Supplemental Figure legends **Li et al.**

Fig. S1. Pairwise identities between the Src homologs presented in Figure 1. Identities were calculated by WU-BLAST (http://www.proweb.org/proweb/Tools/WU-blast.html).

Fig. S2. Immobilized MbSrc1 SH3 and SH2 domains bind to Cas in SYF cell lysates. The blot presented in Figure 2A was stripped and reprobed with anti-Cas antibody.

Fig. S3. Binding of the MbSrc1 SH3 domain to a Pro-rich peptide derived from the sequence of Sam68 (RGAAPPPPPVPRGRG). A representative titration is shown using 20 μM SH3 domain and 5 μl injections of 2 mM peptide. The dissociation constant derived from this titration was 2.1 μM.

Fig. S4. Binding of MbSrc1 to the pYEEI-substrate peptide. The pYEEI-substrate peptide used in Figure 5 was coupled to Affi-Gel 15 resin as described previously (ref. 24). The immobilized peptide (or Affi-Gel resin as control) was incubated with lysate from SYF cells expressing FLAG-MbSrc1 (25 μg total protein). After washing, the bound protein was detected by anti-FLAG Western blotting.

Fig. S5. pYEEI-substrates with shorter linker lengths are not phosphorylated efficiently by MbSrc1. The peptides tested were: the FEEI-substrate control shown in Figure 5; Y(7)pY, a shorter variant of the pYEEI-substrate with a 7-amino acid linker (ref. 42); and Y(3)pY, with a linker length of 3 amino acids. Phosphorylation of the peptides was measured using the phosphocellulose paper assay, as described in Materials and Methods.

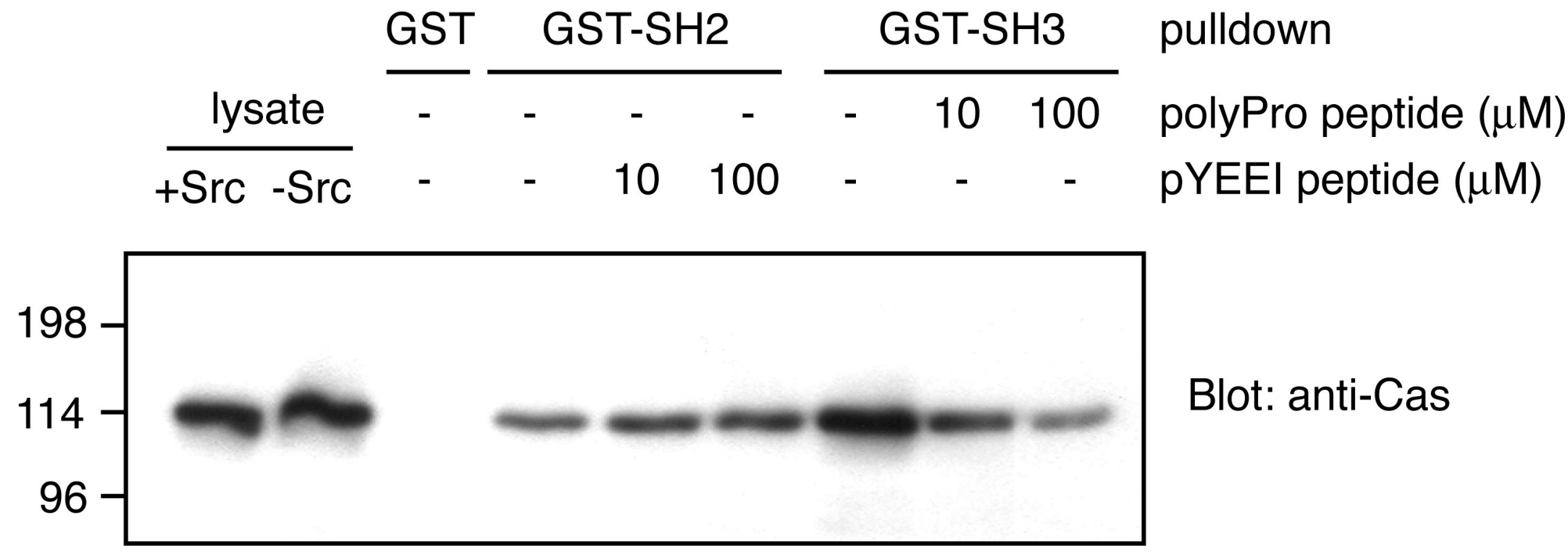
Fig. S6. Identities between MbCsk and the Csk homologs presented in Figure 6. Identities were calculated by WU-BLAST (http://www.proweb.org/proweb/Tools/WU-blast.html).

