Figure S1. High FGFR3 expression is associated with poor NB survival and NB cell lines are dependent on FGFR3 for growth. (A) Kaplan-Meier curves of event-free and overall survival for high/low FGFR3 mRNA expression from a public NB expression cohort (Kosac, n=649). The cut-off between high and low expression was set at the last quartile. P-values were obtained by one-way ANOVA with Bonferroni post-hoc test. Data from R2: Microarray analysis and visualization platform (http://r2.amc.nl) (B) Genome-scale RNAi screening showing dependency of FGFR3 knockdown in cell lines from different cancer diagnoses. For enriched diagnoses two-group comparisons were made across genes using parametric empirical Bayes methods to assess the significance of the observed differences between groups. P-values for each gene were calculated by empirical Bayes moderated t-statistics. Data were retrieved from http://depmap.org. NB, neuroblastoma; FGFR3, fibroblast growth factor receptor 3.

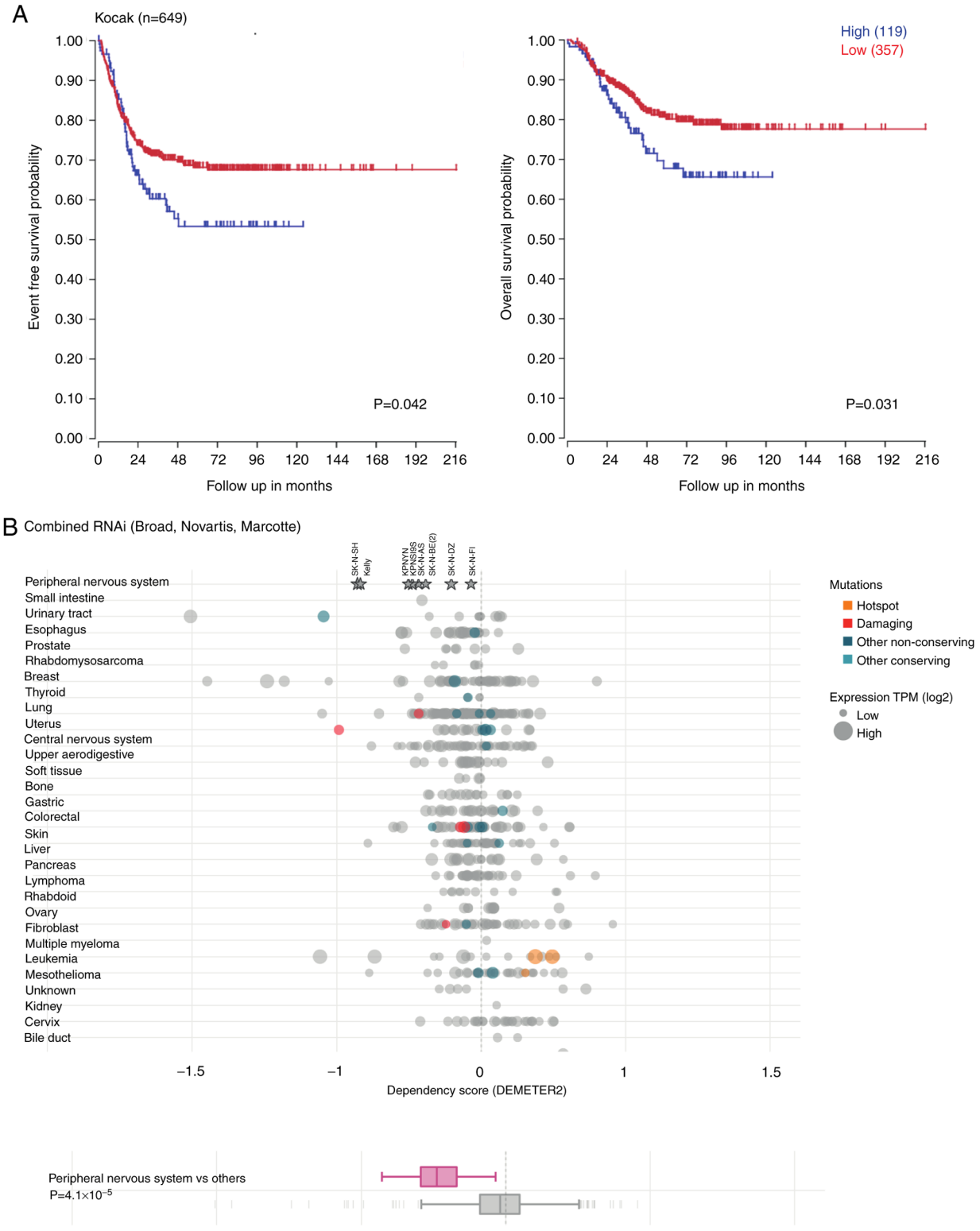

Figure S2. Protein expression of PI3K and FGFR3 in NB cell lines. Western blot analysis showing PI3K p110α (110 kDa), FGFR3 (88 kDa) and loading control GAPDH (36 kDa) protein expression in NB cell lines SK-N-SH, SK-N-FI, SK-N-BE(2)-C and SK-N-AS and in tonsillar and base of tongue cancer cell lines UPCI-SCC-154 and UT-SCC-60A (included as positive controls). NB, neuroblastoma; FGFR3, fibroblast growth factor receptor 3.

