Supporting information for:

MDM2-BCL-X_L PROTACs enable degradation of BCL-X_L and stabilization of p53

NMR and HPLC spectroscopy for BMM 2-4…….....**Page 2**

Original Uncropped Western blots…….....**Page 10**

Preparation of *tert*-butyl (*R*)-(7-(4-(3-((4-(*N*-(4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)-7-oxoheptyl)carbamate (compound **14**). To a solution of 7-((*tert*-butoxycarbonyl)amino)heptanoic acid (13.9 mg, 0.056 mmol) in DCM was added HATU (23.4 mg, 0.062 mmol), TEA (15.6 mg, 0.154 mmol), and compound **12** (50 mg, 0.051 mmol). The reaction was stirred at room temperature for 1 h and quenched by water. The organic layer was collected, washed by brine, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by reverse phase chromatography to afford the title compound (44 mg, 71%). LC–MS (ESI): m/z 1200.4 $[M + H]^+$.

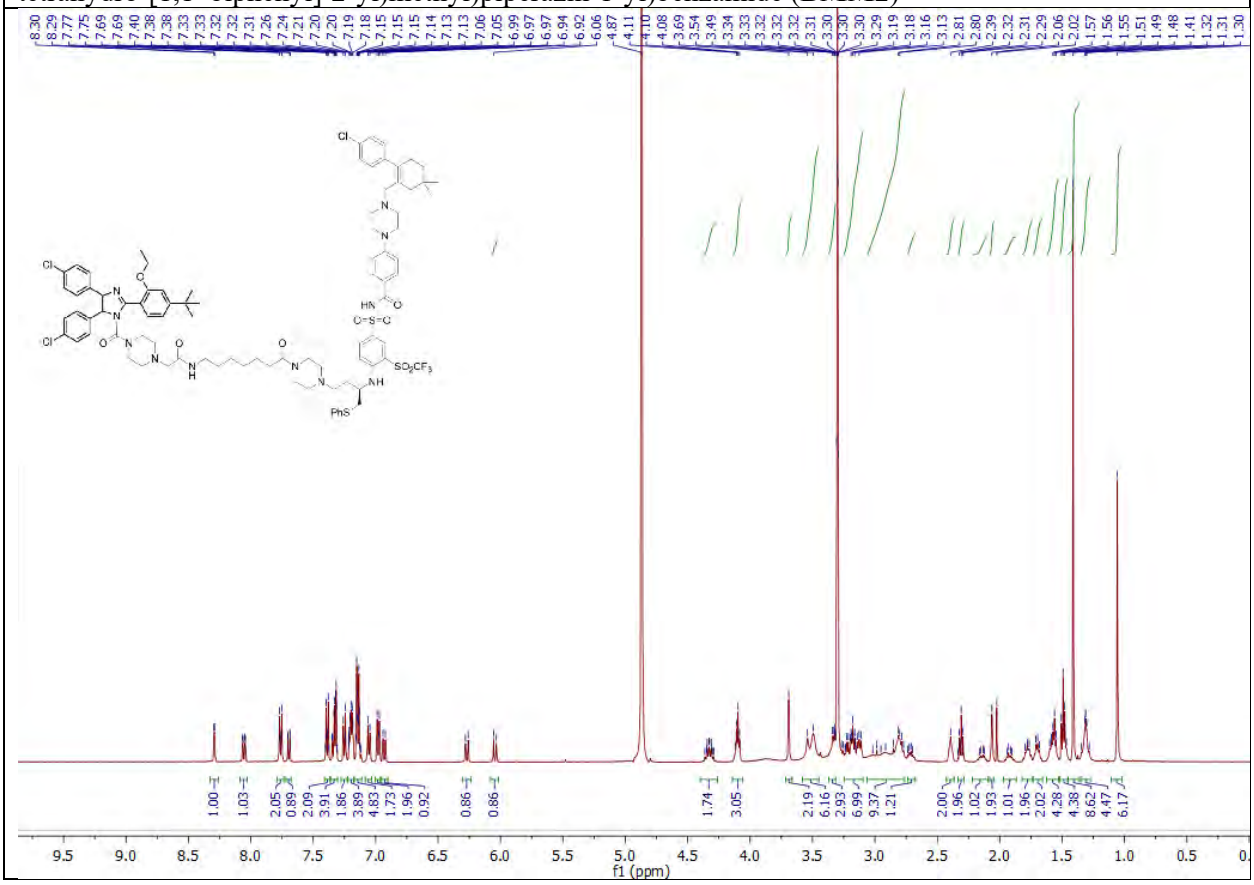
Preparation of (*R*)-*N*-((4-((4-(4-(7-aminoheptanoyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzamide (compound **15**). To a solution of compound **14** (44 mg, 0.037 mmol) in DCM was added TFA (1 mL) and the reaction was stirred at room temperature overnight. The resulting mixture was concentrated under vacuum and used directly for the next step without purification (quantitative). LC–MS (ESI): m/z 1100.4 $[M + H]^+$.

Preparation of *N*-((4-(((2*R*)-4-(4-(7-(2-(4-(2-(4-(tert-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperazin-1-yl)acetamido)heptanoyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzamide (**BMM2**). To a solution of compound 15 (10 mg, 0.016 mmol) in DMF was added HATU (7.2 mg, 0.019 mmol), TEA (4.8 mg, 0.047 mmol) and compound 14 (19.0 mg, 0.17 mmol). The resulting mixture was stirred at room temperature for 4 h and then quenched by water. The aqueous layer was extracted by EA and the combined organic layers were dried over Na2SO4 and concentrated under vacuum. The residue was purified by reverse phase chromatography to afford the title compound (14 mg, 52%). ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.29 (d, J = 2.3 Hz, 1H), 8.05 (dd, J = 9.3, 2.3 Hz, 1H), 7.79 – 7.74 (m, 2H), 7.72 – 7.68 (m, 1H), 7.41 – 7.36 (m, 2H), 7.36 – 7.30 (m, 4H), 7.28 – 7.23 (m, 2H), 7.22 – 7.17 (m, 4H), 7.17 – 7.11 (m, 5H), 7.05 (d, J = 8.2 Hz, 2H), 6.98 (d, J = 9.1 Hz, 2H), 6.93 (d, J = 9.5 Hz, 1H), 6.27 (d, J = 11.2 Hz, 1H), 6.04 (d, J = 11.2 Hz, 1H), 4.38 – 4.27 (m, 2H), 4.14 – 4.07 (m, 3H), 3.69 (s, 2H), 3.57 – 3.45 (m, 6H), 3.37 – 3.32 (m, 3H), 3.25 – 3.10 (m, 7H), 3.06 – 2.76 (m, 9H), 2.74 – 2.68 (m, 1H), 2.43 – 2.37 (m, 2H), 2.33 – 2.27 (m, 2H), 2.22 – 2.09 (m, 1H), 2.06 (s, 2H), 1.97 – 1.87 (m, 1H), 1.82 – 1.74 (m, 2H), 1.73 – 1.66 (m, 2H), 1.62 – 1.53 (m, 4H), 1.52 – 1.45 (m, 4H), 1.41 (s, 9H), 1.35 – 1.27 (m, 4H), 1.06 (s, 6H). LC–MS (ESI): m/z 1718.5 $[M + H]^+$.

