

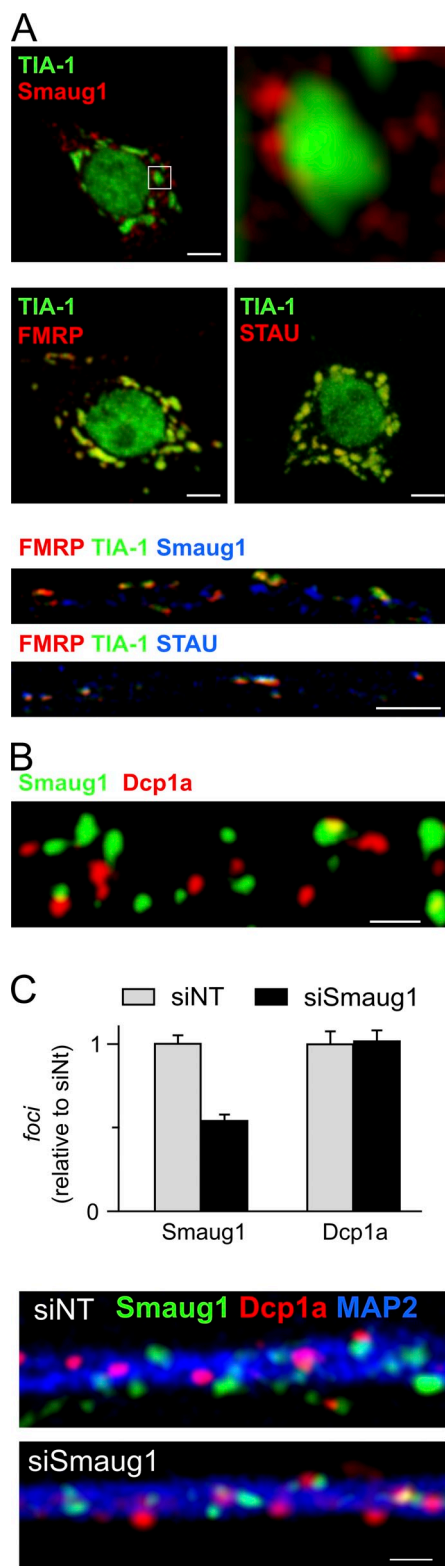
Boccaccio et al., <http://www.jcb.org/cgi/content/full/jcb.201108159/DC1>

Figure S1. Smaug1-foci, SGs, and PBs in neurons. (A) Neurons were exposed to 1 mM arsenite during 90 min, then stained for the SG marker protein TIA1 and the RBPs Smaug1, Staufen, or FMRP. SGs are observed in the cell soma and dendrites. Smaug1 is largely excluded from SGs, whereas Staufen and FMRP are present in all SGs. S-foci are frequently found in close vicinity to SGs. The top right panel shows an enlarged view of the boxed region. (B) Smaug1 and DCP1a were simultaneously detected in neurons. A fraction of the S-foci ($27 \pm 6\%$, averaged from three independent stainings) overlaps or are in close contact with PBs. Bar, 1 μ m. (C) Effect of Smaug1 knockdown in dendritic PBs. Hippocampal neurons were treated with the siSmaug1 pool as described in Materials and methods. The number and size of Smaug1 foci were significantly reduced, whereas PBs identified by DCP1a staining remained unaltered. Normalized values from 25 randomly selected dendritic fragments (total length, 600 μ m) from 10 neurons are plotted. Error bars indicate standard deviation. Bars: (A) 10 μ m; (B and C) 1 μ m;

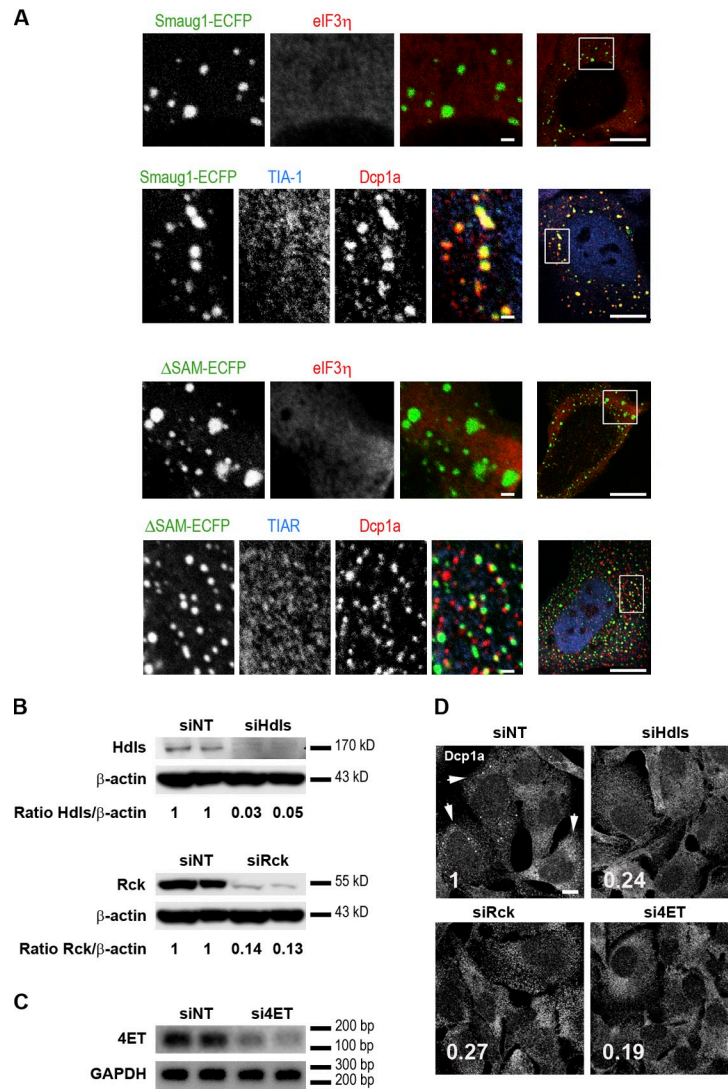


Figure S2. S-foci, SGs, and PBs in cell lines. (A) U2OS cells were transfected with the full-length human Smaug1 or the Δ SAM constructs fused to ECFP, and stained for the indicated molecules. As described previously (Baez and Boccaccio, 2005), the larger granules of Smaug1-ECFP partially colocalize with the SG components TIAR or TIA1. However, eIF3 η , which is an obligate SG component, is always excluded, indicating that Smaug1-ECFP foci are distinct from SGs. A large proportion of these granules are in close contact with PBs, identified by DCP1a staining. Δ SAM-ECFP granules always exclude TIA1/TIAR and eIF3 η . Images on the left show enlarged views of the boxed regions on the right. Bars: (left) 1 μ m; (right) 10 μ m. (B–D) Silencing of PB components in U2OS. (B) U2OS cells were treated with the indicated siRNAs and the corresponding proteins were analyzed by Western blotting. Signal intensity relative to that of control samples is indicated for each treatment. (C) RT-PCR for 4ET after treatment with si4ET. Abundance relative to that of control cells is indicated for each sample. (D) IF for Dcp1a in U2OS cells treated with the indicated siRNAs. PB-positive cells are indicated by arrows and the fraction of cells with PBs relative to NT was evaluated in 100 cells for each treatment. Bar, 10 μ m.

Reference

Baez, M.V., and G.L. Boccaccio. 2005. Mammalian Smaug is a translational repressor that forms cytoplasmic foci similar to stress granules. *J. Biol. Chem.* 280:43131–43140. <http://dx.doi.org/10.1074/jbc.M508374200>