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Supplemental information

**Targeting of c-MET and AXL by cabozantinib is a
potential therapeutic strategy for patients with head
and neck cell carcinoma**

Anais Hagege, Esma Saada-Bouزيد, Damien Ambrosetti, Olivia Rastoin, Julien Boyer, Xingkang He, Julie Rousset, Christopher Montemagno, Jérôme Doyen, Florence Pedoutour, Julien Parola, Isabelle Bourget, Frederic Luciano, Alexandre Bozec, Yihai Cao, Gilles Pagès, and Maeva Dufies

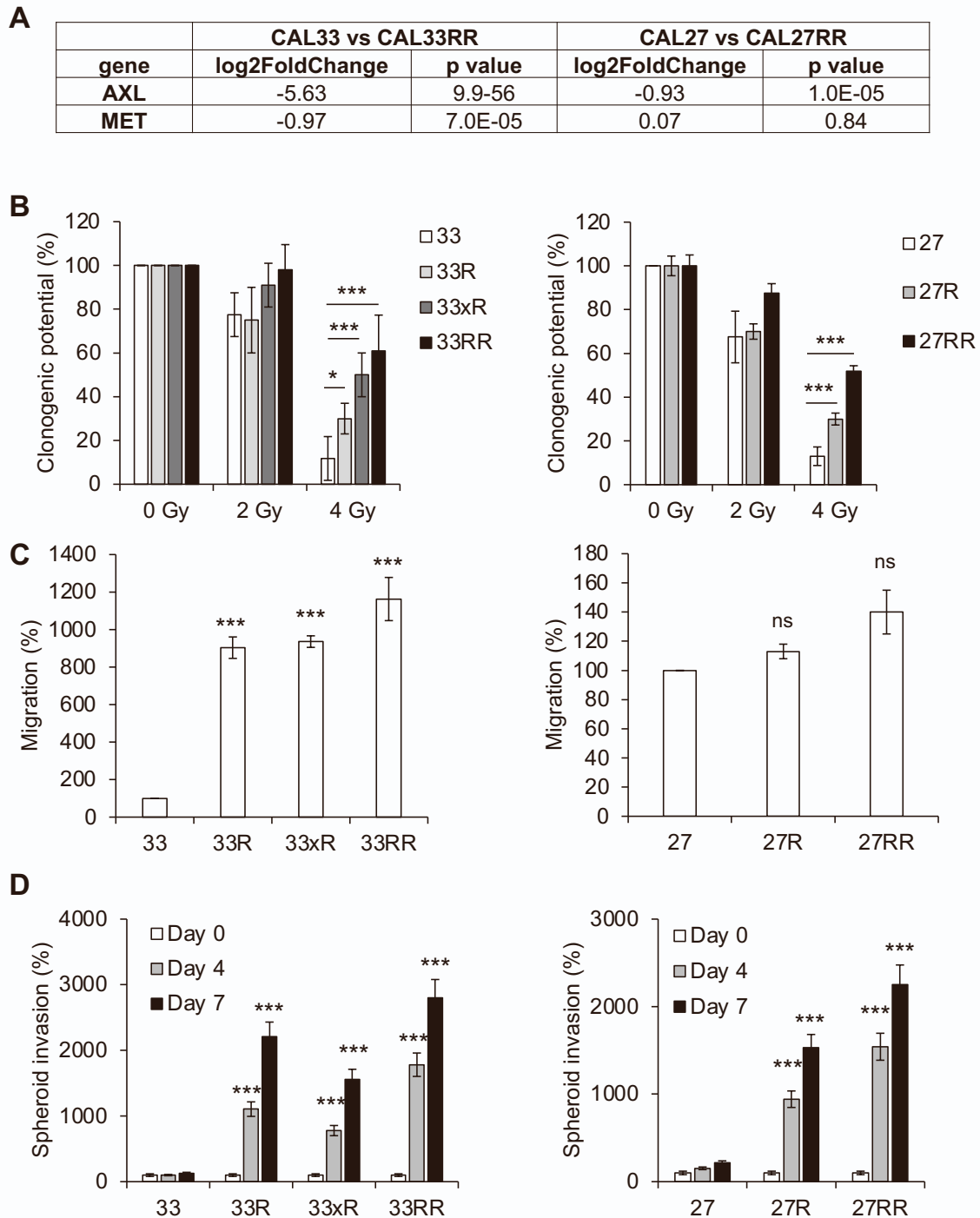


Figure S1. HNSCC resistant cells are more aggressive and overexpress AXL and c-MET.

