Supplemental data

Pellino proteins contain a cryptic FHA domain that mediates interaction with phosphorylated IRAK1.

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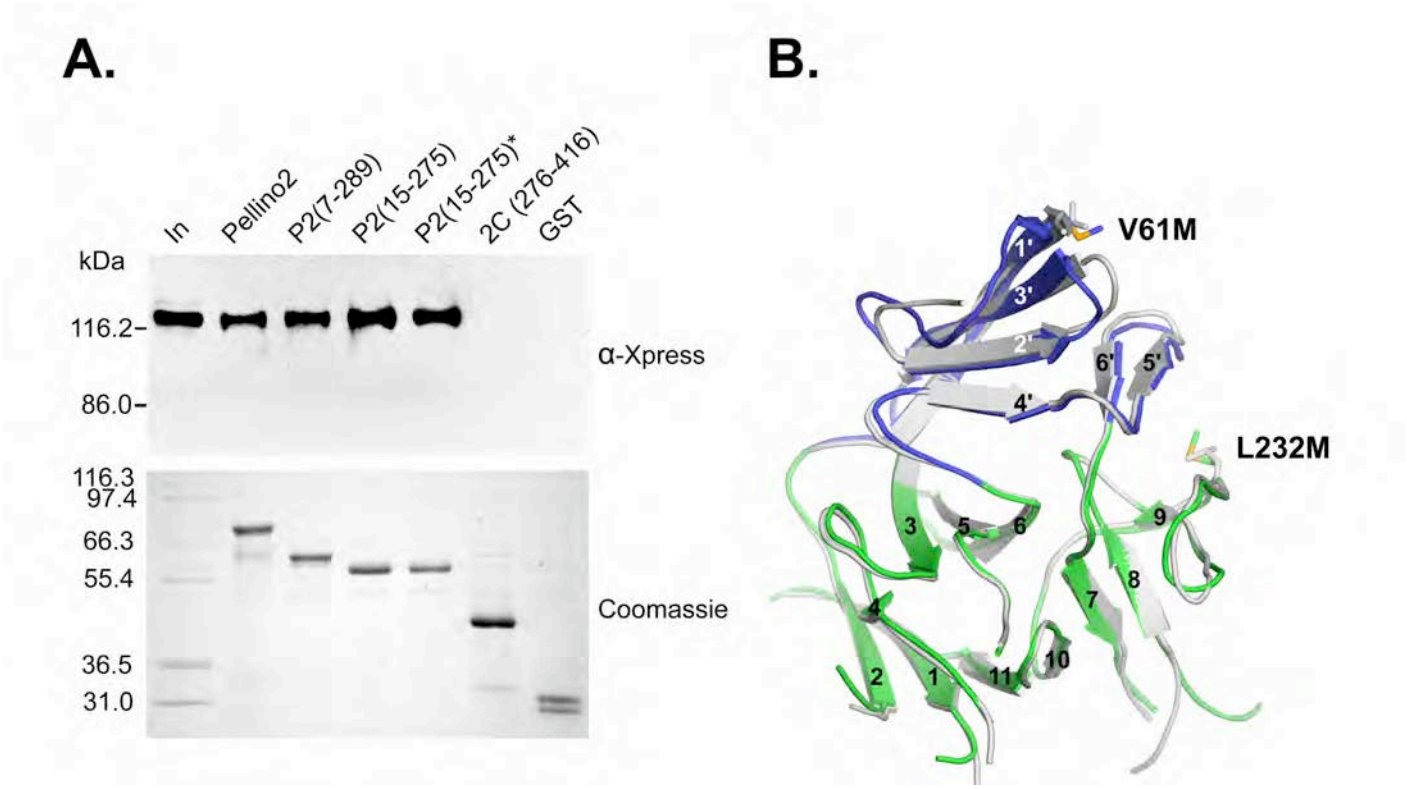

Figure S1. Substitution of methionine at V61 and L232 in Pellino2 does not affect binding to IRAK1 or the structure of Pellino2.

(A) The Pellino2 variants used to generate the crystal structures bind to IRAK1. GST pull-down reactions are shown, performed and analyzed exactly as described in the legend to Fig. 1. Wild type Pellino2 is included as a positive control, and GST alone and the Pellino2 C-terminal region (amino acids 276-416, 2C) as negative controls.

