

Expanded View Figures

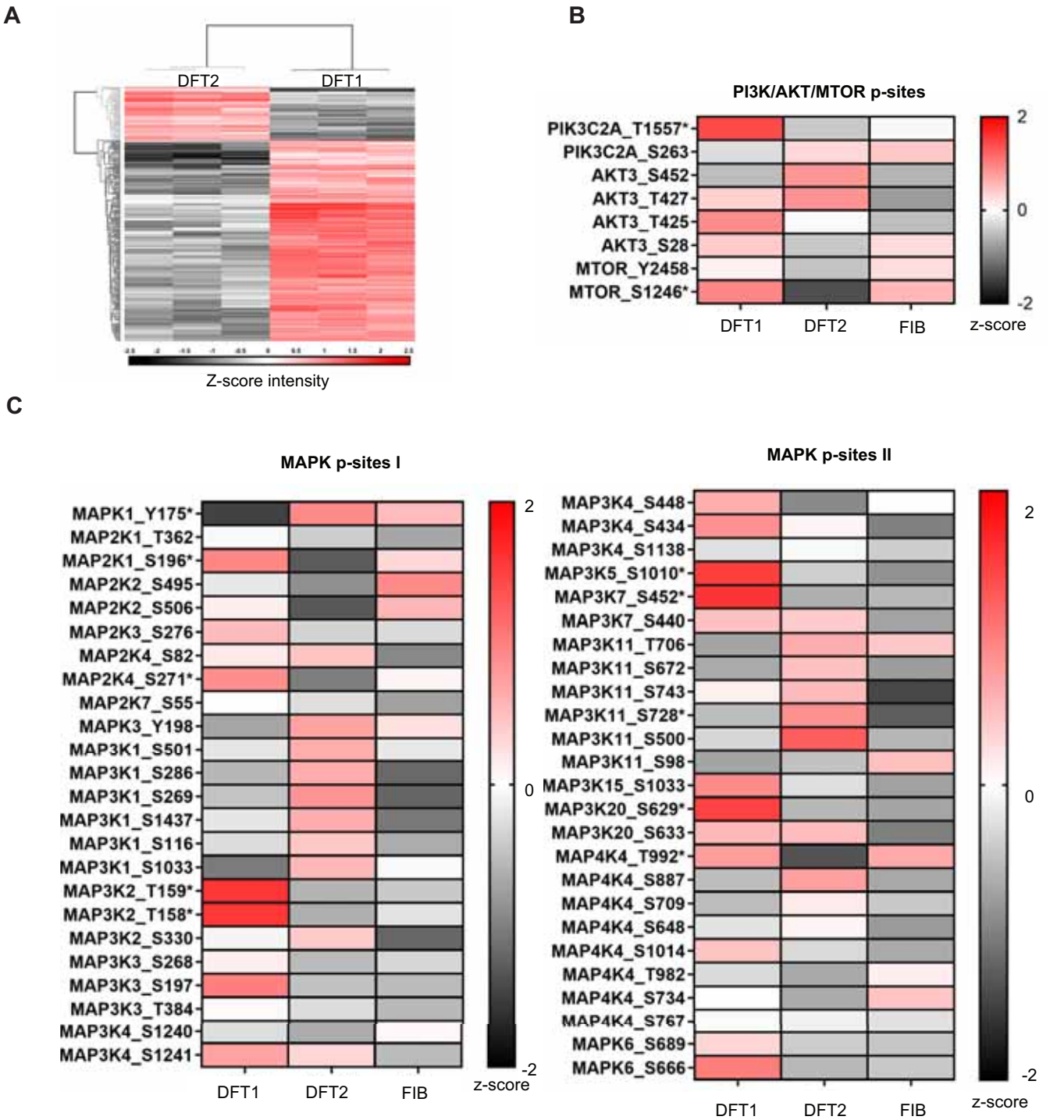
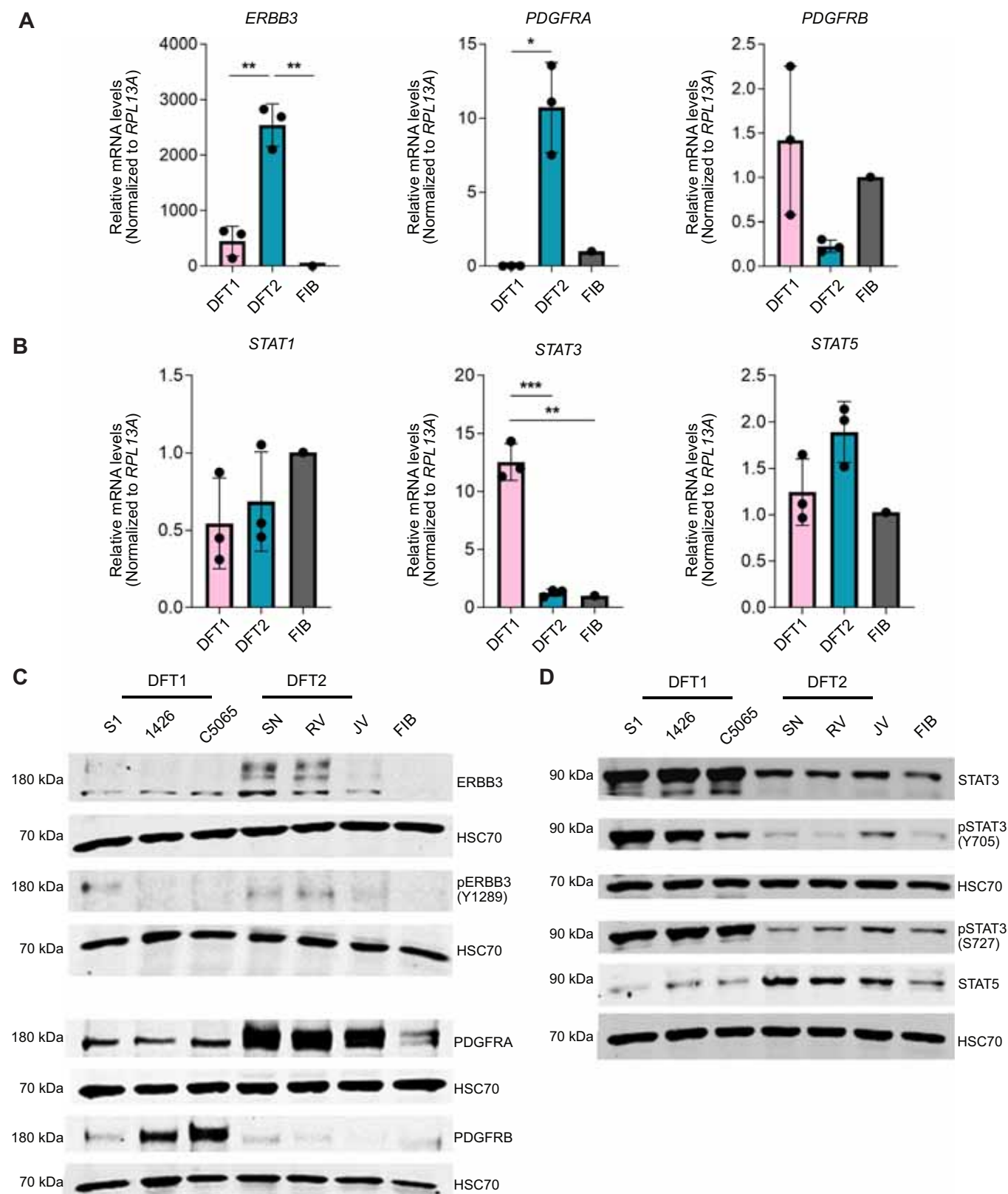


