Review (V1) - The Hallmarks of Cancer

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The Hallmarks of Cancer

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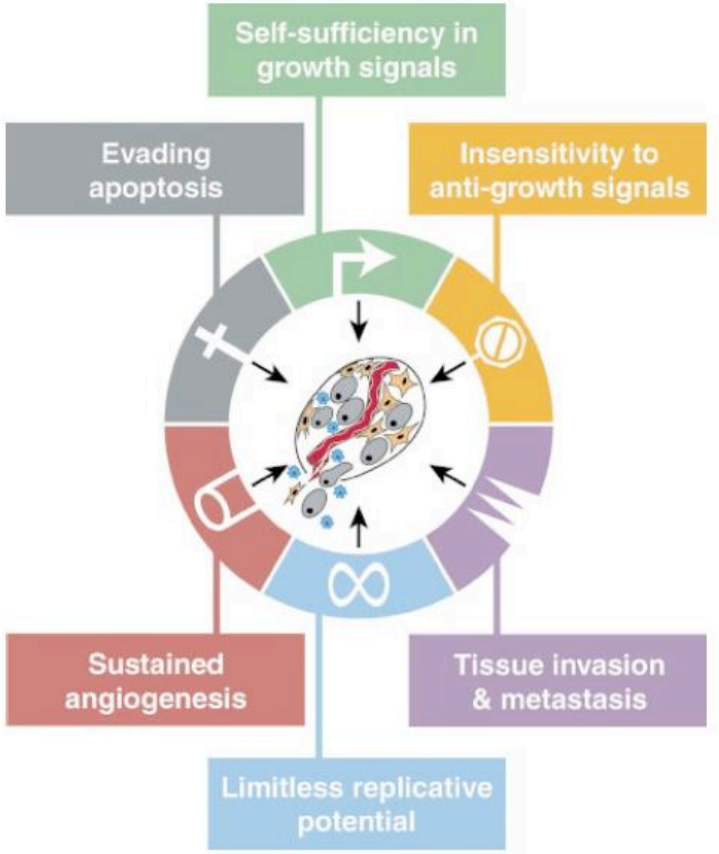

Figure 1. Acquired Capabilities of Cancer

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A

Component	Acquired Capability	Example of Mechanism
	Self-sufficiency in growth signals	Activate H-Ras oncogene
	Insensitivity to anti-growth signals	Lose retinoblastoma suppressor
	Evading apoptosis	Produce IGF survival factors
	Limitless replicative potential	Turn on telomerase
	Sustained angiogenesis	Produce VEGF inducer
	Tissue invasion & metastasis	Inactivate E-cadherin

B

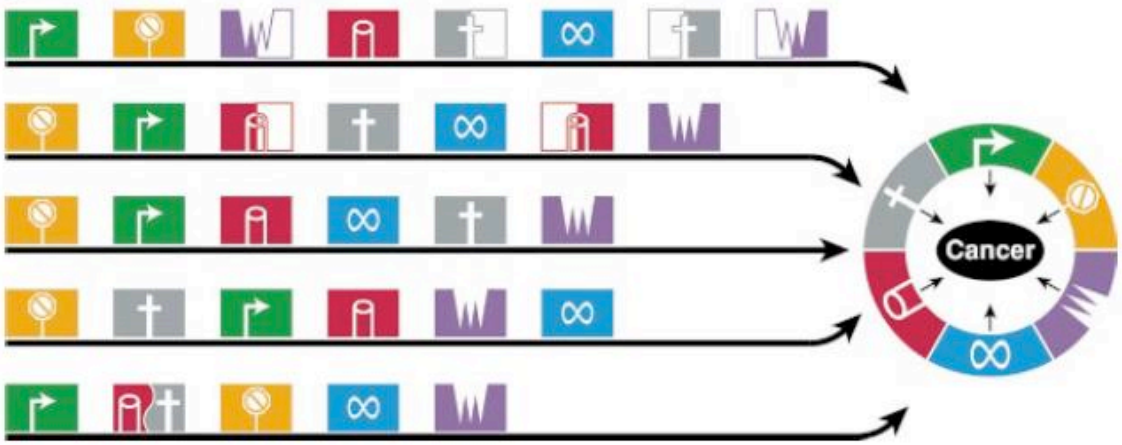
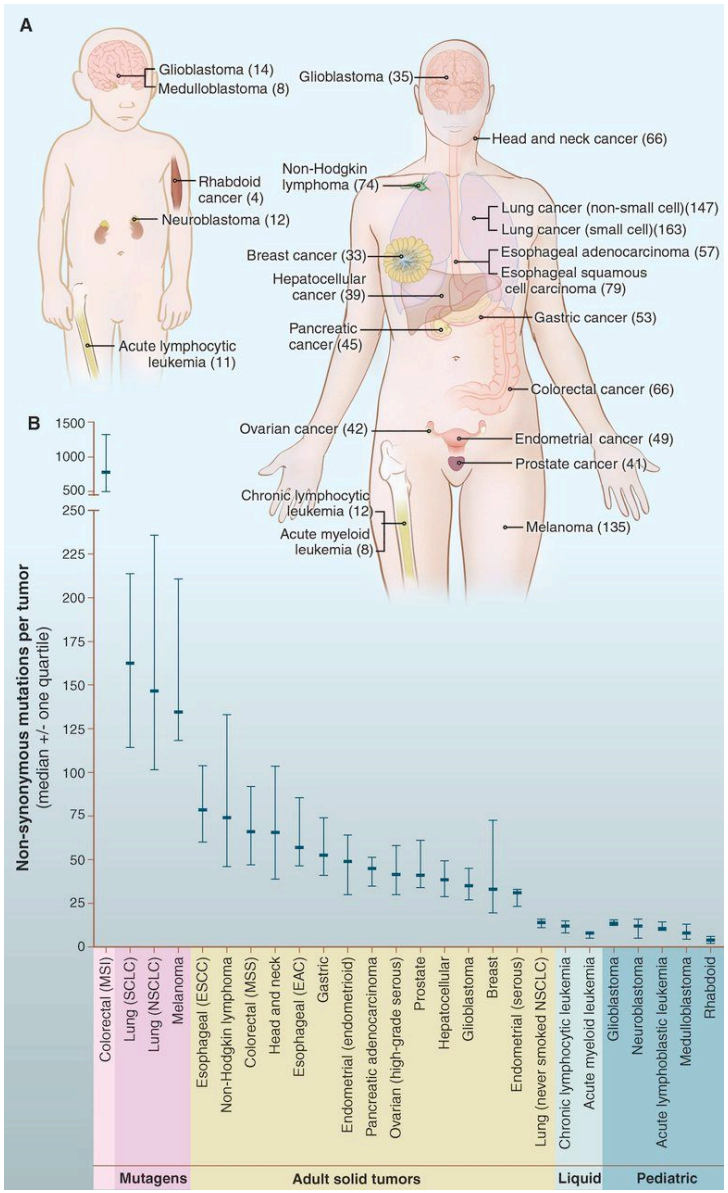


Figure 4. Parallel Pathways of Tumorigenesis

While we believe that virtually all cancers must acquire the same six hallmark capabilities (A), their means of doing so will vary significantly, both mechanistically (see text) and chronologically (B). Thus, the order in which these capabilities are acquired seems likely be quite variable across the spectrum of cancer types and subtypes. Moreover, in some tumors, a particular genetic lesion may confer several capabilities simultaneously, decreasing the number of distinct mutational steps required to complete tumorigenesis. Thus, loss of function of the p53 tumor suppressor can facilitate both angiogenesis and resistance to apoptosis (e.g., in the five-step pathway shown), as well as enabling the characteristic of genomic instability. In other tumors, a capability may only be acquired through the collaboration of two or more distinct genetic changes, thereby increasing the total number necessary for completion of tumor progression. Thus, in the eight-step pathway shown, invasion/metastasis and resistance to apoptosis are each acquired in two steps.

