Supplemental material

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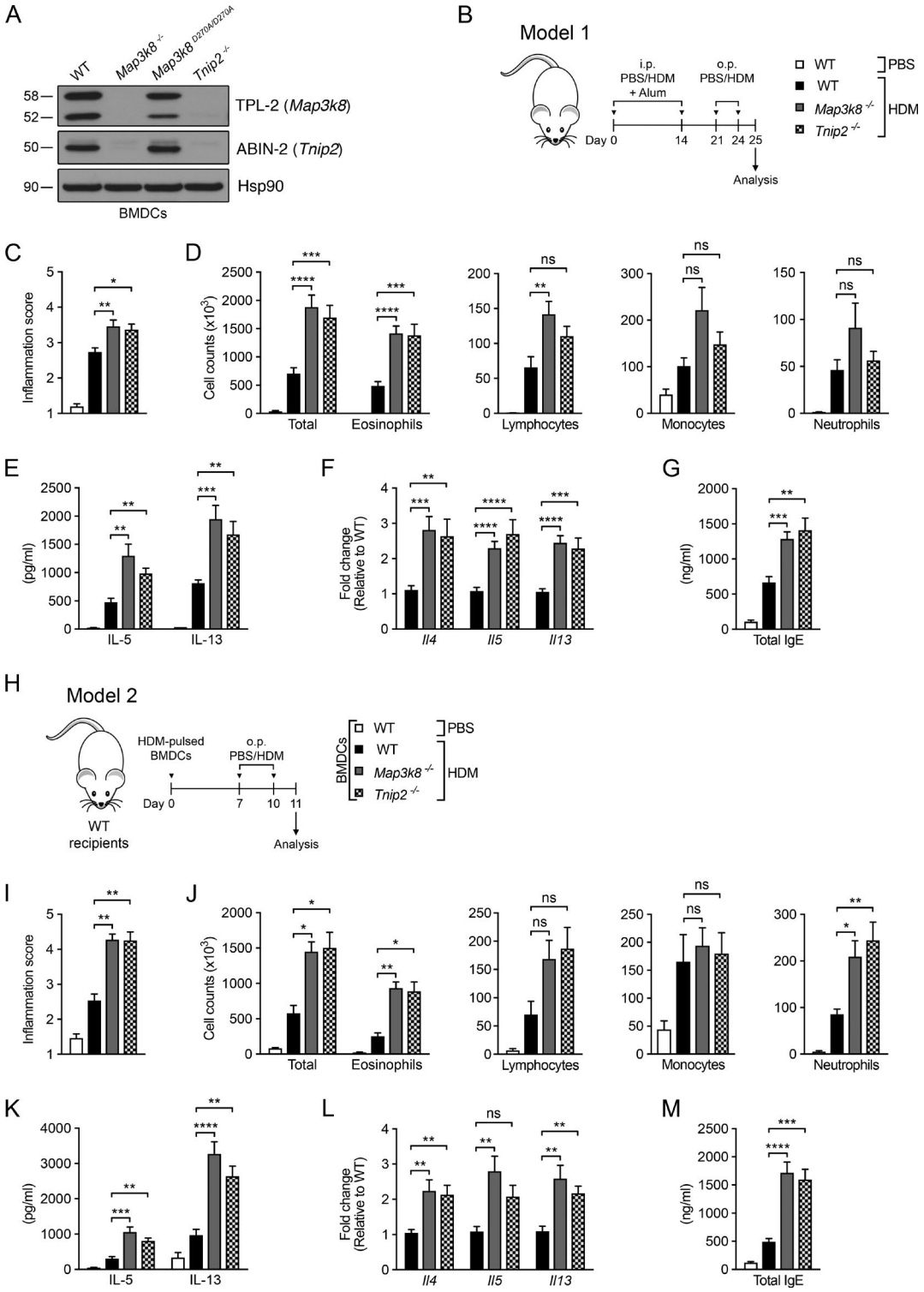

Figure S1. **Deletion of Tnip2 mimics Map3k8$^{-/-}$ phenotype in HDM-induced allergy. (A)** Lysates of BMDCs, generated from the indicated mouse genotypes, were immunoblotted for TPL-2 and ABIN-2. Hsp90 was used as loading control. **(B)** Schematic representation of oropharyngeal HDM sensitization and challenge model in WT, *Map3k8*$^{-/-}$, or *Tnip2*$^{-/-}$ mice. o.p., oropharyngeal. **(C)** Inflammation scores from PBS- and HDM-challenged mice. **(D)** Differential cell counts in BAL fluids of PBS- and HDM-challenged mice. **(E)** Cytokine levels in BAL fluid, as assessed by ELISA. **(F)** Cytokine mRNA expression levels in the lung, measured by qRT-PCR. **(G)** Total IgE levels in blood serum, as assessed by ELISA. **(H)** Schematic representation of BMDC adoptive transfer model of HDM-induced airway allergic inflammation (Model 2). o.p., oropharyngeal. **(I)** Inflammation scores from PBS- or HDM-challenged WT mice after adoptive transfer of HDM-pulsed WT, *Map3k8*$^{-/-}$, or *Tnip2*$^{-/-}$ BMDCs. **(J)** Differential cell counts in BAL fluids of BMDC