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## Supplementary information

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# SKI complex loss renders 9p21.3-deleted or MSI-H cancers dependent on PELO

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In the format provided by the  
authors and unedited

## **SKI complex loss renders 9p21.3-deleted or MSI-H cancers dependent on PELO**

Patricia C Borck<sup>1</sup>, Isabella Boyle<sup>1</sup>, Kristina Jankovic<sup>2</sup>, Nolan Bick<sup>1</sup>, Kyla Foster<sup>1</sup>, Anthony C Lau<sup>1</sup>, Lucy I. Parker-Burns<sup>2</sup>, Daniel A. Lubicki<sup>1</sup>, Tianxia Li<sup>2</sup>, Ashir A Borah<sup>1</sup>, Nicholas J Lofaso<sup>2</sup>, Sohani Das Sharma<sup>2</sup>, Tessla Chan<sup>2</sup>, Riya V. Kishen<sup>2</sup>, Anisah Adeagbo<sup>1</sup>, Srivatsan Raghavan<sup>1,3</sup>, Elisa Aquilanti<sup>1,4</sup>, John R Prensner<sup>1,5</sup>, J Michael Krill-Burger<sup>1</sup>, Todd R Golub<sup>1,6</sup>, Catarina D Campbell<sup>1</sup>, Joshua M Dempster<sup>1</sup>, Edmond M Chan<sup>1,2,7,8#</sup>, Francisca Vazquez<sup>1#</sup>

The following files can be found on Supplementary Information:

- **Supplementary Figure S1:** Western blot images
- **Supplementary Table S1:** Cell line information
- **Supplementary Table S2:** CRISPR guide sequence
- **Supplementary Table S3:** Antibody information
- **Supplementary Table S4:** Plasmids

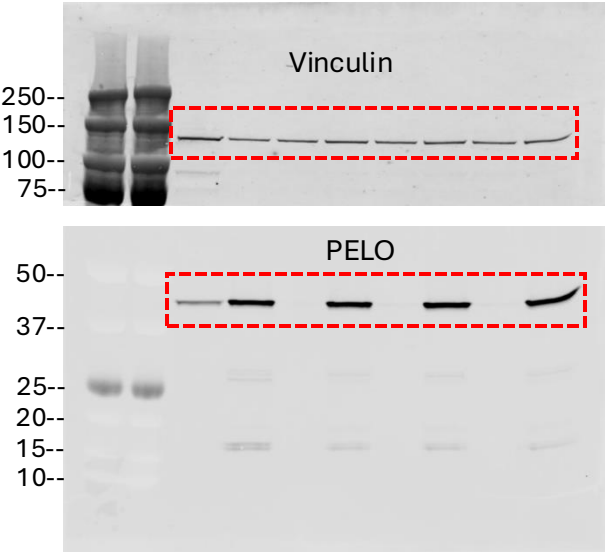
The following files can be found on Figshare:

- **9p21\_3\_relative\_copy\_number.csv** - relative copy number for 9p21.3 cytoband
- **TTC37\_transcript\_expr.csv** - transcript expression for select TTC37 transcripts, taken from DepMap 23Q4 Public
- **microsatellite\_repeats.csv** - repeat lengths extracted from MSISensor2
- **TCGA\_alterations.csv** - alterations of certain genes in TCGA downloaded from cBioPortal
- **readcounts\_9p21\_3.csv** - raw readcounts from CRISPR modifier
- **guide\_map\_9p21\_3.csv** - map of guides to genes in CRISPR modifier
- **sequence\_map\_9p21\_3.csv** - map of sequences (replicates) to conditions (cell line names) in CRISPR modifier screen
- **RSEM\_expected\_count\_RNAseq.csv** - RSEM expected counts for RNAseq
- **sample\_metadata\_RSEMseq.csv** - metadata for samples in RNAseq
- **hallmark\_gene\_sets\_2023.csv** - Hallmark gene sets from mSigDB (h.all.v2023.2.Hs.symbols.gmt) reformatted to CSV
- **Ch2\_KO\_DOXpos\_vs\_neg\_deseq2.csv** - DESeq2 results from KP4 with Ch2 KO for DOX+ vs DOX- condition
- **Ch2\_KO\_DOXpos\_vs\_neg\_gsea\_prerank.csv** - GSEA pre-rank results from KP4 with Ch2 KO for DOX+ vs DOX- condition
- **DOXneg\_FOCAD\_vs\_Ch2\_KO\_deseq2.csv** - DESeq2 results from KP4 with Ch2 KO vs FOCAD KO for DOX- condition
- **DOXneg\_FOCAD\_vs\_Ch2\_KO\_gsea\_prerank.csv** - GSEA pre-rank results with Ch2 KO vs FOCAD KO for DOX- condition
- **FOCAD\_KO\_DOXpos\_vs\_neg\_deseq2.csv** - DESeq2 results from KP4 with FOCAD KO for DOX+ vs DOX- condition

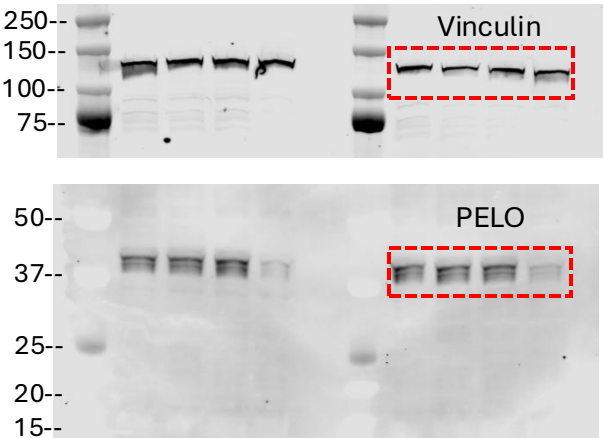
- **FOCAD\_KO\_DOXpos\_vs\_neg\_gsea\_prerank.csv** - GSEA pre-rank results from KP4 with FOCAD KO for DOX+ vs DOX- condition

# Supplementary Figure 1

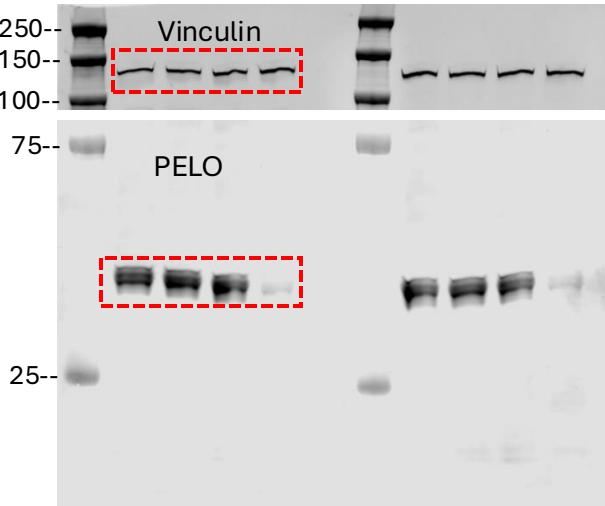
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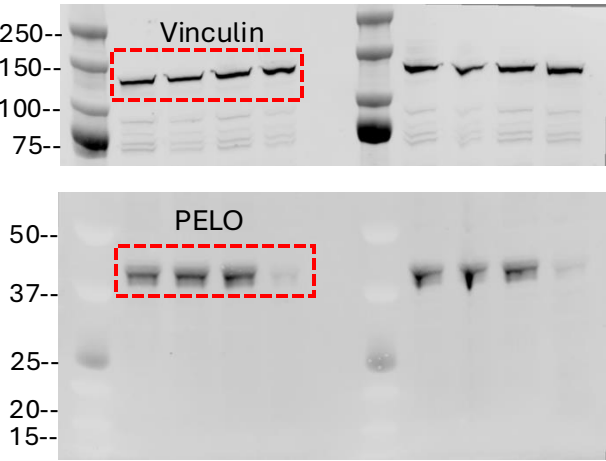
Used in Figure 2c left panel



