Supplementary Table 1 Quantitative Analysis of laminin binding and outgrowth rates.

A. Quantitation of bound laminin after 2 hr incubation from antibody labeling*

Coverslip#	Laminin Concentration (µg/ml)**	Fluorescence intensity	Ratio***
1	9.6	363.9±95	
1	32	685.8±52	.53
2	9.6	392.8±53	
2	32	729.9±59	.54
3	9.6	417.8±80	
3	32	899.9±109	.46

B. Comparison of outgrowth rates on different concentrations of laminin and/or buffer used in medium (from low magnification 2 hr time lapse sequences using $C0_2$ incubation)

Outgrowth rates (µm/hr)	N	Laminin Concentration (µg/ml)	Buffer
39.8±9	8	32	Carbonate
38.9±15	10	9.6	Carbonate
36.2±9	10	9.6	HEPES/Carbonate

*Mab to laminin-1,; ** concentrations used for coating; ***Ratio= Low/high all values represent mean±s.d.

Supplemental Figure Legends

Figure-1. Growth cone area and change in response to Sema 3A treatments. (A) Growth areas are the same on low laminin-1 and high laminin-1 and laminin-10. (B) Sema 3A induced collapse produces greater than 50% (*) decreases in growth cone area. High laminin, Y-27632 treatment (Gallo, 2006) and bath applied latrunculin A (Lat A) prevent full collapse upon Sema 3A treatment.

Figure-2. DRG neuron outgrowth rates are not effected by the concentration or type of substrate bound laminin, but are significantly (*, **) reduced by treatment with Bleb and myosin IIB knockout. Ct, low laminin N=19, Low laminin + Bleb * N=20, p=0.001, Low laminin MIIB KO ** N= 10, p= 10, p= 0.004.

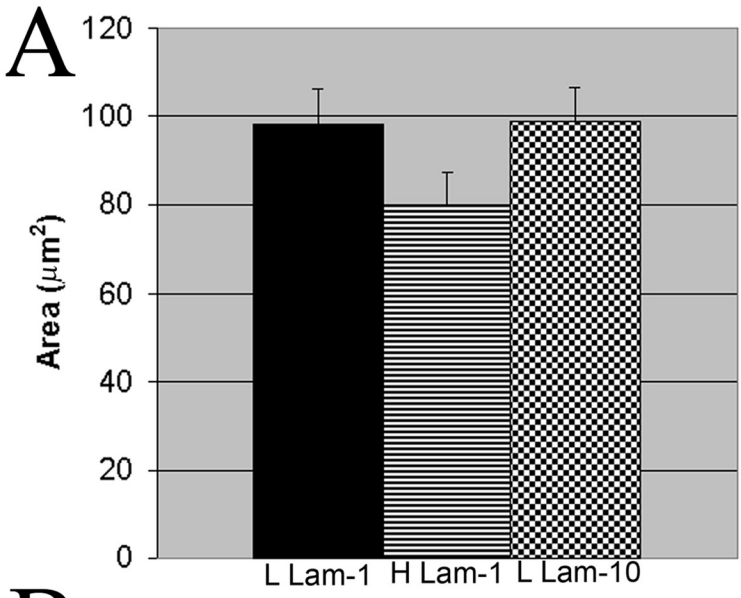
Figure-3. The concentration of laminin used for incubation determines the amount that binds to the substrate over time. Varying concentrations (1.25-32 µg/ml) of fluorescently conjugated laminin mixed 1:4 with unconjugated laminin at the same concentration were incubated for two different amounts of time (2 hrs or 24 hours) with coverslips that had been previously coated with a low concentration of poly-l-lysine (1 µg/ml). The fluorescence intensity was then measured from images taken under identical conditions. The binding curves differ when the amount of time for incubation is changed. The 10 µg/ml shows little change with increased time. However 32 µg/ml increases considerably with longer incubation times. This give rise to a substantial difference between the amount bound to the substrate at the two different concentration (9.6 and 32 µg/ml) used for experiments

