Clockwork Orangutan: microRNAs, Thermoregulatory Tradeoffs, and the Evolution of Brain Size.

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ABSTRACT

In homeothermic mammals the rate of heat dissipation must keep pace with the rate at which heat is generated internally[1]. The brain is metabolically expensive, yet despite decades of effort, the mechanism for offsetting the additional thermogenesis associated with brain expansion in the human lineage remains unknown[2-4]. Consistent with the 'expensive tissue hypothesis', we demonstrate that brain and liver sizes vary inversely when standardized against a thermoregulatory Core (kidney, heart, liver, brain). Instead of log-based specific metabolic rates, we estimate cellular metabolic rates (*cMR*s) by regression on body size and the number of conserved microRNA families (*mirFam*)[5]. While the *cMR*s of kidney, heart and brain vary directly with body mass, liver *cMR*s vary inversely with size, recapitulating the developmental suppression of liver gluconeogenesis by liver growth[6-9] or body growth[10-13]. Thus, while organ sizes track body size between clades, variation in Core composition permits adaptation to thermoregulatory demands within clades. A model assuming that *mirFam* is inversely related to dependence on liver gluconeogenesis detected size adaptation to a low-energy regime among the smallest primates and, among the primates with the largest brains, thermoregulatory adaptation to the physiological limit on the rate of heat dissipation[14].

INTRODUCTION

The diversification of mammalian orders is characterized by two long-term trends: increasing size[15] and decreasing body temperature[16]. Resting *MR*s, in contrast, do not exhibit a long-term trend but, in coordination with variation in thermal conductance (C_{min}), allow mammals of any size to occupy nearly the entire range of climatic zones[17-18], albeit with a narrower range of tolerance to heat than to cold[19-21]. These trends are consistent with a physiological limit on the rate of heat dissipation, initially proposed by Speakman and Król (2010)[14]. Notwithstanding this evidence, current models to account for increasing brain size in the human lineage do not consider thermoregulatory constraints, and there is no satisfactory physiological explanation for the brain's evolutionary expansion[22-23].

The human brain's power consumption of 15W raises the temperature of its venous outflow by ~ 0.2 - 0.3ºC[24-27]. Orangutans are similar to humans in body size, but their brains are ⅔ smaller and generate heat at a rate of only 4W[28]. Given that ~ 15% of human blood volume perfuses the brain

every minute[29], this suggests a significant thermoregulatory challenge that can be addressed in only two ways: a faster rate of heat dissipation or a tradeoff in energy expenditure with another metabolically expensive tissue, such as kidney, heart, liver or intestine[2-4]. Like chimpanzees[30], ancestral humans most likely relied on behavioral thermoregulation to avoid hyperthermia in hot environments which, however, would not have addressed the problem of internal heat generation. Uniquely human adaptations related to heat dissipation include hairlessness, bipedalism, and a 10-fold increase in the density of eccrine sweat glands, relative to densities found in chimpanzees and macaques[31]. This adaptation may have also increased the risk of dehydration[32] in a drier savannah environment that was hotter by day and cooler by night[33-37]. Permanently enlarged breast tissue may also have served a thermoregulatory function[37], and possibly a buffer against infant dehydration.

Despite numerous attempts, the effort to detect the tradeoff predicted by the 'expensive tissue hypothesis' has been abandoned since the publication of a study encompassing 100 species, including 23 primates, that found no significant anticorrelations among the body mass-independent residuals for eight organs[4]. We postulated that the relevant standard for variation of the four most thermogenic organs (kidney, heart, liver and brain) is the mass of a composite thermogenic Core, analogous to that predicted by the thermoregulatory model of Porter and Kearney (2009)[1]. We find that evolutionary increases in the proportion of the brain in this Core are offset by decreases in the proportion of the liver, with a corresponding anticorrelation between their Core mass-independent residuals.

As previously demonstrated, unlike *mirFam*-based cellular *MR*s (*cMR*s) are sensitive to body temperature and more robust to variation in the range of mass than log-based specific *MR*s (*sMR*s) [5]. In the present study, we estimate the cellular *MR* (*cMR*) of each Core organ based on three criteria:

a) correlation with organ size and *mirFam* (physiological relevance)[5];
b) correspondence between the joint *cMR* of these organs and the predicted rate of core thermogenesis ($\propto \sqrt{M}$)[1] (thermodynamic relevance);

c) utility for the unsupervised classification of major primate clades (phylogenetic relevance).

We find that liver *cMR*s are anticorrelated with body mass and with the *cMR*s of kidney, heart and brain. The joint *cMR* of this 4-organ Core ($M^{0.53}$) scales close to the theoretical rate of core thermogenesis, unlike the joint *sMR* derived from the log-based *sMR* of each organ. Since the liver is the primary organ of gluconeogenesis in fasting or starvation, the liver-brain anticorrelation is consistent with an energy-limited, positive feedback model of brain size evolution[22-23].

RESULTS

The proportion of brain mass in the Core varies reciprocally with that of liver

Of the six possible tradeoffs in a Core consisting of four thermogenic organs, one major tradeoff occurred consistently among primate clades, that between brain and liver (Figure 1A, B). A dampened tradeoff between heart and kidney occurred approximately "in phase" with the major tradeoff. The brain - liver tradeoff was conserved among 30 additional Euarchontoglires, along with a diminished heart - kidney tradeoff (Supplementary Figure S1).

Tradeoffs between liver and brain masses were readily demonstrable with log-transformed data, provided that they were ranked according to Core size (Supplementary Figure 2C) rather than body size (Supplementary Figure 2A). Residuals of the regressions exhibited a periodic pattern of variation for each organ, corresponding to the relative proportion of body mass (Supplementary Figure 2B) or Core mass (Supplementary Figure 2D). Since the tradeoff between the residuals of brain and liver mass is apparent only when ranked according to Core mass (Supplementary Figure 2D versus Supplementary Figure 2B), standardization of log *Mbrn* with respect to log *Mbdy* precludes its detection[4].

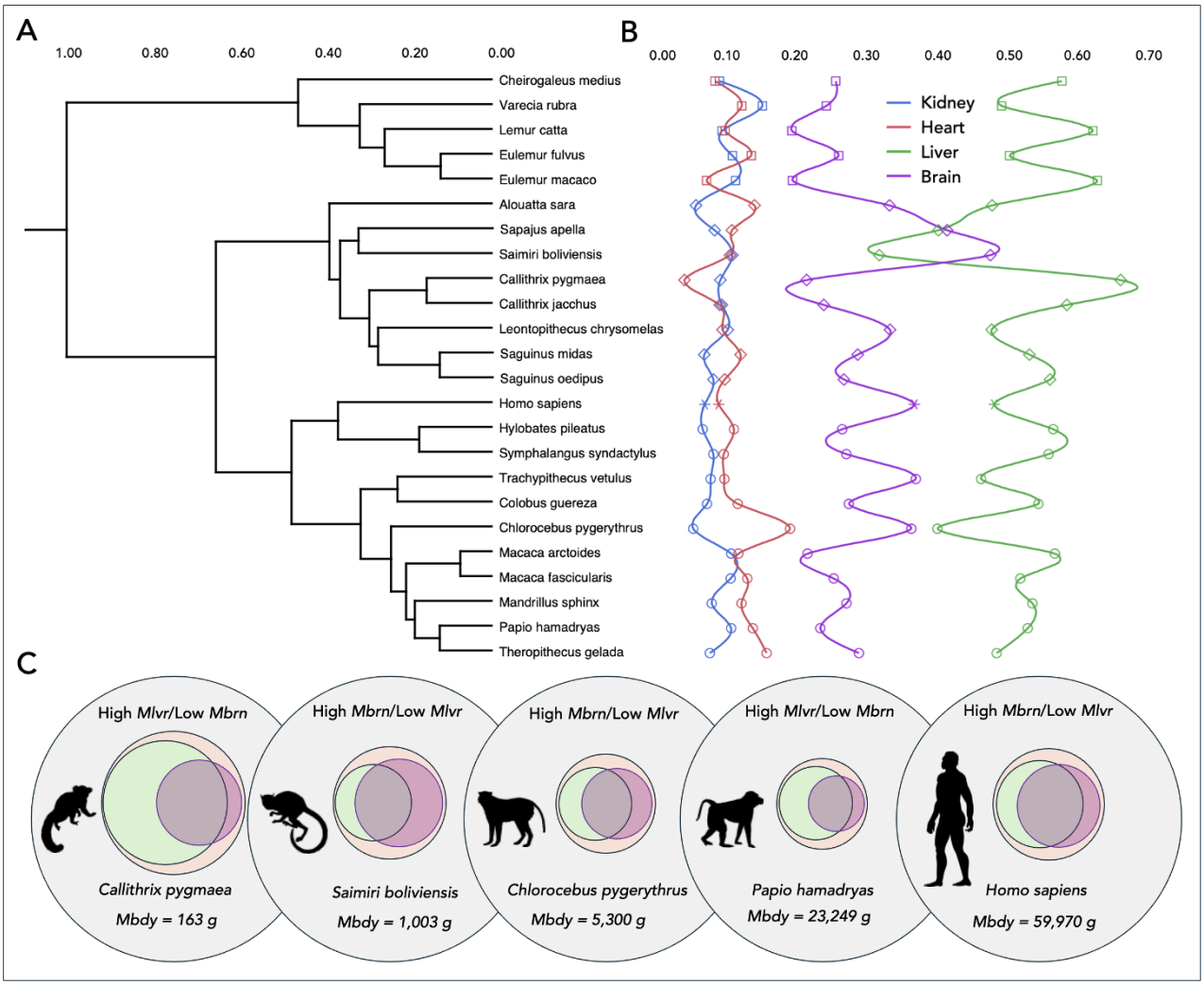

Figure 1. The proportion of brain mass in the Core varies reciprocally with that of the liver.
A. Time tree of 24 primates (scale bar represents the relative time of divergence).
B. Mass proportion of each organ relative to the total Core mass.
C. Summary model comparing representative species with low or high ratios of brain/liver mass. The radius of each circle represents a spherical volume corresponding to relative organ or total Core mass. The relative proportions of liver and brain are represented by green and purple circles, respectively. Silhouttes: *Callithrix jacchus*, *Saimiri* (generic), *Erythrocebus patas*, *Papio* (generic), *Homo sapiens idaltu* (© Phylopic 2024, compiled by T. Michael Keesey).

Physiologically-based cMRs do not correspond to log-based sMRs

Assuming power law models, we distinguish between:

specific *MR* (*sMR*): $sMR = m \times M^b$,

where b is the slope of log *rMR*/log *M*, and

cellular *MR* (*cMR*): $cMR = n \times M^c$,

where c is the allometric exponent obtained by maximum likelihood fitting of rMR/M^c to the distribution of a physiologically-relevant trait, such as *mirFam*[5]. In the present report, "*rMR*" includes metabolic rates measured under basal conditions (*bMR*s). A log-based *sMR* has a fixed value across a set of species, regardless of size; thus, the ratio of *sMR* to a non-logarithmic trait that varies with size is uninformative (Supplemental Figure 3A). A *mirFam*-based *cMR* increases or decreases as a function of size (Supplementary Figure 3B), yielding a biologically informative and phylogenetically consistent rank order for *cMR*/trait ratios (Supplementary Figure S4), with implications for covariance models of trait evolution[38-42].

