Supplementary Table 1. C2C12 cell viability assay values and Comparison of SY-LB-35 vs. SY-LB-57 responses.

Concentration (μM)	SY-LB-35 Cell Viability (% control)	SY-LB-57 Cell Viability (% control)	Comparison 35 vs. 57 (*p*-values)
0.01	265.6 ± 9.4	363.5 ± 65.2	$p = 0.2292$
0.1	248.6 ± 22.3	334.3 ± 62.6	$p = 0.2825$
1	218.1 ± 14.9	303.3 ± 52.5	$p = 0.2054$
10	148.3 ± 15.5	230.9 ± 35.7	$p = 0.0630$
100	68.7 ± 6.5	163.4 ± 21.4	$^{**}p = 0.0047$
1000	12.9 ± 1.5	19.97 ± 3.2	$p = 0.1091$
IC_{50} value	**401.1 μM**	**807.9 μM**	

The mean, SEM and results from Student's two-tailed t-test values for C2C12 cell viability assays. These data were previously reported [30]. $^{**}p < 0.01$.

Supplementary Table 2. WEHI cell viability assay values and Comparison of SY-LB-35 vs. SY-LB-57 responses.

Concentration (μM)	SY-LB-35 Cell Viability (% control)	SY-LB-57 Cell Viability (% control)	Comparison 35 vs. 57 (*p*-values)
0.01	532.3 ± 43.9	621.7 ± 45.3	$p = 0.2291$
0.1	526.6 ± 18.0	615.3 ± 6.1	$^{**}p = 0.0095$
1	537.4 ± 27.5	694.1 ± 4.7	$^{**}p = 0.0050$
10	546.2 ± 32.8	657.5 ± 13.2	$^{*}p = 0.0348$
100	338.5 ± 28.7	178.2 ± 51.2	$p = 0.0524$
1000	71.9 ± 1.6	61.6 ± 6.0	$p = 0.2289$
IC_{50} value	**1074.2 μM**	**1089.5 μM**	

The mean, SEM and results from Student's two-tailed t-test values for WEHI cell viability assays. $^{**}p < 0.01$; $^{*}p < 0.05$.

Supplementary Table 3. Primary PAEC viability assay values and Comparison of SY-LB-35 vs. SY-LB-57 responses.

Concentration (μM)	SY-LB-35 Cell Viability (% control)	SY-LB-57 Cell Viability (% control)	Comparison 35 vs. 57 (*p*-values)
0.01	202.6 ± 3.4	305.5 ± 1.2	****$p = 7.65 \times 10^{-5}$
0.1	204.4 ± 0.8	325.4 ± 3.7	****$p = 4.87 \times 10^{-5}$
1	217.8 ± 5.0	285.6 ± 2.4	**$p = 0.0022$
10	224.1 ± 5.4	246.7 ± 2.3	$p = 0.0894$
100	115.7 ± 2.1	191.1 ± 3.2	***$p = 3.54 \times 10^{-4}$
1000	31.0 ± 1.0	70.0 ± 0.7	****$p = 4.36 \times 10^{-5}$
IC_{50} value	**797.9 μM**	**1148.9 μM**	

The mean ± SEM and results from Student's two-tailed t-test values for primary PAEC viability assays. ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$.

Supplementary Table 4. Quantitative analysis of normalized p-p38 levels: 15-minute and 24-hour stimulation.

p-p38 15 minutes	SAMPLE	MEAN	SD	*p*-value	
	Control	100			
	BMP2	249	34	0.00170	**
SY-LB-35 (μM)	0.01	265	28	0.00055	***
	0.1	343	52	0.00132	**
	1	248	57	0.01098	*
	10	257	19	0.00015	***
SY-LB-57 (μM)	0.01	372	46	0.00052	***
	0.1	330	18	2.38E-05	****
	1	319	50	1.62E-03	**
	10	278	50	3.66E-03	**

p-p38 24 hours	SAMPLE	MEAN	SD	*p*-value	
	Control	100			
	BMP2	276	31	0.00061	***
SY-LB-35 (μM)	0.01	402	45	0.00031	***
	0.1	454	53	0.00033	***
	1	464	59	0.00044	***
	10	438	46	0.00023	***
SY-LB-57 (μM)	0.01	348	41	0.00047	***
	0.1	483	25	1.15E-05	****
	1	499	42	7.76E-05	****
	10	441	29	3.45E-05	****

The mean, SD and results from Student's two-tailed t-test values vs. control for normalized p-p38 levels stimulated by the indicated concentrations of SY-LB-35 and SY-LB-57 for 15 minutes and 24 hours. BMP2 (50 ng/mL) served as a positive control. ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; **$p < 0.05$.

