## Supplementary Figure Legends:

**Supplementary Figure 1. Caloric Restriction in Rats:**

a. Representative photo of F344 rats: young, CR- Caloric restricted, Ad Lib- *Ad Libitum* fed.
b. Weights of old CR and AL F344 rats normalized to the mean weight of sex-matched young adult rats.
c. Representative H&E-stained sections of the testis of the young, old AL and old CR F344 rats, showing extensive Leydig cell hyperplasia in AL-fed old rats. This neoplasm is largely attenuated by CR, but small foci of hyperplasia are still detectable (arrows).

**Supplementary Figure 2. Effect of CR and GHR deficiency on $p16^{INK4a}$ expression with aging**

The absolute copy number (log 10 scale) of *p16*$^{INK4a}$ mRNA molecules per 90 ng total RNA RT-PCR from lung and kidney of young (5 months) and old (21 months) *GHR* +/+ and -/- mice with or without caloric restriction is graphed +/- SEM. Each estimate represents the mean of 8-16 quantitative RT-PCR reactions on independent RNA samples derived from organs of 22 mice.

**Supplementary Figure 3. Expression of regulators of $p16^{INK4a}$ with aging**

a. The ratios of the expression of $p16^{INK4a}$ regulators *Ets-1* and *Bmi-1* in (Old (26 months) / Young (2.5 months)) mice from 15 tissues is graphed +/- SEM. Each estimate represents the mean of 4-8 quantitative RT-PCR reactions on independent RNA samples derived from 4-6 mice.
b. The ratios of the expression of $p16^{INK4a}$ regulators *Ets-1* and *Id1* from 12 tissues derived from (Old (28 months) / Young (3 months)) *ad libitum* fed (AL) or calorically restricted

(CR) F344 rats is graphed +/- SEM. Each estimate represents the mean of 4-8 quantitative RT-PCR reactions on independent RNA samples derived from 4 rats.

**Supplementary Figure 4. Comparison of different housekeeping genes used for normalization in RT-PCR experiments.**

The expression of housekeeping genes in different tissues (four independent assays of each housekeeping gene per tissue) expressed as ratio of expression in individual tissue to the mean expression seen in all tissues. Based on this analysis, each housekeeping gene has limitations. Using Tata-Binding protein (TATA) expression in the testis would overestimate RNA quality, as would normalizing to 18S expression in the pancreas. While GAPDH appears to correlate best across all tissues with the other two housekeeping genes, it demonstrates the lowest reproducibility ($r^2$) when repeated on the same sample. Of note, none of the housekeeping genes appeared to change expression in any tissue with aging, and all three housekeeping genes correlated strongly with one another except in testis and pancreas. As these variations in housekeeping gene expression were tissue-specific, they would not bias O/Y ratios (which measure expression of old versus young within the same tissue), but would affect absolute copy number determination. Therefore, 18S was used for normalization in most tissues, except in the pancreas and testis; where TATA and/or GAPDH were used.

**Supplementary Figure 5. Quantitation of *p16$^{INK4a}$* and *p19$^{ARF}$* in tissues**

a. Standard curve for the quantitation of the expression of $p16^{INK4a}$ and Arf by quantitative RT-PCR. Similar curves were generated for $p21^{CIP}$, $p18^{INK4c}$, and $p19^{INK4d}$. For all assays tested, the PCR reaction was linear over the range studied (19 to 40 cycles of amplification).

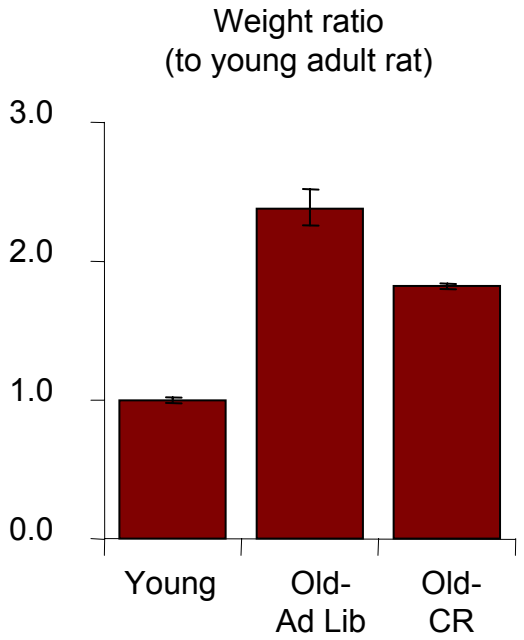
b, c, and d. Real-time $p16^{INK4a}$ amplification curves from cDNA from old versus young murine lung (b), cecum (c), and uterus (d). Change in TaqMan fluorescence per reaction cycle versus cycle number is plotted, and threshold for determination of Ct is indicated by a solid horizontal line.

