SUPPLEMENTAL MATERIALS

Supplemental materials and methods

N-glycosidase treatment of MEGF10. For N-glycosidase treatment, wild type MEGF10 and C774R mutant MEGF10 were overexpressed in HEK293T cells, then 2.5 μg of protein were denatured and treated with *N*-glycosidase (PNGase F, New England BioLabs) at 37°C for 5 hours according to the manufacturer's protocol. Glycosidase treated proteins were lysed with SDS-PAGE sample buffer then subjected to western blot analysis.

Supplemental Table 1. Primers used for cloning and mutagenesis.

	forward	Reverse
human MEGF10 cloning primer	CACCCTCGAG ATGGTTATTTCTTTGAACTCATGCCTG	GGTACCTTCACTGCTGCTGCTGCTGTT
deletion 1 mutagenesis	ATGGGAAAATCCTTAAAAGACCTGG	AATAATGAACAATGCCAGTAGGAAGAGAA
deletion 2 mutagenesis	GACCTCCCAAAGAACAGTCACATC	TTCAGAATTCTTTCCCAGGTCTTTTAAG
Y906F mutagenesis	CGTCAATGCAGATTTTACCATTTCAGGAACCC	GGGTTCCTGAAATGGTAAAATCTGCATTGACG
Y930F mutagenesis	CACCAATCCCAGTTTCCACACGCTCACCC	GGGTGAGCGTGTGGAAACTGGGATTGGTG
Y1016F mutagenesis	CTGGGAAAGAATTCTGAATTTAATTCAAGTAACTGCTC	GAGCAGTTACTTGAATTAAATTCAGAATTCTTTCCCAG
Y1030F mutagenesis	CTGAGAACCCATTTGCCACTATTAAAGACCC	GGGTCTTTAATAGTGGCAAATGGGTTCTCAG
Y1048F mutagenesis	GCTCAGAGTGTGGTTTTGTGGAGATGAAATCG	CGATTTCATCTCCACAAAACCACACTCTGAGC
Y1061F mutagenesis	GAAGAGATTCCCCATTTGCAGAGATCAATAAC	GTTATTGATCTCTGCAAATGGGGAATCTCTTC
Y1075F mutagenesis	CAGGAATGTCTTTGAAGTTGAACCTACAGTG	CACTGTAGGTTCAACTTCAAAGACATTCCTG
Y1099F mutagenesis	CTCCCAGGATCCATTTGACCTCCCAAAG	CTTTGGGAGGTCAAATGGATCCTGGGAG
Y1111F mutagenesis	CATCCCTTGTCATTTTGACCTGCTGCCAG	CTGGCAGCAGGTCAAAATGACAAGGGATG
Y1030D mutagenesis	CTGAGAACCCAGATGCCACTATTAAAGACCC	GGGTCTTTAATAGTGGCATCTGGGTTCTCAG
C759S mutagenesis	GATTGTGCACTGATATCCCAATGTCAAAACGG	CCGTTTTGACATTGGGATATCAGTGCACAATC
C761S mutagenesis	CACTGATATGCCAATCTCAAAACGGAGCTGAC	GTCAGCTCCGTTTTGAGATTGGCATATCAGTG
C767S mutagenesis	CAAAACGGAGCTGACTCCGACCACATTTCTG	CAGAAATGTGGTCGGAGTCAGCTCCGTTTTG
C776S mutagenesis	CTGGGCAGTGTACTTCCCGCACTGGATTC	GAATCCAGTGCGGGAAGTACACTGCCCAG
C785S mutagenesis	CATGGGACGGCACTCTGAGCAGAAGTG	CACTTCTGCTCAGAGTGCCGTCCCATG
c-src K297R mutagenesis	GGTGGCCATCAGAACCCTGAAGCCTGG	CCAGGCTTCAGGGTTCTGATGGCCACC

Supplemental figure 1

(A) Overexpression of wild type MEGF10, C774R mutant MEGF10, and Y1030F mutant MEGF10 in C2C12 cells. Proteins were immunoprecipitated with anti-V5 antibody and subjected to western blotting. Wild type MEGF10 is phosphorylated whereas C774R and Y1030F mutants show defective phosphorylation. (B) Wild type, C326R mutant, and C774R mutant MEGF10 were transfected into C2C12 cells and stained with anti-V5 antibody (green). Wild type and mutant MEGF10 proteins display similar patterns of subcellular localization. Scale bar: 10μm.

Supplemental figure 2

Wild type MEGF10 and empty vector were transfected into HEK293T cells. Cell lysates were immunoprecipitated with anti-P-Tyr antibody (PY20; BD Bioscience) and immunodetected with anti-P-Tyr or anti-V5 antibodies. V5-tagged MEGF10 was immunoprecipitated with anti-P-Tyr antibody.

