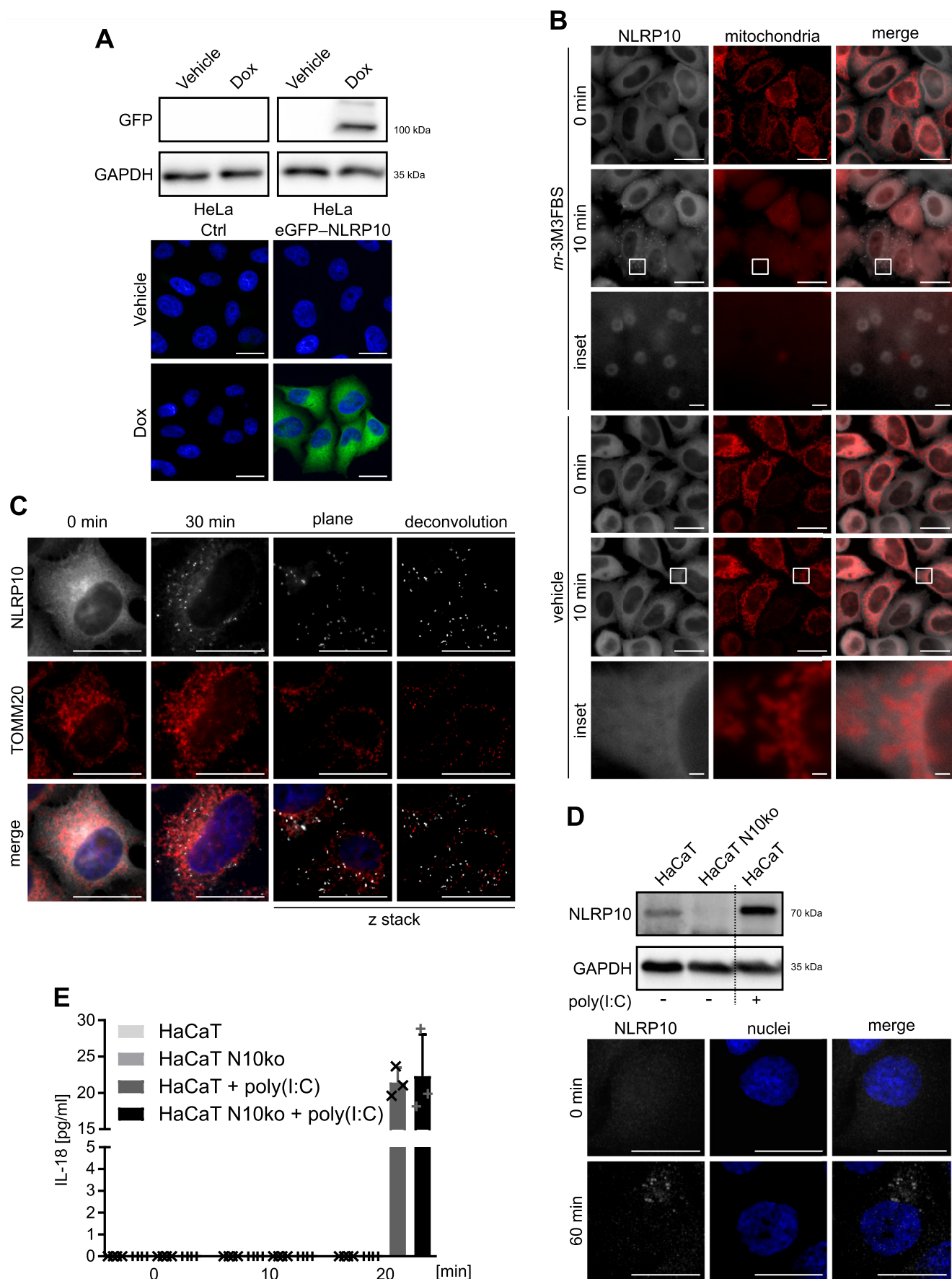


Expanded View Figures

Figure EV1. HeLa and HaCaT cells respond with *m*-3M3FBS-dependent NLRP10 oligomerisation without co-localisation with mitochondrial marker TOMM20.