Preparation of *N*-((4-(((2*R*)-4-(4-(9-(2-(4-(2-(4-(tert-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperazin-1-yl)acetamido)nonanoyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzamide (**BMM4**). The preparation of **BMM4** was similar to compound **BMM2**. ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.29 (d, J = 2.2 Hz, 1H), 8.06 (dd, J = 9.3, 2.3 Hz, 1H), 7.77 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.1 Hz, 1H), 7.42 – 7.37 (m, 2H), 7.36 – 7.30 (m, 4H), 7.25 (d, J = 8.3 Hz, 2H), 7.23 – 7.17 (m, 4H), 7.17 – 7.11 (m, 5H), 7.04 (d, J = 8.2 Hz, 2H), 6.98 (d, J = 8.9 Hz, 2H), 6.93 (d, J = 9.5 Hz, 1H), 6.26 (d, J = 11.2 Hz, 1H), 6.00 (d, J = 11.2 Hz, 1H), 4.36 – 4.29 (m, 2H), 4.14 – 4.07 (m, 3H), 3.69 (s, 2H), 3.51 – 3.37 (m, 6H), 3.29 – 3.13 (m, 8H), 3.12 – 3.06 (m, 2H), 2.88 – 2.50 (m, 10H), 2.43 – 2.37 (m, 2H), 2.30 (t, J = 7.4 Hz, 2H), 2.16 – 2.04 (m, 3H), 1.91 – 1.81 (m, 1H), 1.80 – 1.73 (m, 2H), 1.72 – 1.66 (m, 2H), 1.62 – 1.53 (m, 4H), 1.50 (t, J = 7.0 Hz, 4H), 1.47 – 1.44 (m, 1H), 1.41 (s, 9H), 1.34 – 1.25 (m, 8H), 1.06 (s, 6H). LC–MS (ESI): m/z 1746.6 $[M + H]^+$.

Preparation of *N*-((4-(((2*R*)-4-(4-(11-(2-(4-(2-(4-(tert-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperazin-1-yl)acetamido)undecanoyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzamide (**BMM3**). The preparation of **BMM3** was similar to compound **BMM2**. ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.28 (d, J = 2.2 Hz, 1H), 8.06 (dd, J = 9.3, 2.3 Hz, 1H), 7.77 (d, J = 8.6 Hz, 2H), 7.67 (d, J = 8.1 Hz, 1H), 7.42 – 7.37 (m, 2H), 7.35 – 7.29 (m, 4H), 7.28 – 7.23 (m, 2H), 7.23 – 7.17 (m, 4H), 7.17 – 7.11 (m, 5H), 7.03 (d, J = 8.1 Hz, 2H), 6.98 (d, J = 8.9 Hz, 2H), 6.93 (d, J = 9.5 Hz, 1H), 6.25 (d, J = 11.3 Hz, 1H), 5.99 (d, J = 11.2 Hz, 1H), 4.35 – 4.28 (m, 2H), 4.14 – 4.07 (m, 3H), 3.69 (s, 2H), 3.49 – 3.36 (m, 6H), 3.25 – 3.14 (m, 8H), 3.11 – 3.07 (m, 2H), 2.80 – 2.43 (m, 10H), 2.42 – 2.38 (m, 2H), 2.30 (t, J = 7.5 Hz, 2H), 2.14 – 2.04 (m, 3H), 1.90 – 1.82 (m, 1H), 1.80 – 1.74 (m, 2H), 1.73 – 1.67 (m, 2H), 1.62 – 1.55 (m, 4H), 1.50 (t, J = 7.0 Hz, 3H), 1.47 – 1.44 (m, 1H), 1.41 (s, 9H), 1.31 – 1.24 (m, 12H), 1.06 (s, 6H). LC–MS (ESI): m/z 1774.5 $[M + H]^+$.

N-((4-(((2*R*)-4-(4-(7-(2-(4-(2-(4-(tert-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperazin-1-yl)acetamido)heptanoyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzamide (**BMM2**)



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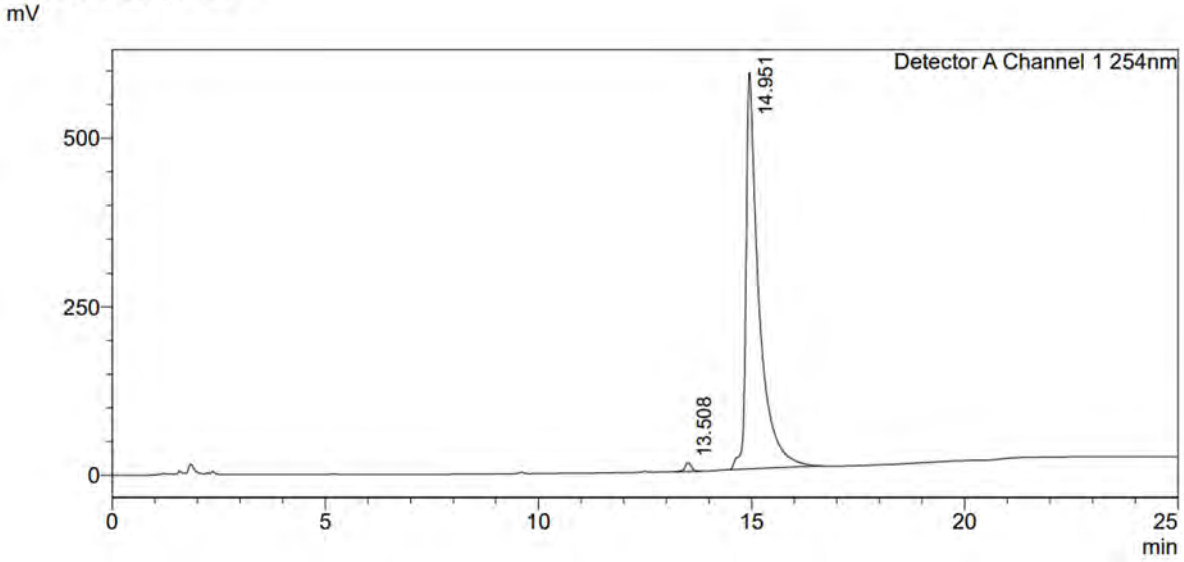

<Peak Table>

Detector A Channel 1 254nm

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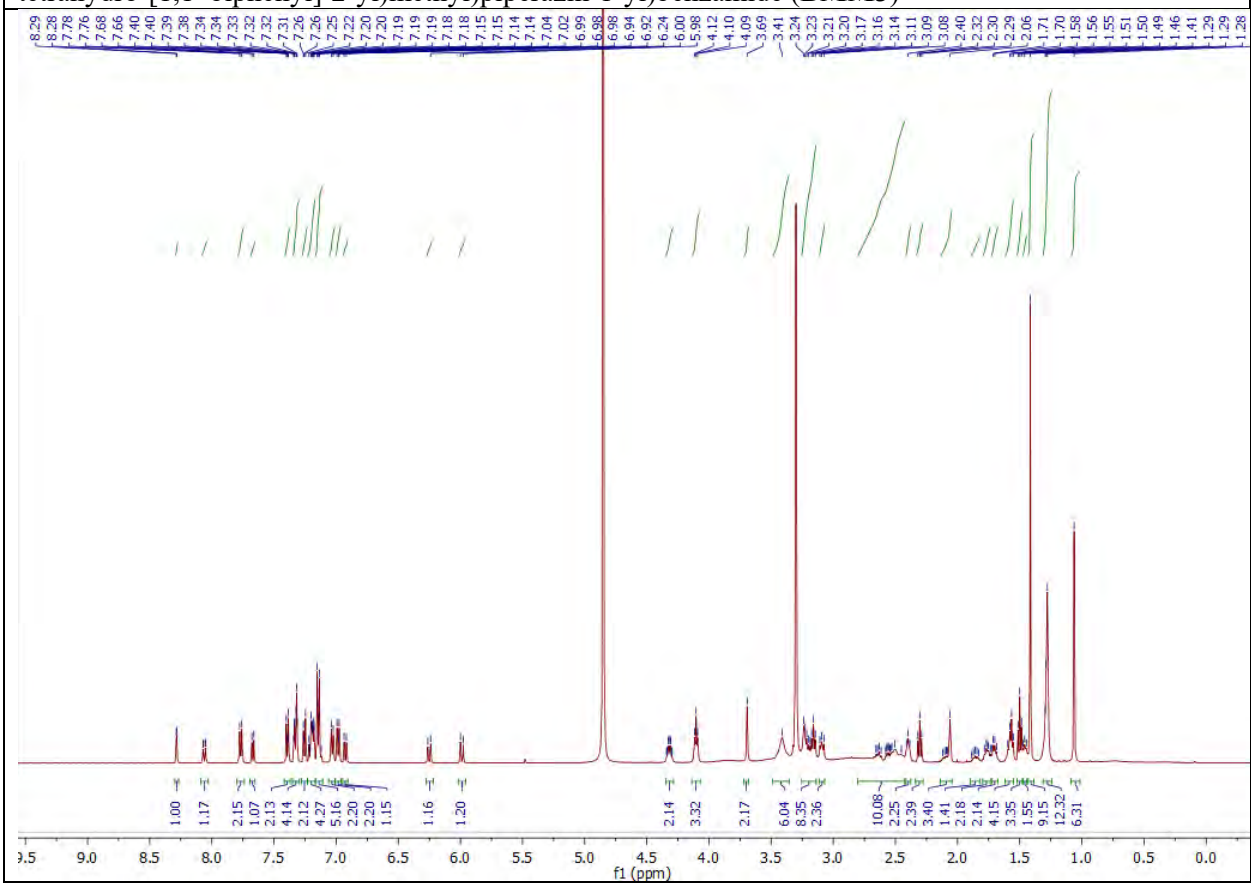
2	14.951	12340480	588386	98.914		98.914
Total		12476001	601481			100.000