Used in Figure 2c middle panel

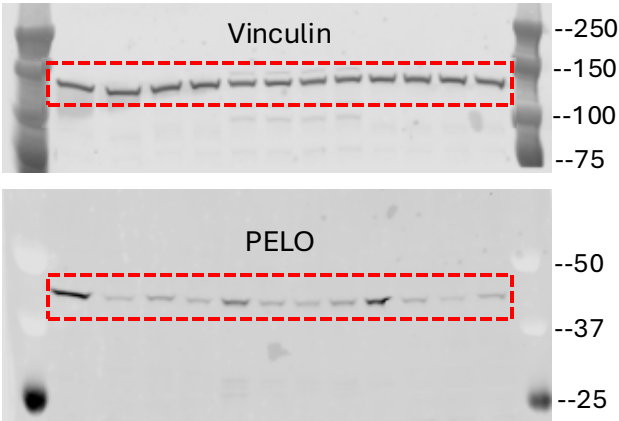


Used in Figure 2c right panel

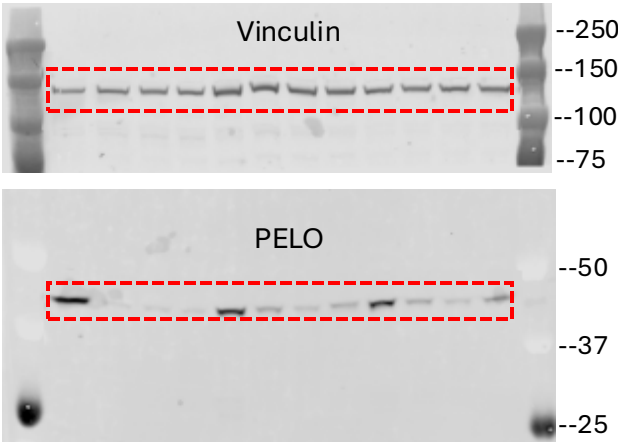


# Supplementary Figure 1

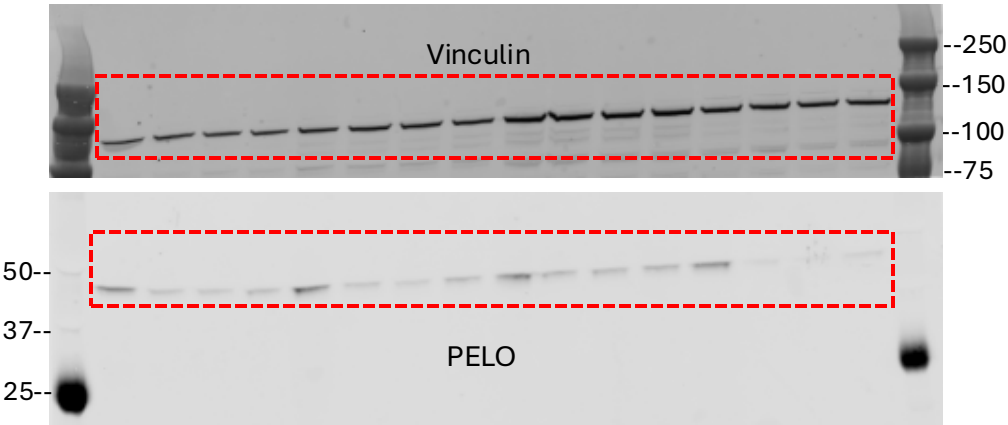
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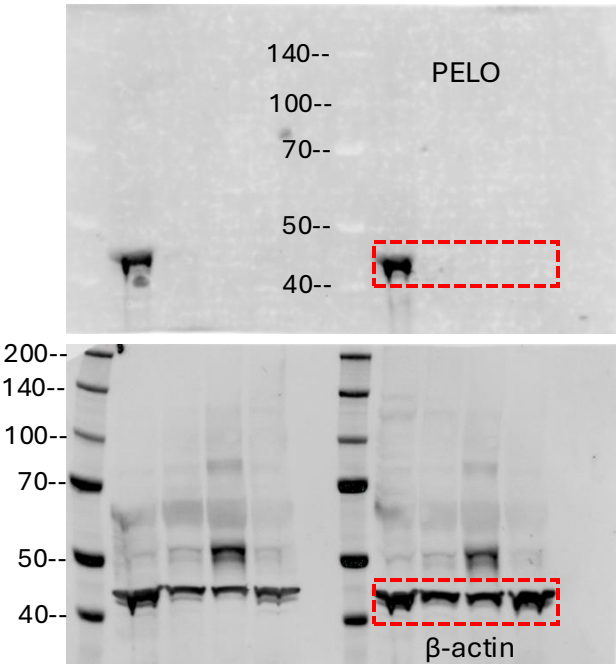
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Used in Ext Figure 2b lower panel

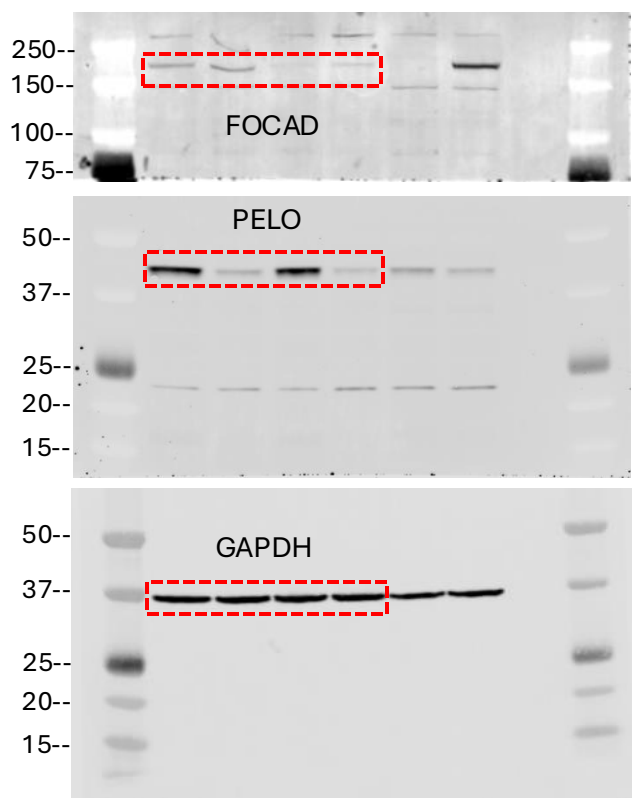


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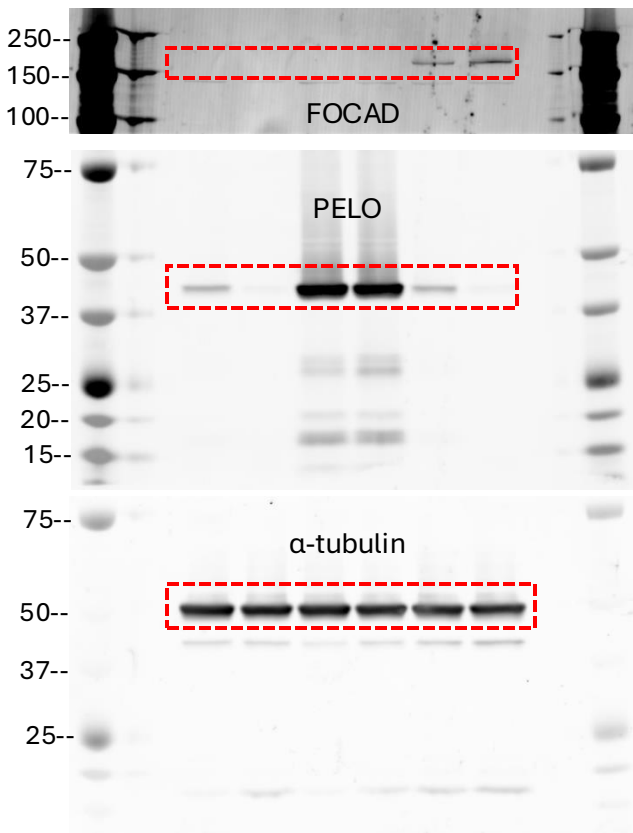


