Type	Ensembl Single	RefSeq Single	UniProtKB Single	Ensembl - RefSeq	Ensembl – UniProtKB	RefSeq - UniProtKB
Alt genome sequence		6	84			
Antisense	7	260	84		96	22
Duplicate (technical)	41			2	1	
IG/TR genes	6		120	33	16	
LncRNA		141	126		1	47
Not in reference	9	160	104		78	3
Other ncRNA		39	40		23	7
Sense overlapping		44				
Pseudogene	2	165	373		101	39
Read-through	38	5		7	358	1
Retroviral gene			26			
Sense intronic	2	31			16	

Table S1. The annotations of genes not classified as coding in all three sets
The table shows the alternative classification for those genes classified as coding by just one or two reference sets. Genes that are not present in other reference sets are labelled as “Not in reference”. Genes annotated, but not in the *reference* set are tagged as “Alt genome sequence”.

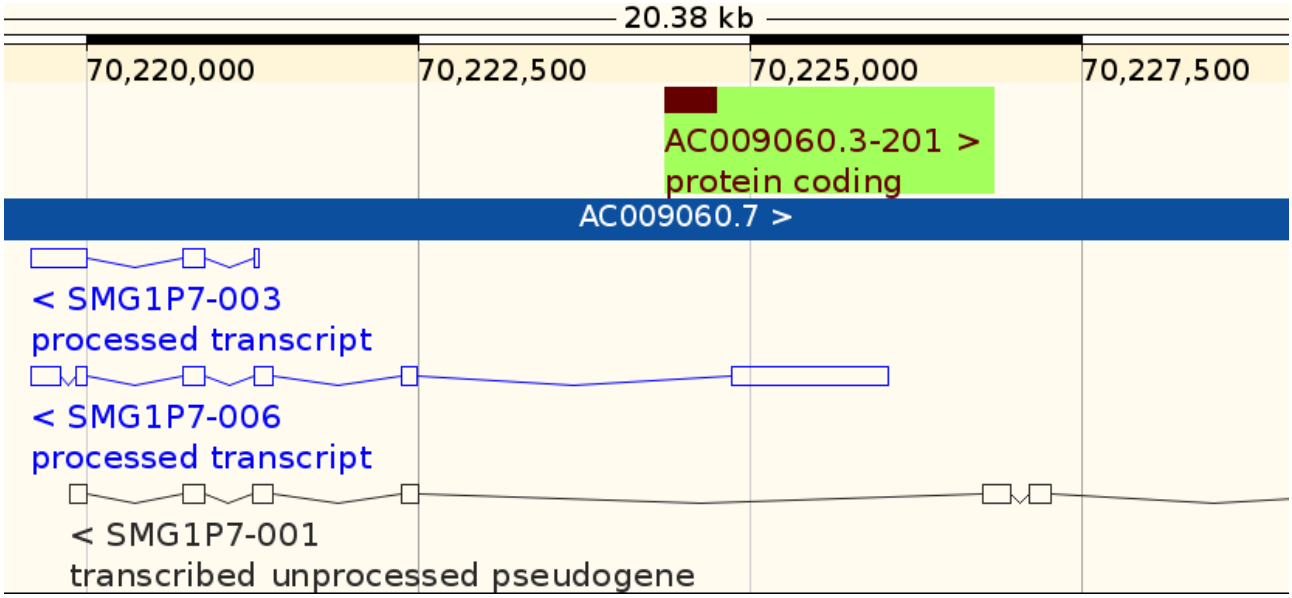


Figure S1. Novel human genes
There are sixteen coding genes that Compara highlights as novel human genes in GENCODE v24. All are single exon genes and are predicted by Ensembl automatic prediction programs. All but one (see *AC009060.3* in the figure) would code for proteins with 124 amino acids. Although many are now "obsolete" in UniProt, some are annotated with the tag "FKSG". UniProt currently annotates 22 FKSG proteins, all with more or less 124 amino acids, while GENCODE v24 annotates 11 of these UniProtKB FKSG genes. None of these novel human genes have their coding status supported by any of the databases or any by reliable peptide or antibody evidence. They do however have 90% identity to "proteins" in *Corethrella appendiculata, Streptococcus pneumonia* and *Bacillus cereus.*

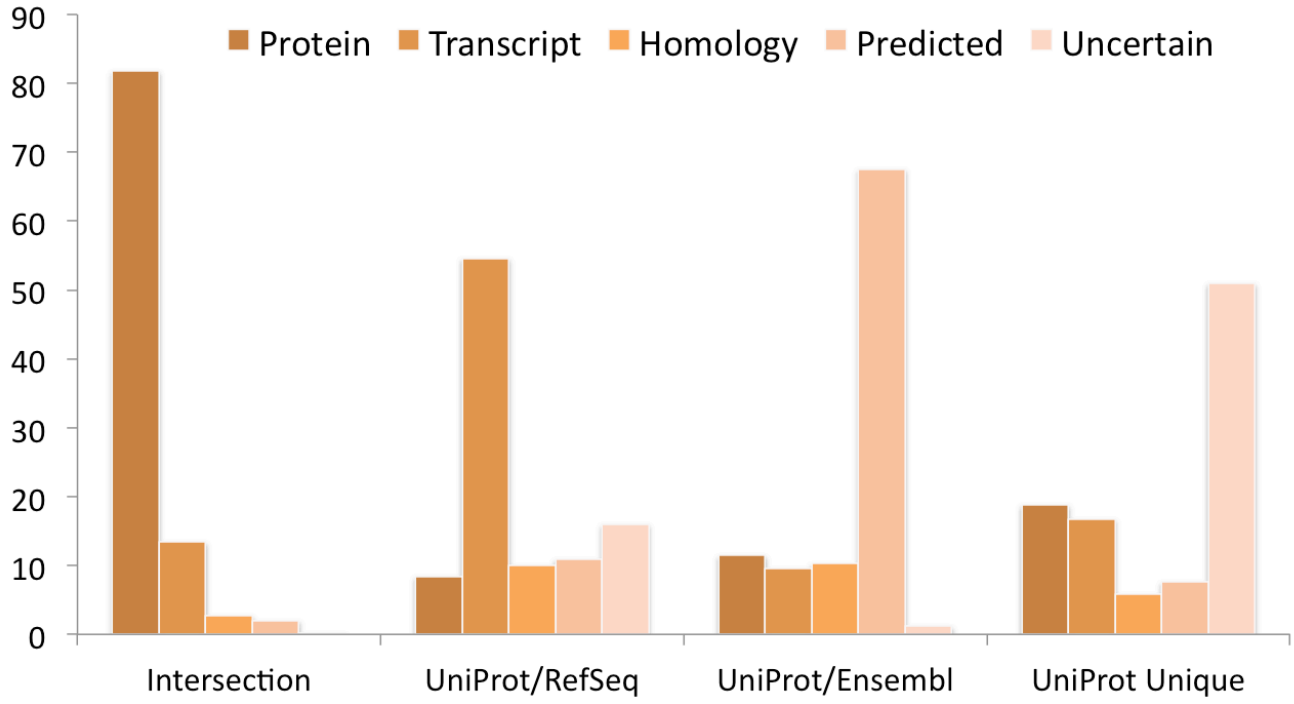


Figure S2. UniProt evidence for genes classified as coding by different sets of manual annotators

The distribution of UniProtKB evidence codes across subsets of UniProtKB genes: those genes that are classified as coding by all three databases (Intersection), genes that are classified as coding by UniProtKB and by RefSeq, genes classified as coding by UniProtKB and by Ensembl/GENCODE, and genes are classified as coding solely by UniProtKB.

