SUPPLEMENTAL FIGURES

Supplemental Figure 1: IGF-1 saturation curves and expression of pan AKT, S6, IGFR in STHdh and PC12 cells. A) Representative western blot and quantification of pan IGFR and pan AKT in STHdh Q7/Q7 cells after 3hr, 50/500µM Mn^{2+} exposure in Media or HBSS. **B**) Representative blot and quantification of p-AKT following 24hr exposure with 0.1-25nM IGF-1 in STHdh Q7/Q7 and Q111/Q111. **C**) Representative western blot of p-AKT, pan AKT, p-S6, and pan S6 in non-HTT induced PC12 cells following 24hr exposures with Mn^{2+} (100µM), IGF (10nM), BMS-536924 (1µM), and/or LY294002 (7µM). Cells were treated with LY294002 (PI3K inhibitor, 7µM) as a negative control but data not quantified **D**) Representative western blot and quantification of p-AKT (Thr308) following 24hr exposure with Mn^{2+} (50µM), IGF (10nM), and/or BMS-536924 (1µM) in STHdh Q7/Q7. **E**) Representative western blot of pan IGFR and pan AKT and quantification of pan AKT in STHdh cells following 24hr exposure with Mn^{2+} (50µM), IGF (10nM), and/or BMS-536924 (1µM). N=3; Error bars= SEM; %= significant difference compared to vehicle by 95% CI *= significant difference by Tukey's multiple comparison test. *P<.05, **P<.01, ***P<.001.

Supplemental Figure 2: Mn uptake after KB-R7943+Mn^{2+} and representative western blot for 3T3 and HEK293 cells. A) Quantification of Mn uptake following 24hr, 25,50uM Mn exposures with or without 10uM KB-R7943 with WT and HD SThdh cells. Two-way ANOVA; treatment= F(3,6)=5.840; p=<0.0326; genotype= F(1,2)=127.9; p=0.0077. n=3. *= significant by Sidak's multiple comparison. *P<.05, **P<.01, ***P<.001. **B)** Representative western blot of p-IGFR, p-AKT, and p-S6 for 3T3 and HEK293 cells following 1hr serum deprivation and 3hr exposure with Mn^{2+} (100µM), IGF-1 (1nM), LY294002 (7µM), and/or Linsitinib (1µM) in HBSS.

Supplemental Figure 3: IGF-1 cannot stimulate pAKT expression in hiPSC-derived neuroprogenitors after 24hr exposure in conventional growth-factor (N2) containing media. **A**) Representative western blot of p-AKT and p-S6 treated with Mn^{2+} (200µM), IGF (10nM), and/or BMS-536924 (100nM) under normal +N2 containing (insulin saturating) media in two control and one HD patient-iPSC-derived neuroprogenitors following 24hr treatment. **B, C**) Quantification of p-AKT (**B)** and p-S6 (**C**). Error bars= SEM; N=4 control; N=3 HD. **D)** Two-way ANOVA statistics from Fig 7.

Supplemental Figure 4: Representative western blots for hiPSC-derived neuroprogenitors. A, B) Representative western blot of of hiPSC-derived neuroprogenitors from a control patient (CC3, **A**) and an HD patient (HD58, **B**) for p-IGFR, p-AKT, p-S6 following 3hr treatment with Mn^{2+} (200/500µM) or Mn^{2+}+IGF (1nM) and BMS-536924 (100nM, 1µM) BMS-754807 (2nm) in serum free media, after 3hr serum withdrawal. **C)** Representative western blot for pan IGFR, pan AKT, and pan S6. Same blot as Panel A. **D-F)** Quantification of pan IGFR (**D**), pan AKT (**E**), and pan S6 (**F**). Left set of conditions (veh, 200µM, 500µM, BMS754807+500µM) is with no IGF, right side is with 1nM IGF. N=4; 2 control patients from 2 separate differentiations.