# Supplementary Figure 1

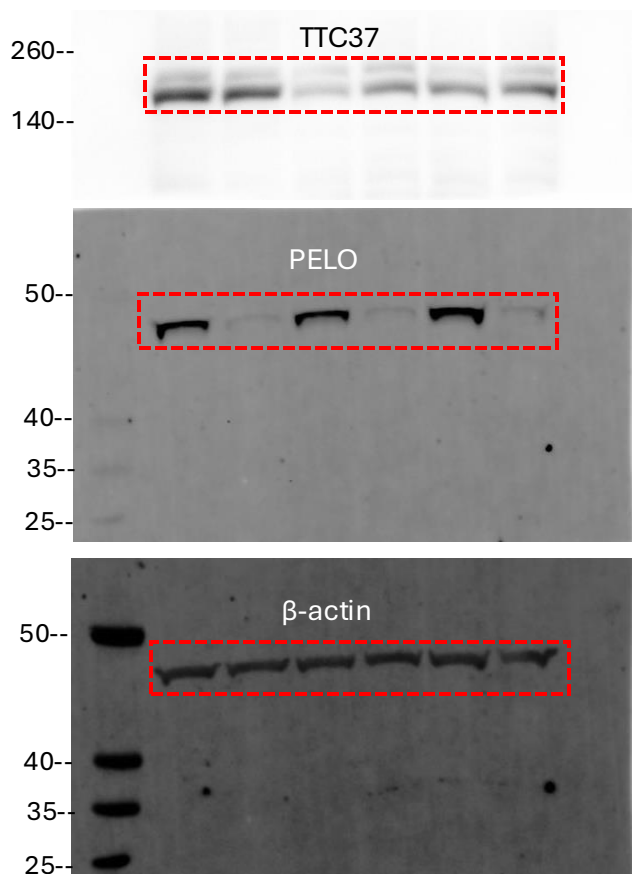
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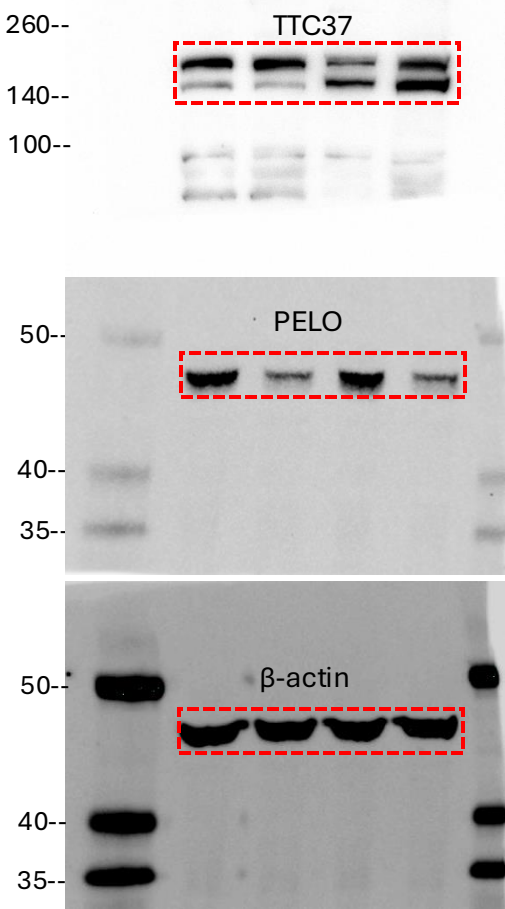
Used in Figure 3c upper panel



