Fulgenzi et al., http://www.jcb.org/cgi/content/full/jcb.201502100/DC1

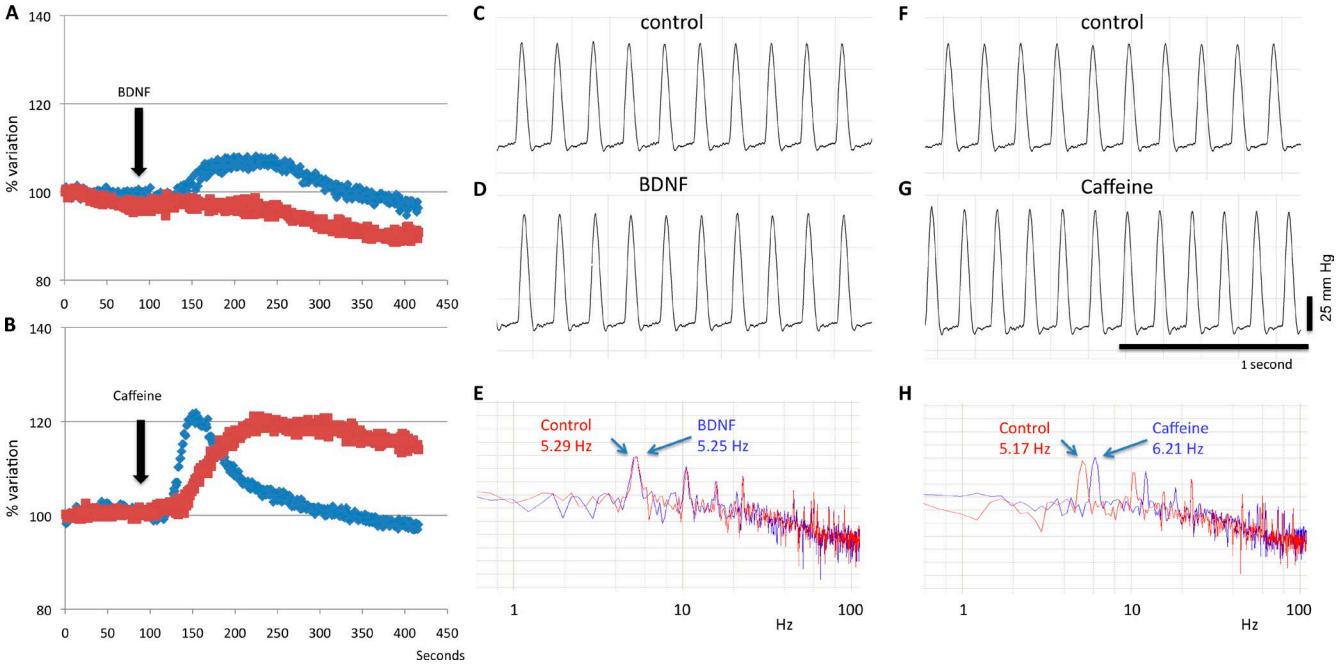

Figure S1. **BDNF does not alter the spontaneous cardiac beating frequency.** (A) BDNF applied (arrow) to a nonpaced Langendorff-perfused heart induces an increase in LVDP (blue trace) but does not alter the spontaneous beating frequency (red trace). (B) Caffeine used as control was injected 5 min after BDNF. Note that the caffeine induces an increase in LVPD and a substantial increase in spontaneous beating frequency whereas BDNF only increases LVPD but not beating frequency. (C and D) Recorded LVPD before and after BDNF application. (E) Frequency power spectrum calculated on the trace showed in C (red trace) and D (blue trace). (F and G) Recorded LVPD before and after caffeine application. (H) Frequency power spectrum calculated on the trace showed in F (red trace) and G (blue trace). Arrows in E and H indicate the spontaneous beating frequency.

