

Supplementary Information

A small-molecule screening strategy to identify suppressors of statin myopathy

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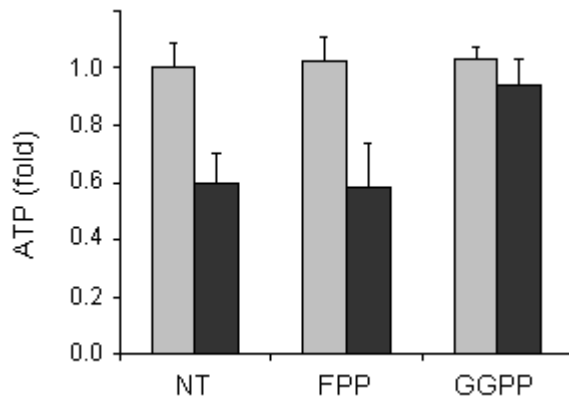
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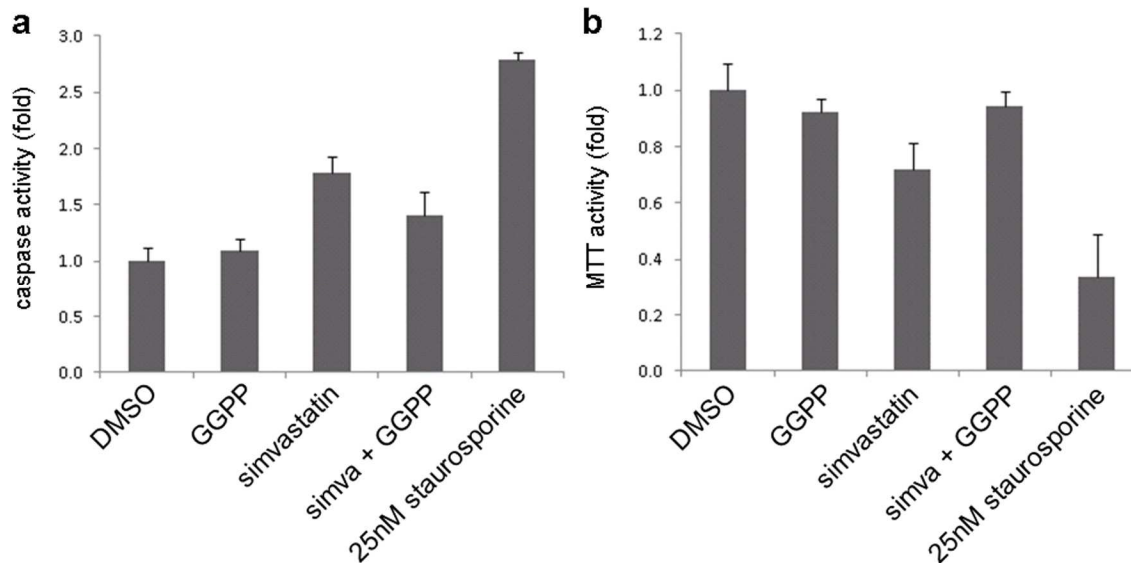
One-sentence summary:

A cell-based assay for skeletal muscle ATP levels resulted in the identification of a small-molecule suppressor of statin-induced toxicity, and suggests a role for Rab prenylation.

Supplementary Figure 1

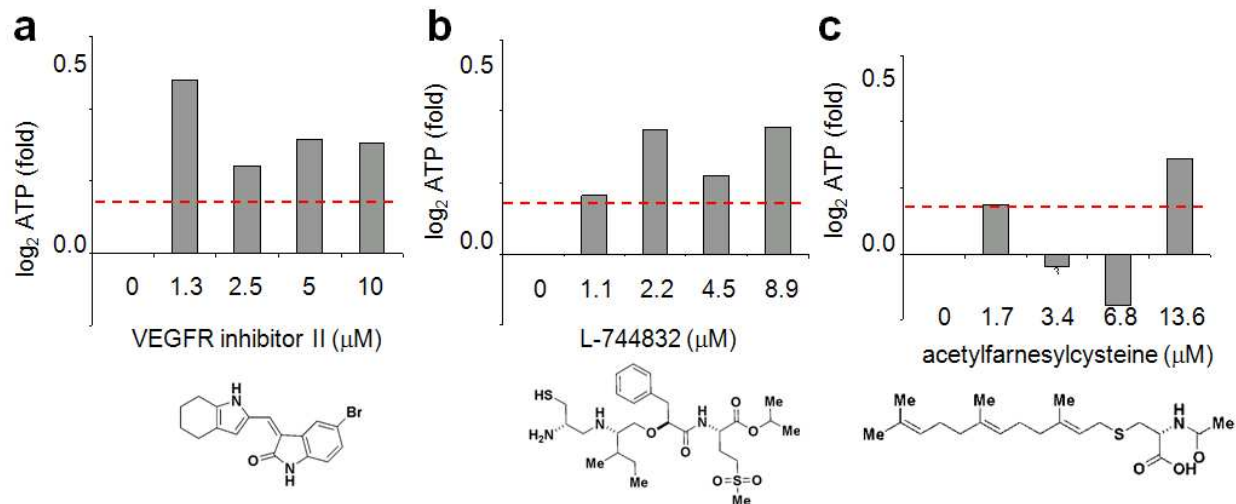
Suppression of the statin-mediated inhibition of ATP levels in C2C12 myotubes by geranylgeranylpyrophosphate (GGPP), but not farnesylpyrophosphate (FPP). Cells were treated with either DMSO (gray bars) or 10 μ M simvastatin (black bars), in the absence or presence of either 10 μ M FPP or 10 μ M GGPP. ATP levels were measured with the CellTiter-Glo kit (Promega).

