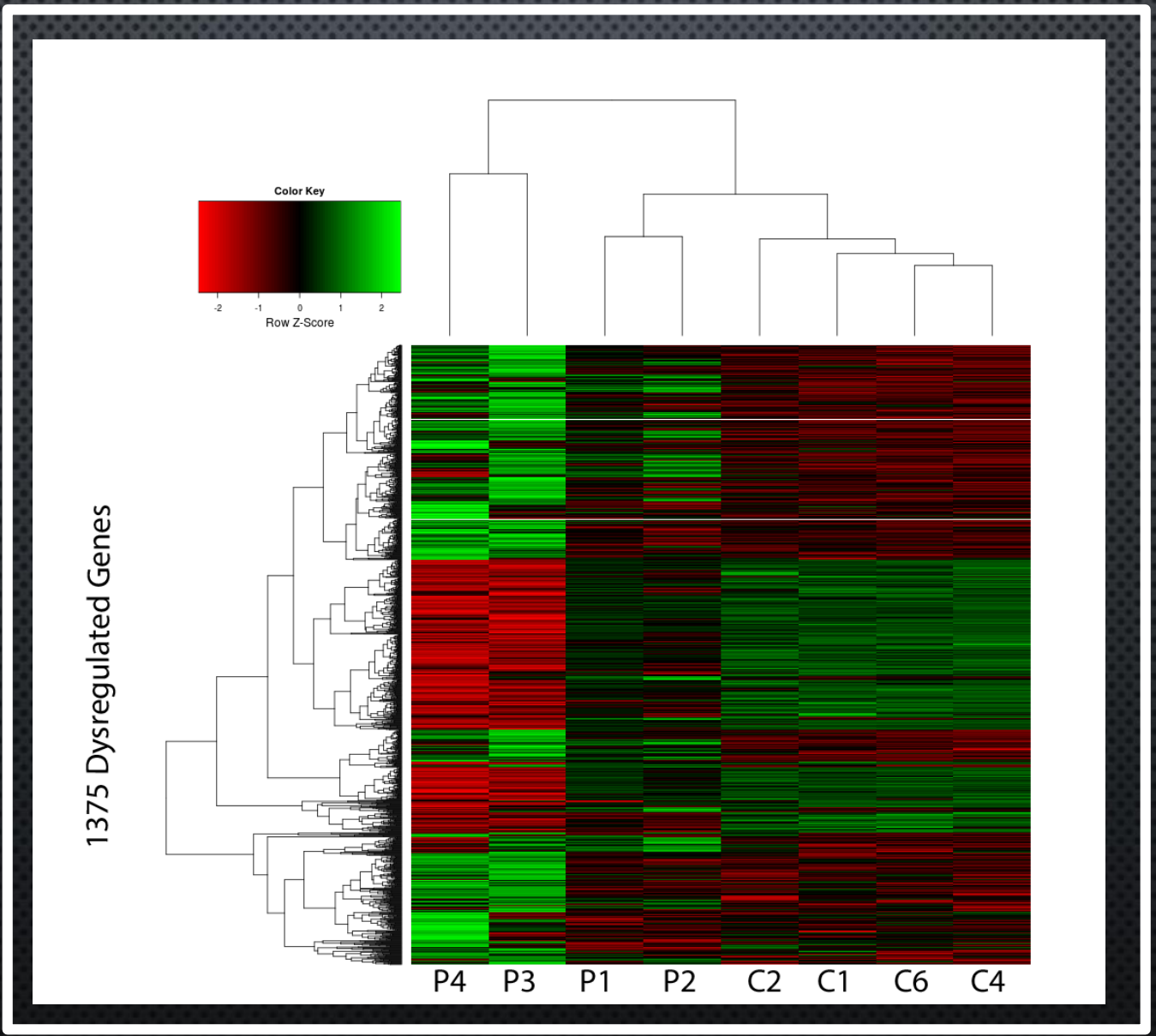
Advantages

Patient ID	Coriell ID	Sex	Ethnicity	Age at Biopsy (years)	Age of Onset (years)	Phenotype	Hospitalizations?	Family History
C1	BJ	M	Causasian	0	-	-	-	unknown
C2	GM03440	M	Causasian	20	-	-	-	unknown
C4	GM04506	F	Causasian	20	-	-	-	unknown
C6	AG09429	F	Causasian	25	-	-	-	unknown
P1	GM02038	M	Causasian	22	6	suicide	?	unknown
P2	GM01792	M	Causasian Jewish / Scandanavian	26	unknown	episodes of agitation, delusions of persecution, and fear of assassination; at age four mild features of pervasive developmental disorder	?	father and sister affected; brother autistic at age four
P3	GM01835	F	Causasian Jewish	27	unknown	drug abuse; schizo-affective disorder	Yes	father and brother affected
P4	GM02497	M	Causasian Jewish	23	15	paralogical thinking, affective shielding, splitting of affect from content, and suspiciousness	Yes	affected father, anorexic /schizoid sister

Experimental Design

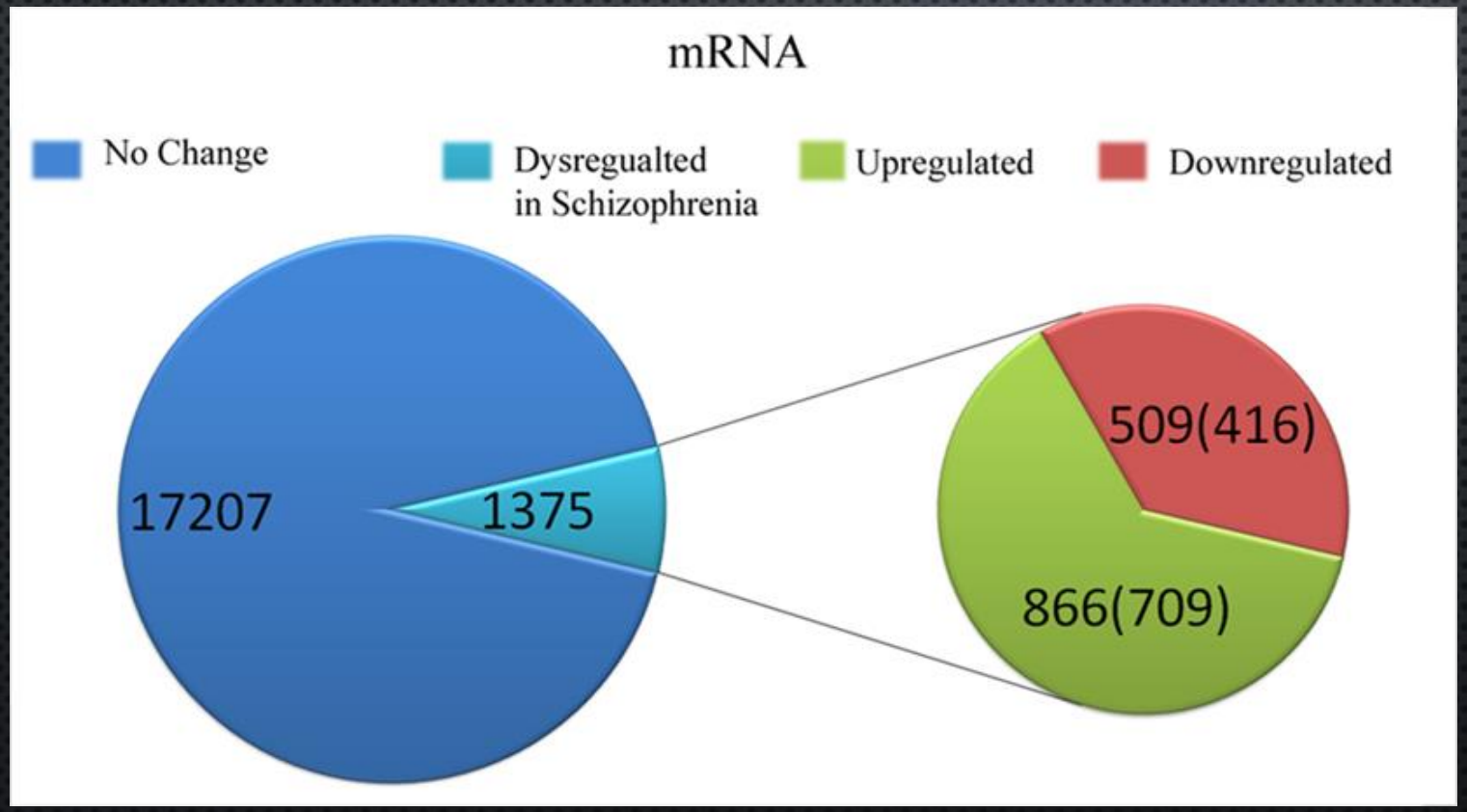
- 4 Patients and 4 Controls
- Preformed RNA-Seq
- Preformed ChIP-Seq with FGFR1

RNA-Seq


Color Key
-2
-1
0
1
2
Row Z-Score
1375 Dysregulated Genes
P4
P3
P1
P2
C2
C1
C6
C4

