Supplementary Materials

Supplementary Material 1. ***In silico analysis for TCGA Head and Neck Squamous Cell Carcinoma (TCGA-HNSCC)***

To evaluate the prognostic significance, the copy number variation of *FGFR1* and mRNA expression (RNA-Seq) from The Cancer Genome Atlas (TCGA) Research Network were analyzed, which was previously opened to public. (https://portal.gdc.cancer.gov/).

In brief, *FGFR1* amplified cases made up 10% of the study population (28 out of 279 patients), all of which were negative for HPV. The primary tumor site was the oral cavity in 65.2 % (15 of 23) of the cases and the larynx in the remaining 34.8% (8 of 23) of cases. High FGFR1 mRNA expression was observed in 5 % (14 of 279) of the patients. A positive correlation was identified between mRNA expression and amplified *FGFR1* gene groups ($p = 0.040$, $r = 0.142$, Supplementary Table 3). All *FGFR1* amplified cases were negative for HPV ISH. *FGFR1* amplification in patients was not correlated with OS rate ($p = 0.924$, log-rank, Supplementary Figure 3A). In addition, high FGFR1 mRNA expression was not correlated with prognosis for OS ($p = 0.254$, log-rank test, Supplementary Figure 3B).

Supplementary Material 2. ***Test for HPV genotyping validation***

We tried to retest for the HPV ISH + cases and HPV ISH - / p16 - cases (n = 72), but, unfortunately, we couldn't performed the HPV genotyping. Because, the genotyping chip kit which was previously used in our study, was disappeared in the market due to their legal problem and couldn't buy the test kit commercially. Thus, we could not test the same method. Another problem was that half of the samples were too old to extract DNA and the quality of

the DNA was very poor. Thus, we couldn't help doing HPV genotyping again with previous method.

Instead, we searched the medical records of tonsillar squamous cell carcinoma patients with HPV genotyping from 2010 to 2014 in ASAN medical center, Seoul, Korea. In that period, our hospital did routinely HPV genotyping test with the same chip kit for diagnosis and treatment in all head and neck squamous cell carcinoma. 102 patients with tonsillar squamous cell carcinoma were performed HPV genotyping. 83 out of 102 patients had the result of immunohistochemistry for p16. Thus, we analyzed these 83 patients as validation group for HPV genotyping. The results described in the supplement figure 2, supplement table 1 & 2. In brief, 79.5 % of TSCC patients showed HPV positivity and most common type was type 16 (n = 65, 63.7 %). We also analyzed the relationship between p16 protein expression and HPV genotyping in supplement table 1. Sensitivity and specificity of p16 for HPV in situ hybridization and HPV genotyping, respectively were 96.6 % *vs* 100 % (sensitivity) and 43.3 % *vs* 29.4 % (specificity). Based on these data, we calculated the estimated HPV genotyping positive rate in our current experimental study group (n = 89). The number of p16 immuno-positivity was 74 patients. The positive predictive value of the p16 for HPV genotyping in validation set was 84.6 %. Thus, estimated number of HPV genotyping positivity in experimental current study group was about 63 patients (74 x 0.846 = 62.6). HPV in situ hybridization patients were 59 in experimental current study group. Although there was some limitation of comparison between HPV in situ and HPV genotyping, HPV genotyping seemed like more sensitive than HPV in situ hybridization.

Supplementary Tables

Supplementary Table 1. Correlation between p16 and HPV status.

Variables	HPV ISH (experimental set, n = 89)			p-value	HPV genotyping (validation set, n = 83)			p-value
	Total No. (%)	positive No. (%)	negative No. (%)		Total No. (%)	positive No. (%)	negative No. (%)	
p16				<0.001*				<0.001*
Positive	74 (83.1)	57 (64.0)	17 (19.1)		78(94.0)	66 (79.5)	12 (14.5)	
Negative	15 (16.9)	2 (2.3)	13 (14.6)		5 (6.0)	0 (0)	5 (6.0)	

HPV ISH represents HPV in situ hybridization.

*Fisher's exact test is applied.