(A) Stable HeLa cells show expression of eGFP-NLRP10 only in response to Dox. The upper panel shows immunoblot analysis with antibodies directed against GFP and GAPDH as a loading control; the lower panel shows micrographs of cells treated with or without Dox. (B) Live-cell imaging micrographs of eGFP-NLRP10 (white) and mts-mCherry (red) expressing HeLa cells treated with 85 μ M *m*-3M3FBS or vehicle DMSO. (C) Micrographs of eGFP-NLRP10 (green) expressing HeLa cells treated with 85 μ M *m*-3M3FBS stained with an antibody directed against TOMM20. Panel 3 shows one plane and panel 4 the deconvoluted z-stack. (D) Immunoblot and micrographs of HaCaT and NLRP10-knockout HaCaT (N10ko) cells stained with an antibody directed against NLRP10 and treated with 10 μ g/ml poly(I:C) or 85 μ M *m*-3M3FBS. (E) Quantification of the IL-18 release represented as mean $\pm$ SD of HaCaT cells treated with 10 μ g/ml poly(I:C) or 85 μ M *m*-3M3FBS (0 min, 10 min and 20 min). Nuclei were stained with HOECHST dye (A, C, D). Scale bar: 20 μ m or 10 μ m (B, inset). $N = 3$ –4, each measured in duplicates (E). Source data are available online for this figure.