Supplementary Figure 2



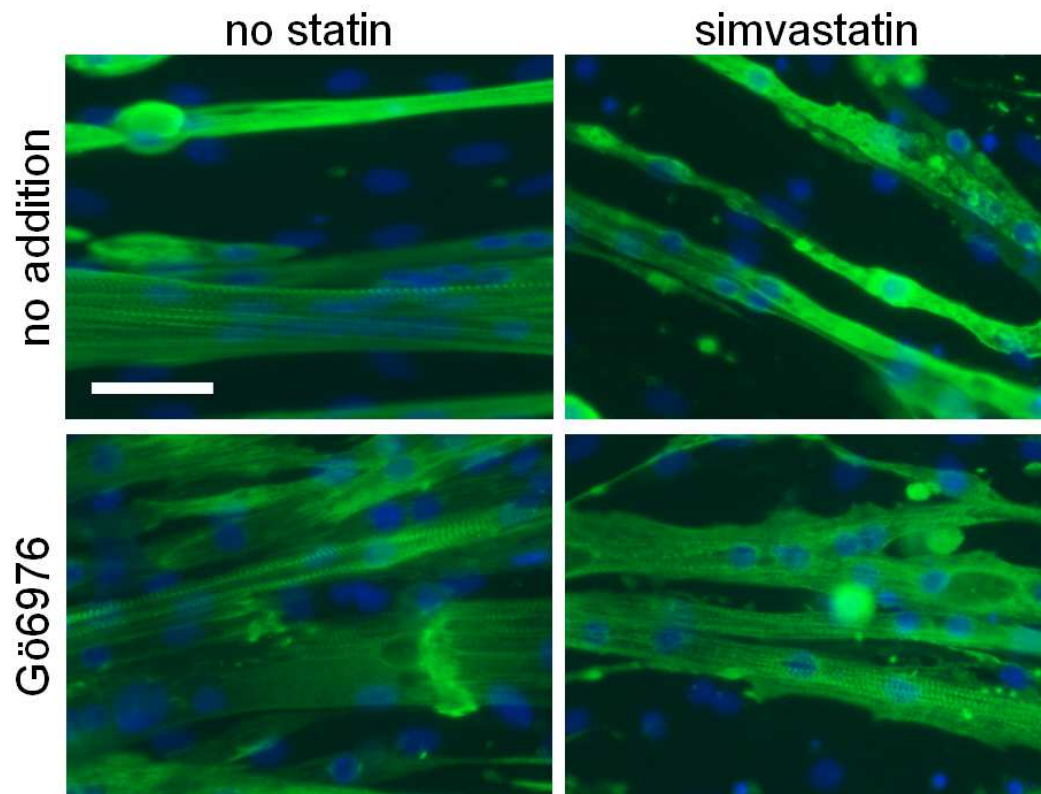
(a) Suppression of the statin-mediated induction of apoptosis in C2C12 myotubes, as measured by caspase-3/7 activity by geranylgeranylpyrophosphate (GGPP). Cells were treated as indicated for 48 hours. Staurosporine was added as a positive control. Caspase activity was measured with the Caspase-Glo kit (Promega). **(b)** Suppression of the statin-mediated decrease in MTT activity in C2C12 myotubes by geranylgeranylpyrophosphate (GGPP). Cells were treated as indicated for 48 hours. Staurosporine was added as a positive control. Data represent the mean and standard deviation of six independent wells.

