

The c-Jun and JunB transcription factors facilitate the transit of classical Hodgkin lymphoma tumour cells through G₁

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Supplementary Figure Legends

Figure S1 – Western blots, growth curves and cell cycle analysis with a distinct control shRNA. Western blots (A-C), growth curves (D), and cell cycle analysis (E) of the indicated cHL cell lines including a control shRNA distinct from shown in **Figures 1-3**. The growth curves represent the average and standard deviation of two independent experiments and the cell cycle represents the average and standard deviation of four independent experiments. ANOVA with Tukey's *post hoc* test was performed in E. Molecular mass markers (in kDa) are indicated to the left of western blots. Note: some of the KM-H2 cell cycle data is included in Figure 3.

Figure S2 –Representative flow plots of control and c-Jun/JunB shRNA–expressing cHL cell lines. A. Gating strategy used to delineate stages of the cell cycle. Representative plots of L-540 (B), L-428 (C), and KM-H2 (D) cells used to generate the results in **Figures 3A-F**.

Figure S3 – Apoptosis measurement in c-Jun/JunB knock-down cells. Apoptosis was measured by TUNEL using the In Situ Cell Death Detection Kit (Roche Applied Science). Positive controls are cells pre-treated with 300 µg/ml DNase, whereas negative controls are permeabilized cells incubated in label solution without TdT enzyme.

Figure S4 – Analysis of ALK+ ALCL cell lines with a distinct control shRNA and representative flow blots from Figure 4. Representative western blots showing c-Jun/JunB expression in Karpas 299 (A) and SUP-M2 (B) cells expressing two control

shRNAs (control 216 and GFP) and the indicated c-Jun/JunB shRNAs. Note: the GFP shRNA targets the GFP protein, which is not expressed in these cells, and is used as a control in these experiments. As well, the seed sequences of JunB#6 shRNA and JunB#1 shRNA overlap and are not distinct. Growth curves (**C**) and BrdU labeling (**D**) of Karpas 299 cells expressing two control shRNAs and the indicated c-Jun/JunB shRNAs. **E**, BrdU/7-AAD labelling of SUP-M2 cells expressing two control shRNAs and the indicated c-Jun/JunB shRNAs. The growth curves (**C**) represent the average and standard deviation of three independent experiments and the data in **D** and **E** represents the average and standard deviation of two independent experiments. ANOVA with Tukey's *post hoc* test was performed in **C**. ** $P < 0.01$, *** $P < 0.001$. Representative BrdU/7-AAD plots of Karpas 299 (**F**) or SUP-M2 (**G**) cells used to generate the results in **Figures 4G, 4H, and 5G**.

Figure S5 – Introduction of a JunB cDNA into JunB shRNA–expressing Karpas 299 cells. A. Protocol for JunB reintroduction **B. i.** Flow plots illustrating the EGFP–positive population **ii.** Histogram plots showing BrdU staining within the EGFP–positive population **C.** Western blot of FLAG-JunB introduced into JunB shRNA–expressing cells.

Figure S6 – c-Jun and JunB levels in cHL and ALK+ ALCL cells used in this study. Western blots showing c-Jun, JunB, or ALK protein levels in lysates of cHL (L-540, L-428, KM-H2), ALK+ ALCL (Karpas 299, SUP-M2, UCONN-L2) and two non-CD30–positive lymphoma cell lines (Ramos and Jurkat).

Figure S1

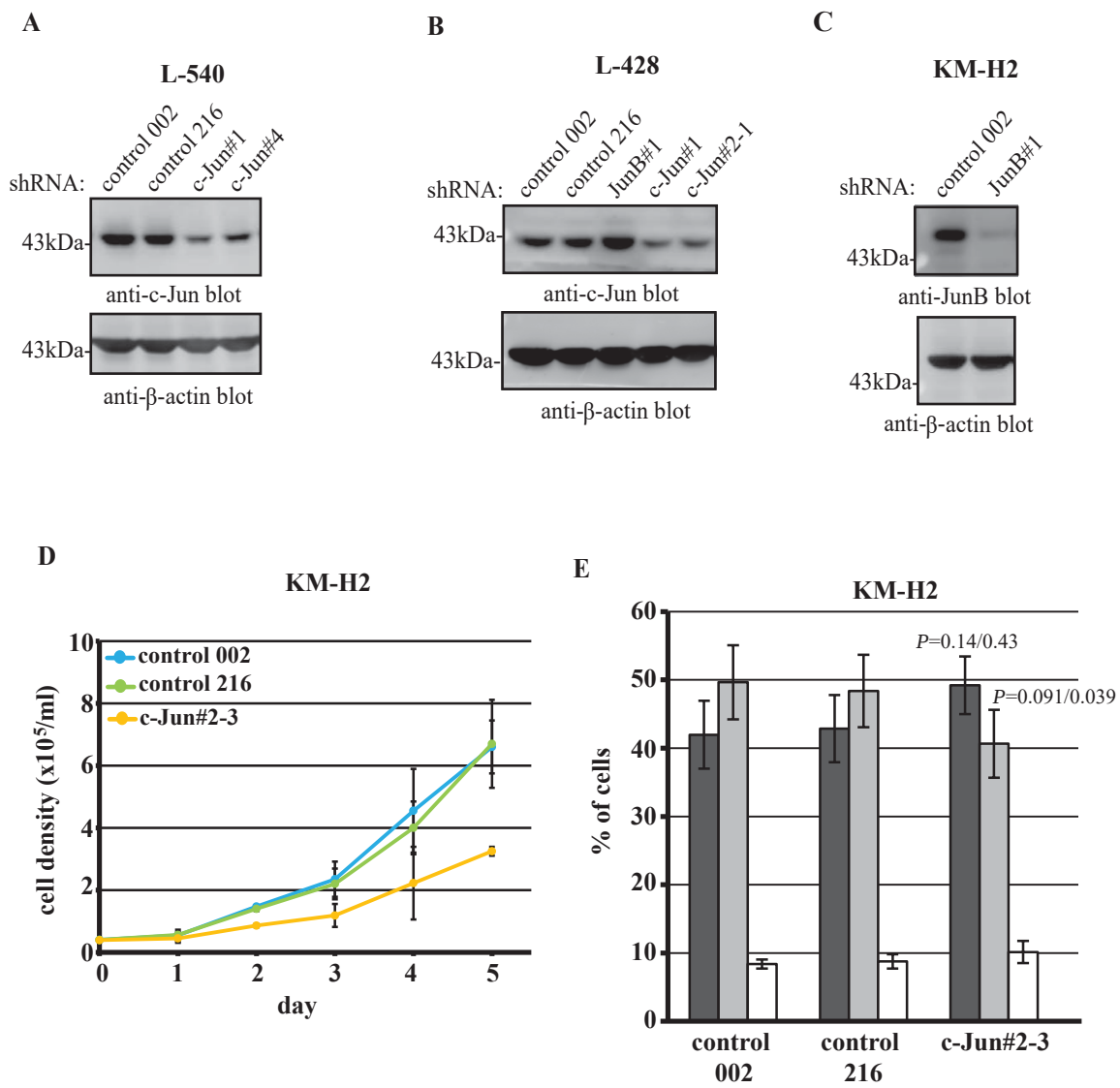
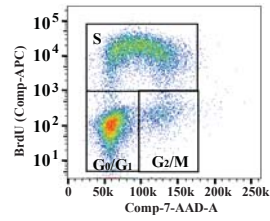
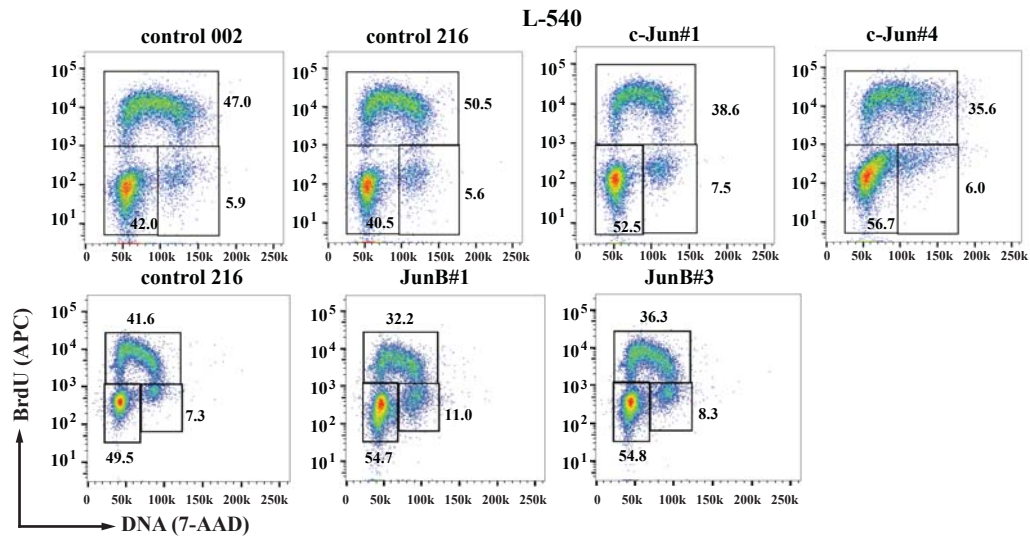


