Supplementary Figures for "An inducible retroviral expression system for tandem affinity purification mass-spectrometry-based proteomics identifies MLKL as an HSP90 client"

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Supporting Information

Supplementary Figure 1

(**A**-**C**) FACS analysis of K-562 RIEP (**A**), HT-29 RIEP (**B**) and KCL-22 RIEP (**C**) GFP cells untreated or treated for 24 h with 2 µg/mL doxycycline.

Supplementary Figure 2

(**A**) Schematic illustration of inducible TRE3G-driven pRSHIC expression vectors with Gateway-cloning cassette fused to N- (upper) or C-terminal (lower) SH-tag. (**B**) Flow cytometry analysis of K-562 RIEP transduced with $pRSHIC_{TREtight}$-GFP or $pRSHIC_{TRE3G}$-GFP untreated or treated for 24 h with 1 µg/mL doxycycline. Corresponding wild-type cells are the baseline control.

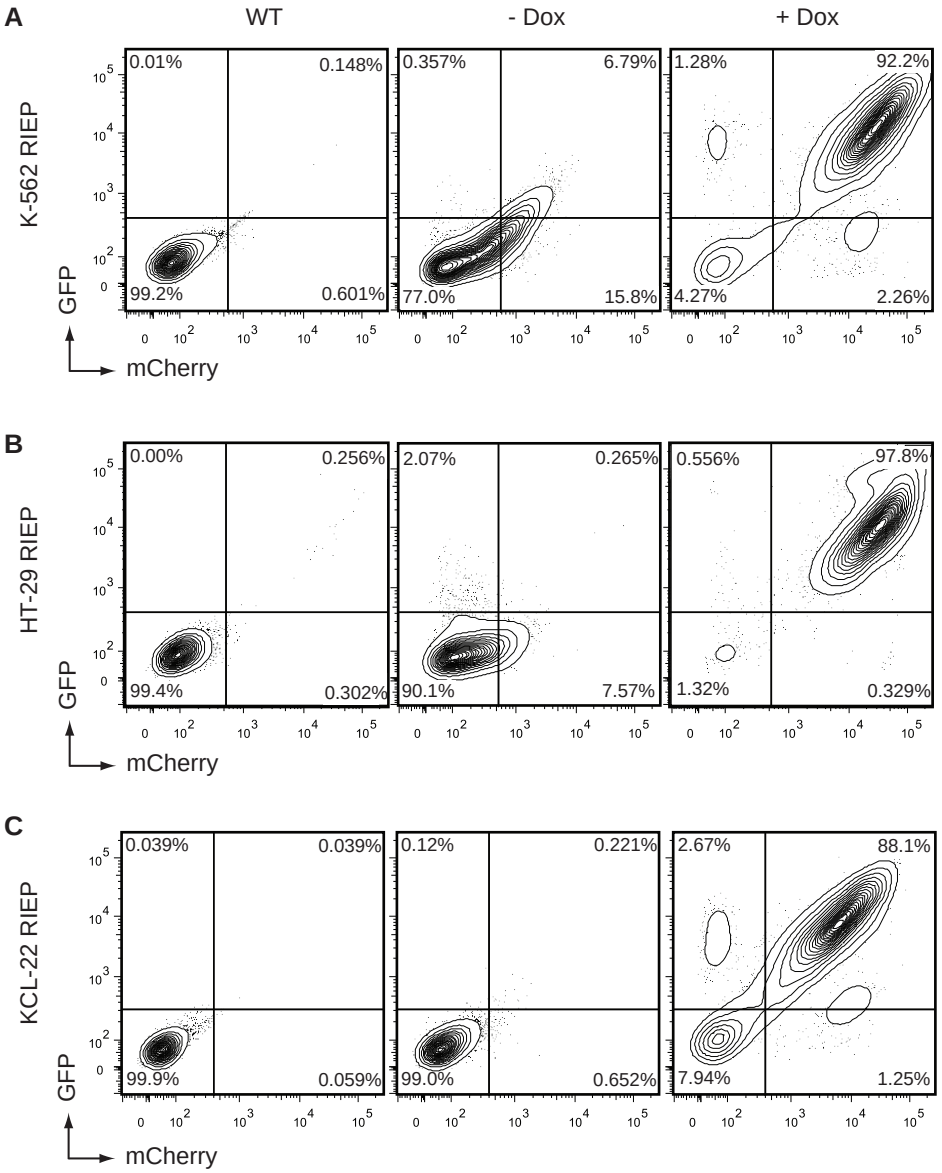
Supplementary Figure 3

