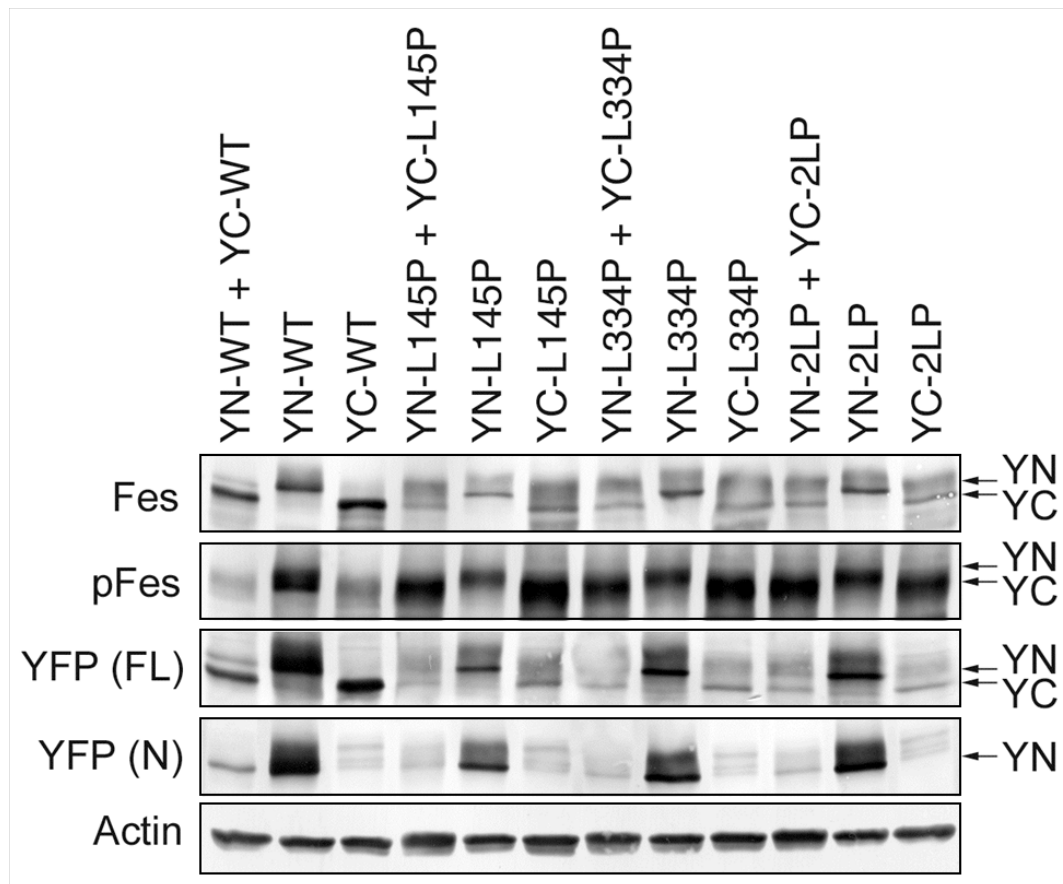


**Supplemental Figure S1. Individual and combined expression of YN-Fes and YC-Fes constructs in COS-7 cells.**



Wild-type (WT) and coiled-coil mutant (L145P, L334P, 2LP) Fes proteins were expressed together in COS-7 cells from the IRES plasmid used for BiFC analysis as shown in the main text, or from individual plasmids using a CMV promoter to verify the migration of each BiFC fusion protein. Cell lysates were then probed with antibodies for Fes protein (Fes); with the phosphospecific antibody (pFes); with a YFP antibody that detects both YN-Fes and YC-Fes proteins [YFP (FL), sc-8334, Santa Cruz]; with a YFP antibody that only detects the N-terminal portion of YFP and hence only the YN-Fes proteins [YFP (N), ab32146, Abcam]; and actin as a loading control. These immunoblots establish that the YC-Fes fusion proteins consistently migrate faster than the YN-Fes proteins. In addition, YN-Fes proteins are expressed at much lower levels in the dual expression construct most likely because of their position after the IRES (see Figure 1 in main text). Because of the low-level expression of YN-Fes proteins from the IRES vector, the activity of these fusion proteins is difficult to detect. Also, because YC-Fes is expressed at higher levels and is much more active in the case of the coiled-coil mutants, its presence overshadows that of YN-Fes in the pFes blots from the co-expression analyses. However, when YN-Fes fusion proteins are expressed alone at higher levels, autophosphorylation is readily apparent.