Supplemental Information

Supplemental figure legends

Figure S1. P7C3A20 protects U2OS cells from doxorubicin-mediated toxicity. Related to Figure 1. U2OS cells were treated with 5μM P7C3-A20 for 2 h prior to incubation with the indicated concentrations of eight toxins for 72h.

Figure S2. P7C3A20 blocks apoptosis, but not DNA damage response induced by doxorubicin. Related to Figure 1. (A) U2OS cells were treated with increasing concentrations of P7C3-A20 for 2 h prior to exposure to 0.5 μM doxorubicin for another 2 h. Cells were harvested and subjected to western blotting with the DNA damage marker, γ-H2AX. Actin was served as loading control. (B) U2OS cells were treated with P7C3-A20 and doxorubicin for the indicated time. Caspase-3 activation was determined by western blotting. Caspase-3 cleavage was observed when treated with doxorubicin alone at 60h, but was prevented in the presence of P7C3-A20.

Figure S3. Twelve-point dose response curves (DRC) of representative tested compounds in doxorubicin toxicity protection assay. Related to Figure 1, Figure 2, and Table S2. Cells were grown in 384-well plates and treated with 5μM-1.7nM (3-fold serial dilution) of various P7C3 derivatives together with 0.5μM doxorubicin for 72h. Cell viability was determined by the Cell Titer Glo assay kit.

Figure S4. Competition for photo-crosslinking of P7C3-S326 to p70 of all tested

compounds. Related to Figure 2. Lysates from crosslinked cells exposed to 0.3 μM P7C3-S326 and 5 μM of various P7C3 derivatives were CLICKed with Alexa 532 and visualized following SDS-PAGE gel electrophoresis and Typhoon scanning.

Figure S5. Five-point dose response curves (DRC) of representative tested compounds in NAD rebound assay. Related to Figure 5 and Table S2. Cells were grown in 384-well plates and treated with 5μM-62nM (3-fold serial dilution) of various P7C3 derivatives together with 0.5μM doxorubicin for 45h. Cellular NAD abundance was determined by the NAD/NADH Glo assay kit.

Figure S6. P7C3-A20 has no effect on the enzymatic activities of either NMNAT or alcohol dehydrogenase. Related to Figure 7. NAMPT was omitted from the enzyme reaction, and nicotinamide was replaced by 150 μM NMN as the substrate. P7C3-A20 showed no ability to increase NAD production at the high dose of 3 μM.

Figure S7. Competition for photo-crosslinking of P7C3-S326 to recombinant NAMPT by P7C3 derivatives. Related to Figure 7. 1 μM recombinant NAMPT was incubated with 1 μM P7C3-S326 and 5 μM of the indicated compounds. Photo-crosslinked NAMPT was labeled by Alexa 532 and visualized following SDS-PAGE gel electrophoresis by Typhoon scanning (upper panel). Coomassie blue staining of the same gel is shown in the lower panel.

Table S1. Compound structures, scores in neurogenesis, dox:tox protection, crosslinking competition, NAD rebound, and in vitro NAMPT assays. Related to Figure 1, Figure 2, Figure 5 and Figure 7.

Table S2. Dose response curves (DRC) of all tested compounds in doxorubicin toxicity protection assay and NAD rebound assay. Related to Figure 1, Figure 2, and Figure 5.

Table S3. List of proteins identified by shotgun mass spectrometry in the four, "green-only" p55 spots excised from the 2D gel. Related to Figure 3.

