# Supplementary Materials: Paclitaxel-Induced Src Activation is Inhibited by Dasatinib Treatment, Independently of Cancer Stem Cell Properties, in a Mouse Model of Ovarian Cancer

**Elif Kadife, Emily Chan, Rodney Luwor, George Kannourakis, Jock Findlay and Nuzhat Ahmed**

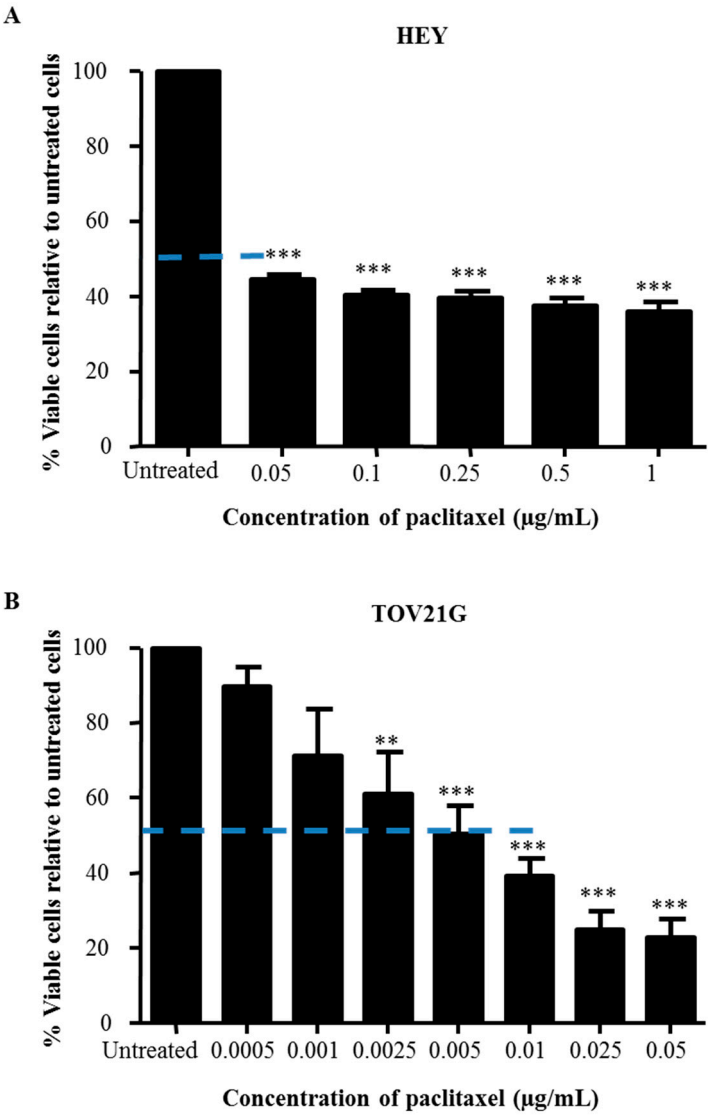


**Figure S1.** Effect of paclitaxel on the viability of ovarian cancer cell lines measured by MTT assay. Ovarian cancer (**A**) HEY and (**B**) TOV21G cell lines were treated with paclitaxel concentration ranging from 0.05–1 µg/mL and 0.0005–0.05 µg/mL respectively for 48 h before analysis by MTT assay. The graph represents four independent experiments performed in triplicate. Results are expressed as the percentage of the average OD reading relative to untreated cells ± SEM. Significance is indicated by ** $p < 0.01$, *** $p < 0.001$ compared to untreated. The intersected line in each graph represents the GI50.

