Low dose dasatinib rescues cardiac function in Noonan syndrome

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Supplemental Data:

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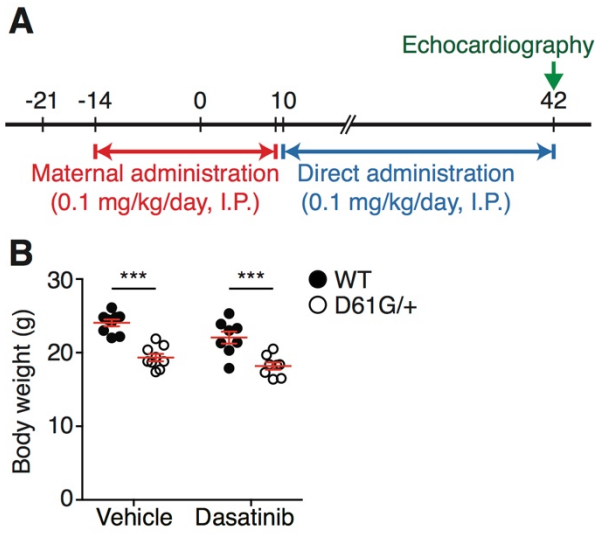
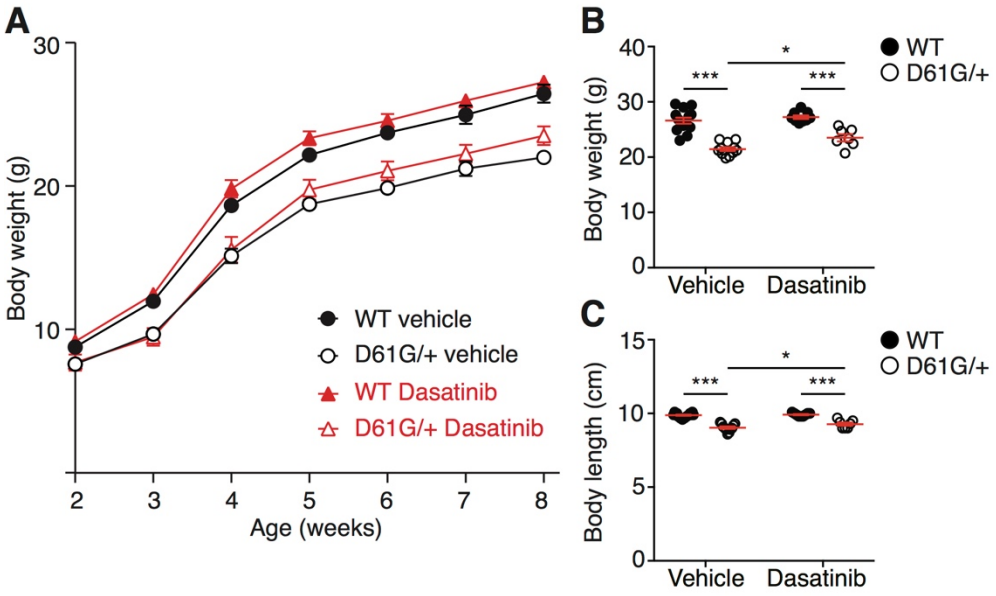
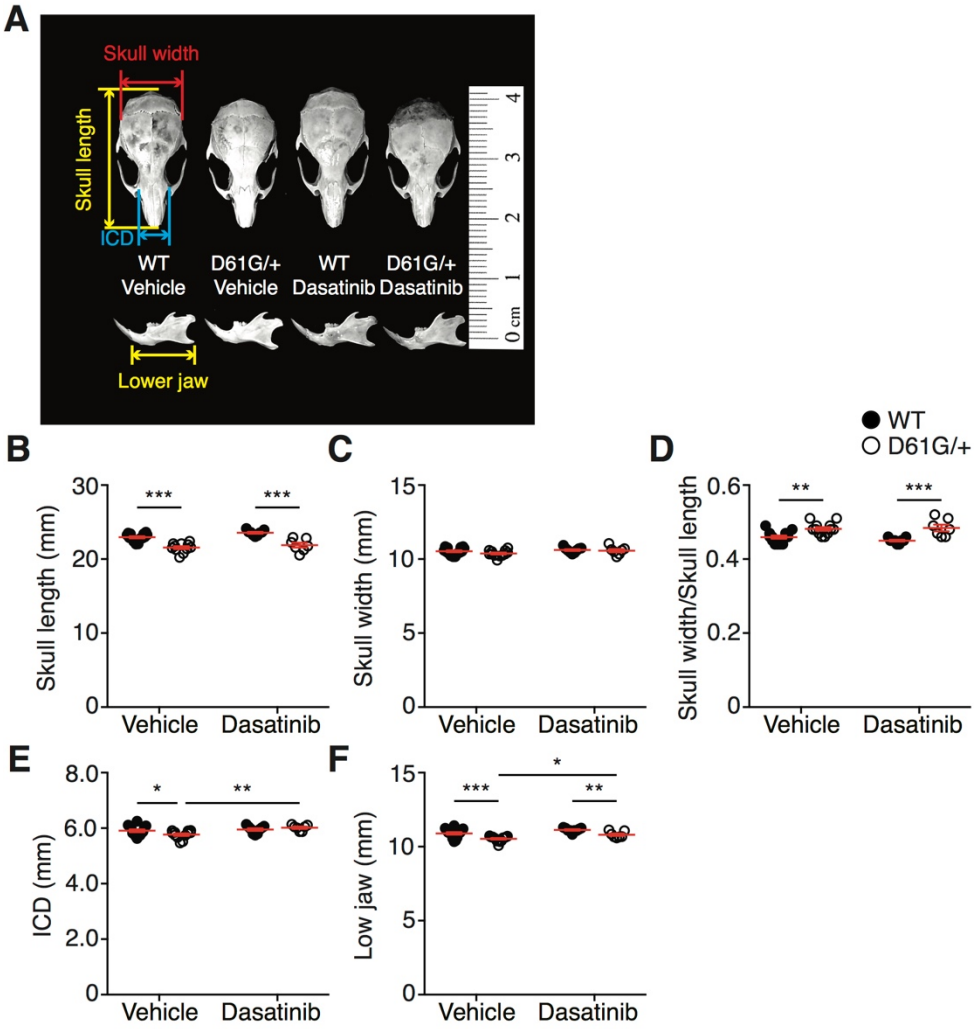


