AACR 2018

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INSPIRED CELL SOLUTIONS

Interrogate your gene of interesting with CRISPR knockout cell lines

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Interrogate your gene of interesting with CRISPR knockout cell lines

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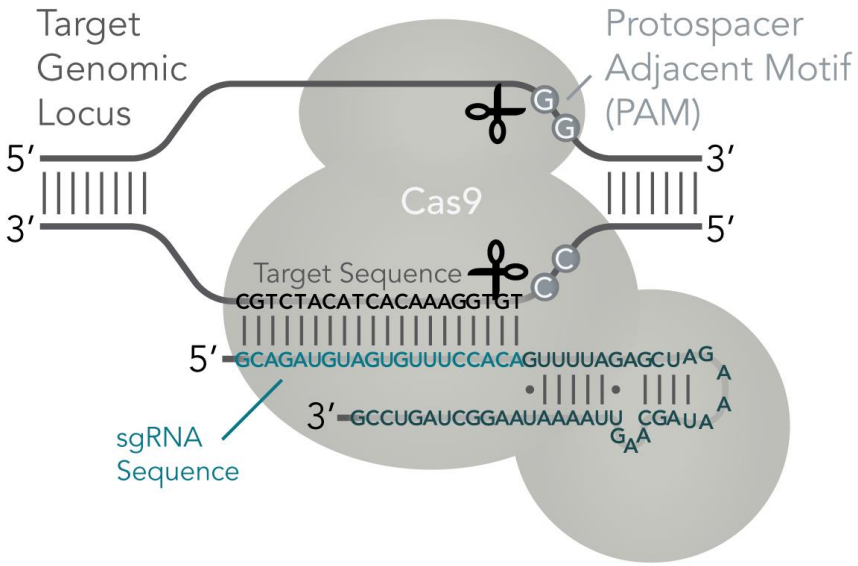
INSPIRED CELL SOLUTIONS

- **Getting the most from your research**

  - ✓ Validating research tools
  - ✓ Identifying key players in a pathway
  - ✓ Using proper controls
  - ✓ Connecting patient relevant mutations to pathology

What is CRISPR-Cas9?



gRNA target sequence PAM

AGCTGGGATCAACTATAGCG

NGG

CRISPR Mediated Genome Editing

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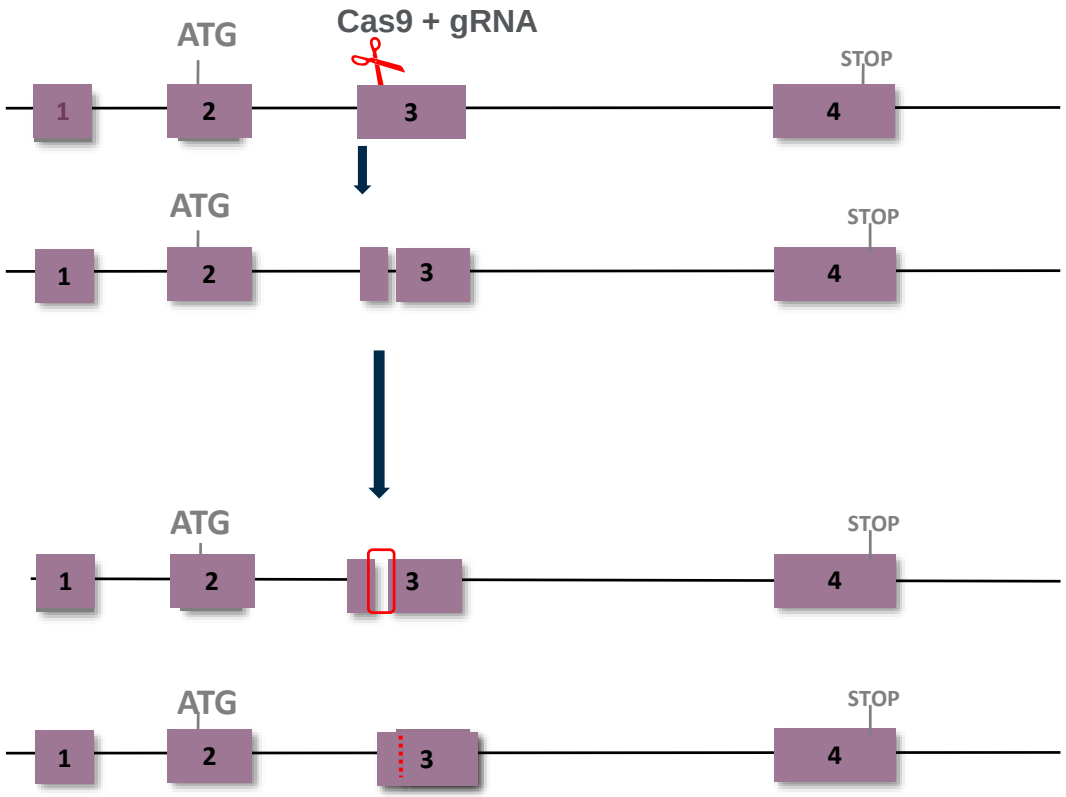
sgRNA to target loci