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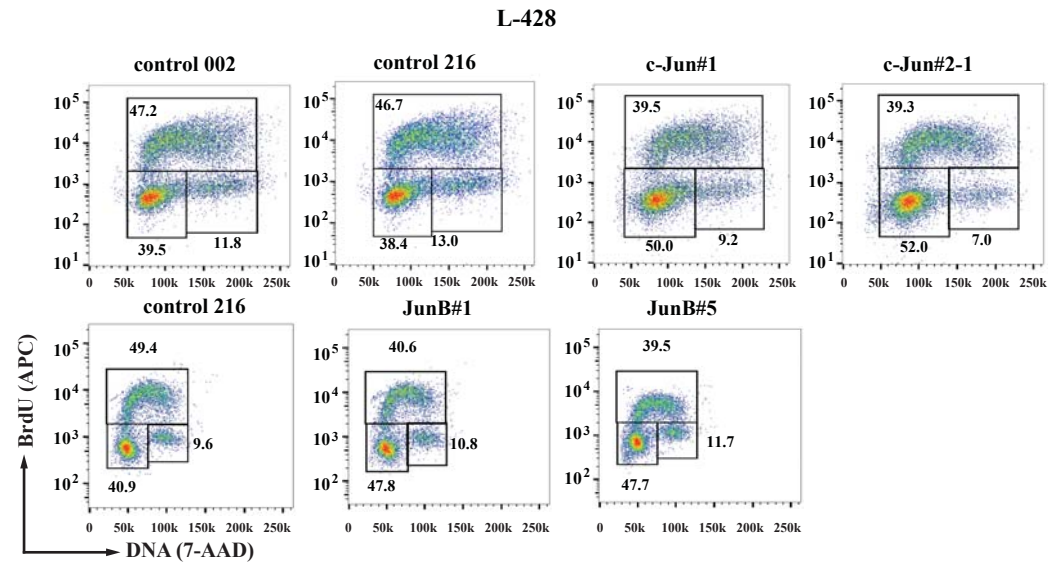
A



B



C



D

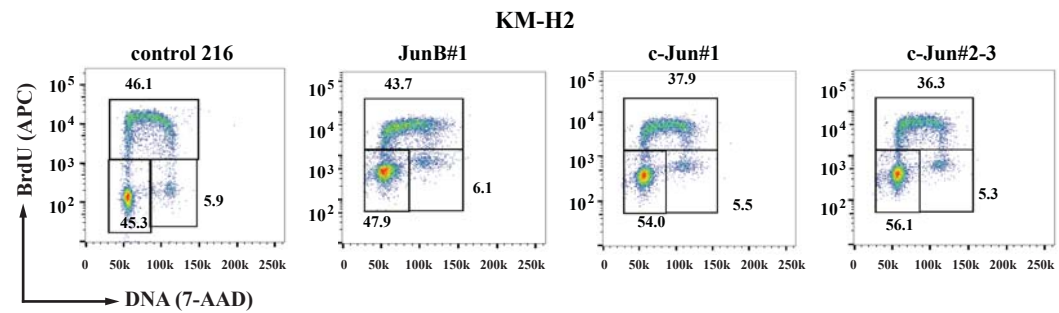


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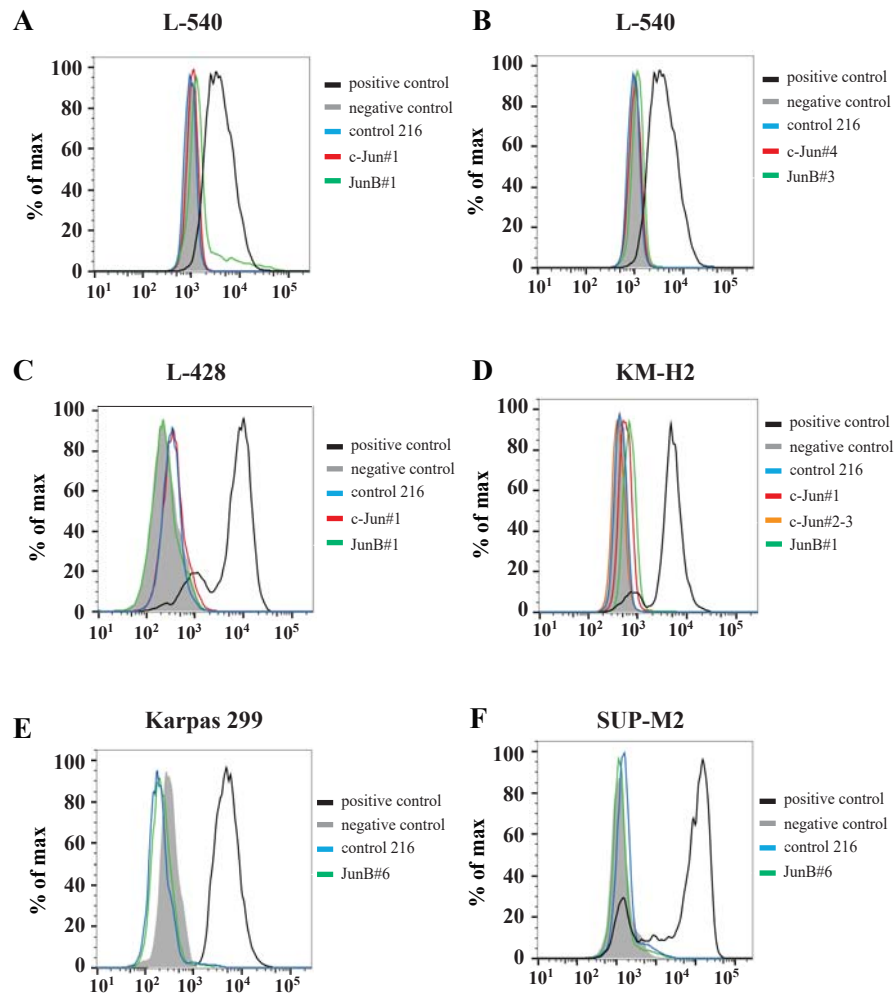