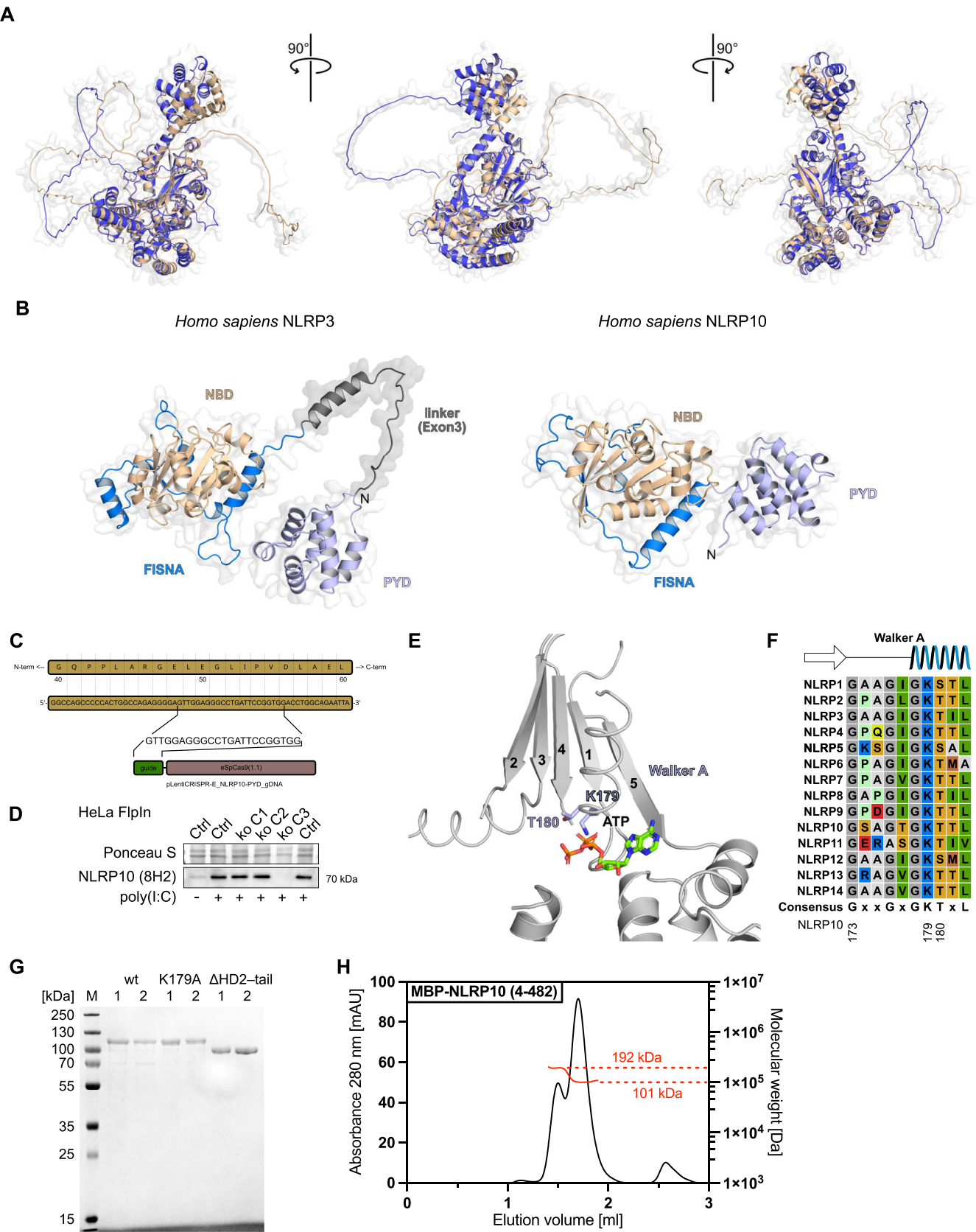


Figure EV2. *Homo sapiens* and *Mus musculus* NLRP10 show high similarity with a different PYD-NACHT linker compared to NLRP3.

(A) Superpositioned AlphaFold models of human (blue) and mouse (beige) NLRP10 at three different angles. (B) The linker between the PYD and NACHT is encoded by an additional Exon in NLRP3, which results in a long stretch (grey), which is absent in NLRP10. (C) Schematics of the gDNA design to target the DNA anti-sense strand of the NLRP10 PYD. (D) Immunoblot analysis of HeLa FlpIn cells transduced with virions produced in Lenti-X 293T cells transfected with the pLentiCRISPR plasmid to target NLRP10 knockout. NLRP10 expression was enhanced using 10 µg/ml poly(I:C). (E) Cartoon of the nucleotide-binding site of NLRP10 modelled with AlphaFold3. The location of ATP refers to the model of NLRP3 (PDB: 7PZC). The Walker A motif is indicated with two catalytically active residues. (F) Sequence alignment of all NLRP family members with consensus for the Walker A motif. (G) SDS-PAGE of the fractions shown in Fig. 2G. (H) SEC-MALS measurements of MBP-NLRP10 (4–482) with determined molecular weights for the concentration-dependent monomer-to-dimer transition. Source data are available online for this figure.