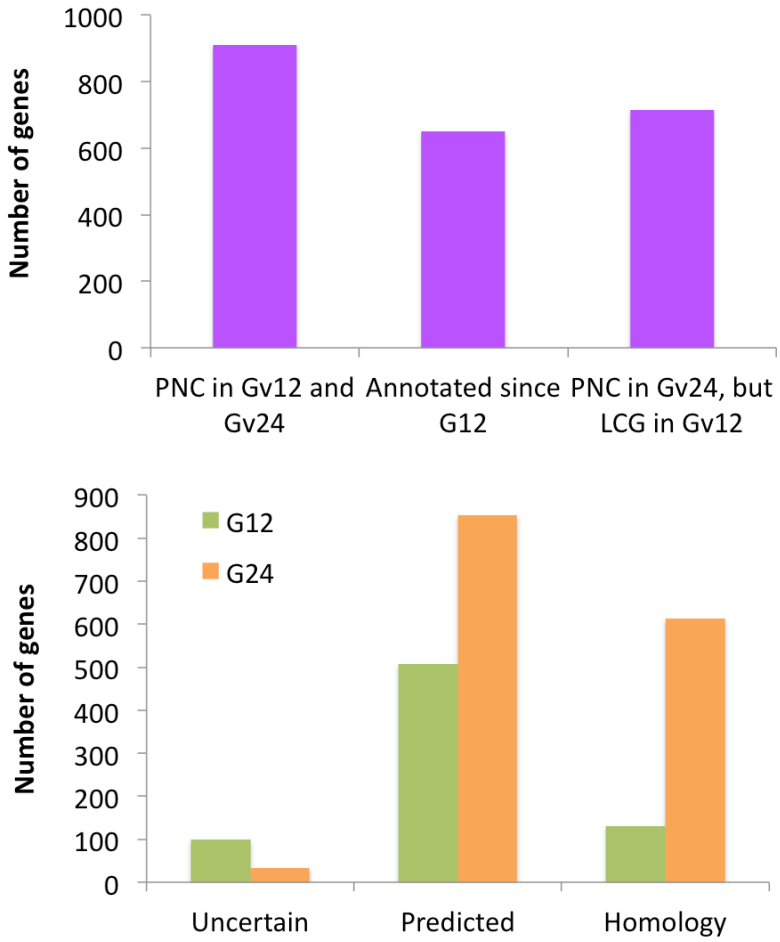


Figure S3. Potential non-coding genes in GENCODE 12 and GENCODE 24
A. The counts of GENCODE 24 potential non-coding genes that were potential non-coding genes in GENCODE 12 too (PNC in Gv12 and Gv24), of GENCODE 24 potential non-coding genes that were likely coding genes in GENCODE 12 (PNC in Gv24, but LCG in Gv12) and of genes that have been annotated in the coding reference set since GENCODE 12. B. The number of genes annotated with protein evidence codes Uncertain, Predicted and Homology by UniProtKB in the GENCODE 12 and GENCODE 24 reference sets. UniProtKB has reviewed the evidence codes for many UniProt entries.

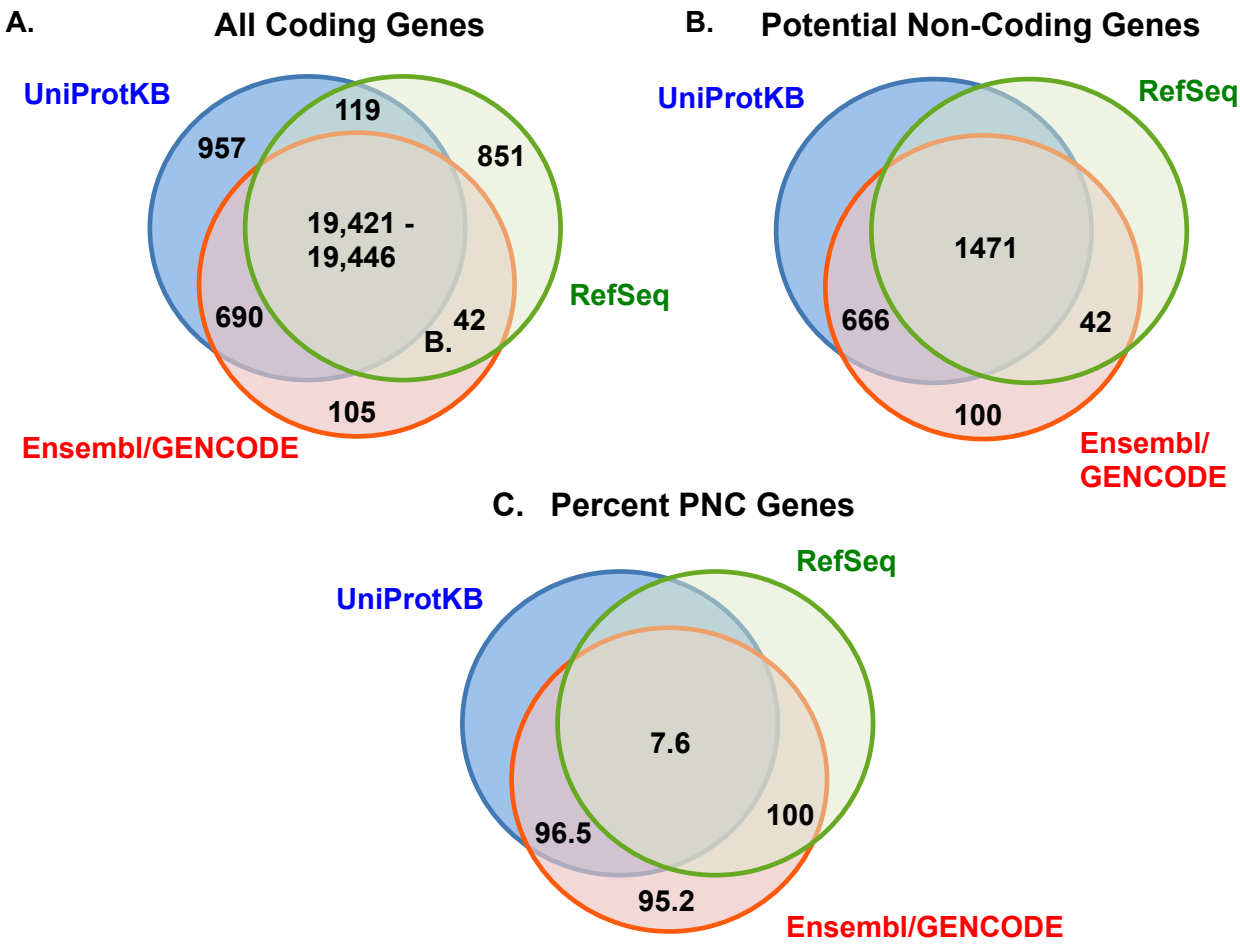


Figure S4. The overlap between genes annotated as coding by three sets of manual annotators

In A the overlap between coding genes in the Ensembl/GENCODE, RefSeq and UniProtKB reference sets. In B the number of coding genes tagged as potential non-coding in the Ensembl/GENCODE reference and how they overlap with the other reference sets. In C the percentage of genes in each of the four relevant sets that are flagged as PNC genes.

