ONLINE SUPPLEMENT

Extracellular Ubiquitin(1-76) and Ubiquitin(1-74) Regulate Cardiac Fibroblast Proliferation

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Running title: *Ubiquitin Affects Cardiac Fibroblast Proliferation*

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TOF MS Spectrum: Ubiquitin(1-76)

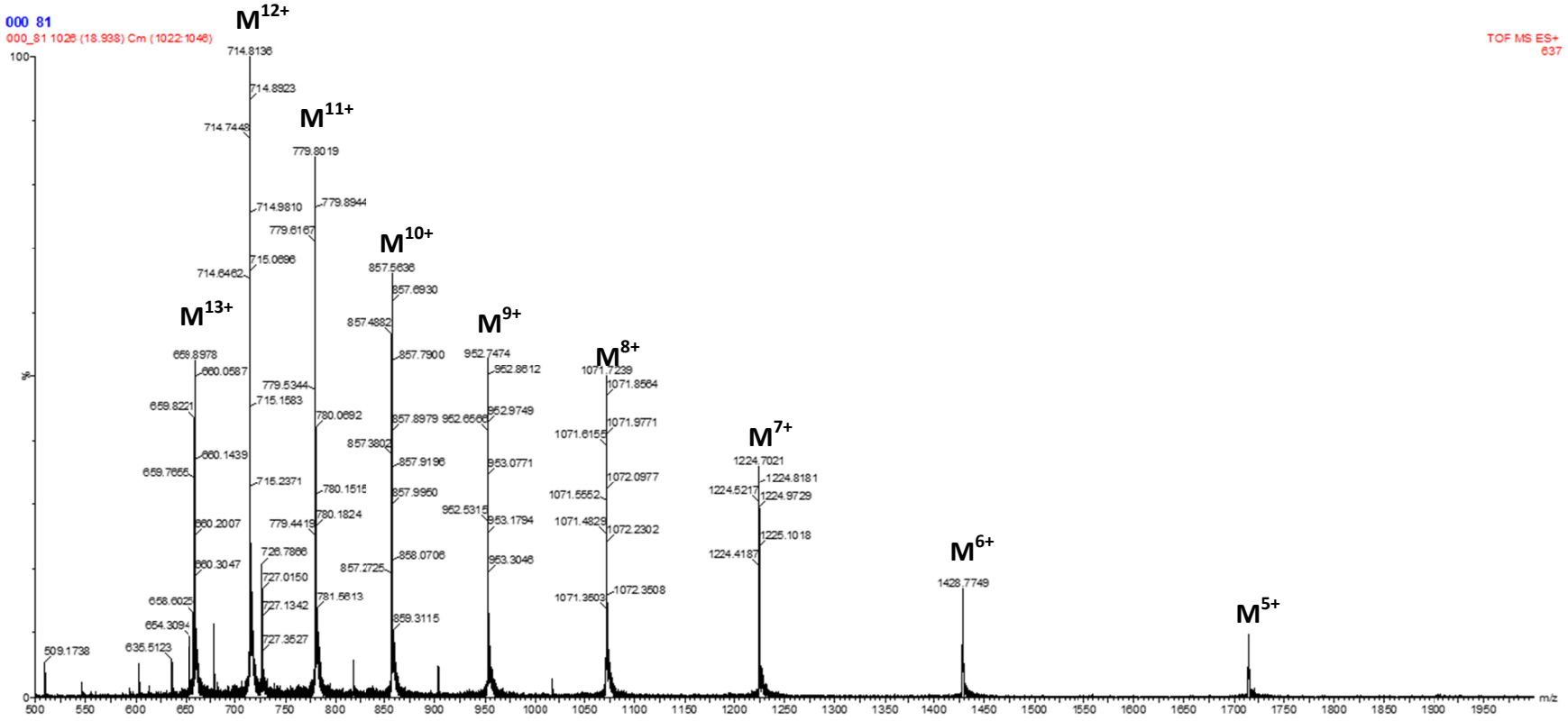

Figure S1

Figure S1. Time-of-flight (TOF) mass spectrum of authentic ubiquitin(1-76). This spectrum illustrates with high-mass resolution the signal strength (y-coordinate) for mass-to-charge (m/z) ratios (x-coordinate) for different species of ionized ubiquitin(1-76). The different m/z signals are due to different levels of ionization of ubiquitin(1-76). The number of positive charges on intact ubiquitin(1-76) is given by M^{x+}, where x is the number of positive charges. There was no evidence of contamination of the ubiquitin(1-76) with ubiquitin(1-74).