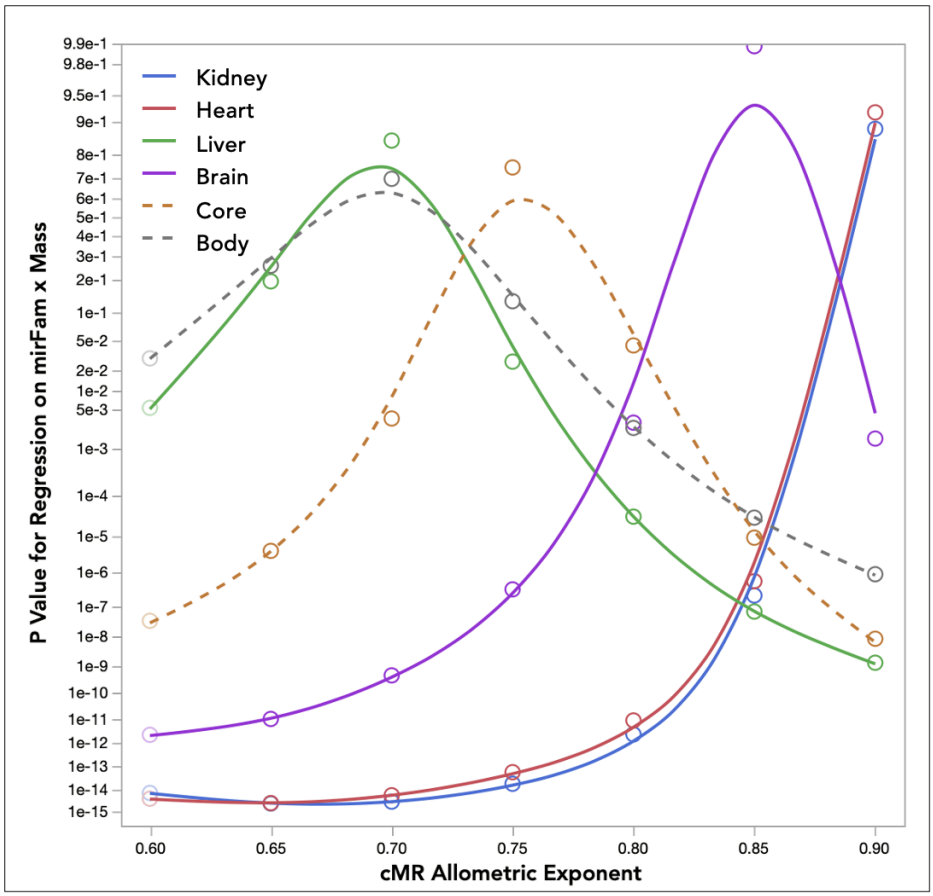

Figure 2. Dependence of *cMR* on *mirFam* and √*Morg* (51 Euarchontoglires).
P values for regressions of organ *cMR* on √*Morg* and *mirFam* (binned–see Methods).
√*Morg* yielded *P* values several orders of magnitude lower than did log *Morg*.
Note that the minimal correlation for the Core *cMR* occurs when scaled to $M^{0.75}$.

Estimation and physiological relevance of thermogenic organ cMRs

We previously demonstrated that variable allometric scaling, in conjunction with a model of stabilizing selection, yielded $M^{0.75}$ as the optimal scaling of *cMRbdy* with respect to *mirFam* among 10 representative mammals[5]: hence "*cMR.75bdy*." In order to estimate *mirFam*-based *cMR*s for each of the four thermogenic organs, we compared the dependence of *cMRorg* in the range *cMR.60* - *cMR.90* on both *mirFam* and organ mass (Figure 2), revealing two distinct patterns of correlation: one for kidney, heart and brain (increasing *P* values), another for the liver (decreasing *P* values), which was tracked by whole body mass (Figure 3B). Assuming an operating range of $M^{0.70}$ - $M^{0.80}$, we estimated each organ's *cMR* based on the lowest *P* value of the multiple regressions, hence *cMR.**70**kdn*, *cMR.**70**hrt*, *cMR.**70**brn* and *cMR.**80**lvr*. These exponents corresponded to the limits of a zone of minimal correlation with the Core *cMR*, centered at $M^{0.75}$ (Figure 2). For each organ, the log-based *sMR* corresponded to the crossover point for the inversion of the *cMR* slopes, *i.e.* the zone of minimal correlation with *mirFam* and body size, and therefore also of minimal constraint by these factors (Supplementary Figure S5). Again, kidney, heart and brain exhibited similar patterns of slope inversion ~ *cMR*.85, while the slopes for liver and whole body inverted at ~ *cMR.70* (Supplementary Figure S5A). Only the Core exhibited a log-based *sMR* and inversion with the conserved Kleiber exponent ~ 0.75 (0.756) (Supplementary Figure S5B)[43-44].

Plotting the estimated *cMR*s as a function of primate whole body size revealed a strict anticorrelation between liver *cMR.80*, on the one hand, body size and the *cMR.70*s of the other organs, on the other, yielding a slightly negative slope for *cMR.75bdy* with respect to *Mbdy* (Figure 3A). Similar slopes were obtained when plotting organ *cMR*s against the minimal thermal conductance (C_{min}) of 16 Euarchontoglires (Figure 3B), pointing to a common origin for these physiological and thermoregulatory relationships. C_{min} is an extensive property that depends primarily on total surface area and secondarily, on the properties of the insulating layer[45]. Like organ size, it is expected to

increase geometrically with body size[1]. Ranking primates according to log C_{min} revealed a tradeoff in *cMR*, but not of mass, between liver and the other Core organs (Supplementary Figure S6).

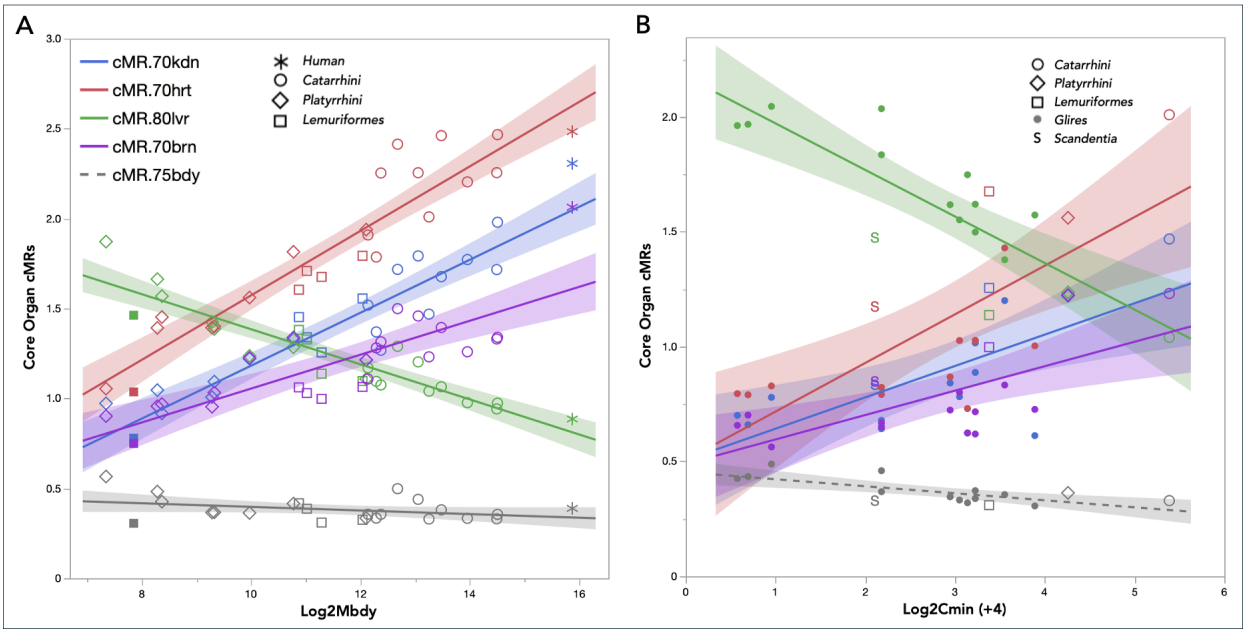

Figure 3. Core organ *cMR*s as a function of body size or thermal conductance.
A. Primates ($N = 24$). Filled symbols represent a heterotherm.
B. Euarchontoglires ($N = 16$). Shaded bands: confidence interval for each regression.

We tested the relevance of these estimated core organ *cMR*s against thermodynamic and phylogenetic models. For a steady state (whole animal) resting *MR* that scales as $M^{0.75}$, the rate of Core thermogenesis has been predicted to scale approximately as √M ($M^{0.50}$)[1]. We applied partial least squares regression[M1] in order to predict the optimal scaling of a joint Core *cMR* composed of either *mirFam*-based *cMR*s or log-based *sMR*s, hence:

$$cMR.Xcor = (rMRkdn + rMRhrt + rMRlvr + rMRbrn) / (Mkdn + Mhrt + Mlvr + Mbrn)^X$$

$$= (a \bullet cMR.\mathbf{70kdn}) + (b \bullet cMR.\mathbf{70hrt}) + (c \bullet cMR.\mathbf{80lvr}) + (d \bullet cMR.\mathbf{70brn})$$

OR

$$sMR.Xcor = (rMRkdn + rMRhrt + rMRlvr + rMRbrn) / (Mkdn + Mhrt + Mlvr + Mbrn)^X$$

$$= (a' \bullet sMR.\mathbf{90kdn}) + (b' \bullet sMR.\mathbf{87hrt}) + (c' \bullet sMR.\mathbf{67lvr}) + (d' \bullet sMR.\mathbf{83brn})$$

The *cMR* model exhibited a broad peak centered at $M^{0.53}$, similar to that predicted by the biophysical model of core thermogenesis[1], with a second peak between $M^{0.80}$ - $M^{0.85}$ (Figure 4A). The log-based

sMR model exhibited a single sharp peak at $M^{0.75}$, incompatible with the biophysical model of thermoregulation, which assumes $M^{0.75}$ scaling for whole body *sMR*[1]. Importantly, the *cMR* and *sMR* models exhibited a critical difference in *VIP* statistics (*Variable Importance of Projection*[M2]), a measure of the degree of interdependence among the variables that is constraining the model (Figure 4A, bottom). While the *cMR* model depended on all four organ *cMR*s over the range *cMR.55cor* - *cMR.70cor*, the *sMR* model depended only on three (excluding kidney) or two organ *sMRs* (excluding kidney and heart) (Figure 4A, bottom). At $M^{0.75}$, where *cMRcor* was minimally correlated with body size and *mirFam* (Figure 2), the *cMR* model also exhibited a lack of constraint by kidney, heart or brain *cMR*, depending only on that of the liver (Figure 4A, bottom).

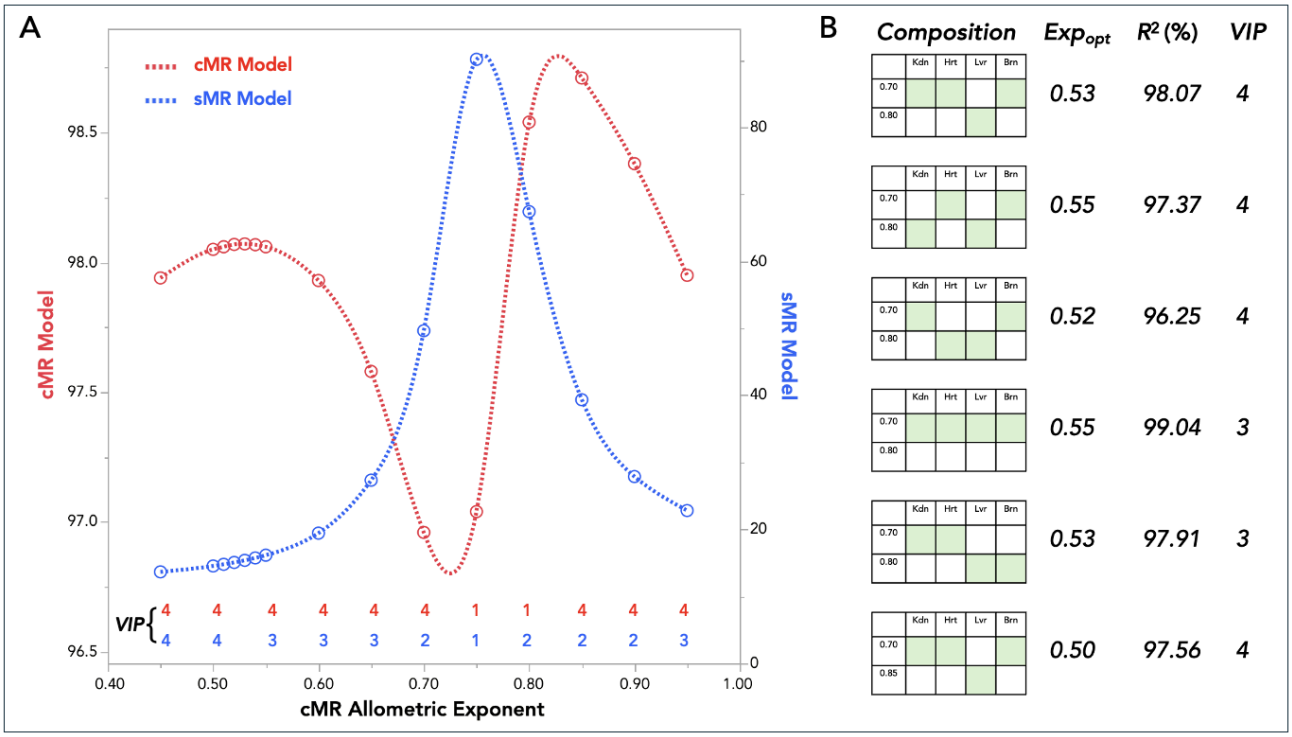

Figure 4. R^2 (% variation) for models of *cMRcor* predicted by linear combinations of *mirFam*-based *cMR*s or log-based *sMR*s of the four thermogenic organs (note the difference in scales; $N = 22$).
A. Comparison between *mirFam*-based organ *cMR*s and log-based organ *sMR*s. Below the plot the table of values for *VIP* (*variable importance of projection*) indicates the number of organs upon which each the model is significantly dependent[M2].
B. Comparison of combinations of *cMR.70* and *cMR.80* among the four thermogenic organs.
Exp_{opt}: the optimally fitting Core allometric exponent for the partial least squares model.

