African Origin of Modern Humans in East Asia: A Tale of 12,000 Y Chromosomes

Yuehai Ke *et al.*

Science **292**, 1151 (2001);
DOI: 10.1126/science.1060011

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

PeeDee Belemnite (VPDB) standard and for ^{15}N = 0.0‰ versus air nitrogen. The first anaylsis (sealed-tube combustion with subsequent measurement on a Finnigan MAT 251 mass spectrometer) has a reproducibility of ± 0.08‰ for organic standards. The second analysis [elemental analyzer–continuous-flow isotope ratio mass spectrometry (EA/CFIRMS)] used a Carlo Erba NC2500 interfaced through a Finnigan CONFLO II to a Finnigan Delta XL mass spectrometer. Reproducibility in this system averages ±0.12‰ for organic standards and homogenous natural samples. The EA/CFIRMS analyses were calibrated as follows: For each sample, $\delta^{15}N$ and $\delta^{13}C$ were measured versus a pulse of 99.999% pure standard gas injected into the mass spectrometer source immediately before or after the sample pulse eluted from the EA. Because the isotope ratios obtained were dependent on mixing ratios of carrier gas and dilutant in the CONFLO, the ratios were normalized to a known organic standard run with the samples under the same conditions. To verify that the corrections were normalized properly, each run also contained four aliquots of a natural sample whose ratios were known from sealed-tube combustion. This natural sample had a precision identical to the organic standard (±0.12‰) and the average of four determinations was within a range of ±0.1‰ of its correct value. Each autosampler run contained seven organic acid standards, four natural sample standards, and 40 samples and blanks. Blanks typically were less than 1% of the sample amount. Samples were prepared for analysis by grinding, followed by weighing into silver boats (measuring 5 mm by 9 mm) and acidification with 20 μl of 50% HCl. After air drying overnight at 50°C, the sample boats were sealed and measured as follows: Isotope analyses were performed by EA/CFIRMS, using a Carlo Erba NC2500 interfaced through a Finnigan CONFLO II to a Finnigan Delta XL mass spectrometer. Sample isotope ratios were normalized in each run to the values obtained for an organic standard with known isotope ratios calibrated in sealed-tube combustions versus NBS-19 for $\delta^{13}C$ = 1.95‰ versus VPDB and for $\delta^{15}N$ = 0.0‰ versus air nitrogen. Precision in this system averages ±0.12‰ for organic standards and homogenous natural samples. Accuracy, as measured by including repeats of a natural sample of known isotopic ratio in each run, was ±0.10‰. All isotope ratios are expressed in delta notation, or parts per thousand deviation from VPDB, where $\delta^{13}C = \{[(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{VPDB}] - 1\} \times 1000$.

12. E. S. Carter, *Mem. Geol.* (*Lausanne*) **11**, 175 (1993).
13. E. S. Carter, unpublished results of 2000 sampling at Kennecott Point.
14. M. J. Orchard, P. J. L. Forster, *Geol. Surv. Can. Pap.* **90-10**, 453 (1991).
15. Supported by a grant from NSF (H. Lane, program administrator) to P.D.W. and by Geological Survey of Canada Project number 870070.

22 December 2000; accepted 28 March 2001

African Origin of Modern Humans in East Asia: A Tale of 12,000 Y Chromosomes

Yuehai Ke,[1]* Bing Su,[2,1,3]* Xiufeng Song,[1] Daru Lu,[1] Lifeng Chen,[1] Hongyu Li,[1] Chunjian Qi,[1] Sangkot Marzuki,[4] Ranjan Deka,[5] Peter Underhill,[6] Chunjie Xiao,[7] Mark Shriver,[8] Jeff Lell,[9] Douglas Wallace,[9] R Spencer Wells,[10] Mark Seielstad,[11] Peter Oefner,[6] Dingliang Zhu,[12] Jianzhong Jin,[1] Wei Huang,[12,13] Ranajit Chakraborty,[3] Zhu Chen,[12,13] Li Jin[1,3,13] †

To test the hypotheses of modern human origin in East Asia, we sampled 12,127 male individuals from 163 populations and typed for three Y chromosome biallelic markers (YAP, M89, and M130). All the individuals carried a mutation at one of the three sites. These three mutations (YAP+, M89T, and M130T) coalesce to another mutation (M168T), which originated in Africa about 35,000 to 89,000 years ago. Therefore, the data do not support even a minimal in situ hominid contribution in the origin of anatomically modern humans in East Asia.

