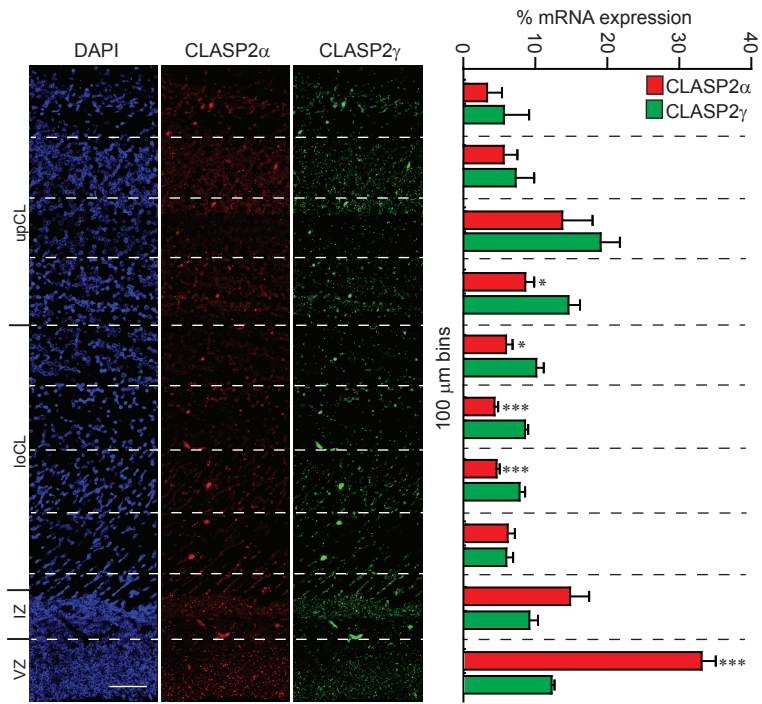
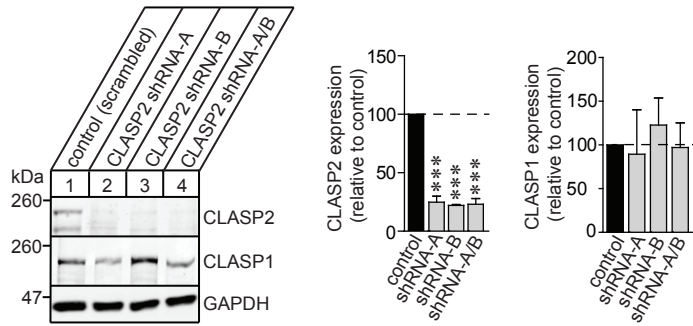


