**Elucidating Carfilzomib's induced cardiotoxicity in an in vivo model of aging: prophylactic potential of metformin.**

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**Supplemental Tables**

**Table S1: Echocardiographic analysis of aged mice in the two-dose protocol at baseline**

| Table S1 | Control | Cfz | Met | Cfz+Met |
|---|---|---|---|---|
| P2_Day 0 | n=7 | n=6 | n=5 | n=6 |
| HR | 524.28±7.19 | 524.33±17.16 | 488.00±16.24 | 525.33±10.66 |
| LVEDD(mm) | 4.06±0.12 | 4.10±0.05 | 3.99±0.11 | 4.03±0.21 |
| LVESD(mm) | 2.63±0.12 | 2.59±0.09 | 2.57±0.11 | 2.55±0.14 |
| PWTd (mm) | 0.77±0.01 | 0.78±0.01 | 0.76±0.01 | 0.78±0.01 |
| FS% | 35.23±1.38 | 36.86±1.68 | 35.62±1.77 | 36.86±1.45 |
| CO (mL/min) | 24.14±1.16 | 26.10±1.38 | 22.46±2.07 | 25.83±3.58 |
| r/h | 2.65±0.11 | 2.63±0.05 | 2.61±0.11 | 2.56±0.12 |

Baseline values of aged mice in the two-dose protocol. Values are presented as mean ± SEM. HR: Heart Rate; LVEDD: Left Ventricular End Diastolic Diameter (mm); LVESD: Left Ventricular End Systolic Diameter (mm); PWTd: Posterior Wall Thickness diastole (mm); FS%: Fractional Shortening %; r/h: ratio of LV radius to PWT-posterior wall thickness.

**Table S2: Echocardiographic analysis of aged mice in the two-dose protocol at endpoint**

| Table S2 | Control | Cfz | Met | Cfz+Met |
|---|---|---|---|---|
| Day 2 | n=7 | n=6 | n=5 | n=6 |
| HR | 554.42±16.75 | 533.33±27.20 | 507.60±16.88 | 507.16±11.35 |
| LVEDD (mm) | 3.72±0.13 | 4.06±0.11 | 4.21±0.08 | 3.86±0.17 |
| LVESD (mm) | 2.43±0.11 | 2.84±0.17 | 2.82±0.07 | 2.54±0.19 |
| PWTd (mm) | 0.75±0.01 | 0.71±0.01 | 0.74±0.01 | 0.77±0.01 |
| FS% | 34.84±1.11 | 30.40±2.42 | 33.04±0.45 | 34.60±2.62 |
| CO (mL/min) | 21.00±0.92 | 22.21±1.59 | 24.99±1.23 | 20.76±1.73 |
| r/h | 2.48±0.09 | 2.84±0.10*† | 2.85±0.08*† | 2.47±0.11 |

Endpoint values of aged mice in the two-dose protocol. Values are presented as mean ± SEM. HR: Heart Rate; LVEDD: Left Ventricular End Diastolic Diameter (mm); LVESD: Left Ventricular End Systolic Diameter (mm); PWTd: Posterior Wall Thickness diastole (mm); FS%: Fractional Shortening %; r/h: ratio of LV radius to PWT-posterior wall thickness. One-Way ANOVA, Bonferroni post-hoc analysis, *P<0.05 vs control, †P<0.05 vs Cfz+Met

**Table S3: Student's T-Test analysis of Cfz Baseline and Endpoint values in the two-dose protocol that were statistically significantly affected.**

| **Table S3** | |
|---|---|
| **Baseline vs Endpoint** | **p value** |
| PWTd (mm) | 0.0047 |
| FS% | 0.0274 |

Student's T-Test analysis of Cfz Baseline and Endpoint values that were statistically significantly affected. PWTd: Posterior Wall Thickness diastole (mm); FS%: Fractional Shortening %. Student's T-Test, paired, two-tailed.