CRISPR/Cas9-induced double strand break

Non-homologous end joining

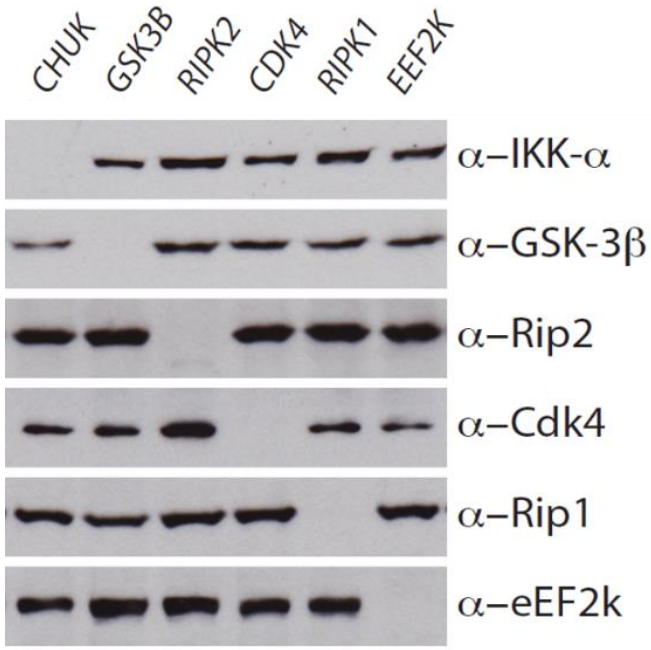
Frameshift knockout mutation



Antibody validation

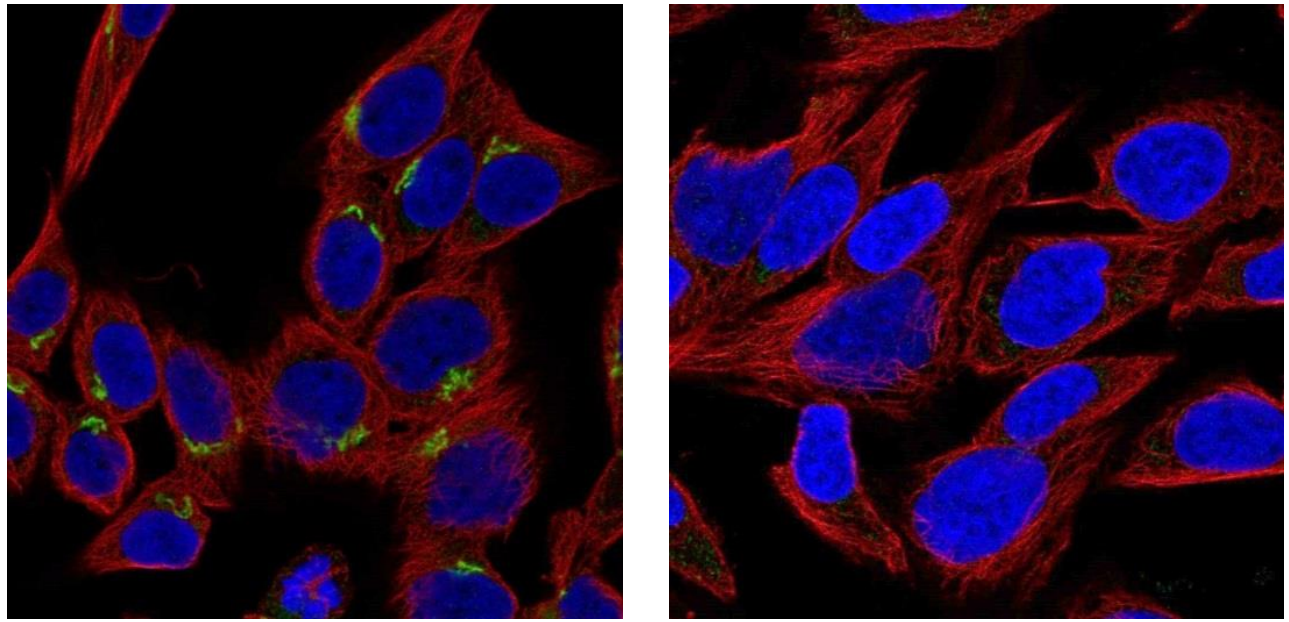
- **Specificity**
- **Reproducibility**
- **Application specific**

Western Blot



A selection of gene edited KO cell lines. Western blot data confirms absence of protein, and so validates the antibody used .

