Supporting Information

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SI Text

Generation of *Ptpn11*$^{N308D/+}$ Mice. The N308D mutation and a unique EcoRV site were introduced by site-directed mutagenesis. To construct the N308D targeting vector, a long arm, containing *Ptpn11* exon 10 (SalI genomic fragment), and a short arm, which includes *Ptpn11* exons 8 and 9 (NotI genomic fragment), were ligated into the vector PGK Neo-HSV-1 TK, as in the generation of D61G/+ mice (1). The targeting vector was linearized with SacII and was used to electroporate J1 ES cells (129Sv background). DNA was isolated from doubly G418/gancyclovir-resistant clones and screened for proper targeting by PCR. The 5′ primer was positioned outside the targeting vector, and the 3′ primer was positioned within the short arm. PCR products were digested with EcoRV to screen for positive clones. Homologous recombination was confirmed by Southern blotting using Neo and external probes. Detailed information about PCR and Southern blotting conditions is available from T.A. upon request.

Correctly targeted ES cells were microinjected into C57BL/6 blastocysts, and the resulting chimeras were mated with CMV-Cre mice on C57BL/6 background (provided by Klaus Rajewsky, Center for Blood Research, Boston), to remove the Neo cassette. Germline transmission was obtained, and N308D/+ offspring were crossed to WT B6 × 129Sv mice to remove the CMV-Cre locus.

Bone Stains. Skulls from 8-week-old mice were stained with Alcian blue and Alizarin red S, followed by treatment with 1.8% and 0.3% potassium hydroxide. Morphometric measurements of skull bones were quantified as described previously (http://craniofacial.jax.org/standard_protocols.html).

Colony Assays. Myeloid colony assays were performed on bone marrow cells from 8-week-old mice as described previously (2).

Protein Tyrosine Phosphatase Assays. For protein tyrosine phosphatase (PTP) assays, lysates were immunoprecipitated with anti-Shp2 antibodies (Santa Cruz Biotechnology) as described (3). Immune complexes were washed twice with PTP reaction buffer (20 mM Hepes, pH 7.4, 150 mM NaCl, 5 mM DTT, and 1 mM EDTA) without pNPP; then PTP reaction buffer with 20 mM pNPP was added, and A_{410} was measured after 1 h incubation at 37 °C.

1. Araki T, et al. (2004) Mouse model of Noonan syndrome reveals cell type- and gene dosage-dependent effects of Ptpn11 mutation. *Nat Med* 10:849–957.
2. Sattler M, et al. (2002) Critical role for Gab2 in transformation by BCR/ABL. *Cancer Cell* 1:479–492.
3. Kontaridis MI, Swanson KD, David FS, Barford D, Neel BG (2006) PTPN11 (Shp2) mutations in LEOPARD syndrome have dominant negative, not activating, effects. *J Biol Chem* 281:6785–6792.
4. Keilhack H, David FS, McGregor M, Cantley LC, Neel BG (2005) Diverse biochemical properties of Shp2 mutants. Implications for disease phenotypes. *J Biol Chem* 280:30984–30993.