Figure S1

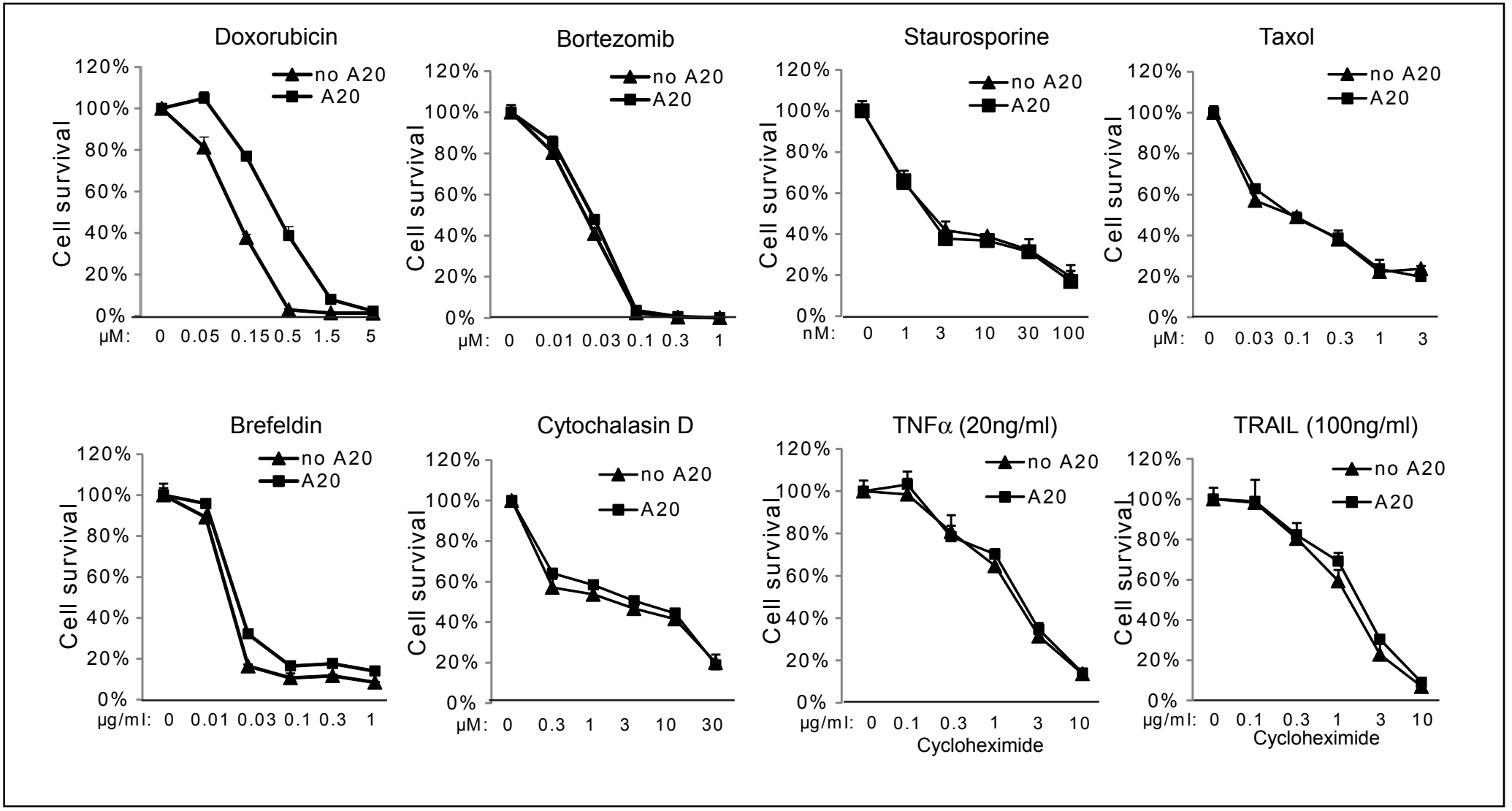
