Supporting Information

A Robust Split-Luciferase-Based Cell Fusion Screening for Discovering Myogenesis-Promoting Molecules

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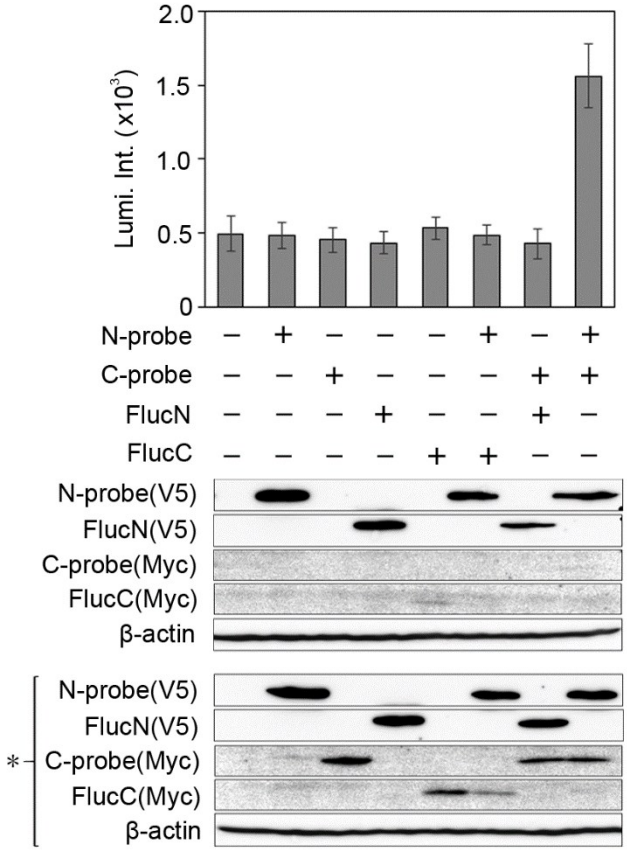
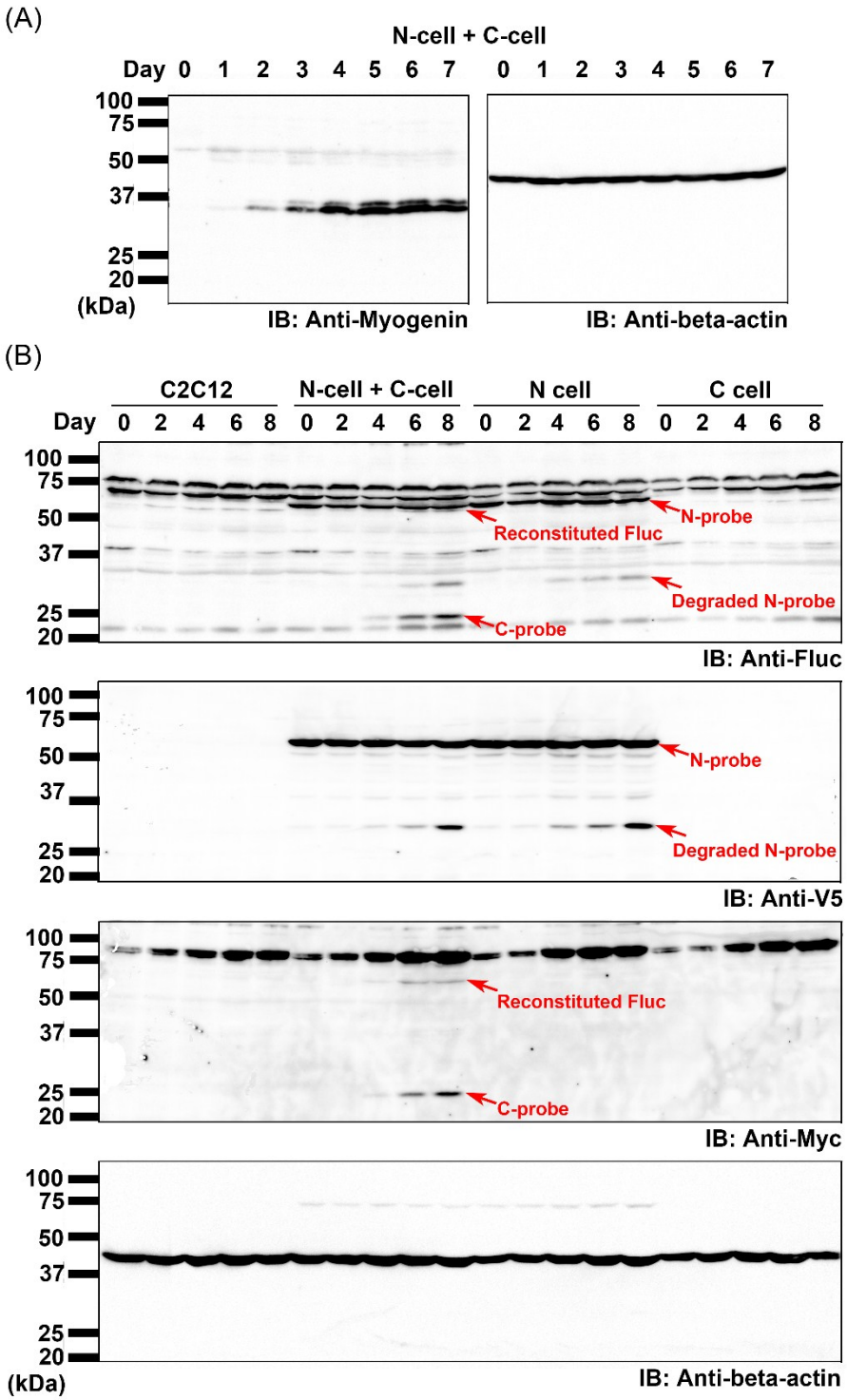