Review (V1) - Number of somatic mutations in human cancers



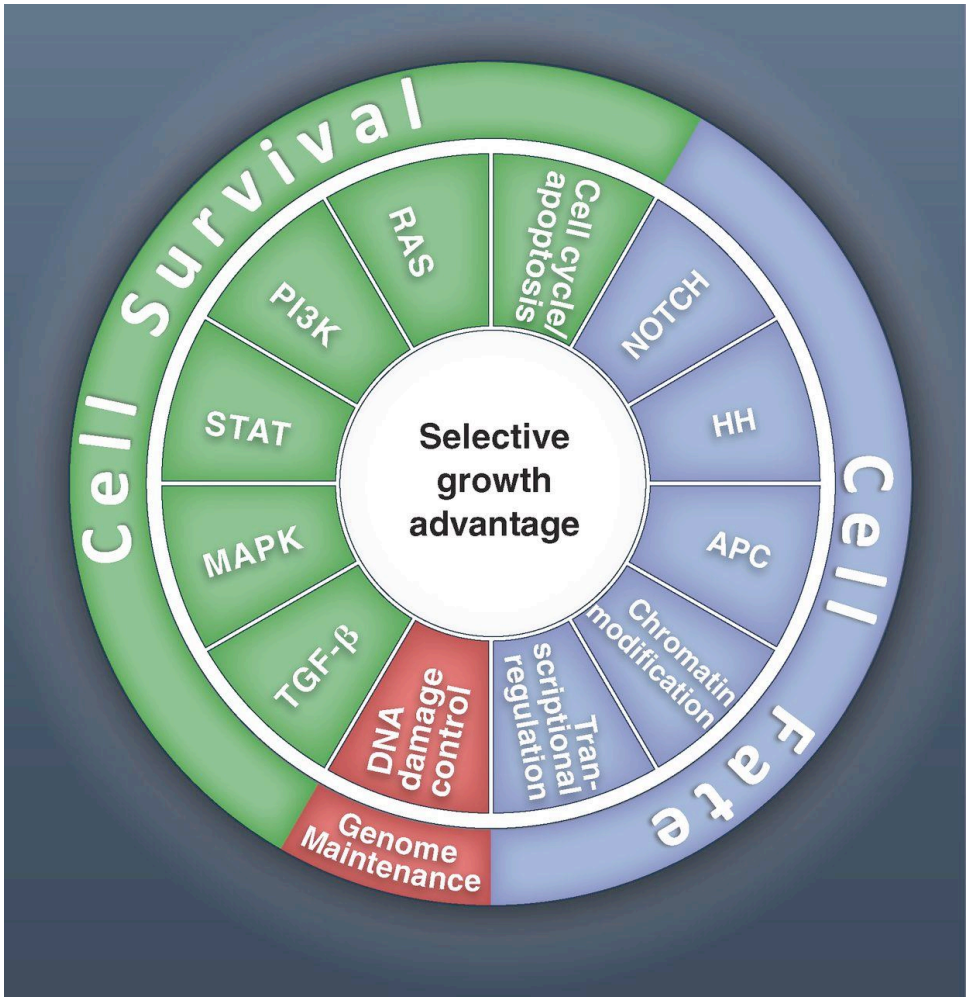
Top: children vs. adults

Numbers in parentheses : median number of nonsynonymous mutations per tumor.

MSI, microsatellite instability;
SCLC, small cell lung cancers;
NSCLC, non–small cell lung cancers;
ESCC, esophageal squamous cell carcinomas;
MSS, microsatellite stable;
EAC, esophageal adenocarcinomas.

B Vogelstein et al. Science 2013; 339:1546-1558

Review (V1) - Cancer driver genes belong to 12 pathways



Cancer cell signaling pathways and the cellular processes they regulate.

All known driver genes can be classified into one or more of 12 pathways (middle ring) that confer a selective growth advantage (inner circle; see main text).

These pathways can themselves be further organized into three core cellular processes (outer ring).

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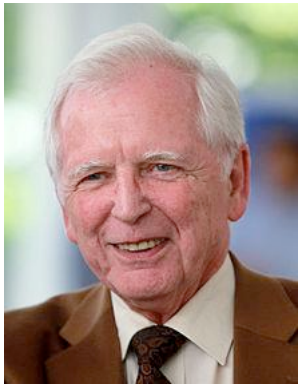
V9 – DNA viruses involved in Cancerogenesis

Human papilloma virus (HPV) causes transformation in cells through interfering with tumor suppressor proteins such as p53.

Interfering with the action of p53 allows a cell infected with the virus to move into S phase of the cell cycle, enabling the virus genome to be replicated.

Some types of HPV increase the risk of, e.g., cervical cancer.



Harald zu Hausen
Noble price for medicine 2008

Epstein-Barr virus

The **Epstein–Barr virus** (EBV), also called **human herpesvirus 4** (HHV-4), is a virus of the herpes family, and is one of the most common viruses in humans.

Most people on earth become infected with EBV and gain adaptive immunity.

EBV infects B cells of the immune system and epithelial cells. While most of the time the infection causes little damage, sometimes the growth activating genes may cause the infected B-cells to turn into cancers in certain people.

Epstein-Barr virus is associated with four types of cancers
- Post-Transplant Lymphoma and AIDS-Associated Lymphoma
- Burkitt's Lymphoma
- Hodgkin's Lymphoma
- cancer of the nasopharynx (the upper part of the throat behind the nose)

The mechanisms how EBV is related to cancerogensis are poorly understood.

Computational systems biology of cancer

LETTER

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Interpreting cancer genomes using systematic host network perturbations by tumour virus proteins

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Working hypothesis:

Authors propose that viruses and genomic variations alter local and global properties of cellular networks in similar ways to cause pathological states.

Study was submitted on June 8, 2011 and accepted only on June 7, 2012!