Fig. S7. MALDI-TOF analysis of the MbCsk reaction on MbSrc1. The reactions are described in the legend to Figure 8A. This figure shows a larger range of m/z. The arrow in the bottom panel points to the peak arising from the phosphorylated C-terminal tail of MbSrc1. The large peak at ca. 2350 likely represents residual unmodified C-tail peptide.

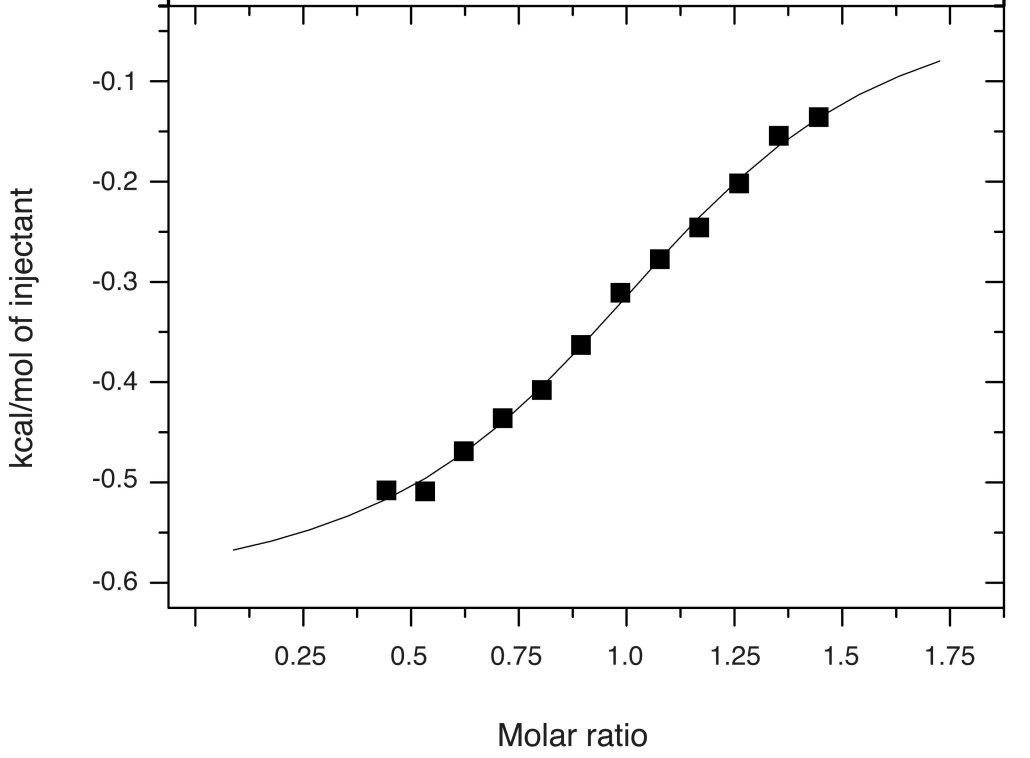
Fig. S8. The indicated FLAG-tagged MbSrc1 constructs were expressed in SYF cells. Lysates were analyzed by SDS-PAGE and Western blotting with anti-phosphotyrosine and anti-FLAG antibodies.

	Query					
Database	Hsap	Dmel	Hvul	Eflu	Mbre	Mova
Hsap		54% Id, 73% Pos	65% Id, 77% Pos	57% Id, 72% Pos	61 % Id, 77% Pos	60% Id, 75 % Pos
Dmel	50% Id, 67% Pos		50% Id, 68% Pos	49% Id, 67%Pos	49% Id, 68% Pos	49% Id, 68% Pos
Hvul	65% Id, 77% Pos	51% Id, 69% Pos		61%Id, 75% Pos	63% Id, 78% Pos	60% Id, 78% Pos
Eflu	56% Id, 71% Pos	49% Id, 67% Pos	57% Id, 72% Pos		58% Id, 75% Pos	58 % Id, 75 % Pos
Mbre	57% Id, 72% Pos	49% Id, 68% Pos	60% Id, 74% Pos	58% Id, 75% Pos		73% Id, 84% Pos
Mova	59% Id, 74% Pos	50% Id, 68% Pos	57% Id, 74% Pos	57% Id, 75% Pos	73%Id, 84%Pos	

