

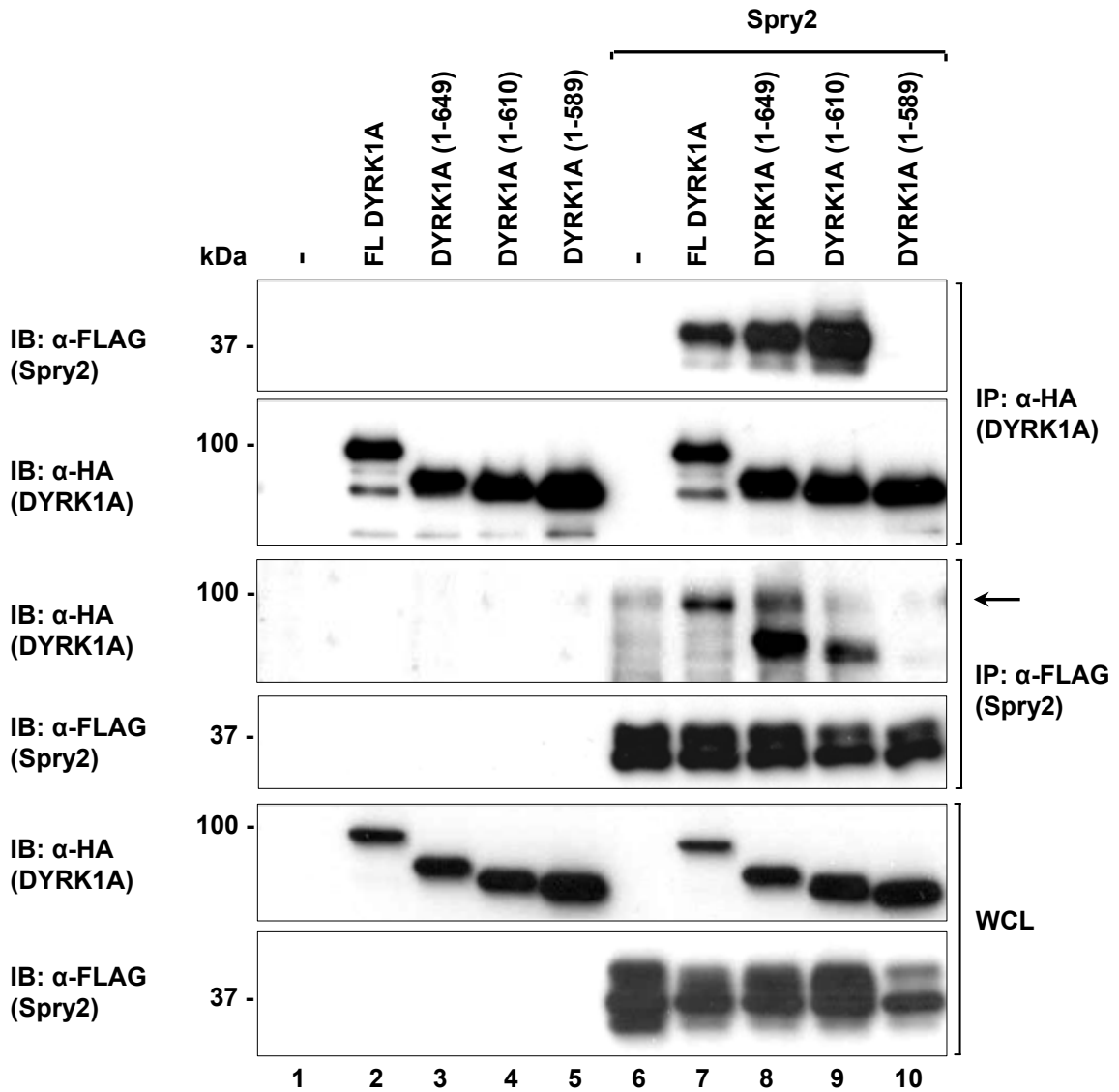
Supplemental figure 1

Proteins	Genebank Accession Number	Number of clones
DYRK1A	BC129889.1	3
Tesk1	NM_01157.3	1
RanBP9	NM_019930	2
Fibrillin	NM_007993	1
Cap1	NM_025548	2
SIN3B	NM_009188.3	3

Table 1 (Supplemental Fig. 1)

Binding partners of Spry2, SPRED1 and 2 isolated in the yeast two-hybrid screens. Human Spry2, mouse SPRED1 and 2 were used as baits for these screens as mentioned in the experimental procedures. This table shows a consolidated summary of interacting partners obtained from three determinations.