Figure EV1. Related to Fig. 1.

(A) Heatmap of hierarchical clustering of z-scored log2 intensity values for significantly differentially abundant phosphopeptides (Fig. 1C). (B, C) Heatmaps of relative abundance (z-scored log2 intensity values) for peptides mapped to PI3K/AKT/mTOR pathway and MAPK pathway proteins. Stars indicate significant phosphorylation differences between DFT1 and DFT2 (t test, FDR < 0.05, s0 = 0.1).



◀ **Figure EV2. Related to Fig. 1.**

(A, B) Gene expression of *ERBB3*, *PDGFRA*, *PDGFRB* and downstream signalling molecules *STAT1*, *STAT3*, *STAT5** measured by qPCR and normalised to *RPL13A*. Data represent three DFT1 and three DFT2 cell lines (each dot corresponds to a cell line), shown as mean $\pm$ SD relative to a fibroblast control. Statistical analysis was performed using one-way ANOVA with Bonferroni's test. Statistical significance was set at $P < 0.05$ and is indicated as follows: $P < 0.0332$ (*), $P < 0.0021$ (**), $P < 0.0002$ (***), and $P < 0.0001$ (****). Exact P values were as follows: *ERBB3* - DFT1 vs. DFT2: $P = 0.0045$; DFT2 vs. FIB: $P = 0.008$. *PDGFRA* - DFT1 vs. DFT2: $P = 0.0110$. *STAT3* - DFT1 vs. DFT2: $P = 0.0008$; DFT1 vs. FIB: $P = 0.0028$. Data shown are from one representative experiment of two independent replicates. (C, D) Western blot analysis of *ERBB3*, p*ERBB3* (Y1289), *PDGFRA*, *PDGFRB*, *STAT3*, p*STAT3* (Y705), p*STAT3* (S727) and *STAT5** in six DFT cell lines and one fibroblast control. HSC70 was the loading control. $n = 3$. *The Tasmanian devil genome contains two genes more homologous to human *STAT5B* than *STAT5A*, suggesting the absence of *STAT5A* protein. Source data are available online for this figure.

