Identification of potential immune targets in controlling Endometrioid Endometrial Carcinoma metastatic progression

a “Sample to Insight” biological exploration

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PLOS ONE

Integrated microRNA and mRNA Transcriptome Sequencing Reveals the Potential Roles of miRNAs in Stage I Endometrioid Endometrial Carcinoma

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Sample to Insight approach: Exploring the Transcriptome in EEC

Using QIAGEN Bioinformatics solutions, three immune proteins were identified as potential therapeutic targets in tumor progression in EEC

We were able to highlight important parameters and to compare transcriptomes of 3 early stage patients:

- ✓ Compared and determined which and how signaling cascades are involved in the 3 patients (EIF2 signaling, ILK signaling, Integrin Signaling)
- ✓ Highlighted which transcriptional program is turned on in these patients (SPDEF, and PPARGC1a, PPARGC1b, SBREF2)
- ✓ Understand which biological processes differ between these 3 patients (cell migration and cell invasion).
- ✓ Identified some splicing variants of importance in the EEC tumor progression (IGTB1, VCAN)
- ✓ Propose new hypotheses that visualize which immune components could be targeted to inhibit cell invasion, EMT and metastasis processes.

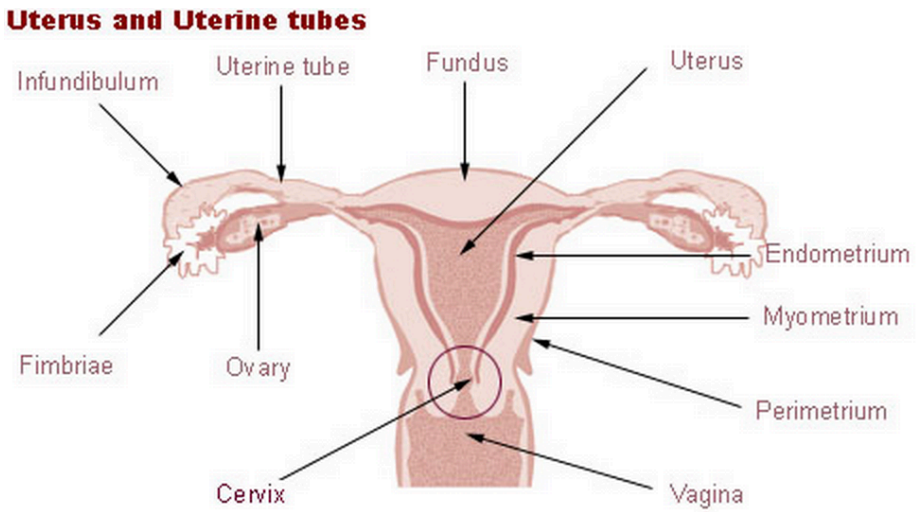
FDA approval of antibodies targeting immune checkpoints

- 2011 Ipilimumab (BMS) - Melanoma
- 2014 Pembrolizimab (Merck) – Melanoma
- 2014 Nivolumab (BMS) – Melanoma
- 2015 Nivolumab (BMS) – Lung
- 2015 Ipilimumab + Nivolumab (BMS) – Melanoma
- 2015 Pembrolizumab (Merck) – Lung
- 2015 Ipilimumab (BMS) – Adjuvant melanoma
- 2015 Nivolumab (BMS) – Renal cell carcinoma

OUTLINE of the presentation

- Endometrioid Endometrial Carcinoma (EEC)
  - ✓ Endometrium: Structure and Function
  - ✓ Background Endometrial Carcinoma and EEC
  - ✓ Description of the study used
- Introduction to QIAGEN Sample to Insight
- Data Analysis
  - ✓ Expression Profile from RNA-seq
- Biological Interpretation of early stage EEC (3 patients)
  - ✓ Analysis of the transcriptome (mRNA profile)
  - ✓ Highlight hypotheses to inhibit tumor progression
- Conclusion

Endometrium: Structure and Function



- Glandular epithelial layer: 3 histologically distinct layers:
  - stratum basalis (deepest layer)
  - stratum spongiosum (intermediate layer)
  - stratum compactum (thin, most superficial layer)
  - spongiosium and compactum = stratum functionalis
- Undergoes cellular and histological changes in the different phases of the cycle and during embryo implantation.
- Functional layer undergoes cyclical growth and tissue remodeling throughout the reproductive years.
- Process regulated by ovarian steroids (estrogen, progesterone), cytokines and growth factors.
- Tissue remodeling shares features with the repair of mucosal injury, characterized by a migratory phenotype with specialized cytoskeletal and matrix-receptor reorganizations and specialized matrix-dependent signaling patterns

Background on Endometrial Carcinoma and EEC

- Common cause of gynecological cancer death
  - ✓ The most common gynecological malignancy in Europe and North America

- Different types and Importance:
  - ✓ Most common type is **Endometrioid Endometrial Adenocarcinoma (EEC) this study**

- Others:
  - ✓ Endometrioid (75%) (secretory, ciliated, papillary or villoglandular), composed of malignant glandular epithelial elements
  - ✓ Adenocarcinoma with squamous differentiation
  - ✓ Adenoacanthoma (benign squamous component)
  - ✓ Adenosquamous (malignant squamous component)
  - ✓ Uterine papillary serous (5%–10%)
  - ✓ Clear cell (1%–5%)
  - ✓ Malignant mixed Mullerian tumours or carcinosarcomas (1–2%)
  - ✓ Uterine sarcomas (leiomyosarcoma, endometrial stromal sarcoma, undifferentiated) (3%)
  - ✓ Mucinous (1%)
  - ✓ Undifferentiated

Background of Endometrioid Endometrial Carcinoma

Summary of EEC

- Estrogen-dependent tumors
- Preceded by hyperplasia, atypical hyperplasia, and endometrial intraepithelial neoplasia, a premalignant outgrowth from hormonally-induced, benign endometrial hyperplasia
- At diagnosis, 75% of women have disease confined to the uterus (early, stage I). Five-year survival for stage I patients is 80-90%, however, about 10–20% develop metastasis
- Most EECs are low-grade tumors (G1 or G2, comprised of moderately to well-differentiated cells) that are early stage (i.e. before extra-uterine spread)
- Risk Factors:
  - ✓ Menopause, but up to 25% of cases premenopausal
  - ✓ Obesity
    - ✓ Estimates are that 50% of endometrial cancer cases in USA attributable to excess adiposity (World Cancer Research Fund 2009, 2013)
    - ✓ Impact of excess body fat on endometrial cancer risk is much greater than it is on breast cancer, likely because circulating estrogens play a much larger role in endometrial cancer development
  - ✓ Nulliparity (having borne no children)
  - ✓ Diabetes mellitus
  - ✓ Prolonged, unopposed estrogen exposure in post-menopause
  - ✓ Tamoxifen and oral contraceptive pills
- Patients are generally treated with surgery, radiation, chemotherapy or hormone therapy

Description of this study and the methods used by the original authors

Material & Methods: Data deposited in the NIH Short Read Archive database (SRP045645)

- Three women diagnosed with Stage I EEC: Two Stage IA and one Stage IB (all Grade 1)

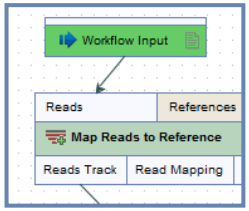
- Sequencing:

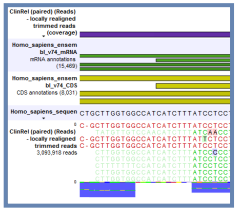
  - ✓ mRNA (100 bp paired-end reads): Illumina HiSeq 2000 of tumor and adjacent normal tissue

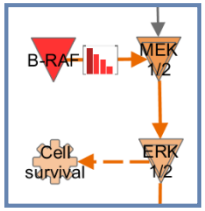
  - ✓ mRNA libraries, an average of 52,716,765 pair-end 100 bp clean , 40X depth

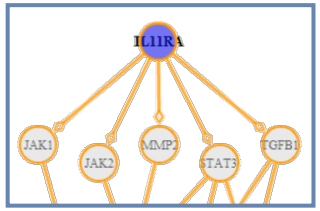
QIAGEN Sample to Insight

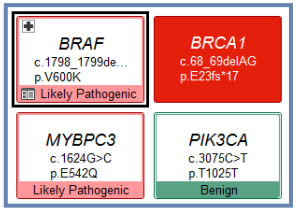
Sample Isolation	Targeted Enrichment	Library Construction	NGS Run	Data Analysis	Interpretation
GeneRead DNA FFPE	GeneRead DNAseq Targeted Panels V2	GeneRead Library Prep Kits	MiSeq®	**Biomedical Genomics Workbench**	**Ingenuity Variant Analysis**
QIAamp FFPE	GeneRead DNAseq Custom Panels V2	GeneRead Library Quant System	NextSeq®	**CLC Genomics Workbench**	**Ingenuity Pathway Analysis (IPA)**
GeneRead REPLI-g single cell	GeneRead Pure mRNA	GeneRead Size Selection Kit	HiSeq®	**CLC Main Workbench**	**HGMD**
GeneRead QuantiMIZE	GeneRead rRNA Depletion		Ion PGM		**QIAGEN Clinical Insight**
QIAamp Circulating Nucleic Acid			Ion Proton		**TRANSFAC**











Sample to Insight: Analyses of EEC tissues

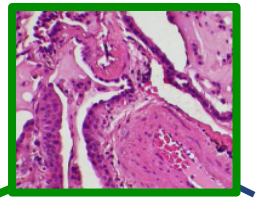
Tumorous vs. adjacent Non-Tumorous tissue after surgical resection

Total RNA extraction

Sequencing

Data Analysis

Interpretation



RNA

DNA

Bx

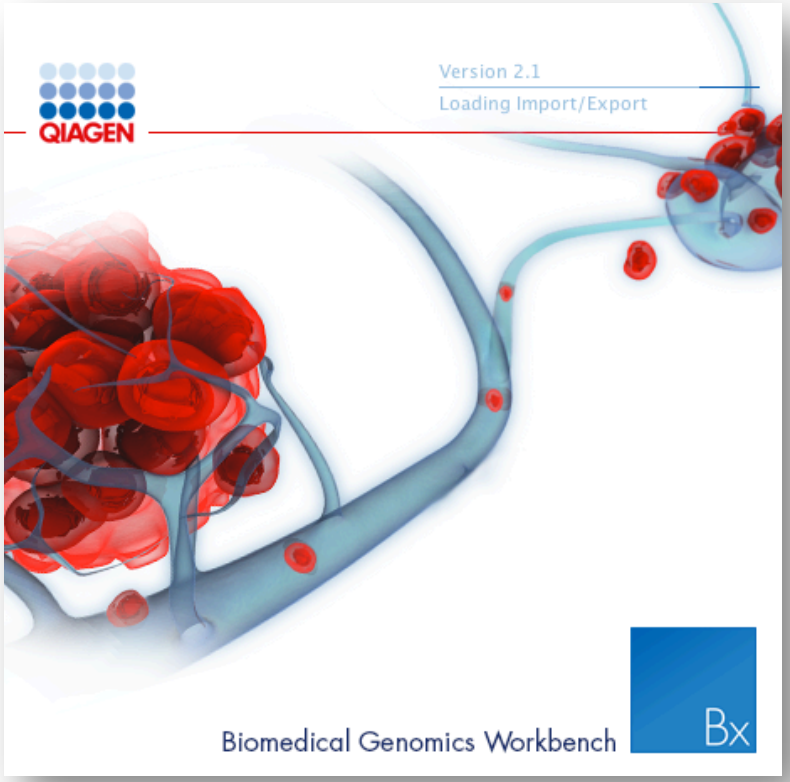
RNA-seq

Variants

INGENUITY PATHWAY ANALYSIS

INGENUITY VARIANT ANALYSIS

Fast and Easy Analysis

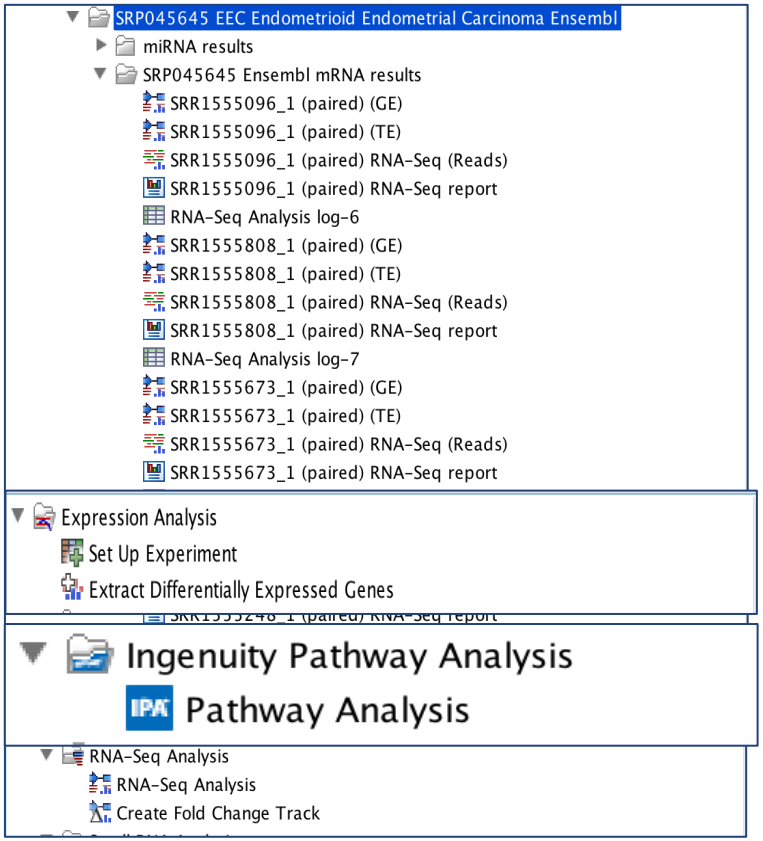


- **Accurate and trustworthy results**
  - ✓ Whole Genome Sequencing
  - ✓ Whole Exome Sequencing
  - ✓ Targeted or Whole Transcriptome Sequencing
  - ✓ ChIP-Seq data
- **Intuitive and easy-in-use**
  - ✓ Comprehensive end-to-end analysis workflows for single samples or cohort studies
  - ✓ One-click analysis of QIAGEN GeneRead DNASeq Amplicon Panels
  - ✓ Streamlined integration with Ingenuity Pathway Analysis (IPA) & Ingenuity Variant Analysis
- **Flexible & customizable**
  - ✓ Ready-to-use workflows can be customized
  - ✓ Build your own workflows