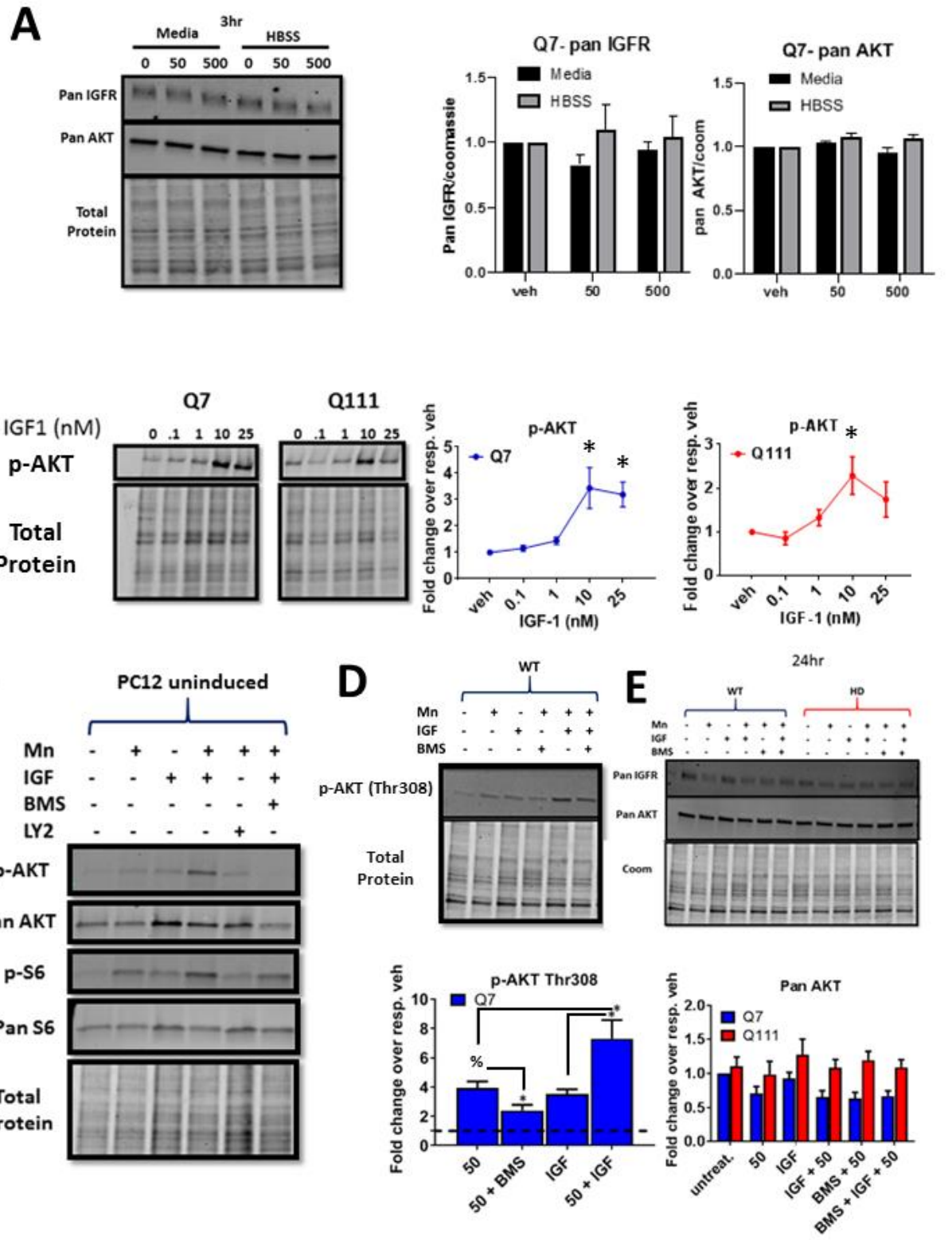
SUPPLEMENTAL FIGURES (Cont'd)

Supplemental Figure 5: Western blot quantification for hiPSC-derived neuroprogenitors. A, B) Quantification of p-IGFR, p-AKT, and p-S6 in control **(A)** or HD **(B)** hiPSC-derived neuroprogenitors after no treatment (trace IGF) or stimulation with IGF-1 alone (1nM) for 3hrs, following 3hr serum deprivation. **C, D**) Quantification of p-IGFR, p-AKT, and p-S6 in control **(C)** or HD **(D)** hiPSC-derived neuroprogenitors after treatment with BMS-536924 (100nM, 1µM) or BMS-754807 (2nM) alone for 3hrs, following 3hr serum deprivation in either trace IGF or 1nM IGF only containing conditions (no Mn^{2+}).

