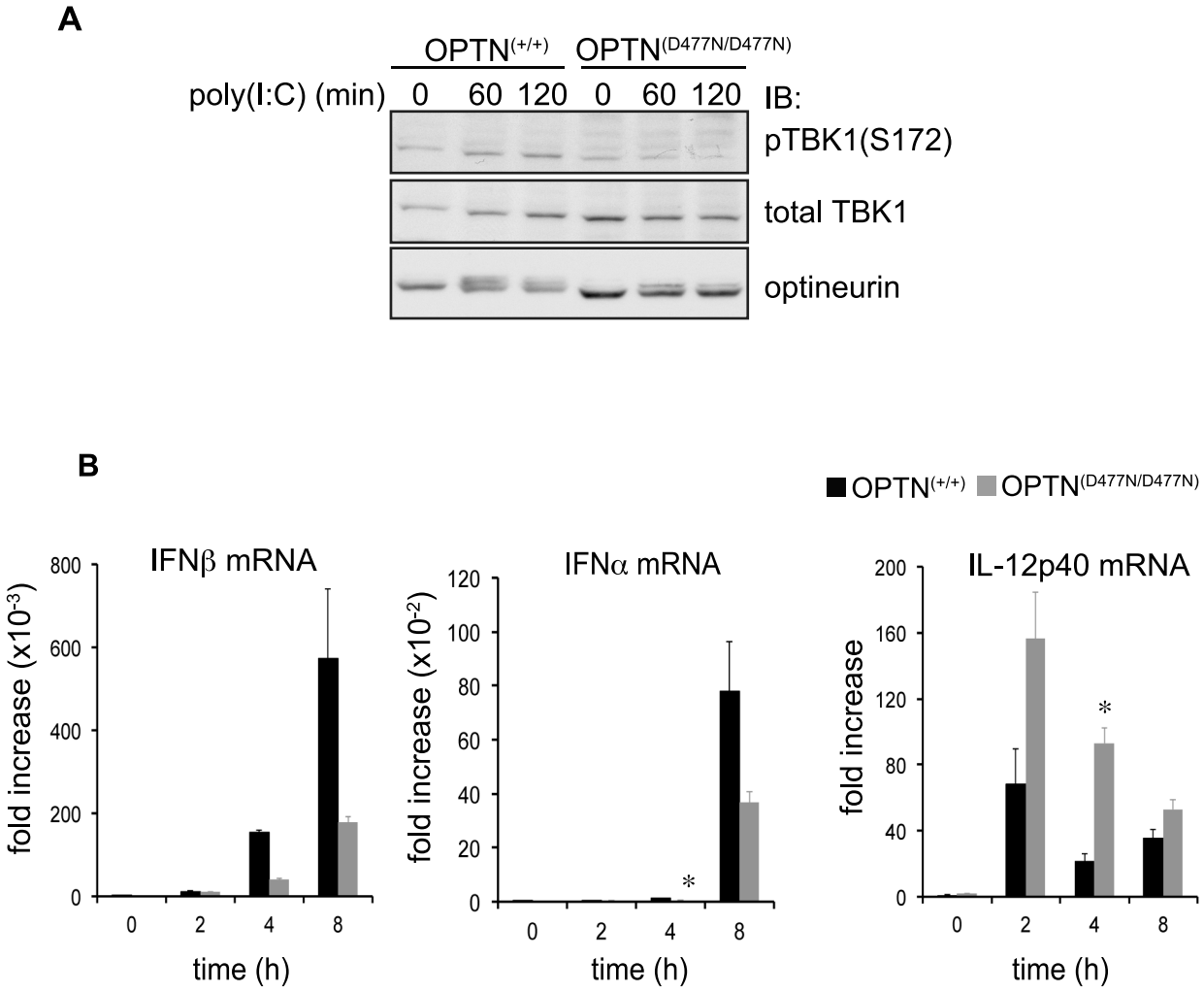
Table S1 Primers used for PCR based genotyping