Supplemental figure 3

Wild type MEGF10 and C774R mutant MEGF10 were transfected into HEK293T cells. Cell lysates were treated with *N*-glycosidase, then subjected to immunodetection with anti-V5 antibody. *N*-glycosidase treated wild type MEGF10 and C774R mutant MEGF10 show similar migration patterns at ~100kDa. PNGase: *N*-glycosidase.

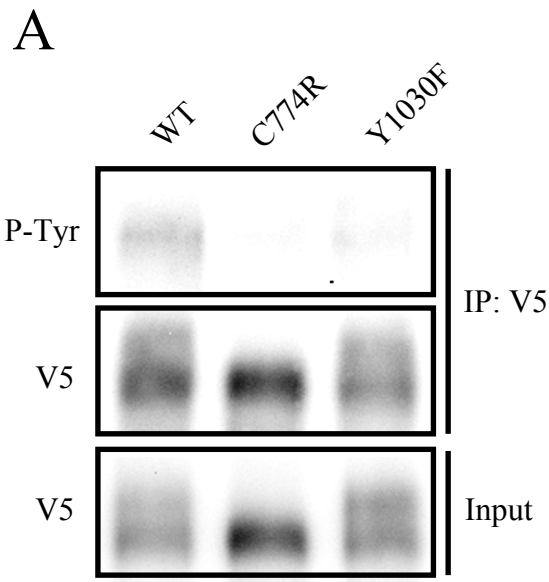
Supplemental figure 4

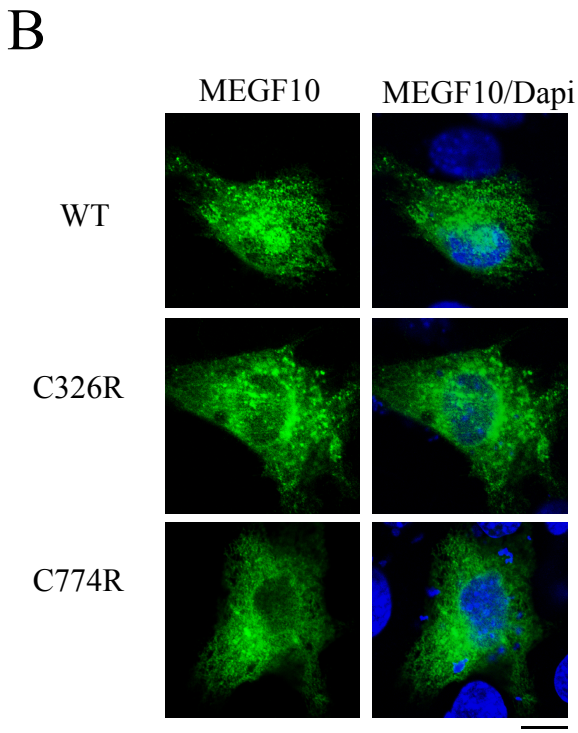
Wild type MEGF10 was co-transfected into HEK293T cells with pcDNA3.1-Myc vector (mock), Myc-tagged c-Src, or Myc-tagged dominant-negative c-Src (dn). Cell lysates were immunoprecipitated with anti-V5 antibody, then subjected to immunodetection with anti-P-Tyr or anti-V5 antibodies. Note that the applied protein amount is half of the amount in the experiments illustrated in Figures 1 and 2, as co-expression of c-Src causes a marked increase in tyrosine phosphorylation. Co-expression of dominant-negative c-Src (dn) with wild type MEGF10 shows decreased tyrosine phosphorylation compared to wild type MEGF10 co-expressed with empty vector. The dominant negative form of c-Src (dn) was not co-immunoprecipitated with MEGF10, whereas wild type c-Src was co-immunoprecipitated with MEGF10.

Supplemental figure 5

(A) C2C12 myoblasts were infected with lentiviral vectors to introduce wild type MEGF10, Y1030F mutant MEGF10, Y1030D mutant MEGF10, or empty vector. We quantified the percentage of Ki-67 positive cells by counting approximately 500 cells. Anti-Ki-67 (BD Pharmingen Inc.) was used for immunofluorescence analysis. Both wild type MEGF10 and Y1030D infected cells showed increased numbers of Ki-67 positive cells. (B) Fluorescent image of Ki-67 positive cells (green) showing the increase of cell proliferation in wild type MEGF10 and Y1030D mutant MEGF10 expressing C2C12 cells.