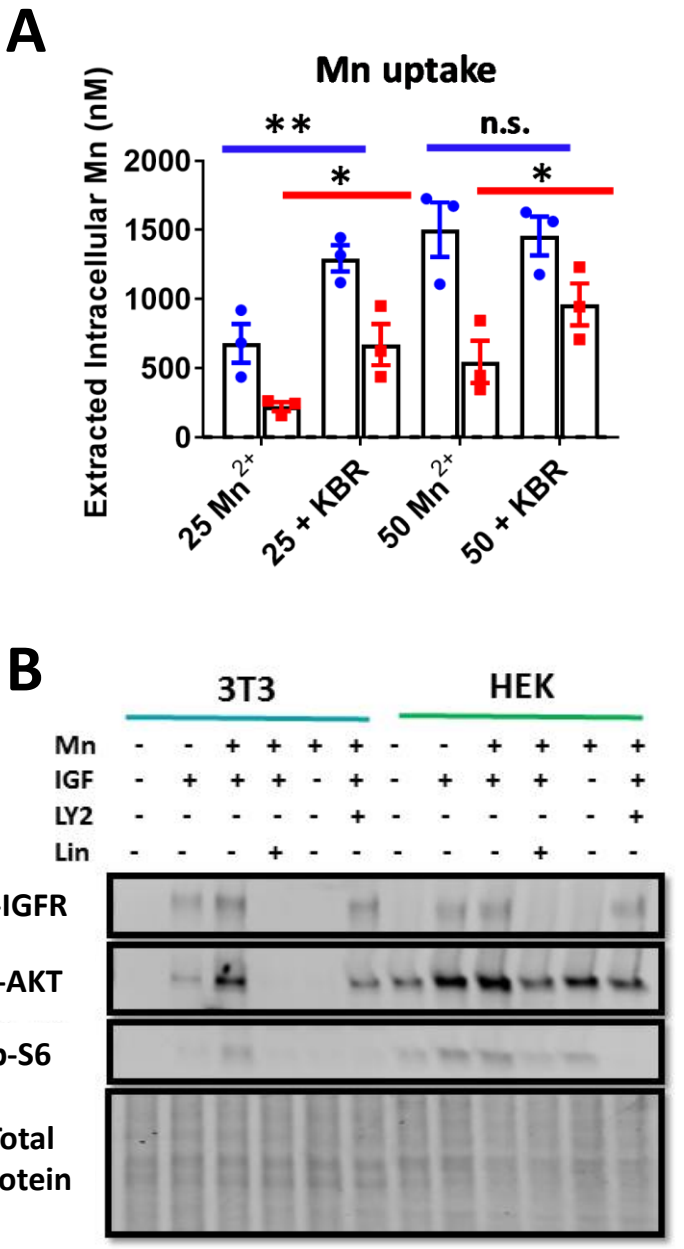
Supplemental Figure 6: **IGFR/IR inhibitors block the majority of Mn-induced p-AKT.** Quantification of degree of Mn^{2+}-induced p-AKT, p-IGFR, and p-S6 inhibition using IGFR/IR inhibitors in WT STHdh (**A**), non-striatal cells (HEK293, 3T3, PC12) (**B**), and hiPSC-derived striatal-like neuroprogenitors (**C**)- quantified from data in Figures 5, 6, and 8. The name, concentration, and IC50 of inhibitors used is listed followed by the percentage of inhibited Mn^{2+}-induced p-AKT, p-IGFR, and p-S6 expression after inhibitor treatment (Mean±Stdev). For B, C an inhibition correlation score is also calculated (%pAKT or p-S6 inhibition)/(%pIGFR/IR inhibition).