adoptively transferred mice. **(K)** Cytokine levels in BAL fluid, as assessed by ELISA. **(L)** Cytokines mRNA expression levels in the lung, as assessed by qRT-PCR. **(M)** Total IgE levels in blood serum measured by ELISA. Data in panels C–G and I–M are shown as mean ± SEM and are pooled from three independent experiments (n = 10–15 mice/genotype). *, P < 0.05; **, P < 0.005; ***, P < 0.001; ****, P < 0.0001. Comparisons assessed by Kruskal-Wallis and Dunn-Bonferroni's post hoc test. ns, not significant.

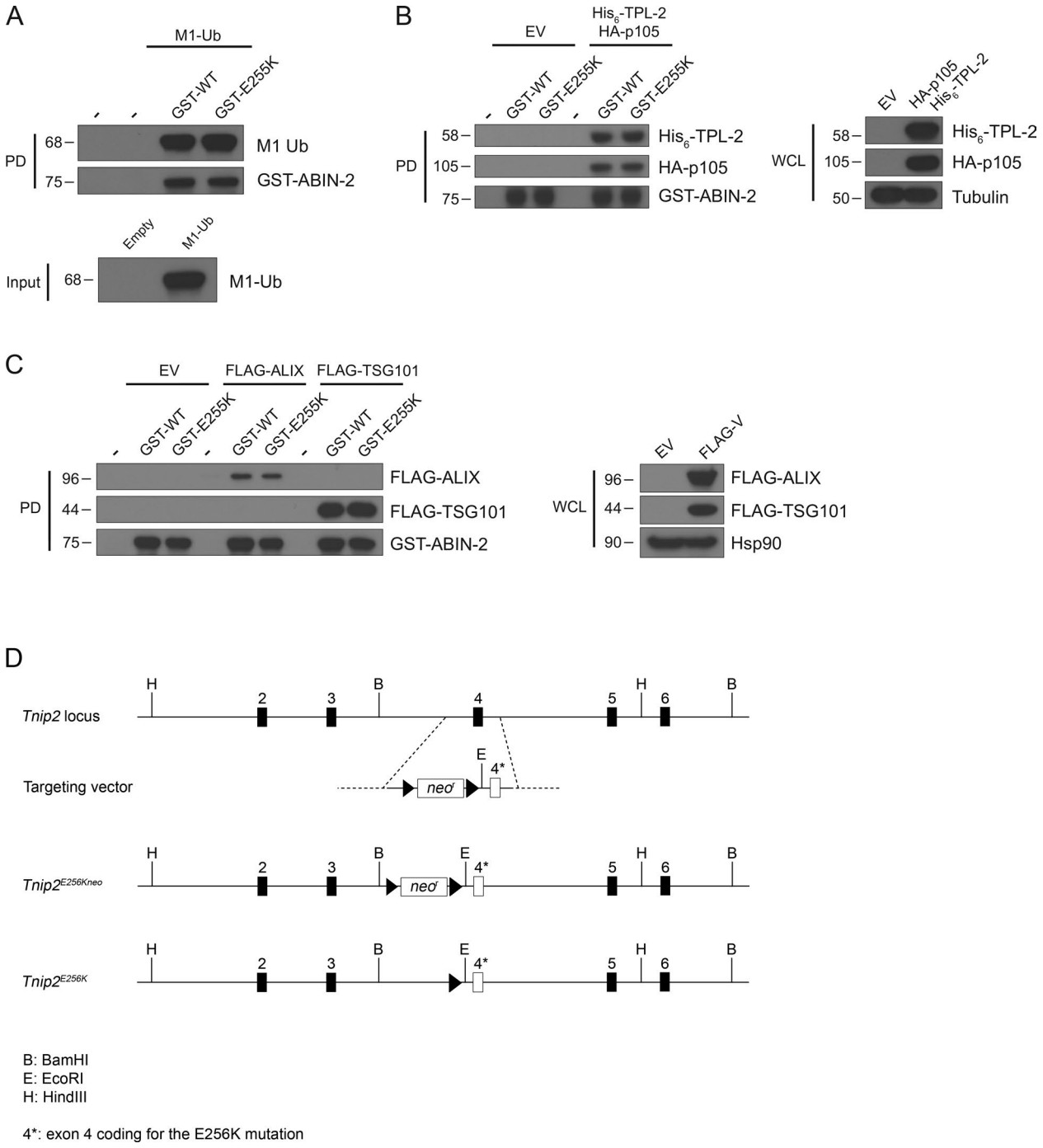

Figure S2. **TNIP2^{E255K} mutation impairs ABIN-2 binding to A20. (A)** Interaction of GST-ABIN-2 and GST-ABIN-2^{E256K} with M1-Ub chains was determined in pulldown assays. Data are representative of three independent experiments. **(B)** His_6-TPL-2 and HA-p105 were transiently coexpressed in HEK293 cells. Interaction of GST-ABIN-2 and GST-ABIN-2^{E256K} with His_6-TPL-2/HA-p105 complexes was determined in pulldown assays. Data are representative of two independent experiments. **(C)** FLAG-ALIX or FLAG-TSG101 were transiently expressed in HEK293 cells. Interaction of GST-ABIN-2 and GST-ABIN-2^{E256K} with these proteins was determined in pulldown assays. Data are representative of two independent experiments. EV, empty vector; PD, pulldown; WCL, whole cell lysate. **(D)** Schematic representation shows the genomic *Tnip2* locus, the targeting vector, and the mutated *Tnip2^{E256K}* allele. Boxes represent exons. The P381-6.1 *Tnip2^{E256K}*-targeting construct was generated by Gene Bridges GmbH. The FRT-flanked neomycin resistance cassette (PGK-gb2-neo) was inserted in intron 3. Exon 4 encoding E256 was mutated to generate an E256K coding allele, incorporating a new EcoRI restriction site. The final targeting vector was linearized with SalI and electroporated into C57BL/6 ES cells by PolyGene AG. An embryonic stem cell clone (5C4) bearing the E256K mutation was injected into C57BL/6 blastocysts by standard techniques. Male chimeras were bred to C57BL/6J female mice, and progeny was screened by digestion of genomic DNA with HindIII or BamHI, followed by Southern blotting with 5′- and 3′-probes, respectively. This allowed the targeted *Tnip2* alleles to be discriminated from the endogenous *Tnip2* allele. *Tnip2$^{E256K/E256K}$* mice were then crossed with FlpE^{+} mice for removal of the PGK-gb2-neo sequence.