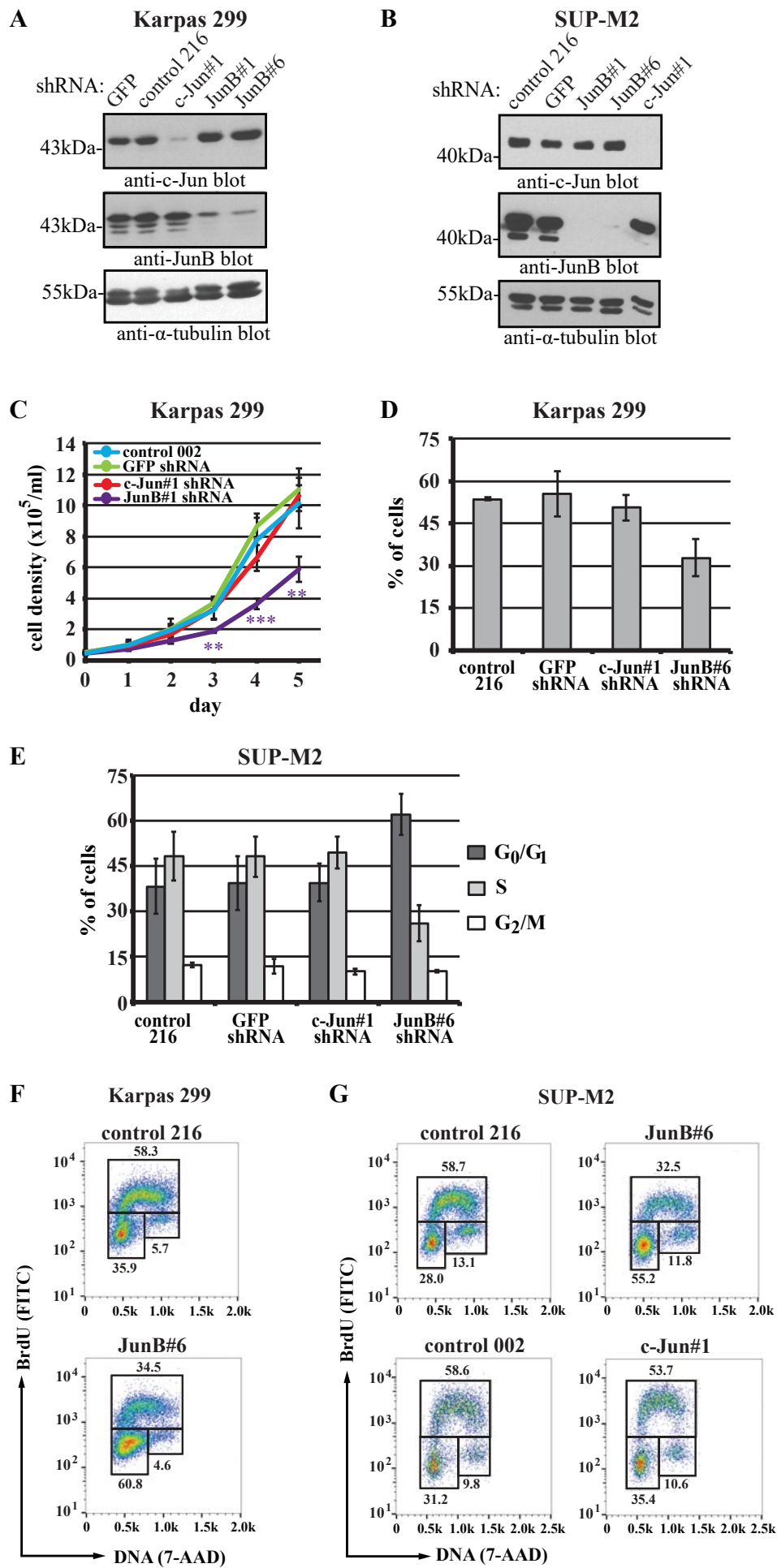
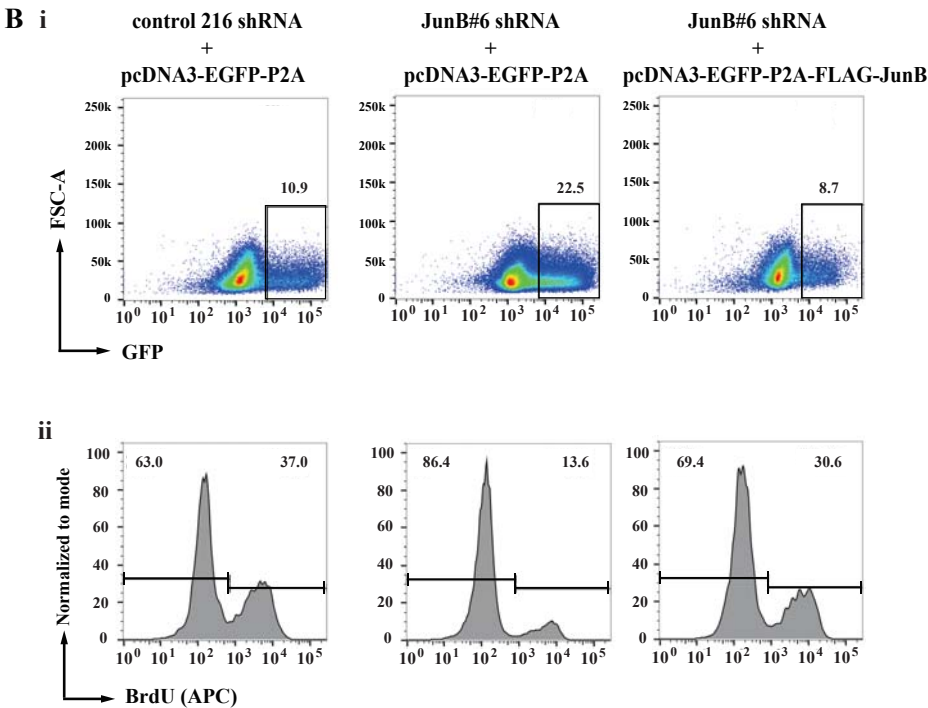
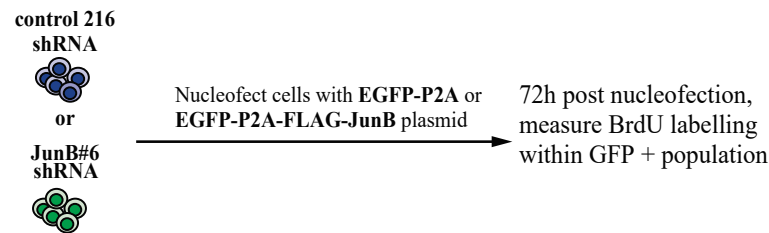
Figure S4