N-((4-(((2*R*)-4-(4-(11-(2-(4-(2-(4-(tert-butyl)-2-ethoxyphenyl)-4,5-bis(4-chlorophenyl)-4,5-dihydro-1*H*-imidazole-1-carbonyl)piperazin-1-yl)acetamido)undecanoyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzamide (**BMM3**)



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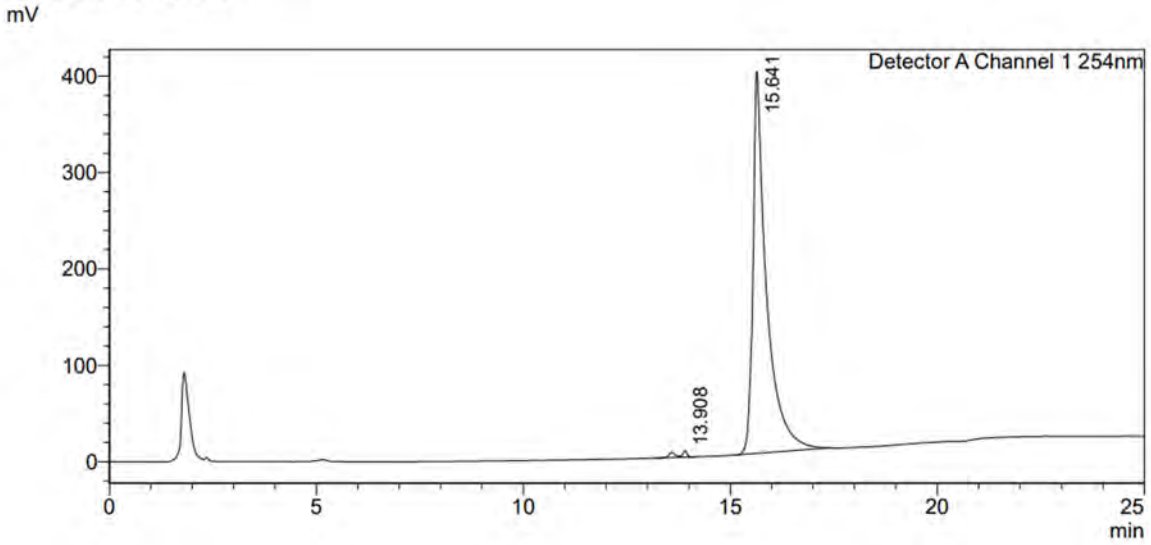

<Peak Table>

Detector A Channel 1 254nm

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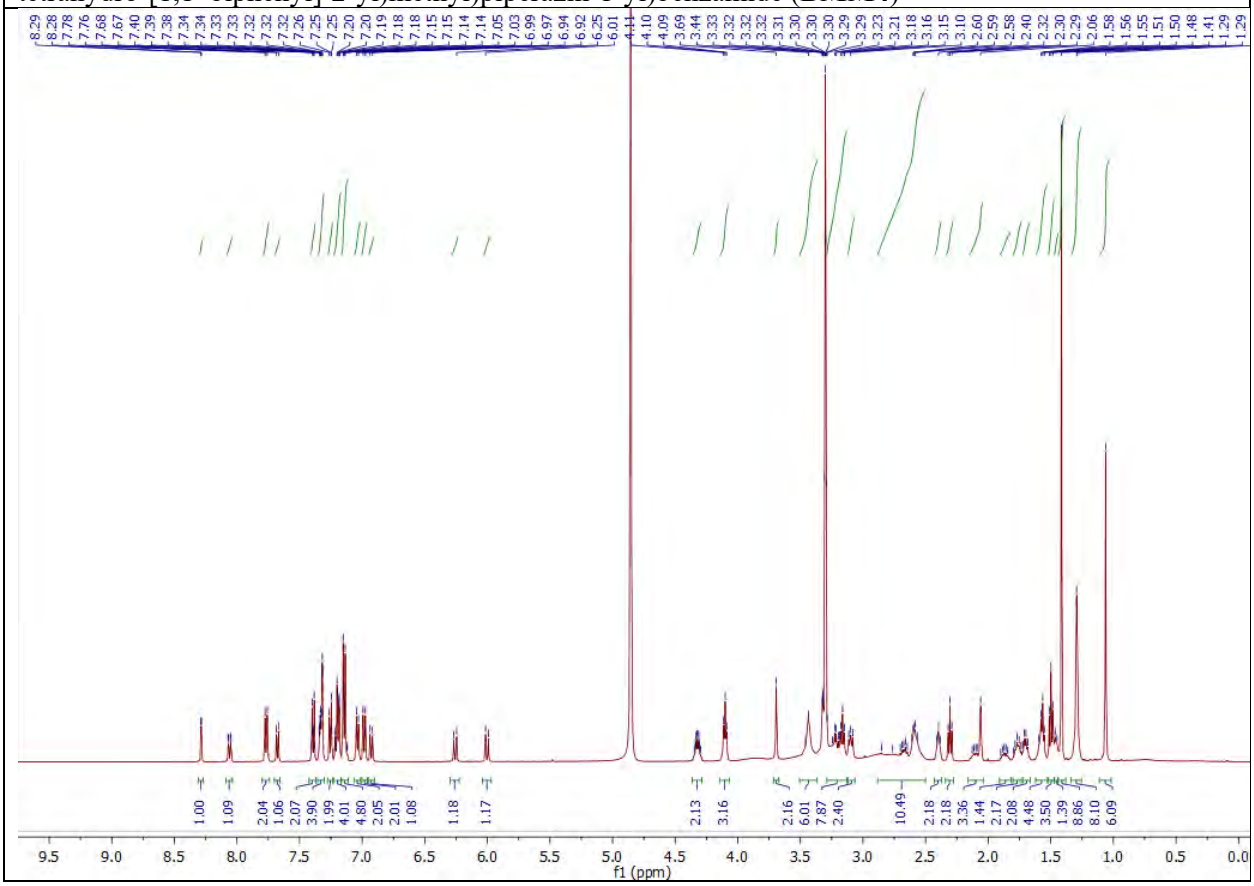

