SUPPLEMENTAL FIGURES

FIGURE S1. Binding of FITC-$K_2Q_{44}K_2$ fibrils to cells deficient in Fn or the Fn receptor components, α5 or β1 integrins.
(A) EDTA-lifted MEFs heterozygous (Fn+/-) or homozygous (Fn-/-) for a null mutation in the Fn gene (1) were treated with 1μM FITC- $K_2Q_{44}K_2$ fibrils in the presence of 1% BSA for 10min at 4°C. The average geometric mean ±SEM of cell-associated FITC fluorescence is shown for 3 independent experiments. (B) AtT-20 mouse pituitary cells or AtT-20 cells (2) stably expressing α5 integrin (AtT-20α5) (3) were plated on tissue culture dishes coated with 0 or 10μg/mL fibronectin. EDTA-lifted cells were treated with 1μM FITC- $K_2Q_{44}K_2$ fibrils in the presence of 1% BSA for 10min at 4°C. The average geometric mean ±SEM of cell-associated FITC fluorescence is shown for 6 (AtT-20), 2 (AtT-20α5) or 1 (AtT-20 or AtT-20α5 plated on Fn) independent experiments. (C) EDTA-lifted wild-type CHO-K1 cells, CHO cells with deficient expression of α5 integrin (CHO-B2) (4), or a CHO-B2 clone stably overexpressing α5 integrin (CHO-B2α27) (5) were treated with 1μM FITC- $K_2Q_{44}K_2$ fibrils in the presence of 1% BSA for 10min at 4°C. The average geometric mean ±SEM of cell-associated FITC fluorescence is shown for 3 independent experiments. (D) EDTA-lifted GD25 mouse cells, which are deficient in β1 integrin expression (6), or GD25 cells overexpressing β1 integrin (GD25-β1A) (7) were treated with 1μM FITC- $K_2Q_{44}K_2$ fibrils in the presence of 1% BSA for 10min at 4°C. The average geometric mean ±SEM of cell-associated FITC fluorescence is shown for 3 independent experiments. (E) 20μg of 1% Triton X-100 lysate from the indicated cell types cultured in normal FBS or FBS depleted of Fn by passing over a gelatin sepharose column were separated by SDS-PAGE and probed for Fn. (F) 20μg of 1% Triton X-100 lysate from the indicated cell types were separated by SDS-PAGE and probed for α5 integrin.

FIGURE S2. Size distribution of polyglutamine fibrils
The length and width of ~ 2830 $htt_{exon1}Q_{44}$ and 2800 Q_{44} fibrils were measured using ~ 50 quantitative negatively stained electron micrographs. The length distribution reveals that the $htt_{exon1}Q_{44}$ and Q_{44} fibrils length ranges from 25 to 100 nm with an average length of ~ 35 nm.

FIGURE S3. Circular dichroism of polyglutamine aggregates
(A) Far-UV circular dichroism spectra of $K_2Q_{44}K_2$ (30μM) in water (red triangles) and in 1.25% TFA, 1.25% HFIP (blue circles) were recorded at 20 °C on a J810 (Jasco) spectropolarimeter as indicated in the supplementary procedures section. The spectra were deconvolved using the algorithm CDSSTR available on the server Dichroweb (http://dichroweb.cryst.bbk.ac.uk/html/home.shtml (B) Absorbance of amorphous and fibrillar $K_2Q_{44}K_2$ at different wavelengths.

SUPPLEMENTAL PROCEDURES

Circular dichroism spectroscopy

Far-UV CD spectra were recorded at 20°C using an AVIV dichrograph (Aviv Biomedical Inc.) equipped with a thermostatic cell holder using a 0.1 cm path length quartz cuvette. Each spectrum was the average of 10 acquisitions recorded in the 260-185 nm range with 0.5 nm steps, a bandwidth of 2 nm, and at a speed of 100 nm min^{-1}. All spectra were solvent corrected. The voltage detected by the dichrograph was automatically gain-adjusted using with the internal photomultiplier. The CD spectra of amorphous $K_2Q_{44}K_2$ assemblies were recorded 3 hours after 40-fold dilution of $K_2Q_{44}K_2$ (1.2 mM) (in 50% TFA, 50% HFIP) in water. The CD spectra of fibrillar $K_2Q_{44}K_2$ assemblies were recorded 3hours after dissolving dried $K_2Q_{44}K_2$ in water (final concentration 30μM). The spectra were deconvolved using the algorithm CDSSTR available on the server Dichroweb (http://dichroweb.cryst.bbk.ac.uk/html/home.shtml).

Electron microscopy

Length distribution measurements were performed manually on quantitative transmission electron micrographs obtained using a Jeol 1400 transmission electron microscope, an airfuge EM90 rotor (Beckman Instruments, Inc., Brea, CA) and carbon-coated 200 mesh grids after negative-staining (1% uranyl acetate). The electron micrographs were recorded with a Gatan Orius CCD camera (Gatan).

