

# SWI/SNF alterations in cancer: molecular targets and therapeutic implications

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## ABSTRACT

### Background:

SWI/SNF chromatin remodelling complex is altered in nearly 20% of all human tumor types, which places it as the most broadly mutated molecular system in human cancer, just after *TP53* [1, 2]. Nevertheless, the molecular mechanisms through which these alterations foster cancer development remain to be fully understood. In addition, any potential susceptibility associated with SWI/SNF alterations might be exploited for the treatment of patients affected by different malignancies.

### Methodology:

We have generated stably-transduced cell lines for a doxycycline-inducible vector that directs the expression of different shRNAs targeting *ARID1A*, *ARID1B* or *ARID2* in different human cancer cell lines. We have studied the transcriptional alterations resulted from the deficiency of these subunits in different cellular contexts by RNA-Seq, as well as the changes in chromatin accessibility by ATAC-Seq. Additionally, we have studied the sensitivity of these cell lines to different antitumoral treatments.

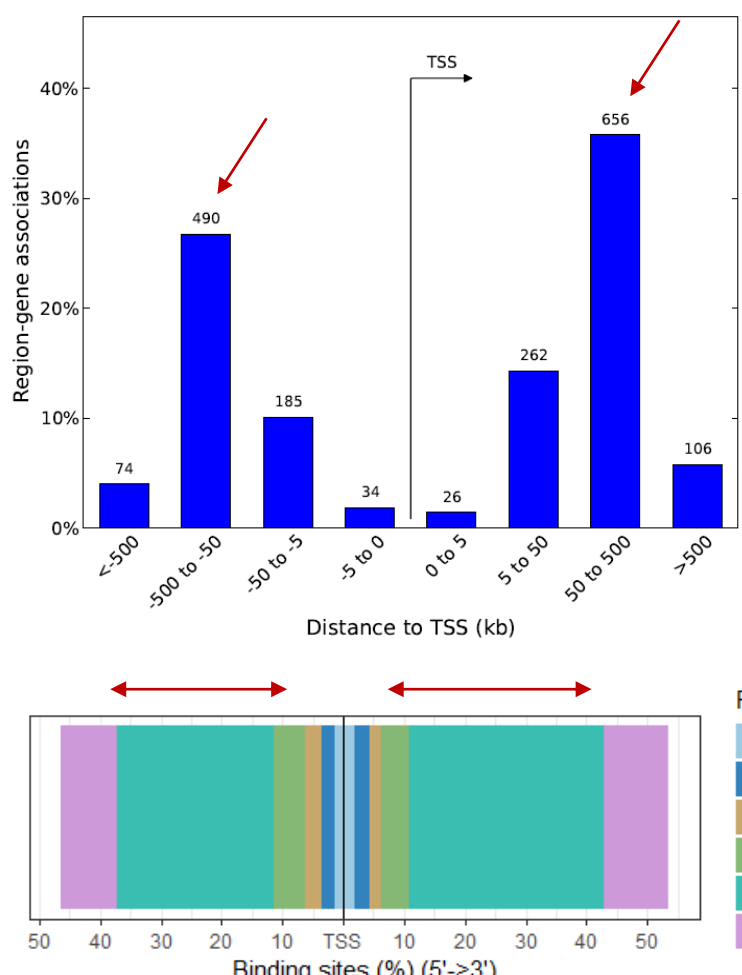
### Results:

We have identified molecular pathways differently expressed in the SWI/SNF-deficient cell lines that might explain some characteristics of the behaviour of these tumor cells. Thus, we have seen an overexpression of genes involved in ROS production, as well as genes encoding metalloproteinases, and a downregulation of genes related to cellular adhesion or tumor suppression. Finally, we have detected different sensitivities in the ARID-deficient cells to some treatments, like cis-platin, and PARP or EGFR inhibitors.

## 2. Chromatin accessibility changes associated with ARID2-deficiency in A549

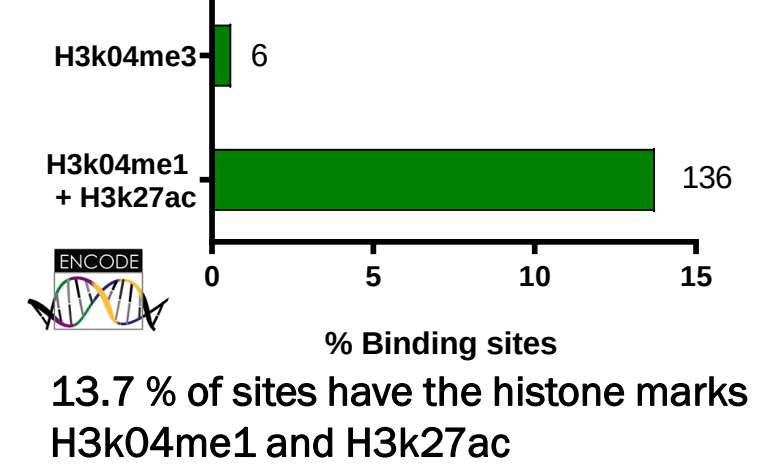
### A) ARID2 is essential to keep an open chromatin structure in enhancers

