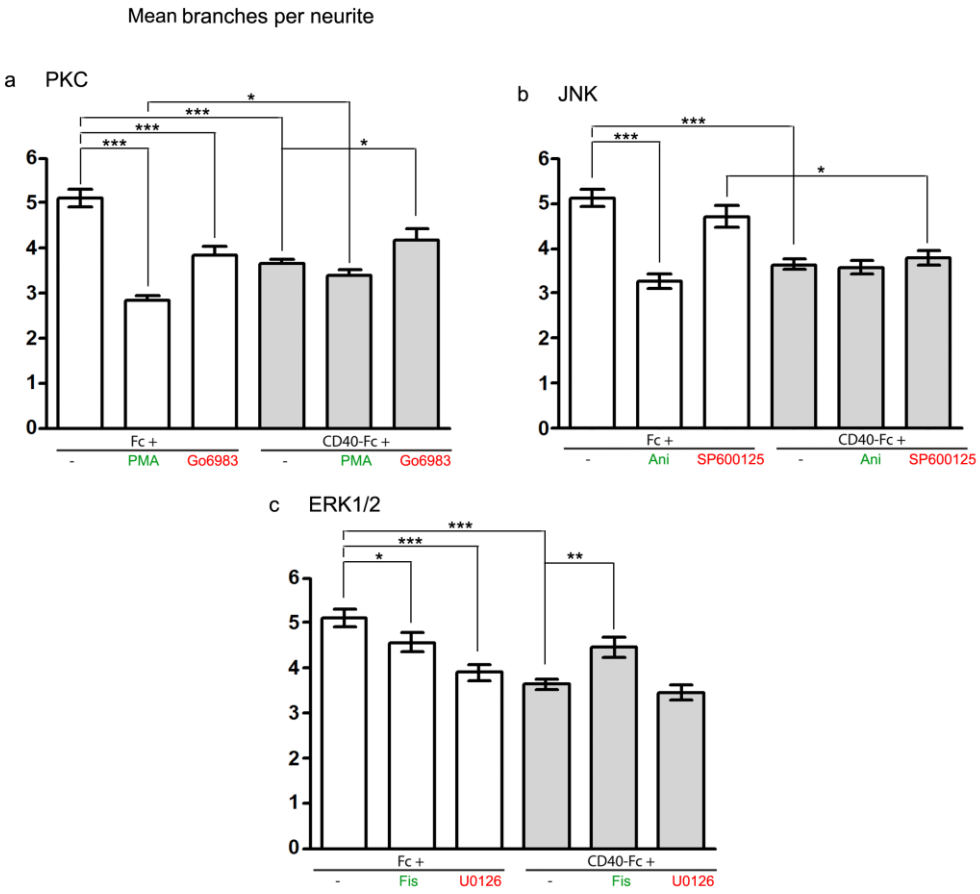

Figure S1. Effect of pharmacological reagents on the mean of branches per neurite from MSNs. (**a**, **b** and **c**) Quantification of the influence of pharmacological reagents on the number of branches per neurite. Cultures of striatal MSNs were established from E14 *Cd40*$^{-/-}$ embryos. The cultures were treated 24h after plating with either 1 µg/ml of Fc (white bars) or 1 µg/ml of CD40-Fc (grey bars) together with pharmacological manipulators of PKC (either 500 nM PMA or 500 nM Go6983) (**a**), JNK (either 50 nM Ani or 1 µM SP600125) (**b**), and ERK1/ERK2 (either 1 µM Fis or 1 µM U0126) (**c**). The activators are labelled in green and the inhibitors in red. Mean ± s.e.m of at least three independent experiments. The number of neurons counted per condition are given below. One-way ANOVA with multiple Newman-Keuls statistical comparison. Key statistical significance differences are indicated (*** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$).