**Table S4: Echocardiographic analysis of aged mice in the four-dose protocol at baseline**

| **Table S4** | **Control** | **Cfz** | **Met** | **Cfz+Met** |
|---|---|---|---|---|
| **P6_ Day 0** | **n=5** | **n=6** | **n=8** | **n=6** |
| **HR** | 541.00±4.47 | 528.16±19.88 | 563.75±10.16 | 542.33±15.88 |
| **LVEDD (mm)** | 3.90±0.06 | 4.00±0.18 | 3.77±0.13 | 3.84±0.11 |
| **LVESD (mm)** | 2.41±0.07 | 2.55±0.14 | 2.42±0.11 | 2.53±0.06 |
| **PWTd (mm)** | 0.76±0.01 | 0.75±0.01 | 0.75±0.01 | 0.76±0.03 |
| **FS%** | 38.00±1.82 | 36.20±1.97 | 35.86±1.00 | 33.87±1.21 |
| **CO (mL/min)** | 24.57±1.61 | 24.08±2.72 | 22.65±1.43 | 21.94±1.44 |
| **r/h** | 2.56±0.08 | 2.67±0.13 | 2.50±0.08 | 2.51±0.10 |

Baseline values of aged mice in the four-dose protocol. Values are presented as mean ± SEM. HR: Heart Rate; LVEDD: Left Ventricular End Diastolic Diameter (mm); LVESD: Left Ventricular End Systolic Diameter (mm); PWTd: Posterior Wall Thickness diastole (mm); FS%: Fractional Shortening %; r/h: ratio of LV radius to PWT-posterior wall thickness.

**Table S5: Echocardiographic analysis of aged mice in the four-dose protocol at endpoint**

| Table S5 | Control | Cfz | Met | Cfz+Met |
|---|---|---|---|---|
| **P6_Day 6** | **n=5** | **n=6** | **n=8** | **n=5** |
| **HR** | 527.40±38.15 | 506.00±20.01 | 561.00±24.18 | 536.77±11.82 |
| **LVEDD (mm)** | 3.78±0.05 | 3.74±0.14 | 3.68±0.10 | 3.71±0.18 |
| **LVESD (mm)** | 2.40±0.11 | 2.59±0.15 | 2.37±0.09 | 2.42±0.13 |
| **PWTd (mm)** | 0.75±0.01 | 0.74±0.01† | 0.74±0.01† | 0.77±0.01 |
| **FS%** | 36.63±2.64 | 30.94±2.14*♯ | 35.67±0.93 | 34.77±1.13 |
| **CO (mL/min)** | 21.64±1.99 | 17.94±1.87 | 21.09±1.05 | 20.47±2.12 |
| **r/h** | 2.49±0.03 | 2.54±0.11 | 2.50±0.06 | 2.40±0.12 |

Endpoint values of aged mice in the four-dose protocol. Values are presented as mean ± SEM. HR: Heart Rate; LVEDD: Left Ventricular End Diastolic Diameter (mm); LVESD: Left Ventricular End Systolic Diameter (mm); PWTd: Posterior Wall Thickness diastole (mm); FS%: Fractional Shortening %; r/h: ratio of LV radius to PWT-posterior wall thickness. One-Way ANOVA, Bonferroni post-hoc analysis, *P<0.05 vs Control, †P<0.05 vs Cfz+Met, ♯P<0.05 vs Met

**Table S6: Student's T-Test analysis of Cfz Baseline and Endpoint values in the four-dose protocol that were statistically significantly affected.**

| Table S6 | |
|---|---|
| **Baseline vs Endpoint** | **p value** |
| FS% | 0.0010 |

Student's T-Test analysis of Cfz Baseline and Endpoint values that were statistically significantly affected. FS%: Fractional Shortening %. Student's T-Test, paired, two-tailed.