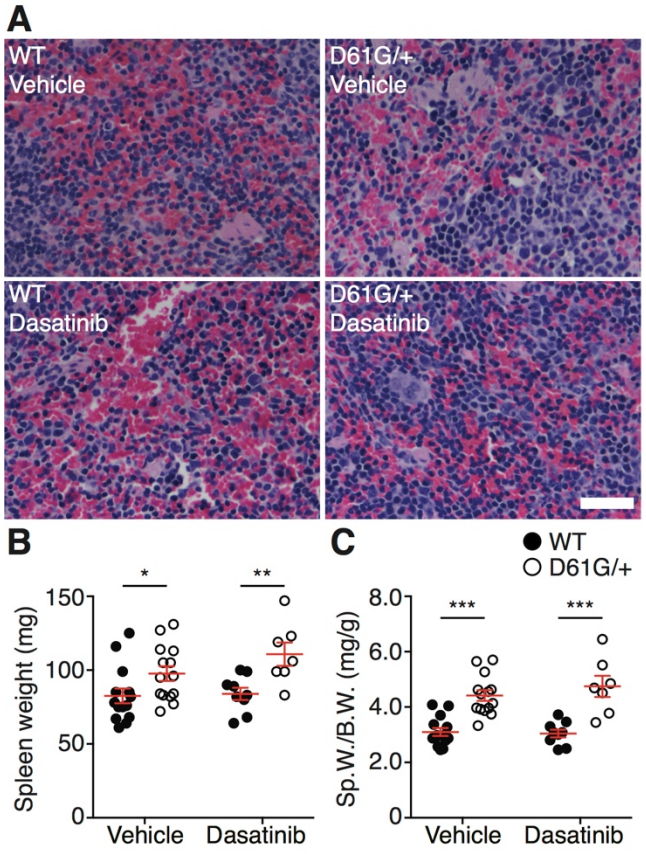
Supplemental Figure 1. Prenatal dasatinib administration into NS mice. (A) Dasatinib was injected i.p. (0.1 mg/kg body weight) into pregnant mice daily, beginning on gestational day 7.5 (E7.5) continuing (in nursing females) until postnatal day 9. Vehicle-injected mice served as a control. Beginning at P10, dasatinib or vehicle alone was injected (i.p.) directly into pups daily, until 6-weeks after birth. Functional cardiac parameters were examined by echocardiography at P42 (**Supplemental Table 4**). (**B**) Body weight were measured at P42. (n = 9 for vehicle-treated WT mice; n = 9 for vehicle-treated NS mice; n = 8 for dasatinib-treated WT mice; n = 8 for dasatinib-treated NS mice). Data represent mean ± SEM. Data were analyzed with two-way analysis of variance (ANOVA) and Tukey's multiple comparison test. ***, $p < 0.001$.



