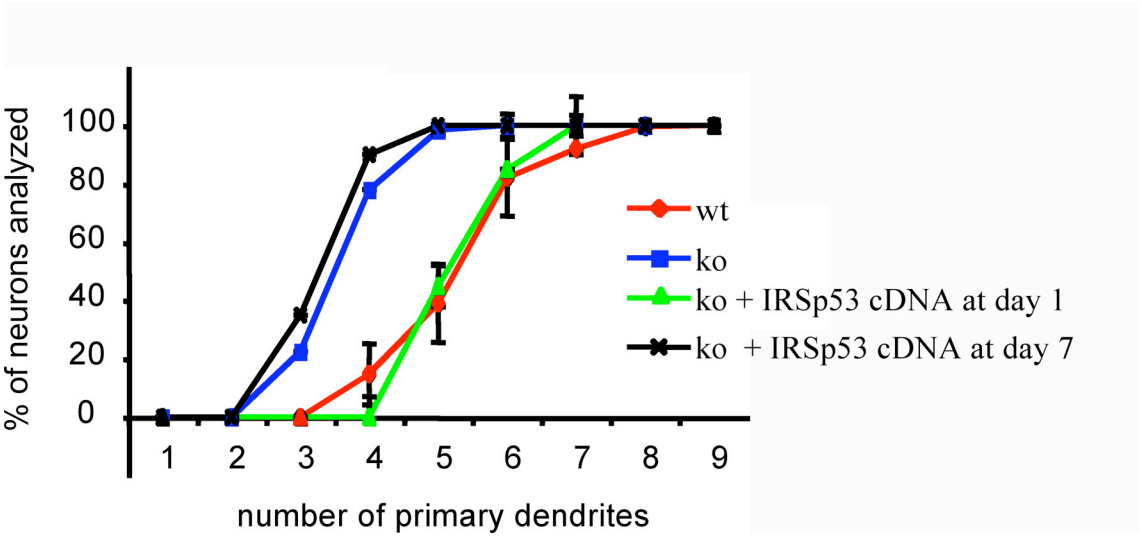


**Figure S1.**
Cumulative frequency plot of the number of primary dendrites in neurons derived from wt and ko mice. Ko neurons were transfected with IRSp53 cDNA at day 1 or day 7, as indicated. All cells were cultured for a total of 14 days in vitro and subsequently immunostained for the dendritic marker MAP2. The number of primary dendrites was determined as described in the legend to Figure 3.