**Supplemental Figures**

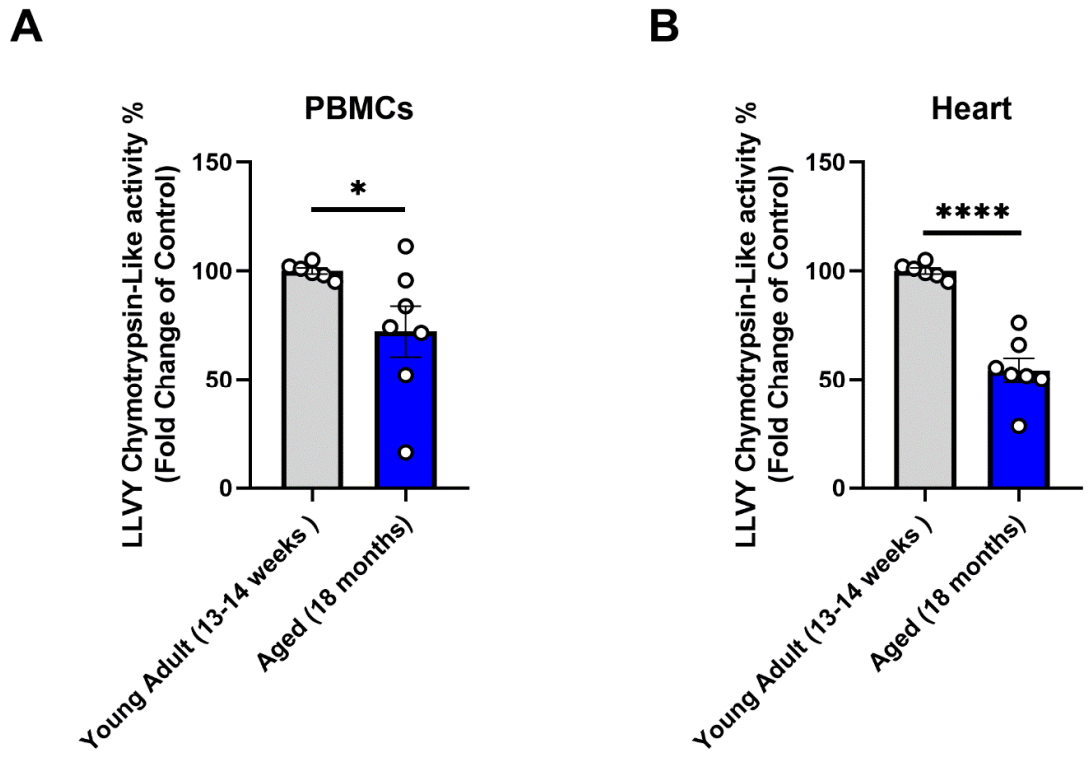


**Figure S1. Aged mice present reduced LVVY proteasome activity compared to young adult mice both in heart and PBMCs.** Graphs of LLVY Chemothrypsin-Like activity % in the A. PBMCs and B. Heart expressed as Fold change of young adult (13 weeks of age) C57BL/6J mice. All data are presented as mean ± SEM. Two Tailed unpaired T-Test; *P<0.05, ****P<0.001. PBMCs, peripheral blood mononuclear cells