Figure S2

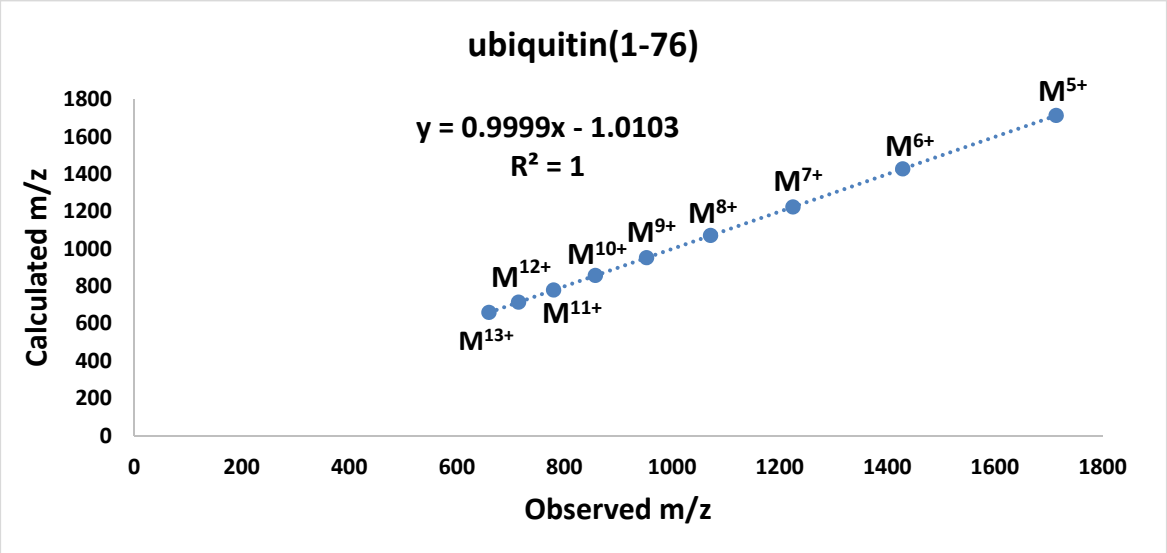
ubiquitin(1-76)
y = 0.9999x - 1.0103
R² = 1
Calculated m/z
Observed m/z
0
200
400
600
800
1000
1200
1400
1600
1800
M5+
M6+
M7+
M8+
M9+
M10+
M11+
M12+
M13+

Figure S2. Plot of calculated versus observed M^{x+} values for ubiquitin(1-76). The observed m/z values for M^{5+} through M^{13+} for ubiquitin(1-76) were obtained from the mass spectrum shown in **Figure S6**. These values were plotted against the theoretical values for M^{5+} through M^{13+} assuming that the material analyzed was indeed ubiquitin(1-76). The straight-line relationship between observed and theoretical values validate this reagent and confirm the lack of contamination by ubiquitin(1-74).

TOF MS Spectrum: Ubiquitin(1-74) plus Ubiquitin(1-76) (1:1 ratio)