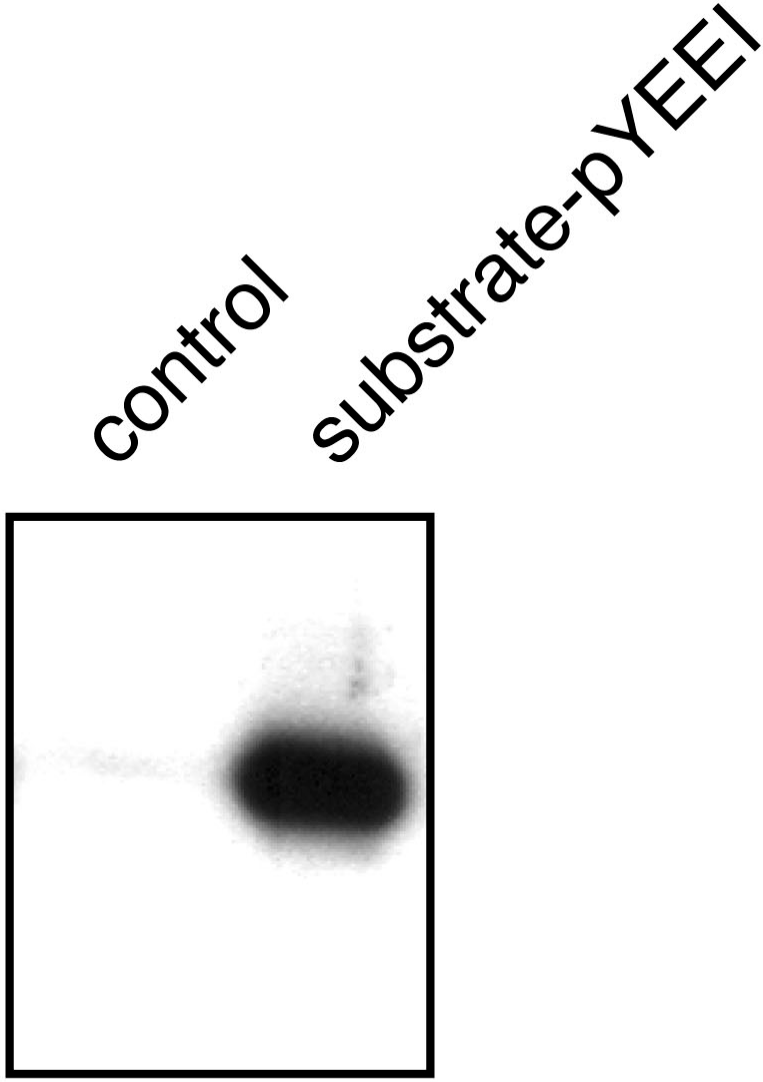
Supplemental Figure S1



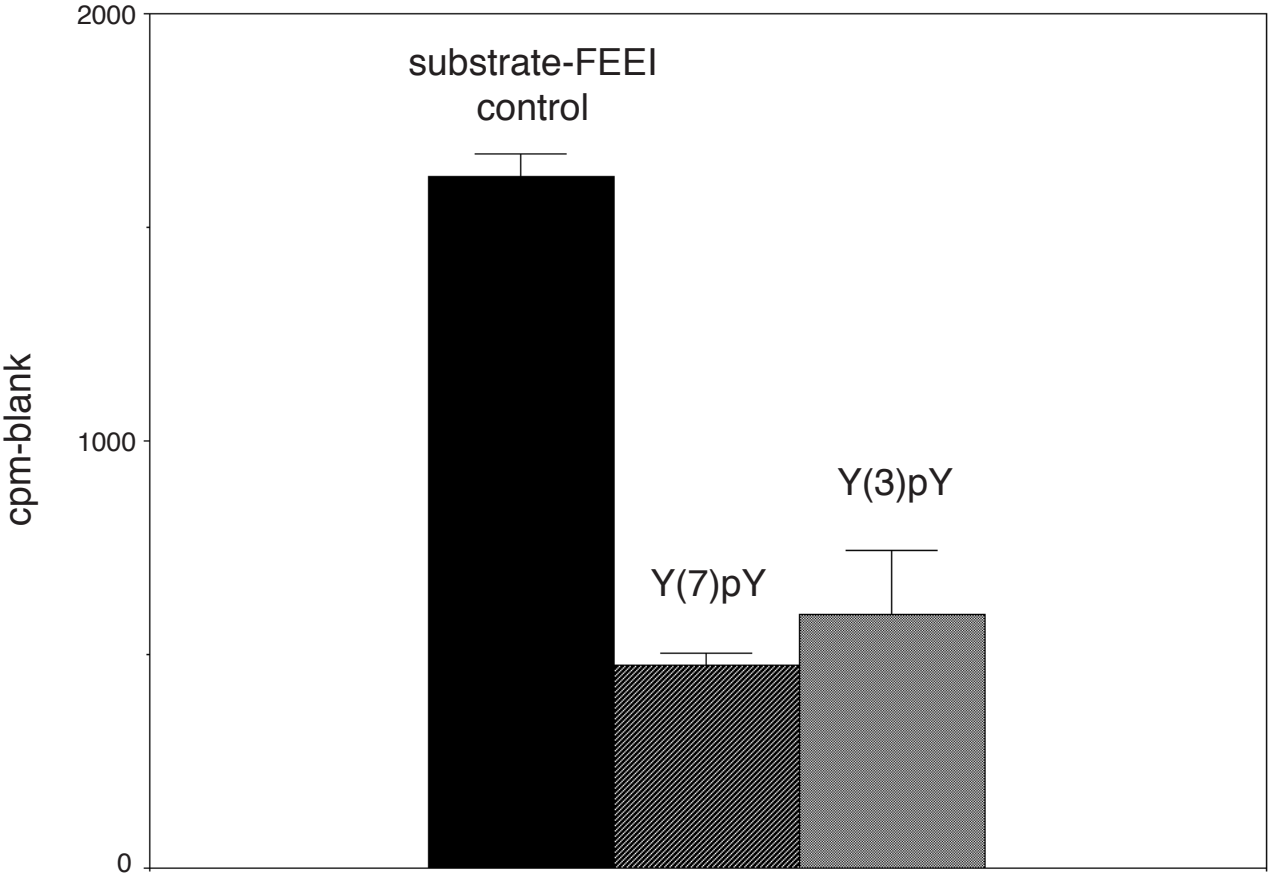
Suppplemental Figure S2



