

Supplementary Materials

Unexpected divergence in magnetoreceptor MagR from robin and pigeon linked to two sequence variations

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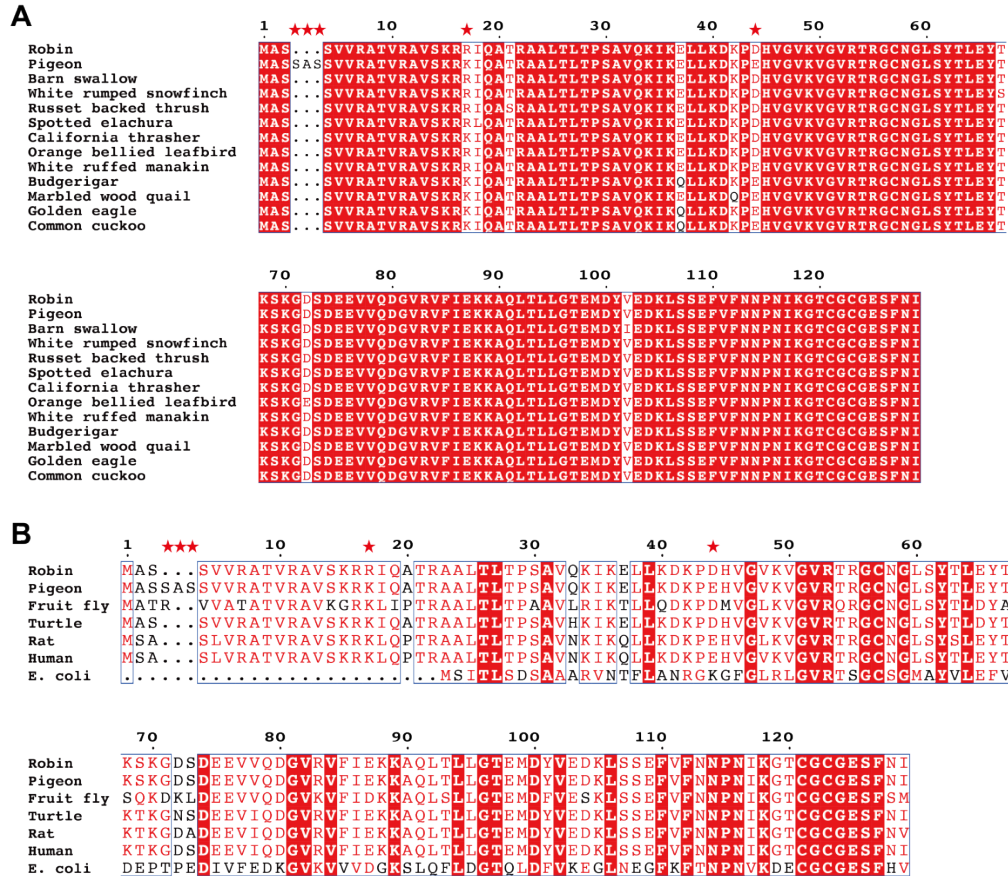
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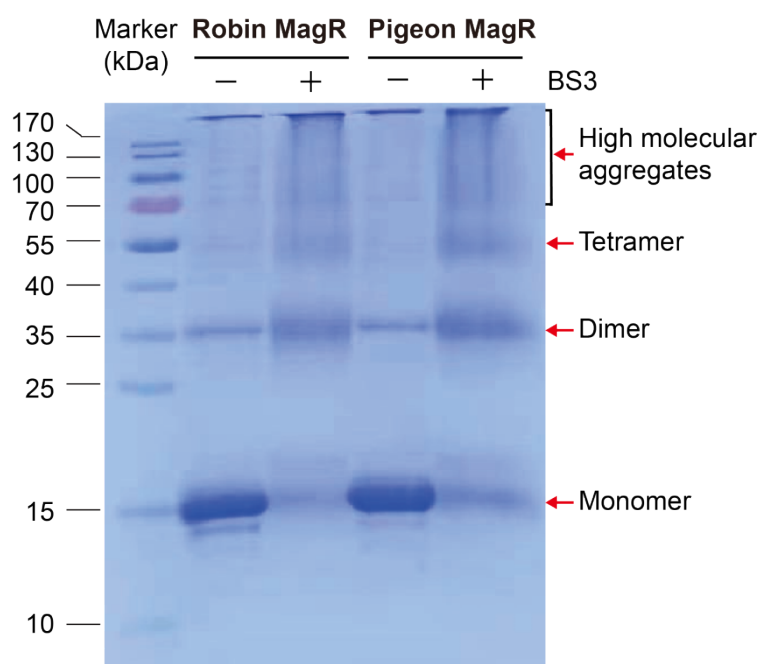
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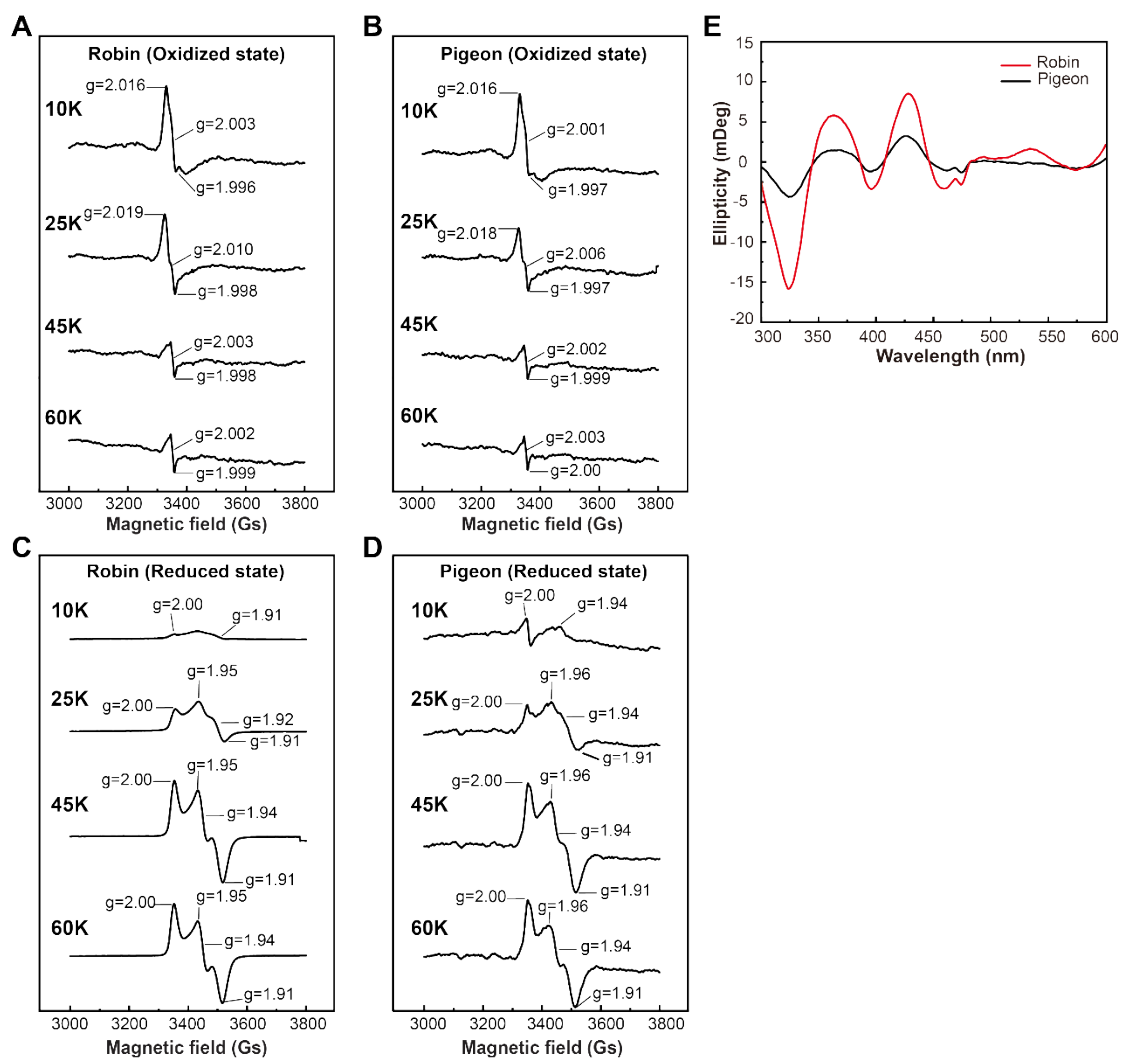
Supplementary Figure S1. Sequence alignment of MagRs from various species.