Fig. S1 The luminescence intensities (Mean±SD, *n*=3) generated by the C2C12 cells being introduced N-probe, C-probe, FlucN and FlucC in varying combinations. The genes of each components were introduced into C2C12 cells by retroviral infection. In the case where the components are introduced in pairs, an equal amount of retrovirus for each component was used. And the total amount of retrovirus used for infection was consistent among the test groups except for the intact C2C12 cell control. After infection, the cells were cultured to full confluence in 3.5-cm dishes and then the luminescence signals were determined for each dish by adding 2 mL of D-luciferin (1.0 mmol L^{-1}) and counting the signal for 30 s. The components expressed in each group were analyzed by western blot via corresponding V5-tag or Myc-tag. The sample treated by MG-132 (20 μmol L^{-1}, 4 hours) were also analyzed

by western blotting (*) to prove the successfully introduction of degraded C-probe and FlucC.



35 **Fig. S2** Raw data of the western blotting analysis of proteins expressed and produced in the

36 C2C12 cells, N-cells and C-cells. (A) Raw data of the western blotting analysis of the

expression level of myogenin in the mixture of N- and C-cells on varying differentiation days (Days 0–7). Myogenin was blotted with anti-myogenin antibody. As a reference for the amounts of the proteins in electrophoresis, beta-actin was stained with its specific antibody; (B) Raw data of the western blotting analysis of the expression of N-probe and C-probe, and the generation of reconstituted Fluc in the intact C2C12 cell, the N-cell, the C-cell and the mixture of the N- and C-cells on varing differentiation days (Days 0, 2, 4, 6, and 8). The N-probe (63 kDa) was blotted with anti-V5. The C-probe (21 kDa) and the reconstituted Fluc (63 kDa) were blotted with anti-Myc antibody. The probes and the reconstituted Fluc were also blotted with anti-Fluc polyclonal antibody for further confirmation. Beta-actin was blotted as a reference for the amounts of the proteins in electrophoresis.