Supplementary Table 5. Quantitative analysis of normalized p-ERK levels: 15-minute and 24-hour stimulation.

p-ERK 15 minutes	SAMPLE	MEAN	SD	*p*-value	
	Control	100			
	BMP2	194	15	0.00041	***
SY-LB-35 (μM)	0.01	221	42	0.00764	**
	0.1	280	64	0.00810	**
	1	225	43	0.00725	**
	10	272	58	0.00659	**
SY-LB-57 (μM)	0.01	215	39	0.00684	**
	0.1	271	24	0.00025	***
	1	252	49	0.00556	**
	10	233	35	0.00279	**

p-ERK 24 hours	SAMPLE	MEAN	SD	*p*-value	
	Control	100			
	BMP2	155	16	0.00446	**
SY-LB-35 (μM)	0.01	220	1	7.56E-10	****
	0.1	299	12	9.39E-06	****
	1	339	40	0.00050	***
	10	317	10	2.71E-06	****
SY-LB-57 (μM)	0.01	154	14	0.00269	**
	0.1	138	6	0.00037	***
	1	221	26	0.00129	**
	10	220	30	0.00239	**

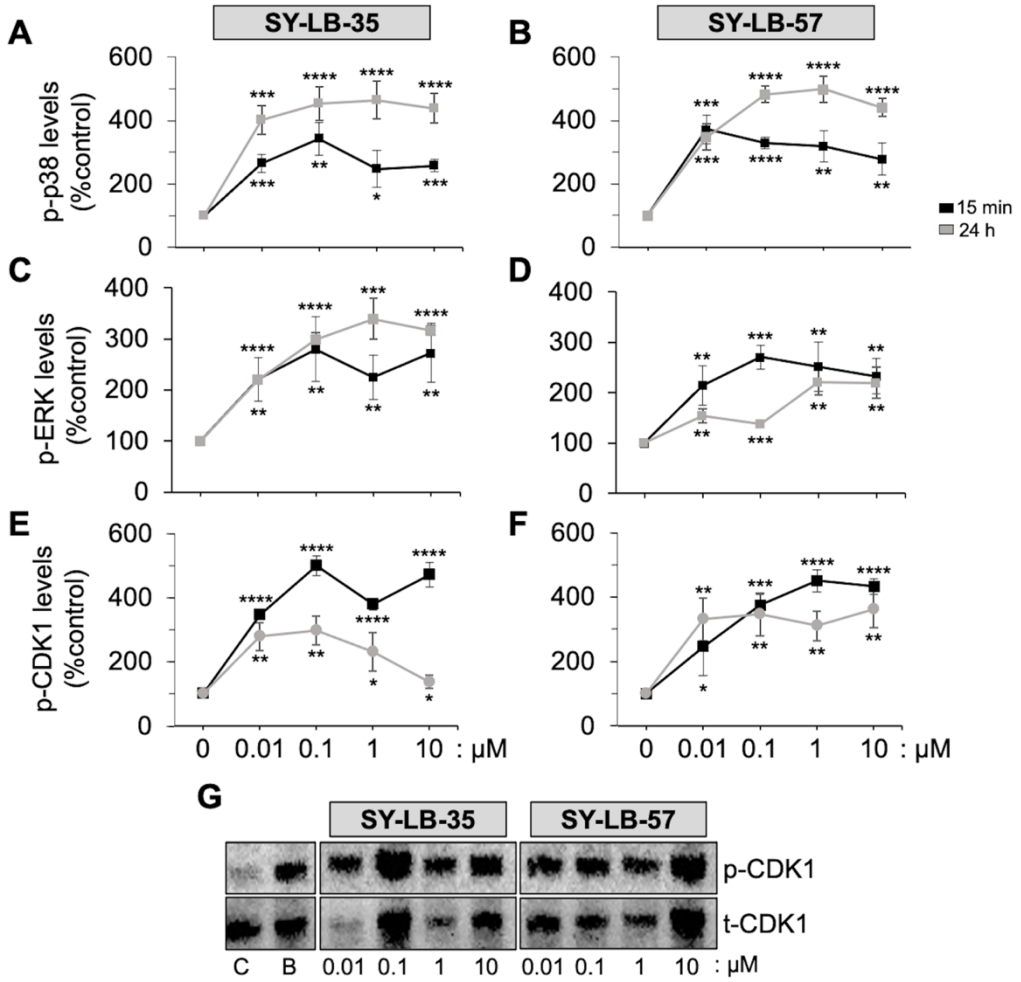
The mean, SD and results from Student's two-tailed t-test values vs. control for normalized p-ERK levels stimulated by the indicated concentrations of SY-LB-35 and SY-LB-57 for 15 minutes and 24 hours. BMP2 (50 ng/mL) served as a positive control. ****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$.

Supplementary Table 6. Quantitative analysis of normalized p-CDK1 levels: 15-minute and 24-hour stimulation.