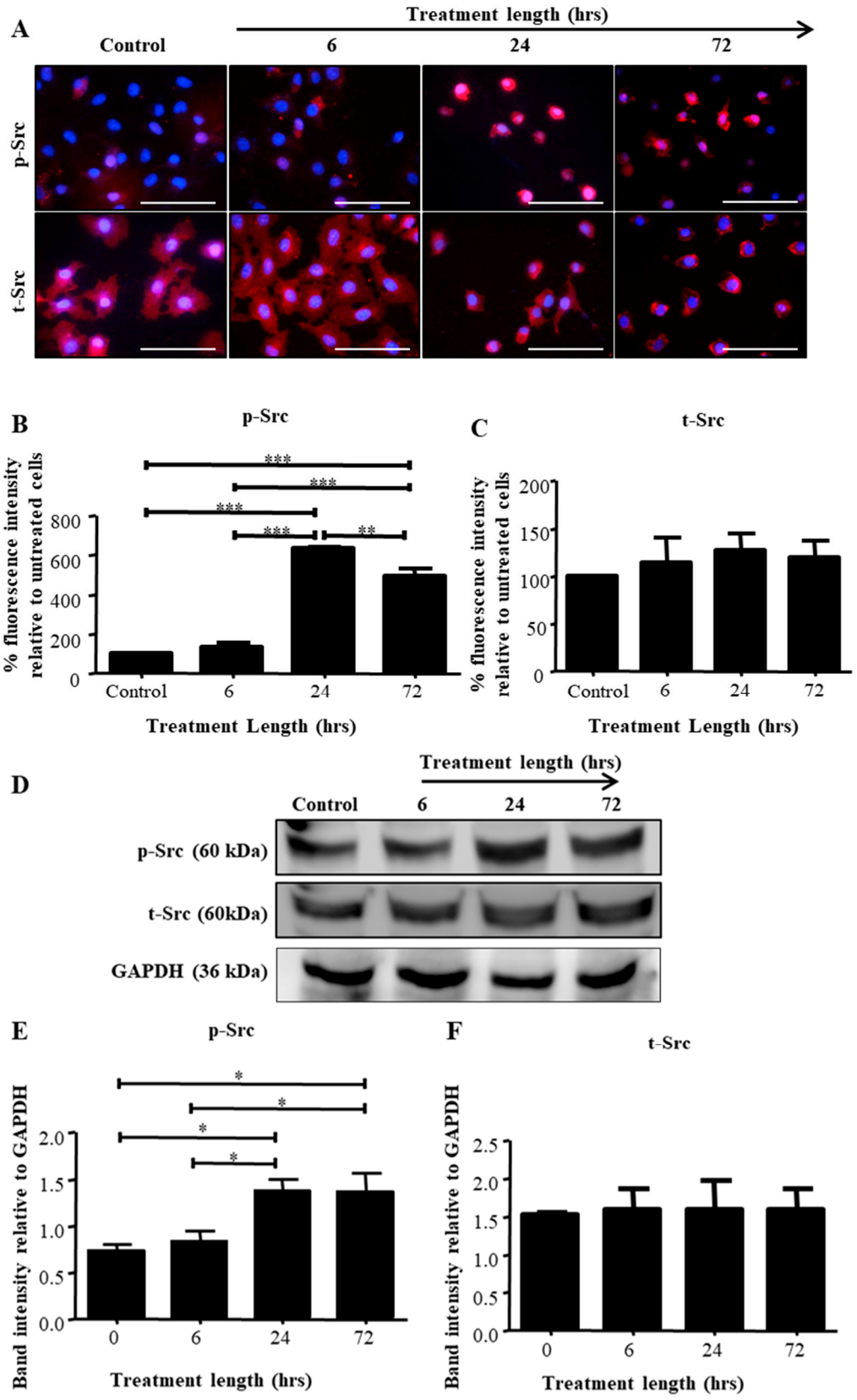


**Figure 2.** Exposure of the TOV21G cells to paclitaxel enhances phosphorylation of Src in a time dependent manner. (**A**) The expression of p-Src and t-Src was assessed by immunofluorescence in untreated and paclitaxel (0.01 μg/mL) treated cells following 6, 24 or 72 h of incubation. Staining was visualized using the secondary Alexa 590 (red) fluorescent-labelled antibody, and nuclei were detected by DAPI (blue) staining. Magnification 400× scale bar = 250 μm. Quantification of (**B**) p-Src and (**C**) t-Src fluorescent intensities was performed using Fiji software. Results are displayed as the

percentage of the average fluorescent intensity relative to untreated cells ± SEM ($n$ = 3/group). (**D**) Total cell lysates of TOV21G cells were collected at 6, 24 and 72 h of paclitaxel treatment and were subjected to immunoblot analysis using antibodies specific for p- or t-Src or GAPDH. Images are representative of three independent experiments. Densitometric analysis of (**E**) p-Src and (**F**) t-Src protein expressions. The values represent the relative mean of band intensity normalized to GAPDH loading control ± SEM. Significance between the groups is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