Supplementary Figure 3



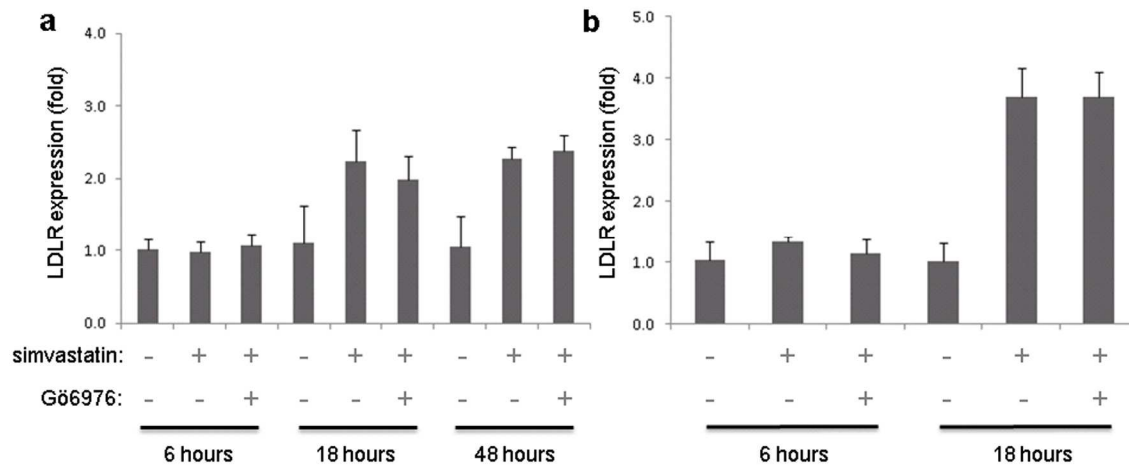
Dose-dependent suppression of ATP inhibition by simvastatin. Four screening positives were identified: **(a)** VEGF receptor inhibitor II; **(b)** L-744832, a farnesyltransferase inhibitor; **(c)** *N*-acetylfarnesylcysteine, an inhibitor of the methylation of endogenously isoprenylated proteins. The data for Gö6976 is shown and discussed in the main text. Dotted red line indicates the noise threshold, represented as the standard deviation of DMSO wells.

Supplementary Figure 4



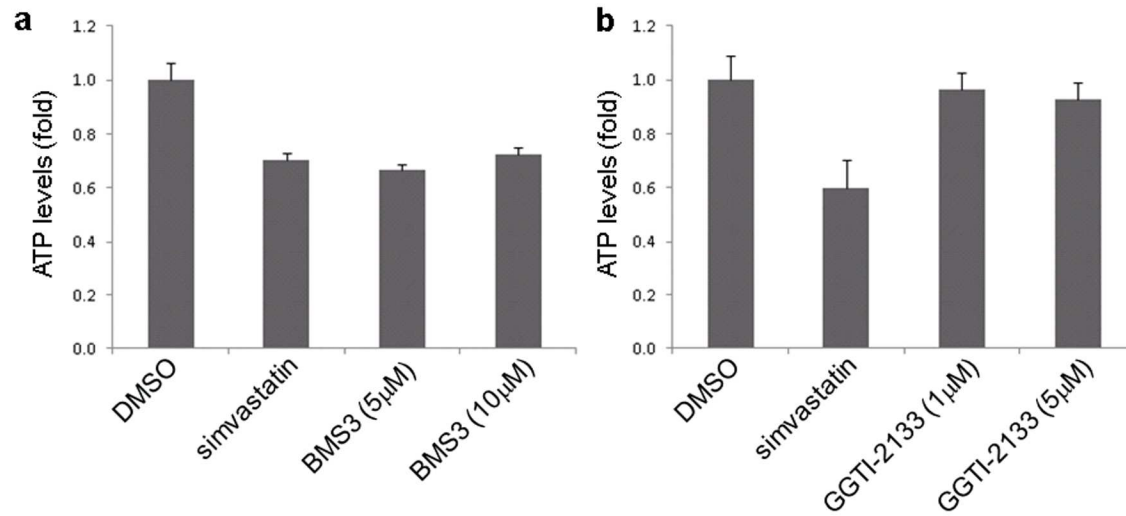
Myosin heavy chain immunofluorescence of C2C12 myotubes treated with or without 5 μ M simvastatin in the absence or presence of 2.5 μ M Gö6976. Scale bar = 50 μ m.