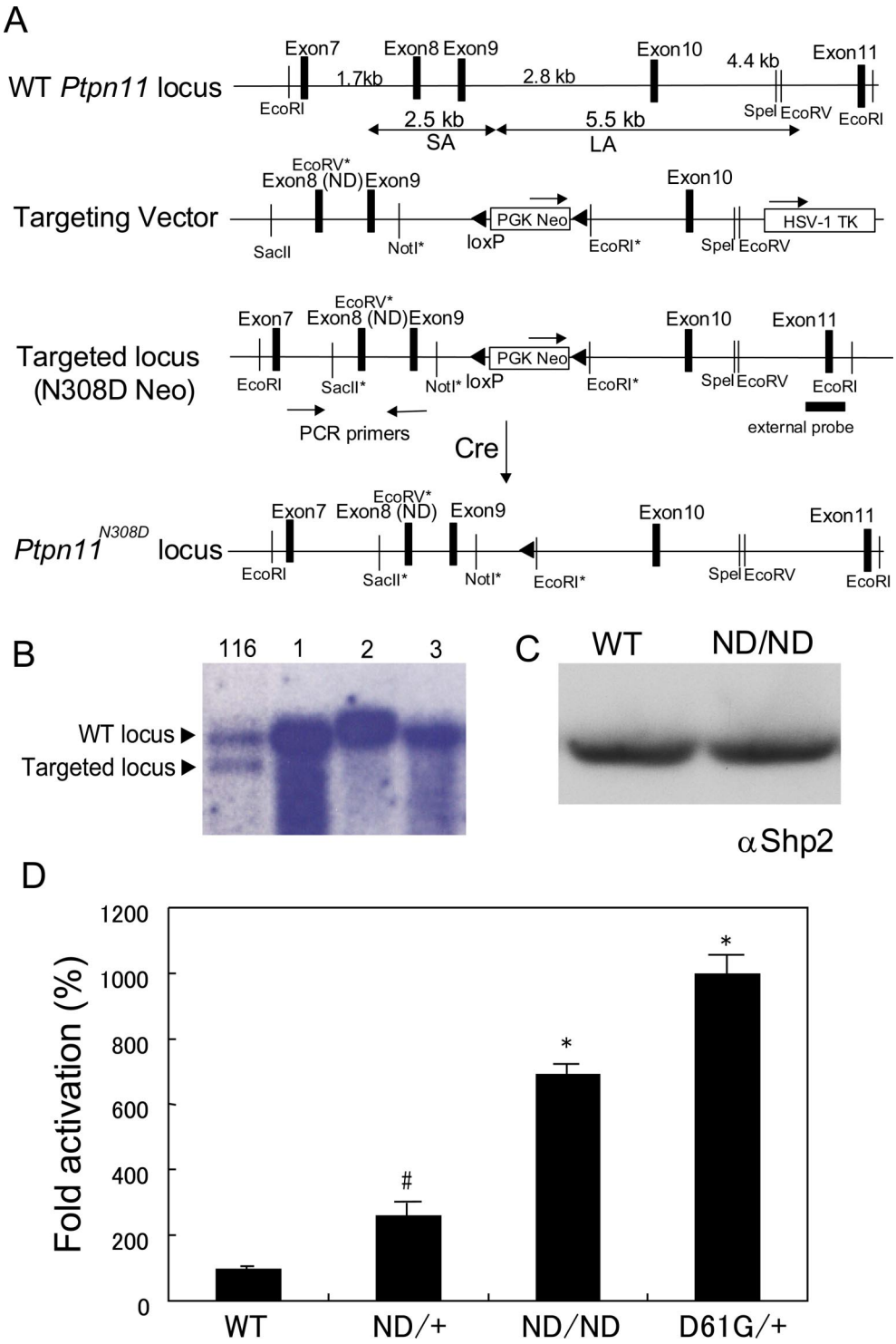


Fig. S1. Generation of *Ptpn11*$^{N308D/+}$ (ND/+) mice. (*A*) Structure of the *Ptpn11* locus, targeting vector, and mutant allele. Protein coding region, loxP sites, PCR primers, and Southern blot external probe are shown. (*B*) Southern blots of ES clones. An exon 11 probe detects a 15-kb EcoRI fragment in the genomic locus and a 13-kb fragment representing the mutant allele of the initial recombinant. (*C*) Shp2 immunoblot of brain lysates from WT and *Ptpn11*$^{N308D/N308D}$ (ND/ND) mice. The same amount of total protein (10 μg) was loaded in each lane. Note that the amount of mutant protein is comparable to WT. (*D*) Immune complex PTP assays of brain lysates. Values are mean $\pm$ SD of triplicates. Statistical significance (compared with WT) was assessed by 2-tailed student's *t* test. #, $P < 0.01$; *, $P < 0.001$. Note that PTP activity was increased by 2.5- and 6-fold in tissue from *Ptpn11*$^{N308D/+}$ (ND/+) and *Ptpn11*$^{N308D/N308D}$ (ND/ND) mice, respectively, consistent with biochemical data using recombinant N308D protein (*4*).

