

Description of Source Data

The source data underlying Figs 1b—f, 2a, 2d—g, 3a—f,4a—e,5a—f ,6a—d and Supplementary Tables and Supplementary Figs 3F-K and from antibody datasheet /FACS gating strategy are provided as a Source Data file.

Page number 2,11,32

Description: They are original pictures.

Related to Figure 1B/2G/6C

Page number 3-9/12-28

Description: They are original WB gel images.

Related to Figure 1C-1F/Fig.2D,2E/Fig 3/Fig.4/Fig.5

Page number 10,29-31,33-37

Description : They are original data.

Related to Figure 2F/Fig.6A,Fig.6B/Fig 6D/ Supplementary Figure 3D,3E/ Supplementary Figure 8

Page number 38-43

Description : They are original WB gel images and raw data.

Related Supplementary Figure3F-K

Page number44-53

Description : They are antibody reporting.

Page number 54-57

Description : They are FACS gating strategy

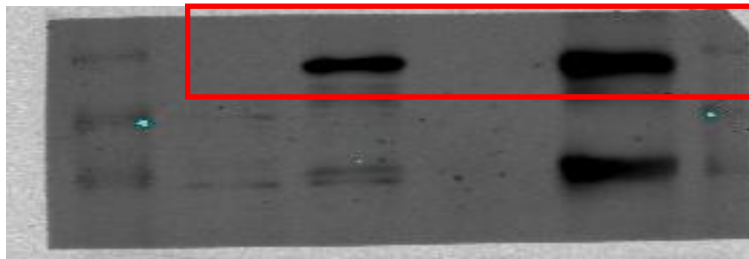
Related Supplementary Figure 6

Uncropped gel images for Figure 1B

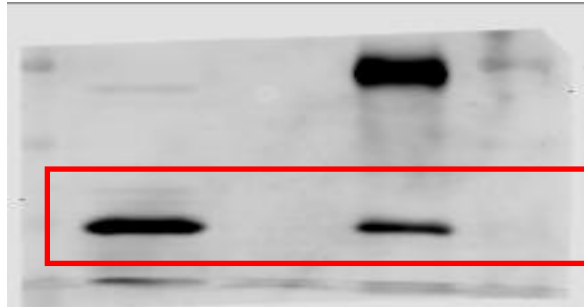


Uncropped gel images for Figure 1C

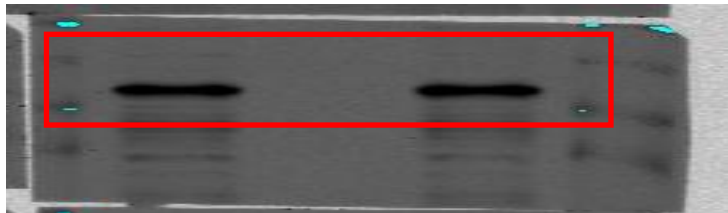
α -NIP30



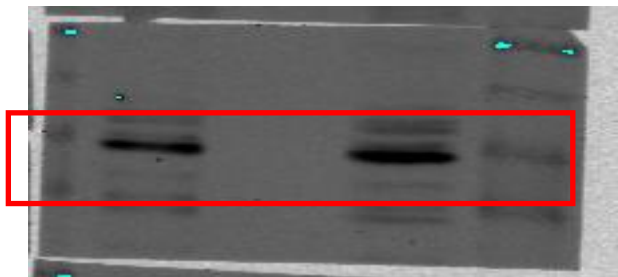
α -REG γ



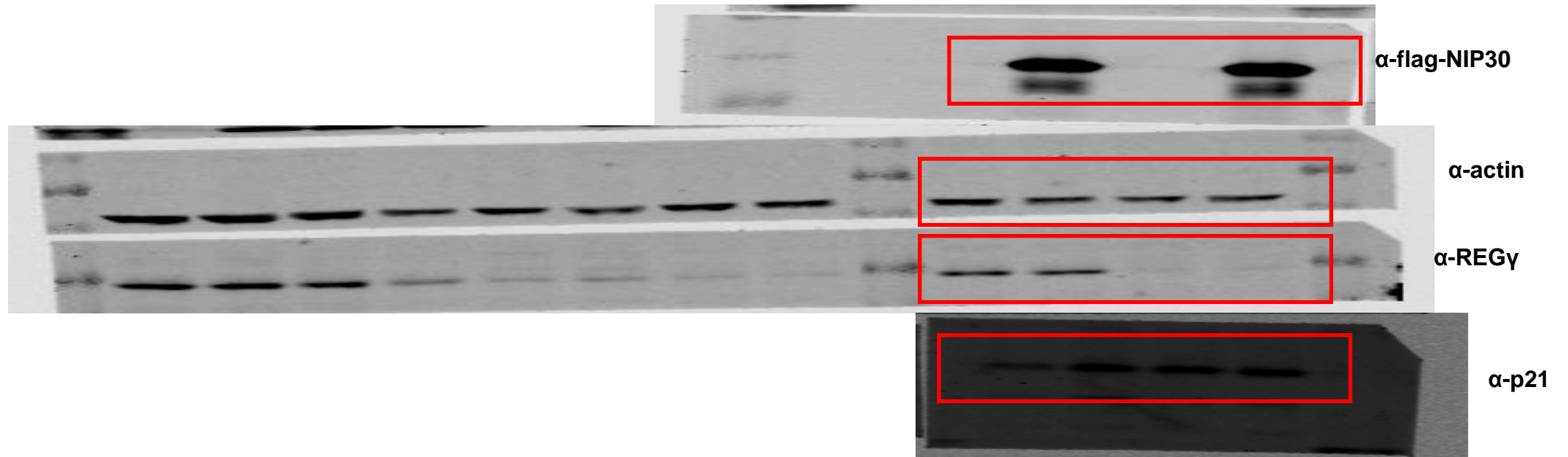
α -NIP30



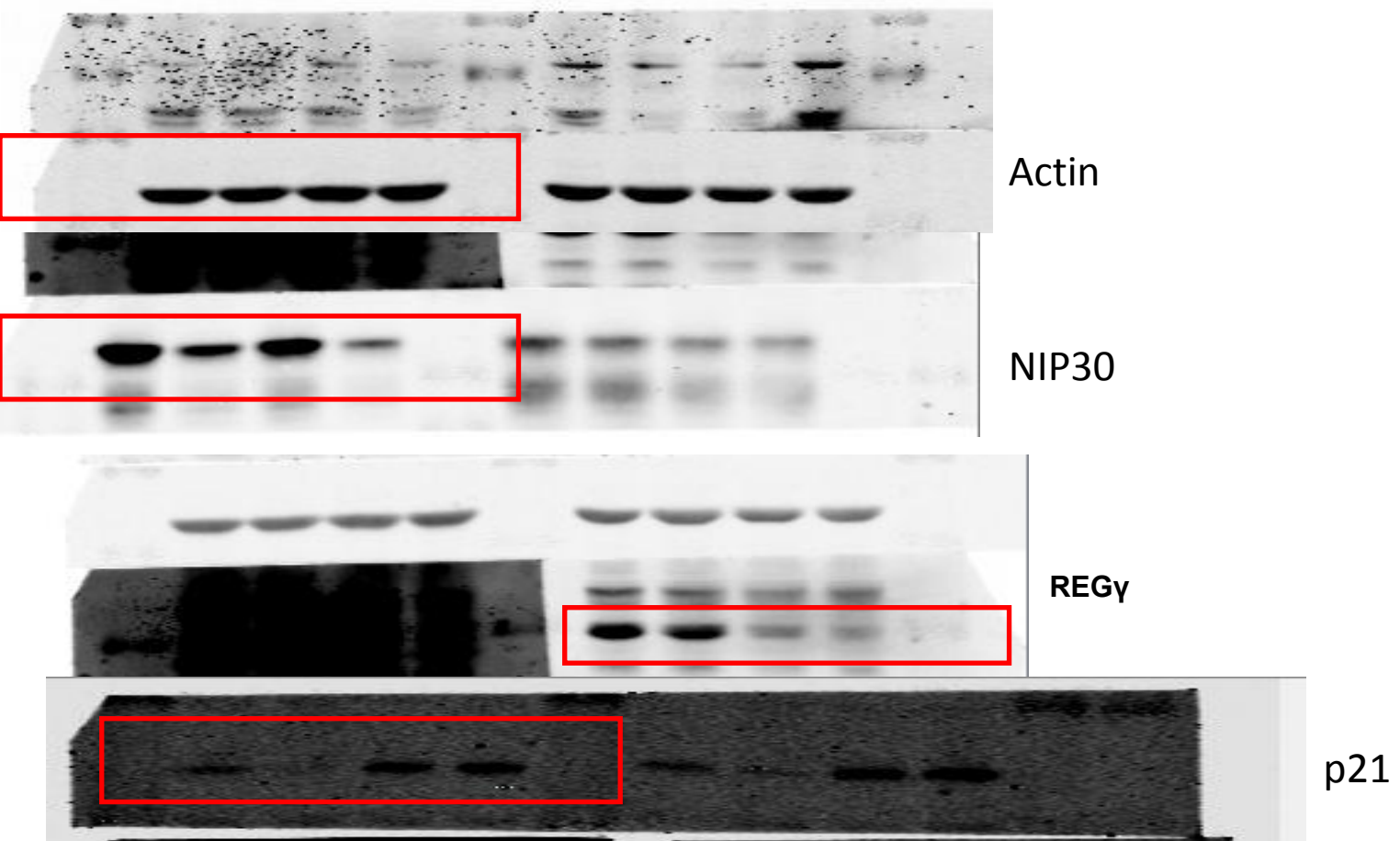
α -REG γ



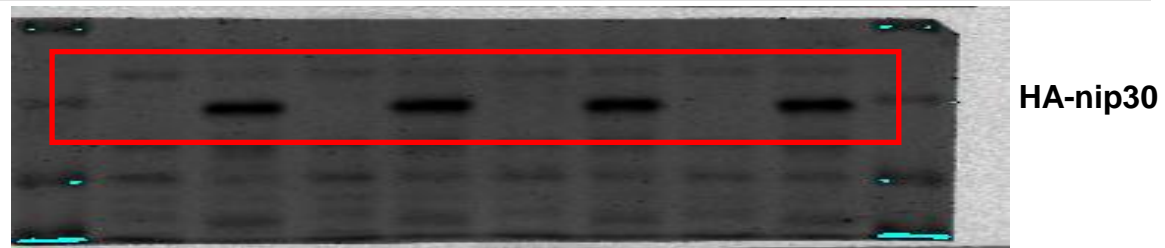
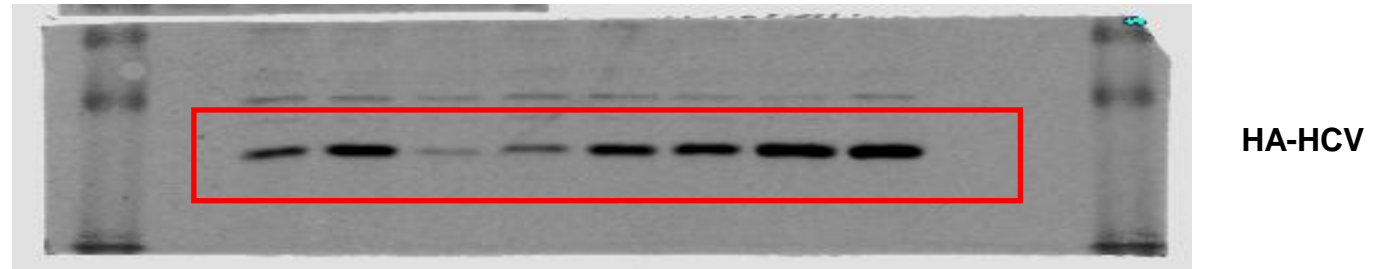
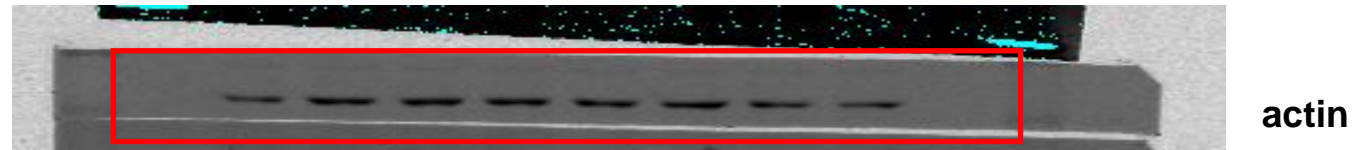
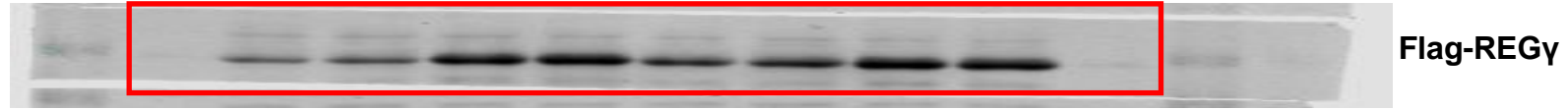
Uncropped gel images for Figure 1D



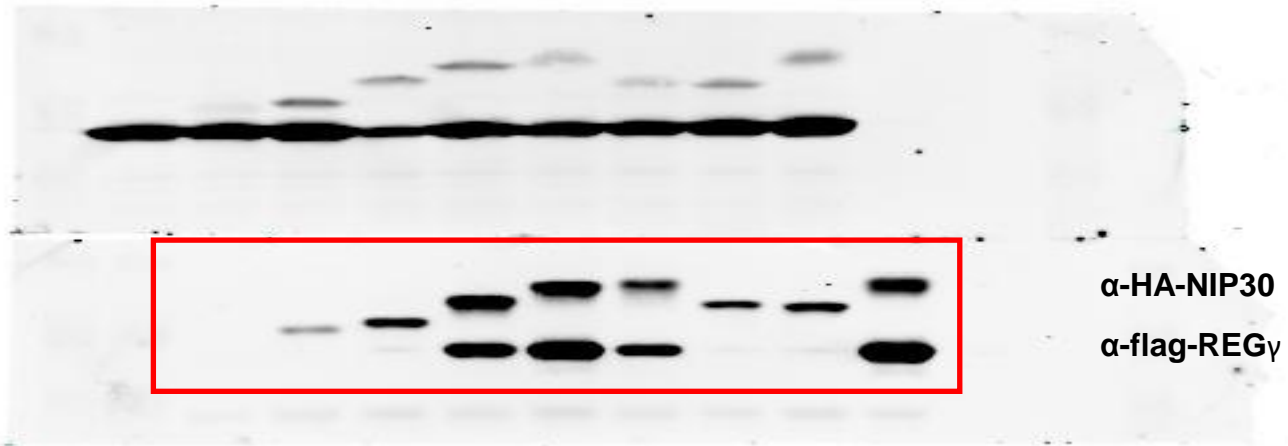
Uncropped gel images for Figure 1E



Uncropped gel images for Figure 1F



Uncropped gel images for Figure 2A



α -HA-NIP30

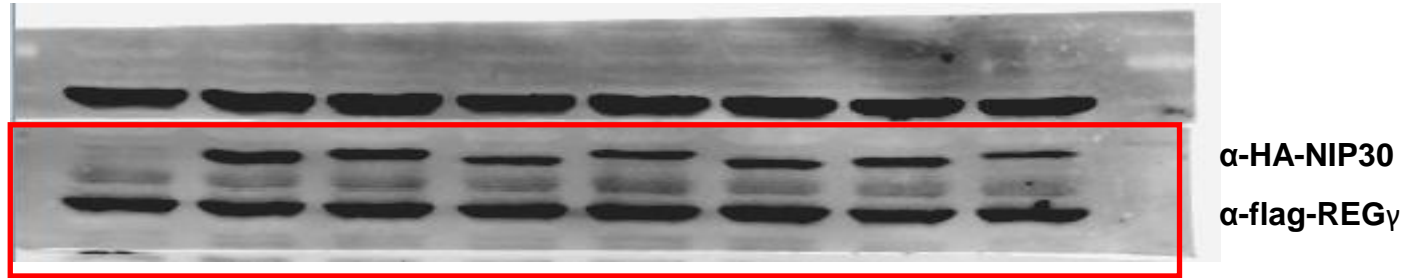
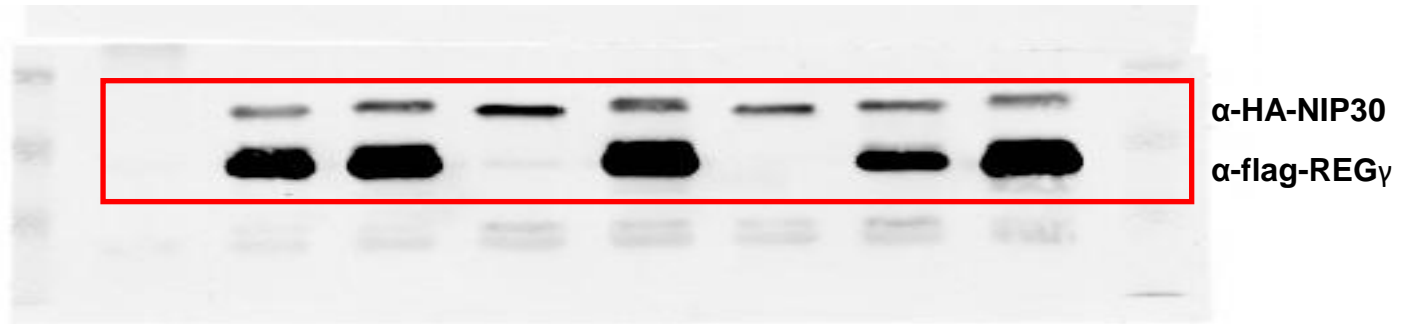
α -flag-REG γ