#### i) A significant proportion of peaks are located far away from TSS



62.5 % of sites located at  $\pm 50$ -500 kb to nearest TSS

#### ii) Overlap with the histone marks found at active enhancers



13.7 % of sites have the histone marks H3K4me3 and H3K27ac

#### iii) Transcription factors of AP-1 family bind to the sequence of DNA of the identified peaks

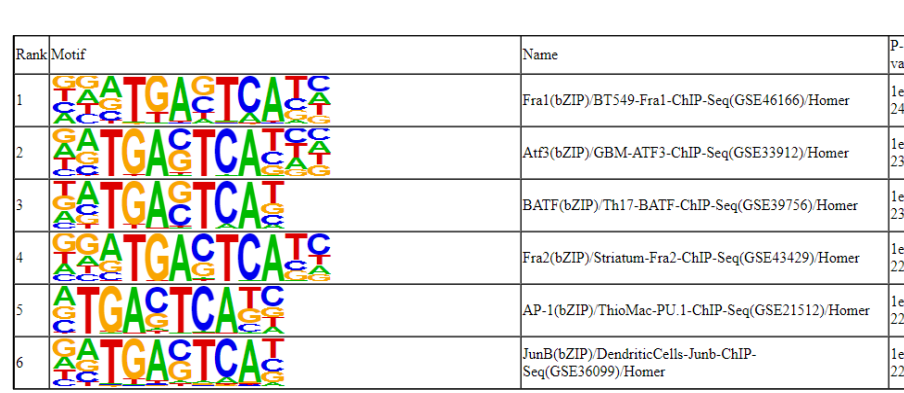
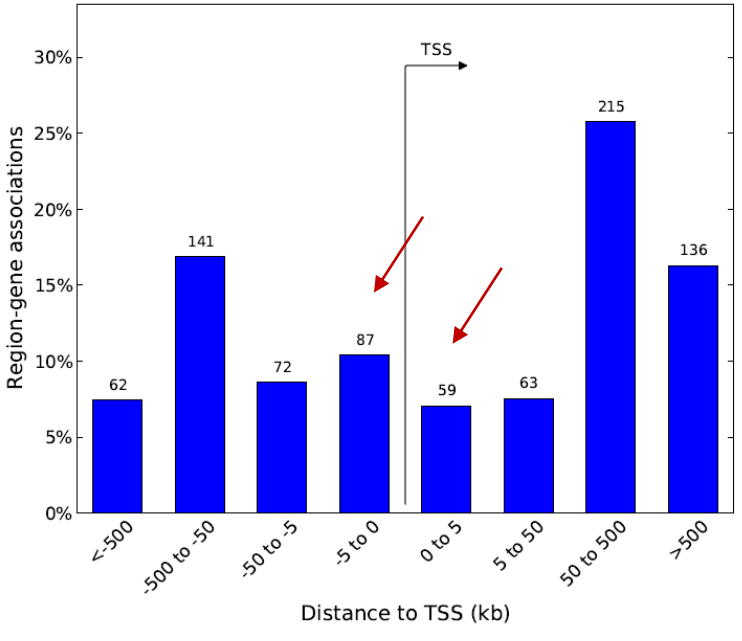


Figure 2A. Characterization of closed regions of DNA as a result of ARID2-deficiency in a lung cancer cell model.

- Representation of the distance to the nearest TSS of the peaks identified by MACS2 and annotated using GREAT and the R package ChIPseeker.
- Overlap of the peaks identified in our analysis with the ones identified in a ChIP-Seq experiment performed in the context of the ENCODE project.
- Identification of motifs of known transcription factors in the close sequences of DNA after ARID2 removal using the software HOMER.

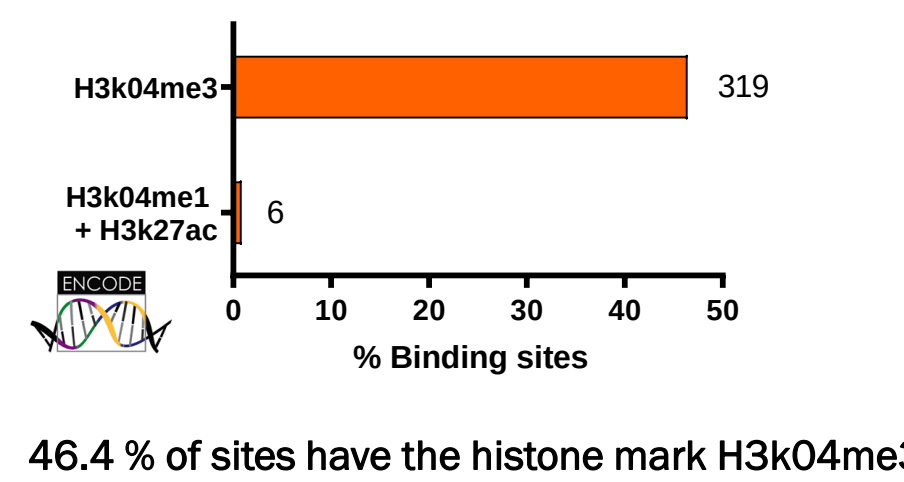
### B) ARID2-deficiency results in the increase of chromatin accessibility in promoters

#### i) A significant proportion of peaks are located near TSS



17.5 % of sites located at  $\pm 0$ -5 kb to nearest TSS

#### ii) Overlap with histone mark found at promoters



46.4 % of sites have the histone mark H3K4me3

Figure 2B. Characterization of open regions of DNA as a result of ARID2-deficiency in a lung cancer cell model

- Representation of the distance to the nearest TSS of the peaks identified by MACS2 and annotated using GREAT and the R package ChIPseeker.
- Overlap of the peaks identified in our analysis with the ones identified in a ChIP-Seq experiment performed in the context of the ENCODE project.