Supplemental Figure 7: Quantification of phosphorylated protein expression by (phospho/Coomassie) or (phospho/pan/Coomassie) do not significantly differ (p-values for difference between normalization methods, Coomassie vs pan, are noted on each graph and **bolded** here in this legend). A) Quantification of p-AKT after 3hr, 50/500µM Mn^{2+} exposure in serum free HBSS (following 1hr serum deprivation) or media containing 10% FBS. Data was normalized as p-AKT/panAKT/Coomassie. Data is taken from the same samples as Fig 1B as a direct comparison. Two-way ANOVA; treatment= F(2,4)=10.64; p=0.0250; normalization type= F(1,2)=0.005268; **p=0.9487** B) Quantification of p-AKT (Thr308, not Ser473) after exposure with 50uM Mn, 10nM IGF, or both for 24hrs. Data is normalized as p-AKT/coomassie or p-AKT/panAKT/coomassie and plotted on the same graph for direct comparison. n=3 for all experiments. Two-way ANOVA; treatment= F(1.432,5.726)=31.57; p=0.0011; genotype= F(3,12)=3.833; p=0.0389; normalization type= F(3,12)=1.183; **p=0.3574** Quantification of p-AKT (C), p-S6 (D), and p-IGFR (E) expression after 3hr exposures in serum-free HBSS with or without 1nM IGF. Data used here is taken from a subset of the same samples used in Fig 7, 8. Phosphorylated expression in control cells is normalized as phos/coomassie (dark blue) or phos/pan/coomassie (light blue) and plotted on the same graph. Two-way ANOVA for C) treatment=F(11, 22)=10.71; p=<0.0001; normalization type=F(1, 2)=2.648 ; **p=0.2452.** D) treatment= F (11, 33) = 4.557; p=0.0003; normalization type= F(1, 3)=1.157; **p=0.3609.** E) treatment= F(11, 22)=3.890 ; p=0.0032; normalization type= F(1, 2)=2.630; **p=0.2463**. **F, G)** Percent of protein remaining after Mn^{2+} exposures (200, 500uM) for 3 hours in STHdh (**F**) and hiPSC-derived neuroprogenitors (**G**) as a cell viability readout. N=4. Error= 95% CI. Normalized to vehicle treated for each genotype. H) Mn uptake in STHdh cells after 24hr Mn^{2+} exposure with 25-100uM Mn^{2+}. Two-way ANOVA for H) treatment=F(3,6)= 236.7; p=<0.0001; genotype=F(1,2)=52.94; p=0.0184. *= significant differences by Sidak's post hoc analysis. All data except F, G are plotted as SEM. N=3 for all experiments except F, G.