BX to IPA: Expression Profile from RNA-seq

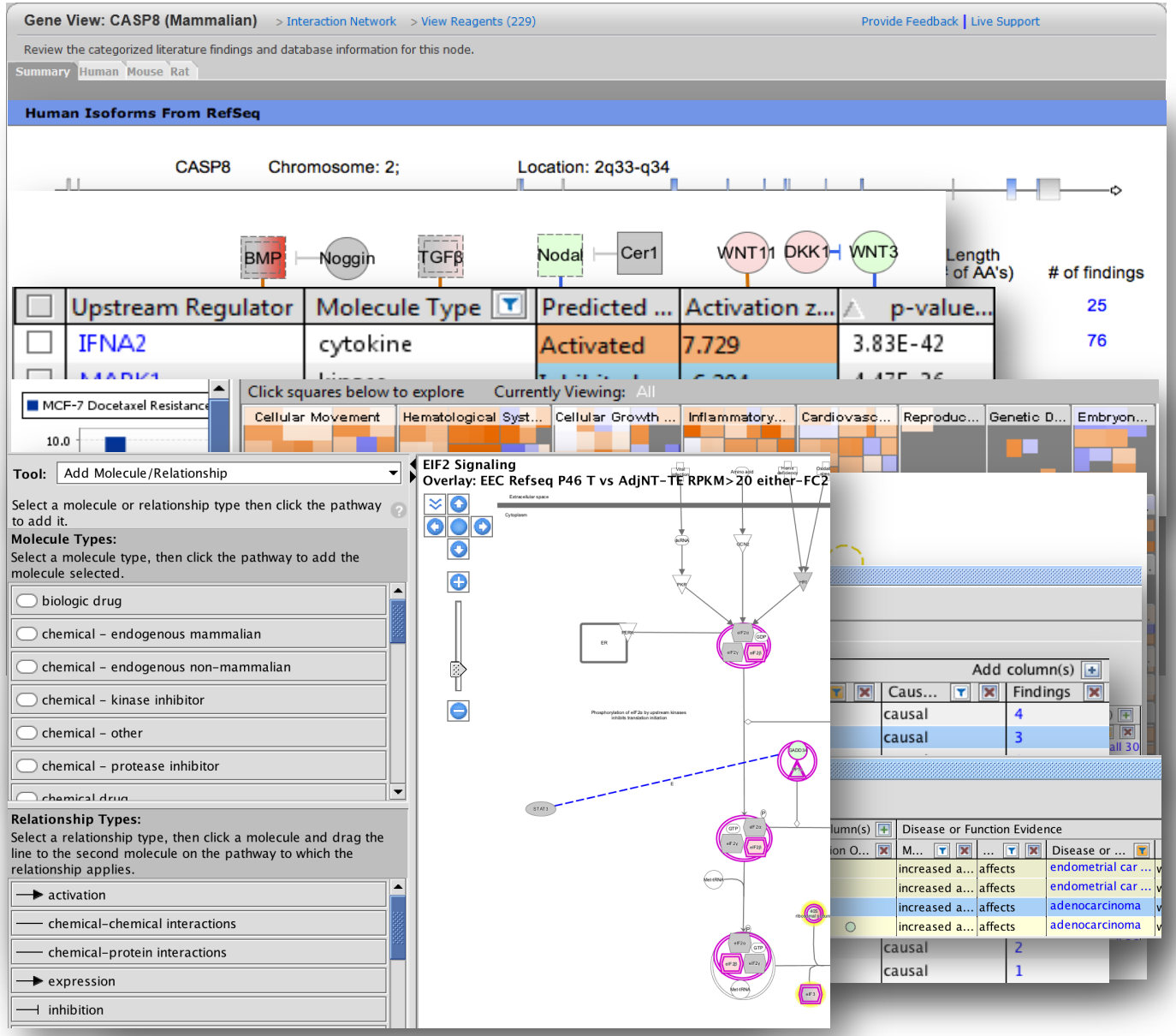
From sequencing to Alignment, Quantitation, Differential Expression

- Selection of Dataset (SR045645)
- Download FASTQ from SRA (convert .sra to FASTQ)
- Import the FASTQ files into BX
- Set up the RNA-seq analysis in BX:
  - **mRNA**
    - Select Reference Genome (human Ensembl or Refseq)
    - Select Mapping options
    - Select Expression Level Options
- Set up the experiment at transcript level (TE): Tumor vs. Adjacent Non-Tumor
- Filter out non-significant transcripts and send dataset to IPA using Plugin from BXWB (Fold Change, p-value, FDR)
- Analyze the processed dataset in IPA (mRNAs)
  - **Dataset**: isoforms with >20 RPKM in either tumor or adjacent non-tumor, |fold change|>1, p<0.05
  - **Analysis**: mRNAs with |fold change|>2 and p<0.05, MicroRNA Target Filter (microRNAs)



Introduction to Ingenuity Pathway Analysis (IPA), Interpretation

- **Gene View, Chem View, and Disease/Function View**
- **Human and Mouse Isoform Views**
- **Canonical Pathways/Molecule Activity Predictor**
- **Upstream Analysis**
  Upstream Regulators/Mechanistic Network/Causal Networks
- **Diseases & Functions**
  Downstream Effects Analysis
- **Regulator Effects**
- **microRNA Target Filter**
- **BioProfiler**
- **IsoProfiler**
- **Interaction Networks, Build and Overlay tools**
- **My Findings**



Three Patients Early Stage EEC (IA/IB), Grade 1

- Understanding the transcriptome in Early Stage EEC

  - ✓ What are the signaling or metabolic pathways involved, are they activated/inhibited? (Canonical Pathways)
  - ✓ What are the underlying transcriptional programs? (Upstream Analysis)
  - ✓ What biological processes are involved and in what way? (Diseases & Functions)
  - ✓ Are there any splicing variants of interest and how regulated are they? (Isoform View/IsoProfiler)
  - ✓ What hypotheses can be drawn further? (Mechanistic Network, Causal Network, Regulator Effects)

Summary of Core Analysis of one of the patients (P47)

EEC Ensembl81 Tr P47 T vs AdjNT-TE RPKM>20 either-FC2-11-04-15

Summary | Canonical Pathways | Upstream Analysis | Diseases & Functions | Regulator Effects | Networks | Lists | My Pathways | Molecules

Export :

Analysis Settings

Top Canonical Pathways

Name	p-value	Overlap
EIF2 Signaling	1.53E-33	28.6 % 53/185
Regulation of eIF4 and p70S6K Signaling	1.91E-13	19.2 % 28/146
Antigen Presentation Pathway	6.13E-13	40.5 % 15/37
mTOR Signaling	3.85E-12	16.0 % 30/188
Agranulocyte Adhesion and Diapedesis	4.42E-12	15.9 % 30/189

1 2 3 4 5 6 7 8 9 >

Top Upstream Regulators

Upstream Regulators

Name	p-value of overlap	Predicted Activation
MYCN	3.25E-60	
TGFB1	9.72E-60	
MYC	3.22E-57	Activated
beta-estradiol	1.57E-51	
dexamethasone	1.93E-51	

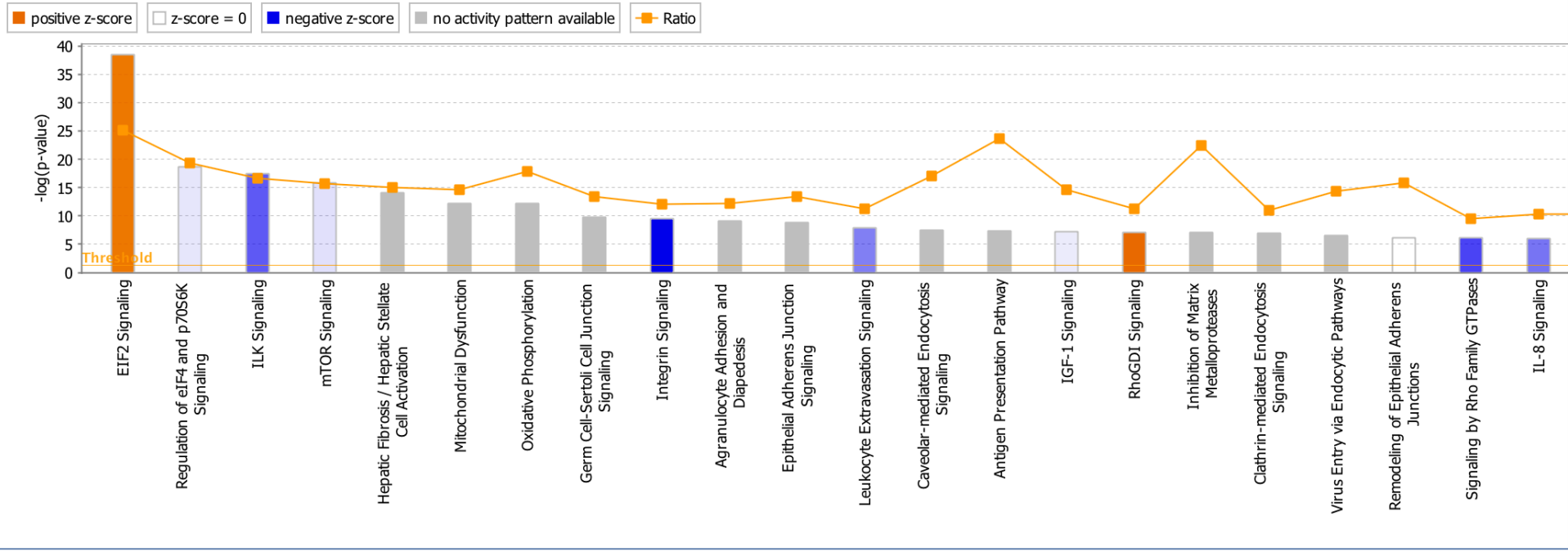
1 2 3 4 5 6 7 8 9 >

Causal Networks

Name	p-value of overlap	Predicted Activation
GRP	1.87E-78	
miR-145-5p (and other miRNAs w/seed UCCAGUU)	4.60E-77	
KLK14	2.06E-76	
TRPS1	3.71E-76	

Canonical Pathways of Patient P32

Canonical Pathways involved in tumor progression (both signaling and metabolic)

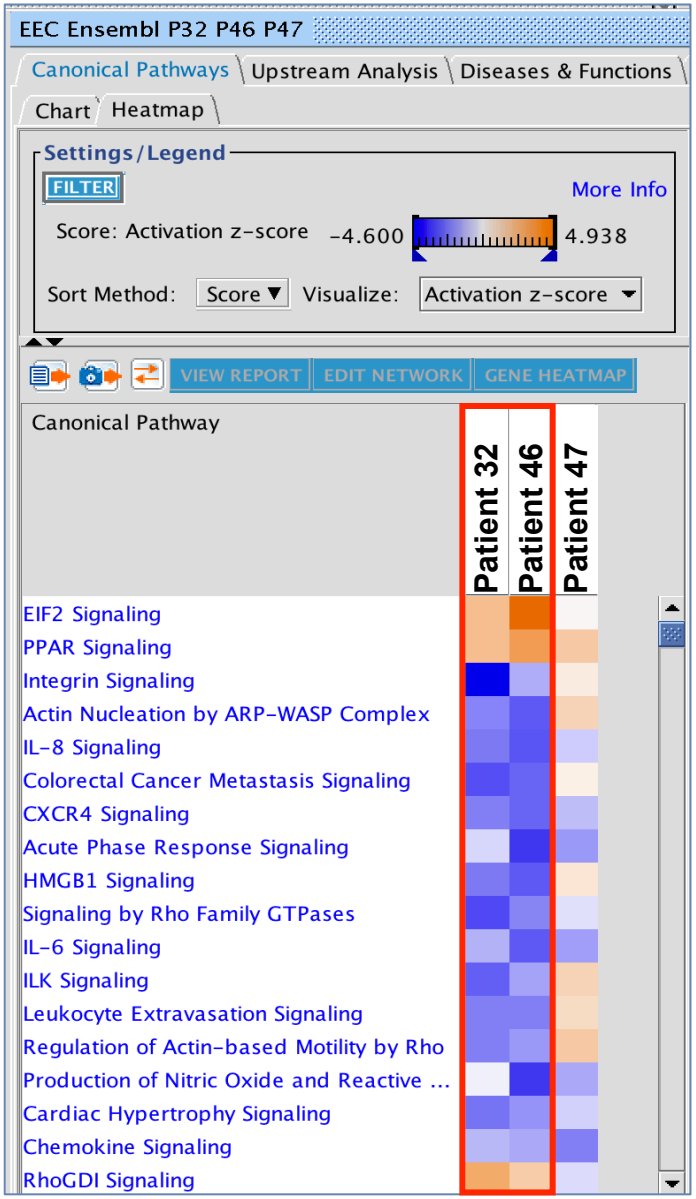


Pathway Activity Analysis:

- ✓ Proliferation pathway (EIF2 signaling) is activated (orange)
- ✓ Cell movement/motility (ILK signaling, Integrin Signaling) are inhibited (blue)

Comparison of Canonical Pathways in the 3 patients P32, P46, P47

- The patients' mRNA expression data indicates activation and inhibition of many of the same Canonical Pathways, involved in tumorigenesis:
  - ✓ Proliferation (EIF2 signaling)
  - ✓ Cell movement (Integrin signaling, ILK signaling, Actin nucleation by ARP-WASP Complex, Signaling by Rho family GTPases, …)
  - ✓ Metabolic pathways (PPAR signaling)
- However two of the three are more alike than the other based on activity pattern:
  - ✓ P32 and P46 are likely Stage IA
  - ✓ P47 is likely Stage IB



Upstream Analysis of Patient 46

Typical Transcriptional Program in tumor progression (early stage)

P46

Summary | Canonical Pathways | Upstream Analysis | Diseases & Functions | Regulator Effects | Networks | Lists | Molecules

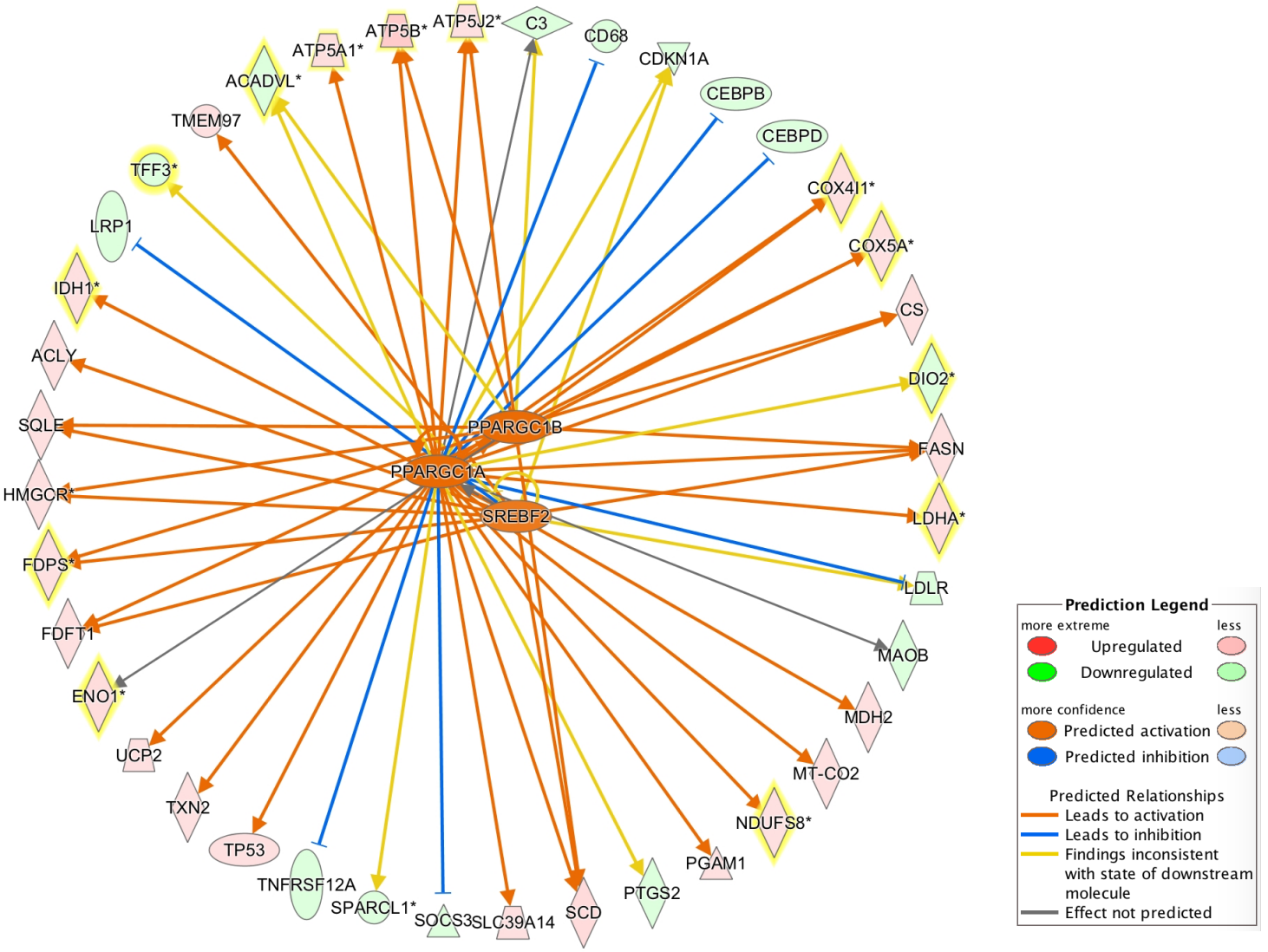
Upstream Regulators | Causal Networks

ADD TO MY PATHWAY | ADD TO MY LIST | DISPLAY AS NETWORK | CUSTOMIZE TABLE | SHOW GRAPHS | MECHANISTIC NETWORKS

Upstream Reg...	Exp Fold ...	Molecule ...	Predicted Acti...	Activation ...	p-value of ov...	Target mo...	Mechanist...
MYCN		transcription regula...	Activated	6.672	1.75E-60	ACTB, ...all 101	317 (7)
MYC	↑6.919	transcription regula...	Activated	6.585	1.92E-85	ACSL4, ...all 240	735 (18)
SMAD7		transcription regula...	Activated	3.954	4.05E-17	ACTA2, ...all 37	416 (19)
PPARGC1A		transcription regula...	Activated	3.390	3.72E-06	ACADVL, ...all 30	620 (21)
SPDEF	↓-1.224	transcription regula...	Activated	3.018	3.12E-13	BIRC3, ...all 24	257 (7)
ZFP36	↓-24.409	transcription regula...	Activated	2.920	4.53E-05	BIRC3, C... ...all 9	310 (12)
GFI1		transcription regula...	Activated	2.894	2.90E-07	BCL3, C... ...all 20	367 (13)
AHR	↓-2.049	ligand-dependent ...	Activated	2.886	1.21E-17	A2M, A... ...all 63	673 (20)
IL1RN		cytokine	Activated	2.809	2.05E-05	ACTA2, ...all 20	445 (15)
SATB1		transcription regula...	Activated	2.750	9.66E-07	ACTG1, ...all 27	
GLIS2		transcription regula...	Activated	2.646	3.90E-05	C3, CTGF, ...all 7	311 (13)
FAS		transmembrane re...	Activated	2.596	5.56E-16	ACTA2, ...all 65	379 (13)
KLF2	↓-13.476	transcription regula...	Activated	2.522	3.14E-12	ADM, B... ...all 33	531 (22)
PPARGC1B		transcription regula...	Activated	2.451	2.53E-07	ACADVL, ...all 14	540 (14)
GMNN	↑6.549	transcription regula...	Activated	2.333	5.18E-03	APCDD1, ...all 11	522 (12)
SOX1		transcription regula...	Activated	2.333	2.48E-02	APCDD1, ...all 9	
SOX3		transcription regula...	Activated	2.333	3.61E-02	APCDD1, ...all 9	
RBPJ		transcription regula...	Activated	2.284	1.98E-02	CD44, ...all 14	
DLX2		transcription regula...	Activated	2.213	1.19E-03	CDKN1A, ...all 5	
CUX1		transcription regula...	Activated	2.200	3.99E-02	CDKN1A, ...all 5	
RCAN1		transcription regula...	Activated	2.176	1.30E-02	CDK4, F3, ...all 5	
HDAC5		transcription regula...	Activated	2.158	1.28E-06	ACTA2, ...all 14	568 (25)
JAG2		growth factor	Activated	2.137	8.83E-06	BCL6, C3 ...all 11	
TBX2		transcription regula...	Activated	2.132	6.82E-02	ATF3, BH... ...all 8	
TAF4		transcription regula...	Activated	2.131	3.28E-08	AREG, ...all 21	
NAB2		transcription regula...	Activated	2.078	2.17E-07	ALOX5AP, ...all 9	313 (11)
WISP2	↓-793.966	growth factor	Activated	2.070	1.01E-10	ACTA2, ...all 18	496 (16)
IKZF1		transcription regula...	Activated	2.064	2.78E-03	ACP1, ...all 16	

Network of three selected transcription regulators in Patient 46

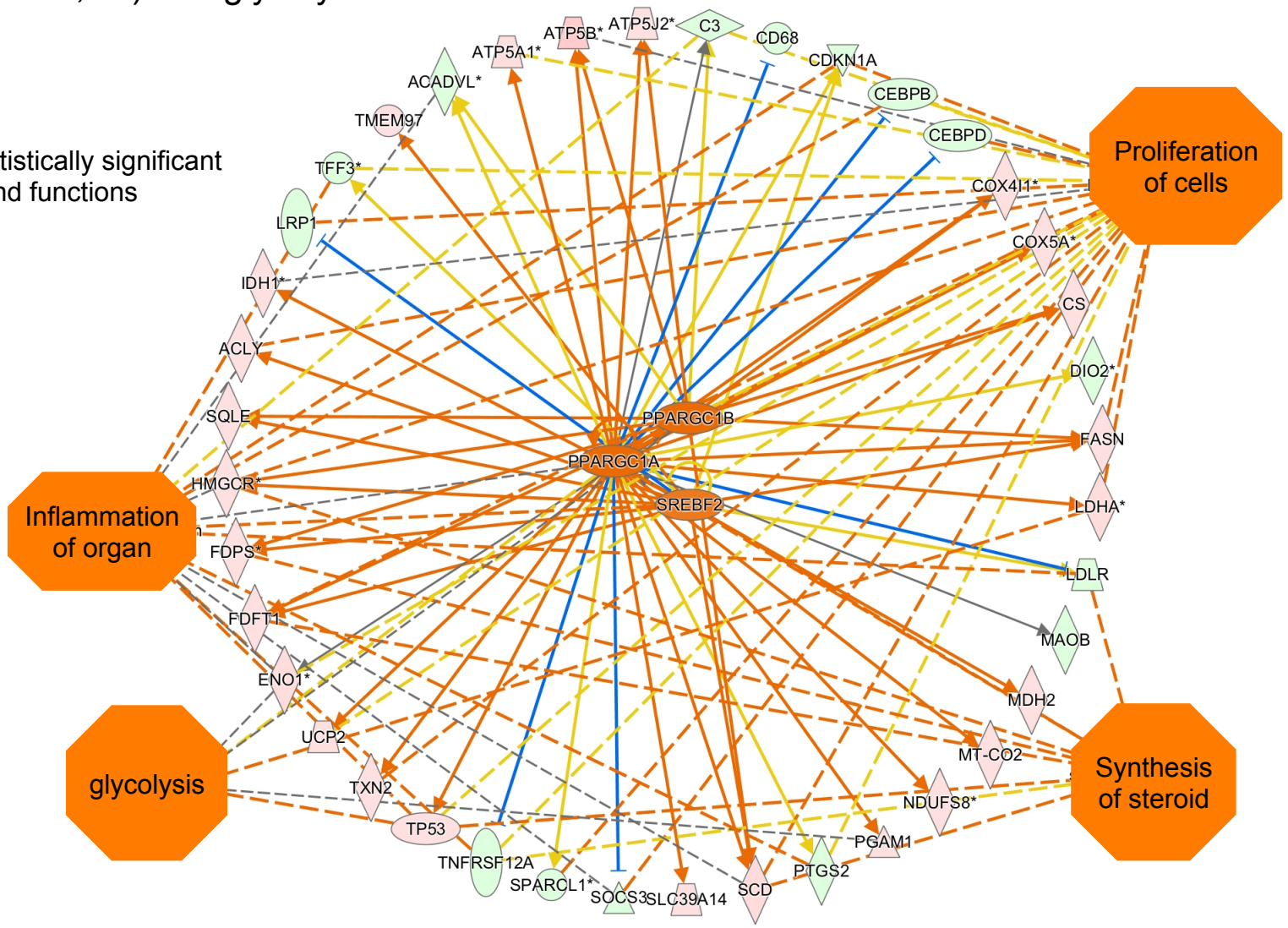
Drivers of Fatty acid and Sterol Metabolism are predicted to be activated



