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Deciphering the radiosensitivity of HPV-positive tumors

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INTRODUCTION

In the last few years, the higher radiosensitivity of HPV-positive tumors (compared to their HPV unrelated counterparts) has been well documented, especially in head and neck cancers where a less restrictive treatment plan has been suggested for HPV-positive patients[1].
Although several explanations were recently proposed to explain this difference in response (i.e. increased angiogenesis, high intratumoral immune cell infiltration, hypoxia), the direct involvement of viral oncoproteins in this phenomenon is still largely unknown.

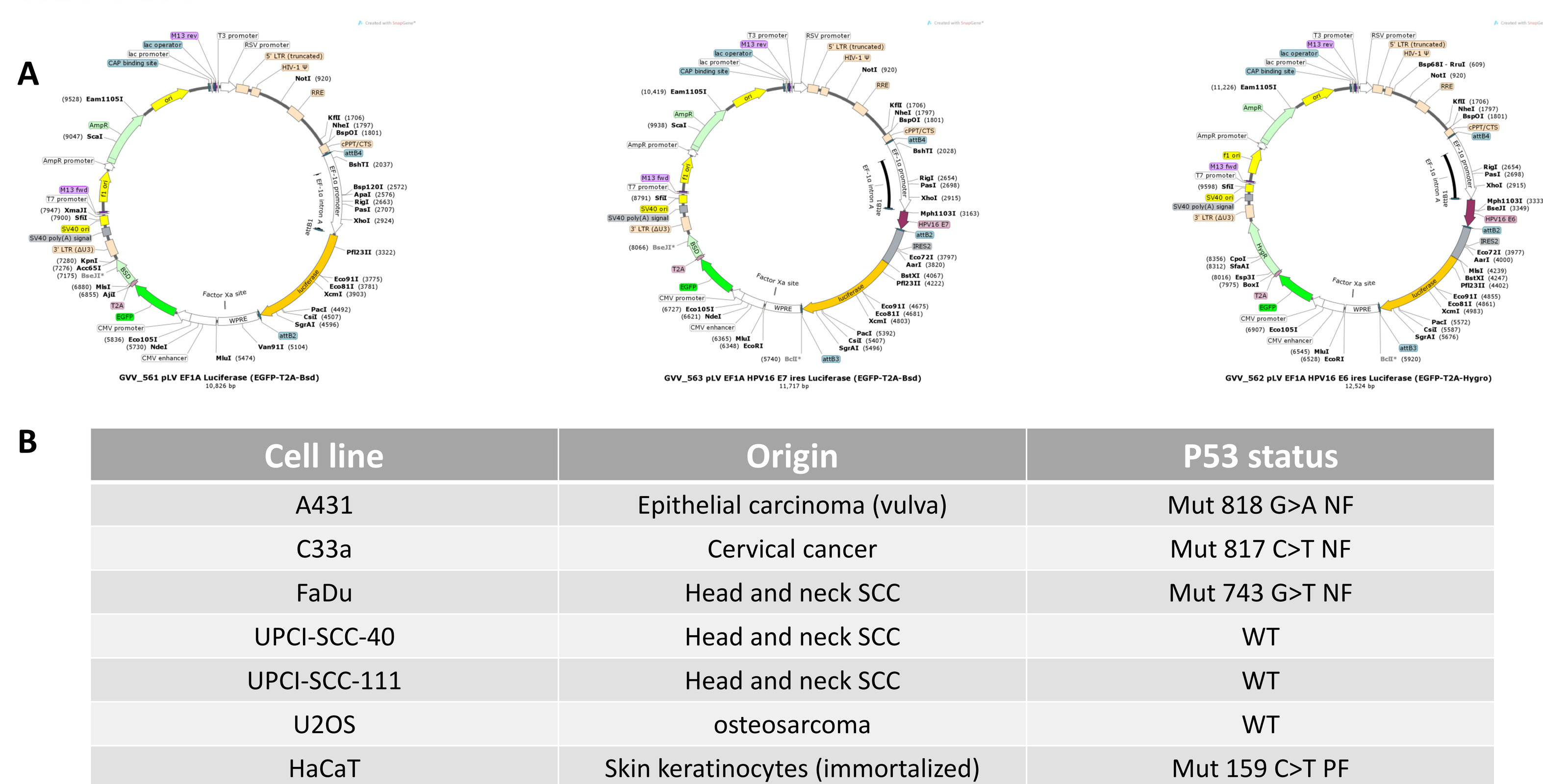
[1] CHEN, Allen M., FELIX, Carol, WANG, Pin-Chieh, *et al.* Reduced-dose radiotherapy for human papillomavirus-associated squamous-cell carcinoma of the oropharynx: a single-arm, phase 2 study. *The Lancet Oncology*, 2017, vol. 18, no 6, p. 803-811

EXPERIMENTAL DESIGN

The present project aims at deciphering the impact of E6 and/or E7 on tumor radiosensitivity. More precisely, we screened a database of around 150 DNA damage repair proteins to highlight potential interaction with these latter viral oncoproteins.