Supplementary Table 2. Diagnostic utility of p16 as a HPV infection Comparison among p16, HPV ISH and HPV genotyping

	HPV ISH (%) (experimental set, n = 89)	HPV genotyping (%) (validation set, n = 83)
sensitivity	96.6	100
specificity	43.3	29.4
Accuracy	78.6	85.5
Prevalence	66.2	79.5
Positive predictive value (PPV)	77.0	84.6
Negative predictive value (NPV)	86.6	100

HPV ISH represents HPV in situ hybridization.

Supplementary Table 3. Correlation between mRNA expression for FGFR1 and copy number variation in the analysis of TCGA data.

Variables	Total No. (%)	FGFR1 mRNA-high expression group, No. (%)	FGFR1 mRNA-low expression group, No. (%)	p-value
CNV	279			0.040*
Amplified	28 (10.0 %)	4 (28.6 %)	24 (9.1 %)	
Non-amplified	251 (90.0 %)	10 (71.4 %)	241 (90.9 %)	

CNV represents copy number variation. More than 2 considered as amplified FGFR1. Z-score of RNA-Seq is 2.

*Fisher's exact test is applied.

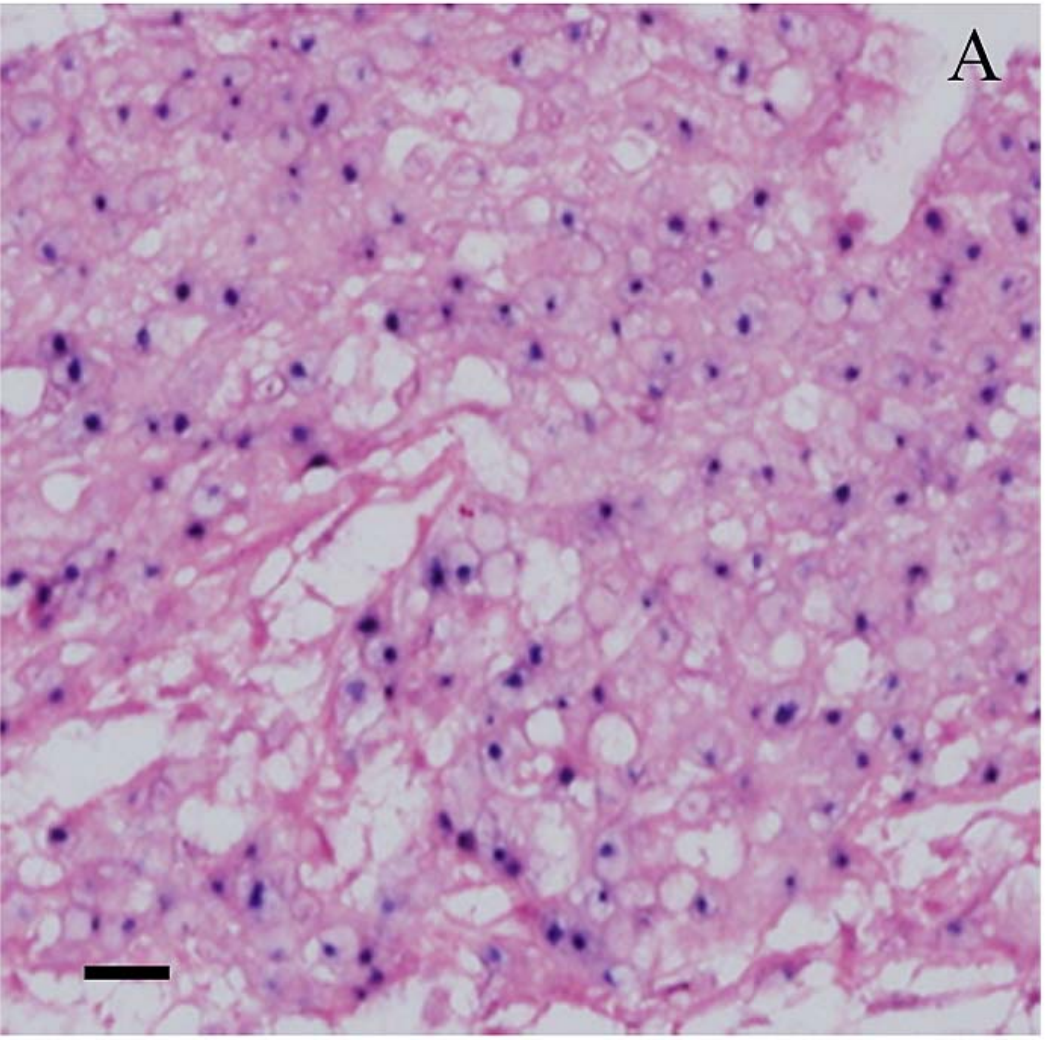
Supplementary Legends

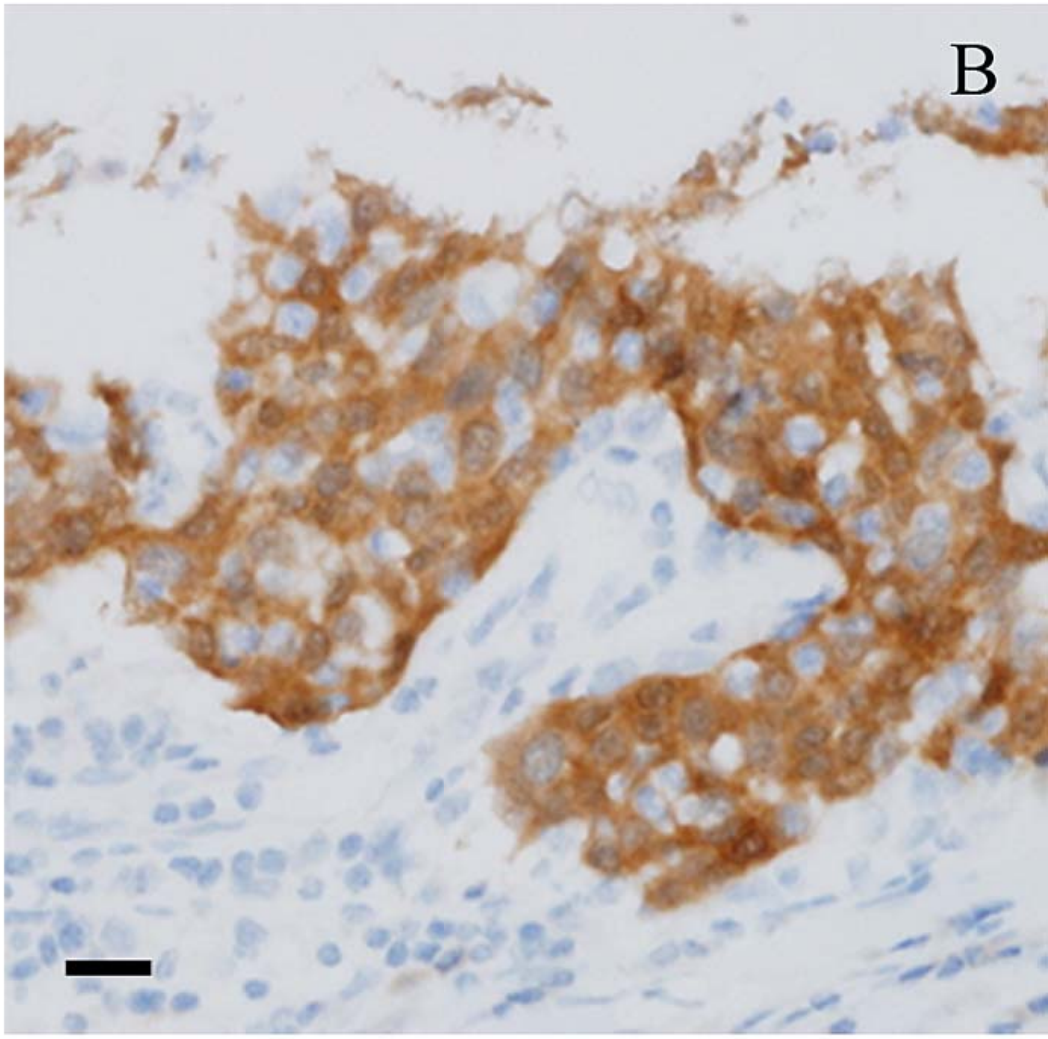
Supplementary Figure 1. Representative images for HPV in situ hybridization (A) and immunohistochemistry for p16 (B). (A) Blue dots represent HPV-positive cells. (B & C) Tumor cells are positive for p16 with a cytoplasmic staining pattern. Scale bar represents 50 ㎛.

