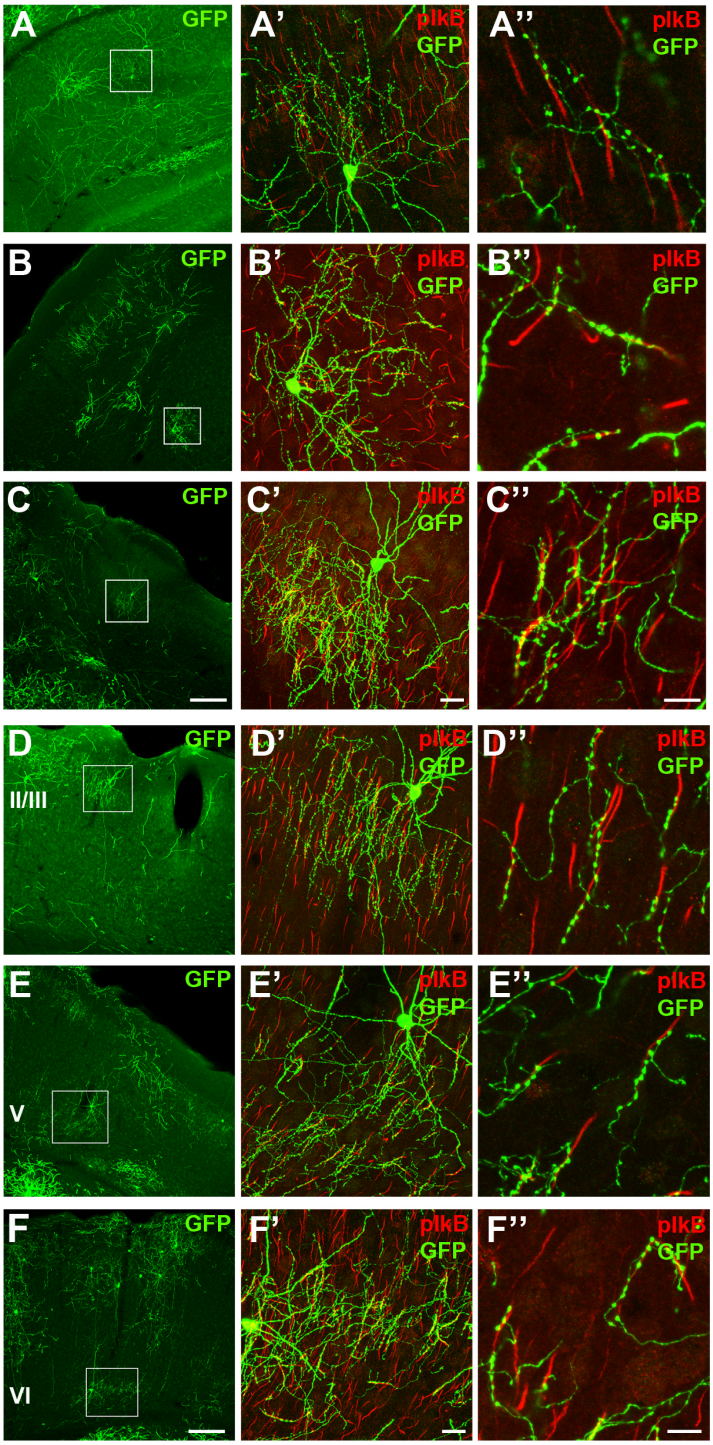

Figure S1. Fluorescent Labeling of ChCs in Different Brain Regions by *In Utero* Electroporation (related to Figure 1)

(A-A'') Representative images of ChC in the hippocampal CA1 area from a mouse electroporated at E12.5 with an EGFP-expressing plasmid and sacrificed at P28. Enlarged view (A') of ChC boxed in (A) with its cartridges (A'').

