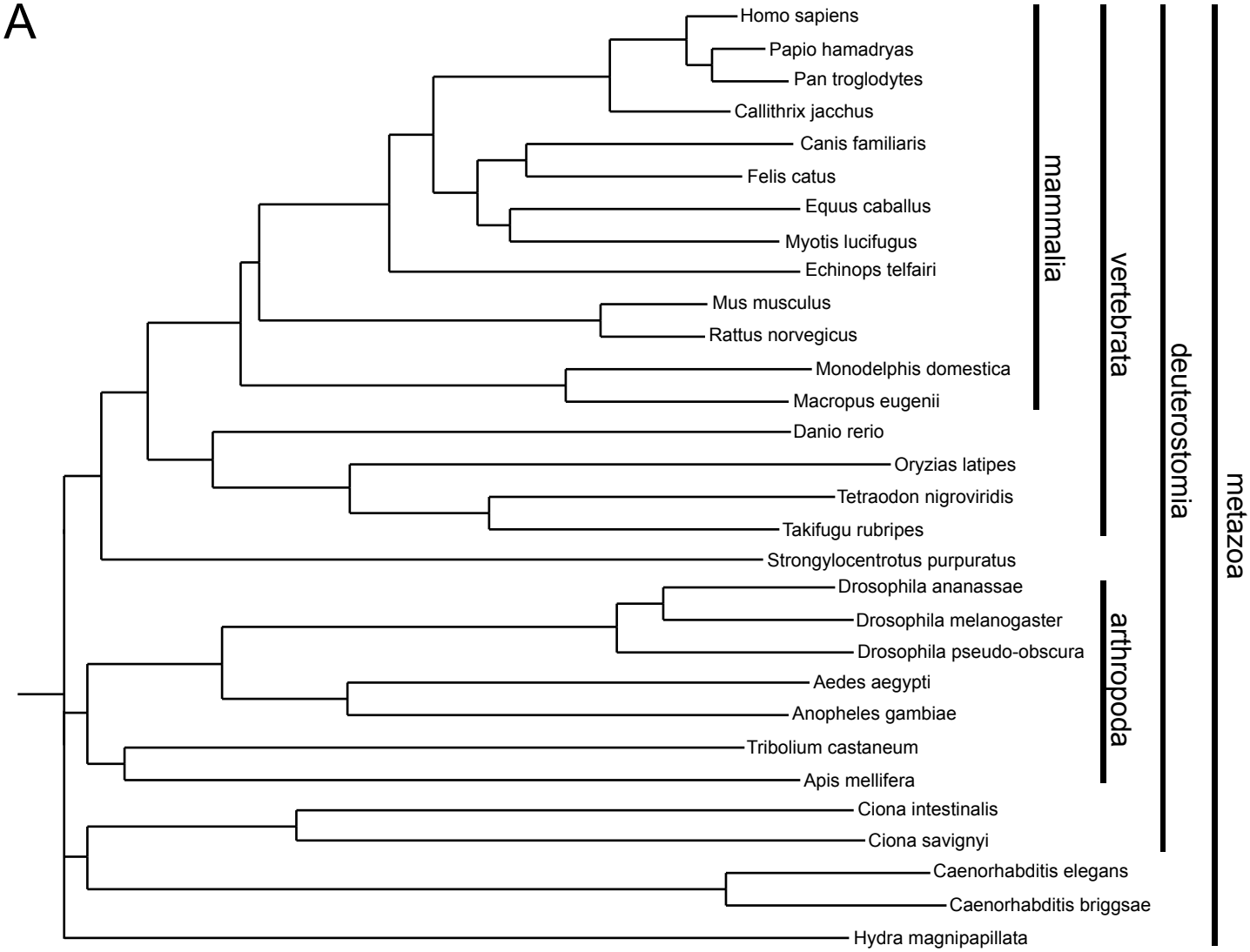
