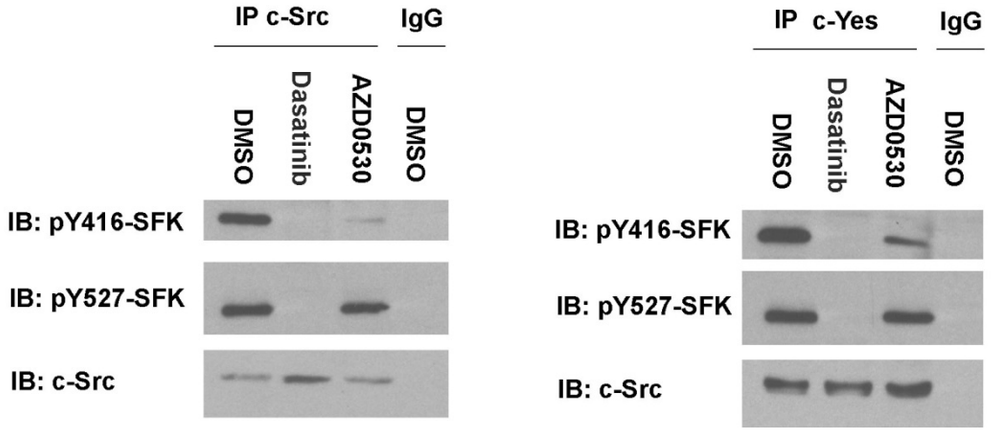
Supplementary Figure 1

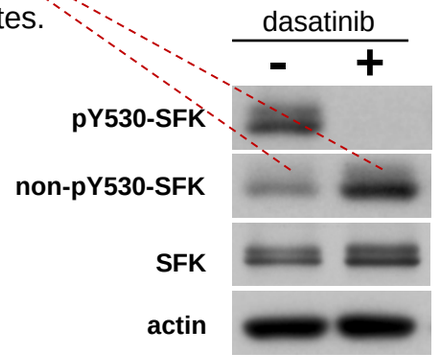
A

MDA-MB-468 cells were treated with the Src inhibitors dasatinib (1uM) or AZD0530 (1uM) for 30 minutes and the state of Y416 and Y527 phosphorylation determined for Src and Yes by IP and immunoblotting as indicated. Dasatinib inhibits CSK as well as SFKs and thus dephosphorylates Y527 of SFKs. AZD0530 is shown for comparison since it is more selective for SFKs and does not inhibit CSK and thus leaves Y527 phosphorylation intact.

