SUPPLEMENTARY MATERIALS

SUPPLEMENTARY FIGURE LEGEND

Supplementary Figure S1. IGF-I-induced activation of ERK1/2 is suppressed by ephrinA/EphA signal, but not ephrinB/EphB signal. (A) Serum-starved C2C12 myoblasts were stimulated for 10 min with IGF-I at the concentrations indicated at the top in the presence (+) or absence (-) of 1.7 nM ephrinA1-Fc (A1-Fc). Cell lysates were subjected to Western blot analysis with anti-phospho-ERK1/2 (p-ERK1/2), anti-ERK1/2 (ERK1/2), anti-phospho-AKT (p-AKT) and anti-AKT (AKT) antibodies as indicated at the left. (B) Serum starved L6 myoblasts were stimulated for 10 min with or without 1 nM IGF-I in the presence or absence of 2.6 nM Fc (Fc) or ephrinA1-Fc (A1-Fc) as indicated at the top. Cell lysates were subjected to Western blot analysis as described in A. (C) Serum-starved C2C12 myoblasts were stimulated for 10 min with or without 10 nM IGF-I in the presence or absence of 1.7 nM Fc (Fc), ephrinB1-Fc (B1-Fc), or ephrinA1-Fc (A1-Fc) as indicated at the top. Cell lysates were subjected to Western blot analysis as described in A. (D) Serum-starved human umbilical vein endothelial cells were stimulated for 15 min with 200 ng/ml COMP-Ang1 (C-Ang1) in the presence or absence of 1.7 nM Fc (Fc), ephrinB1-Fc (B1-Fc), or ephrinA1-Fc (A1-Fc) as indicated at the top. Cell lysates were subjected to Western blot analysis with anti-phospho-ERK1/2 (p-ERK1/2) and anti-ERK1/2 (ERK1/2) antibodies as indicated at the left.

Supplementary Figure S2. IGF-I-induced myogenic differentiation is facilitated by ephrinA/EphA signal. (A) Confluent C2C12 cells were differentiated into myotubes in DMEM containing 1% FBS and IGF-I at the concentrations indicated at the top in the presence of either 6.4 nM Fc (A1-Fc: -) or ephrinA1-Fc (A1-Fc: +) for 3 days. The cells were then fixed, immunostained with anti-MHC antibody, and visualized with Alexa Fluor 488-conjugated secondary antibody. The cells were also post-stained with Hoechst 33342 to visualize the nuclei. Alexa 488 (MHC), bright field (BF), and Hoechst 33342 (Hoechst) images are shown as indicated at the left. Scale bars, 100 μm. (B) Confluent C2C12 myoblasts were differentiated into myotubes as described in A. Cell lysates were subjected to Western blot analysis using anti-MHC (MHC) and anti-AKT (AKT) antibodies as indicated at the left.

