

Ring finger protein 145 (RNF145) is a ubiquitin ligase for sterol-induced degradation of HMG-CoA reductase

Supporting Information

SUPPLEMENTARY FIGURE 1. Screening for ER-localized ubiquitin ligases involved in sterol-induced degradation of HMGCR

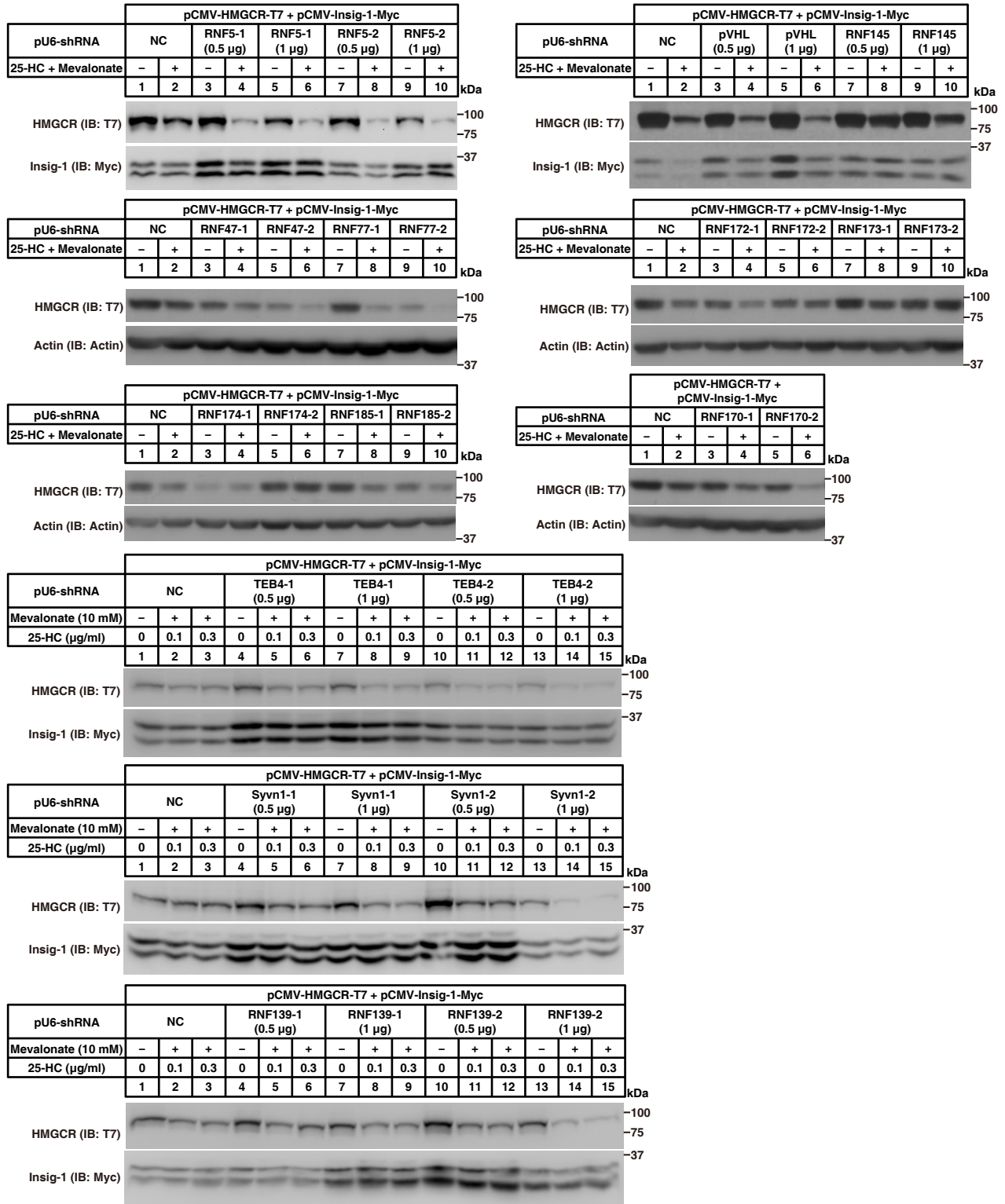
The *gp78*-KO CHO cells were transfected with plasmids encoding HMGCR-T7, Insig-1-Myc and the shRNAs targeting ubiquitin ligases (Supplementary table). After 48 h, the cells were depleted of sterol and then treated with or without 10 mM Mevalonate plus indicated concentrations of 25-HC for 5 hr. Cells were harvested and subjected to SDS-PAGE followed by immunoblot analysis.

