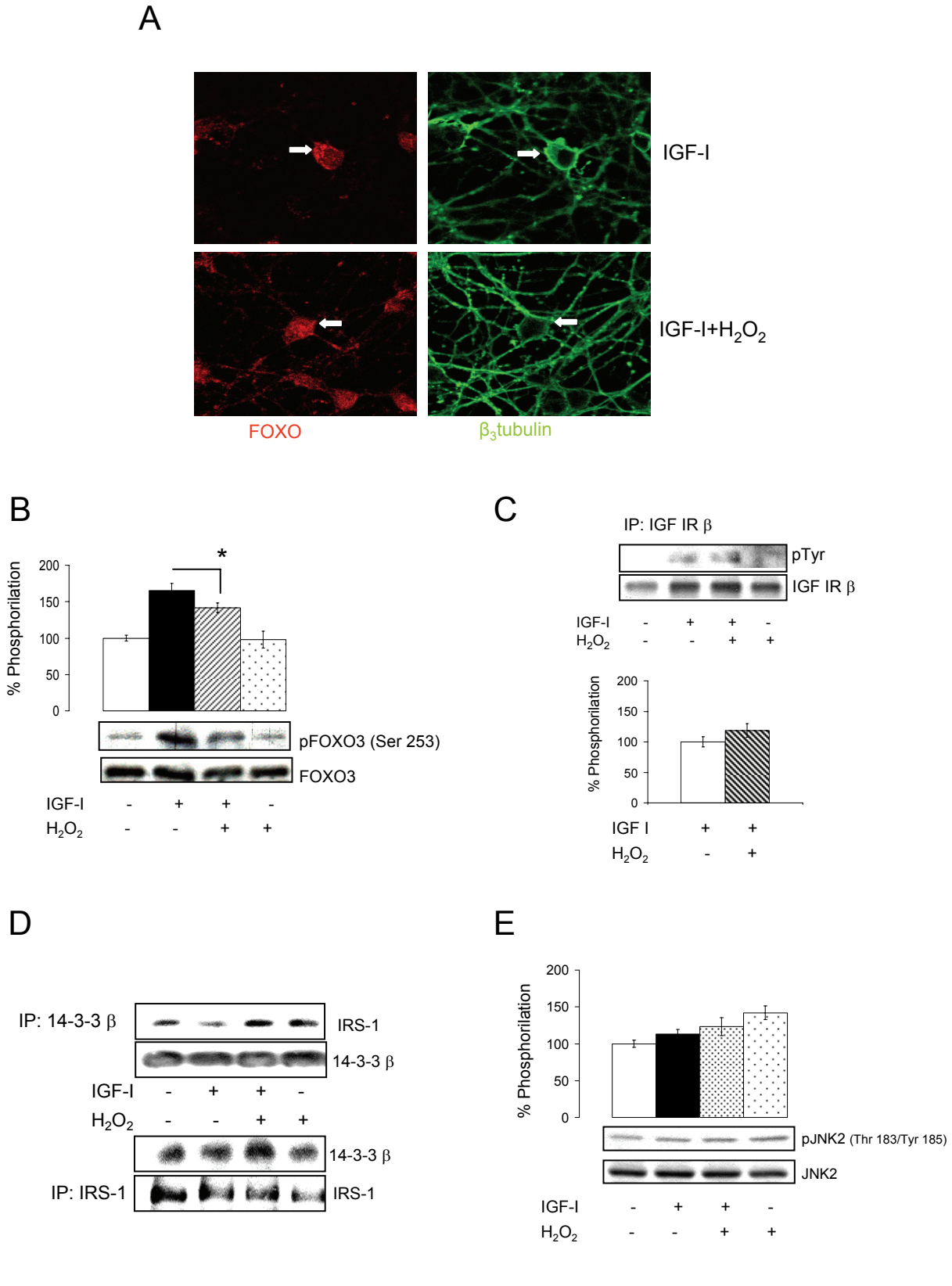
E07-08-0811 Torres-Aleman

Supplemental Figure

Supplemental Figure 1: **A**, Modulation of the subcellular distribution of FOXO by IGF-I and H_2O_2. In the presence of IGF-I, FOXO was located in the neuronal cytoplasm, while when H_2O_2 was added to IGF-I-treated neurons FOXO was retained in the cell nucleus. Immunocytochemical analyses was performed 4 hours after exposure to H_2O_2. **B**, Differential phosphorylation of FOXO by IGF-I and H_2O_2. Enhanced levels of phospho-FOXO3 at the AKT-sensitive residue Ser^{253} 4 hours after IGF-I addition were reduced in the presence of H_2O_2. n= 4. **C**, H_2O_2 does not affect IGF-I receptor phosphorylation. Co-addition of H_2O_2 (50 μM) with IGF-I (100 nM) to neuronal cultures did not affect Tyr-phosphorylation of the IGF-I receptor. A representative blot is shown. Histograms show densitometric quantification of IGF-I and IGF-I+ H_2O_2-treated cultures. No significant differences were appreciated (n= 3). **D,** Differential association of IRS-1 with 14-3-3 after IGF-I and H_2O_2. Addition of IGF-I to cultures reduces the interaction of IRS-1 with 14-3-3β fifteen min later, while H_2O_2 maintains this association. Reciprocal immunoprecipitations (IP) are shown. Upper blot: IP 14-3-3β and WB IRS-1. Lower blots, the opposite (n= 3). **D**, Levels of phospho-JNK2 are not modify at early times after H_2O_2. Addition of 50 μM H_2O_2 to IGF-I-treated cerebellar neurons did not affect JNK2 activity at 15 min as determined by levels of phospho-JNK2. Representative blot is shown (n= 5). Histograms: quantitation of blots.


A
IGF-I
IGF-I+H2O2
FOXO
β3tubulin
B
% Phosphorilation
200
150
100
50
0
*
pFOXO3 (Ser 253)
FOXO3
IGF-I - + + -
H2O2 - - + +
C
IP: IGF IR β
pTyr
IGF IR β
IGF-I - + + -
H2O2 - - + +
% Phosphorilation
200
150
100
50
0
IGF I + +
H2O2 - +
D
IP: 14-3-3 β
IRS-1
14-3-3 β
IGF-I - + + -
H2O2 - - + +
14-3-3 β
IP: IRS-1
IRS-1
E
% Phosphorilation
200
150
100
50
0
pJNK2 (Thr 183/Tyr 185)
JNK2
IGF-I - + + -
H2O2 - - + +