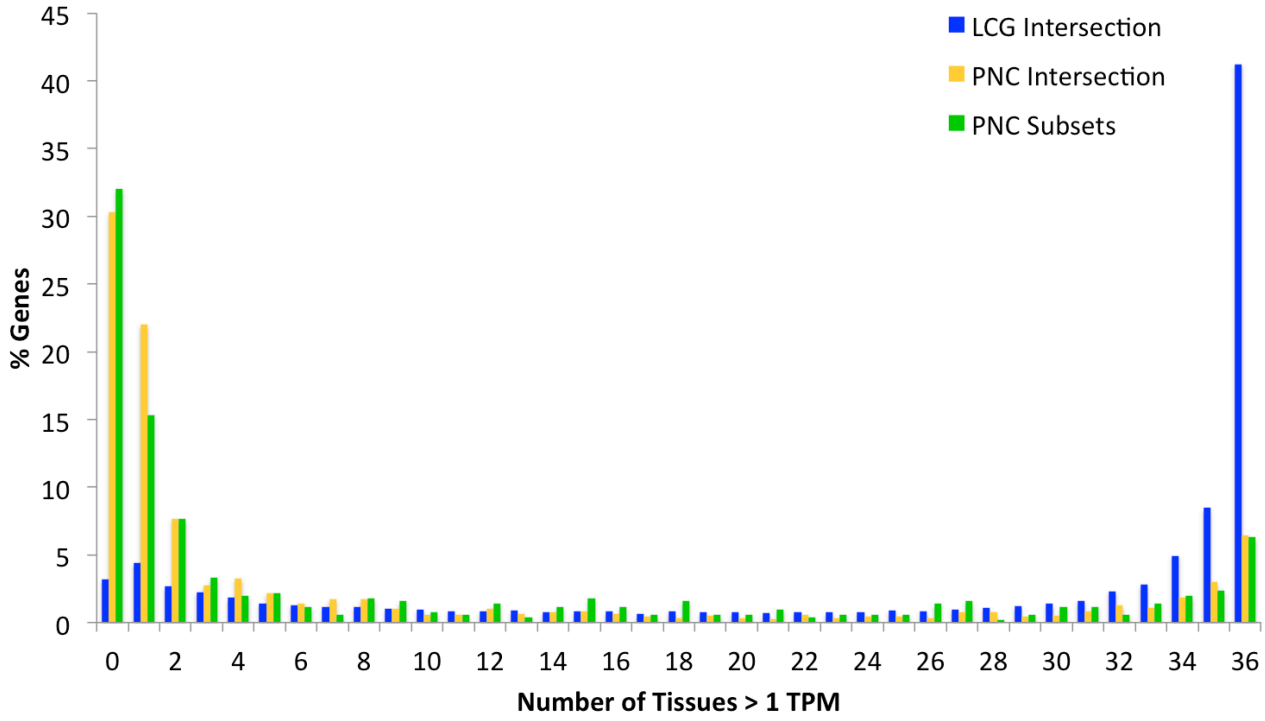


Figure S5. Tissue expression of potential non-coding genes and likely coding genes
Genes binned by number of tissues in which transcripts were detected with a tissue count of more than 1TPM in the 36 tissues of the Human Protein Atlas RNAseq experiments. Tissue distribution shown for the likely coding genes (LCG Intersection), for potential non-coding genes annotated as coding by all three reference sets (PNC Intersection) and for potential non-coding genes annotated by just one or two sets of annotators (PNC Subsets).

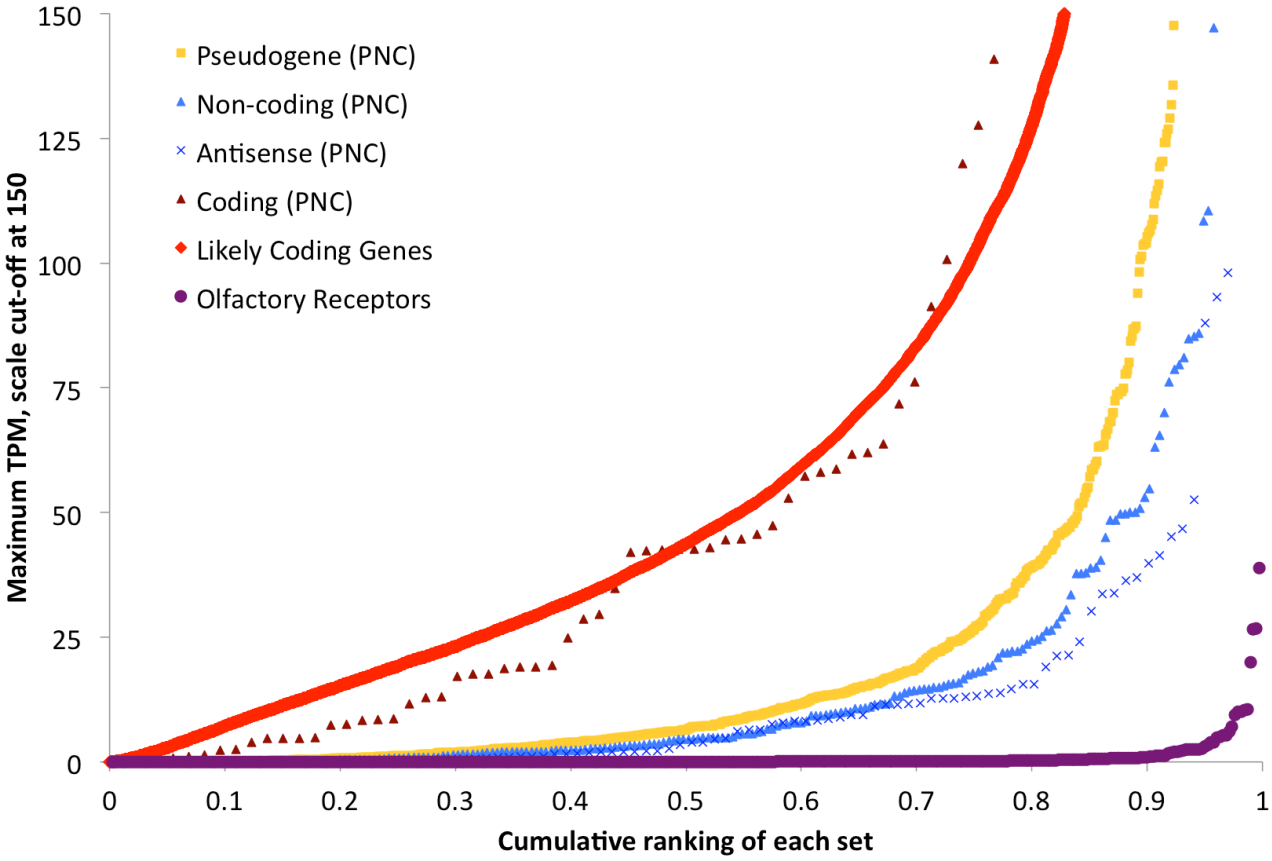


Figure S6. Strength of tissue expression of different types of potential non-coding genes and likely coding genes