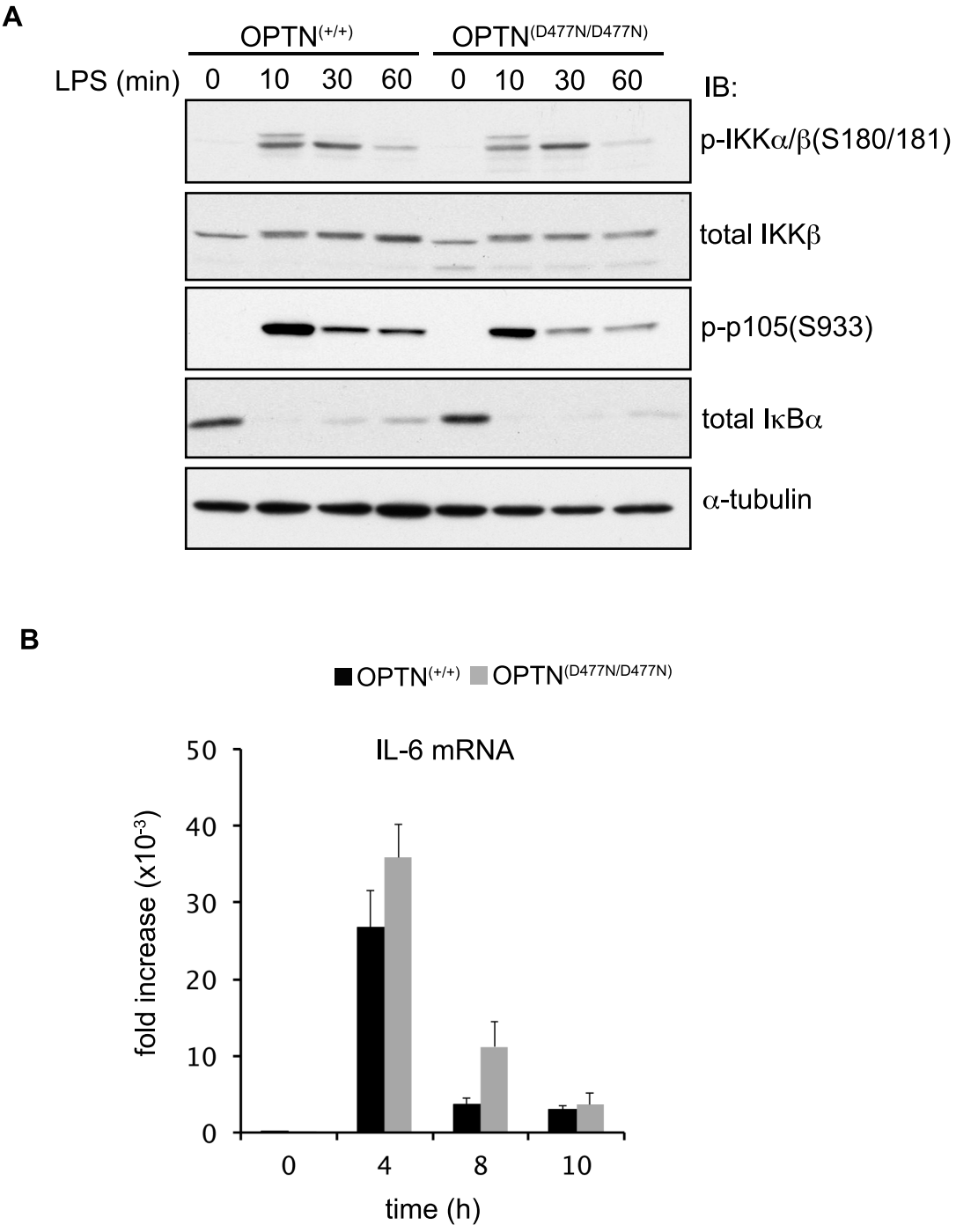
FLP PCR		
FLP sense	CAC CTA AGG TCC TGG TTC GTC A	FLP+ve band: 435bp
FLP antisense	CCC AGA TGC TTT CAC CCT CAC T	
OPTN D477N PCR		
OTPN D477N sense	GATCCGGAGGAGAGCAATGCATG	Wild-type band: ~300bp Knock-in band: ~400bp
OTPN D477N antisense	CTGTAATCTTAGTGTTCCACAGGC AG	

