Supplementary Figure Legends

Supplementary Fig. 1. Sequence alignment indicates that gp74 from the bacteriophage HK97 functions as an HNH endonuclease. Sequences of gp74 from HK97 and related sequences identified in a pBLAST search were aligned using ClustalW (*1, 2*). Residues involved in metal binding (Asp42, His82) are indicated by a closed circle below the sequence. An arrow is used to identify the catalytic base (His43) and the structural Asn of the HNH motif (Asn73) is indicated by a filled diamond. The CXXC and CXXH motifs are also highlighted. The secondary structure of the HNH motif of I-HmuI (*3*) is indicated in grey, with arrows defining β-strands and cylinder defining the α-helix. The secondary structure of the remainder of the gp74 sequence is represented by a dotted line because we do not have any evidence to suggest that is similar to the secondary structure of I-HmuI.

Supplementary Fig. 2. The genome structure of HK97 and related bacteriophages. Schematic diagrams of regions of the genomes of the bacteriophages HK97, E2, and p2 show conservation of the location of the gp74 (HNH) gene in HK97. Although the gene encoding the gp74 is the last gene in the linear chromosome of HK97, circularization of the genome after injection into the host cytoplasm places the gene for gp74 adjacent to small and large terminase subunits and the portal protein, as seen for E2 and p2.

Supplementary Fig. 3. Gp74-mediated digestion of lambda phage DNA in the presence of Mg^{2+} ions. Agarose gel (1%) electrophoresis analysis of the time course of the lambda phage DNA digestion reactions is shown. Lambda DNA (50 μg/ml) incubated

in 20 mM HEPES, pH 7.0 in the presence of gp74 (48 μg/ml) and Mg^{2+} at varying concentrations as indicated. The smear of DNA in these gels indicates that gp74 digestion double-stranded DNA digestion of λ DNA, at multiple sites, in the presence of Mg^{2+}.

Supplementary Fig. 4. Gp74 only requires stoichiometric amounts of Ni^{2+} ions for activity. Agarose gel (1%) electrophoresis analysis of the time course of the lambda phage DNA digestion reactions is shown. Lambda DNA (25 μg/ml) incubated in 20 mM HEPES, pH 7.0 in the presence of gp74 (11 μg/ml = 0.86 μM) and between one to ten equivalents of Ni^{2+} ions. The smear of DNA in these gels, even with one equivalent of Ni^{2+} ions, indicates that stoichiometric amounts of Ni^{2+} are sufficient to cause digestion of λ DNA by gp74.

References

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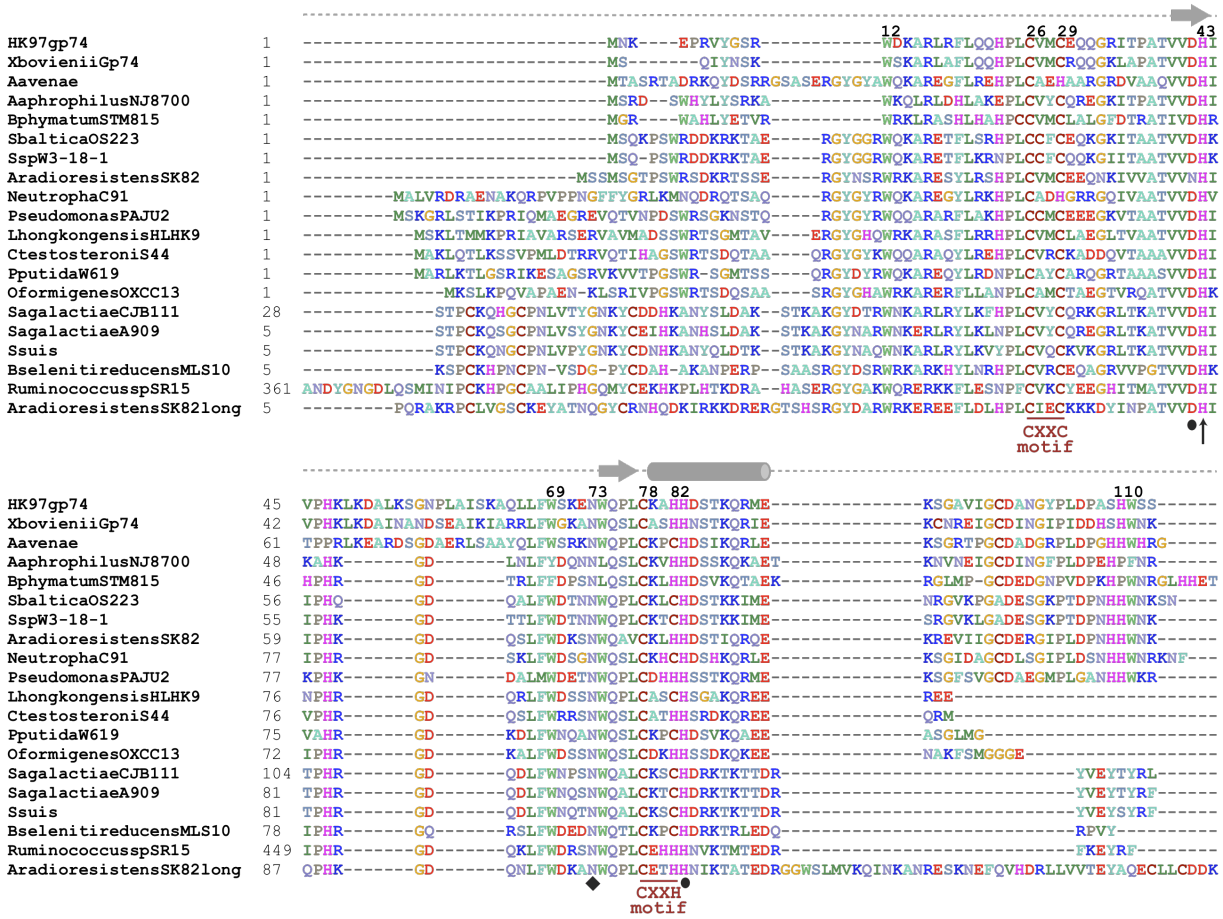