For this figure we separated the olfactory receptors form all other genes. Olfactory receptors here are a control set; even if they do code for proteins almost all should be entirely expressed in the nasal tissue, which was not one of the tissues interrogated in the Human Protein Atlas experiment, so any coding from this tissues can be regarded aas either biological or technical noise. Read-through genes and immunoglobulin and –t-cell receptors were left out of the figure for clarity. Remaining potential non-coding genes were split into four groups, those that were clearly “coding” (those for which we had evidence in PeptideAtlas and the Human Protein Atlas), those that were labelled in RefSeq as “antisense”, and the rest were labelled either “pseudogene” or general “non-coding” based on whether we could detect protein-like features such as protein structural or functional domains, or clear cross-species conservation signals. The pseudogene group was the largest subset of potential non-coding genes with 1,003 members. We plotted the maximum tissue expression of these four sets against the olfactory receptors and the set of likely coding genes. Genes in each set were ordered by their maximum TPM from the 36 tissues in the Human Protein Atlas experiments and we plotted the cumulative ranking of the genes in each set against this maximum TPM. The results can be seen in the figure above. The potential non-coding that were tagged as clearly coding (Coding PNC in dark red) have expression distribution that is highly similar to the known coding genes, while the possible pseudogenes, non-coding and antisense genes from the potential non-coding sets generally have much less expression, though most have much more expression than the olfactory receptors.

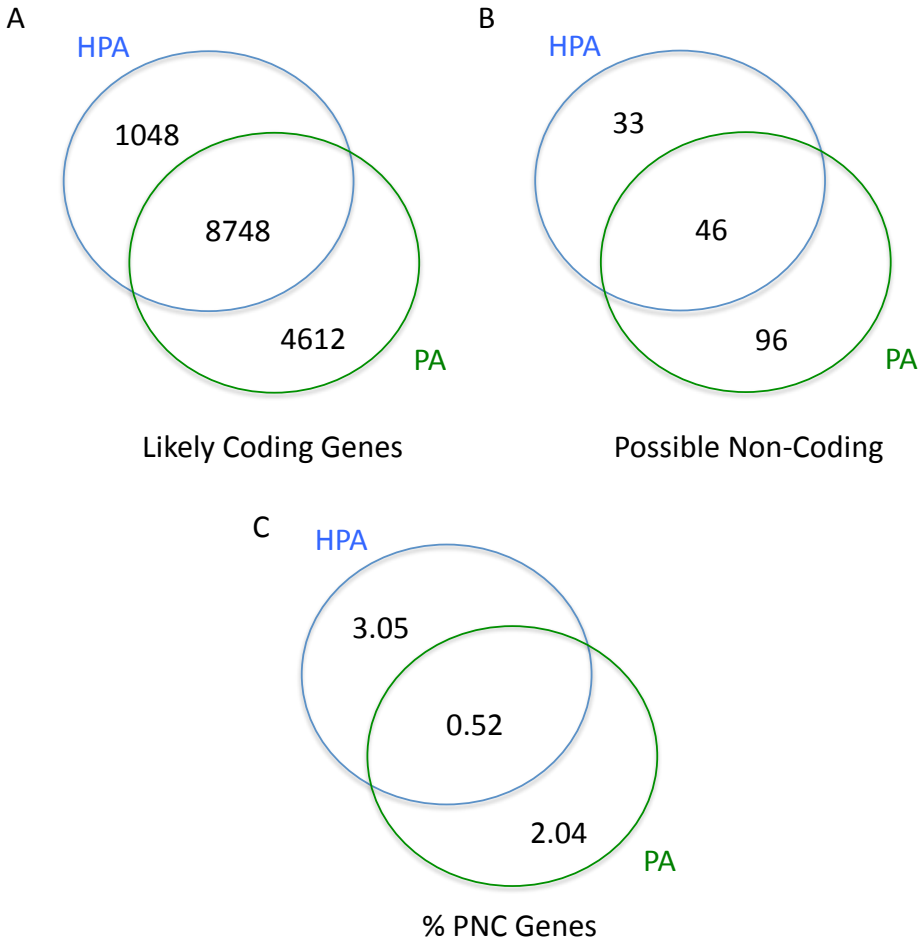


Figure S7. The overlap between three experimental protein detection methods
In A the number of likely coding genes detected in our analysis of the Human Protein Atlas (HPA) and PeptideAtlas (PA) databases. In B the potential non-coding genes detected in the Human Protein Atlas, PeptideAtlas databases. In C the percentage of the genes detected in each experiment that were potential non-coding genes.