Used in Figure 3f upper panel

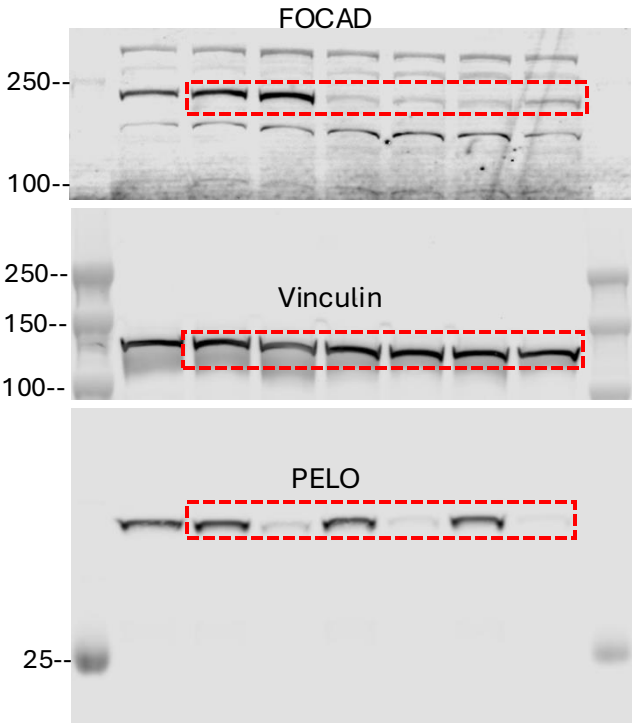


Used in Figure 3g upper panel

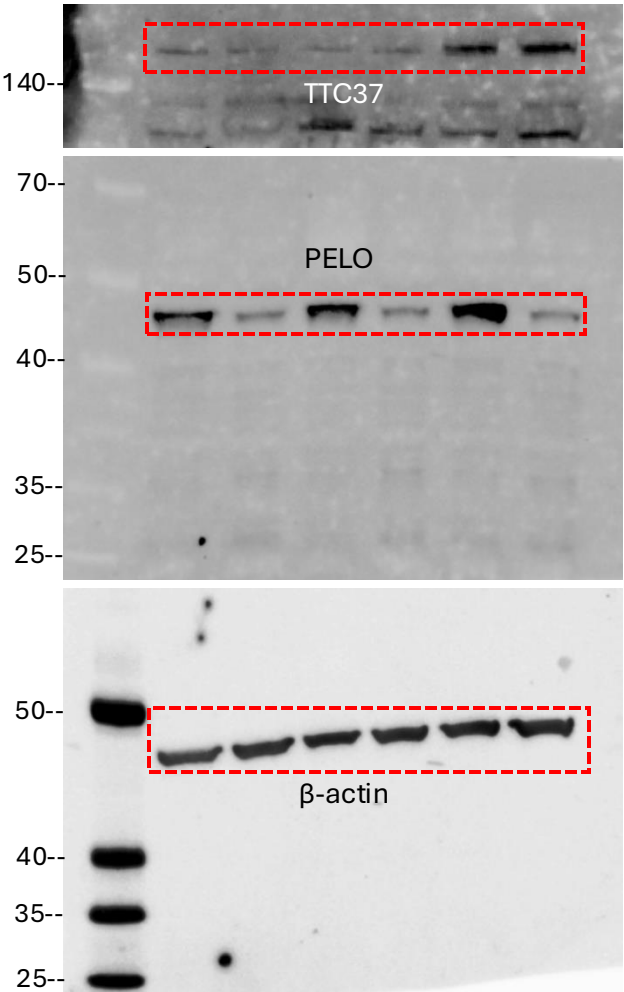


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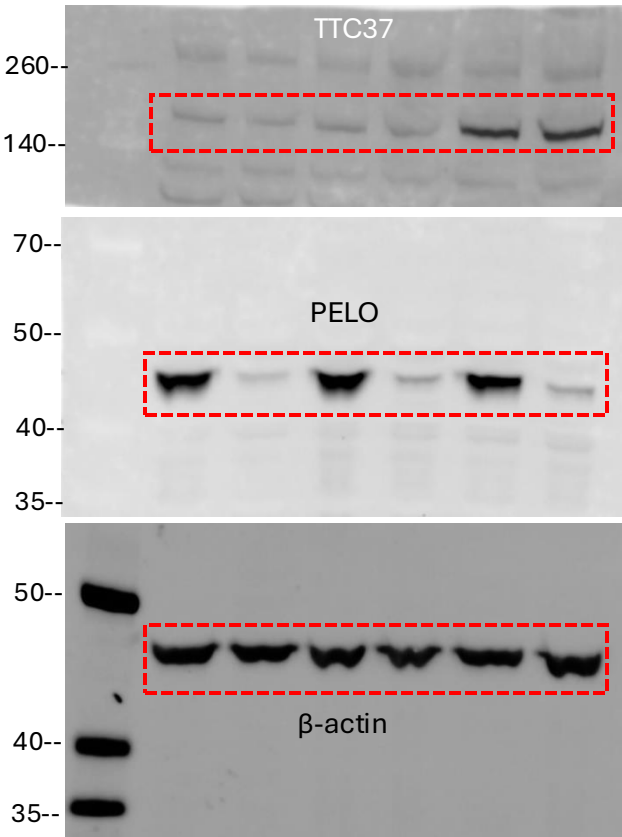
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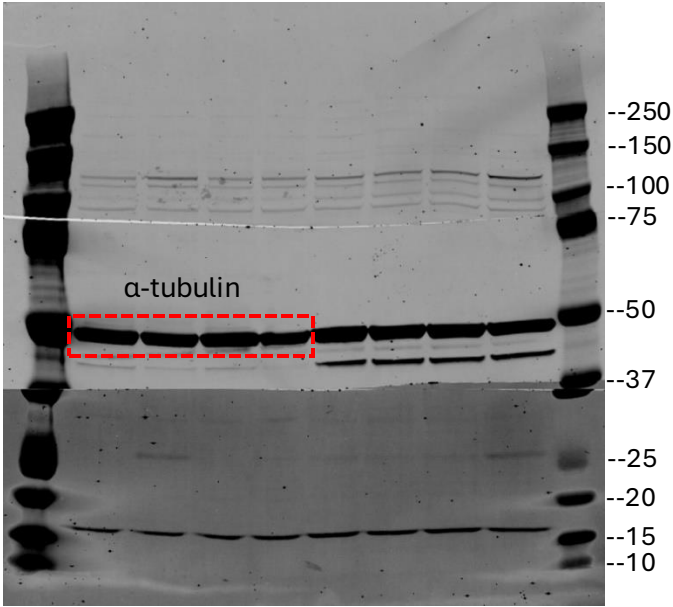


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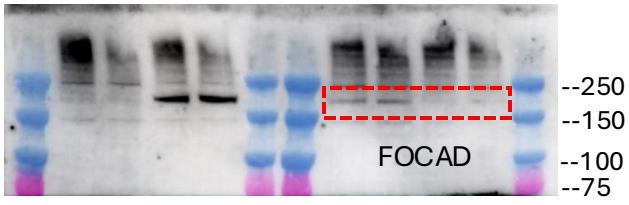
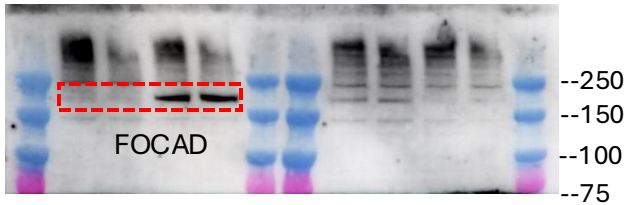
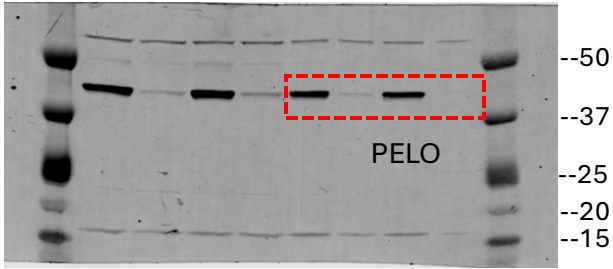
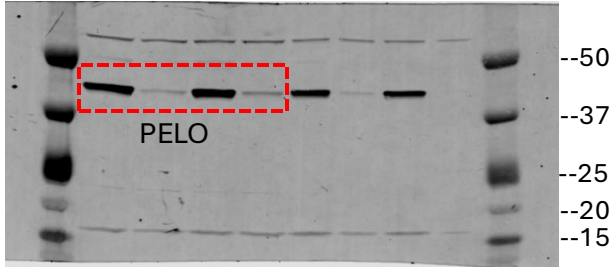
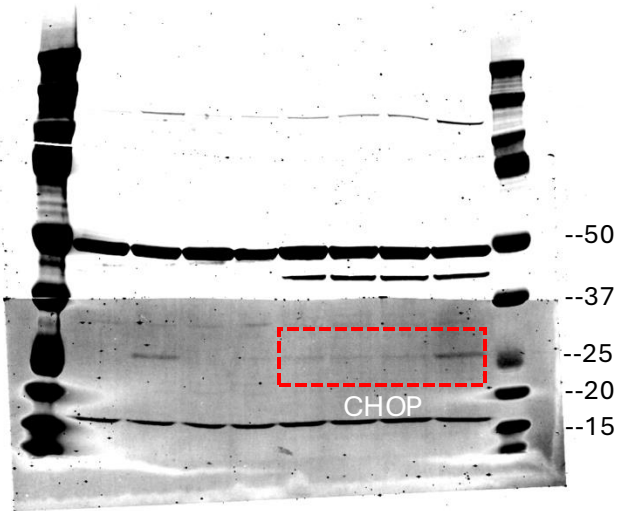
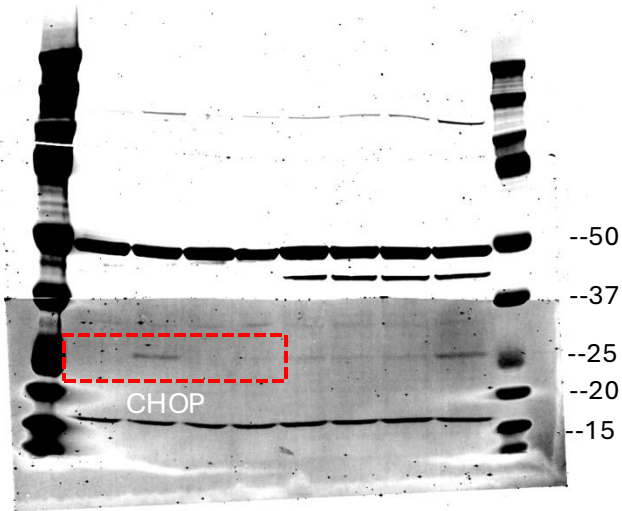
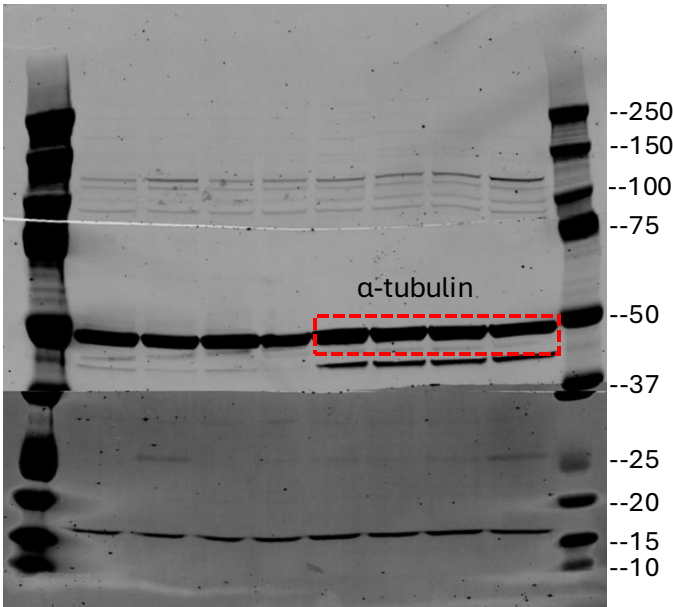


