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Omnibus, with accession code GSE21661. These data, as well as phenotype data, are also available on our laboratory Web site, <http://forde-lab.genetics.utah.edu>. Please contact R.L.G. for access to DNA samples.

#### Supporting Online Material

[www.sciencemag.org/cgi/content/full/science.1189406/DC1](http://www.sciencemag.org/cgi/content/full/science.1189406/DC1)  
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# Sequencing of 50 Human Exomes Reveals Adaptation to High Altitude

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Residents of the Tibetan Plateau show heritable adaptations to extreme altitude. We sequenced 50 exomes of ethnic Tibetans, encompassing coding sequences of 92% of human genes, with an average coverage of 18× per individual. Genes showing population-specific allele frequency changes, which represent strong candidates for altitude adaptation, were identified. The strongest signal of natural selection came from endothelial Per-Arnt-Sim (PAS) domain protein 1 (*EPAS1*), a transcription factor involved in response to hypoxia. One single-nucleotide polymorphism (SNP) at *EPAS1* shows a 78% frequency difference between Tibetan and Han samples, representing the fastest allele frequency change observed at any human gene to date. This SNP's association with erythrocyte abundance supports the role of *EPAS1* in adaptation to hypoxia. Thus, a population genomic survey has revealed a functionally important locus in genetic adaptation to high altitude.

The expansion of humans into a vast range of environments may have involved both cultural and genetic adaptation. Among the most severe environmental challenges to confront human populations is the low oxygen availability of high-altitude regions such as the Tibetan Plateau. Many residents of this region

live at elevations exceeding 4000 m, experiencing oxygen concentrations that are about 40% lower than those at sea level. Ethnic Tibetans possess heritable adaptations to their hypoxic environment, as indicated by birth weight (1), hemoglobin levels (2), and oxygen saturation of blood in infants (3) and adults after exercise (4). These results imply a history of natural selection for altitude adaptation, which may be detectable from a scan of genetic diversity across the genome.

We sequenced the exomes of 50 unrelated individuals from two villages in the Tibet Autonomous Region of China, both at least 4300 m in altitude (5). Exonic sequences were enriched with the NimbleGen (Madison, WI) 2.1M exon capture array (6), targeting 34 Mb of sequence from exons and flanking regions in nearly 20,000 genes. Sequencing was performed with the Illumina (San Diego, CA) Genome Analyzer II platform, and reads were aligned by using SOAP (7) to the reference human genome [National Center for Biotechnology Information (NCBI) Build 36.3].

Exomes were sequenced to a mean depth of 18× (table S1), which does not guarantee confident inference of individual genotypes. Therefore, we statistically estimated the probability of each possible genotype with a Bayesian algorithm (5) that

also estimated single-nucleotide polymorphism (SNP) probabilities and population allele frequencies for each site. A total of 151,825 SNPs were inferred to have >50% probability of being variable within the Tibetan sample, and 101,668 had >99% SNP probability (table S2). Sanger sequencing validated 53 of 56 SNPs that had at least 95% SNP probability and minor allele frequencies between 3% and 50%. Allele frequency estimates showed an excess of low-frequency variants (fig. S1), particularly for nonsynonymous SNPs.

The exome data was compared with 40 genomes from ethnic Han individuals from Beijing [the HapMap CHB sample, part of the 1000 genomes project (<http://1000genomes.org>)], sequenced to about fourfold coverage per individual. Beijing's altitude is less than 50 m above sea level, and nearly all Han come from altitudes below 2000 m. The Han sample represents an appropriate comparison for the Tibetan sample on the basis of low genetic differentiation between these samples ( $F_{ST} = 0.026$ ). The two Tibetan villages show minimal evidence of genetic structure ( $F_{ST} = 0.014$ ), and we therefore treated them as one population for most analyses. We observed a strong covariance between Han and Tibetan allele frequencies (Fig. 1) but with an excess of SNPs at low frequency in the Han and moderate frequency in the Tibetans.

Population historical models were estimated (8) from the two-dimensional frequency spectrum of synonymous sites in the two populations. The best-fitting model suggested that the Tibetan and Han populations diverged 2750 years ago, with the Han population growing from a small initial size and the Tibetan population contracting from a large initial size (fig. S2). Migration was inferred from the Tibetan to the Han sample, with recent admixture in the opposite direction.

Genes with strong frequency differences between populations are potential targets of natural selection. However, a simple ranking of  $F_{ST}$  values would not reveal which population was affected by selection. Therefore, we estimated population-specific allele frequency change by including a third, more distantly related population. We thus examined exome sequences from 200 Danish individuals, collected and analyzed as described for the Tibetan sample. By comparing the three pairwise  $F_{ST}$  values between these three samples, we can estimate the frequency change that occurred in the Tibetan population since its divergence from the Han population (5, 9). We found that this population branch statistic (PBS) has strong power to detect recent natural selection (fig. S3).

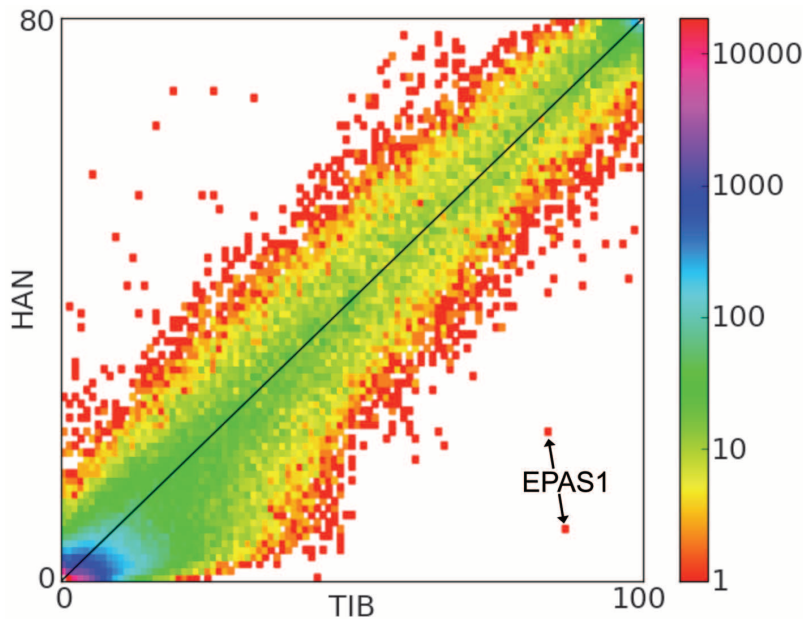
Genes showing extreme Tibetan PBS values represent strong candidates for the genetic basis

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**Fig. 1.** Two-dimensional unfolded site frequency spectrum for SNPs in Tibetan (x axis) and Han (y axis) population samples. The number of SNPs detected is color-coded according to the logarithmic scale plotted on the right. Arrows indicate a pair of intronic SNPs from the *EPAS1* gene that show strongly elevated derived allele frequencies in the Tibetan sample compared with the Han sample.

**Table 1.** Genes with strongest frequency changes in the Tibetan population. The top 30 PBS values for the Tibetan branch are listed. Oxygen-related candidate genes within 100 kb of these loci are noted. For FXYD, F indicates Phe; Y, Tyr; D, Asp; and X, any amino acid.

Gene	Description	Nearby candidate	PBS	P value
<i>EPAS1</i>	Endothelial PAS domain protein 1 (HIF-2α)	(Self)	0.514	<0.000001
<i>C1orf124</i>	Hypothetical protein LOC83932	<i>EGLN1</i>	0.277	0.000203
<i>DISC1</i>	Disrupted in schizophrenia 1	<i>EGLN1</i>	0.251	0.000219
<i>ATP6V1E2</i>	Adenosine triphosphatase (ATPase), H+ transporting, lysosomal 31 kD, V1	<i>EPAS1</i>	0.246	0.000705
<i>SPP1</i>	Secreted phosphoprotein 1		0.238	0.000562
<i>PKLR</i>	Pyruvate kinase, liver, and RBC	(Self)	0.230	0.000896
<i>C4orf7</i>	Chromosome 4 open reading frame 7		0.227	0.001098
<i>PSME2</i>	Proteasome activator subunit 2		0.222	0.001103
<i>OR10X1</i>	Olfactory receptor, family 10, subfamily X	<i>SPTA1</i>	0.218	0.000950
<i>FAM9C</i>	Family with sequence similarity 9, member C	<i>TMSB4X</i>	0.216	0.001389
<i>LRRC3B</i>	Leucine-rich repeat-containing 3B		0.215	0.001405
<i>KRTAP21-2</i>	Keratin-associated protein 21-2		0.213	0.001470
<i>HIST1H2BE</i>	Histone cluster 1, H2be	<i>HFE</i>	0.212	0.001568
<i>TTL3</i>	Tubulin tyrosine ligase-like family, member 3		0.206	0.001146
<i>HIST1H4B</i>	Histone cluster 1, H4b	<i>HFE</i>	0.204	0.001404
<i>ACVR1B</i>	Activin A type IB receptor isoform a precursor	<i>ACVRL1</i>	0.198	0.002041
<i>FXYD6</i>	FXYD domain-containing ion transport regulator		0.192	0.002459
<i>NAGLU</i>	Alpha-N-acetylglucosaminidase precursor		0.186	0.002834
<i>MDH1B</i>	Malate dehydrogenase 1B, nicotinamide adenine dinucleotide (NAD) (soluble)		0.184	0.002113
<i>OR6Y1</i>	Olfactory receptor, family 6, subfamily Y	<i>SPTA1</i>	0.183	0.002835
<i>HBB</i>	Beta globin	(Self), <i>HBG2</i>	0.182	0.003128
<i>OTX1</i>	Orthodenticle homeobox 1		0.181	0.003235
<i>MBNL1</i>	Muscleblind-like 1		0.179	0.002410
<i>IFI27L1</i>	Interferon, alpha-inducible protein 27-like 1		0.179	0.003064
<i>C18orf55</i>	Hypothetical protein LOC29090		0.178	0.002271
<i>RFX3</i>	Regulatory factor X3		0.176	0.002632
<i>HBG2</i>	G-gamma globin	(Self), <i>HBB</i>	0.170	0.004147
<i>FANCA</i>	Fanconi anemia, complementation group A	(Self)	0.169	0.000995
<i>HIST1H3C</i>	Histone cluster 1, H3c	<i>HFE</i>	0.168	0.004287
<i>TMEM206</i>	Transmembrane protein 206		0.166	0.004537

of altitude adaptation. The strongest such signals include several genes with known roles in oxygen transport and regulation (Table 1 and table S3). Overall, the 34 genes in our data set that fell under the gene ontology category “response to hypoxia” had significantly greater PBS values than the genome-wide average ( $P = 0.00796$ ).