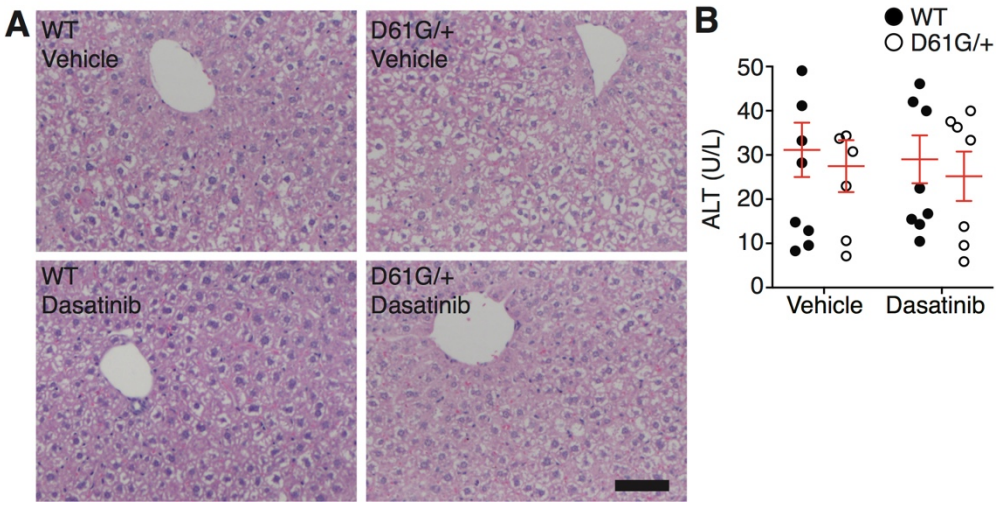
Supplemental Figure 2. Postnatal dasatinib administration does not improve growth of NS mice. (**A**) Growth curves of postnatal vehicle- or dasatinib-treated WT and NS mice (D61G/+). Differences within treatment groups were significant from 3- to 8-weeks. (**B** and **C**), body weight (**B**) and body length (**C**) were measured at P56 (n = 14 for vehicle-treated WT mice; n = 11 for vehicle-treated NS mice; n = 9 for dasatinib-treated WT mice; n = 7 for dasatinib-treated NS mice). Data represent mean ± SEM. Data were analyzed with two-way analysis of variance (ANOVA) and Tukey's multiple comparison test. ***, $p < 0.001$.