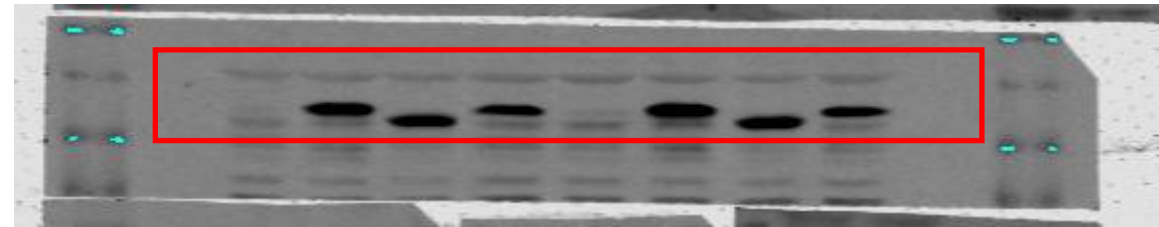
α -HA-NIP30

α -flag-REG γ

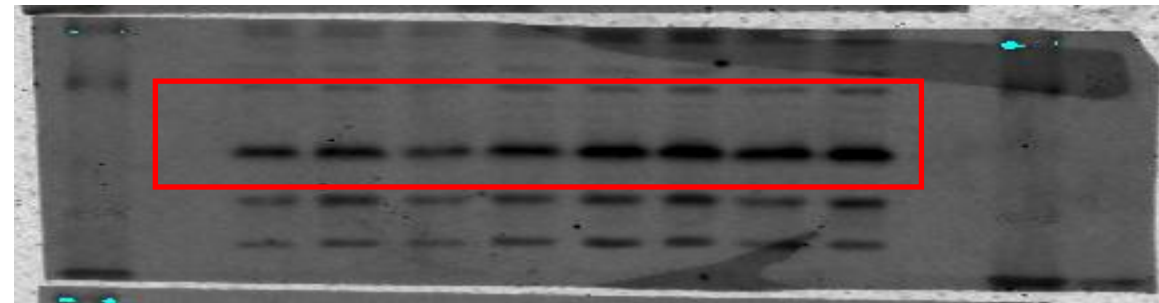
Uncropped gel images for Figure 2D



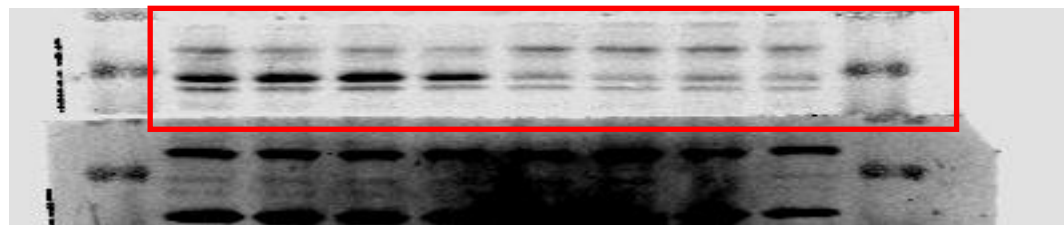
Uncropped gel images for Figure 2E



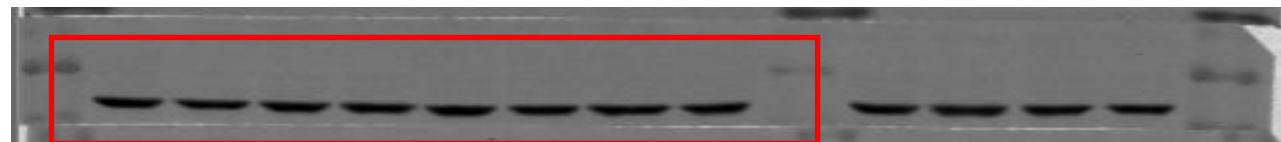
α -flag-NIP30



α -p21



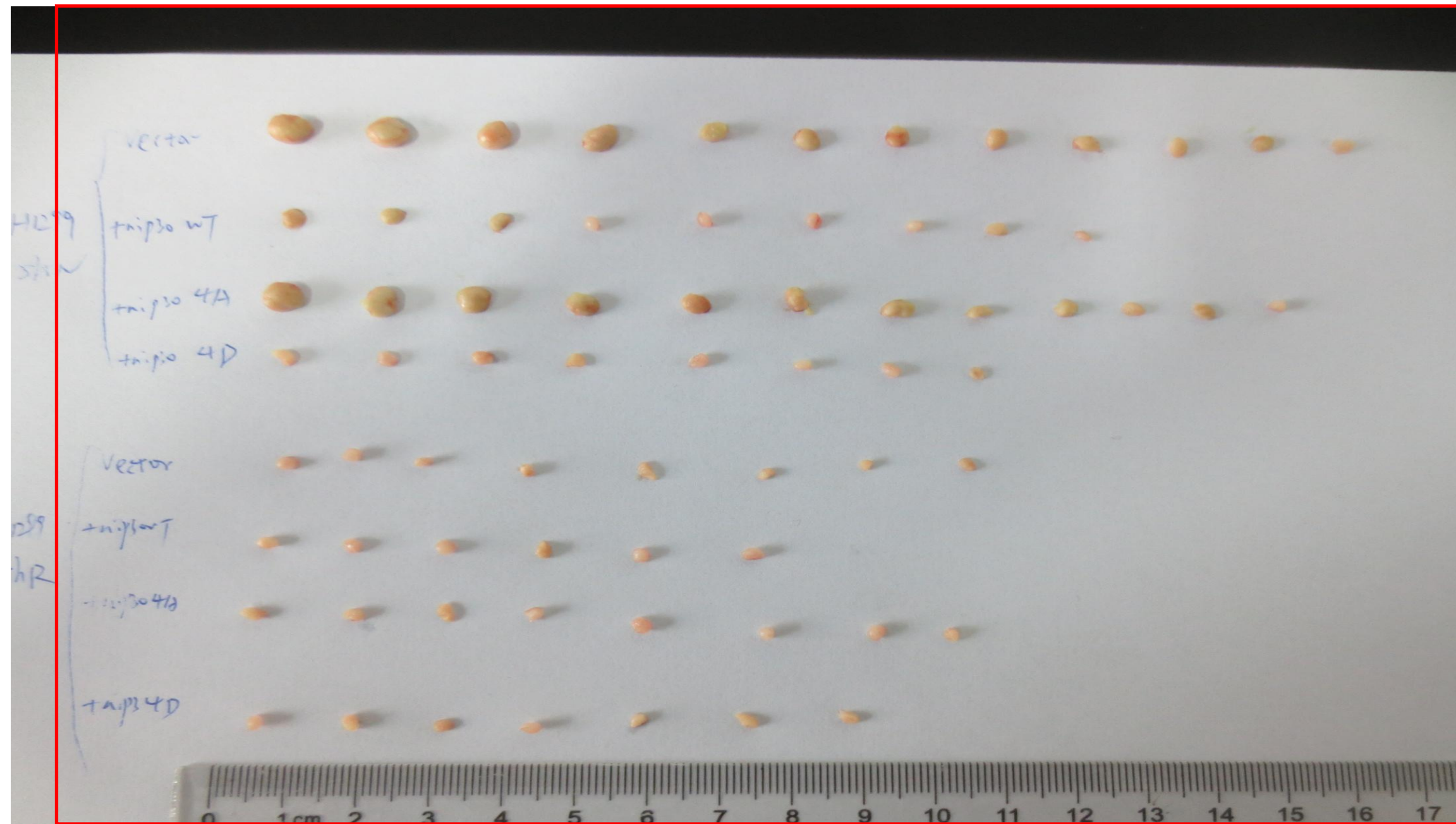
α -REG γ



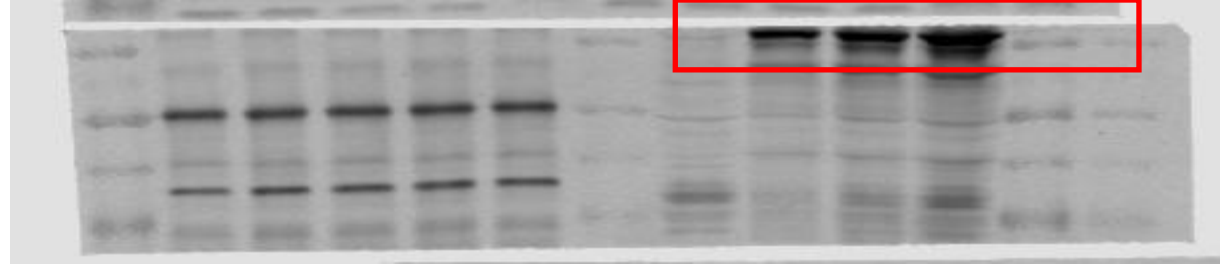
α -actin

Original data for Figure 2F

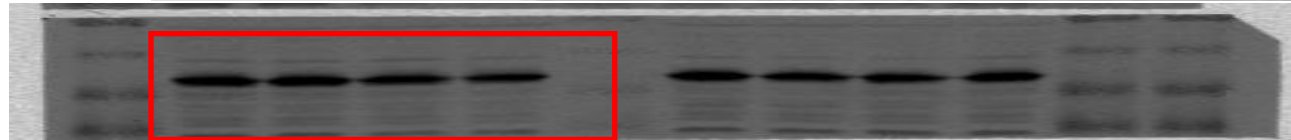
| A | | | B | | | C | |
|------------|----------|---|------------|----------|---|------------|----------|
| Data Set-A | | | Data Set-B | | | Data Set-C | |
| Mean | SEM | N | Mean | SEM | N | Mean | SEM |
| 0.229500 | 0.006062 | 4 | 0.314640 | 0.018488 | 4 | 0.277312 | 0.036836 |
| 0.747360 | 0.029193 | 4 | 0.492141 | 0.012594 | 4 | 0.700417 | 0.029275 |
| 1.761825 | 0.071138 | 4 | 0.777936 | 0.015196 | 4 | 1.461350 | 0.031965 |
| 2.655000 | 0.101550 | 4 | 1.608156 | 0.059478 | 4 | 2.385385 | 0.060161 |
| D | | | E | | | F | |
| Data Set-D | | | Data Set-E | | | Data Set-F | |
| Mean | SEM | N | Mean | SEM | N | Mean | SEM |
| 0.226075 | 0.024897 | 4 | 0.203550 | 0.005353 | 4 | 0.180490 | 0.011751 |
| 0.565477 | 0.060934 | 4 | 0.403000 | 0.027295 | 4 | 0.452250 | 0.011222 |
| 0.831263 | 0.036341 | 4 | 0.704475 | 0.025292 | 4 | 0.730937 | 0.013105 |
| 1.746000 | 0.044202 | 4 | 1.423675 | 0.096436 | 4 | 1.492083 | 0.042914 |
| G | | | H | | | I | |
| Data Set-G | | | Data Set-H | | | Title | |
| Mean | SEM | N | Mean | SEM | N | Mean | SEM |
| 0.196987 | 0.019383 | 4 | 0.170765 | 0.017857 | 4 | | |
| 0.397988 | 0.014991 | 4 | 0.419941 | 0.021998 | 4 | | |
| 0.986200 | 0.052191 | 4 | 0.707296 | 0.019007 | 4 | | |
| 1.573125 | 0.040706 | 4 | 1.466290 | 0.046259 | 4 | | |