**Supplementary Table I:** List of primers and probes used for TaqMan Real-time quantitative PCR.

| Gene | Forward primer | Reverse primer | Probe (5'-Tet, 3'-Tamra) | Reference sequence |
|---|---|---|---|---|
| **Mouse** | | | | |
| *p16$^{INK4a}$* | CCCAACGCCCCGAACT | GCAGAAGAGCTGCTACGTGAA | TTCGGTCGTACCCCGATTCAGGTG | AF044336 |
| *p19$^{ARF}$* | TGAGGCTAGAGAGGATCTTGAGA | GCAGAAGAGCTGCTACGTGAA | CCGCACCGGAATCCTGGACC | NM_009877 |
| *Bmi-1* | AGAAGAGATTTTTATGCAGCTCA | CAACTTCTCCTCGGTCTTCA | AGCTGATGCTGCCAATGGCTCCA | NM_007552 |
| | **ABI assay ID** | | | |
| *p15$^{INK4b}$* | Mm00483241_m1 | | | NM_007670 |
| *p18$^{INK4c}$* | Mm00483243_m1 | | | NM_007671 |
| *p19$^{INK4d}$* | Mm00486943_m1 | | | NM_009878 |
| *p21$^{CIP}$* | Mm00432448_m1 | | | NM_007669 |
| *p27$^{KIP}$* | Mm00438167_g1 | | | NM_009875 |
| *Ets-1* | Mm00468970_m1 | | | NM_011808 |
| *TBP* | Mm00446973_m1 | | | NM_013684 |
| *18S rRNA* | 4333760F | | | X03205 |
| *GAPDH* | Mm99999915_g1 | | | NM_008084 |
| **Rat** | | | | |
| *p16$^{INK4a}$* | ACCAAACGCCCCGAACA | GAGAGCTGCCACTTTGACGT | TCGGTCGTACCCCGATACAGGTGA | L81167 |
| *p19$^{ARF}$* | GAGGGCCGCAGCCACAT | CACCATAGGAGAGCAGGAGAGCT | CGTTGCCCATCATCATCACCTGGT | AF474975 |
| | **ABI assay ID** | | | |
| *p21$^{CIP}$* | Rn00589996_m1 | | | NM_080782 |
| *Ets-1* | Rn00561167_m1 | | | NM_012555 |
| *Id1* | Rn00562985_s1 | | | NM_012797 |
| *18S rRNA* | 4333760F | | | X03205 |

