SUPPLEMENTAL MATERIAL

Massoumi et al., http://www.jem.org/cgi/content/full/jem.20082044/DC1

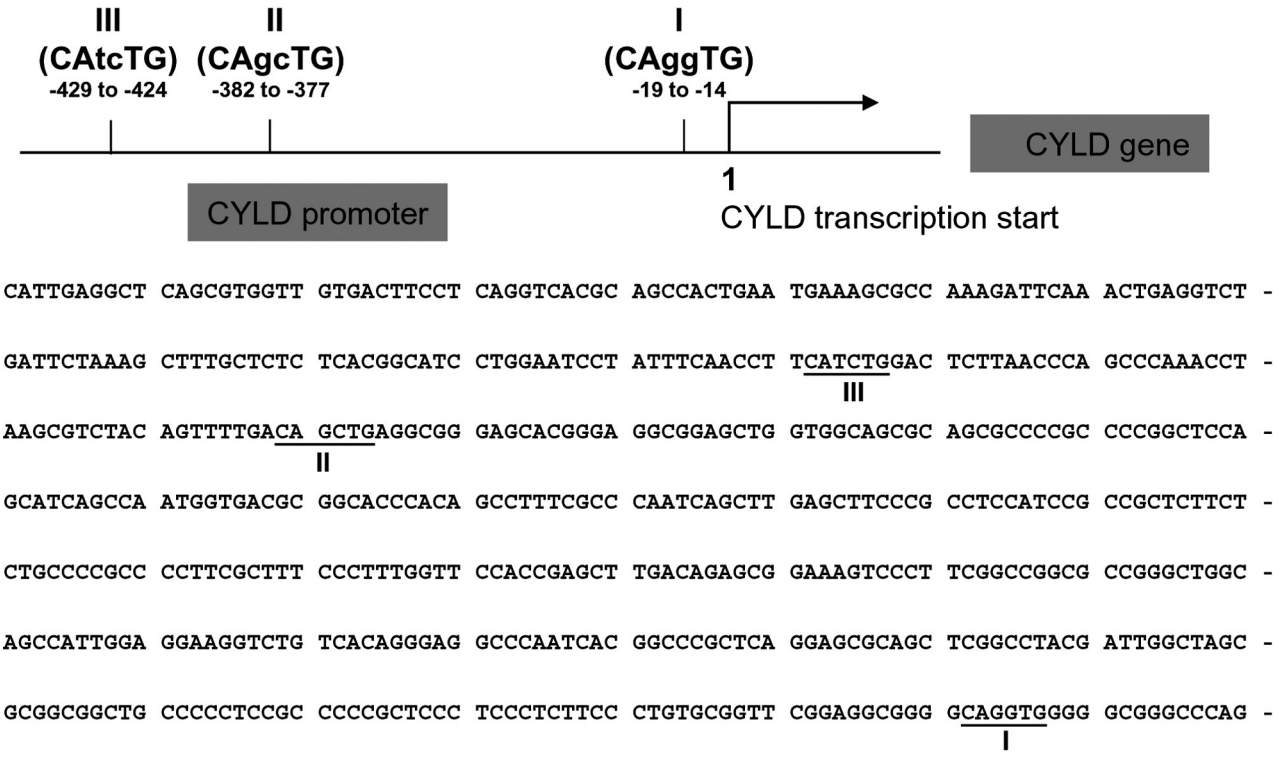

Figure S1. Graph of the *CYLD* promoter and three putative Snail1 binding sites (I, II, and III).

