E08-06-0624 Mattaj

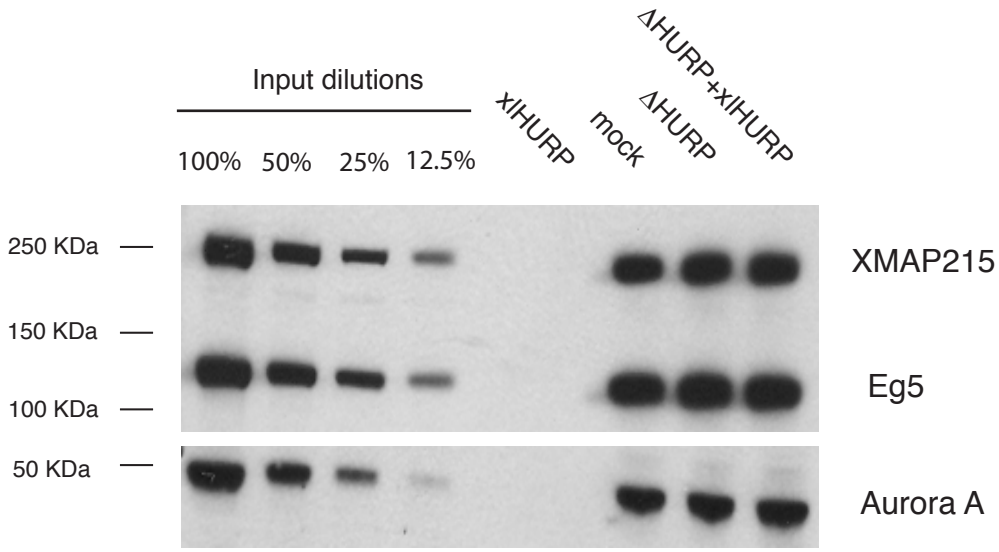
Figure S1. HURP depletion does not cause co-depletion of other MAPs. Western blots were probed for XMAP215, Eg5 and Aurora A.

Figure S2. Determination of HURP endogenous concentration in *Xenopus* egg extracts. Recombinant *xl*HURP was loaded in different dilutions and compared to the non-modified endogenous protein, corresponding to approximately 370 nM. The amount of *xl*HURP used in addback experiments equals the 1:100 dilution and corresponds to ca. 1-1.5 μM. This equals 3-4 times the endogenous concentration of the non-modified HURP and to 1-2 times the endogenous concentration of the two HURP forms.

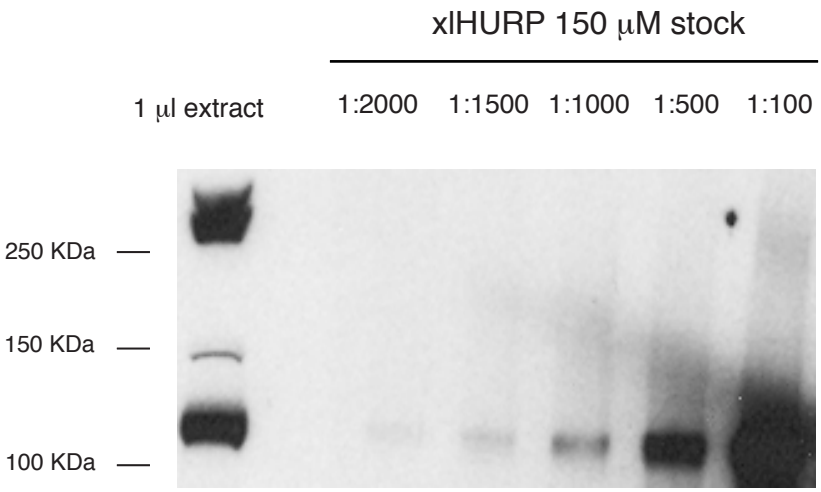
Figure S3. HURP and TPX2 immunoprecipitations. (A) Immunoprecipitation from the MAP fraction. Western blots were probed for HURP and TPX2. **(**B) Immunoprecipitation from total *Xenopus* mitotic egg extracts with the indicated antibodies in the presence or absence of RanQ69L. Western blots were probed for HURP and TPX2. (C) Immunoprecipitation from the NLS protein fraction. Immunoprecipitation was performed with the indicated antibodies in the total NLS protein fraction (NLS) and in NLS protein fraction depleted of TPX2 (NLSΔTPX2). NLS proteins unbound to the antibodies beads (unbound) and NLS proteins eluted from the antibody beads (bound) were run on SDS-PAGE. Western blots were probed for HURP and TPX2.