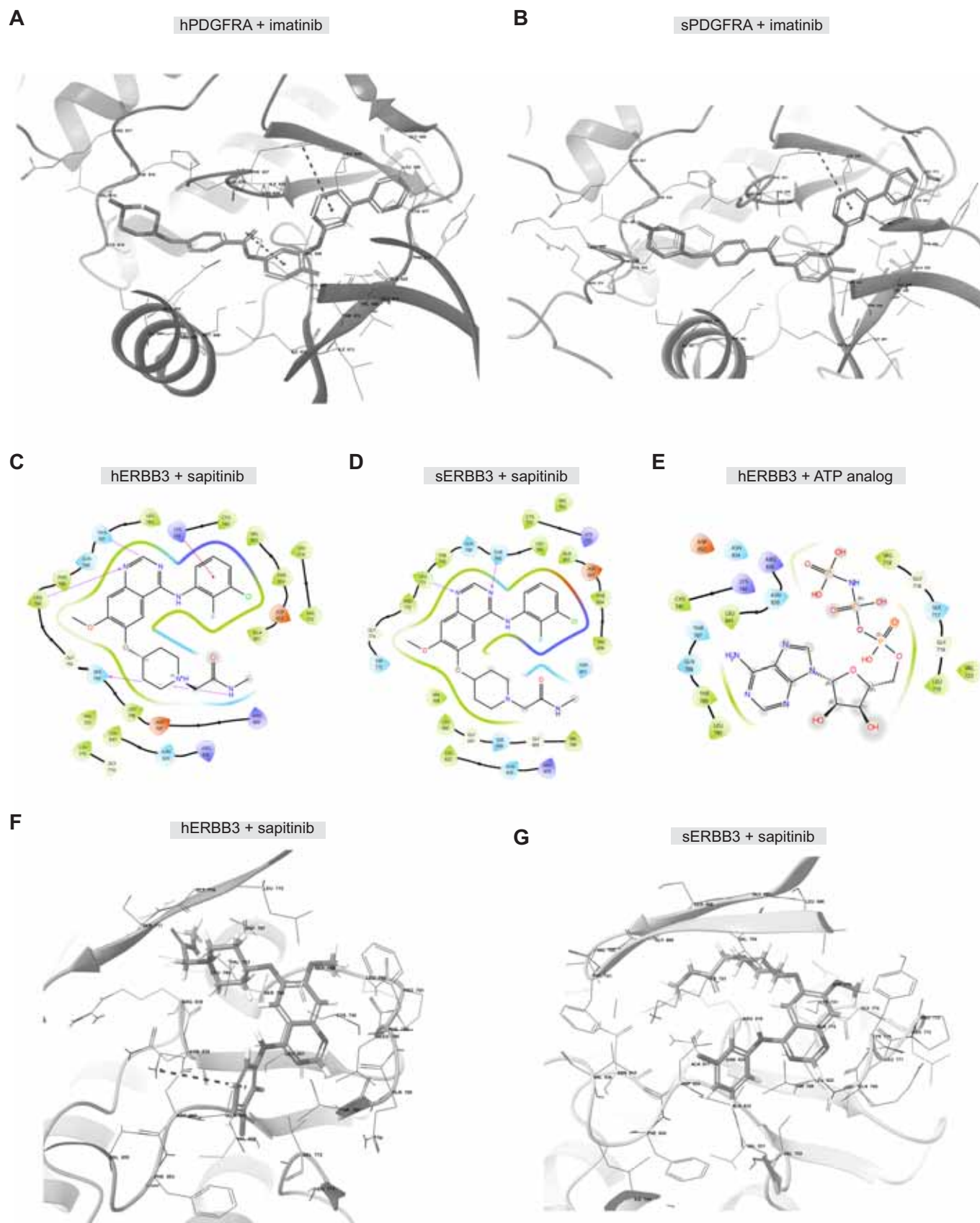