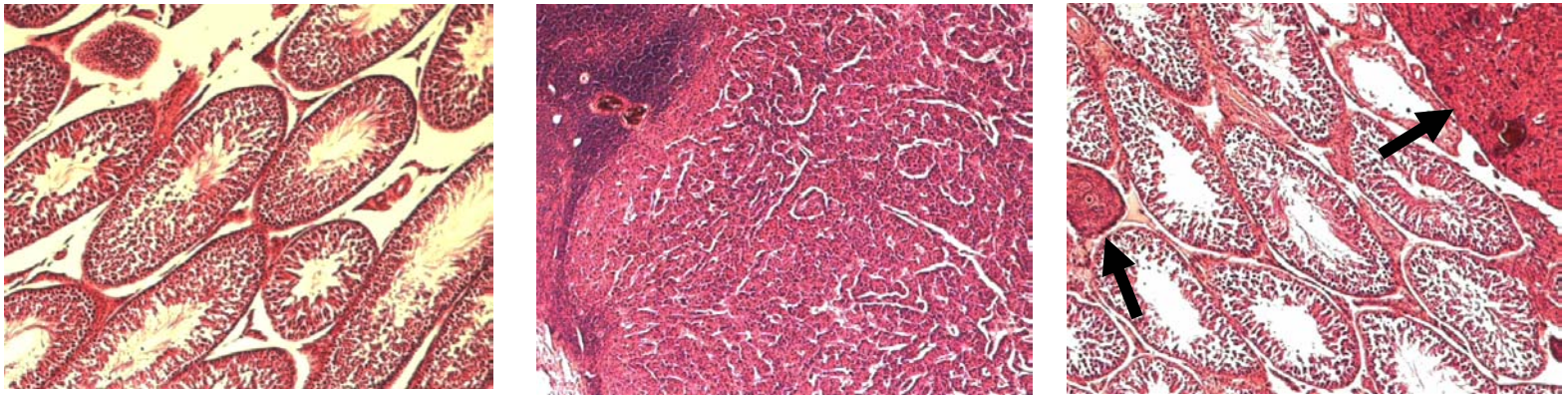
a


b




c

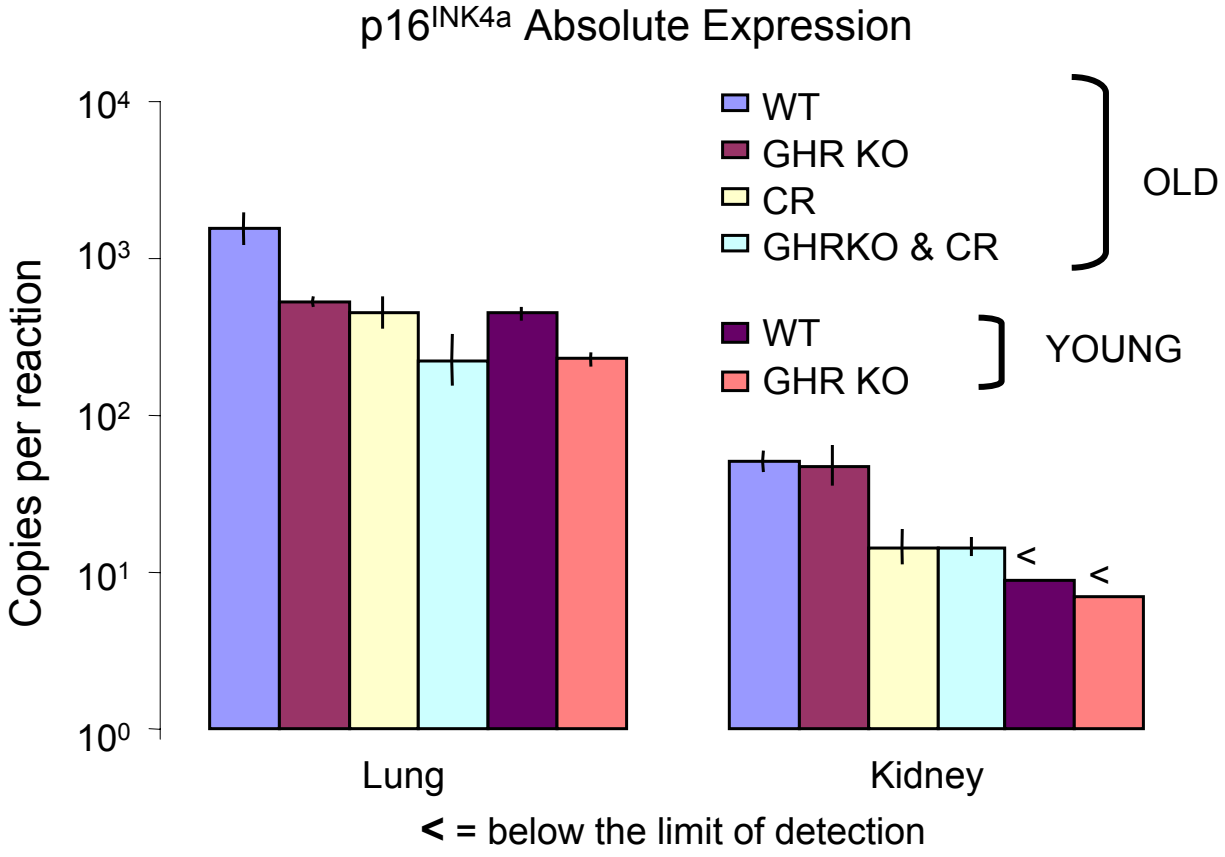
Leydig Cell tumors in aging rat testis