Supplementary Figure 5

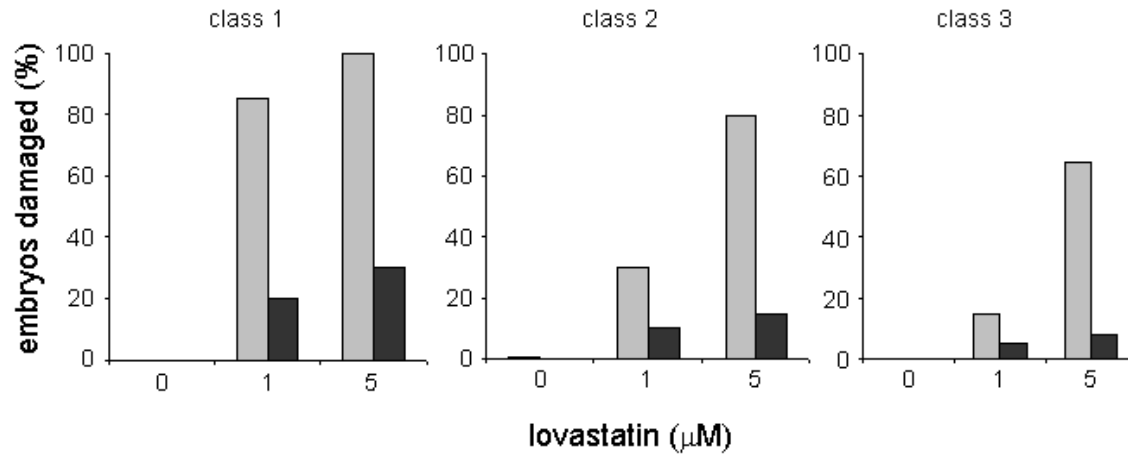


(a) Effect on LDLR expression in HepG2 cells of simvastatin and Gö6976 treatment. Cells were treated for the indicated times, and RNA extracted for qPCR analysis. Actin was used as internal control gene expression. **(b)** Effect on LDLR expression in C2C12 myotubes of simvastatin and Gö6976 treatment. Cells were treated for the indicated times, and RNA extracted for qPCR analysis. Actin was used as internal control gene expression. All data normalized to 6-hour control treatment within each cell type. Data represent the mean and standard deviation of three biological and three technical replicates each.

Supplementary Figure 6

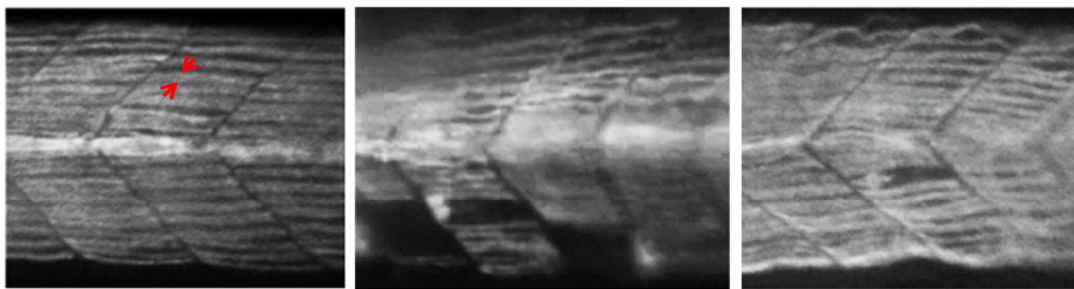


Effect of geranylgeranyltransferase inhibitor treatment in C2C12 myotubes on cellular ATP levels. Cells were treated 48 hours as indicated: **(a)** GGT-II inhibitor BMS3, or **(b)** GGT-I inhibitor GGTI-2133. Data represent the mean and standard deviation of four independent wells.

Supplementary Figure 7

Quantification of muscle-fiber damage in zebrafish caused by 12-hour treatment with the indicated concentrations of lovastatin alone (gray bars), and its suppression in the presence of 10 μM Gö6976 (black bars). Class 1: bowing, gap formation, disrupted fibers; class 2: irregular fibers, diffuse appearance; class 3: irregular somite boundaries. Data represent percentage of embryos with each class defect; 100 embryos/group.

Supplementary Figure 8



lovastatin:	-	+	+
Gö6976:	-	-	+

Enlarged image (from Figure 2, panel e) of zebrafish embryos treated with DMSO alone, 1 μ M lovastatin, or 1 μ M lovastatin and 10 μ M Gö6976.