Figure S5

A Karpas 299



C Karpas 299

shRNA:	control	216	JunB#6
EGFP-P2A:	+	+	-
EGFP-P2A-FLAG-JunB:	-	-	+

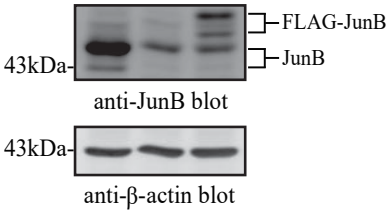
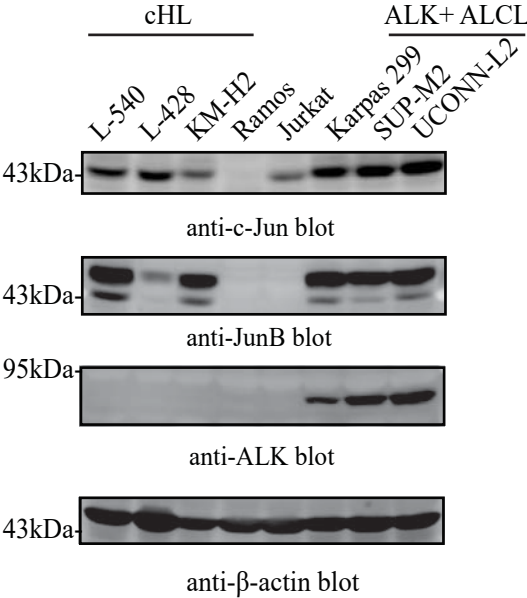
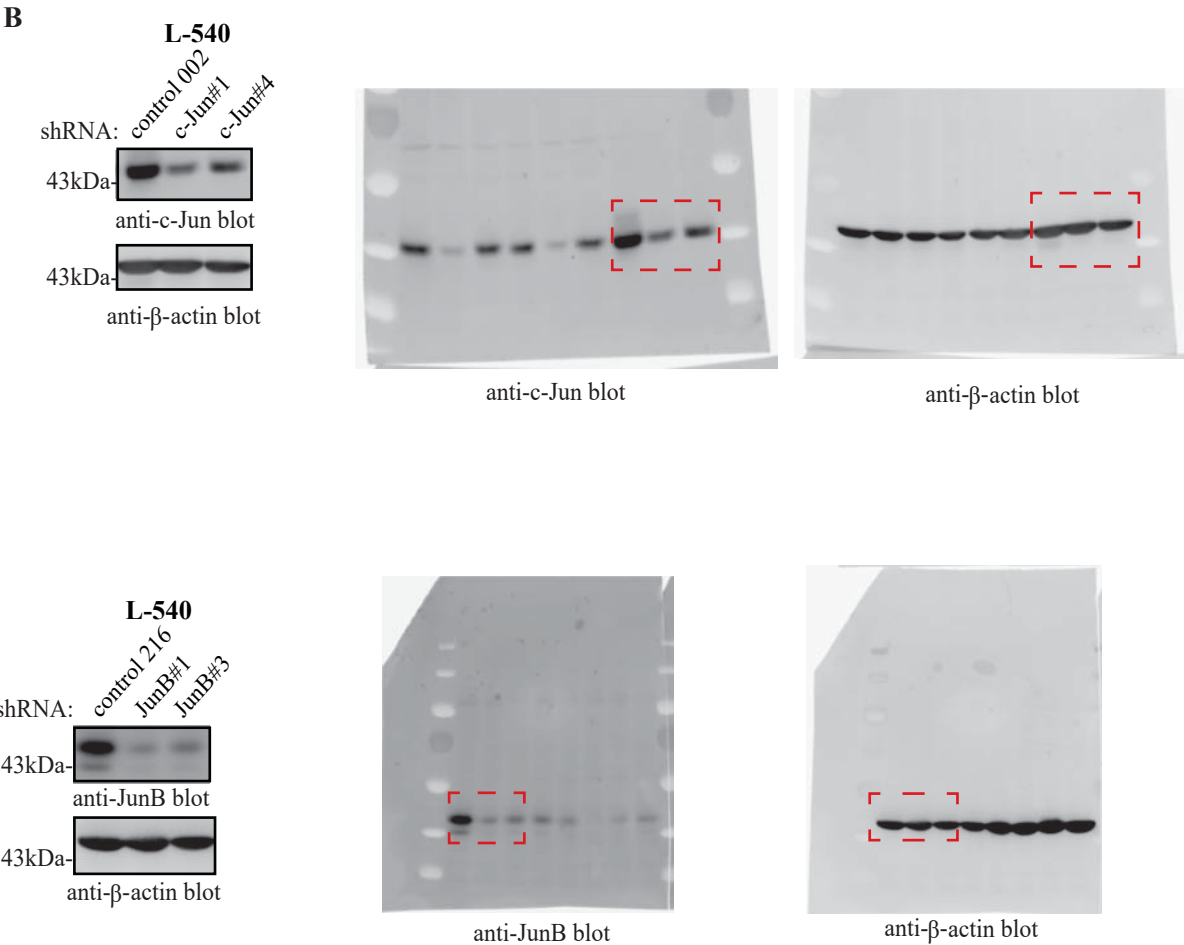
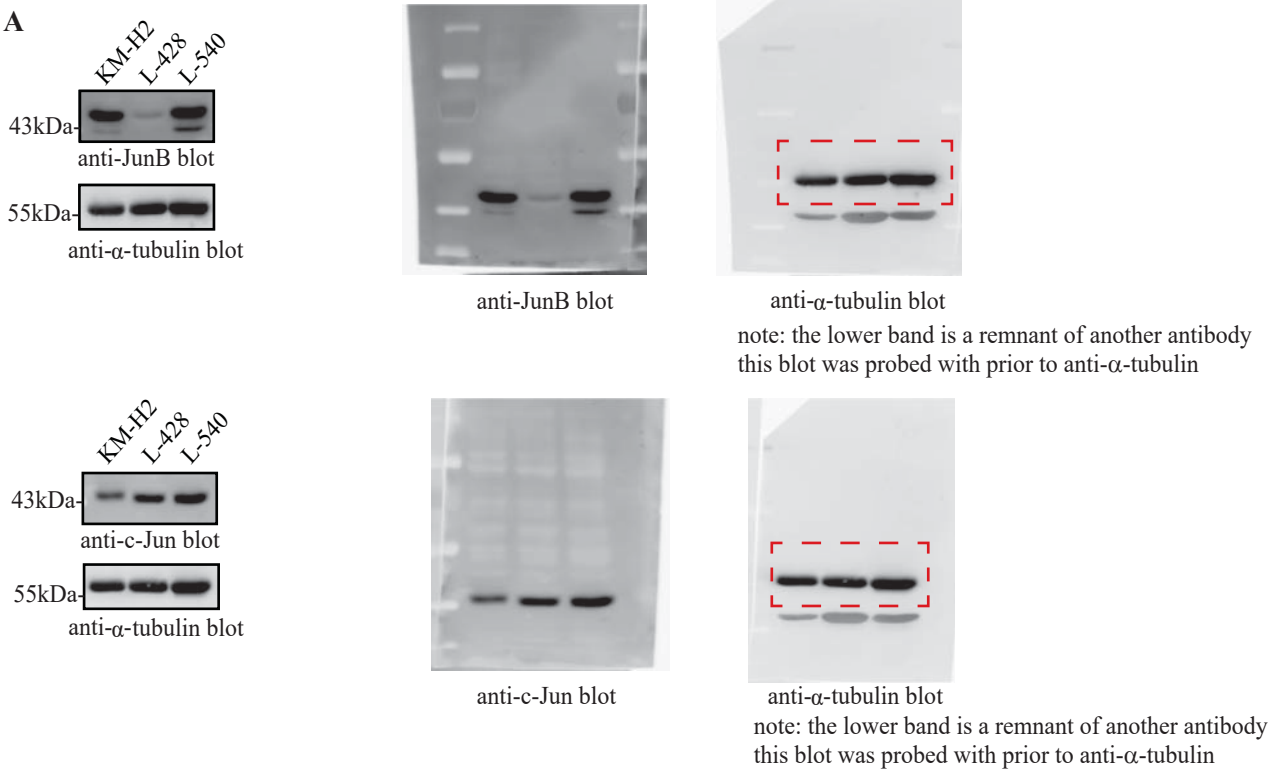


Figure S6

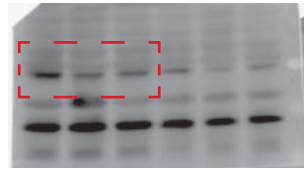
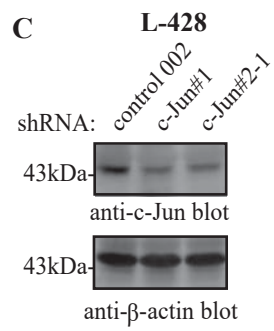


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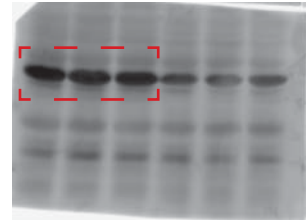
Raw Data for Figures 1C & D