Cell culture

$Fn^{+/-}$, $Fn^{-/-}$ (19) , GD25 and GD25-β1A (24,25) MEFs (kind gifts from D. Mosher, University of Wisconsin-Madison) were grown in Dulbecco's modified minimum essential medium supplemented with 10% FBS and antibiotics. GD25-β1A cells were maintained in 10μg/mL puromycin. CHO-B2 and CHO-B2α27 cells (26,27) (kind gifts from R. Juliano, University of North Carolina) were grown in minimum essential medium (MEMα) containing nucleosides and GlutaMAX (Invitrogen) and supplemented with 10% FBS. AtT-20 pituitary cells were purchased from ATCC and cultured in F-12K medium (Invitrogen) supplemented with 2.5% FBS and 15% horse serum. AtT-20α5 cells (a kind gift from J. Schwarzbauer, Princeton University) (20,21) were grown in DMEM/F-12, HEPES (Invitrogen) supplemented with 10% FBS, 10% horse serum, 4mM L-glutamine and 0.25mg/mL G418. For growing AtT-20 cells on Fn, tissue culture flasks were pre-coated with 10μg/mL fibronectin (Sigma) for 1h and washed three times with PBS before plating the cells. Fn-depleted FBS was prepared by passing the serum over a gelatin-sepharose column (GE Healthcare) twice, and confirming depletion by Western blot.

