VERIFY Tagged Antigen™

Validation Data

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Potential Applications

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1. Protein purifications
2. Protein function studies
3. Antibody validations in Western blot, ELISA, immunoprecipitation, etc.
4. Assay standards
5. Protein-protein interaction
6. Prepare reverse phase protein arrays

Protein Production in HEK293T Cells

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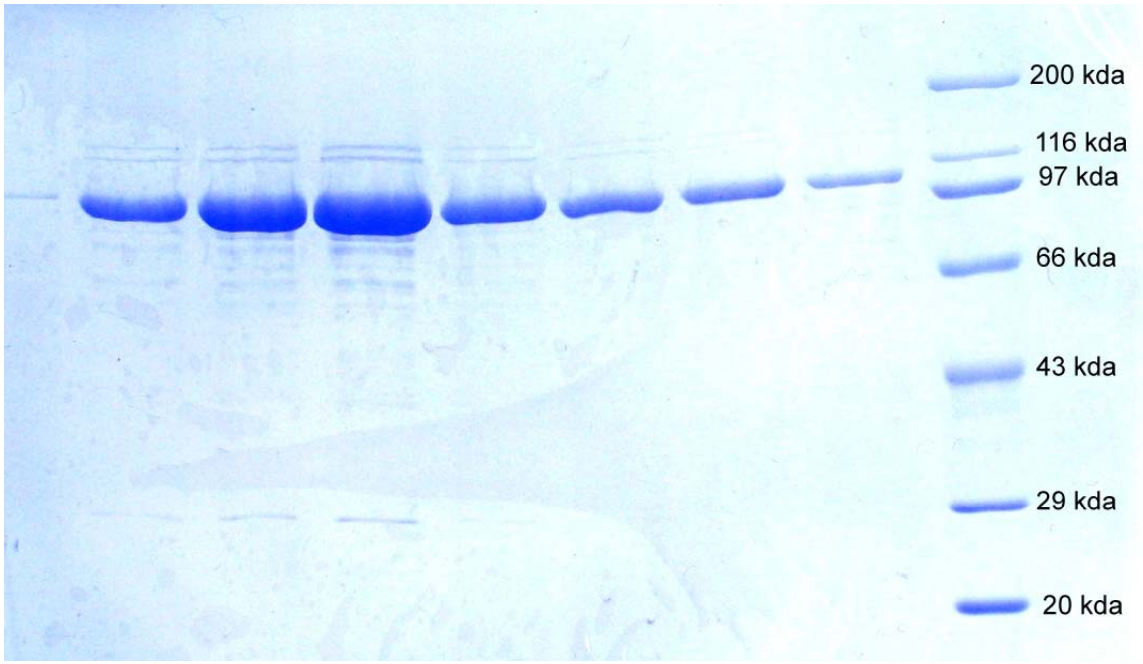

HEK293T cells were transfected with TrueORF INPP5K clone (RC202190). Cells were lysed 48hrs later for protein purification using M2 beads (anti-DDK). Final purified protein is > 80% pure in Comarssie Blue staining.

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Study Protein Functions

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Protein purification using anti-tag antibody and *in vitro* function assays

Vol 456 | 20 November 2008 | doi:10.1038/nature07470 nature

ARTICLES

Identification of Holliday junction resolvases from humans and yeast

Stephen C. Y. Ip[1]*, Ulrich Rass[1]*, Miguel G. Blanco[1]*, Helen R. Flynn[2], J. Mark Skehel[2] & Stephen C. West[1]

- For over 20 years, little was known about eukaryotic Holliday junction resolvase
- GEN1 TrueORF clone with FLAG tag (RC221451) was transfected into HEK293T cells
- GEN1 protein was purified using anti-FLAG M2 affinity column
- Purified GEN1 protein resolved Holliday junction X0 efficiently in vitro
- Both GEN1 and Yen1 were identified as resolvases for eukaryotic cells