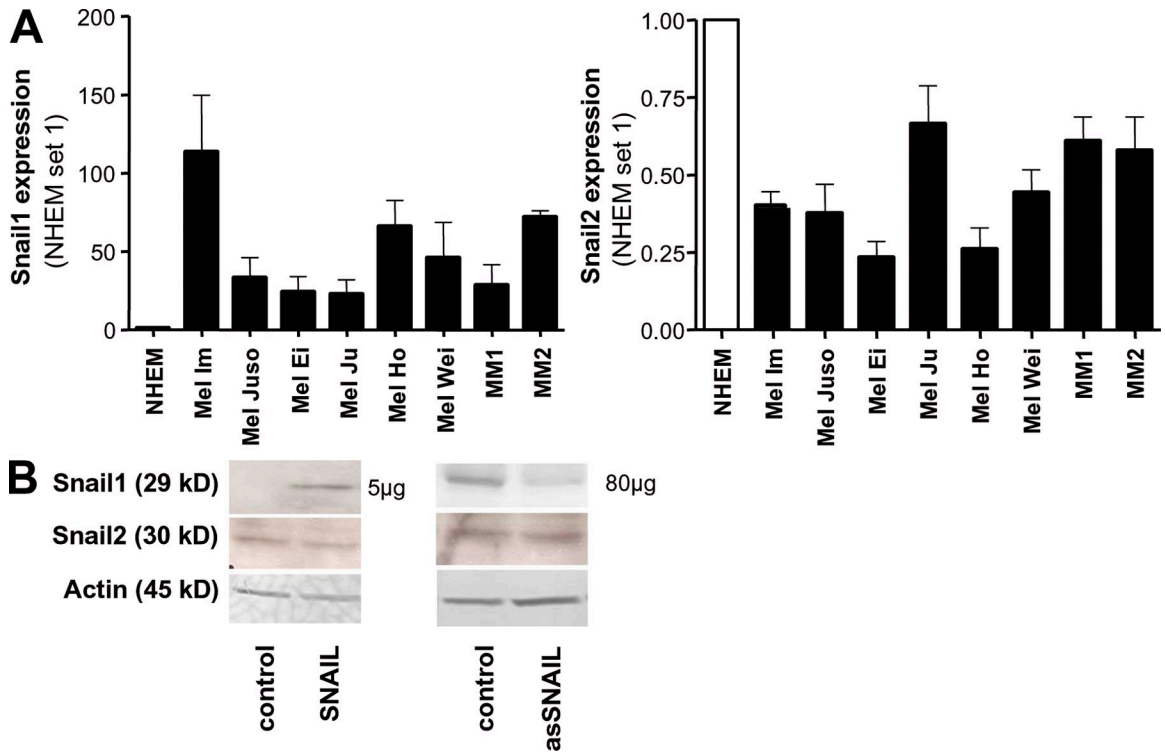

Figure S2. **Expression of Snail and Slug in malignant melanoma.** (A) Quantitative RT-PCR of Snail1 and Snail2 expression in six human melanoma cell lines (Mel Im, Mel Juso, Mel Ei, Mel Ju, Mel Ho, and Mel Wei) and freshly isolated primary melanoma cells from two donors (MM1 and MM2) as compared with NHEMs. (B) Immunoblot analysis of Snail1 and Snail2 after transient transfection of Mel Im cells with sense and antisense snail1 expression constructs. To clearly demonstrate effects on snail1 expression, loading of differential amounts of protein was performed (5 or 80 µg).