C

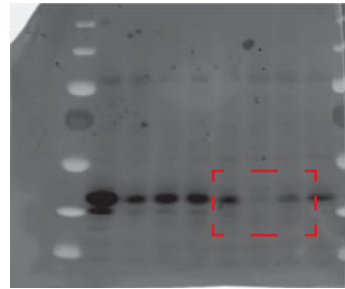
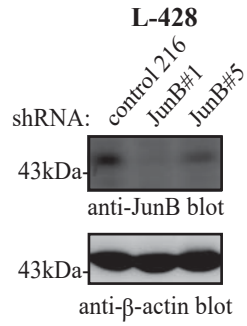


anti-c-Jun blot

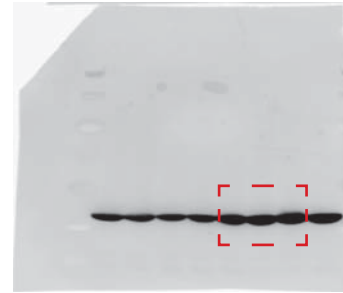
note: the lower bands are remnants of other antibodies this blot was probed with prior to anti-c-Jun



anti- β -actin blot

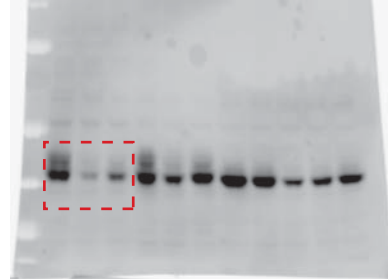
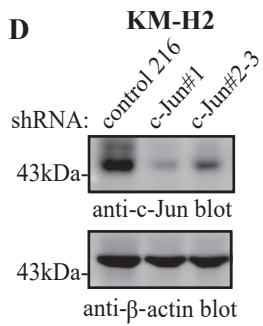


anti-JunB blot

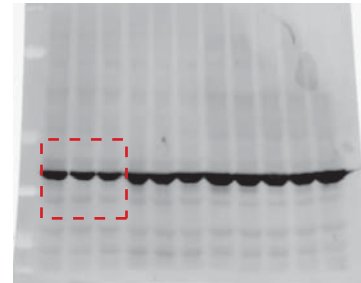


anti- β -actin blot

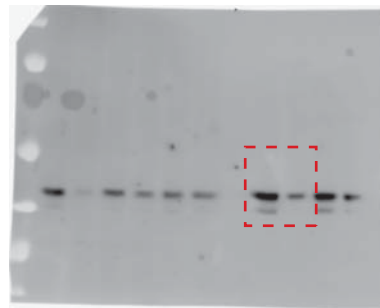
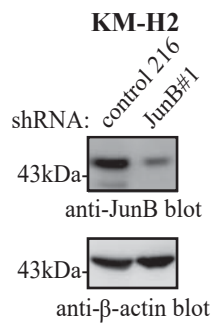
D



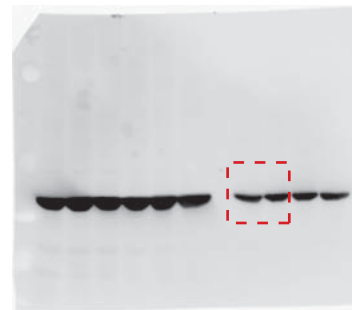
anti-c-Jun blot



anti- β -actin blot

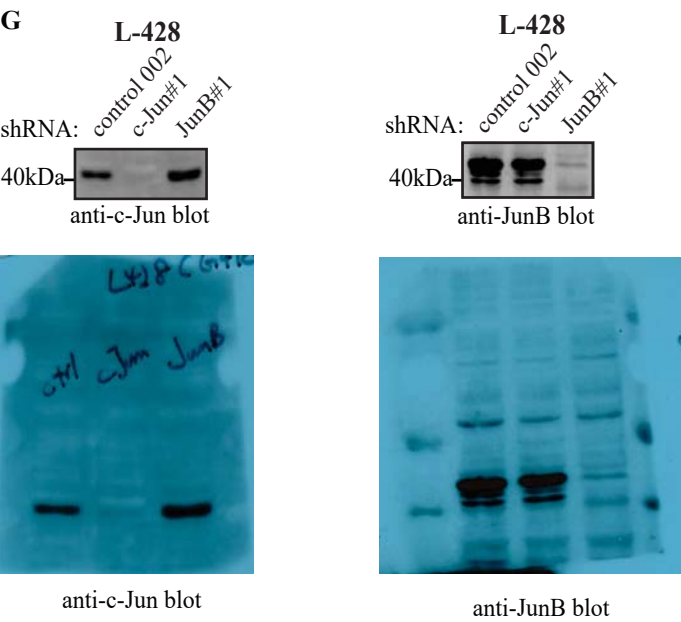


anti-JunB blot



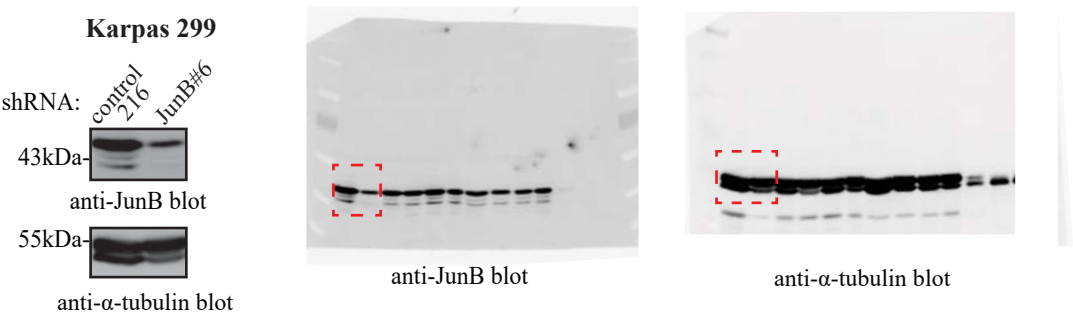
anti- β -actin blot

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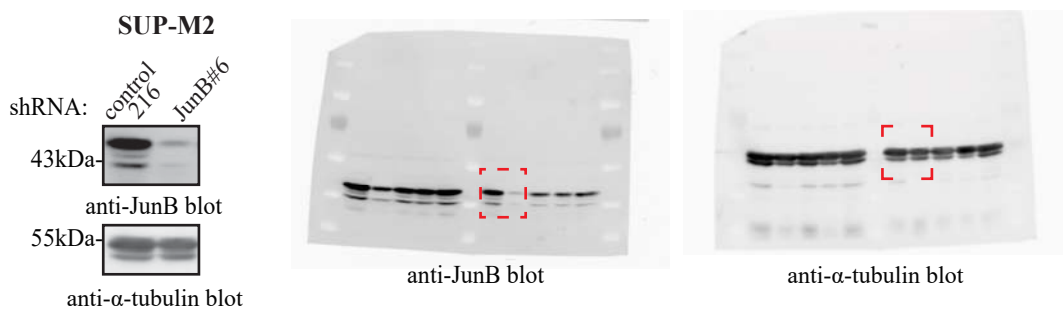


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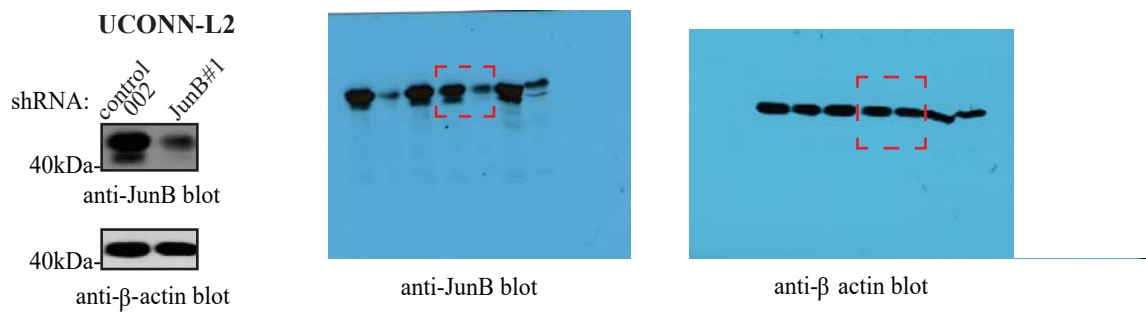
A



B

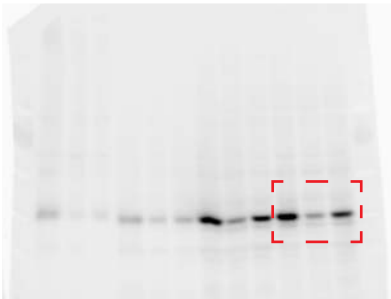
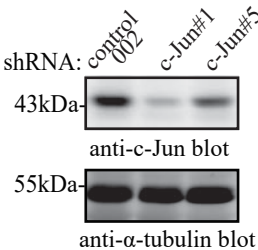