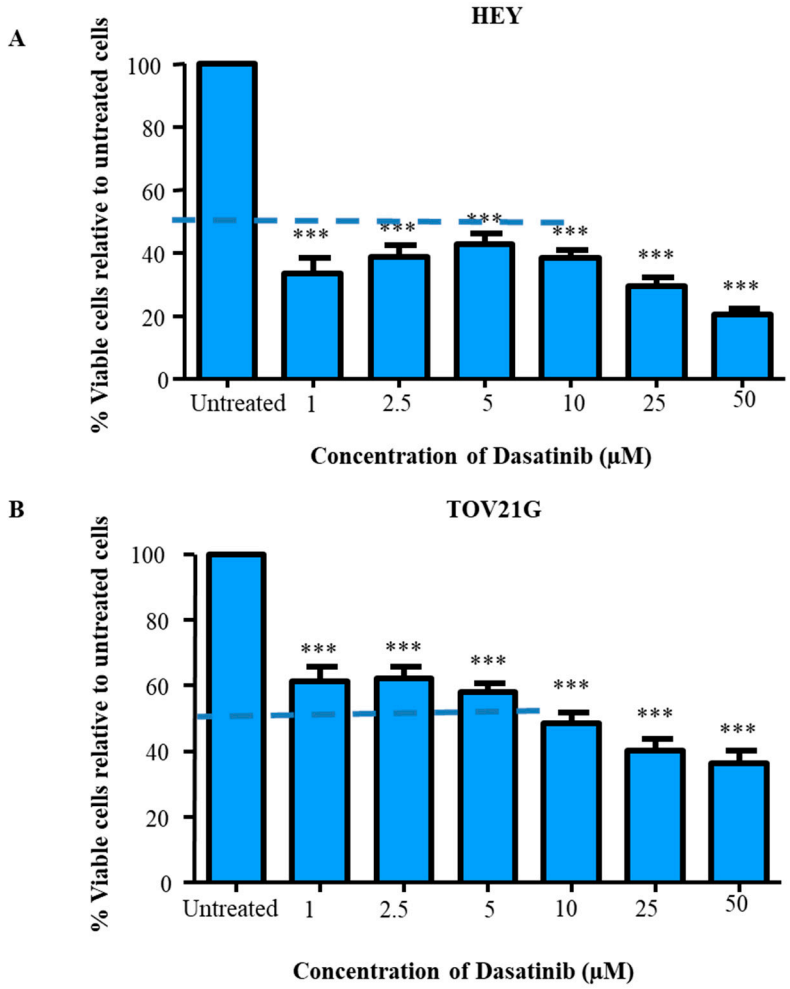


**Figure S3.** Effect of Dasatinib on the viability of ovarian cancer cell lines measured by MTT assay. Ovarian cancer (**A**) HEY and (**B**) TOV21G cell lines were treated with 1–50 μM of Dasatinib for 48 h before analysis by MTT assay. Results are expressed as the percentage of the average OD reading relative to untreated cells ± SEM of three independent experiments performed in triplicate. Significance is indicated by *** $p < 0.001$ compared to untreated. The intersected line in each graph represents the GI50.