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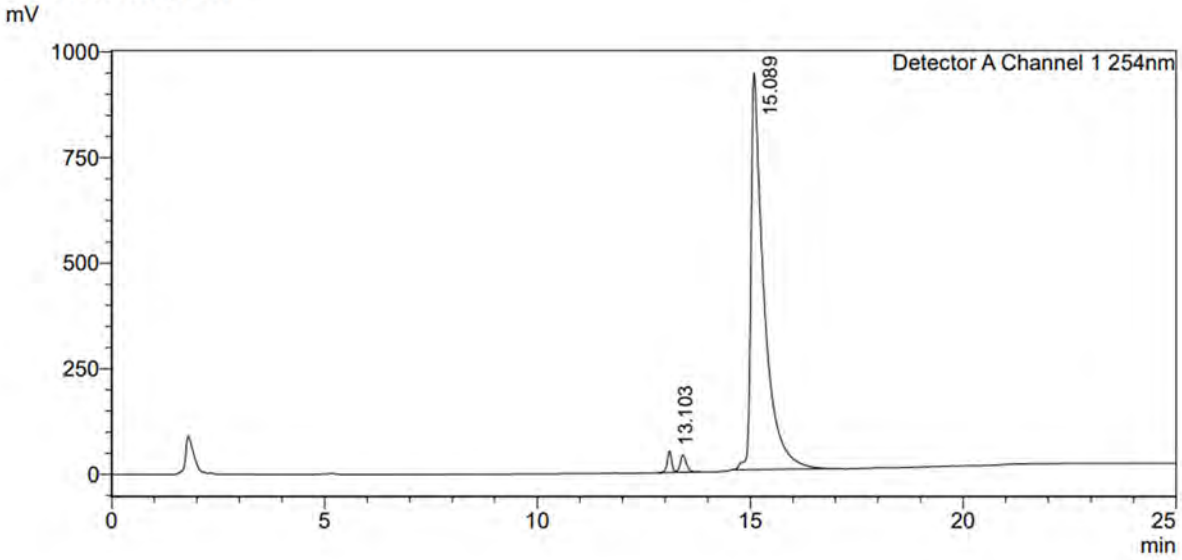

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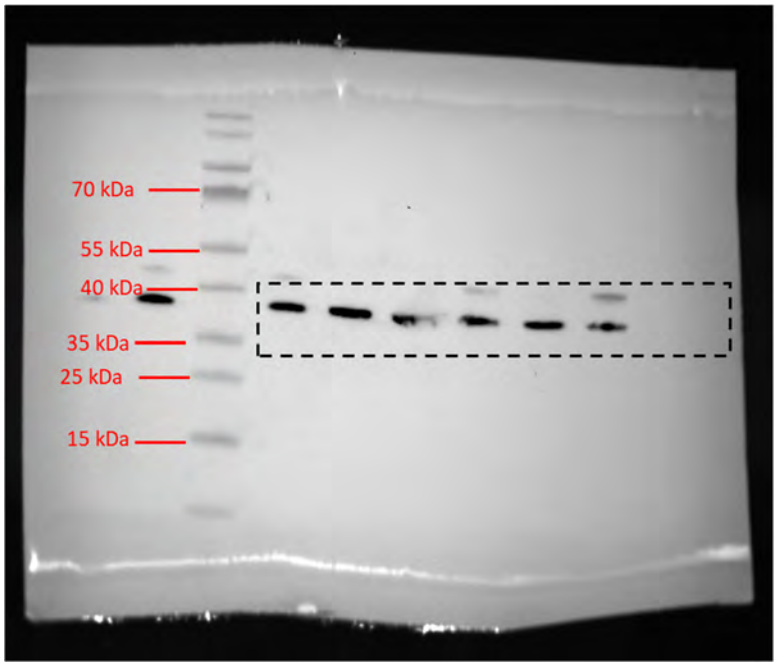
Figures: Original Uncropped Western blots for PROTACs BMM2-4

Uncropped western blots for Fig 2



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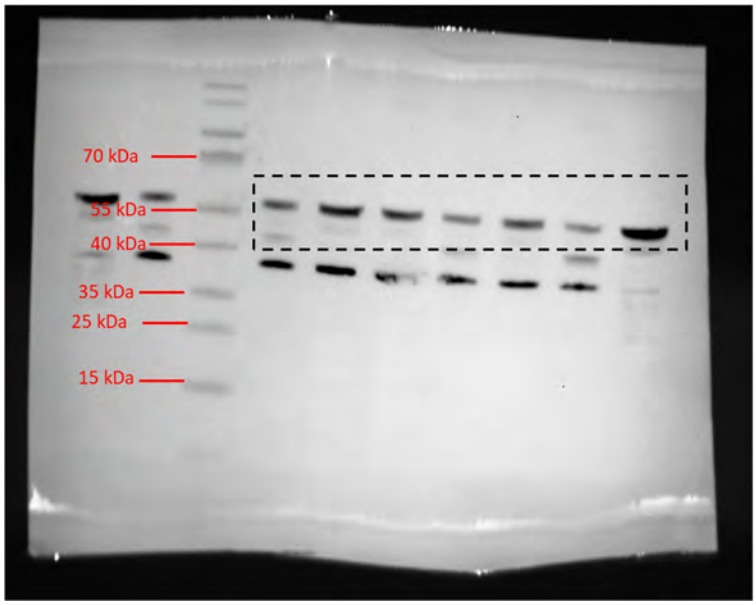

