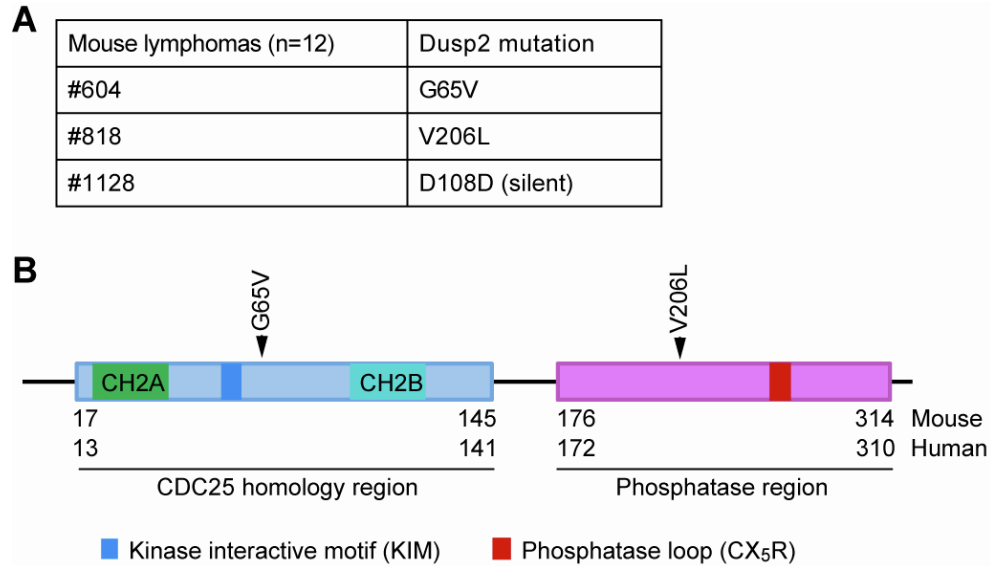


## **Supplemental information**

### **Constitutive DUSP2 expression enhances lymphoid cell proliferation by activating CDK1 and promotes lymphomagenesis**

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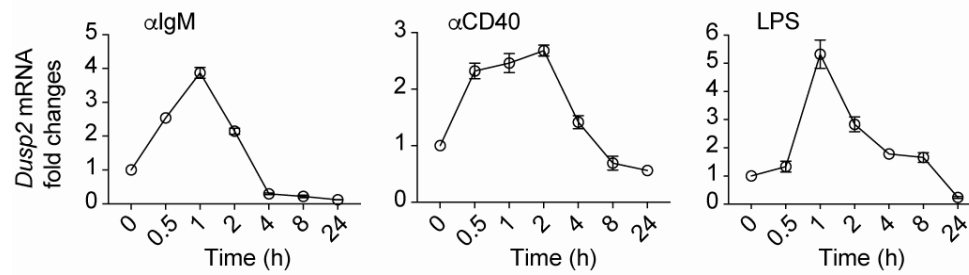


**Figure S1. Sporadic Dusp2 mutations in mouse DLBCLs.**

**A.** Summary of Dusp2 mutations in mouse DLBCLs, discovered by RNA sequencing<sup>1</sup> and confirmed by Sanger sequencing (all mutations are somatic; data not shown).

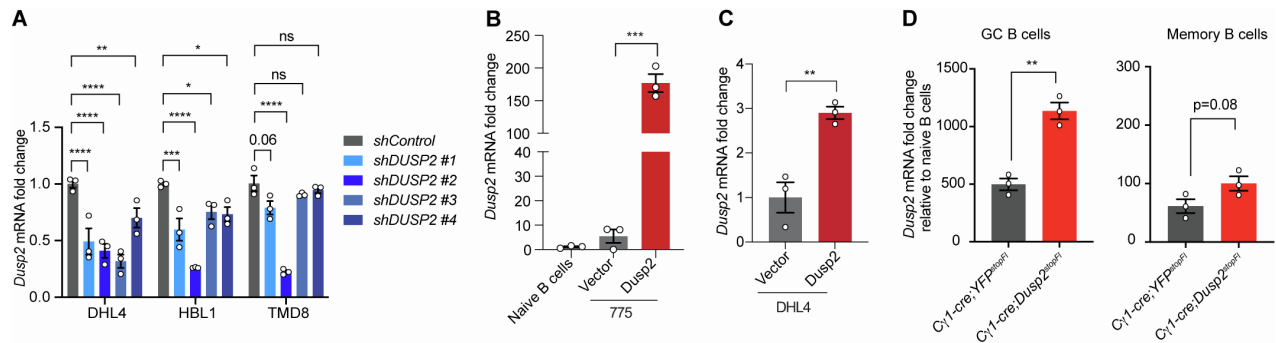
**B.** Distribution of the mouse Dusp2 mutations along the protein sequence, with known functional domains and motifs annotated. CH2A and CH2B, CDC25 homology 2 motifs.

Primary B cells



**Figure S2. *Dusp2* expression in mouse B cells upon antigenic or mitogenic stimulation.**

WT mouse splenic B cells were treated with anti-IgM, anti-CD40 or LPS for the indicated periods of time and then collected for RT-PCR analysis of *Dusp2* expression. Data are representative of two independent experiments. Error bars denote mean  $\pm$  s.d. of 3 replicate assays in a representative experiment.



**Figure S3. *DUSP2* expression upon experimental knockdown or overexpression.**

**A.** *DUSP2* mRNA levels in human DLBCL cell lines transduced with vectors carrying shRNAs against *DUSP2* or the vector control (pLKO.1; Sigma) were assayed by real-time PCR.

**B.** *Dusp2* mRNA levels in the 775 (mouse B-lymphoma) cells transduced with MSCV encoding *Dusp2* or vector control were assayed by real-time PCR, in comparison with that in naïve (resting) B cells from normal mice (set as reference, with a value of 1).

