Supplemental Experimental Procedures

The following siRNAs were used:

human Lbc (hL1) (proto-Lbc Accession AF127481; AKAP13 transcript variant 3 ID:510024) nucleotides 878-896 (sense strand) 5'-GCAGAGUCUUGGAGUCGGA-3'; hL2 nucleotides 2022-2041 (sense strand) 5'-UGUUGGACACCAGAGCCCG-3'; hL3 nucleotides 3227-3248 (sense strand) 5'-AGGACCAGAAGGGCAGAGC-3'

bovine Lbc (L1) (Accession XM_580384) nucleotides 5992-6010 (sense strand) 5'-GCAGAGUCUUGGAGUCAGA-3'; bovine L2 nucleotides 7191-7210 (sense strand) 5'-CUGUAGGUCACGUCGUCGC-3'

scrambled (scr) (sense strand) 5'-GACGGAUUCUGGAUGCGGU-3'

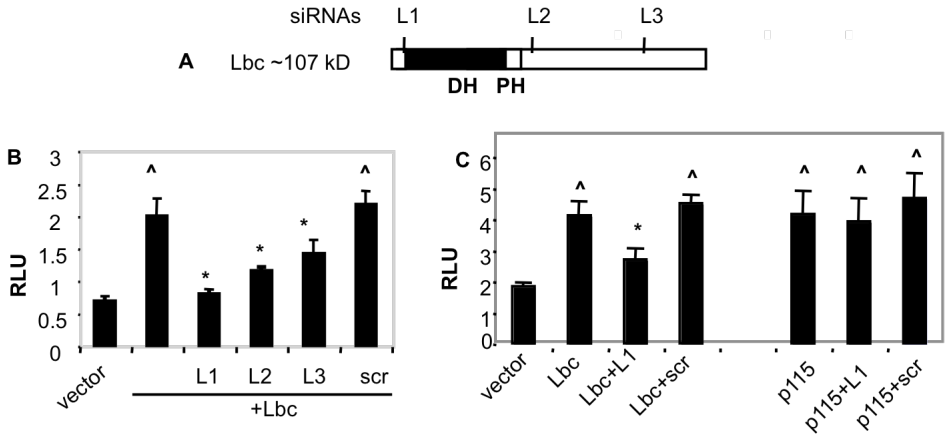
human LARG GEF (LG) (ID:23365; ARHGEF12) nucleotides 1256-1274 (sense strand) 5'-GAAACUCGUCGCAUCUUCC-3'

bovine LARG GEF (DDBJ/EMBL/GenBank: AB188499) 5'-GUUGAGCGCAGUACAUCAC-3'

bovine/human CT α-catulin (CTNNAL1) (Ambion ID#137219) (sense strand) 5'-GCAAUUCAGCGUGUAGGAC-3'

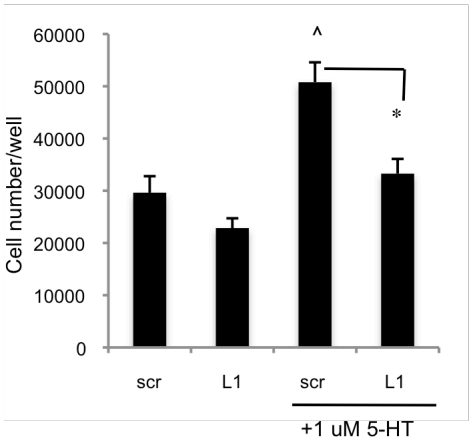
human CT2 α-catulin (CTNNAL1) (Ambion ID#137220) (sense strand) 5'-GCUGUCAACUUGGCAGUUG-3'

Figure S1. Activity and specificity of Lbc siRNAs. A) Schematic showing location of three Lbc-specific siRNAs (L1, L2, L3) targeting different sites on Lbc. DH=DH domain encoding exchange (GEF) activity, PH domain=pleckstrin homology domain. B) L1 and L2 siRNAs effectively inhibit Lbc-induced luciferase reporter activation in HEK293 cells. Cells were transfected with Lbc plasmid (50 ng/well) and Lbc or scrambled (scr) siRNAs (25 nM) along with SRE.L luciferase reporter (10 ng/well) in 48 well dishes, and luciferase levels measured 24 hrs. post transfection. C) L1 siRNA reduces Lbc-, but not p115 GEF-induced reporter activation. Lbc (50 ng/well) or p115 GEF (80 ng/well) plasmids were cotransfected with siRNAs along with reporter, and luciferase levels measured. RLU=relative luciferase units. n=3, bars=S.D.



