IDENTIFICATION OF SMALL MOLECULE INHIBITORS OF PHOSPHATIDYLINOSITOL 3-KINASE AND AUTOPHAGY*

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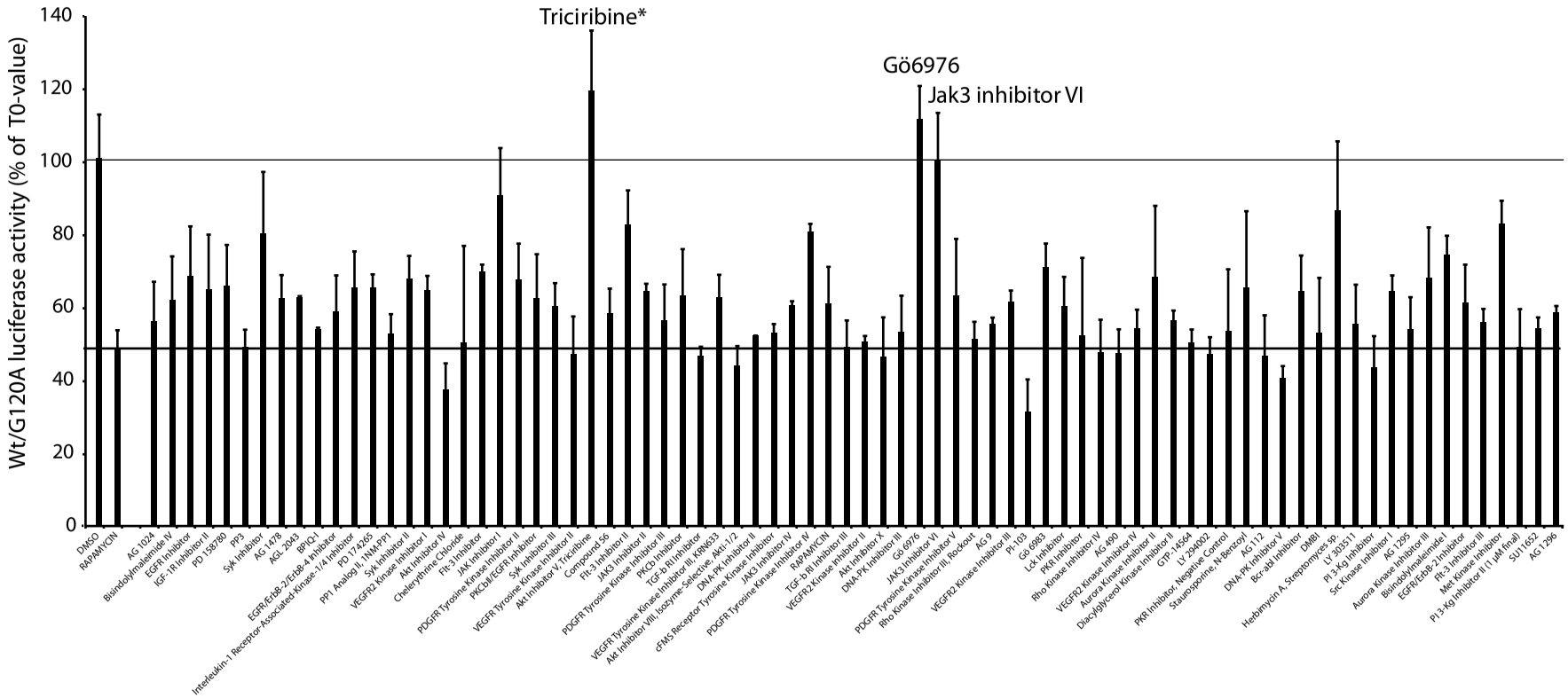
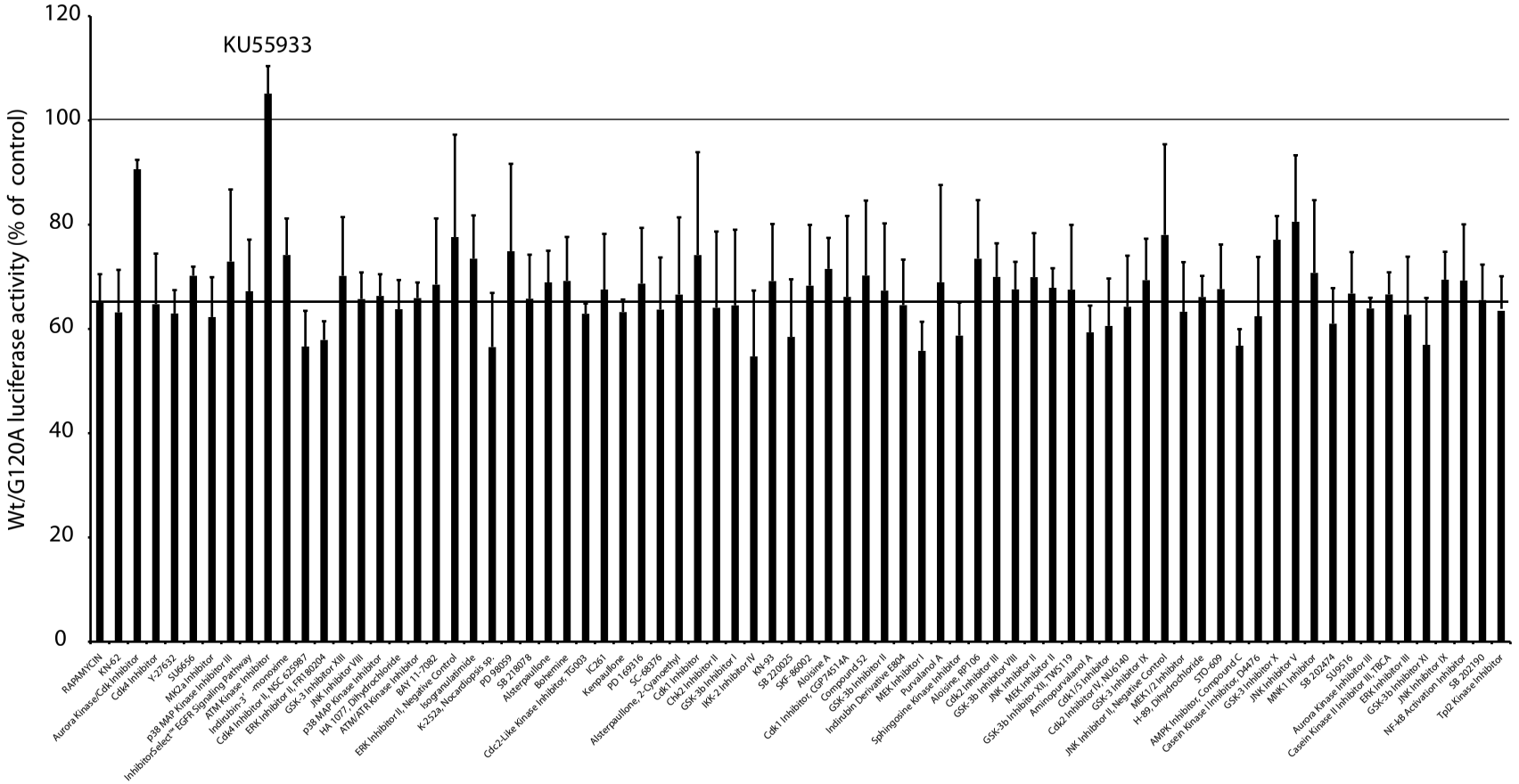
Supplemental data:

Supplemental figures 1-4.



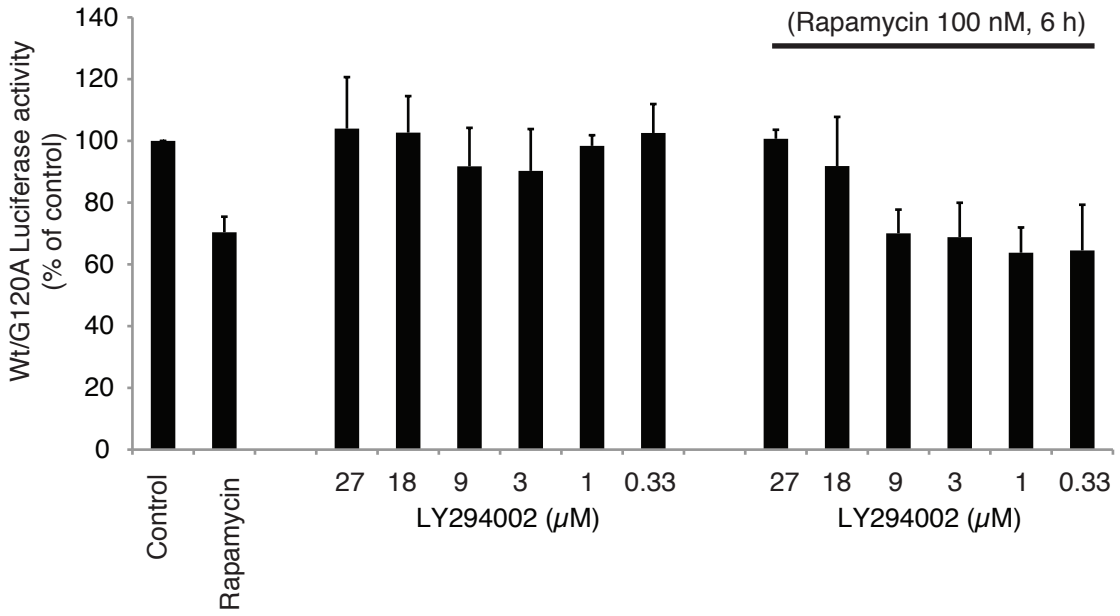
Supplementary Figure S1. Screening of Calbiochem library No. 539744* for inhibition of rapamycin induced autophagy. Cells were treated with DMSO, Rapamycin (100 nM) or Rapamycin (100 nM) + inhibitor (2 µM) for 12h.The data represents the RLucLC3wt/ RLucLC3 G120A reporter-activity ratio at 12 h after the additon of the compounds (T0). The RLucLC3 reporter assay was performed using the live cell substrate Enduren. The numbers represent the average and standard deviation of two independent experiments. Horizontal lines indicate the levels of background (top) and rapamycin-induced (bottom) autophagy. Data on PDK1/Akt/Flt Dual Pathway Inhibitor and Staurosporine were omitted from the chart because of toxicity. A drug was classified as toxic if more than 20% of lactate dehydrogenase was released from the cells after 30 h of exposure (Farkas et al., Autophagy 2009).

*During investigation of the apparent autophagy inhibitory effect of Akt inhibitor V, Triciribine, we encountered a drug-specific difference between results generated with the live cell substrate and corresponding results obtained after extraction of the cells. Performing the RLucLC3-assay in cell extracts revealed that the drug induces autophagy. This was further supported by tandem-fluorescent LC3 data demonstrating translocation of LC3-fusion protein into acidic organelles (Elisabeth Corcelle, unpublished observation).