Supplementary Figure 1



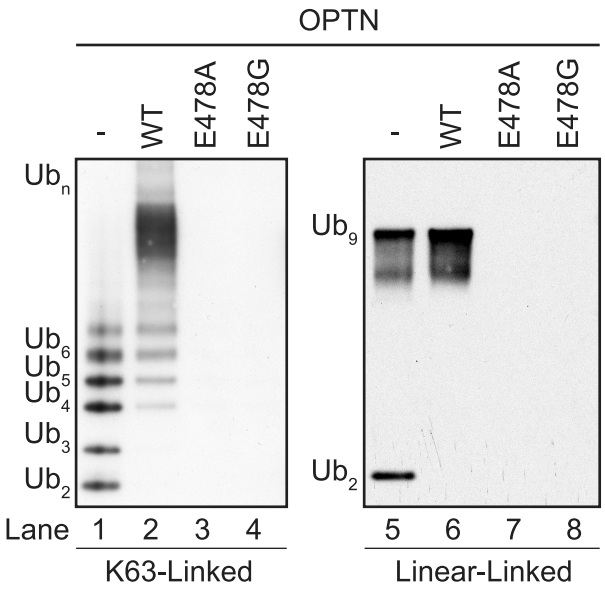
Supplementary Figure 1. Poly(I:C)-induced TBK1 phosphorylation and IFNβ production are diminished in OPTN$^{(D477N/D477N)}$ primary MEFs. (A) OPTN$^{(+/+)}$ and OPTN$^{(D477N/D477N)}$ primary MEFs (passage 3-6) were either mock transfected with lipofectamine alone (0 min) or with lipofectamine and 10 μg/ml poly(I:C) for 60 or 120 min, as indicated. Cell extracts (30 μg) were analyzed by immunoblotting for TBK1 phosphorylated at S172, total TBK1 and total OPTN. The results are representative of two separate experiments. **(B)** Primary MEFs were either mock transfected (0 h) or transfected for the times indicated with 10 μg/ml poly(I:C) as in (A). Total RNA was extracted and analyzed by quantitative RT-PCR for expression of IFNβ, IFNα and IL-12p40. mRNA levels were normalized for 18S rRNA expression and the results are presented as fold-increase relative to the mRNA levels present in wild-type unstimulated cells. Results are representative of 5 separate experiments with duplicate or triplicate determinations in each experiment. The error bars represent the mean +/- S.E.M. Statistical analysis was performed by one-way ANOVA with $p<0.05$ considered statistically significant (*).

Supplementary Figure 2



Supplementary Figure 2. LPS-stimulated NF-κB activation and IL-6 production is similar in BMDMs from OPTN$^{(D477N/D477N)}$ and wild-type mice. BMDMs from OPTN$^{(+/+)}$ and OPTN$^{(D477N/D477N)}$ knock-in mice were stimulated for the times indicated with 100 ng/ml LPS. **(A)** Cell extracts (30 μg) were analyzed by immunoblotting for the phopshoryation of IKKα/IKKβ, p105 and IκBα. α-tubulin was used as an additional loading control. Results are representative of three separate experiments. **(B)** Total RNA was extracted and analyzed by quantitative RT-PCR for expression of IL-6 mRNA. RNA levels were normalized for 18S rRNA expression and the results are presented as fold-increase relative to the mRNA levels present in unstimulated wild-type cells. Results are representative of two separate experiments with n=4 mice per genotype. The *error bars* represent the mean +/- S.E.M.

Supplementary Figure 3



Supplementary Figure 3. A mutation in optineurin that causes amyotrophic lateral sclerosis (ALS) in man abolishes binding to polyubiquitin chains.
The binding of wild type OPTN and OPTN mutants was performed as described in Methods and the legend to Fig 1A. Lanes 1 and 5 show, respectively, the preparations of the K63-linked polyubiquitin chains and the linear polyubiquitin (di-ubiquitin and nona-ubiquitin) oligomers used in the experiment. The K63-linked and linear polyubiquitin chains captured by OPTN are shown in Lanes 2 and 6, respectively. The OPTN[E478G] mutant that causes ALS (lanes 4 and 8), as well as the OPTN[E478A] mutant (lanes 3 and 7) did not bind to either K63-linked or linear polyubiquitin chains under the conditions used. The OPTN[E478A] mutant is equivalent to the NEMO[E315A] mutation that causes greatly increased susceptibility to mycobacteria in humans (Filipe-Santos, O., Bustamante, J., Haverkamp, M. H., Vinolo, E., Ku, C. L., Puel, A., Frucht, D. M., Christel, K., von Bernuth, H., Jouanguy, E., Feinberg, J., Durandy, A., Senechal, B., Chapgier, A., Vogt, G., de Beaucoudrey, L., Fieschi, C., Picard, C., Garfa, M., Chemli, J., Bejaoui, M., Tsolia, M. N., Kutukculer, N., Plebani, A., Notarangelo, L., Bodemer, C., Geissmann, F., Israel, A., Veron, M., Knackstedt, M., Barbouche, R., Abel, L., Magdorf, K., Gendrel, D., Agou, F., Holland, S. M., and Casanova, J. L. (2006) *J Exp Med* **203**, 1745-1759).