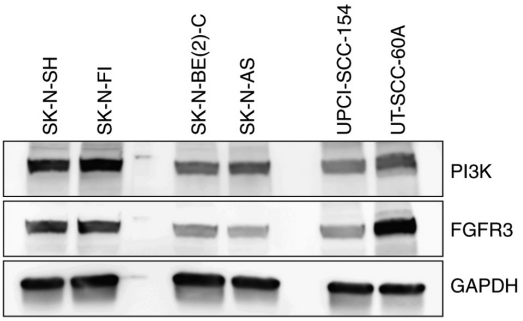

Figure S3. Representative images of wells of the most sensitive (SK-N-SH) and most resistant (SK-N-FI) cell line 72 h after the single and combination treatment. Images in A-a and B-a show the growth of the SK-N-SH and SK-N-FI cell lines without any drug treatment. The effects of various AZD4547 concentrations are shown: For the SK-N-SH cells in the images A-b (5 μM), A-c (10 μM), A-d (25 μM) and for the SK-N-FI cells in B-b (5 μM), B-c (10 μM), B-d (25 μM). The effects of BEZ235 are shown for the SK-N-SH cells in the images A-e (0.25 μM), A-f (0.5 μM), A-g (1 μM), A-h (5 μM) and for the SK-N-FI cells in B-e (0.25 μM), B-f (0.5 μM), B-g (1 μM), B-h (5 μM). The images of the effects of the other PI3K inhibitor, BKM120, for the SK-N-SH cells are shown in A-i (0.25 μM), A-j (0.5 μM), A-k (1 μM), A-l (5 μM), and for the SK-N-FI cells in B-i (0.25 μM), B-j (0.5 μM), B-k (1 μM), B-l (5 μM). The effects of the representative combination treatment are shown for the SK-N-SH cells in A-m (AZD4547 5 μM, BEZ235 0.25 μM), A-n (AZD4547 10 μM, BEZ235 0.5 μM) and A-o (AZD4547 25 μM, BEZ235 1 μM), and for the SK-N-SH cells in B-m (AZD4547 5 μM, BEZ235 0.25 μM), B-n (AZD4547 10 μM, BEZ235 0.5 μM) and B-o (AZD4547 25 μM-BEZ235 1 μM).