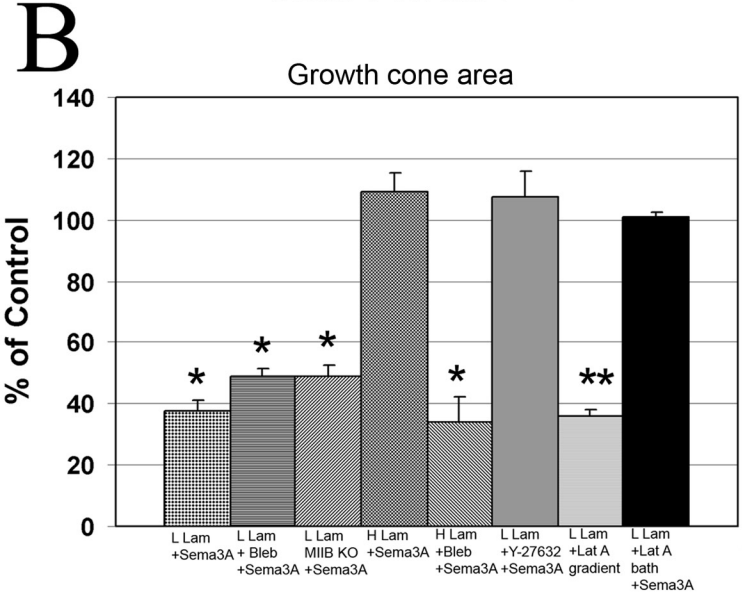
Figure-4. Dose of Sema 3A compared to growth cone collapse response at 30 minutes on low laminin and high laminin. Although high laminin seems to reduce collapse at all doses, but only the 500 ug/ml concentration of Sema 3A produced a significant (* p=0.034) decrease on high laminin.

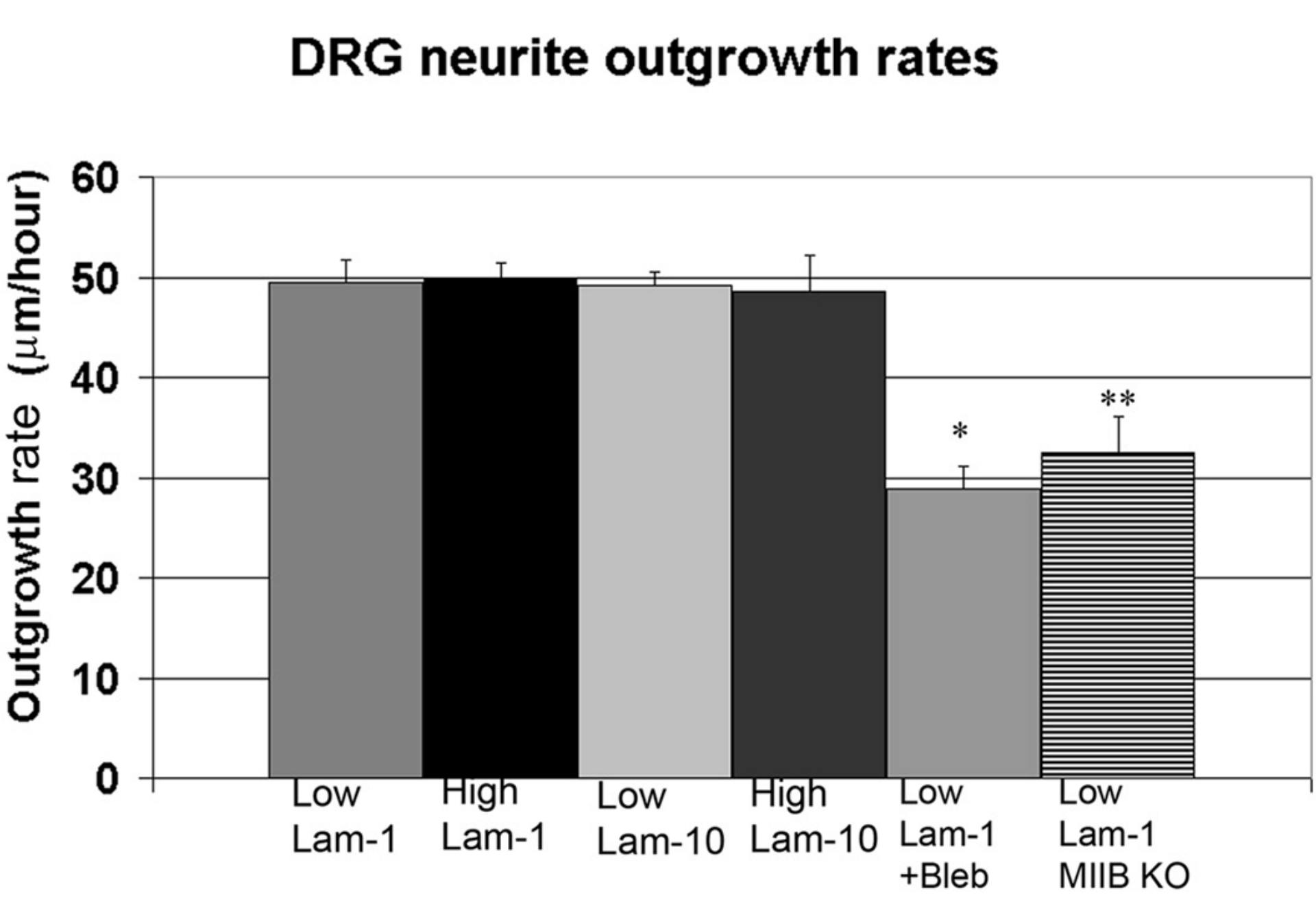
Figure-5. Immunostaining of the different myosin II isoforms in DRG growth cones. (A) Staining for myosin IIA (green) and Cy3 dye for total protein (red). (B) Staining for myosin IIB (green) and total protein (red). (C) Staining for myosin IIC (green) and total protein (red). A-C, Bar=10 μm.

