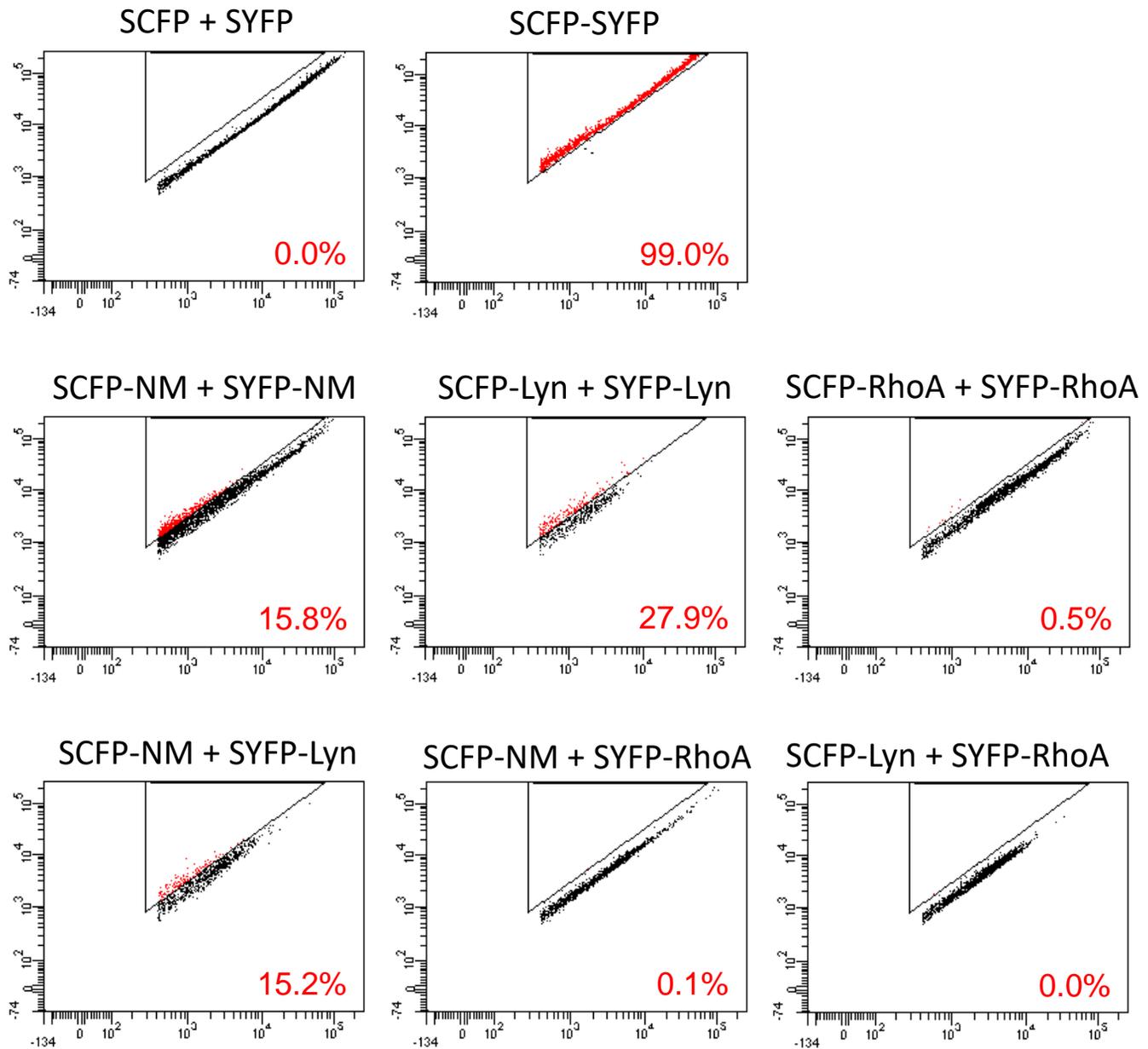
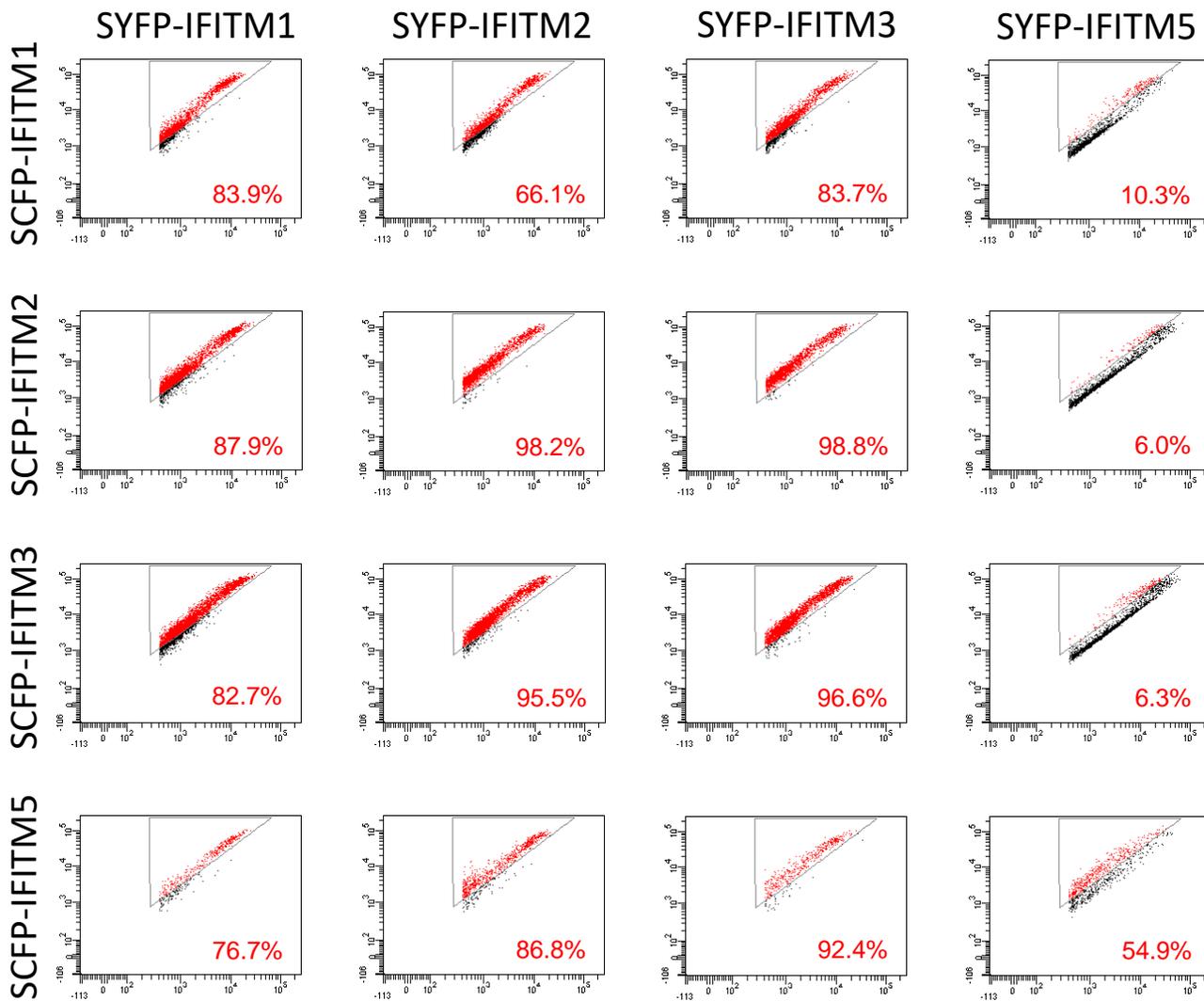


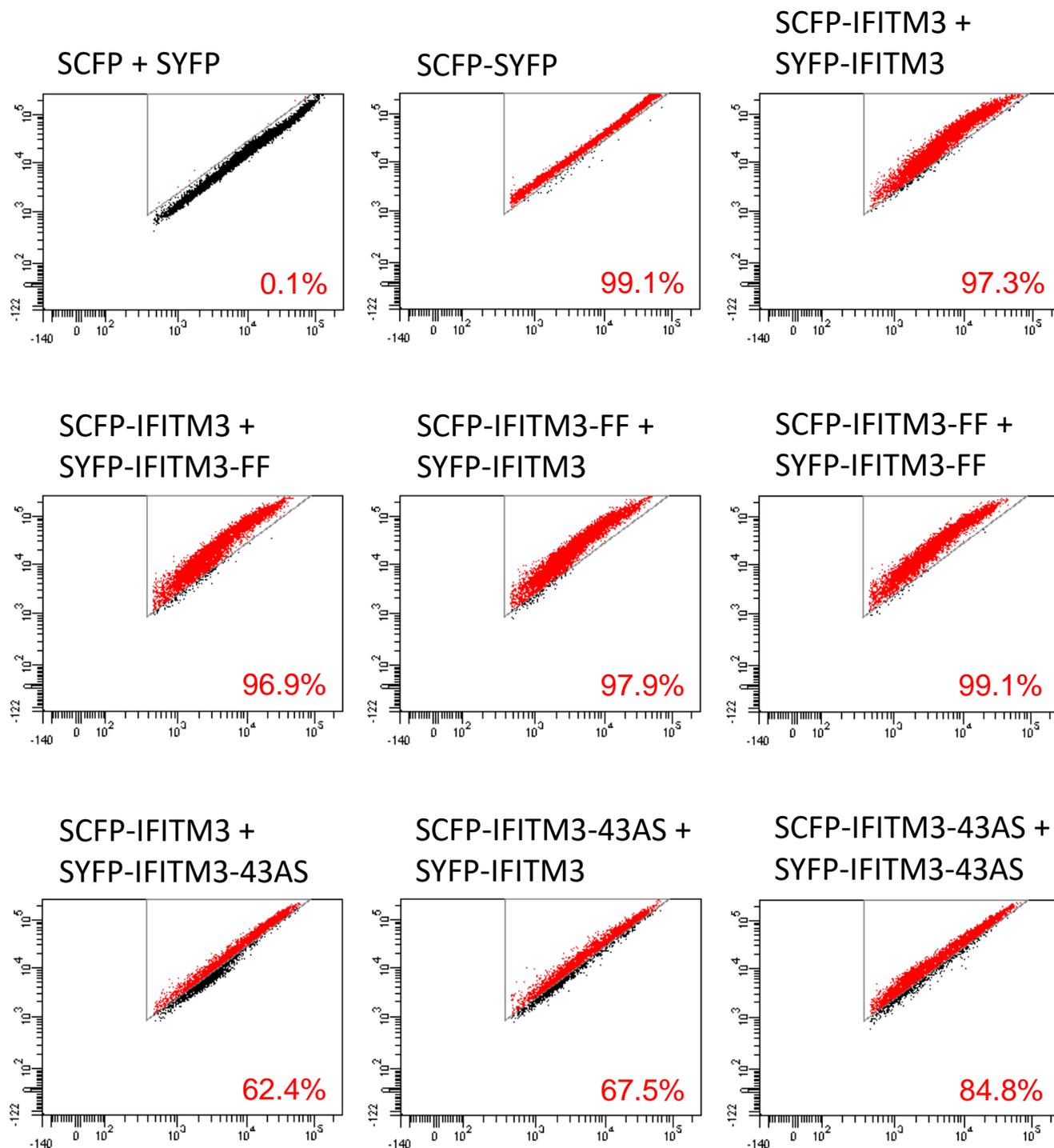
**Suppl. Figure 1.** Gating strategy used for FACS-FRET measurements. 293T cells were transfected with plasmids encoding the proteins indicated on top. Living cells were analyzed in a LSR II flow cytometer. First, in a plot YFP vs. CFP a double positive cell population was gated (top panels). In a second step, cells false positive for FRET signals due to high SYFP signals were excluded in a FRET vs. YFP plot (middle panels). Finally, a FRET vs. CFP plot was used to define a gate (bottom panel) which excludes signals arising from SCFP-RhoA and SYFP-RhoA coexpression (FRET negative) and