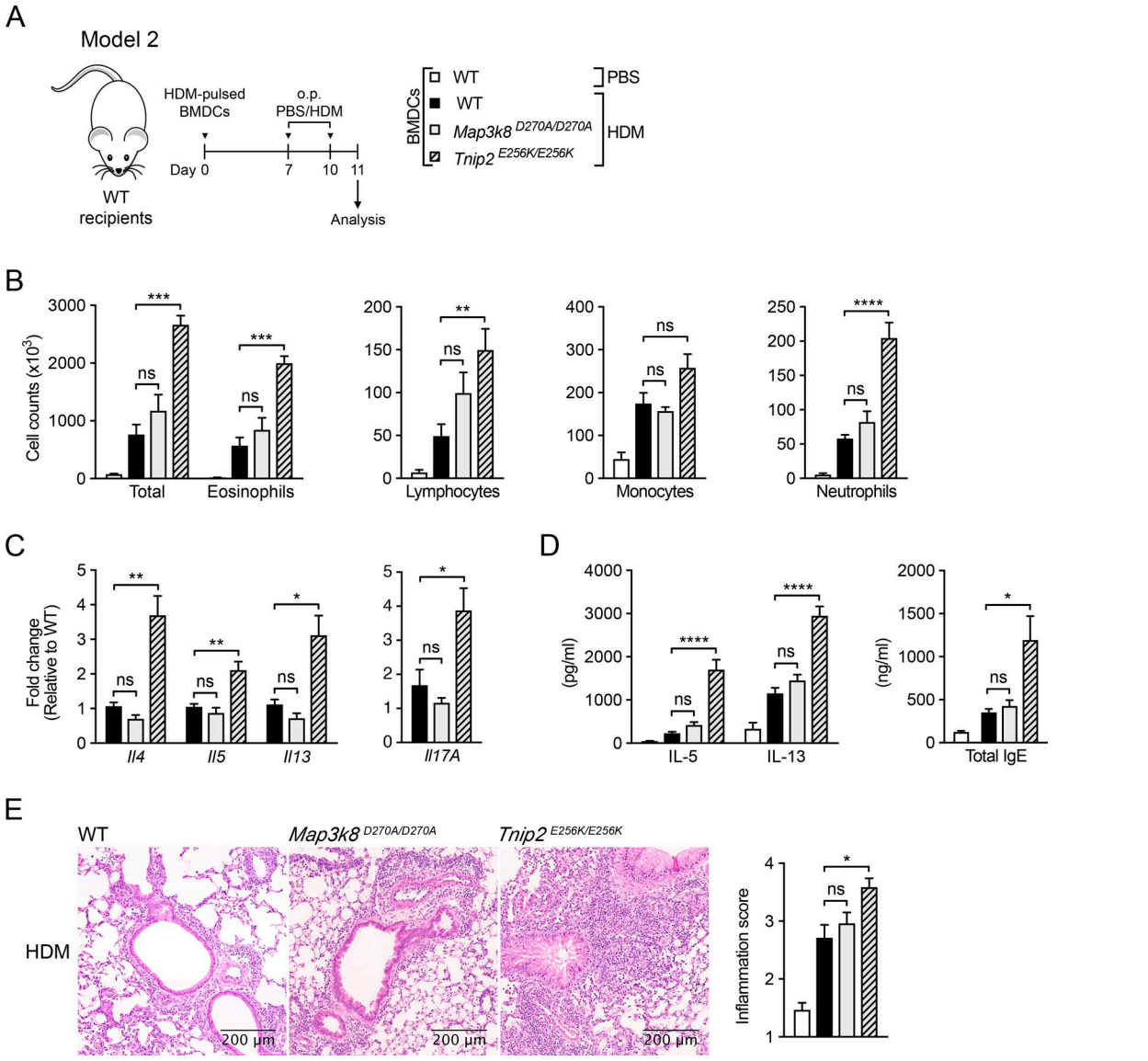

Figure S3. **HDM-pulsed Tnip2$^{E256K/E256K}$ BMDCs induce a severe airway allergic response. (A)** Schematic representation of the BMDC adoptive transfer model of HDM-induced airway allergic inflammation (Model 2). o.p., oropharyngeal. **(B)** Differential cell counts in BAL fluids from PBS- or HDM-challenged WT mice after adoptive transfer of HDM-pulsed WT, *Map3k8*$^{D270A/D270A}$, or *Tnip2*$^{E256K/E256K}$ BMDCs. **(C)** Cytokine mRNA expression levels in the lung, as assessed by qRT-PCR. **(D)** Total IgE levels in blood serum, IL-5, and IL-13 levels in BAL fluid, as assessed by ELISA. **(E)** H&E-stained lung sections (left panel) and inflammation scores (right panel) from PBS and HDM-challenged BMDC adoptively transferred mice. Data in panels B–E are shown as mean ± SEM and are pooled from three independent experiments (n = 9–11 mice/genotype). *, P < 0.05; **, P < 0.005; ***, P < 0.001; ****, P < 0.0001. Comparisons assessed by Kruskal-Wallis and Dunn-Bonferroni's post hoc test. ns, not significant.