1. We stably transduced HPV-negative cell lines from different anatomical sites where HPV infection can be diagnosed. The cells integrated HPV oncogenic proteins E7 and/or E6.
2. On the stably transduced cells, we performed some functional tests and comet assays.
3. We then assembled a library from the Orfeome V7.1 (and soon 8.1) by selecting proteins implicated in DNA damage repair pathways. The created library was screened for plausible interactions with E6 and/or E7.
4. The proteins highlighted by GPCA screening were validated by Co-IP.

RESULTS



Cell line	Origin	P53 status
A431	Epithelial carcinoma (vulva)	Mut 818 G>A NF
C33a	Cervical cancer	Mut 817 C>T NF
FaDu	Head and neck SCC	Mut 743 G>T NF
UPCI-SCC-40	Head and neck SCC	WT
UPCI-SCC-111	Head and neck SCC	WT
U2OS	osteosarcoma	WT
HaCaT	Skin keratinocytes (immortalized)	Mut 159 C>T PF

Figure 1. Plasmid constructs used to integrate E6 and/or E7 into HPV negative cells via lentivirus (A). The two plasmid as well as the control plasmid express luciferase. **List of cell lines used in this project (B).** For each cell line, the origin and the p53 status is specified. WT= wild type. Mut = Mutated. NF = the mutation is non functional. PF = the mutation is partly functional.