Supplementary Figure 1

Used in Figure 4e



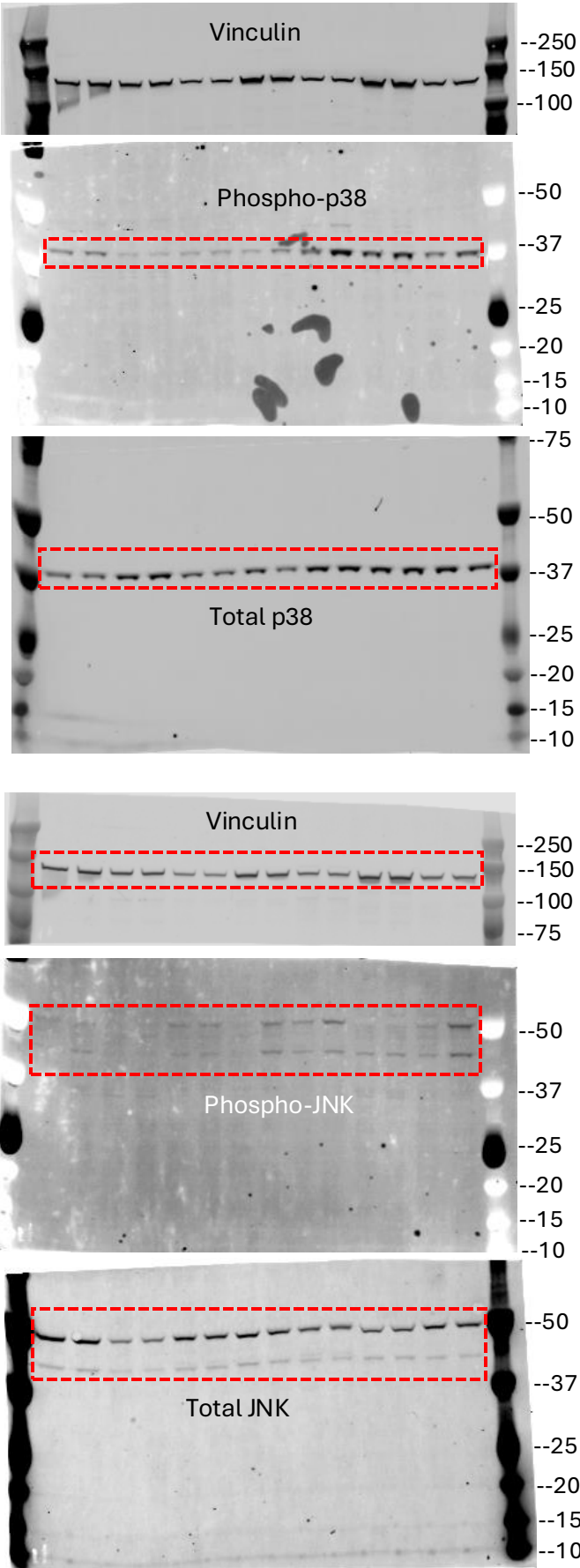
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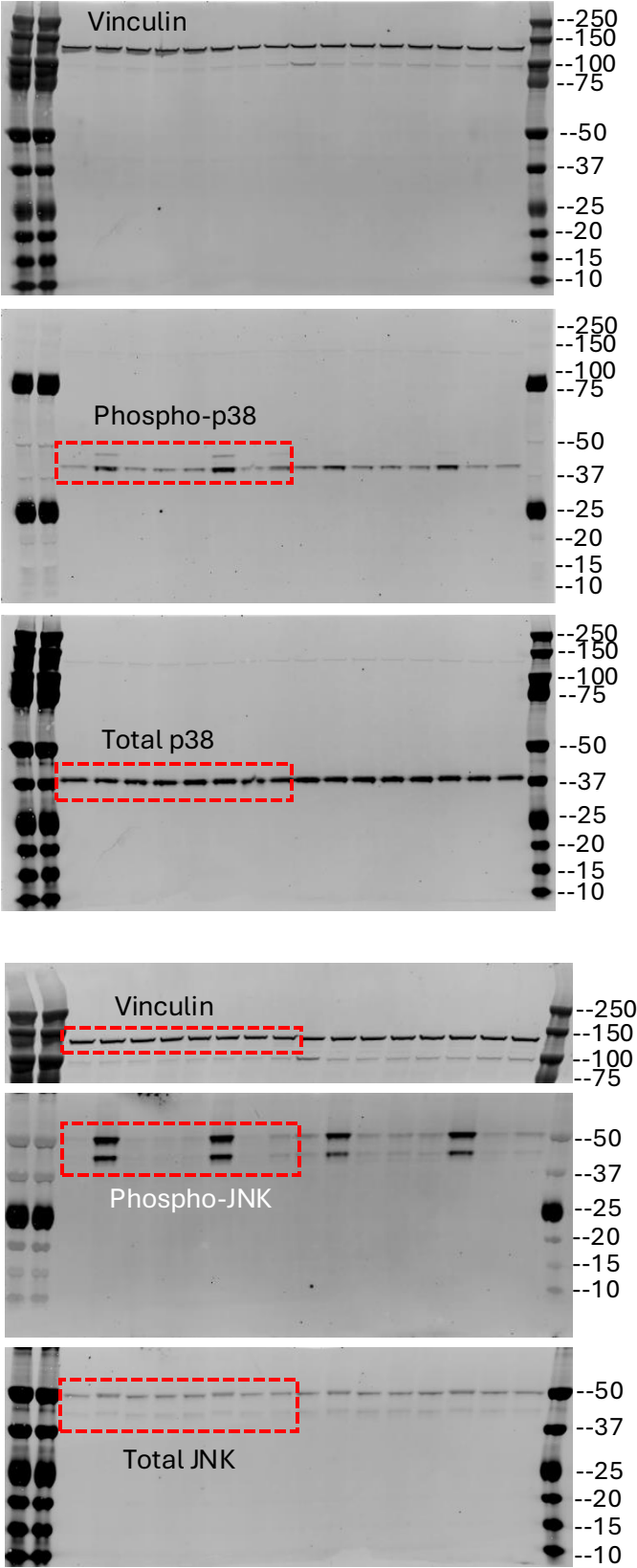


