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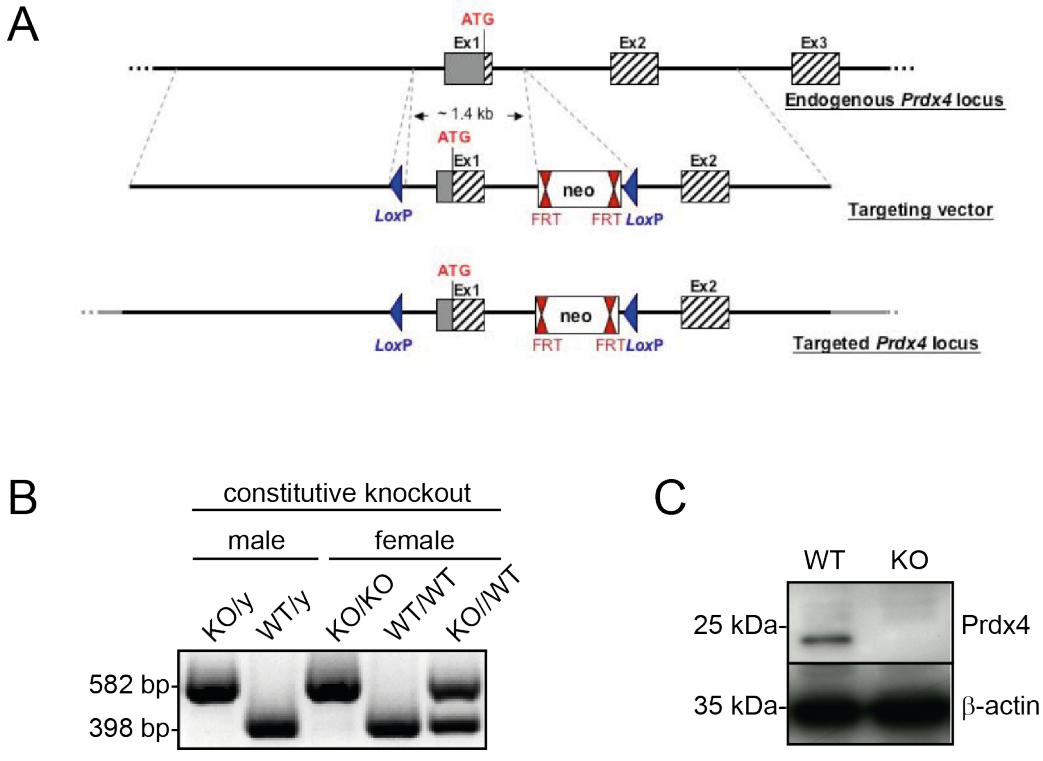
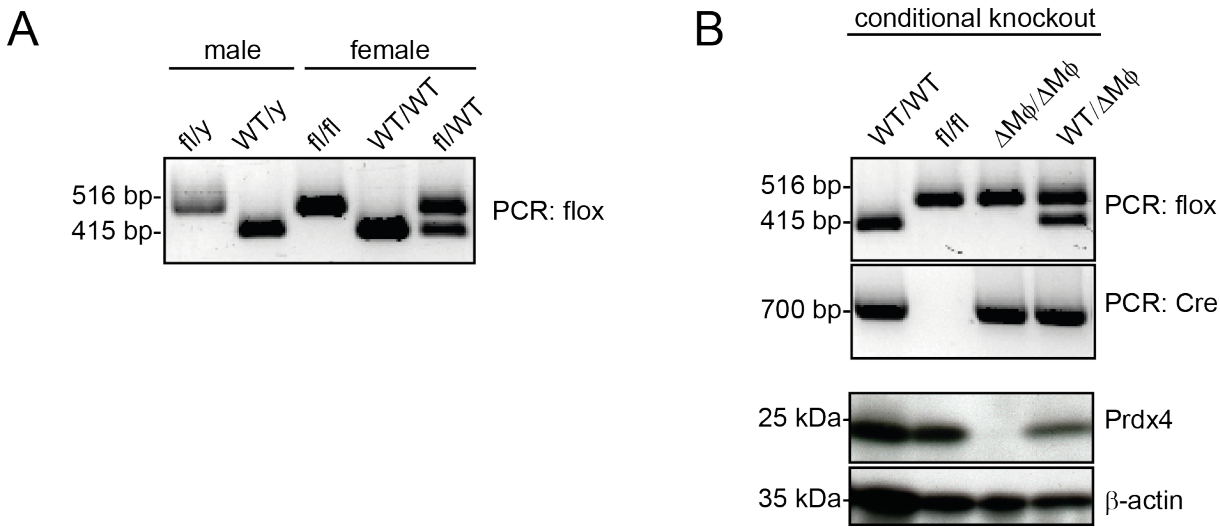