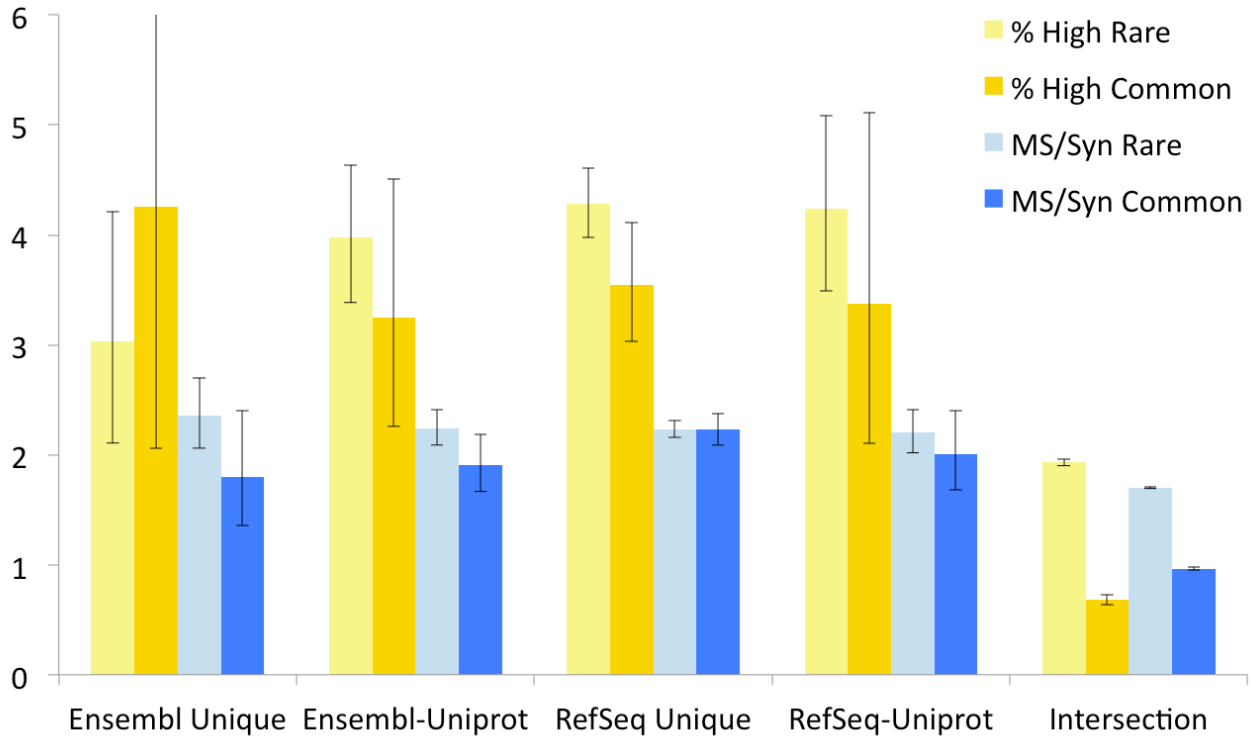


Figure S8. Genomic variation in for subsets of genes in RefSeq and Ensembl/GENCODE
High impact variant percentage (yellow) and non-synonymous/synonymous ratios (blue) for genes classified as coding by all three databases (Intersection), by UniProtKB and by Ensembl/GENCODE only (Ensembl-UniProt), by UniProtKB and RefSeq only (RefSeq-UniProt), by Ensembl/GENCODE (Ensembl Unique) only and by RefSeq only (RefSeq Unique). The darker colours show the values for common variants and the lighter shades show the values for rare variants in each set of bars. 95% confidence intervals are shown.

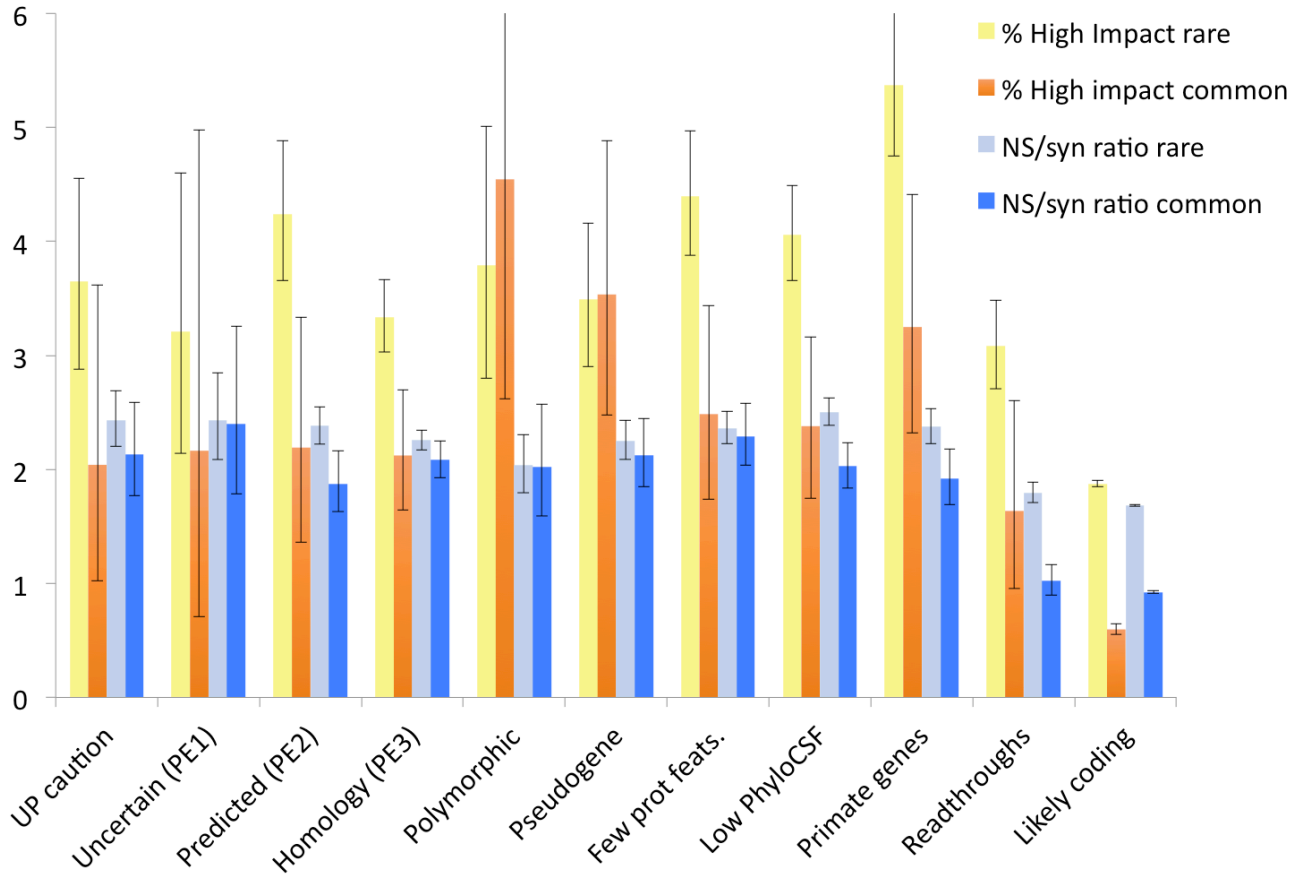


Figure S9 – Human variation and possible non-coding features
The percentage of high impact variants for rare alleles (light yellow) and common alleles (orange) and the non-synonymous/synonymous ratios for rare alleles (light blue) and common alleles (blue) for genes tagged with a range of potential non-coding features. Read-through genes have lower non-synonymous to synonymous ratio in common alleles than in rare alleles and a somewhat lower percentage of high impact variants than the rest of the potential non-coding genes. Since read-through genes are generally composed of exons from two or more coding genes, a certain similarity with likely coding gene variation patterns is to be expected. Read-through genes were excluded from the other sets of potential non-coding features. Where there were fewer than 200 common variants in the genes that had a potential non-coding feature, this feature was excluded from the figure.