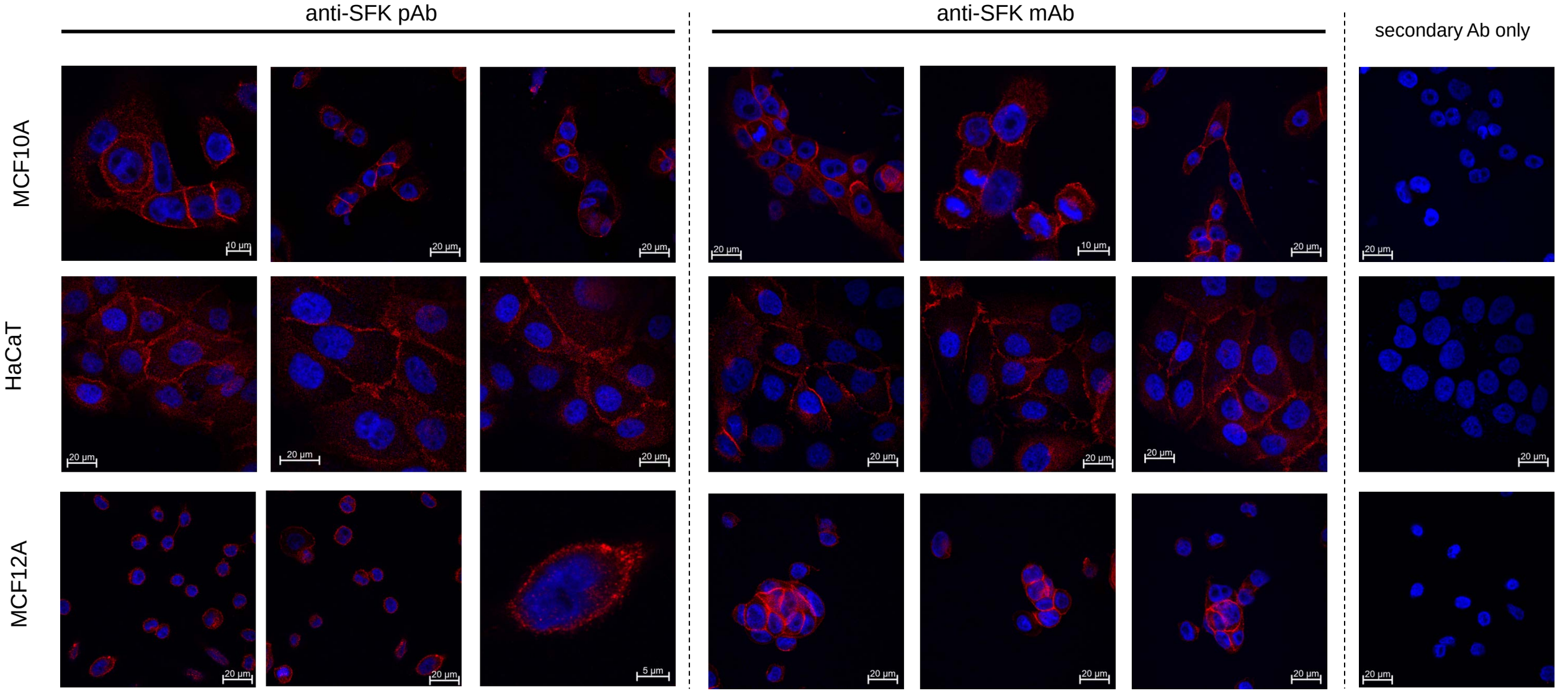


B

MCF10A cells were treated with 1uM dasatinib or DMSO for 30 minutes and total cell lysates immunoblotted as shown. The ratio of these two bands establishes how much of the total SFK is in pY530 vs non-pY530 states.

