Supplementary Materials

Evolutionary Origin of Insulin-Degrading Enzyme and Its Subcellular Localization and Secretion Mechanism: A Study in Microglial Cells

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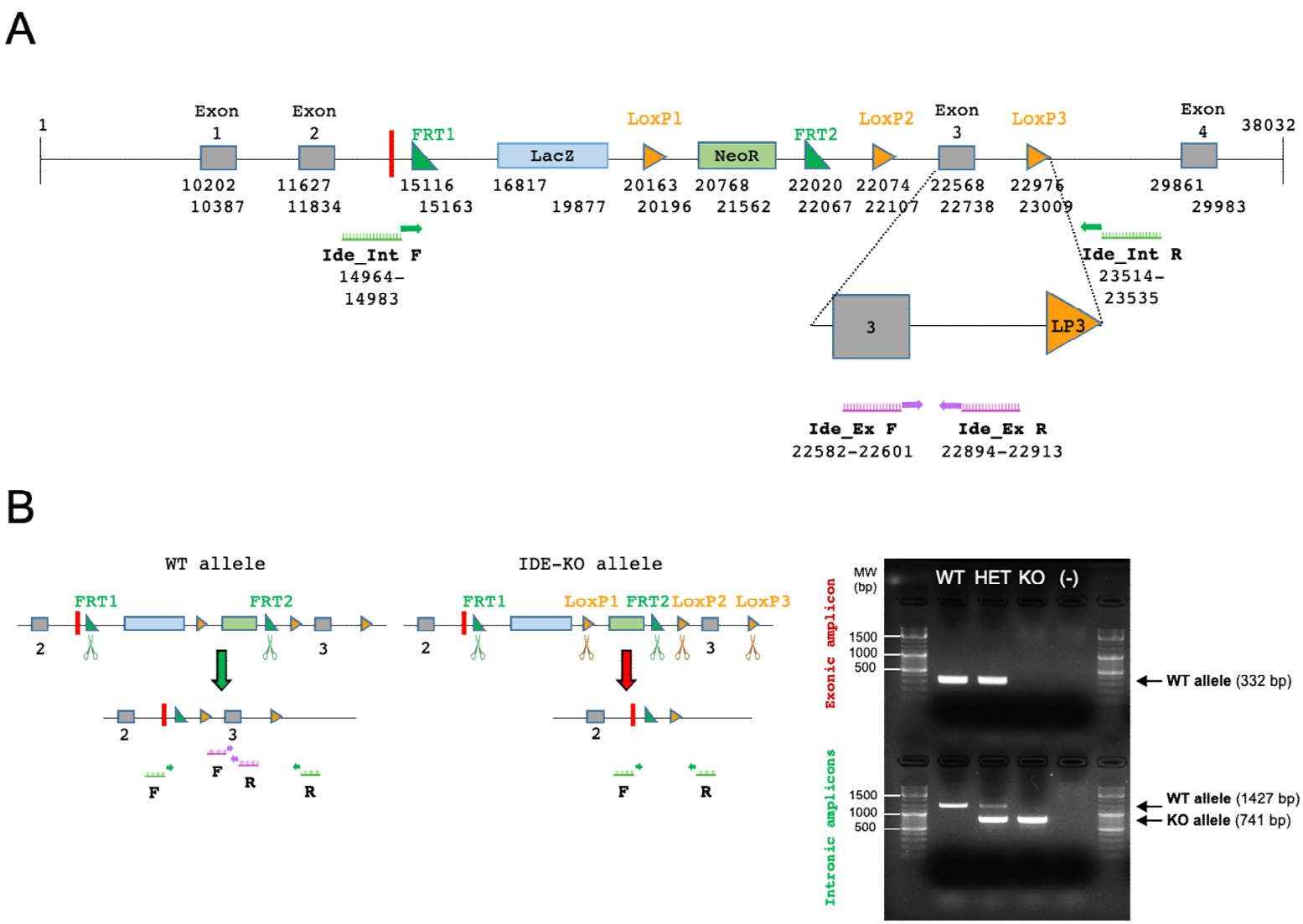

Figure S1. Genotyping of IDE-KO mouse colony. A) Scheme of the genomic map of mutant allele Ide-KO construct (NCBI GenBank JN963136.1) and the genomic localization of the primers designed to genotype IDE-KO mouse colony. **B)** Genetic structure of the WT and IDE-KO alleles. The WT allele produces both exonic and intronic amplicons, while the IDE-KO allele only generates the intronic amplicon, producing a shorter fragment than the WT one due to removal of exon 3. A representative genotyping result is shown (exonic amplicons in the upper part of the gel; intronic amplicons in the lower).

Figure S2. Structural alignments of clan ME metalloendopeptidases. Structural assignments by DSSP, mapped on an underlying sequence alignment, are shown as follows: helix (H/h, in pink), strand (E/e, in blue) and coil (L/l, in green). Uppercase means structurally equivalent positions with human IDE (3CWW) sequence. Lowercase means insertions relative to 3CWW.