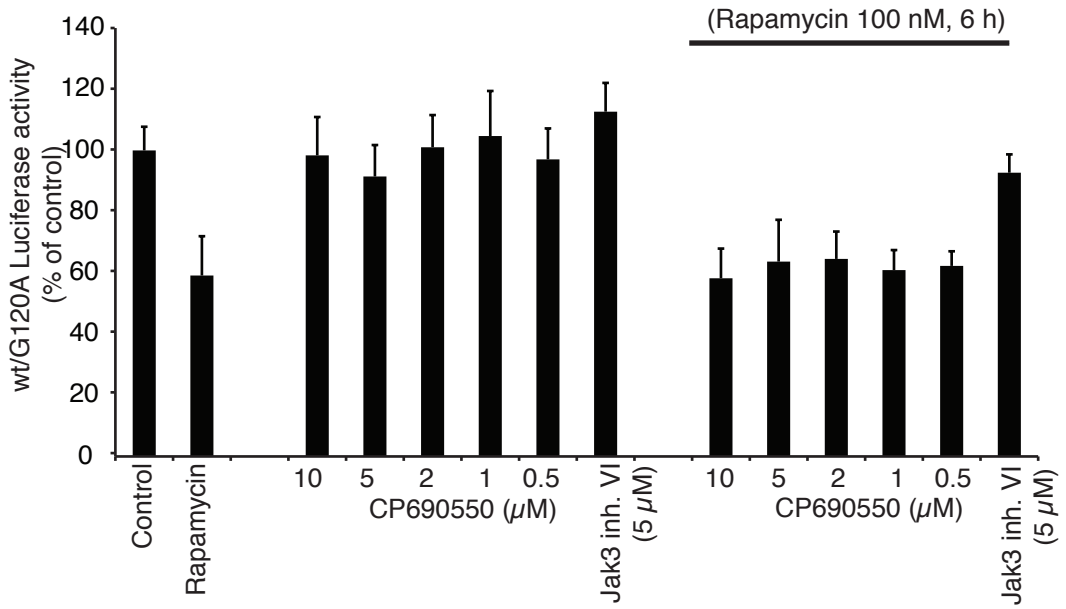
Supplementary Figure S2. Screening of Calbiochem library No. 539745 * for inhibitors of rapamycin-induced autophagy. MCF7 cells were treated with DMSO, rapamycin (100 nM) or rapamycin (100 nM) + an indicated inhibitor (2 μM) for 6 h before extraction and analysis of autophagic flux. The data represent the RLucLC3wt/ RLucLC3 G120A reporter-activity ratio as a percentage of the corresponding ratio in cells treated with DMSO. Mean valuess ± SD from three independent experiments are shown. Horizontal lines indicate the ratios in DMSO- (top) and rapamycin-treated (bottom) cells.

Data on Cdk/Crk Inhibitor, Staurosporine, Streptomyces sp., Cdk1/2 Inhibitor III , Fascaplysin, Synthetic and Cdk4 Inhibitor III were omitted from the chart because of toxicity. A drug was classified as toxic if more than 20% of lactate dehydrogenase was released from the cells after 30 h of exposure (Farkas et al., Autophagy 2009).

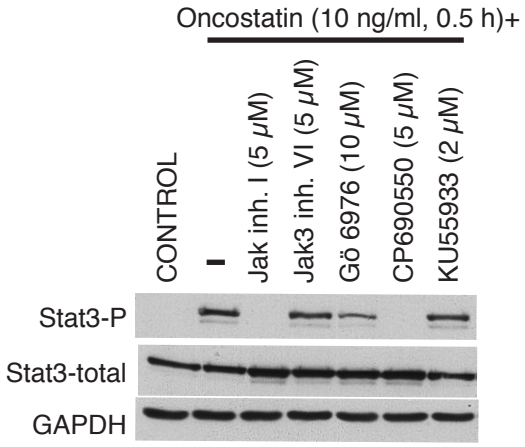


Supplementary Figure S3. Inhibition of autophagy by LY294002. Dose response. MCF7 cells expressing Rluc-LC3wt or RLuc-LC3G120A were incubated for 6h with LY294002 at the indicated concentration in absence or presence of rapamycin. The data represent average ± SD of two independent experiments

A



B



Supplemtary Figure S4. CP690550 inhibits Jak actvity without inhibiting rapamycin-induced autophagy.

A. Autophagic flux was analyzed in MCF7 cells expressing RLuc-LC3wt or RLuc-LC3G120A incubated for 6 h with CP690550 at the indicated concentrations in the absence or presence of rapamycin. The data represent mean ± SD of a representative sextuplicate experiment.

B. Immuno-blotting with antibodies against phospho-Stat3, Stat3 and GAPDH in extracts from MCF-7 cells left untreated or incubated with Oncostatin for 30 min. with or without inhibitors as indicated. Jak inhibitor 1 (Jak inh. I; calbiochem, 420099) serves as a positive control.