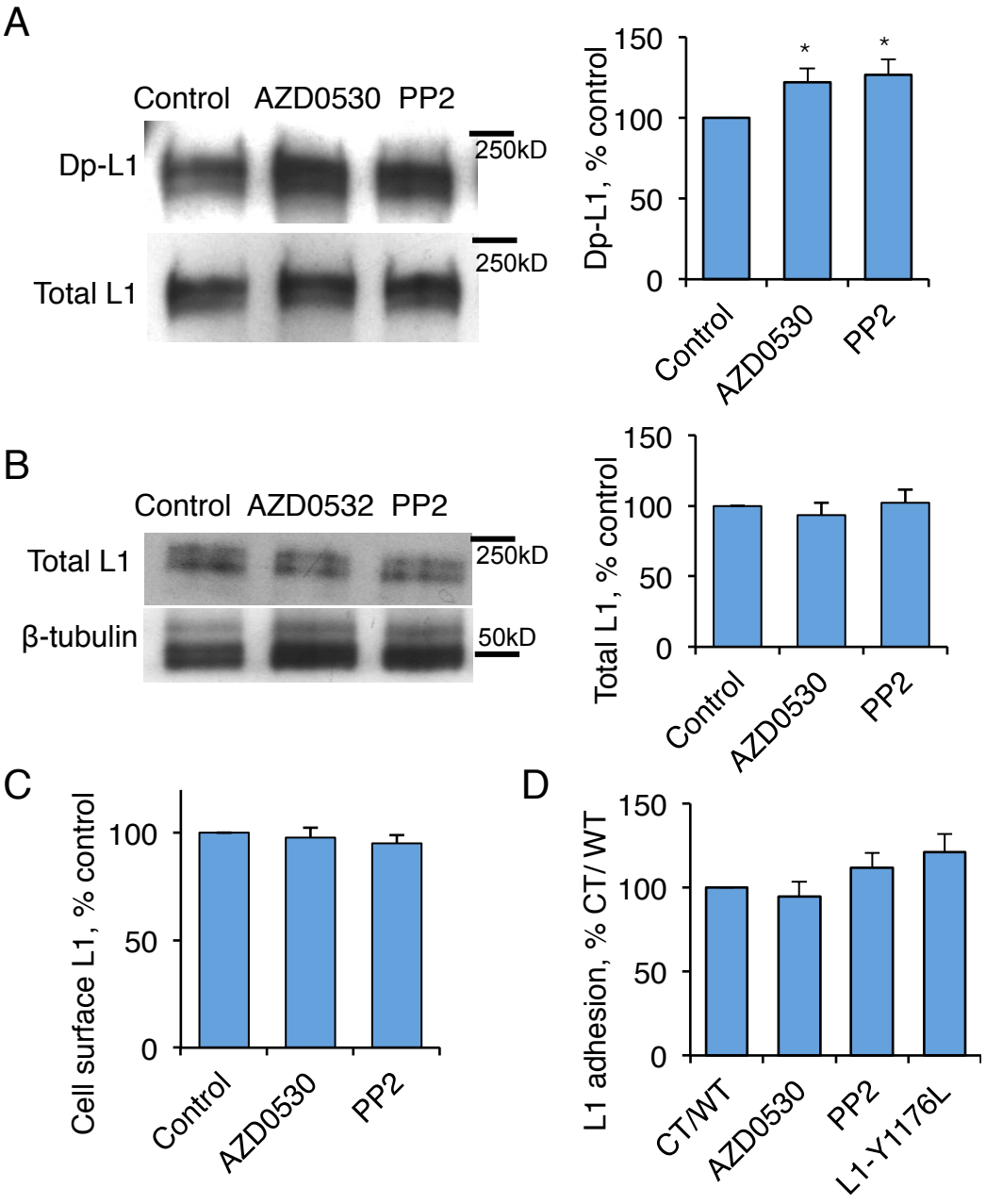
Supplement Table 1

L1 Mutations

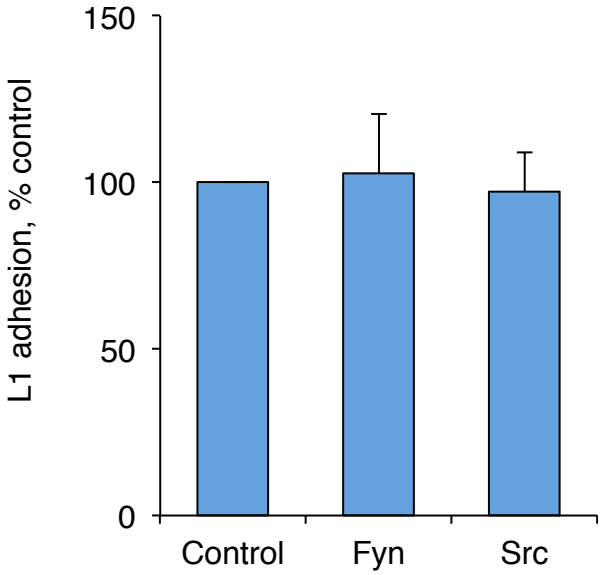
L1 Construct	Mutation
S1152L	Ser1152 → Leu
Y1176L	Tyr1176 → Leu
S1181L	Ser1181 → Leu
S1248L	Ser1248 → Leu
Y1229F	Tyr1229 → Phe
Y1229H	Tyr1229 → His
Y1229E	Tyr1229 → Glu
S1152L/Y1229F	Ser1152 → Leu; Tyr1229 → Phe
Y1176L/Y1229F	Tyr1176 → Leu; Tyr1229 → Phe
S1181L/Y1229F	Ser1181 → Leu; Tyr1229 → Phe
S1248L/Y1229F	Ser1248 → Leu; Tyr1229 → Phe
E33A	Glu33 → Ala
E33A/S1248L	Glu33 → Ala; Ser1248 → Leu

Supplement Fig. 1



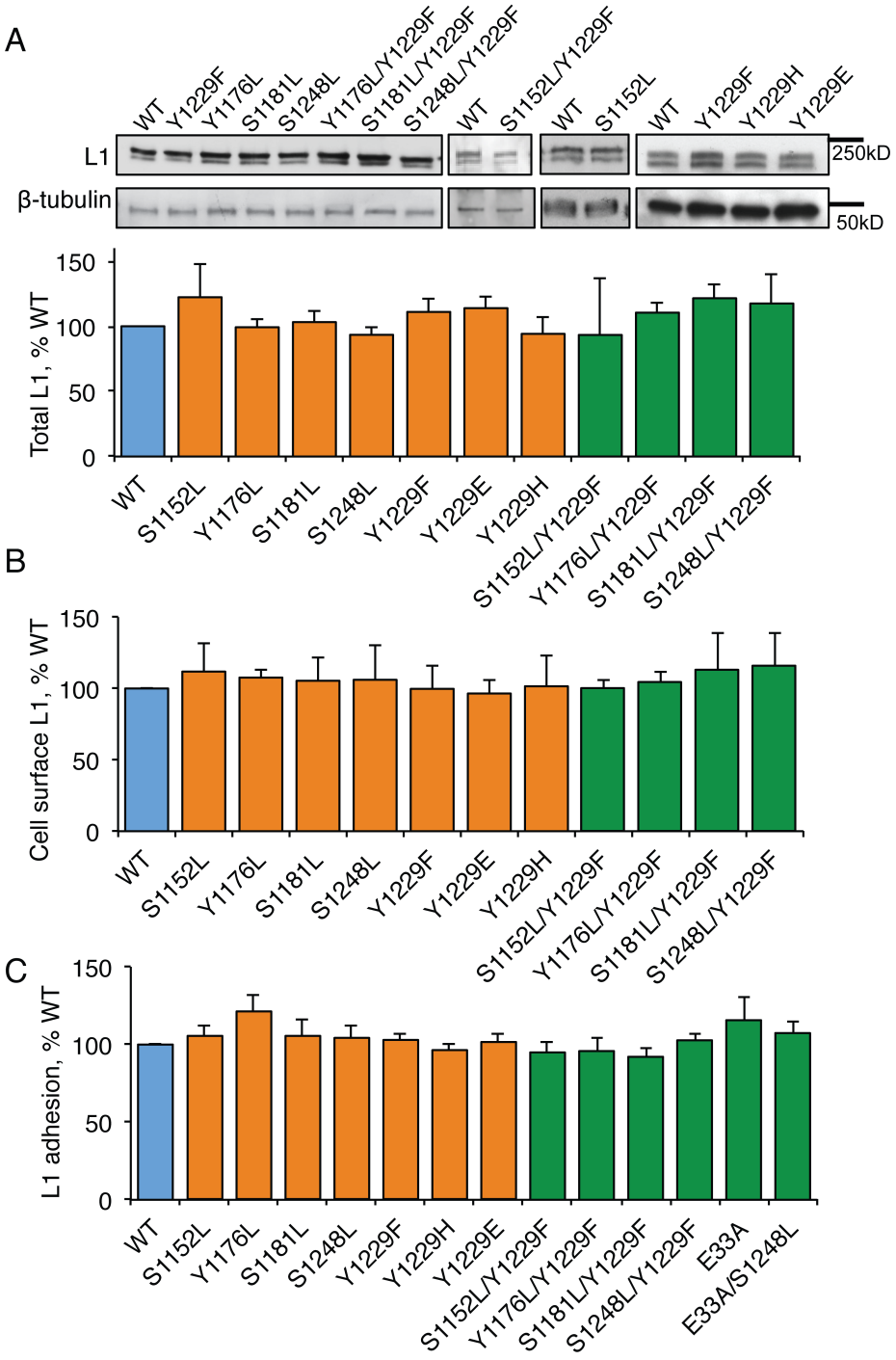
Supplement Figure 1. Effect of Src family kinase inhibitors and mutations of Y1176 on L1 expression and adhesion. 2A2-L1$_s$ cells were incubated with AZD0530 or PP2 and harvested for determination of L1-Y1176 phosphorylation, total L1 expression, cell surface expression of L1, and L1 adhesion, as outlined in Figure 1. (A) Representative Western blot and quantitative densitometry showing L1-dephospho-Y1176 (mAb 74-5H7) and total L1 (mAb UJ127); ($F = 4.00$; $p = 0.0346$), n=7-8; * $p < 0.05$ (B) Total L1 and b-tubulin expression with total L1 expression normalized to b-tubulin (right panel); ($F = 2.17$; $p = 0.1574$), n = 5. (C) L1 cell surface expression determined by FACS using mAb 5G3 against L1; ($F = 1.49$; $p = 0.2574$), n = 6. (D) L1 adhesion of 2A2-L1$_s$ cells in the presence of kinase inhibitors was normalized to values in their absence (CT), and adhesion of NIH/3T3 cells transiently transfected with L1-Y1176L was normalized to values in NIH/3T3 cells transfected with L1-WT (WT); ($F = 0.31$, $p = 0.82$), n = 4-32). All values shown are mean ± SEM, except for FACS data (geometric mean ± SEM).