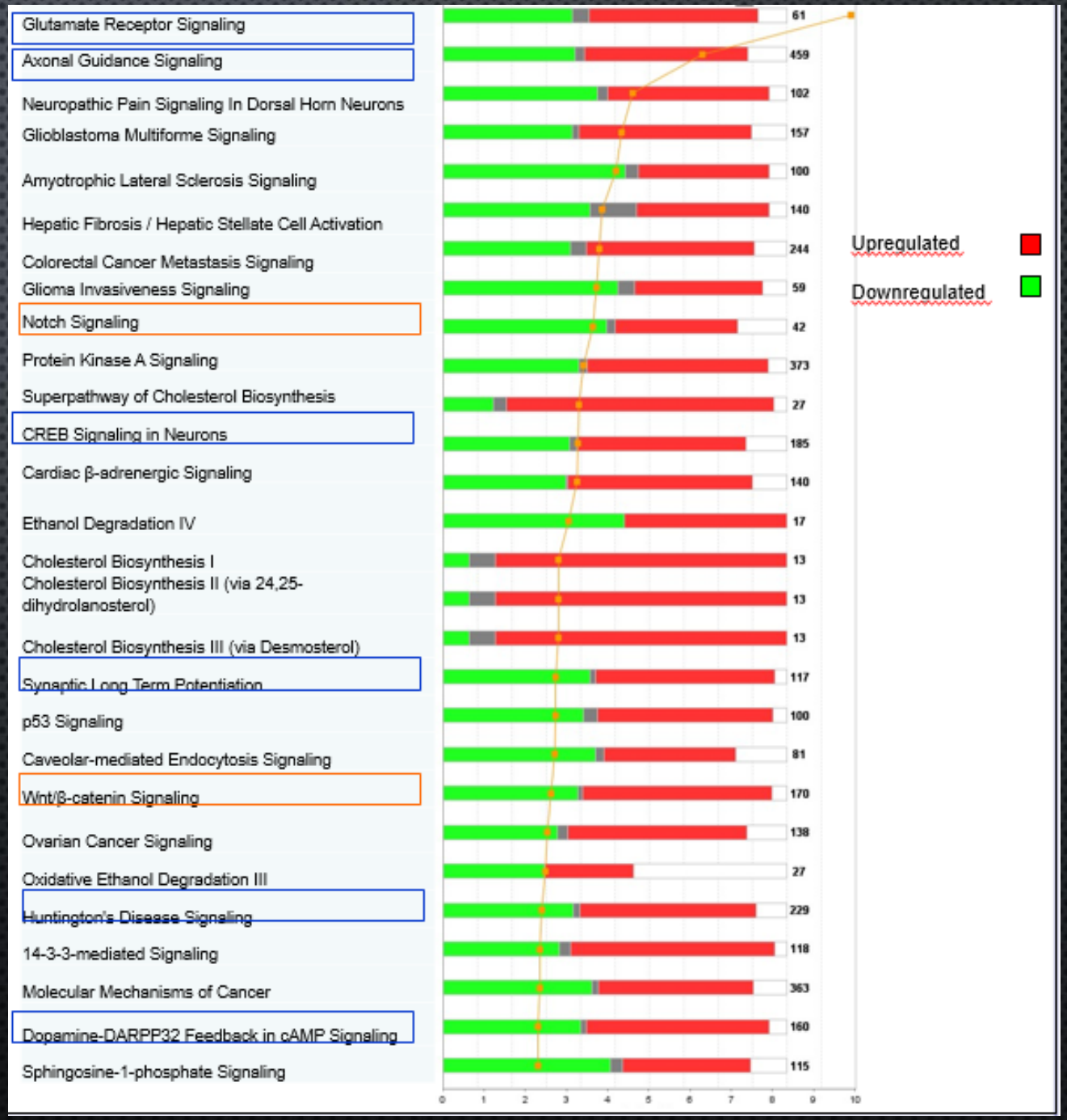
RNA-Seq

Common dysregulated transcriptome in All Patients – 1375 genes



Analysis of Pathways

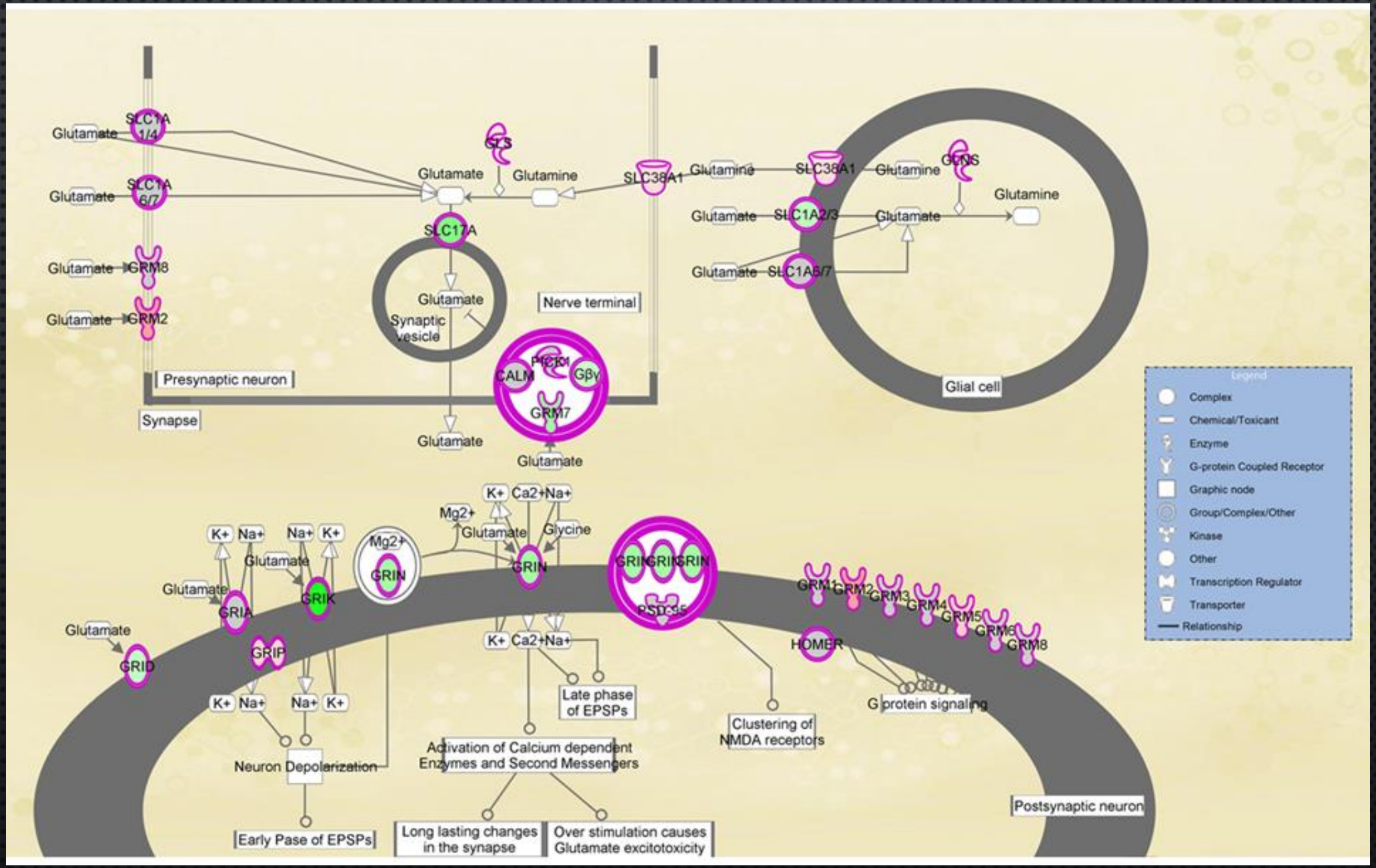
- Developmental pathways
- Neuronal Pathways
- Pathways previously shown that FGFR1 binds



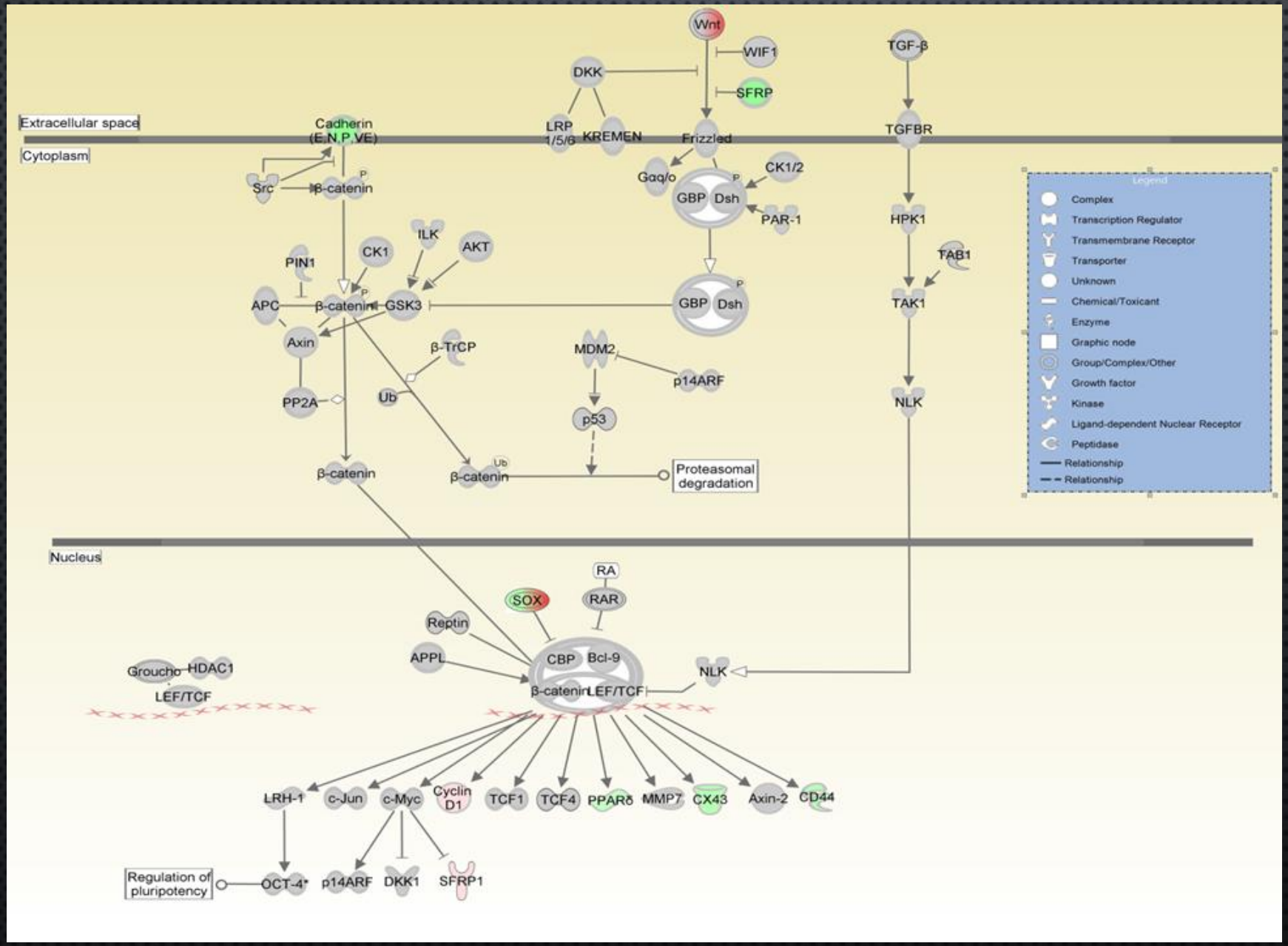
Examples:

the majority of dysregulated genes in glutamate receptor signaling, CREB signaling in neurons, Notch signaling, and dopamine degradation were downregulated, whereas most of those involved in axon guidance, p53 signaling, cholesterol biosynthesis, PI3K/AKT signaling, tight-junction signaling, and STAT3 pathway signaling were upregulated (Figure 1E). Analysis of pathways through Reactome verified segregation of distinct pathways between the up- and downregulated categories. The upregulated genes were involved in neurotransmitter release, axon guidance, (TP53-dependent) transcription of cell cycle genes, and development (Table S6); whereas downregulated genes were involved in cell junction organization, cell-cell junctions, neurotransmitter receptor binding, and cell-cell communication (Table S7).

Glutamatergic Signaling Pathway



WNT Signaling



Dysregulations of Linage

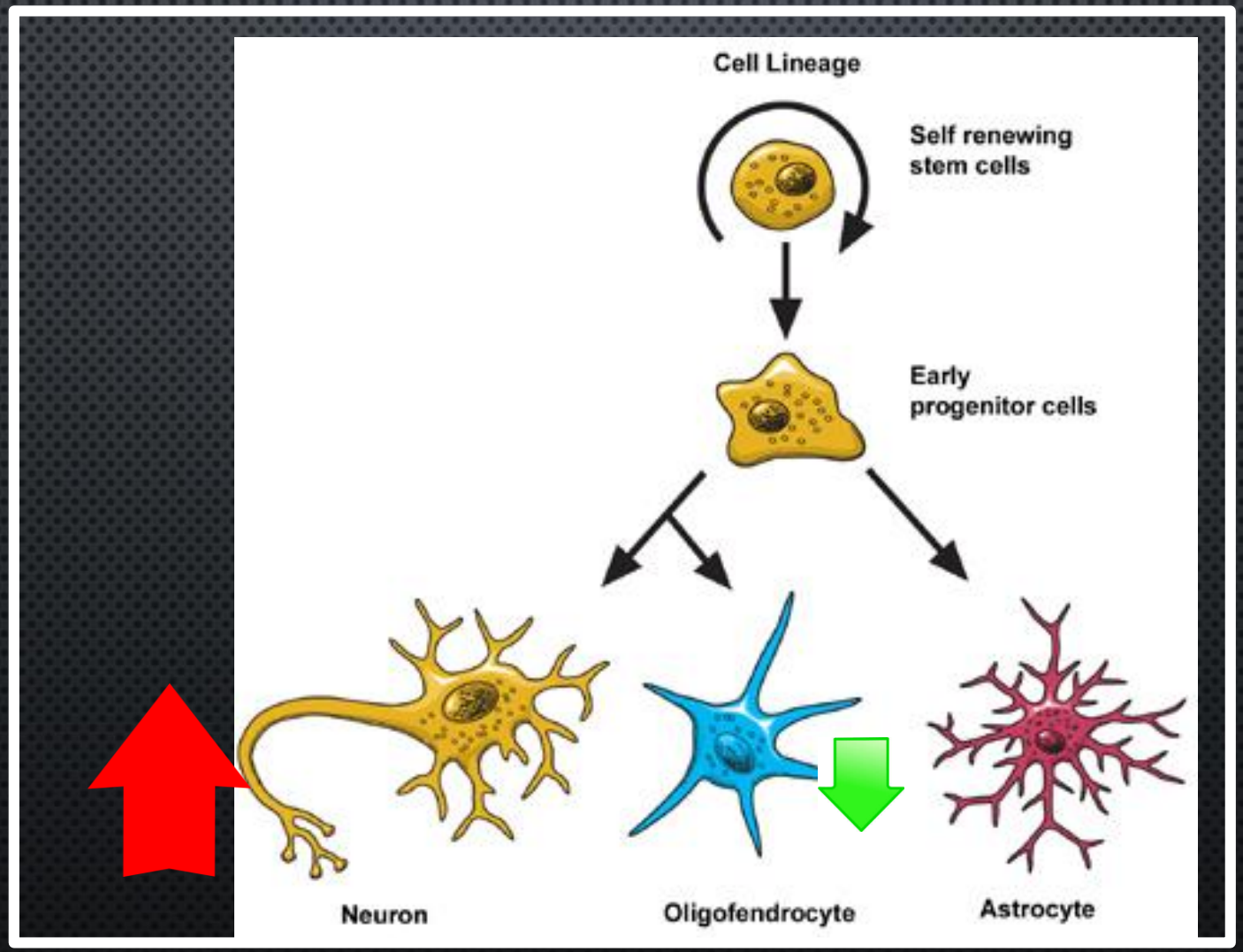
ID	Fold Change	Entrez Gene Name	Type(s)
USP44	55.992	ubiquitin specific peptidase 44	peptidase
HRC	51.410	histidine rich calcium binding protein	other
TYRP1	51.205	tyrosinase-related protein 1	enzyme
IL13RA2	44.156	interleukin 13 receptor, alpha 2	transmembrane receptor
TNFRSF1B	43.655	tumor necrosis factor receptor superfamily, member 1B	transmembrane receptor
NEUROD4	43.247	neuronal differentiation 4	other
FZD10	31.044	frizzled family receptor 10	G-protein coupled receptor
SOX3	30.623	SRY (sex determining region Y)-box 3	transcription regulator
PLAGL1	30.459	pleiomorphic adenoma gene-like 1	transcription regulator
LHX1	30.411	LIM homeobox 1	transcription regulator
PRMT8	28.371	protein arginine methyltransferase 8	enzyme
COL2A1	28.295	collagen, type II, alpha 1	other
SALL4	27.750	spalt-like transcription factor 4	transcription regulator
POU2F2	26.840	POU class 2 homeobox 2	transcription regulator
PAX3	26.369	paired box 3	transcription regulator