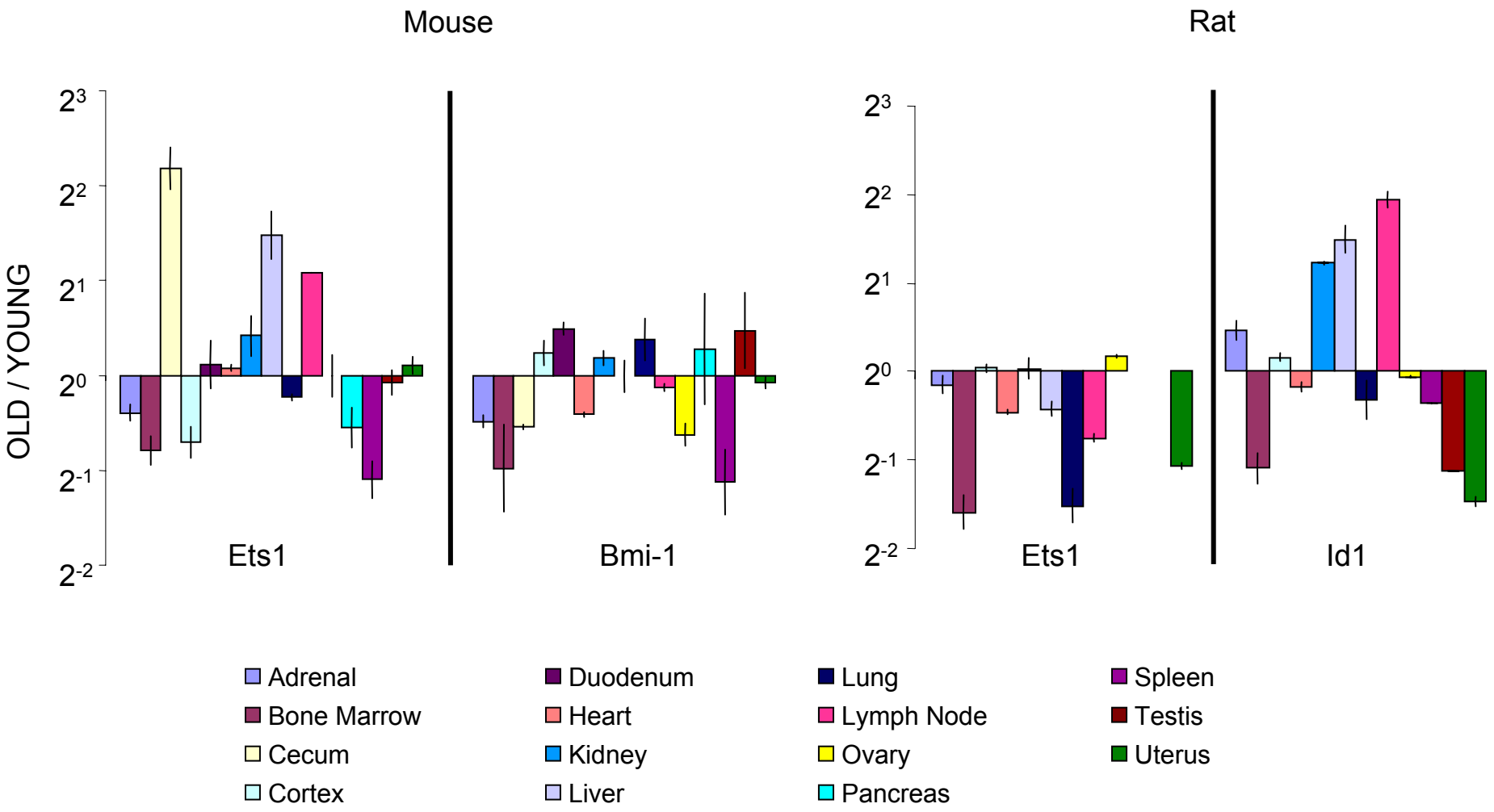
YOUNG OLD-AL OLD-CR

Arrows = Leydig Cell Hyperplasia. Photos with 10x objective.