## 4. ARID2-deficiency is associated with a pro-tumoral and pro-invasive transcriptional programme both *in vitro* and in patients

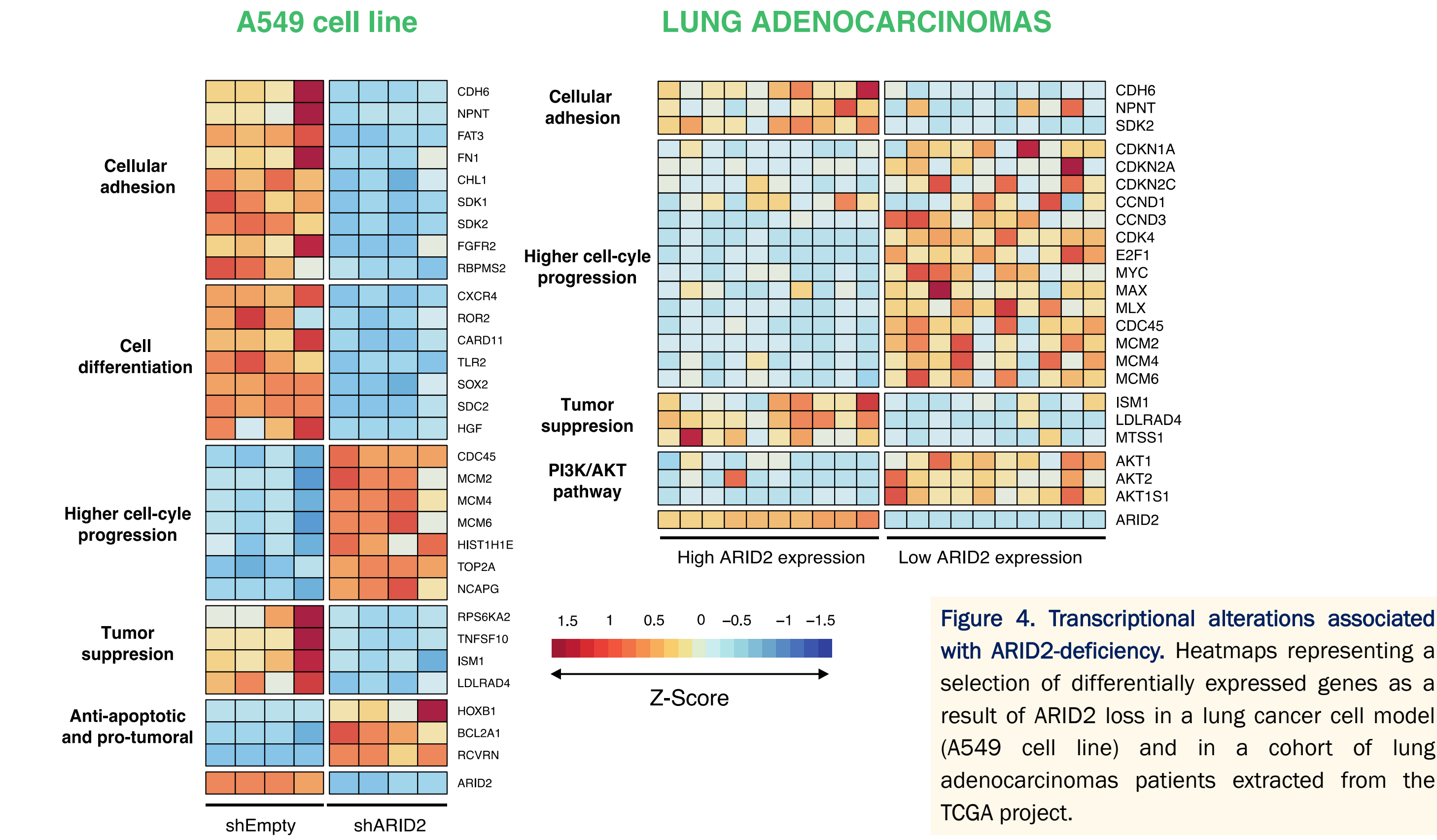


Figure 4. Transcriptional alterations associated with ARID2-deficiency. Heatmaps representing a selection of differentially expressed genes as a result of ARID2 loss in a lung cancer cell model (A549 cell line) and in a cohort of lung adenocarcinomas patients extracted from the TCGA project.

