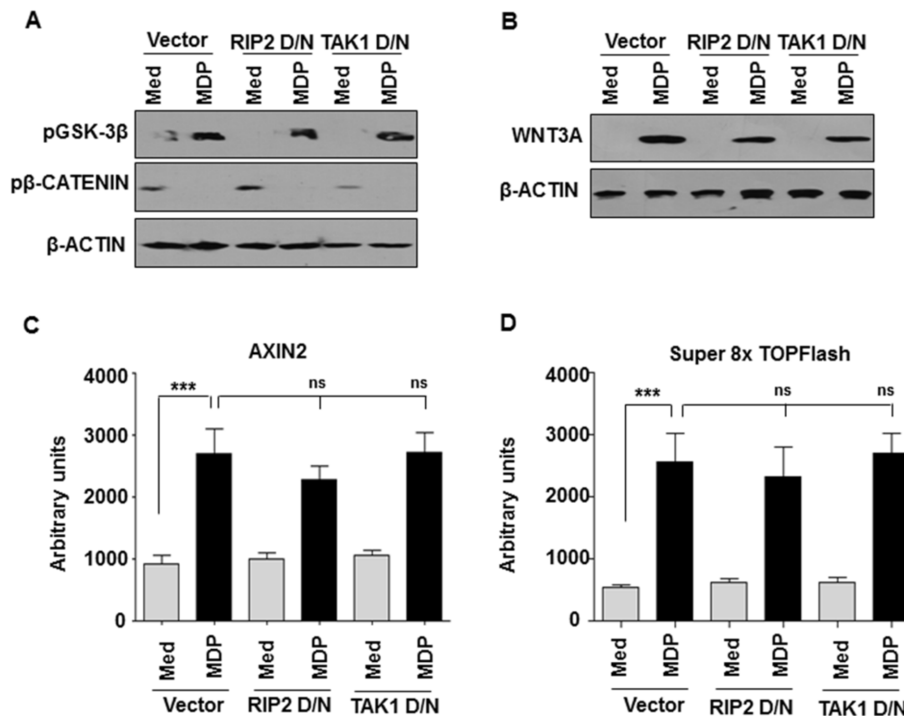
Supplementary Materials



Supplemental Figure S1. NOD2 activates WNT signaling pathway independent of RIP2-TAK1 axis**.** (**A-D**) Murine RAW 264.7 macrophages were transfected with RIP2 and TAK1 D/N constructs. 48 h post-transfection, cells were treated with 100 ng of MDP. Phosphorylation status of GSK-3β and β-CATENIN (A), expression of WNT3A (B) and luciferase activity of AXIN2 (C) and Super 8x TOPFlash (D) was analysed by immunoblotting and luciferase assay respectively. The immunoblotting data shown is representative of results obtained from 3 independent experiments. Luciferase assay data represents the mean ± SEM for 3 independent experiments; ***P < 0.0001 (one-way ANOVA). Med, Medium; D/N, dominant negative; ns, not significant.

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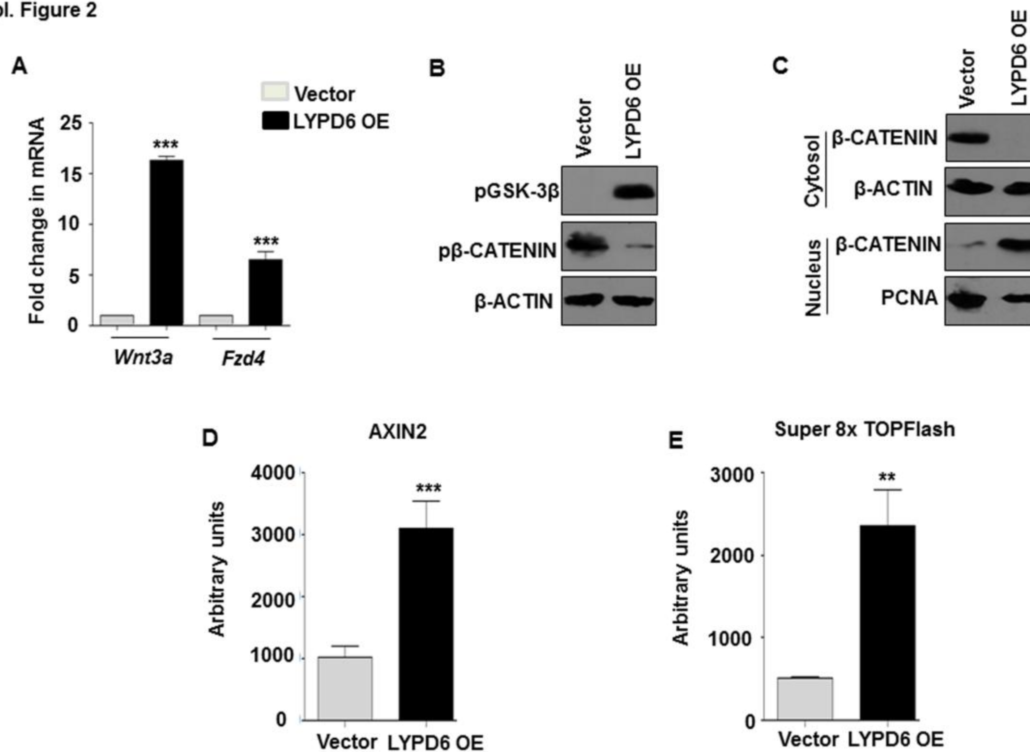