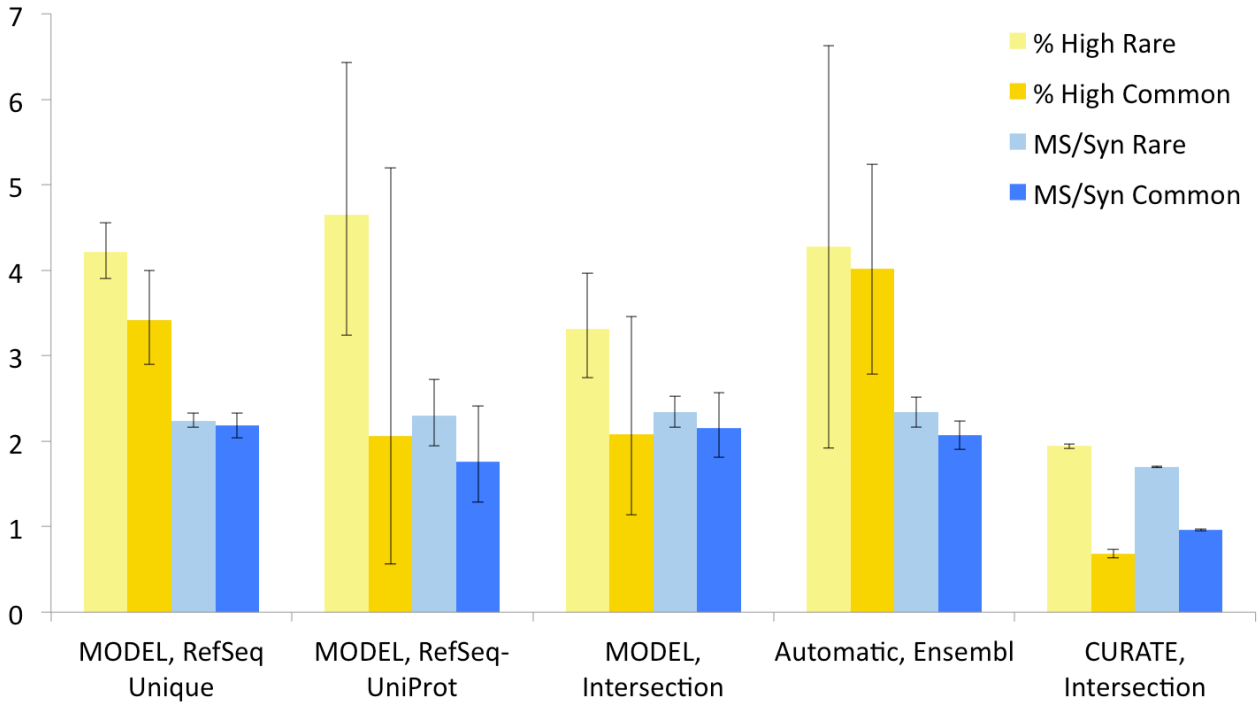
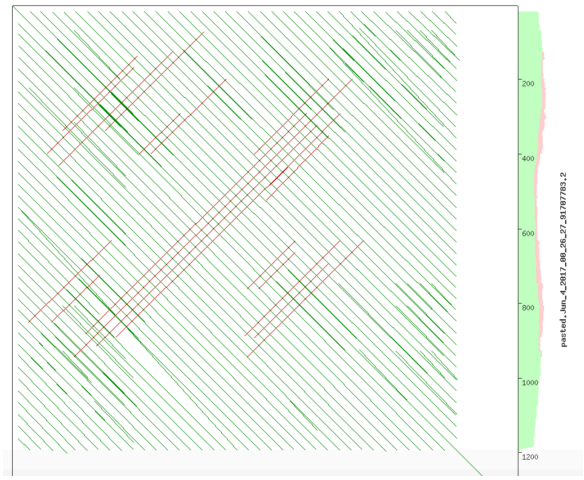


Figure S10. Genomic variation by RefSeq gene type
The percentage of high impact variants (yellow) and non-synonymous/synonymous ratios (blue) from the variants in the 1000 Genomes project for genes with RefSeq type “MODEL” (automatically predicted genes) that were unique to RefSeq (MODEL, RefSeq Unique), with RefSeq type “MODEL” and classified as coding in RefSeq and UniProtKB (MODEL, RefSeq-UniProt), with RefSeq type “MODEL” and coding in all 3 databases (MODEL, Intersection), along with GENCODE 24 automatically predicted genes and as a comparison RefSeq manually curated genes that are present in all three databases (CURATE, Intersection). The darker colours show the values for common variants and the lighter shades show the values for rare variants. 95% confidence intervals are shown. Once again all sets apart from the CURATED RefSeq genes in the intersection appear to have a large proportion of genes that are under neutral selection.

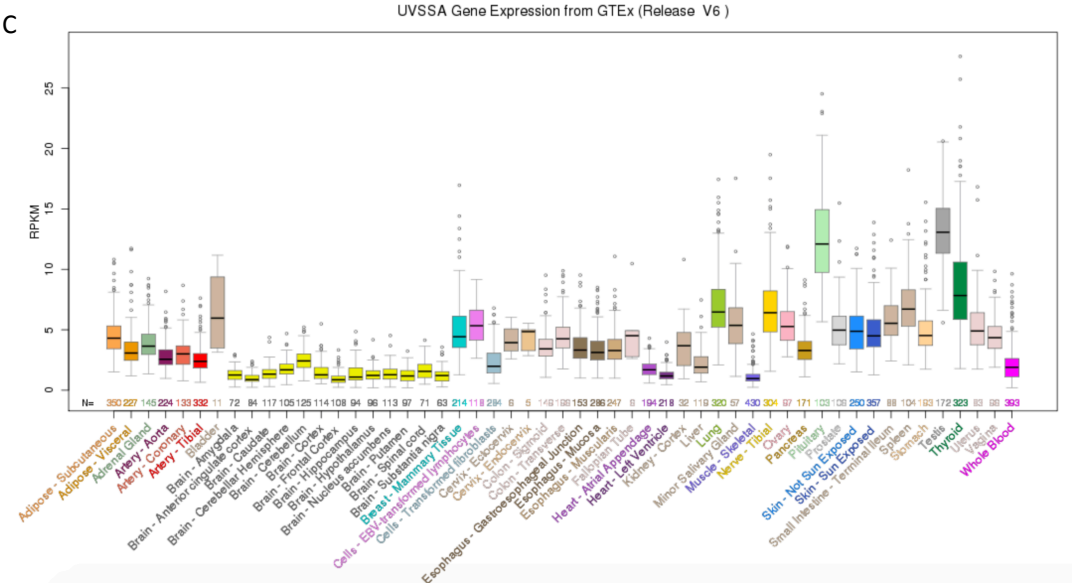
A

```
VECPPAHT--------------------RVH
VECPPAHT--------------------RAH
VECPPAHTCRRGVPACS-----------RAH
MECPPAHTCHCGVPACSHT-------------
VECHLL----------THVPMWSARLLT---
VECPPAHTCPCGVPACS-----------CAH
VECPPAH-----------VPMWSAHLLTCAD
--------CPCGVPACSHVPMRSARLLTRAH
AECPPAHTCPCGVPACSHVPMRSARLLTRAD
VECPPAHTCPCGVPACSHVPTWSARLITRAH
VECPPAHTCPCGVPACSHT-------------
--------CPCGVPACSH--------------
VECSPAHTCRCGVPACSHVPMWSVRLLTRAD
VECPPAHTCRCGVPACSHMPMWSARLLTRAH
AECPPAHTCRCGVPACSHVPMWSARLLT---
--------CRCGVPACSHVPMWSARLLT---
--------CRCGVPACSHVPMWSARLLTRAH
--------CRCGVPACSHVPMWSARLLTRAH
```