Immunofluorescence

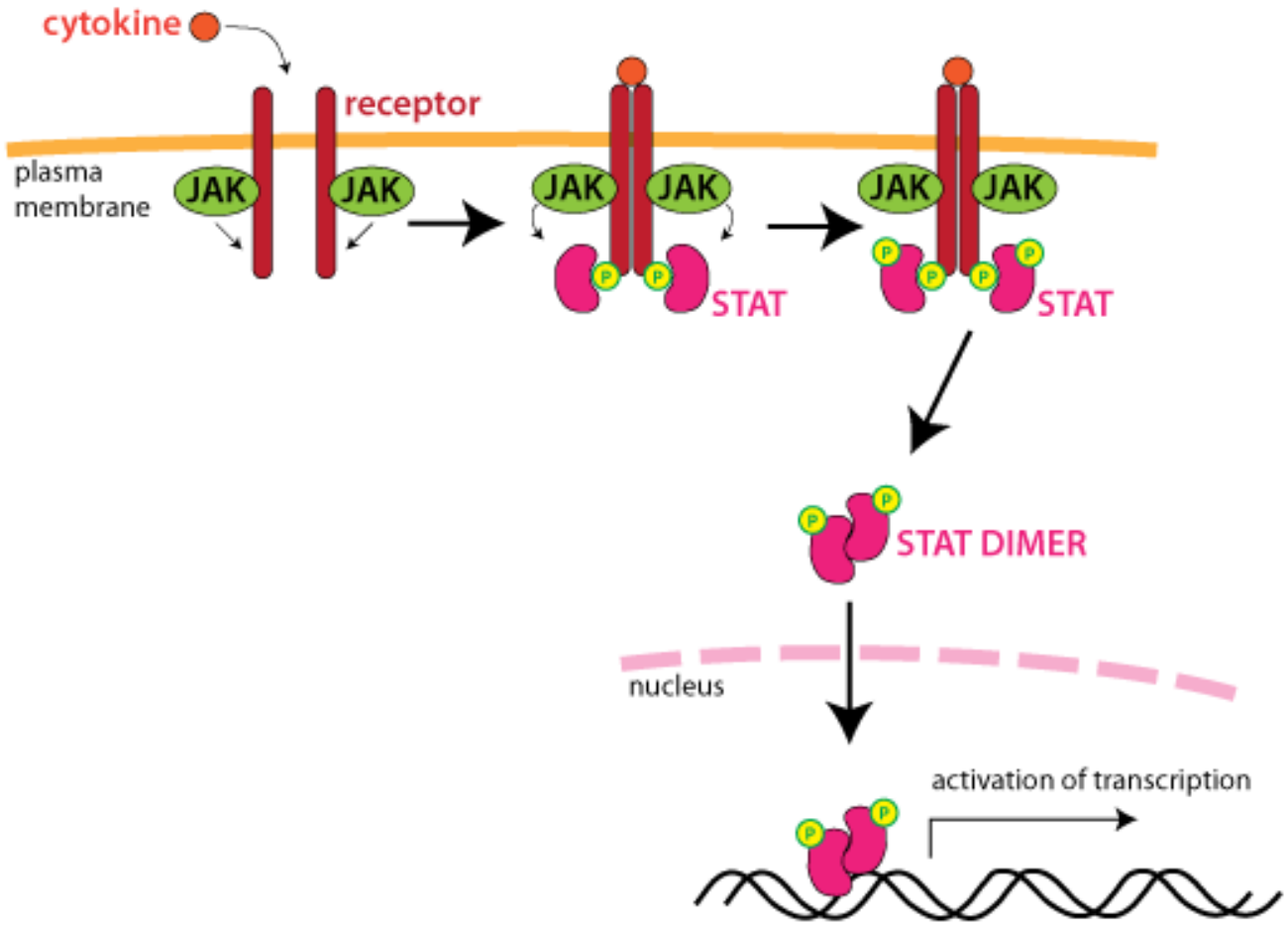


Images curtesy of Dr Emma Lundberg, Cell Profiling facility. KTH Royal Institute of Technology

Green	SLC30A6
Blue	Nucleus
Red	Microtubules

Pathway interrogation: JAK - STAT

- **Often multiple related but distinct molecules and processes are present.**
- **Gene-edited cell lines are a well-established tool to probe the role of a particular gene or mutation**