**C.** *DUSP2* mRNA levels in human DLBCL DHL4 cells transduced to express *DUSP2* were assayed by real-time PCR, and shown as fold change in comparison with vector control.

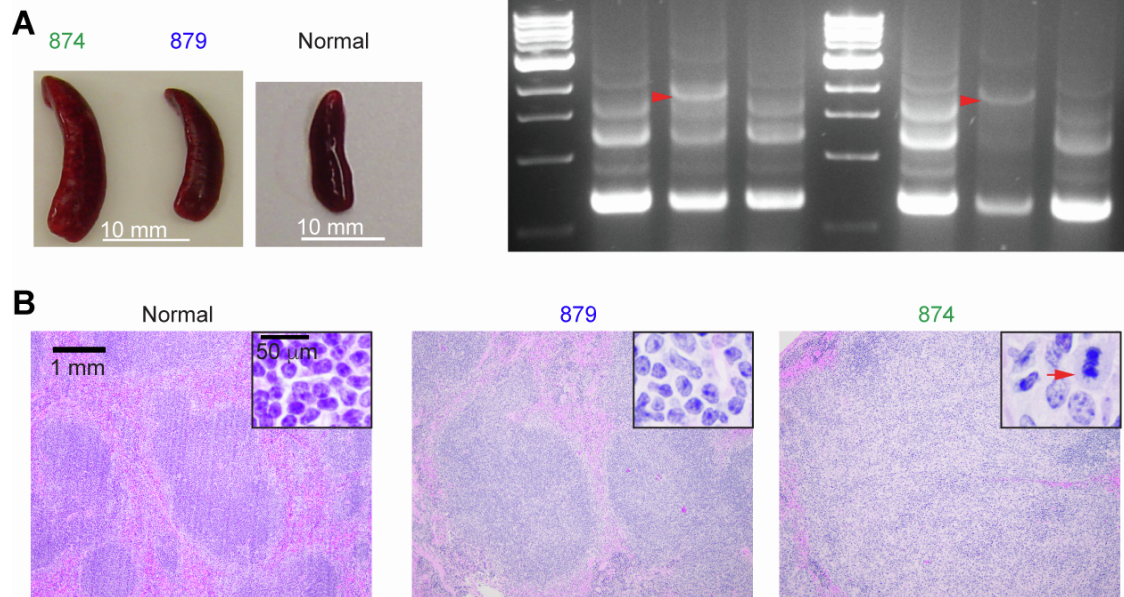
**D.** *Dusp2* mRNA levels in reporter-positive B cells, including germinal center (GC) and memory B cells, from the indicated genotypes of mice were measured by real-time PCR.

Data in **A–D** are representative of two independent experiments. Error bars denote mean  $\pm$  s.d. of 3 replicate assays in a representative experiment. ns, not significant; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ , unpaired two-tailed Student's *t* test.

874, 877: *C $\gamma$ 1-cre;I $\mu$ Bcl6;Dusp2<sup>stopFl</sup>*

879, 876: *C $\gamma$ 1-cre;I $\mu$ Bcl6*

(~2 months after the 2<sup>nd</sup> SRBC immunization)



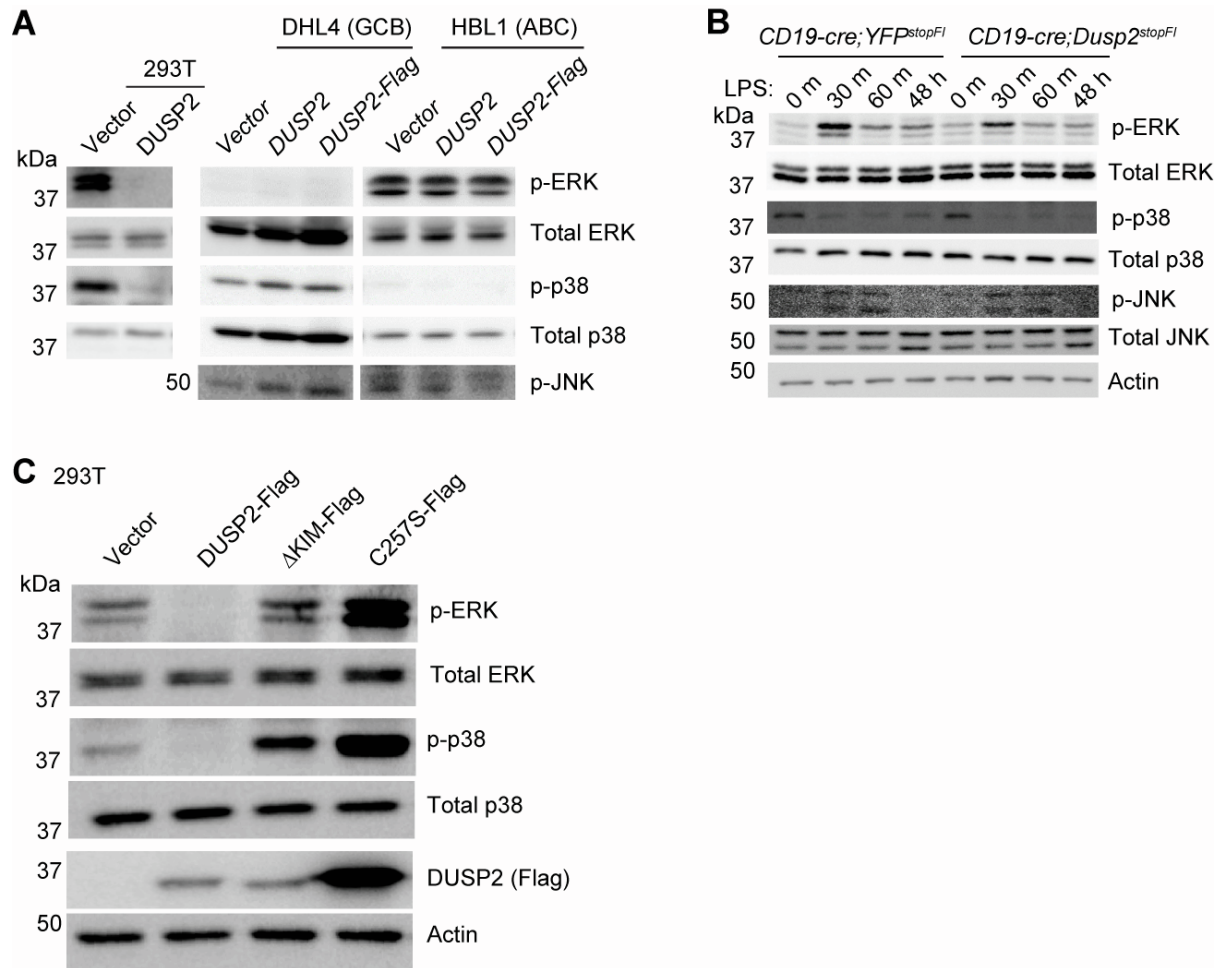
**Figure S4. Detection of clonal B-cell expansion and lymphomagenesis in young mice with enforced B-cell specific *Dusp2* and *Bcl6* expression.**