### Common transcriptional alterations associated with low expression of ARID2 found *in vitro* and in patients

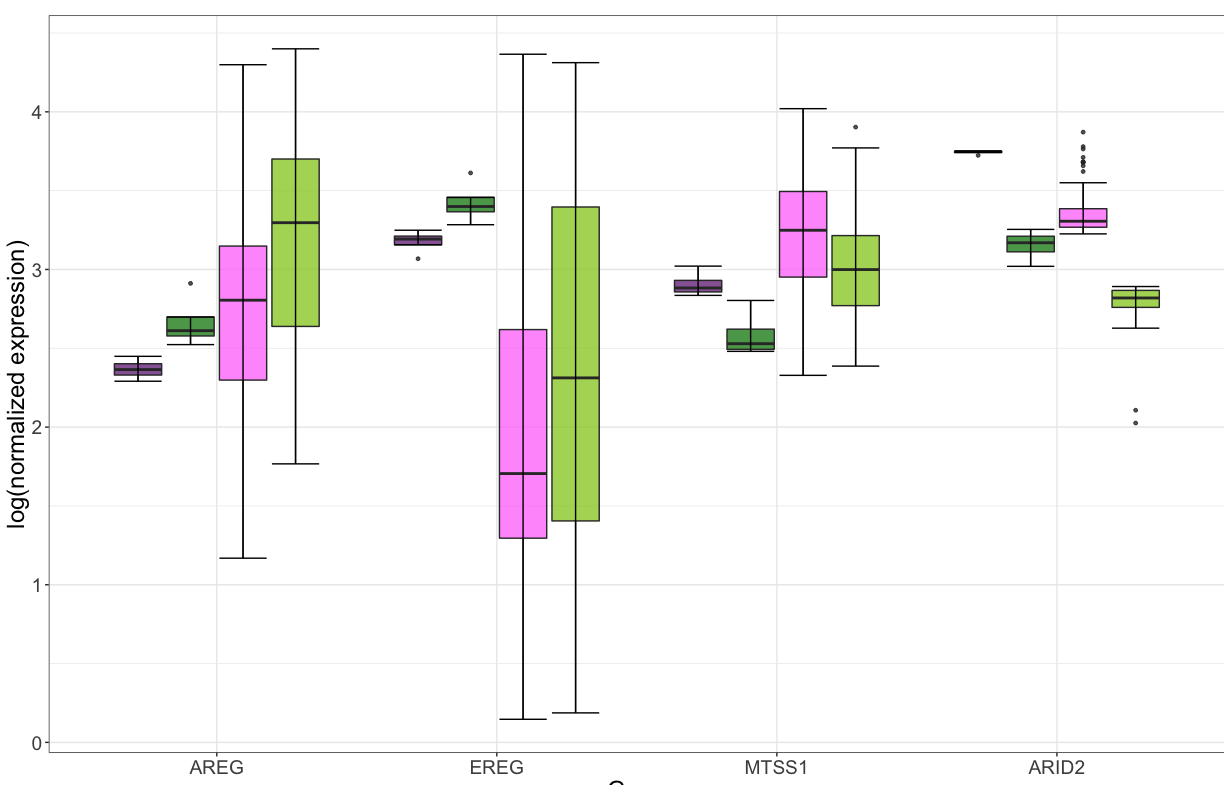


Figure 5. Selection of genes which behaved similarly in cellular models and in patients as a result of ARID2-deficiency. Boxplot representing the logarithmic values of the normalized expression of AREG, EREG and MTSS1 in A549 cells and lung adenocarcinomas

### References

- Wilson, B. G. & Roberts, C. W. M. SWI/SNF nucleosome remodellers and cancer. Nat. Rev. Cancer 11, 481–492 (2011).
- Kadoch, C. & Crabtree, G. R. Mammalian SWI/SNF chromatin remodeling complexes and cancer: Mechanistic insights gained from human genomics. Sci Adv 1, (2015).