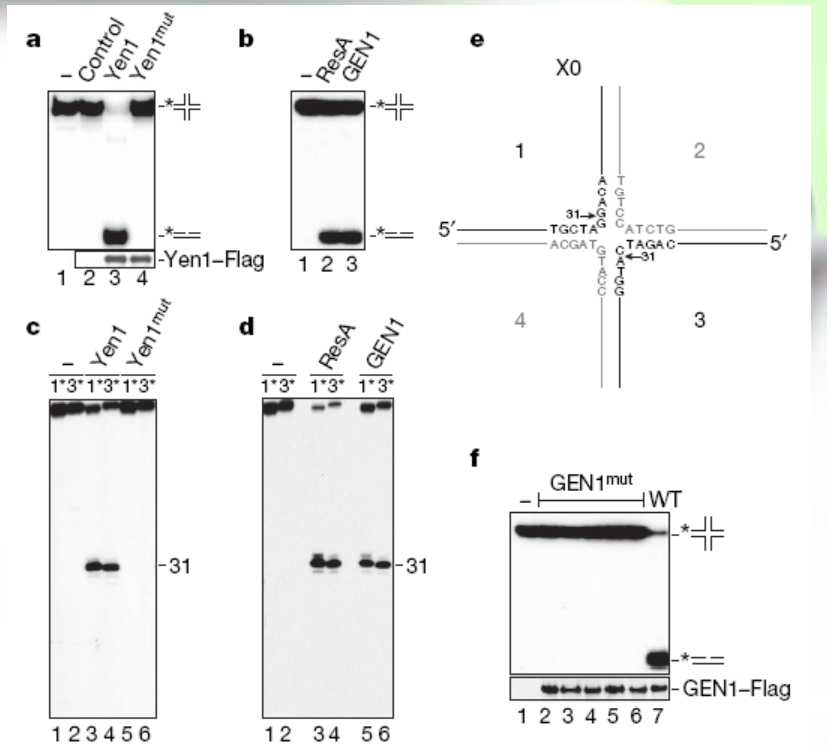

Figure 3 | Resolution of Holliday junctions by recombinant Yen1 and GEN1. **a**, ^{32}P-labelled Holliday junction X0 was incubated with cell-free extracts from yeast overexpressing Flag-tagged Yen1, or a catalytically inactive Yen1(E193A/E195A) mutant (Yen1^{mut}), and the products were analysed by neutral PAGE. Control: extracts from cells transformed with empty expression vector. Yen1–Flag and Yen1^{mut}–Flag were detected by western blotting using anti-Flag antibody. **b**, As **a**, but affinity purified GEN1–Flag, or ResA, was used. **c**, **d**, Holliday junction X0, 5′-^{32}P-end-labelled in strand 1 or 3, was treated as in **a** and **b**, and products were analysed by denaturing PAGE. Asterisks indicate the strand with the radioactive label. **e**, The cleavage sites. **f**, Wild-type and mutant derivatives of GEN1–Flag were assayed using Holliday junction X0. Lane 1, control lane; lane 2, GEN1(D30A); lane 3, GEN1(E134A); lane 4, GEN1(E136A); lane 5, GEN1(D157A); lane 6, GEN1(E134A/E136A); lane 7, wild-type GEN1. In the bottom panel, GEN1–Flag proteins were detected by western blotting.