Supplementary Figure 2. Frequency of HPV type by genotyping in tonsillar squamous cell carcinoma . Seventy eight patients (76.5 %) out of 102 tonsillar squamous cell carcinoma show HPV genotype positivity. Most common type is type 16 (n = 65, 63.7%) and the second is type 35 (n = 7, 6.9 %).

Supplementary Figure 3. Overall survival *in silico* TCGA-HNSCC data. (A) No significant overall survival difference was observed between the *FGFR1* amplified group and non-amplified group (p = 0.924, log-rank). (B) Although the high FGFR1 mRNA expression group appeared to show a good prognosis for the overall survival rate (p = 0.254, log-rank), this finding was statistically insignificant.

Supplementary Figure 4. Survival analysis according to HPV and p16 status. No survival differences according to HPV in situ hybridization (A & B), p16 (C & D), and a combination of HPV and p16 (E & F) were observed.





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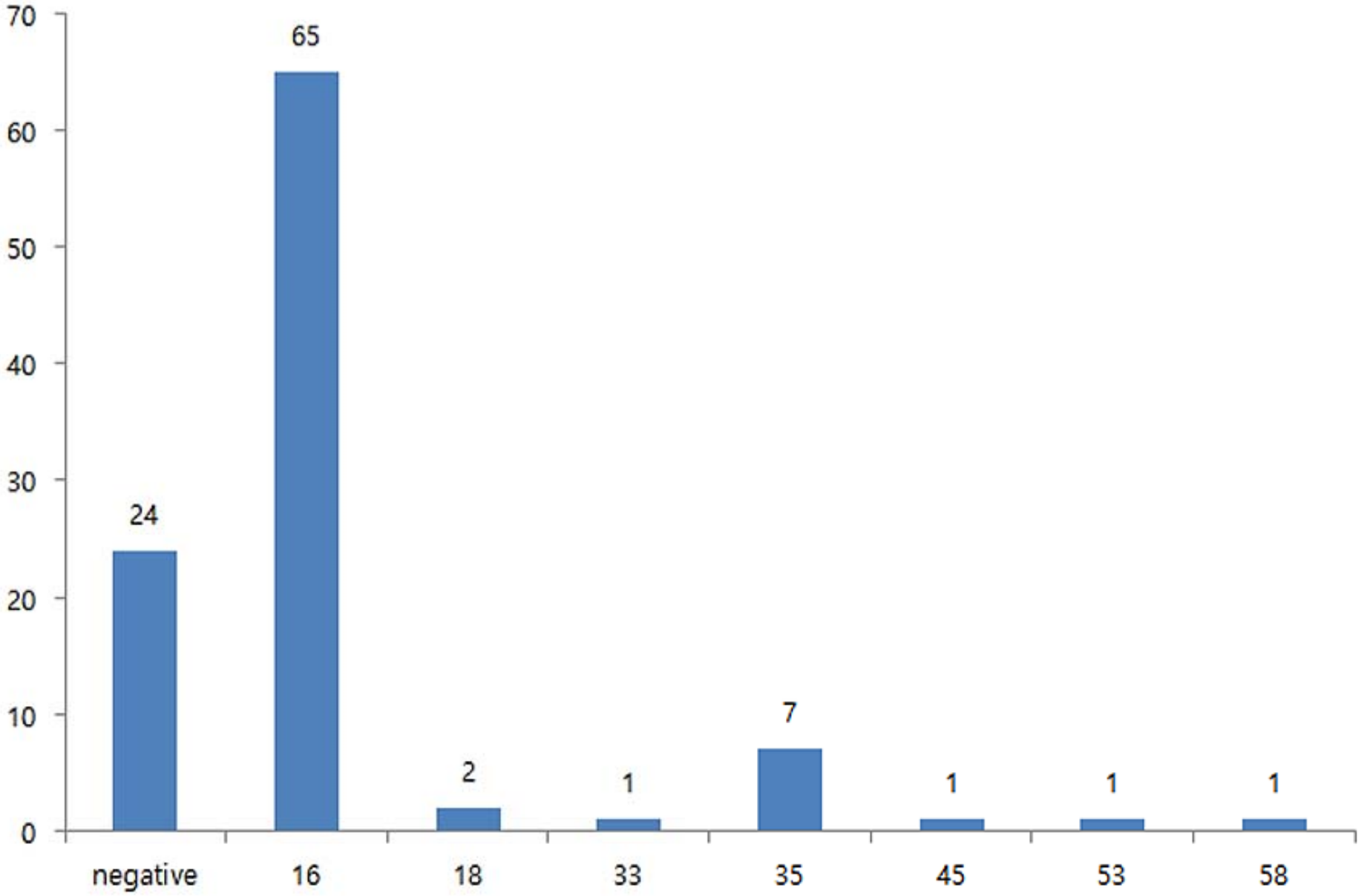