Supplementary Figure 1.
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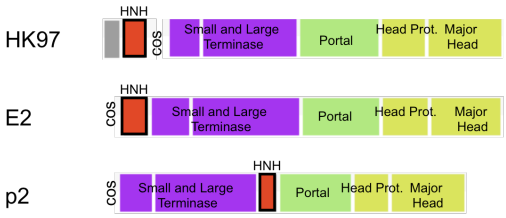

Supplementary Figure 2.
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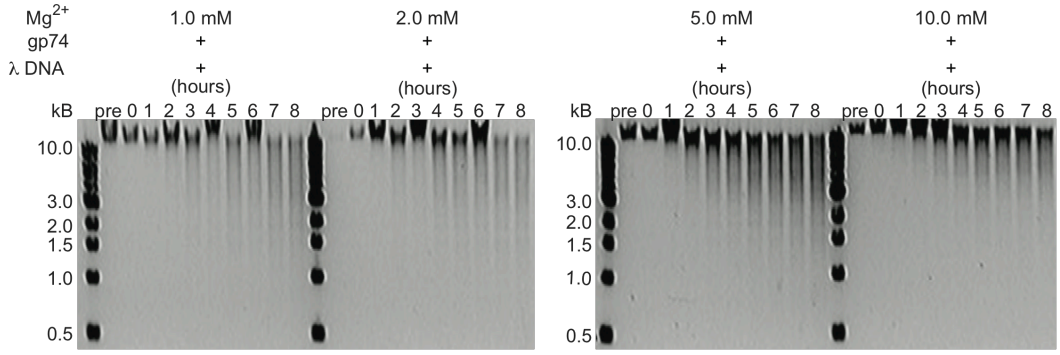

Supplementary Figure 3.
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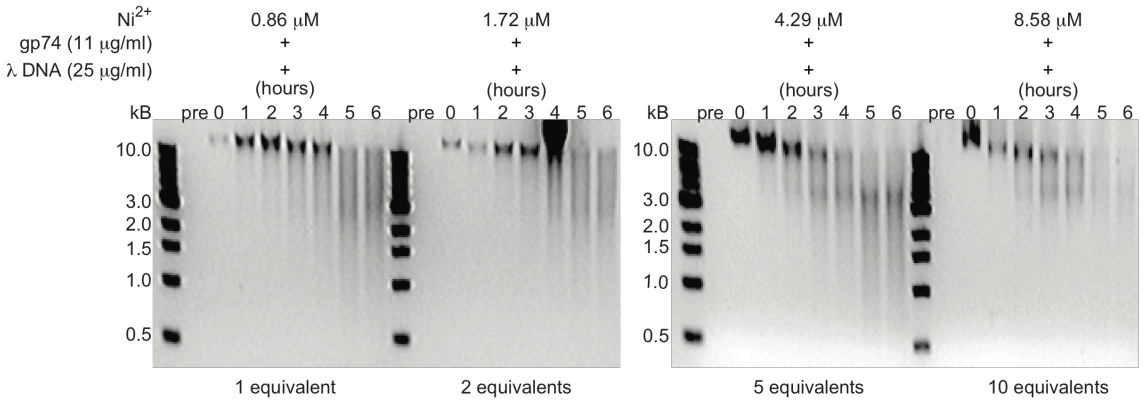

Supplementary Figure 4.
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