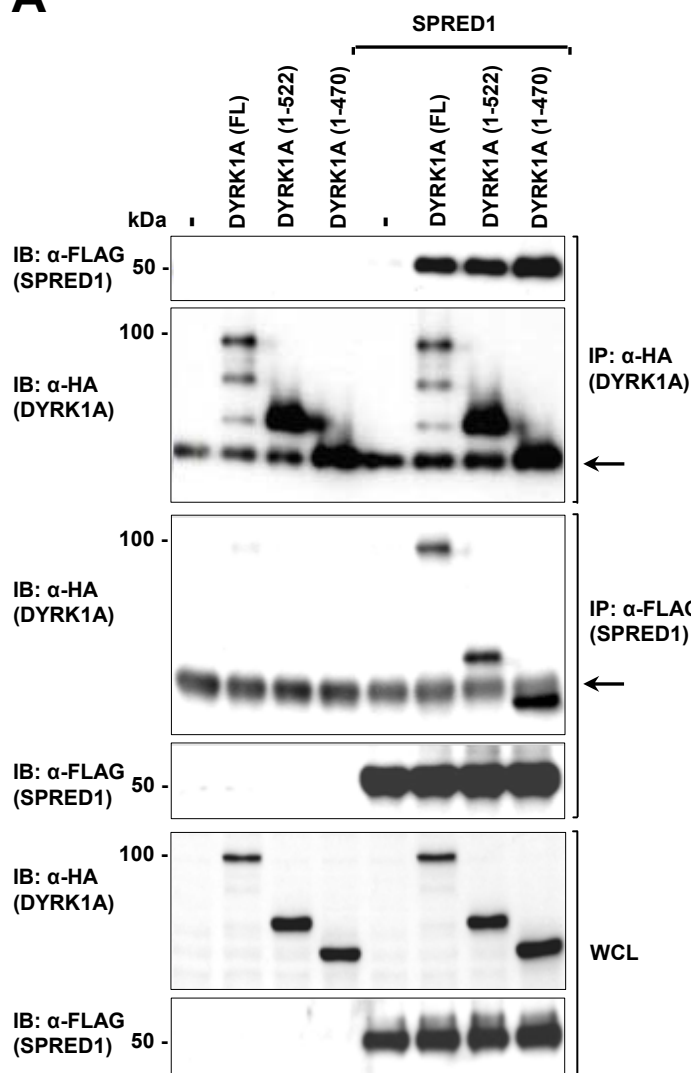
Supplemental figure 2



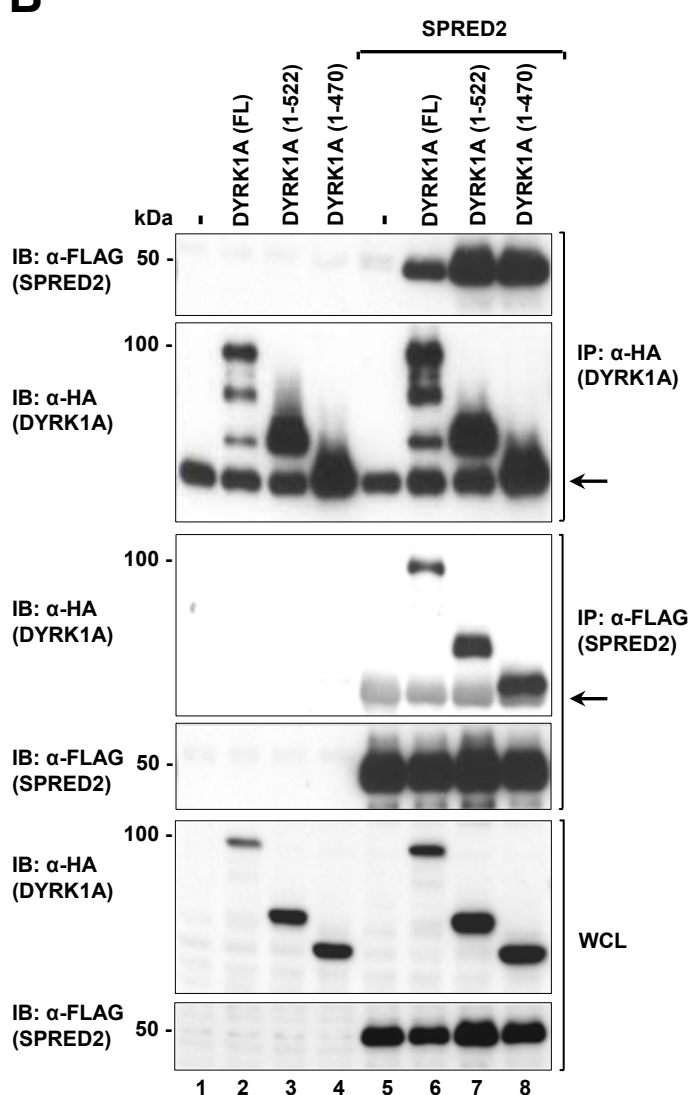
Supplemental Fig. 2 **Spry2 binds to the histidine repeat sequence of DYRK1A.** Full-length FLAG-tagged Spry2 was co-expressed either with full-length HA-DYRK1A or truncated constructs of HA-tagged DYRK1A (1-649, 1-610, and 1-589) in 293 cells. Cell lysates were processed as mentioned in Fig. 1. The arrow indicates the presence of non-specific band in lanes 6, 8 and 9 which is less intense than the FL-DYRK1A band seen in lane 7.