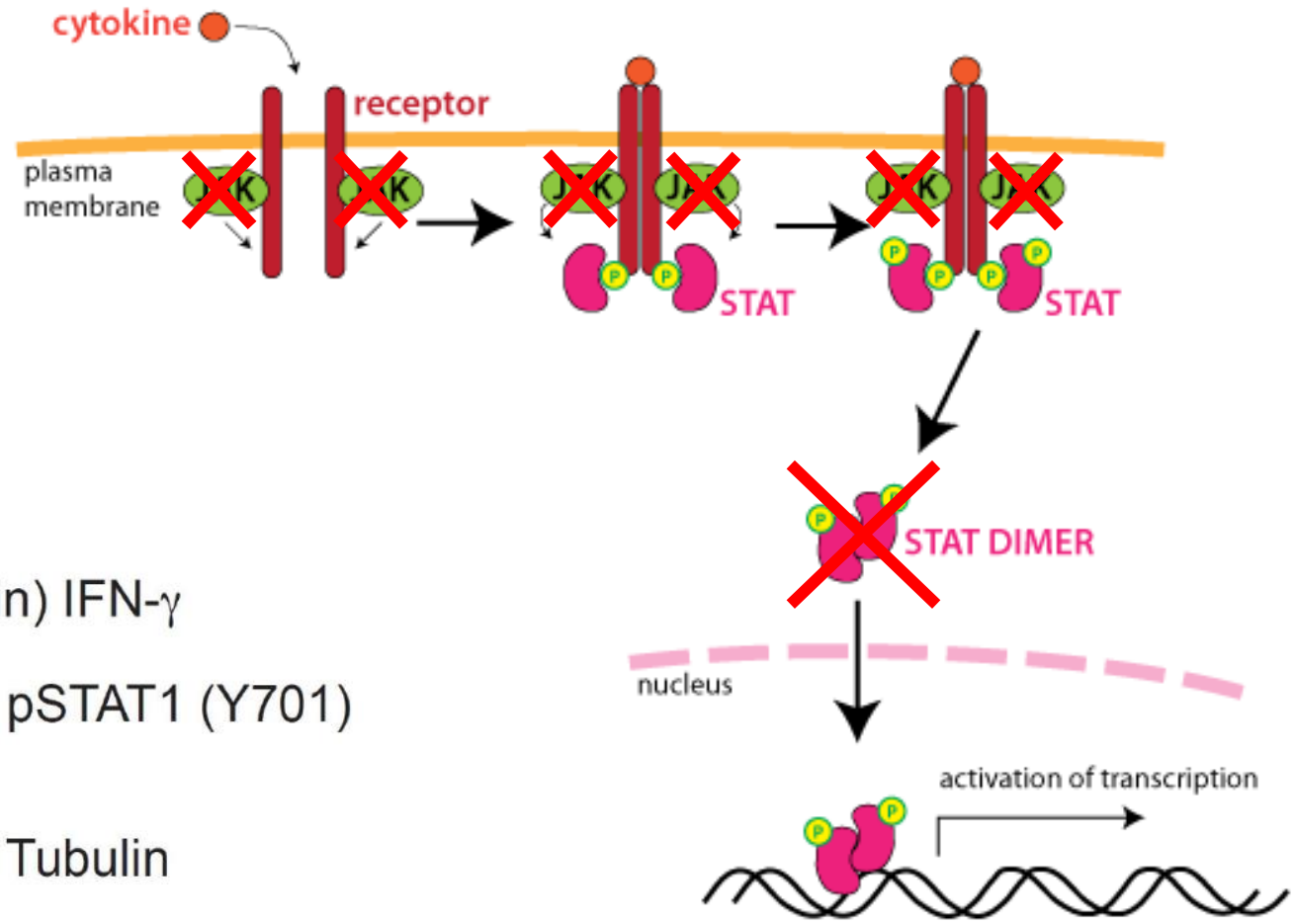


- ✓ **Heterodimerization of INF-g receptor (INFGR1, INFGR2)**
- ✓ **Phosphorylation of STAT**
- ✓ **Expression of target genes**

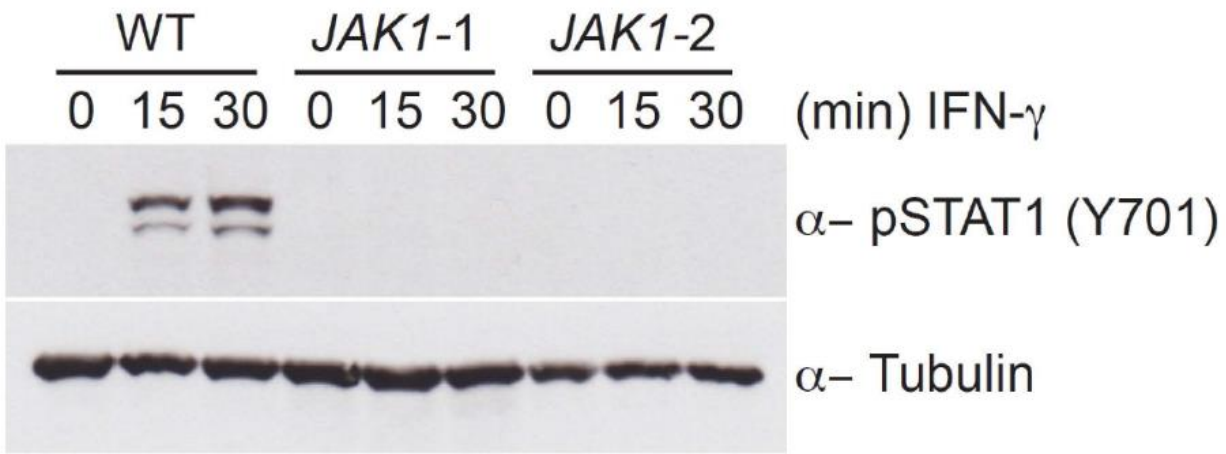
https://courses.washington.edu/conj/bess/jakstat/jakstat.htm

