# Golgi-localized Ring Finger Protein 121 is necessary for MYCN-driven neuroblastoma tumorigenesis

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[#] Joint first authors

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## Supplementary Figure S1

### Chromatograph of *Rnf*121

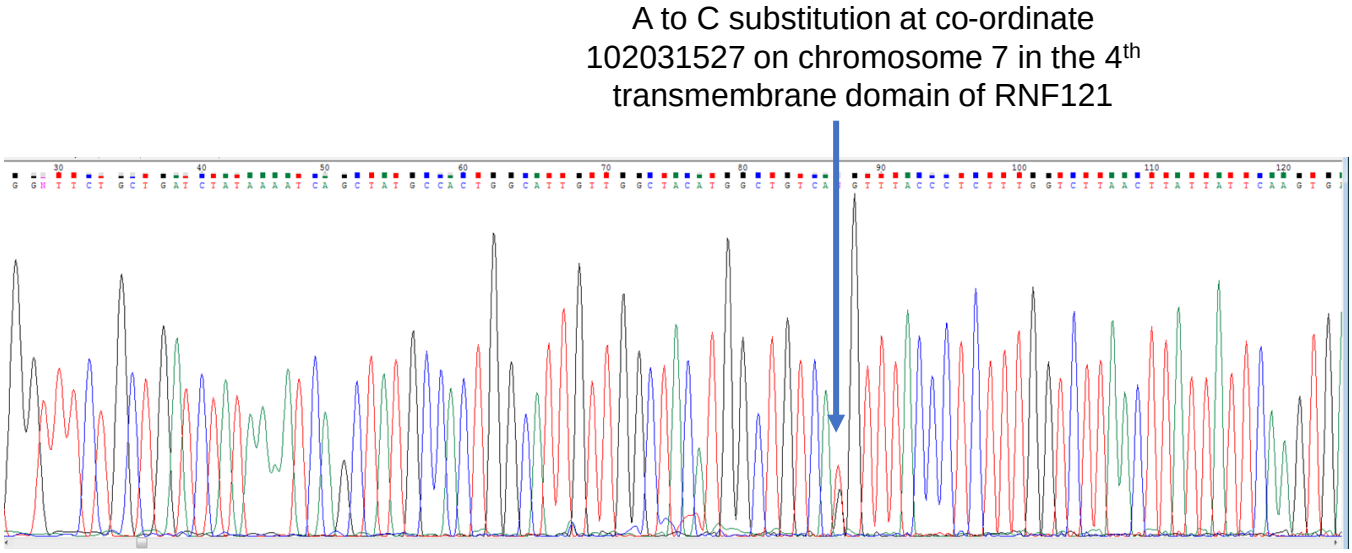
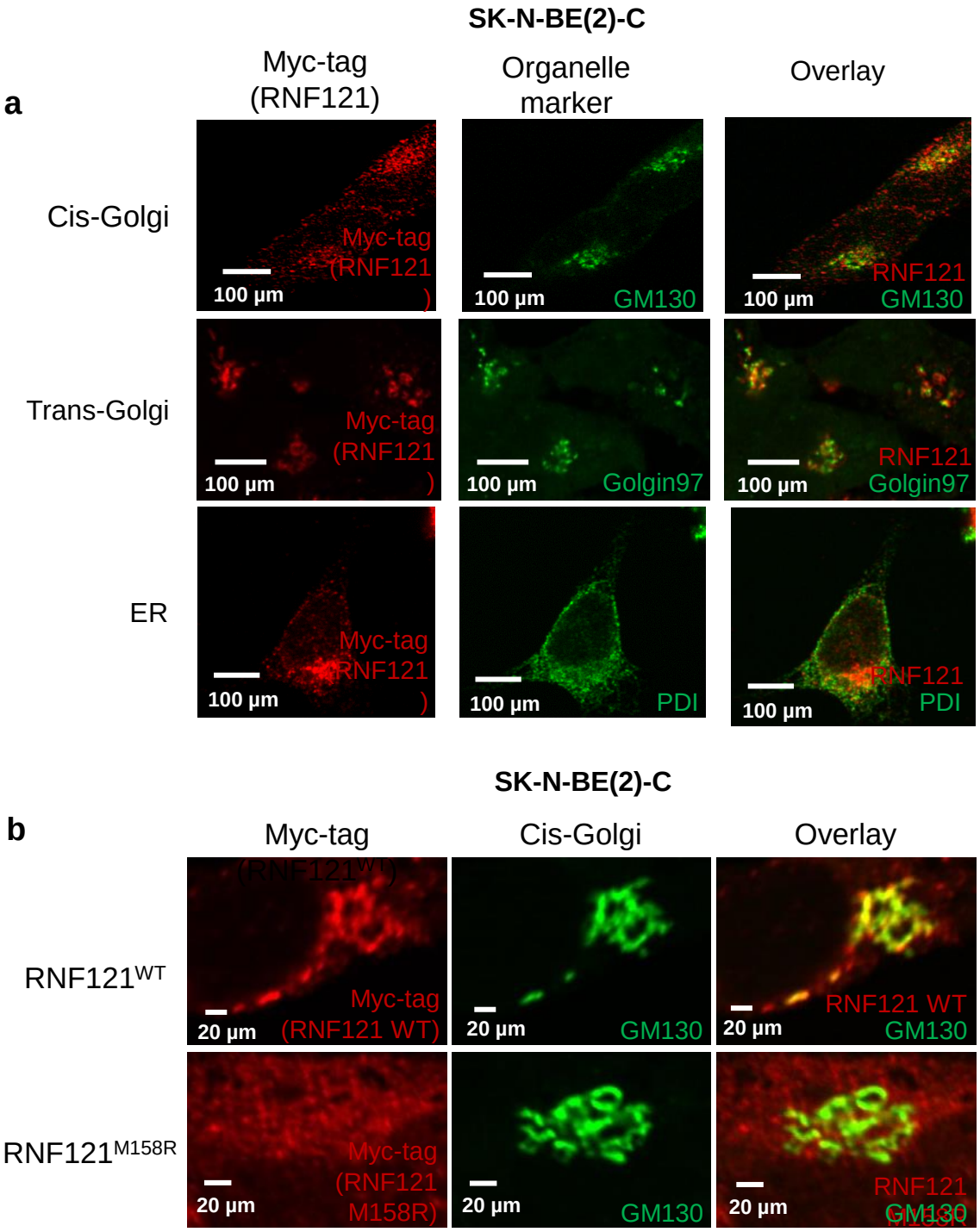



**Fig. S1 A germline RNF121M158R mutation is associated with the tumor suppressed phenotype in Th-MYCN mice.** A representative chromatograph of mouse chromosome 7 DNA encoding the germline Rnf121 gene sequence from a 1929-derived and tumor-suppressed mouse showing heterozygosity of A and C at position 102031527 which is predicted to result in a methionine to arginine amino acid substitution at position 158 in the RNF121 protein.

**Supplementary Figure S2**



**KELLY**

**c**

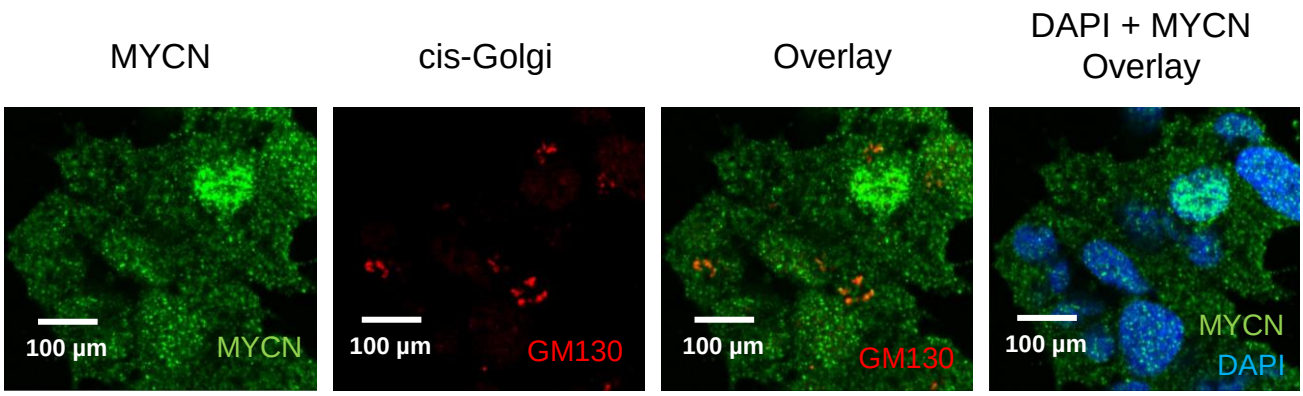



**Fig. S2 *RNF121*$^{M158R}$ mutation disrupts localisation to the *cis*-Golgi. a** Immunofluorescent staining using: an anti-Myc-tag antibody for the transfected Myc-tagged RNF121$^{WT}$ protein, an anti-GM130 antibody as a cis-Golgi marker, an anti-Golgin-97 antibody as a *trans*-Golgi marker, and an anti-PDI antibody as an endoplasmic reticulum (ER) marker in SK-N-BE(2)-C neuroblastoma cells. Scale bar: 100 µm. **b** SK-N-BE(2)-C cells were transiently transfected with Myc-tagged *RNF121*$^{WT}$ or mutant *RNF121*$^{M158R}$ for 48 hours. Representative images of immunofluorescent staining with an anti-Myc-tag antibody for RNF121 protein and a GM130 antibody for *cis*-Golgi are displayed. Scale bar: 20 µm. **c** immunofluorescent staining using an anti-MYCN antibody to detect MYCN protein, an anti-GM310 antibody to detect cis-Golgi, and DAPI to detect nuclei. Scale bar: 100 µM