Some of the Biological Processes Predicted to be Activated in Patient 46

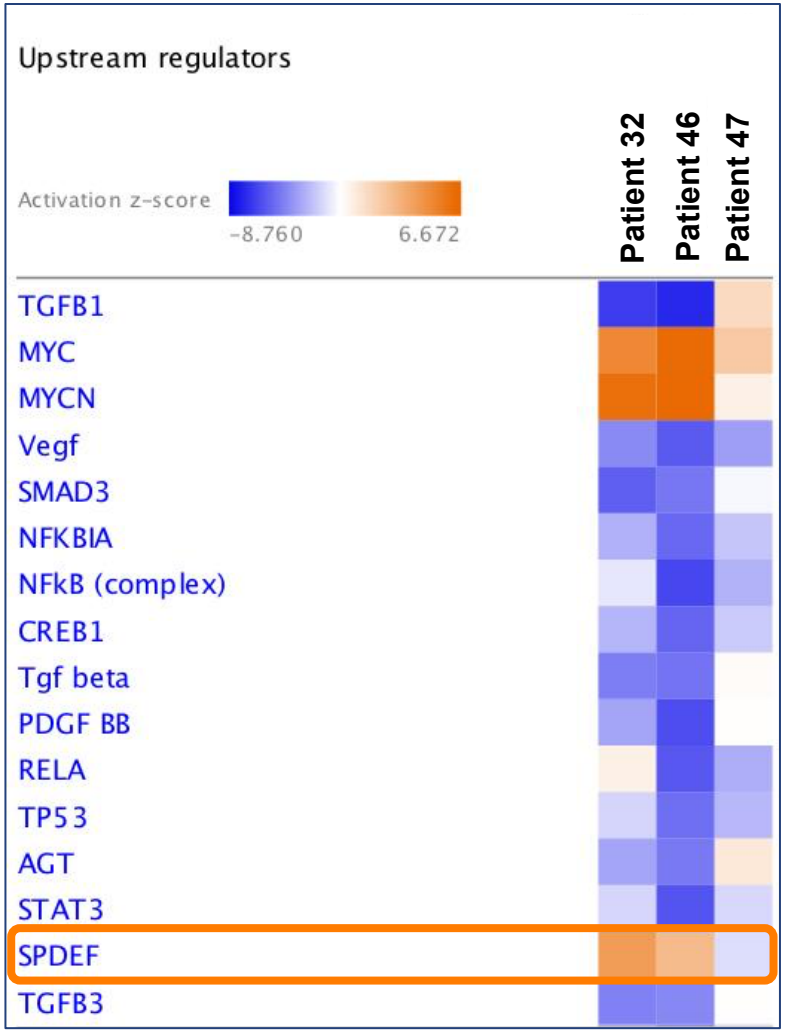
Proliferation of cells and Inflammation are strongly activated, Synthesis of steroid (estrogens, progesterone, …) and glycolysis are activated as well

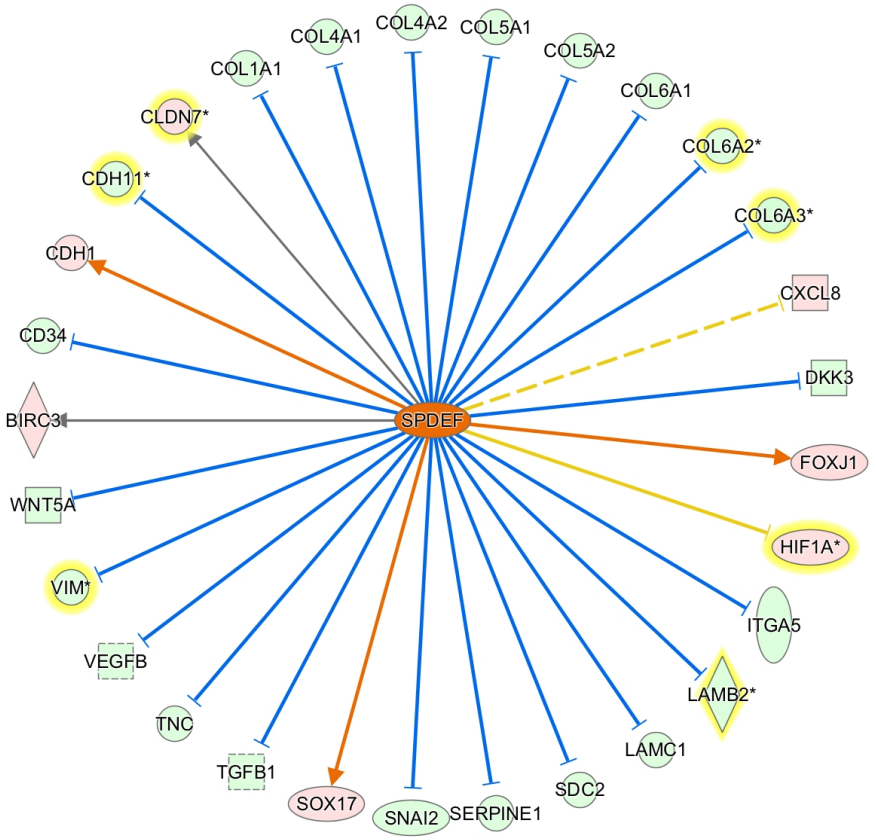
Overlay statistically significant diseases and functions



Comparison of the Upstream Analysis in P32, P46, and P47

Growth Factors and Transcription Regulators also distinguish the patients from one another



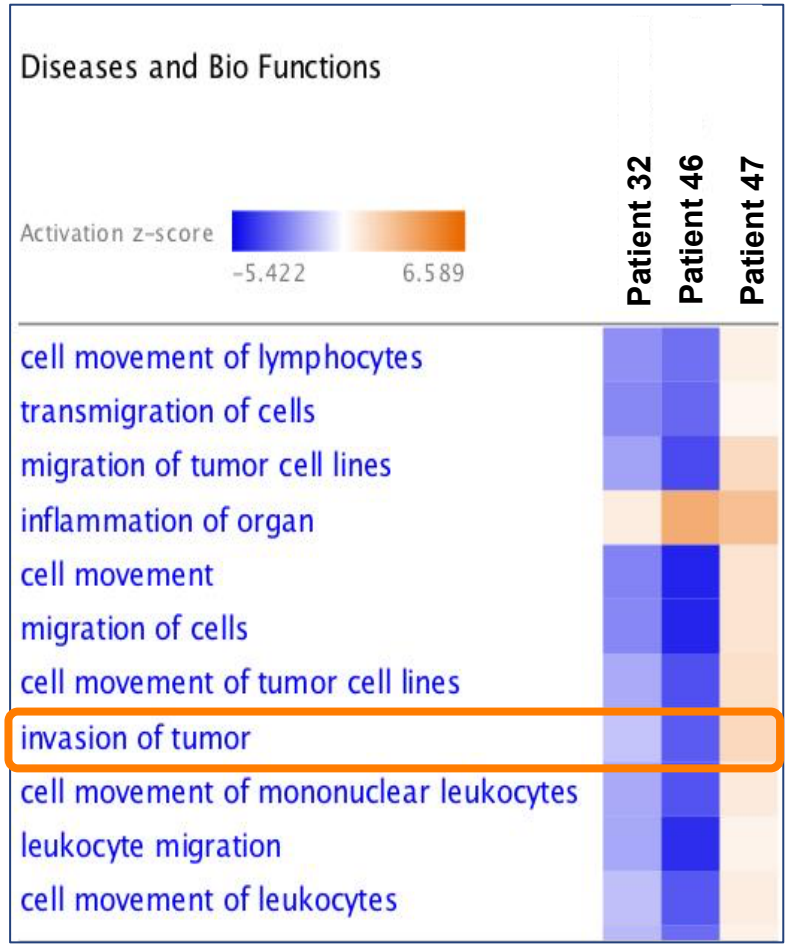


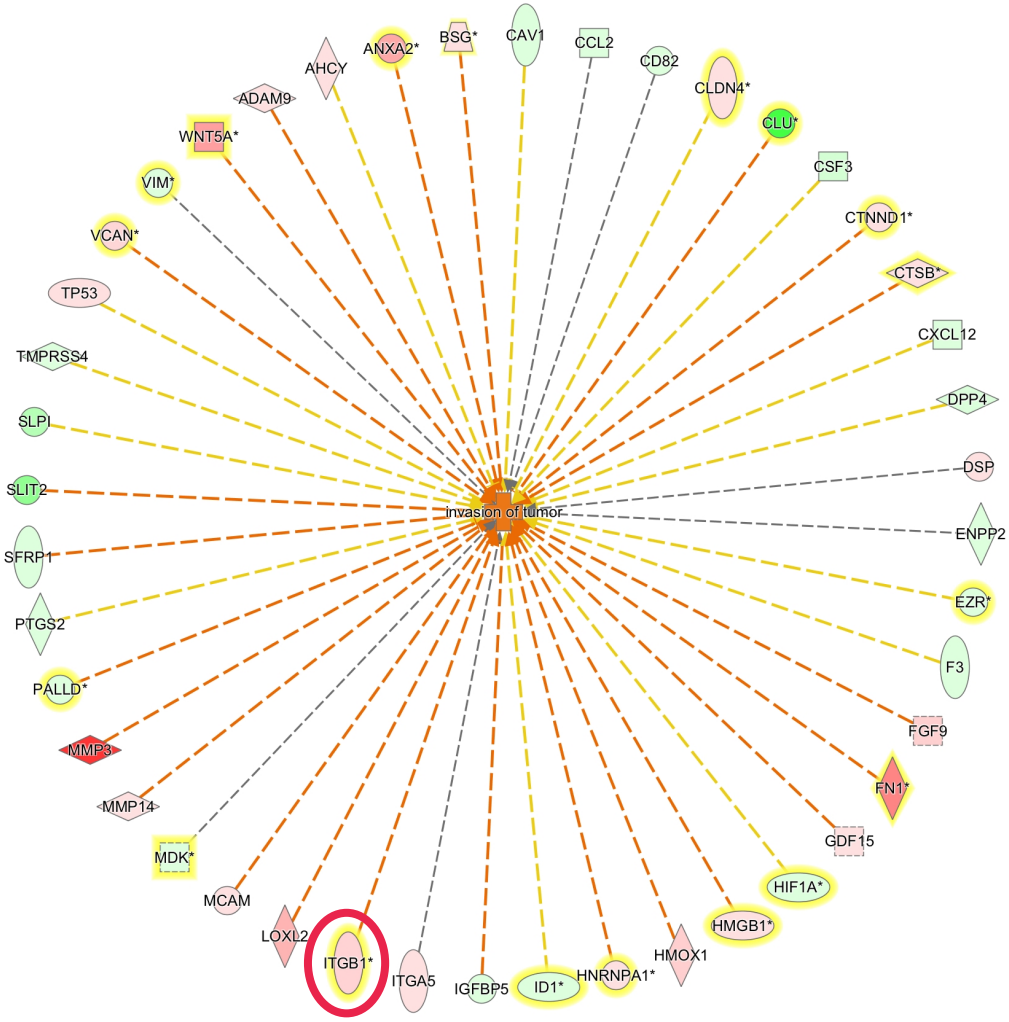
Cell migration & invasion:

- ✓ Inhibited in P32 and P46 (SPDEF activated)
- ✓ Induced in P47 (SPDEF inhibited)

Comparison of the Diseases & Functions in P32, P46, and P47

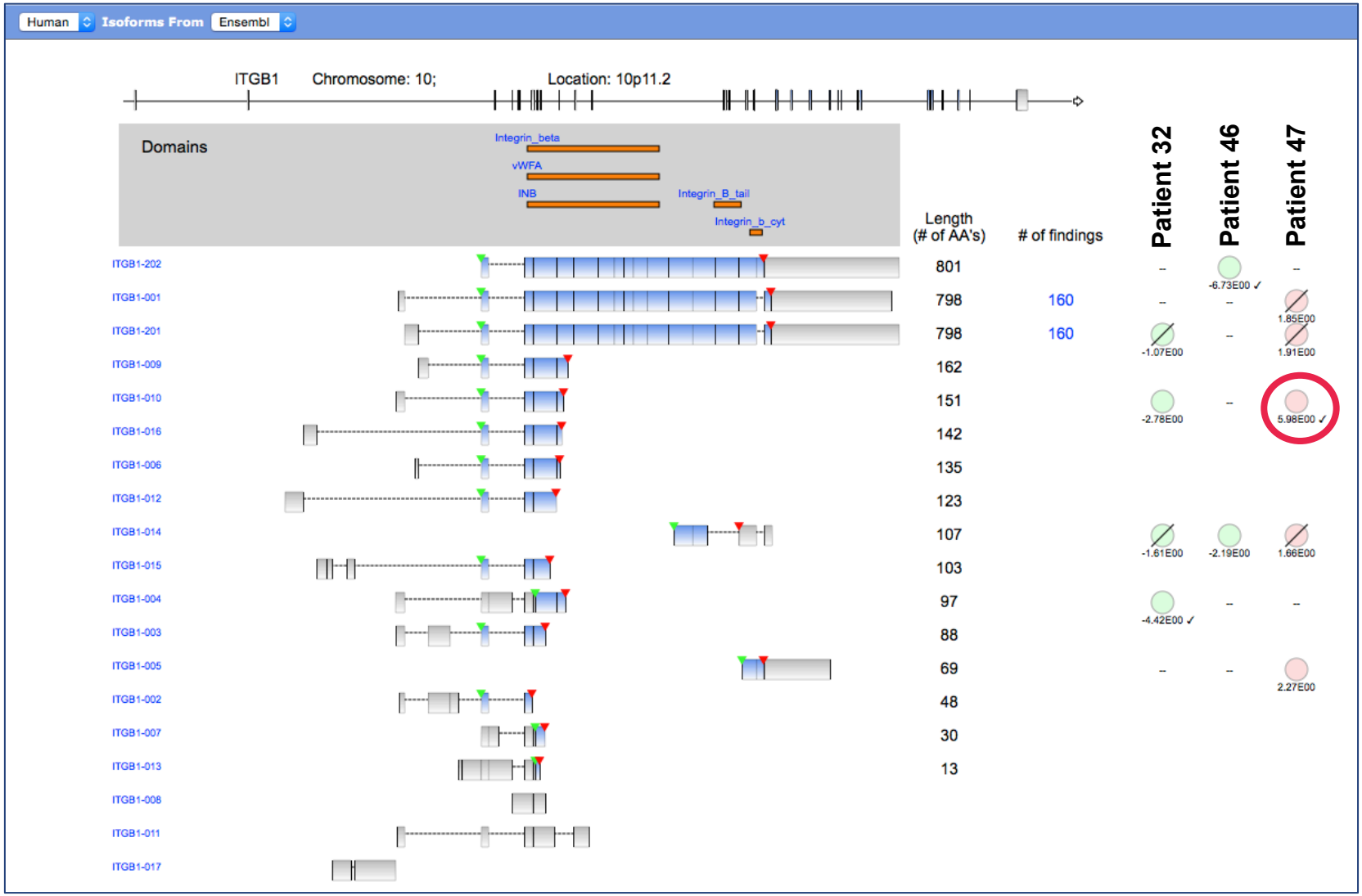
Downstream Effect Analysis indicates increased “invasion of tumor” in P47 compared to P32 and P46