We applied two additional tests of the relevance of the estimated organ *cMR*s, first by comparing the Core *cMR*s obtained after varying the combinations of organ *cMR*s, yielding four models with *VIP* = 4 (Figure 4B). Of these combinations only two, *cMR.70* for heart, kidney and brain, and either *cMR.80* or *cMR.85* for liver, were able to resolve Platyrrhini from Lemuriformes in an unsupervised classification of major primate clades[M3]. After varying the *cMR*s for the liver between *cMR.75* - *cMR.85*, the original set of *mirFam*-based *cMR*s, including *cMR.80lvr*, yielded the lowest value for the information criterion (*AICc*) (Supplementary Figure S7), yielding the linear model:

$$\boldsymbol{cMR.53}\mathbf{cor} = 0.53 \bullet \boldsymbol{cMR.70kdn} + 0.69 \bullet \boldsymbol{cMR.70hrt} + 0.37 \bullet \boldsymbol{cMR.80lvr} + 0.09 \bullet \boldsymbol{cMR.70brn}$$

In the context of the evolution of thermoregulation, the coefficient of each organ *cMR* in the optimal model may be interpreted as reflecting its relative impact on the total rate of internal thermogenesis, and therefore the degree to which it has been constrained by natural selection.

The evolution of organ cMR tradeoffs conforms to the Core model of thermoregulation.

Bayesian inference revealed generally opposing directions of evolution between body size and *cMR.80lvr*, both between Catarrhini and Glires, as well as within each clade (Figure 5). Among primates, an accelerated increase in liver *cMR* accompanied the decrease in size of two of the smallest Platyrrhini, one of which (*Callithrix pygmaea*) may be heterothermic[46]. All five of the smaller (< 1.0 kg) Platyrrhini exhibited elevated levels of *cMR.80lvr*, consistent with adaptation to a low energy regime and increased reliance on gluconeogenesis. Among all Glires, those species that underwent the greatest reductions in size exhibited the highest increases in liver *cMR.80*, while the largest species (Lagomorphs) exhibited the lowest liver *cMR*s (Figure 5).

Brain *cMR.70* increased in intensity and its rate of evolution accelerated with the divergence of Haplorhini, and again in the case of humans, in contrast to its relative stasis in Lemuriformes and Glires (Figure 5). Compared to their ancestor, the five smallest Platyrrhines exhibited an accelerated *reduction* in brain *cMR*, a clade-wide tradeoff that mirrored the size-related tradeoff in Glires between

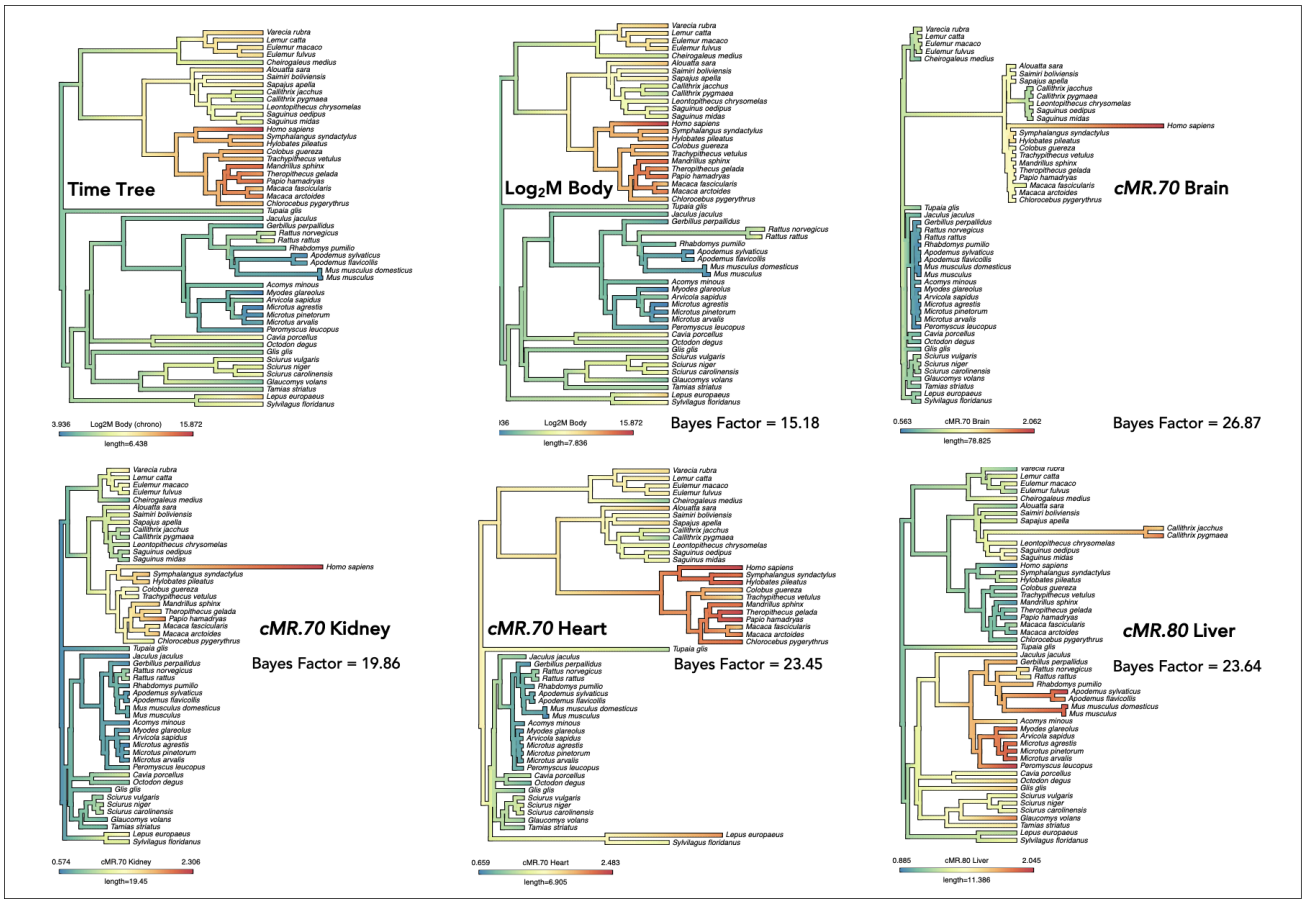

Figure 5. Variable rate phenograms of Log_2M, *cMR.80lvr*, and *cMR.70* of kidney, heart and brain. Bayes factors were computed from the difference between the median log likelihood (*Lh*) of the 1% most likely trees and the median *Lh* of the 1% of trees with the smallest non-zero sum of changes to branch length, *i.e.* those trees most similar to the reference time tree.

brain and liver *cMR*s. The relative levels of heart *cMR.70* generally tracked body size, with the accelerated rates of heart evolution in the clades of the largest Glires (Lagomorpha) and primates (Catarrhini) (Figure 5). In parallel with *cMR.70brn*, an exceptional increase in the rate of evolution of kidney *cMR.70* accompanied the divergence of humans (Figure 5).

DISCUSSION

The recent publication of hundreds of primate genome sequences[47] has been accompanied by ever more fine-grained analysis of the phylogenetic covariance of non-logarithmic traits that may have fueled the evolutionary expansion of brain size in the hominid lineage[48]: diets richer in calories and protein[49-50], more efficient food acquisition and assimilation[51], greater adipose tissue reserves[4, 52-53],

and gains in the reliability of gestation, lactation and infant care[53-55]. However, the disposition of the internal heat generated by larger brains has yet to be addressed: despite having similar mass and surface area, the basal *MR* of humans is 50% higher than than of orangutans[28]. We submit that an implicit assumption of previous studies – that log-transformed brain mass is an accurate index of brain energy expenditure – is not justified. This assumption rests on the observation that the *rMR*s of any related group of species can be made a linear function of *M* by log transformation[38]. However, a thermodynamically-grounded model composed of two logarithmic terms, proportional to mass and surface area, provided even better estimates of log *rMR* than the conventional power law model[39] and, as we have shown here, *cMR*s can be matched to the distributions of relevant physiological and thermodynamic variables without log transformation, in contrast to the fixed distribution of *sMR*s inferred from the slopes of log *rMRorg*/log *Morg*.

Since the seminal analysis by Lande (1979)[38], it has been the convention to base the allometry of brain size on body size[4, 56-57]. Not surprisingly, this approach has not detected significant anticorrelations among organ sizes[4], given that these are developmentally programmed to track body size[58]. The present study underscores the need to consider the evolution of brain size and energy expenditure in the context of the other principal thermogenic organs. Log-based organ *sMR* slopes correspond to the crossover points for the inversion of *cMR* slopes with respect to *mirFam* and organ size, and therefore to the zone of weakest association and constraint by these factors. The Core *sMR* occurred at $M^{0.75}$ and the corresponding *sMR* model of this thermoregulatory core depended only on the liver. Taken together, these observations suggest a hierarchy of organ size regulation: although organ sizes are developmentally programmed between clades to track body size, within clades, the relative proportions of the Core organs may vary in response to thermoregulatory demands.

Of the six possible *cMR* tradeoffs among four thermogenic organs, the brain-liver tradeoff is the most significant and most highly conserved. During the fasting state, once liver glycogen is depleted, the

liver is the primary organ of gluconeogenesis: the glucagon-dependent reduction of amino acid or glycerol carbons, fueled by free fatty acids released from adipose tissue. Gluconeogenesis contributes 40 - 60% of total glucose production after an overnight fast, and greater than 90% after 48 hours of fasting[59]. Liver oxidation of fatty acids also generates ketone bodies that provide 60% of the human brain's energy during a prolonged fast, thereby sparing muscle protein[60]. Elevated levels of circulating FA induce insulin resistance in liver and skeletal muscle[60], an additional mechanism to ensure an uninterrupted supply of glucose to the brain which, unlike these organs, cannot switch to fatty acid oxidation in the fasting state.

Brain glucose utilization peaks around age five in humans, early in the extended period between weaning and sexual maturity, and the period of slowest growth in body mass[11]. Based on the turnover of isotopically-labeled glucose, glucose production was proportional to brain mass over the entire lifespan, and therefore highest among young children[10]. Among prepubertal children (7 - 9 years of age), the rate of gluconeogenesis was still 50% higher than that found among adolescents [12-13]. The inverse relationship between developmental changes in the brain's glucose uptake and body mass growth rate[11] implicate the liver as the pivotal partner in this ontogenetic tradeoff.

Drawing on metabolite fluxes measured in humans, a computational model of the corresponding fluxes in mice found that following an overnight fast, the rates of liver gluconeogenesis, lipolysis and proteolysis were all elevated ~ 10-fold in mice, relative to the corresponding rates in humans[61]. This observation comports well with the opposite directions of evolution of *cMR.80lvr* we found between Glires and primates, which are associated with divergent life history strategies[62-64]. If the evolution of liver sizes depended not only on body size, but also on the capacity for glucose production, then divergence in a setting of high energy reliability might have permitted the observed tradeoffs between liver and other thermogenic organs.

Such evolutionary plasticity appears to co-opt the liver's inherent mechanism of developmental plasticity: in mammals, only the liver retains full regenerative potential[58], and Hippo-YAP, the bilaterian regulator of organ size[65], coordinates liver growth with the suppression of gluconeogenesis[6-9]. Significantly, the global underexpression of microRNAs that is frequently observed in tumor cells has been attributed to aberrant density-dependent regulation of Hippo-YAP signaling[66]: Hippo signaling is normally suppressed at low cell densities, allowing YAP to localize in the nucleus and stimulate cellular proliferation, while YAP translocation and activity are inhibited by Hippo at normal organ size and tissue density[65]. Constitutively active YAP inhibits microRNA biogenesis and causes widespread miRNA suppression in cells and tumors, together with posttranscriptional induction of MYC expression[66-67]. Any significant alteration of overall microRNA biogenesis is expected to cause dysregulation, by either disrupting the timing of transient signals, such as transcription factors[68], or else shifting the repression profile of targets that are regulated by multiple microRNAs. This suggests that expansion of the number of microRNA families, as in the case of the divergence of Boroeutheria and emergence of permanent homeothermy[5], may have been a necessary precursor for resetting the dynamic ranges for regulation of organ size and microRNA biogenesis by Hippo-YAP signaling.

