Supporting Information

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Supporting Materials and Methods

Amplification and Cloning of an Optionally Transcribed Region in Exon 1. T cells were enriched from total splenocytes and lymph node cells using either CD4 or CD8 T Lymphocyte Enrichment Set-DM (BD PharMingen) and activated with plate-bound anti-CD3ε (3 μg/ml) and anti-CD28 antibodies (1 μg/ml) (BD PharMingen). Purity of T cells checked by flow cytometry was >90% of $CD3^+CD4^+$ or $CD3^+CD8^+$. 293FT cells were purchased from Invitrogen. pDC, cDC and T cells were harvested and total cDNA was prepared as described in Methods. PCR was carried out with M5 (forward) and R2 (reverse) (sequence and priming sites are shown in Fig. S1). Two bands appearing in the M5/R2 PCR were cut out from the gel, purified, and cloned into a vector. The sequence of the optionally transcribed region and the insertion site was determined by comparing the sequence results from two clones including either long or short PCR products.

Podosome Formation of pDC. FACS-sorted pDC were infected with lentiviral Opn expression constructs pMLS3 (full Opn) or pMLS5 (artificial ATG/Kozak immediately downstream of the 5′-terminal of signal peptide-encoding sequence) or a control construct expressing GFP. Images were captured 3h after pDC treatment with CpG-1668.