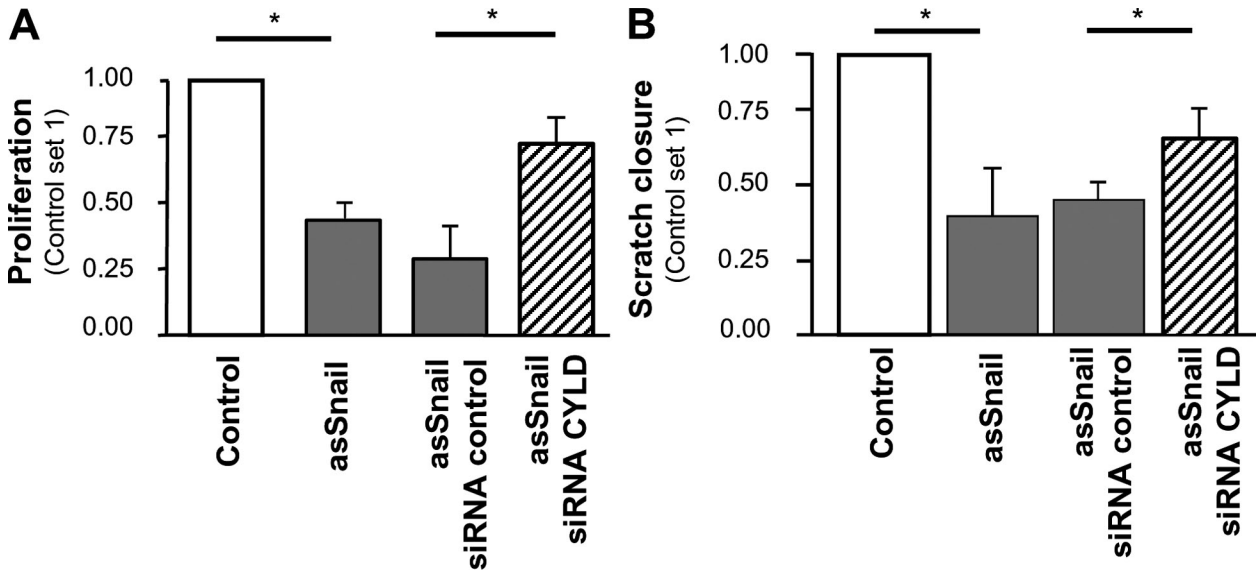

Figure S3. **Effect of CYLD suppression on tumorigenicity of Mel Im as Snail clone 2.** Proliferation after 72 h (A) and migration after 24 h (B) of Mel Im control cells (Control) and Mel Im asSnail clone 2 stably transfected with expression vectors encoding siRNA against CYLD (siRNA CYLD), control siRNA (siRNA control), or without transfection (asSnail). Data given as the mean ± SEM. *, $P < 0.05$.