Appendix Figure S1. ***Generation of constitutive Prdx4-knockout mice*** (relates to **Fig.1**).

(**A**) Schematic representation of *Prdx4* targeting strategy resulting in the deletion of exon Hatched rectangles represent *Prdx4* coding sequences, grey rectangles indicate non-coding exon portions and solid line represents chromosome sequence. The ATG initiation codon is indicated. FRT sites are represented by double red triangles and LoxP sites by blue triangles. The size of the flanked *Prdx4* sequence to be deleted is shown. Diagram is not depicted to scale. Deletion of exon 1 resulted in the deletion of the ATG initiation codon and thus absence of transcription.

(**B**) PCR analysis of Prdx4 genotypes in constitutive knockout and wildtype mice.

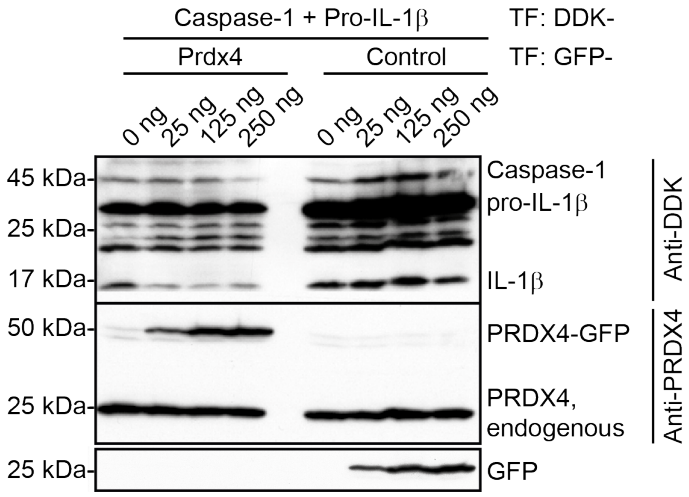
(**C**) Western blot analysis of Prdx4 and β-actin (loading control) in cell lysates from Prdx4 WT and KO BMDMs.



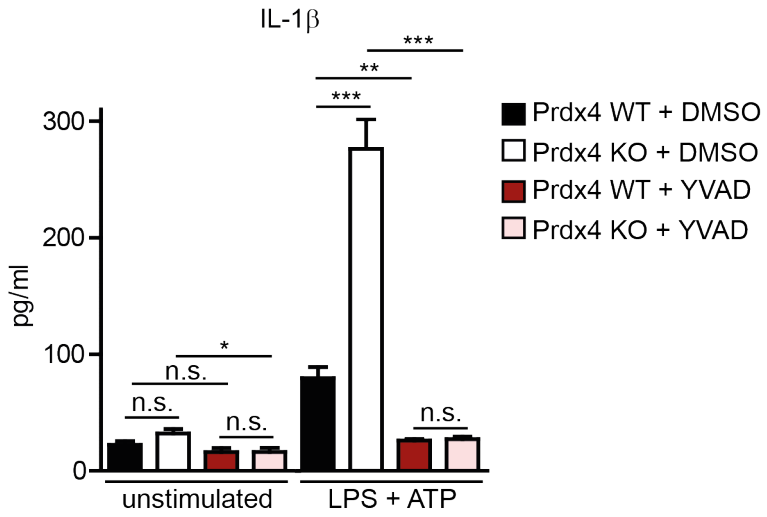
Appendix Figure S2. ***Generation of conditional Prdx4-knockout mice*** (relates to **Fig.2**).