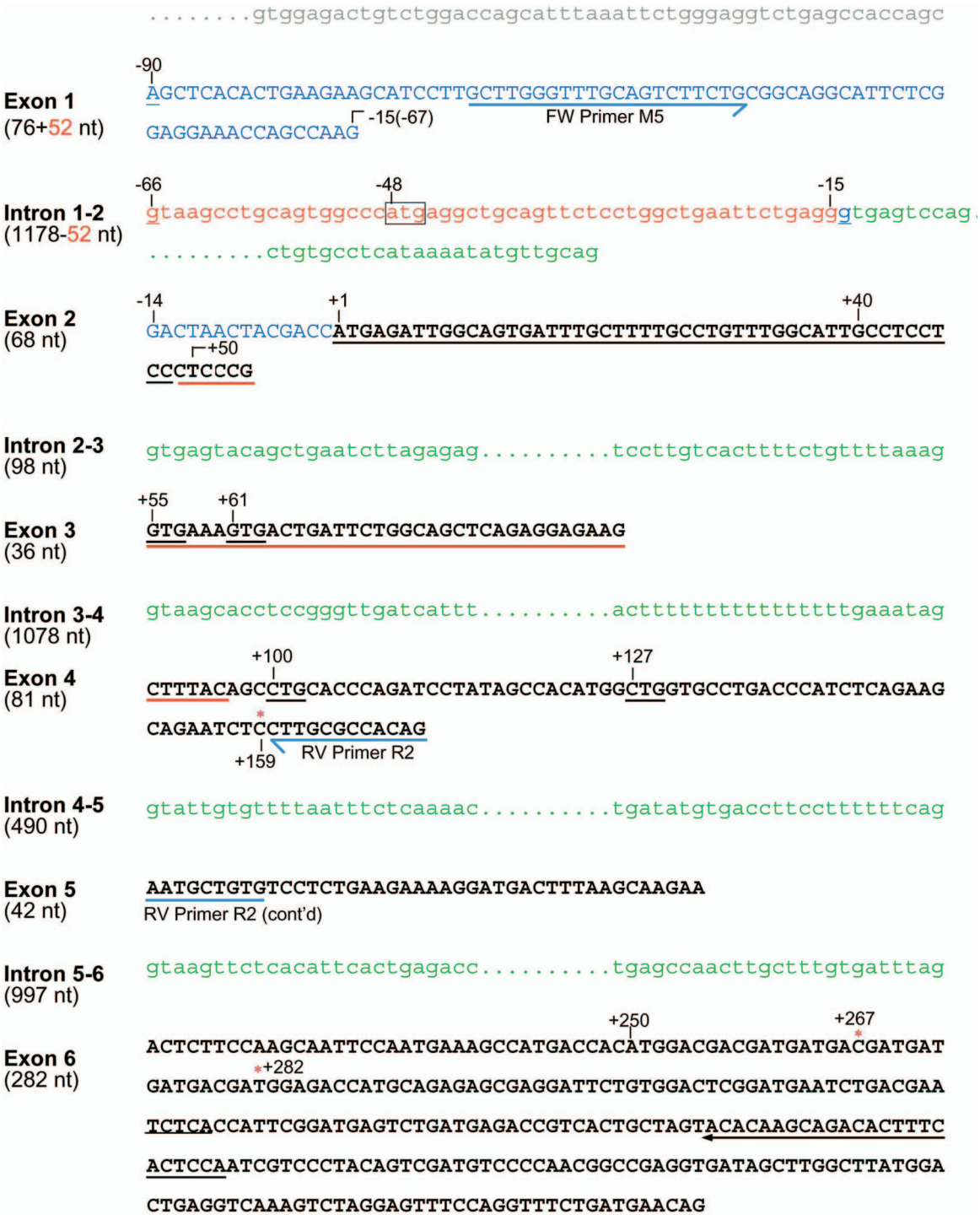

Fig. S1. The 5′ half of *Opn* genomic sequence (from 5′-proximal of the full transcription start sites to Exon 6). Upper- and lowercase letters denote transcribed and untranscribed sequences, respectively. Nucleotide number (+1) is located at the A residue of the canonical ATG translation initiation codon in Exon 2, and transcribed nucleotides alone are numbered. Nucleotides translated into the Opn peptide sequence are shown in capital, bold, black and the 5′-UTR sequence is shown in capital blue letters. Lowercase letters in red denote optional nucleotides (from −67 to −15) added by alternative splicing. Nucleotides translated into signal peptide sequence are underlined from position +1 to +48. Transcription initiation sites by RLM-RACE are at the positions −90 (A residue) for full transcription, and alternative sites, including + 159 (C residue), +267 (C residue), and + 282 (T residue), are indicated with red asterisks. The thick red underlined region (+49 to +96) is translated to be the epitope of anti-Opn antibody, O-17. The annealing site of the Opn inner primer used for RLM-RACE (Fig. 2*A*) is indicated by a black line with an arrowhead in Exon 6. M5 and R5 primer annealing sites (Fig. S2) are shown with the primer names with blue lines. GTG/CTG codons mutated for the experiment in Fig. S3 are underlined in black.