The strongest signal of selection came from the endothelial Per-Arnt-Sim (PAS) domain protein 1 (*EPAS1*) gene. On the basis of frequency differences among the Danes, Han, and Tibetans, *EPAS1* was inferred to have a very long Tibetan branch relative to other genes in the genome (Fig. 2). In order to confirm the action of natural selection, PBS values were compared against neutral simulations under our estimated demographic model. None of one million simulations surpassed the PBS value observed for *EPAS1*, and this result remained statistically significant after accounting for the number of genes tested ( $P < 0.02$  after Bonferroni correction). Many other genes had uncorrected  $P$  values below 0.005 (Table 1), and, although none of these were statistically significant after correcting for multiple tests, the functional enrichment suggests that some of these genes may also contribute to altitude adaptation.

*EPAS1* is also known as hypoxia-inducible factor 2α (*HIF-2α*). The HIF family of transcription factors consist of two subunits, with three

alternate  $\alpha$  subunits (*HIF-1 $\alpha$* , *HIF-2 $\alpha$* /*EPAS1*, *HIF-3 $\alpha$* ) that dimerize with a  $\beta$  subunit encoded by *ARNT* or *ARNT2*. *HIF-1 $\alpha$*  and *EPAS1* each act on a unique set of regulatory targets (10), and the narrower expression profile of *EPAS1* includes adult and fetal lung, placenta, and vascular endothelial cells (11). A protein-stabilizing mutation in *EPAS1* is associated with erythrocytosis (12), suggesting a link between *EPAS1* and the regulation of red blood cell production.

Although our sequencing primarily targeted exons, some flanking intronic and untranslated region (UTR) sequence was included. The *EPAS1* SNP with the greatest Tibetan-Han frequency difference was intronic (with a derived allele at 9% frequency in the Han and 87% in the Tibetan sample; table S4), whereas no amino acid-changing variant had a population frequency difference of greater than 6%. Selection may have acted directly on this variant, or another linked noncoding variant, to influence the regulation of *EPAS1*. Detailed molecular studies will be needed to investigate the direction and the magnitude of gene expression changes associated with this SNP, the tissues and developmental time points affected, and the downstream target genes that show altered regulation.

Associations between SNPs at *EPAS1* and athletic performance have been demonstrated (13). Our data set contains a different set of SNPs, and we conducted association testing on the SNP with the most extreme frequency difference, located just upstream of the sixth exon. Alleles at this SNP tested for association with blood-related phenotypes showed no relationship with oxygen saturation. However, significant associations were discovered for erythrocyte count (F test  $P = 0.00141$ ) and for hemoglobin concentration (F test  $P = 0.00131$ ), with significant or marginally significant  $P$  values for both traits when each

village was tested separately (table S5). Comparison of the *EPAS1* SNP to genotype data from 48 unlinked SNPs confirmed that its  $P$  value is a strong outlier (5) (fig. S4).

The allele at high frequency in the Tibetan sample was associated with lower erythrocyte quantities and correspondingly lower hemoglobin levels (table S4). Because elevated erythrocyte production is a common response to hypoxic stress, it may be that carriers of the “Tibetan” allele of *EPAS1* are able to maintain sufficient oxygenation of tissues at high altitude without the need for increased erythrocyte levels. Thus, the hematological differences observed here may not represent the phenotypic target of selection and could instead reflect a side effect of *EPAS1*-mediated adaptation to hypoxic conditions. Although the precise physiological mechanism remains to be discovered, our results suggest that the allele targeted by selection is likely to confer a functionally relevant adaptation to the hypoxic environment of high altitude.

We also identified components of adult and fetal hemoglobin (*HBB* and *HBBG2*, respectively) as putatively under selection. These genes are located only ~20 kb apart (fig. S5), so their PBS values could reflect a single adaptive event. For both genes, the SNP with the strongest Tibetan-Han frequency difference is intronic. Although altered globin proteins have been found in some altitude-adapted species (14), in this case regulatory changes appear more likely. A parallel result was reported in Andean highlanders, with promoter variants at *HBBG2* varying with altitude and associated with a delayed transition from fetal to adult hemoglobin (15).

Aside from *HBB*, two other anemia-associated genes were identified: *FANCA* and *PKLR*, associated with erythrocyte production and maintenance, respectively (16, 17). We also identified

genes associated with diseases linked to low oxygen during pregnancy or birth: schizophrenia (*DISC1* and *FXYD6*) (18, 19) and epilepsy (*OTX1*) (20). However, the strong signal of selection affecting *DISC1*, along with *C1orf124*, might instead trace to a regulatory region of *EGLN1*, which lies between these loci (fig. S5) and functions in the hypoxia response pathway (21).

Other genes identified in this study are also located near candidate genes. *OR10X1* and *OR6Y1* are within ~60 kb of the *SPTA1* gene (fig. S5), which is associated with erythrocyte shape (22). Additionally, the three histones implicated in this study (Table 1) are clustered around *HFE* (fig. S5), a gene involved in iron storage (23). The influence of population genetic signals on neighboring genes is consistent with recent and strong selection imposed by the hypoxic environment. Stronger frequency changes at flanking genes might be expected if adaptive mutations have targeted candidate gene regulatory regions that are not near common exonic polymorphisms.

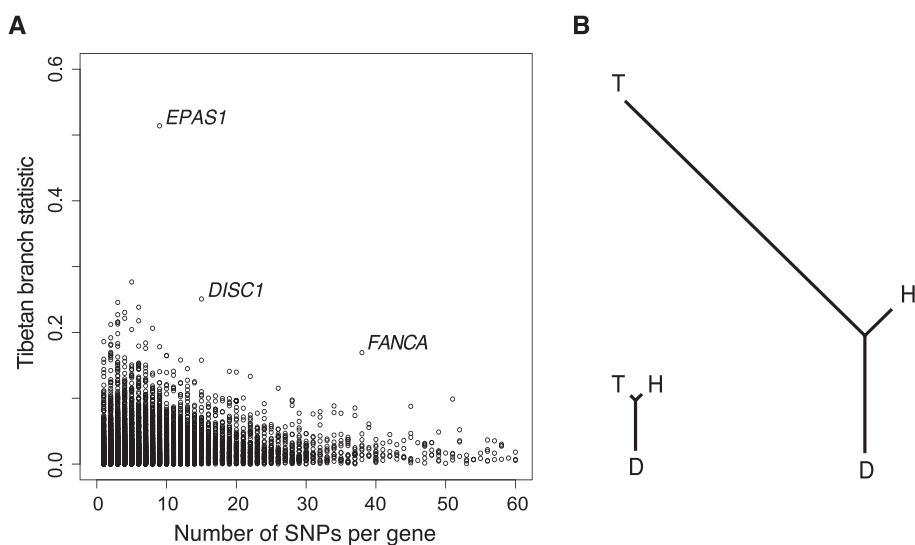
Of the genes identified here, only *EGLN1* was mentioned in a recent SNP variation study in Andean highlanders (24). This result is consistent with the physiological differences observed between Tibetan and Andean populations (25), suggesting that these populations have taken largely distinct evolutionary paths in altitude adaptation.

Several loci previously studied in Himalayan populations showed no signs of selection in our data set (table S6), whereas *EPAS1* has not been a focus of previous altitude research. Although *EPAS1* may play an important role in the oxygen regulation pathway, this gene was identified on the basis of a noncandidate population genomic survey for natural selection, illustrating the utility of evolutionary inference in revealing functionally important loci.

Given our estimate that Han and Tibetans diverged 2750 years ago and experienced subsequent migration, it appears that our focal SNP at *EPAS1* may have experienced a faster rate of frequency change than even the lactase persistence allele in northern Europe, which rose in frequency over the course of about 7500 years (26). *EPAS1* may therefore represent the strongest instance of natural selection documented in a human population, and variation at this gene appears to have had important consequences for human survival and/or reproduction in the Tibetan region.

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**Fig. 2.** Population-specific allele frequency change. (A) The distribution of  $F_{ST}$ -based PBS statistics for the Tibetan branches, according to the number of variable sites in each gene. Outlier genes are indicated in red. (B) The signal of selection on *EPAS1*: Genomic average  $F_{ST}$ -based branch lengths for Tibetan (T), Han (H), and Danish (D) branches (left) and branch lengths for *EPAS1*, indicating substantial differentiation along the Tibetan lineage (right).



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### Supporting Online Material

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Materials and Methods

Figs. S1 to S5

Tables S1 to S6

References

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# Genome-Wide Reprogramming in the Mouse Germ Line Entails the Base Excision Repair Pathway

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Genome-wide active DNA demethylation in primordial germ cells (PGCs), which reprograms the epigenome for totipotency, is linked to changes in nuclear architecture, loss of histone modifications, and widespread histone replacement. Here, we show that DNA demethylation in the mouse PGCs is mechanistically linked to the appearance of single-stranded DNA (ssDNA) breaks and the activation of the base excision repair (BER) pathway, as is the case in the zygote where the paternal pronucleus undergoes active DNA demethylation shortly after fertilization. Whereas BER might be triggered by deamination of a methylcytosine (5mC), cumulative evidence indicates other mechanisms in germ cells. We demonstrate that DNA repair through BER represents a core component of genome-wide DNA demethylation in vivo and provides a mechanistic link to the extensive chromatin remodeling in developing PGCs.

The specification of mouse primordial germ cells (PGCs) at embryonic day 7.25 (E7.25) is accompanied by the initiation of epigenetic changes (1), followed by widespread epigenetic reprogramming at E11.5, which includes genome-wide DNA demethylation, erasure of genomic imprints, and large-scale chromatin remodeling (1–3) (fig. S1). Chromatin remodeling follows the onset of genome-wide DNA demethylation, which suggests that DNA repair might be linked to this process (2). DNA repair-driven DNA demethylation would involve replacement

of a methylcytosine (5mC)-containing nucleotide by an unmethylated cytosine (4, 5). As the epigenetic changes in E11.5 PGCs occur in the G<sub>2</sub> phase of the cell cycle and are thus independent of DNA replication (2), the most likely mechanisms for the replacement of 5mC would be the nucleotide excision repair (NER) or base excision repair (BER) pathways.