B



C



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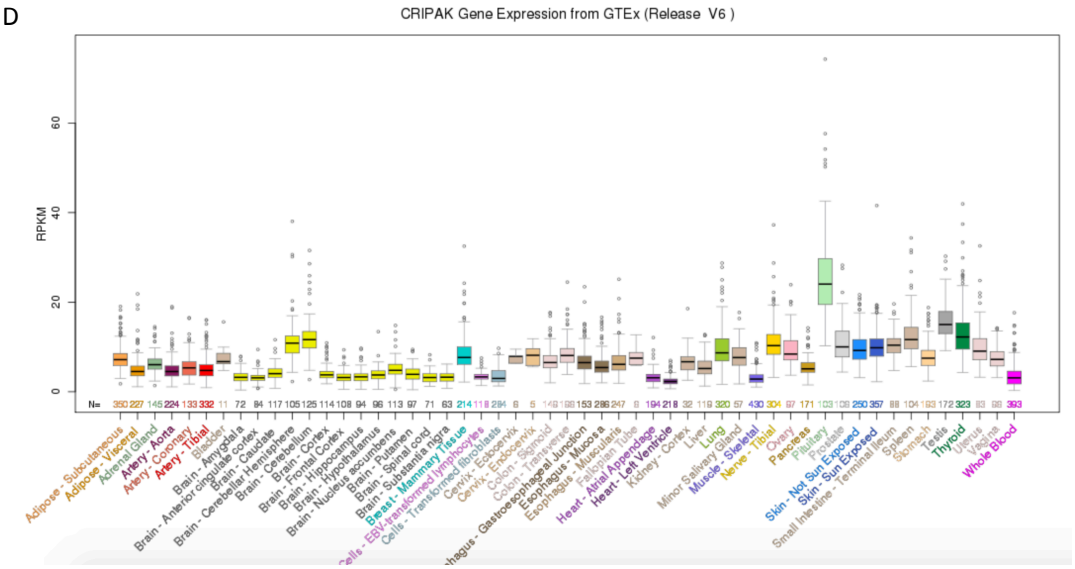
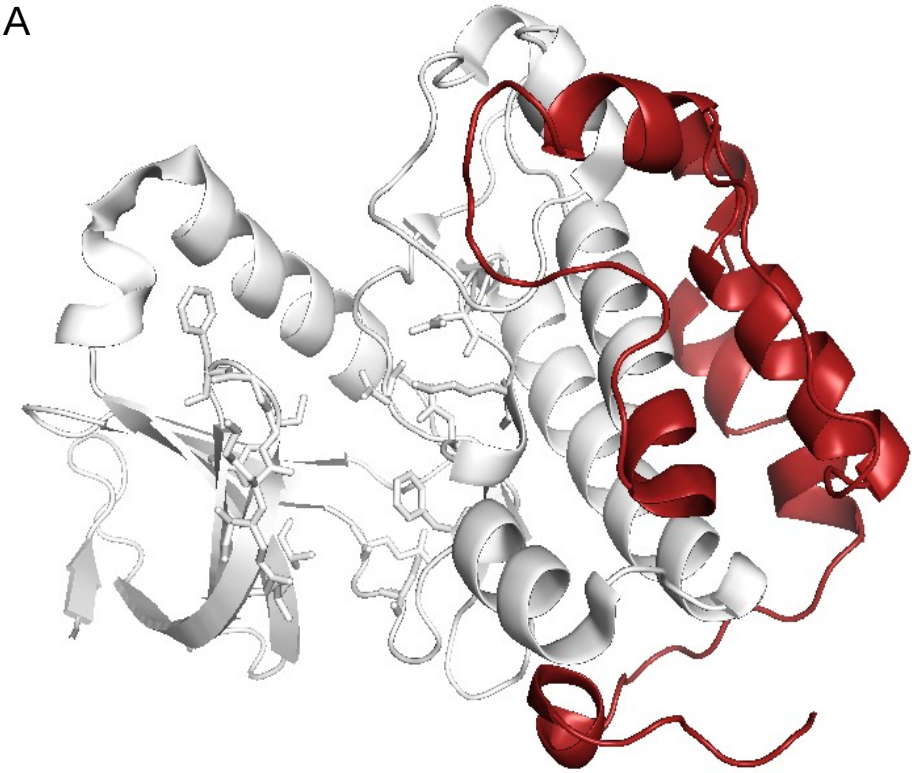


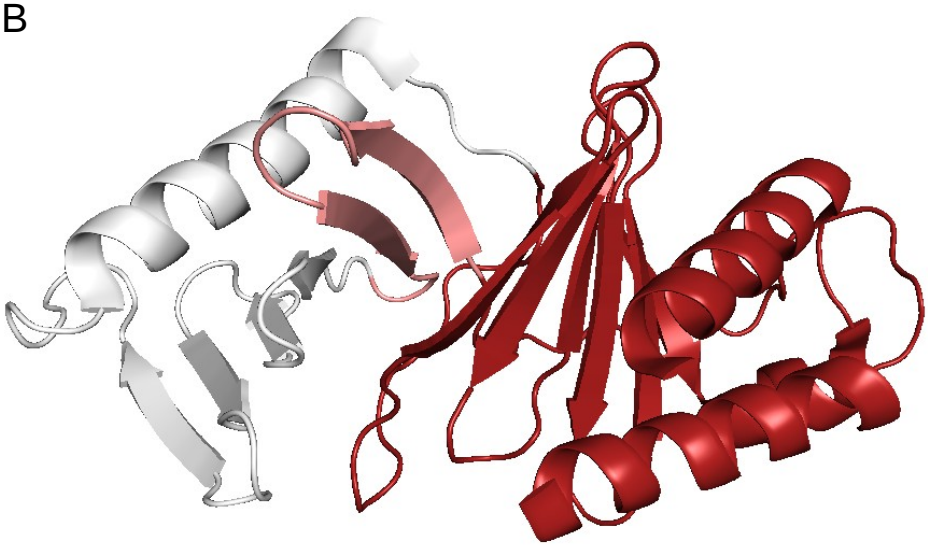
Figure S11. Data supporting the non-coding status of *CRIPAK*
In A the alignment of the "zinc-binding" motifs in *CRIPAK*. In B the dot plot for *CRIPAK* showing the repetitive nature of the sequence. In C the expression patterns of neighbouring gene *UVSSA* from the Human Protein Atlas. In D the expression patterns of *CRIPAK* from the Human Protein Atlas. The *CRIPAK* and *UVSSA* expression patterns are highly similar suggesting a relation or confusion between the two genes.