**Supplementary Figure S3**

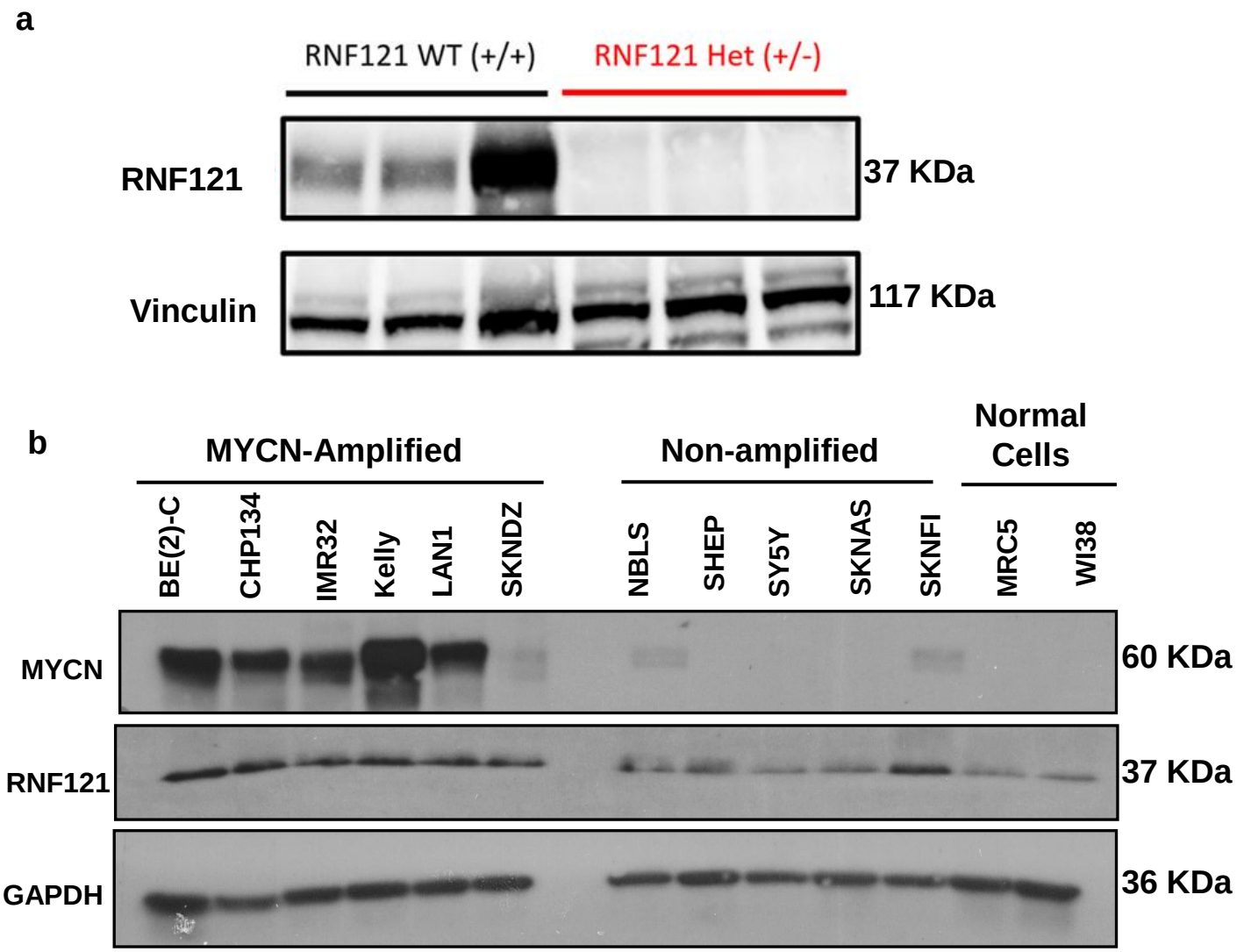
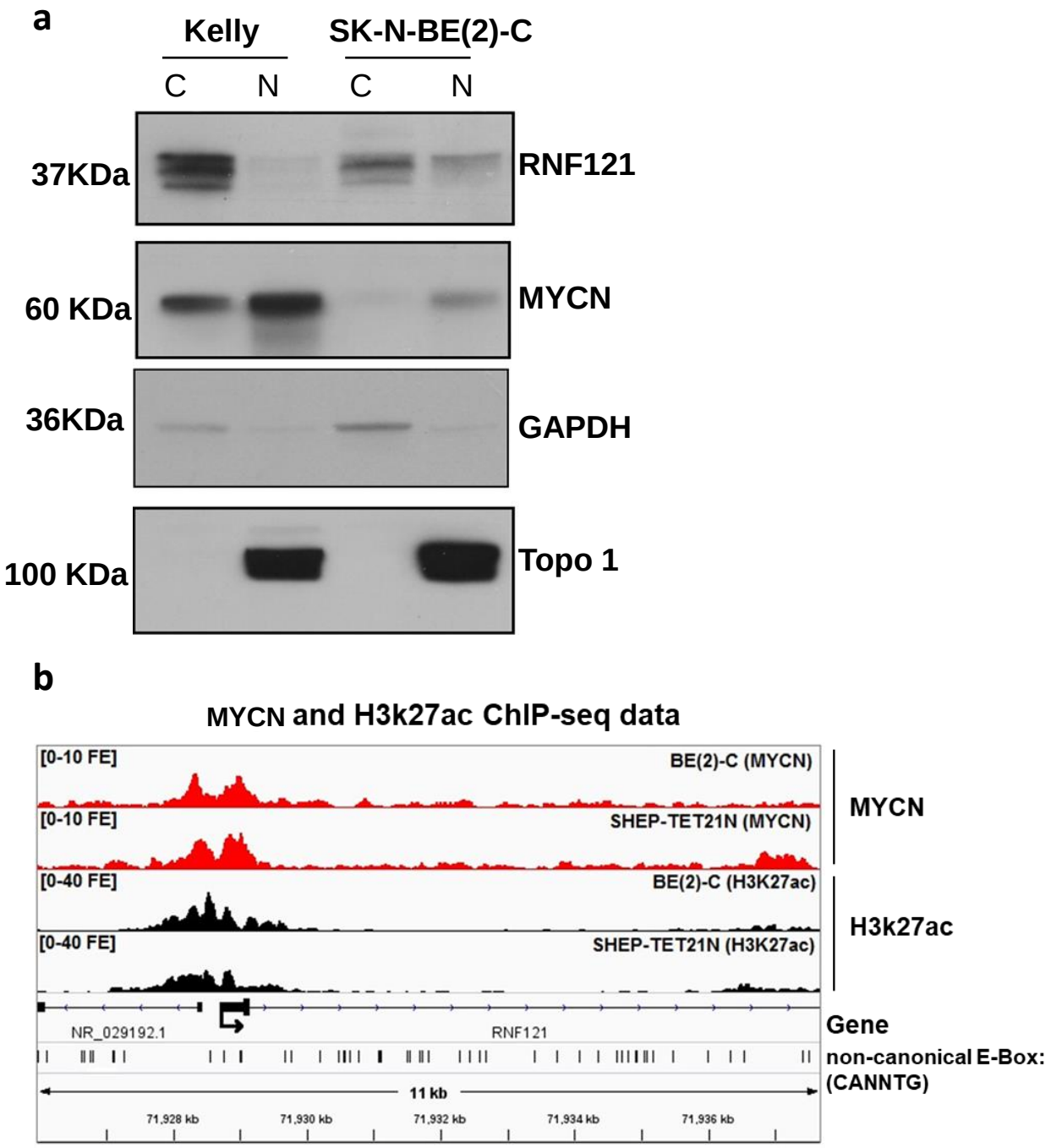



**Fig. S3 The effects of RNF121 deletion, mutation or overexpression in murine and human neuroblastoma cells. a** Immunoblot analysis of whole protein of tumour samples from Hemizygous *RNF121*$^{+/-}$ knockout (n=3) or *RNF121*$^{WT}$ (n=3) mice crossed with *TH-MYCN*$^{+/+}$ *mice* for RNF121 expression using a murine anti-RNF121$^{WT}$ antibody. Vinculin expression was used as a loading control. **b** Immunoblot for MYCN and RNF121 protein expression using specific antibodies in a range of neuroblastoma cell lines and normal human fibroblast cells, MRC-5 and WI-38.

**Supplementary Figure S4**



**Supplementary Figure S4**

**c**

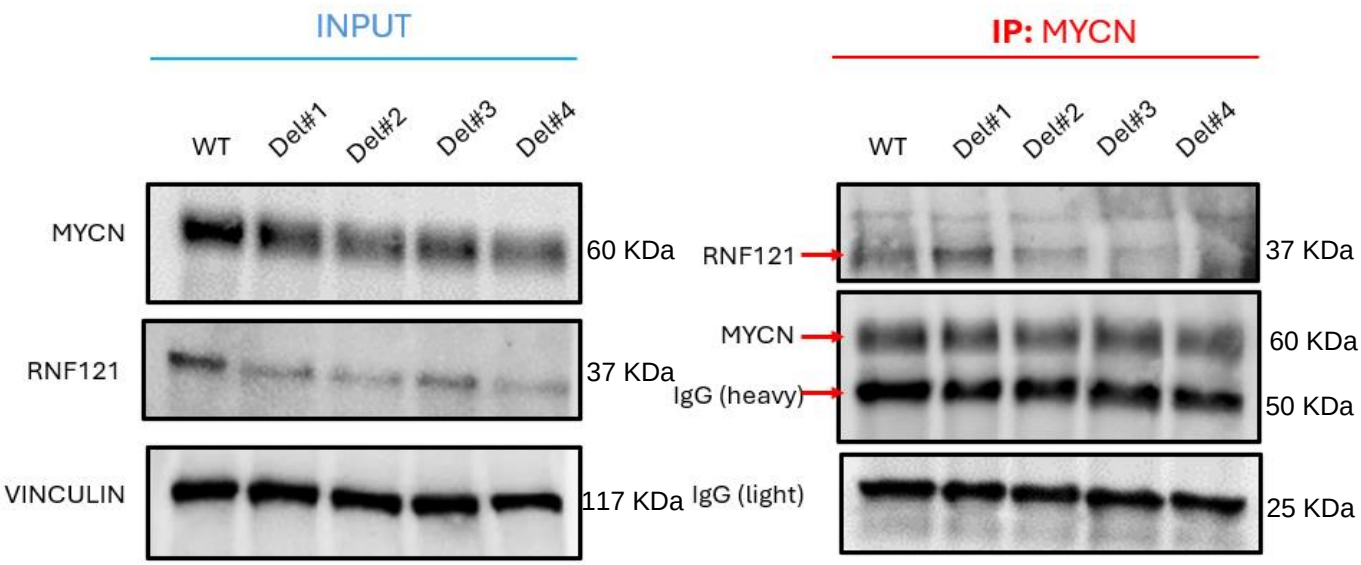
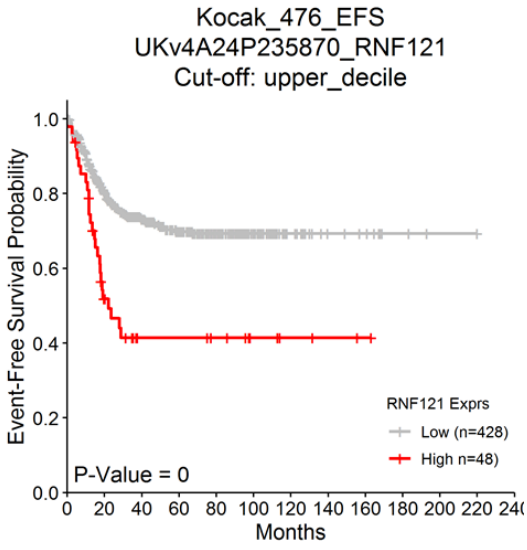
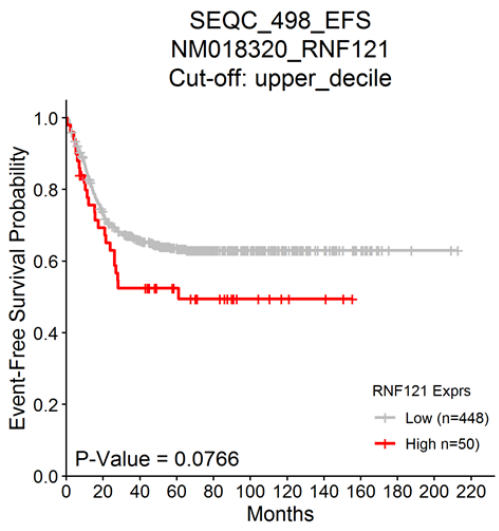



**Fig. S4 RNF121 localises to the cytoplasm and is a transcriptional target for MYCN. a** Immunoblotting showing RNF121 and MYCN expression in Kelly and SK-N-BE(2)-C cells using fractionated protein from the nuclear (N) and cytosolic (C) subcellular fractions. RNF121 and MYCN protein expression were detected using RNF121- and MYCN-specific antibodies. GAPDH- and Topoisomerase 1(Topo1)-specific antibodies were used as loading controls in the cytoplasmic and the nuclear compartments, respectively. **b** MYCN and H3k27ac ChIP-seq profiles covering the *RNF121* gene promoter region for the two neuroblastoma cell lines SK-N-BE(2)-C and SH-EP TET21N[24]. The vertical axes on each track graph represents a scale of fold enrichment (FE) values (compared to genomic input) and the horizontal axes the gene body including the transcription start site (TSS) and proximal promoter. The TSS of RNF121 is marked by a thick arrow in the gene track, exons are indicated by thick rectangles, introns are represented by lines and the directions of transcription are marked by arrows along the gene body. Non-canonical MYCN E-Boxes (CANNTG) are marked under the gene track by vertical lines.