Related to Figure 1. (A) Results from RNAseq of CAL33 vs CAL33RR and CAL27 vs CAL27RR cells. **(B)** Quantification of the clonogenic potential of 33, 33R, 33xR, 33RR, 27, 27R and 27RR cells irradiated with 2 or 4 Gy. **(C)** The quantification of serum-stimulated cell migration after 24 h was analyzed using Boyden chamber assays in 33, 33R, 33xR, 33RR, 27, 27R and 27RR cells. **(D)** 33, 33R, 33xR, 33RR, 27, 27R and 27RR cell invasion after 4 or 7 days was evaluated using spheroid assays. Day 0 was considered as the reference value (1). Statistics were performed using the ANOVA test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

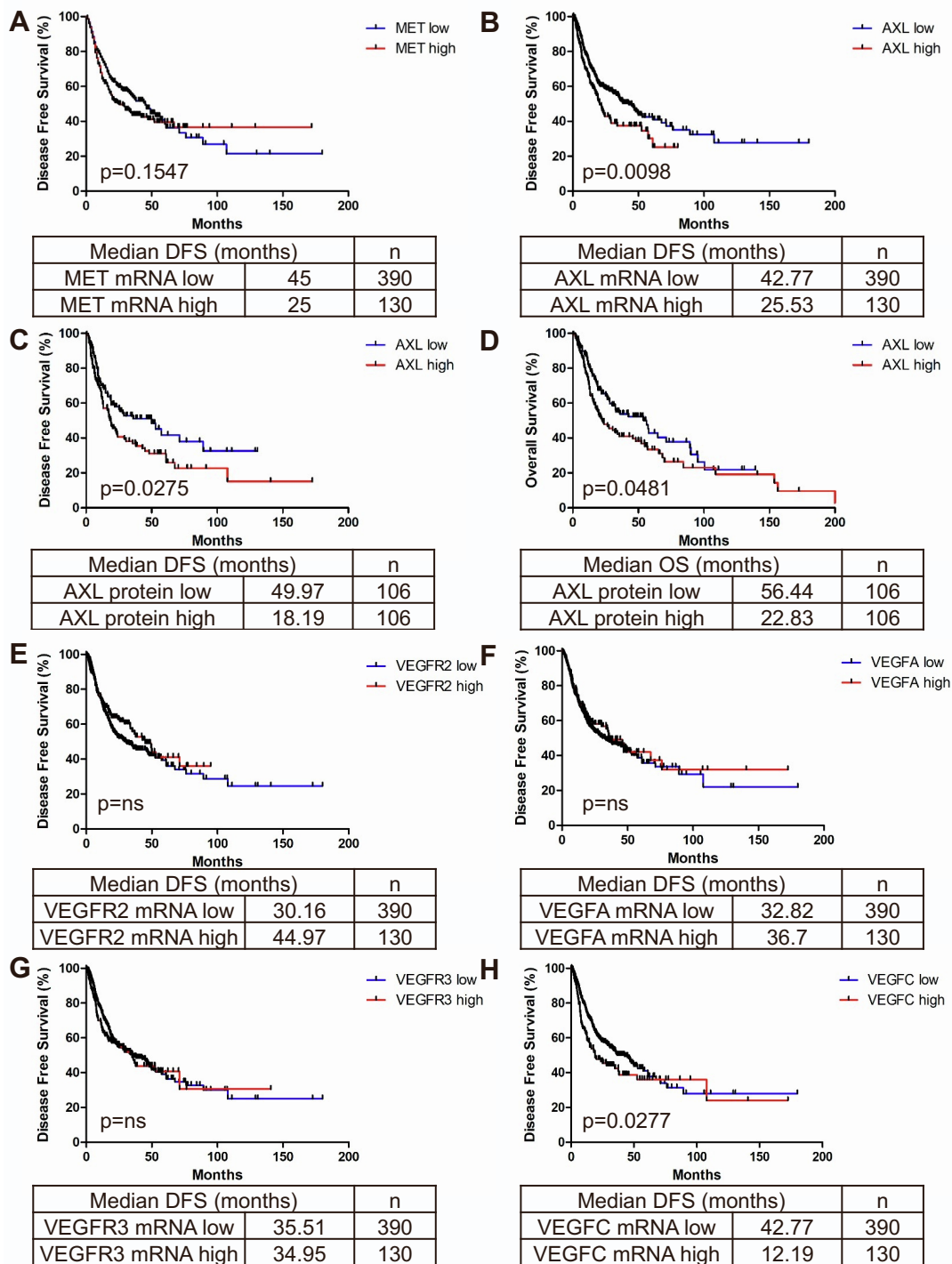


Figure S2. MET and AXL are associated with a poor prognosis of HNSCC.