Antibody Validation in WB

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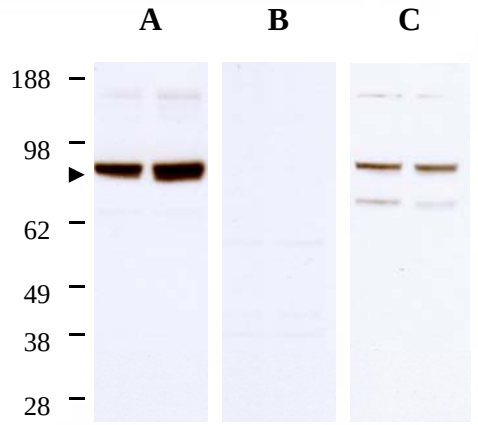
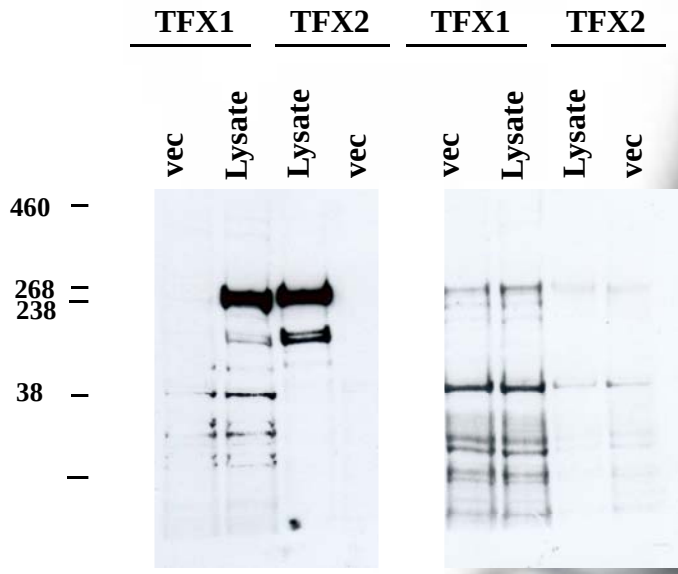
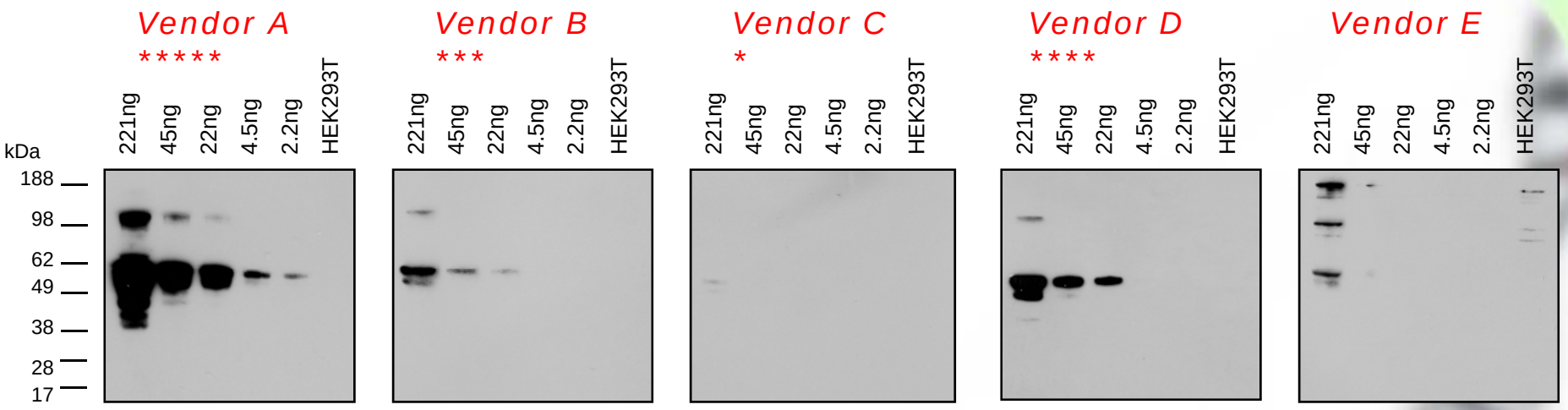
Over-expression cell lysate of human STAT3 (NM_139276) was used to test 3 commercial antibodies. Antibody A shows strong antigen binding. Antibody C shows weak binding, while Antibody B did not react with STAT3 at all.



Over-expression cell lysate for human LRRK2 (NM_198578) was used to validate two anti-LRRK2 antibodies in two rounds of Western blot experiments. The left panel shows positive reaction with a commercial monoclonal antibody. The right panel shows a negative reaction with a polyclonal antibody generated against a protein peptide.