Supplement Fig. 2



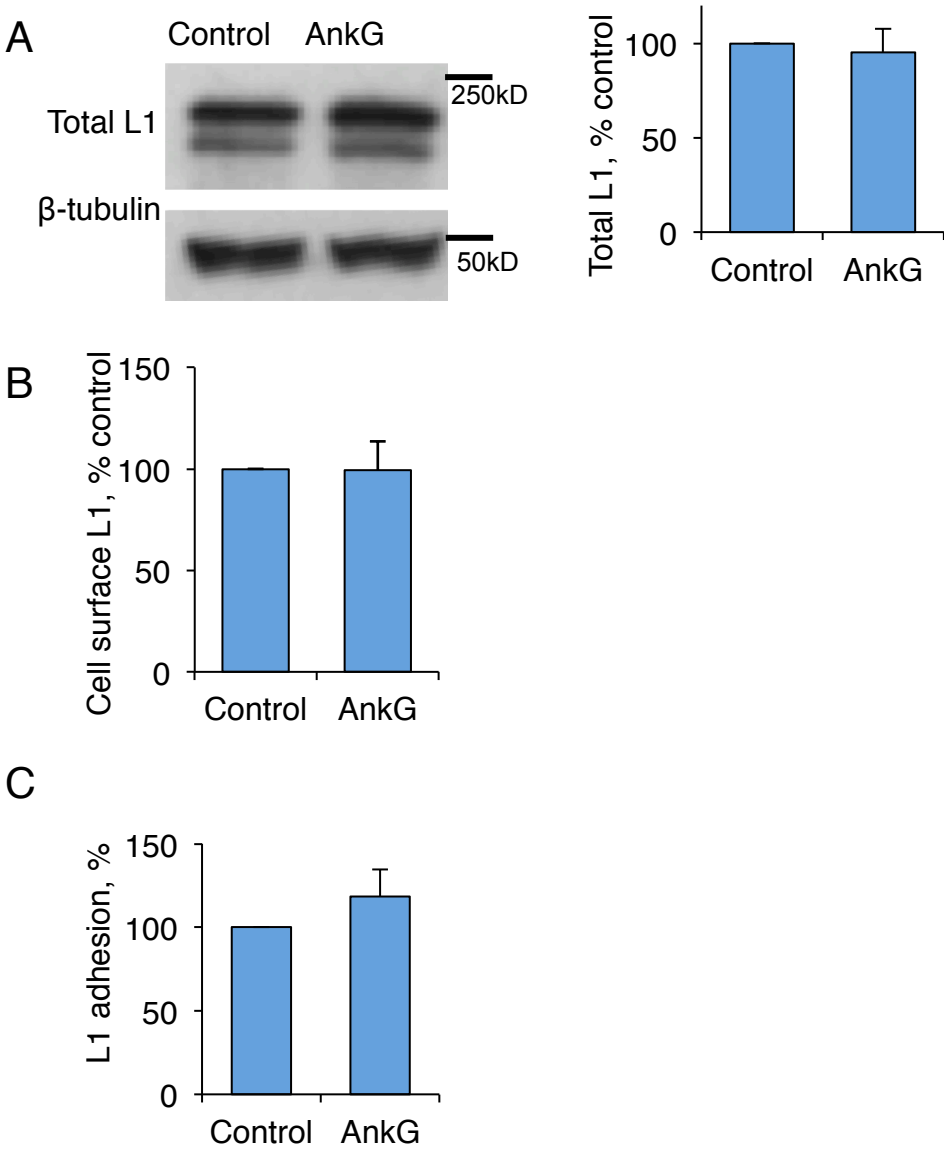
Supplement Figure 2. Effect of siRNA knockdown of Fyn and Src on L1 adhesion in 2A2-L1s cells. Mean ± SEM % adhesion in cells treated with Fyn or Src siRNA was normalized to values in cells treated with a scrambled siRNA (Control); (F = 0.18; p = 0.8396), n = 8.