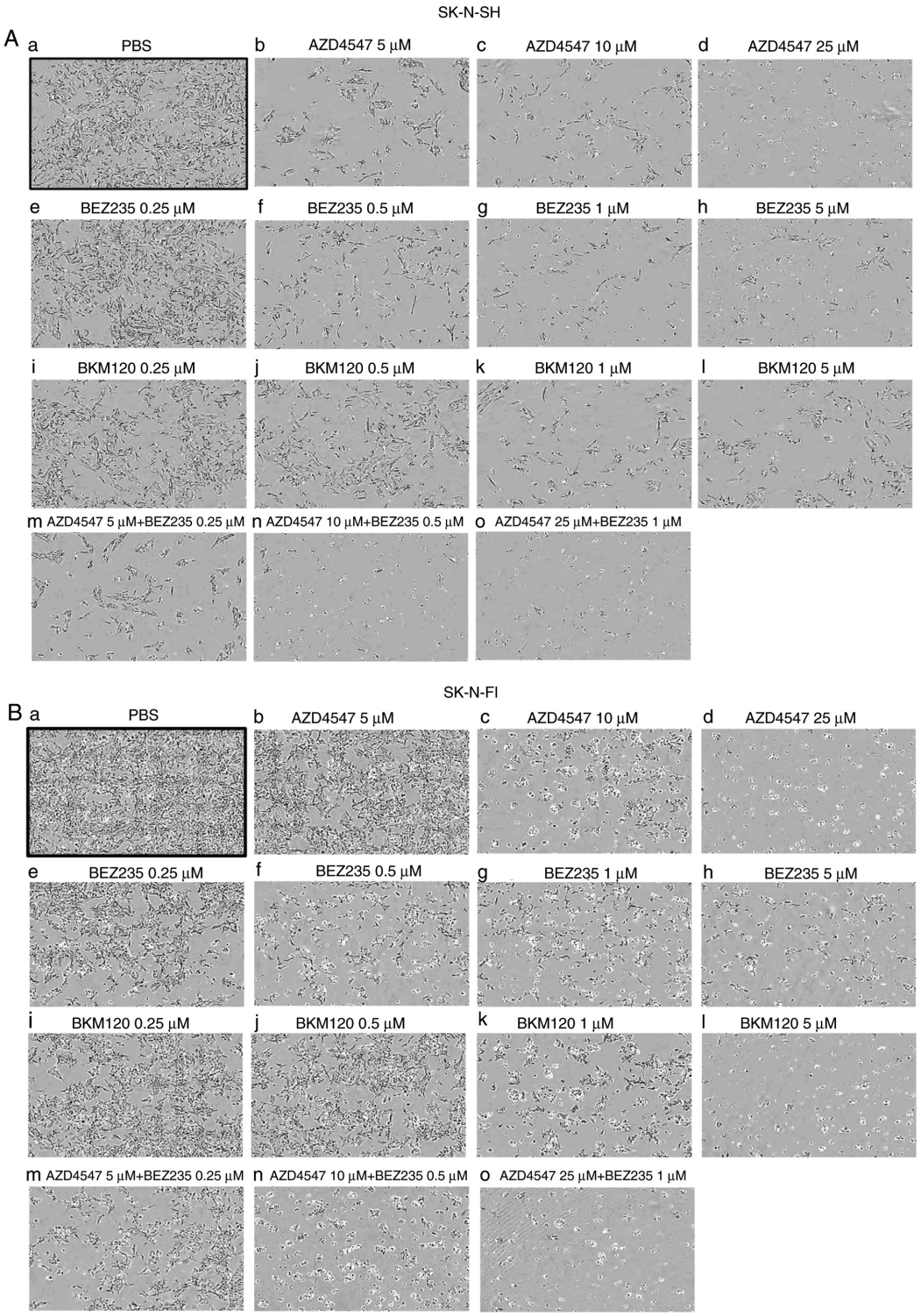

Table SI. Concentrations of FGFR and PI3K inhibitors used for the combination experiments.

Combinations of the FGFR (AZD4547) and PI3K (BEZ235) inhibitors used	
AZD4547 (μM)	BEZ235 (μM)
5	0.25
10	0.25
5	0.5
10	0.5
5	1
10	1
25	1
10	5

FGFR3, fibroblast growth factor receptor 3.