Supp Figure 1



Supp Figure 2



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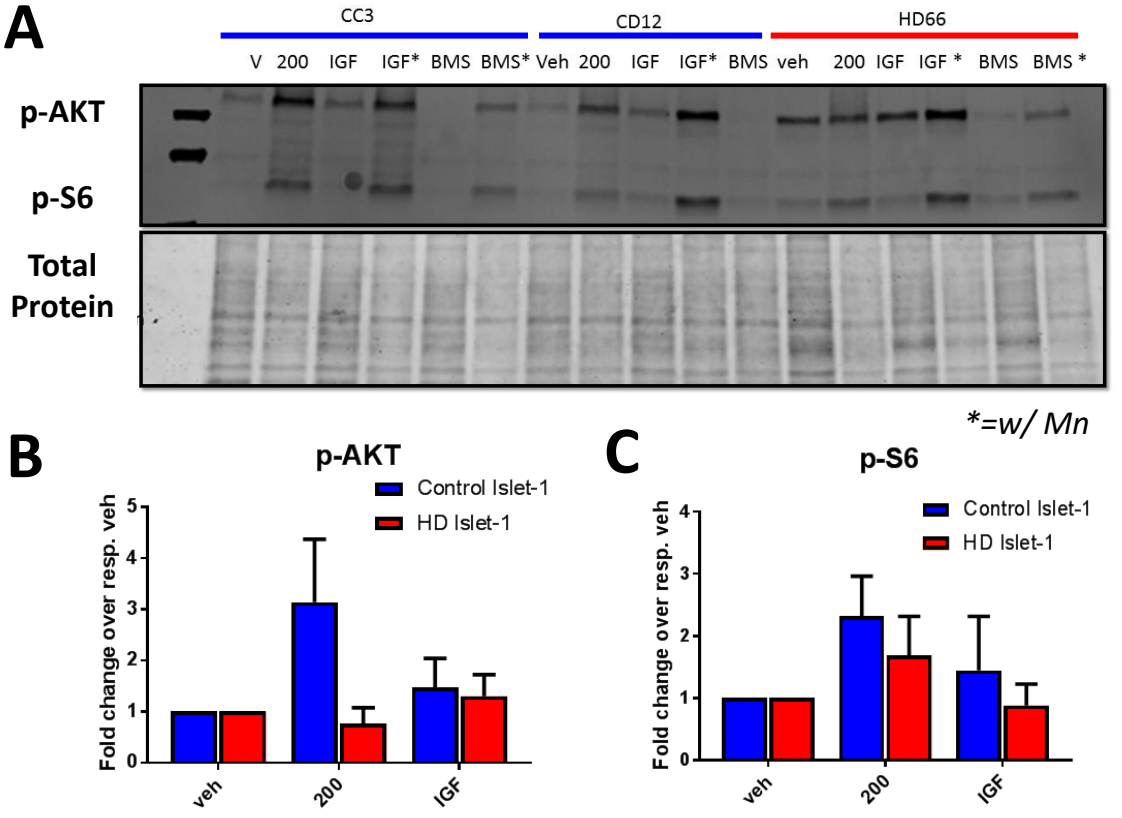
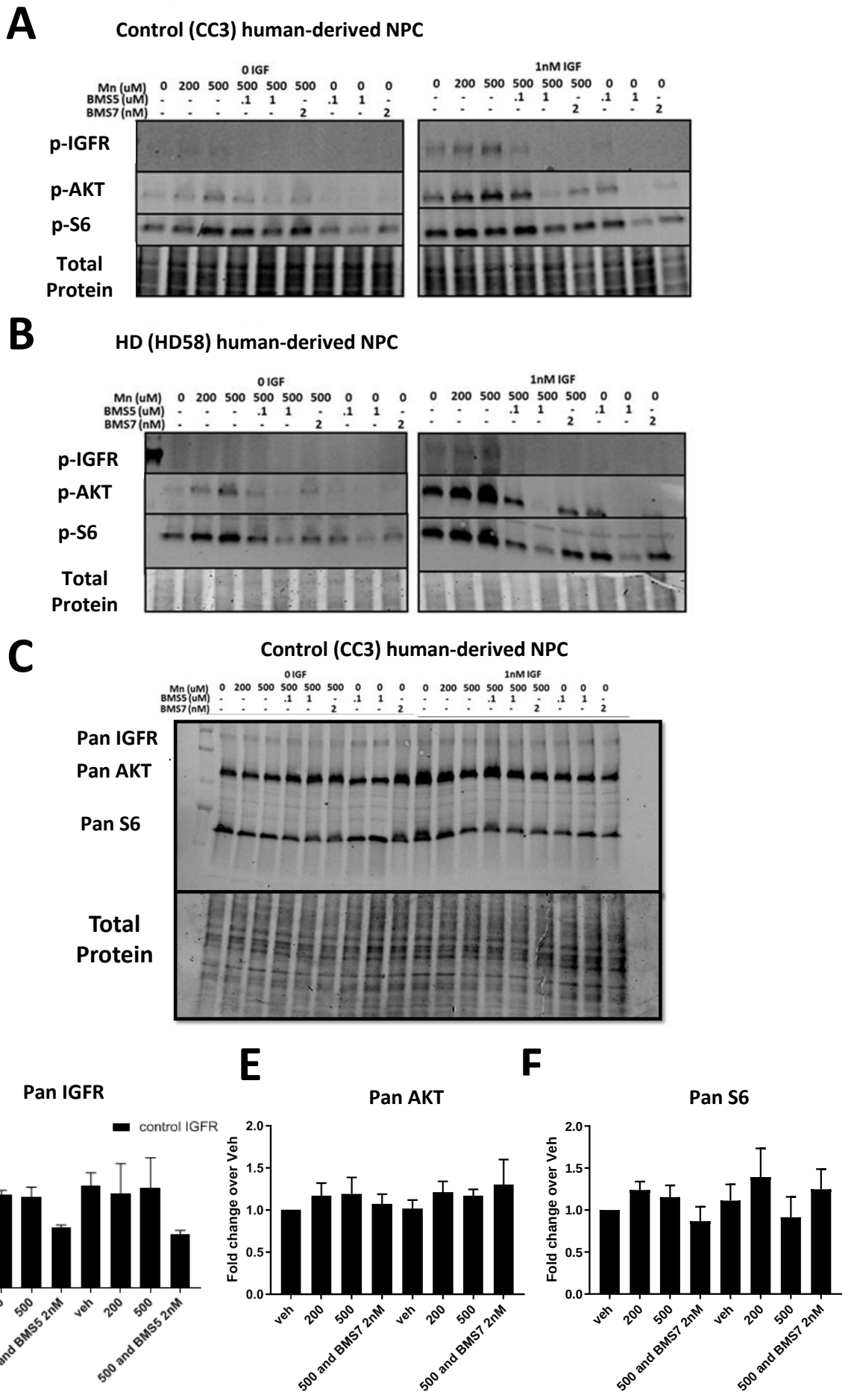