We obtained a quantitative measure of expression of BER and NER components and observed an up-regulation of transcripts of BER components *Parp1*, *Ape1*, and *Xrcc1* in E11.5 PGCs, which was not seen in the neighboring somatic cells (6). By contrast, we observed little expression of NER components *Ercc1* and *Xpa* in these PGCs (Fig. 1A and fig. S2).

Expression of ERCC1 (excision repair cross-complementing rodent repair deficiency, complementation group 1), a core NER component, occurs at low levels in PGCs and neighboring somatic cells at the time of epigenetic reprogramming compared with control ultraviolet light-irradiated primary embryonic fibroblasts (PEFs), where we observed a dose-dependent increase and nuclear localization of ERCC1 (fig.

S3). Although we detected XPA—another NER component—in both somatic cells and PGCs (fig. S4A), it was not chromatin bound and, hence, was inactive (7) (fig. S4B). Thus, the response of the NER pathway is not triggered during the reprogramming process in PGCs.

XRCC1 (x-ray repair complementing defective repair in Chinese hamster cells 1), a core component of the BER pathway (8), is present in PGC nuclei between E10.5 and E12.5 (fig. S5A), as is PARP1 [poly(ADP-ribose) polymerase family, member 1] and APE1 (apurinic/apyrimidinic endonuclease) (fig. S5, B and C). XRCC1 is a soluble nuclear factor, which binds to DNA when single-stranded DNA (ssDNA) breaks occur (8). We determined the amount of chromatin-bound XRCC1 in gonadal PGCs using the preextraction method (9). Whereas we observed an overall enrichment of XRCC1 in PGCs during E10.5 to E12.5, we found an enhancement in chromatin-bound XRCC1, specifically in PGCs at E11.5, which coincides with the stage at which genome-wide DNA demethylation occurs (Fig. 1B), which suggested that ssDNA breaks are present (10). We also detected high levels of PAR polymer, a product of activated PARP1 enzyme and an additional marker of active BER (8, 11), specifically in E11.5 PGCs (Fig. 1C). The presence of activated BER in PGCs during ongoing epigenetic reprogramming suggests that DNA demethylation in PGCs may be linked to the DNA repair pathway (2).

In PGCs spanning a period of ~6 hours, between E11.25 and E11.5, we detected increasing levels of PAR before the loss of signal for histone H1 (Fig. 1C). Because histone H1 is a target for PARP1 ribosylation (11, 12) and PARP1 itself has been shown to displace H1 (13), it is possible that high levels of PARP1-mediated poly(ADP-ribose) (PAR) synthesis in the nuclei of PGCs might be involved in H1 displacement. Additionally, we observed a correlation between high nuclear PAR signals and the disappearance of chromocenters in PGCs (fig. S5D) (2), which is consistent with a proposed role for PARP1 in the regulation of higher-order chromatin structure (11, 14, 15).

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## Supporting Online Material for

### Sequencing of 50 Human Exomes Reveals Adaptation to High Altitude

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## **Materials and Methods**

### **Sample description**

The 50 ethnic Tibetans analyzed in this study were from two villages in the Tibet Autonomous Region, China. Half of these samples were from the town of Zhaxizhong, Dingri (9 females and 16 males), located at the foot of mountain Jomoglangma (4300 meters in altitude). The remainder were from the town of Zaren, Nachu (13 females and 12 males), which is approximately 250 miles northwest of Lhasa (at 4,600 m). All participants gave a self-report of at least three generations living in the sampling site, and provided informed consent for this study.

The peripheral venous blood samples of 50 ethnic Tibetans was collected using the pipelines dictated by the institutional review board of the Beijing Genomics Institute (BGI). In all subjects, oxygen saturation of blood was measured by Fingertip Oximeter: CMS-50DL twice with thirty minutes of interval. Blood testing was done using standard protocols for the BC-3000 Plus Auto Hematology Analyzer (MINDRAY): erythrocyte quantities were assessed by automated cell counting, and hemoglobin was quantified by spectrophotometry following hemolysis (using the SFT method). Comprehensive medical examinations were also conducted for all individuals during sampling to ensure that only healthy subjects were included in our analysis. All samples and measurements were obtained in the home village of each individual.

### **DNA extraction, library construction, exome capture and sequencing**

Genomic DNA was extracted from the blood samples by the use of QIAamp DNA Blood Mini Kit, according to protocol provided by QIAGEN. Following the manufacturer's protocol, genomic DNA of each individual was hybridized with NimbleGen 2.1M-probe sequence capture array (*S1*) (<http://www.nimblegen.com/products/seqcap/>) to enrich the exonic DNA in each library. The array is able to capture 18,654 (92%) of the 20,091 genes that has been deposited in Consensus Coding Sequence Region database (<http://www.ncbi.nlm.nih.gov/projects/CCDS/>).

First, DNA was randomly fragmented by nebulization to an average size of 500bp, and a pair of linkers was ligated to both ends of the resulting fragments. The linker-ligated DNA products were then hybridized to the capture array following NimbleGen's protocol, after which the exome-enriched DNA fragments were eluted from the array and amplified by ligation-mediated PCR, and non-hybridized fragments were then washed out.

Second, the captured DNA fragments were concatenated by DNA ligase and re-sheared to 200bp on average. Thus, we constructed a secondary library from the primary captured DNA library, which enabled the Illumina Genome Analyzer II platform, as previously described (*S2*), with adaptations. We performed sequencing for each captured library independently to ensure each sample had at least ~6-fold coverage. Raw image files were

processed by Illumina Pipeline (version 1.3.4) for base-calling with default parameters and the sequences of each individual were generated as 75bp reads.

## Read Mapping and Data quality analysis

Linker or adapter sequences that may be introduced into raw reads during the experiment process were masked before mapping. More concretely, the small portion of adapter and linker sequences within reads was identified by using a local dynamic programming algorithm, and reads that had more than 12 bp overlap with adapter or linker sequences were identified as contaminated reads. The contaminated sequence in reads was then discarded and the remaining sequence was retained. SOAPaligner (S3, S4) was used to align the clean reads to the NCBI human genome reference assembly (build 36.3), with a maximum of two mismatches, and parameters set as -a -D -o -r 1 -t -c -f 4. Reads that aligned to the designed target region were collected for SNP identification and subsequent analysis. To evaluate exon capture efficiency, the proportions of reads mapping to target regions and to their flanking regions (within 500 bp) were calculated for each individual. 35.5% of reads mapped to target regions (Table S1) and 68.1% of reads were within 500bp of a target region.

## SNP calling and estimation of sample allele frequencies

### *Calculation of genotype likelihoods*

Likelihoods of genotypes of each individual at every genomic location were calculated by SOAPsnp (S4). The observed data in site  $k$  of a particular read,  $d_k$ , contains three elements: (1)  $o_k$ : observed allele type; (2)  $q_k$ : quality score; (3)  $c_k$ : sequencing cycle (coordinate on read); and (4)  $t_k$ , the  $t_k$ -th observation of the same allele from reads with the same mapping location. All three elements in each read are used for the calculation of likelihoods, and sequencing errors are assumed to be independent. The likelihood for genotype  $S$  in site  $k$  is then

$$p(d_k | S) = p(o_k, q_k, c_k | S) = p(o_k, c_k | S, q_k)p(q_k | S)$$

We first estimate a four-dimensional matrix of  $p(o_k, c_k | S, q_k)$  on a grid of values of  $o_k$ ,  $q_k$ , and  $c_k$  for all possible genotypes, based on all of our alignments, using observed mismatch rates. Doing this, we can in effect recalibrate the quality score taking sequencing cycle into account.

$p(q_k | S)$  is the probability of an allele  $S$  to have an observation with quality score  $q_k$ . The quality distribution of each assumed allele is unknown. Here, we assumed that the distributions from A, C, G, and T are the same; then  $p(q_k | S)$  is the function of  $q_k$  only.

The same alleles from reads with the same mapping locations were ordered by the sequencing quality scores from low to high. An empirical treatment was used to reduce the quality of the  $t_k$ -th observation:

$$q'_k = \theta^{t_k} q_k$$

Here,  $\theta$  is called a dependency coefficient. The adjusted quality score  $q'_k$ , instead of the original  $q_k$ , was used in the likelihood matrix.  $\theta$  is set between 0 and 1. Specifically,  $\theta = 0$  means the completely dependent model, and  $\theta = 1$  is the completely independent model. A detailed description of this method is provided elsewhere (S4).

### *Allele frequency estimation*

Population genetic inferences based on called (inferred) SNPs can lead to serious biases and possibly false inferences if the coverage is not so large that the genotypes are known with absolute certainty for each individual. We have therefore developed a series of statistical techniques that can take uncertainty in genotype calls and allele frequency estimation into account.

To call SNPs and to estimate the allele frequencies in the sample, we use a Bayesian approach which is applied jointly to all individuals. SNP calling based on the joint information from all individuals should be more accurate than SNP calling based on independent analyses of single individuals. The same algorithm which estimates the posterior probability that a SNP is variable can also be used to estimate the frequency on an allele. We will first explain how the algorithm works for a single population. We then subsequently describe how the algorithm works for multiple populations.

