

## Supplementary Figure e-1. Standard curve linearity and analytic validation of urinary p75<sup>ECD</sup> ELISA assay.

A. Human p75<sup>ECD</sup> was detected using a sandwich ELISA protocol from 0.01 to 20ng/ml. **B.** The linear range determined by regression was from 0.01 to 2.5ng/ml (from dotted box in **A**). **C.** Plotting the standard curve with a log<sub>10</sub> x-axis shows the detection limit of this ELISA at 0.07ng/ml (70pg/ml). **D.** Urinary p75<sup>ECD</sup> is stable for 4 freeze-thaw cycles for ALS patients with high (•), medium (•) or low ( $\bigstar$ ) concentrations of p75<sup>ECD</sup>. **E.** Storage of urine at 4°C (solid line) and at room temperature (RT, ~23°C; dashed line) (n=3 three controls, •, •,  $\bigstar$ ) did alter urinary p75<sup>ECD</sup> levels after 2 days. **F.** Urinary p75<sup>ECD</sup> (n= 2 controls, •, •, •)) does not vary substantially whether from a random spot (pm), first void (am) or 24-hour urinary sample (solid lines). Adjustment using urinary creatinine concentration to account for dilution, further reduced variability (dashed lines). **G.** Urinary creatinine is correlated with urinary osmolarity (osmol/g) (r=0.88, p<0.0001).