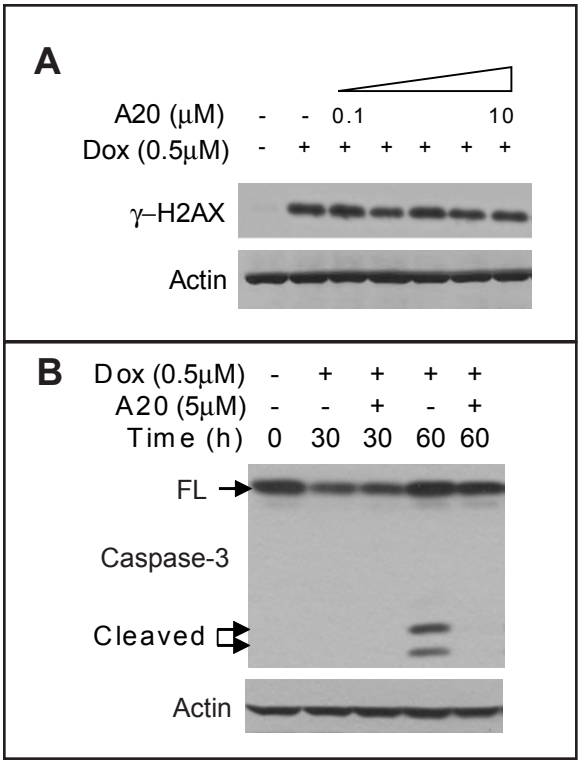
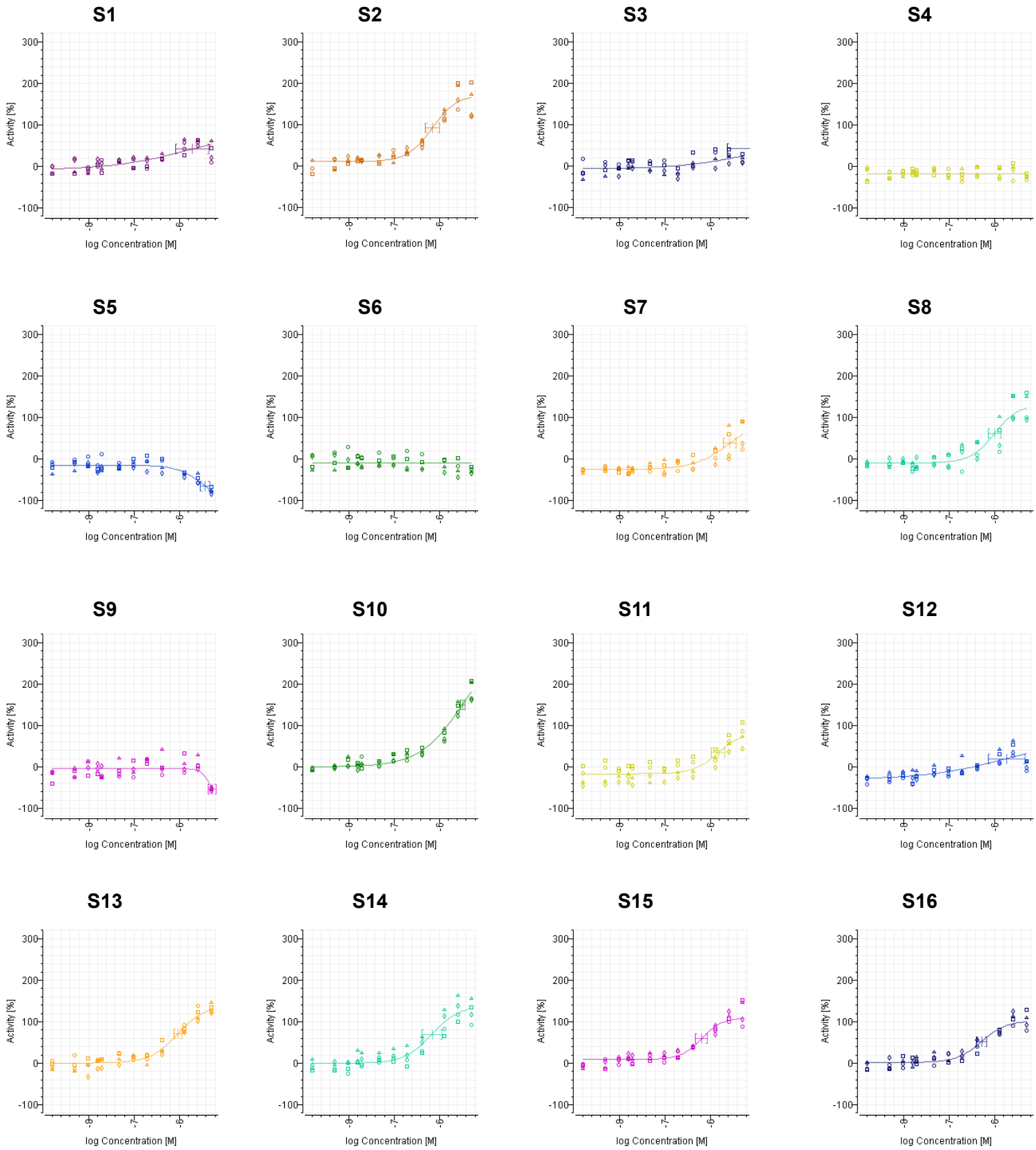