Let  $p_j$  be the posterior probability that a di-allelic SNP has MAF of  $j/2k$ , where  $k$  is the sample size (number of individuals). We assume that a fraction,  $p_{\text{var}}$ , of nucleotide sites are variable in the population (not the sample!). Let the observed sequencing data for the SNP be  $X_i$ , and let  $S = (S_1, S_2, \dots, S_k)$  be a sample configuration where  $S_i \in \Psi$ ,  $\Psi = \{AA, AC, AG, AT, CA, CC, \dots, TT\}$ . Also, assume that the MAF in the population is  $p$ , and let  $\chi(S, j)$  be an indicator function which returns 1 if the sample MAF in configuration  $S$  is  $j/2k$ .  $p_j$  is then, for  $0 < j < k$ , given by

$$\pi_i = \frac{p_{\text{var}} \sum_{S \in \Psi^k} \left( \chi(S, j) \prod_{i=1}^k p(X_i | S_i) p(S_i | p) \right)}{p_{\text{var}} \sum_{S \in \Psi^k} \left( \prod_{i=1}^k p(X_i | S_i) p(S_i | p) \right) + (1 - p_{\text{var}}) \sum_{S \in \Psi^k} \left( \chi(S, 0) \prod_{i=1}^k p(X_i | S_i) p(S_i | p) \right)},$$

and

$$\pi_0 = \frac{\sum_{S \in \Psi^k} \left( \chi(S, 0) \prod_{i=1}^k p(X_i | S_i) p(S_i | p) \right)}{p_{\text{var}} \sum_{S \in \Psi^k} \left( \prod_{i=1}^k p(X_i | S_i) p(S_i | p) \right) + (1 - p_{\text{var}}) \sum_{S \in \Psi^k} \left( \chi(S, 0) \prod_{i=1}^k p(X_i | S_i) p(S_i | p) \right)},$$



for  $i = 0$ .  $p(X_i | S_i)$  is given (up to a scaling factor) by the genotype likelihoods which can be calculated as described above.  $p(S_i | p)$  can be calculated assuming Hardy-Weinberg equilibrium if the allele frequency  $p$  is known. Our algorithm, therefore, proceeds by first estimating  $p$  from the raw sequencing reads. The entire calculation can be done very fast using a dynamic programming algorithm for summing over all elements in  $\Psi^k$ . In the following we give a detailed description of the algorithmic details of the inference method: We first estimate allele frequencies in each site, and we then estimate the Site Frequency Spectrum (SFS).

### *Estimating allele frequencies from reads in one site*

Let the individuals be  $I_1, I_2, \dots, I_k$ , i.e. we assume  $k$  individuals.

(1) For each site in each individual, eliminate all reads with Q score  $< 20$ . Determine which two nucleotides are most common among  $\{A, C, T, G\}$  and let the set of these nucleotides be  $B$ , i.e. if there are 400 A's, 42 C's, 13 T's and 9 G's, then  $B = \{A, C\}$ . Then eliminate all reads that are not elements of  $B$

(2) For  $i=1$  to  $k$

Let  $n_i$  be the number of reads of the minor allele in  $B$  in individual  $I_i$ . Let the total number of reads in  $B$  in individual  $I_i$  be  $n_{iT}$ . Calculate

$$p_i = \frac{n_i - en_{iT}}{n_{iT}(1 - 2e)}$$

This is an error corrected estimate of the allele frequency in individual  $I_i$ , obtained as the solution for  $p_i$  to the equation  $n_i = p_i n_{iT}(1 - e) + (n_{iT} - p_i n_{iT})e$ . The parameter  $e$  is the error rate and is considered a fixed parameter, here assumed to be  $e = 0.005$ . Also calculate  $w_i = \frac{2n_{iT}}{n_{iT} + 1}$ , the inverse of the variance of  $p_i$  (up to a scalar).

(3) The estimate of the MAF is then calculated as

$$\hat{p} = \min\{1, p^*\}, p^* = \max\left\{0, \frac{\sum_{i=1}^k p_i w_i}{\sum_{i=1}^k w_i}\right\},$$

### *Estimating Sample Allele Frequencies*

Likelihood values for all  $G \in \{AA, AC, AG, AT, \dots, TT\}$  have been calculated using the previously described algorithm. We are interested in estimating the posterior probability that the minor allele frequency exists in a frequency  $j$  in the sample of  $2k$  chromosomes. We assume that the prior probabilities of the different genotypes are given by the probabilities predicted under Hardy-Weinberg equilibrium with a MAF of  $\hat{p}$ . This corresponds to using an empirical Bayesian approach where the shared parameter ( $\hat{p}$ ) first is estimated and then provides a prior for each individual. We will denote the minor allele by 'A' and the major by 'a'. Then a dynamic programming algorithm for calculating the posterior probability is given by (for each site):

If  $\hat{p} = 0$ , set  $p_0 = 1$  and  $p_j = 0$  for all  $j > 0$ .

Else

(1) Set  $h_j = 0$ ,  $j = 3, 4, \dots, 2k$ .

(2) For  $i=1$  to  $k$

Set  $P_{AA,i} = g_{AA,i}(\hat{p}^2(1-F) + \hat{p}F)$ ,  $P_{Aa} = c f_i g_{Aa,i} 2(1-\hat{p})\hat{p}(1-F)$  and  $P_{aa} = g_{aa,i}(1-\hat{p})^2(1-F) + (1-\hat{p})F$ .

Here  $g_{G,i}$  is the previously calculated likelihood for genotype  $G$  in individual  $i$ . The parameter  $F_i$  is the inbreeding coefficient and needs to be obtained prior to analyses jointly for all sites. We will assume here that  $F = 0$ .

If  $i=1$

Set  $h_0 = P_{aa}$

Set  $h_1 = P_{Aa}$

Set  $h_2 = P_{AA}$

Otherwise

For  $j = 2i$  to  $2$  (count backwards)

Set  $h_j = P_{AA}h_{j-2} + P_{Aa}h_{j-1} + P_{aa}h_j$

Set  $h_1 = P_{aa}h_1 + P_{Aa}h_0$

Set  $h_0 = P_{aa}h_0$

(3) Set  $\pi_j = \frac{h_j p_{\text{var}}}{p_{\text{var}} \sum_r h_r + (1 - p_{\text{var}}) \prod_{i=1}^k g_{aa}}$ ,  $j = 1, 2, \dots, 2k$

$$\pi_0 = \frac{p_{\text{var}} h_0 + (1 - p_{\text{var}}) \prod_{i=1}^k g_{aa}}{p_{\text{var}} \sum_r h_r + (1 - p_{\text{var}}) \prod_{i=1}^k g_{aa}}$$

The estimated values of  $p_i$ , can then be used for population genetic inferences, either by averaging over  $p_i$ , or by using a Maximum *a posteriori* Probability (MAP) estimate of the sample allele frequency. Notice that this procedure explicitly takes into account differences in sequencing depths between samples when estimating allele frequencies, and quantifies the uncertainty in these estimates. Likewise, SNP calling can proceed in a probabilistic fashion by choosing a cut-off for  $p_0$  ( $p_{2k}$  is so close to zero that it can be ignored because the definition of  $p$  as the minor allele frequency). For example, if we wish to call sites with a probability >95% of being SNPs, we would select all sites with  $p_0 < 0.05$ .

### Extension to multiple populations

We here discuss the extension to two populations, in this case Han ( $H$ ) and Tibetans ( $T$ ). We will use a single estimate of  $p$ , calculated as previously described for both populations. The main motivation for doing this is to avoid situations in which  $\hat{p} = 0$  for one population and  $\hat{p} > 0$  in another population. Another justification for using the shared estimate is that we would rather be conservative with regards to inferences of differences in sample allele frequencies between populations. We therefore prefer to use the same prior for both populations.

The joint posterior probability of a site having allele frequency  $i$  in  $H$  and  $j$  in  $T$ , is then given by

$$\pi_{ij} = \frac{h_j^T h_i^H p_{\text{var}}}{p_{\text{var}} \sum_r \sum_s h_r^T h_s^H + (1 - p_{\text{var}}) \left( \prod_{m=1}^{T_k} g_{aa}^{m,T} \right) \left( \prod_{m=1}^{H_k} g_{aa}^{m,H} \right)}$$

where  $T_k$  is the number of Tibetan individuals and  $H_k$  is the number of Han individuals. All functions sub- or super-scripted with either T or H are calculated as previously described marginally for population  $T$  and  $H$ , respectively. A SNP is then called if  $p_{00}$  is less than some specified cut-off.

### Population genetic inferences

Population genetic statistics that do not use linkage/linkage disequilibrium information into account are all functions of Site-Frequency-Spectrum (SFS). In our case, an estimate of the joint SFS for Tibetans and Han is giving by the matrix  $p = \{p_{ij}\}$ . Statistic such as  $F_{ST}$ , the number of segregating sites, the average number of pairwise differences, etc, can be calculated directly from  $p$  for each gene. This can be done based on the MAP estimate for called SNPs (i.e. SNPs with  $p_{00}$  less than some specified cut-off), or it can be done by summing over the values in  $p$ , thereby taking uncertainty in both SNP calling and inference of allele frequency into account. For example, the number of segregating sites

in the Tibetan population in a gene would be calculated as  $\sum_{\text{sites}} \left( 1 - \sum_{i=1}^{2T_k} \pi_{i0} \right)$  and the total number of segregating sites in a gene would be calculated as  $\sum_{\text{sites}} (1 - \pi_{00})$ . Likewise, the average number of pairwise differences per site can be calculated as

$$\frac{\sum_{\text{sites}} \left( \sum_{i=1}^{2H_k} \sum_{j=1}^{2T_k} \frac{j(2T_k - j) \pi_{ij}}{\binom{2T_k}{2}} \right)}{\sum_{\text{sites}} \left( 1 - \sum_{i=1}^{2H_k} \pi_{i0} \right)}.$$

Any other statistic calculated on a per site basis, which normally for a single variable site with sample allele frequency  $i$  in  $H$  and  $j$  in  $T$  is given by  $f(i, j)$ , can similarly be calculated as

$$\frac{\sum_{sites} \left( \sum_{i=1}^{2H_k} \sum_{j=1}^{2T_k} f(i, j) \pi_{ij} \right)}{\sum_{sites} \left( 1 - \sum_{i=1}^{2H_k} \pi_{i0} \right)}.$$

Our inference of natural selection is primarily based on a new statistic aimed at detecting strong changes in allele in one population. Pairwise differences in allele frequencies can be quantified using  $F_{ST}$ . We use the  $F_{ST}$  estimator of Reynolds *et al.* (S5), based on the MAP estimates for SNP frequencies where sites are considered if they satisfy  $(1.0 - p_{00}) > 0.01$ . We also excluded sites for which the minor allele has MAP frequency estimate of 0 in at least two populations or for which no data was available for the Danish population. . We then use the classical transformation by Cavalli-Sforza (S6),

$$T = -\log(1 - F_{ST})$$

to obtain estimates of the population divergence time  $T$  in units scaled by the population size. For each RefSeq gene, we calculate this value between the Tibetans and Han populations ( $T^{TH}$ ), and between these populations and a Danish population ( $T^{TD}$  and  $T^{HD}$ ), for which data obtained using similar techniques was previously published for 200 individuals, providing very accurate estimates of allele frequencies. The length of the branch leading to the Tibetan population since the divergence from Han, is then obtained as

$$PBS = \frac{T^{TH} + T^{TD} - T^{HD}}{2}$$

A population's  $PBS$  value represents the amount of allele frequency change at a given locus in the history of this population (since its divergence from the other two populations). This approach is similar to the “locus-specific branch lengths” statistic used by Shriver *et al.* (S7), except that by using the above log-transformation, we obtain additive distances that place branches of different magnitudes on the same scale. This statistic should be very powerful to detect selection. It should have power, for example to detect incomplete selective sweeps, a type of selection that is highly relevant here and which most other statistics based on the SFS have little power to detect.