The "Out-of-Africa" hypothesis suggests that anatomically modern humans originated in Africa about 100,000 years ago and then spread outward and completely replaced local archaic populations outside Africa (*1*, *2*). This proposition has been supported by genetic evidence and archaeological findings (*3*–*9*). The replacement in Europe was supported by recent ancient DNA analyses, which ruled out the contribution of Neanderthals to modern Europeans (*10*, *11*). However, it has been argued that the abundant hominid fossils found in China and other regions in East Asia (e.g., Peking man and Java man) demonstrate continuity, not only in morphological characters but also in spatial and temporal distributions (*12*–*16*). In this report, we test the competing hypotheses of modern Asian human origins using Y chromosome polymorphisms.

We sampled 12,127 male individuals from 163 populations across Southeast Asia, Oceania, East Asia, Siberia, and Central Asia and typed for three Y chromosome biallelic markers (YAP, M89, and M130) (*17*, *18*) (Table 1). Being a single-locus multiple-site (i.e., haplotype) system, the Y chromosome is one of the most powerful molecular tools for tracing human evolutionary history (*5*, *9*, *19*–*21*). In previous Y chromosome studies, an extreme geographic structure was revealed in global populations in which the oldest clade represents Africans and the younger ones represent some Africans and all non-African populations (*21*). One Y chromosome polymorphism (C to T mutation) at the M168 locus is shared by all non-African populations and was originally derived from Africa on the basis of a study of 1062 globally representative male individuals (*21*). The age of M168 was estimated at 44,000 years (95% confidence interval: 35,000 to 89,000 years), marking the recent Out-of-Africa migrations (*21*). Under the M168T lineage, there are three major derived sublineages defined by polymorphisms at loci YAP (Alu insertion) (*5*), M89 (C to T mutation), and M130 (C to T mutation, also called RPS4Y) (Fig. 1) (*21*, *22*). Therefore, these three markers can be used to test the completeness of the replacement of modern humans of African origin in East Asia. An observation of a male individual not carrying one of the three polymorphisms would be indicative of a potential ancient origin and could possibly lead to the rejection of such completeness.

Each of the 12,127 samples typed carried one of the three polymorphisms (YAP+, M89T, or M130T) (Table 1). In other words, they all fall into the lineage of M168T that was originally derived from Africa. Hence, no ancient non-African Y chromosome was found in the extant East Asian populations ($P = 5.4 \times 10^{-6}$ assuming a frequency of 1/1000 of local contribution in the extant populations), suggesting an absence of either an independent origin or a 1,000,000-year shared global evolution. This result indicates that modern humans of African origin completely replaced earlier populations in East Asia.

[1]State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Sciences, Fudan University, 220 Handan Road, Shanghai, China 200443, and Morgan-Tan International Center for Life Sciences, Shanghai, China. [2]Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, China. [3]Human Genetics Center, University of Texas–Houston, 1200 Herman Pressler E547, Houston, TX 77030, USA. [4]Eijkman Institute for Molecular Biology, Jakarta, Indonesia. [5]Department of Environmental Health, University of Cincinnati, Cincinnati, OH 45267, USA. [6]Department of Genetics, Stanford University, Stanford, CA 94305, USA. [7]Department of Biology, Yunnan University, Kunming, China. [8]Department of Anthropology, Pennsylvania State University, University Park, PA 16802, USA. [9]Center for Molecular Medicine, Emory University School of Medicine, Atlanta, GA 30322, USA. [10]Wellcome Trust Center for Human Genetics, University of Oxford, UK. [11]Program for Population Genetics, Harvard School of Public Health, Boston, MA 02115, USA. [12]Shanghai Second Medical University, Shanghai, China. [13]National Human Genome Center at Shanghai, China.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: ljin@fudan.edu or ljin@sph.uth.tmc.edu