# Supplementary Figure 1

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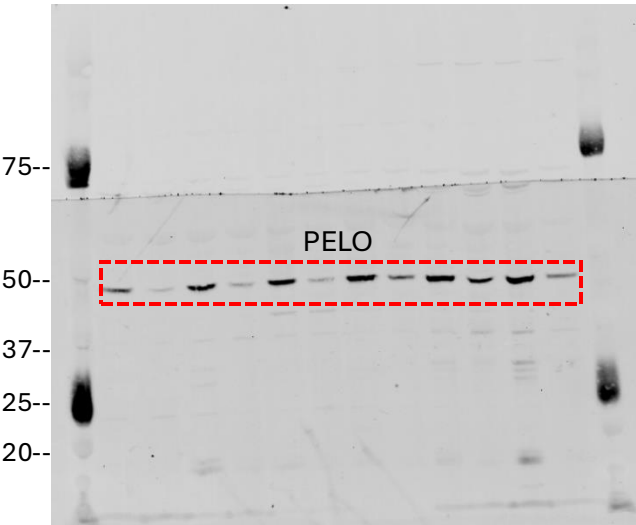
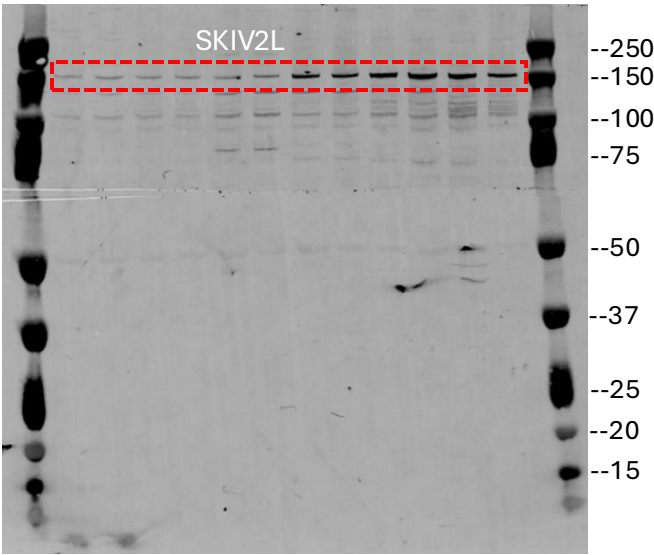
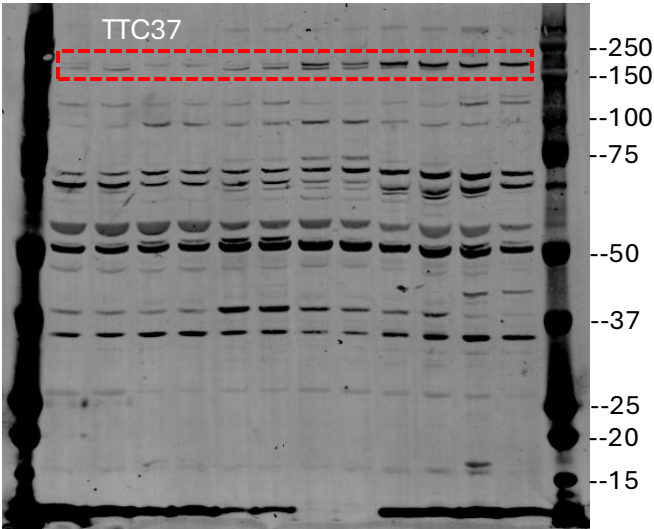


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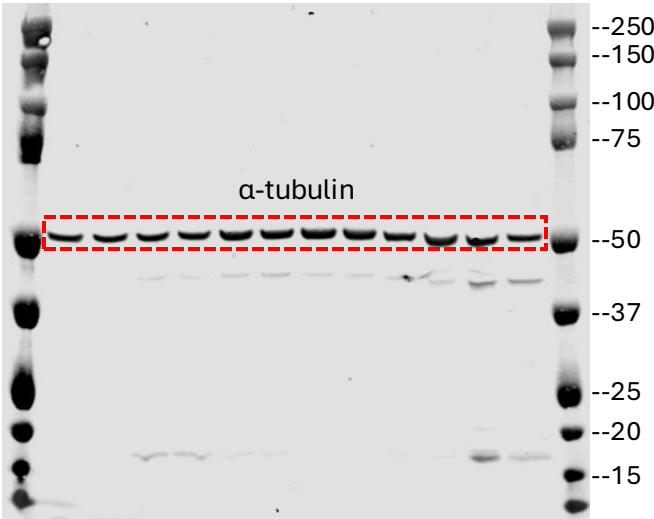


Supplementary Figure 1

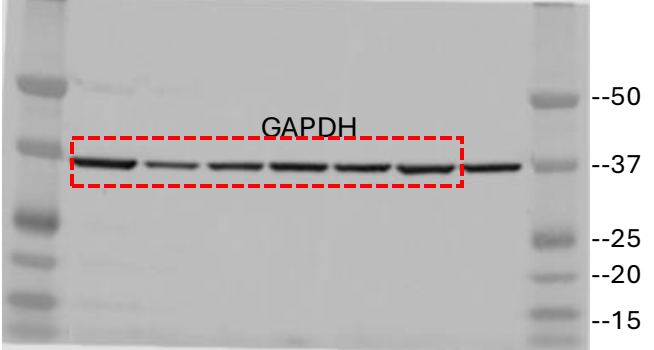
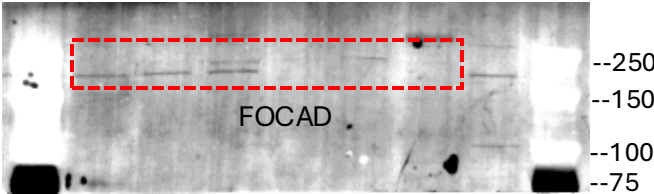
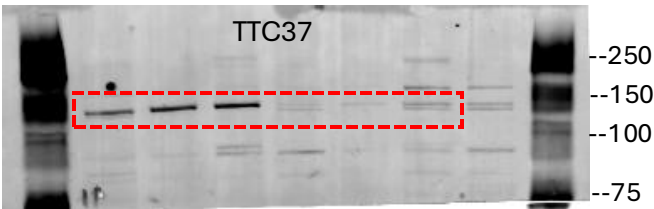
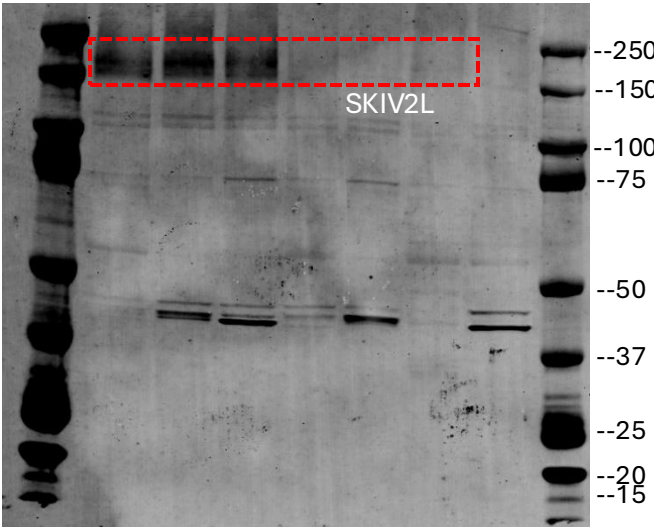
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Used in Ext Figure 4c