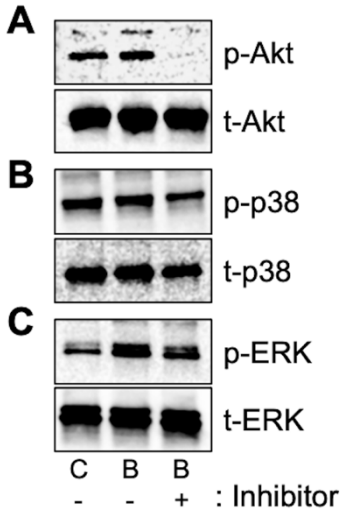
	SAMPLE	AVG	SD	*p*-value	
p-CDK1 15 minutes	Control	100			
	BMP2	261	29	0.00063	***
SY-LB-35 (μM)	0.01	347	11	2.56E-06	****
	0.1	499	32	2.57E-05	****
	1	379	18	1.09E-05	****
	10	471	38	7.44E-05	****
SY-LB-57 (μM)	0.01	246	90	0.04807	*
	0.1	374	34	0.00015	***
	1	451	34	6.04E-05	****
	10	433	25	1.99E-05	****

	SAMPLE	MEAN	SD	*p*-value	
p-CDK1 24 hours	Control	100			
	BMP2	169	31	0.01816	*
SY-LB-35 (μM)	0.01	278	43	0.00202	**
	0.1	297	45	0.00161	**
	1	231	60	0.01916	*
	10	137	20	0.03071	*
SY-LB-57 (μM)	0.01	331	66	0.00368	**
	0.1	346	66	0.00291	**
	1	310	47	0.00146	**
	10	364	58	0.00143	**

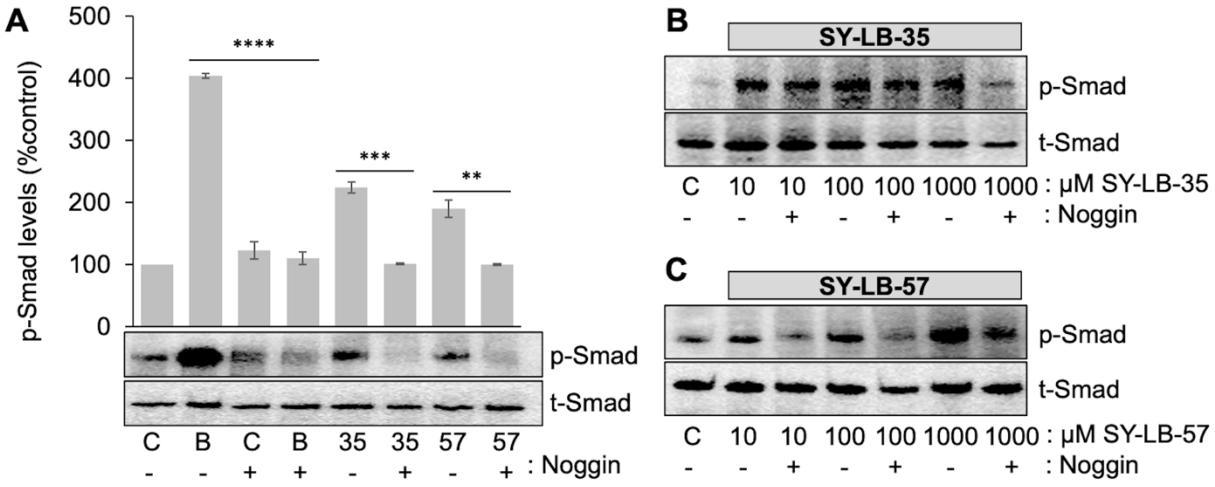
The mean, SD and results from Student's two-tailed t-test values vs. control for normalized p-CDK1 levels stimulated by the indicated concentrations of SY-LB-35 and SY-LB-57 for 15 minutes and 24 hours. BMP2 (50 ng/mL) served as a positive control. ****$p < 0.0001$; ***$p < 0.001$; *$p < 0.05$.