Based on the conjecture that a change in *mirFam* is a necessary precursor to clade-wide change in brain size and overall *rMR*, we modeled a simple feedback loop between brain size and reliability of the energy supply: energy security facilitates an increase in brain size which, in turn, facilitates additional improvement in energy reliability, up to the limits imposed by anatomy and physiology, such as the diameter of the birth canal or the physiological limit on the rate of heat dissipation[14]. Using a logistic function, we fit *mirFam*, a proxy for energy security, to the primate brain size dataset of Grabowski et al (2023)[48], revealing that relative brain size was a predictor of *mirFam*, rather than *vice versa*, and lending credence to the proposition that evolutionary increases in brain size were, more often than not, associated with greater energy reliability:

$$\frac{d\,(mirFam)}{d\,(\log M_{brn}/\log M_{bdy})} = r \bullet mirFam\,(1 - mirFam/mirFam_{max}),$$

where ***r*** is the growth rate and the range of *mirFam* has been scaled to 1.0.

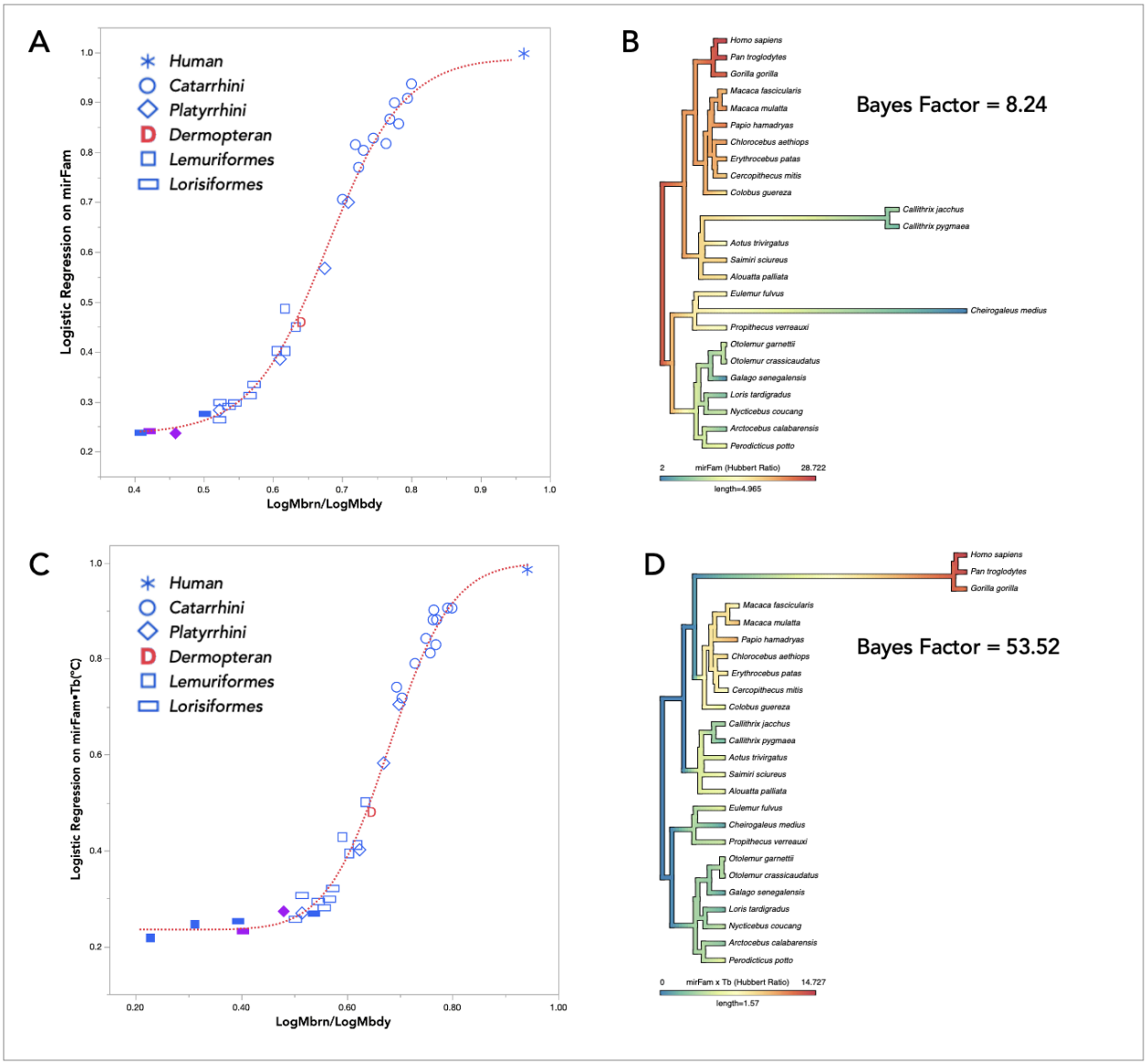

Figure 6. Logistic models of brain size evolution (data from Grabowski et al, 2023)[48].
A, C. For these idealized models (R^2 = 1.00), *mirFam* or *mirFam*•T_b (ºC) was that predicted by the original logistic regression models that excluded the small heterotherm *Cheirogaleus medius*. (original R^2 = 0.88 and 0.85, respectively) (jitter applied). By comparison, logistic regression of Log *Mbdy* on the ratio Log *Mbrn*/Log *Mbdy* yielded R^2 = 0.83. D = *Galeopterus variegatus*. Filled symbols: heterotherms (blue), suspected heterotherms (purple). B, D. Variable rate phenograms of the Hubbert linearization of the corresponding logistic functions.

Given that the relative sizes of the other thermogenic organs can also be fit to logistic models for *mirFam* (Supplementary Figure S8), the question arose as to which relationship might be the

principal driver of evolutionary adaptation. In phylogenetic regressions on *mirFam* of organ *cMR*s ranging from *cMR*.60 - *cMR*.80, kidney and heart *cMR*s, and all but a single liver *cMR* (*cMR.60lvr*) varied independently of phylogeny (Pagel's *lambd*a = 0), while in every case, the variation of brain *cMR*.60 - *cMR*.80 with respect to *mirFam* was secondary to phylogenetic distance (*lambda* = 1)[41], consistent with the proposition that the divergence of primate clades coincided with changes in the dynamic range of *mirFam* with respect to relative brain size (Supplementary Table S3).

A variable rate phenogram corresponding to this model was generated using the ratios from a Hubbert linearization of the logistic function (Figure 7B)[69]. Thus, the color scale represents rank on the logistic curve, which generally tracks body size. The three smallest species, a Lemuriform and two species of Callithrix, exhibited an accelerated descent in rank: *Cheirogaleus medius* is a heterotherm, and *Callithrix pygmaea* may also be heterothermic[46]. This observation aligns with that previously reported for Callithrichines[57], and suggests that the baseline of the logistic function represents a regime of minimal energy reliability, possibly low calorie-density folivory or insectivory, which may also inform the accelerated rate of evolution of liver *cMR.80* in the *Callithrix* clade. Extending this logic, we postulated that the upper plateau of the logistic model, occupied by primates with the largest relative brain sizes, might correspond to a physiological limit on the rate of heat dissipation[14]. During exercise, human brain temperature increases in parallel with that of the body core, leading to hyperthermia and fatigue[24-26, 70]. We have previously reported that body temperatures (T_b) in primates are inversely correlated with *mirFam*[5]. Since the highest *mirFam* are found in the largest primates (*Catarrhini*), this trend is reminiscent of the opposite trends, on a broad mammalian scale, between body size[14] and body temperature[15]. For a given rate of thermogenesis, a larger size and smaller ratio of surface area to volume necessitates a faster rate of heat dissipation, consistent with the accelerated evolution of heart *cMR.70* that we observed in the largest Glires and primates.

Ranking primate species by size revealed conservation of the overall relationship between *mirFam* and body size (Supplementary Figure S9, A), as well as an inverse relationship between *mirFam* and T_b , after correction for phylogeny (Supplementary Figure S9, B). We hypothesized that the product *mirFam* x T_b represents the physiological limit on the rate of heat dissipation limit, given that (i) when ordered according to the rate of Core thermogenesis (*cMR.53cor*), *mirFam* x T_b generally tracked thermal conductance (Supplementary Figure S10, A) and (ii) only among the two earliest-branching clades of primates (lorises and lemurs), did the standardized range of *mirFam* x T_b fall below the range of relative brain size (Supplementary Figure S10, B). Therefore, the accelerated evolution detected among hominid apes near the plateau of the *mirFam* x T_b logistic model (Figure 6D) may signal an adaptation to the physiological limit on the rate of heat dissipation[14].

In keeping with the long-range mammalian trends for body size[14] and body temperature[15], Bayesian inference predicted opposite evolutionary trends for the evolution of the relationship to brain size of *mirFam* and *mirFam* x T_b: descending in rank on the *mirFam* tree, and ascending in rank on the tree for *mirFam x* T_b (Figure 6C and D, respectively). Thus, our logistic model assigns the ancestral primate a high rank with respect to energy reliability and a low rank with respect to the heat dissipation limit, a propitious combination for the founder of a new order of mammals, on a new fitness landscape[71]. In many respects this model, emerging from the reparametrization of rates of energy consumption in terms of *mirFam*-based *cMR*s, fulfills the recent call for an explanation of the distinct pattern of brain size evolution in primates "in terms of the release of a constraint or an adaptive shift that initiated escalating feedback between brain and behavior".[23] Together with the Core model of thermoregulation, it has allowed us to uncover a conserved energy tradeoff that was first proposed 40 years ago in order to account for the expansion of brain size in the human lineage[2]. Since these models are agnostic regarding underlying mechanisms, they may also serve as a null hypothesis for comparison with the evolution of other relevant adaptations.

The *cMR* model also has far-reaching implications for evolutionary medicine: for example, the adaptive increase in human kidney *cMR.70* to a level 30% higher than found in other hominoid apes; by comparison, human kidney *cMR* was 10% *lower* when scaled according to the slope (0.90) of log *rMRkdn*/log*Mkdn*. Since the kidney is responsible for the homeostasis of pH, nitrogen, sodium, water and glucose, the rapid evolution *cMR.70kdn* in humans may have facilitated adaptations to a diet enriched in lipid and protein[49], and an altered landscape of opportunities for hydration *versus* risks of dehydration ("Savanna hypothesis")[33-36]. A simulation of two million years of African and Eurasian climate and habitats has provided evidence that *H. sapiens* evolved from *H. heidelbergensis* in southern Africa during an unusually warm period that would have necessitated adaptation to hotter, drier conditions[36]. A high density of eccrine sweat glands[31] may have enabled early humans to run down game in long distance pursuits[72], but may also have made them uniquely susceptible to dehydration[32], a marathon-enabling tradeoff. As it is, the kidneys of modern humans are susceptible to damage by heat stress: exposure to 40ºC for as little as one hour is sufficient to redirect blood flow to the skin, reduce blood flow to the kidney by one third[73], and trigger responses promoting water conservation that can eventually lead to acute kidney injury, such as activation of the renin-angiotensin-aldosterone system[74-75]. The kidneys of our hominid ancestors may have been even more vulnerable prior to the adaptive increase in *cMR.70kdn*.

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METHODS

Physiological Data

The masses and *rMR*s of the four thermogenic organs were obtained from the dataset of Wang et al (2012)[M4], originally derived from the compilation of Navarette et al (2011)[M5] (Supplementary Table S1). Values for thermal conductance (C_{min}) were obtained from the dataset of Khaliq et al (2014)[M6], substituting congeneric species from the dataset of Wang et al (2012)[M4]. Where C_{min} was available for two or more congeneric species, we chose the one with the mass closest to the species in the Wang dataset. Body temperatures were obtained from the AnAge database[M7].

MicroRNA Families, *Binning*

Complements of conserved microRNA families were obtained from the most complete assembly for each species using the covariance model MirMachine[M8], trained on the manually-curated database of microRNA genes, MirGeneDB2.1[M9] (Supplementary Tables S1 and S2). For Scandentia, Lagamorpha and Rodentia, *mirFam* refers to the number of families common to Euarchontoglires. For Lorisiformes, Lemuriformes and Platyrrhini, it refers to the number of families shared with Catarrhini. For the original regression of organ *cMR*s on *mirFam* and organ size (Figure 2), *mirFam* was binned according to natural divisions in its distribution: 141 - 161 (Scandentia and Glires), 165 - 181 (Glires, Lemuriformes and Platyrrhini), and 194 - 208 (Catarrhini).

