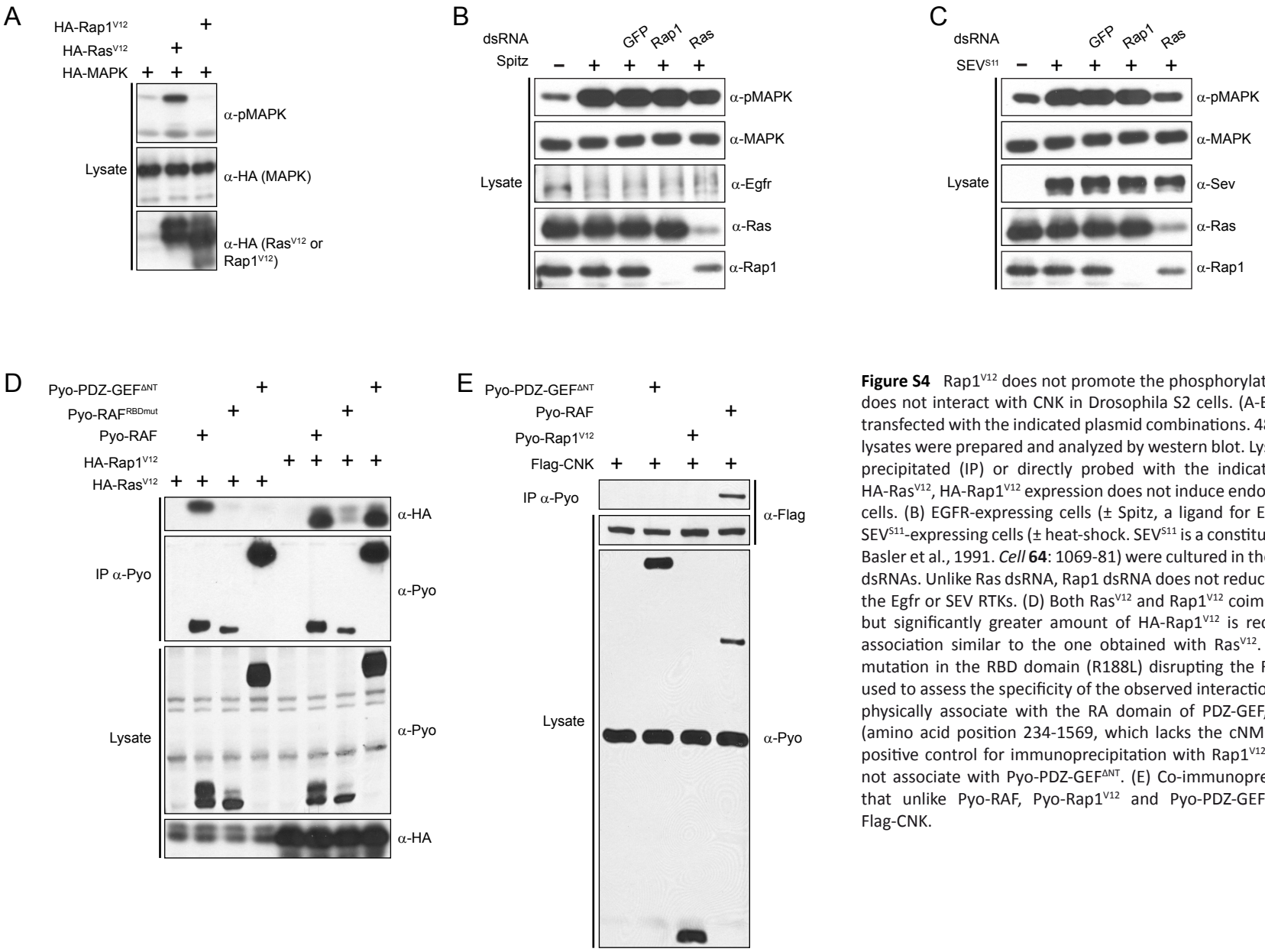

Figure S4 Rap1^{V12} does not promote the phosphorylation of MAPK (pMAPK) and does not interact with CNK in Drosophila S2 cells. (A-E) S2 cells were transiently transfected with the indicated plasmid combinations. 48 hrs post-transfection, cell lysates were prepared and analyzed by western blot. Lysates were either immunoprecipitated (IP) or directly probed with the indicated antibodies. (A) Unlike HA-Ras^{V12}, HA-Rap1^{V12} expression does not induce endogenous pMAPK levels in S2 cells. (B) EGFR-expressing cells (± Spitz, a ligand for EGFR) or (C) heat-inducible SEV^{S11}-expressing cells (± heat-shock. SEV^{S11} is a constitutively active variant of SEV; Basler et al., 1991. *Cell* **64**: 1069-81) were cultured in the presence of the indicated dsRNAs. Unlike Ras dsRNA, Rap1 dsRNA does not reduce pMAPK levels induced by the Egfr or SEV RTKs. (D) Both Ras^{V12} and Rap1^{V12} coimmunoprecipitate with RAF, but significantly greater amount of HA-Rap1^{V12} is required to reach a level of association similar to the one obtained with Ras^{V12}. RAF^{RBDmut} harbors a point mutation in the RBD domain (R188L) disrupting the Ras-RAF association and is used to assess the specificity of the observed interactions. Since Rap1 is known to physically associate with the RA domain of PDZ-GEF, we used Pyo-PDZ-GEF$^{\Delta NT}$ (amino acid position 234-1569, which lacks the cNMP domain; Figure 2F) as a positive control for immunoprecipitation with Rap1^{V12}. Interestingly, Ras^{V12} does not associate with Pyo-PDZ-GEF$^{\Delta NT}$. (E) Co-immunoprecipitation assays revealed that unlike Pyo-RAF, Pyo-Rap1^{V12} and Pyo-PDZ-GEF$^{\Delta NT}$ fail to associate with Flag-CNK.