Dysregulation of Linage

ID	Fold Change	Entrez Gene Name	Type(s)
OLIG2	-10.069	oligodendrocyte lineage transcription factor 2	transcription regulator
AQP4	-10.158	aquaporin 4	transporter
FAM5B	-10.627	bone morphogenetic protein/retinoic acid inducible neural-specific 2	other
GHR	-10.852	growth hormone receptor	transmembrane receptor
LMO2	-10.945	LIM domain only 2 (rhombotin-like 1)	other
EYA4	-11.038	eyes absent homolog 4 (Drosophila)	phosphatase
MYO7B	-11.465	myosin VIIB	peptidase
GRIK3	-11.565	glutamate receptor, ionotropic, kainate 3	ion channel
NEU4	-12.033	sialidase 4	enzyme
P2RX7	-12.190	purinergic receptor P2X, ligand-gated ion channel, 7	ion channel
OLIG1	-13.705	oligodendrocyte transcription factor 1	transcription regulator
PITX2	-16.542	paired-like homeodomain 2	transcription regulator
NKX2-2	-17.396	NK2 homeobox 2	transcription regulator
TMEM132C	-27.072	transmembrane protein 132C	other
PDGFRA	-41.752	platelet-derived growth factor receptor, alpha polypeptide	kinase

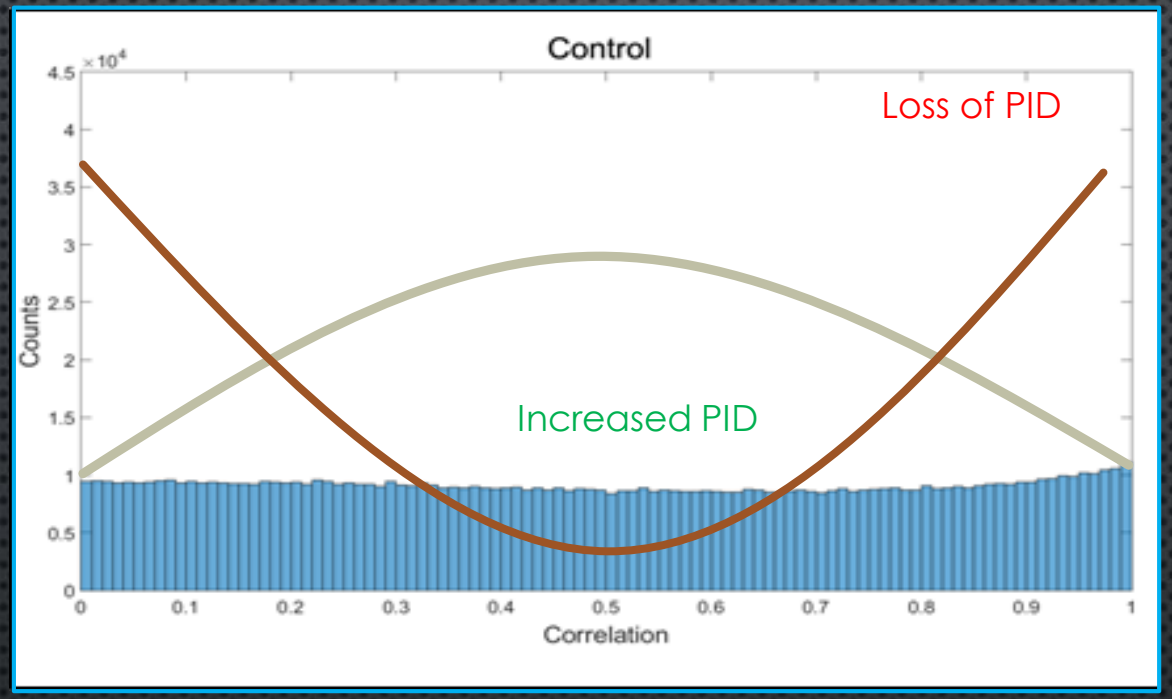
	GO Term
olio-glial Down-regulated	oligodendrocyte differentiation (GO:0048709)
	glial cell differentiation (GO:0010001)
	axon ensheathment (GO:0008366)
	myelination (GO:0042552)
	positive regulation of axonogenesis (GO:0050772)
	extracellular structure organization (GO:0043062)
	regulation of synapse structure or activity (GO:0050803)
Neuronal Up-regulated	neurotransmitter transport (GO:0006836)
	regulation of axonogenesis (GO:0050770)
	learning (GO:0007612)
	stem cell development (GO:0048864)
	synapse organization (GO:0050808)
	stem cell differentiation (GO:0048863)

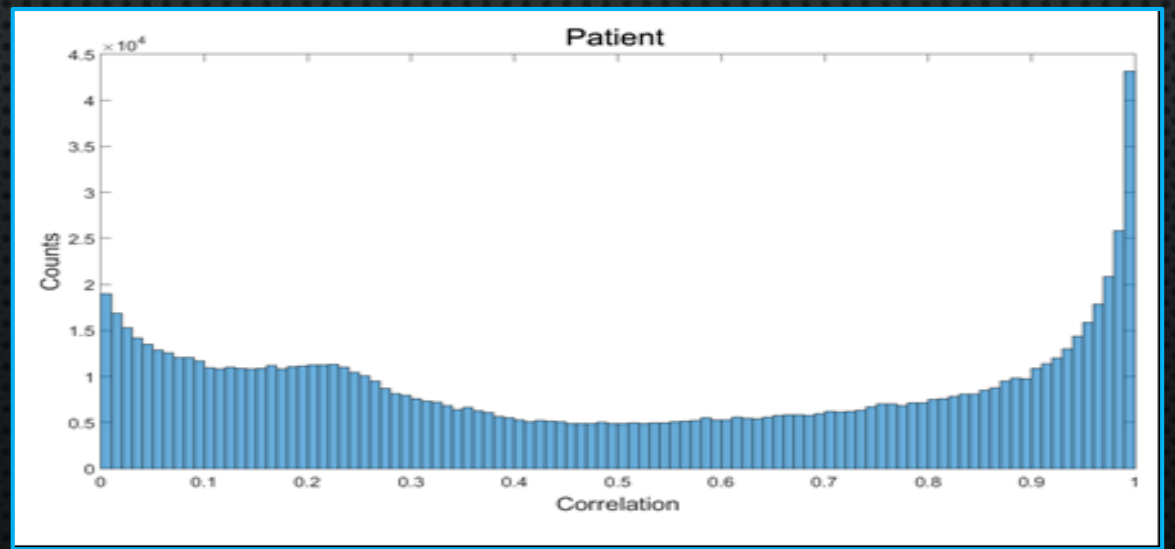
Dysregulation of Lineage Transition



COORDINATED GENE DYSREGULATION IN SCHIZOPHRENIA

PAIRWISE CORRELATION – LOSS OF PROPORTIONAI-INTEGRAL-DERIVATIVE (PID) CONTROLLER FUNCTION

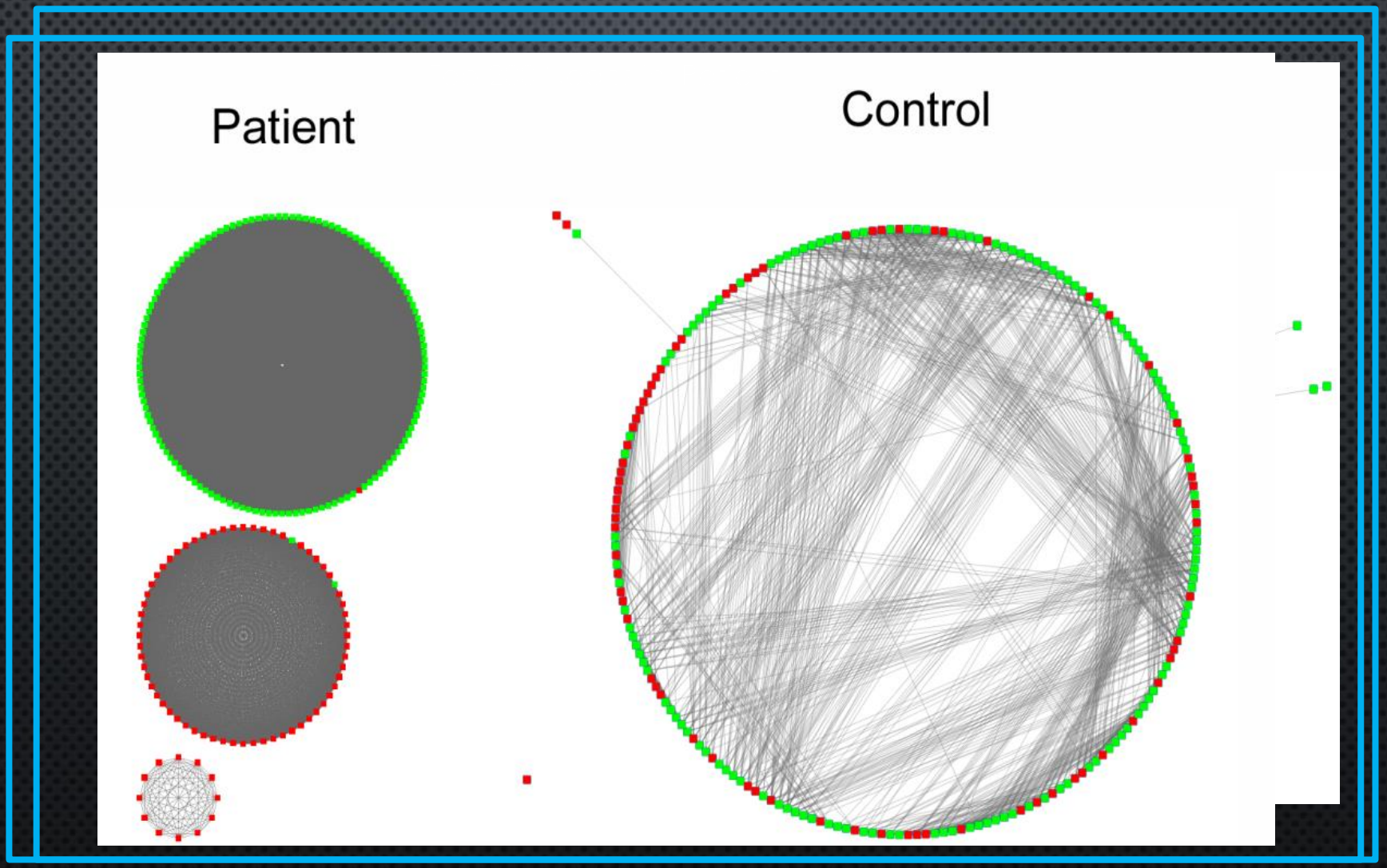




COORDINATE DYSREGULATION OF TRANSCRIPTOME –DISRUPTION OF COORDINATE GENE NETWORKS & FORMATION OF NEW "SCHIZOPHRENIA" GENE NETWORKS