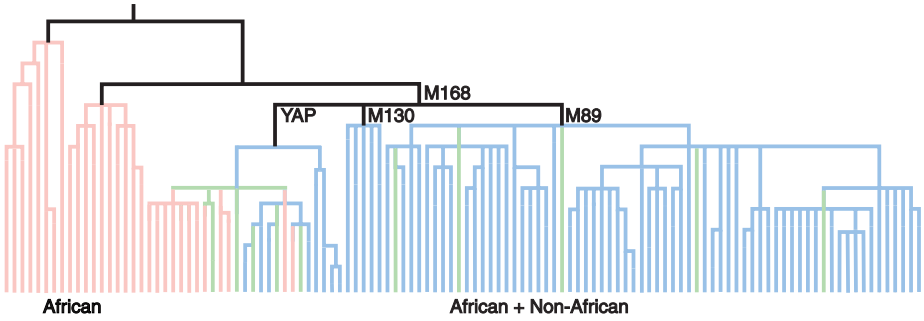

Fig. 1. The phylogenetic relationships of the Y chromosome haplotypes of African and other world populations. The red branches are African-specific haplotypes. The blue branches are non-African–specific haplotypes, and the green ones are shared between Africans and non-Africans [modified from Underhill *et al.* (*21*)].

Table 1. Frequency distribution of the three Y chromosome polymorphisms in 163 Asian populations. N_{Pop}, number of populations; N_{Ind}, number of individuals.

Geographic region	N_{Pop}	N_{Ind}	M89T	M130T	YAP+
Central Asia	13	173	144	25	4
Central Siberia	5	107	70	36	1
Okhotsk/Amur	6	123	46	77	0
Kamchatka/Chukotka	4	102	73	29	0
Northern East Asia	17	578	497	42	39
Northern Han Chinese	14	4592	4296	191	105
Southern Han Chinese	13	5127	4984	97	44
Taiwanese Aborigines	5	58	58	0	0
Southeast Asia	37	620	559	37	24
Indonesia/Malaysia	25	355	333	22	0
Polynesia/Micronesia	11	113	89	23	1
New Guinea/Melanesia	7	120	105	17	0
Northeast India	6	59	57	2	0
Total	163	12,127			

It was argued that the extensive genetic data supporting the Out-of-Africa hypothesis could also be explained by the multiregional hypothesis under a version of the trellis model (*23*). This model suggests that a multiregional evolutionary paradigm is shared across the human range by frequent gene exchanges between continental populations since *Homo erectus* came out of Africa about 1 million years ago (*23*). It is difficult to test the trellis model with markers from mitochondrial hypervariable region (D-loop) and autosome because these markers show frequent recurrent mutations and/or recombination (*24*, *25*), respectively. However, this can be circumvented by the application of a large number of Y chromosome biallelic markers, which escape recombination and have a low mutation rate. It has been shown that all the Y chromosome haplotypes found outside Africa are younger than 35,000 to 89,000 years and derived from Africa (*21*), although this estimation is crude and depends on several assumptions. In addition, if extensive gene flow had occurred between continental populations during the past 1 million years but before the divergence between Africans and non-Africans, as suggested by the multiregionalists, the ancient Y chromosome haplotypes seen in African populations or even much older haplotypes would have been expected in East Asia, which was not observed in our data. However, this observation does not necessarily preclude the possibility of selection sweep that could erase archaic Y chromosomes of modern humans in East Asia. On the other hand, a minor contribution from a female lineage of local origin cannot be excluded either, which should be further studied with the use of mitochondrial DNA (mtDNA) markers. Because the Y chromosome has a relatively small effective population size, it is subject to stochastic process, e.g., genetic drift, which could also lead to extinction of archaic lineages. However, in our study, with 163 populations from different regions of Asia, it is hard to imagine that all of the 163 populations should drift in the same direction.

Inconsistency of age estimations for a common ancestor with the use of mitochondrial/Y chromosome and autosome/X chromosome markers, however, creates confusion. The age estimated with the use of autosome/X chromosome genes ranges from 535,000 to 1,860,000 years (*26*–*29*), much older than those for mtDNA and Y chromosome. However, this difference in age estimation might only reflect the difference in the effective population sizes between Y chromosome/mtDNA and X chromosome/autosome (three to four times as many as the former) in the presence of bottleneck events associated with the outbound migrations from Africa, therefore disqualifying the utility of the latter in distinguishing the competing hypotheses (*24*, *30*).