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Considered virus ORFs

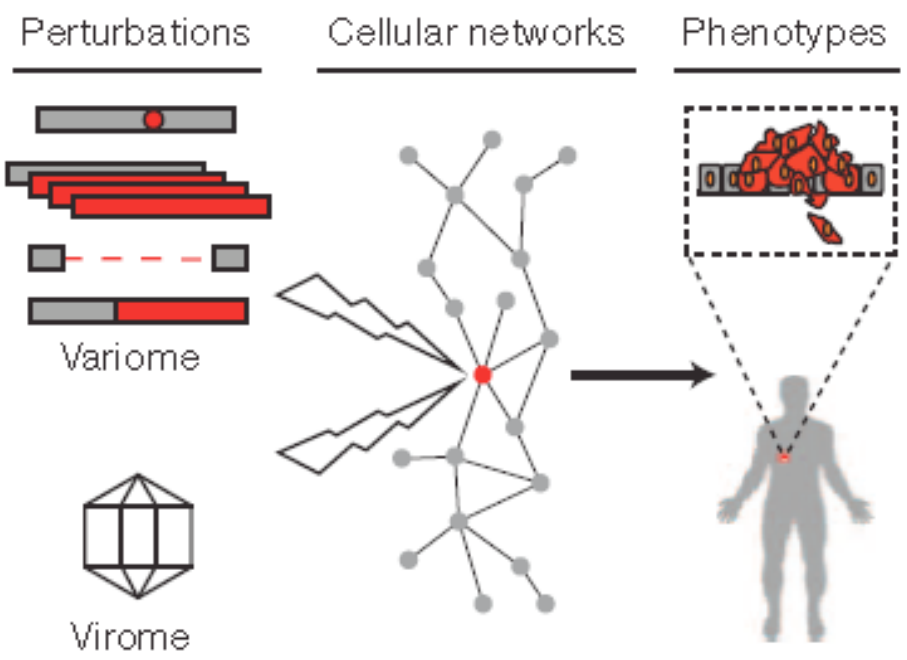
Adenovirus: Nine full length ORFs

Epstein-Barr Virus (EBV): Eighty-one EBV ORFs

Human Papillomaviruses (HPV): Seven HPV types were chosen for this study: HPV6b, 11, 16, 18 and 33 of the alpha genus, and HPV5 and HPV8 of the beta genus

Polyomaviruses: ORF clones were obtained from nine polyomaviruses: BK, HPyV6, HPyV7, JCCY, JCMad1, MCPyV, SV40, TSV and WU.

Virome-to-variome network model



The virome-to-variome network model proposes that genomic variations (point mutations, amplifications, deletions or translocations) and expression of tumour virus proteins induce related disease states by similarly influencing properties of cellular networks.

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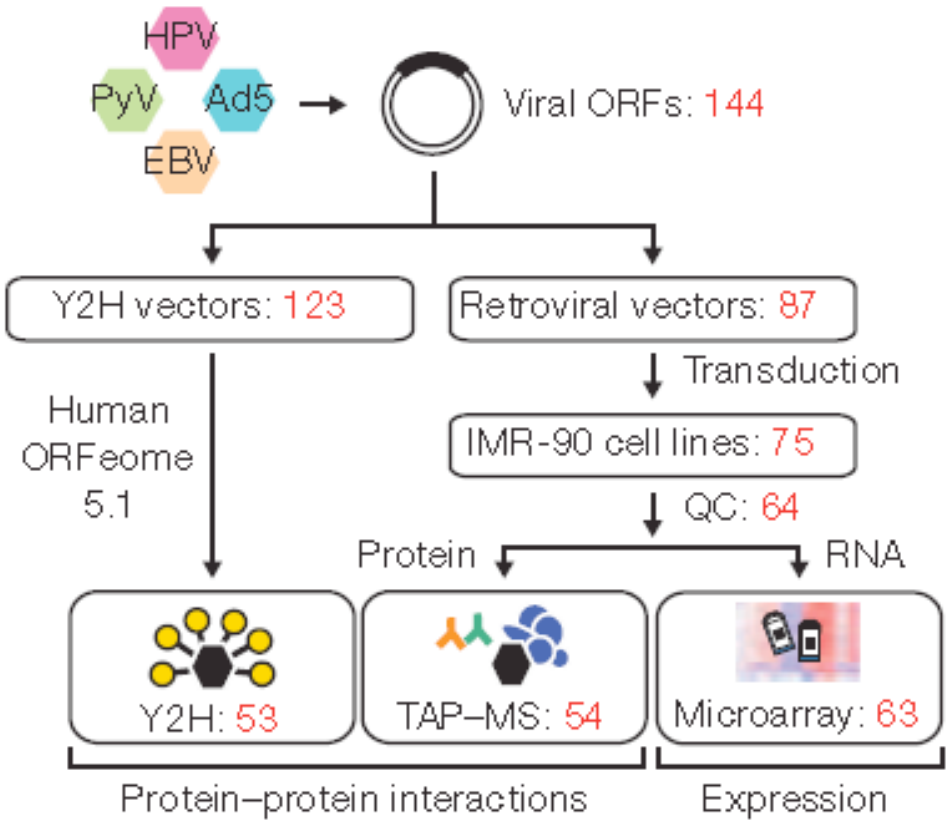
Virus-host protein-protein interactions



Experimental pipeline for identifying virus–host interactions.

123 selected cloned viral ORFs were subjected to Y2H screens against 13000 human ORFs (left), and introduced into cell lines for both TAP–MS and microarray analyses (right).

Numbers of viral ORFs that were successfully processed at each step are indicated in red.

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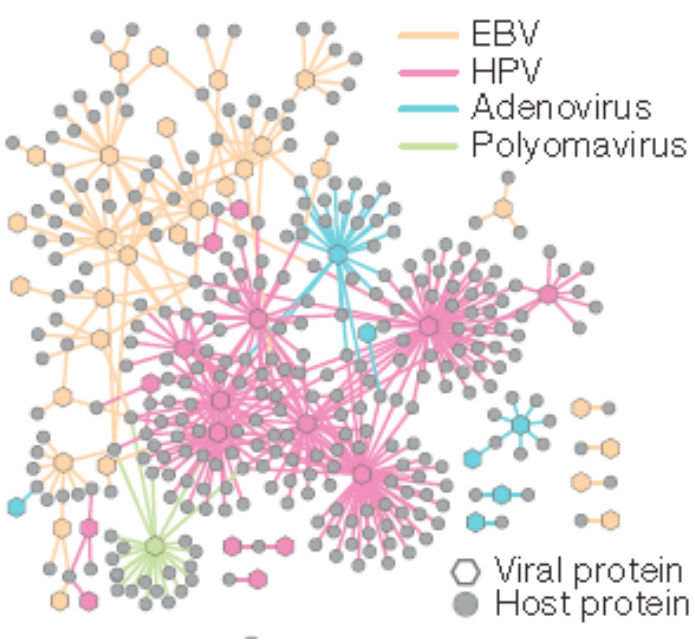
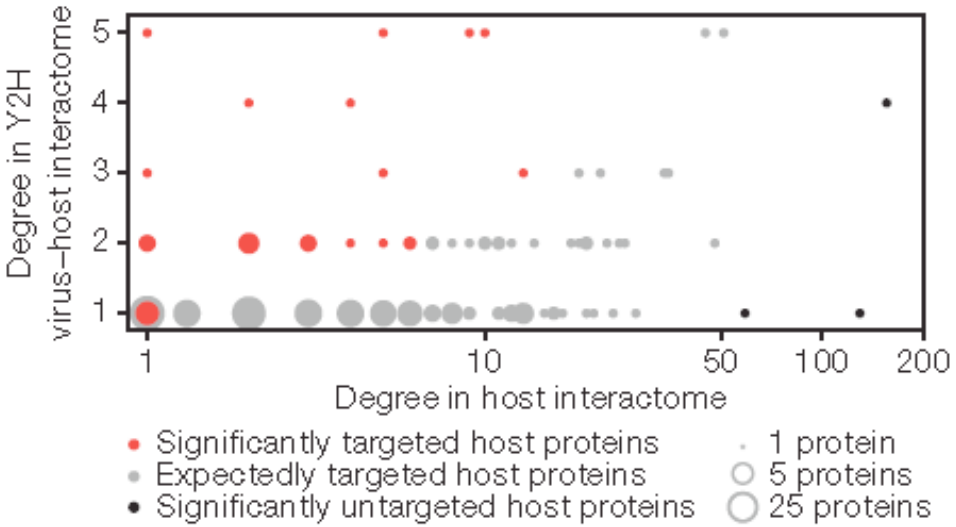
Binary virus-host PPIs identified by Y2H