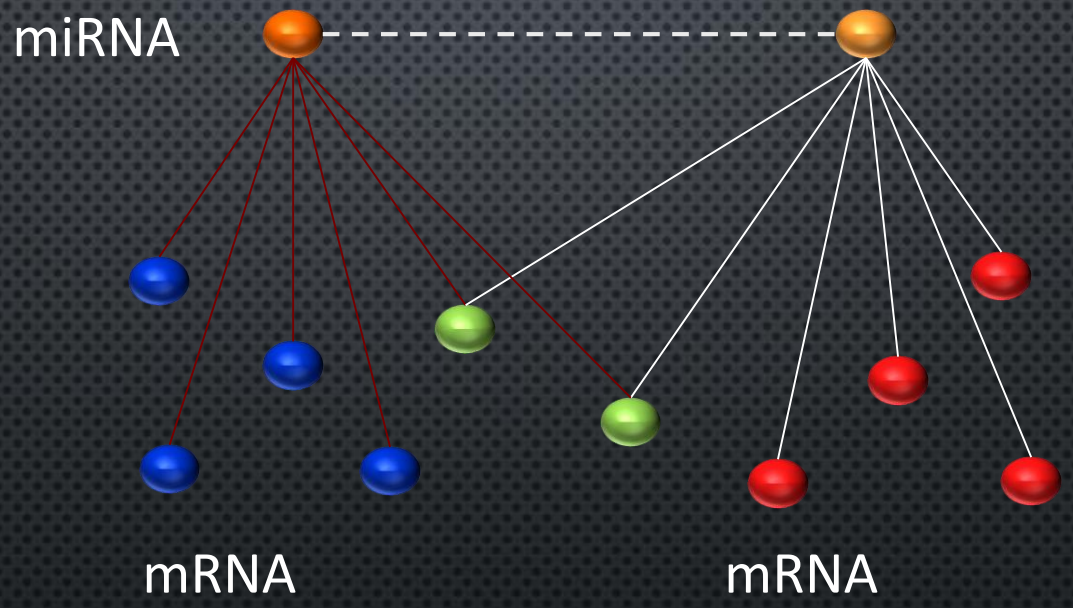
- moderately correlated

- highly correlated

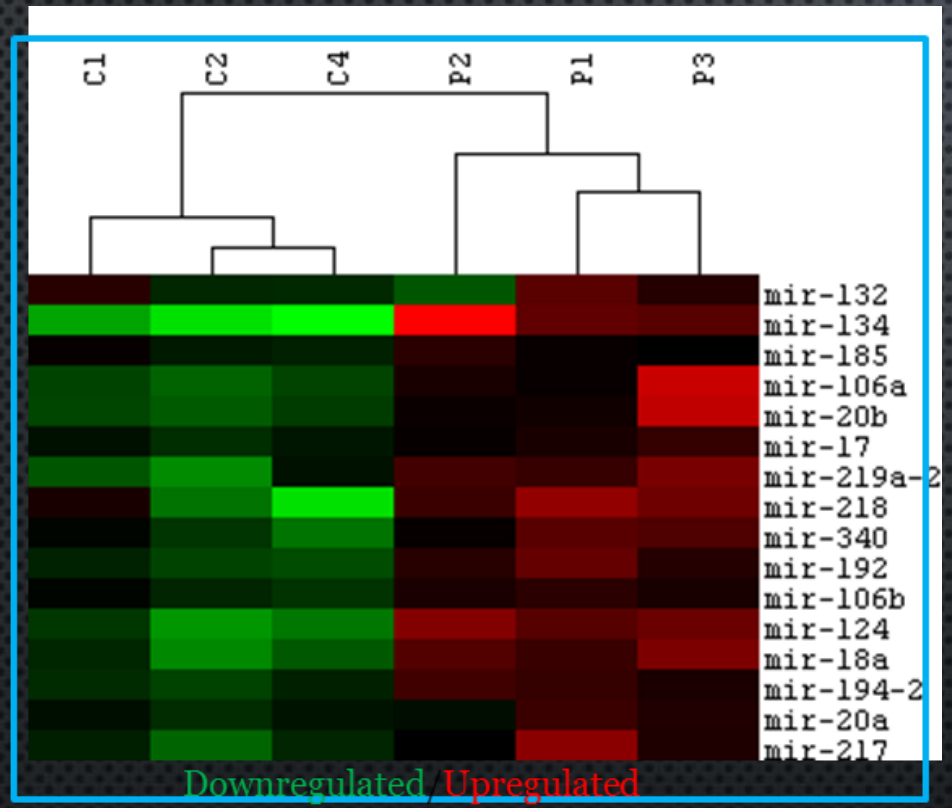


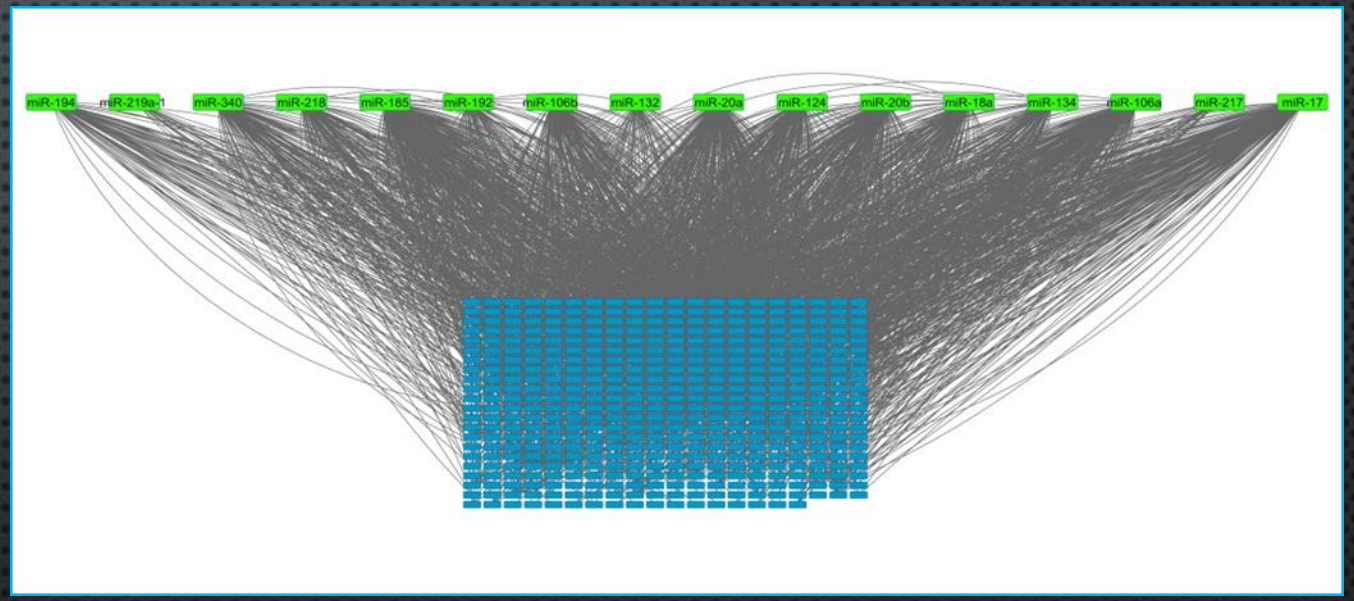
Global Gene Regulators:

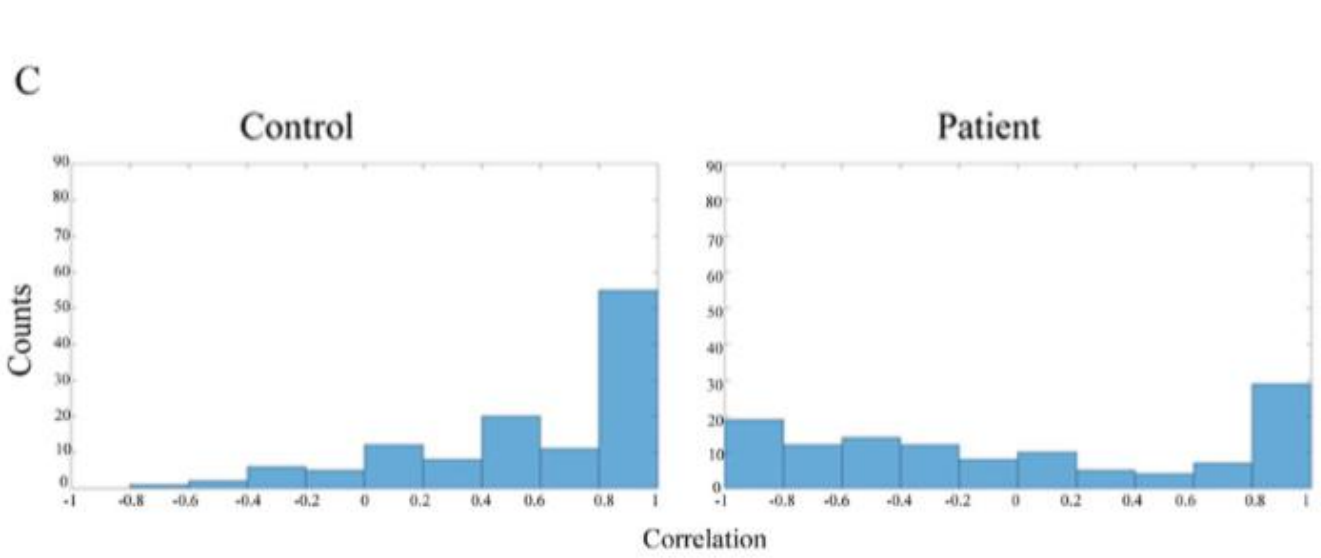
miRNA targeting of mRNA



Dysregulation of coordinated miRNA function in Schizophrenia







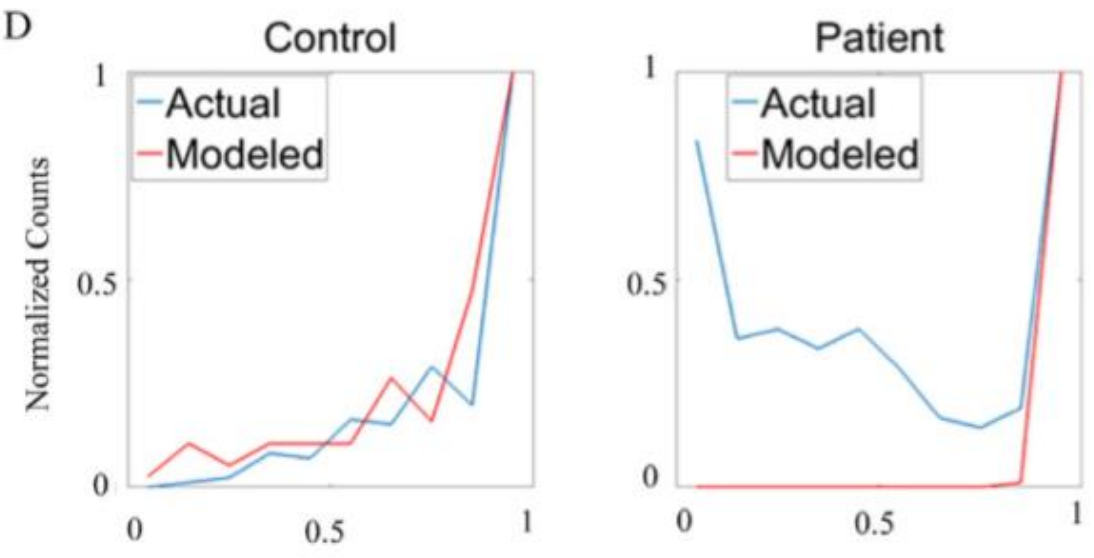


Fig. 3. A) NCCs from three control subjects and three patients were analyzed. Heatmap shows 16 dysregulated miRNA genes (all upregulated) in patient NCCs. Raw expression data were log transformed, and then centered to the median of all 6 samples. Red indicates higher value than median, green indicates, lower value than median. B) 16 dysregulated miRNAs (green) target 440 dysregulated mRNA (Blue). Each gray line indicates a connection between an miRNA and an mRNA. C) In pairwise correlation of dysregulated miRNA genes a high correlation is observed in control NCCs ($n = 3$), but not in patient NCCs ($n = 3$). D) Modeled versus actual miRNA-mRNA correlations. Red line shows correlations for miRNAs predicted based on correlations of their target mRNAs as in (Huttenhower et al., 2009). Blue line shows measured correlations between miRNAs and their target mRNAs. In control NCCs the predicted and actual correlations are similar. In patients, the patterns of predicted and actual correlations differ markedly. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Deconstruction of interactive miRNA-mRNA network in Schizophrenia

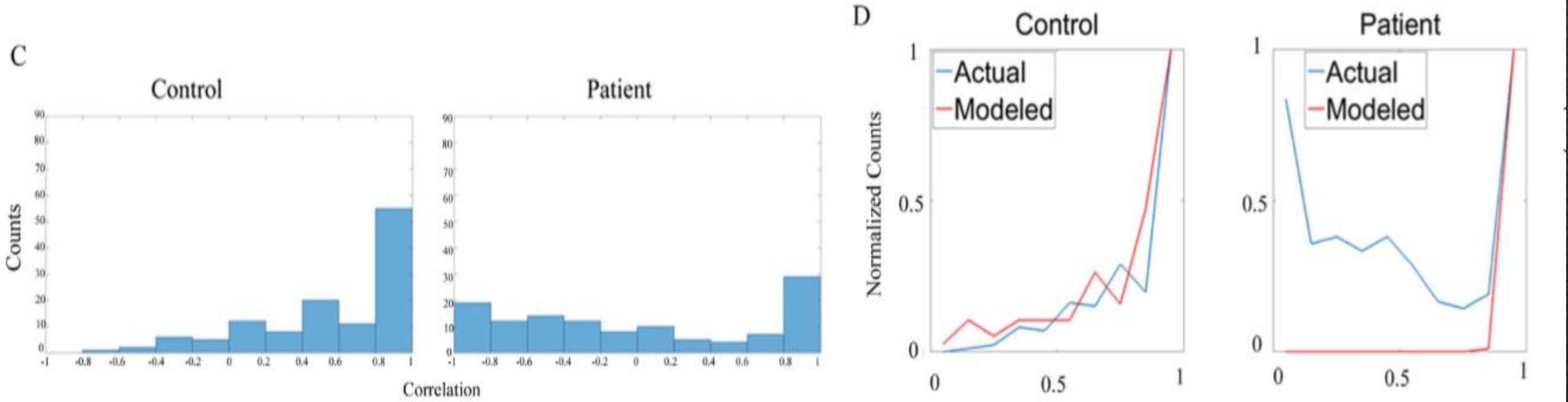


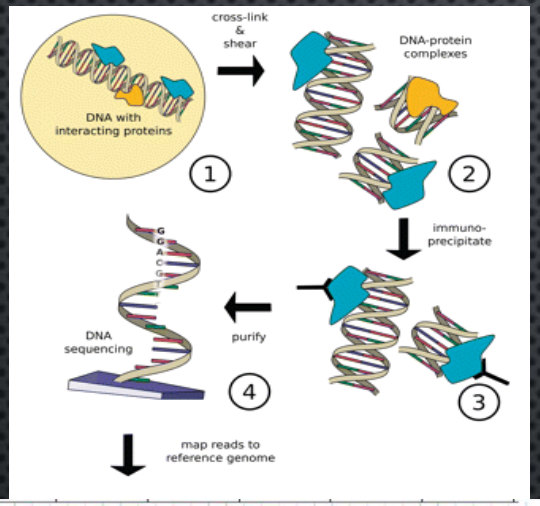
Fig. 3. A) NCCs from three control subjects and three patients were analyzed. Heatmap shows 16 dysregulated miRNA genes (all upregulated) in patient NCCs. Raw expression data were log transformed, and then centered to the median of all 6 samples. Red indicates higher value than median, green indicates, lower value than median. B) 16 dysregulated miRNAs (green) target 440 dysregulated mRNA (Blue). Each gray line indicates a connection between an miRNA and an mRNA. C) In pairwise correlation of dysregulated miRNA genes a high correlation is observed in control NCCs ($n = 3$), but not in patient NCCs ($n = 3$). D) Modeled versus actual miRNA-mRNA correlations. Red line shows correlations for miRNAs predicted based on correlations of their target mRNAs as in (Huttenhower et al., 2009). Blue line shows measured correlations between miRNAs and their target mRNAs. In control NCCs the predicted and actual correlations are similar. In patients, the patterns of predicted and actual correlations differ markedly. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