References and Notes

1. R. L. Cann, M. Stoneking, A. C. Wilson, *Nature* **325**, 31 (1987).
2. L. Vigilant, M. Stoneking, H. Harpending, K. Hawkes, A. C. Wilson, *Science* **253**, 1503 (1991).
3. C. B. Stringer, P. Andrew, *Science* **239**, 1263 (1988).
4. A. M. Bowcock *et al.*, *Nature* **368**, 455 (1994).
5. M. F. Hammer, *Nature* **378**, 376 (1995).
6. S. A. Tishkoff *et al.*, *Science* **271**, 1380 (1996).
7. J. Y. Chu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 11763 (1998).
8. L. Quintana-Murci *et al.*, *Nature Genet.* **23**, 437 (1999).
9. B. Su *et al.*, *Am. J. Hum. Genet.* **65**, 1718 (1999).
10. M. Krings *et al.*, *Cell* **90**, 19 (1997).
11. V. O. Igor *et al.*, *Nature* **404**, 490 (2000).
12. A. S. Brooks, B. Wood, *Nature* **344**, 288 (1990).
13. T. Li, D. A. Etler, *Nature* **357**, 404 (1992).
14. X. Z. Wu, F. E. Poirier, *Human Evolution in China* (Oxford Univ. Press, Oxford, 1995).
15. D. A. Etler, *Annu. Rev. Anthropol.* **25**, 275 (1996).
16. C. C. Swisher *et al.*, *Science* **274**, 1870 (1996).
17. A total of 163 populations were sampled from Central Asia (Crimean Tatar, Iranian, Dungan, Tajik, Turkmen, Karakalpak, Eastern Uzbek, Sinte Romani, Khorezmian Uzbek, Uighur, Kazak, Bukharan Arab, and Kyrgyz); Central Siberia (Tuvan, Tofalar, Yenisey Evenk, Buryat-1, and Buryat-2); Okhotsk/Amur (Okhotsk Evenk, Ulchi/Nanai, Upriver Negidal, Downriver Negidal, Udegey, and Nivkh); Kamchatka/Chukotka (Koryak, Itelman, Chukchi, and Siberian Eskimo); northern East Asia (Ewenki, Manchurian-1, Manchurian-2, Korean, Japanese, Hui-1, Hui-2, Jingpo, Tu, Sala, Mongolian-1, Mongolian-2, Tibetan-Qinghai, Tibetan-Tibet, Tibetan-Yunnan, Kazak-Xinjiang, and Uyghur); northern Han Chinese (Heilongjiang, Liaoning, Hebei, Beijing, Tianjin, Shandong, Shanxi, Gansu, Xinjiang, Henan, Inner-Mongolia, Qinghai, Shaanxi, and Jilin); southern Han Chinese (Anhui, Zhejiang, Jiangsu, Shanghai, Hubei, Sichuan, Jiangxi, Hunan, Fujian, Yunnan, Guangxi, Guangdong, and Guizhou); Taiwan (Bunun, Atayal, Yami, Paiwan, and Ami); Southeast Asia (Tujia, Yao-Nandan, Yao-Jinxiu, Zhuang, Dong, Wa-1, Wa-2, Wa-3, Aini, Blang-1, Blang-2, Lahu-1, Lahu-2, Lahu-3, Lahu-4, Deang, Yi, She, Li, Cambodian, Dai-1, Dai-2, Akha, Karen, Lisu, Jino, Hmong, Yao, Kinh, Muong, Naxi, Ahom, So, Northern Thailand, Northeast Thailand, Bai-1, and Bai-2); Indonesia/Malaysia [Malay CB, Malay KM, Orang Asli, Batak, Malay (Pakanbaru), Minangkabau, Palembang, Bangka, Nias, Dayak, Java, Tengger, Bali, Sasak, Sumbawa, Sumba, Alor, Makassar, Bugis, Torajan, Kaili, Manado, Irian, Kota Kinabalu, and Sakai]; Polynesia/Micronesia (Truk, Guam, Palau, Majuro, Kribati, Pohnpei, Nauru, Kapingamarangi, Tonga, American Somoan, and West Somoan); Papuan and New Guinean Highland (Australian Aborigine, Nasioi-Melanesian, New Guinean-1, New Guinean-2, Bankes and Torres, Santo, and Maewo); and Northeastern India [Adi, Nishi, Assam, Apatani, Rabha(Assam), and Naga]. The numbered populations of the same ethnicity were sampled independently.
18. Genotyping was conducted by a polymerase chain reaction restriction fragment length polymorphism assay. The restriction sites were engineered for M130 (Bsl I) and M89 (Nla III) by designing mismatch primers. The primer sequences are ACAGAAGGATGCTGCTCAGCTT/GCAACTCAGGCAAAGTGAGACAT (M89) and TATCTCCTCTTCTATTGCAG/CCACAAGGGGGAAAAAACAC (M130). The typing of YAP follows previous reports (*5*, *9*). Genotyping was repeated to clarify any equivocal typing results.
19. M. A. Jobling *et al.*, *Trends Genet.* **11**, 449 (1995).