(B-B’’) Representative images of ChC in amygdala from a mouse electroporated at E13.5 with an EGFP-expressing plasmid and sacrificed at P28. Enlarged view (B’) of ChC boxed in (B) with its cartridges (B’’).
(C-C’’) Representative images of ChC in piriform cortex layer II from a mouse electroporated at E12.5 with an EGFP-expressing plasmid and sacrificed at P28. Enlarged view (C’) of ChC boxed in (C) with its cartridges (C’’).
(D-D’’) Representative images of ChC in layer II/III of somatosensory cortex from a mouse electroporated at E12.5 with an EGFP-expressing plasmid and sacrificed at P28. Enlarged view (D’) of ChC boxed in (D) with its cartridges (D’’).
(E-E’’) Representative images of ChC in layer V of somatosensory cortex from a mouse electroporated at E13.5 with an EGFP-expressing plasmid and sacrificed at P28. Enlarged view (E’) of ChC boxed in (E) with its cartridges (E’’).
(F-F’’) Representative images of ChC in layer VI of somatosensory cortex from a mouse electroporated at E13.5 with an EGFP-expressing plasmid and sacrificed at P28. Enlarged view (F’) of ChC boxed in (F) with its cartridges (F’’).
The AISs of postsynaptic target cells are visualized by immunostaining with an antibody to phospho-IkappaB (pIkB) (red). Scale bars, 200 μm (A-F), 20 μm (A’-F’) and 10 μm (A’’-F’’).

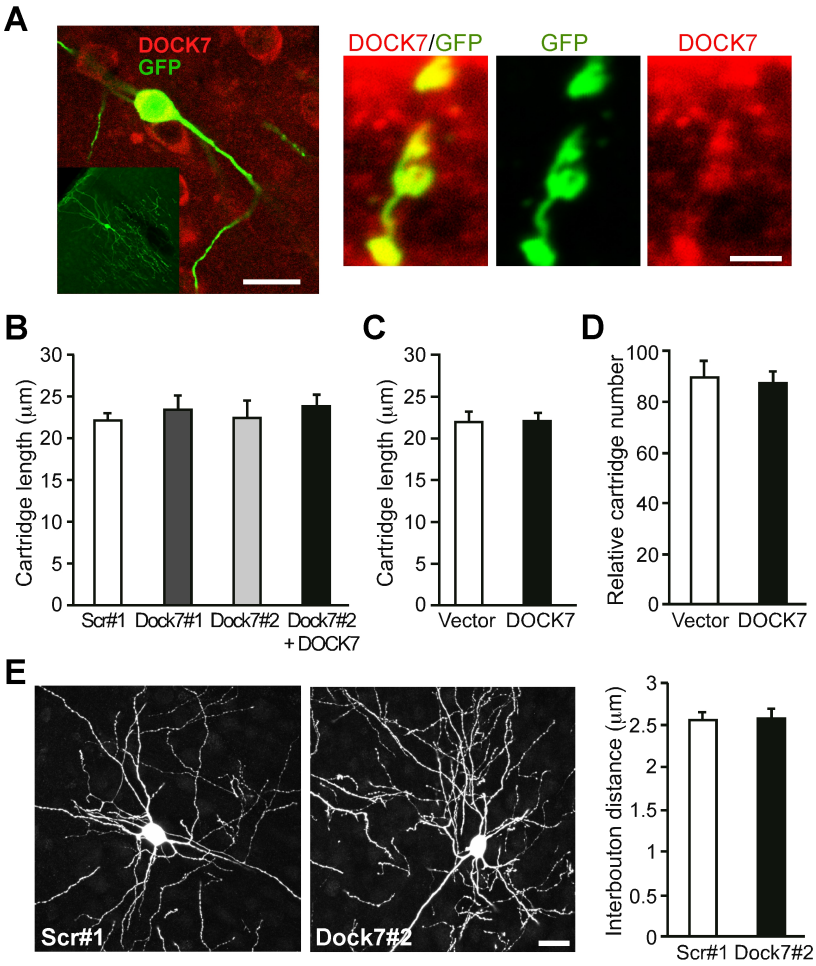

Figure S2. Analysis of DOCK7 Function in ChC Cartridge Development and Basket Cell Bouton Formation (related to Figure 2)

(A) DOCK7 expression in ChCs. Coronal brain sections from P28 mouse electroporated at E12.5 with an EGFP-expressing plasmid to label ChC (see insert, left) and immunolabeled with an antibody to DOCK7. Enlarged view of ChC cartridge with boutons (right panels). Single optical sections are shown for all images. Scale bars, 20 µm (left) and 2 µm (right).