Related to Figure 2E, 2F. HNSCC patients' samples were analyzed for MET, AXL, VEGFR2 and VEGFR3 mRNA or protein (AXL) levels (z-score). These results are in whole or in part based upon data generated by the TCGA Research Network. The levels of MET (A), AXL (B), VEGFR2 (E), VEGFA (F), VEGFR3 (G) and VEGFC (H) mRNA in HNSCCs correlated with DFS. The AXL protein levels correlated with DFS (C) and OS (D). DFS and OS were calculated from patient subgroups with mRNA or protein levels that were less or greater than the third quartile value. The Kaplan-Meier method was used to produce survival curves and analyses of censored data were performed using Cox models. Statistical significance (p value) is indicated.

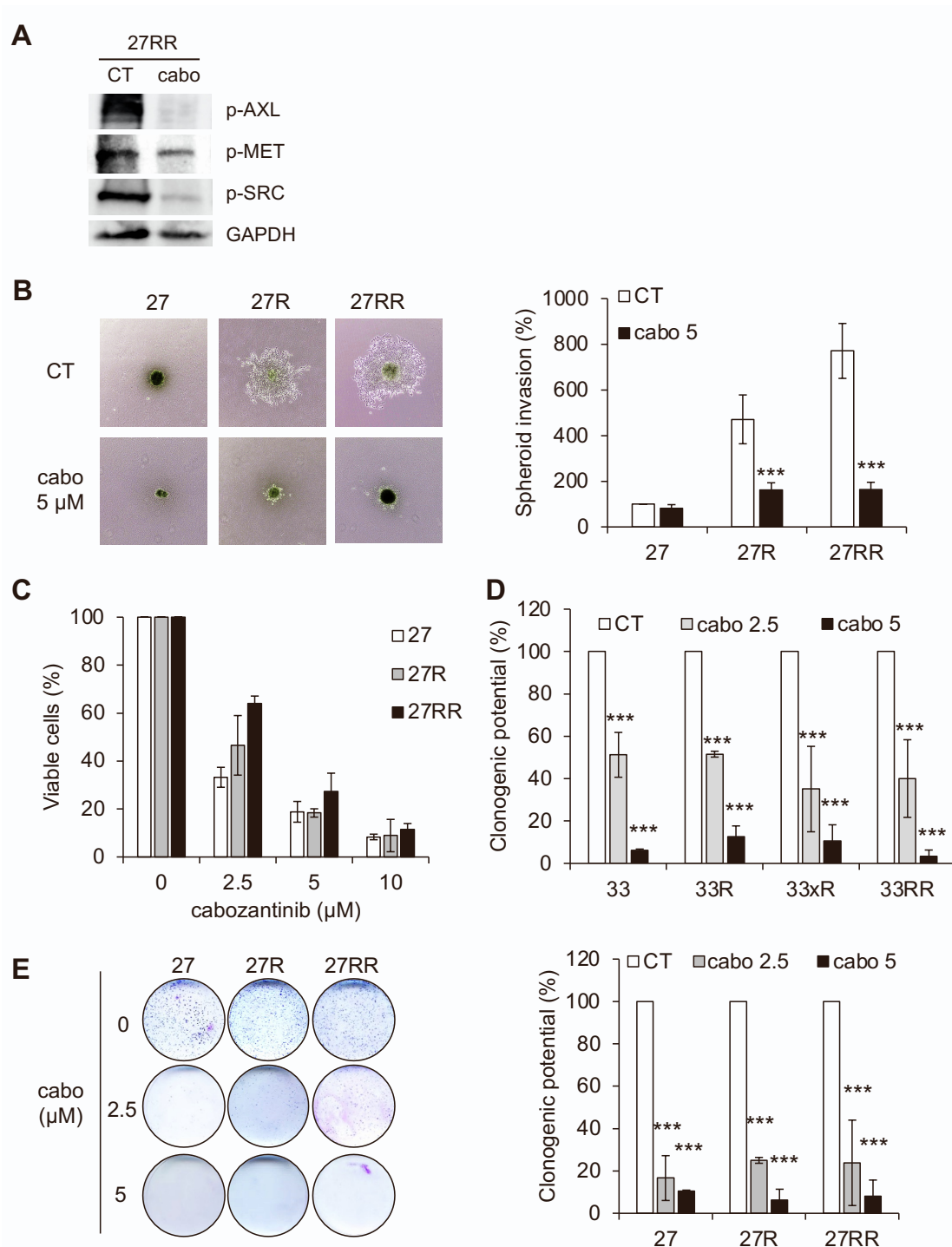


Figure S3. Cabozantinib inhibits migration, invasion, and proliferation of HNSCC cells.

Related to Figure 3. (A) CAL27RR cells were treated with 5μM cabozantinib (cabo) for 1h and phospho-AXL (p-AXL), phospho-MET (p-MET) and phospho-AKT (p-AKT) were evaluated by immunoblotting. GAPDH served as a loading control. **(B)** 27, 27R and 27RR cells were treated with cabozantinib 5 μM (cabo) and cell invasion after 3 days was evaluated using spheroid assays. Representative images are shown. **(C)** 27, 27R and 27RR cells were treated with increasing concentrations of cabozantinib for 48 h. Viable cells were evaluated with ADAM assays. **(D)** 27, 27R and 27RR cells were treated with cabozantinib 2.5 or 5 μM and the clonogenic potential was measured after 10 days. Representative images are shown. **(E)** 27, 27R and 27RR cells were treated with cabozantinib 2.5 or 5 μM and the clonogenic potential was determined after 10 days. Representative images are shown. Statistics were performed using the *ANOVA test*: *** $p < 0.001$.