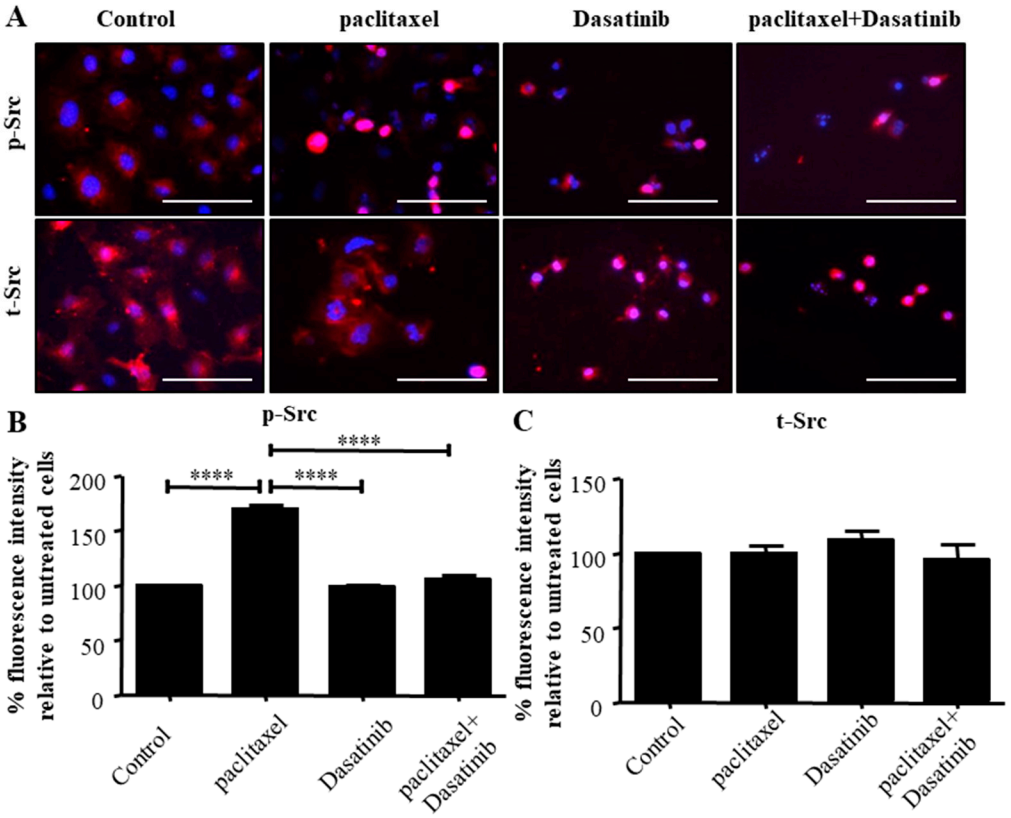


**Figure S4.** Dasatinib inhibits paclitaxel-induced Src activation in TOV-21G cells. (**A**) Immunofluorescent visualisation of **e**xpression and localization of the p- or t-Src proteins in untreated TOV21G cells or following a 24 h treatment with paclitaxel (0.01 μg/mL), Dasatinib (10 μM) or a combination of both. Staining was visualized using the secondary Alexa 590 (red) fluorescent-labelled antibodies, and nuclei were detected by DAPI (blue) staining. Images are representative of three independent experiments. Magnification 400× scale bar = 250 μm. Quantification of (**B**) p-Src and (**C**) t-Src fluorescent intensities was determined using Fiji software. Results are expressed as the percentage of the average fluorescent intensity value relative to untreated cells ± SEM ($n$ = 3/group). Significance is indicated by **** $p < 0.0001$.