Figure S12. The *DLEU1* gene in Ensembl83
Transcripts annotated for the *DLEU1* locus in the Ensembl browser taken from the Ensembl 83 archive (Ensembl 83 is the contemporary to GENCODE v24). Only one transcript (DLEU-033 in gold on the left of the picture) was protein coding, though DLEU-034 is a nonsense-mediated decay transcript.

A



B



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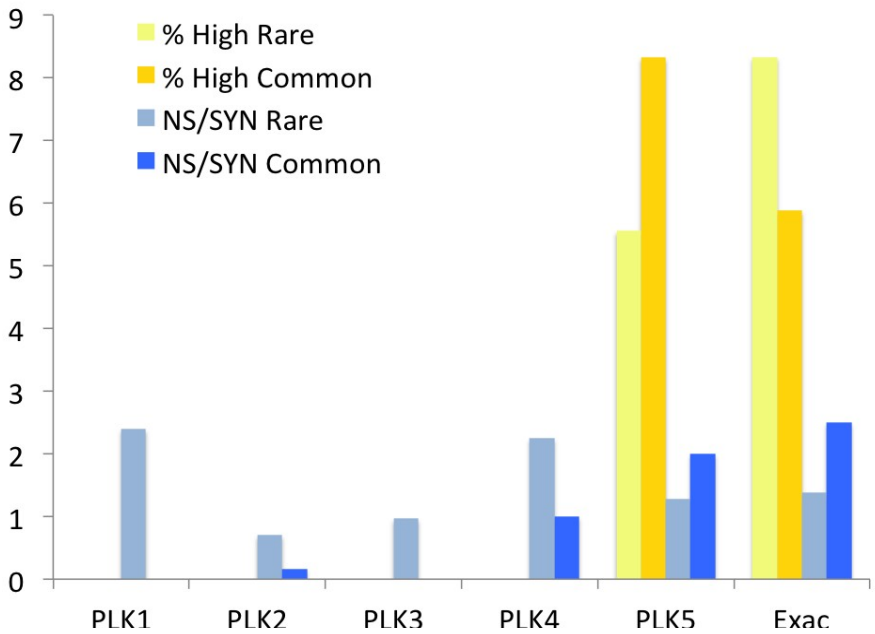
% High Rare
% High Common
NS/SYN Rare
NS/SYN Common
9
8
7
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PLK1
PLK2
PLK3
PLK4
PLK5
Exac

Figure S13. *PLK5* damaged functional domains and genetic variation
Polo-like kinase 5 (*PLK5*) is one of five human polo-like kinases, proteins that a characterized by an N-terminal kinase domain and two polo-box domains. In human *PLK5* all three domains are damaged and two of the three domains have lost whole exons. Human-specific loss of coding exons is a very strong suggestion that human *PLK5* is a classical unitary pseudogene. However, *PLK5* is classified as coding because of a study on the role of mouse *Plk5* that detected antibodies for human *PLK5* [**1**]. Variants from genome-wide variation studies show that while other polo-like kinases have no high impact variants and very few non-synonymous variants, *PLK5* appears to be under very different selection pressures. There are high impact common allele variants for *PLK5* in both the 1,000 Genomes and ExAC [**2**] studies and non-synonymous/synonymous ratios are higher for common alleles than they are for rare alleles. These are small numbers of variants, just 12 common variants in 1,000 Genomes and 17 in ExAC, but they suggest that *PLK5* is not subject to selective pressure and that *PLK5* is probably not functional, even if it does code for a protein as some evidence suggests. In A: the structure of the human *PLK2* kinase domain (PDB code: 4i6h) with the region missing in human *PLK5* shown in white. B. The structure of the second human *PLK2* polo-box domain (PDB code: 4xb0) with the human-specific loss in *PLK5* in white. The important binding strands are shown in pink in the centre; although not lost, these residues will not fold correctly with half the structural domain missing. C. The percentage of high impact variants (yellow) and non-synonymous/synonymous ratios (blue) for the PLK gene family. To confirm the results from the 1000 Genomes for *PLK5*, we have also included the *PLK5* variant proportions from the ExAC consortium. The darker colours show the values for common variants and the lighter shades show the values for rare variants.

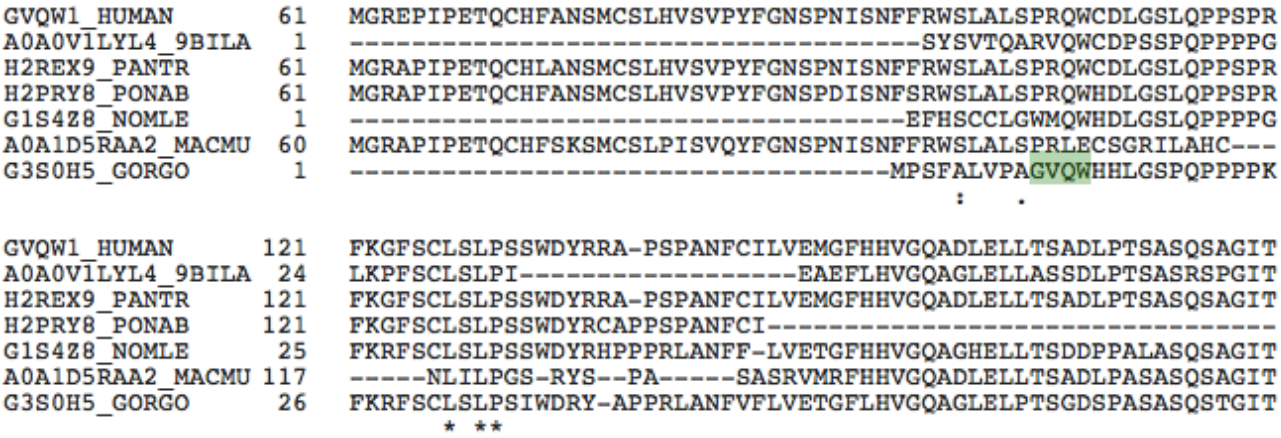

Figure S14. Alignment of *GVQW1* with nearest relatives from UniProtKB
The partial alignment between human *GVQW1* gene product and primate genes annotated as *GVQW1* in UniProtKB. There is little evidence of conservation considering how close the species are in evolutionary time. Only one of the six proteins (G3S0H5_GORGO) has the GVQW motif (shown in green).

Figure S15. Alignment of the 9 UniProtKB proteins used to identify novel coding genes in the CHESS database

We aligned just the most similar regions of each protein. Sequences were aligned with MUSCLE [3]. Seven proteins have a similar C-terminal regions, while there are two different types of N-terminal sequences, one with five sequences (Q8WTZ3, *GVQW2*, Q8N976, *C9orf85*, *LINC00269*) and one with four sequences (*LINC00596*, *C16orf89*, *GVQW1* y UTY_PANTR).

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2. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, *et al*; Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;**536**:285-91.

3. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. 2004;**32**:1792-7.