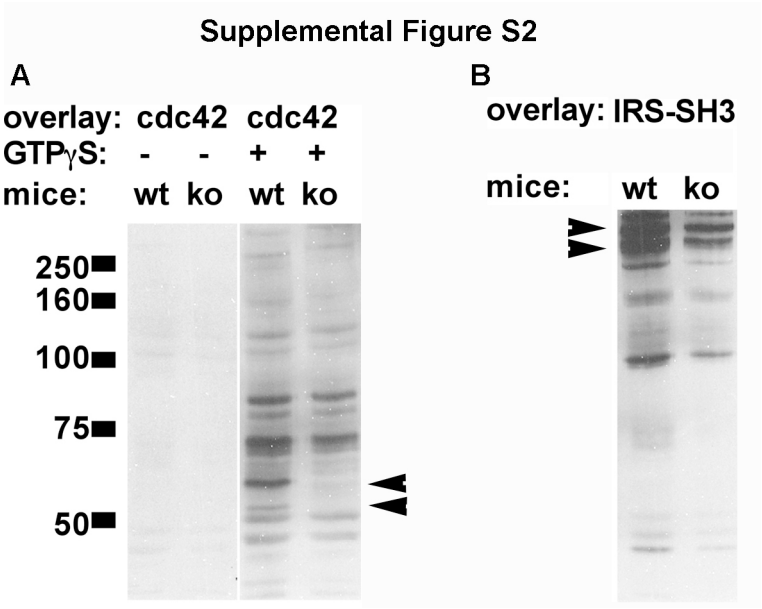


**Figure S2.**

A. Overlay analysis of cdc42 targets. PSD fractions from wt and IRSp53-deficient (ko) animals were separated by SDS-PAGE, blotted on nitrocellulose and analyzed by overlay reactions using GST-cdc42 in the absence or presence of GTPγS. Note that two bands which are recognized by cdc42 in a GTP-dependent manner in PSD derived from wt mice are missing in PSD from ko mice. By molecular weight, comigration with IRSp53 immunoreactive bands (indicated by arrowheads) and their absence in the ko mice these bands can be clearly identified as the 53 kDa and 58 kDa splice variants of IRSp53. Thus IRSp53 constitutes one of the major target molecules of cdc42 in the PSD.

B. Overlay analysis was performed using a GST-fusion of the IRSp53 SH3 domain. The major reactive bands at >250 kDa (arrowheads) comigrate with major isoforms of shank1 and shank3, suggesting that shank proteins constitute the majority of interaction partners of the IRSp53 SH3 domain in the PSD. The molecular weights of all other known interaction partners of the IRSp53 SH3 domain is below 250 kDa.