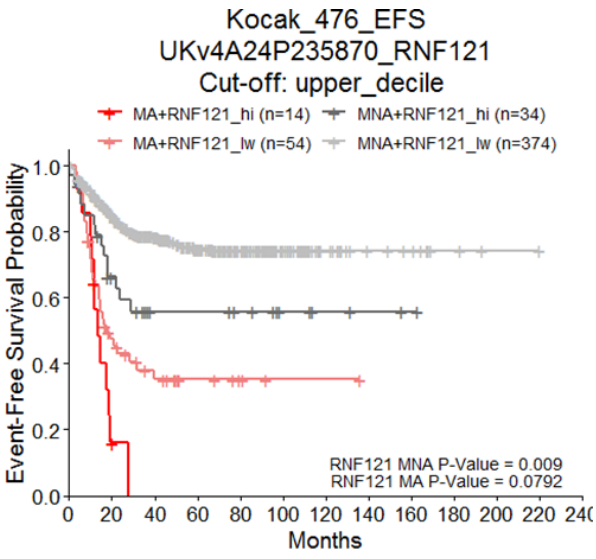
# Supplementary Figure S5

**a**




**b**



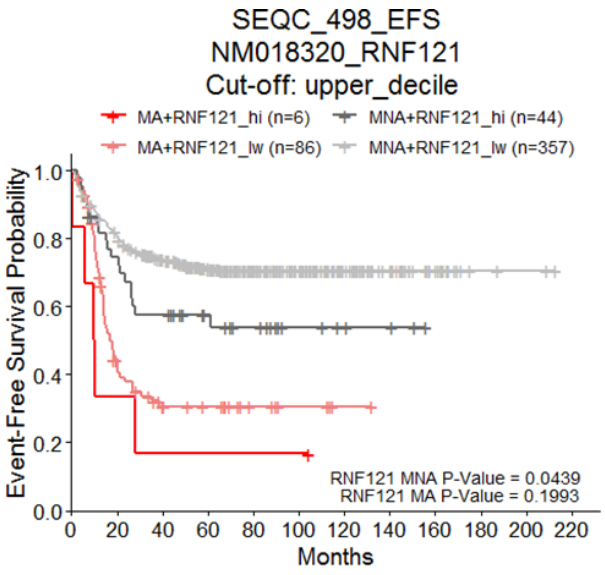
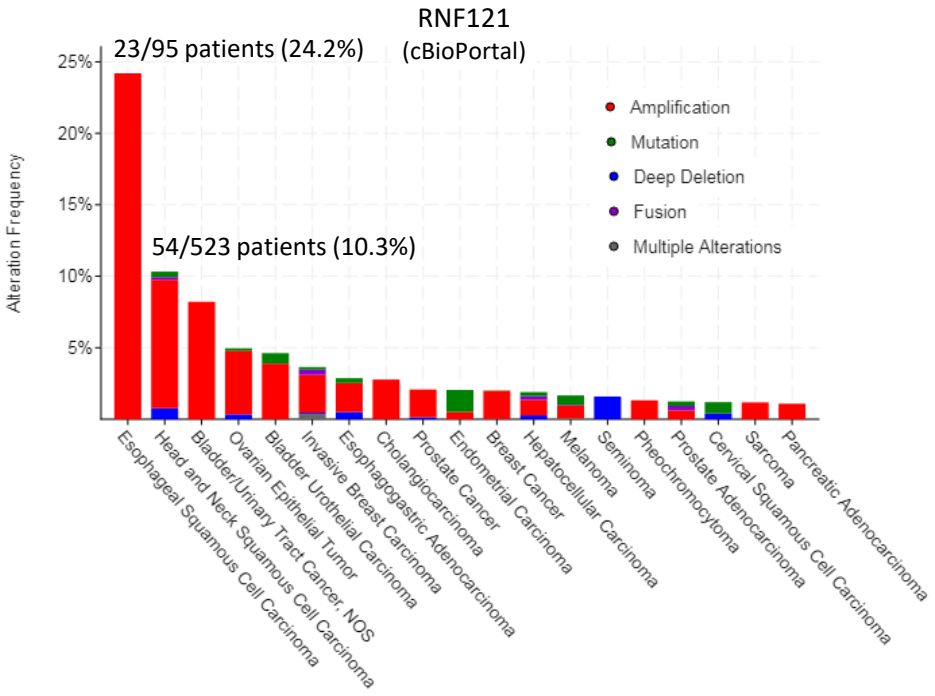



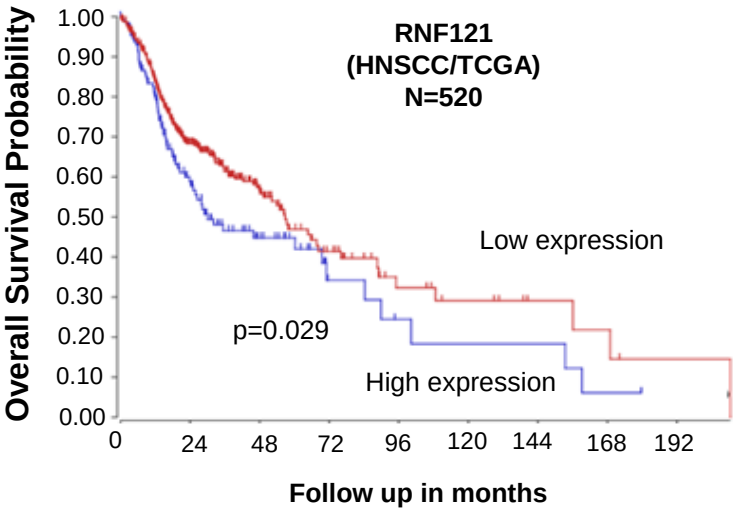
**Fig. S5 High RNF121 mRNA expression associates with poor patient prognosis. a** Kaplan-Meier curves of Event-free Survival probability for patients from the Kocak (n = 476) or SEQC neuroblastoma cohort (n = 498) subgrouped above or below the upper-decile of *RNF121* mRNA expression level. The p-values are from two-sided log-rank tests. **b** Kaplan-Meier curves of Event-free Survival probability for patients from the Kocak (n = 476) or SEQC neuroblastoma cohort (n = 498) subdivided by *RNF121* mRNA expression and *MYCN* amplification status (Amplified; MA, Non-amplified; MNA). Patients were subgrouped above or below the upper decile of *RNF121* expression level (High; *RNF121*_hi, Low; *RNF121*_lw). The p-values are from two-sided log-rank tests between each *RNF121* (lw, hi) expression group after subdividing cohorts by *MYCN* amplification status. P-values were adjusted using the Benjamini–Hochberg method to account for multiple comparisons. **c** Kelly cells were transiently transfected for 48 hours with $RNF121^{WT}$ or the RNF121 deletion mutants 1-4 (with mutants 3 and 4 lacking helix 5). Endogenous MYCN co-IP was performed, followed by immunoblotting with antibodies to recognize MYCN or RNF121 and Vinculin.

# Supplementary Figure S6

**a**




**b**

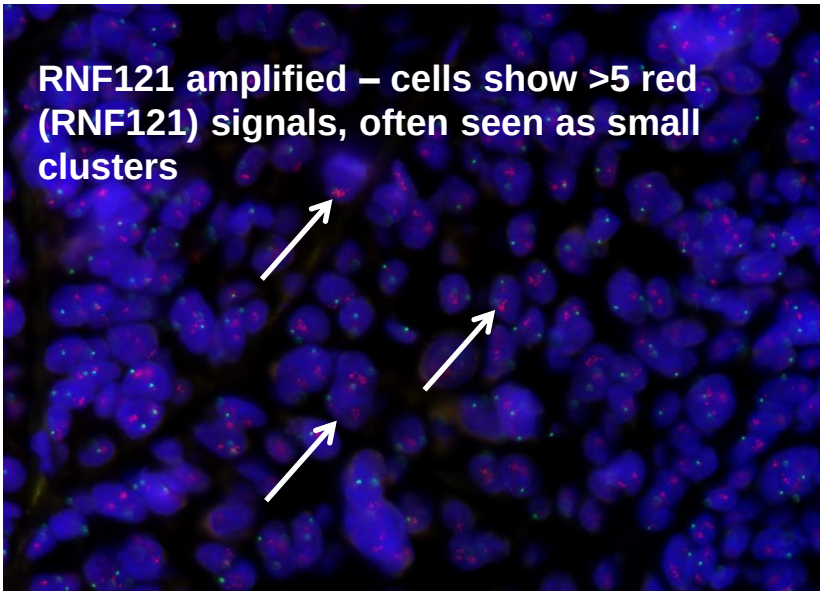


# Supplementary Figure 6

**c**

**12/66 (18.2%) cases scored as RNF121 gene amplified**

| RNF121 FISH results (LSCC TMA's 1A+2A, Empire Genomics probes) | # | % |
|---|---|---|
| **Total # scored** | **66** | |
| **hemi gene loss** | **1** | **1.5** |
| hemi gene loss | 1 | |
| **gene disomy** | **30** | **45.5** |
| disomy | 25 | |
| chr trisomy | 4 | |
| chr polysomy | 1 | |
| **gain** | **23** | **34.8** |
| gain | 16 | |
| gain+chr trisomy | 6 | |
| gain+chr polysomy | 1 | |
| **amplification** | **12** | **18.2** |
| amplification | 7 | |
| amplification+chr trisomy | 2 | |
| amplification+chr polysomy | 3 | |




**d**

**19/46 (41.3%) cases scored as Myc gene amplified**

| Myc FISH results (LSCC TMA's 1A+2A, Vysis probes) | # | % |
|---|---|---|
| **Total # scored** | **46** | |
| **gene disomy** | **12** | **26.1** |
| disomy | 10 | |
| chr trisomy | 1 | |
| chr polysomy | 1 | |
| **gain** | **15** | **32.6** |
| gain | 5 | |
| gain+chr trisomy | 6 | |
| gain+chr polysomy | 4 | |
| **amplification** | **19** | **41.3** |
| amplification | 11 | |
| amplification+chr trisomy | 4 | |
| amplification+chr polysomy | 4 | |

**Fig. S6 *RNF121$^{WT}$* amplification is frequent in head and neck squamous cell cancer (HNSCC).** **a** The frequency of *RNF121$^{WT}$* copy number alterations (Amplification, Mutation, Deep Deletion, Fusion and Multiple Alterations) by cancer type from the cBio Cancer Genomics Portal (cBio portal, http://cbioportal.org). **b** Kaplan-Meier curves of Overall Survival probability of patients from the TCGA HNSCC cohort (n = 520) subdivided by *RNF121* gene expression, where patients were grouped based on the upper-decile of gene expression values. The p-values are from two-sided log-rank tests between each group. **c** Left panel: Fluorescence *in situ* hybridisation (FISH) scoring results for tumour cores from 66 HNSCC patients analysed for *RNF121* gene amplification. Right panel: Representative images of fluorescence in situ hybridisation of *RNF121* amplification. Arrows point to cells with increased number of *RNF121* copies (red signals). **d** FISH scoring results for tumour cores from 46 HNSCC patients analysed for c-*Myc* gene amplification.