Supplementary figure 1





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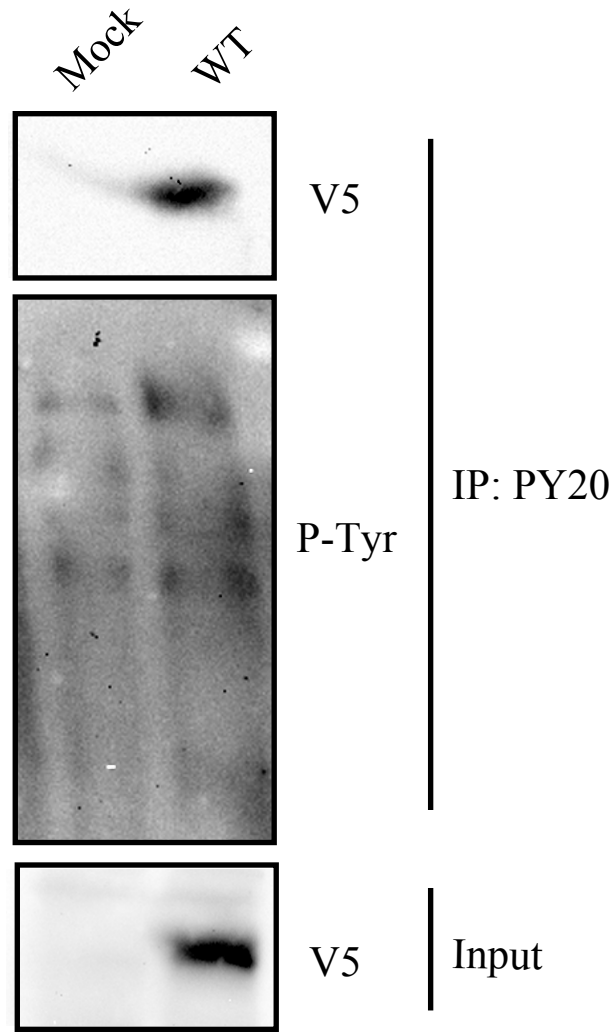

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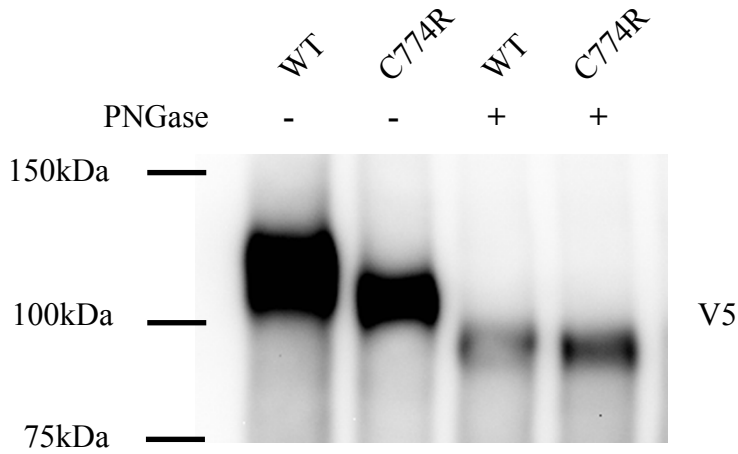

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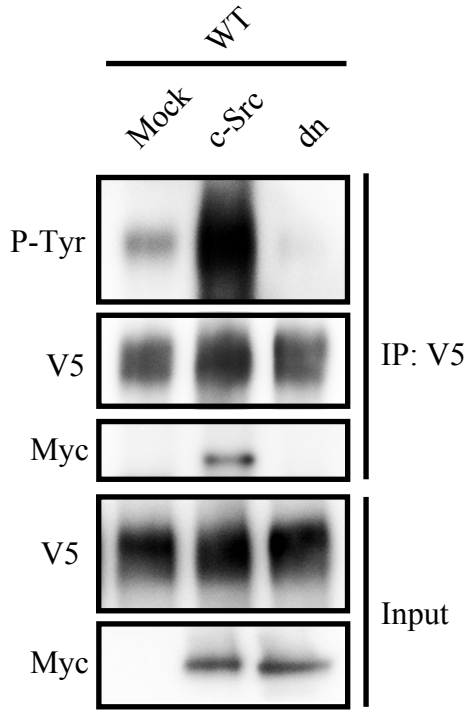
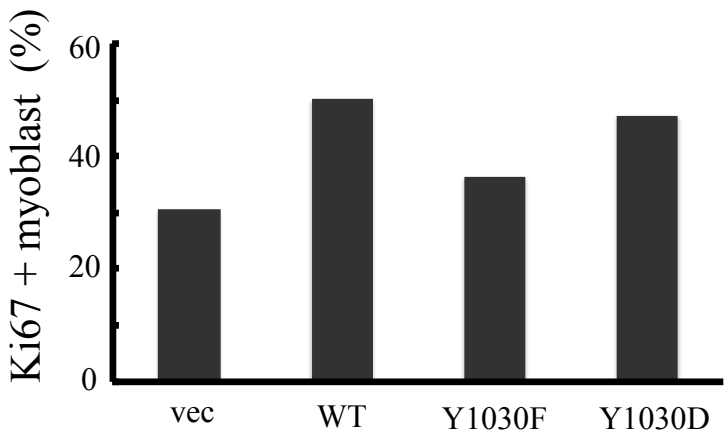


Supplementary figure 5

A



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