

Supplementary figure A - *De novo* assembly metrics. Per-(sub)strain distribution of A) largest contig size, B) N50, C) amount of contigs and D) total contigs length.



Supplementary figure B - The effects of GC-shift and low coverage on the results of novo assembly. Red dots denote the low (<20X) coverage strains: BBDP/Wor, LEW/NCrIBR, SR/Jr, SS/Jr WKY/NHsd and SBN/Ygl. The blue dot is sequenced on Illumina GAII (SHR/Olalpcv).



## Supplementary figure C - Functional classification of putative ORFs.

Panther 9 output of the putative ORFs: the molecular functions "catalytic activity" and "binding" contribute to more than 50% of the found GO-terms. Metabolic processes are the largest biological process in the GO-terms.



Common (M.Musculus)

Common

**Supplementary figure D - Organism-distribution of orthologs.** Based on the found orthologs of OrthoMCL, the majority of found ortologs is found in rodents.



**Supplementary figure E - Klotho beta (KLB).** Large amounts of overlap are visible in both the DNA- and Protein-alignment **A)** Alignment of *Mus musculus* KLB-mRNA and the countigs of WAG/Rij, SHR/NCrIPrin,WKY/NCrI,SHR/OlaIP-cv and F344/Nhsd. The red line denotes coverage (>10X) of SHR/OlaIPcv-liver RNA-seq data. **B)** Predicted proteins, superimposed on mouse KLB structure prediction: Magenta colouring shows areas with high amounts of overlap.