## Supplementary Table 1

### Targeting vector RNF121 sequence:

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***Supplementary Table 1:*** Sequence of the RNF121 targeting vector used to generate the RNF121 conditional allele. LoxP sites are in red text and exon 3 sequence is in blue text.

# Supplementary Figure 7 (unprocessed immunoblots)

Figure **2c**

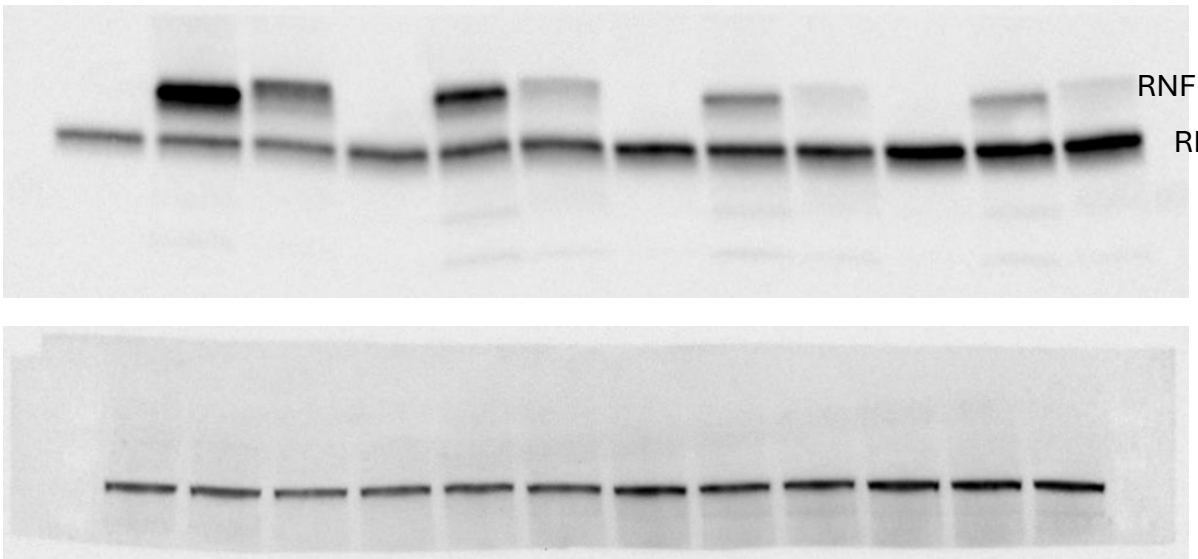
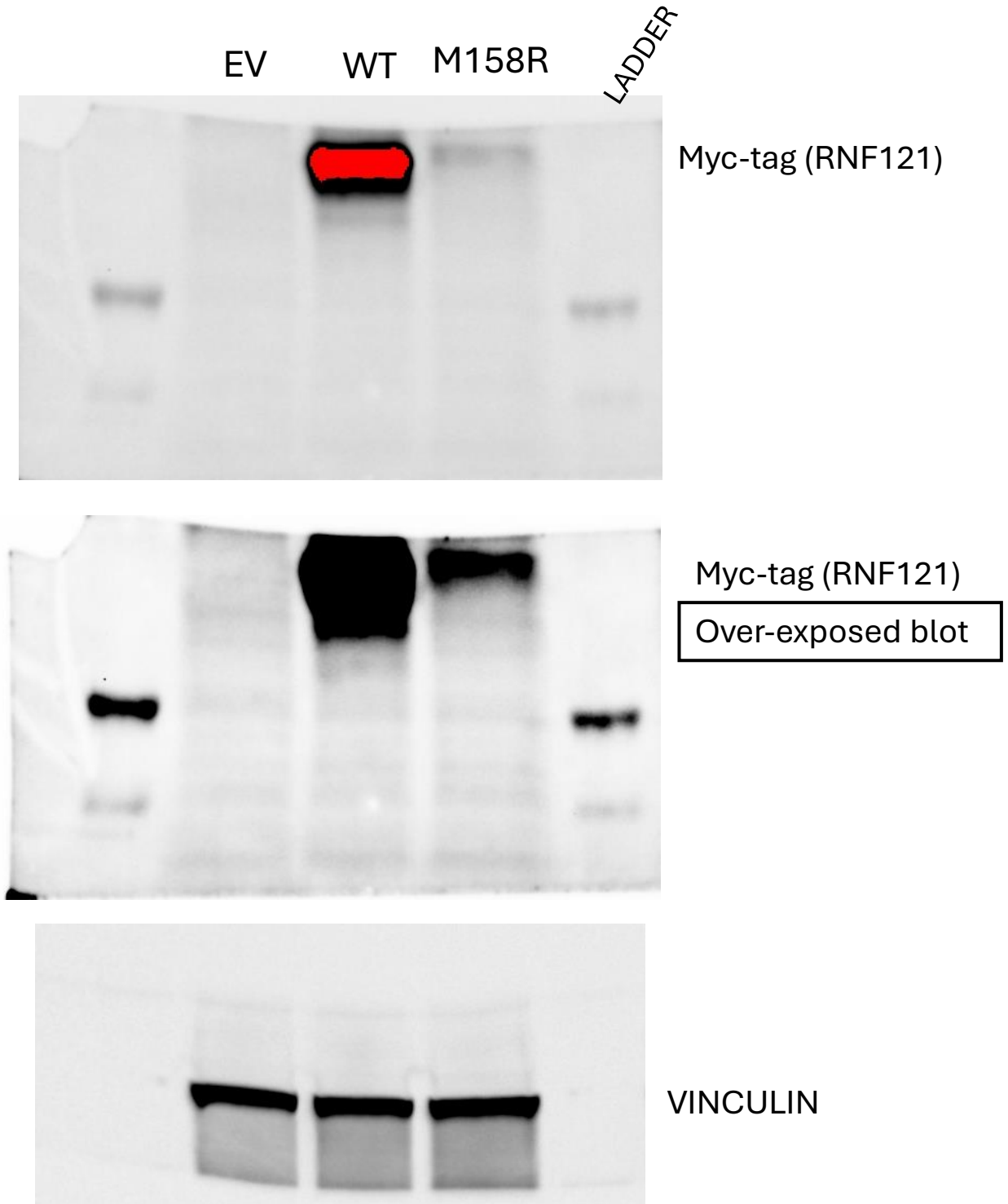



Figure **2d**



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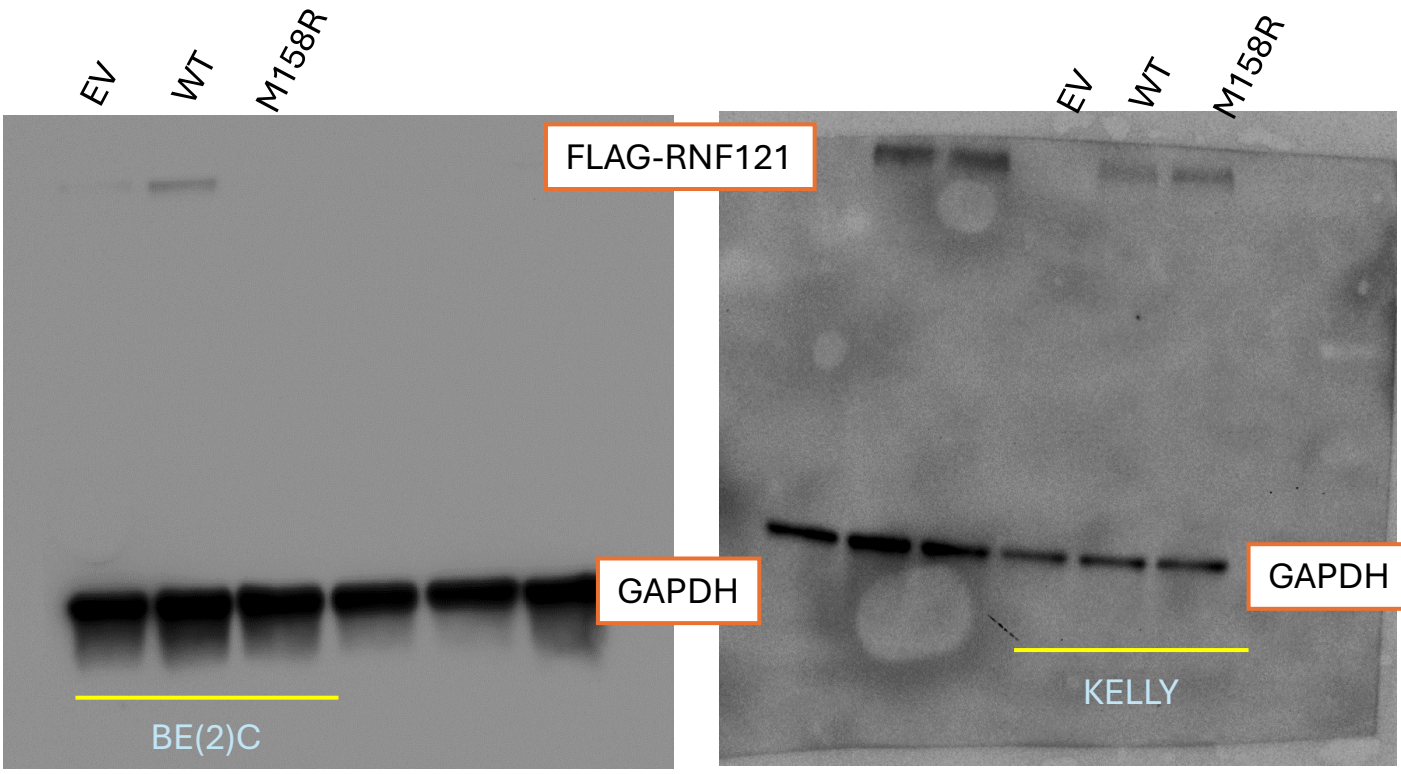