Supplemental figure 3

A

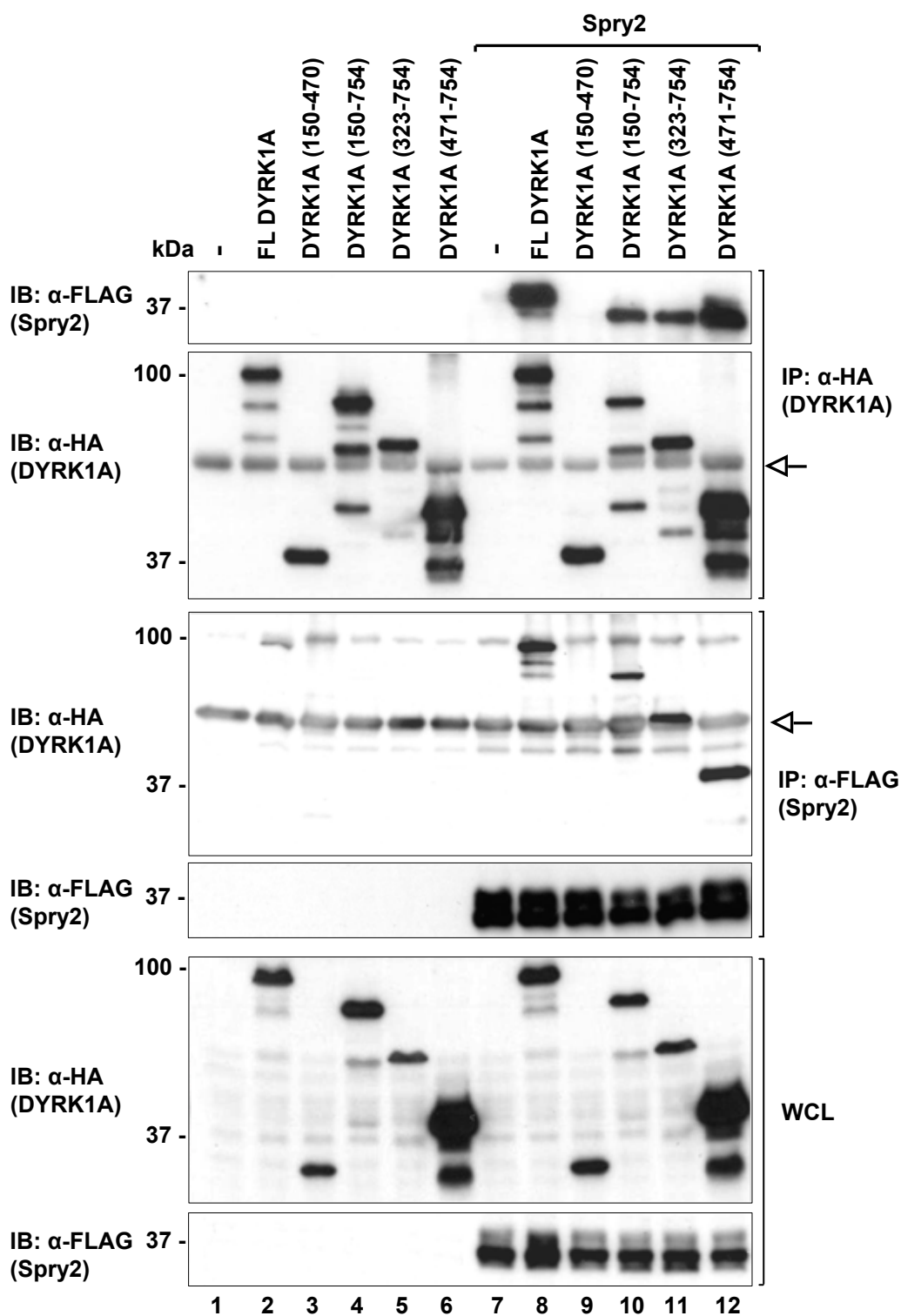


B



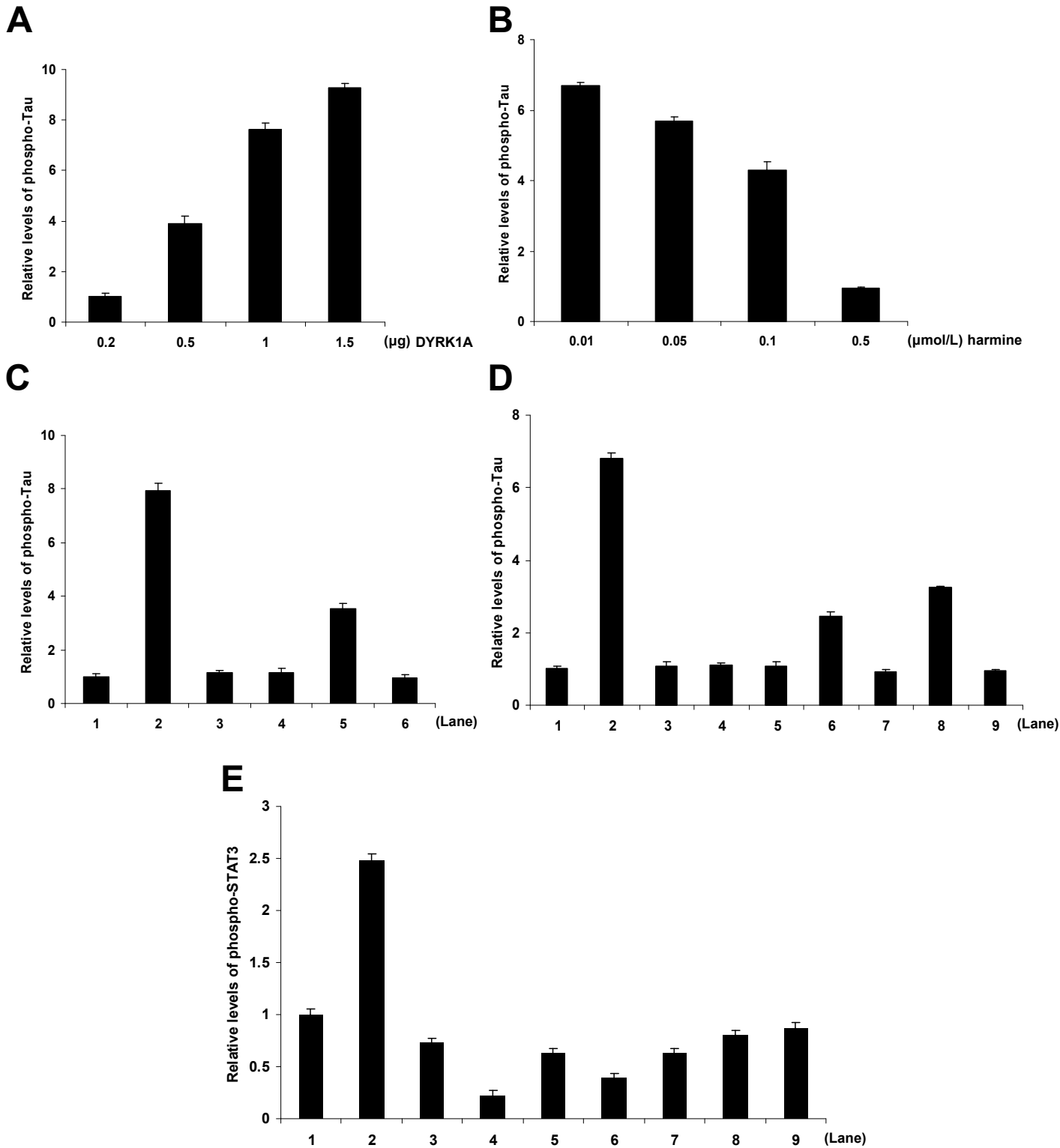
Supplemental Fig. 3 **The interaction between SPRED1 and 2, and DYRK1A does not occur through the His-repeat sequence.** A and B. 293 cells were transfected with full-length HA-tagged DYRK1A or two C-terminal truncated constructs of DYRK1A (1-522 and 1-470) along with FLAG-tagged SPRED1 (A) or 2 (B), as indicated. Cell lysates were processed as mentioned in Fig. 1. The arrows indicate non-specific bands that co-migrate with the actual DYRK1A protein bands (1-470). These are less intense than the actual positive signal.

Supplemental figure 4



Supplemental Fig. 4 **Spry2 does not bind to the kinase domain of DYRK1A.** 293 cells were co-transfected with full-length FLAG-tagged Spry2 and with either full-length HA-tagged DYRK1A, a kinase domain of DYRK1A (150-470) or three truncated constructs of DYRK1A (150-754, 323-754 and 471-754) as indicated. Cell lysates were processed as mentioned in Fig. 1. Open arrow indicates immunoglobulin heavy chain.