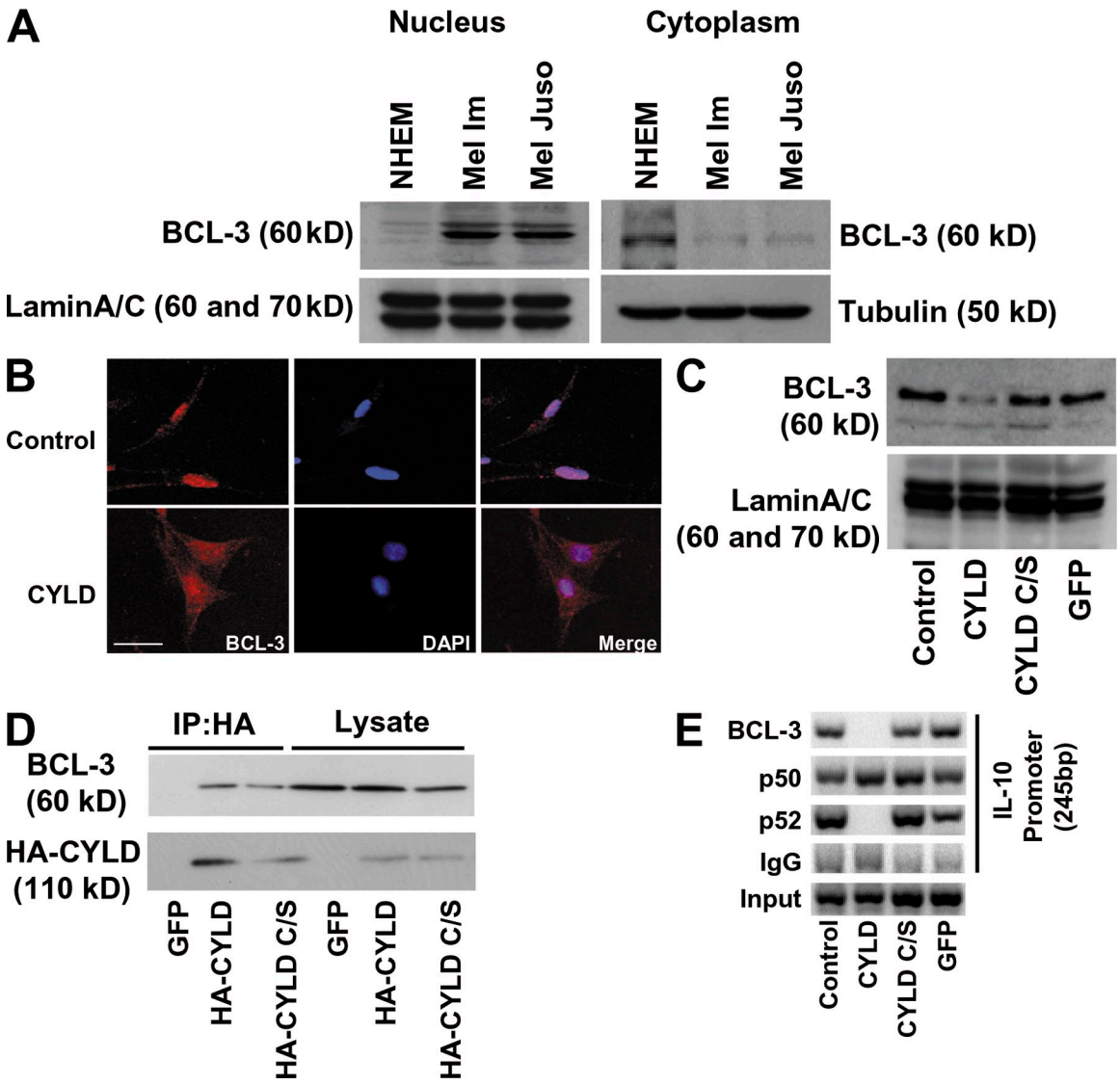

Figure S4. Effect of CYLD on nuclear localization of Bcl-3. (A) Nuclear and cytosolic extracts of NHEMs or melanoma cells (Mel Im or Mel Juso) were immunoblotted with antibodies against BCL-3, laminA/C, or tubulin. (B) Confocal plane of Bcl-3 (red) and DAPI (blue) in Mel Im or Mel Im infected with CYLD. Bar, 5 µm. (C) Nuclear extracts of Mel Im cells transduced with viral vectors expressing CYLD or a catalytic inactive mutant of CYLD (C/S-CYLD) or GFP were immunoblotted with antibodies against BCL-3. Equal loading was confirmed using antibodies against laminA/C. (D) Lysates from CYLD, mutant CYLD (CYLD C/S), or GFP stably expressing Mel Im cells were used for coimmunoprecipitation, revealing binding of BCL-3 to CYLD and CYLD C/S. (E) Lysates from Mel im cells (Control) and CYLD, mutant CYLD (CYLD C/S), or GFP stably expressing Mel Im cells were examined by ChIP assay using specific polyclonal antibodies against BCL-3, p50, or p52, and PCR primer pairs corresponding to the promoter of the *IL-10* gene (245 bp) to analyze recruitment of BCL-3. BCL-3, p50, and p52 IP using polyclonal antibodies as indicated. IgG, negative control rabbit Ig (Dako); Input, 10% of the cell lysate used for the IP is shown.