**A.** Representative picture of the spleen of the indicated mice/genotypes analyzed at 5 months of age (2 months after the 2<sup>nd</sup> SRBC immunization).

**B.** Histological examination of the spleen of the indicated mice. Note the DLBCL-like disease in mouse #874. Red arrow indicates a mitotic cell.

**C.** PCR analysis of rearranged IgH VDJs using DNA from equivalent number of sorted B cells. A normal polyclonal B cell population is denoted by the presence of four major bands, corresponding to rearrangements utilizing the four JH segments; a clonal population is reflected by the preferential amplification of a unique band (red arrowheads).

Data in **A–B** are representative of analyses done in two mice of each indicated genotype.



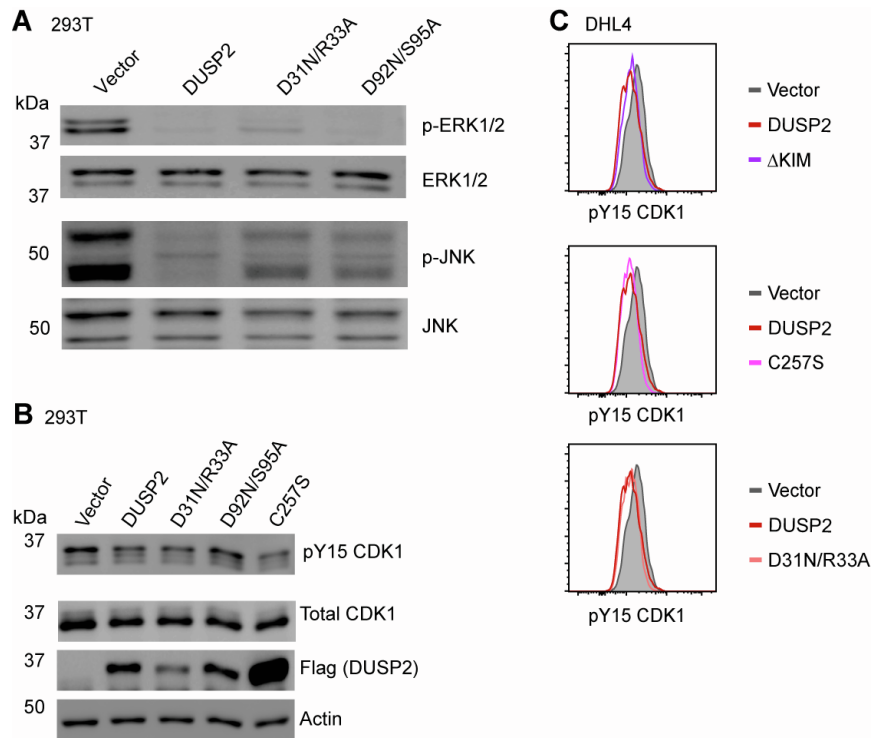
**Figure S5. DUSP2 exerts pronounced regulation of MAPKs in 293T cells, but only modest regulation of these substrates in lymphoma B cells and primary B cells.**

**A.** Phosphorylation status of MAPKs in 293T cells versus DLBCL cells upon ectopic expression of DUSP2 or vector control was assayed by immunoblot analysis.

**B.** Phosphorylation status of MAPKs in primary B cells from *CD19-cre;YFP<sup>stopFl</sup>* or *CD19-cre;Dusp2<sup>stopFl</sup>* mice at the indicated time points of LPS stimulation was assayed by immunoblot analysis.

**C.** Disruption of the KIM motif or the catalytic site C257 abolishes DUSP2's ability to dephosphorylate MAPKs. 293T cells were transfected to express the indicated DUSP2 variants (with a Flag tag) or vector control, and subjected to immunoblot analysis.  $\Delta$ KIM, a DUSP2 variant in which the classical kinase-interactive motif (KIM), required for interaction with ERK/p38/JNK, is functionally abolished by altering LLRRRAR<sub>54-60</sub> to LLAAAAA; C257S, a DUSP2 variant in which the phosphatase active site Cys257 is substituted by Serine, abrogating its phosphatase activity on MAPKs (this mutant also displays a dominant negative effect and enhanced stability)<sup>2</sup>.

Data in **A–C** are representative of two independent experiments.



