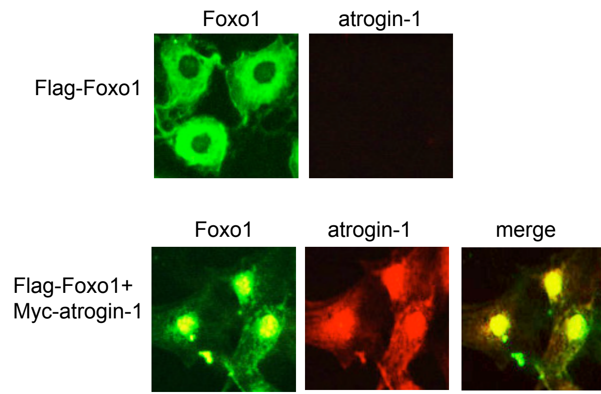


Figure S1

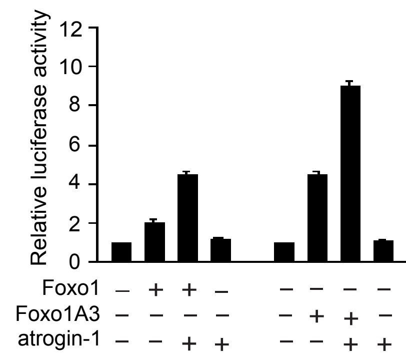
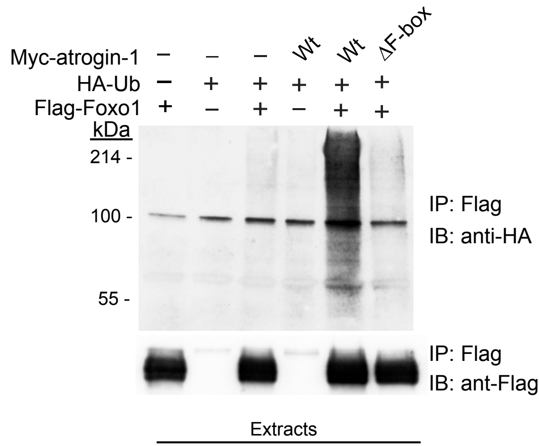
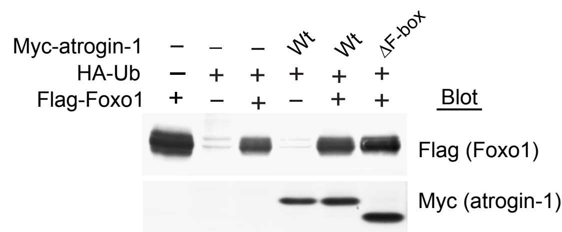
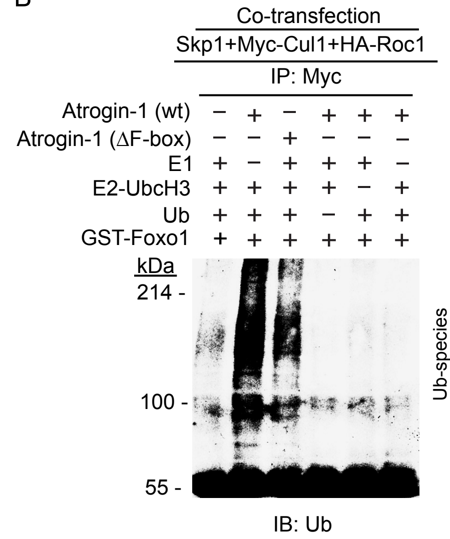