(B-D) Altered DOCK7 expression does not affect ChC cartridge length and number. Quantification of cartridge length (B, C) and relative cartridge number (D) of ChCs in layer II/III of somatosensory cortex from mice co-electroporated at E12.5 with plasmids expressing EGFP and non-targeting shRNA (scr#1), Dock7 targeting shRNAs (Dock7#1, Dock7#2), or Dock7#2 shRNA + Flag-DOCK7 (DOCK7) (B), or with an empty control vector (vector) or Flag-DOCK7 expressing construct (DOCK7) (C, D), and sacrificed at P28. Data are means ± SEM. 7-12 ChCs from 3 animals were analyzed for each condition (B-D), and 6-14 cartridges were analyzed for each cell in B, C. $p = 0.203$ for Dock7#1, 0.801 for Dock7#2, and 0.121 for Dock7#2 + DOCK7, as compared with scr#1; one-way ANOVA, *post hoc* Tukey-Kramer test (B). $p = 0.920$ (C) and $p = 0.714$ (D) for DOCK7, as compared with vector; Student's *t*-test.

(E) DOCK7 knockdown does not affect bouton formation in basket cells. Left panel, representative images of basket cells in layer II/III of somatosensory cortex from mice co-

electroporated at E12.5 with plasmids expressing EGFP and non-targeting shRNA (scr#1) or Dock7 targeting shRNA (Dock7#2), and sacrificed at P28. Scale bar, 20 μm. Right panel, quantification of interbouton distances. Axonal branches of basket cells were randomly chosen within a 150 μm diameter circle centered on the cell body. Interbouton distance was determined by measuring the distance between neighboring boutons along an axonal branch using LSM software. Data are means ± SEM. 10 cells from 3 animals were analyzed for each condition, and 58-96 interbouton distances were determined per cell. p = 0,944, as compared to scr#1; Student's *t*-test.