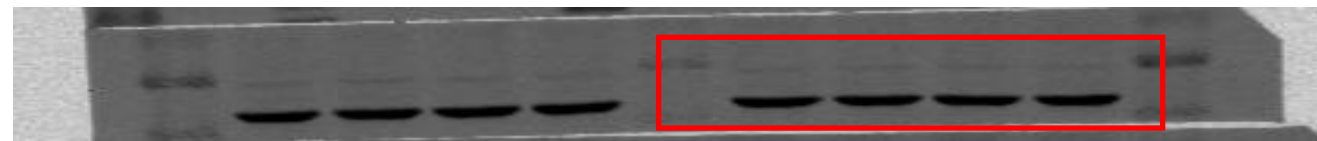
Uncropped gel images for
Figure3A



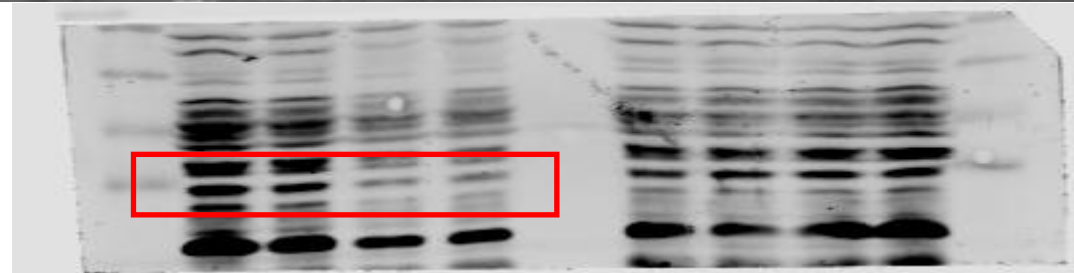
Flag-CDC25A



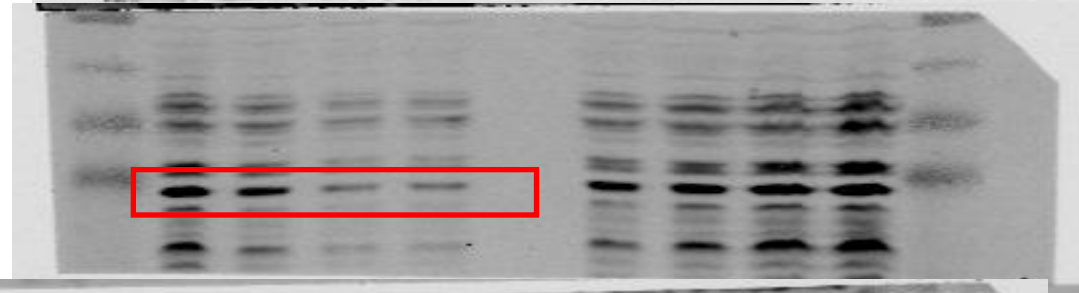
α -NIP30



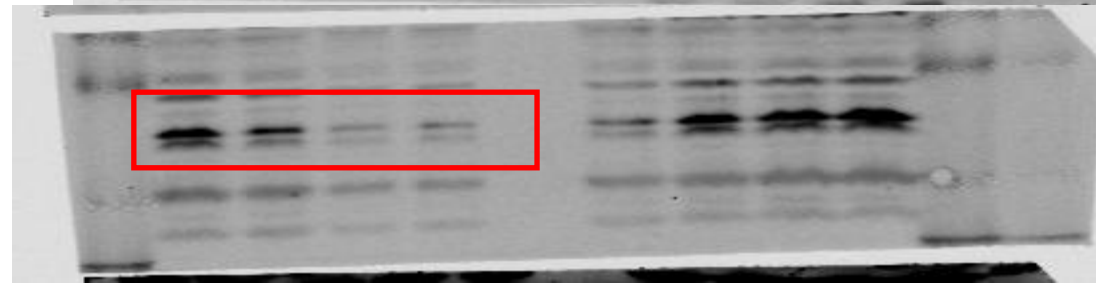
α -actin



α -p-NIP30S228

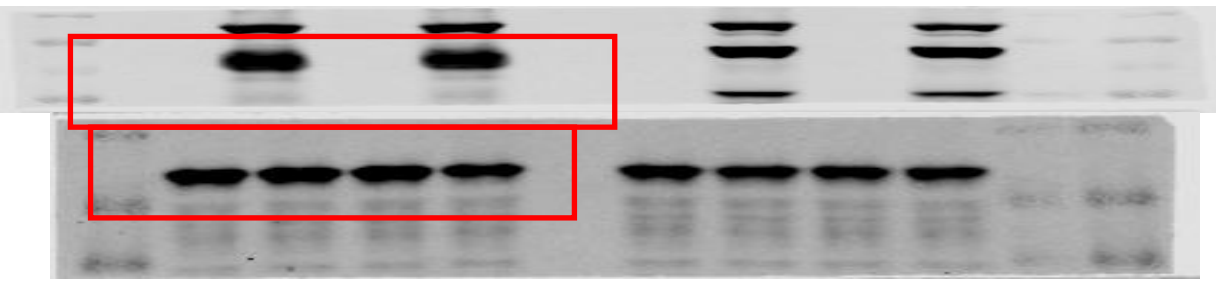


α -p-NIP30S230



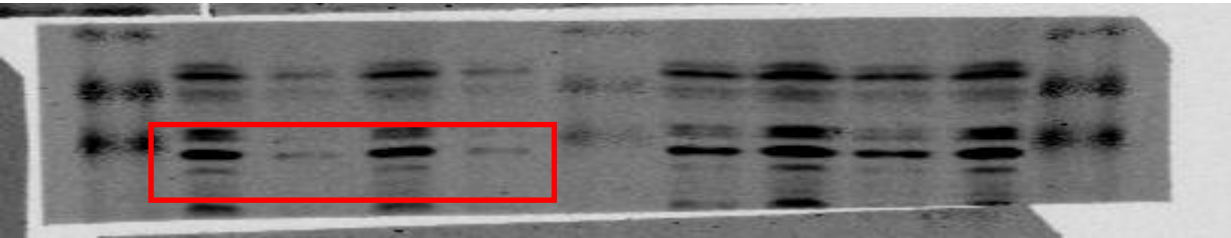
α -p21

Uncropped gel images for Figure3B

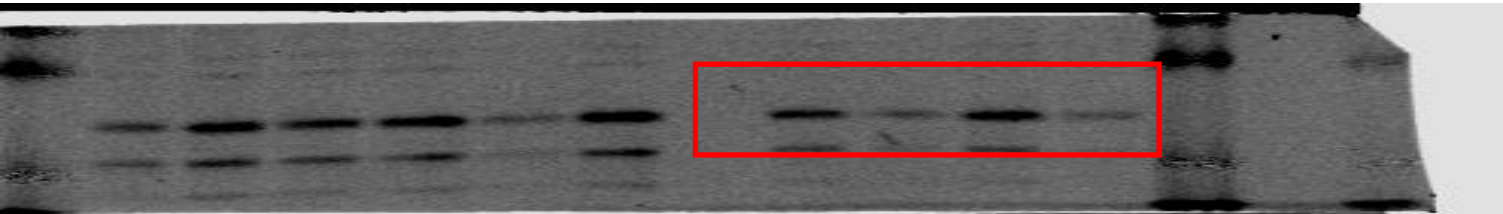


Flag-CDC25A

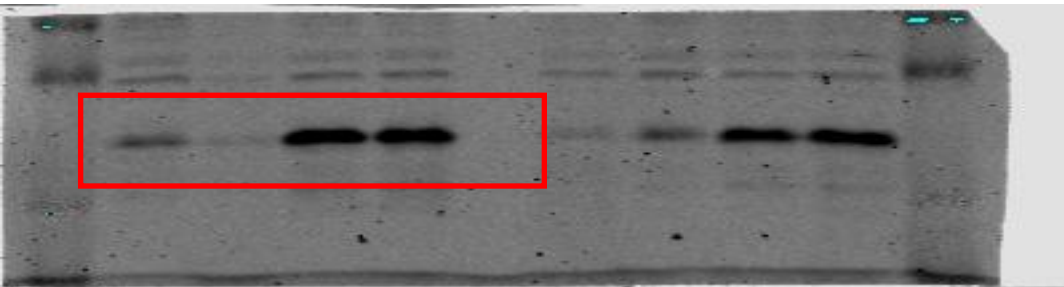
α -NIP30



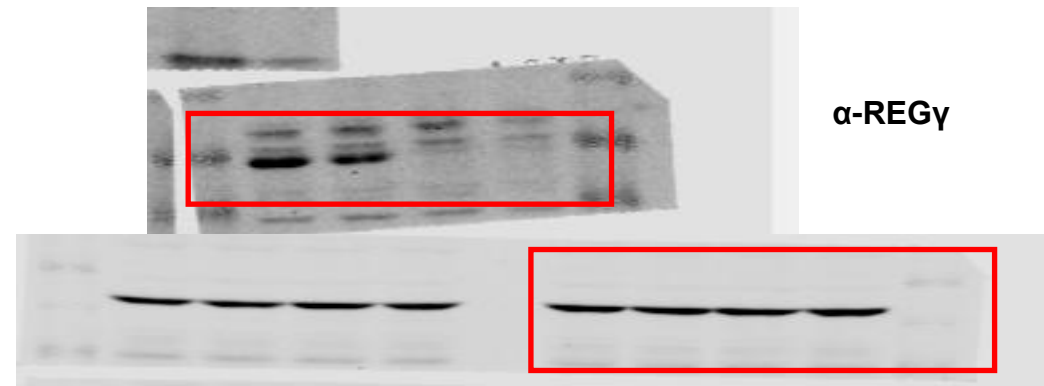
α -p-NIP30S228



α -p-NIP30S230



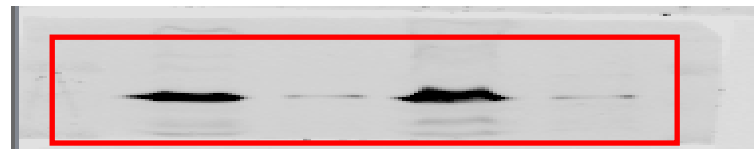
α -p21



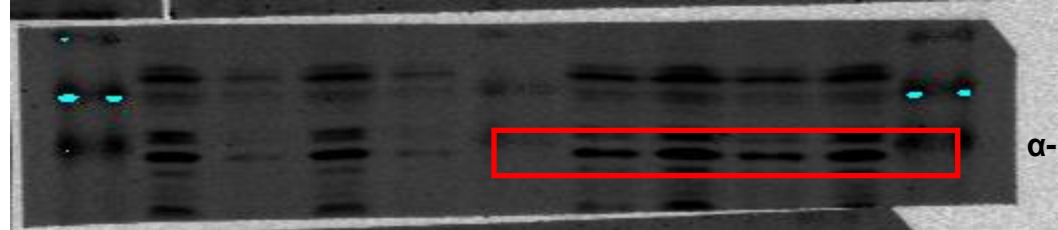
α -REG γ

α -actin

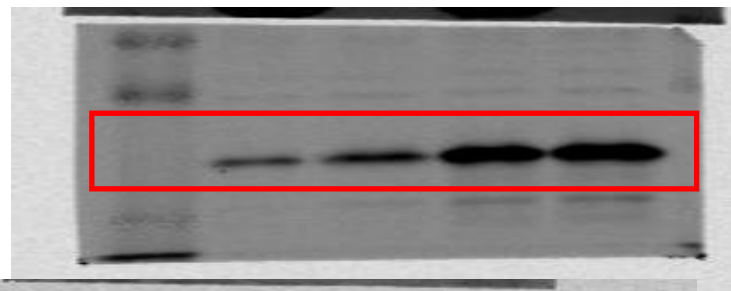
Uncropped gel images for Figure3C



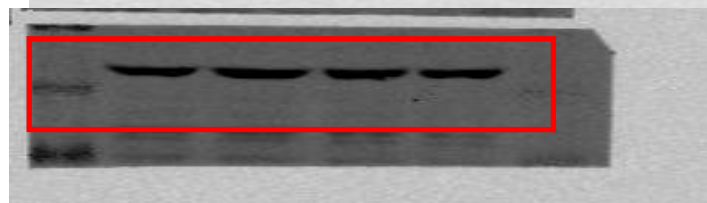
α -CDC25A



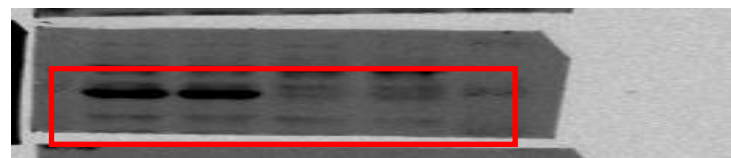
α -p-NIP30S230



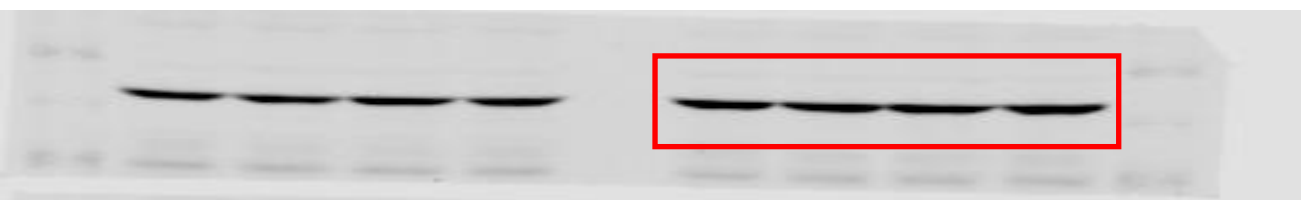
α -p21



α -NIP30



α -REGy

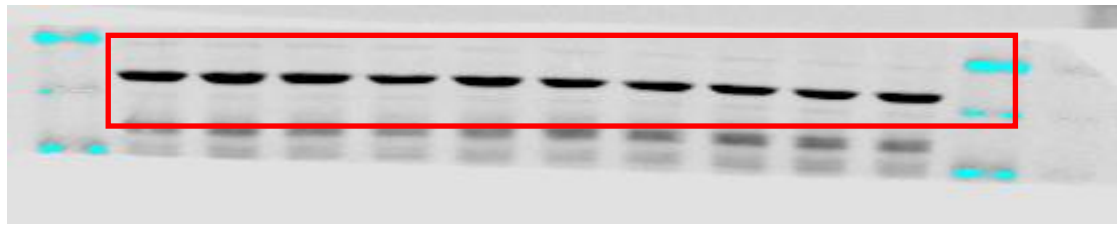


α -actin

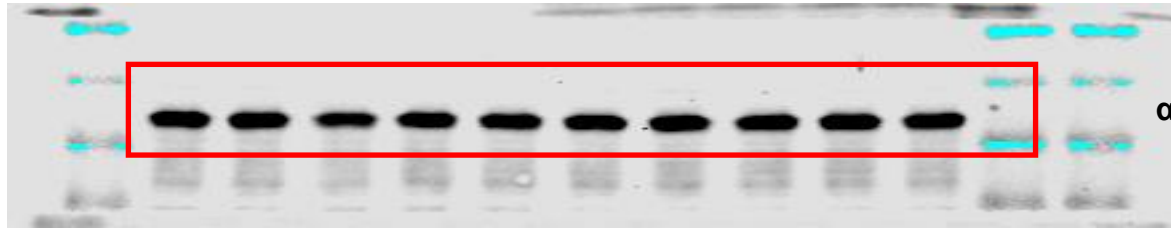


α -p-NIP30S228

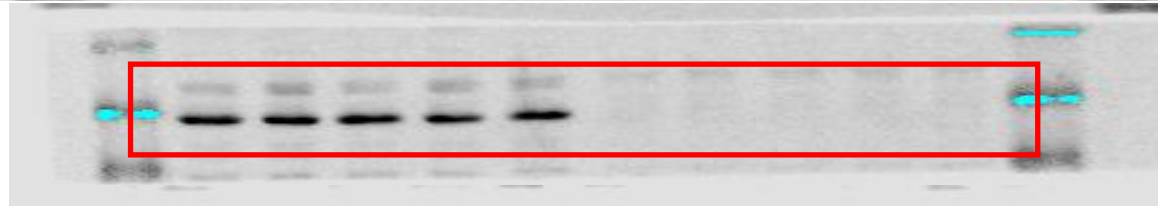
Uncropped gel images for Figure3D



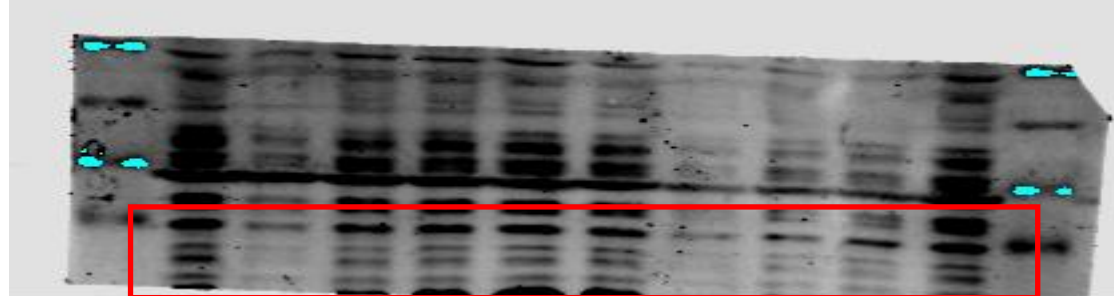
α -actin



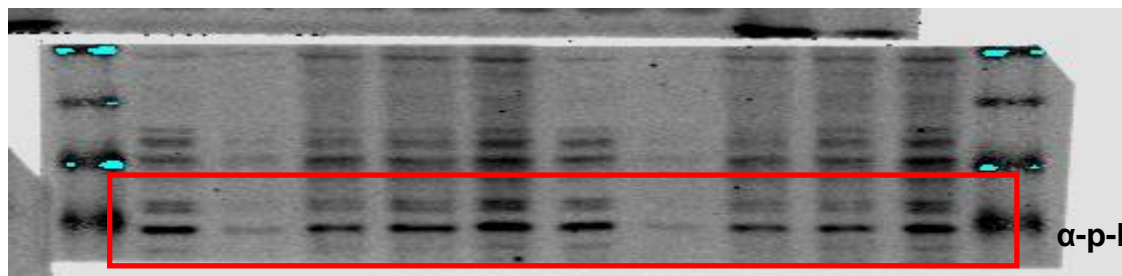
α -NIP30



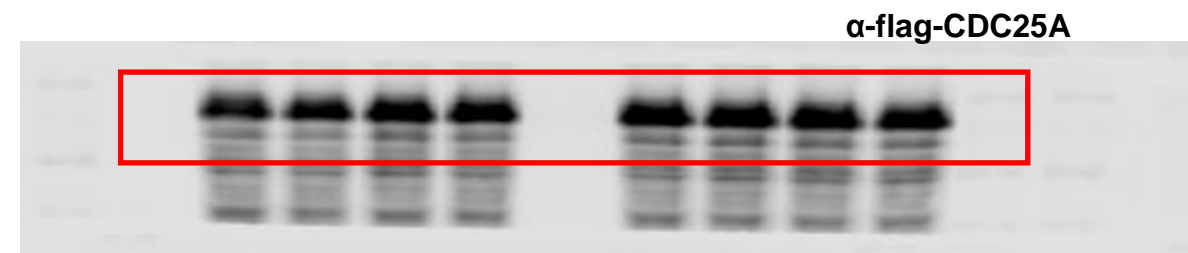
α -REG γ



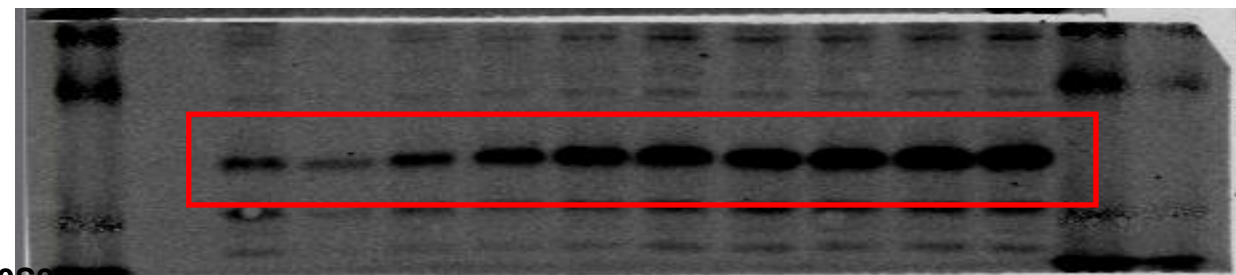
α -p-NIP30S228



α -p-NIP30S250

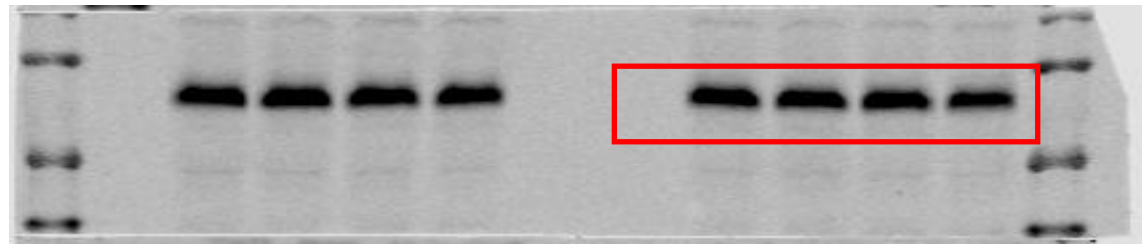


α -flag-CDC25A

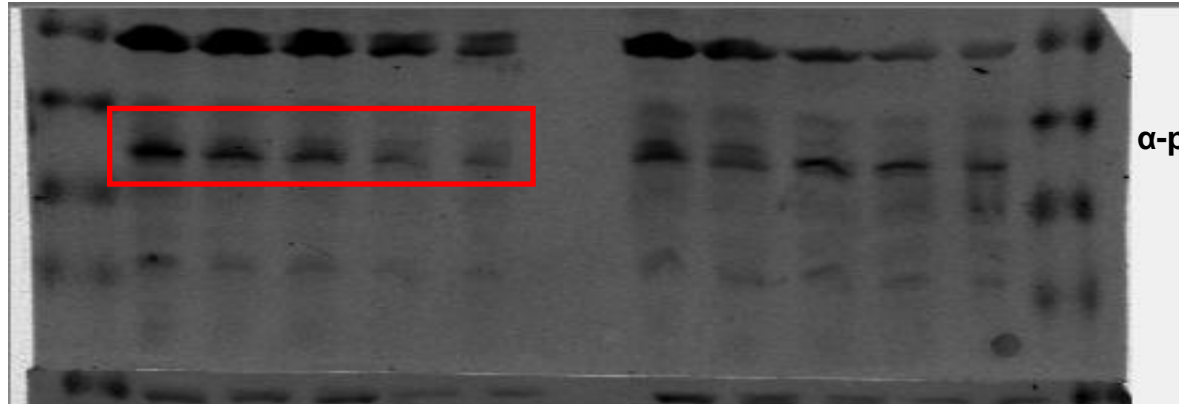


α -p21

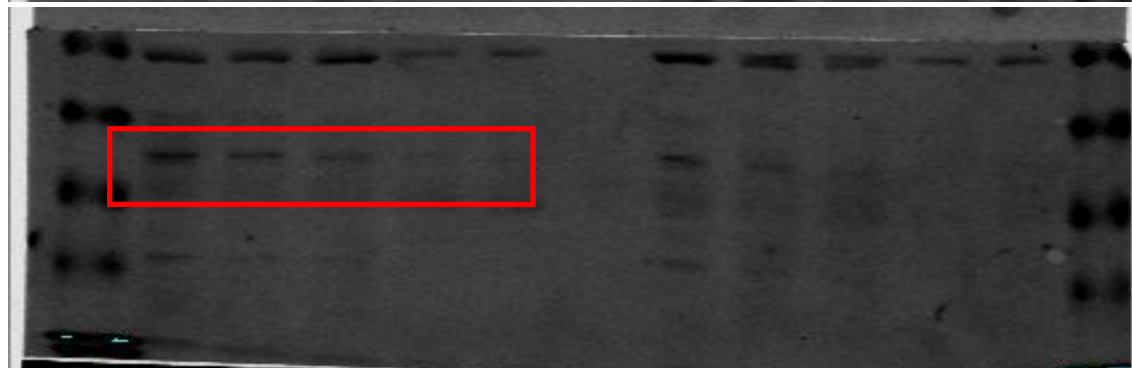
Uncropped gel images for Figure 3E



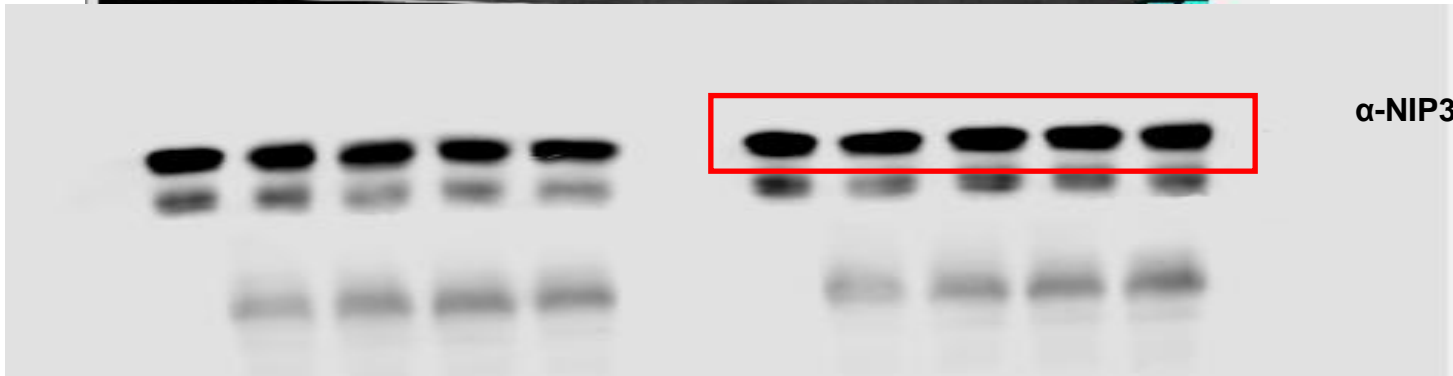
α -CDC25A



α -p-NIP30S230

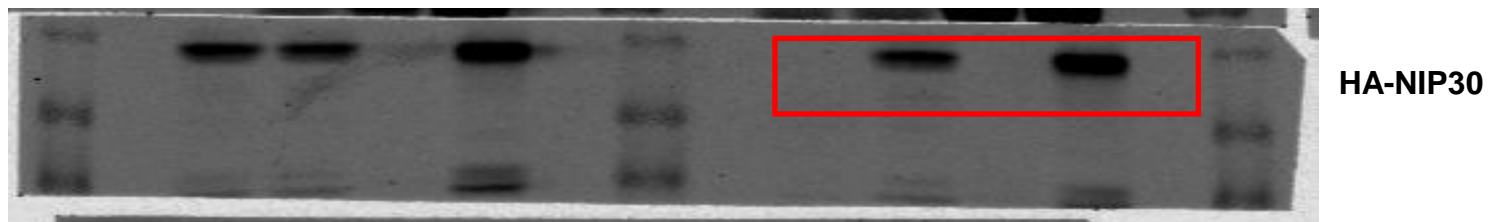
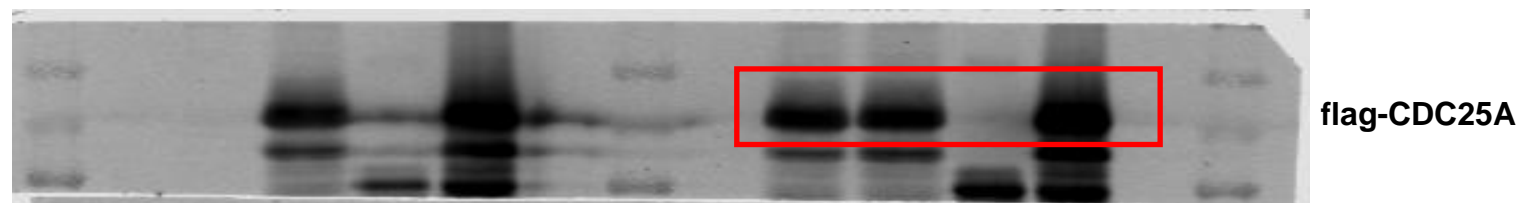
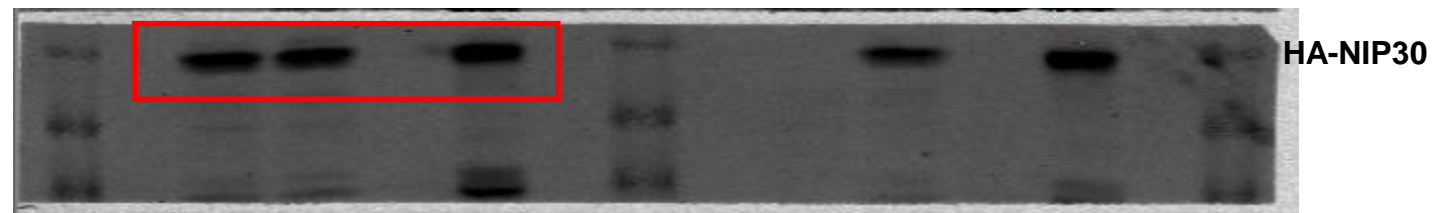
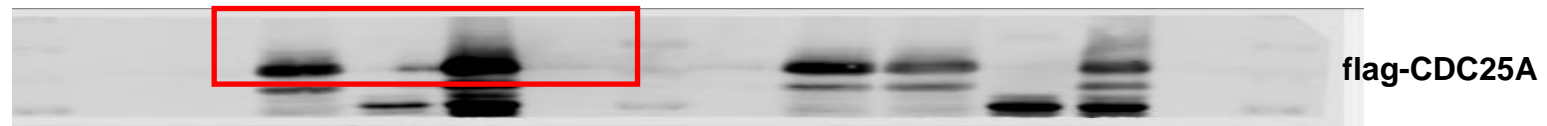