C

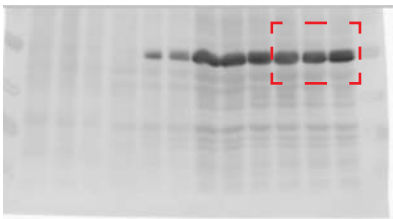


Raw Data for Figure 5

A Karpas 299

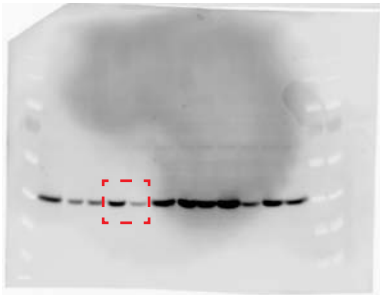
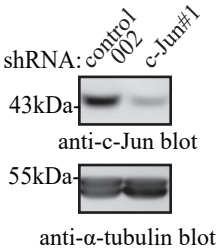


anti-c-Jun blot

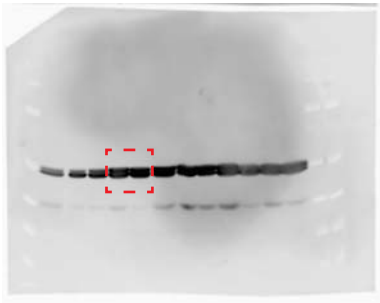


anti- α -tubulin blot

C SUP-M2

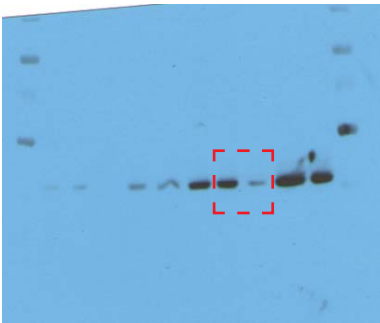
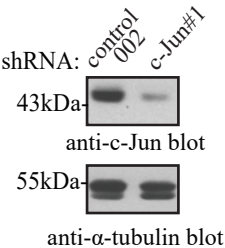


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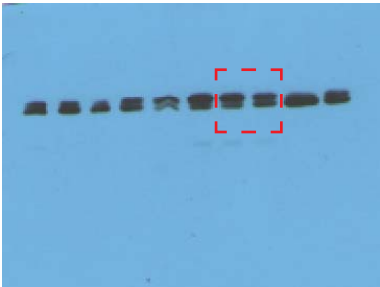


anti- α -tubulin blot

E UCONN-L2



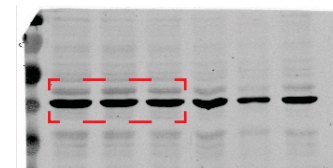
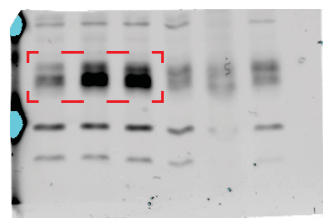
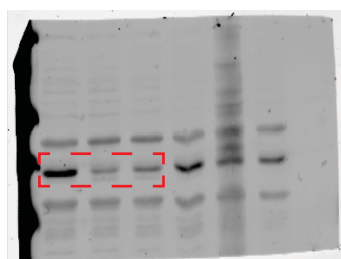
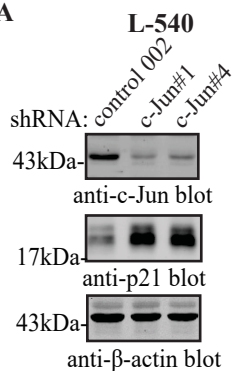
anti-c-Jun blot



anti- α -tubulin blot

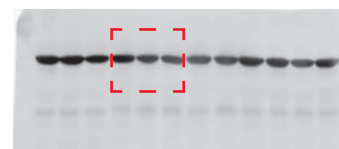
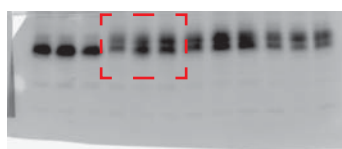
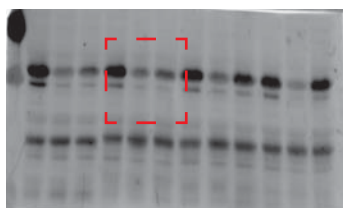
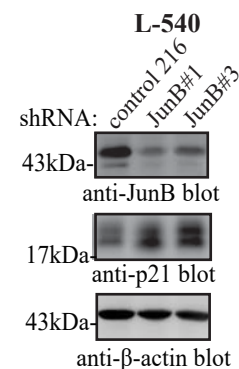
Raw Data for Figures 6A & B

A



note: the additional bands are remnants of other antibodies this blot was probed with prior to anti-c-Jun

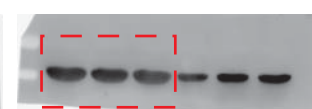
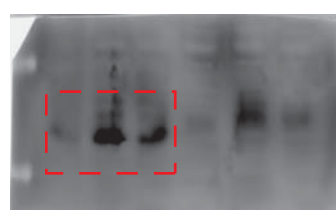
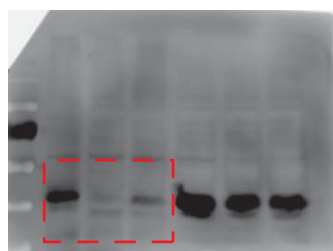
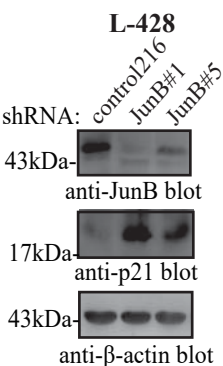
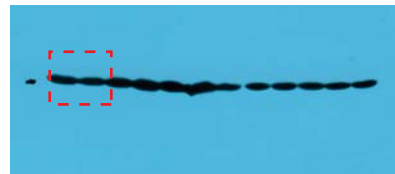
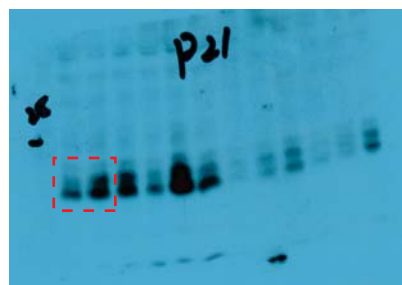
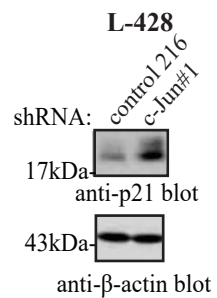
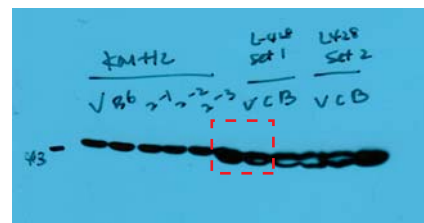
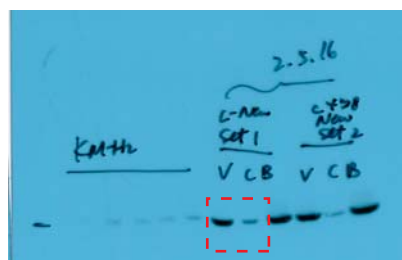
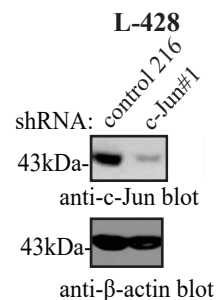
note: all blots are from the same membrane that was cut prior to probing



note: the lower band is a remnant of another antibody this blot was probed with prior to anti-c-Jun