Supplemental Figure S3



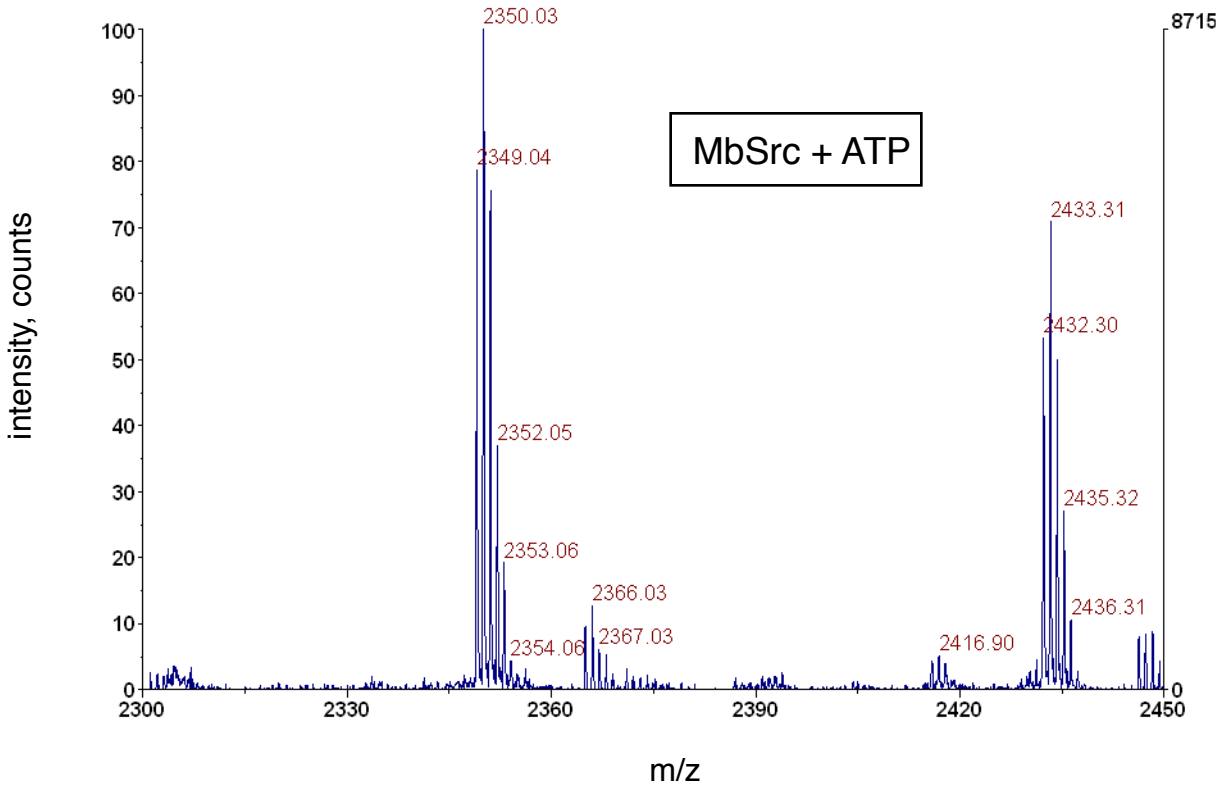
Supplemental Figure S4

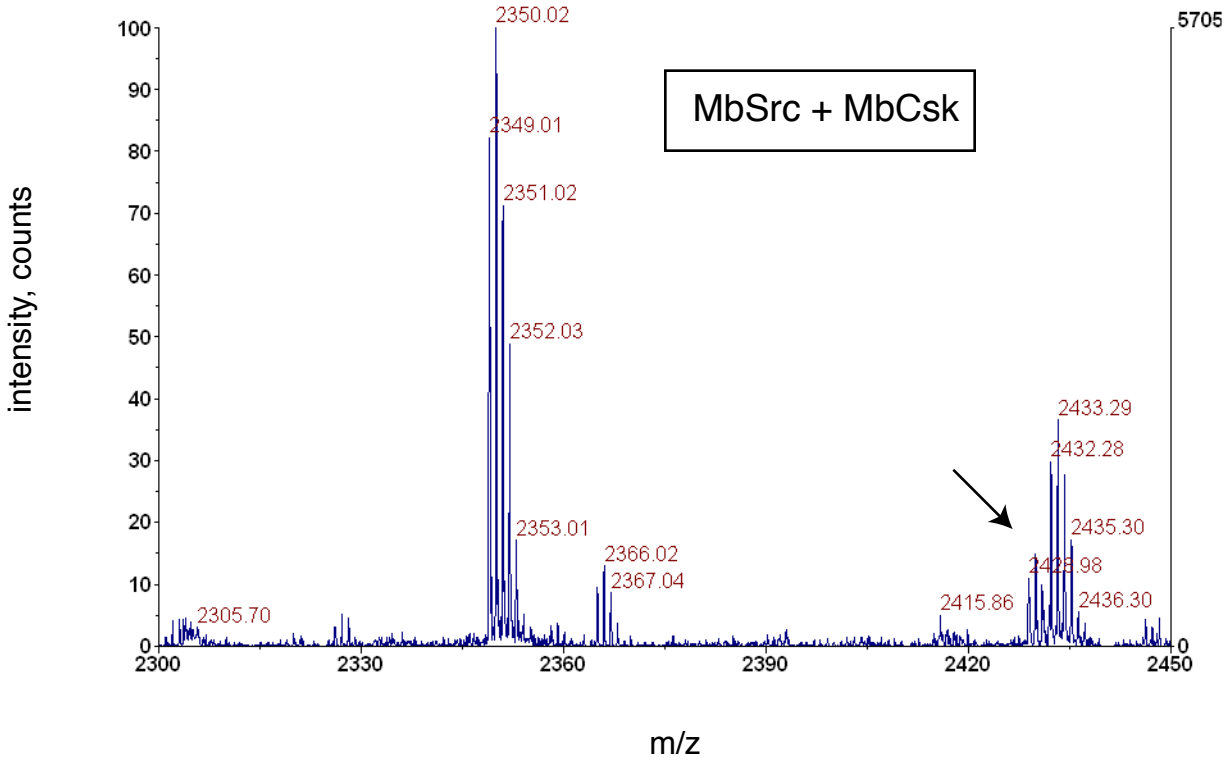