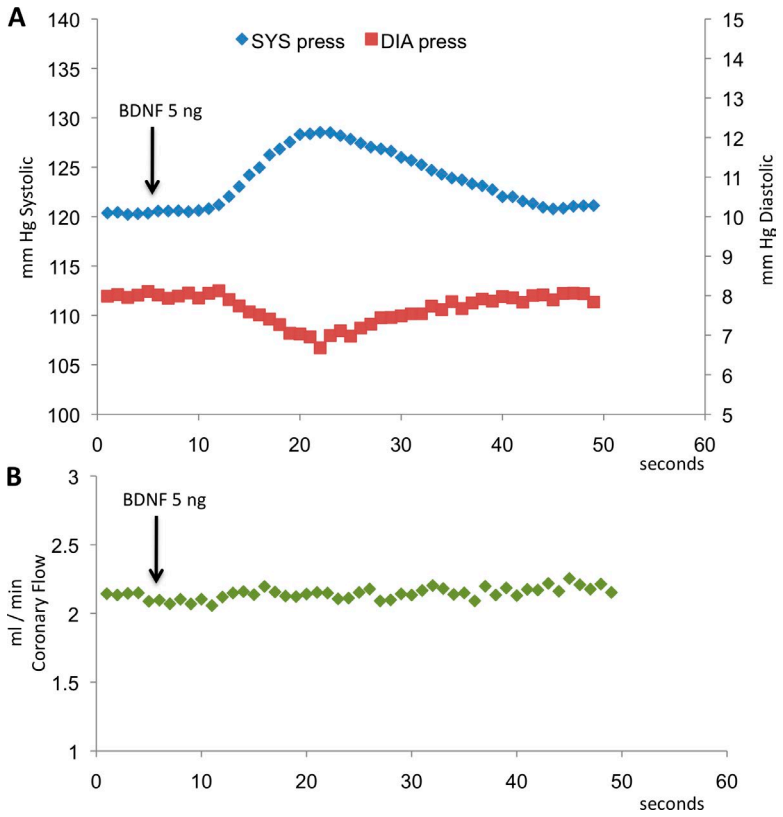

Figure S2. **BDNF increases systolic and decreases diastolic pressure but has no effect on coronary flow.** Systolic (blue) and diastolic (red) ventricular pressure (A) as well as coronary flow (B, green) were measured in a Langendorff-perfused heart electrically paced at 420 bpm after application of BDNF (5 ng; arrow).

