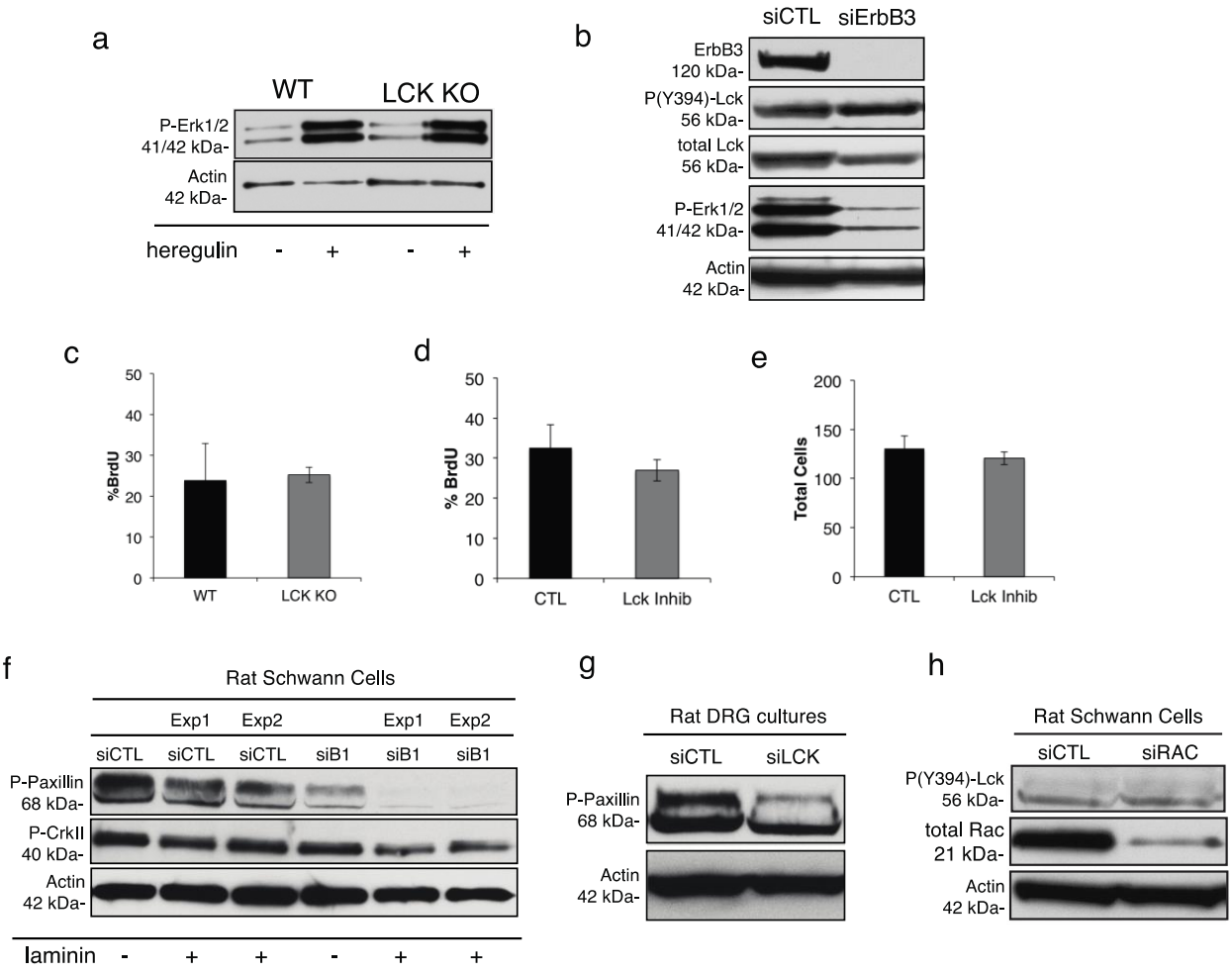
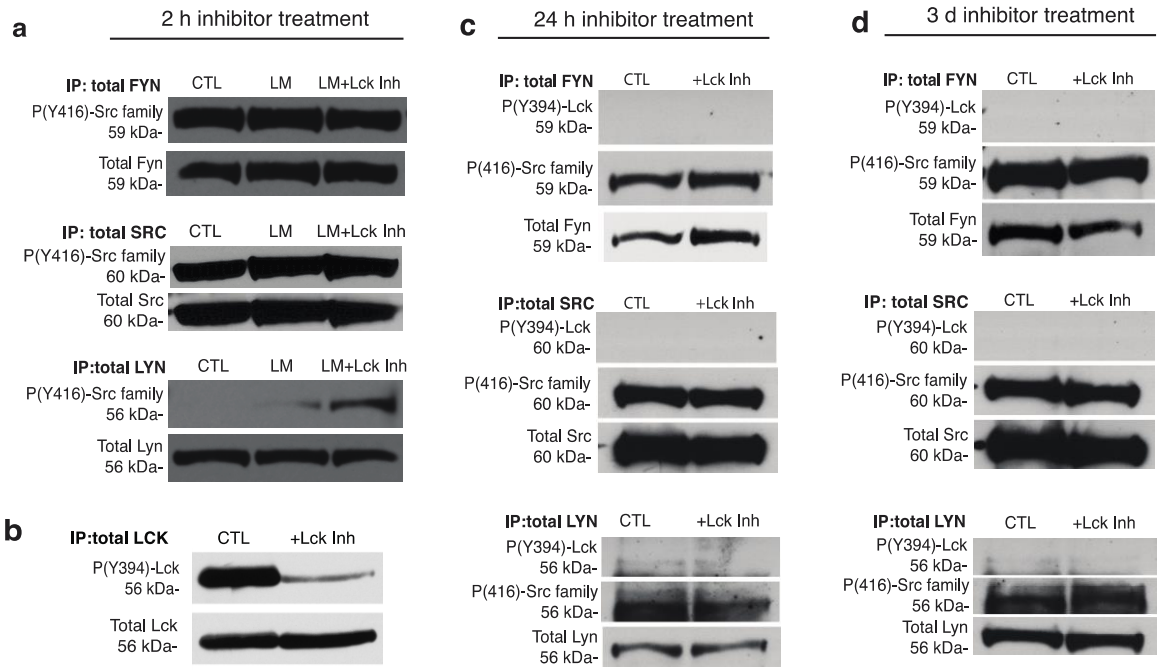
Supplementary Figure S1



