67% of the genes recovered by Oh *et al.* contain one or both of these sequences in their 5-kb upstream regions, we disagree with the authors' conclusion that their procedure was substantially more effective than our microarray analyses in recovering these sequences.

Additionally, a search of all *C. elegans* genes (http://rsat.ulb.ac.be/rsat/) shows that 78% of 5-kb upstream regions contain one or more of these motifs. Thus, a random selection of genes should have met this minimal value, regardless of approach.

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Oh *et al.* reply:

In their original study, Murphy *et al.*[1] listed 514 genes whose expression was affected by DAF-16 activity. For 58 of these genes, canonical DAF-16 binding sites were shown. For the remaining 456 genes, there was no indication that canonical DAF-16 binding sites were present, and we assumed erroneously that these genes lacked a consensus DAF-16 binding site. Based on the new information provided by Kenyon and Murphy, we agree that our estimate of 11% reflected a misunderstanding of their data. We also agree with Kenyon and Murphy that the random occurrence of potential DAF-16 binding in promoters is relatively high (78%, according to their estimate). Thus, the percentage of direct DAF-16 targets included among the list of genes whose expression is affected by DAF-16 cannot be determined solely from microarray and bioinformatic analyses. Indeed, numerous studies, including our own, have shown that not all consensus binding sites for a transcription factor are occupied *in vivo* and that transcription factors can also bind to nonconsensus sites[2–4] (reviewed in refs. 5,6). For these reasons, methods based on transcription factor binding, rather than expression, have been developed to identify direct targets of transcription factors (for example, chromatin immunoprecipitation (ChIP), ChIP-on-chip and ChIP-PET[7]). In our study[4], we identified putative DAF-16 target genes using ChIP, a direct measure of DAF-16 binding, and examined their involvement in DAF-16–dependent processes using a variety of functional assays.

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