# Suppl. Figure 1

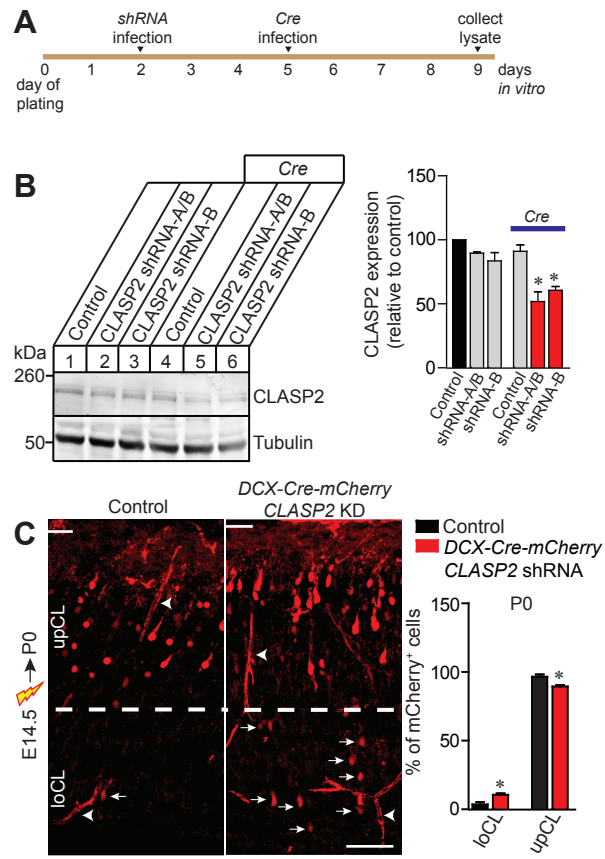


## Suppl Figure 2

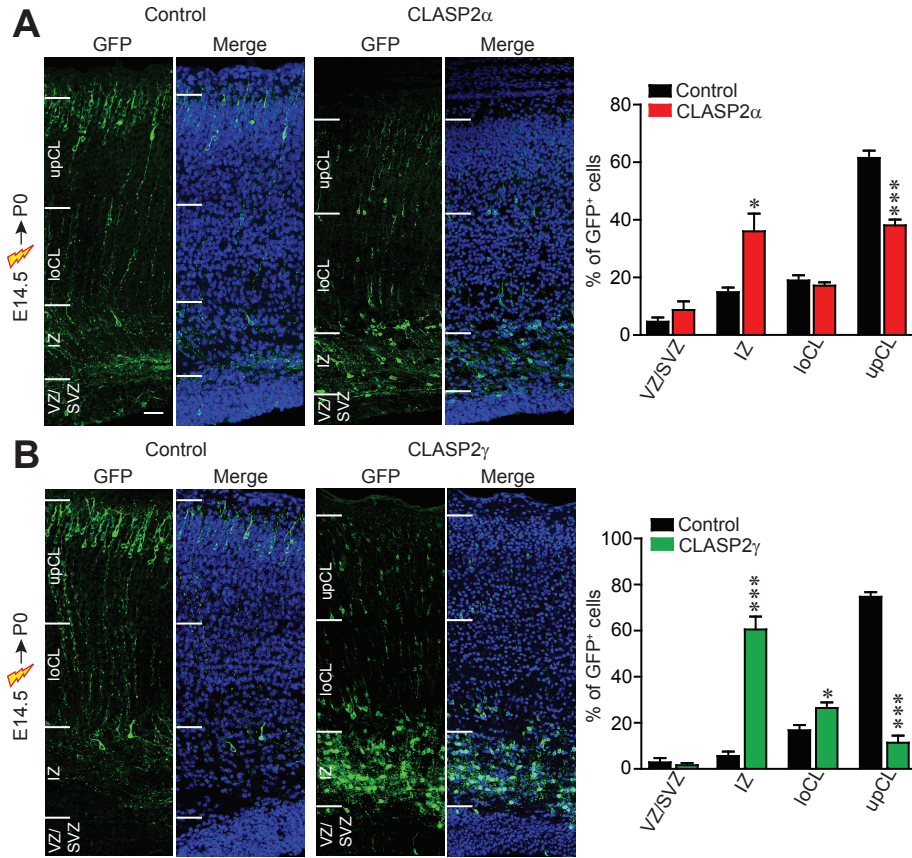




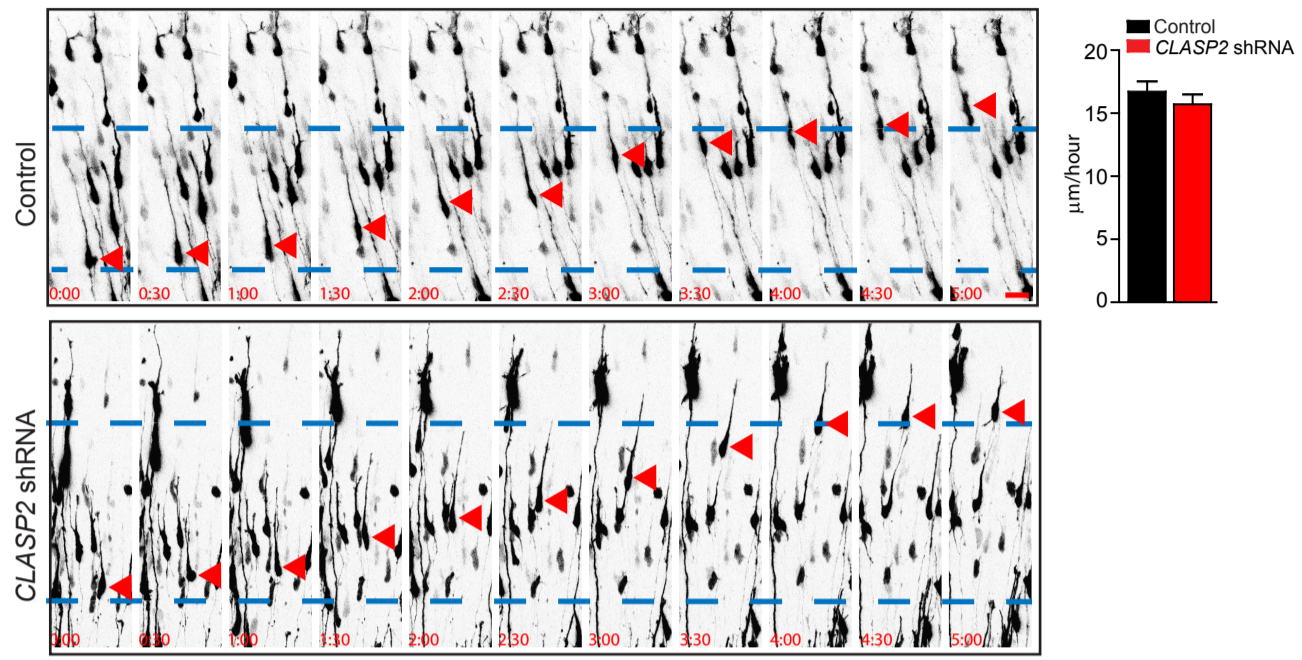
# Suppl Figure 3



# Suppl. Figure 4



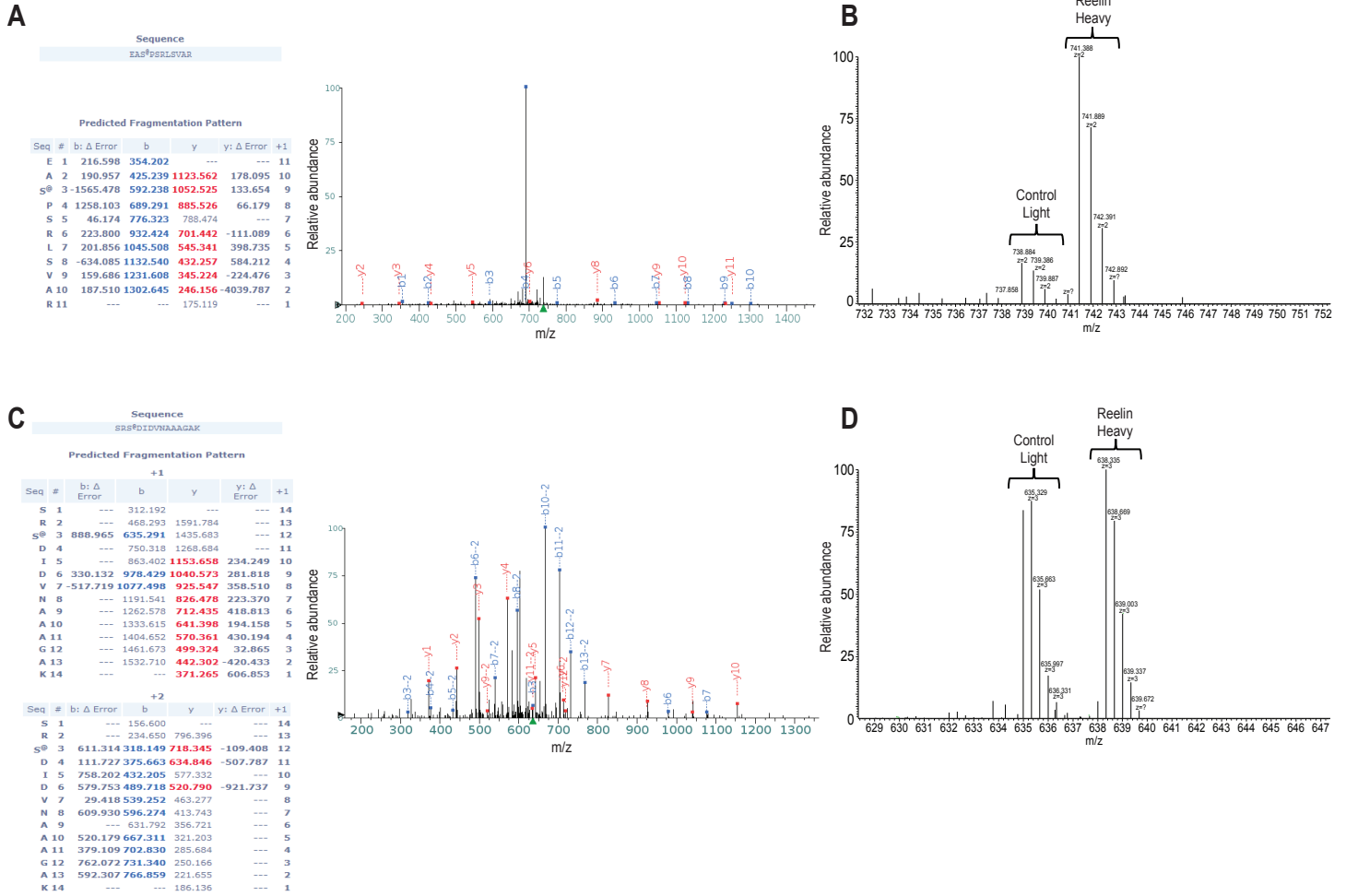
Suppl. Figure 5



## Suppl Figure 6

Amino acids mutated in human CLASP2 $\alpha$	Equivalent amino acid sites in mouse CLASP2 $\gamma$	Phosphosites regulated by Reelin in M/S
568	505	
572	509	
576	513	513
594	531	
598	535	
602	539	
606	543	543
610	547	
614	551	551

# Suppl. Figure 7



## SUPPLEMENTAL INFORMATION

Supplemental information includes seven figures and two movies.

