Supplementary Information for manuscript “Neuroprotective levels of IGF-1 exacerbate epileptogenesis after brain injury” by Y. Song, C. Pimentel, K. Walters, L. Boller, S. Ghiasvand, J. Liu, K.J. Staley, and Y. Berdichevsky.

Supplementary Figure 1. Time course of IGF-1R phosphorylation after addition of IGF-1 to the culture medium of -IGF-1 cultures on DIV 3. Antibody that detects phosphorylated IGF-1R as well as InR was used. No insulin was present in the culture medium. IGF-1R is significantly more phosphorylated after 30 minutes of IGF-1 application (p = 0.011, n = 4). Increase of phospho IGF-1R / total IGF-1R ratio from 30 minutes to 4 hours was not significant (p = 0.463, n = 4). Data represented as mean ± SEM, *p < 0.05.

Supplementary Figure 2. Akt1/2, FR180204, and rapamycin inhibit phosphorylation of their target proteins. Inhibitors were applied on DIV 3 to +IGF-1 cultures, and lysates were collected on DIV 6. 1 µM Akt1/2 significantly reduced phosphorylation of Akt at Thr 308 (p = 0.014) and at Ser 473 (p < 0.001). 10 µM of FR180204 significantly reduced phosphorylation of MAPK (p = 0.037), and 20 nM rapamycin significantly reduced phosphorylation of S6 (p = 0.018). n = 3 cultures, all inhibitors and conditions. Data represented at mean ± standard deviation.
Supplementary Figure 3. Comparison of pyramidal layer length (CA1+CA3b+CA3c) in organotypic hippocampal cultures with different IGF-1 treatments. On DIV 3, vehicle-treated cultures (-IGF-1) had a significantly shorter pyramidal layer (p = 0.009, n = 6). On DIV 14, pyramidal layer in -IGF-1 cultures was also significantly shorter than in +IGF-1 cultures (ANOVA p = 0.005, post-hoc p < 0.001 for +IGF-1 vs. -IGF-1 comparison, n = 7, 3 and 8 for +IGF-1, +/-IGF-1, and -IGF-1 cultures, respectively). No significant differences were found on DIV 25 (ANOVA p = 0.292, n = 10, 10, and 4 for +IGF-1, +/-IGF-1, and -IGF-1 cultures, respectively). Data represented as mean ± SEM, ** p < 0.01, *** p < 0.001.