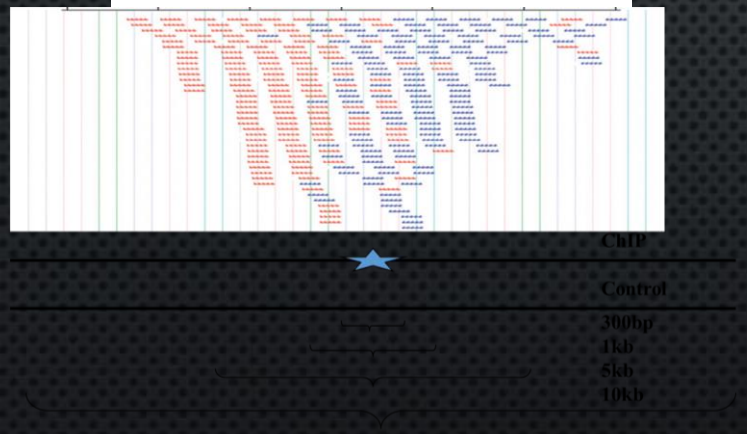
- Modeled miRNA correlation based on mRNA correlation
- Control Similar
- Patient Different

Program developed by Dr. Sarder

Insight into the whole genome !

Cross-linked Chromatin Immunoprecipitation, DNA sequencing (ChIPseq)





RNA and small RNA sequencing (RNAseq)

RNA

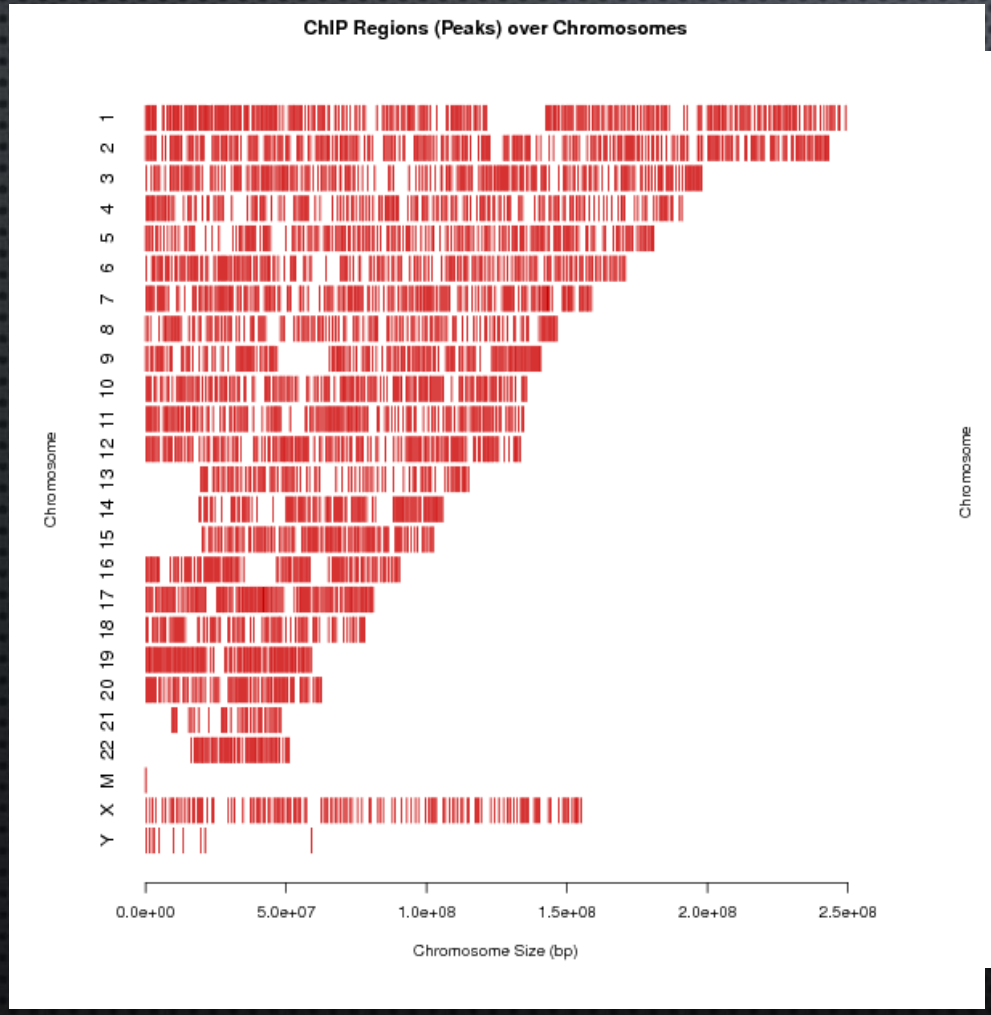
cDNA synthesis

sequencing

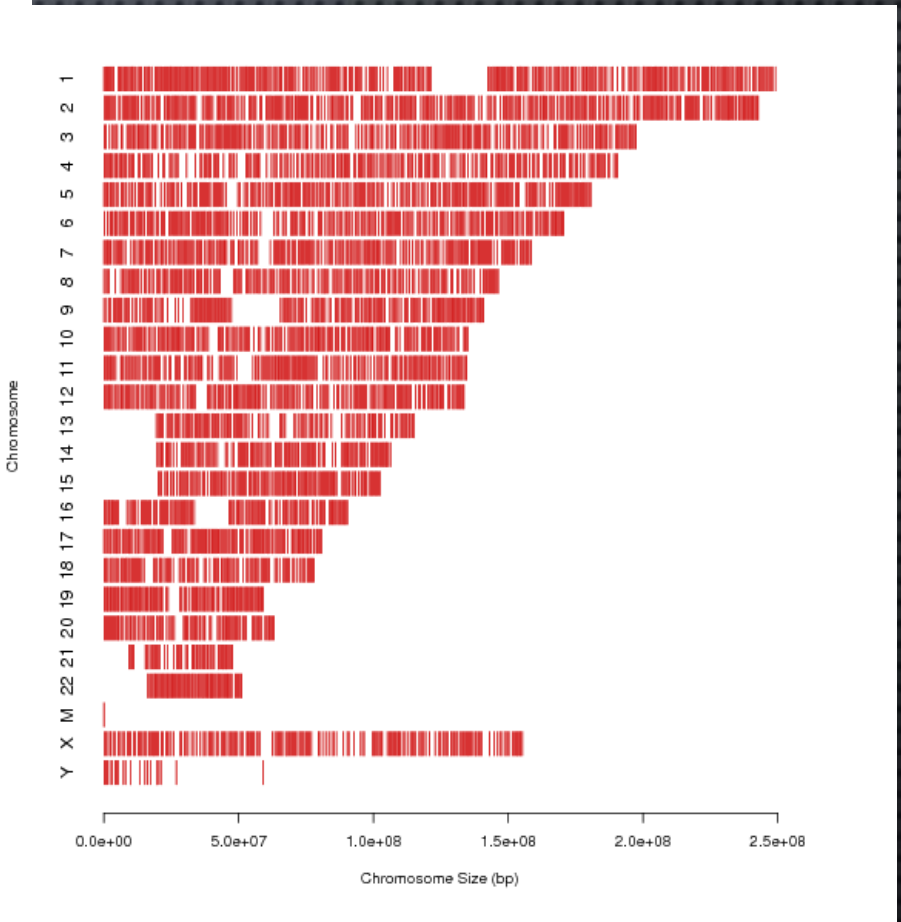
C.Terranova, S. Narla, Yu-Wei Lee, J. Bard, A. Parikh, E.K. Stachowiak, E. S. Tzanakakis, M.J. Buck, B. Birkaya, M.K. Stachowiak, Global developmental gene programing involves a nuclear form of Fibroblast Growth Factor Receptor-1 (FGFR1). 2015, *PLOS One*