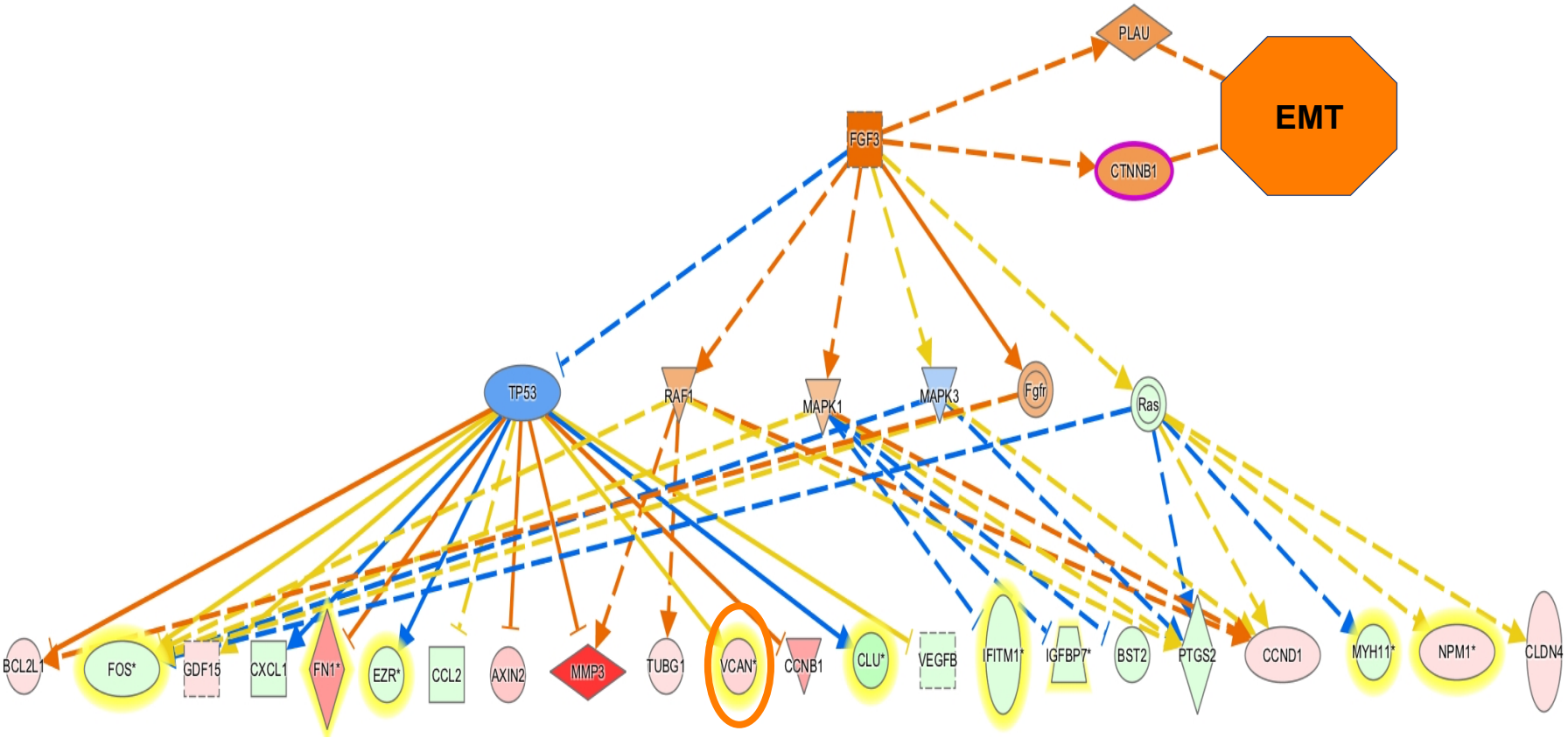
ITGB1 isoforms: potential regulator of invasion of carcinoma cells

Highlight of a key gene and its isoforms: up-regulation of ITGB1-010 (isoform) may promote cell migration/invasion during metastasis to other tissues



FGF3-driven Causal Network linked to Epithelial-to-Mesenchymal Transition

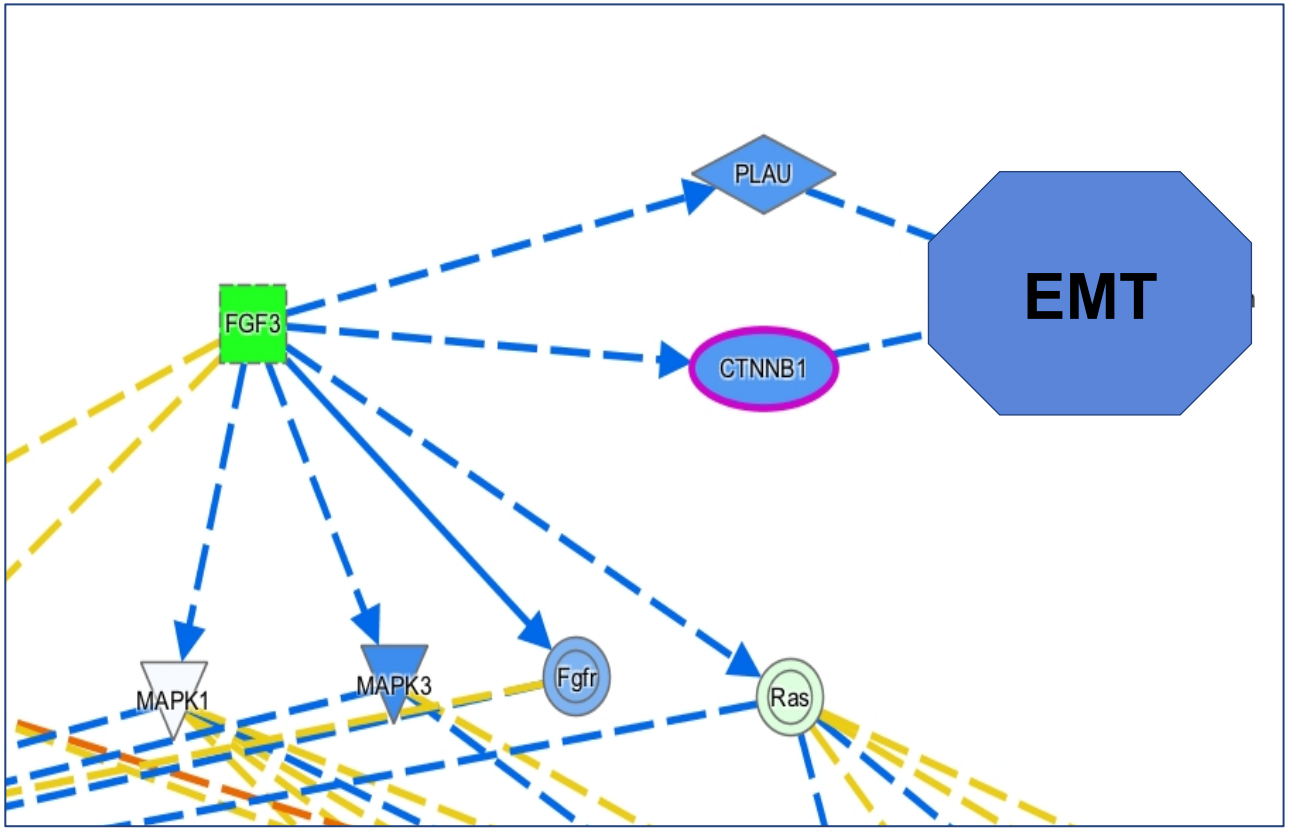
In Patient 47, FGF3 is predicted to be activated and is driving a CN potentially connected to EMT via CTNNB1 and PLAU.



FGF3-driven CN (depth 2) is shown (7 regulators plausibly explaining the expression pattern of 164 downstream targets (22 are shown here). Frequent amplification of this gene has been found in human tumors, which may be important for neoplastic transformation and tumor progression (BrCa). Hypothesis to be tested and validated.

Hypothesis: Inhibition of FGF3 to counteract EMT in EEC

This CN allows to set a new hypothesis in conjunction with MAP (Molecule Activity Predictor).



MAP simulates the inhibition of FGF3 and the impact on the EMT. When FGF3 is inhibited or downregulated, the EMT is decreased (blue circle).

IsoProfiler in IPA to discover isoforms that may drive tumor progression

Versican: upregulation of VCAN-001 is involved in malignant solid tumor (in Breast cancer). This isoform is upregulated in Patient 47

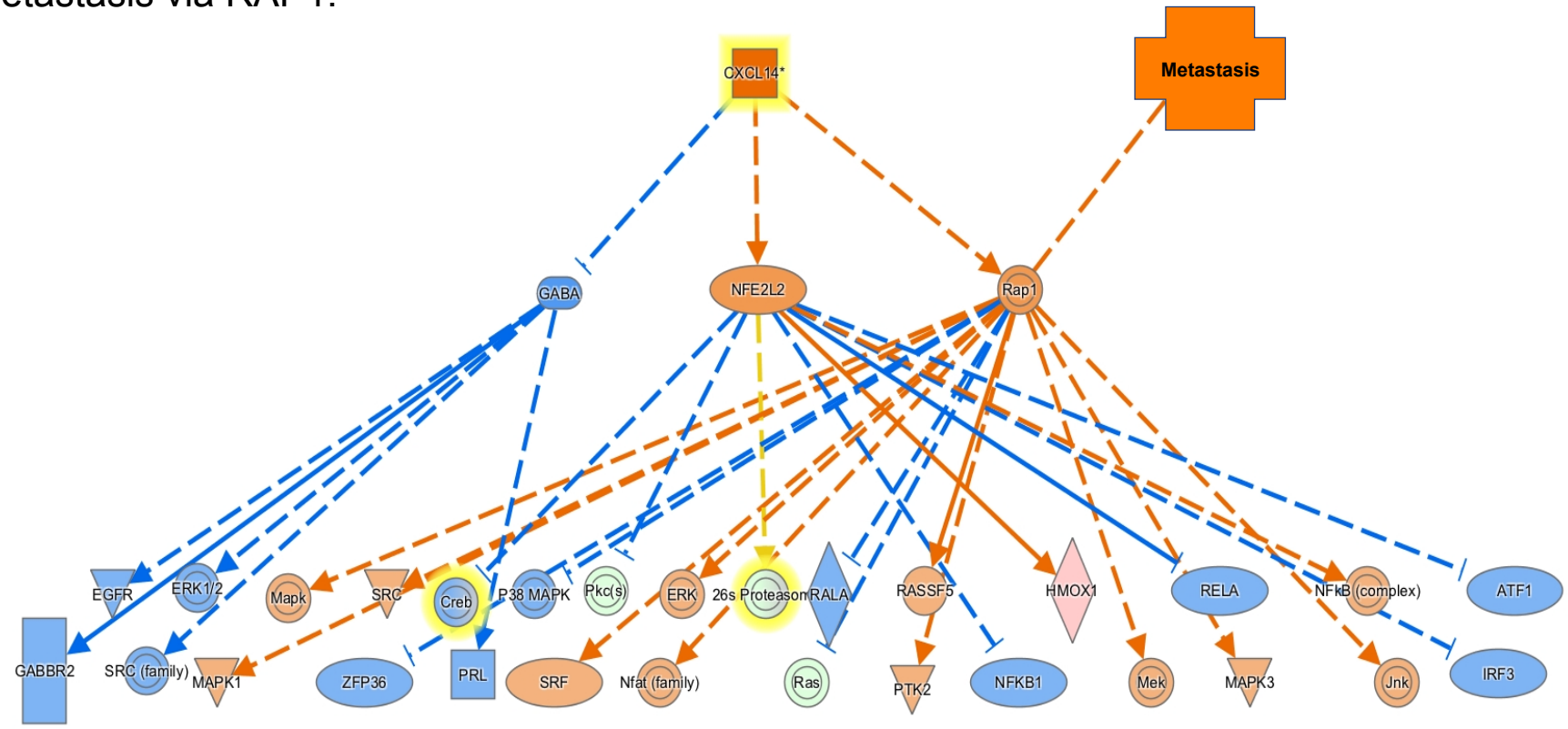
IsoProfiler

ADD TO MY PATHWAY | ADD TO MY LIST | DISPLAY AS NETWORK | CREATE DATASET | EXPAND ROWS | COLLAPSE ROWS | LIMIT TO DATASET | EEC Ensembl P32... | Symbol IGF1 – WNT5A (p2 of 2) | More Inf