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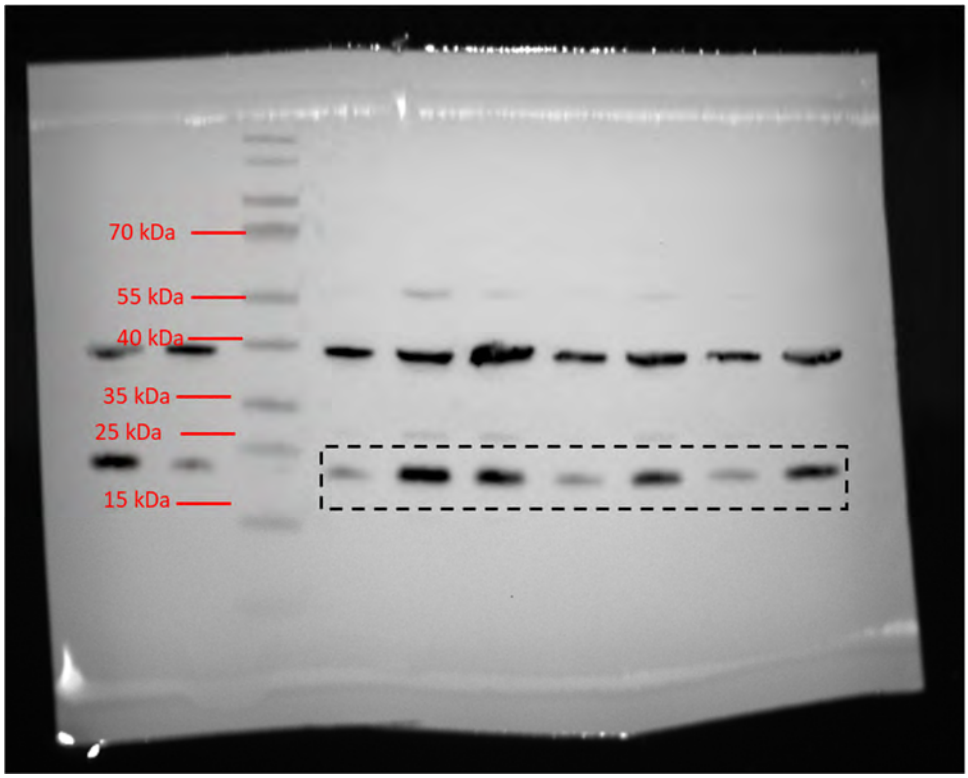
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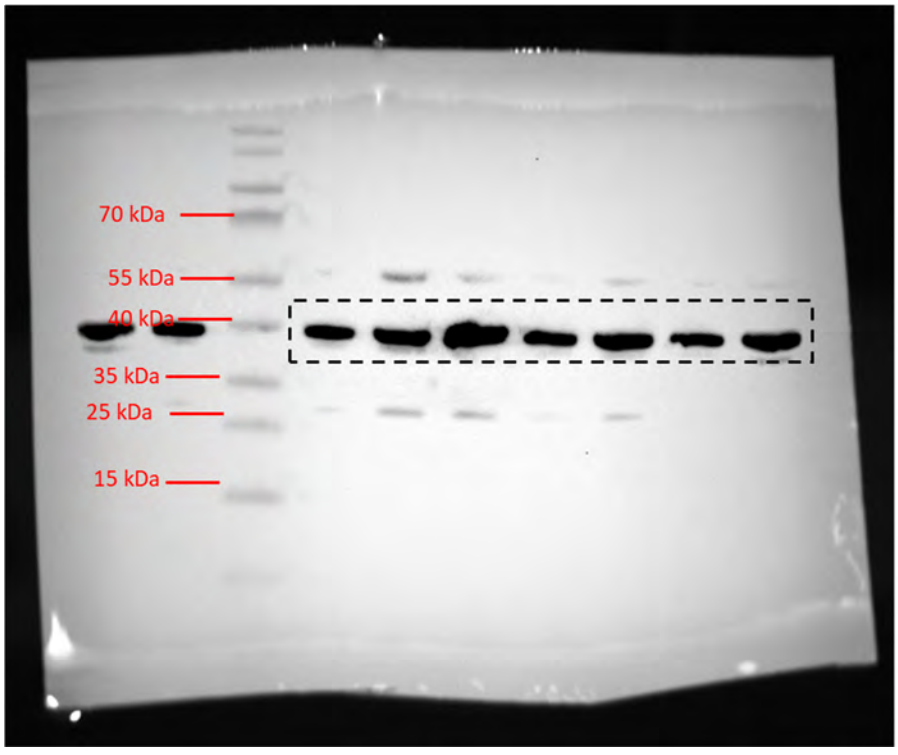
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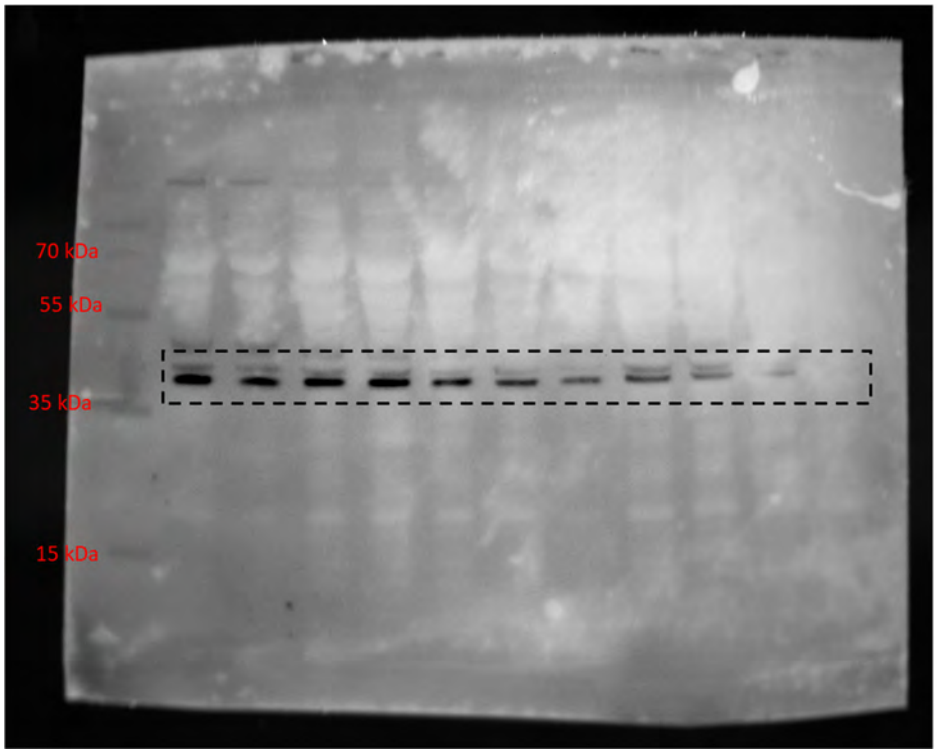
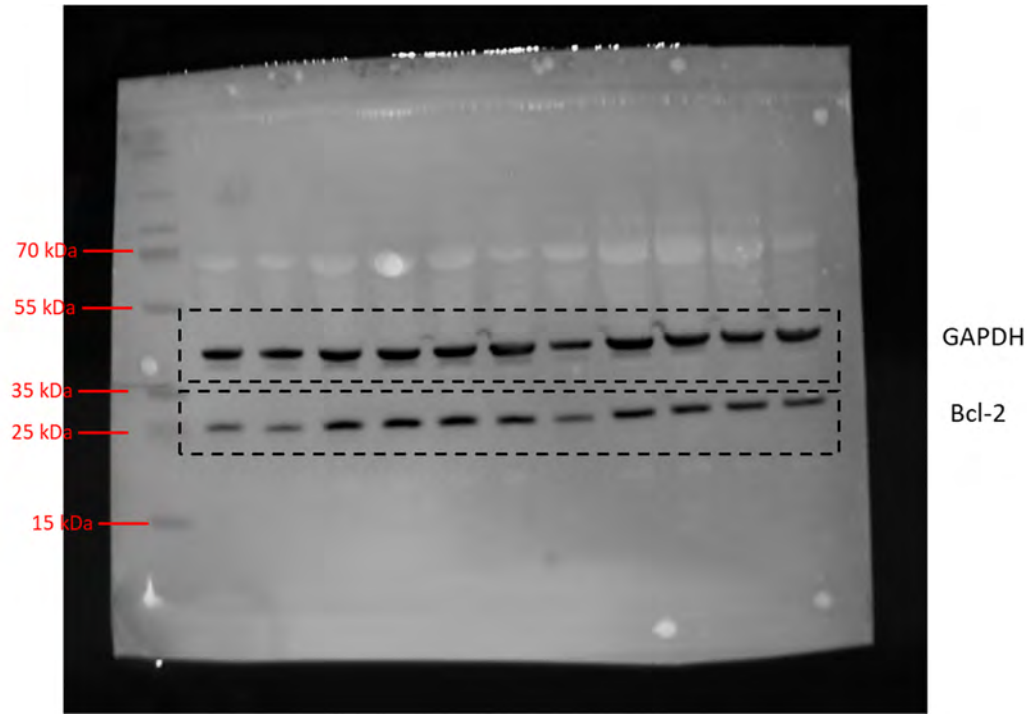
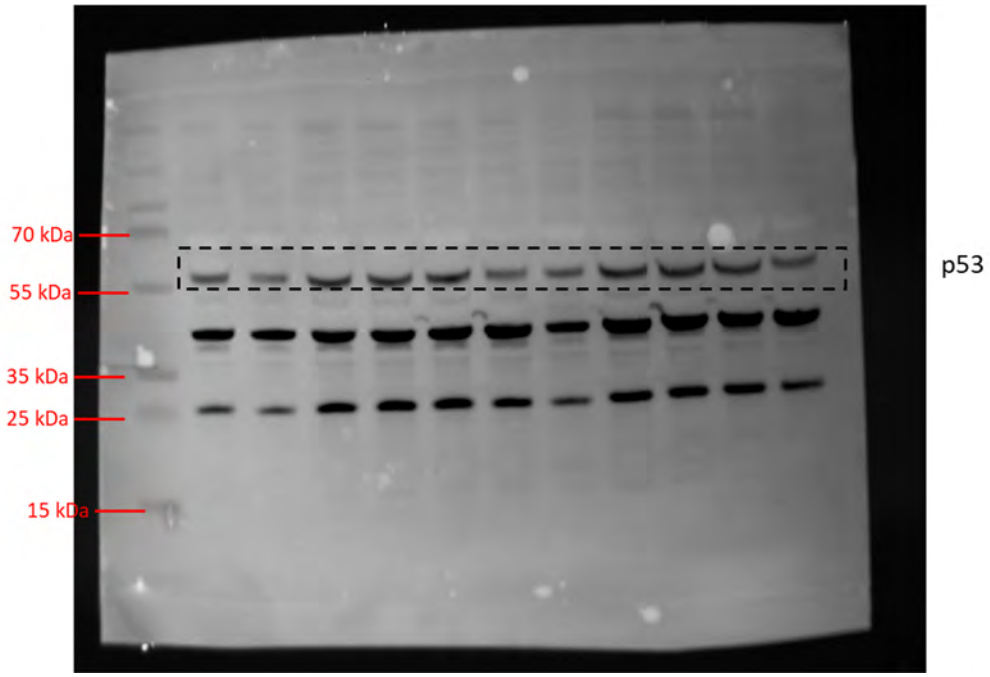
GAPDH