Antibody Validation – P53 Ab



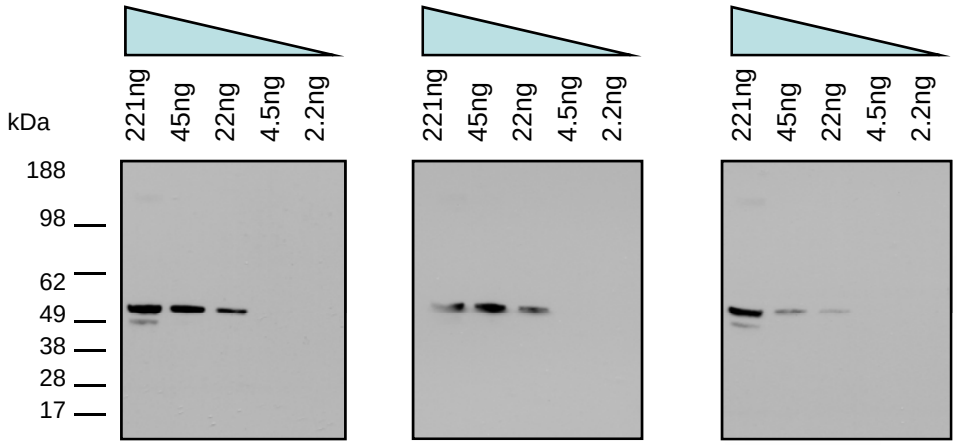
Five commercial antibodies against human P53 were evaluated in Western blot experiments with P53 over-expression cell lysate.

P53 protein level in cell lysate was pre-determined using a purified GST-Myc-DDK standard. Lysate was serially diluted before SDS-PAGE and immunoblotting.

Antibodies A-C are rabbit antibodies. D and E are mouse antibodies.

Antibody quality and star rating is based on P53 protein detection level.

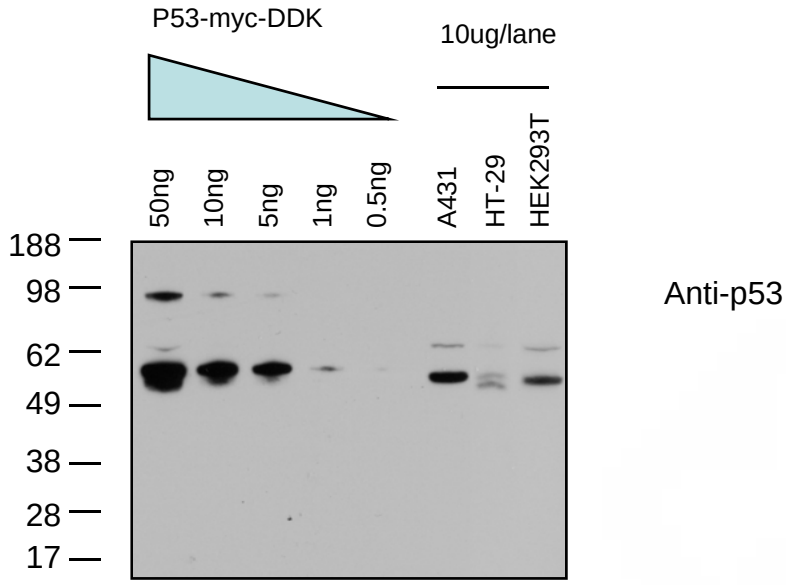
Determining Lot to Lot Consistency



Three different lots of a commercial polyclonal antibody against human P53 were evaluated in Western blot experiments with P53 over-expression cell lysate.

One can see certain lot to lot variation in amount of antigen detection, especially between lot A and C.

Endogenous Protein Expression Level



Estimate the endogenous p53 level in different carcinoma cell line by VERIFY antigen standard. 10 ug whole cell lysates from 3 different cancer cell lines were tested against titrated p53 over-expression lysate. Using over-expression lysate as standard, it is determined endogenous p53 expression level in these 3 cell lines were 3.8 ng (A431), 0.4 ng (HT-29), and 1.3 ng (HEKT293) respectively.