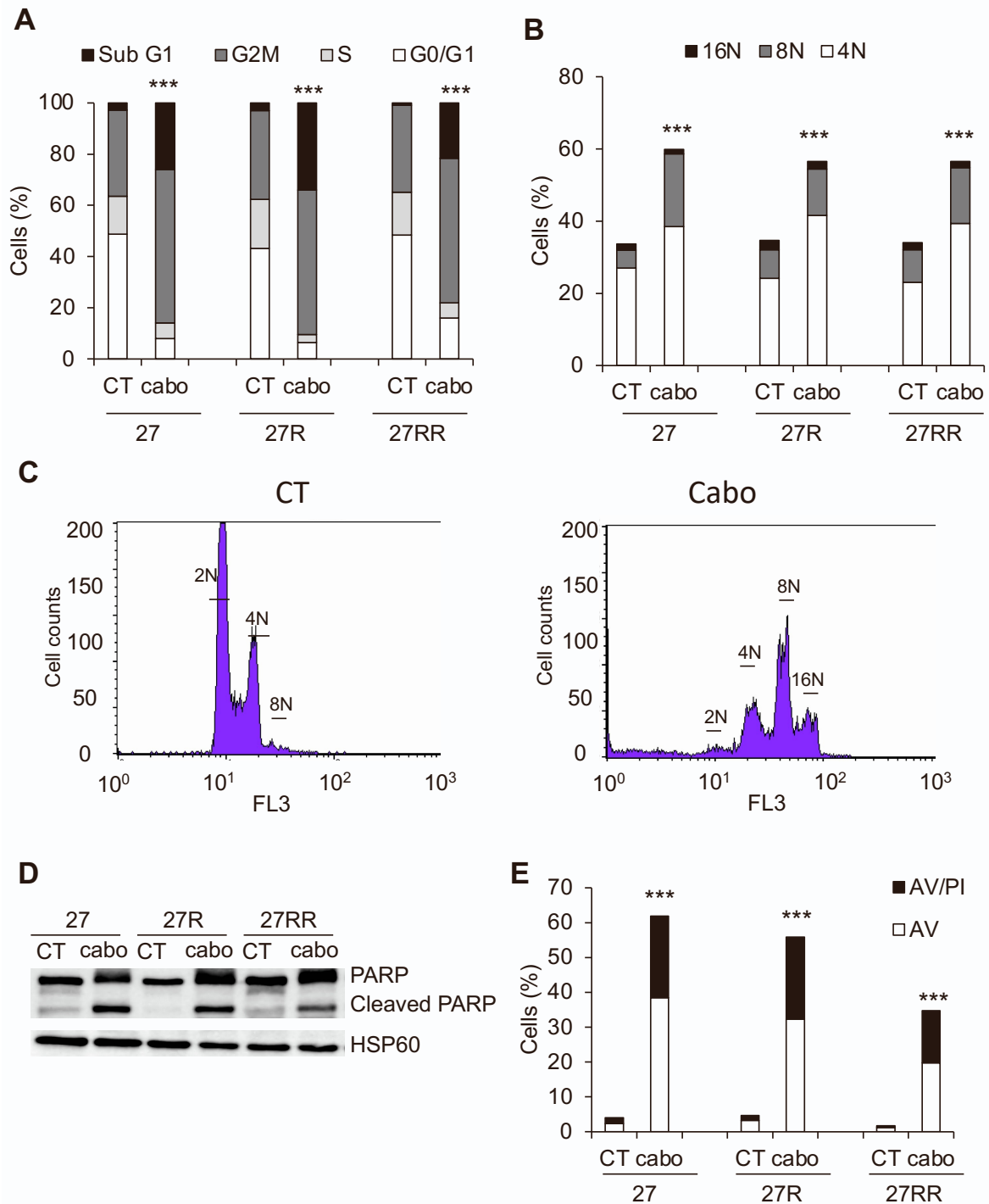


Figure S4. Cabozantinib induces cell cycle arrest, polyploidy, and cell death.

Related to Figure 4. (A) 27, 27R and 27RR cells were treated with cabozantinib 5 μ M for 24 h. The cell cycle was analyzed by cytometry. **(B to E)** 27, 27R and 27RR cells were treated with cabozantinib 5 μ M for 48 h. **(B)** Cells were labeled for 15 min with PI and analyzed by flow cytometry. Histograms represent the percentage of cells with a DNA content of 4N, 8N and 16N. **(C)** Representative cell cycles and polyploidy are shown (27RR cells). **(D)** PARP and cleaved PARP (reflecting apoptosis) were evaluated by immunoblotting. HSP60 served as a loading control. **(E)** Cells were stained with PI/AV. Histograms show AV⁺/PI⁻ cells (apoptosis) and AV⁺/PI⁺ cells (post-apoptosis and/or another cell death). Statistics were performed using the *ANOVA test*: *** $p < 0.001$.