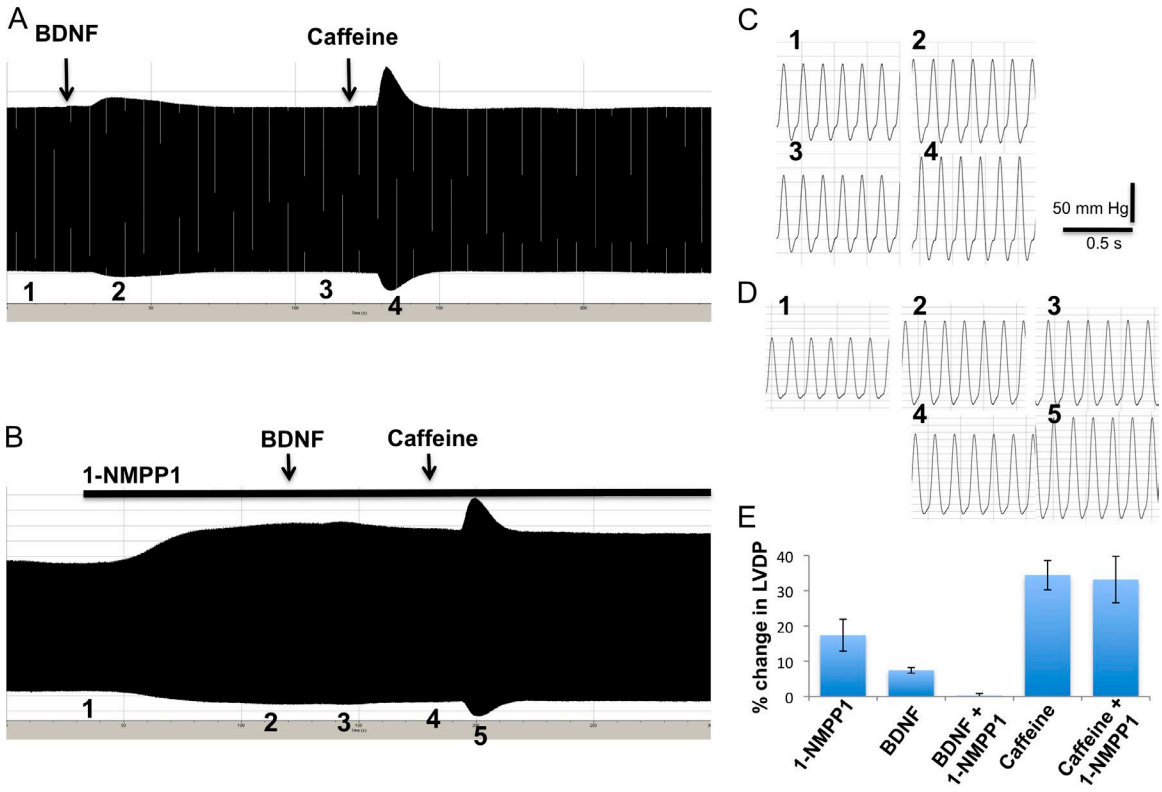

Figure S3. **1-NMPP1 exerts a potent inotropic and lusitropic effect in Langendorff-perfused hearts that occlude BDNF effect on contractility.** (A and B) Representative upper and lower traces showing the LVDP variation of a WT heart in response to a bolus of BDNF (50 µl and 10 ng) and caffeine (50 µl and 5 mM) in the absence (A) or presence (B) of 100 nM 1-NMPP1. Arrows indicate the time of injection in the fluid stream. Note the disappearance of the inotropic and lusitropic effect of BDNF in the presence of 1-NMPP1 (B, black horizontal bar) and the strong inotropic and lusitropic effect exerted by 1-NMPP1 itself. BDNF was applied after the heart reached a stable LVDP and the caffeine was applied 1 min later (arrow) as a positive control. (C and D) Enlargement of the original traces at the indicated time points (1–4, C from panel A; and 1–5, D from panel B). (E) Quantification (mean ± SEM) of percent change in LVPD in response to 1-NMPP1, BDNF, caffeine relative to the baseline or to BDNF, and caffeine relative to the basal value in the presence of 1-NMPP1 (B; $n = 7$ independent experiments).