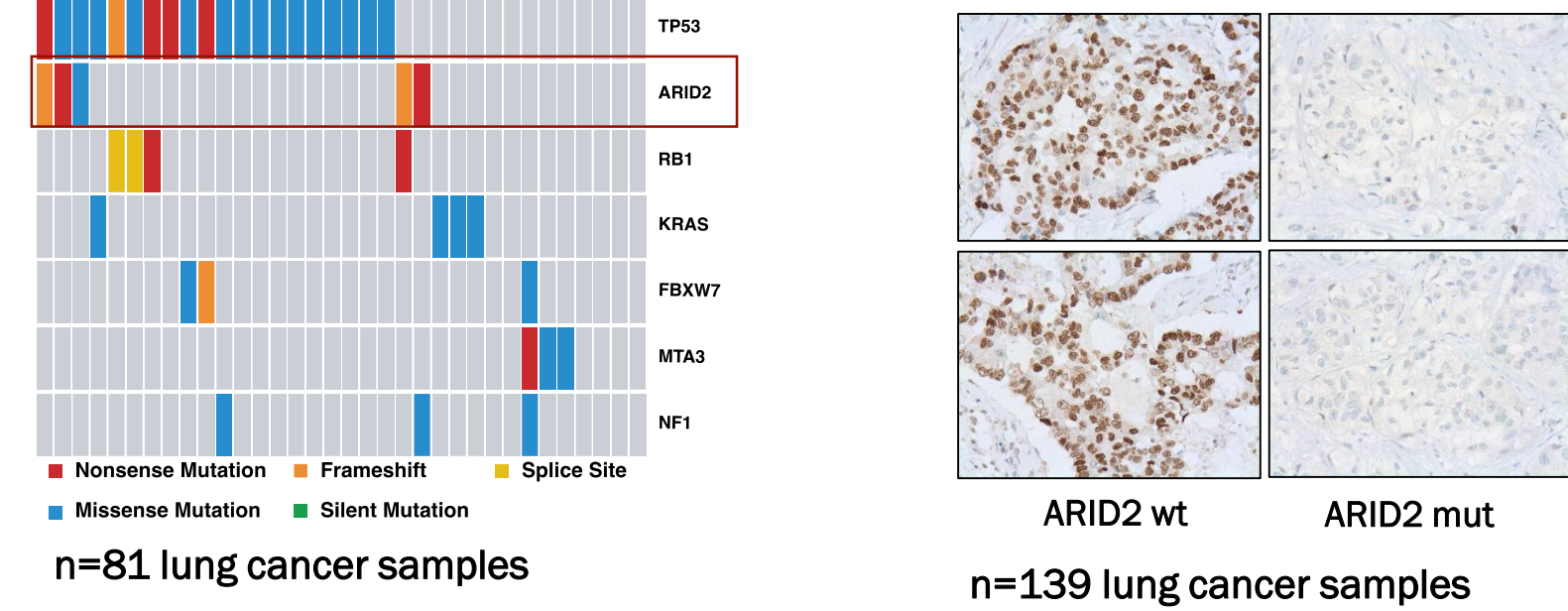
### FUNDING



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## 1. ARID2 loss is associated with poor prognosis in lung cancer

### A) ARID2 mutation frequency: 6.2% B) ARID2 low protein production: 20%



### C) ARID2 low expression correlates with low survival rate D) ARID2 is a driver gene in lung cancer

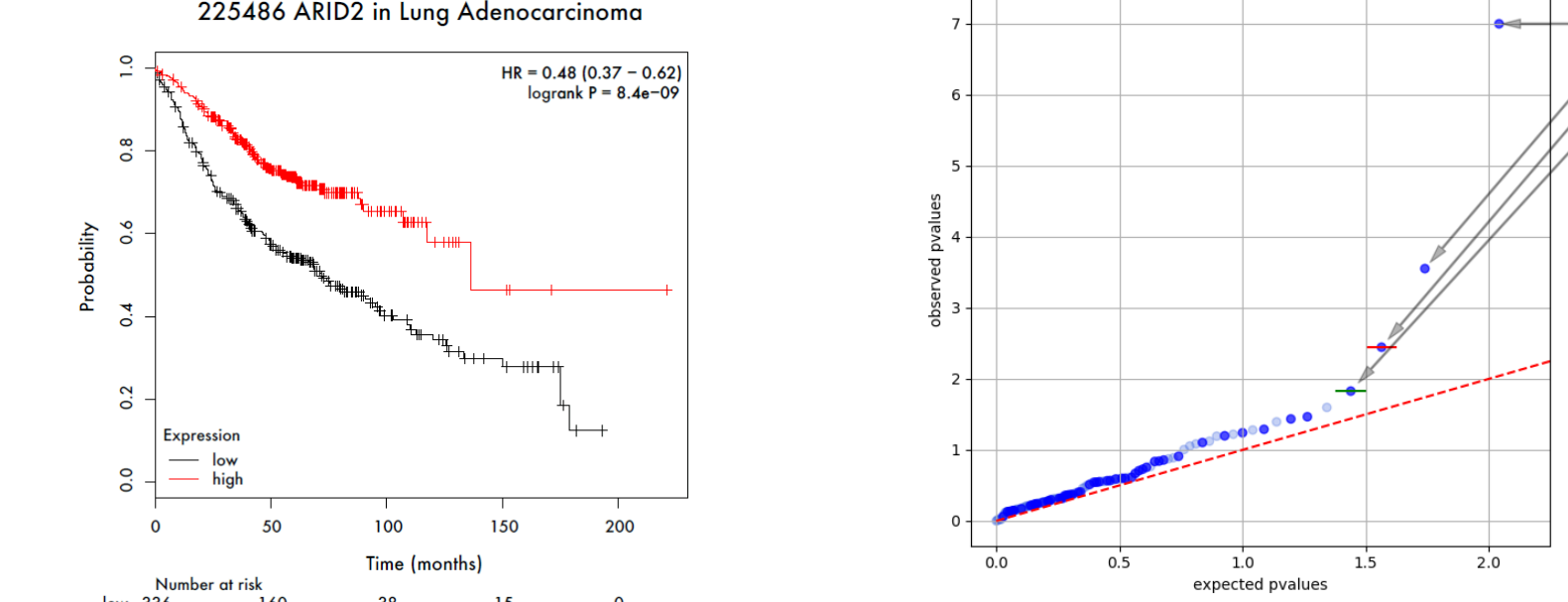


Figure 1. Frequent ARID2 mutations are associated with loss of protein synthesis and worse prognosis in lung cancer patients.

- OncoPrint representation of the most significantly mutated genes according to OncoPrintFML in a cohort of 81 lung adenocarcinomas. Each box represents an independent patient and its coloured according to the type of identified mutation.
- Representative images of ARID2 immunohistochemistry experiments in two ARID2 mutated (left) and two ARID2 wild type (right) lung adenocarcinomas.
- Kaplan-Meier representation of the survival of lung cancer patients with low (black) or high (red) ARID2 expression.
- Identification of driver cancer genes in our cohort of lung adenocarcinomas performed by OncoPrintFML. Genes showed in red presented a q-value < 0.1 after multiple test correction.