Pathway interrogation: JAK - STAT

- **Knockout of the gene of interest disrupts cytokine response**



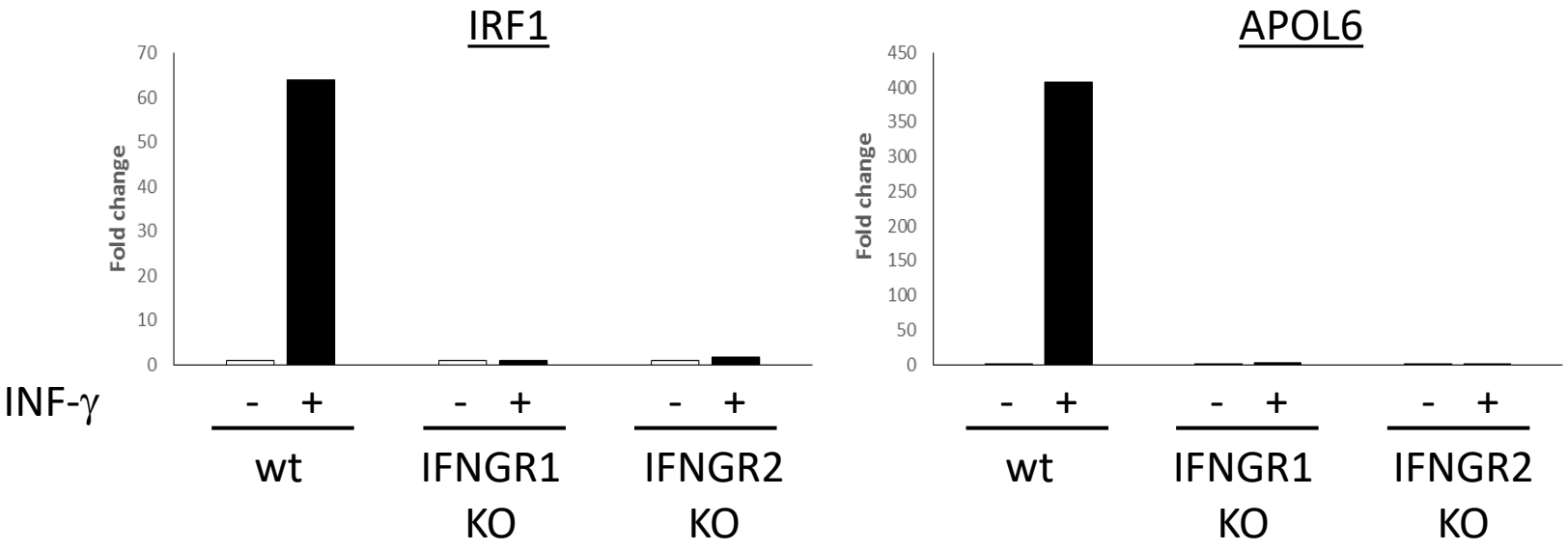
- **Phosphorylation of STAT1 is absent in JAK1 knockout clones**
- **Multiple clones for extra controls**