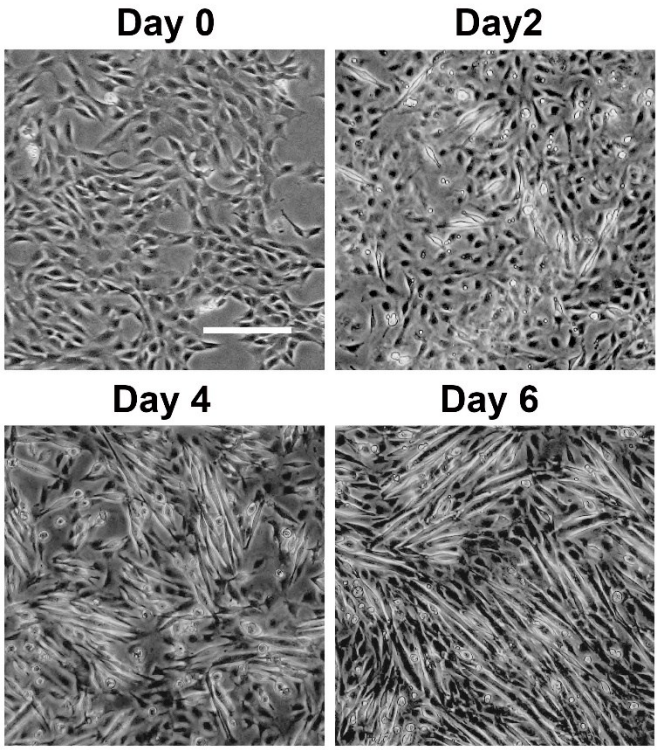


Fig. S3 Myotubes formation in the C2C12 cells on various differentiation days (Days 0, 2, 4, and 6). Scale bar: 100 μm.

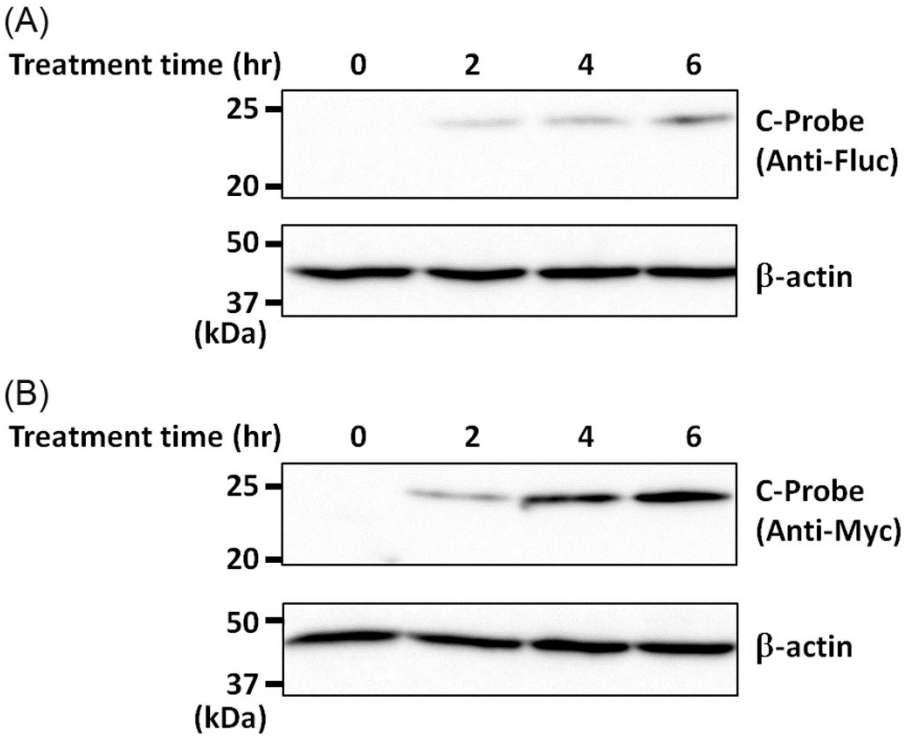


Fig. S4 C-probes detected from the C-cells treated by MG-132. The C-cells were treated by 20 μmol L^{-1} MG-132 for 0, 2, 4 and 6 hours and blotted with anti-Fluc antibody (A) and anti-Myc antibody (B), respectively. β-actin was blotted as a reference for the amounts of the proteins in electrophoresis.