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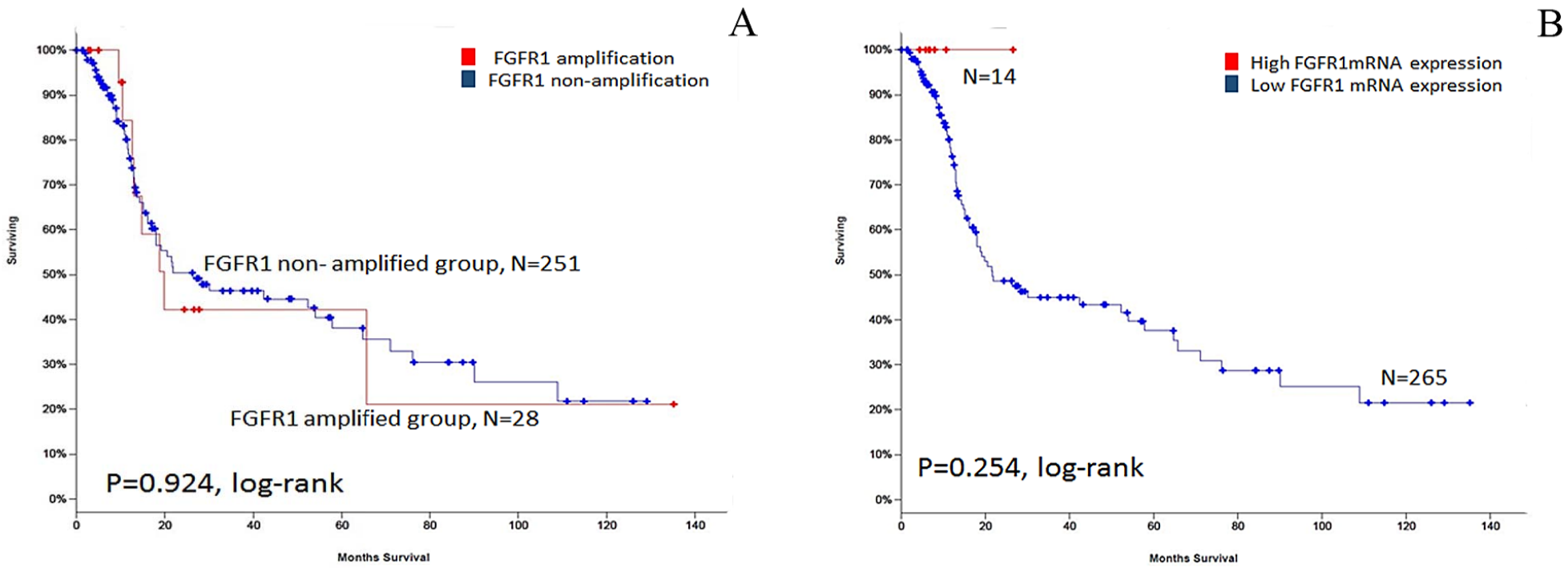