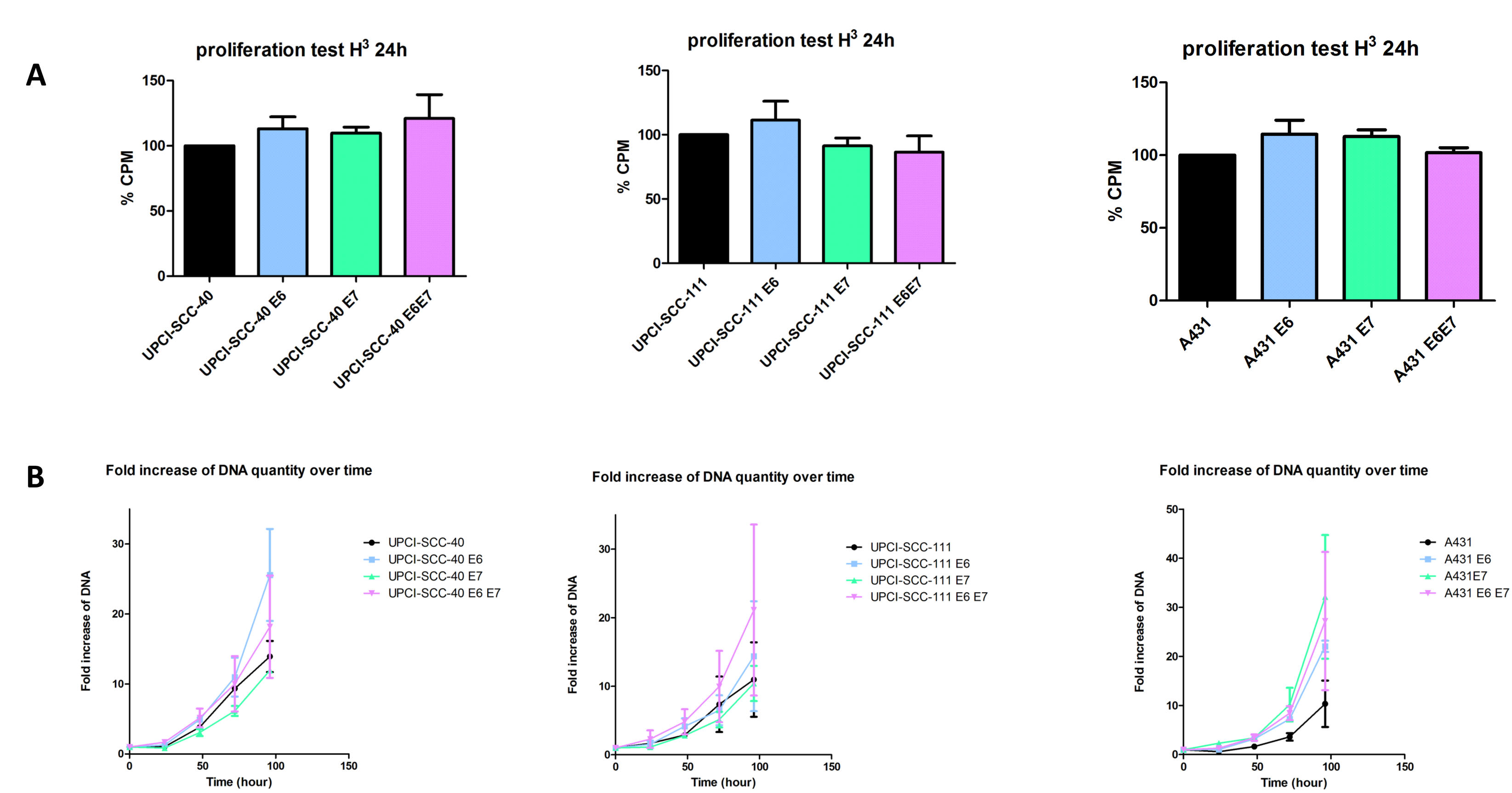


Figure 2.1 Proliferation tests for E6 and/or E7 transduced cell lines. (A) 24 hours H^3 proliferation test on UPCI-SCC-40, UPCI-SCC-111 and A431 cell lines. The results are the mean ± SD of two independent experiments. (B) Proliferation experiment performed by Hoechst on UPCI-SCC-40, UPCI-SCC-111 and A431 cell lines. Measurement has been taken at 0, 24, 48, 72 and 96 hours post adherence of the cells. The results are the means ± SD of two independent experiments.