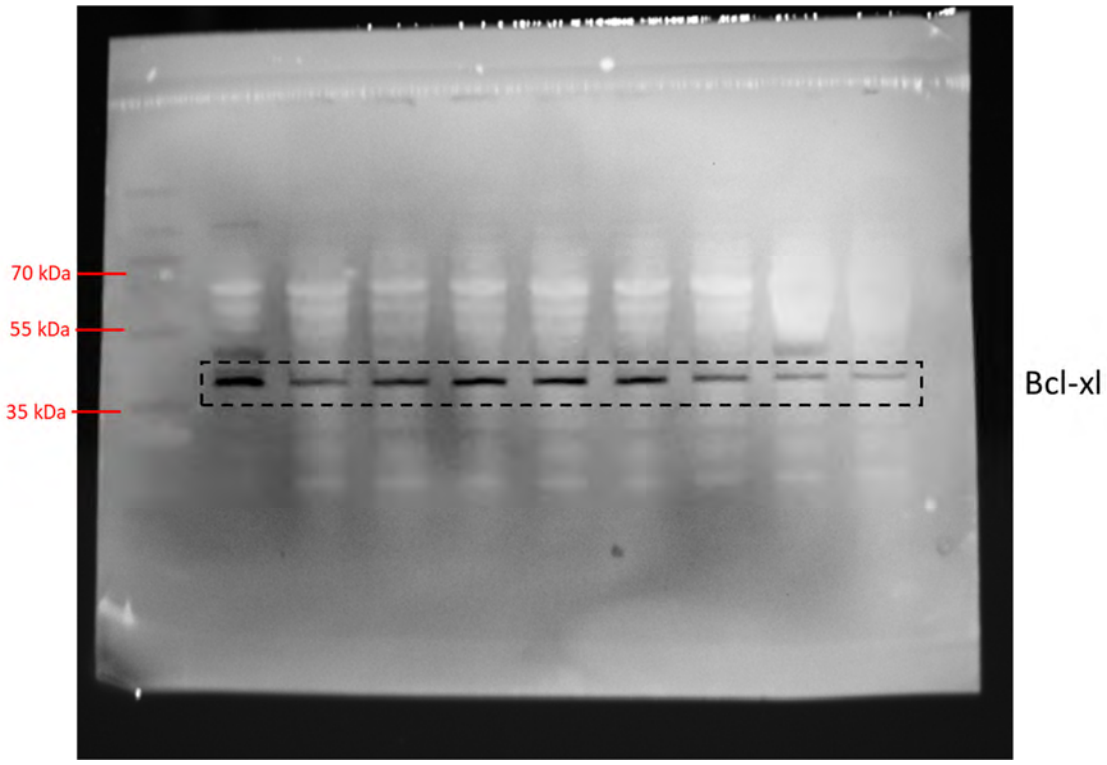
Uncropped western blots for Fig 3 a

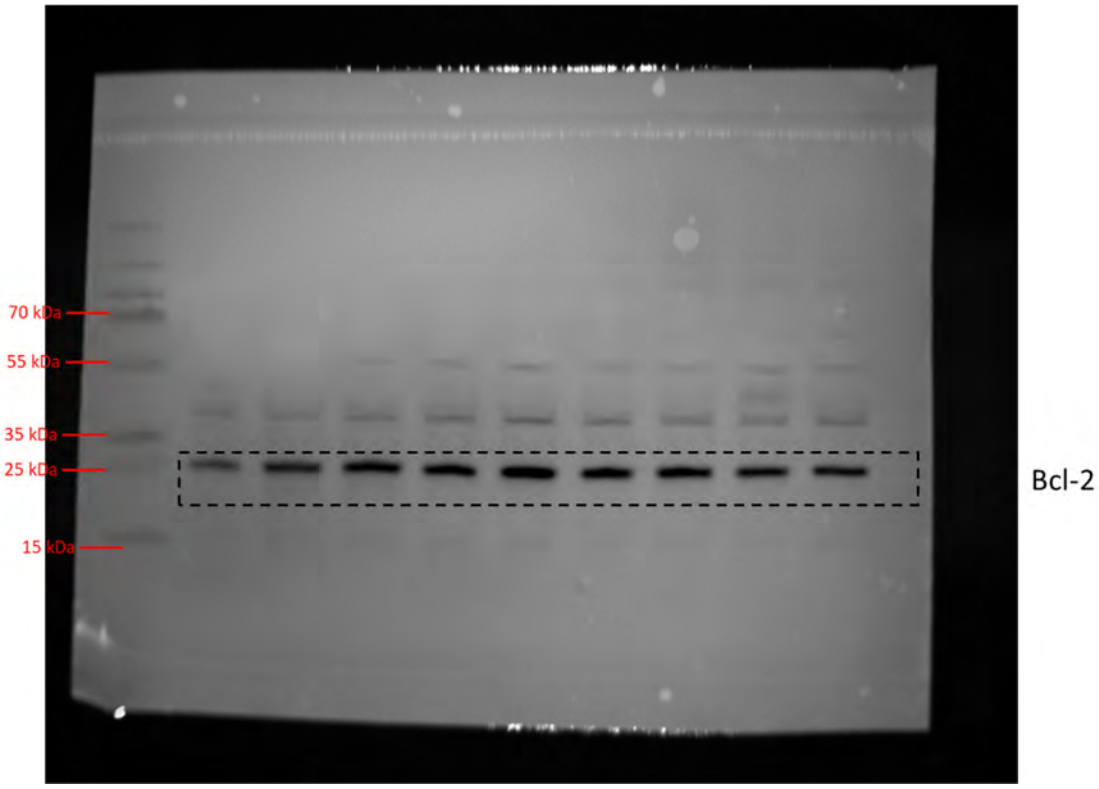






Uncropped western blots for Fig 3 b

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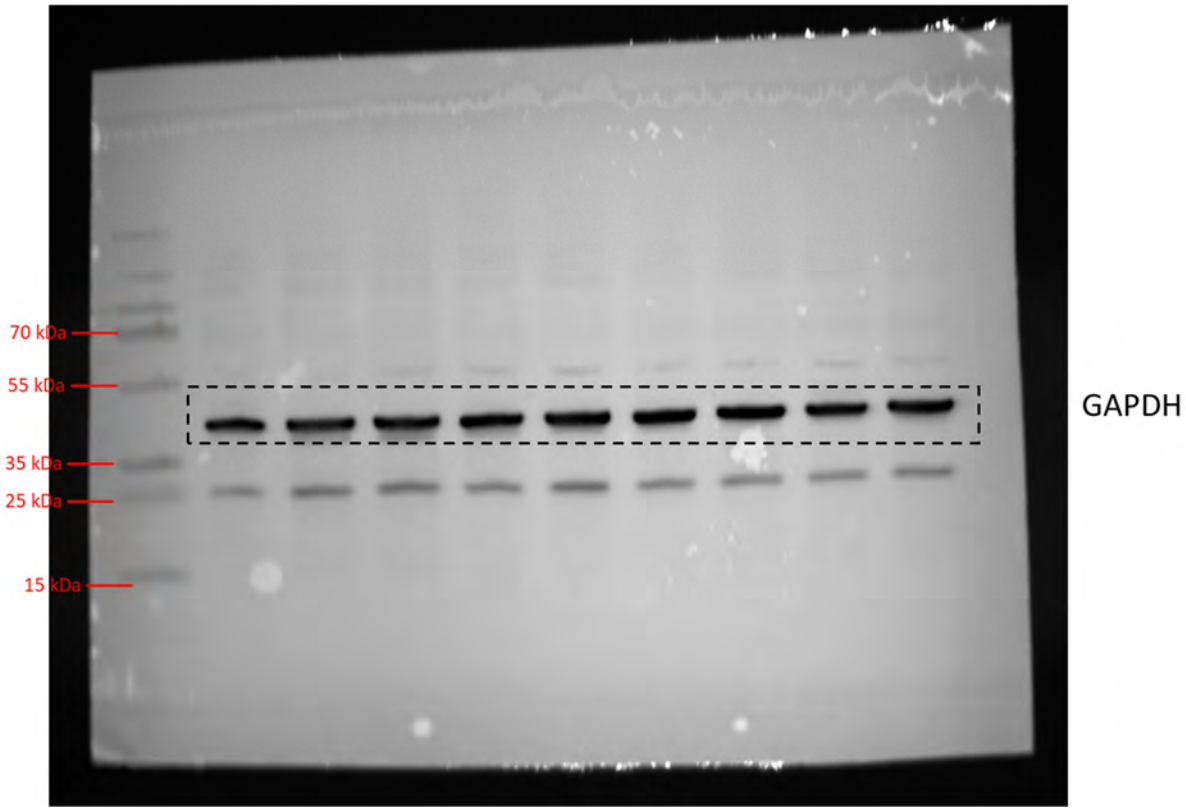