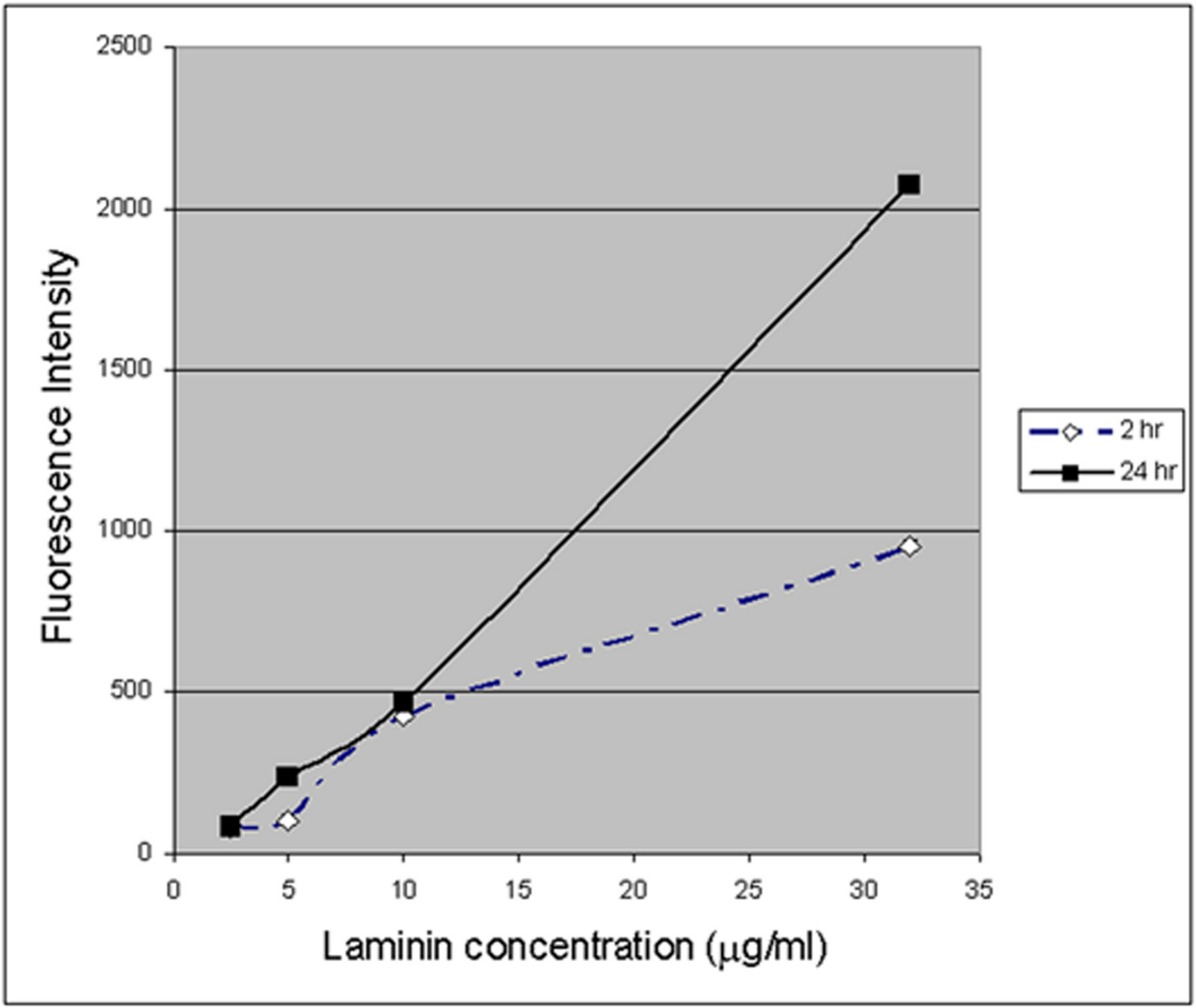
Figure-6. Collapse in response to bath application of Sema3A causes redistribution of myosin IIB. (A) Images from time lapse sequences from a tranfected neuron expressing GFP-myosin IIB growing on low laminin. First panel shows an image prior to Sema3A application. Second and third panels are from 8 and 10 minutes after application. Forth panel is from 16 minutes after start of application. The growth cone narrows and retracts slightly. The neurite first thickens and then narrows (arrowheads). By 16 minutes the myosin IIB has redistributed from a peripheral location to deeper in the cytoplasm. It also seems to concentrate in the slightly retracted growth cones (arrow in forth panel). Four 2 minute time lapse sequences were taken with 10 second intervals. The sequences were: just prior to Sema3A application, at 8 minutes after application, after 16 minutes after application and after 26 minutes. Six additional recordings showed very similar responses. Bar= 20 μm. (B) A neuron cultured from mice expressing GFP-myosin IIB (all endogenous myosin IIB is