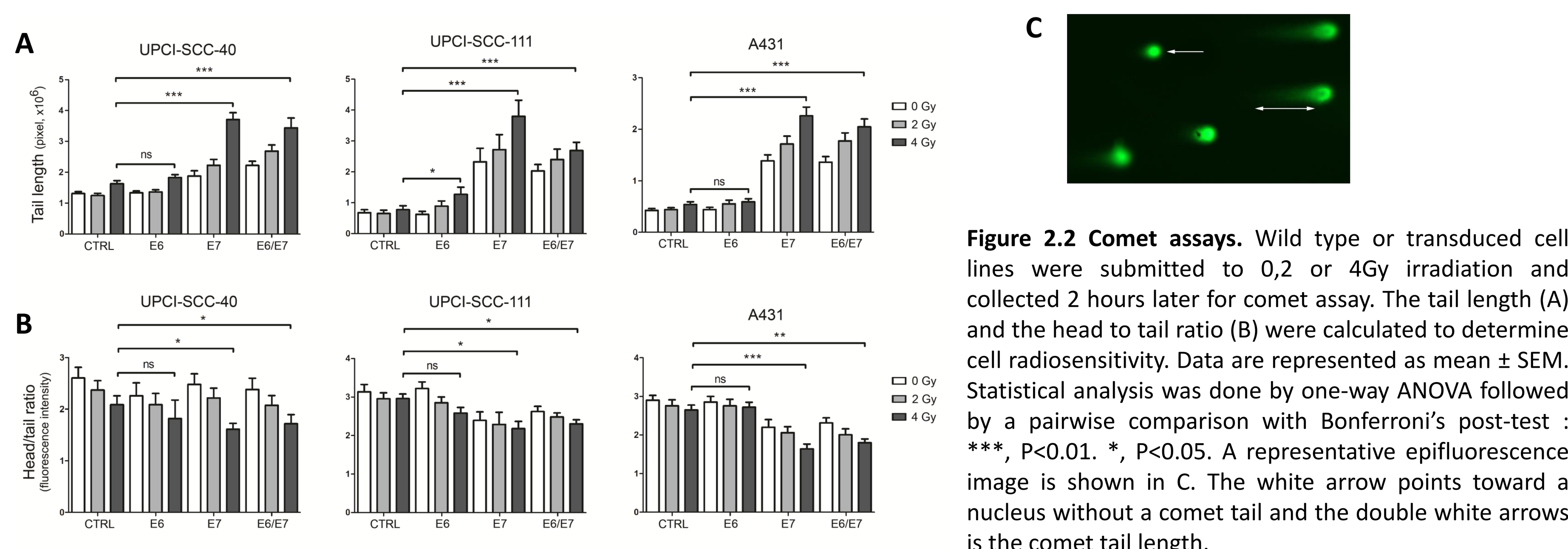


Figure 2.2 Comet assays. Wild type or transduced cell lines were submitted to 0,2 or 4Gy irradiation and collected 2 hours later for comet assay. The tail length (A) and the head to tail ratio (B) were calculated to determine cell radiosensitivity. Data are represented as mean ± SEM. Statistical analysis was done by one-way ANOVA followed by a pairwise comparison with Bonferroni's post-test : ***, P<0.01. *, P<0.05. A representative epifluorescence image is shown in C. The white arrow points toward a nucleus without a comet tail and the double white arrows is the comet tail length.

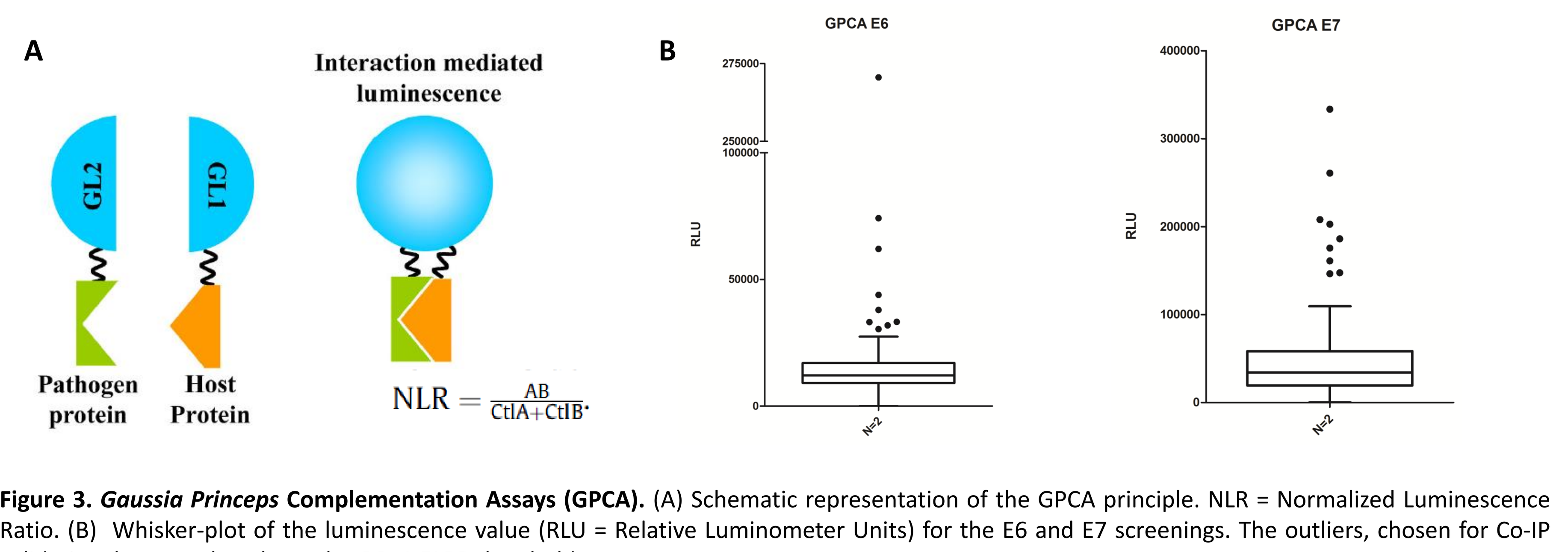
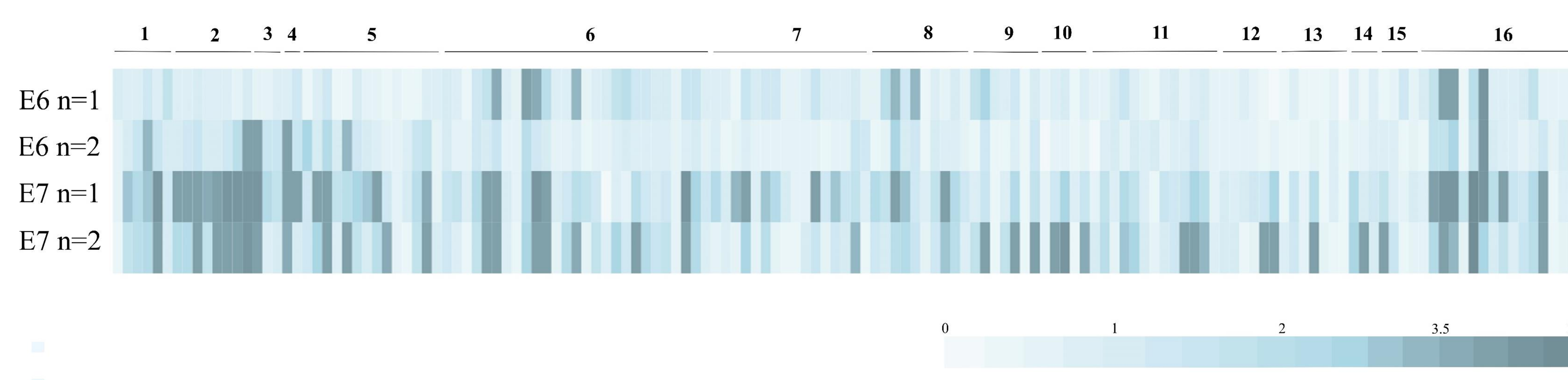
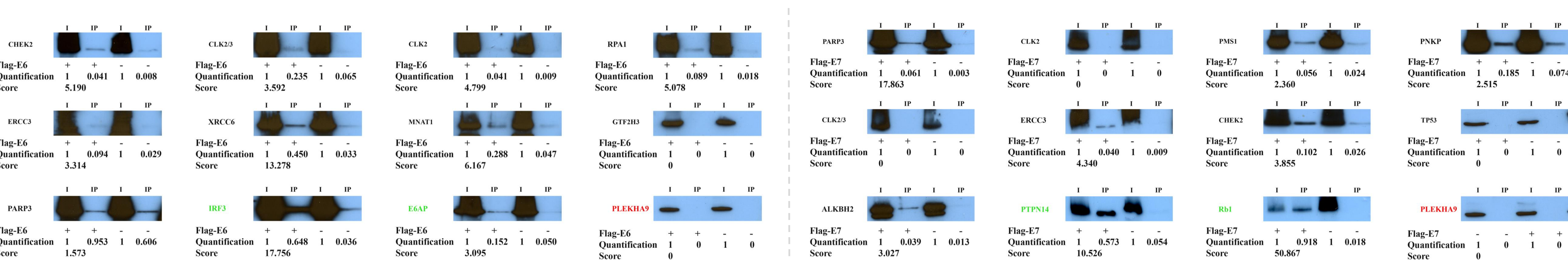