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Figure S1

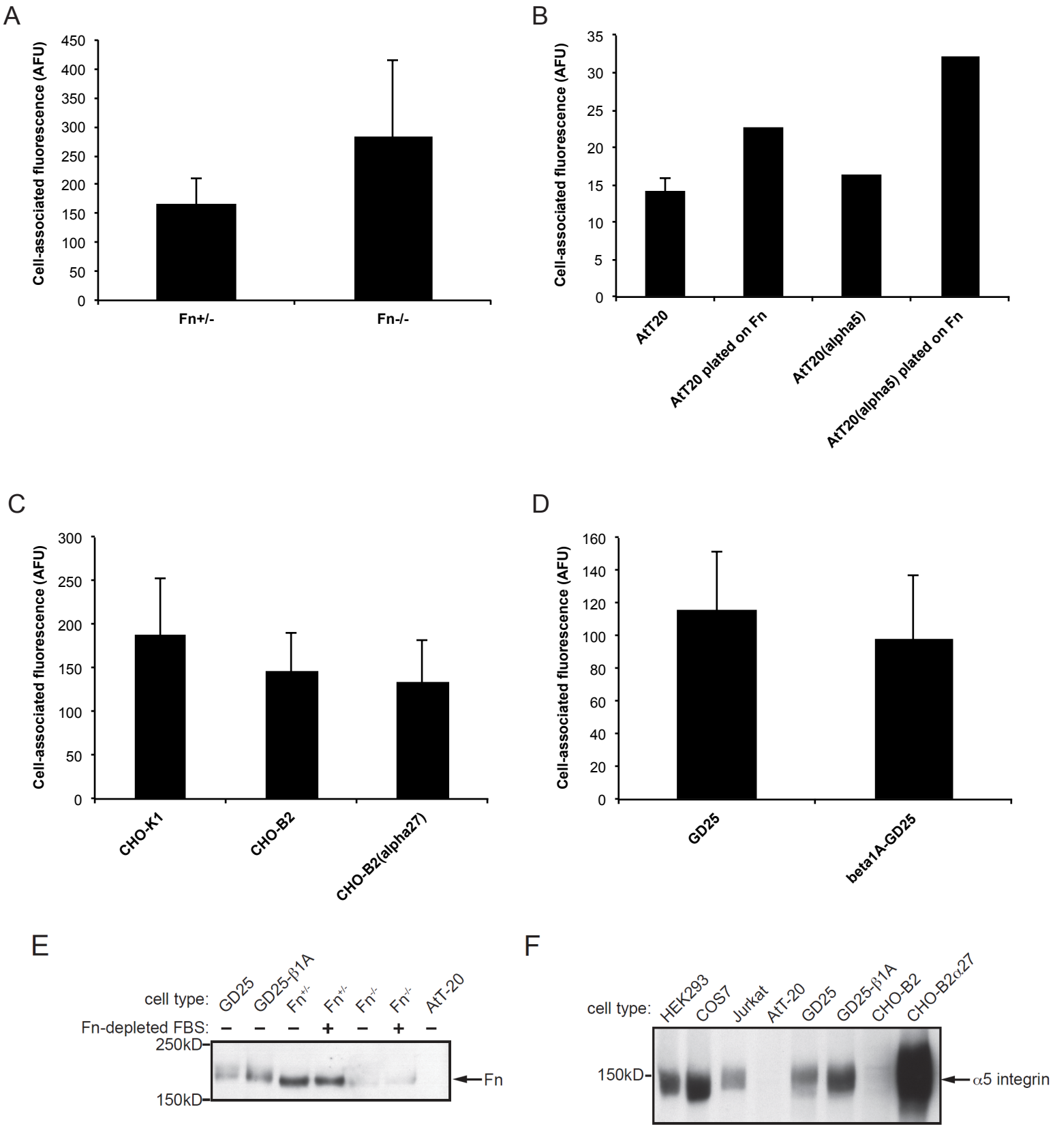

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Figure S2

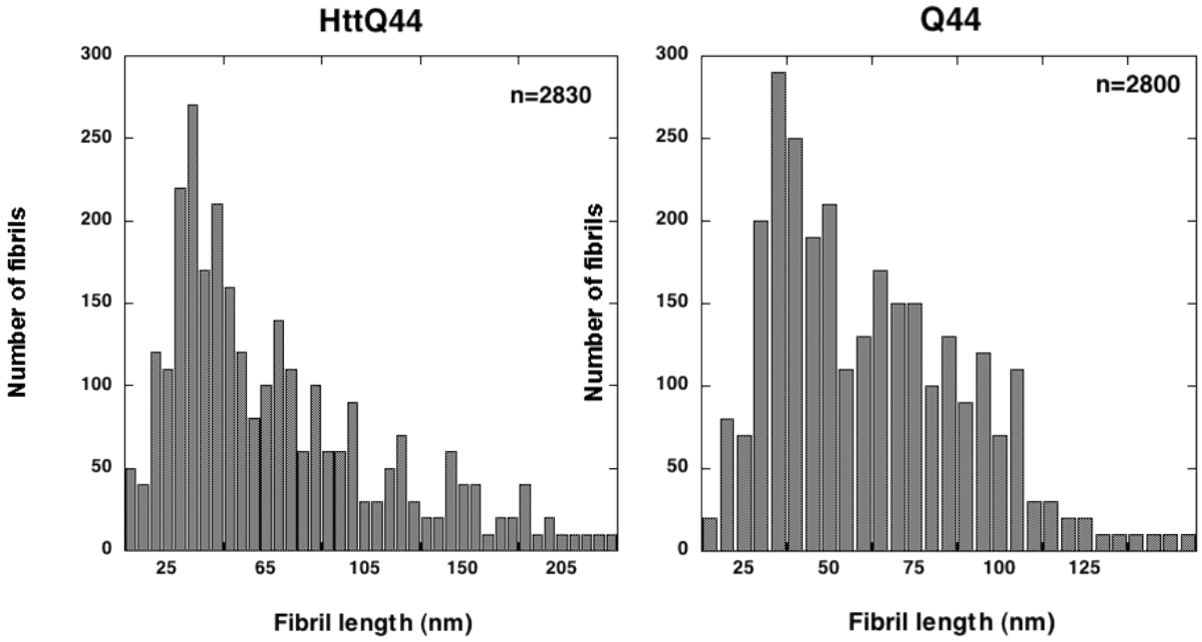

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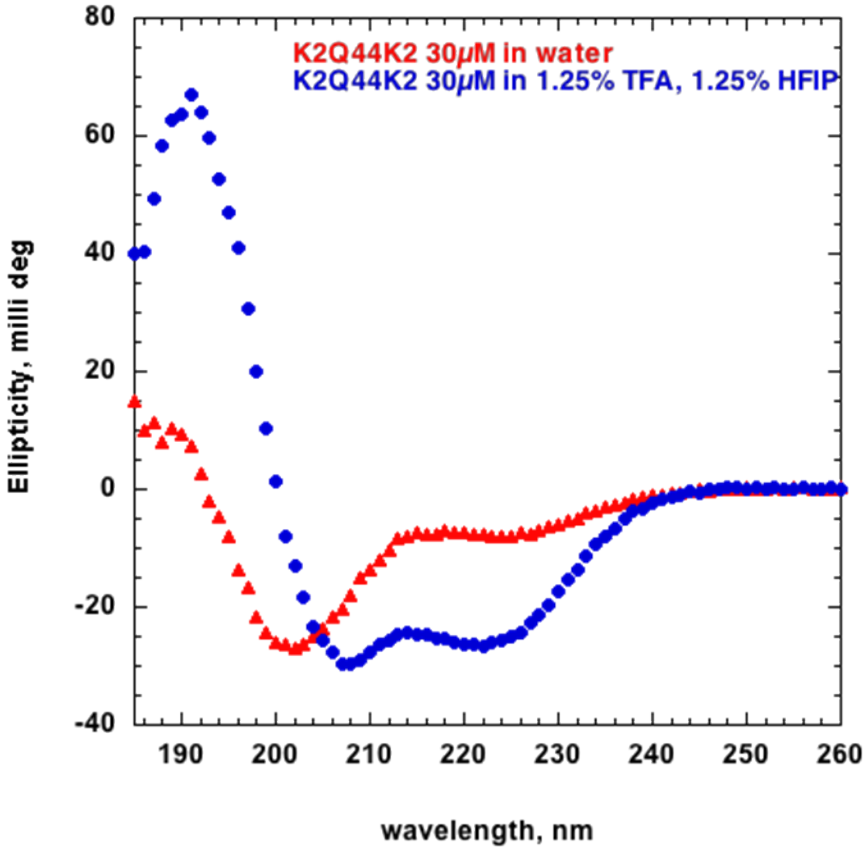
Figure S3

A



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