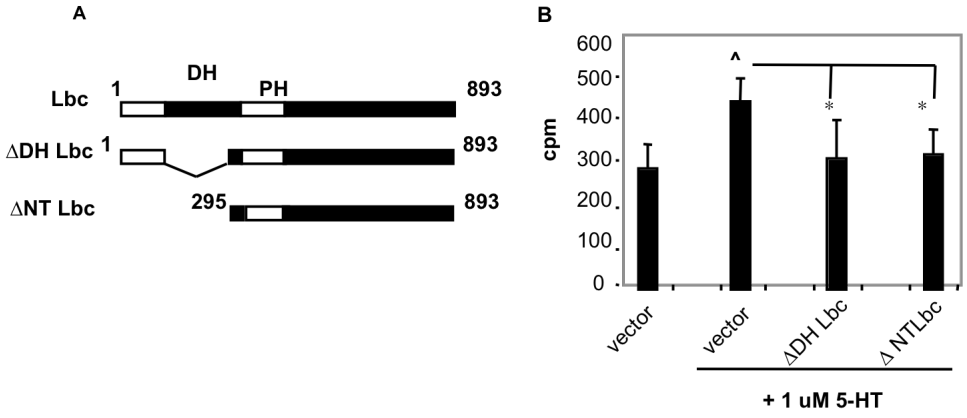
Supplemental Data Figure S1

Figure S2. Attenuation of 5-HT-induced PASMC proliferation by Lbc knock-down. PASMC seeded in 24 well dishes were transfected with 25 nM scrambled (scr) or Lbc (L1) siRNAs and either serum-starved or stimulated with 5-HT. After 72 hrs cells were trypsinized and total cell numbers/well obtained by Coulter counter (n=4). ^p<0.05 for 5-HT + scr vs. scr control; *p<0.05 for 5-HT + L1 vs. 5-HT + scr group.