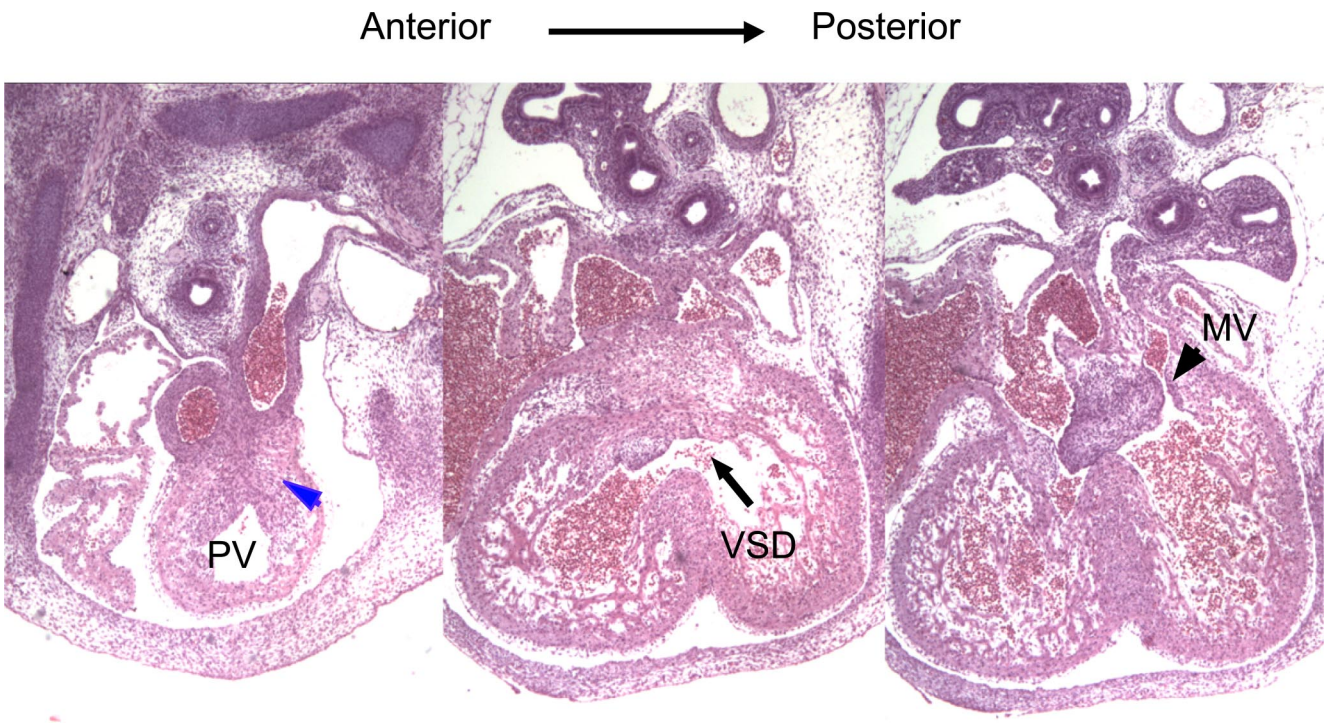


Fig. S2. Effects of *Ptpn11*^{D61Y} expression in epiblast. Heart sections from E13.5 embryos with epiblast-specific expression of D61Y using Mox2-Cre mice. Note the VSD (*black arrow*) and hypertrophy of pulmonary (*blue arrowhead*) and AV valves (*black arrowhead*).