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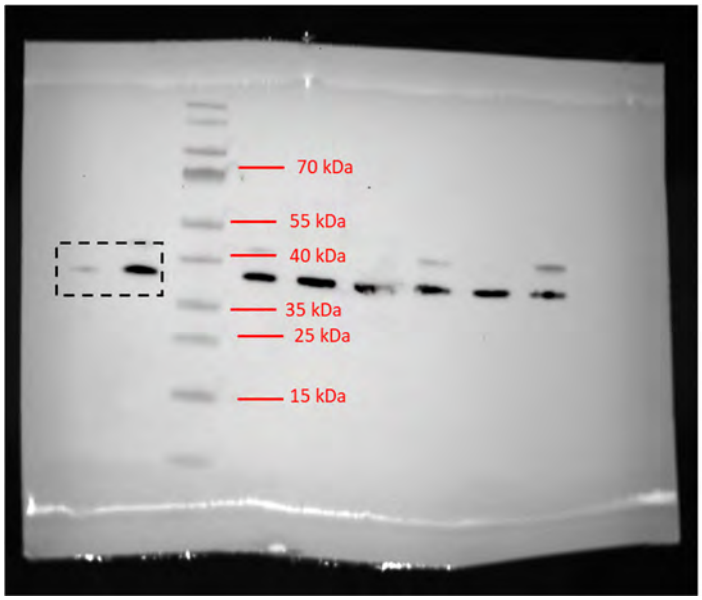
Uncropped western blots for Fig 4 a



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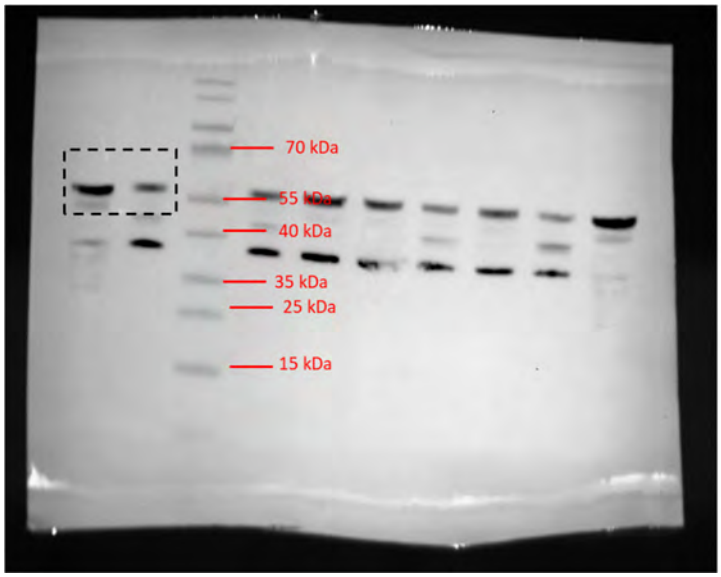

