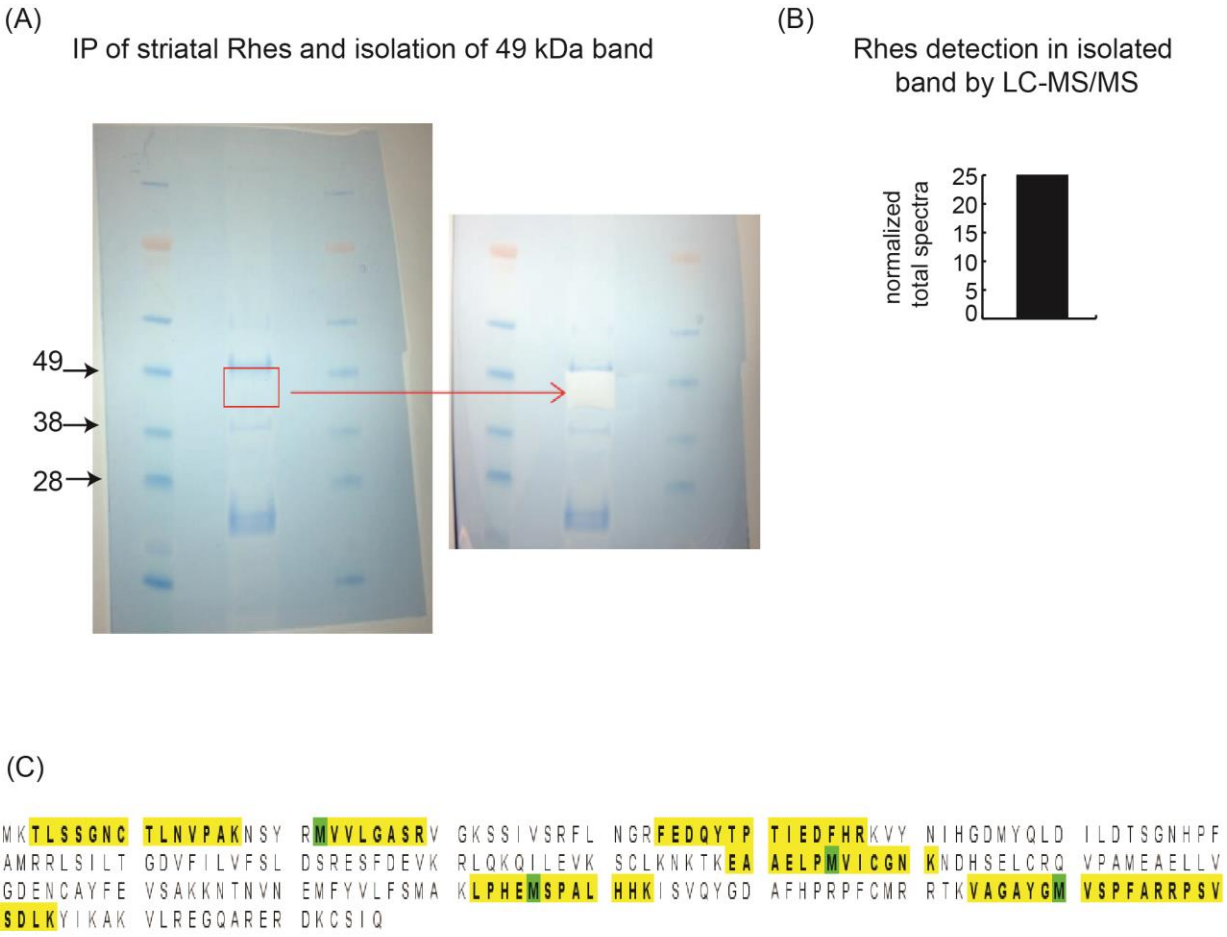

Fig. S1. Identification of 49-kDa Rhes using LC-MS/MS. (**A**) Coomassie-stained gel of Rhes-IP samples from the mouse striatum, and isolation of ~49 kDa band from the gel. (**B**) Rhes detection by LC-MS/MS. (**C**) Number of Rhes peptides detected: 8 exclusive unique peptides, 11 exclusive unique spectra, 30 total spectra and 34% coverage of the Rhes (also known as Rasd2) protein (90 out of 266 amino acids). Data are based on two independent experiments.