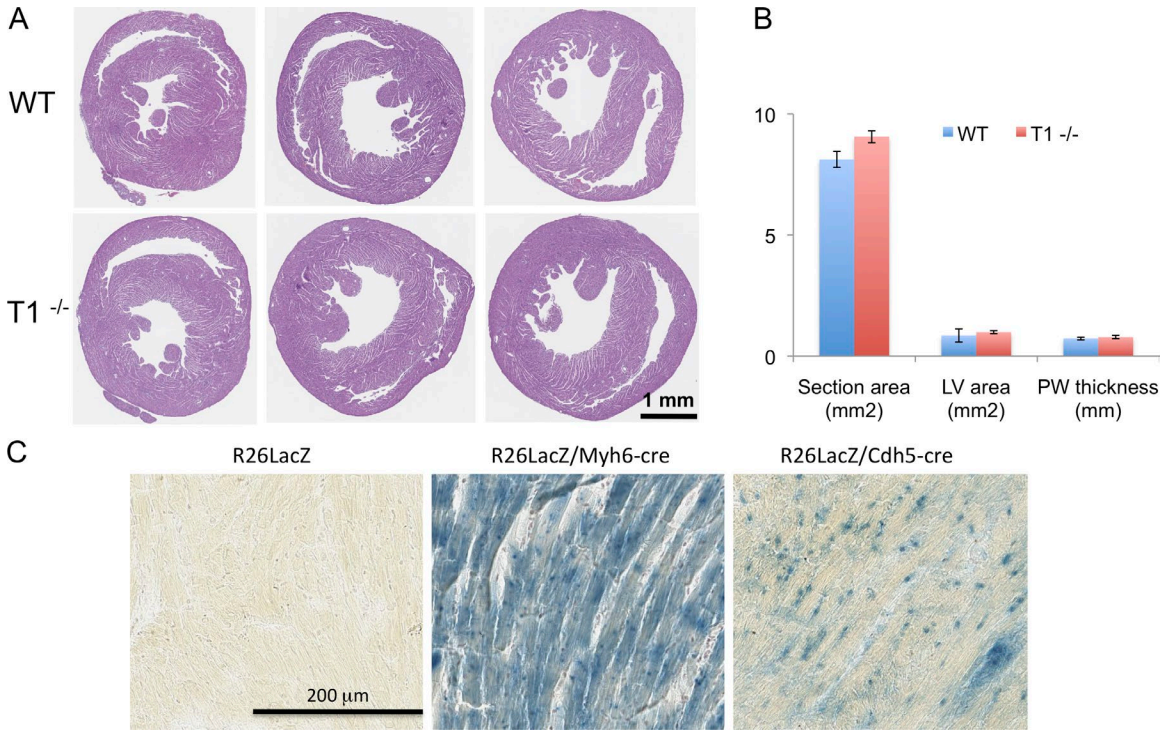

Figure S4. **TrkB.T1 deletion does not cause obvious early postnatal cardiac developmental defects.** (A) Hearts from WT (top) and TrkB.T1 deficient ($T1^{-/-}$; bottom) postnatal day 12 mice were dissected, fixed, sectioned transversally at the level of the papillary muscles, and hematoxylin and eosin stained. Note the similarity and lack of any apparent damage in the mutant mice compared with controls. (B) Histogram showing the quantification (mean ± SEM) of the total section and left ventricle (LV) area and posterior wall (PW) thickness from the mutant and control mouse hearts. (C) Cre recombinase from Myh6-cre and cdh5-cre transgenic mice is active, respectively, in cardiomyocytes and endothelium. Heart sections from a Rosa26floxedLacZ (R26LacZ) mouse used as control, a R26LacZ mouse crossed with a Myh6-cre or a R26LacZ mouse crossed with a Cdh5-cre transgenic mouse and stained for B-galactosidase. Note the specific B-galactosidase staining in the cardiac myofibers and the endothelium cells induced by the activity of the Myh6-cre and Cdh5-cre transgenic mice, respectively.