31 host target proteins showed more binary interactions with viral proteins than would be expected given their ‘degree’ (number of interactors) in the current binary map of the human interactome network (HI-2).

This suggests a set of common mechanisms by which different viral proteins rewire the host interactome network

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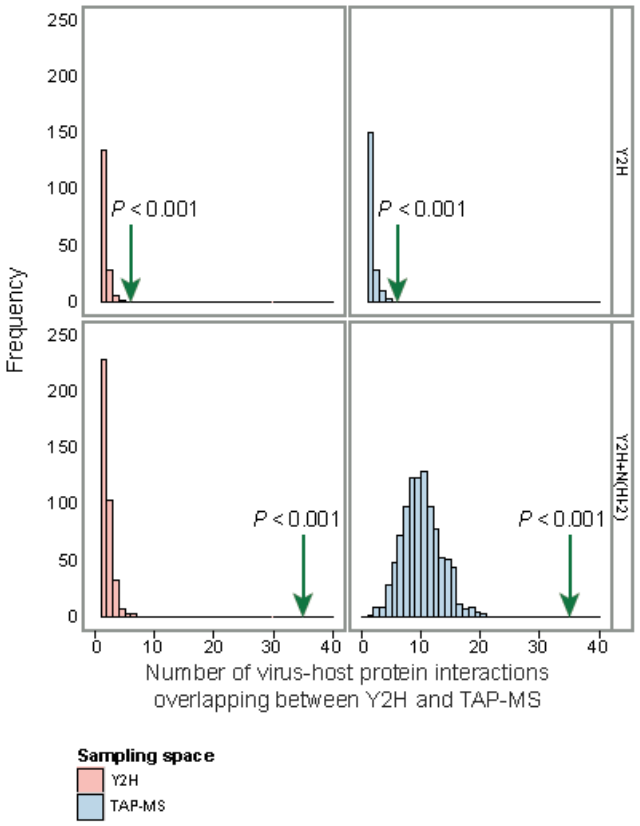
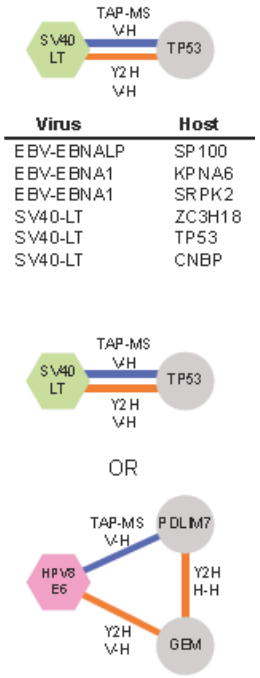
Overlay of Y2H and TAP-MS data sets





Virus	Host
EBV-EBNALP	SP100
EBV-EBNA1	KPNA6
EBV-EBNA1	SRPK2
SV40-LT	ZC3H18
SV40-LT	TP53
SV40-LT	CNBP

Top: number of observed virus-host interactions (arrow) in Y2H and TAP-MS versus those seen by chance through random sampling of the Y2H (red) or TAP-MS (blue) search spaces.
6 interactions were shared (right).

Bottom: corresponding overlaps with expanded (Y2H+N(HI-2)) network, which includes human proteins “one hop” away in the HI-2 human-human interactome network.

Host proteins identified as binary interactors or as members of protein complexes showed a statistically significant overlap (P<0.001) and a statistically significant tendency to interact with each other in HI-2 (P<0.001).
This implies that **host targets** in the virus–host interactome maps tend to fall in the same ‘**neighbourhood**’ of the host network

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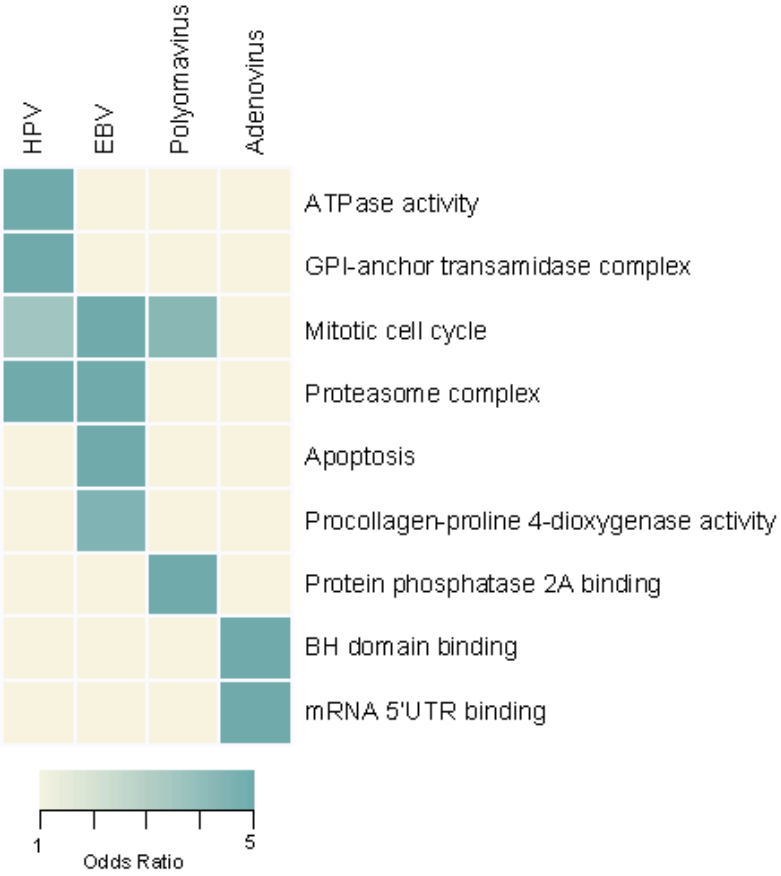
Enriched GO terms for targeted host proteins



With what types of human proteins do viral proteins physically interact?

Enrichment of GO terms for host proteins physically interacting with viral proteins.