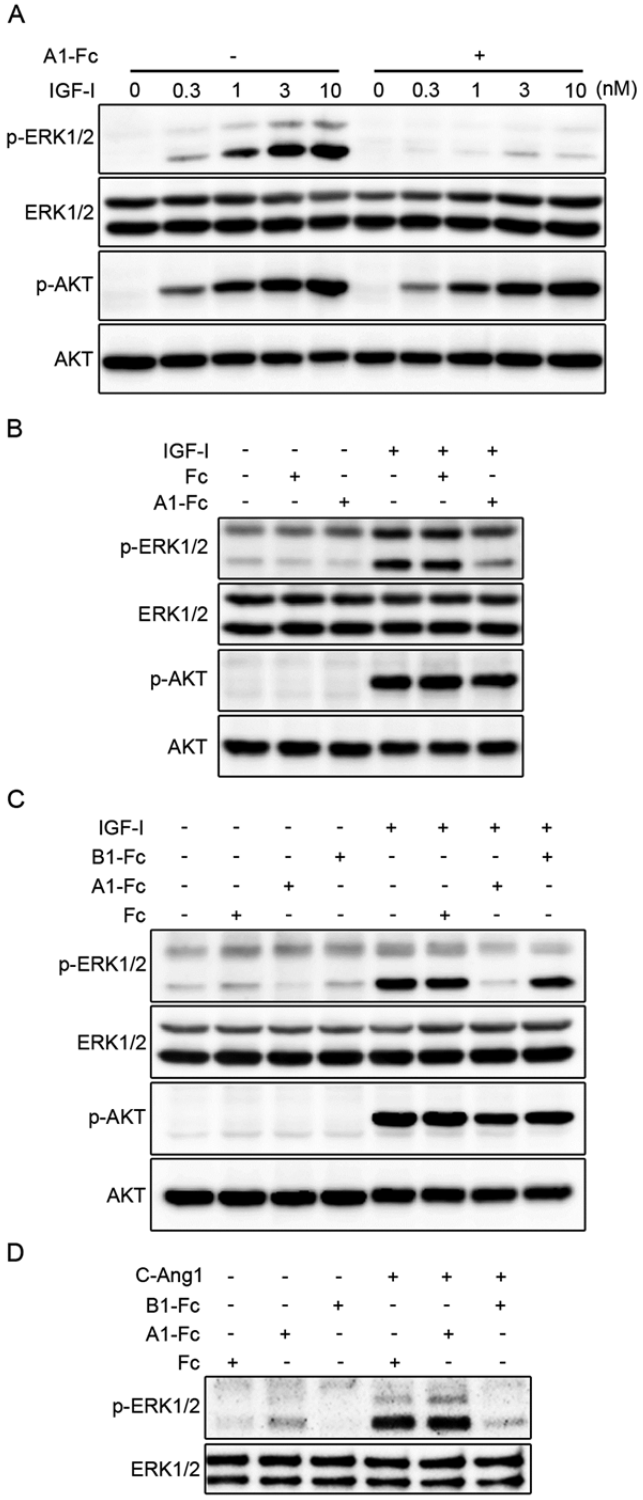
Supplementary Figure S3. IGF-I-induced myogenic differentiation is facilitated by ephrinA/EphA signal. C2C12 myoblasts express multiple members of ephrinA ligands and EphA receptors. Reverse transcription-PCR analysis was performed to examine the expression of ephrinA ligands and EphA receptors in C2C12 myoblasts. Total RNA was isolated from C2C12 myoblasts, and reverse-transcribed to produce cDNA. PCR was performed with (C2C12) or without (-) cDNA template using specific primers for the genes indicated at the left. Sequences of the primers are listed in the Supplementary Table S1.

Supplementary Figure S4. Inhibitory effect of ephrinA1-Fc on IGF-I-induced activation of ERK1/2 is canceled by overexpression of EphA2Δcyto. L6 myoblasts infected with adenoviruses encoding either β-gal or EphA2Δcyto were serum-starved, and stimulated for 10 min with (+) or without (-) 1 nM IGF-I in the presence (+) or absence (-) of 2.6 nM ephrinA1-Fc (A1-Fc) as indicated at the top. Cell lysates were subjected to Western blot analysis with anti-phospho-ERK1/2 (p-ERK1/2), anti-ERK1/2 (ERK1/2), anti-phospho-AKT (p-AKT), anti-AKT (AKT), and anti-HA (EphA2Δcyto) antibodies as indicated at the left.