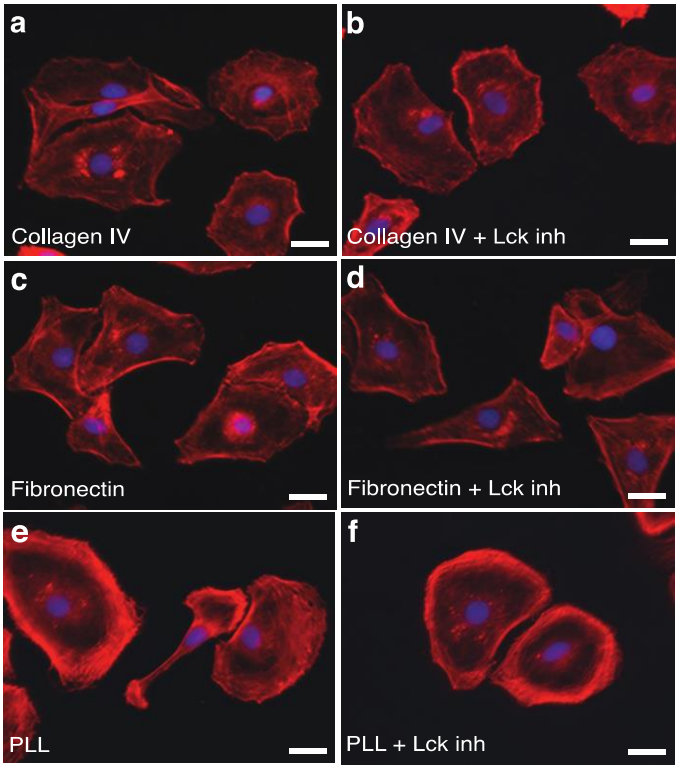
Supplementary Figure S1. Loss of Lck activity has no effect on Schwann cell proliferation rates and the Lck signaling pathway is specific to beta1-integrin, and is not affected by ErbB3 or Rac downregulation. (**a**) Enriched Schwann cells isolated from mouse E18 DRG explants were seeded on laminin-coated dishes overnight. Beta1-heregulin (10 ug/ml) was added to serum-starved Schwann cells for 10 m and lysates were taken. Erk1/2 phosphorylation was equally induced in WT and Lck-/- Schwann cells. (**b**) ErbB3 siRNA and Lck plasmid were co-transfected in RSCs, then the cells were treated with beta1-heregulin for 10 m. Lck phosphorylation was not reduced by ErbB3 downregulation, while Erk1/2 phosphorylation was decreased. (**c**) Mouse Schwann cells were seeded overnight then BrdU was added to the media for 1 h and cells were then fixed and processed for BrdU inmmunocytochemistry. No difference in proliferation rate was seen in WT versus LCK-/- mouse Schwann cells. (**d-e**) RSCs were seeded on poly-l-lysine overnight then treated with Lck inhibitor for 24 h. BrdU was added to the media for 1 h then cells were fixed and immunostained for BrdU. The percentage of BrdU cells was calculated and Lck inhibitor treatment did not significantly affect proliferation rate (**d**). Total number of RSCs did not change with 24 h treatment with Lck inhibitor (**e**) and no cell death was identified by an activated Caspase-3/9 assay (data not shown). (**f**) Phosphorylation of paxillin and CrkII was reduced following down regulation using a β1 integrin specific siRNA but not with a control non-targeting siRNA as in Fig.1. (**g**) Down-regulation of Lck by siRNA results in decreased paxillin phosphorylation in a 3d RSC-DRG co-culture. (**h**) Lck phosphorylation is not affected by down regulation with a Rac specific siRNA, indicating that Lck activation is upstream of Rac activity.