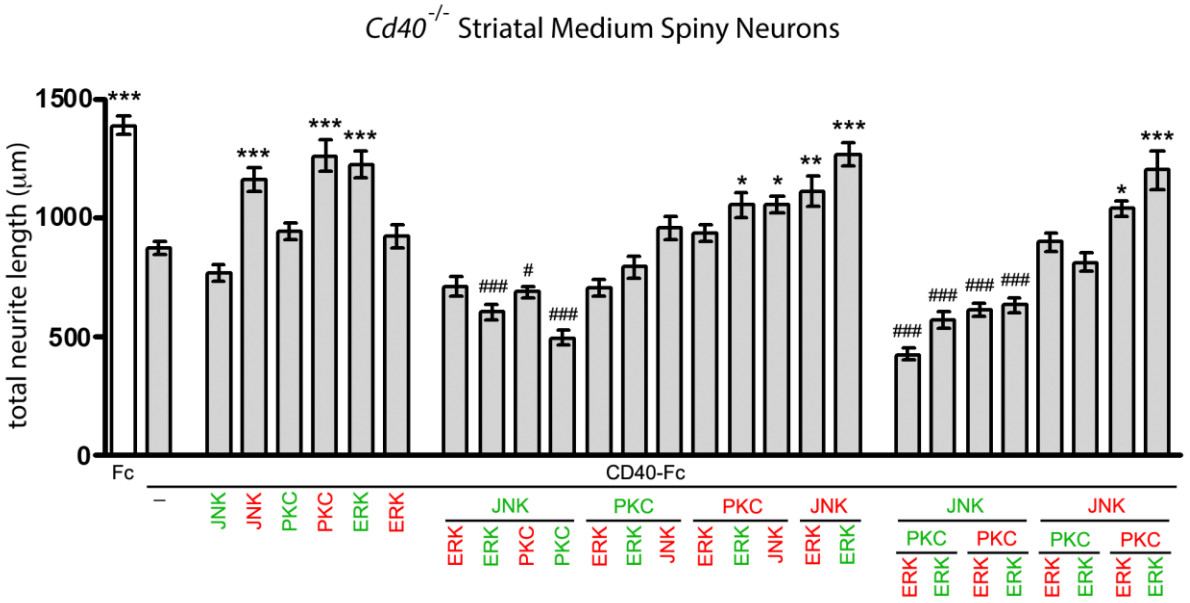

Figure S2. Total neurite length with all pharmacological reagents in combination. Quantification of total neurite lengths of neurons cultured for 10 days *in vitro,* and treated 24 h after plating with the indicating combination of reagents in presence of 1 µg/ml CD40-Fc (grey bars). Control Fc at 1 µg/ml is shown as reference (white bar). The graph shows the mean ± s.e.m of at least three independent experiments. T-test comparisons versus neurons treated with CD40-Fc, *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ (# indicates significant differences but in the opposite direction).