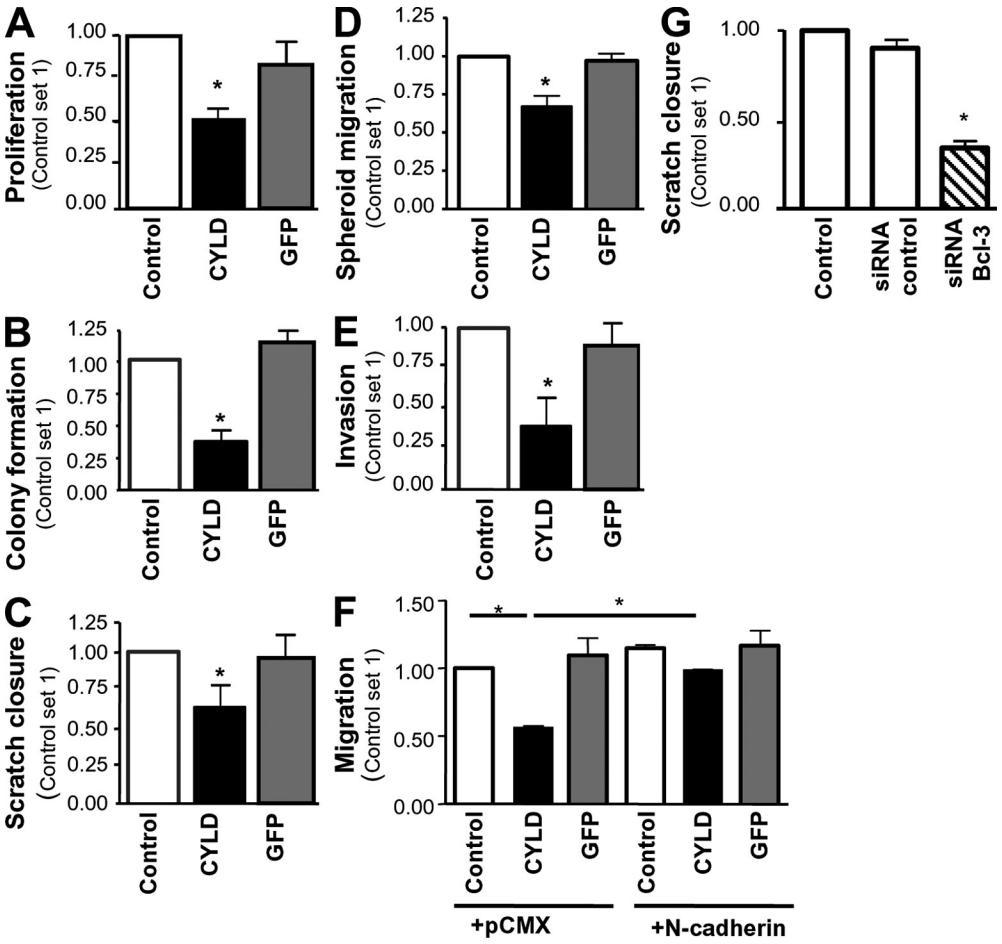

Figure S5. CYLD regulates proliferation and N cadherin–mediated migration and invasion of Mel Juso melanoma cells. Comparison of Mel Juso cells stably transfected with CYLD or GFP and noninfected control cells. (A) Cell proliferation at 72 h (*, $P < 0.05$ compared with control or GFP). (B) Colony formation in soft agar after 3 wk. (C) Migration of melanoma cells in monolayer scratch assays or (D) in spheroid migration assays. (E) Invasion of Mel Juso (Control) cells compared with CYLD or GFP stably transfected cells (after 24 h) in Boyden chamber assays. (F) Migration of melanoma cells in Boyden chamber assays. Comparison of Mel Juso cells stably transfected with CYLD or GFP and transiently cotransfected with N cadherin expression vector or control vector (pCMXpl1). (G) Migration of melanoma cells in monolayer scratch assays untreated (Control) or transfected with Bcl-3 siRNA nucleotides or scrambled siRNA control. Bars represent the mean ± SEM. *, $P < 0.05$ compared with control and GFP.