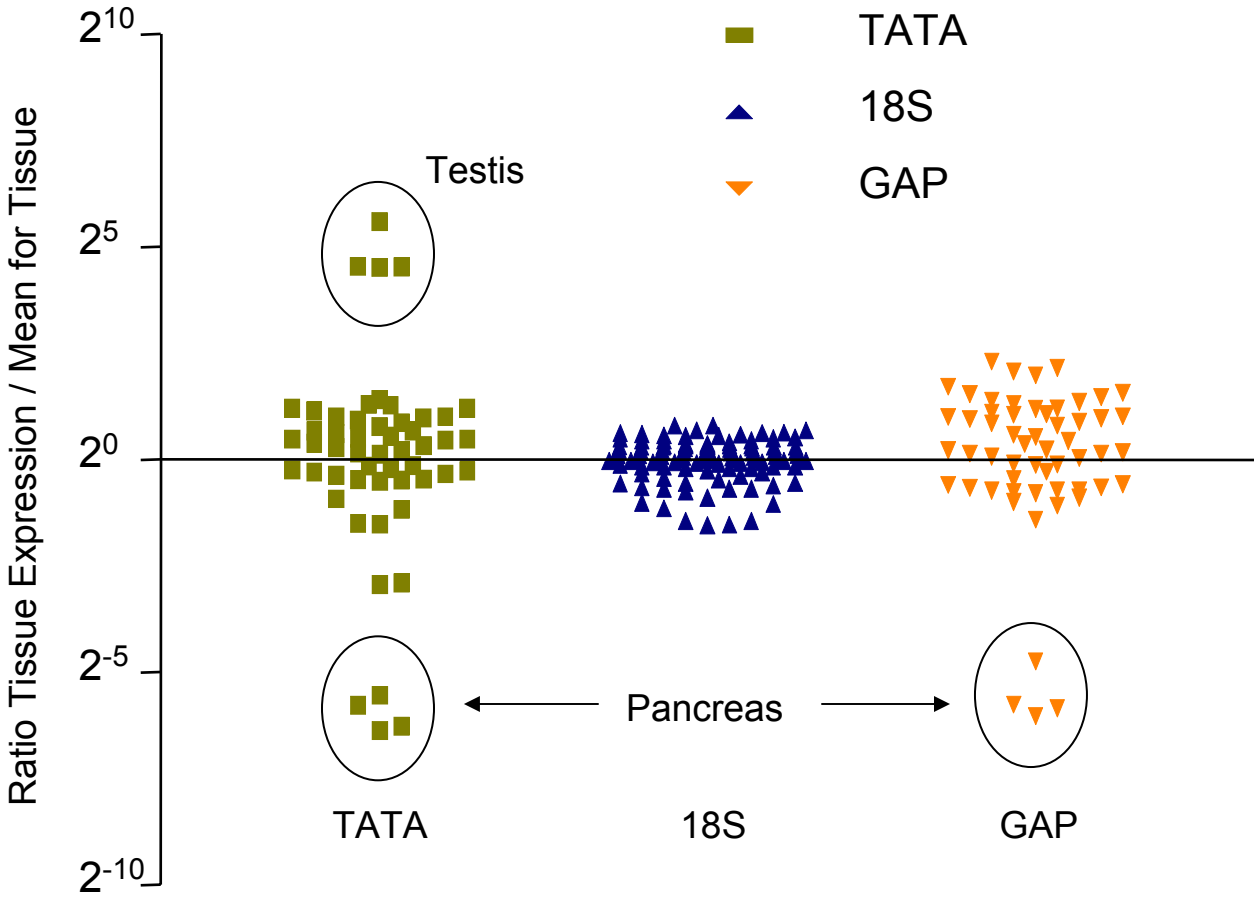
Krishnamurthy et al., Supplementary Figure 1