α -p-NIP30S228

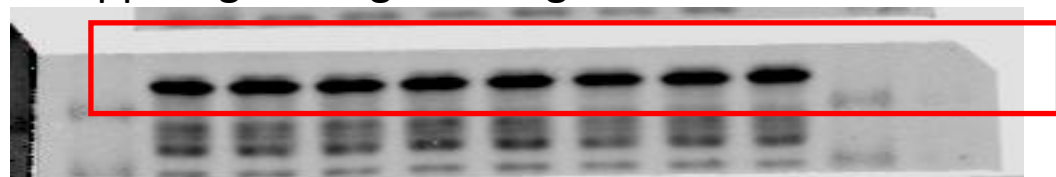


α -NIP30

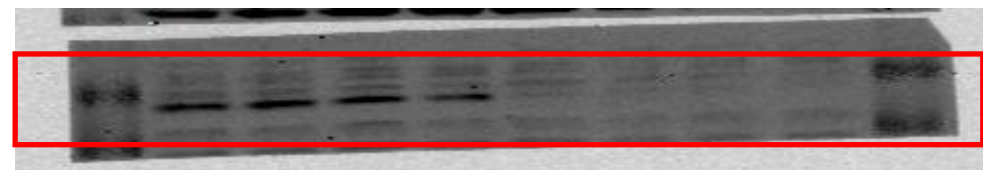
Uncropped gel images for Figure3F



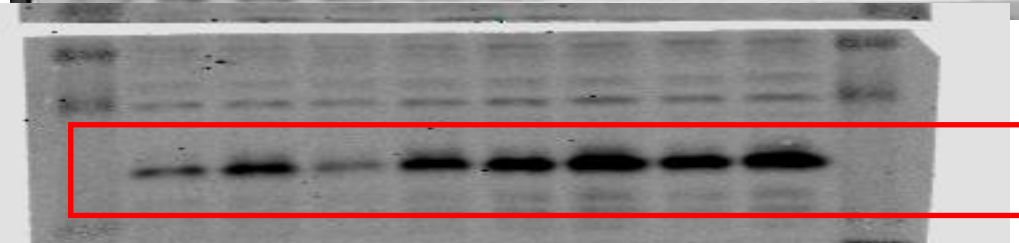
Uncropped gel images for Figure 4A



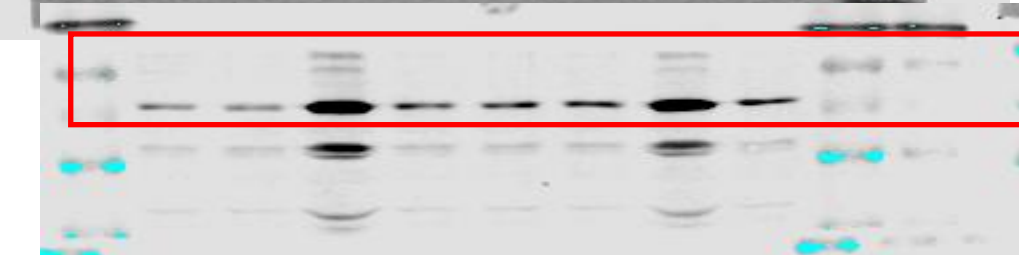
α -actin



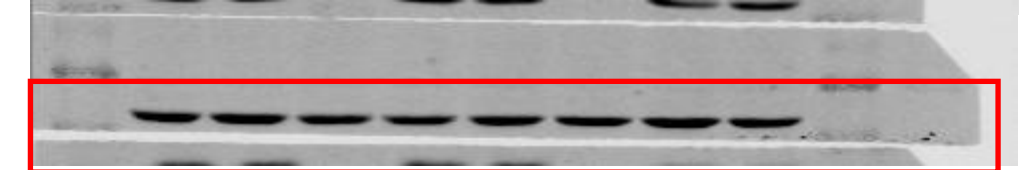
α -REGy



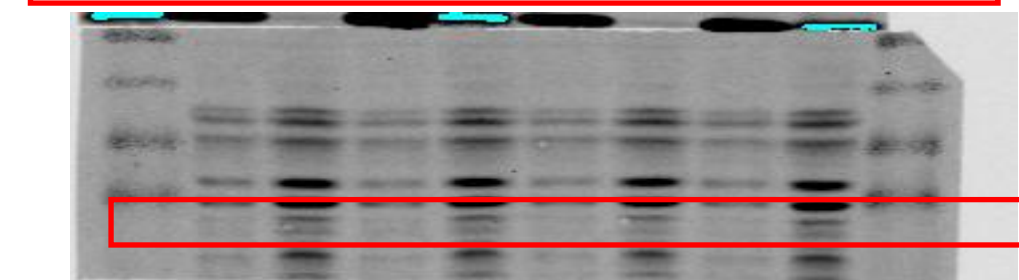
α -p21



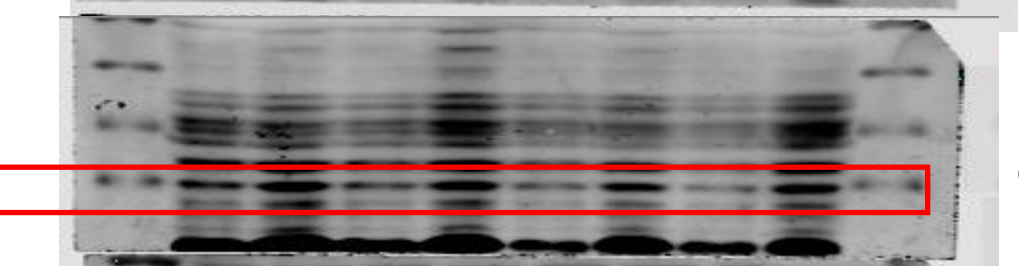
α -CDC25A



α -actin

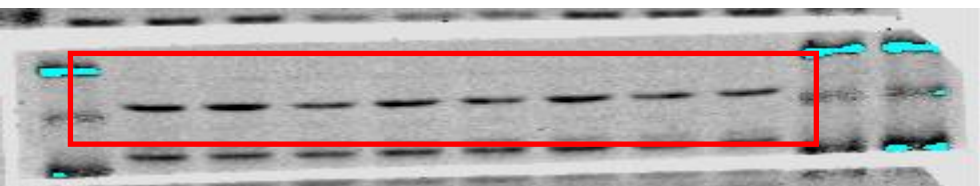


α -p-NIP30S228

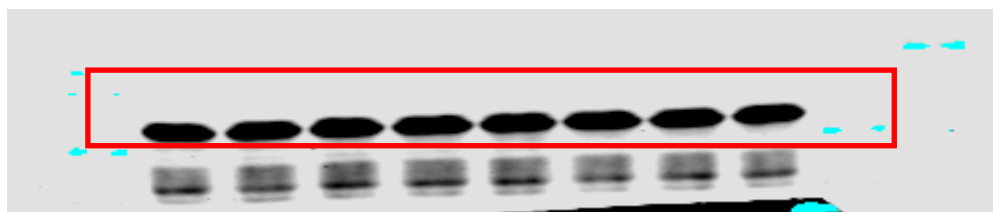


α -p-NIP30S230

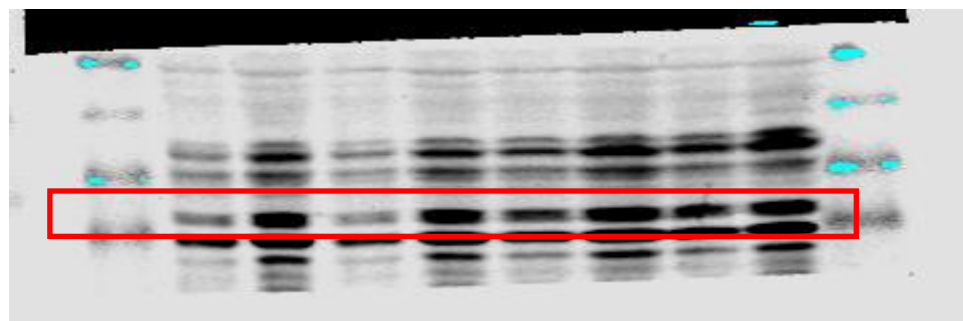
Uncropped gel images for Figure 4B



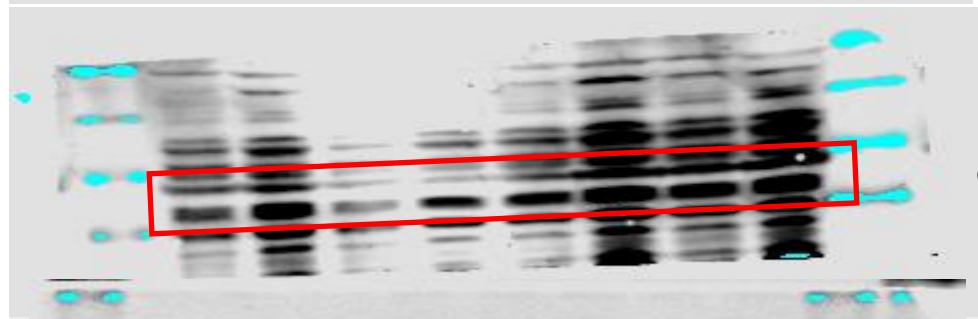
α -actin



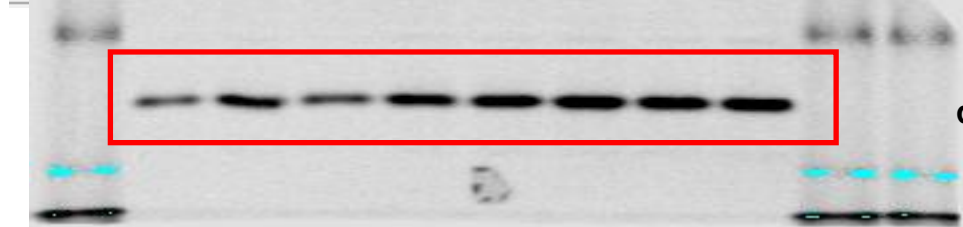
α -NIP30



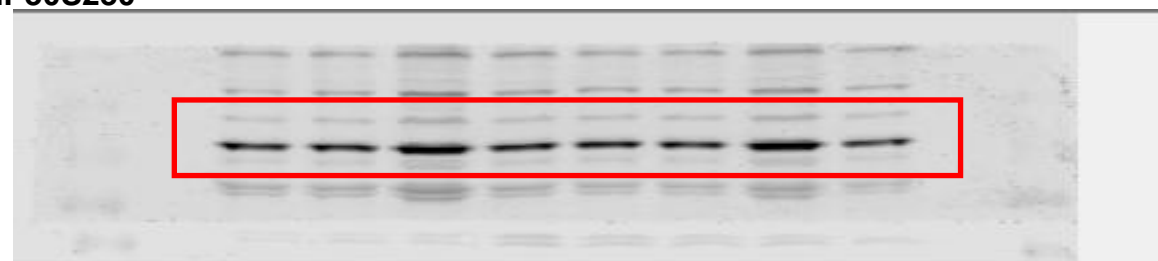
α -p-NIP30S230



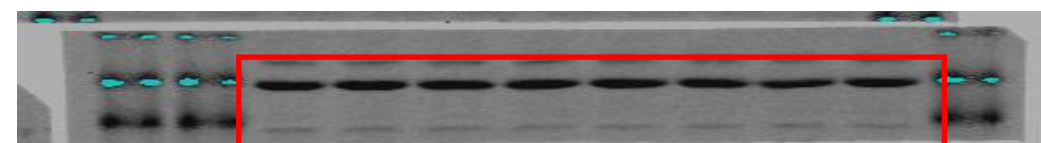
α -p-NIP30S228



α -p21

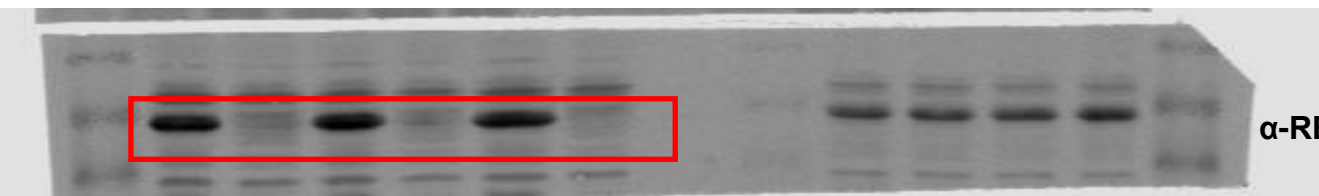


α -CDC25A

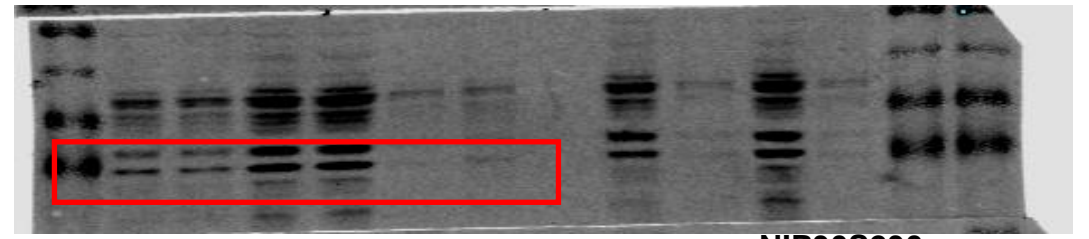


α -actin

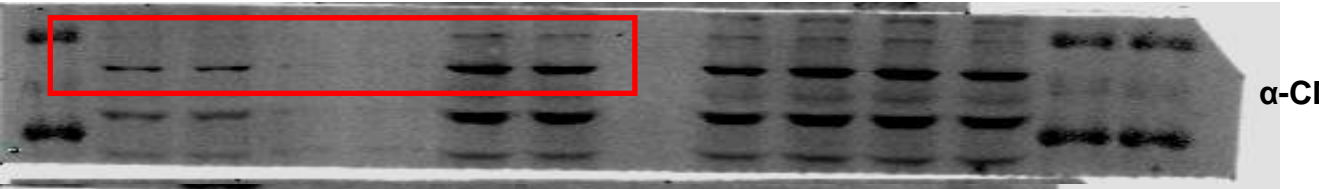
Uncropped gel images for Figure 4C



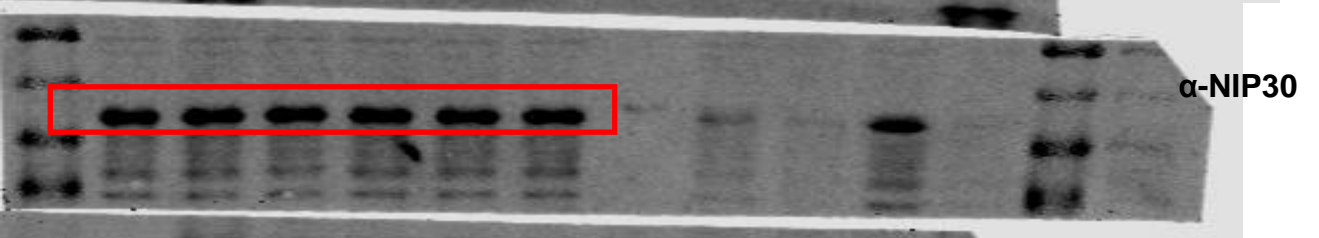
α -REG γ



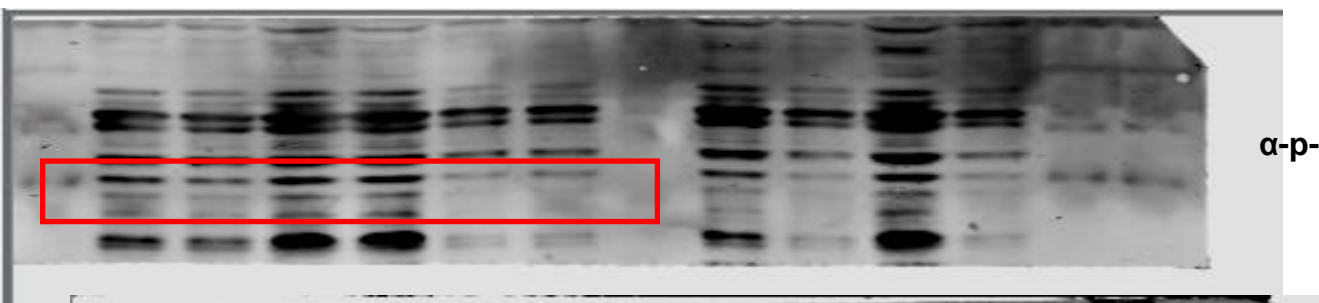
α -p-NIP30S230



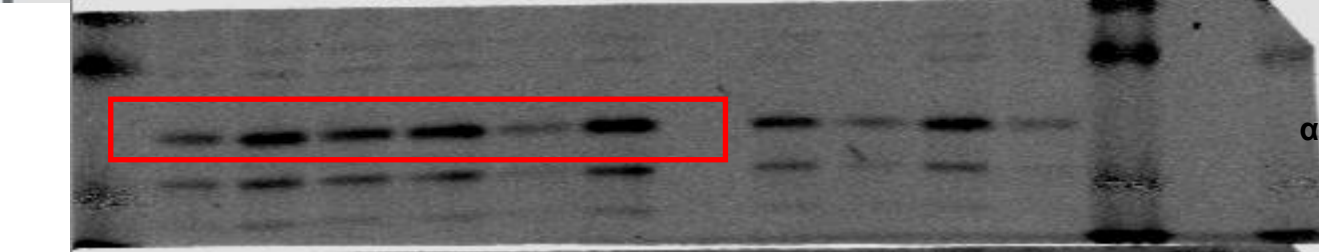
α -CDC25A



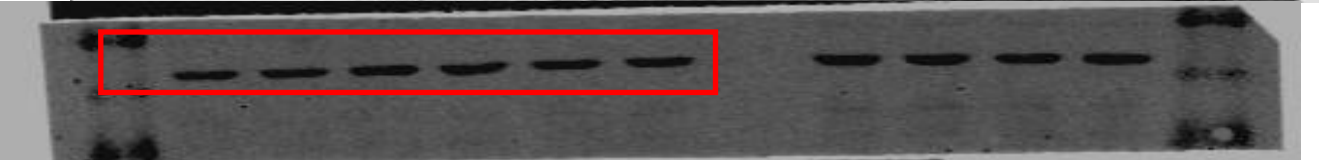
α -NIP30



α -p-NIP30S228

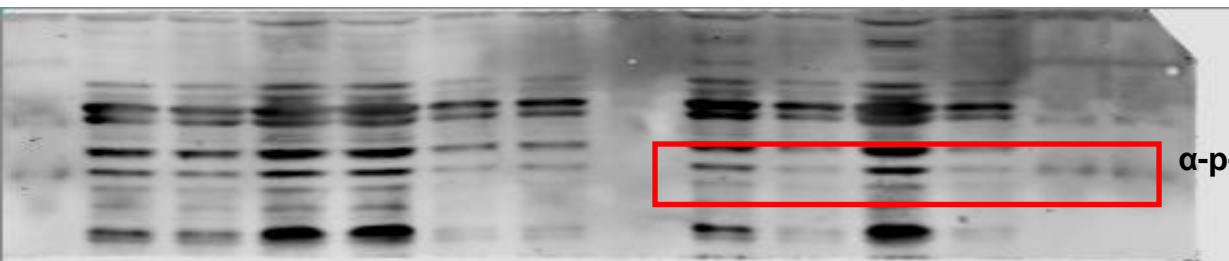


α -p21

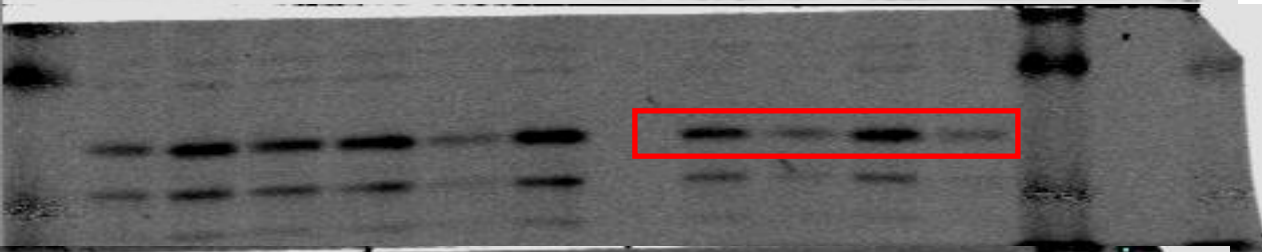


α -actin

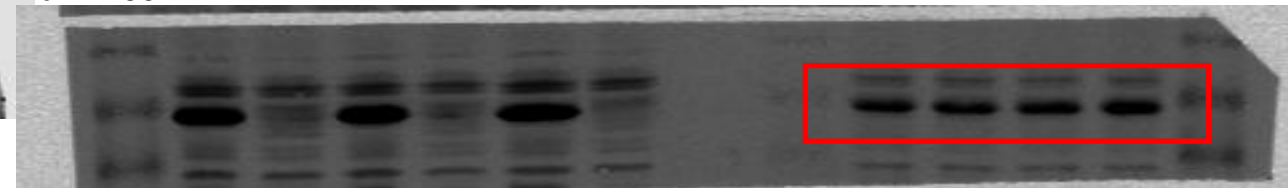
Uncropped gel images for Figure 4D



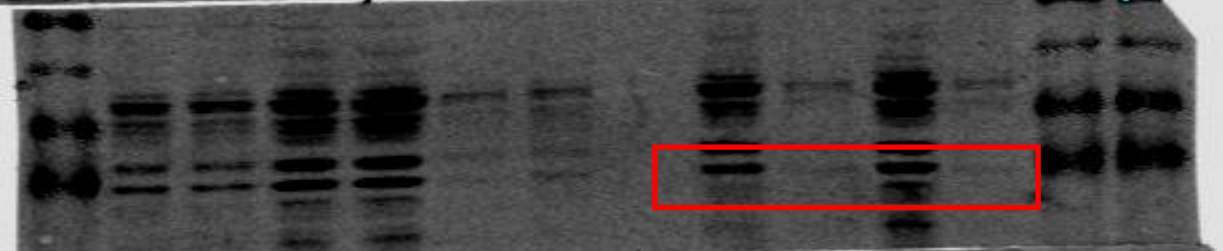
α -p-NIP30S228



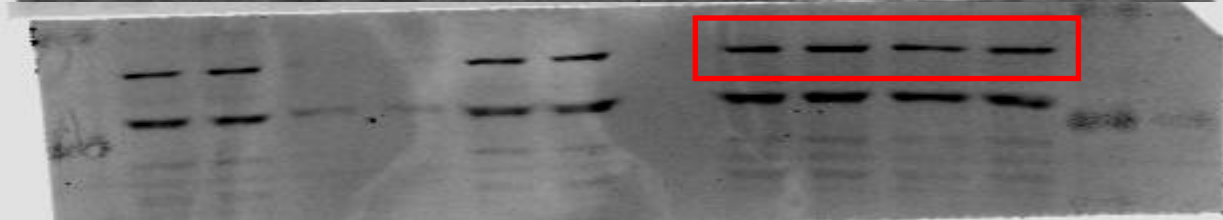
α -NIP30



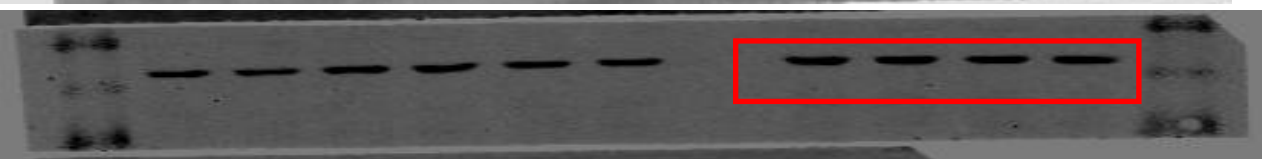
α -REG γ



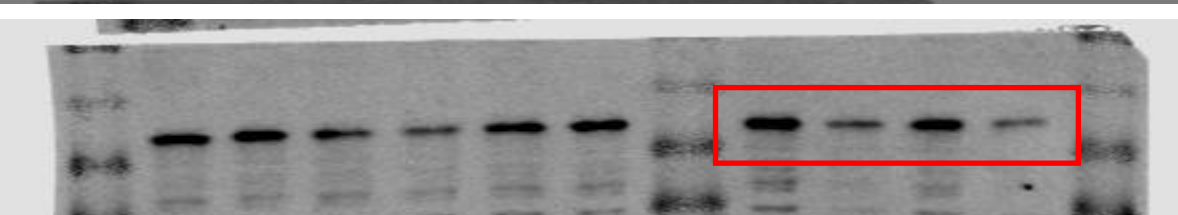
α -p-NIP30S228



α -CDC25A

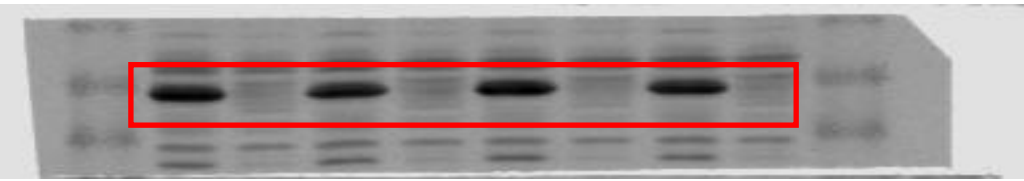


α -actin

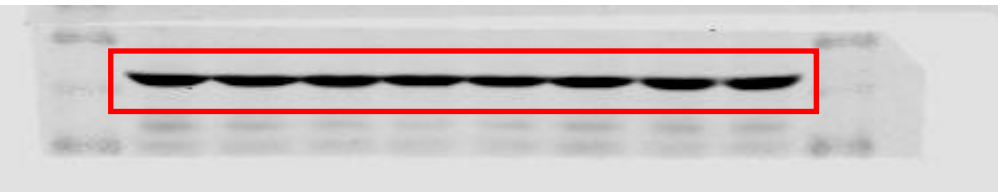


α -p-NIP30S230

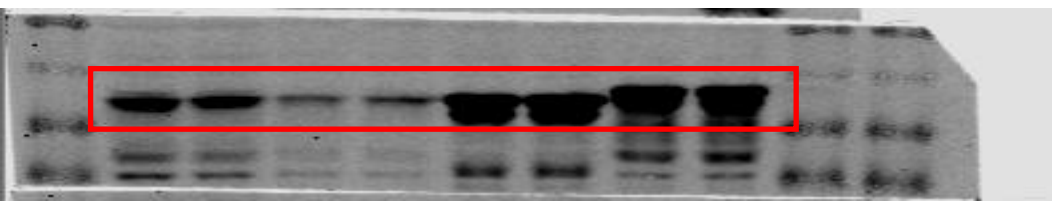
Uncropped gel images for Figure 4E



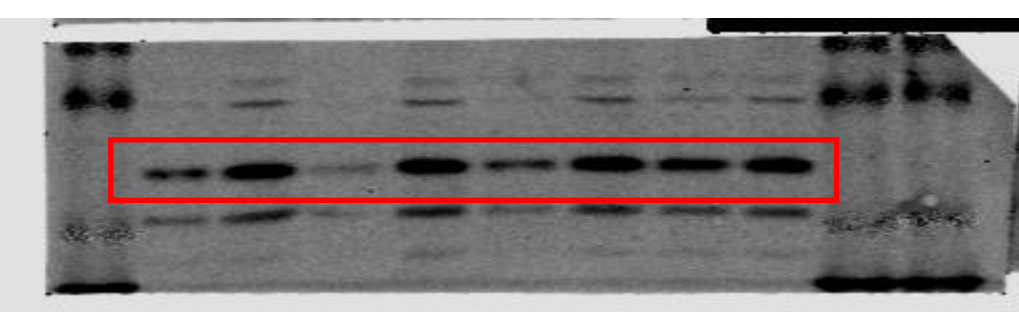
α -REG γ



α -actin

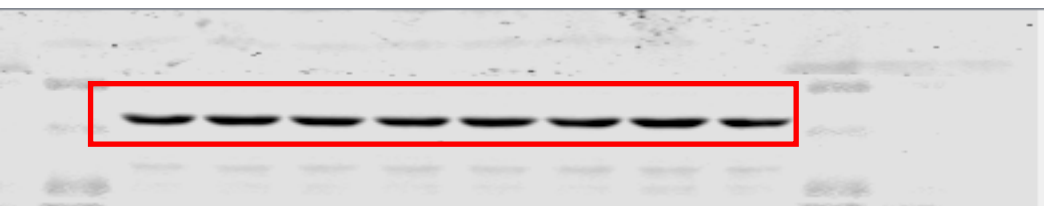


α -nip30

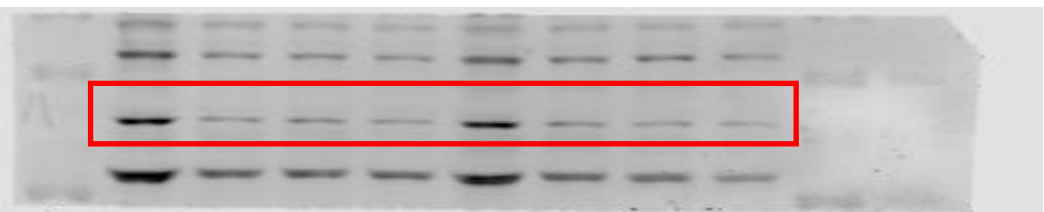


α -p21

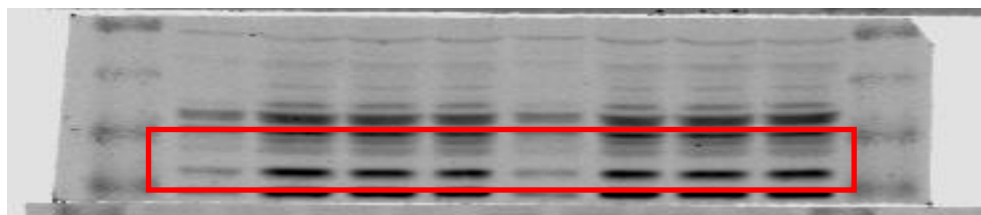
Uncropped gel images for Figure 5A



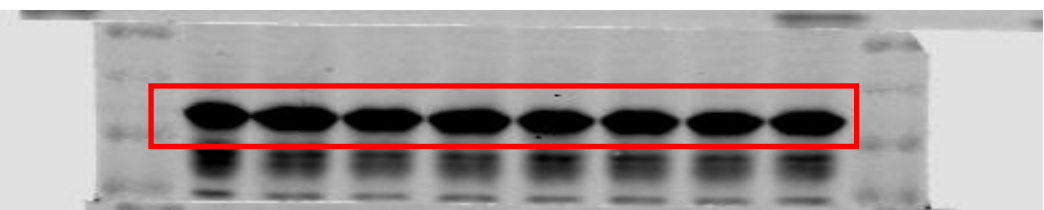
α -actin



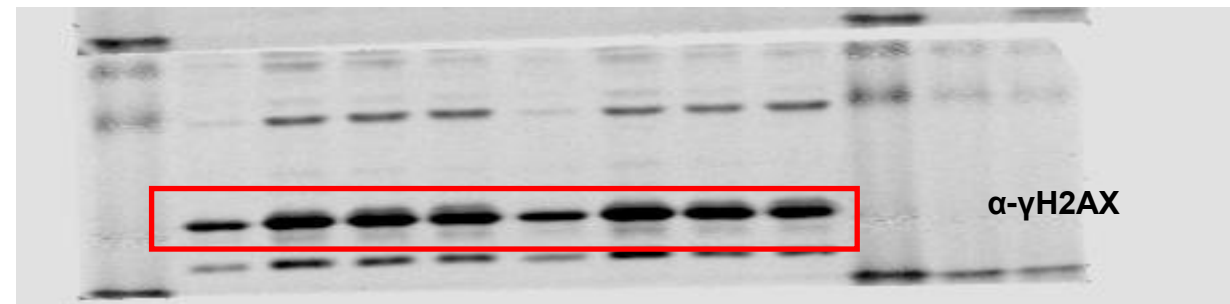
α -CDC25A



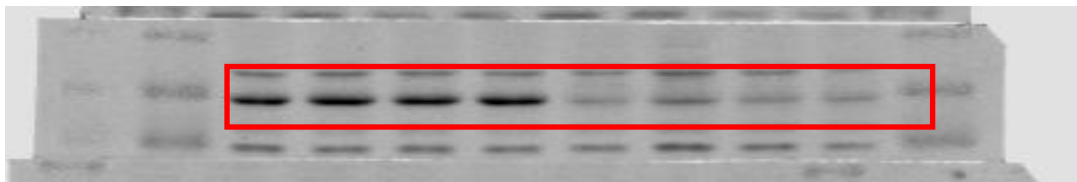
α -p-NIP30S228



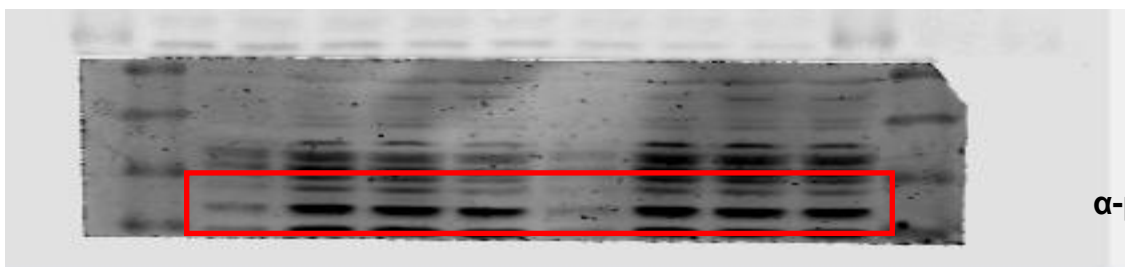
α -NIP30



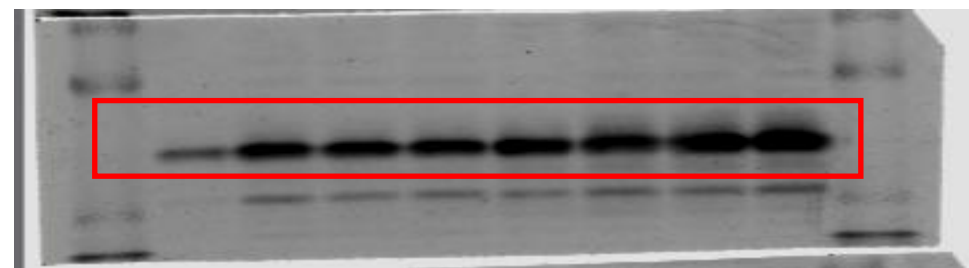
α - γ H2AX



α -REG γ



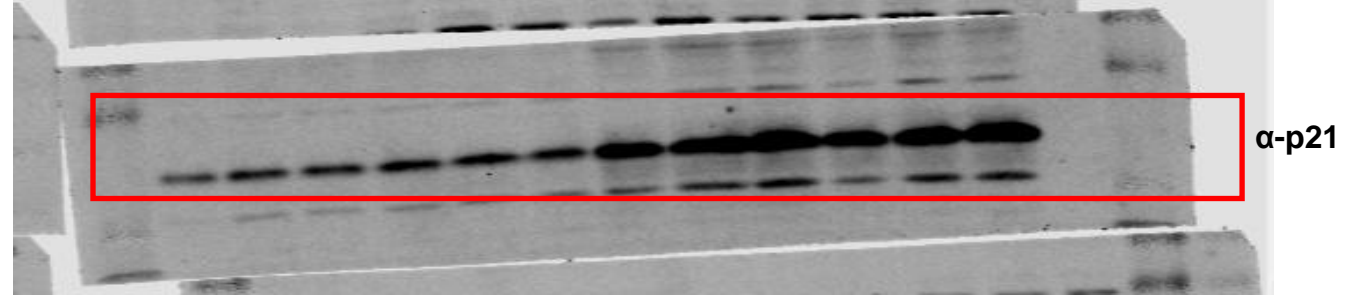
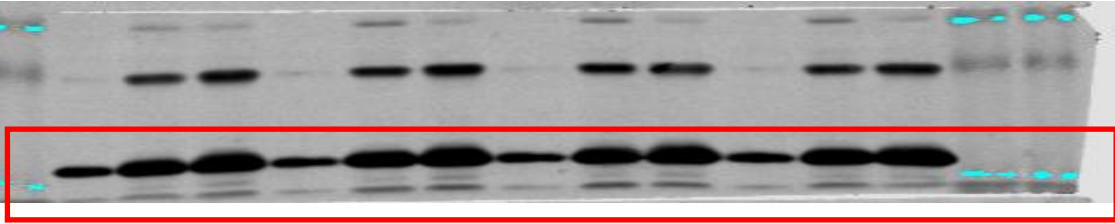
α -p-NIP30S230