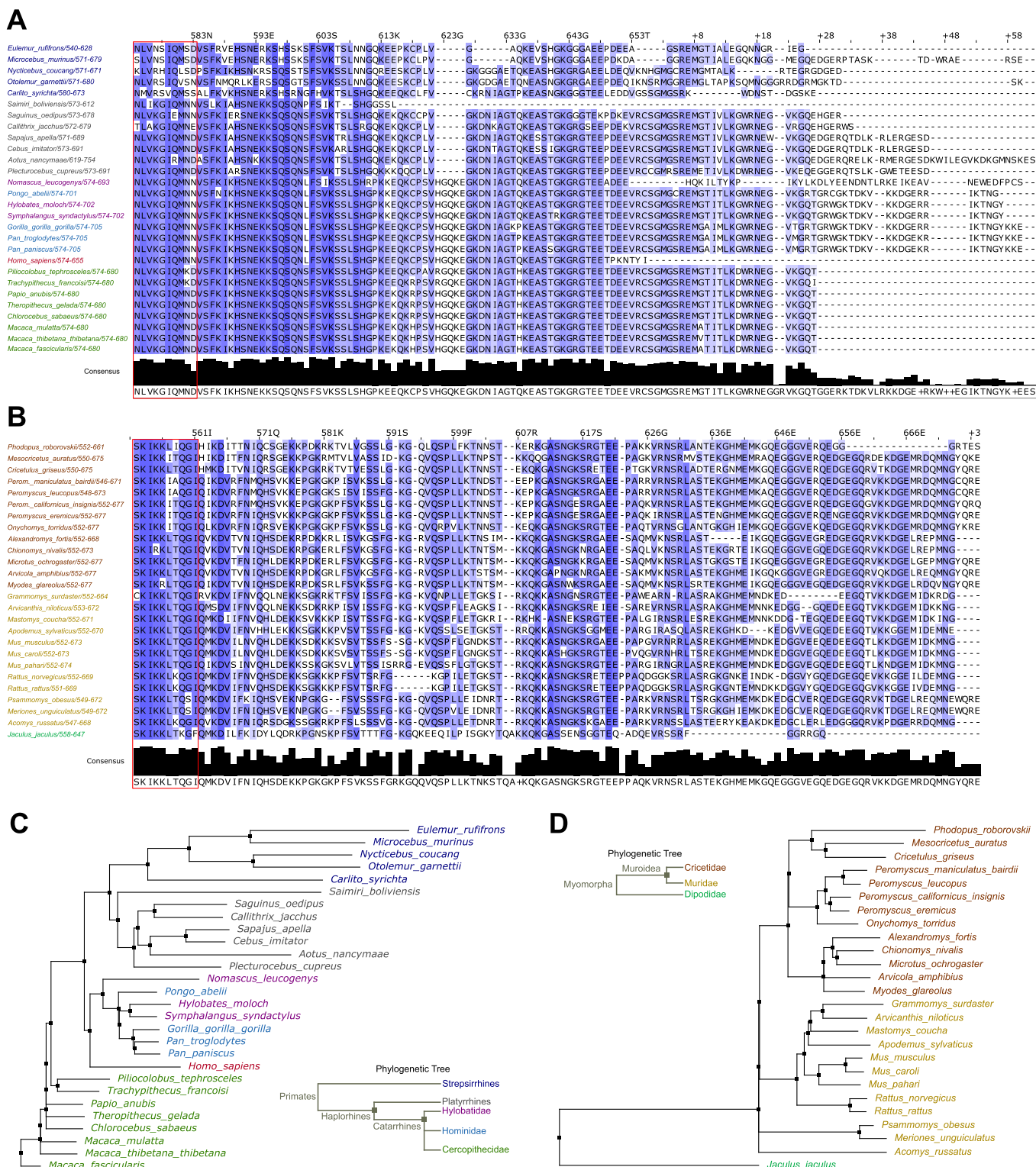


Figure EV3. Evolution of the NLRP10 basic tail: *Homo sapiens* NLRP10 shows divergence among Haplorhines.

(A, B) Multi-sequence alignment of primate NLRP10 tails and Myomorph Nlrp10 tails. The last ten amino acids of the HD2 are shown in a red box. (C, D) Dendrogram according to NLRP10 sequences of primates or Nlrp10 sequences of Myomorph. The dendrogram largely matches the general evolutionary distance of the species. The human NLRP10 (red) separates from all other Catarrhines (C). Among Myomorphs, only *Jaculus jaculus* separates, currently the only high-quality predicted sequence for Dipodidae (D). The colours of (A, B) match those of (C, D) and Appendix Tables. Source data are available online for this figure.