Molecule										Add column(s)	Disease or Function Evidence					Add column
...	Transcri...	...	...	...	ID	...	Exp Fold ...	Exp Fold Ch...	Expression ...		...	...	Disease or F...	...	...	Findi
▶IGF1	IGF1-002	IGF1B	protein is...	growth fa...	ENST000...	↓-58.770					increased...	increases	quantity of cells ...	not applic...	causal	1
▼ITGB1	ITGB1-201	INTEGRI ...	protein is...	transme...	ENST000...	↓-1.073		↑1.912			increased...	increases	cell movement, ...	not applic...	causal	14
	ITGB1-201	INTEGRI ...	protein is...		ENST000...	↓-1.073		↑1.912			increased...	increases	cell movement, ...	not applic...	causal	14
▶ITM2B	ITM2B-001	ADAN	protein is...	other	ENST000...	↓-1.057	↓-3.202	↓-2.049			increased...	affects	Dementia, ...	upregulat...	correlation	1
▶LAMB3	LAMB3-001	LAMB3	protein is...	transporter	ENST000...	↑14.333	↓-2.559	↓-1.422			increased...	increases	cell movement, ...	not applic...	causal	1
▶LAPTM4B	LAPTM4B-001	LAPT ...	protein is...	other	ENST000...	↑2.314	↑1.552	↑2.559			increased...	affects	abdominal cancer	upregulat...	correlation	1
▶LYN	LYN-003	LYNB	protein is...	kinase	ENST000...	↑2.662					increased...	affects	B-cell chronic ly ...	upregulat...	correlation	3
▶MBD2	MBD2-001	MBD2 A i ...	protein is...	transcript...	ENST000...	↑1.417	↓-1.121	↑1.509			increased...	increases	repression of ...	not applic...	causal	1
▼MYH9	MYH9-001	MYH9	protein is...	enzyme	ENST000...	↓-2.983	↓-1.240	↑1.465			unknown ...	affects	hearing loss, ...	not applic...	correlation	1
	MYH9-001	MYH9	protein is...		ENST000...	↓-2.983	↓-1.240	↑1.465			unknown ...	affects	hearing loss, ...	not applic...	correlation	1
▶PBX1	PBX1-001	PBX1A	protein is...	transcript...	ENST000...	↓-4.480		↓-1.602			unknown ...	increases	transactivation, ...	not applic...	causal	2
▶PGR	PGR-001	PGR-B	protein is...	ligand-de...	ENST000...	↓-5.392		↓-1.480			increased...	affects,de...	Ovarian Cancer a ...	upregulat...	correlation	54
▶PPP1R1B	PPP1R1B-002, ...	T-DA ...	protein is...	phosphat...	ENST000...	↑4.300,	↑2.966				increased...	increases	cell survival, ...	not applic...	causal	3
▶RTN4	RTN4-007, ...	NOGO B2	protein is...	other	ENST000...	↓-3.185,	↓-1.989	↓-1.819, ↓-1.695			decrease...	affects,de...	arteriosclerosis, ...	downregu...	causal,co...	2
▶SEPT9	SEPT9-008	SEPT9B	protein is...	enzyme	ENST000...	↓-1.873	↑1.554	↑1.004			increased...	affects,in...	M phase, ...	not applic...	causal	22
▶SET	SET-001	SET al ...	protein is...	phosphat...	ENST000...	↑2.596	↑1.351	↑1.706			unknown ...	decrease...	DNA replication ...	not applic...	causal	2
▶SPP1	SPP1-001, ...	SPP1 iso ...	protein is...	cytokine	ENST000...	↑8.254,	↓-5.091				increased...	affects,de...	Breast Cancer an ...	not applic...	causal	68
▶STAT1	STAT1-001	STAT1 a ...	protein is...	transcript...	ENST000...	↑1.104	↓-1.853	↑1.266			increased...	affects,de...	G0 phase, ...	not applic...	causal	12
▶STAT3	STAT3-001	STAT3 a ...	protein is...	transcript...	ENST000...	↑1.233	↓-1.558	↓-1.655			increased...	affects	Thyroid Cancer a ...	upregulat...	correlation	1
▶SYNPO2	SYNPO2-003	SYNPO2 i ...	protein is...	other	ENST000...	↓-38.657					increased...	affects	development of c ...	not applic...	causal	1
▶TAX1BP1	TAX1BP1-002	TXBP15 ...	protein is...	other	ENST000...	↑1.179	↓-3.356	↓-1.229			increased...	decreases	apoptosis, ...	not applic...	causal	1
▶TSC22D1	TSC22D1-001	TSC22D1	protein is...	transcript...	ENST000...	↓-1.312					increased...	decreases	senescence of cell	not applic...	causal	1
▼VCAN	VCAN-001	VERSICA ...	protein is...	other	ENST000...	↓-12.853		↑2.127			increased...	affects	Breast Cancer an ...	upregulat...	correlation	1
	VCAN-001	VERSICA ...	protein is...		ENST000...	↓-12.853		↑2.127			increased...	affects	breast cancer, ...	upregulat...	correlation	1
▶WFDC2	WFDC2-006	WFDC2- ...	RNA isofo...	other	ENST000...	↑9.597	↓-2.409	↓-6.516			increased...	affects	Uterine Cancer a ...	upregulat...	correlation	2
▶WNT5A	WNT5A-001	WNT5A i ...	protein is...	cytokine	ENST000...	↓-4.727		↑8.093			increased...	decreases	proliferation of b ...	not applic...	causal	3

CXCL14-driven Causal Network is linked to Metastasis in EEC

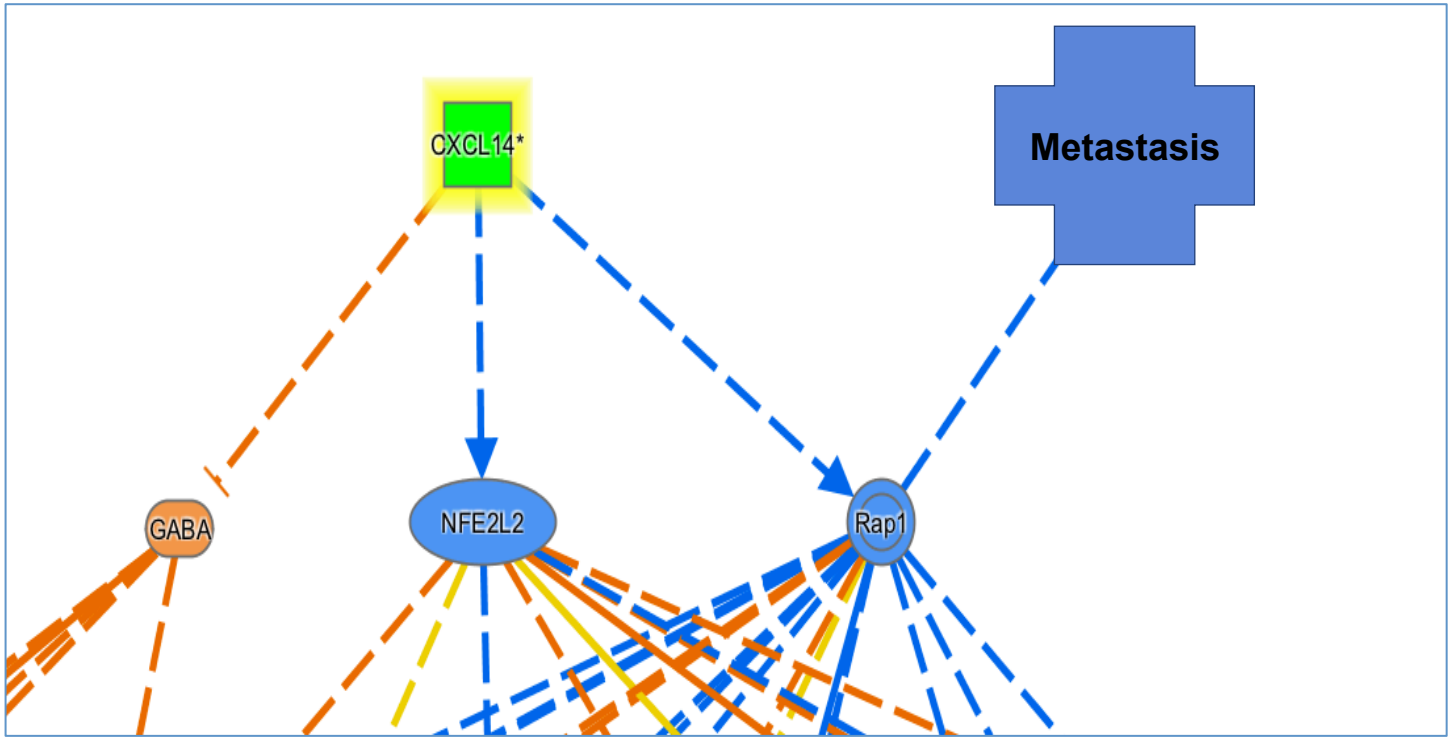
In Patient 47, CXCL14 is predicted to be activated and is driving a CN potentially connected to metastasis via RAP1.



CXCL14-driven CN (depth 3) is shown (4 regulators plausibly explaining the expression pattern of 51 downstream targets (none shown here). Upregulation of CXCL14 has been show to be involved in breast cancer, papillary thyroid carcinoma, prostate cancer, pancreatic cancer.

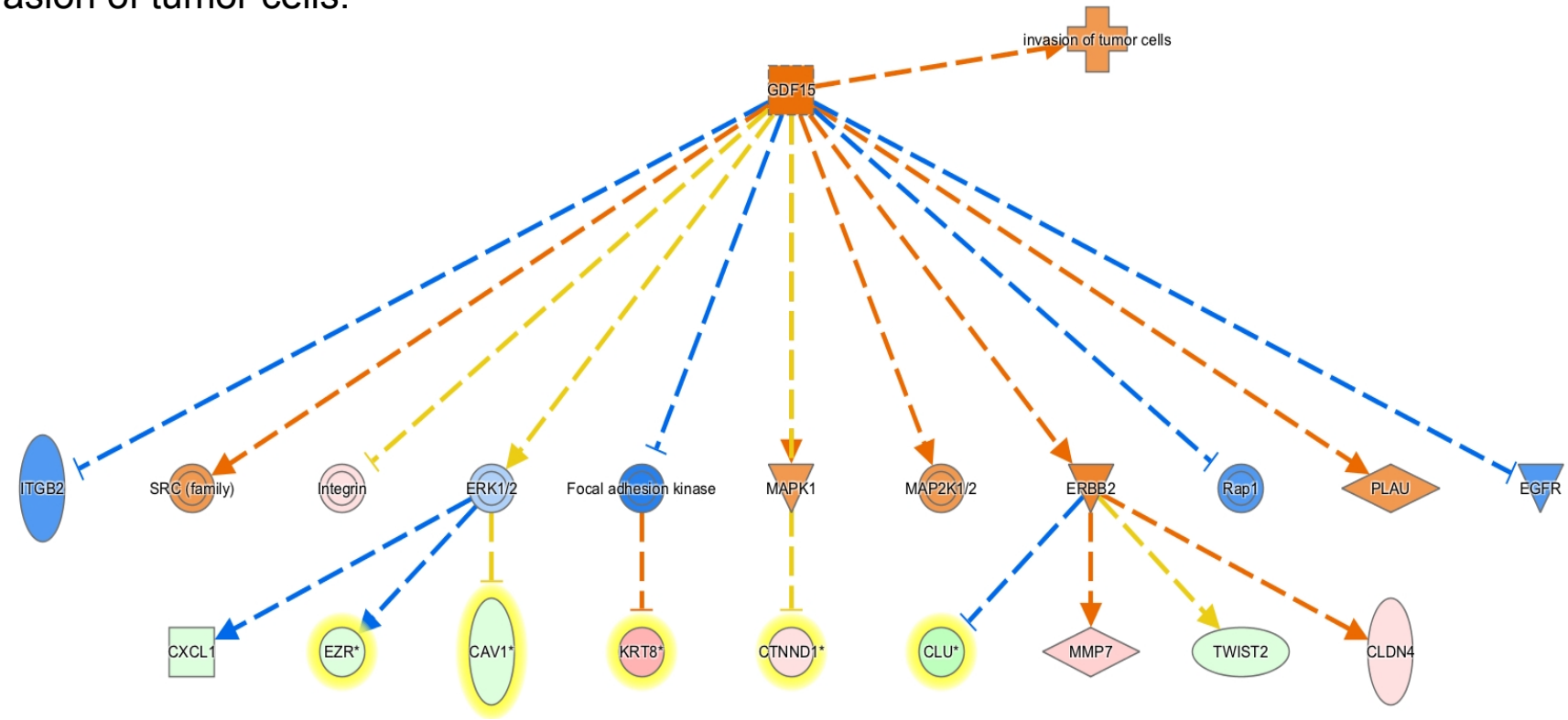
Hypothesis: Inhibition of CXCL14 to decrease Metastasis in EEC

Inhibiting CXCL14 (green) would decrease metastasis (blue).