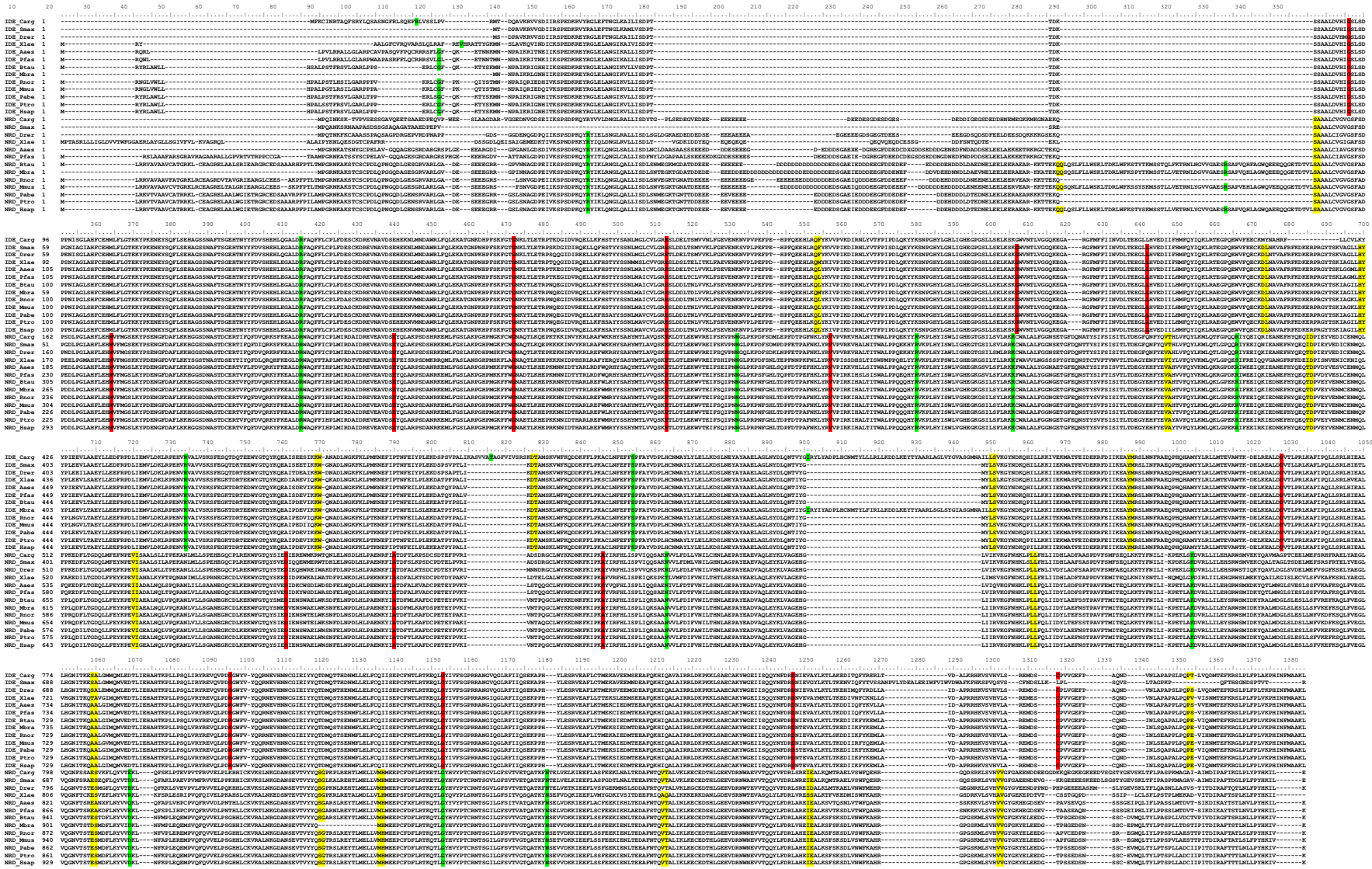

Figure S3. Gene architecture comparison between 26 IDE and NRD proteins. The sequences were retrieved from 13 species of chordates. Introns and their phase (0 = yellow, 1 = red, 2 = green) are depicted on a MSA of protein sequences.