Supplemental Figure S5

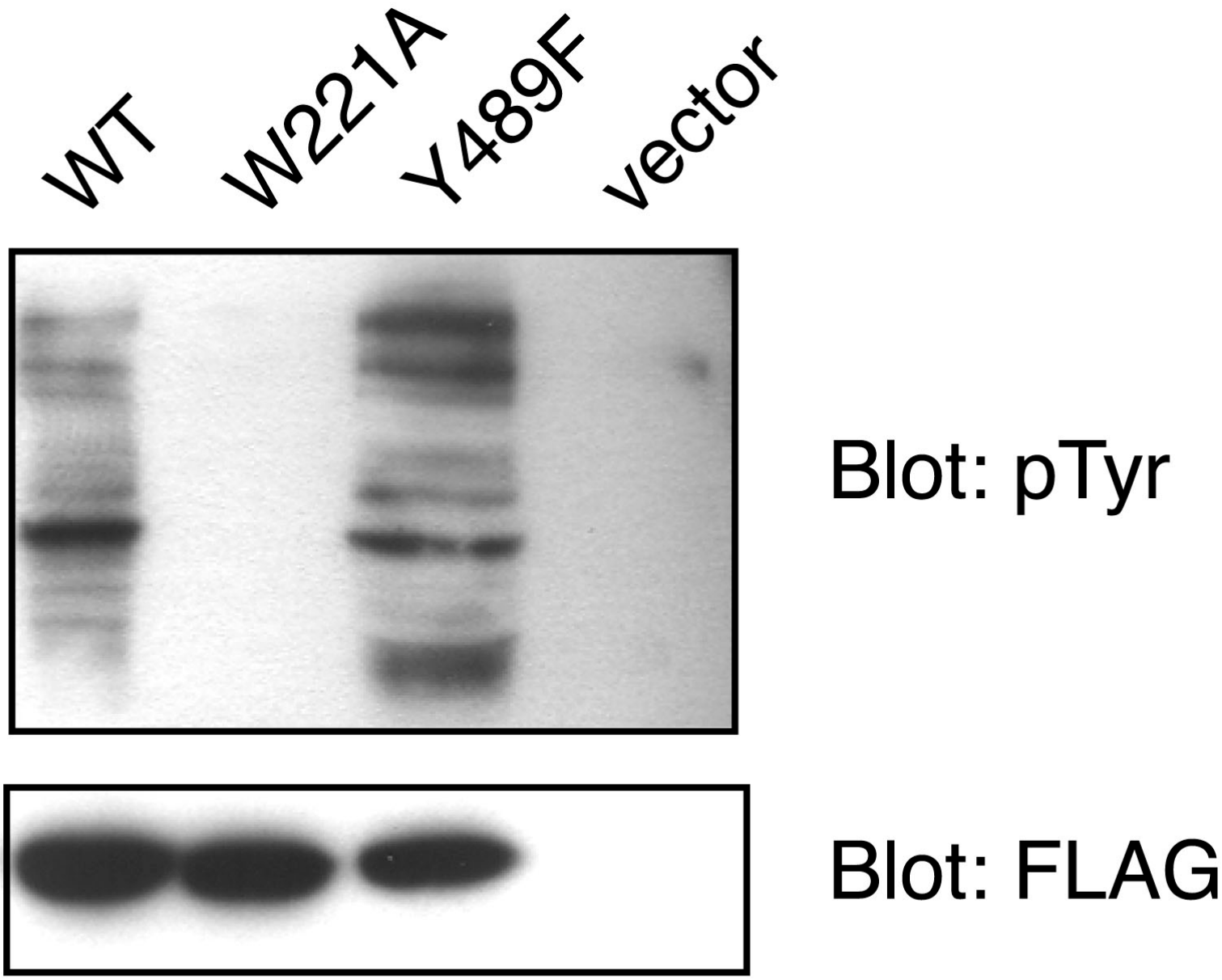
Organism	% Identities	% Positives
H. sapiens	51	66
D. melanogaster	52	68
H. vulgaris	45	66
E. fluviatilis	47	68
M. ovata	56	71

Supplemental Figure S6. Identities with MbCsk.





Supplemental Figure S7



Supplemental Figure S8