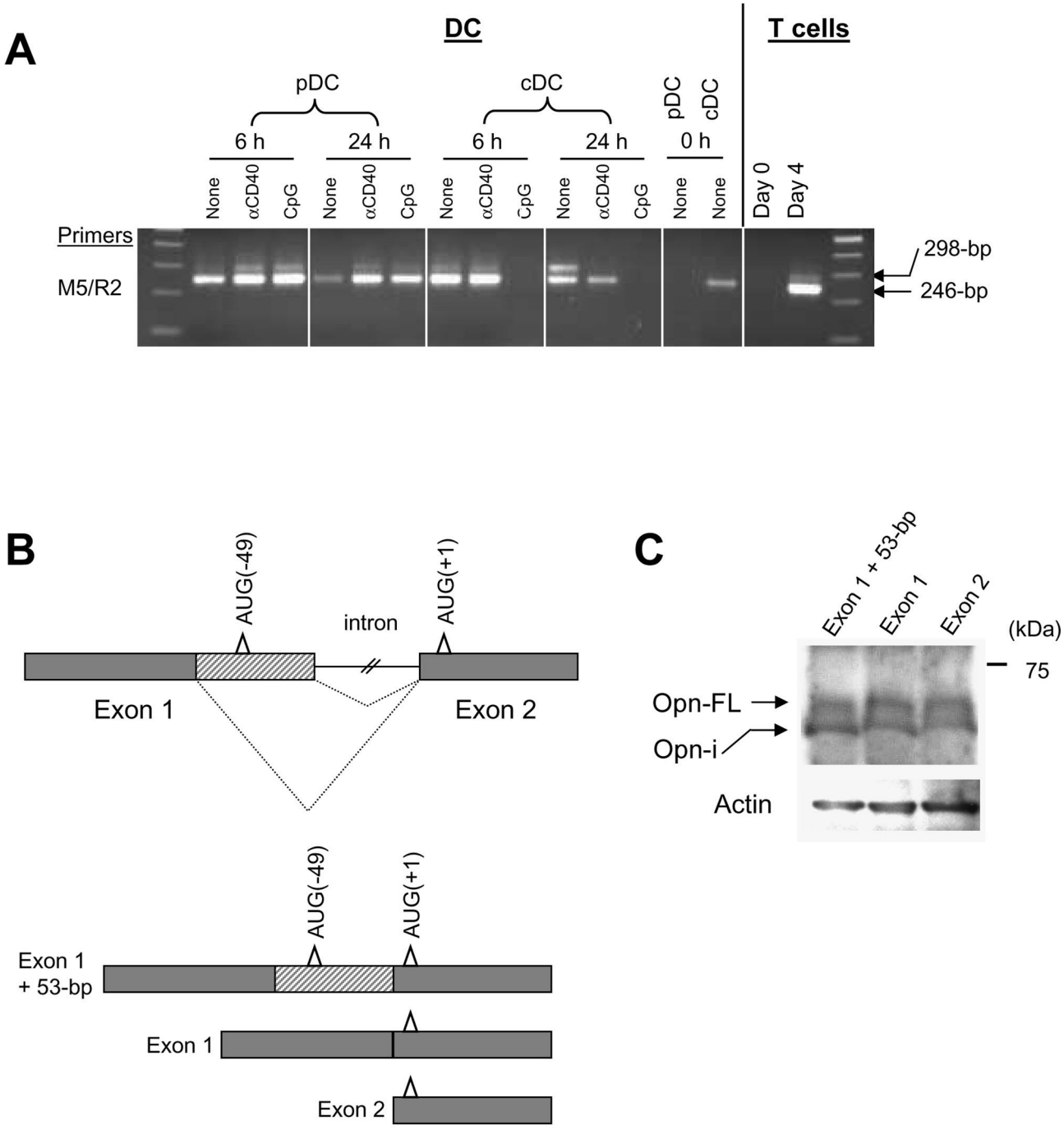

Fig. S2. RT-PCR and cloning of an optional sequence in Exon 1 and Opn peptide expression. (*A*) RT-PCR determines the 5′-proximal of the *Opn* mRNA. Panel shows agarose gel electrophoresis results from PCR with a primer set M5/R2 (the positions of the primers are shown in Fig. S1). All samples were obtained from B6 mice. pDC and cDC were purified by FACS sorting from total BM-DC, then activated with or without anti-CD40 Ab or CpG for indicated time. T cells were enriched by beads from spleens and LN, and activated for 4 days with plate-coated anti-CD3/28 Abs. (*B,C*) Alternative splicing in Exon 1 does not participate in alternative translation. (*B*) Schematic diagram of Exon 1 alternative splicing. The top panel represents arrangement of the 5′-proximal of *Opn*, including Exon 1, Exon 2, and an intron between Exon 1 and 2. The hatched region of Exon 1 is an optional sequence added by alternative splicing that includes an in-frame AUG start codon. (*C*) 293FT cells were transfected with Opn expression constructs shown in (*B*). ''Exon 2'' construct produces the same 5′-end with constructs indicated as full-length *Opn* mRNA throughout this manuscript: starting from the 5′-end of Exon 2. The AUG at −49 nt did not serve as an alternative translation initiation site. Opn in the 293FT lysates was detected by immunoblotting using O-17 Ab.