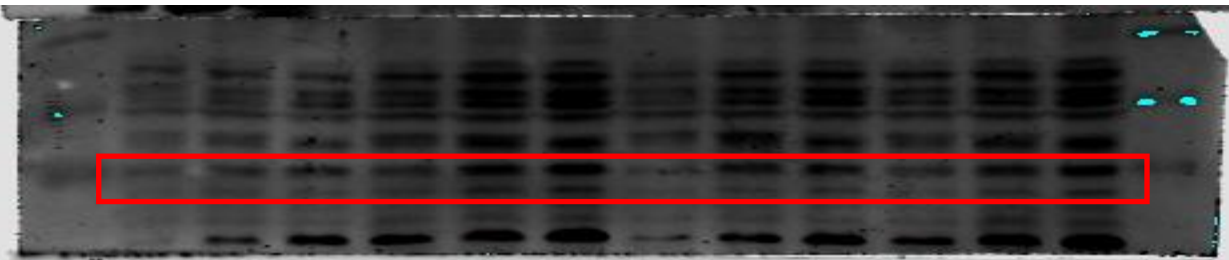
α -p21

Uncropped gel images for Figure 5B

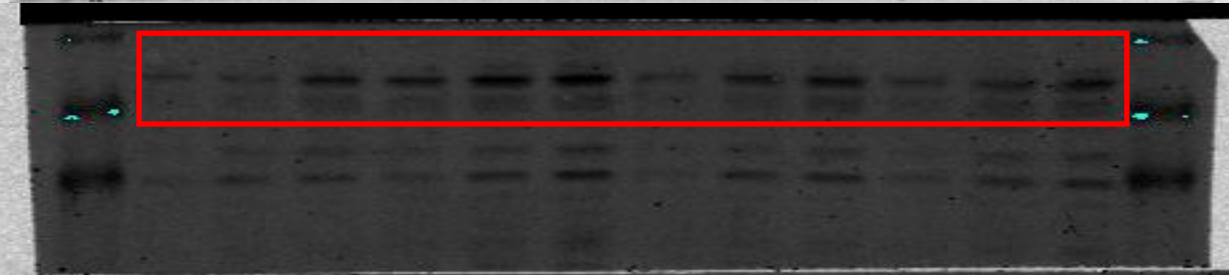
α - γ H2AX



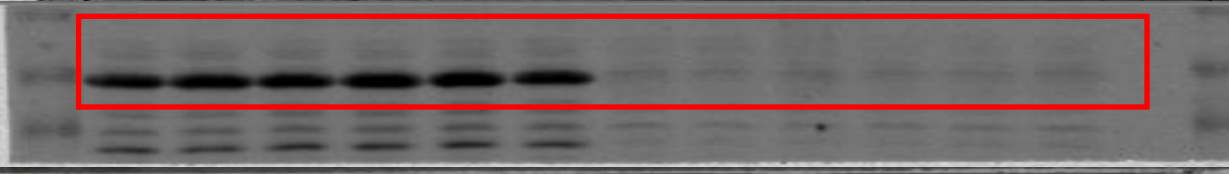
α -p-NIP30S228



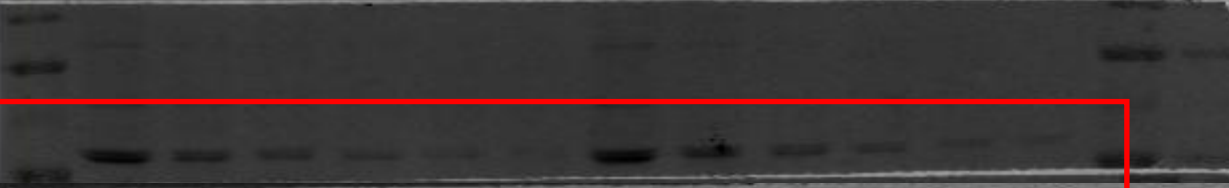
α -p-NIP30S230



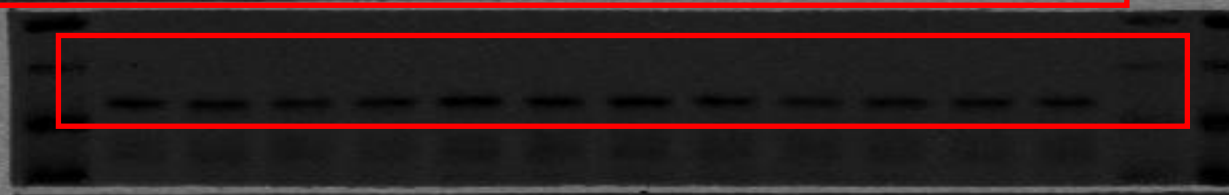
α -REG γ



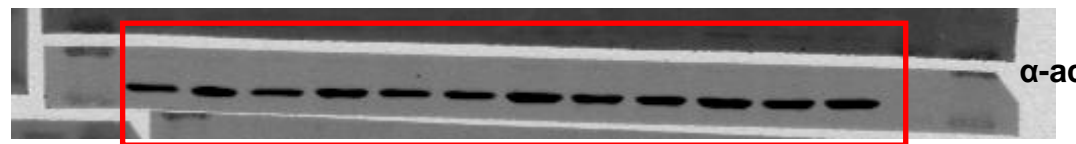
α -CDC25A



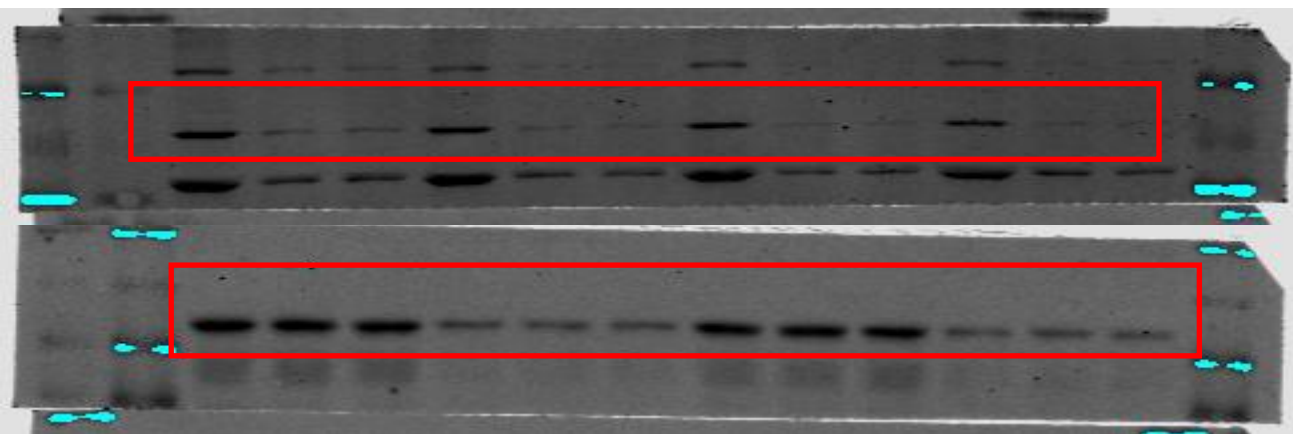
α -NIP30



α -actin

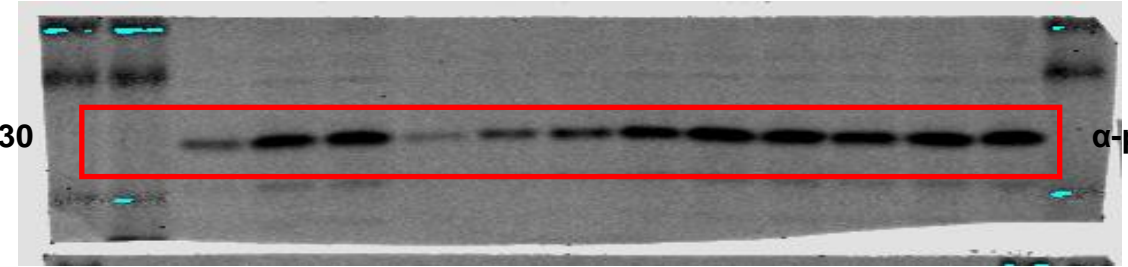


Uncropped gel images for Figure 5C

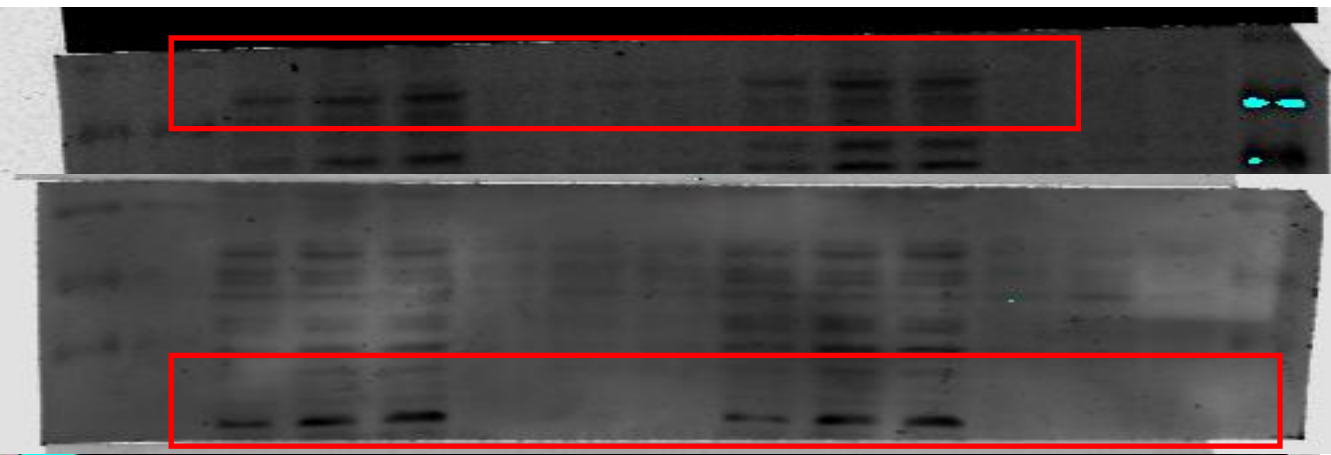


α -CDC25A

α -NIP30

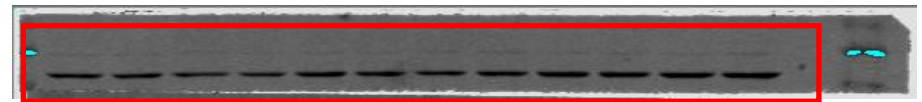


α -p21



α -p-NIP30S230

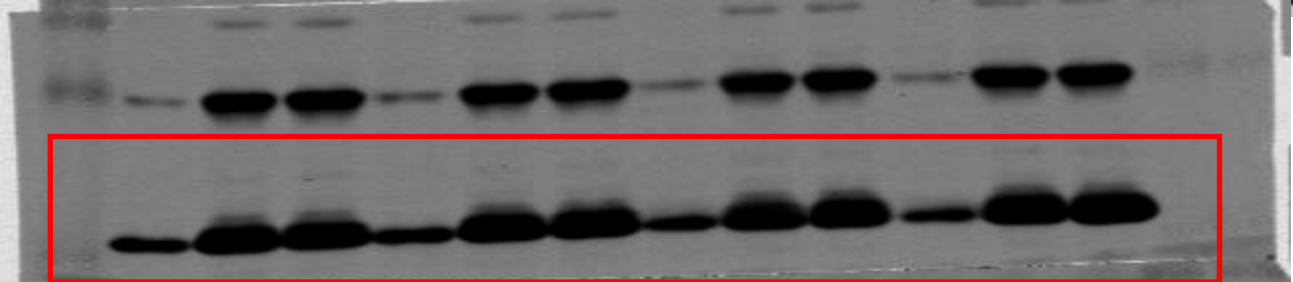
α -p-NIP30S228



α -actin

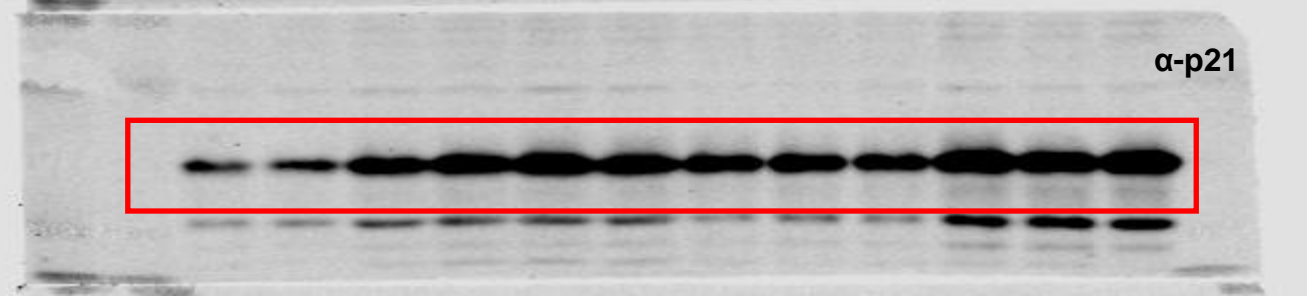
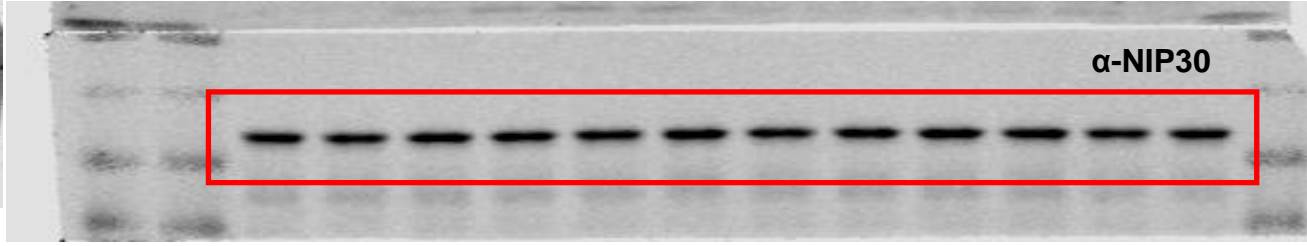
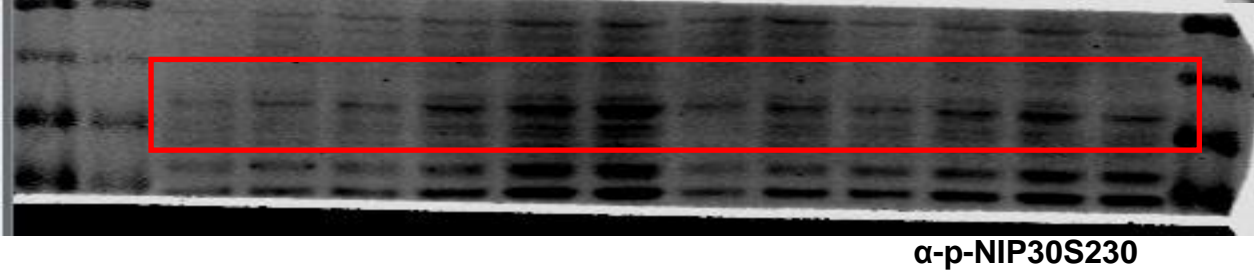
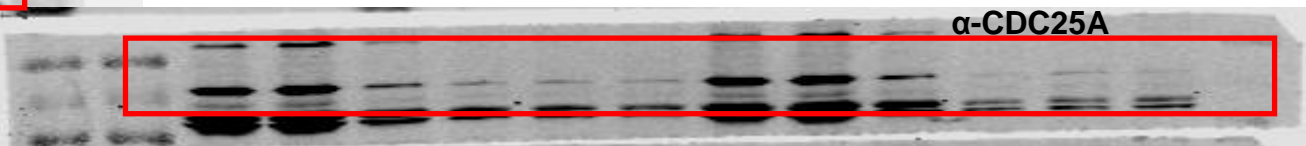
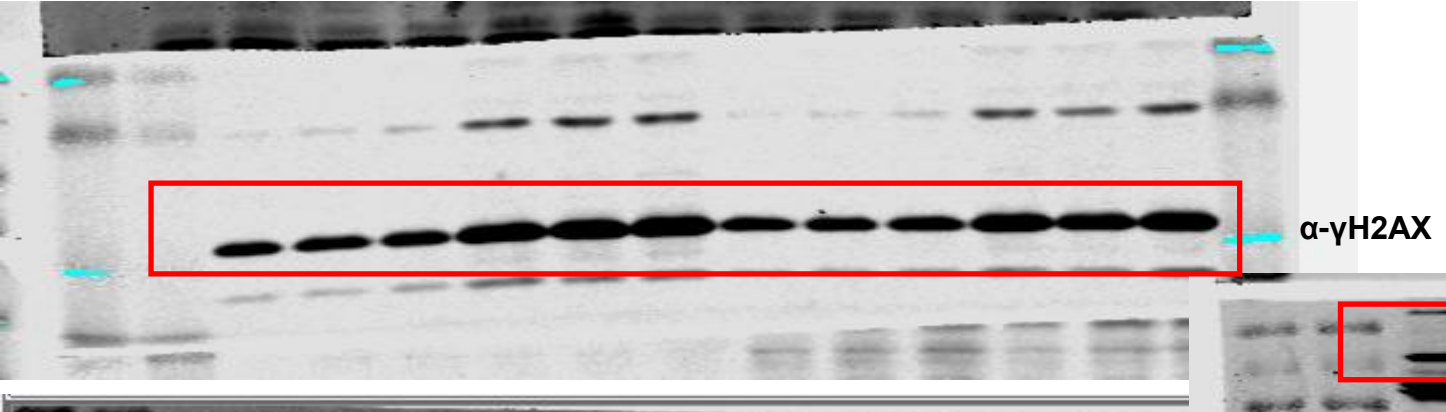
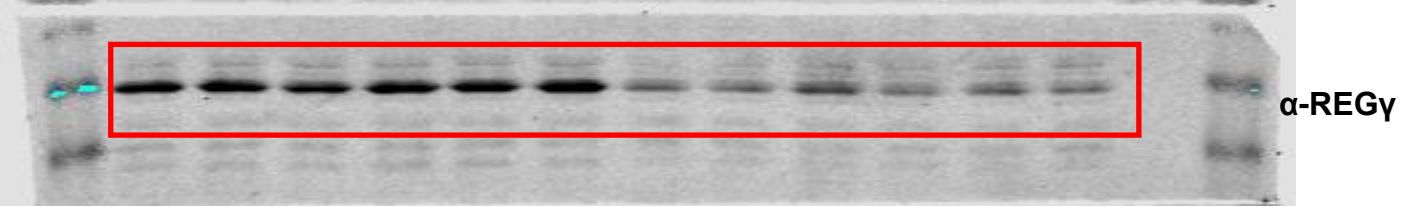
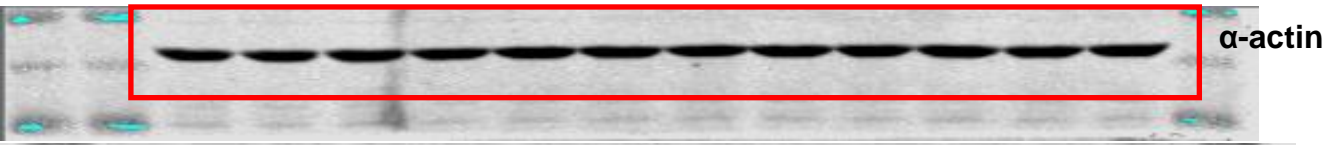


α -REG γ

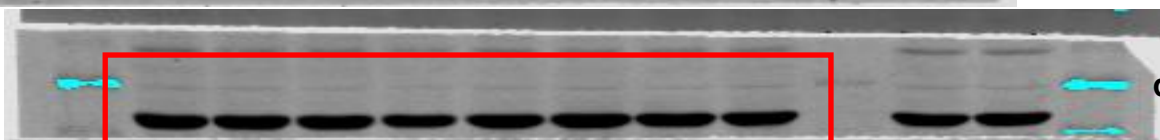
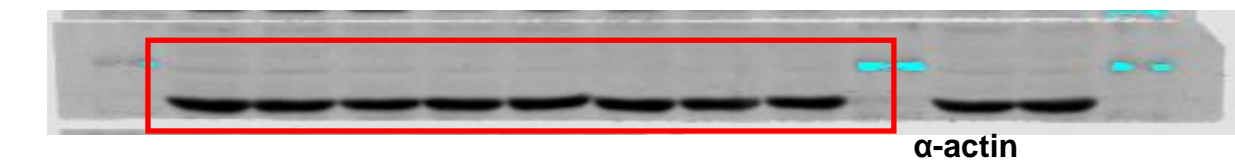
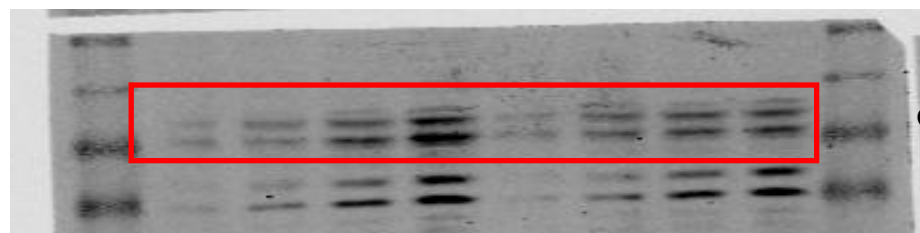
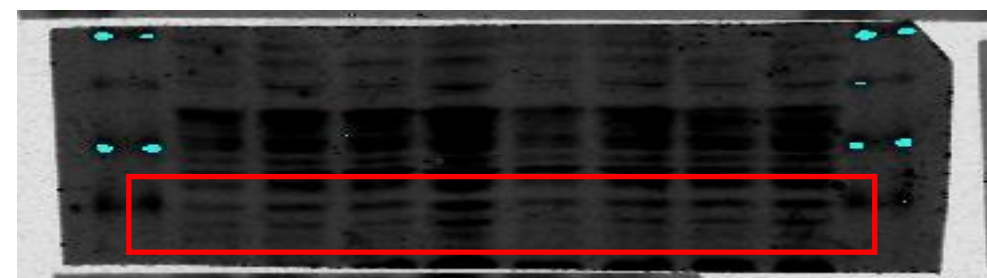
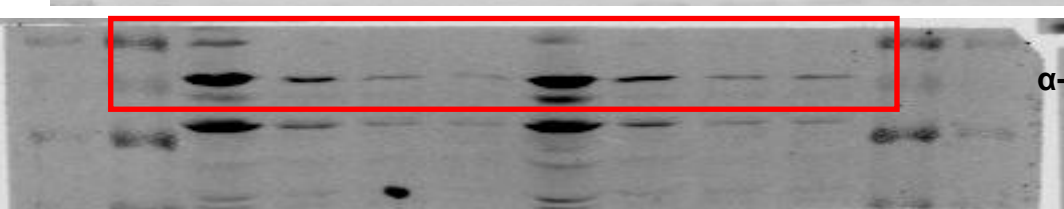
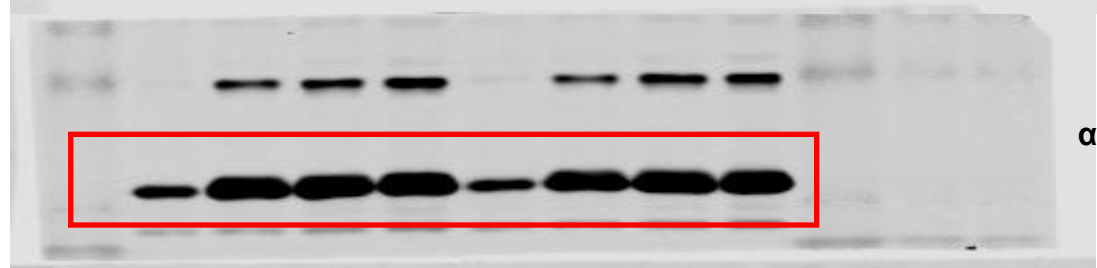
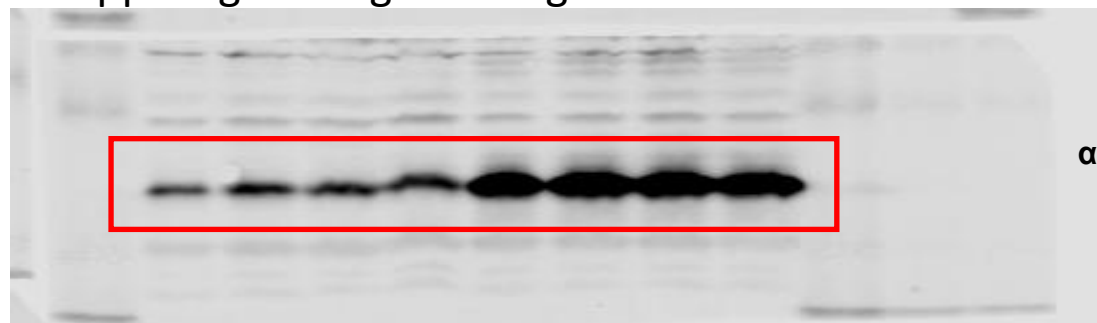