Phylogenetic Trees

Using Mesquite © (2021) we derived ultrametric trees for 51 Euarchontoglires or 24 primates from the phylogenies of Alvarez-Carretero et al (2021)[M10] and Shao et al (2023)[M11] (Figures 5 and 6, respectively).

Variable Rate Phenograms

Variable rate trees and ancestral node values were inferred using BayesTraits.V4[M12-M13] (software available at www.evolution.rdg.ac.uk). Bayes factors for variable rate trees were computed from the difference between the median of the top 1% likelihoods and the median likelihood of the 1% of trees with the smallest non-zero sum of changes to branch length, i.e. those trees most similar to the reference time tree[M14]. Phenograms were generated using Phytools[M15].

Statistics

Linear, logistic and partial least squares regressions and normal mixture clustering were carried out and visualized using JMP16 ®. All R^2 values were adjusted for the number of predictors. Phylogenetic linear regressions were carried out using the R: CAPER package[M16].

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ENDNOTES

Data Availability

All original data with accompanying references are provided in the Supplementary Information Tables S1 and S2.

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Author Contributions

Conceptualization: TS; Methodology: TS; Investigation: BF and TS; Writing – Original Draft: TS; Writing – Review & Editing: BF and TS; Visualization: TS; Funding Acquisition: BF.

Declaration of Interests

The authors declare no competing interests.

SUPPLEMENTARY INFORMATION

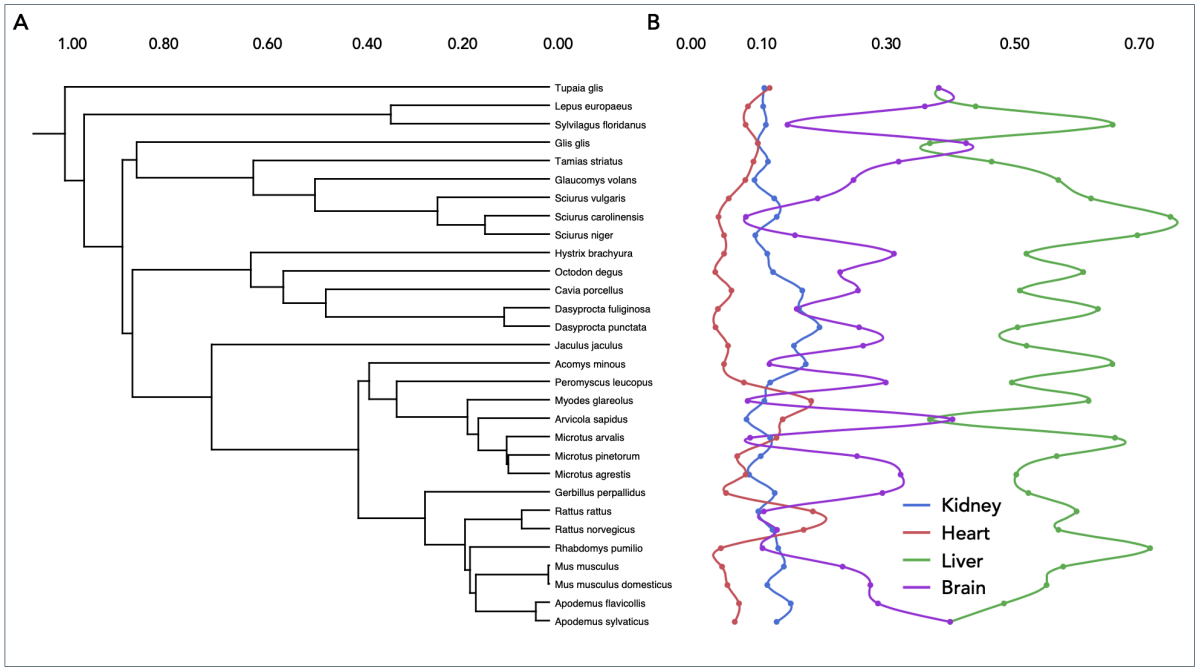

Figure S1. The proportions of brain and liver vary reciprocally in the Euarchontoglires Core. Species include one Scandentia, two Lagomorpha, and 27 Glires, including three Sciuridae.
A. Ultrametric phylogenetic tree. The scale represents relative time of divergence.
B. Mass proportion of each organ relative to the total Core mass.

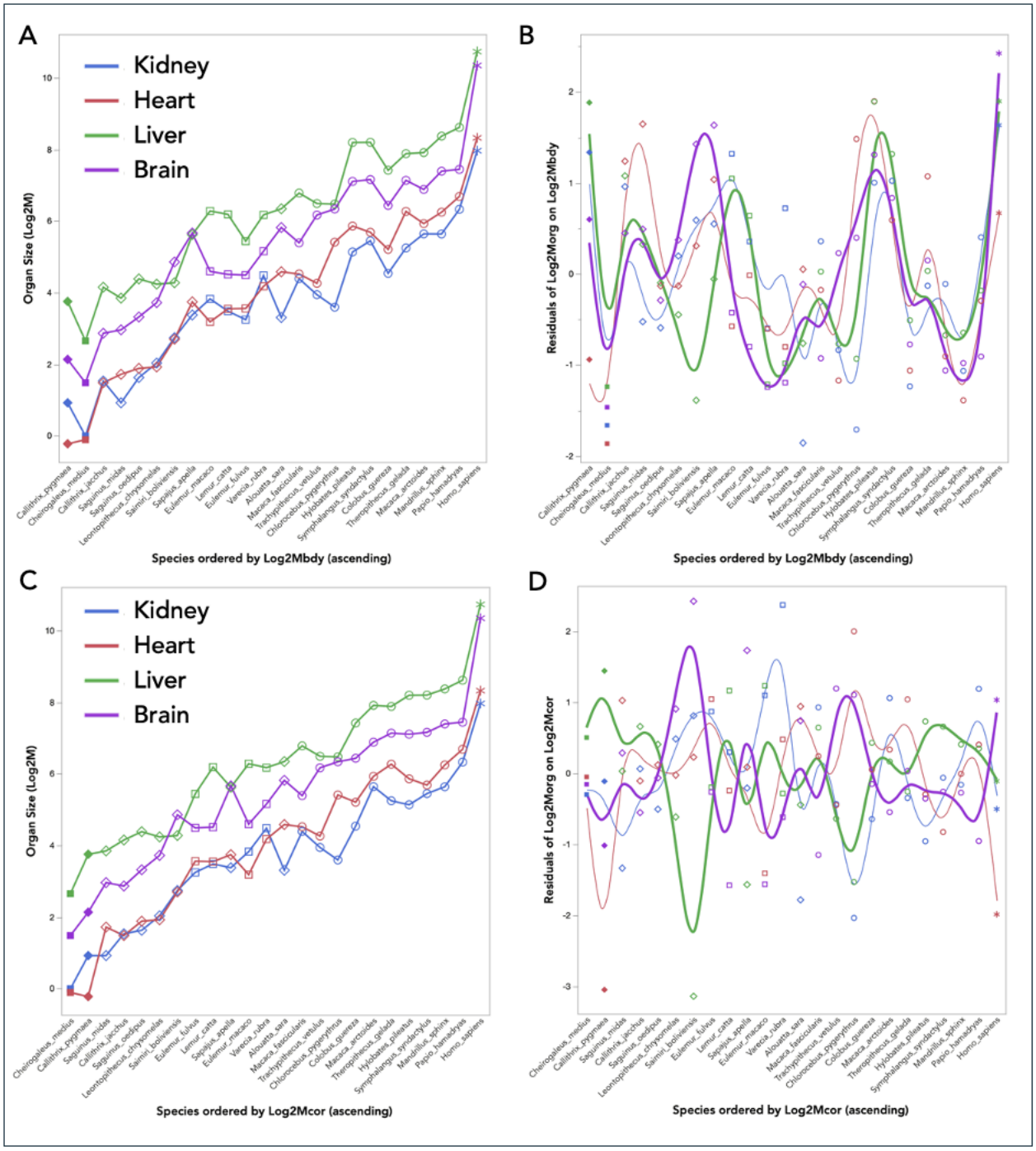

Figure S2. The reciprocal variation between liver and brain mass in the body and the Core.
A and C: Variation of organ size relative to body size and Core size in primates ($N = 24$).
B and D: Mass-independent residual variation relative to body size and Core size in primates.

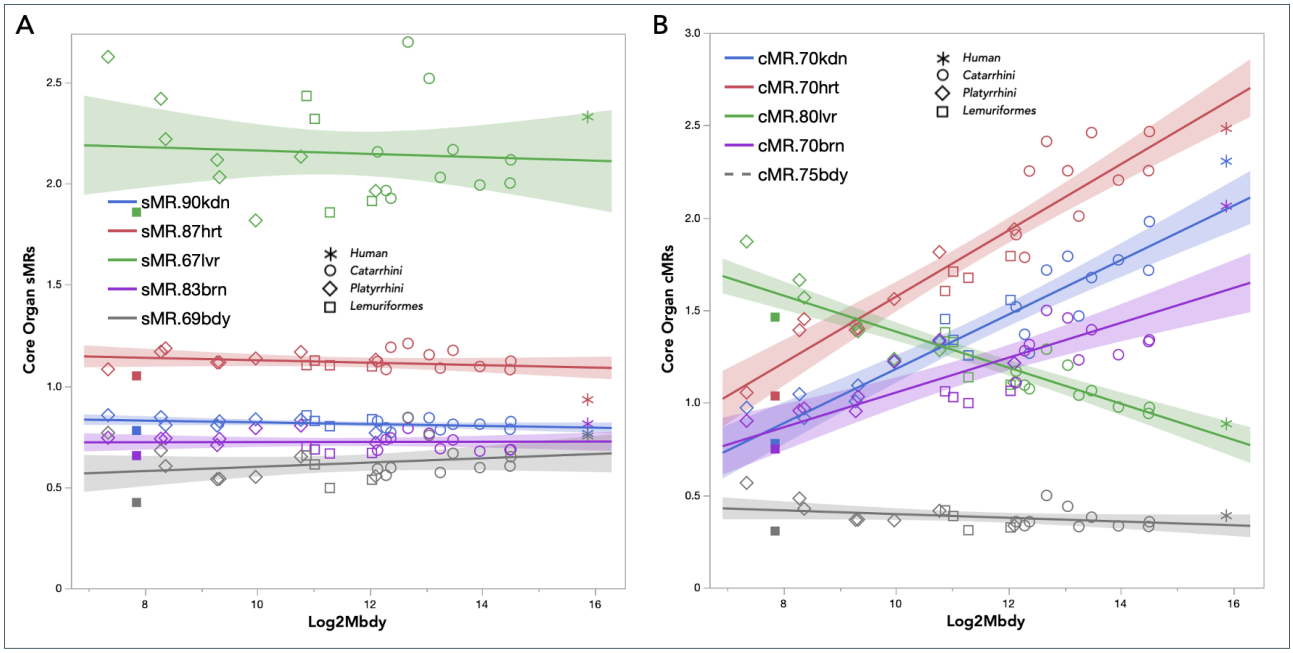

Figure S3. *cMR* slopes vary with size, while an *sMR* slope is independent of size.
A. Log-based *sMR* slopes are independent of organ or body size ($N = 24$).
B. Estimated *cMR* slopes for four thermogenic organs compared to the Kleiber[38] whole-body *sMR*.
See the legend to Figure 3 for details on the estimation of organ *cMR*s.