Supplementary Figure S2



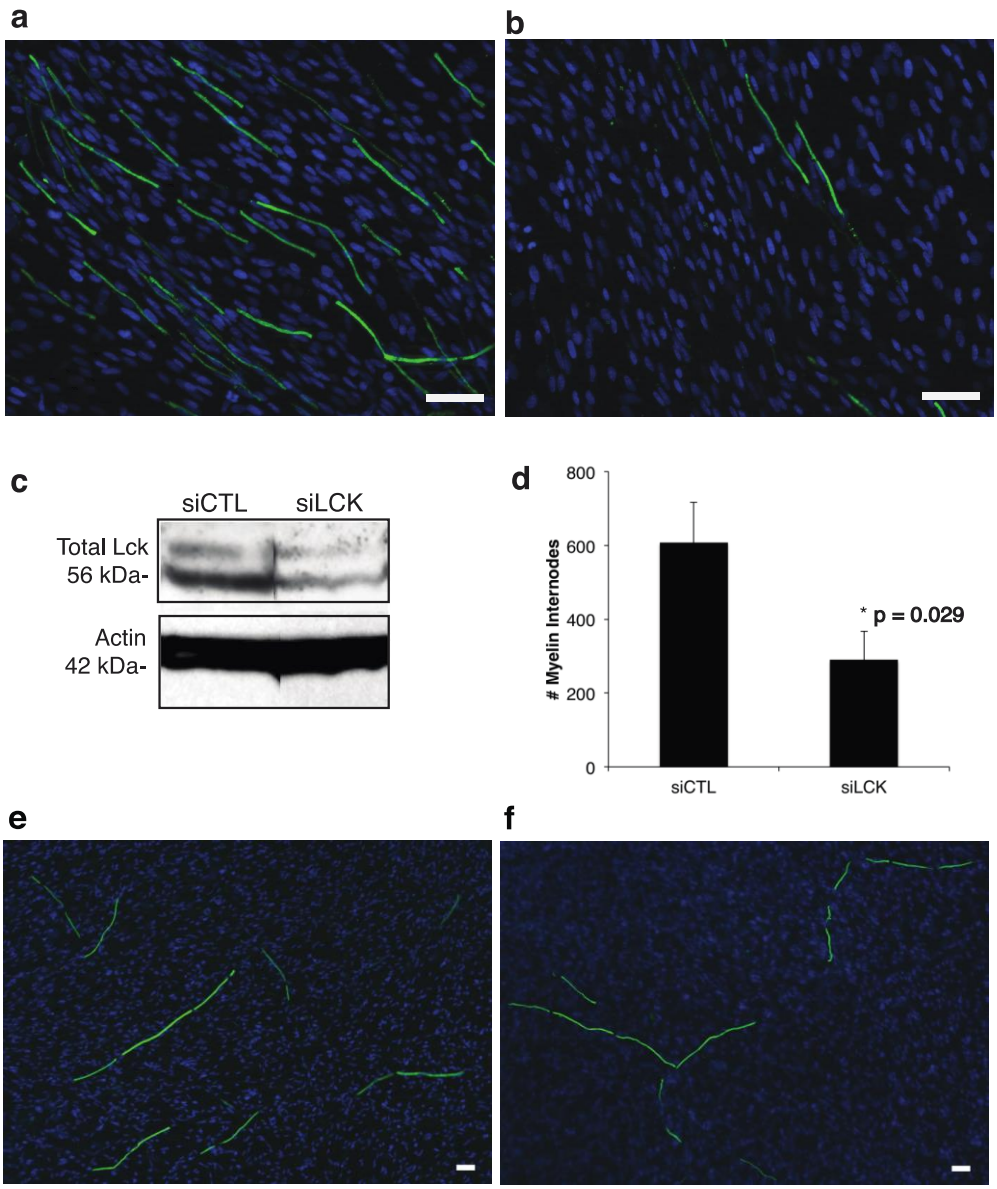
Supplementary Figure S2. Specificity of phospho(Y394)-Lck antibody and Lck inhibitor A770041. (**a**) RSCs seeded on 100mm dishes were serum-starved and pretreated for two hours with Lck inhibitor prior to 10 min treatment with 15 ug/ml laminin. RIPA lysates from three dishes were combined and immunoprecipitated with antibodies (Cell Signaling) to Fyn, Src, or Lyn. Immunoprecipitates were probed for activated Src family (P-Y416) antibody that recognizes the active site of most Src family members, then blots were stripped and reprobed for their respective antibody used for the initial immunoprecipitation. No difference in phosphorylation state was seen for Src or Fyn proteins, but Lyn active site phosphorylation was upregulated following laminin treatment, which was not reduced by Lck inhibitor treatment. (**b**) RIPA lysates of RSCs overexpressing Lck and treated with DMSO (CTL) or Lck inhibitor for 2 hours were immunoprecipitated with antibodies to Lck (3A5, Millipore) and probed for phospho(Y394)-Lck and total Lck. P(Y394)-Lck specifically recognizes