Figure 3. *Gaussia Princeps* Complementation Assays (GPCA). (A) Schematic representation of the GPCA principle. NLR = Normalized Luminescence Ratio. (B) Whisker-plot of the luminescence value (RLU = Relative Luminometer Units) for the E6 and E7 screenings. The outliers, chosen for Co-IP validation, have a value above the Q3+1,5IQR threshold.



Families :
1. DNA glycosylases
2. BER
3. Direct reversal of damage
4. Topoisomerases
5. MMR
6. NER
7. HR
8. Fanconi Anemia
9. NHEJ
10. Modulation of nucleotide pools
11. DNA polymerases
12. Nucleases
13. Ubiquitination enzymes
14. Chromatin structure and modification
15. Genes associated in disease associated DNA damage
16. Others

Figure 4. Heatmap of the GPCA experiments (potential interaction between 147 DNA Damage response proteins and HPV E6 or E7 oncoproteins). HEK293T cells were co-transfected with pSPICA-N2 E6, E7 or empty and pSPICA-N1 with proteins from the DNA damage library. The DNA damage response proteins have been classified by family. The experiment has been performed in duplicates.



Score = IP/I E6 or E7 / IP/I Empty

Figure 5. Co-IP experiments conducted on the main potential targets for E6 and E7 identified by GPCA. For each Co-IP, a score has been calculated based on the Input to IP ratio. An empty plasmid (flagged the same way as the E6 and E7 plasmids) has been used for control. The positive and negative controls are shown in green and red respectively. I = Input. IP = ImmunoPrecipitation.

CONCLUSION

Despite the fact that these results are preliminary, HPV oncoproteins E6 and E7 does not seem to have a strong impact on cell proliferation. In addition, several novel interesting protein interactions between DNA damage repair proteins and HPV oncoproteins (ie E7-PARP3, E6-XRCC6, ..) were highlighted. In the next months, we plan on broadening our screening library by using Orfeome 8.1 and confirm our research in vivo.

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