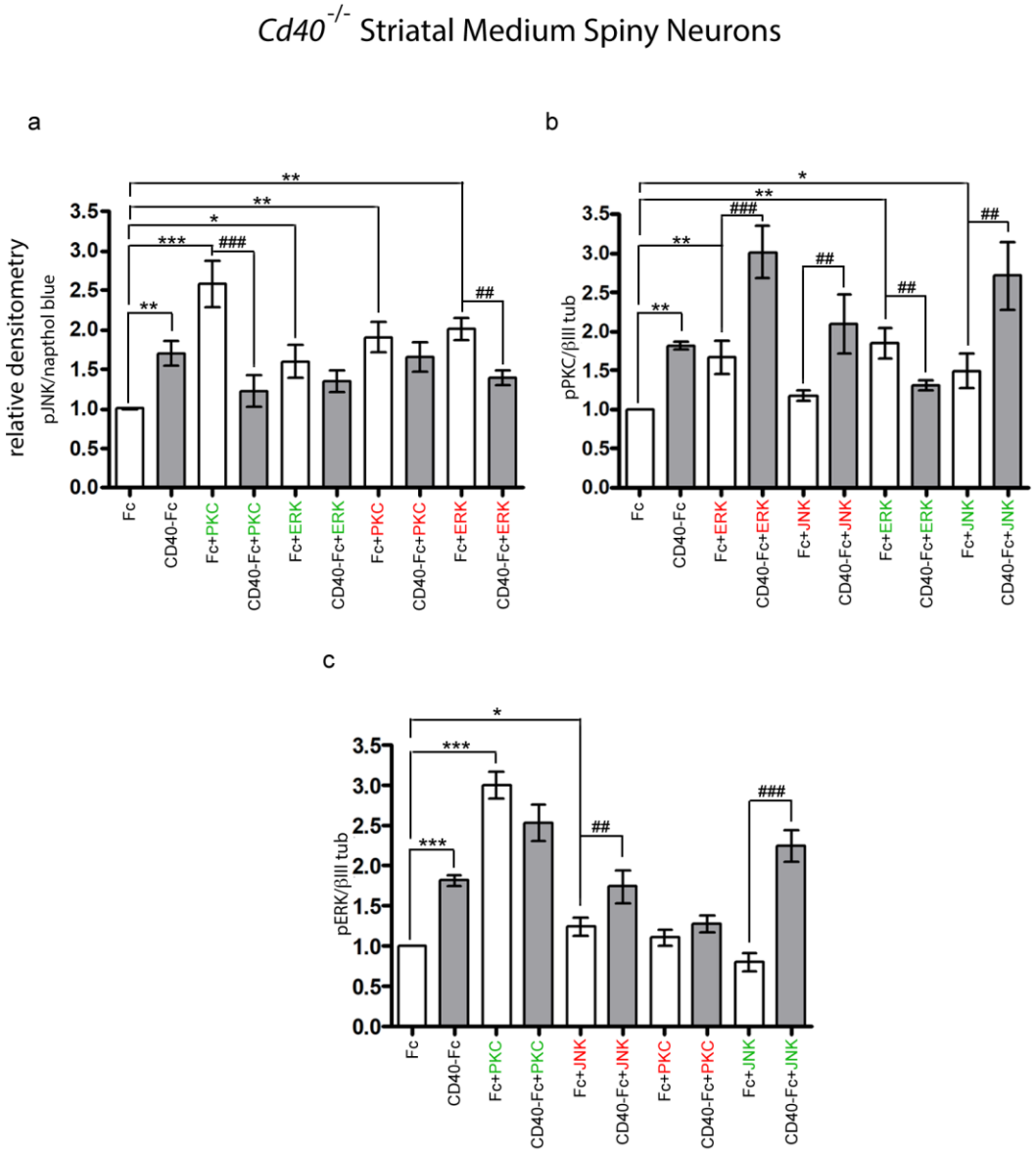

Figure S3. Basal effects on JNK, PKC and ERK1/ERK2 phosphorylation by pharmacological reagents. (a, b, c) Quantification of the basal effect of pharmacological reagents (compared with Fc, significances indicated with *) and in absence and presence of CD40-Fc (significances indicated with #). (a) Effect on the phospho-JNK, (b) phospho-PKC and (c) phospho-ERK1/phospho-ERK2. The concentrations were the same as those indicated in Figure 2. Quantification of at least three independent Western blots. The mean ± s.e.m are indicated (*** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$, one-way ANOVA with multiple Newman-Keuls statistical comparison).

Scheme 1. Protein-protein interactions (PPI) for mouse CD40L and PKCγ and in common between CD40L and PKCγ. The table lists all PPIs with reliable scores of 0.4 and greater for CD40L, PKCγ, and in common. The proteins highlighted indicate the most relevant proteins in common that might be involved in CD40-activated CD40L reverse signalling. These proteins include members of JNK and ERK and associated regulatory proteins like: Map3k1 that activates the ERK1/2 and JNK kinase pathways; Map3k5 that activates JNK but does not have any effect on the activation of MAPK/ERK1/2; and Lyn a protein that phosphorylates Syk and regulates the activation of the ERK1/ERK2 and JNK1/JNK2. .

Number of neurons counted per condition

Figure 2. and Supplemental Figure S1: Fc n=87; CD40-Fc n=93; Fc + PKC n=77; CD40-Fc + PKC n=73; Fc + PKC n=49; CD40-Fc + PKC n=48; Fc + JNK n=53; CD40-Fc + JNK n=53; Fc + JNK n=44; CD40-Fc + JNK n=54; Fc + ERK n=51; CD40-Fc + ERK n=59; Fc + ERK n=45; CD40-Fc + ERK n=56.

Figure 3. and Supplemental Figure S2: Fc n=87; CD40-Fc n=93; CD40-Fc + PKC n=73; CD40-Fc + PKC n=48; CD40-Fc + JNK n=53; CD40-Fc + JNK n=54; CD40-Fc + ERK n=59; CD40-Fc + ERK n=56; CD40-Fc + PKC + JNK n=37; CD40-Fc + PKC + ERK n=43; CD40-Fc + PKC + JNK n=37; CD40-Fc + PKC + ERK n=47; CD40-Fc + JNK + ERK n=43; CD40-Fc + JNK + ERK n=38; CD40-Fc + PKC + ERK n=43; CD40-Fc + PKC + JNK n=37; CD40-Fc + PKC + ERK n=40; CD40-Fc + PKC + JNK n=49; CD40-Fc + JNK + ERK n=54; CD40-Fc + JNK + ERK n=36; CD40-Fc + JNK + ERK n=54; CD40-Fc + PKC + JNK + ERK n=33; CD40-Fc + PKC + JNK + ERK n=34; CD40-Fc + PKC + JNK + ERK n=38; CD40-Fc + PKC + JNK + ERK n=39; CD40-Fc + PKC + JNK + ERK n=35; CD40-Fc + PKC + JNK + ERK n=38; CD40-Fc + PKC + JNK + ERK n=42; CD40-Fc + PKC + JNK + ERK n=78.

Rest of statistical comparisons

Figure 2. PKC: ***p<0.001 (Fc vs CD40-Fc + PKC) (PKC vs PKC) (PKC vs CD40-Fc + PKC) (PKC vs CD40-Fc) (PKC vs CD40-Fc + PKC) (CD40-Fc + PKC vs CD40-Fc + PKC). JNK: ***p<0.001 (Fc vs CD40-Fc + JNK) (Fc vs CD40-Fc + JNK) (JNK vs JNK) (JNK vs CD40-Fc + JNK) (JNK vs CD40-Fc) (JNK vs CD40-Fc + JNK) (CD40-Fc + JNK vs CD40-Fc + JNK); **p<0.01 (JNK vs CD40-Fc); *p<0.01 (CD40-Fc + JNK vs CD40-Fc). ERK: ***p<0.001 (ERK vs CD40-Fc) (CD40-Fc + ERK vs Fc) (CD40-Fc + ERK vs ERK) (CD40-Fc + ERK vs CD40-Fc + ERK); **p<0.01 (Fc vs CD40-Fc + ERK) (CD40-Fc vs ERK) .