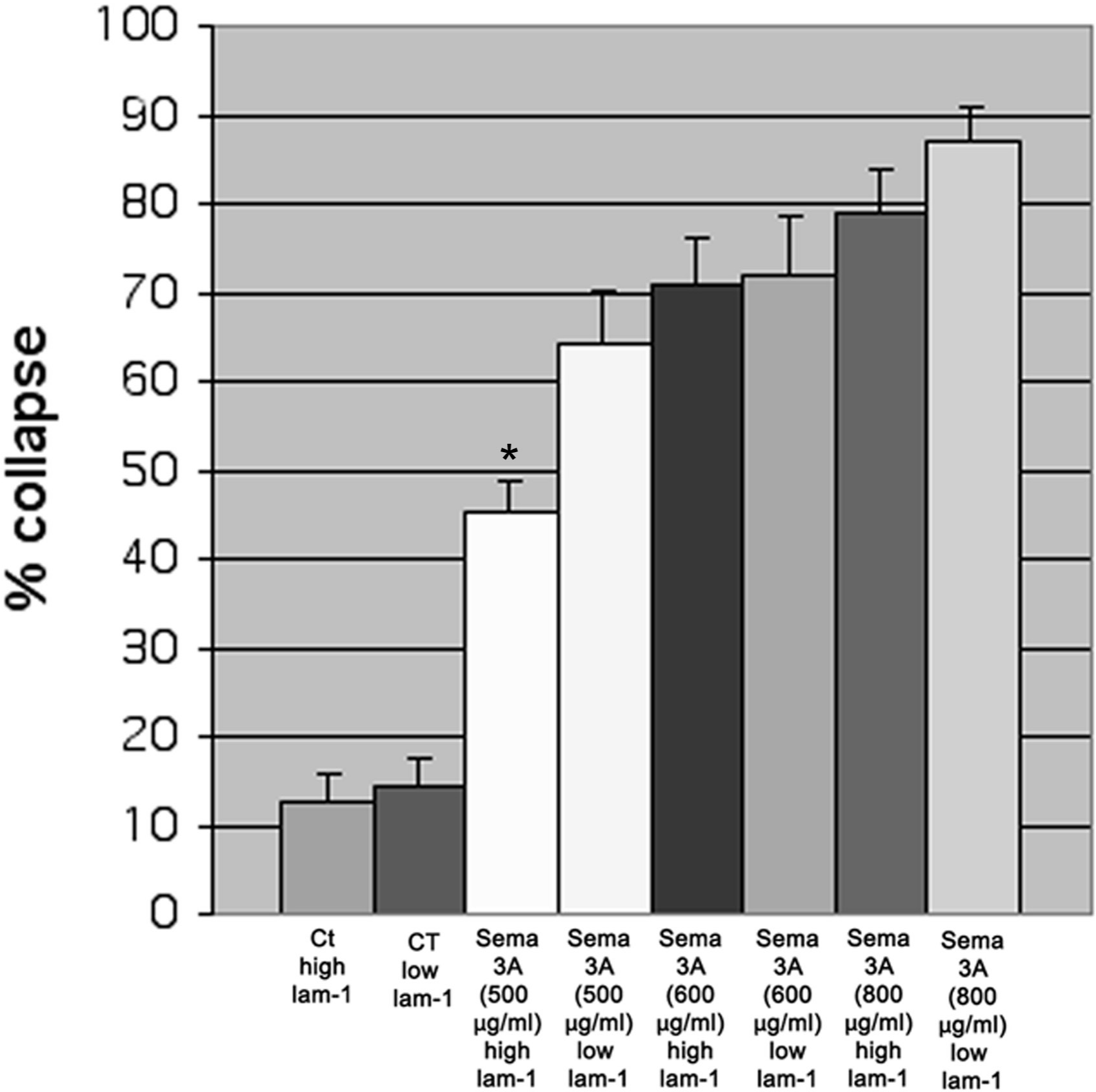
associated with GFP; mice courtesy of Dr. Robert Adelstein, NIH) on high laminin that shows collapse in response to Sema 3A (500 ng/ml added just prior to start of recording). Peripheral myosin IIB (arrowheads) moves centrally and rearward (arrows) during collapse. Panels are at 3 minute intervals except between panel 4 and 5 is 6 minutes. (C and D) Two examples of neurons cultured from mice expressing GFP-myosin IIB growing on high laminin that do not collapse in response to Sema 3A (same condition as in B). Peripheral myosin IIB (arrowheads) is largely retained, although some loss from filopodia is often observed. Panels are at 3 minute intervals. Scale Bar= 25 μm.