Figure S4. HURP and TPX2 do not co-fractionate. (A) NLS protein preparation (upper left panel) and fractionation by ion exchange chromatography (upper right and lower panels). Total mitotic extracts were incubated with RanQ69L beads (activated extracts) and subsequently NLS proteins were depleted using Importin β beads (depleted extracts). NLS proteins were eluted from the Importin β beads by RanQ69L and 500 mM NaCl and applied to a Mono S column. Flow through (f.t.) and eluted fractions

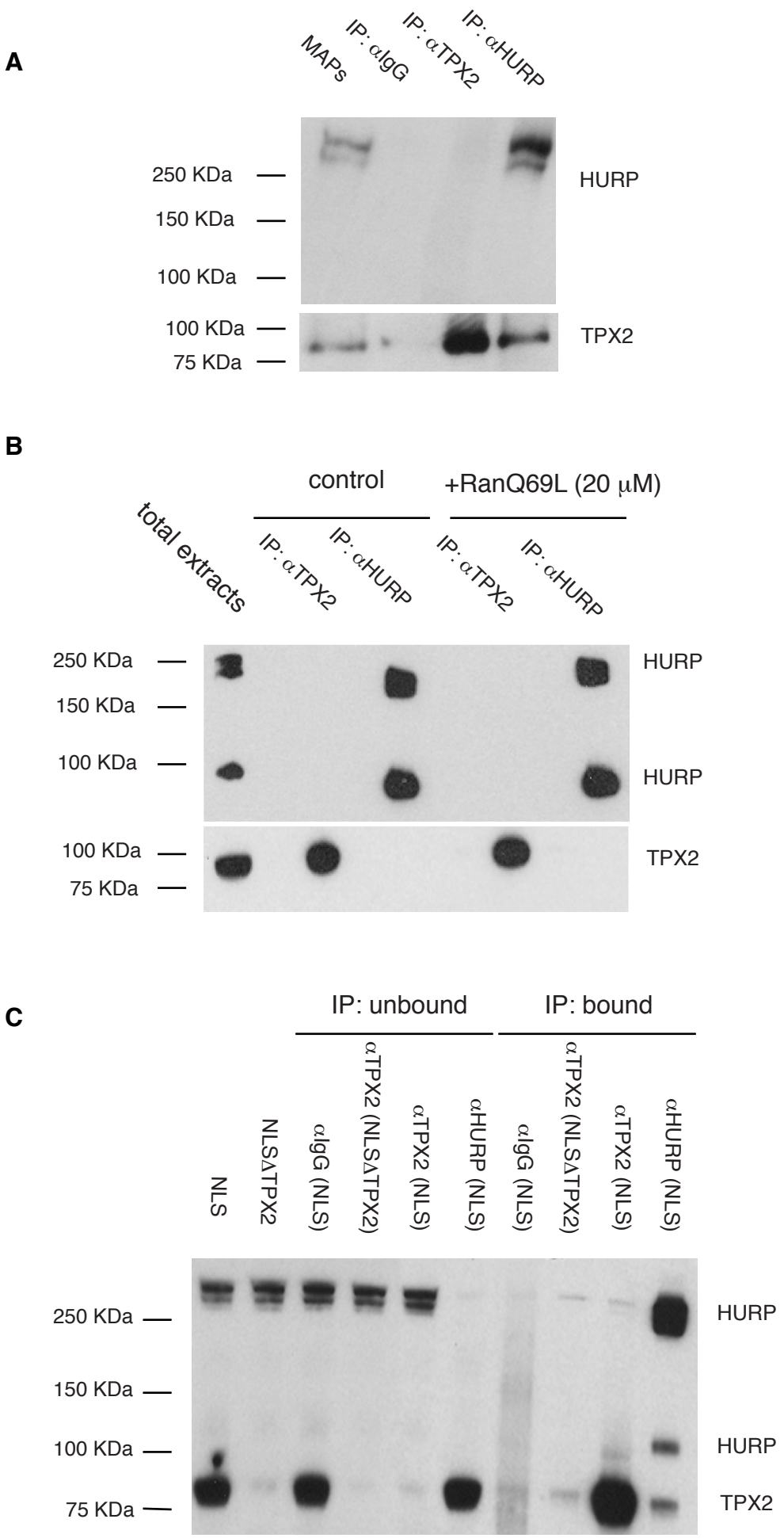
were analyzed by Western blotting (upper right). Fractions 12-22 (input, lower panel) were applied to a Mono Q column. Flow through (f.t.) and eluted fractions were analyzed by Western blotting. Western blots were probed for HURP and TPX2. (B) NLS protein fractionation by gel filtration. NLS proteins (as in A) were applied to a Superose 6 column and fractions eluted were analyzed by Western blotting. Western blots were probed for HURP and TPX2.