Supplement Fig. 3



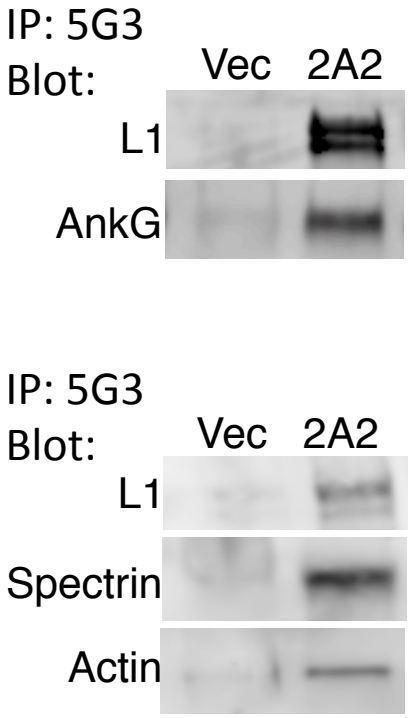
Supplement Figure 3. **Effect of L1 mutations on the expression and adhesion of L1**. All experiments were conducted following transient expression of L1 constructs in NIH/3T3 cells. (A) Total L1 expression, as measured by Western blot, with mean ± SEM relative values in mutant L1-expressing cells expressed as a percentage of values obtained in L1-WT-expressing cells; ($F = 0.52$, $p = 0.8836$), n= 5-37. (B) Geometric mean ± SEM cell surface expression of L1 normalized to values in L1-WT-expressing cells; ($F = 0.19$; $p = 0.9974$), n = 4-13. (C) Mean ± SEM % L1 adhesion expressed as a percentage of values in L1-WT-expressing cells; ($F = 1.66$, $p = 0.0662$), n = 9-151.

Supplement Fig. 4



Supplement Figure 4. Effect of siRNA knockdown of ankyrin-G on the expression and adhesion of L1 in 2A2-L1$_s$ cells. All data from ankyrin-G siRNA-expressing cells are normalized to values in cells expressing scrambled-siRNA. (A) Total L1 expression, as measured by Western blot, with quantitative densitometry normalized to b-tubulin; t = 0.38; p = 0.7176; n = 7. (B) Geometric mean ± SEM cell surface expression of L1, as measured by FACS; t = 0.06; p = 0.9571, n = 5. (C) Mean ± SEM % L1 adhesion; t = 0.13; p = 0.8975; n= 17.

Supplement Fig. 5



Supplement Figure 5. The interaction of L1 with ankyrin-G, spectrin and actin is specific. L1 was immunoprecipated from whole cell lysates of vector-transfected or hL1-transfected NIH/3T3 cells (2A2-$L1_s$) using mAb 5G3. Co-immunoprecipitated proteins were separated and blotted with corresponding antibodies to L1, spectrin, and ankyrin-G (AnkG). Vector-transfected NIH/3T3 cells do not express L1, and mAb 5G3 did not pull down L1, spectrin, or actin from these cells, indicating the specificity of the co-immunoprecipitation procedure. Shown is a representative blot from 6 independent experiments.