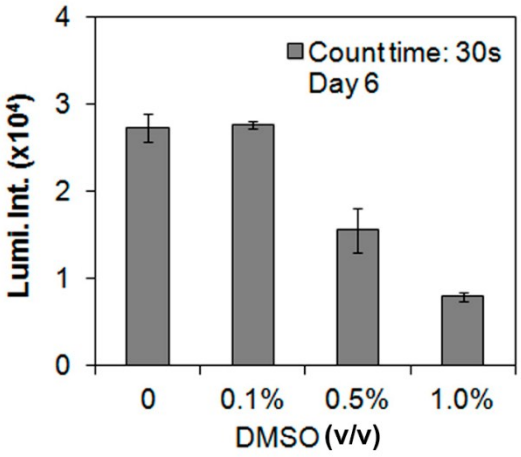
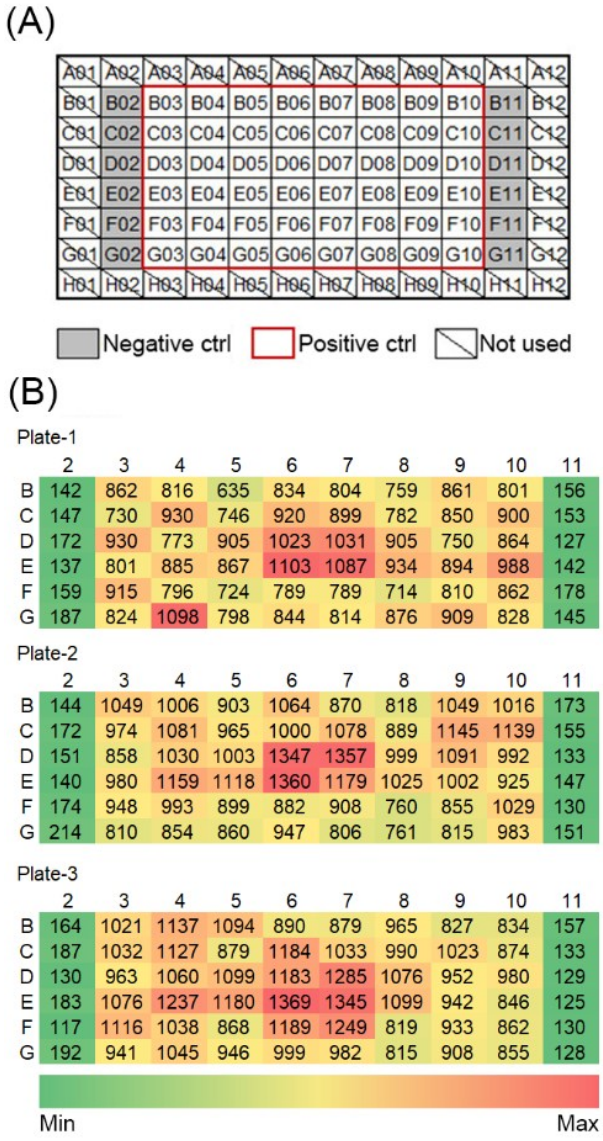


Fig. S5 Luminescence signals generated by the mixed cell cultures of N-cells and C-cells treated with various concentrations of DMSO. DMSO was added to the differentiation medium in final concentrations of 0, 0.1, 0.5, 1.0% (v/v) and luminescence intensities (±SD, *n*=2) were measured on Day 6.



65 **Fig. S6** Validation of a high-throughput screening for myogenesis-promoting compounds.
66 (A) The layout of the 96-well plate for the assay. Gray wells are used for the negative control.
67 Wells within the red box are used for positive controls. The outer wells were not used for the
68 assay; (B) Heatmaps of the luminescence intensity measured on each test plates.