(B) Cartoon representation of a superposition of P2(15-275)*, colored as in Fig. 2, and P2(7-289), in gray. Positions of the methionine substitutions in P2(15-275)* are indicated. The RMSD for all shared main chain atoms is 1.62 Å.

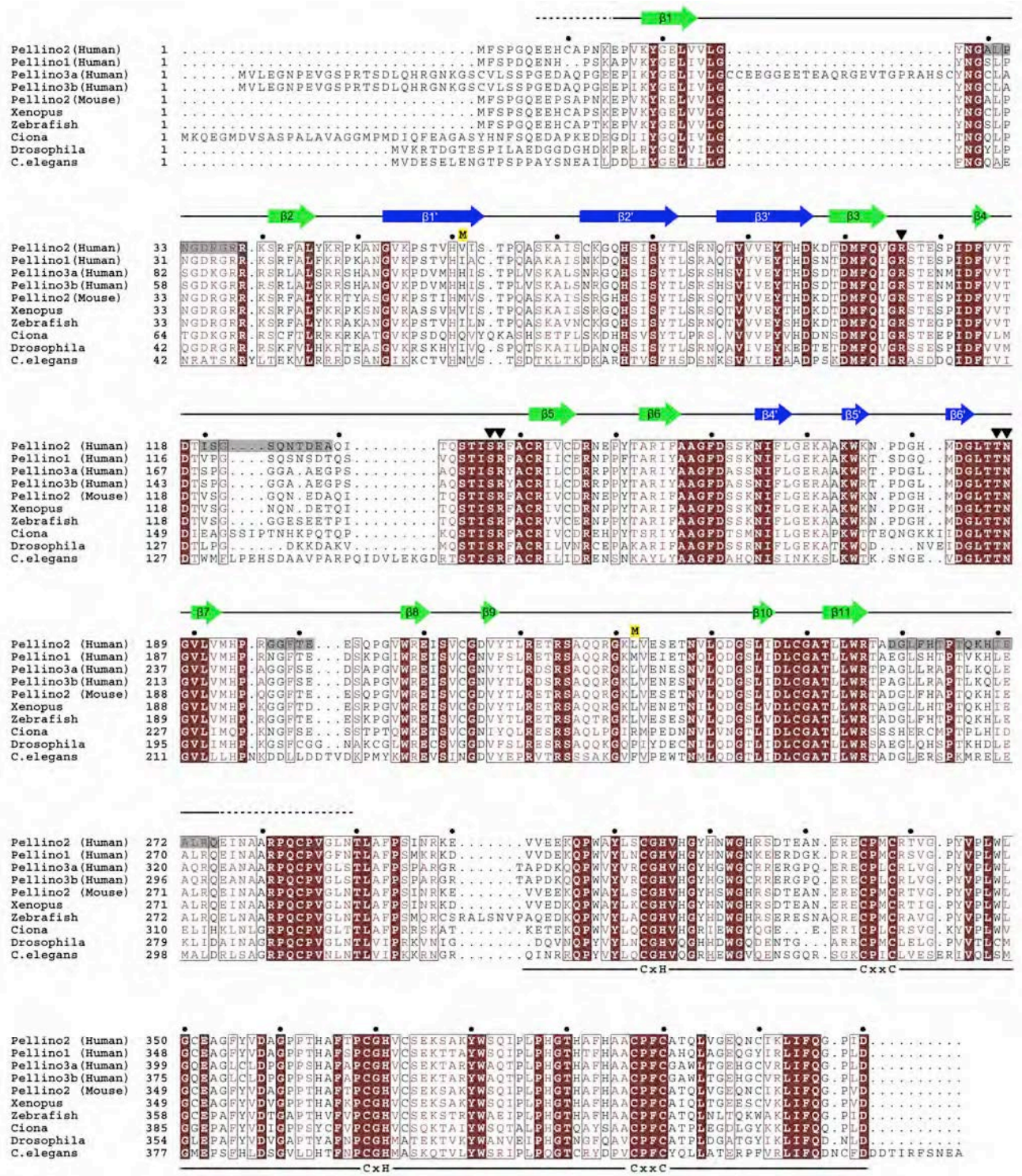

Figure S2. Amino acids sequence alignment of Pellino proteins.
The four human Pellino proteins are shown first, followed by three Pellino2 homologues from mouse, Xenopus and zebrafish. The last three sequences represent more distantly related Pellino homologues, from *Ciona intestinalis*, *Drosophila melanogaster*, and *C. elegans*. The secondary structure elements from the P2(15-275)* structure are shown above the sequence. The dotted lines indicates the additional amino acids in P2(7-289). Gray shading on the Pellino2 sequence indicates the amino acids that are disordered in the P2(15-275)* structure. The positions of the two amino acids that were altered to methionine in P2(15-275)* are indicated, as are the conserved amino acids in the FHA core that are also highlighted in Fig. 3B. The location of the RING motif is indicated under the sequences. Dots above the sequence indicate every 10th amino acids in Pellino2.