### Evaluation of the PBS statistic

Recent simulation studies have shown that  $F_{ST}$ -based statistics (S8) have more power to detect recent adaptation when selection is acting on standing variation. Because of the very short divergence time between Han and Tibetan individuals, and the fact that the waiting time to a new mutation might be large, we expect much local adaptation to be

driven by selection acting on standing variation rather than *de novo* mutations. The test statistic we are using is, therefore, a simple transformation of  $F_{ST}$  designed to take advantage of an outgroup and to identify Tibetan specific selection.

To evaluate if this approach also has power to detect selection on *de novo* mutations, we performed a small scale simulation study. Using the Wright-Fisher model simulator *sfscode* (S9), we simulated 3 populations (representing Danes, Tibetans and Han) introducing one new selected mutation in the Tibetan population at the time of the split of Han and Tibetans. We simulated data sets under a range of scaled selection coefficient, ( $g = 2Ns$ , where  $s$  is the selection coefficient and  $N$  is the population size), assuming the population size  $N = 1000$  for each of the 3 populations, and we assumed divergence times between Danes and Asians, and Han and Tibet, of 1680 and 120 generations, respectively. The locus size was set to 1kb, the population mutation rate and the population recombination rate were set to 0.001 per site. No further complications to the demographic model were used in this analysis, because these simulations were only used for evaluating the power of the *PBS* statistic, and not to generate  $P$  values for empirical observations.

Often the selected mutation in a simulation will be lost after a few generations due to the effect of genetic drift. However, as we were interested in evaluating the power under a complete or incomplete selective sweep, we only examined simulation replicates where the selected mutation was not lost from the Tibetan population, effectively conditioning on the presence of the allele. To determine critical values, we ran neutral simulations (no selected mutation was introduced). The power was then calculated by comparing the simulations with selection to simulations without selection. For comparison, we also calculated Tajima's  $D$  (S10) for each simulation replicate and evaluated the power of Tajima's  $D$  based on the same simulations. Results indicated that *PBS* has strong power to detect a recent selective sweep (Figure S3). The power of Tajima's  $D$ , in contrast, is quite low in this setting, potentially due to low numbers of segregating sites. Because our exonic data contains relatively few SNPs per gene, the high power of *PBS* under these conditions represents an important advantage for our analysis. A similar set of simulations were also conducted with the population recombination rate elevated to 0.01, in order to simulate a locus that is ten times longer, but with only the same number of sampled sites (analogous to our exonic data). Results were qualitatively similar: *PBS* retained high power in these simulations, while Tajima's  $D$  had modestly higher power than it had with shorter loci (data not shown).

## Demographic estimation and neutral simulations

For the inference of demographic parameters we used the unfolded site frequency spectrum (based on ancestral alleles shared by chimpanzee and macaque genomes) of the synonymous sites (61,347 SNPs) in the Han and Tibetan samples. Parameter inference was carried out with the software package *∂a∂i* (version 1.2.3) (S11). We took ancestral population events such as the out-of-Africa bottleneck from the model inferred by Gutenkunst *et al.* (S11), but we estimated parameters pertaining to the two Asian samples studied here. Models were compared via Akaike and Bayesian Information Criteria; the



best fitting-model is shown in Figure S2. As further detailed in the legend of this figure, this model involves a population split 2,750 years ago. The Han size is initially small but grows larger, while the Tibetan size is initially large but contracts with time. Migration occurs from the Tibetan sample to the Han sample, but 20% of the Tibetan gene pool is replaced by Han admixture at the present time. A wide variety of models were tested, but the model shown in Figure S2 fit better, for example, than the same model with symmetric migration, and much better than a similar model lacking the ancestral African time and growth estimates of Gutenkunst *et al.* (S11). The model of European history from Gutenkunst *et al.* (S11) was used for the history of the Danish sample in the simulations described below.

Neutral simulations under the model estimated above were used to calculate *P* values for the *PBS* values inferred for each gene in the ethnic Tibetan sample. Simulations were run using the program *ms* (S12) with demographic parameters from the above model and recombination rates drawn randomly from the map of McVean *et al.* (S13). Gene lengths for simulations were sampled randomly from the lengths of all human genes. One million simulations were performed for each number of SNPs (for 1 to 15 SNPs) or using 5-SNP bins (from 20 to 40 SNPs) and conservatively comparing genes to simulations with slightly fewer SNPs (*e.g.* comparing a gene with 28 SNPs to simulations with 25 SNPs). *P* values were defined simply as the proportion of simulated replicates yielding a higher *PBS* value than empirically observed for a particular gene.

### **Genotyping and association testing for a candidate SNP at EPAS1**

The SNP at *EPAS1* showing the most dramatic frequency difference between ethnic Tibetan and Han samples (located at position 46441523 on chromosome 2) was genotyped in a larger sample of 366 ethnic Tibetans (from the same localities, and collected via the same protocols, as described above. Genotyping was done by use of the mass-spectrometry-based MassArray platform of Sequenom (San Diego, CA, USA). PCR and extension primers were designed using Assay Design v3.1 (Sequenom, San Diego, CA, USA). Forward and reverse PCR primers were ACGTTGGATGTCCATGTCTGACCCTTCCAC and ACGTTGGATGTATTGTGAGGAGGGCAGTTG. Genotyping primers had the unextended sequence GACCCTTCCACGCCTGT, extending to a “C” or “G” for the alternate alleles.

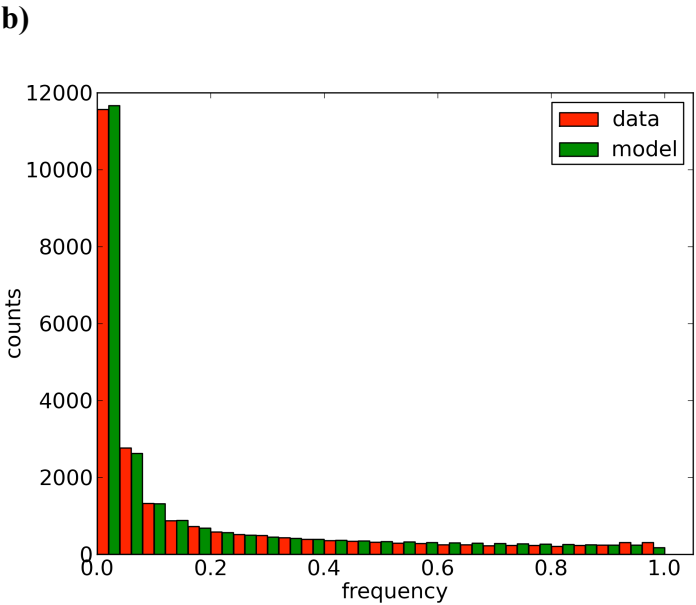
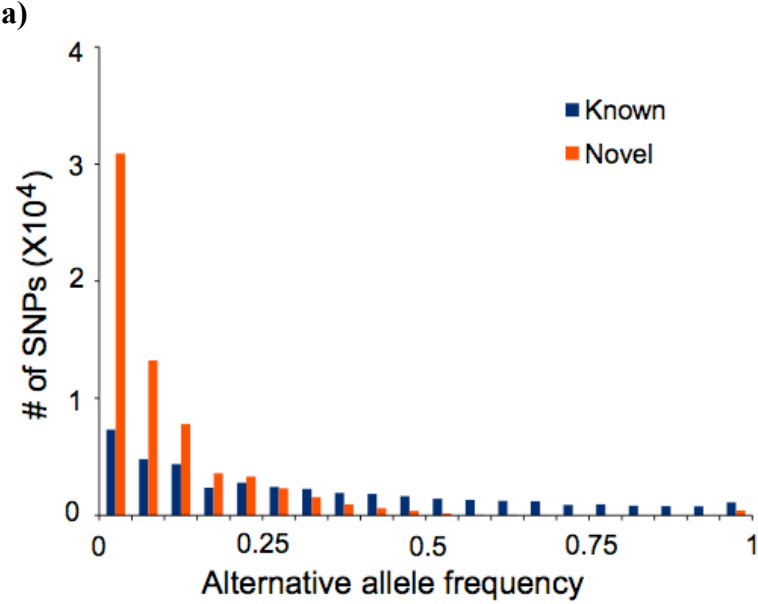
PCR reactions were performed in 5µl PCR cocktail mix, consisting of 1µl DNA template (10-25 ng/µl), 1 × PCR Buffer (including 2 mmol/L MgCl<sub>2</sub>), 2 mmol/L MgCl<sub>2</sub>, 500 µmol/L dNTP mix, 0.1pmol/µl of each PCR primer, and 0.5U Hotstar Taq (Roche). PCR conditions were as follows: incubation at 94°C for 15 min, followed by 45 cycles of 20 sec at 94°C, 30 sec at 56°C, 1 min at 72°C, and a final extension of 3 min at 72°C. Shrimp alkaline phosphatase treatment was performed to dephosphorylate unincorporated dNTPs under the following conditions: 37°C for 40 min, 85°C for 5 min, cooling to 4°C.