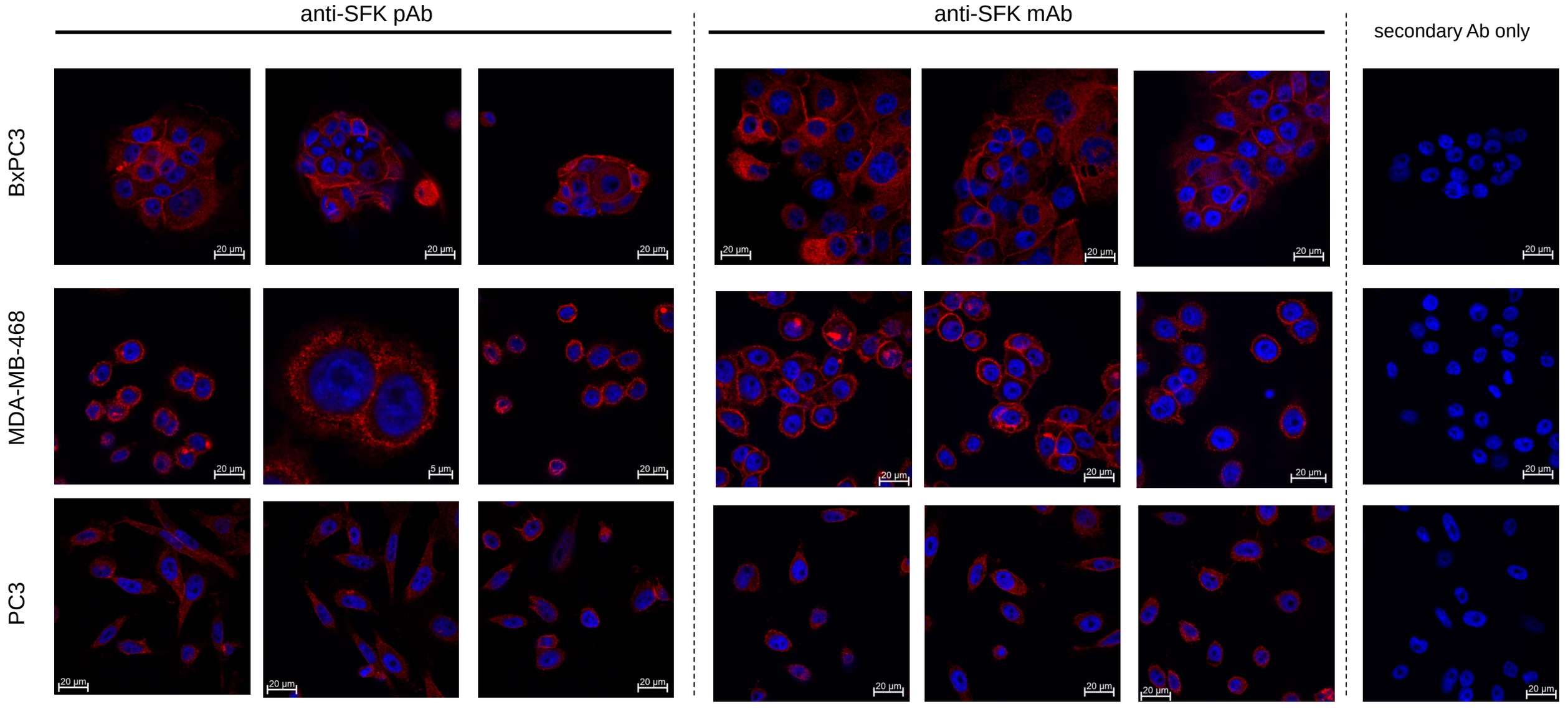


Supplementary Figure 2



The indicated cell types were grown on cover slips, fixed in paraformaldehyde and stained using either anti-SFK polyclonal (left) or monoclonal (right) antibodies. These antibodies target the c-terminal homologous region of SFKs and react with Src, Yes, and Fyn. After application of secondary antibodies conjugated with Alexa Fluor 647 the images were acquired using confocal microscopy with the appropriate laser excitation. We used two different antibodies in order to best account for background staining and we used a far-red fluorophore which exhibits much less endogenous flourescence in most cells. All images were acquired using the 63X objective and 2-3 different fields are shown for each stain. Some images are cropped. The scale bars indicate the field size for each image.