GDF15-driven Causal Network is linked to invasion in EEC

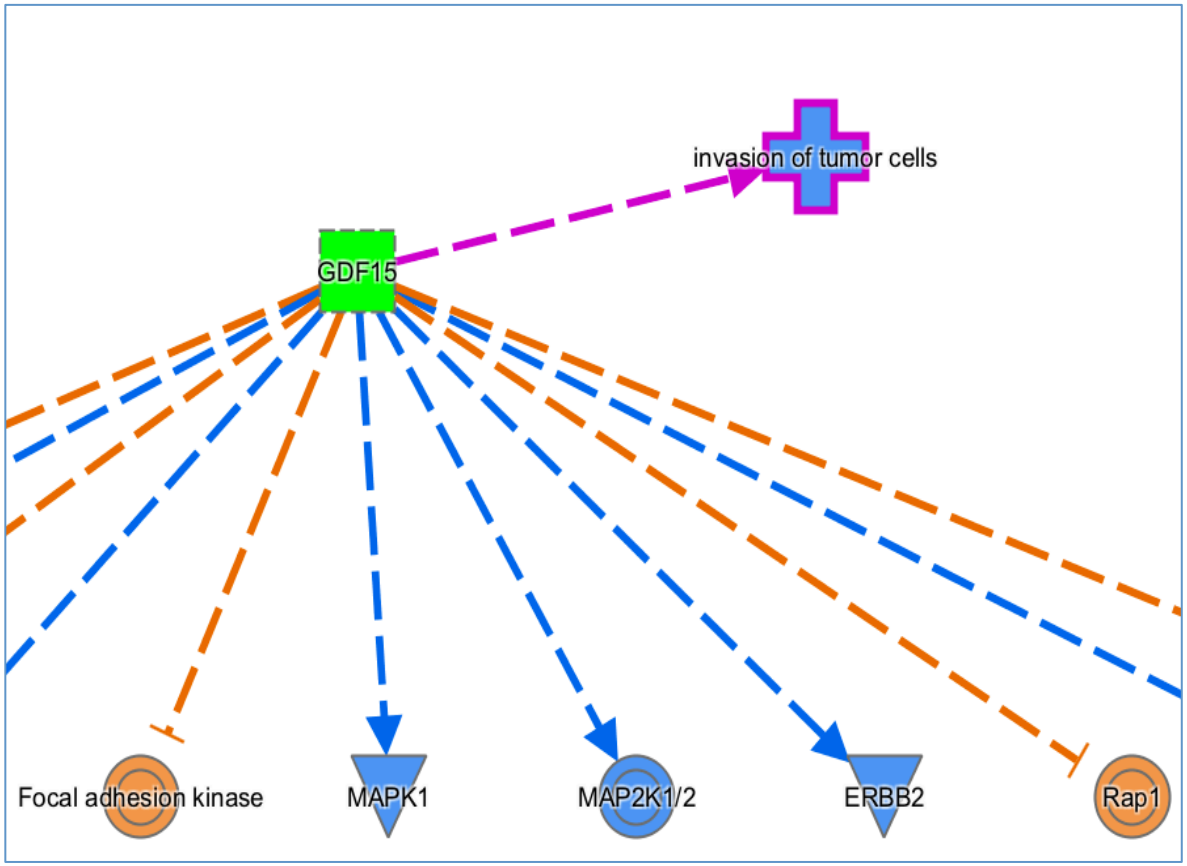
In Patient 47, GDF15 is predicted to be activated and is driving a CN potentially connected to invasion of tumor cells.



GDF15-driven CN (depth 2) is shown below (12 regulators plausibly explaining the expression pattern of 92 downstream targets (9 are shown here).
Overexpression of GDF15 has been show to be involved in many cancers (melanoma, prostate, thyroid, pancreatic, ovarian, colon). Plasma GDF-15 is elevated in patients with endometrial cancer and is a marker for phenotype, including lymph node metastasis and disease-specific survival.

Hypothesis: Inhibition of GDF15 to decrease invasion in EEC

Inhibiting GDF15 (green) would decrease invasion (blue).



Sample to Insight approach: Exploring the Transcriptome in EEC

Using QIAGEN Bioinformatics solutions, three immune proteins were identified as potential therapeutic targets in tumor progression in EEC

We were able to highlight important parameters and to compare transcriptome of 3 early stage patients:

- ✓ We were able to compare and determine which and how signaling cascades are involved in the 3 patients (EIF2 signaling, ILK signaling, Integrin Signaling) (Canonical Pathways, Pathway Activity Analysis)
- ✓ We were able to highlight which transcriptional program is turn on in these patients (SPDEF, and PPARGC1a, PPARGC1b, SBREF2) (Upstream Analysis, Upstream Regulators)
- ✓ We were able to understand which biological processes differ between these 3 patients (cell migration and cell invasion). (Diseases & Functions, Downstream Effects Analysis)
- ✓ We were able to identify some splicing variants of importance in the EEC tumor progression (IGTB1, VCAN) (Isoform View, IsoProfiler)
- ✓ We able to propose new hypotheses that visualize which immune components could be targeted to inhibit key biological processes in tumor progression: cell invasion, EMT and metastasis processes (Upstream Analysis, Causal Networks)

Using QIAGEN Bioinformatics Approach

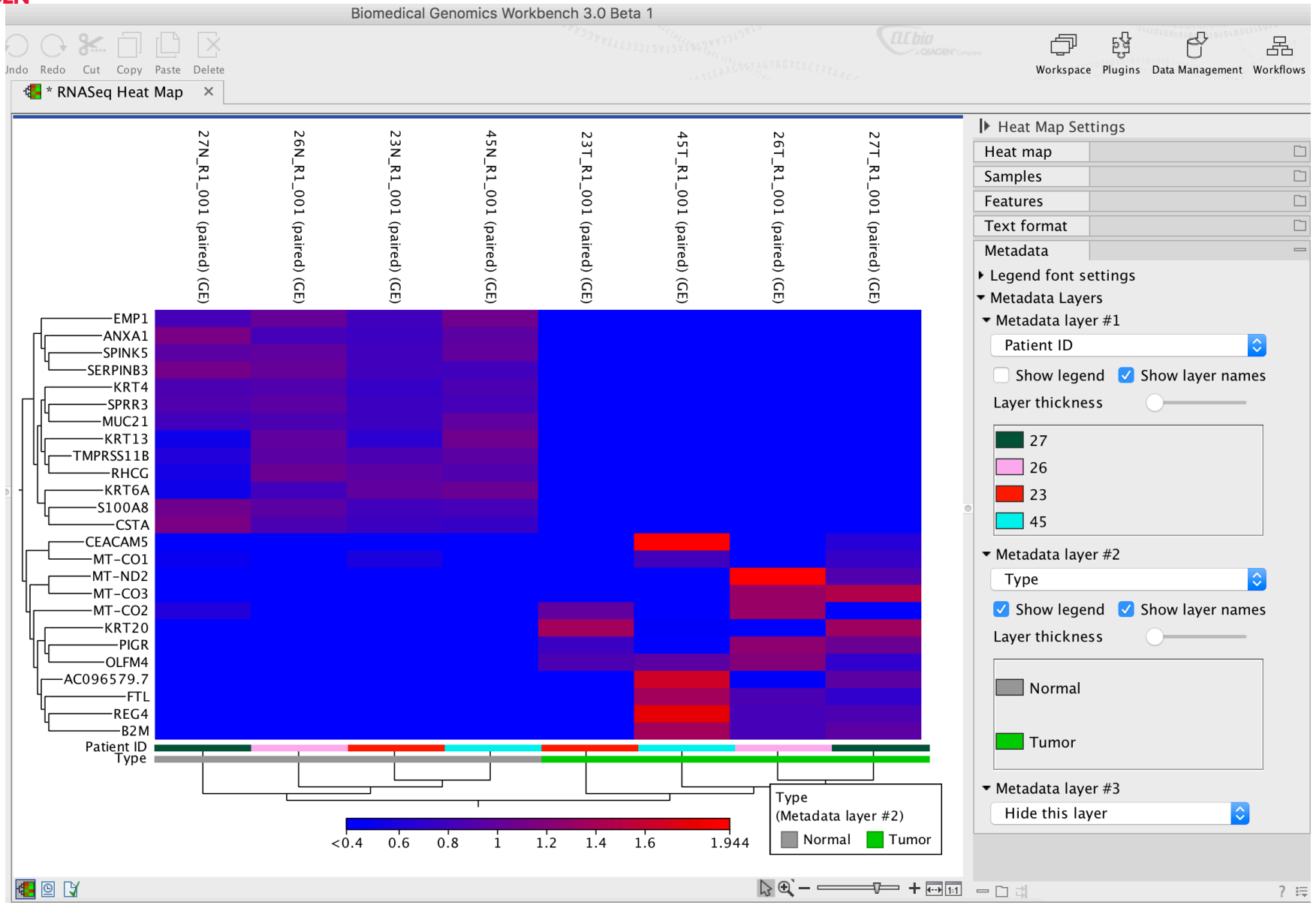
- Using Biomedical Genomics Workbench, we have been able to:

  - ✓ Upload RNA-seq data (FASTQ files from SRA)
  - ✓ Align to the genome of interest (human Ensembl)
  - ✓ Quantitate and obtain differential expression between the samples and Call variants
  - ✓ Seamlessly send data directly into IPA for biological interpretation and to Ingenuity Variant Analysis for variant identification and prioritization

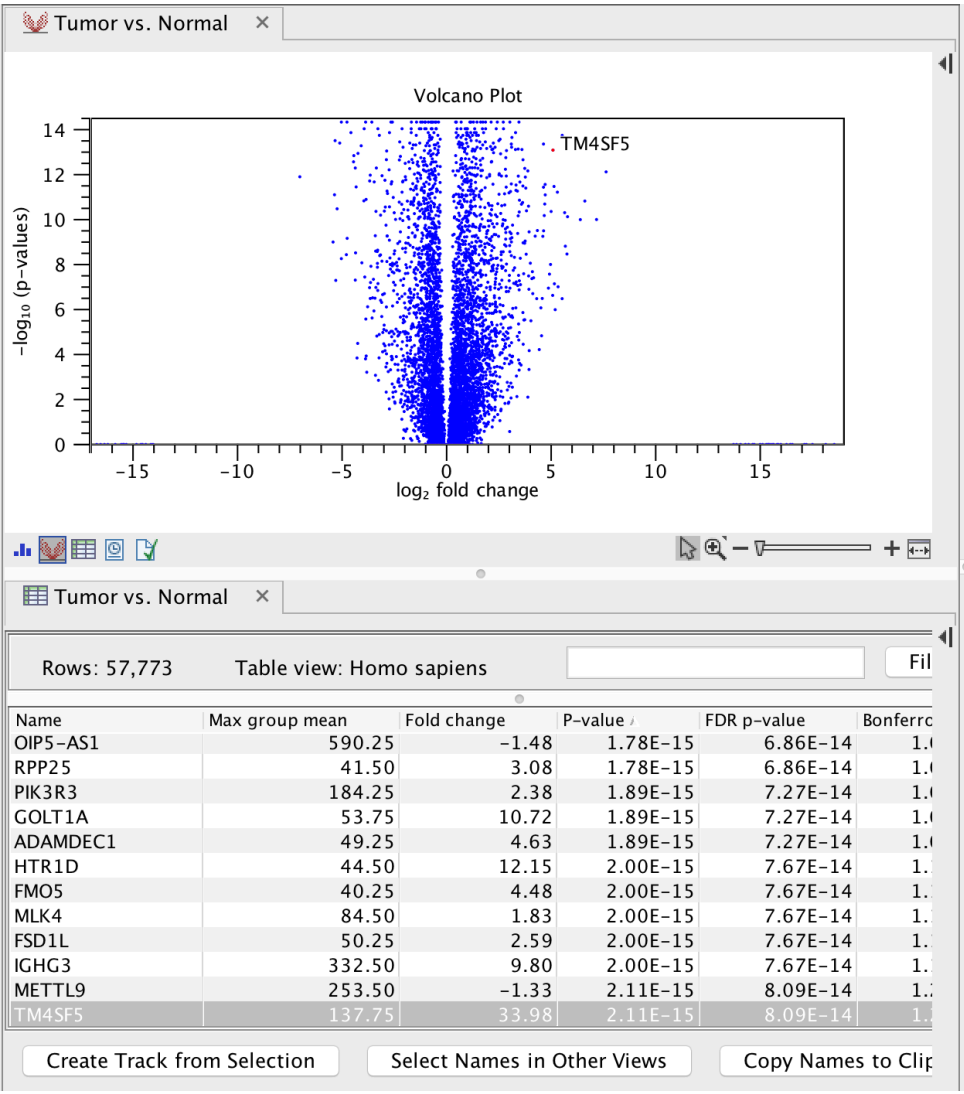
- Using IPA, we have been able to:

  - ✓ Visualize the differentially expressed genes in tumor vs. adjacent non-tumor tissues in three patients
  - ✓ Understand which signaling pathways are involved in tumor progression
  - ✓ Discover potential transcriptional program(s) that are induced or repressed that drives tumorigenesis
  - ✓ Visualize differentially expressed splicing variants
  - ✓ Discover specific biological processes that participate in the tumor progression
  - ✓ Highlight new hypotheses (ready to be tested and validated) that could explain metastasis or invasiveness