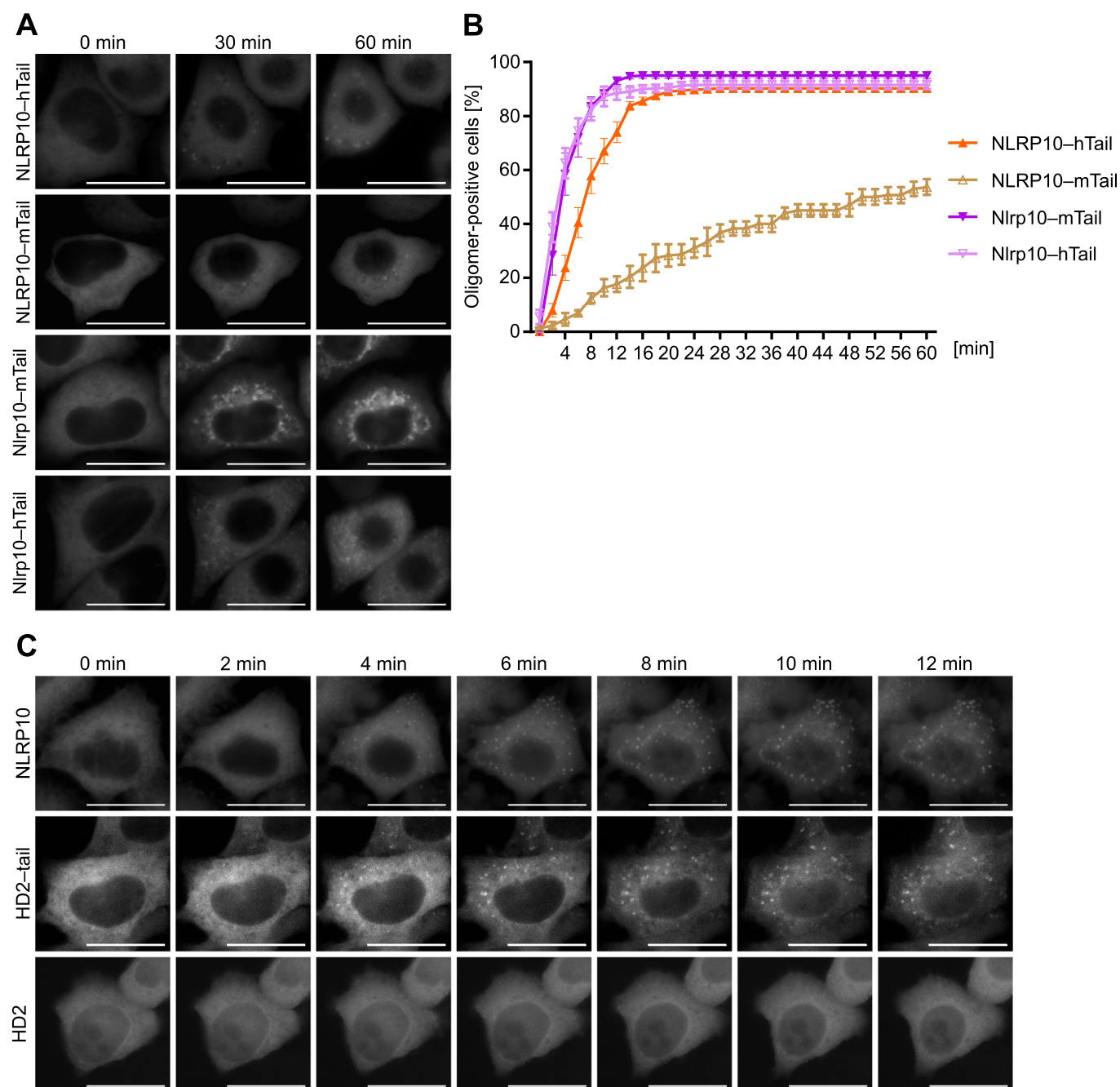


Figure EV4. Human and mouse chimeric NLRP10 can oligomerise, and the tail is sufficient for oligomerisation.

(A) Live-cell imaging micrographs of HeLa cells transiently transfected with chimeric NLRP10 and treated with 85 μ M *m*-3M3FBS. (B) Quantification of the oligomer-positive cells from (A) represented as mean $\pm$ SEM. (C) Live-cell imaging micrographs of HeLa cells treated with 85 μ M *m*-3M3FBS, which were transiently transfected with eGFP fused to the HD2 and HD2-tail of NLRP10 compared to NLRP10 (reused from Fig. 1A). Scale bar 20 μ m. *N* = 3–4 (B). Source data are available online for this figure.