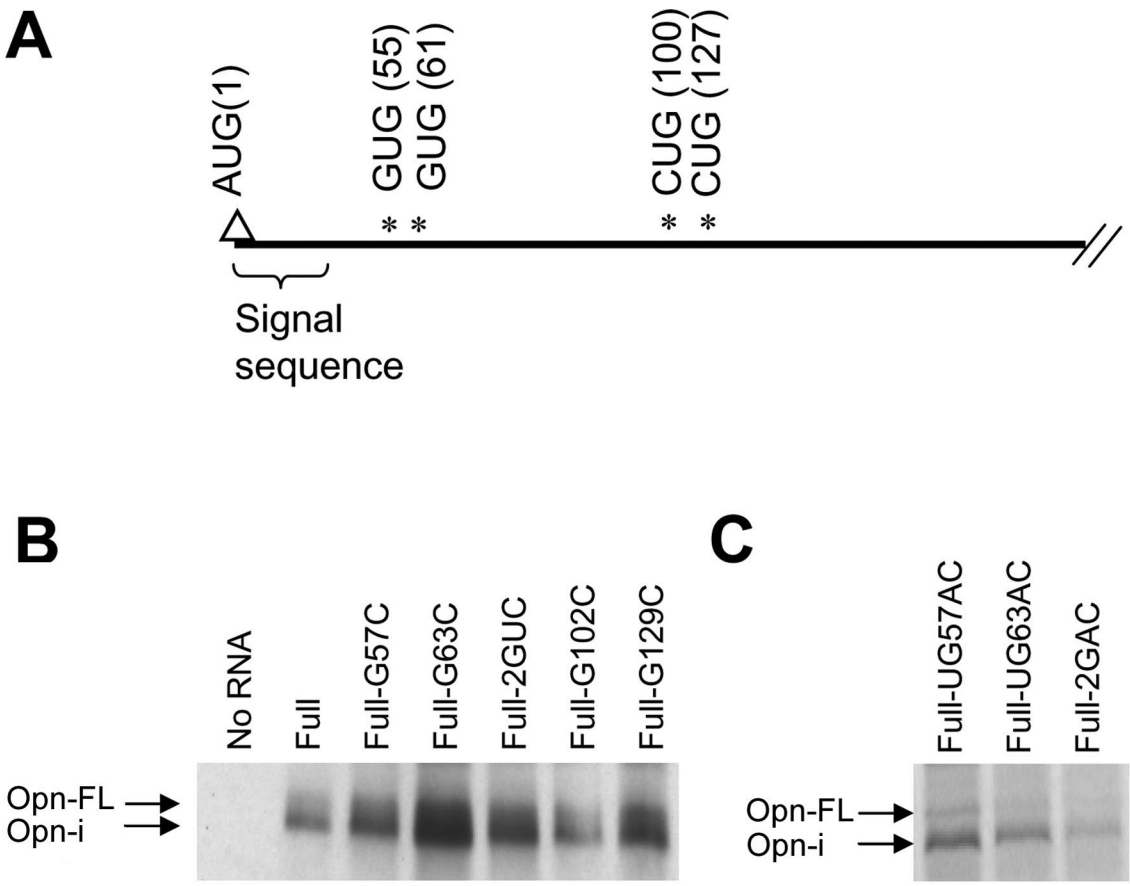

Fig. S3. GUG/CUG codons in Exon 3/4 do not participate in alternative translation. IVT of various Opn expression constructs. (*A*) Schematic diagram of *Opn* mRNA with GUG/CUG codons mutated for IVT analysis. All of the constructs included canonical ATG codon at position +1 with or without point mutation(s) shown with asterisks. (*B*) IVT results of constructs with a single nucleotide mutation in a codon, GTG or CTG (to GTC or CTC, respectively). Full-G57C and Full-G63C are G 224 C mutations at positions +57 and + 63, respectively, to alter the GTG codon to the GTC codon. Full-2GUG construct includes two mutations (GTG to GTC) at position + 57 and + 63. Full-G102C and Full-G129C are G 224 C mutations at positions + 102 and + 129, respectively, to alter the CTG codon to the GTG codon. (*C*) IVT results of constructs with double-nucleotide mutations in GTG to GAC. Full-2GAC construct had both GTG codons mutated to GAC.