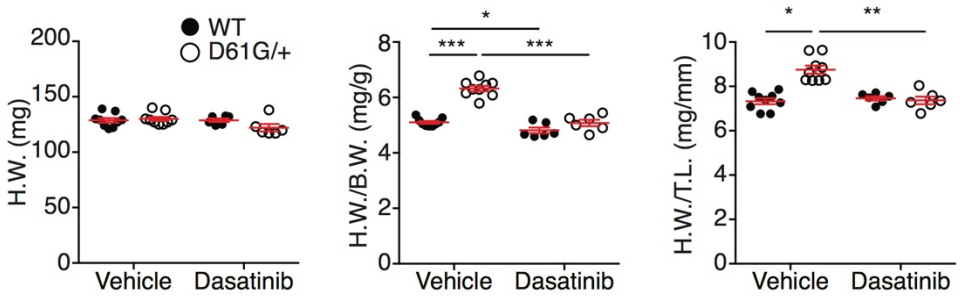
Supplemental Figure 3. No effect on facial dysmorphic features in dasatinib-treated NS mice. (**A**) Representative images of the skull and the lower jaw from postnatally vehicle- or dasatinib-treated WT and NS mice (D61G/+) at P56. Measurements were obtained at the indicated points. (**B-F**) Skull length (**B**), skull width (**C**), ratio of skull length to skull width (**D**), intercantal distance (ICD) (**E**) and lower jaw length (**F**) were measured (n = 14 for vehicle-treated WT mice; n = 11 for vehicle-treated NS mice; n = 9 for dasatinib-treated WT mice; n = 7 for dasatinib-treated NS mice). Graph data represent mean ± SEM. Data were analyzed with two-way analysis of variance (ANOVA) and Tukey's multiple comparison test. **, $p < 0.01$; ***, $p < 0.001$.



Supplemental Figure 4. Spleen phenotypes in dasatinib-treated NS mice. (**A**) Representative H&E-stained histological images of the spleen from postnatally vehicle- or dasatinib-treated WT and NS mice at P56 (bar = 50 μm). (**B-C**) Spleen weight (**B**) and the ratio of spleen weight (Sp.W.) to body weight (B.W.) (**C**) were measured ($n = 14$ for vehicle treated WT mice; $n = 14$ for vehicle-treated NS mice; $n = 9$ for dasatinib-treated WT mice; $n = 7$ for dasatinib-treated NS mice). Graph data represent mean ± SEM. Data were analyzed with two-way analysis of variance (ANOVA) and Tukey's multiple comparison test. ***, $p < 0.001$.



Supplemental Figure 5. Liver phenotypes in dasatinib-treated NS mice. (**A**) Representative H&E-stained histological images of the liver from postnatal vehicle- or dasatinib-treated WT and NS mice (D61G/+) at P56 (bar = 50 μm). (**B**) The enzymatic activities of alanine aminotransferase (ALT) in serum were measured from vehicle- or dasatinib-treated WT and NS mice (n = 10 for vehicle treated WT mice; n = 7 for vehicle-treated NS mice; n = 9 for dasatinib-treated WT mice; n = 7 for dasatinib-treated NS mice). Graph data represent mean ± SEM. Data were analyzed with two-way analysis of variance (ANOVA) and Tukey's multiple comparison test.



Supplemental Figure 6. Gross morphological analysis of dasatinib treated NS mice heart. Heart weight (H.W.), the ratio of H.W. to body weight (B.W.) or tibialis length (T.L.) were measured from 8-week-old vehicle- or dasatinib treated WT and NS mice (D61G/+). Data were analyzed with two-way analysis of variance (ANOVA) and Tukey's multiple comparison test. ∗, $p < 0.05$; ∗∗, $p < 0.01$; ∗∗∗, $p < 0.001$.

Supplemental Table 1. Progeny from NS mice breeding. NS male mice (*Ptpn11*$^{D61G/+;}$ B6129SF2/Tac background) were crossed with wild type B6129SF1/Tac female mice. Progeny were genotyped at postnatal day 10 (P10). Data were analyzed with chi-square goodness of fit test.

	WT	*Ptpn11*$^{D61G/+}$	*n*	χ^2	*p*
P10	135	108	243	3.000	0.0833
(Male)	66	51	117	1.923	0.1655
(Female)	69	57	126	1.143	0.2850

Supplemental Table 2. **Echocardiography parameters of WT and NS mice (D61G/+).**