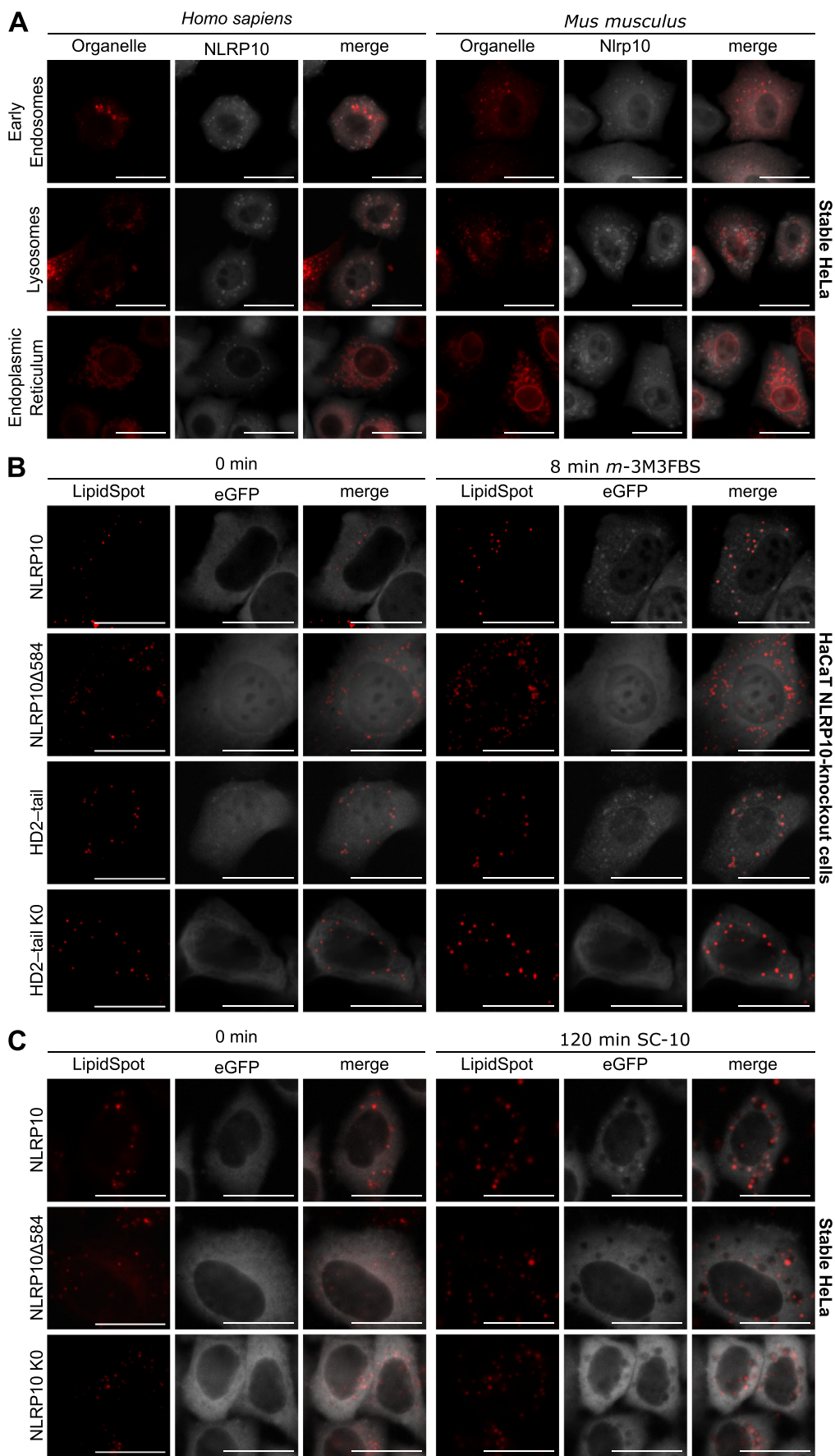


Figure EV5. Human and mouse NLRP10 partially co-localised with early endosomes and lysosomes but not with the endoplasmic reticulum.

(A) Live-cell imaging micrographs (30 min time-point) of HeLa cells expressing human NLRP10 (left) or mouse Nlrp10 (right) treated with 85 μ M *m*-3M3FBS. (B) Micrographs of NLRP10ko HaCaT cells transiently transfected with eGFP-NLRP10, eGFP-NLRP10 Δ 584, eGFP-HD2-tail and eGFP-HD2-tail KO and subsequently treated with 85 μ M *m*-3M3FBS. (C) Live-cell imaging micrographs of HeLa cells expressing NLRP10 (reused from Fig. 1B), NLRP10 Δ 584, or the KO mutant (white) treated with 100 μ M SC-10 and stained with LipidSpot™ 610 (red). Scale bar 20 μ m. Source data are available online for this figure.

