

Table S8. *daf-16* germline mosaic analysis

Category ^a	Number of animals	% of category	Mother	Brood ^b	Progeny	Interpretation(s) regarding segregation of array	Interpretation(s) regarding role of <i>daf-16</i> in brood size
1	20/20	100%	non-Rol	>100	non-Rol, non-GFP	Array absent from maternal soma and germ line.	Large broods in the absence of <i>daf-16(+)</i> in any tissues; background value for <i>daf-16</i> ; <i>daf-2</i>
2A ^b	475/487	97.5%	Rol	10-50 ^c	Rol, GFP ^d	Array present in maternal soma and carried through maternal germ line.	Low brood in the presence of <i>daf-16(+)</i> in all tissues including germ line; background value for <i>daf-16</i> ; <i>daf-2</i> . High correlation of retention of array in germ line with low broods supports hypothesis that <i>daf-16</i> is required in germ line downstream of <i>daf-2</i> .
2B ^b	11/487	2.3%	Rol	60-80	Rol, GFP ^d	Array present in maternal soma and carried through maternal germ line.	Intermediate brood despite presence of <i>daf-16(+)</i> in germ line. Possible loss of array in non-hypodermal lineage in which <i>daf-16(+)</i> affects germline proliferation (e.g., EMS) Alternatively, array may not express as well in these individuals, or they may represent a group of individuals that were slightly older when shifted.
2C ^b	1/487	0.2%	Rol	>100	Rol, GFP ^d	Array present in maternal soma and carried through maternal germ line.	Rare class: Large brood size despite presence of <i>daf-16(+)</i> in germ line. Either loss of <i>daf-16</i> occurred in somatic tissue where <i>rol-6</i> is not required and where <i>daf-16</i> activity affects germline proliferation. Or array may not be expressed in maternal germ line in this individual.
3 (Mosaic)	12/12	100%	non-Rol	40-60	Rol, GFP ^d	Array not present in somatic focus of <i>rol-6</i> activity (including hypodermis lineages, presumed <i>rol-6</i> focus) but carried through maternal germ line. Alternatively, error in non-Rol designation of mother (unlikely, see ^e) or <i>rol-6</i> not expressed well in hypodermis of individual though present.	Low-intermediate brood with <i>daf-16(+)</i> in germ line lineage. Average brood size is somewhat larger than category 2A, suggesting some expression in soma may be responsible for difference.
4A (Mosaic)	17/23	74%	Rol	>100	non-Rol, non-GFP	Array present in maternal soma (particularly hypodermis, presumed focus of <i>rol-6</i> activity) and lost in maternal germline. Alternatively, loss of array in P2 since C contributes to hyp7 (a possible focus of <i>rol-6(+)</i> activity).	Large brood in animals where <i>daf-16(+)</i> is lost from germ line lineage (but not <i>rol-6</i> -requiring lineage). Correlation of loss of <i>daf-16</i> in germ line with large broods is consistent with requirement for <i>daf-16</i> in the germ line

4B (Mosaic)	5/23	22%	Rol	~40	non-Rol, non-GFP	Array present in maternal soma (particularly hypodermis, presumed focus of <i>rol-6</i> activity) and lost in maternal germline. Alternatively, loss of array in P2 since C contributes to <i>hyp7</i> (a possible focus of <i>rol-6(+)</i> activity).	downstream of <i>daf-2</i> . This class may result from loss in P1, which would remove <i>daf-16</i> from muscle lineage as well as germ line (see Fig. 3C). Low-intermediate broods from animals where <i>daf-16(+)</i> is lost from the germ line lineage. This class may result from loss in P1, which would remove <i>daf-16</i> from muscle lineage as well as germ line (see Fig. 3C). Alternatively, loss could have occurred in Z2 or Z3.
4C (Mosaic)	1/23	4%	Rol	~80	non-Rol, non-GFP	Array present in maternal soma (particularly hypodermis, presumed focus of <i>rol-6</i> activity) and lost in maternal germline. Alternatively, loss of array in P2 since C contributes to <i>hyp7</i> (a possible focus of <i>rol-6(+)</i> activity).	<i>daf-16(+)</i> loss from lineage that gives rise to germ line (but not hypodermis) permits large brood. Alternatively, mother may have died prior to producing full brood. Alternatively, loss could have occurred in Z2 or Z3.

^a Animals of the presumed genotype *daf-16(mu86); daf-2(e1370); muEx108[Pdaf-16::DAF-16::GFP]* were synchronized by hatch-off (Pepper et al., 2003) at 20°C and grown to the early L3 stage. Individual Rol or non-Rol 'mothers' were separated onto individual plates and shifted to 25°C (the Rol phenotype was obvious at this stage in this strain and individuals were subsequently checked as adults). Broods were estimated for each individual starting 48 hours later and continued each day until mother was depleted or dead.

^b For 30 broods of the 487 in Category 2, exact broods were counted. The average was 23.2±2.7(±SEM) offspring, similar to the average of 17±1.9 observed in *daf-2(e1370)* animals that underwent the same shift protocol. All other brood sizes were estimated by rough counts. Broods '>100' were noticeably different from those designated as '80'.

^c Animals with broods <10 (253 additional animals) were not included in the analysis as the genotype of the progeny could not be verified.

^d 'Rol, GFP' indicates that ~15% of the progeny were Rol and GFP⁺, consistent with the segregation rate of this particular array.