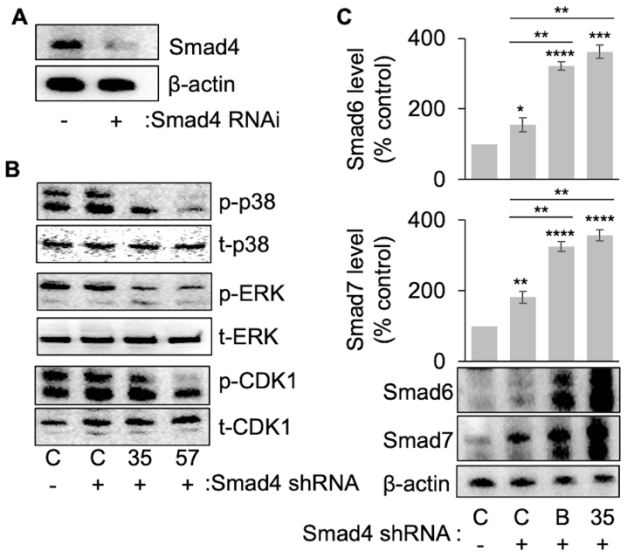
Supplementary Fig. 1. SY-LB compounds stimulate rapid and sustained increases in non-canonical BMP signaling pathways. (A-F) Line graphs representing the concentration-dependence of p-p38 (A, B), p-ERK (C, D) and p-CDK1 (E, F) activation in response to 0.01 μM to 10 μM SY-LB-35 (A, C, E) or SY-LB-57 (B, D, F) at 15 minutes (black lines) and 24 hours (red lines) in C2C12 cells. Levels of phosphorylated proteins were normalized to total protein levels for the respective signaling proteins and are expressed as a percent of control (mean ± SD; (n=3 for all conditions)). All concentrations of SY-LB compounds induced significant increases in p-p38, p-ERK and p-CDK1 levels compared to control, untreated cells. Quantitative data of p-p38, p-ERK and p-CDK1 levels at 15 minutes and 24 hours (mean, SD and *p* values for each condition compared with control) are reported in Supplementary Table 4, Table 5 and Table 6, respectively. The responses for p-p38 and p-ERK at 15 minutes and 24 hours, respectively, were averaged and demonstrate that the responses to p-p38 are significantly elevated at 24 hours compared with p-ERK responses after 24 hours (Averaged p-p38 responses at 15 minutes vs 24 hours, SY-LB-35: 278% ± 22% vs. 440% ± 30%, ***p = 0.00074; SY-LB-57: 307% ± 19% vs. 443% ± 54%, *p = 0.231; Averaged p-ERK responses at 15 minutes vs 24 hours, SY-LB-35: 250% ± 15% vs. 294% ± 26%, p = 0.1922; SY-LB-57: 243% + 12% vs. 183% + 22%, p = 0.0549). Statistical comparisons were carried out using Student's two-tailed, paired t-test. (G) Representative Western blots of C2C12 cell cultures stimulated with 50 ng/mL BMP2 or 0.01 μM to 10 μM SY-LB-35 or SY-LB-57 for 15 minutes. Whole cell lysates were analyzed by Western blot using an anti-p-CDK1 antibody to probe the membrane. The membrane was gently stripped and re-probed with an anti-t-CDK1 antibody. Western blots of the same lysates probed for p-p38 and p-ERK were previously reported [30].



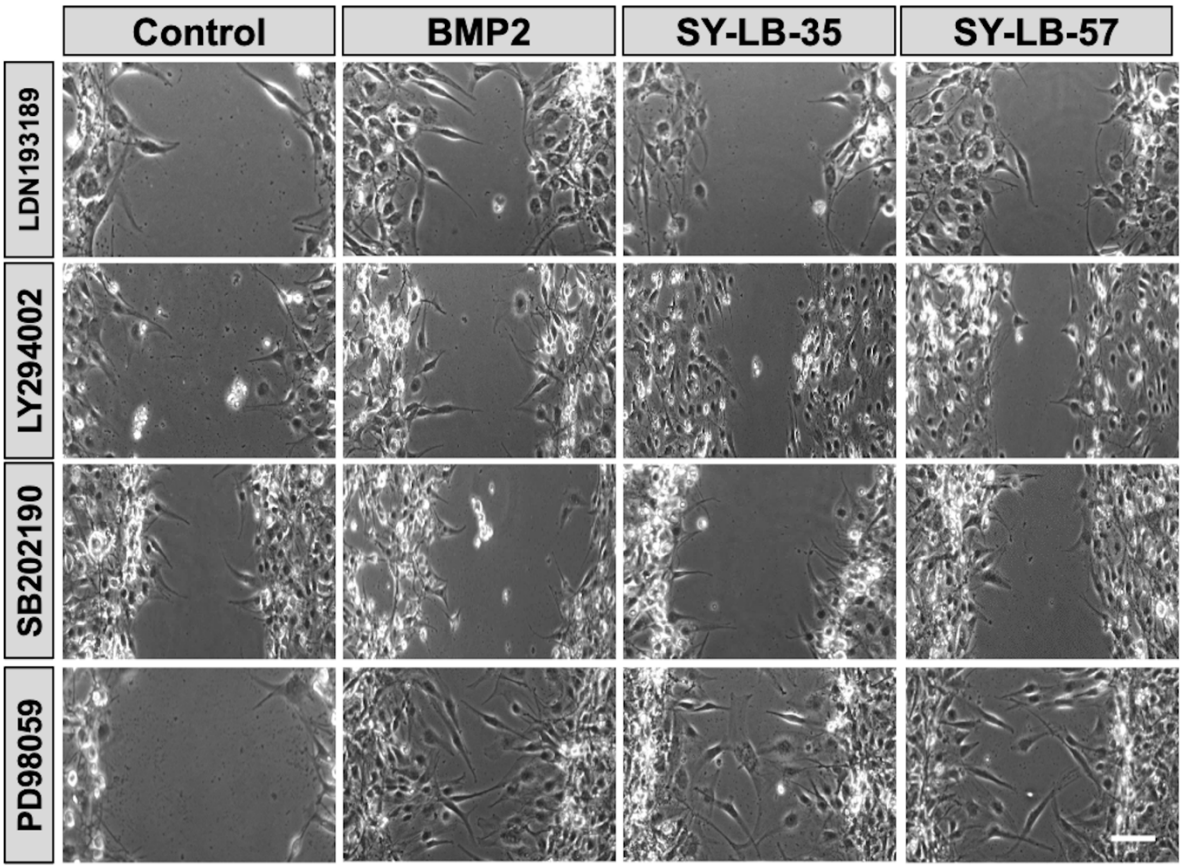
Supplementary Fig. 2. Inhibitors, LY294002, SB202190 and PD98059, suppress BMP-induced phosphorylation of Akt, p38 and ERK. C2C12 cell cultures were serum-starved and pre-treated for 1 hour in the presence or absence of (A) LY294002 (15 μM), (B) SB202190 (10 μM) or (C) PD98059 (5 μM). Next, the cultures were treated with 50 ng/mL BMP9 for 15 minutes and whole cell lysates were prepared. The lysates were probed by Western blot using antibodies against (A) p-Akt, (B) p-p38 and (C) p-ERK. The membranes were gently stripped and re-probed with the respective pan antibodies t-Akt, t-p38 and t-ERK.