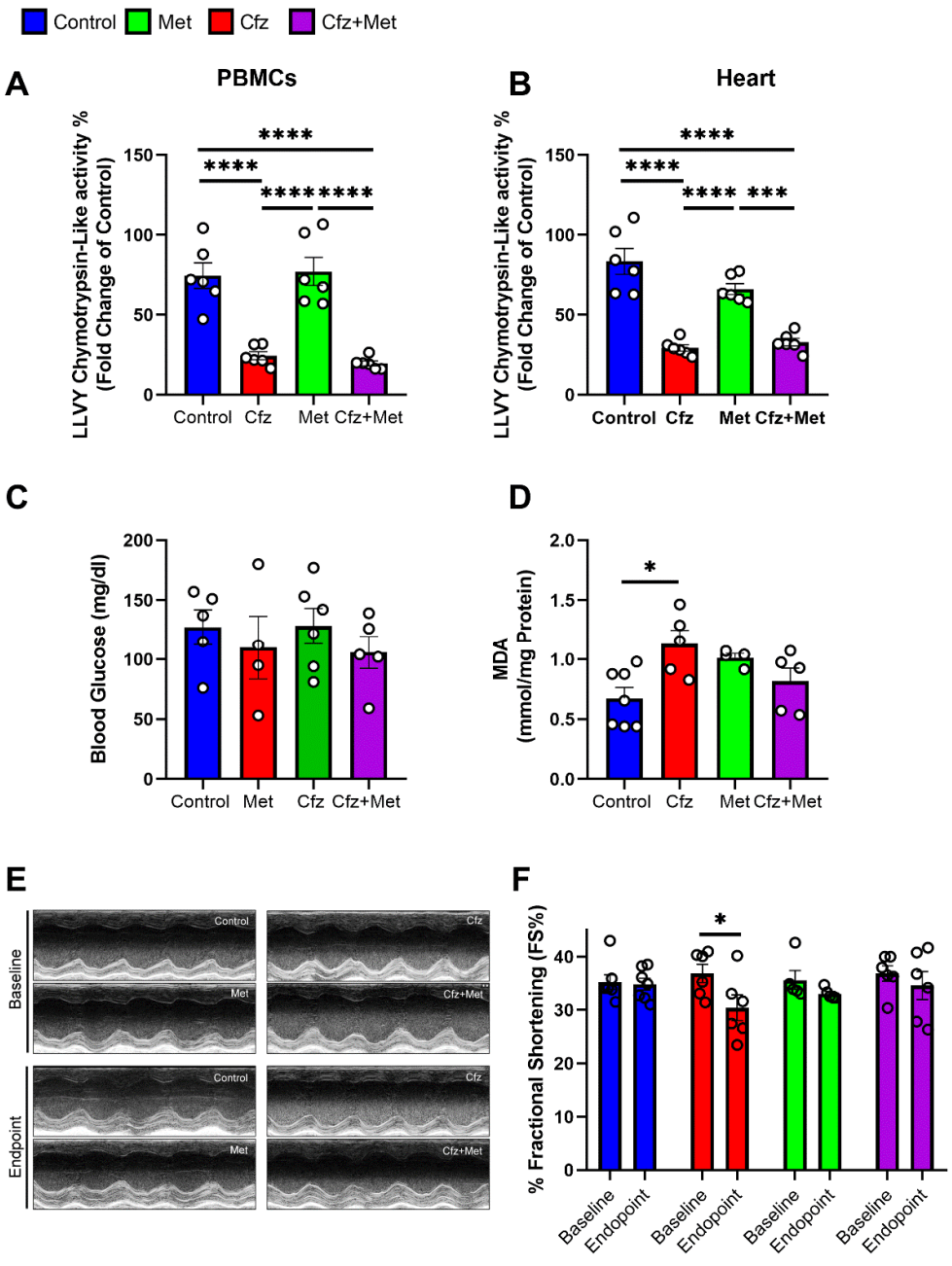


**Figure S2: Metformin in the two doses protocol can successfully mitigate the early mild cardiotoxic phenomena induced by Cfz.** Graphs of LLVY Chemothrypsin-Like activity % in the **A.** PBMCs and **B.** Heart expressed as Fold change of young adult (13 weeks of age) C57BL/6J mice after two doses of Cfz (n=6 per group). **C.** Fasting Blood glucose levels (mg/dl). **D.** Myocardial MDA content (in mmol/mg protein, n=4-7 per group). **E, F**. Representative M-Mode images and graph of Fractional Shortening (FS%) in the two doses protocol (n=5-7 per group). All data are presented as mean ± SEM. One-way ANOVA, Tukey's post-hoc test, *P<0.05, ***P<0.005, ****P<0.001.