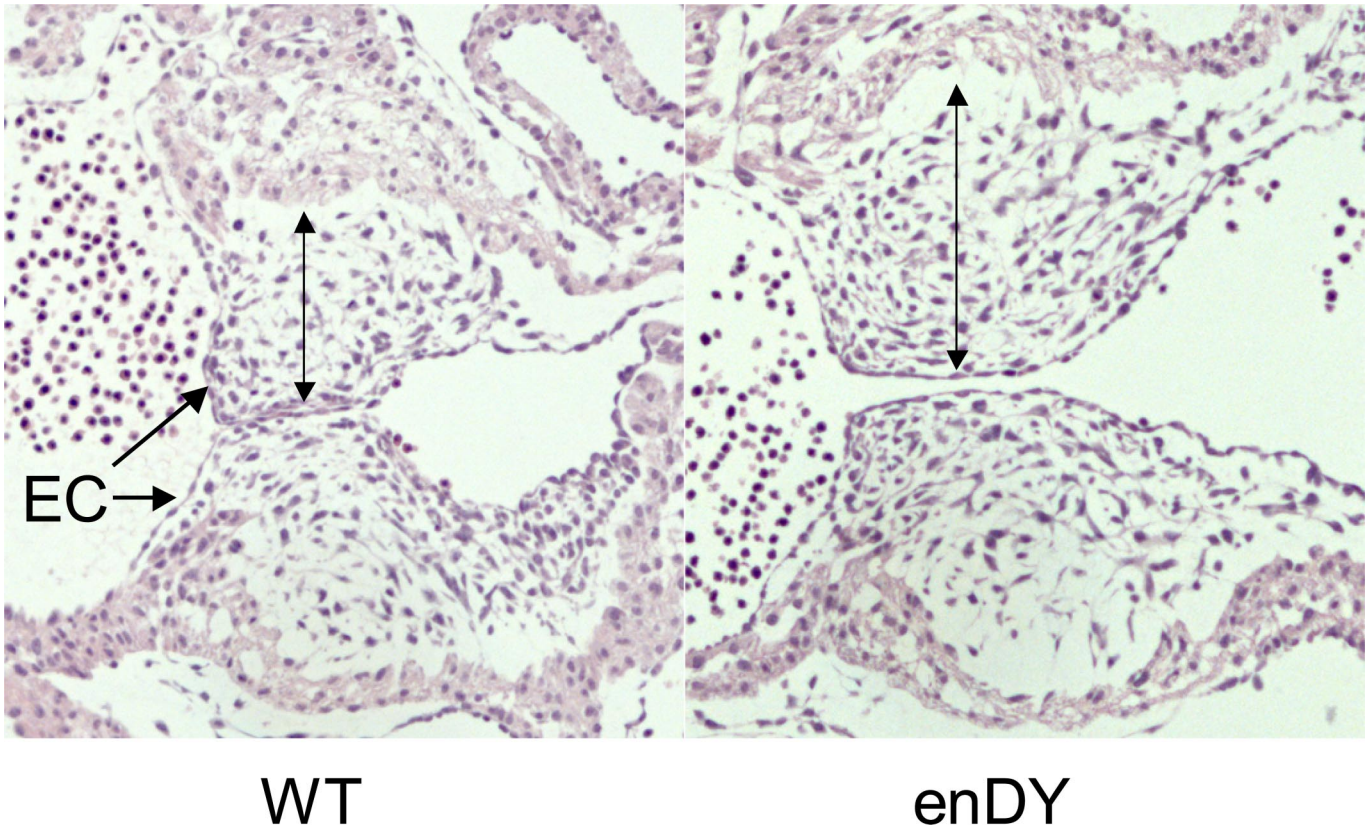
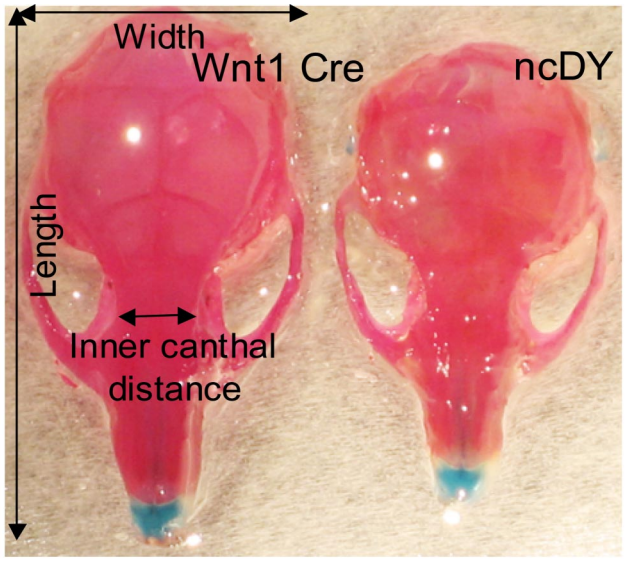