(**A**) PCR analysis of genomic DNA from tail, isolated from Prdx4 WT and floxed mice.

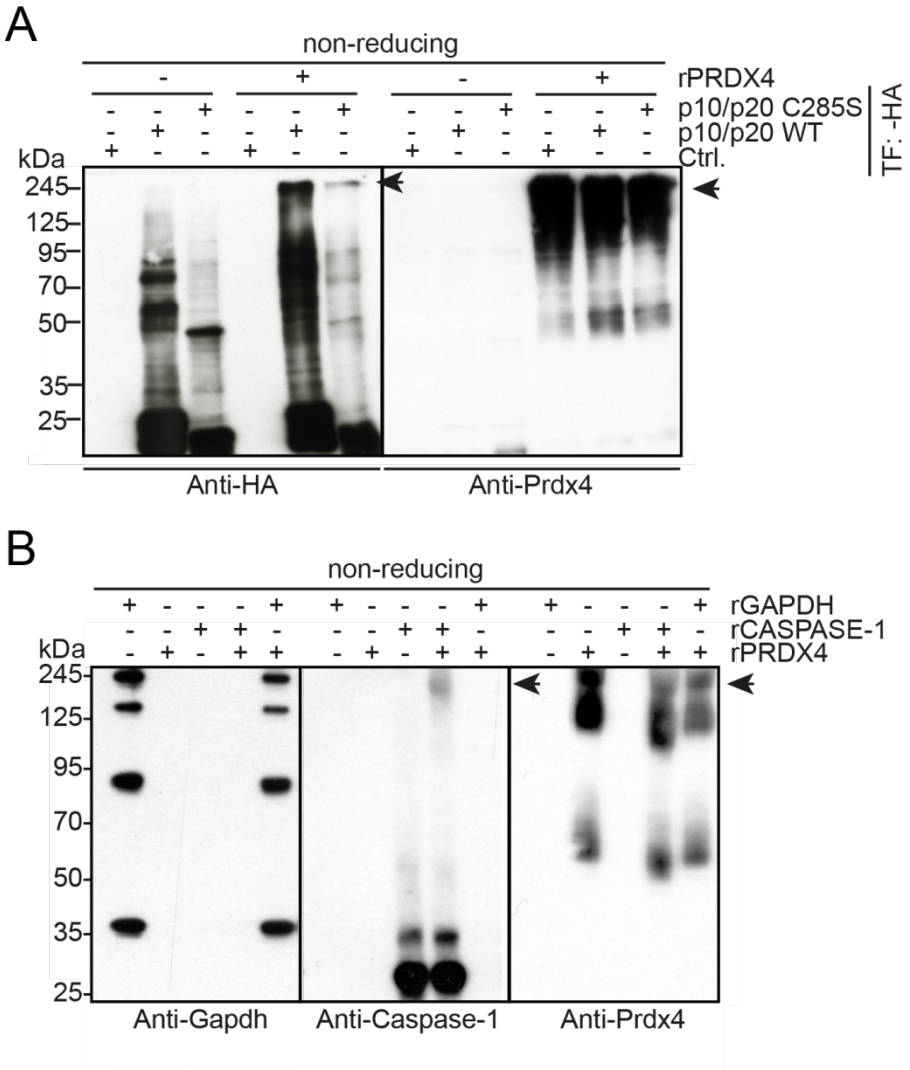
(**B**) PCR analysis of Prdx4 genotypes in conditional knockout and floxed mice (upper panel) and Western blot analysis (lower panel) for Prdx4 and β-actin (loading control), showing deletion of Prdx4 in BMDMs.