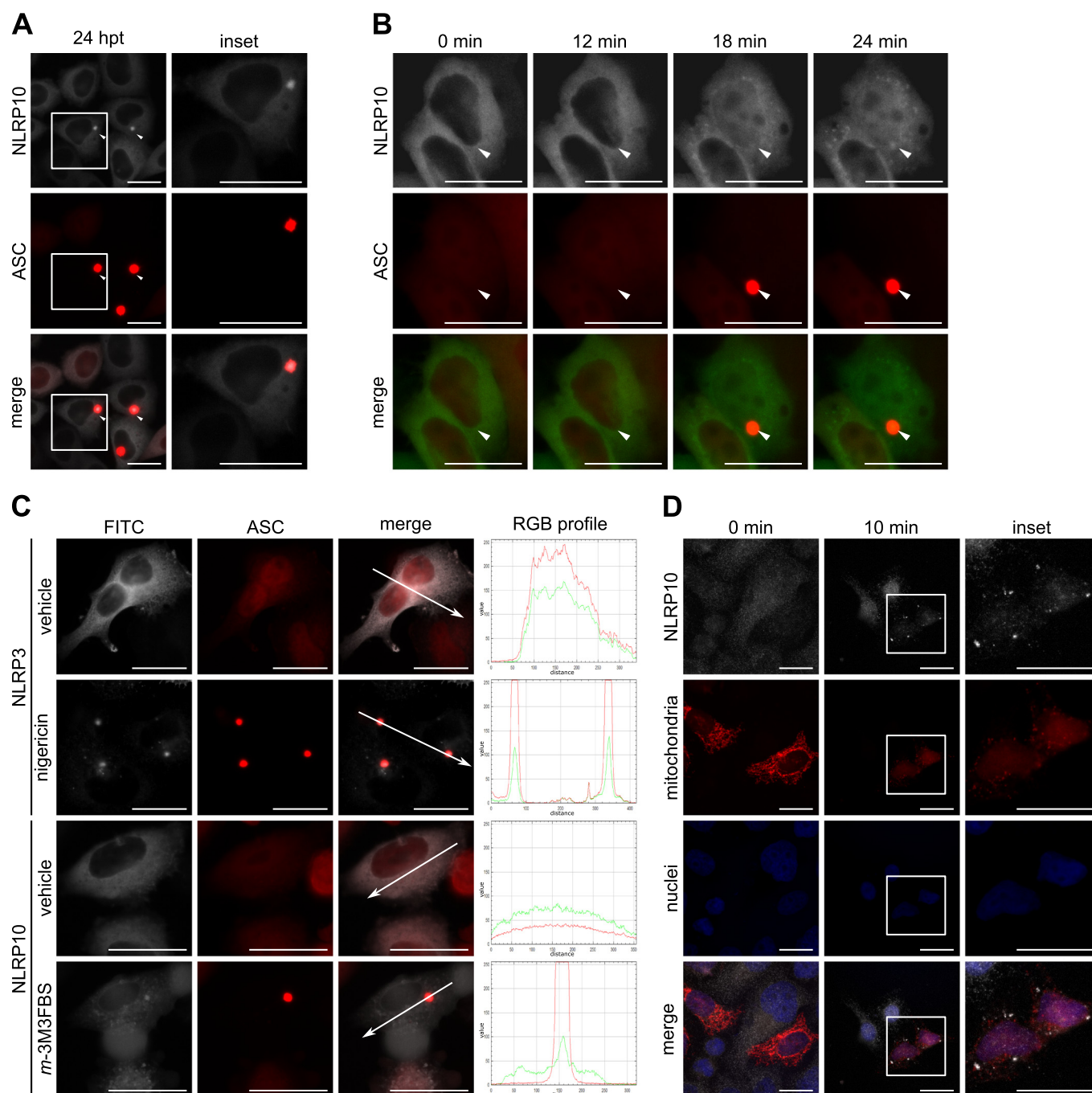


Figure EV6. NLRP10/ASC specks are different to NLRP3/ASC specks.

(A) Live-cell imaging micrographs of stable HeLa cells expressing eGFP-NLRP10 transiently transfected with ASC-RFP (24 h post-transfection). (B) Live-cell imaging micrographs of stable HeLa cells expressing ASC-RFP under a CMVd1 promoter transiently transfected with eGFP-NLRP10 (24 hpt) and treated with 85 μ M *m*-3M3FBS. White arrows indicate specks. (C) Micrographs of stable HeLa cells expressing ASC-RFP under a CMVd1 promoter transiently transfected with myc-NLRP3 or eGFP-NLRP10 (24 hpt) and treated with 10 μ M nigericin (120 min) or 85 μ M *m*-3M3FBS (12 min), respectively. RGB profile of white lines from the third panel showing ASC signal (red) and NLRP3 or NLRP10 signal (green). (D) Micrographs of HeLa cells transiently transfected with mts-mCherry (red) and stained with an antibody (clone 8H2) directed against NLRP10 (white) treated with vehicle or 85 μ M *m*-3M3FBS, nuclei were stained with HOECHST dye. Scale bar 20 μ m. Source data are available online for this figure.