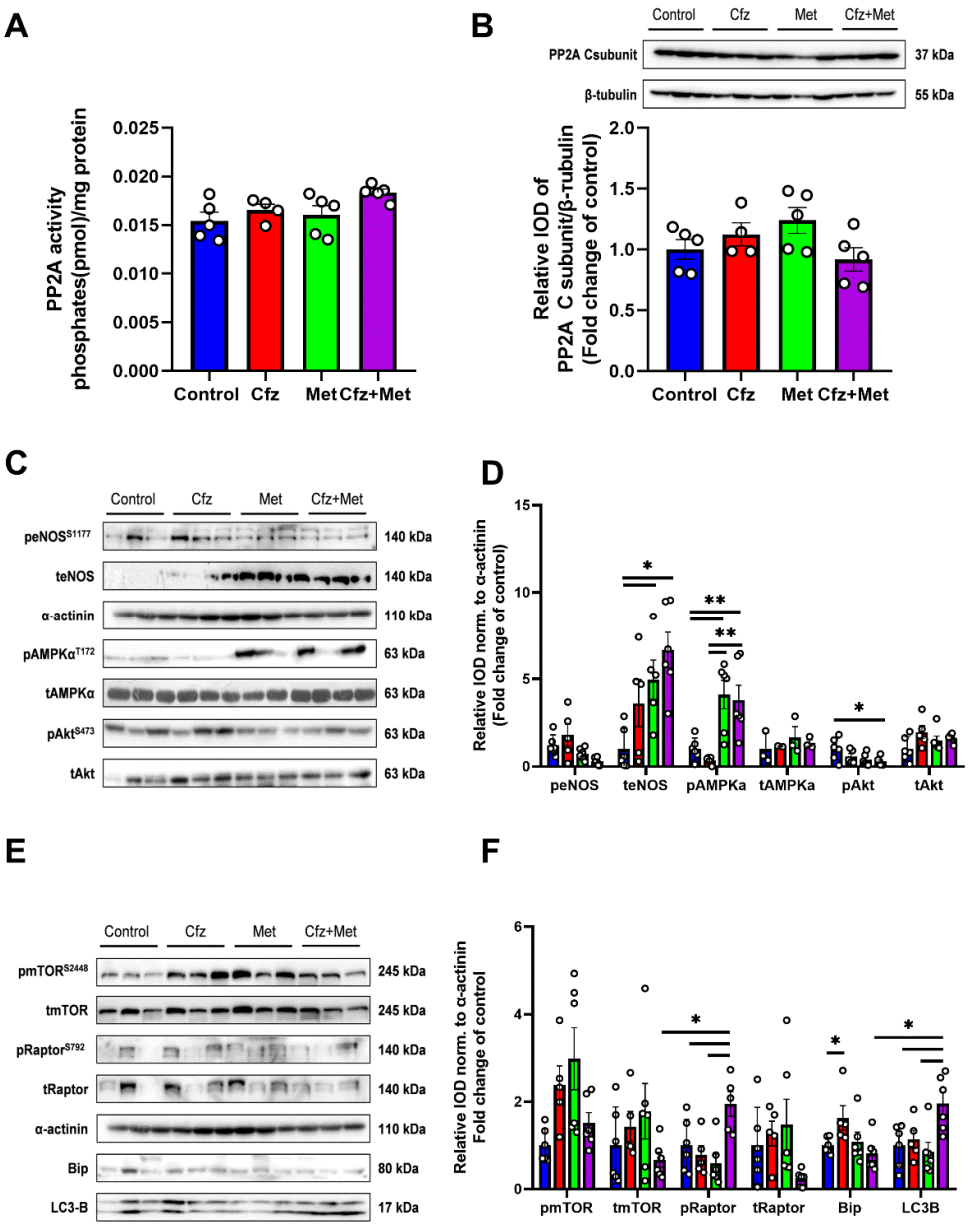


**Figure S3: Metformin protects against early Cfz cardiotoxicity through AMPK$\alpha$ phosphorylation, restoration of Bip expression and synergistic induction of LC3B-dependent autophagy**. Graphs of PP2A **A.** activity (in pmol/mg protein) and **B.** expression (fold change of control) (n=5 per group). **C.** Representative western blots and **D.** Relative densitometry analysis of phosphorylated and total eNOS, AMPK$\alpha$ and Akt levels normalized to $\alpha$-actinin. **E.** Representative Western blots and **F.** Relative densitometry analysis of phosphorylated and total mTOR, Raptor and total Bip and LC3B normalized to $\alpha$-actinin (n=5-6 per group). All data are presented as mean ± SEM. One-way ANOVA, Tukey's post-hoc test, *P<0.05, **P<0.01.