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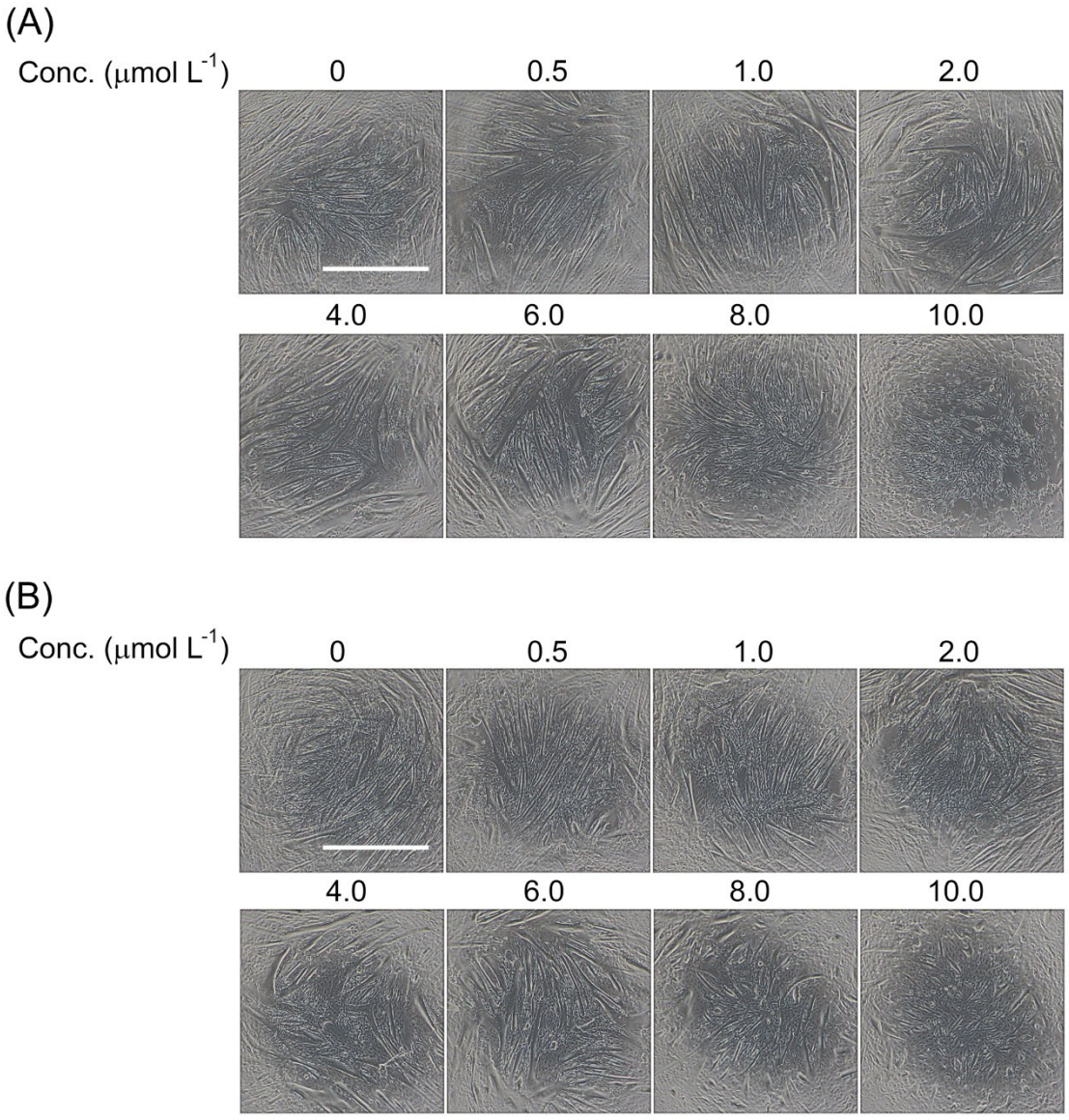

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71 **Fig. S7** Phase-contrast images of the C2C12 cells respectively treated with varying

72 concentrations of Imatinib (A) and Doxazosin mesylate (B). Scale bar: 500 μm.

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Table S1. The parameters evaluated for the 96-well plate-based screening method.

Plate No.		I_{Avg}	I_{SD}	CV (%)	S/B	Z'-factor
1	-	154	18	**11**	**5.6**	**0.50**
	+	859	100	**12**		
2	-	157	23	**15**	**6.3**	**0.41**
	+	991	141	**14**		
3	-	148	27	**18**	**6.9**	**0.42**
	+	1022	143	**14**		

"-": negative ctrl;

"+": positive ctrl;

I_{Avg}: mean of luminescence intensity;

I_{SD}: standard deviation of luminescence intensity

CV: coefficient of variation

S/B: signal to background ratio

$$CV(\%) = \frac{I_{SD}}{I_{Avg}} \times 100\%$$

$$S/B = \frac{I_{Avg}^{C+}}{I_{Avg}^{C-}}$$

$$Z^{'} - factor = 1 - \frac{3(I_{SD}^{C+} - I_{SD}^{C-})}{\left|I_{Avg}^{C+} - I_{Avg}^{C-}\right|}$$