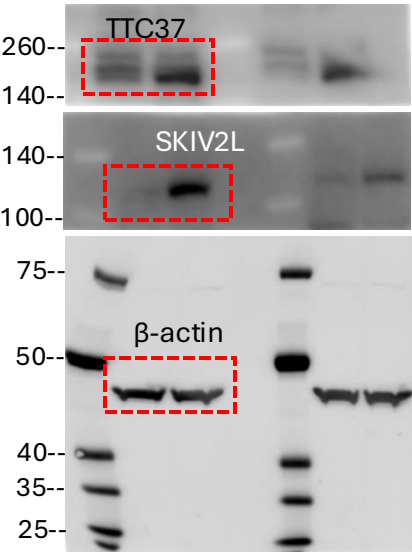


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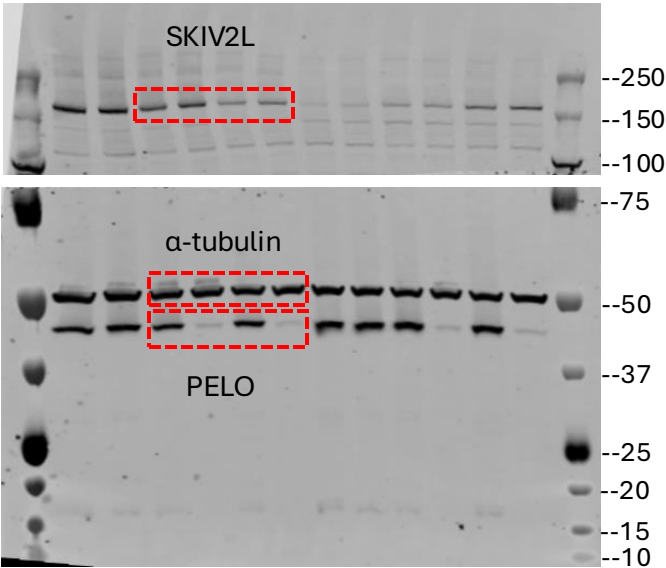


# Supplementary Figure 1

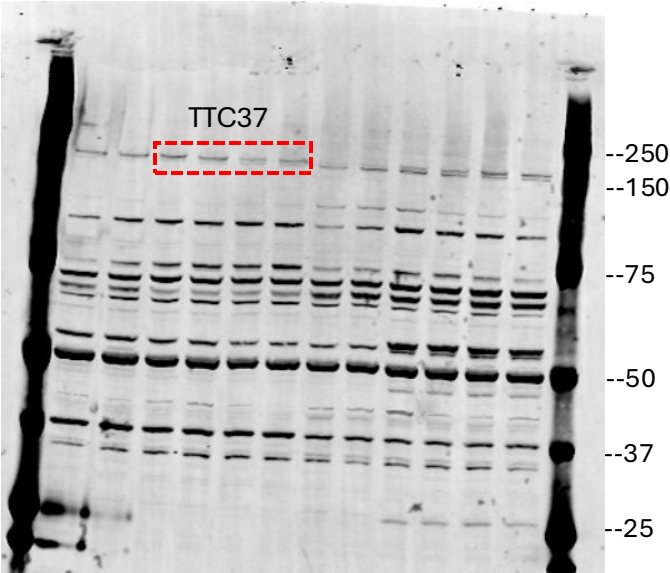
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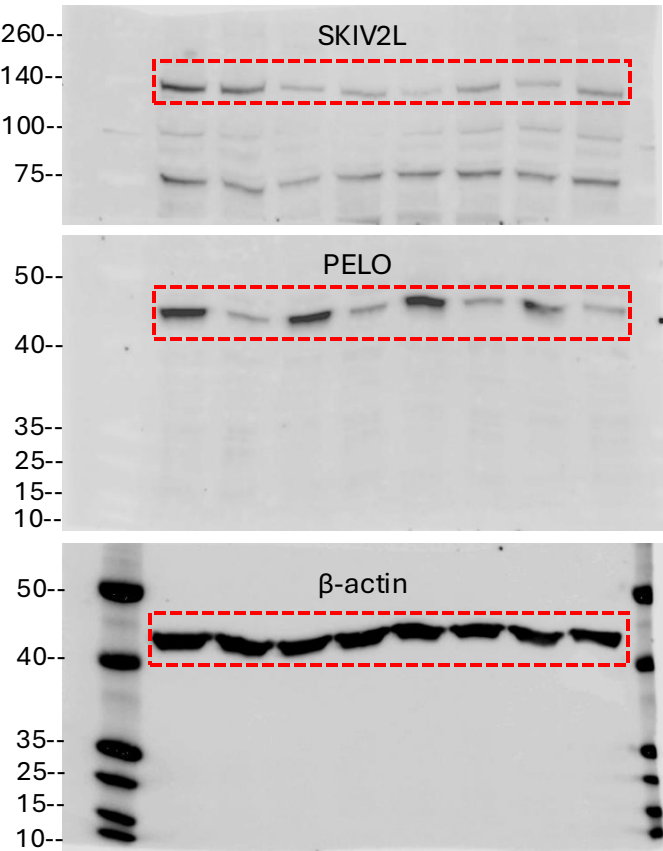
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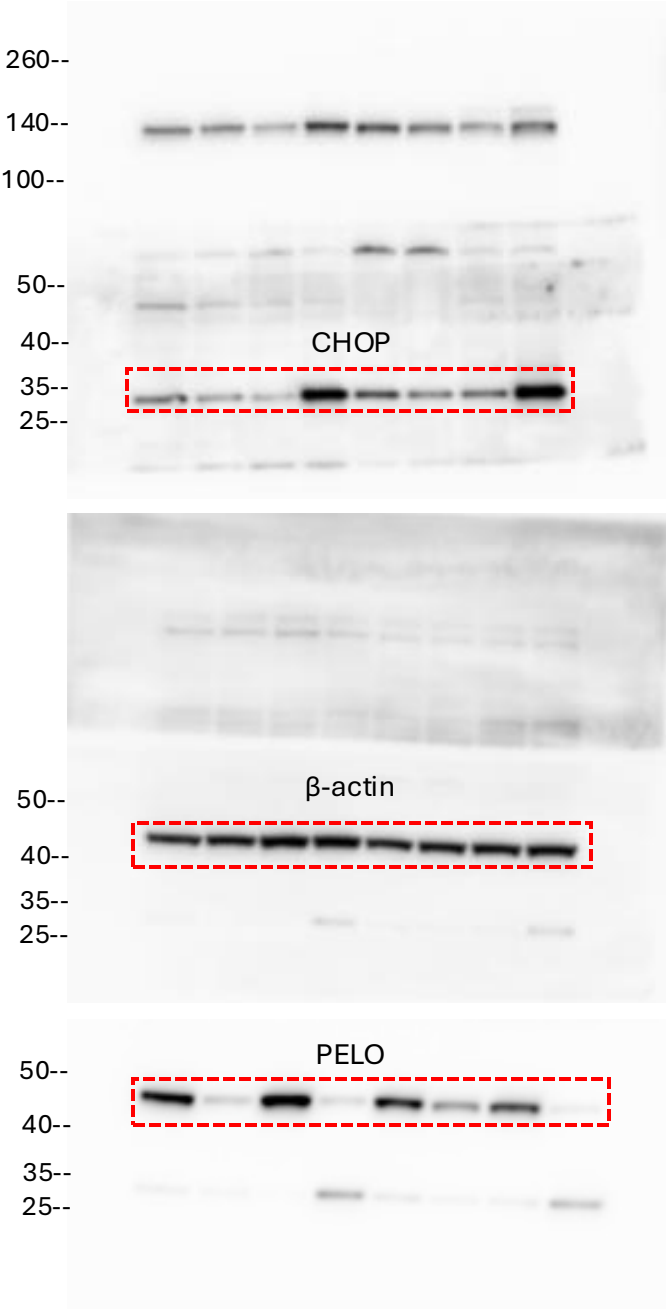