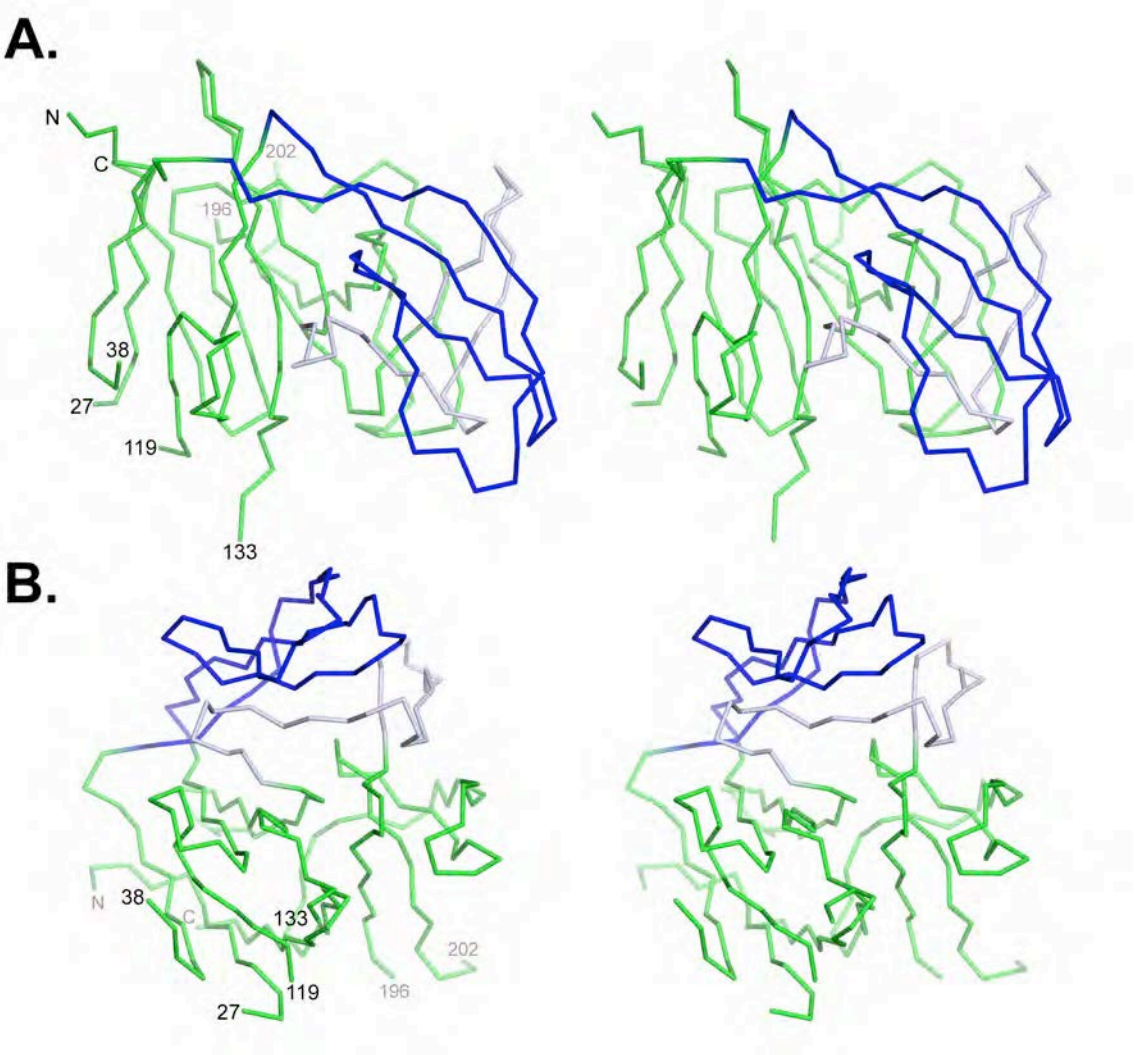

Figure S3. Stereoviews of the Pellino2 FHA domain.