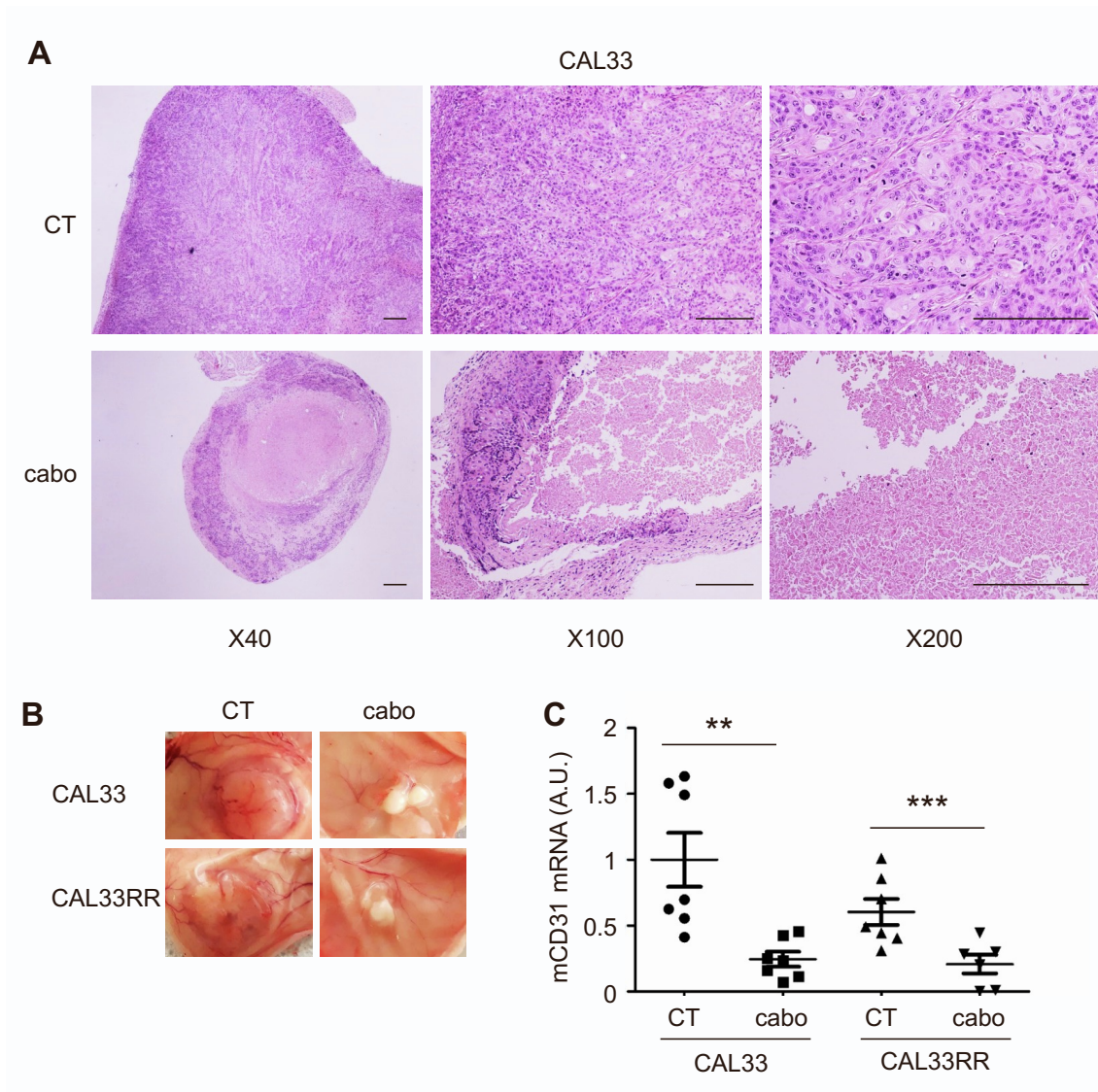


Figure S5. Cabozantinib induces necrosis and acts as an anti-angiogenic drug in experimental HNSCC. Related to Figure 5. (A) Representative images of necrosis of tumors from control and cabozantinib-treated mice at different magnifications (x40, x100, x200). (B) Representative images of tumors with blood vessels are shown. (C) Mouse CD31 mRNA levels were measured by qPCR. Statistics were performed using the *ANOVA test*: ** $p < 0.01$, *** $p < 0.001$.