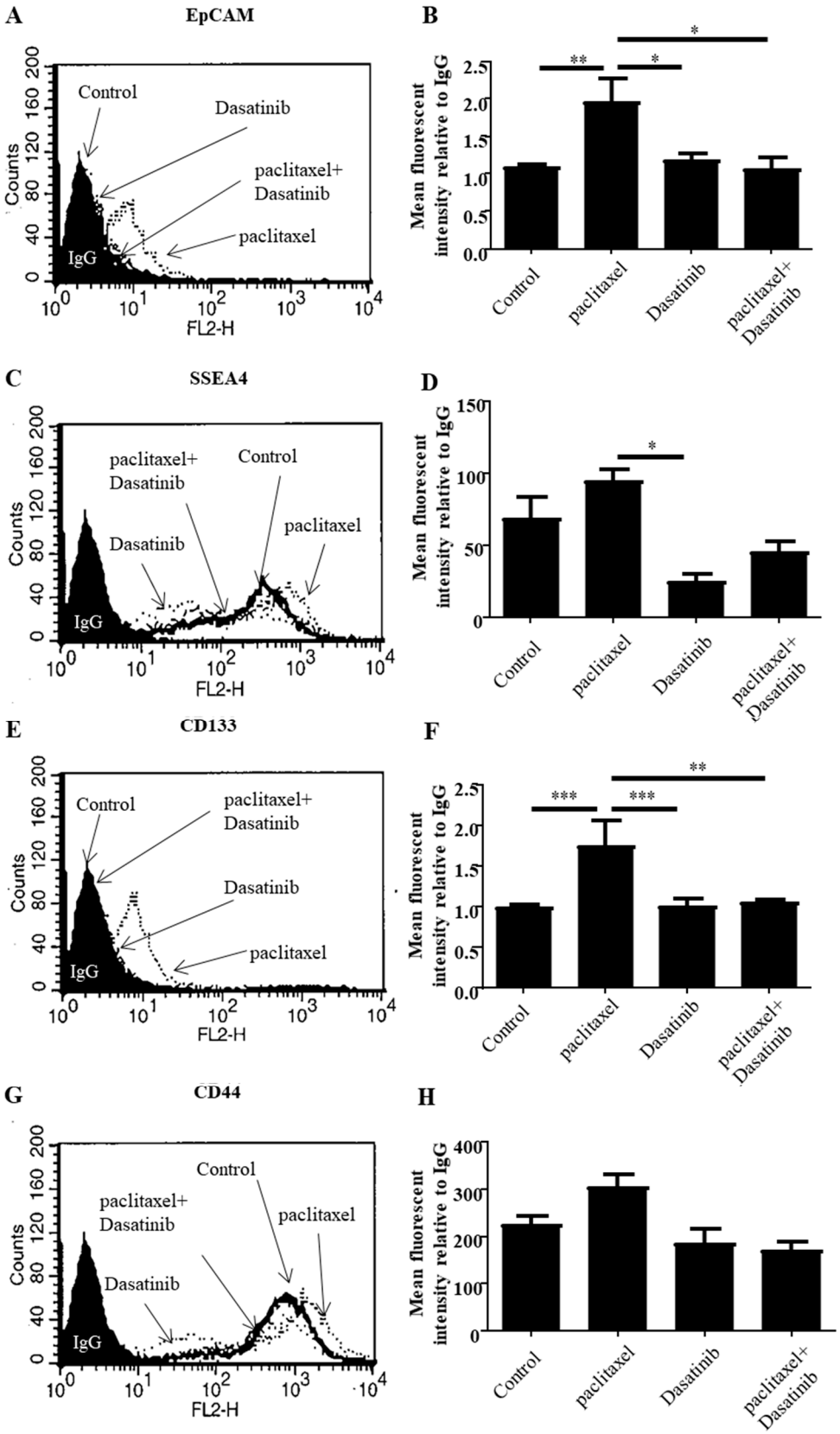


**Figure S5.** CSC-like marker expression is reduced with Dasatinib treatment in the TOV-21G cell line. Surface expression of (**A**,**B**) EpCAM, (**C**,**D**) SSEA-4, (**E**,**F**) CD133 and (**G**,**H**) CD44 in TOV-21G cell line following a 24 h treatment with paclitaxel (0.01 μg/mL), Dasatinib (10 μM) or a combination of both was deduced by Flow cytometry. Histograms are representative of four independent experiments. Semi-quantitative analysis of the arbitrary fluorescent expressions of (**B**) EpCAM, (**D**) SSEA-4, (**F**)

CD133 and (**H**) CD44 standardised to control IgG. Values represent the mean of fluorescent intensity relative to the mean of control IgG ± SEM ($n$ = 4). Significance is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