Supplementary Figure 2



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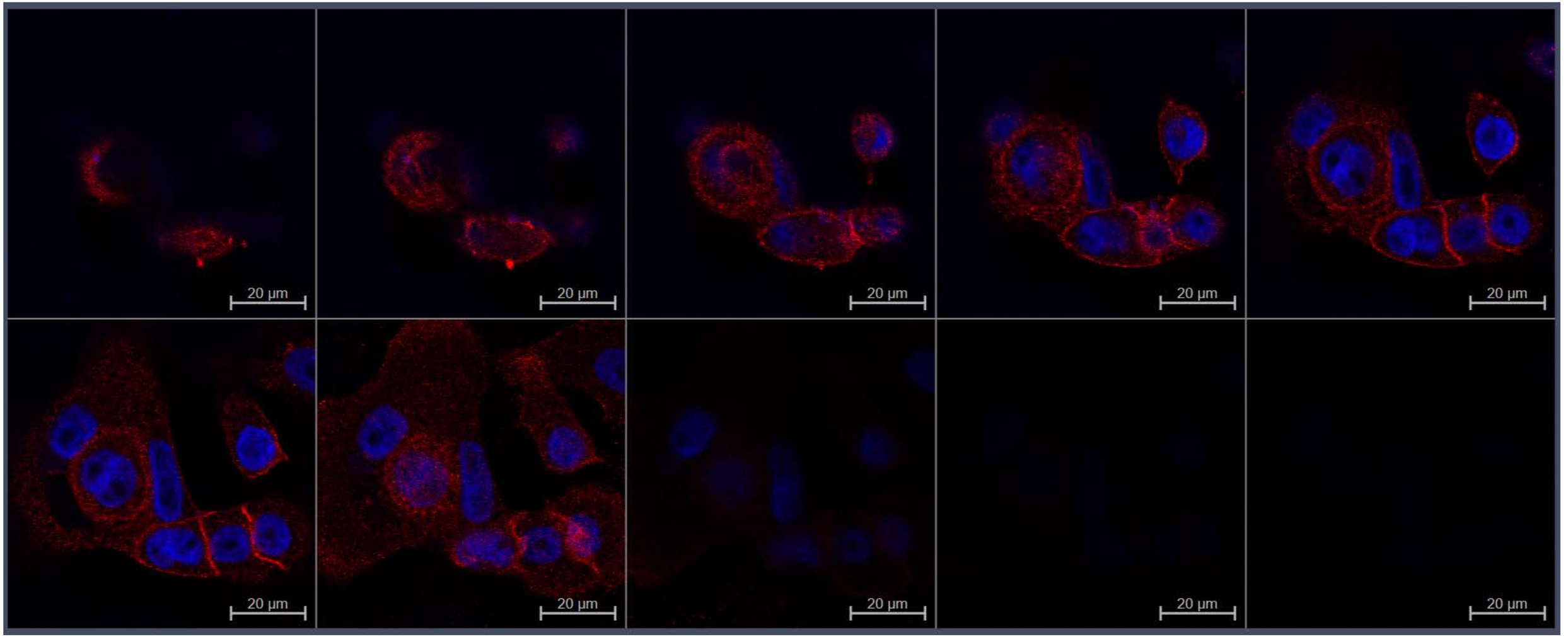
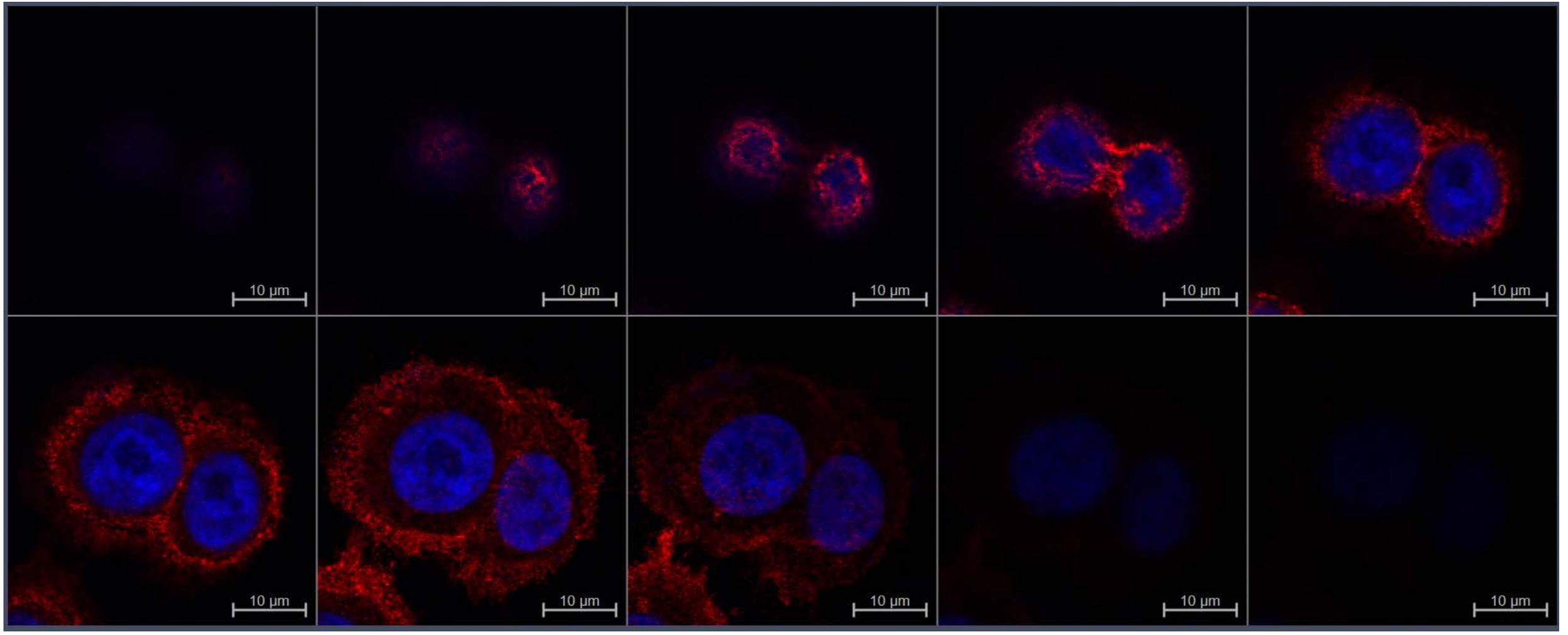