the active site of Lck (see below) and identifies the specific inhibition of Lck active site phosphorylation by Lck inhibitor (see below). (**c, d**) RSCs were seeded and treated with DMSO (CTL) or Lck inhibitor for 24 h (**c**) or 3 days (**d**). Lysates were immunoprecipitated as in (**a**), and probed for antibodies to phospho(Y394)-Lck, phospho(416)-Src family, and the respective immunoprecipitation antibody. P(Y394)-Lck antibody did not identify phosphorylated Fyn, Src, or Lyn, but is specific for endogenous and overexpressed Lck phosphorylation (see S1b, Fig. 1a-1b). Treatment with Lck inhibitor for 2 h, 24 h, or 3 days does not reduce active site phosphorylation of Fyn, Src, or Lyn.

Supplementary Figure S3



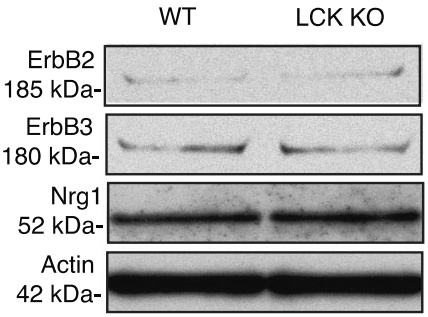
Supplementary Figure S3. Lamellipodia formation on substrates that are not affected by Lck inhibition. RSCs were seeded on a collagen IV (**a-b**), fibronectin (**c-d**), or poly-l-lysine (**e-f**) for 1.5 h then treated with DMSO (**a,c,e**) or 500 nM Lck inhibitor (**b, d, f**) for 2 h. Cell morphology on these substrates was unchanged following Lck inhibition.