## 3. Deficiency in SWI/SNF variant subunits results in pro-tumoral transcriptional programs

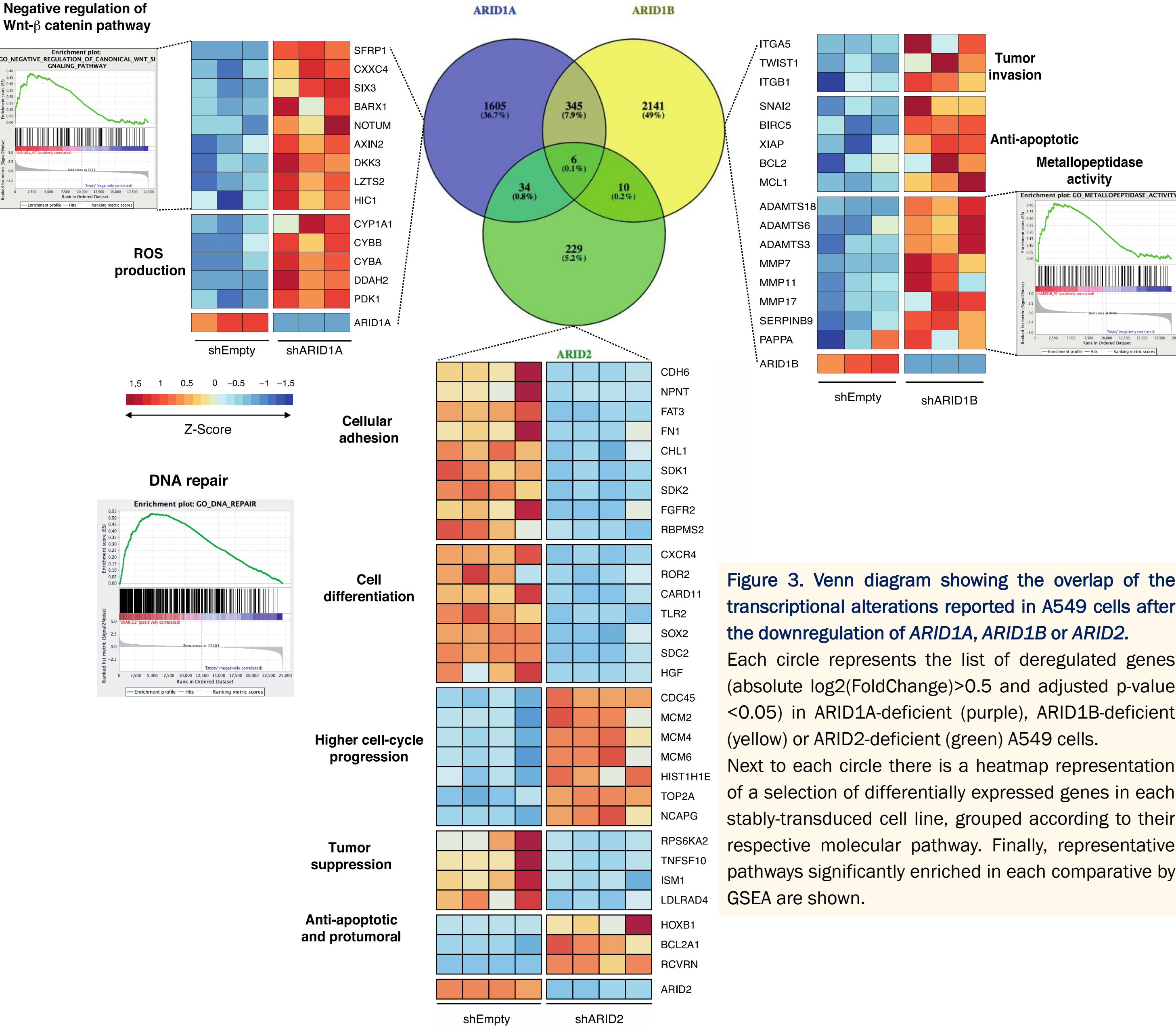
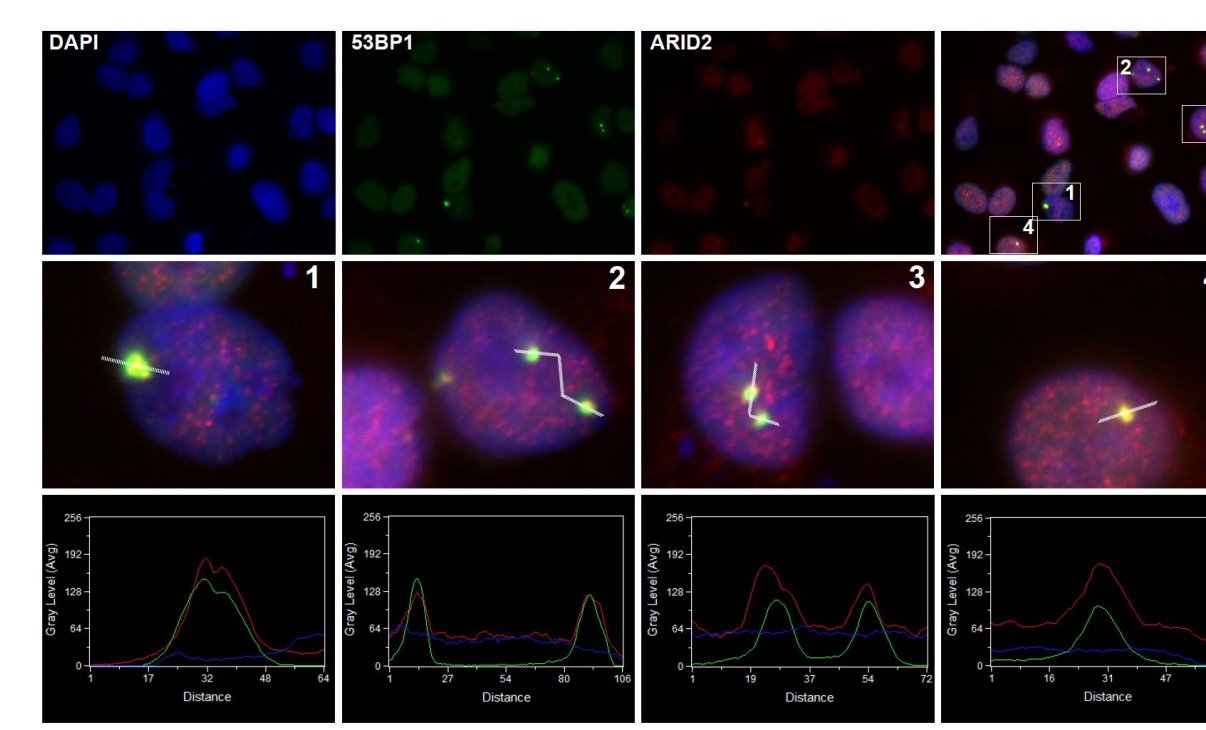