Appendix Figure S3. ***Prdx4 overexpression reduces levels of mature IL-1β*** (relates to **Fig.3**). Western Blot analysis of DDK-tagged Caspase-1, pro-IL-1β /IL-1β, as well as GFP-tagged Prdx4 or empty control in HEK cells. Cells were transiently transfected with NLRP3, ASC, DDK-tagged Caspase-1, pro-IL-1β and increasing amounts (0-250 ng as indicated) of Prdx4-GFP or empty GFP plasmid for 24 h before analysis. Data are representative of three experiments.



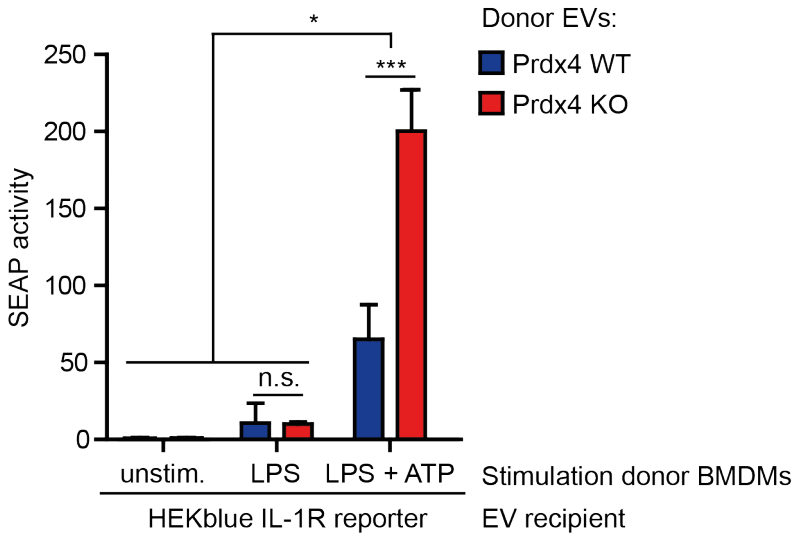
Appendix Figure S4. ***Caspase-1 inhibition by YVAD reduces IL-1β hypersecretion caused by Prdx4-deficiency*** (relates to **Fig.3**). Concentration of IL-1β in supernatants of Prdx4 WT and KO BMDMs pretreated with YVAD or DMSO as control and stimulated with LPS and ATP or left unstimulated. Each bar represents a mean of n=3 biological replicates, vertical lines indicate SD. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; n.s. not significant (two-tailed *t*-test).



Appendix Figure S5. ***Analysis of catalytically inactive p20 C285S active site mutant, rGAPDH and rPRDX4*** (relates to **Fig.4**).

(**A**) Western Blot analysis of HA-tagged WT caspase-1 and C285S active site mutation. Cells were transiently transfected with caspase-1 p10 plus p20 WT-HA and p10 plus p20 C285S-HA or empty HA as control. After cell lysis and purification of HA-tagged proteins, proteins were incubated for 1 h with recombinant Prdx4 or left untreated. SDS-PAGE was performed under non-reducing conditions and Western blot performed using antibodies to HA or to Prdx4.

(**B**) Non-reducing SDS-PAGE and Western blot analysis of rGAPDH, rCASP-1 and rPRDX4 that were incubated either individually or co-incubated as indicated.



Appendix Figure S6. ***Loss of Prdx4 leads to increased IL-1R reporter activity upon EV treatment*** (relates to **Fig.4**). SEAP activity in HEK-Blue IL-1R cells in response to stimulation with EVs derived from LPS, LPS and ATP or control-treated Prdx4 WT or KO BMDMs. Each bar represents a mean of n=6 replicates, vertical lines indicate SD. *, $p < 0.05$; ***, $p < 0.001$; n.s. not significant (two-tailed *t*-test).