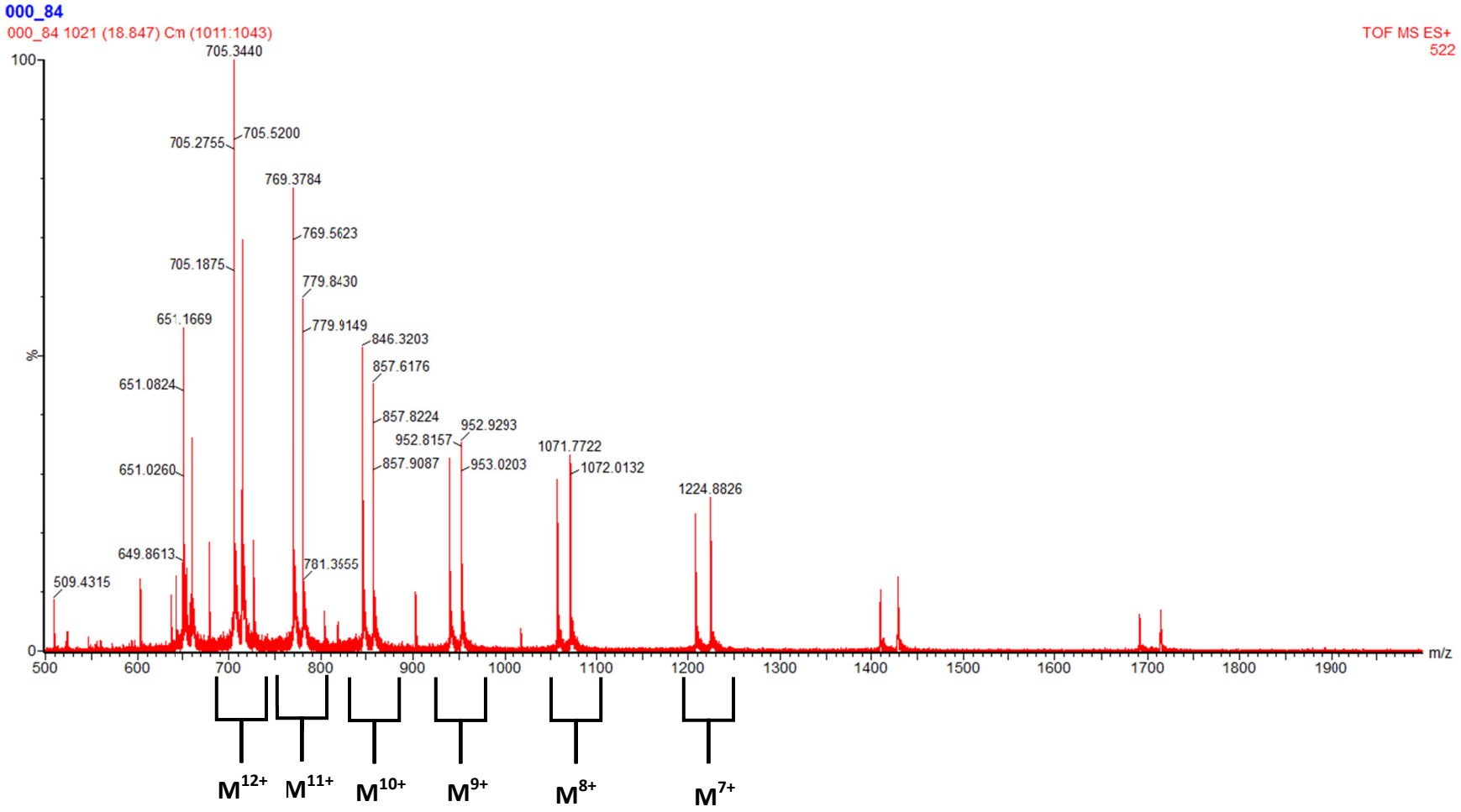

Figure S3

Figure S3. Time-of-flight (TOF) mass spectrum of a solution of equal concentrations of ubiquitin(1-74) and ubiquitin(1-76). This spectrum illustrates with high-mass resolution the signal strength (y-coordinate) for mass-to-charge (m/z) ratios (x-coordinate) for different species of ionized ubiquitin(1-76) and ubiquitin(1-74). The different m/z signals are due to different levels of ionization of the peptides (number of positive charges on intact peptides is given by M^{x+}, where x is the number of positive charges). Note the pairs of signals that correspond to ubiquitin(1-74) (lower m/z) and ubiquitin (1-76) (higher m/z).