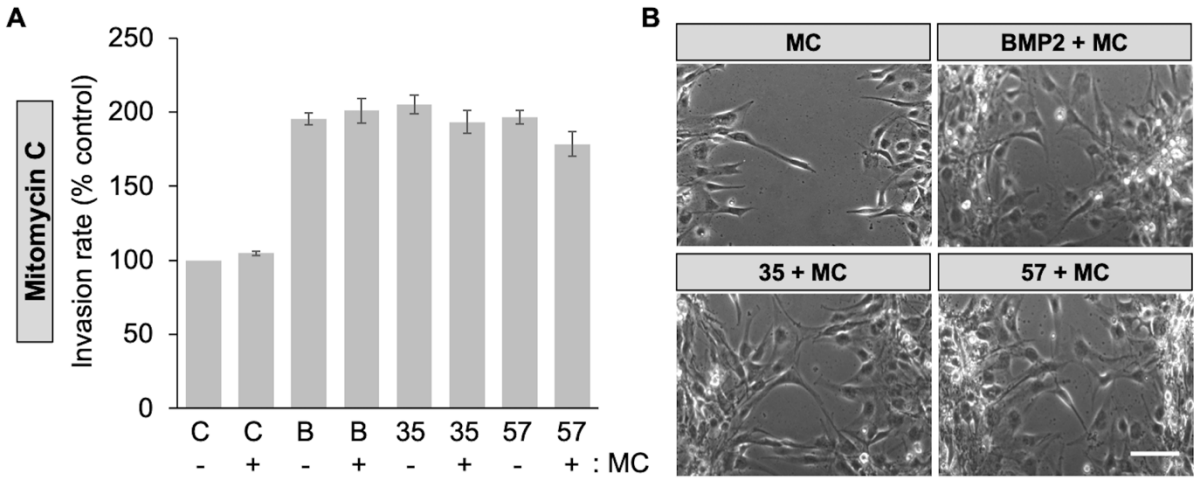
Supplemental Fig. 3. Noggin blocks SY-LB-induced increases in p-Smad levels stimulated by SY-LB compounds. (A) Serum-starved C2C12 cell cultures were treated with BMP2 (B, 50 ng/mL or 2nM) as a positive control and 1 μM SY-LB-35 or SY-LB-57 in absence or presence of Noggin (400 ng/mL) for 30 minutes. Whole cell lysates of treated cultures were analyzed by Western blot using anti-p-Smad and anti-t-Smad antibodies to successively probe the membrane. Quantification of the results demonstrates that pre-treatment of C2C12 cells with Noggin significantly inhibited Smad phosphorylation stimulated by SY-LB-35 and SY-LB-57. The level of p-Smad was normalized to t-Smad levels and the data are expressed as a percentage of control, untreated cultures (mean ± SEM (n=3); Control vs. BMP2, ****$p = 9.38 \times 10^{-8}$; Control vs. SY-LB-35, ***$p = 0.00018$; Control vs. SY-LB-57, **$p = 0.0030$; Noggin(-) vs. Noggin(+), Control: 100% vs. 99% ± 14%, $p = 0.1664$; BMP2: 404% ± 3.4% vs. 130% ± 9.7%, ****$p = 9.05 \times 10^{-6}$; SY-LB-35: 225% ± 9.3% vs. 101% ± 1.8%, ***$p = 0.00021$; SY-LB-57: 190% ± 14% vs. 101% ± 1.3%, **$p = 0.0032$). Statistical comparisons of samples in the absence or presence of Noggin were carried out using Student's two-tailed, paired t-test. (B, C) Serum-starved C2C12 cells were treated with increasing concentrations (10 μM - 1000 μM) of (B) SY-LB-35 or (C) SY-LB-57 in presence or absence of Noggin for 30 minutes (n=3). Western blot analysis of whole cell lysates of the treated cultures was carried out by using anti-p-Smad and anti-t-Smad antibodies to successively probe the membranes. The results demonstrate that concentrations between 10 μM - 1000 μM SY-LB-35 or SY-LB-57 can overcome the block on Smad phosphorylation caused by Noggin observed at 1 μM SY-LB-35 and SY-LB-57, as shown in (A). Representative Western blots are shown.