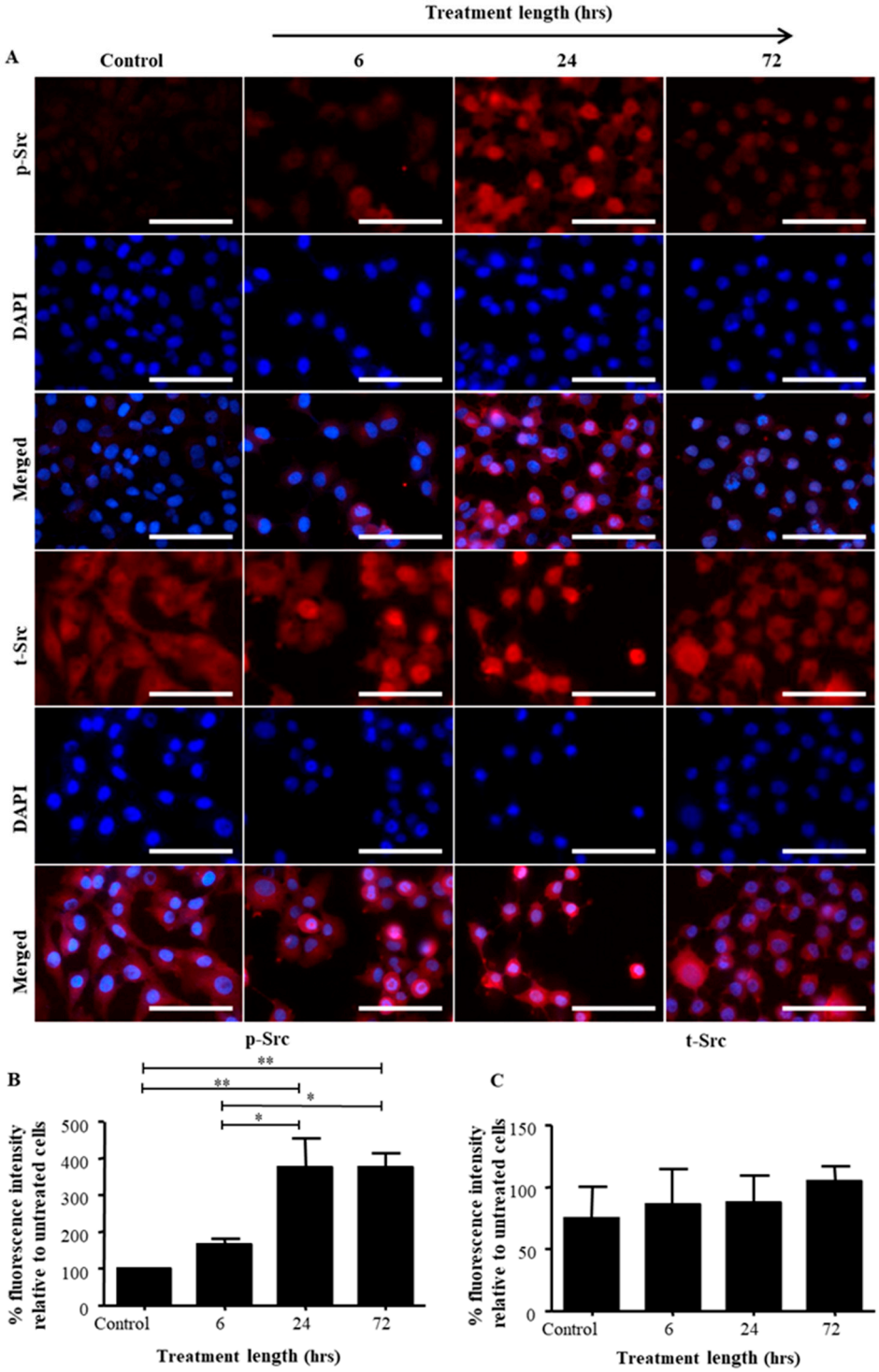


**Figure S6.** Single channel images relating to Figure 2 in the manuscript. (**A**) The expression of p-Src and t-Src was assessed by immunofluorescence in untreated and paclitaxel (0.05 μg/mL) treated HEY cells following 6, 24 or 72 h of incubation. Staining was visualized using the secondary Alexa 590 (red) fluorescent-labelled antibody, and nuclei were detected by DAPI (blue) staining. Magnification 400× scale bar = 250 μm. Quantification of (**B**) p-Src and (**C**) t-Src fluorescent intensities were performed using Fiji software. Results are expressed as the percentage of the average fluorescent intensity relative to untreated cells ± SEM ($n$ = 3/group). Significance between the groups and is indicated by * $p < 0.05$, ** $p < 0.01$.