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Specificity of virus-host relationships: PPIs involving E6

Check protein complex associations mediated by E6 proteins from 6 distinct HPV types representing 3 different disease classes:

- high-risk mucosal (*dt. (Nasen-)schleim*)
- low-risk mucosal
- cutaneous (*dt. kutan, d.h. Haut betreffend*)

E6 and E7 proteins encoded by high-risk mucosal HPVs are strongly oncogenic.

Multiple host proteins associate with E6 proteins from 2 or more different HPV types ($P < 0.001$).

Transcriptional regulators CREBBP and EP300 only associate with E6 proteins from cutaneous HPV types, but not with those from mucosal classes.

In contrast, no group of host proteins showed class-specific targeting by HCV E7 proteins.

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E6 protein

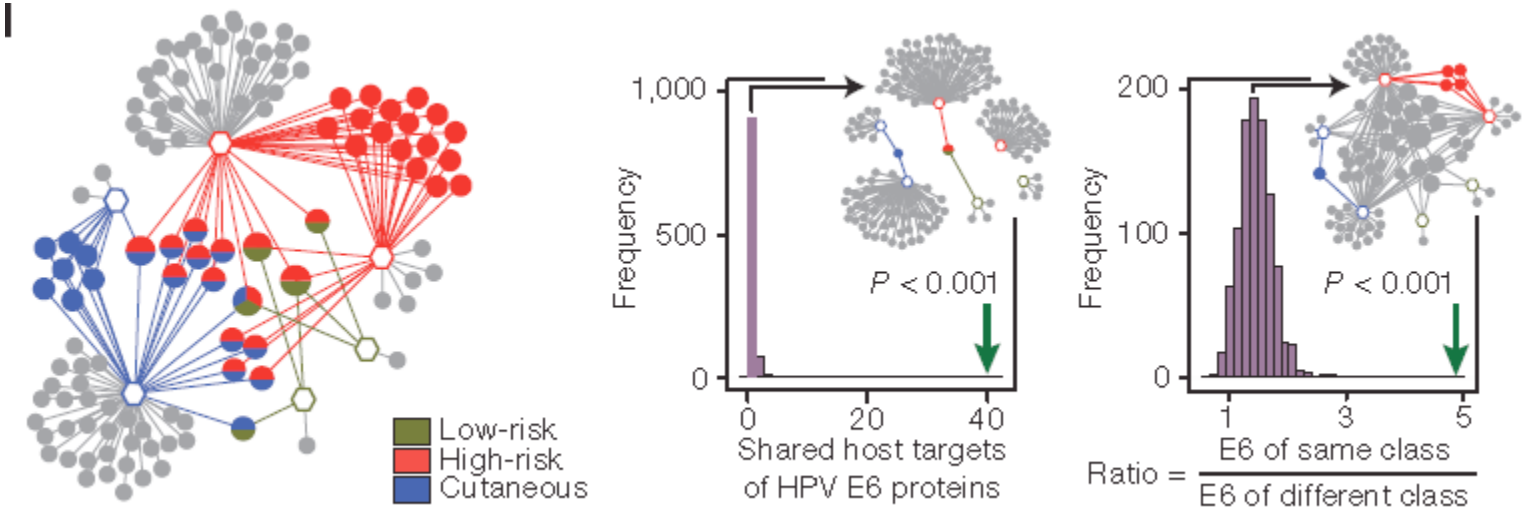
E6 associates with host E6-AP ubiquitin-protein ligase, and **inactivates tumor suppressors TP53 and TP73** by targeting them to the 26S proteasome for degradation.

Other cellular targets including Bak, Fas-associated death domain-containing protein (FADD) and procaspase 8, are degraded by E6/E6AP causing **inhibition of apoptosis**.

E6 also **inhibits immune response** by interacting with host IRF3 and TYK2.

These interactions prevent IRF3 transcriptional activities and inhibit TYK2-mediated JAK-STAT activation by interferon alpha resulting in **inhibition of the interferon signaling pathwa**y.

Protein complex associations involving E6 proteins



Left: Network of protein complex associations of E6 viral proteins from 6 HPV types (hexagons, coloured according to disease class) with host proteins (grey circles).
Host proteins that associate with 2 or more E6 proteins are colored according to the disease class(es) of the corresponding HPV types. Circle size is proportional to the number of associations between host and viral proteins in the E6 networks.
Middle: Distribution plots of 1,000 randomized networks and experimentally observed data (green arrows) for the number of host proteins targeted by 2 or more viral proteins in the corresponding subnetworks.,
Right: ratio of the probability that a host protein is targeted by viral proteins from the same class to the probability that it is targeted by viral proteins from different classes.
Insets: representative random networks from these distributions

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Computational systems biology of cancer

Besides targeting, protein-protein interactions, viral proteins functionally **perturb** their **hosts** through downstream **effects on gene expression**.

→ Profile transcriptome of viral ORF-transduced cell lines to trace pathways through which viral proteins could alter cellular states.

-> 2944 frequently perturbed host genes.

- Clustering gives 31 clusters

- Many of the clusters are enriched for specific GO terms and KEGG pathways ($p < 0.01$)

- Identify enriched TF binding motifs in gene promoters or enhancers from data on cell-specific chromatin accessibility and consensus TF-binding motifs.

