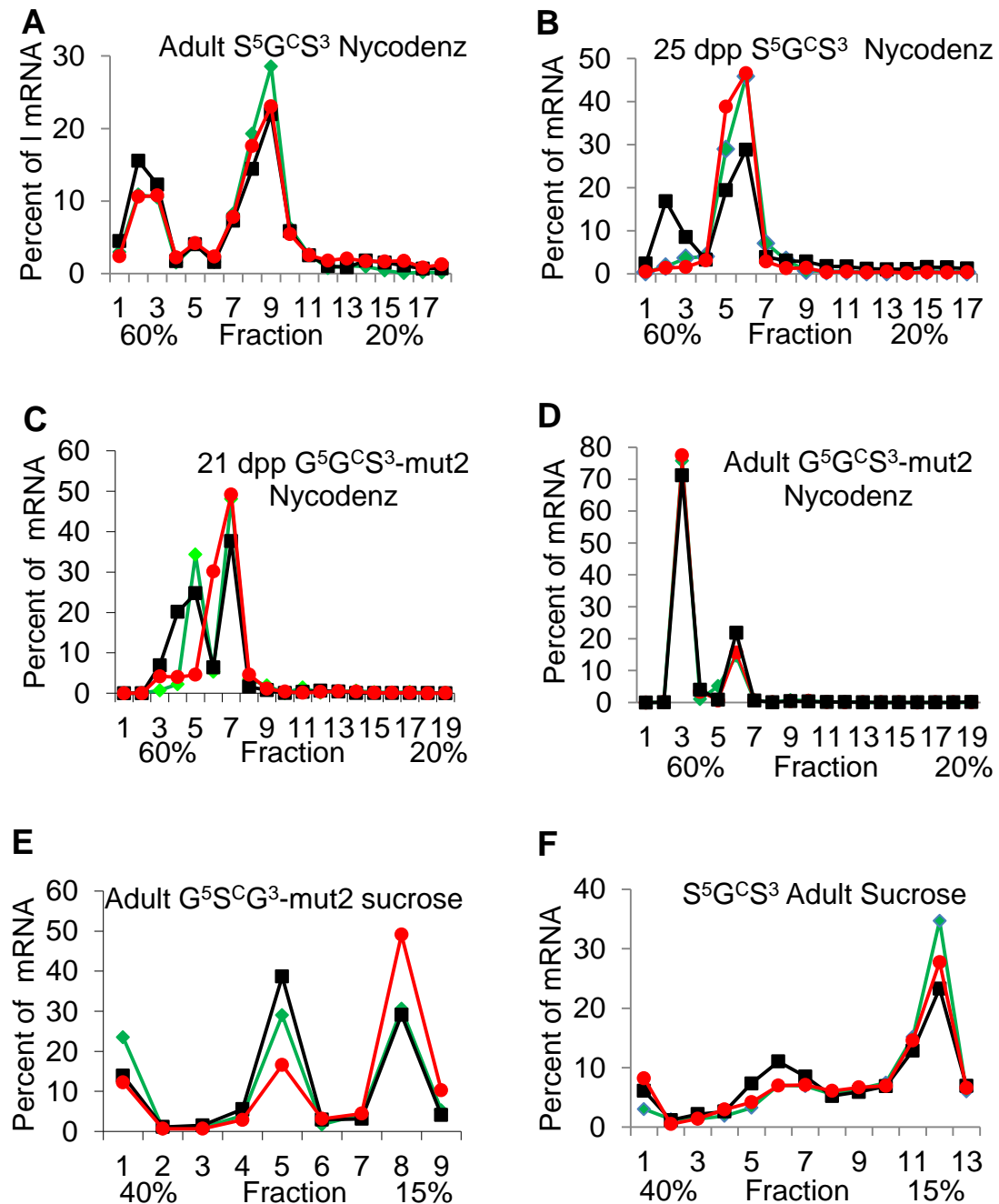


## Supplemental figure 2

$S^5G^CS^3$  and  $G^5G^CS^3$ -mut2,  $\blacklozenge$ — $\blacklozenge$ ; *Smcp*,  $\bullet$ — $\bullet$ ; *Ldhc*,  $\blacksquare$ — $\blacksquare$



**Supplemental Fig. 2** Sucrose and Nycodenz gradient analysis of translational activity of the  $S^5G^CS^3$  and  $G^5G^CS^3$ -mut2 mRNAs in immature and adult testes. Cytoplasmic extracts from 21 dpp, 25 dpp and adult testes were sedimented on 15-40% (w/w) sucrose gradients and 20-60% (w/v) Nycodenz gradients, fractions were collected from the bottom, RNAs were extracted from the pellet

and each fraction, and the amounts of *Ldhc*, *Smcp* and *Smcp-Gfp* transgenic mRNA were determined with RT-qPCR or by phosphorimaging northern blots (Bagarova *et al.* 2010).

Sucrose and Nycodenz gradients separate free-mRNPs and polysomes by different principles (Kleene *et al.* 2010). Sucrose gradients separate polysomes and free-mRNPs by differences in sedimentation velocity according to differences in the sizes of free-mRNPs and polysomes. In contrast, Nycodenz gradients separate free-mRNPs and polysomes by differences in bouyant density. Since the ratio of protein to RNA in free-mRNPs is greater than that in ribosomes, polysomes equilibrate in the dense fractions, free-mRNPs equilibrate in lighter fractions and free proteins equilibrate near the top of the gradient.

The Nycodenz gradients demonstrate that the  $S^5G^CS^3$  and *Smcp* mRNAs equilibrate primarily with translationally repressed free-mRNPs in 21 and 25 dpp testes, and that a substantial proportion of the  $G^5G^CS^3$ -mut2 mRNA equilibrates with polysomes in 21 dpp testes. The sucrose and Nycodenz gradients from adult testes demonstrate that substantial proportions of the *Ldhc*, *Smcp*,  $S^5G^CS^3$  and  $G^5G^CS^3$ -mut2 mRNAs are present in polysomes. In general, these findings corroborate evidence in Fig. 1 and 2 that the  $S^5G^CS^3$  mRNA is translationally repressed in round spermatids, that the  $G^5G^CS^3$ -mut2 mRNA is translationally active in step 3 and 4 round spermatids, and that the *Smcp* and  $S^5G^CS^3$  mRNAs are translationally active in elongated spermatids in adult testes.

## References

- Bagarova J, Chowdhury TA, Kimura M & Kleene KC** 2010 Identification of elements in the *Smcp* 5' and 3' UTR that repress translation and promote the formation of heavy inactive mRNPs in spermatids by analysis of mutations in transgenic mice. *Reproduction* **140** 853-864.
- Kleene KC, Bagarova J, Hawthorne K & Catado LM** 2010 Quantitative analysis of mRNA translation in mammalian spermatogenic cells with sucrose and Nycodenz gradients. *Reprod Biol Endocrinol* **8** 155.