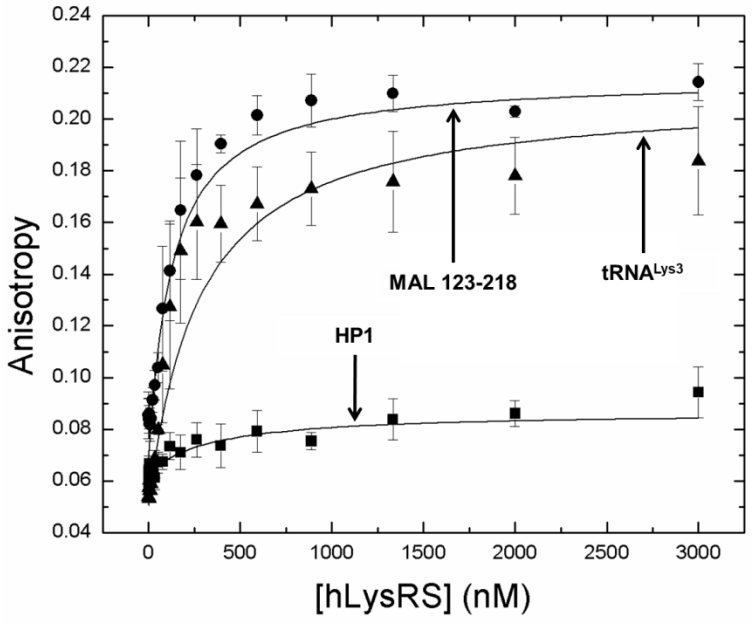

Figure S1. Fluorescence anisotropy binding assay of HP1, MAL 123-218 and $tRNA^{Lys3}$ binding to hLysRS. The fluorescently-labeled RNAs were prepared and folded as described in the Materials and Methods section. RNAs (10 nM) were mixed with varying concentrations of hLysRS in a buffer containing 20 mM Tris-HCl, pH 8, 15 mM NaCl, 35 mM KCl and 1 mM $MgCl_2$. The samples were excited at 485 nm and the emission was measured at 525 nm. The points represent the average of two experiments and the lines represent the best fit to a 1:1 binding equation, with standard deviations shown (Stewart-Maynard et al. 2008). We observe that hLysRS and hLysRSΔN65 interact with the RNAs similarly (compare binding curves in Figure S1 with Figure 3). Human LysRS binds MAL 123-218 and $tRNA^{Lys3}$ with K_d's of 187 ± 84 nM and 124 ± 51 nM, respectively, while no binding was observed for HP1. The ~2-fold enhanced affinity of hLysRS for MAL 123-218 and $tRNA^{Lys3}$ relative to hLysRSΔN65 (see Table 1) is consistent with the presence of the N-terminal basic domain in full-length LysRS, which confers non-specific nucleic acid binding (Francin et al. 2002). However, binding to HP1 is not detected for either protein.