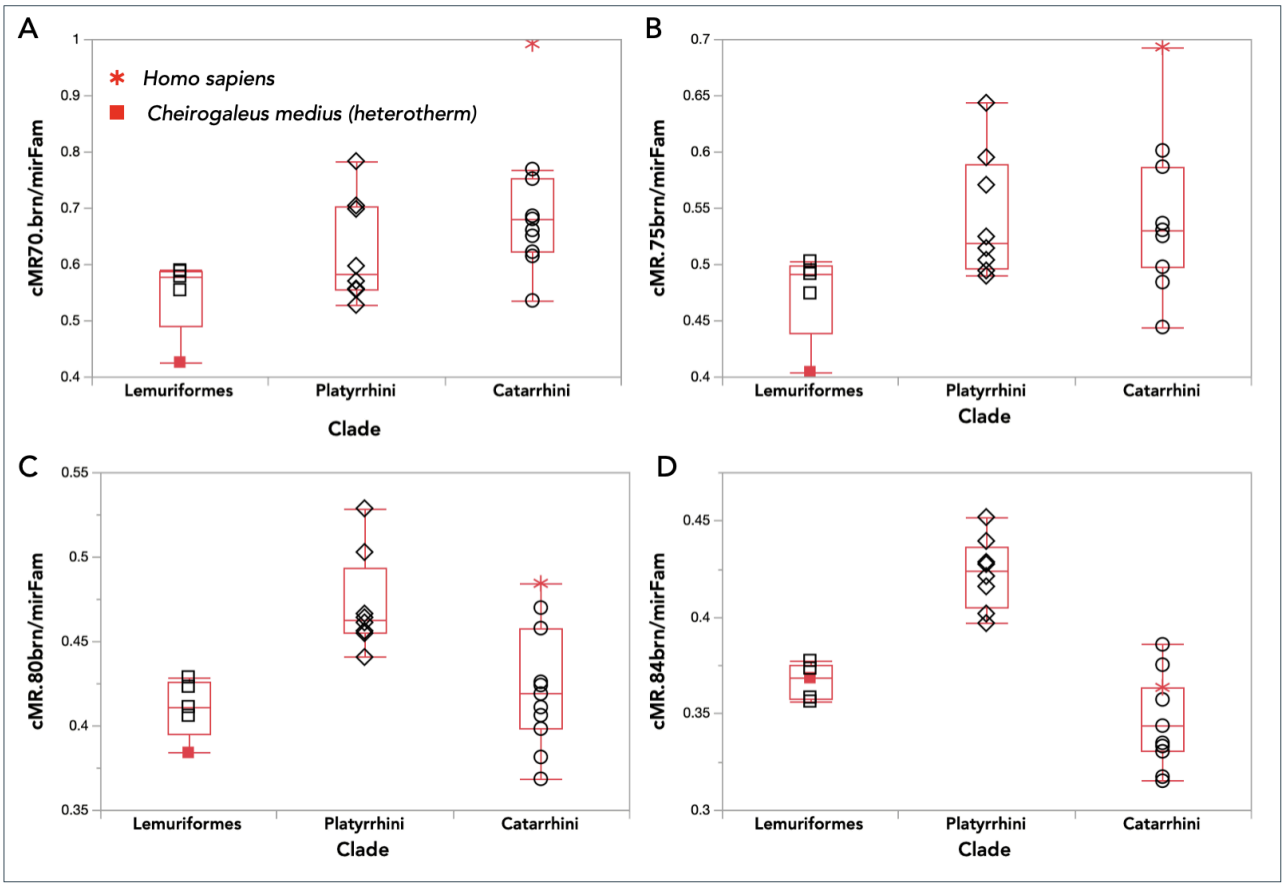

Figure S4. Comparison of the distributions among 24 primates of *cMR*s/*mirFam* or *sMR*/*mirFam*. A. *cMR.70brn*/*mirFam* B. *cMR.75brn*/*mirFam* C. *cMR.80brn*/*mirFam*. D. *sMR.84brn/mirFam*. Log *rMRbrn*/log *Mbrn* = 0.84, hence $sMRbrn = rMRbrn/M^{0.84}$.
Boxplots represent medians, quartiles, minima and maxima.

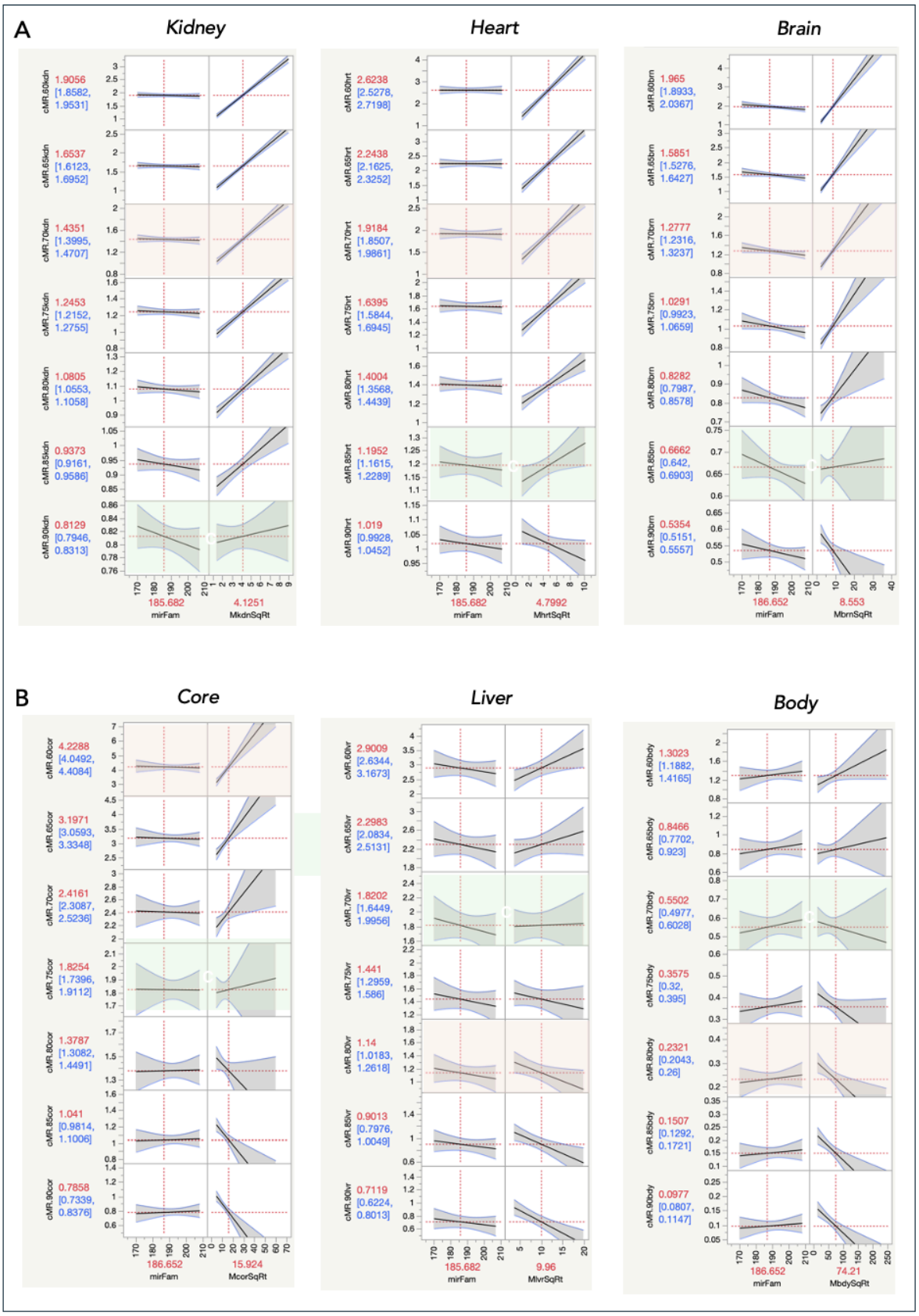

Figure S5. *cMR* slopes with respect to *mirFam* and √*M* as a function of allometric exponent (22 primates). Estimated *cMR* slopes are shaded pink, whereas log-based *sMR* slopes are shaded green. Note that the log-based *sMR*s correspond to crossover points for the inversions of the *cMR* slopes. Values in red represent means (± CI).

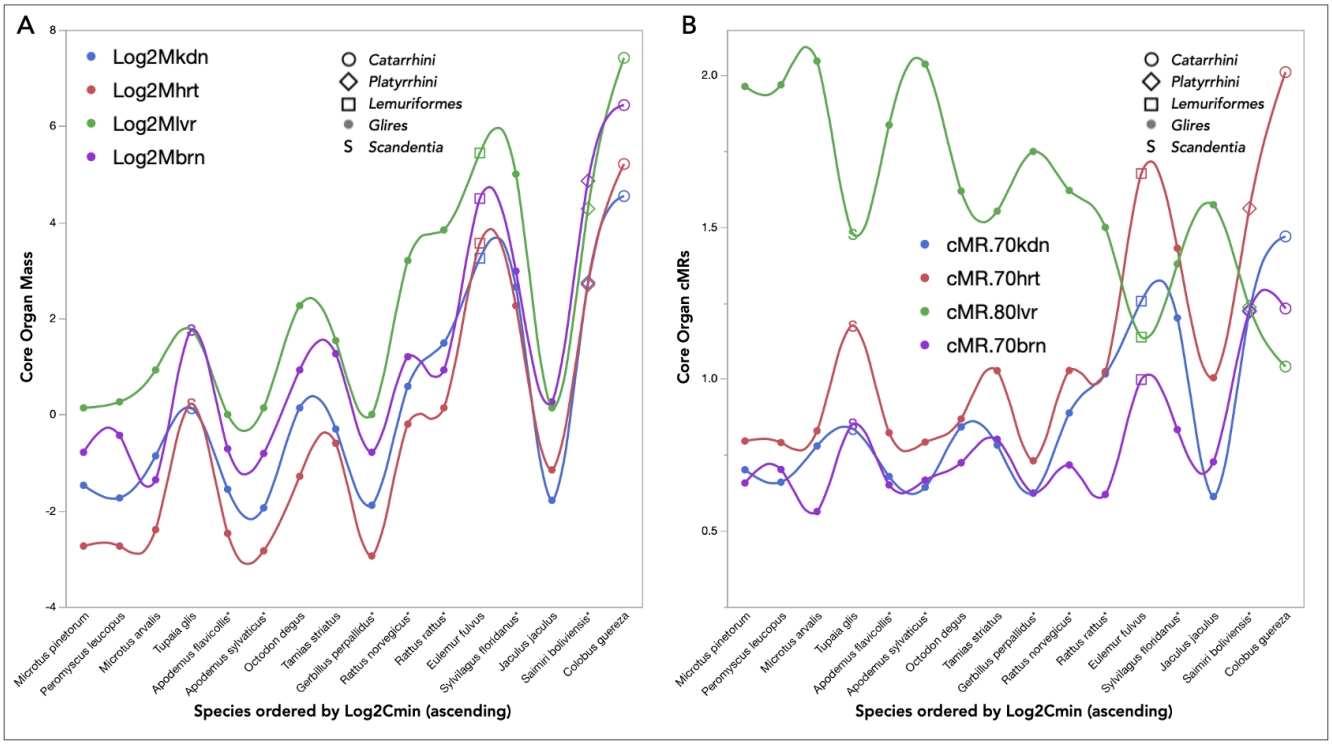

Figure S6. Thermal conductance also covaries with *cMR* tradeoffs between liver and brain.
A. Thermal conductance is a direct function of whole body size, and therefore of organ size.
B. The size-related increase in thermal conductance is related to a compensatory tradeoff in core thermogenesis between liver, on the one hand, and kidney, heart and brain, on the other.
Asterisks (*) represent species in the 4-organ dataset of Wang et al (2012)[M4] that have been substituted for congeneric species in the C_{min} dataset of Khaliq et al (2014)[M6].

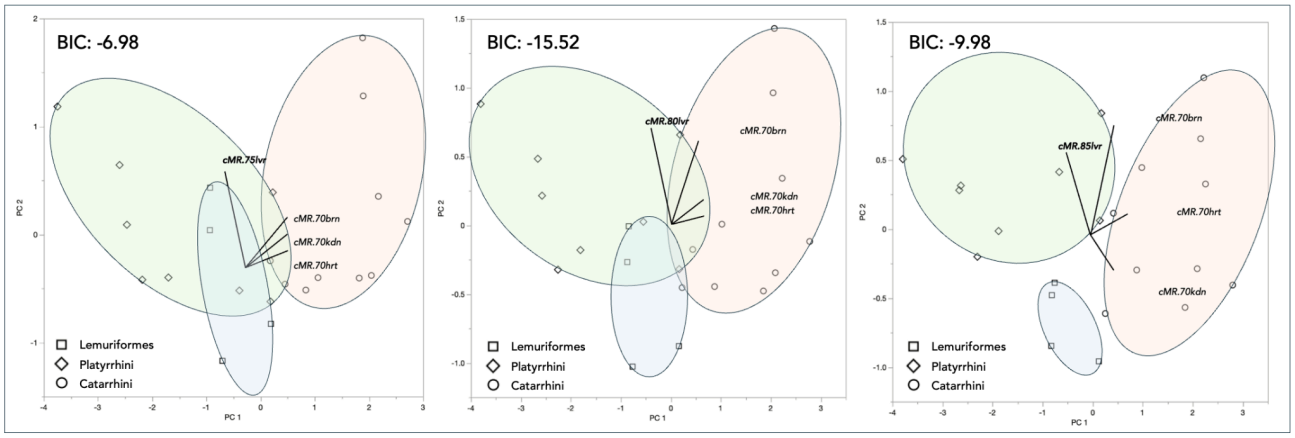

Figure S7. Unsupervised classification of primate clades based on Core *cMR* composition.
Of the four *cMR* models with *VIP* = 4 (Figure 5B), only two (with *cMR.80lvr* and *cMR.85lvr*) resolved Platyrrhini from Lemuriformes. Principal components were calculated from each *cMR* correlation matrix, and membership in two clusters estimated by expectation maximization of their joint probability distributions.
Kidney, heart and brain *cMR* = *cMR.70*; liver *cMR* varied between *cMR.75lvr* - *cMR.85lvr* (bold type). Ellipses were hand-drawn to include all members of the three phylogenetic clades.