**Figure S6. Assessment of DUSP2 variants on regulation of MAPKs and CDK1.**

**A.** DUSP2 mutants D31N/R33A and D92N/S95A retain the ability to dephosphorylate ERK and JNK MAPKs. Phosphorylation status of ERK and JNK in 293T cells transfected to express DUSP2, DUSP2<sup>D31N/R33A</sup>, DUSP2<sup>D92N/S95A</sup> or vector control was assayed by immunoblot analysis. WT DUSP2 and the various DUSP2 variants all carry the Flag tag.

**B.** 293T cells were transfected to express the indicated DUSP2 variants or vector control, and subjected to IB analysis with the indicated antibodies.

**C.** The KIM motif, C257 catalytic site, and the conserved D31/R33 sequence motif are not involved in the regulation of CDK1. DHL4 cells were transduced to express DUSP2, DUSP2 <sup>$\Delta$ KIM</sup>, DUSP2<sup>C257S</sup>, DUSP2<sup>D31N/R33A</sup> or vector control, and subjected to intracellular flow cytometry analysis of pY15 CDK1 levels. The pY15 CDK1 levels in cells expressing each DUSP2 variant are shown in comparison with those expressing WT DUSP2 and vector control in overlay FACS plots. WT DUSP2 and the various DUSP2 variants all carry the Flag tag.

Data in **A–C** are representative of two independent experiments.

**Table S1. Histology of tumors.**

| Genotype                                     | Mice | Organ       | Histology |
|----------------------------------------------|------|-------------|-----------|
| <i>Cγ1-cre/IμBcl6;Dusp2<sup>stopFL</sup></i> | 1018 | Spleen      | DLBCL     |
|                                              |      | mLN         | DLBCL     |
|                                              |      | Inguinal LN | DLBCL     |
|                                              | 1148 | Spleen      | DLBCL     |
|                                              |      | mLN         | DLBCL     |
|                                              |      | Inguinal LN | DLBCL     |
|                                              | 1217 | Spleen      | DLBCL     |
|                                              | 943  | Spleen      | DLBCL     |
|                                              | 1215 | Spleen      | DLBCL     |
|                                              | 1081 | Spleen      | BL        |
|                                              |      | mLN         | BL        |
| <i>Cγ1-cre/IμBcl6</i>                        | 1024 | Spleen      | DLBCL     |
|                                              |      | mLN         | DLBCL     |
|                                              | 1082 | Spleen      | DLBCL     |
|                                              | 1118 | Spleen      | DLBCL     |
|                                              |      | mLN         | DLBCL     |
|                                              | 1212 | Spleen      | DLBCL     |
|                                              |      | mLN         | DLBCL     |
| <i>Cγ1-cre;Dusp2<sup>stopFL</sup></i>        | 670  | mLN         | DLBCL     |
|                                              |      | Renal LN    | DLBCL     |
|                                              | 753  | Spleen      | DLBCL     |
|                                              |      | mLN         | DLBCL     |

LN, lymph node; mLN, mesenteric lymph node.

**Table S2. SHM analysis of tumor samples.**

| Genotype/Mice                                | $V_H$                | $D_H$          | $J_H$        | Nucleotides analyzed (#) | Frequency of mutation (%) |
|----------------------------------------------|----------------------|----------------|--------------|--------------------------|---------------------------|
| <i>Cγ1-cre/lμBcl6;Dusp2<sup>stopFL</sup></i> |                      |                |              |                          |                           |
| #1018                                        | <i>IGHV1S130*01</i>  | -              | <i>J4*01</i> | 540                      | 1.57                      |
| #1117                                        | <i>IGHV1S81*02</i>   | <i>D4-1*01</i> | <i>J2*01</i> | 380                      | 0                         |
| #1148                                        | <i>IGHV5-6-4*01</i>  | <i>D2-3*01</i> | <i>J3*01</i> | 530                      | 0.84                      |
| #1184                                        | <i>IGHV5-12*01</i>   | <i>D1-1*01</i> | <i>J3*01</i> | 530                      | 0.94                      |
| #1199                                        | <i>IGHV1S130*01</i>  | -              | <i>J4*01</i> | 540                      | 0.65                      |
| <i>Cγ1-cre/lμBcl6</i>                        |                      |                |              |                          |                           |
| #1082                                        | <i>IGHV8-10*01</i>   | <i>D6-1*01</i> | <i>J3*01</i> | 540                      | 0.42                      |
| #1118                                        | <i>IGHV5-12-1*01</i> | <i>D2-1*01</i> | <i>J4*01</i> | 540                      | 0                         |
| #1212                                        | <i>IGHV5-6-4*01</i>  | <i>D1-1*01</i> | <i>J1*01</i> | 320                      | 0.36                      |
| <i>Cγ1-cre;Dusp2<sup>stopFL</sup></i>        |                      |                |              |                          |                           |
| #670                                         | <i>IGHV6-3*01</i>    | <i>D4-1*01</i> | <i>J4*01</i> | 540                      | 9.96                      |