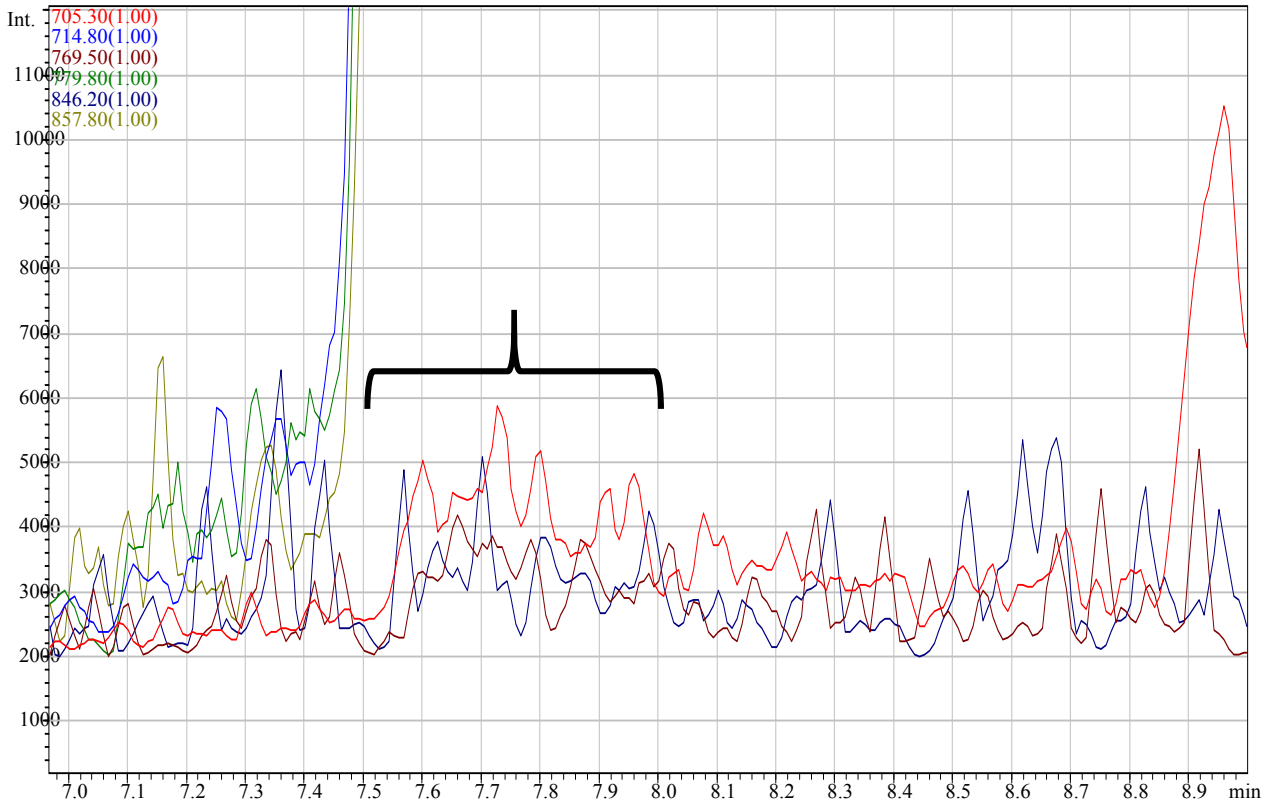
Int.
705.30(1.00)
714.80(1.00)
769.50(1.00)
779.80(1.00)
846.20(1.00)
857.80(1.00)
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
7.0
7.1
7.2
7.3
7.4
7.5
7.6
7.7
7.8
7.9
8.0
8.1
8.2
8.3
8.4
8.5
8.6
8.7
8.8
8.9
min

Figure S4

Figure S4. Ubiquitin(1-74) cannot be detected in cardiac fibroblasts co-incubated with ubiquitin(1-76) plus 6bk (an IDE inhibitor). SHR cardiac fibroblasts were treated with ubiquitin(1-76) (1000 nmol/L) and 6bk (1 μmol/L) for 24 hours, and the medium was collected and processed for analysis by mass spectrometry. Samples were loaded onto a polar reverse phase column connected to a Shimadzu HPLC/LCMS-2010 system. Separation was conducted with linear gradient from 20% acetonitrile to 60% acetonitrile in the presence of 0.1% formic acid at flow rate 0.4 ml/min. The mass spectrometer was operated in the positive ion mode with selected ion monitoring. The bracket indicates the retention time of ubiquitin(1-74). The red, brown, and blue lines represent the signal strength for ubiquitin(1-74) ions at 705.30, 769.50 and 846.20 m/z, respectively. The signals for ubiquitin(1-74) were essentially indiscernible form baseline noise.