Doxorubicin
Bortezomib
Staurosporine
Taxol
Brefeldin
Cytochalasin D
TNFα (20ng/ml)
TRAIL (100ng/ml)
no A20
A20
Cell survival
120%
100%
80%
60%
40%
20%
0%
μM: 0 0.05 0.15 0.5 1.5 5
μM: 0 0.01 0.03 0.1 0.3 1
nM: 0 1 3 10 30 100
μM: 0 0.03 0.1 0.3 1 3
μg/ml: 0 0.01 0.03 0.1 0.3 1
μM: 0 0.3 1 3 10 30
μg/ml: 0 0.1 0.3 1 3 10
Cycloheximide
μg/ml: 0 0.1 0.3 1 3 10
Cycloheximide

Figure S2


A
A20 (μM) - - 0.1 10
Dox (0.5μM) - + + + + + +
γ–H2AX
Actin
B
Dox (0.5μM) - + + + +
A20 (5μM) - - + - +
Time (h) 0 30 30 60 60
FL
Caspase-3
Cleaved
Actin

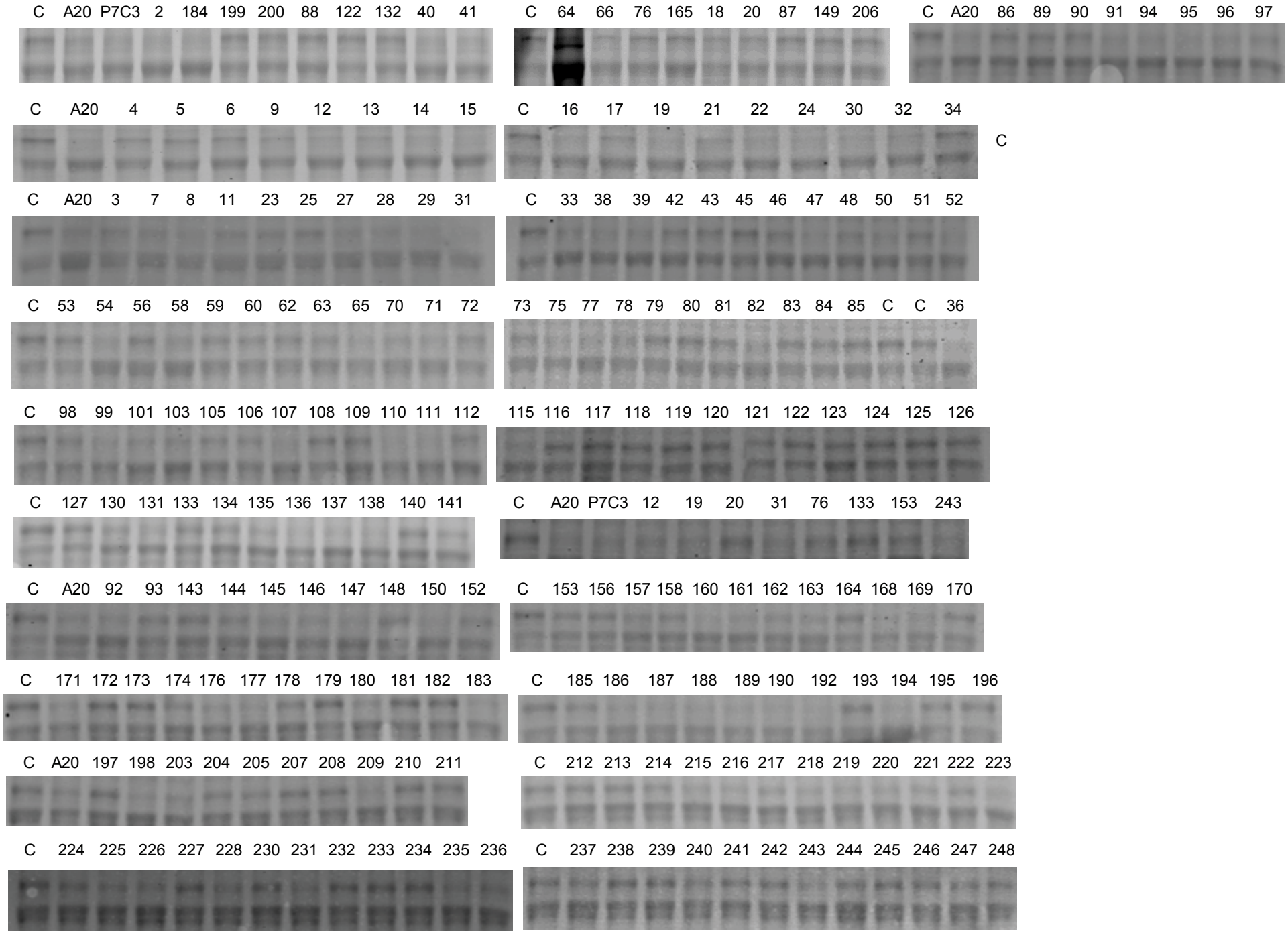
Supplemental Figure

Figure S3



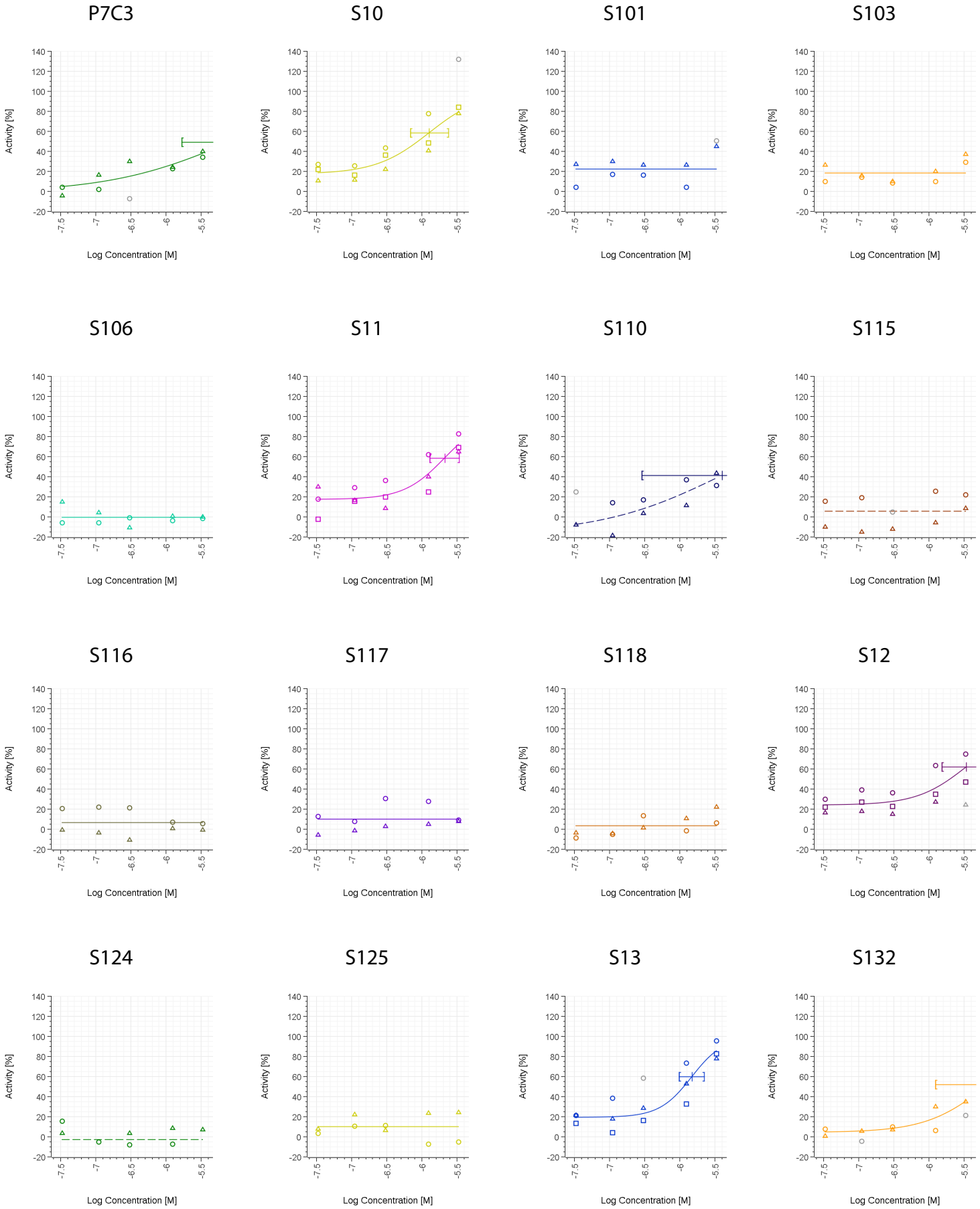