Cα-ribbon representations of P2(15-275)* are shown in the same orientations and colors as in Fig. 2A. The numbers indicate the chain breaks, and the N and C-termini are marked.

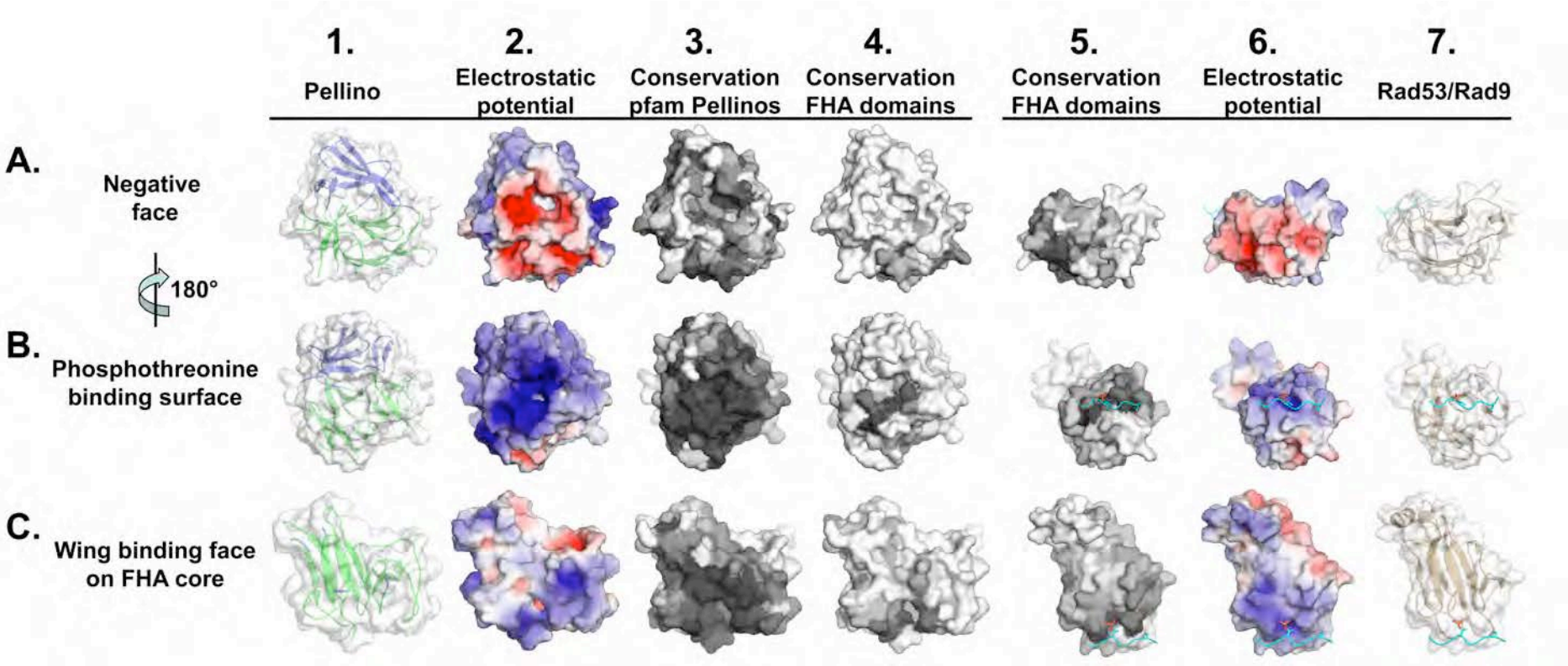

Figure S4. Comparison of surface features of the Pellino2 FHA-like domain with those of canonical FHA domains.
Molecular surface representations of the Pellino2 FHA-like domain (columns 1-4) and of the Rad53 FHA1 (columns 5-7) are shown in three orientations: looking onto (A) the negative face that opposes the phosphothreonine binding site (as in Fig. 5B), (B) the phosphothreonine binding surface (as in Fig. 6) and (C) the wing-binding surface of the Pellino-FHA core. Columns 1 and 7 show a transparent white surface over a cartoon of the structures. In (C) the Pellino wing is not shown and would lie out of the plane of the page. Columns 2 and 6 show the electrostatic potential at the solvent accessible surface, calculated using APBS (Baker et al., 2001) algorithm implemented in Pymol (DeLano, 2004), projected onto the molecular surface, and colored in a gradient (red-white-blue) from -6kT to +6kT. Columns 3, 4 and 5 show amino acid conservation projected onto the same molecular surfaces, using the program CONSURF (Landau et al., 2005). A gradient from gray to white represents most conserved to most divergent positions. Sequence alignments used to generate these gradients from: column 3 - alignment of all Pellino proteins in the Pfam database (Finn et al., 2008), column 4 - a structure based alignment of Pellino2 to all FHA domains of known structure, column 5 - alignment of all canonical FHA domains in the Pfam database. The region on the Pellino FHA core that interacts with the wing is well conserved among Pellino proteins, but not in other FHA domains.

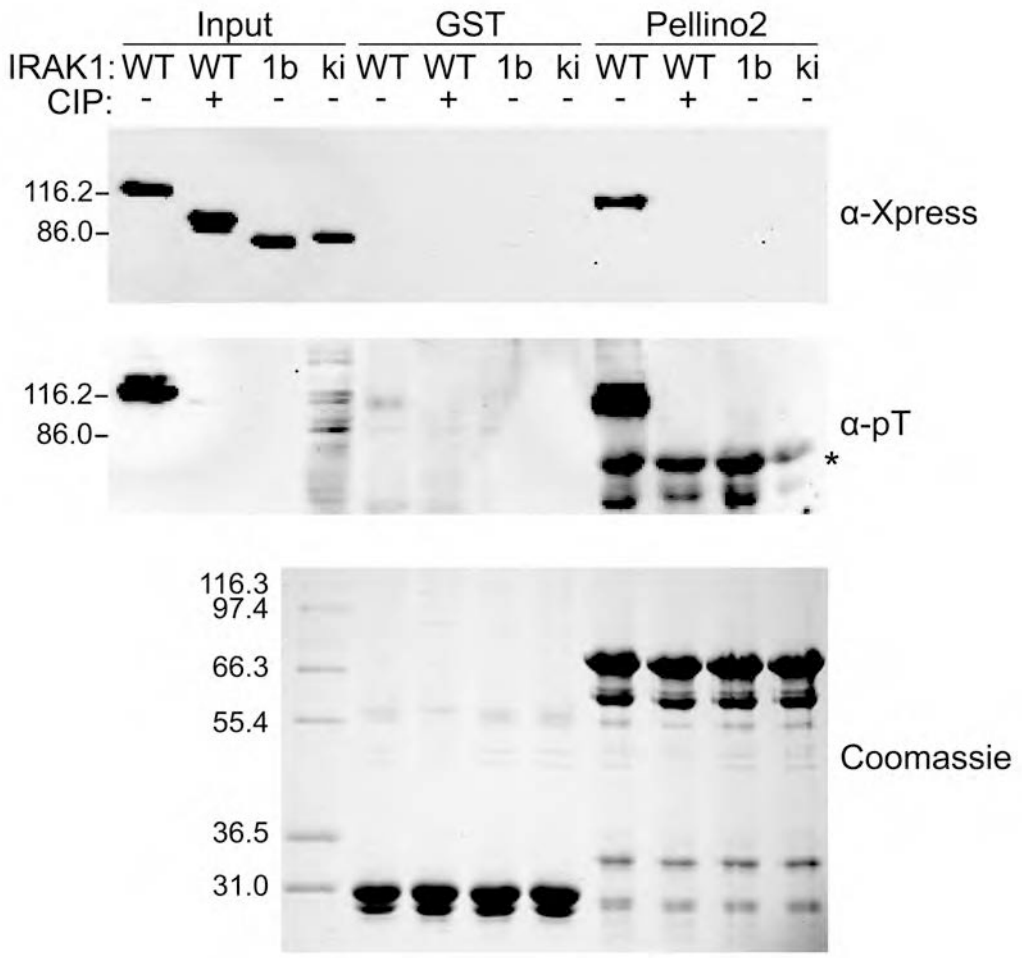

Figure S5. Normalization control for Fig. 4B.

The Western blots shown in Fig. 4B are reproduced above a Coomassie stained gel of these samples to confirm equal loading of the GST and GST-Pellino2 on the agarose beads. The star indicates cross-reaction of the anti-phosphothreonine antibody with the GST-Pellino2 (not shown in Fig. 4B).

Supplemental References

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