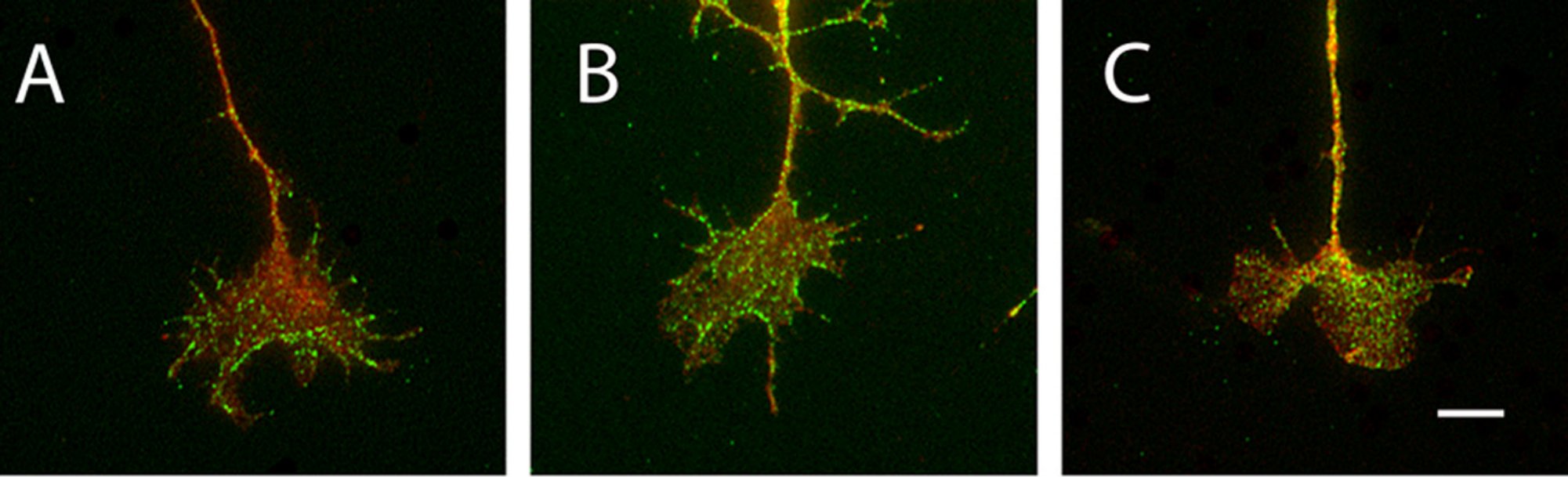

A
120
100
80
60
40
20
0
Area (μm²)
L Lam-1
H Lam-1
L Lam-10

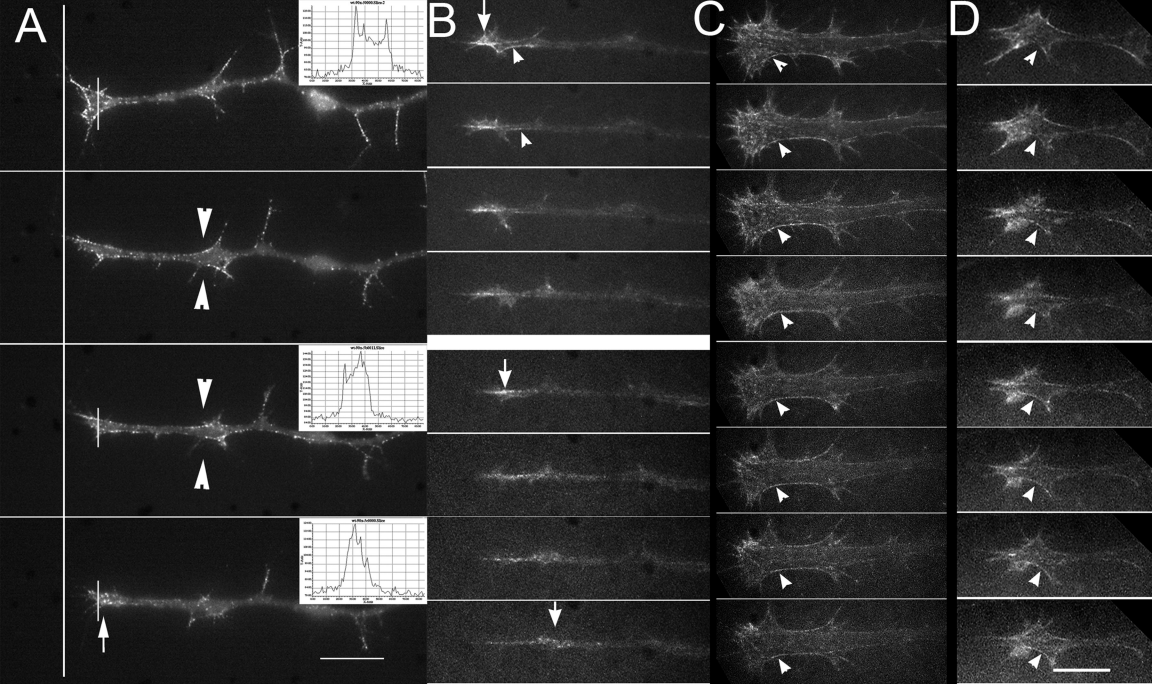

B
Growth cone area
140
120
100
80
60
40
20
0
% of Control
*
*
*
*
**
L Lam +Sema3A
L Lam + Bleb +Sema3A
L Lam MIIB KO +Sema3A
H Lam +Sema3A
H Lam +Bleb +Sema3A
L Lam +Y-27632 +Sema3A
L Lam +Lat A gradient
L Lam +Lat A bath +Sema3A


DRG neurite outgrowth rates
Outgrowth rate (μm/hour)
60
50
40
30
20
10
0
*
**
Low Lam-1
High Lam-1
Low Lam-10
High Lam-10
Low Lam-1 +Bleb
Low Lam-1 MIIB KO


2500
2000
1500
1000
500
0
Fluorescence Intensity
0
5
10
15
20
25
30
35
Laminin concentration (μg/ml)
2 hr
24 hr


100
90
80
70
60
50
40
30
20
10
0
% collapse
*
Ct high lam-1
CT low lam-1
Sema 3A (500 µg/ml) high lam-1
Sema 3A (500 µg/ml) low lam-1
Sema 3A (600 µg/ml) high lam-1
Sema 3A (600 µg/ml) low lam-1
Sema 3A (800 µg/ml) high lam-1
Sema 3A (800 µg/ml) low lam-1


A
B
C


A
B
C
D