**Figure S1. CLASP2 $\alpha$  and CLASP2 $\gamma$  mRNA Expression in the Mouse Cerebral Cortex at Postnatal Day 0.** Representative images showed *in situ* hybridization of cortical sections for CLASP2 $\alpha$  and CLASP2 $\gamma$  at postnatal day 0. Fluorescent expression were quantitated every 100  $\mu\text{m}$  bins from the ventricle to the pial surface regions (VZ = ventricular zone, IZ = intermediate zone, loCL = lower cortical layer, upCL = upper cortical layer). CLASP2 $\alpha$  expression was the highest in the first 100  $\mu\text{m}$  which represents the proliferative ventricular zones (n = 6 brain sections for each group). Data are means  $\pm$  SEM and statistical significance was assessed using one-way ANOVA (\*p < 0.05, \*\*\*p < 0.0001). Scale bar represents 50  $\mu\text{m}$ .

**Figure S2. Efficiency of Individual CLASP2 shRNAs in Knocking Down Expression of Endogenous CLASP2 in Primary Mouse Neurons.** Lentiviral infection of primary cortical neurons of individual (shRNA-A, shRNA-B) and combinations (shRNA-A/B) of CLASP2 shRNAs efficiently downregulated CLASP2 but not CLASP1 protein expression (n = 2 independent experiments).

**Figure S3. Neuron-Specific Knockdown of CLASP2 Affects Neuronal Migration.**

(A) Time line of lentiviral infection of conditional pSico-CLASP2 shRNA and *cre* recombinase to test the levels of CLASP2 knockdown in primary cortical neurons.