Reverse Phase Lysate Arrays

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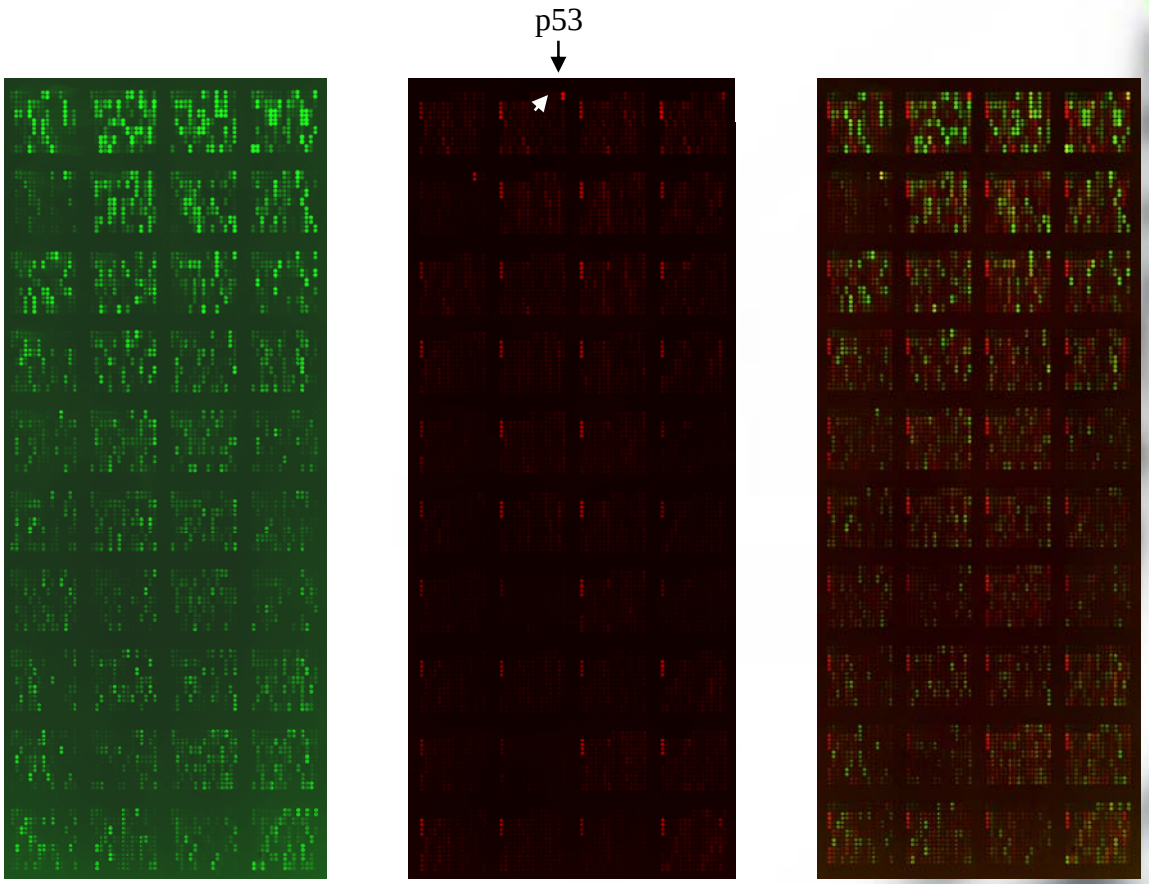
Figure shows a preliminary 4,000 protein array results when spotted lysate arrays are detected using anti-FLAG antibody and anti-P53 antibody each labeled with different fluorescent dyes (Alexa-555 and Alexa-647).

Left two panels are individual antibody staining, while right panel shows combined image to illustrate differential binding.

Protein arrays can be used in detection of autoimmune antibodies, protein-protein interaction, and antibody decoding.