Figure S2







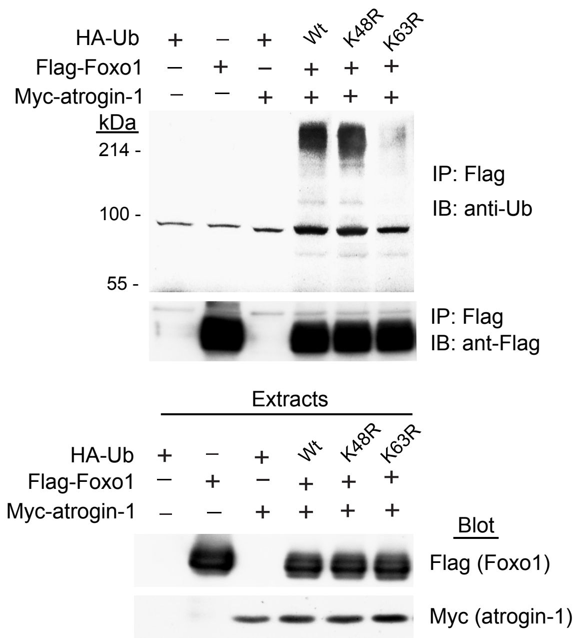


Figure S3

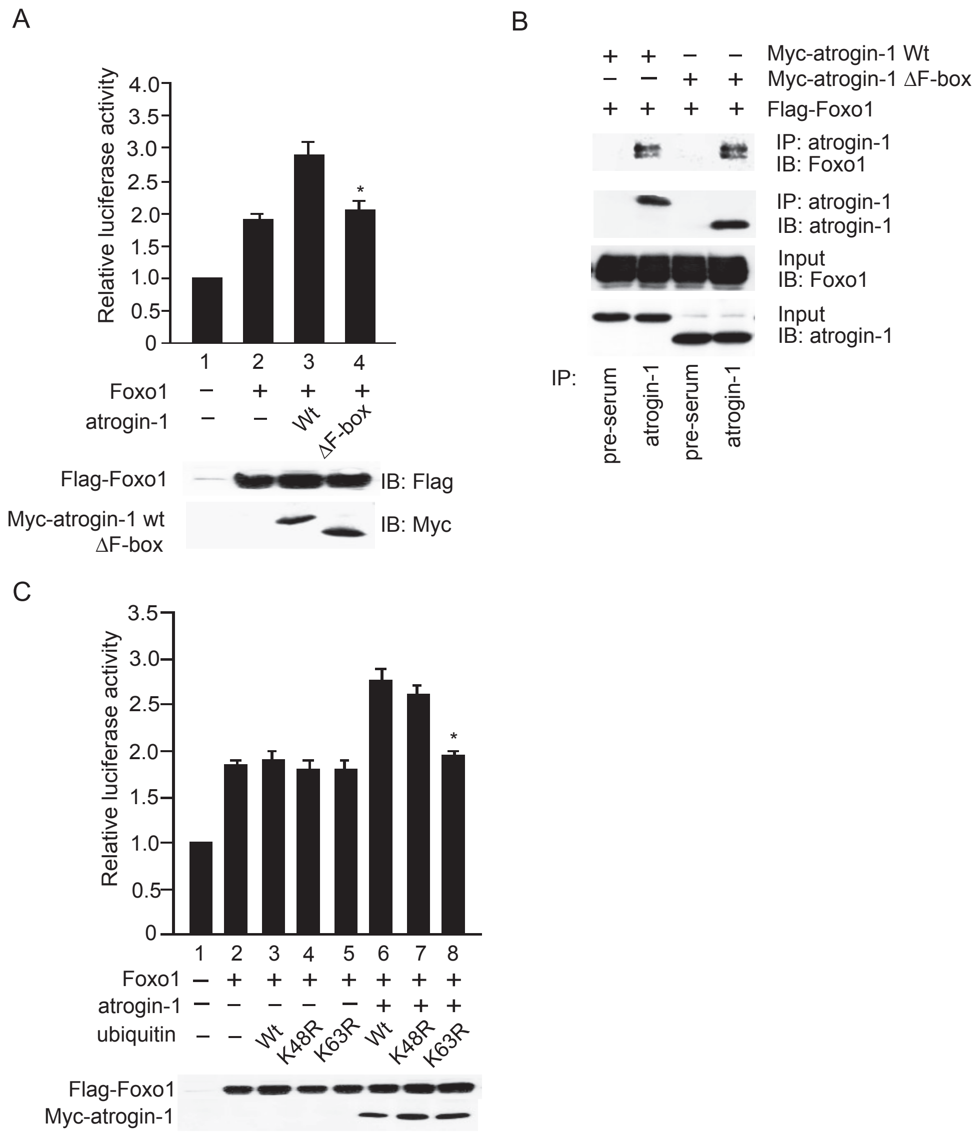
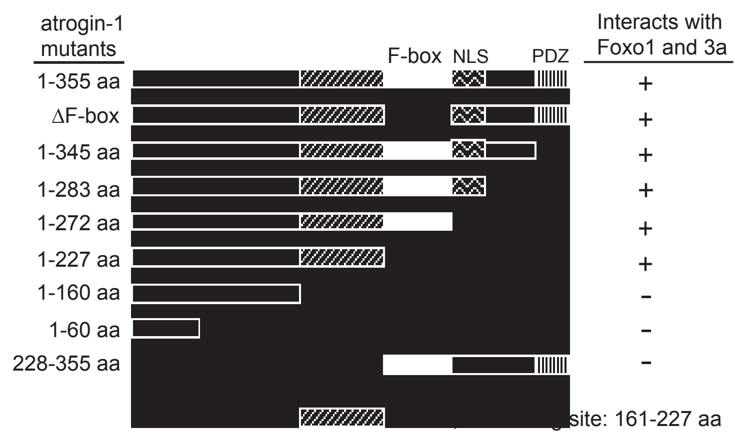