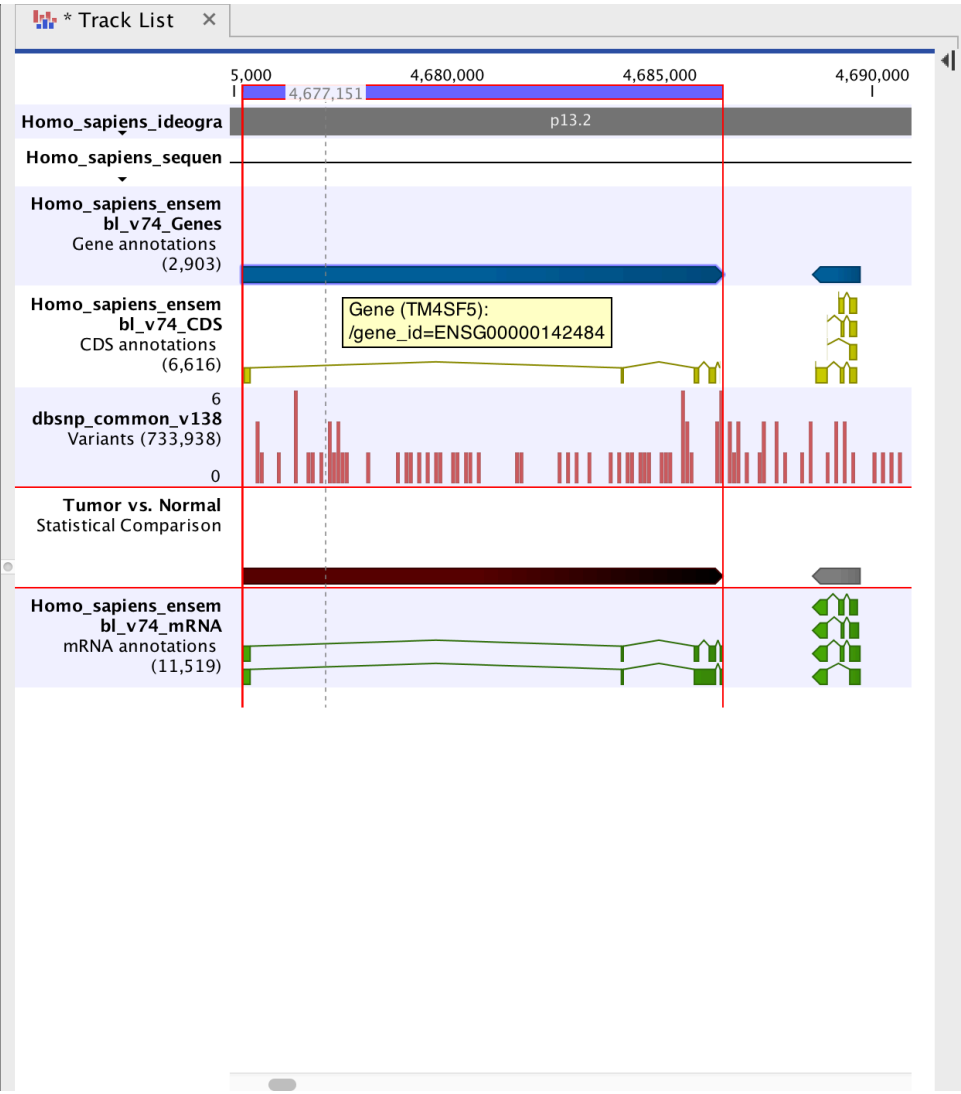
Statistics Pipeline Coming soon…



Statistics Pipeline Coming soon…



Name	Max group mean	Fold change	P-value	FDR p-value	Bonferro
OIP5-AS1	590.25	-1.48	1.78E-15	6.86E-14	1.(
RPP25	41.50	3.08	1.78E-15	6.86E-14	1.(
PIK3R3	184.25	2.38	1.89E-15	7.27E-14	1.(
GOLT1A	53.75	10.72	1.89E-15	7.27E-14	1.(
ADAMDEC1	49.25	4.63	1.89E-15	7.27E-14	1.(
HTR1D	44.50	12.15	2.00E-15	7.67E-14	1.
FMO5	40.25	4.48	2.00E-15	7.67E-14	1.
MLK4	84.50	1.83	2.00E-15	7.67E-14	1.
FSD1L	50.25	2.59	2.00E-15	7.67E-14	1.
IGHG3	332.50	9.80	2.00E-15	7.67E-14	1.
METTL9	253.50	-1.33	2.11E-15	8.09E-14	1.
TM4SF5	137.75	33.98	2.11E-15	8.09E-14	1.



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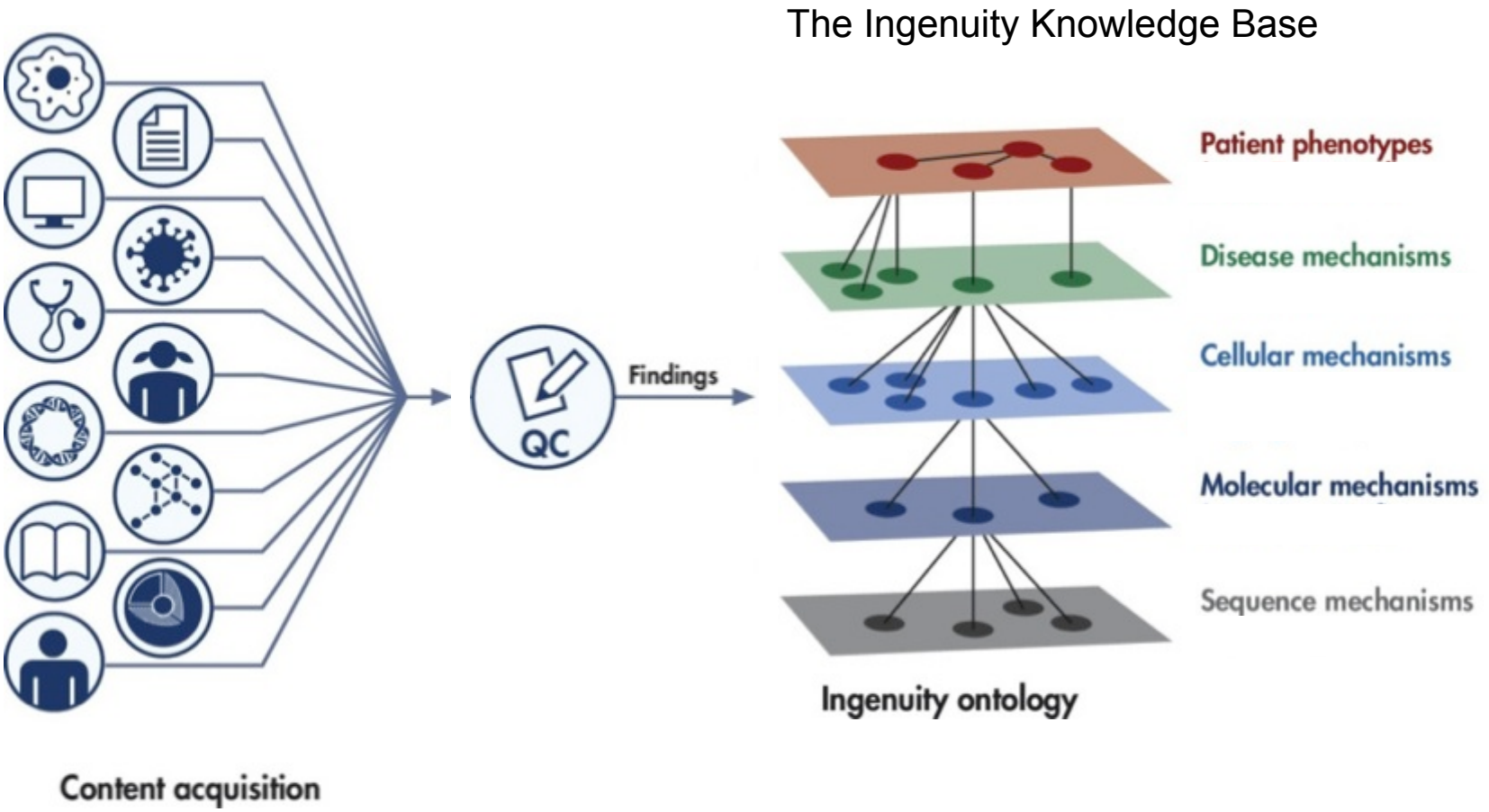
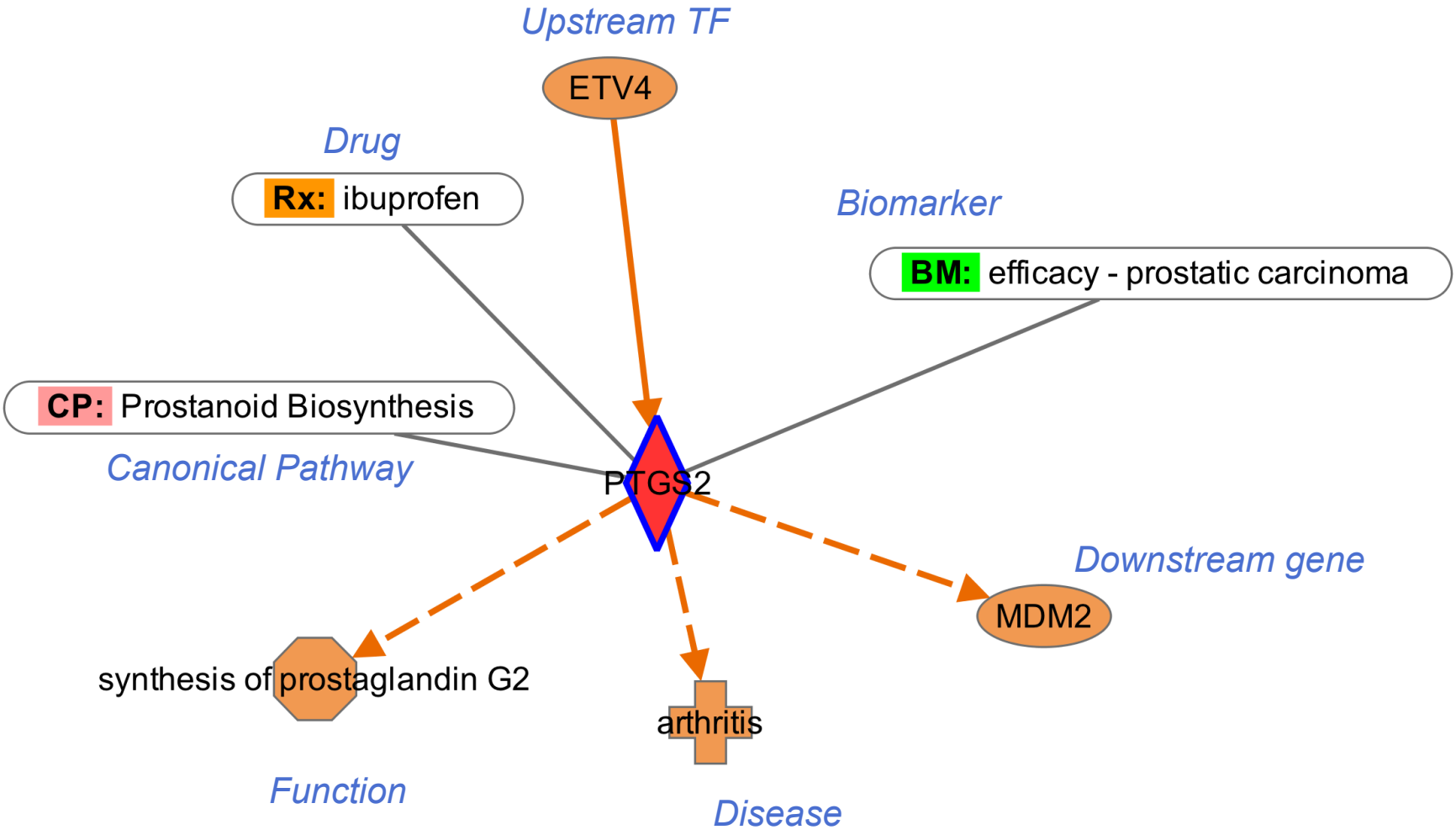
Thank You For Attending

Back Up Slides

The Ingenuity Knowledge Base contains > 5 million findings

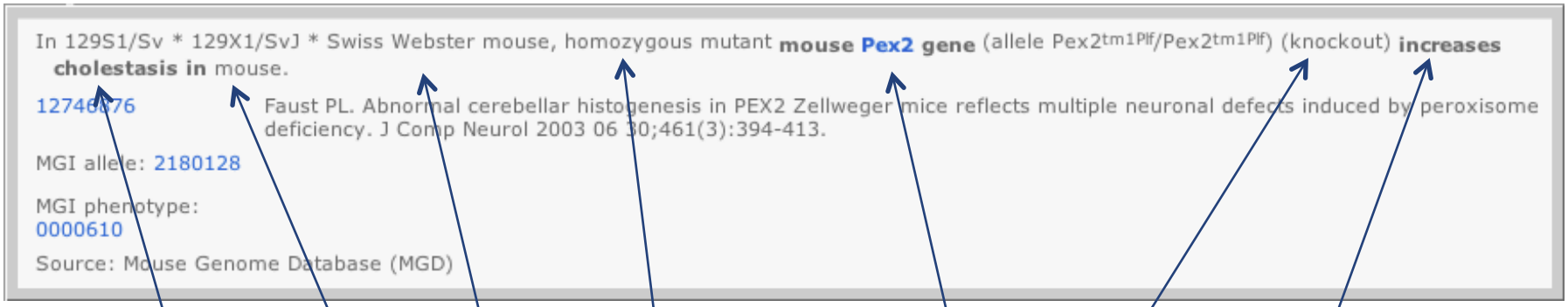


Capturing molecular information from the literature



Gather this information for nearly every gene. Inferences can be made from the resulting networks

Example of a finding: context and direction of effect



disease/ phenotype

species

strain

zygosity

gene

mutation type

Direction of effect on disease / phenotype

Activity of the molecule in this finding (decreased)

This structure provides **rich contextual detail** and powers the algorithms for **causal analysis in IPA**