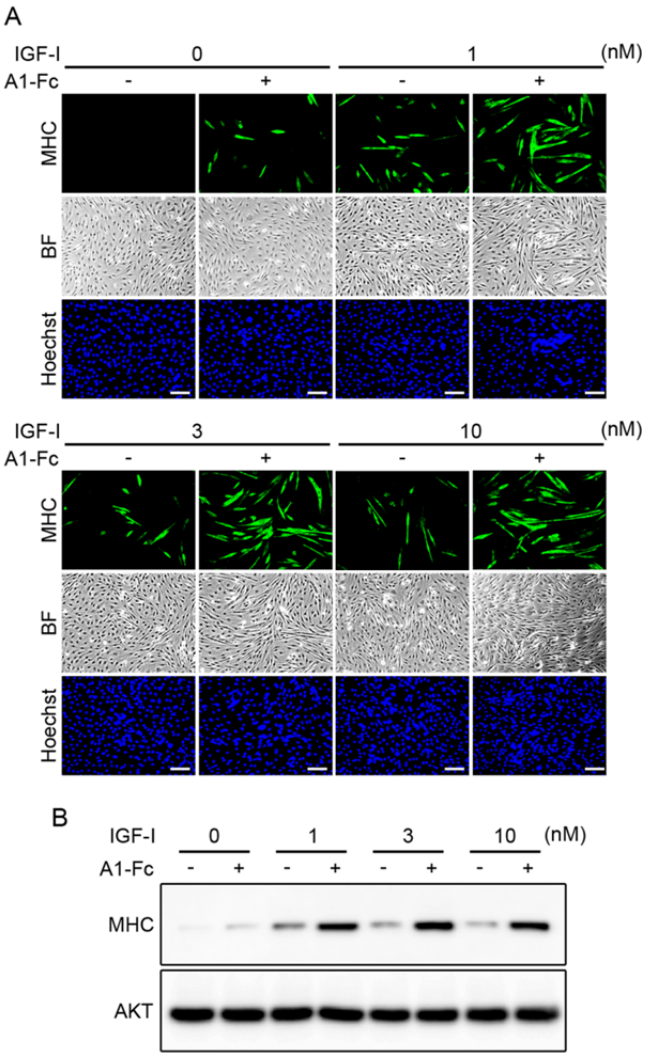
Supplementary Figure S5. EphrinA/EphA signal promotes IGF-I-induced myogenic differentiation of L6 myoblasts by suppressing the ERK1/2 cascade. (A) L6 myoblasts infected without (-) or with adenoviruses encoding either β-gal or EphA2Δcyto were differentiated into myotubes in DMEM containing 1% FBS and 1 nM IGF-I in the presence of vehicle (DMSO) or 3 μM U0126 (U0126) for 3 days. The cells were immunostained with anti-MHC antibody, and visualized with Alexa Fluor 488-conjugated secondary antibody. The cells were also post-stained with Hoechst 33342 to visualize the nuclei. Alexa 488 (MHC), bright field (BF), and Hoechst 33342 (Hoechst) images are shown as indicated at the left. Scale bars, 100 μm. (B) Adenovirus-infected L6 myoblasts were differentiated into myotubes as described in A for time periods (day) indicated at the top. Cell lysates were subjected to Western blot analysis using anti-MHC (MHC), anti-HA (EphA2Δcyto), and anti-AKT (AKT) antibodies as indicated at the left.

Supplementary Figure S6. EphrinA/EphA signal suppresses ERK1/2 activity during IGF-I-induced myogenic differentiation. C2C12 myoblasts infected without (-) or with adenoviruses encoding either β-gal or EphA2Δcyto were differentiated into myotubes in DMEM containing 1% FBS, 10 nM IGF-I and 3.2 nM ephrinA1-Fc for time periods (day) indicated at the top. Cell lysates were subjected to Western blot analysis using anti-phospho-ERK1/2 (p-ERK1/2), anti-ERK1/2 (ERK1/2), anti-phospho-AKT (p-AKT), anti-AKT (AKT), and anti-HA (EphA2Δcyto) as indicated at the left.