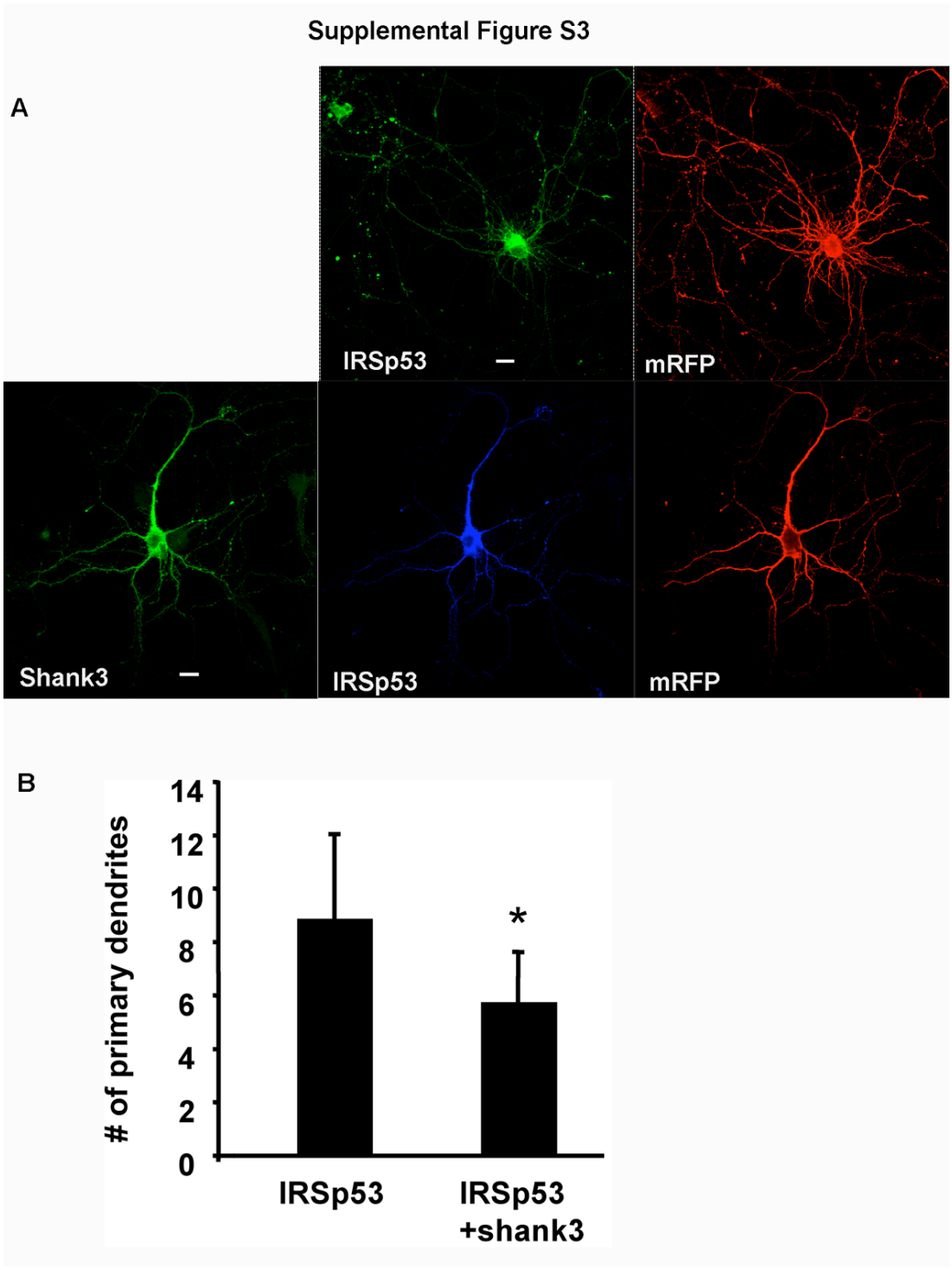


**Figure S3**

Effect of IRSp53 and Shank3 on hippocampal neuronal morphology. A. Rat hippocampal neurons were transfected with expression constructs coding for the monomeric red fluorescent protein (mRFP) in combination with expression plasmids coding for IRSp53 (upper panels), or IRSp53 and Shank3 lower panels. Expressed proteins were first visualized in each cell by immunostaining; mRFP was visualized using its fluorescence. Bar, 10 μm. B. Primary dendrites were counted ased on the mRFP signal in neurons transfected as in A. Whereas IRSp53 expressing cells exhibited an increased number of dendrites when compared to the control situation (mRFP only, not shown), this was reduced by coexpression of Shank3. *, significantly different from IRSp53 transfected cells, $p<0.05$ (n=7; paired t-test).