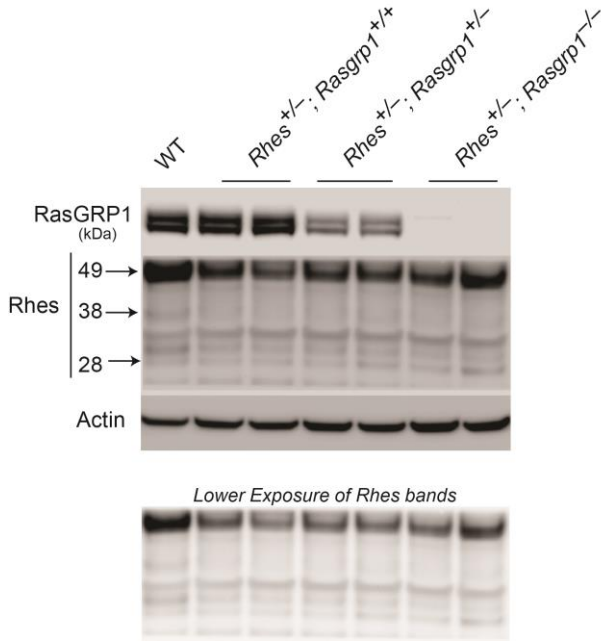

Fig. S2. Rhes protein abundance in the striatum of wild-type and RasGRP1 mutant mice. Western blotting analysis of Rhes protein abundance in lysates from the striatum of wild-type and *Rhes*$^{+/–}$/*Rasgrp1*$^{+/+}$, *Rhes*$^{+/–}$/*Rasgrp1*$^{+/–}$, and *Rhes*$^{+/–}$/*Rasgrp1*$^{–/–}$ mice. Data are representative of three independent experiments.

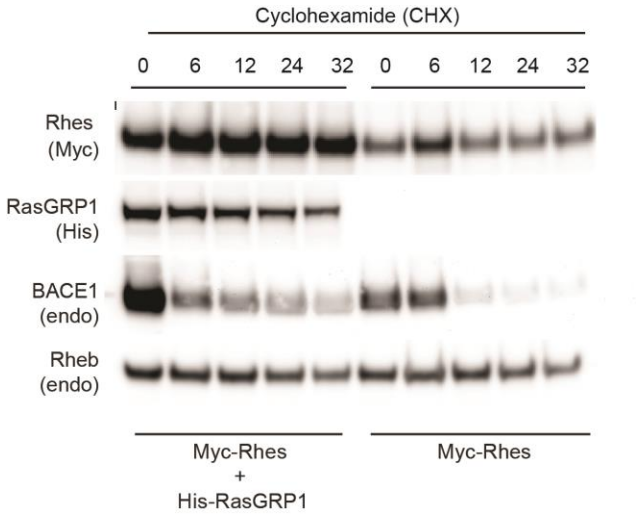

Fig. S3. Half-life of myc-tagged Rhes protein. Western blot analysis of Rhes (Myc), RasGRP1 (His), endogenous BACE1 and endogenous (endo) Rheb in cultured striatal cells (STHdh$^{Q7/Q7}$) transfected with Myc-Rhes in presence or absence of His-RasGRP1. After 36 h (0 h chase), the cycloheximide (100 µM) was added into the culture medium, and the cells were chased for 6, 12, 24 and 32 hours. Data are representative of two independent experiments