α - γ H2AX

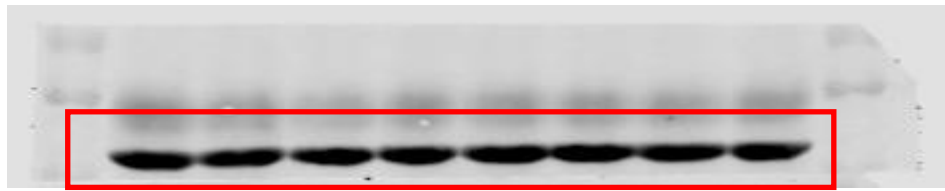
Uncropped gel images for Figure 5D



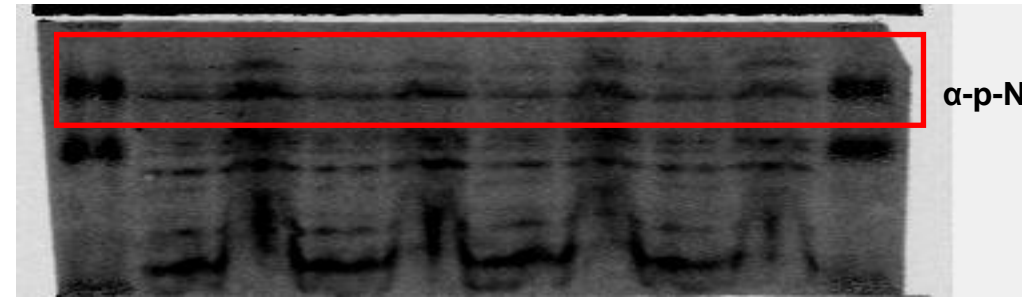
Uncropped gel images for Figure 5E



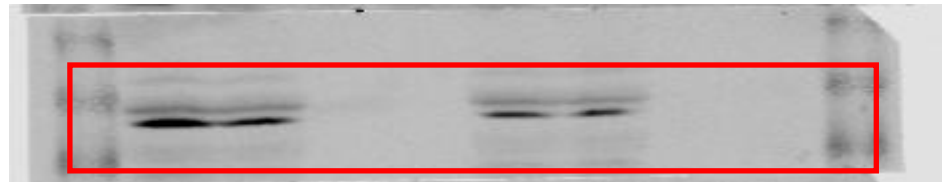
Uncropped gel images for Figure 5F



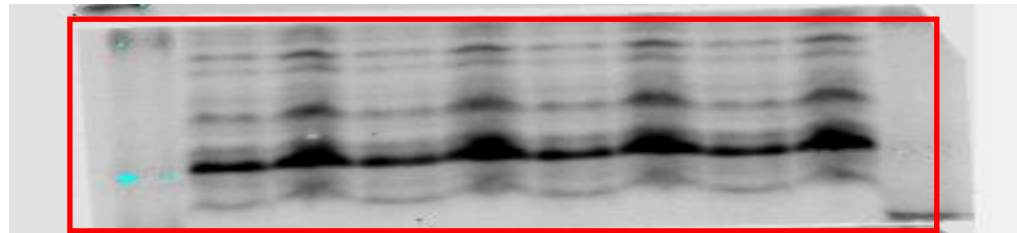
α -actin



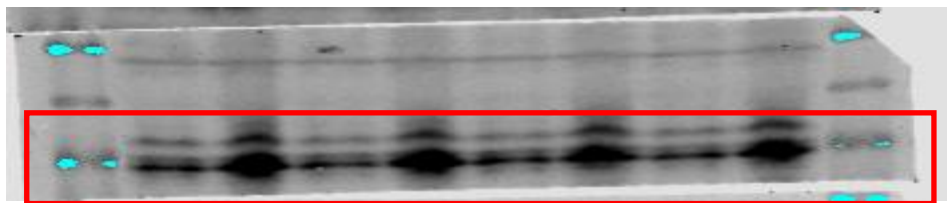
α -p-NIP30S230



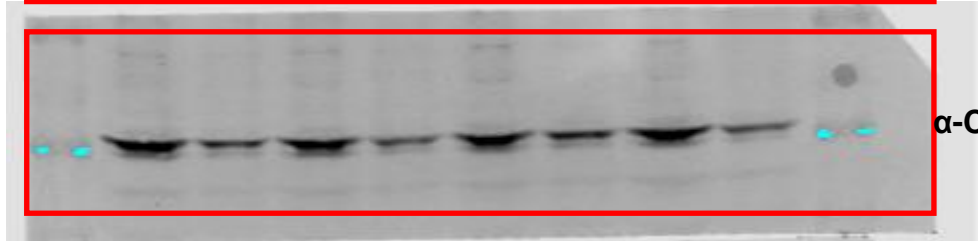
α -REG γ



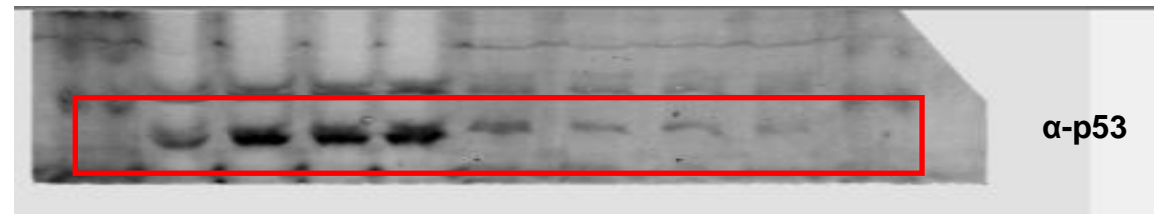
α - γ H2AX



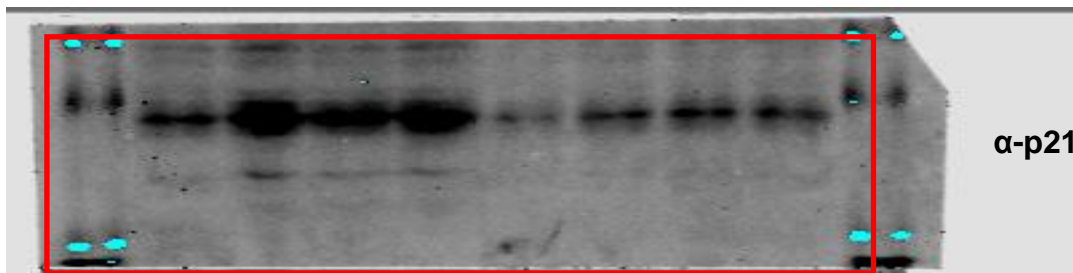
α -p-NIP30S228



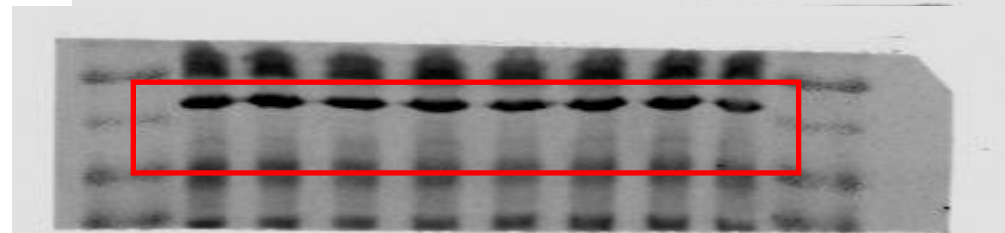
α -CDC25A



α -p53



α -p21



α -NIP30

Original data for Figure 6A

| 1 | Cancer Ty | Protein C | Mutation | Chromosom | Start Pos | End Pos | Ref | Var |
|----|-----------|-----------|-----------|-----------|-----------|----------|-----|-----|
| 2 | Breast Ir | P198L | Missense_ | 16 | 57188374 | 57188374 | G | A |
| 3 | Chromoph | S228L | Missense_ | 16 | 57188284 | 57188284 | G | A |
| 4 | Prostate | D83G | Missense_ | 16 | 57206263 | 57206263 | T | C |
| 5 | Stomach A | S196R | Missense_ | 16 | 57188381 | 57188381 | T | G |
| 6 | Stomach A | A150V | Missense_ | 16 | 57201038 | 57201038 | G | A |
| 7 | Head and | K188T | Missense_ | 16 | 57188404 | 57188404 | T | G |
| 8 | Head and | E108del | In_Frame_ | 16 | 57206187 | 57206189 | TTC | - |
| 9 | Uterine E | E111* | Nonsense_ | 16 | 57206180 | 57206180 | C | A |
| 10 | Uterine E | E101K | Missense_ | 16 | 57206210 | 57206210 | C | T |
| 11 | Uterine S | R24W | Missense_ | 16 | 57207697 | 57207697 | G | A |
| 12 | Uterine E | Q149H | Missense_ | 16 | 57201040 | 57201040 | C | A |
| 13 | Uterine E | K129R | Missense_ | 16 | 57201101 | 57201101 | T | C |
| 14 | Lung Squa | R27M | Missense_ | 16 | 57207687 | 57207687 | C | A |
| 15 | Mucinous | P213S | Missense_ | 16 | 57188330 | 57188330 | G | A |
| 16 | Mucinous | R104Q | Missense_ | 16 | 57206200 | 57206200 | C | T |
| 17 | Renal Cle | X5_splice | Splice_Si | 16 | 57207783 | 57207783 | T | A |
| 18 | Renal Cle | X5_splice | Splice_Si | 16 | 57207783 | 57207783 | T | A |
| 19 | Head and | E231K | Missense_ | 16 | 57188276 | 57188276 | C | T |
| 20 | Hepatocel | D178E | Missense_ | 16 | 57197926 | 57197926 | A | T |
| 21 | Breast Ir | X43_splic | Splice_Re | 16 | 57206793 | 57206793 | G | A |
| 22 | Cutaneous | S224F | Missense_ | 16 | 57188296 | 57188296 | G | A |
| 23 | Cutaneous | D91del | In_Frame_ | 16 | 57206236 | 57206238 | TCA | - |

Original data for Figure 6B

| able format: Grouped | A | | | B | | | C | | |
|-------------------------|------------|----------|---|------------|----------|---|------------|----------|---|
| | Data Set-A | | | Data Set-B | | | Data Set-C | | |
| × | Mean | SEM | N | Mean | SEM | N | Mean | SEM | N |
| 0 | 100.000000 | 5.972148 | 4 | 100.000000 | 4.391329 | 4 | 100.000000 | 4.385837 | 4 |
| 5 | 91.414250 | 4.061247 | 4 | 81.982270 | 6.515805 | 4 | 93.861050 | 4.623367 | 4 |
| 10 | 81.745380 | 2.753384 | 4 | 55.856880 | 8.748584 | 4 | 84.004310 | 4.353581 | 4 |
| 20 | 63.782320 | 3.019455 | 4 | 42.187200 | 4.951428 | 4 | 66.704410 | 1.640015 | 4 |
| 40 | 40.956460 | 1.190171 | 4 | 31.490410 | 3.186132 | 4 | 49.904200 | 3.060161 | 4 |

| D | | |
|------------|----------|---|
| Data Set-D | | |
| Mean | SEM | N |
| 100.000000 | 7.095990 | 4 |
| 76.427410 | 1.727302 | 4 |
| 43.348980 | 3.913222 | 4 |
| 32.576210 | 1.626112 | 4 |
| 22.007980 | 2.542746 | 4 |

| able format: Grouped | A | | | B | | | C | | |
|-------------------------|------------|-----------|---|------------|----------|---|------------|----------|---|
| | Data Set-A | | | Data Set-B | | | Data Set-C | | |
| × | Mean | SEM | N | Mean | SEM | N | Mean | SEM | N |
| 0 | 100.000000 | 13.451470 | 4 | 100.000000 | 3.948328 | 4 | 100.000000 | 2.032693 | 4 |
| 25 | 78.769350 | 7.240422 | 4 | 74.529500 | 7.315637 | 4 | 91.393910 | 6.433432 | 4 |
| 50 | 69.131680 | 7.046267 | 4 | 51.334320 | 6.812981 | 4 | 81.368160 | 6.426945 | 4 |
| 100 | 56.934420 | 8.331352 | 4 | 42.748060 | 6.117490 | 4 | 71.215990 | 7.906507 | 4 |
| 200 | 43.857110 | 3.951880 | 4 | 30.783420 | 6.036523 | 4 | 60.972190 | 6.722385 | 4 |

| D | | |
|------------|----------|---|
| Data Set-D | | |
| Mean | SEM | N |
| 100.000000 | 3.798597 | 4 |
| 69.961360 | 3.882934 | 4 |
| 42.301280 | 3.301097 | 4 |
| 26.210940 | 7.995817 | 4 |
| 21.036270 | 6.738930 | 4 |

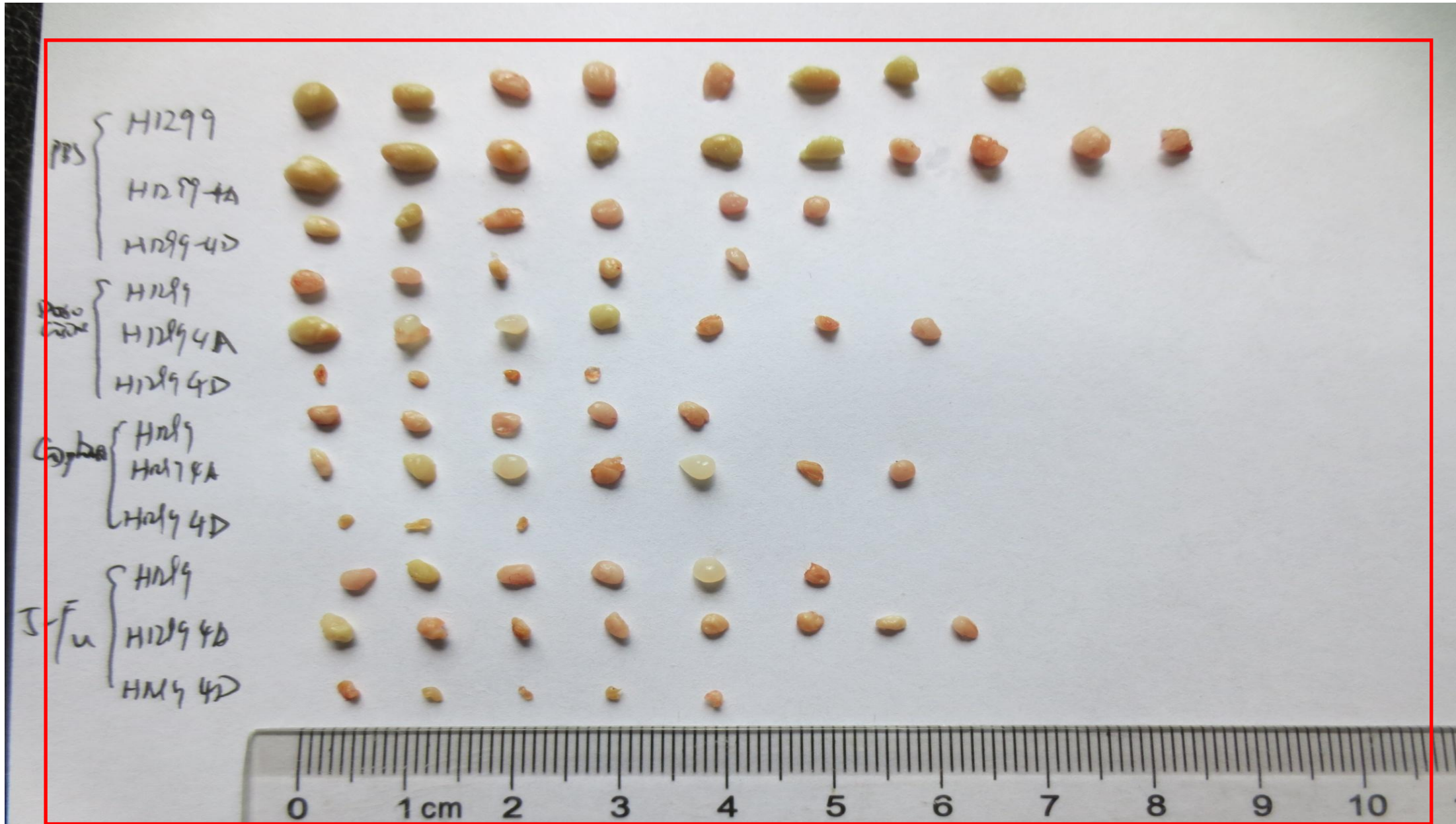
| Table format: Grouped | B | | | C | | | D | | |
|--------------------------|------------|----------|---|------------|----------|---|------------|----------|---|
| | Data Set-B | | | Data Set-C | | | Data Set-D | | |
| | Mean | SEM | N | Mean | SEM | N | Mean | SEM | N |
| 0 | 100.000000 | 3.394450 | 4 | 100.000000 | 4.353409 | 4 | 100.000000 | 7.687875 | 4 |
| 5 | 66.832700 | 4.671573 | 4 | 90.496620 | 4.860917 | 4 | 57.364370 | 9.015898 | 4 |
| 10 | 47.271080 | 4.318573 | 4 | 73.115570 | 9.895278 | 4 | 40.130660 | 3.692994 | 4 |
| 20 | 33.921360 | 3.935569 | 4 | 57.745400 | 8.052529 | 4 | 26.164720 | 4.306086 | 4 |
| 40 | 23.690750 | 3.817653 | 4 | 35.218570 | 4.735401 | 4 | 16.455440 | 4.818836 | 4 |

| D | | |
|------------|----------|---|
| Data Set-D | | |
| Mean | SEM | N |
| 100.000000 | 7.687875 | 4 |
| 57.364370 | 9.015898 | 4 |
| 40.130660 | 3.692994 | 4 |
| 26.164720 | 4.306086 | 4 |
| 16.455440 | 4.818836 | 4 |

| Table format: Grouped | A | | | B | | | C | | |
|--------------------------|------------|-----------|---|------------|----------|---|------------|----------|---|
| | Data Set-A | | | Data Set-B | | | Data Set-C | | |
| | Mean | SEM | N | Mean | SEM | N | Mean | SEM | N |
| 0 | 100.000000 | 5.777298 | 4 | 100.000000 | 6.051938 | 4 | 100.000000 | 6.322669 | 4 |
| 2.5 | 86.865070 | 7.627915 | 4 | 73.636610 | 9.777116 | 4 | 90.919900 | 9.946898 | 4 |
| 5 | 67.070630 | 10.705610 | 4 | 56.749090 | 6.516867 | 4 | 74.251330 | 8.167434 | 4 |
| 10 | 55.840730 | 11.418050 | 4 | 36.269860 | 8.518215 | 4 | 57.548360 | 6.391429 | 4 |
| 20 | 45.085430 | 1.537587 | 4 | 23.098660 | 5.179248 | 4 | 50.676320 | 7.267370 | 4 |

| D | | |
|------------|-----------|---|
| Data Set-D | | |
| Mean | SEM | N |
| 100.000000 | 2.645047 | 4 |
| 66.010610 | 11.053640 | 4 |
| 46.457760 | 10.046310 | 4 |
| 32.469280 | 10.241940 | 4 |
| 19.264430 | 4.124454 | 4 |

Uncropped images for Figure 6C



Supplementary Table 1 NIP30 mutation analysis from TCGA database

| Study | Protein Change | Mutation Type | Chromosome | Start Pos | End Pos | Ref | Var | Mut cases | All samples | Mutant frequency |
|--|----------------|-------------------|------------|-----------|----------|-----|-----|-----------|-------------|------------------|
| Kidney Chromophobe (TCGA, Provisional) | S228L | Missense_Mutation | 16 | 57188284 | 57188284 | G | A | 1 | 66 | 1.515% |
| Lung Squamous Cell Carcinoma (TCGA, Provisional) | R27M | Missense_Mutation | 16 | 57207687 | 57207687 | C | A | 1 | 178 | 0.562% |
| Colorectal Adenocarcinoma (TCGA, Provisional) | P213S | Missense_Mutation | 16 | 57188330 | 57188330 | G | A | 1 | 220 | 0.455% |
| Colorectal Adenocarcinoma (TCGA, Provisional) | R104Q | Missense_Mutation | 16 | 57206200 | 57206200 | C | T | 1 | 220 | 0.455% |
| Kidney Renal Clear Cell Carcinoma (TCGA, Provisional) | X5_splice | Splice_Site | 16 | 57207783 | 57207783 | T | A | 2 | 448 | 0.446% |
| Uterine Corpus Endometrial Carcinoma (TCGA, Provisional) | E111* | Nonsense_Mutation | 16 | 57206180 | 57206180 | C | A | 1 | 242 | 0.413% |
| Uterine Corpus Endometrial Carcinoma (TCGA, Provisional) | E101K | Missense_Mutation | 16 | 57206210 | 57206210 | C | T | 1 | 242 | 0.413% |
| Uterine Corpus Endometrial Carcinoma (TCGA, Provisional) | R24W | Missense_Mutation | 16 | 57207697 | 57207697 | G | A | 1 | 242 | 0.413% |
| Uterine Corpus Endometrial Carcinoma (TCGA, Provisional) | Q149H | Missense_Mutation | 16 | 57201040 | 57201040 | C | A | 1 | 242 | 0.413% |
| Uterine Corpus Endometrial Carcinoma (TCGA, Provisional) | K129R | Missense_Mutation | 16 | 57201101 | 57201101 | T | C | 1 | 242 | 0.413% |
| Skin Cutaneous Melanoma (TCGA, Provisional) | S224F | Missense_Mutation | 16 | 57188296 | 57188296 | G | A | 1 | 287 | 0.348% |
| Skin Cutaneous Melanoma (TCGA, Provisional) | D91del | In_Frame_Del | 16 | 57206236 | 57206238 | TCA | - | 1 | 287 | 0.348% |
| Stomach Adenocarcinoma (TCGA, Provisional) | S196R | Missense_Mutation | 16 | 57188381 | 57188381 | T | G | 1 | 287 | 0.348% |
| Stomach Adenocarcinoma (TCGA, Provisional) | A150V | Missense_Mutation | 16 | 57201038 | 57201038 | G | A | 1 | 287 | 0.348% |
| Liver Hepatocellular Carcinoma (TCGA, Provisional) | D178E | Missense_Mutation | 16 | 57197926 | 57197926 | A | T | 1 | 366 | 0.273% |
| Prostate Adenocarcinoma (TCGA, Provisional) | D83G | Missense_Mutation | 16 | 57206263 | 57206263 | T | C | 1 | 492 | 0.203% |
| Head and Neck Squamous Cell Carcinoma (TCGA, Provisional) | K188T | Missense_Mutation | 16 | 57188404 | 57188404 | T | G | 1 | 504 | 0.198% |
| Head and Neck Squamous Cell Carcinoma (TCGA, Provisional) | E108del | In_Frame_Del | 16 | 57206187 | 57206189 | TTC | - | 1 | 504 | 0.198% |
| Head and Neck Squamous Cell Carcinoma (TCGA, Provisional) | E231K | Missense_Mutation | 16 | 57188276 | 57188276 | C | T | 1 | 504 | 0.198% |
| Breast Invasive Carcinoma (TCGA, Provisional) | P198L | Missense_Mutation | 16 | 57188374 | 57188374 | G | A | 1 | 963 | 0.104% |
| Breast Invasive Carcinoma (TCGA, Provisional) | X43_splice | Splice_Region | 16 | 57206793 | 57206793 | G | A | 1 | 963 | 0.104% |

Supplementary Table 2 NIP30 deletion analysis from TCGA database

| | Deletion cases | All cases | Frequency |
|---|-----------------------|------------------|------------------|
| Bladder Urothelial Carcinoma (TCGA, Provisional) | 13 | 408 | 3.19% |
| Prostate Adenocarcinoma (TCGA, Provisional) | 14 | 492 | 2.85% |
| Lymphoid Neoplasm Diffuse Large B-cell Lymphoma (TCGA, Provisional) | 1 | 48 | 2.08% |
| Uveal Melanoma (TCGA, Provisional) | 1 | 80 | 1.25% |
| Skin Cutaneous Melanoma (TCGA, Provisional) | 3 | 367 | 0.82% |
| Thymoma (TCGA, Provisional) | 1 | 123 | 0.81% |
| Stomach Adenocarcinoma (TCGA, Provisional) | 3 | 441 | 0.68% |
| Esophageal Carcinoma (TCGA, Provisional) | 1 | 184 | 0.54% |
| Acute Myeloid Leukemia (TCGA, Provisional) | 1 | 191 | 0.52% |
| Sarcoma (TCGA, Provisional) | 1 | 257 | 0.39% |
| Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma (TCGA, Provisional) | 1 | 295 | 0.34% |
| Liver Hepatocellular Carcinoma (TCGA, Provisional) | 1 | 370 | 0.27% |
| Lung Adenocarcinoma (TCGA, Provisional) | 1 | 516 | 0.19% |
| Head and Neck Squamous Cell Carcinoma (TCGA, Provisional) | 1 | 522 | 0.19% |
| Uterine Corpus Endometrial Carcinoma (TCGA, Provisional) | 1 | 539 | 0.19% |
| Glioblastoma Multiforme (TCGA, Provisional) | 1 | 577 | 0.17% |
| Ovarian Serous Cystadenocarcinoma (TCGA, Provisional) | 1 | 579 | 0.17% |
| Breast Invasive Carcinoma (TCGA, Provisional) | 1 | 1080 | 0.09% |