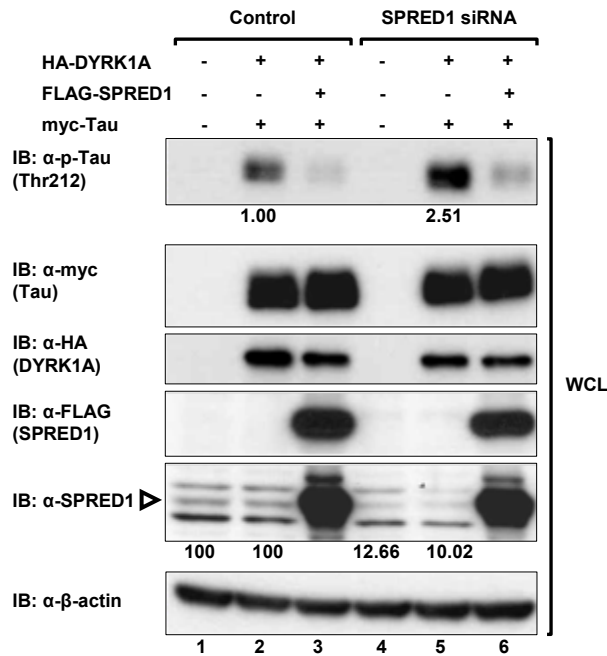
Supplemental figure 5



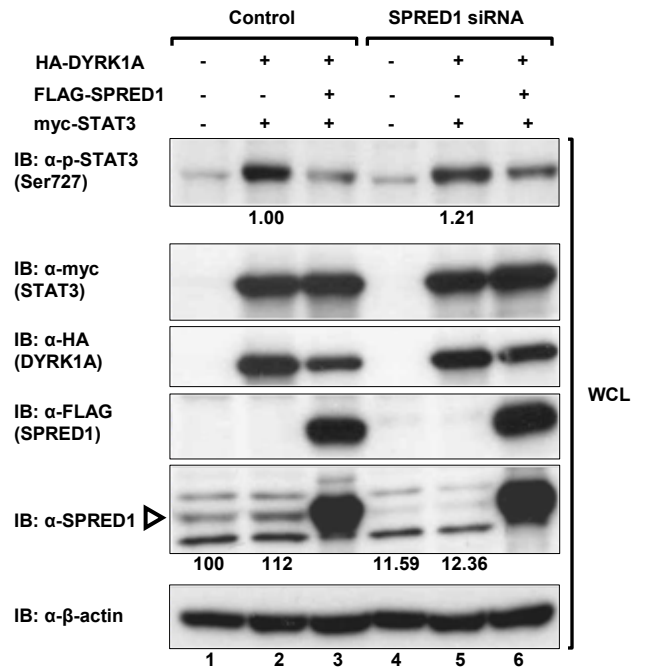
Supplemental Fig. 5 **SPRED1, SPRED2 and Spry2 inhibit the phosphorylation of DYRK1A substrates.** A-D. 293 cells were treated and processed as mentioned in Fig. 5B-E, respectively. The relative levels of phospho-Tau as a proportion of intact Tau protein are indicated in the bar chart (mean \pm S.E., $p < 0.05$, $n = 3$). E. 293 cells were treated and processed as mentioned in Fig. 5F. The relative levels of phospho-STAT3 as a proportion of intact STAT3 protein are indicated in the bar chart (mean \pm S.E., $p < 0.05$, $n = 3$).