Supplemental Figure

Figure S4



Supplemental Figure

Figure S5



Supplemental Figure

Figure S6

Click here to download Supplemental Figure: Figure S6.pdf

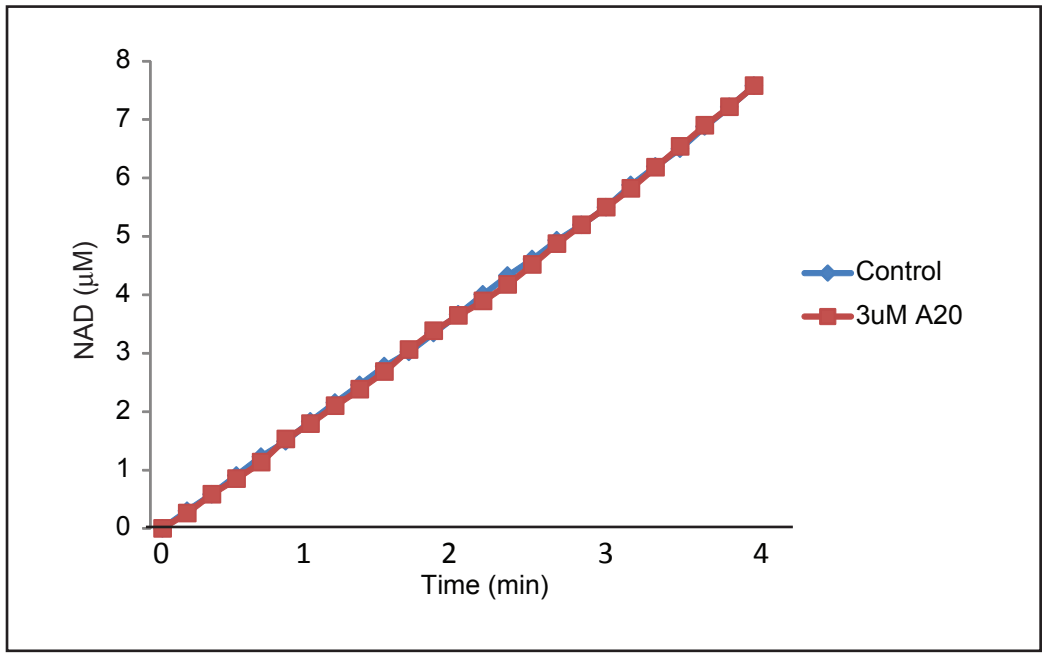
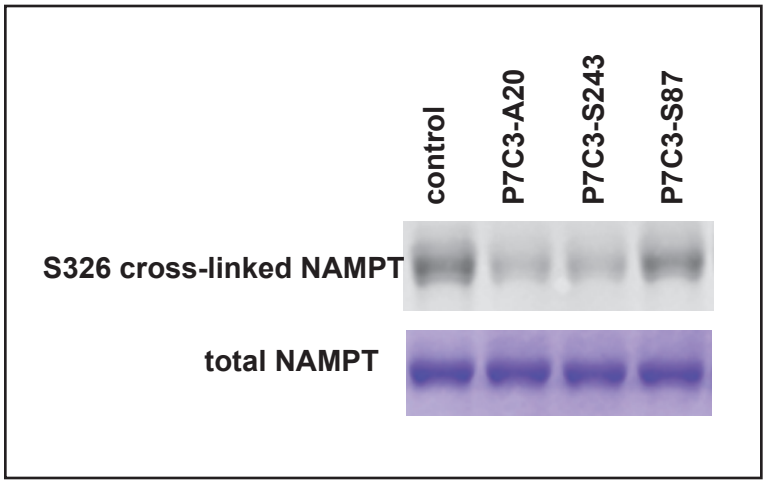


Figure S7


control
P7C3-A20
P7C3-S243
P7C3-S87
S326 cross-linked NAMPT
total NAMPT