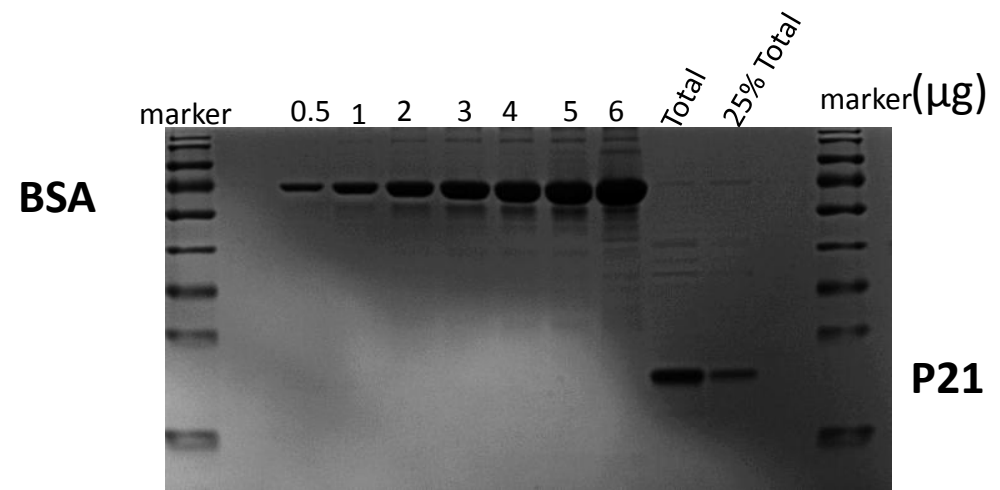
Supplementary Table 3 Phosphorylated at S228 (related Supplementary Figure 3D)

| b-H₃PO₄ | b | | | y | y⁺² | y-H₃PO₄ | y-H₃PO₄⁺² | |
|--------------------------------------|------------------|----|-------------------|----------|-----------------------|--------------------------------------|---|----------|
| --- | --- | 1 | S | 21 | --- | --- | --- | |
| --- | 145.0608 | 2 | G | 20 | <i>1995.7663</i> | 998.3868 | <i>1897.7894</i> | 949.3983 |
| --- | 232.0928 | 3 | S | 19 | <i>1938.7448</i> | 969.876 | <i>1840.7679</i> | 920.8876 |
| --- | 319.1248 | 4 | S | 18 | <i>1851.7128</i> | 926.36 | <i>1753.7359</i> | 877.3716 |
| --- | 434.1518 | 5 | D | 17 | <i>1764.6807</i> | 882.844 | <i>1666.7038</i> | 833.8556 |
| --- | 521.1838 | 6 | S | 16 | <i>1649.6538</i> | 825.3305 | <i>1551.6769</i> | 776.3421 |
| --- | 650.2264 | 7 | E | 15 | <i>1562.6218</i> | 781.8145 | 1464.645 | 732.8261 |
| --- | 737.2584 | 8 | S | 14 | 1433.579 | 717.2932 | 1335.602 | 668.3048 |
| --- | 824.2904 | 9 | S | 13 | 1346.547 | 673.7772 | 1248.57 | 624.7888 |
| 893.3119 | 991.2888 | 10 | S(Phospho) | 12 | 1259.515 | 630.2612 | 1161.538 | 581.2727 |
| 1008.339 | 1106.316 | 11 | D | 11 | 1092.517 | 546.762 | --- | --- |
| 1095.371 | 1193.348 | 12 | S | 10 | 977.4898 | 489.2485 | --- | --- |
| 1224.414 | 1322.39 | 13 | E | 9 | 890.4578 | 445.7325 | --- | --- |
| 1281.435 | 1379.412 | 14 | G | 8 | 761.4152 | 381.2112 | --- | --- |
| 1382.483 | 1480.46 | 15 | T | 7 | 704.3937 | 352.7005 | --- | --- |
| 1495.567 | <i>1593.5436</i> | 16 | I | 6 | 603.3461 | 302.1767 | --- | --- |
| <i>1609.6096</i> | <i>1707.5865</i> | 17 | N | 5 | 490.262 | 245.6346 | --- | --- |
| <i>1680.6467</i> | <i>1778.6236</i> | 18 | A | 4 | 376.2191 | 188.6132 | --- | --- |
| <i>1781.6944</i> | <i>1879.6713</i> | 19 | T | 3 | 305.1819 | 153.0946 | --- | --- |
| <i>1838.7159</i> | <i>1936.6928</i> | 20 | G | 2 | 204.1343 | <i>102.5708</i> | --- | --- |
| --- | --- | 21 | K | 1 | 147.1128 | 74.06 | --- | --- |

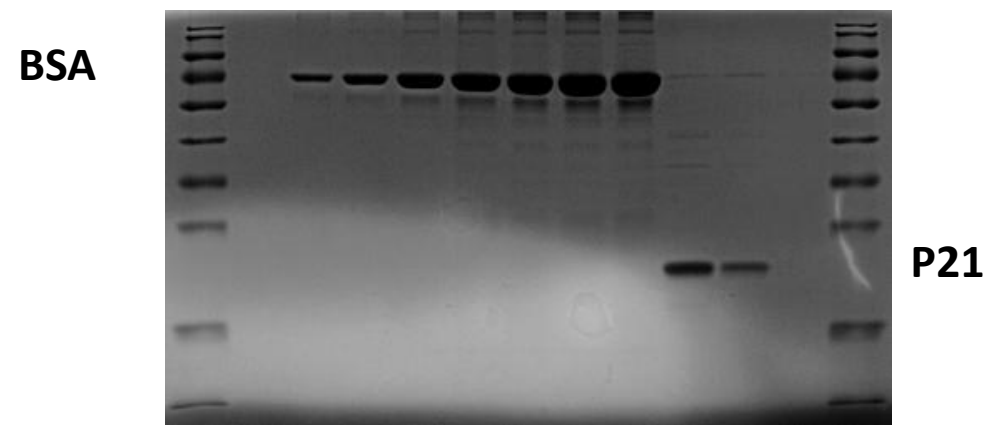
Supplementary Table 4 Phosphorylated at S230 (related Supplementary Figure 3E)

| b-H₃PO₄ | b | | | | y | y⁺² | y-H₃PO₄ | y-H₃PO₄⁺² |
|--------------------------------------|------------------|----|-------------------|----|------------------|-----------------------|--------------------------------------|---|
| --- | --- | 1 | S | 21 | --- | --- | --- | --- |
| --- | <i>145.0608</i> | 2 | G | 20 | <i>1995.7663</i> | 998.3868 | <i>1897.7894</i> | 949.3983 |
| --- | <i>232.0928</i> | 3 | S | 19 | <i>1938.7448</i> | 969.876 | <i>1840.7679</i> | 920.8876 |
| --- | <i>319.1248</i> | 4 | S | 18 | <i>1851.7128</i> | 926.36 | <i>1753.7359</i> | 877.3716 |
| --- | <i>434.1518</i> | 5 | D | 17 | <i>1764.6807</i> | 882.844 | <i>1666.7038</i> | 833.8556 |
| --- | <i>521.1838</i> | 6 | S | 16 | <i>1649.6538</i> | 825.3305 | <i>1551.6769</i> | 776.3421 |
| --- | 650.2264 | 7 | E | 15 | <i>1562.6218</i> | 781.8145 | <i>1464.6449</i> | 732.8261 |
| --- | 737.2584 | 8 | S | 14 | <i>1433.5792</i> | 717.2932 | 1335.602 | 668.3048 |
| --- | 824.2904 | 9 | S | 13 | <i>1346.5471</i> | 673.7772 | 1248.57 | 624.7888 |
| --- | <i>911.3225</i> | 10 | S | 12 | 1259.515 | 630.2612 | 1161.538 | 581.2727 |
| --- | <i>1026.349</i> | 11 | D | 11 | <i>1172.483</i> | 586.7452 | <i>1074.506</i> | 537.7567 |
| <i>1095.371</i> | <i>1193.348</i> | 12 | S(Phospho) | 10 | 1057.456 | 529.2317 | <i>959.4793</i> | 480.2433 |
| <i>1224.414</i> | <i>1322.39</i> | 13 | E | 9 | <i>890.4578</i> | 445.7325 | --- | --- |
| <i>1281.435</i> | <i>1379.4118</i> | 14 | G | 8 | 761.4152 | 381.2112 | --- | --- |
| <i>1382.4826</i> | <i>1480.4595</i> | 15 | T | 7 | <i>704.3937</i> | 352.7005 | --- | --- |
| <i>1495.5667</i> | <i>1593.5436</i> | 16 | I | 6 | <i>603.3461</i> | 302.1767 | --- | --- |
| <i>1609.6096</i> | <i>1707.5865</i> | 17 | N | 5 | 490.262 | 245.6346 | --- | --- |
| <i>1680.6467</i> | <i>1778.6236</i> | 18 | A | 4 | 376.2191 | 188.6132 | --- | --- |
| <i>1781.6944</i> | <i>1879.6713</i> | 19 | T | 3 | <i>305.1819</i> | <i>153.0946</i> | --- | --- |
| <i>1838.7159</i> | <i>1936.6928</i> | 20 | G | 2 | <i>204.1343</i> | <i>102.5708</i> | --- | --- |
| --- | --- | 21 | K | 1 | <i>147.1128</i> | <i>74.06</i> | --- | --- |

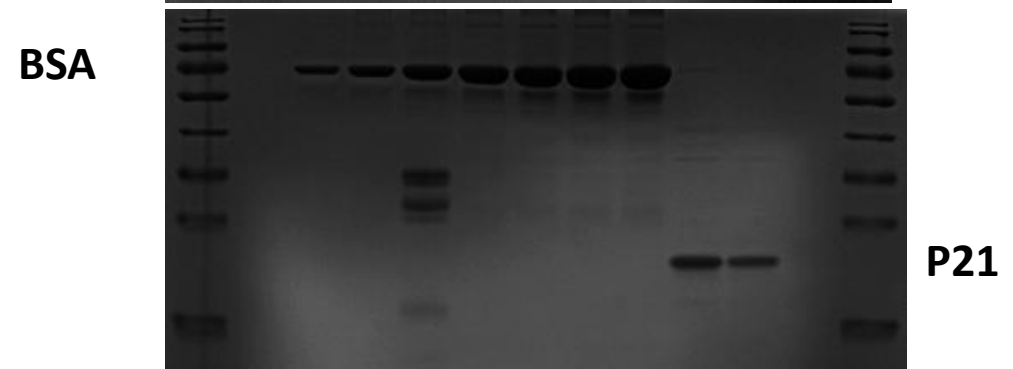
Uncropped gel images for Supplemental Figure 3H



Repeating 1

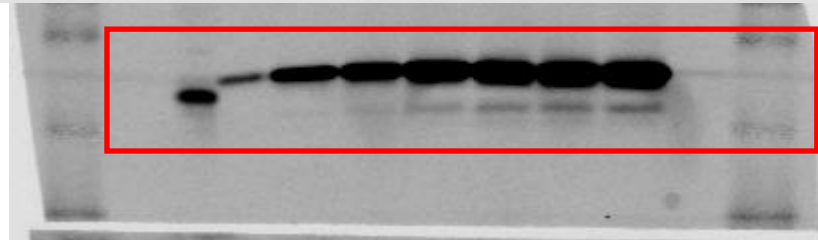
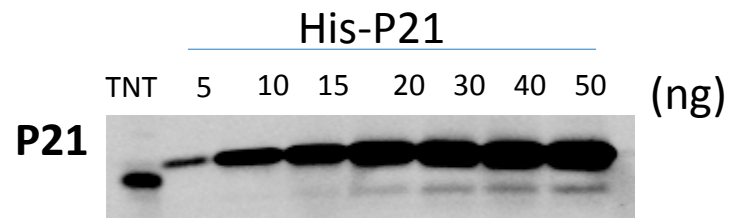
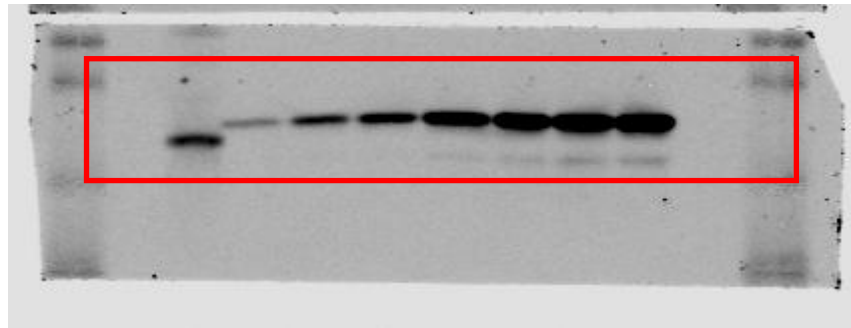
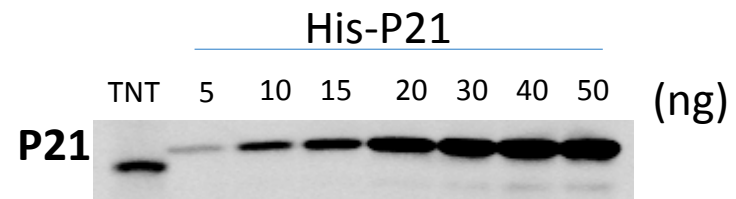
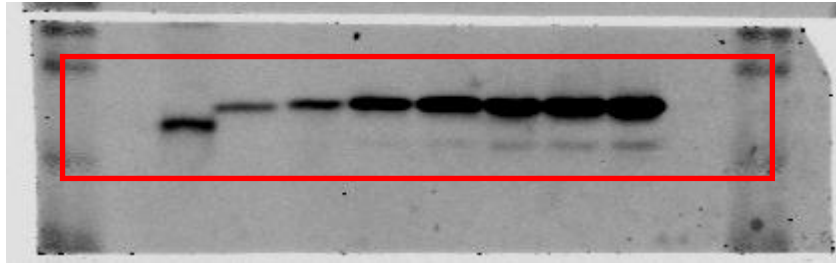
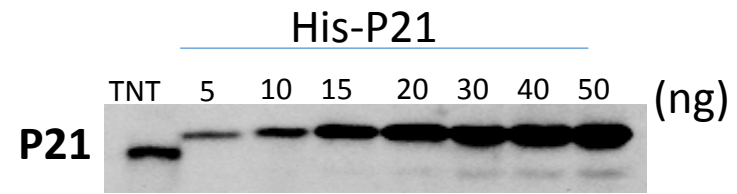


Repeating 2

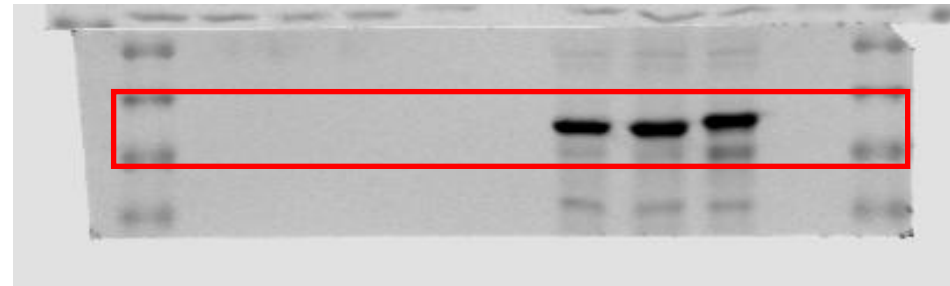
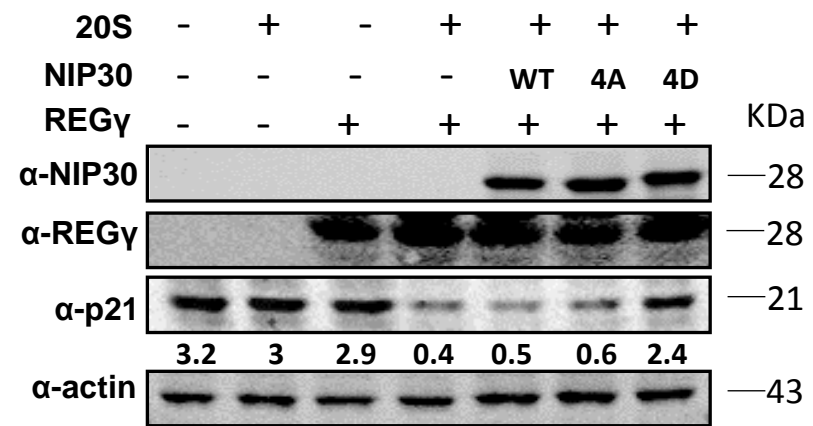


Repeating 3

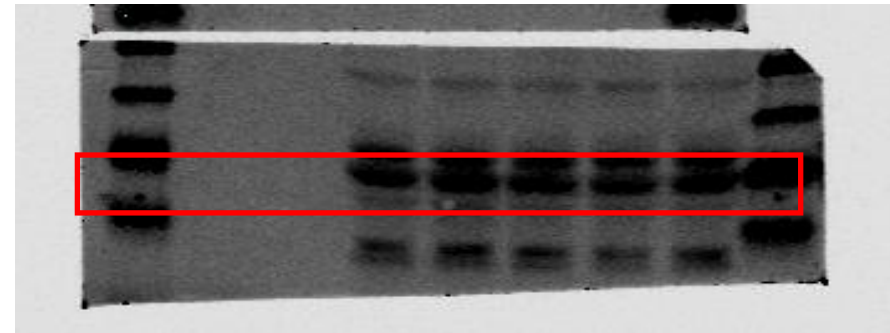
Uncropped gel images for Supplemental Figure 3I



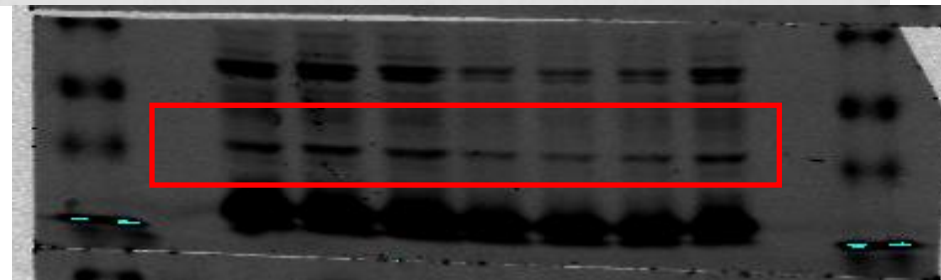
Uncropped gel images for Supplemental Figure3J-K Repeating 1



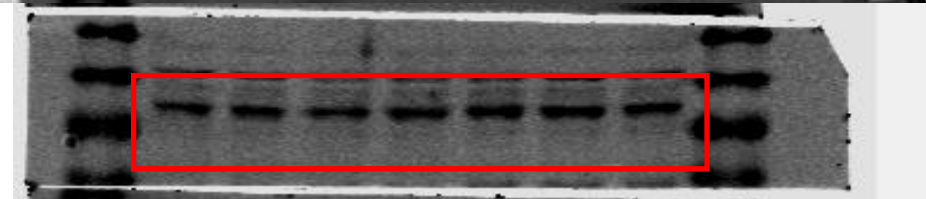
α-nip30



α-REGγ

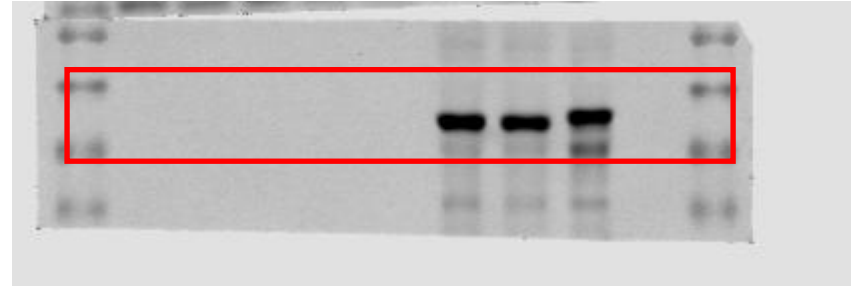
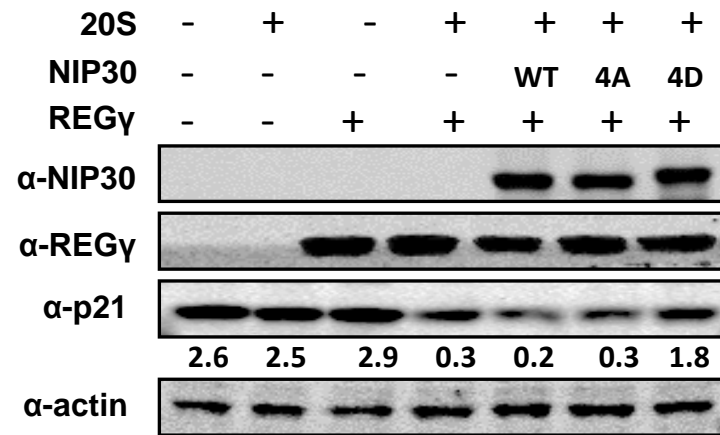


α-p21

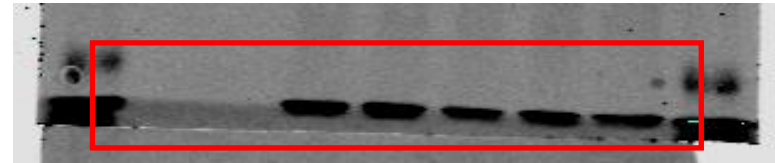


α-actin

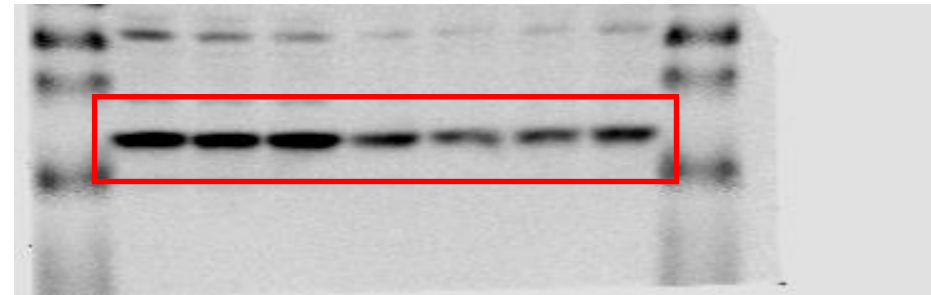
Uncropped gel images for Supplemental Figure3J-K Repeating 2