Figure S4



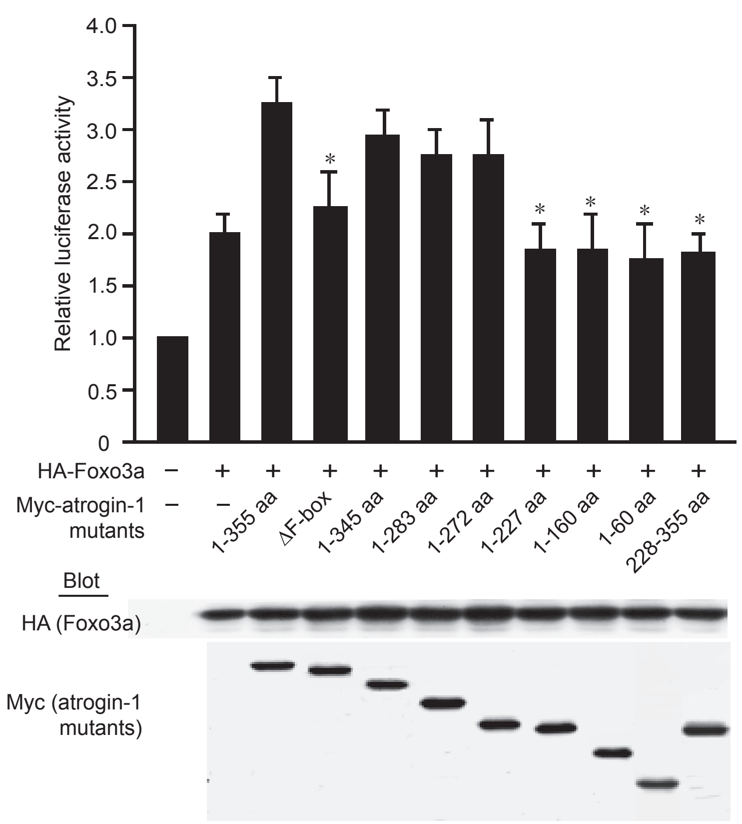


Figure 5

Figure S1.

Atrogin-1 regulates Foxo1 localization. Cardiomyocytes were transiently transfected with expression vectors encoding Ha-Foxo1 and/or Myc-atrogin-1. 24 h after transfection, cells were immunostained with antibodies against HA (*green*) or Myc (*red*). Representative images are shown. Magnification, x200.

Figure S2.

Cultured cells were transfected with Foxo1 Wt or A3 (constitutively active form), atrogin-1 together with the $p27^{kip1}$ reporter and β-galactosidase constructs. 24 h after transfection, cells were lysed and luciferase activity was measured.

Figure S3.

(**a**) 293 cells were transfected with vectors expressing HA-ubiquitin (HA-Ub), Flag-Foxo1, atrogin-1 or atrogin-1 ΔF-box mutant, as indicated. Cell extracts were immunoprecipitated with Flag antibody and analyzed by immunoblotting with the indicated antibodies (*upper panel*). An aliquot of the cell extracts was subjected to direct immunoblot analysis using anti-Flag or anti-Myc antibodies, as indicated (*lower panel*).

(**b**) *in vitro* ubiquitylation reactions were performed with purified ubiquitin, E1, the E2 UBC13, GST-Foxo1 and $SCF^{atrogin-1}$ complex. Reactions were resolved by SDS-PAGE followed immunoblotting with anti-Ub antibody.

(**c**) 293 cells were cotransfected with the indicated plasmids. 24 h after transfection, Ub-conjugated proteins were prepared for immunoprecipitation using anti-Flag. Immunoprecipitates were subject to SDS-PAGE followed by immunoblotting with anti-Ub or anti-Flag antibodies as indicated (*upper panel*). An aliquot of the cell extracts was

subjected to direct immunoblotting analysis using anti-Flag or anti-Myc antibodies, as indicated (*lower panel*).

Figure S4.

(**a**) Cells were transfected with Foxo1, atrogin-1 or atrogin-1 ΔF-box mutant together with the p27^{kip1} reporter and _-galactosidase constructs. 24 h after transfection, cells were lysed and luciferase activity was measured (*upper panel*). Lysates were immunoblotted with anti-Flag or Myc (*lower panel*). *$P < 0.01$ vs. atrogin-1 Wt.

(**b**) 293 cells were transfected with the indicated plasmids. Equal amounts of protein lysates were IP with the anti-atrogin-1 antibody or pre-serum control and analyzed by immunoblotting with the indicated antibodies. WCE, whole cell extract.

(**c**) Cardiomyocytes were cotransfected with the indicated plasmids together with the p27^{kip1} reporter and β -galactosidase constructs. 24 h after transfection, cells were lysed and luciferase activity was measured (*upper panel*). Lysates were immunoblotted with anti-Flag or Myc (*lower panel*). *$P < 0.001$ vs. ubiquitin Wt and K48R.

Figure S5.

(*Upper panel*) Schematic representation of deletion constructs of atrogin-1 used in (Figure 2D) that bind to Foxo1 and 3a.

(*Lower panel*) 293 cells were transfected with Foxo3a, atrogin-1 wt or mutants together with the p27^{kip1} reporter and β -galactosidase construct. 24 hours after transfection, cells were lysed and luciferase activity was measured (top). Lysates were immunoblotted with anti-HA or Myc antibodies (bottom). *$P < 0.01$ vs. Myc-atrogin-1 1-355 aa. The results are expressed relative to the level of expression with the reporter gene alone and are representative of three independent experiments. Error bar indicate ± SEM.