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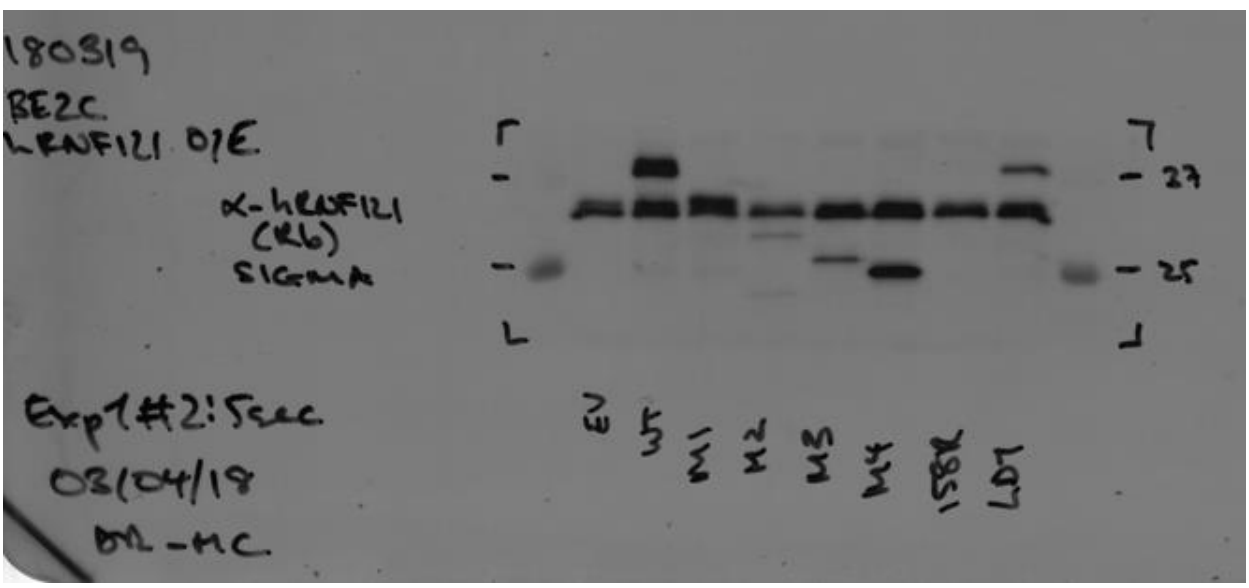


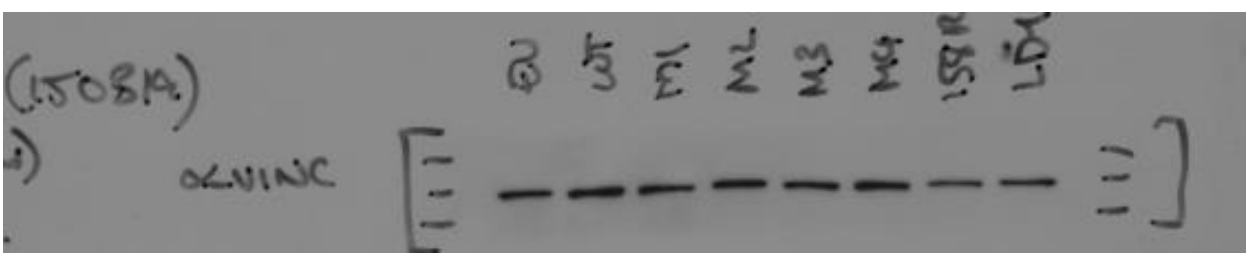
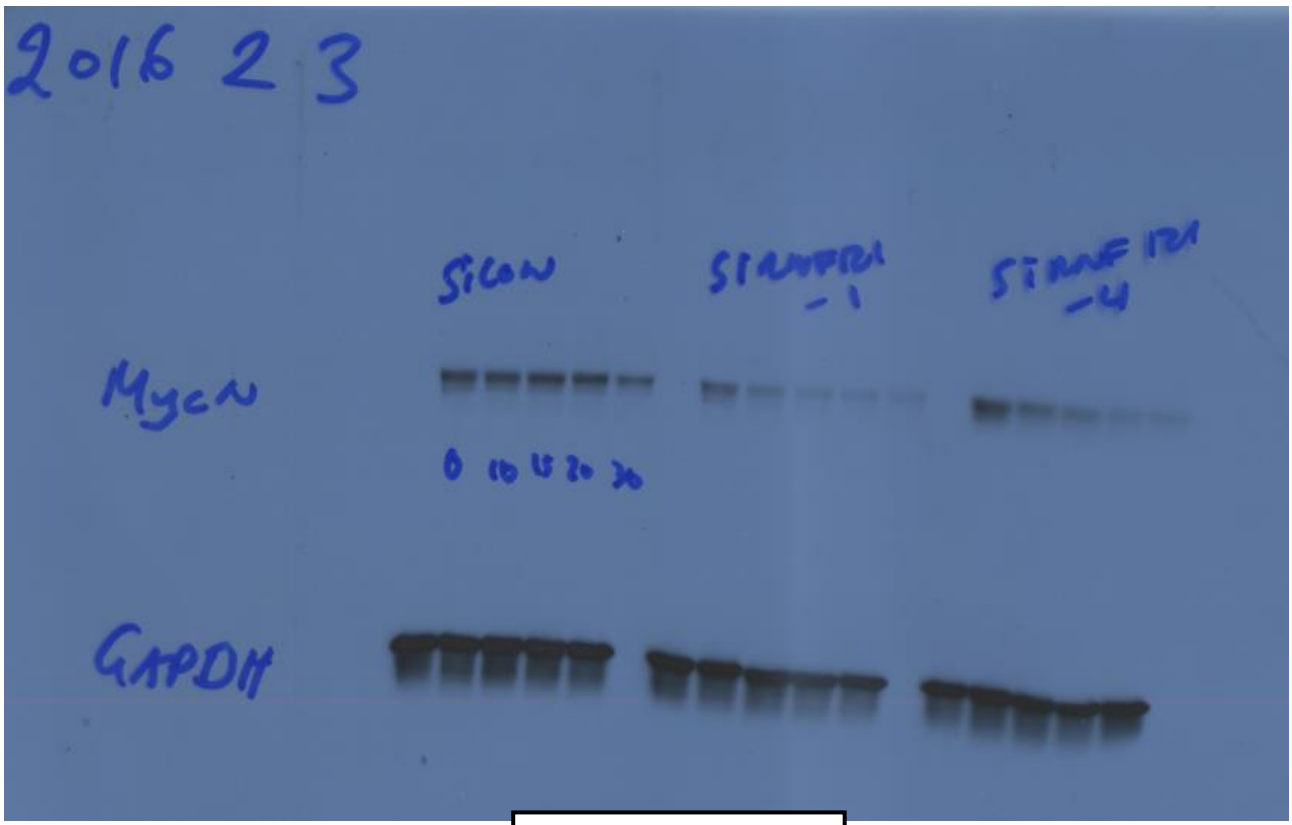



Figure **5a**

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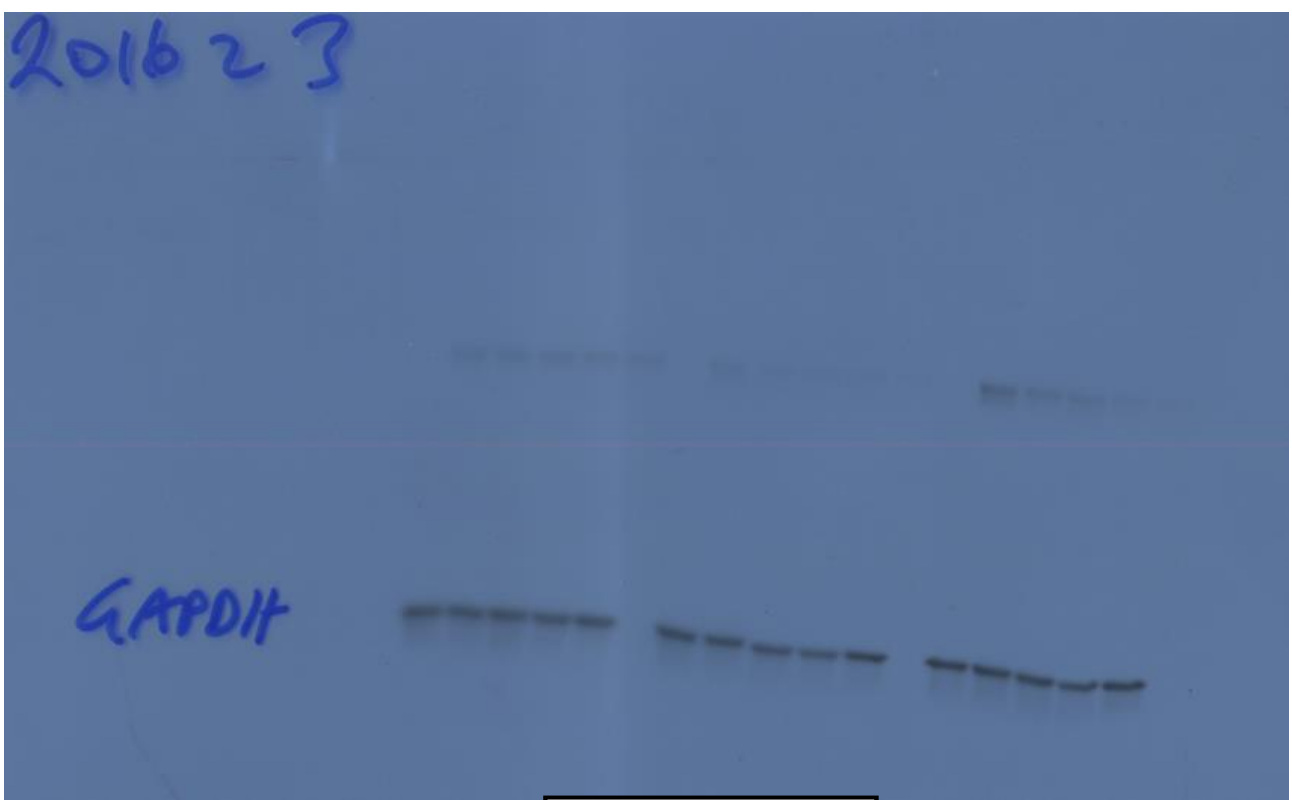