	3-weeks		8-weeks	
	WT (n=9)	D61G/+ (n=9)	WT (n=9)	D61G/+ (n=9)
IVS,d (mm)	0.56±0.02	0.70±0.02***	0.66±0.01	0.65±0.02
IVS,s (mm)	0.95±0.04	1.11±0.05*	1.10±0.03	0.89±0.02
LVID,d (mm)	3.33±0.06	2.95±0.06***	3.86±0.03	3.86±0.03
LVID,s (mm)	2.22±0.04	1.90±0.06***	2.43±0.05	2.95±0.08***
LVPW,d (mm)	0.60±0.02	0.72±0.02***	0.76±0.01	0.75±0.01
LVPW,s (mm)	0.84±0.10	0.95±0.04*	1.16±0.02	0.98±0.01*
LV vol,d (mm^3)	45.30±1.96	33.88±1.78***	64.53±1.05	64.65±1.29
LV vol,s (mm^3)	16.60±0.79	11.30±0.90***	20.93±0.38	30.99±0.97**
%EF	63.28±1.26	66.74±1.72	68.88±1.36	49.80±1.81**
%FS	33.42±0.90	35.72±1.24	36.80±0.62	26.72±0.56***
LV mass (mg)	54.65±1.85	59.64±1.24*	90.97±5.51	89.88±7.37
Heart rate (bpm)	430.00±20.31	466.67±14.46	468.75±18.61	446.50±28.65

Data represents the mean ± SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ denotes significance compared with the same aged WT mice. All p values were derived using unpaired Student's t-test. IVS, Interventricular septum wall thickness; LVID, left ventricular internal dimension; LVPW, left ventricular posterior wall thickness; LV vol, left ventricle volume; EF, ejection fraction; FS, fractional shortening; LV mass, left ventricular mass; d, diatole; s, systole; bpm, beat per minute.

Supplemental Table 3. Progeny from prenatal dasatinib-treated *Ptpn11*$^{D61G/+}$ mice. NS male mice (*Ptpn11*$^{D61G/+;}$ B6129SF2/Tac background) were crossed with wild type B6129SF1/Tac female mice. Pregnant female mice were treated with 0.1 mg/kg of Dasatinib from E7.5 until postnatal day 9. Data are shown for the number of viable pups at postnatal day 10.

	WT	*Ptpn11*$^{D61G/+}$	Dead pup
Vehicle	21	20	-
Dasatinib (0.1 mg/kg)	25	19	4

Supplemental Table 4. Echocardiography parameters of *in utero* for vehicle- or dasatinib-treated WT and NS mice (D61G/+) at P42.

	Vehicle		Dasatinib	
	WT (n=9)	D61G/+ (n=9)	WT (n=8)	D61G/+ (n=8)
IVS,d (mm)	0.69±0.06	0.57±0.03	0.67±0.04	0.65±0.06
IVS,s (mm)	1.10±0.06	1.01±0.06	1.08±0.09	0.97±0.10
LVID,d (mm)	3.95±0.15	3.55±0.10	3.93±0.08	3.61±0.12
LVID,s (mm)	2.45±0.19	3.01±0.07**	2.49±0.09	2.32±0.12††
LVPW,d (mm)	0.74±0.03	0.78±0.02	0.76±0.05	0.70±0.04
LVPW,s (mm)	1.23±0.08	0.97±0.06*	1.13±0.04	1.12±0.07
LV vol,d (mm^3)	68.02±4.23	55.82±2.96	67.51±2.54	60.93±3.75
LV vol,s (mm^3)	20.15±3.15	30.65±1.18**	22.26±1.95	24.21±1.56
%EF	66.84±1.43	43.30±3.38**	64.24±2.52	62.62±2.75††
%FS	36.22±1.21	25.84±3.01**	34.70±1.82	34.29±2.09††
LV mass (mg)	78.62±5.81	76.14±5.28	76.70±4.50	63.54±9.58
Heart rate (bpm)	457.88±26.89	390.386±25.07	474.88±24.08	440.58±29.39

Data represents the mean ± SEM. *, $p < 0.05$; **, $p < 0.01$ denotes significance compared with the vehicle treated WT mice. ††, $p < 0.01$ denotes significance compared with the vehicle-treated *Ptpn11*$^{D61G/+}$ mice. All p values were derived using two-way analysis of variance (ANOVA) and Tukey's multiple comparison test. IVS, Interventricular septum wall thickness; LVID, left ventricular internal dimension; LVPW, left ventricular posterior wall thickness; LV vol, left ventricle volume; EF, ejection fraction; FS, fractional shortening; LV mass, left ventricular mass; d, diatole; s, systole; bpm, beat per minute.