20. P. A. Underhill *et al.*, *Ann. Hum. Genet.* **65**, 43 (2001).
21. P. A. Underhill *et al.*, *Nature Genet.* **26**, 358 (2000).
22. A. W. Bergen *et al.*, *Ann. Hum. Genet.* **63**, 63 (1999).
23. M. H. Wolpoff, J. Hawks, R. Caspari, *Am. J. Phys. Anthropol.* **112**, 129 (2000).
24. L. Jin, B. Su, *Nature Rev. Genet.* **1**, 126 (2000).
25. M. Stoneking *et al.*, *Curr. Opin. Genet. Dev.* **6**, 731 (1996).
26. R. M. Harding *et al.*, *Am. J. Hum. Genet.* **60**, 772 (1997).
27. H. Kaessmann, F. Heissig, A. Haeseler, S. Paabo, *Nature Genet.* **22**, 78 (1999).
28. E. Harris, J. Hey, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 3320 (1999).
29. Z. M. Zhao *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 11354 (2000).
30. J. C. Fay, C. I. Wu, *Mol. Biol. Evol.* **16**, 1003 (1999).
31. We thank all the 12,127 men who donated DNA for this study. This study was supported by the China Natural Science Foundation. B.S., R.C., and L.J. were supported by NIH grants. R.D. was supported by the Center for Environmental Genetics at the University of Cincinnati.

20 February 2001; accepted 20 March 2001

Spermiogenesis Deficiency in Mice Lacking the *Trf2* Gene

Di Zhang,[1] Tarja-Leena Penttila,[3] Patricia L. Morris,[2,3] Martin Teichmann,[1] Robert G. Roeder[1]*

The discovery of TATA-binding protein–related factors (TRFs) has suggested alternative mechanisms for gene-specific transcriptional regulation and raised interest in their biological functions. In contrast to recent observations of an embryonic lethal phenotype for TRF2 inactivation in *Caenorhabditis elegans* and *Xenopus laevis*, we found that *Trf2*-deficient mice are viable. However, *Trf2*$^{-/-}$ mice are sterile because of a severe defect in spermiogenesis. Postmeiotic round spermatids advance at most to step 7 of differentiation but fail to progress to the elongated form, and gene-specific transcription deficiencies were identified. We speculate that mammals may have evolved more specialized TRF2 functions in the testis that involve transcriptional regulation of genes essential for spermiogenesis.

Early studies have suggested that one universal TATA-binding protein (TBP) functions as a central component of the general transcription machineries to mediate transcription by nuclear RNA polymerases I, II, and III in eukaryotes (*1*). However, the identification of two TBP-related factors (TRF1 and TRF2) raised the possibility that TRFs may substitute for TBP in mediating the transcription of specific genes and thus have distinct biological functions (*2–5*). In *Drosophila*, biochemical studies have documented promoter-specific functions of TRF1 (*6, 7*). In both *Caenorhabditis elegans* and *Xenopus laevis*, inactivation of TRF2 results in embryonic lethality and deficiencies in embryonic gene transcription (*8–10*). However, except for the observation that TRF2 is abundantly expressed in the testis of human and mouse (*4, 5*), there has been no information regarding biological functions of TRF2 in mammalian species.

To elucidate the functional role of TRF2, we used homologous recombination in embryonic stem cells to generate mice lacking a functional *Trf2* gene (*11*). We constructed a targeting vector in which a region containing the central four exons of *Trf2* was replaced by a neomycin resistance gene cassette (*11*). This deletion eliminates nearly 80% of the core region of TRF2. Genotyping of 218 F_2 offspring by polymerase chain reaction analysis revealed a *Trf2*$^{+/+}$:*Trf2*$^{+/-}$:*Trf2*$^{-/-}$ distribution (69:109:40) that does not deviate significantly from the expected Mendelian ratio, although there could be some earlier lethality of homozygous embryos. Disruption of the *Trf2* gene was confirmed by Southern blot analysis (*11*). Subsequent Northern blot analyses of testis RNAs from *Trf2* mutant mice showed reduced expression of full-length *Trf2* transcripts in heterozygotes and no expression in homozygotes (*11*).