Supplementary Figure S4



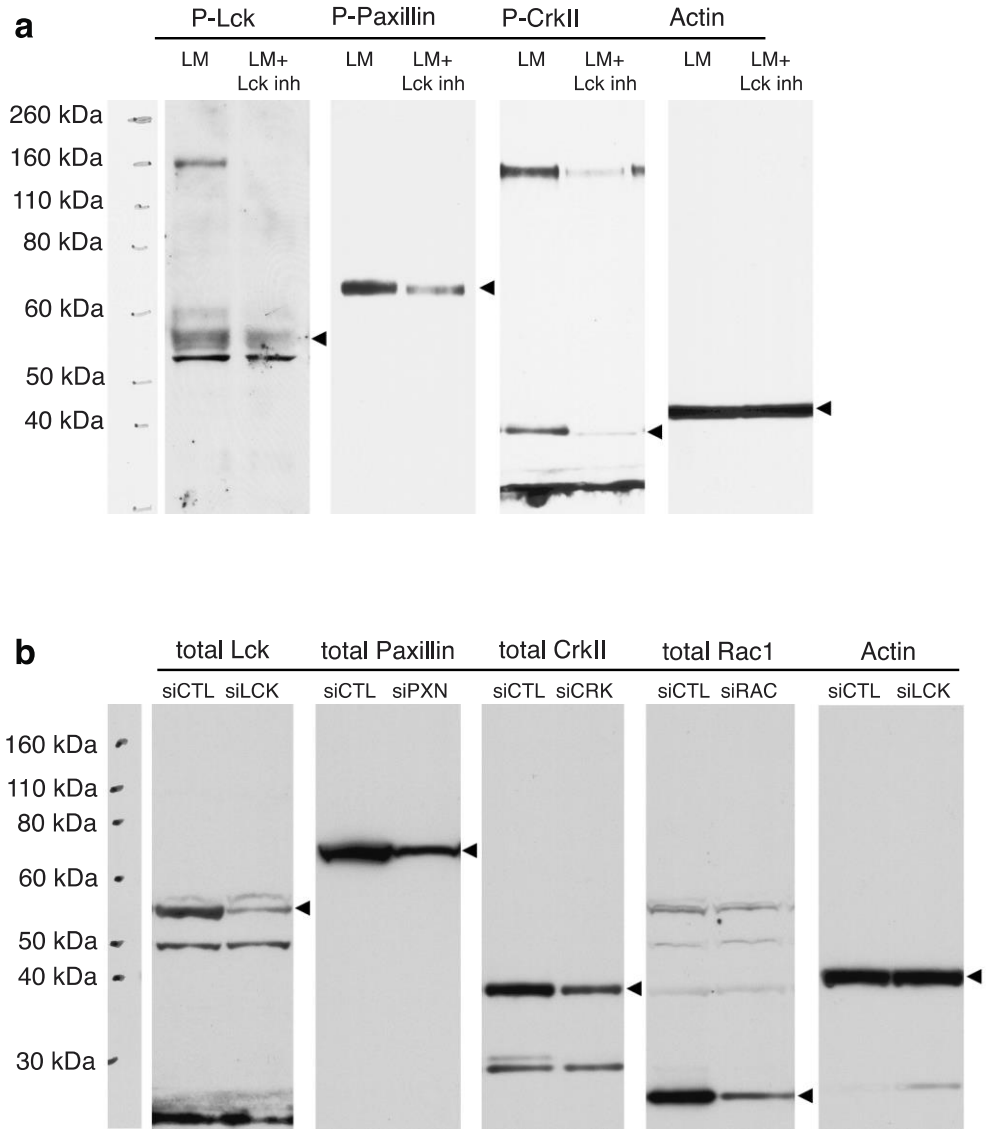
Supplementary Figure S4. Down-regulation of Lck kinase with siRNA inhibits myelination in Schwann cell-DRG co-cultures, while loss of Lck kinase in axons has no effect on myelination. RSCs were transfected with negative control siRNA (**a**) or Lck siRNA (**b**) and plated on DRG cultures after 4 d. Cultures were fixed and immunostained after 13 d of ascorbate treatment. (**c**) Lck protein levels are down 4 d after transfection. (**d**) Total myelin internodes were significantly reduced in Lck knockdown cultures ($p<0.029$, n=9 from two independent experiments, error bars represent +/- s.e.m.). (**e, f**) DRG axons from WT (**e**) or Lck-/- (**f**) mice were seeded with WT mouse Schwann cells and treated with ascorbate for 16 d then immunostained for MBP. Myelination by seeded WT Schwann cells occured on both WT and Lck-/- axons. Scale bars represent 50 microns.