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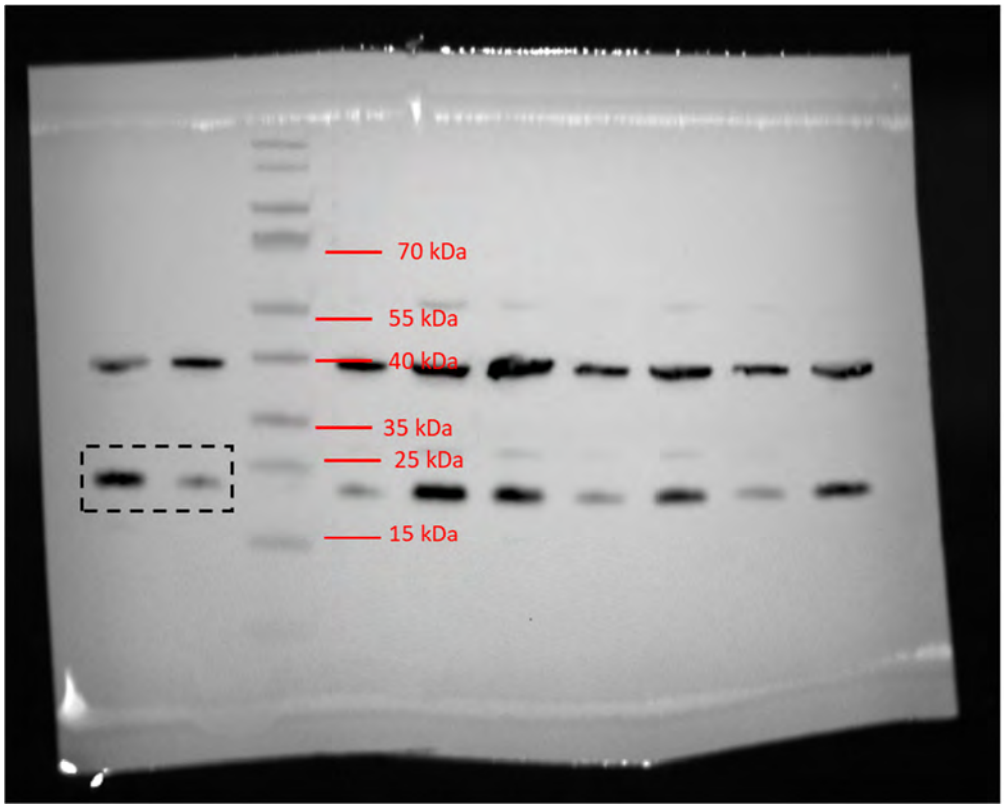
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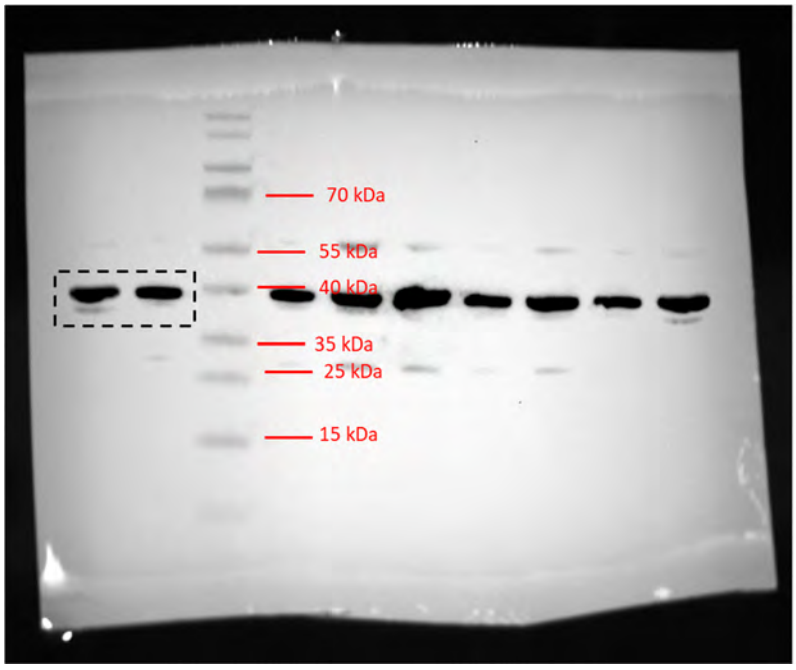
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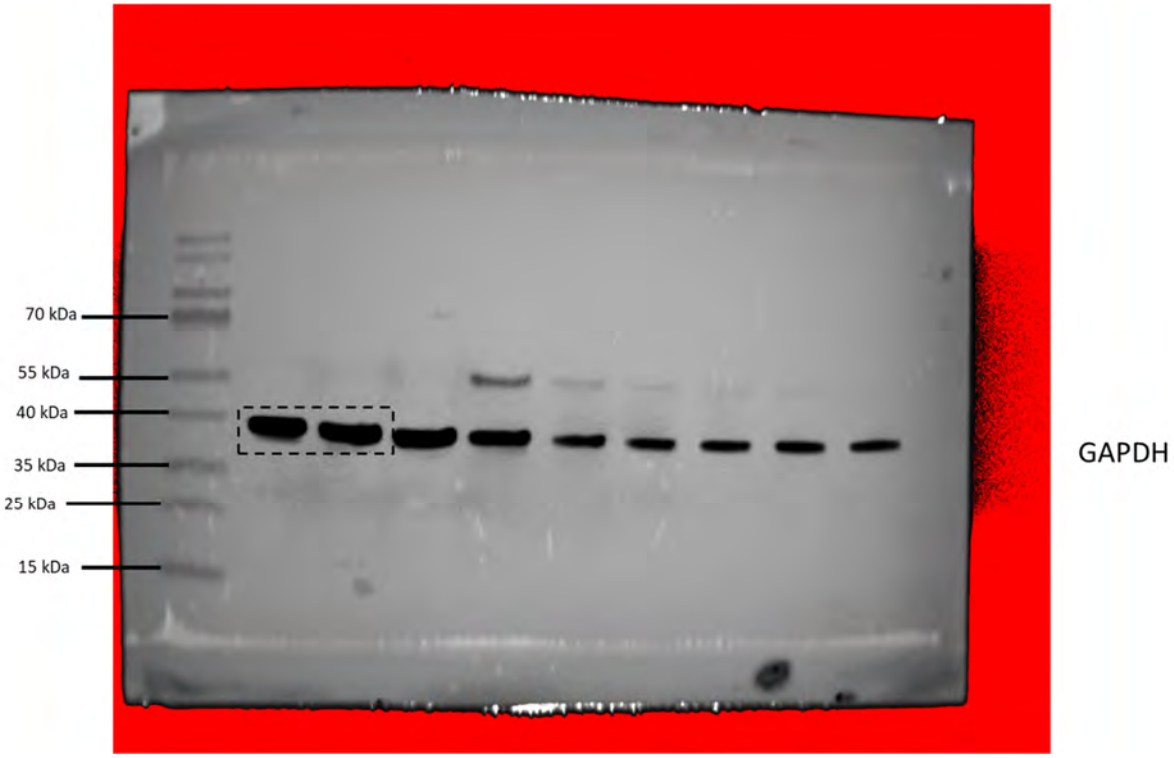
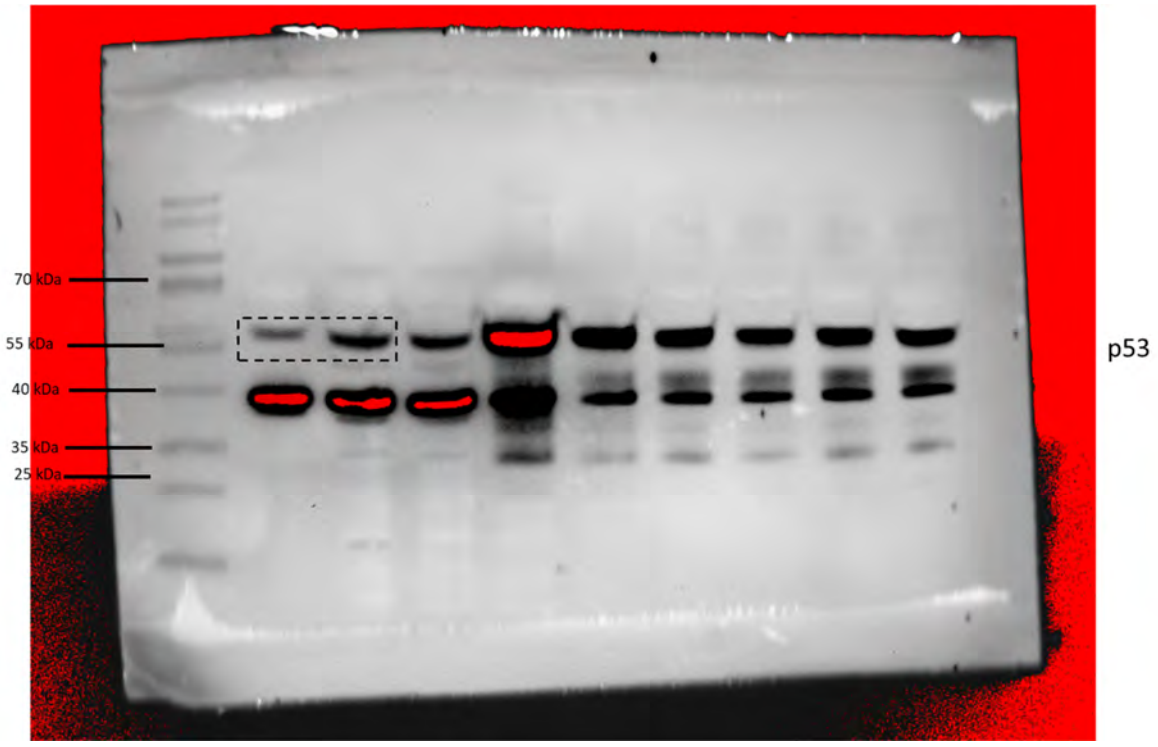
GAPDH

Uncropped western blots for Fig 5 a

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