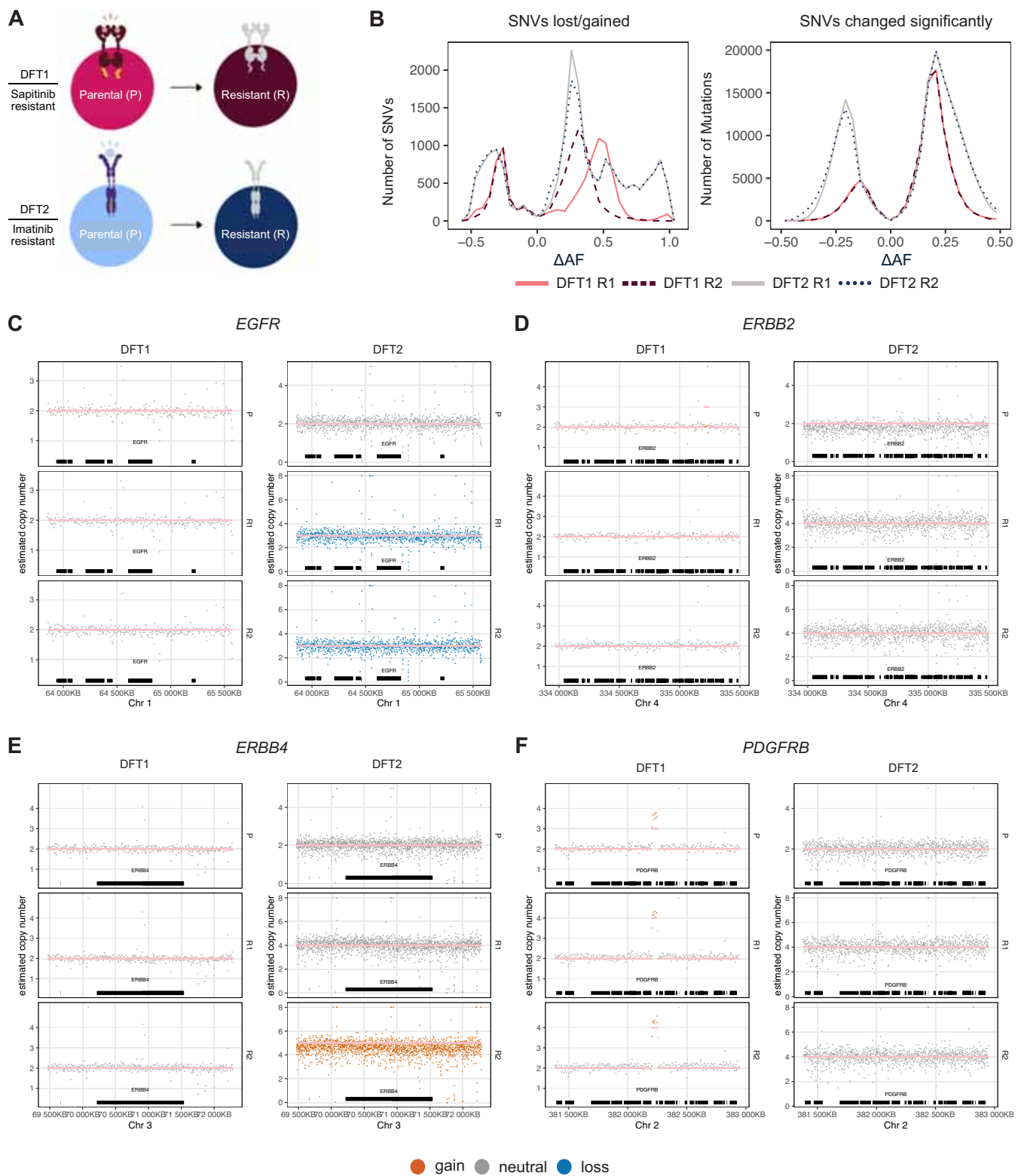