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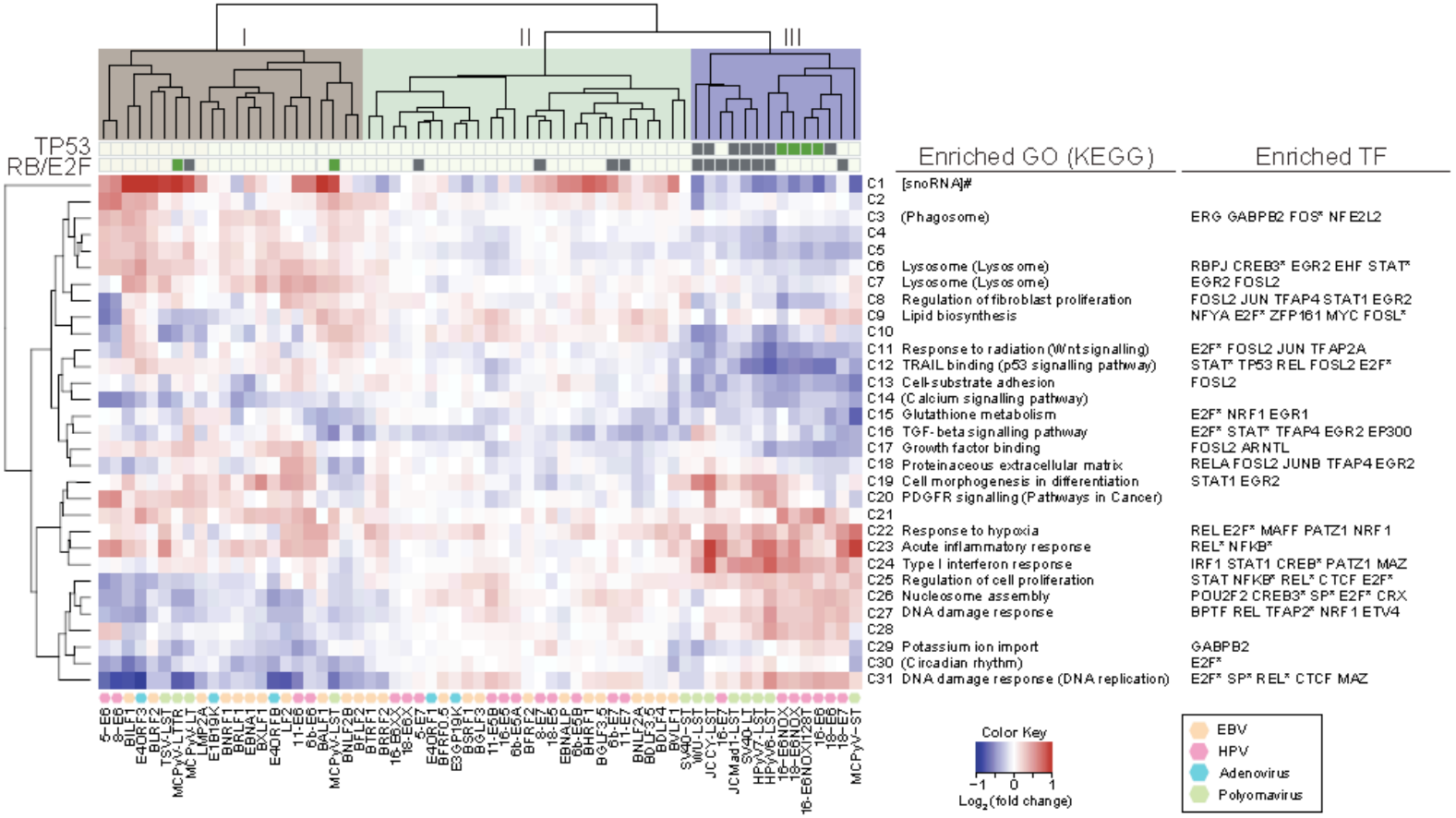
Heatmap of transcriptome perturbations



Enriched GO terms and KEGG pathways are listed adjacent to the numbered expression clusters. TFs with enriched binding sites and gene targets enriched for the listed GO and/or KEGG pathways that are physically associated with or differentially expressed in response to viral proteins are shown, with * denoting multiple members of a TF family. Up to 5 TFs are shown for any cluster. Blocks show which viral proteins associate with the indicated host proteins, as detected in our data set (grey) or manually curated (green).

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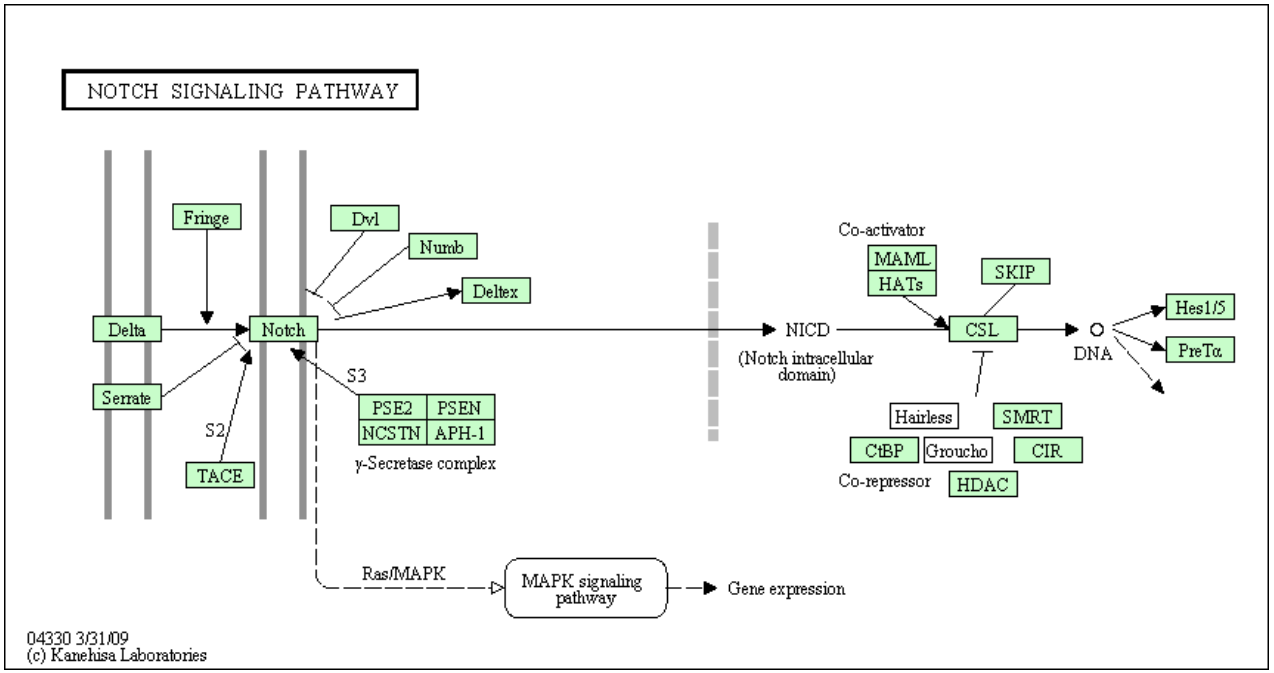
Notch pathway



Perturbations in Notch signalling can confer either oncogenic or tumour-suppressive effects.

Because both inhibition of the Notch pathway and the expression of HPV8 E6 promote squamous cell carcinoma, we reasoned that binding of HPV5 and HPV8 E6 to MAML1 might inhibit Notch signalling.

To test this, examine transcript levels of Notch pathway genes and potential Notch target genes with a predicted RBPJ binding site in their promoter across all HPV E6 cell lines as well as in cells depleted for MAML1.

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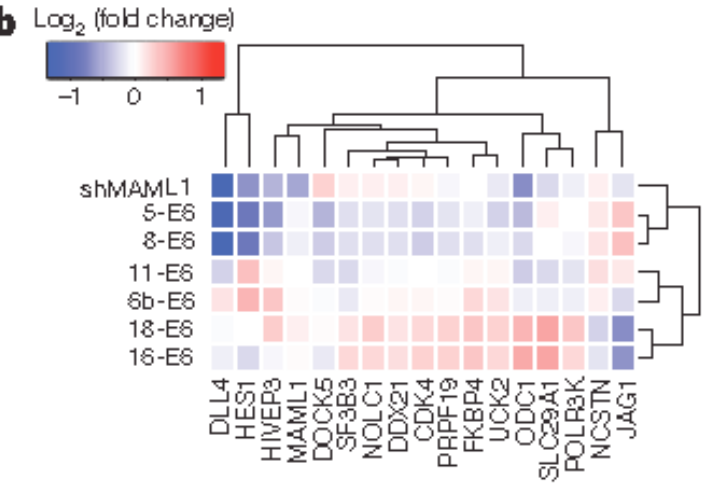
Association of HPV E6 proteins with MAML1 inhibits Notch



Heat map of expression of Notch-pathway-responsive genes in IMR-90 cells on expression of E6 proteins from different HPV types or on knockdown of MAML1, relative to control cells.

