**Figure S1: Proteotoxic stress alters interactions of ataxin-3 with VCP and hHR23B.** HEK293T cells were transfected with myc-Atx3 and either HA-VCP or HA-hHR23B; following treatment with a stressor for the indicated time, cell lysates were immunoprecipitated with polyclonal Atx3 antibody or control antibody (IP Control) and immunoblotted with HA antibody to detect HA-VCP or HA-hHR23B or with monoclonal antibody, 1H9, to confirm the presence of Atx3 in the IP. (A,B) Cells were treated with 10uM MG132 for up to 8 hours; Atx3 interacted with a small amount of VCP and hHR23B under basal conditions; following MG132 treatment the interaction of Atx3 with VCP (A) increased more rapidly than its interaction with hHR23B (B). (C,D) Cells were treated with 1 mM DTT for up to 4 hours; the interaction of Atx3 with VCP (C) increased slightly by 2 hrs and clearly increased by 4 hours of treatment while the interaction of Atx3 with hHR23B (D) decreased by 2 hours and remained lower than the basal interaction after 4 hours of treatment. (E,F) Cells were treated with 10ug/ml tunicamycin for up to 12 hours; the interaction of Atx3 with VCP (E) was unaffected by tunicamycin treatment while the interaction of Atx3 with hHR23B (F) decreased by 4 hours and remained decreased through 12 hours of treatment. (G) The interaction of Atx3 with VCP is increased immediately following a 1 hour heat shock at 42°C, and remained increased through 6 hours of recovery. (H) Heat shock increased the interaction of Atx3 with hHR23B immediately following heat shock and the interaction decreased by 2 hours but remained above the basal interaction level through 6 hours of recovery.

**Figure S2: Proteotoxic stress does not alter the localization of VCP or hHR23B.** HeLa cells transfected with either HA-VCP or HA-hHR23B were treated with 10uM MG132 for 8 hours, 1mM DTT for 4 hours, 10ug/ml tunicamycin for 12 hours, heat-shocked at 42°C for 1 hour, or

maintained as untreated control cells. Following treatment, VCP and hHR23B proteins were localized by immunostaining for the HA tag. Neither VCP nor hHR23B localization was altered by MG132, DTT, tunicamycin, or heat shock.

**Figure S3: The CK2 inhibitor DMAT is not inactivated by exposure to 42°C.** The CK2 inhibitor, DMAT, inhibited nuclear localization of Atx3 under basal conditions but not following 42°C heat shock (see Fig. 2). To rule out the possibility that DMAT was inactivated by 42°C, media containing 20uM DMAT was exposed to 37°C or 42°C for 1 hr and then cooled to 37°C. HEK293T cells were then treated with the media containing DMAT that was exposed to 37°C (DMAT) or 42°C (Heat-Treated DMAT) or DMSO (vehicle). Following a 3hr treatment cells were fractionated and blotted for Atx3, GAPDH, or histone H3. DMAT, heat-treated at 42°C or maintained at 37°C, still inhibited nuclear localization of Atx3 under basal conditions similar to that seen with fresh DMAT (see Fig. 2A,B). This indicates that 42°C does not inactivate DMAT and supports the conclusion that nuclear localization of Atx3 under basal conditions is regulated by CK2 but nuclear localization of Atx3 following 42°C heat shock is not regulated by CK2.

**Figure S4: Endogenous Atx3 and Hsf1 do not co-immunoprecipitate.** Cell lysates from control and heat-shocked (42°C for 1 hour) mouse fibroblasts were used for immunoprecipitations with either an Atx3 polyclonal antibody or control antibody (IP Con), followed by western blots for Atx3 and Hsf1.