Fig. S3. Enlarged endocardial cushions of ecDY embryos at E11.5. Arrows indicate width of endocardial cushions. Note also that a larger number of sections (≈10: 4-μm thickness of paraffin sections) were obtained from ecDY embryos than from WT embryos, indicating cushion hypertrophy.



Genotype	Length (mm)	Width (mm)	Inner canthal distance (mm)
Wnt1 Cre	25.0 ± 0.6	13.8 ± 0.2	3.7 ± 0.2
Wnt1 Cre DY	22.7 ± 0.9 *	13.2 ± 0.6	4.0 ± 0.1 #

Fig. S4. Facial abnormalities in ncDY mice. Alcian blue/Alizarin red-stained skulls of Wnt1-Cre (control) and ncDY mice. Length, width, and inner canthal distances of skulls from 8-week-old WT and ncDY male mice were measured as described in http://craniofacial.jax.org/standard_protocols.html (*Lower*). Values are mean ± SD. $n = 6$. Statistical significance was assessed by 2-tailed student's *t* test, *, # $P < 0.05$.

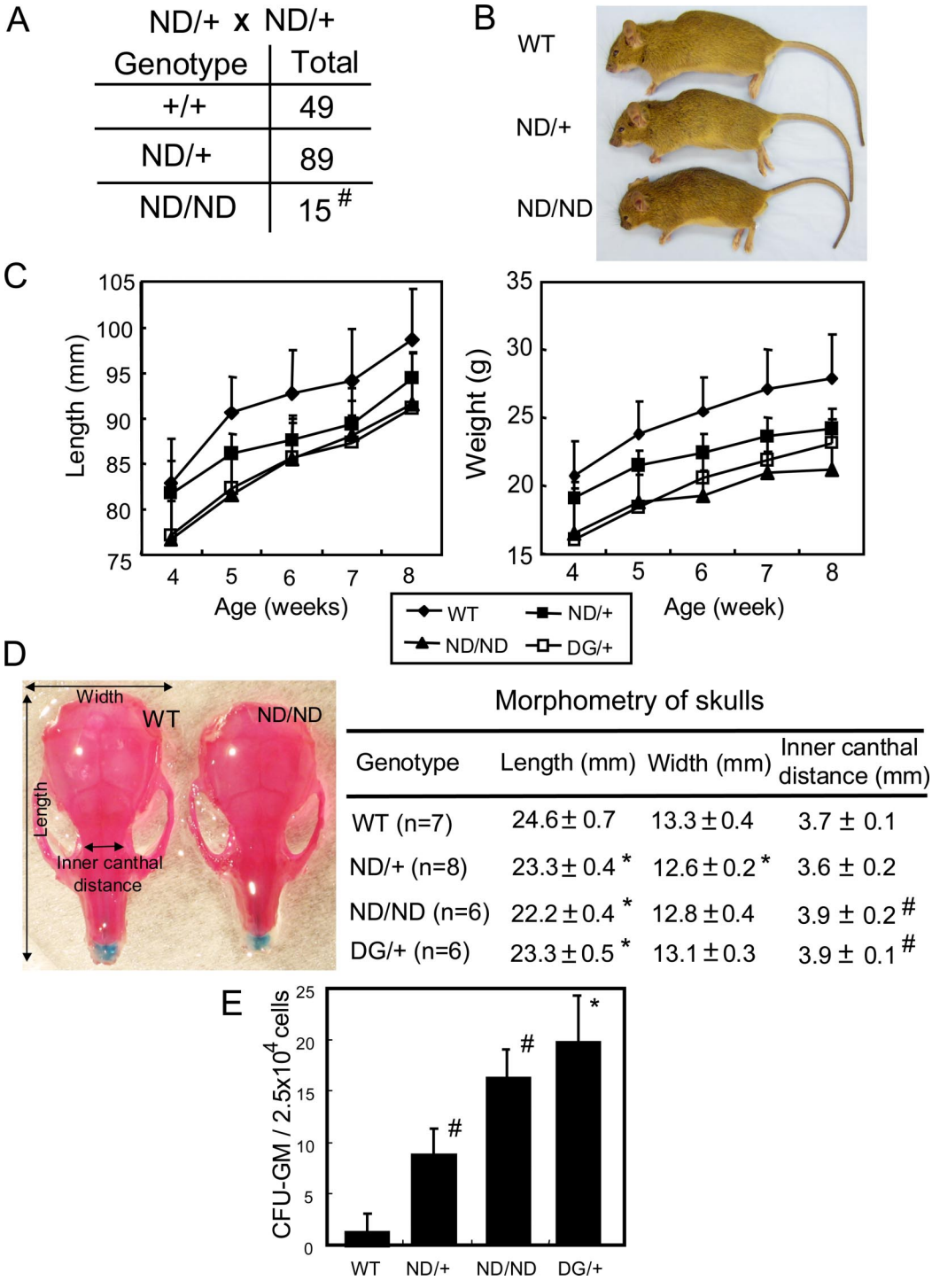


Fig. S5. Genotype/phenotype correlations in surviving WT, *Ptpn11*$^{N308D/+}$, *Ptpn11*$^{N308D/N308D}$, and *Ptpn11*$^{D61G/+}$ mice. (*A*) Progeny from *Ptpn11*$^{N308D/+}$ intercross. Genotypes (at 3 weeks) were determined by PCR. Deviation from Mendelian frequency was assessed by χ^2 test. #, $P < 0.05$). ND/+, *Ptpn11*$^{N308D/+}$; ND/ND, *Ptpn11*$^{N308D/N308D}$. (*B*) Representative