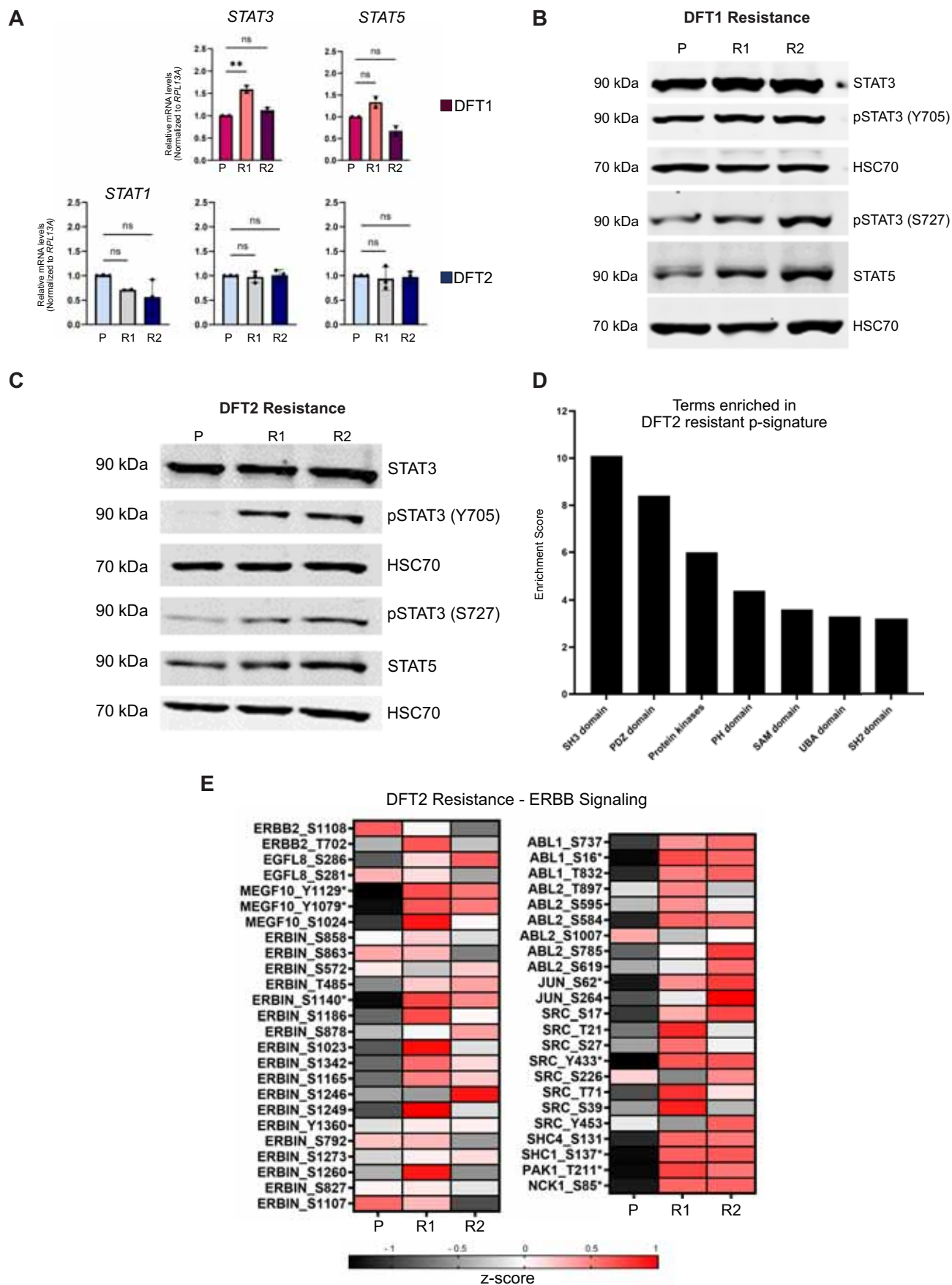
Figure EV3. Associated with Fig. 2.