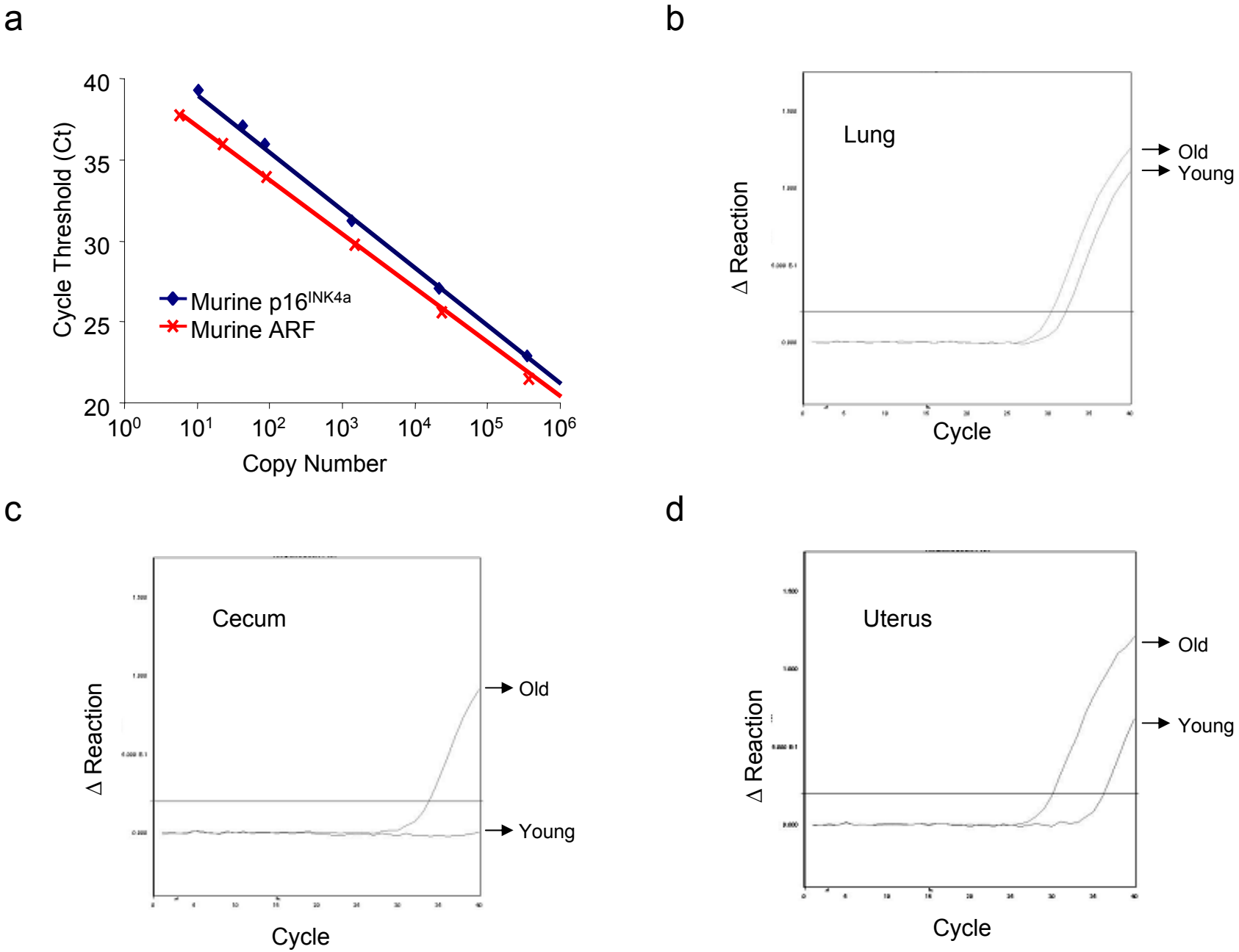

Krishnamurthy et al., Supplementary Figure 2




Krishnamurthy et al., Supplementary Figure 3




Krishnamurthy et al., Supplementary Figure 4




Krishnamurthy et al., Supplementary Figure 5