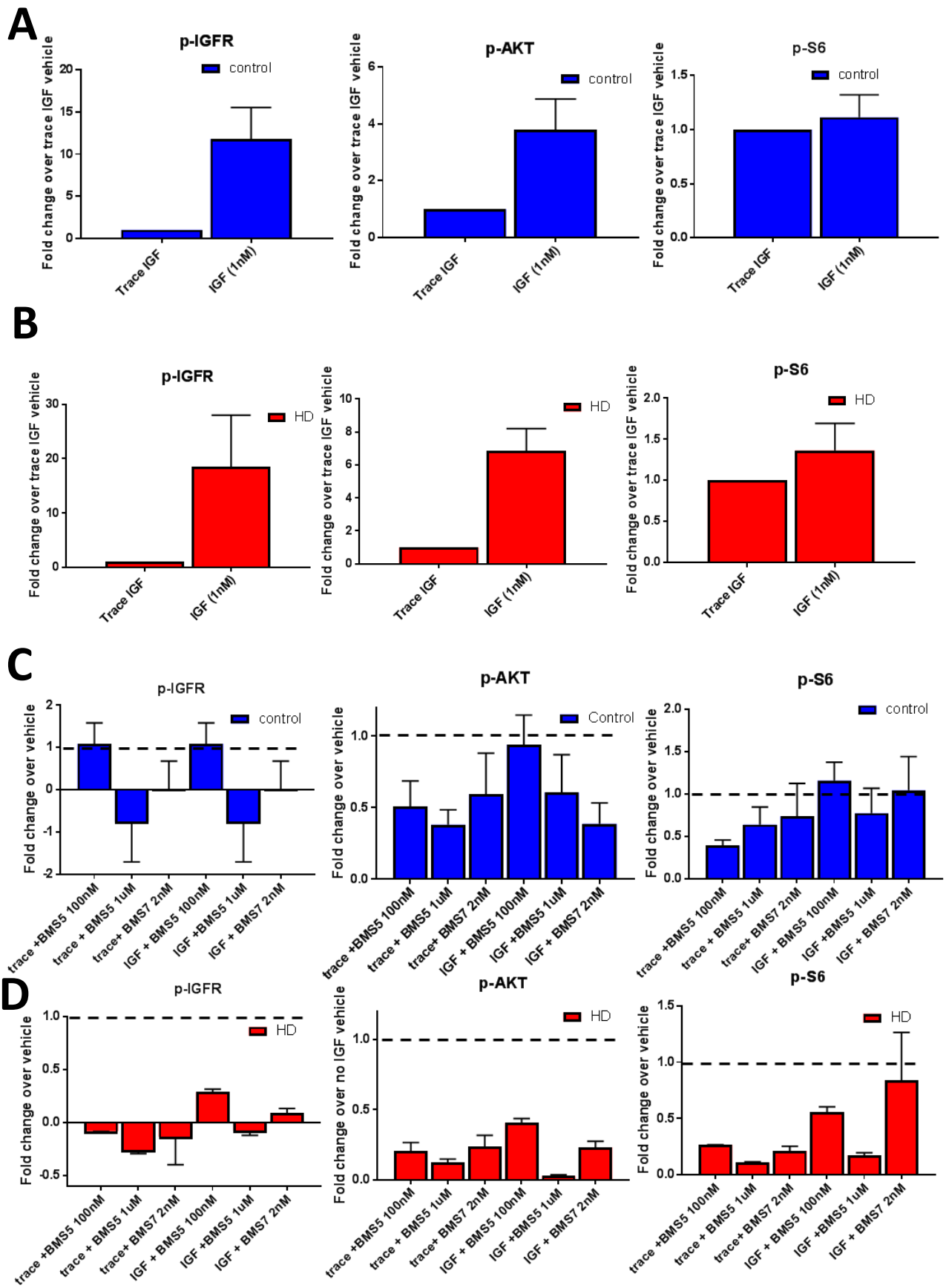
D

Genotype	Probe	Conditions	F (DFn, DFd)	p-value
WT	p-AKT	Mn alone	(2,20)= 29.94	**<.0001**
WT	p-IGFR	Mn alone	(1.1236, 7.416)= 6.378	**0.0334**
WT	p-S6	Mn alone	2,12) = 8.008	**0.0062**
WT	p-AKT	Mn+IGF	(2,20)=29.45	**<.0001**
WT	p-IGFR	Mn+IGF	(1.628, 13.02)=5.347	**0.0249**
WT	p-S6	Mn+IGF	(2,12)=3.756	**0.0541**
HD	p-AKT	Mn alone	(2, 18)= 15.43	**<.0001**
HD	p-IGFR	Mn alone	(1.034, 5.170)=2.607	**0.1657**
HD	p-S6	Mn alone	(2,12)=.2178	**0.8074**
HD	p-AKT	Mn+IGF	(2, 18)= 5.850	**<.0001**
HD	p-IGFR	Mn+IGF	(1.044, 3.132)=2.080	**0.2432**
HD	p-S6	Mn+IGF	(2,12)=.8916	**0.4355**

Supp Figure 4



Supp Figure 5



Supp Figure 6

A STHdh Q7/Q7 (Fig. 5)

Inhibitor	Reported IC50 (nM)	Mn-induced pAKT inhibited (Mn)(%)	Mn-induced AKT inhibited (Mn+IGF)(%)
BMS-536924 (100nM)	100 (IGFR), 73 (IR)	45.5±12.9	24.1±20.3
Linsitinib (100nM)	35 (IGFR), 75 (IR)	71.1±10.9	60.0±9.2
Linsitinib (1uM)	35 (IGFR), 75 (IR)	80.7±6.6	N/A
NVP-AEW541 (100nM)	150 (IGFR), 140 (IR)	54.2±19.5	1.8±28.57
NVP-AEW541 (1uM)	150 (IGFR), 140 (IR)	65.4±32.7	N/A