Transcript levels of several Notch targets were significantly decreased in IMR-90 cells that were either depleted for MAML1 or expressing either HPV5 or HPV8 E6.

This indicates that the association of HPV5 and HPV8 E6 proteins with MAML1 inhibits Notch signalling.

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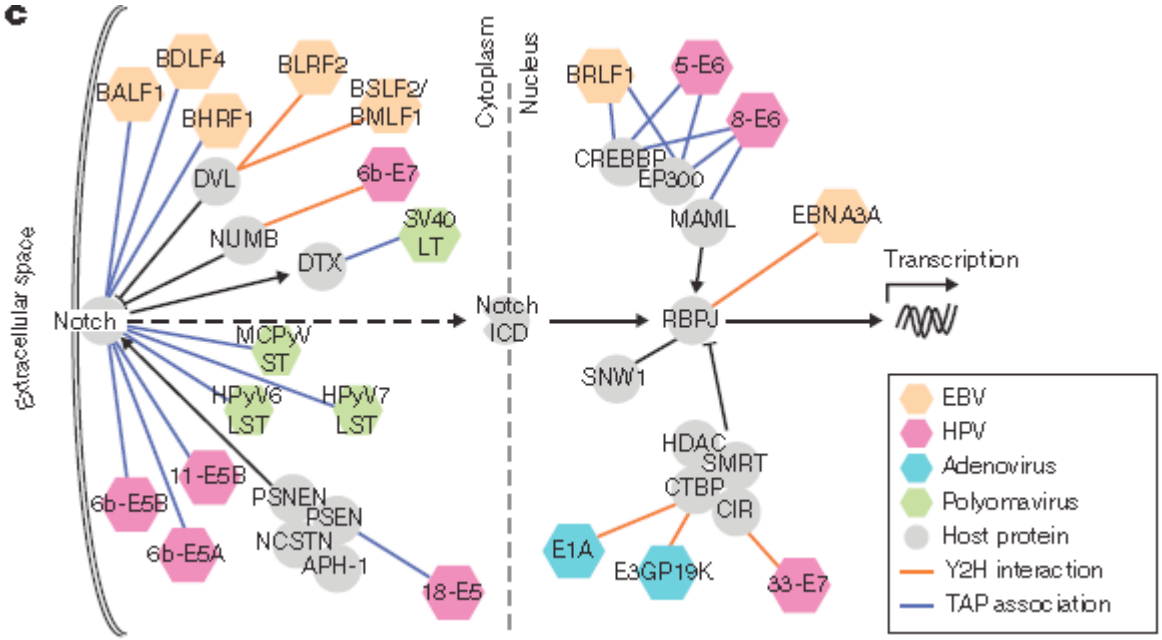
How viral proteins interact with proteins in Notch signaling



Representation of viral protein interactions with components of the Notch signalling pathway.

Notch ICD, Notch intracellular domain.

→ viral proteins from all four DNA tumour viruses target proteins of the Notch pathway ($P < 0.002$).

This highlights the central role of Notch signalling in both virus–host perturbations and tumorigenesis, and support observations that implicate MAML1 in cancer pathogenesis.

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Computational systems biology of cancer

To which extent do viral proteins globally target host proteins causally implicated in cancer?

Compare the viral targets, identified through binary interaction, protein complex associations and TF-binding-site analyses, against a gold standard set of 107 high-confidence causal human cancer genes in the COSMIC Classic (CC) gene set.

Viral targets were significantly enriched among CC genes (P=0.01).

To optimize the stringency of potential cancer enrichment analyses, restrict the set of viral protein targets identified by TAP–MSto those identified by 3 or more unique peptides, a choice corresponding to an experimental reproducibility rate greater than 90%.

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Virus-host network model

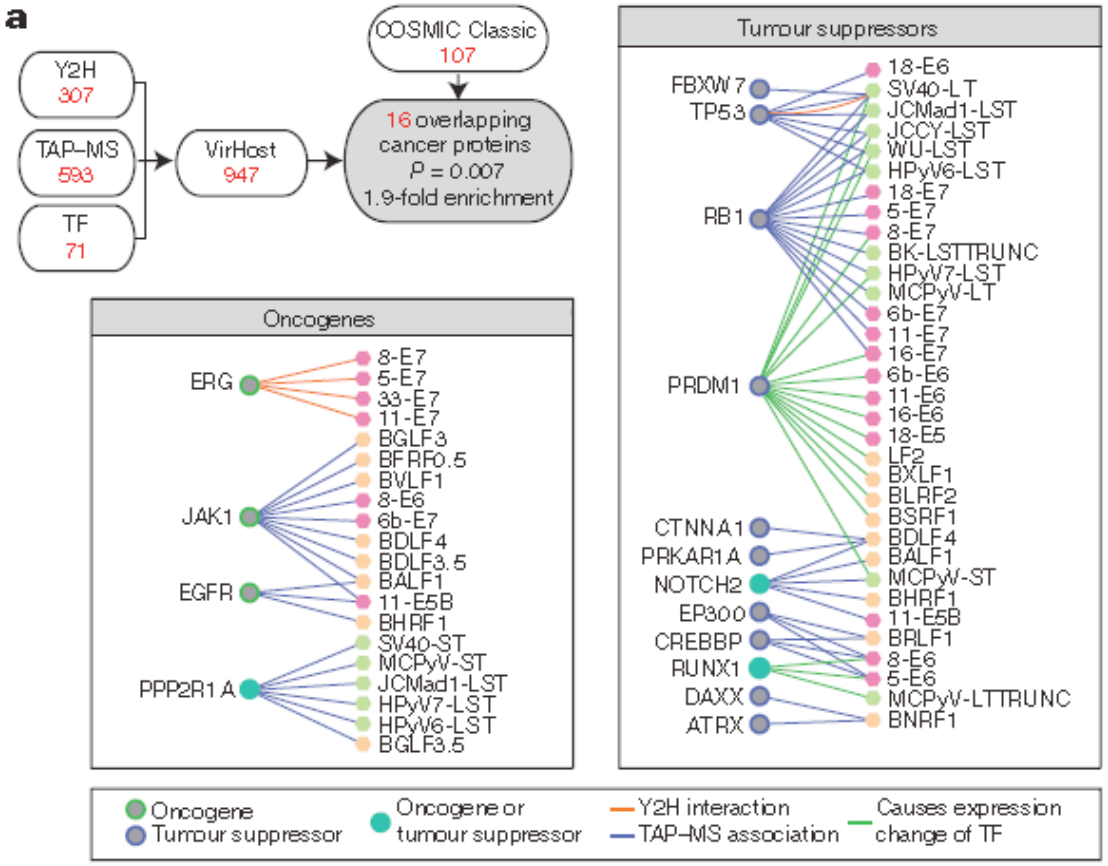

Diagram describing the composition of VirHost (947 proteins identified by TAP–MS with at least 3 unique peptides, Y2H and TF) and overlap with COSMIC Classic (CC) genes.