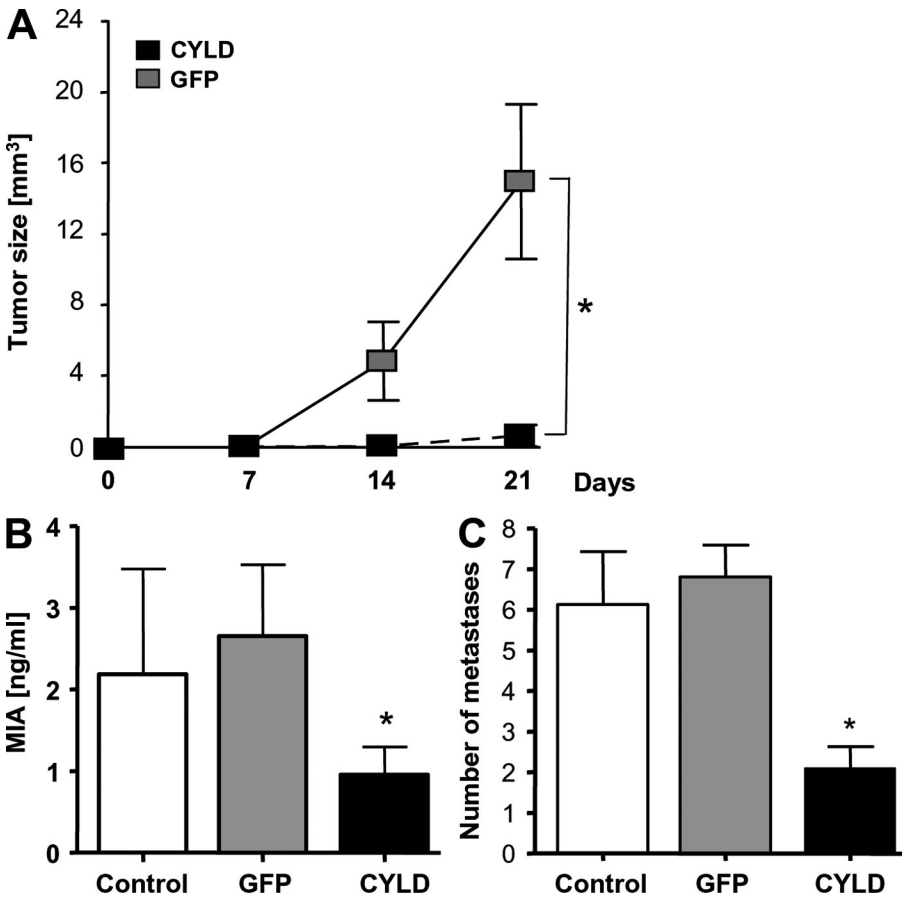

Figure S6. CYLD inhibits proliferation and metastasis of Mel Juso melanoma cells in vivo. (A) Growth kinetic of Mel Juso melanoma cells transduced with viral vectors carrying CYLD or GFP after s.c. implantation into nude mice (10^6 mice/group; 10 mice/group). Data are given as the mean ± SEM. *, $P < 0.05$ compared with GFP. (B) MIA serum levels in nude mice after i.v. injection of Mel Juso control cells (Control) or cells transduced with viral vectors carrying CYLD or GFP (10^6 mice/group; 8–10 mice/group). Data represent mean MIA level (± SEM) 4 wk after injection. *, $P < 0.05$ versus GFP and control. (C) Counting of the number of macrometastatic lesions per one cross section of the lungs from each mouse. Data are given as the mean ± SEM. *, $P < 0.05$ compared with control and GFP.

Table S1. Primers used for amplification of the *CYLD* gene

gene	primer sequence
CYLD forward 223 (exon 3)	5′ - TGC CTT CCA ACT CTC GTC TTG - 3′
CYLD reverse 1898 (exon 9)	5′ - CAG CGA GCA CTT CAT TCA GTC - 3′
CYLD forward 1577 (exon 9)	5′ - GAC CGT TCT TCA CCA CCA CT - 3′
CYLD reverse 2647 (exon 16)	5′ - CAG ACA TGA TGG TGC CTC T - 3′.
beta-actin forward	5'- CTA CGT CGC CCT GGA CTT CGA GC - 3′
beta-actin reverse	5'- GAT GGA GCC GCC GAT CCA CAC GG - 3′

Table S2. Primers used for mRNA amplification by RT-PCR

gene	primer sequence
cyclin D1 forward	5′ - GCC TGT GAT GCT GGG CAC TTC ATC - 3'
cyclin D1 reverse	5′ - TTT GGT TCG GCA GCT TGC TAG GTG - 3'
CYLD forward	5′ - TGC CTT CCA ACT CTC GTC TTG - 3′
CYLD reverse	5′ - AAT CCG CTC TTC CCA GTA GG - 3′
N-cadherin forward	5′ - TGG ATG AAG ATG GCA TGG - 3′
N-cadherin reverse	5′ - AGG TGG CCA CTG TGC TTA C - 3′
SNAIL forward	5′ -AGG CCC TGG CTG CTA CAA G - 3′
SNAIL reverse	5′ -ACA TCT GAG TGG GTC TGG AG - 3′
beta-actin forward	5'- CTA CGT CGC CCT GGA CTT CGA GC - 3′
beta-actin reverse	5'- GAT GGA GCC GCC GAT CCA CAC GG - 3′

Table S3. Primers used in ChIP

ChIP	primer sequence
Snail binding site I (-19)	Snail I Forw: tct tcc ctg tgc ggt tcg ga Snail I Rev: ctc cataagcct gaa ctc at
Snail binding site II and III	Snail II+III Forw: aca gag gtt cgg aaa cag ca Snail II+III Rev: ctg tca aaa ctg tag acg ct
N-cadherin	Forw: aga aca gtc tcc aac tcg cc Rev: agg gag gga gag cgt gtg a
Cyclin D1	Forw: ggt ctt gtc cca ggc aga ggg gac t Rev: gcg gac tct gct gct cgg ctg ctt
IL-10	Forw: cca caa tca agg ttt ccc ggc Rev: cca cag ctg agg gcc tct gc