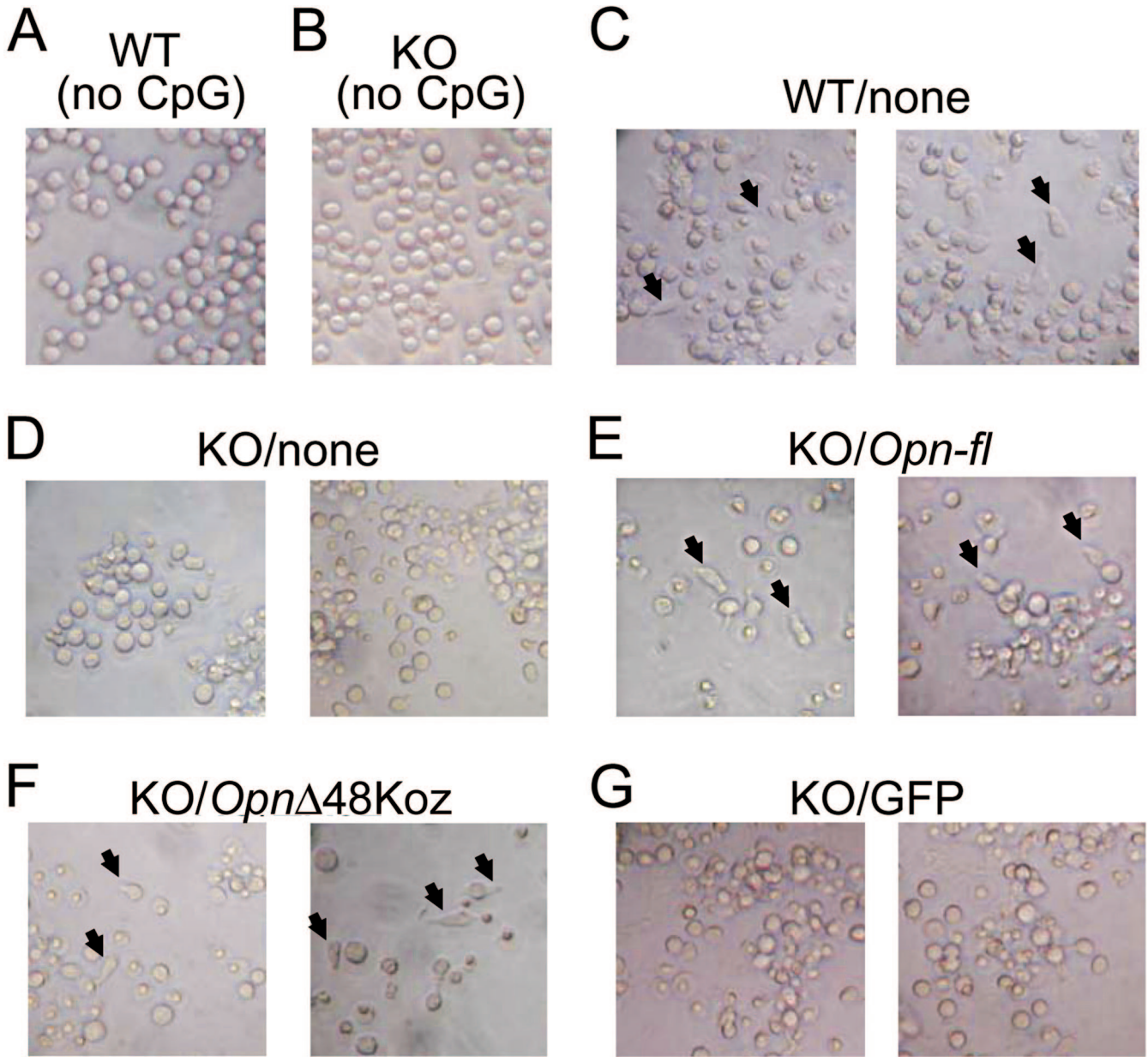

Fig. S4. Podosome formation of pDC. Images corresponding to data presented in Fig. 6*B* were captured 3 h after plating: Opn WT (*A*) and KO (*B*) pDC without CpG. CpG was added to pDC culture supernatants at the time of cell plating: Opn WT (*C*) and KO (*D*) without lentiviral infection; Opn KO with lentivirally transfected Opn WT (*E*), Opn-i (Δ48Koz) (*F*) and GFP (control) (*G*) constructs are shown. Two different fields are shown for samples *C*-*G*. Arrows indicate cells displaying podosomes.

