## Supplemental Fig. 1. R-proIL1β-V expression and processing

(A) NLRP3 inflammasome activation results in a progressive decrease in BRET signal of the sensor. BRET signals were measured in HEK293 cells overexpressing R-proIL1 $\beta$ -V in combination with NLRP3, ASC and caspase-1 after stimulation with nigericin (5  $\mu$ M). (B) BRET signal decrease is due to cleavage by caspase-1. Experiment was performed as described in (A) but cells expressed either R-proIL1 $\beta$ -V or R-proIL1 $\beta$ -D27A/D116A-V. Mutation of the two caspase-1 cleavage sites impairs the decrease on BRET signal observed for R-proIL1 $\beta$ -V after nigericin stimulation. Results are mean  $\pm$  s.e.m. of  $n \geq 3$  experiments and  $n \geq 3$  wells per experiment. (C) Transfection efficiency of immortalized BMDM, J774A.1 cell line and primary BMDM. 24 hours after the transfection with the BRET sensor, percentage of positive cells was determined after excitation of the Venus protein by microscopy. Results are mean  $\pm$  s.e.m. of  $n \geq 3$  transfections.