(B) Lysates of primary cortical neurons infected with individual conditional pSico-shRNA-B and combinations (shRNA-A/B) of CLASP2 shRNAs efficiently downregulated CLASP2 protein expression in *cre*-dependent manner.

(C) Mouse embryonic brains were electroporated *in utero* with pSico-*CLASP2* shRNA-A/B or scrambled control and *DCX-cre-mCherry* at E14.5 and analyzed at P0 (control, n = 3 brains; *CLASP2* shRNA, n = 3 brains). Coronal sections of the cortex were visualized for transfected mCherry-positive neurons (red). Solid and dash white lines indicate the demarcations for pial surface and lower and upper cortical regions (loCL = lower cortical layer, upCL = upper cortical layer), respectively. Arrows indicates the number of mCherry positive neurons in the loCL. Arrowheads indicate blood vessels.

Data are means  $\pm$  SEM and statistical significance was assessed using one-way ANOVA (\*p < 0.05). Scale bar represents 50  $\mu$ m.

#### **Figure S4. CLASP2 Overexpression Alters Neuronal Migration.**

(A-B) Mouse embryos were electroporated *in utero* with control GFP, GFP-*CLASP2 $\alpha$*  or GFP-*CLASP2 $\gamma$*  full-length expression vectors at E14.5 and analyzed at P0 (control, n = 4 brains; *CLASP2 $\alpha$* , n = 4 brains and *CLASP2 $\gamma$* , n = 4 brains). Coronal sections of the cortex were visualized for transfected GFP-positive neurons (green) and cell nuclei (Hoeschst 33342, blue). White lines indicate the demarcations for different cortical regions (VZ = ventricular zone, SVZ = subventricular zone, IZ = intermediate zone, loCL = lower cortical layer, upCL = upper cortical layer).

Data are means  $\pm$  SEM and statistical significance was assessed using one-way ANOVA (\*p < 0.05, \*\*\*p < 0.0001). Scale bar represents 50  $\mu$ m.

#### **Figure S5. CLASP2 Knockdown Does Not Alter the Rate of Neuronal Migration.**

Mouse embryos were electroporated *in utero* with GFP-tagged *CLASP2* shRNAs or scrambled control at E14.5 and brain slices were analyzed at E17.5. Time-lapse imaging of migrating neurons expressing GFP scrambled control or GFP-*CLASP2* shRNA (arrowhead) were captured every 30 seconds (see Supplementary Movie 1 and 2). Average rates of migration showed *CLASP2* knockdown did not alter the rate of neuronal migration (control, n = 82 neurons from 4 brain slices; *CLASP2* shRNA, n = 71 neurons from 3 brain slices).

Data are means  $\pm$  SEM and statistical significance was assessed using unpaired *t* test. Scale bar represents 20  $\mu$ m.

#### **Figure S6. Phosphosites in Murine *CLASP2* $\gamma$ Regulated by Reelin.**

The first column indicates amino acids mutated in human *CLASP2* $\alpha$  phosphomutants within the positively-charged S/A rich region. The second column represents the equivalent amino acid sites in mouse *CLASP2* $\gamma$ . The third column indicates the three amino acid phosphosites in murine *CLASP2* $\gamma$  regulated by Reelin in mouse primary neurons by tandem mass spectrometry.

**Figure S7. Phosphorylation Sites to *CLASP2* in Primary Mouse Neurons in Response to Reelin Stimulation.** Representative tandem mass spectra showing the fragmentation pattern of two phosphopeptides (A and C). The peptide in panel A shows an approximately eight-fold increase in phosphorylation following Reelin treatment, while panel C shows a control peptide with no change following Reelin. Panels B and D depict the full mass scan showing the ratio of the light (control-treated neurons) and heavy (Reelin-treated neurons) peptides for the same peptides.



**Movie S1.** Movie of time-lapse imaging of GFP-positive migrating neurons electroporated with control GFP-scrambled plasmid.

**Movie S2.** Movie of time-lapse imaging of GFP-positive migrating neurons electroporated with GFP-CLASP2 shRNAs.