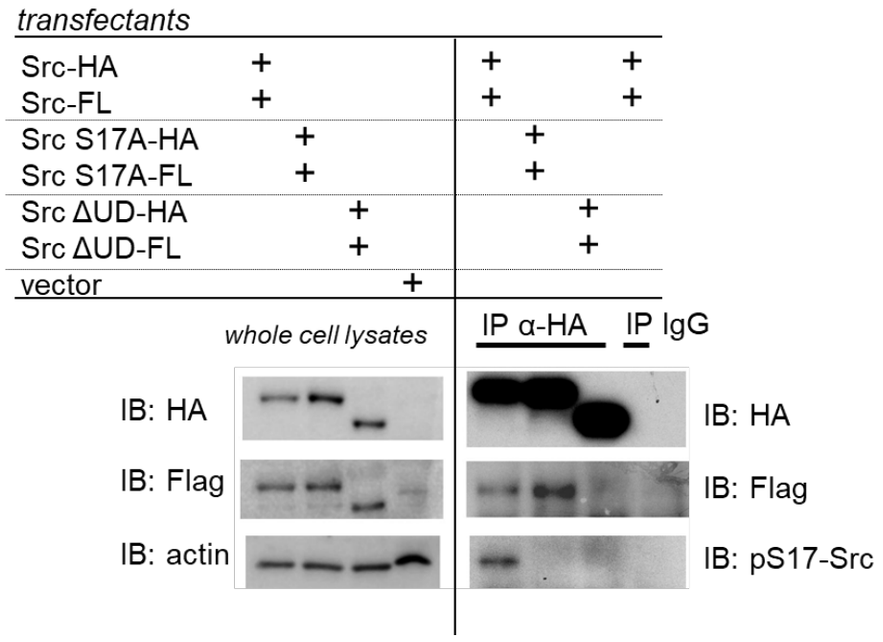
MCF10A cells stained with anti-SFK pAb and Alex Fluor 647 conjugated secondary antibodies. Shown here is a confocal microscope image using a 63X objective cropped around the cells of interest, with 10 Z-stacks spanning a combined thickness of 12µm. Scale bar indicates the size of the field.

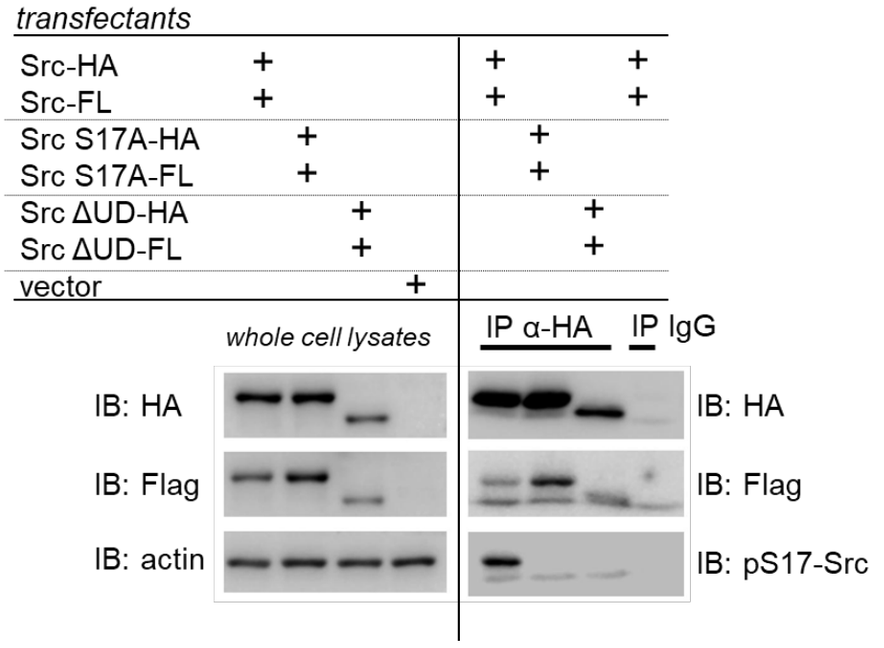
Supplementary Figure 2



MDA-MB-468 cells stained with anti-SFK pAb and Alex Fluor 647 conjugated secondary antibodies. Shown here is a confocal microscope image using a 63X objective cropped around the cells of interest, with 10 Z-stacks spanning a combined thickness of 8.5µm. Scale bar indicates the size of the field.

Supplementary Figure 3





SYF cells were transfected with both HA and Flag tagged Src constructs and their level of dimerization determined by co-immunoprecipitation. This is shown for the wildtype Src, the S17A mutant, and the ΔUD deletion mutant that lacks the entire N-terminal unique domain.