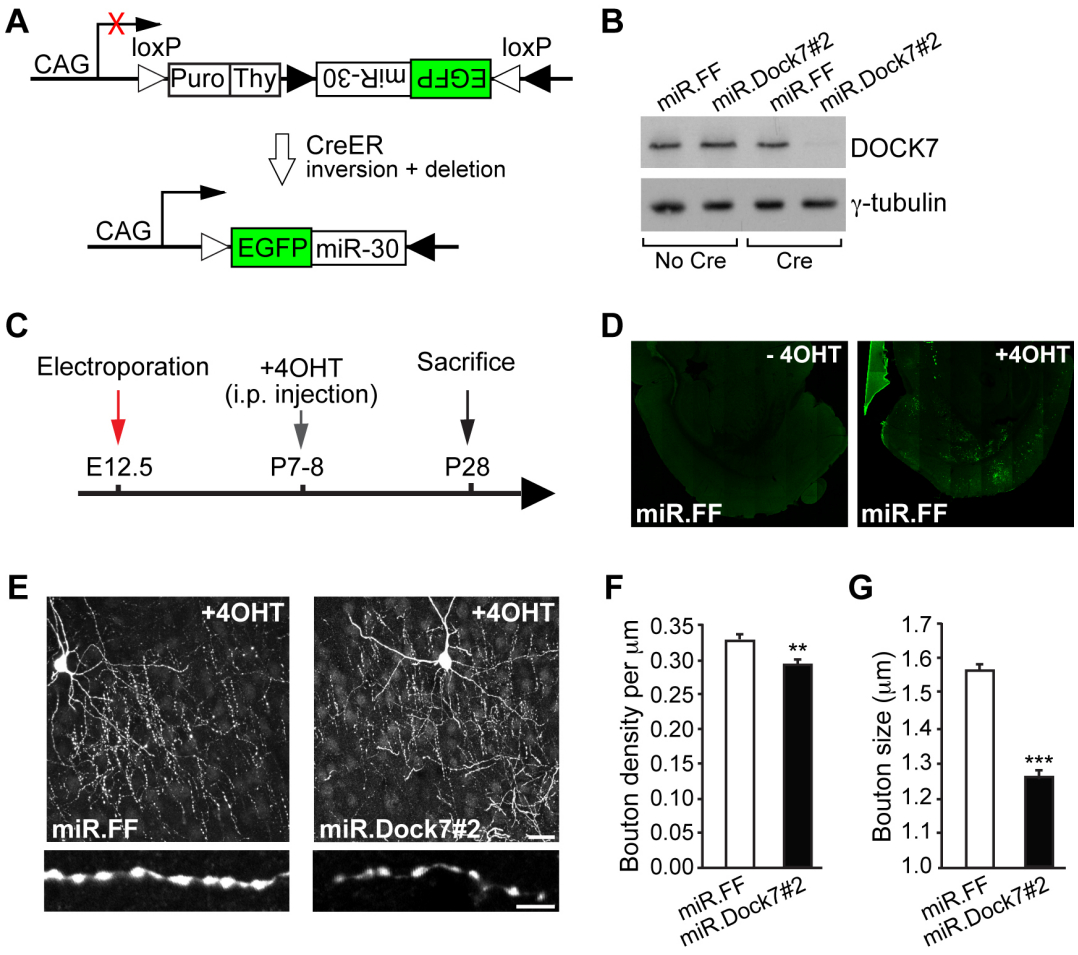

Figure S3. Conditional DOCK7 Knockdown in ChCs at Postnatal Stages Leads to a Decrease in Bouton Size and Density, But No Axonal Phenotype (related to Figure 2**)**

(A) Expression vector for Cre-regulated RNAi expression, based on previously described "FLIP vectors" (Stern et al., 2008). Schematic of a FLIP cassette comprising Puro-2A and Thy1.1 encoding sequences in tandem with the EGFP encoding gene and a miR-30-based shRNA, in the antisense orientation, downstream of the CAG promoter in pCAG-FLIP vector. The cassette is flanked by tandem LoxP sites arranged so that Cre expression inverts the EGFP-miRNA construct, allowing for its regulated expression, and at the same time deletes the Puro-2A-Thy1.1 encoding sequences.

(B) Neuro-2a cells were transfected with pCAG-FLIP vectors containing miR-30-based shRNAs targeting DOCK7 (miR.Dock7#2) or firefly luciferase (miR.FF), alone (no Cre), or together with pCAG-Cre (Cre) to induce inversion. 72 h post-transfection, cells were lysed and subjected to immunoblotting for DOCK7 and γ-tubulin as loading control.

(C) A scheme of the *in vivo* experiment. Embryos were electroporated at E12.5 with pCAG-FLIP-miR.Dock7#2 or pCAG-FLIP-miR.FF, together with pCAG-ER^{T2}CreER^{T2}, which expresses a tamoxifen-inducible double Cre fusion protein (Casanova et al., 2002; Matsuda and Cepko, 2007). The two plasmids were mixed at a ratio of 1:1 (final concentration, 1µg/µl). 4-hydroxytamoxifen *(*4OHT) (100 µg/g body weight) was delivered to pups by intraperitoneal injection at P7-8, and animals were sacrificed at P28.

(D) Representative example of brain slice images demonstrating that $ER^{T2}CreER^{T2}$ has no detectable recombination activity without 4OHT. Mice were co-electroporated with pCAG-FLIP-miR.FF and pCAG-$ER^{T2}CreER^{T2}$ at E12.5, gavaged (right panel) or not (left panel) with 4OHT at P7 and sacrificed at P28. Similar results were obtained when pCAG-FLIP-miR.Dock7#2 construct was co-expressed.
(E) Representative images of ChCs in layer II/III of somatosensory cortex from mice co-electroporated at E12.5 with indicated pCAG-FLIP vectors and pCAG-$ER^{T2}CreER^{T2}$, gavaged with 4OHT at P7-8, and sacrificed at P28. Enlarged view of ChC cartridges with boutons is depicted on the bottom. Scale bars, 20 μm (top) and 5 μm (bottom).
(F, G) Quantification of bouton density (F) and bouton size (G). Data are means ± SEM; 10 ChCs from 2 animals were analyzed for each condition, and for each cell 6-15 cartridges (F) and 98-132 boutons (G) were analyzed. $^{**}p < 0.01$ (F) and $^{***}p < 0.001$(G) (Student's *t*-test), as compared with control.