Figure S1

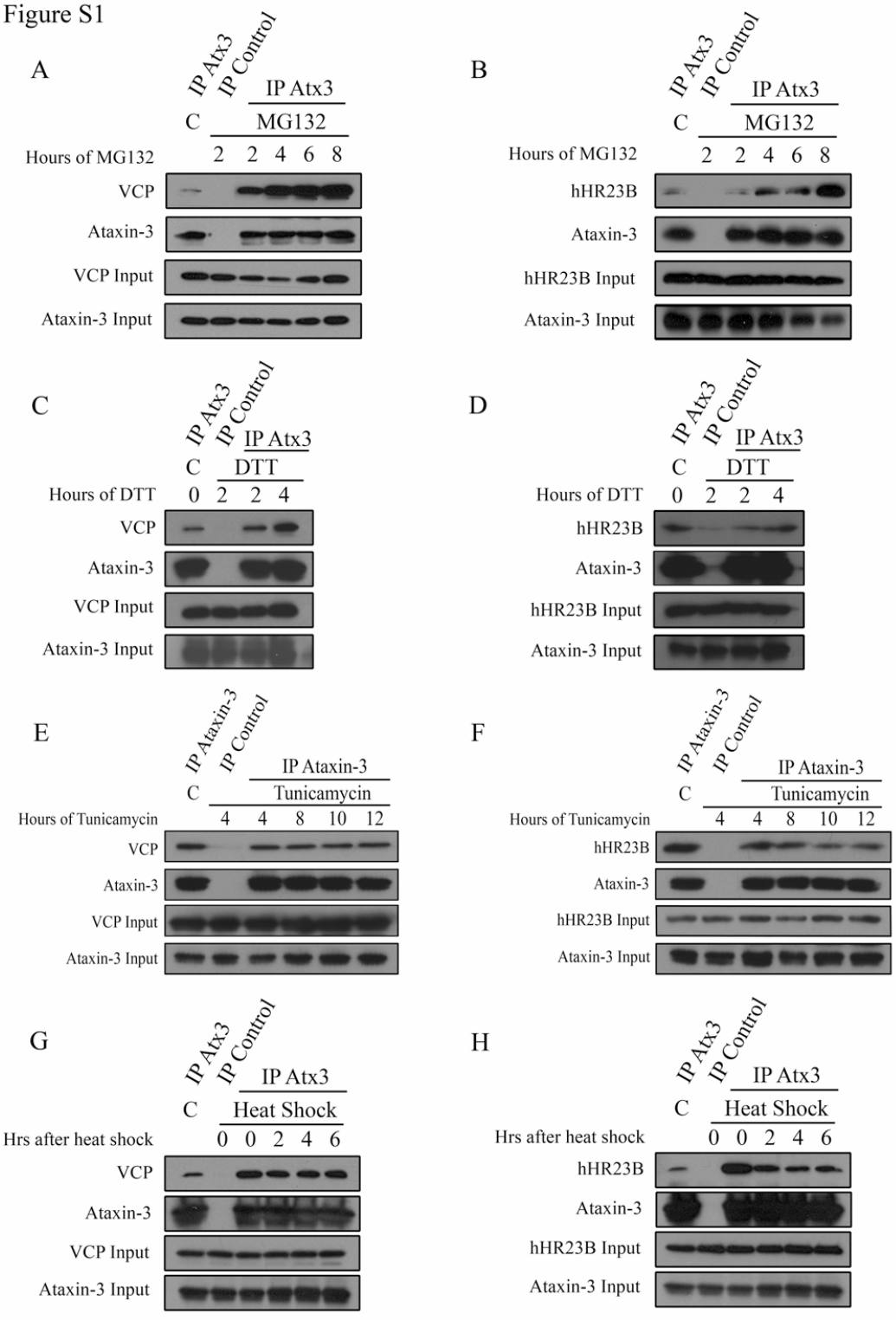
A
IP Atx3
IP Control
IP Atx3
C
MG132
Hours of MG132
2 2 4 6 8
VCP
Ataxin-3
VCP Input
Ataxin-3 Input
B
IP Atx3
IP Control
IP Atx3
C
MG132
Hours of MG132
2 2 4 6 8
hHR23B
Ataxin-3
hHR23B Input
Ataxin-3 Input
C
IP Atx3
IP Control
IP Atx3
C
DTT
Hours of DTT
0 2 2 4
VCP
Ataxin-3
VCP Input
Ataxin-3 Input
D
IP Atx3
IP Control
IP Atx3
C
DTT
Hours of DTT
0 2 2 4
hHR23B
Ataxin-3
hHR23B Input
Ataxin-3 Input
E
IP Ataxin-3
IP Control
IP Ataxin-3
C
Tunicamycin
Hours of Tunicamycin
4 4 8 10 12
VCP
Ataxin-3
VCP Input
Ataxin-3 Input
F
IP Ataxin-3
IP Control
IP Ataxin-3
C
Tunicamycin
Hours of Tunicamycin
4 4 8 10 12
hHR23B
Ataxin-3
hHR23B Input
Ataxin-3 Input
G
IP Atx3
IP Control
IP Atx3
C
Heat Shock
Hrs after heat shock
0 0 2 4 6
VCP
Ataxin-3
VCP Input
Ataxin-3 Input
H
IP Atx3
IP Control
IP Atx3
C
Heat Shock
Hrs after heat shock
0 0 2 4 6
hHR23B
Ataxin-3
hHR23B Input
Ataxin-3 Input

Figure S2

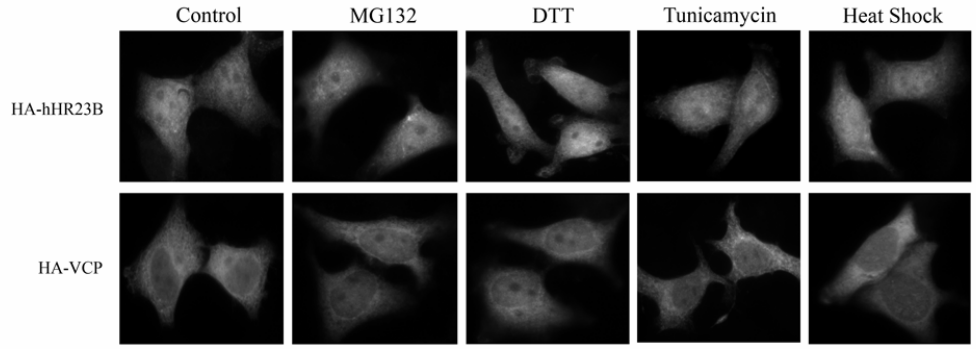

Figure S3

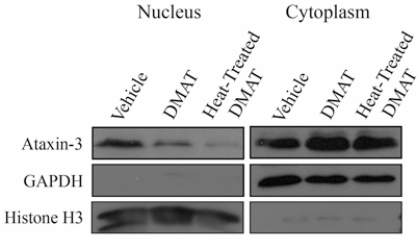
Nucleus
Cytoplasm
Vehicle
DMAT
Heat-Treated DMAT
Vehicle
DMAT
Heat-Treated DMAT
Ataxin-3
GAPDH
Histone H3

Figure S4