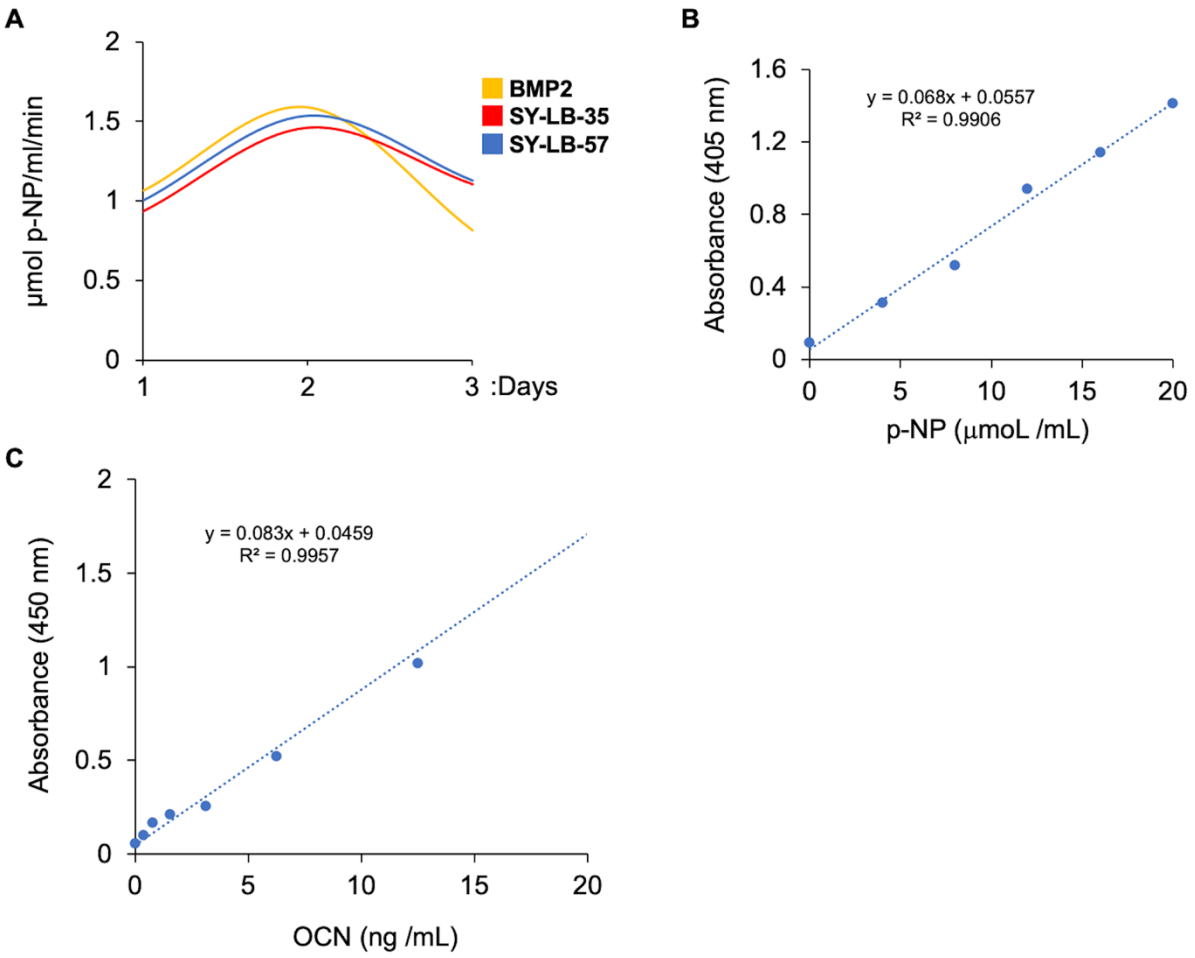
Supplementary Fig. 4. Smad4 RNAi blocks SY-LB-stimulated intracellular signaling and upregulates inhibitory Smad expression in response to SY-LB-35 and SY-LB-57. C2C12 cells were transfected with an shRNA expression construct targeting Smad4. After 48 hours, C2C12 cells were stimulated with 1 μM SY-LB-35 or SY-LB-57 for 30 minutes. Whole cell lysates were prepared and analyzed by Western blot. (A) The membranes were probed with anti-Smad4 antibodies followed by gentle stripping and re-probing of the membrane with antibodies against β-actin (n=3). Transfection of C2C12 cells with the Smad4 shRNA construct results in robust inhibition of Smad4 expression. (B) Western blot analysis of Smad4 shRNA-transfected C2C12 whole cell lysates using antibodies against non-canonical BMP signaling markers p-ERK, p-p38 and p-CDK1. The membranes were gently stripped and re-probed with the respective pan antibodies, t-ERK, t-p38 and t-CDK1 (n=3). Treatment of transfected cells with SY-LB-35 or SY-LB-57 failed to induce phosphorylation of non-canonical markers. (C) Western blot analysis of Smad4 shRNA-transfected C2C12 cell whole cell lysates treated with 50 ng/mL (2 nM) BMP2 (B) or 1 μM SY-LB-35 for 30 minutes. The membrane was probed using antibodies against inhibitory Smad, Smad6. The membrane was successively stripped and re-probed with anti-Smad7 and, then anti-β-actin antibodies (n=3). Quantification demonstrates increased levels of inhibitory Smad6 and Smad7 expression in response to Smad4 shRNA alone (C+) compared with untransfected, unstimulated cells (C-). (shRNA(-) vs. shRNA(+), Smad6: Control vs. Transfected, $*p = 0.0488$; Smad7: Control vs. Transfected, $**p = 0.0072$. shRNA(-) vs. shRNA(+), Smad6: Control vs. BMP2, $****p = 4.69 \times 10^{-5}$; Control vs. SY-LB-35, $***p = 0.00015$; Smad7: Control vs. BMP2, $****p = 8.92 \times 10^{-5}$; Control vs. SY-LB-35, $****p = 7.64 \times 10^{-5}$). Exposure of shRNA transfected cultures to BMP2 or SY-LB-35 significantly increased the expression of Smad6 and Smad7 compared to control (C-) cultures (shRNA(+) vs. shRNA(+), Smad6: Control vs. BMP2, 192% ± 20% vs. 299% ± 12%, $**p = 0.0019$; Control vs. SY-LB-35, 192% ± 20% vs. 362% ± 19%, $**p = 0.0016$; Smad7: Control vs. BMP2, 182% ± 16% vs. 325% ± 14%, $**p = 0.0026$; Control vs. SY-LB-35, 182% ± 16% vs. 357% ± 15%, $**p = 0.0014$). Moreover, levels of Smad6 and Smad7 are significantly greater in BMP2- or SY-LB-35-treated cultures compared with transfected, untreated control (C+) cultures ($**p < 0.01$). Statistical comparisons were carried out using Student's two-tailed t-test.