**Table S4. Sequences of primers used in this work.**

| Genes                     | Primer Sequence                                | Experimental use                       |
|---------------------------|------------------------------------------------|----------------------------------------|
| mDusp2                    | F: 5'-AACCGTCAGCTCCCGCCA-3'                    | Cloning targeted region by CRISPR-Cas9 |
|                           | R: 5'-AGGCAGGGCCTGGGCAGGAG-3'                  |                                        |
| Sg1-mDusp2                | 5'-GTTGCGATGCCGATCGCCATG-3'                    | Guide RNA for mDusp2                   |
| hDUSP2                    | F: 5'-TGCCCCAACCACCTTTGAGG-3'                  | Real-time PCR                          |
|                           | R: 5'-AGTCAATGAAGCCTATGGCCT-3'                 |                                        |
| hGAPDH                    | F: 5'-GCACCGTCAAGGCTGAGAAC-3'                  | Real-time PCR                          |
|                           | R: 5'-TGGTGAAGACGCCAGTGGA-3'                   |                                        |
| mDusp2                    | F: 5'-AGACGGCGTGCGAGCTAGAGT-3'                 | Real-time PCR                          |
|                           | R: 5'-CTTTCATCCAGCACCCAGGCA-3'                 |                                        |
| mHprt                     | F: 5'-GTCATGCCGACCCGCAGTC-3'                   | Real-time PCR                          |
|                           | R: 5'-GTCCTGTCCATAATCAGTCCATGA<br>GGAATAAAC-3' |                                        |
| J <sub>H</sub> 4 intron R | 5'-CTCCACCAGACCTCTCTAGACA-3'                   | Analysis of tumor clonality            |
| V1-FR3                    | 5'-GAGGACTCTGCRGTCTATTWCTGTGC-3'               | Analysis of tumor clonality            |
| V5-FR3                    | 5'-GAGGACACRGCCATGTATTACTGTGC-3'               | Analysis of tumor clonality            |
| V3-FR3                    | 5'-GAGGACACACCCACATATTACTGTGC-3'               | Analysis of tumor clonality            |
| V7-FR3                    | 5'-GAGGACAGTGCCACTTATTACTGTGC-3'               | Analysis of tumor clonality            |
| V9-FR3                    | 5'-ATGAGGACATGGCTACATATTTCTGT-3'               | Analysis of tumor clonality            |

**Supplemental references:**

1. Zhang, B., Calado, D.P., Wang, Z., Frohler, S., Kochert, K., Qian, Y., Koralov, S.B., Schmidt-Suppran, M., Sasaki, Y., Unitt, C., et al. (2015). An Oncogenic Role for Alternative NF-kappaB Signaling in DLBCL Revealed upon Deregulated BCL6 Expression. *Cell reports* 11, 715-726. 10.1016/j.celrep.2015.03.059.
2. Jeffrey, K.L., Brummer, T., Rolph, M.S., Liu, S.M., Callejas, N.A., Grumont, R.J., Gillieron, C., Mackay, F., Grey, S., Camps, M., et al. (2006). Positive regulation of immune cell function and inflammatory responses by phosphatase PAC-1. *Nature immunology* 7, 274-283. 10.1038/ni1310.