Mice deficient for the *Trf2* gene appeared to be healthy and showed no apparent abnormalities in major organs at the gross and histological levels. However, testes from the adult *Trf2*-deficient mice showed size and weight reductions of ~50% in comparison with those from the wild-type and heterozygous controls (*11*). When *Trf2*$^{-/-}$ male mice were mated with *Trf2*$^{+/+}$ female mice, they copulated normally, as evidenced by the formation of vaginal plugs in their mates, but none of the mated female mice became pregnant. In contrast, *Trf2*$^{-/-}$ females were fertile and produced normal average litter sizes (7.3 ± 1.8; $n = 10$). Analyses of serum testosterone levels in *Trf2*$^{-/-}$ male mice revealed no statistically significant difference in comparison to their *Trf2*$^{+/+}$ or *Trf2*$^{+/-}$ littermates (*11*). We next evaluated semen samples extracted from the vas deferens and epididymis. The seminal fluid from *Trf2*$^{-/-}$ mice lacked spermatozoa, whereas there were no apparent differences in sperm number or morphology between *Trf2*$^{+/+}$ and *Trf2*$^{+/-}$ mice (*11*).

In the testis, male germ cells differentiate from spermatogonia into spermatozoa by a complex process referred to as "spermatogenesis." The mouse spermatogenesis cycle is well defined and can be subdivided into 12 stages, with each stage consisting of a specific complement of male germ cells. In determining the nature of the sperm deficiency, we analyzed male germ cell differentiation both in adult mice and in juvenile mice between 8 and 35 days after birth. In the latter case, the first wave of developing germ cells progresses through spermatogenesis with specific mitotic and meiotic cells first appearing according to a well-characterized developmental program (*12*). Inspection of seminiferous tubules in the adult

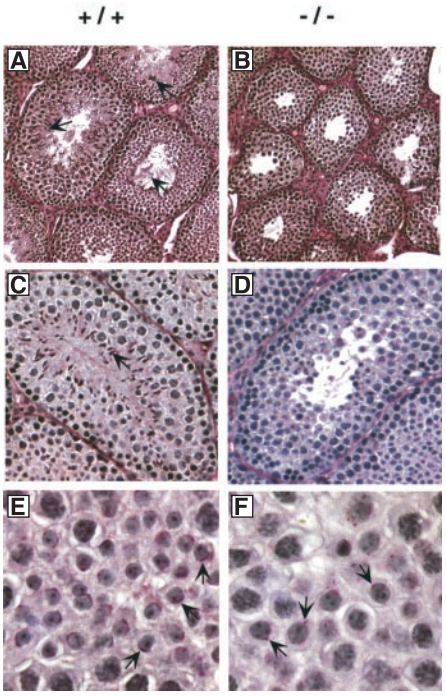

Fig. 1. *Trf2*$^{-/-}$ mice show defects in spermiogenesis. (**A** and **B**) Histological analysis of testis sections from adult *Trf2*$^{+/+}$ (A) and *Trf2*$^{-/-}$ (B) littermates. Magnification, ×200. Arrows indicate the elongated spermatids or spermatozoa that are present in *Trf2*$^{+/+}$ but absent in the *Trf2*$^{-/-}$ testis. (**C** and **D**) Histological analysis of testis sections from *Trf2*$^{+/+}$ (C) and *Trf2*$^{-/-}$ (D) juvenile mice of 28 days of age. Magnification, ×200. The arrow indicates the elongated spermatids that are present in *Trf2*$^{+/+}$ but absent in the *Trf2*$^{-/-}$ testis. (**E** and **F**) Morphology of seminiferous tubules at stage VI from *Trf2*$^{+/+}$ (E) and *Trf2*$^{-/-}$ (F) juvenile mice of 25 days of age. Magnification, ×1000. Arrows indicate the acrosomes of the spermatids, which are stained pink. The acrosomal structures are abnormal in the *Trf2*$^{-/-}$ section, as compared to the *Trf2*$^{+/+}$ section.

[1]Laboratory of Biochemistry and Molecular Biology, [2]The Rockefeller University, New York, NY 10021, USA. [3]Population Council, New York, NY 10021, USA.

*To whom correspondence should be addressed. E-mail: roeder@rockvax.rockefeller.edu