B Non-striatal cells (3T3, HEK293- Fig. 6; PC12- Fig 5)

Linsitinib (1uM) IC50- 35, 75nM (IGFR, IR)	Mn-induced pIGFR/IR inhibited (%)	Mn-induced pAKT inhibited (%)	Mn-induced pS6 inhibited (%)	AKT:IGFR/IR Correlation Score*	S6:IGFR/IR Correlation Score*
3T3	98.3±2.51	97.2±0.56	60.6±32.48	**98.8**	**61.7**
HEK293	98.3±1.80	40.5±22.1	19.1±60.1	**41.1**	**19.4**
PC12	N/A	128.2±42.0	58.7±36.6	**N/A**	**N/A**

**Inhibition correlation score= (%pAKT or pS6 inhibition) / (%pIGFR/IR inhibition)*

C hiPSC-derived neuroprogenitors (Control- Fig. 8)

Inhibitor	Reported IC50 (nM)	Mn-induced pIGFR/IR inhibited (%)	Mn-induced pAKT inhibited (%)	Mn-induced pS6 inhibited (%)	AKT:IGFR/IR Correlation Score*	S6:IGFR/IR Correlation Score*
BMS-536924 (100nM)	100 (IGFR), 73 (IR)	59.9±8.9	47.6±17.1	8.6±37.4	**79.5**	**14.4**
BMS-536924 (1uM)	100 (IGFR), 73 (IR)	96.8±1.3	71.5±18.0	64.5±33.5	**73.9**	**66.6**
BMS-754807 (2nM)	1.8 (IGFR), 1.7 (IR)	84.2±12.7	57.0±24.4	37.8±37.7	**67.7**	**44.9**

**Inhibition correlation score= (%pAKT or pS6 inhibition) / (%pIGFR/IR inhibition)*

Supp Figure 7