Data S1: Original Western blots for all main and supplemental figures

Fig. 5D

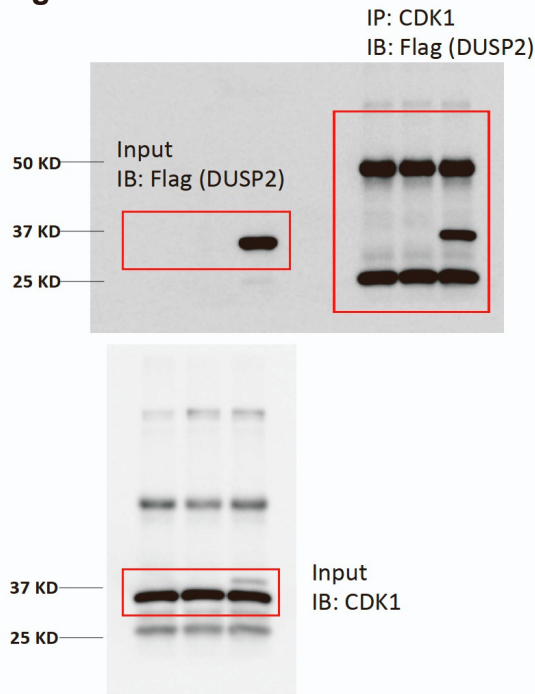


Fig. 5E

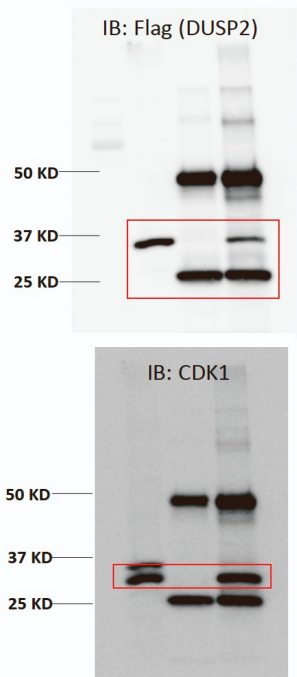


Fig. 5F

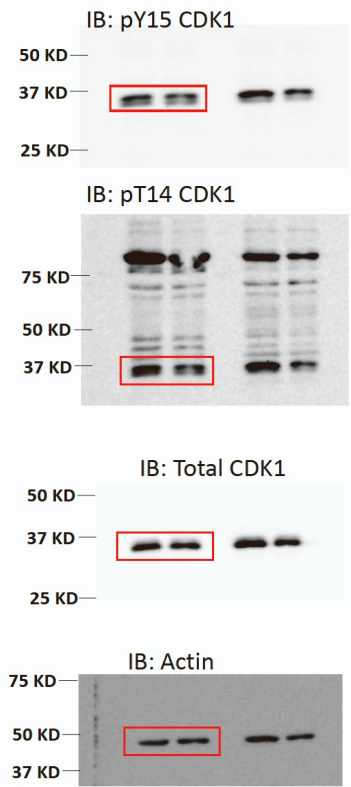
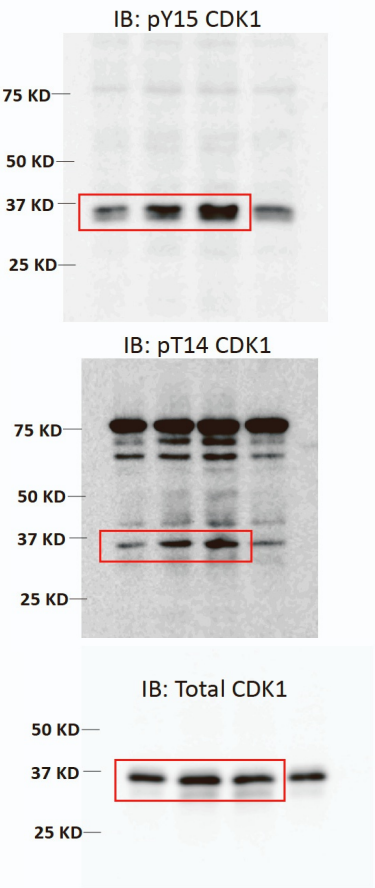
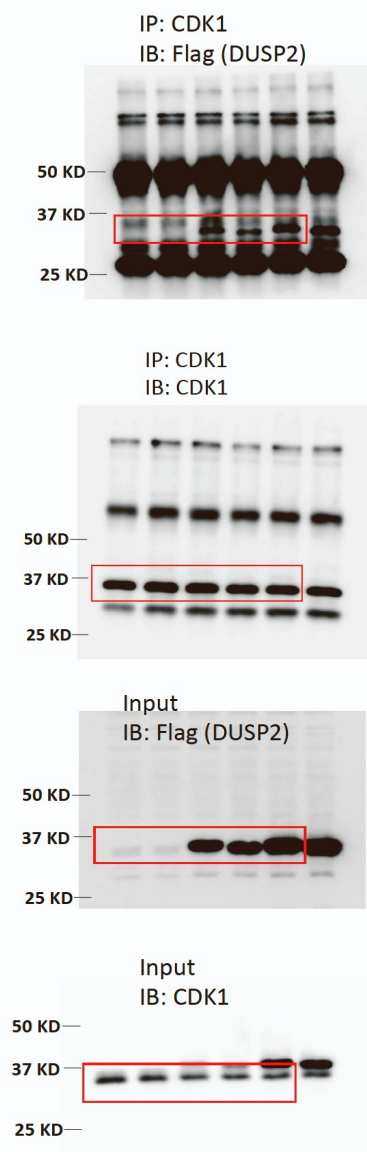