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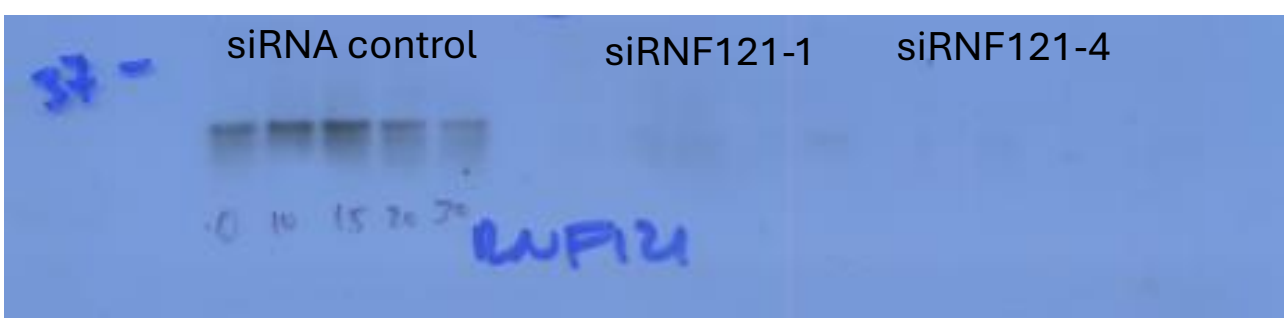
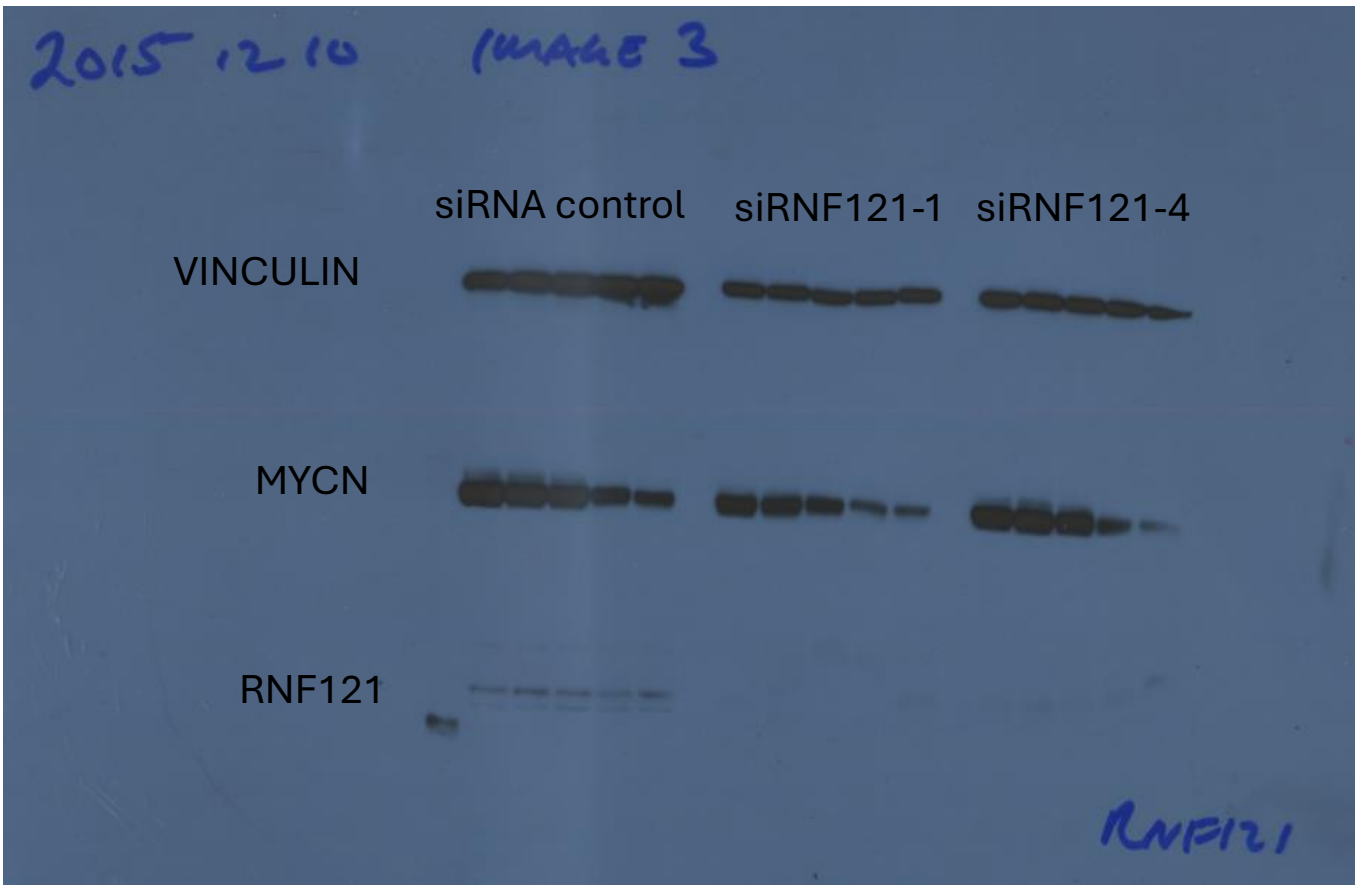


Figure **5b**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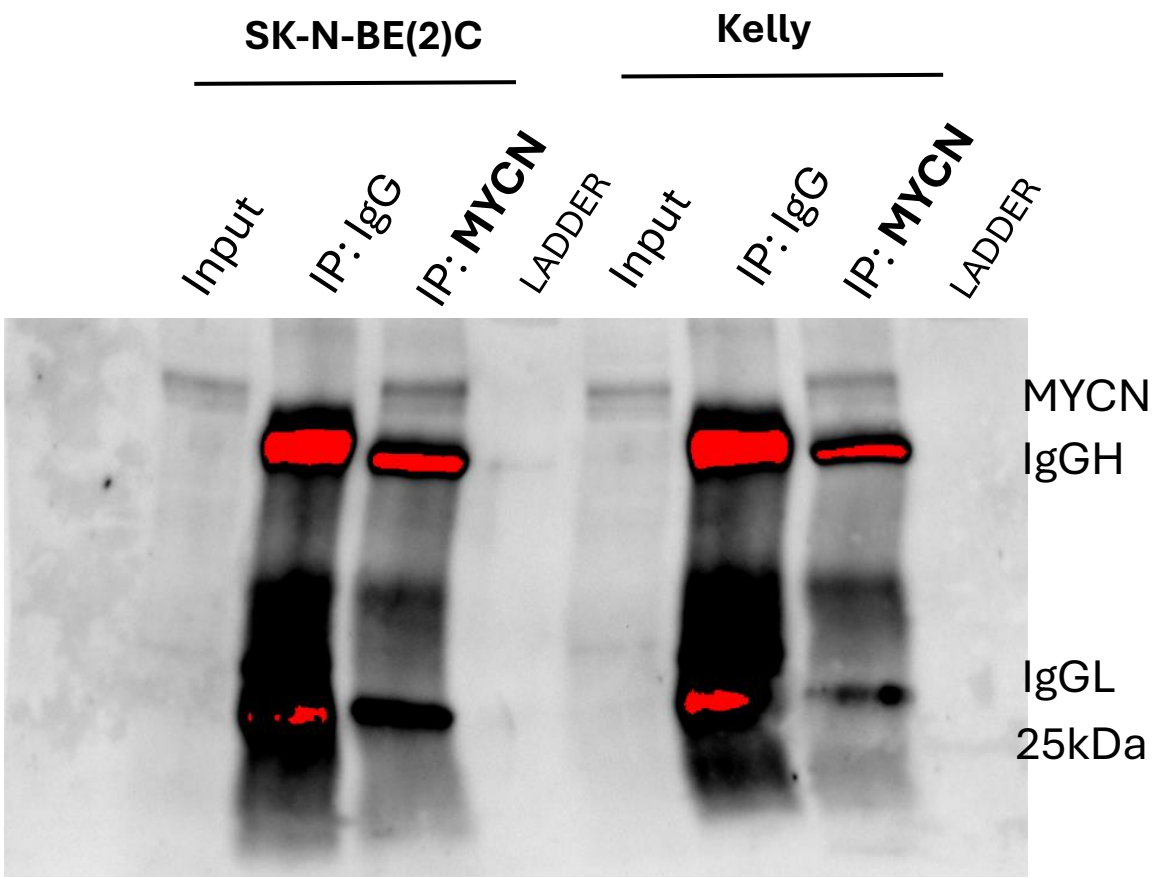
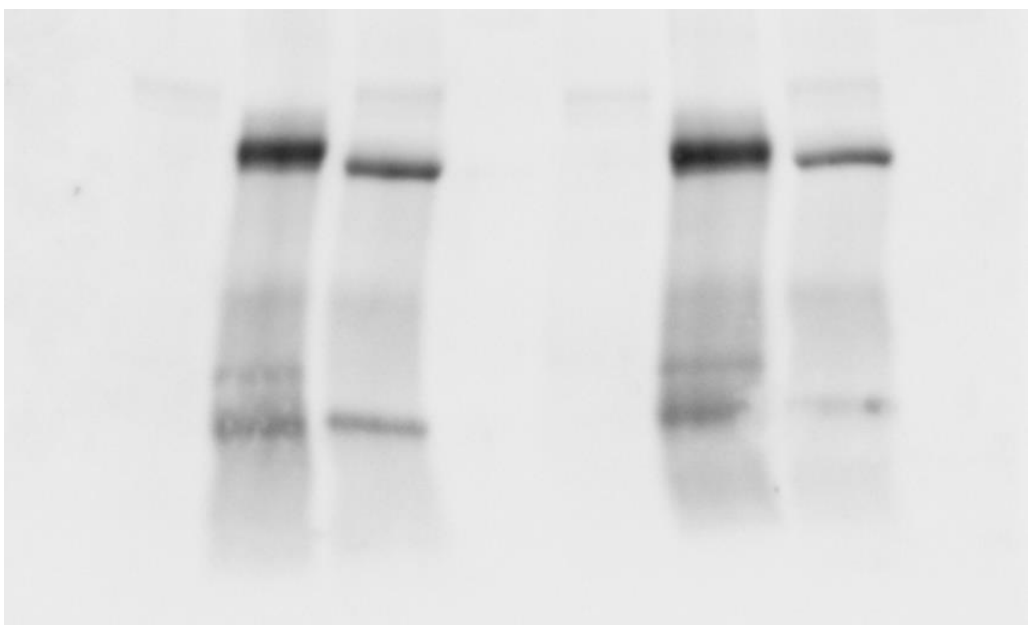
MYCN
RNF121
RNF121

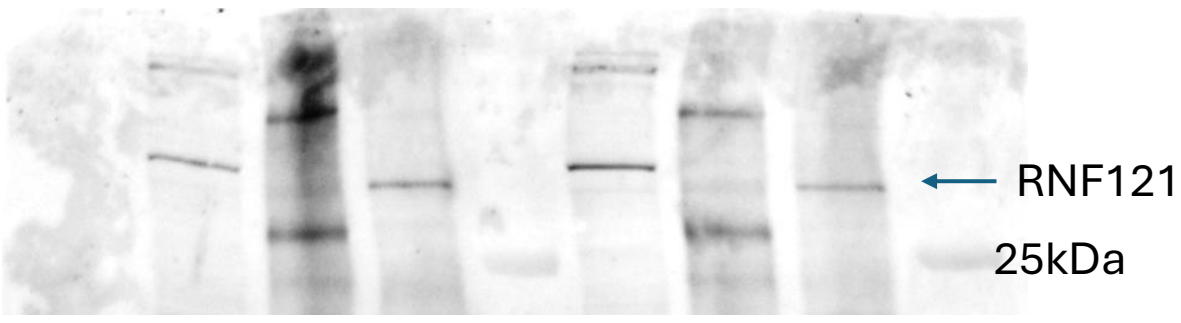
Figure **5g**




Over-exposed blot showing **MYCN pulldown** (red was corrected in processed image)