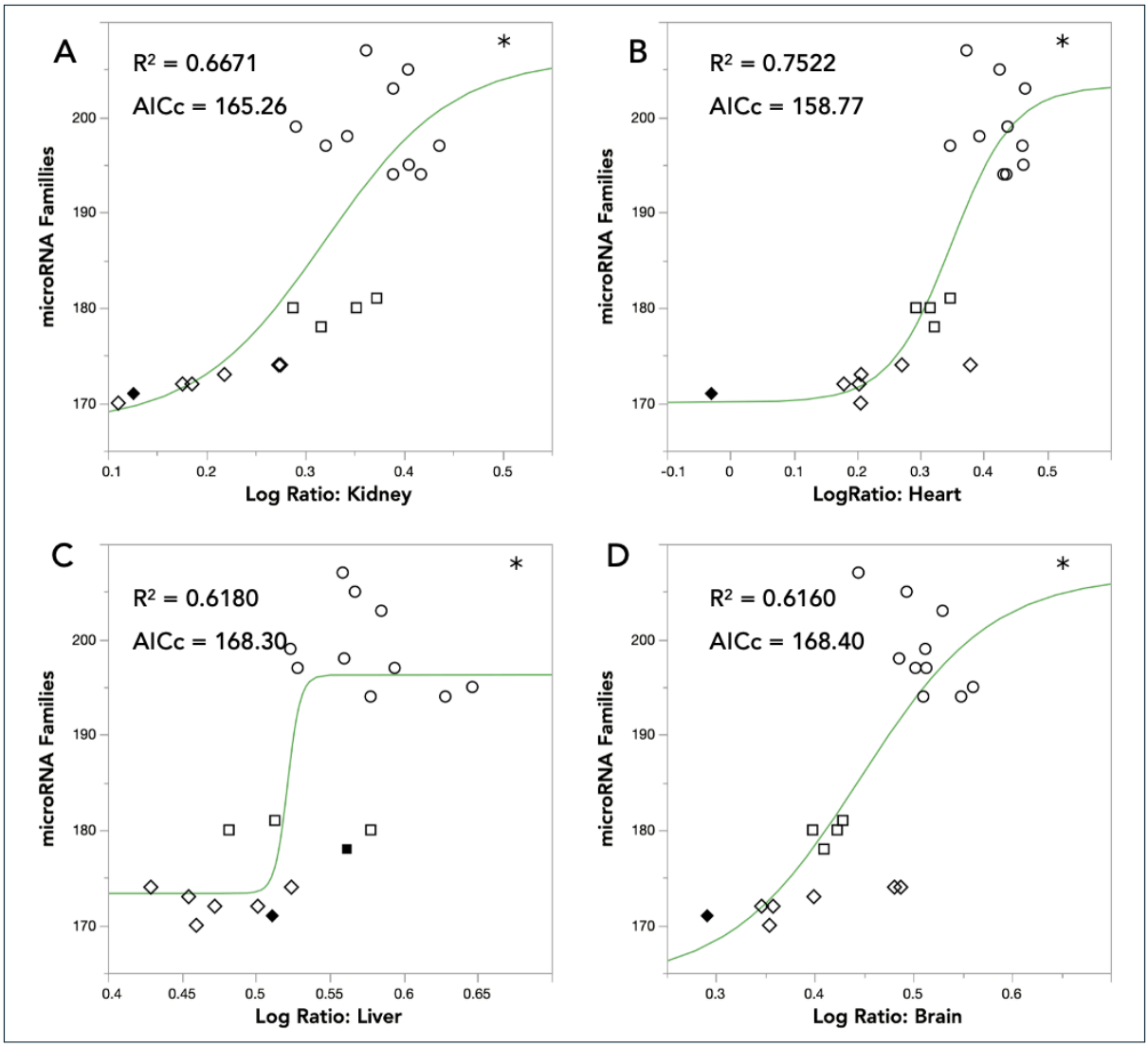

Figure S8. Logistic models for evolution of the relative size of four thermogenic organs. Symbols representing clades as in previous figures, with filled symbols representing heterotherms.

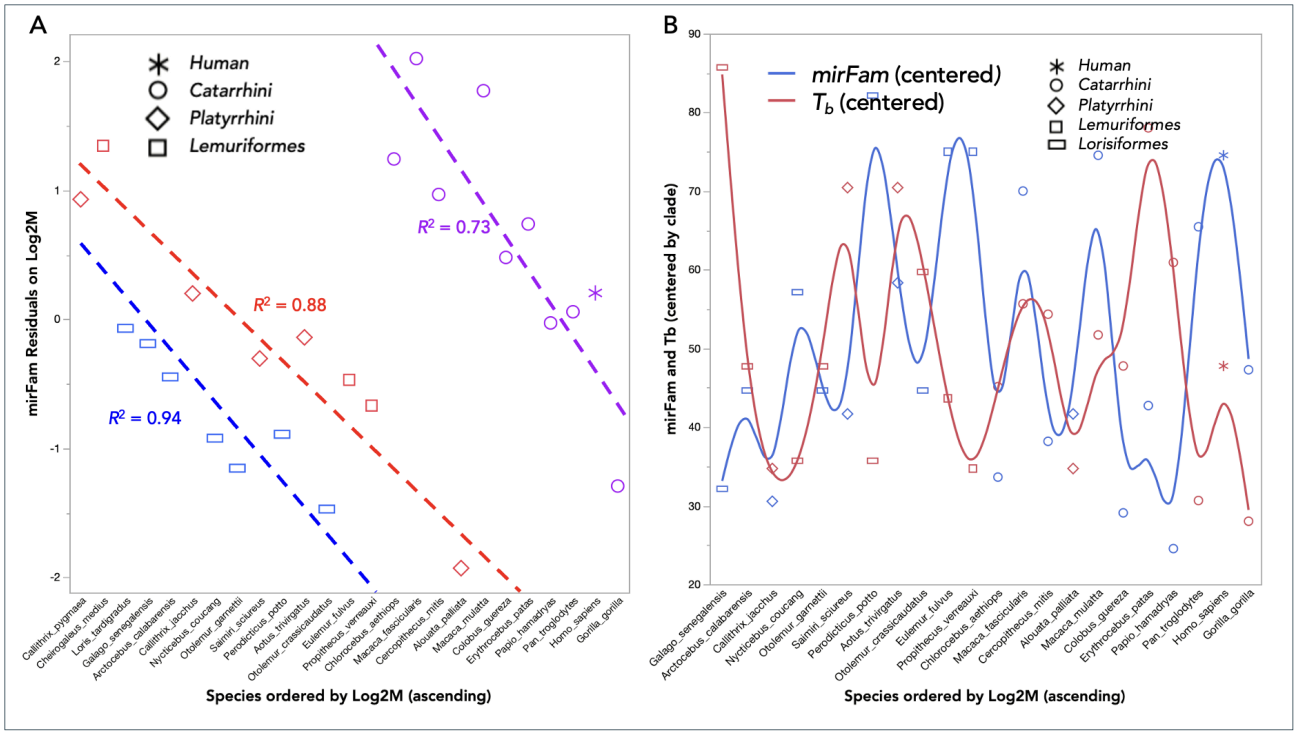

Figure S9. Conservation of *mirFam* and *mirFam* x T_b.
A. The *mirFam*-$\log_2 M$ relationship is the least conserved among Catarrhini (purple).
B. Visualization of the reciprocal variation of *mirFam* and T_b. In order to control for phylogeny, the data were range-standardized for Catarrhini, Lorisiformes, and Platyrrhini+Lemuriformes.

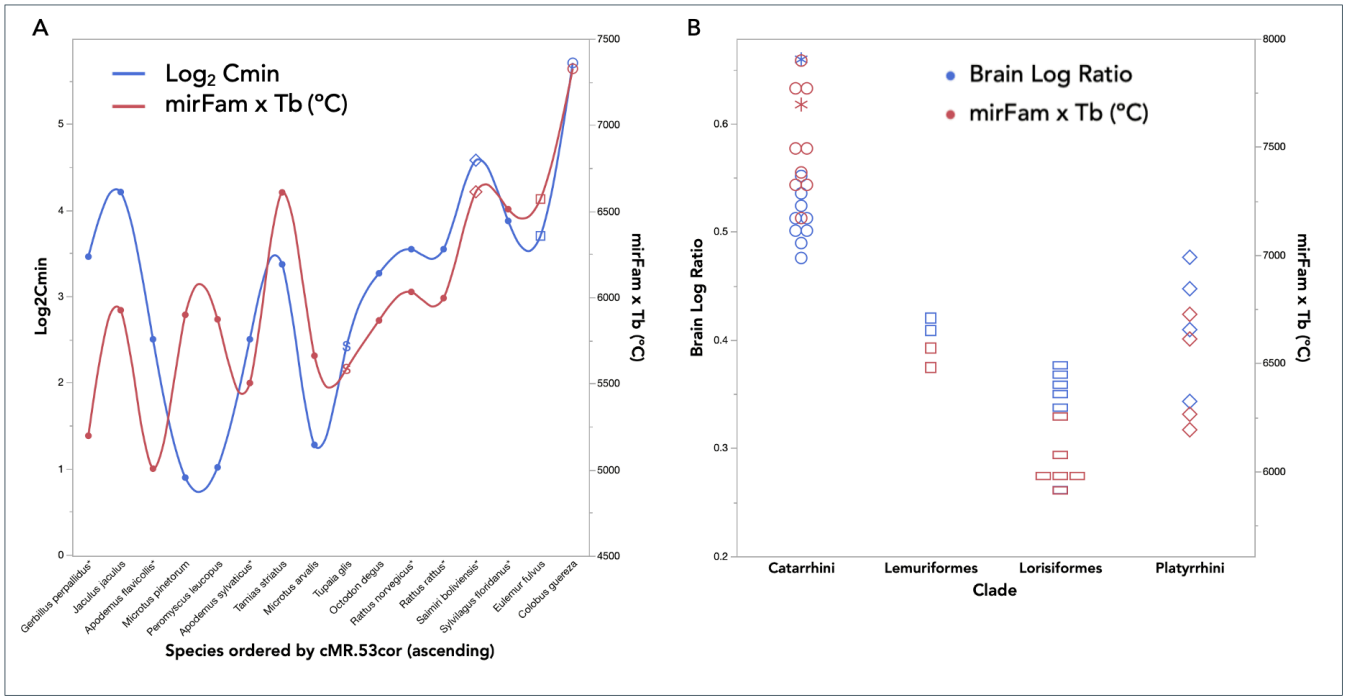

Figure S10. *mirFam* x T_b behaves as a proxy for the physiological limit on heat dissipation.
A. *mirFam* x T_b generally tracks thermal conductance in Euarchontoglires.
B. The range of *mirFam* x T_b falls below the range of relative brain size only in the earliest-branching clades of primates (Lorisiformes and Lemuriformes).

Table S1. Summary of species *mirFam*, mass and resting *MR*s for organs, Core and body. All physiological data were obtained from Wang et al (2012)[M4], most of which had been compiled by Navarette et al (2011)[M5]. The names of the indicated taxa were substituted with congeneric species on the phylogenies of Álvarez-Carretero et al (2022)[M10] and Shao et al (2023)[M11].