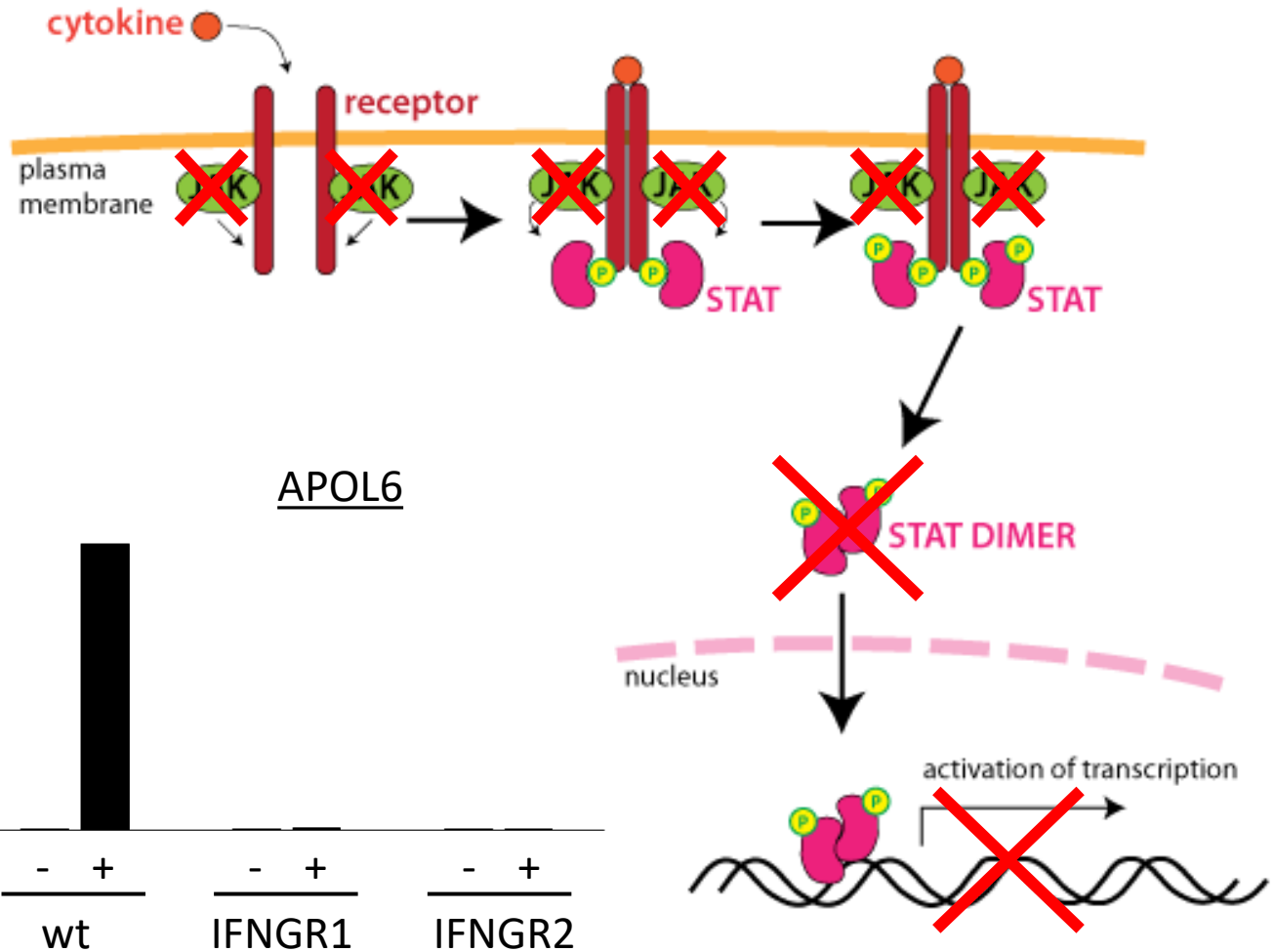


Pathway interrogation: JAK – STAT Downstream

- **IRF1 (Interferon regulatory factor 1) and APOL6 (Apolipoprotein L6)**

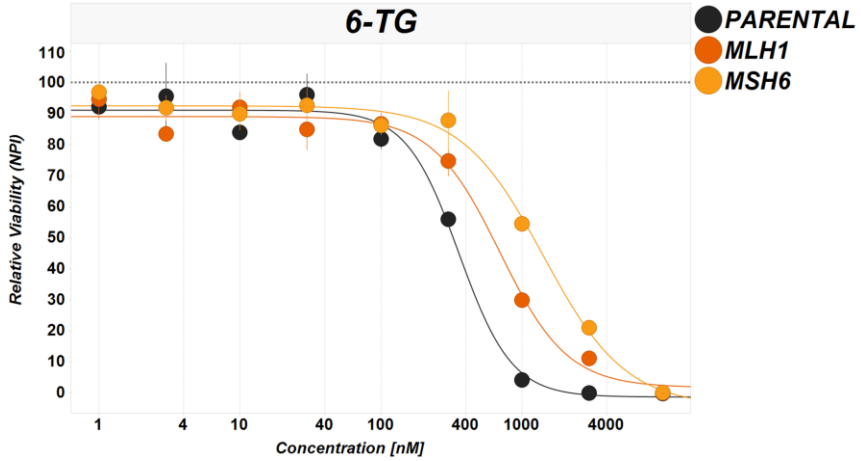
  - **No induction of target genes in INF-g receptor knockout clones**