Fig. 5G



**Fig. 5J**



**Fig. 5K**

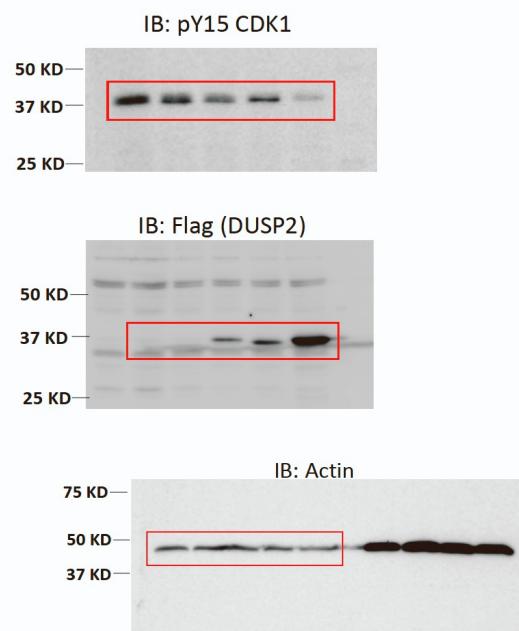


Fig. 6D



Fig. 6F

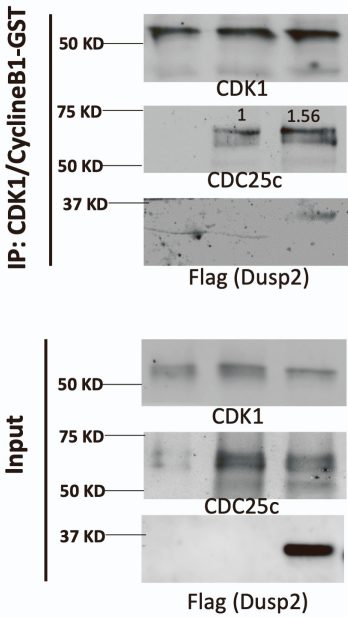
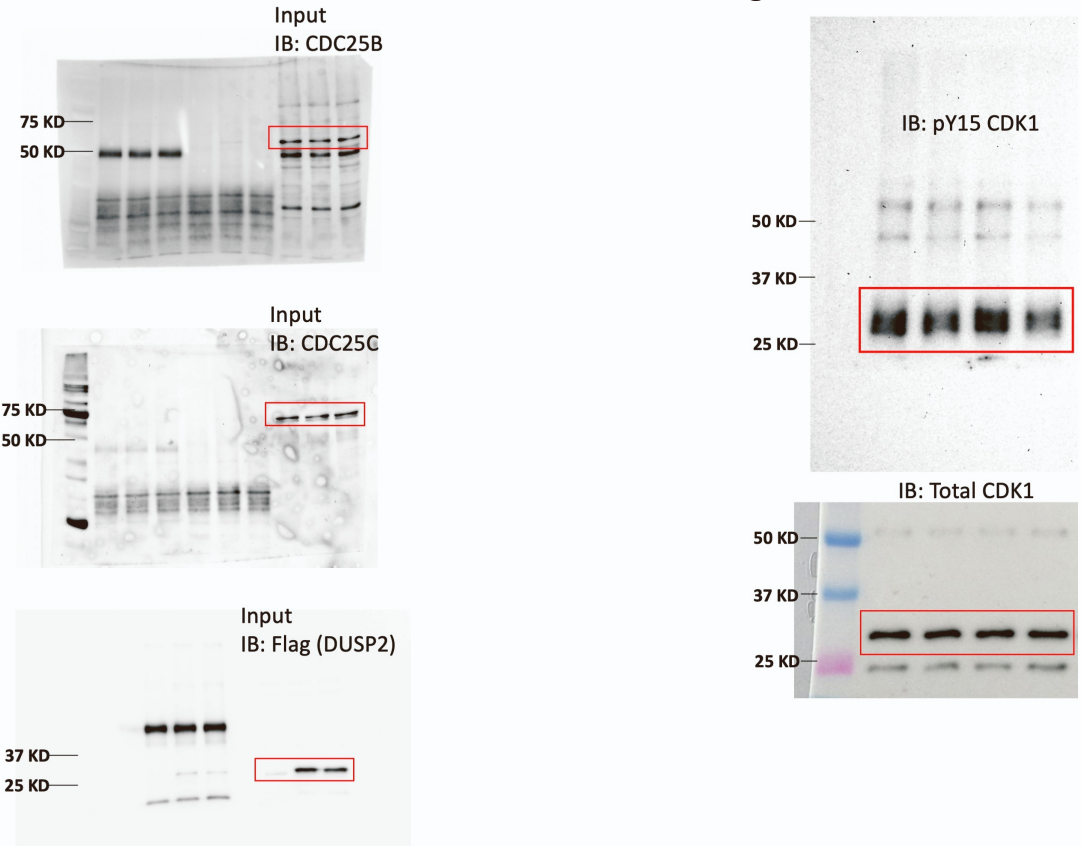
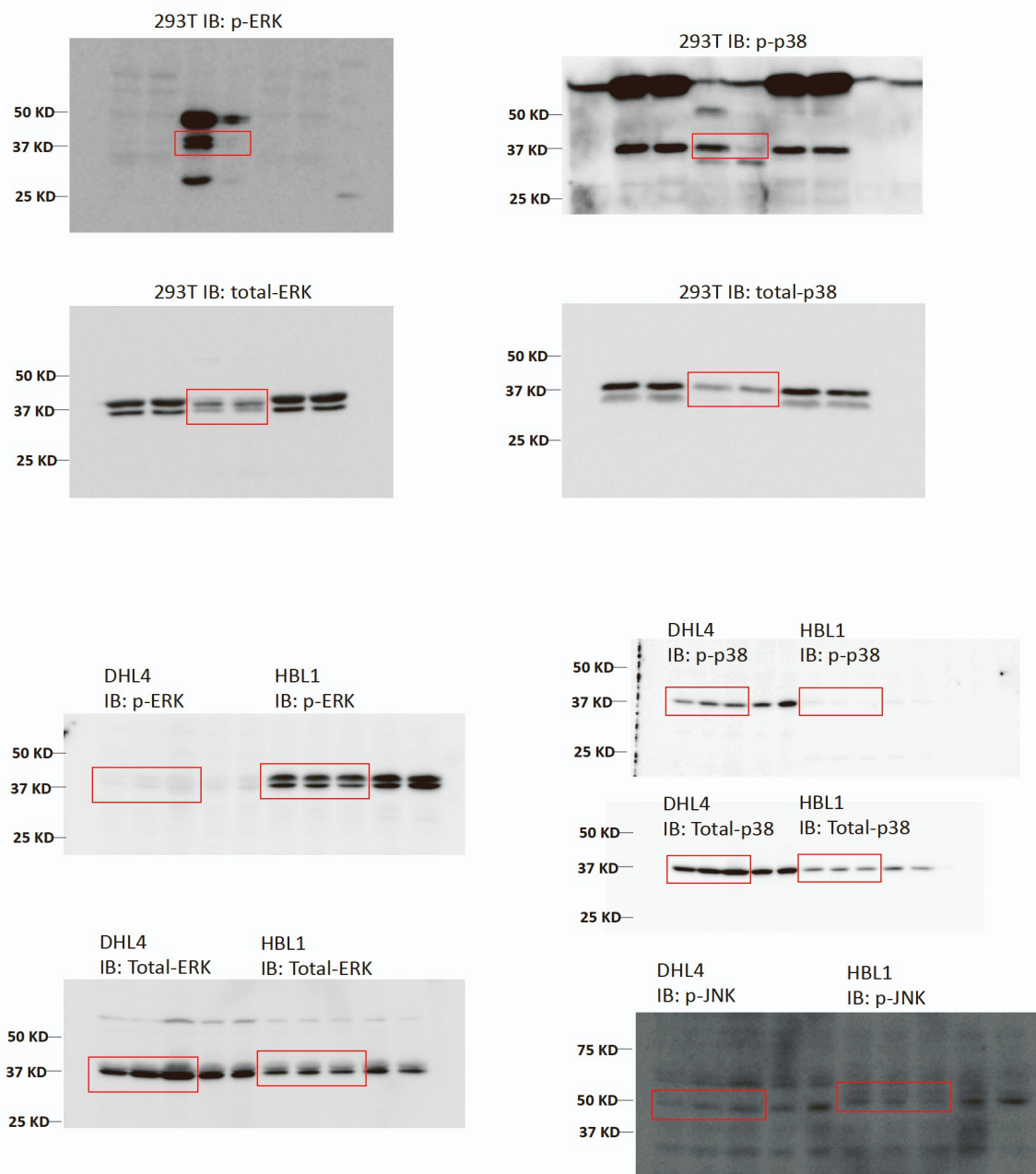