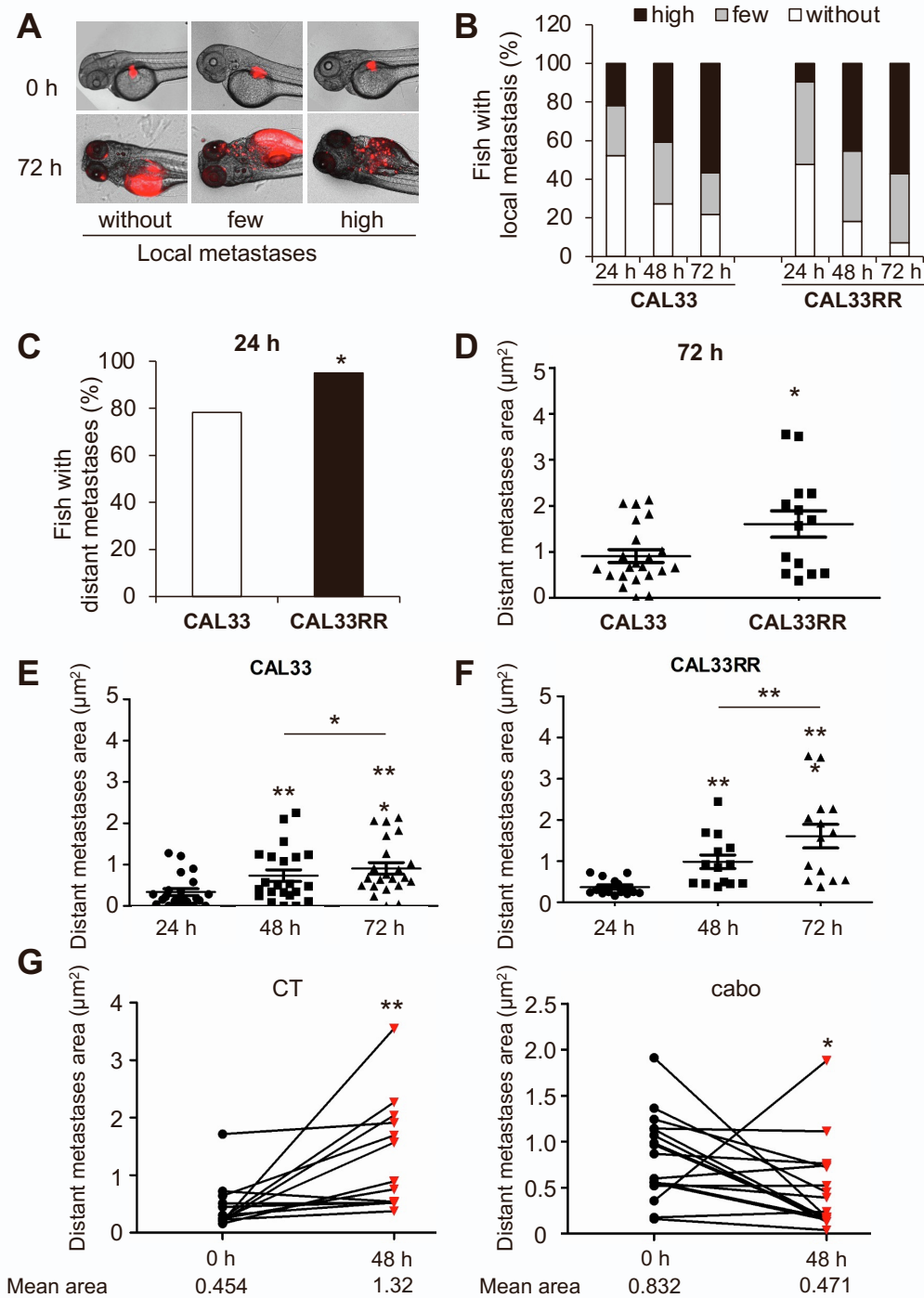


Figure S6. Comparison of the ability of CAL33 and CAL33RR cells to form local and distant metastases in zebrafish. Related to Figure 6. (A to F) Zebrafish embryos ($n=30$) were injected with CAL33 or CAL33RR cells (labeled with red DiD) into the perivitelline space. Zebrafish embryos were monitored for tumor metastases using a fluorescent microscope. (A) Representative images of local metastases (without, few or high local metastases). (B) The appearance and development of local metastases were visualized during 72 h. (C) Percentage of fish with distant metastases after 24 h. (D) Evaluation of the development of distant metastases after 72 h. (E) Determination of the development of CAL33 cell distant metastases during 72 h. (F) Evaluation of the development of CAL33RR cell distant metastases during 72 h. (G) Zebrafish embryos ($n=20$) were injected with CAL33RR cells (labeled with red DiD) into the perivitelline space. 