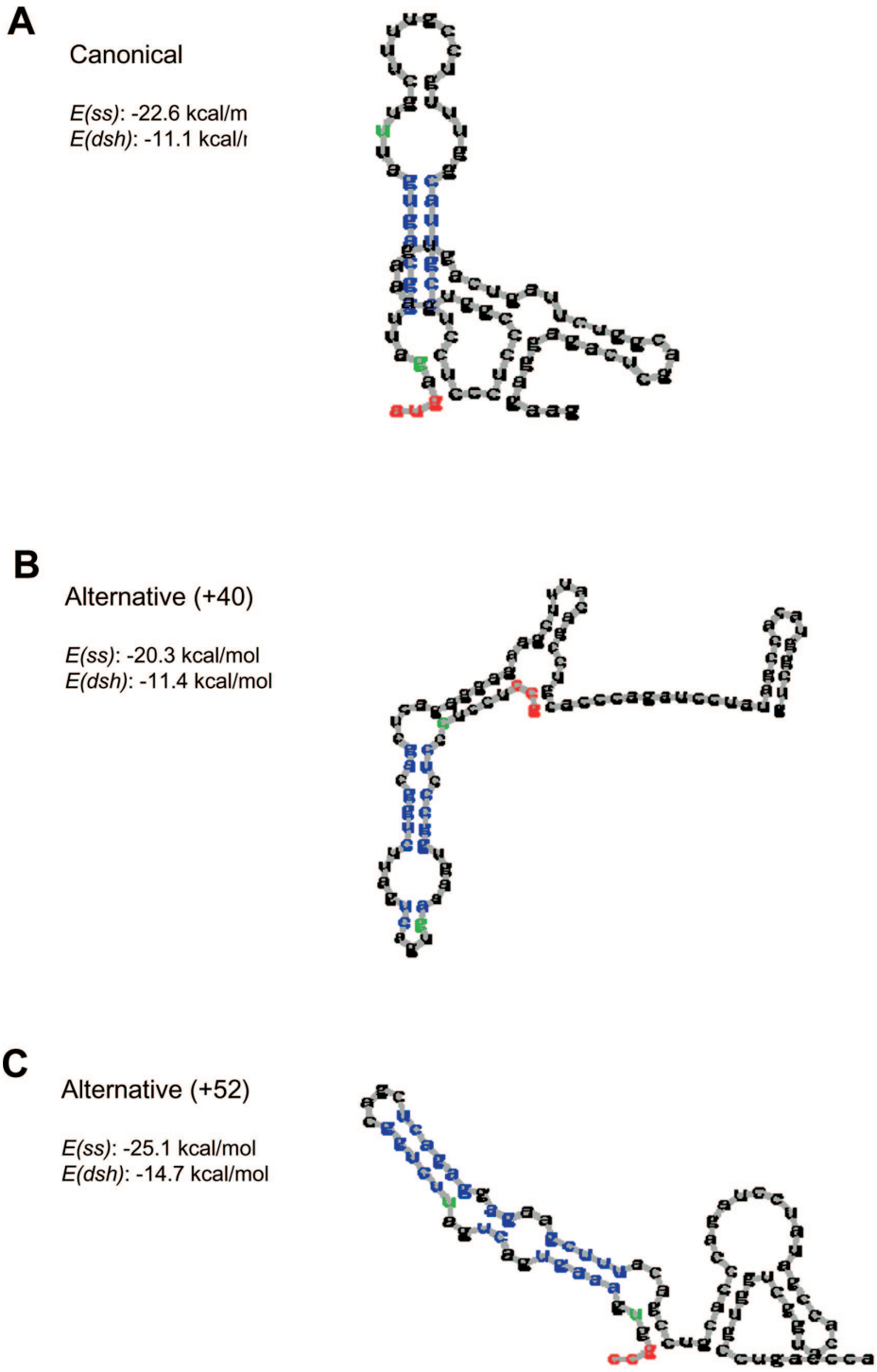

Fig. S5. Downstream RNA secondary structures of conventional and alternative translation initiation sites. RNA downstream secondary structure was calculated using ''AUC_hairpin'' software (wwwmgs.bionet.nsc.ru/mgs/programs/aug_hairpin). Setting to allow positions for hairpin start is between 5 to 18 and one max bulge or interior loop size if imperfect helices were allowed. (*A*) Downstream RNA secondary structure of conventional translation start site between + 1 and + 100. (*B, C*) Downstream RNA secondary structures of alternative translation start sites. Two regions were tested: between + 52 and + 152 (starting from the Asp codon, 3-aa from the last amino acid of the signal sequence) and between + 40 and + 140 (starting from the Pro codon, 2-aa downstream of signal sequence). *E(ss)* and *E(dsh)* denote ''Energy of secondary structure'' and ''Energy of double strands in hairpin,'' respectively.