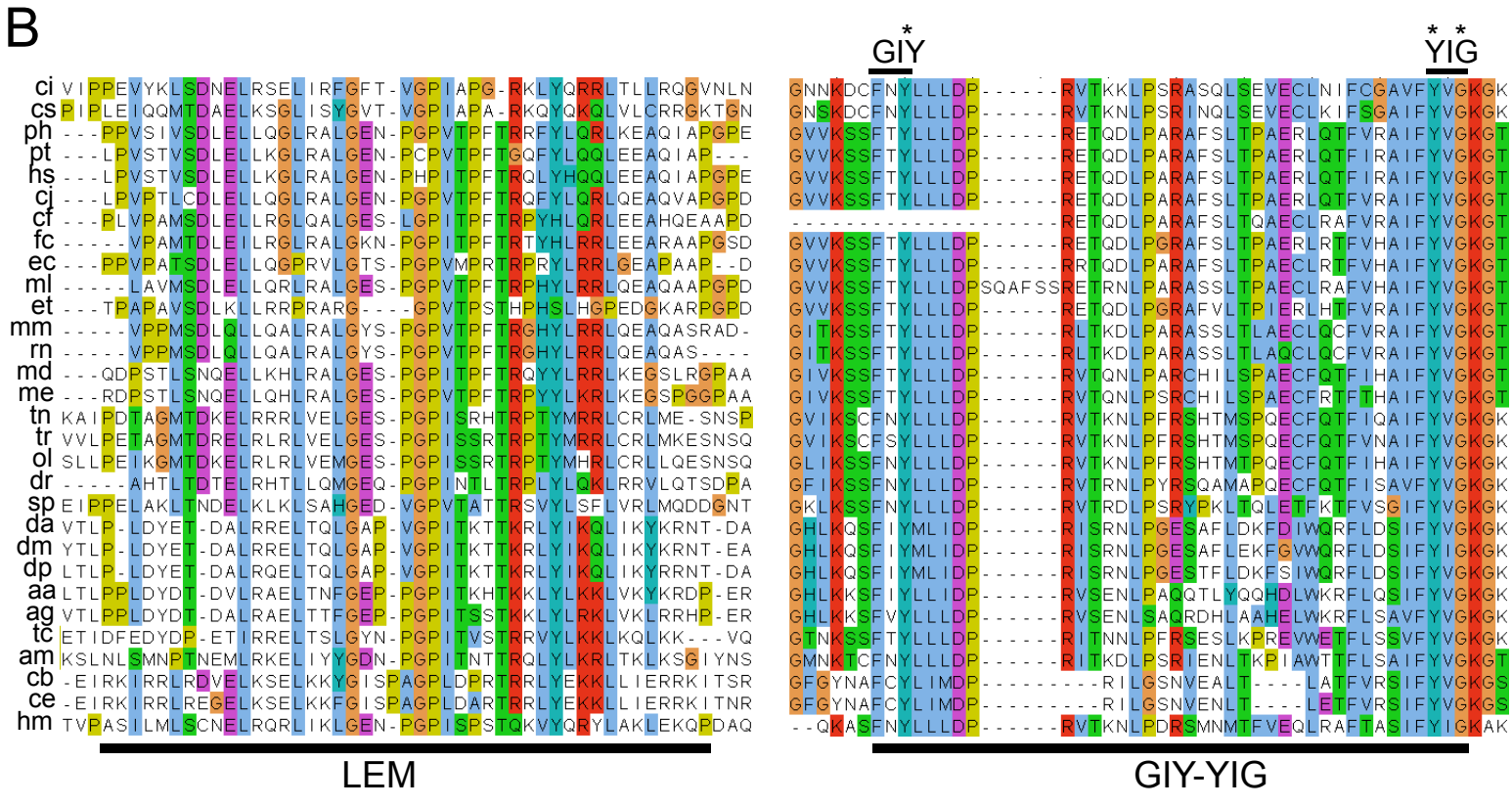
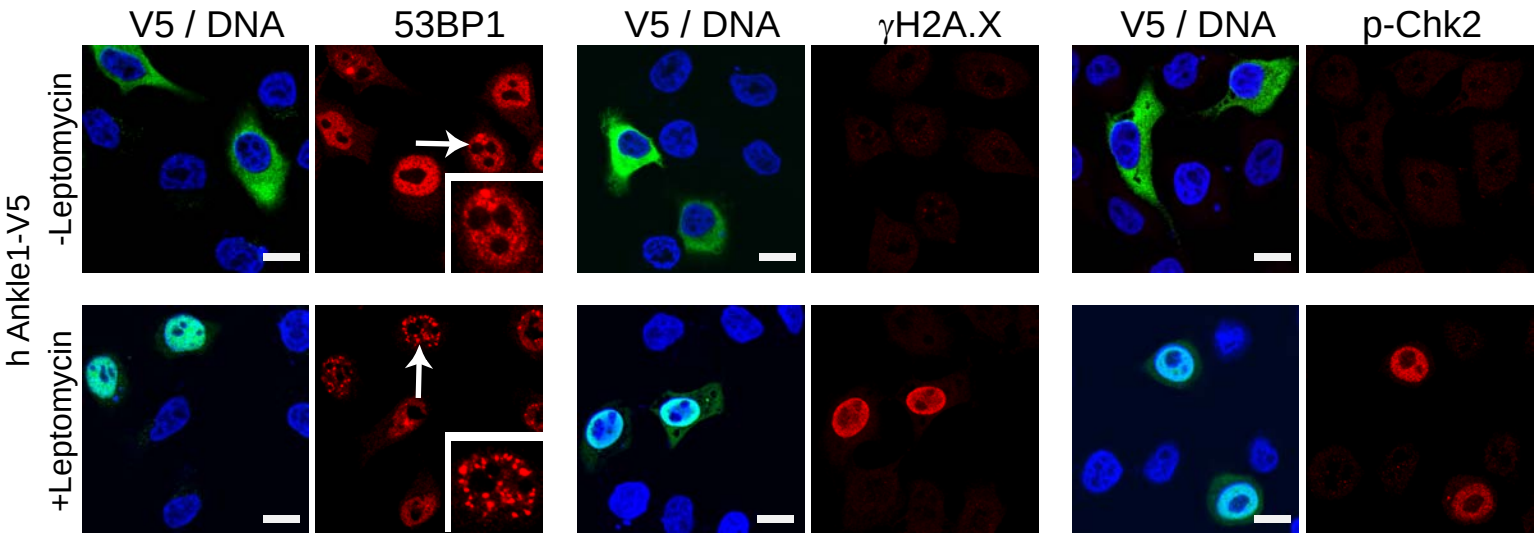
Brachner et al., Supplemental Figure 1