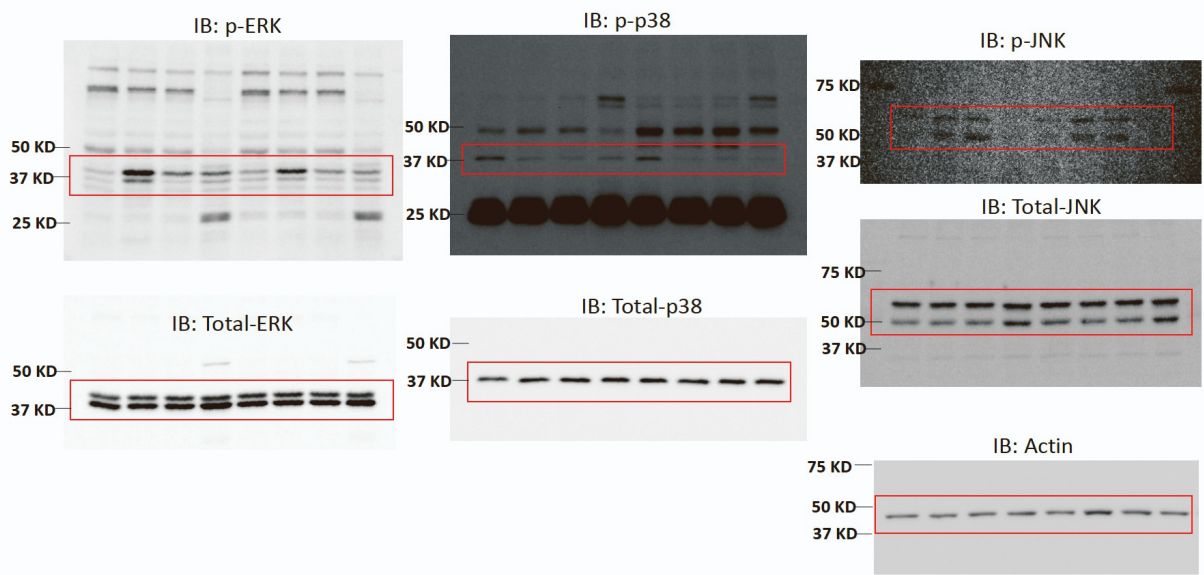
Fig. 6G



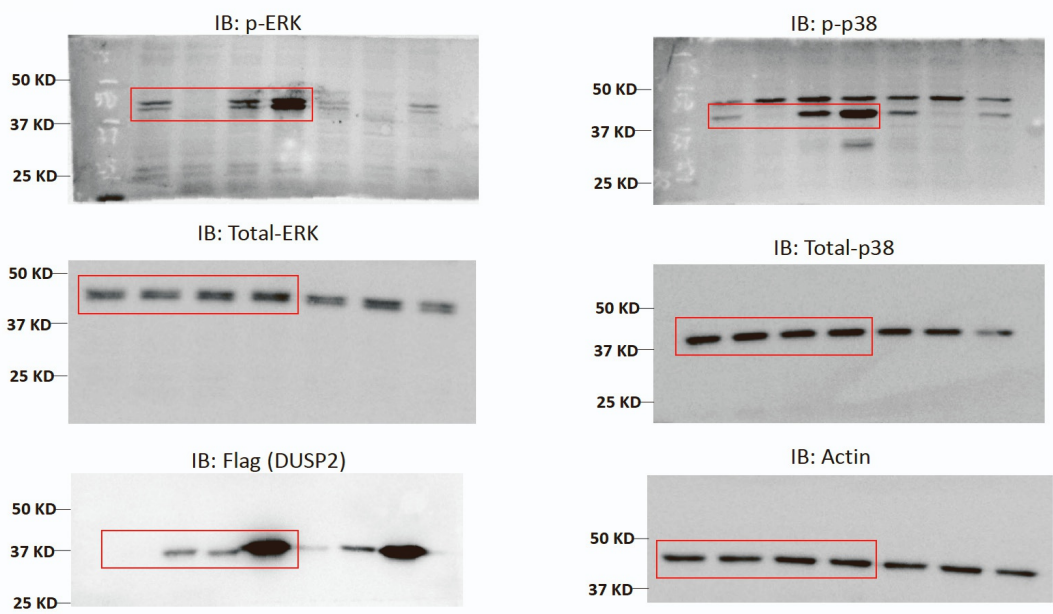
**Fig. S5A**



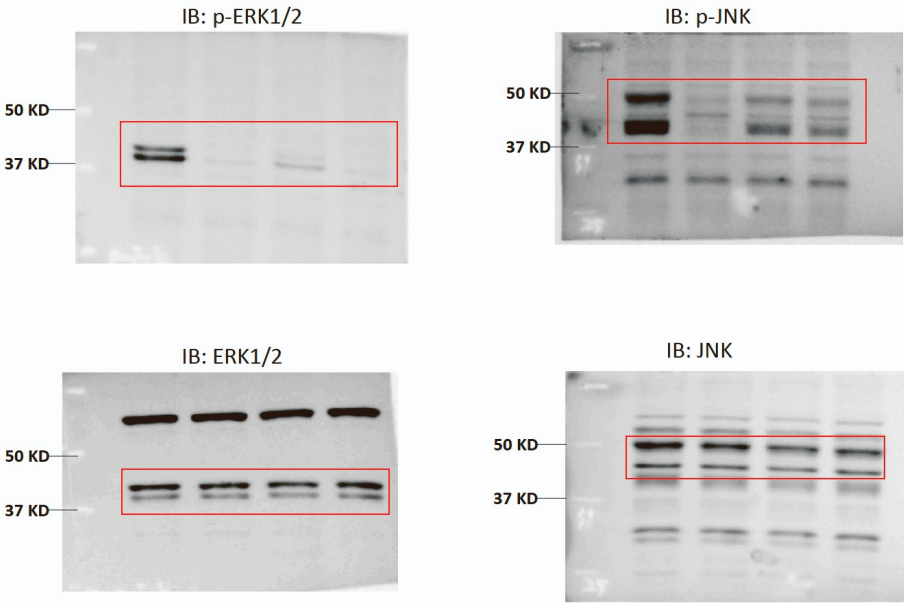
**Fig. S5B**



**Fig. S5C**



**Fig. S6A**



**Fig. S6B**