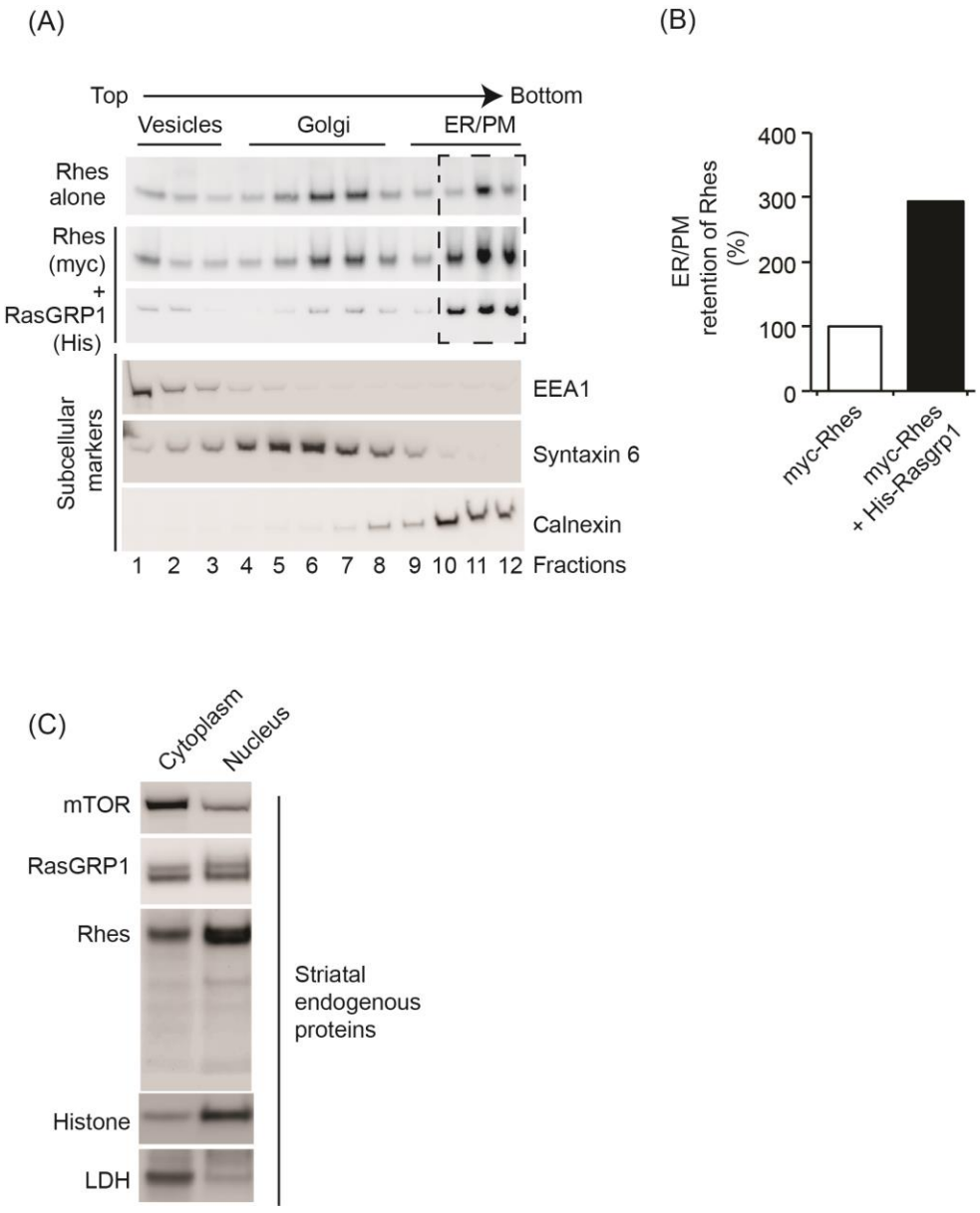

Fig. S4. Intracellular localization of Rhes and RasGRP1. (**A**) Biochemical organelle separation and Western blotting analysis in HEK293 cells overexpressing myc-Rhes alone or His-RasGRP1 and myc-Rhes together. (**B**) Quantification of relative abundance of myc-Rhes in endoplasmic reticulum (ER) and plasma membrane (PM) fractions from A. (**C**) Distribution of endogenous mTOR, Rhes and RasGRP1 in the cytosolic and nuclear fractions of the striatum of wild-type mice. Cytosolic (LDH) and nuclear (histone 3) protein markers are indicated. Data are based on two independent experiments.

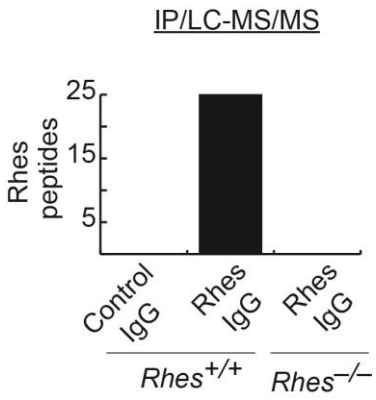

Fig. S5. Immunoprecipitation of Rhes by LC-MS/MS. Immunoprecipitation (IP) of Rhes from the striatum of wild-type or *Rhes*$^{-/-}$ mice using Rhes-IgG or control IgG, followed by Liquid chromatography-mass spectrometry (LC-MS/MS) analysis. Data are representative of three independent experiments.