Supplemental Table 5. Echocardiography parameters of post-developmental vehicle- or dasatinib-treated WT and NS mice (D61G/+) at P42.

	Vehicle		Dasatinib	
	WT (n=7)	D61G/+ (n=6)	WT (n=6)	D61G/+ (n=6)
IVS,d (mm)	0.65±0.06	0.54±0.04	0.71±0.05	0.66±0.05
IVS,s (mm)	1.06±0.06	1.02±0.09	1.13±0.06	1.04±0.07
LVID,d (mm)	3.83±0.17	3.55±0.13	3.80±0.11	3.63±0.07
LVID,s (mm)	2.36±0.18	2.96±0.07**	2.45±0.05	2.50±0.08†
LVPW,d (mm)	0.72±0.03	0.77±0.02	0.81±0.04	0.71±0.03
LVPW,s (mm)	1.19±0.07	0.95±0.05*	1.25±0.07	1.09±0.06
LV vol,d (mm^3)	68.43±6.10	56.10±4.28	65.87±2.78	57.35±2.19
LV vol,s (mm^3)	20.15±3.73	30.90±1.73**	22.24±0.63	24.67±1.18
%EF	65.11±2.42	46.44±5.16**	65.70±0.82	60.93±2.06†
%FS	35.04±1.69	24.39±2.92**	35.52±0.69	33.08±1.33†
LV mass (mg)	80.72±6.62	79.54±4.79	80.66±3.56	67.05±3.86
Heart rate (bpm)	458.00±36.55	375.67±16.49*	476.33±21.10	427.17±10.06

Data represents the mean ± SEM. *, $p < 0.05$; **, $p < 0.01$ denotes significance compared with the vehicle treated WT mice. †, $p < 0.05$ denotes significance compared with the vehicle-treated *Ptpn11*$^{D61G/+}$ mice. All p values were derived using two-way analysis of variance (ANOVA) and Tukey's multiple comparison test. IVS, Interventricular septum wall thickness; LVID, left ventricular internal dimension; LVPW, left ventricular posterior wall thickness; LV vol, left ventricle volume; EF, ejection fraction; FS, fractional shortening; LV mass, left ventricular mass; d, diatole; s, systole; bpm, beat per minute.

Supplemental Table 6. **Echocardiography parameters of post-developmental vehicle- or dasatinib-treated WT and NS mice (D61G/+) at P56.**

	Vehicle		Dasatinib	
	WT (n=9)	D61G/+ (n=9)	WT (n=6)	D61G/+ (n=6)
IVS,d (mm)	0.71±0.03	0.69±0.05	0.69±0.03	0.72±0.04
IVS,s (mm)	1.17±0.10	1.01±0.04	1.19±0.06	1.18±0.05
LVID,d (mm)	3.86±0.08	3.86±0.10	3.92±0.09	3.93±0.08
LVID,s (mm)	2.43±0.06	2.95±0.10***	2.52±0.10	2.60±0.07†
LVPW,d (mm)	0.78±0.02	0.77±0.04	0.80±0.06	0.65±0.03
LVPW,s (mm)	1.22±0.05	0.96±0.04***	1.18±0.05	1.19±0.05†
LV vol,d (mm^3)	69.38±3.40	69.22±5.22	64.06±2.65	65.60±2.44
LV vol,s (mm^3)	20.21±1.05	32.80±2.76***	23.06±2.21	24.85±1.68†
%EF	68.72±2.09	50.92±2.50***	65.89±1.74	64.71±1.67†††
%FS	39.31±1.13	25.64±1.53***	36.63±1.20	34.92±1.23†††
LV mass (mg)	88.38±5.62	89.88±7.38	80.04±4.26	74.90±3.70
Heart rate (bpm)	450.00±24.92	433.67±28.34	495.83±19.60	457.83±13.17

Data represents the mean ± SEM. **, $p < 0.01$; ***, $p < 0.001$ denotes significance compared with the vehicle treated WT mice. †, $p < 0.05$; ††, $p < 0.01$; †††, $p < 0.001$ denotes significance compared with the vehicle-treated *Ptpn11*$^{D61G/+}$ mice. All p values were derived using two-way analysis of variance (ANOVA) and Tukey's multiple comparison test. IVS, Interventricular septum wall thickness; LVID, left ventricular internal dimension; LVPW, left ventricular posterior wall thickness; LV vol, left ventricle volume; EF, ejection fraction; FS, fractional shortening; LV mass, left ventricular mass; d, diatole; s, systole; bpm, beat per minute.