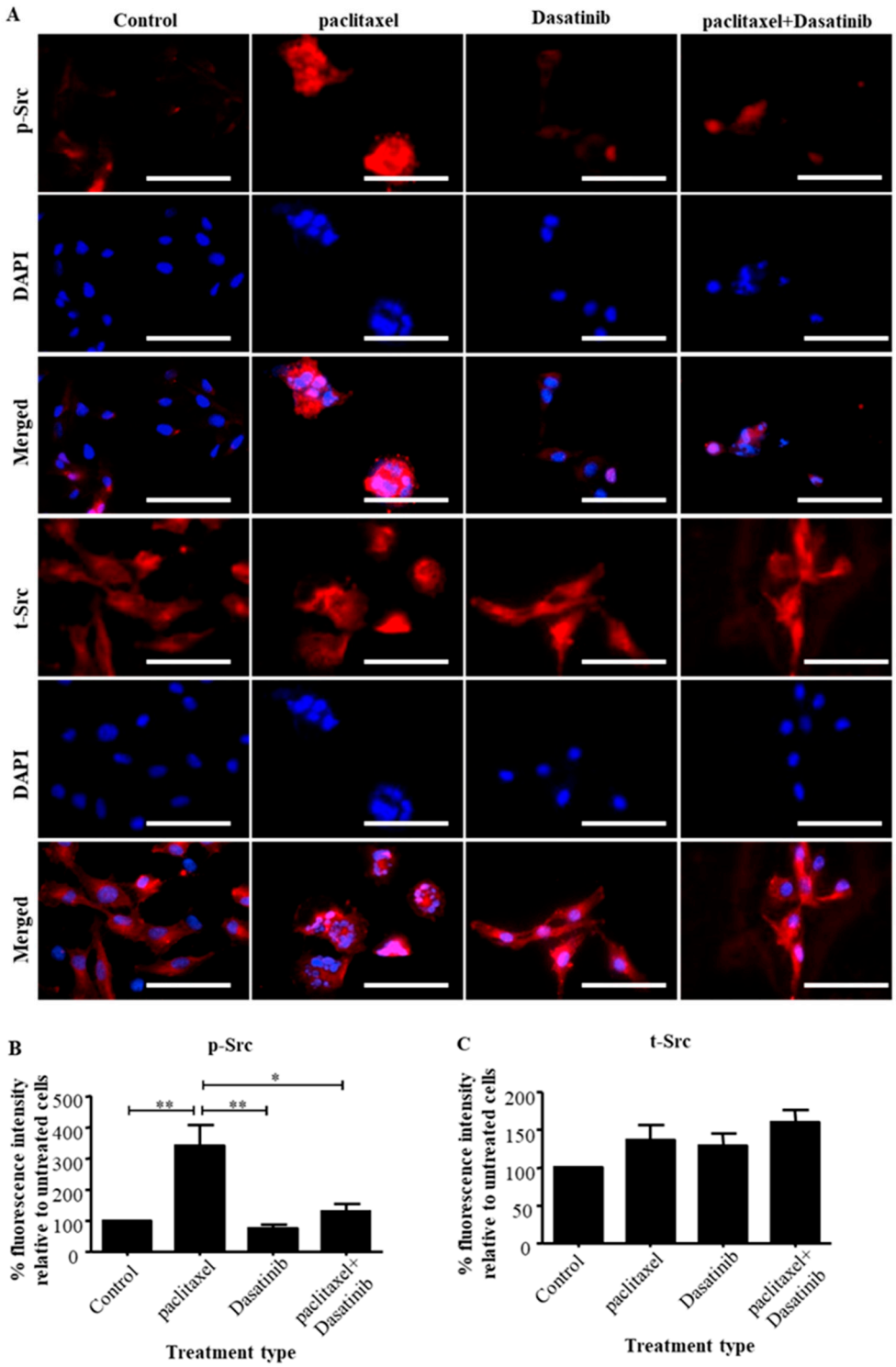


**Figure S7.** Single channel images relating to Figure 3 in the manuscript. (**A**) Expression and localization of the p- or t-Src in untreated, paclitaxel (0.05 μg/mL), Dasatinib (10 μM) or a combination of both treated HEY cells by immunofluorescence. Staining was visualized using the secondary Alexa 590 (red) fluorescent-labelled antibodies and nuclei were detected by DAPI (blue) staining. Images are representative of three independent experiments. Magnification 400× scale bar = 250 μm. Quantification of (**B**) p-Src and (**C**) t-Src fluorescent intensities were determined using Fiji software. Results are expressed as the percentage of the average fluorescent intensity value relative to untreated cells ± SEM ($n$ = 3/group). Significance between the groups and is indicated by * $p < 0.05$, ** $p < 0.01$.