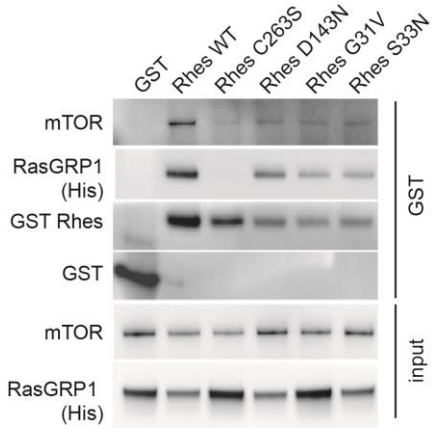

Fig. S6. Differential interaction of wild-type and mutant GST-Rhes with His-RasGRP1. Immunoprecipitation of His-RasGRP1 or endogenous mTOR with wild-type or mutant (C263S, D143N, G31V, or S33N) GST-Rhes in HEK293 cells. Data are representative of two independent experiments.

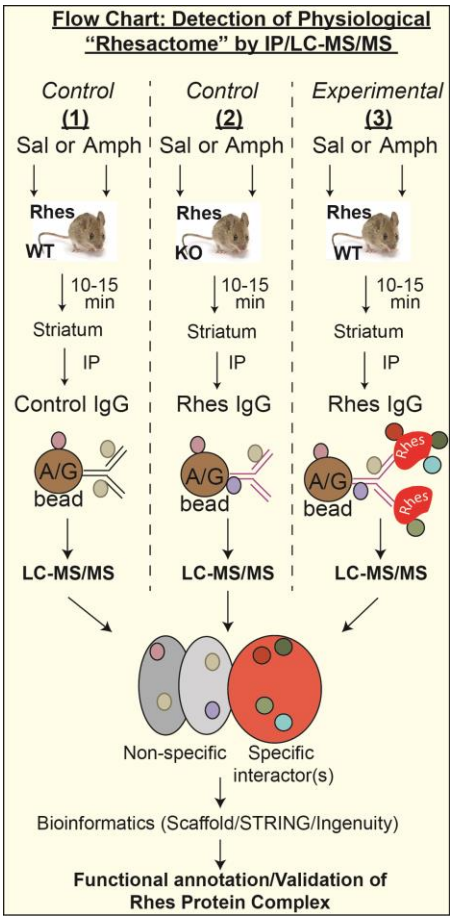

Fig. S7. Flow chart for IP–LC-MS/MS for the detection of a physiological Rhesactome. After 15 minutes of saline or amphetamine injection the striatal lysates of indicated mice were immunopreciptated (IP) with (1) control IgG (control) or (2) Rhes IgG in Rhes KO (control) or (3) Rhes wild-type (experimental) followed by LC-MS/MS analysis. Amphetamine-induced Rhes interactors were analyzed using bioinformatics sources (Scaffold, STRING and Ingenuity pathway analysis).

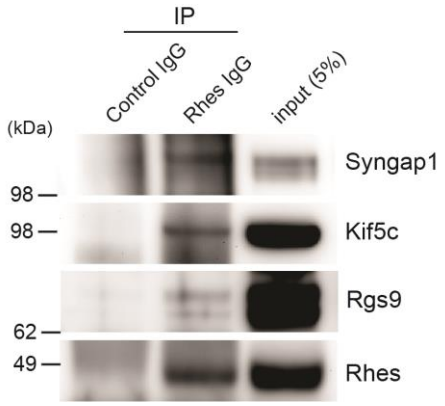

Fig. S8. Validation of selected Rhes interactors. Immunoprecipitation (IP) of Rhes with Rhes IgG or control IgG and Western blotting for endogenous of syngap1, kif5c and Rgs9 in striatal lysates from wild-type mice. Data are representative of three independent experiments.