(**A**) Ba/F3 rtTA3 cells inducibly expressing NRAS G12D or GFP were treated with 1 µg/mL doxycycline for 24 h. Cells were lysed and immunoblotted with the indicated antibodies. (**B**) Flow cytometry analysis of Ba/F3 rtTA3 cells expressing NRAS G12D or GFP treated with 1 µg/mL doxycycline for 24 h. (**C**) Ba/F3 rtTA3 NRAS G12D cells were induced with 1 µg/mL doxycycline in the presence of IL-3 for 48 h. Cells were then washed once and cultured in the presence of 1 µg/mL doxycycline and increasing concentrations of trametinib with or without IL-3 for 3 h. DMSO-treated cells were the baseline control. Subsequently cells were lysed and immunoblotted with the indicated antibodies.

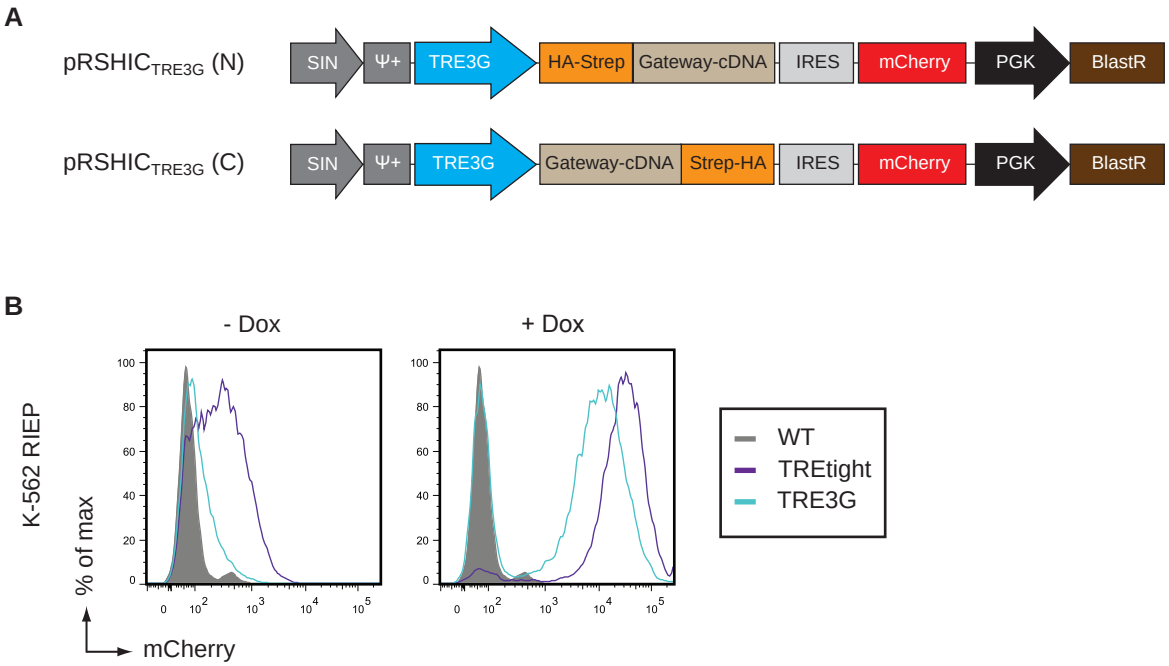
Supplementary Figure 4

(**A**) Flow cytometry analysis of HT-29 RIEP MLKL S358D cells induced with 2 µg/mL doxycycline for the indicated times. (**B**) Microscopy (brightfield, 10×) of HT-29 RIEP MLKL S358D cells uninduced or induced with 2 µg/mL doxycycline for 24 h (scale bar: 100 µm). (**C**) Flow cytometry analysis of HT-29 RIEP MLKL S358D cells treated with 2 µg/mL doxycycline and Nec-1 (10 µM), NSA (10µM) or geldanamycin (GA, 1 µM) for 6 h.