note: all blots are from the same membrane that was cut prior to probing

B



note: all blots are from the same membrane that was cut prior to probing

Raw Data for Figures 6C & D

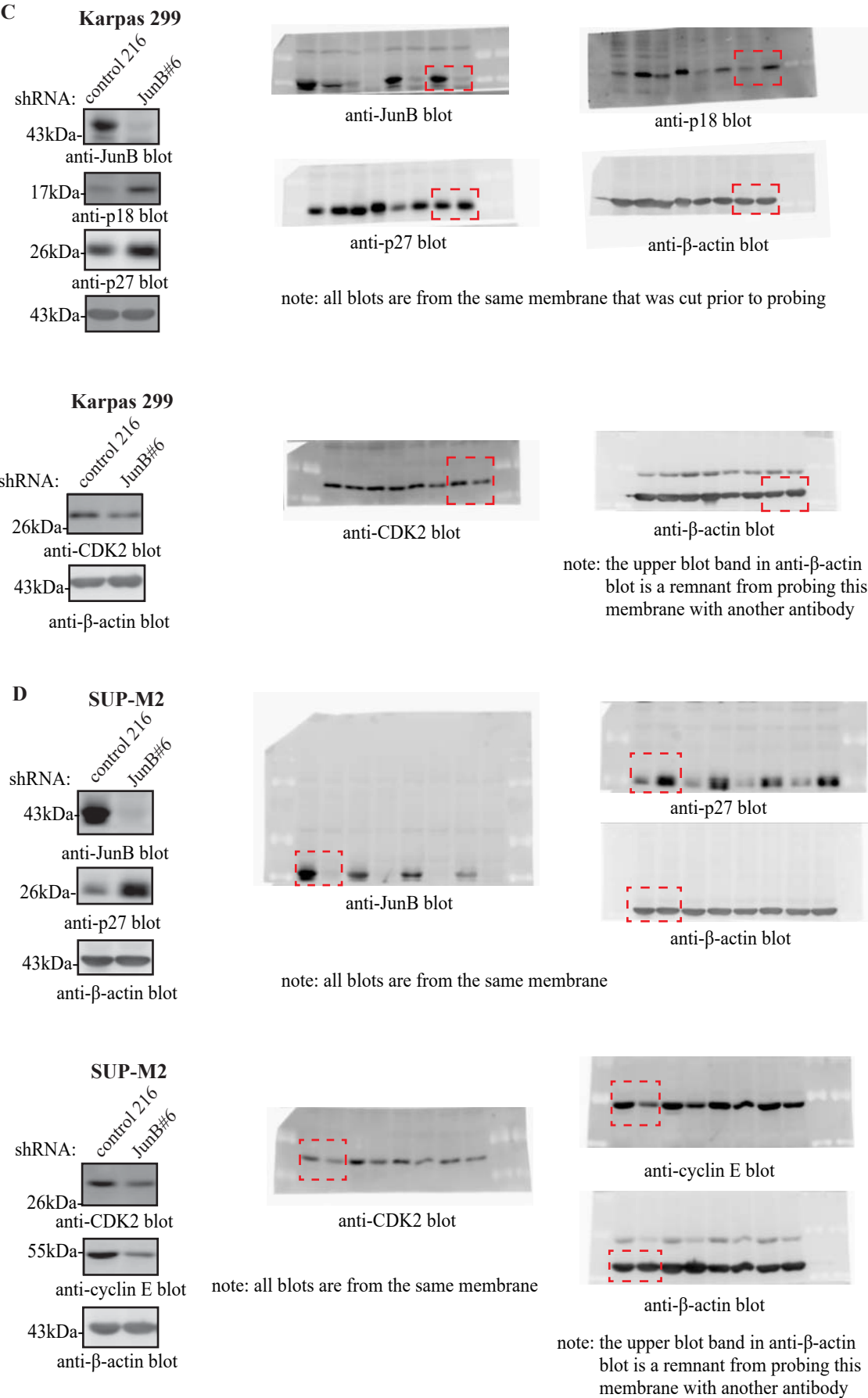


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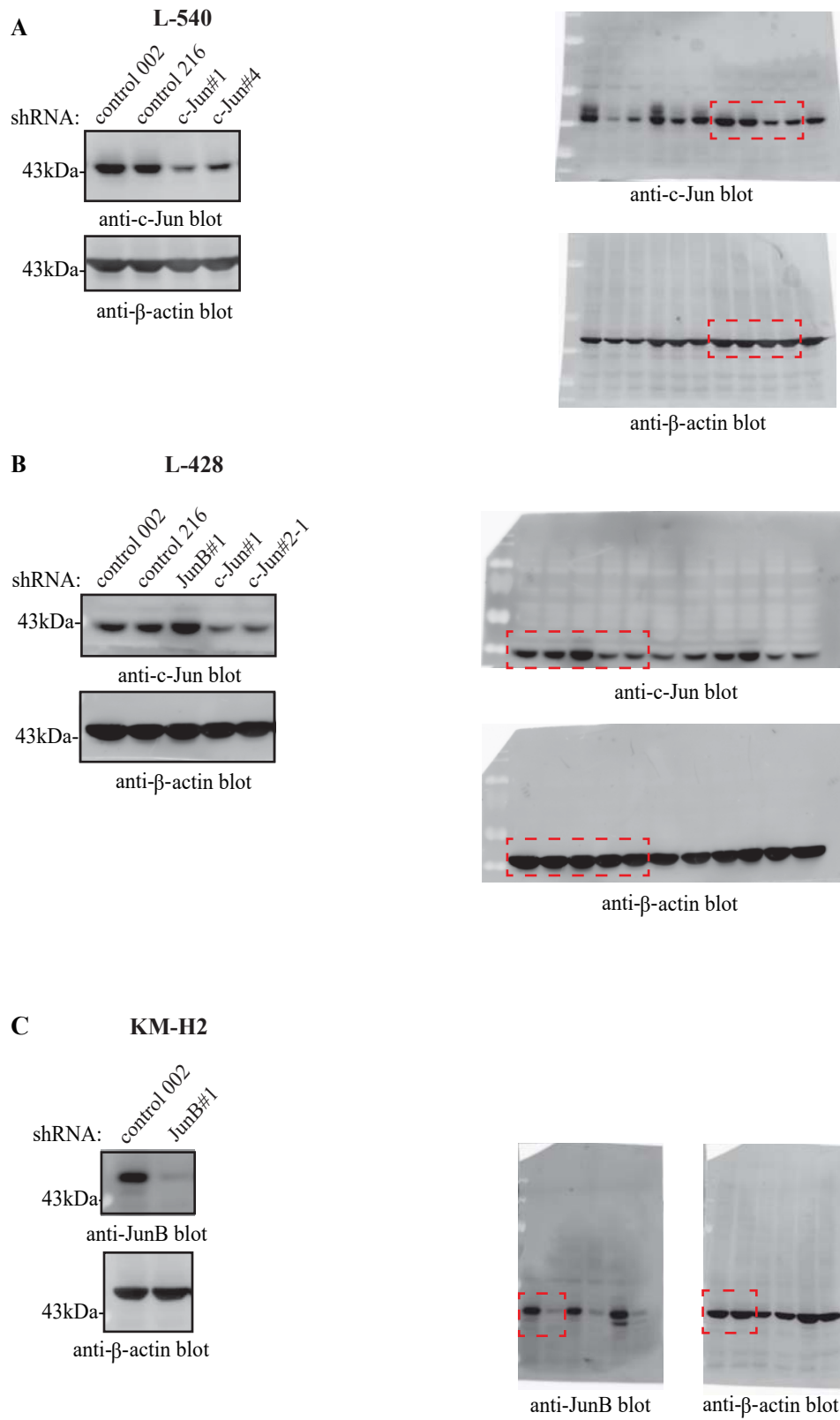
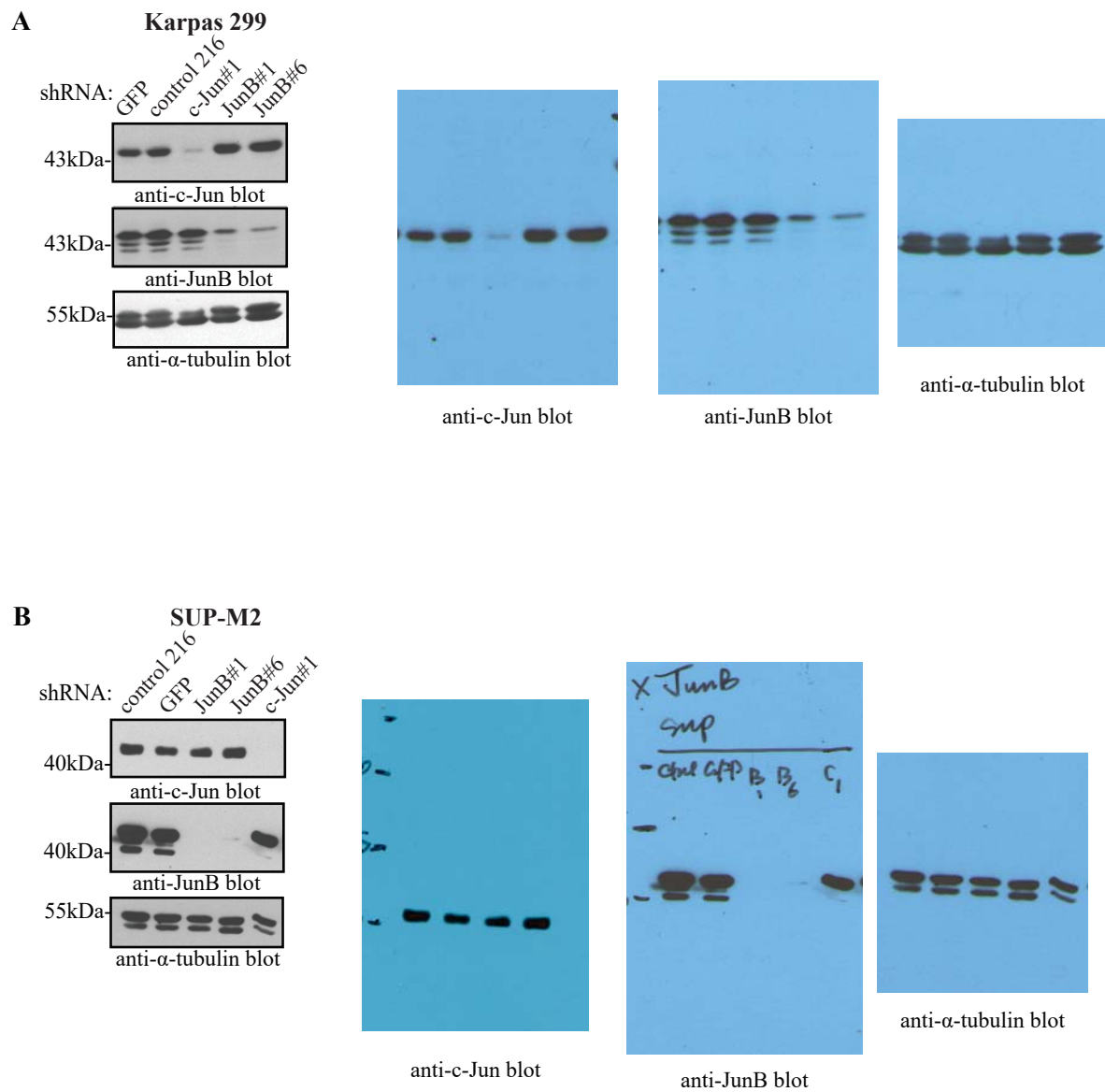
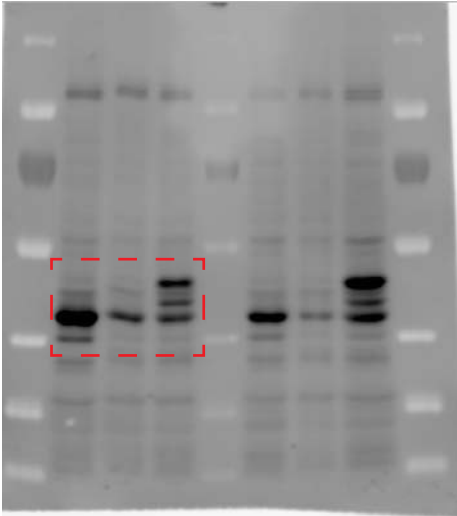
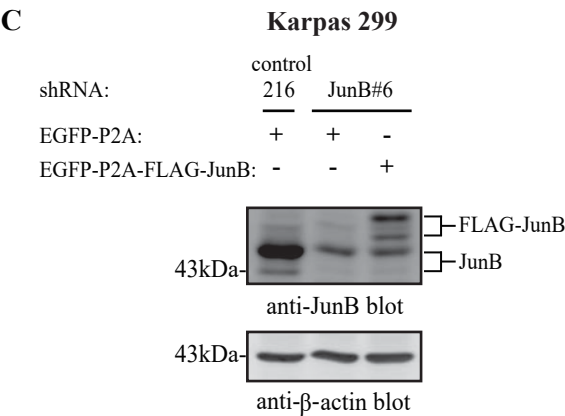