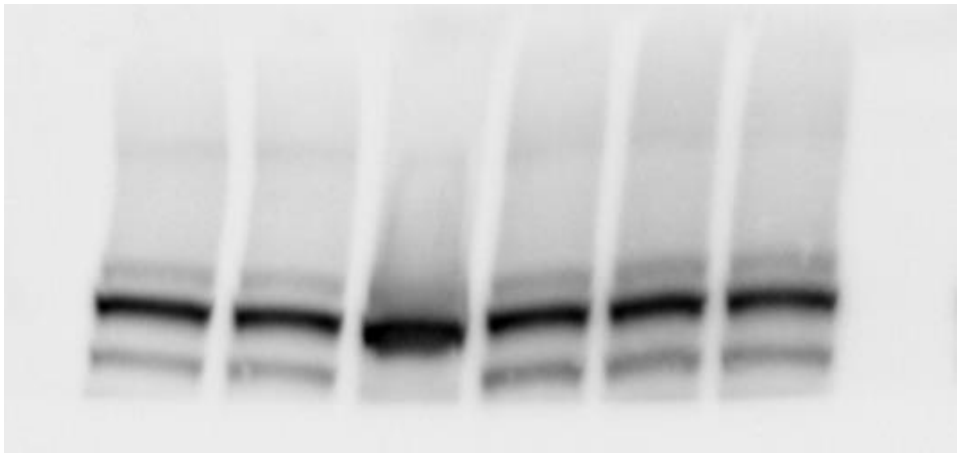


Low-exposure whole blot showing MYCN pulldown

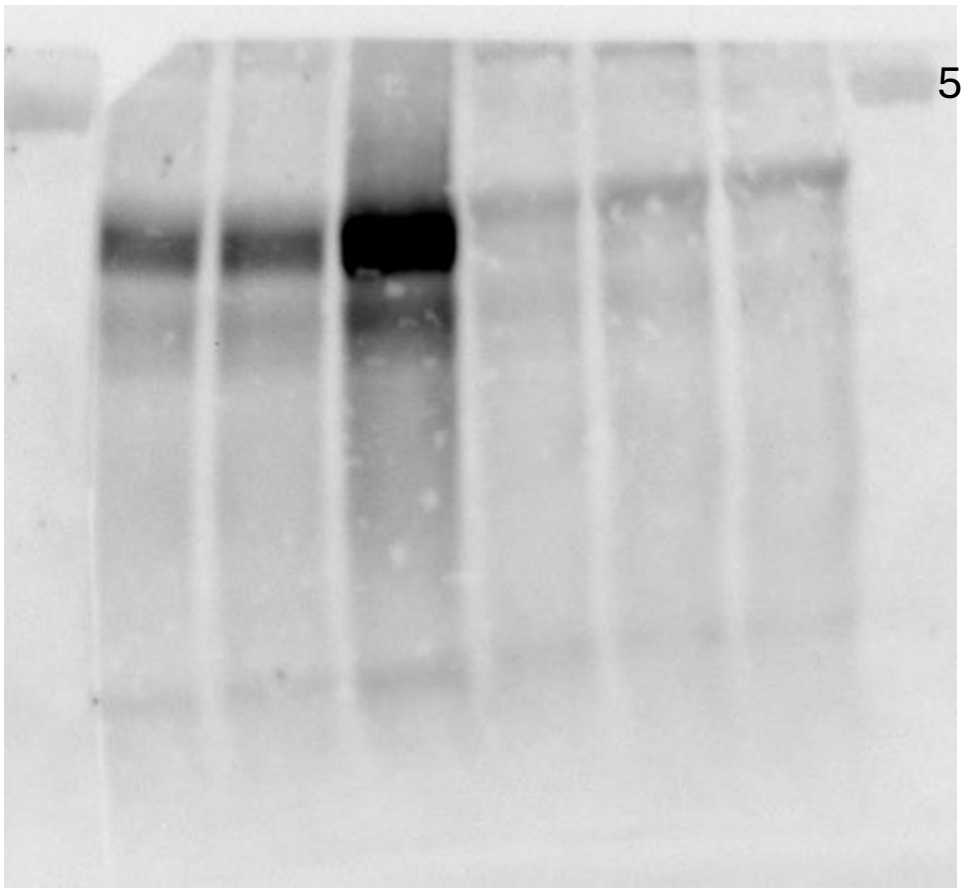



Blot was cut, stripped, and **re-probed for RNF121** to check for co-immunoprecipitation

Supplementary figure **S3a**



Vinculin

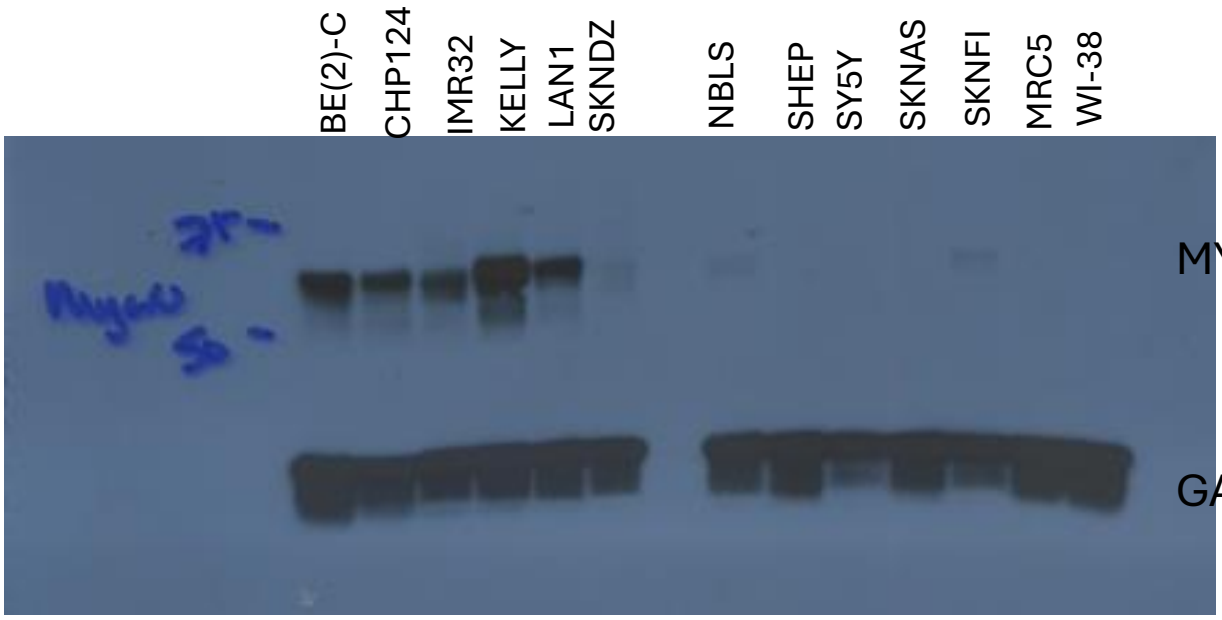


50kDa

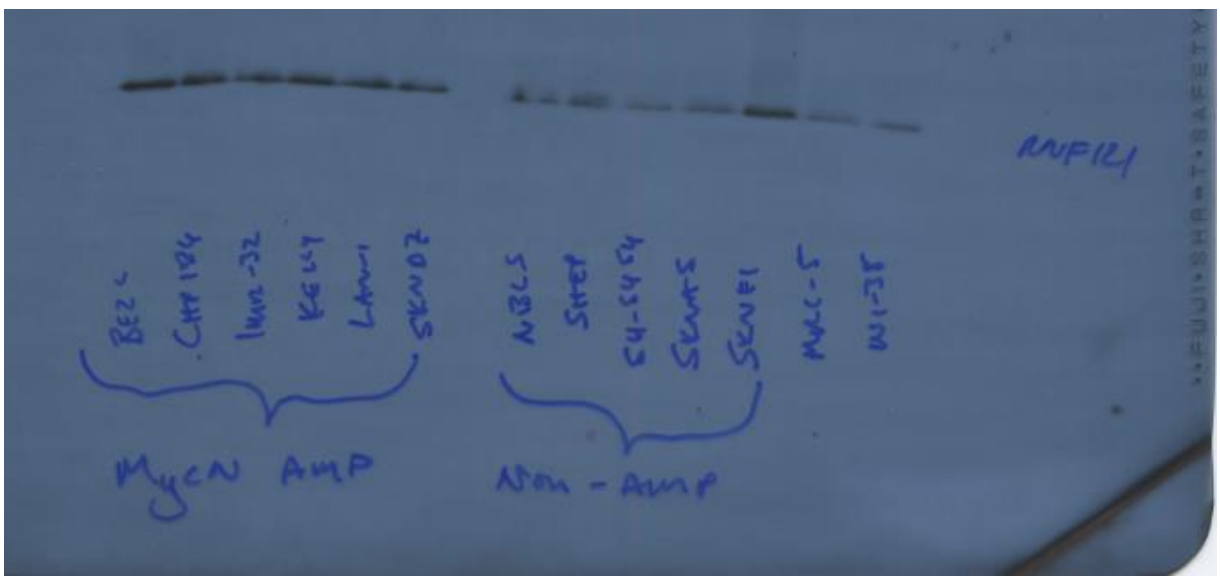
RNF121

Whole blot cut and probed for Vinculin (on top) and RNF121 in bottom half

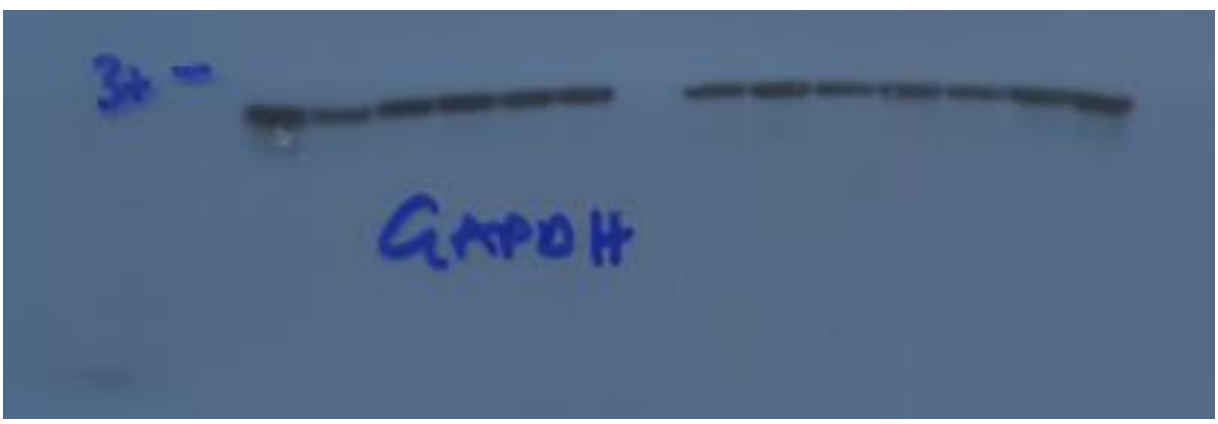
Supplementary figure **S3b**




MYCN expression across cell line panel (with GAPDH **over-exposed**)