SUPPLEMENTARY FIGURE 2. Knockout of *gp78* stabilized exogenous HMGCR and Insig-1

The WT and *gp78*-KO CHO cells were transfected with indicated plasmids, depleted of sterol and treated with or without 1 μ g/ml 25-HC plus 10 mM Mevalonate for 5 h. Cells were harvested and subjected to SDS-PAGE followed by immunoblot analysis.

SUPPLEMENTARY FIGURE 1

Screening for ER-localized ubiquitin ligases involved in sterol-induced degradation of HMGCR



SUPPLEMENTARY FIGURE 2
Knockout of *gp78* stabilized exogenous HMGCR and Insig-1

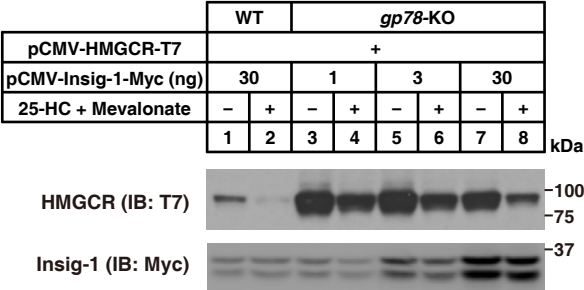


Table S1
Primers used to generate the plasmids expressing shRNAs

	Sequence
March6-1-F	CCGGGGAAAGAATGCTTGGGCTTGACTCGAGTCAAGCCAAGCATTCTTTCCTTTTTG
March6-1-R	AATTCAAAAAGGAAAGAATGCTTGGGCTTGACTCGAGTCAAGCCAAGCATTCTTTC
March6-2-F	CCGGGCTGGGAGTCTGCTATATTGTCTCGAGACAATATAGCAGACTCCCAGCTTTTTG
March6-2-R	AATTCAAAAAGCTGGGAGTCTGCTATATTGTCTCGAGACAATATAGCAGACTCCCAGC
Syvn-1-F	CCGGGCAGCTGGTGTGTTGGCTTTGACTCGAGTCAAAGCCAAACACCAGCTGCTTTTTG
Syvn-1-R	AATTCAAAAAGCAGCTGGTGTGTTGGCTTTGACTCGAGTCAAAGCCAAACACCAGCTGC
Syvn-2-F	CCGGGCTCTACACGGAGCTGTTTACCTCGAGGTAACAGCTCCGTGTAGAGCTTTTTG
Syvn-2-R	AATTCAAAAAGCTCTACACGGAGCTGTTTACCTCGAGGTAACAGCTCCGTGTAGAGC
RNF5-1-F	CCGGGCGCGACCTTCGAATGTAATACTCGAGTATTACATTCTGAAGGTCGCGCTTTTTG
RNF5-1-R	AATTCAAAAAGCGCGACCTTCGAATGTAATACTCGAGTATTACATTCTGAAGGTCGCGC
RNF5-2-F	CCGGGCTAGAGGAGAATCAGTATTGCTCGAGCAATACTGATTCTCCTCTAGCTTTTTG
RNF5-2-R	AATTCAAAAAGCTAGAGGAGAATCAGTATTGCTCGAGCAATACTGATTCTCCTCTAGC
RNF47-1-F	CCGGGCCCTCAGATTTCTGTGAATGCTCGAGCATTACAGAAATCTGAGGGCTTTTTG
RNF47-1-R	AATTCAAAAAGCCCTCAGATTTCTGTGAATGCTCGAGCATTACAGAAATCTGAGGGC
RNF47-2-F	CCGGGCCCTAGCTTTATGGTGGATGTCTCGAGACATCCACCATAAAGCTAGGCTTTTTG
RNF47-2-R	AATTCAAAAAGCCTAGCTTTATGGTGGATGTCTCGAGACATCCACCATAAAGCTAGGC
RNF77-1-F	CCGGGGATGTGATGGAGCTGCTTGACTCGAGTCAAGCAGCTCCATCACATCCTTTTTG
RNF77-1-R	AATTCAAAAAGGATGTGATGGAGCTGCTTGACTCGAGTCAAGCAGCTCCATCACATCC
RNF77-2-F	CCGGGGGAATGTGCGGAATTCATTGCTCGAGCAATGAATCCGCACATTCCCTTTTTG