(A, B) Structural depiction of human PDGFRA (hPDGFRA, A) bound to imatinib (PDB ID: [6JOL](#)) and Tasmanian devil PDGFRA (sPDGFRA, B) modeled into the 6JOL structure, showing binding poses and interacting residues at the catalytic site of the kinase domain. (C–E) Residues of human ERBB3 (hERBB3, C) and Tasmanian devil ERBB3 (sERBB3, D) predicted to interact with sapitinib at the catalytic site of the kinase domain. For comparison, residues involved in binding an ATP analog ligand in the hERBB3 crystal structure (PDB ID: [4RIX](#)) are shown (E). Note that residues in the 4RIX structure are truncated at the N-terminus. (F, G) Induced-fit docking of sapitinib with hERBB3 (F) and sERBB3 (G), illustrating predicted binding poses and interacting residues at the catalytic site.



◀ **Figure EV4. Associated with Fig. 4.**

(A) Schematic showing the generation of sapitinib-resistant DFT1 and imatinib-resistant DFT2 cell lines. (B) Distribution of SNVs gained or lost in resistant lines. Left panel: ΔAF represents the change in minor allele frequency relative to the parental line. SNVs with $\Delta AF < 0$ were considered lost; $\Delta AF > 0$ indicates novel SNVs. Y-axis shows the number of mutations at each ΔAF bin. Filtering was performed using an FDR-corrected binomial test ($P < 0.1$). Right panel: SNVs showing statistically significant frequency changes between parental and resistant lines, identified using Fisher's exact test ($P < 0.05$). (C-F) Copy number (CN) profiles for *EGFR*, *ERBB2*, *ERBB4* and *PDGFRB*. Normalised CN ratios were calculated by Control-FREEC using 5 kbp or 1 kbp genomic windows for DFT1 and DFT2 respectively and multiplied by the assumed ploidy. Genes are indicated as black bars. Copy number gains are shown in orange and losses in blue. Data are displayed in 5 kbp or 1 kbp windows. The pink line represents estimated copy number inferred by Control-FREEC.



**Figure EV5. Associated with Fig. 5.**

(A) Gene expression of *STAT1*, *STAT3*, and *STAT5* in parental (P) and resistant (R1, R2) DFT1 (top) and DFT2 (bottom) cell lines, quantified by real-time PCR and normalised to *RPL13A*. Data are shown relative to the respective parental control and presented as mean $\pm$ SD ($n = 3$ replicates). Statistical analysis was performed using one-way ANOVA with Bonferroni's multiple comparisons test, comparing each group to the parental (P) group. Statistical significance was set at $P < 0.05$ and is indicated as follows: $P < 0.0332$ (*), $P < 0.0021$ (**), $P < 0.0002$ (***), and $P < 0.0001$ (****). *STAT3* (DFT1) P vs. R1; $P = 0.0058$. (B, C) Western blot analysis of *STAT3*, p*STAT3* (Y705), p*STAT3* (S727), and *STAT5* in parental and resistant DFT1 (B) and DFT2 (C) cell lines. HSC70 served as a loading control. $n = 2$. (D) Bar plot showing annotation terms enriched in the DFT2 resistant p-peptide signature. Terms were determined by functional annotation clustering using DAVID Bioinformatics, with selection based on enrichment score < 3 and P value < 0.05 (Bonferroni correction). (E) Heatmaps of the relative abundance (z-scored log₂ intensity values) of ERBB pathway p-peptides in DFT2 parental and imatinib-resistant cell lines. *Significant differences in phosphorylation between parental and resistant cells lines (P value < 0.05). Source data are available online for this figure.