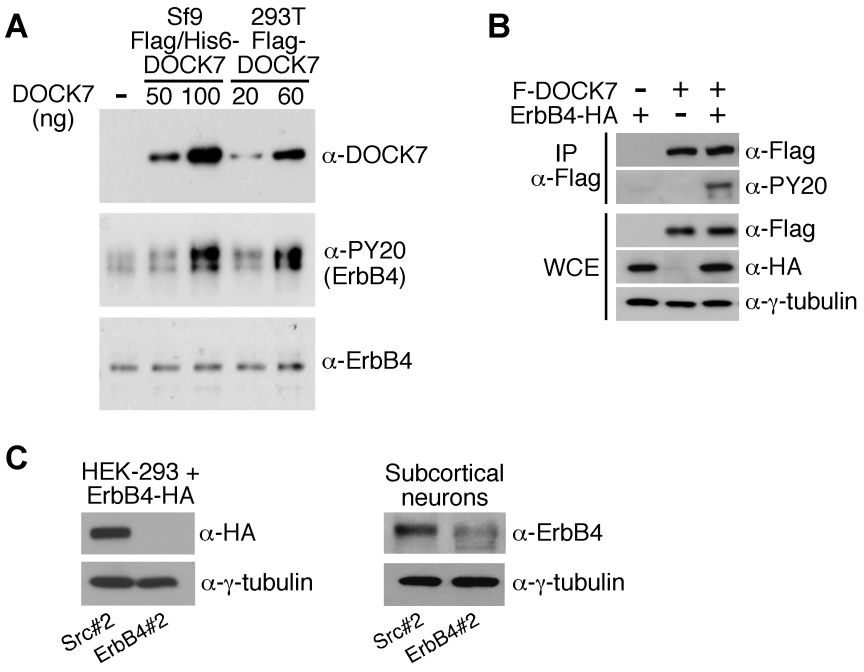

Figure S4. Biochemical Characterization of DOCK7-ErbB4 Interaction and Efficient ErbB4 Knockdown by RNAi (related to Figures 3 and 4)

(A) DOCK7 enhances ErbB4 phosphorylation in a cell free system. ErbB4-Myc (20 ng) was incubated with the indicated amounts of recombinant DOCK7 protein that was generated in insect Sf9 cells by recombinant baculovirus expressing Flag/His6-DOCK7 or obtained from HEK-293T cells expressing Flag-DOCK7 in the presence of ATP in a 15 µl reaction volume for 30 min at 30°C. The reaction was terminated by the addition of Laemmli buffer, and the proteins were resolved on SDS-PAGE and analyzed by Western blotting with antibodies to DOCK7, phosphotyrosine (PY20), and to ErbB4.

(B) ErbB4 elicits tyrosine phosphorylation of DOCK7. Extracts from HEK-293 cells transiently expressing Flag-DOCK7 (F-DOCK7) and/or ErbB4-HA were immunoprecipitated with anti-Flag antibody. Immunprecipitates (IP) and whole cell extracts (WCE) were analyzed by Western blotting with indicated antibodies. γ-tubulin was included as a loading control.

(C) Efficient knockdown of ErbB4 by RNAi. Extracts from HEK-293 cells co-transfected with plasmids expressing ErbB4-HA and non-targeting shRNA (scr#2) or ErbB4 targeting shRNA (ErbB4#2) were analyzed by Western blotting with antibodies to HA and to γ-tubulin as a loading control (left panel). Extracts from cultured subcortical neurons transfected with a plasmid expressing non-targeting shRNA (scr#2) or ErbB4 targeting shRNA (ErbB4#2) using the Amaxa Nucleofection system were analyzed by Western blotting with antibodies to ErbB4 and to γ-tubulin as a loading control (right panel).

Extended Experimental Procedures

DNA and RNAi Constructs

pCAGGS-Flag-DOCK7 and pCAGGS-Flag-DOCK7ΔDHR2 constructs have been described previously (Watabe-Uchida et al., 2006). pVL1392-Flag/His6-DOCK7 was obtained by cloning DOCK7 cDNA with N-terminal Flag/His6 epitope tags into the pVL1392 baculovirus transfer vector (BD Biosciences). pcDNA3.1-ErbB4-WT, -ErbB4-K751M, and -ErbB4-E836K encoding wild type, kinase-dead, and kinase-active forms, respectively, of ErbB4 JM-a/CYT-1 were obtained from AddGene (#29527, #29533 and #29534). ErbB4-WT, ErbB4-K751M, and ErbB4-E836K cDNAs were amplified by PCR and cloned into pCAGGS-IRES-GFP. ErbB4-WT and ErbB4-K751M cDNAs were also cloned into pCAGGS with the addition of a C-terminal HA epitope tag. cDNAs encoding the cytoplasmic domain of ErbB4 (amino acids 676-1308; ErbB4-C) or a C-terminal deletion mutant of ErbB4 lacking most of its intracellular domain (amino acids 1-724; ErbB4ΔC) were amplified by PCR and cloned into pCAGGS with the addition of a C-terminal HA epitope tag. For RNAi experiments, DNA fragments encoding short hairpin RNAs (shRNAs) directed against mouse *DOCK7* mRNA (Dock7#1: 5'-GCTAATCGGGATGCAAAGA-3'; Dock7#2: 5'-GGTACAGTACACATTTACA-3') (Watabe-Uchida et al., 2006), or against mouse *ErbB4* mRNA (ErbB4#2: 5'-CCAGACTACCTGCAGGAATAC-3') (Li et al., 2007), were cloned into pSUPER (Oligoengine), and in the case of Dock7#2 shRNA also into the pTRIPΔU3-EF1α-EGFP lentiviral vector (Janas et al., 2006; Kasri et al., 2008). For Cre-regulated RNAi, we used the previously described "FLIP-based" system (Stern et al., 2008). pCAG-FLIP-miR.FF was generated by cloning the FLIP cassette containing miR-30 based shRNA targeting Firefly luciferase (FF), which was amplified by PCR from MSCV-FLIP FF (AddGene #19744, provided by Richard O. Hynes), into the PacI and SalI sites of pCAGGS. pCAG-FLIP-miR.Dock7#2 was generated by excising the miR.FF encoding sequence from pCAG-FLIP-miR.FF with EcoRI and XhoI and replacing it with the miR.Dock7#2 encoding sequence. For the latter, an siRNA targeting *DOCK7* (5'-GCCTGCAGTATCTTTGACTTA-3') was incorporated into the miR-30 background (Chang et al., 2013). The pCAG-ER^{T2}CreER^{T2} vector (Casanova et al., 2002; Matsuda and Cepko, 2007) was obtained from AddGene (#13777). All constructs used in this study were sequence verified.