The iPLEX primer extension reaction was performed using the iPLEX cocktail mix (Sequenom, San Diego, CA, USA), which contains buffer, iPLEX termination mix,

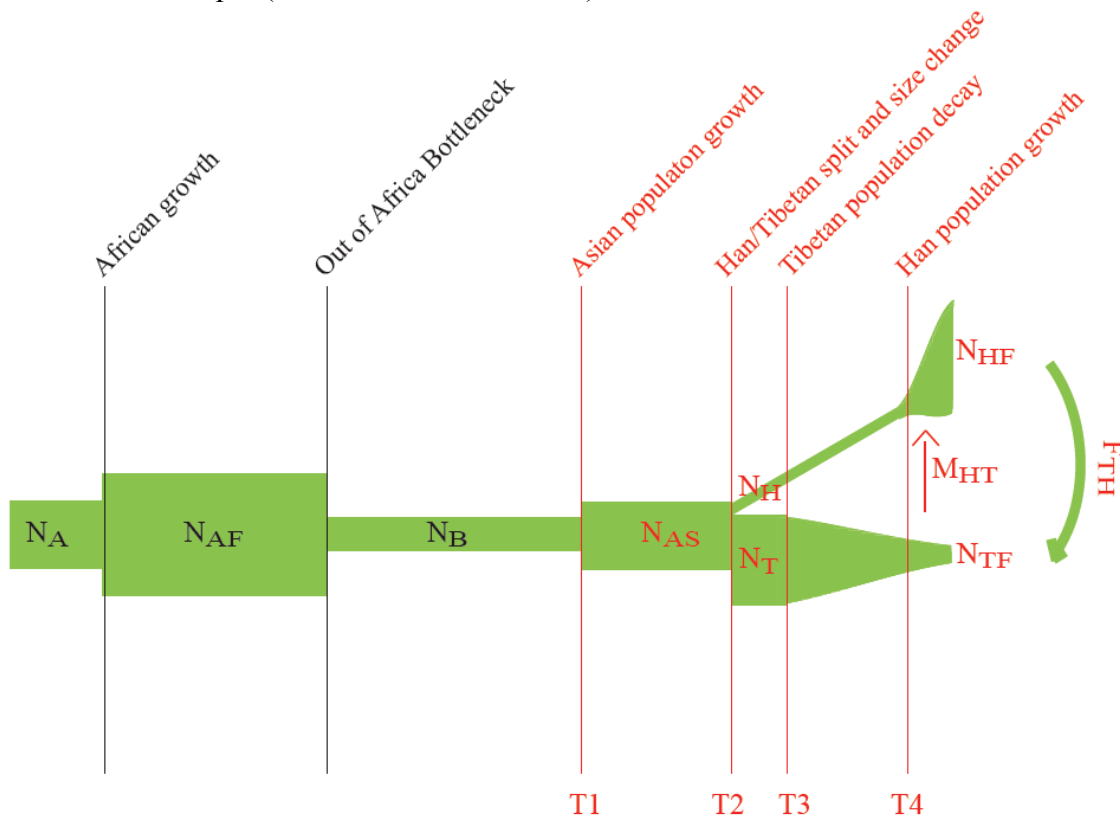
iPLEX enzyme and extension primers, under the following conditions: the DNA sample is denatured at 94°C, Strands are annealed at 52°C for 5 seconds and extended at 80°C for 5 seconds, The annealing and extension cycle is repeated four more times for a total of five cycles and then looped back to a 94°C denaturing step for 5 seconds and then enters the 5 cycle annealing and extension loop again. The five annealing and extension steps with the single denaturing step are repeated an additional 39 times for a total of 40. A final extension is done at 72°C for three minutes and then the sample is cooled to 4°C. Six milligram clean resin was added into 384-well PCR plate to desalt the iPLEX extension products before mass spectrometric analysis. An average of 3-10 nl products were dispensed onto a 384-element SpectroCHIP bioarray (Sequenom) by a nanodispenser. MassARRAY Workstation version 3.4 software (Sequenom) was used to process and analyze iPLEX SpectroCHIP bioarrays. Positive and negative control samples were run at each step and on each chip.

Association testing was performed using simple linear regressions of the measurements oxygen saturation, erythrocyte count, and hemoglobin concentration on the genotypes of the focal SNP at *EPAS1*. The genotypes were encoded as numerical values 0, 1, 2 corresponding to homozygous, heterozygous and homozygous (for the other allele) genotypes. We used the model  $E[Y|X_i] = \beta_0 + \beta_1 X_i$  and tested whether the slope ( $\beta_1$ ) is different from zero. Here Y is the quantitative trait and  $X_i$  takes the values {0, 1, 2} for the genotypes at SNP site  $i$ . The regressions were performed for the full sample of 366 individuals, for each of the two villages separately (Table S5). The analysis made use of the linear regression function from the R programming language and F-test  $P$ -values were recorded. Genotypes at the focal *EPAS1* SNP were uncorrelated with gender. To further control for gender-related phenotypic differences, we also performed association testing in females only, and in males only. Results were very similar to the overall results: associations for erythrocyte count and hemoglobin quantity remained statistically significant or marginally significant, and associations for oxygen saturation did not approach statistical significance. Since population stratification may be an issue, we calculated the inflation factor from non-associated SNPs (*SI4*) in the full sample, and used this inflation factor to compute *EPAS1* association  $P$ -values corrected for population stratification for our most differentiated SNP. The results remained statistically significant. The phenotypic associations observed for focal SNP at *EPAS1* were also compared against 48 additional SNPs from around the genome, genotyped in the same large sample. The  $P$  value observed for *EPAS1* was a clear outlier from this set (Fig. S5). Positions for these “genomic control” SNPs were as follows: chr1-12491677, chr1-27151140, chr1-45846284, chr1-52675081, chr1-53448299, chr1-65630810, chr1-110567989, chr1-154829060, chr1-194962365, chr1-201404410, chr2-43955048, chr2-71215412, chr2-178202419, chr2-218391384, chr3-19936334, chr3-57113459, chr4-77284346, chr5-35912031, chr5-96357847, chr5-172274635, chr5-172274640, chr6-25881584, chr6-26164595, chr6-133146813, chr6-151715282, chr7-6032976, chr8-101796892, chr8-105430170, chr10-29931167, chr11-1934128, chr11-3642019, chr11-61767439, chr11-74793531, chr11-89541802, chr11-106702850, chr12-9208040, chr14-64267910, chr17-39581008, chr17-64702135, chr19-1005255, chr19-1776926, chr19-6864707, chr19-8097184, chr19-46314107, chr19-59665806, chr20-33678648, chr20-36217869, chr22-40816669.

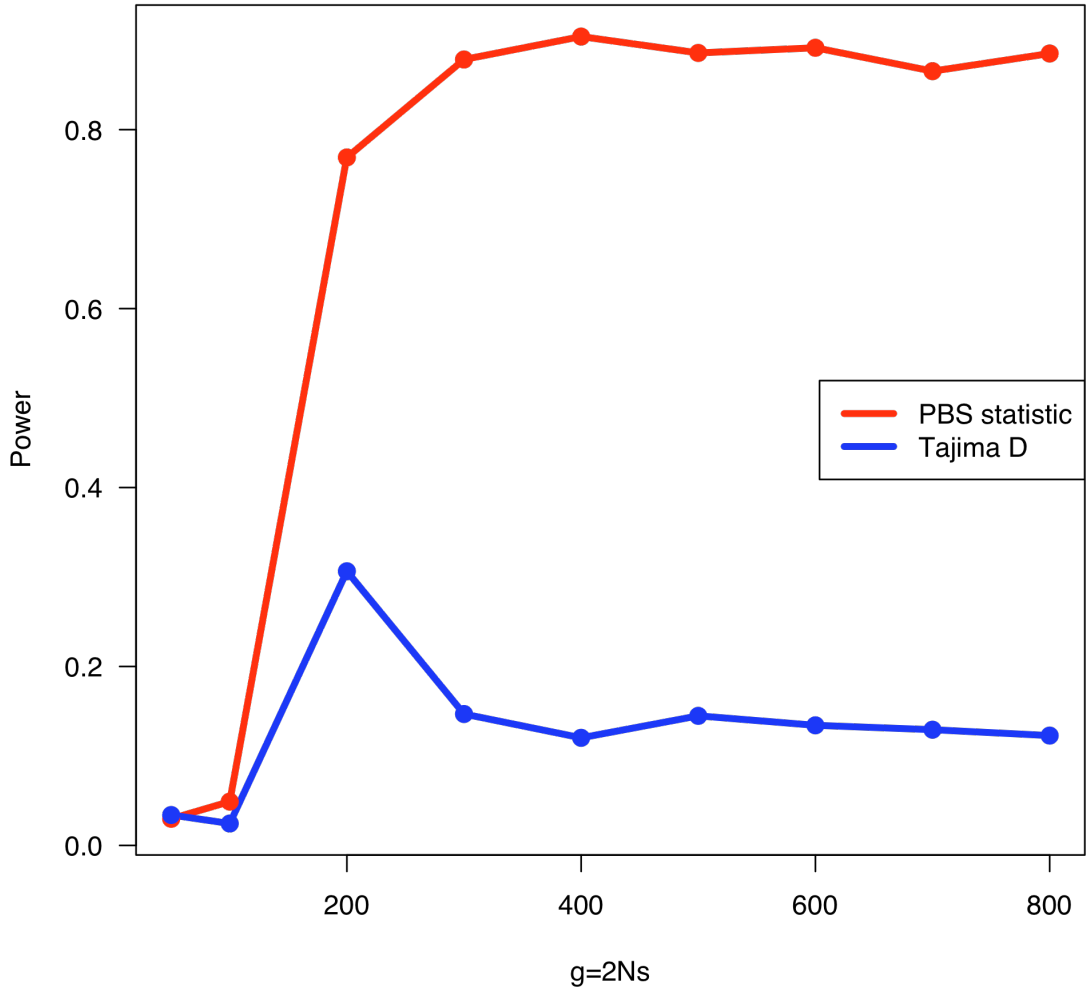
**Figure S1.** Alternative site frequency spectra (SFS) for Tibetan exome data.  
a) Comparison between AFS of known (blue; in dbSNP v129) and novel SNPs (red).  
b) Comparison between AFS of empirical data and the estimated demography model.



**Figure S2.** Illustration of best-fitting demographic model according to Akaike Information Criterion and Bayesian Information Criterion. Parameters in red were estimated; parameters in black were fixed according to the model of Gutenkunst *et al.* (2009). Estimates for inferred parameters were as follows: The ancestral non-African population grows to a size of  $N_{AS} = 7360$  at time  $T_1 = 42,955$  years ago (all time estimates assume 25 years per generation). At time  $T_2 = 2,750$  years ago, the Han and Tibetan lineages split, with the Han population having initial size  $N_H = 288$  and the Tibetan population having initial size  $N_T = 22,642$ . At time  $T_3 = 1,973$  years ago, the Tibetan population begins exponential decline to a final size of  $N_{TF} = 1,270$ . At time  $T_4 = 1,813$  years ago, the Han population begins exponential growth to a final size of  $N_{HF} = 12,850$ , and migration from the Tibetan to the Han population occurs at rate  $m_{HT} = 0.00526$ . Finally, at the present time, a proportion  $F_{TH} = 0.2$  of the Tibetan gene pool is drawn from the Han sample (instantaneous admixture).