24 h later, only zebrafish with metastases were chosen and treated for 48 h with cabozantinib (cabo, $1 \mu\text{M}$). Zebrafish embryos were monitored for tumor metastasis using a fluorescent microscope. Areas of distant metastases were quantified. Statistics were performed using the ANOVA test: * $p < 0.05$, ** $p < 0.01$.

HNSCC	Diagnostic	Relapse after radiotherapy + platin treatment
Number	20	
Sex		
Female	5 (15%)	
Male	15 (75%)	
pT		
1/2	7 (77.8%)	6 (54.5%)
3/4	2 (22.2%)	5 (45.5%)
x	11	9
pN		
0	3 (33.3%)	10 (90.9%)
≥ 1	6 (66.7%)	1 (9.1%)
x	11	9
pM		
0	20 (100%)	16 (80%)
1	0 (0%)	4 (20%)
Location		
Oral cavity	10 (50%)	5 (25%)
Oropharynx	7 (35%)	13 (65%)
Larynx	3 (15%)	0 (0%)
Lung	0 (0%)	2 (10%)
DFS (median)	37 months	
OS (median)	119.1 months	

Table S1. The characteristics of the HNSCC patients (French cohort) included in the study. Related to Figure 2B, 2C, 2D.

HNSCC patients

Number	5
Tumor from	
Diagnostic	2 (40%)
Relapse after after radiotherapy + platin treatment	3 (60%)
Sex	
Female	2 (40%)
Male	3 (60%)
pT	
1/2	1 (20%)
3/4	4 (80%)
pN	
0	3 (75%)
≥ 1	1 (25%)
x	1
pM	
0	5 (100%)
1	0 (0%)
Location	
Oral cavity	3 (60%)
Oropharynx	2 (40%)

Table S2. The characteristics of the HNSCC patients included in the study. Related to Figure 7.