SIMILAR PATTERN OF BINDING BETWEEN CONTROL AND PATIENT

C4

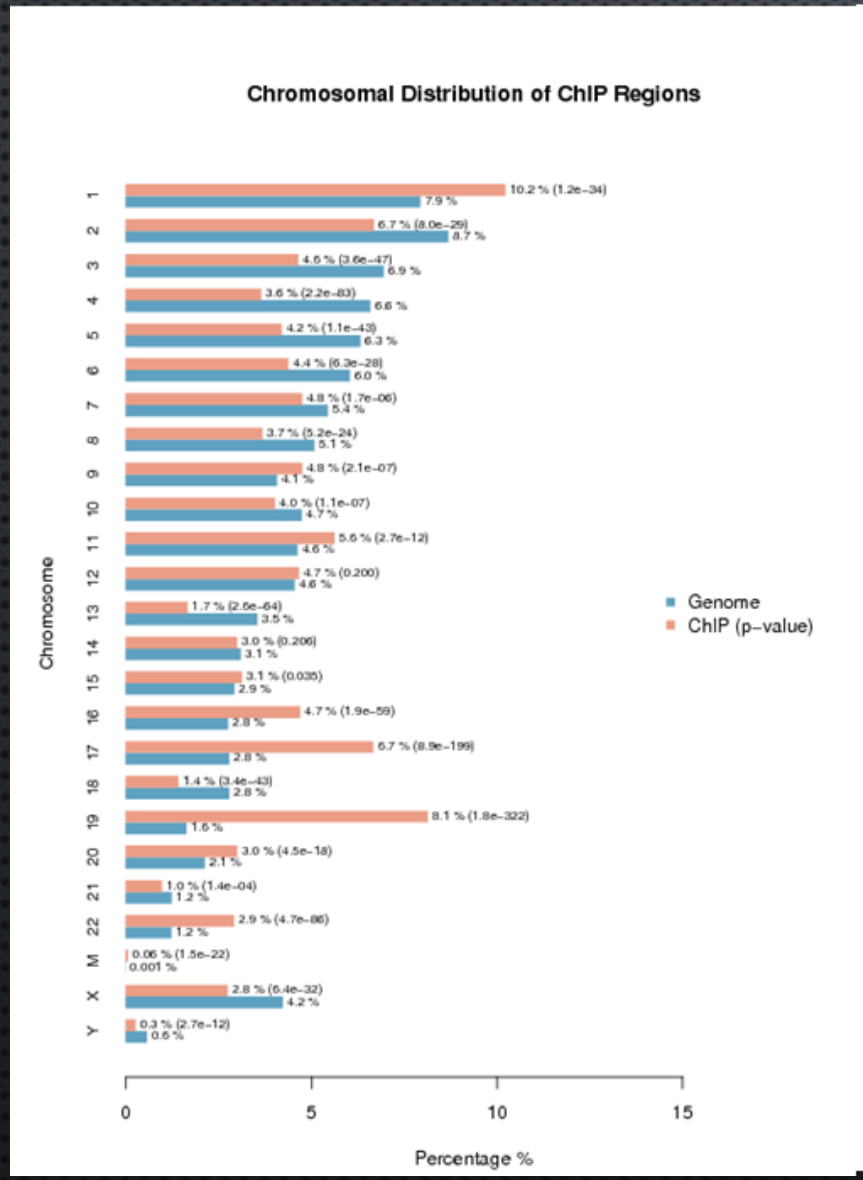


P3

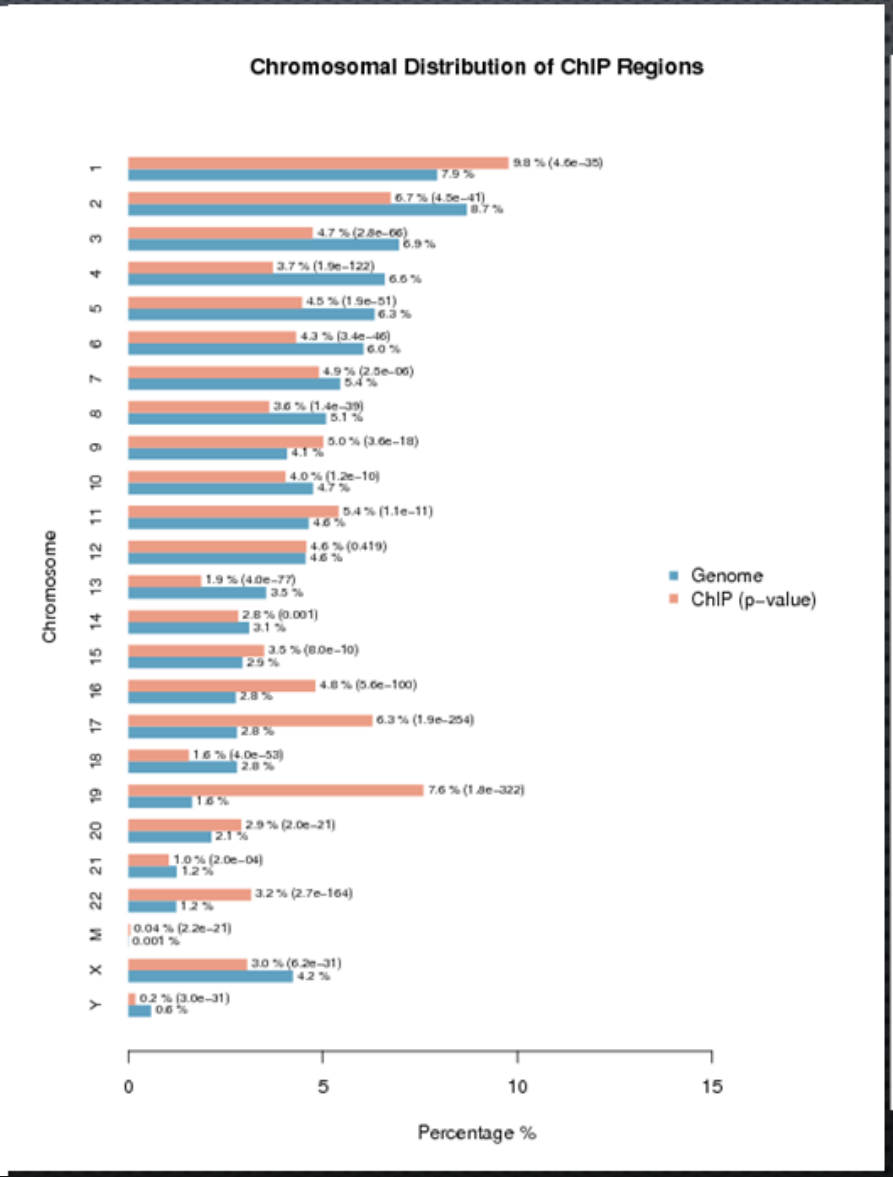


DISTRIBUTION OF PEAKS

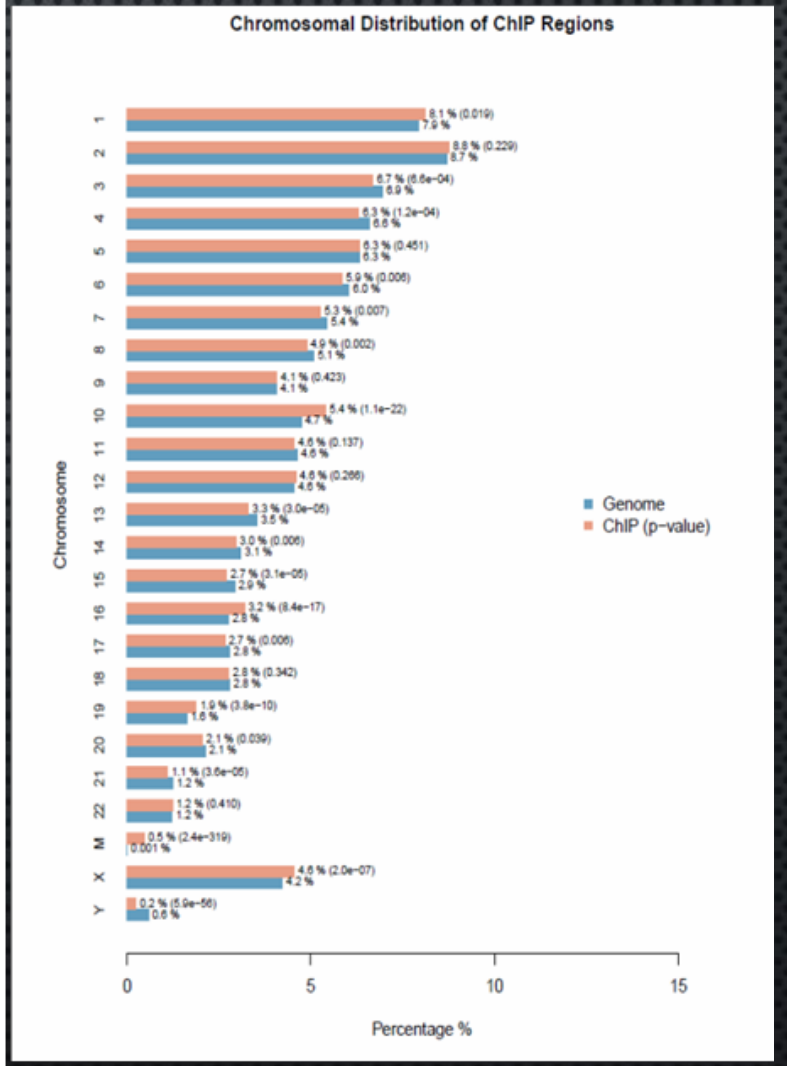
Control 4



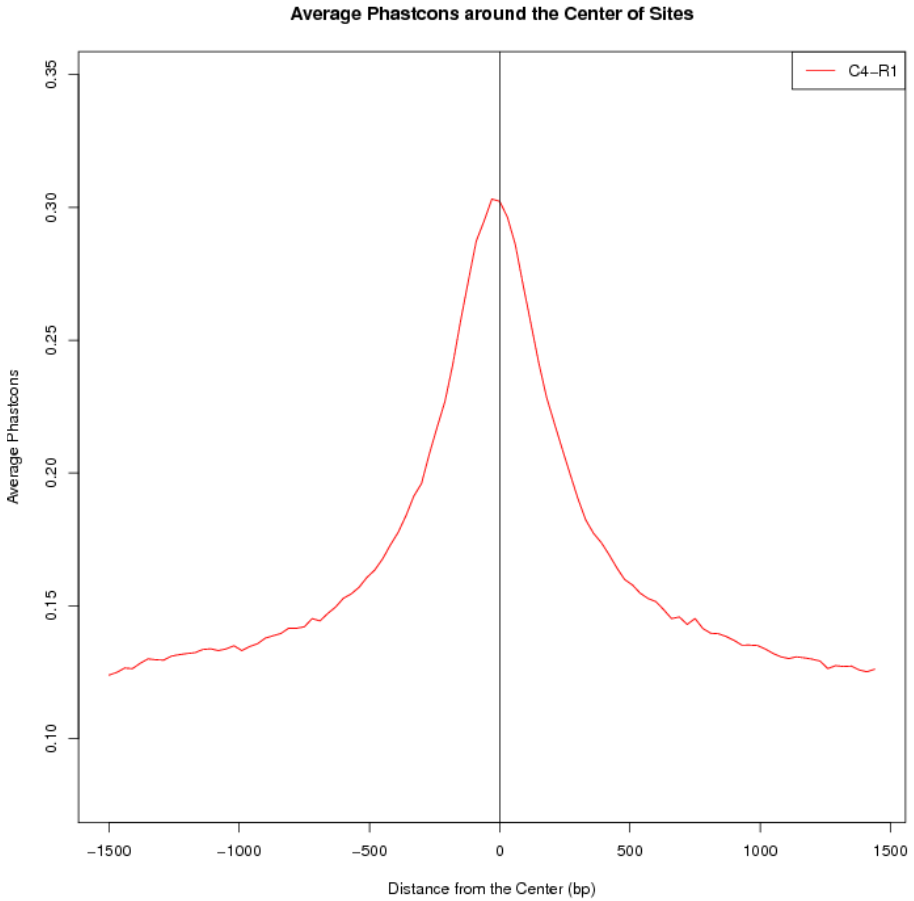
Patient 3

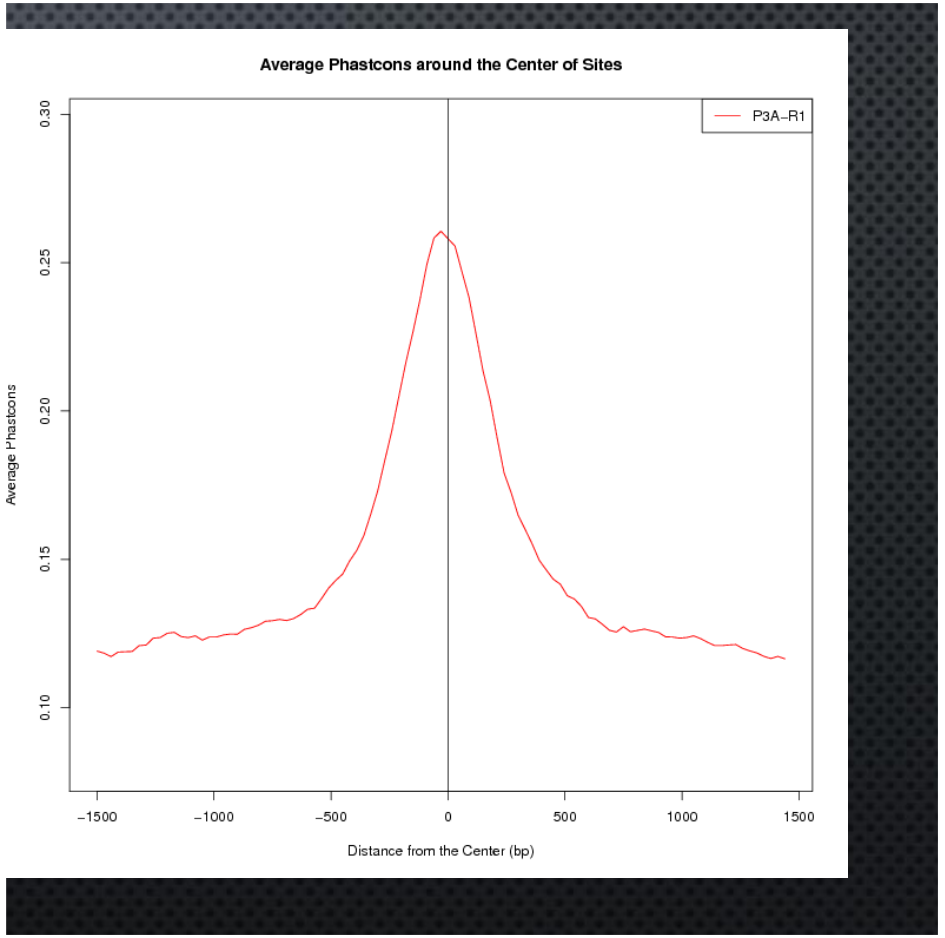


Input



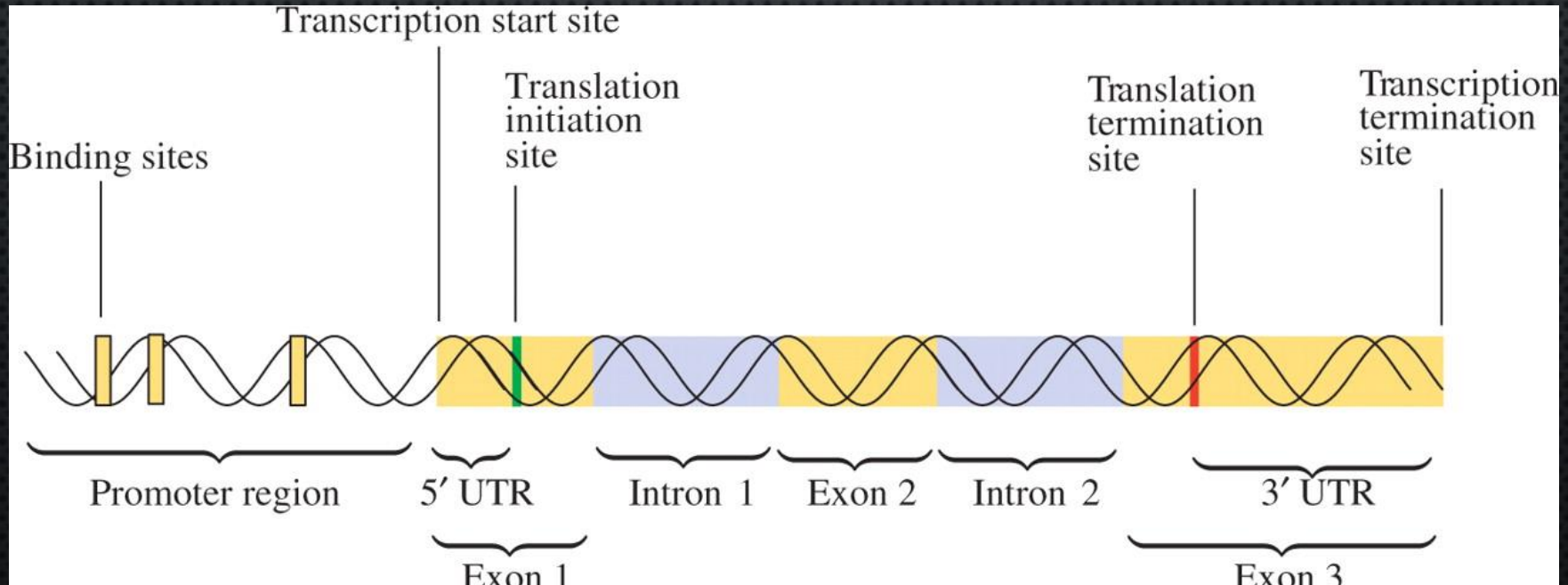
CONSERVATION PLOT





Comparison of FGFR1 peaks

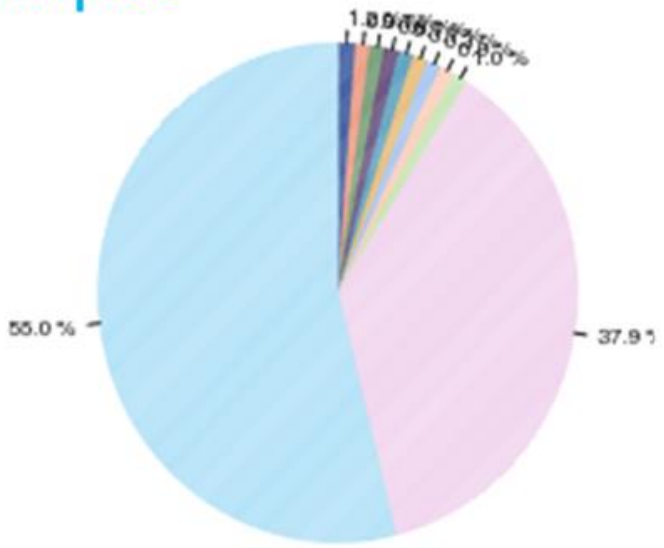
- Similar Binding locations in Control and Schizophrenia
- 80% overlap in genes



Enriched Binding of FGFR1 in Promoter and 5’ UTR

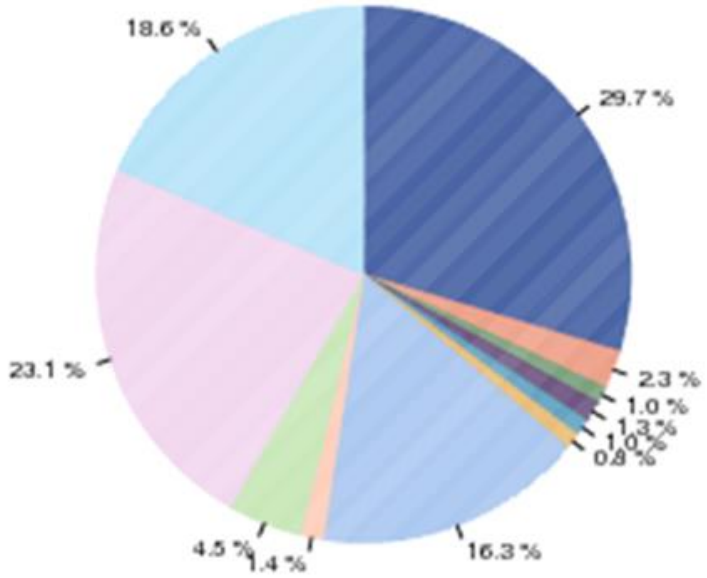
Schizophrenia – reduced 5’UTR increased intergenic nFGFR1

Input



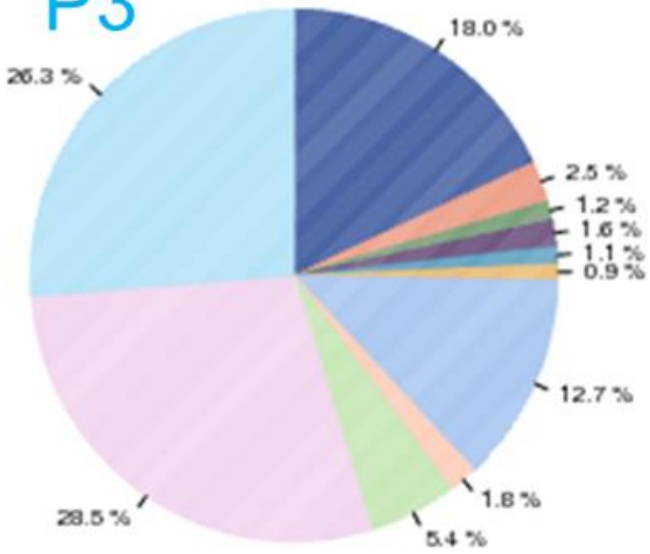
- Promoter (<=1000 bp): 1.2 %
- Promoter (1000–2000 bp): 0.9 %
- Promoter (2000–3000 bp): 0.7 %
- Downstream (<=1000 bp): 0.8 %
- Downstream (1000–2000 bp): 0.7 %
- Downstream (2000–3000 bp): 0.7 %
- 5'UTR: 0.3 %
- 3'UTR: 0.9 %
- Coding exon: 1.0 %
- Intron: 37.9 %