Supplemental Figure S2. NOD2-triggered WNT signaling activation is regulated by LYPD6. (**A- E**) RAW 264.7 macrophages were transfected with LYPD6 OE as indicated. 48 h post- transfection, *Wnt3a* and *Fzd4* transcript (A), phosphorylation status of GSK-3β and β-CATENIN (B), nuclear translocation of β-CATENIN (C), AXIN2 (D) or Super 8x TOPflash (E) luciferase activity was analysed by quantitative real-time RT-PCR, immunoblotting or luciferase assay respectively. The immunoblotting data shown is representative of results obtained from 3 independent experiments. Quantitative real-time RT-PCR data and luciferase data represents the mean ± SEM for 3 independent experiments; $^{**}P < 0.005$, $^{***}P < 0.0001$ (one-way ANOVA for A, *t*-test for D and E). Med, Medium; NT, nontargeting; OE, overexpression; D/N, dominant negative.

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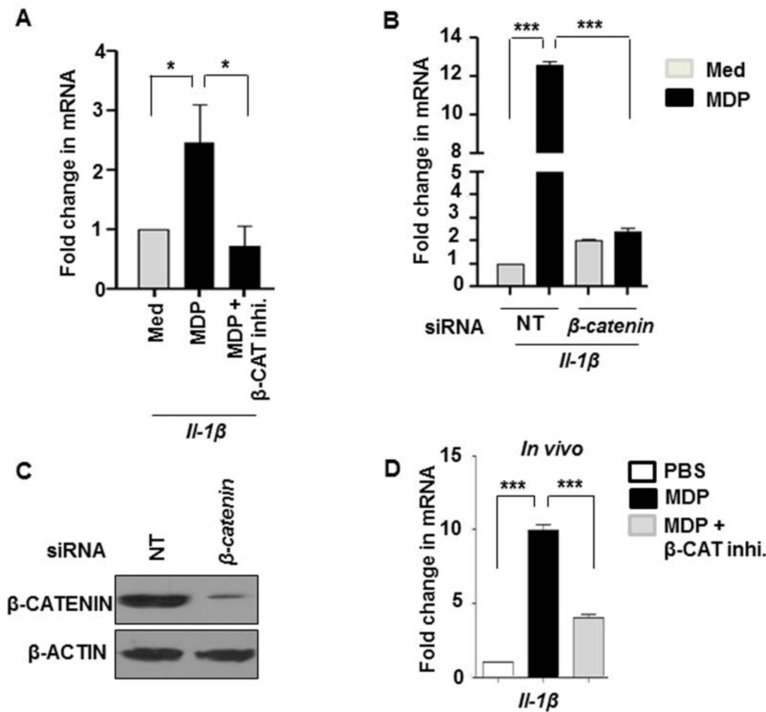

Supplemental Figure S3. WNT signaling orchestrates NOD2-induced inflammasome activation**.** (**A**) Mouse peritoneal macrophages were treated with β-CAT inhi for 1 h prior to treatment of MDP for 12 h and inflammasome activation was analysed by quantitative real-time RT-PCR. (**B**) RAW 264.7 macrophages were transfected with *β-catenin* siRNA followed by MDP treatment. Activation of inflammasome was assessed by quantitative real-time RT-PCR. (**C**) Validation of *β-catenin* siRNA. (**D**) Mice were intravenously challenged with β-CAT inhi. (50 mg/mice) for 2 h prior to MDP (100 μg/mice) injection for 12 h. mRNA level of *Il-1β* was analysed by quantitative real-time RT-PCR. The immunoblotting data shown is representative of results obtained from 3 independent experiments. Quantitative real-time RT-PCR data represents the mean ± SEM for 3 independent experiments; *$P < 0.05$, ***$P < 0.0001$ (one-way ANOVA). Med, Medium; NT, nontargeting; β-CAT inhi, β-CATENIN inhibitor.

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