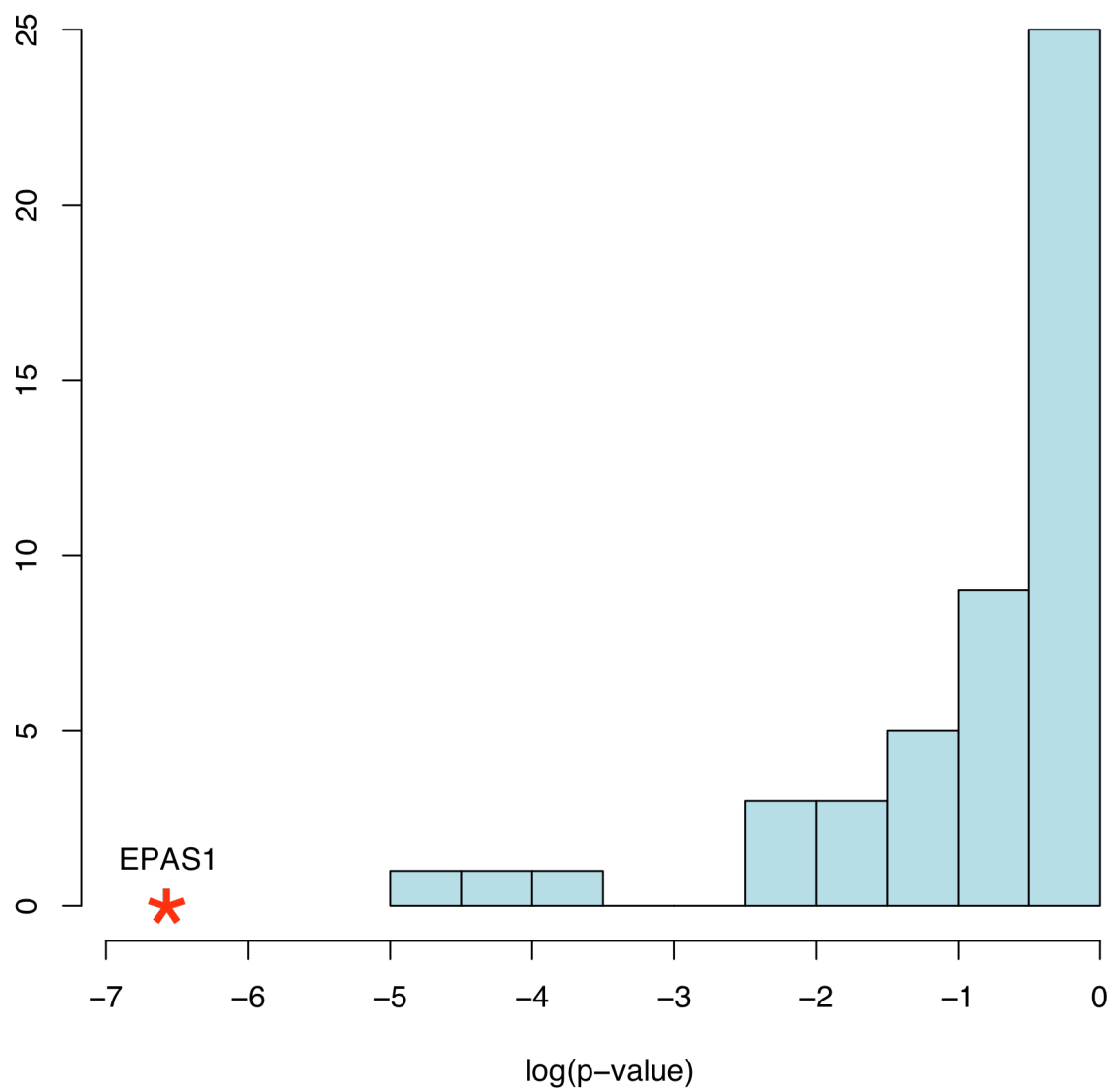


**Figure S3.** Power of the *PBS* and Tajima’s *D* statistics to detect a recent selective sweep, depending on the strength of selection (X-axis). Simulations were conducted as described in the Supplemental Text.

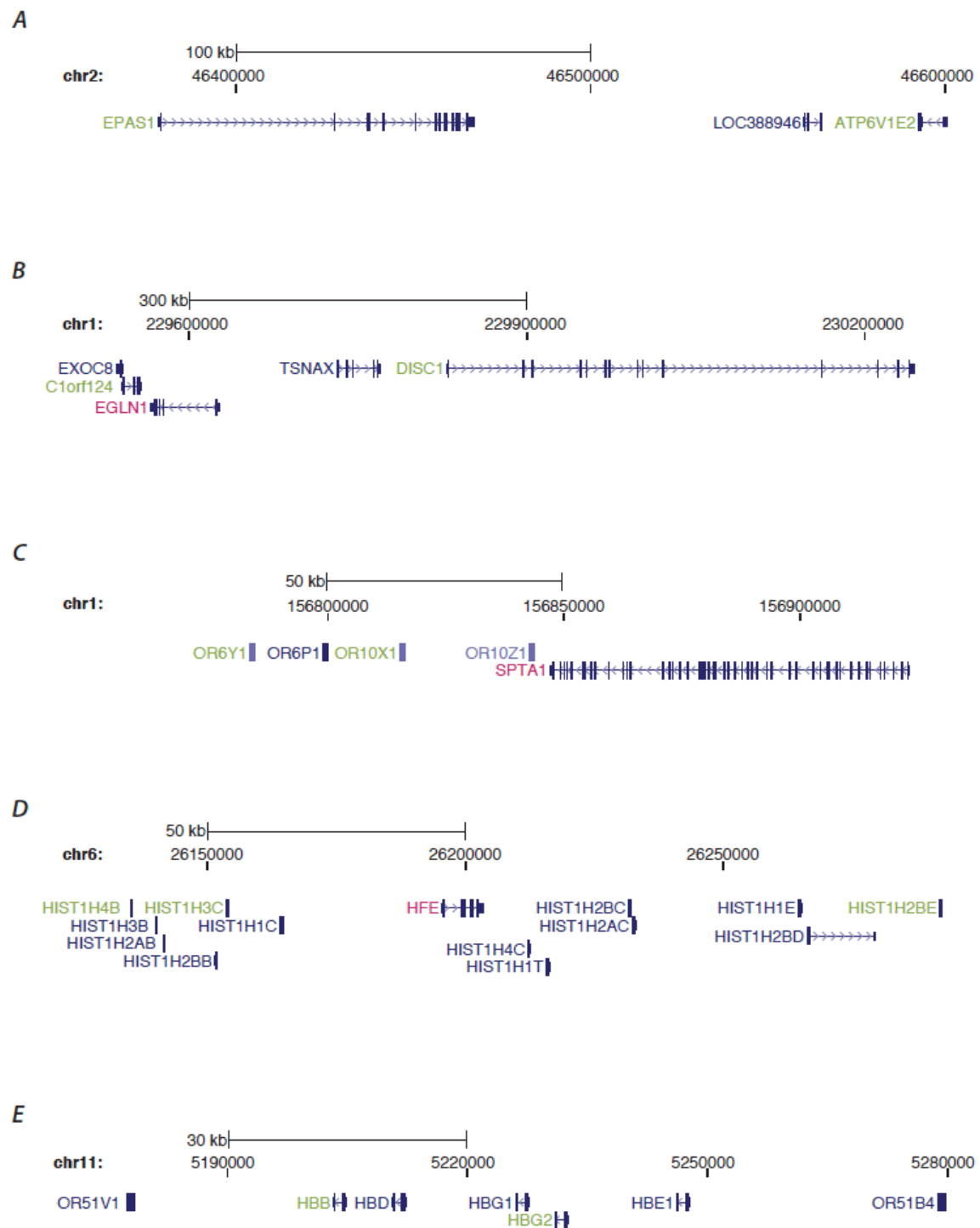




**Figure S4.** Distribution of  $\log_e$  p-values for 48 genomic control SNPs regressed against erythrocyte count, and for the genotyped *EPAS1* SNP (red star)



**Figure S5.** Linked pairs or groups of genes that appear on the list of most extreme *PBS* values (Table 2) are shown in green. Nearby candidate genes are marked in red.



**Table S1.** Data production by individual sample

Sample ID	Raw reads (x1e+6)	Raw data yield (Mb)	Reads mapped to genome (x1e+6)	Reads mapped to target region (x1e+6)	Data mapped to target region (Mb)	Mean depth of target region	Coverage of target region (%)	Average read length (bp)	Rate of nucleotide mismatch (%)
DR-F11	44.75	3,356	13.7	4.08	201.92	5.92	87.01	62.07	2.04
DR-F14	35.51	2,663	31.38	11.15	614.64	18.02	95.25	69.93	0.96
DR-F17	38.23	2,867	32.23	11.6	638.52	18.72	95.26	70.32	0.9
DR-F18	47.41	3,556	18.88	6.48	316.87	9.29	90.13	61.47	2.17
DR-F19	42.30	3,172	12.37	4.02	199.88	5.86	88.05	62.32	2.03
DR-F35	44.19	3,314	29.94	11.92	596.22	17.48	97.55	61.43	1.29
DR-F40	33.01	2,476	29.07	10.4	557.68	16.35	96.37	69.78	1.08
DR-F6	31.84	2,388	28.48	10.08	556.31	16.31	94.94	69.98	0.96
DR-F8	46.18	3,463	19.08	6.7	328.47	9.63	92.75	61.55	2.24
DR-M10	39.01	2,926	34.29	12.85	704.01	20.64	95.97	69.76	1
DR-M19	40.06	2,404	34.51	8.06	398.73	11.69	96.21	61.5	0.76
DR-M22	41.24	1,526	21.79	9.54	440.34	12.91	97.09	56.16	1.26
DR-M23	21.06	1,685	17.91	7.34	424.31	12.44	95.1	73.92	0.89
DR-M24	40.76	3,057	36.47	14.18	785.87	23.04	96.05	70.68	0.85
DR-M28	40.58	3,043	27.67	11.63	578.14	16.95	97.89	60.69	1.21
DR-M30	41.19	3,089	28.34	11.82	587.35	17.22	97.95	60.64	1.21
DR-M31	36.41	2,731	31.49	10.95	587.69	17.23	95.51	69.66	1.1
DR-M38	40.10	3,007	25.97	12.1	642.27	18.83	96.9	65.67	1.1
DR-M42	40.14	3,010	26.38	10.77	536.19	15.72	97.39	60.96	1.28
DR-M43	40.80	3,060	25.85	10.52	526.3	15.43	97.4	61.27	1.1
DR-M44	40.91	1,514	22.67	9.41	435.23	12.76	97.22	56.28	1.1
DR-M46	37.95	2,846	16.9	7.47	372.13	10.91	96.55	60.77	1.18
DR-M7	45.35	3,401	17.43	5.88	298.79	8.76	90.37	64.51	1.84
DR-M8	46.58	2,795	39.98	11.85	597.59	17.52	97.14	62.83	0.72
DR-M9	41.28	3,096	27.96	11.49	573.37	16.81	97.63	60.92	1.22
NQ-F15	66.40	4,980	47.15	15.59	819.98	24.04	96.01	66.33	1.15
NQ-F16	38.01	2,851	30.67	10.97	603.04	17.68	96.71	69.86	0.99