Clade	Wang Taxon Names	Names on Phylogeny	*mirFam*	*Mkdn* (g)	*Mhrt* (g)	*Mlvr* (g)	*Mbrn* (g)	*Mcor* (g)	*Mbdy* (g)	*MRkdn* (W)	*MRhrt* (W)	*MRlvr* (W)	*MRbrn* (W)	*MRcor* (W)	*MRbdy* (W)
Primates	*Alouatta sara*	*Alouatta palliata*	174	9.9	24	81.2	56.5	171.6	4399.6	13.7088	13.6112	33.6452	12.9948	73.96	169.5872
Primates		*Callithrix jacchus*	172	2.9	2.8	17.8	7.3	30.8	311.8	6.039	17.928	37.352	20.453	81.772	180.7072
Primates	*Cebualla pygmaea*	*Callithrix pygmaea*	171	1.9	0.86	13.5	4.4	20.66	162.66	6.8432	11.1622	29.0377	20.9296	67.9727	112.3625
Primates		*Cheirogaleus medius*	176	1	0.93	6.3	2.8	11.03	231.03	1.5238	0.94858	15.012	2.5432	20.02758	25.77858
Primates		*Chlorocebus pygerythrus*	199	12.1	42.6	89	80.8	224.5	5300.5	2.204	2.8616	16.643	3.8471	25.5557	35.7841
Primates		*Colobus guereza*	198	23.3	37	171	86.5	317.8	9749.8	2.9356	3.5644	14.553	6.2832	27.3362	46.7808
Primates		*Eulemur fulvus*	180	9.5	11.8	43.4	22.5	87.2	2500.2	1.4364	3.3495	13.248	4.0794	22.1133	33.0213
Primates		*Eulemur macaco*	180	14.2	9.1	77.8	24.2	125.3	1875.3	2.2227	3.4817	16.2184	4.78	26.7028	45.6892
Primates	Human	*Homo sapiens*	208	250	320	1700	1300	3570	59970	9.2862	14.1312	40.05	25.56	89.0274	199.0161
Primates	*Hylobates concolor*	*Hylobates pileatus*	195	35.2	58.2	293	137.8	524.2	6550.2	13.3043	25.16	63.612	27.9395	130.0158	323.3718
Primates		*Lemur catta*	178	11.2	11.7	72.9	22.8	118.6	2074.6	12.789	17.135	50.27	15.162	95.356	195.6392
Primates		*Leontopithecus chrysomelas*	173	4.1	3.8	18.9	13.2	40	642	27.4	39.162	78.566	35.518	180.646	471.706
Primates	*Macaca nigra*	*Macaca arctoides*	205	50	61	241	118	470	15870	42.6393	63.242	115.64	49.59	271.1113	669.3613
Primates	Monkey	*Macaca fascicularis*	207	21	23	110	42	196	4500	21.394	51.5696	84.252	44.5244	201.74	419.94
Primates		*Mandrillus sphinx*	194	49.9	76	331	168	624.9	23024.9	0.8932	1.31274	3.9304	2.006	8.14234	13.62034
Primates		*Papio hamadryas*	197	80.3	103	392	174	749.3	23249.3	2.229	2.4725	9.2769	3.8025	17.7809	31.2792
Primates	*Saguinus lagonotus*	*Saguinus midas*	170	1.9	3.3	14.4	7.8	27.4	330.4	0.2841	0.2094	2.2764	0.56832	3.33822	4.47022
Primates		*Saguinus oedipus*	172	3.1	3.7	20.9	10	37.7	623.7	0.23424	0.1456	1.39829	0.2828	2.06093	2.90093
Primates		*Saimiri boliviensis*	174	6.7	6.5	19.4	29	61.6	1002.6	0.609	0.34636	3.7466	0.7304	5.43236	8.15126
Primates		*Sapajus apella*	171	10.4	13.4	49.3	50.8	123.9	1749.9	0.5126	0.25916	3.4181	0.29133	4.48119	5.79399
Primates		*Symphalangus syndactylus*	194	43.7	51.5	294	143	532.2	8500.2	1.179	0.93177	9.5588	1.2834	12.95297	20.57737
Primates		*Theropithecus gelada*	203	38	77.2	236	140.9	492.1	11402.1	0.27412	0.10241	1.43344	0.29232	2.10229	2.94789
Primates		*Trachypithecus vetulus*	197	15.4	19.2	90	72	196.6	4999.6	0.47379	0.21728	3.2058	0.3715	4.26837	5.62837
Primates		*Varecia rubra*	181	22.4	18.1	72.2	35.7	148.4	4200.4	0.25038	0.19992	2.1967	0.44973	3.09673	4.03113
Scandentia		*Tupaia glis*	151	1.1	1.17	3.4	3.4	9.07	141.07	0.31892	0.24768	1.835	0.46055	2.86215	4.13635
Glires		*Acomys minous*	157	0.32	0.18	0.9	0.9	2.3	42.3	0.779	0.98487	6.3756	1.54	9.67947	18.10547
Glires		*Apodemus flavicollis*	141	0.34	0.18	1	0.61	2.13	25.13	7.2688	9.5589	41.0427	9.1884	67.0588	118.8928
Glires		*Apodemus sylvaticus*	155	0.26	0.14	1.1	0.57	2.07	18.07	6.0705	9.4282	23.219	8.82	47.5377	109.5518
Glires		*Arvicola sapidus*	149	0.7	0.28	2.6	1.1	4.68	61.68	9.301	7.5257	44.9684	9.8736	71.6687	118.9187
Glires	Guinea Pig	*Cavia porcellus*	174	5.6	2.3	27	4.7	39.6	799.6	4.623	5.785	13.2502	12.934	36.5922	64.7281
Glires		*Gerbillus perpallidus*	147	0.27	0.13	1	0.58	1.98	29.98	110	140.8	340	312	902.8	1489.36
Glires		*Glaucomys volans*	176	0.59	0.56	2.9	1.9	5.95	54.95	20.768	41.4966	121.302	47.1276	230.6942	362.6636
Glires		*Glis glis*	168	0.68	0.48	3.2	1.5	5.86	83.86	25.2149	35.5865	113.484	47.047	221.3324	388.6604
Glires		*Jaculus jaculus*	158	0.29	0.45	1.1	1.2	3.04	48.04	7.26	31.1406	38.982	28.4416	105.8242	221.0494
Glires		*Lepus europaeus*	173	18.5	28.9	90.4	14.8	152.6	3338.6	26.4969	46.74	97.645	48.048	218.9299	617.6499
Glires		*Microtus agrestis*	148	0.17	0.12	0.63	0.39	1.31	15.31	11.544	22.3108	44.748	5.5648	84.1676	162.2246
Glires		*Microtus arvalis*	153	0.55	0.19	1.9	0.39	3.03	27.03	4.3533	4.2864	22.048	3.5392	34.2269	61.949
Glires		*Microtus pinetorum*	154	0.36	0.15	1.1	0.58	2.19	21.19	0.57664	0.57312	4.256	0.954	6.35976	9.89316
Glires		*Mus musculus*	167	0.28	0.07	0.68	0.36	1.39	15.39	0.67473	0.76758	3.6366	1.4784	6.55731	10.79621
Glires	Mouse	*Mus musculus domesticus*	167	0.51	0.16	1.8	0.5	2.97	27.97	0.51802	0.70224	4.3094	1.2825	6.81216	9.19356
Glires		*Myodes glareolus*	143	0.24	0.1	0.67	0.35	1.36	15.36	1.3056	1.7629	5.3185	3.3831	11.7701	21.4049
Glires		*Octodon degus*	156	1.1	0.41	4.8	1.9	8.21	129.21	2.304	2.6488	12.8904	3.6075	21.4507	39.9589
Glires		*Peromyscus leucopus*	160	0.3	0.15	1.2	0.74	2.39	22.39	0.8998	0.46494	5.6736	1.1343	8.17264	13.26674
Glires		*Rattus norvegicus*	168	1.5	0.87	9.2	2.3	13.87	209.87	3.9368	2.1022	19.602	2.1667	27.8077	51.4437
Glires	Rat	*Rattus rattus*	167	2.8	1.1	14.3	1.9	20.1	390.1	0.25752	0.57375	1.6973	0.8256	3.35417	5.59067
Glires		*Rhabdomys pumilio*	161	0.41	0.21	1.8	0.6	3.02	50.02	0.28704	0.23274	1.4337	0.63	2.58348	4.61148
Glires		*Sciurus carolinensis*	175	3.2	2.8	16.4	7.5	29.9	595.9	0.34272	0.21075	2.1175	0.44892	3.11989	4.20479
Glires		*Sciurus niger*	165	3	2.5	10.7	7.5	23.7	412.7	0.16592	0.17472	1.31481	0.31512	1.97057	2.81057
Glires		*Sciurus vulgaris*	175	1.7	1.7	5.5	6.3	15.2	274.2	0.24948	0.17511	1.748	0.4263	2.59889	4.10249
Glires		*Sylvilagus floridanus*	170	6.3	4.8	32	7.9	51	972	2.0888	1.0945	12.584	0.9709	16.7382	29.7252
Glires		*Tamias striatus*	173	0.81	0.66	2.9	2.4	6.77	103.77	0.36285	0.26607	2.7414	0.4098	3.78012	6.09722

Table S2. Primate microRNA families, brain volume (ECV), body mass and temperature. Endocranial volume (ECV) and body mass were obtained from Grabowski et al (2023)[48]. Body temperatures were retrieved from the AnAge database (Magalhães et al, 2024)[M7].

Order	Species	*mirFam*	ECV	Mass (g)	T_b (°C)
Catarrhini	*Homo sapiens*	208	1252.00	50000	37.0
Catarrhini	*Pan troglodytes*	206	368.35	44967	35.7
Catarrhini	*Gorilla gorilla*	202	490.41	120950	35.5
Catarrhini	*Macaca fascicularis*	207	63.98	4251	37.6
Catarrhini	*Macaca mulatta*	208	88.98	6793	37.3
Catarrhini	*Papio hamadryas*	197	146.17	14150	38.0
Catarrhini	*Chlorocebus aethiops*	199	65.00	3720	36.8
Catarrhini	*Erythrocebus patas*	201	97.73	9450	39.3
Catarrhini	*Cercopithecus mitis*	200	71.33	6109	37.5
Catarrhini	*Colobus guereza*	198	74.39	8589	37.0
Platyrrhini	*Callithrix jacchus*	172	7.24	320	36.0
Platyrrhini	*Callithrix pygmaea*	171	4.17	116	35.2
Platyrrhini	*Aotus trivirgatus*	177	16.85	989	38.0
Platyrrhini	*Saimiri sciureus*	174	24.14	799	38.0
Platyrrhini	*Alouata palliata*	174	49.88	6250	36.0
Lemuriformes	*Eulemur fulvus*	180	25.77	2292	36.5
Lemuriformes	*Cheirogaleus medius*	176	2.60	140	38.0
Lemuriformes	*Propithecus verreauxi*	180	26.21	2955	36.0
Lorisiformes	*Otolemur garnettii*	166	11.50	764	36.0
Lorisiformes	*Otolemur crassicaudatus*	166	11.78	1150	36.6
Lorisiformes	*Galago senegalensis*	165	3.96	194	37.9
Lorisiformes	*Loris tardigradus*	166	5.87	193	35.5
Lorisiformes	*Nycticebus coucang*	167	10.13	653	35.4
Lorisiformes	*Arctocebus calabarensis*	166	6.92	309	36.0
Lorisiformes	*Perodicticus potto*	169	12.42	835	35.4

Supplementary Table S3. Phylogenetic regressions of primate organ *cMR*s on *mirFam*. For comparison, see the logistic regressions of *mirFam* on relative organ size (Supplementary Figure S8).

	Allometric Exponent	Pagel's *lambda*	R^2	*P*	*AICc*
KIDNEY	0.80	0	0.5366	6.39E-05	-42.7
	0.75	0	0.5834	2.14E-05	-20.09
	0.70	0	0.5958	1.56E-05	-0.93
	0.65	0	0.5946	1.61E-05	16.22
	0.60	0	0.5859	2.00E-05	32.13
		Pagel's *lambda*	R^2	*P*	*AICc*
HEART	0.80	0	0.4937	1.60E-04	-38.02
	0.75	0	0.6476	3.83E-06	-17.88
	0.70	0	0.6962	8.44E-07	0.63
	0.65	0	0.7133	4.69E-07	17.65
	0.60	0	0.7163	4.21E-07	33.66
		Pagel's *lambda*	R^2	*P*	*AICc*
LIVER	0.80	0	0.5724	2.79E-05	-15.22
	0.75	0	0.4011	9.28E-04	-10.98
	0.70	0	0.0854	1.01E-01	-2.86
	0.65	0	-0.0344	5.89E-01	10.24
	0.60	0.962	0.0145	2.66E-01	27.34
		Pagel's *lambda*	R^2	*P*	*AICc*
BRAIN	0.80	1	0.0329	2.05E-01	-63.9
	0.75	1	0.107	7.55E-02	-35.75
	0.70	1	0.1336	5.07E-02	-10.58
	0.65	1	0.151	4.17E-02	11.76
	0.60	1	0.1596	3.71E-02	32.24