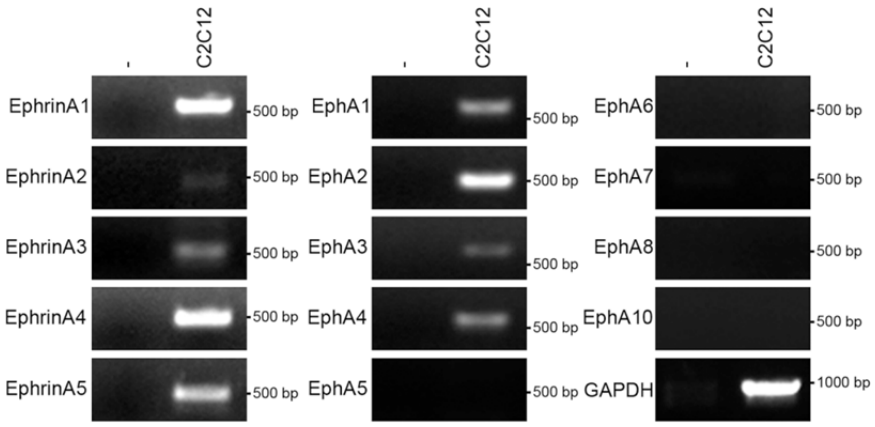
Supplementary Figure S1. Minami et al.



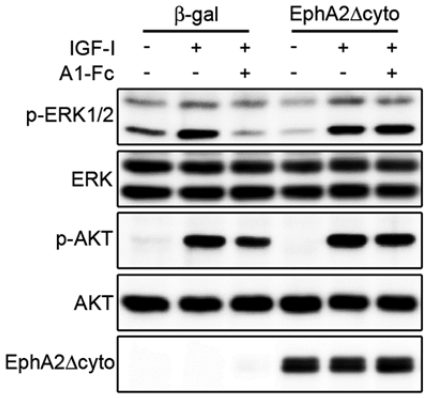
Supplementary Figure S2. Minami et al.



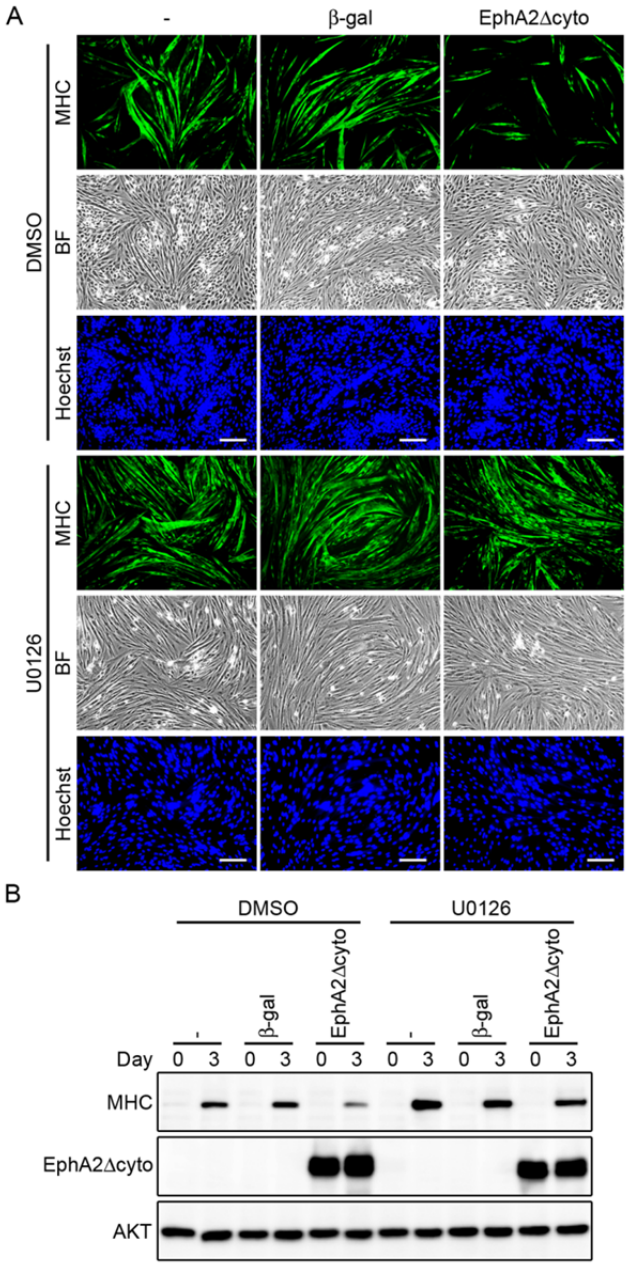
Supplementary Figure S3. Minami et al.