A: Sequence alignment of MagRs in birds. Fully conserved residues are shaded in red. Residues mutated in this study are marked by red stars. Species common name, Latin name, and sequence ID in NCBI are listed as follows: Robin (*Erithacus rubecula*), Pigeon (*Columba livia*), XP_005508102.1; Barn Swallow (*Hirundo rustica*), XP_039945980.1; White-rumped Snowfinch (*Onychostruthus taczanowskii*), XP_041255423.1; Russet-backed Thrush (*Catharus ustulatus*), XP_032941987.1; Spotted Elachura (*Spelaornis formosus*), NXD29669.1; California Thrasher (*Toxostoma redivivum*), NWS83727.1; Orange-bellied Leafbird (*Chloropsis hardwickii*), NWH32334.1; White-ruffed Manakin (*Corapipo altera*), XP_027490836.1; Budgerigar (*Melopsittacus undulatus*), XP_005154986.1; Marbled Wood-quail (*Odontophorus gujanensis*), NXJ10518.1; Golden Eagle (*Aquila chrysaetos canadensis*), XP_011576125.1; Common Cuckoo (*Cuculus canorus*), XP_009559927.1. **B:** Sequence alignment of MagRs in seven representative species. Fully conserved residues are shaded in red. Residues mutated in this study are marked by red stars. Species common name, Latin name, and sequence ID in NCBI are listed as follows: Robin (*Erithacus rubecula*) NWY76935.1; Pigeon (*Columba livia*), XP_005508102.1*; Fly (*Drosophila melanogaster*), NP_573062.1*; Turtle (*Chelonia mydas*), XP_037757680.1; Rat (*Rattus norvegicus*), NP_853657.1; Human (*Homo sapiens*), NP_112202.2; *E. coli* (*Escherichia coli*), RIL20818.1.



Supplementary Figure S2. Chemical crosslinking assay of robin MagR and pigeon MagR.

Coomassie-stained SDS-PAGE of untreated and BS3 (suberic acid bis (3-sulfo-N-hydroxysuccinimide ester) sodium salt)-treated cross-linked MagRs are shown. Arrowheads depict detected signals of MagR monomer, dimer, and tetramer as well as higher molecular aggregates.



Supplementary Figure S3. Type of iron-sulfur clusters in robin MagR and pigeon MagR.

A-D: X-band EPR spectra of as-purified robin MagR (**A** and **C**) and pigeon MagR (**B** and **D**) at oxidized (top) and reduced (bottom) states. Spectra were collected at various temperatures (10, 25, 45, and 60 K), 2 mW power, and 9.4 GHz. **E:** CD Spectra of as-purified robin MagR (red line) and pigeon MagR (black line) at a concentration of 100 μM .