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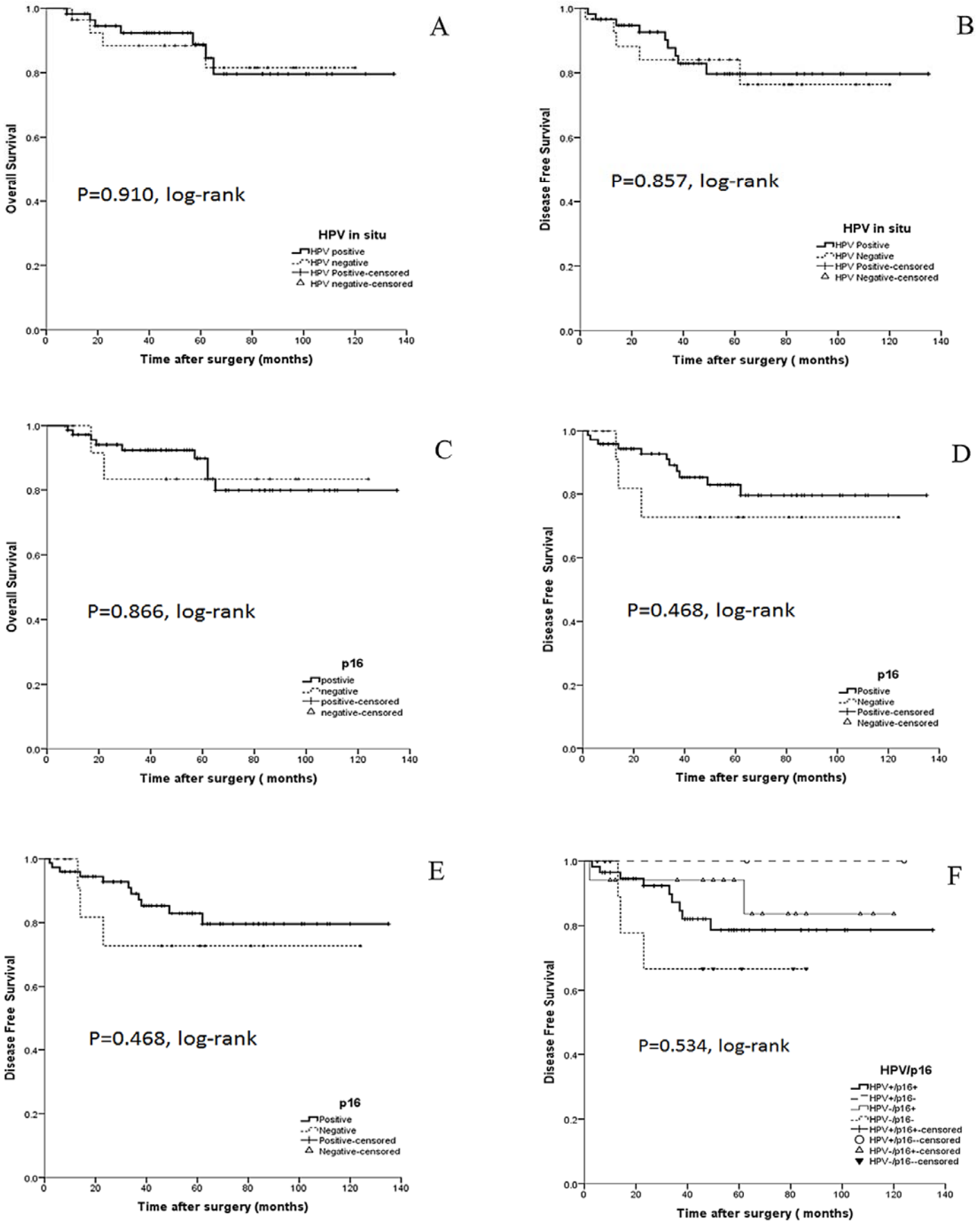

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