- Distal intergenic: 55.0 %

C4



- Promoter (<=1000 bp): 29.7 %
- Promoter (1000–2000 bp): 2.3 %
- Promoter (2000–3000 bp): 1.0 %
- Downstream (<=1000 bp): 1.3 %
- Downstream (1000–2000 bp): 1.0 %
- Downstream (2000–3000 bp): 0.8 %
- 5'UTR: 16.3 %
- 3'UTR: 1.4 %
- Coding exon: 4.5 %
- Intron: 23.1 %
- Distal intergenic: 18.6 %

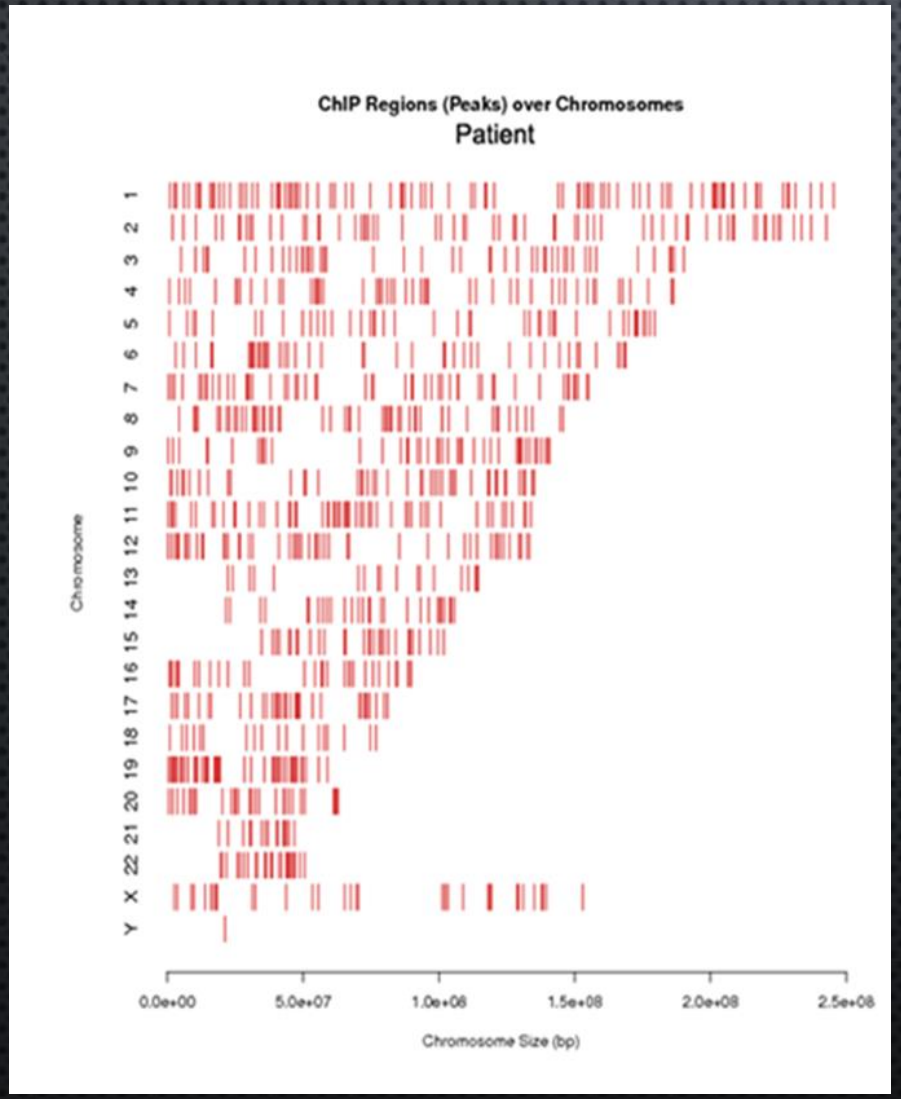
P3

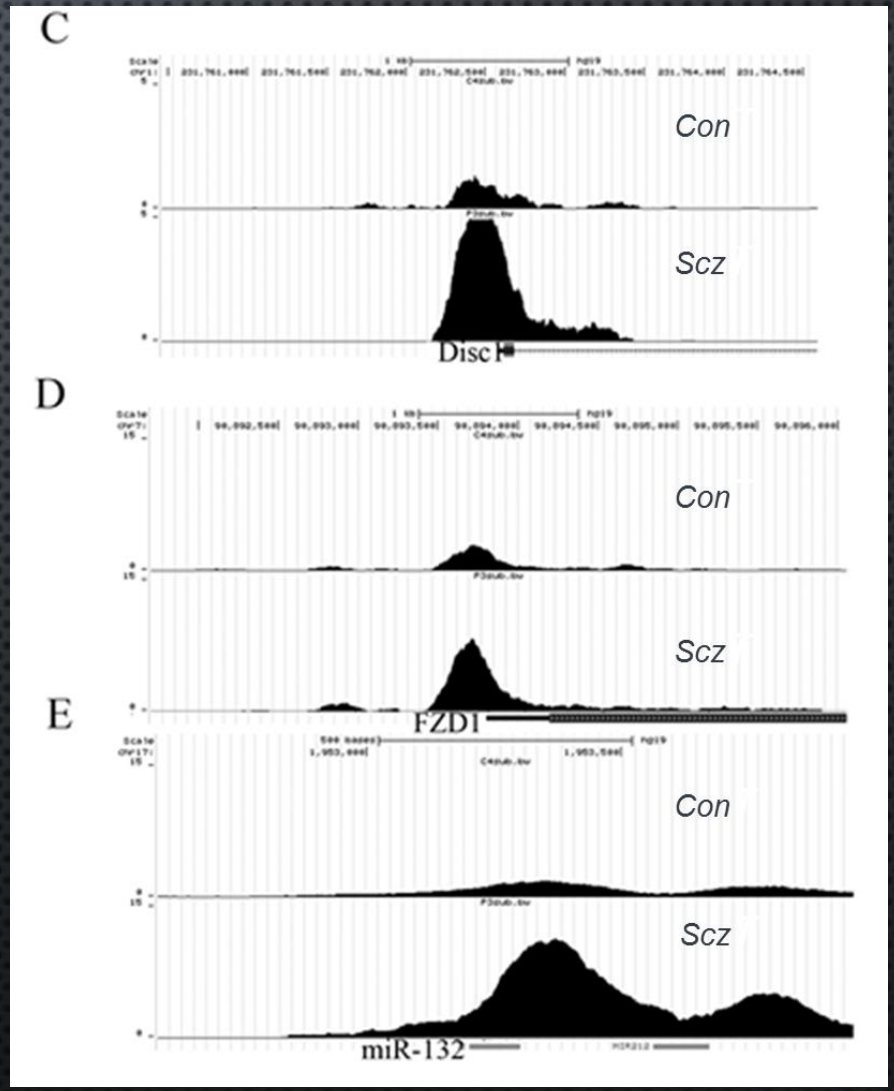


- Promoter (<=1000 bp): 18.0 %
- Promoter (1000–2000 bp): 2.5 %
- Promoter (2000–3000 bp): 1.2 %
- Downstream (<=1000 bp): 1.6 %
- Downstream (1000–2000 bp): 1.1 %
- Downstream (2000–3000 bp): 0.9 %
- 5'UTR: 12.7 %
- 3'UTR: 1.8 %
- Coding exon: 5.4 %
- Intron: 28.5 %
- Distal intergenic: 26.3 %

Global Genome Dysregulation via Panontogenic INFS

Chromatin Immunoprecipitation (ChiPseq) -nFGFR1 binds to ~85% of dysregulated genes in schizophrenia cells





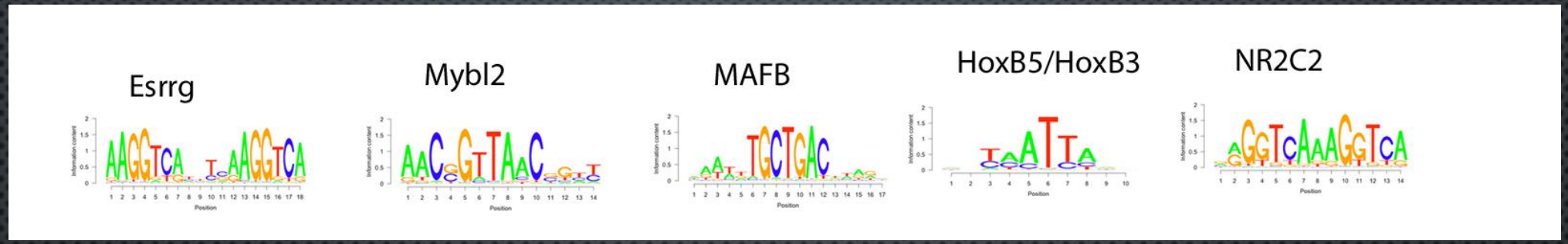
Categories of dysregulated genes targeted by nFGFR1 were identified using GO, IPA, and Reactome.