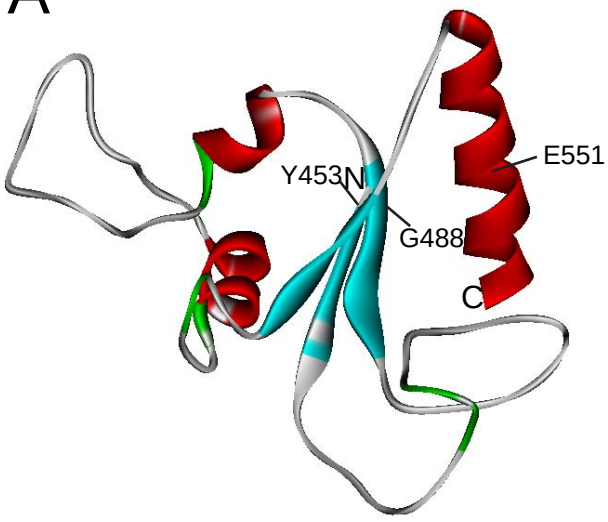


Brachner et al., Supplemental Figure 2

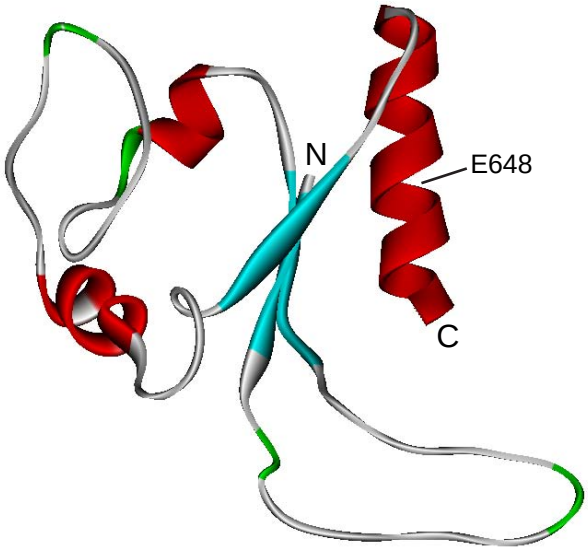


Brachner et al., Supplemental Figure 3

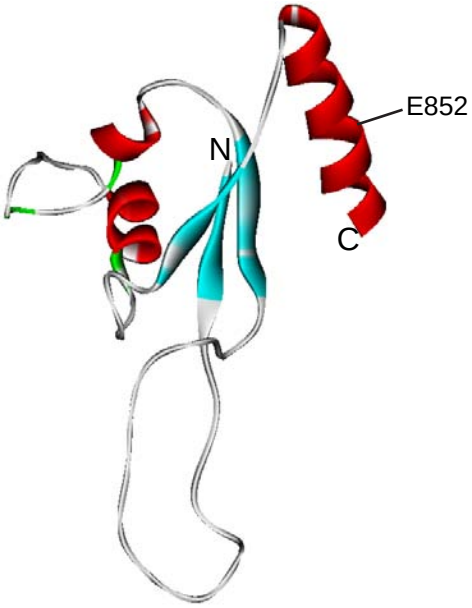
A



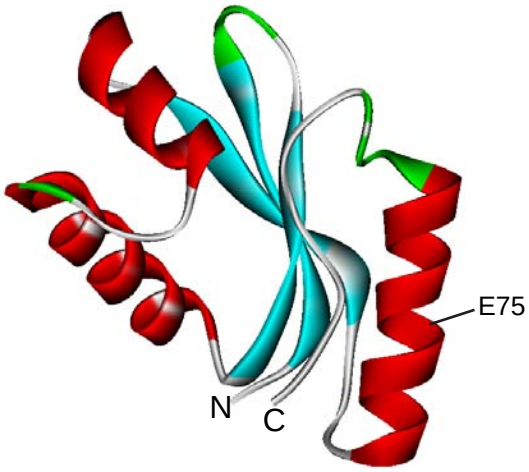
human Ankle1 CT (452-573)



ce Ankle1 CT (558-654)

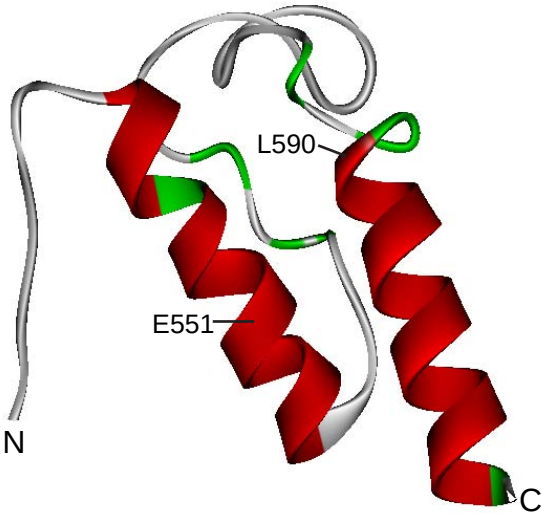


hydra Ankle1 (761-859)