appearances of WT, *Ptpn11*$^{N308D/+}$ (ND/+), and *Ptpn11*$^{N308D/N308D}$ (ND/ND) mice at 8 weeks. (*C*) Growth curves of WT, *Ptpn11*$^{N308D/+}$ (ND/+), *Ptpn11*$^{N308D/N308D}$ (ND/ND), and *Ptpn11*$^{D61G/+}$ male mice. Differences between WT and ND/ND or WT and DG/+ were significant at all times. $P < 0.05$ for length and weight, assessed by repeated-measure ANOVA; Newman-Keuls multiple range test. Differences between WT and ND/+ were significant only at 8 weeks. (*D*) Facial characteristics of WT, *Ptpn11*$^{N308D/+}$ (ND/+), *Ptpn11*$^{N308D/N308D}$ (ND/ND), and *Ptpn11*$^{D61G/+}$ (DG/+) mice. Alcian blue/Alizarin red-stained skulls of WT and ND/ND (*Left*). Morphometric characteristics of WT, ND/+, ND/ND, and DG/+ skulls (*Right*). Statistical significance was assessed by 1-way ANOVA; Newman-Keuls multiple range test. #, $P < 0.05$; *, $P < 0.01$. (*E*) Myeloid colony assays in the absence of cytokines. BM cells were prepared from the indicated mice at 8 weeks and plated in methylcellulose-based media without cytokines. Values are mean ± SD of triplicates. Statistical significance compared with WT was assessed by 1-way ANOVA; Newman-Keuls multiple range test. #, $P < 0.01$; *, $P < 0.001$.