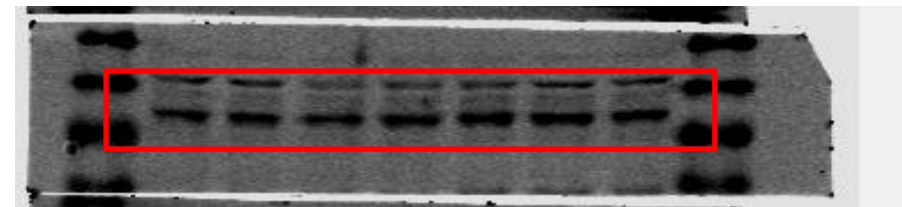
α-nip30



α-REGγ



α-p21



α-actin

Uncropped gel images for Supplemental Figure3J-K Repeating 3

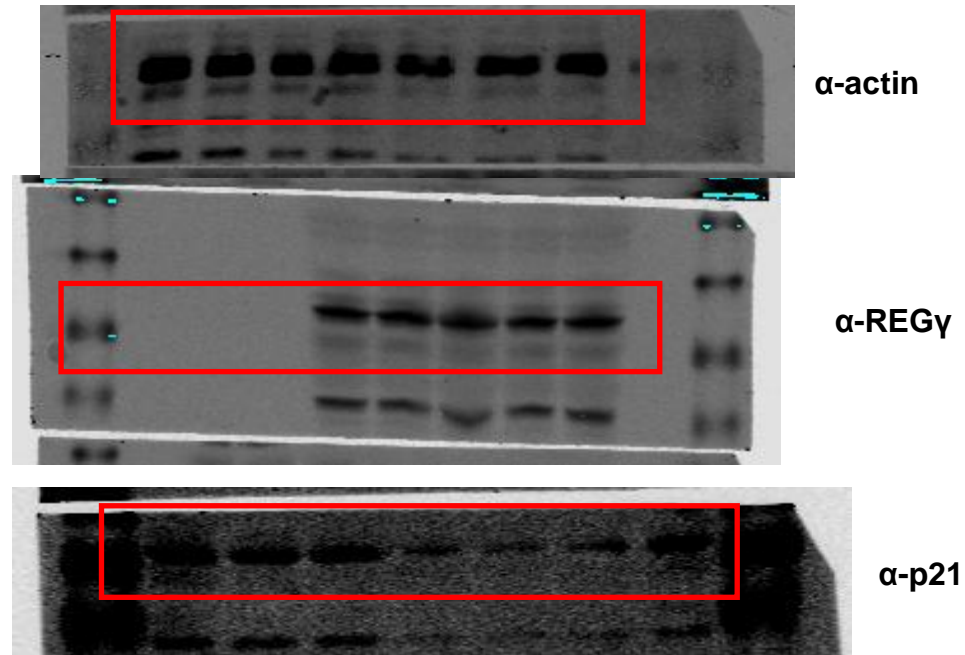
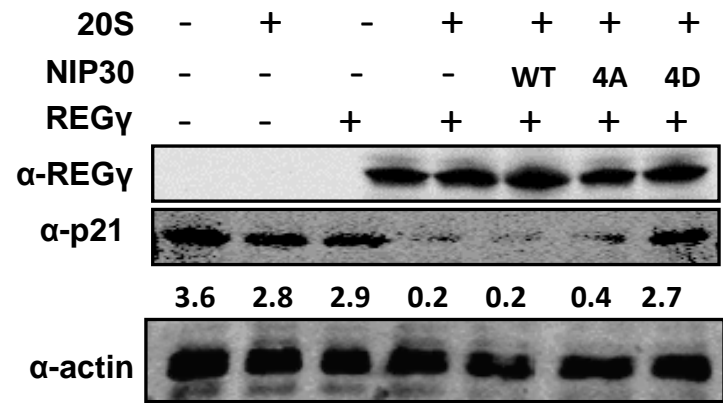


Image J value for each lane per experiment for actin and p21
(S-fig3K-table1)

| | Grayscale Value by Image J | | | | | | | |
|-------|----------------------------|-------|---------|-------|---------|----------|-------|-----|
| | Repeat1 | | Repeat2 | | Repeat3 | | | p21 |
| | actin | p21 | actin | p21 | actin | actin/20 | | |
| lane1 | 10570 | 33974 | 10113 | 26052 | 189740 | 9487 | 33807 | |
| lane2 | 10666 | 32192 | 9958 | 24399 | 178525 | 8926.25 | 25106 | |
| lane3 | 10787 | 31012 | 9994 | 28486 | 176434 | 8821.7 | 25561 | |
| lane4 | 10618 | 4620 | 11475 | 2946 | 179131 | 8956.55 | 2158 | |
| lane5 | 11615 | 5846 | 10331 | 2309 | 177536 | 8876.8 | 2097 | |
| lane6 | 11386 | 7178 | 10419 | 3627 | 183433 | 9171.65 | 3366 | |
| lane7 | 10082 | 24568 | 10237 | 18746 | 167203 | 8360.15 | 22468 | |

Note.*actin/20 refers to normalization by 20 fold reduction in grayscale values for all actin levels in repeating experiment 3. We did so with the intention to reduce variations among the 3 experiments. We have carefully calculated and concluded that with or without normalization by 20 fold reductions, the final results in the table s3 are the same for the relative p21 expression levels. For example, we got the lane 1 and lane 7 values in repeat 3 as 0.178175 and 0.134376 respectively by p21/actin without normalization of 20-fold reductions. When we normalize lane 7 in repeat3 to 3.2142, we use this formula ($Y=0.134376 / 0.178175 * 3.2142=2.4241$), which is the same as the calculation with normalization by 20-fold reduction of actin [$p21/(actin/20)$] as shown in S-fig3K-table3.

P21 value / actin value
(S-fig3K-table2)

| | Repeat1 | Repeat2 | Repeat3 |
|-------|---------|---------|---------|
| lane1 | 3.2142 | 2.5761 | 3.5635 |
| lane2 | 3.0182 | 2.4502 | 2.8126 |
| lane3 | 2.8749 | 2.8503 | 2.8975 |
| lane4 | 0.4351 | 0.2567 | 0.2409 |
| lane5 | 0.5033 | 0.2235 | 0.2362 |
| lane6 | 0.6304 | 0.3481 | 0.367 |
| lane7 | 2.4368 | 1.8312 | 2.6875 |

Normalized to the first P21 band value(3.2142)
of the first repeating assay
(S-fig3K-table3)

| Normalized data | | |
|-----------------|----------|----------|
| Repeat1 | Repeat2 | Repeat3 |
| 3.2142 | 3.2142 | 3.2142 |
| 3.0182 | 3.057115 | 2.536904 |
| 2.8749 | 3.556319 | 2.613482 |
| 0.4351 | 0.320285 | 0.217287 |
| 0.5033 | 0.278861 | 0.213047 |
| 0.6304 | 0.434324 | 0.331026 |
| 2.4368 | 2.284788 | 2.424067 |

When normalizing the data to the 1st band in repeat2, we use this formula:
 $Y=N/2.5761*3.2142$, (N is the value of repeat2 in S-fig3K-table2); when normalizing the data to the 1st band in repeat3, we use the following formula: $Y=N/3.5635*3.2142$, (N is the value of repeat3 in S-fig3K-table2).

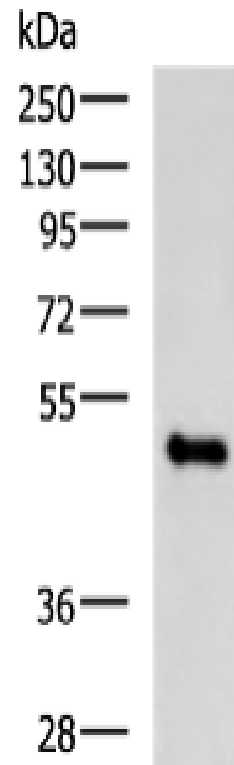
1 .Anti-Flag-mouse MBL (anti-DDDDK-mouse) reference from antibody Datasheet

Applications

Western blotting

Positive control: Fusion protein containing the Flag Tag

Recommended dilution: 1000-5000



Gel: 8%SDS-PAGE

Lysate: 40 µg

Lane: Fusion protein containing the Flag Tag

Primary antibody: D190828(Flag Tag Antibody) at dilution 1/1000

Secondary antibody: Goat anti mouse IgG at 1/5000 dilution

Exposure time: 30 seconds

2.β-actin-mouse MBL reference from antibody Datasheet

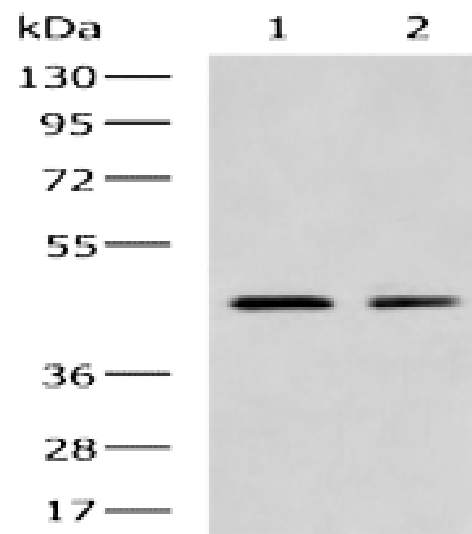
Applications

Western blotting

Predicted band size: 42 kDa

Positive control: Corn leaves lysate and Nicotiana tabacum leaves lysates

Recommended dilution: 500-2000



Gel: 8%SDS-PAGE

Lysate: 40 µg

Lane 1-2: Corn leaves lysate and Nicotiana tabacum leaves lysates

Primary antibody: D191048(plant-actin Antibody) at dilution 1/1000

Secondary antibody: Goat anti mouse IgG at 1/5000 dilution

Exposure time: 5 minutes

3.anti-HA-mouse reference from antibody Datasheet

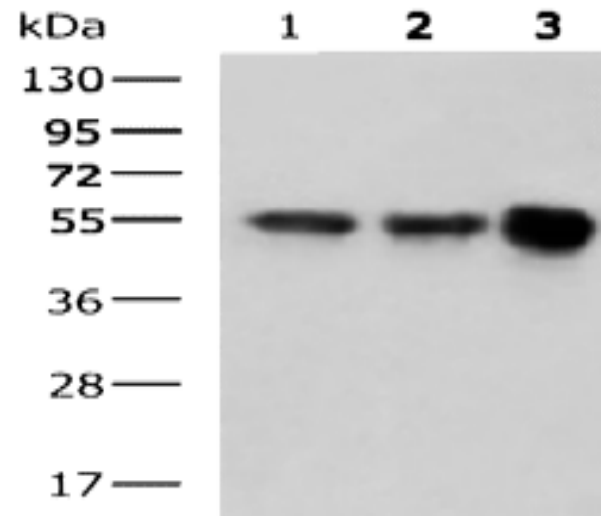
Applications

Western blotting

Predicted band size:55 kDa

Positive control: Fusion proteins containing the HA Tag

Recommended dilution: 20000-100000



Gel: 8%SDS-PAGE

Lysate: 0.01/0.02/0.04 μ g

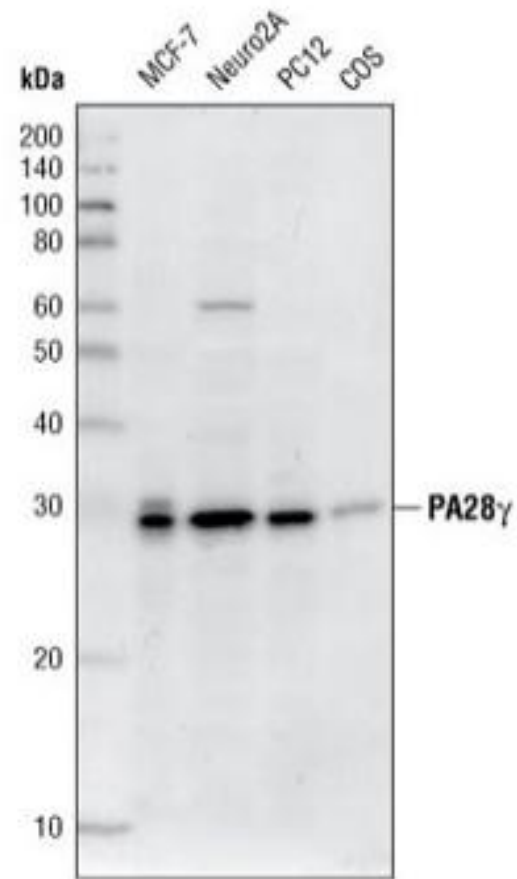
Lane 1-3: 0.01/0.02/0.04 μ g Fusion proteins containing the HA Tag

Primary antibody: D191044(HA tag Antibody) at dilution 1/50000

Secondary antibody: Goat anti mouse IgG at 1/5000 dilution

Exposure time: 30 seconds

4. anti-REG γ -rabbit(γ) reference from antibody Datasheet



Western blot analysis of extracts from MCF-7, Neuro2A, PC12 and COS cells using PA28 γ Antibody.

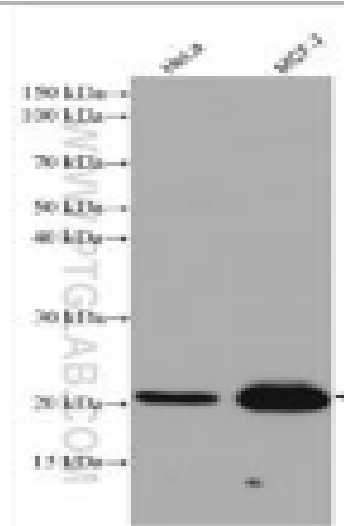
5. anti-NIP30-rabbit Proteintech Rabbit reference from antibody Datasheet

56kDa
36kDa
28kDa
17kDa
6kDa



HeLa cells were subjected to SDS PAGE followed by western blot with 16830-1-AP(NIP30 antibody) at dilution of 1:500 incubated at room temperature for 1.5 hours

6. anti-p21-rabbit reference from antibody Datasheet



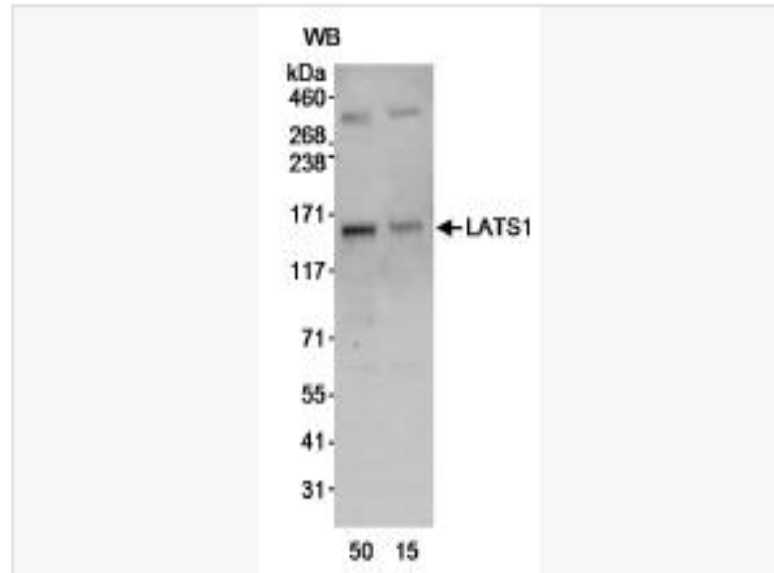
Various lysates were subjected to SDS PAGE followed by western blot with 10355-1-AP (P21 antibody) at dilution of 1:800 incubated at room temperature for 1.5 hours

7. anti-CDC25A-rabbit reference from antibody Datasheet



HepG2 cells were subjected to SDS PAGE followed by western blot with 55031-1-AP(CDC25A antibody) at dilution of 1:1500 incubated at room temperature for 1.5 hours

8. anti-LAST1 –rabbit reference from antibody Datasheet



Western blot - Anti-LATS1/WARTS antibody (ab70562)

All lanes : Anti-LATS1/WARTS antibody (ab70562) at 0.4 $\mu\text{g/ml}$

Lane 1 : HeLa whole cell lysate at 50 μg

Lane 2 : HeLa whole cell lysate at 15 μg

Developed using the ECL technique.

Predicted band size: 127 kDa

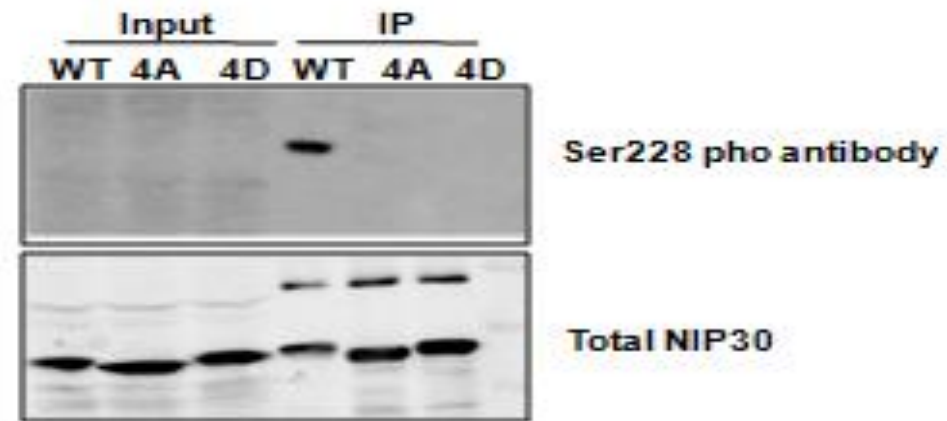
Observed band size: 160 kDa

why is the actual band size different from the predicted?

Additional bands at: 350 kDa. We are unsure as to the identity of these extra bands.

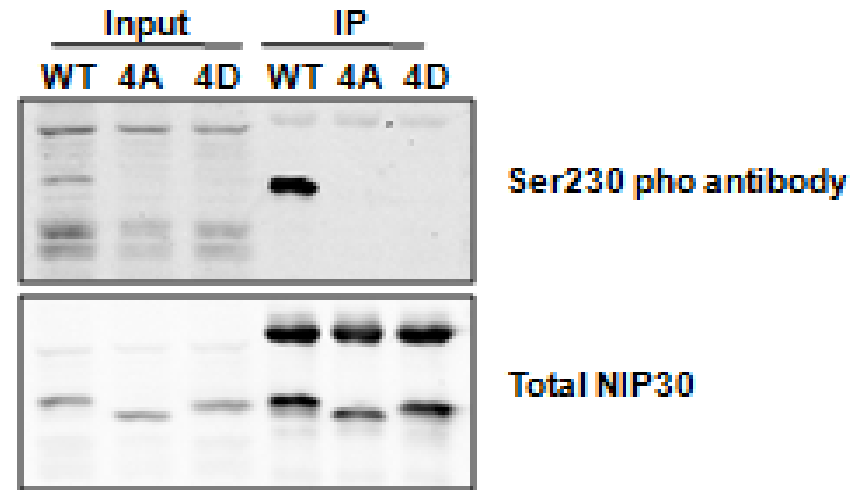
9. pNIP30Ser228 was tested in Supplemental information 4A

A



10. pNIP30Ser230 was tested in Supplemental information 4B

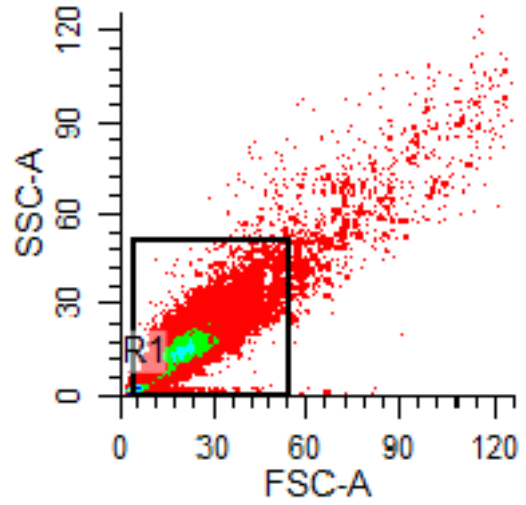
B



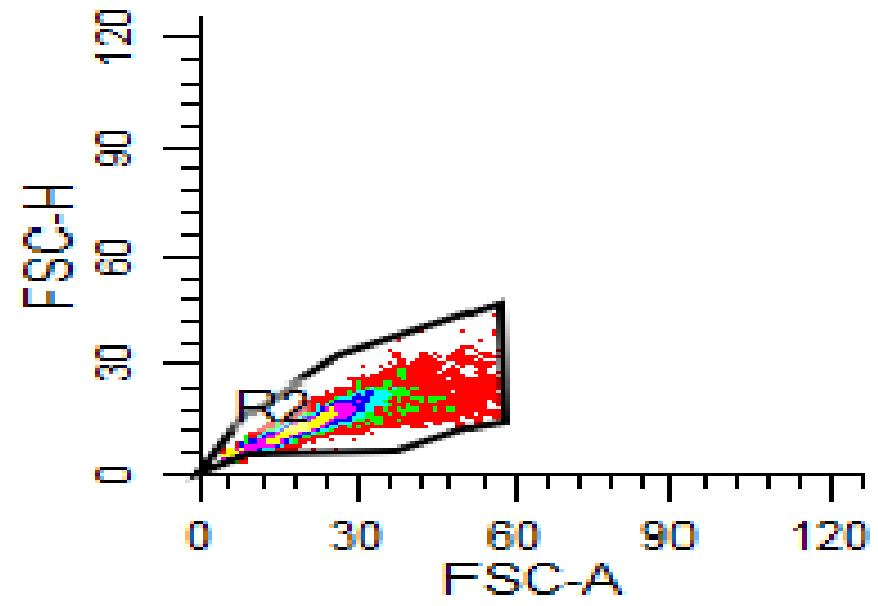
FACS gating strategy

ModFitLT software was used to analysis the FACS data.

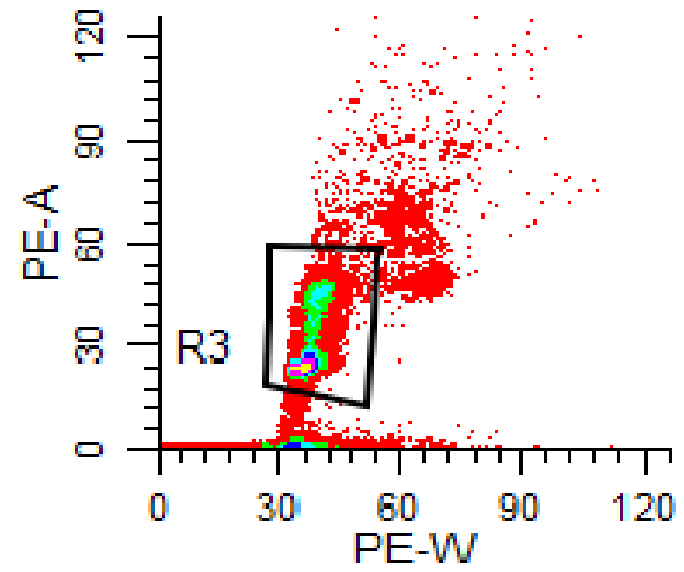
Step 1.



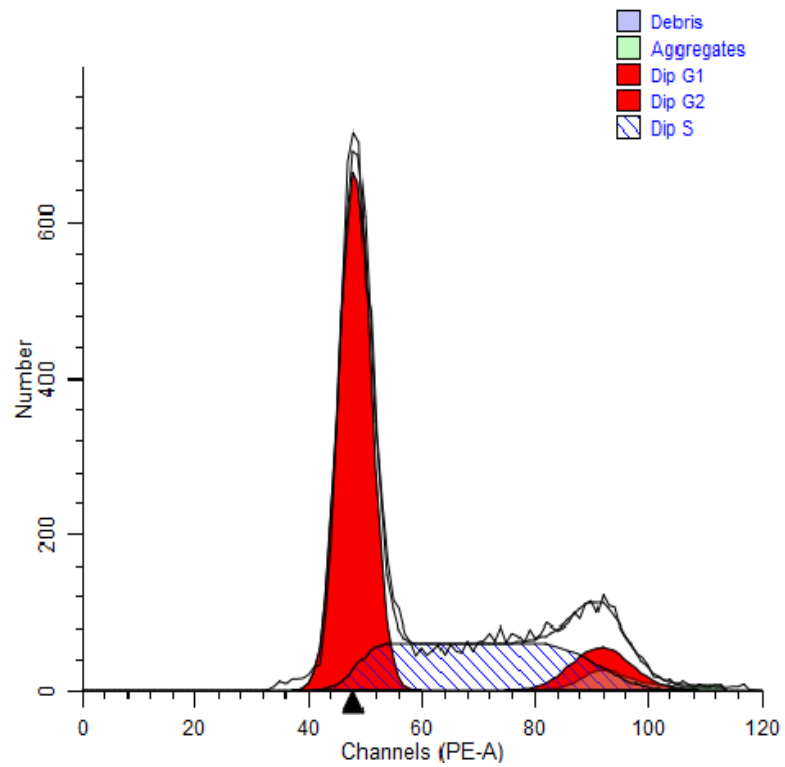
Step2.



Step3.



Step4.



File analyzed: 2019-09-03_1_013.fcs

Date analyzed: 3-Sep-2019

Model: 1DA0n_DSD

Analysis type: Manual analysis

Ploidy Mode: First cycle is diploid

Diploid: 100.00 %

Dip G1: 58.03 % at 48.35

Dip G2: 9.19 % at 91.86

Dip S: 32.78 % G2/G1: 1.90

%CV: 5.81

Total S-Phase: 32.78 %

Total B.A.D.: 1.84 %

Debris: 0.15 %

Aggregates: 4.05 %

Modeled events: 8522

All cycle events: 8164

Cycle events per channel: 183

RCS: 1.868