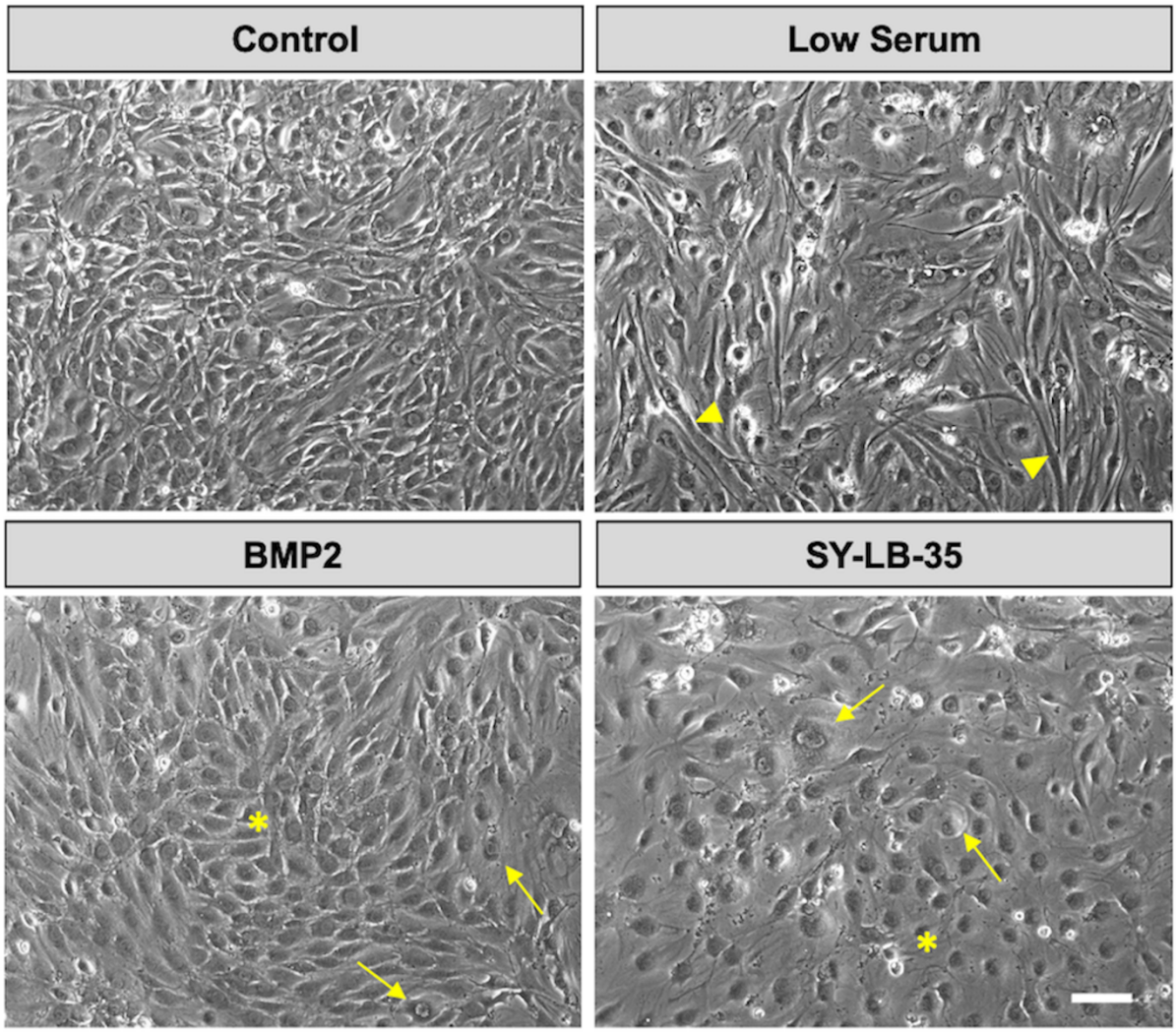
Supplementary Fig. 5. Scratch-wounded C2C12 cell cultures in the presence of various signaling inhibitors. Representative 20x phase contrast images of scratch-wounded C2C12 cell monolayers after 18 hours in the presence of the ALK2 inhibitor, LDN193189 (5 μM), the pan-PI3K inhibitor, LY294002 (15 μM), an inhibitor of p38 activity, SB203190 (10 μM), and the ERK inhibitor, PD98059 (5 μM) in combination with unsupplemented medium, 50 ng/mL BMP2, 1 μM SY-LB-35 or 1 μM SY-LB-57. Inhibition of ALK2, PI3K and p38 activity inhibited the Invasion rate compared with the respective control. Scale bar = 100 μm.