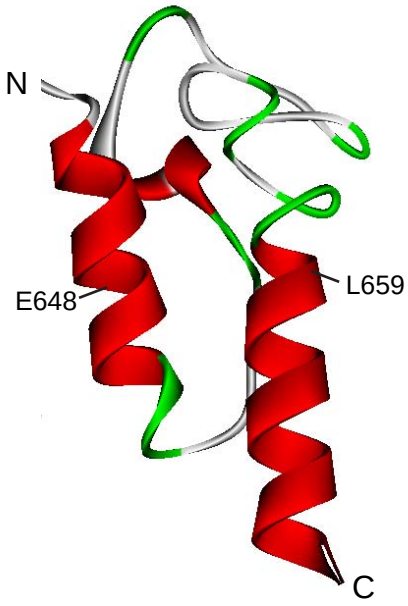
T4 TevI (1-97)

B



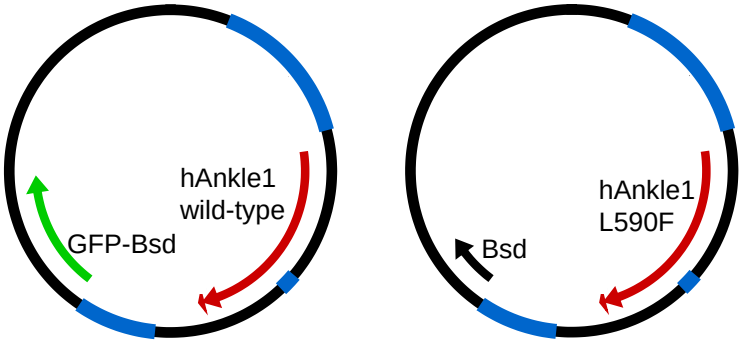
human Ankle1 CT (532-604)

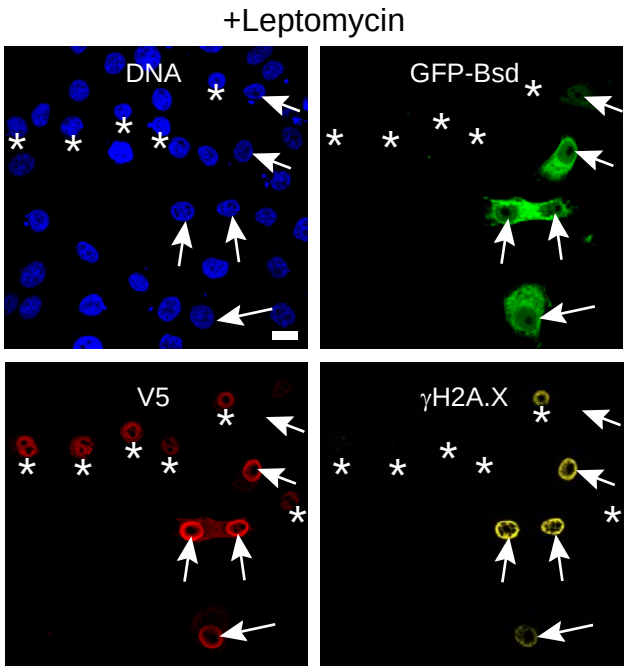
C



ce Ankle1 CT (639-701)