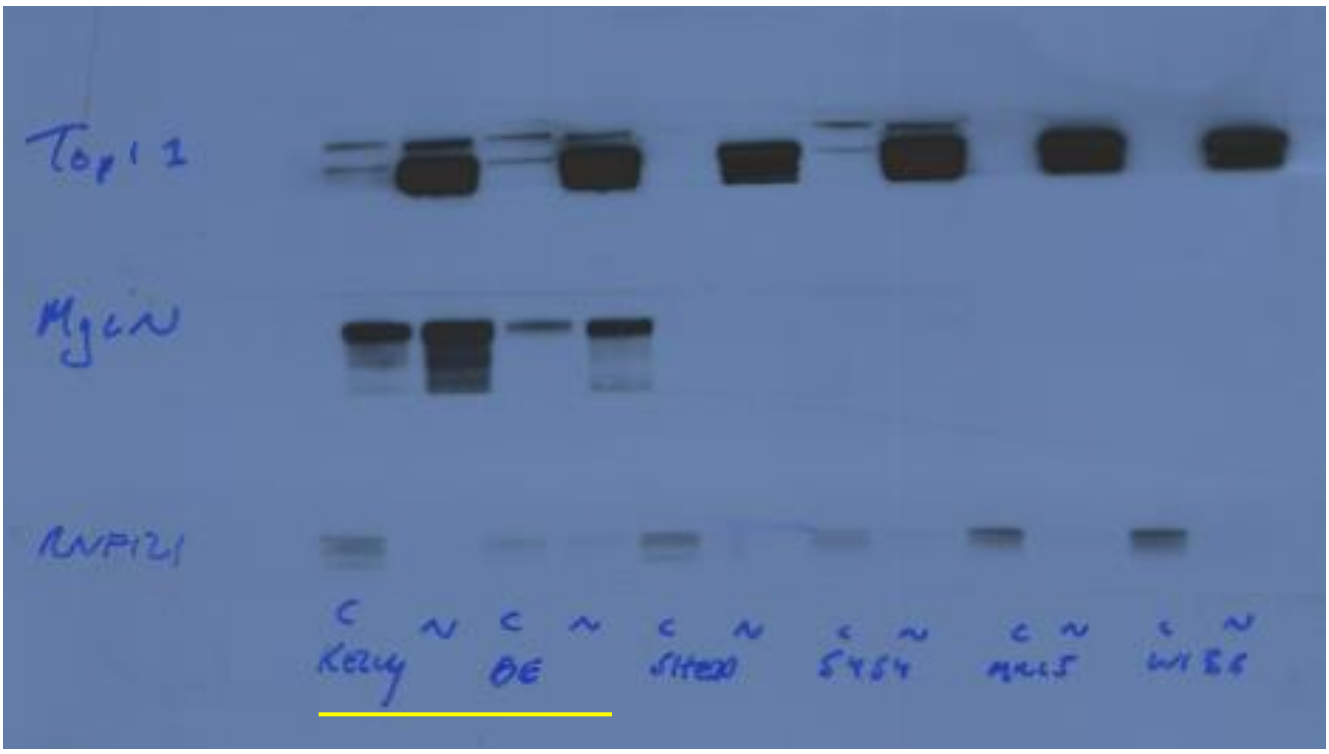


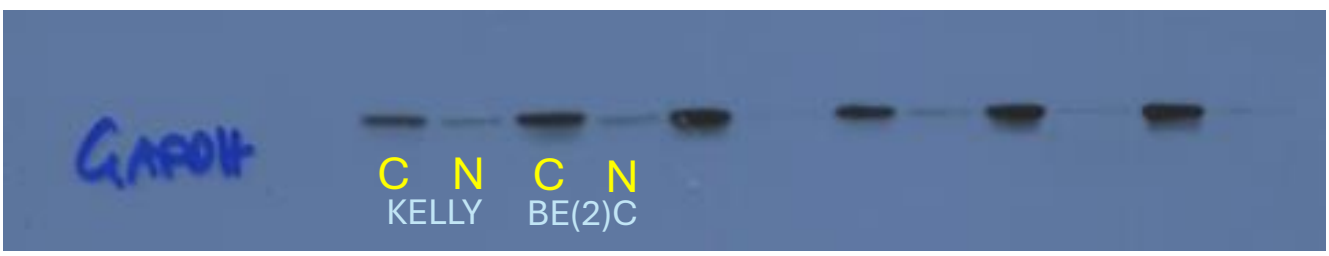

RNF121 expression across cell line panel




GAPDH low-exposure

Supplementary figure **S4a**



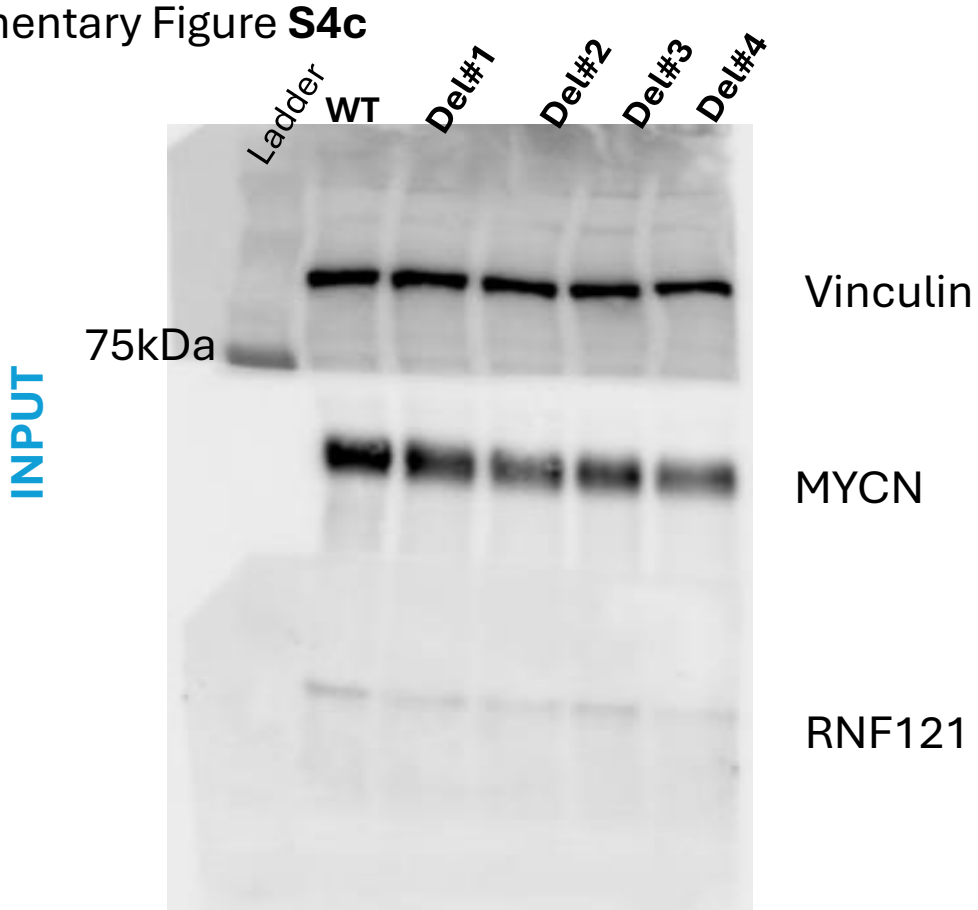




Only Kelly and BE(2)-C data was taken for processed figure

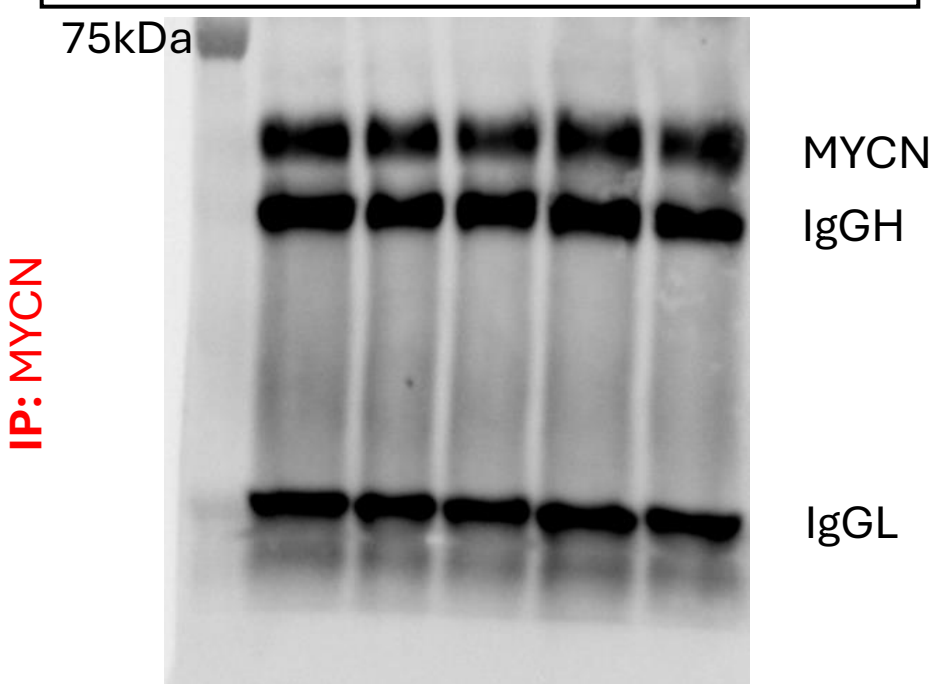

RNF121

Over-exposed for RNF121 expression

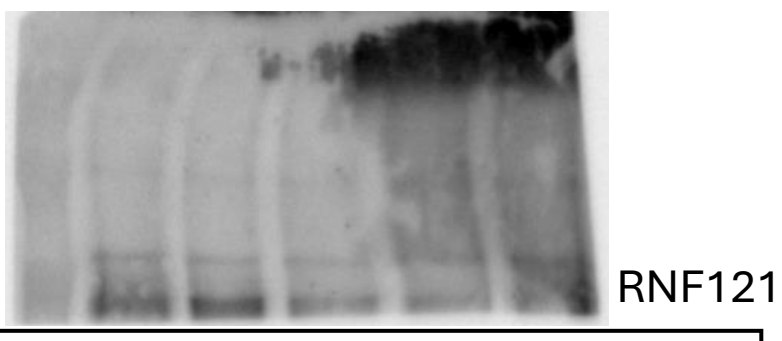
Supplementary Figure **S4c**




INPUT immunoblot **strips put together** (cut to probe for different antibodies – MYCN ladder caused over-exposure and was cut before imaging)




Immunoblot probed for **MYCN pulldown** (high-exposure)




Immunoblot cut, stripped, and probed for **RNF121 pulldown** (highest-exposure)