

GENETICS

Supporting Information

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**Activation of Sterol Regulatory Element Binding Proteins
in the Absence of Scap in *Drosophila melanogaster***

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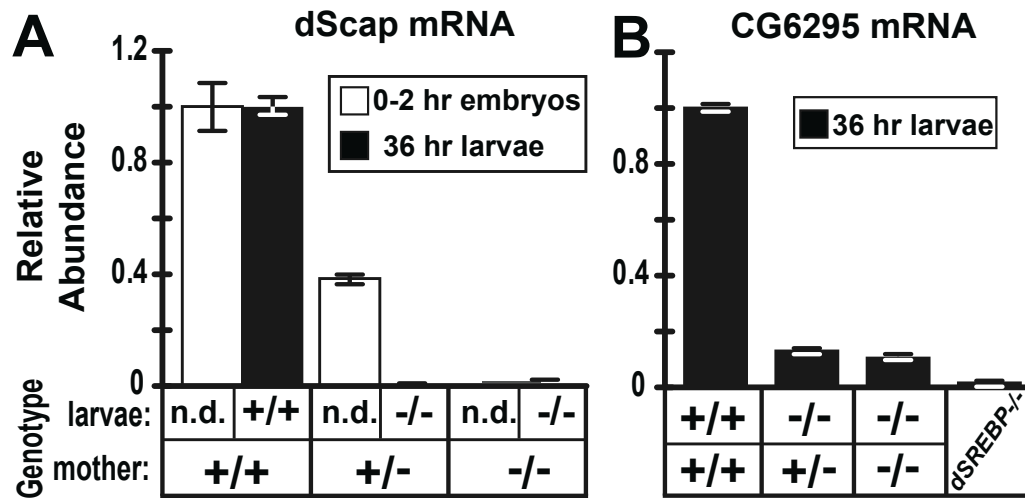


FIGURE S1.—Maternal contribution of dScap mRNA. (A) Quantitative RT-PCR analysis of dScap mRNA in 0-2 hour embryos (white bars) and first instar larvae (black bars). Embryos from wild-type or virgin *dscap⁴/dscap⁴* females crossed to *dscap⁴/Cyo*, act-GFP males were collected for 2 hours. Embryos were either collected for immediate RNA isolation or seeded (10 mg/ dish) onto a dish containing semi-defined media. After 36 hours, larvae were genotyped based on GFP fluorescence and total RNA was isolated. (B) Activation of dSREBP was determined in first instar larvae from above by analysis of CG6295 transcript levels. *dsrebp¹⁸⁹* nulls are included for comparison. Quantitative RT-PCR was performed as described in *Materials and Methods*. The relative abundance of embryonic and larval transcripts was calculated relative to wild-type 0-2 hour embryos and 36 hour larvae RNA, respectively.

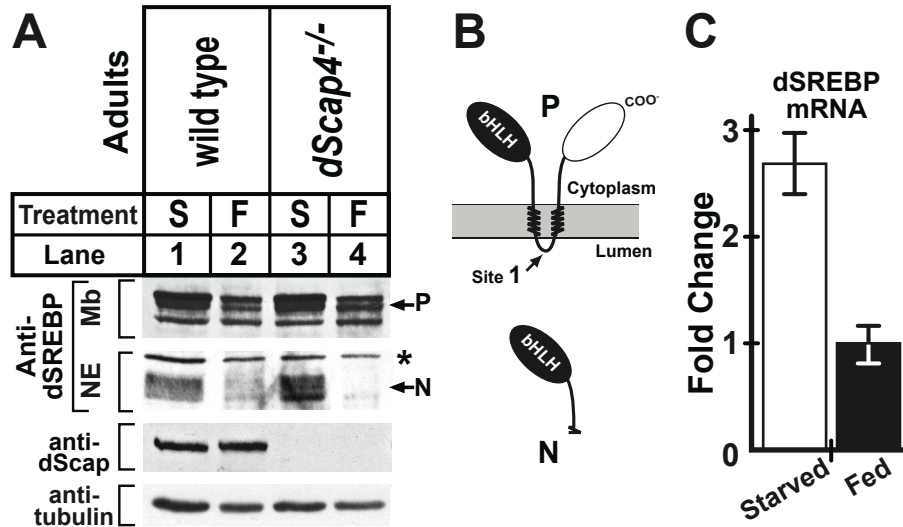


FIGURE S2.—The cleaved amino-terminal transcription factor domain of dSREBP accumulates in the nuclei of *dscap* null flies. (A) Immunoblot analysis of membrane fraction and nuclear extracts from adult flies of the indicated genotypes. Adults were either starved or starved and refed (indicated by S and F respectively) as described in *Materials and Methods*. The membrane fractions (75 μ g) and nuclear extracts (25 μ g) were subjected to immunoblot analysis as described in *Materials and Methods*. A parallel membrane blot was probed with IgG-7A8 against dScap. A membrane fraction blot was stripped and re-probed with anti-tubulin as a loading control. P, precursor; N, nuclear form. The asterisk indicates a cross-reacting band. (B) Schematic showing the topology of dSREBP and its cleavage fragments. bHLH, the transcription factor domain; COO-, carboxy terminal regulatory domain; Site 1, site of cleavage by S1P. The cytoplasm and lumen are indicated. (C) Quantitative RT-PCR analysis of dSREBP transcripts in starved (white bars) and refed (black bars) wild-type flies. RNA was prepared and transcripts quantified as described in *Materials and Methods*. Error bars represent the SEM.