Cell Culture and Transfection/Infection

HEK-293, HEK-293T, and Neuro 2A cells were cultured in DMEM containing 10% fetal bovine serum (HyClone), 4 mM L-glutamine (Gibco BRL), 100 I.U./ml penicillin (Gibco BRL), and 100 μg/ml streptomycin (Gibco BRL). For the preparation of subcortical neuronal cultures, tissues were dissected from subcortical brain areas, including thalamus, hypothalamus and neostriatum of E15.5 CD1 mouse brains, and then incubated with trypsin solution for 6 min at 37ºC. After trypsin inactivation cells were mechanically dissociated and cultured in Neurobasal medium (Gibco BRL) containing 2% B27 and 0.5 mM glutamine. HEK-293 and HEK-293T cells were transfected using the calcium phosphate co-precipitation method. Neuro 2A cells were transfected using the Amaxa Nucleofection system (Amaxa, Lonza) (Yang et al., 2012). Cultured subcortical neurons

were transfected using the Amaxa Nucleofection system or were infected with lentiviruses, which were generated as previously described (Janas et al., 2006; Kasri et al., 2008).

Immunohistochemistry

Animals were deeply anesthetized with isoflurane and perfused transcardially with 4% paraformaldehyde. Brains were post-fixed and sectioned into 50-µm thick coronal sections using a Vibratome (Leica VT1000S). Sections were blocked and permeabilized with 10% normal goat serum (NGS) and 0.3% Triton X-100 in PBS at RT for 1 h, and then incubated with the appropriate primary antibodies diluted in 3% NGS and 0.3% Triton X-100 in PBS overnight at 4 °C. Sections were subsequently incubated with the appropriate fluorescently-conjugated secondary antibodies diluted in the same buffer for 1 h at RT. The following primary antibodies were used: anti-GFP (chicken, 1:1000, Aves Labs); anti-pIkB (rabbit, 1:1000, Cell Signaling); anti-DOCK7 (rabbit, 1:500) (Watabe-Uchida et al., 2006). Secondary antibodies used were: Alexa Fluor 488 goat anti-chicken (1:1000, Invitrogen), Alexa Fluor 594 goat anti-rabbit (1:1000, Invitrogen), and Alexa Fluor 594 goat anti-mouse (1:1000, Invitrogen).