includes signals arising from SYFP-SCFP fusion proteins (FRET positive). FRET positive cells are colored red and the percentage of FRET-positive cells is given for negative and positive controls and a SCFP-IFITM3 + SYFP-IFITM3 sample.



**Suppl. Figure 2.** Representative primary FACS-FRET plots complementing Figure 1D. Plots showing FRET vs. CFP signals and the gate used for evaluation of FRET<sup>+</sup> cells are shown for individual samples used in figure 1D. The proteins coexpressed in 293T cells are indicated on top. FRET-positive cells are colored red and the percentage of FRET-positive cells is indicated.



**Suppl. Figure 3.** Representative primary FACS-FRET plots complementing Figure 2C. Plots showing FRET vs. CFP signals and the gate used for evaluation of FRET-positive cells are shown for individual samples used in figure 2C. The plots are arranged as an array with the proteins coexpressed indicated on the left and on top. FRET-positive cells are colored red and the percentage of FRET-positive cells is indicated.



**Suppl. Figure 4.** Representative primary FACS-FRET plots complementing Figure 3E. Plots showing FRET vs. CFP signals and the gate used for evaluation of FRET-positive cells are shown for individual samples used in figure 3E. The proteins coexpressed in 293T cells are indicated on top. FRET-positive cells are colored red and the percentage of FRET-positive cells is given.