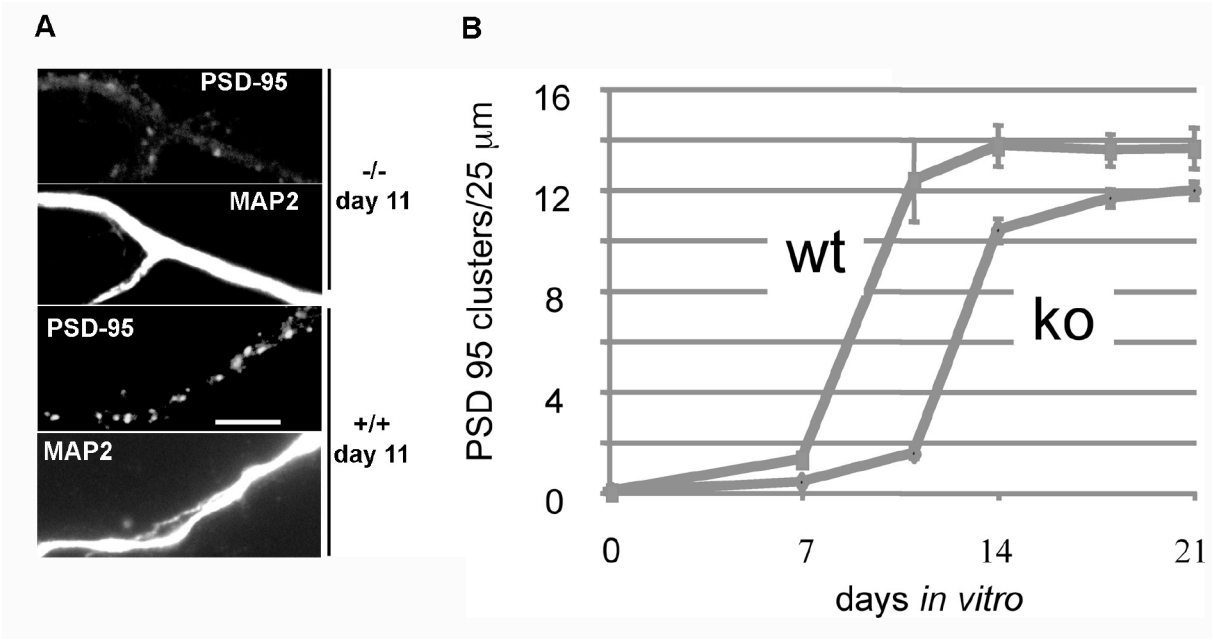


**Figure S4**

Developmental appearance of postsynaptic clusters in cultured neurons derived from wt and IRSp53 deficient animals. A. Neurons were cultured for 11 days in vitro and stained for MAP2 (to outline dendrites; lower panels) and PSD-95 (upper panels). B. Quantification of PSD-95 clusters during time course of in vitro neuronal differentiation. Note that though the total number of PSD-95 clusters after 21 days in culture was not changed between ko and wt neurons, cluster formation in ko neurons occurred later, such that at day eleven in culture ko neurons displayed only about 15 % of the number of clusters obtained by their wild type counterparts.

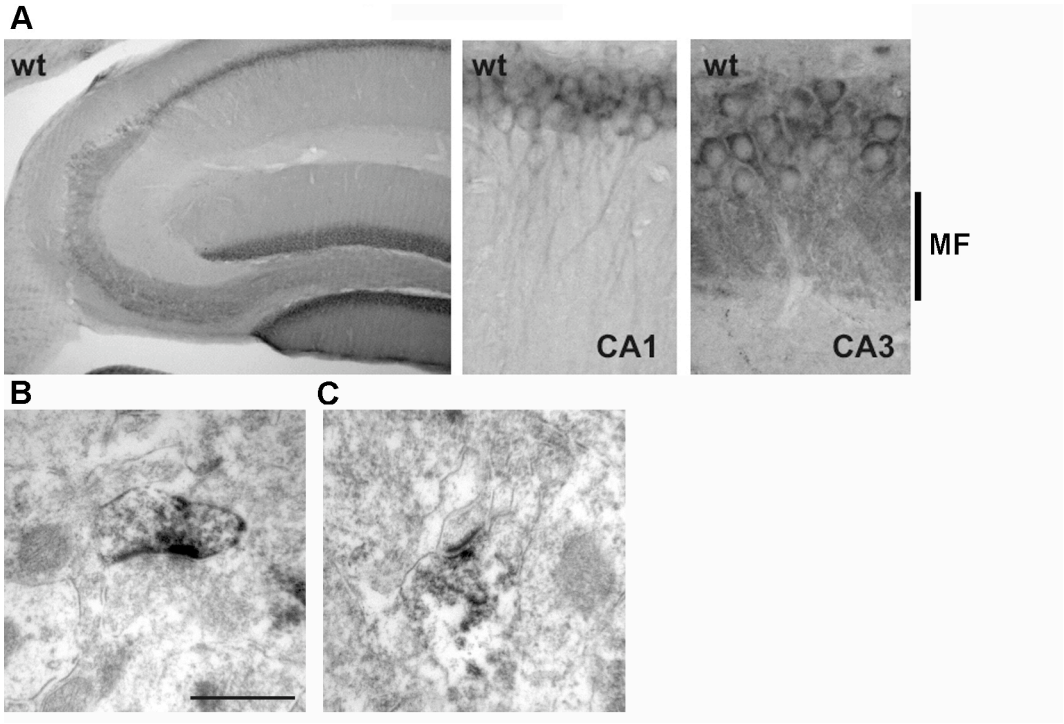


**Figure S5.**
Localization of IRSp53 in the hippocampus. A. Details of anti-IRSp53 immunostaining in the hippocampus of wt mice. Staining is prominent in cell somata and dendrites; in addition, diffuse staining of the mossy fiber area (MF) in CA3 indicates possible axonal labeling. B,C: Whereas most immunoreactivity was observed postsynaptically (B), some presynaptic terminals were also labeled in CA3 at the electron microscopic level (C). Scale Bar: 0.5 μm