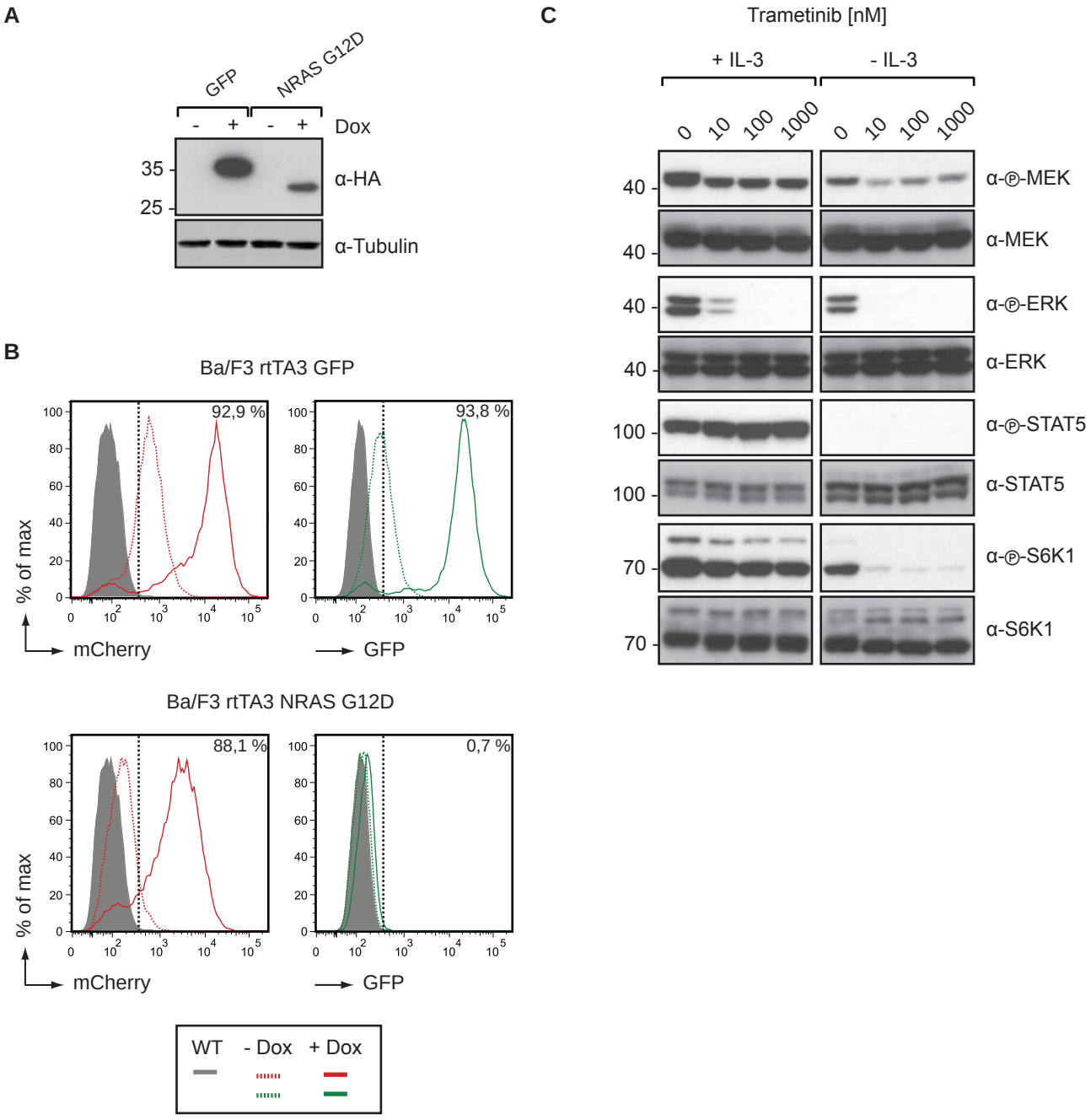
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4