Used in Ext Figure 5c



# Supplementary Figure 1

Used in Ext Figure 5h



**Supplementary Table S1**

| <b>Cell Line:</b> | <b>Media Condition:</b>                                                                                                                        | <b>Source</b>               |
|-------------------|------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| MIA PaCa-2        | DMEM - 4.5g/l D-glucose, L-glutamine, 110mg/L sodium pyruvate (Gibco, 11995073 or Corning, 10-013CV)                                           | ATCC                        |
| KM12              | RPMI1640 - L-glutamine (Corning, 10-040-CV)                                                                                                    | JCRB                        |
| DLD1              | RPMI1640 (Corning, 10-040-CV)                                                                                                                  | Academic: Schvartzman's Lab |
| HCT 116           | McCoy's 5A - L-glutamine (Gibco, 16600082)                                                                                                     | ATCC                        |
| KP4               | DMEM/F12 - L-glutamine, 2.43 g/L sodium bicarbonate (Gibco, 11320033)                                                                          | RIKEN                       |
| 293T              | DMEM (Gibco; 11995073); 10% FBS ONLY; no PSG                                                                                                   | ATCC                        |
| SW620             | Leibovitz's L-15 - L-glutamine (Gibco, 11415-064) 10% FBS                                                                                      | ATCC                        |
| SW837             | RPMI-1640 - L glutamine (Corning, 10-040-CV) 10% FBS                                                                                           | ATCC                        |
| SF295             | RPMI-1640 - L glutamine (Corning, 10-040-CV) 10% FBS                                                                                           | Academic lab: NCI/DCTD      |
| GB1               | DMEM - 4.5g/l D-glucose, L-glutamine, 110mg/L sodium pyruvate (Gibco, 11995073 or Corning, 10-013CV)                                           | HSRRB                       |
| SU.86.86          | RPMI-1640 - L glutamine (Corning, 10-040-CV) 10% FBS                                                                                           | ATCC                        |
| IGROV-1           | RPMI-1640 - L glutamine (Corning, 10-040-CV) 10% FBS                                                                                           | Academic lab: NCI/DCTD      |
|                   |                                                                                                                                                |                             |
|                   |                                                                                                                                                |                             |
|                   | <b>All media was supplemented with:</b>                                                                                                        |                             |
|                   | PSG (penicillin 100 µg ml <sup>-1</sup> , streptomycin 100 µg ml <sup>-1</sup> , and l-glutamine 292 µg ml <sup>-1</sup> ; Corning, 30-009-CI) |                             |
|                   | 10% FBS (Gemini Bio-products, 100-106, or Sigma, F4135)                                                                                        |                             |

| Supplementary Table S2 |                             |                         |
|------------------------|-----------------------------|-------------------------|
| <u>gRNA</u>            | <u>CRISPR KO or CRISPRi</u> | <u>Sequence</u>         |
| gRNA Ch2-2             | CRISPRi                     | GGTGTGCGTATGAAGCAGTG    |
| gRNA PELO#1            | CRISPRi                     | CGCTGCGTCTAACAGGCTCG    |
| gRNA PELO#2            | CRISPRi                     | TGCCAGCGGGAAGTGTGTAG    |
| gRNA PELO#8            | CRISPRi                     | CCGCTGGCAGCACGCAACAG    |
| gRNA POLR2D            | CRISPRi                     | CGGCGGGTGGCAGCGATCCG    |
| gRNA SF3B1             | CRISPRi                     | AAGATCGCCAAGACTCACGA    |
| gRNA Ch2               | AsCas12a CRISPR KO          | GTAAAACCCTACTAATATTCCAC |
| gRNA FOCAD#2           | AsCas12a CRISPR KO          | AATCACCCCAACTAACCTCCAGG |
| gRNA FOCAD#5           | AsCas12a CRISPR KO          | AAGTGGAGCGAATGCAAACAACC |
| gRNA SKIV2L#1          | AsCas12a CRISPR KO          | GCCTCACGTACACTATGATCCTC |
| gRNA SKIV2L#2          | AsCas12a CRISPR KO          | TTGCTGGACTCCCGAGGAGCCTT |
| gRNA SKIV2L#5          | AsCas12a CRISPR KO          | TCTCTCCCATAGTGGGCATTTGA |
| gRNA TTC37#6           | AsCas12a CRISPR KO          | AAATAGTATAGTCCTCGCCTAAG |
| gRNA TTC37#9           | AsCas12a CRISPR KO          | GTTGTTACAGACTGCAGAAGACC |

**Supplementary Table S3**

| <b>Antibody</b>                                | <b>Brand/Catalog Number</b>       | <b>Dilution</b> |
|------------------------------------------------|-----------------------------------|-----------------|
| PELO                                           | Abcam 140615                      | 1:500           |
| FOCAD                                          | Novus NBP2-49163                  | 1:500           |
| FOCAD                                          | Millipore Sigma HPA055015         | 1:500           |
| Vinculin                                       | ThermoFisher Scientific MA5-11690 | 1:4000          |
| GAPDH                                          | Cell Signaling Technology, 5174S  | 1:2000          |
| TTC37 "Ski3"                                   | Proteintech 24594-1-AP            | 1:500           |
| SKIV2L                                         | Proteintech 11462-1-AP            | 1:1000          |
| phospho-p38 (Thr180/Tyr182)                    | Cell Signaling Technology 9211S   | 1:1000          |
| total-p38                                      | Cell Signaling Technology 9212S   | 1:1000          |
| phospho-JNK/SAPK (Thr183-Tyr185)               | Cell Signaling technology 4668T   | 1:1000          |
| total-JNK/SAPK                                 | Cell Signaling Technology 9252S   | 1:1000          |
| alpha-TUBULIN                                  | Millipore Sigma T6793             | 1:4000          |
| β-Actin                                        | Millipore Sigma, A5441            | 1:2000          |
| Vinculin                                       | Proteintech, 66305-1-Ig           | 1:2000          |
| DDIT3/CHOP                                     | Abcam, ab11419                    | 1:1000          |
| DDIT3/CHOP                                     | ABclonal, A21902                  | 1:500           |
|                                                |                                   |                 |
| <b>Protein Ladder</b>                          | <b>Brand/Catalog Number</b>       |                 |
| Spectra™ Multicolor Broad Range Protein Ladder | Thermo Scientific - 26634         |                 |
| Precision Plus Protein Dual Color Standards    | Bio-Rad - 1610374                 |                 |

| Supplementary Table S4             |                         |
|------------------------------------|-------------------------|
| Plasmid:                           | Addgene Deposit Number: |
| pRDA355_sgCiPELO #2                | 229020                  |
| pRDA355_sgCiCh2-2                  | 229021                  |
| pRDA355_sgCiPOLR2D                 | 229022                  |
| pRDA355H_sgCiPELO #2               | 228430                  |
| pRDA355H_sgCiCh2-2                 | 228431                  |
| pRDA355H_sgCiPOLR2D                | 228432                  |
| pLenti_Puro_T2A_Firefly Luciferase | 228433                  |
| pLenti_Puro_T2A_TTC37              | 228434                  |
| pRDA052H_sgCh2                     | 229023                  |
| pRDA052H_sgFOCAD #2                | 229024                  |
| pRDA052H_sgTTC37 #6                | 228437                  |
| pRDA052H_sgSKIV2L #5               | 228438                  |
| pLX313_FOCAD                       | 229025                  |
| pRDA052H_sgSKIV2L #1               | 228440                  |
| pRDA052H_sgSKIV2L #2               | 228441                  |
| pRDA052H_sgTTC37 #9                | 228442                  |
| pRDA_052_HygroR                    | 228937                  |
| pXPR_051_dCas9-KRAB-MeCP2          | 228936                  |
| pXPR_051d_sgCiChr2-2               | 228940                  |
| pXPR_051d_sgCiPELO #1              | 229017                  |
| pXPR_051d_sgCiPELO #2              | 229018                  |
| pXPR_023_dCas9-KRAB                | 228931                  |
| pXPR_023d_sgCiPELO #1              | 228932                  |
| pXPR_023d_sgCiPELO #2              | 228933                  |
| pXPR_023d_sgCiPELO #8              | 228934                  |
| pRDA_174_neoR                      | 228935                  |
| pLX313 PELO                        | 228938                  |