Figure 3. Venn diagram showing the overlap of the transcriptional alterations reported in A549 cells after the downregulation of ARID1A, ARID1B or ARID2. Each circle represents the list of deregulated genes (absolute log2(FoldChange)>0.5 and adjusted p-value <0.05) in ARID1A-deficient (purple), ARID1B-deficient (yellow) or ARID2-deficient (green) A549 cells.

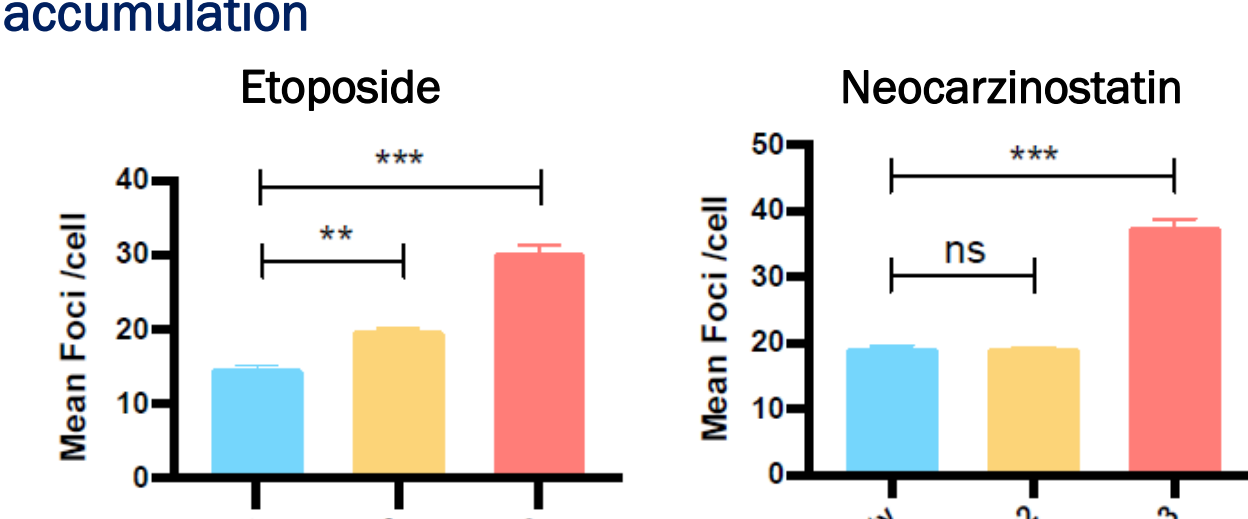
Next to each circle there is a heatmap representation of a selection of differentially expressed genes in each stably-transduced cell line, grouped according to their respective molecular pathway. Finally, representative pathways significantly enriched in each comparative by GSEA are shown.