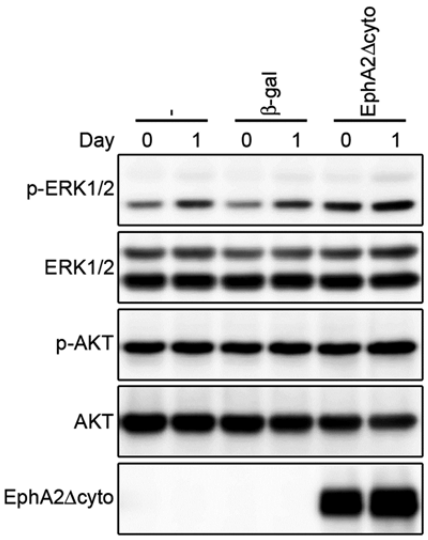
Supplementary Figure S4. Minami et al.



Supplementary Figure S5. Minami et al.



Supplementary Figure S6. Minami et al.



Supplementary Table S1. Minami et al.

Gene	Sequence
EphrinA1	5′-GTTCAAATCCCAAGTTCCGTGAG-3′ 5′-CAGCAGCAGTGGTACGAGCAATAC-3′
EphrinA2	5′-ACCGATACGCAGTCTACTGGAACC-3′ 5′-GTTACTGGTGAAGATGGGCTCTGG-3′
EphrinA3	5′-TGCAGGTGAACGTGAACGACTATC-3′ 5′-GTCCCACTGATGCTCTTCTCAAGC-3′
EphrinA4	5′-GTGGAGCTGGGCTTCAACGATTAC-3′ 5′-AACTCTCAGGAGACGGAGGATTGG-3′
EphrinA5	5′-GAGGACTCTGTCCCAGAAGACAAG-3′ 5′-AAAGCATCGCCAGGAGGAACAGTA-3′
EphA1	5′-CAGCCTTACGCCAACTACACATTTAC-3′ 5′-GTCCACATAGGGTTTTAGCCACAG-3′
EphA2	5′-ACTGGGAACTGTCAAACCACGAGG-3′ 5′-GCTCTTCAAGTATTGTTGGGCACG-3′
EphA3	5′-GATTGTCACCTCTCCATCCTCGTC-3′ 5′-GCCAGATTCTTCACTGTAAACGGG-3′
EphA4	5′-AGAACGGCTCCTTGGATGCTTTCC-3′ 5′-CTGTTGGGATTGCGGATGAGTTTG-3′
EphA5	5′-GCTCTGGTTTCTGTCCGTGTCTAC-3′ 5′-CACTGCCAACAAGATGACTCCCAC-3′
EphA6	5′-AGGAGAAGAGAACCAACCAGAGCC-3′ 5′-AAAACTCGGACTGAGACCAGAGCG-3′
EphA7	5′-AAGCGACAGCAGTCTCCAGTGAACAG-3′ 5′-ATCCCAGCGGCAATACCTCTCAAC-3′
EphA8	5′-CGACATCACCTACAACGCAGTGTG-3′ 5′-TCTCTTCATCCGAGTCCTGGAACG-3′
EphA10	5′-CCGAAGAAGTTATCCTCCTGGACTC-3′ 5′-ACTGCTTGTAGTAGACACGCACCG-3′
GAPDH	5′-TGAAGGTCGGTGTGAACGGATTTGG-3′ 5′-CATGTAGGCCATGAGGTCCACCAC-3′