Anti-FLAG Anti-p53 Merged

Over-expression Cell Lysate QC

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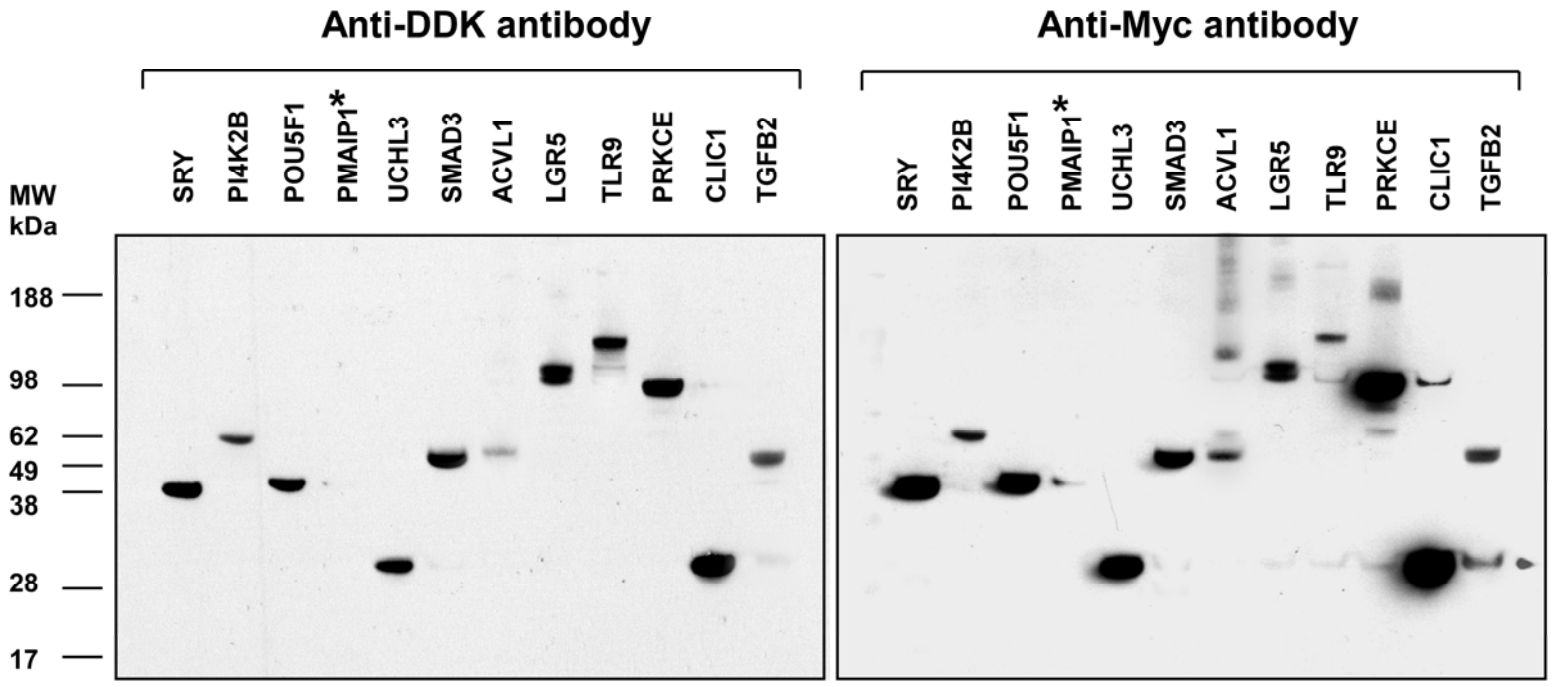
Figures show typical Western blot images when over-expression cell lysates were tested with anti-tag antibodies. Figure 4 shows a comparison using either anti-myc antibody (TA100010) or anti-DDK antibody (TA100011). Figures 5 and 6 were done with anti-myc antibody. Most proteins are detected as a single band. Some proteins are detected as two or multiple bands due to various reasons, such as post-translation modification, protein degradation, etc.



* Molecular weight is smaller than 10 kDa.