Supplementary Figure S5



Supplementary Figure S5. Protein expression of Lck KO sciatic nerves at postnatal day 5. Sciatic nerves from 5 d WT and Lck KO mice were isolated and lysates were analyzed for ErbB2, ErbB3, Nrg1 protein expression with actin as a loading control. No change in expression of the neuregulin pathway proteins was seen with lack of Lck expression.

Supplementary Figure S6



Supplementary Figure S6. Representative full-length Western blot images. **(a)** Full-length Western blot images are shown for Fig. 2a. Arrowheads identify specific bands. **(b)** Full-length Western blot images are shown for Fig. 4d. Arrowheads identify specific bands. Actin Western blots showed equal protein loading for all lanes.

Supplementary Methods

Mouse DRG explants and migration studies.

Mouse DRG explants were isolated from E13.5 or E14.5 mouse embryos, placed onto thick Collagen-I coated Aclar discs, and overlaid in defined N2+B27+NGF medium. The following day recombinant human heregulin-β1 was added. On day 4 the DRG explants were changed to C (10% serum) medium for 2 days, then to N2+B27+NGF from day 6 to day 11, and back to C medium for the duration. Treatment with Lck inhibitor (500nM) was started in some cultures on day 4 and continued every other day with medium change until time of harvest. For myelination studies, ascorbate was added to the media. At appropriate time points, cultures were fixed with 3% paraformaldehyde for 10 minutes and processed for indirect immunofluorescence staining.

Mouse neuron-Schwann cell co-cultures

Mouse DRG explants were isolated from E13.5 or E14.5 embryos and placed onto thick collagen-1 coated Aclar discs. To produce enriched DRG axons, cultures were maintained in serum-free N2+B27+NGF medium to restrict the proliferation and migration of Schwann cells out of the explant while promoting neuronal survival and axonal outgrowth. Enriched mouse Schwann cells were isolated from DRG explants maintained in N2 with heregulin for 15 d, after which the ganglia was removed and remaining Schwann cells were detached from the dish with trypsin then seeded on axons. Co-cultures were then maintained in serum-containing media with ascorbate to induce myelination. Isolated mouse Schwann cells were also used as monocultures in some experiments.

Bimolecular fluorescence complementation constructs

Fluorescent fusion proteins were produced using ITGB1 (GeneCopeia) and Lck (Origene) after amplification by PCR and subcloning into YFP(1-158)pcDNA1/Amp and YFP(159-238)pcDNA1/Amp (a generous gift from C. H. Berlot PhD). The PCR for ITGB1 introduced a HindIII site and linker sequence at the 5' end and a BamHI site at the 3' end. An internal HindIII site in the ITGB1 cDNA was removed using the QuikChange site-directed mutagenesis kit (Stratagene). The PCR product was digested with HindIII and BamHI and subcloned into the multiple cloning site on YFP(159-238)pcDNA1/Amp such that the YFP fragment was fused to the C-terminus of ITGB1. The PCR for Lck introduced a XhoI site at the 5' end and a linker sequence with a XhoI site at the 3' end. The PCR product was digested with XhoI and subcloned into YFP(1-158)pcDNA1/Amp such that the YFP fragment was fused with the N-terminus of Lck.

Chemiluminescence ligand binding assay

Increasing concentrations of recombinant human paxillin or human beta1-integrin were passively adsorbed onto high-bind plates overnight at 4^0C (Meso Scale Discovery). The wells were blocked in 5%BSA (one hour, room temperature) and washed three times in TBS buffer. Ligand binding was initiated by the addition of saturating (6.25 μM) concentrations of his-tagged recombinant active Lck for two h, then wells were washed and Lck binding was detected by consecutive addition of anti-penta-his antibody (Qiagen) and anti-mouse Sulfo-Tag antibody (Meso Scale Discovery). Wells were washed and reading buffer was added (Meso Scale Discovery). Saturation binding data were collected as duplicates using a SECTOR Imager 2400A (Meso Scale Discovery)

and analyzed by nonlinear curve fitting using GraphPad Prism 6.0 software to determine K_D values.