RNF77-2-R	AATTCAAAAAGGGAATGTGCGGAATTCATTGCTCGAGCAATGAATCCGCACATTCCC
RNF139-1-F	CCGGGCTCTTCAGACTGGCTTAAGTCTCGAGACTTAAGCCAGTCTGAAGAGCTTTTTG
RNF139-1-R	AATTCAAAAAGCTCTTCAGACTGGCTTAAGTCTCGAGACTTAAGCCAGTCTGAAGAGC
RNF139-2-F	CCGGGGGCCTATACCATGATGTTTGCTCGAGCAAACATCATGGTATAGGCCCTTTTTG
RNF139-2-R	AATTCAAAAAGGGCCTATACCATGATGTTTGCTCGAGCAAACATCATGGTATAGGCC
VHL-F	CCGGGGTGTCTAAGGATAAACATCACTCGAGTGATGTTTATCCTTAAGCACCTTTTTG
VHL-R	AATTCAAAAAGGTGCTTAAGGATAAACATCACTCGAGTGATGTTTATCCTTAAGCAC
RNF145-F	CCGGGCAGCATCTGGTTCAGCTTTACTCGAGTAAAGCTGAACCAGATGCTGCTTTTTG
RNF145-R	AATTCAAAAAGCAGCATCTGGTTCAGCTTTACTCGAGTAAAGCTGAACCAGATGCTGC
RNF170-1-F	CCGGGGATGATTCAGTTATAGAAGGCTCGAGCCTTCTATAACTGAATCATCCTTTTTG
RNF170-1-R	AATTCAAAAAGGATGATTCAGTTATAGAAGGCTCGAGCCTTCTATAACTGAATCATCC
RNF170-2-F	CCGGGCTCGACAGCAGTTCTATACTCTCGAGAGTATAGAAGTCTGCTGTCGAGCTTTTTG
RNF170-2-R	AATTCAAAAAGCTCGACAGCAGTTCTATACTCTCGAGAGTATAGAAGTCTGCTGTCGAGC
RNF172-1-F	CCGGGCACTGAGAAGCGGACATTGTCTCGAGACAATGTCCGCTTCTCAGTGCTTTTTG
RNF172-1-R	AATTCAAAAAGCACTGAGAAGCGGACATTGTCTCGAGACAATGTCCGCTTCTCAGTGC
RNF172-2-F	CCGGGGAGGAAGACCAATCAGAAAGCTCGAGCTTTCTGATTGGTCTTCTCCTTTTTG
RNF172-2-R	AATTCAAAAAGGAGGAAGACCAATCAGAAAGCTCGAGCTTTCTGATTGGTCTTCTCCTCC
RNF173-1-F	CCGGGCCACAGTATGTCATGCAAGTCTCGAGACTTGCATGACATACTGTGGCTTTTTG
RNF173-1-R	AATTCAAAAAGCCACAGTATGTCATGCAAGTCTCGAGACTTGCATGACATACTGTGGC
RNF173-2-F	CCGGGGACCAATCAGAGGGTATTCTCGAGGAATCACCTCTGATTGGTCTTTTTG
RNF173-2-R	AATTCAAAAAGGACCAATCAGAGGGTATTCTCGAGGAATCACCTCTGATTGGTCTCC
RNF174-1-F	CCGGGCCTCCTTCTGTGGAAGATGACTCGAGTCATCTTCCACAGAAGGAGGCTTTTTG
RNF174-1-R	AATTCAAAAAGCCTCCTTCTGTGGAAGATGACTCGAGTCATCTTCCACAGAAGGAGGC
RNF174-2-F	CCGGGCTGTGAGCTGTGTTACTACACTCGAGTGTAGTAACACAGCTCACAGCTTTTTG
RNF174-2-R	AATTCAAAAAGCTGTGAGCTGTGTTACTACACTCGAGTGTAGTAACACAGCTCACAGC
RNF185-1-F	CCGGGCTTCTCCATTAGAGCTTTGACTCGAGTCAAAGCTCTAATGGAGAAGCTTTTTG
RNF185-1-R	AATTCAAAAAGCTTCTCCATTAGAGCTTTGACTCGAGTCAAAGCTCTAATGGAGAAGC
RNF185-2-F	CCGGGCTGTAACACCCTCTATAACCTCGAGGTTATAGAGGGTGTTCACAGCTTTTTG
RNF185-2-R	AATTCAAAAAGCTGTAACACCCTCTATAACCTCGAGGTTATAGAGGGTGTTCACAGC