Group	IgG	Genotype	Number of mice	Number of (IP) repeats	Total Number of mice
Saline	Control IgG	WT	**6**	**2**	**12**
Amphetamine	Control IgG	WT	**6**	**2**	**12**
Saline	Rhes IgG	WT	**4**	**3**	**12**
Amphetamine	Rhes IgG	WT	**6**	**3**	**18**
Saline	Rhes IgG	*Rhes*$^{-/-}$	**6**	**1**	**6**
Amphetamine	Rhes IgG	*Rhes*$^{-/-}$	**6**	**1**	**6**
Saline	Rhes IgG	*Rhes*$^{+/-}$/ *Rasgrp1*$^{+/+}$	**5**	**2**	**10**
Amphetamine	Rhes IgG	*Rhes*$^{+/-}$/*Rasgrp1*$^{+/+}$	**6**	**2**	**12**
Saline	Rhes IgG	*Rhes*$^{+/-}$/ *Rasgrp1*$^{-/-}$	**5**	**2**	**10**
Amphetamine	Rhes IgG	*Rhes*$^{+/-}$/ *Rasgrp1*$^{-/-}$	**4**	**2**	**8**

Table S1. Group and number of samples analyzed by IP–LC-MS/MS. List of all the groups and number of mice per IP per group analyzed in IP/LC-MS/MS.

Rhes interactor	Experimental	Interactors or related proteins in "Rhesactome"
GNB1/2/3	Two-hybrid (*97*)	Gnb1/2/5
LRP5/6	Affinity Capture-MS (*98*)	Lrp1
PI3K3CA	In vitro (*36*)	Pip5k1c
RABAC1	Two-hybrid (*99*)	Rab11
RAP1GDS1	Affinity Capture-MS (*98*)	Rap1gap1, Rap1gap2
SLC4A7	Affinity Capture-MS (*98*)	Slc1a2, Slc1a3, Slc25a4, Slc25a1

RasGRP1 interactor	Experimental	Related proteins in "Rhesactome"
ABCB8	Co-fractionation (*100*)	Abcd3
DGKZ	Affinity Capture-Western (*101*)	Dgkz, Dgkβ
DLG4	Protein-peptide (*102*)	Dlg4, Dlg2, Dlg3, Dlgap2, Dlgap3, Dlgap4
PPM1B	Affinity Capture-MS (*102*)	Ppp1ca, Ppp1cc

Table S2. Comparison of known Rhes/RasGRP1 interactors to the striatal Rhesactome. List of the known Rhes or RasGRP1 interactors and their comparison to the interactors or related proteins in the striatal "Rhesactome" (Source: BioGRID is an interaction repository, thebiogrid.org).

Data file S1. Rhesactome of wild-type mice striatum in response to amphetamine. List of all the specific striatal interactors of Rhes (Log_2 fold change) in amphetamine (3mg/kg i.p. at 10μl/g) compared to saline conditions in wild-type mice striatum. File is provided as an Excel file in the online supplementary materials. Data are based on three IP-LC-MS/MS experiments.

Data file S2. Rhesactome of *Rhes*$^{+/-}$/*Rasgrp1*$^{+/+}$ and *Rhes*$^{+/-}$/*Rasgrp1*$^{-/-}$ mice striatum in response to amphetamine. List of all the specific striatal interactors of Rhes (Log_2 fold change) in *Rhes*$^{+/-}$/ *Rasgrp1*$^{+/+}$ or *Rhes*$^{+/-}$/ *Rasgrp1*$^{-/-}$ in amphetamine vs. saline conditions (3mg/kg i.p. at 10μl/g). File is provided as an Excel file in the online supplementary materials. Data are based on two IP-LC-MS/MS experiments.

Data file S3. Proteins identified in control IgG IP LC-MS/MS in the wild-type mice striatum (control 1). List of all proteins (log_2 fold change) identified in control IgG IP LC-MS/MS in the wild-type mice striatum in amphetamine vs. saline conditions (3mg/kg i.p. at 10μl/g). File is provided as an Excel file in the online supplementary materials. Data are based on two IP-LC-MS/MS experiments.

Data file S4. Proteins identified in Rhes IgG IP LC-MS/MS in the *Rhes* KO mice striatum (control 2). List of all proteins (log_2 fold change) identified in Rhes IgG IP LC-MS/MS in the *Rhes*$^{-/-}$ mice striatum in amphetamine vs. saline conditions (3mg/kg i.p. at 10μl/g). File is provided as an Excel file in the online supplementary materials. Data is based on one IP-LC-MS/MS experiments.