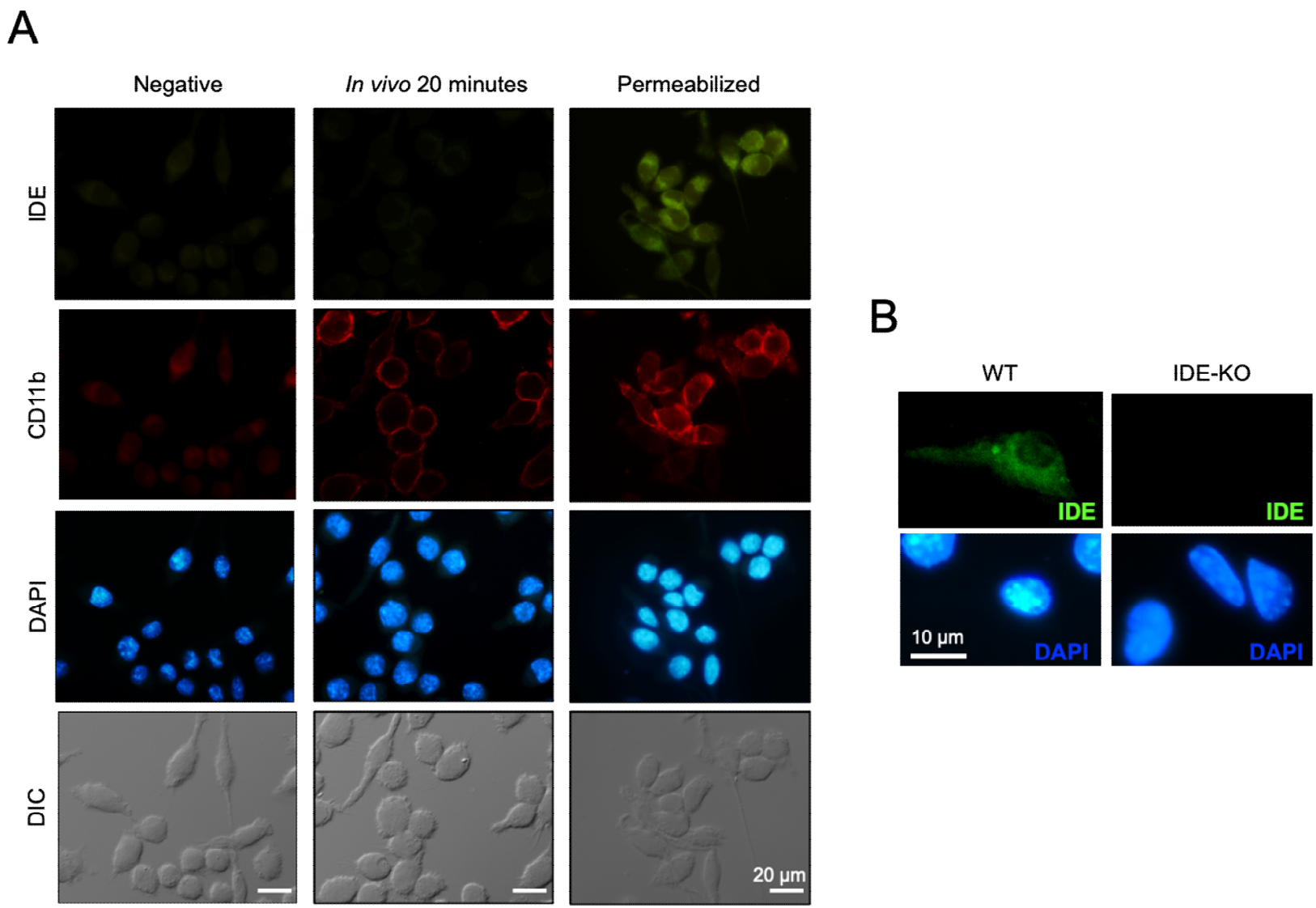

Figure S4. Controls for microscopy experiments. A) Double immunolabeling of IDE and CD11b in BV-2 microglial cell line. Negative control: All steps performed, but no primary antibody added. **B)** Validation of the labeling protocol with anti-IDE antibody in WT and IDE-KO microglial cells.

Molecular phylogeny data S2. Trees output (text format)

Figure 1A

(Eupitrilysin_Hsap:1.0191103455,(PreP1_Atha:0.7225082131,Falcilysin_Pfal:1.3415617952)84:0.2457498546,((MPPA_Hsap:1.0363378193,(MPPB_Hsap:0.7195480079,YmxG_Rpro:1.2491304481)61:0.2537924513)100:1.0639391740,(((((NRDC_Hsap:1.0268046682,((Ste23_Scer:0.8832602370,IDE_Hsap:0.5509579495)94:0.1756842058,Plinsulysin_Slyc:0.7650341070)67:0.1260218911)89:0.2364566254,Pitrilysin_Ecol:1.3800937785)67:0.2110329985,Axl1p_Scer:1.7584369669)87:0.4037858439,G1L_MP44_Vvir:2.8498386663)82:0.3549218325,SPP_Atha:1.8855216901)71:0.1808566439)100:1.1295031830);

Figure 1B

(((((((3CWWA:9.1,s005A:9.6):7.95,Ste23:18.05):3.51666666666666,1Q2LA:22.8666666666667):5.38333333333333,NRD:27.15):4.21,Axl1p:30.66):5.556666666666667,(((MPPB:19.2,s008A:21.1):5.3,MPPA:17.3):15.5333333333333,SPP:38.3333333333333):0.68333333333333):5.23333333333333,((4L3TA:23.6,2fgeA:25.1):3.05,3S5MA:21.85):15.8):4.38846153846154,G1L:20.1384615384615);

Figure 2A

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Figure 2C

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Figure 3A

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