'VirHost' set includes 16 proteins encoded by CC genes (P=0.007), among which tumour suppressor genes were significantly over-represented (P=0.03).

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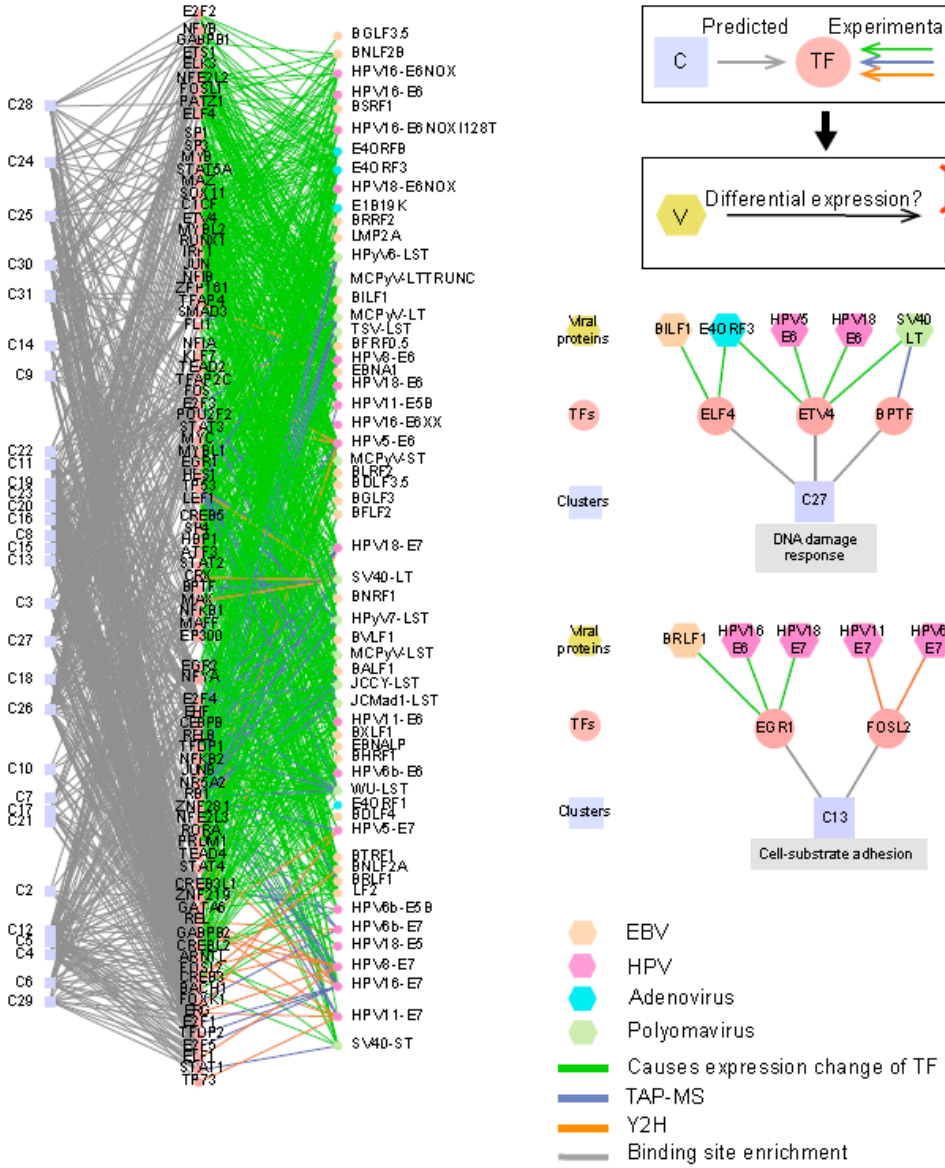
Viral proteins, transcription factors and clusters



(Left) Network representation of all predicted viral protein-TF-cluster cascades.

(Right) Schematic shows how viral protein-TF-target gene network was constructed

(Below) representative networks.

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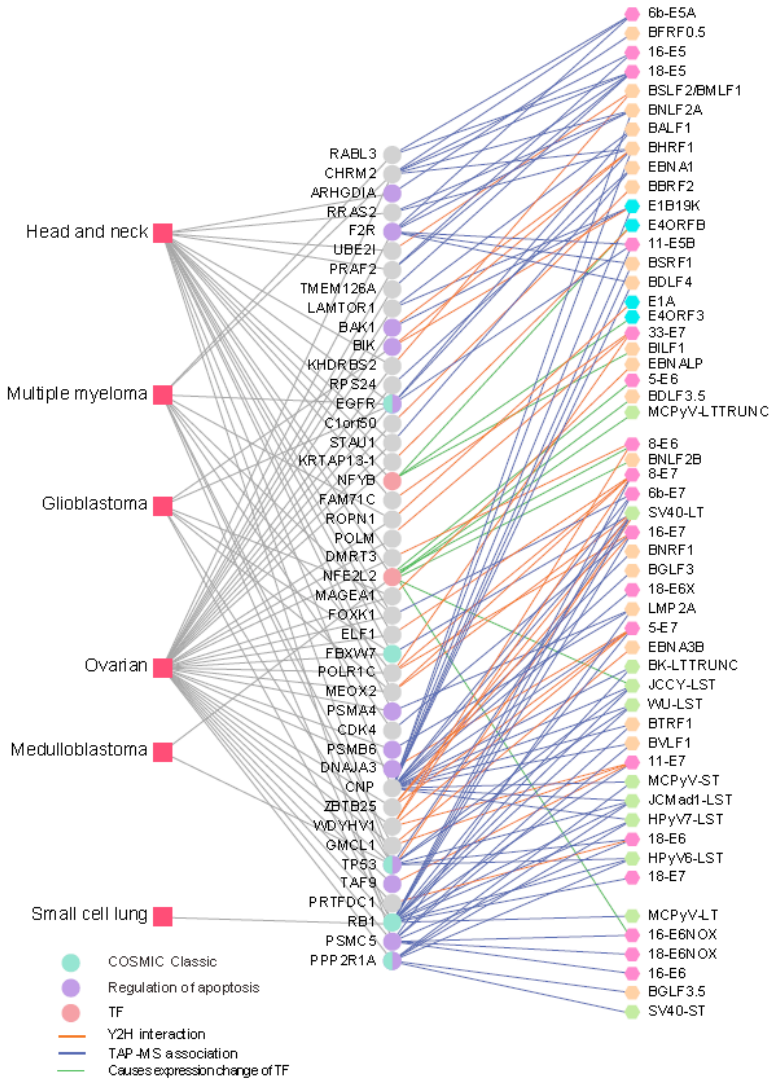
Network of VirHostSM to host targets and cancers

Mapping of VirHostSM gene products to both tumours in which they are mutated (left) and to viral interactors (right).

Proteins annotated with the GO term “regulation of apoptosis” indicated in purple.



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Summary

If the mechanisms of cancer formation induced by genetic mutations and by DNA viruses are indeed similar,
this opens up interesting possibilities to study cancerogeneis by controlled viral infection.

Network view correponds to modern field of cancer systems biology.

Important for drug design.

Study which individuals are susceptible to viral infection and which ones are not?