Figure 6

Over-expression Cell Lysate QC

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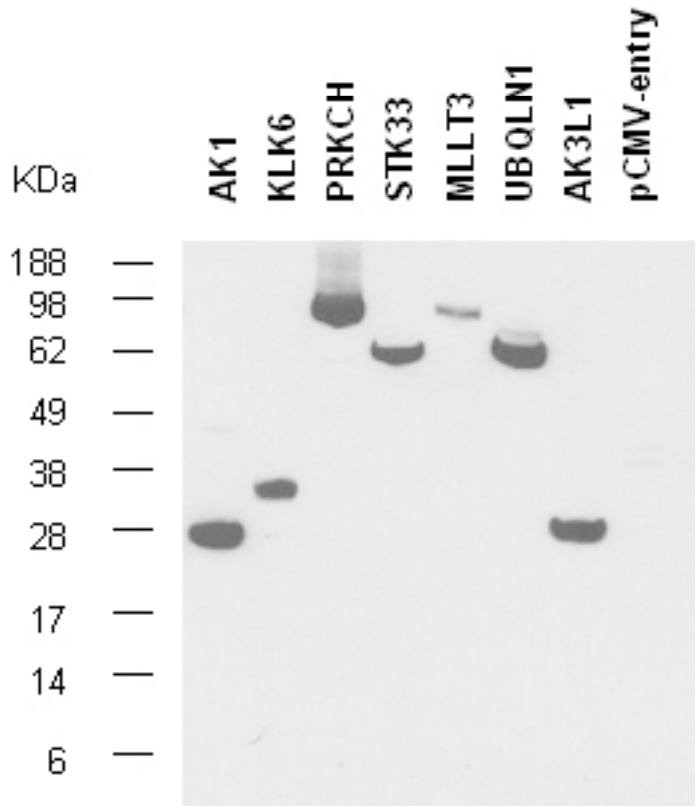

Figure 7

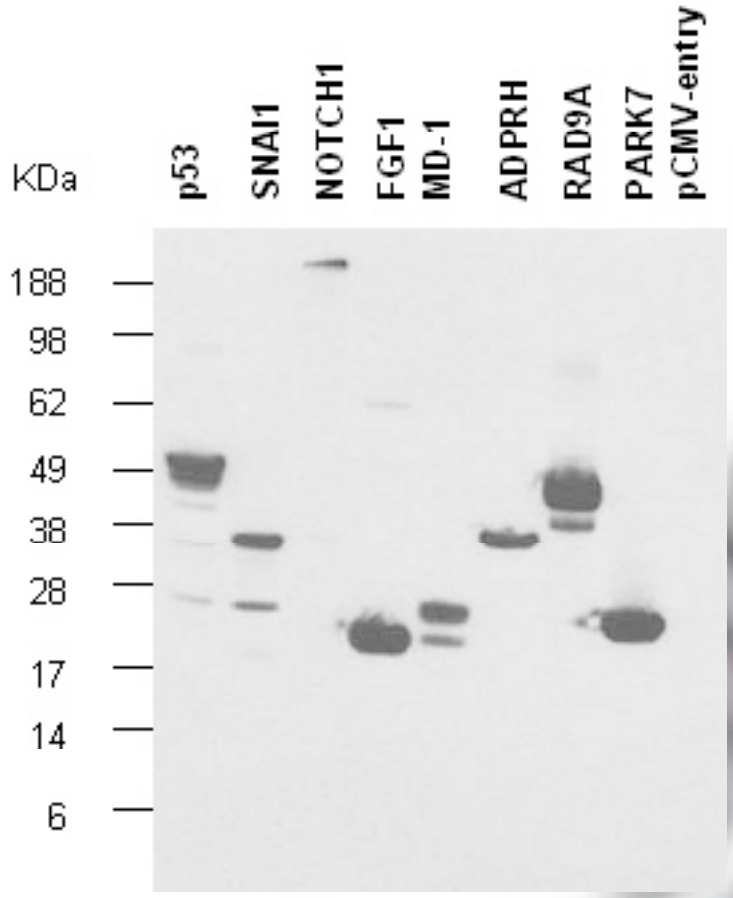

Figure 8

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