NQ-F17	65.31	4,898	44.84	13.82	717.99	21.05	96.92	65.33	1.21
NQ-F19	70.48	3,947	48.48	15.33	819.98	24.04	95.38	67.93	1.17
NQ-F20	41.22	1,525	22.94	8.88	411.69	12.07	97.14	56.51	1.04
NQ-F24	62.88	4,716	49.48	18.6	977.9	28.67	97.68	65.93	1.52
NQ-F25	64.36	4,827	45.8	16.34	845.22	24.78	97.15	65.19	1.26
NQ-F26	59.85	4,489	48.25	18.59	978.24	28.68	97.53	65.85	1.56
NQ-F32	67.11	5,033	47.13	14.32	753.46	22.09	94.68	66.49	1.13
NQ-F34	46.40	3,480	15.25	4.97	242.51	7.11	90.47	61.21	2.11
NQ-F35	57.04	4,278	43.41	15.01	794.74	23.3	97.61	66.08	1.14
NQ-F36	58.83	4,412	39.95	13.27	689.68	20.22	97.27	64.98	1.29
NQ-F7	58.93	4,420	40.16	12.39	647.73	18.99	97.44	65.41	1.23
NQ-M12	48.98	3,674	19.23	5.8	294.7	8.64	89.62	64.17	1.76
NQ-M13	39.68	2,976	33.82	13.64	758.92	22.25	92.71	70.03	0.99
NQ-M20	64.05	4,803	47.25	16.55	875.91	25.68	96.39	66.44	1.3
NQ-M21	46.41	3,481	18.97	5.47	268.1	7.86	87.6	61.04	2.15
NQ-M26	41.56	3,117	28.48	10.24	524.59	15.38	96.27	64.15	1.58
NQ-M31	39.38	1,457	17.59	7.51	362.24	10.62	96.66	59.19	0.98
NQ-M32	62.55	4,691	49.82	19.97	1043.05	30.58	97.75	65.45	1.59
NQ-M33	58.68	4,401	47.42	15.04	788.25	23.11	97.08	65.29	1.41
NQ-M35	55.80	4,185	47.4	17.67	978.92	28.7	97.06	70.58	0.87
NQ-M5	59.33	4,450	46.85	17.76	925.37	27.13	97.87	64.84	1.61
NQ-M7	59.36	4,452	47.69	15.65	816.22	23.93	96.62	65.26	1.44
NQ-M9	63.90	3,578	43.68	10.55	551.2	16.16	96.73	65.39	1.25

**Table S2. Variation detection from Tibetan exomes**

SNP discovery for functional classes of sites

Genomic features		Known	Novel	Total
		# of SNPs	# of SNPs	# of SNPs
CDS	synonymous	14,439	12,312	26,751
	nonsynonymous	11,421	26,634	38,055
	nonsense	73	541	614
Intron		14,547	23,623	38,170
5'UTR		848	1,129	1,977
3'UTR		895	1,100	1,995
Intergenic		15	16	31



**Table S3.** Additional statistics for the 30 genes with highest Tibetan *PBS* values.

Gene	refseq ID	S Tibetan	$\Pi$ Tibetan	S Han	$\Pi$ Han	T <sub>TH</sub>	T <sub>TD</sub>	T <sub>HD</sub>
<i>EPAS1</i>	NM_001430	12.64	0.10	8.86	0.17	0.57	0.70	0.24
<i>C1orf124</i>	NM_032018	3.37	0.16	4.15	0.27	0.14	0.53	0.12
<i>DISC1</i>	NM_018662	18.31	0.13	11.99	0.16	0.16	0.49	0.15
<i>ATP6V1E2</i>	NM_080653	1.02	0.24	3.00	0.19	0.12	0.50	0.12
<i>SPP1</i>	NM_001040060	6.15	0.18	4.17	0.28	0.13	0.59	0.25
<i>PKLR</i>	NM_000298	8.91	0.10	5.04	0.20	0.06	0.85	0.45
<i>C4orf7</i>	NM_152997	3.43	0.24	2.11	0.10	0.20	0.27	0.01
<i>PSME2</i>	NM_002818	4.15	0.12	3.79	0.17	0.09	0.56	0.21
<i>OR10X1</i>	NM_001004477	5.11	0.21	5.04	0.37	0.10	0.49	0.15
<i>FAM9C</i>	NM_174901	4.00	0.07	2.22	0.21	0.14	0.36	0.07
<i>LRRC3B</i>	NM_052953	3.81	0.14	1.08	0.23	0.19	0.25	0.00
<i>KRTAP21-2</i>	NM_181617	3.00	0.45	3.11	0.22	0.26	0.23	0.07
<i>HIST1H2BE</i>	NM_003523	2.39	0.09	2.41	0.20	0.09	0.62	0.29
<i>TTLL3</i>	NM_001025930	7.84	0.08	6.38	0.19	0.09	0.56	0.24
<i>HIST1H4B</i>	NM_003544	3.71	0.15	5.02	0.13	0.12	0.43	0.14
<i>ACVR1B</i>	NM_004302	4.09	0.19	3.81	0.19	0.13	0.29	0.03
<i>FXYP6</i>	NM_022003	2.07	0.20	2.01	0.07	0.18	0.24	0.04
<i>NAGLU</i>	NM_000263	5.11	0.13	3.60	0.10	0.16	0.23	0.02
<i>MDH1B</i>	NM_001039845	6.23	0.19	6.36	0.20	0.07	0.61	0.31
<i>OR6Y1</i>	NM_001005189	3.10	0.32	2.08	0.46	0.10	0.34	0.08
<i>HBB</i>	NM_000518	2.32	0.39	2.14	0.47	0.08	0.46	0.17
<i>OTX1</i>	NM_014562	3.57	0.18	2.38	0.18	0.12	0.30	0.05
<i>MBNL1</i>	NM_207292	6.96	0.17	3.75	0.08	0.18	0.18	0.01
<i>IFI27L1</i>	NM_206949	3.14	0.25	2.55	0.11	0.18	0.18	0.01
<i>C18orf55</i>	NM_014177	7.95	0.17	4.68	0.11	0.15	0.24	0.03
<i>RFX3</i>	NM_134428	6.24	0.16	4.47	0.07	0.20	0.16	0.00
<i>HBG2</i>	NM_000184	2.47	0.17	1.46	0.06	0.17	0.17	0.00
<i>FANCA</i>	NM_000135	40.40	0.08	33.13	0.23	0.11	0.62	0.39
<i>HIST1H3C</i>	NM_003531	2.47	0.23	2.05	0.35	0.05	0.72	0.43
<i>TMEM206</i>	NM_018252	2.22	0.16	0.68	0.04	0.17	0.16	0.00

**Table S4:** Population frequencies and mean phenotypes at the focal *EPAS1* SNP

Allele/genotype	Tibetan frequency	Han frequency	Danish frequency	mean hemoglobin concentration	mean erythrocyte count	mean oxygen saturation
C	0.13	0.9125	1	n/a	n/a	n/a
G	0.87	0.0875	0	n/a	n/a	n/a
CC	10	n/a	n/a	178	5.3	87.5
CG	84	n/a	n/a	178.9	5.6	86.68
GG	272	n/a	n/a	167.5	5.2	86.42

**Table S5.** Association testing  $P$  values for the focal *EPAS1* SNP, for the full sample and for each village separately (Dingri and Naqu). For phenotypes with significant  $P$  values, regression coefficients ( $\beta_I$ ), standard errors, and sample sizes (n) for the linear regressions are also given.

Sample	SaO2 $P$	Erythrocyte $P$	Erythrocyte $\beta_I$	Erythrocyte SE	Erythrocyte n	Hemoglobin $P$	Hemoglobin $\beta_I$	Hemoglobin SE	Hemoglobin n
All Tibetans	0.726	0.00145	-0.236	0.0734	314	0.00127	-9.23	2.84	358
Dingri only	0.805	0.00188	-0.284	0.0901	198	0.00458	-9.14	3.19	240
Naqu only	0.467	0.0609	-0.214	0.113	116	0.00166	-13.6	4.21	118

**Table S6.** Population genetic statistics for selected *a priori* candidate genes for altitude adaptation in the Tibetan sample.

Gene	refseq ID	Description	S Tibetan	$\Pi$ Tibetan	S Han	$\Pi$ Han	T <sub>TH</sub>	T <sub>TD</sub>	T <sub>HD</sub>	PBS Tibetan
<i>NOS3</i>	NM_000603	nitric oxide synthase 3 (endothelial cell)	26.07	3.37	17.76	3.22	0.01	0.09	0.06	0.02
<i>HIF1A</i>	NM_181054	hypoxia-inducible factor 1, alpha subunit	13.65	0.73	6.13	0.25	0.02	0.01	0.02	0.01
<i>MB</i>	NM_203377	myoglobin	6.44	2.94	6.27	2.61	0.02	0.04	0.09	-0.02
<i>ACE</i>	NM_000789	angiotensin I converting enzyme isoform 1	23.00	3.94	18.15	3.06	0.02	0.08	0.18	-0.04
<i>CYP11B2</i>	NM_000498	cytochrome P450, subfamily XIB polypeptide 2	13.43	2.83	12.44	2.47	0.03	0.14	0.31	-0.07

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