Supplemental Figure Legend

Figure S1. MW7, Happ1 and Happ3 bind Htt in a PRR-dependent manner. 293 cells were transfected with HDx-1 (PQ25) or HDx-1ΔPRR (Q23). After 48 hours, cell lysates were separated by SDS/PAGE and blotted with intrabodies. Non-transfected cells (NEG) were used as a negative control. While V_L12.3 binds both forms of HDx-1, MW7, Happ1 and Happ3 bind only the form containing the PRR. CV_L does not bind HDx-1.

Figure S2. VL12.3 increases the level of nuclear HDx-1. ST14A cells were co-transfected with mHDx-1-GFP and intrabody at optimal ratios. (A) At 48 hours post-transfection, cells were fixed, stained for the appropriate intrabody (iAb) and cell nuclei, and analyzed by confocal microscopy. (B) Mean whole cell fluorescence intensity (int.) and mean nuclear fluorescence intensity of HDx-1 were compared. While MW7, Happ1 and Happ3 have no effect on HDx-1 localization, V_L12.3 significantly increases nuclear HDx-1. * = Differs from HDx-1 at $p<.01$. (C) Mean whole cell fluorescence intensity and mean nuclear fluorescence intensity of the intrabodies themselves were compared. MW7 is slightly more cytoplasmic than the other intrabodies, possibly due to its larger size.

Supplemental Methods

Expression and purification of intrabody proteins. Intrabodies in pGEX-6p1 vector were transformed into ArcticExpress competent bacteria (Stratagene), and cells induced according to the manufacturer's protocol. GST fusion proteins were harvested from bacterial cultures as described above. Intrabody protein was verified by Coomassie staining of PAGE gels and comparison to protein molecular weight standards (data not shown). Relative expression levels of intrabodies were determined by intensity of Coomassie staining, and slurry volumes normalized to obtain approximately equal concentrations of intrabody protein. Intrabodies were eluted from glutathione beads and suspended in 1 ml PBS (Ausubel, 1993).

Intrabody binding assays. HEK 293 cells in 10 cm dishes at ~80% confluency were transfected with 10 μg 23Q-HDx-1-CFP lacking the PRR (Q23) or 25Q-HDx-1-GFP (PQ25) in pcDNA3.1 vector (Invitrogen). A non-transfected dish was used as a negative control. Cells were dislodged by mechanical dissociation and pipetting 48 hours post-transfection, harvested by centrifugation, washed with PBS and lysed by sonication in 500 μl whole cell lysis buffer (0.2 M Tris-HCl, 140 mM SDS, 50% glycerol). Samples were analyzed by SDS/PAGE and immunoblotting. Intrabody protein eluates dissolved in 15 ml 3% milk were used as primary antibody. Secondary antibodies were HRP-conjugated M2 anti-flag (1:1000 Sigma, St. Louis, MO.) for detecting MW7, and HRP-conjugated 3F10 anti-HA (1:1000 Roche, Indianapolis, IN.) for detecting V_L12.3, Happ1, Happ3 and CV_L.