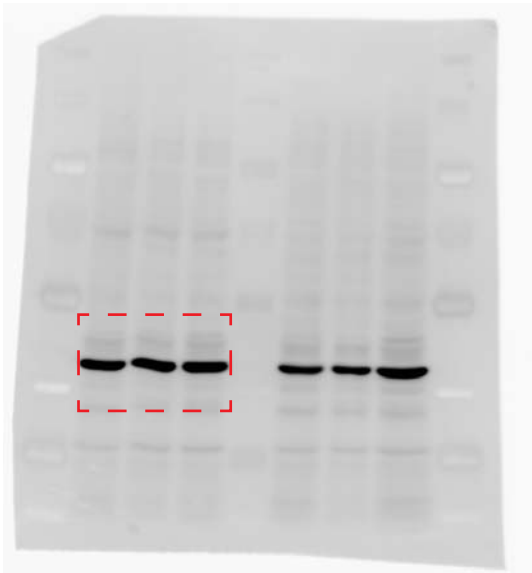
Figure S4 Raw Data



Raw Data for Figure S5

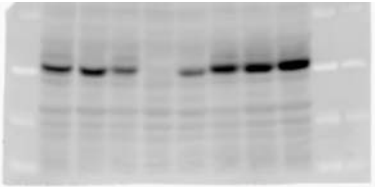
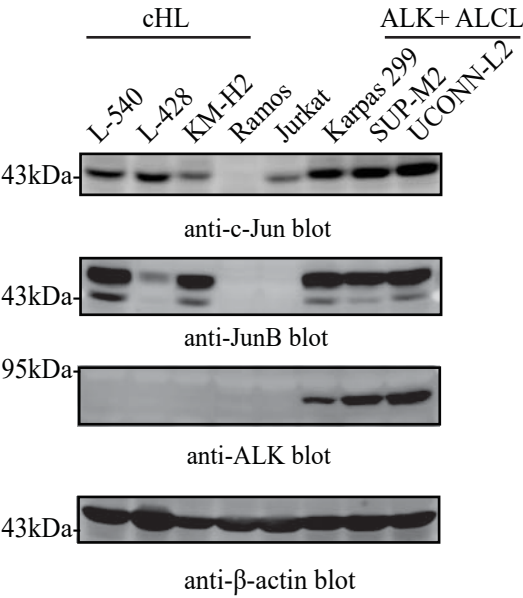


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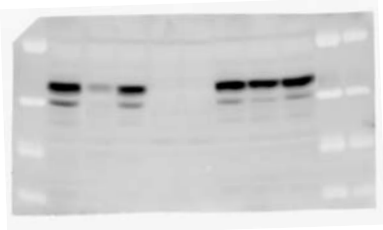


anti-β-actin reprobe

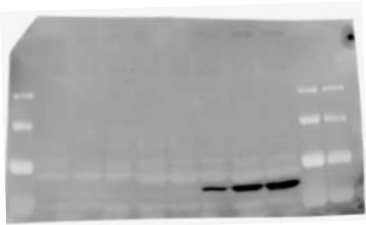
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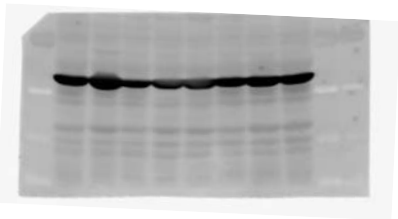
anti-c-Jun blot



anti-JunB blot



anti-ALK blot



anti-β-actin blot

note: all blots are from the same membrane