Supplementary Fig. 6. Wound healing stimulated by SY-LB-35 and SY-LB-57 does not rely on proliferation. Confluent cultures of C2C12 cells serum-starved for 2 hours were scratch-wounded, then treated with Mitomycin (MC, 30 nM) for 2 hours to inhibit proliferation. Next, the cultures were stimulated with BMP2 (B, 50 ng/mL) as a positive control or SY-LB compounds (1 μM) for 18 hours. The wounds were examined at 0- and 18-hour time points to assess wound closure over time. (A) The Invasion rate is expressed as the number of cells invading the wound as a percentage of invading cells in control (C-), untreated cultures. Inhibition of proliferation had no effect on the invasion rate compared with the respective control sample (mean ± SEM (n=3)). Proliferation inhibitor, MC(-) vs. MC(+), Control: 100% vs. 105% ± 1.7%, $p = 0.0501$; MC(-) vs. MC(+), BMP2: 195% ± 4.1% vs. 201% ± 8.3%, $p = 0.5751$; SY-LB-35: 205% ± 6.3% vs. 193% ± 7.9%, $p = 0.3137$; SY-LB-57: 197% ± 4.6% vs. 178% ± 8.2%, $p = 0.1242$. (B) Representative 20x phase contrast images for each condition. Scale bar = 100 μm.



Supplementary Fig. 7. Time course of ALP activity in response to SY-LB compounds, ALP assay standard curve and OCN ELISA standard curve. (A) Confluent cultures of C2C12 cells were treated with BMP2 (200 ng/mL), 10 µM SY-LB-35 or 10 µM SY-LB-57 and incubated for 72 hours. Samples of culture medium were collected at 24, 48 and 72 hours and subjected to ALP assays using soluble p-nitrophenol phosphate (p-NPP) as a substrate. ALP enzymatic activity is represented as the amount of p-nitrophenol (p-NP) product per mL of medium per minute. The time course of SY-LB-35- (red curve) and SY-LB-57-induced (blue curve) ALP activity demonstrates sustained secretion of active ALP enzyme into the culture medium mimicking the time course of BMP2 (yellow curve). (B) Standard curve of p-NP versus absorbance at 405 nm. The amount of p-NP in each treatment group was calculated using the standard curve of p-NP versus absorbance at 405 nm. (C) Standard curve of known concentrations of OCN (0, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25 ng/mL) versus absorbance at 450 nm.



Supplementary Fig. 8. SY-LB-35 induces morphological changes in C2C12 cells similar to BMP2-induced responses. Phase contrast images (20x) of serum-starved, confluent C2C12 cell cultures stimulated with BMP2 (200 ng/mL) or SY-LB-35 (10 μM) for 72 hours (treated every 24 hours) in Low Serum (2% FBS in DMEM) (n=3). Control cultures grown in complete growth medium (10% FBS) exhibit typical C2C12 cell morphology. Under reduced serum conditions, unstimulated cells develop into elongated myofiber bundles (yellow arrowheads). Daily treatment with BMP2 or SY-LB-35 suppressed myofiber development in the cultures and produced enlarged cells (yellow arrows) and areas of cobblestone-like clusters (yellow asterisks). Scale bar = 100 μm.