Brachner et al., Supplemental Figure 4





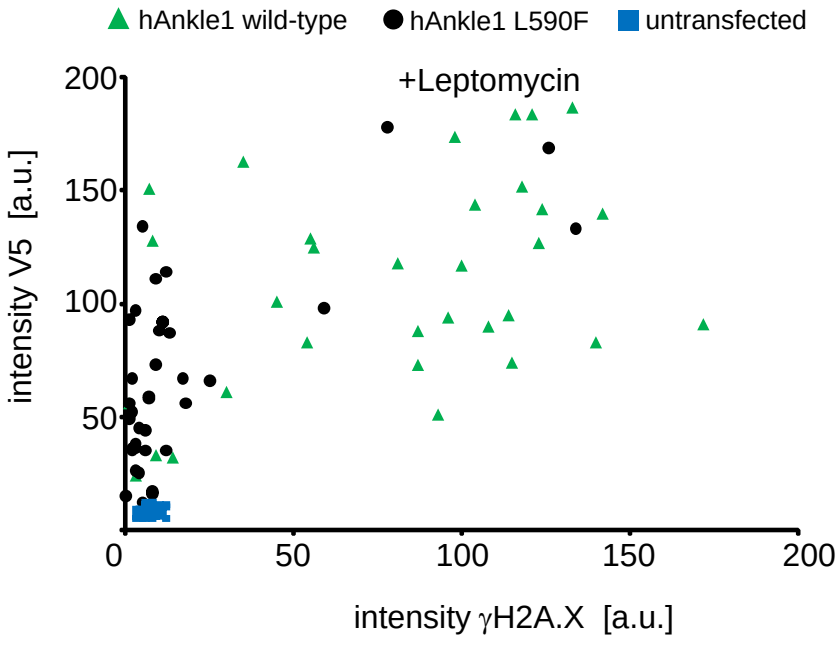


Figure Legends:

Suppl. Fig. 1. Ankle1 is conserved throughout metazoan evolution. (**A**) Ankle1 protein sequences were retrieved from ENSEMBL and NCBI databases or predicted from genomic sequences using in silico gene prediction software. Complete Ankle1 sequences were aligned using ClustalW2 software and phylogenetic prediction data were visualized as a cladogram using Phylodendron software. (**B**) Alignment of Ankle1's predicted LEM and GIY-YIG motifs. Amino acid residue conservation is indicated by ClustalX color code. Invariable residues of the GIY-YIG motif are marked with a *.

Suppl. Fig. 2. Human Ankle1-V5 shuttles between cytoplasm and nucleus and induces DNA damage response. HeLa cells were transiently transfected with Ankle1-V5, treated with Leptomycin and processed for immunofluorescence. Cells were co-stained with anti-V5 antibodies and either 53BP1, γH2A.X or p-Chk2. Arrows indicate nuclei shown in insert. Bars, 10 μm.

Suppl. Fig. 3. In silico modelling of 3D structure of Ankle1 C-terminus. (**A**) C-terminal domains of human, *C.elegans* and hydra Ankle1, and a region in T4 TevI containing the predicted GIY-YIG motifs and ~100 residues downstream were modeled employing the CPH models server (http://www.cbs.dtu.dk/services/CPHmodels). hAnkle1 point mutants used in Figure 5A are indicated. (**B**) Structure models of the very C-termini of human Ankle1 and *C.elegans LEM-3* are depicted, including the conserved mutation L590F in hAnkle1, identified originally in *C.elegans* (L659F). The highly conserved glutamic

acid (E551 in human Ankle1) is indicated in all models. All structural models were visualized using Discovery Visualizer Studio software.

Suppl. Fig. 4. Mutation of a conserved leucine residue in human Ankle1 impairs ability to induce DNA damage response. A previously identified point mutation leading to DNA damage hypersensitivity in *C.elegans* was introduced into human Ankle1, and wild type and mutated Ankle1 were expressed in HeLa cells. V5-tagged wild-type Ankle1 was expressed from a plasmid also expressing a CMV-driven GFP-Blasticidin marker gene (GFP-Bsd), whereas the plasmid encoding the mutated V5-tagged Ankle1 expressed Blasticidin alone (plasmid maps). A mixed culture of cells expressing one of these constructs was treated with Leptomycin, and analyzed by immunofluorescence microscopy using antibodies to V5 (red) and γH2A.X (yellow). DNA was stained with DAPI (blue), GFP-Blasticidin is shown in green. Representative confocal fluorescence microscopic images are shown. Cells marked with asterisks are GFP-negative, hence express Ankle1-L590F, while GFP-Bsd positive cells (arrow) express wild-type Ankle1. Relative fluorescence intensities of V5 and γH2A.X signals within the nucleus of untransfected, GFP-positive and GFP-negative transfected, and Leptomycin-treated cells (n>30 each) were measured and plotted using Graphpad Prism software. Bar, 10 μm.