**Supplementary Figure and Table Legends**

**Supplementary Fig. 1. Alignment of Olduvai-associated pG4 motifs in human genome.**

DNA sequence alignment is shown for 70 copies of the pG4 motif in the human genome and specific sequence variations are indicated. The *NBPF* gene and specific Olduvai subtype to which each pG4 corresponds is indicated at the left.

**Supplementary Fig. 2. Alignment of introns involved in NAHR events.** Grey regions indicate sequence that is identical to the intron of CON3 from *NBPF12 (*shaded with a long horizontal bar in the figure). Colored vertical lines represent nucleotide differences from that sequence. The introns of amplified HLS1 sequences (labels shaded purple) resemble those of CON3 sequences (labels shaded red) on their 5’ end and transition to resembling the introns of ancestral HLS1 sequences (labels shaded blue) towards their 3’ end.

**Supplementary Fig. 3. The effect of varying the required number of supporting molecules on conclusions regarding structural variation in the HLS region of *NBPF* genes.**

Plots show size calls for the HLS region when the minimum number of supporting molecules is varied. Data is shown for thresholds of 2,3,4,6,8, and 14 molecules. The average size of an HLS domain (1570 bps) is used as the minor grid and the average size of an Olduvai triplet (4710 bps) is used as the major grid. Boxed numbers along the bottom of each plot denote the number of pG4-containing triplets in that gene in hg38. The number of samples for which data is shown is indicated following each gene name. If only one allele was detected for a sample, the data point was duplicated so that alleles in homozygous individuals are not underrepresented. The color of the data point denotes whether the gene has a pG4-containing triplet in hg38 (red = yes, blue = no).

**Supplementary Fig. 4.** **The abundance of DNA fragment sizes after incubation with KCl for 36 hours at 37o** **C supports G4 formation under these conditions**. Data points represent the relative quantity of DNA fragments (indicated by relative fluorescent units) of different lengths following incubation with the specified ion and treatment with the single-strand specific endonuclease. Data is shown for three replicates (run 1, run 2, run 3) for the KCl and LiCl (negative control) conditions. Shaded regions denote the expected locations of single stranded DNA during G4 formation. The G-rich strand is expected to have 3 single stranded loop regions (from left to right, loop 1, loop 2, loop 3). Peaks at the far right of the x-axis represent full-length template DNA.

**S1 File. Olduvai annotation in panTro3.**

**S2 File. Olduvai annotation in panTro4.**

**S3 File. Olduvai annotation in panTro5.**

**S4 File. Olduvai annotation in gorGor3.**

**S5 File. Olduvai annotation in gorGor4.**

**S6 File. Olduvai annotation in gorGor5.**

**S7 File. List of 154 samples from 1000 Genomes collection**