Supplemental Table 7. Hemodynamic analysis parameters of post-developmental vehicle- or dasatinib-treated WT and NS mice (D61G/+) at P56.

	Vehicle		Dasatinib	
	WT (n=8)	D61G/+ (n=9)	WT (n=7)	D61G/+ (n=9)
Systolic pressure (mmHg)	142.2±9.3	102.8±3.1***	146.2±5.1	129.3±6.2†
Diastolic pressure (mmHg)	89.1±5.0	61.8±2.2***	95.1±4.2	84.0±3.5††
Pulse pressure (mmHg)	67.2±5.3	53.0±1.5	65.2±4.6	63.7±4.4
Mean arterial pressure (mmHg)	106.8±6.3	75.5±2.4***	112.1±4.0	96.8±4.2††
Left ventricle pressure (mmHg)	137.9±8.0	105.6±3.6**	136.5±2.5	127.9±4.5†
End diastolic pressure (mmHg)	7.8±1.8	10.5±0.9	8.7±1.3	11.1±0.7
+*dP/dp* (mmHg/s)	8497±556	5850±282**	8626±476	7718±498†
-*dP/dt* (mmHg/s)	-6075±235	-5354±538	-6529±847	-6189±626

Data represents the mean ± SEM. **, $p < 0.01$; ***, $p < 0.001$ denotes significance compared with the vehicle treated WT mice. †, $p < 0.05$; ††, $p < 0.01$ denotes significance compared with the vehicle-treated *Ptpn11*$^{D61G/+}$ mice. All p values were derived using two-way analysis of variance (ANOVA) and Tukey's multiple comparison test.

Supplemental Table 8. Ca^{2+} excitation-contraction coupling parameters of cardiomyocytes isolated from the heart of post-developmental vehicle- or dasatinib-treated WT and NS mice (D61G/+) at P56.

	Vehicle		Dasatinib	
	WT (n=131 cell, n= 3 mice)	D61G/+ (n=128 cells, n=3 mice)	WT (n=111 cells, n=3 mice)	D61G/+ (n=162 cells, n=3 mice)
Diastolic Ca^{2+} (Min $F_{340/380}$)	1.05±0.01	1.00±0.01***	0.94±0.01***	0.98±0.01
Systolic Ca^{2+} (Max $F_{340/380}$)	1.21±0.01	1.24±0.01	1.06±0.01***	1.12±0.01†††
Rmag Ca^{2+} ($\Delta F_{340/380}$)	0.16±0.01	0.25±0.01***	0.11±0.01***	0.14±0.01†††
Ca^{2+} TTP (ms, $F_{340/380}$)	42.99±0.95	40.41±0.76	42.75±1.21	43.22±0.90
Tau Ca^{2+} (ms)	109.18±2.67	123.13±1.88***	105.85±2.35	116.52±2.36
Peak Shortening (%)	5.22±0.19	4.09±0.20***	6.04±0.23*	6.59±0.18†††
Shortening TTP (ms)	64.45±0.82	67.75±0.97*	71.52±0.90***	69.32±0.79
Shortening RT50 (ms)	35.20±0.70	38.74±1.03*	41.11±0.96***	37.02±0.62†
Shortening RT90 (ms)	96.47±3.60	112.16±4.35*	113.38±4.18*	99.24±3.23

Data represents the mean ± SEM. *, $p < 0.05$; ***, $p < 0.001$ denotes significance compared with the vehicle treated WT mice. †, $p < 0.05$; †††, $p < 0.001$ denotes significance compared with the vehicle-treated *Ptpn11*$^{D61G/+}$ mice. All p values were derived using two-way analysis of variance (ANOVA) and Tukey's multiple comparison test. TTP, Time to peak; RT50, Time from peak tension to 50% relaxation; RT90, Time from peak tension to 90% relaxation.