915 genes in which nFGFR1 was bound in both control and schizophrenia NCCs overrepresented the pathways involved in axon guidance, neurotransmitter release, and glial-cell differentiation, Notch signaling, and Wnt/β-catenin signaling, TP53-regulated cell cycle genes,

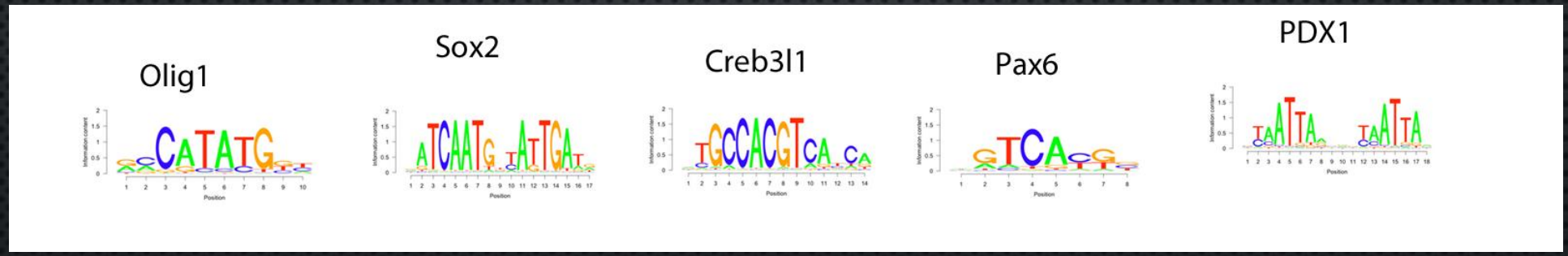
203 genes bound in patient cells but not in controls - neuron development, axon guidance, extracellular matrix, RHO GTPase pathways, p75 NTR axonogenesis pathway, MHC Class 1 Antigen processing and presentation, urea cycle.

The 203 genes, which were targeted by nFGFR1 only in schizophrenia cells, were engaged in the same functions as the remaining genes targeted by nFGFR1 in both control and patient cells

MOTIFS ENRICHING IN UPREGULATED



Motifs enriched in downregulated



NOTCH BINDING SITES MANY SHARED WITH FGFR1

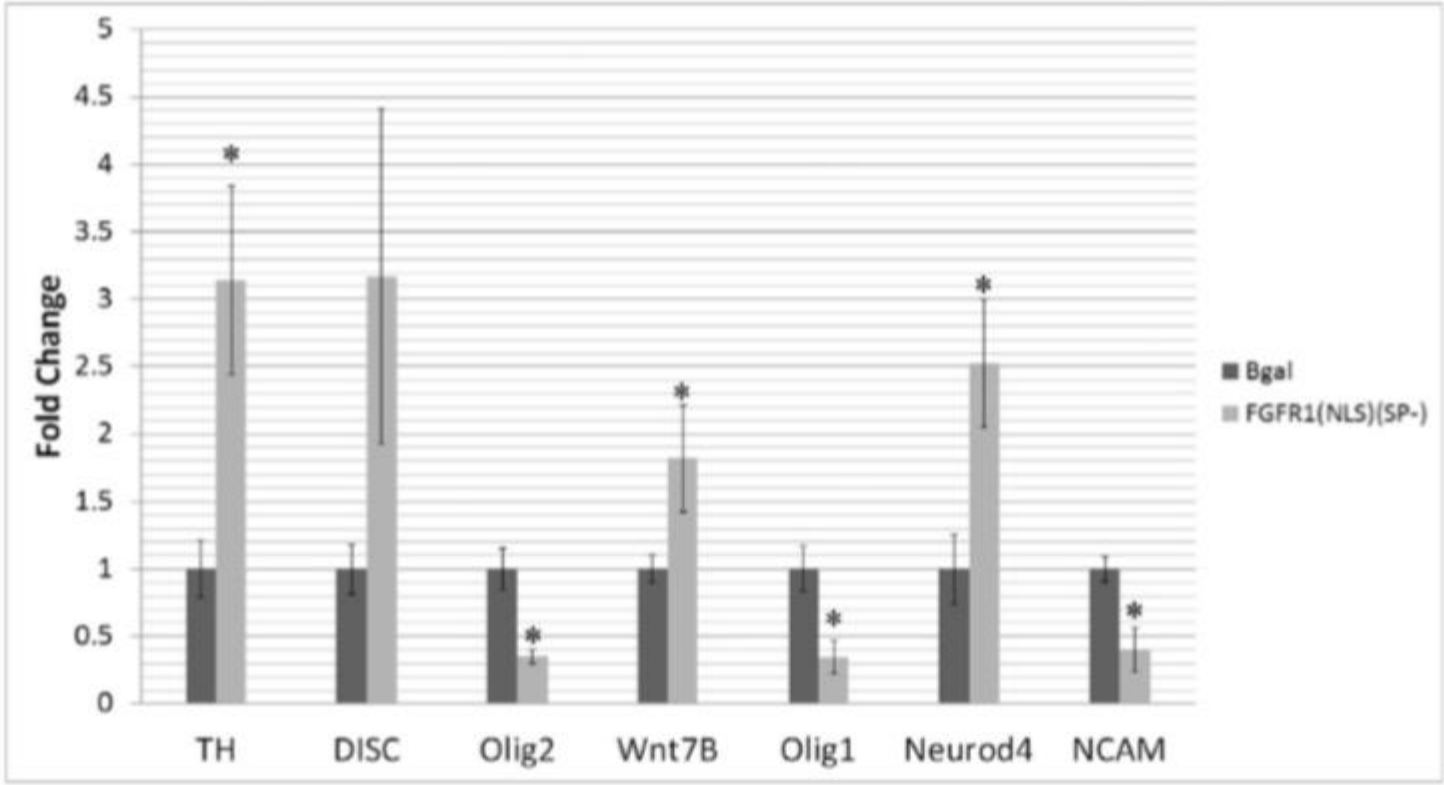


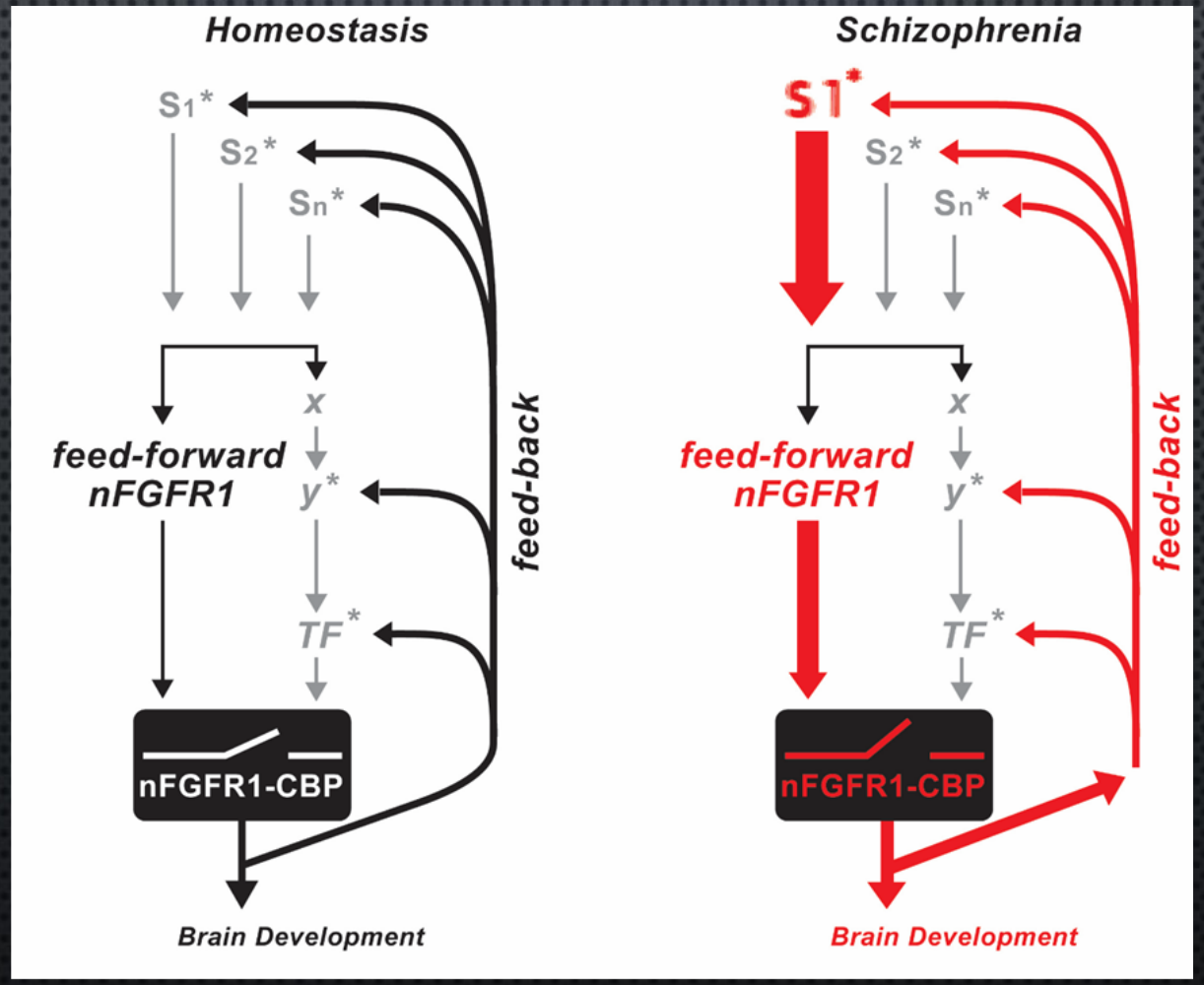
Fig. 7. Overexpression of constitutively active nuclear FGFR1 (NLS/SP-) affects expression of selected schizophrenia dysregulated genes. Human ESCs were stimulated to differentiate into NPCs and transfected with constitutively active nuclear FGFR1 (NLS/SP-) or control β-galactosidase (β-gal). Transfected NPCs were induced to commit to a neuronal lineage (NCCs) within 2 days of treatment, and specific mRNAs were analyzed by RT-qPCR. FGFR1 (NLS/SP-) transfection upregulates TH, Wnt7B, and Neurod4 mRNAs and downregulates Olig2, Olig1, and NCAM mRNAs.

SUMMARY OF GENOMIC STUDIES

1. Early (pre-neuronal) genomic etiology of schizophrenia
2. Dysregulated genes are common to multiple patients with schizophrenia (watershed mechanism)
3. Gene dysregulations are accompanied by changes in nFGFR1-genome interactions and coordinate genome deprogramming - a common mechanism in schizophrenia
4. Disasociation of miRNA> mRNA networks

HYPOTHESIS – "FEED FORWARD AND GATE" SIGNALING BY INFS DURING DEVELOPMENT – DEVELOPMENTAL DEPROGRAMMING IN SCHIZOPHRENIA

* marks signaling pathways in which schizophrenia-linked genes have been found.



Stachowiak and Stachowiak, JCPhysiol, 2016

From genes to brain: Schizophrenia untangled

U. BUFFALO (US) — An early defect in a critical neurological pathway may be responsible for the onset of schizophrenia later in life.

"INFS functions like the conductor of an orchestra …….
It doesn't matter which musician is playing the wrong note, it brings down the conductor and the whole orchestra. With INFS, we propose that when there is an alteration or mutation in a single schizophrenia-linked gene, the INFS system that controls development of the whole brain becomes un-tuned. That's how schizophrenia develops…"