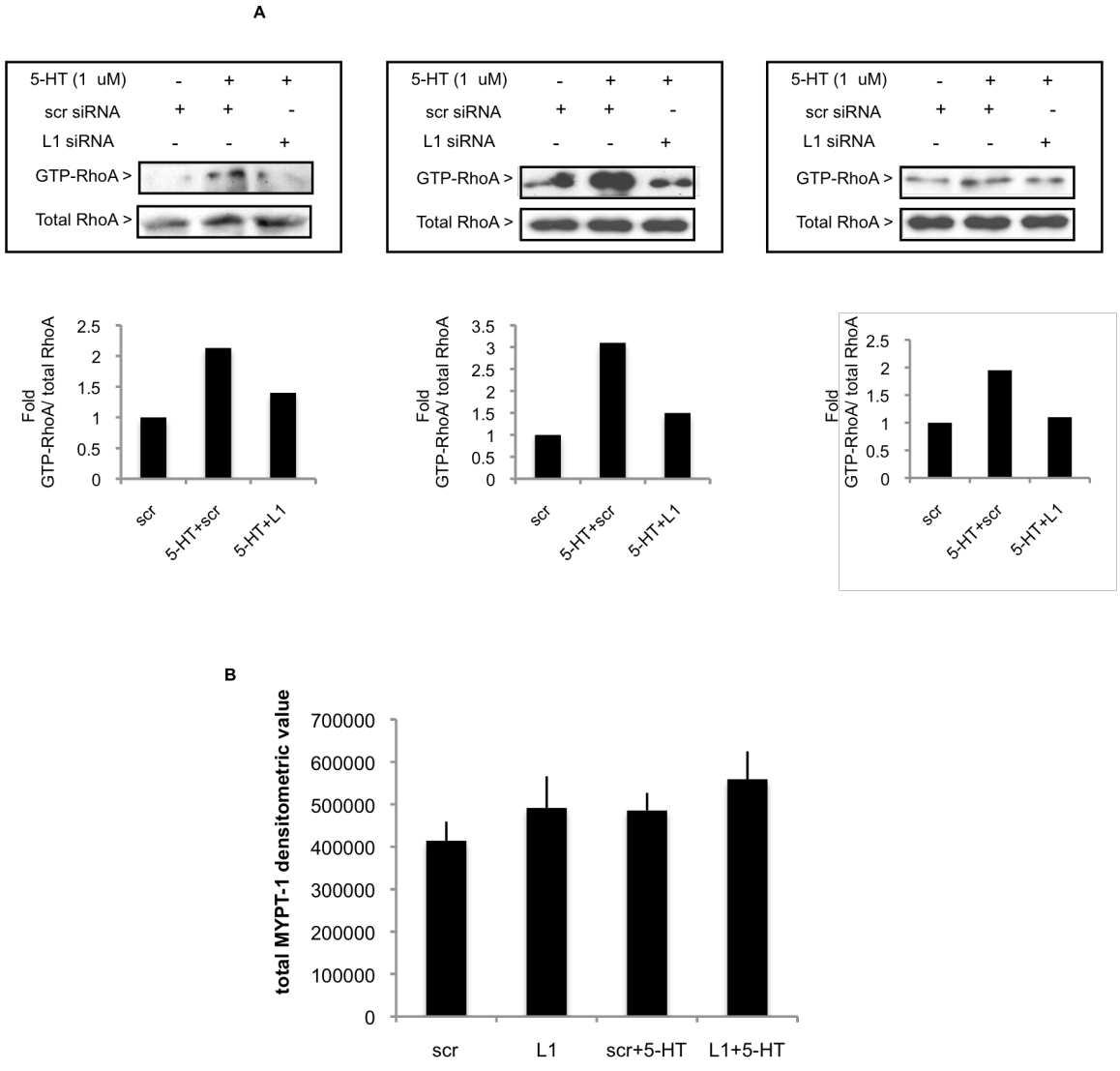
Supplemental Data Figure S2

Figure S3. Lbc dominant negative mutants inhibit 5-HT-induced PASMC mitogenesis. A) Schematic of Lbc mutants lacking the DH (GEF) domain; these were previously described and their activities characterized in (27). B) Growth-arrested PASMC transfected with Lbc mutants showed reduced thymidine incorporation induced by 5-HT. ^p<0.05 for vector + 5-HT vs. vector alone group, *p<0.05 for 5-HT + Lbc mutant vs. 5-HT + vector groups.



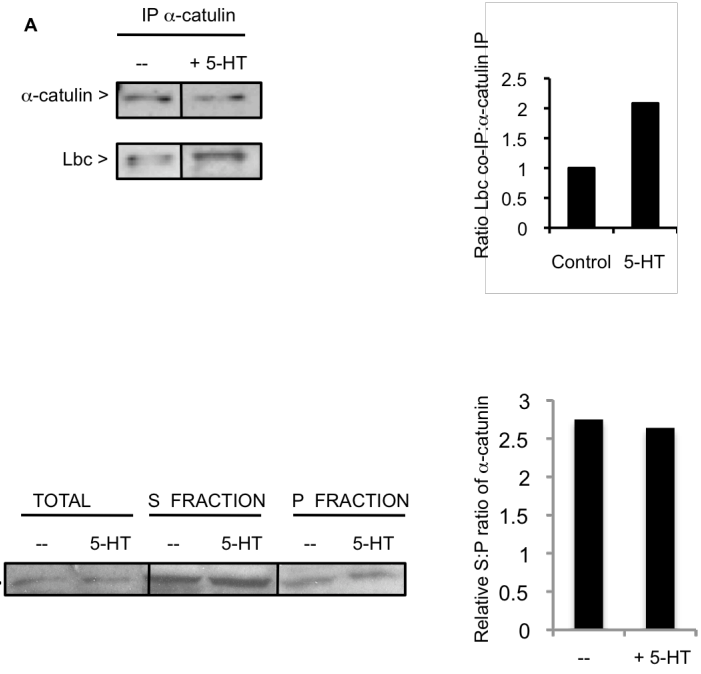
Supplemental Results Figure S3

Figure S4A. Lbc knock-down inhibits 5-HT-induced GTP-RhoA formation. PASMC were transfected with siRNAs, serum-starved, and treated with 5-HT for 10 min. Relative GTP-RhoA levels were assayed by pull-down, and pull-down material and total lysates immunoblotted for RhoA. Three separate experiments are shown, with accompanying relative densitometric quantitation. S4B. Quantification of relative total MYPT-1 densitometric values from Fig 3C immunoblot showing lack of effect on total MYPT-1 values of L1 siRNA + 5-HT group vs. L1 alone.



Supplemental Results Figure S4

Figure S5A. 5-HT treatment induces increased endogenous Lbc/α-catulin complex formation. Serum-starved cells were stimulated with 5-HT, then lysates immunoprecipitated with α-catulin-conjugated protein G sepharose, and precipitates immunoblotted for α-catulin (top panels) and Lbc (bottom panels). Precipitate:co-precipitate material was loaded on gel at a 1:4 ratio. S5B. Lack of effect of 5-HT on α-catulin subcellular localization as assayed by high-speed fractionation. Serum-starved PASMC were stimulated with 1 uM 5-HT, then fractionated into cytosolic (S) or membrane-rich (P) fractions, and immunoblotted for α-catulin. Graph quantitates the S:P ratio of the relative densitometric values of α-catulin in each fraction.



Supplemental Results Figure S5