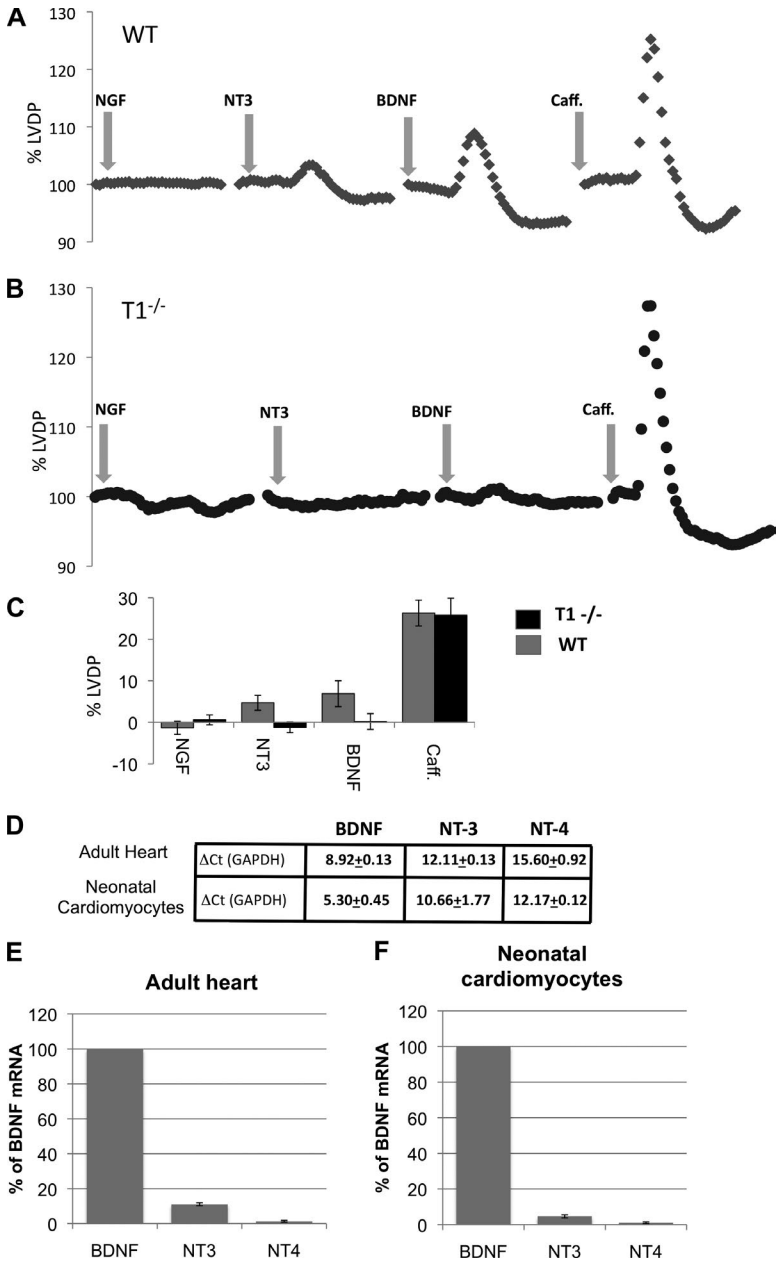

Figure S5. **NT3 but not NGF causes an increase in the contraction force of WT but not TrkB.T1-deficient hearts.** 1 ng of NGF, NT3, and BDNF in a 50-µl bolus solution was injected in the perfusion system of a WT (A) or a TrkB.T1 (B) heart spaced by a period of 5 min from each other. Caffeine (5 mM) was injected 5 min after the last neurotrophin injection as positive control. (C) Quantified from three different experiments (mean ± SEM). (D–F) BDNF is the main neurotrophin expressed in neonatal and adult cardiomyocytes. (D) Quantification of real-time PCR analysis as expressed by the number of PCR cycles at which BDNF, NT-3–, or NT-4—pecific PCR product are equal to GAPDH level (Δ Ct) from total adult heart and cardiomyocyte RNA. Note that BDNF is expressed at a significantly higher level than the other neurotrophins with BDNF PCR product appearing in the adult heart after only 9 cycles of GADPH detection versus 12 and 15 cycles of NT-3 and NT-4, respectively. The differences in neonatal cardiomyocytes were even more dramatic as NT3 and NT4 transcripts appeared, respectively, five and seven cycles later than BDNF. $n = 3$. (E and F) Histograms showing the values of NT-3– and NT-4–specific mRNA represented as a percentage of BDNF transcripts in adult heart (E) and neonatal cardiomyocytes (F). Data are presented as the mean ± SEM.

Table S1. **Functional parameters recorded in Langendorff-perfused hearts from WT and T1$^{-/-}$ animals**

Mice	HBR	basal flow	min slope	max slope[a]	LVDP[b]
	bpm	*ml/g × min*	*mmHg/s*	*mmHg/s*	*mmHg*
WT (*n* = 17)	376.0	19.16	−3,144	5,047	96
T1$^{-/-}$ (*n* = 11)	394.2	27.01	−2,906	3,794	73

After 10 min of stabilization, 1 min of recording was averaged from each animal studied. Only the spontaneous beating frequency (bpm) was calculated after the initial stabilization but before the pacing at 420 bpm was initiated. Student's *t* test value is reported for significantly different parameters. Values are ±SEM. HBR; heart beat rate.
[a]P = 0.043.
[b]P = 0.035.