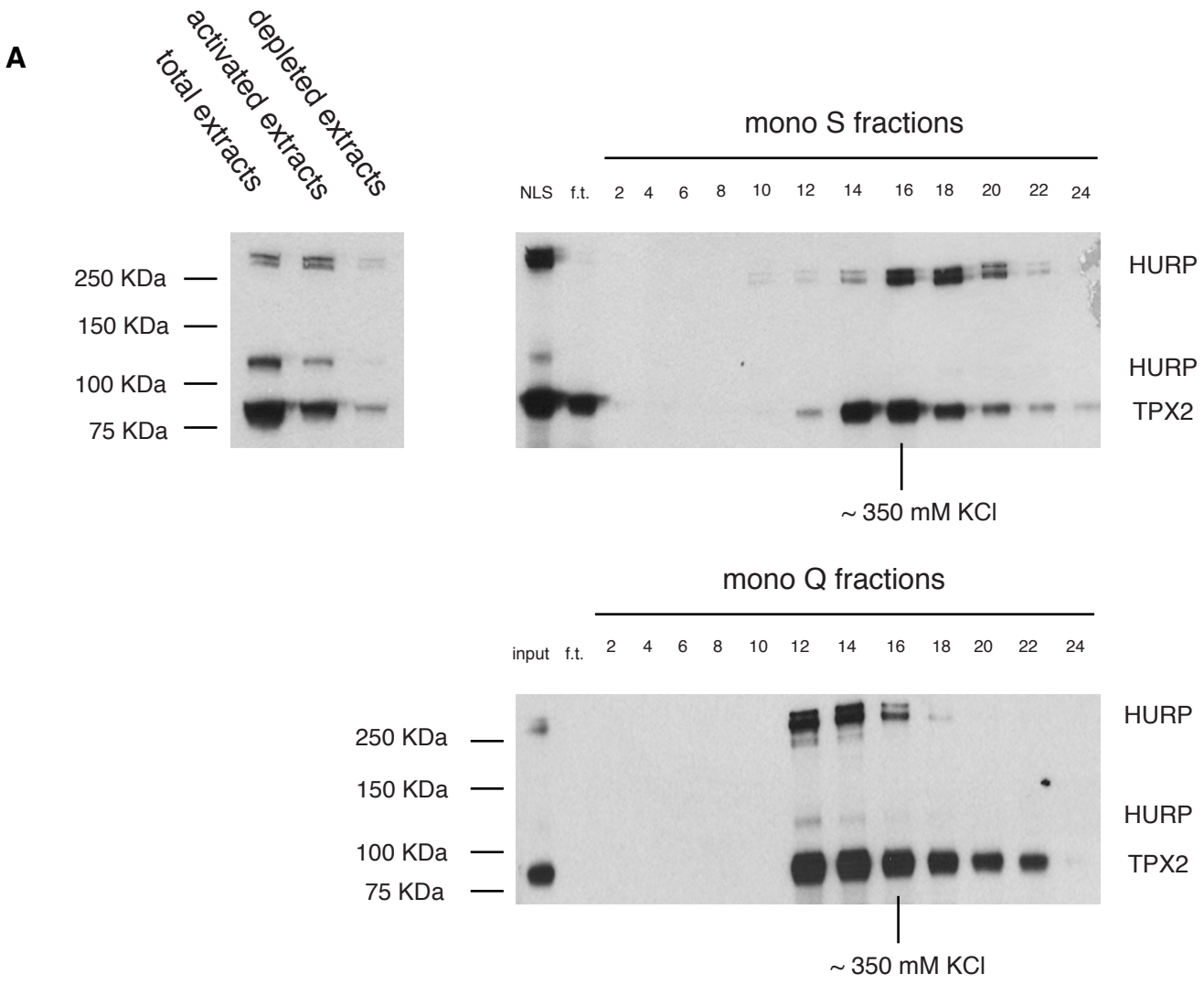
Supplemental Figure 1

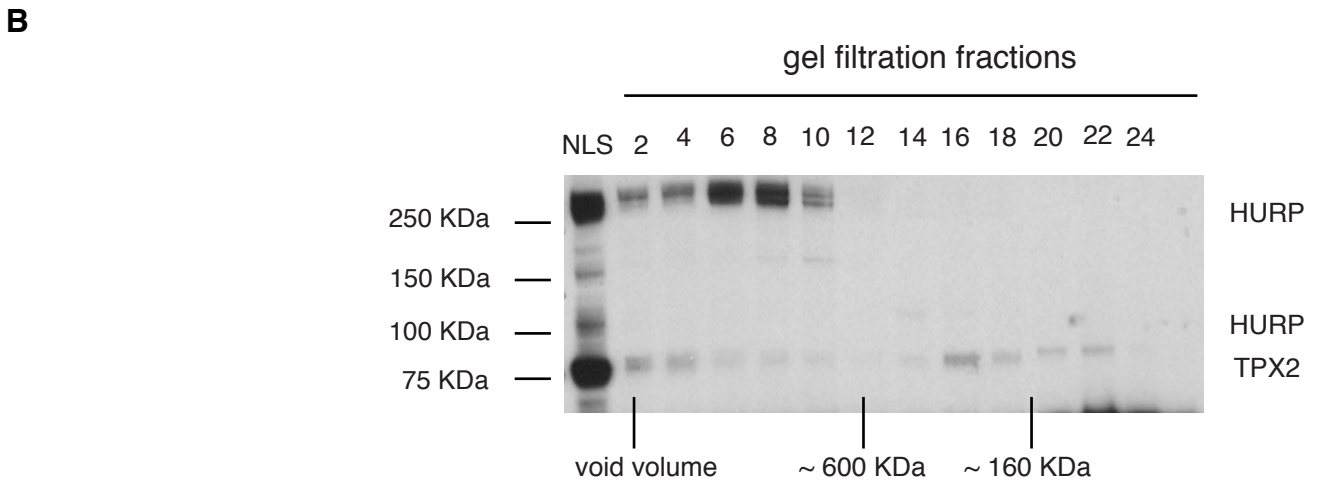


Supplemental Figure 2



Supplemental Figure 3





Supplemental Figure 4