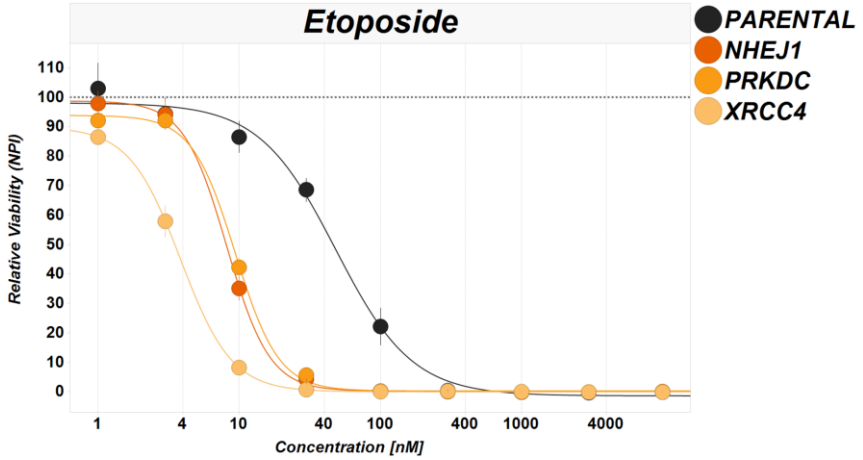


Probing DNA Damage Response Pathways

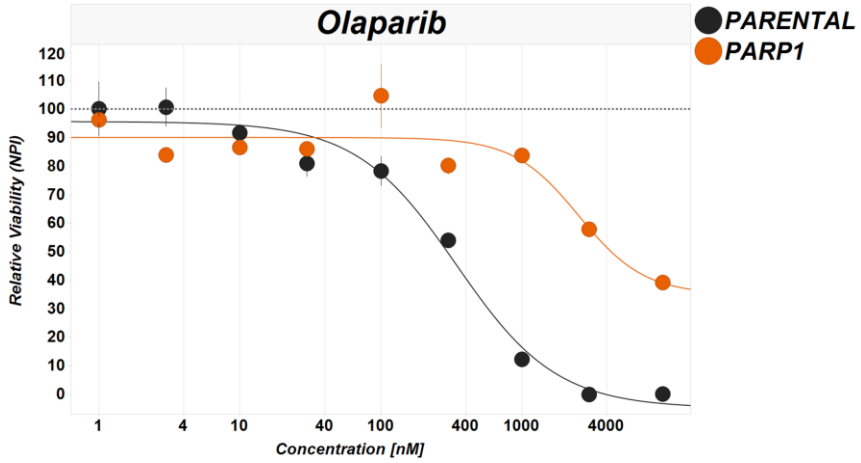
DNA mismatch repair



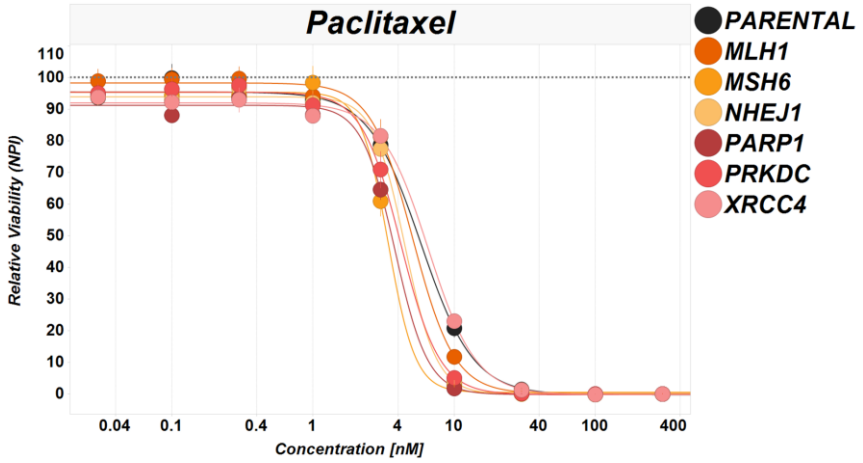
DNA double strand breaks



Single strand break (SSB) repair

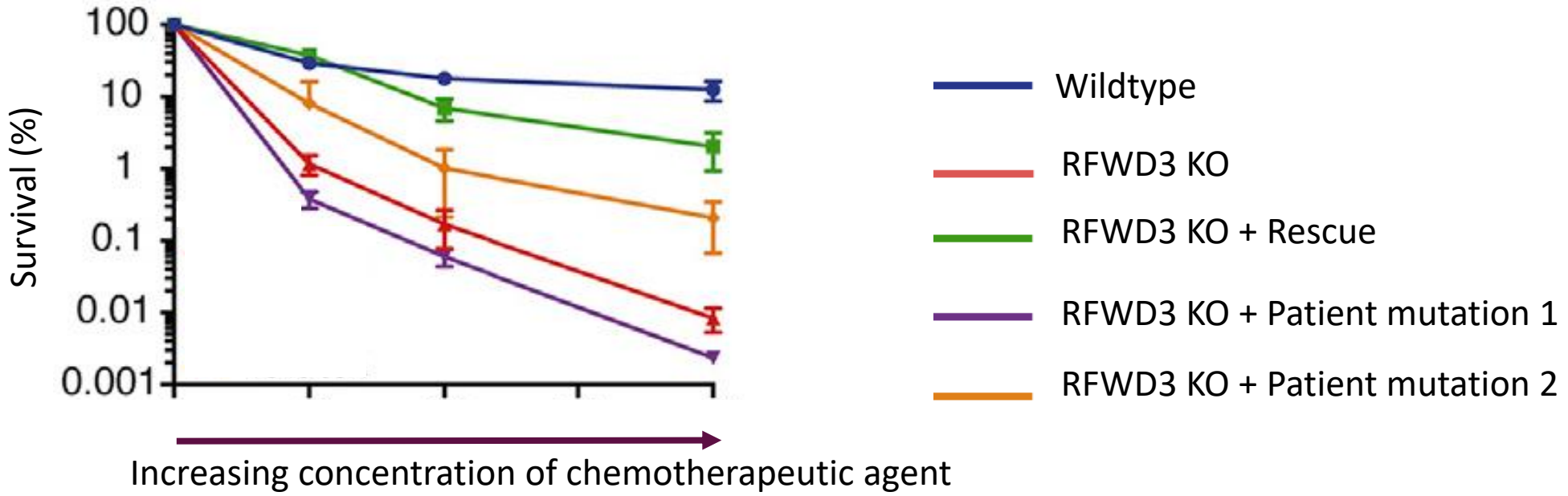


Control



Connecting patient relevant mutations to pathology

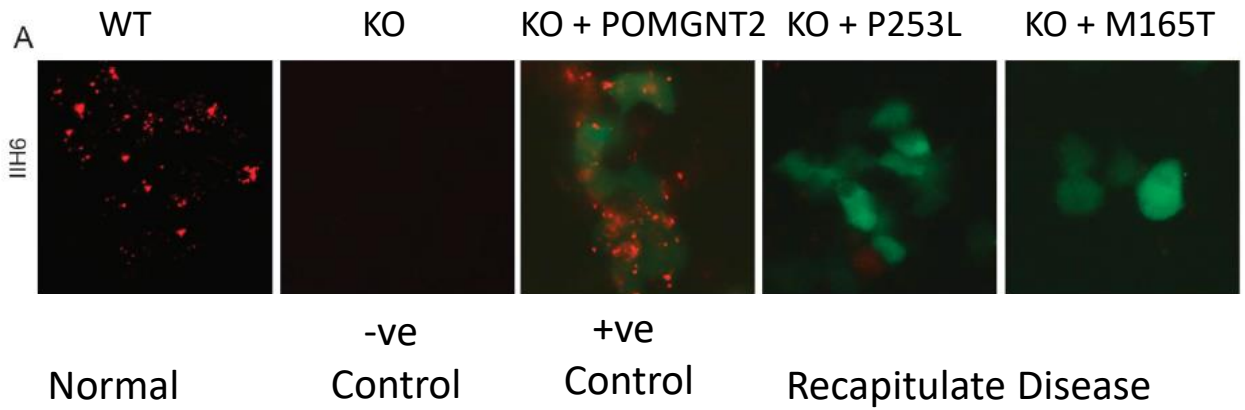
- **Probe the biological mechanisms that result in the disease phenotype, using patient mutations**
- **The Knockout Cell Lines collection combined with simple molecular biology techniques allows you to**
  - ✓ Rescue the gene of interest to restore phenotype
  - ✓ Complement the Knockout cell line with a functional mutation