Co-immunoprecipitation and Western Blotting Analysis

For co-immunoprecipitation experiments, HEK-293 or Neuro 2A cells expressing wild type (WT) or mutant versions of ErbB4-HA or ErbB4 and/or Flag-DOCK7 were lysed in ice-cold lysis buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 50 mM NaF, 40 mM β-glycerophosphate, 2 mM Na_3VO_4, and complete protease inhibitors (Roche). Cell extracts were subjected to immunoprecipitation with anti-Flag M2-agarose beads (Sigma), or with polyclonal anti-ErbB4 antibody (GeneTex) followed by capture of immunocomplexes using Protein A Agarose (Roche). Beads were washed 6 times with ice-cold lysis buffer. Immunoprecipitates were eluted with Laemmli buffer, resolved by SDS-PAGE (using 8 or 10% acrylamide gels), transferred to an Immobilon PVDF membrane (Millipore), and analyzed by immunoblotting with the indicated antibodies. The following antibodies were used: monoclonal anti-Flag (M2, Sigma), polyclonal anti-Flag (Sigma), monoclonal anti-HA HRP conjugate (Roche), polyclonal anti-ErbB4 (C1C3, GeneTex), monoclonal anti-ErbB4 (HFR1; Thermo Scientific), monoclonal anti-phosphotyrosine HRP conjugate (PY20; BD Transduction Laboratories).

For the analysis of ErbB4 autophosphorylation, cells were either left unstimulated or stimulated with neuregulin-1 (NRG1, R&D Systems) (50 ng/ml) for 5 min. Subsequently, cells were washed with PBS and lysed in lysis buffer. Cell extracts were resolved by SDS-PAGE (using 8 or 10% acrylamide gels), transferred to an Immobilon PVDF membrane (Millipore), and analyzed by immunoblotting using the following antibodies: polyclonal anti-phospho-ErbB4 (pTyr1284) (Cell Signaling), polyclonal anti-ErbB4 (C1C3, GeneTex), polyclonal anti-Flag (Sigma) or polyclonal anti-DOCK7 (Watabe-Uchida et al., 2006), and monoclonal anti-γ-tubulin (Sigma).

Purified/Recombinant DOCK7 Protein

Two strategies were used to produce recombinant DOCK7 protein. In the first one, Flag-tagged DOCK7 protein was expressed and purified from transiently transfected HEK-293T cells, largely as described previously (Yamauchi et al., 2008). In brief, cells were washed

with ice-cold PBS and lysed in lysis buffer (LB) containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 10% glycerol and complete protease inhibitors (Roche). Lysates were cleared by centrifugation and incubated with anti-Flag M2-agarose beads (Sigma) for 8 h at 4°C. The beads were then washed three times with LB modified to contain 250 mM NaCl, 0.1% Triton X-100, 5% glycerol, followed by three washes with LB modified to contain no Triton X-100. Anti-Flag immunoprecipitates were eluted in buffer containing 50 mM Tris-HCl, pH 7.5, 250 mM NaCl, 5% glycerol and supplemented with Flag peptide (0.2 mg/ml), and then dialyzed at 4°C in dialysis buffer containing 50 mM Tris-HCl, pH 7.5, 250 mM NaCl, 20% glycerol, 1 mM DTT and protease inhibitors. As a second strategy, recombinant DOCK7 protein containing N-terminal Flag/His6 epitope tags was produced in Sf9 insect cells using the BD BaculoGold^{TM} expression system following the manufacturer protocol. The cells were lysed in LB modified to contain 40 mM imidazole and lysates were incubated with Ni-nitrolotracetic acid (NTA) resin (Qiagen) for 8 h at 4°C. Resin was then extensively washed with wash buffer containing 50 mM Tris-HCl, pH 7.5, 250 mM NaCl, 0.1% Triton X-100, 5% glycerol, 40 mM imidazole and protease inhibitors, followed by three washes with wash buffer containing no Triton X-100. Bound proteins were eluted in wash buffer supplemented with 300 mM imidazole, and then dialyzed as described above.

In Vitro ErbB4 Phosphorylation Assay

An equal amount (20 ng) of Myc-tagged recombinant ErbB4 protein (Origene) was incubated with 12.5 to 100 ng of Flag/His6-tagged DOCK7 or Flag-tagged DOCK7 recombinant protein in buffer containing 50 mM HEPES-NaOH, pH 7.5, 250 mM NaCl, 10 mM $MgCl_2$, 5 mM $MnCl_2$, 1 mM DTT, 5 μM Na_3VO_4, 100 μg/μl BSA, 30 μM ATP and complete protease inhibitors, in a 15 μl total reaction volume, at 30°C for 30 min. The reactions were stopped by the addition of Laemmli buffer. The proteins were resolved by SDS/PAGE and analyzed by immunoblotting with monoclonal anti-phosphotyrosine antibody (PY20, BD Transduction Laboratories), polyclonal anti-ErbB4 antibody (C1C3 GeneTex) and polyclonal anti-DOCK7 antibody (Watabe-Uchida et al., 2006).

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