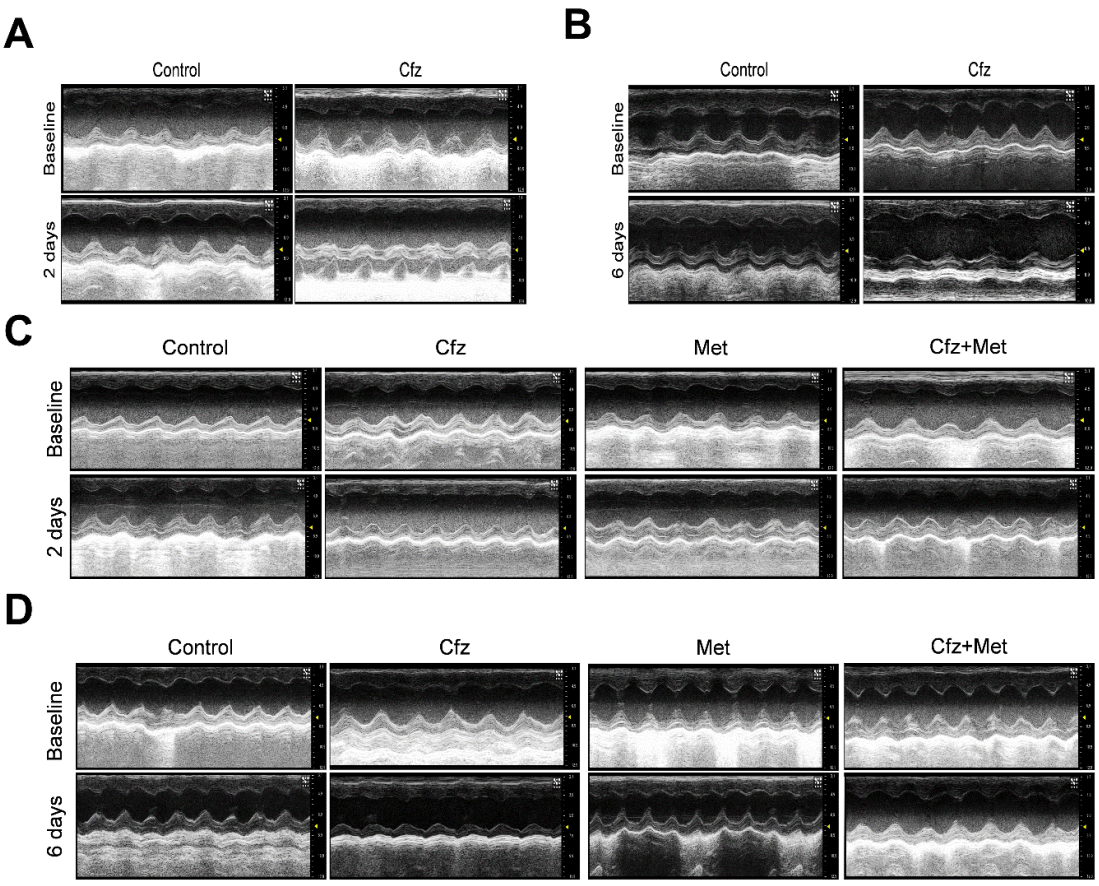


**Figure S4: Panel of original echocardiography images.** Panel of original echocardiography images including the scale bar corresponding to **A-B.** Figure 1 **C.** Figure S1 and **D.** Figure 4.

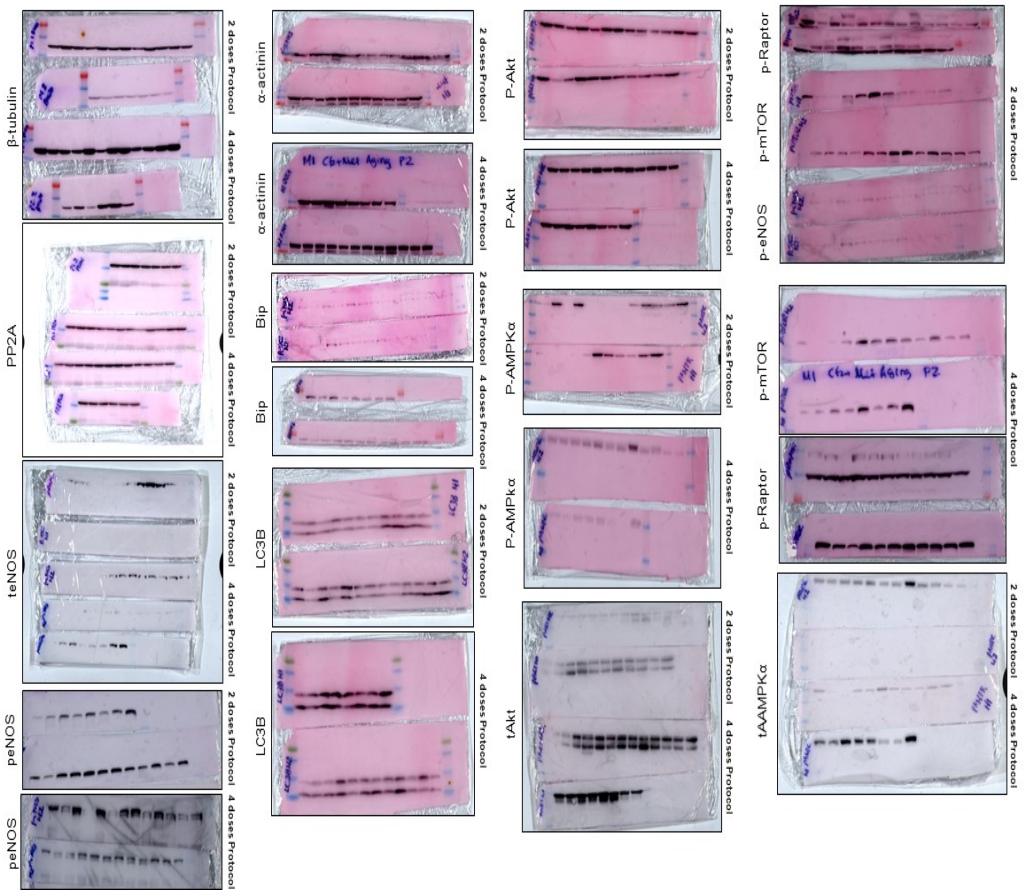


**Figure S5: Panel of original western blots.** Panel of original western blot images of 2 doses and 4 doses protocols.