Adapted from Inano et al., 2017, Molecular Cell 66, 622–634

Connecting patient relevant mutations to pathology

Milder forms of muscular dystrophy associated with POMGNT2 mutations



- Mutant POMGNT2 exhibits defects in reactivity to the anti-a-dystroglycan antibody, IIH6
- The POMGNT2-KO HAP1 cells were rescued by WT cDNA
- By contrast, mutant POMGNT2-KO HAP1 cells, fail to rescue the IIH6 positive phenotype

Adapted from: Milder forms of muscular dystrophy associated with POMGNT2 mutations; Neurol Genet. 2015 Dec 10;1(4):e33 Endo et al

Connecting patient relevant mutations to pathology

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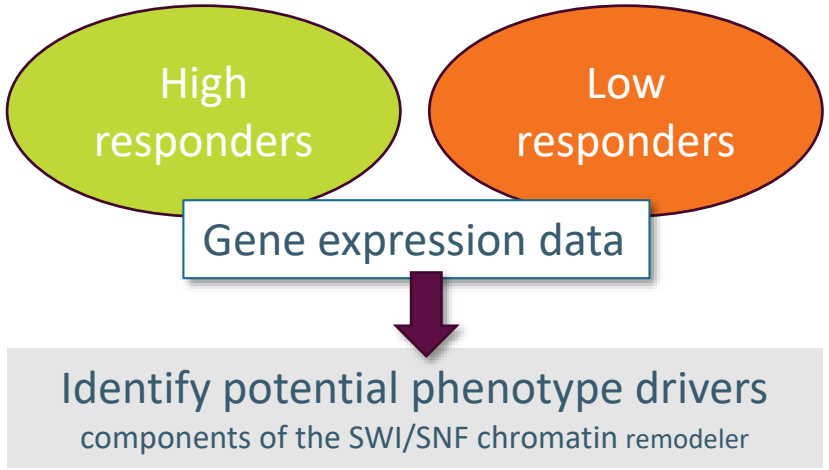
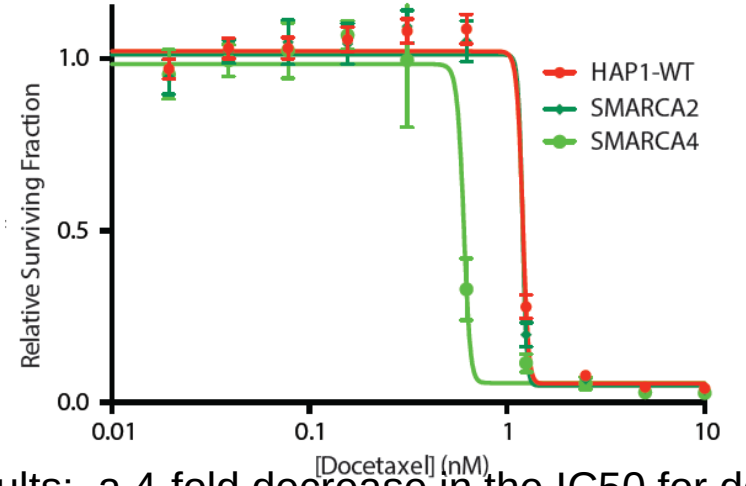
1. Patient samples



2. Extend findings to 60 diverse human cancer cell lines:

Expression of SWI/SNF components inversely correlates with docetaxel sensitivity.

3. Confirm these finding using HAP1 KO cell lines



Results: a 4-fold decrease in the IC50 for docetaxel in the SMARCA4-KO cell line relative to wildtype

Summary:
Functional association between the SWI/SNF chromatin remodeler and the docetaxel response shown in:

- Patient material
- NCI-60 database
- And **confirmed** in KO cell line

Interrogate your gene of interesting with CRISPR knockout cell lines

- **Horizon's Knockout Cell Line Collection**
  - ✓ CRISPR Knock out cell lines
  - ✓ Multiple clones available
  - ✓ 1000 gene targets available
  - ✓ 3000 gene targets ready to ship

- **Getting the most from your research**
  - ✓ Validating research tools
  - ✓ Identifying key players in a pathway
  - ✓ Using proper controls
  - ✓ Connecting patient relevant mutations to pathology

Thank you for your attention

Have a question?

Get in touch:

www.horizondiscovery.com/contact-us