## 5. Increased sensitivity of ARID2-deficient A549 cells to antitumor treatments

### A) ARID2 co-localizes with 53BP1 at DNA repair foci



### B) ARID2-deficiency is associated with DNA-damage accumulation



### C) ARID2-deficient cells are more sensitive to DNA-damage drugs

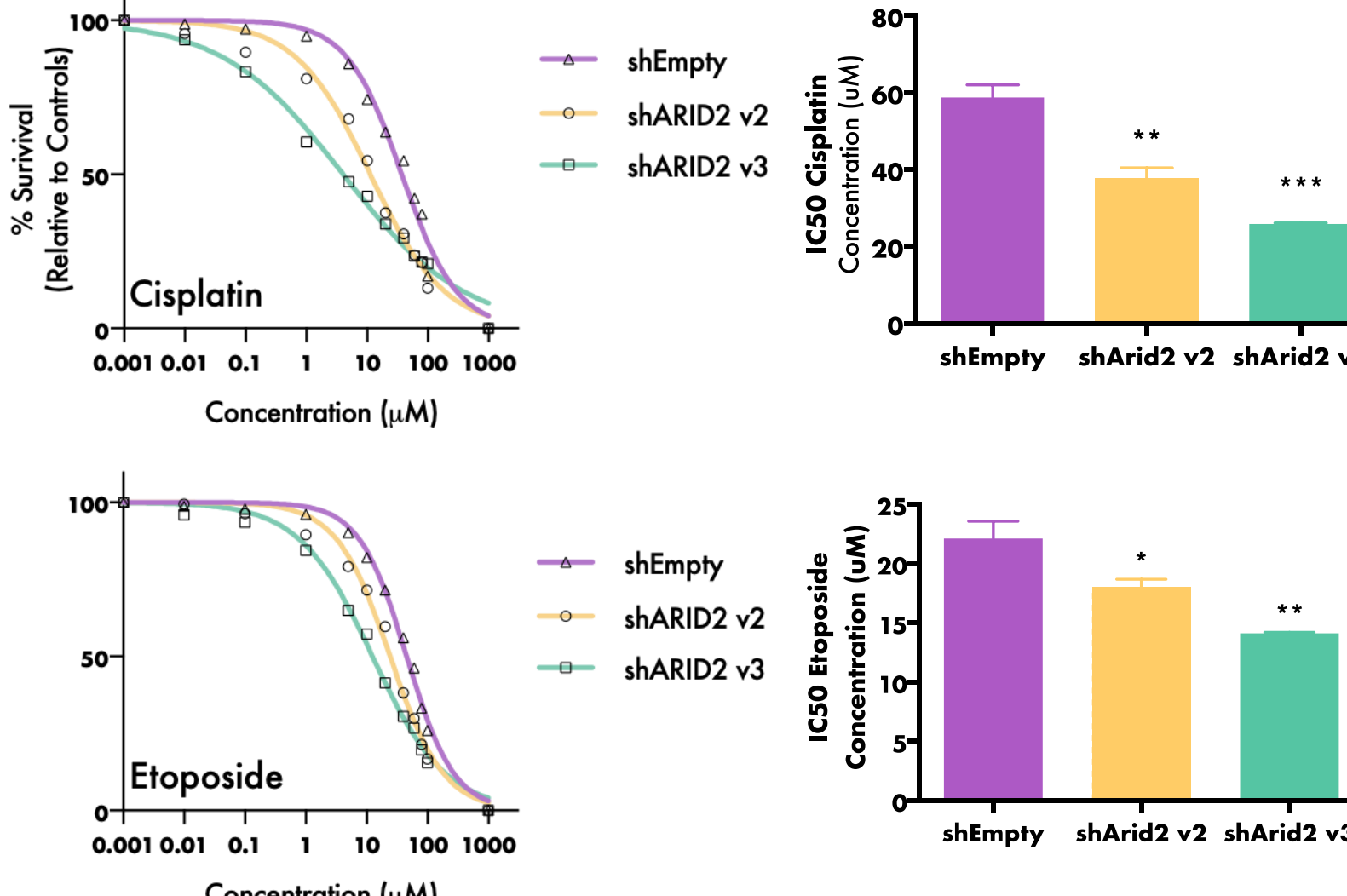


Figure 6. ARID2-deficiency affects DNA repair and increases sensitivity to antitumor therapies.

- Representative images of immunofluorescence experiments proving the colocalization of 53BP1 (green) and ARID2 (red) in A549 cells treated with neocarzinostatin.
- Bar representation of the foci quantification in each transduced cell line, treated with drugs that cause DNA-damage (etoposide and neocarzinostatin).
- Graphs of representative experiments measuring cell survival to increasing concentrations of different antitumor treatments (cisplatin and etoposide) in A549 cells transduced with shEmpty (purple), shARID2v2 (yellow) or shARID2v3 (green) vectors. Bar representation of the calculated IC50 of each cell line are also displayed. In all cases, the results are represented as mean  $\pm$  SE of three independent results (one or two-tailed t-test \*p < 0.05, \*\* p < 0.01 and \*\*\* p < 0.001).

## CONCLUSIONS

- ARID2 low expression is associated with worse prognosis in lung cancer.
- ARID2 is preferentially bound to enhancers through the collaboration of AP-1 family transcription factors.
- ARID2-deficiency is associated with a pro-tumorigenic, pro-invasive transcriptional program.
- The expression of AREG or MTSS1 seems to be directly regulated by ARID2 and might play an important role in ARID2 tumor suppression role.
- ARID2-deficiency gives rise to an enhanced sensitivity to drugs that cause DNA damage.