Supplemental figure 6

A



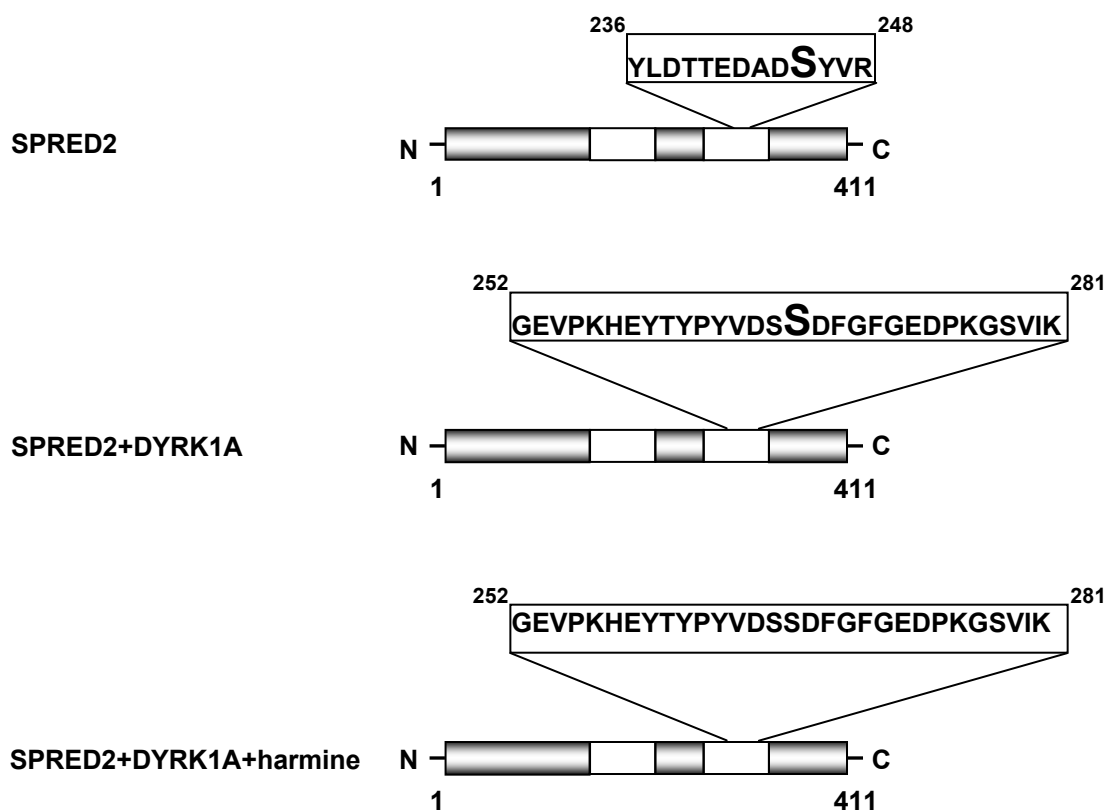
B



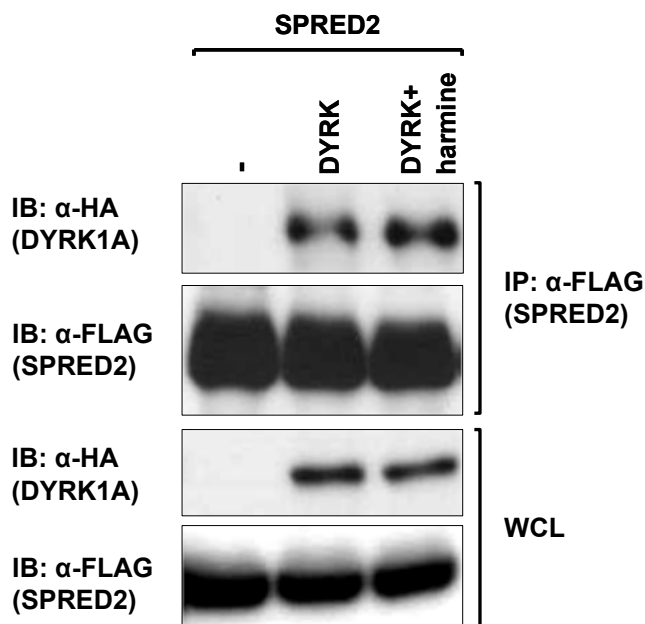
Supplemental Fig. 6 Knockdown of SPRED1 increases the DYRK1A-dependent phosphorylation of Tau and STAT3. A, 293 cells were transfected with vector control, non-specific siRNA, or a pool of four SPRED1 siRNAs using Lipofectamine 2000 as described in the experimental procedures. 48 hours post-transfection of the siRNA, the cells were transfected with the other cDNA plasmids as indicated. Cells were harvested 24 hours after the second transfection. Cell lysates were separated on SDS-PAGE and immunoblotted with antibodies indicated on the left. B. 293 cells were treated in the same way as described in (A), but with the replacement of myc-Tau with myc-STAT3. Cell lysates were subsequently treated similarly as in (A). Open triangle indicates SPRED1.

Supplemental figure 7

A



B



Supplemental Fig. 7 SPRED2 is a likely substrate of DYRK1A.

A, Summary of mass spectrometry analysis of the immunoprecipitates of 293 cells transfected with mSPRED2, mSPRED2+DYRK1A, and mSPRED2+DYRK1A+harmine, respectively. SPRED2 is constitutively phosphorylated on Serine 245; a new Serine at 267 of SPRED2 is phosphorylated in the presence of DYRK1A; this Serine 267 on SPRED2 is dephosphorylated with harmine. B, Western analysis of the samples that were subjected to the Liquid Chromatography-MS/MS analysis in A. Methods for this analysis is as described by Lao *et al* (62).